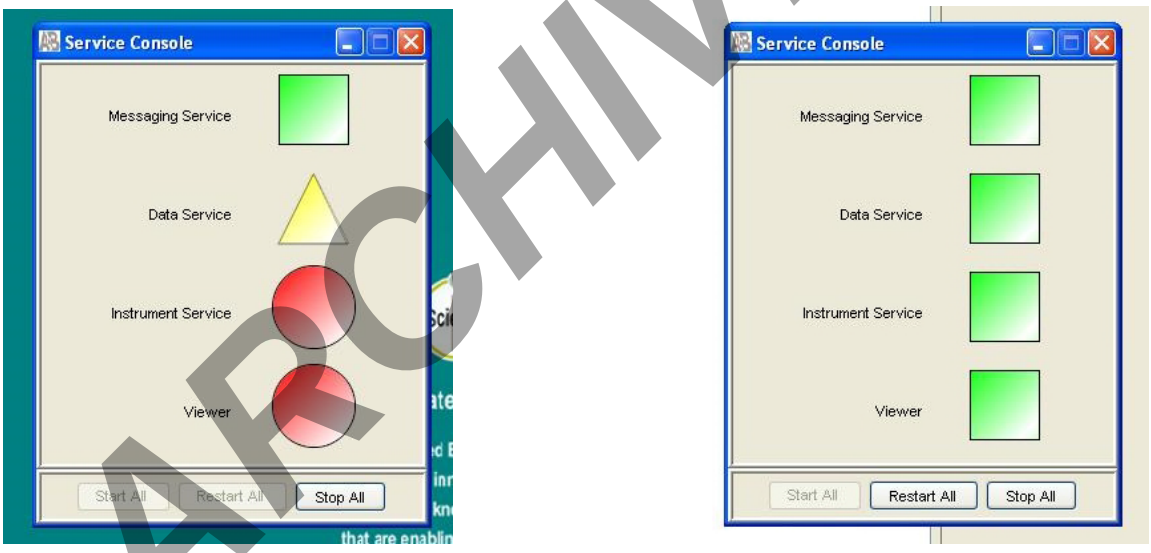
PowerPlex Fusion – Capillary Electrophoresis

1 Setting Up A 3130*xl* Run

1.1 Go to the computer attached to the instrument.

1.2 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 3130*xl* Data Collection v3.0 to display the Service Console.

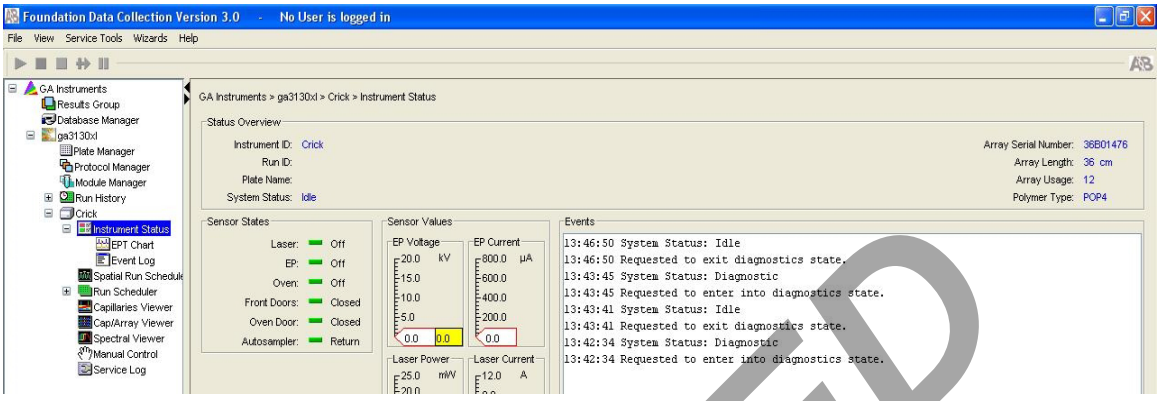
1.3 By default, all applications are off, indicated by the red circles. As each application activates, the red circles (off) change to yellow triangles (activating), eventually progressing to green squares (on) when they are fully functional.



