FORENSIC BIOLOGY
PROTOCOLS FOR FORENSIC STR ANALYSIS

Approving Authority:
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Procedures	Effective Date	Revision History
General Guidelines for DNA Casework	3/24/2010	Initial Version
DNA Extraction		
Chelex Extraction from Blood and Buccal Swabs	3/24/2010	Initial Version
Chelex Extraction from Soft Tissue (e.g., Fetus Samples)	3/24/2010	Initial Version
Chelex Extraction from Epithelial Cells (Amylase Positive Samples)	3/24/2010	Initial Version
Chelex Extraction from Semen Stains or Swabs (Non-Differential)	3/24/2010	Initial Version
Chelex Extraction from Semen Stains or Swabs (Differential)	3/24/2010	Initial Version
Chelex Extraction from Hair	3/24/2010	Initial Version
Organic Extraction	3/24/2010	Initial Version
High Sensitivity Extraction	3/24/2010	Initial Version
Extraction of Exogenous DNA from Nails	3/24/2010	Initial Version
MagAttract DNA Extraction from Bloodstains and Exemplars	3/24/2010	Initial Version
MagAttract DNA Extraction from Other Casework Samples	3/24/2010	Initial Version
Microcon YM100 DNA Concentration and Purification	3/24/2010	Initial Version

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Estimation of DNA Quantity Using the RotorGene™	3/24/2010	Initial Version
General Guidelines for Fluorescent STR Analysis	3/24/2010	Initial Version
Identifiler Kit and Y-Multiplex 1 (YM1) Kit		
Generation of Amplification Sheets	3/24/2010	Initial Version
Amplification – Sample Preparation	3/24/2010	Initial Version
STR Analysis	3/24/2010	Initial Version
STR Data Conversion and Archiving	3/24/2010	Initial Version
GeneScan Analysis	3/24/2010	Initial Version
Genotyper Analysis	3/24/2010	Initial Version
PowerPlex Y Kit		
Amplification	3/24/2010	Initial Version
Capillary Electrophoresis	3/24/2010	Initial Version
Analysis	8/2/2010	Initial Version
MiniFiler Kit		
Amplification	3/24/2010	Initial Version
Capillary Electrophoresis	3/24/2010	Initial Version
STR Analysis with GeneMapper ID	3/24/2010	Initial Version
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Editing Codes	3/24/2010	Initial Version
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Analysis Method Editor	3/24/2010	Initial Version
Troubleshooting	3/24/2010	Initial Version
References – Allelic Ladders, Controls, and Size Standards	8/2/2010	Initial Version
Default Table and Plot Settings	3/24/2010	Initial Version
STR Results Interpretation	3/24/2010	Initial Version
Additional Interpretations of Y-STR Results and Complex Y-STR Results	3/24/2010	Initial Version
Population Frequencies for STR's	3/24/2010	Initial Version
Paternity Analysis	3/24/2010	Initial Version
References	3/24/2010	Initial Version
DNA-VIEW For Paternity and Kinship Analysis	3/24/2010	Initial Version
Appendix	3/24/2010	Initial Version

Laboratory organization

1. To minimize the potential for carry-over contamination, the laboratory is organized so that the areas for DNA extraction, PCR set-up, and handling amplified DNA are physically isolated from each other. Each of the three areas is in a separate room.

2. Based on need, microcentrifuge tube racks have been placed in sample handling areas. These racks should only leave their designated area to transport samples to the next designated area. Immediately after transporting samples, the racks should be cleaned and returned to their designated area.

3. Dedicated equipment such as pipetters should not leave their designated areas. Only the samples in designated racks should move between areas.

4. Analysts in each work area must wear appropriate personal protective equipment (PPE). Contamination preventive equipment (CPE) must be worn where available. All PPE and CPE shall be donned in the bio-vestibules.

   Required PPE and CPE for each laboratory are posted conspicuously in each bio-vestibule.

Work Place Preparation

1. Apply 10% bleach followed by water and/or 70% Ethanol to the entire work surface, cap opener, and pipettes.

2. Obtain clean racks and cap openers, and irradiated microcentrifuge tubes, and irradiated water from storage. **Arrange work place to minimize crossover.**

3. Position gloves nearby with 10% Bleach/70% Ethanol/water in order to facilitate frequent glove changes and cleaning of equipment.

Microcentrifuge tube and pipette handling

1. Microcentrifuge tubes, Microcon collection tubes, Dolphin tubes, and M48 tubes must be irradiated prior to use.

2. Avoid splashes and aerosols. Centrifuge all liquid to the bottom of a closed microcentrifuge tube before opening it.

3. Avoid touching the inside surface of the tube caps with pipetters, gloves, or lab coat sleeves.

4. Use the correct pipetter for the volume to be pipetted. For pipetters with a maximum volume of 20µL or over, the range begins at 10% of its maximum volume (i.e., a 100µL pipette can be used for volumes of 10-100µL). For pipetters with a maximum volume of 10µL or under, the range begins at 5% of its maximum volume (i.e., a 10µL pipette can be used for volumes of 0.5-10µL).

5. Filter pipette tips must be used when pipetting DNA and they should be used, whenever possible, for other reagents. Use the appropriate size filter tips for the different pipetters; the tip of the pipette should never touch the filter.

6. Always change pipette tips between handling each sample.

7. Never "blow out" the last bit of sample from a pipette. Blowing out increases the potential for aerosols, this may contaminate a sample with DNA from other samples. The accuracy of liquid volume delivered is not critical enough to justify blowing out.

8. Discard pipette tips if they accidentally touch the bench paper or any other surface.

9. Wipe the outside of the pipette with 10% bleach solution followed by a 70% ethanol solution if the barrel goes inside a tube.

Sample handling

1. Samples that have not yet been amplified should never come in contact with equipment in the amplified DNA work area. Samples that have been amplified should never come in contact with equipment in the unamplified work area.

2. The DNA extraction and PCR setup of evidence samples should be performed at a separate time from the DNA extraction and PCR setup of exemplars. This precaution helps to prevent potential cross-contamination between evidence samples and exemplars.

3. Use disposable bench paper to prevent the accumulation of human DNA on permanent work surfaces. 10% bleach followed by 70% ethanol should always be used to decontaminate all work surfaces before and after each procedure.

4. Limit the quantity of samples handled in a single run to a manageable number. This precaution will reduce the risk of sample mix-up and the potential for sample-to-sample contamination.

5. Change gloves frequently to avoid sample-to-sample contamination. Change them whenever they might have been contaminated with DNA and whenever exiting a sample handling area.

6. Make sure worksheets and logbooks are completely filled out.

All worksheets must have the handwritten initials of the individual performing the test.

Body fluid identification

1. The general laboratory policy is to identify the stain type (i.e., blood, semen, or saliva) before individualization is attempted on serious cases such as sexual assaults, homicides, robberies, and assaults. However, circumstances may exist when this will not be possible. For example, on most property crime cases when a swab of an item is submitted for testing, the analyst will cut the swab directly for individualization rather than testing the swab for body fluid identification.

2. A positive screening test for blood followed by the detection of a real-time PCR quantitation value greater than or equal to 0.1 pg/µL is indicative of the presence of human blood.

3. High Copy Number (HCN) testing is performed when the samples have a quantitation value ≥7.5 pg/uL for YM1 (at least 195 pg per amp), ≥20 pg/µL for Identifiler 28 cycles (at least 100 pg per amp), ≥10 pg/uL for Minifiler (at least 100pg per amp), or ≥5 pg/uL for PowerPlex Y (at least 100pg per amp).

   High Sensitivity DNA testing (Identifiler 31 cycles) can be performed if samples have a quantitation value of less than 7.5 pg/µL (or 20 pg/µL) and greater than 1 pg/µL.

DNA Extraction Guidelines

Slightly different extraction procedures may be required for each type of specimen. Due to the varied nature of evidence samples, the user may need to modify procedures.

1. All tube set-ups must be witnessed/confirmed **prior** to starting the extraction (**NOTE:** For differential extractions, the tube set-up should be witnessed after the incubation step.)

2. Use Kimwipes or a tube opener to open tubes containing samples; only one tube should be uncapped at a time.

3. When pouring or pipetting Chelex solutions, the resin beads must be distributed evenly in solution. This can be achieved by shaking or vortexing the tubes containing the Chelex stock solution before aliquoting.

4. For pipetting Chelex, the pipette tip used must have a relatively large bore – 1 mL pipette tips are adequate.

5. Be aware of small particles of fabric, which may cling to the outside of tubes.

6. With the exception of the Mitochondrial DNA Team and the High Sensitivity Team, two extraction negative controls (E-neg) must be included with each batch of extractions to demonstrate extraction integrity. The first E-Neg will typically be subjected to a micro-con and will be consumed to ensure that an E-neg associated with each extraction set will be extracted concurrently with the samples, and run using the same instrument model and under the same or more sensitive injection conditions as the samples. The second E-Neg will ensure that the samples in that extraction set can be sent on for further testing in another team or in a future kit. In the Mitochondrial DNA Team and the High Sensitivity Team, only one extraction negative control is needed.

   Refer to the end of this section for flow charts.

The extraction negative control contains all solutions used in the extraction process but no biological fluid or sample. For samples that will be amplified in YM1, the associated extraction negative control should be re-quantified to confirm any quantitation value of 1.00 pg/µL or greater. For samples that will be amplified in Identifiler (28 or 31 cycles), PowerPlex Y, or MiniFiler, the associated extraction negative should be re-quantified to confirm any quantitation value of 0.2 pg/µL or greater.

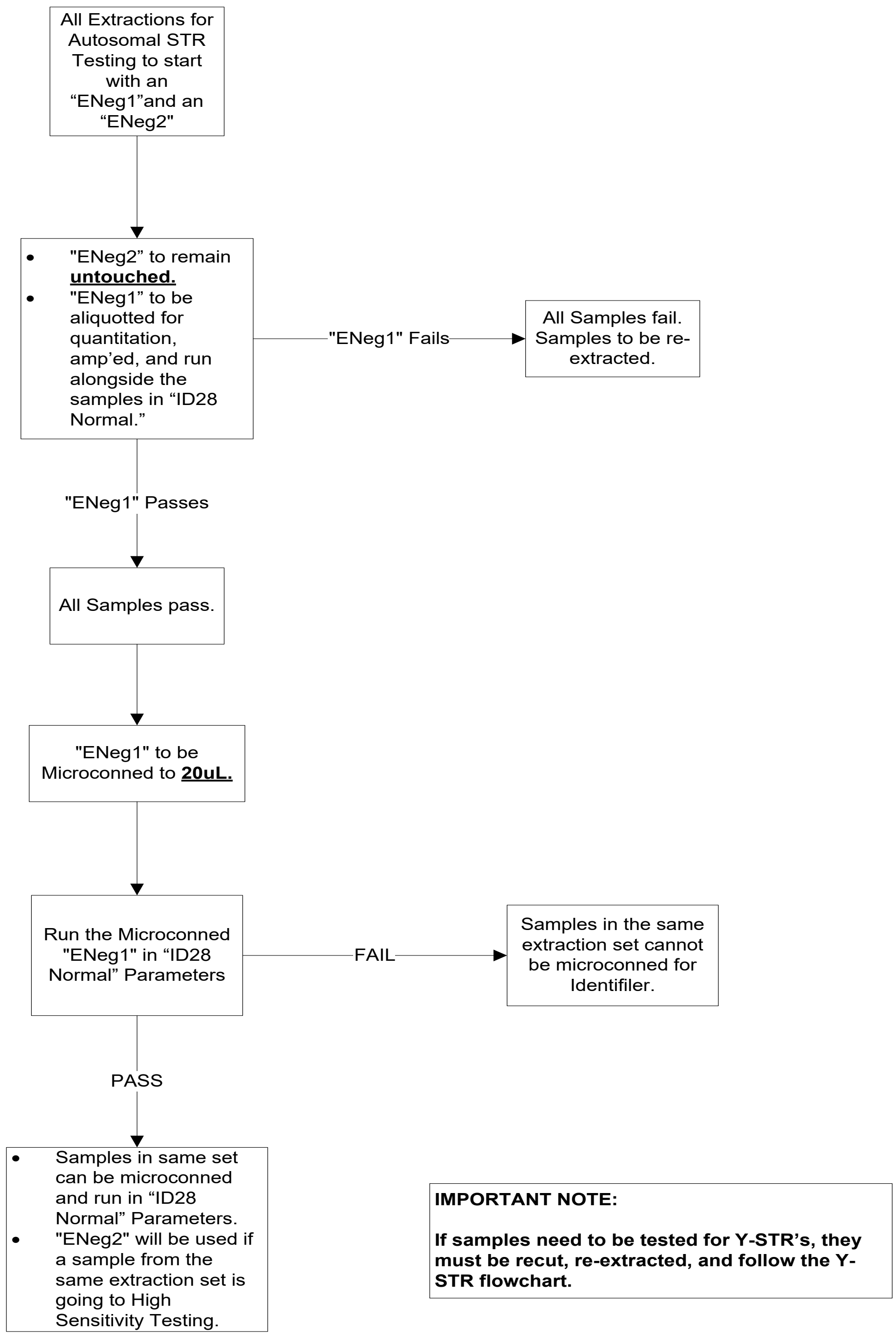
7. If a sample is found to contain less than 7.5 pg/µL of DNA, then the sample should <u>not</u> be amplified in YM1. If a sample is found to contain less than 20 pg/µL of DNA, then the sample should <u>not</u> be amplified in Identifiler (28 cycles), PowerPlex Y, or MiniFiler. It can be re-extracted, reported as containing insufficient DNA, concentrated using a Microcon-100 (see Section 3 of the STR manual), or possibly submitted for High Sensitivity testing. The interpreting analyst shall consult with a supervisor to determine how to proceed. Other DNA samples may also be concentrated and purified using a Microcon-100 if the DNA is suspected of being degraded or shows inhibition or background fluorescence during quantitation. Samples that are 1 pg/µL to 20pg/µL may be submitted for high sensitivity testing with a supervisor's permission.

8. After extraction, the tubes containing the unamplified DNA should be transferred to a box and stored in the appropriate refrigerator or freezer. The tubes should not be stored in the extraction racks.

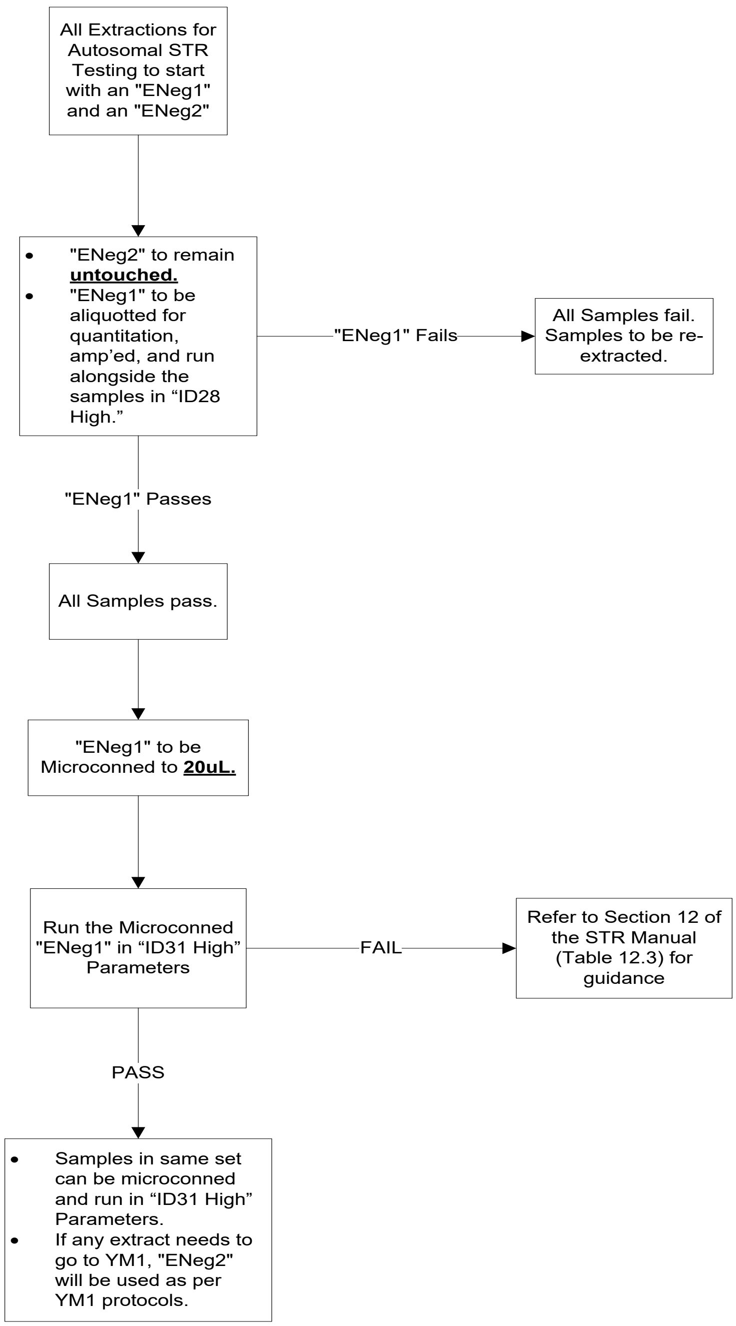
9. All tubes must have the complete case number, sample identifier and IA initials on the side of the tube. This includes aliquots submitted for quantitation.

10. Extract tracking sheets are created for each case within an extraction set. Any aliquots subsequent to the first quantitation attempt should be recorded on this tracking sheet.

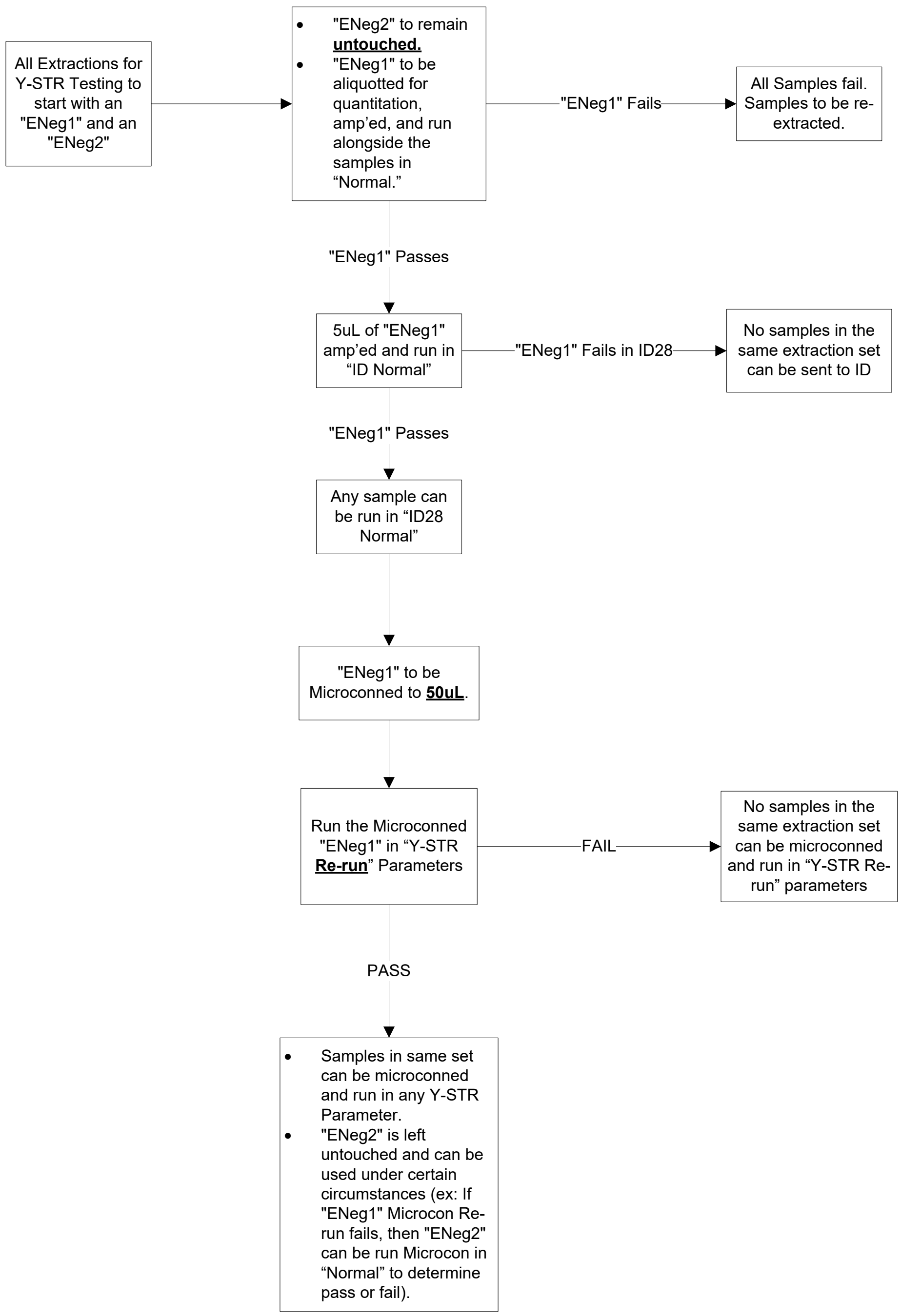
HSC, PC, and X-TEAMS – EXTRACTION NEGATIVE FLOW AUTOSOMAL STR TESTING



HYBRID TEAM – EXTRACTION NEGATIVE FLOW
AUTOSOMAL STR TESTING



Y-STR TESTING (ALL APPLICABLE TEAMS)
EXTRACTION NEGATIVE FLOW



Controls for PCR analysis

The following controls must be processed alongside the sample analysis:

1. A positive control is a DNA sample where the STR alleles for the relevant STR loci are known. The positive control tests the success and the specificity of the amplification, and during the detection and analysis stage the correct allele calling by the software.

2. An extraction negative control consists of all reagents used in the extraction process and is necessary to detect DNA contamination of these reagents. **Note:** Since the Y STR system only detects male DNA, one cannot infer from a clean Y STR extraction negative the absence of female DNA. Therefore, an extraction negative control originally typed in Y STRs must be retested if the samples are amped in Identifiler.

3. Samples that were extracted together should all be amplified together, so that every sample is run parallel to its associated extraction negative control.

4. An amplification negative control consists of only amplification reagents without the addition of DNA, and is used to detect DNA contamination of the amplification reagents.

Failure of any of the controls does not automatically invalidate the test. Under certain circumstances it is acceptable to retest negative and positive controls. **See STR Results Interpretation Procedure for rules on retesting of control samples.**

Concordant analyses and "duplicate rule"

The general laboratory policy is to confirm DNA results either by having concordant DNA results within a case, or (for 28-cycle systems) by duplicating the DNA results with a separate aliquot, amplification, and electrophoresis plate. The most common situations are confirmation of a match or exclusion within a case and confirming DNA results when less than the optimal amount of DNA is amplified. Concordant and duplicate analyses are also used to detect sample mix-up and confirm the presence of DNA mixtures.

1. For evidence samples, the following guidelines apply:

    a. Identical DNA profiles among at least two items (two evidence samples or one evidence sample plus an exemplar) within a case are considered internally concordant results ("duplicate rule").

b. If a sample does not match any other sample in the case, it must be duplicated by a second amplification. If the only result was obtained using Y-STRs, this must be duplicated in the Y system.

c. If after the first DNA analysis there is an indication that the sample consists of a mixture of DNA, several scenarios must be considered. Further analysis steps have to be decided based on the nature of each case. Consult with your supervisor if you encounter a situation that is not represented in the following examples:

   1) If all alleles in a mixture are consistent with coming from any of the known or unknown samples in the case, e.g. a victim and a semen source, no further concordance testing is needed. Further testing could be performed if needed (e.g., to obtain a CODIS profile).

   2) If two or more mixtures in a case are consistent with each other and display the same allele combinations, they are considered duplicated.

   3) If one or more alleles cannot be accounted for by other contributors in the case, the presence of the foreign component must be confirmed by a second amplification.

   4) If there is only one sample in a case and this happens to be a mixed sample, the results need to be confirmed by a second amplification.

   5) Inconclusive samples (as defined in the STR Results Interpretation Procedure) that cannot be used for comparison do not require duplication.

d. Another reason for duplication is to confirm results when a low amount of DNA is obtained from an evidence sample and/or less than optimal amounts of DNA are amplified to account for possible stochastic effects.

   Duplicate Identifiler 28 amplifications are required when there is less than 1000 pg of DNA in the total extraction volume (e.g., calculate total yield by multiplying DNA concentration by the 200 uL in a Chelex extraction); any duplicate amplification done for this reason should be performed as soon as possible after extraction to minimize loss of DNA in the extract.

Another method to satisfy this policy is if two different kits with overlapping loci are used. At least two (2) autosomal loci must be duplicated to confirm results. (For example, using Cofiler/Profiler Plus or Identifiler/MiniFiler on the same evidence sample.)

e. Automatic duplications designed to streamline testing of any evidence samples is also permitted.

2. For exemplar samples, duplication is designed to rule out false exclusions based on sample mix-up, and also to streamline testing. Duplication must start with a second independent extraction, with the exemplar cut and submitted for extraction at a different time. The two resulting extracts must be aliquotted for amplification separately at different times, and aliquotted for electrophoresis separately and run on separate plates. If there is no additional exemplar material available for extraction, the duplication may begin at the amplification stage.

To streamline testing, all suspect and victim exemplars may be duplicated.

The following guidelines apply for required duplications:

a. If the DNA profile of a **victim's exemplar** does not match any of the DNA profiles of evidence samples in the case, including mixtures, the victim's exemplar must be duplicated to eliminate the possibility of an exemplar mix-up. *This is because it is highly likely that an exemplar mix-up would generate a false exclusion.*

b. Duplication of a victim's DNA profile is not necessary in a negative case (no alleles detected in evidence samples).

c. Since duplicate exemplar analyses are performed to confirm the exclusion, a partial DNA profile (at least one complete locus) that demonstrates an exclusion is sufficient.

d. If the DNA profile of a **victim's exemplar** matches any of the DNA profiles of evidence in the case, or is present in a mixture, the exemplar does not have to be duplicated. *This is because it is highly unlikely that a sample mix-up would generate a false inclusion.*

e. If the DNA profile of a **suspect's exemplar** (or other non-victim elimination exemplar) does not match any of the DNA profiles in the case, or in the local database, the exemplar does not have to be duplicated. *This is meant to streamline the process similar to convicted offender testing*.

f. If the DNA profile of a **suspect's exemplar** matches any of the DNA profiles in the case, or in the local database, the suspect's exemplar has to be duplicated to eliminate the possibility of an exemplar mix-up. *This is meant to streamline the process similar to convicted offender testing*.

g. **Pseudo exemplars** do not have to be duplicated, regardless if the DNA profile matches any of the DNA profiles in the case.

3. For evidence samples or exemplar samples analyzed in DNA systems containing overlapping loci, the DNA results for the overlapping loci must be consistent. If no or partial results were obtained for some of the overlapping loci, this amplification is still valid if consistent results were obtained for at least one overlapping locus (Amelogenin is not considered an overlapping locus in this context). If the partial amplification confirms a match or an exclusion of an exemplar or another evidence sample, it does not have to be repeated.

4. Partial profiles can satisfy the duplication policy. Consistent DNA typing results from at least one overlapping locus in a different amplification is considered a concordant analysis.

5. For Y-STR testing, the sample does not have to be reamplified if the internal duplication rule applies or if the Y-STR results are concordant with the autosomal results: confirming an exclusion or inclusion, confirming the presence of male DNA, confirming the number of semen donors. Based on the case scenario it might be necessary to reamplify in order to confirm the exact Y-STR allele calls. There might not be sufficient autosomal data to establish concordance.

Exogenous DNA Policy

Exogenous DNA is defined as the addition of DNA/biological fluid to evidence or controls subsequent to the crime. Sources of exogenous DNA could be first responders, EMT's, crime scene technicians, MLI's, ME's, ADA's, NYPD personnel, or laboratory personnel.

1. Medical treatment and decontamination of hazardous materials are the first priority. Steps should be taken to minimize exogenous DNA as much as possible.

2. The source of any exogenous DNA should be identified so that samples can be properly interpreted. It may be possible to identify the source by:

    a. Examining other samples from the same batch for similar occurrences.

    b. Examining samples from different batches, handled or processed at approximately the same time for possible similar occurrences (such as from dirty equipment or surfaces).

    c. Processing elimination samples to look for exogenous DNA occurring in the field or by laboratory personnel

    Samples should be routinely compared to case specific elimination samples, personnel databases, and the local CODIS database for possible matches. Mixtures may have to be manually compared.

    If a negative or positive control contains exogenous DNA, all the associated samples are deemed inconclusive and their alleles are not listed in the report. The samples should be re-extracted or re-amplified, if possible.

3. If a clean result cannot be obtained or the sample cannot be repeated then the summary section of the reports should state **"The following sample(s) can not be used for comparison due to quality control reasons."**

4. Once exogenous DNA has been discovered, the first step is to try to find an alternate sample.

a. As appropriate, a new extraction, amplification, or electrophoresis of the same sample can serve as an alternate for the affected sample. For this type of alternate sample the discovery of exogenous DNA is not noted in the report. However all case notes related to the discovery of exogenous DNA are retained in the case file for review by the quality assurance group, forensic biology staff, attorneys and outside experts. A form is inserted on the right side of the case file identifying the source of the exogenous DNA by Lab Type ID Number, if known, and stating which samples were affected.

b. If there are other samples from the crime scene which would serve the same purpose, they could be used as an alternate sample. For example, in a blood trail or a blood spatter, another sample from the same source should be used. Another swab or underwear cutting should be used for a sexual assault. In this scenario, the sample containing the exogenous DNA should be listed in the summary section of the report as follows: **"The [sample] can not be used for comparison because it appears to contain DNA consistent with a {NYPD member, OCME [laboratory] member, medical responder}. Instead please see [alternate sample] for comparison".** No names for the possible source(s) of the exogenous DNA are listed in the report. All case notes related to the event are retained in the case file for review by attorneys and their experts. A form is inserted on the right side of the case file identifying the source of the exogenous DNA by Lab Type ID Number, if known, and stating which samples were affected.

5. If an alternate sample cannot be found then only samples containing a partial profile of the exogenous DNA can be interpreted. Interpreting samples containing a full profile of the exogenous DNA could lead to erroneous conclusions due to the masking effect of significant amounts of DNA.

a. If a sample has a single source of DNA and this DNA appears to be exogenous DNA then the following should be listed in the summary section of the report: **"The [sample] will not be used for comparison because it appears to contain DNA consistent with a {NYPD member, OCME [laboratory] member, medical responder}."** No names for the possible source(s) of exogenous DNA are listed in the report. All case notes related to the event are retained in the case file for review by the quality assurance group, forensic biology staff, attorneys, and outside experts. A form is inserted on the right side of the case file identifying the source of the exogenous DNA by Lab Type ID Number and stating which samples were contaminated.

b. If a sample contains a mixture of DNA and **ALL** of the alleles from the source of the exogenous DNA appear in the mixture then the following should be listed in the summary section of the report. **"The [sample] contains a mixture of DNA. The mixture is consistent with a {NYPD member, OCME [laboratory] member, medical responder} and at least [#] other individual(s)." The [sample] will not be used for comparison."** No names for the possible source(s) of exogenous DNA are listed in the report. All case notes related to the event are retained in the case file for review by the quality assurance group, forensic biology staff, attorneys, and outside experts. A form is inserted on the right side of the case file identifying the source of the exogenous DNA by Lab Type ID Number and stating which samples were affected.

DNA storage

1. Store evidence and unamplified DNA in a separate refrigerator or freezer from the amplified DNA.

2. During analysis, all evidence, unamplified DNA, and amplified DNA should be stored refrigerated or frozen. Freezing is generally better for long term storage.

3. Amplified DNA is discarded after the Genotyper analysis is completed.

4. DNA extracts are retained refrigerated for a period of time, then frozen for long-term storage.

CHELEX DNA EXTRACTION FROM BLOOD AND BUCCAL SWABS		
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Sample sizes for Chelex extraction should be approximately 3µL of liquid blood or saliva, 1/3 of a swab, or a 3x3mm cutting of a bloodstain.

1. Remove the extraction rack from the refrigerator. Extract either evidence or exemplars. Obtain a tube for the extraction negative and label it.

2. Have a witness confirm the order of the samples.

3. Pipet 1 mL of sterile deionized water into each of the tubes in the extraction rack.

4. Mix the tubes by inversion or vortexing.

5. Incubate in a shaker (at approx. 1000 rpm) at room temperature for 15 to 30 minutes.

6. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g (13,200 rpm).

7. Carefully remove supernatant (all but 30 to 50 µL). If the sample is a bloodstain or swab, leave the substrate in the tube with pellet.

8. Add 175 µL of 5% Chelex (from a well-resuspended Chelex solution).

9. Incubate at 56°C for 15 to 30 minutes.

10. Vortex at high speed for 5 to 10 seconds.

11. Incubate at 100°C for 8 minutes using a screw-down rack.

12. Vortex at high speed for 5 to 10 seconds.

13. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g (13,200 rpm).

14. Pipet aliquots of neat and/or diluted extract (using TE^{-4}) into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

15. Store the extracts at 2 to 8°C or frozen.

16. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

Revision History:
March 24, 2010 – Initial version of procedure.

CHELEX DNA EXTRACTION FROM SOFT TISSUE (E.G., FETUS SAMPLES)		
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Sample sizes for this Chelex extraction should be approximately a 3x3mm cutting of tissue.

1. Remove the extraction rack from the refrigerator. Extract either evidence or exemplars. Obtain a tube for the extraction negative and label it. Have a witness confirm the order of the samples.

2. Pipet 1 mL of sterile deionized water into each of the tubes in the extraction rack. Mix the tubes by inversion or vortexing.

3. Incubate at room temperature for 15 to 30 minutes. Mix occasionally by inversion or vortexing.

4. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g (13,200 rpm).

5. Carefully remove supernatant (all but 30 to 50 µL).

6. To each tube add: 200 µL of 5% Chelex (from a well-resuspended Chelex solution).
   1 µL of 20 mg/mL Proteinase K

7. Mix using pipette tip.

8. Incubate at 56°C for 60 minutes.

9. Vortex at high speed for 5 to 10 seconds.

10. Incubate at 100°C for 8 minutes using a screw down rack.

11. Vortex at high speed for 5 to 10 seconds.

12. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g (13,200 rpm).

13. As needed, pipet aliquots of a neat, 1/100 dilution and a 1/10,000 dilution (using TE^{-4}) into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

14. Store the extracts at 2 to 8°C or frozen.

15. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

Revision History:
March 24, 2010 – Initial version of procedure.

(FOR AMYLASE POSITIVE STAINS OR SWABS, CIGARETTE BUTTS, SCRAPINGS)

Sample sizes for this Chelex extraction should be approximately a 5x5mm cutting or 50% of the scrapings recovered from an item.

1. Remove the extraction rack from the refrigerator. Extract either evidence or exemplars. Obtain a tube for the extraction negative and label it.

2. Have a witness confirm the order of the samples.

3. To each tube add: 200 µL of 5% Chelex (from a well-resuspended Chelex solution).
   1 µL of 20 mg/mL Proteinase K

   (Note: For very large cuttings, the reaction can be scaled up to 4 times this amount. This must be indicated on the extraction sheet. Scaling up any higher requires permission from the supervisor and/or IA of the case. The final extract may need to be Microcon concentrated.)

4. Mix using pipette tip.

5. Incubate at 56°C for 60 minutes.

6. Vortex at high speed for 5 to 10 seconds.

7. Incubate at 100°C for 8 minutes using a screw down rack.

8. Vortex at high speed for 5 to 10 seconds.

9. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g (13,200 rpm).

10. As needed, pipet neat and a 1/100 dilution (using TE^{-4}) into microcentrifuge tubes for Real-Time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

11. Store the remainder of the supernatant at 2 to 8°C or frozen.

12. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

Revision History:
March 24, 2010 – Initial version of procedure.

NON-DIFFERENTIAL CHELEX DNA EXTRACTION FROM SEMEN STAINS OR SWABS		
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NOTE: For very large cuttings 200 µL of Chelex might not be enough to provide enough suspension of the sample. The reaction can be scaled up and reconcentrated using Microcon concentrators.

Sample sizes for non-differential Chelex extractions depend on the circumstances of the case. Regularly 1/3 of a swab or a 3x3mm cutting of a stain should be used. For cases where semen is present but no sperm cells were detected, the sample size can be increased.

1. Remove the extraction rack from the refrigerator. Obtain a tube for the extraction negative and label it.

2. Have a witness confirm the order of the samples.

3. To each tube add: 200 µL of 5% Chelex (from a well-resuspended Chelex solution).
   1 µL of 20 mg/mL Proteinase K
   7 µL of 1 M DTT

4. Use the pipette tip when adding the DTT to thoroughly mix the contents of the tubes.

5. Incubate at 56°C for approximately 2 hours.

6. Vortex at high speed for 10 to 30 seconds.

7. Incubate at 100°C for 8 minutes using a screw down rack.

8. Vortex at high speed for 10 to 30 seconds.

9. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g (13,200 rpm).

10. Pipet aliquots of neat and 1/100 dilution (using TE^{-4}) into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

11. Store the extracts at 2 to 8°C or frozen.

12. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

Revision History:
March 24, 2010 – Initial version of procedure.

DIFFERENTIAL CHELEX DNA EXTRACTION FROM SEMEN STAINS OR SWABS		
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Approximately 1/3 of a swab or a 3x3mm cutting of a stain should be used for this type of extraction.

1. Remove the extraction rack from the refrigerator.

2. Pipet 1 mL of PBS into each tube, including a tube for an extraction negative control, in the extraction rack.

3. Mix by inversion or vortexing.

4. Incubate at room temperature overnight or for a minimum of 1 hour using a shaking platform (at approx. 1000 rpm).

5. Have a witness confirm the order of the samples.

6. Vortex or sonicate the substrate or swab for at least 2 minutes to agitate the cells off of the substrate or swab. At this point, label the extraction negative control with the date and time.

7. Label new tubes to hold the swab or substrate remains. Sterilize tweezers with 10% bleach, distilled water, and 70% ethanol before the removal of each sample. Remove the swab or other substrate from the sample tube, one tube at a time, using sterile tweezers and close tube. Place swab or substrate in the sterile labeled substrate remains fraction tube.

8. Spin in a microcentrifuge for 5 minutes at 10,000 to 15,000 x g (13,200 rpm).

9. Without disturbing the pellet, remove and discard all but 50 µL of the supernatant.

10. Resuspend the pellet in the remaining 50 µL by stirring with a sterile pipette tip.

11. To the approximately 50 µL of resuspended cell debris pellet, add 150 µL sterile deionized water (final volume of 200 µL).

12. Add 1 µL of 20 mg/mL Proteinase K. Vortex briefly to resuspend the pellet.

13. Incubate at 56°C for about 60 minutes to lyse epithelial cells, but for no more than 75 minutes, to minimize sperm lysis.

14. During the incubation step do the following:

    a. Label a new tube for each sample, including an epithelial cell extraction negative control. Mark each tube as an epithelial cell fraction.
    b. Add 50 µL of 20% Chelex (from a well-resuspended Chelex solution) to each epithelial cell fraction tube.
    c. Close tubes.

15. Spin the extract in a microcentrifuge at 10,000 to 15,000 x g (13,200 rpm) for 5 minutes.

16. Add 150 µL of the supernatant from each sample and the extraction negative to its respective epithelial cell fraction sample tube. Store at 4°C or on ice until step 20.

17. Wash the sperm pellet with Digest Buffer as follows:
    a. Resuspend the pellet in 0.5 mL Digest Buffer.
    b. Vortex briefly to resuspend pellet.
    c. Spin in a microcentrifuge at 10,000 to 15,000 x g (13,200 rpm) for 5 minutes.
    d. Remove all but 50 µL of the supernatant and discard the supernatant.
    e. Repeat steps a-d for a total of 5 times.

18. Wash the sperm pellet once with sterile dH_2O as follows:
    a. Resuspend the pellet in 1 mL sterile dH_2O.
    b. Vortex briefly to resuspend pellet.
    c. Spin in a microcentrifuge at 10,000 to 15,000 x g (13,200 rpm) for 5 minutes.
    d. Remove all but 50 µL of the supernatant and discard the supernatant.

19. Resuspend the pellet by stirring with a sterile pipette tip.

20. To the approximately 50 µL resuspended sperm fraction and to the tubes containing the substrate remains and the sperm fraction extraction negative, add 150 µL of 5% Chelex, 1 µL of 20 mg/mL Proteinase K, and 7 µL of 1M DTT. Mix gently.

21. Vortex both the epithelial cell and sperm fractions. The following steps apply to all fractions.

22. Incubate at 56°C for approximately 60 minutes.

23. Vortex at high speed for 5 to 10 seconds.

24. Incubate at 100°C for 8 minutes using a screw down rack.

25. Vortex at high speed for 5 to 10 seconds.

26. Spin in a microcentrifuge for 2 to 3 minutes at 10,000 to 15,000 x g (13,200 rpm).

27. Pipet aliquots of neat and a 1/100 dilution (using TE^{-4}) into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

28. Store the extracts at 2 to 8°C or frozen.

29. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

Revision History:
March 24, 2010 – Initial version of procedure.

DNA EXTRACTION FROM HAIR		
DATE EFFECTIVE 03-24-2010	APPROVED BY EUGENE LIEN	PAGE 1 OF 1

Refer to the following sections of the Protocols for Forensic Mitochondrial DNA Analysis:

Hair Examination
Mitochondrial and Nuclear DNA Hair Extraction
Mideo Macro/Microscopic Digital Imaging

Revision History:
March 24, 2010 – Initial version of procedure.

A. Sample Preparation

Liquid/dry blood, bone marrow, oral swab and tissue sample preparation

Stained substrates and oral swabs should be cut into small pieces (3 x 3 mm). Tissues should be minced into small pieces in a weigh boat using a sterile scalpel or razor blade. Place samples in 1.5mL microcentrifuge tubes or conical tubes when appropriate. See table below for various sample types.

Proceed to Section B: Sample Incubation

Sample type	Amount
Liquid blood	100 to 500 µL
Bone marrow	0.5 x 0.5 cm to 1.5 x 1.5cm
Oral swab	1/3 to a whole swab
Blood stain	0.5 x 0.5 cm to 1.5 x 1.5cm
Soft tissue	0.5 x 0.5 cm to 1.5 x 1.5cm
Paraffin embedded tissue	0.3 x 0.3 cm to 1.0 x 1.0 cm

Bone preparation

Before extraction, a bone or tooth specimen should be cleaned entirely of soft tissue and dirt using a range of methods, such as scraping, rinsing and sonication. A combination of sterile scalpels, sterile toothbrushes and running water should be used to clean the specimen. For a sonication bath, the sample is placed in a conical tube and covered with a 5% Terg-a-Zyme solution. For additional cleaning, the sonication step may be repeated multiple times by decanting the liquid and replacing with fresh Terg-a-zyme solution. After cleaning, the sample is usually rinsed with distilled water and dried using a 56°C incubator (drying time may vary from a few hours to overnight).

Note: Terg-a-zyme is an enzyme-active powdered detergent. A 5% solution should be made fresh prior to bone preparation and cleaning. Refer to Appendix A in the Quality Assurance Manual. Once prepared, the reagent will only be effective for up to 16 hours.

1. Photograph bone or tooth sample after cleaning. Measure and weigh specimen prior to sampling.

2. If several bones are available, generally compact bone is preferred, such as humerus, femur, or tibia.

   ****WARNING****
   Protective eyewear, lab coats, cut resistant gloves, sleeve protectors, and HEPA-filtered facial masks should be worn when cutting bone. Avoid breathing bone dust. All cutting of bone must be done under a biological hood.

3. Using an autopsy saw or a Dremel tool equipped with a 409 or 420 cutting wheel, cut the bone specimen into approximately 5x5x5mm size pieces. Take enough cuttings for an end weight of approximately 2g. For older or compromised bones, several aliquots of 2g can be extracted and combined during the Microcon step. For tooth samples, the whole root should be taken. **Note: The cutting wheel should be disposed of after each use and the Dremel and hood should be completely wiped down with bleach and ethanol**.

4. Place bone cuttings in 50mL conical tubes labeled with the FB case number, ME#, PM item #, initials, and date.

5. Cover bone cuttings with 5% Terg-a-zyme solution and sonicate samples for 30-45 minutes. **Note: Ensure water level in the sonicator is 1-2 inches from the top.**

6. Decant the Terg-a-zyme and wash with distilled water until no detergent bubbles remain.

7. If necessary, repeat with fresh changes of 5% Terg-a-zyme and water washes until the dirt has been removed.

8. Place the clean cuttings in a weigh boat on a small Kim Wipe. Cover with another weigh boat. Label the weight boat with the FB case number, ME#, PM item #, initials, and date.

9. Seal with evidence tape.

10. Dry in a 56°C incubator for a few hours or overnight. After sufficient drying, weigh bone cuttings. **The bone sample must be completely dry before milling.**

Sample milling with the SPEX Certiprep 6750 Freezer Mill

All freezer mill parts that come into contact with bone specimens, such as the cylinders, metal end plugs and impactors, should be cleaned, dried and sterilized prior to use. See Step 22 for appropriate cleaning procedure.

1. Assemble specimen vials in the following order: metal bottom, plastic cylinder, impactor, and metal top.

2. Place under UV light for a minimum of 15 minutes.

3. Label metal bottoms with a case identifier using a blue ink Sharpie.

4. Add bone cuttings to specimen vial around impactor using decontaminated forceps. Cover with metal top. **Note: Shake specimen vial and ensure that the impactor can move back and forth.**

5. Wipe down inside of mill with a wet paper towel. **Do not use bleach or ethanol.**

6. Plug in mill and switch ON.

7. Obtain liquid nitrogen from tank by filling transfer container. Be aware that the liquid nitrogen tank may be empty when the detector level reads anywhere from "¼" to "empty".

   ****WARNING****
   Liquid Nitrogen can be hazardous. Use cryogenic gloves, protective eyewear/face shield and lab coats when handling. Avoid liquid nitrogen splashes to face and hands.

8. Open the freezer mill lid. Add liquid nitrogen slowly into the mill up to the **FILL LINE** to avoid splashing and boiling over.

9. Place the specimen vial into the round chamber. If processing more than one bone sample it is possible to save pre-cooling time by placing up to two vials in the mesh container inside the mill.

10. Change cycle number to match total number of samples plus two (n + 2).

11. Adjust mill settings as follows:

Cycle	**set to # of samples + 2**	
Time	**T1 (milling)** **T2 (pause)** **T3 (pre-cool)**	**2.0 min** **2.0 min** **15.0 min**
Rate	**Bones – 8-10** **Teeth – 6-8**	

12. Close cover slowly to avoid any liquid nitrogen splashes and press **RUN** to start the mill. Pre-cooling will begin followed by the milling cycle.

13. During the 2-minute pause phase, it is now possible to open the mill and remove the finished sample using cryogenic gloves.

14. Place one of the pre-cooled specimens waiting in the dock in the round chamber.

15. If liquid nitrogen level is below the **FILL LINE**, refill. A loud noise during milling means that the liquid nitrogen level is low. If liquid nitrogen is not refilled, damage to the mill, mill parts, and cylinder can occur.

16. Close the lid and press **RUN** again. Repeat from Step 11 until all samples are processed.

17. Inspect each sample after removal from the mill. If sample is sufficiently pulverized, remove the metal top using the Spex Certi-Prep opening device. **Note: Samples may be reinserted into the mill for additional grinding.**

18. Using decontaminated tweezers, remove impactor from vial and submerge in 10% bleach.

19. Empty bone dust into labeled 50mL Falcon tube. Ensure complete dust transfer by tapping bottom of cylinder. Weigh bone dust and document.

20. Soak metal end parts and plastic cylinder in 10% Bleach.

21. When milling is complete, switch mill to **OFF** and unplug. Leave cover open for liquid nitrogen to evaporate. The next day, lower cover and place in storage until next use.

22. **Mill Parts -Clean Up**: Mill parts must be cleaned immediately after processing. If this is not possible, steps a-b must be completed before leaving overnight.
    a. Rinse off with 10% bleach.
    b. Soak all parts in 0.1% SDS.
    c. Brush parts with a new toothbrush to remove any residual bone dust.
    d. Rinse with water.
    e. Soak parts in 10% bleach and brush each part in bleach individually.
    f. Rinse with water.
    g. Separate the plastic cylinders from the metal parts.
    h. Rinse in 100% ethanol. **ONLY** the metal top, metal bottom, and compactor can be rinsed in 100% ethanol. **DO NOT** rinse the plastic cylinder in ethanol as it will cause the plastic cylinder to break.
    i. Use isopropanol to remove any identifying marks made with a Sharpie on the tops or bottoms of the cylinders.
    j. Dry and expose the parts to UV light for a minimum of 2 hours. The UV light in a biological hood or a StrataLinker can be used.

23. Proceed to Section B: Sample Incubation.

Laser Microdissection of Products of Conception

1. Initial processing

   The product of conception (POC) can be received in different stages of preparation:

   a) POC scrapings in saline buffer:

      Remove tissue from liquid either by filtration or centrifugation:
      - Transfer liquid to 50mL falcon tube
      - Spin sample in a bench top Eppendorf or IEC Centra CL3R at 1000 RPM for 5 minutes
      - Discard liquid supernatant

Submit sample to the Histology department for tissue processing according to the OCME Histology Procedure Manual section E. Then proceed as for b).

b) POC fixated and embedded in paraffin blocks:

Contact histology department and ask them to prepare microscope slides from the paraffin block using the following precautions:

- Use disposable blades for the microtome and discard after each case.
- Clean working surface on microtome by wiping with 10% bleach and alcohol before and after each case.
- Use individual floating chambers for each case
- Use uncharged microscope slides

The slides then should be stained with hematoxylin and eosin-phloxine (H&E technique) as described in the OCME Histology Procedure Manual. But again during the staining procedure, separate sets of jars have to be used for each case.

c) Stained or unstained microscope slides from POC blocks:

If the slides are unstained, ask the histology department to stain them as described above. Otherwise proceed with the microdissection technique. **Attention:** for slides that were prepared by a histology laboratory outside of the OCME, foreign DNA not from the mother and the fetus might be present on the slide.

2. PixCell IIe Laser Capture Microdissection

A trained pathologist has to be present to distinguish decidual tissue from chorionic villi and operate the laser. After the slide has been placed on the microscope platform the pathologist will visually identify the area of interest, mark this area for the laser, and activate the laser. The laser setting is specified in the Arcturus instrument manual. The Forensic Biology Criminalist needs to be present during the complete procedure to maintain chain of custody of the evidence.

An area of chorionic villi and an area of maternal tissue should be collected on separate CapSure caps. The caps can be stored and transported in 50 ml Falcon tubes. A third unused CapSure cap should be extracted as an extraction negative control.

Use new scalpel and clean forceps to remove the film from the cap and transfer the film to a fresh 1.5mL microcentrifuge tube containing 500µL of organic extraction buffer, DTT, SDS and Proteinase K as described below.

B. Sample Incubation

1. Process an extraction negative with every batch of extractions.

2. Prepare the master mix in microcentrifuge tube or conical tube and mix thoroughly by swirling or vortexing *very briefly*.

For liquid blood, dry blood and bone marrow samples:

	1 Sample	**5 Samples**	**10 Samples**	**15 Samples**
Organic extraction buffer	400 µL	2.0 mL	4.0 mL	6.0 mL
20% SDS	10µL	50 µL	100µL	150 µL
Proteinase K (20 mg/mL)	13.6 µL	68 µL	136 µL	204 µL
Total Incubation Volume *per sample*:				*400 µL*

For bone samples:

	Per bone (~2g dust)	**1 sample (N+ 2)**	**3 samples (N+ 2)**	**5 samples (N+ 2)**
Organic Extraction Buffer	2370 µL	7.11 mL	11.85 mL	16.59 mL
20% SDS	300 µL	900 µL	1.5 mL	2.1 mL
1.0 M DTT	120 µL	360 µL	600 µL	840 µL
Proteinase K (20 mg/mL)	210 µL	630 µL	1.05 mL	1.47 mL
Total Incubation Volume *per sample*:				*3000 µL*

For teeth samples:

	Per tooth	**1 sample (N+ 2)**	**3 samples (N+ 2)**	**5 samples (N+ 2)**
Organic Extraction Buffer	790 µL	2.37 mL	3.95 mL	5.53 mL
20% SDS	100 µL	300 µL	500 µL	700 µL
1.0 M DTT	40 µL	120 µL	200 µL	280 µL
Proteinase K (20 mg/mL)	70 µL	210 µL	350 µL	490 µL
Total Incubation Volume *per sample*:				*1000 µL*

For tissues and paraffin embedded tissue (e.g. microdissection) samples:

	Per tissue	**1 sample (N+ 2)**	**3 samples (N+ 2)**
Organic extraction buffer	395 µL	1185 µL	1975 µL
20% SDS	50 µL	150 µL	250 µL
1.0 M DTT	20 µL	60 µL	100 µL
Proteinase K (20 mg/mL)	35 µL	105 µL	175 µL
Total Incubation Volume *per sample*:			*500 µL*

3. Add the appropriate incubation volume of master mix to each sample tube and eneg tube. Vortex tubes briefly. Make certain the substrate, tissue, or swab is totally submerged. **Note: Reagent volumes may be adjusted in order to accommodate the size or nature of a particular sample.**

4. Place tubes in a shaking 56°C heat block and incubate overnight.

5. Proceed to Section C: Phenol Chloroform Extraction and Microcon® cleanup.

C. Phenol Chloroform and Microcon Clean up

<u>Set Up</u>

Remove the Phenol:Chloroform:Isoamyl Alcohol (25:24:1) (PCIA) from the refrigerator.

Obtain organic waste jug for disposal of any tubes or pipette tips that come in contact with PCIA.

****WARNING****
Phenol Chloroform is toxic. Protective eyewear, mask, lab coat, and nitrile gloves should be worn when handling. All work must be conducted under a chemical fume hood.

For samples possibly needing mtDNA or High Sensitivity DNA testing: Place one Microcon® YM100 collection tube and one 1.5 mL microcentrifuge tube for each sample, including the extraction negative, in the StrataLinker for at least 15 minutes. **Note: Irradiate multiple tubes (4-6) per bone sample to accommodate the total volume of incubation buffer.**

1. Vortex and centrifuge the incubated microcentrifuge tube samples at high speed for 1 minute. Vortex and centrifuge bone dust, incubated in 50 mL conical tubes, for 5-10 minutes at 1000 RPM in Eppendorf Centrifuge Model 5810.

2. Obtain and label one prepared Eppendorf Phase Lock Gel (PLG) tube per sample, including the extraction negative. PLG tubes make phase separation easier and are optional.

   NOTE: For bone samples, label as many tubes to accommodate the total volume of incubation buffer per sample. For example, if you incubated 2g of bone dust with 3 mL of incubation buffer, you will need 6 PLG tubes.

   NOTE: See section D for PLG tube preparation instructions.

3. Centrifuge PLG tubes at maximum speed for 30 seconds.

4. Label Microcon® YM100 filters for each sample. Prepare the Microcon® YM100 concentrators by adding 100 μL of TE^{-4} to the filter side (top) of each concentrator. Set aside until step 11.

5. Add a volume of Phenol:Chloroform:Isoamyl Alcohol 25:24:1 (PCIA) to the PLG tube which is equal to the volume of incubation buffer (typically 400 µL) to be added from the sample. **Note: When pipetting PCIA, you must penetrate the top buffer layer and only aliquot the desired amount from the lower, clear organic layer. Place used pipette tips in the organic waste bottle.**

6. Have someone witness your sample tubes, PLG tubes, and Microcon® YM100 tubes.

7. Pipet the sample supernatant (typically 400 µL) to the PLG tube already containing PCIA. For bone dust samples, pipet several aliquots of the supernatant into multiple PLG tubes. **Note: Do not disturb bone pellet.**

8. Shake the PLG tube vigorously by hand or by inversion to form a milky colored emulsion. **Note: Do NOT vortex the PLG tube.**
9. Centrifuge samples for 2 minutes at maximum speed to achieve phase separation. (On Eppendorf Centrifuge Model 5415D, spin at 16.1 RCF or 13.2 RPM).

10. If the sample is discolored, contains particles in the aqueous phase, or contains a lot of fatty tissue, transfer the top layer (aqueous phase) to a new PLG tube and repeat Steps 7-9. Note: The aqueous layer from bone and teeth will usually be discolored. Only repeat the phenol-chloroform clean-up steps if any dust or particles are present in the aqueous layer. If it is not necessary to repeat the clean-up step, go to Step 11.

11. Carefully transfer the aqueous phase (top layer) to the prepared Microcon® YM100 concentrator. Be careful not to let the pipette tip touch the gel. **Note: Discard used PLG tubes into the organic waste bottle.**

12. Spin the Microcon® YM100 concentrators for 15-30 minutes at 500 x g, which is approximately 2500 RPM. (On Eppendorf Centrifuge Model 5415D, spin at 0.6 RCF or 2600 RPM). **Note: Ensure that all fluid has passed through filter. If it has not, spin for additional time, in 10-minute increments. If fluid still remains, transfer sample to a new filter and microcon again.**

13. Discard the wash tubes and place the concentrators into a new collection tube.

14. Add 400 µL of TE^{-4} to the filter side of each Microcon® YM100 concentrator.

15. Spin again for 15 minutes at 500 x g for 15 minutes. (On Eppendorf Centrifuge Model 5415D, spin at 0.6 RCF or 2600 RPM). **Note: Ensure that all fluid has passed through filter. If it has not, spin for additional time, in 10-minute increments. If fluid still remains, transfer sample to a new filter and microcon again.**

16. Add 40 µL of TE^{-4} to the filter side of each Microcon® YM100 concentrator. **Note: For bone samples, add only 10-20 µL of TE^{-4} to each filter side to ensure smallest elution volume.**

17. Invert sample reservoir and place into a new labeled collection tube. *(For samples possibly needing mtDNA or High Sensitivity DNA testing, invert sample reservoirs into irradiated collection tubes).* Spin at 1000 x g, which is approximately 3500 RPM, for 3 minutes. (On Eppendorf Centrifuge Model 5415D, spin at 1.2 RCF or 3600 RPM).

18. Measure the approximate volume recovered and record on the organic extraction worksheet. **Note: Combine bone elutants before measuring volume.**

19. Discard sample reservoir and adjust sample volume depending on the starting amount and expected DNA content as follows using TE^{-4}. **Note: Samples may be microcon'ed again to further concentrate low DNA content samples.**

Sample type	**Final Volume**
High DNA content (Large amounts of blood, fresh tissue, bone marrow, oral swabs, and dried bloodstains)	400 µL
Medium DNA content (Small amounts of blood, fresh tissue, bone marrow, oral swabs, and dried bloodstains); differential lysis samples	200 µL
Low DNA content (Formalin fixed tissue, dried bone, teeth, samples from decomposed or degraded remains, some reference samples)	100 µL

20. Transfer samples to newly labeled 1.5mL microcentrifuge tubes for storage. *(For samples possibly needing mtDNA or High Sensitivity DNA testing, transfer samples to irradiated 1.5 mL microcentrifuge tubes).* Record the approximate final volume on the organic extraction worksheet.

21. As needed, pipet aliquots of neat and/or diluted extract (using TE^{-4}) into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

22. Store the extracts at 2 to 8°C or frozen.

23. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

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NOTE: See Microcon® troubleshooting (in the appropriate section of the STR manual) as needed.

D. Preparation of Phase Lock Gel (PLG) tubes

Make sure the plasticware being used is resistant to phenol and chloroform.

1. Without putting pressure on the plunger, twist off the **orange cap** and discard. Attach the **gray dispensing tip** (supplied) to the syringe and tighten securely. (NOTE: Use of gray tip is optional for a smoother application of PLG. Less force is necessary when gray tip is NOT used.)

2. Apply firm pressure on the plunger to dispense PLG until it reaches the end of gray tip. Add heavy PLG based on Table below. NOTE: 325µL = 3.25 cc corresponds to 3 lines on the syringe

Tube size	**PLG heavy**	**Tube size**	**PLG heavy**
0.5mL	100µL	15mL	3mL
1.5mL	325µL	50mL	5mL
2.0mL	325µL		

3. Pellet the PLG by spinning the tubes prior to use. See table below.

Tube size	Centrifuge model	Speed	Time
0.5 to 2.0mL	Eppendorf 5415C Eppendorf 5415D	14 x 1000 RPM 13.2 x 1000RPM/16.1 x 1000RCF	30s
15 and 50mL	Sigma 4-15 C	1500 RCF	2m

Revision History:
March 24, 2010 – Initial version of procedure.

A. Preparation

1. Extraction sets consist of 9 samples and one or two extraction negatives. Additional extractions may continue sequentially during incubations.

2. In cell H1 of the appropriate extraction sheet, type in the name of the extraction assay as follows: "E" for extraction, month, day, and year, "period", hour and minute. For example, E040905.1330. (Note: The HI Team extraction sheet can be found in the "HighSens_Data\Templates in Use\2 extraction" folder, and the PC Team extraction sheet can be found in the "Fbiology_Main\Forms\PC\Extraction" folder on the main drive.)

3. Manually enter OR copy and paste the sample names into the appropriate extraction sheet. The worksheet will automatically calculate the requisite amount of reagents needed for the extraction.

4. **Follow the procedures for Work Place Preparation (refer to the General Guidelines Procedure of this manual).**

B. Digestion

1. **Self-Witnessing Step:** Confirm the sample names on the extraction sheet with the names on the sample tubes.

2. Prepare digestion buffer in an UV irradiated tube (1.5 mL, 2.0 mL Dolphin, or 15 mL).

3. Prepare the digestion buffer according to the calculated volumes on the extraction sheet. The volume for one sample is shown below.

Stock Solution	Concentration	1 sample
0.05% SDS (or 0.01% SDS when using Poly A RNA at a later step)	**0.05%** (or 0.01%)	192 µL
Proteinase K 20 mg/mL	0.80 mg/mL	8 µL

4. Vortex solution well. Add **200 µL** of the digestion buffer to each sample. Open only one sample tube at a time using the cap opener. Ensure that the swabs are submerged in the digestion fluid. If necessary, add an additional 200 µL of the digest buffer (including the Proteinase K) to the sample in order to submerge a large sample.

5. Record the temperatures of the heat shakers on the extraction worksheet. Temperatures must be within $\pm$ 3°C of the set temperature.

6. Incubate on the heat shaker at 56ºC for 30 minutes with shaking at 1400 rpm.

7. Incubate on the heat shaker at 99ºC for 10 minutes with no shaking (0 rpm).

8. Place sample in cold block at 4ºC for 10 minutes with no shaking (0 rpm).

9. Centrifuge the samples at full speed, briefly.

10. During the digestion period label the Microcon®, elution, and storage tubes.

C. Purification and Concentration

1. Prepare Microcon® 100 tubes and label the membrane tube and filtrate tube cap.

2. **Witness step:** Confirm the sample names on the extraction sheet with the names on the sample and Microcon® tubes.

3. Pre-coat the Microcon® membrane with Fish Sperm DNA or a 1/1000 dilution of Poly A RNA prepared as follows in an irradiated microcentrifuge tube or 15 mL tube:

    a. Fish Sperm DNA Preparation

        i. Add 1 uL of stock Fish Sperm DNA solution (1mg/mL) to 199uL of water for each sample on the extraction sheet.

        ii. Aliquot 200 uL of this Fish Sperm DNA solution to each Microcon® tube. Avoid touching the membrane. The volume for one sample is shown below. Refer to the extraction worksheet for calculated value.

b. Poly A RNA Preparation

i. Make a 1/10 dilution of 1mg/mL of Poly A RNA as follows: add 2 µL of Poly A RNA to 18 µL of irradiated water and mix the solution well. This is a final concentration of 100µg/mL.

ii. Using the 1/10 dilution, make a 1/100 dilution with 2 uL of 100ug/mL Poly A RNA in 198 uL of irradiated water and mix the solution well. The solution has a final concentration of 1 ng/uL.

iii. Add 1 uL of the 1ng/uL Poly A RNA solution to 199uL of water for each sample on the extraction sheet.

iv. Aliquot 200 uL of this Poly A RNA solution to each Microcon® tube. Avoid touching the membrane. The volume for one sample is shown below. Refer to the extraction worksheet for calculated value.

Reagent	1 sample
Water	199 µL
Fish Sperm DNA (1mg/mL) or Poly A RNA (1ng/µL)	1 µL

NOTE: For samples with 400 µL of digest solution, make a 20 µL solution of 1 uL of Fish Sperm DNA (1mg/mL) or 1 µL of Poly A RNA (1 ng/µL) with 19 µL of water. Mix well and add this solution to the membrane. Ensure that the entirety of the membrane is covered. In this manner, all of the digest may be added to the Microcon® membrane for a total volume of 420 uL.

4. Filtration

a. Add the entirety of each extract to its pretreated Microcon® membrane. Aspirate all of the solution from the sample tube by placing the pipet within the swab. The sample tubes may be discarded.

b. Centrifuge the Microcon® tube at 2400 rpm for 15 minutes. An additional 5 minutes may be required to ensure that all the liquid is filtered. However, do not centrifuge too long such that the membrane is dry.

If indicated on the evidence examination schedule sheet or by a supervisor, or if the filtrate is not clear, perform a second wash step applying 400 µL of water onto the membrane and centrifuging again at 2400 rpm for 15 minutes or until the all the liquid is filtered. However, do not centrifuge to dryness. This process may be repeated, as necessary. Document the additional washes on the extraction sheet. All samples extracted with 0.05% SDS should be washed at least twice; however, for samples with clear filtrates, 200 µL of water may be added.

c. Visually inspect each Microcon® membrane tube. If it appears that more than 5 µL remains above the membrane, centrifuge that tube for 5 more minutes at 2400 rpm.

5. Elution

a. Open only one Microcon® tube and its fresh collection tube at a time.

b. Add 20 µL of irradiated water to the Microcon® and invert the Microcon® over the new collection tube. Avoid touching the membrane.

c. Centrifuge at 3400 rpm for 3 minutes.

d. Transfer the eluant to an irradiated and labeled 1.5 mL tube. Measure and record the approximate volume. The total volume should not exceed 30 uL and should not be less than 20 uL. Adjust the final volume to 20 uL using irradiated water (if less). Discard the Microcon® membrane.

e. If the eluant appears to be a dark color or is not clear, it may be necessary to purify the sample again. Prepare a fresh Microcon® tube and repeat steps 4-5.

f. Store the extracts at 2 to 8ºC or frozen.

g. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

A. Preparation

1. Extraction sets consist of 10 samples and two Extraction Negatives. Additional extractions may continue sequentially during incubations.

2. In cell H1 of the appropriate nail extraction sheet (found for example in the "Templates in Use\2 extraction" folder on the Hi Sens Data or in the "Forms\Extract" folder on the FBIOLOGY_MAIN drive), type in the name of the extraction assay as follows: month (MM), day (DD), and year (YY), "period", hour (HH) and minute (MM). For example, 040905.1330 for an extraction performed on April 9th, 2005 at 1:30pm. Save the sheet with "E" for extraction followed by the name of the extraction assay. For example, E040905.1330.

3. Manually enter OR copy and paste the sample names into the appropriate extraction sheet. The worksheet will automatically calculate the requisite amount of reagents needed for the extraction.

4. **Follow the procedures for Work Place Preparation in the General Guidelines Section of this manual.**

B. Digestion

1. From evidence exam, each nail (or group of nails) should be placed in an irradiated tube.

2. Add 200 µL of irradiated 25 mM EDTA/PBS solution to each sample.

3. Sonicate the samples for one hour at room temperature.

4. Label a new set of irradiated microcentrifuge tubes with the sample identifiers.

5. Remove the supernatants from the samples and place in the labeled irradiated microcentrifuge tubes.

C. Extraction

1. Prepare the digestion buffer according to the calculated volumes on the nail extraction sheet. The volumes for one sample are shown below:

Stock Solution	Concentration	1 sample
1.0% SDS	1.0% (0.96%)	2.3 (2.25) µL
Proteinase K 20 mg/mL	0.80 mg/mL	9 µL
Irradiated water	N/A	13.7 µL

2. Prepare Microcon® 100 tubes and label the membrane tube and filtrate tube cap with the sample identifiers. Prepare and label the Microcon® collection tubes, sample storage microcentrifuge tubes as well as post-sonication nail collection tubes. The identifier for the post sonication nail collection tubes should include "PS" as a suffix. For example, the post sonication tube for left nail ring finger could be "nail L4 PS".

3. **Witness step:** Confirm the sample names on the extraction sheet with the names on all labeled tubes.

4. Vortex solution well. Add 25 µL of the nail digestion buffer to each sample. Open only one sample tube at a time using the cap opener.

5. Record the temperatures of the heat shakers on the extraction worksheet. Temperatures must be within ± 3°C of the set temperature.

6. Incubate on the heat shaker at 56ºC for 30 minutes with shaking at 1400 rpm.

7. Incubate on the heat shaker at 99ºC for 10 minutes with no shaking (0 rpm).

8. After removing from the shaker, centrifuge the samples at full speed, briefly. Allow the samples to cool for a few minutes while preparing for next steps or chill for 10 minutes at 4°C.

9. During the digestion period remove the nails using clean tweezers and dry them in a hood. When dry, place the nails in the labeled, post-sonication nail collection tubes.

D. Purification and Concentration

1. **Self-witness step:** Confirm the sample names on the extraction sheet with the names on the sample and Microcon® tubes.

2. Pre-coat the Microcon® membrane with Fish Sperm DNA or a 1/1000 dilution of Poly A RNA prepared as follows in an irradiated microcentrifuge tube or 15 mL tube:

   a. Fish Sperm DNA Preparation

      i. Add 1 uL of stock Fish Sperm DNA solution (1mg/mL) to 199uL of water for each sample on the extraction sheet.

      ii. Aliquot 200 uL of this Fish Sperm DNA solution to each Microcon® tube. Avoid touching the membrane. The volume for one sample is shown below. Refer to the extraction worksheet for calculated value.

   b. Poly A RNA Preparation

      i. Make a 1/10 dilution of 1mg/mL of Poly A RNA as follows: add 2 µL of Poly A RNA to 18 µL of irradiated water and mix the solution well. This is a final concentration of 100µg/mL.

      ii. Using the 1/10 dilution, make a 1/100 dilution with 2 uL of 100ug/mL Poly A RNA in 198 uL of irradiated water and mix the solution well. The solution has a final concentration of 1 ng/uL.

      iii. Add 1 uL of the 1ng/uL Poly A RNA solution to 199uL of water for each sample on the extraction sheet.

iv. Aliquot 200 uL of this Poly A RNA solution to each Microcon® tube. Avoid touching the membrane. The volume for one sample is shown below. Refer to the extraction worksheet for calculated value.

Reagent	1 sample
Water	199 µL
Fish Sperm DNA (1mg/mL) or Poly A RNA (1ng/µL)	1 µL

NOTE: For samples with 400 µL of digest solution, make a 20 µL solution of 1 uL of Fish Sperm DNA (1mg/mL) or 1 µL of Poly A RNA (1 ng/µL) with 19 µL of water. Mix well and add this solution to the membrane. Ensure that the entirety of the membrane is covered. In this manner, all of the digest may be added to the Microcon® membrane for a total volume of 420 uL.

3. Filtration

a. Add the entirety of each extract to its pretreated Microcon® membrane. The sample tubes may be discarded.

b. Centrifuge the Microcon® tube at 2400 rpm for 15 minutes.

c. **Repeat this wash step two more times applying 400uL of water onto the membrane and centrifuging again at 2400 rpm for 15 minutes for a total of three washes to remove any residual EDTA.**

d. Visually inspect each Microcon® membrane tube after the third wash. If it appears that more than 5 µL remains above the membrane, centrifuge that tube for 5 more minutes at 2400 rpm.

4. Elution

   a. Open only one Microcon® tube and its fresh collection tube at a time.

   b. Add 20 µL of irradiated water to the Microcon® and invert the Microcon® over the new collection tube. Avoid touching the membrane.

   c. Centrifuge at 3400 rpm for 3 minutes.

   d. Transfer the eluant to an irradiated and labeled 1.5 mL tube. Measure and record the approximate volume. The total volume should not exceed 30 uL and should not be less than 20 uL. Adjust the final volume to 20 uL (if necessary) with irradiated water. Discard the Microcon® membrane.

   e. If the eluant appears to be a dark color or is not clear, it may be necessary to purify the sample again. Prepare a fresh Microcon® tube and repeat steps 3-4.

   f. As needed, pipet aliquots of neat and/or diluted extracts (using TE^{-4}) into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

   g. Store the extracts at 2 to 8°C or frozen.

   h. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

Revision History:
March 24, 2010 – Initial version of procedure.

Sample size for the extraction should be approximately 1/3 of a swab or a 3x3 mm cutting of the stain**. This extraction is not applicable to cigarette butts.**

All bloodstain and exemplar cuttings should be placed in 2.0mL screw cap sample tubes.

A. Setting up M48 Spreadsheet and Saving Sample Name List

1. Collect the M48 Sample Submission Sheets for the extraction. On these sheets, assign each sample a sample rack position, remembering that the extraction negative will occupy Position 1 (and position 25, if extracting >24 samples). Also fill in the initials of the analyst performing the extraction and the extraction date(s) and time(s). This date and time will be used throughout the extraction.

2. Open the appropriate M48 spreadsheet, evidence (M48EV) or exemplar (M48EX) depending on your sample set.

2. Click the “Input Sample Names” tab and enter the sample names for the extraction, including the extraction negative(s), into the appropriate positions in column B.

3. Save this sheet by going to File → Save As and save the sheet to the “SampleName” folder on the desktop with “File Name:” in MMDDYY.HHMM format and “Save As Type:” set to CSV (Comma delimited)(*.csv). For instance an extraction performed at 2:20pm on May 23, 2006 would be saved, with date and time in military format, as 052306.1420.csv.

4. Click “Save”.

5. A window stating “The selected file type does not support workbooks that contain multiple sheets” will open. Click “OK”.

6. A second window asking "Do you want to keep the workbook in this format?" opens. Click "Yes".

7. Click the Ext Sheet 24 or Ext Sheet 48 tab depending on the batch size of the extraction.

8. Once the appropriate extraction sheet is open, finish the sheet by entering the tube label, target date, and IA initials for each sample.

9. Print the extraction sheet.

10. **Minimize** the M48 spreadsheet (do not close Excel or hit the "X" in the upper right-hand corner!).

B. Sample Preparation and Incubation

1. Remove the extraction rack from the refrigerator. Extract either evidence or exemplars. Do not extract both together.

2. Sample preparation should be performed under a hood.

3. Obtain an empty tube for the extraction negative and label it.

4. Have a witness verify your samples.

5. For large runs, prepare master mix for N+2 samples as follows, vortex briefly, and add 200µL to each of the tubes in the extraction rack and the pre-prepared extraction negative tube. For smaller runs, you may add Proteinase K and G2 Buffer to each tube individually:

Reagent	1 sample	6 samples	12 samples	18 samples	24 samples
Digestion Buffer (Buffer G2)	190 µL	1520 µL	2660 µL	3800 µL	4940 µL
QIAgen Proteinase K	10 µL	80 µL	140 µL	200 µL	260 µL

6. Shake at 1000 rpm at 56° C for a minimum of 30 minutes.

C. BioRobot M48 Software and Platform Set-Up

1. Double click on the "BioRobot M48" icon on the desktop.

2. Click the "Start" button. **Note: The door and container interlock must be closed to proceed.**

3. "F Trace MTL" protocol should be selected. If not, click on the arrow in the middle of the screen and then select "New Dev" → "gDNA" → and "F Trace MTL"**.**

4. Click on the "select" button and select "1.5 ml" for the size of the elution tubes.

5. Select the number of samples 6, 12, 18, 24, 30, 36, 42, or 48.

6. Set sample volume to 200 uL (cannot and should not change).

7. Set elution volume to 200 uL.

8. The next prompt asks to ensure the drop catcher is clean. In order to check this, click on "manual operation" and select "Drop Catcher Cleaning". The arm of the robot will move to the front of the machine, and the drop catcher (a small plastic tray) will be right in front of you. Remove and clean with 70% ethanol. When the catcher is clean, replace the tray, close the door, and click "OK" in the window.

9. Make sure that the chute to the sharps container bin is clear for the tips to be discarded. Click "Next".

10. The software will calculate the number of tips necessary for the run. Place tips in the tip rack(s) if necessary. **When filling racks, make sure that the pipette tips are correctly seated in the rack and flush with the robotic platform.** Tips are located in three racks. These racks may be filled one at a time, BUT you must fill a **whole rack** at a time. After a rack is filled, reset the tip rack by clicking on "Yes tip rack ...", If no new tips are being added to the robot click "No".

    NOTE: When opening a new tip bag, ALL tips should be placed onto the robotic platform. Open tip bags should not be returned to the drawer. Racks may be used for tip storage. When adding tips, spilling into the next empty rack is OK, just do not **reset** the rack until it is **completely** full.

    Tips needed for a run:

# Samples	6	12	18	24	30	36	42	48
# Tips	30	42	54	66	78	90	102	114

    After you are finished, click "Next".

11. Fill the reagent reservoirs as stated below. All reagents are stored in their respective plastic reservoirs in the metal rack, covered with Parafilm, **EXCEPT** the magnetic resin. The resin is stored between runs in its original stock bottle to prevent evaporation. Vortex the magnetic resin solution well, both in the stock bottle and in the reservoir, before adding it to the metal rack. If you notice crystallization in any of the solutions, discard the solution, rinse the container out with distilled water, and start again with fresh reagent.

12. Remove the Parafilm and lids from the reagents, and fill the reservoirs to the appropriate level using solutions from the working solution bottles using the same lot as labeled on the reservoir. If not enough of the same lot of a solution remains, discard the remaining solution from the reservoir, rinse and re-label the reservoir with the new lot number. When filling the reservoirs **add approximately 10% to the volumes recommended below to account for the use of the large bore pipette tips:**

    Note: Bottles of MW1 require the addition of ethanol prior to use. See bottle for confirmation of ethanol addition and instructions for preparation if needed.

# of samples	Large reservoir Sterilized Water (mL)	Large reservoir Ethanol (mL)	Large reservoir Buffer MW1 (mL)	Large reservoir Buffer MTL (mL)	Small reservoir Buffer MW2 (mL)	Elution buffer (TE^{-4}) (mL)	Small reservoir Magnetic Resin (mL)
6	10.0	11.8	7.2	5.9	3.5	2.5	1.5
12	18.4	22.6	12.9	10.3	5.9	3.7	1.7
18	26.9	33.4	18.6	14.7	8.4	4.9	1.9
24	35.3	44.2	24.3	19.0	10.8	6.1	2.1
30	43.7	55.0	30.0	23.4	13.3	7.3	2.3
36	52.2	65.8	35.7	27.8	15.7	8.5	2.5
42	60.6	76.6	41.4	32.1	18.2	9.7	2.7
48	69.0	87.4	47.0	36.5	20.6	10.9	2.9

Place each reservoir into the metal rack in the following locations. The plastic reservoirs only fit into the rack one way. Check the directions of the notches which should point **into** the robot:

Size Container	Rack Position	Software Tag	Reagent
Large Container	L4	Rea_4	Sterilized Water
Large Container	L3	Rea_3	Ethanol (100%)
Large Container	L2	Rea_2	Wash Buffer 1 (Buffer MW1)
Large Container	L1	Rea_1	Lysis and Binding Buffer (Buffer MTL)
Small Container	S6	ReaS6	(empty)
Small Container	S5	ReaS5	(empty)
Small Container	S4	ReaS4	(empty)
Small Container	S3	ReaS3	Wash Buffer 2 (Buffer MW2)

Size Container	Rack Position	Software Tag	Reagent
Small Container	S2	ReaS2	Elution Buffer (TE^{-4})
Small Container	S1	ReaS1	Magnetic Particle Resin

13. Flip up the "container interlocks" and place the metal reservoir holder onto the left side of the robotic platform in the proper position. **DO NOT force the holder into place and be careful not to hit the robotic arm.** After correctly seating the metal holder, flip down the "container interlocks" and press "next".

14. Click "Next" when you are prompted to write a memo.

15. Place the sample preparation trays on the robot. One tray for every 6 samples. Click "Next".

16. Place empty, unlabeled 1.5mL elution tubes in the 65 degree (back) hot block, located on the right side of the robotic platform. Click "Next".

17. Label 1.5 mL screw top tubes for final sample collection in the robot.

18. Place **labeled**, empty 1.5 mL sample collection tubes in the 8 degree (front) cold block for collection of final samples.

19. At this point, the samples should be near the end of the incubation period (From Section E, Step 6). Spin all tubes in a microcentrifuge for 1 minute at 10,000 to 15,000 x g.

20. **Have a witness confirm the order and labels of both the sample tubes and the labeled 1.5 mL final sample collection tubes. The robot setup witness should also verify that all plasticware is in the correct position and correctly seated in the platform.**

21. Remove caps and place the samples for extraction on the robot. Discard the caps. **For empty positions, add a 2.0 mL sample tube filled with 200 uL of sterile water.**

22. Click "Yes" when asked to input sample names.

D. Importing Sample Names

1. At the sample input page, click "Import".

2. The Open window will appear. "Look in:" should automatically be set to a default of "SampleName". If not, the correct pathway to the folder is My Computer\C:\Program Files\GenoM-48\Export\SampleName. (The SampleName folder on the desktop is a shortcut to this file.)

3. Select your sample name file and click "Open". Verify that your sample names have imported correctly. Do not be concerned if a long sample name is not completely displayed in the small window available for each sample.

4. Manually type in the word "Blank" for all empty white fields.

5. Click "Next".

E. Verifying Robot Set-Up and Starting the Purification

1. In addition to confirming the *position* of all plasticware and samples, check the following conditions before proceeding:

All plasticware (tips, sample plates, tubes) is seated properly in the robotic platform	✔
Metal reservoir rack is seated properly, UNDER the interlocks	✔
Interlocks are down	✔
Sample tubes, elution tubes and sample collection tubes have been added to the platform in multiples of 6 as follows:	
Empty 1.5 mL tubes are filling empty positions for both sets of elution tubes in the cold and hot blocks	✔
2.0 mL sample tubes filled with 200uL of sterile H_2O are in empty positions of the sample rack	✔

2. After confirming the position and set-up of the plasticware click "Confirm".

3. Click "OK" after closing the door.

4. Click "Go" to start the extraction.

5. The screen will display the start time, remaining time, and the completion time.

6. Monitor the extraction until the transfer of DNA sample from the sample tubes to the first row of sample plate wells to ensure proper mixing of magnetic resin and DNA sample.

7. At the end of the extraction, a results page will be displayed indicating the pass/fail status of each set of six samples. See Section F for instructions for printing out the report page.

F. Saving and Printing Extraction Report Page

1. At the results page click the “Export” button at the bottom center of the screen. The Save As window will appear. “Save In:” should be set to the “Report” folder on the desktop. This is a shortcut to the following larger pathway: My Computer\C:\Program Files\GenoM-48\Export\Report.

2. In “File Name:”, name the report in the format, MMDDYY.HHMM. Set “Save As Type:” to Result Files (*.csv). For instance an extraction performed at 4:30pm on 5/14/06 would be saved as 051406.1630.csv.

3. Click “Save”.

4. Maximize the M48 spreadsheet by clicking its icon on the bottom tool bar.

5. At the bottom of the spreadsheet, click the “Import Run Results” tab.

6. Highlight cell “A1” and in the pull-down menus go to Data → Get External Data → Import Text File…

7. In the Import Text File window select:

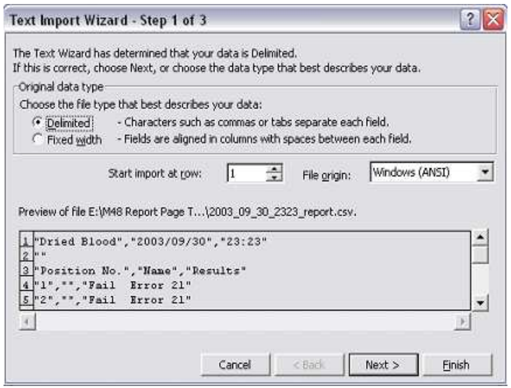
   Look in: Report (For specific pathway refer to Section F Step 1)
   Files of Type: All files
   File Name: Select your extraction run results by date and time

8. Click “Open”.

9. In the Text Import window Step 1 of 3, check the following settings:

   Original Data Type: Delimited
   Start Import at Row: 1
   File Origin: WINDOWS (ANSI)

The window should appear as below:

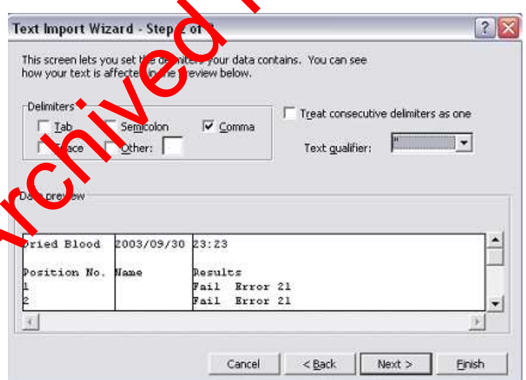


10. Click “Next”.

11. In Text Import window Step 2 of 3, select the following:

Delimiters: Place a check by comma. Make sure no other options are checked.
Text qualifier: “

Verify that the settings and data preview corresponds to those in the window below:



12. Click “Next”.

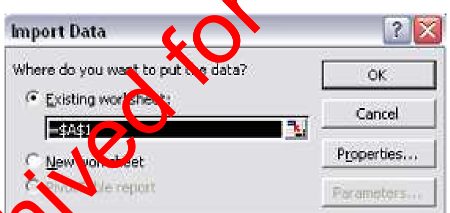
13. In Text Import window Step 3 of 3, select the following:

    Column Data Format: General

    The window should appear as below:

14. Click “Finish”.

15. In the Import Data window “Existing Worksheet” should be selected and the data input cell should read “=$A$1”. See below:



16. Click “OK”. Data will import into spreadsheet.

17. Click on the “Report” tab and verify that the run data has correctly imported into the report page.

18. Manually enter the analyst’s initials and extraction date (MM/DD/YY) and time (HH:MM AM/PM) in the highlighted cells.

19. Print the run report page.

20. Close the spreadsheet by going to File → Exit. A window asking “Do you want to save changes you made to…?”. Click “No”.

21. Proceed with clean-up and sterilization.

G. Post-Extraction Clean Up and UV Sterilization

1. Remove samples (from the 8 degree (front) cold block) from the robotic platform and cap with newly labeled screw caps.

2. Discard used pipette tips, sample tubes, and sample preparation plate(s). Remove reservoir rack.

3. Replace the lid on the magnetic resin reservoir and vortex remaining resin thoroughly. Transfer the Magnetic resin to the stock bottle immediately with a 1000uL pipetteman. Rinse the reagent container with de-ionized water followed by ethanol and store to dry.

4. Cover all other reagents and seal with Parafilm for storage. **LABEL RESERVOIRS WITH THE LOT NUMBER OF THE REAGENT THEY CONTAIN and record lot numbers on the worksheet.**

5. Wipe down the robotic platform and waste chute with 70% ethanol. **DO NOT USE SPRAY BOTTLES.**

6. Click “Next”.

7. When prompted, “Do you want to perform a UV sterilization of the worktable?”, click “Yes”.

8. Select 1 Hour for the time of “UV sterilization” then click “yes” to close the software upon completion.

9. As needed, pipet aliquots of neat and/or diluted extract into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

10. Store the extracts at 2 to 8°C or frozen.

11. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

12. Submit the run report and extraction paperwork to the supervisor for review.

13. **COMPLETE THE M48 USAGE LOG WITH THE TIME AND DATE OF THE EXTRACTION, USER INITIALS, AND ANY COMMENTS ARISING FROM THE RUN.**

H. BioRobot M48 Platform Diagram

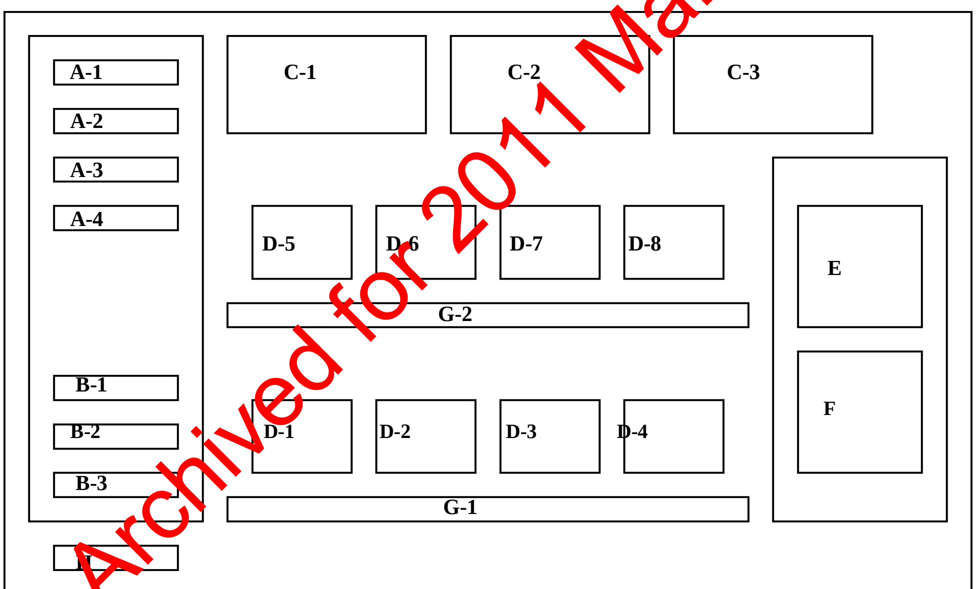


Figure 1. Diagram of Robotic Platform of the QIAGEN BioRobot M48.

A	(1-4)	Large Reagent Reservoir Positions
B	(1-3)	Small Reagent Reservoir Positions
C	(1-3)	Tube Racks 1, 2, and 3
D	(1-8)	Sample Plate Holders
E		Hot Elution Block (65 degrees)
F		Cold Final Elution Block (8 degrees)
G	(1-2)	Sample Tube Racks
H		Waste Disposal Chute

I. Troubleshooting

ERROR	CAUSE/REMEDY
Resin/sample is being drawn up into pipette tips unequally	**Report problem to QA.** Resin buffer has evaporated. O-rings are leaking and need service.
Crystallization around 1st row of wells in sample plate	Forgot to fill empty sample tubes with 200uL of sterile H_20.
BioRobot M48 cannot be switched on	BioRobot M48 is not receiving power. Check that the power cord is connected to the workstation and to the wall.
Computer cannot be switched on	Computer is not receiving power. Check that the power cord is connected to the computer and to the wall power outlet.
BioRobot M48 shows no movement when a protocol is started	BioRobot M48 is not switched on. Check that the BioRobot M48 is switched on.
BioRobot M48 shows abnormal movement when a protocol is started	The pipettor head may have lost its home position. In the QIAsoft M software, select "Manual Operation/ Home".
Aspirated liquid drips from disposable tips.	Dripping is acceptable when ethanol is being handled. For other liquids: air is leaking from the syringe pump. **Report problem to QA.** O-rings require replacement or greasing. If the problem persists, contact QIAGEN Technical Services

Revision History:
March 24, 2010 – Initial version of procedure.

Sample size for the extraction should be approximately 1/3 of a swab or a 3x3 mm cutting of the stain**. This extraction is applicable for all casework samples EXCEPT semen samples.**

All bloodstain cuttings should be placed in 2.0mL screw cap sample tubes.

A. Setting up M48 Spreadsheet and Saving Sample Name List

1. Collect the M48 Sample Submission Sheets for the extraction. On these sheets, assign each sample a sample rack position, remembering that the extraction negative will occupy Position 1 (and position 25, if extracting >24 samples). Also fill in the initials of the analyst performing the extraction and the extraction date(s) and time(s). This date and time will be used throughout the extraction.

2. Open the M48 evidence spreadsheet (M48EV).

3. Click the "Input Sample Names" tab and enter the sample names for the extraction, including the extraction negative(s), into the appropriate positions in column B.

4. Click the Ext Sheet 24 or Ext Sheet 48 tab depending on the batch size of the extraction.

5. Once the appropriate extraction sheet is open, finish the sheet by entering the tube label, target date, and IA initials for each sample.

6. Save this sheet by going to File → Save As and save the sheet to a flash drive with "File Name:" in MMDDYY.HHMM format and "Save As Type:" set to XLS (Microsoft Office Excel Workbook)(*.xls). For instance an extraction performed at 2:20pm on May 23, 2006 would be saved, with date and time in military format, as 052306.1420.xls.

7. Close out of the file completely by going to File → Exit. Print the extraction sheet.

8. After printing, reopen the file on the M48 computer and return to the "Input Sample Names" tab. Save this sheet by going to File → Save As and save the sheet to the "SampleName" folder on the desktop with "File Name:" in MMDDYY.HHMM format and "Save As Type:" set to CSV (Comma delimited)(*.csv).

9. Click "Save".

10. A window stating "The selected file type does not support workbooks that contain multiple sheets" will open. Click "OK".

11. A second window asking "Do you want to keep the workbook in this format?" opens. Click "Yes".

12. **Minimize** the M48 spreadsheet (do not close Excel or hit the "X" in the upper right-hand corner!).

B. Sample Preparation and Incubation

1. Remove the extraction rack from the refrigerator. Extract either evidence or exemplars. Do not extract both together.

2. Sample preparation should be performed under a hood.

3. Obtain an empty tube for the extraction negative and label it.

4. Have a witness verify your samples.

5. For large runs, prepare master mix for N+2 samples as follows, vortex briefly, and add 200uL to each of the tubes in the extraction rack and the pre-prepared extraction negative tube. For smaller runs, you may add Proteinase K and G2 Buffer to each tube individually:

Reagent	**1 sample**	**6 samples**	**12 samples**	**18 samples**	**24 samples**
Digestion Buffer (Buffer G2)	190 µL	1520 µL	2660 µL	3800 µL	4940 µL
QIAgen Proteinase K	10 µL	80 µL	140 µL	200 µL	260 µL

6. Shake at 1000 rpm at 56° C for a minimum of 30 minutes. Record the thermomixer temperature in the appropriate log book.

C. BioRobot M48 Software and Platform Set-Up

1. Double click on the “BioRobot M48" icon on the desktop.

2. Click the “Start” button. **Note: The door and container interlock must be closed to proceed.**

3. “Trace TD v1.1C1” protocol should be selected for casework samples. If not selected, click on the arrow in the middle of the screen and then select “Forensic” → “gDNA” → and “Trace TD v1.1C1”

4. Click on the “select” button and select “1.5 mL” for the size of the elution tubes.

5. Select the number of samples: 6, 12, 18, 24, 30, 36, 42, or 48.

6. Set sample volume to 200 µL (can not and should not change).

7. Set elution volume to 50 µL.

8. The next prompt asks to ensure the drop catcher is clean. In order to check this click on "manual operation" and select "Drop Catcher Cleaning". The arm of the robot will move to the front of the machine, and the drop catcher (a small plastic tray) will be right in front of you. Remove and clean with ethanol. When the catcher is clean, replace the tray, close the door, and click "OK" in the window.

9. Place a bag for the tips to be discarded. Click "Next".

10. The software will calculate the number of tips necessary for the run. Place tips in the tip rack(s) if necessary. **When filling racks make sure that the pipette tips are correctly seated in the rack and flush with the robotic platform.** Tips are located in three racks. These racks may be filled one at a time, BUT you must fill a **whole rack** at a time. After a rack is filled, reset the tip rack by clicking on "Yes tip rack ...", If no new tips are being added to the robot click "No".

    NOTE: When opening a new tip bag, ALL tips should be placed onto the robotic platform. Open tip bags should not be returned to the drawer. Racks may be used for tip storage. When adding tips, spilling into the next empty rack is OK, just do not **reset** the rack until it is **completely** full.

    Tips needed for a run:

# samples	6	12	18	24	30	36	42	48
# tips	30	42	54	66	78	90	102	114

    After you are finished, click "Next"

11. Fill the reagent reservoirs as stated below. All reagents are stored in their respective plastic reservoirs in the metal rack, covered with Parafilm, **EXCEPT** the magnetic resin. The resin is disposed of after every extraction. Vortex the magnetic resin solution well, both in the stock bottle and in the reservoir, before adding it to the metal rack. If you notice cystallization in any of the solutions, discard the solution, rinse the container out, and start again with fresh reagent.

12. Refer to the table below for amounts of 1000ng/uL Poly A RNA stock solution to add for resin preparation:

Samples	Volume of 1000ng/uL stock PolyA RNA solution added to resin (uL)	Volume of Untreated MagAttract Resin (uL)	Total Volume of RNA Treated MagAttract Resin (uL)
6 samples	**4.4**	**1497.8**	1500.0
12 samples	**5.0**	**1697.5**	1700.0
18 samples	**5.6**	**1897.2**	1900.0
24 samples	**6.2**	**2096.9**	2100.0
30 samples	**6.8**	**2296.6**	2300.0
36 samples	**7.4**	**2496.3**	2500.0
42 samples	**7.9**	**2696.0**	2700.0
48 samples	**8.5**	**2895.7**	2900.0

13. The pretreated resin may be prepared in a 15mL conical tube and then added to the appropriate reservoir for addition to the platform in the amount dictated by the protocol.

14. Remove the Parafilm and lids from the reagents, and fill the reservoirs to the appropriate level using solutions from the working solution bottles, adding approximately 10% to the volumes recommended below to account for the use of the large bore pipette tips:

Note: Bottles of MW1 require the addition of ethanol prior to use. See bottle for confirmation of ethanol addition and instructions for preparation if needed.

# of samples	Large reservoir Sterilized Water (mL)	Large reservoir Ethanol (mL)	Large reservoir Buffer MW1 (mL)	Large reservoir Buffer MTL (mL)	Small reservoir Sterile Water (mL)	Elution buffer (TE^{-4}) (mL)	Small reservoir Poly A RNA - Magnetic Resin (mL)
6	10.0	11.8	7.2	5.9	3.5	1.6	1.5
12	18.4	22.6	12.9	10.3	5.9	1.9	1.7
18	26.9	33.4	18.6	14.7	8.4	2.2	1.9
24	35.3	44.2	24.3	19.0	10.8	2.5	2.1
30	43.7	55.0	30.0	23.4	13.3	2.8	2.3
36	52.2	65.8	35.7	27.8	15.7	3.1	2.5
42	60.6	76.6	41.4	32.1	18.2	3.4	2.7
48	69.0	87.4	47.0	36.5	20.6	3.7	2.9

Place into the metal rack in the following locations. The plastic reservoirs only fit into the rack one way. Check the directions of the notches which should point **into** the robot:

Size Container	Rack Position	Software Tag	Reagent
Large Container	L4	Rea_4	Sterilized Water
Large Container	L3	Rea_3	Ethanol (100%)
Large Container	L2	Rea_2	Wash Buffer 1 (Buffer MW1)
Large Container	L1	Rea_1	Lysis and Binding Buffer (Buffer MTL)
Small Container	S6	ReaS6	(empty)
Small Container	S5	ReaS5	(empty)
Small Container	S4	ReaS4	(empty)
Small Container	S3	ReaS3	Sterilized Water
Small Container	S2	ReaS2	Elution Buffer (TE^{-4})
Small Container	S1	ReaS1	Magnetic Particle Resin

15. Flip up the "container interlocks" and place the metal reservoir holder onto the left side of the robotic platform in the proper position. **DO NOT force the holder into place and be careful not to hit the robotic arm.** After correctly seating the metal holder, flip down the "container interlocks" and press "next".

16. Click "Next" when you are prompted to write a memo.

17. Place the sample preparation trays on the robot. One tray for every 6 samples. Click "Next".

18. Place empty, unlabeled 1.5mL elution tubes in the 65 degree (back) hot block, located on the right side of the robotic platform. Click "Next".

19. Label 1.5 mL screw top tubes for final sample collection in the robot.

20. Place **labeled**, empty 1.5 mL sample collection tubes in the 8 degree (front) cold block for collection of final samples.

21. At this point, the samples should be near the end of the incubation period (From Section B, Step 6). After incubation, spin the samples down briefly and pretreat with Poly A RNA prior to placing on the robot. To each sample lysate add 250ng of Poly A RNA. A dilution of the stock Poly A RNA solution may be prepared for a final concentration of 250ng/uL and 1uL of this dilution should be added to each sample lysate. Prepare the 250ng/uL solution by adding 15uL of the stock 1000ng/uL Poly A RNA solution to 45uL of irradiated water.

    NOTE: For cigarette butts, if the sample submitted is a strip of the filter paper, the lysate must be transferred to a new 2.0mL screw cap tube while leaving behind the cigarette strip. This is important to avoid the clogging of the M48 tips.

22. Spin all tubes in a microcentrifuge for 1 minute at 10,000 to 15,000 x g. **When they are ready, have a witness confirm the order and labels of both the sample tubes and the labeled 1.5 mL final sample collection tubes. The robot setup witness should also verify that all plasticware is in the correct position and correctly seated in the platform.**

23. Remove caps and place the samples for extraction on the robot. Discard the caps. **For empty positions, add a 2.0 mL sample tube filled with 200 uL of sterile water.**

24. Click "Yes" when asked to input sample names.

D. Importing Sample Names

1. At the sample input page, click "Import".

2. The Open window will appear. "Look in:" should automatically be set to a default of "SampleName". If not, the correct pathway to the folder is My Computer\C:\Program Files\GenoM-48\Export\SampleName. (The SampleName folder on the desktop is a shortcut to this file.)

3. Select your sample name file and click "Open". Verify that your sample names have imported correctly. Do not be concerned if a long sample name is not completely displayed in the small window available for each sample.

4. Manually type in the word "Blank" for all empty white fields.

5. Click "Next".

E. Verifying Robot Set-Up and Starting the Purification

1. In addition to confirming the *position* of all plasticware and samples, check the following conditions before proceeding:

All plasticware (tips, sample plates, tubes) is seated properly in the robotic platform	✔
Metal reservoir rack is seated properly, UNDER the interlocks	✔
Interlocks are down	✔
Sample tubes, elution tubes and sample collection tubes have been added to the platform in multiples of 6 as follows:	
Empty 1.5 mL tubes are filling empty positions for both sets of elution tubes in the cold and hot blocks	✔
2.0 mL sample tubes filled with 200uL of sterile H2O are in empty positions of the sample rack	✔

2. After confirming the position and set-up of the plasticware click "Confirm".

3. Click "OK" after closing the door.

4. Click "Go" to start the extraction.

5. The screen will display the start time, remaining time, and the completion time.

6. Monitor the extraction until the transfer of DNA sample from the sample tubes to the first row of sample plate wells to ensure proper mixing of magnetic resin and DNA sample.

7. At the end of the extraction, a results page will be displayed indicating the pass/fail status of each set of six samples. See Section F for instructions for printing out the report page.

F. Saving and Printing Extraction Report Page

1. At the results page click the "Export" button at the bottom center of the screen. The Save As window will appear. "Save In:" should be set to the "Report" folder on the desktop. This is a shortcut to the following larger pathway: My Computer\C:\Program Files\GenoM-48\Export\Report.

2. In "File Name:", name the report in the format, MMDDYY.HHMM. Set "Save As Type:" to Result Files (*.csv). For instance an extraction performed at 4:30pm on 5/14/06 would be saved as 051406.1630.csv.

3. Click "Save".

4. Maximize the M48 spreadsheet by clicking its icon on the bottom tool bar.

5. At the bottom of the spreadsheet, click the "Import Run Results" tab.

6. Highlight cell "A1" and in the pull-down menus go to Data → Get External Data → Import Text File…

7. In the Import Text File window select:

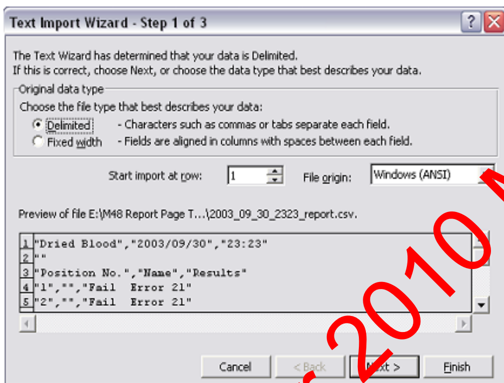
   Look in: Report (For specific pathway refer to Section F Step 1)
   Files of Type: All files
   File Name: Select your extraction run results by date and time

8. Click “Open”.

9. In the Text Import window Step 1 of 3, check the following settings:

Original Data Type: Delimited
Start Import at Row: 1
File Origin: WINDOWS (ANSI)

The window should appear as below:

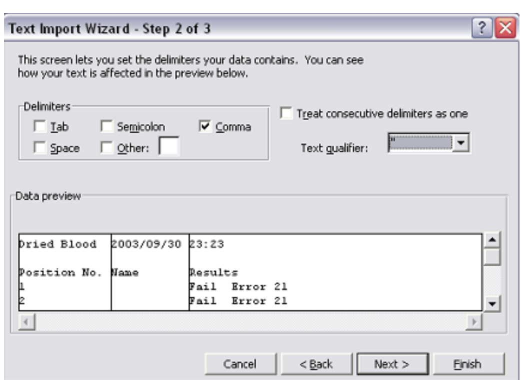


10. Click “Next”.

11. In Text Import window Step 2 of 3, select the following:

Delimiters: Place a check by comma. Make sure no other options are checked.
Text qualifier: “

Verify that the settings and data preview corresponds to those in the window below.

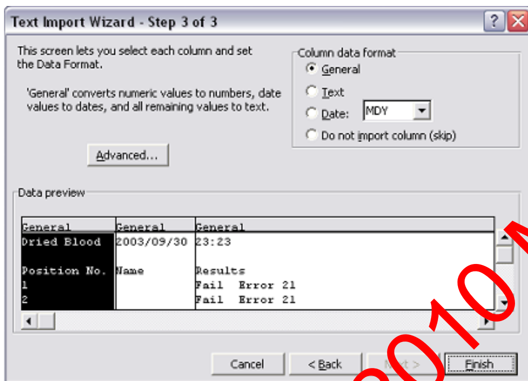


12. Click “Next”.

13. In Text Import window Step 3 of 3, select the following:

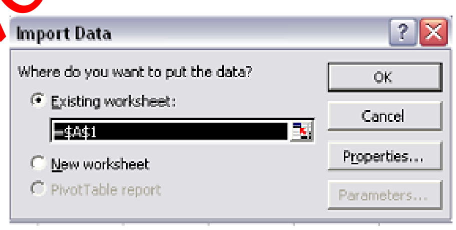
    Column Data Format: General

    The window should appear as below:



14. Click “Finish”.

15. In the Import Data window “Existing Worksheet” should be selected and the data input cell should read “=$A$1”. See below:



16. Click “OK”. Data will import into spreadsheet.

17. Click on the “Report” tab and verify that the run data has correctly imported into the report page.

18. Manually enter the analyst's initials and extraction date (MM/DD/YY) and time (HH:MM AM/PM) in the highlighted cells.

19. Save this sheet by going to File → Save As and save the sheet to a flash drive with "File Name:" in MMDDYY.HHMM format and "Save As Type:" set to XLS (Microsoft Office Excel Workbook)(*.xls). This may require you to write over the original file saved by that name on the flash drive.

20. Close out of the file completely by going to File → Exit. Print the run report page.

21. Proceed with clean-up and sterilization.

G. Post-Extraction Clean Up and UV Sterilization

1. Wipe down the robotic platform and waste chute with Ethanol. **DO NOT USE SPRAY BOTTLES.**

2. Discard used pipette tips, sample tubes, and sample preparation plate(s).

3. Replace the lid on the magnetic resin reservoir and vortex remaining resin thoroughly. Discard the Magnetic resin immediately with a 1000uL pipetteman. Rinse the reagent container with de-ionized water followed by ethanol and store to dry.

4. Cover all other reagents and seal with Parafilm for storage. **LABEL RESERVOIRS WITH THE LOT NUMBER OF THE REAGENT THEY CONTAIN and record lot numbers on the worksheet.**

5. Click "Next".

6. When prompted, "Do you want to perform a UV sterilization of the worktable?", click "Yes".

7. Select 1 Hour for the time of "UV sterilization" then click "yes" to close the software upon completion.

8. Have a supervisor sign-off on the run report, and submit samples at 1/10 and/or 1/100 dilutions, as needed for real-time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

9. Store the extracts at 2 to 8°C or frozen.

10. Samples should be added to the next available Rotorgene Summary Sheet, saved to the appropriate folder on the network pertaining to your casework group.

11. **COMPLETE THE M48 USAGE LOG WITH THE TIME AND DATE OF THE EXTRACTION, USER INITIALS, AND ANY COMMENTS ARISING FROM THE RUN.**

H. BioRobot M48 Platform Diagram

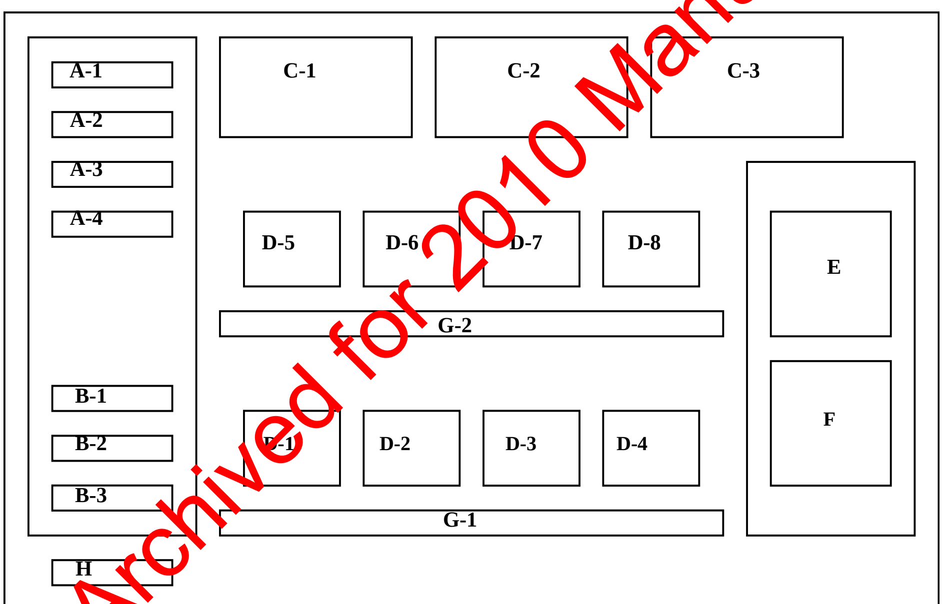


Figure 1. Diagram of Robotic Platform of the QIAGEN BioRobot M48.

A	(1-4)	Large Reagent Reservoir Positions
B	(1-3)	Small Reagent Reservoir Positions
C	(1-3)	Tube Racks 1, 2, and 3
D	(1-8)	Sample Plate Holders
E		Hot Elution Block (65 degrees)
F		Cold Final Elution Block (8 degrees)
G	(1-2)	Sample Tube Racks
H		Waste Disposal Chute

I. Troubleshooting

Error	Cause/ Remedy
Resin/sample is being drawn up into pipette tips unequally	**Report problem to QA.** Resin buffer has evaporated. O-rings are leaking and need service.
Crystallization around 1st row of wells in sample plate	Forgot to fill empty sample tubes with 200uL of sterile H_20
BioRobot M48 cannot be switched on	BioRobot M48 is not receiving power. Check that the power cord is connected to the workstation and to the wall
Computer cannot be switched on	Computer is not receiving power. Check that the power cord is connected to the computer and to the wall power outlet.
BioRobot M48 shows no movement when a protocol is started	BioRobot M48 is not switched on. Check that the BioRobot M48 is switched on.
BioRobot M48 shows abnormal movement when a protocol is started	The pipettor head may have lost its home position. In the QIAsoft M software, select "Manual Operation/ Home".
Aspirated liquid drips from disposable tips.	Dripping is acceptable when ethanol is being handled. For other liquids: air is leaking from the syringe pump. **Report problem to QA.** O-rings require replacement or greasing. If the problem persists, contact QIAGEN Technical Services

Revision History:
March 24, 2010 – Initial version of procedure.

In order to allow for duplicate amplifications, the final volume should be between 20 :L and 50 :L. See Table 1 for minimum sample concentration requirements.

1. Fill out a Microcon worksheet. Label a sufficient number of blue Microcon YM100 sample reservoirs and insert each into a labeled collection tubes.

   A. Pipet 100 :L of TE^{-4} solution into each labeled sample reservoir including the Microcon negative control.

   B. Alternatively, pre-coat the Microcon® membrane with Fish Sperm DNA or a 1/1000 dilution of Poly A RNA in an irradiated microcentrifuge tube or 15 mL tube:

      a. Fish Sperm DNA Preparation

         i. Add 1 uL of stock Fish Sperm DNA solution (1mg/mL) to 199uL of irradiated water for each sample on the microcon sheet.

         ii. Aliquot 200 uL of this Fish Sperm DNA solution to each Microcon® tube. Avoid touching the membrane. The volume for one sample is shown below. Refer to the microcon worksheet for calculated value.

      b. Poly A RNA Preparation

         i. Make a 1/10 dilution of 1mg/mL of Poly A RNA as follows: add 2 µL of Poly A RNA to 18 µL of irradiated water and mix the solution well. This is a final concentration of 100µg/mL.

         ii. Using the 1/10 dilution, make a 1/100 dilution with 2 uL of 100ug/mL Poly A RNA in 198 uL of irradiated water and mix the solution well. The solution has a final concentration of 1 ng/uL.

         iii. Add 1 uL of the 1ng/uL Poly A RNA solution to 199uL of water for each sample on the microcon sheet.

         iv. Aliquot 200 uL of this Poly A RNA solution to each Microcon® tube. Avoid touching the membrane. The volume for one sample is shown below. Refer to the microcon worksheet for calculated value.

Reagent	1 sample
Water	199 µL
Fish Sperm DNA (1mg/mL) or Poly A RNA (1ng/µL)	1 µL

NOTE: For samples with 400 µL of digest solution, make a 20 µL solution of 1 uL of Fish Sperm DNA (1mg/mL) or 1 µL of Poly A RNA (1 ng/µL) with 19 µL of water. Mix well and add this solution to the membrane. Ensure that the entirety of the membrane is covered. In this manner, all of the digest may be added to the Microcon® membrane for a total volume of 420 uL.

2. Process 50 :L of TE^{-4} solution or irradiated water as a Microcon negative control. Make sure to use the same lot that will be used to dilute the samples, and don't forget to label the final negative control tube with the Microcon date and time.

