

11 Genetic Analyzer Sample Preparation

Amplified samples can be analyzed by injection into a capillary on the Applied Biosystems Genetic Analyzer 3130xl. An internal lane size standard is loaded with each sample to allow for automatic sizing of the PCR products and to normalize differences in electrophoretic mobility between injections. GenemapperID software is then used for automatic analysis and genotyping of alleles in the collected data.

Safety

Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Exposure to formamide may have chronic health effects, and may be toxic to internal organs; avoid ingestion or inhalation.

Related Forms – 3130 Maintenance, 3130 As-Needed Maintenance

Equipment, Materials, and Reagents

- Applied Biosystems 3130xl Genetic Analyzer (instrument, computer and appropriate software)
- Manual: Applied Biosystems 3130/3130xl Genetic Analyzers: Getting Started Guide
- Manual: ABI PRISM® 3100-Avant Genetic Analyzer: User Guide: Rev B
- 3130XL & 3100 capillary array 36 cm (PN 4315931) MicroAmp® Optical 96-Well Reaction Plate (PN N801-0560) Plate septa 96-well (PN 4315933)
- MicroAmp splash free 96-well base (PN 4312063)
- MicroAmp® 8-Cap Strip (PN N801-0535)
- Genetic Analyzer Buffer Reservoirs
- 3130 & 3100 series reservoir septa (PN 4315932)
- AmpF/STR® Identifiler® PCR Amplification kit ladder (PN 4322288)
- AmpF/STR® Yfiler™ PCR Amplification kit ladder (PN 4359513)
- **AmpF/STR® Identifiler® Plus PCR Amplification kit ladder (PN 4427368)**
- GeneScan 500 Liz size standard (PN 4322682)
- Matrix Standard Set DS-33 (P/N 4345833)
- HiDi formamide (PN 4311320) 3130 POP-4 Polymer 7ml (PN 4352755)
- Buffer 10X w/ EDTA (25ml) (PN 40284)
- Deionized water
- Genetic Analyzer 4 ml Buffer Vials (P/N 401955)
- vortex
- microcentrifuge tubes 1.5 ml
- microtube racks

- pipets
- pipet tips
- GeneAmp® PCR System 9700 Thermalcycler

Standards, Controls, and Calibration

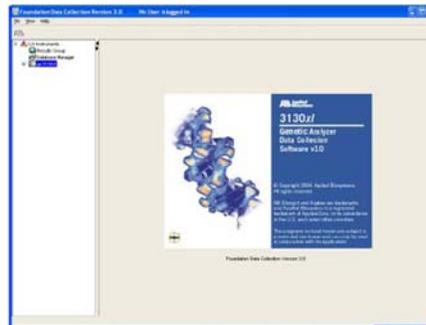
An appropriate allelic ladder, an amplification positive control and amplification negative control will be included with each Genetic Analyzer run. A “run” refers to the collection of injections that are part of an individual plate record. An internal size standard (GS500 LIZ) will be added to each sample.

11.1 Instrument Maintenance for the 3130xl Genetic Analyzer

Record all maintenance tasks in the log book each time maintenance is performed on the instrument.

11.1.1 Starting the AB Prism® 3130xl Genetic Analyzer

- Turn on the computer. When the “Log On to Windows dialog box” appears, type “Administrator” in the “User Name” and “Trouble” in the “Password:” box. Click OK. Ensure the computer has finished booting up before turning on the AB Prism® 3130xl Genetic Analyzer.
- While the computer is logging on, check and ensure the oven door is locked and the instrument doors are closed. Once the main screen appears and the “hour glass” symbol disappears, start the AB Prism® 3130xl Genetic Analyzer by pressing the on/off button on the front left side of the instrument. Ensure the solid green light is on before proceeding.
- Start the 3130xl Data Collection Software v 3.0 by either clicking on the shortcut icon on the computer desktop or by selecting Start>All programs>Applied Biosystems>Data Collection>Run 3130xl Data Collection v3.0 (>= then).
- The service console will appear on the screen. The image will turn from a red circle to a green square as each application is activated; the yellow triangle indicates the function is in the process of activating. Those functions include: Messaging Service, Data Service, Instrument Service, and Viewer.
- Once all of the applications are activated, the AB Prism® 3130xl Genetic Analyzer operation screen will appear.



- F. The Data collection software is based on an Explorer tree format. Click the “+” symbol next to each folder and subfolder heading on the left tree pane to view all applications associated with the software. Selecting any item in the left side tree will allow you to view that operational screen on the right side of the screen.
- G. The instrument and computer are now ready for use. If the computer gives you a blue screen or the AB Prism® 3130xl Genetic Analyzer has a red blinking light at anytime during these steps, make a note of the error message before restarting the instrument or the computer.

Note: If the AB Prism® 3130xl Genetic Analyzer freezes, you must restart the instrument and computer.

11.1.2 Schedule

- A. Daily when the instrument is in use:
 - 1) Replenish 1X buffer and water in reservoirs unless no runs have been performed between weekly maintenance and current run.
 - 2) Inspect block and polymer channels for dried polymer.
 - 3) Remove bubbles if present.
 - 4) Inspect capillary tips in loading-end header.
 - 5) Clean or replace capillary as needed.
 - 6) Inspect volume of polymer in polymer supply bottle to ensure enough is available for all scheduled runs
 - 7) Check for leaks around all fittings on the pump and lower polymer block
 - 8) Clean 3130xl surfaces.
- B. Weekly when the instrument is in use:
 - 1) Restart the computer.
 - 2) Clean the water and buffer reservoirs.
 - 3) Perform water flush of the pump chamber.
 - 4) Clean drip trays.
 - 5) Replace polymer bottle. Select the Replenish Polymer or Change Polymer Type Wizard to replace polymer older than one week.
 - 6) Check number of injections for the capillary array. Replace as needed.
- C. As needed:
 - 1) Replace capillary array.
 - 2) Replace blocks.
 - 3) Perform spatial calibration
 - 4) Perform spectral calibration
 - 5) Defragment the computer hard drive

11.1.3 Daily Preventive Maintenance on the 3130xl for DNA Fragment Analysis when the instrument is in use

Note: Refer to Appendix 1 for a diagram of the interior of the instrument and an explanation of functions.

- A. Check and make sure that the maintenance log has been filled out for that day. If the daily maintenance log has not been completed, perform the required daily

- instrument maintenance. Daily maintenance should be performed by the first person using the AB Prism® 3130xl Genetic Analyzer each day.
- B. With the AB Prism® 3130xl Genetic Analyzer doors closed, press the “Tray” button on the outside of the instrument to bring the autosampler deck to the forward position.
 - C. When the autosampler has stopped moving, open the AB Prism® 3130xl Genetic Analyzer doors.
 - D. Remove any 96 well optical plates from previous runs and discard or store in the freezer. **Note:** Do not store HiDi-Formamide re-suspended plates in the refrigerator. Storage in the refrigerator will cause the HiDi-Formamide to break down into Formic acid, which hydrolyzes to negatively charged formate ions that compete for injection with DNA. If the plate is accidentally stored in the refrigerator, store the plate in the -20°C freezer for at least 3 hours before re-injecting the samples.
 - E. Ensure the polymer supply bottle attached next to the lower anode buffer chamber (see Appendix 1) has enough POP4 (fragment analysis) in it. The polymer supply bottle should have at least 1ml of polymer in it (See image below). When the POP4 level is equal with this line, there is approximately 1ml in the bottle).



