QUALITY ASSURANCE MANUAL
VERSION 4.0

Effective date: January 7, 2005

APPROVED BY			
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FORENSIC BIOLOGY QUALITY ASSURANCE MANUAL

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1. INTRODUCTION

As of this date, the Quality Assurance Manual, Version 4.0 supersedes all previous Quality Assurance (QA) and/or Quality Control (QC) Manuals in the Department of Forensic Biology at the New York City Office Of Chief Medical Examiner (OCME). Where appropriate, references have been made to the Forensic Biology Administrative Manual, Case Management Manual, Forensic Biochemistry Methods Manual, and Protocols for Forensic STR Analysis.

The Quality Assurance Manual consists of various sections that address the current FBI Quality Assurance Standards and the ASCLD/LAB Manual. Its appendices contain reagent sheets (Appendix A), Quality Control procedures (Appendix B), and a list of usage and maintenance logs (Appendix C) that are currently being used in the laboratory.

A. Section 1 through Section 7

These sections address the current FBI Quality Assurance and ASCLD/LAB Standards and specify the policies and procedures followed by the Department of Forensic Biology. These sections are controlled and must be approved by the Director or his/her designee prior to being implemented and/or changed.

B. Reagent sheets (Appendix A)

The Department of Forensic Biology documents the preparation of all internal critical reagents. This documentation is in the form of a reagent sheet that lists the chemical makeup and procedures necessary for the preparation of a given reagent. All current reagent sheets are filed in a series of **Reagent Sheet Binders**. A copy of each reagent sheet has also been included in this manual as Appendix A. Reagent sheets are worksheets, and do not require the Director or his/her designee's approval prior to being implemented and/or changed, but must be reviewed by the Quality Assurance Manager.

C. Quality Control Testing Procedures (Appendix B)

The purpose of a Quality Assurance Program is to ensure that the laboratory meets a specified standard of quality. The Quality Assurance Program does this through the monitoring, verifying, and documenting of the performance of the laboratory. To accomplish these tasks, the Forensic Biology Quality Assurance Program has established a series of Quality Control Testing Procedures that are designed to monitor critical aspects of forensic sample analysis in order to ensure that the resulting product conforms to the current standards set forth by the ASCLD/LAB Manual, FBI Quality Assurance Standards, and Scientific Working Group for DNA Analysis Methods (SWGDAM). These Quality Control Testing Procedures are contained in Appendix B and are identified by specific QC numbers. As an appendix, Quality Control Testing Procedures do not require the Director or his/her designee's approval prior to being implemented and/or changed, but must be reviewed by the Quality Assurance Manager.

D. Miscellaneous (Appendix C)

Appendix C lists miscellaneous items associated with the Quality Assurance Program of the laboratory. As an appendix, they do not require the Director or his/her designee's approval prior to being implemented and/or changed, but must be reviewed by the Quality Assurance Manager.

Appendix C-1 lists the usage and maintenance logs used by the laboratory to provide documentation of equipment use, calibration, and maintenance. This documentation aids the QA program in identifying trends in equipment operation and analyst performance. This information can also assist the QA program in identifying potential or existing problems of quality.

Appendix C-2 shows a list of quality control testing "procedures" used in the Department of Forensic Biology. Each procedure may be a combination of several quality control tests listed in Appendix B. If a reagent sheet lists a "procedure" for its quality control, then the reagent must pass all the quality control tests listed. If it lists a specific "QC" number, then the reagent must pass that quality control tests only.

2. ASCLD/LAB MANUAL

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In accordance to the Quality Assurance Manual guidelines (See Discussion of Standard 1.4.2.1) set forth by the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB), this manual contains or references the documents or policies/procedures pertaining to the following:

- **A quality policy statement including objectives and commitments by management.**
  This is listed in the Forensic Biology Administrative Manual.

- **The organization and management structure of the laboratory, its place in any parent organization, and relevant organizational charts.**
  This is diagrammed and discussed in the Forensic Biology Administrative Manual.

- **The relationships and responsibilities of management, technical operations, and support services in implementing the quality system.**
  This is presented in the Forensic Biology Administrative Manual.

- **Job descriptions, education, and up-to-date training records of laboratory staff.**
  Job descriptions for all laboratory personnel are described in the Forensic Biology Administrative Manual. In addition, Civil Service job specifications for each job title are located in a filing cabinet containing ASCLD/LAB and FBI QAS criterion files. Training records of laboratory staff are kept in a filing cabinet located near the departmental administrative office.

- **Control and maintenance of documentation of case records and procedure manuals.**
  The control and maintenance of documentation of case records is discussed in the Forensic Biology Administrative Manual.

The Laboratory Director, or his/her designee, has the ultimate responsibility for all procedural manuals and assigns the writing and editing of manuals to the Deputy Director, Assistant Directors, Quality Assurance Manager and/or Criminalist IVs on a regular basis. Minor revisions to each manual are made when necessary. The finalization of each revision occurs when (i) the Director or his/her designee, and if necessary, the Technical Manager, Deputy/Assistant Directors, Quality Assurance Manager or other laboratory members have reviewed the change(s), and (ii) the Director, or his/her designee, signs an approval to indicate that a newly revised manual will be in effect. The Quality Assurance Manager shall maintain the original signed approval for each procedural manual and keeps track of all changes that have been made. The original controlled version of each procedural manual shall remain on the Departmental network drive. Every effort will be made to inform the laboratory of changes to the procedural manuals, however, it is the responsibility of each analyst to ensure that if they have a personal (uncontrolled) copy of a manual that it corresponds to the most up-to-date version.

- **The laboratory's procedures for ensuring that measurements are traceable to appropriate standards, where available.**
  These are listed in the "NIST Standards" and "Equipment Calibration and Maintenance" sections of this manual.

- **The type and extent of examinations conducted by the laboratory.**
  These are listed and described in detail in the Forensic Biology Biochemistry Manual and the Forensic Biology Protocols for Forensic STR Analysis.

- **Validation of test procedures used.**
  This is described in the Forensic Biology Administrative Manual.

- **Handling evidence.**
  This is described in the Forensic Biology Administrative Manual and the Forensic Biology Case Management Manual.

- **The use of standards and controls in laboratory procedures.**
  These are discussed in the "Reference Standards" and "Equipment Calibration and Maintenance" sections of this manual. These are also discussed in the Forensic Biology Biochemistry Manual and the Forensic Biology Protocols for Forensic STR Analysis under each analytical procedure.

- **Calibration and maintenance of equipment.**
  This is presented in the Forensic Biology Administrative Manual and in the "Equipment Calibration and Maintenance" section of this manual.

- P**ractices for ensuring continuing competence of examiners including interlaboratory comparisons, proficiency testing programs, and internal quality control schemes (e.g., technical peer review).**
  Proficiency testing, sample re-analysis, and technical review procedures are discussed in the Forensic Biology Administrative Manual. External proficiency testing for DNA analysis is conducted in the laboratory according to the FBI Quality Assurance Standards and the National DNA Index System (NDIS) standards for the operation of the Combined DNA Index System (CODIS).

- T**aking corrective action whenever analytical discrepancies are detected.**
  This is discussed in the Forensic Biology Administrative Manual.

- **Monitoring court testimony to ensure the reporting of scientific findings in an unbiased and effective manner.**
  This is discussed in the Forensic Biology Administrative Manual. All documents monitoring the court testimony of Criminalists, Assistant Directors, and Director are filed in a binder located in a designated area of the Forensic Biology Laboratory.

- **Laboratory protocol permitting departures from documented policies and procedures.**
  The specific procedures for analytical techniques done in this laboratory are thoroughly presented in the Forensic Biology Biochemistry Manual and the Forensic Biology Protocols for Forensic STR Analysis. Any deviations from the procedures must be clearly documented on the data sheets (eg. worksheets, electropherograms, etc.) that are generated.

- **Dealing with complaints.**
  This is discussed in the Forensic Biology Administrative Manual.

- **Disclosure of information.**
  This is discussed in the Forensic Biology Administrative Manual.

- **Audits and quality system review.**
  The Department of Forensic Biology Laboratory conducts audits annually in accordance to the standards dictated by ASCLD/LAB, the FBI Quality Assurance Standards, and NDIS; this is further discussed in the Forensic Biology Administrative Manual.

3. FBI QUALITY ASSURANCE STANDARDS		
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In accordance to the FBI Quality Assurance Standards (See Standard 3.1.1), this manual contains or references the documents or policies/procedures pertaining to the following:

- **Goals and Objectives**
  The goals and objectives of the Department of Forensic Biology are listed in the Forensic Biology Administrative Manual

- O**rganization and management structure**
  The organization and management structure of the laboratory are diagramed and discussed in the Forensic Biology Administrative Manual.

- **Personnel qualifications and training**
  Job descriptions for all laboratory personnel are described in the Forensic Biology Administrative Manual. In addition, the Civil Service specifications for each job title are kept in the laboratory along with personnel transcripts, resumes, and documentation of continuing education and training.

- **Facilities**
  This is presented in the subsequent sections of this manual.

- **Evidence Control**
  Evidence control, handling, and documentation procedures are discussed in the Forensic Biology Administrative Manual and the Forensic Biology Case Management Manual. These procedures have been designed to ensure the integrity of all physical evidence that enters the laboratory.

- **Validation**
  Validation is conducted according to the FBI Quality Assurance Standards and is described in the Forensic Biology Administrative Manual.

- **Analytical Procedures**
  This is presented in the subsequent sections of this manual and in various procedural manuals of the laboratory.

- **Calibration and Maintenance**
  This is presented in the subsequent sections of this manual and in the Forensic Biology Administrative Manual.

- **Proficiency Testing**
  Proficiency testing is discussed in the Forensic Biology Administrative Manual. External proficiency testing for DNA analysis is conducted in the laboratory according to the FBI Quality Assurance Standards and the National DNA Index System (NDIS) standards for the operation of the Combined DNA Index System (CODIS).

- **Corrective Action**
  This is discussed in the Forensic Biology Administrative Manual.

- **Reports**
  Written procedures for writing and issuing reports are presented in the Forensic Biology Case Management Manual, the Forensic Biology Administrative Manual, and the Forensic Biology Protocols for Forensic STR Analysis.

- **Review**
  Case review and related issues are discussed in the Forensic Biology Administrative Manual and the Forensic Biology Case Management Manual.

- **Safety**
  The Department of Forensic Biology has a documented environmental health and safety program as listed in the Forensic Biology Administrative Manual. This documentation is kept in the **Safety Binder**. The OCME building safety officer conducts at least three inspections each year of the laboratory.

- **Audits**
  The Department of Forensic Biology Laboratory conducts audits annually in accordance to the standards dictated by ASCLD/LAB, the FBI Quality Assurance Standards, and NDIS; this is further discussed in the Forensic Biology Administrative Manual.

4. FACILITIES

A. Security

Laboratory and building security are discussed in the Forensic Biology Administrative Manual.

B. Contamination

1. Prevention

Several measures have been taken to prevent contamination within the Department of Forensic Biology. The laboratory is divided into physically isolated areas for evidence examination, DNA extraction, pre-amplification (amplification setup) and post-amplification (amplification and DNA typing). Each area has its own dedicated equipment. Once samples are accepted into the laboratory, they move through these areas in one direction only. Samples are first processed in the evidence examination area. They are then moved to the DNA extraction area. Following DNA extraction, aliquots of each sample are quantitated in the DNA quantitation area. Following DNA quantitation, aliquots of each sample are moved into the pre-amplification area. Here fresh kit reagents are stored and samples are prepared for amplification. Finally, the samples are amplified and typed in the post-amplification area. This laboratory setup helps eliminate cross contamination from amplified DNA areas back into non-amplified DNA areas.

To avoid cross contamination between specimens, exemplar samples are processed separately from evidence samples. Also, only one sample is processed at a time using single-use disposable supplies whenever possible (eg. pipet tips), and scissors/tweezers are thoroughly cleaned between each sample (see Protocols for Forensic STR Analysis and Case Management Manuals for additional procedures to avoid cross contamination).

By far, the best defense against contamination is training for the analysts. The analysts must understand what is happening to the DNA at every step of the procedure. They must understand the rationale behind the laboratory setup and the methods of sample handling, so they are able to prevent problems before they arise. In this way, they are equipped to assess and to modify their individual habits as they practice each test of the training program.

2. Identification

Contamination can be identified as 1) the presence of signal in QuantiBlot, P30, and Amylase negatives (reagent blanks), 2) presence of ***alleles*** in extraction negatives (reagent blanks) or amplification negatives (reagent blanks), 3) presence of extraneous alleles in positive controls, or 4) presence of extraneous alleles in case samples. Contamination problems reflect a system failure or contamination of the samples by an outside source. The source may be equipment, reagents, the working environment, laboratory personnel, or an analytical error. Contamination can either be a single isolated event such as cross contamination between two samples or it can be persistent, such as contamination of a reagent or equipment. To remedy contamination caused by a single isolated event, the appropriate extraction, quantitation, amplification and/or STR analysis is repeated (also see the STR Results Interpretation section in the Forensic Biology Protocols for Forensic STR Analysis).

The Quality Assurance Manager must be notified if contamination occurs. The source of contamination should be identified, if possible, and eliminated. To demonstrate the elimination of the persistent contamination, a clean run (see QC155) may be performed. During a clean run, control samples are processed along with a series of negative controls. Negative controls are run at the extraction, amplification, and typing steps. The results from these samples will indicate the area in which contamination appears. By focusing attention on one area at a time, the source or sources of contamination can be systematically eliminated. In addition, recent casework may be reviewed and selected samples may be repeated later to verify the results. The analysts will be informed of any corrective action adopted to prevent the recurrence of the problem.

3. Troubleshooting

Often, the source of a contamination problem can be identified on the basis of experience. For example, in a Quantiblot run, a persistent appearance of a light signal in the extraction negative control or the standard negative control (slot 1H) indicates (i) contamination of the reagents used during the extraction procedure, (ii) contamination of the solutions used during the Quantiblot run, (iii) consistent contamination by the analyst during extraction, or (iv) equipment contamination by improper cleaning. In the former case, this contamination may represent a build up of DNA in the reagents over the course of many extractions. The weak signal appears when the concentration of DNA in the extraction negative is greater than the threshold of detectability for the hybridization. Generally, fresh reagents will eliminate this problem. In the latter case, if necessary, corrective action in the form of counseling and/or retraining will be given to the identified analyst(s).

Electrophoresis runs, which appear to have the same mixture of DNA types across all the samples, indicate a more serious contamination problem at the level of the instrument or amplification step. If tubes or reagents are contaminated during the pre-amplification set up, the contaminant DNA will be amplified along with the sample. The sample signals may even be overwhelmed by the contaminant. To solve this problem, the pre-amplification room must be cleaned out and the bench washed with a 10% bleach solution. All of the kit reagents must be changed and new reaction tubes must be aliquoted.

Documentation resulting from troubleshooting experiments is kept in the **QA/QC Troubleshooting/Issues** binder**.**

4. Quality Control Testing Procedures

In addition to proper technique on the part of the analyst, care must also be taken in the preparation of all in-house reagents and in keeping all apparatus that come in contact with forensic samples free of contamination. To this end, various QC procedures have been developed and are part of routine laboratory operation (see Appendix B).

a. Reagent Preparation

Clean laboratory glassware is an essential in reagent preparation (see QC175). Furthermore, all aliquots of deionized water and Tris-EDTA (TE^{-4}) buffer are first sterilized using an autoclave (see QC115) prior to distribution throughout the laboratory. This procedure protects these reagents from possible bacterial contamination that could later result in the degradation of sample DNA. In addition, autoclaving conditions help to keep these solutions DNA-free. Other working reagents that are kept in the laboratory for long periods of time (e.g. 0.5M EDTA) may also be autoclaved to increase their shelf life.

b. Equipment Decontamination

Various QC procedures have also been developed to help maintain a DNA-free environment at the points of sample contact with the various apparatus used in DNA analysis. A 10% bleach solution is extremely effective in degrading DNA and is thus used for general cleanup procedures of equipment and the laboratory environment (e.g. laboratory desks and benches). Regular decontamination procedures with 10% bleach are used for the disinfection of the P30 ELISA Plate Washer (QC235), micropipetman (QC215), microcentrifuges (QC140), thermocyclers (QC290), and biosafety/fume hoods (QC125).

Documentation of these various decontamination procedures is kept in the Plate Washer Maintenance Log Binder, Micropipette Calibration Log Binder, Centrifuge Maintenance Log Binder, Thermocycler Calibration and Maintenance Log Binder and Biosafety/Fume Hood Maintenance Log Binder, respectively.

A. Introduction

Analytical procedures that are used by the Forensic Biology Laboratory are described in the Biochemistry Methods Manual and Protocols for Forensic STR Analysis Manual. These manuals also include general guidelines for the interpretation of data. References to scientific literature on which these procedures are based are also included in these manuals.

B. Reagents

Reagents used to perform various analytical procedures in the laboratory are purchased from commercial vendors or prepared in the laboratory. Reagents that are purchased from commercial vendors (e.g. calibrator standards for quantitation of human DNA, 30% hydrogen peroxide, sodium dodecyl sulfate, sodium hydroxide, etc.) are used either directly in a given analytical procedure (eg. calibrator standards for quantitation of human DNA, 30% hydrogen peroxide) or in the preparation of in-house reagents (e.g. sodium dodecyl sulfate, sodium hydroxide).

Each reagent has a corresponding **reagent sheet** which may include the identity and application of the reagent, date of preparation, identity of individual preparing the reagent, reagent lot number (if critical reagent), standard batch size, ingredients of the reagent, procedure to follow when preparing the reagent, data log section, and the quality control procedures to be performed before the reagent is released for use into the laboratory (see Appendices A and B). Working copies of the reagent sheets are kept in the **Reagent Binders**.

At a minimum, every reagent (or its container) that is prepared by the Department of Forensic Biology is labeled with the identity of the reagent, the date of preparation or expiration, and the identity of the individual preparing the reagent. The reagent sheets may further dictate what, in addition, must be indicated on the label.

1. Lot Numbers

All critical reagents are assigned a lot number. Subsequent lots increase in numerical order (e.g. 51, 52, 53, etc.). Some reagents that are usually made fresh for a given procedure and/or are not critical reagents are not assigned lot numbers. Where applicable, the reagent sheet indicates the lot number of that reagent and the lot numbers of the ingredients that were used for making the reagent. The reagent sheets for each lot are also filed in the Reagent Binders along with any supporting quality control documentation.

2. Standard Batch Size

Each reagent sheet indicates the standard batch size routinely prepared for each lot. The quantities listed in the ingredients section have been calculated for this standard batch. Occasionally, it may be convenient to prepare a batch larger or smaller than the standard batch size. In such cases, the preparer must note the adjusted amount of each ingredient added for preparation of the reagent. If changes in demand persist over time, the reagent sheet may be modified to reflect the new batch size.

3. Ingredients

An ingredient may be either purchased from an outside vendor or prepared in-house. The ingredients required for the preparation of the reagent and the amounts of each ingredient required for the standard batch size are listed at the top of the reagent sheet. When suitable, final concentrations, and/or a tolerance of measurement are also listed next to the amount of a given ingredient. The tolerance of measurement is calculated to define an acceptable range of variation that will not significantly change the final concentration of a given reagent. Also, certain ranges have been adopted based upon recommendations for optimum performance. Volume measurements, which are made in the appropriate, size graduated cylinders and which appear to the eye to be exact, fall well within the range of tolerance listed in the ingredients section.

4. Procedure

The procedure describes how to prepare the solution step by step and includes important notes regarding the safe handling of hazardous chemicals. The completed sheets must document exactly how the solution was prepared. Any deviation from the printed procedure must be clearly documented on the reagent sheet.

5. Data Log

The **Data Log** records information regarding the ingredients used in the preparation of reagents. This information includes the source of the ingredient, lot number of the ingredient, amount of ingredient used, date of preparation, and the identity of the individual preparing the reagent. Reagents prepared in the laboratory may also be listed as ingredients (eg. 20X SSPE which is used in the preparation of Quantiblot Hybridization Solution). In those cases, the source is listed as FB (Forensic Biology) and the laboratory lot number is recorded.

6. Quality Control

The quality control section lists the appropriate QC tests to be performed, if any, before the solution is released for use in the laboratory. These QC test procedures have been assigned QC numbers and names (eg. QC145 Chelex Extraction).

The type and number of quality procedures required to be done on a given reagent is dictated by the nature of that reagent. For example, QC250 Quantiblot Hybridization, is listed in the quality control section for Quantiblot Wash Solution (see Quantiblot Wash Solution reagent sheet in Appendix B). To evaluate the performance of this component, it is not necessary to amplify and type test samples. Only the Quantiblot hybridization procedure is necessary to establish quality of the Quantiblot Wash Solution. On the other hand, the QC procedure for 5% Chelex (QC145) requires an extraction, human DNA quantitation, amplification, and STR analysis of the appropriate controls. The newly prepared 5% Chelex solution is released into the laboratory when all the tests have been passed.

More than one solution may be tested with a given QC procedure. In this case, the quality test must be sufficient for all of the components. For example, if a single run is to be performed for 5% Chelex and Quantiblot Wash Solution, the quality test must begin with the extraction. QC145 Chelex Extraction is the appropriate test for the Chelex, and the procedure encompasses the hybridization necessary for the wash solution.

7. Documentation

After a quality test has been performed, the supporting documentation is attached to the original solution sheet and submitted for review. If the reagent performance is satisfactory, it will be released for general use in the laboratory. If the reagent fails to meet the standards set forth in the QC procedure, it may be submitted for further testing or discarded.

After a reagent has passed quality control and been released, the reagent sheet and quality control documentation are filed in the appropriate QC reagent binder. If more than one reagent has been tested for quality control in a single test run, the original quality control documents will be filed with one solution sheet and cross referenced on the reagent sheet of the other.

C. Critical Reagents

By definition, "critical reagents are determined by empirical studies or routine practice to require testing on established samples before use on evidentiary samples in order to prevent unnecessary loss of sample." (FBI QAS, 7/2004). Thus, all critical reagents in the Forensic Biology Laboratory have a QC test procedure listed on each respective reagent sheet. This QC test procedure must be performed in order for the reagent to be released for use in routine casework analysis.

D. Reference Standards

The laboratory must check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available National Institute of Standards and Technology (NIST) standard reference material (SRM) or controls that are traceable to a NIST standard.

Standard reference materials (SRM) for STR analysis may be purchased from the National Institute of Standards and Technology (NIST) and tested annually as a quality check on the equipment and procedures that are used by the lab for STR typing. The laboratory determines the DNA profiles of the given SRM samples. The results of these experiments are compared to the allele identification results that are also provided by NIST. Secondary standards may be created by identify controls and running them against NIST SRM's, which in turn makes these controls NIST traceable. The laboratory determines the DNA profiles of the controls. The results of these experiments are compared to the allele identification results of the original run. This information is filed in the **PCR NIST Standards Binder**.

Positive and negative controls are run for every analytical procedure that is done in the laboratory. A discussion of the purpose for various types of negative controls used in the laboratory is presented in the Forensic Biology Protocols for Forensic STR Analysis. A list of the correct DNA profiles for various positive controls used in STR typing is presented in the same section of the Protocols for Forensic STR Analysis Manual (see subsection Amplification Positive Control)

6. EQUIPMENT CALIBRATION AND MAINTENANCE

Equipment calibration and maintenance is essential for establishing confidence in the results that are generated during routine testing of forensic DNA samples. Equipment calibration and maintenance procedures can be subdivided into three (3) separate categories:

A. Weights and Measures

1. Temperature

The Department of Forensic Biology monitors the temperatures of all freezers, refrigerators, heat blocks, incubators, and water baths that are used for storage of evidence and all types of casework samples on a daily basis, when the laboratory is open. Temperature and humidity readings are taken from several areas in the laboratory. Acceptable temperature readings for each specific apparatus are noted below.