3. Spin each DNA sample briefly. Have a witness confirm the order of the samples and Microcons.

4. Add each sample (0.4 mL maximum volume) to the buffer in the reservoir. Don't transfer any Chelex beads, or in case of an organic extraction sample, any organic solvent! Seal with attached cap. ***Avoid touching the membrane with the pipette tip!***

6. Return the original extraction tubes to their storage location. Do not discard the empty tubes.

7. Place the Microcon assembly into a variable speed microcentrifuge. Make sure all tubes are balanced! ***To prevent failure of device, do not exceed recommended g-forces.***

8. Spin at 500 x g (2400 RPM, Eppendorf) for 15 minutes at room temperature.

<u> FOR CONCENTRATION ONLY, SKIP STEP 9 AND PROCEED TO STEP 10 **</u>**

**** FOR CONCENTRATION ONLY, SKIP STEP 9 AND PROCEED TO STEP 10 ****

9. **FOR PURIFICATION** of the DNA sample add 200 :L of TE^{-4} solution or irradiated water and repeat Steps 7-8. Do this as often as necessary to generate a clear extract, and then continue with Step 10. When performing multiple wash steps it is necessary to empty the bottom collection tube intermittently.

   NOTE: When purifying samples with a low DNA concentration it may be advantageous to use several wash steps and to also reduce the volume to achieve both, a cleaner sample and an increased DNA concentration.

10. Remove assembly from centrifuge. Visually inspect each Microcon 100 membrane tube. If it appears that more than 20 µL remains above the membrane, centrifuge that tube for 5 more minutes at 2400 rpm. This process may be repeated as necessary.

11. Open the attached cap using a tube opener and add 20:L TE^{-4}. ***Avoid touching the membrane with the pipette tip!*** Separate collection tube from sample reservoir.

12. Place sample reservoir upside down in a new **labeled** collection tube, then spin for 3 minutes at 1000 x g (3400 RPM Eppendorf). Make sure all tubes are balanced!

13. Remove from centrifuge and separate sample reservoir. Measure resulting volume using an adjustable Micropipette, record volume on worksheet; adjust volume to desired level using TE^{-4} or irradiated water.

    A. Clean-up for high DNA concentrations: reconstitute to starting volume.
    B. Low DNA samples (clean-up and/or concentration): adjust to 20-50 :L (depending on amplification system)

14. Transfer the DNA extracts and the Microcon negative control to newly labeled 1.5mL Eppendorf tubes and store extract for later use. Note storage location on worksheet.

15. Calculate resulting concentration or submit to real-time PCR analysis to find the new DNA concentration.

    ATTENTION: **Do not store the DNA in the Microcon vials! The lids are not tight enough to prevent evaporation**.

Troubleshooting:

Lint, bone dust and other particles can clog the membrane. If the liquid does not go down, collect the sample from the filter and redistribute the supernatant to multiple filters or a new filter. Pipet off the clear supernatant without disturbing the particle pellet. Microcon negative controls should be treated accordingly.

If the problem persists, the specific Microcon lot number might be faulty. Notify the QA Unit and try a different lot number.

TABLE 1:

	Identifiler™ 28 cycles	**Identifiler™ 31 cycles**
Minimum Desired Template	100.00 pg	^20.00 pg
Template volume for amp	5 µL	5 µL
Minimum Sample Concentration in 200 µL	20 pg/µL	^4 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning* to 50 µL	5 pg/µL	N/A
Minimum Sample Concentration in 200 µL prior to Microconning** to 20 µL	2 pg/µL	0.40 to ^0.10 pg/µL
For LCN samples: Minimum Sample Concentration in 20 µL	20.00 pg/µL	**4.00 to ^1.00** pg/µL

* Sample concentration **prior** to processing with a Microcon 100 and elution to 50 µL
** Sample concentration **prior** to processing with a Microcon 100 and elution to 20 µL
^ Samples with less than 20 pg per amplification may be amplified upon referral with the LCN supervisor

Revision History:
March 24, 2010 – Initial version of procedure.

A. Paperwork Preparation

1. Open the "RG summary sheet" Excel file template in the Rotorgene - RG sheets folder.

2. In cell D3 of the assay sheet tab, type in the name of the quantitation assay as follows: RG# and "Q" or the name of the RG, month, day, and year, period, hour and minutes. For example, RG1Q040905.1330 or GertyQ051707.1500.

3. Exemplar and evidentiary samples may be quantitated simultaneously. However, exemplar extracts must be diluted prior to performing the assay. In other words, only the aliquots and/or dilutions of the exemplars may be present with the evidentiary samples.

4. Create a Rotorgene "sample sheet" by using one of the following steps:

   a. Type sample names from the extraction sheet and/or "to be quanted sheet" into the Rotorgene "sample sheet" (second sheet of the Excel workbook). For samples requiring dilutions, the dilution factor should be entered in decimal form following a comma after the sample name. For instance, for bloodstain 1A a 1/10 dilution is required. This sample should be entered into the RG sample sheet as "bloodstain 1A, 0.1". For neat samples, no additional info should be added.
   b. Or open the "Rotorgene generation macro".
      i. Copy/Type sample names into the appropriate extraction type section
      ii. Click on the appropriate button to create dilutions for that extraction type.
      iii. Click the "Complete & Save" button.

NOTE: Type sample names in 3130xl format. Letters, numbers, and only the following characters: - _ . ( ){ }[ ] + ^ may be used. Do not use commas (except to separate sample and dilution info), colons, or quotes. Use the character ^ instead of quotes.

5. Three calibrators and 15 standards are measured with each assay; therefore, 54 samples may be measured on each RG assay.

6. If applicable, enter the initials of the analysts to whom paperwork should be directed, the target date, and the top tube label under the "IA", "target date", and "tube label" columns, respectively. If not available or not applicable, type a dash in the cell. For quant results going directly to the analyst rather than the auto-aliquot system, enter an "A" in the A column.

7. In cell D4 of the assay sheet tab, enter the name of the extraction assay. If multiple extraction sets are being run, enter "misc".

8. The number of samples that are being measured will be automatically calculated and shown in cell E7 as samples are added to the "sample sheet". Verify that this number is correct. The spreadsheet will automatically calculate how much of each reagent to aliquot.

9. Save the sheet in the appropriate Rotorgene folder using the quantitation name.

10. Print the assay and sample sheets.

B. Work Place Preparation

1. Retrieve clean racks, cap openers, Rotorgene 0.1 mL tubes and caps, microcentrifuge tubes, and irradiated GIBCO™ ULTRA PURE™ distilled water from storage or the Stratalinker.

2. Apply 10% bleach followed by water and/or 70% Ethanol to the entire work surface. Cap openers, racks, and pipettes may be cleaned in a similar manner. **For LCN samples, all Rotorgene setup steps should be carried out under a hood.**

    a. For LCN samples, the 1.5 mL microcentrifuge tubes and water aliquots in 1.5 mL tubes must be irradiated for 30 and 45 minutes, respectively.
    b. Rotorgene tubes and caps are used as packaged.

C. Sample Dilution

If necessary, dilute the sample extracts (as with HCN samples).

1. Label microcentrifuge dilution tubes with sample name and dilution.

2. Place each dilution tube directly behind the corresponding extract tube in a rack.

3. Add the appropriate amount of diluant (irradiated water or TE) to each dilution according to Table 1.
    a. Sexual assault semen and saliva samples, scrapings and other samples that are extracted with the "Chelex other" or M48 method, and bone samples should be measured with a neat and a 1/100 dilution.
    b. Blood and buccal samples and all burglary samples may be measured with a 1/10 dilution only. This will capture most concentrations. If necessary, a second measurement may be taken with either a neat or a 1/100 dilution.
    c. LCN samples should be measured with a neat dilution. If necessary, a 1/10 dilution may be made if one suspects inhibition.
    d. Pipet tips do not need to be changed to add water/TE to empty tubes. Close all caps.

4. Open only one sample and its corresponding dilution tube at one time.

5. Thoroughly mix each extract, prior to aliquotting.

6. Immediately following each dilution, return the original sample extract tube to its cryobox. Return the original samples to 4ºC storage.

7. Once the dilutions are completed, evidentiary samples may join exemplar dilutions on the benchtop.

TABLE 1:

	Submission 1			**Submission 2**		
	Dilution 1	**Sample**	**Water or TE**	**Dilution 2**	**Sample**	**Water or TE**
HCN Semen and saliva (amylase positive) samples	**Neat**	**5 µL**	**0**	**1/100**	**2 µL**	**198 µL**
HCN Scrapings or “other” extractions	**Neat**	**5 µL**	**0**	**1/100**	**2 µL**	**198 µL**
HCN exemplars Bone	**Neat**	**5 µL**	**0**	**1/100**	**2 µL**	**198 µL**
HCN exemplars Blood or Saliva	**1/10**	**2 µL**	**18 µL**	**1/100 or neat (if necessary)**	**2 µL or N/A**	**198 µL or N/A**
HCN Blood Samples	**1/10**	**2 µL**	**18 µL**	**1/100 or Neat (if necessary)**	**2 µL or N/A**	**198 µL or N/A**
Touched objects and/or LCN Samples	**Neat**	**N/A**	**N/A**	**1/10 (if necessary)**	**2 µL**	**18 µL**

In order to conserve, neat LCN samples may be taken from the extract tube and added to the quantitation tube directly (no neat submission tube is necessary). However, 1/10 dilutions should be prepared in advance as specified above.

D. Remove reagents for the master mix from the reagent freezer/refrigerator

1. Retrieve $MgCl_2$, 10X PCR buffer, BSA, dNTPs, TAQ GOLD, unlabeled “EB1” and “EB2” primers, and SYBR Green I from the freezer, irradiated GIBCO™ ULTRA PURE™ distilled water from the refrigerator, and DMSO from the cabinet.

2. Store reagents, except DMSO and water, in a Nalgene cooler on the bench.

3. Record lot numbers of reagents.

4. Just before initiating "sample preparation", place $MgCl_2$, 10X PCR buffer, BSA, dNTPs, and unlabeled "EB1" and "EB2" primers on a 48-position microcentrifuge rack in order to thaw these reagents.

E. Standard Curve Preparation

1. Retrieve 1600 pg/µL standard DNA from the freezer and record lot #.

2. Ensure that the contents of the 1600 pg/µL standard DNA tube are thawed and removed from the cap, by centrifuging the tube.

3. Label tubes as follows: 400, 100, 25, 6.25, 1.56, 0.39, and NTC (no template control or 0 pg/µL).

4. Add 15 µL of irradiated water to tubes 400, 100, 25, 6.25, 1.56, 0.39, and the NTC. Pipet tips do not need to be changed to add water to empty tubes. Close all caps.

5. 0.25 Serial dilution

   In order to mix each dilution thoroughly, either pipet the dilution up and down several times or vortex each dilution and subsequently centrifuge the tube at no more than 3000 rpm for 3 seconds.

   a. Open only two consecutive standard DNA tubes at once starting with the 1600 and the 400 pg/µL tubes.
   b. Mix the DNA solution in the 1600 pg/µL. Take 5 µL of standard DNA at 1600 pg/µL and add to the 400 pg/µL tube, and thoroughly mix the contents.
   c. With a new pipet tip, take 5 µL of standard DNA at 400 pg/µL and add to the 100 pg/µL tube, and thoroughly mix the contents.
   d. With a new pipet tip, take 5 µL of standard DNA at 100 pg/µL and add to the 25 pg/µL tube, and thoroughly mix the contents.
   e. With a new pipet tip, take 5 µL of standard DNA at 25 pg/µL and add to the 6.25 pg/µL tube, and thoroughly mix the contents.
   f. With a new pipet tip, take 5 µL of standard DNA at 6.25 pg/µL and add to the 1.56 pg/µL tube, and thoroughly mix the contents.

g. With a new pipet tip, take 5 µL of standard DNA at 1.56 pg/µL and add to the 0.39 pg/µL tube, and thoroughly mix the contents.
h. Do not add anything to the NTC tube.

F. Sample Preparation

1. Remove 1500 pg/µL calibrator from freezer and record lot number.

   a. Vortex the calibrator thoroughly and centrifuge the tube at 3000 rpm for approximately 3 seconds.

   b. Make three 0.166 dilution (1/6) of the calibrator with 4 µL of the calibrator and 20 µL of irradiated water.

2. Vortex all samples including the standards, NTC, calibrator, and the dilution and/or extract tubes.

3. Centrifuge all samples briefly for 3 seconds at no greater than 3000 rpm; this will prevent the DNA from aggregating at the bottom of the tube.

4. **Witness Step:**
   Arrange samples in order according to the sample sheet in a 96 well rack.
   a. Place samples in exactly the same place on the rack as they will appear vertically positioned in the rotor.
   b. Label the top of the sample tubes with rotor well identifier or tube labels.
   c. Have a witness confirm the sample locations.

G. Master Mix preparation

1. Remove the SYBR Green I from the Nalgene cooler and prepare a 1/100 dilution. Take 2 µL of SYBR Green I in 198 µL of irradiated water, vortex, and tap the tube on the bench to consolidate the reagent at the bottom of the tube.

2. Mix each reagent before adding.
   a. After each reagent has thawed, vortex each reagent, with the exception of TAQ GOLD.
   b. Centrifuge reagents in the table top centrifuge at 3000rpm for approximately 3 seconds.

3. Add each reagent in the order as it appears on the worksheet. Thoroughly mix each tube reagent by pipetting up and down, or vortexing briefly. If vortexing, afterwards tap the tube on the bench to prevent the reagent from being trapped in the cap.

4. For total reagent volumes above 20 µL, use a P200 even for multiple dispenses as opposed to one dispense with a P1000. To ensure accurate pipetting, aspirate and dispense the reagent as specified on the run sheet.

5. After adding each reagent, check that it has been added on the quantitation sheet, and place the reagent back in the Nalgene cooler, or for water and DMSO, in the opposite corner of the 48 well microcentrifuge rack.

6. Thoroughly mix the master mix by vortexing. Tap the tube on the bench to prevent the reagent from being trapped in the cap and/or centrifuge briefly for approximately 3 seconds.

7. Add 23 µL of master mix to the appropriate number of Rotorgene tubes. Fill tubes in a vertical fashion (positions 1-16 or A1 to A8, and B1-B8 in older rotors). After adding master mix to 16 tubes, re-vortex the master mix and ensure all of the master mix is consolidated by tapping the tube on the bench and centrifuging briefly for approximately 3 seconds. Use a new pipette tip.

   See Table 2 below for reagent concentrations, the spreadsheet will calculate amounts for n+10%n samples and will display rounded values for pipetting.

TABLE 2:

Reagent	Concentration	µL[#] for 1 Rx
Irradiated GIBCO™ ULTRA PURE™ distilled water		8.3 (8.26)
10X PCR Buffer	10mM Tris/50mM KCL	2.5
25 mM $MgCl_2$	275 µM	2.8 (2.75)
5 mg/mL BSA	0.525µg/µL	4.0
2.5 mM dNTPs	200 µM each	2.0
DMSO	8%	2.0 (1.96)
1/100 dilution of 10,000X SYBR Green I	100X	0.3 (0.28)
20 pmol/µL Primer EB1	0.4 µM	0.5
20 pmol/µL Primer EB2	0.4 µM	0.5
5U/µL ABI Taq Gold	1.25U	0.3 (0.25)
Total volume		23.00

[#]The spreadsheet calculates the values using two significant figures. However, for the purposes of manual addition, only one significant digit is shown.

H. Sample Addition

1. In order to avoid the creation of aerosols, thoroughly mix the contents of each tube by pipetting up and down repeatedly.

2. Add 2 µL of each sample, including the standards, NTC, the calibrator dilution, and the sample dilutions and/or extracts, to each tube with master mix.
   a. If necessary, in order to conserve sample, only 1 µL of sample may be measured. Note this on the sample sheet and double the resultant value to accurately reflect the sample's concentration per microliter.
   b. Every four reaction tubes, place caps on the tubes. (The caps are attached in sets of four.)

c. Number the first cap in every set of four as they will appear in the rotor. (1 for 1, 2 for 2, etc. For the older rotors, 1 for A1, 5 for A5, 9 for B1 etc.) **DO NOT** label the tube itself, as this may interfere with fluorescent detection.

d. Open the machine. Remove the circular rotor from the instrument by either pressing in the middle silver stem in the RG6000 or unscrewing the center piece in the RG3000. Remove either the silver clip from the RG6000 rotor or the silver ring from the RG3000 rotor. Add tubes to the rotor. Ensure that tube 1 is in position 1, etc. or in older rotors, 1 is in position A1 etc.

e. Ensure that all positions on the rotor are filled (using blanks if necessary).

f. In the RG6000, add the silver clip to the rotor, lock into the Rotorgene, and close machine. In the RG3000, add the silver ring and screw the rotor into the Rotorgene, locking the rotor in place. Ensure the silver ring is in place and sitting securely in the rotor on all sides. Close machine.

I. Software Operation

1. Open Excel and the relevant sample sheet to the sheet with the sample names, and then collapse the window.

2. Open Rotorgene 6 software on the desktop.

3. Click File, New, Casework, and click "new"

4. In the wizard:
   a. Ensure that the "Rotorgene 72 well rotor" is highlighted
   b. Make sure that the box next to "locking ring attached", is checked.
   c. Click "Next."
   d. Type initials for Operator and add any notes (extraction date/time)
   e. Reaction volume should be "25 µL"
   f. Sample layout should be "1, 2, 3…"
   g. Click "Next."

h. In the RG3000s, click "Calibrate". In the RG6000, click "gain optimisation".
   i. "Perform Calibration before 1st acquisition"
   ii. Click on "calibrate acquiring" (RG3000) or "optimize acquiring" (RG6000).
   iii. "This will remove your existing setting for auto gain calibration?" The window appears, click YES. A green gain window will open. Click "ok", then "close".
   iv. Note selecting "calibrate all" will attempt to calibrate for all channels known by the software whereas "calibrate acquiring" will instead only calibrate those that have been used in the thermal profile defined in the run such as FAM or Green.
   v. Click next in wizard and "start run"

5. "Save as" the RG#, date and time (for example, "RG1Q112904.1400" for a run on RG1 on Nov 29, 2004 at 2:00pm) in Log Archive folder.

6. Sample sheet window
   a. Expand the Excel sample sheet window. Copy the sample names.
   b. Paste sample names in the appropriate rows in the Rotorgene sample window by right clicking and selecting paste.
   c. Settings:
      i. Given concentration format: 123,456.78 unit pg/µL
      ii. Type category
         1) Standards: std
         2) Zero standard: NTC
         3) Samples and calibrator: unk
      iii. In all wells with standard, calibrator or sample, select "YES"

   d. Hit "Finish"

See below for cycling parameters that should not be changed:

95ºC	10 min	
94ºC	15 sec	35 cycles
68ºC	60 sec	
72ºC	30 sec	
72ºC	15 sec	

7. Enter run information in the Rotorgene log book.

8. The run will approximately require 1 hour and 40 minutes for completion.

9. Following the initial heating to activate the TAQ and the gain calibration, the raw data will appear on the screen. With this information, one can monitor the progress of the run. Fluorescence for the highest standard should be apparent from ~ cycle 15.

10. Previous run files may be examined while the computer is collecting data.
    a. Collapse the window.
    b. Double click on the Rotorgene icon on the desktop.
    c. The computer will prompt that another version of the software is running and ask if you want to run an analysis version only. Click yes.

J. Clean Up

1. Return water, dNTPs, $MgCl_2$, 10X PCR buffer, BSA, DMSO, EB1 primer, EB2 primer, TAQ GOLD and water tubes with any remaining reagents to the working reagents box.

2. Dispose of all dilution tubes of the standard, calibrator, and SYBR Green I. Sample aliquots may be stored until assay success is confirmed.

K. Sample and Data Storage

1. Store extracts in a cryobox in the DNA refrigerator. For LCN, the extracts should be stored in the DNA refrigerator in the pre-amp room in the designated area.

2. Ensure that the final Rotorgene sheet is stored on the network in the folder labeled “RG sheets” and that the data from the assay is in the folder labeled “RG data” under the appropriate Rotorgene folder.

3. To transfer over the Rotorgene data to the network:
    a. After the run is done, save and exit out of the Rotorgene software.
    b. In the Log Archive, go to the appropriate run folder.
    c. Copy the run onto a flash drive and transfer the run into the appropriate Rotorgene folder under the “RG data” folder on the network.

4. Pass the assay and sample sheets to the rotation supervisor for review.

L. Analysis

1. Analysis may be performed on the instrument computer or any computer that has access to the software.

2. Open Rotorgene software on the desktop. If the computer is not connected to an instrument, when the software indicates that the computer cannot connect to the instrument on serial port COM1, select "run in virtual mode".

3. Click "Open" and click on the run to be analyzed in the "RG data" folder

4. Click "Analysis" on the toolbar.
   a. Select **"Quantitation", "Show".**
      i. Three windows will open with the standard curve, the samples, and fluorescence.
      ii. If a "Calculate Automatic Threshold" window opens up, click ok.
      iii. Ensure that **"dynamic tube"** and **"slope correct"** are selected on the tool bar.
      iv. Select the tab **"more settings".**
         1) Ensure that the NTC threshold is set to 10%.
         2) The box under the **"reaction efficiency threshold" should NOT be selected** however.
         3) Click "OK"
      v. If any of the settings need to be corrected, "auto find threshold" must be performed again. ("Auto find threshold" can be found in the lower right corner of the screen if the "Quantitation Analysis" graph is selected.)

   b. Check if any sample curve crosses the threshold at an early cycle due to background fluorescence. The sample in question would have no value, but the normalized data would display a curve that crosses the threshold both at an early and at a later cycle.

In order to avoid disabling the dynamic tube normalization setting, move the threshold to the right, ignoring the first few cycles, so that the sample does not cross the threshold. This can be achieved by the following:

i. In the normalized data windowpane, on the lower right side, under CT calculation, change the number for “Eliminate Cycles before:” from 0 to 1-5. Chose the smallest number where the threshold does not cross the data curve in question.

ii. Alternatively, select the grid immediately to the right of “Eliminate cycles before”. This allows manual manipulation of the starting cycle number of the threshold.

iii. Reanalyze the data by selecting “auto find threshold”.

c. One may also manually manipulate the vertical position of the threshold on the standard curves.

i. Select the grid to the right of the threshold value and then click on the red threshold line and adjust the line. Moving this line vertically will make the threshold cross the standards’ curves at different cycles and thus will change the efficiency, Ct, and sample values.

ii. Position the line to optimize the distance between the Ct values of the standards and thus the calibrator values, while maintaining a passing efficiency value.

5. Save the RG data project.

M. Report

1. On the “Quant results” screen, (by right clicking the table heading with the mouse and un-checking certain columns) only pick the following columns: No., Name, Ct., and Calc. Conc.

2. If the No. column shows the well location instead of the number, select “Samples” from toolbar. Under “format”, select “Toggle Sample ID Display”. Click “OK”.

3. Select "Reports" from toolbar
   a. Select "Quantitation, cycling A FAM"
   b. Select "full report" double click
   c. Generate report

4. Supervisors must initial all pages of the report after reviewing the assay.

N. Assay Interpretation

Standards and Controls

1. Check the raw data for cycling. (If the raw data graph is not seen, click on "Cycling A.FAM" in the tool bar and then "Arrange".) If the fluorescence is below 80 RFUs, yet the reaction efficiency is acceptable (see 5), determine if the SYBR Green I was thawed more than once. If not, notify QC in order to test stock. The assay still passes as long as conditions 2b and 3 are fulfilled.

2. Confirm that the following settings are correct:
   a. standard curve imported "no"
   b. Start normalizing from cycle "1"
   c. noise slope correction "yes"
   d. reaction efficiency threshold "disabled"
   e. normalization method "dynamic tube normalization"
   f. digital filter "light"
   g. no template control threshold "10%"

3. Slope optimum: -3.322

4. R^2 value optimum: 0.999

5. **Reaction efficiencies should range from 0.80 to 1.15.** Efficiencies are **rounded down. (For example, 0.799 fails.)**

6. **Two of the three calibrator values must be between 400 pg/µL and 100 pg/µL.**

7. **No template controls or zero standards should be < 0.1 pg/µL.**

   If the no template control is > 0.1 pg/µL, LCN samples may be amplified since there may not be sufficient sample to retest. However, HCN samples must be re-quantitated.

8. **The difference between the average Ct values of each consecutive duplicate standard concentration should be approximately two cycles.**

9. **At least one of each duplicate standard concentration should be apparent ("clicked on"). (If #10 is exercised, at least one of each duplicate standard concentration should be apparent for 5 of the 7 remaining standards.)** If one duplicate of a standard does not yield the expected Ct value, but the other duplicate is within the expected range, the aberrant standard may be excluded from the standard curve calculation. Unselect the sample on the right side of the screen, and reanalyze.

10. Similarly, if both replicates of a standard are not within the expected range, they may both be excluded from the standard curve calculation, and if all the other parameters of the assay are satisfactory, the assay passes. **However, no more than two standard pairs may be absent.**

11. The assay fails if the reaction efficiency, calibrator and/or non-template control values are unacceptable.

12. For LCN samples, in order to preserve sample, if the quantitation assay fails twice, proceed to amplification without a third quantitation.

13. Initiate retesting of all samples in a failed run. Although a quantitation assay may fail, the resultant values may be used to estimate the need for further dilutions for the re-quantitation assay.

TABLE 3:

Required Settings		Required Results	
Parameter	**Value**	**Parameter**	**Value**
Start normalizing from cycle	**1***	NTC	**< 0.10 pg/µL**
Noise slope correction	**yes**	Calibrator	**100 to 400 pg/µL**
Reaction Efficiency threshold	**Disabled**	Reaction Efficiency	**0.80 to 1.15**
Normalization Method	**Dynamic tube Normalization**	Ct values of standards	**~2 cycles between each concentration**
Digital Filter	**Light**	Standards analyzed	**No more than 2 pairs may be absent**
No template control threshold	**10%**	Samples	**<1000 pg/µL or dilute and re-quantitate**
		Sample Notes	**"*" if background fluorescence "Δ" if inhibited**

* May change if a sample curve crosses the threshold early (refer to Section M.4.b.ii. of this section).

Sample Interpretation

1. Samples that are 1000 pg/µL and above should be requantitated at a 1/100 or a 1/1000 dilution.

2. For amplification with Identifiler™, PowerPlex Y, or MiniFiler, if the extraction negative is > 0.2 pg/µL it should be re-quantitated. If it fails again, the sample set must be re-extracted prior to amplification.

3. For the YM1 system, if the extraction negative is > 1 pg/µL it will need to be re-quantitated.

TABLE 4:

Amplification System	Sensitivity of Amplification	Extraction Negative Control Threshold
YM1	20 pg	1.00 pg/µL in 20 µL
Identifiler™ 28/31 cycles	1 pg	0.20 pg/µL in 5 µL
PowerPlex Y	1 pg	0.20 pg/µL in 5 µL
MiniFiler	1 pg	0.20 pg/µL in 5 µL

4. If a sample appears to be inhibited, i.e. the curve initially increases and then plateaus, note this with a triangle, "Δ", in the "Comments" column of the Summary sheet.

5. If a sample displays background fluorescence, indicate such samples with an asterisk, "*", on the Summary sheet in the "Comments" column.

6. If a sample displays low background fluorescence, i.e. approximately 10% or less of the total fluorescence, indicate this with a double asterisk, "**", on the Summary sheet in the "Comments" column.

7. The neat and the value calculated from the 1/100 dilutions of the samples should differ by no more than a factor of 2.5. If the dilutions are not within a factor of 2.5, the samples should be re-quantitated.

8. Table 5 (next page) summarizes which concentration should be selected for entry into the automated Amp Macro.

TABLE 5:

Samples	Resolution
N = x pg/uL 1/100 = within +/- 2.5x	Select neat value
N = x pg/uL 1/100 = +/- >2.5x No indication of inhibition or background fluorescence	Re-quant samples.
N = >1000 pg/uL 1/100 = <1000 pg/uL	Select dilution
N = >1000 pg/uL Dilution >1000 pg/uL	Requant sample at a greater dilution
N = < 20 pg/uL, NO inhibition or fluoresence dilution within +/- 2.5 fold	Not suitable for amplification with Identifiler 28
N = < 10 pg/uL, NO inhibition or fluoresence dilution within +/- 2.5 fold	Not suitable for amplification with MiniFiler
N = <7.5 pg/uL, NO inhibition or fluoresence Dilution within +/- 2.5 fold	Not suitable for amplification with YM1
N = < 5 pg/uL, NO inhibition or fluoresence dilution within +/- 2.5 fold	Not suitable for amplification with PowerPlex Y
N = <1 pg/uL, NO inhibition or fluoresence dilution within +/- 2.5 fold	Not suitable for amplification with Identifiler 31
N = *, **, or Δ Dilution NO *, **, or Δ and yields sufficient DNA for HCN amplification	Select dilution
N = **, dilution **	Select dilution
N = * or Δ dilution * or Δ	Send to analyst
N= <7.5 pg/uL, NO *, **, or Δ Dilution not within 2.5 fold	Not suitable for amplification with YM1, Identifiler 28, MiniFiler, or PowerPlex Y, no further testing
N = * or Δ 1/100 Dilution <0.1 pg/uL	Re-quantitate at 1/10 dilution
1/10 dilution only = **	Amplify if sufficient DNA for HCN DNA testing.

Samples	Resolution
1/10 dilution only = * or Δ	If sample quant dictates a greater than 1/10 dilution factor for amp, proceed with amp. Otherwise, send to analyst.
Any value less than 0.1 pg/uL	Do not interpret

O. Creating a Rotorgene Summary Page

1. Open the Rotorgene summary sheet Excel file for the Rotorgene run being analyzed and reviewed. The run will be saved with the run name in the folder for that instrument, such as RG3Q011707.1100 saved in the RG3 folder. Go to the "RG values" tab.

2. On the Rotorgene Software (main screen after analysis), go to the "Quant. Results - Cycling FAM" table (lower left window).

3. Maximize the screen. By right-clicking the table heading with the mouse and un-checking certain columns, eliminate all columns except the following:
   No
   Name
   Ct
   Calc. Conc.

4. Select all remaining cells (left click and drag across all column headings until all cells are highlighted blue). Then, right-click mouse and select copy.

5. In the Rotorgene summary sheet Excel file in the "RG values" sheet, place cursor in cell C1. Right click on cell C1 and paste values. In row 1, the column headings should be visible.

6. If the extraction negative does not cross the threshold, the sample is not inhibited, and there is no value, ensure a value of zero is entered into the calculated concentration column.

7. If applicable, fill in tube labels for respective cases in column B of the "RG values" sheet. (Enter "-" for standards, negative controls and calibrators.) The tube labels may also be copied and pasted from the "sample sheet" or typed in manually.

8. Enter the target dates and IA initials for the respective cases in column G and H, if applicable. The IAs and the target dates may also be copied and pasted from the "sample sheet" or typed in manually.

9. Go to the "Summary Sheet" tab, and check to make sure all sample names fit in respective cells.

10. The RG summary sheet will automatically place an "RQ" next to samples with quant values greater than 1000pg/uL. Inspect these samples to ensure that a re-quant is in order. If the dilution can be used, right click on the "Comments" cell for that sample and "clear contents".

11. Schedule samples for re-quantitation if needed, by placing an "RQ" in the comments section next to those samples. Any sample with a lowest dilution quant value of greater than 1000 pg/uL should be re-quantified. Also, any sample pair with values for the neat and diluted samples that do not correlate should also be re-quanted.

12. Inhibited and/or fluorescent samples should be noted in the "Comments" column of the summary sheet as described in Section N Sample Interpretation # 5-7. These symbols and some common combinations of them are included as buttons to the right of the RG Summary sheet in the electronic file. Click on the cell in which you would like to insert these symbols and click the appropriate button. Additional notes may be added manually. (Note: Clicking these buttons will overwrite any info previously in the cell.)

13. Enter the reaction efficiency and any comments pertaining to the run in the "Comments" section at the top of the summary sheet.

14. For LCN casework, the supervisor may indicate whether a sample requires purification by inserting a "P" in the comments section.

15. For PC casework, the supervisor may indicate whether a sample requires purification by inserting a "M" in the "A" column.

16. Save the excel workbook.

17. Print the summary sheet page(s).

18. Copy and Paste all sample info for those samples requiring re-quantitation into a "post-quantitation resolutions" sheet. This sheet is found in each casework group's folder. This sheet may then be maintained electronically or printed out and used as a hard copy. Samples can also be added directly to the next available RG sheet.

19. The reviewer must initial and date both pages of the summary sheet and indicate whether the assay has passed or failed.

P. Paperwork Distribution

Distribute only the Rotorgene summary sheet(s) to analysts.

References:

Nicklas, J. A., Buel, E. Development of an *Alu*-based, Real-Time PCR Method for Quantitation of Human DNA in Forensic Samples

Nicklas, J. A., Buel, E. Development of an *Alu*-based, QSY 7-Labeled Primer PCR Method for Quantitation of Human DNA in Forensic Samples

Revision History:
March 24, 2010 – Initial version of procedure.

Batch processing

1. Exemplars and evidence samples must be handled separately at all times. These samples must never be together on the same sample tray.

2. For the ABI 3130*xl*, an exemplar and evidence plate may be in the same instrument. Two separate plates are the equivalent of two consecutive runs.

3. Samples from one amplification sheet should be processed together, so that the samples are accompanied by the appropriate controls.

4. Use the correct worksheet for the specific sample type and make sure the sample preparation set-up is witnessed properly.

5. Controls must be run using the same instrument model and under the same, or more sensitive, injection conditions as the samples to ensure that no exogenous DNA is present. Therefore, samples that must be run at higher injection parameters must have an associated control run concurrently with the samples, or have previously passed under the same, or more sensitive, injection parameters. Controls do not have to be run at the same injection parameters as the samples if it previously passed at a higher injection parameter.

   <u>NOTE:</u> Each run that is performed must have at least one correct positive control.

Sample handling

1. Prior to loading on the capillary, the amplified samples are stored at 4°C in the amplified DNA area. The tubes containing the amplified product must never leave the amplified DNA area.

2. Amplified samples that have been loaded on an instrument should be stored until the electrophoresis results are known. After it has been determined that the amplified samples do not require repeated testing, they may be discarded.

Instrument and computer maintenance

1. Be gentle with all instrument parts and instruments. Keep everything clean.

2. It is good practice to monitor initial instrument performance. This enables the user to detect problems such as leaks, air bubbles or calibration issues.

3. Hard disks should be regularly defragmented to improve

4. Data files and other non-essential files from the computer hard disk should be deleted at least once a week to improve performance.

5. Notify the Quality Assurance Unit if any problems are noted.

Revision History:
March 24, 2010 – Initial version of procedure.

GENERAL INFORMATION

The Identifiler Kit is a PCR Amplification Kit manufactured, sold, and trademarked by Applied Biosystems (ABI). The YM1 Kit is a PCR Amplification Kit manufactured in-house that test for four (4) Y-STR Loci.

Target DNA template amounts are as follows:

- Identifiler, 28 amplification cycles (ID28) – 500 pg in sample aliquot of 5 µL
- Identifiler, 31 amplification cycles (ID31) – 100 pg in sample aliquot of 5 µL
- YM1 – 2000 pg in sample aliquot of 26 µL

To calculate the amount of template DNA and diluant to add, the following formula is used:

$$\text{Amt of DNA extract } (\mu L) = \frac{\text{Target Amount (pg)}}{(\text{sample concentration, pg}/\mu L)(\text{dilution factor})}$$

The amount of diluant to add to the reaction (µL) =
Volume of sample aliquot (µL) - amount of DNA extract (µL)

GENERATION OF AMPLIFICATION SHEETS

To determine the appropriate system for amplification of samples, refer to Table 1.

TABLE 1: PCR amplification input based on Rotorgene values

RG value at 1:10 dilution pg/µL	RG value neat pg/µL	Amplification Sheet	Dilution
LCN extraction ≥ 0.4 pg/µL	≥ 4.0* to 20 pg/µL	Amplify with ID for 31 cycles*	Neat = 1
LCN/HSC extraction ≥ 2.0 pg/µL	≥ 20 pg/µL	Amplify with ID for 28 cycles	As appropriate
HSC extraction ≥ 0.7 pg/µL	≥ 7.5 pg/µL	Amplify with YM1 or Microcon and amplify with ID 28	As appropriate

* Samples providing less than 20 pg per amplification can only be amplified with the permission of a supervisor.

A. HSC Team Amp Macro (Evidence samples) for paperwork preparation for amplification with Identifiler 28 Cycles and YM1

1. Open the "RGAMP Macro HSC" and the "RG summary sheet" Excel files for samples ready to be amplified. The "RG summary sheet" is saved as the assay name.
   a. If a window opens stating " "…RGAmp Macro HSC.xls" contains macros. Macros may contain viruses…", click "Enable Macros".
   b. If a window opens stating "Macros are disabled because the security level is set to High…", do the following: Select Tools in the toolbar. Click Macro, Security, and set the level to Low. The file must be closed and reopened.

2. Copy the sample information (without the standards or calibrators) from the "summary sheet" of the "RG summary sheet" file including the tube label, sample name, Ct value, the calculated concentration, the target date, and the IA, and paste special as values into the corresponding columns of the "RG value" sheet of the "RGAMP Macro HSC" file.

3. In the last column, entitled "Type", enter the type of amplification according to the following abbreviations next to the samples to be amplified:

   a. "V" for ID28 Evidence
   b. "Y" for YM1 Evidence

   Selecting neat samples versus diluted samples can be done here.

4. Check the sample names to ensure that commas are only located after the full sample name and before the dilution value (i.e. FB01-1234_vag_SF, 0.1).

5. Hit Ctrl+R or click the "Split dilutions & sample info" button to run the dilution macro. A window asking "Do you want to replace the contents of the destination cell?" will appear. Click "OK".

   The dilution macro will separate the dilution factors from the samples names to facilitate the calculation of the neat concentration of the samples.

   a. If the dilution column does not contain the correct dilutions, the file must be closed and reopened. Check for commas in the wrong location in the sample names.

b. If the macro will not run, follow the instructions in the box and select tools, macro, security, and low. The file must be closed and reopened.

6. Hit Ctrl+G or click the "Sort samples" button to run the sample sorting macro.

a. The macro will filter and eliminate all values that are less than 20 pg/µL or 7.5 pg/µL for Identifiler 28 or YM1, respectively. The macro will also sort the samples by system/type and sample concentration in the "Sort" sheet.

b. Inspect the samples sorted in the appropriate columns according to system/type and sample concentration.

<u>For Identifiler 28 samples, proceed to Step 7.</u>
<u>For YM1 samples, proceed to Step 8.</u>

7. <u>For Identifiler 28 samples:</u>

a. Samples with concentrations between or equal to 20 pg/µL and 100 pg/µL (less than or equal to 500 pg amplified) may be automatically amplified in duplicate; see the concordant analysis policy (section 1).

If you have not done so already, select the samples that require amplification now (i.e. amplifying neat sample versus diluted sample).

b. Copy and Paste Special as values all samples to be amplified from the appropriate columns on the "Sort" sheet to the associated columns on the "Samples" sheet.

NOTE:
- Samples <100pg/µL will be sorted into a different section. Copy them into the amp sheet as well.
- If applicable, copy the Identifiler duplication samples (for samples <100pg/µL) to the "Identifiler 28 Evidence Dup" sections. This amplification sheet may be used for automatic duplication of samples, depending on the team.

<u>Proceed to step 9.</u>

8. **For YM1 samples:**

a. Copy and Paste Special as values all samples to be amplified from the appropriate columns on the "Sort" sheet to the associated columns on the "Samples" sheet.

b. For samples being sent on for YM1 amplification from P30 values, on the "Samples" sheet, change the Calculated Values column to the appropriate letter associated with the P30 value and sample type.

For Non-Differential semen or differential swab/substrate remain samples:

Orifice swab, P30 value, 2ng subtract	**Stains P30 value, 0.05 A subtract**	**Type this letter in the Calculated Value column**
HIGH	HIGH	A
1.1 - 3.0	1.1 - 3.0	B
>0 - 1.0	>0 - 1.0	C

For vaginal swab samples sent for Amylase Positive Extractions, two concentrations must be sent for amplification:

Amounts sent to amplification		**Type this letter in the Calculated Value column**
DNA Target	**TE^{-4}**	
10	16	B
26	0	C

c. For samples being sent on for YM1 amplification from Quantification values, the amplification sheet should calculate the appropriate DNA and TE^{-4} target amount on the amplification sheet.

9. If there are more than 28 samples for amplification, the overflow samples will automatically be transferred into a second amplification sheet (i.e. "ID2", "ID DUP2" or "YM1 2").

10. When all samples to be amplified have been organized on the “Samples” sheet, click on the appropriate amplification sheet(s) and check all entries for errors.

    All changes, except for the amount of extract submitted during low and high sample submission, should be made in the “Samples” sheet.

11. Save the entire macro workbook in the appropriate folder.

Saving Amplification Sheets on the Network for Additional Samples

1. Partially full or completed amplification sheets may be saved as independent sheets for subsequent sample additions by clicking the “Samples” and amp sheet tab (via holding the ctrl button down). Both sheets should now be highlighted white. Right click and select “move or copy”.

2. In this window, select “(new book)” in the “to book” window and check “create a copy”. Click “OK”. Go to File, Save As and save into the appropriate folder.

3. Samples may be manually added to these sheets by the rotation supervisor from the Aliquot Request form or copied and Paste Special from re-quantification sheets or consolidated from additional amplification sheets of the same type at the end of each Rotorgene run.

4. If any samples need to be submitted to amplification with a DNA amount other than the optimal amount, the rotation supervisor can change the amount of DNA submitted by changing the value in the DNA column in the amplification sheet.

    Be aware that once the DNA amount is manually added to the amplification sheet, the sheet will not be able to calculate the value from the quantification value.

    All other changes should be done in the “Samples” sheet.

5. When a macro amplification sheet is full the rotation supervisor will add tube labels and fill in the amplification date and time in the appropriate blue cell in the “Samples” sheet. This should automatically populate the appropriate cells in the Amplification sheet.

    Any changes to the amplification sheet should be done in the “Samples” sheet.

6. Save the sheet as the time and date of the amplification as follows: "ID041207.1100" for Identifiler28 amplifications, or "YV041207.1100" for YM1 amplifications, performed on April 12, 2007 at 11:00am in the appropriate folder.

7. A supervisor should review all entries were entered correctly before printing the Amplification sheet.

B. RG Amp Macro X (exemplar samples) for Paperwork Preparation for Amplification with Identifiler 28 and YM1

1. Open the "RGAmpMacro X".

2. For ID 28 samples, open the "RG summary sheet" Excel file for samples ready to be amped. Copy the information from the "summary sheet" of the "RG summary sheet" file including the tube label, sample name, Ct value, the calculated concentration, the target date, and the IA, and paste special as values into the corresponding columns of the "RG value" sheet of the "RGAmpMacro X" file.

3. In the last column, entitled "type", the following information is already added:

   "IDX" for ID28 exemplars

4. Click the "Separate dilutions and sample info" button to run the dilution macro. A window asking "Do you want to replace the contents of the destination cell?" will appear. Click "OK".

   a. If the macro will not run, follow the instructions in the box and select tools, macro, security, and low. The file must be closed and reopened.
   b. The dilution macro will separate the dilution factors from the sample names to facilitate the calculation of the neat concentration of the samples.

5. Click the "Sort samples" button to run the sample sorting macro.

   a. The macro will filter and eliminate all values that are less than 20 pg/µL for Identifiler 28.
   b. Inspect the samples sorted in the appropriate columns and select the samples that require amp. For instance, determine whether you will be using the calculated concentration derived from the neat sample or the dilution.

c. Samples may be added or deleted to or from the columns following the macro's execution.

   To delete a sample do the following:
   i. On the "sort" sheet in the "RGAmpMacro X" file, locate the columns relevant to the amplification system and sample type.
   ii. Select the cells relevant to the sample you would like to delete.
   iii. Select edit and clear contents.
   iv. Do not simply delete, always use the "clear contents" function.

   To add a sample, do the following:
   i. Copy sample info from the "RG values revised" sheet in the "RGAmpMacro X" file: the tube label, sample name, Ct value, the calculated concentration, the target date, and the IA.
   ii. Paste special these values into the appropriate columns of the "sort" sheet in the "RGAmpMacro X" file.

6. Copy and paste all samples to be amped from the appropriate column on the "sort" sheet to the associated column on the "samples" sheet. This is the sheet on which you are building your amp.

7. Ensure that all samples to be amped have been organized correctly on the "samples" sheet and select the appropriate amplification worksheet tab.

   The sheet will calculate the dilution factor necessary for the samples as well as the amount of sample and TE^{-4} or irradiated water to add.

8. Save the macro sheet in the appropriate folder.

9. For YM1 samples, copy all information directly from the aliquot request form. Paste special as values into the "paste Ys" tab of the "RGAmpMacro X".

10. Once all samples are added, click on the "YM1" tab.

    The sheet will calculate the dilution factor necessary for the samples as well as the amount of sample and TE^{-4} or irradiated water to add.

11. Save the macro sheet in the appropriate folder.

C. Aliquot Request and Amp Sheets for HCN evidence and exemplar samples only

Aliquot request sheets have been created for evidence and exemplar submission.

1. Open the correct aliquot request sheet. The sheet can be found in M:\FBIOLOGY_MAIN\Amp Sheets\ALIQUOT REQUEST FORMS\(either EVIDENCE or EXEMPLAR)
2. Fill out the next empty line. Type the case information in 3130 format.
3. Refer to the calculation in this section of the Manual to determine the volume of extract to be aliquotted, based on DNA concentration and target for amplification. If you want to amp your sample at a condition different than normal (reamp high, low/opt/high, etc.) indicate this in the "Sample Information" section.
4. Save the sheet.
5. The person that aliquots the samples will type their initials and the date they aliquot the samples in the last column. That person will email all analysts listed on the sheet indicating that samples have been aliquotted. It is up to the analyst to fill out the extract tracking form with the aliquotting information.
6. The rotation supervisor is responsible for preparing amplification sheets, determining when the samples will be aliquotted and that information that is typed onto the amp sheets is correct.

D. RG Amp Macro HI (High Sensitivity samples) for Paperwork preparation for Amplification with Identifiler 28 and 31

1. Open the current version of the "RGAMP MACRO HI" Excel workbook and the "RG summary sheet" Excel files for samples ready to be amped. These files can be found in the "TEMPLATES IN USE" folder on the High Sensitivity Data drive. The RG Summary Sheets are saved as the assay name in the "Rotorgene" folder on the FBiology Main drive.

2. Copy the information for samples and controls only from the "summary sheet" of the "RG summary sheet" file including the tube label (if applicable), sample name, Ct value, the calculated concentration, the target date, and the IA. Paste special as values into the corresponding columns of the "RG value" sheet of the "RG Amp macro" file. The standards and calibrators need not be copied.

3. In the column entitled "type" enter the type of amplification according to the following abbreviations:
   a. "X" for exemplars
   b. "V" for evidence

4. Note whether any sample has a comma in its name. If not, add a comma after one sample's name so that the macro will work. Click the "Separate Dilution and Sample Info" button to run the dilution macro. A window asking "Do you want to replace the contents of the destination cell?" will appear. Click "OK".
   a. If the macro will not run, follow the instructions in the box and select tools, macro, security, and low. The file must be closed and reopened.
   b. The dilution macro will separate the dilution factors from the sample name to facilitate the calculation of the neat concentration of the sample

5. Click the "Sort Samples" button to run the sample sorting macro.
   a. The sort macro will filter values according to the following specifications which differ depending upon the amount of template DNA.
      i. The macro eliminates all values that are less than 1 pg/µL
      ii. Values between 1 pg/µL and 20 pg/µL are sorted for LCN amplification with Identifiler for 31 cycles.
      iii. All values greater than 20 pg/µL are sorted for HCN amplification with Identifiler for 28 cycles.
      iv. Note, for samples with greater than 100 pg/µL and less than 124 pg/µL, the macro will indicate to add 5 µL of template DNA. (In order to avoid pipetting less than 1 µL, slightly more than 500 pg of DNA will be added to the reaction.)

b. The extraction negatives will be sorted independently so that they may be inspected and placed at the top of the list with the associated samples when setting up the amp sheets.

c. Samples will be sorted into groups for ID31 evidence and exemplar amp, and ID28 evidence amp. Samples amplified with Identifiler for 31 cycles are amplified in triplicate concurrently whereas samples amplified with Identifiler for 28 cycles are amplified in duplicate in two separate amplifications.

6. Select samples for amplification and copy and paste those samples to the appropriate column on the "samples" sheet. The sample information is then automatically populated into the amplification and 3130 run sheets. Samples may also be added or deleted to or from the amp sheets as described below. For example, samples with less than 4 pg/µL or 20 pg/amp require supervisor approval for LCN amplification, and depending upon the case, may not be amplified. Refer to the amplification guidelines and the RG interpretation manual to select samples and the appropriate dilutions to use for amplification calculations.

To delete a sample do the following:

a. Go to the "sort" sheet in the RG AMP MACRO HI file and locate the columns relevant to the amplification system and sample type.

b. Select the cells relevant to the sample you would like to delete.

c. Select edit and clear contents.

d. Do not simply delete, always use the "clear contents" function.

To add a sample, do the following:

a. Copy the tube label, sample name, Ct value, the calculated concentration, the target date, and the IA from the "RG values revised" sheet in the "RG AMP MACRO HI" file.

b. Paste special as values into the appropriate columns for the amplification system of the "samples" sheet in the "RG AMP MACRO HI" file.

c. Alternatively, a sample may be manually added by typing the sample information into the appropriate column in the "samples" sheet.

7. Select the appropriate amplification worksheet, verify the sample information and calculations, and type the name of the amplification in cell B1 as follows: month**date**year.time for example, 011106.1000.

   a. The sheet will automatically calculate the number of samples that are to be amplified. This will populate cell B2 of the worksheet.
   b. The sheet will also calculate the amount of reagents required, and the dilution factor necessary for the samples. Verify these calculations.

8. Save the sheet in the amplification sheets folder (as A**month**date**year**.time) and review.

9. Print the amplification sheet. Have the sheet reviewed by a supervisor prior to set-up.

E. RG Amp Macro PC (Property Crimes Samples) for Paperwork Preparation for Amplification with Identifiler 28.

1. Open the "RGAmp MacroPC.xls" and the "RG summary sheet" Excel files for samples ready to be amplified. The "RG summary sheet" is saved as the assay name.
   a. If a window opens stating "…RGAmp MacroPC" contains macros. Macros may contain viruses…," click "Enable Macros".
   b. If a window opens stating "Macros are disabled because the security level is set to High…," do the following: Select Tools in the toolbar. Click Macro, Security, and set the level to Low. The file must be closed and reopened.

2. Copy the sample information (without the standards or calibrators) from the "summary sheet" tab of the "RG summary sheet" file including the tube label, sample name, Ct value, the calculated concentration, the target date, and the IA, and paste special as values into the corresponding columns of the "RG value" sheet of the "RGAmp MacroPC" file.

3. In the last column, entitled "Type", enter a "V" for Evidence.

   The decision to sort neat samples versus diluted samples can be done at this point.

4. Check the sample names to ensure that commas are only located after the full sample name and before the dilution value (i.e. FB01-1234_^bottle_swab^, 0.1).

5. Press Ctrl+R or click the "Split dilutions and sample info" button to run the dilution macro. A window asking "Do you want to replace the contents of the destination cell?" will appear. Click "OK".

   The dilution macro will separate the dilution factors from the samples names to facilitate the calculation of the neat concentration of the samples.

6. If the macro does not sort, this may be because no samples containing dilutions are available to sort. In this case, clear the Dilution column and try sorting again.

7. Press Ctrl+G or click the "Sort samples" button to run the sample sorting macro.
   a. The macro will filter and eliminate all values that are less than 20.0 pg/µL for Identifiler 28.
   b. Samples will be sorted into four columns: Negative Controls, ID28 samples, ID28 Immediate Dups, and ID28 Negative.

8. For Identifiler 28 samples (Property Crimes):
   a. **ALL** samples will be amplified twice; once as an initial amplification and the second time as a duplicate amplification.

      If you have not done so already, select the samples that require amplification now (i.e. amplifying neat sample versus diluted sample).

   b. Copy and Paste Special as values all samples to be amplified from the appropriate columns on the "Sort" sheet to the associated columns on the "Samples" sheet.

   c. Note: Extraction Negatives do not need to be duplicated.

9. If there are more than 28 samples for amplification, the overflow samples will spill into the highlighted area of the Samples sheet, prompting you to make a new amplification sheet.

10. Once satisfied that all samples to be amplified have been organized on the "Samples" sheet, check both the initial and duplicate amplification sheets for errors.

    All changes, except for the amount of extract submitted during low and high sample submission, should be made in the "Samples" sheet.

Saving Amplification Sheets on the Network for Additional Samples

1. Once complete save each amp (initial and dup) in its respective folder.

2. If any samples need to be submitted to amplification with a DNA amount other than the optimal amount, the amount of DNA submitted can be adjusted by changing the value in the DNA column in the amplification sheet.

   Please be aware once the DNA concentration or dilution value is manually added to the amplification sheet, the sheet will not be able to calculate the volume of DNA needed for amplification from the quantification value.

   All other changes should be done in the "Samples" sheet.

F. Saving Amp Sheets to the Network for Additional Samples

1. Amp sheets may be saved as independent sheets for subsequent sample additions by right-clicking the corresponding tab and selecting "move or copy". In this window, select "(new book)" in the "to book" window and check "create a copy". Click "OK". Go to File – Save-As and save into the appropriate folder.

2. Samples may be manually typed into these sheets or copied and pasted special from re-quant sheets or consolidated from additional amp sheets of the same type at the end of each Rotorgene run.

3. When a sheet is full the analyst may fill in the appropriate information (cells shaded blue) and save the sheet as the time and date of the amp.

Revision History:
March 24, 2010 – Initial version of procedure.

A. Preparing DNA aliquots for amplification (if applicable)

1. Follow applicable procedures for preparation for testing.

2. Obtain reviewed amp worksheet from supervisor or network folder.

3. For each sample to be amplified, label a new tube. Add DNA and irradiated water or TE^{-4} as specified by the amplification sheet. (Samples amplified with Identifiler reagents should be prepared with irradiated water. Samples amplified with YM1 reagents should be prepared with TE^{-4}.)

4. Prepare dilutions for each sample, if necessary, according to Table 1.

TABLE 1: Dilutions

Dilution	Amount of DNA Template (uL)	Amount of TE^{-4} or Irradiated Water (uL)
0.25	3 or (2)	9 or (6)
0.2	2	8
0.1	2	18
0.05	2	38
0.04	4 or (2)	96 or (48)
0.02	2 or (1)	98 or (49)
0.01	2	198
0.008	4 or (2)	496 or (248)

a. Centrifuge samples at full speed briefly.
b. Label tubes appropriately for dilutions. Add the correct amount of irradiated water or TE^{-4} as specified by the amplification sheet and Table 1.
c. Pipet sample up and down several times to thoroughly mix sample.
d. Set the sample aside until you are ready to aliquot it for amplification.

B. Identifiler – Sample and Amplification Set-up

Samples and Controls

1. The target DNA template amount for Identifiler™ 28 cycles is 500 pg.
   The target DNA template amount for Identifiler™ 31 cycles is 100 pg.

   To calculate the amount of template DNA and irradiated water (diluent) to add, the following formulas are used. The sample concentration is the RotorGene quantitation value:

   $$\text{DNA extract added } (\mu L) = \frac{\text{Target DNA Template Amount (pg)}}{(\text{sample concentration, pg}/\mu L)(\text{dilution factor})}$$

   The volume of diluent to add (µL) = 5 µL - DNA extract added (µL)

   For samples with RotorGene values ≤ 100 pg/uL aliquot 5 uL extract.

2.
   a. **For an Identifiler™ 28 cycle amplification, make a 0.5 (1/2) dilution of the ABI Positive (A9947) control at 100 pg/ µL (5 µL in 5 µL of water).**

      This yields 50 pg/µL of which 5 µL or 250 pg will be used.

   b. **For an Identifiler™ 31 cycle amplification, make a 0.2 (1/5) dilution of the ABI Positive (A9947) control at 100 pg/µL (4 µL in 16µL of water).**

      This yields 20 pg/µL of which 5 µL or 100 pg will be used.

3. 5 µL of irradiated water will serve as an amplification negative control.

4. Arrange samples in precisely the positions they appear on the sheet.

5. **Witness step.** Have another analyst witness the sample set-up.

Master Mix Preparation

1. Retrieve **Identifiler™** primers and reaction mix from the refrigerator and Taq Gold from the freezer. Store in a Nalgene cooler, if desired. Record the lot numbers of the reagents.

2. Vortex or pipet the reagents up and down several times. Centrifuge reagents at full speed briefly. **Do not vortex TAQ GOLD**.

3. Consult the amplification sheet for the exact amount of Identifiler™ primers, reaction mix, and Taq Gold, to add. The amount of reagents for one amplification reaction is listed in Table 2.

TABLE 2: Identifiler™ PCR amplification reagents for one sample

Reagent	Per reaction
Primer mix	2.5 µL
Reaction mix	5 µL
AmpliTaq Gold DNA Polymerase (5U/µL)	0.5 µL
Mastermix total:	8 µL
DNA	5 µL

Reagent and Sample Aliquot

1. Vortex master mix. After vortexing, briefly centrifuge or tap master mix tube on bench.

2. Add **8 µL** of the Identifiler™ master mix to each tube that will be utilized, changing pipette tips and remixing master mix as needed.

3. Prior to immediately adding each sample or control, pipet each sample or control up and down several times to thoroughly mix. The final aqueous volume in the PCR reaction mix tubes will be 13µL. After addition of the DNA, cap each sample before proceeding to the next tube.

4. After all samples have been added, return DNA extracts to storage and take the rack to the amplified DNA area for Thermal Cycling (continue to section D).

An alternative method for amplification is to use a 96-well plate.

1. Positive Control
   a. If only half a plate of samples are amplified, only one PE is necessary, however, to encompass all of the injections required for a full plate of samples, amplify two or more PEs (10 µL in 10µL of water).
   b. The amp sheet will automatically populate these PEs.

2. Sealing the Plate
   a. If using a PCR plate, place a super pierce strong seal on top of the plate, and place the plate in the plate adapter on the ABgene heat sealer.
   b. Push the heat sealer on top of the plate for 2 seconds.
   c. Rotate the plate and reseal for 2 additional seconds.
   d. Label the plate with "A" for amplification and the date and time. (A011104.1300)

C. Sample and Amplification Set-up for YM1

The amplification of exemplars and sperm cell fractions of differential lysis samples is based on the quantitation results (see Table 3). Semen positive swabs taken from females, that were extracted using the non-differential semen extraction, and the substrate remains fractions of differential lysis samples, are amplified using the amounts specified in Table 4. Amylase positive samples are amplified using the amounts specified in Table 5.

TABLE 3: PCR amplification input based on Rotorgene values

RG value at 1:10 dilution pg/µL	RG value neat pg/µL	Amplification Sheet	Dilution
HSC extraction $\geq$ 0.7 pg/µL	$\geq$ 7.5 pg/µL	Amplify with YSTR	*As appropriate

* Add TE^{-4} to a final volume of 26 µL.

The target DNA template amount for YM1 is 2 ng (2000 pg).

To calculate the amount of template DNA and TE^{-4} (diluant) to add, the following formulas are used. The sample concentration is the Rotorgene quantitation value:

$$\text{DNA extract added } (\mu L) = \frac{\text{Target DNA Template Amount (pg)}}{(\text{Sample concentration, pg}/\mu L)(\text{Dilution factor})}$$

The volume of diluant to add (uL) = 26 µL – DNA extract added (µL)

For samples with Rotorgene values $\leq$ 50 pg/uL but $\geq$ 7.5 pg/uL aliquot 26 uL extract.

TABLE 4: Increased amount of DNA extract from a non-differential semen extraction or from the substrate remains fraction of a differential lysis sample to be amplified for YM1. Never amplify less than 2 ng of DNA based on P30 or sperm search results.

P30 result for the 2ng subtraction (Body cavity swabs)	P30 result for the 0.05A units subtraction (Stains or penile swabs)	DNA Volume (µL) to be amplified	TE^{-4} (µL)	Range of Volumes (µL) which can be amplified
$\geq$ 1.1	$\geq$ 1.1	10	16	2 - 26*
> 0 - 1.0	> 0 - 1.0	26	0	5 - 26*
Sperm Seen Not sent to P30	Sperm Seen Not sent to P30	10	16	2 - 26*

* Add TE^{-4} to a final volume of 26 µL.

TABLE 5: Amount of DNA extract to be amplified for Amylase positive samples **

<table>
<tr><th>Type of item</th><th></th><th>DNA Target Volume (µL)</th><th>TE⁻⁴ (µL)</th><th>Range (µL)</th></tr>
<tr><td>Orifice swab</td><td>Initially try two amounts</td><td>10
26</td><td>16
0</td><td>1 - 26*</td></tr>
<tr><td>Dried secretions swab (External)</td><td>Based on Quantitation result</td><td colspan="3" rowspan="2">See Table 3</td></tr>
<tr><td>Stain</td><td>Based on Quantitation result</td></tr>
</table>

* Add TE^{-4} to a final volume of 26 µL.

** Rotorgene does not reflect male DNA (keep in mind for orifice swabs). Try more or less if negative.

TABLE 6: Control samples Y STR multiplex YM1

Sample	DNA	TE^{-4}
male positive control	26 µL	---
female negative control	26 µL	---
amplification negative control	---	26 µL
extraction negative control	26 µL	0 µL

Master Mix Tube Preparation - YM1

1. Fill out the amplification worksheet and record the appropriate lot numbers.

2. Determine the number of samples to be amplified, including controls, and label a PCR reaction mix tube for each sample.

3. Ensure that the solution is at the bottom of each PCR reaction mix tube by tapping the tube down on the bench or by centrifuging briefly.

Reagent and Sample Aliquot - YM1

1. Pipet 4 µL of $MgCl_2$ in the solution at the bottom of the tube. Use a fresh sterile pipette tip for each tube. Close all of the tubes.

2. Arrange samples in precisely the positions they appear on the sheet.

3. **Witness step.** Have another analyst witness the sample set-up.

4. Prior to immediately adding each sample or control, pipet each sample or control up and down several times to thoroughly mix. The final aqueous volume in the PCR reaction mix tubes will be 50µL. After the addition of the DNA, cap each sample before proceeding to the next tube.

5. After all samples have been added, return DNA extracts to storage and take the rack to the amplified DNA area for Thermal Cycling (continue to section D).

D. Thermal Cycling – all amplification systems

1. Turn on the ABI 9700 Thermal Cycler.

2. Choose the following files in order to amplify each system:

Identifiler 28	Identifiler 31	YM1
user: **hisens** or **casewk** file: **id28**	user: **hisens** or **casewk** file: **id31**	user: **casewk** file: **ym1**

3. The following tables list the conditions that should be included in each file. If the files are not correct, bring this to the attention of the Quality Assurance Team and a supervisor.

Identifiler PCR Conditions for the Applied Biosystems GeneAmp PCR System 9700

9700 **Identifiler 28 or 31** user: **hisens** or **casewk** file: **id28** or **id31**	The Identifiler file is as follows: Soak at 95°C for 11 minutes 28 or 31 Cycles : Denature at 94°C for 1 minute : Anneal at 59°C for 2 minutes : Extend at 72°C for 1 minute 60 minute incubation at 60°C. Storage soak indefinitely at 4°C

YM1 PCR Conditions for the Perkin Elmer GeneAmp PCR System 9700

9700 **YM1** user: **casewk** file: **ym1**	The YM1 file is as follows: Soak at 95°C for 10 minutes 30 Cycles: : Denature at 94°C for 45 seconds : Anneal at 58°C for 58 seconds : Extend at 72°C for 1 minute 15 seconds 30 minute incubation at 60°C Storage soak indefinitely at 4°C

9700 Instructions

1. Place the tubes in the tray in the heat block, slide the heated lid over the tubes, and fasten the lid by pulling the handle forward. Make sure you use a tray that has a 9700 label.

2. Start the run by performing the following steps:

3. The main menu options are RUN CREATE EDIT UTIL USER. To select an option, press the F key (F1…F5) directly under that menu option.

4. Verify that user is set to “casewk.” If it is not, select the USER option (F5) to display the “Select User Name” screen.

5. Use the circular arrow pad to highlight “casewk.” Select the ACCEPT option (F1).

6. Select the RUN option (F1).

7. Use the circular arrow pad to highlight the desired STR system. Select the START option (F1). The “Select Method Options” screen will appear.

8. Verify that the reaction volume is set to **13µL** for **Identifiler** and **50µL** for **YM1**. The ramp speed is set to **9600**.

9. If all is correct, select the START option (F1).

10. The run will start when the heated cover reaches 103°C. The screen will then display a flow chart of the run conditions. A flashing line indicates the step being performed, hold time is counted down. Cycle number is indicated at the top of the screen, counting up.

11. Upon completion of the amplification, remove samples and press the STOP button repeatedly until the "End of Run" screen is displayed. Select the EXIT option (F5). Wipe any condensation from the heat block with a Kimwipe and pull the lid closed to prevent dust from collecting on the heat block. Turn the instrument off. Place the microtube rack used to set-up the samples for PCR in the container of 10% bleach in the Post-Amp area.

    After the amplification process, the samples are ready to be loaded on the fluorescent instruments. They may be stored in the appropriate refrigerator at 2-8°C for a period of up to 6 months.

    <u>NOTE</u>:

    Turn instruments off ONLY when the Main Menu is displayed, otherwise there will be a "Power Failure" message the next time the instrument is turned on. If this happens, it will prompt you to review the run history. Unless you have reason to believe that there was indeed a power failure, this is not necessary. Otherwise, press the STOP button repeatedly until the Main Menu appears.

    In case of an actual power failure, the 9700 thermal cycler will automatically resume the run if the power outage did not last more than 18 hours. The history file contains the information at which stage of the cycling process the instrument stopped. Consult the Quality Assurance Team on how to proceed.

E. Amplification Troubleshooting

PROBLEM: No or only weak signal from both the positive control and the test samples

Possible Cause	Recommended Action
Mistake during the amplification set up such as not adding one of the components or not starting the thermal cycler	Prepare new samples and repeat amplification step
Thermal cycler defect or wrong program used	Check instrument, notify QA team, prepare new samples and repeat amplification step

PROBLEM: Positive control fails but sample signal level is fine

Possible Cause	Recommended Action
Mistake during the amplification set up such as not adding enough of the positive control DNA	Prepare new samples and repeat amplification step
Positive control lot degraded	Notify QA team to investigate lot number, prepare new samples and repeat amplification step with a new lot of positive control

PROBLEM: Presence of unexpected or additional peaks in the positive control

Possible Cause	Recommended Action
Contamination by other samples, contaminated reagents	Notify QA team to investigate the amplification reagents, prepare new samples and repeat amplification step
Non-specific priming	Notify QA team to check thermal cycler for correct annealing settings, prepare new samples and repeat amplification step

PROBLEM: Strong signal from the positive controls, but no or below threshold signal from DNA test sample

Possible Cause	Recommended Action
The amount of DNA was insufficient or the DNA is severely degraded	Amplify a larger aliquot of the DNA extract Concentrate the extracted DNA using a Microcon 100 ultrafiltration device as described in the Microcon section Re-extract the sample using a larger area of the stain or more biological fluid to ensure sufficient high molecular DNA is present
Test sample contains PCR inhibitor (e.g. heme compounds, certain dyes)	Amplify a smaller aliquot of the DNA extract to dilute potential Taq Gold polymerase inhibitors Purify the extracted DNA using a Microcon 100 ultrafiltration device as described in the Microcon section Re-extract the sample using a smaller area of the stain to dilute potential Taq Gold polymerase inhibitors Re-extract the samples using the organic extraction procedure

The decision on which of the above approaches is the most promising should be made after consultation with a supervisor.

Revision History:
March 24, 2010 – Initial version of procedure.

A. Setting Up A 3130*xl* Run

1. Collect amp sheets that are ready to run.

2. Go to the computer attached to the instrument.

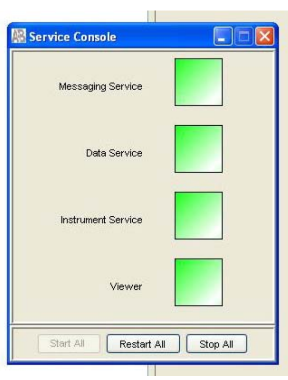
3. If needed, press "CTRL-ALT-DEL" to login.

4. User should be "Administrator", password should be left blank.

5. Click OK.

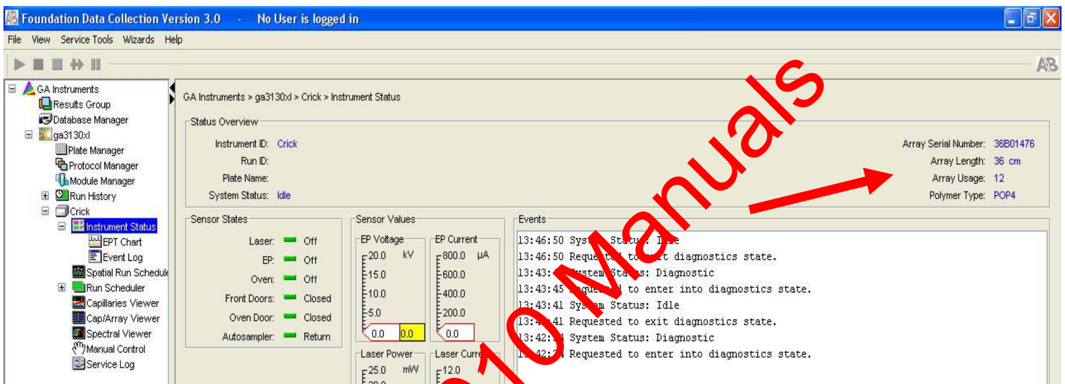
6. Open the 3130*xl* Data Collection v3.0 software by double clicking on the desktop Icon or select **Start > All Programs > AppliedBiosystems > Data Collection > Run 3130*xl* Data Collection v3.0** to display the **Service Console**.

   By default, all applications are off, indicated by the red circles. As each application activates, the red circles (off) change to yellow triangles (activating), eventually progressing to green squares (on) when they are fully functional.



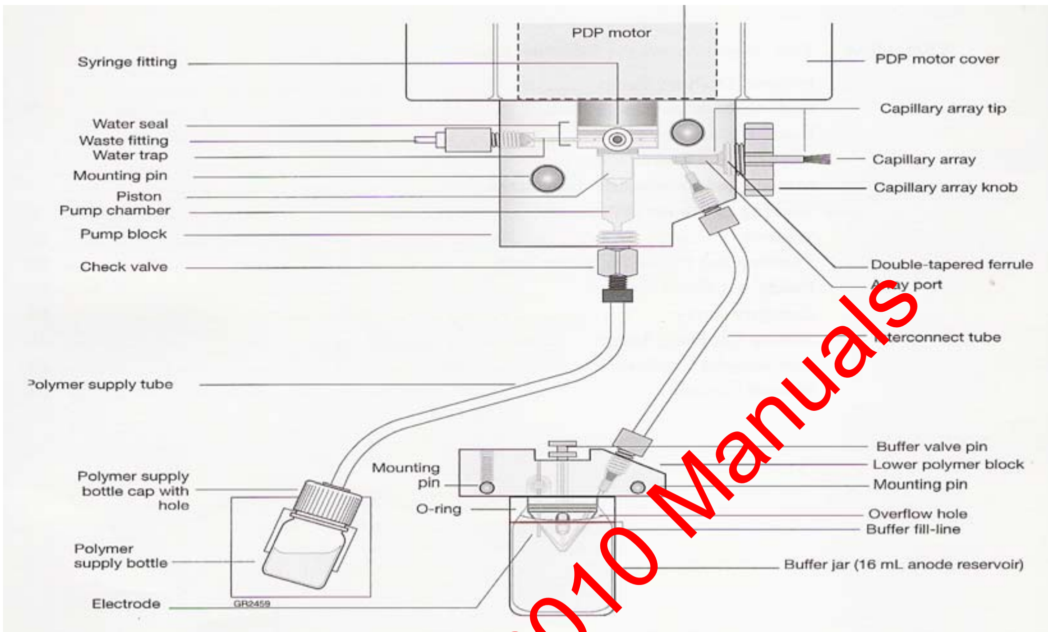
   Once all applications are running, the **Foundation Data Collection** window will be displayed at which time the **Service Console** window may be minimized.

7. Check the number of injections on the capillary in the 3130*xl* binder and in the **Foundation Data Collection** window by clicking on the **ga3130*xl* > *instrument name* > Instrument Status**. If the numbers are not the same, update the binder. If the number is ≥140, notify QA. Proceed only if the number of injections that will be running plus the usage number is ≤150.



8. Check the binder to see when the POP4 was last changed. If it is >7 days, proceed with POP4 change (See Part K. of this section) and then return to Step 11. The POP4 does not need to be changed if it is the 7th day.

9. Check the level of POP4 in the bottle to ensure there is enough for the run (~450 µL for 6 injections). A full piston chamber is approximately 200ul. If not enough, proceed with POP4 change (See Part K. of this section) and then return to Step 11.



10. If it is the first run of the day on the instrument, proceed with steps 11-20. If a run has already been performed on the instrument that day and the "buffer changed" column has been checked off in the binder, skip to Part B. of this section.

11. Close the instrument doors and press the tray button on the outside of the instrument to bring the autosampler to the forward position.

12. Wait until the autosampler has stopped moving and the light on the instrument turns green, and then open the instrument doors.

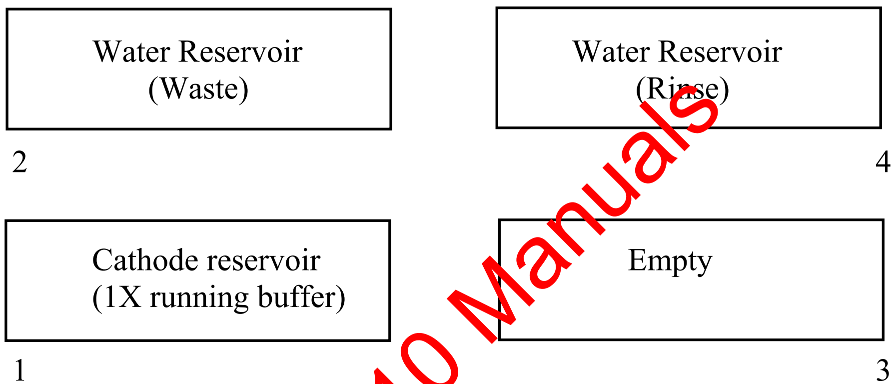
13. Remove the three plastic reservoirs in front of the sample tray and anode jar from the base of the lower pump block and dispose of the fluids.

14. Rinse, dry thoroughly, and then fill the "water" and "waste" reservoirs to the line with deionized water such as GIBCO®.

15. Make a batch of 1X buffer (45 ml Gibco® water, 5 ml 10X buffer) in a 50 mL conical tube. Record the lot number of the buffer, date of make, and your initials on the side of the tube. Rinse and fill the "buffer" reservoir and anode jar with 1X buffer to the lines.

16. Dry the outside <u>and inside rim</u> of the reservoirs/septa and outside of the anode jar using a Kimwipe and replace the septa strip snugly onto each reservoir.

17. Place the reservoirs in the instrument in their respective positions, as shown below:



18. Place the anode jar at the base of the lower pump block.

19. Close the instrument doors.

In the binder, check the "buffer changed" column and record the lot number.

B. Creating a Plate Record through Excel

3130Macro for HSC, Exemplar, and Property Crime Teams

1. Open the 3130Macro and the amp sheets ready to be run.

2. On the amp sheets, copy only the following columns:

   - Label
   - Sample Description
   - pg/µL
   - Dilution
   - DNA
   - H_2O/TE^{-4}
   - IA

   Copy from the controls to the last sample waiting to be run.

3. On the 3130Macro "Samples" tab, "Paste Special" "Values" the copied information from the amp sheets in the appropriate injection.

   If samples need to be rerun:

   - In the Samples tab, type in the necessary information, or copy and paste the information from the rerun log.

   - Click on the buttons available in the Samples tab to describe the reason for rerun in the 3130sheet comments column.

     In **comment1** column, type the run name from which the sample is being rerun.
     In **comment2** column, type in the dilution (if applicable) or click one of the buttons available for reason of rerun.

     In **comment3** column, click one of the buttons available for reason of rerun if not already done.

     Make sure the correct cell is selected before clicking the buttons.

Table 1: Rerun Legend

Symbol	Description
Δ	Rerun to confirm off ladder
dil	Rerun at a dilution
#	Rerun due to bad size standard

- Any other comments can be manually typed in the comment column.
- For rerun normal samples, fill up the end of the injection with any normal reruns before starting a new injection.
- Rerun high samples should have a seperate injection from samples run under normal conditions.

Samples cannot contain more than 50 characters or the sheet will not import.

Any changes made to the Label, Sample Description, IA, or Comments columns MUST be done on the "Samples" tab.

4. Go to the 3130Macro "3130Sheet" tab and type the sample sheet name in cell D1.

   Sample sheets should be named indicating the instrument, the year, and the consecutive run number for the multiplex. For example: "Mendel06-021ID" or "Kastle07-058ID-014Y."

   Sample sheet names cannot be more than 50 characters or the sheet will not import.

5. Save the sample sheet in M:\STR_Data\CASEWORK\SAMPLE SHEETS archive by selecting File, Save As in the format of *yoursamplesheetname.xl*s.

6. On the "3130Sheet" tab, type the appropriate System into the "Sys" column of the first row of the injection. Once the first row of the injection is filled, the rest of the injection should automatically populate with the same System code.

Table 2

Amplification System/Cycle	Specification	Run Module Code	Parameters
Identifiler 28	Normal	I	1 kV for 22 sec
	High	IR	5 kV for 20 sec
YM1	Normal	M	3 kV for 10 sec
	High	MR	3 kV for 20 sec

7. In the "Type" column, fill in the appropriate letter(s) for the type of sample:

Table 3

Sample Type	Designation
Allelic Ladder	AL
Positive Control	PC
Negative Control	NC
Sample	S

8. If there are more than two injections of Identifiler samples, Allelic Ladder should automatically fill into the first rows (colored in grey) of the injection in the "3130Sheet" tab once samples are added to the injection.

   To add a second allelic ladder to an injection, the allelic ladder must be typed in the "Samples" tab.

   If running a system with no Allelic Ladder (ie.YM1), the first sample can be typed into the grey color row in the 3130Sheet tab.

9. Do a final check of the sample sheet. Make sure to check the following:

   - No tube label is duplicated.
   - All necessary columns are filled out.
   - The samples are in correct 3130 format: -_.(){}[]+^ only (and no spaces).

If any changes need to be made in the Label, Sample Description, IA or Comments columns, changes MUST be done in the "Samples" tab (with the exception of Allelic ladders in the first row of the injection)**.**

10. Re-save and print out the sample sheet. If the sample sheet is only one page, highlight the entire area of page 1. Go to File, select "Print Area" and then "Set Print Area."

11. On the 3130Macro "Pre Record" tab, click the "Create Plate Record" button on the top center of the sheet. The macro will automatically jump to the "Plate Record" tab.

12. On the 3130Macro "Plate Record" tab, click the "Remove Empty Rows" button on the top center of the sheet.

13. Staying on the "Plate Record" tab, select File, Save As and do the following:

    a. Change *Save as file type* to "Text (Tab-delimited)".
    b. Save onto a flash drive.

14. Click OK to prompt: The selected file type does not support workbooks that contain multiple sheets.

15. Click Yes to prompt: Do you want to keep the workbook in this format?

16. Insert the flash drive into the USB port of the instrumental computer. Drag-and-drop the plate record from the flash drive to the instrument's plate record folder.

3130*xl* Sample Sheet and Plate Record Macro For High Sensitivity Team

1. Transfer the workbook containing the amplification to be run to the 3130*xl* instrument that will be used. This can be done with a USB flash drive.

2. Open the 3130*xl* sample sheet associated with the amplification, it can be found as a tab labeled with the amplification type (i.e. ID28V for Identifiler 28 evidence) and "3130 sheet" in the appropriate RGAmp Macro workbook of the associated amplification date and time. All information from the amplification will have been automatically imported into the 3130*xl* sheets. However, if changes need to be made to the sheet or samples manually added or moved, follow the instructions below:

    a. The negative controls may be set up in a separate injection from the samples, and injected using "high" run parameters so that they only need to be run once.

    b. For ID31, samples with less than 20 pg amped may be injected high immediately to reduce the number of reruns necessary.

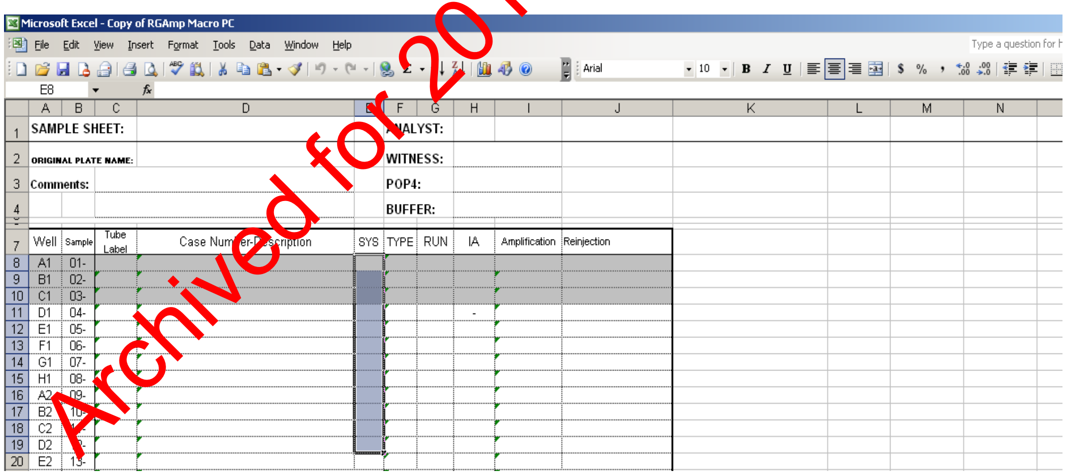
    c. For ID28, samples with less than 200 pg amped may be injected at rerun parameters immediately as well.

NOTE: When using Excel worksheets, DO NOT "copy" and "paste". "Copy" must be followed by "paste special" and "values" when needed.

3. Below is a description of the fields in the sample sheet:

Table 4

Tube Label	label given to each sample for amplification
Case Number- Sample Description	sample name
Sys.	Identifiler (see #6 for abbreviations and associated injection parameters)
Type	sample type (see #8 below)
Run	the injection or run number
RA	the reporting analyst assigned to the case
Amplification	the corresponding amplification date and time
Reinjection	if the plate is re-injected, the original or previous run name



4. Name the sample sheet as follows: ***Instrument name & date_Run folders*** for example: Athena042407_70-76. If the plate is being reinjected, the original plate name is recorded underneath the new sample sheet name.

5. Sample information will automatically populate from amp sheets into the "Tube Label", "**Case Number-Sample Description", "IA", and "Amplification"** columns. Allelic Ladders and Positive Controls will populate the first, second, ninth and tenth wells of each injection. It is mandatory that there be a ladder and Positive Control included with each injection set for Identifiler.

6. In the “Sys.” column, fill in the appropriate letter for the correct run or rerun module code:

Table 5: Identifiler Injection Parameters for the High Sensitivity Team

Amplification Cycle	Specification	Run Module Code	Parameters
Identifiler 31	Low	L	1 kV for 22 sec
	Normal	N	3 kV for 20 sec
	High	H	6 kV for 30 sec
Identifiler 28	Normal	I	1 kV for 22 sec
	High	IR	5 kV for 20 sec

7. In the “Run” column, fill in the appropriate injection or run number referring to the instrument log. (This number can be verified in later stages by opening “Run View” after linking the plate.)

8. In the “Type” column, fill in the appropriate letter(s) for the type of the sample:

Table 6

Sample Type	Designation
Allelic Ladder	AL
Positive Control	PC
Negative Control	NC
Sample	S

9. Proofread sample sheet, make corrections and re-save as necessary.

   <u>IMPORTANT</u>: Remember that all names must consist of letters, numbers, and only the following characters: **- _ . ( ){ }[ ] + ^ (no spaces)**.

10. Save the sample sheet by selecting Save As from the File menu and save the sheet in the format of: *yoursamplesheetname.xl*s. Save in: **D:\AppliedBiosystems\Sample Sheets** (.*xl*s files.)

11. On the 3130Macro "Pre Record" tab, click the "Create Plate Record" button in the top center of the sheet. The Macro will automatically forward to the "Plate Record" tab, copying all of the run information to that sheet.

12. On the 3130Macro "Plate Record" tab, click the "Remove Empty Rows" button in the top center of the sheet. All rows not containing an instrument protocol will be deleted.

13. Select File, Save As and do the following:
    a. Change *Save as file type* to "Text (Tab-delimited)".
    b. Save in the appropriate Plate Record folder.

14. Click OK to prompt: "The selected file type does not support workbooks that contain multiple sheets".

15. Click Yes to prompt: "Do you want to keep the workbook in this format?"

16. While importing the plate record into the ABI 3130*xl* software, minimize the Excel file until the record has been successfully imported.

17. After successfully importing the plate record, exit Excel by going to **File** > **Exit**. A prompt will appear to save again; this is not necessary select **NO**.

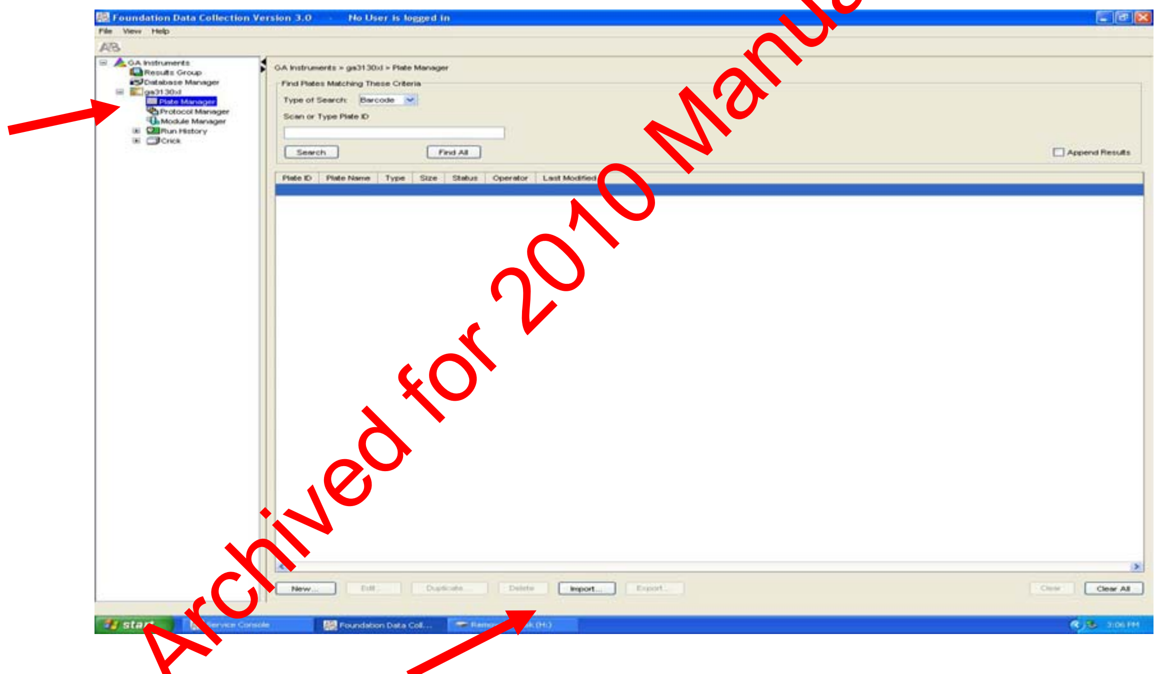
C. Foundation Data Collection (Importing Plate Record)

1. Maximize the Foundation Data Collection window.

2. Click + to expand subfolders in the left tree pane of "**ga 3130*xl***".

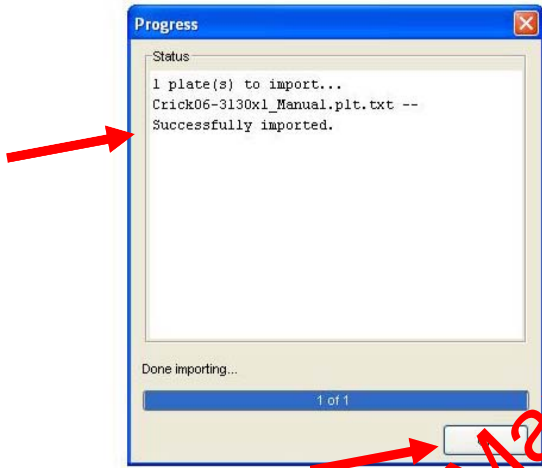
3. Click on "**Plate Manager**".

4. In the Plate Manager window click on "**Import…**"



5. Browse for the plate record in **D:\AppliedBiosystems\Plate Records**. Double click on the file or highlight it and click **Open**.

6. A window will prompt the user that the plate record was successfully imported. Click **OK**.



If the Plate Record will not import, a window will prompt the user where changes are needed. Go back to edit the sample sheet and resave the corrected Plate Record and Sample Sheet with the same file name. Reprint the sample sheet if necessary.

D. Preparing and Running the DNA Samples

1. Retrieve amplified samples from the thermal cycler or refrigerator. If needed, retrieve a passing positive control from a previous passing run.

2. If condensation is seen in the caps of the tubes, centrifuge tubes briefly.

Mastermix and Sample Addition for Identifiler 28 for HSC, Exemplar, and Property Crime Teams:

1. Mastermix preparation:

   a. Prepare one mastermix for all samples, negative and positive controls, and allelic ladders as specified in Table 7 and on the sample sheet (26.625 µL of HIDI + 0.375 µL of LIZ per sample)

TABLE 7: Identifiler 28

# Samples + 2	**HiDi Form** (26.6 µL per sample)	**LIZ500 Std** (0.375 µL per sample)
16	480 µL	7 µL
32	906 µL	13 µL
48	1332 µL	19 µL
64	1758 µL	25 µL
80	2184 µL	31 µL
96	2610 µL	37 µL
112	3036 µL	43 µL
128	3462 µL	49 µL

NOTE: HiDi Formamide must not be re-frozen.

b. Obtain a reaction plate and label the side with the name used for the Sample Sheet with a sharpie. Place the plate in an amplification tray or the plate base.

c. Aliquot **27 µL** of **mastermix** to each well.

d. If an injection has less than 16 samples, add at least 12 uL of either dH_2O, formamide, HiDi, buffer or mastermix to all unused wells within that injection.

Adding Samples:

a. Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96-well reaction plate. Sample order is as follows: A1, B1, C1... A2, B2, C2...etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1-H2, the second A3-H4 and so on.

b. **Witness step.** Have another analyst witness the sample set-up.

c. For sample sets being run at normal parameters: Aliquot **1 µL** of **allelic ladder**.

d. For sample sets being run at normal parameters: Aliquot **3 µL** of the **positive control**.

e. Aliquot **3 µL** of **each sample** and **negative control**.

f. When adding PCR product, make sure to pipette the solution directly into the mastermix and gently flush the pipette tip up and down a few times to mix it.

g. Skip to Part E (Denature/Chill) of this section.

<u>Mastermix and Sample Addition for Identifiler 28 for High Sensitivity Team:</u>

1. Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96-well reaction plate. Sample order is as follows: A1, B1, C1... A2, B2, C2...etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1-H2, the second A3-H4 and so on.

2. **Witness step.** Have another analyst witness the sample set-up.

3. Obtain a reaction plate and label the side with the name used for the Sample Sheet with a sharpie. Place the plate in an amplification tray or the plate base.

4. The Sample Sheet automatically calculates the amount of HiDi Formamide and LIZ Standard needed per sample. This information can be found at the top of the second page of the Sample Sheet.

   NOTE: HiDi Formamide cannot be re-frozen.

   Mastermix for 28 Cycles:

   a. Prepare one mastermix for all samples, negative and positive controls, allelic ladders as specified in Table 8 and on the sample sheet

      i. Add 26.625 µL of HIDI per sample
      ii. Add 0.375 µL of LIZ per sample
      iii. Aliquot **27 µL** of **mastermix** to each well

b. If an injection has less than 16 samples, add 12ul of either dH_2O, buffer or formamide/LIZ mix to all unused wells within that injection.

Add samples to the plate, adhering to the following guidelines:

NOTE: Multichannel pipettes may be used to load samples. If pipetting from a 96 well PCR plate, pierce the seal.

5. **Adding Samples for 28 Cycles:**

a. Aliquot **3 µL** of **each sample** and **negative control** and the **positive control**.

b. Aliquot **0.5 µL** of **positive control** or **1 µL** of **1/2 dilution** (4 uL positive control in 4uL of water) into the wells labeled **"PEH".** This is the positive for the "high" injection parameters.

c. Aliquot **0.7 uL** of **allelic ladder**. If a full plate will be used, mix 6 µL of ladder with 2.4 µL of water and aliquot 1 µL per ladder well.

d. Alternatively, **1 µL** and **0.5 µL** of **allelic ladder** can be used for the normal and the rerun parameters for each injection to account for differences in lots of allelic ladder.

i. For a full plate, add 3.5 µL of ladder to 3.5 µL of water, mix, and and aliquot 1 µL of this dilution.
ii. For a half plate, add 2 µL of ladder to 2 µL of water, mix and aliquot 1 µL of this dilution.
iii. A P2 pipet must be used to make 0.7 and 0.5 µL aliquots to avoid making dilutions and to conserve ladder.

e. Skip to Part E (Denature/Chill) of this section.

TABLE 8: Identifiler 28 Samples for High Sensitivity Team

Injection Parameters	Samples and negs	LIZ	HIDI	Allelic Ladder	Positive Control
I	3 µL	0.375 µL	26.6 µL	1.0 µL or (0.7 µL)*	3 µL
IR	3 µL	0.375 µL	26.6 µL	0.5 µL or (0.7 µL)*	0.5 µL

* Two amounts of allelic ladder, 1 µL and 0.5 µL, may be used for the normal and the rerun parameters to account for differences in lots of ladder rather than 0.7 µL, which is satisfactory for both parameters in most situations.

Mastermix and Sample Addition for Identifiler 31 for High Sensitivity Team

1. Prepare pooled samples: **IDENTIFILER 31 ONLY**

   a. Centrifuge all tubes at full speed briefly.
   b. Label one 0.2 mL PCR tube with the sample name and "abc" to represent the pooled sample injection for the corresponding sample set.
   c. Take 5 µL of each sample replicate, after mixing by pipeting up and down, and place each aliquot into the "abc" labeled tube.
   d. Place each pooled sample directly next to the third amplification replicate labeled "c" of each sample set.

2. Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96-well reaction plate. Sample order is as follows: A1, B1, C1..., A2, B2, C2...etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1-H2, the second A3-H4 and so on.

3. **Witness step.** Have another analyst witness the sample set-up.

4. Obtain a reaction plate and label the side with the name used for the sample sheet with a sharpie. Place the plate in an amplification tray or the plate base.

5. The sample sheet automatically calculates the amount of HiDi Formamide and LIZ Standard needed per sample. This information can be found at the top of the second page of the Sample Sheet.

   NOTE: HiDi Formamide must not be re-frozen.

6. **Mastermix for 31 CYCLES**:

   a. Prepare the following **mastermix** for **samples**, and **negative controls** as specified in Table 8 and on the sample sheet
      i. 44.6 µL of HIDI per sample
      ii. 0.375 µL of LIZ per sample
      iii. Aliquot **45 µL** of **mastermix** to each **sample** and **negative control** well

   b. Prepare a separate **mastermix** for **allelic ladders** and **positive controls**
      i. Add 14.6 µL of HIDI to each AL and PE
      ii. Add 0.375 µL of LIZ per AL and PE
      iii. Aliquot **15 µL** of **mastermix** to each **Allelic Ladder** and **Positive Control** well

7. If an injection has less than 16 samples, add 12ul of either dH_2O, buffer or formamide/LIZ mix to all unused wells within that injection.

8. Add samples to the plate, adhering to the following guidelines:

   NOTE: Multichannel pipettes may be used to load samples. If pipetting from a 96 well PCR plate, pierce the seal.

9. **Adding Samples for Identifiler 31:**

   a. Aliquot **5 µL** of **each sample (including pooled)** and **negative control**.

   b. Aliquot **1 µL** of a **1/10 dilution** of **positive control** into each well labeled **"PE".** (Make the 1/10 dilution by mixing 2 uL of Positive Control with 18 uL water). This is the positive for the "normal" injection parameters.

   c. Aliquot **1 µL** of a **1/20 dilution** of **positive control** into each well labeled **"PEH".** (Make the 1/20 dilution by mixing 2 uL of Positive Control with 38 uL water). This is the positive control for the "high" injection parameters.

   d. Aliquot **0.5 uL** of **allelic ladder** into each well labeled **"AL".** Alternatively, make a 1/2 dilution of ladder and aliquot 1 uL per "AL" well. Make this dilution by mixing 2 uL ladder with 2 uL of water for 1-2 injections, 3 uL ladder with 3 uL of water for 3-4 injections or 4 uL ladder with 4 uL water for 5-6 injections. This is the allelic ladder for the "normal" injection parameters.

   e. Aliquot **0.3 uL** of **allelic ladder** into each well labeled **"ALH".** Alternatively, make a 3/10 dilution of ladder and aliquot 1 uL per "ALH" well. Make this dilution by mixing 1 uL of ladder with 2.3 uL of water for 1-2 injections, 2 uL of ladder and 4.6 uL of water for 3-4 injections, or 3 uL of ladder with 6.9 uL water for 5-6 injections. This is the allelic ladder for "high" injection parameters.

TABLE 9: 31 Cycle Samples for High Sensitivity Team

Injection Parameters	Samples and negs	LIZ for samples and negs	HIDI for samples and negs	Allelic Ladder	Positive Control	LIZ for ALs And PEs	HIDI for ALs And PEs
L	5 µL	0.375 µL	44.6 µL	0.5 µL	1µL of 1/10 dil	0.375 µL	14.6 µL
N	5 µL	0.375 µL	44.6 µL	0.5 µL	1µL of 1/10 dil	0.375 µL	14.6 µL
H	5 µL	0.375 µL	44.6 µL	0.3 µL	1µL of 1/20 dil	0.375 µL	14.6 µL

10. Proceed to Part E (Denature/Chill) in this section.

Mastermix and sample addition for YM1

Refer to the table below to determine the amount of HiDi Formamide and standard to use for the number of samples on the run. To prepare mix for (n+2) samples: For YM1: 9.5 µL of HiDi Formamide + 0.5 µL of Liz Standard is mixed per sample**.**

Table 10: YM1

# Samples + 2	**HiDi Form**	**LIZ Std**
16	171 µL	9 µL
32	323 µL	17 µL
48	475 µL	25 µL
64	627 µL	33 µL
80	779 µL	41 µL
96	931 µL	49 µL
112	1083 µL	57 µL
128	1235 µL	65 µL

1. Aliquot **10 µL** of the **formamide/standard mixture** into each well being used on the 96-well reaction plate.

2. If an injection has less than 16 samples, add 12ul of either dH_2O, buffer or formamide/standard mix to all unused wells within that injection.

3. Rerun "high" samples **cannot** be on the same injection as non rerun samples. Rerun "normal" samples may be integrated with non rerun samples.

4. **Witness step.** Have another analyst witness the sample set-up.

5. Add samples to the plate, adhering to the following guidelines:

   For samples being run at normal parameters:
   a. add **2 µL** of **each sample** (including the positive control)

   For samples being run at rerun high parameters:
   a. add **4 µL** of a **1/10 dilution** of the **positive control**
   b. add **4 µL** of **each sample**

6. When adding PCR product, make sure to pipette the solution directly into the formamide/standard mixture and gently flush the pipette tip up and down a few times to mix it.

7. Proceed to Part E (Denature/Chill) of this section.

E. Denature/Chill - For All Systems After Sample Addition

1. Once all of the samples have been added to the plate, place a new 96-well septa over the reaction plate and firmly press the septa into place.

2. Spin plate in centrifuge at 1000 RPM for one minute.

3. For Denature/Chill:

   a. 9700 Thermal Cycler

      i. Place the plate on a 9700 thermal cycler (Make sure to keep the thermal cycler lid off of the sample tray).
      ii. Select the "denature/chill" program.
      iii. Make sure the volume is set to 12 µL for YM1, 30 µL for Identifiler 28, and 50 µL for Identifiler 31. If more than one system is loaded on the same plate, use the higher value.
      iv. Press **Run** on the thermal cycler. The program will denature samples at 95^{o}C for 5 minutes followed by a chill at 4^{o}C (the plate should be left to chill for at least 5 min).
      v. While the denature/chill is occurring, the oven may be turned on.

b. Heat Block

i. Place the plate on a 95°C heat block for 5 minutes.
ii. Place the plate on a 4°C heat block for 5 minutes.

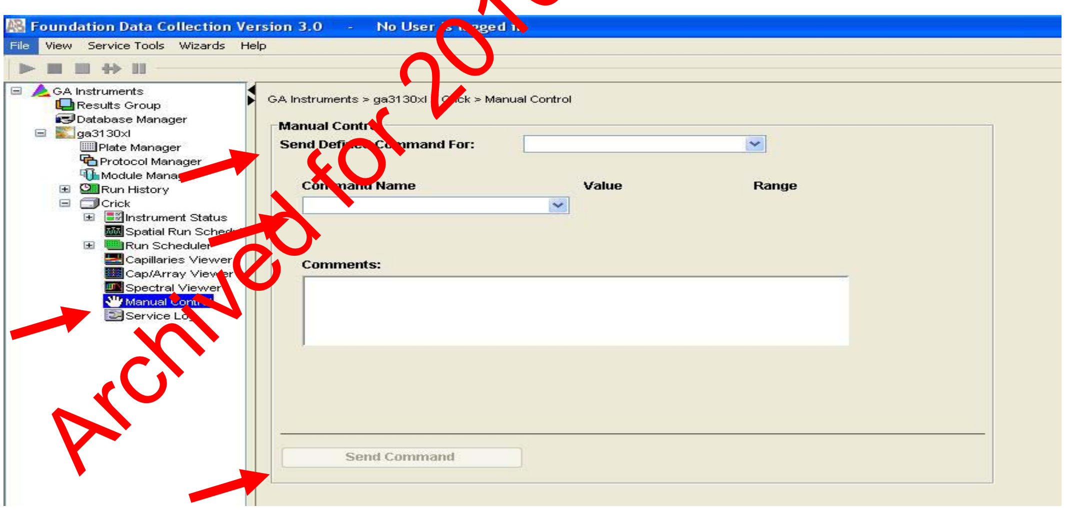
F. Turning the Oven on and Setting the Temperature

1. In the tree pane of the Data Collection v3.0 software click on **GA Instrument > ga3130*xl* > *instrument name* > Manual Control**

2. Under Manual Control "**Send Defined Command For:**" click on **Oven.**

3. Under "**Command Name**" click on "**Turn On/Off oven**".

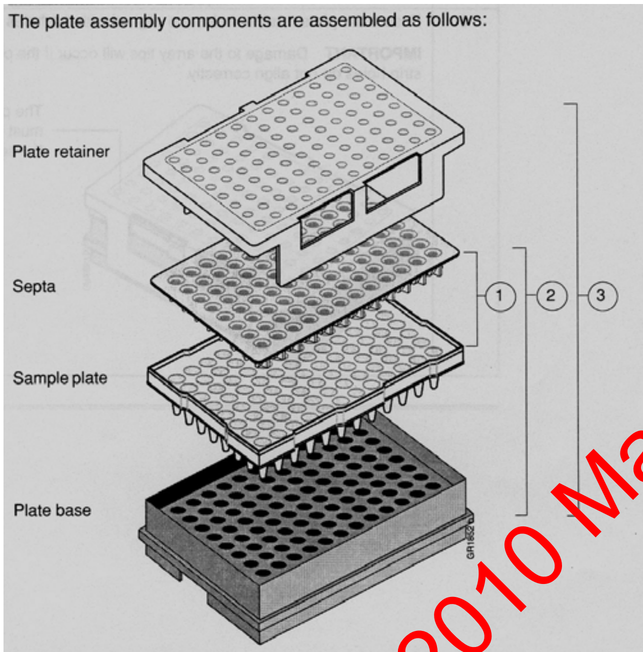
4. Click on the "**Send Command**" button.



5. Under "**Command Name**" click on "**Set oven temperature**" and Under "**Value**" set it to **60**.

6. Click on the "**Send Command**" button.

7. Once denatured, spin the plate in centrifuge at 1000 RPM for one minute before placing the reaction plate into the plate base. Secure the plate base and reaction plate with the plate retainer.



G. **Placing the Plate onto the Autosampler (Linking and Unlinking Plate)**

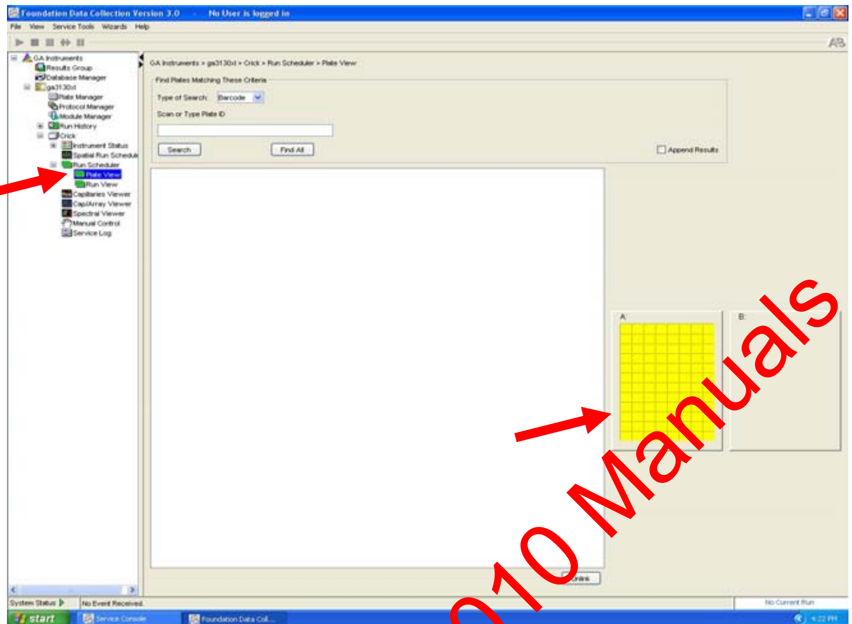
1. In the tree pane of the Foundation Data Collection v3.0 software click on **GA Instrument > ga3130*xl* > *instrument name* > Run Scheduler > Plate View**

2. Push the tray button on the bottom left of the machine and wait for the autosampler to move forward and stop at the forward position.

3. Open the doors and place the tray onto the autosampler in the correct tray position, A or B. There is only one orientation for the plate. (The notched end faces away from the user.)

4. Ensure the plate assembly fits flat in the autosampler.

   When the plate is correctly positioned, the plate position indicator on the **Plate View** window changes from gray to yellow. Close the instrument doors and allow the autosampler to move back to the home position.



<u>Linking/Unlinking the Plate Record to Plate</u>

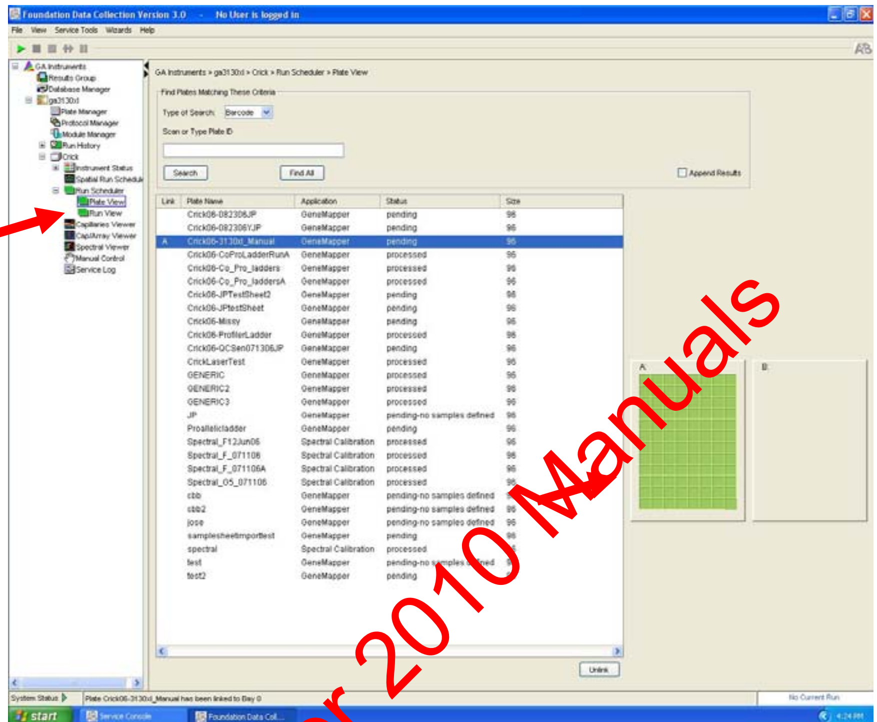
5. Type the exact plate name in the Plate ID window and click **"Search."** Or, click the "**Find All**" button and select the desired plate record.

   NOTE: If the plate name is not typed in correctly, your plate will not be found. Instead, a prompt to create a new plate will appear. Click "No" and retype the plate name correctly.

   Click the plate position indicator corresponding to the plate position in the instrument. The plate position (A or B) displays in the link column.

   If two plates are being run, the order in which they are run is based on the order in which the plates were linked.

6. The plate position indicator changes from yellow to green when linked correctly and the green run button becomes active.



7. To unlink a plate record just click the plate record to be unlinked and click "**Unlink**".

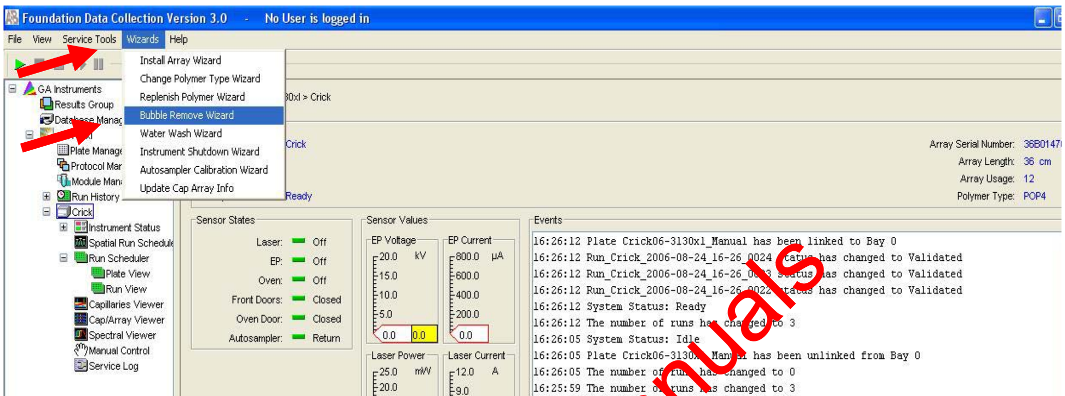
H. Viewing the Run Schedule

1. In the tree pane of the Foundation Data Collection software, click **GA Instruments** > **ga3130*xl*** > ***instrument name*** > **Run Scheduler** > **Run View.**

2. The **RunID** column indicates the folder number(s) associated with each injection in the run (e.g. Run_ Venus_2006-07-13_**0018-0019**). These folder number(s) should be recorded in the 3130*xl* Usage Log binder along with the run control sheet name.

3. Click on the run file to see the Plate Map or grid diagram of the plate on the right. Check if the blue highlighted boxes correspond to the correct placement of the samples in the injections.

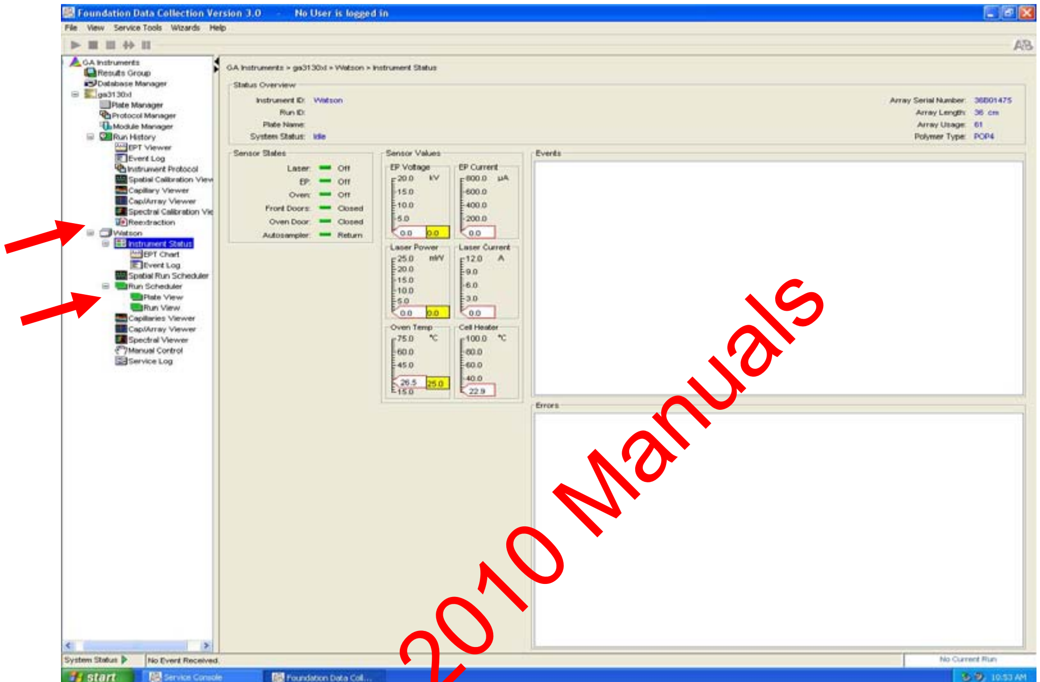
NOTE: Before starting a run, check for air bubbles in the polymer blocks. If present, click on the **Wizards** tool box on the top and select "**Bubble Remove Wizard**". Follow the wizard until all bubbles are removed.



4. Click on green **Run** button in the tool bar when you are ready to start the run. When the **Processing Plate** dialog box opens (You are about to start processing plates…), click **OK**.

