**4.1 PURPOSE**

To amplify and detect DNA samples using Identifiler Plus (IDP), Minifiler (MF), and Yfiler (YF) systems.

**4.2 RESPONSIBILITY**

DNA Section Personnel.

**Note: These processes should occur in an amplification hood.**

**4.3 AmpF*l*STR Identifiler Plus Amplification**

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

4.3.2 The total reaction volume is 25µL; 15µL of Master Mix (MM) plus optimally ~0.5-1.5ng of purified human DNA (volume of DNA + $dH_2O$ = 10µL) as determined by qPCR for evidentiary samples.

To obtain the targeted amount of DNA (~0.5-1.5ng), dilutions with $dH_2O$ may have to be made. This step is documented on DNA QR-22a and maintained in the batch file.

The master mix is prepared per manufacturer's instructions (AmpF*l*STR Identifiler Plus PCR Amplification Kit user's Manual): Make enough master mix for the number of reactions needed plus 1-3 additional reactions to compensate for pipetting volume variations.

| | |
| --- | --- |
| Reaction Mix | 10µL/reaction |
| Primer Set | 5µL/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 20 sec, 59°C for 3 min), then 60°C for 10 min, and finally 12°C until the samples are removed from the thermal cycler.

4.3.5 Identifiler Plus Amplification of DNA from single source samples (database samples and casework knowns).

4.3.5.1 This protocol may be used for known sample processing only. Please refer to DNA SOP-12 and SOP-19 for further details.

4.3.5.2 Half reactions may be used with a total volume of 12.5µL. Prepare the master mix. Use the volumes listed on the extraction worksheets (QR-14g or QR-14f) or the amplification worksheet.

In general, the reaction is as follows:

| | |
| --- | --- |
| Reaction Mix | 5µL/reaction |
| Primer Set | 2.5µL/reaction |
| DNA + $dH_2O$ | 5µL/reaction |
| Total volume | 12.5µL/reaction |

4.3.5.3 Positive Amplification Control. The positive control supplied by the kit manufacturer is purified genomic DNA (liquid). Therefore, an appropriate quantity of master mix (the number of reactions needed plus 1-3 additional reactions to compensate for pipetting volume variations) without water is generated first with 7.5 ul of this being added to the positive amplification control.

4.3.5.4 Then, depending on the input volume of DNA, add the appropriate amount of water for each reaction for the number of reactions made in 4.4.3, minus one reaction, to the master mix and vortex and briefly spin down. The master mix (plus water) is dispensed into the 0.2mL amplification tubes or into 0.2mL wells of a 96-well plate. Finally, the DNA is added to the tube/well. The final volume of the reaction should be 12.5µL.

4.3.5.5 Note: Quality and quantity of sample may be taken into consideration when determining the volume of input DNA used for amplification.

4.3.5.6 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 as in 4.3.4.

**4.4 AmpF*l*STR Minifiler PCR Amplification Kit**

4.4.1 The Minifiler Kit uses primers closely flanking the STR regions of the DNA. This amplification results in amplicons that are significantly shorter in length than those produced in Identifiler Plus. It has been shown that Minifiler has a higher success rate for DNA analysis of degraded DNA samples. This kit is to be used for select evidentiary samples* and only with approval of the DNA Technical Leader.

*In general, Minifiler is used only when significant sample degradation would account for dropout at the larger Identifiler Plus amplicons and to generate additional results from degraded samples for CODIS entry. Given that Minifiler loci are in the same size range as the smaller Identifiler Plus amplicons, Minifiler is not indicated if the potential dropout would be occurring at the smaller Identifiler Plus loci (D8, D21, D3, TH01, D19, vWA, D5).

4.4.2 This amplification kits is a 5 dye STR multiplex assay that amplifies 8 tetranucleatide repeat loci and the Amelogenin marker. These loci and their respective dye tag are D7S820 and D13S317 (6-FAM); D2S1338 and D21S11 (VIC), D18S51 and D16S539 (NED); CSF1PO and FGA (PET).

4.4.3 Where appropriate, known samples will be amplified by Minifiler, in addition to evidentiary samples. For example, a known sample will be amplified by Minifiler when there is a potential for non-concordance between the Identifiler Plus profile (of the known) and the Minifiler profile of the question sample (i.e. one allele does not match).

4.4.4 Minifiler uses the GeneScan-500 or 600 Size Standard labeled with the LIZ dye used also in Identifiler Plus Kits.

**4.5 AmpF*l*STR Minifiler Amplification**

Minifiler master mix is created by combining the reagents in the following ratios:
Minifiler Master Mix: 10µL/reaction
Minifiler Primer Set: 5µL/reaction

4.5.1 Dispense 15µL of master mix into 0.2mL amplification tubes.

4.5.2 Add to each tube the sample of purified human DNA up to 10µL (volume of DNA + $dH_2O$ = 10µL) as determined by qPCR. Total reaction volume is 25µL.

4.5.3 To obtain the targeted amount of DNA (~0.5-2ng), 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.

4.5.4 Minifiler Kit Amplification Positive Control (0.10ng/µL male 007 DNA) is added at a target of 1ng/µL. 10µL+15µL of master mix.