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

1.5 Check the number of injections on the capillary in the 3130*xl* usage log and in the **Foundation Data Collection** window by clicking on the **ga3130*xl* > *instrument name* >Instrument Status**.

1.5.1 If the numbers are not the same, update the usage log. If the number is **≥145,** notify QA. Proceed only if the number of injections that will be running plus the usage number is ≤150.

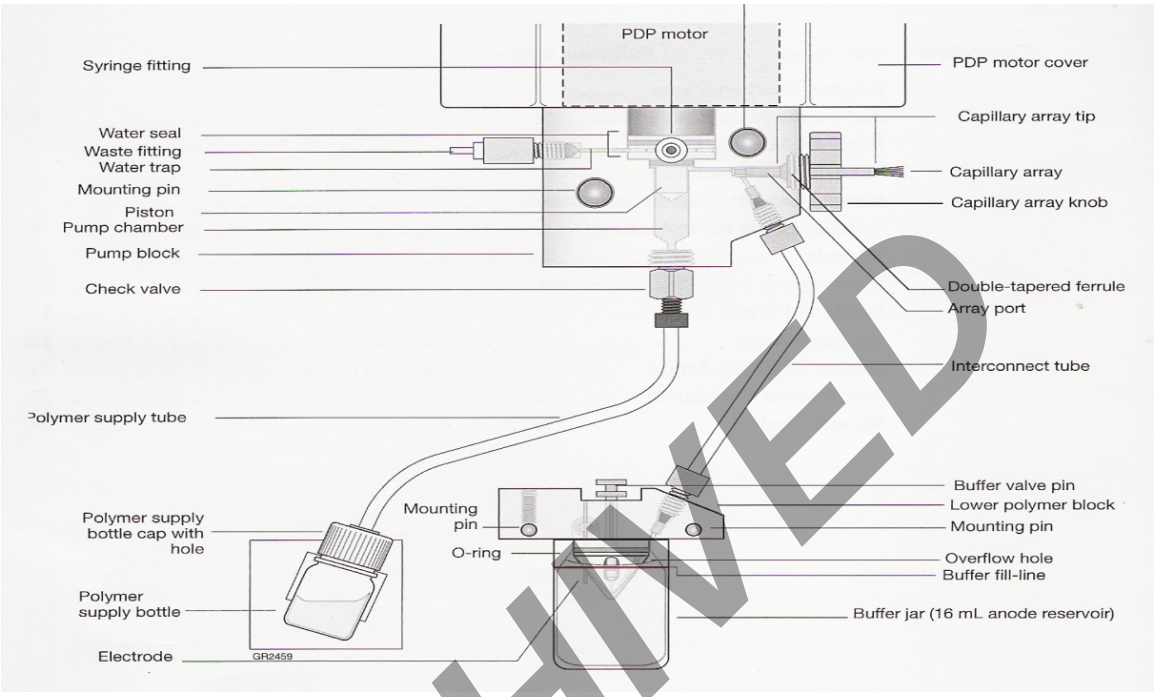


1.6 Determine if POP needs to be changed.

1.6.1 Check the usage log to determine when the POP4 was last changed. If it is >7 days, proceed with POP4 change. The POP4 does not need to be changed if it is the 7th day.

1.6.2 Check the level of POP4 in the bottle to ensure there is enough for the run (~450 µL for 6 injections). A full piston chamber is approximately 600ul.

1.6.3 If POP needs to be changed, see (**See Water Wash & POP Change in the QC 135-ABI 3130xl Maintenance Procedure**).



1.7 If it is the first run of the day on the instrument, Change the Buffer, Water and Waste. If a run has already been performed on the instrument that day and the "buffer changed" column displays that day's date, buffer does not need to be changed again, continue to step 1.9.