5. To check the progress of a run, click on the **Capillary Viewer** or **Cap/ArrayViewer** in the tree pane of the Foundation Data Collection software. The **Capillary Viewer** will show you the raw data of the capillaries you select to view whereas the **Cap/Array Viewer** will show the raw data of all 16 capillaries at once.

IMPORTANT: Always exit from the **Capillary Viewer** and **Cap/Array Viewer** windows. During a run, do not leave these pages open for extended periods. Leave the **Instrument Status** window open.



The visible settings should be: EP voltage 15kV
EP current (no set value)
Laser Power Prerun 15 mW
Laser Power During run 15mW
Laser Current (no set value)
Oven temperature 60°C

Expected values are: EP current constant around 120 to 160µA
Laser current: 5.0A ± 1.0

It is good practice to monitor the initial injections in order to detect problems.

Table 11

	I/L	IR	N	H
Oven Temp	60°C	60°C	60°C	60°C
Pre-Run Voltage	15.0 kV	15.0 kV	15.0 kV	15.0 kV
Pre-Run Time	180 sec	180 sec	180 sec	180 sec
Injection Voltage	1 kV	5 kV	3 kV	6 kV
Injection Time	22 sec	20 sec	20 sec	30 sec
Run Voltage	15 kV	15 kV	15 kV	15 kV
Run Time	1500 sec	1500 sec	1500 sec	1500 sec

Table 12

	M	MR
Oven Temp	60°C	60°C
Pre-Run Voltage	15.0 kV	15.0 kV
Pre-Run Time	180 sec	180 sec
Injection Voltage	3 kV	3 kV
Injection Time	10 sec	20 sec
Run Voltage	15 kV	15 kV
Run Time	1500 sec	1500 sec

I. Converting Run for GeneScan Analysis

When a run is complete, it will automatically be placed in **D:/AppliedBio/Current Runs** folder, properly labeled with the *instrument name, date and runID* (e.g. Run_Venus_2006-07-13_0018). Proceed to Section 9 for instructions on how to convert this data for GeneScan analysis.

J. Re-injecting Plates

1. Plates should be re-injected as soon as possible, preferably the same day.

2. If a plate is being re-injected the same day on which it was originally run, it does not require an additional denature/chill step before being rerun.

3. Create a new sample sheet and plate record using the original sample sheet as a guide. Select only those samples that need to be rerun by re-assigning "Sys". For example, assign "IR" for an ID28 sample that needs to be re-run high.

   NOTE: See Section 7 for information on which controls need to be run.

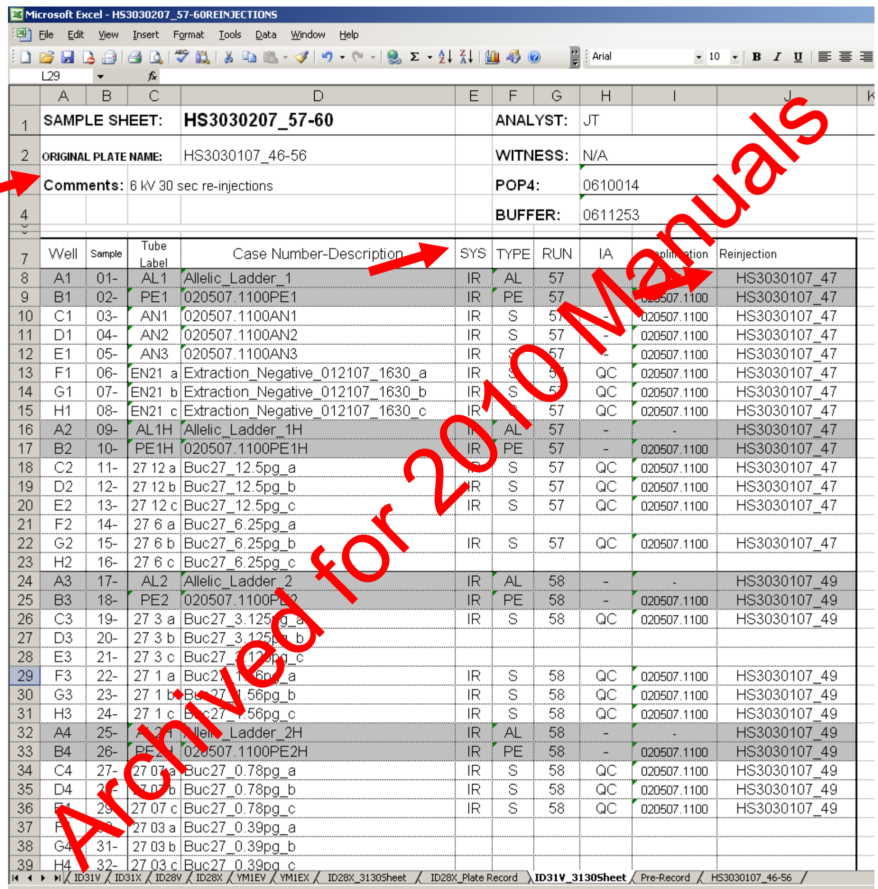
4. For High Sensitivity Team

   a. Next to **Sample Sheet**, type the new run name. Next to **Original Plate Name**, insert the original run name (e.g. Venus041706_35-39).

   b. Under **Reinjection** insert the original run date and run number (e.g. Venus041706_35).

5. Follow the instructions for saving a sample sheet and creating a plate record. Re-import the plate record.

6. Re-denature/chill the plate (if needed) as described in Part E.



SAMPLE SHEET:			HS3030207_57-60		ANALYST:		JT		
ORIGINAL PLATE NAME:			HS3030107_46-56		WITNESS:		N/A		
Comments:	6 kV 30 sec re-injections				POP4:		0610014		
					BUFFER:		0611253		
Well	Sample	Tube Label	Case Number-Description	SYS	TYPE	RUN	IA	Amplification	Reinjection
A1	01-	AL1	Allelic_Ladder_1	IR	AL	57	[illegible]	[illegible]	HS3030107_47
B1	02-	PE1	020507.1100PE1	IR	PE	57	[illegible]	020507.1100	HS3030107_47
C1	03-	AN1	020507.1100AN1	IR	S	57	-	020507.1100	HS3030107_47
D1	04-	AN2	020507.1100AN2	IR	S	57	-	020507.1100	HS3030107_47
E1	05-	AN3	020507.1100AN3	IR	S	57	-	020507.1100	HS3030107_47
F1	06-	EN21 a	Extraction_Negative_012107_1630_a	IR	S	57	QC	020507.1100	HS3030107_47
G1	07-	EN21 b	Extraction_Negative_012107_1630_b	IR	S	57	QC	020507.1100	HS3030107_47
H1	08-	EN21 c	Extraction_Negative_012107_1630_c	IR	S	57	QC	020507.1100	HS3030107_47
A2	09-	AL1H	Allelic_Ladder_1H	IR	AL	57	-	-	HS3030107_47
B2	10-	PE1H	020507.1100PE1H	IR	PE	57	-	020507.1100	HS3030107_47
C2	11-	27 12 a	Buc27_12.5pg_a	IR	S	57	QC	020507.1100	HS3030107_47
D2	12-	27 12 b	Buc27_12.5pg_b	IR	S	57	QC	020507.1100	HS3030107_47
E2	13-	27 12 c	Buc27_12.5pg_c	IR	S	57	QC	020507.1100	HS3030107_47
F2	14-	27 6 a	Buc27_6.25pg_a						
G2	15-	27 6 b	Buc27_6.25pg_b	IR	S	57	QC	020507.1100	HS3030107_47
H2	16-	27 6 c	Buc27_6.25pg_c						
A3	17-	AL2	Allelic_Ladder_2	IR	AL	58	-	-	HS3030107_49
B3	18-	PE2	020507.1100PE2	IR	PE	58	-	020507.1100	HS3030107_49
C3	19-	27 3 a	Buc27_3.125pg_a	IR	S	58	QC	020507.1100	HS3030107_49
D3	20-	27 3 b	Buc27_3.125pg_b						
E3	21-	27 3 c	Buc27_3.125pg_c						
F3	22-	27 1 a	Buc27_1.56pg_a	IR	S	58	QC	020507.1100	HS3030107_49
G3	23-	27 1 b	Buc27_1.56pg_b	IR	S	58	QC	020507.1100	HS3030107_49
H3	24-	27 1 c	Buc27_1.56pg_c	IR	S	58	QC	020507.1100	HS3030107_49
A4	25-	AL2H	Allelic_Ladder_2H	IR	AL	58	-	-	HS3030107_49
B4	26-	PE2H	020507.1100PE2H	IR	PE	58	-	020507.1100	HS3030107_49
C4	27-	27 07 a	Buc27_0.78pg_a	IR	S	58	QC	020507.1100	HS3030107_49
D4	[illegible]	27 07 b	Buc27_0.78pg_b	IR	S	58	QC	020507.1100	HS3030107_49
[illegible]	29-	27 07 c	Buc27_0.78pg_c	IR	S	58	QC	020507.1100	HS3030107_49
F[illegible]	[illegible]	27 03 a	Buc27_0.39pg_a						
G4	31-	27 03 b	Buc27_0.39pg_b						
H4	32-	27 03 c	Buc27_0.39pg_c						

K. Water Wash and POP Change

Refer to Section A for schematic of 3130*xl* while proceeding with the water wash and POP change procedure.

1. Remove a new bottle of POP4 from the refrigerator.

2. Select **Wizards** > **Water Wash Wizard** and follow the wizard.

3. When the "Fill Array" step has completed, remove the anode buffer jar, empty, and fill with 1x TBE Buffer (~15 mL).

4. Close instrument doors and wait for the steady green light.

5. Click "Finish."

L. Cleanup Database Utility (QA Team)

1. Open the *Foundation Data Collection Window* of the 3130 software.
2. In the left hand panel, click on "GA Instruments".
3. Click on "Database Manager".
4. Click the "Cleanup Processed Plate" button.
5. This will erase the database and reset the run number to 0. Therefore, the next plate run after this process will be labeled run number 1. Verify this information for the usage log.

TROUBLESHOOTING GUIDE

This section is provided as a guide. The decision on which of the recommended actions is the most promising should be made after consultation with a supervisor.

PROBLEM: Many artifacts in sample.

Possible Cause	Recommended Action
Secondary structure present. Sample not denatured properly.	1. Clean pump block and change polymer to refresh the urea environment. 2. Denature/chill samples.

PROBLEM: Decreasing peak heights in all samples.

Possible Cause	Recommended Action
Poor quality formamide or sample environment very ionic.	Realiquot samples with fresh HIDI.

PROBLEM: Individual injections run at varying speeds. For example, the scan number where the 100 bp size standard appears differs significantly from one injection to another, usually appearing earlier.

Possible Cause	Recommended Action
Warm laboratory temperatures.	1. Redefine size standard. 2. If this fails, reinject.

PROBLEM: Loss of resolution of peaks.

Possible Cause	Recommended Action
Loss of resolution of peaks.	1. Clean pump block and change polymer to refresh the urea environment. 2. Denature chill samples.

PROBLEM: An off ladder peak appears to be a pull up, but it is not exactly the same basepair as the true peak.

Possible Cause	Recommended Action
1. Matrix over-subtraction. Usually in the green channel, the true peak is overblown and is split. 2. Pull up peaks appear in the blue and the red channels. 3. In the yellow channel, there is a negative peak at the base pairs of the true peak, however immediately to the right and to the left are off ladder peaks.	Remove off ladder peaks as matrix over-subtraction.

PROBLEM: Peaks overblown and running as off ladder alleles.

Possible Cause	Recommended Action
More than the optimum amount of sample amplified.	1. Rerun samples at lower injection parameters. 2. Or rerun samples with less DNA.

PROBLEM: Pull up peaks.

Possible Cause	Recommended Action
Colors bleeding into other colors.	Run a spectral.

PROBLEM: Spikes in the electropherogram.

Possible Cause	Recommended Action
Crystals in the polymer solution due to the polymer warming and congealing from fluctuations in the room temperature.	Change the polymer.

PROBLEM: Spikes in electropherogram and artifacts.

Possible Cause	Recommended Action
Arcing: water around the buffer chambers.	Clean chambers; beware of drops accumulating around the septa.

PROBLEM: Split peaks.

Possible Cause	Recommended Action
Lower pump block is in the process of burning out due to the formation of a bubble.	Clean the block.

PROBLEM: Increasing number of spurious alleles.

Possible Cause	Recommended Action
Extraneous DNA in reagents, consumables, or instrument.	1. Stop laboratory work under advisement of technical leader. 2. Implement a major laboratory clean-up.

GENERAL PROBLEMS

Problems	Recommended Action
1. Fatal Errors.	1. Close collection software. 2. Restart collection software.
2. 3130*xl* not cooperating.	3. Restart Computer and Instrument.
3. Shutter problems.	4. Call Service.

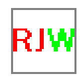
Revision History:
March 24, 2010 – Initial version of procedure.

A. Converting Run for GeneScan Analysis

Prior to importing *.fsa files into GeneScan, the files must have been converted using the HIDDyeBUtility conversion tool.

1. Make sure the run (injection) has completed.

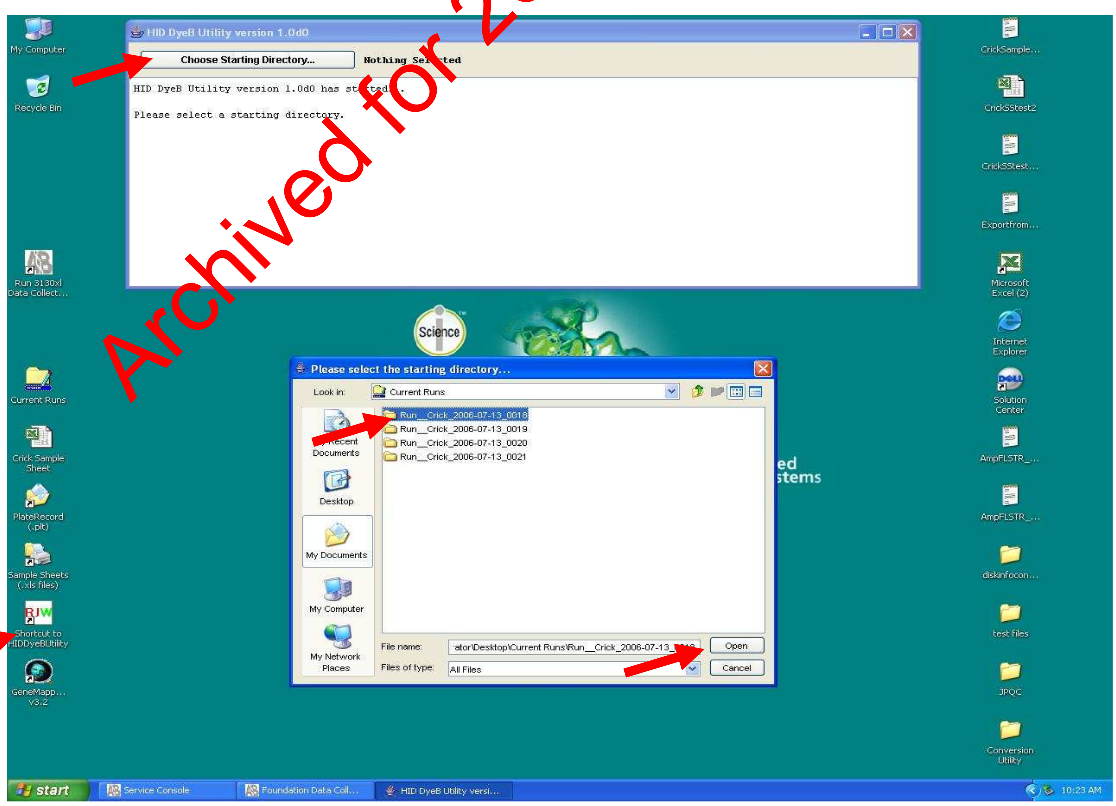
2. On the desktop, click on the shortcut for the **RJW** conversion program.



HIDDyeBUtility.exe

3. On the top of the **RJW** conversion program window click on the "**Choose Starting Directory**" button.

4. Browse the **Current Runs** Folder (located in the D drive, Applied Biosystems folder) for your run folder(s) (e.g. Run_Venus_2006-07-13_0018).



5. Double click on your run folder(s) then hit **Open.** The run folder(s) are now converted and are now ready to be analyzed in **GeneScan**.

B. Archiving Converted Data and Sample Sheets

For the HSC, Exemplar, and Property Crime Teams:

1. When a run is finished, locate the run folders on the 3130*xl* instrumental computer. These folders are in the **Current Runs** folder (on the desktop) and have previously been converted.

2. Insert a flash drive into the USB port of the instrumental computer.

3. Copy the run folder(s) onto the flash drive. Eject the flash drive from the instrumental computer and take it over to the network computer.

4. The STR data folders (located in **M:\STR_Data\CASEWORK**) are organized on the network by instrument name, year, and amplification system. Within these folders they are organized by amplification set (see Example #2 below).

   On the network computer, create a new folder for each run and put it in the appropriate location. Name this folder(s) with the file name according to your sample sheet (e.g. Stripes09-005ID, saved in M:\STR_Data\CASEWORK\Stripes\2009\Identifiler).

   <u>Example #1</u>: An Identifiler and a YM1 amplification set were run on Stripes with the following sample sheet name: Stripes09-005ID-003Y. Two run folders will be created with the following names: Stripes09-005ID and Stripes09-003Y

   <u>Example #2</u>: Two Identifiler amplification sets were run on Stripes with the following sample sheet name: Stripes09-006ID. Two folders will be created inside this folder, with the folder names corresponding to each amplification set as follows: Stripes09-006IDA and Stripes09-006IDB..

5. Copy the run folders from the flash drive into their corresponding amplification set folders on the network. Once saved to the network, delete the files from the flash drive.

6. Proceed with GeneScan and Genotyper analysis.

For the High Sensitivity Team:

1. When an injection is complete, the data will automatically be placed in the **D:/AppliedBio/Current Runs** folder, properly labeled with the *instrument name, date and runID* (e.g. Run_Venus_2006-07-13_0018).

2. After conversion of the data in each run folder, copy the relevant run folders as well as the sample sheets to a flash drive.

3. Transfer the run files from the flash drive to the appropriate data drive on the network.

   a. The run folders should be stored on the network in the run folder of the instrument on which they were run.

   b. The sample sheets should be stored on the network in the sample sheets folder of the instrument on which they were run.

4. After confirming that the files are on the network, delete the files from the flash drive.

5. Proceed with GeneScan and Genotyper analysis.

C. Backup of Data

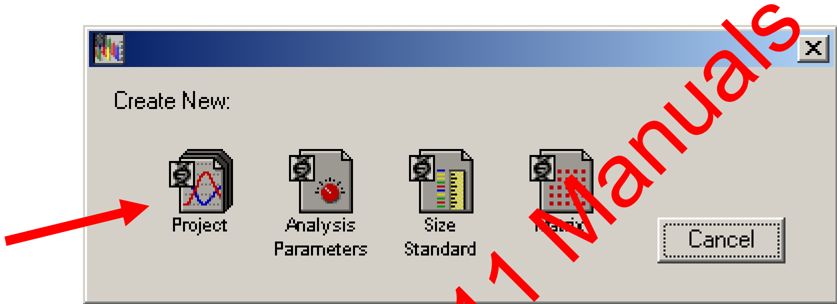
All of the 3130*xl* data, once loaded on the network drive, will be backed up in a process by DoITT, and stored in archives on and off site of the OCME building.

Revision History:
March 24, 2010 – Initial version of procedure.

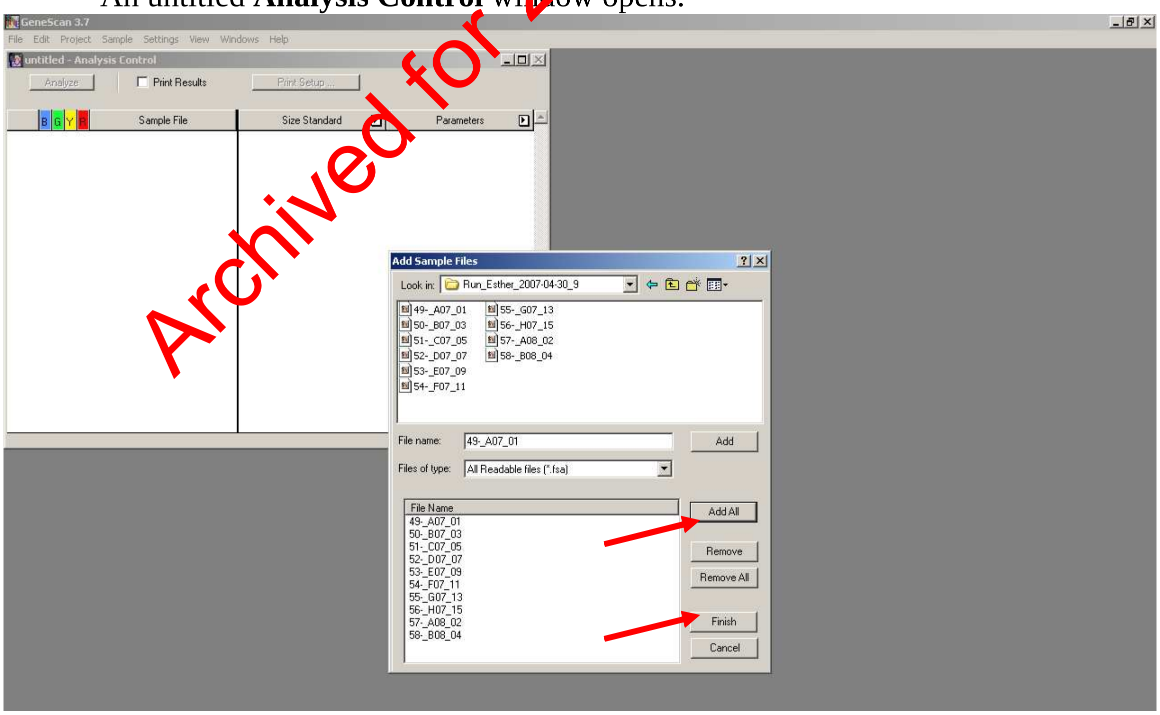
A. Access to GeneScan

1. Click on the GeneScan shortcut located on the desktop of the analysis station computer.

2. Create a new GeneScan project by clicking **File→ New**. A dialog box with several icons will pop up. Click on the project icon.



An untitled **Analysis Control** window opens.



3. To add sample files to the open analysis control window, click on **Project** from the menu options and select **Add Sample Files**.

4. When the **Add Sample Files** dialog window appears, go to **M: → STR_Data → Casework** and select the corresponding instrument's folder. Find the run folders with the samples that you want to add to the project. Once you click on the specific run folder, you will see icons representing each individual sample, all belonging to one injection.

   To add samples to a project, take the following action:

If you want to...	Then...
Select a single sample file	Double-click the file OR select the file and click **Add**
Select all the sample files	Click **Add All**
Add a continuous list of sample files	a. Click the first sample that you want to add. b. Press the **Shift key** and click the last sample you want to add. Click **Add**.
Add a discontinuous list of samples	a. Click the first sample that you want to add b. Press the **Control key** and then click on the other sample(s) you want to add. Click **Add**.

5. Click **Finish** when you have added all of the samples.

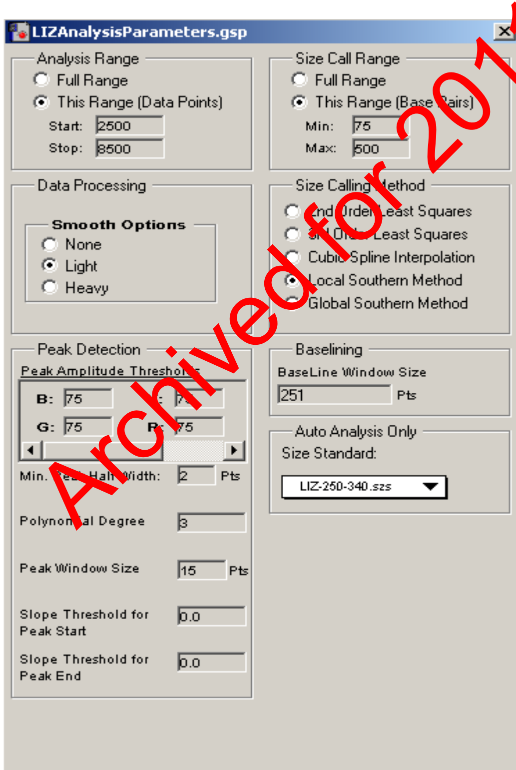
B. Analysis Settings

The analysis should then be performed using the following predefined files:

System	Size Standard File	Analysis Parameter File
YM1	Ystr.szs	YM1.gsp
Identifiler	LIZ-250-340.szs	LIZAnalysisParameters.gsp

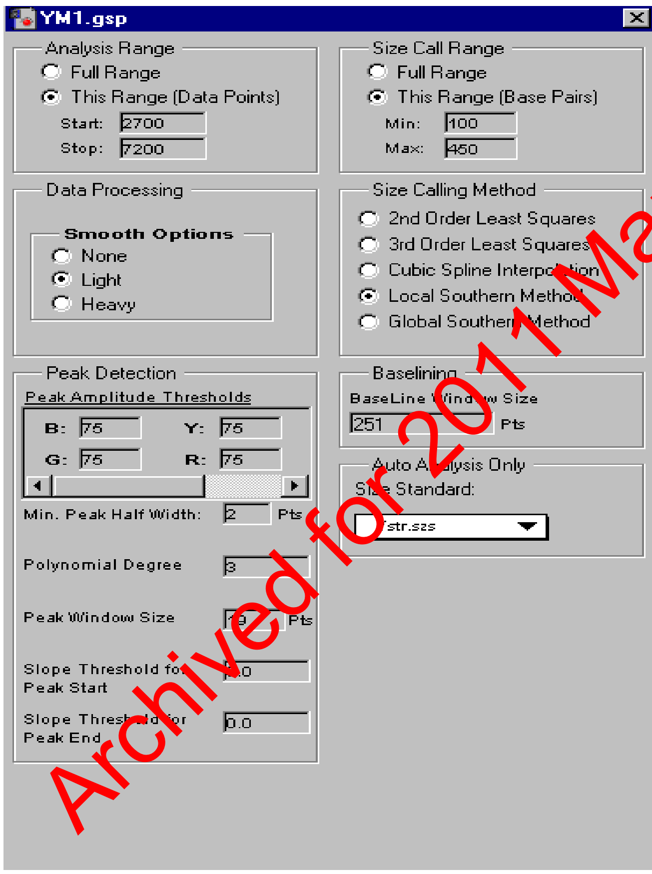
1. Identifiler Analysis Parameters

Do not change any of the settings except the range or the peak amplitude threshold for Orange (O), which may be lowered to 25 rfu.



2. YM1 Analysis Parameters

Do not change any of the settings except the range or the peak amplitude threshold for Orange (O), which may be lowered to 25 rfu.



Once the correct parameters have been chosen, the samples can be analyzed by clicking the **Analyze** button.

When the samples are analyzed, the boxes will change from colored to dark grey in the Analysis Control window. If a sample does not analyze, see Section D: Analysis Troubleshooting.

C. Analysis

To ensure that all the sizing results are correct, check the labeling of the size standard peaks for each sample.

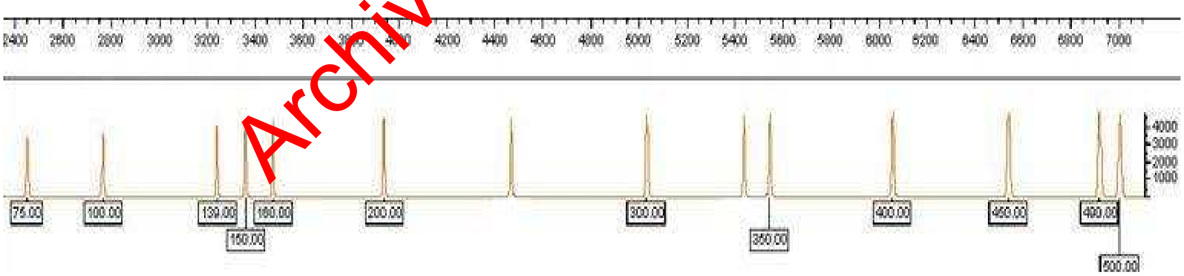
1. To view the analysis results, select **Windows** from the main menu and click on **Results Control**.

2. The raw data can be seen in up to 8 display panels, by changing the **# of panels** to **8**. To view each color separately, check **Quick Tile** to **On**.

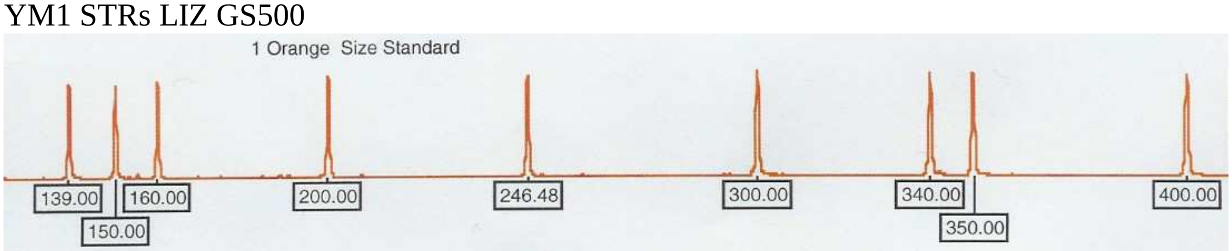
3. 3. Select the first 8 size standard dye lanes by clicking on them and then click **Display**. Each sample standard will be displayed in its own window. To view all 8 standards, you must scroll through all of the windows. Make sure that all peaks are correctly labeled. Continue checking your size standard for the entire tray by going back to the **Results Control** window, clicking on **Clear All** and selecting the next 8 samples.

   <u>**IMPORTANT:**</u> For ABI 3130 runs, the 250bp fragment in the Identifiler LIZ Orange Size Standard may not be labeled as 250. In Identifiler, the 340bp fragment is also not labeled.

Identifiler LIZ Orange Size Standard



YM1 STRs LIZ GS500



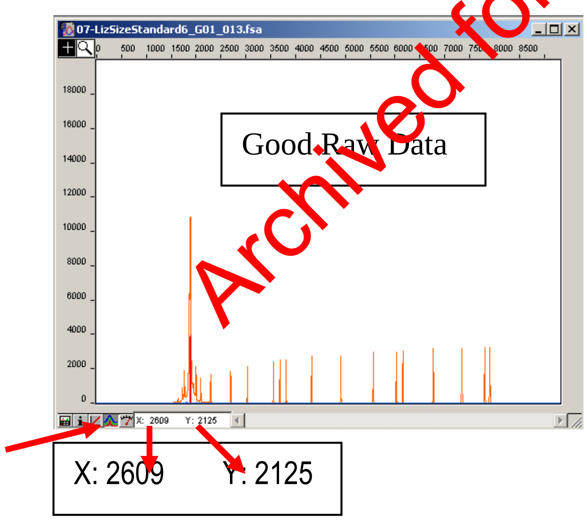
Before proceeding with the Genotyper analysis, under **File** select **Save Project As.** The project will be named according to the Sample Sheet name. This file will save as a *.prj file in the run folder.

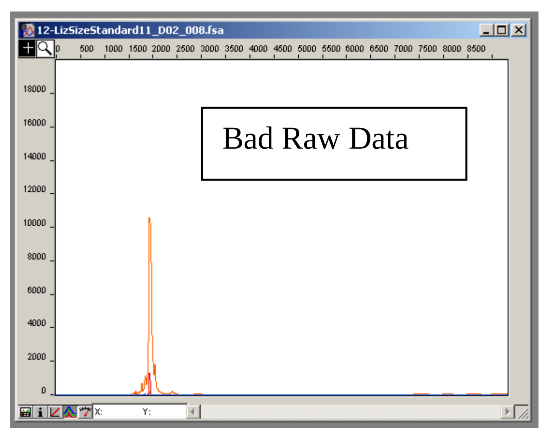
D. Analysis Troubleshooting

The error message for a failed analysis is: "Analysis failed on Dye B, G, Y, R. Repeat the above choosing another scan range."

If the sample fails to be analyzed, examine the **Raw Data**. Click to highlight the sample under the **Sample File** column in the **Analysis Control** window and go the **Sample** tool bar and choose **Raw Data**. Alternatively, click and highlight a sample and hit **Ctrl+R** or double click on a sample and click on the raw data symbol on the bottom left hand side of the **Raw Data** window that pops up. If there is no evidence of size standard peaks, the sample fails. Note on the editing sheet that the sample needs to be rerun. If peaks are present take the following steps.

Raw Data Window:





1. Check the height of the size standard

    a. Examine the **Raw Data** to check the peak height of the size standard fragments. The peak height is indicated by the datapoint value of the **Y**, located on the bottom of the Raw Data window, when the cursor is placed on top of the peak. See instructions and diagram above.

    b. In the **Analysis Control** window under the **Parameter** column click and highlight the parameter of the sample that needs to be adjusted and click the small arrow ▶ on the right side of the cell and select the predefined parameter "**LIZAnalysisParameterOrange25**".

    c. Reanalyze samples. There should be a ♦ on the size standard column.

2. Change the analysis parameters

    a. It is also possible, that the run was either to fast or to slow. The analysis range may need to be changed. Examine the **Raw Data** to see the scan range. See instructions and diagram above.

    b. Observe where the first size standard is located in the sample by moving the cursor to the peak. Take note of the datapoint value of the **X** located on the bottom of the **Raw Data** window.

    c. From the **Analysis Control** window**,** go to the **Parameter** column, click and highlight the parameter that needs to be adjusted and click on the small arrow ▶ on the right side of the cell and select **Define New.**

    d. From here an **untitled** analysis parameter window will appear. Make sure all the default settings are correct as indicated above. Under **Analysis Range** adjust the **start** value to approximately 25 bp less than the datapoint value of the **X** as indicated in step 2b. (eg. X:2400 adjust Start: 2375)

    e. Exit out of the window by hitting $\boxed{\text{X}}$ and click **Save.** Save the file in the folder **C:\AppliedBio\Shared\Analysis\Sizecaller\Params** that can only be accessed through the desktop shortcut **AppliedBio** folder.

f. Reanalyze samples. There should be a ♦ on the size standard column.

g. After reanalyzing the samples go back to the Parameter folder and drag the parameter you created to the **Archive Parameter Folder**. The default predefined analysis parameters indicated above should be the only choice in the drop down menu.

<u>**NOTE**</u>: For Identifiler, if the last two orange size standards, 490 and 500, are not visible, change the size call range to "this range" and adjust the maximum to 450. At least the 100 bp to 450 bp size standards must be apparent.

3. If the baseline of the size standard is noisy, raise the RFU threshold of the red or orange to above the noise level.

a. Alternatively, **redefine the size standard**. In the **Analysis Control** window under the **Size Standard** column click and highlight the size standard of the sample. Click on the small arrow ▶ on the right side of the cell and select **Define New.** The size standard peaks will appear and at the appropriate peak, type the label in the column (see above for correct values).

NOTE: For Identifiler LIZ runs do not define the 250 bp and the 340bp size standards.

b. When you are done defining the new size standard, exit out of the window by hitting X and click **Save.** Save the size standard file in the folder **C:\AppliedBio\Shared\Analysis\Sizecaller\SizeStandards** that can only be accessed through the desktop shortcut **AppliedBio** folder. Name the size standard whatever you wish. Select this size standard for the analysis of all the failed samples.

c. Reanalyze samples. There should be a ♦ on the size standard column

d. After reanalyzing the samples go back to the **SizeStandard** folder and drag the size standard you created to the **Archive SizeStandards Folder**. The default predefined size standards indicated above should be the only choice in the drop down menu.

<u>ATTENTION:</u> all reanalysis results and parameter changes are automatically written to the individual sample files, even if the changes to the project are not saved. Do not reanalyze casework data without a reason.

Revision History:
March 24, 2010 – Initial version of procedure.

For 3130*xl* instruments, multiple sets of amplifications can be run in one tray. If the amplifications were done in different multiplex systems, it is necessary to perform the Genotyper analysis separately using the appropriate template. For two amplifications in the same system it is optional to process them together or separately.

I. **YM1**

A. Importing Data and Allele Call Assignment

1. Open the Genotyper macro for the desired amplification system by clicking on the appropriate Genotyper shortcut on the desktop of the analysis station computer.

2. Under **File→Import** and select **From GeneScan File**. If the Current Runs folder does not already appear in the window, scroll to find it from the pull-down menu and double-click on it. Double-click on the folder containing the project that was created in GeneScan.

3. Click **Add** or double-click on the project icon to add the project for analysis. When the project has been added, click **Finish**.

4. Under **View→Show Dye/Lanes window** a list of the samples imported from GeneScan analysis can be seen. If samples need to be removed, highlight the lanes for these samples and select **Cut** from the **Edit** menu.

5. Under **File→Save As**, save the Genotyper template to the user's initials and the casework run file name. (Under **File** select **Save As)**.

   For example: "Stars09-001Y JLS" for YM1 runs saved by "JLS."

6. After importing the project and saving the Genotyper file, run the first Macro by pressing Ctrl+1 or double clicking "**kazam**".

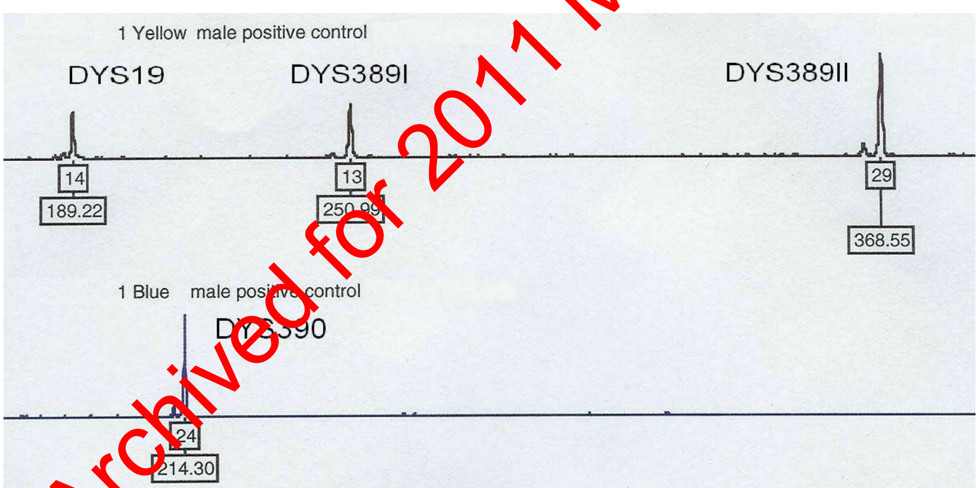
7. The plot window will appear automatically when the macro is completed. Check the results for the positive control. The plots will also display the orange size standard.

Table 1

Multiplex System	Necessary LIZ GS500 standard peaks
YM1	9 fragments from 139-400 bp

Table 2 YM1 Positive Control

	DYS19	DYS389 I	DYS389 II
Yellow label	14	13	29
	DYS390		
Blue label	24		



8. Fill out an STR 3130*xl* Control Review Worksheet indicating the status of all controls.

9. Under Analysis→Change labels, select size in bp, peak height and category name. Click Ok.

10. Check all lanes. Labels for extra peaks can be manually deleted by placing the cursor on the peak above the baseline and clicking.

    Shortcut: If a label was mistakenly deleted, press **Ctrl+Z** and the allele name label will reappear. **Ctrl**+Z will only undo the last action.

11. To zoom into a desired region of an electropherogram, hold the left mouse click down and draw a box around the desired region.

12. Under **View→Zoom→Zoom In (selected area)**.

    Shortcut: Zoom in by holding down the left mouse click button and dragging the cursor across the area to zoom in on. Then, press **Ctrl+R** or Ctrl+ + to zoom in on that region.

13. To revert to the correct scan range, go to **View→Zoom →Zoom To**. Set the plot range to **range listed in Table 3.** Click **OK**.

    Table 3

System	Range
YM1	120 - 410

    Compare the orange electropherograms with the other color lanes by:

    a. holding down the shift key and clicking on the orange "O" box in the upper left hand corner

    b. under **edit** go to **select** +orange

14. Fill out the Genotyper Editing Sheet for each Electrophoresis run indicating the following:

    a. no editing required

b. sample(s) requiring manual removal of non allelic peaks. Refer to STR Results Interpretation Section.

c. sample(s) requiring rerun and/or re-injection. Refer to STR Results Interpretation Section

Each sample listed on the Genotyper Editing Sheet must be indicated by sample number. The reason for the edit must be indicated using a number code and/or symbol.

15. After the editing has been finished, scroll through the plot window to double-check.

B. Genotyper Table

1. Press **Ctrl+2 to create** table

2. Compare the sample information in the table with the amplification and the 3130*xl* run control sheet. If an error is detected at this point it can be corrected as follows:

a. Open the dye/lane window or “sample info box”

b. Place the cursor in the sample info box and correct the text

c. Under Main Menu→**Analysis,** select **Clear Table to clear table**

d. Select the appropriate colors by shift clicking on the dye buttons or using edit.

e. Run **Create Table Macro** again

f. Continue to Step 4 and print according to the directions.

C. Viewing and Printing Electropherograms

1. Controls

a. Under View→Dye Lane Window and select **blue and yellow** for all lanes containing controls including microcon controls

b. To select multiple labels, press **Ctrl** while clicking on the lanes

c. Go to **View** and open the **Plot Window**

d. Under **Analysis→Change Labels and** select s**ize in bp** and c**ategory name**.

Click **ok**. Save.

e. Continue to Step 4 and print the controls according to the directions.

2. Evidence Samples

a. Under **View→Dye Lane** Window and select **blue and yellow** for all lanes containing casework samples

b. To select multiple labels, press **Ctrl** while clicking on the lanes

c. Go to **View** and open the **Plot Window**

d. Under **Analysis→Change Labels and** select **size in bp, peak height** and c**ategory name**. Click **ok**. Save.

e. Continue to Step 4 and print according to the directions.

3. Exemplar Samples

a. Under View→Dye Lane Window and select **blue and yellow** for all lanes containing casework samples

b. To select multiple labels, press **Ctrl** while clicking on the lanes

c. Go to **View** and open the **Plot Window**

d. Under **Analysis→Change Labels and** select s**ize in bp** and c**ategory name**. Click **ok**. Save.

f. Continue to Step 4 and print according to the directions.

2. Printing Electropherograms

a. Make sure the file is named properly, including initials.

b. Set Plot window zoom range as shown in Table 4. The active window will be printed so open Table or Plot as needed.

c. Under File→ Print→ Properties button→ Finishing tab→ Document, set the parameters below.

Table 4 YM1 Print parameters:

	Table	**Plot**
Orientation	Portrait	Portrait
Scale	100% 2 per page	100% 2 per page
Zoom range	n/a	120 - 410

NOTE: The Genotyper printout for YM1 should have a standard format: yellow lanes, then blue lanes. The table should have 2 columns for each locus. The controls are not needed in the table.

d. Click OK, OK.

e. After the printing is finished, under **file**, **quit** Genotyper. Click **save**. Make sure that the Genotyper file is saved in the appropriate **Common runs folder**.

f. Initial all Genotyper pages.

g. List rerun samples on the rerun sheet

h. Place rerun samples into the designated rerun crybox.

i. Have a supervisor review the analyzed run

j. For **Troubleshooting** see the last Section V- Multiplex Kit Toubleshooting**.**

II. Identifiler, 28 Cycles for High Copy Number

A. Importing data and allele call assignment

1. Open the Identifiler 28 macro by clicking on the Genotyper shortcut on the desktop of the analysis station computer.

2. Under **File→Import** and select **From GeneScan File**. If the Current Runs folder does not already appear in the window, scroll to find it from the pull-down menu and double-click on it. Double-click on the folder containing the project that was created in GeneScan.

3. Click **Add** or double-click on the project icon to add the project for analysis. When the project has been added, click **Finish**.

4. Under **View→Show Dye/Lanes window,** a list of the samples imported from GeneScan analysis can be seen. If samples need to be removed, highlight the lanes for these samples and select **Cut** from the **Edit** menu.

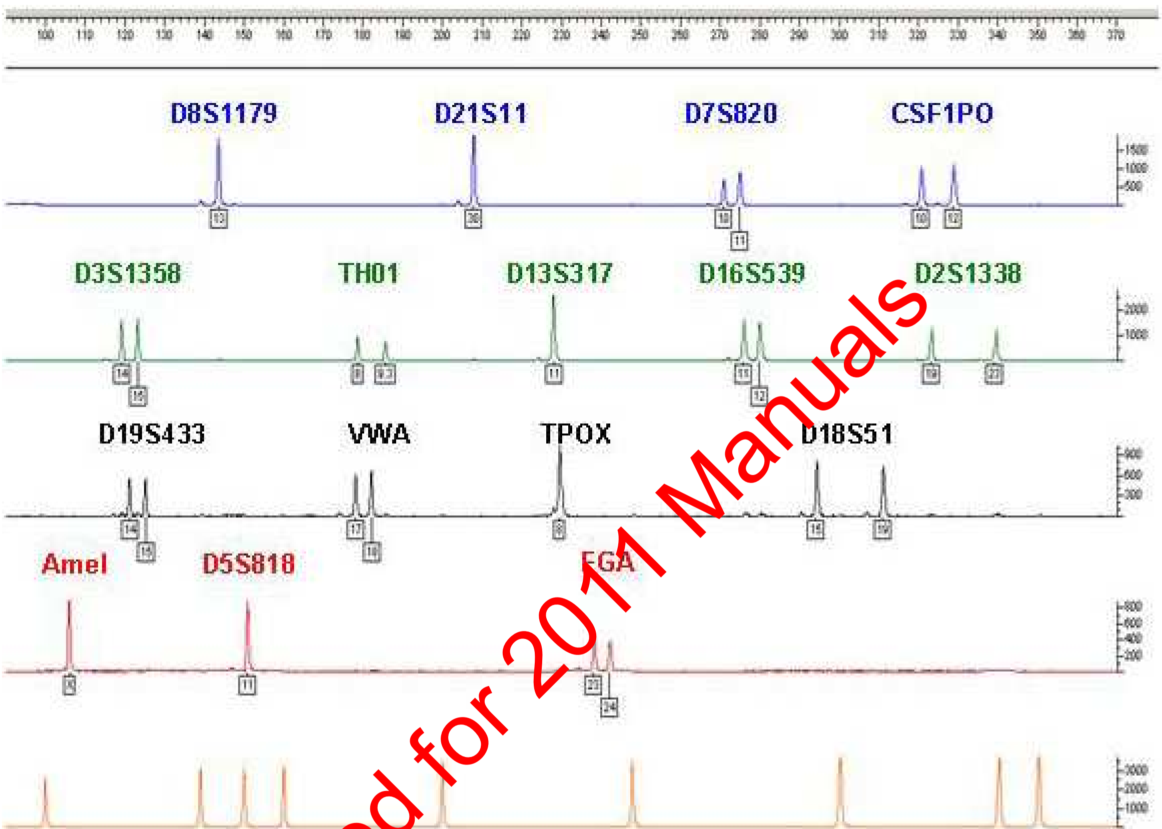
5. After importing the project and saving the Genotyper file, run the first Macro by pressing **Crtl+9**, or double click the **ID 28: Identifiler 28** macro

6. Under **File→Save As,** save the Genotyper template as the casework run file and initials. For example: “Kastle09-108ID JLS”

7. The plot window will appear automatically when the macro is completed. Check to make sure that the ladders match the allele sequence shown below. Also check the results for the positive control. The plots will display the orange size standard.

TABLE 5 IDENTIFILER™ POSITIVE CONTROL

	D8S1179	**D21S11**	**D7S820**	**CSF1PO**	
Blue (6-FAM)	13	30	10, 11	10, 12	
	D3S1358	**TH01**	**D13S317**	**D16S539**	**D2S1338**
Green (VIC)	14, 15	8, 9.3	11	11, 12	19, 23
	D19S433	**VWA**	**TPOX**	**D18S51**	
Yellow (NED)	14, 15	17, 18	8	15, 19	
	AMEL	**D5S818**	**FGA**		
Red (PET)	X	11	23, 24		



D8S1179
D21S11
D7S820
CSF1PO
D3S1358
TH01
D13S317
D16S539
D2S1338
D19S433
VWA
TPOX
D18S51
Amel
D5S818
FGA

IDENTIFILER™ ALLELIC LADDER

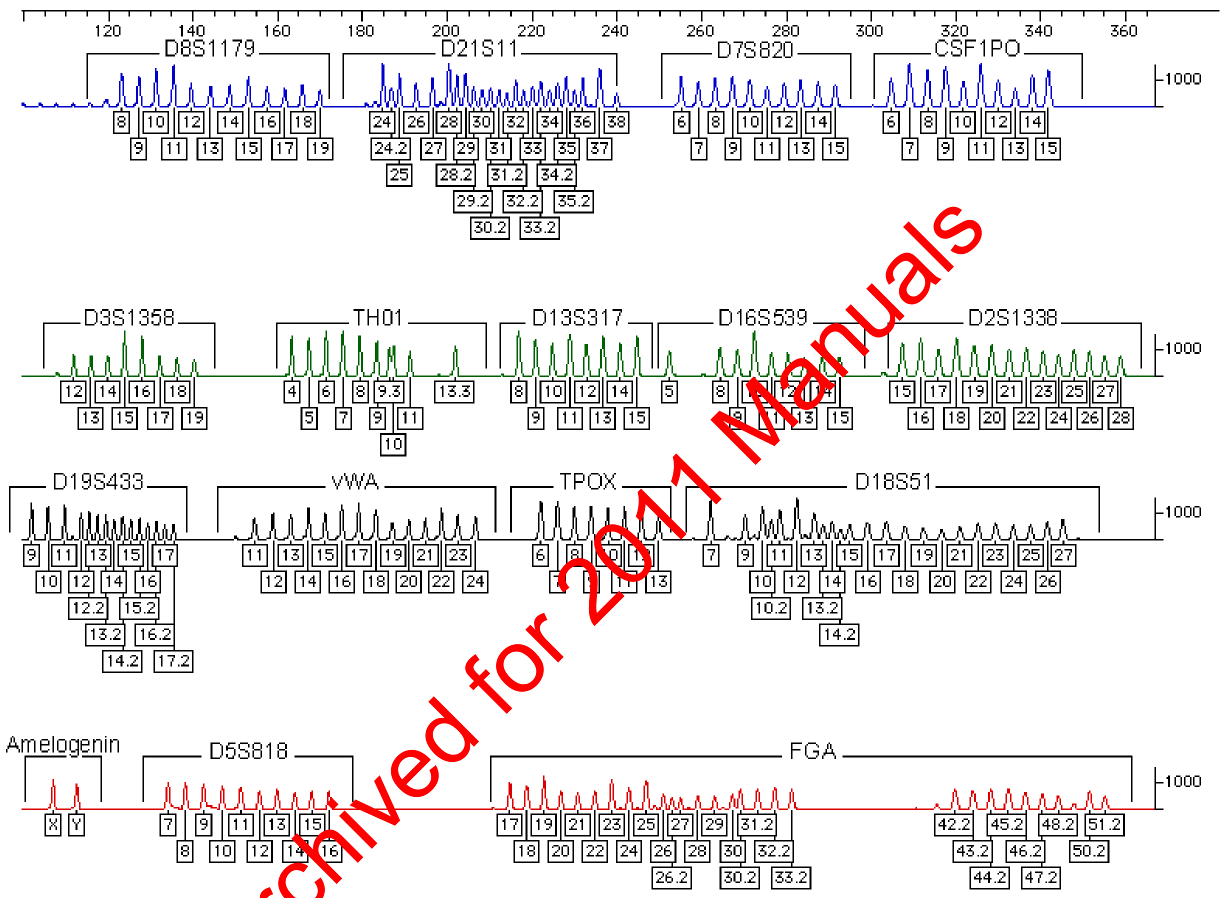


Table 6

Multiplex System	Necessary LIZ GS500 standard peaks
Identifiler™	12 fragments from 75 - 450 bp

8. Fill out an STR 3130*xl* Control Review Worksheet indicating the status of all controls.

9. Under Analysis→Change labels, select size in bp, peak height and category name. Click Ok.

10. Check all lanes. Labels for extra peaks can be manually deleted by placing the cursor on the peak above the baseline and clicking.

    Shortcut: If a label is mistakenly deleted, press **Ctrl+Z** and the allele name label will reappear. Ctrl+Z will only undo the last action.

11. To zoom into a desired region of an electropherogram, hold the left mouse click down draw a box around the desired region.

12. Under **View→Zoom**, select **Zoom In (selected area)**.

    Shortcut: Zoom in by holding down the left mouse click button and dragging the cursor across the area to zoom in on. Then, press **Ctrl+R or Ctrl + +** to zoom in on that region. To zoom out in a stepwise fashion, press **Ctrl+ -**.

13. To revert to the correct scan range, go to **View→Zoom →Zoom To**. Set the plot range to **range listed in Table 7.** Click **OK**.

Table 7

System	**Range**
Identifiler	90- 370

Compare the orange electropherograms with the other color lanes by either:

a. holding down the shift key and clicking on the orange "O" box in the upper left hand corner

b. under **edit** go to **select** +orange

14. Fill out the Genotyper Editing Sheet for each Electrophoresis run to indicate the following:

    a. no editing required

    b. sample(s) requiring manual removal of non allelic peaks. Refer to STR Results Interpretation Section.

    c. sample(s) requiring rerun and/or re-injection. Refer to STR Results Interpretation Section.

    Each sample listed on the Genotyper Editing Sheet must be indicated by sample number. The reason for the edit must be indicated using a number code and/or symbol.

15. After the editing has been finished, scroll through the plot window to double-check.

B. **Viewing and Printing Electropherograms**

1. Controls

    a. Under View→Dye Lane Window and select **blue, green, yellow, red and orange** for all lanes containing the allelic ladder.

    b. To select multiple labels, press **Ctrl** while clicking on the lanes

    c. Go to **View** and open the **Plot Window**

    d. Under **Analysis→Change Labels and** select s**ize in bp** and c**ategory name**.

    Click **ok**. Save.

    e. Repeat steps 1a - c for all lanes containing controls including microcon controls

f. To select multiple labels, press **Ctrl** while clicking on the lanes

g. Go to **View** and open the **Plot Window**

h. Under **Analysis→Change Labels and** select s**ize in bp, peak height** and c**ategory name**. Click **ok**. Save.

g. Continue to Step 3 and print the controls according to the directions.

2. Evidence and Exemplar Samples

a. Under View→Dye Lane Window and select **blue, green, yellow, red and orange** for all lanes containing casework samples

b. To select multiple labels, press **Ctrl** while clicking on the lanes

c. Go to **View** and open the **Plot Window**

d. Under **Analysis→Change Labels and** select s**ize in bp, peak height** and c**ategory name.** Click **ok**. Save.

e. Continue to Step 3 and print according to the directions.

3. Printing Electropherograms

a. Make sure the file is named properly, including initials.

b. Set Plot window zoom range as shown in Table 8. The active window will be printed so open Table or Plot as needed.

c. Under File→ Print → Properties button→ Finishing tab→ Document, set the parameters below.

Table 8 Identifiler Print parameters:

	Plot
Orientation	Portrait
Scale	100% 2 per page
Zoom range	90 - 370

d. Click OK, OK.

e. After the printing is finished, ensure that all alleles in the ladder, controls and samples are labeled. Manually enter the base pair size if necessary and initial and date.

f. Under **file**, **quit** Genotyper. Click **save**. Make sure the Genotyper file is saved in the appropriate **Common runs folder**.

g. Initial all Genotyper pages.

h. List rerun samples on the rerun sheet

i. Place rerun samples into the designated rerun crybox

j. Have a supervisor review the analyzed run

k. For **Troubleshooting** see Section V- Multiplex Kit Troubleshooting**.**

C**.** Genotyper Tables for Identifiler 28 samples

1. Genotyper Table

a. Select all relevant samples in the main window

b. Under Analysis→Clear table

c. Under Analysis→Change Labels select category name

d. Under Table→Set up table→Labels →Options

e. Set the number of peaks per category to “6”. Next to “Text if >N”, click on “Options”. Set the number of peaks to “6” and the text to “Overflow”

f. Click OK. Under Table→Append to table. Save.

g. Click on the table window panel view.

h. Under Edit→Select All, Copy.

2. Identifiler 28 Profile Generation

a. Go to M:\FBIOLOGY_MAIN\FORMS\STRS\ID 28 Profile Generation Table and paste into the Instructions tab. .

b. Refer to the specific instructions on the first tab of that workbook for creation of the profile table.

c. Save ID 28 Profile Generation table as casework run name and initials. Print and store with the electropherogram.

3. The table must be saved in the appropriate folder containing the raw data and the GeneScan project.

4. Have a supervisor review the analyzed run.

5. For **Troubleshooting** see Section V- Multiplex Kit Troubleshooting

III. Identifiler – High Sensitivity Testing

A. Importing data and allele call assignment

1. Open the HS Identifiler 10% Macro by clicking on the Genotyper shortcut on the desktop of the analysis station computer.

2. Under **File→Import** and select **From GeneScan File**. If the Current Runs folder does not already appear in the window, scroll to find it from the pull-down menu and double-click on it. Double-click on the folder containing the project that was created in GeneScan.

3. Click **Add** or double-click on the project icon to add the project for analysis. When the project has been added, click **Finish**.

4. Under **View→Show Dye/Lanes window,** a list of the samples that were imported from GeneScan analysis can be seen. If samples need to be removed, highlight the lanes for these samples and select **Cut** from the **Edit** menu.

5. After importing the project and saving the Genotyper file, run the first Macro by pressing **Crtl+9**, or double click the following according to the macro:

   a. ID 28: Identifiler 28

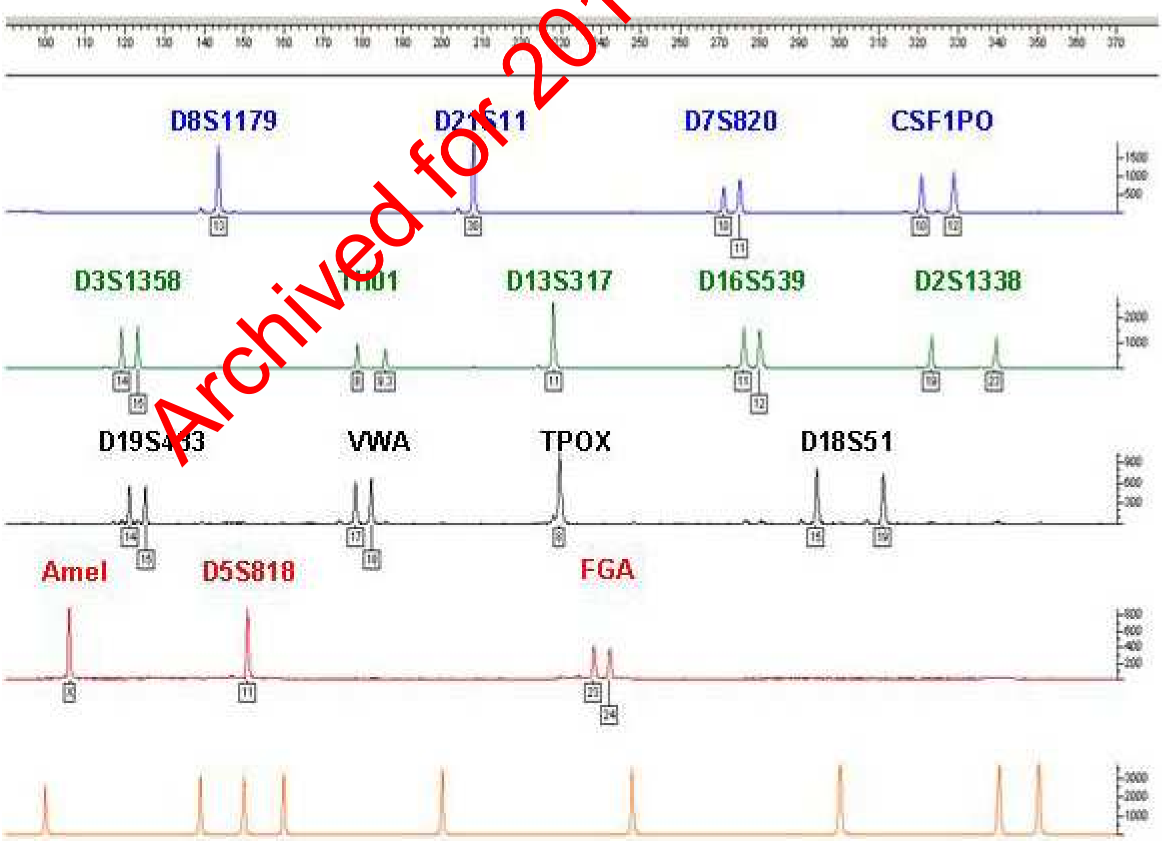
   b. ID 31: HS Identifiler 10%.

6. Under **File→Save As**, save the Genotyper template as the plate record, the run folder and injection parameter. For example: Venus042507_25L.

7. The plot window will appear automatically when the macro is completed. Check to make sure that the ladders match the allele sequence shown below. Also check the results for the positive control. The plots will also display the orange size standard.

TABLE 9 IDENTIFILER™ POSITIVE CONTROL

	D8S1179	**D21S11**	**D7S820**	**CSF1PO**	
Blue (6-FAM)	13	30	10, 11	10, 12	
	D3S1358	**TH01**	**D13S317**	**D16S539**	**D2S1338**
Green (VIC)	14, 15	8, 9.3	11	11, 12	19, 23
	D19S433	**VWA**	**TPOX**	**D18S51**	
Yellow (NED)	14, 15	17, 18	8	15, 19	
	AMEL	**D5S818**	**FGA**		
Red (PET)	X	11	23, 24		



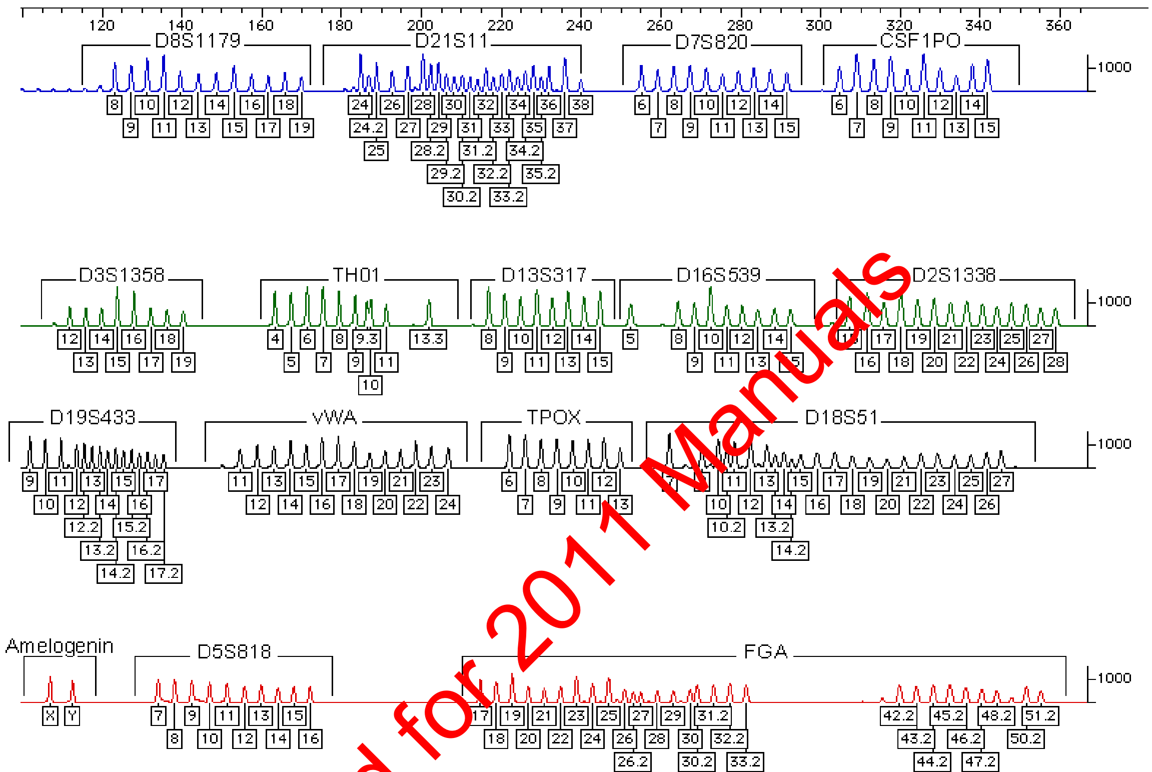


Table 10

Multiplex System	Necessary LIZ GS500 standard peaks
Identifiler™	12 fragments from 75 - 450 bp

8. Fill out an STR 3130*xl* Control Review Worksheet indicating the status of all controls.

9. Under **Analysis→Change** labels, select size in bp, peak height and category name. Click Ok.

10. Check all lanes. Labels for extra peaks can be manually deleted by placing the cursor on the peak above the baseline and clicking.

    Shortcut: If a label was mistakenly deleted, press **Ctrl+Z** and the allele name label will reappear. Ctrl+Z will only undo the last action.

11. For samples that need to be viewed in triplicate by color (31 cycles only) under **Views→Dye Lane Sorting**, the first precedence should be set to Dye Color and the second to File Name, both in ascending order.

12. To zoom into a desired region of an electropherogram, hold the left mouse click down draw a box around the desired region.

13. Under **View→Zoom**, select **Zoom In (selected area)**.

    Shortcut: Zoom in by holding down the left mouse click button and dragging the cursor across the area to zoom in on. Then, press **Ctrl+R or Ctrl + +** to zoom in on that region. To zoom out in a stepwise fashion, press **Ctrl+ -**.

14. To revert to the correct scan range, go to **View→Zoom →Zoom To**. Set the plot range to **range listed in Table 11.** Click **OK**.

Table 11

System	Range
Identifiler	90- 370

Compare the orange electropherograms with the other color lanes by either:

a. holding down the shift key and clicking on the orange "O" box in the upper left hand corner

b. under **edit** go to **select** +orange

15. Fill out the Genotyper Editing Sheet for each Electrophoresis run to indicate the following:

    a. no editing required

    b. sample(s) requiring manual removal of non allelic peaks. Refer to STR Results Interpretation Section.

    c. sample(s) requiring rerun and/or re-injection. Refer to STR Results Interpretation Section .

    Each sample listed on the Genotyper Editing Sheet must be indicated by sample number. The reason for the edit must be indicated using a number code and/or symbol.

16. After the editing has been finished, scroll through the plot window to double-check.

B. Viewing and Printing Electropherograms

1. Controls

    a. Under View→Dye Lane Window and select **blue, green, yellow, red and orange** for all lanes containing the allelic ladder.

    b. To select multiple labels, press **Ctrl** while clicking on the lanes

    c. Go to **View** and open the **Plot Window**

d. Under **Analysis→Change Labels and** select s**ize in bp** and c**ategory name**. Click **ok**. Save.

e. Repeat steps 1a - c for all lanes containing controls including microcon controls

f. To select multiple labels, press **Ctrl** while clicking on the lanes

g. Go to **View** and open the **Plot Window**

h. Under **Analysis→Change Labels and** select s**ize in bp, peak height** and c**ategory name**. Click **ok**. Save.

g. Continue to Step 3 and print the controls according to the directions.

2. Evidence and Exemplar Samples

a. Under View→Dye/Lane Window and select **blue, green, yellow, red and orange** for all lanes containing casework samples

b. To select multiple labels, press **Ctrl** while clicking on the lanes

c. Go to **View** and open the **Plot Window**

d. Under **Analysis→Change Labels and** select s**ize in bp, peak height** and c**ategory name.** Click **ok**. Save.

e. To print the electropherograms for 31 cycle samples, select each sample (triplicates (a, b, c) and pooled (abc)) and sort by Dye Color, then File Name. Each sample will have to be printed separately. Follow steps 2a - d.

f. Continue to Step 3 and print according to the directions.

3. Printing Electropherograms

   a. Make sure the file is named properly, including initials.

   b. Set Plot window zoom range as shown below. The active window will be printed so open Table or Plot as needed.

	Plot
Orientation	Portrait
Scale	100% 2 per page
Zoom range	90 - 370

   c. Under File→ Print → Properties button→ Finishing tab→ Document, set the parameters above.

   d. Click OK, OK.

   e. After the printing is finished, ensure that all alleles in the ladder, controls and samples are labeled. Manually enter the base pair size if necessary and initial and date.

   f. Under **file**, **quit** Genotyper. Click **save**. Make sure the Genotyper file is saved in the appropriate **Common runs folder**.

   g. Initial all Genotyper pages.

   h. List rerun samples on the rerun sheet

   i. Place rerun samples into the designated rerun crybox

   j. Have a supervisor review the analyzed run

   k. For **Troubleshooting** see Section V- Multiplex Kit Troubleshooting**.**

C. Genotyper Tables

1. Identifiler 28 Profile Generation Table

   a. Select all relevant samples in the main window

   b. Under **Analysis→Clear table**

   c. Under **Analysis→Change Labels** select **category name**

   d. Under **Table→Set up table→Labels→Options**

   e. Set the number of peaks per category to “6”. Next to “Text if >N”, click on “Options”. Set the number of peaks to “6” and the text to “Overflow”

   f. Click OK. Under **Table→Append to table**. Save.

   g. Click on the table window panel view.

   h. Under **Edit→Select All**, Copy.

2. Identifiler 31 Profile Generation Table

   a. Ensure that all relevant samples are selected in the main window

   b. Under **Analysis→Clear table**

   c. Under **Analysis→Change Labels**, ensure only “category name” is selected

   d. Under **Table→Set up table→Labels→Options**

   e. Set the number of peaks per category to “6”. Next to “Text if >N”, click on “Options”. Set the number of peaks to “6” and the text to “Overflow”

   f. **OK→OK→Table→Append** to table

g. **View→Show Table Window**

h. **Edit→Select All→Edit→Copy**

2. Open the Profile Generation spreadsheet macro found in HIGHSENS\TEMPLATES IN USE\ANALYSIS\ID31 Profile Generation Sheet-STR. Click **Don't Update**.

3. Paste into cell A12 of "extra sheet" and delete rows containing the Allelic Ladders.

a. Starting at row 12, ensure that samples are in the following order:

i. Sample info and Loci names
ii. Positive controls
iii. Amp Negatives
iv. Extraction negatives and Microcon negatives (triple amps)
v. Samples begin in row 25 (triple amp plus pooled).
vi. Sample triplicates and pooled samples should be consecutive.

b. Two rows are to be skipped between each sample (three between each control inserted after row 25). Insert or delete rows if necessary.

For example: the first sample is in row 25-28, then rows 29 and 30 are skipped, and the second sample is in rows 31-34, and so on.

c. Alternatively, sample info may be copy and pasted directly into the appropriate rows in the "Copy Geno Triple" sheet of the Excel workbook.

4. Compilation of triple amplifications

a. On the "extra sheet", Edit→select all→copy

b. Paste into cell A1 of the copy geno triple sheet. (The geno db sheet is for double amplifications that would not be utilized for casework.)

5. “NIKE” macros to filter and sort

    a. Macro 4: Select the control and the “n” keys to filter sample sheets 1-14.

    b. Macro 4b: Select the control and the “i” keys to filter sample sheets 15-29.

    c. Profiles macro: Select the control and the “k” keys to sort sample sheets 1-14.

    d. ProfilesB macro: Select the control and the “e” keys to sort sample sheets 15-29.

6. Arrow to the right to the triple chart.

    a. Each amplification replicate is shown in the white rows, and the composite profile containing alleles that repeat in two of the three amplifications is in the row below the 3 amplifications.

    b. The pooled injection is located beneath the composite profile.

    c. Loci with more than 6 alleles will not be accurately reflected. However, the word “overflow” will appear in the cell as a signal to check the alleles on the electropherogram. Additional alleles may be manually entered into the cell.

    d. Print and store table with the electropherogram.

7. The table must be saved in the appropriate folder containing the raw data and the GeneScan project.

8. Have a supervisor review the analyzed run.

9. For **Troubleshooting** see Section V- Multiplex Kit Troubleshooting

IV. Re-injection Guidelines – YM1

A. YM1 Controls

1. Refer to the following procedure sin this manual before making a decision to rerun/re-inject a control:

   a. Genotyper Analysis Section V – Multiplex Kit Troubleshooting

   b. STR Results Interpretion Section V – Interpretation of Controls

2. If a complete injection fails, rerun with the same parameters.

3. Rerun/ re-inject normal if the following applies:

   a. Positive Control fails

   b. Amplification Negative fails

   c. Extraction Negative fails

   d. No size standard

   NOTE: All reruns/ re-injections must be accompanied by a passing positive control.

B. YM1 Samples

1. Rerun normal if the following applies:

   a. No orange size standard

   b. New allele/Off-ladder allele

   c. Overamplified single source samples (rfus >6000) with plateau shaped or misshaped peaks, numerous labeled stutter peaks and artifacts – remove all peaks and rerun with a dilution

d. Overamplified mixed samples (rfus >6000) – remove all peaks and rerun with a dilution

2. Rerun with high parameters if there are peaks below threshold

NOTE: All reruns/ re-injections must be accompanied by a passing positive control.

V. Re-injection Guidelines – Identifiler, 28 Cycles

A. Identifiler 28 Controls

1. Refer to the following sections before making a decision to rerun/ re-inject a control:

a. Genotyper Analysis Section V – Multiplex Kit Troubleshooting

b. STR Results Interpretation Section V – Interpretation of Controls

2. If a complete injection fails, rerun with the same parameters.

3. Rerun/ re-inject normal if the following applies:

a. Positive Control fails

b. Amplification Negative fails

c. Extraction Negative fails

d. No size standard

NOTE: All reruns/ re-injections must be accompanied by a passing positive control.

B. Identifiler 28 Samples

1. Rerun normal if the following applies:

   a. No orange size standard
   b. New allele/ Off ladder allele

   c. Overamplified single source samples (rfus >7000) with plateau shaped or misshaped peaks with numerous labeled stutter peaks and artifacts – remove all peak and run with a dilution

   d. Overamplified mixed samples (rfus >7000) – remove all peaks and run with a dilution or follow steps in section 3 below.

2. Samples may be rerun high on the approved High Sensitivity CEs or samples may be injected high on these instruments initially if appropriate

   a. All relevant controls must be re-injected at the high parameter

   b. For mixed samples at these parameters, overblown peaks (>7000 RFUs) as well as peaks from loci within the same basepair range in the other colors should be removed and deemed inconclusive. However, data from the other loci should be retained. Data from both injections may be used for interpretation. For consistency, confirm that the injections at different parameters generate overlapping loci.

V. Re-injection Guidelines – Identifiler, 31 Cycles

A. Identifiler 31 Controls

1. Refer to the following sections before making a decision to rerun/ re-inject a control:

   a. Genotyper Analysis Section V – Multiplex Kit Troubleshooting

   b. STR Results Interpretation Section V – Interpretation of Controls

2. If a complete injection fails, rerun with the same parameters.

3. Rerun/ re-inject normal if the following applies:

   a. Positive Control fails

   b. Amplification Negative fails

   c. Extraction Negative fails

   d. No size standard

   NOTE: For reruns that are lower than the original injection, only a positive control must be re-injected.

B. Identifiler 31 Samples

1. Rerun at the same injection parameters if the following applies:

   a. No orange size standard

   b. New allele/Off ladder allele

2. Samples may be rerun with higher parameters if peaks are below threshold Samples may be initially injected at a high parameter if appropriate

   NOTE: All controls must be re-injected for all rerun conditions that are at a higher parameter

3. Rerun at a lower injection parameter and/or with a dilution if the following applies

   a. Overamplified single source samples (rfus >7000) with plateau shaped or misshaped peaks with numerous labeled stutter peaks and artifacts
   b. Overamplified mixed samples (rfus >7000)

4. For Mixed samples run at more than one injection parameter or concentration
   a. Remove overblown peaks (>7000 RFUs) as well as peaks from loci within the same basepair range in the other colors and deem these loci inconclusive.
   b. Retain data from the other loci.
   c. Data from both injections may be used for interpretation. For consistency, confirm that the injections at different parameters generate overlapping loci.

VI. Troubleshooting

A. Genotyper Macro 1 produces an error message that reads: **"Could not complete your request because no dye/lanes are selected"**.

1. Make sure the ladder was imported from the project.

   Solution: If the ladder was not imported into the project, import the ladder and rerun the macro.

2. Check the spelling of "ladder" and the sample information in the **dye/lanes window**.

   Solution: Spell correctly and/or correct sample information. Then, rerun the macro.

B. Genotyper Macro 1 produces an error message that reads: **"Could not complete your request because the labeled peak could not be found".**

This message indicates that the ladder cannot be matched to the defined categories. There are three possibilities:

1. There may be peaks in the ladder that are too low to be recognized by the program.

   Solution: Two options:

   a. **One**: If another ladder in the run is more intense, alter or delete the name of the first ladder in the Genotyper Dye/Lane window. Then, rerun **Macro 1**. Now the macro will use the first backup ladder for the off-set calculation.

   b. **Two**: The **minimum peak height** can be lowered for the off-set in the categories window by:

      i. Under View→**Show Categories Window**. In the "offset" categories the first allele is defined with a scaled peak height of 200 or higher. The high value is meant to eliminate stutter and background.

      ii. Change this to 75 for the 3130*xl* by clicking on the first category that it highlights.

      In the dialogue box locate the **Minimum Peak Height** and change it to the appropriate value.

      Click **Add,** and then click **Replace** when given the option. This must be done for each locus. Do not use values less than the instrument threshold.

      DO NOT CHANGE THE MINIMUM PEAK HEIGHT FOR ANY OTHER CATEGORY EXCEPT THE OFF-SET.

   After the macro is rerun, make sure the ladder begins with the correct allele and that the first allele is not assigned to a stutter which might precede the first peak.

2. The first ladder peak of each locus is outside of the pre-defined size range window.

   Solution: Expand the search window in the categories window by:

   i. Under **View→Show Categories Window.** In the "offset" categories the first allele is defined with a certain size +/- 7bp.

   ii. Change the 7 to 10 or higher, by clicking on the first category which highlights it.

   iii. In the dialogue box locate, the **+/- box** and change the value

   iv. Click **Add,** and then click **Replace** when given the option.

   v. This can be done for each locus that gave the error message.

3. There are no peaks at all in any of the allelic ladders.

   Solution: Rerun all samples with freshly prepared Allelic Ladders.

C. Off Ladder (OL) allele labels

1. A run with a large number of samples may have a high incidence of OL allele labels toward the end of the run. This is due to a shift during the run.

   Solution: Try to reanalyze the run by using the second allelic ladder as the off-set reference by:

   i. removing the word "ladder" from the name of the first ladder in the dye lane window.

   ii. This ladder will not be recognized by the macro program

   iii. Rerun **Macro 1** and evaluate the results

iv. Determine which one of both allelic ladders causes fewer "OL allele?" labels.

v. Complete the Genotyping process using this ladder. Any remaining samples displaying OL alleles have to be rerun.

2. If all or most of the samples have "OL allele?" labels, it may be that the samples were automatically analyzed with an ill-defined size standard.

Solution: Redefine the size standard (see GeneScan analysis for 3130*xl*). Reanalyze the run

D. Incorrect positive control type

The Genotyper has shifted allele positions during the category assignment to the ladder.

Ensure that a sample mix-up did not occur.

Check the ladder and make sure the first assigned allele is assigned to the first real peak and not to a stutter peak, which may precede it. If the stutter peak is designated with the first allele name, the peak height must be raised in the categories window in order to force the software to skip the stutter peak and start with proper allele.

1. Determine the height of the stutter peak by placing the cursor on the peak in question (as if editing).

2. The information displayed on the top of the window refers to the peak where the cursor is located and contains the peak height. Make a note of the peak height.

3. Under **View→Show Categories Window** and highlight the first allele in the offset category (e.g., 18 o.s.) of the polymorphism that needs to be corrected.

4. In the dialogue box change the height for the minimum peak height to a few points above the determined height of the stutter.

5. Rerun the macro and then check to make sure everything is correct by looking at the first allele in each locus in the ladder and by comparing the result for the positive control.

E. Lining up unlabeled peaks

1. In order to place samples next to each other for comparison purposes, mark them by double clicking.

2. A black bullet appears in front of the lane number.

3. If this happens accidentally, a lane can be unmarked by either double clicking on it again or, under **Edit→unmark**.

4. To be able to align an unlabeled allele with a labeled allele in the same run, you must select **View→ View by Scan**.

NOTE: Unsized peaks cannot be placed according to size on the electropherogram. Therefore, when comparing an unlabeled allele (unlabeled because it is too low to be sized, but high enough to be detected visually) to a labeled allele (e.g., in the ladder) you cannot determine the allele type and size by visual comparison while the results are viewed by size.

F. Too many samples

If you see the same sample listed several times in the dye/lanes window or you see more samples than you have imported, you have most likely imported your samples more than once or you have imported your samples into a Genotyper template that already contained other samples.

1. Under **Analysis→Clear Dye/Lanes** window.

2. Under **Analysis→Clear Table**.

3. Re-import your file(s).

G. Typographical error in the sample information and/or sample comment

If you detect a mistake in the sample information, this can be corrected for the Genotyper file by:

1. Opening the dye lane list window

2. Highlighting the lane

3. Retyping the sample information for all colors

NOTE: The short sample name cannot be changed here. It can only be changed on the sample sheet level.

H. Less samples in Table than in Plots

Samples with the same sample information are only listed once in the Table. Add modifier to the sample information (see above) of one of the samples and rerun Macro.

I. Too many background peaks labeled

If peaks are still labeled in the plot even though they are listed as having been removed or they appear to be below the stutter filter threshold, the following mistake could have happened:

1. Instead Analysis→**Change labels;** the analyst clicked Analysis→**Label peaks**

2. The **Change labels** command labels the valid peaks with the allele name and the size in basepairs prior to printing the plot.

3. The **Label peaks** command labels all peaks above threshold independent of any Macro stutter and background filters.

4. This **Label peaks** command will also re-label peaks that were edited out.

<u>Solution</u>: Rerun the macro, repeat the documented editing steps and reprint the Table and the Plot.

Revision History:
March 24, 2010 – Initial version of procedure.

I. General Information for Amplification

The PowerPlex® Y Amplification System from Promega targets eleven (11) locations on the Y chromosome. The system includes loci with tri-nucleotide, tetra-nucleotide and penta-nucleotide repeats.

LOCUS	REPEAT
DYS391	tetra-nucleotide
DYS389I	tetra-nucleotide
DYS439	tetra-nucleotide
DYS389II	tetra-nucleotide
DYS438	penta-nucleotide
DYS437	tetra-nucleotide
DYS19	tetra-nucleotide
DYS392	tri-nucleotide
DYS393	tetra-nucleotide
DYS390	tetra-nucleotide
DYS385	tetra-nucleotide

The target DNA concentration for amplification using the PowerPlex Y system is 500 pg. The minimum DNA concentration required for amplification in this system is 100 pg (minimum quantitiation value of 5 pg/ul). If a sample is found to contain less than 5.0 pg/µL of DNA, then the sample should not be amplified in PowerPlex® Y. It can be re-extracted, reported as containing insufficient DNA, concentrated using a Microcon-100 or possibly submitted for High Sensitivity testing. (see Table 1)

TABLE 1: For PowerPlex Y

Minimum Desired Template	100.00 pg
Template volume for amp	20 µL
Minimum Sample Concentration in 200 µL	5.0 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning* to 50 µL	1.25 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning** to 20 µL	0.50 pg/ul

* Sample concentration **prior** to processing with a Microcon 100 and elution to 50 µL
** Sample concentration **prior** to processing with a Microcon 100 and elution to 20 µL

Since PowerPlex® Y samples often require further testing in Identifiler, the extraction negative must also have a quantitation value of < 0.2 pg/ul. Thus, if the extraction negative is > 0.2 pg/µL it should be re-quantitated. If it fails again, the sample set must be re-extracted prior to amplification. (see Table 2)

TABLE 2:

Amplification System	Sensitivity of Amplification	Extraction Negative Control Threshold
PowerPlex® Y	5 pg	0.20 pg/µL in 20 µL

II. Generation of Amplification Sheets

Amp sheets are generated by supervisors following review of quantification results. Furthermore, samples may be submitted for amplification through aliquot request sheets. Excel macros may be employed to generate of these sheets. Different sheets may be used as described below depending upon the throughput of each team.

A. HSC Team Amp Macro for paperwork preparation from RotorGene values for amplification of evidence samples with PowerPlex Y

1. Open the "RGAMP Macro HSC" and the "RG summary sheet" Excel files for samples ready to be amplified. The "RG summary sheet" is saved as the assay name.

2. Copy the sample information (without the standards or calibrators) from the "summary sheet" of the "RG summary sheet" file including the tube label, sample name, Ct value, the calculated concentration, the target date, and the IA, and paste special as values into the corresponding columns of the "RG value" sheet of the "RGAMP Macro HSC" file.

3. In the last column, entitled "Type", enter "Y" for PowerPlex Y Evidence next to the samples to be amplified. Selecting sending neat samples versus diluted samples can be done here.

4. Check the sample names to ensure commas are not located in the wrong areas. There can only be one comma in the sample name. The comma should be located after the full sample name and before the dilution value (ie. FB01-1234_vag_SF, 0.1).

5. Hit Ctrl+R or click the "Separate dilutions and sample info" button to run the dilution macro. A window asking "Do you want to replace the contents of the destination cell?" will appear. Click "OK".

   The dilution macro will separate the dilution factors from the samples names to facilitate the calculation of the neat concentration of the samples.

6. Hit Ctrl+G or click the "Sort" button to run the sample sorting macro.
   a. The macro will filter and eliminate all values that are less than 5.0 pg/ul for PowerPlex Y.
   b. Inspect the samples sorted in the appropriate columns according to system/type and sample concentration.

7. For PowerPlex® Y samples:

   a. Copy and Paste Special as values all samples to be amplified from the appropriate columns on the "Sort" sheet to the associated columns on the "Samples" sheet.
   b. For samples being sent on for PowerPlex® Y amplification from P30 values, on the "Samples" sheet, change the Calculated Values column to the appropriate letter associated with the P30 value and sample type:

   For Non-Differential semen or differential swab/substrate remain samples:

Orifice swab, P30 value, 2ng subtraction	**Stains P30 value, 0.05 A subtraction**	**Type this letter in the "Calculated Value" column**
Sperm Seen; No P30 ELISA Done		B
1.1 - 3.0	1.1 - 3.0	B
>0 - 1.0	>0 - 1.0	C

   For vaginal swab samples sent for Amylase Positive Extractions, two concentrations must be sent for amplification:

Amounts sent to amplification		**Type this letter in the Calculated Value column**
DNA Target	**TE^{-4}**	
8	12	B
20	0	C

c. For samples being sent on for PowerPlex® Y amplification from Quantification values, the amplification sheet should calculate the appropriate DNA and TE^{-4} target amount on the amplification sheet.

8. Each amplification sheet can hold up to 28 samples. Since there are 54 samples on a full RotorGene run, it is possible that more than one amplification sheet is necessary. If this is the case, the overflow samples will automatically be transferred into a second amplification sheet (i.e. “PowerPlex® Y 2”).

9. When all samples to be amplified have been organized on the “Samples” sheet, click on the appropriate amplification sheet(s) and check all entries for errors.

   All changes, except for the amount of extract submitted during low and high sample submission, should be made in the “Samples” sheet.

10. Save the entire macro workbook in the appropriate folder.

B. MACRO X for Paperwork Preparation for Amplification with PowerPlex Y

1. Open the “RGAMP MACRO X” and the “Aliquot Request Form for PPY” Excel files for samples ready to be amped.

2. Copy all of the information from the “Aliquot Request Form for PPY” and paste as values into the “RGAMP MACRO X” under the “Paste PPY” worksheet.

3. Click on the “PPY” tab to see the Amp worksheet and check all entries for errors

   All changes, except for the amount of extract submitted during low and high sample submission, should be made in the “Paste PPY” sheet.

4. Save the entire macro workbook in the appropriate folder.

C. Saving Amplification Sheets on the Network for Additional Samples

1. Partially full or completed amplification sheets may be saved as independent sheets for subsequent sample additions by clicking the "Samples" and amp sheet tab (via holding the ctrl button down). Both sheets should now be highlighted white. Right click and select "move or copy".

2. In this window, select "(new book)" in the "to book" window and check "create a copy". Click "OK". Go to File, Save As and save into the appropriate folder with the amplification system followed by "waiting for amp" or "ready to amp".

3. Samples may be manually added to these sheets by individual analysts or copied and Paste Special from re-quantification sheets or consolidated from additional amplification sheets of the same type at the end of each RotorGene run.

4. If any samples need to be submitted to amplification with a DNA amount other than the optimal amount, the analyst can change the amount of DNA submitted by changing the value in the DNA column in the amplification sheet.

   Be aware that once the DNA amount is manually added to the amplification sheet, the sheet will not be able to calculate the value from the quantification value.

   All other changes should be done in the "Samples" sheet.

5. When a macro amplification sheet is full the analyst may then fill in the amplification date and time in the appropriate blue cell in the "Samples" sheet. This should automatically populate the appropriate cells in the Amplification sheet.

   Any changes to the amplification sheet should be done in the "Samples" sheet.

6. Save the sheet as the time and date of the amplification as follows: "PPY090909.1100" for PowerPlex Y amplifications, performed on September 9, 2009 at 11:00am. These completed amplification sheets should be saved in the "Amp Sheets", "Amp Sheet Archive" folder.

7. A supervisor should review all entries were entered correctly before printing the Amplification sheet.

III. PCR Amplification – Sample Preparation

A. Samples amplified with PowerPlex Y reagents should be prepared with TE^{-4}.

Prepare dilutions for each sample, if necessary, according to Table 3.

TABLE 3: Dilutions

Dilution	Amount of DNA Template (uL)	Amount of TE^{-4} (uL)
0.25	3 or (2)	9 or (6)
0.2	2	8
0.1	2	18
0.05	2.5	47.5
0.04	4 or (2)	96 or (48)
0.02	2 or (1)	98 or (49)
0.01	2	198
0.008	4 or (2)	496 or (248)

The target DNA template amount for PowerPlex® Y is 500 pg.

To calculate the amount of template DNA and diluent to add, the following formulas are used:

$$\text{Amt of DNA } (\mu L) = \frac{\text{Target Amount (pg)}}{(\text{Sample concentration, pg}/\mu L)(\text{Dilution factor})}$$

The amount of diluant to add to the reaction = 20 µL – amt of DNA (µL)

The amplification of exemplars, sperm cell fractions of samples extracted by differential lysis and semen stains, where no epithelial cells were seen during the differential lysis, is based on the quantitation results. Semen positive swabs taken from female individuals that were extracted using the non-differential semen extraction and the swab remains fractions of differential lysis samples are amplified using the amounts specified in Table 4. Amylase positive samples should be amplified based on Table 5.

Table 4: Amount of DNA extract from a non-differential semen extraction or from the swab/substrate remains fraction of a differential lysis sample to be amplified in PowerPlex® Y.

P30 result for the 2ng subtraction (Body cavity swabs)	**P30 result for the 0.05A units subtraction (Stains or penile swabs)**	**DNA Volume (µL) to be amplified**	**TE^{-4} (µL)**
Sperm Seen; Not Sent to P30 ELISA		8	12
≥ 1.1	≥ 1.1	8	12
> 0 - 1.0	> 0 - 1.0	20	0

Table 5: **Amount of DNA extract to be amplified for Amylase positive samples.****

Type of item		**DNA Target Volume (µL)**	**TE^{-4} (µL)**
Orifice swab	Initially try two amounts	8 20	12 0
Dried secretions swab (External)	Based on Quantitation result		
Stain	Based on Quantitation result		

** RotorGene does not reflect male DNA, especially for vaginal swabs. Try more or less if negative.

B. Male Positive Control

1. If using the Promega PowerPlex Y 9948 Male Positive Control that comes with the Kit, make a 1/100 dilution (2 µL Control in 198 µL of TE) of this control. Only 5 µL of this dilution will be added to the amplification tube. The remainder of the solution can be used if another PowerPlex® Y amplification is needed.

2. If using the Forensic Biology in-house Male Positive Control, remove a tube of MPC from the freezer and thaw. Once thawed, 20 µL of the male positive control may be added directly to the amplification tube.

C. Female Negative Control

For the Promega Female Negative Control, make a 1/100 dilution (2 µL Control in 198 µL of TE). Only 5 µL of this dilution will be used. The remainder of the solution can be used if another PowerPlex® Y amplification is needed.

D. Amplification Negative Control

TE^{-4} will serve as an amplification negative control.

E. Master Mix Preparation

1. Retrieve PowerPlex® Y primers, PowerPlex® Y reaction mix and ABI Taq Gold from the freezer and store in a Nalgene cooler on bench. **Record the lot numbers of the reagents.**

2. Vortex or pipet the reagents up and down several times to thoroughly mix the reagents. **Do not vortex Taq Gold** as it may degrade the enzyme.

   After vortexing, centrifuge reagents (**except the primers)** briefly at full speed to ensure that no sample is trapped in the cap. Primers tubes may be tapped on the benchtop or may be centrifuged at 3000 rpm for 3 seconds if necessary.

3. Consult the amplification sheet for the exact amount of PowerPlex® Y primers, reaction mix and ABI Taq Gold to add. The amount of reagents for one amplification reaction is listed in Table 6.

Table 6 - PowerPlex® Y PCR amplification reagents for one sample

Reagent	Per reaction
10X Primer mix	2.5µL
Gold Star 10X Buffer	2.5µL
AmpliTaq Gold DNA Polymerase (5U/µL)	0.55uL
Mastermix total in each sample:	5.55µL
DNA	20µL

F. Reagent and Sample Aliquot

1. Vortex master mix to thoroughly mix. After vortexing, briefly tap or centrifuge the master mix tube to ensure that no reagent is trapped in the cap.

2. Add **5.55 µL** of the PowerPlex® Y master mix to each tube that will be utilized, changing pipette tips and remixing master mix as needed.

   NOTE: Use a new sterile filter pipet tip for each sample addition. Open only one tube at a time for sample addition.

3. Arrange samples in a rack in precisely the positions they appear on the sheet.

4. **Witness step.** Ensure that your samples are properly positioned.

5. Prior to adding sample or control, pipet each sample or control up and down several times to thoroughly mix. The final aqueous volume in the PCR reaction mix tubes will be 25.55µL. After addition of the DNA, cap each sample before proceeding to the next tube.

6. After all samples have been added, take the rack to the amplified DNA area for Thermal Cycling.

IV. Thermal Cycling

A. Turn on the ABI 9700 Thermal Cycler. (See manufacturer's instructions).

B. Choose the following files to amplify in PowerPlex Y:

PowerPlex Y
user: casewk file: powery

PCR Conditions for the Perkin Elmer GeneAmp PCR System 9700

9700 PowerPlex® Y user: casewk file: PowerY	The PowerPlex® Y file is as follows: Soak at 95° for 11 minutes. Soak at 96° for 1 minute. Ramp 100% Denature at 94°C for 30 seconds Ramp 29% Anneal at 60°C for 30 seconds Ramp 23% Extend at 70°C for 45 seconds For 10 cycles then… Ramp 100% Denature at 90°C for 30 seconds Ramp 29% Anneal at 58°C for 30 seconds Ramp 23% Extend at 70°C for 45 seconds For 20 cycles then… 30 minute incubation at 60°C. Storage soak indefinitely at 4°C.

C. 9700 Instructions

1. Place the tubes in the tray in the heat block **(do not add mineral oil)**, slide the heated lid over the tubes, and fasten the lid by pulling the handle forward. Make sure you use a tray that has a 9700 label.

2. Start the run by performing the following steps:

3. The main menu options are RUN CREATE EDIT UTIL USER. To select an option, press the F key (F1…F5) directly under that menu option.

4. Verify that user is set to "casewk." If it is not, select the USER option (F5) to display the "Select User Name" screen.

5. Use the circular arrow pad to highlight "casewk." Select the ACCEPT option (F1).

6. Select the RUN option (F1).

7. Use the circular arrow pad to highlight the desired STR system. Select the START option (F1). The "Select Method Options" screen will appear.

8. Verify that the reaction volume is set to **25**µL For PowerPlex Y and the ramp speed is set to **9600 (very important)**.

9. If all is correct, select the START option (F1).

10. The run will start when the heated cover reaches 103°C. The screen will then display a flow chart of the run conditions. A flashing line indicates the step being performed, hold time is counted down. Cycle number is indicated at the top of the screen, counting up.

11. Upon completion of the amplification, remove samples and press the STOP button repeatedly until the "End of Run" screen is displayed. Select the EXIT option (F5). Wipe any condensation from the heat block with a Kimwipe and pull the lid closed to prevent dust from collecting on the heat block. Turn the instrument off.

    Note: **Place the microtube rack used to set-up the samples for PCR in the container of 10% bleach container in the Post-Amp area in Room 714A.**

Revision History:
March 24, 2010 – Initial version of procedure.

I. General Information for Amplification

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DYS437	tetra-nucleotide
DYS19	tetra-nucleotide
DYS392	tri-nucleotide
DYS393	tetra-nucleotide
DYS390	tetra-nucleotide
DYS385	tetra-nucleotide

The target DNA concentration for amplification using the PowerPlex Y system is 500 pg. The minimum DNA concentration required for amplification in this system is 100 pg (minimum quantitiation value of 5 pg/ul). If a sample is found to contain less than 5.0 pg/µL of DNA, then the sample should not be amplified in PowerPlex® Y. It can be re-extracted, reported as containing insufficient DNA, concentrated using a Microcon-100 or possibly submitted for High Sensitivity testing. (see Table 1)

TABLE 1: For PowerPlex Y

Minimum Desired Template	100.00 pg
Template volume for amp	20 µL
Minimum Sample Concentration in 200 µL	5.0 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning* to 50 µL	1.25 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning** to 20 µL	0.50 pg/ul

* Sample concentration **prior** to processing with a Microcon 100 and elution to 50 µL
** Sample concentration **prior** to processing with a Microcon 100 and elution to 20 µL

Since PowerPlex® Y samples often require further testing in Identifiler, the extraction negative must also have a quantitation value of < 0.2 pg/ul. Thus, if the extraction negative is > 0.2 pg/µL it should be re-quantitated. If it fails again, the sample set must be re-extracted prior to amplification. (see Table 2)

TABLE 2:

Amplification System	Sensitivity of Amplification	Extraction Negative Control Threshold
PowerPlex® Y	5 pg	0.20 pg/µL in 20 µL

II. Generation of Amplification Sheets

Amp sheets are generated by supervisors following review of quantification results. Furthermore, samples may be submitted for amplification through aliquot request sheets. Excel macros may be employed to generate of these sheets. Different sheets may be used as described below depending upon the throughput of each team.

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3. In the last column, entitled "Type", enter "Y" for PowerPlex Y Evidence next to the samples to be amplified. Selecting sending neat samples versus diluted samples can be done here.

4. Check the sample names to ensure commas are not located in the wrong areas. There can only be one comma in the sample name. The comma should be located after the full sample name and before the dilution value (ie. FB01-1234_vag_SF, 0.1).

5. Hit Ctrl+R or click the "Separate dilutions and sample info" button to run the dilution macro. A window asking "Do you want to replace the contents of the destination cell?" will appear. Click "OK".

   The dilution macro will separate the dilution factors from the samples names to facilitate the calculation of the neat concentration of the samples.

6. Hit Ctrl+G or click the “Sort” button to run the sample sorting macro.
   a. The macro will filter and eliminate all values that are less than 5.0 pg/ul for PowerPlex Y.
   b. Inspect the samples sorted in the appropriate columns according to system/type and sample concentration.

7. For PowerPlex® Y samples:

   a. Copy and Paste Special as values all samples to be amplified from the appropriate columns on the “Sort” sheet to the associated columns on the “Samples” sheet.
   b. For samples being sent on for PowerPlex® Y amplification from P30 values, on the “Samples” sheet, change the Calculated Values column to the appropriate letter associated with the P30 value and sample type:

   For Non-Differential semen or differential swab/substrate remain samples:

Orifice swab, P30 value, 2ng subtraction	**Stains P30 value, 0.05 A subtraction**	**Type this letter in the “Calculated Value” column**
Sperm Seen; No P30 ELISA Done		B
1.1 - 3.0	1.1 - 3.0	B
>0 - 1.0	>0 - 1.0	C

   For vaginal swab samples sent for Amylase Positive Extractions, two concentrations must be sent for amplification:

Amounts sent to amplification		**Type this letter in the Calculated Value column**
DNA Target	**TE^{-4}**	
8	12	B
20	0	C

c. For samples being sent on for PowerPlex® Y amplification from Quantification values, the amplification sheet should calculate the appropriate DNA and TE^{-4} target amount on the amplification sheet.

8. Each amplification sheet can hold up to 28 samples. Since there are 54 samples on a full RotorGene run, it is possible that more than one amplification sheet is necessary. If this is the case, the overflow samples will automatically be transferred into a second amplification sheet (i.e. "PowerPlex® Y 2").

9. When all samples to be amplified have been organized on the "Samples" sheet, click on the appropriate amplification sheet(s) and check all entries for errors.

   All changes, except for the amount of extract submitted during low and high sample submission, should be made in the "Samples" sheet.

10. Save the entire macro workbook in the appropriate folder.

B. MACRO X for Paperwork Preparation for Amplification with PowerPlex Y

1. Open the "RGAMP MACRO X" and the "Aliquot Request Form for PPY" Excel files for samples ready to be amped.

2. Copy all of the information from the "Aliquot Request Form for PPY" and paste as values into the "RGAMP MACRO X" under the "Paste PPY" worksheet.

3. Click on the "PPY" tab to see the Amp worksheet and check all entries for errors

   All changes, except for the amount of extract submitted during low and high sample submission, should be made in the "Paste PPY" sheet.

4. Save the entire macro workbook in the appropriate folder.

C. Saving Amplification Sheets on the Network for Additional Samples

1. Partially full or completed amplification sheets may be saved as independent sheets for subsequent sample additions by clicking the "Samples" and amp sheet tab (via holding the ctrl button down). Both sheets should now be highlighted white. Right click and select "move or copy".

2. In this window, select "(new book)" in the "to book" window and check "create a copy". Click "OK". Go to File, Save As and save into the appropriate folder with the amplification system followed by "waiting for amp" or "ready to amp".

3. Samples may be manually added to these sheets by individual analysts or copied and Paste Special from re-quantification sheets or consolidated from additional amplification sheets of the same type at the end of each RotorGene run.

4. If any samples need to be submitted to amplification with a DNA amount other than the optimal amount, the analyst can change the amount of DNA submitted by changing the value in the DNA column in the amplification sheet.

   Be aware that once the DNA amount is manually added to the amplification sheet, the sheet will not be able to calculate the value from the quantification value.

   All other changes should be done in the "Samples" sheet.

5. When a macro amplification sheet is full the analyst may then fill in the amplification date and time in the appropriate blue cell in the "Samples" sheet. This should automatically populate the appropriate cells in the Amplification sheet.

   Any changes to the amplification sheet should be done in the "Samples" sheet.

6. Save the sheet as the time and date of the amplification as follows: “PPY090909.1100” for PowerPlex Y amplifications, performed on September 9, 2009 at 11:00am. These completed amplification sheets should be saved in the “Amp Sheets”, “Amp Sheet Archive” folder.

7. A supervisor should review all entries were entered correctly before printing the Amplification sheet.

III. PCR Amplification – Sample Preparation

A. Samples amplified with PowerPlex Y reagents should be prepared with TE^{-4}.

Prepare dilutions for each sample, if necessary, according to Table 3.

TABLE 3: Dilutions

Dilution	Amount of DNA Template (uL)	Amount of TE^{-4} (uL)
0.25	3 or (2)	9 or (6)
0.2	2	8
0.1	2	18
0.05	2.5	47.5
0.04	4 or (2)	96 or (48)
0.02	2 or (1)	98 or (49)
0.01	2	198
0.008	4 or (2)	496 or (248)

The target DNA template amount for PowerPlex® Y is 500 pg.

To calculate the amount of template DNA and diluent to add, the following formulas are used:

$$\text{Amt of DNA } (\mu L) = \frac{\text{Target Amount (pg)}}{(\text{Sample concentration, pg}/\mu L)(\text{Dilution factor})}$$

The amount of diluant to add to the reaction = 20 µL – amt of DNA (µL)

The amplification of exemplars, sperm cell fractions of samples extracted by differential lysis and semen stains, where no epithelial cells were seen during the differential lysis, is based on the quantitation results. Semen positive swabs taken from female individuals that were extracted using the non-differential semen extraction and the swab remains fractions of differential lysis samples are amplified using the amounts specified in Table 4. Amylase positive samples should be amplified based on Table 5.

Table 4: Amount of DNA extract from a non-differential semen extraction or from the swab/substrate remains fraction of a differential lysis sample to be amplified in PowerPlex® Y.

P30 result for the 2ng subtraction (Body cavity swabs)	**P30 result for the 0.05A units subtraction (Stains or penile swabs)**	**DNA Volume (µL) to be amplified**	**TE^{-4} (µL)**
Sperm Seen; Not Sent to P30 ELISA		8	12
≥ 1.1	≥ 1.1	8	12
> 0 - 1.0	> 0 - 1.0	20	0

Table 5: Amount of DNA extract to be amplified for Amylase positive samples.**

Type of item		**DNA Target Volume (µL)**	**TE^{-4} (µL)**
Orifice swab	Initially try two amounts	8 20	12 0
Dried secretions swab (External)	Based on Quantitation result		
Stain	Based on Quantitation result		

** RotorGene does not reflect male DNA, especially for vaginal swabs. Try more or less if negative.

B. Male Positive Control

If using the Promega PowerPlex Y 9948 or Forensic Biology in-house Male Positive Control, remove a tube of MPC from the freezer and thaw. Once thawed, 20 µL of the male positive control may be added directly to the amplification tube.

C. Female Negative Control

For the Promega Female Negative Control, make a 1/100 dilution (2 µL Control in 198 µL of TE). Only 5 µL of this dilution will be used. The remainder of the solution can be used if another PowerPlex® Y amplification is needed.

D. Amplification Negative Control

TE^{-4} will serve as an amplification negative control.

E. Master Mix Preparation

1. Retrieve PowerPlex® Y primers, PowerPlex® Y reaction mix and ABI Taq Gold from the freezer and store in a Nalgene cooler on bench. **Record the lot numbers of the reagents.**

2. Vortex or pipet the reagents up and down several times to thoroughly mix the reagents. **Do not vortex Taq Gold** as it may degrade the enzyme.

   After vortexing, centrifuge reagents (**except the primers)** briefly at full speed to ensure that no sample is trapped in the cap. Primers tubes may be tapped on the benchtop or may be centrifuged at 3000 rpm for 3 seconds if necessary.

3. Consult the amplification sheet for the exact amount of PowerPlex® Y primers, reaction mix and ABI Taq Gold to add. The amount of reagents for one amplification reaction is listed in Table 6.

Table 6 - PowerPlex® Y PCR amplification reagents for one sample

Reagent	Per reaction
10X Primer mix	2.5µL
Gold Star 10X Buffer	2.5µL
AmpliTaq Gold DNA Polymerase (5U/µL)	0.55uL
Mastermix total in each sample:	5.55µL
DNA	20µL

F. Reagent and Sample Aliquot

1. Vortex master mix to thoroughly mix. After vortexing, briefly tap or centrifuge the master mix tube to ensure that no reagent is trapped in the cap.

2. Add **5.55 µL** of the PowerPlex® Y master mix to each tube that will be utilized, changing pipette tips and remixing master mix as needed.

   NOTE: Use a new sterile filter pipet tip for each sample addition. Open only one tube at a time for sample addition.

3. Arrange samples in a rack in precisely the positions they appear on the sheet.

4. **Witness step.** Ensure that your samples are properly positioned.

5. Prior to adding sample or control, pipet each sample or control up and down several times to thoroughly mix. The final aqueous volume in the PCR reaction mix tubes will be 25.55µL. After addition of the DNA, cap each sample before proceeding to the next tube.

6. After all samples have been added, take the rack to the amplified DNA area for Thermal Cycling.

IV. Thermal Cycling

A. Turn on the ABI 9700 Thermal Cycler. (See manufacturer's instructions).

B. Choose the following files to amplify in PowerPlex Y:

PowerPlex Y
user: casewk file: powery

PCR Conditions for the Perkin Elmer GeneAmp PCR System 9700

9700 PowerPlex® Y user: casewk file: PowerY	The PowerPlex® Y file is as follows: Soak at 95° for 11 minutes. Soak at 96° for 1 minute. Ramp 100% Denature at 94°C for 30 seconds Ramp 29% Anneal at 60°C for 30 seconds Ramp 23% Extend at 70°C for 45 seconds For 10 cycles then… Ramp 100% Denature at 90°C for 30 seconds Ramp 29% Anneal at 58°C for 30 seconds Ramp 23% Extend at 70°C for 45 seconds For 20 cycles then… 30 minute incubation at 60°C. Storage soak indefinitely at 4°C.

C. 9700 Instructions

1. Place the tubes in the tray in the heat block **(do not add mineral oil)**, slide the heated lid over the tubes, and fasten the lid by pulling the handle forward. Make sure you use a tray that has a 9700 label.

2. Start the run by performing the following steps:

3. The main menu options are RUN CREATE EDIT UTIL USER. To select an option, press the F key (F1…F5) directly under that menu option.

4. Verify that user is set to "casewk." If it is not, select the USER option (F5) to display the "Select User Name" screen.

5. Use the circular arrow pad to highlight "casewk." Select the ACCEPT option (F1).

6. Select the RUN option (F1).

7. Use the circular arrow pad to highlight the desired STR system. Select the START option (F1). The "Select Method Options" screen will appear.

8. Verify that the reaction volume is set to **25**µL For PowerPlex Y and the ramp speed is set to **9600 (very important)**.

9. If all is correct, select the START option (F1).

10. The run will start when the heated cover reaches 103°C. The screen will then display a flow chart of the run conditions. A flashing line indicates the step being performed, hold time is counted down. Cycle number is indicated at the top of the screen, counting up.

11. Upon completion of the amplification, remove samples and press the STOP button repeatedly until the "End of Run" screen is displayed. Select the EXIT option (F5). Wipe any condensation from the heat block with a Kimwipe and pull the lid closed to prevent dust from collecting on the heat block. Turn the instrument off.

    Note: **Place the microtube rack used to set-up the samples for PCR in the container of 10% bleach container in the Post-Amp area in Room 714A.**

Revision History:

March 24, 2010 – Initial version of procedure.

March 29, 2011 – Revised Step III.B. Preparation is the same using either the Promega 9948 or in-house made Male Positive Control.

A. Preparation of 3130xl sheet

On the "3130Sheet" tab, type the appropriate System into the "Sys" column of the first row of the injection. Once the first row of the injection is filled, the rest of the injection should automatically populate with the same System code.

Table 1

Amplification System/Cycle)	Specification	Run Module Code	Parameters
PowerPlex Y	Normal	Y	3 kV for 5 sec
	High	YR	3 kV for 10 sec

B. Mastermix and Sample Addition for PowerPlex® Y

1. Prepare one mastermix for all samples, negative and positive controls, allelic ladders as specified in the table below (mastermix calculation, add 9.5µL HiDi + 0.5µL ILS 600 standard per sample).

# Samples + 2	HiDi Form (9.5 µL per sample)	ILS600 Std (0.5 µL per sample)
16	171 µL	9 µL
32	323 µL	17 µL
48	475 µL	25 µL
64	627 µL	33 µL
80	779 µL	41 µL
96	931 µL	49 µL
112	1083 µL	57 µL
128	1235 µL	65 µL

NOTE: HiDi Formamide cannot be re-frozen.

2. Obtain a reaction plate and label the side with the name used for the Sample Sheet with a sharpie and place the plate in an amplification tray or the plate base. Aliquot **10µL of mastermix** to **each** well.

C. Adding Samples:

1. Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96- well reaction plate. Sample order is as follows: A1, B1, C1, D1... G1, H1, A2, B2, C2...G2, H2, A3, B3, C3, etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1-H2, the second A3-H4 and so on.

2. Have someone witness the tube setup by comparing the tube labels and positions indicated on the sample sheet with the tube labels and positions of the tubes themselves.

3. For samples being run at normal parameters: Aliquot the following:

   Allelic Ladder: 1 µL
   Positive/Negative Controls: 1 µL
   Samples: 1 µL

4. For samples being run at high parameters: Aliquot the following:

   Allelic Ladder: 2 ul of a 1/10 dilution
   Positive Control: 1 ul
   Samples: 2 ul

5. When adding PCR product, make sure to pipette the solution directly into the formamide and gently flush the pipette tip up and down a few times to mix it.

6. If an injection has less than 16 samples, add at least 12µL of either dH_2O, formamide, HiDi, buffer or mastermix to all unused wells within that injection.

D. Denature/Chill - For PowerPlex Y After Sample Addition:

1. Once all of the samples have been added to the plate, place a new 96-well Septa over the reaction plate and firmly press the septa into place.

2. Spin plate in centrifuge at 1000 RPM for one minute.

3. For Denature/Chill:

   a. Place the plate on a 9700 thermal Cycler (Make sure to keep the Thermal Cycler lid off of the sample tray to prevent the septa from heating up.)
   b. Select the "dechillppy" program for PowerPlex Y (95°C for 3 minutes followed by 4°C for 3 minutes). Make sure the volume is set to 12 µL.
   c. Press **Run** on the Thermal Cycler.
   d. While the denature/chill is occurring, you can turn on the oven on the ABI 3130xl.

   3130xl visible settings:
   EP voltage 15kV
   EP current (no set value)
   Laser Power Prerun 15 mW
   Laser Power During run 15mW
   Laser Current (no set value)
   Oven temperature 60°C

   Expected values are:
   EP current constant around 120 to 160µA
   Laser current: 5.0A ± 1.0

It is good practice to monitor the initial injections in order to detect problems.

Table 2

	Y	**YR**
Oven Temp	60°C	60°C
Pre-Run Voltage	15.0 kV	15.0 kV
Pre-Run Time	180 sec	180 sec
Injection Voltage	3 kV	3 kV
Injection Time	5 sec	10 sec
Run Voltage	15 kV	15 kV
Run Time	2000 sec	2000 sec

Revision History:
March 24, 2010 – Initial version of procedure.