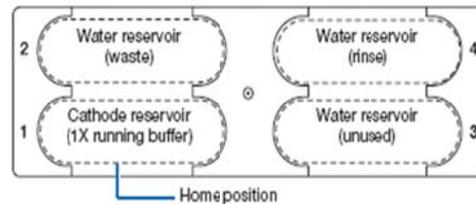
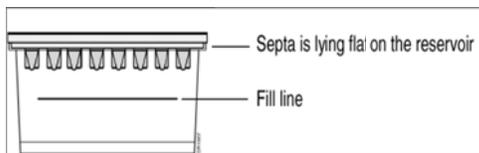
Note:

- 1. The amount of polymer should be checked during the daily maintenance as well as before every run.
 - 2. One 96 well optical plate equals at least 6 runs on the AB Prism® 3130xl Genetic Analyzer. One run is equal to up to 16 samples (16x6=96).
 - 3. Approximately 80µl of POP4 to fill the capillary array (16 capillaries) is needed for each run.
- F. If there is sufficient polymer, click the instrument status icon on the left side of the 3130xl data collection software to see the date that polymer was last added. If the polymer is older than 7 days, it will need to be replaced.
 - G. If there is not enough polymer (POP4) to run the AB Prism® 3130xl Genetic Analyzer for the number of designated runs and the polymer on the instrument is less than seven days old, add polymer from the same lot to the supply bottle. If the new polymer is from a different lot, then replace the polymer supply bottle.
 - H. If polymer needs to be added to the instrument, take a new bottle of polymer out of the refrigerator, loosen the cap and allow the polymer to warm to room temperature (15 minutes). The reason for this is that as the polymer warms up, it will let off gas in the form of bubbles. If degassing occurs within the pumping

mechanism, the bubbles will cause the AB Prism® 3130xl Genetic Analyzer to arc during a run. Arcing will eventually cause the lower buffer chamber electrode to melt, which will cause the instrument to stop running, and the lower buffer chamber will have to be replaced.

- I. As the polymer is warming up, remove the cathode buffer reservoir as well as the two water reservoirs. Discard the old buffer and water. Wash each reservoir with deionized water and thoroughly dry the inside and outside with a kimwipe.
- J. Refill the water reservoirs with deionized water and the buffer reservoir with 1X Genetic Analyzer buffer with EDTA. Prepare 1x buffer by diluting 10x buffer-1 part buffer to 9 parts water with deionized water. Store 1x buffer at 2-8° C for 1 month or at room temperature for 1 week.
- K. Place the 16 well septa on each reservoir. Ensure the septa are firmly seated and flat with the chamber. If they are not, the capillary may bend or break. If you notice that a capillary is bent or broken, refer to changing out the array in section 11.4. Place the reservoirs back onto the autosampler as seen below.

NOTE: It is critical that each reservoir is returned to its original location. The reservoirs and the position on the autosampler are labeled.



- L. If there is sufficient polymer in the supply bottle, proceed to step 11.1.4.B. If additional polymer is needed in the polymer supply bottle, proceed to step M. If expired polymer needs to be discarded or a different lot of polymer needs to be added to the polymer supply bottle, proceed to step 11.1.4.A.
- M. To replenish the polymer in the polymer supply bottle:
 - 1) Click on the instrument status icon to pull up the instrument status screen on the right side of the data collection software.
 - 2) Select the “Wizards” tab from the top of the instrument status window and select the Replenish Polymer Wizard.



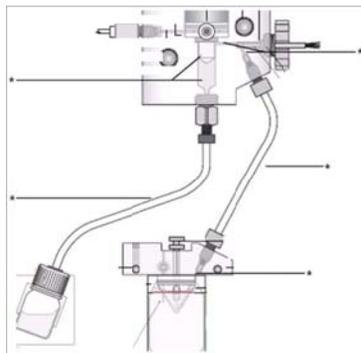
- 3) Follow the instructions to add new polymer to the supply bottle with the same lot number. The wizard will also aid you in removing any air bubbles introduced into the system.
- 4) At the end of the wizard, it will ask if the array needs to be refilled. Choose to fill the array in order for old polymer to be removed from the array.

11.1.4 Weekly and Quarterly Preventive Maintenance of the 3130xl when the instrument is in use

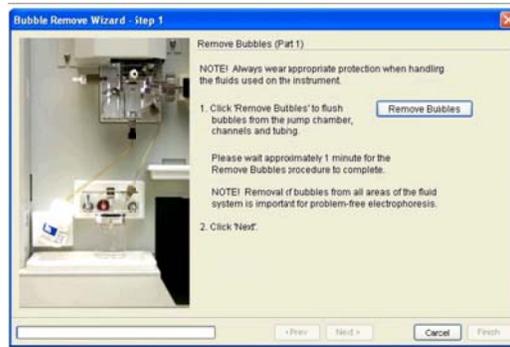
- A. To install new polymer after 7 days or a different lot of polymer:
 - 1) Select the “Water Wash Wizard.” Follow directions on how to flush the system with distilled water before adding the new polymer.



- 2) Select the “Replenish Polymer Wizard.” Follow the instructions to add the new bottle of polymer and to remove the air bubbles introduced into the system.
 - 3) At the end of the wizard, it will ask if the array needs to be filled. Chose to fill the array to remove the old polymer from the array.
- B. Check to see if there are any bubbles present in the pump delivery system (the 5 areas to look at are designated by an “*”).



- C. If bubbles are present at any of the positions indicated above, click the “Wizard” tab and select the “Bubble Remove Wizard.” Follow the instructions on how to remove bubbles from the system.



Note: If the bubble is larger than 0.2mm (about the size of the pointed end of a pin), then remove that bubble from the instrument with the “Bubble Remove Wizard”. The operational software has a voltage tolerance setting that helps with preventing blown blocks, but one should not rely on it.

D. To flush and fill the water seal trap:

- 1) Fill the supplied 20 mL, all-plastic Luer lock syringe (in the PDP Cleaning kit) with deionized water. Expel any bubbles from the syringe.
- 2) Do not use a syringe smaller than 20 mL. Doing so may generate excessive pressure within the trap.
- 3) Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.
- 4) Open the Luer fitting by grasping the body of the fitting and turning it and the attached syringe approximately one-half turn counterclockwise.
- 5) Open the exit fitting at the top left side of the pump block by turning it approximately one-half turn counterclockwise.
- 6) Hold an empty tube or beaker under the exit fitting to receive approximately 5mL of waste. Flush the trap by pushing steadily on the syringe plunger.
- 7) Close the fittings in this order by turning each clockwise until the fittings seal against the block:
 - a) Luer fitting
 - b) Exit fitting
 - c) Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

Dell Personal Computer (PC) and AB Prism® 3130xl Genetic Analyzer

- A. The Dell computer and AB Prism® 3130xl Genetic Analyzer should be re-booted when a fatal error has occurred as indicated by the red light status. Optional: The computer can be re-booted once a week, preferably on the day of the weekly maintenance.
- B. To reset the instrument, close the doors and turn the instrument off by pressing the on/off button located on the front left side of the instrument. Restart the

computer by pressing shutdown from the Start menu and wait until the computer has completely shutdown.

