ABI 3130xl Sequencing

PURPOSE: The 3130*xl* 16-capillary array system is used to electrophoretically analyze samples following cycle sequencing and cleanup. The system uses 96-well plates containing the samples of interest, and can process 16 separate samples with each injection. Sequence data is generated at the end of the run for downstream sequencing analysis.

A. Setting up a 3130*xl* Run

1. Turn on the computer. Make sure computer is fully booted to the Windows desktop. To login, the User should be "ocmelims" and the password should be "passw0rd". If the instrument is not on, turn it on. The status bar light will change from solid yellow (indicates instrument is booting) to blinking yellow (indicates machine is communicating with computer) and then to solid green (indicates instrument is ready for command).

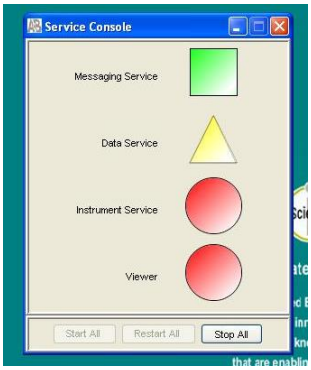
2. On the desktop, click on the shortcut for the respective instrument's data file. The main path to this data file is:

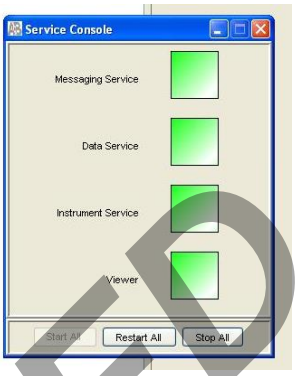
   E:\Applied Biosystems\UDC\data collection\data\ga3130xl*Instrumentname*

3. Once there, create a master file using the following format: "*InstrumentnameYear-Run Number* Files" (e.g. Batman08-015 Files) within the appropriate archive folder (e.g. Batman 2008). Move the 3130xl mtDNA files into this master file.

4. Open the 3130*xl* Data Collection v3.0 software by double clicking on the desktop Icon or select **Start > All Programs > AppliedBiosystems > Data Collection > Run 3130xl Data Collection v3.0** to display **the Service Console.**.

   By default, all applications are off indicated by the red circles. As each application activates, the red circles (off) change to yellow triangles (activating), eventually progressing to green squares (on) when they are fully functional.

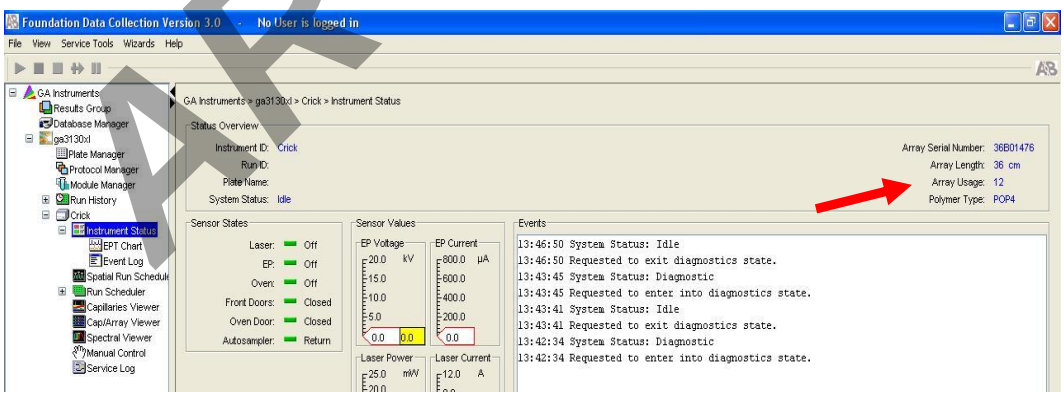




NOTE: This process could take several minutes. The Service Console must <u>not</u> be closed or it will shut down the application.