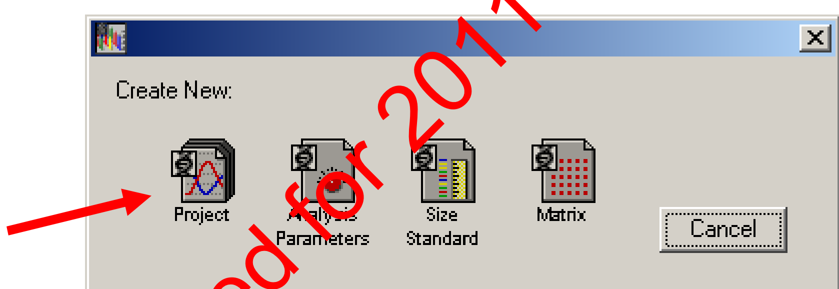
When a run is complete, it will automatically be placed in **D:/AppliedBio/Current Runs** folder, properly labeled with the *instrument name, date and runID* (e.g. **Run_Venus_2006-07-13_0018**).

Prior to importing *.fsa files into GeneScan, the files must have been converted using the conversion tool. Refer to the "STR Data Conversion and Archiving" Section of the STR manual.

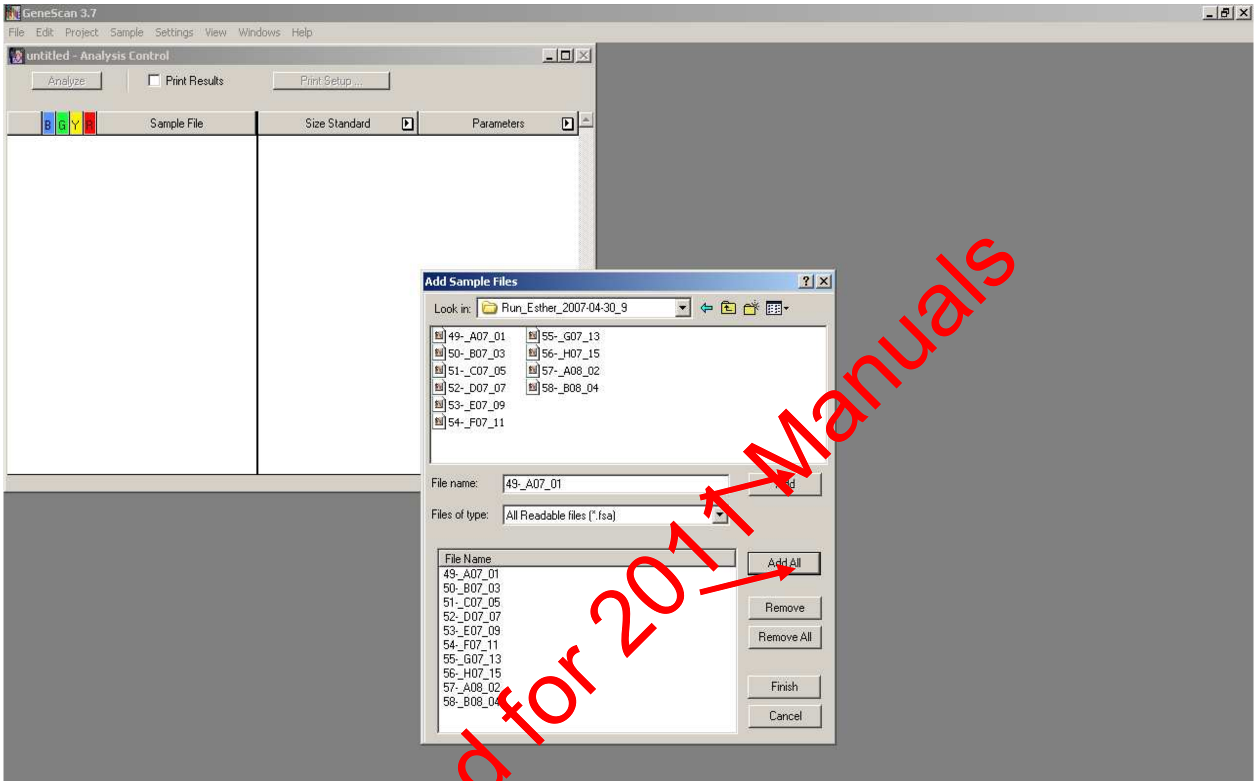
A. Access to GeneScan

1. Click on the GeneScan shortcut located on the desktop of the analysis station computer.

2. Create a new GeneScan project by clicking **File→New (Ctrl+N)**. A dialog box with several icons will pop up. Click on the project icon.



An untitled **Analysis Control** window opens.



3. To add sample files to the open analysis control window, click on **Project** from the menu options and select **Add Sample Files**.

4. When the **Add Sample Files** dialog window appears, find the **Current Run** folder containing the injection folders with the samples that you want to add to the project. Add your samples to the project.

To add samples to a project, take the following action:

If you want to...	Then...
Select a single sample file	Double-click the file OR select the file and click **Add**
Select all the sample files	Click **Add All**
Add a continuous list of sample files	a. Click the first sample that you want to add. b. Press the **Shift key** and click the last sample you want to add. Click **Add**. All the files between the first and last file are selected.
Add a discontinuous list of samples	a. Click the first sample that you want to add b. Press the **Control key** and then click on the other sample(s) you want to add. Click **Add**. All the files you selected will be highlighted and selected.

5. Click **Finish** when you have added all of the samples.

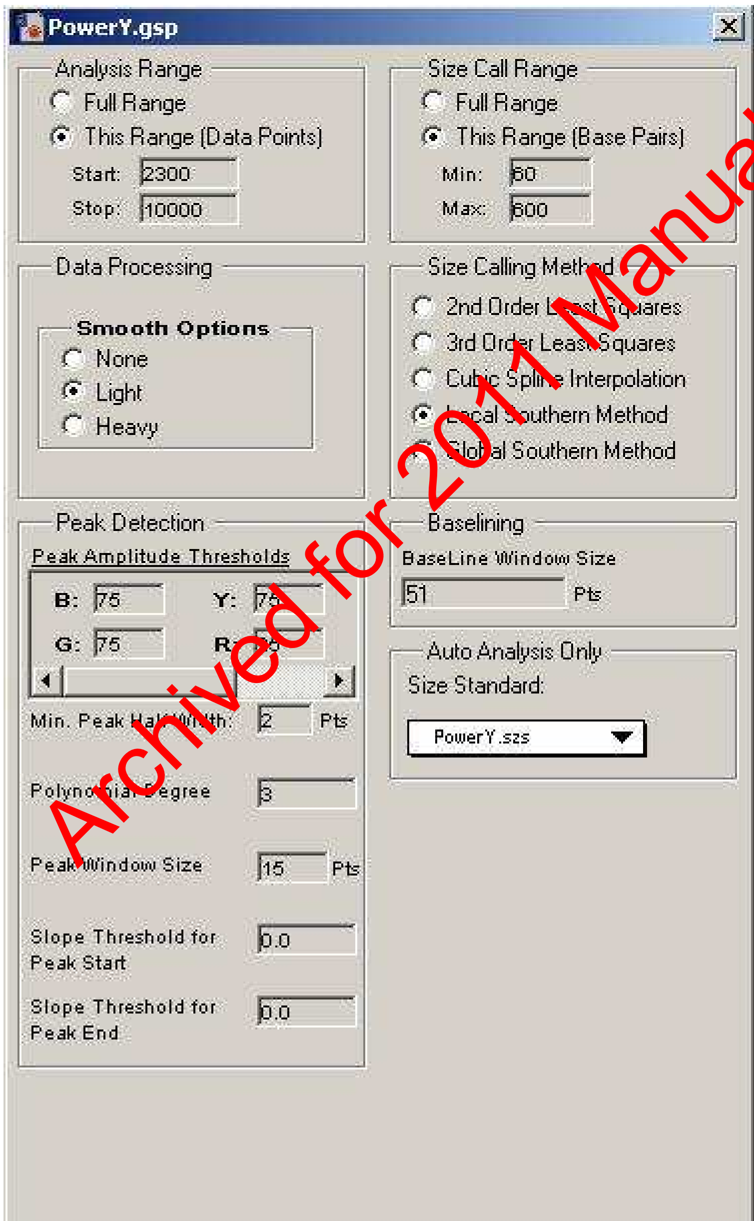
B. Analysis Settings

The **Analysis Control** window shows in separate columns the dye lanes, sample file names, size standard options, and analysis parameters to choose for each lane (See options for PowerPlex Y analysis below). Boxes for the red dye lane should be marked with diamonds to indicate that this is the color for the PowerPlex Y size standard.

System	Size Standard File	Analysis Parameter File
PowerPlex Y	PowerY.szs	PowerY.gsp

PowerPlex Y Analysis Parameters

Do not change any of the settings except the range or the peak amplitude threshold for Red (R).



C. Analysis

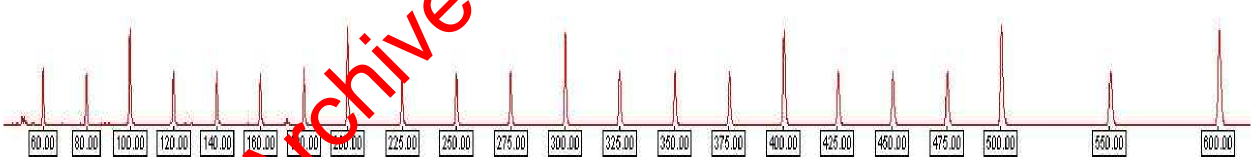
To ensure that all the sizing results are correct, check the labeling of the size standard peaks for each sample.

1. To view the analysis results, select **Windows** from the main menu and click on **Results Control**. The analyzed colors for each lane are shown in dark grey. The white squares mean that this color has not been analyzed.

2. The raw data can be seen in up to 8 display panels, by changing the **# of panels** to **8**. To view each color separately, check **Quick Tile** to **On**.

3. Select the first 8 size standard dye lanes by clicking on them and then click **Display**. Each sample standard will be displayed in its own window. To view all 8 standards, you must scroll through all of the windows. Make sure that all peaks are correctly labeled. Continue checking your size standard for the entire tray by going back to the **Results Control** window, clicking on **Clear All** and selecting the next 8 samples. Repeat these steps until all of the sample size standards have been checked.

For PowerPlex Y, at least the 60 – 375 bp size standards must be apparent.



Before proceeding with the Genotyper analysis, under **File** select **Save Project As.** The project will be named according to the Sample Sheet name. This file will save as a *.prj file in the run folder.

D. GenoTyper Analysis for PowerPlex® Y

1. Open the Genotyper macro for PowerPlex® Y by clicking on the PowerPlex® Y Genotyper shortcut on the desktop of the analysis station computer. Under **File** go to **Import** and select **From GeneScan File**. Double-click on the folder containing the PowerPlex® Y project that you created in GeneScan. Click **Add** or double-click on the project icon to add the project for analysis. When the project has been added, click **Finish**.

2. Under **View** select **Show Dye/Lanes window** you will see a list of the samples you have imported from GeneScan analysis. If samples need to be removed, highlight the lanes for these samples and select **Cut** from the **Edit** menu.

3. Change the name of the PowerPlex® Y Genotyper template to your initials and the casework run file name (under **File** select **Save As)**.

   For example: "Stripes04-001PPY EL" for PowerPlex Y runs

4. After importing the project and saving the Genotyper file run the first Macro by simultaneously press **Control key** and the **number 1**, or double clicking "**Power**".

5. The plot window will appear automatically when the macro is completed. Check to make sure that the ladders that were run match the allele sequence shown below. Also check the results for the positive control.

Table 1

Multiplex System	Necessary LIZ GS500 standard peaks
PowerPlex® Y	15 fragments from 60 - 375 bp

PowerPlex® Y ALLELIC LADDER

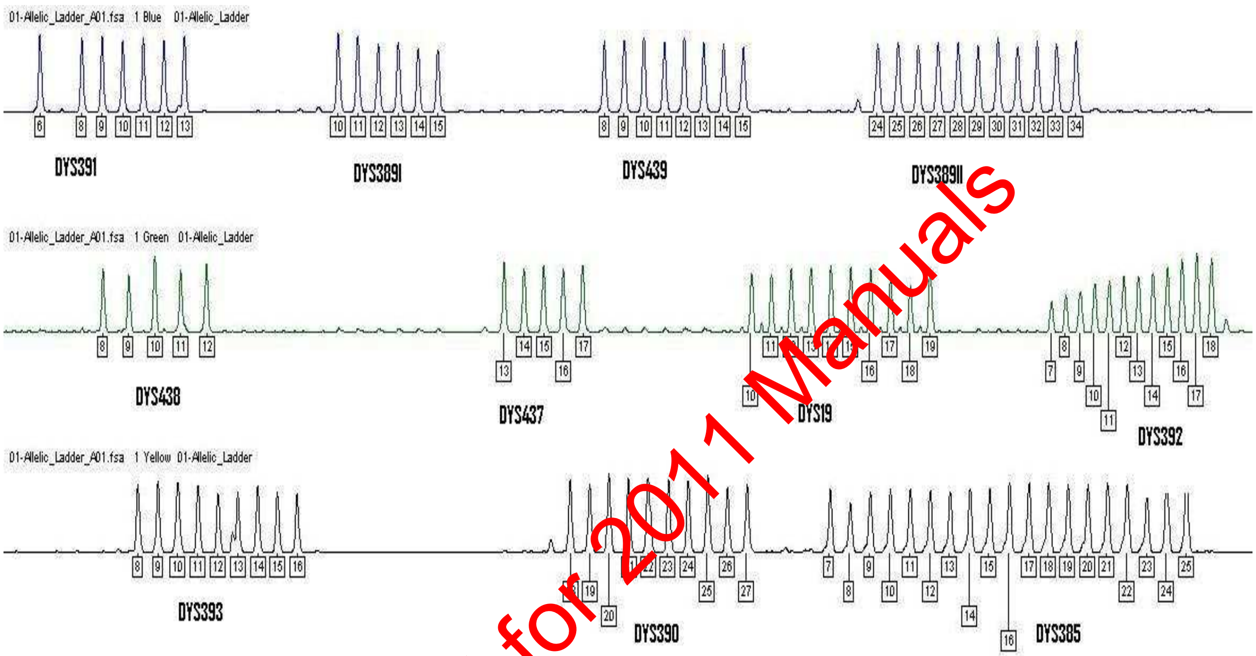


TABLE 2 PowerPlex® Y 9948 Male Positive Control

Blue (FL)	DYS391	DYS389I	DYS439	DYS389II
	10	13	12	31
Green (JOE)	**DYS438**	**DYS437**	**DYS19**	**DYS392**
	11	15	14	13
Yellow (TMR)	**DYS393**	**DYS390**	**DYS385**	
	13	24	11, 14	

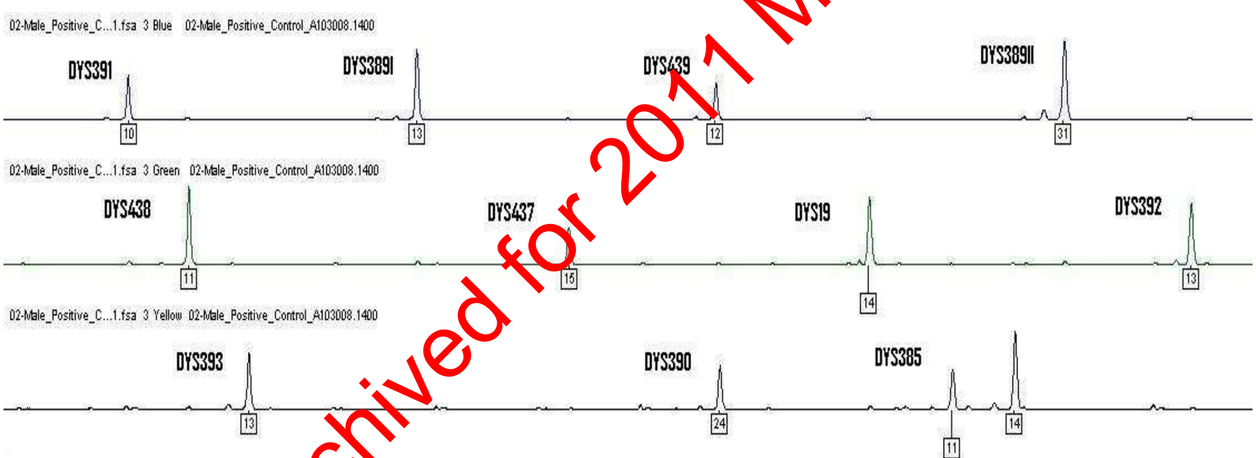
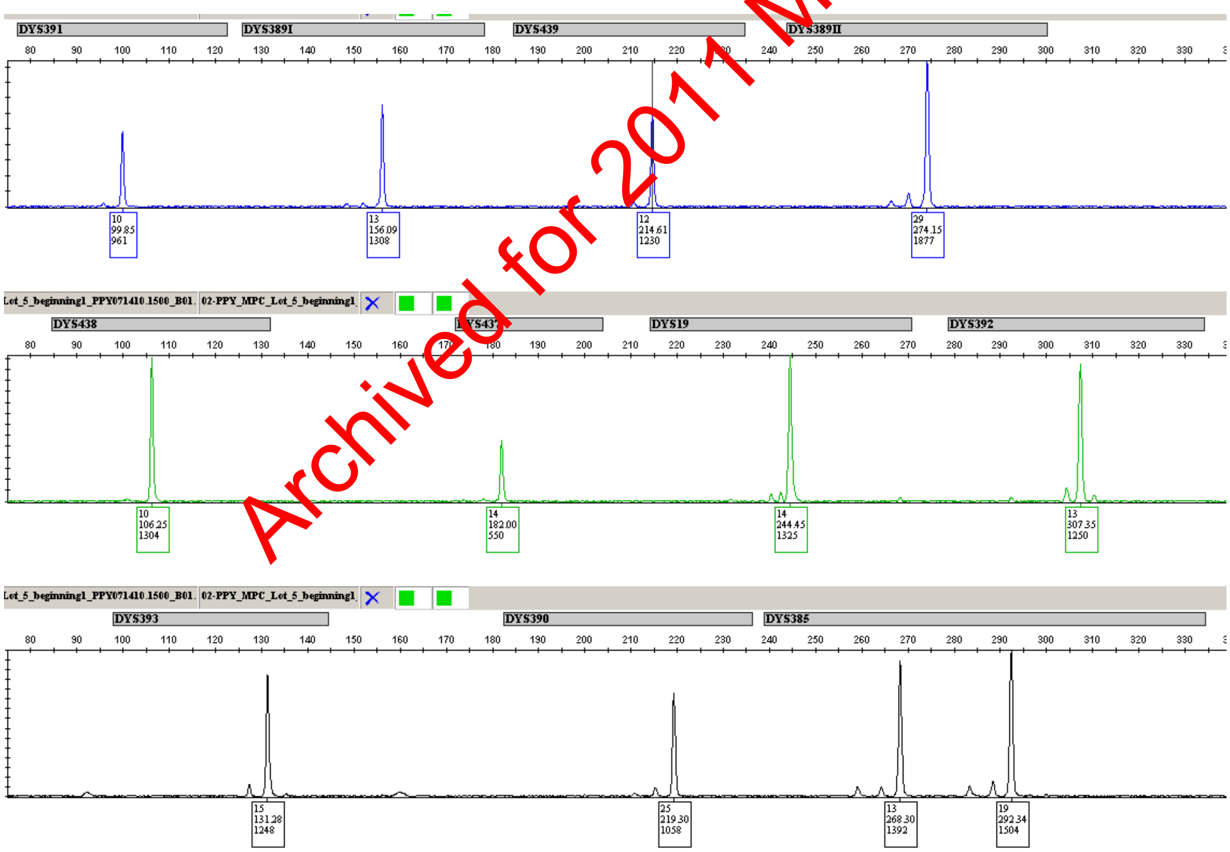


TABLE 2b Forensic Biology In-House Male Positive Control
(As of August 2, 2010; NIST Traceable)

Blue (FL)	**DYS391**	**DYS389I**	**DYS439**	**DYS389II**
	10	13	12	29
Green (JOE)	**DYS438**	**DYS437**	**DYS19**	**DYS392**
	10	14	14	13
Yellow (TMR)	**DYS393**	**DYS390**	**DYS385**	
	15	25	13, 19	



F. Viewing samples

1. Check all lanes or Under Views→Show Main Window and highlight the appropriate samples. Under View→Show Plot Window (Ctrl+Y) or click on the plots icon to view the electropherogram.

2. The plot scan range for PowerPlex Y should be set in the plots window, under Views→Zoom To… type 75 and 340 in the dialog box.

G. Editing of Genotyper files

Peaks can be removed if they meet one of the criteria listed in the editing section (12.II of the STR Manual). Labels for extra peaks can be manually deleted by placing the cursor on the peak above the baseline and clicking. This removal must be documented on an editing sheet.

Based on the validation and on the Promega PowerPlex® Y System Technical Manual, for PowerPlex Y, known artifacts tend to occur at the following locations and may be edited out as “specific artifacts.”

- **DYS19 and DYS389II can display low-level products in the n-2 and n+2 positions**.
- **DYS437 and DYS385 can display low-level peaks in the n-5, n-9 and n-10 positions.**
- **DYS393 can display low-level peaks in the n-9 and n-10 position.**

At this stage it is also necessary to make decisions about samples that should be rerun with either more or less amount of amplification product.

If a sample displays allele peaks just below the instrument detection threshold there is a distinct possibility that the alleles can be identified after a repeated run with increased amplification product or higher injection parameters. Place the sample on a rerun sheet. **For PowerPlex Y, use 2 ul of amplified sample with the Rerun Module (3 kV 10 sec).**

H. Preparing Samples for Printing

1. Display all samples and the positive and negative controls with basepairs, peak heights, and category names. The relevant allelic ladder is labeled with basepairs and category names only.

2. Highlight all samples except the Ladder and under Analysis→ Change Labels. Select peak heights, basepairs, and category names.

3. Highlight the relevant Allelic Ladder under Analysis→ Change Labels. Select basepairs and category names.

   Ensure that the view is set to 75 to 340 bp prior to printing.

I. Printing Controls

1. In the main view window, highlight the ladder, and all the controls.

2. Highlight all colors.

3. Make sure that the view is set to 75-340.

4. Under File→ Print → Properties button→ Finishing tab→ Document Options→ Pages per Sheet→ select "2 pages per sheet"→ Orientation→click on "Portrait"→ click OK→ OK

5. File→ print→ OK→OK

6. Once printed, ensure that all alleles in the ladder are labeled. Manually enter the basepair size if necessary and initial and date.

J. Printing Samples

To print the electropherograms for samples, select all samples and print following steps 2-5 from the "Printing Controls" section.

K. Create a table by running the Create Table Macro.

1. Double click "**Make Allele Table**" or press "Control +8". The table will open once the macro has completed.

2. Compare the sample information in the table with the amplification and the run control sheet. If an error is detected at this point it can be corrected as follows:

   a. Open the dye/lane window or "sample info box"
   b. Place the cursor in the sample info box and correct the text
   c. Clear the table by going to **Analysis** on the main menu, select **Clear Table**
   d. Select the appropriate colors by shift clicking on the dye buttons or using edit
   e. Run Create Table Macro again.

3. Before printing the results make sure the file is named properly, including initials. Print the table with the "Pages per Sheet" set to 4 and with the orientation set to Landscape.

4. After the printing is finished, under **file → quit** Genotyper. Click **save**. Normally the software will place the Genotyper file to the folder from which the data were imported. Make sure that the Genotyper is saved in the appropriate folder.

5. Initial all Genotyper pages. Pull the rerun samples and list on the appropriate rerun sheet.

6. Have a supervisor review the analyzed run and get a signature on the editing and control review sheets.

Revision History:
March 24, 2010 – Initial version of procedure.
August 2, 2010 – The profile of the in-house Male Positive Control was changed (Table 2b, Page 9)

I. General Information for AmpFℓSTR® MiniFiler™ PCR Amplification

The MiniFiler™ PCR Amplification Kit from Applied Biosystems is a miniature STR (miniSTR) test that utilizes reduced size primers to target Amelogenin and eight of the larger STR loci amplified with Identifiler® (D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA). The MiniFiler™ amplification results in amplicons that are significantly shorter in length than those produced with Identifiler® (see **Figure 1**). MiniFiler™ can be used in conjunction with Identifiler® to recover the larger loci that typically drop-out due to sample degradation. It can also be used for samples that may be inhibited and show no amplification with Identifiler®.

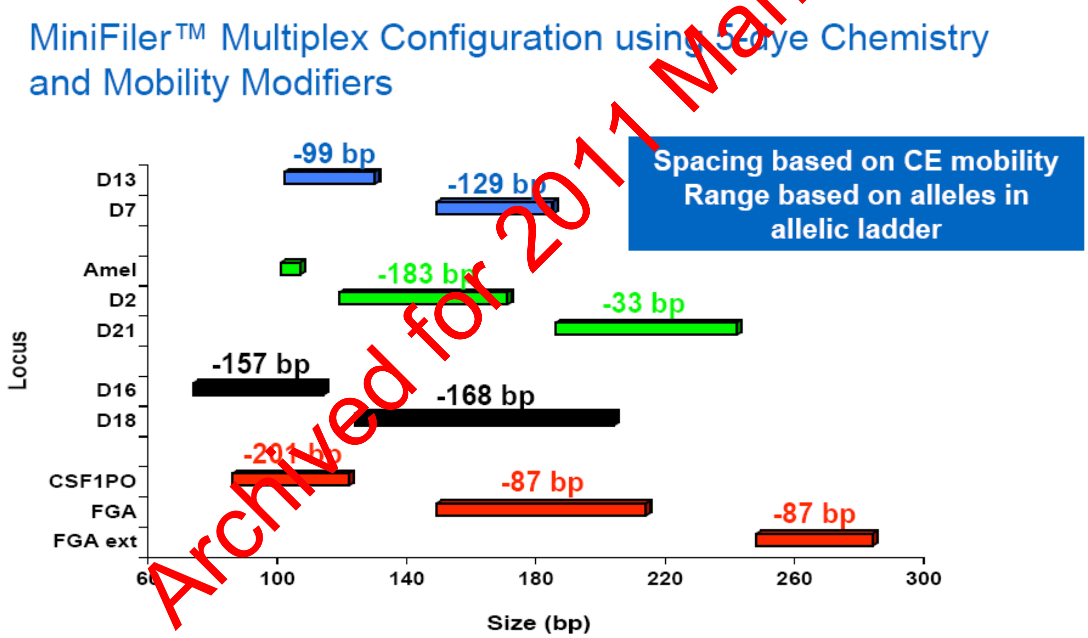


Figure 1. Amplicon size reduction of MiniFiler™ compared to the same STR loci in Identifiler®. Image from Applied Biosystems's "MiniFiler™ Kit Multiplex Configuration," 2006.
http://marketing.appliedbiosystems.com/images/Product_Microsites/Minifiler1106/pdf/MplexConfig.pdf

The target DNA concentration for amplification using the MiniFiler™ system is 500 pg. The minimum DNA concentration required for amplification in this system is 100 pg (minimum quantitiation value of 10 pg/µL). If a sample is found to contain less than 10 pg/µL of DNA, then the sample should not be amplified in MiniFiler™. It can be re-extracted, reported as containing insufficient DNA, concentrated using a Microcon-100, or possibly submitted for High Sensitivity testing (see **Table 1**).

TABLE 1: For MiniFiler™

Minimum Desired Template	100 pg
Template Volume for Amp	10 µL
Minimum Sample Concentration in 200 µL	10.0 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning* to 50 µL	2.5 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning** to 20 µL	1.0 pg/µL

* Sample concentration **prior** to processing with a Microcon 100 and elution to 50 µL

** Sample concentration **prior** to processing with a Microcon 100 and elution to 20 µL

Since MiniFiler™ has a template amplification volume of 10 µL, the extraction negative **must have a quantitation value of < 0.1 pg/µL**. Thus, if the extraction negative is > 0.1 pg/µL, it should be re-quantitated. If it fails again, the sample set must be re-extracted prior to amplification (see **Table 2**).

TABLE 2:

Amplification System	Sensitivity of Amplification	Extraction Negative Control Threshold
MiniFiler™	10 pg	0.10 pg/µL in 10 µL

II. Generation of Amplification Sheets

Amp sheets are generated by supervisors following review of quantification results. Furthermore, samples may be submitted for amplification through aliquot request sheets. Microsoft Excel macros may be employed to generate of these sheets. Different sheets may be used, as described below, depending upon the throughput of each team.

A. MACRO X for Paperwork Preparation for Amplification with MiniFiler™

1. Open the "RGAMP MACRO X" and the "Aliquot Request Form for MiniFiler" Excel files for samples ready to be amped.

2. Copy all of the information from the "Aliquot Request Form for MiniFiler" and paste as values into the "RGAMP MACRO X" under the "Paste MiniFiler" worksheet.

3. Click on the "MiniFiler" tab to see the Amp worksheet and check all entries for errors

   All changes, except for the amount of extract submitted during low and high sample submission, should be made in the "Paste MiniFiler" sheet.

4. Save the entire macro workbook in the appropriate folder.

B. Saving Amplification Sheets on the Network for Additional Samples

1. Partially full or completed amplification sheets may be saved as independent sheets for subsequent sample additions by clicking the "Samples" and amp sheet tab (via holding the ctrl button down). Both sheets should now be highlighted white. Right click and select "move or copy".

2. In this window, select "(new book)" in the "to book" window and check "create a copy". Click "OK". Go to File, Save As and save into the appropriate folder with the amplification system followed by "waiting for amp" or "ready to amp".

3. Samples may be manually added to these sheets by individual analysts or copied and Paste Special from re-quantification sheets or consolidated from additional amplification sheets of the same type at the end of each RotorGene run.

4. If any samples need to be submitted to amplification with a DNA amount other than the optimal amount, the analyst can change the amount of DNA submitted by changing the value in the DNA column in the amplification sheet.

   Be aware that once the DNA amount is manually added to the amplification sheet, the sheet will not be able to calculate the value from the quantification value.

   All other changes should be done in the "Samples" sheet.

5. When a macro amplification sheet is full the analyst may then fill in the amplification date and time in the appropriate blue cell in the "Samples" sheet. This should automatically populate the appropriate cells in the Amplification sheet.

   Any changes to the amplification sheet should be done in the "Samples" sheet.

6. Save the sheet as the time and date of the amplification as follows: "mini090909.1100" for Minifiler amplifications, performed on September 9, 2009 at 11:00am. These completed amplification sheets should be saved in the "Amp Sheets", "Amp Sheet Archive" folder.

7. A supervisor should review all entries were entered correctly before printing the Amplification sheet.

III. PCR Amplification – Sample Preparation

A. Samples amplified with MiniFiler™ reagents should be prepared with irradiated TE^{-4}.

Prepare dilutions for each sample, if necessary, according to **Table 3**.

TABLE 3: Dilutions

Dilution	Amount of DNA Template (µL)	Amount of Irradiated TE^{-4} (µL)
0.25	3 or (2)	9 or (6)
0.2	2	8
0.1	2	18
0.05	2.5	47.5
0.04	4 or (2)	96 or (48)
0.02	2 or (1)	98 or (49)
0.01	2	198
0.008	4 or (2)	496 or (248)

The target DNA template amount for MiniFiler™ is 500 pg.

To calculate the amount of template DNA and diluent to add, the following formulas are used:

$$\text{Amt of DNA } (\mu L) = \frac{\text{Target Amount (pg)}}{(\text{Sample concentration, pg}/\mu L)(\text{Dilution factor})}$$

The amount of diluent to add to the reaction = 10 µL – amt of DNA (µL)

For samples with RotorGene values ≤50 pg/µL but ≥10 pg/µL, aliquot 10 µL extract.

B. Positive Control

For MiniFiler™, DO NOT make a dilution of the 100 pg/µL AmpF*l*STR Control DNA 007. Instead, combine 5 µL of the Control DNA with 5 µL of irradiated TE^{-4}. This yields a total volume of 10 µL with 500 pg in the amplification.

C. Amplification Negative Control

10 µL of irradiated TE^{-4} will serve as an Amplification Negative Control.

D. Master Mix Preparation

1. Retrieve the MiniFiler™ Primer Set and MiniFiler™ Master Mix from the refrigerator and store in a Nalgene cooler on the bench. **Record the lot numbers of the reagents.**

2. Vortex or pipet the reagents up and down several times to thoroughly mix the reagents. After vortexing, centrifuge reagents at full speed briefly to ensure that no sample is trapped in the cap.

3. Consult the amplification sheet for the exact amount of MiniFiler™ Primer Set and Master Mix to add. The amount of reagents for one amplification reaction is listed in **Table 4**.

TABLE 4: MiniFiler™ PCR amplification reagents for one sample

Reagent	Per reaction
MiniFiler™ Primer Set	5.0 µL
MiniFiler™ Master Mix	10.0 µL
Reaction Mix Total:	15.0 µL
DNA	10.0 µL

E. Reagent and Sample Aliquot

1. Vortex master mix to thoroughly mix. After vortexing, briefly tap or centrifuge the master mix tube to ensure that no reagent is trapped in the cap.

2. Add 15 µL of the MiniFiler™ reaction mix to each of the stratalinked PCR tubes that will be utilized, changing pipette tips and remixing reaction mix as needed.

   NOTE: Use a new sterile filter pipet tip for each sample addition. Open only one tube at a time for sample addition.

3. Arrange samples in a rack in precisely the positions they appear on the sheet.

4. **Witness step.** Ensure that your samples are properly positioned.

5. Prior to adding sample or control, pipet each sample or control up and down several times to thoroughly mix. The final aqueous volume in the PCR reaction mix tubes will be 25 µL. After addition of the DNA, cap each sample before proceeding to the next tube.

6. After all samples have been added, take the rack to the amplified DNA area for Thermal Cycling.

IV. Thermal Cycling

1. Turn on the ABI 9700 Thermal Cycler. (See manufacturer's instructions).

2. Choose the following files in order to amplify in MiniFiler™:

MiniFiler
User: casewk File: mini

PCR Conditions for the Perkin Elmer GeneAmp PCR System 9700

9700 **MiniFiler** User: casewk File: mini	The mini file is as follows: Soak at 95°C for 11 minutes 30 Cycles: : Denature at 94°C for 20 seconds : Anneal at 59°C for 2 minutes : Extend at 72°C for 1 minute 45 minute incubation at 60°C. Storage soak indefinitely at 4°C

3. 9700 Instructions

   a. Place the tubes in the tray in the heat block **(do not add mineral oil)**, slide the heated lid over the tubes, and fasten the lid by pulling the handle forward. Make sure you use a tray that has a 9700 label.

   b. Start the run by performing the following steps:

   c. The main menu options are RUN CREATE EDIT UTIL USER. To select an option, press the F key (F1…F5) directly under that menu option.

   d. Verify that user is set to "casewk." If it is not, select the USER option (F5) to display the "Select User Name" screen.

   e. Use the circular arrow pad to highlight "casewk." Select the ACCEPT option (F1).

   f. Select the RUN option (F1).

   g. Use the circular arrow pad to highlight the desired STR system. Select the START option (F1). The "Select Method Options" screen will appear.

   h. Verify that the reaction volume is set to **25**µL for MiniFiler™ and the ramp speed is set to **9600 (very important)**.

   i. If all is correct, select the START option (F1).

   j. The run will start when the heated cover reaches 103°C. The screen will then display a flow chart of the run conditions. A flashing line indicates the step being performed, hold time is counted down. Cycle number is indicated at the top of the screen, counting up.

k. Upon completion of the amplification, remove samples and press the STOP button repeatedly until the "End of Run" screen is displayed. Select the EXIT option (F5). Wipe any condensation from the heat block with a Kimwipe and pull the lid closed to prevent dust from collecting on the heat block. Turn the instrument off.

NOTE: **Place the microtube rack used to set-up the samples for PCR in the container of 10% bleach container in the Post-Amp area in Room 714A.**

Revision History:
March 24, 2010 – Initial version of procedure.

A. Preparation of 3130xl sheet

On the "3130Sheet" tab, type the appropriate System into the "Sys" column of the first row of the injection. Once the first row of the injection is filled, the rest of the injection should automatically populate with the same System code.

Table 1

Amplification System/Cycle)	Specification	Run Module Code	Parameters
MiniFiler™	Normal	F	3 kV for 10 sec

B. Master Mix and Sample Addition for MiniFiler™

1. Prepare one master mix for all samples, negative and positive controls, and allelic ladders as specified in the table below (master mix calculation: add 8.7 µL HiDi + 0.3 µL LIZ500 standard per sample).

# Samples + 2	HiDi Form (8.7 µL per sample)	LIZ500 Std (0.3 µL per sample)
16	157 µL	6 µL
32	296 µL	11 µL
48	436 µL	16 µL
64	575 µL	20 µL
80	714 µL	25 µL
96	853 µL	30 µL
112	992 µL	35 µL
128	1132 µL	40 µL

NOTE: HiDi Formamide cannot be re-frozen.

2. Obtain a reaction plate and label the side with the name used for the Sample Sheet with a sharpie and place the plate in an amplification tray or the plate base. Aliquot **9 µL of mastermix** to **each** well.

C. Adding Samples:

1. Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96- well reaction plate. Sample order is as follows: A1, B1, C1, D1... G1, H1, A2, B2, C2...G2, H2, A3, B3, C3, etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1-H2, the second A3-H4 and so on.

2. Have someone witness the tube setup by comparing the tube labels and positions indicated on the sample sheet with the tube labels and positions of the tubes themselves.

3. Aliquot the following:

Allelic Ladder:	1 µL
Positive/Negative Controls:	1 µL
Samples:	1 µL

4. When adding PCR product, make sure to pipette the solution directly into the formamide and gently flush the pipette tip up and down a few times to mix it.

5. If an injection has less than 16 samples, add 10µL of either dH_2O, HiDi formamide, or master mix to all unused wells within that injection.

D. Denature/Chill – For MiniFiler™ After Sample Addition:

1. Once all of the samples have been added to the plate, place a new 96-well Septa over the reaction plate and firmly press the septa into place.

2. Spin plate in centrifuge at 1000 RPM for one minute.

3. For Denature/Chill:

   a. Place the plate on a 9700 Thermal Cycler (Make sure to keep the Thermal Cycler lid off of the sample tray to prevent the septa from heating up).
   b. Select the "denature/chill" program. Make sure the volume is set to 10 µL.
   c. Press **Run** on the Thermal Cycler. The program will heat denature samples at 95°C for 5 minutes followed by a quick chill at 4°C (this will run indefinitely, but the plate should be left on the block for at least **5 min**).
   d. While the denature/chill is occurring, you can turn on the oven on the ABI 3130*xl*.

E. 3130*xl* Settings

3130*xl* visible settings:
EP voltage 15kV
EP current (no set value)
Laser Power Prerun 15 mW
Laser Power During run 15mW
Laser Current (no set value)
Oven temperature 60°C

Expected values are:
EP current constant around 120 to 160µA
Laser current: 5.0A ± 1.0

It is good practice to monitor the initial injections in order to detect problems.

Table 2

	F
Oven Temp	60°C
Pre-Run Voltage	15.0 kV
Pre-Run Time	180 sec
Injection Voltage	3 kV
Injection Time	10 sec
Run Voltage	15 kV
Run Time	1500 sec

Revision History:
March 24, 2010 – Initial version of procedure.

A. CREATING A NEW PROJECT

1. Double click on the GeneMapper ID v3.2.1 icon on the analysis station desktop.

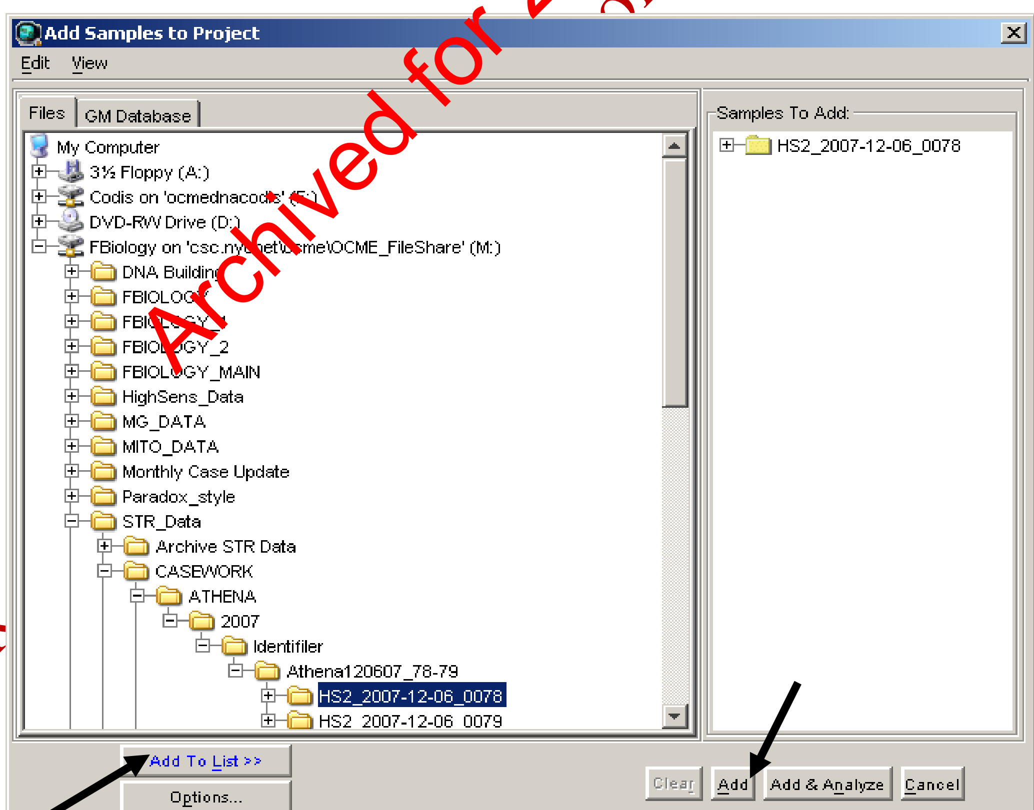
2. When prompted, enter your username and password.

3. The program will automatically open a new (blank) project. This main window is called the **"Project Window"**.

4. Click on **File→Add Samples to Project…**or **Ctrl+K**. A new window will open, listing the drives or folders from which to add the samples on the left.

5. Navigate to the proper drive, and choose the folder that contains the run folders or samples that need to be analyzed. Select the run folder(s) and click on **Add to List**.

6. On the bottom right Click **Add**. The chosen samples will now populate the project.

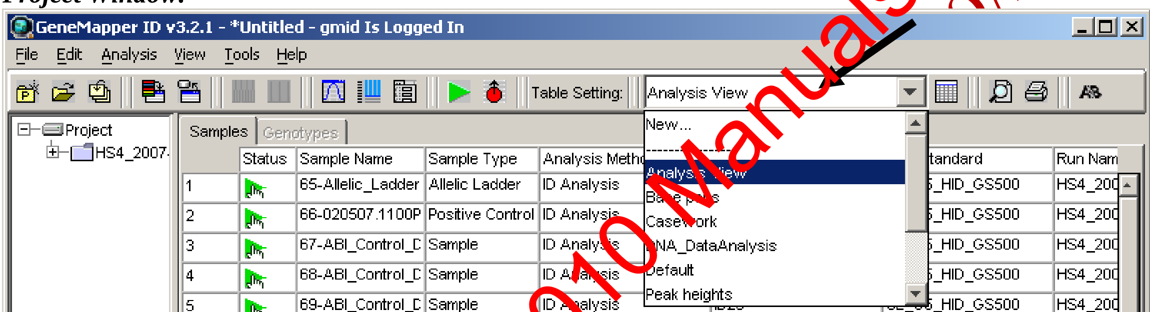


B. ANALYSIS SETTINGS

1. All defined settings must be used and can be referenced in *Appendix D. Analysis Method Editor* and *Appendix G. Default Table and Plot Settings*.

2. From the **"Table Setting"** drop-down menu in the toolbar, select "**Analysis View**".

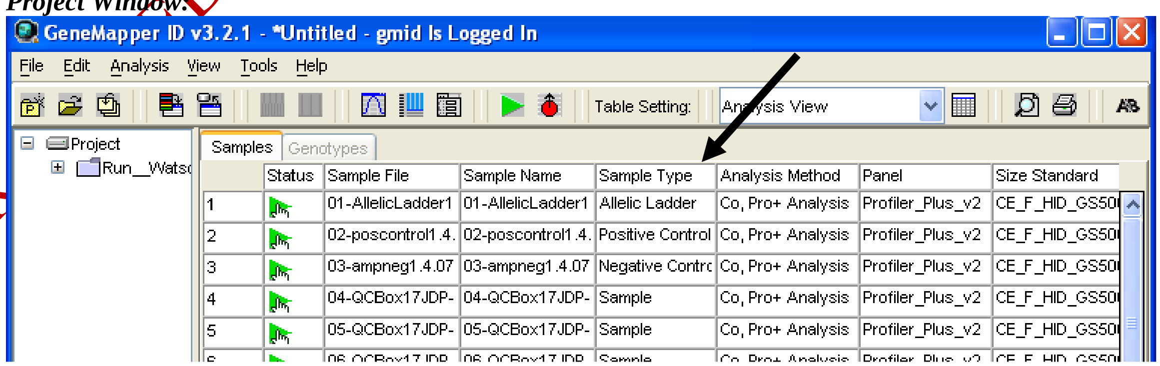
Project Window:



3. If the ladders, positive control, and negative control have not yet been designated, do so now under "***Sample Type***".

4. When there is more than one ladder in a project, make sure each is designated as "Allelic Ladder" in the ***Sample Type*** column. In this manner the software will average the ladders and use this average to assign alleles. Therefore, all allelic ladders must pass analysis. For projects containing more than one injection (and therefore more than one ladder) where the analysis fails, designate the ladder associated with the Positive Control injection as "Allelic Ladder" and any others as "Sample" and re-analyze the project. Schedule failing samples for rerun as necessary.

Project Window:



5. Fill in the correct analysis method, panel, and size standard following the table below. Once the analysis method, panel, and size standard have been chosen for the first sample, you can fill down the same information by selecting all three columns. Do this by selecting the title row of the columns and then while holding down the left mouse button drag across the three columns, the selected columns will be highlighted blue. Next, click on **Edit → Fill Down** or **Ctrl+D**.

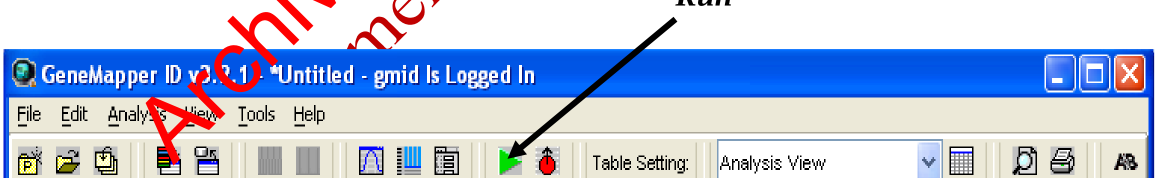
System	Analysis Method	Panel	Size Standard
Identifiler 28 Cycles	ID Analysis	ID28	LIZ-250-340
Identifiler 31 Cycles	ID Analysis	ID31	LIZ-250-340
MiniFiler	MiniFiler Analysis	MiniFiler_GS500_v1	LIZ-250-340
PowerPlexY	PowerPlexY	PowerY	ILS600

6. The last two columns on the right of the ***Project Window*** are user defined columns with information that is carried over from the 3130xl run sheet. If these columns are blank fill them in with the appropriate information.

   a. In UD2 type the tube label.
   b. In UD3 type the IA name for that sample.

7. A green arrow in the **Status** column of each sample means that the data is ready to be analyzed. Click on the **green arrow** in the **toolbar**. A "save project" prompt will pop-up asking for the run to be named.

Run



8. Name the project with the same name of the run i.e. Stripes09-098ID or HS3030607_78N. Click **OK** to start analysis.

9. The progress of the analysis can be seen at the bottom of the project window in the progress status bar. Once analysis is finished the blue progress bar will stop, and the bottom left corner of the screen will read "Analysis Completed."

C. VIEWING ANALYZED DATA

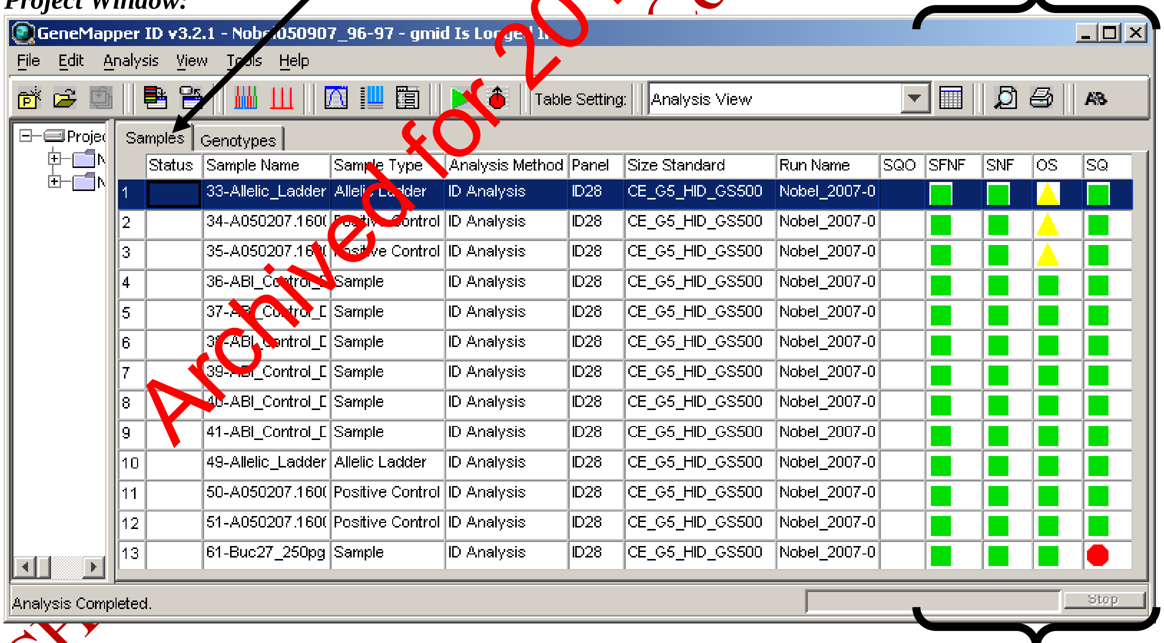
Samples View – Overall Sample Quality Flags

1. In the ***Project Window*** under the ***Samples*** tab, the columns to the right side with colored shapes are Process Quality Value (PQV) flags. These flags do not replace our method for editing samples. Each sample must still be viewed and edited. The flags are simply a tool to draw your attention to samples that have analysis problems therefore assisting you with initial analysis, and editing.

2. The **Pass** (green square) symbol indicates that no problem exists. If a yellow "check" flag, or a red "low quality" flag result in any of the columns, refer to the appendix A – "Quality Flags" for a description of the flags and the problems they identify. Whether a problem is flagged or not, proceed to the sizing section of the manual to individually check each size standard.

Samples tab ***Quality Flags***

Project Window:



D. SIZING

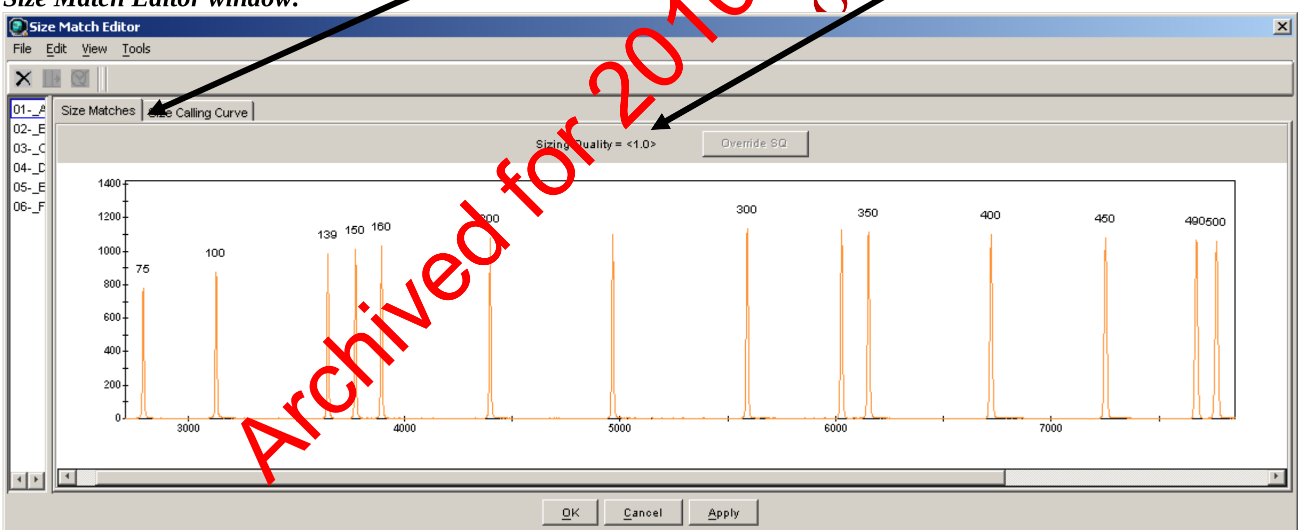
1. Select all of the samples in the ***Samples*** tab by clicking on **Edit→ Select All**.

2. Next, click on the ***Sizing*** icon and the ***Size Match Editor*** window will open.

Sizing icon

Size Matches tab ***Sizing Quality value***

Size Match Editor window:



3. Using the arrow keys, scroll through the samples on the left column and check the sizing for each sample in the ***Size Matches*** tab. The sizing is displayed as a plot with the base pairs displayed above each peak. See Appendix F for a reference of size standards.

   a. Identifiler samples are run with LIZ 500 and should not have the 250 bp or 340 bp size standard labeled. At least the 100bp to 450bp peaks must be present for proper sizing.

b. MiniFiler samples are run with LIZ 500 and should not have the 250 bp or 340 bp size standard labeled. At least the 75bp to 400bp peaks must be present for proper sizing.

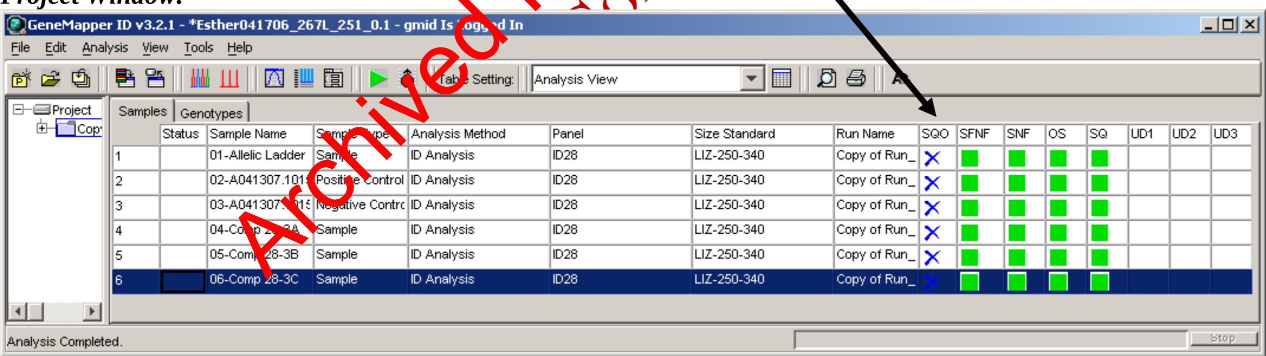
c. PowerPlexY samples are run with ILS600 and at least the 60 – 375bp size standard peaks must be present for proper sizing.

4. **Red octagon symbol in the SQ column of the project window**:

In some cases you may still be able to use this data by redefining the size standard for that sample. For instructions on how to re-label peaks which have been incorrectly labeled, see the Appendix E – Troubleshooting section of this manual.

5. While still in the Size Match Editor window document that each sample size standard has been inspected by selecting **Edit → "Override All SQ"** or **Ctrl+Shift+O**; Click **Apply** and then **OK**. The Size Match Editor window will then automatically close. A blue "X" will appear in the sizing quality check box (SQO) for each sample, signaling that the size standard for each sample has been reviewed.

Project Window:



6. If a green triangle appears in the status column for any of the samples after you applied the SQO, press the green analyze button in the toolbar to finish the sizing quality override.

E. PLOT VIEWS

Samples Plot – Reviewing Ladders, Controls, and Samples

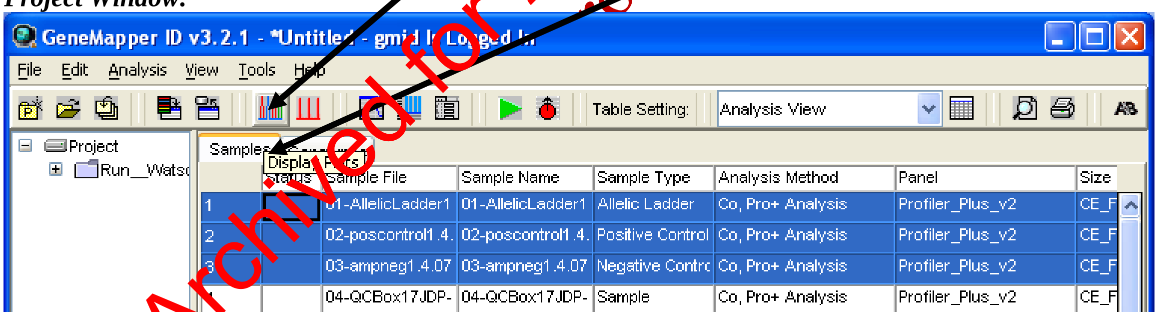
1. First, check the ladders and controls in the project using the following steps. If a project contains more than one allelic ladder, each ladder must be reviewed and pass analysis. Then repeat the steps for the samples. See Appendix F for a reference of allelic ladders and positive controls.

2. If there are two positive controls of the same date and time (i.e. high and normal), you can remove one by selecting it in the ***Samples*** tab of the ***Project Window***, then from the pull down menu select Edit → Delete from Project → OK.

3. In the ***Samples*** tab of the ***Project Window***, select the sample rows you want to view (i.e. ladders, controls, or samples) then click the plot button to display the plots (Analysis → Display Plots or Ctrl+L). Use the shift key or the ctrl key to select multiple samples.

Plot button ***Samples tab***

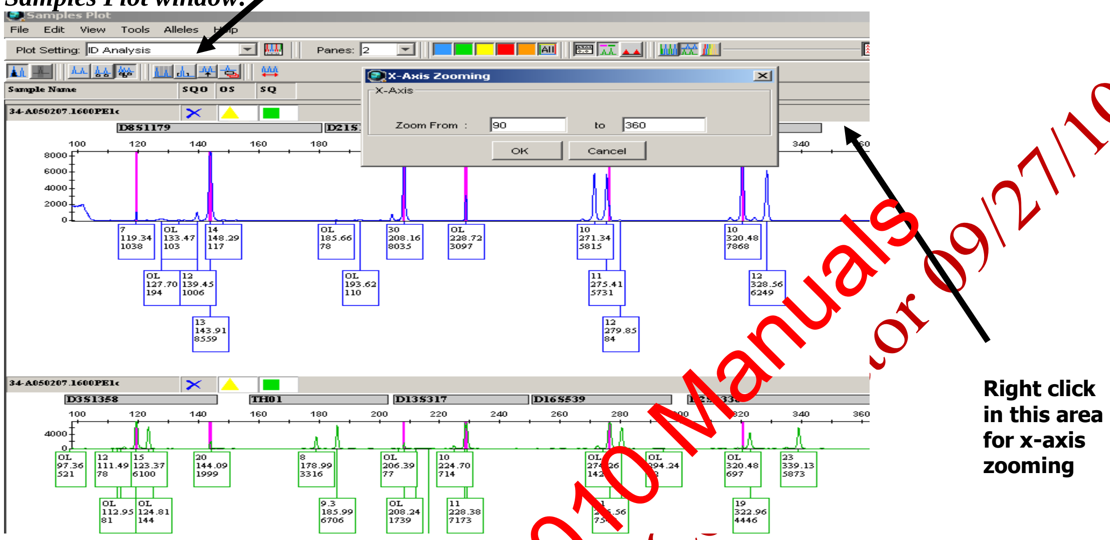
Project Window:



4. In the "*Samples Plot*" window toolbar there is a **Plot Setting dropdown list**. For Identifiler and PowerPlexY, select *"Analysis View."* For Minifiler, select *"Mini Analysis."* This will label the peaks with base pairs, RFUs and allele name.

“Plot Setting” dropdown list

Samples Plot window:



5. Adjust the window zoom by right clicking above the plot pane and using the X Axis Zooming dialog box to zoom into a specific range. Alternatively, hover the mouse above the panel; it will change into a magnifying glass that can be used to draw a box around a selected area to zoom in.

6. If you still have “no room for labels”, for example when you have many alleles per locus such as the Allelic Ladder, it may be easier to review the sample in the “Genotypes Plot” as described in *Appendix E – Troubleshooting Guide, 3. Genotypes Plot – Locus Specific Quality Flags*. The Genotypes Plot is an alternate view option showing each locus in a separate pane. The locus specific quality flags can only be viewed in the ***Genotypes Plot*** window.

NOTE: Refer to the Appendix A – “Quality Flags” for a description of the flags and the problems they identify.

F. EDITING

Electronic Editing – First Analysis

1. You can view the sample in the ***Samples Plot*** window or the ***Genotypes Plot*** window or minimize back and forth between these views to facilitate analysis. Just ensure that you are using the correct view settings ("Analysis View" or "Mini Analysis.")

2. Left click on the allele in question to select it.

3. To edit the allele you must right click on it while it is highlighted and you will see a list of three choices – Delete Allele(s); Rename Allele; History.

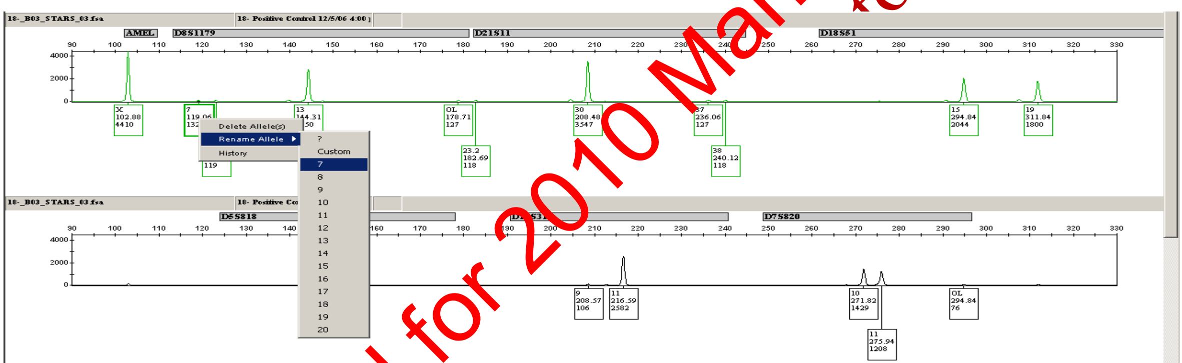
4. Select ***Rename Allele***; another drop down menu will appear listing all of the possible choices for alleles at that locus including ***"?"*** and ***Custom***.

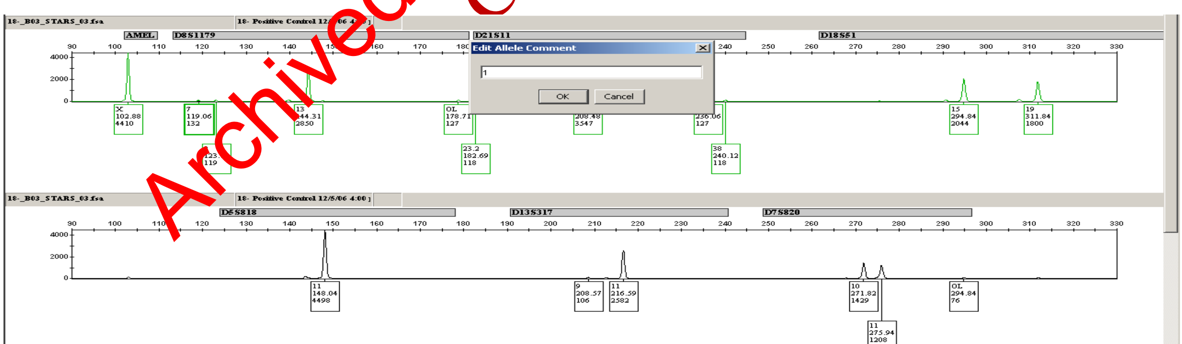
5. If the sample has been labeled an Off Ladder (OL), choose “?”. If the peak has been given an allele call, chose that same allele call from the drop-down list.

   For example, if a pull-up peak has been labeled a 7, highlight the 7 then right click and rename the allele 7 from the drop-down menu. This is done so that the reviewer can see what the allele was originally called.

6. A dialog box will then prompt you for an Edit Allele Comment. In the box enter the code for the allele edit (see Appendix B for a list of editing codes).

7. Click OK.





8. You will notice on the electropherogram that the peak has been labeled as follows: “changed”, the allele call, base pair, and RFU, followed by the corresponding edit code.

9. If you are removing all the peaks in the entire sample because it needs to be rerun, for example, when a sample is completely overblown, then you can delete all the peaks together without renaming each peak. The rerun is documented in the electronic rerun sheet.
   a. To delete a range of peaks, select the first peak of the range, and while the first peak is still highlighted, drag a box across the range of peaks to select everything. Right click on the selection and click Delete Allele(s). When doing so, a box may pop-up with a message that more than one allele will be deleted. Click OK then enter the edit type in the allele comment box.
   b. If the removed peaks need to be put back in, highlight the necessary samples from the ***Samples*** tab in the project window. From the ***Analysis*** drop down menu, select ***"Analyze Selected Samples."*** A pop up window will ask for confirmation and state the action cannot be undone. Click OK. Edit the sample(s) appropriately. If this action is done as a change to the original project, there is no need to change the project name. Create new tables and re-export the project.

NOTE: If you are removing several alleles at a single locus but not from the entire sample, do not select the Delete Allele(s) option. You must still select the Rename option as this is the only way to electronically track the edits after you export the project to Excel. Only the reviewer will actually delete the alleles.

IMPORTANT: The changed alleles must be conserved for the reviewer.

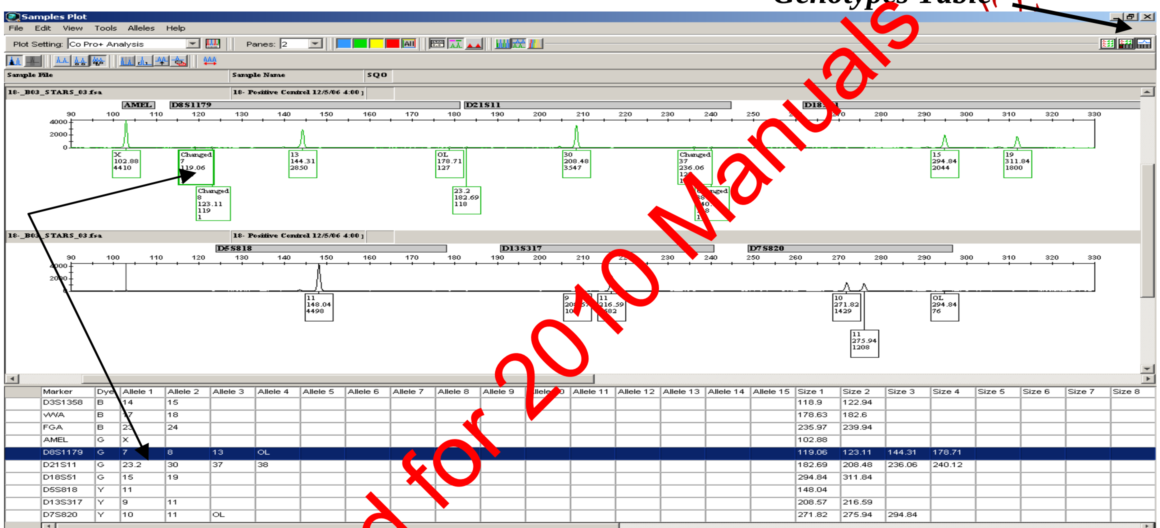
10. If you mistakenly delete a peak instead of renaming it first try to undo by selecting ***edit*** from the drop down menu then select ***undo***. You can undo as many changes as you made while that plot window was open, but if you close and re-open the plot window you will not be able to undo.

11. To revert a deleted peak back to the original allele call, select the peak, right click, then choose ***add allele call*** when prompted for an ***add allele comment*** leave it blank.
   a. The original allele call will be added to the peak but the word "changed" will still appear in the label.
   b. The word "changed" will not appear in the printed electropherogram, but it will appear in the electronic editing sheet as a sample entry with no edit comment.

c. When the editing sheet is generated, scan through the sheet for any sample entries without edit comments these are the peaks that were added back in. **Manually remove them from the worksheet before you print.**

12. Once editing has been completed you can view the edits in the Genotypes table. This table contains all of the alleles, sizes, and edits for all of the samples. Up to 15 edits can be captured per locus.

Genotypes Table

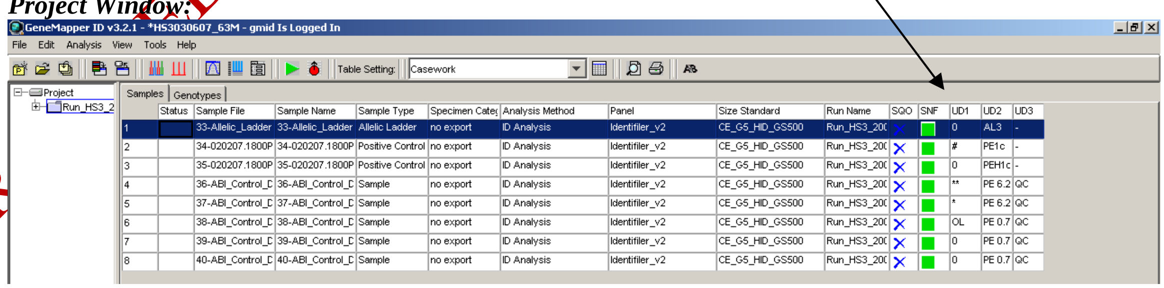


Electronic Rerun Sheet

1. If a sample needs to be rerun, this too is electronically noted. Close the ***Sample Plots*** window and return to the ***Samples*** tab in the ***Project Window***.

enter rerun code in UD1

Project Window:



2. Each sample scheduled for rerun must contain a code in column UD1. The first figure of the code stands for the **sample status**, the second figure stands for the **multiplex system** of the sample, and the third figure stands for the **rerun parameter**. The following are a few examples:
    a. A sample was overblown and all peaks were removed. It should be rerun at a 1/10 dilution in Identifiler. Rerun Code: *ID
    b. An ID28 sample contained an off-ladder allele and needs to be rerun normal in Identifiler. Rerun Code: ^I.
    c. An ID31 sample has a poor size standard and needs to be rerun at the normal parameter. Rerun Code: #IN
    d. A sample has already been rerun once and the second time still produces an off ladder allele, therefore it will **not** be rerun. Rerun code: ^N/A

3. After entering a code, click outside of the cell for the data to export properly.

4. See the Appendixes B and C for a complete list of edit, system, and rerun codes.

Exporting Data for Tables

1. To export this information for use in the **Combined Tables** excel workbook:

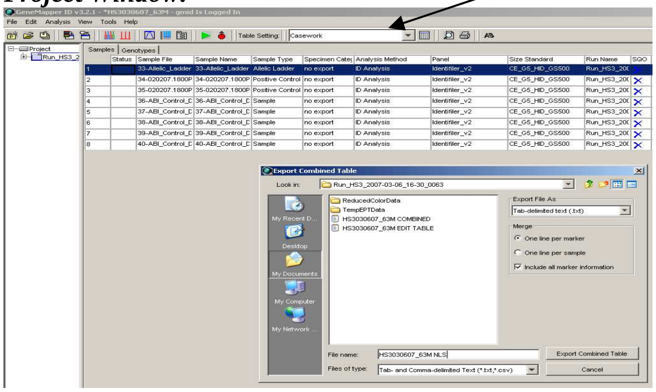
    a. First, in the ***Project Window***, make sure the table setting drop down menu is set to **"Casework"**. In this view you will notice an additional category column "Specimen Category" this column should be set to "no export" for all the samples.
    b. Then Go to ***File*** → ***Export Combined Table***. This table combines the rerun information from the ***Samples*** table and the editing information from the ***Genotypes*** table.

2. Select the appropriate run folder and check the run name contains the initials of the person analyzing the run.

3. The file must be exported as Text-tab delimited (.txt). Ensure this is selected and click "Export Combined Table."

Casework table setting

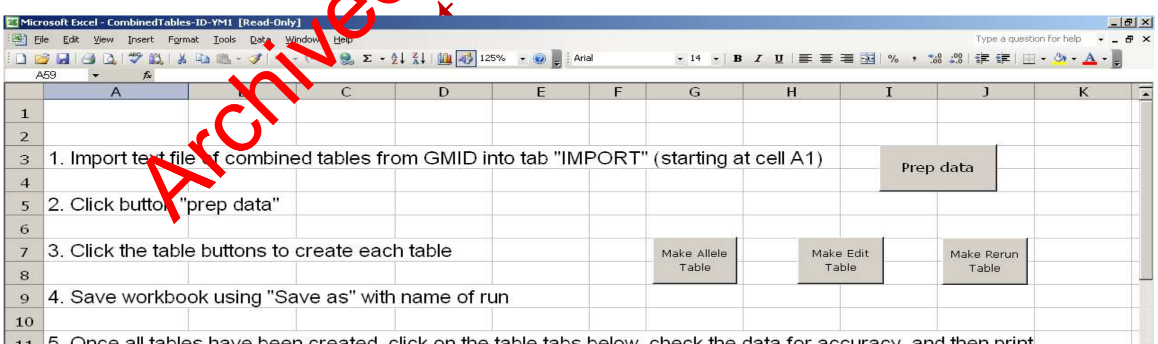
Project Window:



Creating the Allele, Edit, and Rerun Tables

1. Minimize the GeneMapper® *ID* program while you create the tables from the Excel worksheet.

2. Open the “Combined Tables” excel workbook specific for the system you are analyzing. For Identifiler or PowerPlex Y, you will open the workbook named “CombinedTables-ID-PXY”. For MiniFiler the workbook is named “Combined Tables-Mini”.



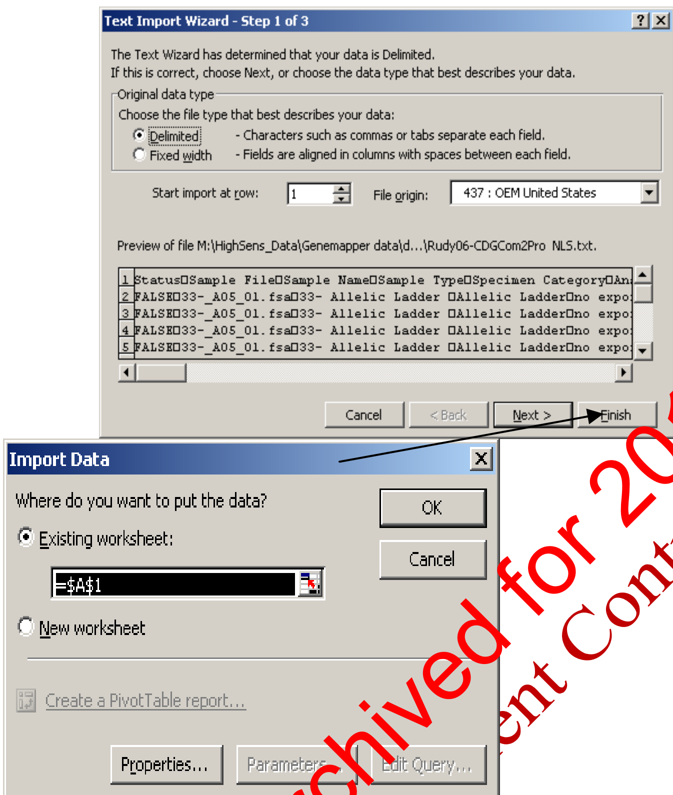
3. The worksheet opens to the ***Instructions*** tab. Before pressing any of the buttons you need to import your data into the “Import” sheet.

   a. On the bottom of the worksheet select the “IMPORT” tab. Then in the menu bar, select ***Data→Import External Data→Import Data***.

b. Navigate to the folder that contains the **.txt* file that you exported. Click ***Open***.

c. In the *Text Import Wizard* box, click ***Finish***.

d. In the *Import Data* box, click ***OK***.



step c

4. After the data has been imported, select the ***Instructions*** tab then click on the "Prep Data" button. This re-sorts the data into the format needed to create the tables.

5. To make the Allele table, Editing table, and Rerun table, click on the appropriate buttons.

6. Review the data in the Allele table; make sure each allele lines up under the correct column corresponding to its locus. Each cell accommodates 15 alleles per locus. Resize the cells if necessary to view all the alleles present. If a sample has more than 15 alleles present at a locus, you must manually enter the remaining alleles.

7. Review the edits in the Edit table; scan through the sheet and make sure all the sample entries have edit comments. If there are entries with no edit comment it is possible that the code was mistyped or inadvertently left out by the analyst. Also, peaks that were mistakenly deleted and subsequently re-labeled will appear as an edit entry without an edit comment. Manually make these corrections to the worksheet before you print.

8. Finally, make sure that the sample names are legible, and not cut-off in any of the worksheets. Resize the cells or shrink the font to fit if necessary.

9. Print the Allele table, Edit table, and Rerun table, then paperclip them to the 3130x*l* run sheet to submit for second review. Initial and date where appropriate.

10. To make the data available for review, the project needs to be exported from the Oracle database and placed on the network. Once on the network, the reviewer will have to re-import the project into a local Genemapper station before being able to review.

Exporting a Project

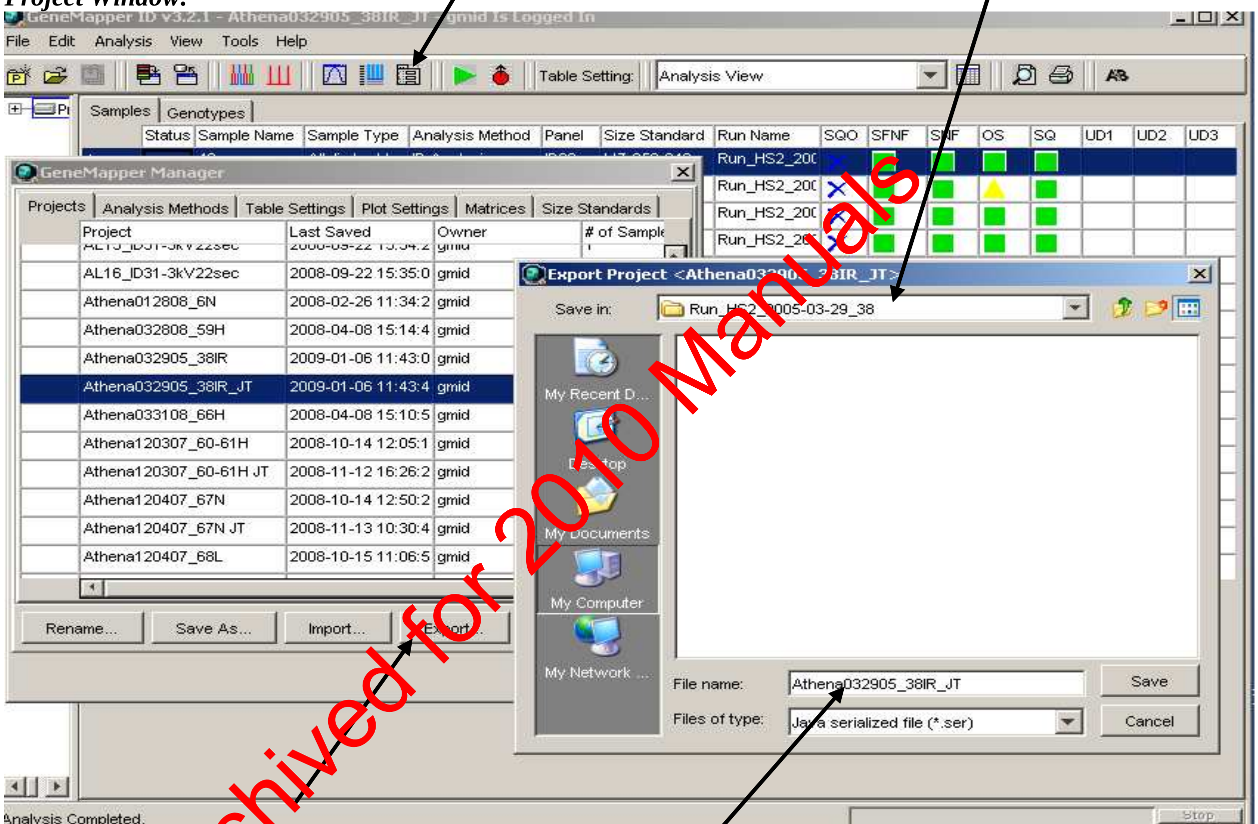
1. Click on Tools → GeneMapper Manager (Ctrl+M) or click on the GeneMapper Manager icon.

2. Select the project to export and click the **"Export"** button. A new window will open. Navigate to the 3130x*l* run folder through the "Save in" drop down box. In the *"File name"* box type in the name of the run. The *"Files of type"* box should be defaulted to Java serialized file (*.ser).

GeneMapper Manager icon

Save in your 3130xl run folder

Project Window:



Export

Type in the name of your run

G. EDITING - REVIEWER

Importing a Project

1. To import the project, open the GeneMapper Manager and click Import.

2. A new window will open asking for the file name. Navigate to the appropriate run folder, select the project and click **Import**. The project will be imported into GeneMapper.

3. To open the project you just imported, click *File* → *Open Project (Ctrl + O).* Select your project and click **Open**.

Electronic Editing - Reviewer

1. The reviewer should check the edits on the editing sheet against the electronic data.

2. To display the sample plots, highlight all samples and click the "Plot View" button or click "Analysis à Display Plots". For more detailed information, refer to Section E *"Plot Views"*.

3. The software always keeps the original allele assignments and a list of all the changes made. If desired, the allele history can be viewed. See *"Appendix E – Troubleshooting Guide, 6. Allele History"* for instructions.

4. To change, revert, or add an edit into the printed sheet, the reviewer should handwrite the correction into the edit table, then initial and date the correction.

5. In the GMID project, to revert an edited peak back to the original allele call, left click on the allele to select it, then right click to ***Rename Allele***; another drop down menu will appear listing all of the possible choices for alleles at that locus. Select the correct allele assignment to re-label the peak. This change will still be added to the history of that allele.

   NOTE: Peaks can be selected and deleted together. For example when a sample is overblown, and you need to remove many peaks in a range, simply select the first peak of the range, and while the first peak is still highlighted, drag a box across the range of peaks to select all. Press the delete key.

   If the reviewing analyst disagrees with the removal of all peaks made during the first analysis, the reviewer should not complete the review. Have the analyzing analyst go back to the project and reanalyze the affected sample(s), re-export the data and create new allele, edit and rerun tables and re-submit for review. The reviewer should then review the entire project again.

6. Once the reviewer approves all the edits, the peaks that are slated to be removed should be deleted by selecting the peaks individually and using the Delete key.

7. A "Delete Allele Comment" box will pop-up. This can be left blank if you agree with the edit. If you made a change to the edit on the editing table, enter the new edit code. Click OK.

8. Once the changed alleles are deleted, the electronic editing sheet cannot be recreated. Therefore, **Re-Save the project as the run name with "Reviewed"** after the analyst's initials so the original edited project is not lost.

9. Print out the electropherograms using the instructions in the next section, Section H *Printing*. The reviewer will sign off on the editing and rerun tables, the control review sheet, and initial the electropherogram pages. If necessary, electronically correct, reprint, and initial the allele table if editing changes were made that affect this table.

10. Export the new project to the run folder on the network as described in the previous section.

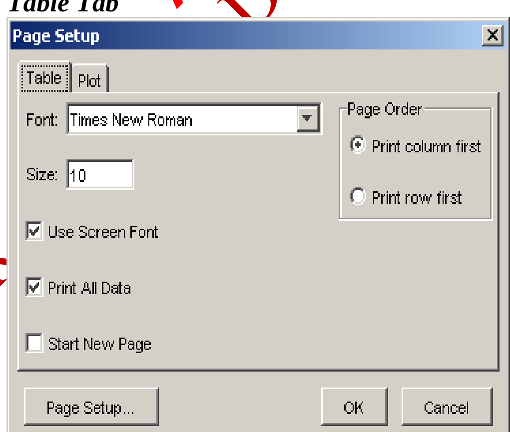
11. Once the project is exported, delete it from the project window in the GeneMapper Manager.

12. Changes to any reviewed project can be saved under the same "reviewed" name. However, the affected pages must be hand initialed by the analyst making the changes.

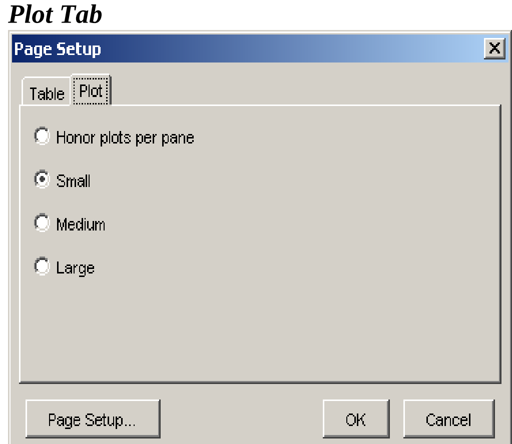
H. PRINTING

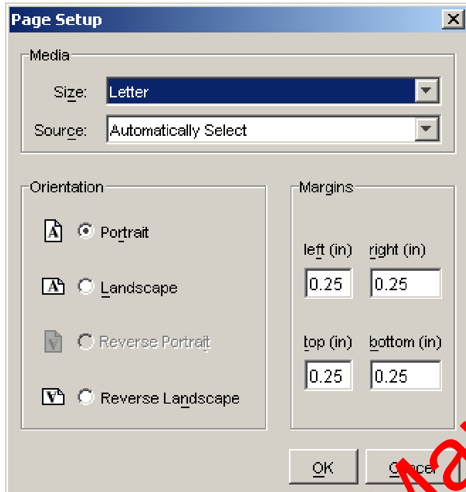
The following are the page settings for the printer that can be checked by selecting *File* from the drop down menu, then *Page Setup* while in the *Samples Plot* view.

Table Tab



Plot Tab





Printing: ID28, PowerPlexY, and MiniFiler

1. Printing is done separately for the allelic ladders, controls, and samples. Each allelic ladder in a project must be printed.

2. In the ***Project Window*** under the ***Samples*** tab, select only the rows you want to print.

3. Click the plots button.

4. In the Samples Plot window, select the plot setting from the drop down list according to the system and sample type you need:

Print - ID Allelic Ladder	Print - ID Controls	Print - ID 28 Samples
Print - PPY Allelic Ladder	Print - PPY Controls	Print - ID 31 Samples
Print - Mini Allelic Ladder	Print - Mini Controls	Print - PPY Samples
		Print - Mini Samples

5. Notice that the font size is reduced to accommodate the print setting. This setting will add the appropriate labels to each peak for printing.

6. Zoom to the appropriate range by using the X-Axis Zooming dialog box to set the plot to the correct range listed in the table below:

X-Axis Zooming:

Identifiler	Zoom from 90 to 370
PowerPlexY	Zoom from 75 to 340
MiniFiler	Zoom from 68 to 300

7. Select *File* from the drop down menu, and then *print* (ctrl+P).

8. If the peaks appear unusually small against the baseline in the printed electropherogram, follow the additional instructions in *Appendix E - Troubleshooting, 4. Printing*, and re-print the affected pages.

Printing: ID31 Samples

1. For ID31 Allelic Ladders and Controls, use the ID print views. Continue below for printing the Samples.

2. In the ***Project Window*** under the ***Samples*** tab, select the replicates of one sample and its corresponding pooled sample (i.e. “trigger_swab_a”, “trigger_swab_b”, “trigger_swab_c”, and “trigger_swab_abc”).

3. Click the plots button.

4. In the Samples Plot window, select the plot setting from the drop down list titled “Print – ID31 Samples”.

5. Notice that in the Samples Plot tool bar only the blue dye is selected. This is because one color will be printed at a time for these sample replicates.

6. Using the X-Axis Zooming dialog box, set the plot to zoom from 90 to 370.

7. Select *File* from the drop down menu, and then *print* (ctrl+P).

8. If the peaks appear unusually small against the baseline in the printed electropherogram, follow the additional instructions in *Appendix E. Troubleshooting Guide, 4. Printing*, and re-print the affected pages.

9. In the Samples Plot tool bar, unselect the blue dye by clicking it, and select the green dye. With only the green dye selected repeat steps 5 and 6 for the green dye. Then repeat steps 5 and 6 for the yellow dye, red dye and orange dyes individually.

10. After all colors have been printed for one triplicate sample, repeat steps 1 through 7 for the next sample in the injection until all samples in that run have been printed.

Revision History:

March 24, 2010 – Initial version of procedure.

The **Pass** (green square) symbol indicates that no problem exists. The **Check** (yellow triangle) symbol appears when there are problematic components such as missing size standards, or off-scale data. The **Low Quality** (red octagon) symbol appears when the result falls below the defined threshold.

Whether you identify a size standard problem or not, proceed to the sizing section of the manual to individually check each size standard.

The following flags are visible in the **Project Window** with the **"Samples"** tab selected:

Quality Flag in "Samples" tab	Code
Sizing Quality Override – This check box marks the samples that have had the size standard quality score overridden. This box can also be used to indicate if the size standard has been reviewed.	**SQO**
Sample File Not Found – if the software cannot locate the .fsa files that correspond to a project, a yellow "check" flag is displayed. Re-import the run into the GeneMapper® *ID* software.	**SFNF**
Size Standard Not Found – A yellow "check" flag is displayed when no size standard is found in the sample. If a size standard has failed, it will be assigned an SQ value of 0.0 and "no sizing data" will be displayed in the "samples plot" window.	**SNF**
Off scale – This flag directs your attention to overblown peaks whose height [RFU] exceeds the range of the collection instrument.	**OS**
Sizing Quality – Values closest to 1.0 are denoted by a green "pass" flag. Questionable data is within the range of 0.25 and 0.75, and indicated with a yellow "check" flag. Low quality data is within the range of 0.0 – 0.25 and denoted by a red flag. If the RFU of the size standard falls below our detection threshold, it will be assigned an SQ value of 0.0, and the corresponding sample will display "no sizing data" in the "samples plot" window.	**SQ**

These flags are intended to draw your attention to samples that have analysis problems. These flags do not replace our method for editing samples. Each sample must still be viewed and edited. If you identify a problem in a sample that can be edited, proceed to the editing section of this manual.

The following flags are visible in the **Plot View** with the **"Genotypes"** tab selected:

Quality Flag in "Genotypes" tab	Code
Allele Display Overflow – This check box indicates that there are more alleles at this locus than are displayed in the current window view.	**ADO**
Allele Edit – This box is checked when the allelic calls have been edited by the analyst in the plot view page.	**AE**
Off scale – This flag directs your attention to overblown peaks whose height [RFU] exceeds the range of the collection instrument for each locus.	**OS**
Out of bin allele – Displays a yellow "check" flag when peaks are outside of the bin boundary. These peaks are called OL.	**BIN**
Peak Height Ratio – Displays a yellow "check" flag if the ratio between the lower allele height and the higher allele height are below 70%. This value can be set in the Analysis Methods Peak Quality window.	**PHR**
Allele Number – This flag is a useful indicator of mixture samples, locus dropout, and extraneous alleles in the positive and negative controls. A yellow "check" flag is displayed when the number of alleles exceeds the number of expected alleles at a locus for the individual, or if no alleles are found. This number can be set in the Analysis Methods Peak Quality window.	**AN**

Quality Flag in "Genotypes" tab	Code
Control Concordance – Serves as quality assurance during STR analysis. A yellow "check" flag appears when the designated control sample (positive or negative) does not exactly match the defined alleles at each locus.	**CC**
Overlap – It is possible to have two allele size ranges that overlap, therefore a yellow "check" flag is displayed when a peak in the overlapped region is called twice.	**OVL**

Revision History:
March 24, 2010 – Initial version of procedure.

GENEMAPPER ID – EDITING CODES		
DATE EFFECTIVE 03-24-2010	APPROVED BY EUGENE LIEN	PAGE 1 OF 1

Reason for Edit	Edit Code
Pull-ups of peaks in any color caused by a very high peak of another color in the same basepair range of a sample	**1**
Shoulder peaks approx. 1-4 bp bigger or smaller than main peak	**2**
Split peak due to "N" bands	**3a**
Split peak due to matrix over-subtraction	**3b**
stutter in non-mixtures +	**4a**
stutter preceding shoulder in a mixture ++	**4b**

Reason for Edit	Edit Code
>20% stutter w/main peak plateau in non-mixtures	**4c**
Non specific artifacts +++	**5**
Labels placed on elevated baselines	**6**
Spikes or peaks present in all colors in one sample	**7**
Dye artifact occurring at a constant scan position	**8**
Peak outside of printed scan range	**9**

\+ This edit is applicable for stutter peaks in non-mixtures in +/-4 bp positions for both Identifiler® and Power Plex® Y and in +/-3 bp positions at DYS392 and +/-5 bp positions at DYS438 for Power Plex® Y only.

++ This edit is applicable for stutter peaks preceding a shoulder in a mixture in the -4 bp position for Identifiler and the -3, -4, and -5 bp positions for Power Plex® Y.

+++ For Power Plex® Y, this edit is applicable for artifacts in the +/-2 bp position for DYS389II and DYS19, the -9 and -10bp position at DYS393 and the -5, -9, and -10 bp positions at DYS437 and DYS385.

Revision History:
March 24, 2010 – Initial version of procedure.

GENEMAPPER ID – RERUN CODES		
DATE EFFECTIVE 03-24-2010	APPROVED BY EUGENE LIEN	PAGE 1 OF 1

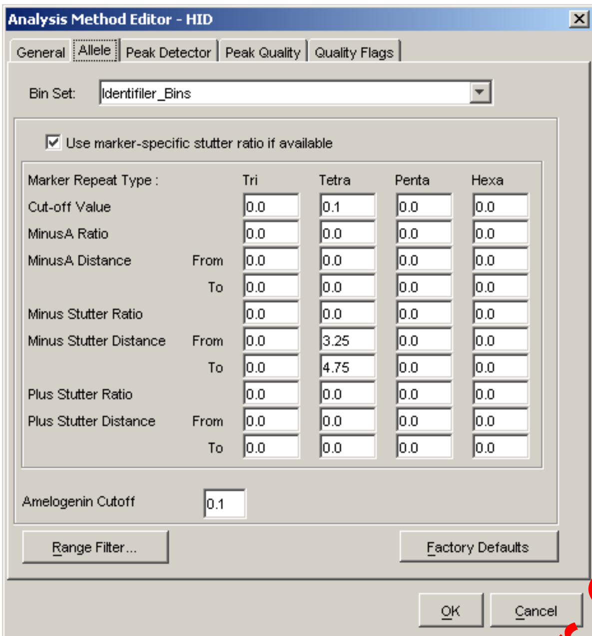
Sample Status	Code
Sample inconclusive, all peaks removed.	*
Sample shows presence of OL allele	^
No or poor size standard	#

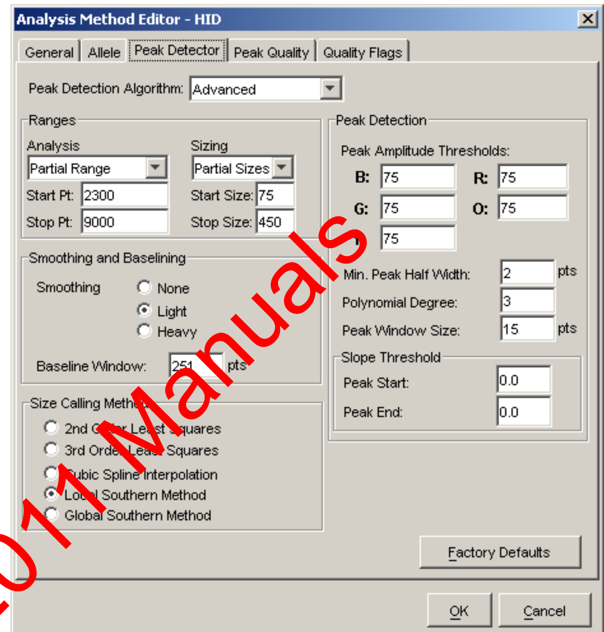
System for Rerun	Code
PowerPlexY	**Y**
Identifiler	**I**
MiniFiler	**F**
Do not rerun	**N/A**

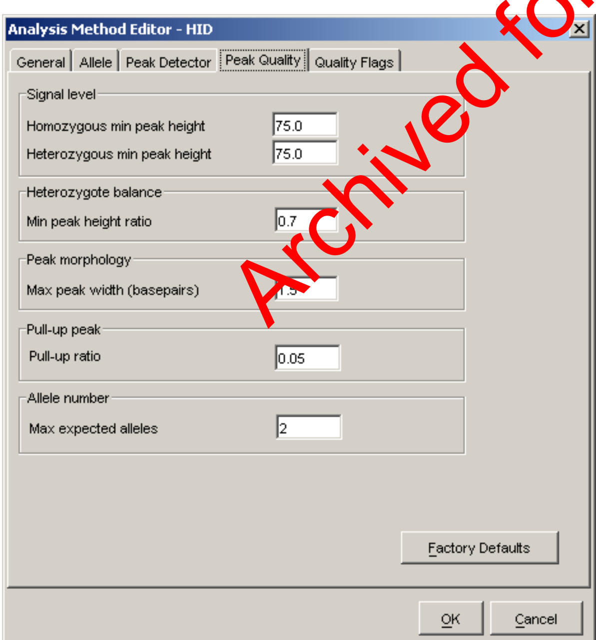
Parameter for Rerun	Code
Normal (HCN)	**no code**
High (HCN)	**R**
1/5 dilution	**D.2**
1/10 dilution	**D.1**
1/20 dilution	**D.05**
1/100 dilution	**D.01**
Re-aliqout 1 ul	**1ul**
Re-aliqout 2 ul	**2ul**
1 kV 22 s (LCN)	**L**
3 kV 20 s (LCN)	**N**
6 kV 30 s (LCN)	**H**

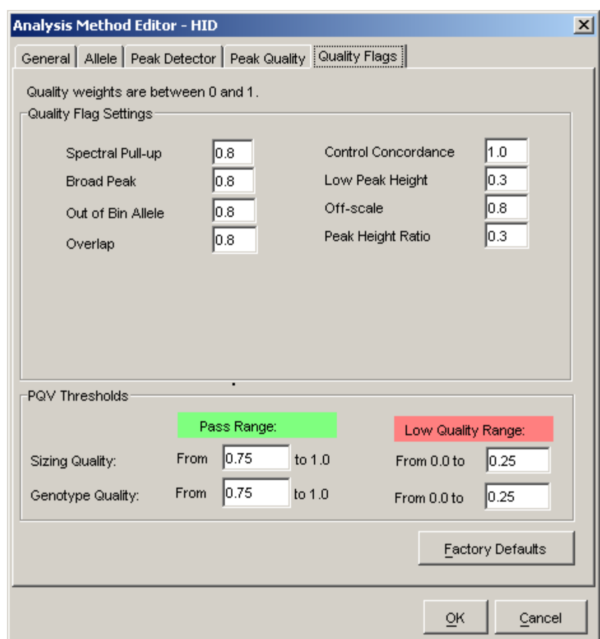
Revision History:
March 24, 2010 – Initial version of procedure.

Identifiler Analysis Settings:

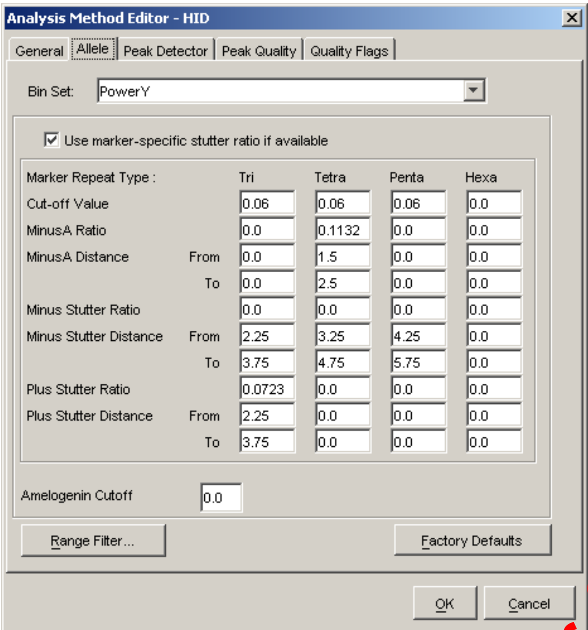


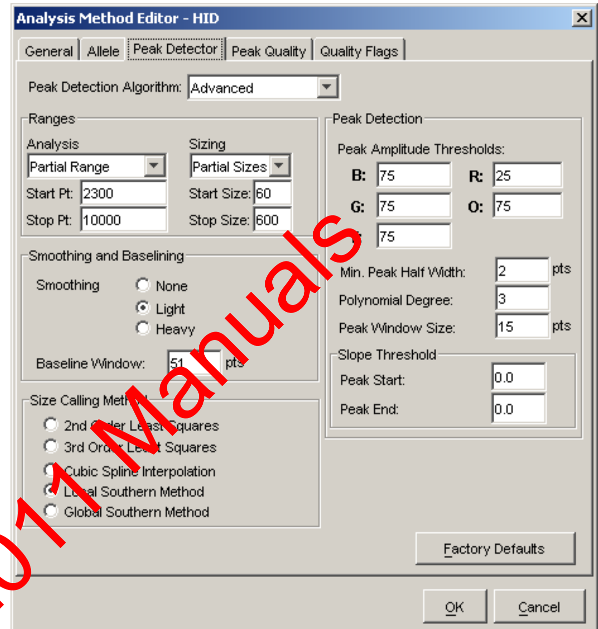


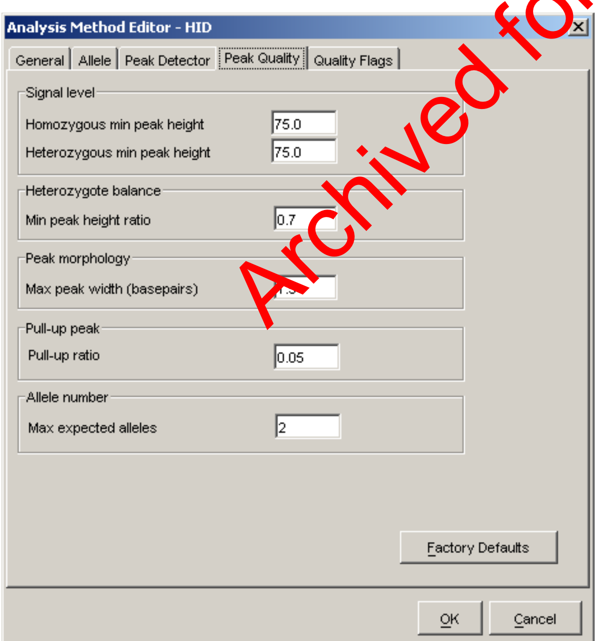


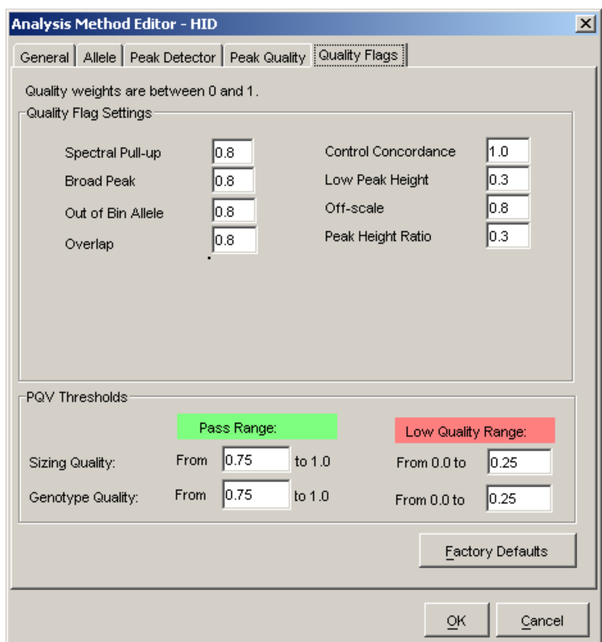


PowerPlexY Analysis Settings:

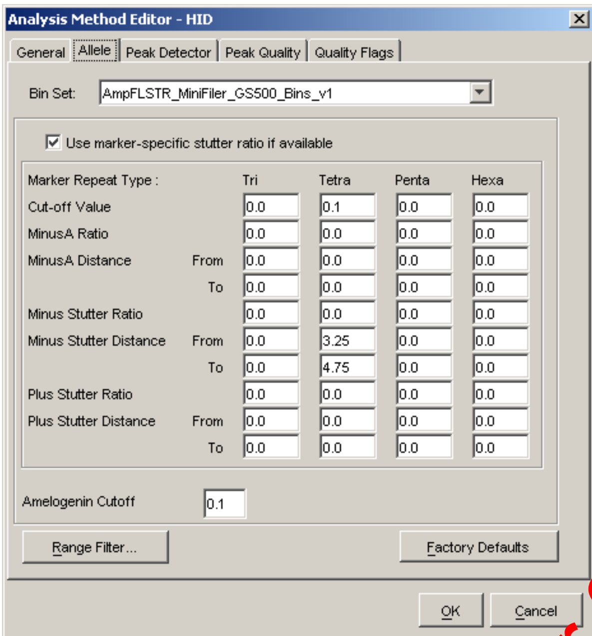


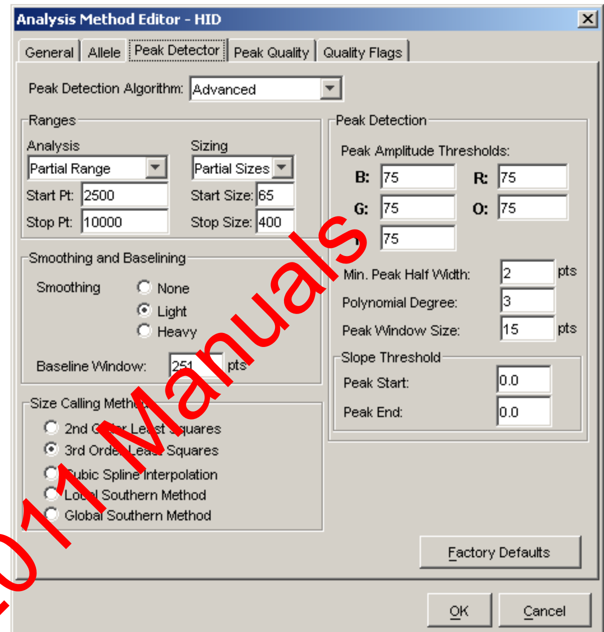


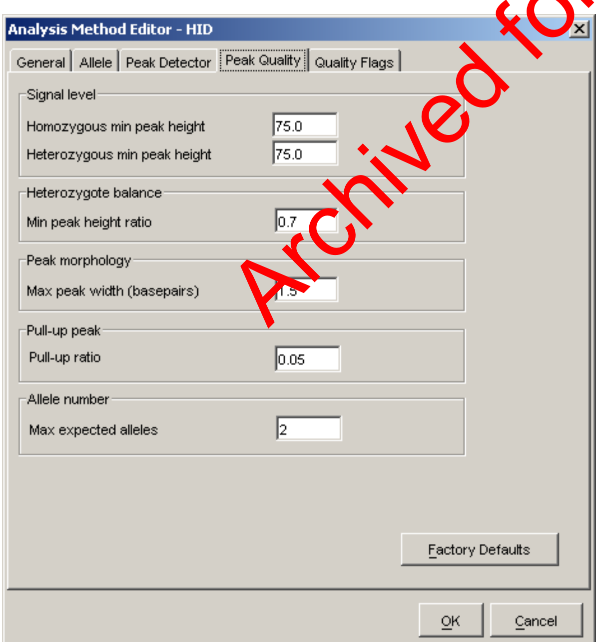


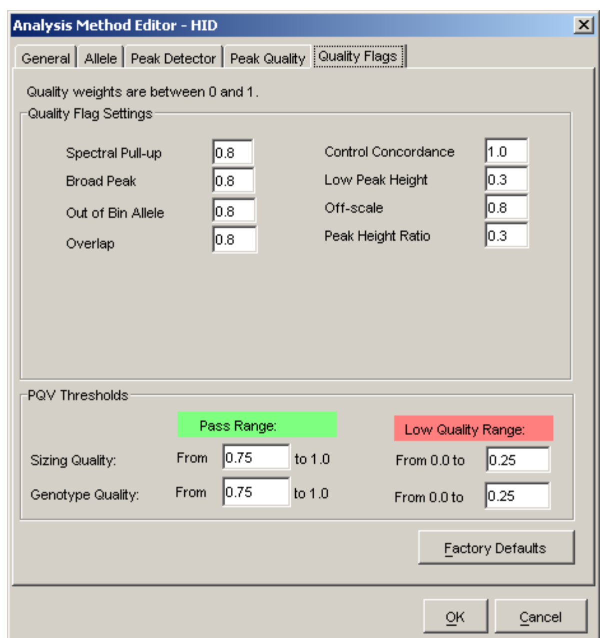


MiniFiler Analysis Settings:







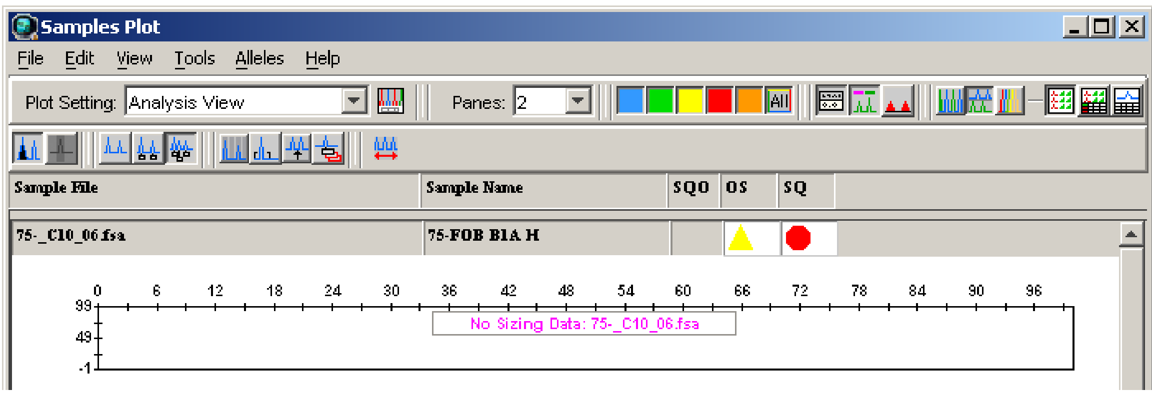


Revision History:

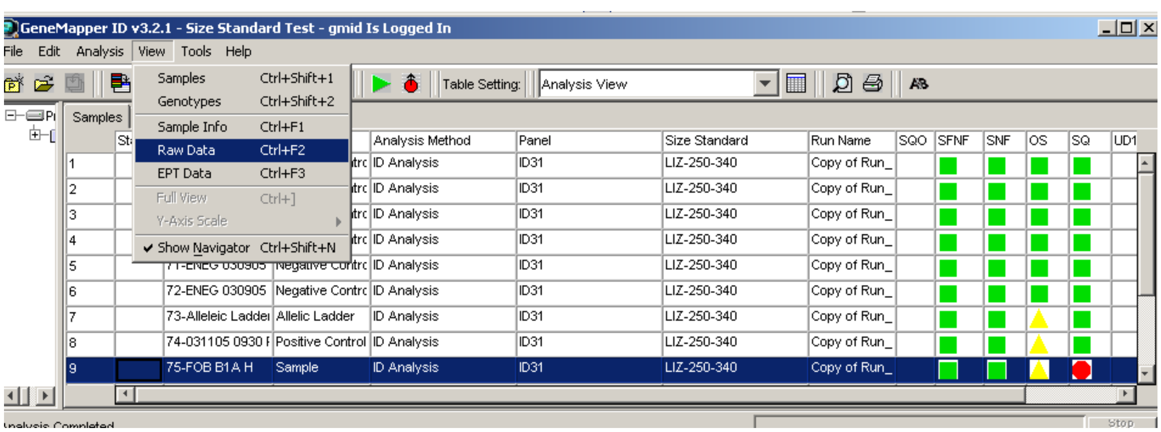
March 24, 2010 – Initial version of procedure.

1. REDEFINING THE SIZE STANDARD

1.1. PROBLEM: "No Sizing Data" message; red octagon in SQ column

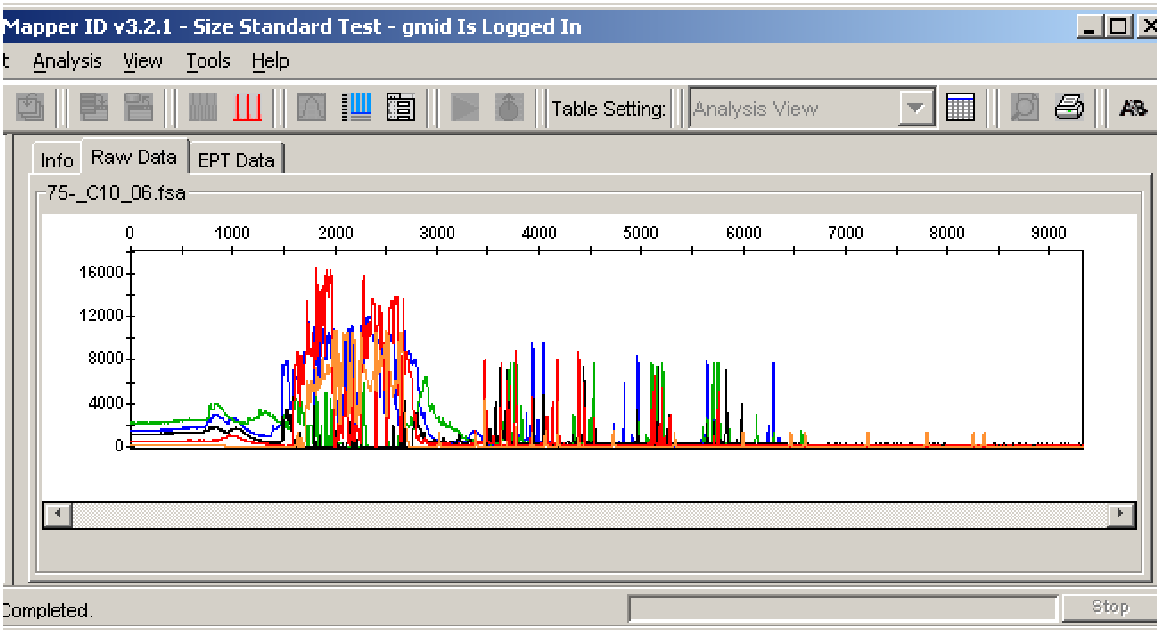


a. Select the flagged sample in the ***Samples*** tab of the ***Project Window*** as shown in the picture below.