- C. To restart the AB Prism® 3130xl Genetic Analyzer, you must first start the computer, wait for the “Log On to Windows” dialog box to appear. In the “User Name” box type “Administrator” and in the “Password” box type “Trouble” and then click OK. Make sure that it has finished booting up before turning the AB Prism® 3130xl Genetic Analyzer on.
- D. While the computer is logging on, check and ensure that the oven door is locked and the instrument doors are closed. Once the main screen appears and the “hour glass” symbol disappears, start the AB Prism® 3130xl Genetic Analyzer by depressing the on/off button on the front left side of the instrument. Ensure that the solid green light is lit before proceeding.
- E. Start 3130xl Data Collection Software v 3.0 by either clicking on the 3130xl Data Collection icon on the desktop or by selecting Start>All programs>Applied Biosystems>Data Collection>Run 3130xl Data Collection v3.0 (>= then) and wait for the 3130xl operation screen to appear.
- F. The Data collection software is based on an Explorer tree format. Select the “+” symbol next to each folder and subfolder heading on the left tree pane to view all applications associated with the software. Selecting any item in the left side tree will enable the operational screen on the right side of the screen to be viewed (refer to “Operating the AB Prism® 3130xl Genetic Analyzer for STR Fragment Analysis and Mitochondrial DNA Sequencing” SOP for pictures).
- G. The instrument and computer are now ready for use. At anytime during these steps if the computer exhibits a blue screen or the AB Prism® 3130xl Genetic Analyzer has a red blinking light, make a note of the error message before proceeding. It is important that the error messages are observed before restarting the instrument or the computer.

Note: If the instrument freezes, the instrument and computer must be restarted.

Dell Personal Computer (PC) Maintenance

- A. Defragmenting the hard drive

Frequency: As needed when the instrument is in use

- 1) From Windows desktop, right click on **My Computer**
- 2) Select **Manage**
- 3) Click **Computer Management (Local) > Disk Defragmenter**
- 4) Select **E Drive**
- 5) Click **Defragment**

- B. Check Available Disk Space

Frequency: As needed when the instrument is in use.

- 1) From tree pane of **Data Collection software > GA Instruments > Database Manager.**
- 2) Click on **Disc Space Status Window bar** to reveal available space.

- 3) Archive data and delete archived data as needed.
- C. Delete Archived Data from Hard Drive
- 1) In tree pane of Data Collection Software, select GA Instrument > Database manager.
 - 2) Select Cleanup Processed Plates.
 - 3) Select OK after reading dialog box.

11.1.5 Annual Preventive Maintenance of the 3130xl when the instrument is in use

An annual calibration performed by an outside vendor is required for the 3130xl Genetic Analyzer. After the calibration is performed, a performance check is required before release back into casework.

11.2 Running the AB Prism® 3130xl Genetic Analyzer

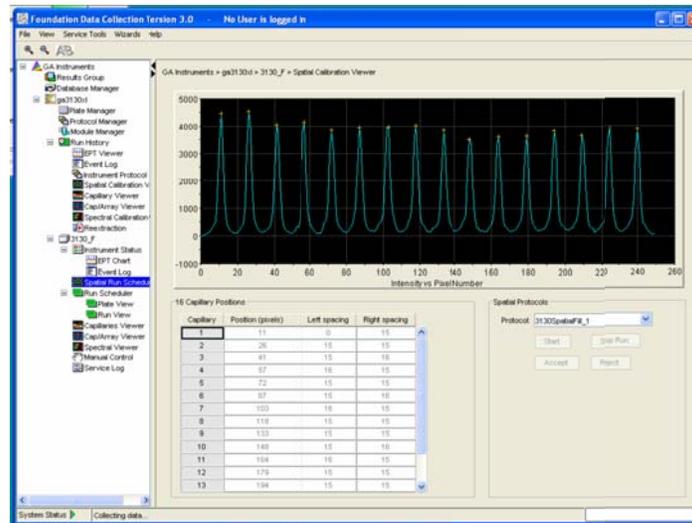
- A. Prior to running the instrument, ensure that the daily preventive maintenance tasks have been performed according to 11.1.3 of this manual. .
- B. Clean out the drip trays and any crystallized polymer with deionized water.
- C. To flush the instrument and add a new bottle of polymer:
 - 1) Select the “Water Wash Wizard” and follow directions on how to flush the system with distilled water before adding the new polymer. See image from daily maintenance log.
 - 2) Replace the water wash bottle with a new bottle containing POP4 when prompted. Continue with the wizard.
 - 3) At the end of the wizard, it will ask you if you would like to fill the array. Choose to fill the array to flush the old polymer out of the array.
- D. Check to see if there are any bubbles present in the pump delivery system (areas to look at designated by *). See image from 11.1.4.B -Weekly and Quarterly Preventive Maintenance.
- E. If bubbles are present at any of the positions noted, select the “Wizard” tab and then the “Bubble Remove Wizard.” Follow the instructions on how to remove bubbles from the system.
- F. The instrument is ready to run the spatial calibration if needed.

11.3 Running a Spatial Calibration on the 3130xl

- A. A spatial calibration must be performed each time a new capillary array is installed, if the capillary is temporarily removed from the detection block, or if the instrument is moved.

Note: A spatial calibration provides information on each capillary’s fluorescence position for the CCD camera, but does not provide information about the capillary’s performance.

- B. From the left side of the Data collection software, double click on the **Spatial Run Scheduler** to perform a spatial run.



- C. Under the Protocol drop down, select 3130SpatialFill_1 or 3130SpatialNoFill_1 and then click the start button. The run will take approximately 2 minutes if not filling the array and 6 minutes if filling the array.

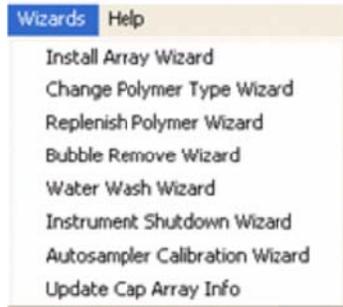
Note:

- 1) Always chose **3130SpatialFill_1** when running the spatial the first time after weekly duties have been performed, or when a new capillary has been placed on the instrument.
 - 2) The **3130SpatialNoFill_1** option is used if it is not a new capillary or if the spatial is being rerun.
- D. As the spatial is run, the intensity vs. Pixel Number graph will update until the spatial calibration is finished. When the spatial calibration is finished, the Accept and Reject buttons will be active (lower right).
- E. When evaluating a passed spatial (pictured above), each capillary should have a single sharp peak with small shoulders, and the peak heights should be greater than 2000 relative fluorescence units (rfus) and similar for all 16 capillaries. There should be one orange cross above the center of each peak. The capillary position (pixels) values between one capillary and the next should be greater than the previous value by increments of 15 or 16, with 15 being the theoretical spacing between each capillary. For example, in the profile that passed, capillary one has a position value of 11, capillary two has a position value of 26, and the spacing between capillary one and two is 15.
- F. Make sure that the right and left side spacing for each capillary is 15 or 16, except the left side spacing for capillary one should be zero.
- G. After evaluating the profile, click the **OK** button. The spatial calibration run window will appear. Click the **OK** button to accept the new spatial array and to overwrite the old spatial. The instrument is now ready to run.
- H. If you reject the spatial due to high shouldering or the pixels being less than 15 or 16, re-run the spatial using the **3130SpatialNoFill_1** option. Evaluate the spatial using the guidelines in steps E through G.

- I. If the spatial has failed a second time, but is better than the first, run the spatial a third time using the **3130SpatialNoFill_1** option again.
- J. If the spatial fails a third time, open the laser detection window and clean the capillary mirror with a kimwipe to remove any dust or fingerprints. Repeat steps A through I.
- K. If the spatial still does not pass, notify the Technical Leader.

11.4 Installing and Removing the Capillary Array on the 3130xl

- A. A capillary array should be changed if any capillary fails multiple times during a single plate. In addition, if any one of the following conditions is observed before approximately 100 runs based upon AB's recommendation, a new array should be installed: poor resolution, poor allele calling or decreased signal intensity.
- B. Fill in the appropriate information in the log book: the date, array serial number, and the number of runs (found under the status window in the 3130xl collection software).
- C. Depress the tray button and allow the autosampler to move completely to the forward position before opening the instrument doors.
- D. From the 3130xl data collection software, click on the **Instrument Status** icon on the left side of the screen and then select the **Wizards** tab from the top of the Data Collection window and the following dialog box should appear.