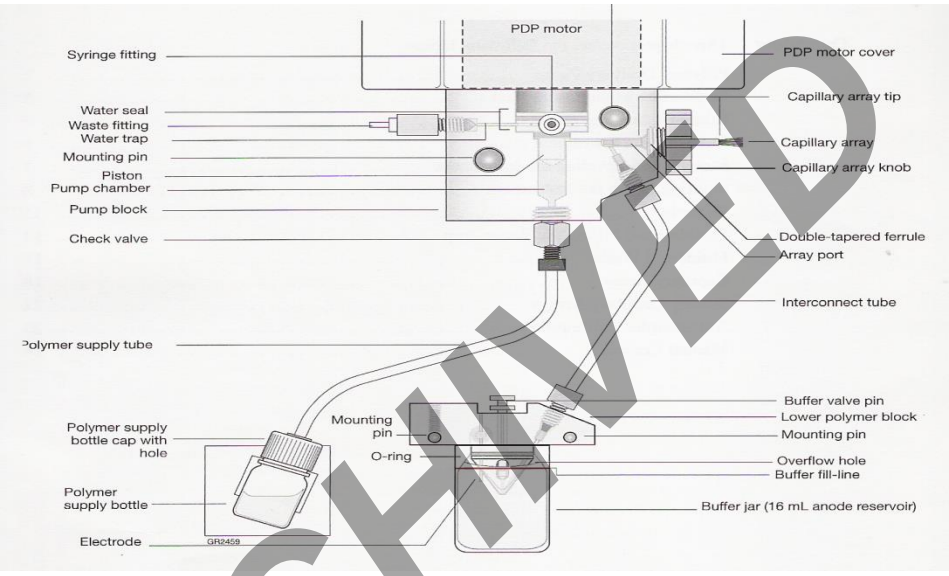
Once all applications are running, the **Foundation Data Collection** window will be displayed at which time the **Service Console** window may be minimized.

5. Check the number of injections on the capillary in the LIMS and in the **Foundation Data Collection** window by clicking on the **ga3130xl > *instrument name* > Instrument Status.** If the numbers are not the same, update the LIMS system. If the number is ≥ 140, notify QC. Proceed only if the number of injections you are running plus the usage number is ≤ 150.



6. Check the LIMS to see when the POP6 was last changed. If it is >7 days, proceed with POP6 change (See part F of this Section) and then return to Step 9.

7. Check the level of POP6 in the bottle to ensure there is enough for your run (approximately 600 µL is needed per injection). If there is not, proceed with POP6 change (See part F of this section) and then return to Step 9.



8. If you are the first run on the instrument of the day, proceed with steps 9 - 17. If a run has already been performed on the instrument that day, skip to "**Creating a Plate ID"**

9. Close the instrument doors and press the tray button on the outside of the instrument to bring the autosampler to the forward position.

10. Wait until the autosampler has stopped moving and then open the instrument doors.

11. Remove the three plastic reservoirs from the sample tray and anode jar from the base of the lower pump block and dispose of the fluids.

12. Rinse and fill the "water" and "waste" reservoirs to the line with Gibco® water.

13. Make a batch of 1X buffer (45 ml Gibco® water, 5 ml 10X buffer) in a 50mL conical tube. Record the lot number of the buffer, date of make, and initials on the side of the tube. Rinse and fill the "buffer" reservoir and anode jar with 1X buffer to the lines.

14. Dry the outside and inside rim of the reservoirs/septa and outside of the anode jar using a Kimwipe and replace the septa strip snugly onto each reservoir. **If these items are not dry, arcing could occur thus ruining the capillary and polymer blocks.**

15. Place the reservoirs in the instrument in their respective positions, as shown below:

Water Reservoir (waste) 2	Water Reservoir (rinse) 4
Cathode Reservoir (1X buffer) 1	Empty 3

16. Place the anode jar at the base of the lower pump block.

17. Close the instrument doors

B. Creating a Plate ID

1. Click on the **Plate Manager** line in the left window.

2. Select **Import** from the bottom of the screen. Find the text file that was previously saved in the master file for the 3130xl run data (e.g. B08-015.txt file present in the **Batman08-015 files** folder)

3. Click on **OK.**

C. Preparing the DNA Samples for Sequencing

Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96- well reaction plate. Sample order is as follows: A1, B1, C1, D1... G1, H1, A2, B2, C2... G2, H2, A3, B3, C3, etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1 to H2, the second injection corresponds to wells A3 to H4 and so on. Label the side of the reaction plate with the name used for the Plate ID with a sharpie.