1.8 Change Buffer, Water and Waste:

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

1.8.2 Wait until the autosampler has stopped moving and the light on the instrument turns green, and then open the instrument doors.

1.8.3 Remove the three plastic reservoirs in front of the sample tray and anode jar from the base of the lower pump block and dispose of the fluids in the sink.

1.8.4 Rinse with the distilled water from a water station or squirt bottle, dry thoroughly.

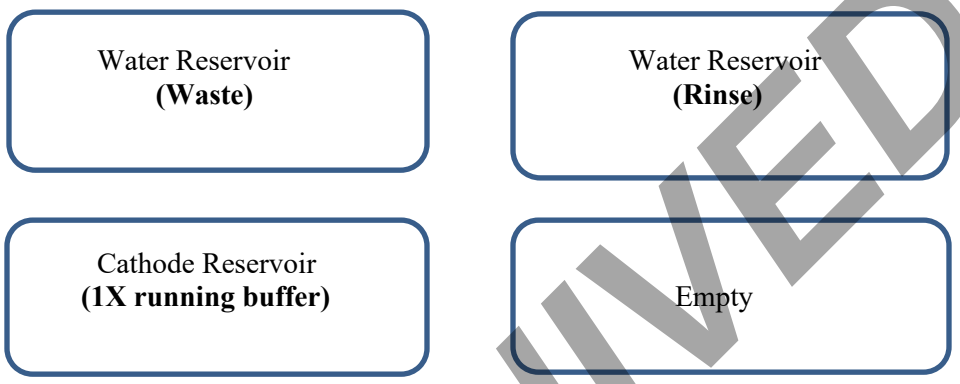
1.8.5 Fill the "water" and "waste" reservoirs to the line with sterile deionized water such as INVITROGEN®.

1.8.6 Make a batch of 1X buffer (45 ml sterile deionized water, 5 ml 10X buffer) in a 50 mL conical tube. Rinse and fill the "buffer" reservoir and anode jar with 1X buffer to the lines.

1.8.6.1 If keeping excess buffer for additional runs, label the tube with your initials, date of make and lot number.

1.8.7 Dry the outside and inside rim of the reservoirs/septa and outside of the anode jar using a lint free wipe and replace the septa strip snugly onto each reservoir.

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



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

1.8.10 Close the instrument doors.

1.9 Record lot numbers for POP4 and buffer in the Usage log as a separate entry if a run is not being performed, otherwise this is logged with the Usage log entry in section 3.24.

2 Creating a Test Batch

2.1 3130*xl* Test Batch Creation (additionally, see the LIMS Manual, Test Batch Creation if needed)

2.1.1 Sample names and run names cannot be longer than 50 characters, and must be in correct 3130 format: -_.(){}[]+^ only.

2.1.2 Allelic Ladder(s) must be individually added to the test batch. Allelic Ladder should be positioned as the first sample of each injection during the plate loading step. (1 Injection is two rows / 16 samples).

2.1.3 Ensure that the correct System is in the “Sys” column in the Data Entry Screen.

2.1.3.1 PowerPlex Fusion sample cannot be run on the same plate as Yfiler samples due to the need to use a different spectral.

2.1.4 Name the test batch and plate name as follows: ***Instrument name & date injection number(s)*** for example: Athena042419 70-76.

2.1.4.1 LIMS automatically changes the "space" to an underscore; therefore, if naming the test batch outside of LIMS, the plate name should not use spaces. For Example: Athena042419_70-76

2.1.5 If samples on the test batch are being rerun, confirm that the dilution (if applicable), suffix, comments, or any other necessary information is present.

2.1.6 For rerun of samples, fill up the end of the injection with any reruns of the before starting a new injection.

3 Preparing and Running the DNA samples

3.1 Retrieve amplified samples from the thermal cycler or refrigerator.

3.2 Prepare thermal cyclers for snap dechill step.

3.2.1 Set one thermal cycler to 95°C (heat program) and one thermal cycler to 4°C (chill program).

3.2.2 Thermal cyclers should be turned off after each use.

3.3 Spin down samples at 1000 RPM for one minute.

3.4 Prepare dilutions of amplified samples, if necessary. 0.1X TE^{-4} should be used to make the dilutions. Pipette mix prior to aliquoting for dilution. Ensure that TE lot number is recorded.