- E. Click on **Install Array Wizard** and follow the directions given in the wizard to replace or install an array.
- F. Once the array has been installed, you must run a spatial calibration. Follow the **Running a Spatial Calibration** procedure outlined above in section 3.

11.5 Performing Spectral Calibrations on the 3130xl

A spectral calibration must be performed at least once every 6 months, or when there is a need such as excessive pull-up or increased baseline.

Dyes contained within the dye set, G5, are:

- 6-FAM – blue
- VIC – green
- NED – yellow

- PET – red
- LIZ – orange

11.5.1 Overview of the Spectral Calibration Procedure:

A. Spectral Calibrations Requirements:

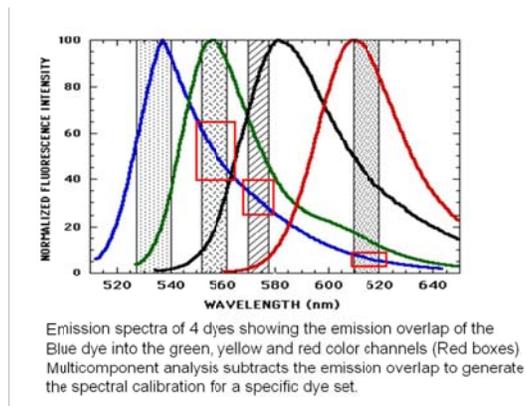
Spectral Calibrations are required to be performed when the instrument is first installed in the lab, if the capillary array length changes, if the laser has been replaced, if the mirrors or CCD camera is realigned, and if the quality of the data decreases (i.e. elevated baselines, increased pull-up between the color channels).

B. Software Setup:

Begin the procedure by preparing the instrument and calibration standards. Next, set up the run using the Plate Manager window of the 3130xl Data Collection software. When prompted during the software setup, select a specific Instrument Protocol: the Dye set (the Spectral parameter file and algorithm to use), Polymer type, Chemistry (matrix Standard), Array length and run module (run conditions). All instrument protocols have been predefined.

C. Standards Calibration:

During the calibration, dye-labeled DNA fragments are electrophoresed. During data collection, the fluorescent signals are separated by a diffraction grating according to their wave lengths and projected onto the CCD camera. Through multicomponent analyses, the instrument generates a spectral calibration that removes the emission overlap between dyes (See figure below).



D. Data Analyses:

After the calibration run, the software analyzes the spectral for each capillary and passes the spectral if the fluorescence emission maxima for each dye color is between 750 to 3000 rfus and the quality (Q-value) and condition (C-value) values are within the defined parameters. If a capillary's spectral fails, the software automatically replaces the failed spectral with a passed spectral from the next nearest capillary, the left side taking priority over the right side. Even though the instrument has passed a spectral calibration, it does not mean that the calibration data should necessarily be used in data analysis. All 16 spectral

calibrations must be evaluated before saving and using them in data analysis. Ideally, each capillary should have its own passed spectral.

11.5.2 Performing Spectral Calibration using default parameters

Preparation of DNA AmpFISTR®-Identifiler Matrix Standard Set D33 (G5 matrix dyes):

- A. Ensure that the Daily and/or Weekly maintenance has been performed before starting this procedure.
- B. Obtain the AmpFISTR®-Identifiler Matrix Standard Set D33 (G5 matrix dyes)

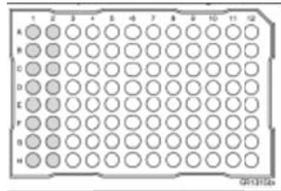
Note: There will be instrument-to-instrument variation in the sensitivity of detection. Matrix sample dilutions described here may need to be optimized for each AB Prism® 3130xl Genetic Analyzer.

- 1) Thaw and thoroughly mix the contents of the Matrix Standard Set DS-33 tube, then spin briefly in a microcentrifuge.
- 2) Combine 5 µl of DS-33 and 195 µl of HiDi-Formamide in a 1.5 ml microcentrifuge tube vortex to mix, and spin briefly in a centrifuge.

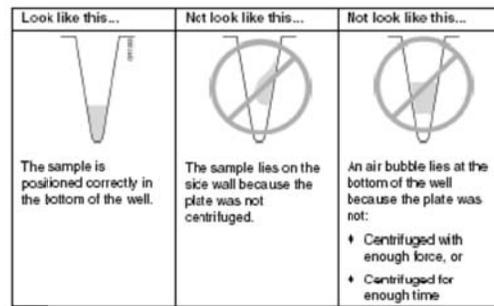
Note: Matrix sample dilution recipe may be increased as necessary.

11.5.3 Preparation of Spectral Load Plate and Loading on Instrument

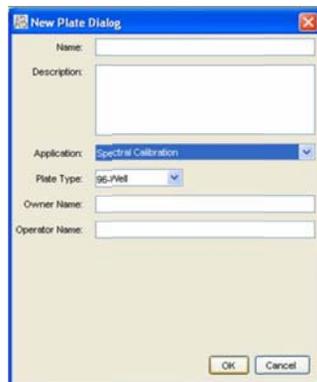
- A. To load the standards, dispense 10µl of Matrix standard set D-33 into wells A1-H2 of a 96 well optical plate as seen in the figure below.



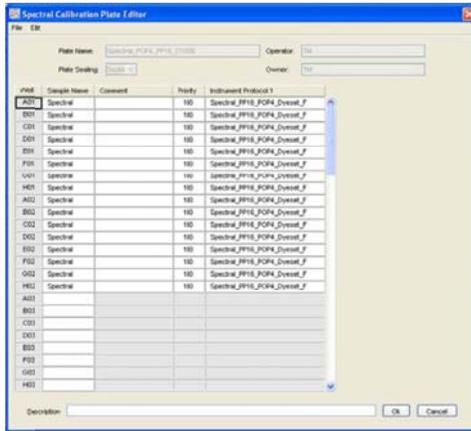
- B. Cover the wells with strip caps, briefly spin down, heat denature the samples for 3 minutes at 95°C, and chill in the freezer for 3 minutes.



- C. Remove the strip caps and place a clean, 96 well septa over the 96 well optical plate and make sure that it is completely flat. Then place the optical plate into the plate base and cover with the plate retainer.
- D. Place the plate assembly onto the autosampler platform. When the plate is on correctly, the plate indicator in the Plate View window will turn yellow.
- E. Turn the oven on by clicking the Manual Control icon on the left side of the screen. In the manual control box select oven and then select oven temp. Type in "60" for STR spectral dyes and click the set button. Next select the set state and turn on, then click the set button. Click on the instrument status on the left side of the screen and the temperature should be highlighted in yellow.
- F. Click the Plate Manager on the left side of the 3130xl Data Collection software to go to the Plate Manager View page.
- G. Click the "New..." tab to initiate the New Plate Dialog box.
 - 1) Name your plate (no spaces or slashes in plate OR sample names).
 - 2) Add any comments necessary in the description box.
 - 3) Select Spectral Calibration from the Application drop down box.
 - 4) Specify the plate as 96-well plate form the Plate Type drop down.
 - 5) Type your initials in the owner box and the operator box.
 - 6) Click OK.



- H. The Spectral Calibration Plate Editor will open :
 - 1) Type Spectral for the sample name (wells A1-H2).
 - 2) Leave comments blank.
 - 3) Priority is 100.
 - 4) Select Instrument Protocol: SpectralG5:
 - 5) Click OK.

