4.1 PURPOSE

To amplify and detect DNA samples using the AmpF*l*STR Yfiler (YF) system.

4.2 RESPONSIBILITY

DNA Section Personnel.

Note: This process should occur in an amplification hood.

4.3 AmpF*l*STR Yfiler Amplification

4.3.1 These procedures generally follow those outlined in AmpF*l*STR Yfiler PCR Amplification Kit User's Manual.

4.3.2 The total reaction volume is 25µL; 15µL of Master Mix (MM) plus optimally ~1.0-2.0ng of purified male DNA (volume of DNA + dH_2O = 10µL) as determined by qPCR for evidentiary samples.

To obtain the targeted amount of DNA (~1.0-2.0ng), dilutions with dH_2O may have to be made. This step is documented on DNA QR-22 or QR-22a and maintained in the batch file.

The master mix is prepared per manufacturer's instructions (AmpF*l*STR Yfiler PCR Amplification Kit User's Manual): Make enough master mix for the number of reactions needed plus additional reactions to compensate for pipetting volume variations.

Reaction Mix	9.2µL/reaction
Primer Set	5.0µL/reaction
AmpliTaq Gold Polymerase	0.8µL/reaction
Aliquot 15µL of MM per tube	
DNA + dH_2O	10µL/reaction

4.3.3 An aliquot of the master mix is dispensed into 0.2mL amplification tubes, strip tubes, or into 0.2mL 96 well plates (15µL) along with the appropriate volume of DNA + dH_2O. Multiple quantities of DNA from one sample may be amplified when Taq inhibitors are present in the sample or when the sample DNA is significantly degraded.

4.3.4 The samples are amplified in a GeneAmp PCR System 9700 thermal cycler using the 9600 emulation mode or other suitable thermal cycler with the ramping speeds set to 1°C/sec. The amplification parameters are 95°C for 11 min, followed by 28 cycles of (94°C for 1 min, 61°C for 1 min, 72°C for 1 min), then 60°C for 80 min, and finally 12°C until the samples are removed from the thermal cycler.

4.3.5 A previous laboratory positive control (KJL-female DNA—which can be used for older cases) is not amplified by the AmpF*l*STR Yfiler kit. Where possible, the male laboratory positive control (EP1, RKO or TMP) can be amplified by AmpF*l*STR Yfiler. When not available, amplifying the AmpF*l*STR Yfiler kit male positive control is sufficient.

4.4 Detection of PCR Amplification Products on the ABI Prism 3130*xl* Genetic Analyzer:

4.4.1 Starting the Data Collection Software

4.4.1.1 Turn on the monitor and computer. Log on to the local computer with the appropriate login ID and password. Turn on the 3130 machine. Wait for a solid green light before launching collection software.

4.4.1.2 Select **Start > All Programs > Applied Biosystems > Data Collection > Run 3130 Data Collection v3.0** or **double click the shortcut located on the desktop.** The pop-up window, "Service Console", will launch from red circles to yellow triangles to green squares to indicate whether all the applications are running.

4.4.2 Sample Sheet Preparation

4.4.2.1 On the left menu pane, select **Plate Manager**. Select **New** and complete the information in the **New Plate Dialog** box. Select Generic Genemapper for the **Application** drop-down box. For **Owner/Operator**, type in the initials of the analyst performing the injection.

4.4.2.2 When the **Plate Editor** pops up, under the **Sample Name** column, enter a sample name in the appropriate well. For wells that will not have a sample, do not fill out the information for sample name. For ladders, the word **ladder** must appear in the **Sample Type** column box. Otherwise, the analyst must make the correction during analysis. There should be at least one ladder run per module, due to slight differences in migration of amplification products between modules. Each injection module has 16 wells (2 adjacent columns of 8 wells, A1-H2, A3-H4, A5-H6, A7-H8, A9-H10, or A11-H12).

4.4.2.3 To help prevent injection carryover, high-level samples (i.e. samples with ideal peak heights at a 5 second injection or less) are not to be placed in a module expected to be injected for the maximum time. Injections on a 3130 instrument used only for knowns are an exception to this.

