Department of Forensic Biology

Protocols for Forensic STR Analysis v 6.0

Revision Sheets

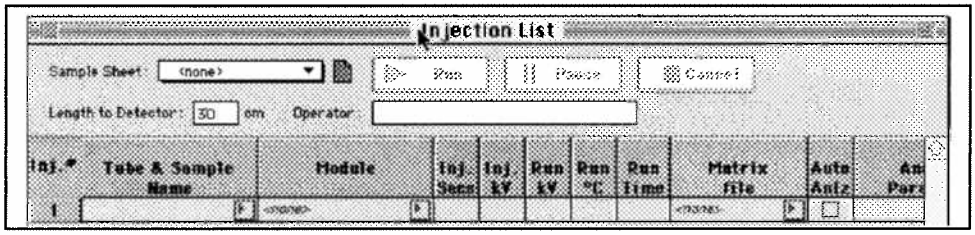
The following table must be filled out when changes to the Quality Assurance Manual are made. The following definitions apply:

Date:	The date the revision went into effect.
Revision #:	The Revision number of the manual affected.
Change:	This column is checked if the revision reflects a change in procedures.
Addition:	This column is checked if the revision reflects an addition to the manual.
Initials:	Initials of the laboratory director.

Date	Rev. #	Page #	Change	Addition	Initials
5/3/99		63		sample comment column	RD
5/3/99		88		load AL on Pro⊕ see	RD
5/3/99		131	removed 25.2 label in FGA		RD
5/3/99		126	removed 11 label in D3		RD
5/3/99		134	expanded scan range to 350		RD
5/6/99		64		added 2 sec injection	RD
5/6/99		64	matrix not only NED POP 4 anymore		RD

Initials: ROS Date: 5/6/99

2. Click the **Genescan Injection List** icon. A blank Injection list appears.



3. Click the arrow in the **Sample Sheet** field to display a pop-up menu and select your sample sheet.

   The information recorded in the Sample Sheet will appear in the Sample Name Column of the Injection List. The Default module is "**GS STR POP4 1mL, F**" and should appear for each sample in the MODULE column. The run conditions are: Inj sec 5, Inj KV 15.0, Run KV 15.0, Run 60°C , Run time 24. Change the injection time to 2 seconds if necessary; do not touch the other settings. The matrix standard must be the most recent matrix made for the instrument. Size standard and Analysis Parameters are both preset analysis default settings.

Reinjecting a Sample

For each run it is advisable to inject the first ladder 2X. To accomplish this highlight, by clicking #1 on the injection list, injection #1, then from the **Edit** menu select **Insert**. A new row will appear at the top of the injection list. Click on the injection # of the sample you want to reinject (in this case the ladder), this will highlight the row. Under **Edit** select **Copy**. Then click on the blank row and under **Edit** select **Paste**.

4. Enter your initials in the OPERATOR box.

5. From the **Window** menu select **manual control**, choose **Temperature Set** from the **Function** pop-up window

6. Set the temperature to 60°C, then click **Execute**. The instrument takes up to 30 minutes to reach the 60°C run temperature. Samples can be prepared while the instrument is heating.

7. Under **Windows** open the **Status** window to observe the temperature.

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In the STD column make sure a diamond is present next to the red box, this denotes that the standard dye is RED (this is the default setting).

5. Fill in the your first sample name in the first space of the **Sample Name** column (Your first sample must be an allelic ladder). The number of the sample (A1, A3, A5..etc) corresponds to the position of the sample in the autosampler tray. Your second sample name should be entered in the A3 position, and so on. ENTER ONLY THE NAMES OF THE SAMPLES THAT ARE PRESENT IN THE RUN. DO NOT FORGET ANY SAMPLES. Use a short name, e.g. the tube labels noted on the amplification sheet, as the sample "name". If you are going to inject a sample more than once **do not** enter the information in the sample sheet twice. For a reinjection see section F, step 3.

6. In the **Sample Info** column enter the complete sample identification, including the FB number in the box(es) corresponding to the dye colors present in your sample; the checkbox in the column labeled "**pres**" (present) automatically becomes selected.

   The **copy**, **paste, and fill down** functions should be used to fill out the sample info column. Fill out the sample information accurately because, when the data is imported into Genotyper, the sample names will appear on the Genotyper dye/lane list. The sample info has to be filled out for all colors that are present.

   COFILER ONLY: copy and paste all the entries from the **sample info** column also into the **sample comment** column

7. Save the sample sheet by selecting **Save As...**from the **File** menu. Enter the name of the run, using the following format :

   CE1/99-001, CE2/99-001, CE3/99-001 respectively,

For QC and validation runs use descriptive names, click **Save.** The sample sheet is automatically saved to the sample sheet folder. Note the sample sheet name and the run folder date on the run control sheets.

F. Preparing a Genescan Injection List and presetting the temperature

The injection list specifies the order in which each sample will run and the modules to be used for the run. Each row in the injection list corresponds to a single operation - an injection. The left column is the injection number and implies these operations are ordered (sequential).