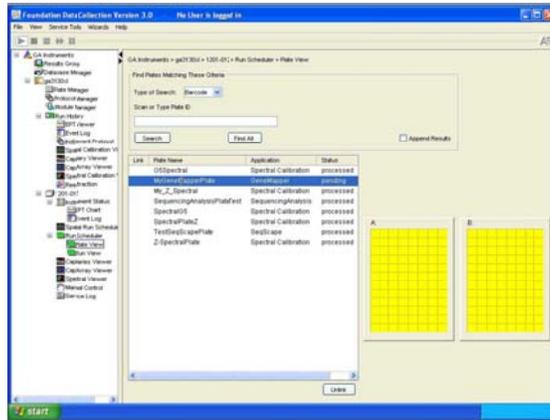


Note: Instrument Protocols are predefined during instrument set up and should not be altered without notifying the Technical Leader or designee. (See representative picture below).



11.5.4 To Link a Plate Record to a Sample Plate

- A. To link the Spectral plate record to your spectral sample plate, click the Run Scheduler folder on the 3130xl Data Collection software window to open the Run Scheduler view.



- B. On the Run scheduler view: Select your Plate Record, which should have a status of “**pending**” in the Status column and Spectral Calibration in the Application Column, then select the plate to be linked to the sample sheet.
- C. Verify that the plate has been linked. Once the plate has been linked, the plate position indicator for the linked plate becomes green, the Run Instrument button on the toolbar is enabled, and the position A or B is placed next to the sample sheet in the Link column.
- D. Select the Instrument Status folder on the left side of the 3130xl Data Collection Software and click the green Run arrow to start the instrument.

Note: The pre-run must be started before the instrument is left unattended to ensure that there are no air bubbles in the array that will cause the instrument to arc. During the pre-run, the EP current should be between 140-160 and stable. If it is fluctuating or dropping quickly, click the stop button on the tool bar and look for bubbles in the instrument. Use the Bubble Remove Wizard to remove bubbles. The sample sheet will need to be edited in the Plate Manager Window to initiate another run for the one that was terminated. This can be done while the plate is linked.

- E. At the end of the run, open the Event Log under the Instrument Status folder. In the event status dialog box, the number of capillaries that passed will be listed. At least 14 out of 16 capillaries have to pass.

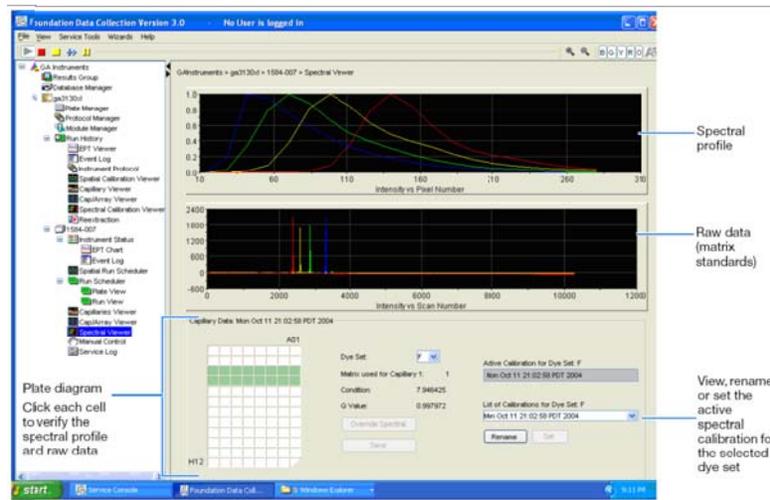
When a Capillary Fails

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 (e.g., “Array View Page” on page 3-53).

For applications where pull-up and pull-down peaks will cause critical errors, we recommend that you repeat the spectral calibration and use a unique spectral for each capillary.

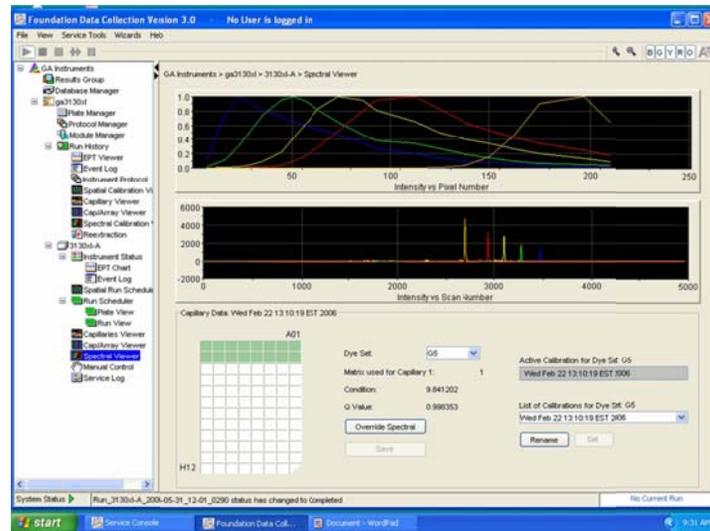
11.5.5 Displaying and Reviewing a Spectral Calibration:

- A. To evaluate the spectral calibration, select the **Spectral Viewer** from the 3130xl Data collection software.
- B. The following data box will appear:



Note: The default Dye Set on the screen will be the last Dye Set that was run on that instrument.

- C. For Dye Set G5 (Matrix Standard D-33):



- 1) Select the G5 from the Dye Set drop down.
- 2) A green square indicates the spectral for that capillary passed; a brown square indicates that the spectral for that capillary failed.

- 3) Click on the individual capillaries to verify the spectral profile, the Q value, Condition value, and the raw data.
 - 4) Verify order of peaks in spectral viewer: 5-dye: blue-green-yellow-red-orange.
 - 5) Verify peaks in raw data so the spectral profile does not contain gross overlaps, dips or other irregularities.
- D. To accept spectral for Dye Set G5 Matrix Dyes
- 1) Q-values above 0.95.
 - 2) Condition numbers (C-value) 7-12.
 - 3) The fluorescent peaks should be pointed at the top of the curve, unlike the blue channel in the picture above that has a flat peak. If flat peaks occur, dilute the standards further and re-run the spectral.
 - a) Re-dilution of standards that generated flat peaks is not empirical but should be based on the rfus to the left of the screen and previously generated matrix results.
 - b) The initial dilution was 1:40. If the rfus are less than 1000, a 1:30 dilution should be tried. If the rfus are greater than 4000 and there is a flattening of the peaks, a 1:50 dilution should be tried.
 - c) Further dilutions may be necessary. The better the quality of matrix, the higher quality the data will be (less elevated baseline and bleed through).
 - 4) If all of the Q-values and C-values are within specifications, and 14 out of 16 capillaries have passed, no further action is required.
 - 5) If the Q and C-values are below the acceptable limits or less than 14 capillaries have passed, the spectral calibration will have to be re-run starting from the beginning. The same plate for Matrix standard D-33, cannot be used; a new plate must be made.

11.5.6 Activating a Spectral Calibration

- A. Click Spectral Viewer
- B. In Dye Set drop-down list, select dye set G5
- C. In the List of Calibrations for Dye Set drop-down list, select the spectral calibration you want to activate.
- D. Click Set.

11.5.7 Evaluation of the new Spectral Calibration

- A. For STR Fragment spectral calibrations:
 - 1) It is recommended that a set of allelic ladders or positives be run to evaluate the Spectral calibration. Results will be assessed for baseline integrity, peak resolution and the degree of pull-up between color channels.
 - a) Pull up should be approximately 2% of the true allele peak for DNA samples under 2.5 ng. For example, when looking at a 3000 rfu peak, the pull-up into the other color channels should not exceed 60 rfus.

- B. If for any reason, the data is not consistent with previously run spectral calibration data for that instrument, or it does not match results from the validation or performance check of the instrument, the spectral should be re-run.