4. Remove the Hi-Di formamide from the freezer and allow it to thaw. Add 10µL of formamide to each dried sample and mix to bring the sample into solution.

   Once formamide is thawed and aliquoted, discard the tube. Do not re-freeze opened tubes of Hi-Di formamide.

5. If single Centri-Sep columns were used, load the entire 10 µL of the resuspended samples into the 96-well tray in the appropriate wells. The injections are grouped into 16 wells starting with A1, B1, and so on moving down two columns ending with 2G, 2H, for a total of 16 wells. Fill any unused wells that are part of an injection set (eg. containing <16 samples) with 10 µL of Hi-Di formamide.

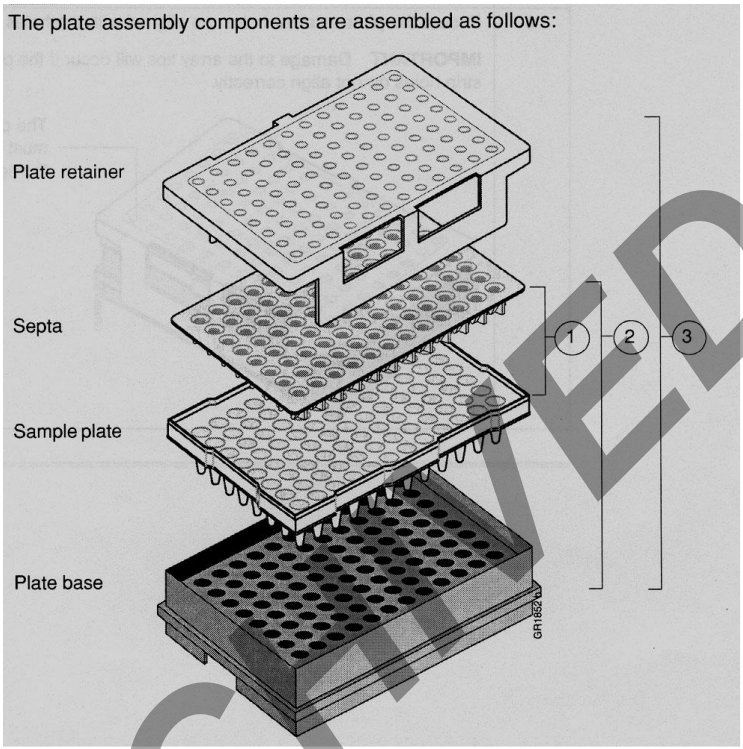
6. Once all of the samples have been added to the plate, place the 96-well septa over the reaction plate and firmly press the septa into place. Spin plate in the centrifuge for one minute.

7. Remove the reaction plate from the base and heat denature samples in the 95ºC heatblock for 2 minutes followed by a quick chill in the 4ºC chill block for 5 minutes. Centrifuge the tray for one minute after the heat/chill.

8. Once denatured, place the plate into the plate base. Secure the plate base and plate with the plate retainer.

   IMPORTANT: **Damage to the array tips will occur if the plate retainer and septa strip holes do not align correctly.**

   Do not write on the septa with pen, markers, sharpies, etc. Ink may cause artifacts in samples. Any unnecessary markings or debris on the septa may compromise instrument performance.



D. Placing the Plate onto the Autosampler (Linking and Unlinking Plate)

The Autosampler holds up to two, 96-well plates in tray positions A and B. To place the plate assembly on the autosampler, there is only one orientation for the plate, with the notched end of the plate base away from you.

9. In the tree pane of the Foundation Data Collection v3.0 software click on **GA Instrument > ga3130xl > *instrument name* > Run Scheduler > Plate View**

10. Push the tray button on the bottom left of the machine and wait for the autosampler to move forward and stop at the forward position.

11. Open the doors and place the tray onto the autosampler in the correct tray position, A or B. **There is only one orientation for the plate.**

12. Ensure that the plate assembly fits flat in the autosampler. Failure to do so may allow the capillary tips to lift the plate assembly off the autosampler.

When the plate is correctly positioned, the plate position indicator on the **Plate View** page changes from gray to yellow. Close the instrument doors and allow the autosampler to move back to the home position.