4.4.2.4 Correspondingly, for that sample row, select the appropriate value from the drop-down box under **Results Group** and **Instrument Protocol 1**. Based on internal validation and performance checks, the following injection time ranges are recommended for use on specific 3130 instruments that are online for casework:

3130-1 2sec – 35sec (CO/knowns only)
3130-2 2sec – 22sec
3130-3 2sec – 20sec
3130-4 2sec – 22sec
3130-6 2sec – 16sec

4.4.2.5 For Example: Sample Name (NEG), Results Group (Run Data), Instrument Protocol 1 (HID_G5_3kV10sec).

4.4.2.6 Negative controls (Neg and RB) must be injected at the most sensitive injection time used for any samples for which they control.

4.4.2.7 Click **OK** to save the Plate Record.

4.4.2.8 Alternatively to steps 4.4.2.1- 4.4.2.3, a **macro** stored in the Injection Worksheet Excel file may be used to generate a plate record which is then imported into the **Plate Manager**. Follow instructions in the Excel file.

4.4.3 Sample Preparation and Plate Assembly

4.4.3.1 Use the following formula to generate a **Yfiler** master mix for your samples: GS500-LIZ [(# of samples + 1) x 8.7 – 8.9µL formamide] + [(# of samples + 1) x 0.3 – 0.1µL size standard], i.e. if using 8.7µL formamide then use 0.3µL size standard, if using 8.9µL formamide then use 0.1µL size standard; GS600-LIZ [(# of samples + 1) x 8.0 – 8.7µL formamide] + [(# of samples + 1) x 1.0 – 0.3µL size standard.

4.4.3.2 Wells for negative controls (Neg and RB) must contain the lowest volume of size standard used for any sample for which they control.

4.4.3.3 Dispense 9µL of master mix to the appropriate wells for Yfiler samples. If a well does not contain any sample within a module, dispense 10µL of formamide to that well.

4.4.3.4 Dispense 0.5-2µL (as needed) of amplification product or ladder (use the appropriate Yfiler kit ladder) to the appropriate well.

4.4.3.5 Wells for negative controls (Neg and RB) must contain the highest volume of amp product used for any sample for which they control.

4.4.3.6 Cover the plate with the appropriate septa, mix briefly (via the plate vortexer), and centrifuge. Alternatively to mixing by the vortex, heat the plate for ~3 minutes at 95ºC, then incubate for ~3 minutes at 4°C or immediately snap freeze for ~2 minutes in the metal 96-well block stored in the -20°C freezer.

4.4.3.7 Carefully attach the sample plate to the plate base. Snap the plate retainer onto the plate and plate base. Verify that the holes of the plate retained and septa holes are aligned.

4.4.4 Plate Loading and Running

4.4.4.1 An extra flush of fresh polymer must be pushed through the array if running a maximum injection time run following a maximum injection in which one or more wells contained high-level samples (regardless of whether recorded or not), or if your first injection is at maximum time for the instrument (so you don't know what was run previously). This flush is incorporated into the run programs for maximum injections.

Should the need arise to perform an array flush manually, the procedure is: select manual control and change the "Send Defined Command For:" field to "Autosampler", under "Command Name" select "Move Auto Sample to Site", then under "Value" select "Waste". Then click send command. Next change the "Send Defined Command For:" field to "Polymer Delivery Pump". Then under "Command Name" select "Fill 36 cm capillary array". Then click send command. After the capillary array fill completes, change the "Send Defined Command For:" field to "Autosampler", under "Command Name" select "Move Auto Sample to Site", then under "Value" select "Water 2". Finally, repeat this last step except with the "Value" set to "Buffer".

4.4.4.2 Select the **TRAY** button on the machine. The autosampler will move from its home position to the load position. Open the machine door and put the plate assembly onto the autosampler, making sure that it lies flat in the autosampler. Close the instrument doors.

4.4.4.3 On the left menu pane, select **Plate View**. In the row **Plate Name** and under column **Condition**, choose the search criteria "contains", and type the analyst initials (or other appropriate qualifier to find the plate record) in the **Value 1** column. Hit the **Search** button to locate plate record names with the analyst initials. Select the sample plate record, and click on the appropriate plate (A or B) to link the plate record to the position on the autosampler. The color will change from yellow to green. Select **Run View** to make sure that all samples are correctly selected and will collect data for analysis.