11.6 Preparing Samples for loading on the 3130xl

Internal size standards and allelic ladders, along with amplification positive and negative controls, are included in every run on a genetic analyzer. **It is advisable that at least two, well-spaced ladders are included on each run. The manufacturer recommends at least one ladder every 16 samples.**

- A. STR Fragment analysis sample preparation for **Identifiler®**, **Identifiler® Plus**, or **Yfiler**:

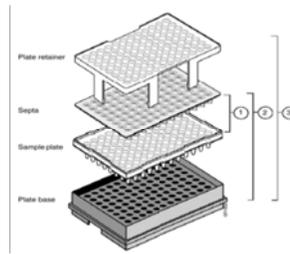
- 1) Bring GeneScan-500 LIZ, Identifiler®, **Identifiler® Plus**, or Yfiler™ Ladder and Hi-Di formamide to room temperature.
- 2) Label the plate.
- 3) Prepare a loading cocktail for Identifiler®, **Identifiler® Plus**, or Yfiler™ amplification reactions as follows:

(0.15-0.3 µl LIZ_GeneScan500) x (# of samples)

+ (8.85-8.7 µl HiDi-Formamide) x (# of samples)

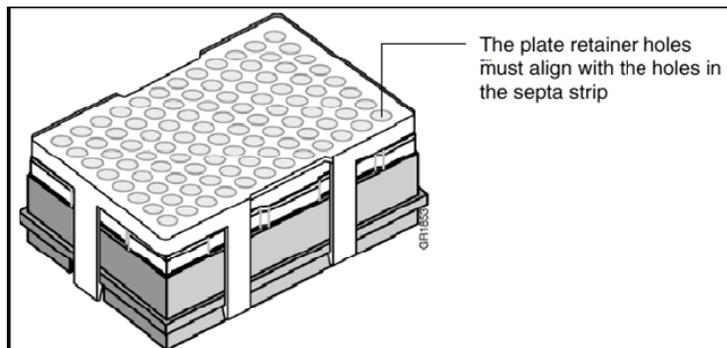
Note: Be sure to include the allelic ladders in the calculations.

- 4) Pipette 9µl of loading cocktail into each well that will contain a sample. Fill any wells that do not contain a sample with 10µl of HiDi Formamide or extra Master Mix as calculated by the workbook(s). Since 16 capillaries will be injecting DNA simultaneously, an even number of columns must contain liquid (e.g. if 7 columns contain samples, the 8th column must contain HiDi Formamide).
 - 5) Add 1µl of amplified product or Allelic Ladder to the appropriate well, place a septa on the plate. Centrifuge the plate briefly. Heat denature for 3 minutes at 95°C on a 9700, using the “Denature” program. Snap cool the plate in the freezer for 3-5 minutes.
- B. To place the plate on the instrument, assemble the plate with the plate base and the plate retainer as pictured and described below:

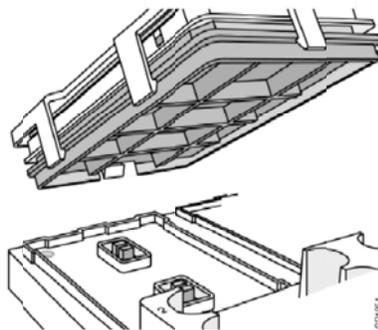


- 1) Secure a clean dry septa strip on the sample plate.
- 2) Place the sample plate into the plate base.
- 3) Snap the plate retainer onto the plate and plate base.

C. Ensure the plate retainer holes are aligned with the holes in the septa strip.

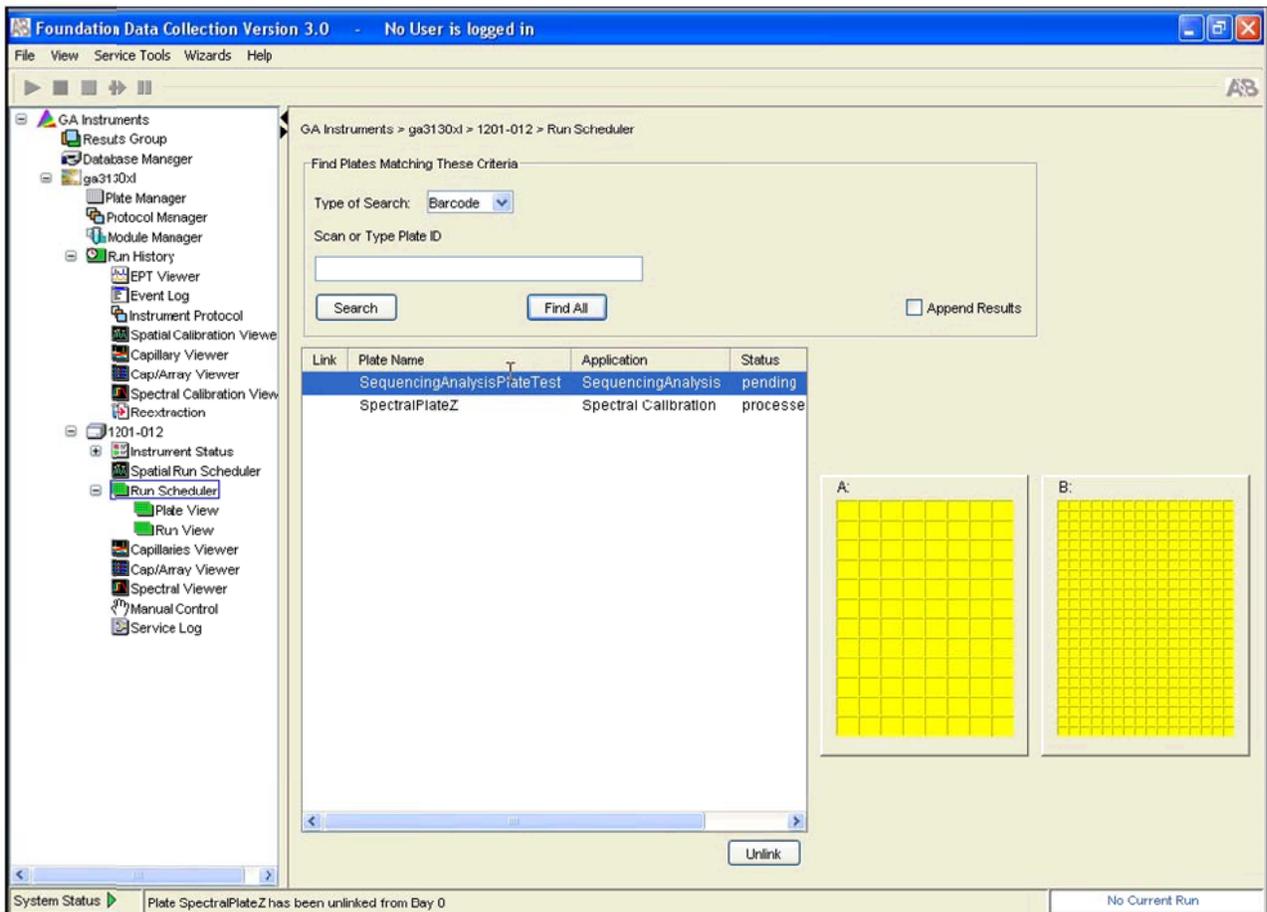
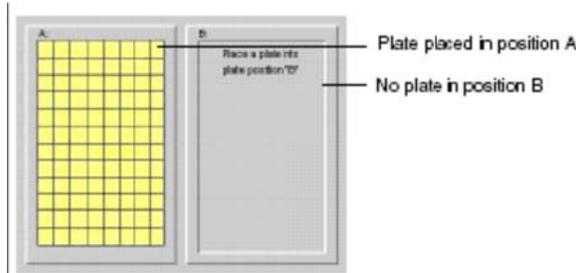


D. Select the **Run Scheduler** option on the left hand tree of the operating software. Press the tray button to bring the autosampler to the front of the instrument. When the autosampler finishes moving, open the door and place the plate assembly on the autosampler as shown below:



Note: There is only one orientation for the plate: the notched end of the plate base away from the open door.