NOTE: When removing a plate from the autosampler, be careful not to hit the capillary array. Plate B is located directly under the array, so be especially careful when removing this tray.

Linking/Unlinking the Plate record to Plate

13. On the plate view screen, click on the plate ID that you are linking. If the plate ID is not available click **Find All**, and select the plate ID created for the run.

14. Click the plate position (A or B) that corresponds to the plate you are linking.

    NOTE: It may take a minute for the plate record to link to the plate depending on the size of the sample sheet.

    If two plates are being run, the order in which they are run is based on the order in which the plates were linked.

    Once the plate has been linked, the plate position indicator changes from yellow to green when linked correctly and the green run button becomes active.

15. To unlink a plate record just click the plate record you want to unlink and click "**Unlink".**

E. Viewing Run Schedule and Starting Run

1. In the tree pane of the Foundation Data Collection software, click **GA Instruments > ga3130xl > *instrument name* > Run Scheduler > Run View.**

2. The **RunID** column indicates the folder number(s) associated with each injection in your run (e.g. *Batman-2008-0114-1600-0197)*. The folder number(s) and the run ID should be recorded in the **LIMS**.

3. Click on the run file to see the Plate Map or grid diagram of your plate on the right. Check if the blue highlighted boxes correspond to the correct placement of the samples in

the injections.

4. **NOTE: Before starting a run, check for air bubbles in the polymer blocks. If bubbles are present, click on the Wizards tool box on the top and select "Bubble Remove Wizard". Follow the wizard until all bubbles are removed.**

5. Click on the green **Run** button in the tool bar when you are ready to start the run. When the **Processing Plate** dialog box opens (You are about to start processing plates…), click **OK**.

6. To check the progress of a run, click on the **Cap/Array Viewer** or **Capillaries Viewer** in the left window. The **Cap/Array Viewer** window will show the raw data of all 16 capillaries at once. The **Capillaries Viewer** window will show you the raw data of the capillaries you select to view.

   IMPORTANT: Always exit from the Capillary Viewer and Cap/Array Viewer windows. During a run, do not leave these pages open for extended periods. This may cause unrecoverable screen update problems. Leave the Instrument Status window open.

   The visible setting should be:

EP voltage 12.2 kV	EP current (no set value)
Laser Power prerun 15 mW	Laser Power during run 15mW
Laser current (no set value)	Oven temperature 50°C

   Expected values are: EP current constant around 40-60 µA starting current
   EP current constant around 70-80 µA running current
   Laser current: 5.0 A ± 1.0 A

It is good practice to monitor the initial injections in order to detect problems.

F. Water Wash and POP Change

Refer to Section A for schematic of 3130*xl* while proceeding with the water wash and POP change procedure.

1. Remove a new bottle of POP6 from the refrigerator.
2. Select **Wizards** > **Water Wash Wizard**
3. Click "Close Valve"
4. Open instrument doors and remove the empty POP bottle.
5. With a dampened Kimwipe®, wipe the polymer supply tube and cap. Dry.
6. Replace POP bottle with the water bottle filled to the top with Gibco® Water.
7. Remove, empty, and replace the anode buffer jar on the lower polymer block.
8. Click "Water Wash." This procedure is will take approximately 4 minutes.
9. When the water wash is finished click "Next"
10. Select "Same Lot" or "Different Lot"
11. Remove water bottle from the lower polymer block. Dry supply tube and cap with a Kimwipe®.
12. Replace with a new bottle of room temperature POP.
13. Click "Next."
14. Click "Flush." This will take approximately 2 minutes to complete.
15. Inspect the pump block, channels, and tubing for air bubbles.
16. Click "Next."

3130xl Genetic Analyzer Troubleshooting

Instrument Startup

Observation	Possible Cause	Recommended Action
No communication between the instrument and the computer (yellow light is blinking).	Instrument not started up correctly.	Make sure the oven door is closed and locked and the front doors are closed properly. If everything is closed properly, start up in the following sequence: a. Log out of the computer. b. Turn off the instrument. c. Boot up the computer. d. After the computer has booted completely, turn the instrument on. Wait for the green status light to come on. e. Launch Data Collection software.
Red light is blinking.	Incorrect start up procedure.	Start up in the following sequence: a. Log out of the computer. b. Turn off the instrument. c. Boot up the computer. d. After the computer has booted completely, turn the instrument on. Wait for the green status light to come on. e. Launch the Data Collection Software.
Computer screen is frozen.	Communication error. This may be due to leaving the user interface in the Capillary View or Array View window.	There will be no loss of data. However, if the instrument is in the middle of a run, wait for the run to stop. Then, exit the Data Collection software and restart as described above.

