

**Department of Forensic Science**

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**FORENSIC BIOLOGY  
PROCEDURES MANUAL  
OF  
CE FOR AMPFSTR® YFILER™**

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## 1 CAPILLARY ELECTROPHORESIS

All sample preparation work surfaces will be cleaned with 10% bleach (or a solution that will degrade DNA) followed by cleaning with isopropyl alcohol and/or ethanol before sample preparation.

For the purpose of this manual, the following definitions apply:

**Injection** - all samples that were separated via CE at a single time. Thus, for one injection on the 3130xl, up to 16 samples can be separated at a single time.

**Run** - all samples that were defined on a single plate and loaded onto the instrument at a single time for separation, regardless of the number of injections utilized to separate each sample contained on the plate. Thus, a run could contain up to 96 samples for separation on a single plate. At least 2 ladder samples are needed per original run. At least 1 ladder sample is needed per reinjection run if set up as a new plate record.

### 1.1 Equipment

- Applied Biosystems 3130xl Genetic Analyzer
- Heat block, capable of 95 °C
- 96-well plate centrifuge
- Pipettes – 2 µL, 10 µL, 100 µL, 200 µL, and 1000 µL
- 8-Channel Pipette – Range 0.5 µL to 10 µL
- Repeater pipette

### 1.2 Materials

- 36-cm capillary array (Applied Biosystems, Inc. P/N 4315931 (16-capillary))
- MicroAmp® Optical 96-Well Reaction Plate (Applied Biosystems, Inc. P/N N801-0560)
- 96-well plate septum (Applied Biosystems, Inc. P/N 4315933)
- 96-well plate retainer and base (Applied Biosystems, Inc. P/N 403081 and N801-0531)
- 50 mL conical tube
- Freezer block
- Sterile ART tips for pipettes – 10 µL, 20 µL, 100 µL, 200 µL, and 1000 µL

### 1.3 Reagents

- 10X Genetic Analyzer Buffer with EDTA (Applied Biosystems, Inc. P/N 402824)
- Hi-Di Formamide (Applied Biosystems, Inc. P/N 4311320)
- Yfiler™ Allelic Ladder (Applied Biosystems, Inc. P/N 4373675)
- GeneScan™ 600 LIZ® Size Standard (Applied Biosystems, Inc. P/N 4366589)
- POP-4 Polymer (Applied Biosystems, Inc. P/N 4363752 (3.5 mL) or P/N 4352755 (7 mL))

### 1.4 Safety Considerations

The Applied Biosystems 3130xl Genetic Analyzer uses an Argon-ion laser. Under normal operating conditions, the instrument laser is categorized as a Class I laser. All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating, (during service with safety interlocks disabled) you may be exposed to laser emissions in excess of the Class 3B rating. Do not remove safety labels or disable safety interlocks.

Performance Optimized Polymer (POP) is an irritant, always wear gloves when handling or cleaning liquid or dried polymer. It may cause skin, eye, and respiratory tract irritation.

Exposure to Hi-Di formamide causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.

## 1.5 References

- Applied Biosystems. AmpflSTR® Yfiler™ User's Manual, rev C (08/2006).
- Applied Biosystems 3130 and 3130xl Genetic Analyzers, System Profile.

## 1.6 Computer

- 1.6.1 Power on the computer.
- 1.6.2 Power on the 3130xl instrument. Wait for the solid green light on the front of the instrument.
- 1.6.3 Select the Data Collection icon from the screen to start the Data Collection software. The service console opens. When all of the applications are running, the Data Collection Viewer window opens and green squares appear in all fields of the service console.

## 1.7 Sample and Instrument Preparation

- 1.7.1 Ensure that sufficient volume of polymer is present in the bottle for the run and that the polymer was loaded onto the instrument  $\leq 14$  days (2 weeks) prior. (Note: approximately 0.5 mL of polymer is sufficient to run approximately a plate of samples without introduction of bubbles into the pump block. Additional polymer may be necessary to clear bubbles, etc.)
  - 1.7.1.1 If the polymer must be changed, follow the Replenish Polymer Wizard in the instrument software.
- 1.7.2 Ensure that the pump block and lower polymer block are securely fit on the instrument.
- 1.7.3 Ensure that the instrument surfaces are clean; always use Type I water to clean.
- 1.7.4 Check for leaks around the array knob, interconnecting tube nuts, and check valve, all of which are shown in the Figure below.