Equipment	Set Temperature	Acceptable
Freezers	-20°C	-1 to -25°C
	-80°C	-60 to -85°C
Refrigerators	4°C	1 to 13°C
56°C heat block	56°C	56 ± 3°C
65°C heat block	65°C	65 ± 3°C
95°C heat block	95°C	95 ± 3°C
100°C heat block	100°C	100 ± 3°C
37°C incubator	37°C	37 ± 3°C
Quantiblot H_2O bath	50°C	50 ± 1°C

The laboratory may choose to use more stringent values. However, the above minimum acceptable values must be observed.

Digital thermometers that are used to monitor the temperature of laboratory refrigerators, freezers, cold rooms, incubators, heat blocks, water baths, and air temperature are calibrated or are replaced by new units according to the vendor specifications (e.g., recalibration date; see QC270). Digital thermometers and dedicated RTD probes used in calibrating thermocyclers are calibrated annually to National Institute of Standards and Technology (NIST) traceable standards. Each of these measuring instruments or probes (e.g., thermocouples with the exception of the Type T-brown[1]) are calibrated yearly to National Institute of Standards and Technology (NIST) traceable standards (see QC270 and QC280). The date of calibration is documented on the appropriate log sheet and filed in the **Temperature Equipment Maintenance Log Binder**. All new temperature measuring instruments/probes must have proof of calibration (e.g. documentation of traceability to NIST standards) prior to being used in the laboratory.

Any additional maintenance performed on refrigerators and freezers is documented in the **Temperature Equipment Maintenance Log Binder**.

NOTE: At the time of writing of this section, the Rees Centron Temperature Monitoring System is being installed. Once installation is complete, the System equipment and log shall replace all monitoring equipment currently used. The System shall be calibrated according to manufacturer's recommendations.

2. Balances

Analytical balances are used to weigh chemicals for the preparation of all laboratory reagents. At a minimum, balances must be calibrated annually to NIST traceable standards (see QC120). Documentation of each calibration is kept in the **General Equipment Maintenance Binder**.

3. pH Meter

The pH meter is used to measure the pH of reagents. A two-point calibration and verification of the pH meter is performed at least weekly (see QC245) and is documented in the **pH Log & Water System Binder**.

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[1] Type T-brown thermocouples are used in the measurement of ultra-low low temperature freezers (-80°C). A verification of these thermocouples is done yearly (see QC285) since an exact low temperature for the storage of DNA extracts, tissue samples, etc., is not critical, and NIST traceable thermometers are not made for this low temperature range.

4. **Micropipettes**

Micropipettes are used routinely in the laboratory to measure and dispense accurate volumes of reagents used for a given protocol. All micropipettes are calibrated twice each year by an outside vendor (see QC215). In addition, if at any time, there is reason to suspect that a micropipette may not be performing to its specifications, a quick gravimetric check may be done by weighing specific volumes of water on an analytical balance. If the micropipette differs significantly from specifications, the Quality Assurance Manager must be notified and the micropipette under question will be removed from laboratory operations and will be sent for calibration with the next outgoing shipment. When possible, spare calibrated micropipettes will be used as temporary replacements for any micropipettes that have been removed by this manner from regular operation. Micropipette calibration is documented in the **Micropipette Calibration QC Log Binder.**

B. Analytical Methods

Equipment that is used for specific analytical methods in the laboratory is also calibrated on a regular basis according to the specific QC procedure indicated below.

Documentation of calibration and maintenance procedures performed for equipment is done on specific equipment log sheets that are filed in each specific equipment logbook. Each logbook is located near the equipment under consideration.

Equipment	Analytical Procedure	Calibration/ Maintenance Protocol
ABI 310 Genetic Analyzer	STR Capillary Electrophoresis (310)	QC135
ABI 3100 Genetic Analyzer	STR Capillary Electrophoresis (3100)	QC360
BioRad Benchmark Microplate Reader	P30 ELISA	QC230
GeneAmp PCR System 9700	STR PCR	QC302

C. Lab Personnel Safety

The laboratory has chemical fume hoods and biological containment hoods that are inspected annually by an outside vendor (see QC125). Documentation of inspections is kept in the **Chemical Fume Hood & Biological Cabinet Maintenance Log Book**.

This section shows a list of reagents used in the Department of Forensic Biology. They are further classified as "Critical" or "Non-Critical" reagents. As per the FBI Quality Assurance Standards, a "Critical reagent" requires testing on established samples before use in order to prevent unnecessary loss of sample. In addition, the Department of Forensic Biology may quality control test "Non-Critical" reagents to reduce the chances that analyses are rerun.

REAGENT	CRITICAL
Acid Phosphatase Test Reagent	Y
Alkaline Substrate Buffer	Y
AmpFℓSTR Cofiler PCR Amplification Kit	Y*
AmpFℓSTR Profiler Plus PCR Amplification Kit	Y*
Amylase Gel Buffer	Y
BSA Solution, 5 mg/mL	Y
Casein Stock Solution	Y
Cell Lysis Buffer (CLB)	Y
Chelex, 20%	Y
Chelex, 5%	Y
Chloroform-Isoamyl Alcohol	N
Chromogen Solution	N
Coomassie Blue Stain	N
Deoxynucleotide Triphosphates, 2.5 mM (dNTPs)	Y
Destain Solution	N
Digest Buffer	Y
Dithiothreitol (DTT), 1M	Y
EDTA, 0.5 M	N
Formamide and Loading Buffer	N
Formamide, Deionized	N
Hydrogen Peroxide, 3%	N

REAGENT	CRITICAL
Iodine Solution, 0.01 N	N
Kastle-Meyer (KM) Reagent	Y
Leucomalachite Green (LMG) Reagent	Y
Negative female control DNA for Y STR analysis	Y
Nuclear Fast Red	Y
Organic Extraction Buffer	Y
PBS for Chelex Extraction	Y
PBS Solution for P30 ELISA (PBS tablets)	Y*
PBS-BSA Solution	N
Polydimethylsiloxane (PDMS) Positive Control	Y
Picric Indigo Carmine (PIC)	Y
Positive Male Control DNA for Y STR Analysis	Y
Potassium Cyanide Solution (KCN), 0.05%	N
Primer, DYS19/1	Y
Primer, DYS19/2	Y
Primer, DYS389/1	Y
Primer, DYS389/2	Y
Primer, DYS390/1	Y
Primer, DYS390/2	Y
Quantiblot Citrate Buffer	N
Quantiblot DNA Standards	Y
Quantiblot Hybridization Solution	N
Quantiblot Pre-Wetting Solution	N
Quantiblot Spotting Solution	N
Quantiblot Wash Solution	N

REAGENT	CRITICAL
Saline (0.85% NaCl)	N
Sarkosyl, 20%	N
SDS, 0.1%	N
Sequencing Loading Buffer	Y
Sodium Acetate, 0.1 M	N
Species Agarose Gel	N
Species Tank Buffer	N
SSPE, 20X	N
Stain Extraction Buffer	Y
Sterile Deionized Water	Y
TNE, 10X	N
TNE, 1X	N
Tris-EDTA, 1X	Y
Tris-HCl, 1M (pH 8.0)	N
Urea, 10.8 g	Y*
Urea Diffusion Test and Blank Plates	Y*
Urease	Y*
YM1 STR/PCR Reaction Mixture	Y*

***Tested for each new vendor lot/shipment**.

Effective Date: 01/07/2005

Appendix A

1. Forensic Biochemistry Methods: Reagent Sheets

Acid Phosphatase Spot Test Reagent
Alkaline Substrate Buffer
Amylase Gel Buffer
Casein Stock Solution
Coomassie Blue Stain
Destain Solution
Iodine Solution
Kastle-Meyer (KM) Reagent
Nuclear Fast Red
PBS Solution
PBS-BSA Solution
Picric Indigo Carmine
Positive PDMS Control
Potassium Cyanide (KCN) Solution, 0.05%
Saline (0.85% NaCl)
Sodium Acetate, 0.1 M
Species Agarose Gel
Species Tank Buffer
Urea Diffusion Test and Blank Plates
Urease, 3 U/ml

2. Forensic STR Analysis: Reagent Sheets

BSA Solution
Chelex, 5%
Chelex, 20%
Chromogen
Deoxynucleotide Triphosphate (dNTPs), 2.5 mM
Digest Buffer
Dithiothreitol, 1 M
Ethylenediaminetetracetate (EDTA), 0.5 M
Formamide, Deionized
Negative Female Control DNA
Organic Extraction Buffer
PCR Reaction Mixture (Cofiler and Profiler Plus)
Phosphate Buffered Saline (PBS), Chelex
Positive Male Control DNA
Primer, DYS19/1
Primer, DYS19/2
Primer, DYS389/1
Primer, DYS389/2
Primer, DYS390/1
Primer, DYS390/2
QuantiBlot Citrate Buffer
QuantiBlot DNA Standards
QuantiBlot Hybridization Solution
QuantiBlot Pre-wetting Solution
Quantiblot Spotting Solution
QuantiBlot Wash Solution
Sodium Dodecyl Sulfate (SDS), 0.1%
SSPE, 20X
Sterile Deionized Water
Tris EDTA, 1X
Tris-HCl, 1 M
Urea, 10.8g
YM1 STR/PCR Reaction Mixture

In the event that an identical reagent is slated for use between Forensic STR Analysis and Forensic Mitochondrial DNA Analysis, the additional steps and quality control tests required in the Forensic Mitochondrial DNA Analysis reagent sheets must be conducted prior to its use for Forensic Mitochondrial DNA Analysis.

3. Forensic Mitochondrial DNA Analysis: Reagent Sheets

Chelex 5%
Dithiothreitol, 1M
Ethidium Bromide
FBI Primer A1 (100 µM)
FBI Primer B1 (100 µM)
FBI Primer C1 (100 µM)
FBI Primer D1 (100 µM)
FBI Primer A2 (100 µM)
FBI Primer B2 (100 µM)
FBI Primer C2 (100 µM)
FBI Primer D2 (100 µM)
FBI Primer A4 (100 µM)
FBI Primer B4 (100 µM)
FBI Primer HVIF (100 µM)
FBI Primer HVIR (100 µM)
FBI Primer HVIIF (100 µM)
FBI Primer HVIIR (100 µM)
Human Leukemia 60 (HL60), 0.1ng/20µL
Orange G Loading Dye
Organic Extraction Buffer
Proteinase K, ~400u/mL
Sterile Deionized Water
TRIS-EDTA, 1X

Effective Date: 01/07/2005

ACID PHOSPHATASE TEST REAGENT

Standard batch size: 2 x 500 ml Lot Number: _____

Application
Acid Phosphatase presumptive test for semen (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Sodium Acetate, 0.1 M (pH 5.5)	0.1 M	1000 ml
Alpha-Naphthyl Phosphate (disodium)	0.1%	0.5 g
o-Dianisidine Tetrazotized Fast Blue Salt BN	0.1%	0.5 g

Procedure

1) Prepare two separate 500 ml flasks, each containing 500 ml of 0.1 M sodium acetate. Add sodium alpha-naphthyl phosphate to one bottle. Mix well. Add fast blue B salt to the other flask and wrap with aluminum foil to minimize its exposure to light. Mix well.

2) Aliquot 10 ml of each reagent into 15 ml conical tubes. Wrap fast blue B salt tubes with aluminum foil.

3) Store at -20°C.

Data Log

	source	lot	amount
Sodium Acetate, 0.1 M	_____	_____	_____
Sodium Alpha-Naphthyl Phosphate	_____	_____	_____
Fast Blue B Salt	_____	_____	_____

Quality Control Test
QC100 - Acid Phosphatase Spot Test Reagent

semen dilution	result
N	____
1/2	____
1/4	____
1/8	____
1/16	____
1/32	____
1/64	____
Negative	____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

ALKALINE SUBSTRATE BUFFER

Standard batch size: 8 L Lot Number: _____

Application
p30 ELISA (see Forensic Biology Methods Manual)

Ingredients

	final concentration	amount
Diethanolamine	1.0 M	776 ml
Sodium Azide	0.02%	1.6 g
Magnesium Chloride ($MgCl_2 \bullet 6H_2O$)	0.5 mM	0.8 g
Hydrochloric Acid (concentrated)	N/A	As needed

Procedure

1) Dissolve the diethanolamine, sodium azide, and magnesium chloride in 6400 ml deionized water.

2) Adjust to pH 9.8 (+/- 0.1) with hydrochloric acid (approximately 40-80 ml)

3) Bring to 8 L volume with deionized water.

4) Store between 2-8°C in brown bottle or wrap clear bottle with aluminum foil.

Data Log

	source	lot	amount
Diethanolamine	_____	_____	_____
Sodium Azide	_____	_____	_____
Magnesium Chloride	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control
QC225 - p30 ELISA

Final pH value: _____ (9.8 +/- 0.1)

Results: ☐ Pass ☐ Fail Initials: _____

Cross-reference (date): _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

AMYLASE GEL BUFFER

Standard batch size: 8 L Lot Number: _____

Application
Amylase diffusion presumptive test for saliva (see Forensic Biology Methods Manual)

Ingredients	final concentration	amount
Sodium Phosphate, anhydrous, monobasic (NaH_2PO_4)	0.05 M	12.4 g
Sodium Phosphate, monohydrate, dibasic ($Na_2HPO_4 \cdot H_2O$)	0.05 M	15.6 g
Sodium Chloride	7 mM	0.8 g
10 N NaOH	N/A	As needed
Hydrochloric Acid (concentrated)	N/A	As needed

Procedure

1) Add the ingredients to 7.5 L of deionized water.

2) Adjust pH to 6.9 (+/- 0.1), if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

3) Adjust the final volume to 8 L with deionized water.

4) Store between 2-8°C.

Data Log	source	lot	amount
NaH_2PO_4, anhydrous	_____	_____	_____
Na_2HPO_4, anhydrous	_____	_____	_____
Sodium Chloride	_____	_____	_____
NaOH, 10 N	_____	_____	_____
Hydrochloric Acid (concentrated)	_____	_____	_____

Quality Control
QC105 - Alpha-amylase gel radial diffusion

Standard	Diameter
20 units	_____
2 units	_____
0.2 units	_____
0.02 units	_____
0.002 units	_____
Negative	_____

Final pH value: _____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

CASEIN STOCK SOLUTION

Standard batch size: 3 L Lot Number: _____

Application
p30 ELISA (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Hammerstein Casein	1%	30 g
NaOH, 10 N	N/A	as needed
Phosphate Buffered Saline	50%	1.5 L
Sodium Azide	0.1%	0.3 g

Procedure

1) In an Erlenmeyer flask, thoroughly dissolve the Hammerstein casein with 1.5 L deionized water. Adjust the pH to 8.0 (+/- 0.1) by adding NaOH (drop by drop) to help casein go into solution. Take care to ensure that the solution does not go over pH 8.0. **Do not add acid to lower pH!**

2) Add the PBS and sodium azide.

3) Transfer to a graduated cylinder and increase volume to 3 L.

4) Transfer back into the Erlenmeyer flask and after the solution has mixed, record the final pH.

5) Store at -20°C in 40 ml aliquots.

Data Log

	source	lot	amount
Hammerstein Casein	_____	_____	_____
Sodium Hydroxide	_____	_____	_____
Phosphate Buffered Saline	_____	_____	_____
Sodium Azide	_____	_____	_____

Quality Control
QC225 - p30 ELISA

Final pH value: _____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

COOMASSIE BLUE STAIN

Standard batch size: 1 L

Applications

Ouchterlony radial diffusion-species determination and cross-over electrophoresis-species determination (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Methanol	50%	500 ml
Glacial Acetic Acid	10%	100 ml
Brilliant Blue R	0.1% (w/v)	1.0 g

Procedure

1) Mix together methanol, glacial acetic acid, and 400 ml deionized water.

2) Add brilliant blue R to the solution and stir for several minutes.

3) Filter the solution directly into a storage bottle.

4) Store at room temperature

5) Write your initials and date of make (DOM) on reagent label.

6) Make fresh as needed

Effective Date: 01/07/2005

DESTAIN SOLUTION

Standard batch size: 4 L

Applications
Ouchterlony radial diffusion-species determination and cross-over electrophoresis-species determination (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Methanol	45.5%	1820 ml
Glacial Acetic Acid	9%	360 ml

Procedure

1) Mix together methanol, glacial acetic acid, and 1820 ml deionized water.

2) Transfer to a 4 L storage bottle.

3) Store at room temperature.

4) Write your initials and date of make (DOM) on reagent label.

5) Make fresh as needed.

Effective Date: 01/07/2005

IODINE SOLUTION, 0.01 N

Standard batch size: 500 ml

Application
Amylase diffusion presumptive test for saliva (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
1 N Iodine (Iodine-Iodide Solution)	0.01 N	5 ml

Procedure

1) Mix 5 ml of 1 N iodine with 495 ml deionized water.

2) Store at room temperature in a brown bottle or aluminum foiled glass bottle.

3) Write your initials and date of make (DOM) on reagent label.

Effective Date: 01/07/2005

KASTLE-MEYER (KM) REAGENT

Standard batch size: 3 L Lot Number: _____

Application
Kastle-Meyer (KM) presumptive test for blood (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Phenolphthalin	0.2%	6.0 g
Potassium Hydroxide	0.18 M	30.0 g
Absolute Ethanol (100%)	80%	2400 ml
Zinc Dust	N/A	variable

Procedure
1) In an aluminum-foiled flask, dissolve the phenolphthalin in 600 ml deionized water.
2) Add potassium hydroxide. The phenolphthalin will dissolve.
3) Stir until clear (very light pink is OK)
4) Add the ethanol.
5) Add enough zinc dust to cover the bottom of bottle.
6) Store between 2-8°C in a dark or foiled bottle.

Data Log	source	lot	amount
Phenolphthalin	_____	_____	_____
Potassium Hydroxide	_____	_____	_____
Ethanol	_____	_____	_____
Zinc Dust	_____	_____	_____

Quality Control Test
QC200 - Kastle-Meyer presumptive test for blood (reagent does not have to be sensitive to 1/1,000,000)

Reagent Sensitivity whole blood dilution	Before 3% H_2O_2	After 3% H_2O_2
N	_____	_____
1/10	_____	_____
1/100	_____	_____
1/1,000	_____	_____
1/10,000	_____	_____
1/100,000	_____	_____
1/1,000,000	_____	_____
Negative	_____	_____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

NUCLEAR FAST RED (RED CHRISTMAS TREE STAIN)

Standard batch size: 4 L Lot Number: _____

Application
Christmas Tree stain for spermatoazoa (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Aluminum Sulfate	0.07 M	100.0 g
Nuclear Fast Red	0.05%	2.0 g

Procedure

1) Dissolve the aluminum sulfate in 4 L of warm deionized water and add the nuclear fast red. Stir over low heat overnight. Allow to cool, then filter.

2) Label with a nine (9) month expiration date.

3) Store between 2-8°C.

Data Log	source	lot	amount
Aluminum Sulfate	_____	_____	_____
Nuclear Fast Red	_____	_____	_____

Quality Control
QC150 - Christmas tree stain for spermatozoa

Results: ☐ Pass ☐ Fail Initials: _____

EXPIRATION DATE:

Made By: _____ Date: _____

Effective Date: 01/07/2005

PBS SOLUTION

Standard batch size: 1 L

Application
P30 ELISA (see Forensic Biochemistry Methods Manual)

Ingredients

	amount
Phosphate Buffered Saline (PBS) Tablets	5

Procedure

1) Dissolve the tablets in 1 L of deionized water.

2) Store between 2-8°C**.**

Data Log

	source	lot	amount
PBS Tablets	_____	_____	_____

Quality Control

QC225 - P30 ELISA done only on new shipments of tablets. Subsequent preparations are made at the bench by analysts and do not require completing this sheet.

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

PBS-BSA SOLUTION

Standard batch size: 100 ml

Application
P30 ELISA (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Phosphate Buffered Saline (PBS)	99.99%	100 ml
Bovine Serum Albumin (BSA, Molecular Biology Grade)	0.01%	0.01 g

OR

Phosphate Buffered Saline (PBS)	99.99%	100 ml
Bovine Serum Albumin, 5mg/ml	0.01%	2 ml

Procedure

1) Dissolve the BSA in PBS.

2) Use immediately to prepare stock solution of P30 antigen.

Effective Date: 01/07/2005

PICRIC INDIGO CARMINE (GREEN CHRISTMAS TREE STAIN)

Standard batch size: 2 L Lot Number: _____

Application

Christmas Tree stain for spermatoazoa (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Picric Acid	0.06 M	26 g
Indigo Carmine	0.34%	6.8g

Procedure

CAUTION: PICRIC ACID IS EXPLOSIVE WHEN DRY AND SHOULD BE MAINTAINED WITH MORE THAN 10% dH_2O. WEIGH OUT PICRIC ACID WITH NEGLIGIBLE AMOUNT OF WATER IN WEIGH BOAT.

1) Dissolve the picric acid in 2 L of warm deionized water, add the indigo carmine and stir overnight, then filter.

2) Label with a nine (9) month expiration date.

3) Store between 2-8℃.

Data Log	source	lot	amount
Picric Acid, Saturated	_____	_____	_____
Indigo Carmine	_____	_____	_____

Quality Control

QC150 - Christmas Tree stain for spermatazoa

Results: ☐ Pass ☐ Fail Initials: _____

EXPIRATION DATE:

Made By: _____ Date: _____

Effective Date: 01/07/2005

POSITIVE PDMS CONTROL

Standard batch size: 50 swabs Lot Number: _____

Application
Lubricant Analysis (see Condom Trace Evidence Analysis Manual)

Ingredients		final concentration	amount
Polydimethylsiloxane	(PDMS)	1.0%	0.5 ml
Methylene Chloride		N/A	49.5 ml

Procedure

CAUTION: **Methylene chloride is mutagenic, teratogenic, and carcinogenic. All work with methylene chloride must be done under a chemical fume hood and proper safety equipment (neoprene gloves, lab coat, etc.) must be worn.**

1. Stock solution:

A. Transfer between 0.75mL to 1mL of Polydimethylsiloxane into a 1.5mL microcentrifuge tube using a disposable 3 mL transfer pipette. It may be necessary to cut the bottom of the transfer pipette to achieve a larger hole.

B. Dispense 49.5 mL of QC'd methylene chloride into a 125 mL Erlenmeyer flask. Label the flask as "1% PDMS."

C. Transfer approximately 0.5 mL of PDMS into the Erlenmeyer flask using a disposable 3 mL transfer pipette and rinse pipette tip in the solution. Swirl flask and cover.

2) Preparation of Swabs:

A. Obtain 25 Sterile Cotton-Tipped Applicators. Remove applicators from packaging and place in a suitable holder.

B. Dip each swab, one at a time, into the 1% PDMS stock solution and let dry for 30 minutes. Discard any unused portion of the 1% PDMS stock solution into an organic waste container.

C. Label 50 micro centrifuge tubes with the DOM, lot number, and initials

D. After the swabs have dried, use disinfected scissors and pincers to cut two small pieces off the tip of each swab. Be careful not to cut near the wood stick that the swab is attached to during this process. Place each piece into an individual microcentrifuge tube.

E. Cap all tubes tightly and store in a rack. Label rack with lot number, initials, and date of manufacture (DOM).

Data Log	source lot		amount
Polydimethylsiloxane	_____	_____	_____
Methylene Chloride	_____	_____	_____

Quality control

FTIR analysis - Follow the procedures found in the Condom Trace Evidence Analysis Manual to obtain a FT-IR spectrum of the positive control. Perform a library search. Search results must indicate PDMS as the first "hit." Print and include the results of this search.