3.4.1 When manually recording lot numbers, include the entire series of letters and numbers (i.e., 0.1XTE1612155668) in the Notes section of the test batch.

3.5 Retrieve the following reagents from the associated refrigerator and/or freezer.

PowerPlex Fusion® WEN ILS 500
PowerPlex Fusion® Allelic Ladder
HiDi Formamide*

3.5.1 HiDi Formamide must not be re-frozen.

3.6 Record lot numbers of reagents.

3.7 Prepare one mastermix for all samples, negative and positive controls, and allelic ladders as specified below:

(9.5 µL of HiDi + 0.5 µL of WEN ILS500 per sample)

# Samples + 2	HiDi Form	ILS500
16	171 uL	9 uL
32	323 uL	17 uL
48	475 uL	25 uL
64	627 uL	33 uL
80	779 uL	41 uL
96	931 uL	49 uL

3.8 Obtain a reaction plate and label the side with the run name.

3.9 Aliquot **10µL** of **mastermix** to each well.

3.9.1 If an injection has less than 16 samples, add at least 10 uL of either dH_2O, formamide, HiDi, buffer, or mastermix to all unused wells within that injection.

3.10 **Witness step.** Have another analyst verify the tube setup by comparing the tube labels and positions indicated on the Load Plate Screen in the LIMS system with the tube labels and positions of the amp tubes. **The entire amp tube label must be read for each sample.**

3.10.1 For samples being transferred from a Fusion Direct amplification plate:

3.10.1.1 The witness must verify that each individual sample well is in the same order by comparing the amplification test batch Load Plate screen against the STR test batch Load Plate screen. This must be verified by hovering the cursor over the wells and corresponding samples on the plate images, not by using the load plate sample list.

3.10.1.2 The analyst must state the test batch ID on the amplification plate and amplification time; the witness must verify this information in the amplification test batch in LIMS.

3.10.1.3 Controls- verify that the positive control date and time from the amplification test batch is the same as the positive control date and time in the STR test batch.

3.10.1.4 Sample wells- analyst setting up the plate must state which wells hold amplified product and are being transferred from the amplification plate. Analyst must also state the wells being loaded in the STR plate. Witness must verify that the wells are correct in the Load Plate screens of the amplification test batch and the STR test batch.

3.11 Aliquot **1µL** of allelic ladder, positive control, negative control, and sample into their appropriate well.

3.11.1 Sample order is as follows: A1, B1, C1… A2, B2, C2, etc.

3.11.2 Samples may be loaded from a PowerPlex Fusion Direct amplification plate into the CE plate by using a multichannel pipette. **1ul of allelic ladder must be loaded separately into the appropriate wells.**

3.12 Once all of the samples have been added to the plate, place a new 96-well septa over the reaction plate and firmly press the septa into place.

3.12.1 After aliquoting from a PowerPlex Fusion Direct amplification plate, discard the amplification plate.

3.13 Denature/Chill the plate:

3.13.1 Spin the plate in the centrifuge at 1000 RPM for one minute.

3.13.2 Denature plate for **3 minutes** with the thermal cycler set to 95°C.

3.13.3 Chill plate for **3 minutes** with the thermal cycler set to 4°C**.**

3.14 While plates are denature/chilling, set up the 3130 for run.

3.14.1 Turn on oven and set for 60°C

3.14.1.1 Manual Control → Send Defined Command For: click on Oven.

3.14.1.2 Command Name click on Turn On/Off oven → Send Command

3.14.1.3 Command Name click on Set Oven Temperature→60→Send Command

3.14.2 Import the Instrument Plate Record

3.14.2.1 Use the Download to Instrument Button on the Plate Record screen to have LIMS create the plate record. This will be created in the LIMS file share drive.