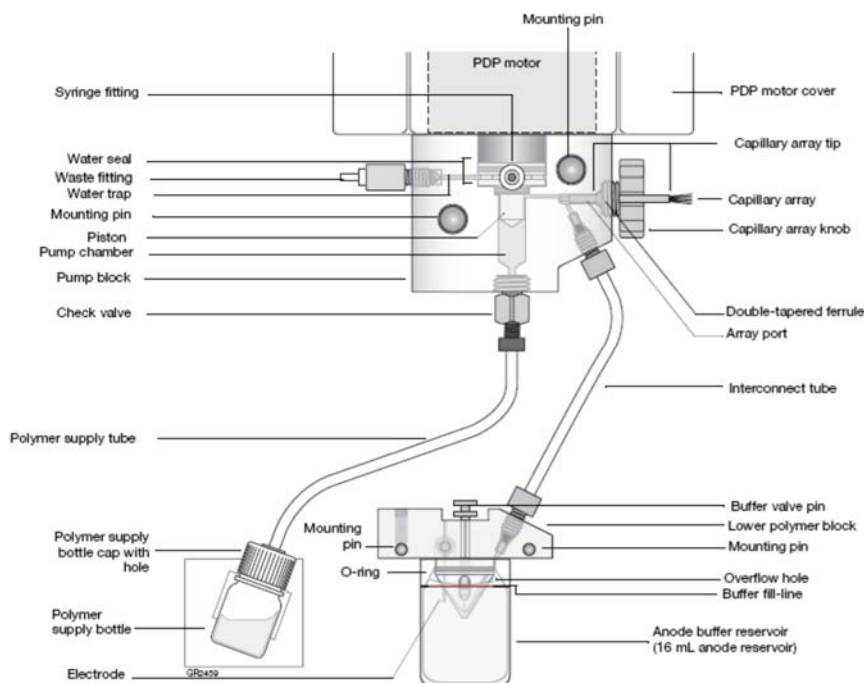


Figure reproduced from Applied Biosystems 3130/3130xl Genetic Analyzers (2004) Maintenance and Troubleshooting Guide

## 1.7.5 Install the Capillary Array

1.7.5.1 If a capillary array has already been installed on the instrument and does not need to be changed, proceed to 1.7.6 for continued preparation of the instrument.

1.7.5.1.1 The capillary array may need to be changed if poor sizing precision or allele calling results are noted; if poor resolution and/or decreased signal intensity is observed.

1.7.5.2 In the Data Collection Software, select the Wizards menu and choose Install Array. Follow the on-screen directions for installing a capillary array.

## 1.7.6 Change the buffer

1.7.6.1 If the buffer on the instrument is <48 hours old, proceed to 1.7.7 for continued preparation of the instrument.

1.7.6.2 Make a 1X dilution of the 10X Genetic Analyzer Buffer with EDTA:

Add 3.5 mL 10X Genetic Analyzer Buffer with EDTA to 31.5 mL Type I water and mix.

1.7.6.3 With the instrument doors closed and the green light illuminated, press the TRAY button to bring the autosampler forward and wait until completely forward.

1.7.6.4 Open the instrument doors and:

1.7.6.4.1 Remove the anode buffer jar at the base of the block. Discard buffer, rinse and dry the jar with a lint-free wipe. Fill to the fill line with newly made buffer, replace the septa strip (or replace with a new one as needed) and replace the jar on the instrument.

1.7.6.4.2 Remove the cathode buffer reservoir. Discard buffer, rinse and dry the reservoir with a lint-free wipe. Fill to the fill line with newly made buffer, replace the septa strip (or replace with a new one as needed) and replace the reservoir on the instrument.

1.7.6.4.3 Remove the water reservoirs. Discard the water, rinse and dry the reservoirs with lint-free wipes. Fill to the fill line with new Type I water, replace the septa strips (or replace with new ones as needed) and replace the reservoirs on the instrument.

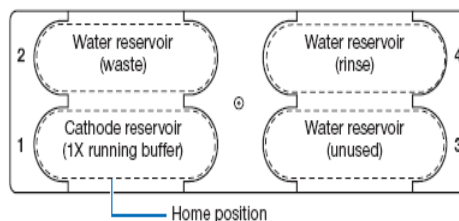


Figure reproduced from Applied Biosystems 3130/3130xl Genetic Analyzers (2004) Maintenance and Troubleshooting Guide.

1.7.6.4.4 Close the instrument doors.

- 1.7.7 Set the oven temperature to 60 °C
- 1.7.7.1 In the Data Collection software, select Manual Control in the tree pane to bring up the manual control dialog box.
- 1.7.7.2 Select “Oven” in the drop down menu for “Send Defined Command For:”
- 1.7.7.3 Select “Set oven temperature” from the drop down menu under “Command Name”.
- 1.7.7.4 Type 60 for the value and click “Send Command”.
- NOTE:** The instrument default oven temperature is set at 50 °C. Therefore, the temperature must always be set to 60 °C for the pre-warming.
- 1.7.7.5 Turn on the oven. The oven should be warming up as samples are being prepared, or for approximately 30-45 minutes.
- 1.7.7.6 Select “Turn On/Off oven” under command name and “On” from the drop down list under Value.
- 1.7.7.7 Click “Send Command”.