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

POTASSIUM CYANIDE SOLUTION (KCN), 0.05%

Standard batch size: 200 ml

Application
Hemoglobin (Hb) by IEF (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Potassium Cyanide	0.05%	0.1 g

Procedure

CAUTION: POTASSIUM CYANIDE IS A TOXIC COMPOUND THAT CAN BE ABSORBED BY CONTACT WITH SKIN OR BY INHALATION. USE ADEQUATE PROTECTION, INCLUDING LAB COAT, GLOVES, AND EYE PROTECTION, WHEN HANDLING THIS COMPOUND.

1) Dissolve the potassium cyanide in 200 ml of deionized water.

2) Store at room temperature.

3) Make fresh as needed.

4) Write your initials and date of make (DOM) on reagent label.

Effective Date: 01/07/2005

SALINE (0.85% NaCl)

Standard batch size: 10 L

Application
Ouchterlony radial diffusion-species determination and cross-over electrophoresis-species determination (See Forensic Biolochemistry Methods Manual).

Ingredients	final concentration	amount
Sodium Chloride	0.85%	85.0 g

Procedure

1) Dissolve the sodium chloride in 10 L of deionized water in a carboy.

2) Store at room temperature.

3) Make fresh as needed.

4) Write your initials and date of make (DOM) on reagent label.

Effective Date: 01/07/2005

SODIUM ACETATE, 0.1M (pH 5.5)

Standard batch size: 1 L Lot Number: _____

Application
Acid Phosphatase presumptive test for semen (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Sodium Acetate, Anhydrous	0.1 M	8.21 g
Glacial Acetic Acid	-----	as needed

Procedure

1) Dissolve the sodium acetate in 900 ml of deionized water.

2) Adjust pH to 5.5 (+/- 0.1) with glacial acetic acid.

3) Transfer solution to a graduated cylinder and bring the volume up to 1 L. Mix well.

4) Store at room temperature.

Data Log	sourse	lot	amount
Sodium Acetate, Anhydrous	_____	_____	_____
Glacial Acetic Acid	_____	_____	_____

Final pH Value _____ (5.5 +/- 0.1)

Made By: _____ Date: _____

Effective Date: 01/07/2005

SPECIES AGAROSE GEL

Standard batch size: 150 ml (variable number of aliquots)

Application
Ouchterlony radial diffusion-species determination and cross-over electrophoresis-species determination (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Species Tank Buffer	50%	150 ml
Sigma Type I Agarose (or equivalent)	1%	3 g

Procedure

1) Mix species tank buffer with 150 ml deionized water.

2) Dissolve Sigma type I agarose (or equivalent) in the solution by heating on a stir plate.

3) Once solution is clear, dispense 7 ml aliquots into 20 x 150 mm test tubes.

4) Gel can be used immediately or may be stored covered with Parafilm at 2-8^{o}C.

Effective Date: 01/07/2005

SPECIES TANK BUFFER

Standard batch size: 1 L

Application

Ouchterlony radial diffusion-species determination and crossover electrophoresis-species determination (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Barbital (sodium salt)	0.05 M	8.76 g
Barbital (free acid)	7 mM	1.28 g
Calcium Lactate	0.07 M	0.38 g
10 N NaOH	----	as needed
Hydrochloric Acid (concentrated)	----	as needed

Procedure

1) Dissolve barbital (sodium salt and free acid), and calcium lactate in 800 ml deionized water.

2) Adjust the pH to 8.6, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

3) Dilute to 1 L with deionized water.

4) Store at room temperature.

5) Make fresh as needed.

6) Write your initials and date of make (DOM) on reagent label.

Effective Date: 01/07/2005

UREA DIFFUSION TEST AND BLANK PLATES

Standard batch size: 613.5 ml (10 plates)

Application
Urea diffusion presumptive test for urine (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Agarose, type 1	1%	6 g
Bromothymol Blue, 1.5%	1%	6 ml
Urease (3 U/ml)	1.2%	7.5 ml

Procedure
1) Dissolve the agarose into 600 ml of boiling deionized water.
2) Add the bromothymol blue solution to the dissolved agarose.
3) Allow the solution to cool to 50^{o}C.
4) Separate the solution into two 300 ml portions.
5) To one portion, add the urease solution.
6) Dispense 30 ml aliquots of both solutions into 10 cm^2 petri dishes and allow to solidify.
7) Store between 2-8°C.

Data Log	source	lot	amount
Agarose, Type 1	_____	_____	_____
Bromothymol Blue	_____	_____	_____
Urease	_____	_____	_____

Quality Control
QC305 - Urea Gel Diffusion is done on new shipments of urease.

Standard	Diameter	Concentration
Urea, 5%	_____	
Urea, 0.5%	_____	
Urea, 0.05%	_____	
Urea, 0.005%	_____	
Negative	_____	
Urine stain, N	_____	_____
Urine stain, 1/10 dilution	_____	_____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

UREASE, 3 U/ml

Standard batch size: 100 ml Lot Number: _____

Application
Urea diffusion presumptive test for urine (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Urease	3 U/ml	~10 mg (see calculation)
Deionized Water	----	100 ml

Procedure

1) Add the Urease to 100 ml of deionized water.
2) Mix so that all of the Urease is dissolved into solution.
3) Make fresh for each batch of urea diffusion plates.

Calculation
300 U (units) x concentration of vendor urease (g/U) = amount of Urease to add.

Quality Control
QC305 - Urea Gel Diffusion (done only on new vendor lot/shipment of urease)

Note: Use "Urea Diffusion Test and Blank Plates" reagent sheet for documentation.

Effective Date: 01/07/2005

BSA SOLUTION, 5 mg/ml

Standard batch size: ~50 tubes x 0.1g Lot Number: _____

Application

YM1 STR Reaction Mix (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Bovine Serum Albumin (BSA, molecular biology grade)	2.5% (w/v)	125 mg
Sterile Deionized Water	N/A	25 ml (guideline)

Procedure

1) Autoclave a 50 ml glass beaker with a stir bar in it.
2) Add the BSA to 20 ml of sterile water in the glass beaker.
3) Stir gently over **very low** heat until the BSA is completely dissolved.
4) Add the solution to a 50 ml disposable conical tube.
5) Add sterile water to a final volume of 25 ml.
6) Aliquot approximately 0.5 ml of BSA solution into 1.5 ml microcentrifuge tubes.
7) Label each tube with "BSA" and the lot number.
8) Store at -20°C.

Data Log

	source	lot	amount
BSA	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

Procedure 1 (test 20 µL of solution)

Results: ☐ Pass☐ Fail Initials: _____

QC240 - YM1 STR/PCR Amplification
QC165 - STR gel electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

CHELEX, 5%

Standard batch size: 800ml Lot Number: _____

Application
DNA Extraction (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Chelex 100	5%	40 g
Sterile Deionized Water	----	600 ml

Procedure

1) Filter sterilize approximately 900 ml deionized water. The water will evaporate off in step 4.
2) Pour the water into a 1L bottle.
3) Save the bottom container from the disposable filter unit.
4) Autoclave the water at 250°F for 30 minutes.
5) Add 40g of the Chelex 100 to the bottom container of the filter unit.
6) Allow the water to cool after autoclaving.
7) Add sterile water to the Chelex 100 to a volume of 800 ml using the graduation markings on the disposable filter container.
8) Mix on a magnetic stir plate.
9) While the stock solution is mixing, aliquot 10 ml each into 50 ml conical tubes.
10) Label each tube with its contents, date of make (DOM), your initials, and date.
11) Store at 2-8°C.

Data Log	source	lot	amount
Chelex 100	_____	_____	_____

Quality Control

Procedure 4

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

CHELEX, 20%

Standard batch size: 500ml Lot Number: _____

Application
DNA Extraction (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Chelex 100	20%	100 ± 2 g
Sterile Deionized Water	----	450 ± 50 ml (guideline)

Procedure

1) Filter sterilize approximately 600 ml deionized water. The water will evaporate off in step 4.
2) Pour the water into a 500 ml bottle.
3) Save the bottom container from the disposable filter unit.
4) Autoclave the water at 250°F for 30 minutes.
5) Add the Chelex to the bottom container of the filter unit.
6) Allow the water to cool after autoclaving.
7) Add sterile water to the Chelex to a volume of 500 ml using the graduation markings on the disposable filter container.
8) Mix on a magnetic stir plate.
9) While the stock solution is mixing, aliquot 10 ml each into 50 ml conical tubes.
10) Label each tube with its contents, date of make (DOM), your initials, and date.
11) Store at 2-8°C.

Data Log	source	lot	amount
Chelex 100	_____	_____	_____

Quality Control
Procedure 4 – Differential Extraction

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

CHROMOGEN SOLUTION

Application
QuantiBlot Hybridization (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Chromogen:TMB	0.2%	60 mg
Ethanol, 100% Reagent Grade	--------	30 ml

Procedure

CAUTION: DO NOT USE ETHANOL STORED IN A METAL CONTAINER; ONLY USE 100% REAGENT GRADE ETHANOL.

1) Bring bottle of Chromogen:TMB to room temperature.
2) Before opening, lightly tap the bottle on the counter to bring its contents to the bottom.
3) Carefully remove the stopper and reconstitute the chromogen:TMB with the room temperature ethanol.
4) Recap the bottle and seal with Parafilm.
5) Tilt the bottle several times to ensure that all the powder is removed from within the rubber cap.
6) Shake on an orbital shaker for 30 minutes or longer.
7) Write your name and Date Of Make (DOM) on the reagent label.
8) Store at 2-8°C and away from rust.
9) The solution is stable for six months.

Quality Control
Procedure 1 – QuantiBlot Hybridization is done on new lots/shipment of Chromogen and is documented on F183 Raw Material Quality Control Test Form.

Effective Date: 01/07/2005

DEOXYNUCLEOTIDE TRIPHOSPHATES, 2.5 mM (dNTPs)

Standard batch size: ~32 tubes x 1000 µL Lot Number: _____

Application
YM1 STR reaction mix

Ingredients	final concentration	amount
dATP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
dCTP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
dGTP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
dTTP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
Autoclaved, microcentrifuge tubes		~32 tubes

Procedure

NOTE: ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USE ONLY FILTER-PIPET TIPS OR A REPEAT PIPETTOR FOR ALL PIPETTING.

1) Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.
2) Pool together the manufacturers' shipment of all dNTPs into a 50 ml falcon tube. Mix well.
3) While wearing clean gloves, remove all 1.5 ml microcentrifuge tubes from the bag and place them in a clean rack designated for the PCR preparation room only.
4) Aliquot 1000 µL of dNTP mix into each tube.
5) Once aliquotting is complete, cap all tubes and store in a labeled rack away from all sources of DNA. Label each tube with lot number and item description.
6) Store frozen at -20°C.

Data Log	source	lot	amount
dATP	_____	_____	_____
dCTP	_____	_____	_____
dGTP	_____	_____	_____
dTTP	_____	_____	_____

Quality Control
Procedure 2

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

DIGEST BUFFER

Standard batch size: 4L Lot Number: _____

Application
Organic Extraction procedure (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
EDTA, 0.5 M	10 mM	80 ± 4 ml
TRIS	10 mM	4.8 ± 0.4 g
Sodium Chloride	50 mM	11.6 ± 0.8 g
SDS, 20%	2.0%	400 ± 4 ml
Hydrochloric Acid	-----	As needed

Procedure

1) Add the EDTA, TRIS, sodium chloride, and SDS to approximately 1.5 L deionized water.
2) Adjust the pH to 7.5 (± 0.1) with hydrochloric acid.
3) Bring up to the final volume with deionized water and mix well.
4) Measure and record the final pH.
5) Aliquot into 50 ml conical tubes.
6) Label each tube with its contents, date of make (DOM), your initials, and date.
7) Store at room temperature.

Data Log	source	lot	amount
EDTA, 0.5 M	_____	_____	_____
TRIS	_____	_____	_____
Sodium Chloride	_____	_____	_____
SDS, 20%	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control
Procedure 1 (Test 20 µL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

Final pH: _____ (7.5 ± 0.1)

Made By: _____ Date: _____

Effective Date: 01/07/2005

DITHIOTHREITOL (DTT), 1M

Standard batch size: 20 ml Lot Number: _____

Application
Differential Extraction (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
Dithiothreitol	1.0 M	3.06 ± 0.05 g
Sterile Deionized Water	----	15 ml

Procedure

1) Add the DTT to approximately 15 ml sterile deionized water in a 50 ml conical tube.
2) Mix well by vortex agitation.
3) When the DTT is dissolved, bring up to 20 mL with sterile deionized water.
4) Filter sterilize.
5) Dispense 250 µL aliquots into sterile 1.5 ml microcentrifuge tubes.
6) Label with a four-month expiration date, contents, and the lot number.
7) Store at -20°C.

Data Log	source	lot	amount
Dithiothreitol	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

Procedure 1 (Test 20 µL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

EXPIRATION DATE:

Made By: _____ Date: _____

Effective Date: 01/07/2005

ETHLYENE-DIAMINE-TETRA-ACETIC ACID (EDTA), 0.5M

Standard batch size: 3L Lot Number: _____

Application
Preparation of Tris-EDTA, 1X and Digest Buffer (see Quality Manual)

Ingredients	final concentration	amount
EDTA	0.50 M	558 ± 1 g
Sodium Hydroxide, 10 N	------	variable

Procedure

1) Add the EDTA to approximately 1500 ml deionized water.
2) Adjust the pH to 8.0 (± 0.1) with sodium hydroxide solution.
3) Mix well.
4) The EDTA will dissolve as the pH reaches 8.0.
5) Bring up to volume with deionized water by using a graduated cylinder.
6) Check and record the final pH.
7) Autoclave at 250°F for 20 minutes.
8) Store at room temperature.

Data Log	source	lot	amount
EDTA	_____	_____	_____
Sodium Hydroxide, 10 N	_____	_____	_____

Quality Control

Final pH: _____ (8.0 ± 0.1)

Made By: _____ Date: _____

Effective Date: 01/07/2005

FORMAMIDE, DEIONIZED

Standard batch size: ~36 tubes x 1400 µL Lot Number: _____

Application
STR Analysis on the ABI Prism 310 Capillary Electrophoresis Genetic Analyzer (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Formamide (super pure grade)	N/A	50 ml

Procedure

CAUTION: THIS PROCEDURE MUST BE PERFORMED UNDER THE CHEMICAL FUME HOOD! FORMAMIDE IS HARMFUL BY INHALATION, INGESTION, AND SKIN ABSORPTION. WEAR GLOVES, EYE GLASSES, AND LAB COAT.

1) Make sure that you are using a super pure grade of formamide. Super pure grade formamide has been pre-treated with a mixed-bed resin (available from commercial supplier).

2) Dispense the deionized formamide into 1.5 ml reaction tubes in aliquots of 500µL and store at -15 to -20°C.

3) Label the tube rack with the lot number, the date of make (DOM), and initials.

Data Log	source	lot	amount
Formamide	_____	_____	_____

Quality Control
Procedure 6

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

NEGATIVE FEMALE CONTROL DNA

Standard batch size: 10 ml Lot Number: _____

Application - YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Procedure

1) Stock solution:

For the stock solution, extract a 3mm x 3mm portion of a dried bloodstain from a female or 1/3 of an oral swab also from a female following the organic extraction procedure in the Protocols for Forensic STR Analysis Manual. Adjust the final volume to 200µL. Submit a 1/100 and a 1/ 1000 dilution for QuantiBlot.

Data Log	Source	Date prepared	DNA concentration
Bloodstain or Oral Swab	_____	_____	_____

2) Working solution:

Based on the Quantiblot results prepare a conical tube with 10 ml of a dilution with a concentration of 2.5ng/20µL.

Use the following formula: **C1 x V1 = C2 x V2**
(z)(DNA concentration) = (10,000 µL)(2.5 ng/20 µL)
z = required volume of DNA per ml

Prepare a 15 ml conical tube with TE^{-4} (10 mL - the req. DNA vol.). Add the DNA to the tube.

Submit 20µL from the tube for QuantiBlot. The Quantiblot result should be 2.5ng. Discard the tube if the reading is <1.25. A tube with a reading of 1.25 or 5 ng should be amplified and checked if the expected peak heights of 500-3000 RFUs can be achieved. Discard the tubes with peak heights outside of this peak height range.

Data Log	source	lot	amount
DNA stock	_____	_____	_____
TE^{-4}	_____	_____	_____

Quality control
Procedure 3

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

ORGANIC EXTRACTION BUFFER

Standard batch size: 1 L Lot Number: _____

Application
Organic Extraction (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
Tris	10 mM, pH 8.0	1.2 g
EDTA, 0.5M	50 mM, pH 8.0	100 mL
NaCl	100 mM	5.8 g

Procedure

1. Measure 500 ml deionized water into a beaker.
2. Add 1.2 g Tris and dissolve with agitation and mild heat.
3. Check the pH and adjust to approximately 7.5 (+/- 0.1) by adding either HCl or NaOH dropwise.
4. Add 100 mL EDTA and allow it to dissolve.
5. Add 5.8 g NaCl to the solution and allow it to dissolve.
6. Adjust the volume to 1 L with ddH_2O.
7. Autoclave the solution for 25 minutes.
8. Transfer into labeled 50 mL conical vials.

Data Log	source	lot	amount
Tris	_____	_____	_____
EDTA	_____	_____	_____
NaCl	_____	_____	_____

Quality Control

Final pH: _____ (7.5 ± 0.1)

Procedure 1 (Test 20 µL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

PCR REACTION MIXTURE (COFILER AND PROFILER PLUS)

Standard batch size: 100-1000 tubes x 20 µL Lot Number: _____

Application
Cofiler and Profiler PCR Amplification (see Protocols for Forensic STR Analysis)

Ingredients	Final Conc	1 Tube Amount	100 Tubes	400 Tubes	800 Tubes	1000 Tubes
PCR Reaction Mix	1x	20µL	2,000µL	8,000µL	16,000µL	20,000µL
AmpliTaq Gold	5U	1µL	100µL	400µL	800µL	1,000µL

Procedure

NOTE: ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

1) Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.
2) Add the ingredients to either a 1.5 ml microcentrifuge tube or a 15 ml conical tube using pipetmen dedicated to PCR preparation area only. Vortex and spin the reaction mixture briefly.
3) While wearing clean gloves, remove a sufficient amount of 0.2 ml PCR reaction tubes from the bag and place them in a clean rack designated for the PCR prep room only.
4) Add 20 µL per tube using a designated repeat pipettor or tips with hydrophobic filters.
5) Cap all tubes and store in a labeled rack away from all sources of DNA.
6) Store at 2-8℃.

Data Log	source	lot	amount
Reaction Mix	_____	_____	_____
AmpliTaq Gold	_____	_____	_____

Quality Control
Procedure 2 – Only for the first kit of each shipment/lot

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

PHOSPHATE BUFFERED SALINE (PBS)

Standard batch size: 4L Lot Number: _____

Application
Chelex Extraction (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Phosphate Buffered Saline (PBS) Tablets	N/A	20

Procedure

1) Dissolve the tablets in 4 liters of deionized water.
2) Measure and record the final pH (should be 7.5 ± 0.1).
3) Autoclave at 250°F for 20 minutes. Allow to cool.
4) Dispense into 50 ml conical tubes. Label each tube with contents, DOM, and your initials.
5) Store at room temperature.

Data Log	source	lot	amount
PBS Tablets	_____	_____	_____

Quality Control

Final pH: _____ (7.5 ± 0.1)

Procedure 1

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

POSITIVE MALE CONTROL DNA

Standard batch size: 10 ml Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Procedure

1) Stock solution:
Extract a 3mm x 3mm portion of a dried bloodstain following the organic extraction procedure in the "Protocols for Forensic STR Analysis" manual. Adjust the final volume to 200µL. Submit a 1/100 and a 1/1000 dilution for QuantiBlot.

Data Log	Source	Date prepared	DNA concentration
Bloodstain	RCS	_____	_____

2) Working solution:

Based on the Quantiblot results prepare a conical tube with 10 ml of a dilution with a concentration of 2.5 ng/20µL.

Use the following formula: **C1 x V1 = C2 x V2**
(z)(DNA concentration) = (10,000 µL)(2.5 ng/20 µL)
z = required volume of DNA per ml

Prepare a 15 ml conical tube with TE^{-4} (10 mL - the req. DNA vol.). Add the DNA to the tube.

Submit 20µL from the tube for QuantiBlot. The Quantiblot result should be 2.5ng. Discard the tube if the reading is <1.25. A tube with a reading of 1.25 or 5 ng should be amplified and checked if the expected peak heights of 500-3000 RFUs can be achieved. Discard the tubes with peak heights outside of this peak height range.

Data Log	source	lot	amount
DNA stock	_____	_____	_____
TE^{-4}	_____	_____	_____

Quality control
Procedure 3

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

PRIMER, DYS19/1 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence NED - 5' CTA CTG AGT TTC TGT TAT AGT 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS19/1 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure
1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log

	source	lot	amount
Primer DYS19/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculations checked by _____

Quality Control
Procedure 2

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

PRIMER, DYS19/2 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data
Sequence 5' ATG GCA TGT AGT GAG GAC A 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS19/2 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure
1) Add the sterile deionized water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log

	source	lot	amount
Primer DYS19/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
Procedure 2

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

PRIMER, DYS389/1 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data
Sequence NED - 5' CCA ACT CTC ATC TGT ATT ATC T 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS389/1 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer DYS389/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
Procedure 2

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

PRIMER, DYS389/2 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data
Sequence 5' TCT TAT CTC CAC CCA CCA GA 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS389/2 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer DYS389/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
Procedure 2

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

PRIMER, DYS390/1 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data
Sequence 6-FAM - 5' TAT ATT TTA CAC ATT TTT GGG CC 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS390/1 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer DYS390/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
Procedure 2

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

PRIMER, DYS390/2 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data
Sequence 5' TGA CAG TAA AAT GAA CAC ATT GC 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS390/2 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer DYS390/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
Procedure 2

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

QUANTIBLOT CITRATE BUFFER

Standard batch size: 8 L Lot Number: _____

Ingredients

	final concentration	amount
(Tri)sodium Citrate	0.06 M	147.2 ± 0.2 g
Citric Acid	0.025 M	43.4 ± 2 g (guideline)

Procedure

1) Dissolve the sodium citrate in approximately 6 L deionized water in a carboy.
2) Adjust the pH to 5.0 (± 0.2) by addition of citric acid (approximately 40 g).
3) Adjust the final volume to 8 liters with deionized water.
4) Mix well.
5) Measure and record the final pH (must be 5.0 ± 0.2).
6) Aliquot into amber bottles and store at room temperature.

Data Log	source	lot	amount
Trisodium Citrate	_____	_____	_____
Citric Acid	_____	_____	_____

Quality Control

Final pH: _____ (5.0 ± 0.2)

Made By: _____ Date: _____

Effective Date: 01/07/2005

QUANTIBLOT DNA STANDARDS

Standard batch size: variable Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
DNA Standard A	varies	1000 µl
TE^{-4}, 1X	1X	3000 µl

Procedure
1. Pool the contents of four DNA Standard A tubes (use all from one lot number). Each tube contains 250 µL of standard. Vortex tubes to mix thoroughly and centrifuge briefly.
3. Label seven sterile1.5 ml microcentrifuge tubes, A - G.
4. Aliquot 500 µL of 1X TE^{-4} into the six tubes labeled B - G.
5. **Tube A:** Transfer 1000 µL of DNA Standard A into the tube labeled A. This is now DNA Standard A. **Tube B:** Add 500 µL of DNA Standard A to the 500 µL of 1X TE^{-4} in tube B. Vortex to mix thoroughly then centrifuge briefly. **Tube C:** Add 500 µL of DNA Standard B to the 500 µL of 1X TE^{-4} in tube C. Vortex to mix thoroughly then centrifuge briefly. Continue the serial dilution through tube 1G.
6. Store at 2° to 8°C. Label with an expiration date of 3 months.