4.5.5 Amplification Negative Control is 10µL of dH2O + 15µL of master mix.

4.5.6 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 20 sec, 59°C for 2 min, 72°C for 1 min), then 60°C for 45 min, and finally 12°C until the samples are removed from the thermal cycler.

**4.6 AmpF*l*STR Yfiler Amplification**

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

4.6.2 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 1-3 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 |

4.6.3 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.

4.6.4 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.

4.6.5 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 (RKO) will be amplified by AmpF*l*STR Yfiler. When not available, amplifying the AmpF*l*STR Yfiler kit male positive control is sufficient.

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

4.7.1 **Starting the Data Collection Software**

4.7.1.1 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.

4.7.1.2 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.

4.7.2 **Sample Sheet Preparation**

4.7.2.1 On the left menu pane, select **Plate Manager**. Select **New** and complete the information in the **New Plate Dialog** box. **Plate Name** may contain relevant information such as the amp system-machine#-BatchID, i.e., IDP-3-MGR090915, YF-2-NCSY090915. Select Generic Genemapper for the **Application** drop-down box. For **Owner/Operator**, type in the initials of the analyst performing the injection.

4.7.2.2 When the **Genemapper 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 Genemapper 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).

4.7.2.3 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, the following injection time ranges are recommended for use on specific 3130 instruments that are online for casework:

3130-1 2sec – 35sec
3130-2 2sec – 30sec
3130-3 2sec – 20sec
3130-4 2sec – 20sec
3130-6 2sec – 20sec

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

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

4.7.2.6 Alternatively to steps 4.8.2.1-4.8.2.3, 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.

### 4.7.3 Sample Preparation and Plate Assembly

4.7.3.1 Use the following formula to generate an **Identifiler Plus, Minifiler, or Yfiler** mastermix 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.

4.7.3.2 Dispense 9µL of master mix to the appropriate wells for Identifiler Plus, Yfiler, or Minifiler samples. If the wells do not contain any sample, dispense 10µL of formamide to that well.

4.7.3.3 Dispense 0.5-2µL (as needed) of amplification product or ladder (use the appropriate Identifiler Plus, Minifiler, Yfiler, kit ladder) to the appropriate well.

4.7.3.4 Cover the plate with the appropriate septa, mix briefly (via the plate vortexer), and centrifuge. Alternatively to mixing by the vortex, denature 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.

4.7.3.5 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.

**4.7.4 Plate Loading and Running**

4.7.4.1 If your first injection is going to be at the maximum injection time for that 3130 instrument, push an extra flush of fresh polymer through the array. If running back to back maximum injections, push an extra flush of fresh polymer through the array in between the maximum injection runs. To do this, 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".

4.7.4.2 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.

4.7.4.3 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.

4.7.4.4 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.

4.7.4.5 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 4.12.2**.

4.7.4.6 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).

**4.7.5 3130 Maintenance and Cleaning**

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

4.7.5.2 Replace the water and 1X CE run buffer in the reservoirs on the instrument as needed (e.g.., after 10-12 runs 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 every 3 months which is documented on DNA-QR-222 3130xl Maintenance Log.

4.7.5.3 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.

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

4.7.5.5 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.

4.7.5.6 Check the pump block and the lower polymer block to ensure a secure fit on the instrument.

4.7.5.7 Check for leaks around the array knob, interconnecting tube nuts, and check valve.

4.7.5.8 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.

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

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

4.7.5.11 Flush the water trap as needed. 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 to receive about 5mL of waste. Flush the trap by pushing steadily (apply even force) on the syringe plunger. It takes about 30 seconds to flush 5mL. Do Not Use Excessive Force. After flushing, tighten the fittings and remove the syringe.

4.7.5.12 Run the Water Wash Wizard as needed.

4.7.5.13 Fill out the DNA QR-222 3130*xl* Maintenance log as needed.

**4.7.6 Computer Maintenance**

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

4.7.6.2 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.

### 4.7.7 Performing, Creating, and Evaluating a Spatial Calibration

4.7.7.1 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.

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

4.7.7.3 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**.

4.7.7.4 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).

4.7.7.5 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.

### 4.7.8 Performing and Creating a Spectral Calibration

4.7.8.1 The spectral calibration creates a matrix that is used to subtract out overlapping spectral fluorescence in raw data, using either the 4-dye or 5-dye chemistry kits, and storing matrix applied data in the generated sample files.

4.7.8.2 A spectral calibration can be performed when a new dye set is used on the instrument, a change in capillary length or polymer type, a realignment/replacement of the laser or CCD camera, or a decrease in spectral separation in the raw/analyzed data.

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

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

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

4.7.8.6 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.

4.7.8.7 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.

4.7.8.8 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 or F) as the **Sample Name** for all 16 samples of the module. Select the appropriate **Instrument Protocol 1** (i.e. for G5 or F dye sets). Link the plate record to the correct plate position and start the run.

### 4.7.9 Evaluating and Activating the Spectral Calibration

4.7.9.1 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.

4.7.9.2 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.

4.7.9.3 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.

4.7.9.4 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