1.7.8 Preparation of the sample plate

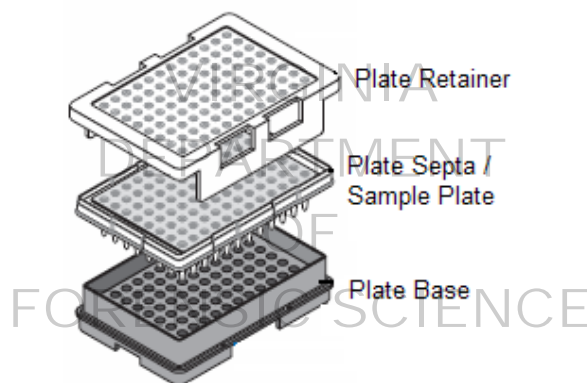
**NOTE:** When loaded onto the same plate, all evidence samples pertaining to a case must be loaded on to the plate prior to loading reference samples for the same case. Samples may not be added to empty wells previously subjected to injection.

- 1.7.8.1 Prepare a master mix of formamide and size standard as follows:
- 1.7.8.1.1 Thaw the Hi-Di formamide to room temperature, vortex to mix, and pipet 8.7 µL of Hi-Di formamide for each sample (as well as extra samples to account for pipetting variation which will vary depending on the number of samples) to a clean microcentrifuge tube.
- 1.7.8.1.2 Vortex the GeneScan™ 600 LIZ® Size Standard and pipet 0.3 µL per sample (as well as the same number of extra samples used in 1.7.8.1.1) to the microcentrifuge tube.
- 1.7.8.1.3 Vortex this master mix to ensure the contents are thoroughly mixed.
- NOTE:** The master mix may be transferred to a sterile trough for use with an 8-channel pipette, if desired.
- 1.7.8.2 Pipet 9 µL of the master mix into each well of the 96-well reaction plate to be used.
- 1.7.8.3 9 µL of Hi-Di formamide or master mix must be pipetted into unused wells of the plate that will be injected to ensure that air is not injected into any of the capillaries. (That is, if one injection (16 samples total) will be completed on the 16-capillary 3130xl, it is necessary to ensure that all sample wells in the two columns to be injected contain liquid.)
- 1.7.8.4 Pipet PCR product (for samples, reagent blanks, positive controls, and negative controls) into the corresponding well of the 96-well plate. 0.5 µL, 1 µL or 2 µL of the PCR product may be used at the analyst’s discretion. For over-amplified samples, it may also be necessary to dilute the PCR product prior to placement into the injection cocktail.

**NOTE:** The same volume of PCR product (or more) used for the samples must also be used for the negative controls and associated blanks.

- 1.7.8.5 Pipet 1  $\mu\text{L}$  of allelic ladder into at least two wells per run.
- 1.7.8.6 After addition of all samples and ladders, the plate septum should be placed on top of the sample wells and the plate heated at 95 °C for  $\geq 2$  minutes.
- 1.7.8.7 Following heating, place the plate in a freezer block or on ice for at least two minutes prior to loading.
- 1.7.8.8 Ensure the plate assemblies are properly assembled (as shown below) and positioned on the deck properly.

**NOTE:** If the plate assembly is properly assembled, the gray septa will not be visible when looking down through the holes of the plate retainer. Instead, the wells will appear black. If the plate assembly is not properly assembled, damage to the capillary array is likely to occur.



*Figure reproduced from Applied Biosystems 3130/3130xl Genetic Analyzers (2004) Maintenance and Troubleshooting Guide*


- 1.7.8.9 Check again for bubbles in the pump block, lower polymer block, interconnect tube, polymer supply tube, and channels. If bubbles are present, remove all bubbles with the Bubble Remove wizard.