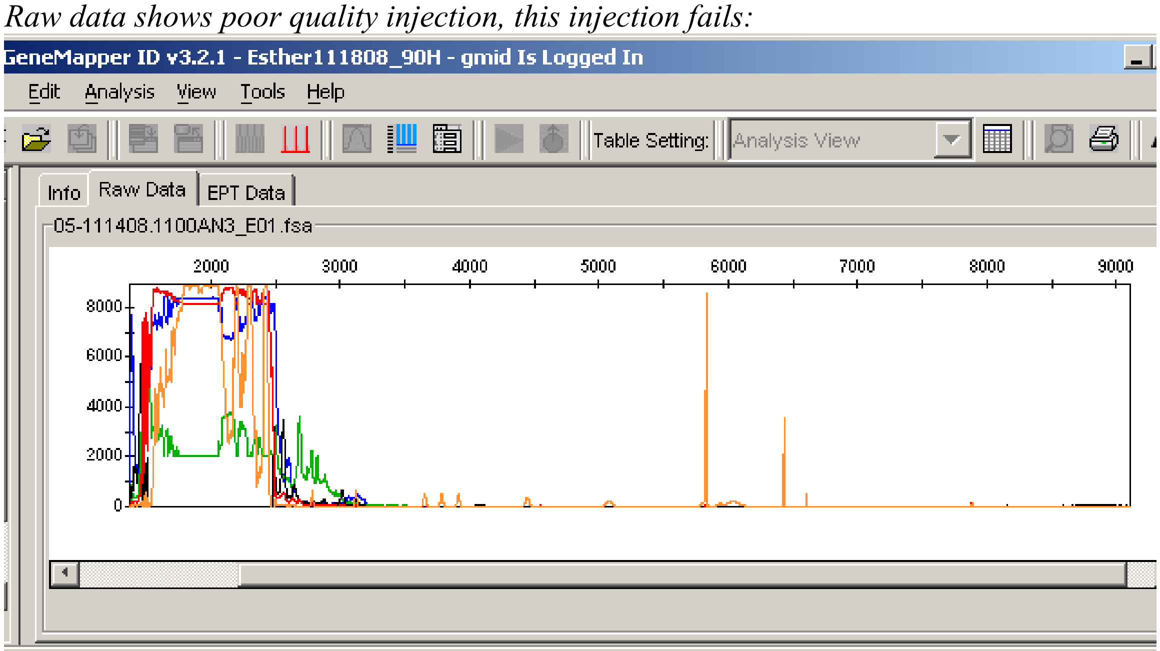


b. From the ***View*** drop down menu, select ***Raw Data* -** this will show what the sample looks like. If raw data is visible, and after analysis there is "No Sizing Data", most likely the size standard is mislabeled. If no raw data is visible, the injection for that capillary failed or no sample was loaded in to the well.

Raw data view shows usable data:

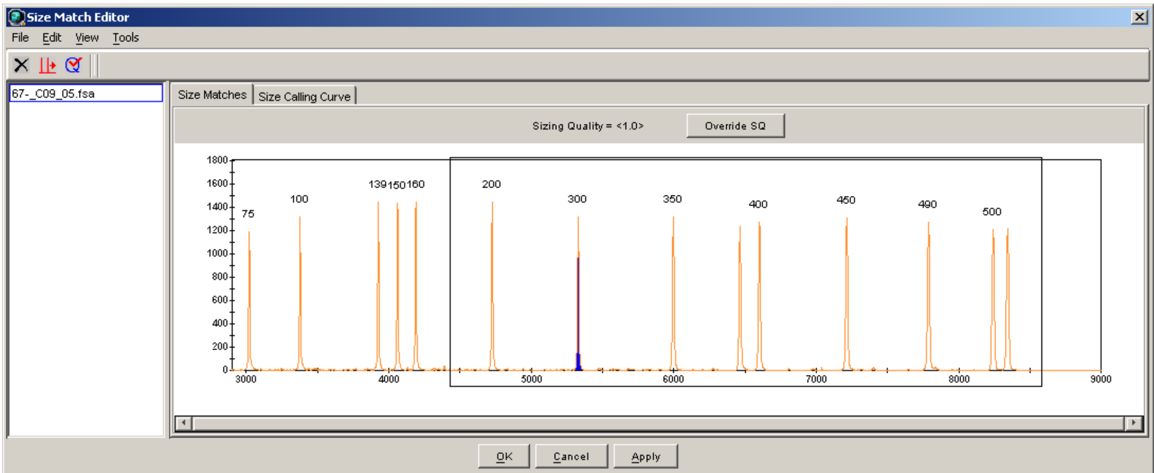


Raw data shows poor quality injection, this injection fails:



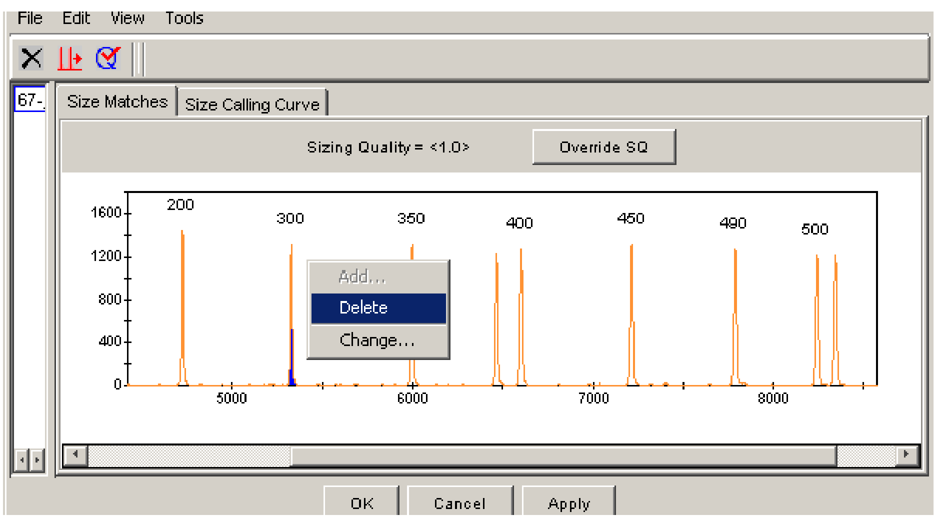
c. Click on the Size Match Editor icon in the toolbar to open the sizing window. Here you can see the labels that the macro assigned to each peak in the size standard for that sample.

d. Using the magnifying tool, zoom in on the area that appears to be mislabeled.



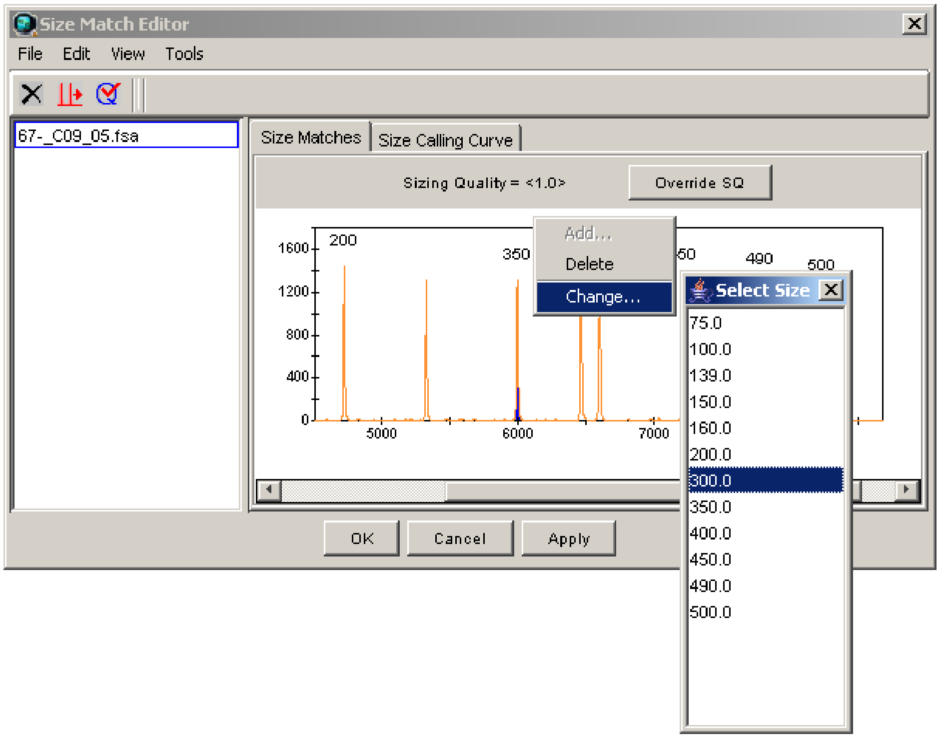
e. Left click to select the peak that needs to be changed. The peak will be highlighted in blue.

f. Right click on the peak which is mislabeled, a menu pops up, with add, delete or change.



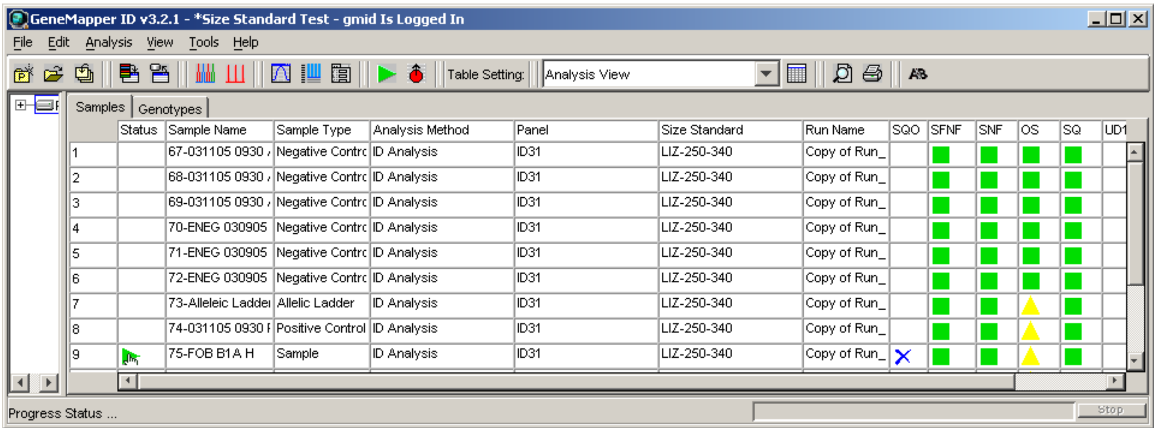
g. If a peak is labeled which is not supposed to be (the 250 or 340 peaks), select delete and the peak is unlabeled.

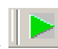
h. To re-label a peak correctly, select ***change***, a dropdown list appears with the choices for that size standard. Choose the correct one. The peak will be re-labeled.



i. Once all the changes are made, click on ***Apply*** to apply the changes. And then ***Ok*** to close the window.

j. From the ***View*** drop down menu, select ***Samples*** to return to the ***Samples*** tab. In the ***Analysis View*** table setting, notice that the SQO box for that sample has a blue "X", the SQ box has turned to a green square, and the status box for that sample has a green arrow. The green arrow indicates that a setting (in this case it's the size standard) has been modified and it needs to be re-analyzed.



k. Click on the green analyze button  in the toolbar to re-analyze that sample with the redefined size standard.

2. ADJUSTING THE ANALYSIS DATA START POINT AND STOP POINT RANGE

2.1. PROBLEM: The data is too far to the left or right of the injection scan range, or the size standard is cut out of the analysis range and therefore labeled incorrectly.

a. From the ***View*** drop down menu, select ***Raw Data.***

b. In the raw data view, choose a ***start point*** between the dye blob region that appears at the beginning of every injection, and the first required peak of the size standard by hovering the mouse pointer over that peak on the x-axis. At the bottom of the screen you will see that the data point and RFU is displayed for the area you are hovering with the mouse. Try not to include any of the blobs in the beginning of the run as they tend to be very high RFUs and the software uses the highest signal in each color to determine the Y axis cut-off in the plot view.

c. Choose a ***stop point*** anywhere after the last peak in the size standard.

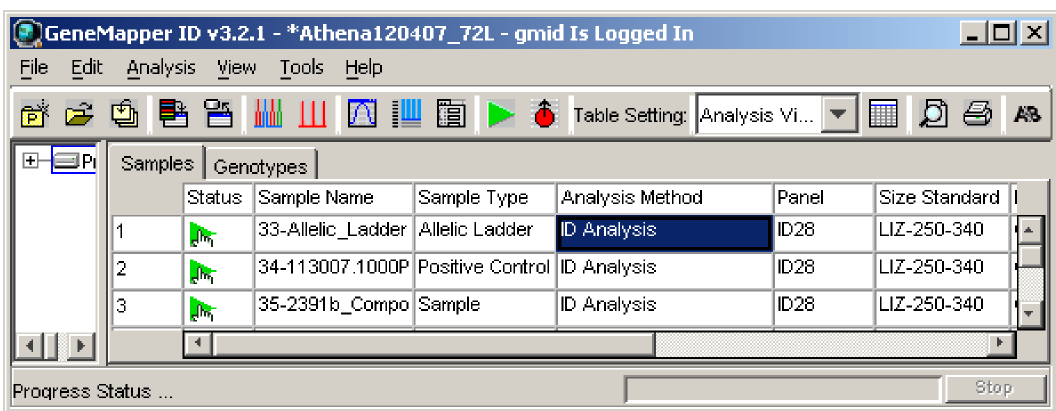
d. At a minimum the following size standard peaks must be present for proper analysis:

- For Identifiler, 100bp to 450bp minus the 250bp and 340bp peaks.
- For PowerPlexY, 60bp to 375bp.
- For Minifiler, 75bp to 400bp minus the 250bp and 340bp peaks. (The Analysis Methods peak detector tab must start at 65bp and not 75bp in order to properly size peaks. This is because the 3rd Order Least Squares is the size calling method used.)

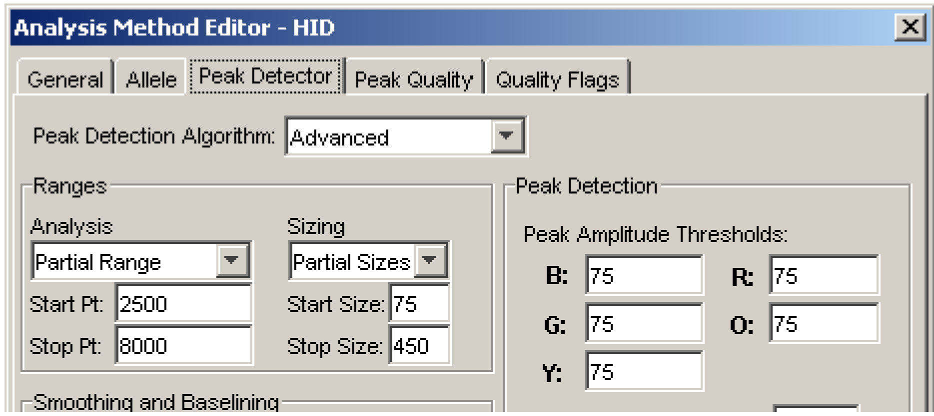
NOTE: If the data in an Identifiler run is too far to the right and the last two peaks of the size standard (490 bp and 500 bp) are cut out of the visible range (as seen in the raw data view), the run can still be analyzed by selecting the size standard named "LIZ-250-340-490-500". In this case your ***stop point*** for the analysis range should be set to 10,000. Additionally, QC should be notified to inspect the instrument as this occurrence is usually indicative of a polymer leak.

e. From the ***View*** drop down menu, select ***Samples*** to return to the ***samples*** tab.

f. Select the analysis method in the project window to highlight it blue, and then double click to open it.



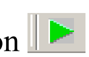
g. The ***Analysis Method Editor*** window will automatically open to the ***Peak Detector*** tab.



h. In the ***Ranges*** section, change the ***start point*** and ***stop point*** as necessary. The only other setting that can be changed in this window is the ***Peak Amplitude Thresholds*** for the color of the size standard. If the size standard produced a low RFU signal this setting can be lowered to 25 RFU only in orange for Identifiler and MiniFiler and only in red for PowerPlexY.

i. Click ***OK***.

j. When you return to the ***samples*** tab, you will see that the samples have a green arrow in the status column signaling that a setting has been modified and it needs to be re-analyzed.

k. Click on the green analyze button  in the toolbar to re-analyze with the modified setting.

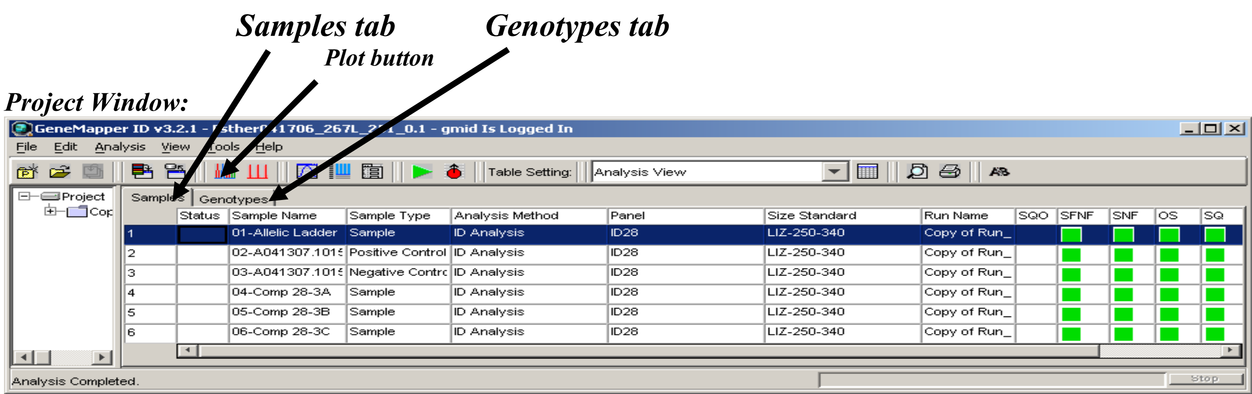
3. Genotypes Plot – Locus Specific Quality Flags

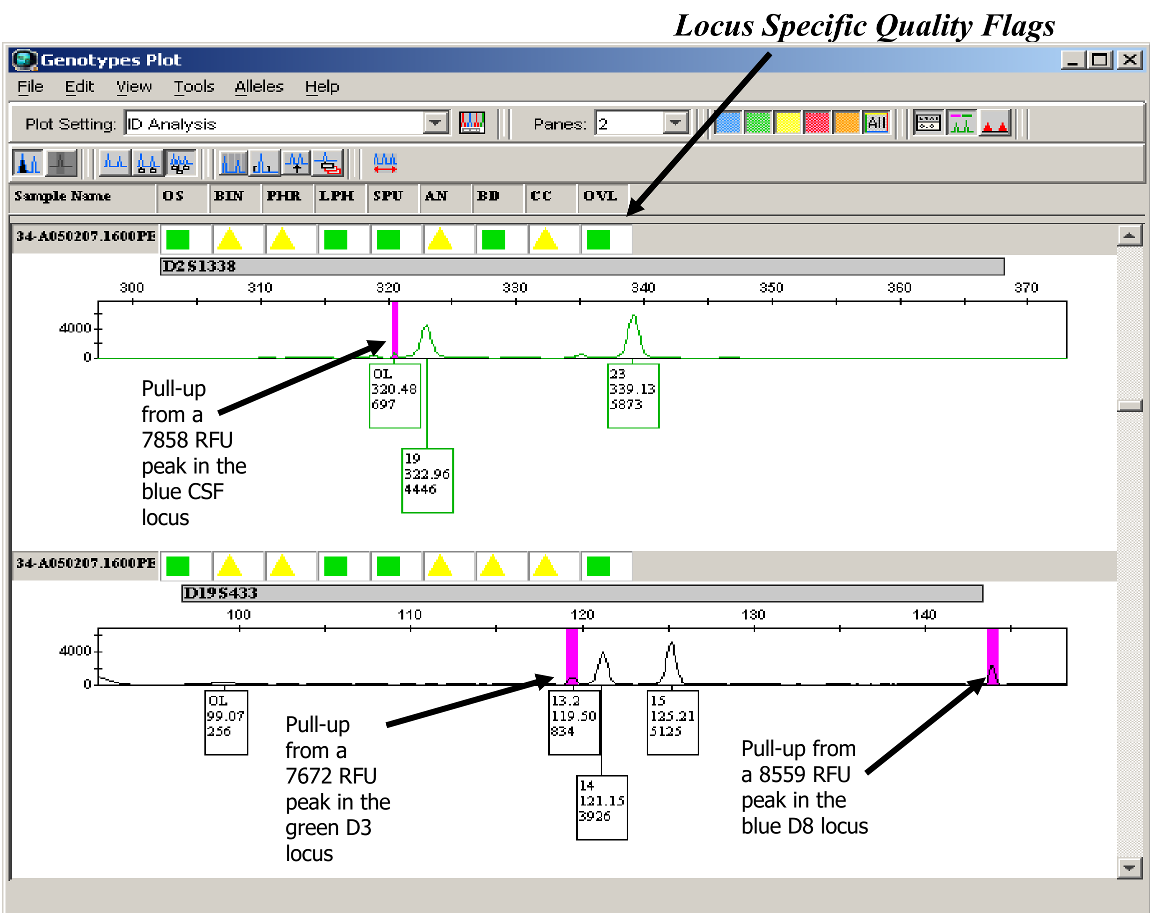
3.1. PROBLEM: You see "no room for labels" in the panes of the *Samples Plot* window.

a. In the ***Project Window*** select the ***Genotypes*** tab, and then click the plot button (Analysis → Display Plots or Ctrl+L). This plot window displays each locus in a separate pane; this is called the "***Genotypes Plot***". Here you can clearly view each locus with its relevant quality flags. Once you are in the plot view you can toggle between the ***Samples Plot*** and the ***Genotypes Plot*** by going to the ***Project Window*** and selecting the ***Samples*** tab or ***Genotypes*** tab.

b. If a locus contains a peak that exceeds the saturation threshold of the 3130x*l* a pink line will indicate the affected basepair range in every color, and draw attention to areas where the off-scale peaks have created pull-up.

c. These pink lines can be turned on or off from the plot window by selecting View → "Off-scale peak indicator" from the pull down menu. Ensure that the off scale peak indication is checked on.





d. Regardless of peak height, if the pink off scale indicator is not triggered, the sample does not need to be rerun.

e. For High Copy Number testing:

i. Mixtures: If there is an overblown peak in one of the loci with a pink line region then remove all peaks and run with a dilution.

ii. Oversaturated single source samples with plateau shaped or misshaped peaks with numerous labeled stutter peaks and artifacts should have all peaks removed and run with a dilution.

f. For High Sensitivity testing:

i. Mixtures: If there is an overblown peak in one of the loci with a pink line region then edit all alleles at that locus, and also edit all the peaks at all other loci within that base pair range as a * (all peaks removed edit). These other loci will also be easily identifiable because they have the pink line indicating where the overblown peak from the other color has interrupted that entire base pair range. Rerun at a lower parameter (if applicable) or with a dilution.

ii. Oversaturated single source samples with plateau shaped or misshaped peaks with numerous labeled stutter peaks and artifacts should have all peaks removed and run with a dilution.

g. The quality flags in the ***Genotypes*** window indicate locus specific problems. If a yellow "check" flag, or a red "low quality" flag result in any of the columns, refer to the appendix A – "Quality Flags" for a description of the flags and the problems they identify.

NOTE: The locus specific quality flags can only be viewed in the ***Genotypes Plot*** window.

4. PRINTING

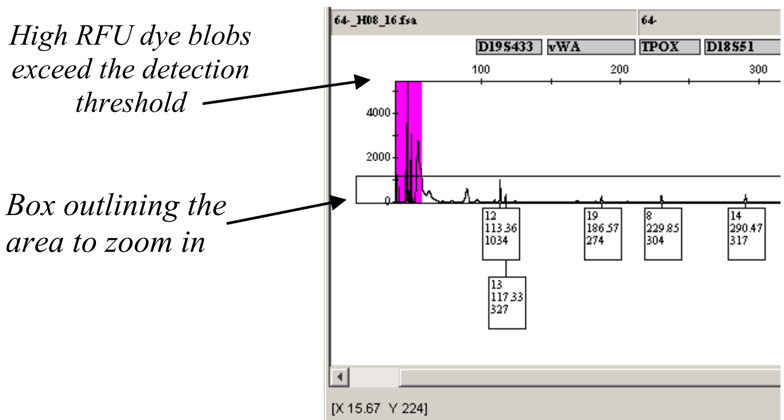
4.1. PROBLEM: The peaks in the printed electropherogram appear unusually small.

a. The maximum RFU signal in each color is used to calculate the Y axis cut-off value for the plot display.

b. When the analysis range includes too much of the dye blob region that appears at the beginning of each run, the Y axis cut-off will be very high because the blobs in the beginning of the run generally have high RFUs. As a result, the true peaks will appear really small in the plot display.

c. To adjust the Y axis cut-off, move the mouse pointer over the numbers on the Y axis. Notice that the pointer will turn into a magnifying glass. While holding the left mouse button down you can move the magnifying glass up and down the Y axis and a box will form outlining the area to be zoomed in. Choose a level directly above the tallest peak. When you release the left mouse button, the area will automatically zoom in.

d. If you need to zoom back out to the full range, double click on the Y axis while the mouse pointer is in the magnifying glass form.



e. Do this individually for each color where the peak display is affected by the high RFU blob region.

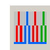
f. Print the electropherogram as described in section H. *Printing*.

5. ALLELIC LADDER

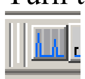
5.1. PROBLEM: All of the peaks in the ladders and my samples are labeled "OL".

Make sure that only the allelic ladders are designated as "Allelic Ladder" in the ***Sample Type*** column in the project window and rerun the analysis.

5.2. PROBLEM: There is a confirmed off-ladder in my sample, how do I determine the closest allele call?

a. Select the ladder with your sample and view the plot by clicking on the ***Display Plots*** button in the toolbar. 

b. Turn off all colors except the color in which the OL appears using the quick select color buttons in the toolbar. 

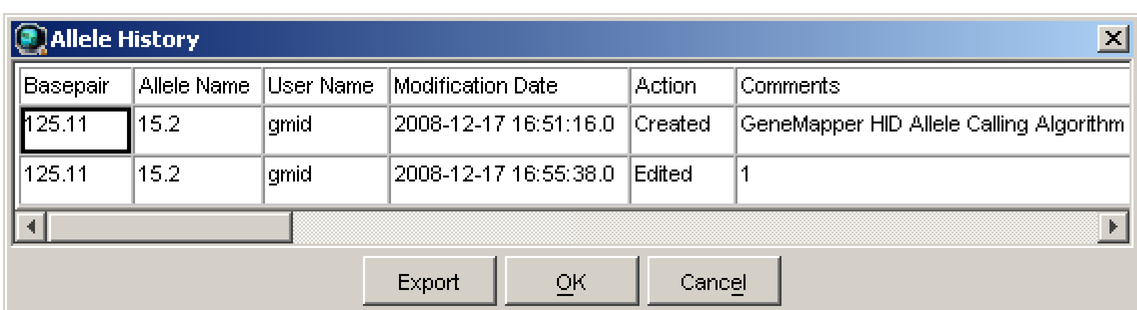
c. Turn the bins on by clicking on the ***Show Bins*** button in the toolbar.


d. Zoom in to the locus where the OL appears. The bins for that locus will be shaded in grey and you can determine what the true allele would be.

6. ALLELE HISTORY

6.1. PROBLEM: How do I know the history of an allele that was edited?

a. Double click on the allele and a window opens with the allele history of that peak. When an allele is created by the macro, it will read "GeneMapper HID Allele Calling Algorithm" in the comments section. The rest of the table describes the action taken on that peak. In this example allele 15.2 was edited as pull-up. The action column describes what was done to the peak and the comments column contains the editing code.


Allele History

Basepair	Allele Name	User Name	Modification Date	Action	Comments
125.11	15.2	gmid	2008-12-17 16:51:16.0	Created	GeneMapper HID Allele Calling Algorithm
125.11	15.2	gmid	2008-12-17 16:55:38.0	Edited	1

Export OK Cancel

b. If when you double click on a peak, a button pops up that reads "add allele call", it means that the peak was not labeled by the GeneMapper macro.

6.2. PROBLEM: How do I view all deleted peak calls in a project?

Select all the samples in the ***samples*** tab of the ***project window***. Click the Samples Plot button to view the electropherogram. In the ***View*** dropdown menu, select ***Allele Changes***. Any peak that was called and subsequently deleted will appear with a strike out as depicted below.

7. SAMPLE HISTORY

7.1. PROBLEM: How can I see the run log for a sample to determine how the run was injected and analyzed?

a. In the ***project window*** under the ***samples*** tab, select the sample(s) of interest.

b. From the ***View*** drop down menu, select ***Sample Info***

c. This view contains all of the information pertaining to the sample including error messages, current settings, run information, data collection settings, and capillary information.

8. TYPOGRAPHICAL ERROR IN SAMPLE

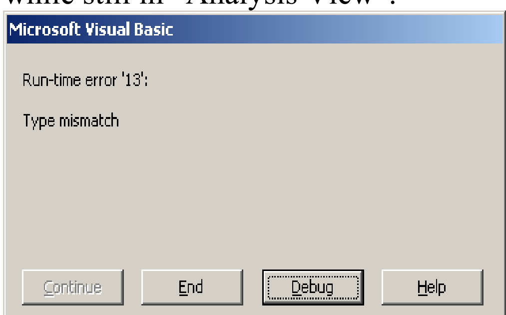
8.1. PROBLEM: There is a typo in the sample name.

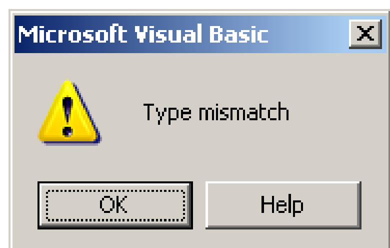
In the ***project window*** under the ***Samples*** tab, click on the sample name in the ***Sample Name*** column and correct the error.

9. TABLE ERRORS

9.1. PROBLEM: An error message occurs when making the allele table.

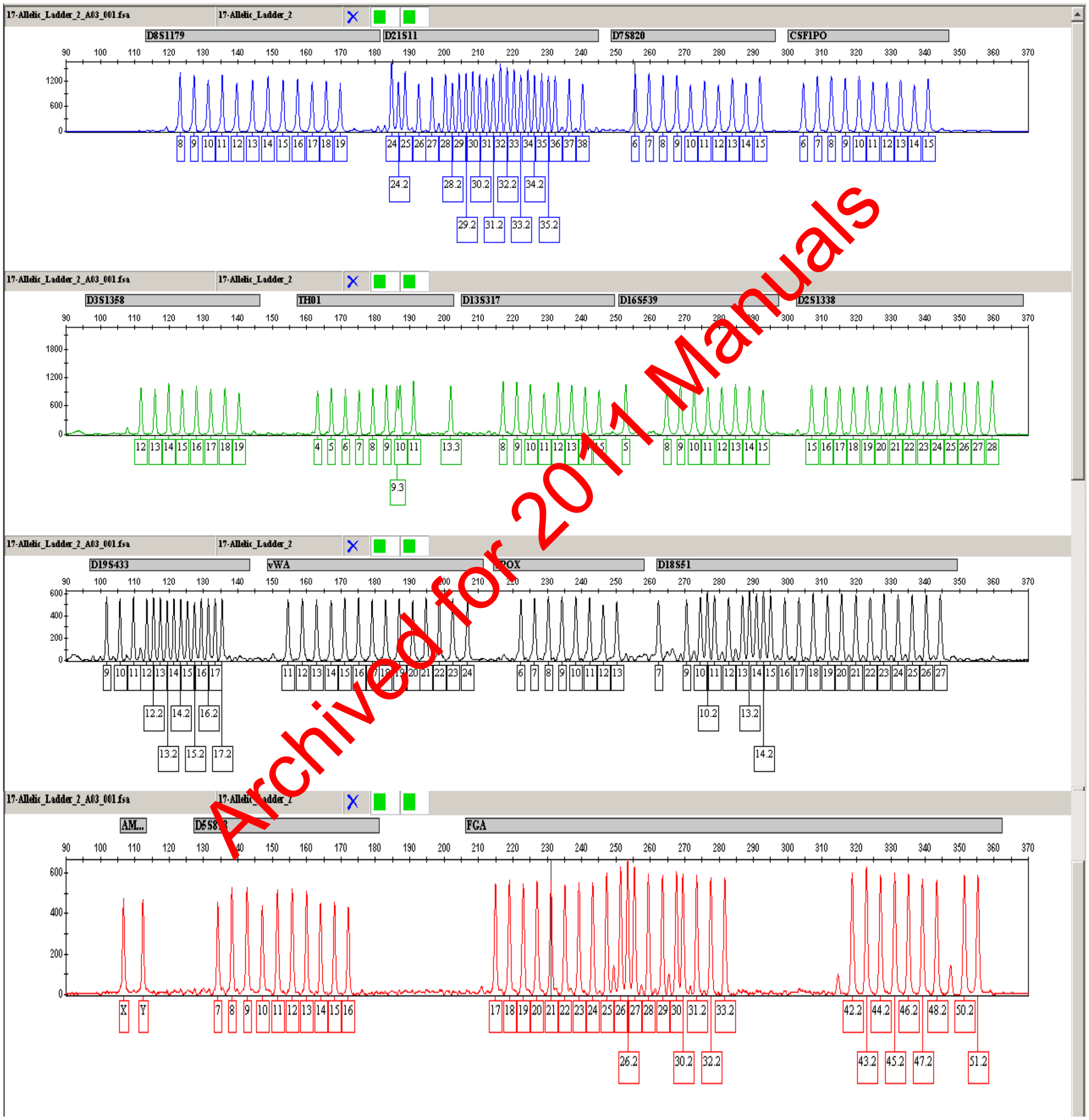
If you get an error message, this means that you have exported the combined table while still in "Analysis View".



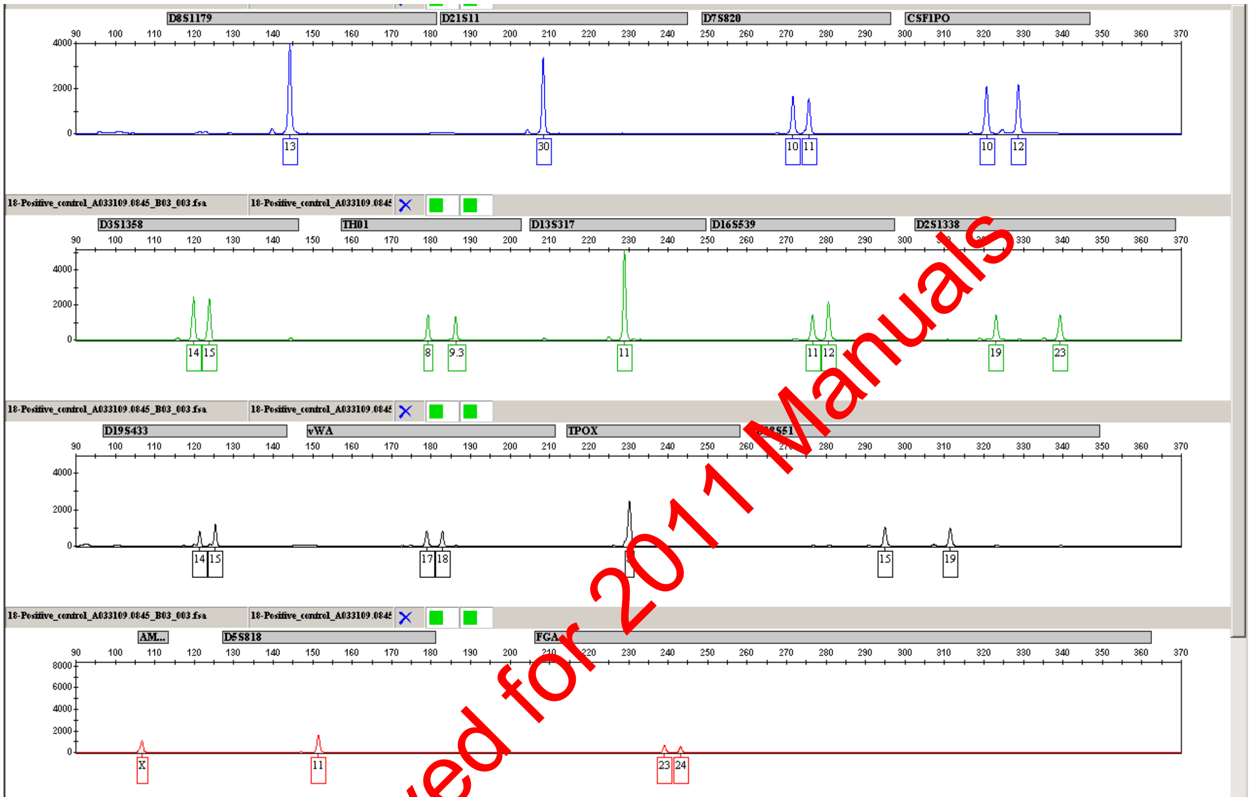


Click "End" or "OK" to close the error window, and close the excel worksheet without saving. Go back to your project in GeneMapper® *ID*. In the ***Project Window*** change the table setting drop down menu to **"Casework"**. Re-export the combined tables, then re-import into a new excel worksheet.

Identifiler Allelic Ladder

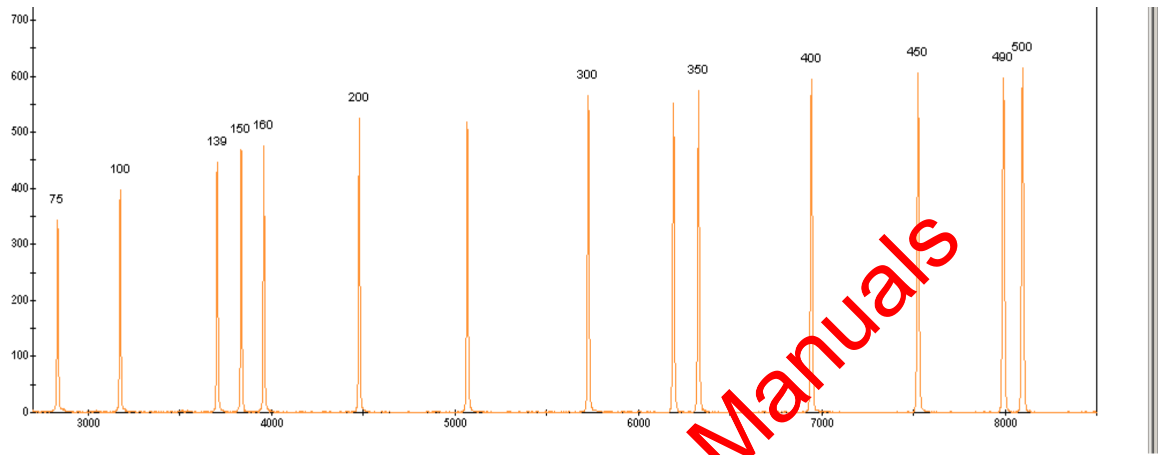


Identifiler Positive Control



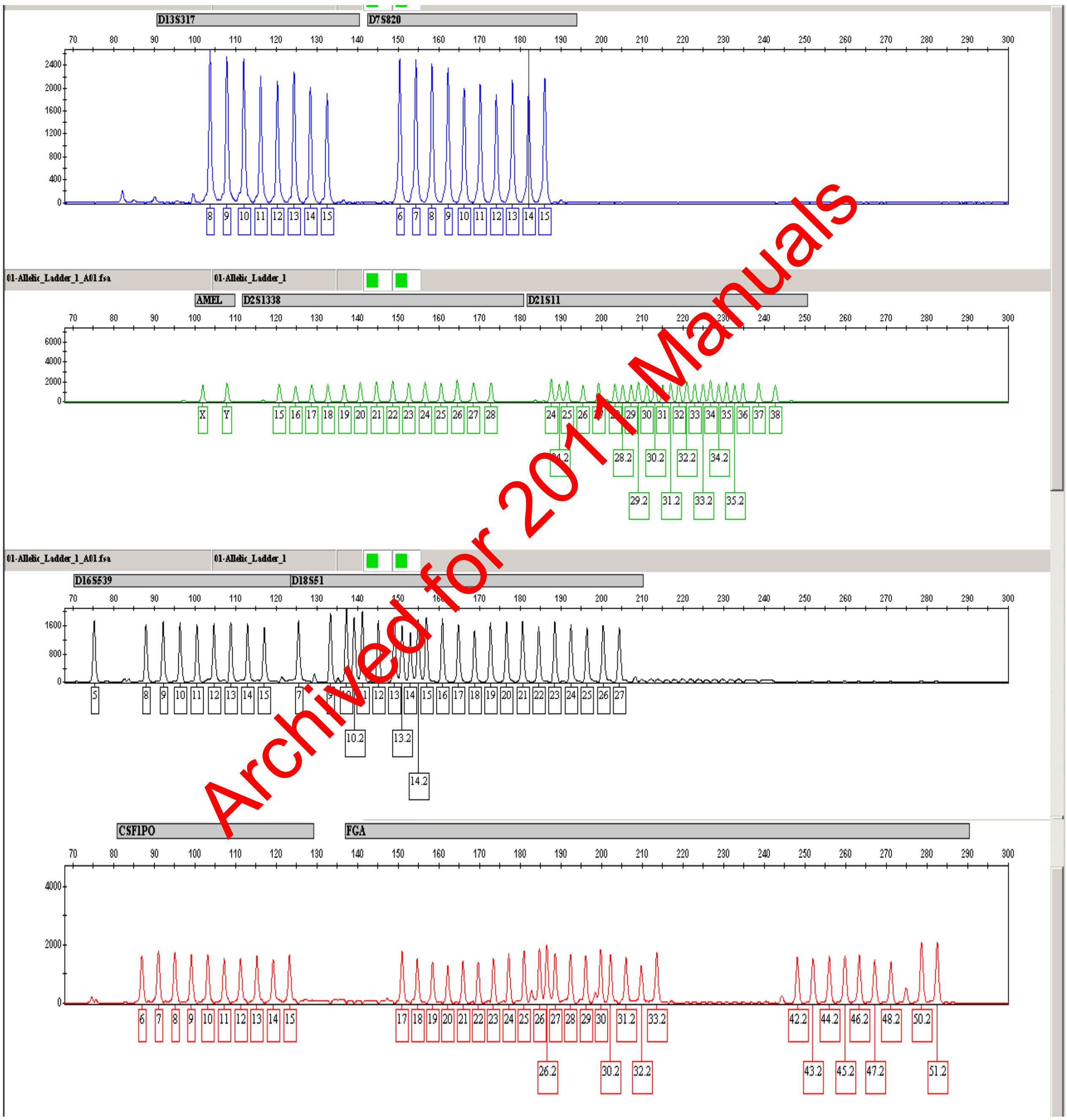
Blue (6-FAM)	**D8S1179**	**D21S11**	**D7S820**	**CSF1PO**	
	13	30	10, 11	10, 12	
Green (VIC)	**D3S1358**	**TH01**	**D13S317**	**D16S539**	**D2S1338**
	14, 15	8, 9.3	11	11, 12	19, 23
Yellow (NED)	**D19S433**	**VWA**	**TPOX**	**D18S51**	
	14, 15	17, 18	8	15, 19	
Red (PET)	**AMEL**	**D5S818**	**FGA**		
	X	11	23, 24		

LIZ-250-340

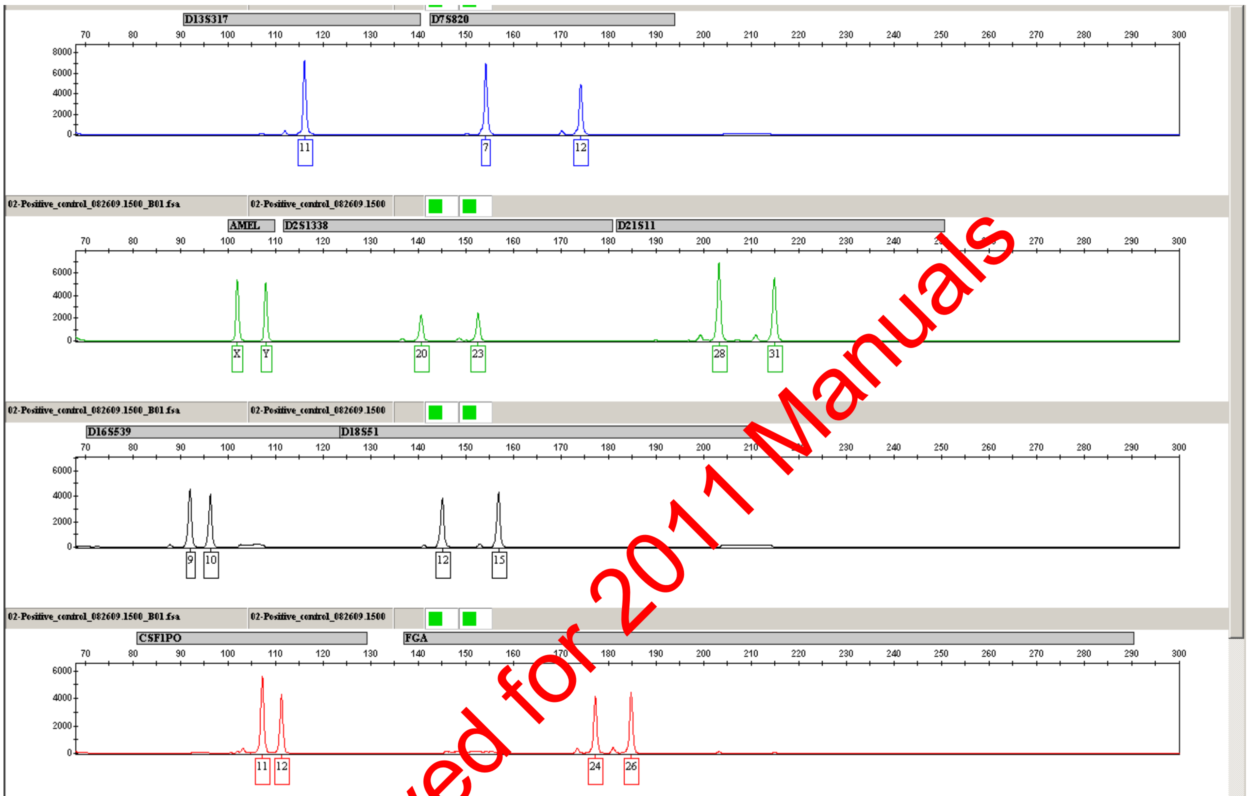


700
600
500
400
300
200
100
0
75
100
139
150
160
200
300
350
400
450
490
500
3000
4000
5000
6000
7000
8000

MiniFiler Allelic Ladder

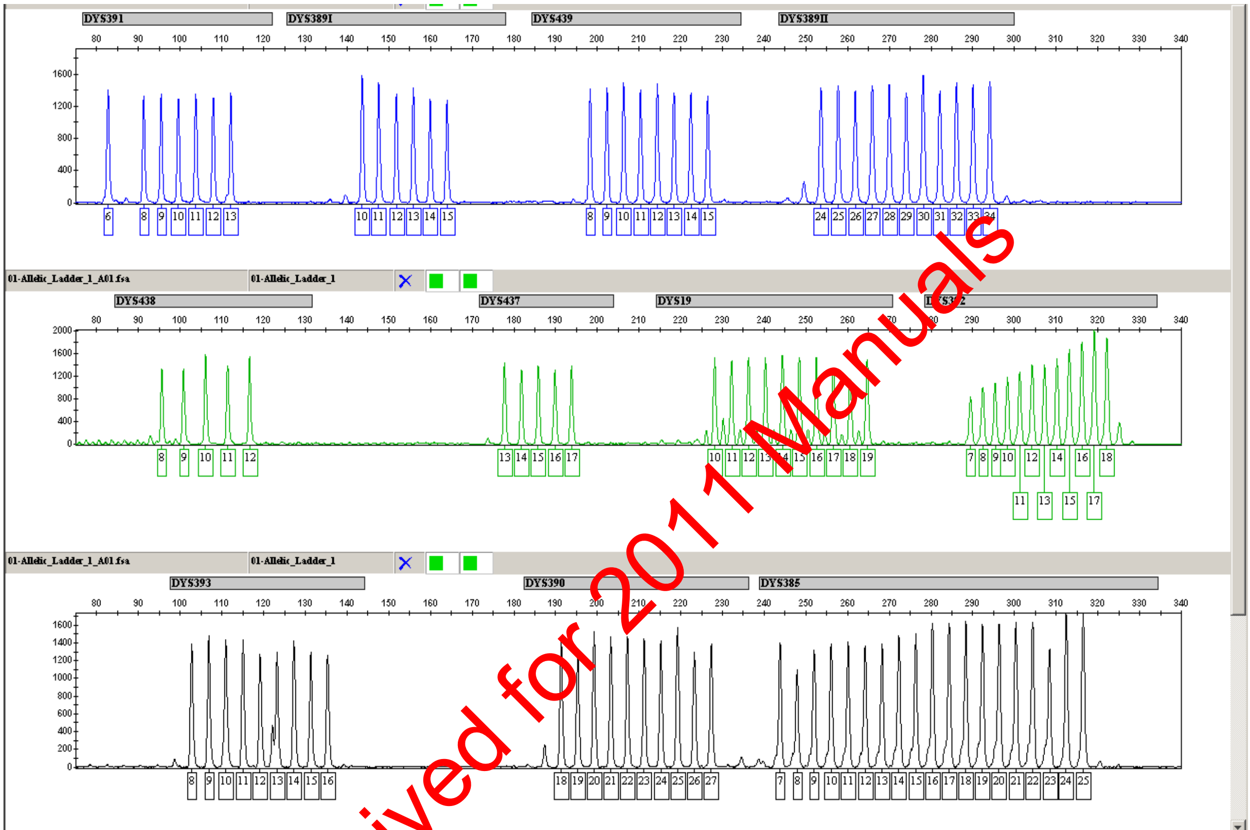


MiniFiler Positive Control

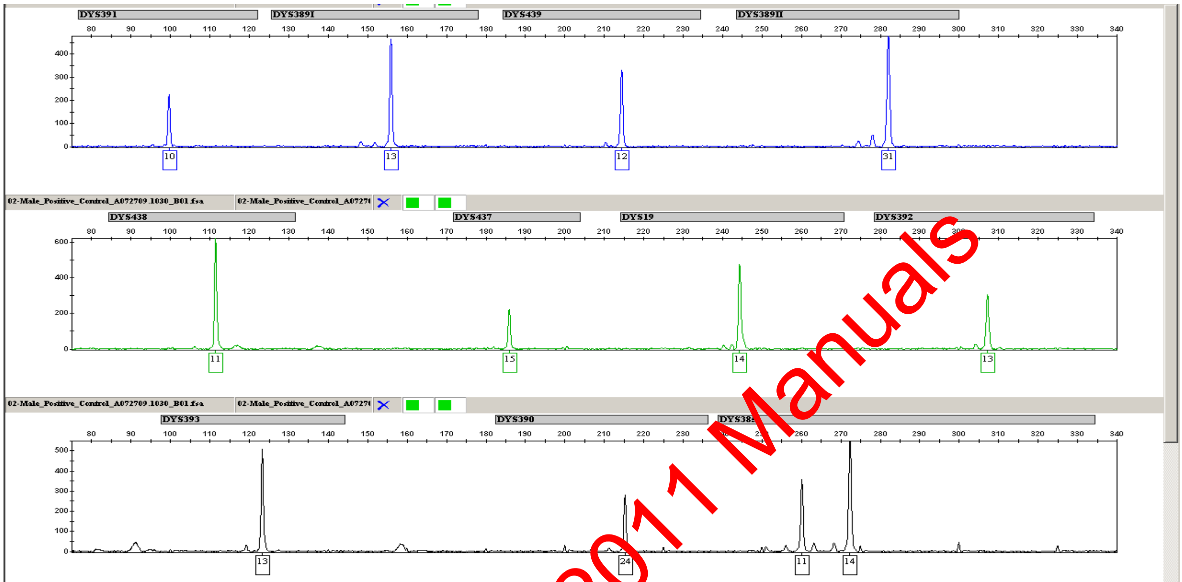


Blue (6-FAM)	**D13S317**	**D7S820**	
	11	7, 12	
Green (VIC)	**AMEL**	**D2S1338**	**D21S11**
	X, Y	20, 23	28, 31
Yellow (NED)	**D16S539**	**D18S51**	
	9, 10	12, 15	
Red (PET)	**CSF1PO**	**FGA**	
	11, 12	24, 26	

PowerPlex Y Allelic Ladder



PowerPlex Y Male Positive Control – Promega

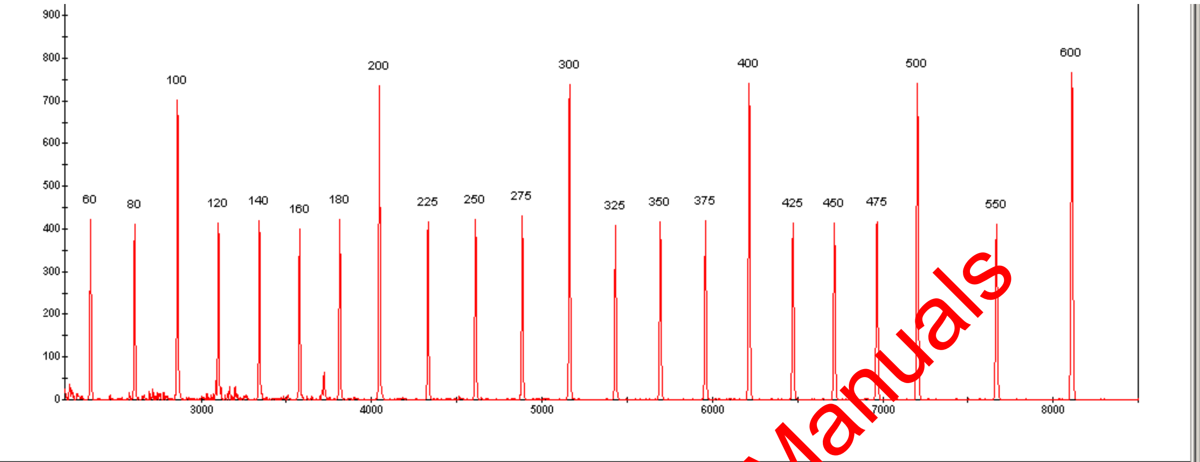


Blue (FL)	DYS391	DYS389I	DYS439	DYS389II
	10	13	12	31
Green (JOE)	**DYS438**	**DYS437**	**DYS19**	**DYS392**
	11	15	14	13
Yellow (TMR)	**DYS393**	**DYS390**	**DYS385**	
	13	24	11, 14	

PowerPlex Y Male Positive Control – In-House

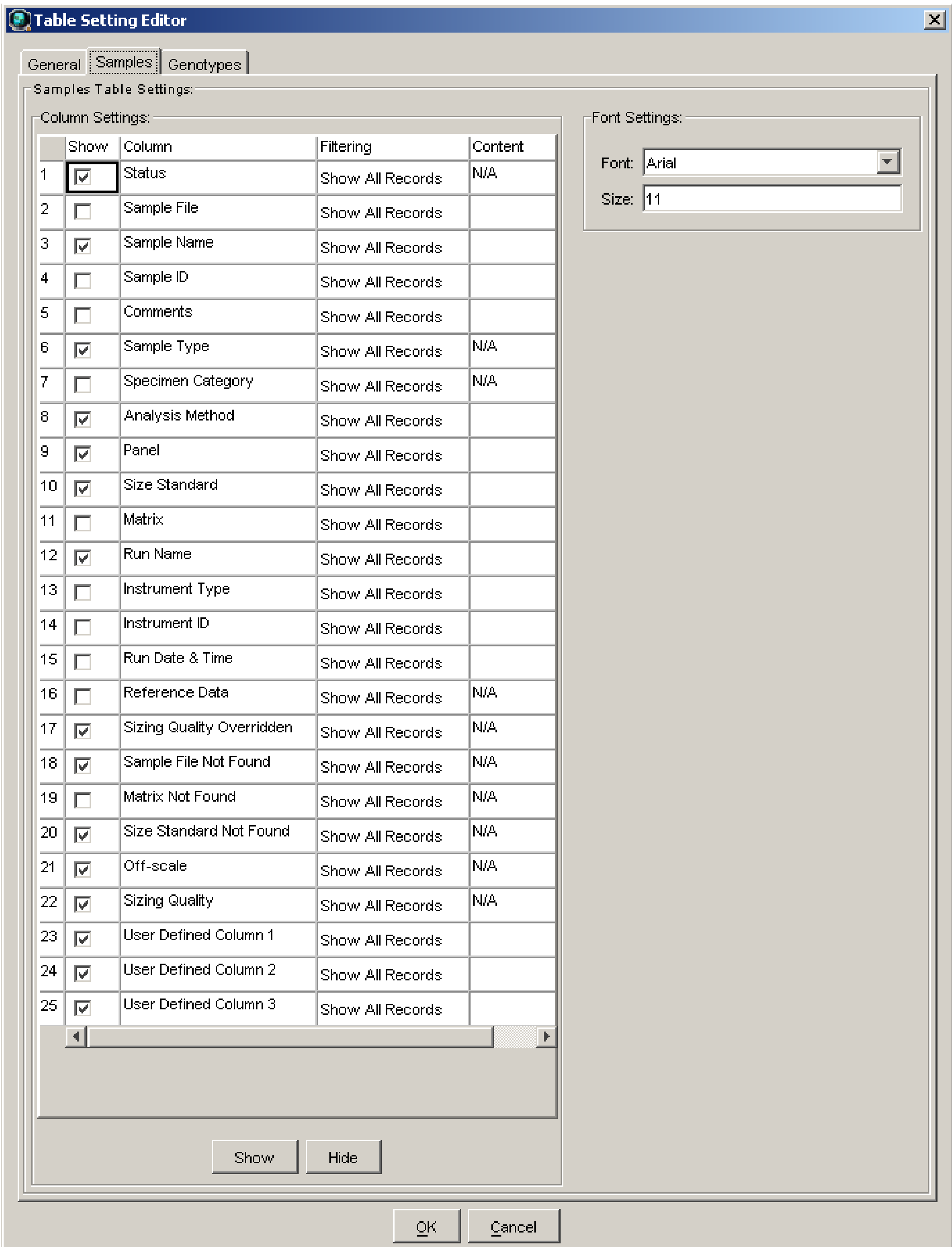
Blue (FL)	**DYS391**	**DYS389I**	**DYS439**	**DYS389II**
	10	13	12	29
Green (JOE)	**DYS438**	**DYS437**	**DYS19**	**DYS392**
	10	14	14	13
Yellow (TMR)	**DYS393**	**DYS390**	**DYS385**	
	15	25	13, 19	

ILS600



Revision History:
March 24, 2010 – Initial version of procedure.
August 2, 2010 – The profile of the in-house Male Positive Control was changed

TABLE SETTINGS – ANALYSIS VIEW: SAMPLES SETTINGS



	Show	Column	Filtering	Content
1	☑	Status	Show All Records	N/A
2	☐	Sample File	Show All Records	
3	☑	Sample Name	Show All Records	
4	☐	Sample ID	Show All Records	
5	☐	Comments	Show All Records	
6	☑	Sample Type	Show All Records	N/A
7	☐	Specimen Category	Show All Records	N/A
8	☑	Analysis Method	Show All Records	
9	☑	Panel	Show All Records	
10	☑	Size Standard	Show All Records	
11	☐	Matrix	Show All Records	
12	☑	Run Name	Show All Records	
13	☐	Instrument Type	Show All Records	
14	☐	Instrument ID	Show All Records	
15	☐	Run Date & Time	Show All Records	
16	☐	Reference Data	Show All Records	N/A
17	☑	Sizing Quality Overridden	Show All Records	N/A
18	☑	Sample File Not Found	Show All Records	N/A
19	☐	Matrix Not Found	Show All Records	N/A
20	☑	Size Standard Not Found	Show All Records	N/A
21	☑	Off-scale	Show All Records	N/A
22	☑	Sizing Quality	Show All Records	N/A
23	☑	User Defined Column 1	Show All Records	
24	☑	User Defined Column 2	Show All Records	
25	☑	User Defined Column 3	Show All Records	

TABLE SETTINGS – ANALYSIS VIEW: GENOTYPES SETTINGS

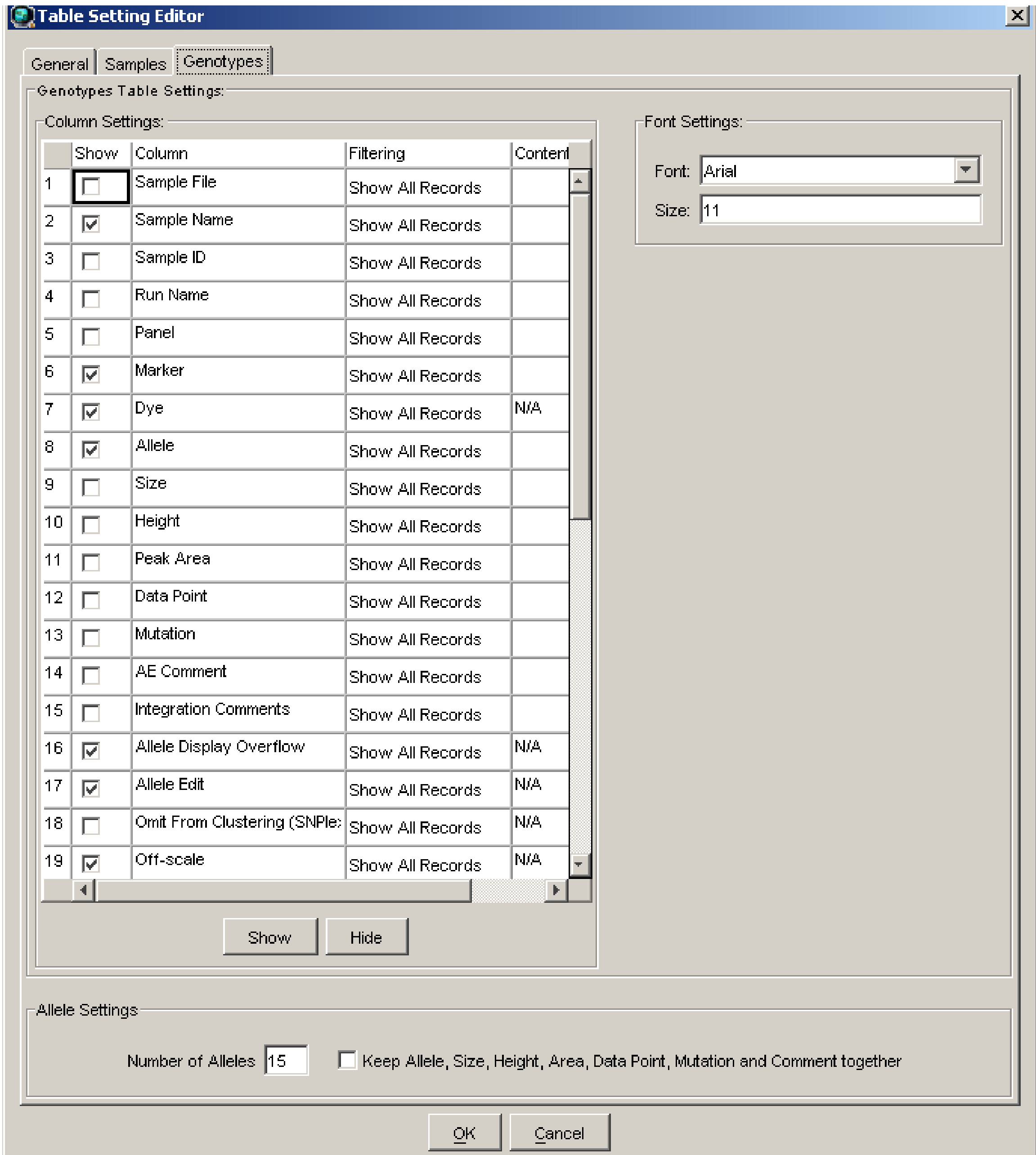


TABLE SETTINGS – ANALYSIS VIEW: GENOTYPES SETTINGS (continued)

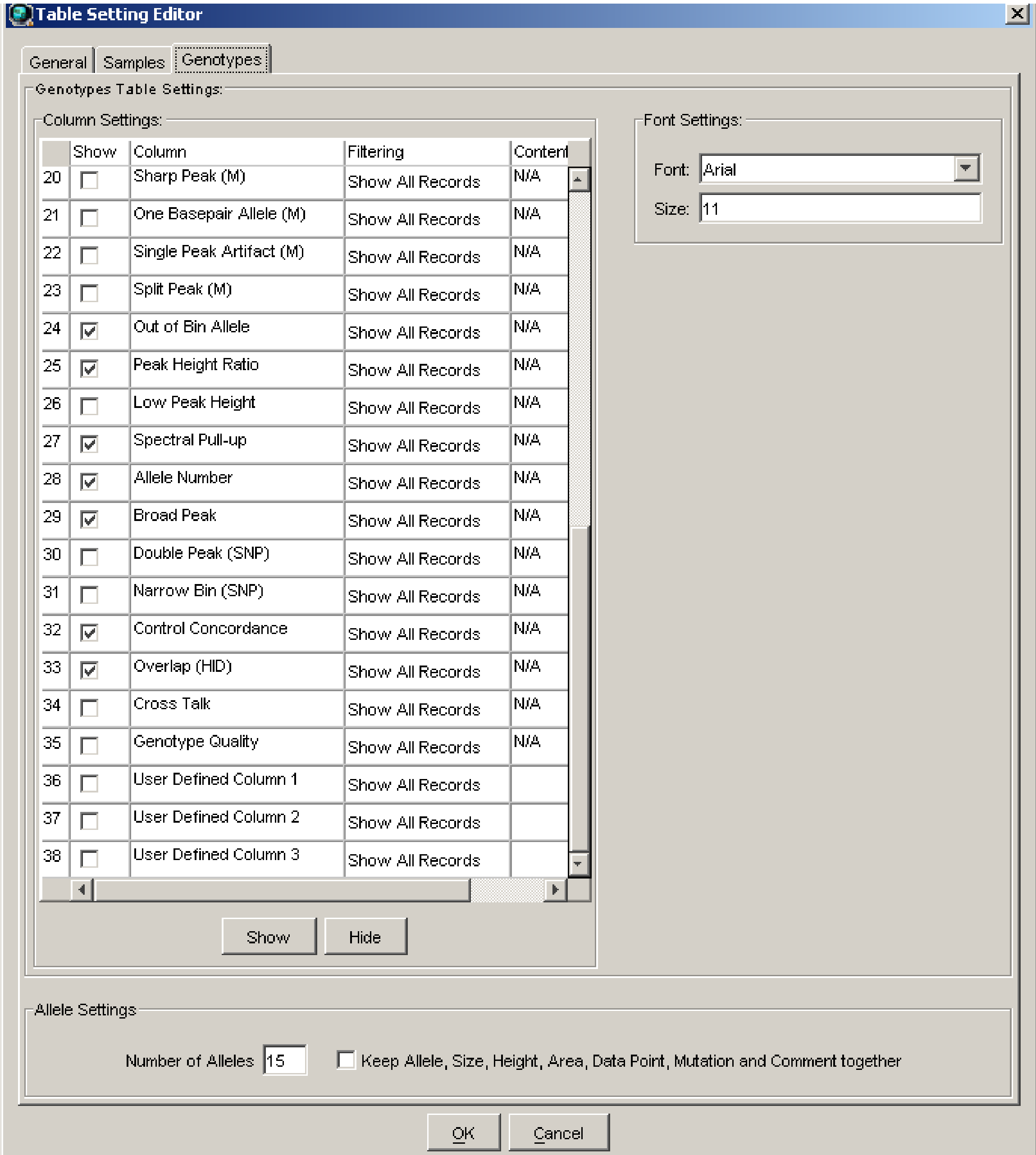
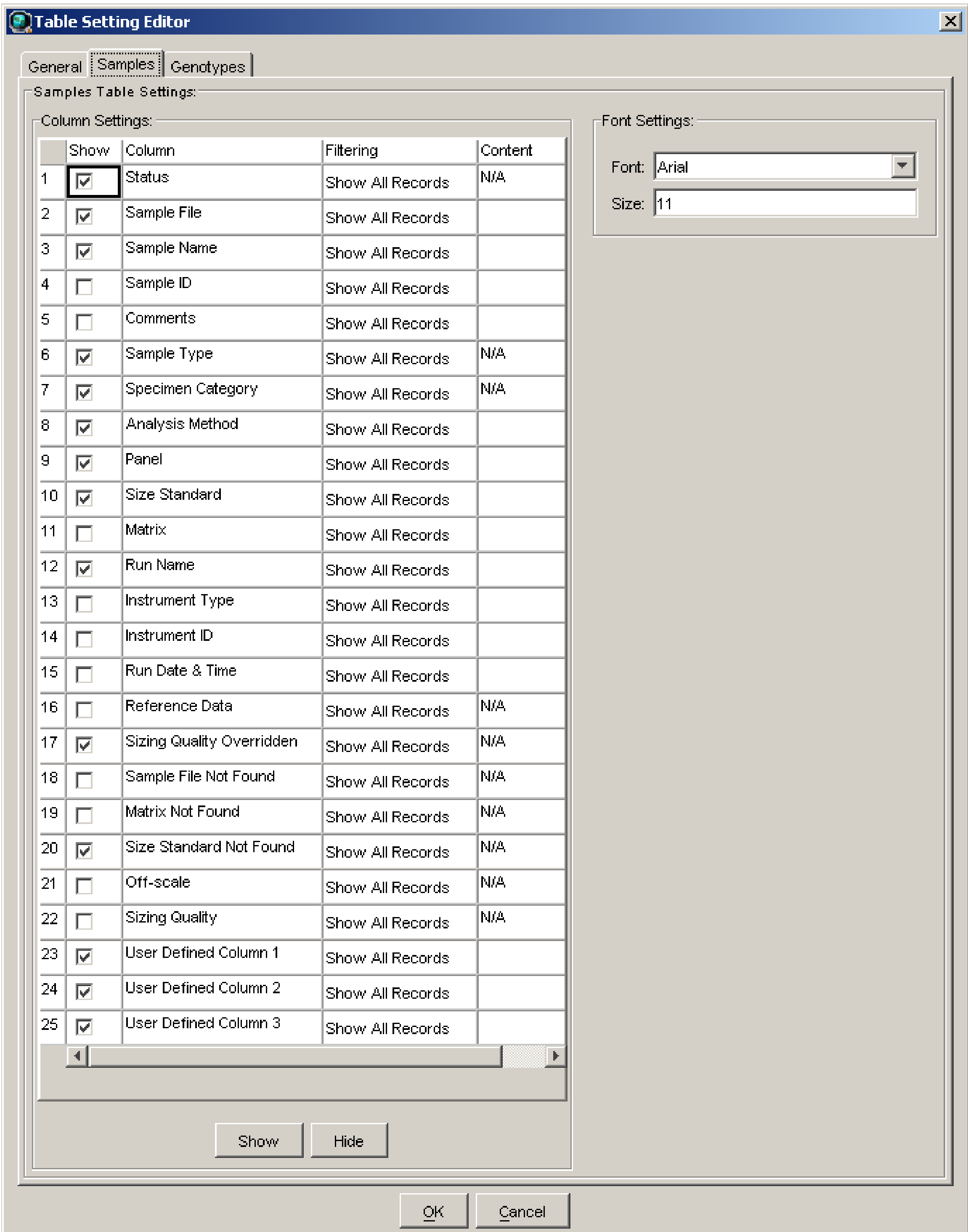


TABLE SETTINGS – CASEWORK VIEW: SAMPLES SETTINGS



	Show	Column	Filtering	Content
1	✓	Status	Show All Records	N/A
2	✓	Sample File	Show All Records	
3	✓	Sample Name	Show All Records	
4		Sample ID	Show All Records	
5		Comments	Show All Records	
6	✓	Sample Type	Show All Records	N/A
7	✓	Specimen Category	Show All Records	N/A
8	✓	Analysis Method	Show All Records	
9	✓	Panel	Show All Records	
10	✓	Size Standard	Show All Records	
11		Matrix	Show All Records	
12	✓	Run Name	Show All Records	
13		Instrument Type	Show All Records	
14		Instrument ID	Show All Records	
15		Run Date & Time	Show All Records	
16		Reference Data	Show All Records	N/A
17	✓	Sizing Quality Overridden	Show All Records	N/A
18		Sample File Not Found	Show All Records	N/A
19		Matrix Not Found	Show All Records	N/A
20	✓	Size Standard Not Found	Show All Records	N/A
21		Off-scale	Show All Records	N/A
22		Sizing Quality	Show All Records	N/A
23	✓	User Defined Column 1	Show All Records	
24	✓	User Defined Column 2	Show All Records	
25	✓	User Defined Column 3	Show All Records	

TABLE SETTINGS – CASEWORK VIEW: GENOTYPES SETTINGS

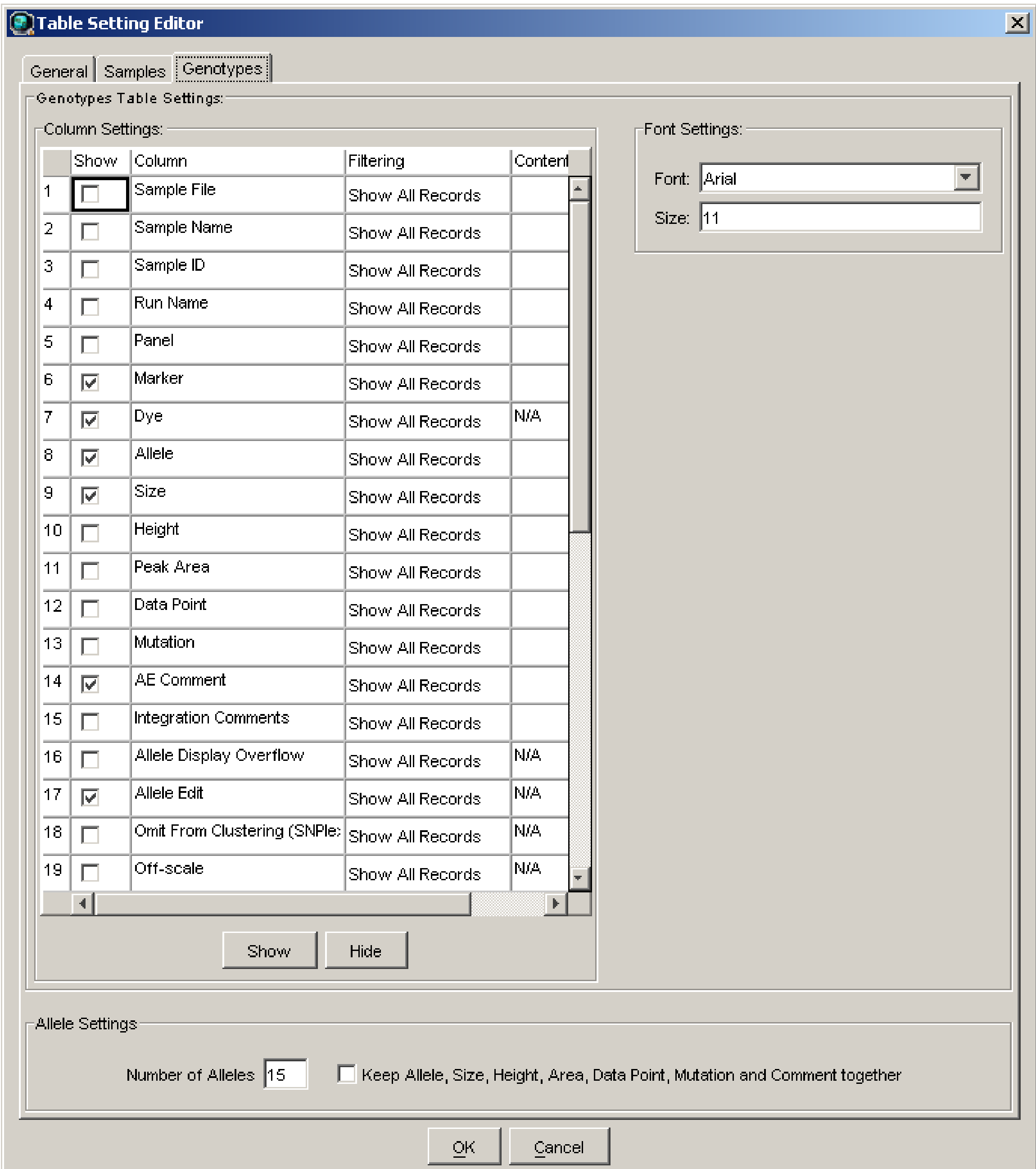
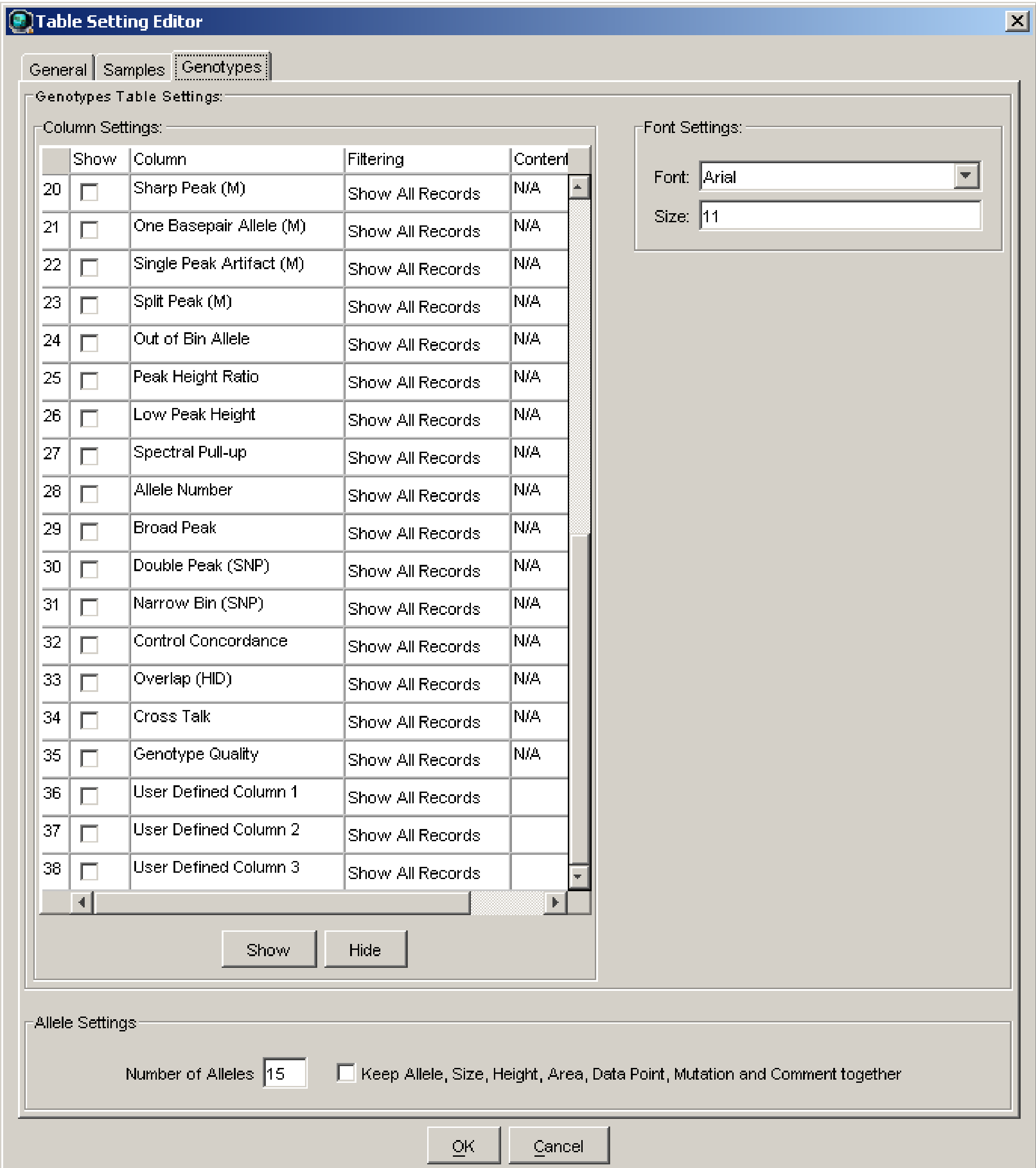


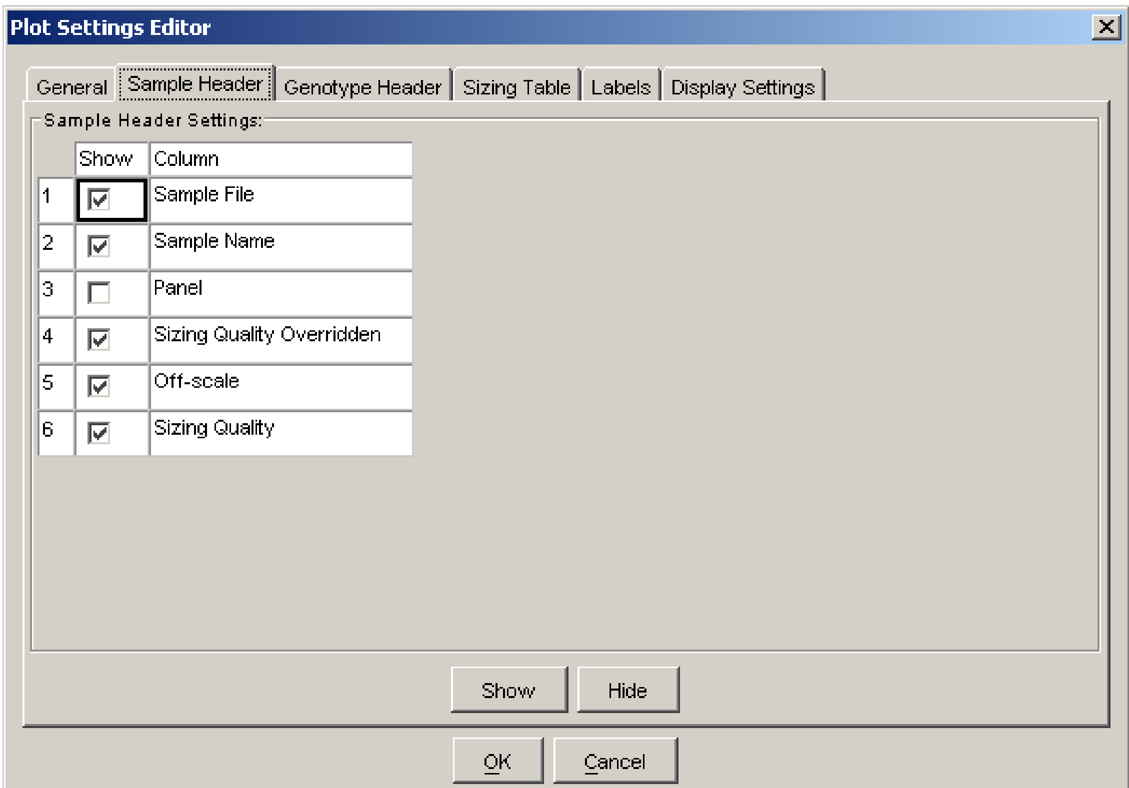
TABLE SETTINGS – CASEWORK VIEW: GENOTYPES SETTINGS (continued)



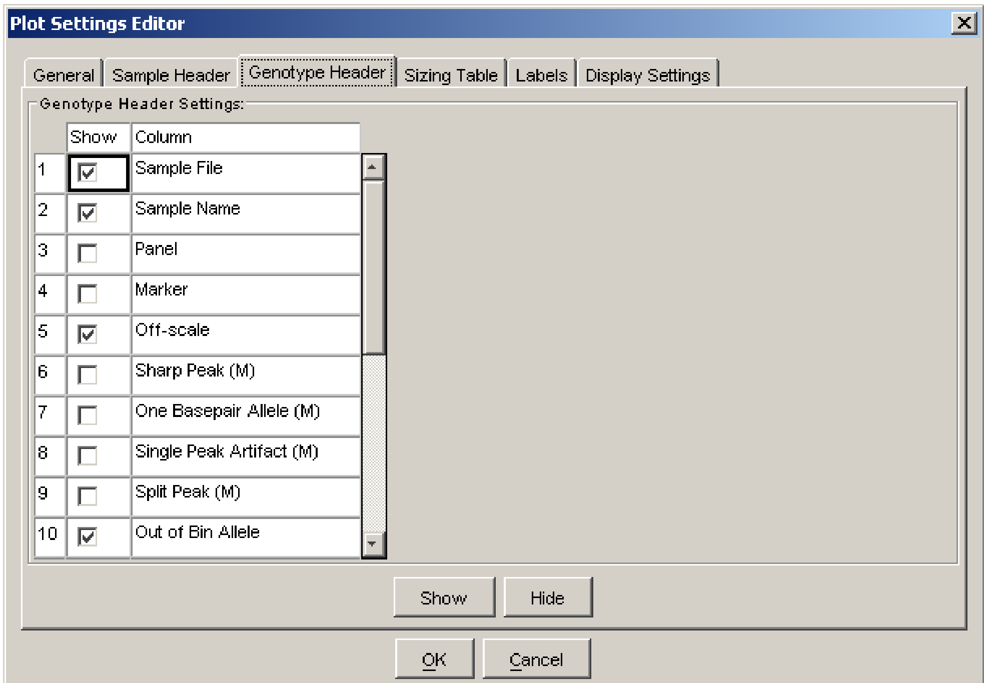
	Show	Column	Filtering	Content
20	☐	Sharp Peak (M)	Show All Records	N/A
21	☐	One Basepair Allele (M)	Show All Records	N/A
22	☐	Single Peak Artifact (M)	Show All Records	N/A
23	☐	Split Peak (M)	Show All Records	N/A
24	☐	Out of Bin Allele	Show All Records	N/A
25	☐	Peak Height Ratio	Show All Records	N/A
26	☐	Low Peak Height	Show All Records	N/A
27	☐	Spectral Pull-up	Show All Records	N/A
28	☐	Allele Number	Show All Records	N/A
29	☐	Broad Peak	Show All Records	N/A
30	☐	Double Peak (SNP)	Show All Records	N/A
31	☐	Narrow Bin (SNP)	Show All Records	N/A
32	☐	Control Concordance	Show All Records	N/A
33	☐	Overlap (HID)	Show All Records	N/A
34	☐	Cross Talk	Show All Records	N/A
35	☐	Genotype Quality	Show All Records	N/A
36	☐	User Defined Column 1	Show All Records	
37	☐	User Defined Column 2	Show All Records	
38	☐	User Defined Column 3	Show All Records	

PLOT SETTINGS: ANALYSIS VIEW

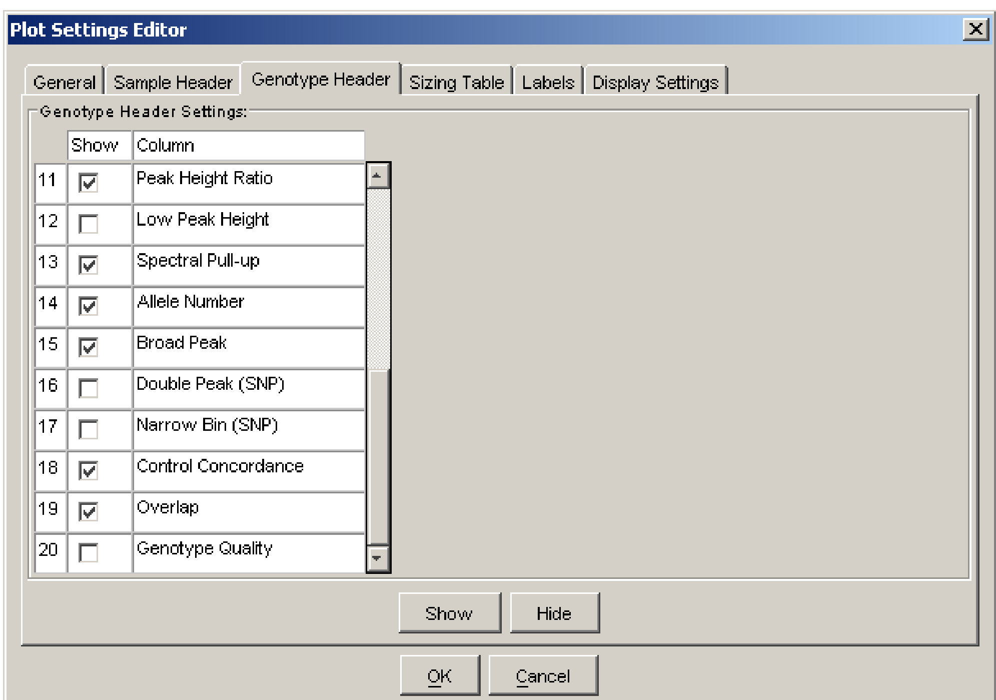
Analysis View: Sample Header



Analysis View: Genotype Header

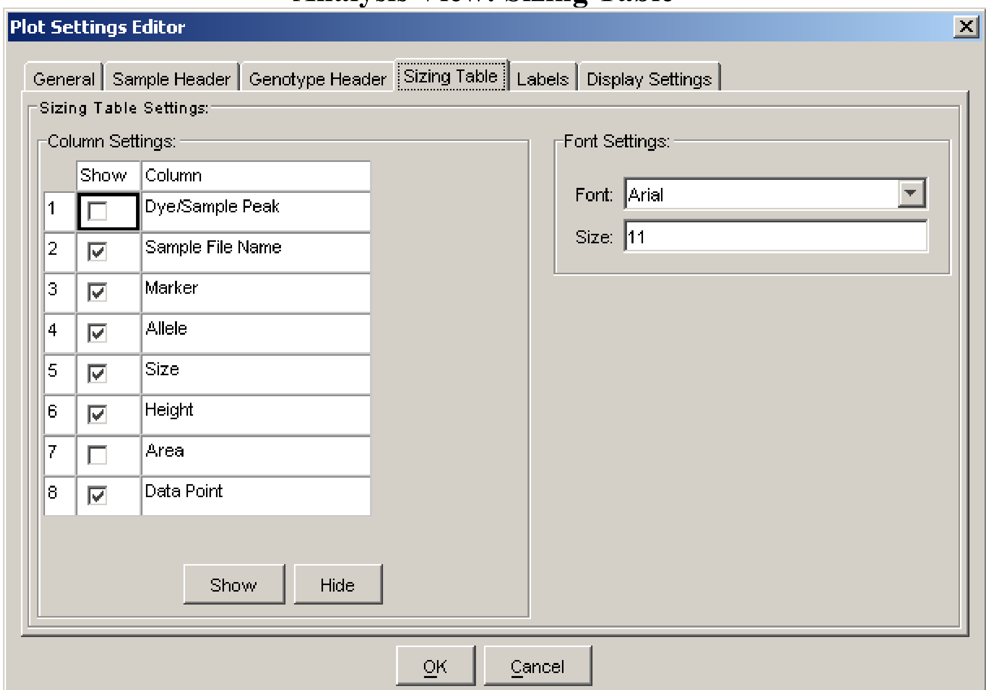


Plot Settings Editor
General
Sample Header
Genotype Header
Sizing Table
Labels
Display Settings
Genotype Header Settings:
Show
Column
1 Sample File
2 Sample Name
3 Panel
4 Marker
5 Off-scale
6 Sharp Peak (M)
7 One Basepair Allele (M)
8 Single Peak Artifact (M)
9 Split Peak (M)
10 Out of Bin Allele
Show
Hide
OK
Cancel

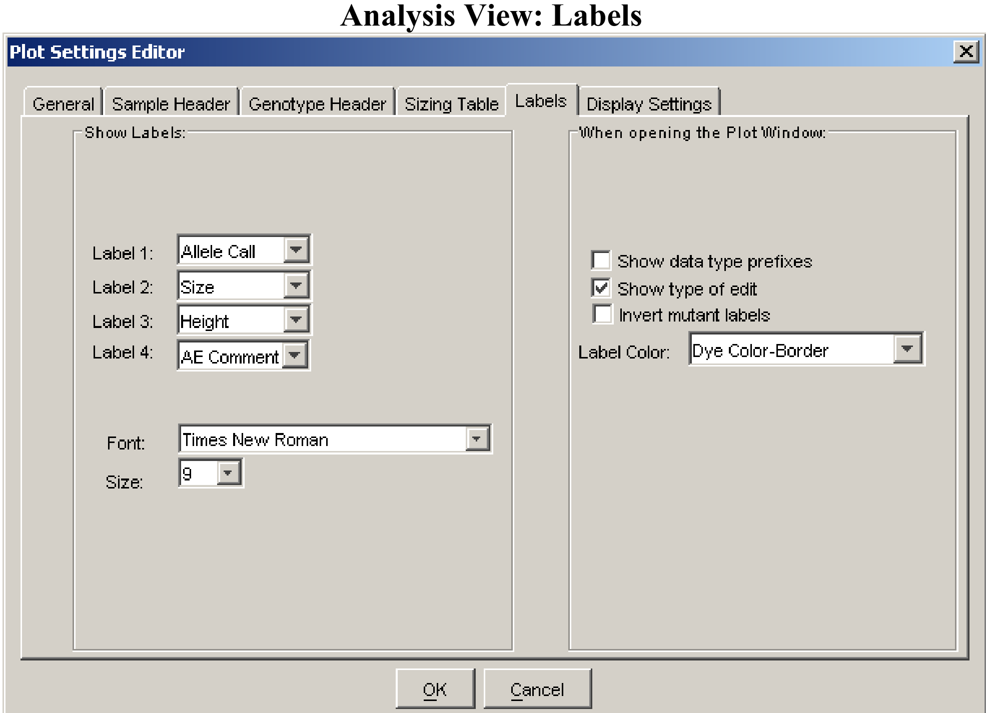


Plot Settings Editor
General
Sample Header
Genotype Header
Sizing Table
Labels
Display Settings
Genotype Header Settings:
Show
Column
11 Peak Height Ratio
12 Low Peak Height
13 Spectral Pull-up
14 Allele Number
15 Broad Peak
16 Double Peak (SNP)
17 Narrow Bin (SNP)
18 Control Concordance
19 Overlap
20 Genotype Quality
Show
Hide
OK
Cancel

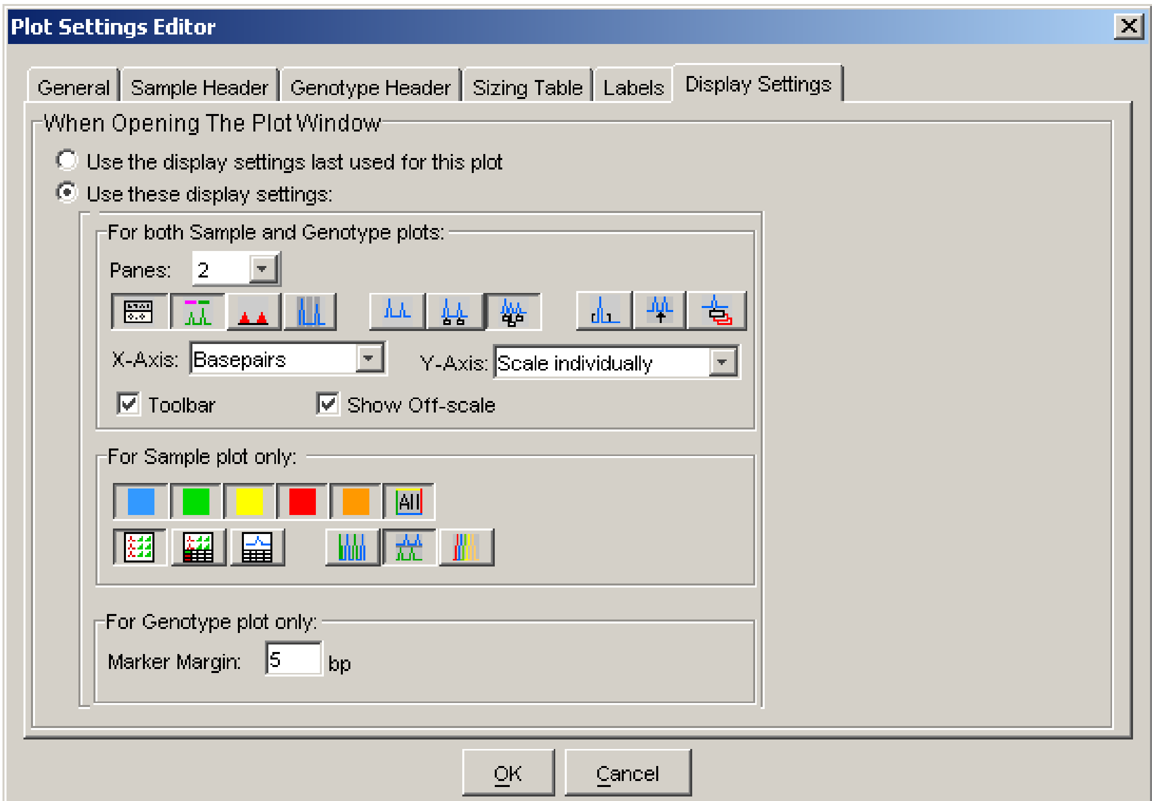
Analysis View: Sizing Table



Analysis View: Labels

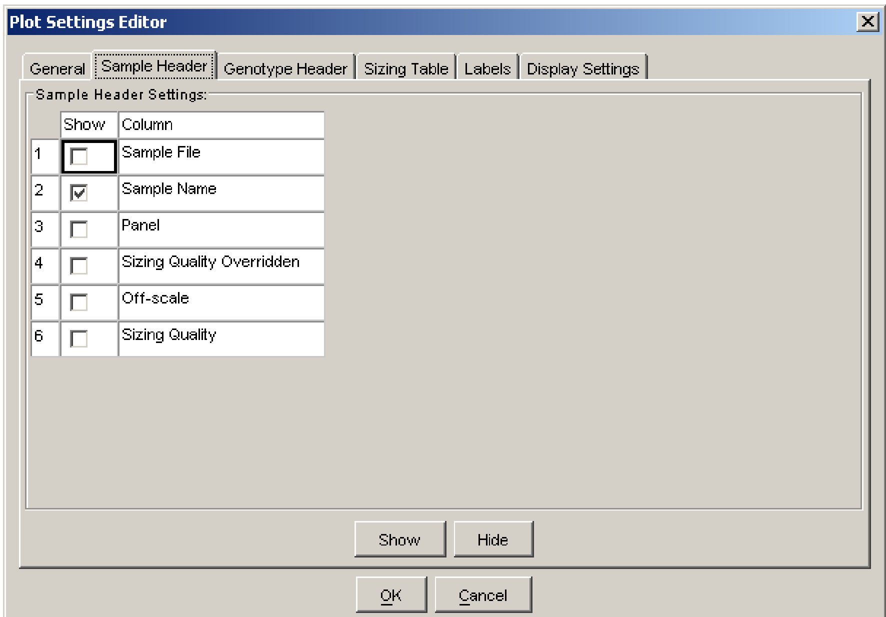


Analysis View: Display Settings

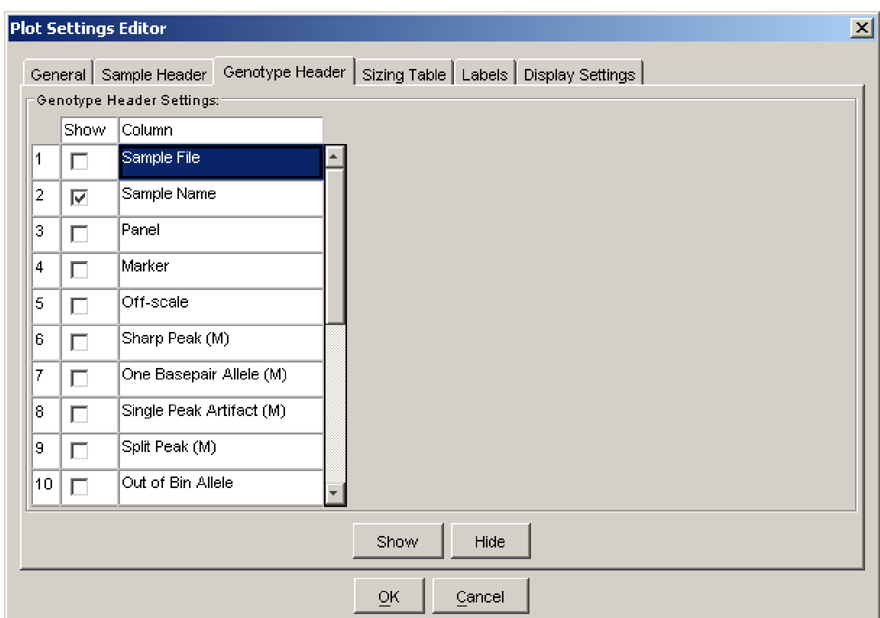


PLOT SETTINGS: PRINT – IDENTIFILER ALLELIC LADDER

Print – Identifiler Allelic Ladder: Sample Header

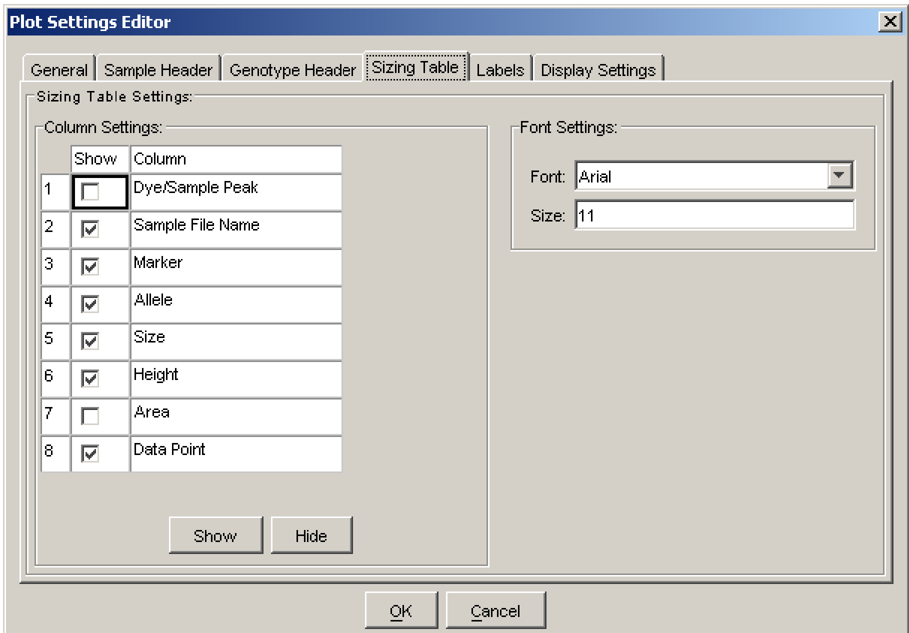


Print – Identifiler Allelic Ladder: Genotype Header

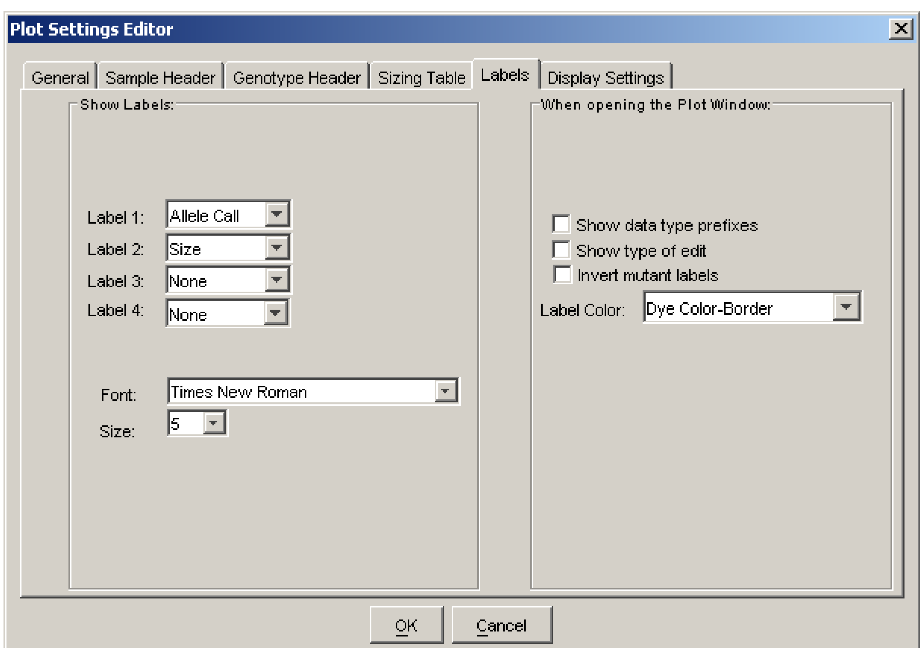


Boxes 3 – 20 are unchecked

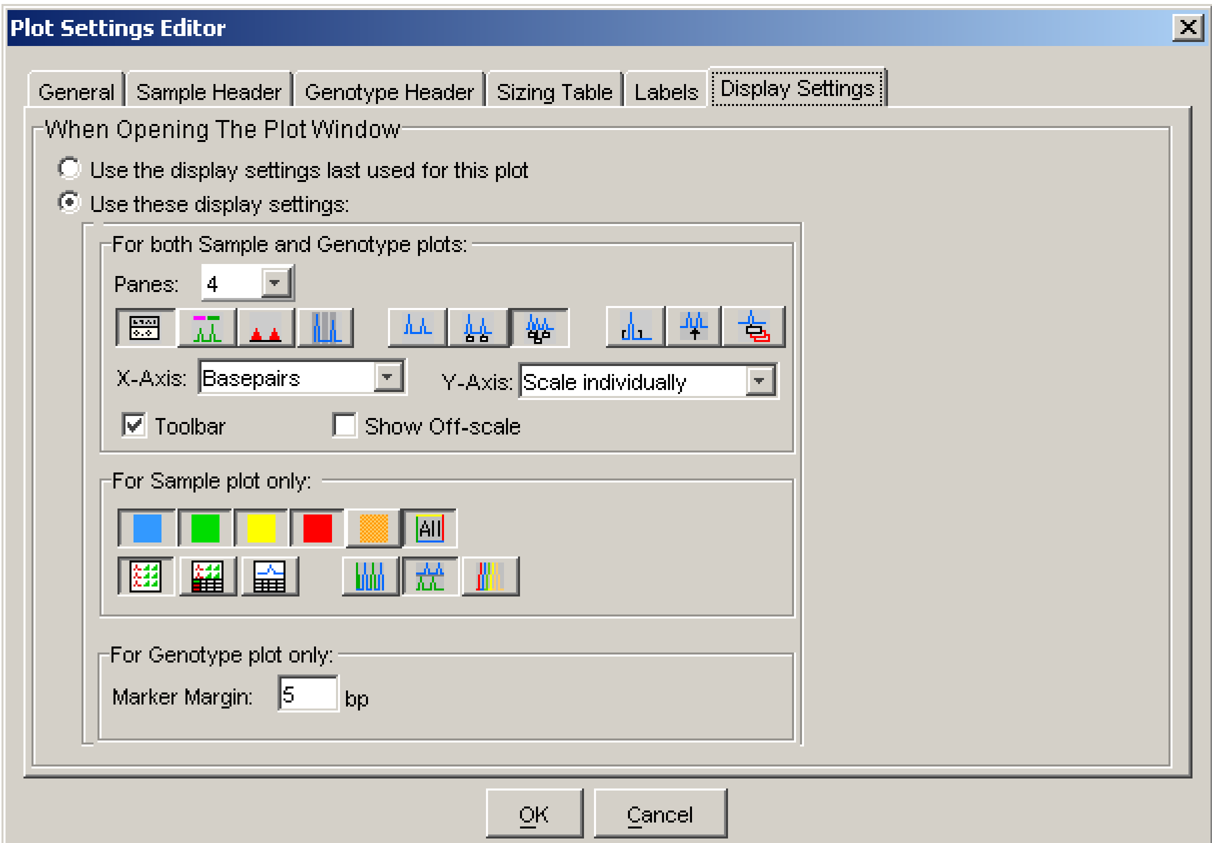
Print – Identifiler Allelic Ladder: Sizing Table



Print – Identifiler Allelic Ladder: Labels

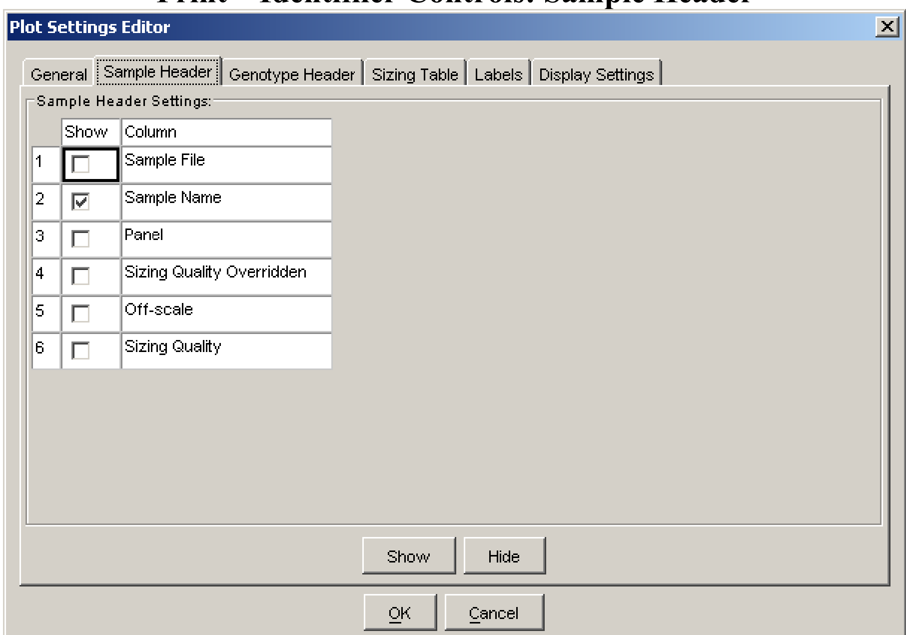


Print – Identifiler Allelic Ladder: Display Settings



PLOT SETTINGS: PRINT – IDENTIFILER CONTROLS

Print – Identifiler Controls: Sample Header



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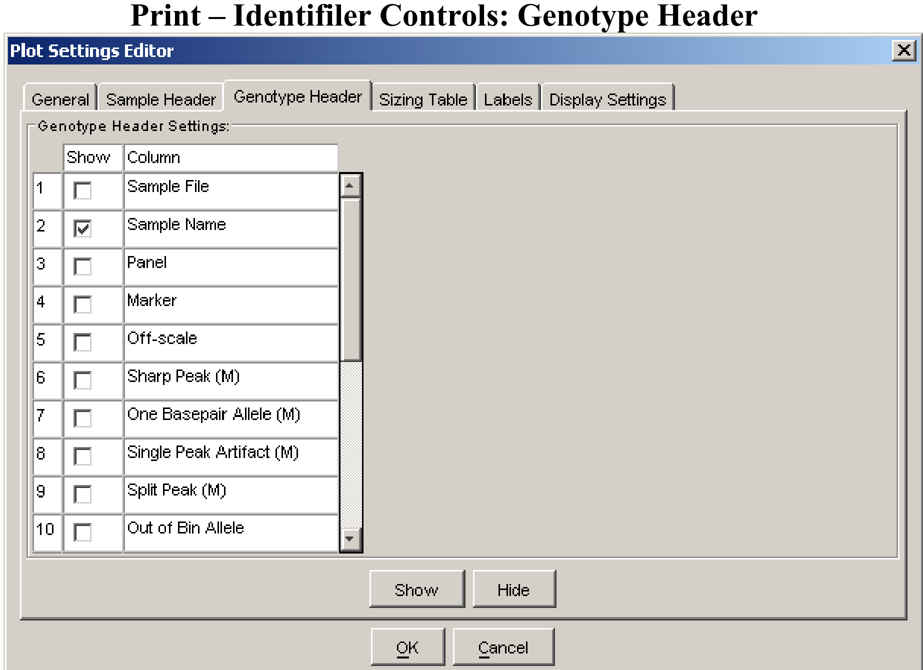

Boxes 3 – 20 are unchecked

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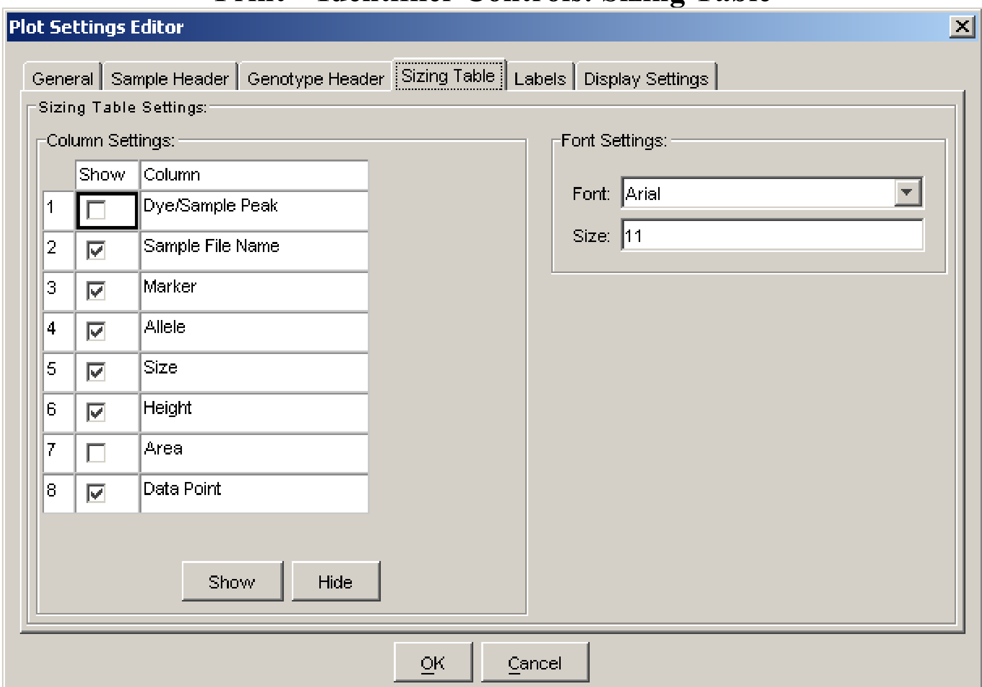