1. Choose **New** from the **File** menu. The **Create New** Dialogue box appears.

Asian DB 1-30. The gel name will automatically be used as a project name during gel analysis.

Do not forget to note the **Runfolder date and time** on the gel sheet (see step 3 under B create a run file). The runfolder date and time are found on top of the GS run window.

2. Make a mastermix of 5μl blue formamide and 0.55μl of Genescan 500 standard per sample for n+2 samples referring to the following table:

sample no.+ 2	Blue Formamide	GS 500
12	60 μL	6.6 μL
16	80 μL	8.8 μL
20	100 μL	11 μL
26	130 μL	14.3 μL
36	180 μL	19.8 μL

Profiler Plus only: include one tube of allelic ladder in the above calculation.

3. Label a sufficient number of 0.5mL sample tubes. Have another analyst witness your tube set up. Add 5μl of the mastermix to each tube, then add 4μl of amplified product. The amplified product must be pipetted directly into the formamide, otherwise the sample will not be denatured correctly.
Profiler Plus only: add 4μl of allelic ladder to the mastermix. Load the allelic ladder on the gel twice from that one tube.

4. Heat the samples at 95°C for two minutes and put on ice immediately. The samples can stay chilled for a maximum of 30 minutes. If they have not been loaded in that time span, the samples must be reheated.

F. Sample Loading and Starting the Run

1. Remember: the run cannot be started before the gel temperature is at least 48°C. Check the status window for the temperature readings.

2. Interrupt the prerun by clicking **pause.**
NOTE: Do not cancel and terminate the run because this will cause the temperature to drop!!!

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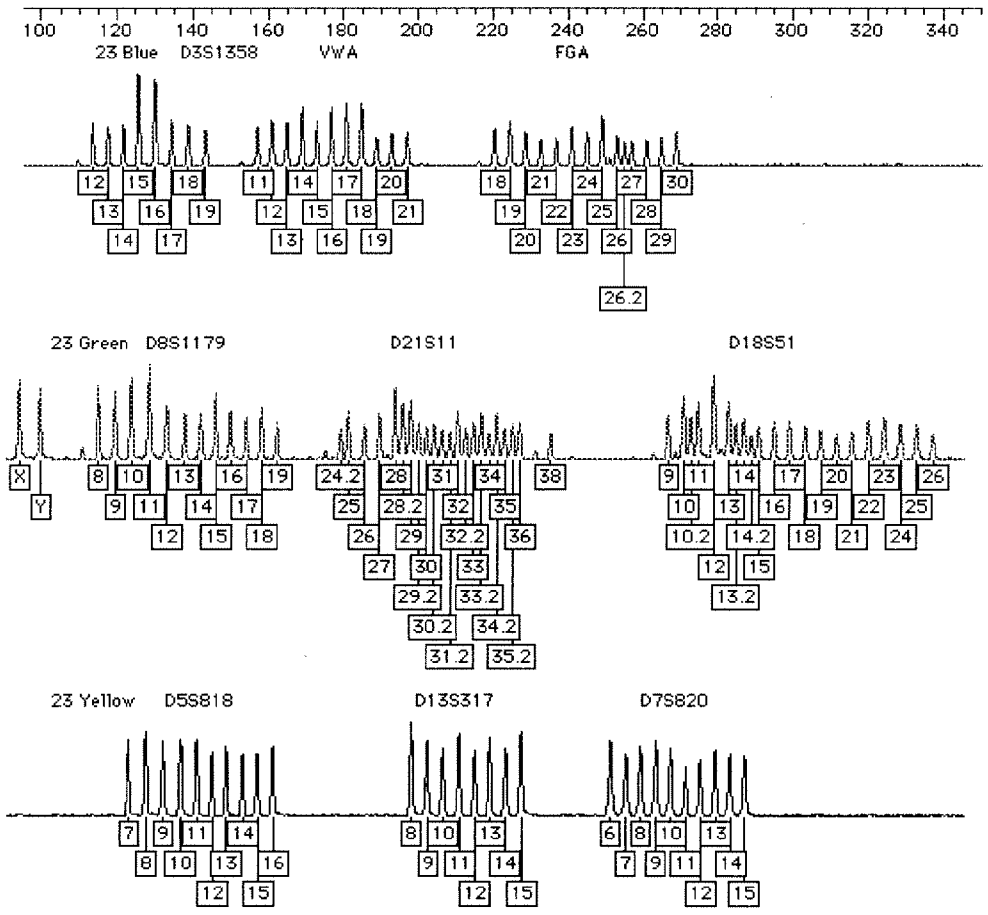
If the alleles for the positive control are shifted one step towards a higher allele number, this is an indication that the first allele for the allelic ladder has been assigned incorrectly (see step 7.).