## 1.8 Instrument Operation

- 1.8.1 Ensure that the instrument doors are closed and the green light on the front panel of the instrument is on. If not already open, open the 3130xl data collection software. The data collection software sets up the instrument for operation and data collection and manages the database for every aspect of the capillary electrophoresis. The software has two basic components: Data Management (e.g., ga3130xl) and Instrument Control (e.g., 3130xl), both of which are found in the tree pane on the left side of the software screen.
- 1.8.2 **Plate Manager** - Sample information is entered into corresponding plate locations along with the individual protocol, results group, and analysis method for each sample loaded on the plate. Sample information can be entered manually within the plate manager or imported from an external worksheet. Samples should be organized on the plate in order to maximize efficiency considering the capillaries labeled 1-16 correspond to A1-H2, A3-H4, A5-H6, A7-H8, A9-H10, and A11-H12 on the sample plate for each injection.
  - 1.8.2.1 Click on the Plate Manager from the ga3130xl dropdown list.

- 1.8.2.2 The sample information may be entered in a manual or automated fashion.
- 1.8.2.3 Manual Sample Input:
- 1.8.2.3.1 Click the “New” button at the bottom of the display to open the New Plate Dialog box.
- 1.8.2.3.2 Complete the information in the New Plate Dialog: Type a name and description for the plate (description is optional).
- 1.8.2.3.3 Select your fragment analysis application in the “Application” drop-down list. Typically, this will be “3130xl\_1”.
- 1.8.2.3.4 Select 96-well or 384-well in the “Plate Type” drop-down list.
- 1.8.2.3.5 Type DFS for the owner and a name or initials for the operator.
- 1.8.2.3.6 Click “OK” to open the Plate Editor, as shown in Figure 8.
- 1.8.2.3.7 For each sample, fill in the following:
- Sample Name
- The Priority column will fill in automatically. Lowering the number (100 is default) for each set of 16 samples increases the priority meaning lower priority samples will be injected prior to higher priority samples.
- Comments column – Enter any comments or notations for the sample (optional).
- Sample Type – Choose the appropriate sample type from the drop down list (it is optional to designate positive and negative controls as such).
- Size Standard – Choose GS LIZ 600 from the drop down menu.
- Panel – choose the Yfiler™ panel from the options listed. Typically, this will be “CODISYfiler\_v2”.
- Analysis Method – Choose the appropriate analysis method. Typically, this will be “CODISYfiler”, found under a sub-menu.
- Results Group - Select a group from the drop-down list or create a new one.
- Instrument Protocol – Select a protocol from the drop-down list. This will be “Yfiler\_5s” for a 5 second injection, “Yfiler\_12s” for a 12 second injection and “Yfiler\_20s” for a 20 second injection.
- 1.8.2.3.8 When finished entering the information in the plate manager, click “OK”.
- 1.8.2.4 Automated Sample Input
- 1.8.2.4.1 The plate data must be created from an outside program such as Microsoft Excel and be in a tab-delimited, .txt, format before it can be imported.
- 1.8.2.4.2 Click on the “Import” button on the bottom of the display to open the “Import Plate” dialog box.



- 1.8.2.4.3 Select the location and file containing the plate data and click on “OK”. The plate will be added to the list of plate records.
- 1.8.2.4.4 Check the plate record by selecting the plate and clicking “Edit” to ensure the plate samples, results groups, protocols, and analysis methods are correct.
- 1.8.3 **Run Scheduler** – Two plates may be loaded onto the autosampler at a time in positions A and B. The run scheduler designates which plates are located in which position on the autosampler. When loading the autosampler, ensure the plate is properly positioned in either position A or B. If two plates are loaded into the autosampler, the first plate linked to its plate map will be the first plate that is injected, independent of whether it is loaded into position A or B (unless the priority column of the plate map has been used to re-prioritize the injections). The plate on the left in the autosampler is defined as position A and the plate on the right is defined as position B, as described below.
- 1.8.3.1 Click “Run Scheduler” in the tree pane of the Data Collection software to open the Run Scheduler window.
- 1.8.3.2 Click “Find All” and find the plate map to be linked. Highlight the plate map and the position on the autosampler picture to link the map to that position.
- 1.8.3.2.1 Repeat 2.8.3 to indicate the location of a second 96-well plate, if necessary.
- 1.8.3.3 Once the plates have been linked, the run can be started. Click the green button  in the toolbar.
- 1.8.3.4 The Processing Plates dialog box opens. Click OK.
- 1.8.3.5 Once an injection has been completed, the data may be viewed in GeneMapper® ID.
- 1.8.3.6 At the discretion of the scientist, reinjections of samples may be initiated in one of two ways:
- 1.8.3.6.1 If the overall run is not complete, a reinjection of a sample can be carried out by opening the current plate record, choosing the “Add a sample run” in the file menu and filling in the Results Group and Instrument Protocol for each appropriate sample.
- NOTE:** This will create a sample file with the designation “.2” in the sample file name for each reinjected sample.
- 1.8.3.6.2 If the overall run is complete, a new plate record must be created as if a new run is set up. The original plate record can be duplicated, renamed, and the wells not being reinjected cleared with the “Clear Row” command.