NOTE: Ensure that the Batch Setup Review Performed By step is filled out prior to using the Download to Instrument button. This will trigger the renaming of any ladders in the batch, ex. from "Allelic Ladder-Fusion" to "Allelic Ladder_1".

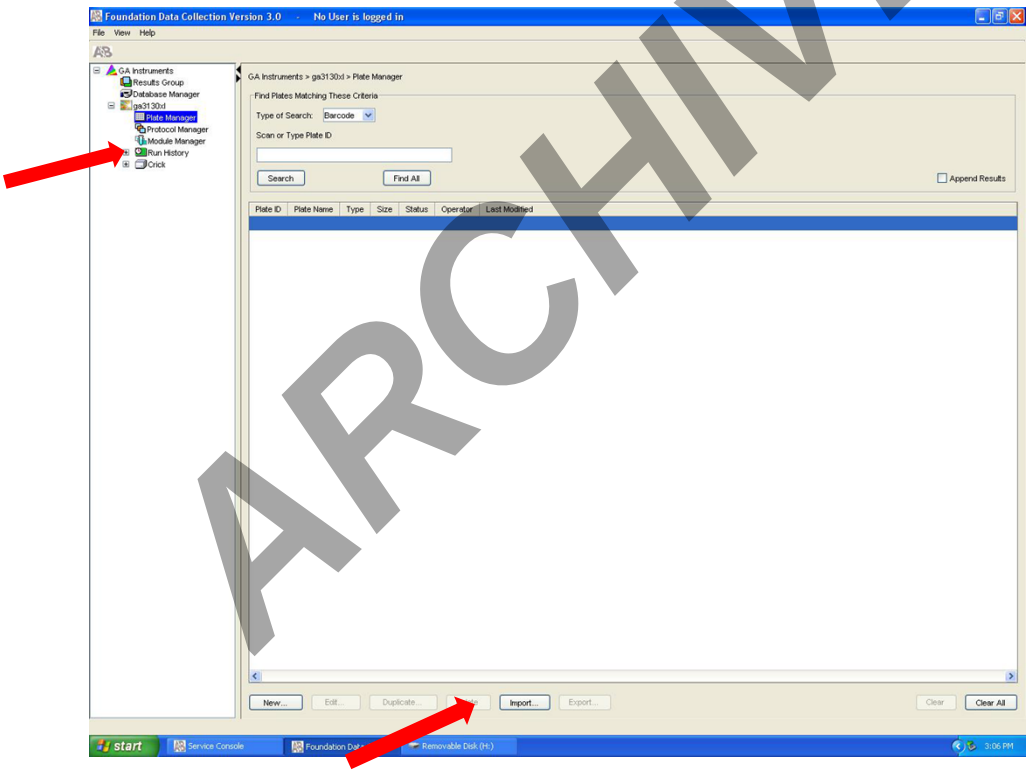
3.14.2.2 From the LIMS file share drive, on the instrument computer, or by using a USB drive, drag-and-drop the plate record to the instrument's plate record folder.

3.14.2.3 Maximize the Foundation Data Collection window.

3.14.2.4 Click + to expand subfolders in the left tree pane of "ga 3130*xl*".

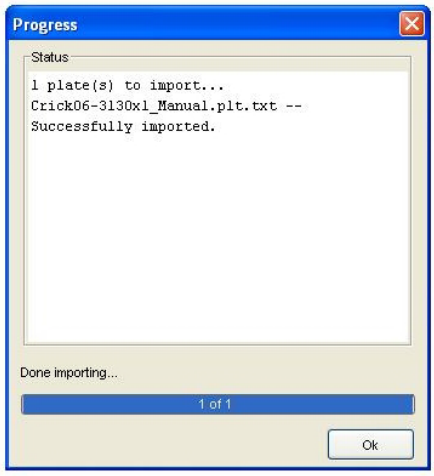
3.14.2.5 Click on "Plate Manager".