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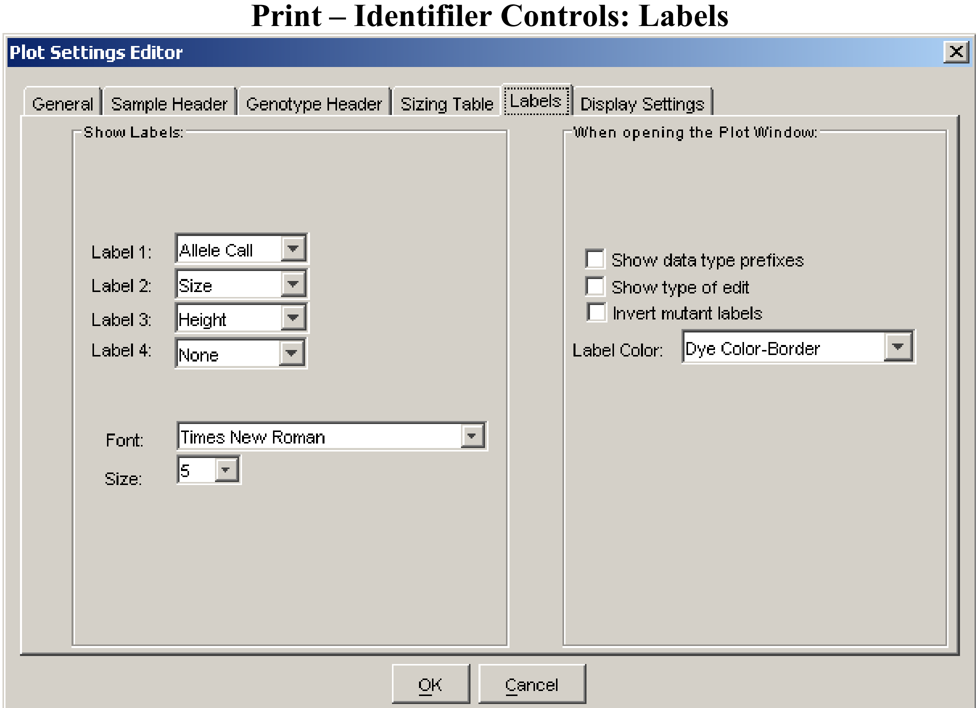
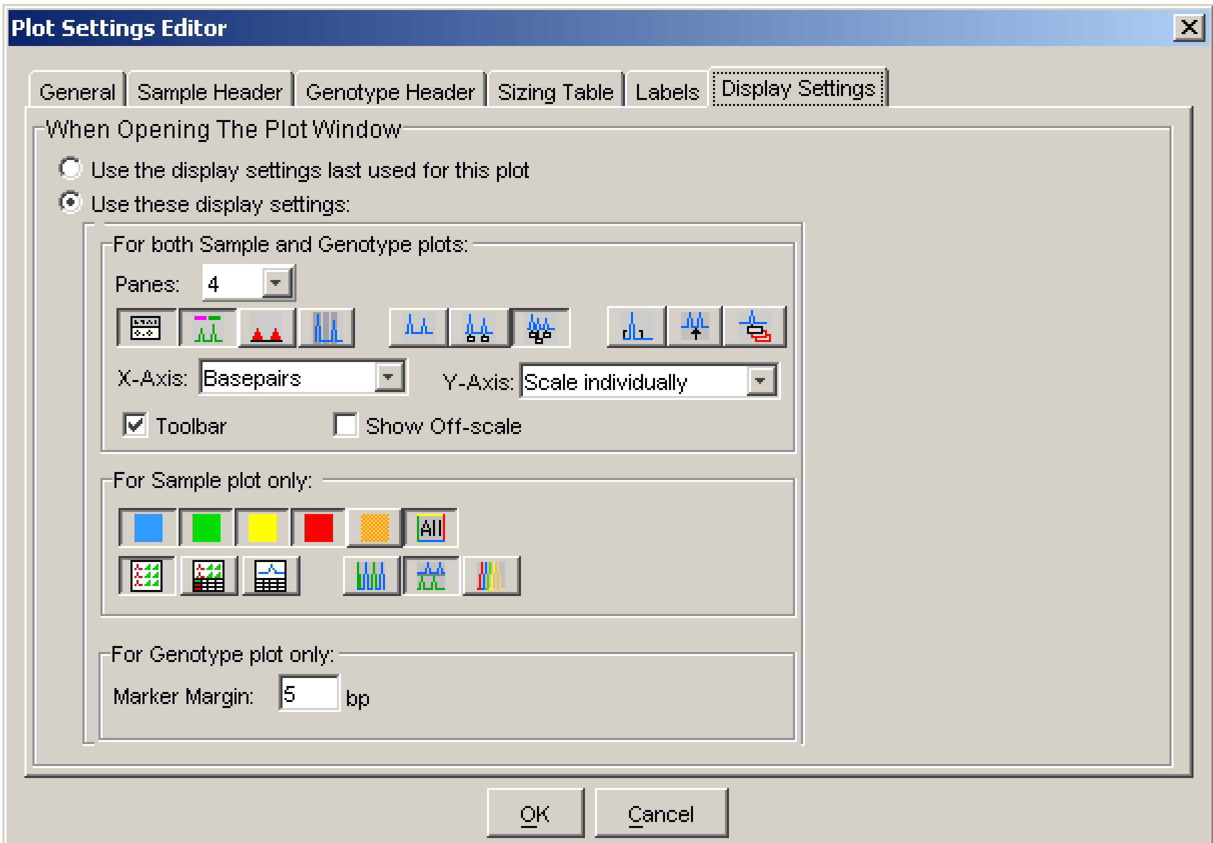
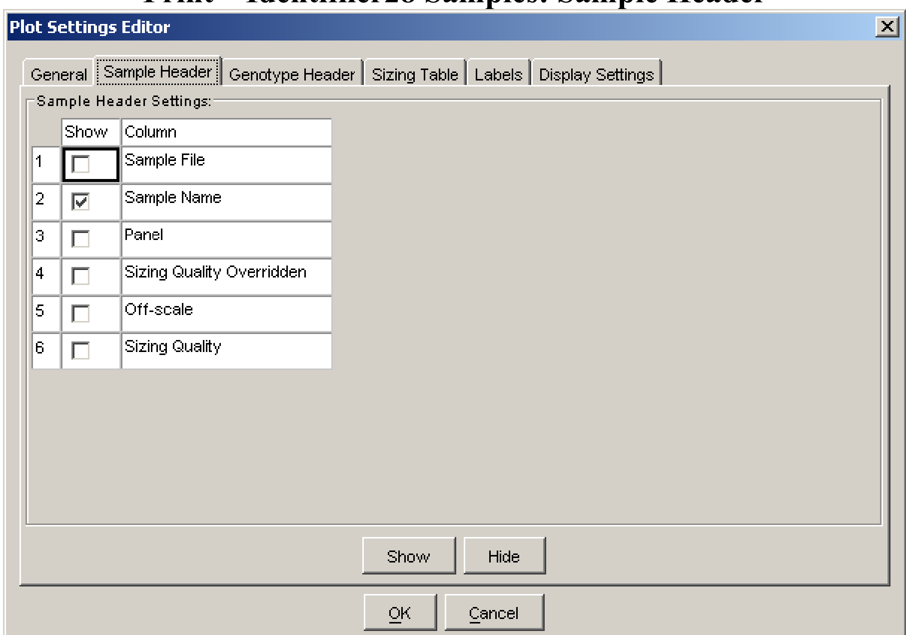


Print – Identifiler Controls: Display Settings



PLOT SETTINGS: PRINT – IDENTIFILER 28 SAMPLES

Print – Identifiler28 Samples: Sample Header



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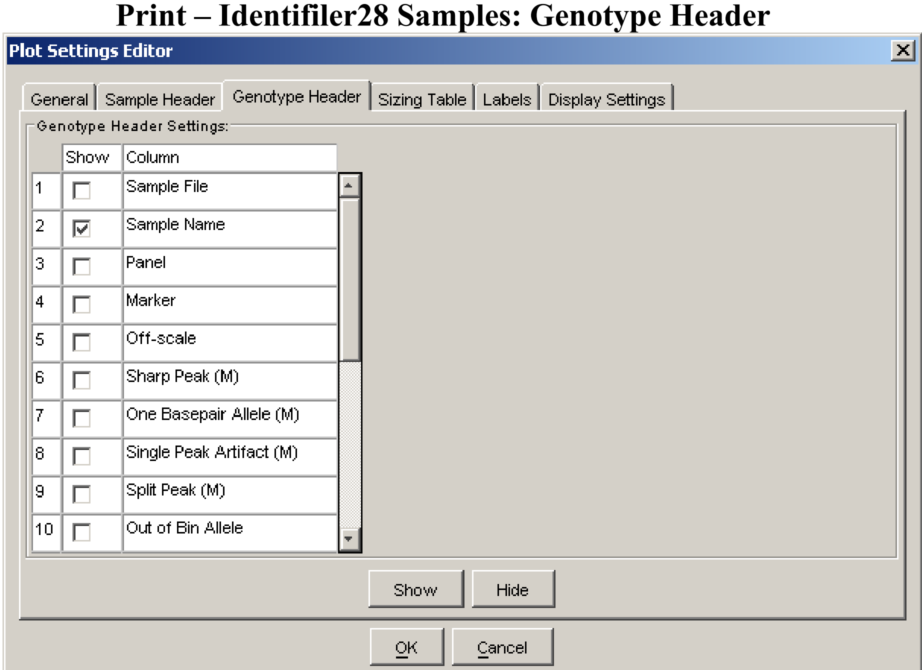

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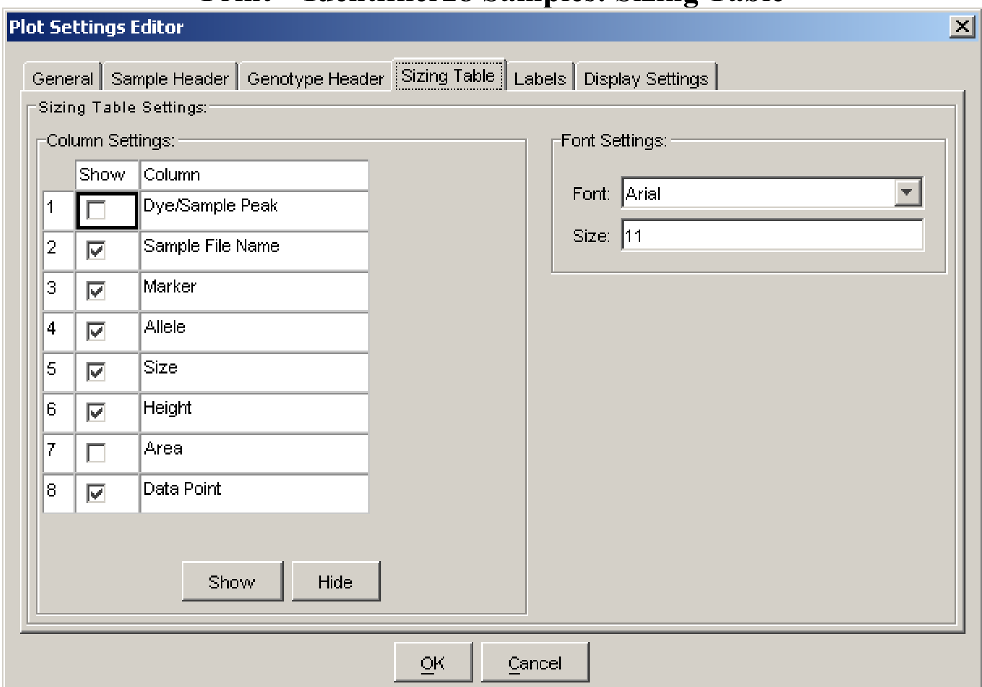

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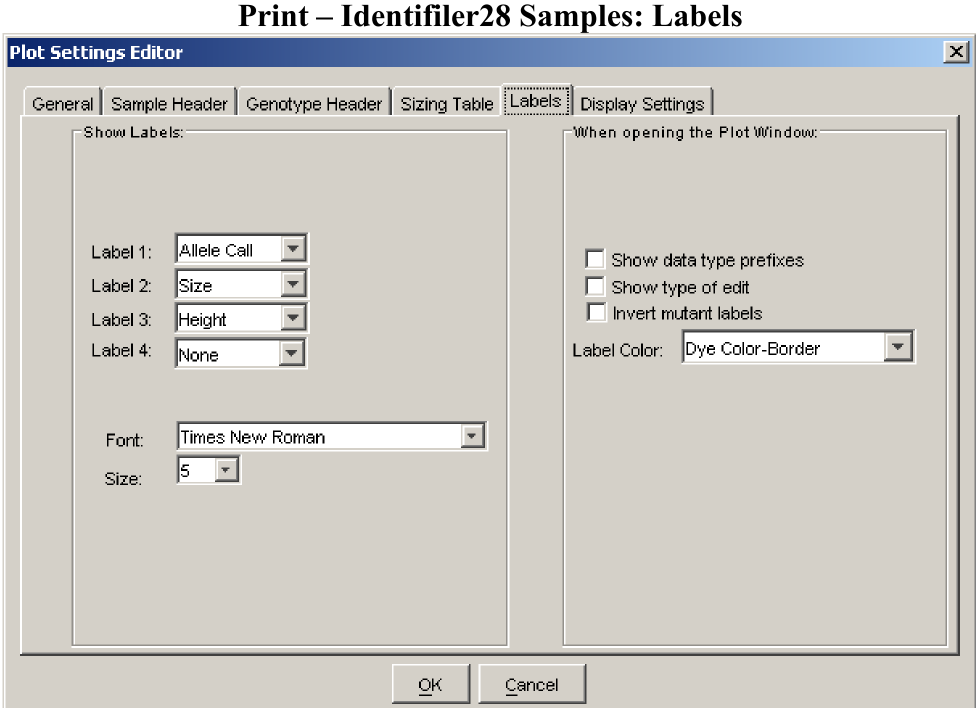

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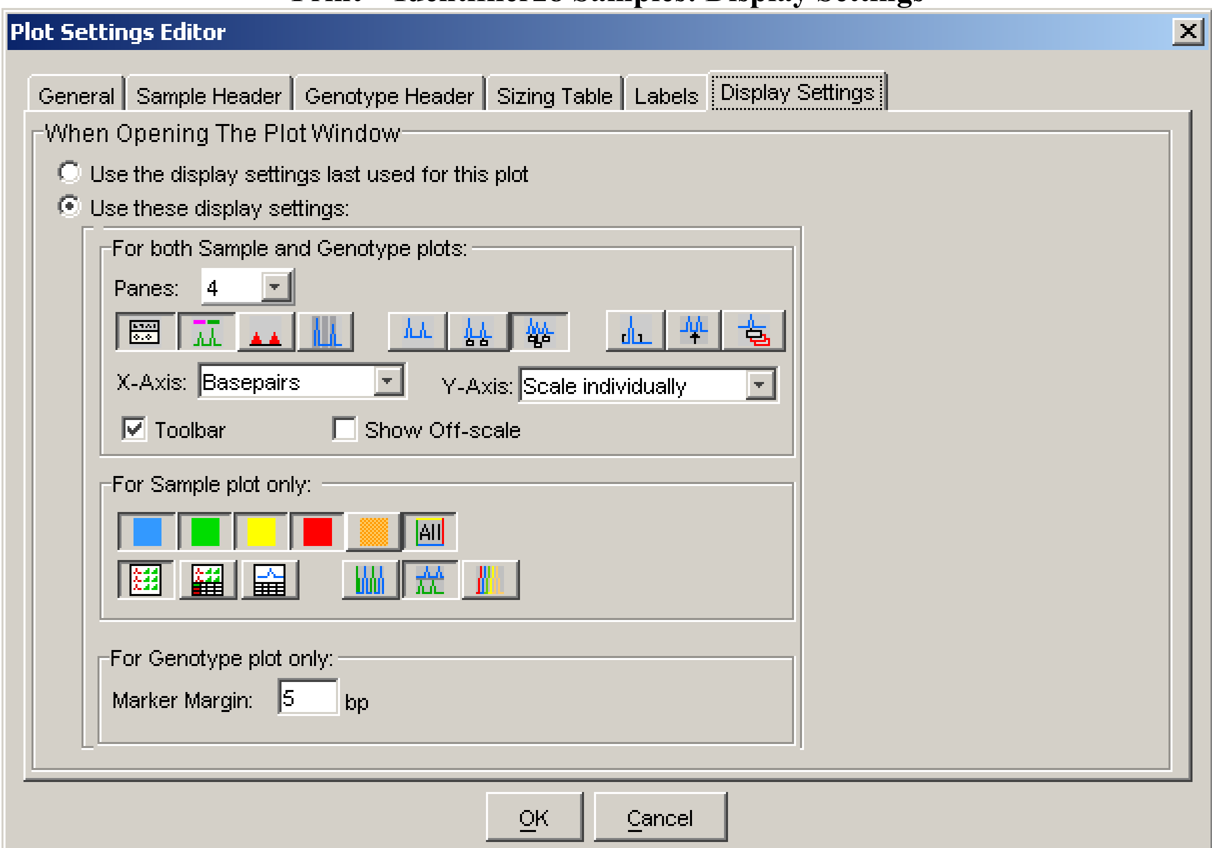
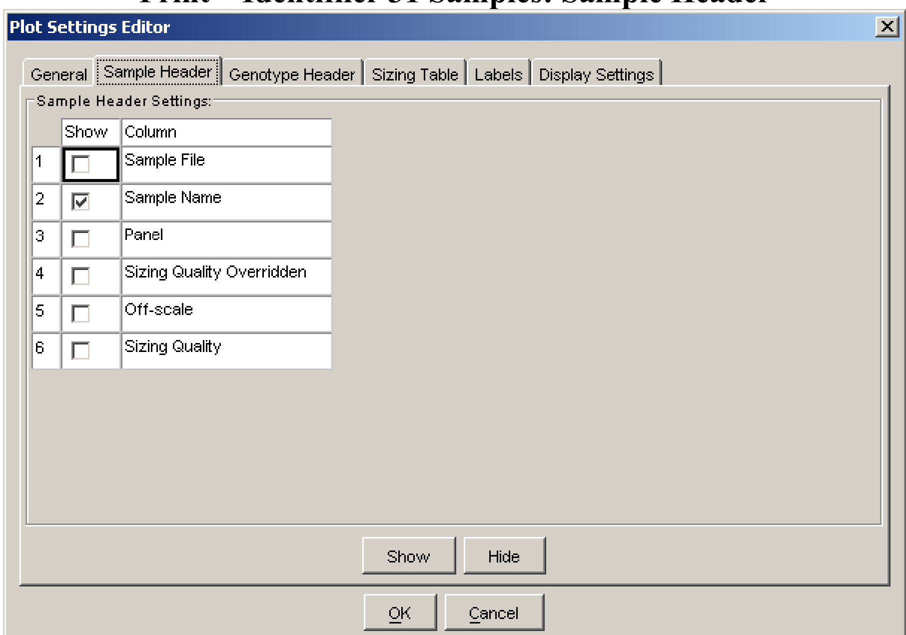


PLOT SETTINGS: PRINT – IDENTIFILER 31 SAMPLES

Print – Identifiler 31 Samples: Sample Header



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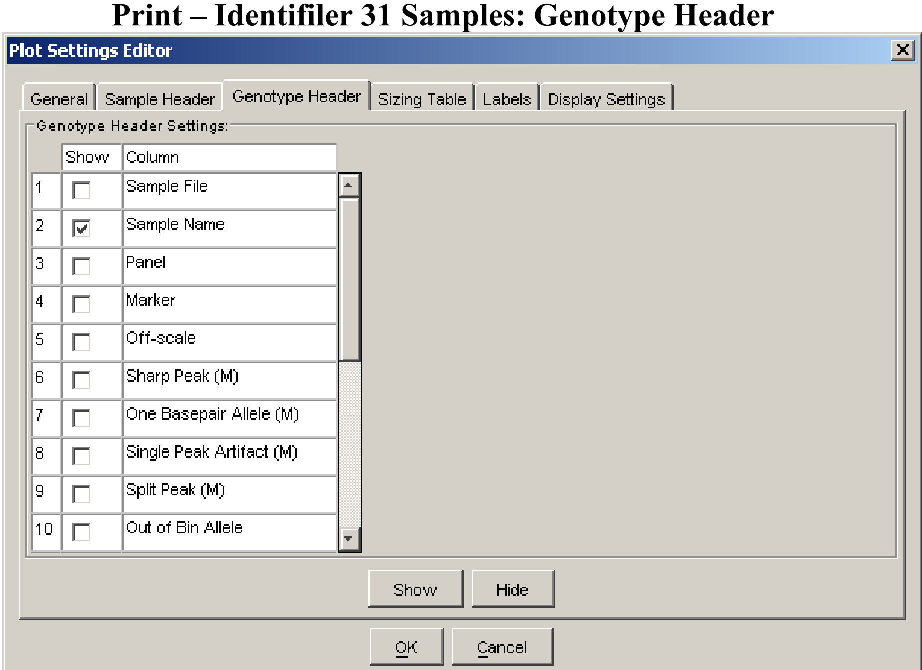

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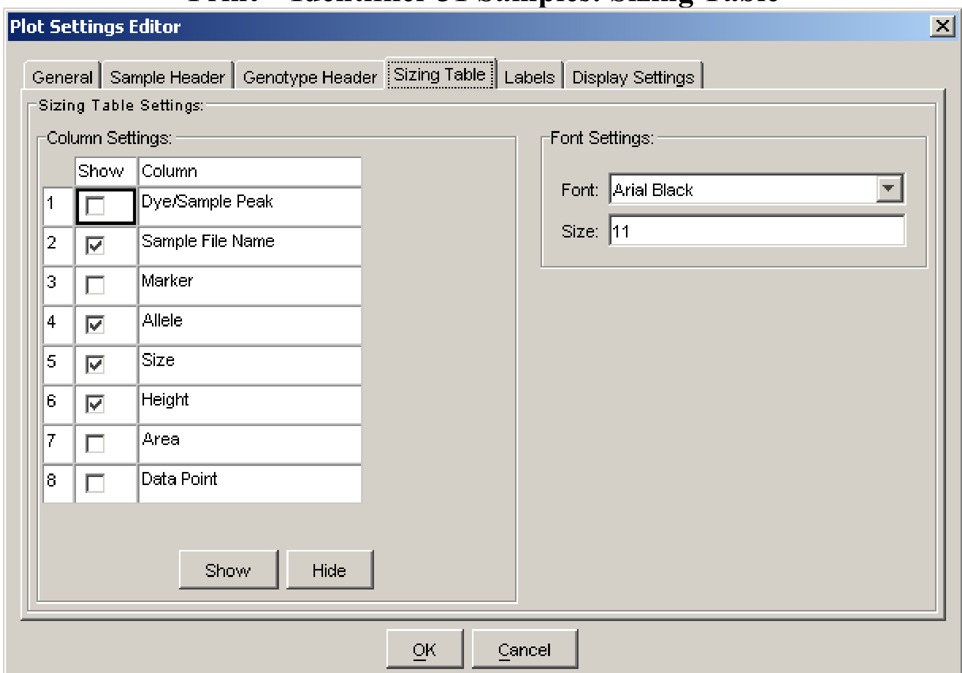

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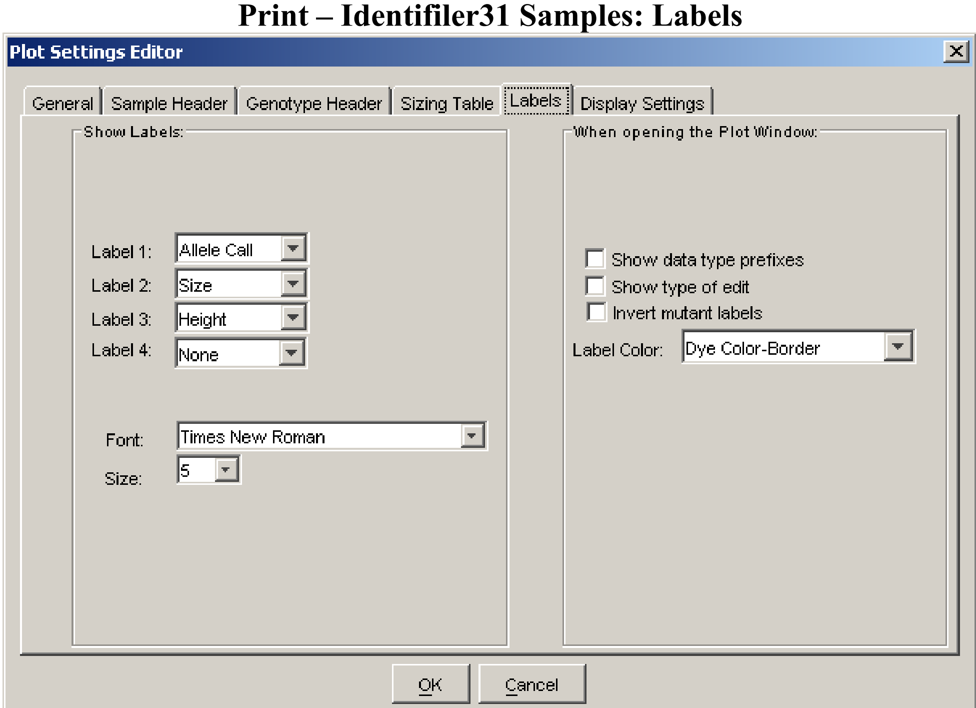
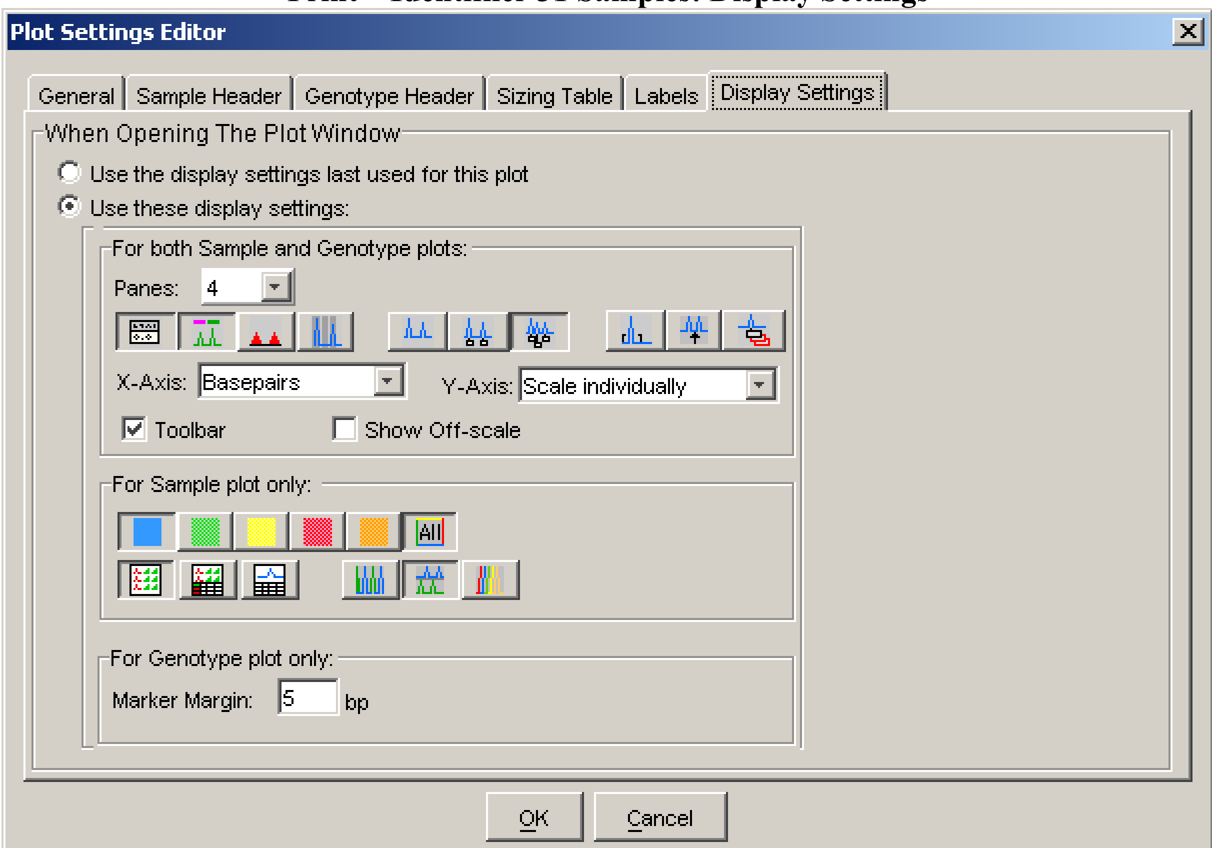
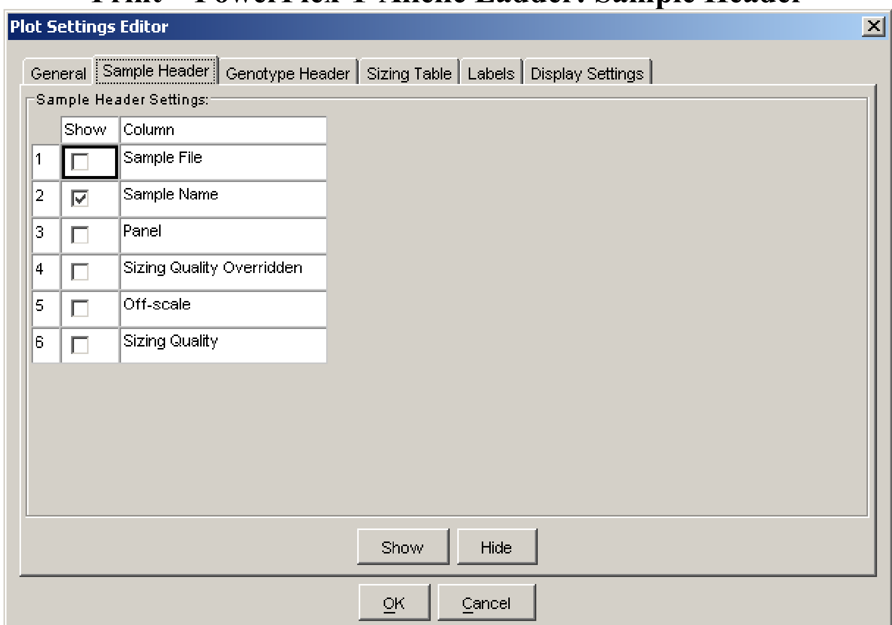


Print – Identifiler 31 Samples: Display Settings

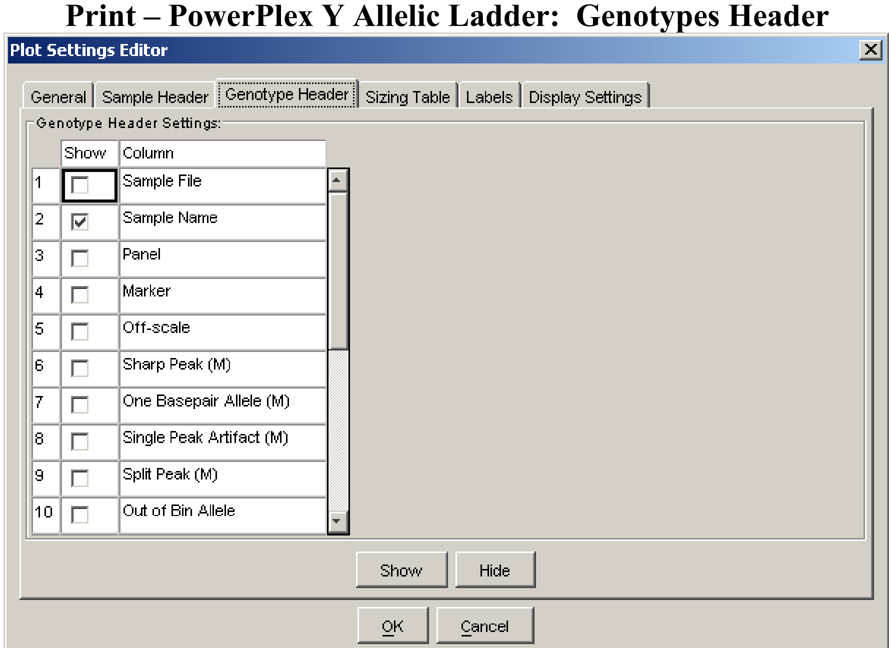


PLOT SETTINGS: PRINT – POWERPLEX Y ALLELIC LADDER

Print – PowerPlex Y Allelic Ladder: Sample Header



Print – PowerPlex Y Allelic Ladder: Genotypes Header



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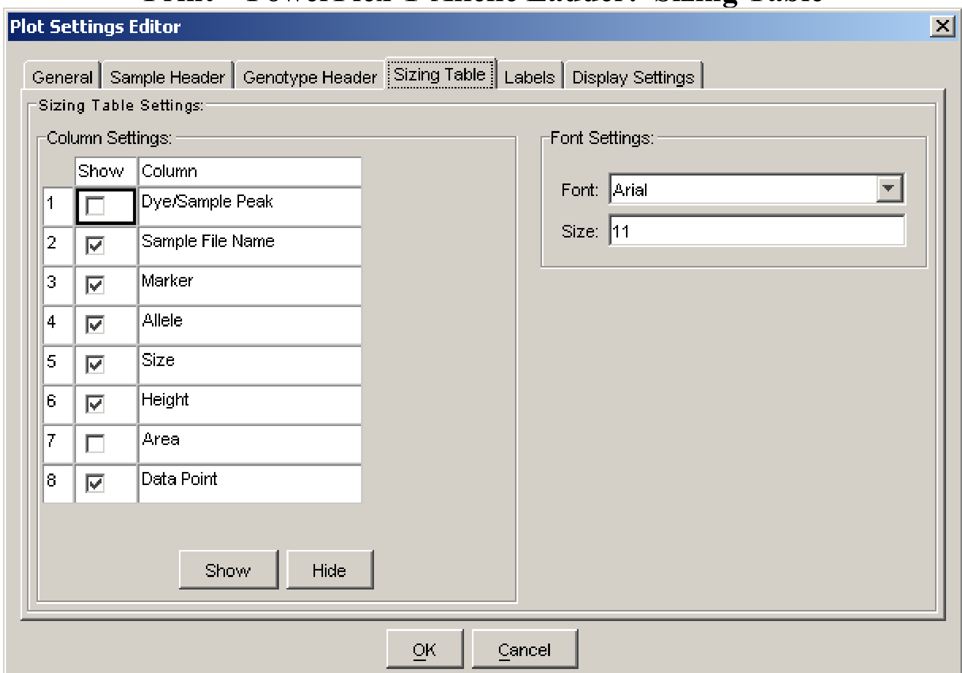
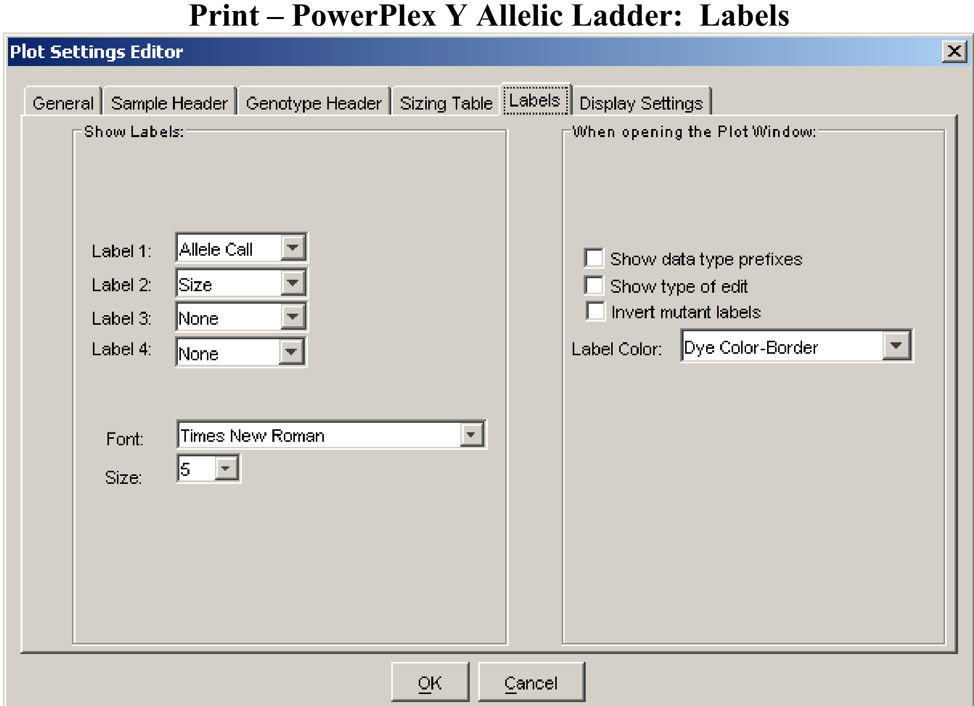
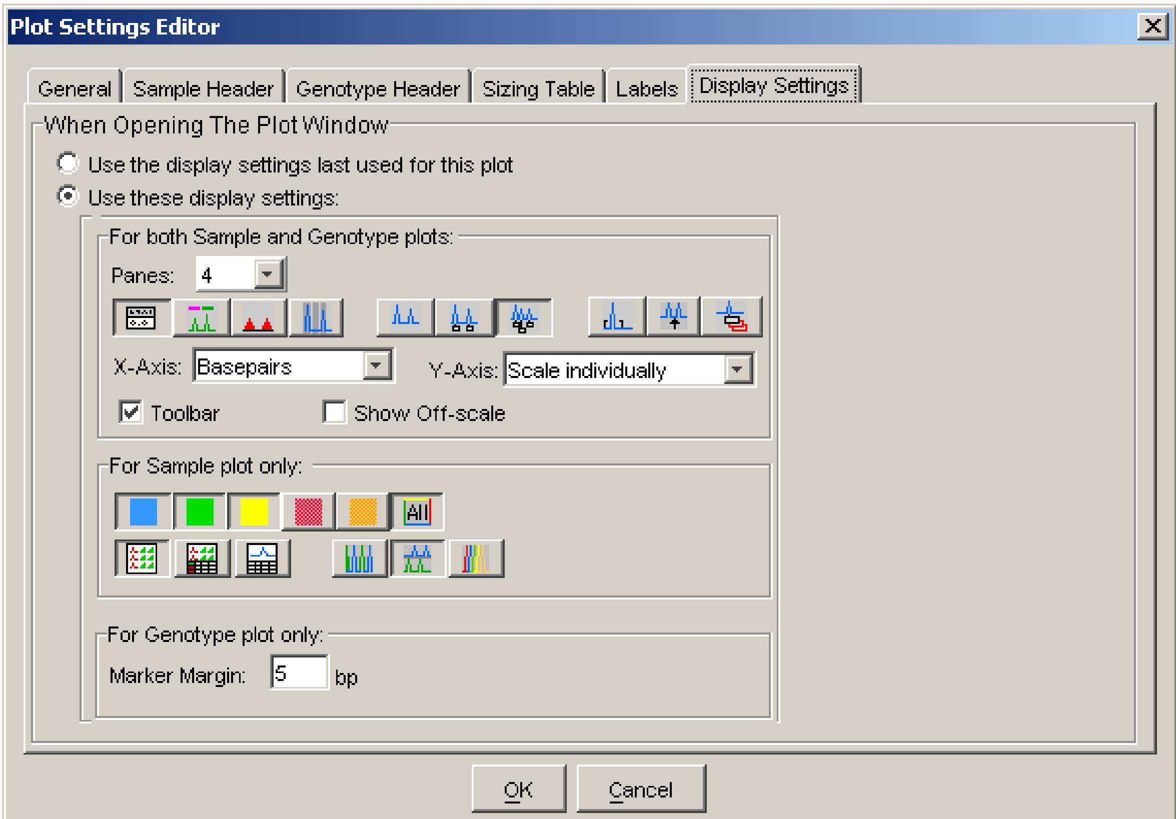
Print – PowerPlex Y Allelic Ladder: Sizing Table



Print – PowerPlex Y Allelic Ladder: Labels

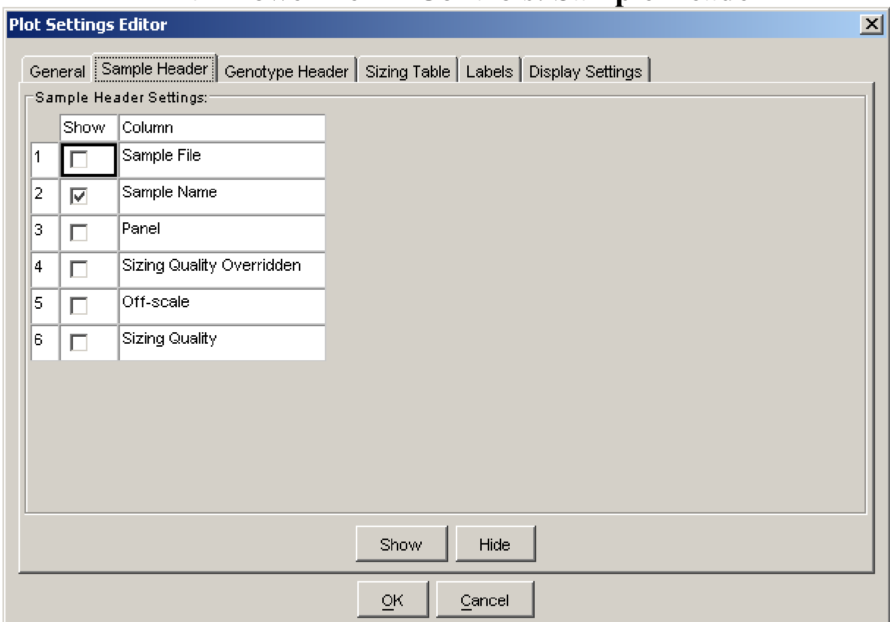


Print – PowerPlex Y Allelic Ladder: Display Settings



PLOT SETTINGS: PRINT – POWERPLEX Y CONTROLS

Print – PowerPlex Y Controls: Sample Header



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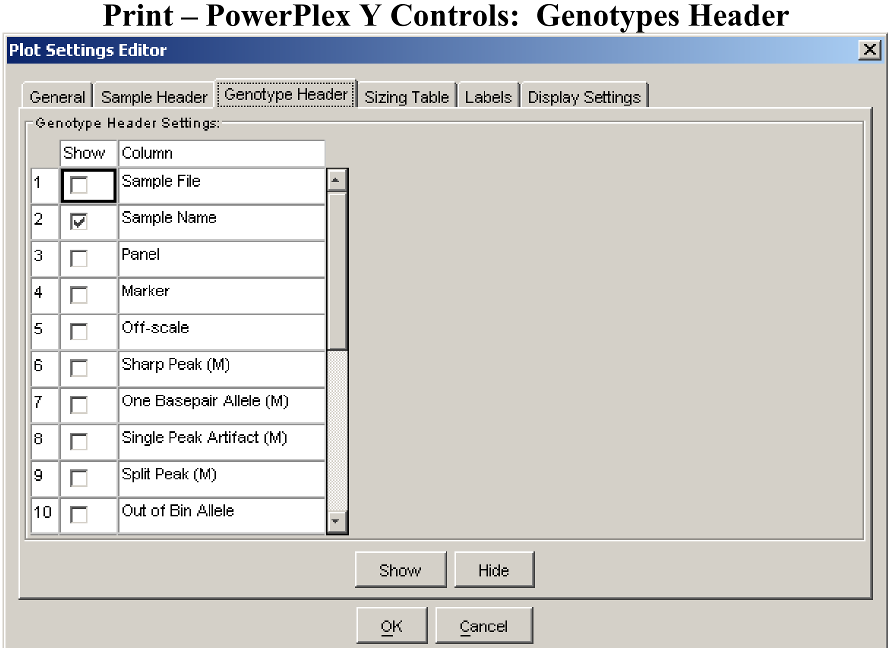

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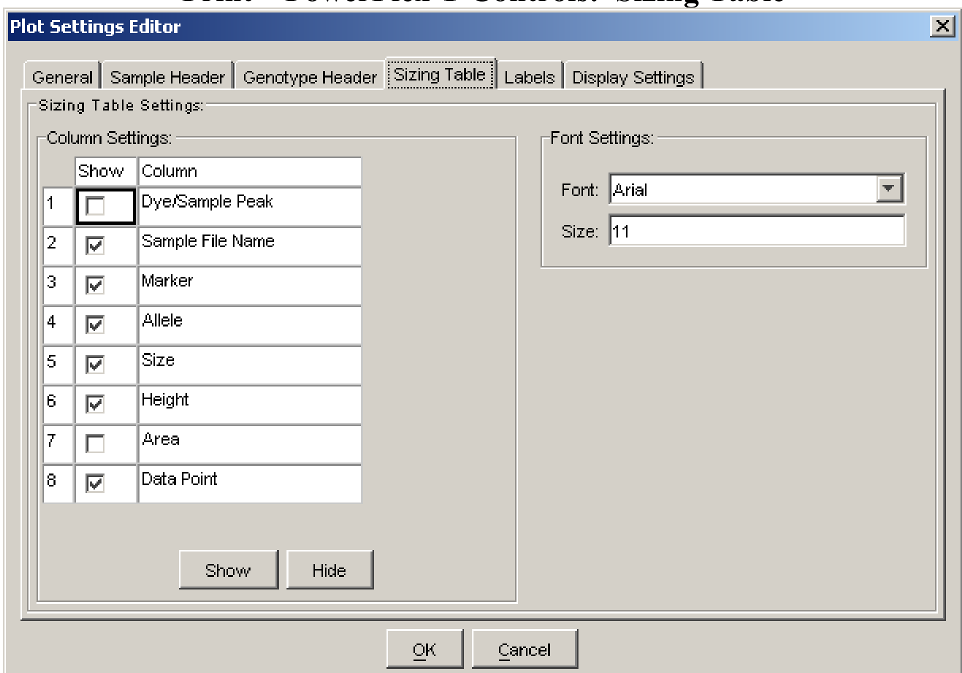
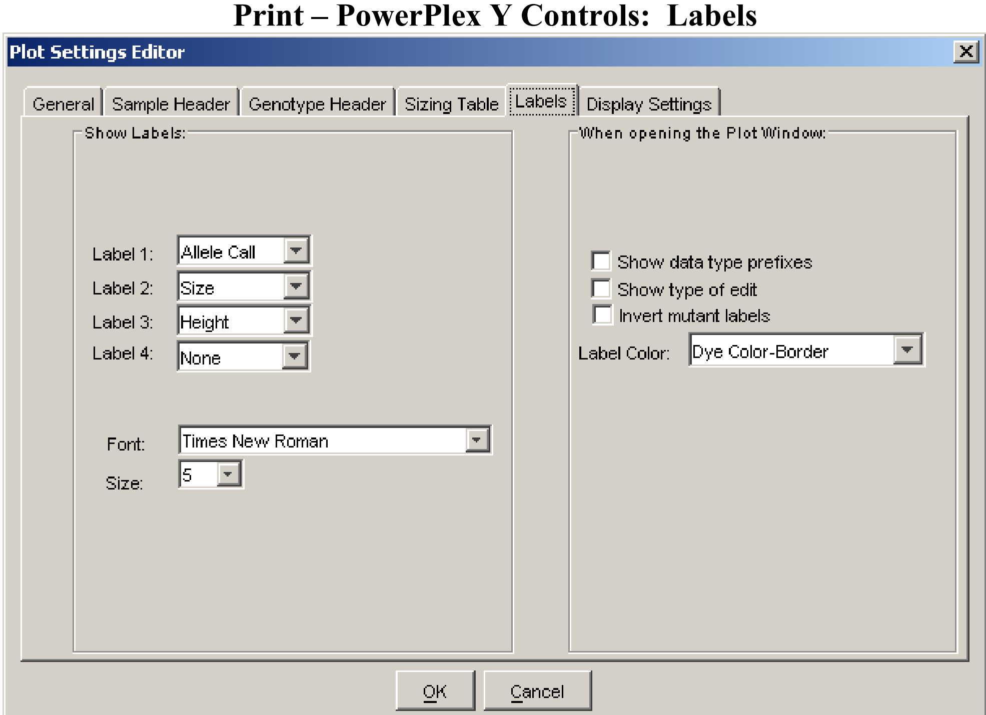
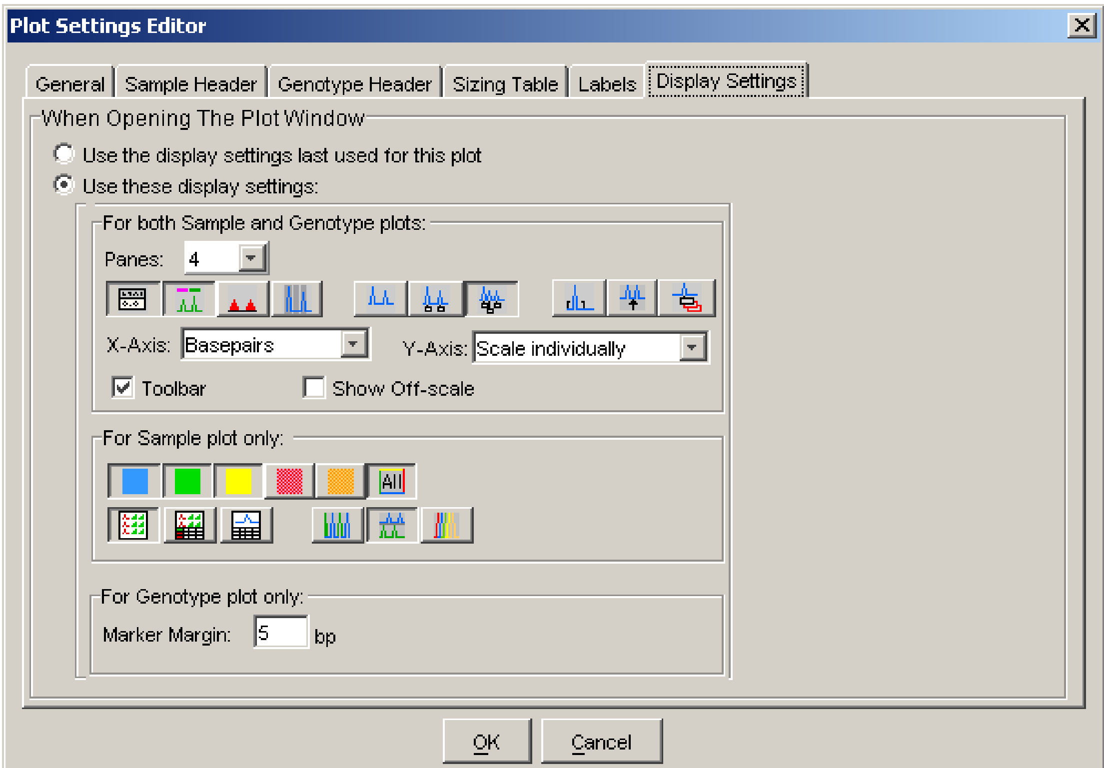
Print – PowerPlex Y Controls: Sizing Table



Print – PowerPlex Y Controls: Labels

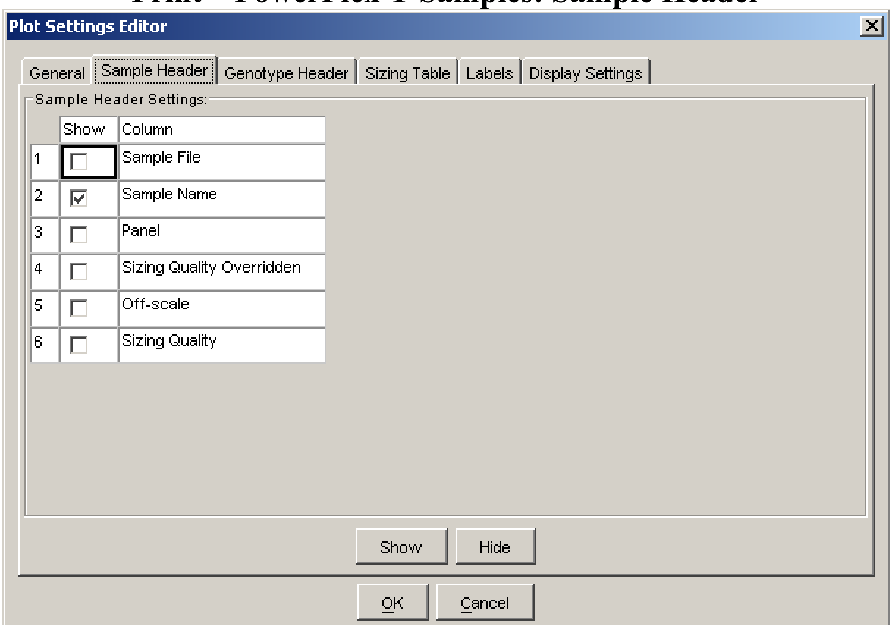


Print – PowerPlex Y Controls: Display Settings

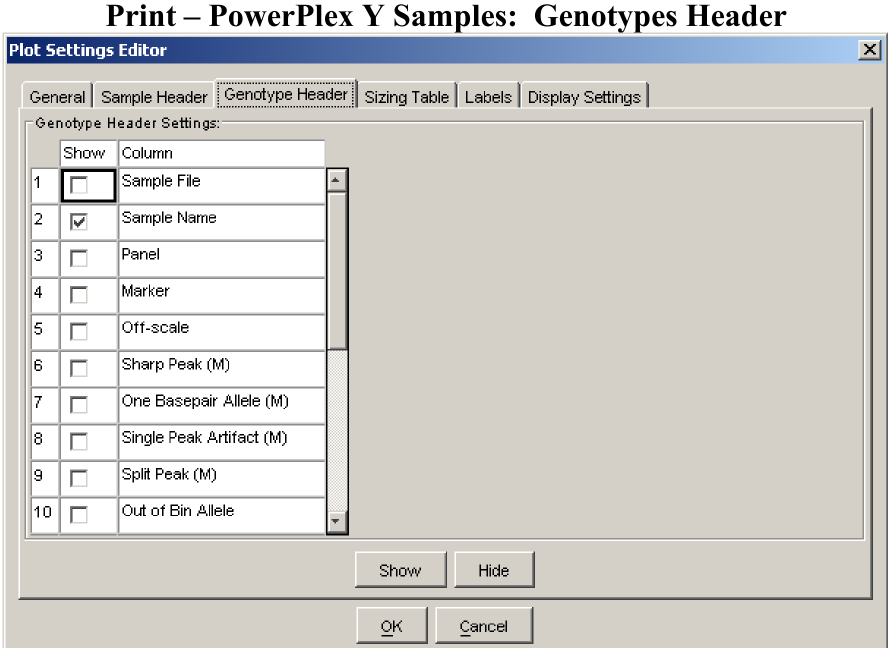


PLOT SETTINGS: PRINT – POWERPLEX Y SAMPLES

Print – PowerPlex Y Samples: Sample Header



Print – PowerPlex Y Samples: Genotypes Header



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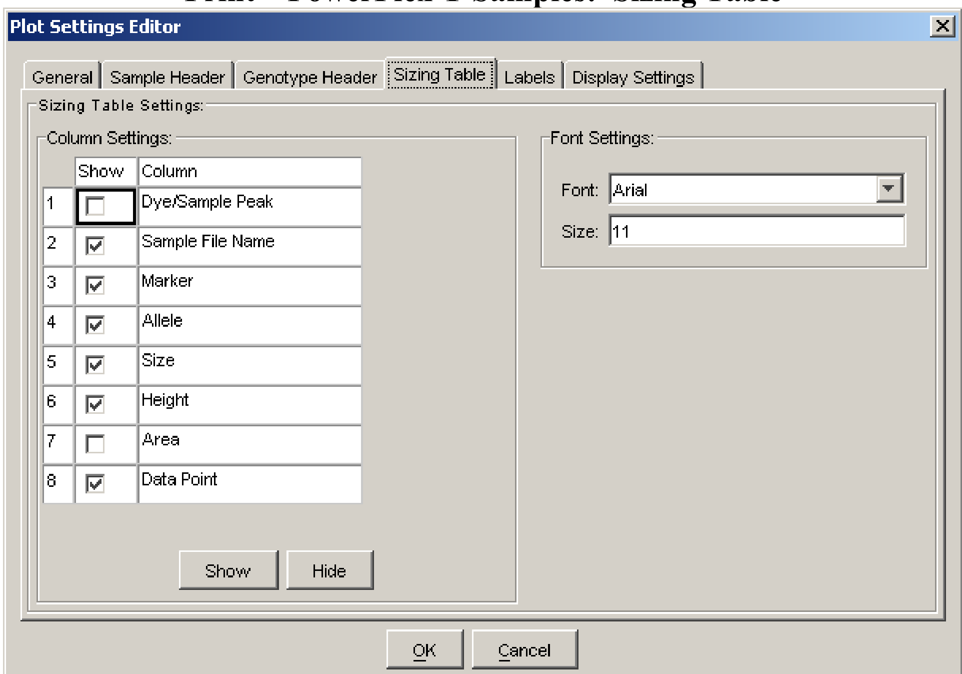
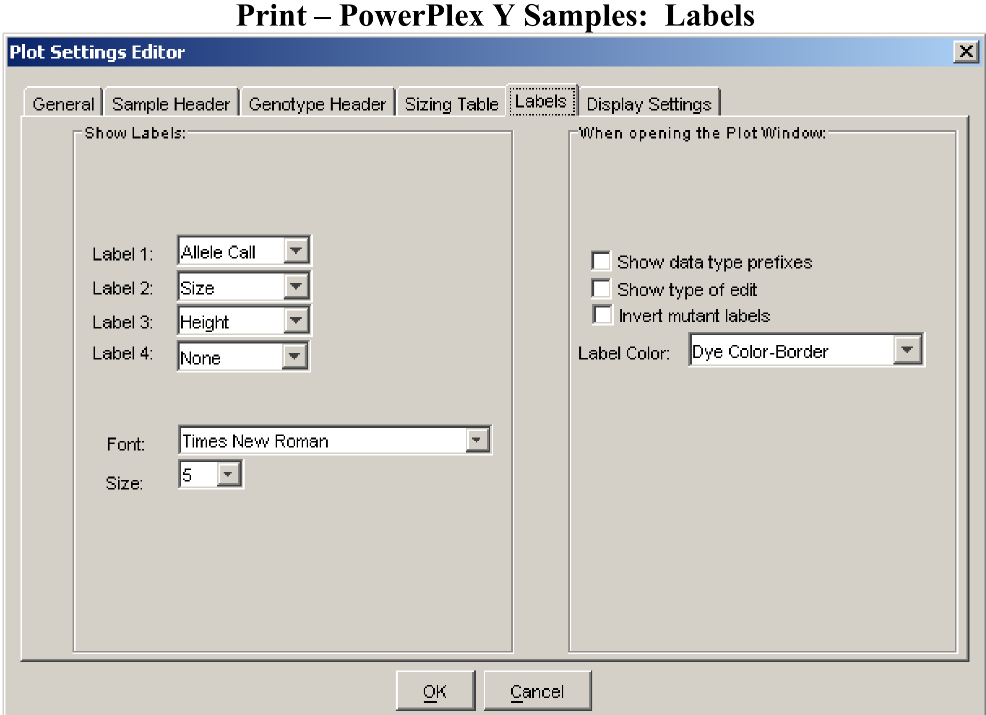
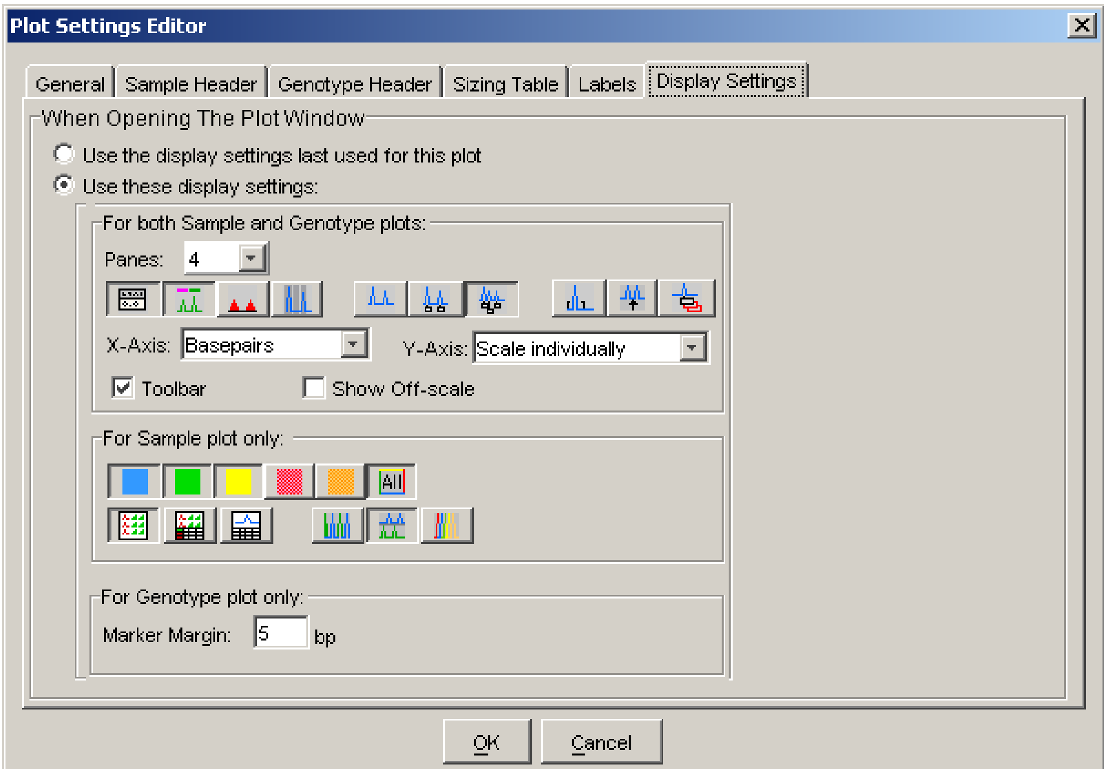
Print – PowerPlex Y Samples: Sizing Table



Print – PowerPlex Y Samples: Labels

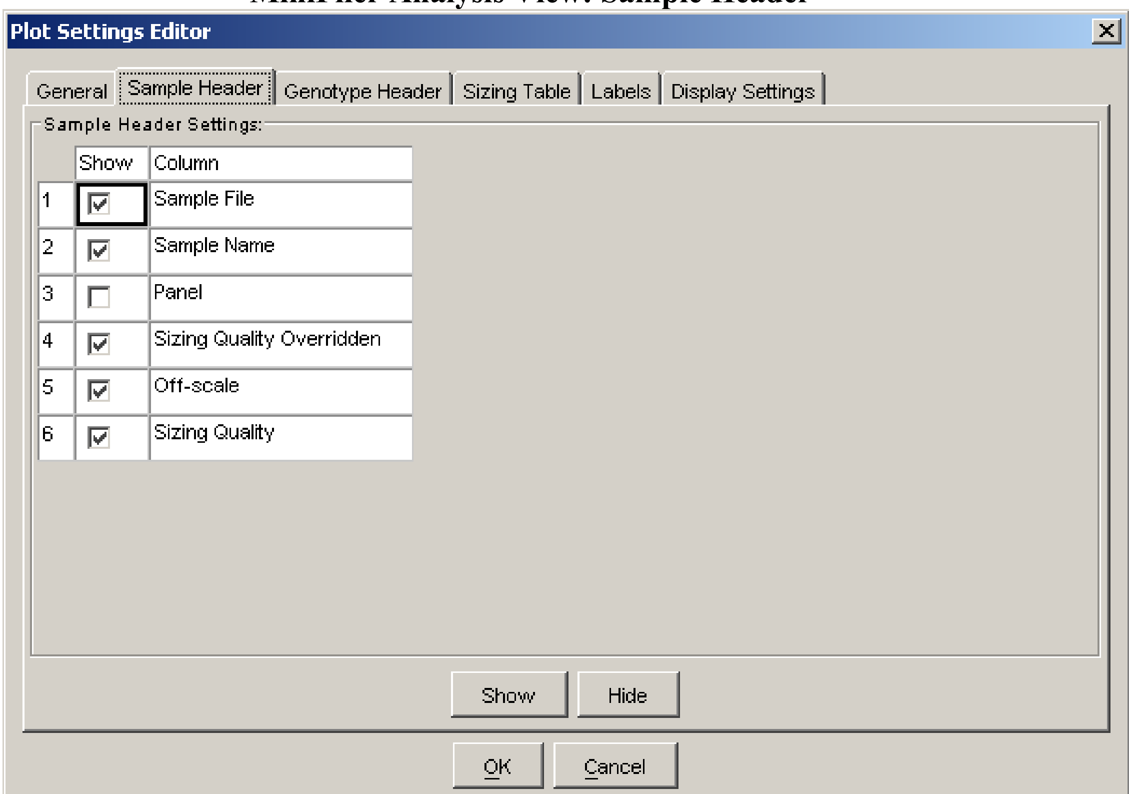


Print – PowerPlex Y Samples: Display Settings

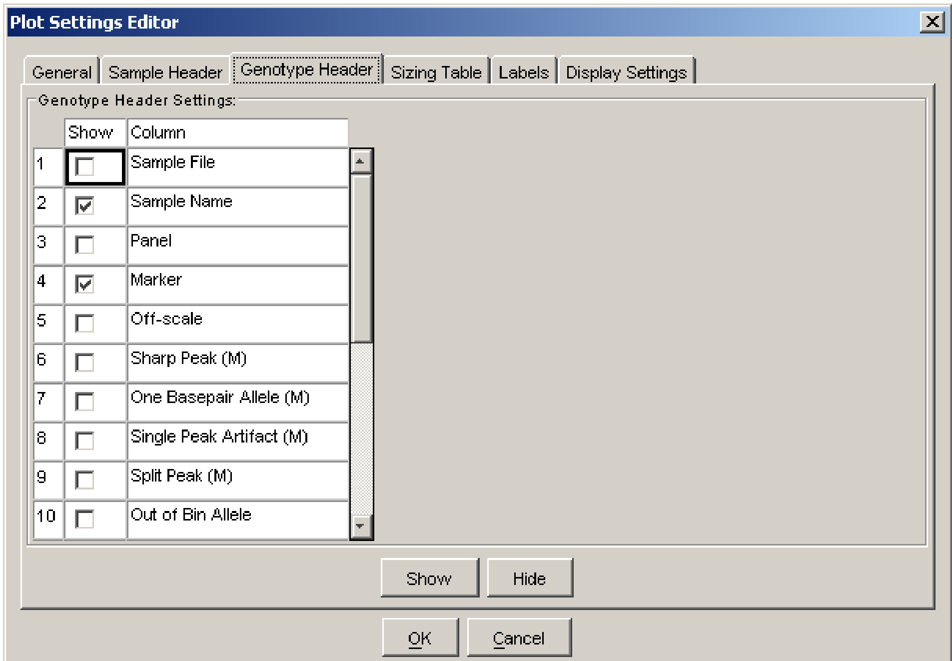


PLOT SETTINGS: MINIFILER ANALYSIS VIEW

MiniFiler Analysis View: Sample Header



MiniFiler Analysis View: Genotype Header



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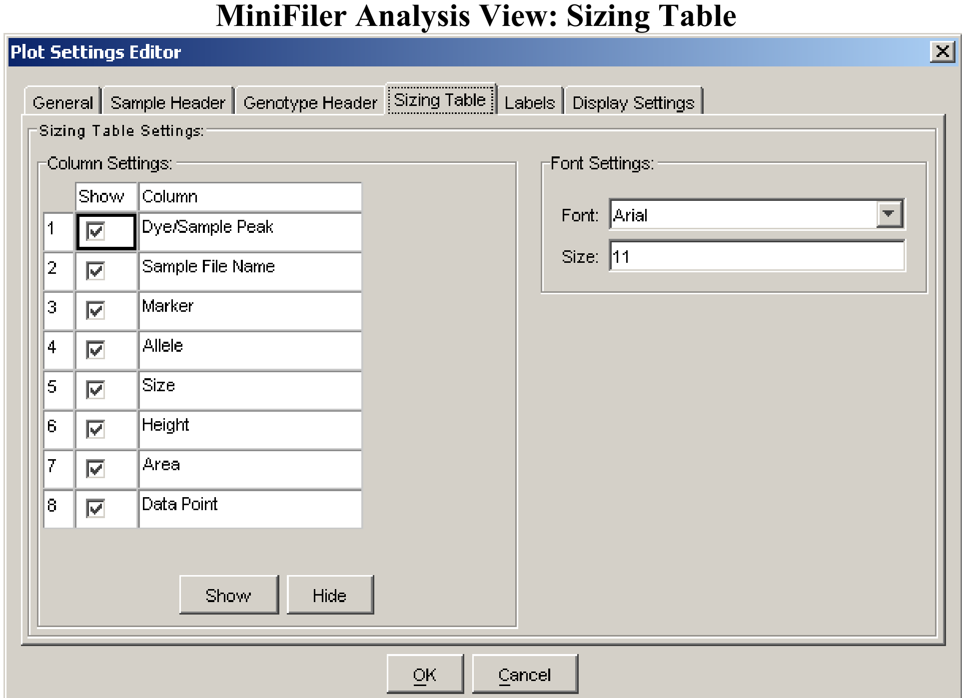
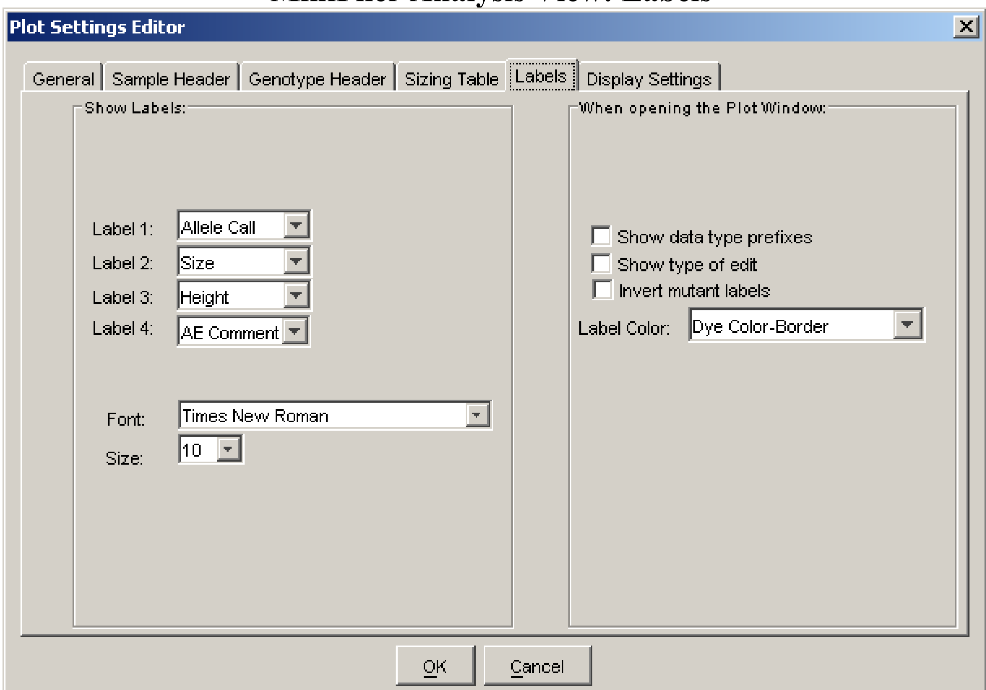
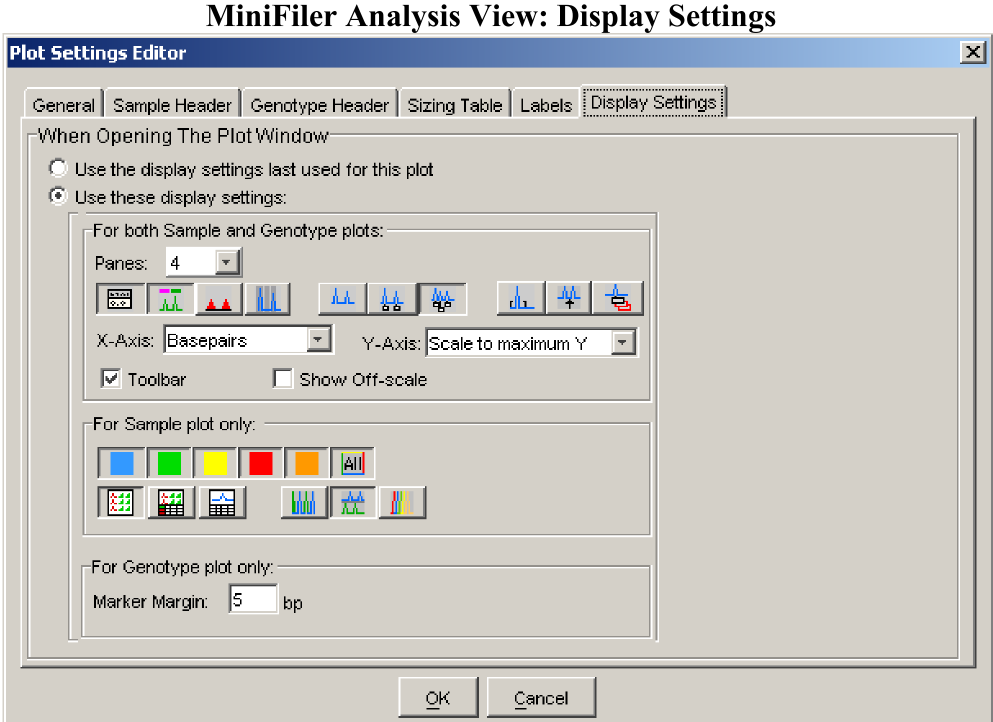
MiniFiler Analysis View: Sizing Table



MiniFiler Analysis View: Labels

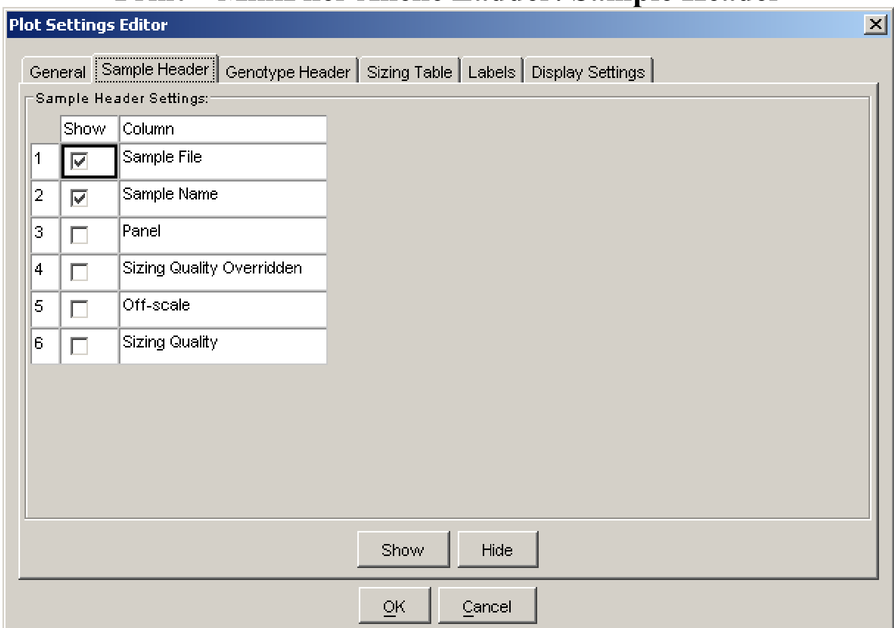


MiniFiler Analysis View: Display Settings

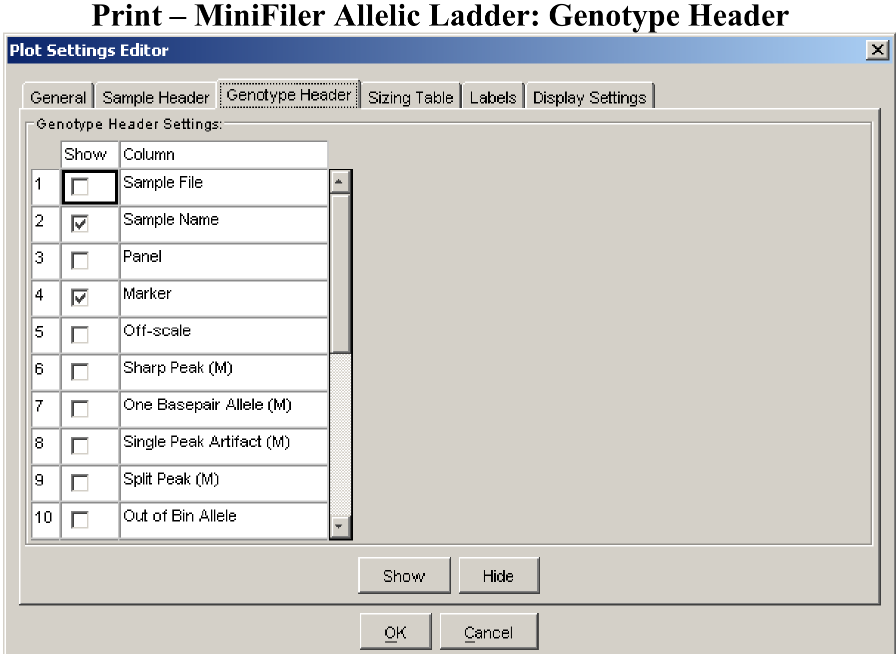


PLOT SETTINGS: PRINT – MINIFILER ALLELIC LADDER

Print – MiniFiler Allelic Ladder: Sample Header



Print – MiniFiler Allelic Ladder: Genotype Header



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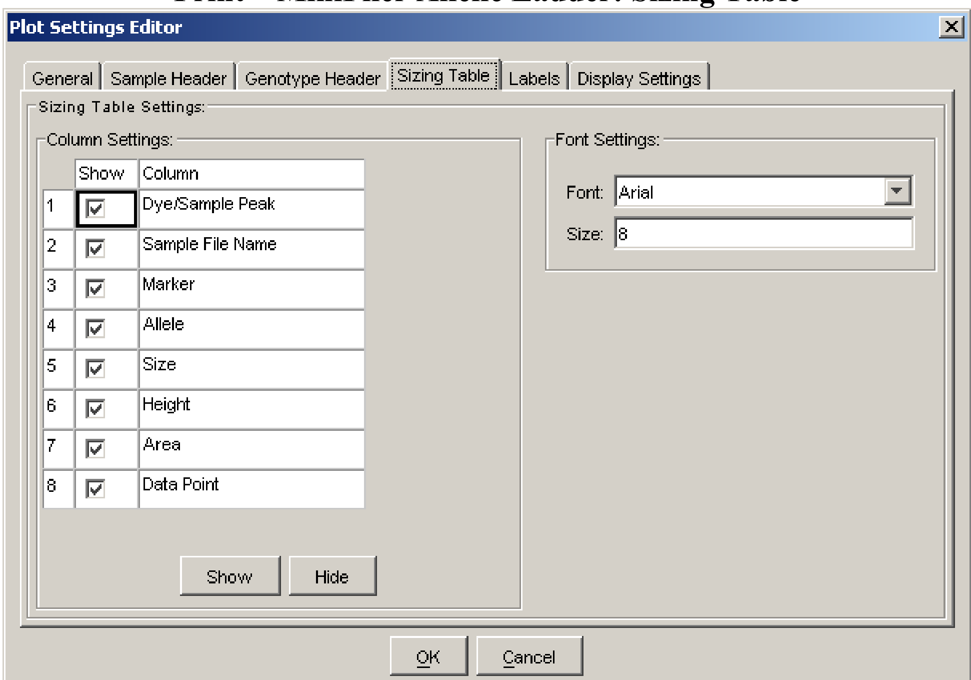
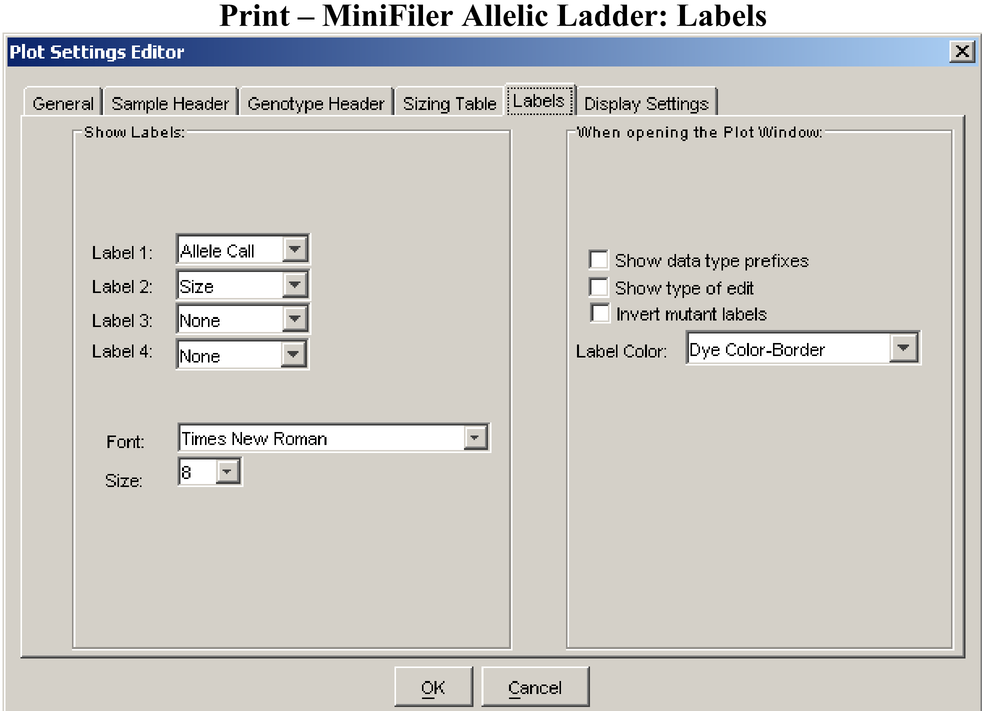
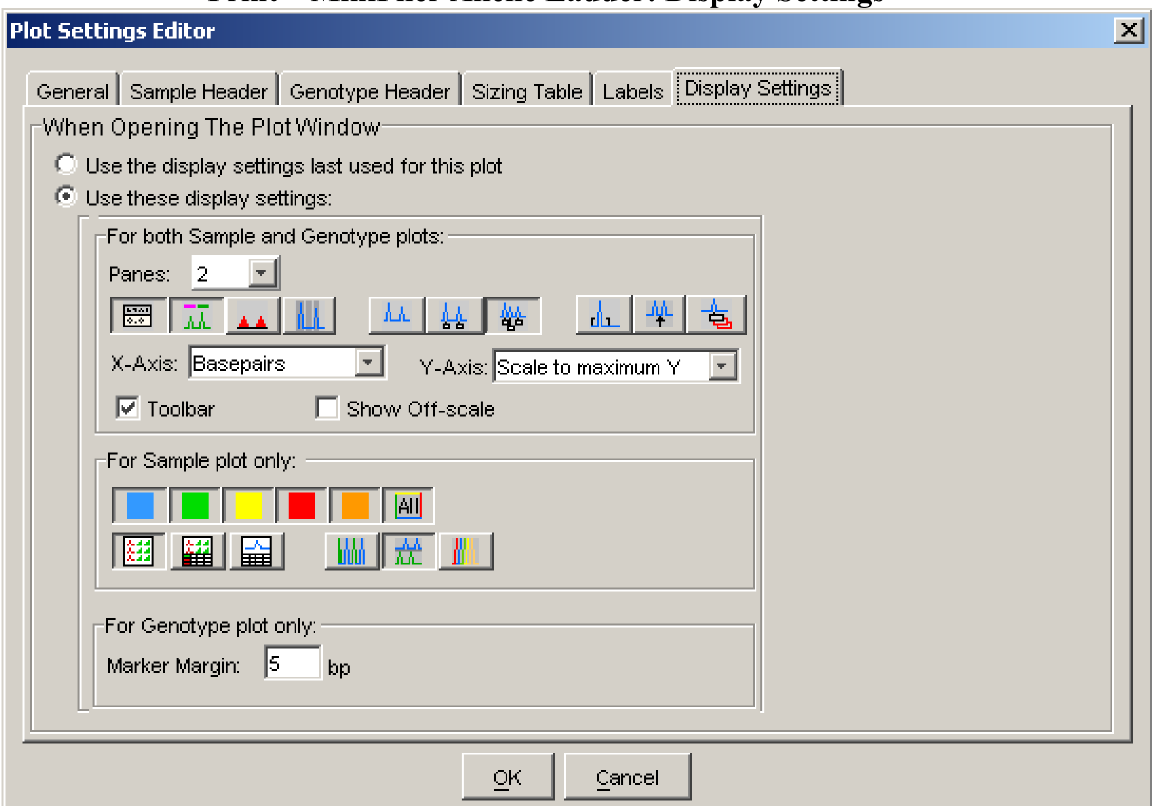
Print – MiniFiler Allelic Ladder: Sizing Table



Print – MiniFiler Allelic Ladder: Labels

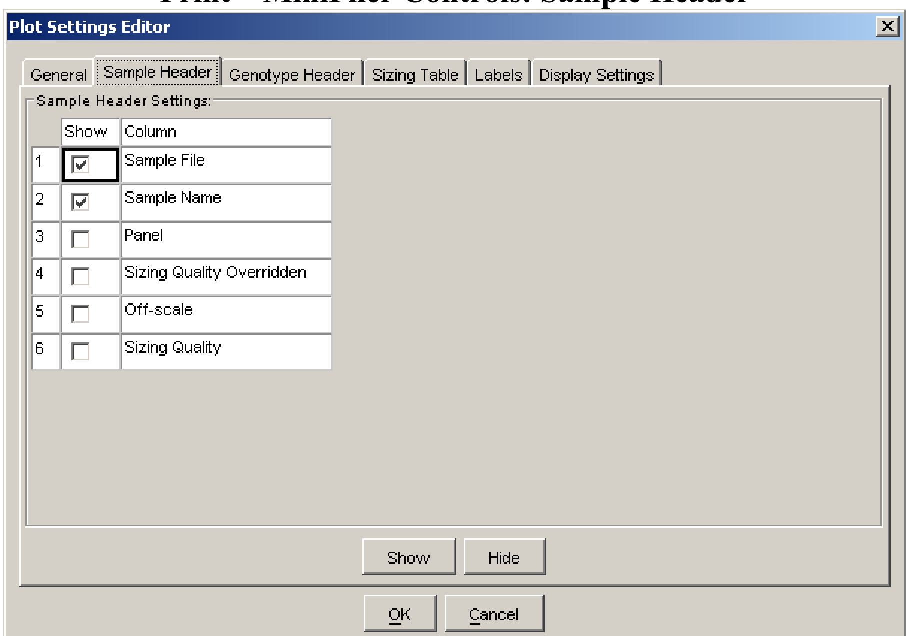


Print – MiniFiler Allelic Ladder: Display Settings

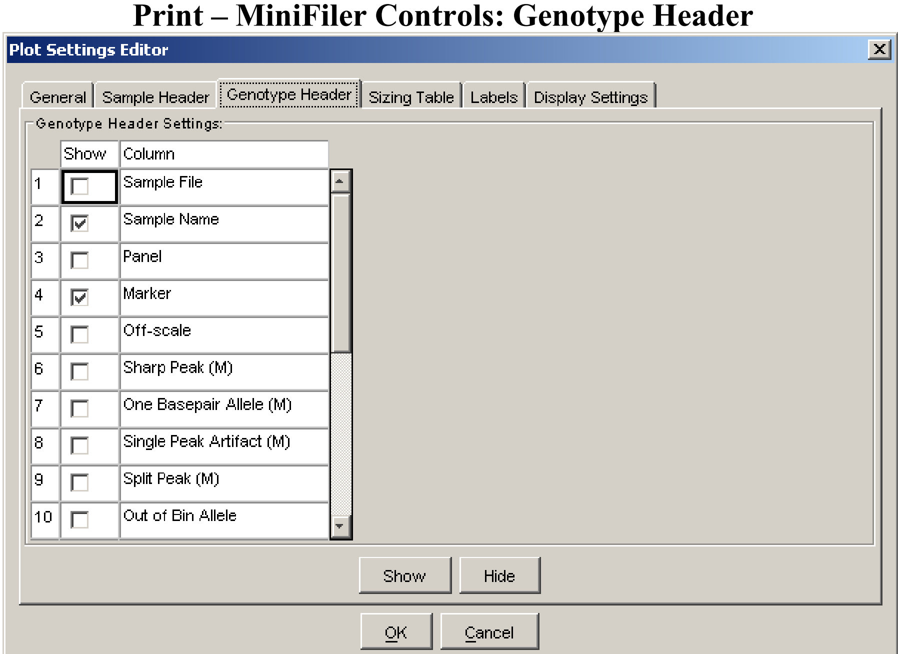


PLOT SETTINGS: PRINT – MINIFILER CONTROLS

Print – MiniFiler Controls: Sample Header



Print – MiniFiler Controls: Genotype Header



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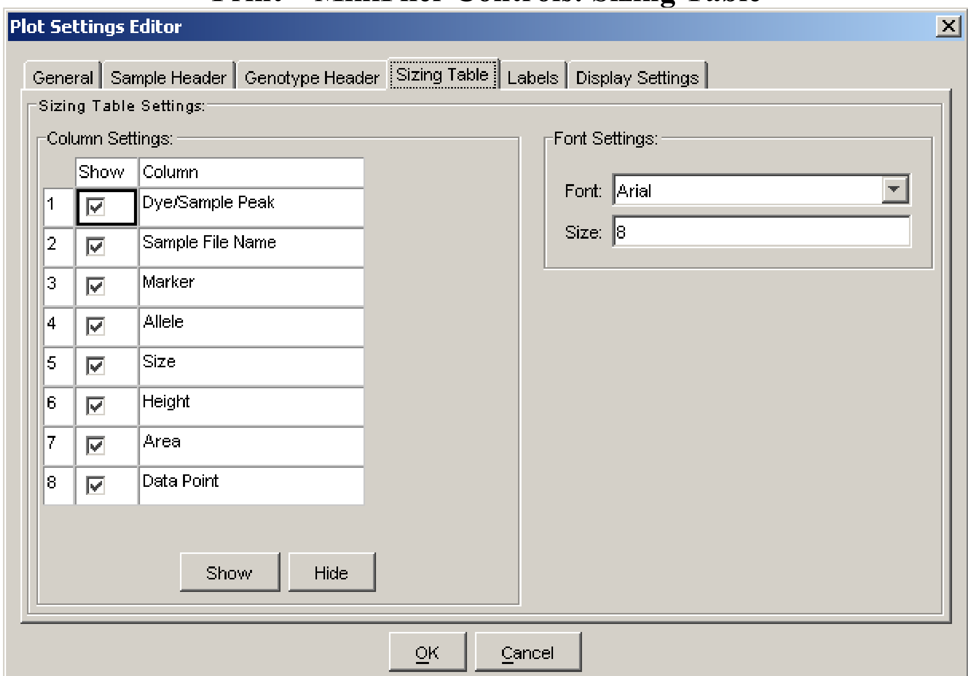
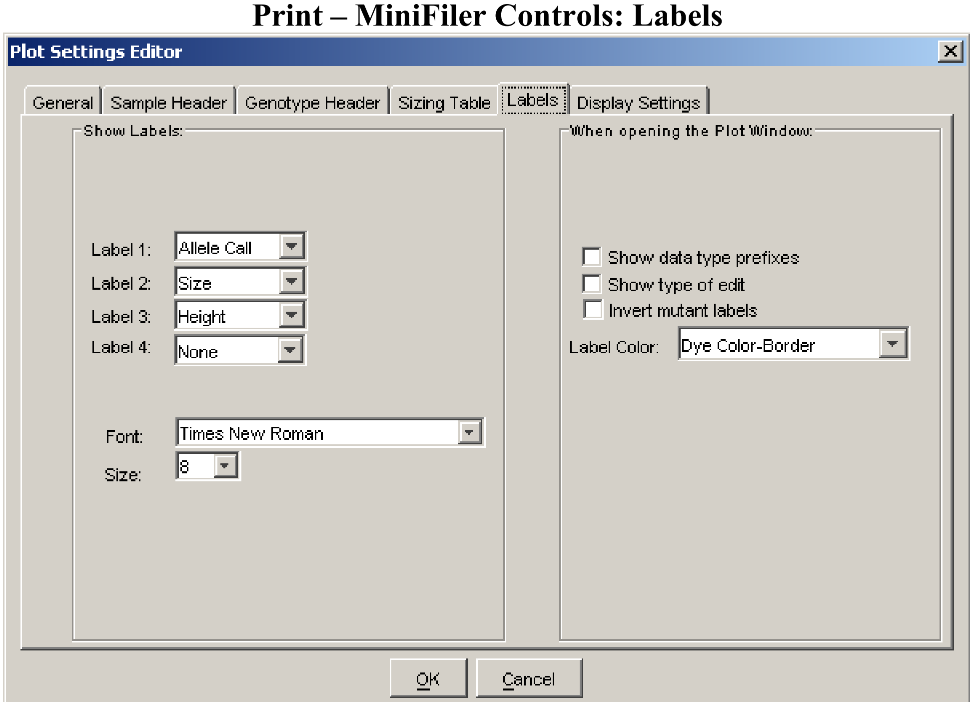
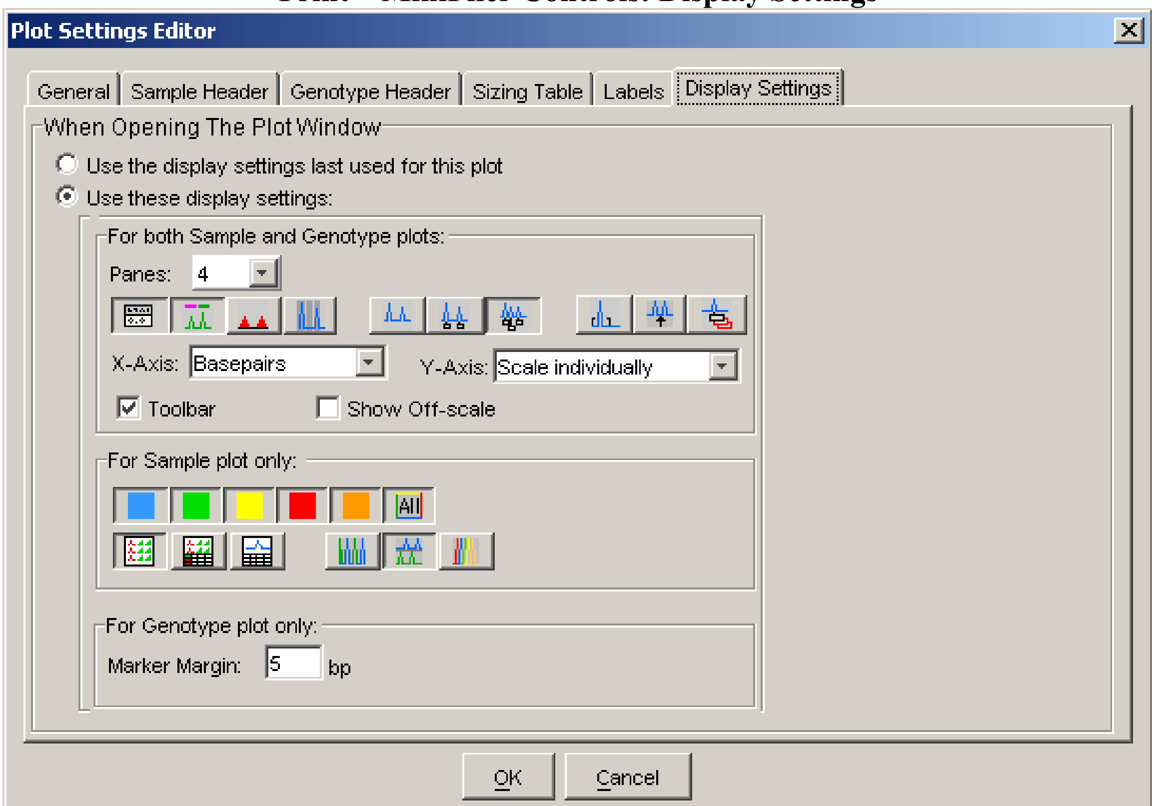
Print – MiniFiler Controls: Sizing Table



Print – MiniFiler Controls: Labels

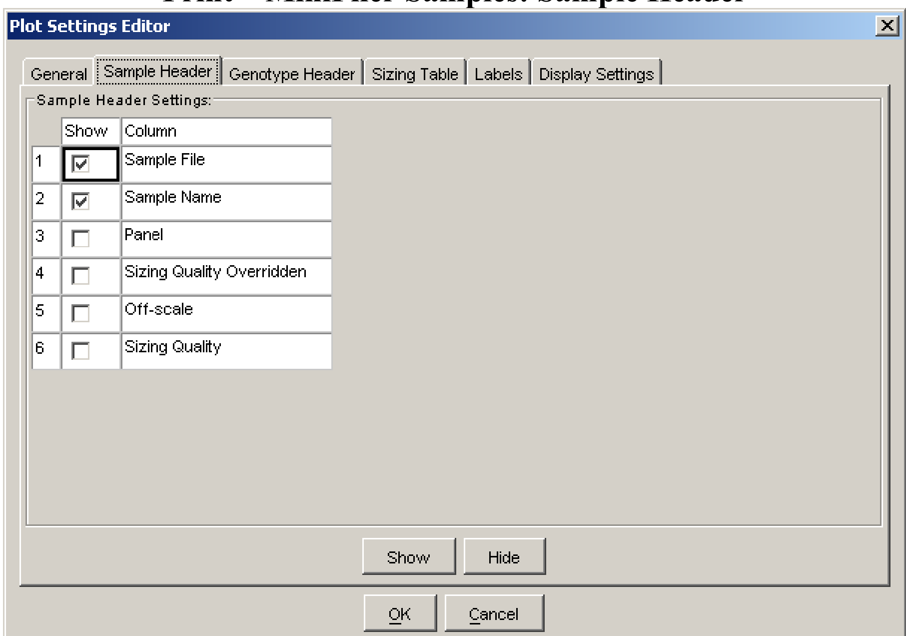


Print – MiniFiler Controls: Display Settings

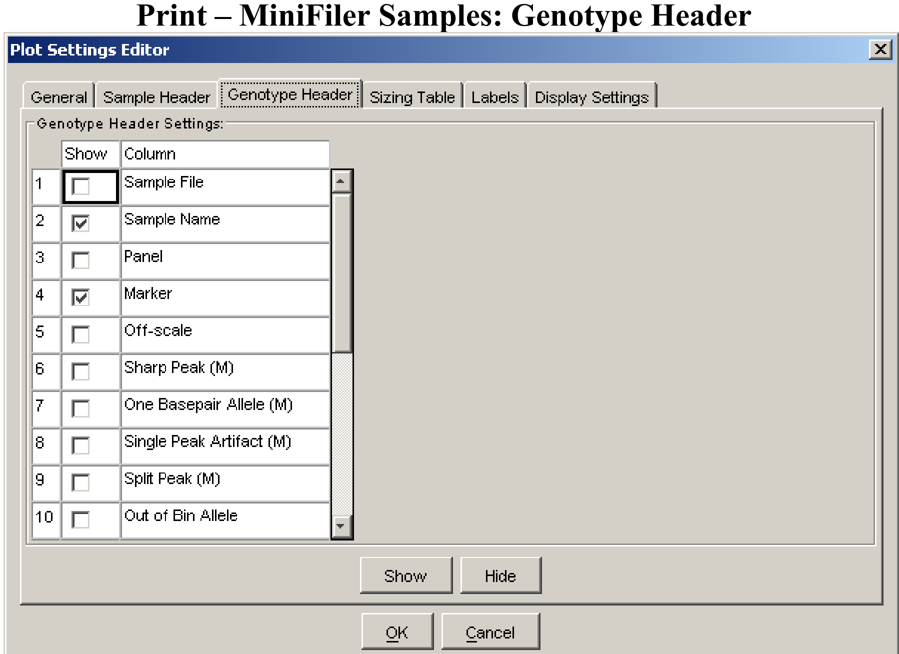


PLOT SETTINGS: PRINT – MINIFILER SAMPLES

Print – MiniFiler Samples: Sample Header



Print – MiniFiler Samples: Genotype Header



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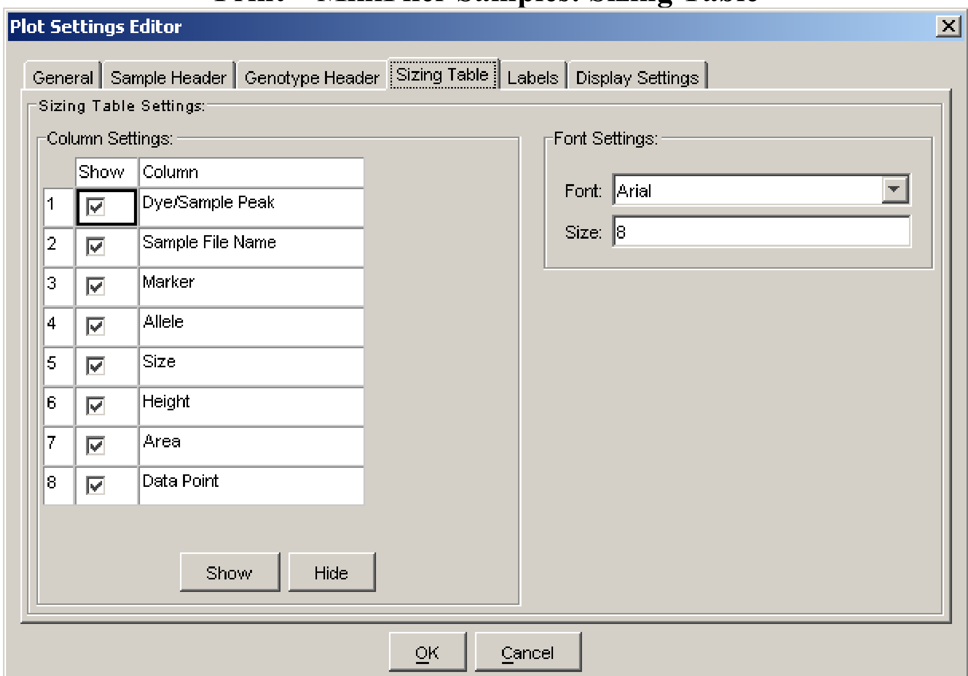
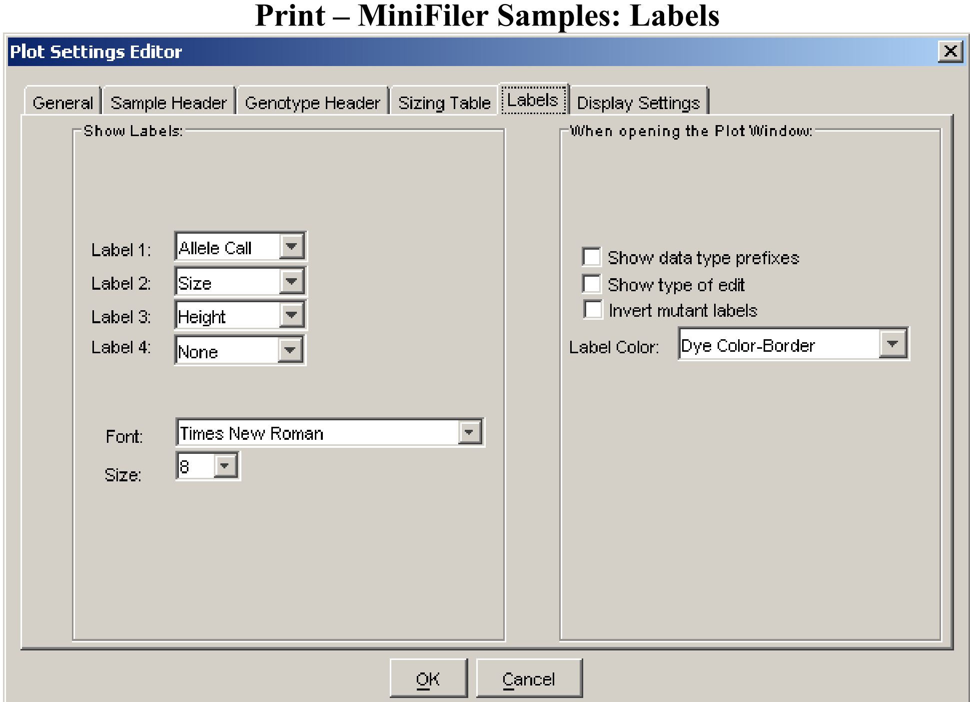
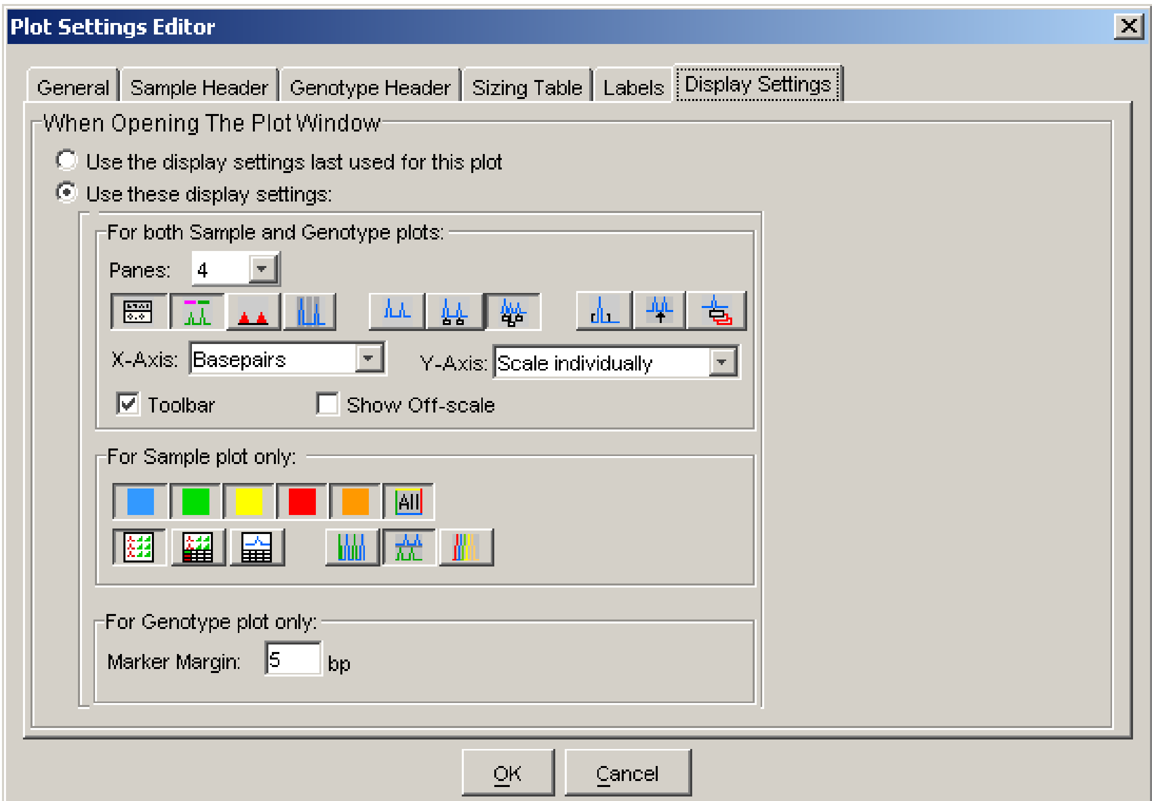
Print – MiniFiler Samples: Sizing Table



Print – MiniFiler Samples: Labels



Print – MiniFiler Samples: Display Settings



I. Allele Calling Criteria

Results are interpreted by observing the occurrence of electropherogram peaks for the loci that are amplified simultaneously. The identification of a peak as an allele is determined through comparison to the allelic ladder or for YM1 by the Genotyper categories. An allele is characterized by the labeling color of the locus specific primers and the length of the amplified fragment. See the Appendix for a listing of each locus in each multiplex.

For each locus an individual can be either homozygous and show one allele, or heterozygous and show two alleles. In order to eliminate possible background and stutter peaks, only peaks that display intensity above the minimum threshold based on validation data – 75 Relative Fluorescent Units (RFU's) – are labeled as alleles.

A. Computer program processing steps for raw data:

1. Recalculating fluorescence peaks using the instrument-specific spectral file in order to correct for the overlapping spectra of the fluorescent dyes.

2. Calculating the fragment length for the detected peaks using the known in-lane standard fragments.

3. For YM1 (a system without an allelic ladder) – labeling of all sized fragments that are >75 RFU fall within the locus size range and match to an allele size average within a ∀ 1.0bp tolerance window. Labels are automatically removed from minor peaks based on the background and stutter filter functions outlined in the YM1 Genotyper section.

4. For Identifiler 28, Identifiler 31, Cofiler, and Profiler Plus (systems with an allelic ladder) – comparing and adjusting the allele categories to the sizing of the co-electrophoresed allelic ladder by calculating the off sets (the difference between the first allele in a category and the first allele in the allelic ladder at each locus).

5. For, Identifiler 28, Identifiler 31, Cofiler, and Profiler Plus – labeling of all sized fragments that are above threshold and fall within the locus specific size range (see Appendix). Removing the labels from minor peaks (background and stutter) according to the filter functions detailed in the appendix of this manual.

II. Manual Removal of Non Allelic Peaks

Additional **non-allelic peaks** may occur under the following instances (Clark 1988, Walsh et al. 1996, Clayton et al. 1998), which may be manually edited. Make sure not to remove any labels for potential DNA alleles. All edits must have a reference point on the editing sheet. When in doubt leave the peak labeled for review. Mixture samples must be edited conservatively and only electrophoresis artifacts can be eliminated. Peaks in stutter positions cannot be edited for mixtures, except when masked, (see D4).

A. Pull-up

1. Pull-up of peaks in one color may be due to very high peaks in another color. Pull-up is a spectral artifact that is caused by the inability of the software to compensate for the spectral overlap between the different colors if the peak height is too high.

2. The label in the other color will have a basepair size very close to the real allele in the other color. The peak that is considered an artifact or “pull up” will always be shorter than the original, true peak. It is possible to for a particularly high stutter peak in for example blue or green, to create pull up in red or orange.

3. Spectral artifacts could also be manifested as a raised baseline between two high peaks or an indentation of a large peak over another large peak. Labels placed on such artifacts can be removed and is known as “spectral over-subtraction”.

B. Shoulder

Shoulder Peaks are peaks approximately 1-4 bp smaller or larger than main alleles. Shoulder Peaks can be recognized by their shape; they do not have the shape of an actual peak, rather they are continuous with the main peak.

C. Split peaks ("N" Bands)

Split peaks are due to the main peak being split into two peaks caused by the Taq polymerase activity that causes the addition of a single "A" to the terminus of the amplified product ("N+1" band). Since allele calling is based on N+1 bands, a complete extra "A" addition is desired.

1. Split peaks due to incomplete non nucleotide template A addition should not occur for samples with low amounts of DNA

2. Split peaks can also be an electrophoresis artifact and attributed to an overblown allele. Additional labels can be edited out.

3. Split peaks may occur in overblown samples or amplicons due to matrix over-subtraction. For example, an overblown green peak may dip at the top where a pull up peak is present in blue and in red. The yellow peak will also display over-subtraction with a dip at the peak's crest.

D. Stutter – 4bp smaller than the main allele
(Peaks one repeat unit longer or multiple units shorter than the main allele may be stutter, but is rare.)

1. The macro for each system has an automated stutter filter for each locus (see appendix for stutter values)

2. In addition, for single source samples, potential stutter peaks may be removed if they are within 15% of the larger peak for Cofiler, Profiler Plus, and YM1, and 20% of the larger peak for Identifiler.

3. Identifiler 31 samples have been shown to occasionally display peaks 4 bp longer than the main allele.

4. If the main allele has an additional label prior to the main allele label (e.g. a shoulder peak, 1bp less in size) this peak will be used for stutter

percentage calculation and the stutter might not have been automatically removed. In this case, the stutter peak can also be removed for mixtures.

5. Peaks that are overblown with RFUs above 7000 (and thus their peak height has plateaued), will often have a stutter peak that will be more than 20% of the main peak. If the sample is not a mixture, the stutter peaks for the alleles above 7000 RFUs may be removed.

E. Non specific artifacts

This category should be used if a labeled peak is caused by a not-previously categorized technical problem or caused by non-specific priming in a multiplex reaction. These artifacts are usually easily recognized due to their low peak height and their position outside of the allele range.

F. Elevated baseline

Elevated or noisy baseline may be labeled. They do not resemble distinct peaks. Sometimes, an elevated baseline may occur adjacent to a shoulder peak.

G. Spikes

1. Generally, a spike is an electrophoresis artifact that is usually present in all colors.

2. Spikes might look like a single vertical line or a peak. They can easily be distinguished from DNA peaks by looking at the other fluorescent colors, including red or orange. For Identifiler™, a spike may appear in the red or green, but not be readily apparent in the other colors. However, you can zoom in and confirm the spike.

3. Spikes may be caused by power surges, crystals, or air bubbles traveling past the laser detector window during electrophoresis.

H. Dye Artifacts

1. Constant peaks caused by fluorescent dye that is not attached to the primers or is unincorporated dye-labeled primers. These "color blips" can occur in any color. Dye artifacts commonly occur in the beginning of the green, blue, and the yellow loci right after the primer peaks (Applied Biosystems 2004 a and b).

2. These artifacts may or may not appear in all samples, but are particularly apparent in samples with little or no DNA such as the negative controls.

I. Removal of a range of alleles

Mixed samples which contain overblown peaks must be rerun. Refer to the Genotyper Analysis Section for more information.

All manual removals of peak labels must be documented on the editing sheet. This sheet also serves as documentation for the technical review. Check the appendix for the correct peak assignments to each allelic ladder and the expected genotype of the positive control.

III. Detection of Rare Alleles

A. New Allele/Off Ladder Allele

1. A peak defined outside the defined allele range or is not present in the allelic ladder.

2. If an OL allele could be a true allele, the sample must be rerun.

3. If multiple samples from the same case within the same run all show the same OL allele, only one sample needs to be rerun to confirm the OL allele.

4. Off-ladder alleles that are within the range of the ladder and are called by the software need not be rerun (i.e., a "19.2" at FGA).

5. If an assigned allele is either larger or smaller than the smallest or largest allele in the ladder, it should be rerun.