**APPENDIX A - REAGENTS****GENETIC ANALYZER BUFFER WITH EDTA (10x)**

Expiration date: Manufacturer's expiration date  
 Purchased from Applied Biosystems, Foster City, CA. PN 402824, 25 mL each.

**HI-DI™ FORMAMIDE**

Expiration date: Manufacturer's expiration date  
 Purchased from Applied Biosystems, Inc., Foster City, CA, PN 4311230, 25 mL each.

Aliquot into 250 µL, 500 µL, 1000 µL, or other suitable volume aliquots and store at -20°C.

**PERFORMANCE OPTIMIZED POLYMER – 4 (POP 4)**

Expiration date: Manufacturer's expiration date  
 Purchased from Applied Biosystems, Foster City, CA. PN 4363752 (3.5 mL) OR PN 4352755, (7 mL)

**AMPF $\ell$ STR Yfiler™ KIT**

Expiration date: Manufacturer's expiration date or 6 months from date of opening, whichever comes first  
 Purchased from Applied Biosystems, Inc., Foster City, CA., Catalog Number 4359513 for 100 reaction kit.

Kit components included (expiration date same as kit unless specified):

- AMPF $\ell$ STR Yfiler™ PRIMER SET
- AMPF $\ell$ STR Yfiler™ PCR REACTION MIX
- AMPF $\ell$ STR Yfiler™ ALLELIC LADDER
- AMPLITAQ® GOLD DNA POLYMERASE – 5U/µL
- CONTROL DNA (007 MALE DNA STANDARD-0.10 ng/µL, 9947A FEMALE CELL LINE- 10 ng/µL)

**NOTE:** Only the Allelic Ladder is stored and used in the post-amplification room for Capillary Electrophoretic Detection.

**MATRIX STANDARD – DS-33 (Dye Set G5)**

Expiration date: Manufacturer's expiration date  
 Applied Biosystems, Foster City, CA, PN 4345833.

**GENESCAN™ 600 LIZ® SIZE STANDARD**

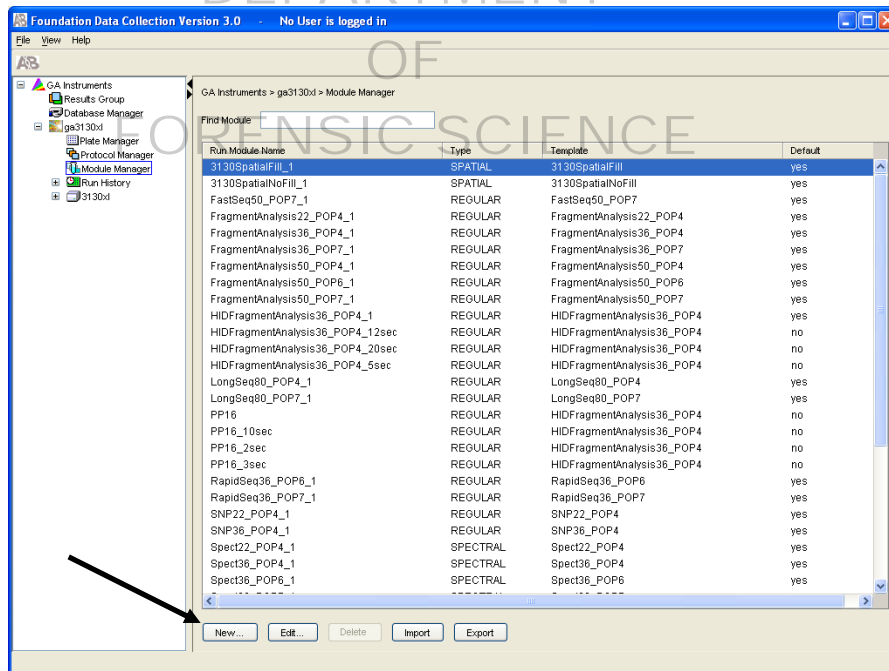
Expiration date: Manufacturer's expiration date or 6 months from date of receipt, whichever comes first  
 Applied Biosystems, Inc., Foster City, CA, PN 4366589

**APPENDIX B – INITIAL SETUP OF GENEMAPPER® ID AND DATA COLLECTION SOFTWARE**

After installation of a new instrument and installation of the GeneMapper™ ID software on a new computer, a number of customizations must be made. These include defining: panels and bin sets, the size standard, plot settings, and table settings. If the new computer will also be used to control the instrument, appropriate protocols, modules, and results groups must also be created. This appendix describes how to make all of these settings modifications.