Standard Tube	Concentration (ng/µL)	Quantity (ng/5µL)
1A	2	10
1B	1	5
1C	0.5	2.5
1D	0.25	1.25
1E	0.125	0.625
1F	0.0625	0.3125
1G	0.03125	0.15625

Data Log	source	lot	amount
DNA Standard A	_____	_____	_____
TE^{-4}, 1X	_____	_____	_____

EXPIRATION DATE:

Quality Control
Procedure 1

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

QUANTIBLOT HYBRIDIZATION SOLUTION

Standard batch size: 6 L Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
SSPE, 20X	5.0 X	1500 ± 10 ml
SDS, 20%	0.50 %	150 ± 1 ml

Procedure

1) Combine the SSPE and 4350 ml deionized water into a carboy.
2) Add the SDS.
3) Warm the solution until all solids are dissolved.
4) Mix well.
5) Dispense into 1 L pre-labeled bottles.
6) Store at room temperature.

Data Log	source	lot	amount
SSPE, 20X	_____	_____	_____
SDS, 20%	_____	_____	_____

Quality Control

Procedure 1

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

QUANTIBLOT PRE-WETTING SOLUTION

Standard batch size: 8 L Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
NaOH, 10 N	0.4 N	320 ± 10 ml
EDTA, 0.5 M	25 mM	400 ± 10 ml

Procedure

1) Measure 7280 ml deionized water into a carboy.
2) Add 320 ml NaOH and 200 ml EDTA.
3) Mix well.
4) Dispense into 1 L pre-labeled bottles.
5) Store at room temperature.

Data Log	source	lot	amount
NaOH, 10 N	_____	_____	_____
EDTA, 0.5 M	_____	_____	_____

Made By: _____ Date: _____

Effective Date: 01/07/2005

QUANTIBLOT SPOTTING SOLUTION

Standard batch size: 6 bottles Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Pre-Wetting Solution	-----	449 ml (± 1ml)
Bromothymol Blue, 0.04%	0.00008%	900 µL (± 1 µL)

Procedure

1) Measure 449 ml Pre-Wetting Solution in a graduated cylinder and pour into a 1L Erlenmeyer flask.

2) Add 900 µL bromothymol blue.

3) Mix well.

4) Aliquot 75 mL of the solution into 125 mL bottles.

5) Store at room temperature.

Data Log

Bottles made: _____

	source	lot	amount
Pre-Wetting Solution	_____	_____	_____
Bromothymol Blue, 0.04%	_____	_____	_____

Made By: _____ Date: _____

Effective Date: 01/07/2005

QUANTIBLOT WASH SOLUTION

Standard batch size: 20 L Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
SSPE, 20X	2.5 X	2500 ± 50 ml
SDS, 20%	0.10 %	100 ± 5 ml

Procedure

1) Add 2500 ml SSPE and 17.4 L deionized water into a carboy.
2) Add in 100 ml 20% SDS.
3) Mix well.
4) Aliquot into five 4L brown, pre-labeled bottles.
5) Store at room temperature.

Data Log	source	lot	amount
SSPE, 20X	_____	_____	_____
SDS, 20%	_____	_____	_____

Quality Control

Procedure 1

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

SODIUM DODECYL SULFATE (SDS), 0.1%

Standard batch size: 20 L

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
Sodium Dodecyl Sulfate (SDS), 20%	0.1 %	100 ± 10 ml

Procedure

NOTE: This solution is "made at the bench," no reagent sheet is required.

1) Add approximately 15 L of deionized water into a 20 L carboy.

2) Add 100 ml 20% SDS. Mix.

3) Bring up to a final volume of 20 L with deionized water. Mix.

4) Store at room temperature.

Effective Date: 01/07/2005

SSPE, 20X

Standard batch size: 8 L Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
EDTA, Disodium Salt	20 mM	59.6 ± 1.4 g
Sodium Hydroxide, 10 N	-----	150 ± 10 ml (guideline)
Sodium Phosphate, Monobasic	200 mM	220 ± 6 g
Sodium Chloride	3.6 M	1680 ± 20 g

Procedure

1) Dissolve the EDTA in approximately 6 liters deionized water (use SSPE carboy).
2) Add 80 mL 10N sodium hydroxide to help dissolve the EDTA.
3) Add the sodium phosphate first and then the sodium chloride.
4) Adjust the pH to 7.4 with 10N sodium hydroxide (about 70ml).
5) Adjust the final volume to 8 liters with deionized water.
6) Measure and record the final pH.
7) Store at room temperature.

Data Log	source	lot	amount
EDTA, Disodium Salt	_____	_____	_____
Sodium Hydroxide, 10N	_____	_____	_____
Sodium Phosphate, Monobasic	_____	_____	_____
Sodium Chloride	_____	_____	_____

Quality Control

Final pH: _____ (7.4 ± 0.2)

Made By: _____ Date: _____

Effective Date: 01/07/2005

STERILE DEIONIZED WATER

Standard batch size: 4L Lot Number: _____

Application
DNA Extraction (see Protocols for Forensic STR Analysis)

Procedure

1) Filter sterilize 4 L of deionized water.

2) Autoclave at 250°F for 20 minutes.

3) Aliquot 30 ml each into 50 ml centrifuge tubes (133 tubes). Label each tube with its contents, the date of make (DOM), and your initials.

4) Store at room temperature.

Quality Control

Procedure 1 (test 20 µL of Solution)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

TRIS-EDTA (TE^{-4}), 1X

Standard batch size: 1 L Lot Number: _____

Ingredients	final concentration	amount
TRIS-HCl, pH 8.0, 1 M	10 mM	10.0 ± 0.3 ml
EDTA, 0.5 M	0.1 mM	200± 2µL

Procedure

1) Add the TRIS and EDTA to 990 ml deionized water. Mix well and filter.
2) Autoclave at 250°F for 20 minutes.
3) Dispense into 50 ml sterile centrifuge tubes. Label each tube with its contents, the date of make (DOM), and your initials.
4) Store at room temperature.

Data Log	source	lot	amount
TRIS-HCl, pH 8.0, 1 M	_____	_____	_____
EDTA, 0.5 M	_____	_____	_____

Quality Control

Final pH: _____ (8.0 ± 0.2)

Procedure 1 (test 20 µL of Solution)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

TRIS-HCl, 1M - pH 8.0

Standard batch size: 500 ml Lot Number: _____

Ingredients

	final concentration	amount
TRIS	1.00 M	60.5 ± 0.1 g
Hydrochloric Acid	------	variable

Procedure

1) Add the TRIS to approximately 400 ml deionized water.

2) Mix well.

3) Adjust the pH to 8.0 with concentrated hydrochloric acid.

4) Bring up to final volume with deionized water.

5) Measure and record the final pH.

6) Prepare a 1/100 dilution (10 mM TRIS-HCl) by mixing 1 ml TRIS-HCl solution and 99 ml deionized water.

7) Autoclave at 250°F for 20 minutes.

8) Store at room temperature.

Data Log	source	lot	amount
TRIS	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control

Final pH: _____ (8.0 ± 0.1)

Made By: _____ Date: _____

Effective Date: 01/07/2005

UREA (10.8 g)

Standard batch size: ~ 25 tubes x 10.8 g Lot Number: _____

Application
Isoelectric Focusing Hemoglobin Plates (see Quality Assurance Manual)

Ingredients	Aliquot	Total Amount
Urea (Electrophoresis Grade)	10.8 ± 0.1 g	270 ± 2.5 g

Procedure

CAUTION: WHEN WORKING WITH POWDERED UREA WEAR GLOVES, EYE PROTECTION, LAB COAT, AND RESPIRATOR FOR SAFETY.

1) Using small weigh boat, weigh 10.8 ± 0.1 g aliquots of urea.

2) Transfer the aliquots to labeled 50 ml conical tubes.

3) Cap all tubes tightly and store in a rack. Label with contents, lot number, date, and your initials.

4) Store at room temperature.

Data Log	source	lot	amount
Urea	_____	_____	_____

Quality Control

Isoelectric Focusing Hemoglobin Plates

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

YM1 STR/PCR REACTION MIXTURE (Page 1 of 2)

Standard batch size: 50 to 200 tubes Lot Number: _____

Ingredients:

	Final Concentration	1 Tube Amount	50 Tubes	100 Tubes	200 Tubes
10X PCR Buffer II	1X	5 µL	250 µL	500 µL	1000 µL
dNTP's (2.5 mM)	200 µM	4 µL	200 µL	400 µL	800 µL
sterile dH_20	--------	7.4µL	370 µL	740 µL	1480µL
BSA (5 mg/ml)	160µg/ml	1.6 µL	80 µL	160 µL	320 µL
DYS19/1 (50 pM/µL)	0.24 µM	0.24 µL	12 µL	24 µL	48 µL
DYS19/2 (50 pM/µL)	0.24 µM	0.24 µL	12 µL	24 µL	48 µL
DYS390/1 (50 pM/µL)	0.24µM	0.24 µL	12 µL	24 µL	48 µL
DYS390/2 (50 pM/µL)	0.24 µM	0.24 µL	12 µL	24 µL	48 µL
DYS389/1 (50 pM/µL)	0.12 µM	0.12 µL	6 µL	12 µL	24 µL
DYS389/2 (50 pM/µL)	0.12 µM	0.12 µL	6 µL	12 µL	24 µL
AmpliTaq Gold (5u/µL)	4 U	0.8 µL	40 µL	80 µL	160 µL
TOTAL		20 µL	1 ml	2 ml	4 ml

Procedure

NOTE: ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

1) Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

2) Add the ingredients to either a microcentrifuge tube or a 15 ml centrifuge tube using pipetmen dedicated to PCR preparation area only.

3) While wearing clean gloves, remove sufficient amount of tubes from the bag and place them in a clean rack designated for the PCR prep room only.

(Next Page)

YM1 STR/PCR REACTION MIXTURE **(Page 2 of 2)**

Procedures (continued)

4) Vortex and spin briefly. Add 20 µL per 0.2ml tube using a dedicated repeat pipettor or tips with hydrophobic filters.

5) Cap all tubes and store in a labeled rack away from all sources of DNA.

6) Store at 2-8℃.

Data Log	source	lot	amount
10X PCR Buffer II	_____	_____	_____
dNTP's (2.5 mM)	_____	_____	_____
Sterile dH_20	_____	_____	_____
BSA (5 mg/ml)	_____	_____	_____
DYS19/1 (50 pM/µL)	_____	_____	_____
DYS19/2 (50 pM/µL)	_____	_____	_____
DYS390/1 (50 pM/µL)	_____	_____	_____
DYS390/2 (50 pM/µL)	_____	_____	_____
DYS389/1 (50 pM/µL)	_____	_____	_____
DYS389/2 (50 pM/µL)	_____	_____	_____
AmpliTaq Gold (5u/µL)	_____	_____	_____

Made By: _____ Date: _____

Effective Date: 01/07/2005

CHELEX, 5% (for Mitochondrial DNA Analysis)

Standard batch size: 800mL Lot Number: _____

Application
Mitochondrial DNA Analysis

Ingredients

	final concentration	amount
Chelex 100	5%	40 g
Sterile Deionized Water	----	600 mL

Procedure

1)Filter sterilize approximately 900 mL deionized water. The water will evaporate off in step 4.
2)Pour the water into a 1L bottle.
3)Save the bottom container from the disposable filter unit.
4)Autoclave the water at 250□F for 30 minutes.
5)Add 40g of the Chelex 100 to the bottom container of the filter unit.
6)Allow the water to cool after autoclaving.
7)Add sterile water to the Chelex 100 to a volume of 800 ml using the graduation markings on the disposable filter container.
8)Mix on a magnetic stir plate.
9)While the stock solution is mixing, aliquot 10 mL each into 50 mL conical tubes.
10)Label each tube with its contents, date of make (DOM), your initials, and date.
11)Store at 2-8□C.

Data Log	source	lot	amount
Chelex 100	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control
QC500 Chelex Extraction (Mitochondrial DNA Analysis)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

DITHIOTHREITOL (DTT), 1M (for Mitochondrial DNA Analysis)

Standard batch size: 20 mL Lot Number: _____

Application
Differential Extraction

Ingredients	final concentration	amount
Dithiothreitol	1.0 M	3.06 ± 0.05 g
Sterile Deionized Water	----	19 mL

Procedure
1) Add the DTT to approximately 19 ml sterile deionized water in a 50 mL conical tube.
2) Mix well by vortex agitation.
3) When the DTT is dissolved, bring up to volume with sterile deionized water.
4) Filter sterilize.
5) Dispense 250 µL aliquots into sterile 1.5 mL microcentrifuge tubes.
6) Label with a four-month expiration date, contents, and the lot number.
7) Store at -20^{O}C.

Data Log	source	lot	amount
Dithiothreitol	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

Results: ☐ Pass ☐ Fail Initials: _____

EXPIRATION DATE:

Made By: _____ Date: _____

Effective Date: 01/07/2005

ETHIDIUM BROMIDE

Standard batch size: 1.5L (0.5 ug/mL) Lot Number: _____

Application
Product yield gel

Ingredients

	final concentration	amount
Ethidium Bromide	10mg/mL	75 µL
Deionized Water	—	1.5 L

Procedure

CAUTION: Ethidium Bromide is a known CARCINOGEN!! Protective gloves, eye protection and lab coat must be worn when handling!!

1) Add 75µL of 10mg/mL Ethidium Bromide stock to 1.5L deionized water.
2) Dispense into Ethidium Bromide bath tray and gently rock to mix.
3) All Ethidium Bromide waste must be disposed of properly!

Data Log	sourse	lot	amount
Ethidium Bromide	_____	_____	_____
Deionized Water	_____	_____	_____

Made By: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, A1 (100 µM) Lot number: _____

Physical data

Sequence 5' CAC CAT TAG CAC CCA AAG CT 3'

Ingredients	amount in nmoles	final concentration	volume dH_2O (µL)
FBI A1 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation:

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at –20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer A1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, B1 (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' GAG GAT GGT GGT CAA GGG AC 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI B1 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer B1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: ☐ Pass ☐ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, C1 (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' CTC ACG GGA GCT CTC CAT GC 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI C1 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer C1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: ☐ Pass ☐ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, D1 (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' CTG TTA AAA GTG CAT ACC GCC A 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI D1 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer D1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, A2 (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' TAC TTG ACC ACC TGT AGT AC 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI A2 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer A2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, B2 (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' GGC TTT GGA GTT GCA GTT GAT 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI B2 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer B2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, C2 (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' TTA TTT ATC GCA CCT ACG TTC AAT 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI C2 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer C2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, D2 (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' GGG GTT TGG TGG AAA TTT TTT G 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI D2 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer D2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, A4 (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' CCC CAT GCT TAC AAG CAA GT 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI A4 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer A4	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, B4 (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' TTT GAT GTG GAT TGG GTT T 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI B4 primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer B4	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, HVIF (100 µM) (9/8/04) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' CTC CAC CAT TAG CAC CCA A 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI HVIF primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer HVIF	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, HVIR (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' ATT TCA CGG AGG ATG CTG 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI HVIR primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer HV1R	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, HVIIF (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' CAC CCT ATT AAC CAC TCA CG 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI HVIIF primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer HVIIF	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: ☐ Pass ☐ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

FBI PRIMER, HVIIR (100 µM) Lot number: _____

Application
Mitochondrial DNA Analysis

Physical data
Sequence 5' CTG TTA AAA GTG CAT ACC GC 3'

Ingredients	Amount in pmoles	Final concentration	Volume dH_2O (µL)
FBI HVIIR primer		100 µM	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

(dH_2O volume) = (amount in nmoles) x 10

Record the water volume above. Have another analyst check the calculation.

Procedure
Add the sterile water to the original primer tube. Mix well.
In order to achieve a final concentration of 1 µM needed for sequencing, add 2 µL of stock primer (100 µM) to 198 µL dH_2O in a 1.5 mL microcentrifuge tube.
Store at -20^{O}C and label tube with concentration, initials, and date.

Data Log	source	lot	amount
Primer HVIIR	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: □ Pass □ Fail Initials: _____

Made by: _____ Date: _____

Effective Date: 01/07/2005

HUMAN LEUKEMIA 60 (HL60), 0.1ng/20µL

Standard batch size: 1 mL Lot Number: _____

Application
Positive Control (see Protocols for Forensic mtDNA Analysis)

Ingredients	final concentration	amount
HL60	0.025ng/µL	20 µL
TE^{-4}	----	As needed

Procedure

1) Make a 1000-fold dilution of the stock solution by diluting 1µL of stock HL60 into 999µL of TE.
2) This becomes the working stock solution. Depending on desired amount, dilute working stock down to 0.025ng/µL. Document calculations (attach additional sheet if necessary)
3) Label with reagent name, lot number, initials, and date of manufacture.
4) Store at -20^{O}C.

Data Log	source	lot	amount
HL60	_____	_____	_____
TE	_____	_____	_____

Quality Control

QC600 Linear Array Sequence Detection

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

ORANGE G LOADING DYE

Standard batch size: 50mL Lot Number: _____

Application
Product yield gel (see Protocols for mtDNA Analysis)

Ingredients

	final concentration	amount
Sucrose	30% (w/v)	15 g
Orange G (Sigma)	0.35% (w/v)	0.175 g

Procedure

1) Dissolve the Sucrose in 50mL dH_20
2) Add the Orange G to the solution
3) Mix well
4) Dispense into sterile 1.5ml eppendorf tubes
5) Store at -20^{O}C

Data Log	sourse	lot	amount
Sucrose	_____	_____	_____
Orange G	_____	_____	_____

Made By: _____ Date: _____

Effective Date: 01/07/2005

ORGANIC EXTRACTION BUFFER

Standard batch size: 1 L Lot Number: _____

Ingredients

	final concentration	amount
Tris	10 mM, pH 8.0	1.2 g
EDTA, Disodium Salt, Dihydrate	50 mM, pH 8.0	18.6 g
NaCl	100 mM	5.8 g

Procedure

1. Measure 500 mL deionized water into a beaker.
2. Add 1.2 g Tris and dissolve with agitation and mild heat.
3. Check the pH and adjust to approximately 7.5 (+/- 0.1) by adding either HCl or NaOH dropwise.
4. Add 18.6 g EDTA and allow it to dissolve.
5. Again, adjust the pH to approximately 7.5 (+/- 0.1) by adding either HCl or NaOH dropwise.
6. Add 5.8 g NaCl to the solution and allow it to dissolve.
7. Adjust the volume to 1 L with dH_2O.
8. Autoclave the solution for 25 minutes.
9. Transfer into labeled 50 mL conical vials.

Data Log

	source	lot	amount
Tris	_____	_____	_____
EDTA	_____	_____	_____
NaCl	_____	_____	_____

Quality Control

Final pH: _____ (7.5 ± 0.1)

1. Nuclear DNA: QC250 Quantiblot Hybridization (test 20uL)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date:_____

Effective Date: 01/07/2005

PROTEINASE K, ~400u/mL

Standard batch size: 7.5mL to 50mL Lot Number: _____

Ingredients	**Amount (Invitrogen purchased – 20units/mg)**	**Amount (Promega purchased – 30units/mg)**
Proteinase K	1g	100mg
Incubation Buffer	50mL	7.5mL

Procedure with Proteinase K purchased from Invitrogen

1. Add 25mL of Incubation Buffer to a 1g bottle of Invitrogen Proteinase K and dissolve.
2. Pour into a 50mL conical tube and add remaining 25mL of Incubation Buffer.
3. Replace cap and mix by inverting several times.
4. Place 10mL aliquots of the solution into 15mL conical tubes. Label with name, lot number, initials and date of manufacture (DOM).
5. Store tubes at -20^{O}C. Indicate every time the tube is thawed with a line on the cap. Discard after 5 freeze thaw cycles.

Procedure with Proteinase K purchased from Promega

1. Add 7.5mL of Incubation Buffer to a 100mg bottle of Promega Proteinase K.
2. Replace cap and mix by inverting several times until dissolved.
3. Place 1mL aliquots into 1.5mL microcentrifuge tubes. Label with name, lot number, initials and date of manufacture (DOM).
4. Store tubes at -20^{O}C. Indicate every time the tube is thawed with a line on the cap. Discard after 5 freeze thaw cycles.

Data Log	source	lot	amount
Proteinase K	_____	_____	_____
Incubation Buffer	_____	_____	_____

Quality Control:
QC250 - Quantiblot Hybridization (test 20uL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

STERILE DEIONIZED WATER (for Mitochondrial DNA Analysis)

Standard batch size: 2L Lot Number: _____

Procedure

1. Filter sterilize 2 L of deionized water.
2. Autoclave at 250^{O}F for 20 minutes.
3. Aliquot 10 mL each into 15 mL centrifuge tubes (200 tubes). Label each tube with its contents, the date of make (DOM), and your initials.
4. Store at room temperature.
5. Further aliquot 500uL water into 1.5mL microcentrifuge tubes.
6. Place tubes into Stratalinker for a minimum UV exposure of 30min.

Quality Control

QC250 - Quantiblot Hybridization (test 20uL of solution)
QC550 - DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

TRIS-EDTA (TE^{-4}), 1X (for Mitochondrial DNA Analysis)

Standard batch size: 500 mL Lot Number: _____

	Ingredients	final concentration	amount
1)	TRIS-HCl, pH 8.0, 1 M	10 mM	5.0 ± 0.3 mL
2)	EDTA, 0.5 M	0.1 mM	100± 2µL
	OR		
1)	TE, 100X	1.0X	5.0 mL

Procedure

1. Add the TRIS and EDTA to 495 mL deionized water. Mix well and filter.
2. Autoclave at 250°F for 20 minutes.
3. Dispense into 15 mL sterile centrifuge tubes. Label each tube with its contents, the date of make (DOM), and your initials.
4. Store at room temperature.
5. Aliquot 500uL into 1.5mL microfuge tubes and expose to UV light in a Stratalinker for a minimum of 30min.

OR

1. Add TE, 100X to 495 mL deionized water.
2. Autoclave at 250°F for 20 minutes.
3. Dispense into 15 mL sterile centrifuge tubes. Label each tube with its contents, the date of make (DOM), and your initials.
4. Store at room temperature.
5. Aliquot 500uL into 1.5mL microfuge tubes and expose to UV light in a Stratalinker for a minimum of 30min.

Data Log	source	lot	amount
TRIS-HCl, pH 8.0, 1 M	_____	_____	_____
EDTA, 0.5 M	_____	_____	_____
TE, 100X	_____	_____	_____

Quality Control

Final pH: _____ (7.4 ± 0.2)

QC250 - Quantiblot Hybridization (test 20uL of solution)
QC550 - DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Effective Date: 01/07/2005

Appendix B

Quality Control testing procedures used in the OCME Forensic Biology Laboratory are contained in this appendix. These procedures are divided into two parts: 1) General and Analytical Methods, and 2) Calibration and Maintenance. The General and Analytical Methods section refers to QC procedures for the testing of reagents that are used in various analytical methods in the laboratory. Also included in this section are general QC procedures that are used to insure an appropriate laboratory environment for the performance of the various analytical methods. The Calibration and Maintenance section includes QC procedures that are done to monitor and insure the optimum performance of various instruments and apparatus used in the laboratory.