6. Use the following table for guidance if off-ladder alleles occur in samples that are injected with the same or different parameters:

Table 1 Retesting Strategies for Rare Alleles

Injection 1	Injection 2 at same or higher injection parameter	Course of Action
Allele called	Allele labeled as "OL"	No rerun necessary report called allele.
Allele labeled as "OL"	Allele called	No rerun necessary report called allele
Allele not called	Off Ladder	Rerun high
Allele labeled as "OL"	Allele labeled as "OL"	No rerun necessary report allele relative to position in the allelic ladder

7. After the second rerun, the allele is still off ladder, examine the allele closely. If it is not at least one basepair from a true allele, it is likely not a real off-ladder allele. In this case, a third injection on another instrument may be done to rule out the possibility of migration. If the locus is small and the peak heights are high, the sample may be re-aliquotted and re-injected.

IV. Interpretation of STR Data

A. Allele Table

1. After the assigning of allele names to the remaining labeled peaks, the software prepares a result table where all peaks that meet the above listed criteria are listed as alleles.

2. The allele nomenclature follows the recommendations of the International Society for Forensic Haemogenetics (ISFH), (DNA recommendations, 1994) and reflects the number of 4bp core repeat units for the different alleles.

3. Subtypes displaying incomplete repeat units are labeled with the number of complete repeats and a period followed by the number of additional bases.

4. The Y chromosome allele nomenclature is also based on the number of 4bp core repeats and follows the nomenclature suggested in Evaluation of Y Chromosomal STRs (Kayser et al 1997) and the one used in the European Caucasian Y-STR Haplotype database (Roewer et al 2001).

B. Electropherograms

1. Printouts of capillary electrophoresis runs containing case specific samples are part of each case file.

2. The table reflects the number and allele assignments of the labeled peaks visible on the plot print out. **The plot printouts are the basis for results interpretation.**

3. The plot will display peak height information, unlabeled peaks, intensity differences that may indicate the presence of a mixture, and will show all peaks at each locus.

4. Looking at the plots also serves as a control for the editing process.

5. In certain instances it may be necessary to view the electropherogram electronically:

    a. No peak is above the minimum threshold but unlabeled peaks are visible. Refer to Genotyper Analysis Procedure.

    b. High peaks and very minor peaks present in the same color lane

        i. Since the RFU scale of the electropherogram is based on the highest peak in each color, alleles at weak loci will not be clearly visible if the loci are imbalanced.

        ii. Access the file for mixture interpretation or allelic dropout detection.

        iii. Go to **View** menu enter a fixed y-scale for **Plot Options, Main Window Lower Panel**. Print pages. Do not save changes.

    c. Plot states "no size data available"

        i. None of the peaks were above threshold

        ii. The original data which may be visible in GeneScan, displays visible peaks below the sizing threshold.

    d. Distinct unlabeled peak in locus with similar height as "homozygous" allele. Refer to Section III – Detection of Rare Alleles.

V. Interpretation of controls

A. Electrophoresis Controls

1. Allelic Ladder

   Evaluate the allelic ladder for expected results – Refer to Genotyper Analysis Section.

2. Amplification Positive Control

   a. Evaluate the positive control for the expected type

      i. YM1 – Refer to Genotyper Analysis Section I

      ii. Identifiler 28 – Refer to Genotyper Analysis Section II

      iii. Identifiler 31 – Refer to the Guidelines for reporting samples amplified with Identifiler for 31 cycles section IX to assign alleles to the positive control profile, and to Genotyper Analysis section II for the expected profile.

   b. If the positive control has been shown to give the correct type, this confirms the integrity of the electrophoresis run and amplification set.

   c. The amplification positive control may be run at a different (lower or higher) injection parameter or dilution than the corresponding samples and the amplification set can pass.

3. Electrophoresis Run with Failed Positive Control

    a. Electrophoresis Run containing one Positive Control

        i. fill out an Electrophoresis Failure Report or a Resolution Sheet and indicate the Positive Control will be rerun

        ii. Retest the Positive Control

            a) If the Positive Control passes, then rerun the complete Amplification Set with the retested Positive Control. (The entire amplification set, including the positive control, may be rerun together as determined by the analyst.)

            b) If the Positive Control fails; the Amplification Set fails. Fill out an Electrophoresis Failure Report or a Resolution Sheet and indicate the Amplification Set will be re-amplified.

    b. Electrophoresis Run containing more than one Positive Controls

        i. use another Positive Control to analyze the run

        ii. Complete the STR Control Review Sheet indicating the failed Positive Control "will be rerun"

        iii. Add the sample number corresponding to the (failed) Positive Control to the Editing sheet

        iv. Retest the (failed) Positive Control

            a) If the Positive Control passes; the Amplification Set passes

            b) If the Positive Control fails; the Amplification Set fails. Complete the STR Control Review Sheet indicating the "sample set will be re-amplified"

e. Reruns / Re-injections

An injection set consisting of reruns or re-injections must have at least one Positive Control

Table 2 Interpretation of Electrophoresis Runs

Controls / Status	Resolution
Allelic Ladder – Pass Positive Control – Pass	Run passes
Allelic Ladder – Pass Positive Control – Fail	Refer to Section 3
Allelic Ladder(s) – Fail Positive Control – Fail	Run fails Fill out Electrophoresis Failure Report/ Resolution sheet

Table 3 Retesting Strategies for Positive Control

Positive Control Result	Course of action
No Data Available - No orange size standard in lane	Rerun
No amplification product but orange size standard correct Rerun with same result	Rerun Re-amplify amplification set
Incorrect genotype - Could be caused by ill-defined size standard, other Genotyper problems or sample mix-up Rerun fails to give correct type	Reanalyze sample, if not able to resolve, rerun amplification product Re-amplify amplification set
OL alleles - possibly Genotyper problem	Rerun amplification product

B. Extraction Negative and Amplification Negative Controls

1. YM1 negative controls and Identifiler 28 negative controls injected at “I” parameters

   a. Evaluate the extraction negative and/or amplification negative control for expected results

   b. If peaks attributed to DNA are detected in an extraction negative and/or amplification negative control

      i. retest the extraction negative control and/or amplification negative control

      ii. Refer to Table 4 and/or 5 for Retesting Strategies

Table 4 Retesting Strategies for Extraction Negative Control

Extraction Negative Result	Course of action
No data available - No orange size standard in lane	Rerun
Misshaped orange size standard peaks	Control passes if no peaks are present
Run artifacts such as color blips or spikes	Edit Rerun only if the artifacts are so abundant that amplified DNA might be masked
Alleles detected – Initial Run	Rerun
Alleles detected – Rerun	Re-amplify control
Alleles detected – Re-amplification	Extraction set fails All samples must be re-extracted

Table 5 Retesting Strategies for Amplification Negative Controls

Amplification Negative Result	Course of action
No data available - No orange size standard in lane	Rerun
Misshapen orangesize standard peaks	Control passes if no peaks are present
Run artifacts such as color blips or spikes	Edit Rerun only if artifacts are so abundant that amplified DNA might be masked.
Peaks detected – Initial Run	Re-run
Peaks detected – Rerun	Amplification set fails Re-amplify amplification set

2. **Identifiler 28 negative controls injected at "IR" parameters**
   a. Evaluate the extraction negative, amplification negative, and/or microcon negative control for expected results

   b. If peaks attributed to DNA are detected in a negative control, refer to Table 7 for retesting strategies.
      i. Re-aliquot and rerun the control at the same injection conditions to confirm failure. If the realiquot still fails, the control (either the original aliquot so one can re-inject the sample plate) or the second aliquot must be re-injected with a lower injection parameter.
      ii. If a negative control fails following injection with "IR" parameters but passes with injections at "I" parameters, data from samples in the amplification set injected with "IR" parameters fails accordingly, whereas data from samples injected with "I" parameters passes.

3. Identifiler 31 Controls

   Negative controls can display spurious allele peaks and still pass, unless:

   a. The allele occurs in two of the two or three amplifications, which indicates potential contamination instead of drop-in. If this happens for only one or two loci, the affected loci must be evaluated for all samples. The locus is inconclusive for samples that display the same allele, which is present in the negative control, at this locus.

b. If more than two repeating peaks are present in a negative control, the amplification or extraction fails.

c. Even if none of the spurious allele peaks repeat in two amplifications, a control fails if too many spurious alleles are present. The cut off is > 9 drop-in peaks distributed over at least two of the three amplification aliquots for three amplifications.

d. If a negative control fails, it must be realiquotted and rerun at the same injection conditions to confirm failure. If the realiquot still fails, the control (either the original aliquot so one can re-inject the sample plate) or the second aliquot must be re-injected with a lower injection parameter.

e. If a negative control fails following injection with "high" parameters but passes with injections at "optimal" or "low" parameters, data from samples in the amplification set injected with "high" parameters fails accordingly, whereas data from samples injected with "optimal" or "low" parameters passes.

f. Refer to the Table 6 to determine whether data for ID28 and ID31 samples may be used with respect to the pass/fail status of the associated controls at ID28 and ID31 injection parameters

TABLE 6 Interpretation of samples and Retesting Strategies for Negative Controls amplified with Identifiler 31.

Treatment of E-Neg/M'con Negative Controls	**Result**	**Course of action**	**Interpretation**	
			Samples may be amped/run in:	**Samples may NOT be amped/run in:** (All peaks should be removed from electropherograms)
Amplified in Identifiler 31; Run on H parameters	PASS	None	Identifiler 31, Identifiler 28 and YM1 (any parameter).	N/A
Amplified in Identifiler 31; **<u>First</u>** run on H parameters	FAIL	Controls should be re-aliquoted and injected at H parameters again	N/A	N/A
Amplified in Identifiler 31; **<u>Second</u>** run on H parameters	FAIL	Controls should be re-injected at N parameters	N/A	N/A
Amplified in Identifiler 31; Run on N parameters	PASS	None	Identifiler 31 injected at N or L, Identifiler 28 injected at I or IR and YM1	Identifiler 31 injected at H
Amplified in Identifiler 31; Run on N parameters	FAIL	Controls should be re-injected at L parameters	N/A	N/A
Amplified in Identifiler 31; Run on L parameters	PASS	None	Identifiler 31 injected at L, Identifiler 28 injected at I and YM1	Identifiler 31 injected at H and N Identifiler 28 injected at IR
Amplified in Identifiler 31, Run on L parameters	FAIL	Controls may be amped in Identifiler 28 , or YM1	N/A	Identifiler 31, Identifiler 28 and YM1 (any parameter).

H = High injection for Identifiler 31 samples at 6 kV 30 sec
N = Normal injection for Identifiler 31 samples at 3 kV 20 sec
L = Normal injection for Identifiler 31 samples at 1 kV 22sec

TABLE 7 Interpretation of samples and Retesting Strategies for Extraction/Microcon Negative Controls amplified with Identifiler 28.*

Treatment of E-Neg/M'con Negative Controls	Result	Course of action	Interpretation	
			Samples may be amped/run in:	**Samples may NOT be amped/run in:** (All peaks should be removed from electropherograms)
Amplified in Identifiler 28; Run on IR Parameters	PASS	None	Identifiler 28 injected at I or IR and YM1 samples	Identifiler 31
Amplified in Identifiler 28; **First** run on IR Parameters	FAIL	Controls should be re-aliquoted and injected at IR again	N/A	N/A
Amplified in Identifiler 28; **Second** run on IR Parameters	FAIL	Controls should be re-injected at I	N/A	N/A
Amplified in Identifiler 28; Run on I Parameters	PASS	None	Identifiler 28 injected at I and YM1	Identifiler 31 and Identifiler 28 injected at IR
Amplified in Identifiler 28; Run on I Parameters	FAIL	Controls may be amped in YM1 as needed	N/A	Identifiler 31 and Identifiler 28 (all injection parameters)

IR = High injection for Identifiler 28 samples at 5 kV 20 sec
I = Normal injection for Identifiler 28 samples at 1 kV 22 sec
* If a negative control is amplified in Identifiler 28 initially, there may not be enough volume for Identifiler 31 amplification

VI. Reporting Procedures

Evidence samples will be duplicated (single source and mixture samples) according to the concordant analyses and "duplicate rule." To improve workflow, the Property Crimes and High Sensitivity/Hybrid teams may automatically duplicate evidence samples regardless of DNA concentration.

A. Guidelines for Reporting Allelic Results

1. Items listed in allele typing tables should be limited to samples that are used to draw important conclusions of the case. Genotypes are not reported and should not be inferred, i.e., if only a "7" allele is found; it should be reported as 7. Alleles and/or peaks are listed in the results tables regardless of intensity differences, based on the reporting criteria below.

2. If an allele meets the above reporting thresholds and fulfills the concordant analyses and the duplicate rule as stated in the General PCR Guidelines, then the allele will be evaluated for the report and/or summary table in the file.

3. In cases where a mixture sample was re-amplified in the same multiplex system consult Table 8 about how to report the alleles:

4. If no alleles are detected in a locus, then the locus may be reported as "NEG" (no alleles detected).

B. **Previously unreported rare alleles**

1. A distinct peak of the same labeling color outside the allelic range could be a rare new allele for this locus. This possibility should be considered if:

    a. The overall amplification for the other loci displays distinct peaks >75 (or 100 if applicable) and does not show artifacts

    b. The same color locus closest to the new size peak does not have more than one allele peak, and

    c. The new size peak is also detected in the duplicate run.

2. All alleles that are not present in the allelic ladder should be identified by their relative position to the alleles in the allelic ladder. The peak label should show the length in base pairs and this value can be used to determine the proper allele nomenclature. A D7S820 allele of the length 274 bp in Identifiler, is located between alleles 10 (271 bp) and 11 (275) and has to be designated 10.3. The off-ladder allele should be reported using this nomenclature.

3. Off-ladder alleles which fall outside the range of the allelic ladder at that locus should be reported as < or > the smallest or largest allele in the ladder.

4. New alleles observed for YM1 where no allelic ladder is available should be reported with their rounded base pair size. The base pair value should also appear in the footnotes, e.g. 128 = Allele is reported as size in base pairs.

C. The following samples and/or components of samples are considered "inconclusive" and should not be interpreted or used for comparison.

1. Single source samples showing less than 8 labeled alleles over four autosomal loci or six loci for low-template DNA samples.

2. In a deduced mixture, where the major contributor was determined: Further comparisons to the minor component may only be made when at least 8 STR alleles over 4 autosomal loci are attributed to the minor component.

3. Non-deducible mixtures are deemed inconclusive if any of the following circumstances apply:
   a. Fewer than 12 labeled STR alleles over 6 autosomal loci.
   b. 7 STR alleles at two or more loci. (For samples amplified with 31 cycles, these alleles must be present in the composite profile.)
   c. Indication of multiple contributors to mixtures with low amounts of DNA for a system (For example, <30 pg/µL amplified with ID28 or 4 pg/µL amplified with ID31).
   d. For high-copy samples, drastic stochastic effects between duplicate amplifications.
   e. All or many loci with peak heights below 200 RFUs
   f. Excessive number of peaks below threshold over many loci

VII. Guidelines for Interpretation of Results

Occasionally typing results may appear markedly different from the standard patterns. Such results could be due to a procedural error, mixtures of DNA (multiple contributors to the sample), or DNA degradation.

Non-Mixtures

A locus may be assigned a "Z" to indicate that another allele may be present, particularly for potential false-homozygote.

1. The possibility of allelic dropout should be considered for low peak heights, especially when below 250 RFUs. This is particularly important for samples amplified with less than 250pg and/or show a pattern of degradation.

2. Larger and/or less efficient loci are usually affected in samples that are degraded or otherwise compromised. In Identifiler, these loci are: CSF1PO, D2S1338, D18S51, FGA, and sometimes TH01 and D16S539.

3. Moreover, for degraded samples, the last labeled loci of each color may be a potential false homozygote. For example, in Identifiler, if no alleles in CSF were labeled, and only one allele is labeled and visible at D7S820, this allele could be a false-homozygote.

4. For samples injected with higher parameters or at a dilution, false-homozygote peaks could be higher than 250 RFUs. Additional caution must be used when interpreting these samples.

Mixtures of DNA

1. General Mixtures

a. Evidence samples may contain DNA from more than one individual. The possibility of multiple contributors should be considered when interpreting STR typing results. For HCN DNA samples for any typing system in which heterozygous genotypes are analyzed, the detection of more than two alleles in at least two loci indicates a mixed sample.

b. In Identifiler validation studies, heterozygote peak height imbalance was measured at 67% (OCME validation) and was noted to go as low as 61% (Collins, et al, 2004); however, greater peak height imbalance has been observed in casework.

c. Degradation or primer binding site mutations are other possible causes for peak height ratio imbalance. Low DNA amounts are more likely to show uneven heterozygote peak heights due to stochastic effects. For this reason, mixtures resulting from amplifications with low amounts of DNA (<200 pg), or with RFU values below 250 (when injected normal) should be interpreted with caution.

d. A single locus might not be helpful in detecting a mixture. Even though a mixture is present, a locus might only reveal two alleles. For example, in a 1:1 mixture there is a possibility that a phenotype (e.g. VWA 15, 17), is a mixture of a homozygous 15, 15 individual and a homozygous 17,17 individual. Other possible combinations that would result in a two allele pattern are mixtures of individuals with 15,15 + 15,17 or 17,17 + 15,17. In these cases, the electropherogram should reveal unequal peak heights caused by the triplicate presence of one of the alleles. It is, therefore, best to use the results for all tested loci to determine the presence of a mixture.

e. Results for all tested loci, other than Identifiler locus D2, should be interpreted in order to determine the presence of a mixture.

2. Mixtures with different levels of starting DNA

Another scenario that could lead to unequal peak heights is the presence of unequal amounts of heterologous DNA in a sample (Gill et al. 1995, Clayton et al., 1998). A VWA typing profile 18>16>14 can be caused by unequal amounts of 14, 16 and 18, 18 but also by a mixture of two individuals with 14, 18 and 16, 18. Here, different scenarios must be considered:

a. Mixture has a known component (e.g. a vaginal swab), or a component that may be inferred within the context of the case

i. After identifying the alleles that could have come from the victim, it can be stated that the remaining alleles must have come from the unknown DNA source. To deduce the complete allele combination of the foreign DNA, the results and allele peak heights must be taken into consideration for each locus.

ii. If two foreign alleles of similar peak height are present at a locus, these two alleles are likely to comprise the genotype of the unknown contributor.

iii. If the alleles foreign to the victim constitute the major component of a mixture, the allele combination can be deduced by combining all major allele peaks (also see section (2) below). All peak height inconsistencies for heterozygote loci should be accounted for by overlap with the known component.

iv. If the alleles foreign to the victim are the minor component and only one foreign allele is visible at a locus, it might not be possible to determine the complete allele composition for this STR. The foreign type might either be homozygous or heterozygous with one allele overlapping with the known component. For heterozygous types of the known component, peak height differences between the two alleles indicate the presence of an overlapping allele in the minor component. For homozygous patterns and very small peak height differences a decision cannot be made. In these cases it is possible to indicate that a second allele might be present without identifying the allele.

b. The major and the minor component of the mixture can clearly be distinguished

i. Using a locus where four alleles are present, it is possible to determine the ratio of the two DNA components in a mixture. This ratio can then be used to interpret the amount of copies of each allele that must be present at other loci with less than four alleles. Therefore, if there is a large difference in peak heights, the genotype of the major component can be inferred without having one known contributor and without four alleles being present at each locus. Be careful to eliminate the possibility of more than two contributors before interpreting the mixture.

ii. It might not be possible to unambiguously deduce the DNA type for the minor component. See above for a discussion of the limitations.

3. Very small additional allele peaks are detected at only a few loci

a. The major DNA profile can be interpreted. The presence of additional alleles should be noted, but deduction of the minor component should not be attempted.

b. If sufficient DNA is available, and based on the peak heights of the major alleles, consider concentrating the sample or amplifying the mixture sample with more DNA.

4. **Possible mixture components masked by -4bp stutter**

   a. Due to enzyme slippage when replicating repetitive DNA stretches, an additional peak of a length exactly -4bp shorter than the main allele peak is a frequent occurrence for STR polymorphisms (Gill et al. 1995, Walsh et al 1996, Holt et al 2002). Some of the STR loci are very prone to stutter and almost always show stutter peaks e.g. DYS19 or VWA. The Amelogenin locus is not based on a repetitive STR sequence and doesn't show any stutter.

   b. Over all loci the average stutter peak height ranges from 2.5% to 9.5%, with maxima from 17.4% - 24.1% (in house validation for HCN DNA samples). Therefore peaks in a -4bp position from a main peak and less than a certain percentage (differs per locus, see Appendix) of the main peak's height are not reported as true alleles.

   c. In a mixture the -4bp stutter could mask a real mixture component. Therefore individuals cannot be excluded from being a minor contributor to a mixture if their alleles are in the -4bp position of an allele from another individual.

VIII. Partial Profiles: not all loci display allele peaks

1. **Degradation**

   a. DNA degradation is the process of a very long (>40,000 bp) DNA double strand being broken down into smaller pieces. With increasing degradation, the DNA fragments get very short, until the target sequences for the PCR reaction which at least have to contain both primer annealing sites are also broken down. For example, Identifiler with FGA (350bp) and YM1 with DYS389II (362-386bp).

   b. The longer alleles are more likely not to be present in partially degraded DNA (Gill et al. 1995, Sparkes et al. 1996, Holt et al 2002). An Identifiler result that displays only D3S1358 and Amelogenin but none of the higher molecular weight loci, can be explained as being caused by DNA degradation. A profile with no D3S1358 result but callable FGA alleles cannot be caused by degradation but must have other reasons (e.g., see the following paragraph).

c. Due to the allele size differences within a locus, degradation can also cause partial profiles for heterozygous DNA types, e.g., for the FGA type 19, 29, allele 19 (220 bp) can be present while allele 29 (260 bp) drops out. Parallel to the disappearing of the larger size allele, an imbalanced peak height with the larger allele peak being smaller, can be explained by DNA degradation.

d. The possibility of an allelic drop out has to be considered especially for amplification with low DNA input, degraded DNA, or low peak heights (200 RFU's or below with the normal injection parameters).

e. For degraded samples amplified in Identifiler 31 or amplified in Identifiler 28 and run with the 5kV/20sec injection parameter (such as those in the High Sensitivity Team), small loci may be overblown in order to visualize larger loci. In these instances, use the data from an injection with lower parameters for the small loci whereas data from injections with higher parameters may be used for allelic assignments for larger loci. In this manner, a complete or near complete profile may be assigned. Regarding the small loci at high injection parameters, remove the peaks if they are overblown and consider the locus inconclusive at the high injection parameters.

2. Detection limit

Due to the different detection sensitivity of the dyes, the yellow peaks are generally lower than the blue and green peaks. If the DNA sample is at the lower limit of the testing sensitivity it is therefore possible to get a partial profile where one or all of the yellow loci are missing. Additionally, blue is slightly more sensitive than the green dye, so that it is possible to see more blue loci than green loci above the detection threshold.

IX. Guidelines for reporting samples amplified with Identifiler for 31 cycles

After samples are amplified in triplicate, the alleles which repeat in at least two of three amplifications are considered part of the Composite (or consensus profile). When data is copied into a profile generation sheet (or table), the composite profile is displayed in a row below the three rows of the replicate amplifications. These are termed “repeating or confirmed alleles”. Only confirmed alleles may be assigned to the most likely DNA profile of a sample interpreted as a single source, whereas only alleles that are detected in all three amplifications may be assigned to the most likely major DNA profile of a mixed DNA sample. However, in order to be assigned to a profile, termed “Assigned Alleles” for single source samples or the “Assigned Major” for mixed samples, the confirmed alleles must meet the criteria described below. Non-repeating alleles may only be used for comparison. These non-repeating alleles may be an allele from a minor contributor or may be a PCR artifact.

1. Low Template DNA (LT-DNA) Profile Production

For each case file, a final profile generation sheet should be created from the profile generation sheet(s) from the relevant STR runs. This may include injections from different runs particularly if a replicate sample had required re-injection due to a failed size standard for example.

a. The three individual amplifications and the composite profile should be copied from the STR table for each sample from the case.

i. The a, b, c or pooled injections do not need to be copied.

ii. If a sample was re-injected due to a poor injection, only include the data from the successful run.

iii. If a sample was injected with low, normal and/or high parameters, but the high or low injection yielded the better profile for all loci, the normal injection does not need to be placed in the table.

iv. However, if some loci, for example small loci, were apparent in the normal injection but were deemed inconclusive in the high injection whereas other longer loci were not apparent in the normal injection but were evident in the high injection, the appropriate loci from all injections should be used and combined in the table.

v. The relevant run names should be listed in the table for each replicate after the sample name.

b. In the row beneath the composite profile, termed the "Assigned Alleles or Assigned Major", list alleles from the composite profile that can be assigned to the single source profile or to the major component of the mixture profile, respectively. If no such profile can be assigned based on the guidelines below, list "mixture for comparison only" or "inconclusive", if applicable. Refer to the section of the manual entitled "Allele Confirmation and Profile Determination" for detailed instructions regarding allelic assignment.

c. Copy the chart sheet to a new file.

i. Right Click on the triple chart sheet

ii. Select Move or Copy, create a copy, and under "To book" select "newbook".

iii. Save the Newbook with the case number to the profile sheets folder in the case management folder within the Highsens data folder on the network.

iv. Add this sheet to the sample's case file.

2. Sample Interpretation

a. Samples or components of samples with less than eight repeating alleles over six autosomal loci will not be interpreted or used for comparison. Samples with more than 6 repeating alleles at at least two loci in the composite profile will also not be used for interpretation or comparison.

b. When examining a triplicate amplification result, one must decide if the sample will be treated as a mixture of DNA or can be treated as a single source DNA profile.

   i. Samples with 3 repeating alleles at at least three loci must be interpreted as mixtures.

   ii. Samples with 3 repeating alleles at less than 3 loci may be interpreted as single source profiles. Refer to the interpretation section below for allelic assignment.

   iii. In some cases, a sample should be interpreted as a mixture even if there are not 3 repeating alleles at at least 3 loci. For example, this may be evident when results at multiple loci are inconsistent among replicate amplifications.

c. A locus in the assigned profiles may be assigned a “Z” to indicate that another allele may be present.

d. ID 31 samples treated as **single source** DNA profiles are interpreted as follows:

   i. The heterozygote type for a locus is determined based on the two tallest repeating alleles in two amplifications. The heterozygote peaks do not have to show a specific peak balance with the following exceptions:

   ii. If two repeating alleles are clearly major alleles, any additional repeating alleles, which are consistently minor, are not assigned to the single source profile.

   iii. When the same repeating allele is in the plus or minus 4 bp stutter position, and is less than 30% of the major peak in two out of three amplifications, and is less than 50% of the major peak in the third amplification, the allele in the stutter position may not be part of the heterozygote pair. Therefore, a Z is assigned.

iv. If repeating alleles are present, and one allele is consistently major such that all alleles are less than 30% of this allele in all amplifications, the major allele may be assigned a homozygote if the criteria described below are met.

v. Homozygotes must be interpreted carefully.

1) An allele must appear in all three amplifications to be considered a homozygote.

2) The presence of an additional allele in one of the three amplifications can be indicative of allelic dropout.

- But if one allele is clearly the major allele and the minor allele(s) (even if they repeat) are less than 30% of the major allele in all three amplifications, the major allele can be assigned as a homozygote.

- Alternatively, if the non-repeating minor allele(s) are >30% of the repeating major allele, allelic drop out should be suspected and the locus is marked with a Z, to indicate the possibility of a heterozygote.

- For following scenarios, loci should always be assigned a Z:

    - ➢ High molecular weight or less efficient loci: CSF1PO, THO1, D16S539, D2S1338, D18S51, and FGA if only one allele could be called

    - ➢ The largest locus <u>with repeating alleles</u> in each color

    - ➢ All loci in samples amplified with less than 20 picograms in each replicate

3) If alleles in one of three amplifications are completely different from the other two amplifications, **the assigned allele call for that locus** is inconclusive. For example,

	Example 1	**Example 2**
Replicate a	8	8
Replicate b	8	8
Replicate c	11, 12	11
Composite Profile	8, Z	8, Z
Assigned Alleles	**INC**	**8, Z**

e. **ID 31 Mixture** Sample Interpretation

i. Determine the number of contributors to the mixture. A sample may be considered to have at least three or more contributors if five or more repeating alleles are present at at least two loci. Consider whether the repeating peaks appear to be true alleles or are PCR artifacts.

ii. Determine the mixture ratio**.** Examination of the profile from the injection of the pooled amplification products is often indicative of the mixture ratio.

iii. Mixture samples with apparently equal contribution from donors can only be used for comparison. Data generated for all replicates may be used for comparison.

iv. Mixtures may be deduced or deconvoluted as follows:

a) Major alleles can be assigned to a major component if they appear **in all three amplifications** and if they are the major alleles in **two out of the three.** A heterozygote pair can be called if two out of the three amplifications show allelic balance ≥ 50%.

b) Homozygote types must be deduced carefully. If one allele is clearly the major allele and the minor allele(s) (even if they repeat) are less than 30% of the major allele in all three amplifications, the major allele can be assigned as a homozygote.

c) When the shorter allele is within 30 to 50% of the taller allele, in at least two amplifications, it cannot be concluded if the major component is heterozygote or homozygote. In this case, a major peak can be assigned to the major component with a Z.

d) If only one allele could be confirmed, loci should always be assigned a Z in the following scenarios:

- High molecular weight or less efficient loci such as CSF1PO, THO1, D16S539, D2S1338, D18S51 and FGA

- The largest locus with repeating alleles in each color.

- TPOX, a locus prone to primer binding mutations- This is relevant for mixtures that contain a homozygote and a heterozygote which share the same allele.

- All loci in samples amplified with less than 20 picograms in each replicate

v. Note that mixture ratios may vary between the smaller and the larger loci and in some cases larger loci may not be resolvable particularly if only two alleles are apparent.

vi. When deducing a mixture, if none of the alleles can be assigned to the major component at one particular locus, that locus is not deduced and is called inconclusive in the Assigned Major profile.

vii. Minor components from non-intimate samples are not deduced. Alleles that may be attributed to the minor components may only be used for comparison.

viii. For intimate samples, alleles that are confirmed but do not belong to the known component may be assigned.

f. In addition to applying the above protocols to the replicates, the pooled sample (which is a combined sample of amplification products from replicates a, b, and c) should be considered. Although the pooled sample is not evaluated independently, if it does not confirm the allelic assignments from the replicates, caution should be exercised.

I. Y-STR Mixtures of Male DNA

Other than at the DYS385 locus, the occurrence of more than one allele peak at one or more Y-STR loci indicates the presence of a mixture of male DNA.

A. In General

If the additional allele peaks are of similar height at one or more loci, the different components are present in similar levels. If only either DYS19 or DYS390 displays two alleles, and the other three loci show single peaks, the presence of an allele duplication event should be considered.

Mixtures of male DNA with different levels of starting DNA will lead to unequal peak heights for the different alleles for one system. If the ratio of the lower peak to the higher peak is consistent for all loci with two allele peaks, the haplotypes of the major and minor component can be inferred. If this is not the case, the possible presence of three contributors must be considered.

It is unreliable to solely use the alleles present at the DYS385 locus to determine whether or not a mixture is present or estimating the ratios of a determined mixture.

C. Possible mixture component masked by -4bp stutter

Peaks within a -4bp position from a main peak and less than 20% of the peak heights are not reported as true alleles. In a mixture the -4bp stutter could mask a real mixture component. Therefore individuals cannot be excluded from being a minor contributor to a mixture if their alleles are in the -4bp position of an allele from another individual.

D. Refer to the “STR Results Interpretation” section. Follow the procedures outlined in the appropriate section.

1. Partial Profiles
2. Detection of Previously Unreported Rare Alleles
3. Samples with High Background Levels

Revision History:
March 24, 2010 – Initial version of procedure.

To interpret the significance of a match between genetically typed samples, it is necessary to know the population distribution of alleles at the loci that were typed. If the STR alleles of the relevant evidence sample are different from the alleles of a subject's reference sample, then the subject is "excluded," and cannot be the donor of the biological evidence being tested. An exclusion is independent of the frequency of the alleles in the population.

If the subject and evidence samples have the same alleles, then the subject is "included," and could be the source of the evidence sample. The random match probability, or the probability that another, unrelated, individual would also match the evidence sample, is equal to the frequency of the evidence profile genotypes in the relevant population. Population frequencies are estimated separately for the Asian, Black, Caucasian and Hispanic populations. Additional population frequencies may be used for other population groups. If a source contains more than one frequency for a single population group, then the highest frequency is used for calculations. Allele frequencies are used for all calculations. Profile frequency estimates are calculated according to the National Research Council report entitled *The Evaluation of Forensic DNA Evidence* (National Academy Press 1996, pp. 4-36 to 4-37).

Spreadsheets are used to automate the calculation of the population specific genotype and profile frequency estimates. The spreadsheets are located in the "POPSTATS" subdirectory on the network and explanations for their use are included with the spreadsheets.

The population allele frequencies of the 13 core CODIS loci and D2S1338 and D19S433 are derived from the FBI and OCME Databases.

I. Random Match Probability for Autosomal STRs

A. Enter the evidence profile alleles in the Identifiler worksheet of the POPSTATS spreadsheet. Off-ladder alleles can be entered as decimals (for example, "12.2") or as ">" or "<" for values above or below the ladder, respectively.

B. For loci assigned a "Z" to indicate the possible presence of another allele, only one allele is entered in the calculation spreadsheet. In this manner, the locus is not treated as a true homozygote whose statistical values are determined by squaring the allele frequency (p^2). Rather "Z" loci utilize the probability only of the one assigned allele (2p), which allows the second allele to be anything.

C. The overall profile frequency estimate for each group is calculated by multiplying the individual locus genotype frequency estimates together.

D. In the standard scenario, homozygote genotype frequencies are estimated for each population using the formula $p^2+p(1-p)\theta$ for $\theta= 0.03$ and heterozygote genotype frequencies are estimated using the formula $2p_ip_j$.

E. Genotype and profile frequencies are also estimated for isolated populations (i.e., "evidence and subject from the same subgroup (isolated village)") and for relatives using the formulas in the National Research Council Report.

F. For each population, the overall profile frequency estimate under the standard scenario of $\theta=0.03$ unless there is reason to suspect that the "evidence DNA and subject are from the same subgroup" or a relative of the subject left the biological sample.

G. Calculations and allele frequencies are retained in the case file for referral at a later date if necessary.

II. Random Match Probability for Y STRs

A. The frequency for a Y STR haplotype is estimated by counting the number of times the haplotype occurs in each of the population databases and dividing by the total number of individuals in the database.

1. A haplotype that has not been previously observed in the Asian database, which includes 196 individuals, would be reported as "less than 1 in 196 Asians".

2. A haplotype that has been observed once in the Asian database would be reported as "1 in 196 Asians".

3. A haplotype that has been observed 5 times in the Asian database is reported as "1 in 39 Asians" (5 in 196 is equal to 1 in 39).

B. **For YM1 haplotypes**, use the POPSTATS spreadsheet to estimate haplotype frequencies.

1. Enter the YM1 alleles into the Identifiler worksheet of the POPSTATS spreadsheet. Partial profiles cannot be entered into the spreadsheet. Instead, haplotype frequency estimates must be calculated manually for partial profiles.

2. Refer to the Y-STR tab of the POPSTATS spreadsheet for YM1 haplotype frequency estimates. Print this page for the case file.

3. If both autosomal and YM1 STRs are typed for a sample, then the combined frequency can be estimated by multiplying the autosomal profile frequency estimate by the larger of either a) the YM1 haplotype frequency estimate, or b) the YM1 haplotype frequency estimate if the haplotype had been observed one time in the database. This calculation is done automatically by the POPSTATS spreadsheet.

C. For **PowerPlex Y (PPY)** haplotypes, use the US Y-STR database to estimate haplotype frequencies.

1. Using Internet Explorer, navigate to www.usystrdatabase.org

2. Enter the alleles from the PPY profile into the drop-down boxes on the screen.

3. To specify a value not listed in the drop-down box, enter the value in the text box next to the drop-down box.

4. The following value types are allowed:

   a) Standard ladder allele such as "12"
   b) Off-ladder allele value such as "12.2"
   c) Off-ladder low- or high-value such as "<15" or ">21"
   d) Null allele: enter "0" if the sample is believed to contain a legitimate null allele, for example, due to a primer binding site mutation.
   e) No data: "*" is the default value. Loci with * are treated as wild cards.

5. Click "Search"

6. Scroll down for the results. The website reports the number of times the haplotype was observed in the database, the observed frequency of the haplotype, and the upper bound of the 95% confidence interval. These values are reported for each of the populations in the database (African American, Asian, Caucasian, Hispanic, and Native American) and for all of the populations combined.

7. Click "Show Details" for a summary table.

8. Adjust the margins of the page by selecting "Page Setup" from the printer menu at the top of the page and changing the top and bottom margins to 0.5, then choosing "OK".

9. Print the screen by selecting "Print" from the printer menu at the top of the page and selecting a printer.

10. Verify on the printout that the Y-haplotype alleles were correctly entered into the website.

11. If both autosomal and PPY STRs are typed, the results are reported separately.

III. Combined Probability of Inclusion (CPI) for Mixtures

The combined probability of inclusion (CPI) is defined as the probability that a randomly selected individual would be a contributor to a mixture of labeled DNA alleles. In other words, it is the expected frequency of individuals who could be included as potential contributors to the mixture because all of their alleles are labeled in the evidence profile.

CPI can only be used if all of the following circumstances are met:

- When the evidence sample contains a non-deducible mixture.
- When the alleles of the associated known sample are labeled at all of the conclusive loci in the evidence sample.

A conclusive locus is a locus with concordant or repeating alleles. If an evidentiary sample is amplified more than once, loci with concordant alleles (HT-DNA samples) or repeating alleles (LT-DNA samples) are determined. Loci that are designated as "NEG" (for negative) or "INC" (for inconclusive) are not used in the CPI calculation. To avoid the possibility of bias, the determination to deem a locus inconclusive in the evidence profile must be made prior to viewing the comparison sample profile.

CPI is calculated (if necessary) after the DNA profile of the comparison sample(s) is determined to be included in the evidence sample. The CPI is calculated for informative samples. If RMP values have been generated, the CPI may not need to be calculated. The CPI is reported in the evidence report.

The comparison is based on the previously determined allele calls. If any of the alleles of a comparison sample are missing from the evidence profile at conclusive loci, CPI is not appropriate.

A. Computing CPI

1. Open CPI worksheet named "CPI.xls"

2. In cells A9 through P9 of the Data Entry worksheet, enter each allele that is labeled in the evidence profile at conclusive loci, up to 10 alleles per locus. Alleles should be separated by commas and/or spaces. A profile from a PG sheet may be pasted into cells A9 through P9. **All alleles that are labeled at conclusive loci in all amplifications must be entered**.

3. Press the blue "Run CPI macro" button. The CPI for the Black, Caucasian, Hispanic, and Asian populations appears at the bottom of the Results worksheet.

4. Print the results by selecting File > Print while in the Results worksheet. The printout will include the alleles entered and the results.

Note:
Off-ladder alleles may be entered in either 15.x format or as "<" or ">". 5/2N will be used as the frequency for an off-ladder allele.

B. Interpretation

Results are presented for each of the four populations: Black, Caucasian, Hispanic, and Asian. The probability of inclusion is stated in the report.

Combined Probability of Inclusion is the expected frequency of individuals who are carrying only alleles that are labeled in the mixture in question, and if tested could potentially be included as contributors to this mixture. It is the expected frequency of individuals who could be included as potential contributors to the mixture because they do not carry any alleles that are not labeled in the evidence profile.

Revision History:
March 24, 2010 – Initial version of procedure.

I. Comparison of Samples Based on STR Results

A. Determine whether it is likely that a sample contains a mixture of DNA (i.e. more than two alleles at two loci, intensity differences between alleles within a locus, or a reproducible pattern of visible but unlabeled peaks).

B. State in the report if a sample contains a single donor or a mixture of DNA.

C. Determine the minimum number of individuals who could have contributed to a mixture and the likely source of each component of the mixture

D. Compare all possible evidence and exemplar pairs to determine inclusions and exclusions.

E. Assuming a single physiological fluid donor, two samples could derive from a common biological source (inclusion) if all the alleles in the evidence sample are accounted for by the alleles in the exemplar sample.

If however a mixture is possible in the evidence sample, there may be alleles that are not accounted for by the exemplar sample.

F. Random Match Probabilities (RMP) are calculated for evidence samples only where:

i. The sample is apparently unmixed.

ii. The sample appears to be a mixture of two components and the source of a known component is used to deduce the foreign component. RMP can be calculated for the foreign component (i.e., when the victim's DNA is present in the sperm fraction of a vaginal swab and the foreign component is deduced).

iii. There is a large difference in peak heights between the major and minor components and then the genotype of the major component is easily inferred. The minor component genotype can be determined if four alleles are present at a locus. If less than four alleles are present one has to be very careful because other alleles of the minor component may be masked by the major component alleles. A deduction may be possible based on peak height imbalances. See below for the calculation of statistics.

iv. When one (or more) component of a mixture must be assumed in order to interpret the mixture, state the assumption directly in the report.

G. Statistics are not calculated for expected inclusions such as epithelial cells from a vaginal swab, underwear or (for non blood only) the victim's own bedding.

NOTE: Do not forget to evaluate the significance of a match for epithelial cell fractions for items not connected to the victim such as condoms or the suspect's clothes.

II. Reporting partial profiles

A. Duplicated alleles at a single locus can be used for comparison purposes even if not all loci could be typed for this sample.

B. If only one allele meets the reporting criteria at a locus and the result of a weaker allele cannot be improved, the called allele can be used for comparison purposes. The presence of the weaker allele in the exemplar does not exclude this individual.

III. Reporting previously unreported rare alleles

A. A match based on the presence of a new size allele in both the exemplar and the evidence DNA can be reported.

B. The new allele may be included in the statistical evaluation of a match.

C. An exclusion based on the presence of a new size allele, where there is a match for all other tested polymorphisms, has to be reported as inconclusive.

IV. Samples with High Background Levels

A. A sample which has more than two allele peaks per locus and a high background with multiple extra peaks of unknown origin outside of the allelic range has to be interpreted extra carefully and can be reported as inconclusive.

B. If the high background levels are low in a degraded sample, the sample should be microconned and amplified with more DNA.

C. In order to fully resolve components of mixtures with peak heights above 6000 RFUs in YM1 and 7000 RFUs in Identifiler 28, samples must be repeated.

D. This reanalysis is not always necessary for clean DNA samples if, in spite of the peak heights, all peaks show the proper shape and no major background is present.

V. Discrepancies for overlapping loci in different multiplex systems

A. The primer-binding site of an allele may contain a mutation, which renders the annealing phase of its amplification less efficient, or if the mutation is near the 3' end completely blocks the extension (Clayton et al. 1998)

i. This may result in a pseudo-homozygote type, which is reproducible for the specific primer pair. These mutations are extremely rare, approximately estimated between 0.01 and 0.001 per locus (Clayton et al. 1998)

ii. A comparison between evidence and exemplar samples based on a locus where both samples were amplified with the same primer sequence is no problem.

iii. If the same locus is amplified using different multiplex systems, it is possible to obtain a heterozygote type in one multiplex and the pseudo-homozygote in the second, because the primer sequences for the same loci may differ.

iv. The heterozygote type should be the correct type and should be reported. It is important to have typing results for evidence and exemplars based on the same multiplex.

VI. Identifiler 31 - Profile Comparison and Statistical Evaluation

A. Based on the triple amplifications, only repeating alleles in the composite profile will be used for database entry. All allelic assignments are part of the most likely DNA type for the DNA source.

B. Samples, including major components of samples, must contain at least six apparent loci in order to be used for comparisons.

C. Consider all possible evidence and exemplar samples to determine inclusions and exclusions.

D. For comparisons, the amount of DNA amplified, the number of contributors to the sample, the loci characteristics which were empirically defined, and the length of the repeat in question should be considered as well as electronic data.

E. Allelic dropout caused by stochastic effects is a common occurrence for low copy number DNA samples, and a mismatch between a heterozygote exemplar and an apparent homozygote locus is not necessarily an exclusion, even if no Z was assigned (a second allele may be present).

i. For example, a comparison sample cannot be excluded from an evidentiary sample if all alleles in the comparison sample are either called or can be explained by uncalled peaks or dropouts.

ii. Regarding inclusions, one should evaluate whether a particular mixture is what you would expect to see had a comparison sample contributed to the mixture. For example, the comparison sample's alleles may be apparent in all of the replicates of loci that are designated in the composite profile.

F. In some cases, the presence or absence of a contributor to a mixture may be inconclusive.

G. Non-mixtures or deduced major components may be used for a statistical evaluation.

H. For loci assigned a "Z," only one allele is entered in the calculation spreadsheet. In this manner, the locus is not treated as a true homozygote whose statistical values are determined by multiplying the frequency of the allele in the database by itself (p^2). Rather Z loci utilize the probability only of the one assigned allele (2p).

Revision History:
March 24, 2010 – Initial version of procedure.

Kinship Analysis tests alternate or competing hypotheses of kinship. In the forensic context, it is useful for determining familial relationships, the identification of unknown bodies, and the identification of the donor of bloodstains when the donor/body is missing or unavailable, and the identification of the biological father or mother of products of conception/babies, which result from a sexual assault or are abandoned. All calculations are performed according to the Parentage Testing Standards of the American Association of Blood Banks. The DNA from the subject/stain in question is compared to the DNA of close biological relatives.

For parent(s)/child comparisons, the loci are first evaluated to determine whether the individual in question can be excluded as a biological relative of the other individual(s) (see below). If the individual cannot be excluded, or for comparisons not involving a parent(s)/child relationship, a PI (traditionally called a paternity index, but this could be a maternity or kinship index), is calculated for each locus using the DNAVIEW program of Dr. Charles Brenner. The formulas for parent/child comparisons are listed in Appendices 6 and 11 of Parentage Testing Accreditation Requirements Manual, 3rd edition, AABB.

If there is an exclusion at a single locus in a parent/child comparison, The PI is calculated according to the formula in Appendix 11 (PI=μ/PE) where

> μ (locus specific mutation rate) is obtained from Appendix 14 of Parentage Testing Accreditation Requirements Manual, Fourth Edition, AABB and
>
> **PE** = h^2 ($1-2hH^2$) where H is the frequency of homozygosity and h is the frequency of heterozygosity. PE is calculated by the DNAVIEW program.

An overall CPI (combined paternity index) is calculated by multiplying all of the individual PIs. A probability of paternity (maternity/kinship) is then calculated using Bayes' theorem and assuming a prior probability of 50%. The individual loci PI, the CPI, and probability of paternity (W) are calculated by the DNAVIEW program. The report printed out from DNAVIEW should be included in the case file as the statistics sheet. The DNAVIEW calculations should be performed for each race.

The Forensic Biology case report should report the results for ONE race, preferably the race of the individual in question (e.g., the race of the tested man in a paternity case). The case report must list the PI for each locus, the race used for the calculations, the CPI, the probability of paternity, and the assumed prior probability. It must also state the final conclusion. The three possible final conclusions are exclusion, inconclusive, or inclusion, of the tested hypothesis of kinship.

Exclusions occur when either 2 or more loci exclude in a parent/child comparison, or when the CPI < 0.1.

Inconclusive occurs when the CPI is between 0.1 and 10, and for individual loci in mixtures of parent/child combinations when there are other peaks visible which could potentially exclude or include but can not be genotyped by the software.

Inclusions occur when either 0 or 1 loci exclude in parent/child combinations, and when for all cases the CPI > 10. The analyst should bear in mind and report the strength of the inclusion based on the CPI. When the CPI is greater than 2000 (probability of paternity > 99.95%, 50% prior probability), the hypothesis of kinship should be accepted (considered proven). When the CPI is between 100 and 2000, the hypothesis is supported by the data. When the CPI is between 10 and 100, the hypothesis should not be rejected, and should be considered a weak inclusion.

Revision History:
March 24, 2010 – Initial version of procedure.

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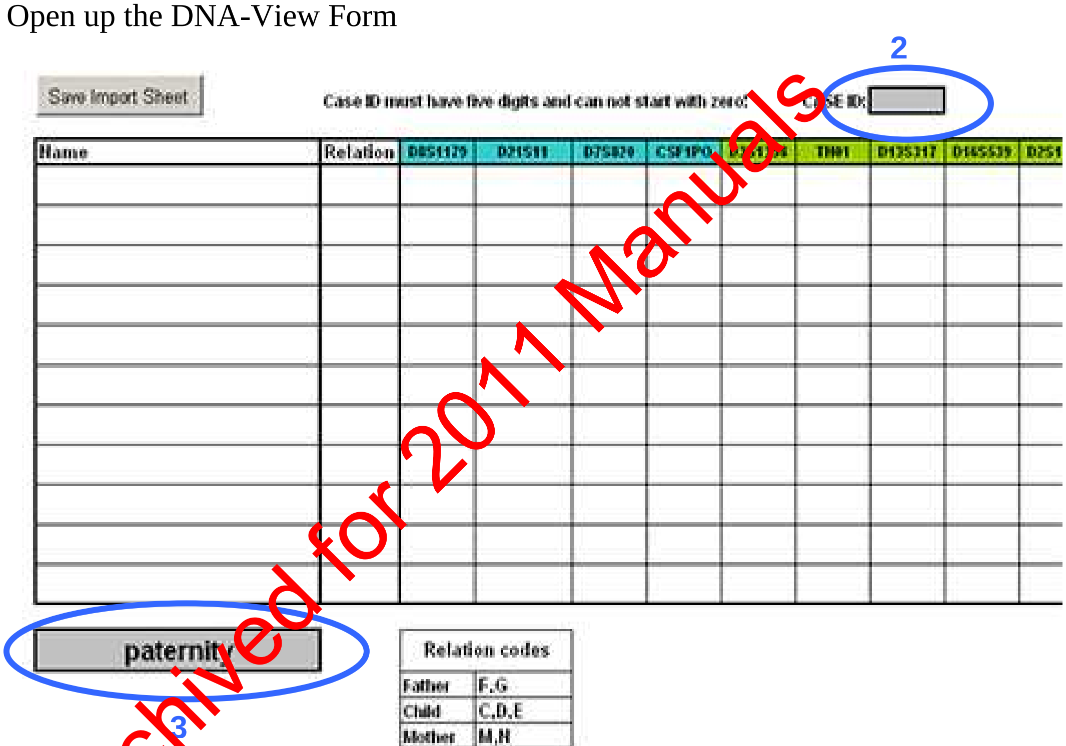
Morling N, Allen R, et al (2003) Paternity testing commission of the International Society of Forensic Genetics: recommendations on genetic investigations in paternity cases. Int J Leg Med 117:51-61.

Revision History:
March 24, 2010 – Initial version of procedure.

DNA-View is software created by Dr. Charles Brenner and is used for the performing paternity and kinship analysis. The following instructions are guidelines as to the use of DNA-View and interpretation of the results.

I. Creating a DNA-View Worksheet and Import Record

1. Open up the DNA-View Form



2. On the **DNAView Worksheet**, fill in a 5-digit **Case ID** (i.e., if your case is FB04-1345, then the case ID will be 41345). Note the Case ID cannot start with zero.

3. Select the **Case Type** from the drop down menu: **Paternity** or **Kinship**.

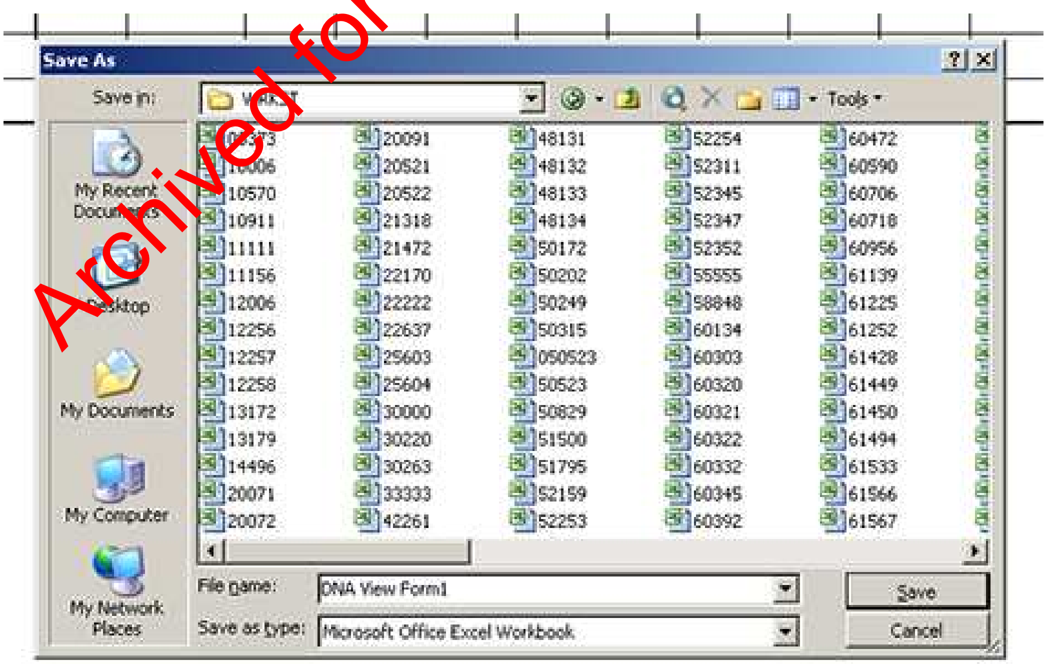
4. Fill in **Name** section with sample names. Don't use quotes because DNA-VIEW will place double quotes around those sample names at the import step.

5. Assign a **Relation** to each sample using the designation codes from the **Paternity** or **Kinship** table below the spreadsheet (i.e., if the person is a mother, enter **M** for relation. If the person is a sibling, enter **U** for relation, if there are additional siblings, enter **A**, then **B**. There are only a standard number of designation codes for each relationship. If additional sibling relationships are required, for example, use the designations for Other: X, Y, Z, as needed. This convention also holds true for other relationships in the table).

6. Enter the DNA profiles for each sample. This can be done by typing them in by hand or by copy and pasting directly from an STR profile table.

   For both homozygote and heterozygote profiles, **enter both alleles at each locus, separated by a space**, not a comma. If there is allelic dropout at a locus, leave the entire locus blank.

7. Once the sheet is completely filled out, save it in the **DNAVIEW \ WRKST** folder. Use the **case ID** as the file name and "save as" type **Microsoft Office Excel Workbook**. See below:



8. Click on the **Save Import Sheet** button on the top left corner of the worksheet. This will save the sheet in a format that DNA-View can import. The filename will be the five-digit case ID and the file will be saved in the **DNAVIEW \ IMPORT** folder.

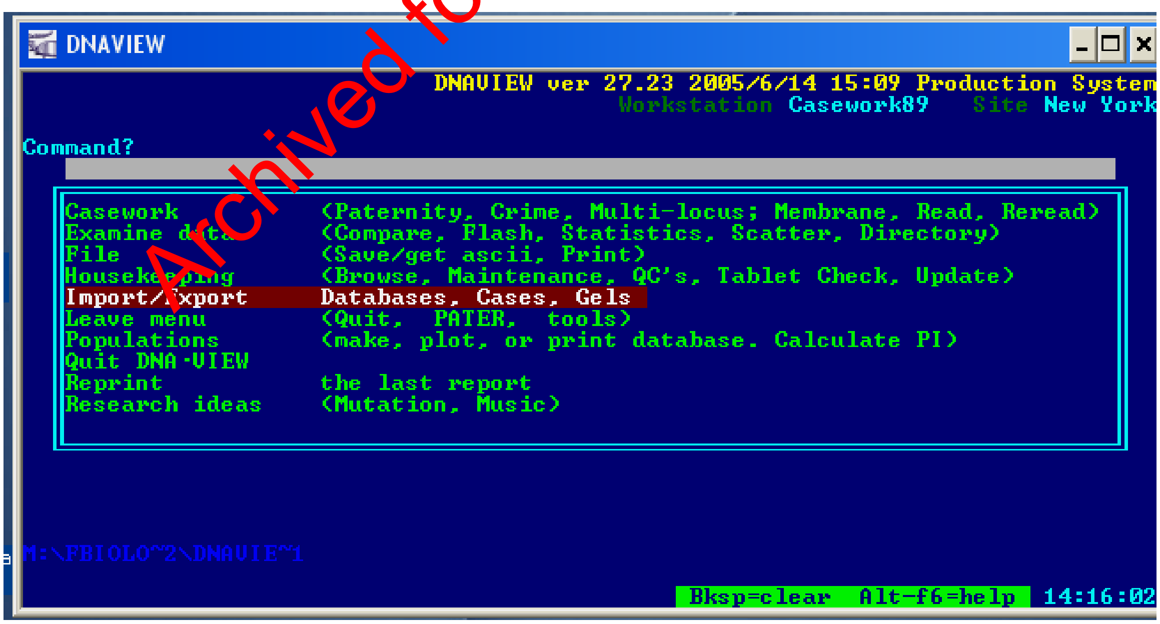
9. Exit from Microsoft Excel. Another Microsoft Excel alert will pop-up asking if you want to save the changes. Click **No**.

II. Importing profiles into DNA-View

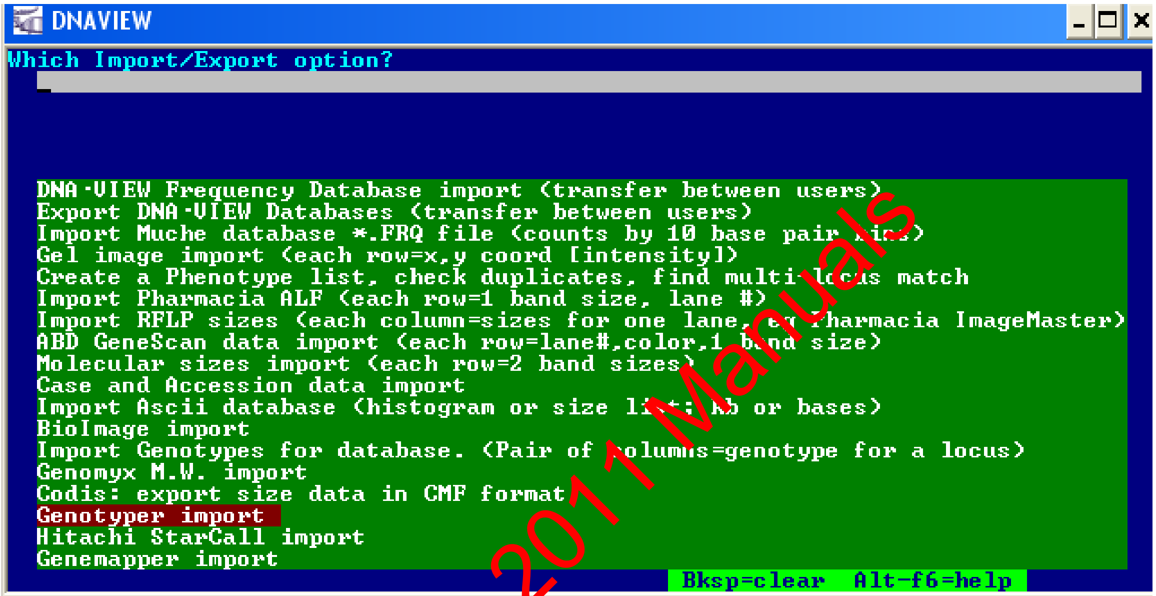
YOU CAN ALWAYS RETURN TO THE MAIN MENU FROM ANY STAGE OF THE PROGRAM (AND WITHOUT LOSING MUCH INFORMATION) BY HITTING the **Ctrl+C** KEYS SIMULTANEOUSLY. THIS MAY COME IN HANDY IF YOU MISTYPE ANY ENTRY.

YOU CAN ALSO USE THE MOUSE, SCROLL USING KEYBOARD ARROWS OR TYPE IN COMMANDS TO SELECT FROM THE MENU.

1. Open DNA-View, select **Import/Export** (by either typing it in the **Command** field or clicking it with a mouse), hit Enter.



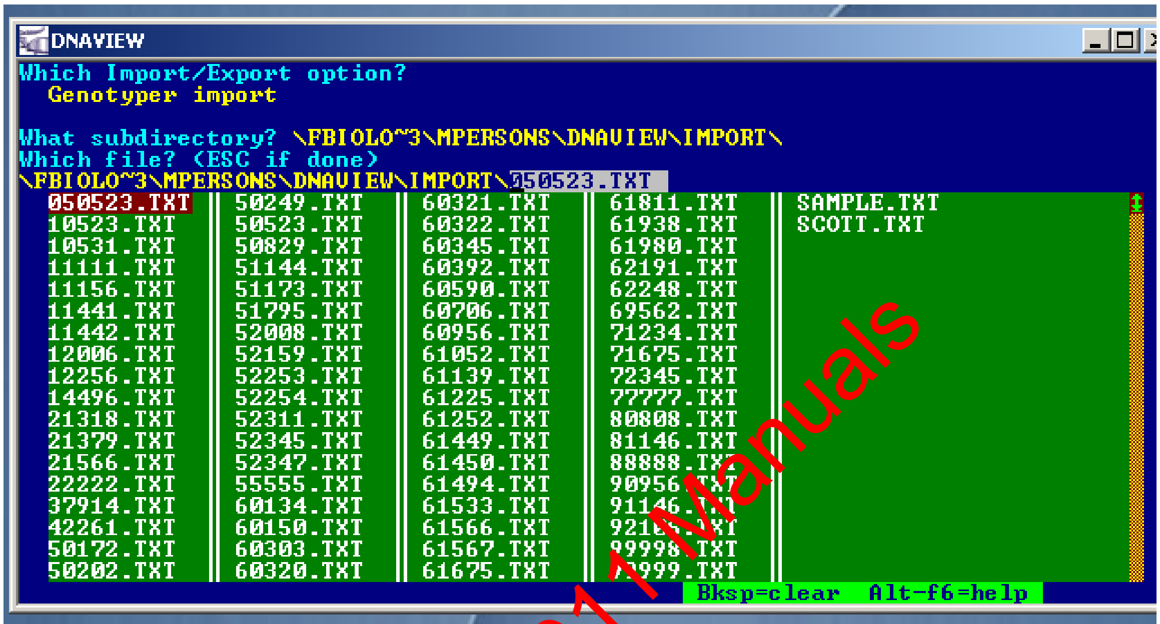
2. At next screen, there is field that says **Which Import/Export option?** select **Genotyper import**, hit **Enter**.



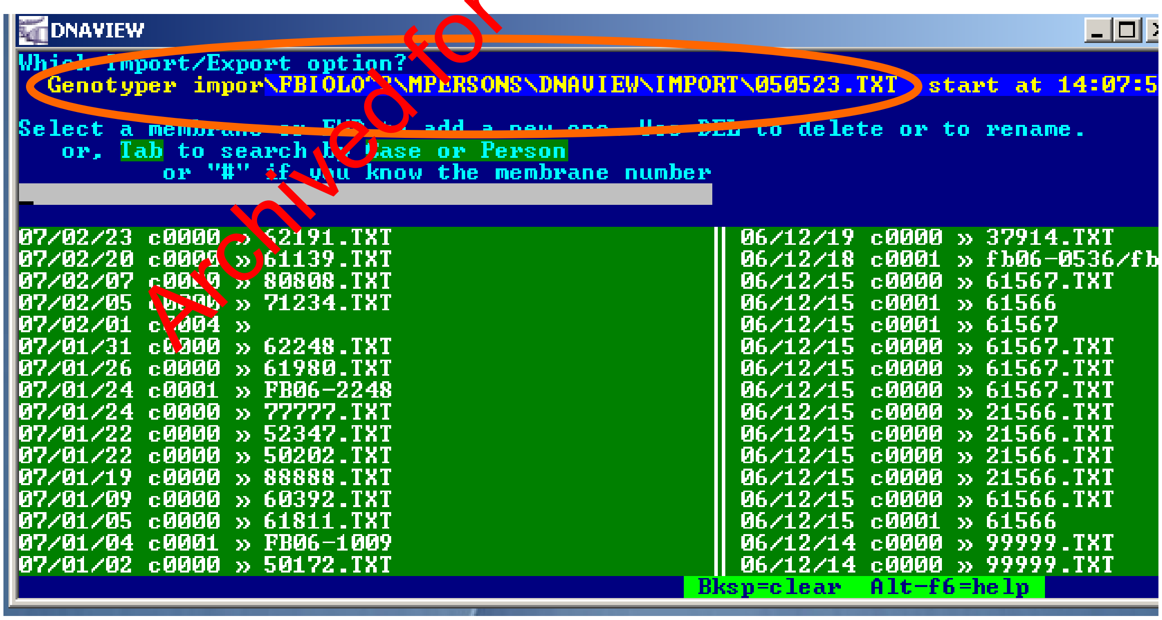
3. In the field that says "**What subdirectory?",** a path **(\FBIOLO~3\MPERSONS\DNAVIEW\IMPORT\)** will already be specified. Hit **Enter**.

   If the field is blank, see the Troubleshooting section for specifying the subdirectory.

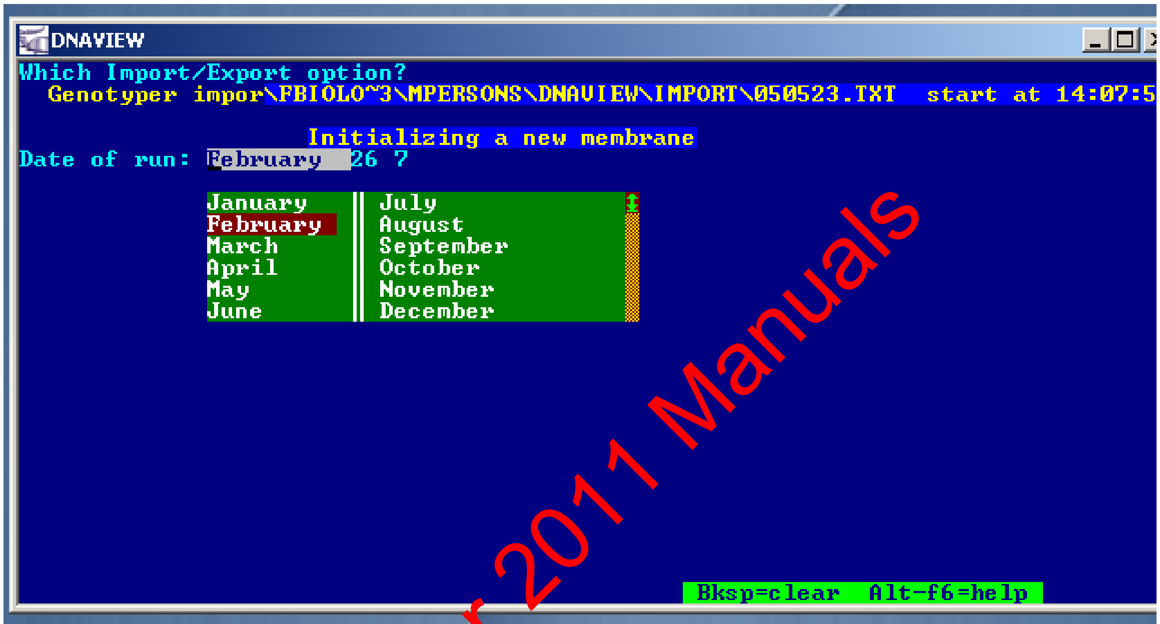
4. Select your Case ID from the list. Hit Enter.



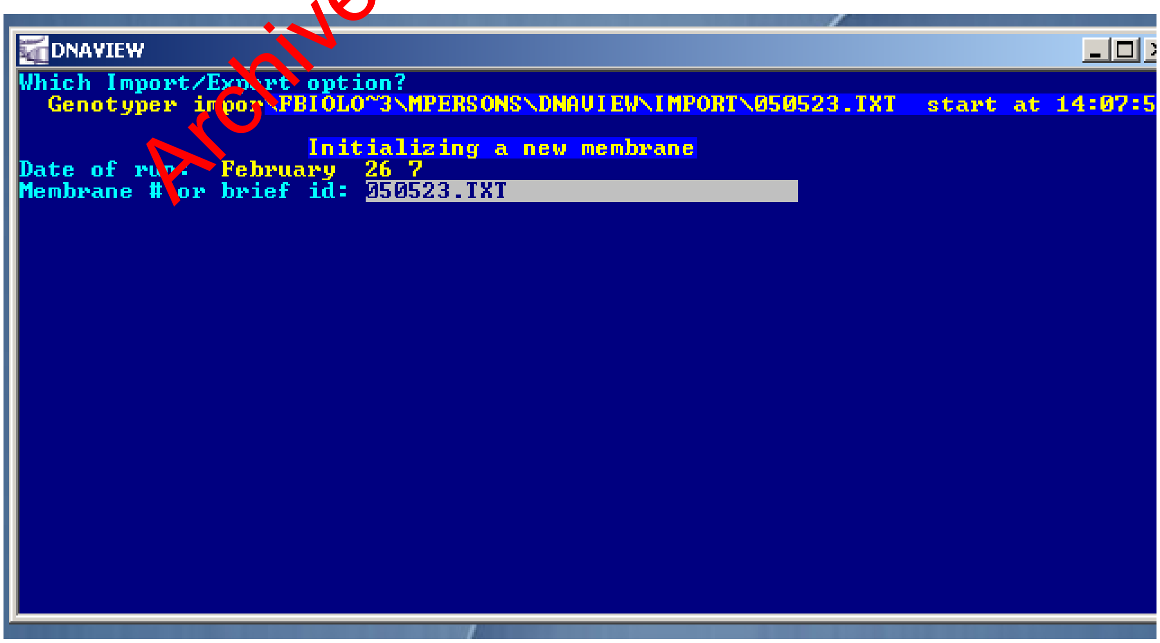
5. At the following window, path with selected **Case ID** will appear, hit Enter.



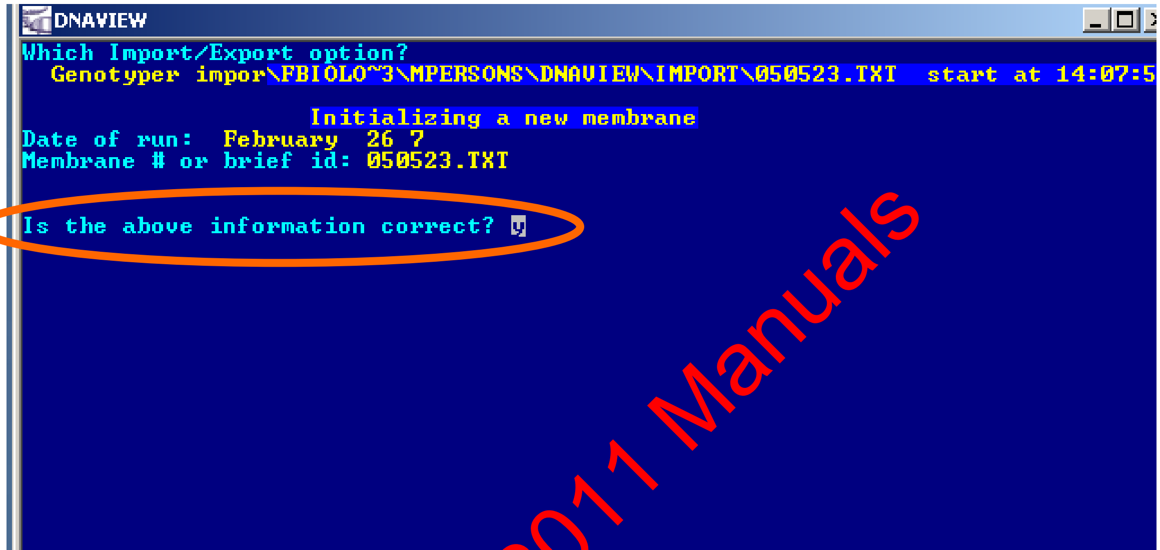
6. Now that **Case ID** has been selected, screen will say **Initializing a new membrane**. **Date of run** will default to the current date, hit **Enter**.



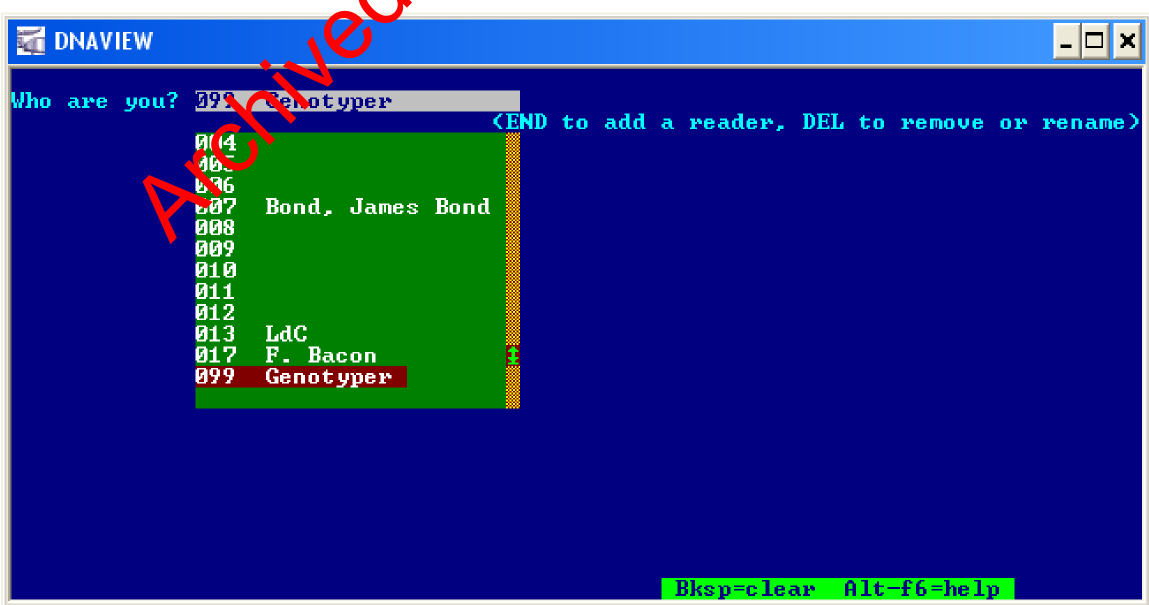
7. **Membrane # or brief id** will list the selected **Case ID** in the format of **#####.txt**. Hit **Enter**.



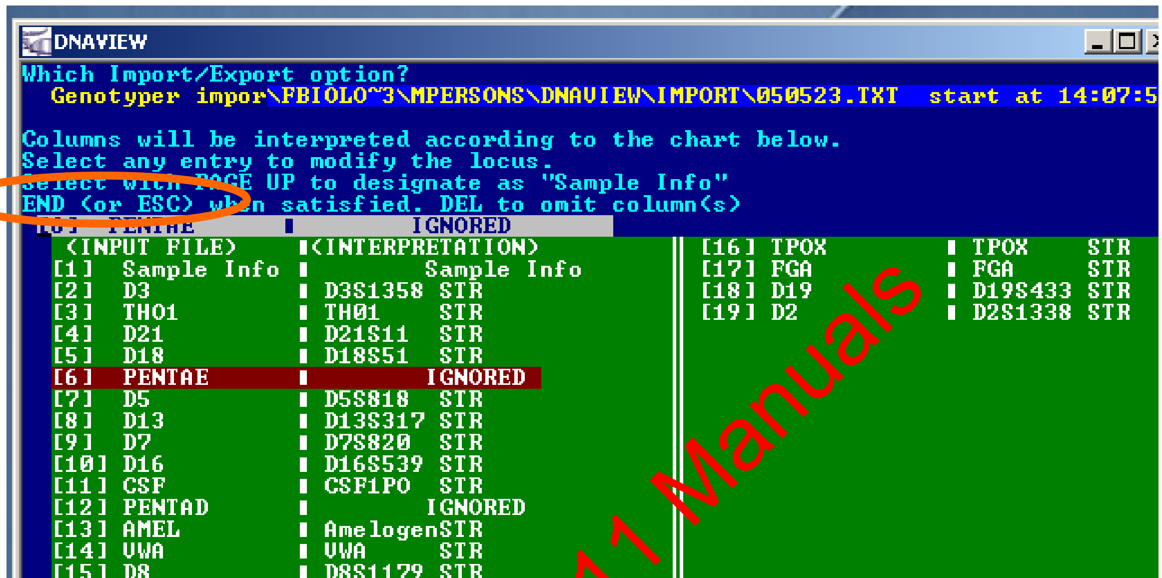
8. You will be asked, **Is the above information correct?** Verify the **Date of run** and the **Case ID** and hit **Enter**.



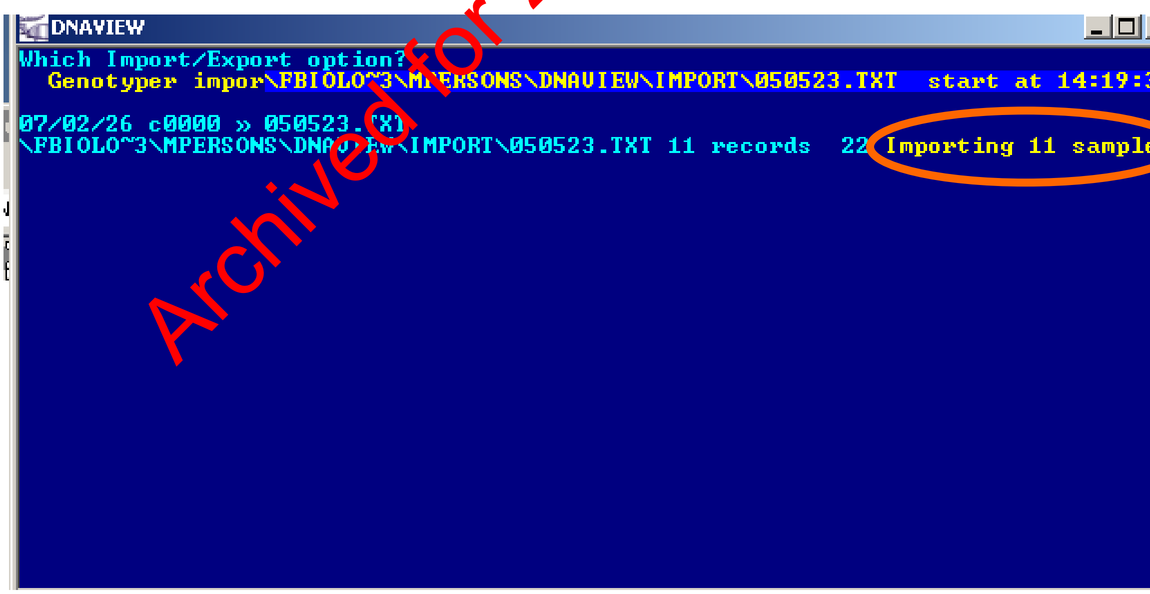
9. You will be asked **Who are you?** The program defaults to **099 Genotyper** (and unless you want to be someone else, such as secret agent, James Bond, or father of inductive reasoning, Francis Bacon) hit **Enter**.



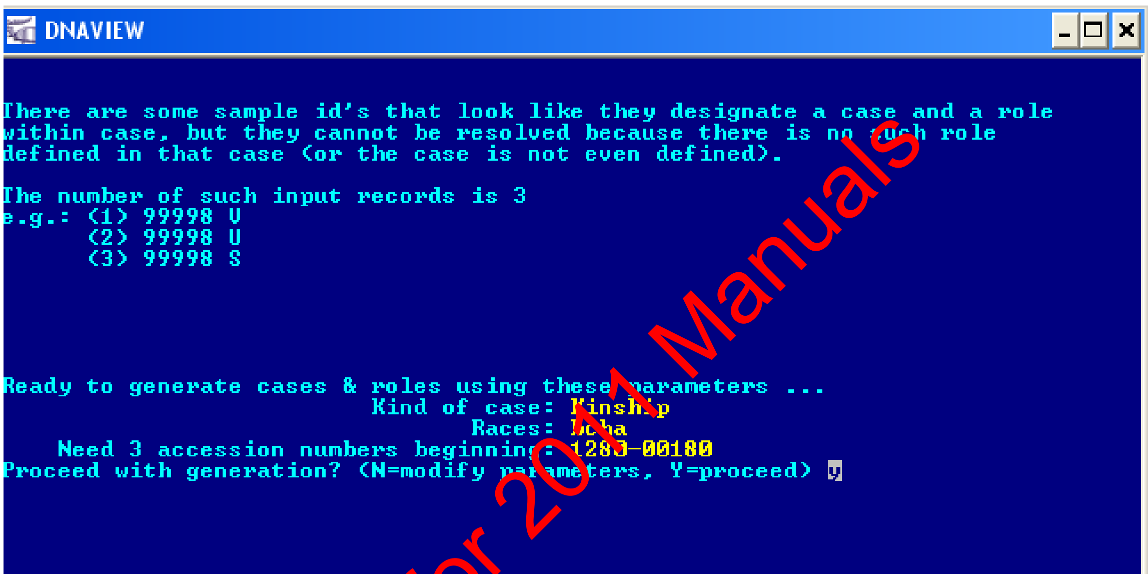
10. The following window displays the entered loci, hit **End** or **Esc**, not **Enter**.



11. Wait for a few seconds for the DNA profiles to import.

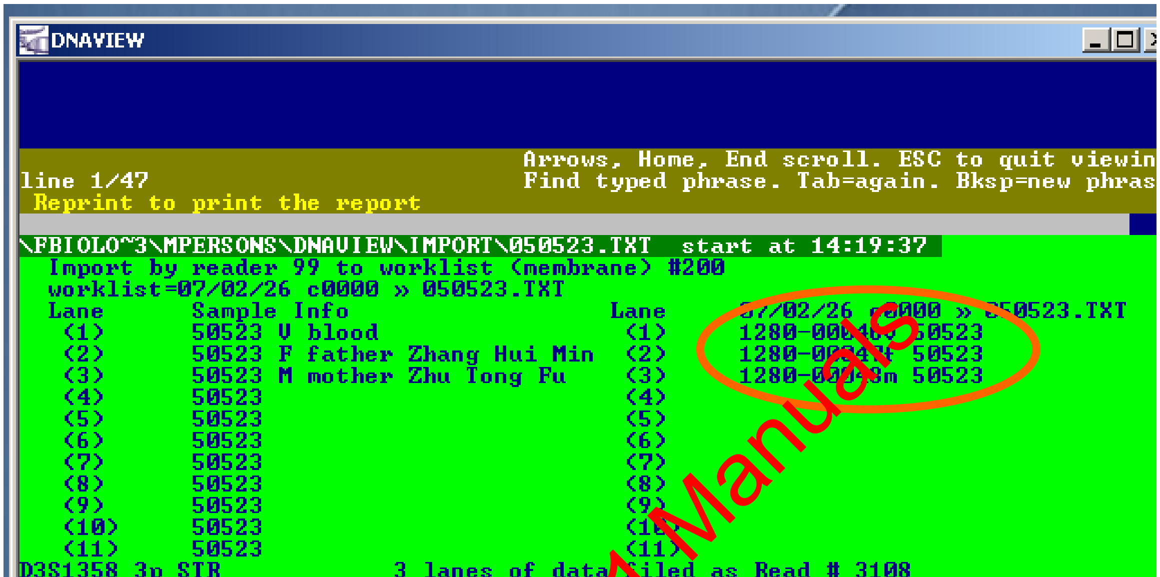


12. Note: A screen ***may*** appear that says "There are some samples id's…". At the bottom of this screen, the program asks **Proceed with generation? (N=modify parameters, Y=proceed).** **Y** will appear, hit **Enter**. ***If this screen does not appear, do not be alarmed, the import will still work.***



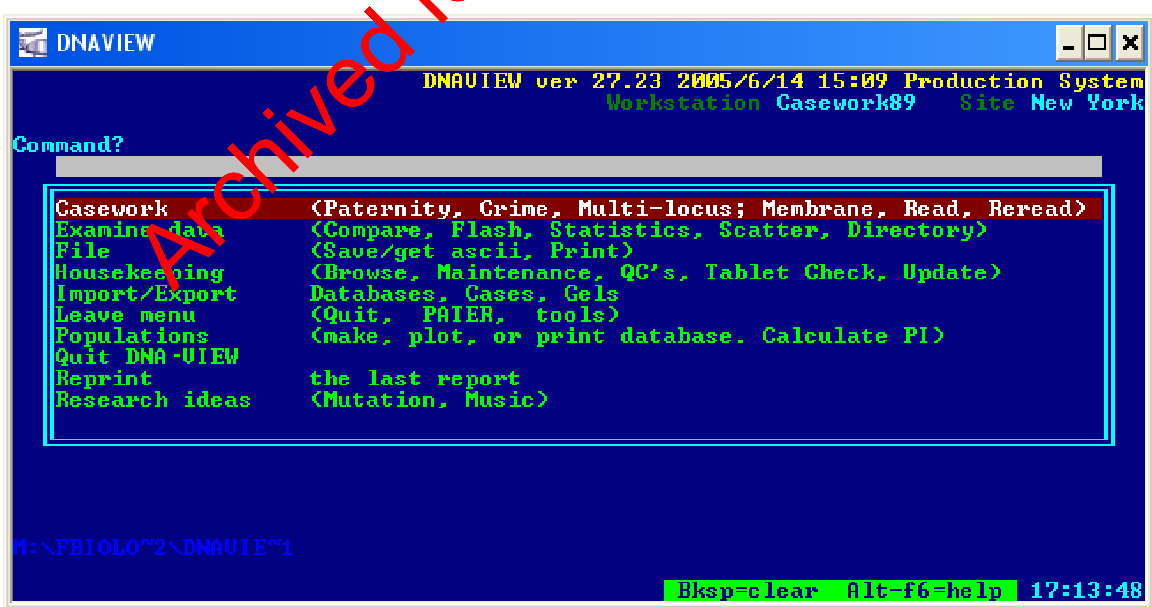
If you are using paternity instead of kinship, answer "N" to modify the parameters and type in "paternity." If the order of races are incorrect or if you only want to test one race, you can change the order here or type in one letter for the race.

13. A green screen will appear, indicating a successful import. At this step, unique identifiers (circled below) are also added to each profile. Hit **Esc** to quit viewing this screen, and **Esc** again to get back to main menu.

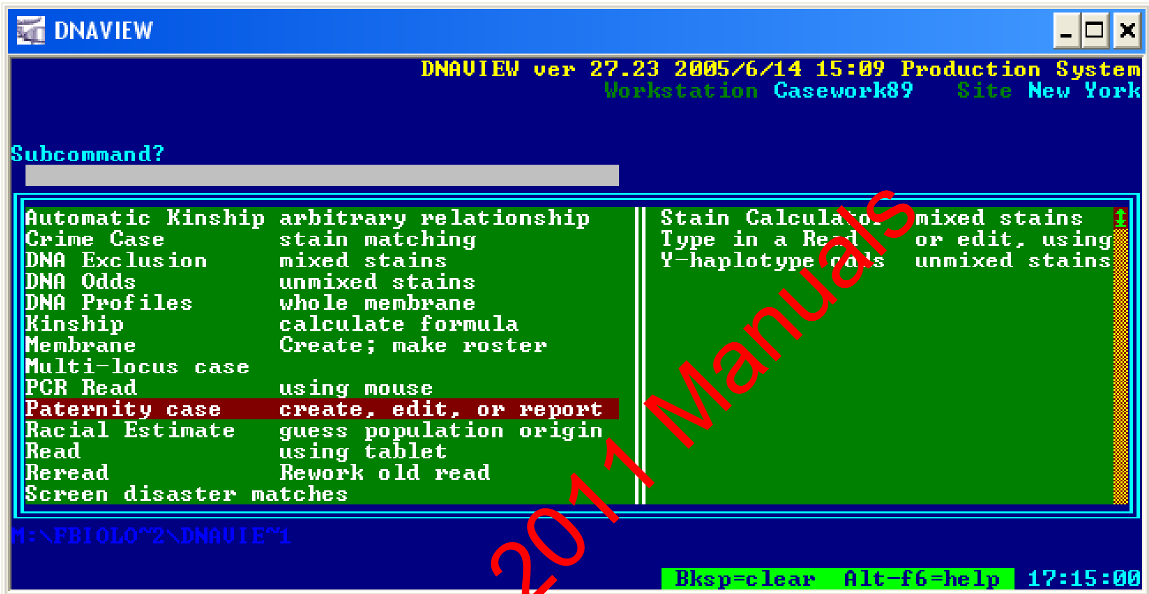


III. Performing Paternity or Kinship Analysis

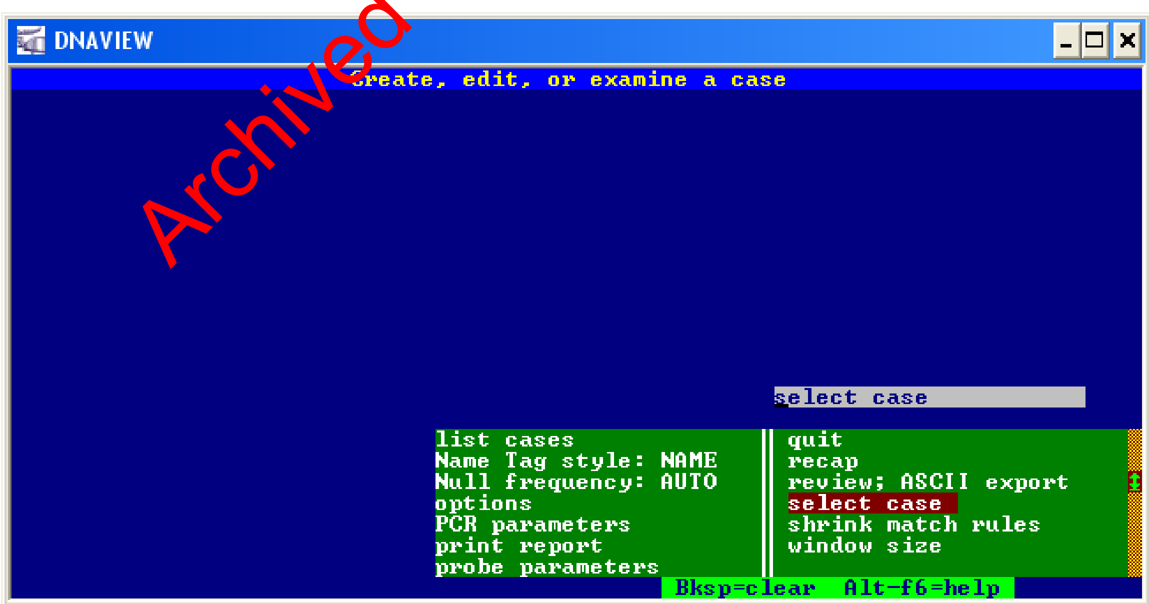
1. Select **Casework**, hit Enter.



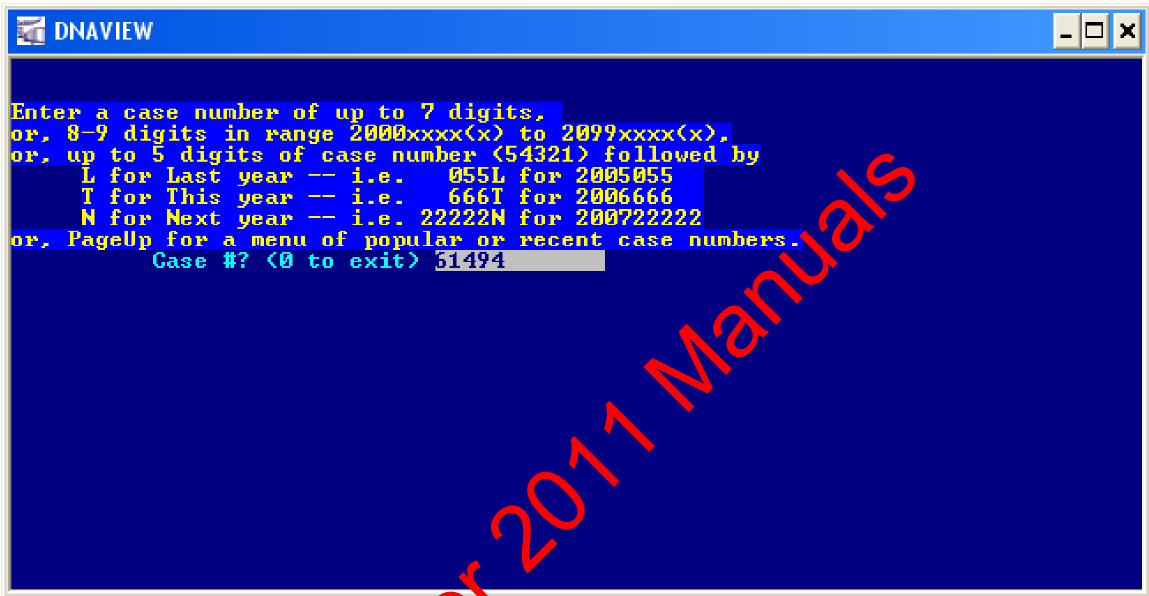
2. Select **Paternity case**, hit **Enter**. (This will be used whether a paternity or a kinship case is being done).



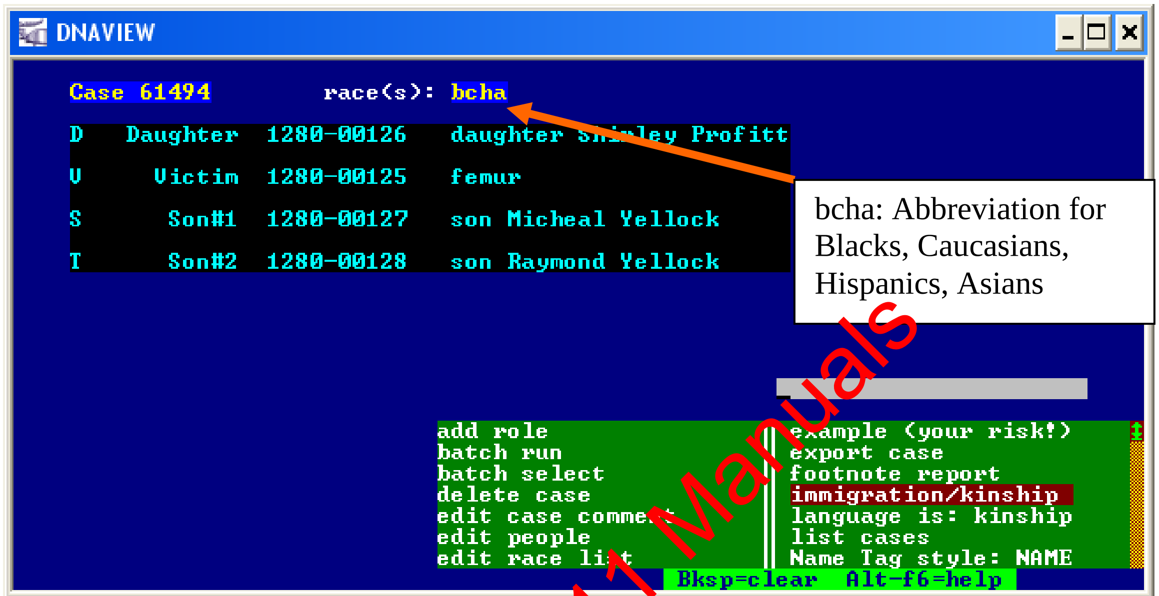
3. **Select case** should be highlighted. Hit **Enter**.



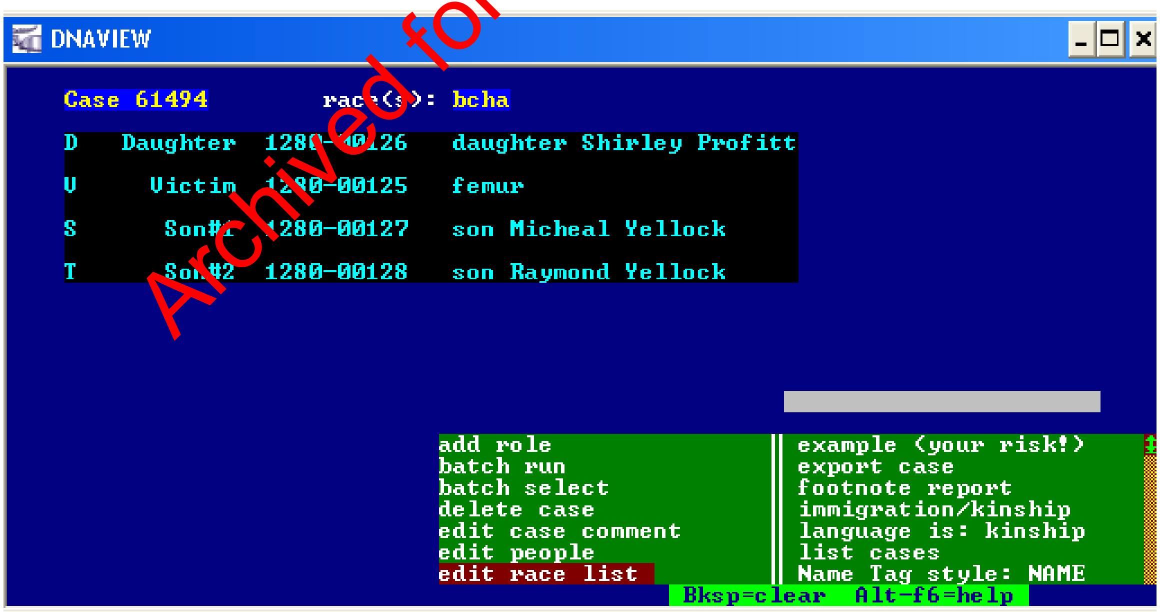
4. At the next screen, at the field **Case # (0 to exit)** look for the 5 digit **Case ID** that was imported. If it is there, Hit Enter. If it is not there, the import step may need to be repeated (Refer to II. Importing profiles into DNA-VIEW).



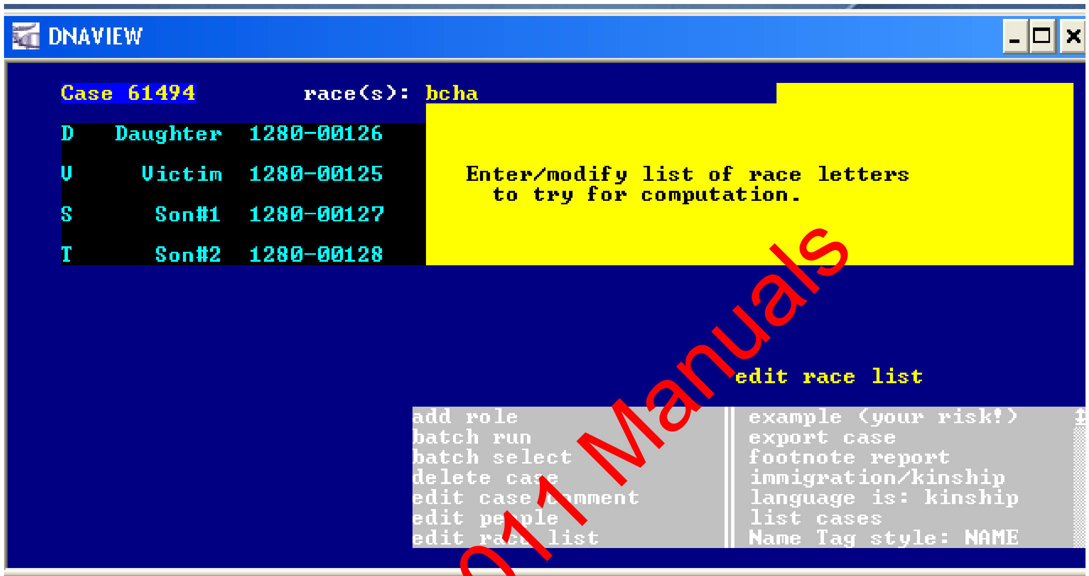
5. Select **immigration/kinship**, hit Enter. Verify that the imported case information is correct such as the **Case ID** and all sample information, including relationships (*if not, see section V.2. for changing case language)*, and that, in the **race(s):** field, **bcha** is indicated. Go to step 8. If **bcha** is not indicated, the race list needs to be edited. ***See steps 6-8 for editing race list.***



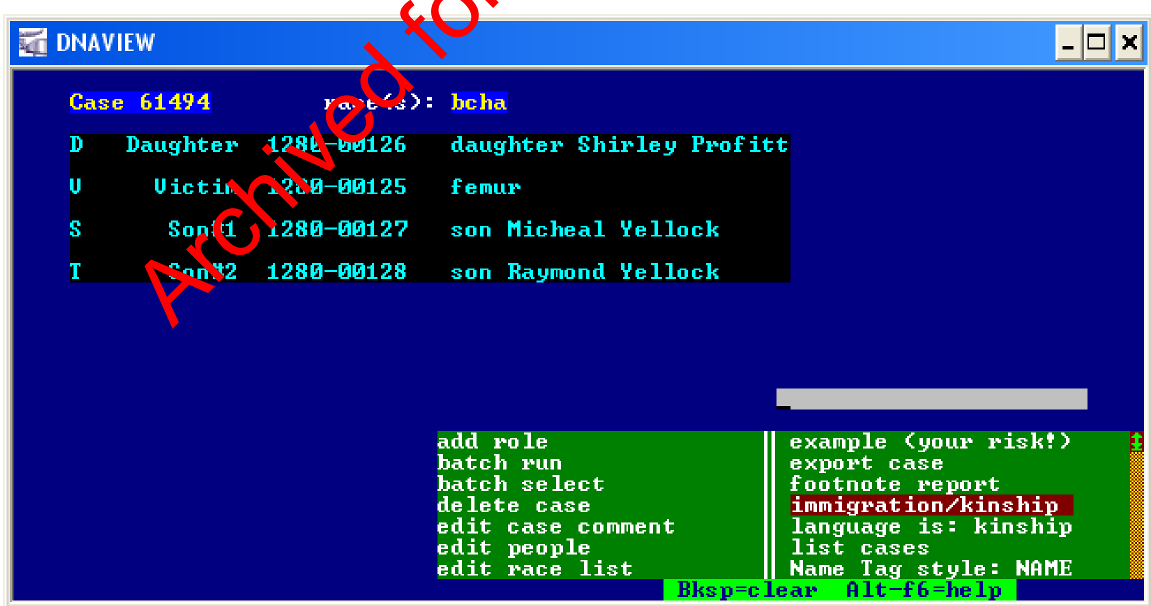
6. Use arrow keys to select **edit race list** in green menu on lower right corner of screen. Hit **Enter.**



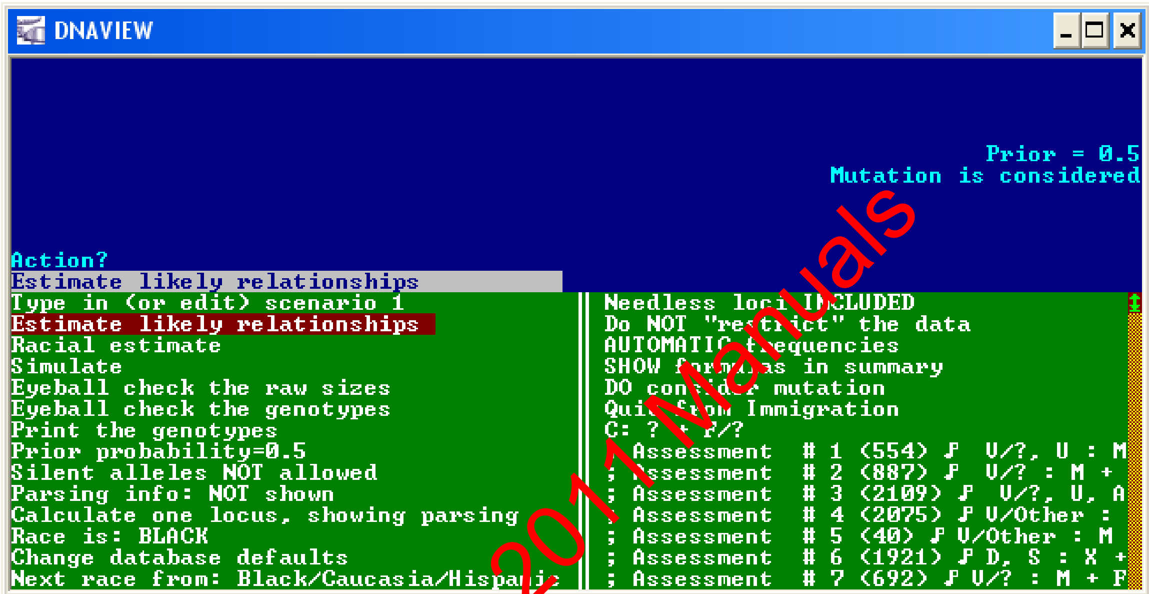
7. Type **bcha** in the **race(s):** field. Hit **Enter**. The changes will be saved.



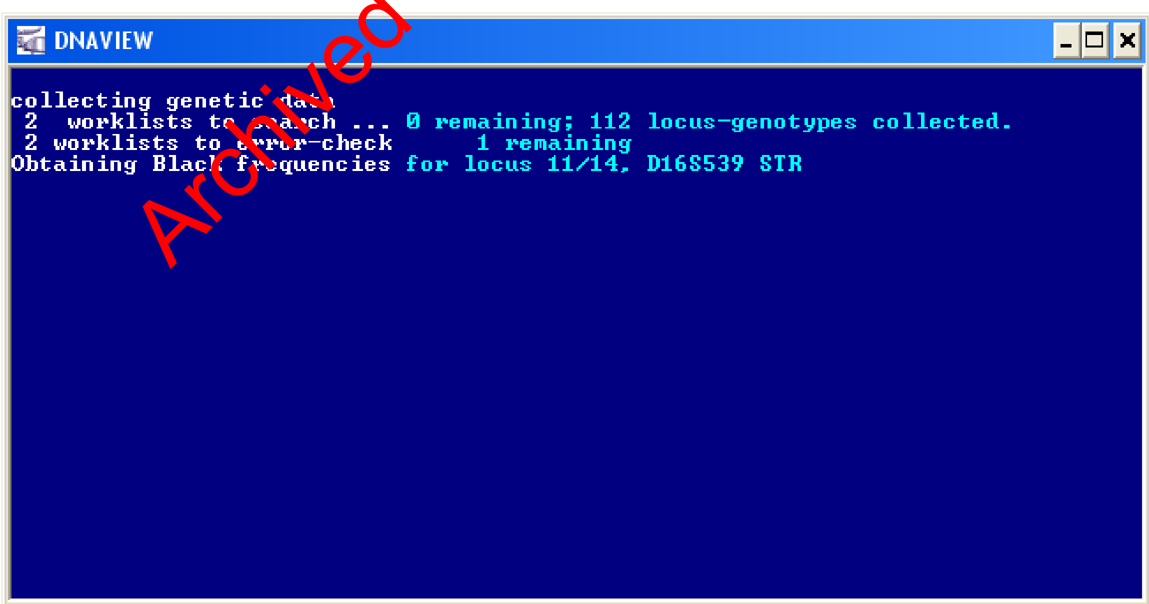
8. After editing race list, select **immigration/kinship**, hit **Enter**.



9. **Estimate likely relationships** should be highlighted already. If not, select it and then hit **Enter**.



10. Wait for program to obtain allele frequencies for the four races.

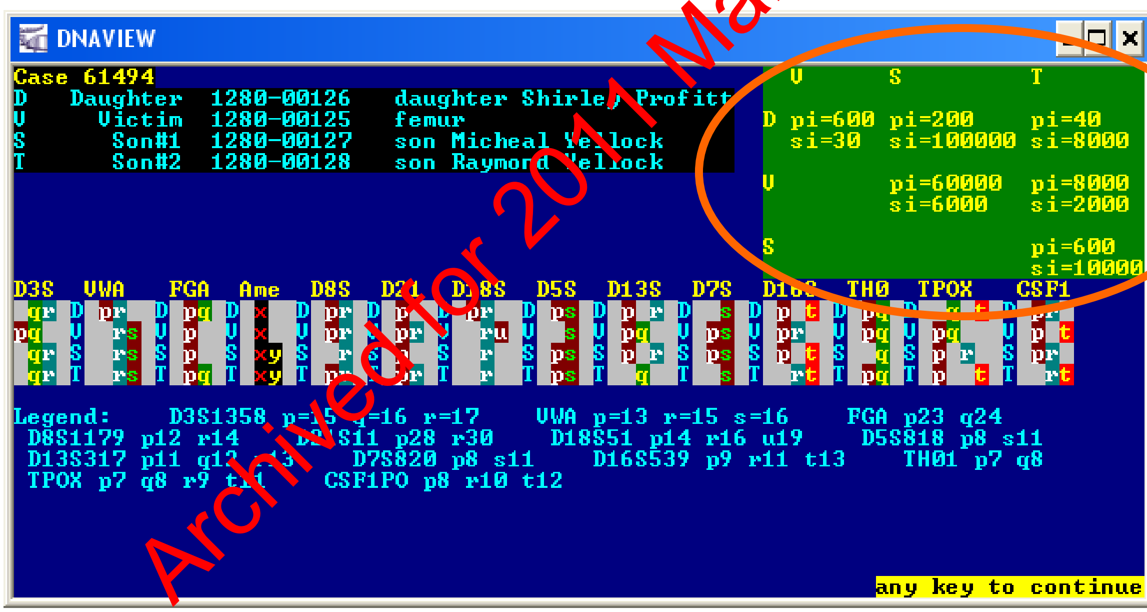


11. The **Estimate likely relationships** screen will display the following information:

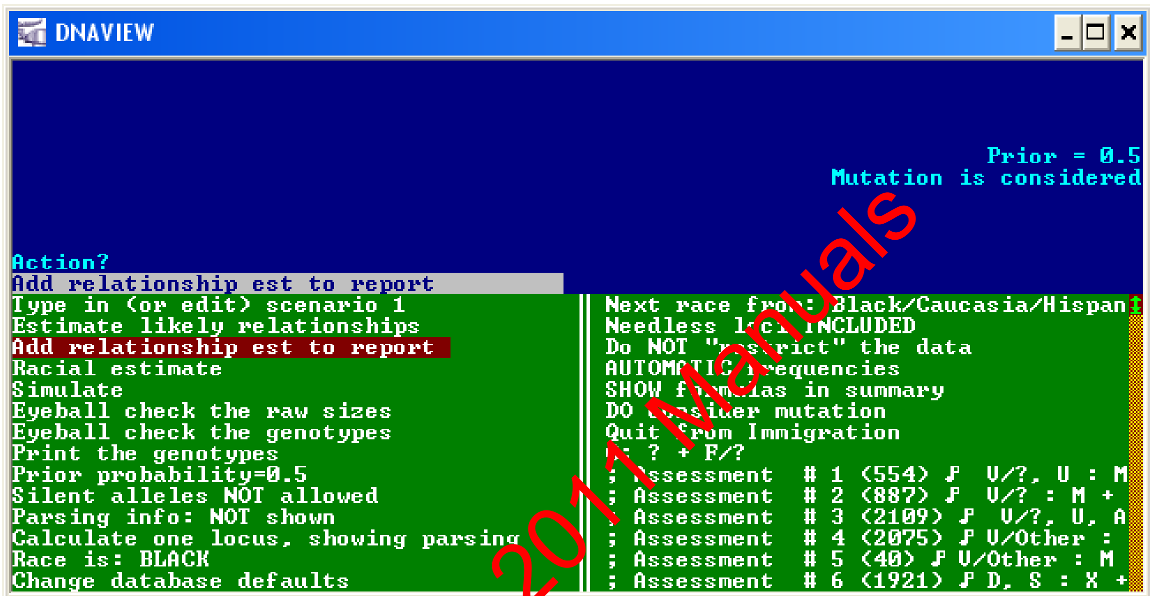
  a. DNA profiles for each sample with a corresponding legend (alleles are expressed in letters)

  b. A green *likely relationships* table (circled below) that lists PI (paternity indices) and SI (sibship indices) generated from calculations comparing every pair of individuals in the case. The numbers in each cell evaluate the corresponding pair of people as potential parent-children (PI), and as potential siblings (SI). Numbers are omitted if very small. (As per Dr. Charles Brenner's DNA-VIEW Newsletter #17, http://dna-view.com/news17.htm)

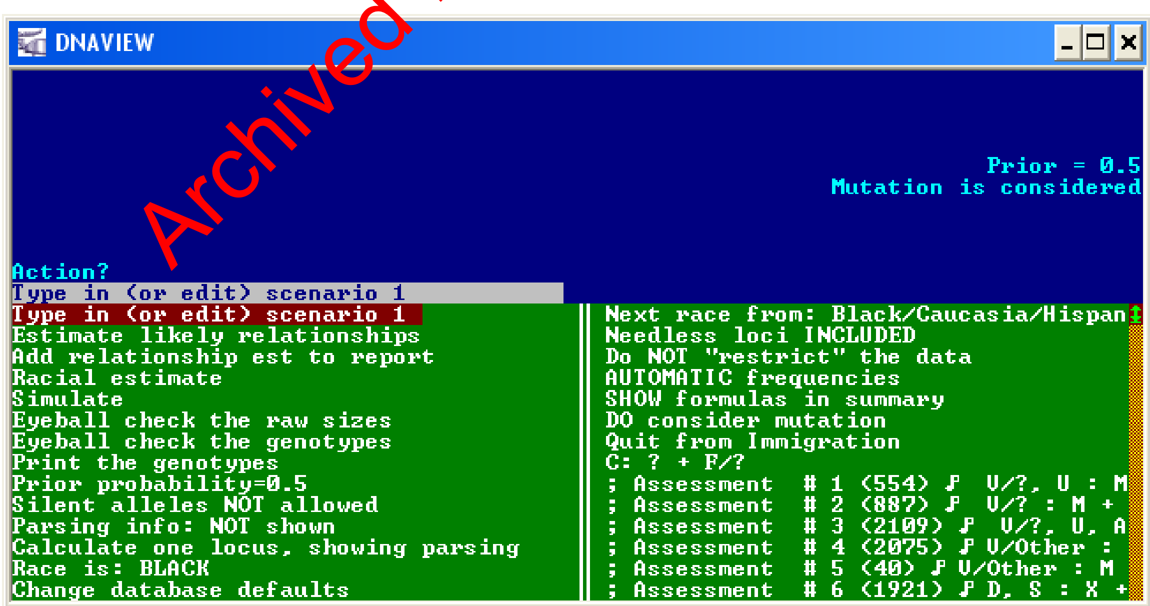
  c. After viewing this information, Hit **Enter**.



12. Select **Add relationship est to report**, hit **Enter** to add the *likely relationships table* to the final report that will be placed in the casefile.