**1 Creation of new run modules**

- 1.1 Instrument procedures are controlled by the module manager. The control of operations such as injection time, oven temperature, voltage during electrophoresis, data collection interval and z-axis (not adjustable by user) can be modified. Modules must be created or modified from the templates provided.
- 1.2 While setting up a new instrument computer, it will be necessary to create a new module. For use with the AmpFℓSTR® Yfiler™ System, the module “Yfiler\_5s” will be used for a 5s injection. For a 12s injection, “Yfiler\_12s” will be used, and “Yfiler\_20s” will be used for the 20s injection.
- 1.3 To create a new module, navigate to Instruments>ga3130xl>Module Manager in the tree pane view on the left side of the screen (in the Data Collection software) to open the Module Manager window as shown in Figure 1. Click on “New” and a Run Module Editor window appears.
  - 1.3.1 A previously made module can be exported from another computer and imported into the Module Manager rather than creating a new module, if desired. The import and export buttons are shown at the bottom of Figure 1.



**Figure 1.** Module Manager.

- 1.4 Fill in the name and select the type of module (Regular) as well as a template (HIDFragmentAnalysis36\_POP4) from the drop-down list. Make modifications to the run conditions as necessary to satisfy the run requirements.
- 1.5 After the settings have been verified, save the new module by clicking “OK”.

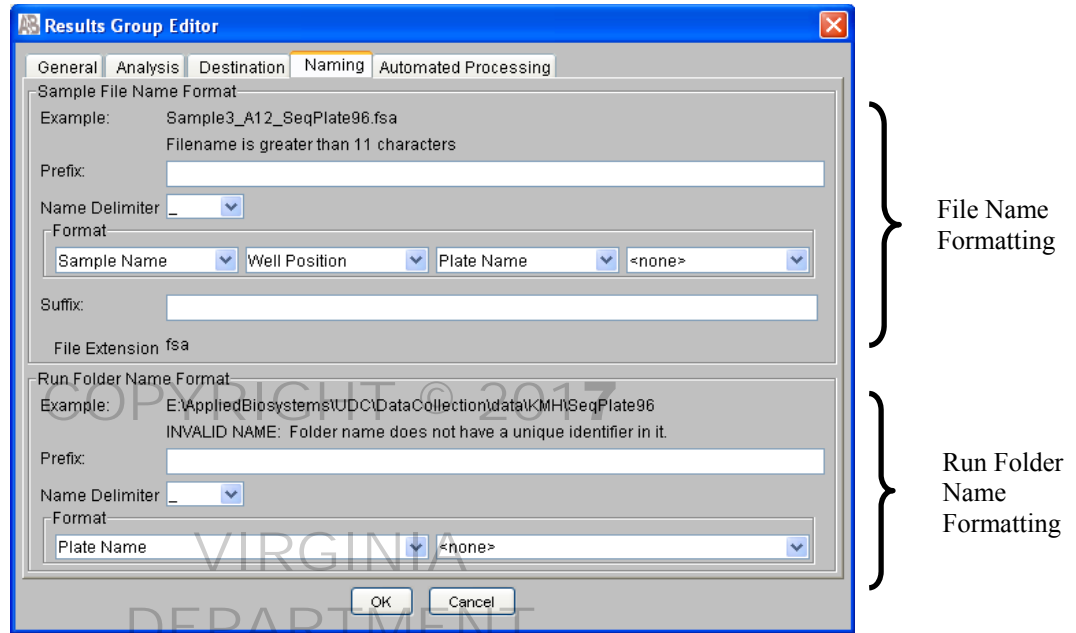
## 2 Creation of New Protocols

- 2.1 The instrument protocol is used to tell the instrument all of the necessary run conditions for the sample, including the type of run, the run module, and the dye set. Typically, once a protocol is defined for a particular multiplex, it will be used repeatedly and it will not be necessary to create a new one. If a new protocol needs to be generated, proceed for directions.
- 2.2 On the left side of the Data Collection Software, select GA Instruments>ga3130xl>Protocol Manager.
- 2.3 In the Instrument Protocols section, click “New” to open the Protocol Editor and create a new protocol.
  - 2.3.1 A previously-made instrument protocol can be exported from another computer and imported into the Protocol Manager rather than creating a new protocol, if desired.
- 2.4 Type a name for the protocol. Typically, this names the amplification kit or dye set utilized for the samples of interest.
- 2.5 Writing a description is optional.
- 2.6 Select “Regular” in the “Type” drop-down list.
- 2.7 Select the run module for your run. Typically, this will be: HIDFragmentAnalysis36\_POP4\_1, as this is the default run module for Yfiler™. A custom run module may be selected.
- 2.8 Select the Dye Set for your samples. Typically, this will be “G5”, which is the dye set for Yfiler™.
- 2.9 Click OK.