1. QC Procedures: General and Analytical Methods

QC100 Acid Phosphatase Spot Test Reagent
QC105 Alpha-Amylase Gel Radial Diffusion
QC115 Autoclaving
QC130 Capillary Electrophoresis (ABI 310)
QC140 Centrifuge Cleaning
QC145 Chelex Extraction
QC145A Organic Extraction
QC150 Christmas Tree Stain for Spermatazoa
QC155 Clean Run
QC160 Differential Extraction
QC175 Glassware Cleaning
QC190 Isoelectric Focusing: Hemoglobin
QC200 Kastle-Meyer Presumptive Test for Blood
QC205 Leucomalachite Green Presumptive Test for Blood
QC210 Matrix/Spectral File
QC220 Ouchterlony Radial Diffusion-Species Determination
QC225 P30 ELISA
QC240 PCR Amplification
QC250 QuantiBlot Hybridization
QC255 Species Crossover Electrophoresis
QC305 Urea Gel Diffusion
QC350 Capillary Electrophoresis (ABI 3100)
QC500 DNA Extraction (Mitochondrial DNA Analysis)
QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

2. QC Procedures: Calibration and Maintenance

Effective Date: 01/07/2005

QC100 ACID PHOSPHATASE SPOT TEST REAGENT

Test Materials
Acid Phosphatase Spot Test Reagent

Samples
Whole human semen
Deionized water

Procedure
1. Prepare 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 dilutions of whole human semen with deionized water or saline.

2. Prepare dried stains of each dilution (including a neat semen stain) on stain cards. Fresh dilutions should be prepared every 3 months.

3. Perform the spot test on each stain and on a negative control (deionized water) stain as specified in the Forensic Biology Biochemistry Methods Manual.

Specifications
Positive results should be obtained on each semen dilution stain.
Negative results must be obtained with the negative control stain.

Documentation
Write test results on the reagent sheet and file into the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC105 α-AMYLASE GEL RADIAL DIFFUSION

Test Materials
Amylase Gel Buffer
α-Amylase Standard (only for new shipments)

Samples
α-Amylase Standards
Deionized Water Negative Control

Procedure
1. Prepare a set of ten-fold serial dilutions of α-Amylase standards consisting of 20, 2, 0.2, 0.02, and 0.002 units each per 10 µL of deionized water as described in the Forensic Biochemistry Methods Manual.

2. Test 10 µL of each standard and a deionized water negative control as per the Amylase Diffusion Presumptive Test for Saliva method specified in the Forensic Biochemistry Methods Manual.

Specifications
The amount of diffusion for the standards (eg., diameter of the clear circles around standard wells) needs to be linear with respect to the amylase activity expressed logarithmically. Perform a linear regression analysis on the data samples to determine the correlation coefficient (r^2). The r^2 value should be greater than 0.95.

The values of diffusion for the 0.02 and 0.002 unit standards should fall in the ranges of 7-15 and 4-10 mm, respectively. In addition, the amount of diffusion of the 0.02 unit standard must be greater than that of the 0.002 unit standard.

The negative control must be negative.

Documentation
Write the test results on the reagent sheet.

Attach the Amylase Diffusion worksheet and Amylase Diffusion Assay spreadsheet to the reagent sheet and file into the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC115 AUTOCLAVING

Glassware/equipment

All glassware must be clean and dry prior to autoclaving (refer to QC175 for standard glassware cleaning procedure).

Cover glassware openings with aluminum foil.

Attach a strip of autoclave time tape to the aluminum foil on each piece.

Bottles should be loosely capped.

Small items may be autoclaved inside a beaker covered with foil.

Solutions

Conical tubes and glass bottles should be loosely capped. Small tubes are autoclaved inside a beaker.

Attach a strip of autoclave time tape to the object being autoclaved.

Do not fill bottles and tubes more than 75% of capacity.

Operation

The drain should be closed. The chamber should be filled with deionized water to the fill line (approximately 4 L). Load the chamber and close the door. Select exhaust, temperature and set the timer. Use fast exhaust for glassware and equipment and slow exhaust for solutions. The autoclave starts automatically and should not be opened until all of the pressure is released. If additional autoclaving is needed, refill water chamber and repeat procedure.

Maintenance

Once all autoclaving has been done, the chamber should be drained of water by opening the drain knob and the door should be left open.

Specification

Lettering on autoclave time tape should turn color (black).

Effective Date: 01/07/2005

QC130 CAPILLARY ELECTROPHORESIS (ABI 310)

Test Materials

Performance Optimized Polymer 4
310 Genetic Analyzer Buffer with EDTA
Formamide (Deionized)
CXR Size Standard
Cofiler Kit Reagents (see QC110)

Samples

Run amplified products from two DNA samples; an allelic ladder, amplified positive control DNA, and a reagent blank (amplification negative control).

Procedure

1. Electrophorese samples according to the capillary electrophoresis protocol.

2. Analyze samples according to the Genescan Analysis and Genotyper protocols as described in the Protocols for Forensic STR Analysis Manual.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Document on appropriate capillary electrophoresis run worksheets.

Attach the completed worksheets to a Raw Material Quality Control Test Form (F183).

File reagent sheet and CE run worksheets together in the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC140 CENTRIFUGE CLEANING

Centrifuges are cleaned with a 10% bleach solution on a monthly basis. This insures that the centrifuge surface will be relatively clean of DNA that may have built up through normal laboratory use.

Both the inside chamber, rotor, and outside of the centrifuge should be wiped with the 10% bleach solution. This first wipe is then followed by another wipe, now using 70% ethanol. The ethanol is used to clean the surfaces from bleach and to complete the decontamination/disinfection process.

Cleaning of centrifuges is recorded on a Maintenance Log Sheet (F165) and filed in the Centrifuge Maintenance Log Binder.

Effective Date: 01/07/2005

QC145 CHELEX EXTRACTION

Test Materials
Chelex, 5%
Proteinase K

Samples
Two whole blood or bloodstain samples of known type
One negative control sample

Procedure
1. Extract the two known samples and the extraction negative control sample according to the Chelex extraction procedure for whole blood and bloodstains as described in the Protocols for Forensic STR Analysis Manual.

2. Amplify the samples according to the appropriate amplification protocol.

3. Electrophorese the samples according to the appropriate protocol.

Specifications
Each sample must match the assigned type within the current interpretation guidelines.
The extraction negative control sample must show no evidence of contamination.

Documentation
Fill out the appropriate worksheets.

Attach the completed worksheets to the appropriate reagent sheet.

File the reagent sheet and the worksheets in the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC145A ORGANIC EXTRACTION

Test Materials
YM1 Male Positive Control
YM1 Female Negative Control
Phenol: Chloroform: Isoamyl alcohol (P.C.I.A)
Phenyl:Chloroform: Asoamyl Alcohol 25:24:1
Proteinase K Solution for Bone Extraction

Samples
One extraction negative control sample.
One positive DNA control sample from the DNA typing kit (if applicable)

Procedure
1. Extract the known swab and the extraction negative control sample according to the organic extraction procedure in the Protocols for Forensic STR Analysis manual.

2. Amplify the samples and a DNA positive control from the kit according to the appropriate amplification protocol.

3. Electrophorese the samples according to the appropriate protocol.

Specifications
Each sample fraction must match the assigned type within the current interpretation guidelines.
The negative control sample must show no evidence of contamination.

Documentation
Document on a set of appropriate worksheets.

Attach the completed worksheets to the reagent sheet.

File the reagent sheet and worksheets in the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC150 CHRISTMAS TREE STAIN FOR SPERMATAZOA

Test Materials
Nuclear Fast Red
Picric Indigo Carmine

Samples
One positive control sperm sample heat fixed to a slide.

Procedure
Apply the Nuclear Fast Red and Picric Indigo Carmine to the cells and view the slide as described in the Forensic Biochemistry Methods Manual.

Specifications
There should be a visible acrosome and nucleus stained red.
The tail should be stained green.

Documentation
The slide should be enclosed in a slide mailer with all pertinent information listed on the front, encased in a plastic Kapak bag and attached to the appropriate reagent sheet.

File the reagent sheet and slide mailer in the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC155 CLEAN RUN

This procedure is used to pinpoint sources of contamination when a typing problem arises.

Samples
Two whole blood or bloodstain samples of known type
One extraction negative
One amplification negative
One electrophoresis negative
One positive control sample from the DNA typing kit (if applicable)

Procedure
1. Extract the control samples and the extraction negative according to the Chelex extraction procedure for whole blood and bloodstains as described in the Protocols for Forensic STR Analysis Manual. The extraction negative control is a reagent control containing deionized water in place of sample. This sample should be handled the same way as the other samples, but no substrate is added.

2. Amplify the samples with the appropriate positive control and an amplification negative according to the appropriate amplification protocol. No Chelex extract is added to the amplification negative. This negative is used to evaluate contamination from the reagents and equipment in the amplification area.

3. Electrophorese the samples with an electrophoresis negative control, according to the appropriate protocol. No amplified or chelex extract is added to the electrophoresis or amplification negative controls.

Evaluation
If only the extraction negative shows contamination, the problem has occurred during the extraction step.

If the amplification negative shows contamination while the extraction negative is clean, the problem has occurred during the amplification setup.

If only the positive control appears contaminated, the problem might be a contaminated positive control.

Individual clean runs have to be evaluated on a case by case basis. It may be useful to determine what components have been changed since the last successful typing and to work from there.

Documentation
Document the clean run on a set of appropriate worksheets and place into the QC Troubleshooting/ Investigative Binder.

Effective Date: 01/07/2005

QC160 DIFFERENTIAL EXTRACTION

Test Materials
Chelex, 20%
Dithiothreitol, 1M

Samples
One swab with epithelial and sperm cells of known type.
One extraction negative control sample.
One positive DNA control sample from the DNA typing kit (if applicable).

Procedure
1. Extract the known swab and the extraction negative control sample according to the differential extraction procedure in the Protocols for Forensic STR Analysis manual.

2. Amplify the samples and a DNA positive control from the kit according to the appropriate amplification protocol.

3. Electrophorese the samples according to the appropriate protocol.

Specifications
Each sample fraction must match the assigned type within the current interpretation guidelines.
The negative control sample must show no evidence of contamination.

Documentation
Document on a set of appropriate worksheets.

Attach the completed worksheets to the reagent sheet.

File the reagent sheet and worksheets in the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC175 GLASSWARE CLEANING

General Procedure

Most pieces of laboratory glassware can be cleaned by washing and brushing with a solution of detergent. Detergent is available from the OCME stockroom.

Rinse each piece at least three times with tap water to remove all detergent residue.

Rinse each piece several times with deionized water. If the surface is clean, the water will wet the surface uniformly. On soiled glass the water stands in droplets. If spotting is observed during the deionized water rinse, the detergent wash should be repeated. If spotting is observed after a second detergent wash, an acid rinse may be necessary (see below).

Allow the glassware to dry at room temperature on a drying rack.

Dishwasher

Load the dishwasher with glassware and put a scoop (approximately 42 g) of non-foaming, laboratory dishwasher detergent in the detergent cup. **Do not use regular laboratory detergent!**

Turn on the dishwasher using the steam scrubbing cycle. When the cycle is finished, remove the clean glassware.

Alternative Cleaning Procedures

When glassware cannot be completely cleaned by scrubbing with a detergent solution, other cleaning methods must be used.

<u>Agarose</u>

Solidified agarose in flasks can be redissolved by adding water to the flask and heating in the microwave. Solidified agarose in graduated cylinders can be removed with a brush. It is best not to use boiling water to redissolve solidified agarose in graduated cylinders, since this may affect the calibration of the cylinder over time.

<u>Acid Rinse</u>

Stubborn films and residues which adhere to the inside of flasks and bottles may often be removed by rinsing with dilute (approx 1-10 M) acetic or nitric acid . Some glassware may need to soak in dilute acid overnight. Any acid rinse must be followed by multiple rinses with deionized water to remove any acid residue.

Effective Date: 01/07/2005

QC190 ISOELECTRIC FOCUSING: HEMOGLOBIN

Test Materials
pH 3-10, 6-8, 7-9 Ampholytes
AFSC Standard

Samples
AFSC Standard
Potassium Cyanide

Procedure
1. Dilute 5uL of the AFSC hemoglobin control with 45 µL 0.05% potassium cyanide.

2. Ten microliter (10uL) aliquot of the diluted standard is tested as per the hemoglobin IEF method as specified in the Forensic Biochemistry Methods Manual.

Specification
All four bands must be visible and sharply defined in at least one standard. The volume giving optimal banding will be used in casework.

Band separation must be as follows:

Bands	Allowable Separation
A to F	>2mm
F to S	>3mm
S to C	>6mm

Documentation
Document on the appropriate worksheet and attach photographic documentation.

File in the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC200 KASTLE -MEYER PRESUMPTIVE TEST FOR BLOOD

Test Materials
Kastle-Meyer Reagent

Samples
Whole Blood
Deionized Water Negative Control

Procedure
1. Prepare serial dilution of whole blood in deionized water beginning with 1/10 and ending with a 1/1,000,000 dilution.

2. Place one drop of each dilution on a stain card (including a neat sample) and deionized water and allow to dry.

3. Test each dried drop with Kastle-Meyer reagent as per the Forensic Biochemistry Methods Manual.

Specifications
Reagent sensitivity must not be less that 1/1000 dilution of whole blood.

The deionized water must give a negative result.

Positive reactions must be observed in any dilution only after the addition of 3% hydrogen peroxide.

Documentation
Write test results on Reagent Sheet.

Effective Date: 01/07/2005

QC205 LEUCOMALACHITE GREEN PRESUMPTIVE TEST FOR BLOOD

Test Materials
Leucomalachite Green Reagent

Samples
Whole Blood
Deionized Water Negative Control

Procedure
1. Prepare serial dilution of whole blood in deionized water beginning with 1/10 and ending with a 1/1,000,000 dilution.

2. Place one drop of each dilution on a stain card (including a neat sample) and deionized water and allow to dry.

3. Test each dried drop with Leucomalachite Green reagent as per the Forensic Biochemistry Methods Manual.

Specifications
Reagent sensitivity must not be less than 1/1000 dilution of whole blood.

The deionized water must give a negative result.

Positive reactions must be observed in any dilution only after the addition of 3% hydrogen peroxide.

Documentation
Write test results on Reagent Sheet.

Effective Date: 01/07/2005

QC210 MATRIX/SPECTRAL FILE

Making a matrix/spectral

Introduction

A matrix file is required by the ABI 3100 and ABI 310 fluorescent fragment detection software in order to subtract overlapping wavelength components from the different color signals (for the ABI 3100 platform, a matrix file is referred to as a "spectral calibration"). Therefore the matrix consists of a table of numbers that quantitatively reflect the amount of each dye detected in each color filter.

The necessity to make a matrix arises anything might change the optical properties of an instrument; this might be a repair or replacement of a component of the optical system or a change in the gel composition. Since there are subtle differences between the different instruments each instrument has to have its own matrix file and gels or runs performed have to be analyzed with the matrix belonging to the instrument that was used.

Due to minor shifts in the quality of the CCD camera, the laser, the glass plates, or the reagents, it can become necessary to make a new matrix, even though no changes were made. The following occurrences are indications that the old matrix does not achieve the correct amount of spectral overlap:

- Pull up peaks underneath peaks of a height less than 2000fu
- Pull down events in a different color caused by peaks in another color
- Elevated baseline of a different color between two peaks in another color

The matrix file is made by running the pure dyes and then performing the Genescan software step "New Matrix" that is described below. Different labeling chemistries of course require different matrices to be used during the analysis.

The table below shows the different labels used for fluorescent system employed by the Department of Forensic Biology for casework and research. The table also displays how the matrix standards are supplied by either Applied Biosystems or Promega, and which virtual filterwheel on the instrument corresponds to which dye.

When making a new matrix **select the appropriate four samples for each system**. Standards for different systems can be run together. The matrix standards have to be run under the regular conditions, but with no matrix applied to the run. Matrix standards can be coloaded with other samples, which can be analyzed separately afterwards.

Effective Date: 01/07/2005

Table 1: Available Matrix Standards

Multiplex systems	Color	Label	Kit needed	Filterwheel required
YM1	Blue	6-FAM	ABI Matrix Standard Set DS-33	G5
	Green	VIC		
	Yellow	NED		
	Red	PET		
	Orange	LIZ		
Cofiler and Profiler Plus	Blue	5-FAM	ABI Matrix Standard Set DS-32	A or F
	Green	JOE		
	Yellow	NED		
	Red	ROX		
Powerplex systems	Blue	Fluorescein	Promega Matrix Standards, 3100	Z
	Green	JOE		
	Yellow	TMR		
	Red	ROX		

Effective Date: 01/07/2005

3100 Spectral Calibration preparation for Cofiler/Profiler Plus systems

NOTE: Matrix standards must be mixed with Hi-Di Formamide.

1. Thoroughly mix the contents of each tube and spin briefly.

2. Combine 1.25ul of standard from each of the 4 tubes supplied (5FAM, JOE**,** NED, ROX) and 195ul of Hi-Di Formamide in a 1.5ml centrifuge tube.

3. Mix thoroughly and spin briefly. Dispense 10ul of matrix standard/formamide mixture into two columns of a 96 well plate. Denature by placing 96 well plate onto the 9700 thermal cycler at 95 C for 5 minutes. Immediately place on ice. Or, use the denature/chill option programmed in the thermal cycler.

3100 Spectral Calibration preparation for PowerPlex 16 systems

NOTE: Matrix standards must be mixed with Hi-Di Formamide.

1. Initial dilution of concentrated fragments: before mixing the dye fragments together, dilute an aliquot of each separate dye fragment 1:10 in Nuclease-Free Water. Vortex to mix.

	JOE	**FL**	**TMR**	**CXR**
Concentrated Dye	2 µl	2 µl	2 µl	2 µl
Nuclease-Free Water	18 µl	18 µl	18 µl	18 µl

2. Fragment mix (using 1:10 dilutions of dye fragments): After the initial dilution, the dye fragments are mixed together as listed below:

JOE Standard	5 µl
FL Standard	5 µl
TMR Standard	5 µl
CXR Standard	5 µl
Nuclease-Free Water	480 µl

3. On the ABI Prism® 3100 Genetic Analyzer, 16 wells are used for matrix detection on the 16 capillaries (wells A1 through H2 of a 96 well tray). Load 25µl of the fragment mix into each of the 16 wells.

4. Denature samples at 95°C for 3 minutes, then chill on ice for 3 minutes before loading into the 3100 instrument. Or, use the denature/chill option programmed in the thermal cycler.

Effective Date: 01/07/2005

3100 Spectral Calibration preparation for YM1 system

NOTE: Matrix standards must be mixed with Hi-Di Formamide.

1. Thoroughly mix the contents of the Matrix Standard Set DS-33 tube and spin briefly in a microcentrifuge.
2. Prepare the Matrix Standard by combining 5 µl of the tube labeled "Matrix Standard DS-33 for 3100 system" supplied in the kit and 195 µl of Hi-Di Formamide in a 1.5 mL microcentrifuge tube.
3. Mix thoroughly and spin briefly in a microcentrifuge.
4. Dispense 10 µl of the Matrix Standard/Hi-Di Formamide mixture into two columns (16 wells) of a 96-well microtiter plate.
5. Cover the plate and denature at 95°C for 5 minutes. Immediately place on ice. Or, use the denature/chill option programmed in the thermal cycler

310 Matrix Standard preparation for Cofiler/Profiler Plus systems

NOTE: Matrix standards must be mixed with formamide and denatured, but **DO NOT** add the red size standard.

1. Mix 1μL of each matrix standard with 25 μL of deionized formamide only. Denature at 95°C for 2-3 minutes, then chill on ice and place in the 48-well sample tray. Do two injections each.

Electrophoresis and Making a Matrix file

1. For 3100 Place the 96 well plate onto the 3100 autosampler. Within "Plate View" of the 3100 Data Collection software, click "New". In the "Plate Editor" dialog box: a) name the plate, b) select "spectral calibration", c) select 96 well for plate type, d) click "Finish". Complete the "Plate Editor" spreadsheet: a) assign sample names b) select dye set "F", c) select run module "Spect36_POP4DefaultModule," select the spectral parameter "MtxStd{GeneScanSetF}.par."

   Follow the Department of Forensic Biology Protocols for Forensic STR Analysis manual for instructions on how to run samples.

At the end of the run, while the data is being analyzed, the Spectral Calibration Result dialog box opens to indicate which capillaries have passed and which have failed. An "X" represents failed capillaries. Passed capillaries are represented by a "." dot. Click "OK."

If a capillary fails, it is automatically assigned the spectral profile of its nearest passing capillary to the left. If there are no passing capillaries to the left, it will be assigned the profile of the nearest passing capillary to the right. These capillaries are marked yellow instead of green in the Array View. It is recommended that each capillary have a passing spectral. Repeat the calibration if necessary.

Review the spectral calibration profile by choosing "Tools," "Display Spectral Calibration," "Dye Set." Select the dye set that corresponds to the correct matrix run (dye set F is for Cofiler/Profiler.) Click "OK."

Use the arrow buttons to review the data for each capillary. For a good quality calibration for dye set F, the condition number should fall between 4 and 7. The Q-value has to be greater than 0.95. Once each capillary has been reviewed, click "OK."

The spectral is automatically saved as the default, and there is no need to print out the profiles.

To archive the spectral file, open the directory: D:\AppliedBio\3100\DataCollection\Spectral Cal Logs and drag the "Spectral Cal" folder with the correct date and run folder name onto an archive CD for spectral runs.

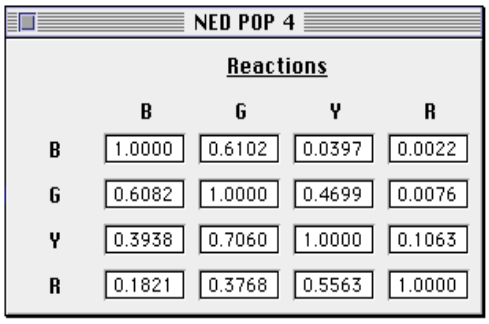
2. For 310 Set up sample sheet, injection list as usual (see STR Manual). The only modification is that in the injection list under Matrix file you have to select "none". Prepare the samples a stated above and start the run.

The duplicates of the standards are only meant as backup. It is not necessary to use both sets. For each standard select the more intense one of the duplicates.

After the run is complete the Genescan analysis software should be open already. Under **File** select **New** and there select **Matrix**.

In the window that appears indicate the sample file that corresponds to each dye color. Refer to **Table 1** for which color has which name and in order to decide which colors to combine for each system. It may be necessary to browse and open the run folder. Select starting scan numbers of 3300 for each sample. This starting number is intended to exclude the primer peaks.

Under points enter 10,000 and click O.K. The computer makes the matrix and the following window appears:



Under **File** select **Save**. Save the new matrix twice: once in the GS Matrix folder in the Genescan analysis folder (on hard drive), and **IMPORTANT** in the ABI folder in the Macintosh System folder (on hard drive). In order to save a copy in each of these folders, highlight the icon after it has been saved once, under **File** select **Duplicate**. Then drag one of the copies in the other folder. Only if the matrix is saved in the system folder it will be available as an option in the injection list.

As a filename use the instrument name and the creation date:
e.g. CE3 5/03

Proceed with the section **Quality Control Testing of Genescan Matrix Files** (see next section) in order to test the new matrix and print out the documentation.

If runs are analyzed on separate terminals the matrix for the different instruments have to be made available. Copy the file in the GS Matrix folder in Genescan folder on the hard drive.