4.4.4.4 Click the green button in the tool bar. A **Process Plates** dialog box opens, and click OK. The software will automatically perform a run validation. If it passes, then, the run starts. If it does not pass, the run does not start. Check the event log for information.

4.4.4.5 When re-running the same module on the plate, select **Plate Manager** on the left pane menu. Perform a search for the plate record. Select the plate record, and hit the **Duplicate** button. When a dialog box appears, edit the original plate name, for example, from IDP-2-MGR090915 to IDP-2-MGR090915-20sec. Delete the samples names from the sample sheet for data not being collected or not used for analysis. Modify the Instrument Protocol 1 and other parameters as needed. Click OK. Alternatively, create a new plate record with the appropriate samples to be rerun as in the previous section **Sample Sheet Preparation 4.4.2**.

4.4.4.6 After the module run is finished, transfer a copy of the data files from E:\AppliedBiosystems\UDC\DataCollection\data **to** U:\3130 NUCLEAR DNA CASEWORK & QC\To Be Analyzed\primary analyst's folder (identified by the analyst's initials).

4.4.5 3130 Maintenance and Cleaning

4.4.5.1 Before using the ABI 3130*xl*, ensure adequate levels of buffer and water in the reservoirs.

4.4.5.2 Replace the water and 1X CE run buffer in the reservoirs on the instrument as needed (approximately once a week or if levels are low due to evaporation). Allow 1X run buffer in the reservoirs to warm to near room temperature (approximately 20 minutes) before starting first run with replaced buffer to avoid split peaks in Amelogenin.

Each time the deionized water and 1X CE are replaced, take the reservoir septa and rinse them in de-ionized water. These septa are replaced with new septa approximately every 3 months which is documented on DNA-QR-222 3130xl Maintenance Log.

4.4.5.3 Check for bubbles in the pump block, lower polymer block, interconnect tube, polymer supply tube, and channels. Use the **Bubble Remove Wizard** under the **Wizard** drop-down menu as needed.

4.4.5.4 Check the loading-end header to ensure the capillary tips are not crushed, damaged, or bent.

4.4.5.5 Check the level of polymer in the polymer fill chamber and the polymer bottle to ensure sufficient volume for runs (the fill chamber holds ~ 0.5mL, which is enough for ~10 module runs). Otherwise, remove a bottle of polymer from the refrigerator and allow it to equilibrate to room temperature (~20 minutes). Use the **Replenish Polymer Wizard** or **Water Wash Wizard** (if the current bottle of polymer has been on the instrument for more than a week) found under the **Wizard** drop-down menu.

4.4.5.6 If applicable:

Clean off any dried polymer build-up (crystals) on the instrument including the capillary tips and the stripper plate with dH_2O and lint-free swabs.

Clean the array port knob, plug, or opening threads of these parts with lint-free swabs.

Clean out the drip trays with dH_2O and lint-free tissue.

Flush the water trap. Fill a 20mL Luer Lock Syringe with dH_2O and expel any bubbles from the syringe. Attach the syringe to the Luer fitting. Open the exit fitting at the top left side of the pump block. Hold an empty beaker under the exit fitting. Flush the trap by pushing slowly and steadily (apply even force) on the syringe plunger. Do Not Use Excessive Force. After flushing, tighten the fittings and remove the syringe.

Fill out the DNA QR-222 3130*xl* Maintenance log appropriately.

4.4.6 Computer Maintenance

4.4.6.1 Restart the computer and instrument as needed, e.g. monthly.

4.4.6.2 To delete records from the database, select **GA Instruments > Database Manager** from the left menu pane. Click **Cleanup Processed Plates**. Click **OK** on the pop-up dialog box. You will have to do this if you attempt to save a plate record and you get an error message telling you that the plate record cannot be saved because the Database is full. Note: the spectral and spatial calibrations are not deleted. Spectral calibrations can only

be deleted after all processed plates associated with that spectral calibration are deleted. A spectral calibration can be re-created using the archived run on CD-ROM.

4.4.7 Performing, Creating, and Evaluating a Spatial Calibration

4.4.7.1 The spatial calibration establishes a relationship between the signal emitted by each capillary and the position where that signals falls on and is detected by the CCD camera.

4.4.7.2 A spatial calibration is required when a capillary array is installed or when the capillary array is briefly removed from the detection block.