## 3 New Results Groups

- 3.1 The results group defines the file type, file name, analysis parameters, and file save location and run folder name preferences for the samples separated on the instrument.
- 3.2 Create a results group by clicking on “Results Group” in the Tree Pane and click on the “New” button. Alternatively, select a previously created results group to modify and click “Duplicate” and rename the results group. The results group editor opens.
  - 3.2.1 A previously made results group can be exported from another computer and imported rather than creating a new results group, if desired.
- 3.3 Complete the general tab by entering a name for the results group and owner; the comment is not required.
- 3.4 Select the Analysis tab, choose “GeneMapper-3130xl\_1” (or the name the instrument was assigned) as the analysis type, and ensure that Autoanalysis is not selected.
- 3.5 Select the Destination tab, and choose a destination folder for the results. The default location is: E:\AppliedBiosystems\udc\datacollection\Data\Folder Name. Click “Use Custom Location” and browse to a location by clicking the “Browse” button.
- 3.6 Select the Naming tab to customize sample file and run folder names.

- 3.6.1 Sample file names shall be formatted as: <WellPosition>\_<SampleName>\_<Plate Name>. The figure below shows how one would select this option in the Results Group Editor. A potential filename produced by the selection shown is: A01\_AllelicLadder\_2009-000.fsa



- 3.6.2 In a similar manner, the run folder name format shall be formatted as: <Plate Name>.fsa. The figure above also shows how one would select this option in the Results Group Editor. An example of a run folder name is: 2009-000.fsa
- 3.6.3 Select the Automated Processing tab and confirm that “only when the results group is complete” is selected.
- 3.6.4 Click on “OK” to save the Results Group. This results group will be used during the sample data input in the Plate Manager.

#### 4 New Size Standard

- 4.1 In the GeneMapper® ID software, choose “GeneMapper Manager” in the “Tools” pull-down menu. The GeneMapper Manager dialog box appears.
- 4.2 Choose the “Size Standards” tab.
- 4.3 Choose “New...” (near bottom of dialog box) to create a new size standard.
- 4.3.1 Alternatively, a previously made size standard can be imported by choosing “Import...”
- 4.4 In the dialog box that opens, choose “Basic” or “Advanced” and click OK.
- 4.5 In the Size Standard Editor dialog box that opens, name the new size standard “GS LIZ 600” and enter the size standard dye (Orange) and fragment sizes. At a minimum, the 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400 sizes will be defined.

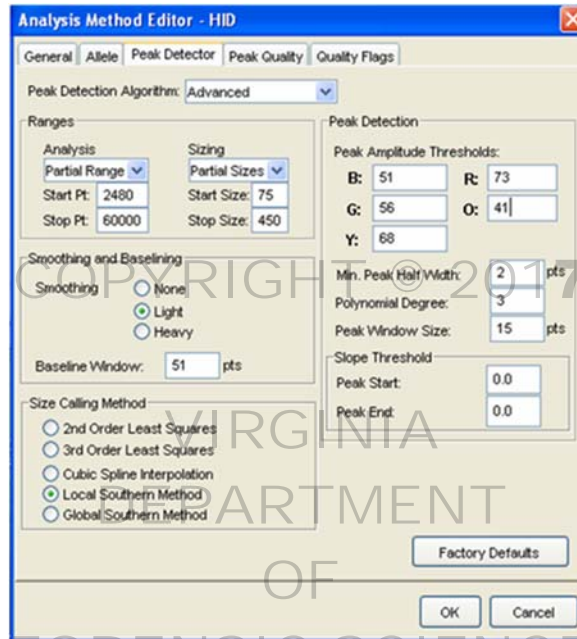
#### 5 Analysis Method Editor

- 5.1 In the GeneMapper Manager, select the “Analysis Methods” tab and click “New”. In the dialog box that

opens, choose “HD” and click OK. Give it the name, “CODISYfiler”. In the GeneMapper™ ID software, select Analysis>Analysis Method Editor.