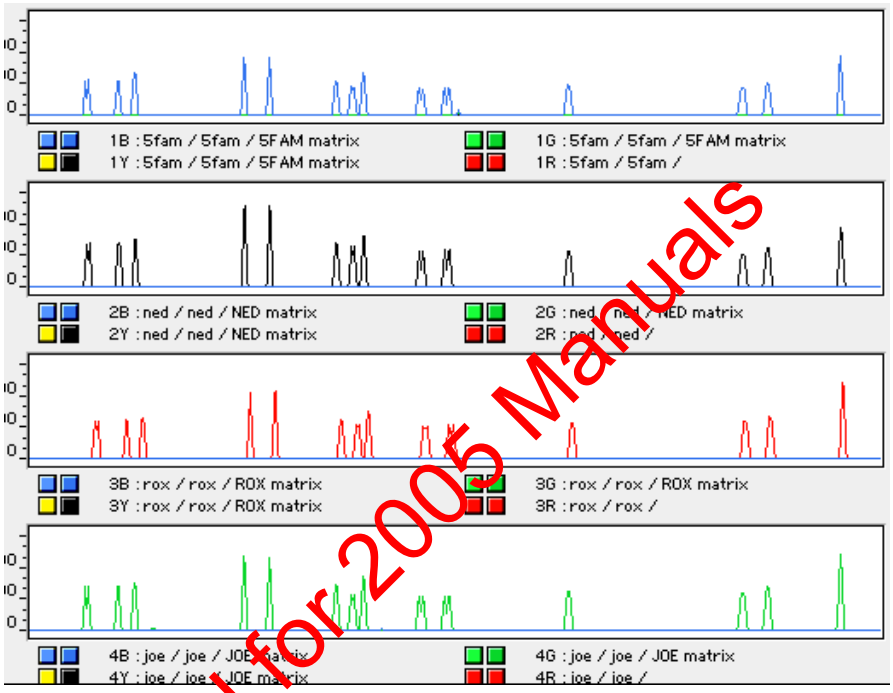
Quality control testing of Genescan STR matrix files

In order to test, if the new matrix is working correctly, it should be applied to the matrix standard sample files.

Open the project with the extracted matrix standards. Under **Samples** choose **Install new matrix**. Install the matrix you just made.

Click on the top blue, green, yellow, and red boxes to select the all colors for the analysis for all lanes. Click on the **Analyze** button in the upper left corner. All selected samples will be analyzed. There will be an error message in the analysis log window because the samples do not have a size standard. Ignore this message.

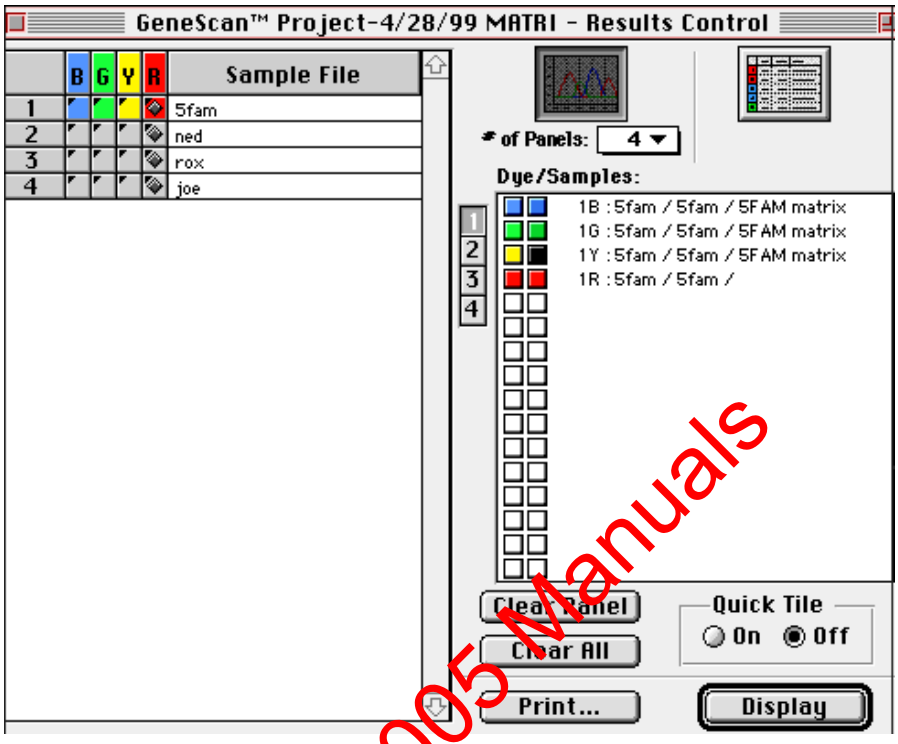
Open the results control window.



In the upper right hand corner, deselect the **Display Table** option by clicking on the icon, so that it is not indented anymore. Also switch **Quick Tile** to **Off**.

Display all colors in sample one in field one, sample two in filed two, and so on...

If the matrix is correct, no pull-up peaks should be visible; all colors should only consist of one color. See example on the next page.



Print out the following documentation for the **Matrix Log Book**:

For STRs: the Matrix number box (double click on the icon in the Matrix Folder in Genescan analysis folder to open the file and select print) , the electropherogram of the analyzed matrix standards (see above).

For Sequencing: the three Matrix number boxes

File these sheets together with the run control or gel sheets in the Matrix Log book.

Effective Date: 01/07/2005

QC220 OUCHTERLONY RADIAL DIFFUSION: SPECIES DETERMINATION

Test Materials
Serum
α-Serum

Samples
One serum sample positive control.
One corresponding α-serum sample.
One negative control (deionized water or saline).

Procedure
Prepare the tank buffer and agarose gel as described in the Quality Manual.
Punch holes in the solidified gel, load samples and develop gel as described in the Forensic Biochemistry Methods Manual.

Specifications
The positive control must give a positive result.
The negative control must give a negative result.

Documentation
Document on an Ouchterlony Test Worksheet and attach it to the appropriate reagent sheet.

Note: Either QC220 or QC255 may be used to QC serum and α-serum.

Effective Date: 01/07/2005

QC225 P30 ELISA

Test Materials

P30 Antigen
Polyclonal Anti-human P30
IgG1, Kappa Chain (MOPC 21)
Alkaline Substrate Buffer
Casein Stock Solution
Monoclonal Anti-human P30
Alkaline Phosphatase Conjugate
p-Nitrophenol Phosphate Tablets
Phosphate Buffered Saline Tablets

PROCEDURE - MOPC QC

NOTE: All dilutions are contingent upon the concentration of reagent.

Prepare 1/5,000 - 1/10,000 dilutions of MOPC with phosphate buffered saline. Set up a microtiter plate as diagramed below and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3 C-D, 3 G-H & 4 C-D: 1/5,000 MOPC
4 G-H, 5 C-D & 5 G-H: 1/6,000 MOPC
6 C-D, 6 G-H & 7 C-D: 1/7,000 MOPC
7 G-H, 8 C-D & 8 G-H: 1/8,000 MOPC
9 C-D, 9 G-H & 10 C-D: 1/9,000 MOPC
10 G-H, 11 C-D & 11 G-H: 1/10,000 MOPC

Note: 2-12, A-B and E-F are coated with the current dilution of monoclonal anti-human as described in the Biochemistry Methods Manual.

Effective Date: 01/07/2005

Specifications
Determine the dilution of antisera that gives a result at approximately the midpoint of the specification for the 2ng P30 standard. Choose that as the working titer. Once the proper working titer has been established, also perform specificity procedure (see below).

Documentation
Document test on a P30 ELISA worksheet.
Fill out a P30 Antisera and Reagents QC sheet (including working titer).
Attach P30 ELISA worksheet to QC sheet and file into the appropriate QC binder.

PROCEDURE - MONOCLONAL ANTI-HUMAN P30 QC

NOTE: All dilutions are contingent upon the concentration of reagent.

Prepare 1/2500- 1/5000 dilutions of monoclonal anti-human P30 with phosphate buffered saline. Set up a microtiter plate as diagramed below and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3 C-D, 3 G-H & 4 C-D:	1/2,500	monoclonal anti-human P30
4 G-H, 5 C-D & 5 G-H:	1/3,000	monoclonal anti-human P30
6 C-D, 6 G-H & 7 C-D:	1/3,500	monoclonal anti-human P30
7 G-H, 8 C-D & 8 G-H:	1/4,000	monoclonal anti-human P30
9 C-D, 9 G-H & 10 C-D:	1/4,500	monoclonal anti-human P30
10 G-H, 11 C-D & 11 G-H:	1/5,000	monoclonal anti-human P30

Note: 2-12, A-B and E-F are coated with the current dilution of MOPC as described in the Biochemistry Methods Manual.

Effective Date: 01/07/2005

Specifications
Determine the dilution of antisera that gives a result at approximately the midpoint of the specification for the 2ng P30 standard. Choose that as the working titer. Once the proper working titer has been established, also perform specificity procedure (see below).

Documentation
Document test on a P30 ELISA worksheet.
Fill out a P30 Antisera and Reagents QC sheet (including working titer).
Attach P30 ELISA worksheet to QC sheet and file into the appropriate QC binder.

PROCEDURE - POLYCLONAL RABBIT ANTI-HUMAN P30 QC

NOTE: All dilutions are contingent upon the concentration of reagent.

Prepare 1/500 - 1/3000 dilutions of polyclonal anti-human P30 with phosphate buffered saline. Set up a microtiter plate as diagramed below and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3 C-D, 3 G-H & 4 C-D: 1/500 polyclonal anti-human P30
4 G-H, 5 C-D & 5 G-H: 1/1,000 polyclonal anti-human P30
6 C-D, 6 G-H & 7 C-D: 1/1,500 polyclonal anti-human P30
7 G-H, 8 C-D & 8 G-H: 1/2,000 polyclonal anti-human P30
9 C-D, 9 G-H & 10 C-D: 1/2,500 polyclonal anti-human P30
10 G-H, 11 C-D & 11 G-H: 1/3,000 polyclonal anti-human P30

Note: 2-12, A-B and E-F are coated with the current dilutions of MOPC and monoclonal anti-human as described in the Biochemistry Methods Manual.

Effective Date: 01/07/2005

Specifications
Determine the dilution of antisera that gives a result at approximately the midpoint of the specification for the 2ng P30 standard. Choose that as the working titer. Once the proper working titer has been established, also perform specificity procedure (see below).

Documentation
Document test on a P30 ELISA worksheet.
Fill out a P30 Antisera and Reagents QC sheet (including working titer) .
Attach P30 ELISA worksheet to QC sheet and file into the appropriate QC binder.

PROCEDURE – GOAT ALKALINE PHOSPHATASE CONJUGATE QC

NOTE: All dilutions are contingent upon the concentration of reagent

Prepare 1/500 - 1/3,000 dilutions of alkaline phosphatase conjugate with phosphate buffered saline. Set up a microtiter plate as diagramed below and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

PBS = phosphate buffered saline W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3 C-D, 3 G-H & 4 C-D: 1/500 alkaline phosphatase conjugate
4 G-H, 5 C-D & 5 G-H: 1/1,000 alkaline phosphatase conjugate
6 C-D, 6 G-H & 7 C-D: 1/1,500 alkaline phosphatase conjugate
7 G-H, 8 C-D & 8 G-H: 1/2,000 alkaline phosphatase conjugate
9 C-D, 9 G-H & 10 C-D: 1/2,500 alkaline phosphatase conjugate
10 G-H, 11 C-D & 11 G-H: 1/3,000 alkaline phosphatase conjugate

Note: 2-12, A-B and E-F are coated with the current dilutions of MOPC and monoclonal anti-human as described in the Biochemistry Methods Manual.

Effective Date: 01/07/2005

Specifications
Determine the dilution of alkaline phosphatase conjugate that gives a result at approximately the midpoint of the specification for the 2ng P30 standard. Choose that as the working dilution. Once the proper working dilution has been established, also perform specificity procedure (see below).

Once the proper working titer has been established, also perform specificity procedure (see below).

Documentation
Document test on a P30 ELISA worksheet.
Fill out a P30 Antisera and Reagents QC sheet (including working titer).
Attach P30 ELISA worksheet to QC sheet and file into the appropriate QC binder.

SPECIFICITY PROCEDURE - ALL OTHER REAGENTS

Prepare a 1/25 dilution of stains prepared from semen, blood, urine, and saliva from healthy males.

Prepare concentrations of standard P30 antigen as follows:

0.5ng/mL: 2.5 uL P30 (1 ug/mL) + 5mL PBS-casein
1ng/mL: 5 uL P30 (1 ug/mL) + 5mL PBS-casein
2ng/mL: 10 uL P30 (1 ug/mL) + 5mL PBS-casein
6ng/mL: 30 uL P30 (1 ug/mL) + 5mL PBS-casein
10ng/mL: 50 uL P30 (1 ug/mL) + 5mL PBS-casein
14ng/mL: 70 uL P30 (1 ug/mL) + 5mL PBS-casein
18ng/mL: 90 uL P30 (1 ug/mL) + 5mL PBS-casein

Effective Date: 01/07/2005

Set up a microtiter plate as diagramed below and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	PBS	W	2ng	1ng	6ng	14ng	sem	u				
B	PBS	W	2ng	1ng	6ng	14ng	sem	u				
C	PBS	W	2ng	1ng	6ng	14ng	sem	u				
D	PBS	W	2ng	1ng	6ng	14ng	sem	u				
E	PBS	W	0.5ng	2ng	10ng	18ng	b	sal				
F	PBS	W	0.5ng	2ng	10ng	18ng	b	sal				
G	PBS	W	0.5ng	2ng	10ng	18ng	b	sal				
H	PBS	W	0.5ng	2ng	10ng	18ng	b	sal				

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
0.5ng, 1ng, 2ng, etc. - quantity of standard P30 antigen
sem = 1/25 semen
b = 1/25 blood
u = 1/25 urine
sal = 1/25 saliva

Specifications

All samples of blood, urine, and saliva must give negative results.

Semen results must yield positive results.

P30 standard results must reflect standard quantities with values indicative of its concentration.

Documentation

Fill out and attach P30 ELISA worksheet to an appropriate reagent sheet and file into the appropriate QC binder.

Effective Date: 01/07/2005

QC240 PCR AMPLIFICATION

Test Materials
BSA
Cofiler Kit Reagents
dNTPs set
$MgCl_2$
10X PCR Buffer
Profiler Plus Kit Reagents
Quad and Y STR Primers
Quad STR Positive Control
Taq Gold
Y STR Male Positive and Female Negative Controls

Samples
Two whole blood or stain samples of known type.
One amplification negative.
One positive control sample from amplification materials.

Procedure
1) Amplify the samples and a positive control using reaction mixture according to the amplification protocol. No extract is added to the amplification negative.

2) Electrophorese samples according to the gel electrophoresis protocol.

3) Analyse samples according to the STR Analysis and Genotyper Instructions protocols.

Specifications
Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation
Document on an appropriate amplification and STR gel worksheets.

Attach the completed worksheets to the appropriate reagent sheet or raw material log sheet (F183).

File the reagent sheet or raw material log sheet and the worksheets in the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC250 QUANTIBLOT HYBRIDIZATION

Test Materials

BSA, 5 mg/ml
Chromagen
dNTPs Set
Digest Buffer
DTT, 1 M
$MgCl_2$ (25 µL)
PCR Buffer (25 µL)
Phosphate Buffered Saline (PBS)
Primers Used for Quad & Y STR Analysis
Proteinase-K Enzyme, 20 mg/ml
QuantiBlot DNA Standards
QuantiBlot Hybridization Solution
QuantiBlot Kits
- Calibrators 1 & 2
- DNA Probe
- Enzyme Conjugate

QuantiBlot Wash Solution
Sterile Water
Taq DNA Polymerase (20 µL)
TE^{-4}, 1X

Samples

Solution to be tested for the presence of DNA at the volume indicated above or in the QC section of the reagent sheet.

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each QuantiBlot Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The tested solution must show no evidence of contamination. There must be no hybridization to the slot containing the tested solution.
The negative control must show no evidence of contamination.

Documentation

Document on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the appropriate reagent sheet or raw material log sheet.
File the reagent sheet or raw material log sheet and the worksheets in the appropriate QC reagent binder.

Note: Chromagen and components of the QuantiBlot Kits (with the exception of the QuantiBlot DNA Standards which are tested for each new lot) should be tested for each new vendor lot/ shipment.

Effective Date: 01/07/2005

QC255 SPECIES CROSSOVER ELECTROPHORESIS

Test Materials:
Serum
α-Serum

Samples
One positive control serum sample.
One corresponding α-serum sample.
One negative control (distilled water or saline).

Procedure
Prepare tank buffer and agarose gel as described in the Quality Manual: Appendix A.
Punch holes in solidified gel, load samples and develop gel as described in the Forensic Biochemistry Methods Manual.

Specifications
The positive control must give a positive result.
The negative control must give a negative result.

Documentation
Document on Crossover Electrophoresis Worksheet and attach the completed sheet to the appropriate reagent sheet.

Note: Either QC220 or QC255 may be used to QC serum and α-serum.

Effective Date: 01/07/2005

QC305 UREA GEL DIFFUSION

Test Materials
Urease standard

Samples
Urea standards
Dried urine stain

Procedure
1. Prepare urea standards containing 5g/100ml, 0.5g urea/100ml, 0.05g urea/100ml, and 0.005g urea/100ml respectively, in deionized water.

2. Extract a 1cmx1cm urine stain in 200ml deionized water and prepared a 1/10 dilution of the extract in deionized water.

3. Test each urea standard, the neat and 1/10 urine stain extract dilution, and a deionized water blank as per the urine gel diffusion procedure specified in the Forensic Biochemistry Methods Manual.

4. Prepare a standard curve of urea concentration (expressed logarithmically on x axis) versus the adjusted diffusion radius (determined by subtracting the mean diffusion radius of each standard on the blank plate from the mean diffusion radius on the test plate).

5. Plot the adjusted diffusion radius of the neat and 1/10 diluted extracts of the known urine stain on the standard curve.

Specifications
The adjusted diffusion radius of the standard needs to be linear with respect to the urea concentration expressed logarithmically.

The adjusted diffusion radius of the neat and 1/10 diluted urine stain extracts needs to fall between the highest and lowest points on the standard curve.

The calculated urea concentration of the neat and 1/10 diluted urine stain extracts needs to differ by an approximate factor of 10.

Documentation
Write test results on the appropriate reagent sheet.
Attach appropriate worksheets to the reagent sheet.

Effective Date: 01/07/2005

QC350 CAPILLARY ELECTROPHORESIS (ABI 3100)

Test Materials

Performance Optimized Polymer 4
310/3100 Genetic Analyzer Buffer with EDTA
Cofiler, Profiler Plus, PowerPlex 16 Kit Reagents (see QC110)
CXR or ILS 600 Size Standard
HI-DI Formamide

Samples

Run amplified products from two DNA samples; an allelic ladder, amplified positive control DNA, and a reagent blank (amplification negative control).

Procedure

1. Electrophorese samples according to the capillary electrophoresis protocol.

2. Analyze samples according to the Genescan Analysis and Genotyper protocols as described in the Protocols for Forensic STR Analysis Manual.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Document on appropriate capillary electrophoresis run worksheets.

Attach the completed worksheets to a Raw Material Quality Control Test Form (F183).

File reagent sheet and CE run worksheets together in the appropriate QC reagent binder.

Effective Date: 01/07/2005

QC500 DNA Extraction (Mitochondrial DNA Analysis)

Test Materials

Chelex, 5%
Dithiothreitol, 1M (DTT)
Organic Extraction Buffer
TE^{-4}
Phenol Chloroform Isoamyl Alcohol (PCIA)
Proteinase K, 20mg/mL (ProK)
Deionized water (dH_2O)

Samples

Two known samples (hair and blood)
One amplification negative control sample
One extraction negative control sample

Procedure

Extract the two known samples and the extraction negative control sample according to the (i) Organic extraction procedure (as necessary) for whole blood and bloodstains as described in the Protocols for Forensic STR Analysis manual or (ii) mtDNA hair extraction procedure as described in the protocols for Forensic Mitochondrial DNA Analysis manual.

Amplify the two known samples along with the extraction negative and amplification negative controls and sequence HVI or HVII as described in the Protocols for Forensic Mitochondrial DNA Analysis manual.

The extraction negative control must also be subjected to sequence analysis as described in QC550 mtDNA: DNA Cycle Sequencing.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The extraction negative control sample must show no evidence of contamination according to the current interpretation guidelines.

Documentation

Fill out the appropriate worksheets. Attach the completed worksheets to the appropriate reagent sheet.

File the reagent sheet and the worksheets in the appropriate mtDNA QC reagent binder.

Effective Date: 01/07/2005

QC550 DNA Cycle Sequencing (Mitochondrial DNA Analysis)

Test Materials

BigDye Terminator Ready Reaction Mix
BigDye Terminator Sequencing Buffer
ExoSAP-IT
HL60 Positive Control
Oligonucleotide Primers

Samples

One known sample (BigDye kit components and oligonucleotides only)
One positive control (HL60)
One amplification negative control sample

Procedure

Subject required samples (as listed above) to DNA sequence analysis as described in the Protocols for Forensic Mitochondrial DNA Analysis manual. See individual reagent sheets and table below for the extent of sequencing that is required for each reagent.

Test material	Region(s) to be sequenced*	Samples
BigDye terminator ready reaction mix, BigDye terminator sequencing buffer, and Oligonucleotide primers	HVI or HVII	Known sample Positive control Amp. Negative control
ExoSAP-IT	HVI or HVII	Amp. Negative control
HL60 positive control	HVI and HVII	HL60 positive control

* Each region will be sequenced with complementing forward and reverse primers. When running a QC test for a specific oligonucleotide primer, its complementing oligonucleotide primer will be included as part of the QC test.

Specifications

The positive control and known sample must yield the correct type within the current interpretation guidelines.

The extraction negative control sample must pass according to the current interpretation guidelines.

The amplification negative control sample must show no evidence of contamination.

Documentation

Fill out the appropriate worksheets.

Attach the completed worksheets to the appropriate reagent sheet.

File the reagent sheet and the worksheets in the appropriate mtDNA QC reagent binder.

Effective Date: 01/07/2005

QC120 BALANCES: VERIFICATION AND MAINTENANCE

Routine Weight Measurements

1. Press the control bar once to turn on the power. Allow the readout to stabilize to 0.000.
2. Place the weigh paper or weigh boat on the pan of the balance. Allow the readout to stabilize.
3. Press the control bar once to tare the balance.
1. Make the desired measurement.
5. When finished, pull the control bar up to turn off the power. Clean out the weighing chamber with the small brush or a damp paper towel, being careful not to disturb the pan.

Mettler AE260 Analytical Balance Two-point Calibration

A two-point standardization should be performed each calendar month using the protocol described below:

1. Press the control bar once to turn on the power.
2. Close all the doors surrounding the weighing chamber and allow the readout to stabilize to 0.000.
3. Press and hold the control bar until the readout says CALIB.
4. When the readout flashes 100, slide the lever on the right side back to release the internal 100 gram standard weight. Allow the balance to calibrate at 100 grams.
5. When the readout flashes 0, slide the lever forward. Allow the readout to stabilize.

The balance is calibrated and ready for use.

Balance Four-point Weight Verification

Each calendar month, the balance is verified using four standard weights.

Do not handle the weights directly. Use Kimwipes or forceps to handle weights.

1. Weigh the first standard. Record the standard weight and the measured weight on the Balance Verification and Maintenance Log (F100).
2. Repeat the measurements for the other three standard weights. Record all measurements.
3. File Balance Verification and Maintenance Logs into the Scale Log Binder.

Calibration and Maintenance

Balances must be calibrated at least yearly by an outside contractor.

QC120 BALANCES: VERIFICATION AND MAINTENANCE (CONT.)

Specification
Specification for weight verification should be +/- 0.1%.

Standard (g)	Range of tolerance (g)
4000	3996.0 - 4004.0
1000	999.0 - 1001.0
500	499.5 - 500.5
100	99.9 - 100.1
50	49.95 - 50.05
20	19.98 - 20.02
2	1.998 - 2.002

If a value falls out of range, repeat. If still out of range for the AE260 Analytical Balance, then perform calibration using the internal 100 g weight. Repeat verification. If still out of range, phone for instrument calibration by an outside vendor.

Effective Date: 01/07/2005

QC125 BIOLOGICAL SAFETY CABINET/FUME HOOD: OPERATION AND MAINTENANCE

Routine Use

Turn the blower on and **WAIT** 15 minutes before using the hood. Leave the blower on while you are working in the hood.