Observation	Possible Cause	Recommended Action
Autosampler does not move to the forward position**.**	Possible communication error, OR Oven or instrument door is not closed.	Restart the system, and then press the Tray button. OR a. Close and lock the oven door. b. Close the instrument doors. c. Press the Tray button.
Communication within the computer is slow.	Database is full.	Old files need to be cleaned out of the database. Follow proper manual procedures described in the ABI Prism 3130xl Genetic Analyzer User's Manual.

Spatial Calibration

Observation	Possible Cause	Recommended Action
Unusual peaks or a flat line for the spatial calibration.	The instrument may need more time to reach stability. An unstable instrument can cause a flat line with no peaks in the spatial view.	Check or repeat spatial calibration.
	Improper installation of the detection window.	Reinstall the detection window and make sure it fits in the proper position.
	Broken capillary resulting in a bad polymer fill.	Check for a broken capillary, particularly in the detection window area. If necessary, replace the capillary array using the Install Array Wizard.
	Dirty detection window.	Place a drop of METHANOL onto the detection window, and dry. Use only light air force.
Persistently bad spatial calibration results.	Bad capillary array.	Replace the capillary array, and then repeat the calibration. Call Technical Support if the results do not improve.

Spectral Calibration

Observation	Possible Cause	Recommended Action
No signal.	Incorrect preparation of sample. Air bubbles in sample tray.	Replace samples with fresh samples prepared with fresh formamide. Centrifuge samples to remove air bubbles.
If the spectral calibration fails, or if a message displays "No candidate spectral files found".	Clogged capillary Incorrect parameter files and/or run modules selected. Insufficient filling of array. Expired matrix standards	Refill the capillaries using manual control. Look for clogged capillaries during capillary fill on the cathode side. Correct the files and rerun the calibration. Check for broken capillaries and refill the capillary array. Check the expiration date and storage conditions of the matrix standards. If necessary, replace with a fresh lot.
Spike in the data.	Expired polymer. Air bubbles, especially in the polymer block tubing. Possible contaminant or crystal deposits in the polymer.	Replace the polymer with fresh lot using the change Polymer Wizard. Refill the capillaries using manual control. Properly bring the polymer to room temperature; do not heat to thaw rapidly. Swirl to dissolve any solids. Replace the polymer if it has expired.

Run Performance

Observation	Possible Cause	Recommended Action
No data in all capillaries	Bubbles in the system.	Visually inspect the polymer block and the syringes for bubbles. Remove any bubbles using the Change Polymer Wizard. If bubbles still persist, perform the following: a. Remove the capillary array. b. Clean out the polymer bottle. c. Replace polymer with fresh polymer.
No signal.	Dead space at bottom of sample tube. Bent capillary array. Failed reaction. Cracked or broken capillary	Centrifuge the sample tray. Replace the capillary array Repeat reaction. Visually inspect the capillary array including the detector window area for signs of breakage.
Low signal strength.	Poor quality formamide. Insufficient mixing. Weak amplification of DNA Instrument/Laser problem	Use a fresh lot of formamide Vortex the sample thoroughly, and then centrifuge the tube to condense the sample. Re-amplify the DNA. Run instrument diagnostics.