5.2 Click on the “Peak Detector” tab.

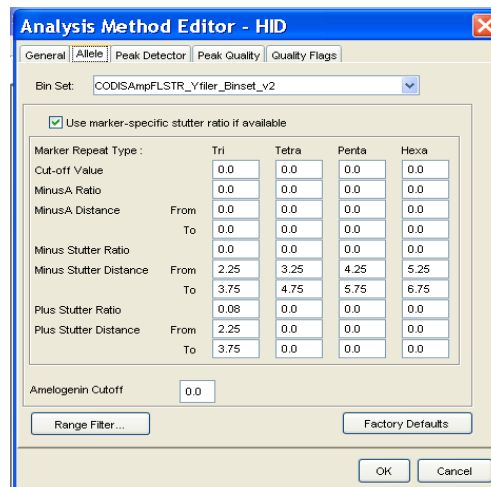
5.3 Ensure that all selections are properly designated as shown below. The size calling method used for the AmpFLSTR® Yfiler™ System is the Local Southern method.



5.3.1 The analysis range starting and stopping points may be altered on a run-by-run basis to accommodate migration times that may vary between injections.

5.3.2 The values for the peak amplitude thresholds are based upon empirical data derived from the validation performed at the Department. The threshold for the orange dye (ILS) may be adjusted to capture all the necessary peaks or to limit artifact peaks in the size standard peak pattern. It is important to note that ONLY the orange dye (ILS 600 size standard) may be adjusted.

5.4 The settings under the “Allele” tab should remain as shown in the figure below. The Bin Set is defined as “CODISampFLSTR\_Yfiler\_Binset\_v2” and the marker specific stutter ratio is used.



5.5 The default settings for the Peak Quality and Quality Flags tabs are used.

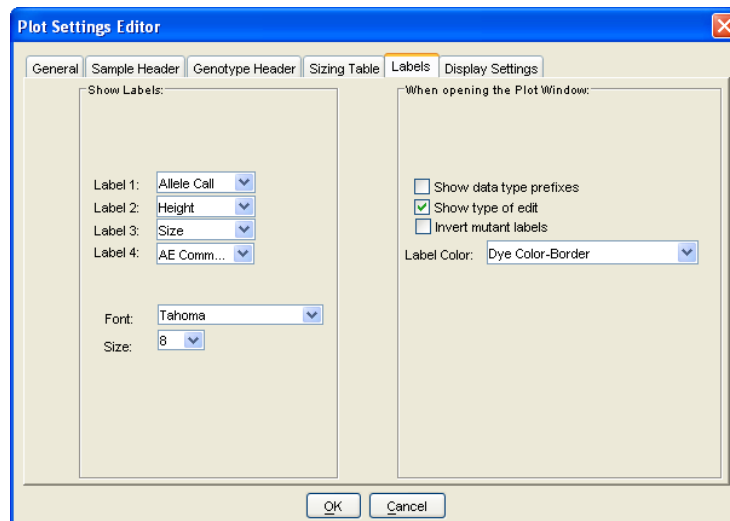
6 Panels and Bins

- 6.1 Panels and bins must be created or imported for allele calling of Yfiler™ data. In the GeneMapper® ID software, navigate to Tools>Panel Manager. In the tree pane view on the left side of the dialog box, choose Panel Manager.
- 6.2 Choose File>Import Panels and browse to the file entitled CODISYfiler\_v2 which has been created and named by the Department.
- 6.3 Navigate to “CODISYfiler\_v2” of the treepane view (at the left side of the screen), choose File > Import Bin Set and browse to the bins file entitled “CODISampFLSTR\_Yfiler\_Binset\_v2”.
- 6.4 Navigate to CODISYfiler\_v2 > CODISampFLSTR\_Yfiler\_Binset\_v2 of the treepane view (at the left side of the screen). The resulting Yfiler™ panel/bin set is shown. The marker specific stutter ratios must be updated to reflect the validation data obtained at the Department. The new stutter ratios should be edited to reflect those shown below:

| Locus     | % Stutter | Locus     | % Stutter |
|-----------|-----------|-----------|-----------|
| DYS456    | 14        | DYS391    | 10        |
| DYS389I   | 9         | DYS439    | 10        |
| DYS390    | 13        | DYS635    | 11        |
| DYS389II  | 18        | DYS392    | 14        |
| DYS458    | 14        | Y GATA H4 | 10        |
| DYS19     | 11        | DYS437    | 8         |
| DYS385a/b | 16        | DYS438    | 5         |
| DYS393    | 13        | DYS448    | 4         |

7 Plot Settings

- 7.1 In GeneMapper® ID, navigate to Tools > GeneMapper Manager. In the tabs near the top of the GeneMapper Manager dialog box, choose Plot Settings to create custom plot settings.
- 7.2 Create a new plot setting by clicking New and the Plot Settings Editor will appear. In the General tab, type a name for the plot settings.
- 7.3 In the Labels tab in the Plot Settings Editor, the following settings should be used for samples. The ladders, if viewed, may be labeled differently (allele call only, for example).



7.4 Click OK.

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