4.4.7.3 On the left menu pane, select **GA Instruments** > **ga3130*xl*** > ***instrument name*** > **Spatial Run Scheduler**. In the Spatial Protocol section, select either **Protocol > 3130SpatialNoFill_1** (old polymer) or **Protocol > 3130SpatialFill_1** (fresh polymer). Click **Start**.

4.4.7.4 Evaluate the spatial calibration profile by examining peak heights (similar for all peaks), orange cross at the top of each peak, single sharp peak for each capillary, and spacing between 15 to 16 pixels (theoretical spacing of 15 pixels between capillaries).

4.4.7.5 If the Spatial profile passes, click **Accept** to write the calibration data to the database. If the spatial fails, retry the spatial calibration. If the spatial continues to fail, a new capillary array will need to be installed. Follow the **Change Capillary Array Wizard** found under the **Wizard** drop down menu.

4.4.8 Performing and Creating a Spectral Calibration

4.4.8.1 The spectral calibration creates a matrix that is used to subtract out overlapping spectral fluorescence in raw data, using the 5-dye chemistry kits, and storing matrix applied data in the generated sample files.

4.4.8.2 In addition to when required by chemistry or equipment changes, a spectral calibration may be performed when there is a decrease in spectral separation in the raw/analyzed data.

4.4.8.3 Prepare amount of calibration standard accordingly to each kit; dye set G5 (Applied Biosystems, Matrix standards set DS-33) for Yfiler].

4.4.8.4 Add spectral standards to one entire module to run, i.e., A1-H2 or A3-H4

4.4.8.5 Cover with appropriate septa, mix briefly, and centrifuge.

4.4.8.6 Denature the spectral standards for ~3 minutes at 95ºC, then incubate for ~3 minutes at 4°C or immediately snap freeze for ~2 minutes in the metal 96-well block stored in the -20°C freezer.

4.4.8.7 Attach the sample plate to the plate base. Snap the plate retainer onto the plate and plate base. Verify that the holes of the plate retained and septa holes are aligned. Load the plate assembly into the ABI 3130.

4.4.8.8 Create a plate record (sample sheet) for the spectral calibration run using the **Plate Manager**. Select **New** from the menu, type in a name for the plate, select **Spectral Calibration** for the application, and type in your initials as the owner and operator. Type in the dye set (i.e. G5) as the **Sample Name** for all 16 samples of the module. Select the appropriate **Instrument Protocol 1** (i.e. for G5 dye sets). Link the plate record to the correct plate position and start the run.

4.4.9 Evaluating and Activating the Spectral Calibration

4.4.9.1 On the left menu pane, select **GA Instruments** > **ga3130*xl*** > ***instrument name*** > **Spectral Viewer**. In the dye set drop-down list, select the dye set that was just created.

4.4.9.2 In the plate diagram, select each well to view the capillary spectral results. Verify for the electropherogram labeled "Intensity vs Pixel Number" that the order of the peaks in the spectral profile from left to right are blue-green-yellow-red (4-dye) or blue-green-yellow-red-orange (5-dye). Verify for the electropherogram labeled "Intensity vs Scan Number" that the order of the peaks in the spectral profile are in reverse to that listed above. Peaks less than 750 RFU will cause a spectral for that capillary to fail. The peaks will typically be in the ~2000 – 3000 scan range. Extra peaks detected in the < 2000 scan range can cause a spectral for that capillary to fail. Repeat these steps for all capillaries in that module.

4.4.9.3 Capillaries that have failed spectral calibrations will be assigned a spectral profile from an adjacent capillary. 12 or more of the 16 capillaries must pass for a successful spectral calibration.

4.4.9.4 To set an active spectral calibration, on the left menu pane, select **GA Instruments** > **ga3130*xl*** > ***instrument name*** > **Spectral Viewer**. In the dye set drop-down list, select a dye set. In the **List of Calibrations for Dye Set**, select the spectral calibration to be

used, which will display the spectral profile and raw data. Click **Set** to accept the spectral profile.

Also referenced is:

ABI Prism 3130 Genetic Analyzer User's Manual.
ABI Prism 3130 Genetic Analyzer Quick start guide for fragment analysis
ABI Prism 3130 and 3130-Avant Genetic Analyzers User Bulletin 01/03