Profiler Plus Allelic Ladder

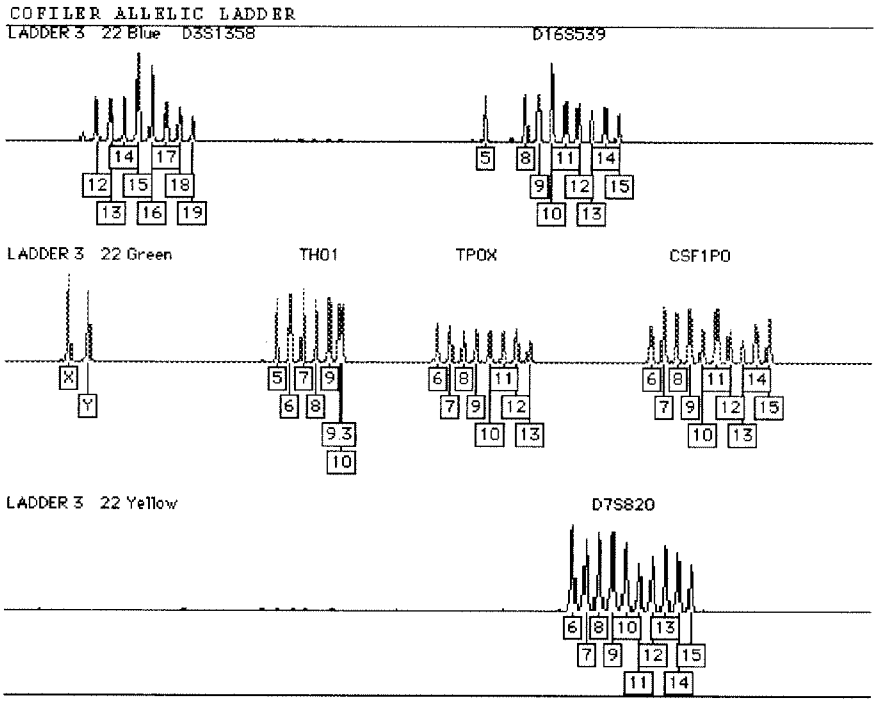


7. If the first allele of the ladder has been assigned incorrectly in one of the systems, in most cases, this is because the preceding stutter peak is designated with the first allele name. If this is the case, you must raise the peak height in the categories window in order to force the software to skip the stutter peak.

- First determine the height of the stutter peak by placing the cursor on the peak in question (as if you are editing). The information displayed on the top of the window

If the alleles for the positive control are shifted one step towards a higher allele number, this is an indication that the first allele for the allelic ladder has been assigned incorrectly (see step 7.).

Cofiler Allelic Ladder



7. If the first allele of the ladder has been assigned incorrectly in one of the systems, in most cases, this is because the preceding stutter peak is designated with the first allele name. If this is the case, you must raise the peak height in the categories window in order to force the software to skip the stutter peak.

- First determine the height of the stutter peak by placing the cursor on the peak in question (as if you are editing). The information displayed on the top of the window refers to the peak where the cursor is located and contains the peak height. Make a note of the peak height.

- Open the categories window (under views on the menu) and highlight the first allele in the offset category (e.g 18 o.s.) of the polymorphism that needs to be corrected.

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12. Before printing plots, check that the zoom range shows 90-350. Change the page set-up back to 100% and letter. Under **file** select **print**. Click **okay** in the print dialogue box.

13. After the printing is finished, under **file**, **quit** Genotyper. Click **save**.
The Genotyper file will automatically be saved in the run folder from which you imported your data; it can be located there and re-edited at a later date.

14. As outlined in the General Guidelines for fluorescent STR analysis after running the Genotyper the run folder should contain the following items:
    - a run file (65K ABI Prism 377 Collection document)
    - the run log (65K ABI Prism 377 Collection document)
    - the gel file (ca. 12 MB Genescan document)
    - sample files for all samples (33K Genescan documents)
    - a project file (65K Genescan document)
    - the genotyper file (ca. 400K Genotyper document)

Archive data as described in the General Guidelines for fluorescent STR analysis.

Genotyper Trouble Shooting for AmpFlSTR Blue and Green, Profiler Plus and Cofiler

A. If you get an Error Message when you try to run the Genotyper Macro 1 that reads: **"Could not complete your request because no dye/lanes are selected"**.

Make sure you have actually imported the ladder from the project. Make sure "ladder" is spelled correctly in the **dye/lanes window**. If there is a mispelling or the sample information for the ladder is absent, the macro will not recognize the ladder and will not be able to complete the procedure. Correct the spelling and rerun the macro.

B. If you get an Error Message when you try to run the Genotyper Macro 1 that reads: **"Could not complete your request because the labeled peak could not be found".**

This message indicates that the ladder cannot be matched to the defined categories. There are three possibilities.

1. The wrong ladder is being typed, (i.e. You are trying to type a Green ladder in the Blue Genotyper or vice versa).