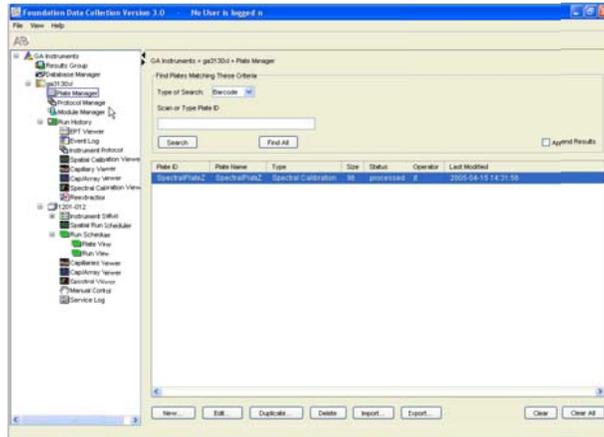
- E. The plate designator on the right side of the screen will turn from gray to yellow when the plate is detected by the autosampler deck. Check to see that this has happened.



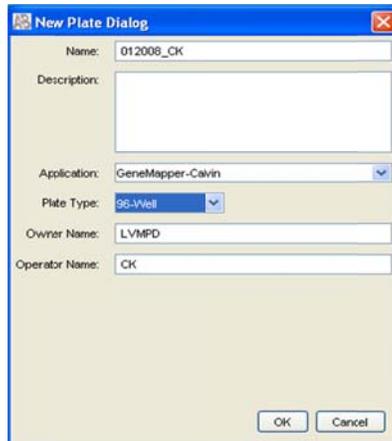
F. Close the instrument doors.

11.7 Creating an STR Fragment Analysis Plate Record

A. Click the **Plate Manager** on the left side of the 3130xl Data Collection software to go to the Plate Manager View page.



- B. Click the New tab to initiate New Plate Dialog box.
- 1) Name your plate (no spaces or slashes in plate OR sample names).
 - 2) Add any comments necessary in the description box
 - 3) Select GeneMapper-3130xl (e.g. 3130-Beta) from the Application drop down box.
 - 4) Specify the plate as 96-well plate form the Plate Type drop down.
 - 5) Type your initials in the owner box and the operator box.



- 6) Click "OK" when finished.
- C. In the GeneMapper-3130xl plate editor spread sheet, complete the plate record spreadsheet for the wells you have loaded.
 - 1) Type the names of all the samples in the Sample Name column.
Note: Do not exceed 29 characters. Letters, numbers and dots may be used. Do not use spaces or the "/" or "\".
 - 2) In the "Comments" section: Notations may be added here. This is optional.
 - 3) For each sample, a priority of 100 is automatically assigned, which means that all runs have the same priority and injections will proceed from A1-H12 in groups of 16 samples. You are able to rank the priority of your runs by lowering the priority value for that sample (the lower the number the higher the priority). However, all 16 samples associated with that run will be injected along with that sample.

For example if the sample in well D7 needed to be run first, one could change the priority to 50 for that sample and then wells A7-H8 would be injected first followed in order by A1-H2, A3-H4, A5-H6, A9-H10 etc.

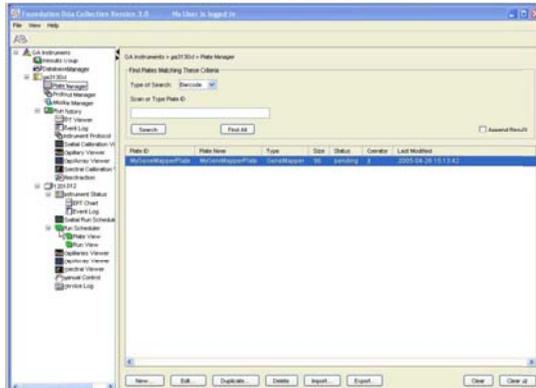
- 4) Select the correct sample type for each sample from the Sample Type drop-down list:
 - a) For Ladder, choose "Allelic Ladder"
 - b) For all other Samples, choose "Sample". Amplification positive and negative controls may be identified accordingly.
- 5) Select either Identifiler® or Yfiler™ related items from the Size Standard, Panel, and Analysis Method drop down menus. These can also be left blank as the appropriate settings will be selected in the Genemapper ID software when analyzing data. E.g.:
 - a) Size Standard: CE_G5_HID_GS500
 - b) Panel: Identifiler_v2
 - c) Analysis Method: HPD3130
- 6) Select/insert the following:
 - a) SNP set: select "none"
 - b) Users Defined 1: indicate the injection seconds
 - c) Users Defined 2: optional: insert identifying information, such as the batch to which the sample belongs, or the analyst who will ultimately interpret the sample, if known
- 7) Select the 3130xl_beta or 3130xl_gamma from the Results Group 1 drop down.
- 8) Select the appropriate Instrument Protocol from the Instrument Protocol 1 drop-down list. (e.g. 5_Second_Injection, 10_Second_Injection, or 20_Second_Injection) **Note: Identifiler Plus samples may only use the 5 second or 10 second injection times.**
- 9) Verify that the plate record is correct and then click OK.

Note: It may take a while for the new plate record to be saved to the database and added to the Pending Plate Records table.

11.8 Importing a Plate Record for the 3130xl

A. From the Excel workbook

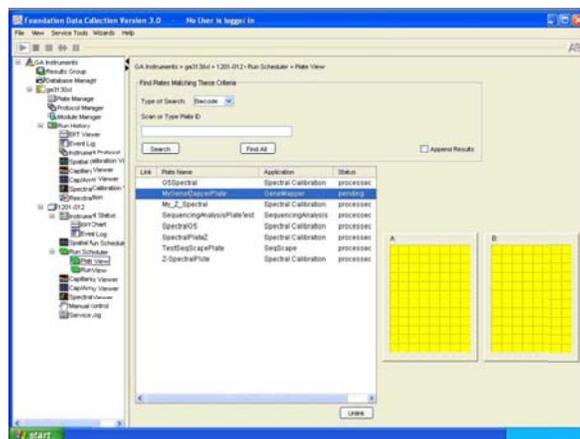
- 1) Click the Plate Manager folder on the 3130xl Data Collection software to go to the Plate Manager View page.
- 2) Click the Import tab on the bottom of the Plate Manger View screen and browse to the desired record. The record will be the plate name.
- 3) Select record, and wait while 3130xl Data Collection software imports the load sheet into the plate manager window. Once imported, all of the other buttons at the bottom of the Plate Manager View window are enabled.



- 4) Highlight the plate in the Plate Manager Window and then select the edit button to verify that all of your settings, and sample names are correct.