13. Select **Type in (or edit) scenario 1**, hit **Enter**.

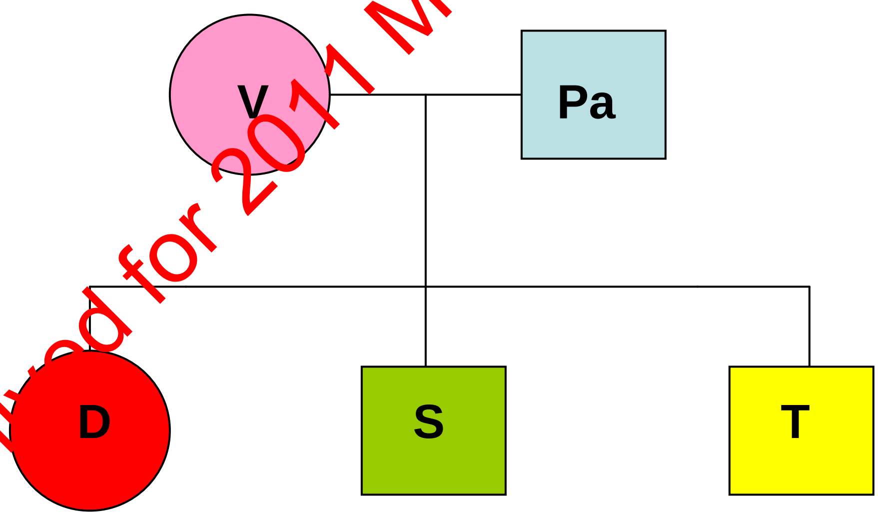


14. In the blue field, enter a kinship or maternity/paternity statement that expresses two hypotheses (or ways people are related), then hit **Esc**, not **Enter**. See below for examples of Kinship and Paternity scenarios.

    a. In the case example featured in the screen captures, there is a typed femur, **V**, that may *or may not* be from the mother of the typed daughter, **D**, son **S**, and son **T**

    The format for this KINSHIP case is as follows:

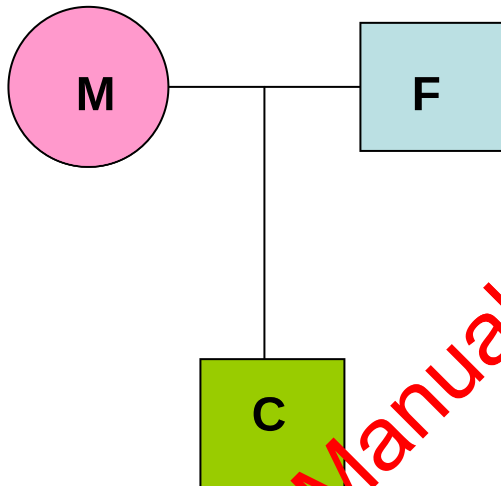
    1) D,S,T:V/Other+Pa (as seen in screen capture below)
    2) This means daughter, **D**, son, **S**, and son, **T** are a product of the typed femur donor, **V,** or another unknown individual, **Other**, and some untested man, **Pa**.

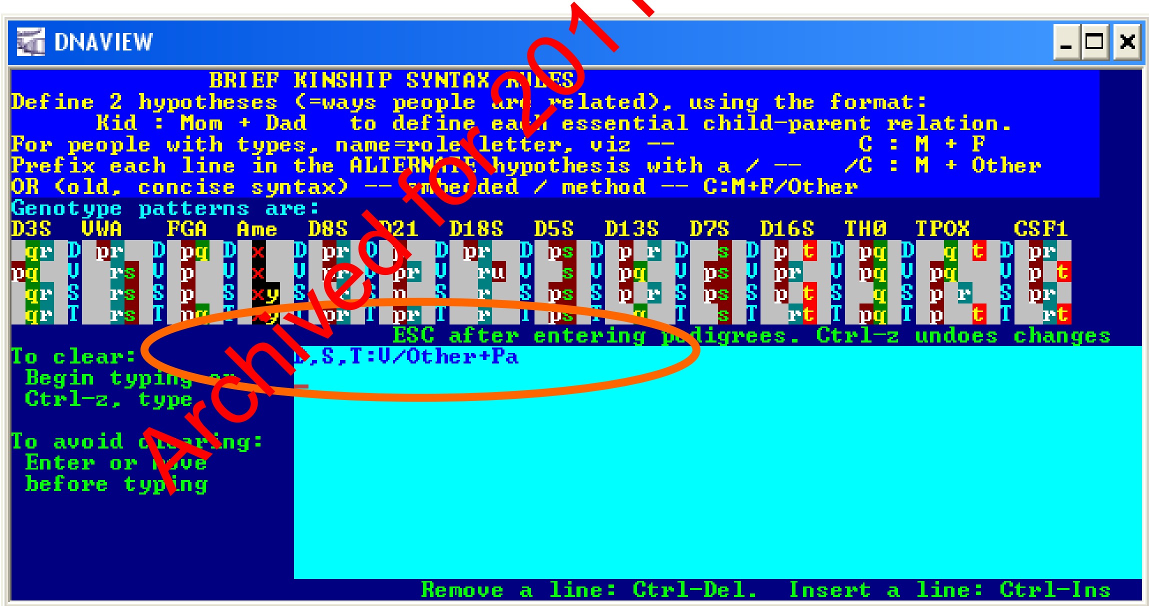


    b. Another option is a case of with a trio of typed individuals, a child, **C,** a mother, **M,** and a tested man that may *or may not* be the father, **F**

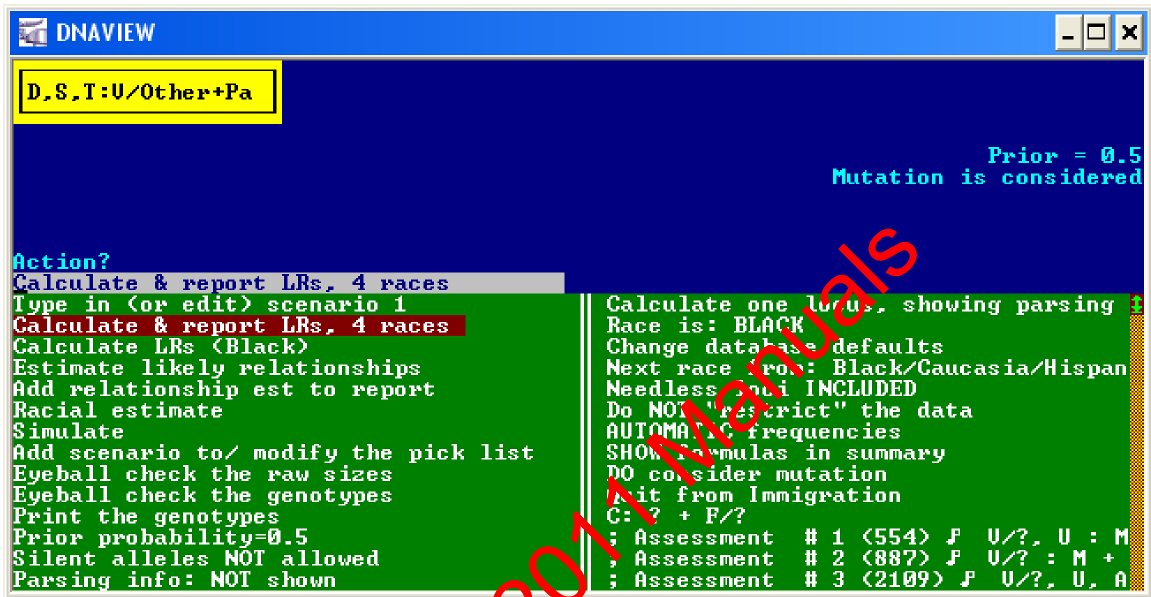
    The format for this PATERNITY case is as follows:

    1) C:M+F/Other
    2) This means that the child, C, is a product of the typed mother, **M**, and the tested man, **F**, or another unknown man, **Other**.

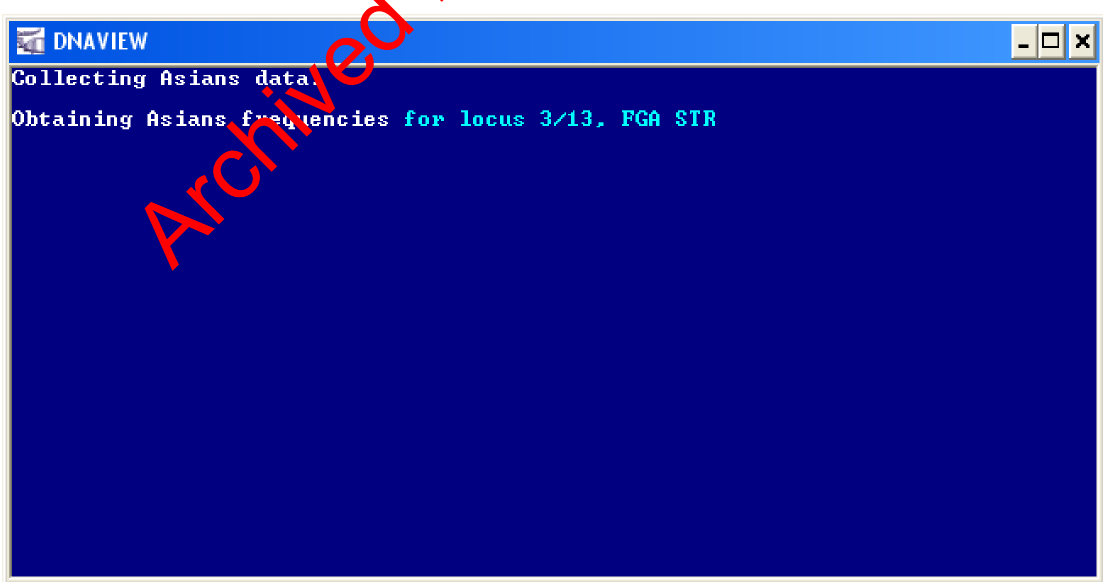

M
F
C


DNAVIEW
BRIEF KINSHIP SYNTAX
Define 2 hypotheses (=ways people related), using the format:
Kid : Mom + Dad
essential child-parent relation.
For people with types, name=role letter, viz --
C : M + F
Prefix each line in the
hypothesis with a / --
/C : M + Other
OR (old, concise syntax) --
method -- C:M+F/Other
Genotype patterns are:
D3S VWA FGA Ame D8S D21 D18S D5S D13S D7S D16S TH0 TPOX CSF1
ESC after entering
Ctrl-z undoes changes
To clear:
Begin typing
Ctrl-z, type
To avoid
Enter or
before
Remove a line: Ctrl-Del. Insert a line: Ctrl-Ins

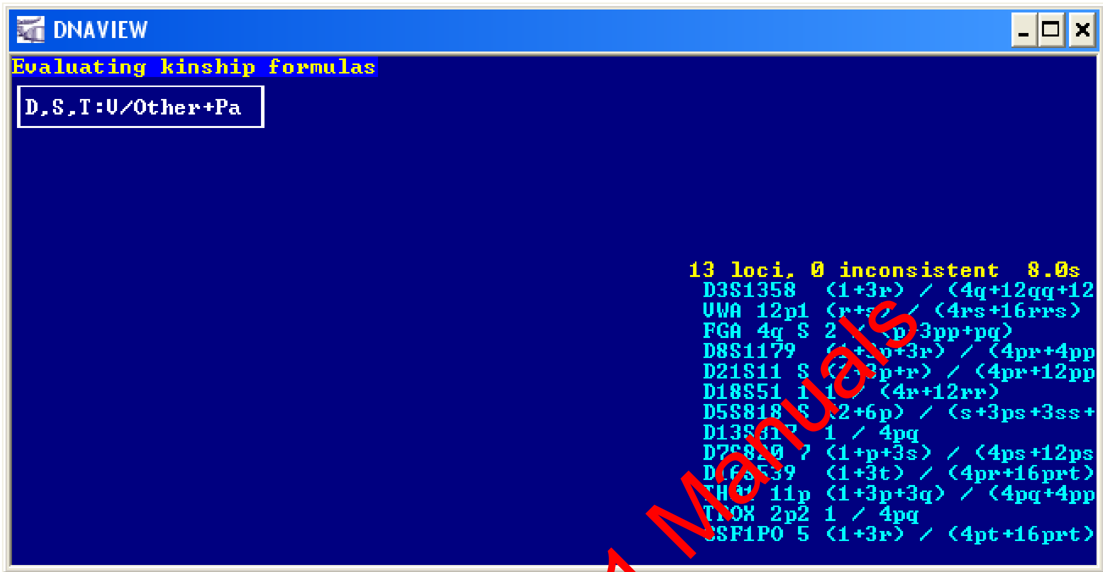
15. Select **Calculate & report LRs, 4 races**, hit **Enter.**



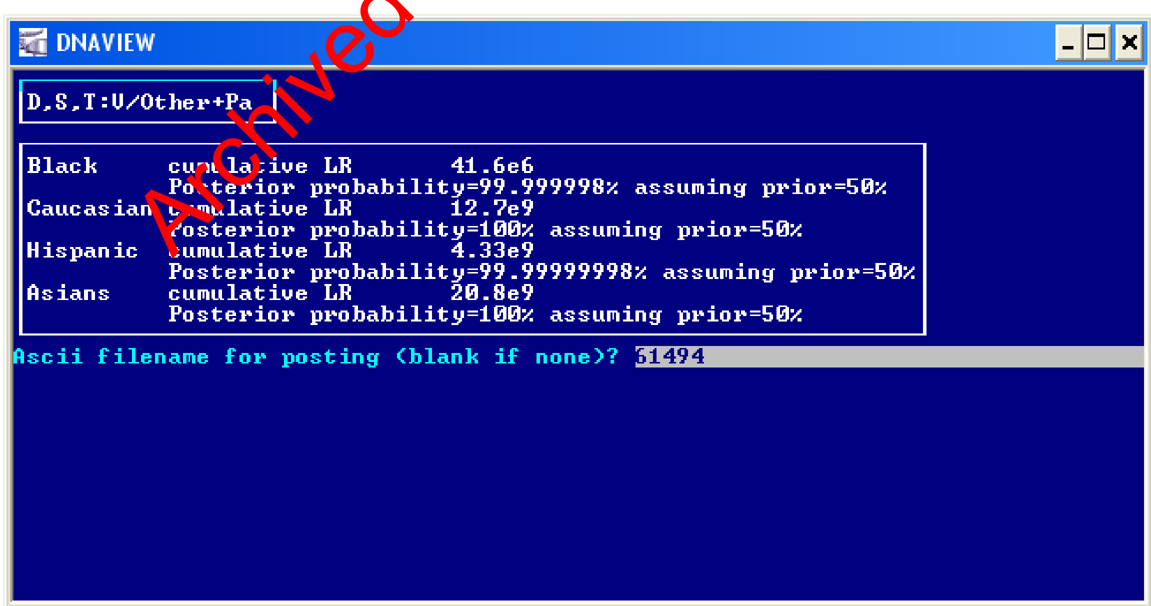
16. Wait for the program to collect allele frequencies and calculate kinship equations. A series of screens will appear, see examples below.



Wait…



17. A table with cumulative LRs for each race will appear. These are the statistics that will be presented in the Forensic Biology report. **In the field that says Ascii file name for posting (blank if none)?**, enter the filename: first letter is a P or K (Paternity or Kinship) followed by the five digit ID number, and ending with **.txt** (*e.g.* **P91125.txt**, **K80144.txt**). Hit **Enter** to save the file.



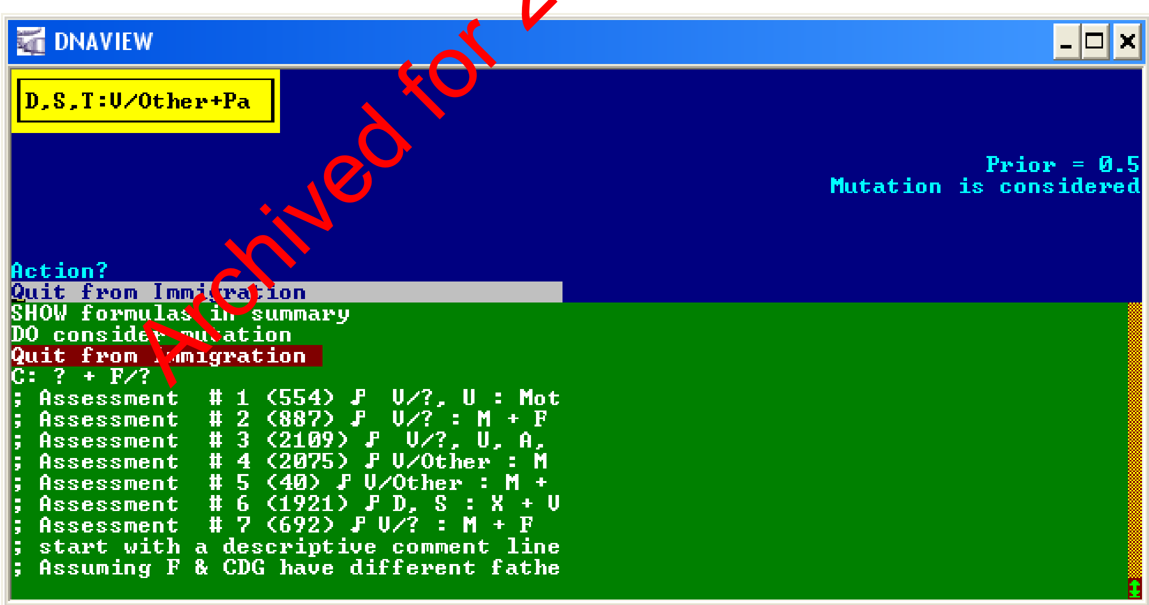
a. Displayed in this screen capture is the following:

Cumulative LR
This is a likelihood ratio, also known as the combined kinship index (CKI) or combined paternity index (CPI) which evaluates the assumptions spelled out in the proposed kinship or paternity scenarios from step 14 and determines which is more genetically likely.

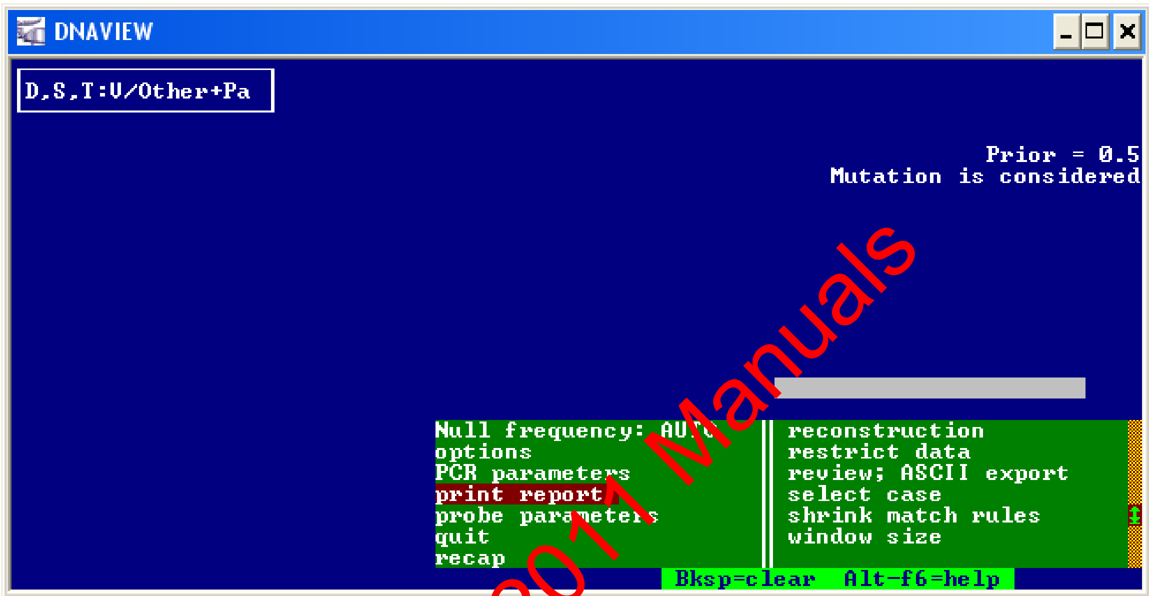
Posterior probability
Posterior probability is also the **relative chance of paternity** (mentioned in Forensic Biology paternity report)

Prior probability
Prior probability is always 50% (both hypotheses equally plausible) for paternity and kinship cases (mentioned in Forensic Biology paternity report)

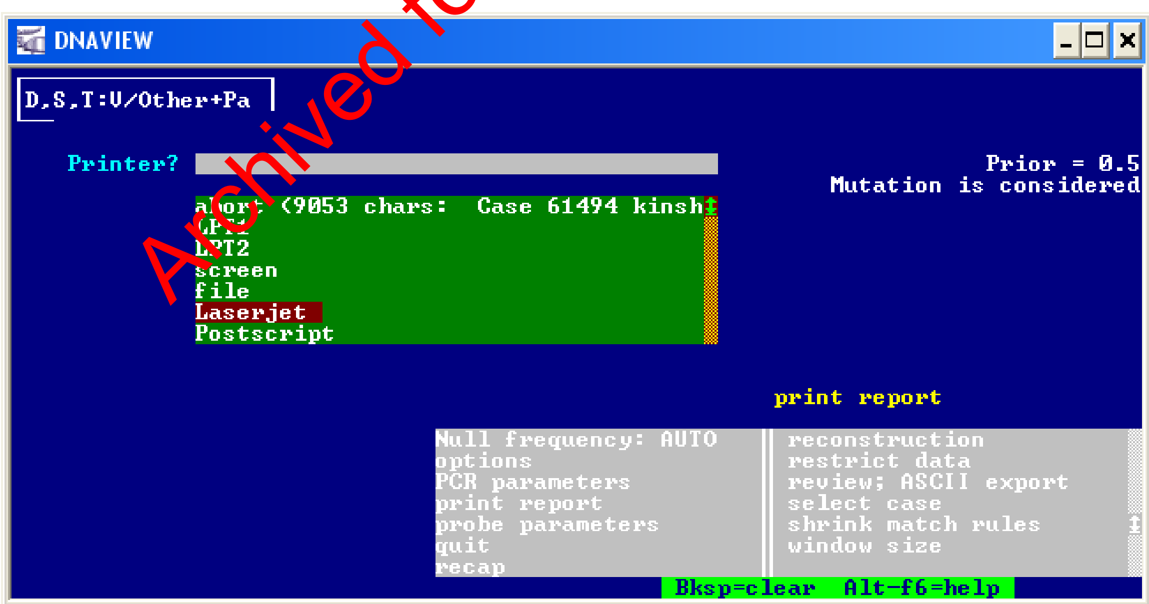
18. Select Quit from Immigration (should already be highlighted) and hit **Enter**.



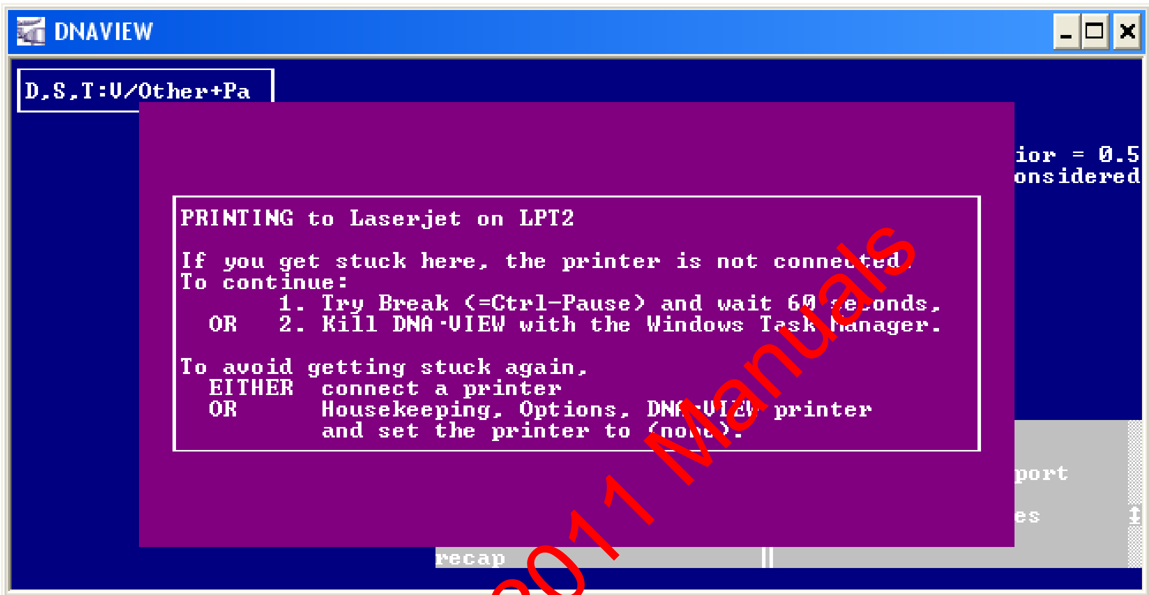
19. Select **print report**, hit **Enter**.



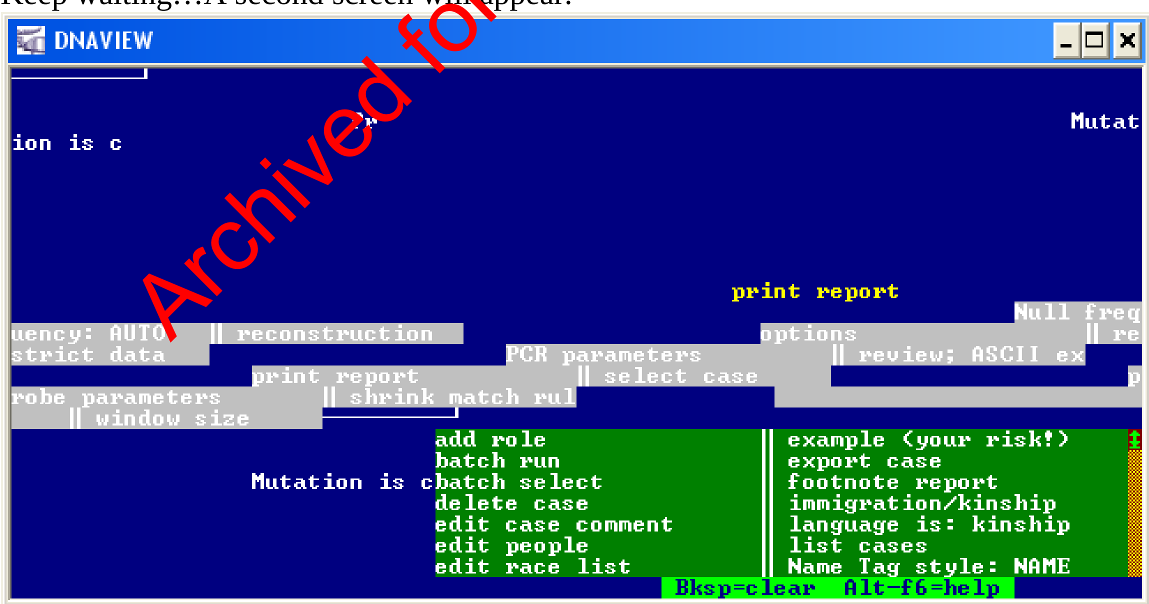
20. Select Laserjet and hit **Enter**.



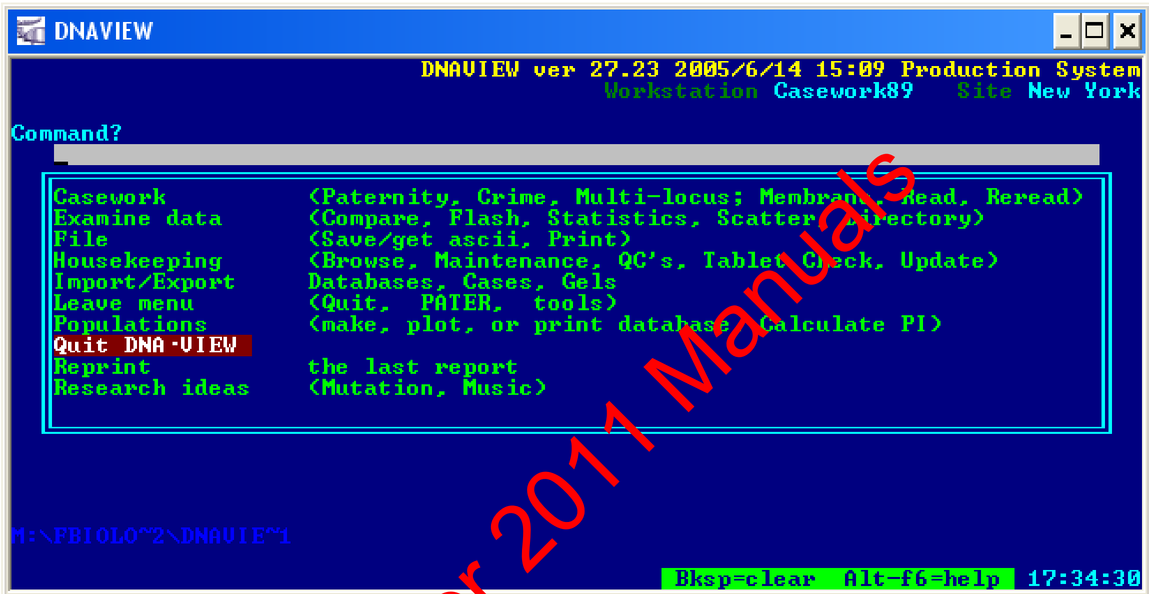
21. The following screens will appear. Just wait for the file to print.



Keep waiting…A second screen will appear.



22. After you obtain printed report, hit **Ctrl+C** to get back to the main menu. Select **Quit DNA-VIEW** and hit **Enter**. If report is not printing, see Section IV for troubleshooting.



IV. Importing Raw Data

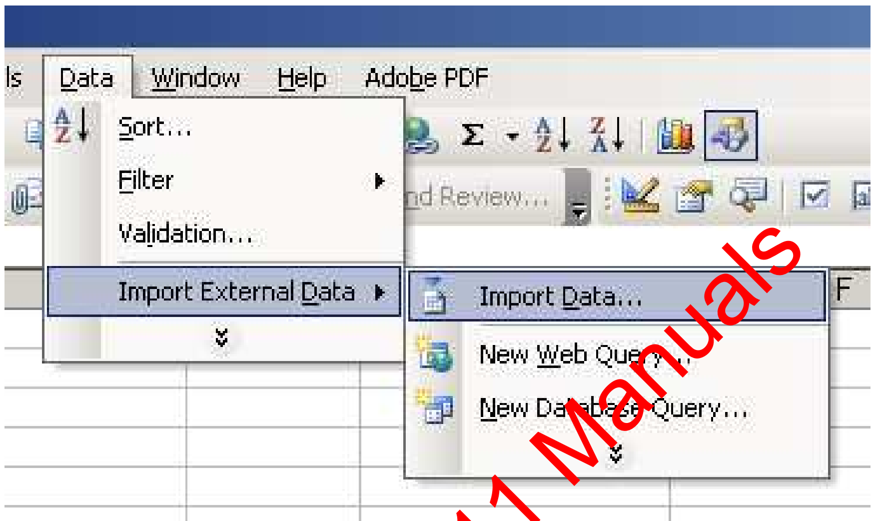
The next step is to convert the raw data to a format that is easier to read and can be pasted into a report. You also have the option to type in the raw data into your report tables by hand.

1. Open the workbook you saved earlier. It can be found in the **DNAVIEW \ WRKST** folder.

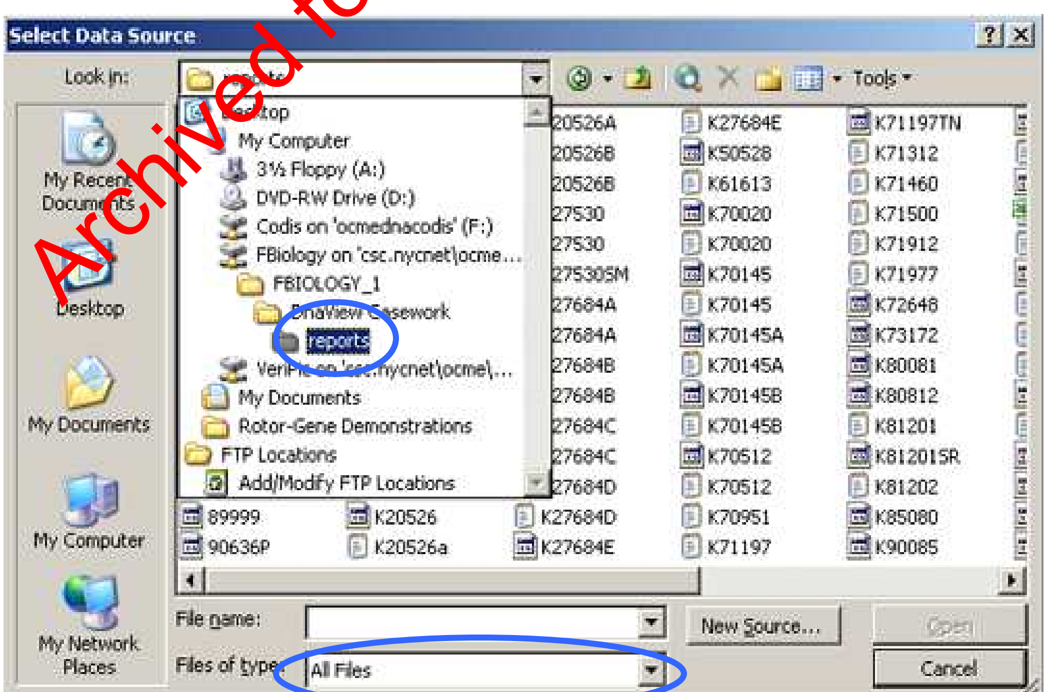
2. Click on the **Paste Report** tab at the bottom of the worksheet

3. Select cell **A1**. Failure to select this cell may lead to improper results.

4. From the top menu, select Data → Import External Data → Import Data

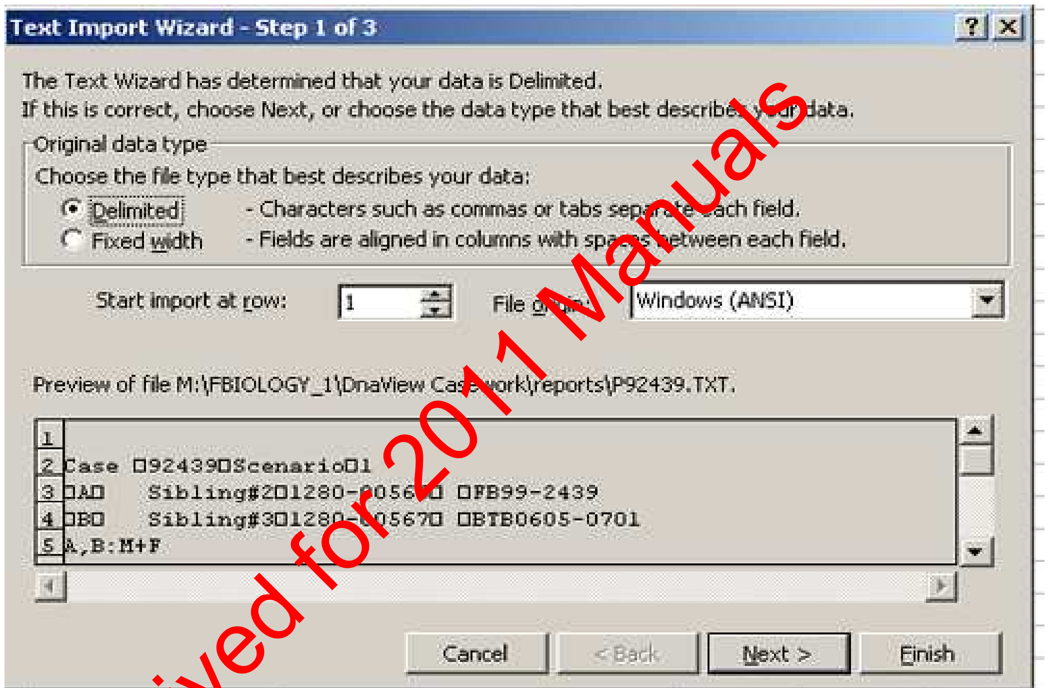


5. Select the **FBIOLOGY_1 / DnaView Casework / reports** folder from the **Look in:** menu

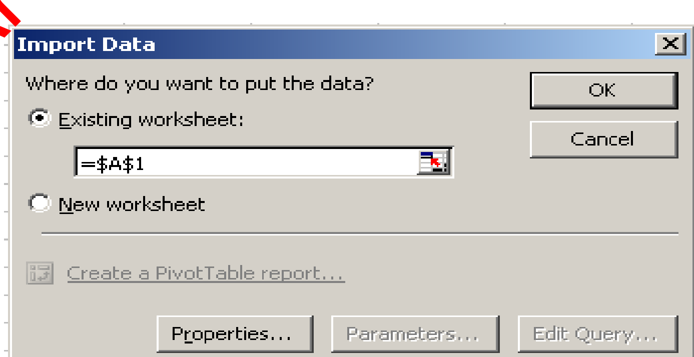


6. This folder contains the ASCII file you saved in Section III Step 17. Change the **Files of** type select **All** Files. Select the file and click **Open**.

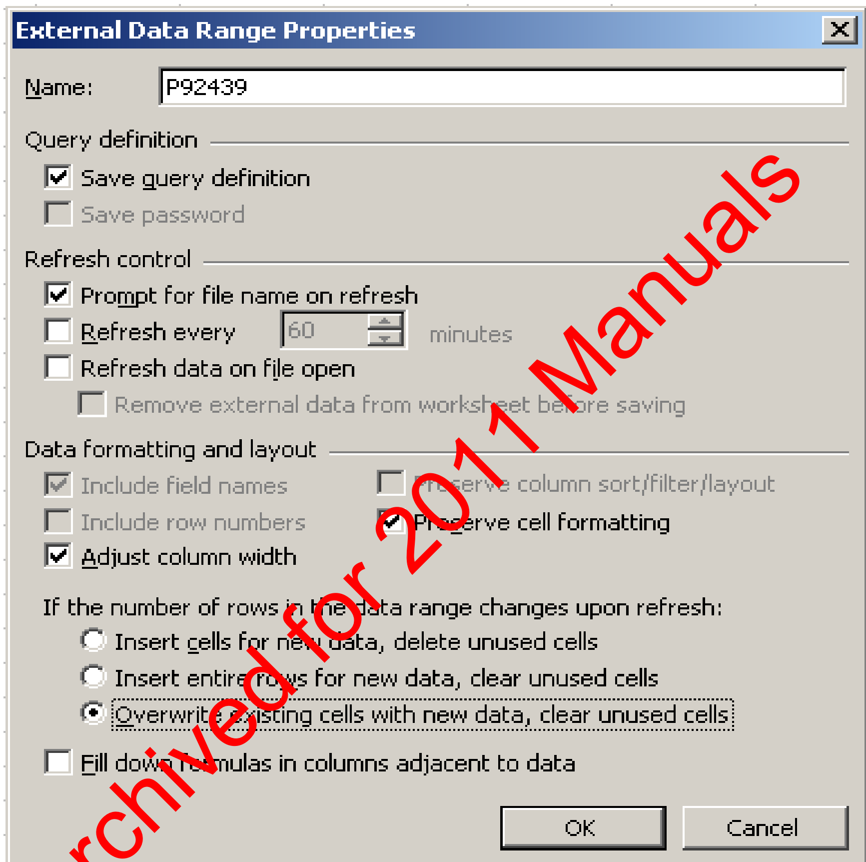
7. The **Text Import Wizard** window will appear. The default settings should be as seen above, correct them if they are not, and click **Finish**.



8. The **Import Data** window will appear. Select **Properties…**



9. The default settings in the **External Data Range Properties** window are correct but you need to select **Overwrite existing cells with new data, clear unused cells**. When the window has the settings shown above click **OK.**



10. You will be taken back to the **Import Data** window. Make sure **Existing worksheet** is selected and the window below it has **=$A$1**. Click **OK.**

11. The raw data has now been imported and your worksheet should look something like this:

	A	B	C	D	E	F	G	H
1								
2	Case	90439	Scenario	1				
3		V	Victim	1280-00819		humerus		
4		M	Mother	1280-00821		Jane Doe		
5		F	Father	1280-00820		John Doe		
6	V/other:M+F							
7		------------------------------						
8		Black	cumulative LR	1.02E+17	Posterior probability=	100%	assuming prior=	50%
9		Caucasian	cumulative LR	1.03E+14	Posterior probability=	100%	assuming prior=	50%
10		Hispanic	cumulative LR	2.25E+14	Posterior probability=	100%	assuming prior=	50%
11		Asians	cumulative LR	6.28E+14	Posterior probability=	100%	assuming prior=	50%
12		------------------------------						
13	---	Black				M	V	F
14		D8S1179 STR	40.7	1 / 2pp	p=0.111	12 13	12	12
15		D21S11 STR	17.7	1 / 4ap	p=0.181 a=0.078	31û2 32û2	30 31û2	30
16		D7S820 STR	32.3	1 / 8ap	p=0.325 a=0.0[illegible]	10 12	10 10û3	10 10û3
17		CSF1PO STR	14.5	1 / 4pq	p=0.302 q=0.0[illegible]	12 13	12 13	12
18		D3S1358 STR	11.7	1 / pp	p=0.292	15	15	15
19		TH01 STR	15.9	1 / 4ar	r=0.1[illegible] a=0.107	9	9 9û3	7 9û3
20		D13S317 STR	17	1 / 8pr	p=0.030[illegible] r=0.24	9 12	9 11	9 11
21		D16S539 STR	2.65	1 / 4rr	r=0.296	11 12	11	9 11
22		D2S1338 STR	24.3	1 / 4ss	s=0.101	23 25	23	20 23
23		D19S433 STR	15	1 / 8qr	q=0.2 r=0.0418	13 15	14 15	13 14
24		VWA STR	5.65	1 / 4pr	p=0.238 r=0.186	15 16	15 17	17
25		TPOX STR	3.65	1 / 2pp	p=0.37	8 11	8	8
26		D18S51 STR	1[illegible].9	1 / 8rv	r=0.0582 v=0.166	11 13	13 17	15 17
27		D5S818 STR	14.4	1 / pp	p=0.263	11	11	11
28		FGA STR	23.8	1 / 4rr	r=0.102	23 25	25	24 25
29		cumulative LR	1.02E+17					
30		------------------------------						
31	---	Caucasian				M	V	F
32		D8S1179 STR	23	1 / 2pp	p=0.148	12 13	12	12
33		D21S11 STR	10.5	1 / 4ap	p=0.234 a=0.102	31û2 32û2	30 31û2	30
34		D7S820 STR	34.8	1 / 8ap	p=0.292 a=0.0123	10 12	10 10û3	10 10û3
35		CSF1PO STR	10.4	1 / 4pq	p=0.327 q=0.0737	12 13	12 13	12
36		D3S1358 STR	16.2	1 / pp	p=0.248	15	15	15
37		TH01 STR	4.87	1 / 4ar	r=0.167 a=0.307	9	9 9û3	7 9û3
38		D13S317 STR	4.94	1 / 8pr	p=0.0789 r=0.321	9 12	9 11	9 11
39		D16S539 STR	3.33	1 / 4rr	r=0.274	11 12	11	9 11
40		D2S1338 STR	13.2	1 / 4ss	s=0.138	23 25	23	20 23
41		D19S433 STR	2.69	1 / 8qr	q=0.338 r=0.138	13 15	14 15	13 14
42		VWA STR	8.25	1 / 4pr	p=0.115 r=0.265	15 16	15 17	17
43		TPOX STR	1.68	1 / 2pp	p=0.545	8 11	8	8
44		D18S51 STR	6.35	1 / 8rv	r=0.125 v=0.158	11 13	13 17	15 17
45		D5S818 STR	5.90E+00	1 / pp	p=0.412	11	11	11
46		FGA STR	49.3	1 / 4rr	r=0.0712	23 25	25	24 25
47		cumulative LR	1.03E+14					

Allele Entry / Paste Report / Table

12. Click on the **Table** tab at the bottom, and you will see a cleaned up version of the data you just imported:

Import OK | Data OK

Cumulative Likelihood Ratios

B	[illegible]	100 quadrillion	
C	[illegible]	100 trillion	<- most conservative
H	[illegible]	220 trillion	
A	[illegible]	620 trillion	

Posterior Probability (assuming prior probability = 50%)

B	100.00%
C	100.00%
H	100.00%
A	100.00%

Sample	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	AMEL	D5S818	FGA
Jane Doe mother	12, 13	30.2, 31.2	10, 12	12, 13	15	9	8, 12	11, 12	23, 25	13, 15	[illegible]	[illegible]	11, 13	X	11	23, 25
Unknown victim	12	30, 31.2	10, 10.3	12, 13	15	9, 9.3	9, 11	11	23	14, 15	[illegible]	8	13, 17	X, Y	11	23
John Doe (alleged) father	12	30	10, 10.3	12	15	7, 9.3	9, 11	9, 11	20, 2[illegible]	[illegible]	17	8	15, 17	X, Y	11	24, 25
Paternity Index - Black	[illegible]	17.7	32.3	14.5	11.7	15.9	17	2.85	24.3	15	5.65	5.65	12.9	-	14.4	23.8
Paternity Index - Caucasian	[illegible]	10.5	34.8	10.4	16.2	4.87	4.94	3.33	13.2	2.69	8.25	1.68	6.35	-	5.9	49.3
Paternity Index - Hispanic	40.2	9.38	28	14.5	8.13	6.78	4.91	3.82	15.7	3.15	8.1	2.55	7.75	-	10.5	17
Paternity Index - Asian	15.8	12.5	34.8	9.81	6.95	9.14	3.27	3.91	6.5	6.32	36.3	1.98	14.2	-	9.07	30.3

This table has sorted the data you provided in the **Allele Entry** tab, as well as the raw data from DNA-View, into a format that is easy to read.

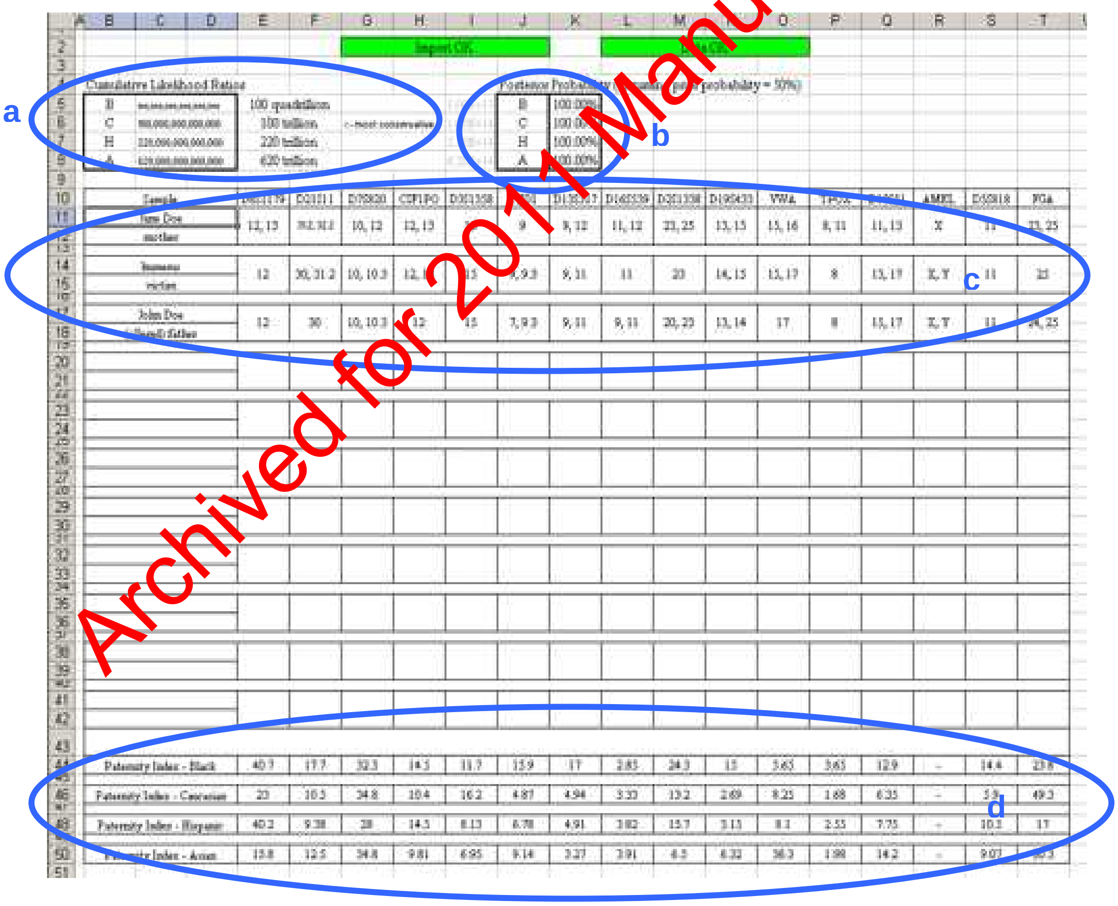
13. The top of the sheet has two indicators which let you know the status of the import and the data.
    a. **No data imported –** Data has not been imported
    b. **Import OK** – The import was successful
    c. **Data OK –** The order of the loci in the imported data is usable

d. The following two errors are common when older files are imported:

- **Imported data not in correct order** – Data has been imported but the order of the loci in the report is not in the correct order to use this table.
- **Imported data is in Co Pro order** - Data has been imported but the order of the loci in the report is in Co Pro order.

Create a new report in DNA-View to fix this problem.

14. The rest of the table contains all of the information from the DNA-View report.



a. **Cumulative Likelihood Ratios –** listed numerically and with words. The most conservative (lowest) value is indicated. Values are truncated at two significant figures.

b. **Posterior Probability –** listed to two decimal places

c. **Allele table –** names, loci and alleles listed in FBio report format

d. **Paternity/Kinship Index Table –** the paternity/kinship indices of each locus' genotype is listed below the locus for four major races

15. The allele table and paternity/kinship index table can be copied and pasted directly into the table of the report template. Blank rows should be omitted from the copy. Adjust wording from paternity to kinship as necessary.

V. Troubleshooting DNA-View

1. **Printing problems**

a. Re-establish communication between DNA-View and the printer

1) Go to **My Computer** from the Start menu or the desktop icon.

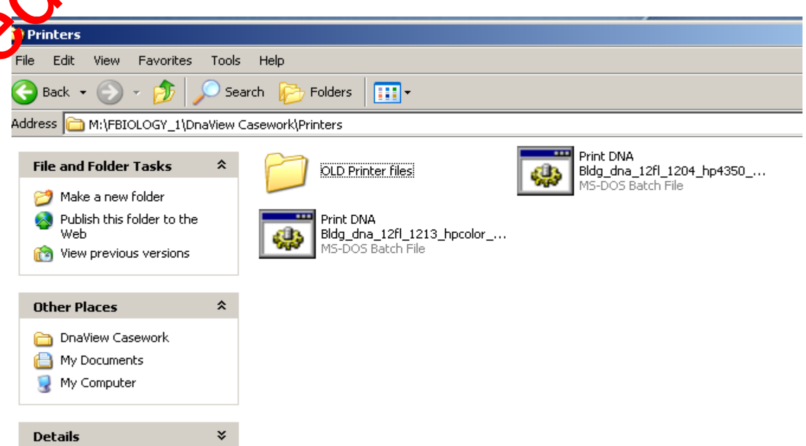
2) Double click on **M:** drive.

3) Double click on **FBiology_1** folder.

4) Double click on the **DnaView Casework** folder.

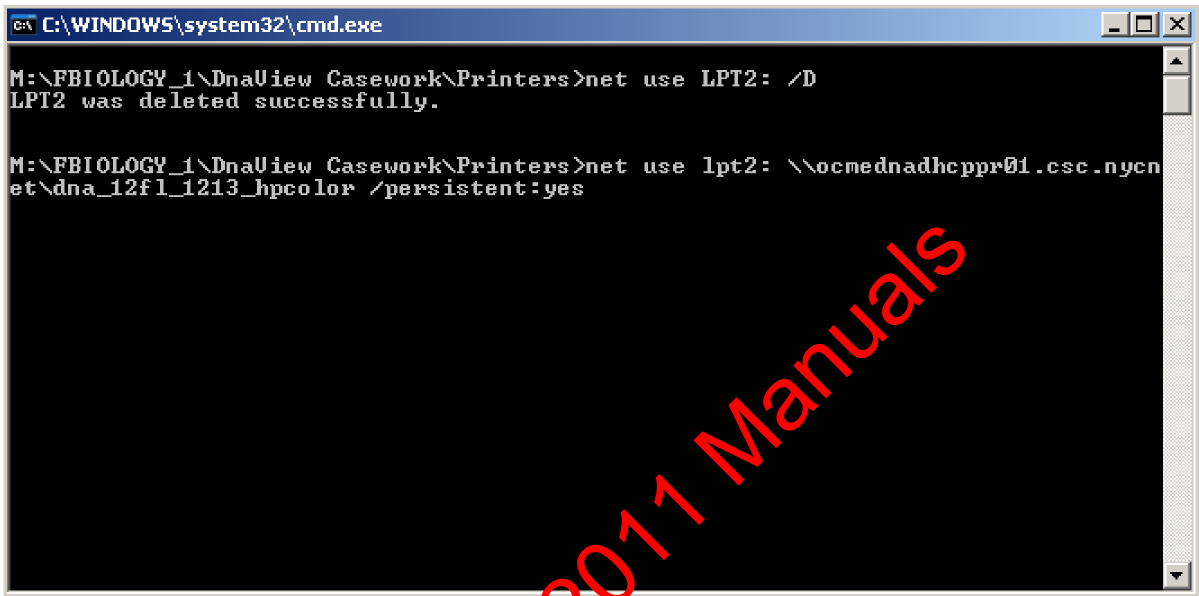
5) Double click on the **Printers** folder.

6) A list of MS-DOS batch files appears similar to those depicted below:



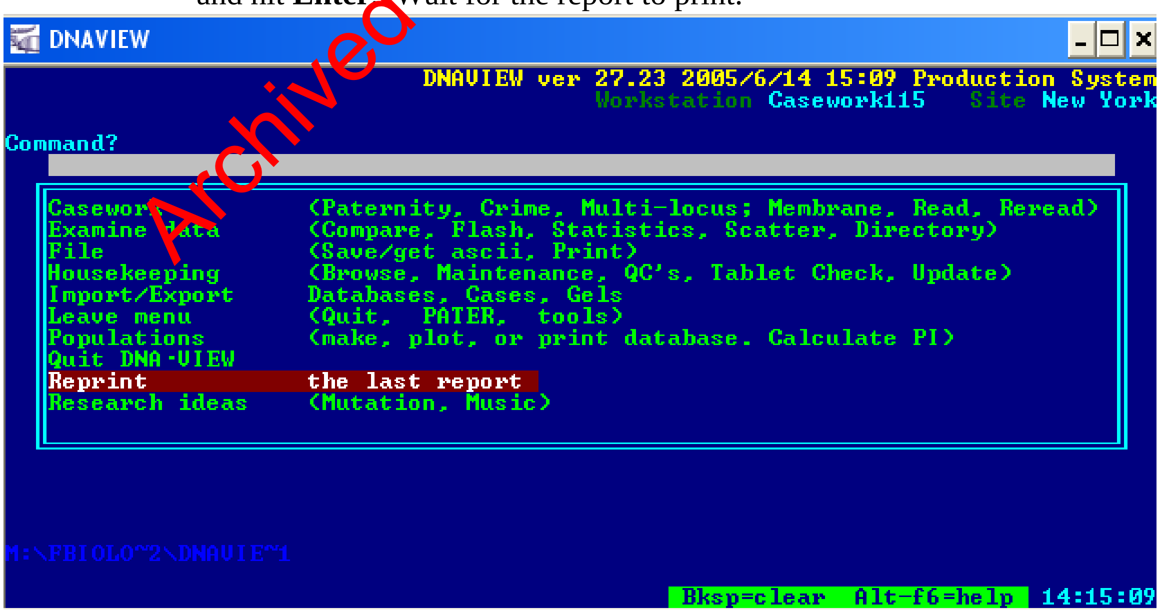
7) Double click on the file that corresponds with your printer. (i.e., If you are trying to print to the printer on the 12th flr, click on **Print DNABldg_dna_12fl_1204_hp4350_LPT2**)

8) A black screen will appear and disappear quickly, this is normal. See below:

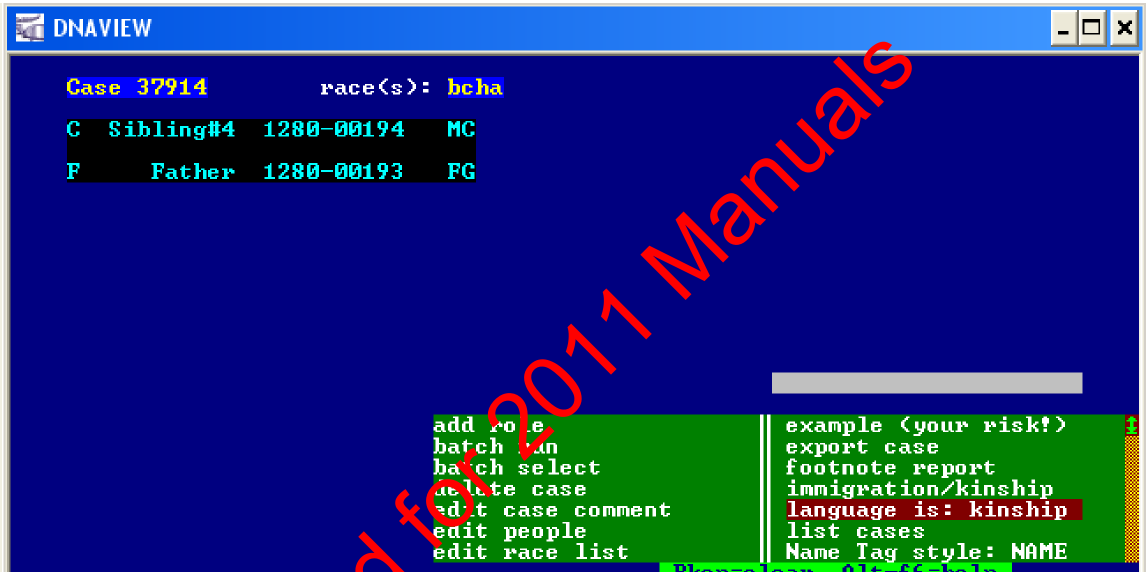


b. Communication has now been established successfully and printing should work.

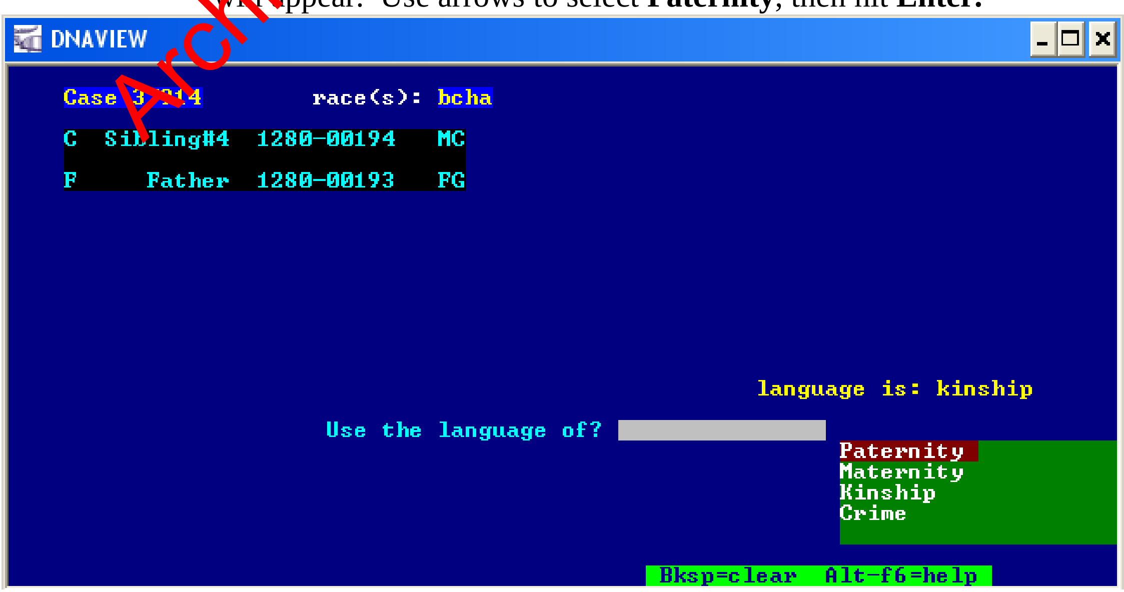
c. Go back to DNA View. In the main menu, select **Reprint the last report** and hit **Enter**. Wait for the report to print.



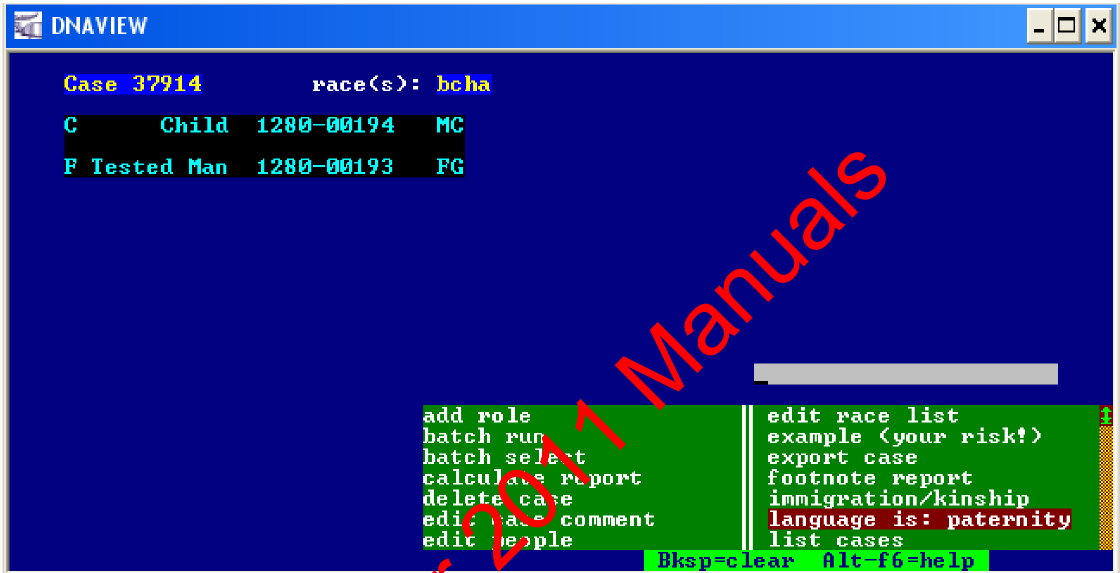
2. **Changing Language from Kinship to Paternity**
   a. This is useful for paternity cases where **C** is indicated as **Sibling #4**, instead of **Child** and **F** is indicated as **Father** instead of **Tested Man**
   b. Change case language from **kinship** to **paternity**
      - After **selecting case** in step III.3., a menu will appear. Use arrows to select **language is: kinship**. Hit **Enter.**



      - A field will appear that says **Use the language of?** and four options will appear. Use arrows to select **Paternity**, then hit **Enter.**



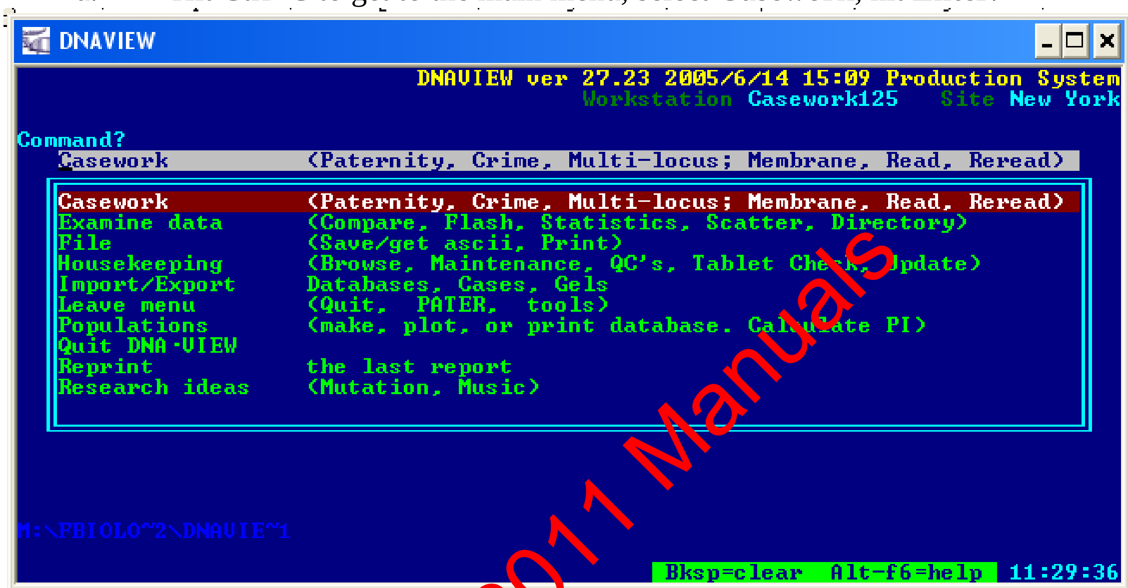
- Relationships have now been changed from **Sibling #4** to **Child** and **Father** to **Tested Man**.



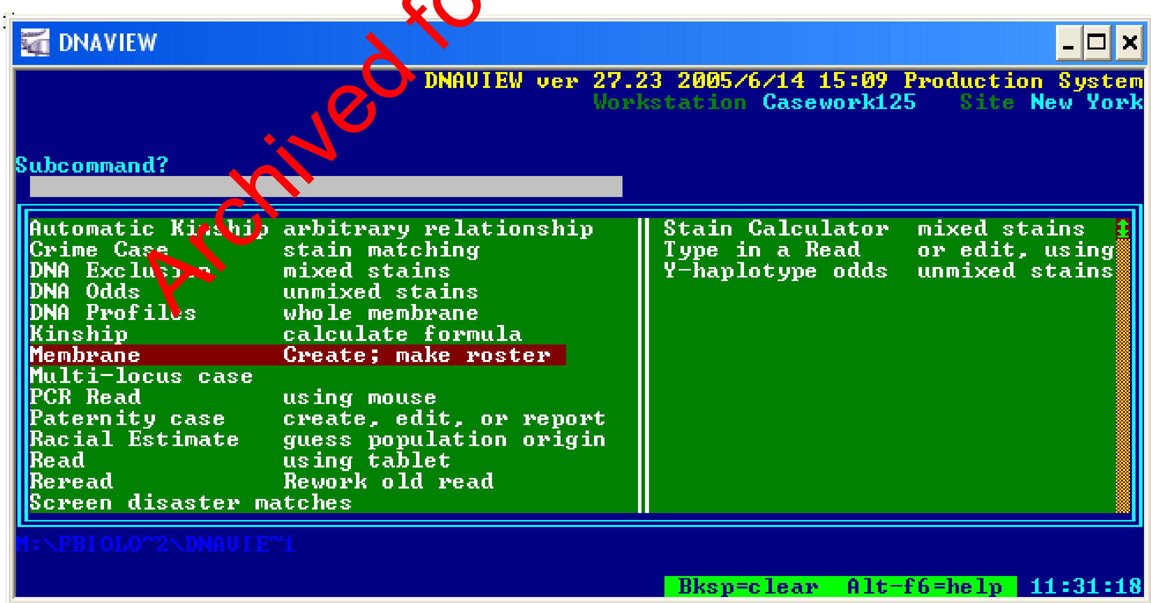
- Language will now be changed to **paternity** until the next user changes it to **kinship**.

3. **Deleting records from DNA-View (in case of import problems, etc.)**

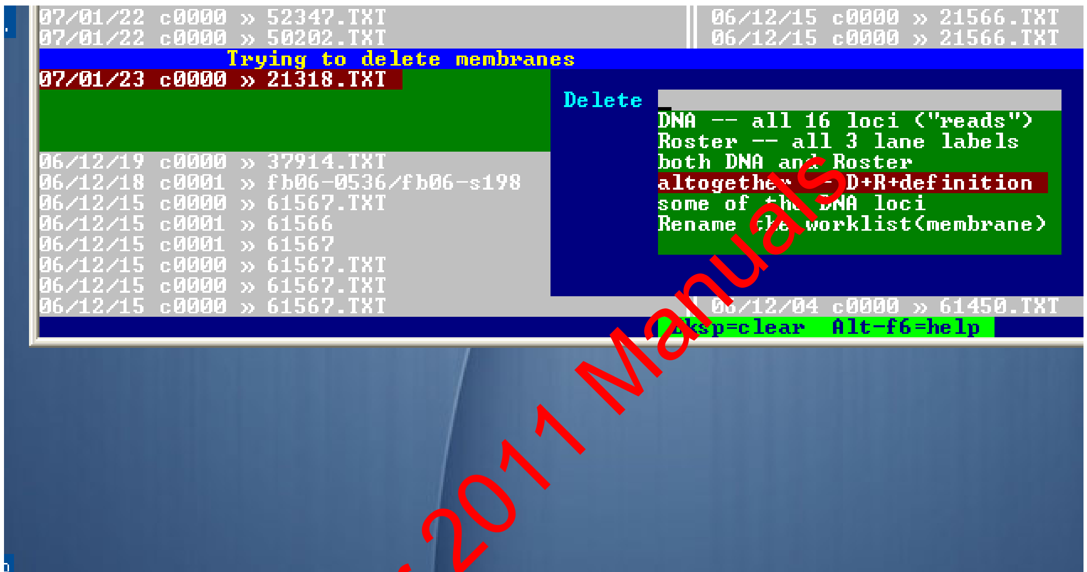
a. Hit Ctrl+C to get to the main menu, select **Casework**, hit **Enter**.



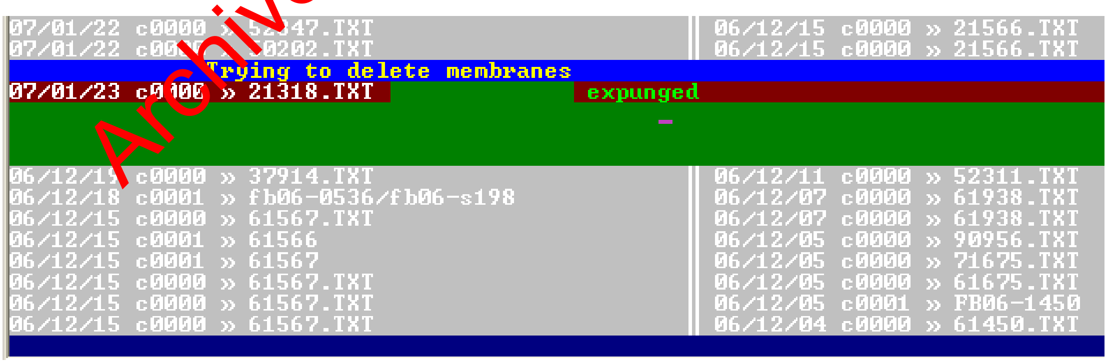
b. Select **Membrane**, hit **Enter.**



c. Use arrows to highlight case that you want to delete, hit **Delete**. Screen will say **Trying to delete** membranes. A list will appear with a blank field that says **Delete**, select **altogether-- D +R+ definition**, hit **Enter**.



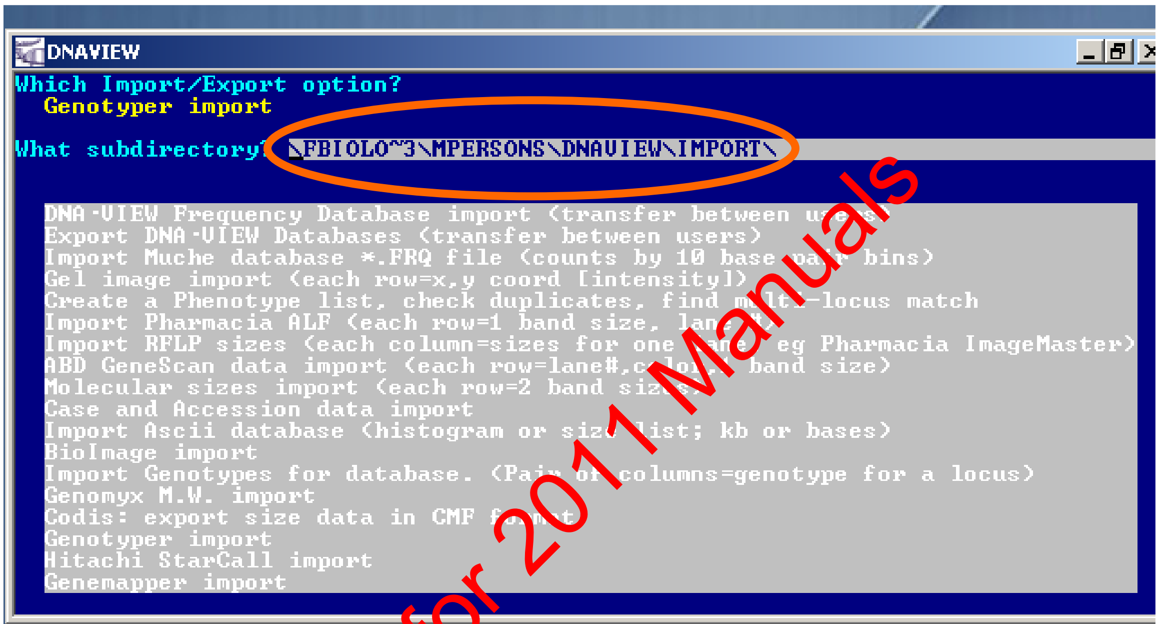
d. Wait for data to be deleted. When successful, a screen that says **Trying to delete membranes** (highlighted in blue) and **expunged** (in green) will appear, then disappear quickly.



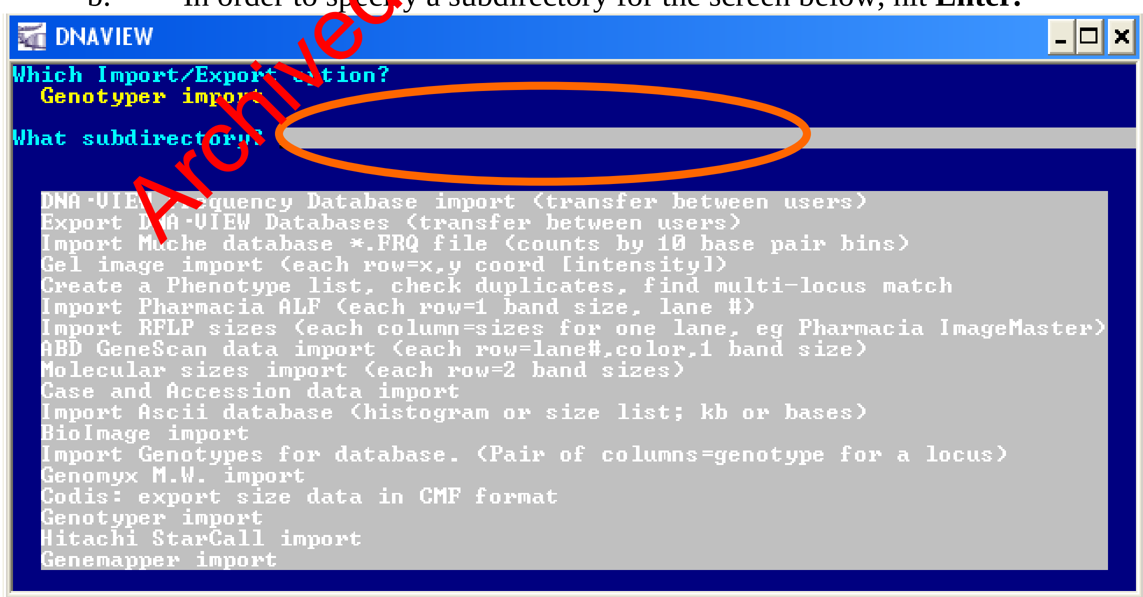
e. The import list will then display (not pictured). The case that was deleted will no longer be in the import list. Hit **Esc** or **Ctrl-C** to get back to the main menu.

4. **Designating a subdirectory if the subdirectory field is blank**

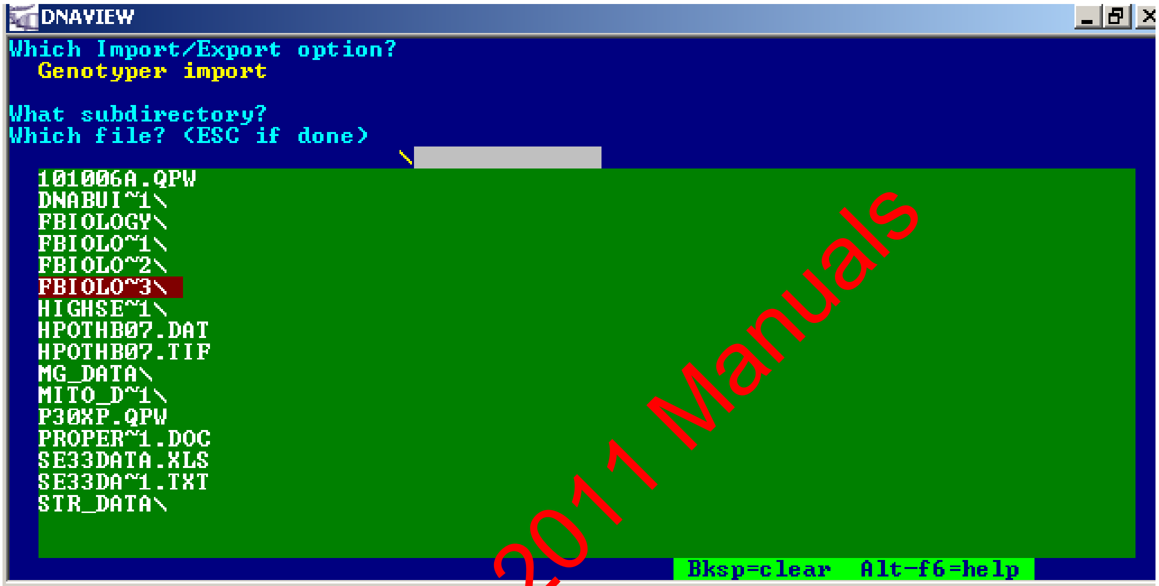
a. Normally, the subdirectory field contains the following pathway:



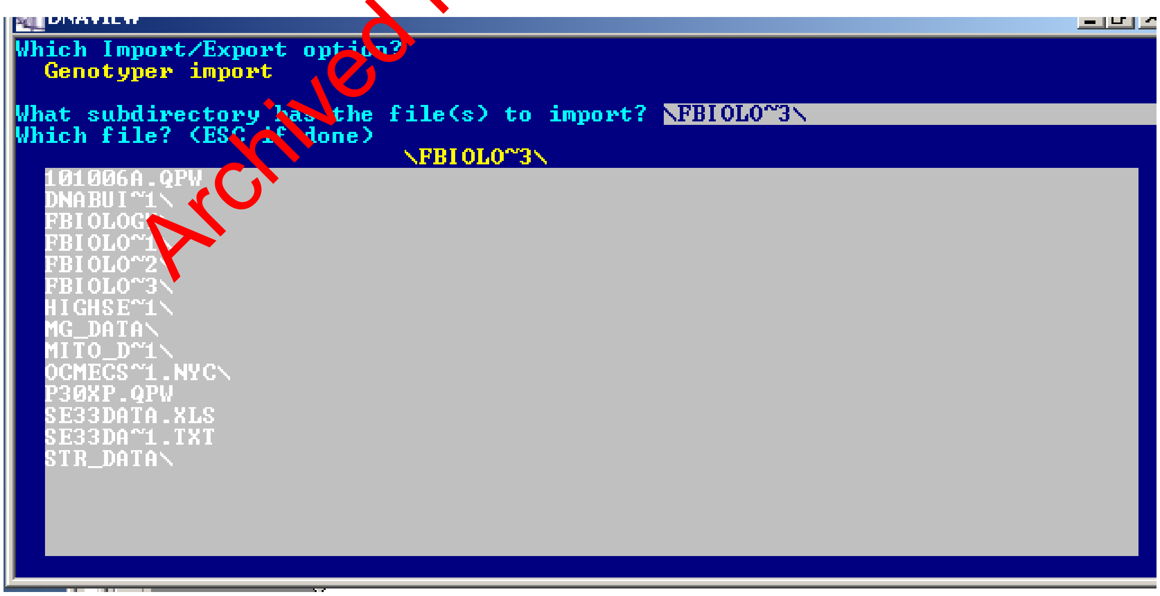
b. In order to specify a subdirectory for the screen below, hit **Enter.**



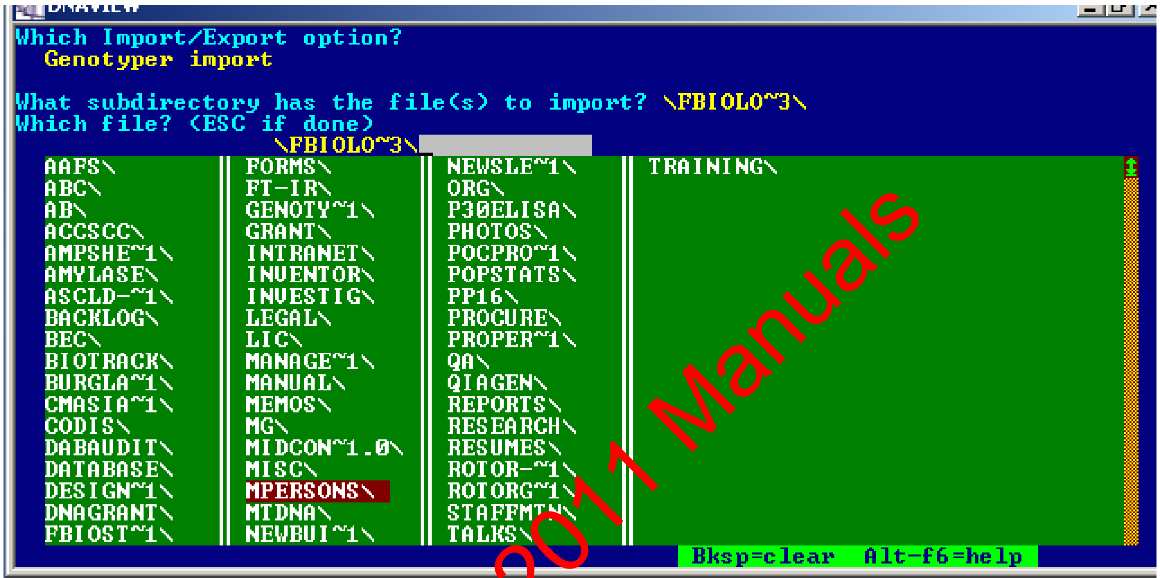
c. On the next screen, a list of folders will appear. You will be asked **Which file? (Esc if done)** Select **FBIOLO~3** from the list. Then hit **Enter**.



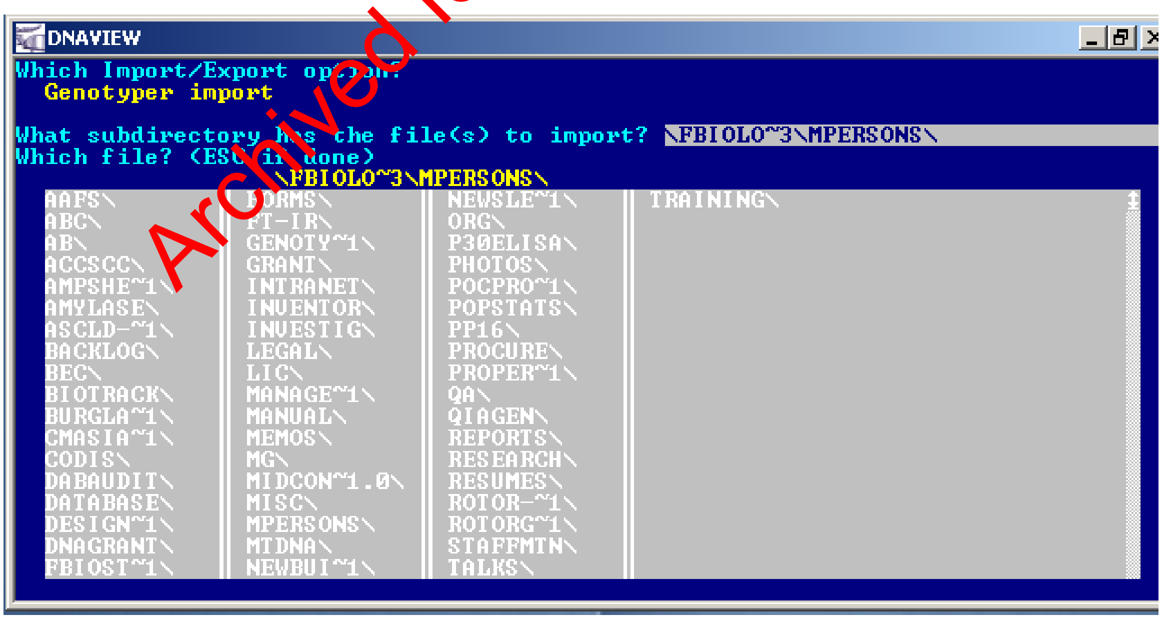
d. At this screen, hit **Esc**.



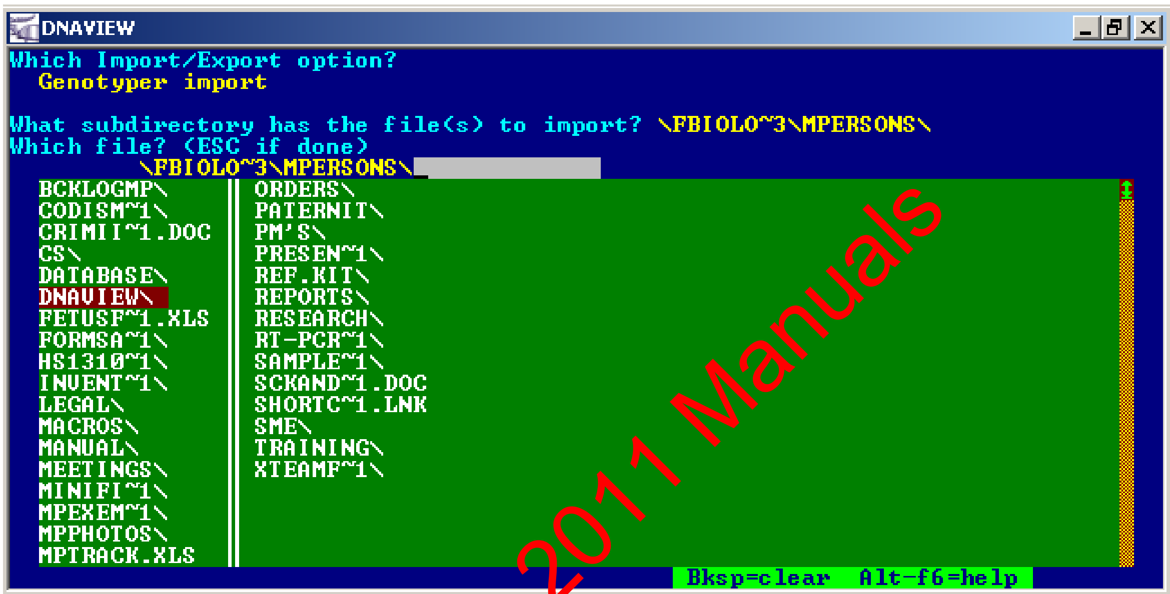
e. A list of folders contained in the main Forensic Biology folder will appear. Select **MPERSONS** and then hit **Enter**.



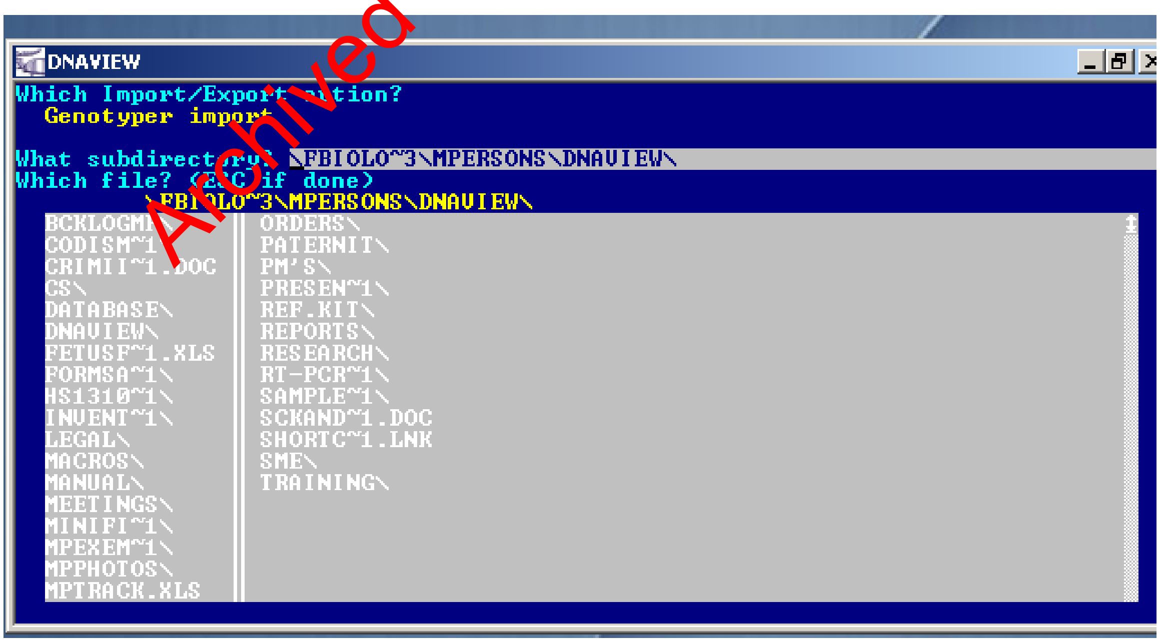
f. This folder has now been added to the path. Hit **Esc**.



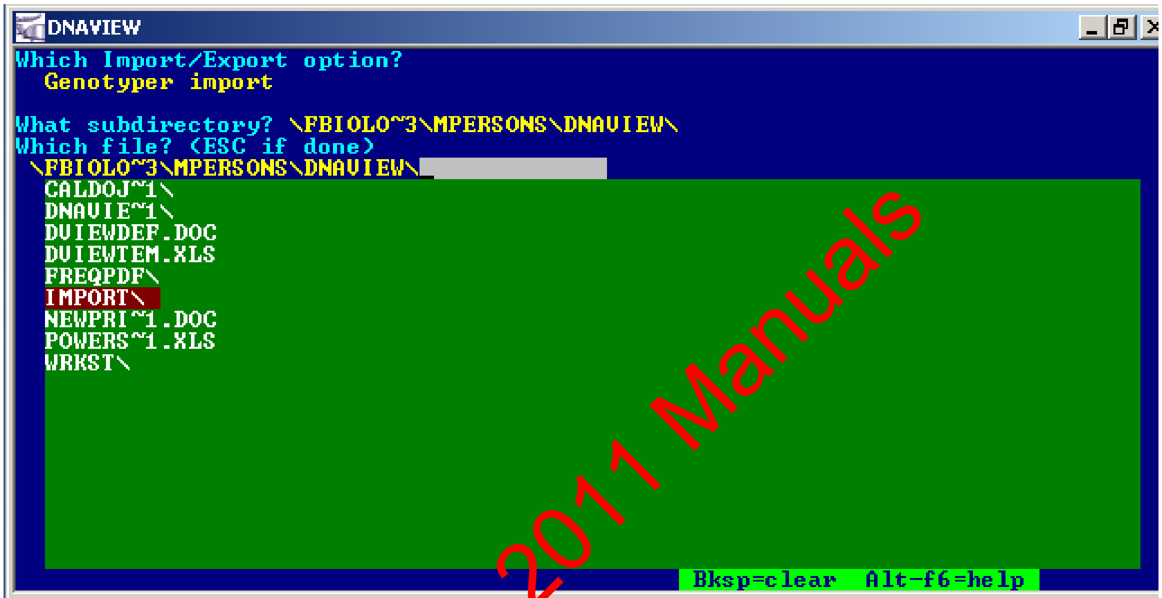
g. A list of folders in the MPersons folder will appear. Select **DNAVIEW** then hit **Enter**.



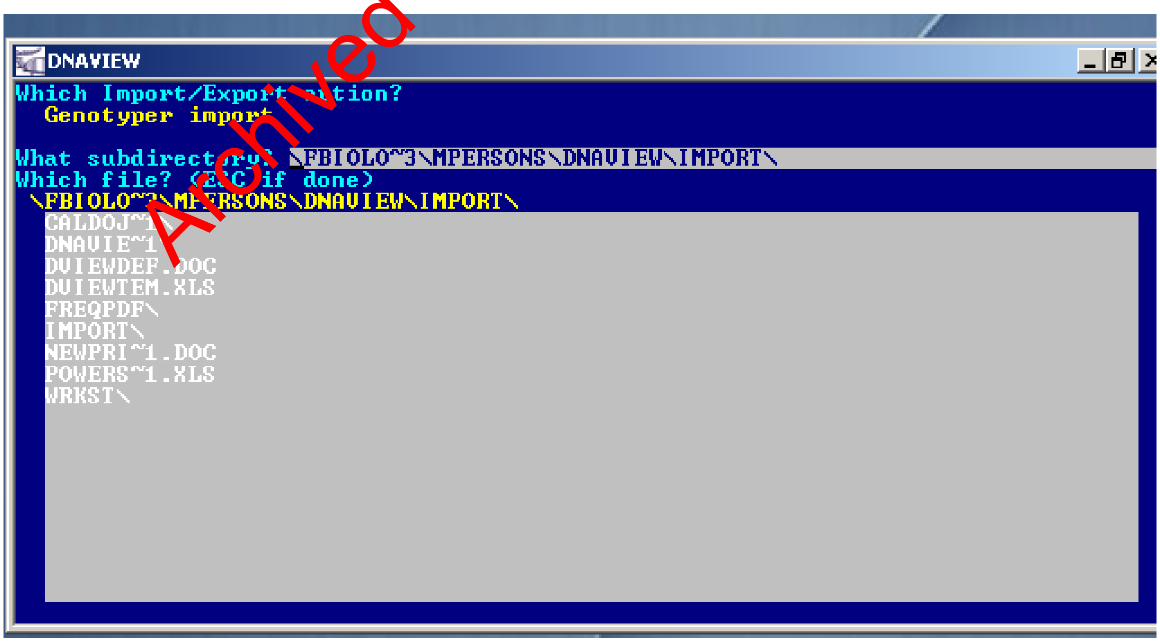
h. This folder has now been added to the path. Hit **Esc**.



i. A list of folders in the DNAVIEW folder appears. Select **IMPORT**\ and hit **Enter**.



j. This folder has now been added to the path. Hit **Esc**.



k. The folder has now been added and the subdirectory path is complete. It will be automatically saved by the program. Hit **Esc**. Hit **Esc** again to return to the main menu.

5. **Interpretation of DNA-View Report**

Page 1 features (see sample next page):

a. Case #
b. Sample names with one letter relation code (i.e., M), relationship (i.e., mother), unique identifier, typed subject's name
c. DNA profiles. Alleles are displayed in letter format. The letters are decoded in succeeding legend.
d. Likely relationships table displays paternity and sibling indices (PI and SI) to numerically evaluate plausible relationships between each tested subject
e. Kinship/Paternity scenario contains the tested assumption and an alternate hypothesis
f. LR/CPI/CKI is cumulative likelihood ratio (also known as combined paternity index or combined kinship index) or the genetic odds in favor of paternity or kinship. This number will be indicated in Forensic Biology paternity and kinship reports for all 4 races (Blacks, Caucasians, Hispanics, and Asians).
g. Posterior and prior probabilities. Posterior probability is also known as the relative chance of paternity. Prior probability is always 50% (meaning that both hypotheses are equally plausible). Both relative chance of paternity and prior probability are indicated in Forensic Biology paternity reports.

Office of the Chief Medical Examiner -- DNA·VIEW Casework 2

Casework77 -- DNAVIEW ver 27.23 2005/6/14 15:09

```
Case 61494 kinship analysis

        D   Daughter  1280-00126   daughter Shirley Profitt
        V     Victim  1280-00125   femur
        S      Son#1  1280-00127   son Micheal Yellock
        T      Son#2  1280-00128   son Raymond Yellock

Genotype patterns are:
D3S  VWA   FGA  Ame  D8S  D21  D18S  D5S  D13S  D7S  D16S  TH0  TPOX   CSF1
 qr D pr  D pq D x  D pr D p  D pr  D ps D p r D  s D p t D pq D  q t D pr
pq  V  rs V p  V x  V pr V pr V  ru V  s V pq  V ps V pr  V pq V pq   V p t
 qr S  rs S p  S xy S  r S p  S  r  S ps S p r S ps S p t S  q S p r  S pr
 qr T  rs T pq T xy T pr T pr T  r  T ps T  q  T  s T  rt T pq T p  t T  rt
 Legend:   D3S1358 p=15 q=16 r=17   VWA p=13 r=15 s=16   FGA p23 q24
  D8S1179 p12 r14    D21S11 p28 r30    D18S51 p14 r16 u19    D5S818 p8 s11
  D13S317 p11 q12 r13    D7S820 p8 s11    D16S539 p9 r11 t13    TH01 p7 q8
  TPOX p7 q8 r9 t11    CSF1PO p8 r10 t12

PLAUSIBLE RELATIONSHIP CHART
  pi,si,id are paternity, sibling, and identity indices.
    V      S         T

  D pi=600 pi=200    pi=40
    si=30  si=100000 si=8000

  V        pi=60000  pi=8000
           si=6000   si=2000

  S                  pi=600
                     si=10000

**** Kinship scenario
D,S,T:V/Other+Pa

D,S,T:V/Other+Pa

Black     cumulative LR        41.6e6
          Posterior probability=99.999998% assuming prior=50%
Caucasian cumulative LR        12.7e9
          Posterior probability=100% assuming prior=50%
Hispanic  cumulative LR        4.33e9
          Posterior probability=99.99999998% assuming prior=50%
Asians    cumulative LR        20.8e9
          Posterior probability=100% assuming prior=50%
```

a. case #

b. sample names

c. Profiles in letter format with legend below

d. Likely Relationships Table, also known as Plausible Relationship Chart

e. Kinship/Paternity scenario

f. LR/CPI/CKI

g. Posterior and prior probabilities

Page 2 (see sample below):

```
Case 61494 kinship analysis                                      2006/9/22 16:

Black
D3S1358 3p STR       0.396    (1+3r) / (4q+12qq+12qr+48qqr)    q=0.309 r=0.202
VWA 12p13.3 STR      1.01     (r+s) / (4rs+16rrs)               r=0.238 s=0.271
FGA 4q STR           9.99     2 / (p+3pp+pq)                    p=0.127 q=0.188
D8S1179 STR          7.44     (1+3p+3r) / (4pr+4ppr+12prr)      p=0.111 r=0.335
D21S11 STR           7.05     (1+3p+r) / (4pr+12ppr)            p=0.217 r=0.181
D18S51 18q21.33 STR  0.831    1 / (4r+12rr)                     r=0.191
D5S818 STR           4.16     (2+6p) / (s+3ps+3ss+12pss)        p=0.0526 s=0.263
D13S317 STR          2.15     1 / 4pq                           p=0.24 q=0.485
D7S820 7q11 STR      6.96     (1+p+3s) / (4ps+12pss)            p=0.176 s=0.226
D16S539 16q24 STR    3.79     (1+3t) / (4pr+16prt)              p=0.2 r=0.296
                                                                t=0.167
TH01 11p15.5 STR     4.34     (1+3p+3q) / (4pq+4ppq+12pqq)      p=0.442 q=0.1[illegible]8
TPOX 2p25-p24 STR    28.3     1 / 4pq                           p=0.0239 q=[illegible]
CSF1PO 5q33-34 STR   8.2      (1+3r) / (4pt+16prt)              p=0.0879 r=[illegible].273
                                                                t=0.30[illegible]
cumulative LR        41.6e6
Posterior probability=99.999998% assuming prior=50%

Caucasian
D3S1358 3p STR       0.598    (1+3r) / (4q+12qq+12qr+48qqr)    q=0.233 r=0.214
VWA 12p13.3 STR      2.34     (r+s) / (4rs+16rrs)               r=0.115 s=0.204
FGA 4q STR           7.7      2 / (p+3pp+pq)                    p=0.16 q=0.14
D8S1179 STR          9.72     (1+3p+3r) / (4pr+4ppr+12prr)      p=0.148 r=0.204
D21S11 STR           7.35     (1+3p+r) / (4pr+12ppr)            p=0.168 r=0.234
D18S51 18q21.33 STR  1.72     1 / (4r+12rr)                     r=0.109
D5S818 STR           2.16     (2+6p) / (s+3ps+3ss+12pss)        p=0.0128 s=0.412
D13S317 STR          2.51     1 / 4pq                           p=0.321 q=0.31
D7S820 7q11 STR      8.21     (1+p+3s) / (4ps+12pss)            p=0.165 s=0.204
D16S539 16q24 STR    7.74     (1+3t) / (4pr+16prt)              p=0.106 r=0.274
                                                                t=0.165
TH01 11p15.5 STR     13.7     (1+3p+3q) / (4pq+4ppq+12pqq)      p=0.174 q=0.128
TPOX 2p25-p24 STR    37.3     1 / 4pq                           p=0.0123 q=0.545
CSF1PO 5q33-34 STR   54.4     (1+3r) / (4pt+16prt)              p=0.0123 r=0.256
                                                                t=0.327
cumulative LR        12.7e9
Posterior probability=100% assuming prior=50%

Hispanic
D3S1358 3p STR       0.411    (1+3r) / (4q+12qq+12qr+48qqr)    q=0.302 r=0.2
VWA 12p13.3 STR      1.76     (r+s) / (4rs+16rrs)               r=0.134 s=0.295
FGA 4q STR           8.41     2 / (p+3pp+pq)                    p=0.151 q=0.125
D8S1179 STR          12.7     (1+3p+3r) / (4pr+4ppr+12prr)      p=0.111 r=0.2
D21S11 STR           1[illegible].3     (1+3p+r) / (4pr+12ppr)            p=0.128 r=0.22
D18S51 18q21.33 STR  1.46     1 / (4r+12rr)                     r=0.125
D5S818 STR           3.32     (2+6p) / (s+3ps+3ss+12pss)        p=0.0328 s=0.308
D13S317 STR          3.24     1 / 4pq                           p=0.243 q=0.318
D7S820 7q11 STR      6.85     (1+p+3s) / (4ps+12pss)            p=0.151 s=0.262
D16S539 16q24 STR    4.35     (1+3t) / (4pr+16prt)              p=0.203 r=0.256
                                                                t=0.154
TH01 11p15.5 STR     10       (1+3p+3q) / (4pq+4ppq+12pqq)      p=0.239 q=0.134
TPOX 2p25-p24 STR    34.5     1 / 4pq                           p=0.0164 q=0.443
CSF1PO 5q33-34 STR   33.6     (1+3r) / (4pt+16prt)              p=0.0197 r=0.285
                                                                t=0.328
cumulative LR        4.33e9
Posterior probability=99.99999998% assuming prior=50%
```

← Allele frequencies as per OCME STR database (Hispanics, Asians), FBI database (Blacks, Caucasians) JFS 1999; 44(6): 1277-1286

Kinship equations

Individual LRs for each locus, all multiplied together to give cumulative LR (CPI/CKI) which goes into FBIO report

Page 3 (see sample below):

```
Case 61494 kinship analysis                                    2006/9/22 16:2

Asians
D3S1358 3p STR      0.389    (1+3r) / (4q+12qq+12qr+48qqr)   q=0.312 r=0.217
VWA 12p13.3 STR     9.43     (r+s) / (4rs+16rrs)             r=0.0277 s=0.174
FGA 4q STR          6.47     2 / (p+3pp+pq)                  p=0.178 q=0.206
D8S1179 STR         9.54     (1+3p+3r) / (4pr+4ppr+12prr)    p=0.178 r=0.178
D21S11 STR          18.9     (1+3p+r) / (4pr+12ppr)          p=0.0632 r=0.253
D18S51 18q21.33 STR 1.15     1 / (4r+12rr)                   r=0.15
D5S818 STR          2.99     (2+6p) / (s+3ps+3ss+12pss)      p=0.0198 s=0.3[illegible]
D13S317 STR         6.93     1 / 4pq                         p=0.277 q=0.13
D7S820 7q11 STR     6.04     (1+p+3s) / (4ps+12pss)          p=0.138 s=0.32
D16S539 16q24 STR   3.89     (1+3t) / (4pr+16prt)            p=0.233 r=0.253
                                                             t=0.12
TH01 11p15.5 STR    11.9     (1+3p+3q) / (4pq+4ppq+12pqq)    p=[illegible]324 q=0.0909
TPOX 2p25-p24 STR   25.2     1 / 4pq                         p=0.0198 q=0.502
CSF1PO 5q33-34 STR  29       (1+3r) / (4pt+16prt)            p=0.0198 r=0.277
                                                             t=0.379

cumulative LR       20.8e9
Posterior probability=100% assuming prior=50%

                        RAW FRAGMENT SIZES


membrane: 06/09/22 c0000 » 61494.TXT
                   lane 1      lane 2      lane 3      lane 4
                   1280-00125v 1280-00126d 1280-00127s 1280-00128t
locus     Rdr Read
D3S1358ST  99 1981 15,16       16,17       16,17       16,17
VWA    ST  99 1991 15,16       13,15       15,16       15,16
FGA    ST  99 1994 23          23,24       23          23,24
AmelogeST  99 1990 X           X           X,Y         X,Y
D8S1179ST  99 1992 12,14       12,14       14          12,14
D21S11 ST  99 1983 28,30       28          28          28,30
D18S51 ST  99 1984 16,19       14,16       16          16
D5S818 ST  99 1985 11          8,11        8,11        8,11
D13S317ST  99 1986 11,12       11,13       11,13       12
D7S820 ST  99 1987 8,11        11          8,11        11
D16S539ST  99 1988 9,11        9,13        9,13        11,13
TH01   ST  99 1982 7,8         7,8         8           7,8
TPOX   ST  99 1993 7,8         8,11        7,9         7,11
CSF1PO ST  99 1989 8,12        8,10        8,10        10,12
```

Imported DNA profiles

Revision History:

March 24, 2010 – Initial version of procedure.

Identifiler loci and approximate size range

Identifiler locus	Color	Size Range 3130*xl* GS500 Std.	Allele range in Ladder
D8S1179	Blue	123.0bp ± 0.5bp To 169.0 ± 0.5bp	8 to 19
D21S11	Blue	185.0bp ± 0.5bp To 216.0 ± 0.5bp	24 to 38
D7S820	Blue	255.0bp ± 0.5bp To 291.0 ± 0.5bp	6 to 15
CSF1PO	Blue	305.0bp ± 0.5bp To 342.0 ± 0.5bp	6 to 15
D3S1358	Green	112.0bp ± 0.5bp To 140.0 ± 0.5bp	12 to 19
THO1	Green	163.0bp ± 0.5bp To 202.0 ± 0.5bp	4 to 13.3
D13S317	Green	217.0bp ± 0.5bp To 244.0 ± 0.5bp	8 to 15
D16S539	Green	252.0bp ± 0.5bp To 292.0 ± 0.5bp	5 to 15
D2S1338	Green	307.0bp ± 0.5bp To 359.0 ± 0.5bp	15 to 28
D19S433	Yellow	102.0bp ± 0.5bp To 135.0 ± 0.5bp	9 to 17.2
vWA	Yellow	154.0bp ± 0.5bp To 206.0 ± 0.5bp	11 to 24
TPOX	Yellow	222.0bp ± 0.5bp To 250.0 ± 0.5bp	6 to 13
D18S51	Yellow	262.0bp ± 0.5bp To 345.0 ± 0.5bp	7 to 27
Amelogenin	Red	106.0bp ± 0.5bp To 112.0 ± 0.5bp	X and Y
D5S818	Red	134.0bp ± 0.5bp To 172.0 ± 0.5bp	7 to 16
FGA	Red	214.0bp ± 0.5bp To 355.0 ± 0.5bp	17 to 51.2

MiniFiler loci and approximate size range

MiniFiler locus	Color	Size Range 3130*xl* GS500 Std.	Allele range in Ladder
D13S317	Blue	90.0bp ± 0.5bp To 139.0 ± 0.5bp	8 to 15
D7S820	Blue	141.5bp± 0.5bp To 193.5 ± 0.5bp	6 to 15
Amelogenin	Green	99.3bp ± 0.5bp To 109.3 ± 0.5bp	X and Y
D2S1338	Green	110.9bp ± 0.5bp To 179.9 ± 0.5bp	15 to 28
D21S11	Green	180.6bp ± 0.5bp To 250.6 ± 0.5bp	24 to 38
D16S539	Yellow	70.0bp ± 0.5bp To 122.0 ± 0.5bp	5 to 15
D18S51	Yellow	122.4bp ± 0.5bp To 210.4 ± 0.5bp	7 to 27
CSF1PO	Red	84.6bp ± 0.5bp To 132.6 ± 0.5bp	6 to 15
FGA	Red	136.4bp ± 0.5bp To 296.4 ± 0.5bp	17 to 51.2

PowerPlex Y loci and approximate size range

PowerPlex Y locus	Color	Size Range 3130*xl* GS500 Std.	Allele range in Ladder
DYS391	Blue	79.0bp ± 0.5bp To 123.0 ± 0.5bp	6 to 13
DYS389I	Blue	127.0bp± 0.5bp To 179.0 ± 0.5bp	10 to 15
DYS439	Blue	186.0bp ± 0.5bp To 236bp ± 0.5bp	8 to 15
DYS389II	Blue	245.0bp ± 0.5bp To 301.0 ± 0.5bp	24 to 34
DYS438	Green	86.75bp ± 0.5bp To 133.0 ± 0.5bp	8 to 12
DYS437	Green	174.0bp ± 0.5bp To 206.0 ± 0.5bp	13 to 17
DYS19	Green	216.0bp ± 0.5bp To 272.0 ± 0.5bp	10 to 19
DYS392	Green	280.0bp ± 0.5bp To 336.0 ± 0.5bp	7 to 18
DYS393	Yellow	98.0bp ± 0.5bp To 144.0 ± 0.5bp	8 to 16
DYS390	Yellow	183.0bp ± 0.5bp To 237.0 ± 0.5bp	18 to 27
DYS385	Yellow	239.0bp ± 0.5bp To 334.0 ± 0.5bp	7 to 25

YM1 Genotyper Categories Table for ABI 3130*xl*

DYS19

12	Highest peak at 180.70 ± 1.00 bp in yellow with height ≥75
13	Highest peak at 184.70 ± 1.00 bp in yellow with height ≥75
14	Highest peak at 188.80 ±1.00 bp in yellow with height ≥75
15	Highest peak at 192.60 ±1.00 bp in yellow with height ≥75
16	Highest peak at 196.70 ±1.00 bp in yellow with height ≥75
17	Highest peak at 200.50 ±1.00 bp in yellow with height ≥75
18	Highest peak at 204.50 ±1.00 bp in yellow with height ≥75

DYS389 I

10	Highest peak at 238.60 ±1.00 bp in yellow with height ≥75
11	Highest peak at 242.60 ±1.00 bp in yellow with height ≥75
12	Highest peak at 246.50 ±1.00 bp in yellow with height ≥75
13	Highest peak at 250.70 ±1.00 bp in yellow with height ≥75
14	Highest peak at 254.70 ±1.00 bp in yellow with height ≥75
15	Highest peak at 258.70 ±1.00 bp in yellow with height ≥75

DYS389 II

26	Highest peak at 356.60 ±1.00 bp in yellow with height ≥75
27	Highest peak at 360.60 ±1.00 bp in yellow with height ≥75
28	Highest peak at 364.60 ±1.00 bp in yellow with height ≥75
29	Highest peak at 368.50 ±1.00 bp in yellow with height ≥75
30	Highest peak at 372.40 ±1.00 bp in yellow with height ≥75
31	Highest peak at 376.40 ±1.00 bp in yellow with height ≥75
32	Highest peak at 380.50 ±1.00 bp in yellow with height ≥75
33	Highest peak at 384.40 ±1.00 bp in yellow with height ≥75

DYS390

20	Highest peak at 197.90 ±1.00 bp in blue with height ≥75
21	Highest peak at 201.90 ±1.00 bp in blue with height ≥75
22	Highest peak at 205.80 ±1.00 bp in blue with height ≥75
23	Highest peak at 209.90 ±1.00 bp in blue with height ≥75
24	Highest peak at 213.90 ±1.00 bp in blue with height ≥75
25	Highest peak at 217.90 ±1.00 bp in blue with height ≥75
26	Highest peak at 221.90 ±1.00 bp in blue with height ≥75
27	Highest peak at 225.90 ±1.00 bp in blue with height ≥75

Macro Filter functions

Identifiler 28 cycles	**Allele Filters**
Locus	Stutter Filter 3130*xl* (OCME validation @ 500pg)
D8S1179	11.2%
D21S11	14.7%
D7S820	11.0%
CSF1PO	10.4%
D3S1358	10.8%
THO1	7.7%
D13S317	9.3%
D16S539	9.7%
D2S1338	10.5%
D19S433	19.1%
vWA	18.1%
TPOX	3.0%
D18S51	13.6%
Amelogenin	none
D5S818	13.3%
FGA	24.6%

Identifiler 31 cycles	**Allele Filters**
Locus	Stutter Filter 3130*xl* (ABI default)
D8S1179	12%
D21S11	13%
D7S820	9%
CSF1PO	9%
D3S1358	11%
THO1	6%
D13S317	10%
D16S539	13%
D2S1338	15%
D19S433	17%
vWA	11%
TPOX	6%
D18S51	16%
Amelogenin	none
D5S818	10%
FGA	11%

MiniFiler	**Allele Filters**
Locus	Stutter Filter 3130*xl* (ABI default)
D13S317	14 %
D7S820	11 %
Amelogenin	None
D2S1338	18 %
D21S11	16 %
D16S539	15 %
D18S51	18 %
CSF1PO	14 %
FGA	15 %

PowerPlex Y	**Allele Filters**
Locus	Stutter Filter 3130*xl* (OCME validation @ 500pg)
DYS391	8.39 %
DYS389I	8.41 %
DYS439	8.61 %
DYS389II	14.81 %
DYS438	3.49 %
DYS437	7.31 %
DYS19	5.64 %
DYS392	13.10 %
DYS393	11.38 %
DYS390	11.39 %
DYS385	15.43 %

For PowerPlex Y, a 6 % general filter is also applied to all loci.

See Y M1 Genotyper section for **Y M1** filter functions.

Revision History:
March 24, 2010 – Initial version of procedure.