Turn on the fluorescent light (NOT the UV light of the Biological Safety Cabinet).

Wipe all exposed hood surfaces with 70% ethanol. This must be done by every individual, each time they start to work in the hood.

Line the work surface with absorbent pads. Put the plastic side down and the paper side up. Do not block the vents.

Work on the absorbent pads following all of the safety precautions listed above.

In case of a spill onto the hood surface, decontaminate with 10% bleach for 10 minutes. Absorb the bleach onto a paper towel and rinse the surface with 70% ethanol.

NOTE: All the bleach must be rinsed from the hood surface with the ethanol. Otherwise the hood will corrode.

If the blower stops running, DISCONTINUE all work and safely seal up all samples. **The hood no longer offers any protection.**

When you are done working, discard the absorbent pads and change your top layer of gloves.

Wipe all exposed surfaces with 70% ethanol and then discard your gloves layer by layer in the red biohazard bags.

If using a Biological Safety Cabinet that is equipped with a UV light, turn the UV light on for 1 hour. Do not expose yourself to the UV.

Shut off the blower and UV (if applicable). Do NOT leave on overnight.

NOTE: **Do not work with any organic solvents (except ethanol) in the biosafety hood.** Use the Fume Hood for this purpose.

Effective Date: 01/07/2005

QC126 BIOLOGICAL SAFETY CABINET/FUME HOOD: OPERATION AND MAINTENANCE (MISONIX FE-2620 WORKSTATION)

Routine Use

Turn the blower on and adjust air speed (if necessary).

Line the work surface with absorbent pads. Put the plastic side down and the paper side up.

Work on the absorbent pads following all of the safety precautions of the laboratory.

In case of a spill onto the surface, decontaminate with 10% bleach for 10 minutes. Absorb the bleach onto a paper towel and rinse the surface with 70% ethanol.

If the blower stops running, DISCONTINUE all work and safely seal up all samples. **The hood no longer offers any protection.**

When you are done working, discard the absorbent pads and change your gloves.

Wipe all exposed surfaces with 70% ethanol and then discard your gloves in the red biohazard bags.

Shut off the blower. Do NOT leave on overnight.

NOTE: Organic solvents can be used in the workstations as long as they contain an "A/C" level carbon filter.

Maintenance

The Misonix FE-2620 Work Station contains two filters - a Pre-filter and a Carbon Filter. It is recommended by Misonix that the carbon filters be changed once a year and the pre-filters as often as necessary. Consult the Misonix FE-2620 Operating Manual for instructions on how this can be done. An outside company will inspect the workstations once a year.

Effective Date: 01/07/2005

QC135 CAPILLARY ELECTROPHORESIS (ABI 310): MAINTENANCE

Basic cleaning of the instruments should be done once a week by simply wiping down the inside with Kimwipes lightly dampened with deionized water. According to the ABI manual, organic solvents should not be used to clean the instrument. Be sure to wipe down the entire inside of the instrument including inside the oven, under the autosampler, the syringe holder and drip trays as well as the doors. Check for leaks around the syringe and clean any dried polymer.

The polymer blocks should be cleaned every time casework analysts change the capillary. They can be cleaned more often as needed if there are leaks.

Once a month, the buffer and water reservoirs should be soaked in warm water or changed, and the septa for these reservoirs should be changed.

Be sure that everything is dry when done.

When problems are experienced with the ABI 310 Capillary Electrophoresis unit, there are two diagnostic tests that may be done according to the protocols presented below. The purpose of these tests is to check the operation of the laser and CCD camera.

The test results are recorded on a 310 Capillary Electrophoresis Diagnostic Log sheet. These tests can be run while there is a capillary in the instrument. Make sure that the capillary is not damaged during the testing. Especially since the second test requires the removal of the capillary from the laser window.

The first test cannot be run with the 310 Collection Software open!

LASER TEST

1) Quit 310 Collection Software if necessary.

2) To access the diagnostic test files, open the **310 diagnostics** folder located on the hard drive. And click on the 310 diagnostics icon. At this point you will receive a warning, that the 310 diagnostics software cannot run if the Prism collection software is already running. You can check this by going to the upper left hand corner, and clicking on the finder icon. If it is not running, click **Continue,** otherwise click **Quit** and start with step1).

   At this point you may receive the message “Establishing serial communication link with 310 instrument. This may take several seconds. Do not click Abort!!! Afterwards you might get the message “Instrument is not responding. Wait 10 seconds and then click o.k.” Do wait and click **o.k.**.

   From the first menu of options choose **Test Components.** From the second menu of test components choose **Laser Power**.

3) Click on **start**. The values for the laser power mW and the laser power Amps will appear on the screen, ignore the first two readings and record the 3rd, the 4th, and the 5th reading on the Capillary Electrophoresis Diagnostic Log. Also record the pass or fail status.

4) After the 5th set of values appeared, wait till the indicator on the left side shows 100% done, then click on **Done**. The message that will appear says results not logged. To the question "log now" click **no**.

5) On the 310 components menu press **Return.** On the main diagnostics menu press **Quit.**

If the laser fails readings 3-5 take the instrument out of service and call the PE/ABD technical service representative.

CCD CAMERA SENSITIVITY TEST

For this test the regular capillary is replaced with a sensitivity standard capillary and a mock run is performed. The capillary does not have to be taken out, it is sufficient to temporarily remove it from the CCD camera lens window.

1) Open the 310 Collection Software.

2) Under **file** select **new** then select **sequence sample sheet for 48 tubes**. In the first row (A1) put one sample name e.g. CCD test. If there is no module and no matrix selected, import any of the existing possibilities. The sections have to be filled, but the files will not be applied and are just fake. Close the sample sheet and save it as e.g. CCD test.

3) Under **file** select **new** then select **sequence injection run.** Import the sample sheet that was created under 2. Select **Test CCD sensitivity** as run module. Deselect Autoanalyze if necessary.

4) Open the 310 instrument door, open the heat plate cover door, and the laser window door. Be careful not to damage the regularly installed capillary during the next steps. Move the capillary out of the laser window notch and bend it out of the way so that the laser window door and the heat plate cover can be closed without damaging the capillary.

5) Take the sensitivity standard capillary provided by ABD/PE (part # 401928) and place its window in front of the camera lens. The yellow tag should be on top. Carefully close the laser window door, the heat plate cover and the instrument door.

6) Click on **Run**. Under **Window** open **Status** to observe the progress. The program will collect data for 5 min. Then a second data collection set for 3 min will start. An alert message "EP current is zero" will pop up, click **o.k.**. Data collection will continue.

7) When the alert prompt " Remove capillary" appears, open the instrument door, open the heat plate cover and the laser window door and remove the sensitivity standard. Do not put the old capillary back yet!! Close all doors, click **o.k.**, the run will resume automatically. Data will be collected for 3 minutes. Click **o.k.** to the alert prompt that the EP current is zero.

8) After the data collection is completed, close the run, save the injection list, and quit the data collection program.

9) On the hard drive open the **310 diagnostics** folder and click on the **310 diagnostics icon**. From the main menu select **Analysis**. From the Analysis menu select **Signal to Noise Auto**.

QC135 CAPILLARY ELECTROPHORESIS (ABI 310): MAINTENANCE (CONT.)

10) Click on **Start**. Import the mock run from before, which should be in the current run folder. Highlight the sample file and click ok. The data will be analyzed automatically. Record the relevant values on the 310 Capillary Electrophoresis Diagnostic Log; the relevant values are 586 S/N ratio, 625 S/N ratio, 586 signal w/cap, and 586 signal net. These are the only ones listed on this form.

11) Click on **done**. On the 310 components menu press **Return**. On the main diagnostics menu press **Quit.**

12) Open the instrument door, the heat plate door, and the laser window door and place the regular capillary in front of the camera lens. Close all doors.

If any of the values fail call technical service.

Effective Date: 01/07/2005

QC215 MICROPIPETTE CALIBRATION AND MAINTENANCE

Calibration & Maintenance
Micropipettes are sent to an outside vendor twice a year for calibration.

Each station is equipped with a set amount of pipetters. During the time of calibration, complete sets of pipetters are replaced with a substitute set consisting of pre-calibrated pipetters that are reserved for this particular function. Pipetters from several stations can be removed and sent for calibration at one time.

Any micropipette transfer to or from service for any reason (i.e. repair, calibration, return from calibration) must be documented on the respective Micropipette Maintenance Log (F170). These sheets are located in the Micropipette Calibration QC Log binder. This binder is organized by type of pipette (e.g. p20, p10, etc.).

Micropipettes are prepared by wiping the outer shaft with 10% bleach and then followed with a final wipe using 95% ethanol.

Package micropipettes in protective packaging material before they are shipped out.

Gravimetric Check of Pipetter Accuracy
The table on the following page shows the performance specifications for the various pipetters that are being used in the laboratory. These specifications show levels of tolerance at various points on a given pipetter's range. If measured values differ significantly from the specifications, the pipetter in question will be removed from laboratory use and included in the next shipment of pipetters for calibration.

Effective Date: 01/07/2005

QC215 MICROPIPETTE CALIBRATION AND MAINTENANCE

Table: Pipette Performance Specifications

Type	Volume Setting (µL)	Percent Error	Allowable Range (µL)
P-1000	1000	≤ ±2.0	980-1020
	500	≤ ±2.0	490-510
	200	≤ ±2.0	196-204
P-200	200	≤ ±2.0	196-204
	100	≤ ±2.0	98-102
	50	≤ ±2.0	49-51
P-100	100	≤ ±2.0	98-102
	50	≤ ±2.0	49-51
	20	≤ ±2.0	19.6-20.4
P-20	20	≤ ±2.0	19.6-20.4
	10	≤ ±2.0	9.8-10.2
	2	≤ ±10	1.8-2.2
E-10	10	≤ ±2.0	9.8-10.2
	5	≤ ±5.0	4.75-5.25
	2	≤ ±10	1.8-2.2
Repeater	10 (500µL tip)	≤ ±2.0	9.8-10.2
	30 (500µL tip)	≤ ±2.0	29.4-30.6
	50 (500µL tip)	≤ ±2.0	49-51
	50 (2.5ml tip)	≤ ±2.0	49-51
	250 (12.5ml tip)	≤ ±2.0	245-255

P - Rainin Pipetters
E - Microcentrifuge Ultra-micropipette
Repeater - Microcentrifuge Repeater Pipette

Effective Date: 01/07/2005

QC230 P30 PLATE READER DIAGNOSTIC TESTS

Microwell (microtiter) plate reader(s) should be tested monthly for **linearity**, **repeatability** of readings, and **calibration**.

Linearity is determined by the relationship of the calibrator absorbance (well No. 2) to the p-nitrophenol (PNP) concentrations in the remaining wells.

Repeatability is determined by comparing the absorbance of a given well in the strip when the strip is read twice in succession.

Calibration is determined by measuring the absorbance of the calibration well (well No. 2) and comparing it to the acceptable absorbance range assigned to the Microwell reader. The acceptable range is determined by the Microwell reader manufacturer.

NOTE: PNP IS TOXIC. IT IS HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. IT IS IRRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. IT IS ALSO A POSSIBLE MUTAGEN. USE APPROPRIATE PRECAUTIONS WHEN HANDLING. WASH HANDS THOROUGHLY AFTER USE.

Test Materials/Supplies
AccuChrome™ 405 Microwells Kit
Deionized Water
Parafilm™
Linearity/Repeatability and Calibration Record Sheets (found in Microwell kit)

Procedure

1) Remove one Microwell strip from the kit. Gently tap the bottom of the strip on the counter to settle PNP in the wells (this is to prevent loss of powder on opening). DO NOT remove the tab on the Microwell strip.

2) Gently remove plastic and paper covering the strip. Keep the strip right side up.

3) Reconstitute each well with 200 µl of deionized water. Pipet carefully to avoid splashing, bubbles, or overfill. Use a calibrated micropipet. **DO NOT** touch the bottom of the microwell with the pipet tip. **DO NOT MIX.**

4) Place the wells strip into row A of the microtiter plate designed for the wells strip (supplied with kit). Notice that the wells strip has a tab on one side of the strip. The correct placement of the wells strip into the microtiter plate is so that the tab is positioned next to column 12 of the microtiter plate.

QC230 P30 PLATE READER DIAGNOSTIC TESTS (CONT.)

5) Gently cover all wells of the strip with Parafilm™ to prevent evaporation. Let stand on benchtop for two hours at room temperature (18-26°C). **DO NOT** disturb during incubation. Warm up the microtiter plate reader the required amount of time before the end of the two hour incubation time. After two hours, remove the Parafilm™, being careful not to splash any of the samples.

6) Place the microtiter plate with the test wells into the plate reader. Read the test samples according to the standard plate reader protocol used for casework samples (measurement filter = 405 nm; reference filter = 655 nm) and print the results.

7) Repeat the reading of the wells a second time and then print the second set of results as well.

Calculations

1. **Linearity Data Record (measures accuracy)**

   a. Calculate the average concentrations for replicate wells. Then calculate the average concentration of wells 3,4; of wells 5,6; of wells 7,8; and wells 9,10,11.

      Example:

      Average Concentration of well 3 = 25.4
      Average Concentration of well 4 = 25.6
      Average concentration of wells 3 & 4: (25.4 + 25.6) / 2 = 25.5

   b. Using the Linearity Graph Paper provided with the kit, plot the calculated average concentration on the vertical axis and the assigned concentration (see below) on the horizontal axis for each set of replicate wells.

QC230 P30 PLATE READER DIAGNOSTIC TESTS (CONT.)

Well No.	**PNP Concentration (Units)**
Well1:	0 (blank)
Well2:	50 (calibrator)
Well3:	25
Well4:	25
Well5:	50
Well6:	50
Well7:	100
Well8:	100
Well9:	200
Well10:	200
Well11:	200
Well12:	0 (blank)

1. All values must fall within the shaded area on the Linearity Graph Paper. This means the instrument has acceptable linearity (+/- 10%) variation.

Specifications

Loss of linearity is an indicator of stray light due to filter deterioration.
If the values fall outside the shaded area on the Linearity Graph Paper, the test must be repeated. If the repeat test values are still outside the shaded area on the Linearity Graph Paper, the instrument must be serviced and not allowed to be used for casework until it has passed the test.

2. **Repeatability Data Record (measures precision)**

a. Calculate the difference between the absorbance readings for each of the strips.

Example:

READING	WELL #	ABSORPTION	DIFFERENCE
1st	3	.243	0.000
2nd	3	.243	
1st	4	.244	0.001
2nd	4	.255	

QC230 P30 PLATE READER DIAGNOSTIC TESTS (CONT.)

b. Record the difference for each well in the appropriate space on the second page of the report (the Repeatability Record Sheet on the back of the Linearity Record Sheet).

Specifications

To ensure repeatability of readings, the difference in absorbance of each well between the two readings must be within the acceptable range as indicated on the Linearity Graph Paper (Repeatability section). If the difference is not within the acceptable range, there is a loss of repeatability of the readings.

If the repeatability is not within the accepted range, the test must be repeated. If the repeat test results are still out of the accepted range, the instrument must be serviced and not be used for casework.

3. **Calibration Data Record**

a. AccuChrome™ Microwell strips calibration assignments are lot specific. Use calibration ranges assigned on the Calibration Sheet included in each kit.

b. Recorded absorbance of the calibrator (well No. 2) of the first strip in the column labeled Strip 1 if you are using the first strip in a new kit. If previous strips have already been used, record the average absorbance of well number two for this run in the appropriate strip # column on the Calibration Record Sheet.

c. When the first strip in a kit is used set upper and lower limits for absorbance by drawing a line 0.040 absorbance units above and below the observed absorbance for the calibrator (well No.2). Absobances of all remaining strips should fall within the drawn absorbance limits.

Specifications

If the absorbance of the calibrator (well No.2) falls within the range on the Calibration Record Sheet contained in the kit (as established by Sigma Diagnostics) there is no significant change in the calibration performance of the instrument. The acceptable range incorporated the expected variation due to the strips, the dye, and run-to-run variation.

If the calibrator does not fall within the range on the Calibration Record Sheet, the test must be repeated. If the repeat test value falls outside the range on the Calibration Record Sheet, the instrument must be serviced and is not to be used for casework.

QC230 P30 PLATE READER DIAGNOSTIC TESTS (CONT.)

Documentation

File the Linearity/Repeatability Record Sheet that was filled out for this QC run with the Calibration Sheet that accompanied the kit for this lot of microwells. All sheets should be filed together in the P30 Plate Reader Maintenance Binder.

Effective Date: 01/07/2005

QC235 P30 ELISA DISINFECTION

Disinfection of the P30 plate washer should be done weekly to insure good working order of this instrument. Documentation for the performance of this procedure is recorded on the Plate Washer Maintenance Log Sheet (F180) and filed in the Plate Washer Maintenance Log Binder.

Procedure

1) Prepare a 10% solution of bleach (100 ml of bleach, 900 ml of dH_2O).

2) Under the SELECT function press the up arrow to reach the DISINFECTION program. Press YES.

3) The machine will prompt the connection of the disinfectant (the 10% bleach solution). Place the designated wash hose into the bottle of prepared bleach mixture (DO NOT pour the bleach mixture into the designated wash container that came with the machine or it will have to be thoroughly rinsed when disinfection is complete). Press YES.

4) The machine will indicate that the pump is priming. Disinfection will then occur for 30 minutes.

5) The machine will prompt the connection of the rinse. Place the wash hose into either the washer's designated rinse bottle filled with dH_2O or a plain bottle filled with dH_2O. Press YES.

6) The machine will indicate that the pump is priming. Prime the plate washer multiple times to ensure that the machine and the wash hose are free of the 10% bleach solution.

7) The SELECT function will return at the RUN program. You may now turn the plate washer off.

Effective Date: 01/07/2005

QC245 pH METER

A two-point calibration is done weekly using the pH meter and standard pH solutions. This information is documented on a pH Meter Calibration Log sheet and filed in the pH Log & Water System Binder.

Two-point Calibration

1. Fill the electrode with saturated KCl solution if necessary. Then choose standard buffer solutions for a two-point calibration that bracket the expected final pH of the solution to be measured. (i.e. use pH 7 and 10 standard buffers for a solution with final pH of 8.) Press STNDBY/MEAS button before the electrode is removed from any solution. Do not allow electrode to dry out.

2. Press the MODE button until the pH mode is displayed. (Most likely it will already be in this mode.)

3. Press the SETUP button TWICE and then the ENTER button. This clears the existing standardization values.

4. Immerse the rinsed electrode into one of the buffers (4, 7, or 10) and stir moderately.

5. Press the STD button to access the standardize mode. (The selected buffer group is displayed briefly.)

6. Press the STD button again to initiate standardization. The meter will automatically recognize the buffer and flash the value on the screen.

When the stable icon appears the buffer value is entered (automatically) and the meter returns to the measure screen. RECORD the value on the log sheet once it has stabilized.

7. Repeat steps 3-5 with a second buffer.

If the electrode is within range, the GOOD ELECTRODE message appears. If the electrode is outside the range, the ELECTRODE ERROR message appears.

Routine pH Measurements

Fill the electrode with saturated KCl solution if necessary. When fresh KCl is added, it is a good idea to mix the solution in the electrode by slowly inverting the electrode several times before continuing.

Calibrate the pH meter.

Rinse the electrode with deionized water. Blot dry outside of electrode.

Place the electrode in the solution. When the measurement has stabilized and 3 asterisks appear, record the measurement.

Calibration & Maintenance

The pH electrode must be kept filled with saturated KCl solution. This solution is approximately 30% KCl. The electrode is stored in a 2% KCl solution made from the saturated KCl filling solution (NOT deionized water or pH 7.00 standard solution). Do not leave electrode in deionized water for long periods of time.

When measuring the pH of large volumes, the pH electrode must be held in place. The electrode can be damaged if it is hung over the edge of the container and allowed to stir with the solution.

If the pH reading drifts or requires a long time to stabilize, the electrode bulb may need to be rejuvenated in 1 M HCl or the electrode may need to be replaced. Refer to the Beckman insert for further details of electrode maintenance.

Specification

During a two-point calibration the pH meter calculates the slope for the given two standards. If the slope does not pass meter specifications an error message - EFFICIENCY OUT OF TOLERANCE - flashes on the display.

Effective Date: 01/07/2005

QC270 TEMPERATURE CONTROL

Refrigerators & -20°C Freezers

A digital thermometer is used to measure refrigerators and -20°C freezers. The refrigerator and -20°C freezer temperatures are recorded daily during the work week.

Each refrigerator/freezer has its own dedicated temperature probe.

Measure the temperature and document in the respective Refrigerator and Freezer (-20°C) Temperature Control Log sheet for that unit.

-80°C Freezers

An Omega thermocouple thermometer and an Omega thermocouple probe (type T-Brown) is used to measure -80°C freezers. The -80°C freezers are monitored daily during the work week.

Measure the temperature and record reading in the monthly Freezer (-80°C) Temperature Control Log (F120) sheet for that unit.

Air Humidity & Temperature

A digital hygrometer/thermometer is used to measure the north, south, and southeast rooms of the laboratory. The room temperature and percent humidity is recorded daily during the workweek.

Place the probe on any surface and allow it to equilibrate for 5 - 10 minutes. Measure the temperature and percent humidity and log in the Temperature Control Log sheet for that room.

Water Baths & Heat Blocks

An Omega thermocouple thermometer and an Omega thermocouple probe (type T-blue) are used to measure the temperature of the water baths and heat blocks. Each probe is calibrated before use (see QC280). Temperature measurements are recorded each day the water bath is used. Temperatures are recorded daily during the workweek for the heat block.

NOTE: Rees Scientific temperature monitoring system will be used to monitor temperatures of all equipment once properly validated.

QC270 TEMPERATURE CONTROL (CONT.)

To measure the temperature, turn the water bath or heat block on (if necessary) and allow it to equilibrate for at least 15 minutes. The probe is mounted in the water bath or positioned in the heat block.

When the temperature has stabilized, record the temperature reading on the appropriate Temperature Control Log sheet or Water Bath Temperature Control Log (F230). To measure the thermocouple temperature, plug the probe into the correct position in the meter (silver-colored constantan wire on the left, copper wire on the right). Record the reading. The thermocouple reading can be corrected using the slope and y-intercept values calculated from the probe calibration (see QC280).

Unit	Acceptable Thermocouple Reading
QuantiBlot Water Bath	50 ± 1°C
56°C Heat Block	56 ± 3°C
65°C Heat Block	65 ± 3°C
95°C Heat Block	95 ± 5°C
100°C Heat Block	100 ± 5°C

Calibration

Digital thermometers with the exception of Omega Model HH21 (see below) and hygrometer/thermometers are sent out for calibration against a NIST traceable standard to an outside vendor once a year. Documentation of calibration is recorded on an appropriate log sheet (F165) and filed in the Temperature Equipment Maintenance Log Binder.

Type T-Blue thermocouples which are used to monitor waterbath and heat block temperatures, are calibrated with designated Omega (Model HH21) digital thermometers against an NIST traceable mercury thermometer (see QC280) annually. After calibration, Type T-Blue thermocouples are always used with the Omega meter that they were used with for calibration.

Type T-Brown thermocouples are used to measure temperatures of the -80°C low temperature freezers. Since an exact low temperature of these freezers is not critical (eg. for storage of forensic DNA extracts), Type T-Brown thermocouples are not calibrated. However, the performance of the Type T-Brown thermocouple is verified yearly as described in QC285.

If a suspicion arises of the performance of any of the digital thermometers, hygrometer/ thermometers, or probes during use, that particular temperature measuring device will be taken offline and recalibrated or reverified to insure that it meets proper specification.

Effective Date: 01/07/2005

QC285 THERMOCOUPLE VERIFICATION (TYPE T-BROWN)

Temperature probe operation is verified once a year.