Observation	Possible Cause	Recommended Action
Elevated baseline	Possible contamination in the polymer path.	Wash the polymer block with hot water. Pay particular attention to the pump block, the ferrule, the ferrule screw, and the peek tubing. Dry the parts by vacuum pump before replacing them onto the instrument.
	Possible contaminant or crystal deposits in the polymer.	Bring the polymer to room temperature, swirl to dissolve any deposits. Replace polymer if expired.
	Poor spectral calibration.	Perform new spectral calibration.
	Detection cell is dirty.	Place a drop of methanol onto the detection cell window.
Loss of resolution.	Too much sample injected.	Dilute the sample and reinject.
	Poor quality water.	Use high quality, ultra pure water.
	Poor quality or dilute running buffer.	Prepare fresh running buffer.
	Poor quality or breakdown of polymer.	Use a fresh lot of polymer.
	Capillary array used for more than 150 injections.	Replace with new capillary array.
	Degraded formamide.	Use fresh formamide and ensure correct storage conditions.
	Improper injection and run conditions.	Notify QA to check default settings.

Observation	Possible Cause	Recommended Action
Poor resolution in some capillaries.	Insufficient filling of array.	Refill array and look for cracked or broken capillaries. If problem persists contact Technical Support.
No current	Poor quality water. Water placed in buffer reservoir position 1. Not enough buffer in anode reservoir. Buffer is too dilute. Bubbles present in the polymer block and/or the capillary and /or peek tubing.	Use high quality, ultra pure water. Replace with fresh running buffer. Add buffer up to fill line. Prepare new running buffer. Pause run and inspect the instrument for bubbles. They may be hidden in the peek tubing.
Elevated current.	Decomposed polymer. Incorrect buffer dilution. Arcing in the gel block.	Open fresh lot of polymer and store at 4°C. Prepare fresh 1X running buffer. Check for moisture in and around the septa, the reservoirs, the oven, and the autosampler.

Observation	Possible Cause	Recommended Action
Fluctuating current	Bubble in polymer block.	Pause the run, check the polymer path for bubbles, and remove them if present.
	A slow leak may be present in the system.	Check polymer blocks for leaks. Tighten all fittings.
	Incorrect buffer concentration.	Prepare fresh running buffer.
	Not enough buffer in anode.	Add buffer up to the fill line.
	Clogged capillary.	Refill capillary array and check for clogs.
	Arcing.	Check for moisture in and around the septa, the reservoirs, the oven, and the autosampler.
Poor performance of capillary array used for fewer than 150 runs.	Poor quality formamide	Prepare fresh formamide and reprep samples.
	Incorrect buffer.	Prepare new running buffer.
	Poor quality sample, possible cleanup needed.	Desalt samples using a recommended purification protocol (e.g., microcon).
Migration time becomes progressively slower.	Leak in the system.	Tighten all ferrules, screws and check valves. Replace any faulty parts.
	Improper filling of polymer block.	Check polymer pump force. If the force needs to be adjusted, make a service call.
	Expired polymer.	If necessary, change the lot of polymer.

Observation	Possible Cause	Recommended Action
Migration time becomes progressively faster.	Water in polymer bottle resulting in diluted polymer.	Replace the polymer, making sure the bottle is clean and dry.
Arcing in the anode – lower polymer block.	Moisture on the outside of the lower polymer block.	Dry the lower block. If damaged, replace lower polymer block.
Error message, "Leak detected" appears. The run aborts.	Air bubbles in the polymer path. Pump block system is loose/leaking. Lower pump block has burnt out. When there is condensation in the reservoir(s) this will cause electrophoresis problems and burn the lower block	Check for bubbles and remove if present, then check for leaks. Make sure all ferrules, screws, and tubing is tightly secure. Ferrule in capillary end of block may be positioned wrong or missing. Check for this ferrule. Replace the lower block.
Buffer jar fills very quickly with polymer.	Air bubbles in the polymer path. Lower polymer block is not correctly mounted on the pin valve.	Check for bubbles and remove if present. Then, look for leaks. Check to make sure the metal fork is in between the pin holder and not on top or below it.

Observation	Possible Cause	Recommended Action
Detection window pops out while replacing the capillary array. Replacing the window in the correct orientation is difficult.	Tightening of the array ferrule knob at the gel block causes high tension.	Loosen the array ferrule knob to allow the secure placement of the window. Re-tighten and close the detection door.
Detection window stuck. It is difficult to remove when changing the capillary array.		To loosen the detection window: a. Undo the array ferrule knob and pull the polymer block towards you to first notch. b. Remove the capillary comb from the holder in the oven. c. Hold both sides of the capillary array around the detection window area, and apply gentle pressure equally on both sides. d. Release.