11.8.1 To Link a Plate Record to a Sample Plate

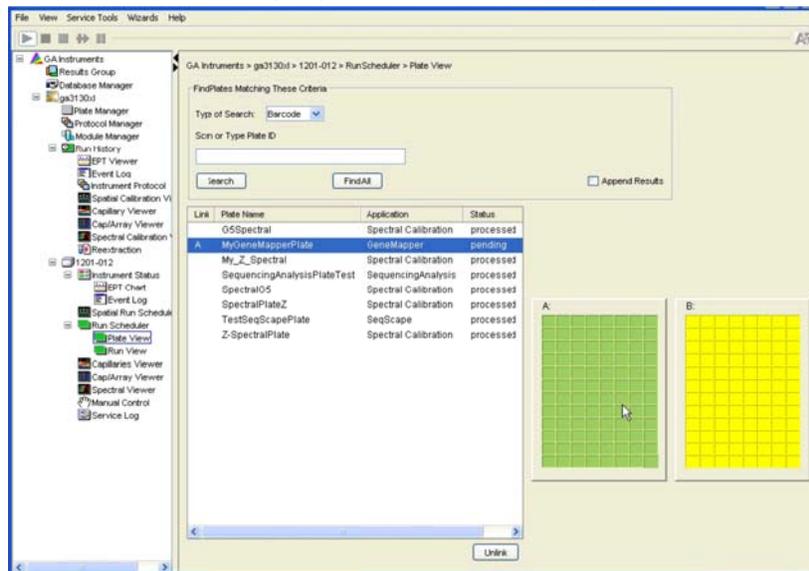
- A. To link the plate record to your sample plate, click the Run Scheduler folder on the 3130xl Data Collection software window to open the Run Scheduler view.



- B. On the Run scheduler view: Select the Plate Record, which should have a status of “**pending**” in the status column, and then select the desired plate to link to the sample sheet.
- C. Verify that the plate has been linked. Once the plate has been linked, the plate position indicator for the linked plate becomes green, the Run Instrument button on the toolbar is enabled, and the position A or B is placed next to the sample sheet in the Link column.

Note: To unlink a plate, click the **Unlink** button.

Note: To view a scheduled run, click the Run View window to verify runs are scheduled correctly. Select a row for any run and the corresponding wells to be injected for that run are highlighted in the plate diagram.



- D. Repeat steps 2 and 3 to link a second plate, if applicable.
- E. Select the Instrument Status folder on the left side of the 3130xl Data Collection Software and click the **Green Run Arrow** to start the instrument.

Note: The pre-run should be started before the AB Prism® 3130xl Genetic Analyzer is left unattended to ensure there are no air bubbles in the array that will cause the instrument to arc. During the pre-run the EP current should be stable between 140-160. If it is fluctuating or dropping quickly, click the “stop” button on the tool bar and look for bubbles in the instrument. Use the “Bubble Remove Wizard” to remove bubbles. In the “Plate Manager” window, edit the sample sheet to initiate another run for the one that was terminated. This can be done while the plate is linked.

11.9 Instrument Status of the 3130xl

- A. Clicking on Instrument Status allows a person to monitor the status of the instrument or current run. Under Instrument Status, a person can view EPT Chart and Event Log. Event log itemizes events such as errors and general information for all data collection steps.
 - 1) Capillaries Viewer - Cap/Array Viewer
 - a) Click on Capillaries Viewer to examine the quality of raw data during a run for several capillaries at once. Select checked boxes of capillaries for which you want electropherograms displayed. An electropherogram is a graph of relative dye concentration against time, plotted for each dye. The raw data displayed has been corrected for spectral overlap.
 - b) To zoom in and out, click on magnifying glass (with a + on it) and then place pointer over area of interest and click to expand view. Click on magnifying glass (with a - on it) to return to full view.
 - c) Click on Cap/Array Viewer to examine quality of your data, which is displayed as color data for entire capillary array. You can view all capillaries (vertical axis) as a function of time/scan numbers (horizontal axis). To zoom in and out, follow preceding paragraph.

Note: The capillary viewer should not be left on during a run as it may cause the computer to freeze.

11.10 Re-injecting while the instrument is running

- A. If you should choose to re-inject a sample from a plate that is running:
 - 1) Click the Plate Manager folder on the 3130xl Data Collection software to go to the Plate Manager View page.
 - 2) Select the correct plate ID.
 - 3) Select Edit>Edit>Add Sample Run (Alt + A)
 - 4) In the "Results Group" for the sample(s) being re-injected, select the appropriate results group from the dropdown menu.
 - 5) In "Instrument Protocol 2" for the sample(s) being re-injected, select the appropriate module from the dropdown menu.
 - 6) Select OK.

11.11 Re-injecting from an already created plate and plate ID

- A. To re-inject from a plate that is already made, but the run has completed:
 - 1) Click the Plate Manager folder on the 3130xl Data Collection software to go to the Plate Manager View page.
 - 2) Select Plate ID that is to be reused.
 - 3) Select Duplicate.
 - 4) Rename the plate accordingly. (If same day, add a suffix to distinguish from original run; if new date, use the new date in the plate ID name)

- 5) Select OK.
- 6) Modify the plate accordingly (e.g., delete rows, change the injection module, etc.)
- 7) Select OK
- 8) Return to step 11.8.

11.12 Loading Sample Files on the 3130xl

- A. When a run is finished, analyzed sample files are extracted into run folder located in:

E:\AppliedBiosystems\UDC\DataCollection\Data\Run Folder Name

- B. After the run has been extracted to sample files, you can use GeneMapper Software to view electropherogram data, both raw and analyzed. Copy the files to your mass storage device and transfer them to a location off the instrument's computer.

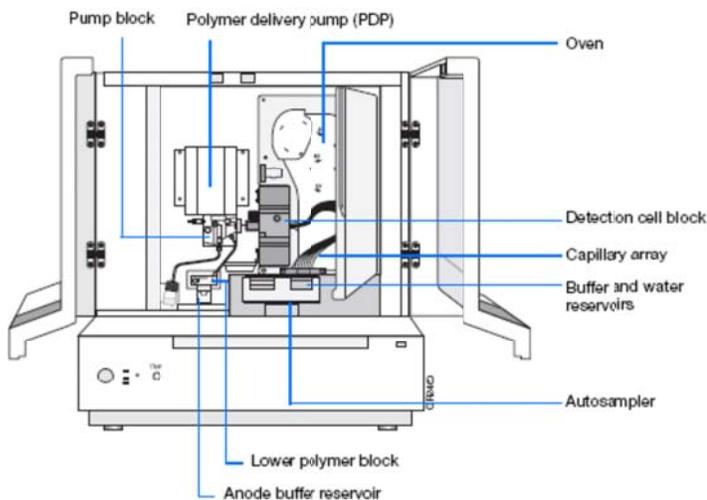
11.13 Transferring run folder

Select and copy the run folder from the data folder desktop shortcut, or E:\AppliedBiosystems\UDC\DataCollection\data. Paste this folder in the appropriate year and month folder at S:\BiologyInstruments\3130Beta or 3130Gamma. This data is archived monthly. A copy of all archived data will be retained off-site.

11.14 Reference:

3130/3130xl Getting Started Guide by Applied Biosystems

Appendix 1: Instrument Overview



Part	Function
Anode buffer reservoir	Contains 16 mL of 1X running buffer.
Buffer and water reservoirs (four)	Each contain 16 mL of 1X running buffer or water.
Autosampler	Holds the sample plates and reservoirs and moves to align the samples, water, or buffer with the capillaries.
Capillary array	Enables the separation of the fluorescent-labeled DNA fragments by electrophoresis. It is a replaceable unit composed of 4 or 16 silica capillaries.
Detection cell block and heater	Holds the capillaries in place for laser detection.
Lower polymer block	Contains the buffer valve, anode electrode, and anode buffer reservoir.
Oven	Maintains uniform capillary array temperature.
Polymer delivery pump (PDP)	Pumps polymer into the array and performs maintenance procedures.
Pump block	Includes the displacement pump chamber, piston water seal, array attachment point (array port), and connection to the lower polymer block through the interconnect tube.

Appendix 1: (Continued)

Polymer Delivery Pump

Components of the polymer delivery pump (PDP) are identified in the drawing below.