Before beginning the verification procedure, the NIST traceable thermometer is checked by measuring two standard temperatures.

Mercury Thermometer Standardization

Place the NIST traceable thermometer in an ice-water slurry. The etched line around the bottom of the thermometer must be at or below the level of the liquid. Allow the temperature to equilibrate. The thermometer must read between -0.2 and 0.2°C.

Place the thermometer in a boiling water bath. The etched line around the bottom of the thermometer must be at or below the level of the liquid. The thermometer must read between 99.8 and 100.2°C.

Record the results of the temperature check on the Thermocouple (Type T-Brown) Verification Log (F205).

Verification

Place the temperature probe in an ice-water slurry along with a NIST traceable thermometer that has been previously standardized. Allow the temperature to equilibrate. The probe must read between -1 and 1°C.

If the probe is going to be used in the 0 to 100°C range, place the temperature probe in a boiling water bath. Allow the temperature to equilibrate. The probe must read between 99 and 101°C.

If the probe is going to be used in the -80 to 0°C range, place the temperature probe in a dry ice ethanol slurry. Allow the temperature to equilibrate. The probe must read between -78 and -74°C.

Record the results of the temperature check on the Thermocouple (Type T-Brown) Verification Log (205). If the type T-brown probe fails verification, it is removed from service. The probe must meet the above specifications to be certified for use.

Effective Date: 01/07/2005

QC290 THERMOCYCLER BLOCK CLEANING

The wells of the sample block must be cleaned each month. Dirt, oil, and other contaminating agents collect in the sample wells, preventing the reaction tubes from seating properly. Maximum contact ensures optimum heat transfer from the block to the sample.

Documentation of Thermocycler Block Cleaning is kept in the Thermocycler Calibration and Maintenance Log Binder.

Procedure

NOTE: PROTECTIVE EYEWEAR MUST BE WORN WHEN CLEANING THE SAMPLE BLOCK. LIQUID MAY SPRAY OUT OF THE SAMPLE WELLS AS THEY ARE CLEANED WITH COTTON SWABS.

Prepare a 50% v/v isopropanol/water solution.

Clean excess oil out of the wells using kimwipes or cotton swabs.

Add one or two drops of the isopropanol solution to each well and carefully clean using cotton swabs. Rotating the swab helps to loosen material caked in the bottom. Wash the sides of each well with the isopropanol solution.

Remove excess liquid using a kimwipe or a dry cotton swab.

Check that there are no deposits left in the sample wells.

Clean the channels between the rows of the block using the same procedure.

If the deposits of dirt are heavy, it may be difficult to clean the wells. In this case, set the thermocycler to soak at 37°C. At a slightly warmer temperature, hardened deposits are easier to remove.

If the sample block has been contaminated with biological material, clean the wells using a 10% bleach solution, followed by a deionized water rinse. Dry the sample wells with dry cotton swabs or kimwipes.

Effective Date: 01/07/2005

QC302 THERMAL CYCLER DIAGNOSTIC TESTS (ABI 9700)

There are three monthly diagnostic tests that are run on the Gene Amp PCR System 9700 to check temperature calibration and verify the integrity of the cooling and heating system. The tests are as follows:

1. Temperature Verification Test
2. Rate Test
3. Cycle Test

In addition, a temperature non-uniformity (TNU) test is done yearly to test the temperature uniformity of the sample block in the Gene Amp PCR System 9700.

The temperature verification and TNU tests are performed using a digital thermometer with probe and a 9700 probe tray. The rate and cycle tests require a 96-well plate with full plate cover. The thermal cycler must pass specifications set by the manufacturer to be used on line in forensic STR analysis.

1. Temperature Verification

This test requires the 96-well 0.2 ml Temperature Verification System. Two types of verification systems, cat. #N8010435 and #4317939 can be used for performing this test. The major difference between the two verification systems is whether the probe contains one or two cones.

The temperature verification system cat. #N8010435 consists of two cones, one of which measures the temperature of the sample well. The first cone that the wire is attached to does not measure the temperature of the sample well; this cone is the dummy probe. The other cone measures the well temperature. Temperature verification system cat. #4317939 consists of one cone that measures the well temperature.

Procedure

1. Place a probe tray on the 9700 sample block so that the notch faces the front of the instrument. Thread the probe wire through the notch in the probe tray. Make sure the probe is connected to the digital thermometer.
2. Coat well A6 lightly with mineral oil. Also coat well B6 with mineral oil if using the two-cone temperature verification system.
3. Place the temperature measuring probe of the temperature measuring system into well A6. If using a two cone temperature verification system, also place the dummy probe into well B6.
4. Turn on the digital thermometer by moving the ON-OFF/RANGE switch to the 200 position.

5. Access the temperature verification screen by following this path:

Util (F4)	➜	Diag (F1)	➜	TempVer (F3)

The 9700 thermal cycler has 5 function keys (F1 to F5) that you will be pressing to access various instrument functions. The above schematic shows what function key you will be pressing (in parentheses) to access the indicated function.

6. Press Run. The System 9700 screen will look as follows:

Calibration Verification	
Block temp= xx.x°C	Cover temp=xxx°C
Setpoint is 85°C	
Cover must be w/i 10° C of Setpoint	

7. When the block temperature reaches 85° C the instrument will begin a countdown. When this value reaches zero enter the actual block temperature (read from the external digital meter of the temperature verification system) on the 9700 instrument using the numeric keypad.

8. Repeat the temperature entry for the 45° C setpoint as prompted by the instrument.

9. When the System 9700 completes calibration verification one of two screens appear:

Calibration Verification Calibration is Good
OR
Calibration Verification Instrument may Require Service Contact PE/Applied Biosystems Technical Support

10. Complete this test by removing probe and cleaning the oil from the sample block.

Specification

Instrument must indicate that calibration is good. Contact Applied Biosystems if the other screen is displayed. Instrument must be taken off line if the test has failed.

Documentation

Document the test results on F217 Thermal Cycler (AB 9700) Diagnostic Log and file in the Thermal Cycler Maintenance Log Binder.

2. Rate Test

Before beginning the rate and cycle tests, place an empty 96 well plate with full plate cover on the sample block (this test does not require the 96-well 0.2 ml Temperature Verification System). Slide the heat cover forward and pull down the lever. Access the rate test function by following the path shown below.

Util (F4)	➜	Diag (F1)	➜	System (F2)	➜	Rate (F1)

After accessing the rate test function, the instrument will prompt you to install an empty microplate with a microamp full plate cover. Press the CONTINUE (F1) function key.

The instrument then runs a series of tests stabilizing the sample block at 35° C, 94° C, and 4° C. At the conclusion of the test, the test results appear on the screen and whether the test passes or fails.

Specification

The instrument must indicate on the screen that it passes this test according to the following specifications: heating >3.0° C/second; cooling >3.0° C/second. If the instrument does not pass this test, contact Applied Biosystems. Instrument must be taken off line if the test has failed.

Documentation

Document the test results on F217 Thermal Cycler (AB 9700) Diagnostic Log and file in the Thermal Cycler Maintenance Log Binder.

3. Cycle Test

Access the cycle test function by following the schematic shown below:

Util (F4)	➜	Diag (F1)	➜	System (F2)	➜	Cycle (F2)

After accessing the rate test function, the instrument will prompt you to install an empty microplate with a microamp full plate cover. Press the CONTINUE (F1) function key.

Note: Pressing pause will generate false test results. Test must be allowed to run in its entirety. At the conclusion of the test, the screen displays the test results and whether or not it passes or fails.

At the conclusion of this test, the screen displays the test results and whether or not the instrument passes or fails.

Specification

The instrument must indicate on the screen that it passes this test according to the following specifications: Average Cycle Time < 160 seconds; Cycle Time Standard < 5 seconds. If the instrument does not pass this test, contact Applied Biosystems. Instrument must be taken off line if the test has failed.

Documentation

Document the test results on F217 Thermal Cycler (AB 9700) Diagnostic Log and file in the Thermal Cycler Maintenance Log Binder.

4. Temperature Non-uniformity (TNU) Test

This test requires the 96-well 0.2 ml Temperature Verification Systems (see the Temperature Verification section above for a discussion of temperature verification systems).

Procedure

1. Place a probe tray on the 9700 sample block so that the notch faces the front of the instrument. Thread the probe wire through the notch in the probe tray. Make sure the probe is connected to the digital thermometer.
2. Coat well A1 lightly with mineral oil. Also coat well A2 if using the two-cone temperature verification system.
3. Place the temperature measuring probe of the temperature measuring system into well A1. If using a two cone temperature verification system, also place the dummy probe into well A2.
4. Turn on the digital thermometer by moving the ON-OFF/RANGE switch to the 200 position.
5. Slide heat cover forward and bring lever down to lock in place.
6. Access the TNU screen by the following path:

Util (F4)	➜	Diag (F1)	➜	TempVer (F3)	➜	TNU (F2)

7. When prompted to put probe in well A1, press RUN.
8. When sample block reaches 94° C, the TNU performance screen will show that the block is stabilizing for 30 seconds and will ask for block temperature.
9. Record block temperature from the digital thermometer and using the instrument numeric keypad enter this value. Also, record this value on F218 Thermal Cycler (AB 9700) Diagnostic Log.
10. The sample temperature then approaches the next temperature point, 37° C by shutting off the heat cover.
11. The message “stabilizing block at set point... 00:30" will appear on the screen.
12. When the block has stabilized at 37 °C (e.g., timer has counted down to 0:00), record the block temperature from the digital thermometer and enter this value using the instrument’s numeric keypad. Record this value on F218 Thermal Cycler (AB 9700) Diagnostic Log. Press ENTER.

Note: Prompts appear for you to move the probe assembly to the respective sample well to be tested.

	TNU performance
sample temp= xxx°C	Cover temp= xxx°C
place probe in well xx,	dummy in xx

13. Slide heat cover back. Remove probe(s) from wells and move to the next prompted well(s)
14. Slide heat cover forward and pull lever down.

Repeat these steps for the wells prompted by the instrument. They are as follows: A1/A2, A12/A11, C4/C3, C9/C10, F4/F3, F9/F10, H1/H2, and H12/H11. The first well of each pair indicates the well the measuring probe is placed in. The second well number indicates the well the dummy probe is placed into when using a two cone probe.

The instrument will prompt you to move the probe(s) through this sequence of wells twice, once for the higher temperature (94° C) and the second time for the lower temperature (37° C).

Specification

When the System 9700 completes the TNU test, the screen will display all of the TNU values at 94°C and 37°C. If all of the values are correct press ACCEPT. If not, repeat the test. The instrument will then display the final TNU values on the screen and will indicate pass or fail according to this specification: ≤ 0.5. This value reflects whether the range of temperature values at a given temperature does not exceed +/- 1 °C. If the instrument fails this specification call Applied Biosystems. Instrument must be taken off line if the test has failed.

Documentation

Document the test results on F218 Thermal Cycler (AB 9700) Diagnostic Log and filed in the Thermal Cycler Maintenance Log Binder.

Effective Date: 01/07/2005

QC310 WATER QUALITY MAINTENANCE

Changing Water Filters

Water filters should be changed once every two weeks. This is documented on a Maintenance Log (F165) and filed in the pH Log & Water Systems Binder. Use the procedure that follows to change filters:

1. Turn off the main water valve. Open deionized water valve and depress pressure release button (red button on dispenser) to relieve pressure in the housing.
2. Unscrew filter housing from cap, discard used cartridge and insert new cartridge (1 and 5 um).
3. Screw the housing onto the cap and hand tighten.
4. Open the main water valve slowly. Let the water run for 1-2 min. through the dispenser.
5. Turn off the deionized water dispenser.

Checking Water Quality

Water quality is checked weekly to include readings of total chlorine, free chlorine, total hardness, total alkalinity, pH and resistivity of the water using an Aquacheck strip and Myron L conductivity meter. Information is recorded on a Maintenance Log (F165) along with water filter information (if necessary) and filed together in the pH Log & Water Systems Binder.

Procedure

1. Take one strip from the bottle.
2. Turn on the deionized water.
3. Pass the strip under water system
4. Remove (do not shake).
5. Compare total hardness, total alkalinity and pH to the color chart shown on the bottle.
6. Record the readings on the log.
7. Again hold the strip under water system for 10 seconds.
8. Compare chlorine pads to the color chart.
9. Record readings on the log.

Specification

Readings should show a neutral pH (approx pH 7), and very low (total chlorine < 1 ppm; free chlorine <1 ppm; total hardness < 50 ppm; total alkalinity <80 ppm) or no traces of ions. The detection of ions indicates a reduced efficiency of ion removal by the deionizing tanks. A red light on top of the tanks indicates that tank replacement is necessary.

Effective Date: 01/07/2005

QC310 WATER QUALITY MAINTENANCE (CONT.)

Checking Water Resistivity

1. Check batteries of the meter by pressing the button at the lower right corner of the meter. If the light is not visible, change the batteries.
2. Select range by turning the range knob at the lower left corner (x .1).
3. Rinse the cell cup three times with deionized water.
4. Then fill with deionized water to at least 1/4" above upper electrode.
5. Push button to read directly in microohms or megaohms.

Specification

Record the readings on the same Maintenance Log as for checking the Water Quality. File the Maintenance Log into the pH Log & Water System Binder.
The resistivity reading should be greater than 10 megaohms (on the red lettered scale). When readings fall to 1 megaohm, call vendor for ion exchange tank replacement.

Effective Date: 01/07/2005

QC320 PERFORMANCE CHECK FOR ADDITIONAL ABI 3100 GENETIC ANALYZERS

This procedure only refers to new instruments of the same model number and from the same manufacturer as the current data collection platform. For a change of model or manufacturer a more extensive validation is required.

The laboratory has been utilizing the ABI 3100 genetic analyzer for a couple of years and reproducibility and precision data were established for each platform during the different multiplex validations. The main objective for testing new additional instruments prior to casework is to compare the performance and sensitivity to the current equipment.

For each multiplex system, run a batch of previously amplified and analyzed samples according to the following chart. Include negative controls and allelic ladders where applicable. Compare the new results to the old runs in regard to 1) allele calls, 2) peak intensities, and 3) absence of artifacts.

The new instrument must yield the same allele calls and similar peak intensities without unspecific signals.

ORIGINAL 3100 (NEW PLATFORM) VALIDATION	ADDITIONAL 3100 PERFORMANCE CHECK
Reproducibility	
1. COfiler and Profiler Plus allelic ladders were each run on all 16 capillaries. The same samples were re-injected for a total of 4 times.	1. Same, but re-inject for a total of 2 times only
2. Precision Statistics: Done on the first injection of the allelic ladders from above. Size of smallest and largest allele at each locus determined for each capillary; mean and standard deviation determined.	2. Same
3. 1 ng of known DNA was amplified and run in all 16 capillaries; this was repeated for a total of 5 individuals. Entire procedure was also done for 0.2 ng of DNA from the same 5 individuals.	3. Not necessary
4. Typing of different sample types from same individual: Buccal, blood, and semen samples from one individual. Tissue and blood samples were typed from another individual.	4. Not necessary

ORIGINAL 3100 (NEW PLATFORM) VALIDATION	ADDITIONAL 3100 PERFORMANCE CHECK
Sensitivity	
1. Titration analysis: 10, 5, 2.5, 1.25, 1.0, 0.75, 0.5, 0.25, 0.125, 0.075, and 0.05 ng of known DNA was amplified and run. This was repeated for 3 individuals in duplicate amps for each individual.	1. Same, but only for 2 individuals.
2. Mixture analysis: DNA from 2 individuals were run at ratios of 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, and 1:20. The total amount of DNA in each mixture was constant at 1 ng.	2. Not necessary
Concordance	
1. Typing of 28 tissue, 26 blood/semen, 29 exemplar, and 43 lab types was done on STARS and compared to the same samples run on 377 and/or 310. Typing of 24 tissue, 26 blood/semen, 29 exemplar, and 43 lab types was done on STRIPES and compared to the same samples run on 377 and/or 310.	1. Not necessary

Effective Date: 01/07/2005

QC325 INSTALLATION VALIDATION FOR ADDITIONAL 9700 THERMOCYCLERS

This procedure only refers to new instruments of the same model number and from the same manufacturer as thermocyclers that were previously put in service. For a change of model or manufacturer, a more extensive validation is required.

Amplification conditions for all casework multiplexes were previously established and validated on the 480, 9600, and 9700 thermocyclers. A new instrument has to pass the diagnostics test and yield satisfactory amounts of specific PCR product.

Perform diagnostics test as outlined in QC302.

It is necessary to make sure both the instrument and the different multiplex programs are in proper working order. Check the instrument and the Cofiler-Profiler program simultaneously. After the instrument has been proven to function properly, amplify the other multiplex systems.

Cofiler and Profiler: Amplify 24 positive control samples in every other well or every fourth well of the thermocycler block. Insert an amplification negative between two positive control samples.

Other Multiplexes: Amplify 24 positive control samples in every fourth well of the thermocycler block. Insert an amplification negative between two positive control samples.

The following guidelines apply:

- All samples must yield the correct type
- No sample should display additional alleles
- All samples should be of similar peak intensity

Effective Date: 01/07/2005

QC330 PERFORMANCE TEST AFTER MAJOR REPAIRS FOR ABI 310 and ABI 3100 INSTRUMENTS

This procedure only applies for repairs affecting the optical system and/or computer parts essential for data collection. Neither a performance test nor a new matrix is required for minor repairs such as the syringe for the 310.

Create a new matrix/spectral following QC 210. On the same run, include the amplification product of at least one known sample, one negative control, if not previously run, and if applicable an allelic ladder.

Compare the new results to the old runs in regard to:

- Allele calls
- Peak intensity
- Absence of artifacts

The new instrument must yield the same allele calls and similar peak intensities without unspecific signals. Even if the instrument type is used for more than one kind of casework multiplex it is not necessary to test each multiplex. A performance test in one of the systems is sufficient.

Effective Date: 01/07/2005

QC335 PERFORMANCE TEST AFTER MAJOR REPAIRS FOR 9700 THERMAL CYCLERS

This procedure applies to instruments that have been shipped out for service and have to be tested before reinstating them for use in casework.

Perform diagnostics test as outlined in QC295, QC300 and QC302.

If the thermocycler passes the diagnostics test, amplify a positive control sample in every other well of the thermocycler block. One well should contain the amplification negative control.

The following guidelines apply:

- All samples must yield the correct type
- No sample should display additional alleles
- All samples should be of similar peak intensity

Even if the instrument type is used for more than one kind of casework multiplex it is not necessary to test each multiplex. A performance test in one of the systems is sufficient.

Effective Date: 01/07/2005

QC340 PERFORMANCE TEST FOR MISCELLANEOUS EQUIPMENT FOLLOWING REPAIR

Instruments such as heat blocks, water baths, freezers, balances, pH meters, refrigerators, freezers, ice machines, incubators, microplate washers, microplate readers, and water stations do not require specific performance tests other than the QC tests that are done routinely or as needed (eg., verifying that the water bath temperature is in range) to demonstrate that the instruments are performing to specification. Where applicable, diagnostic tests (eg., linearity and reapeatability tests for the microplate reader) will also be run to demonstrate that the instrument is performing to specification.

Effective Date: 01/07/2005

QC360 CAPILLARY ELECTROPHORESIS (ABI 3100): MAINTENANCE

Basic cleaning of the instruments should be done once a week by simply wiping down the inside with Kimwipes lightly dampened with deionized water. According to the ABI manual, organic solvents should not be used to clean the instrument. Be sure to wipe down the entire inside of the instrument including inside the oven, under the autosampler, the syringe holder and drip trays as well as the doors. Check for leaks around the syringes and clean any dried polymer.

The polymer blocks should be cleaned every time casework analysts change the capillary. They can be cleaned more often as needed if there are leaks.

Once a month, the air filters should be checked to see if they need to be cleaned or changed, the buffer and water reservoirs should be soaked in warm water or changed, and the septa for these reservoirs should be changed.

Be sure that everything is dry when done.

When problems are experienced with the ABI 3100 Capillary Electrophoresis unit, there are limited diagnostic tests that may be done according to the protocols presented below. The purpose of these tests is to check the operation of the laser and the power supply. The diagnostics software is primarily for use by the ABI service engineers. Do not perform tests other than the ones listed below.

LASER TEST OR POWER SUPPLY TEST

1. Make sure the doors of the instrument are closed.

2. To access the diagnostic test files, select the **PE Biosystems** folder from the start menu. And click on the "3100 service" option. From this menu, select "3100diagnostics.exe," then select "Diagnostics Menu." Here you have several options to choose from. Only choose the laser power or EP power options by clicking on the appropriate box. Click on the start button to run the test. Once the test is finished, a pass/fail grade will be given to each tested item. If any test fails, take the instrument offline and place a service call.

3. A message will appear to log the results. Click "no." Click **Return** to exist out of screen until you reach the main diagnostics menu then press **Exit.**

If it is necessary to shut down the instrument, close the instrument doors and press the ON/OFF button on the front of the instrument. Next, turn off the computer.

To restart the instrument, first restart the computer (let it completely restart before proceeding) then press the ON/OFF button on the front of the instrument. The firmware and calibration files will reload.

Effective Date: 01/07/2005

Appendix C-1

This appendix shows a list of log usage and maintenance forms that are used in the Department of Forensic Biology to provide records of equipment use, calibration, and maintenance. All of these forms can be accessed on the Forensic Biology computer network.

Usage and Maintenance Log List

F036 3100 Usage Log
F100 Balance Verification and Maintenance Log
F105 Capillary Electrophoresis Diagnostic Log
F110 Capillary Electrophoresis (ABI 310) Usage Log
F115 Freezer (-20°C) Temperature Control Log
F120 Freezer (-80°C) Temperature Control Log
F135 Heat Block (56°C) Temperature Control Log
F140 Heat Block (65°C) Temperature Control Log
F145 Heat Block (95°C) Temperature Control Log
F150 Heat Block (100°C) Temperature Control Log
F157 Incubator Control Log (37°C)
F160 Kit Control Log
F163 Lab Clean-up Sheet
F165 Maintenance Log
F170 Micropipette Maintenance Log
F172 P30 ELISA Raw Material Quality Control Test Form
F175 pH Meter Calibration Log
F180 Plate Washer Maintenance Log
F182 QA/QC Activity Summary Log Corrective Action Form
F183 Raw Material Quality Control Test Form
F187 Reagents/Machine Verification Quality Control Log
F190 Temperature Control Log-520
F195D Temperature Control Log-Bellevue
F205 Thermocouple (Type T-Brown) Verification Log
F217 Thermocycler Diagnostic form for 9700
F218 Thermocycler 9700 Temperature Nonconformity Log
F220 Thermocycler File Log
F225 Thermocycler Usage Log
F230 Water Bath Temperature Control Log
F235 Water Maintenance Log
F245 Notification of Case Reanalysis Form

Effective Date: 01/07/2005

Appendix C-2

This appendix shows a list of quality control testing "procedures" used in the Department of Forensic Biology. Each procedure may be a combination of several quality control tests listed in Appendix B. If a reagent sheet lists a "procedure" for its quality control, then the reagent must pass all the quality control tests listed. If it lists a specific "QC" number, then the reagent must pass that quality control tests only.

	QC Tests Included	Analysis
Procedure 1	QC250	QuantiBlot
Procedure 2	QC130, QC240, QC350	PCR Amplification and STRs
Procedure 3	QC145A, QC240, QC250, QC130, QC350	Organic Extraction, QuantiBlot, PCR Amplification, and STRs
Procedure 4	QC130, QC145, QC160, QC240, QC250, QC350	Chelex Extraction, QuantiBlot, PCR Amplification, and STRs
Procedure 5	QC350	3100 STRs
Procedure 6	QC130	310 STRs