3.14.2.6 In the Plate Manager window click on "Import…"



3.14.2.7 Browse for the plate record in **D:\AppliedBiosystems\Plate Records**. Double click on the file or highlight it and click **Open**.

3.14.2.8 A window will prompt the user that the plate record was successfully imported. Click **OK**.



3.14.2.9 If the Plate Record will not import, a window will prompt the user where changes are needed. Go back to edit the documentation and resave the corrected Plate Record and Sample Sheet with the same file name.

3.14.3 Set G5 spectral to most recent PPFusion spectral.

3.14.3.1 Spectral viewer → Dye Set drop down select G5 → List of Calibrations for Dye Set: G5 select PPFusionG5 spectral with the most recent date → click Set

3.14.3.2 This spectral must be changed to the Yfiler G5 spectral before running a Yfiler plate on this instrument. Due to the need to change spectrals between Fusion and Yfiler, Yfiler samples CANNOT be run on the same plate, or as the second plate on an instrument running a Fusion plate.

3.15 Spin down plate at 1000RPM for 1 minute.

3.16 Place plate into plate assembly and ensure white top is secured to the black base. The notch in the plate will align to the same corner as the notch in the plate assembly.

3.17 Linking plate to instrument

3.17.1 Run Scheduler →Plate View

3.17.2 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.

3.17.3 Open the doors and place the plate assembly onto the autosampler in the correct tray position, A or B. There is only one orientation for the plate. (The notched end faces away from the user.

3.17.3.1 Ensure the plate assembly fits flat in the autosampler. The Plate View window will change from gray to yellow. Close the instrument doors and allow the autosampler to move back to the home position.

3.17.3.2 Type the exact plate name in the Plate ID window and click "Search." Or, click the "Find All" button and select the desired plate record.

NOTE: If the plate name is not typed in correctly, your plate will not be found. Instead, a prompt to create a new plate will appear. Click "No" and retype the plate name.

3.17.4 Click the plate position indicator corresponding to the plate position in the instrument. The plate position (A or B) displays in the link column. If two plates are being run, the order in which they are run is based on the order in which the plates were linked.

3.17.5 Click on plate record and then corresponding plate position in the Plate View screen. The indicator will change from yellow to green when linked correctly, and the green run button becomes active.

3.17.5.1 To unlink a plate record, click the plate record to be unlinked and click "**Unlink**".

3.18 Check Run View to ensure correct number of samples and injections are being run.

3.18.1 GA Instruments > ga3130*xl* > *instrument name* > Run Scheduler > Run View

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

3.19 Ensure instrument is ready to go by checking the reservoirs are filled properly, the plate is linked correctly, and no bubbles are present in the polymer block.

3.19.1 If bubbles are present proceed to **Bubble Removal Wizard**. (Wizards→Bubble Removal Wizard) and follow the on-screen prompts.

3.19.1.1 See Bubble Removal Wizard in the QC 135 ABI 3130xl Maintenance Procedure.

3.19.1.2 Very small bubbles that do not move or disappear with the bubble removal wizard will not affect the run and the run may proceed without further removal.

3.19.1.3 While performing **the** Bubble Removal Wizard, DO NOT turn the array port knob unless bubbles are present in the small tubing in the array port.

3.19.1.3.1 If the knob needs to be turned, turn it one turn counterclockwise to open it. Turn the knob one turn clockwise to tighten. Usually an arrow is present on the knob. Make sure to align the arrow to the capillary shaft to ensure the knob is tightened correctly, preventing pop leaks.

3.19.1.4 Contact QA if large persistent bubbles are still seen.

3.20 Start run by pressing green **Run** button (play button). When the Processing Plate dialog box opens (You are about to start processing plates…), click OK.

3.21 Parameters are as follows:

	U
Oven Temp	60°C
Pre-Run Voltage	15.0 kV
Pre-Run Time	180 sec
Injection Voltage	3 kV
Injection Time	5 sec
Run Voltage	13 kV
Run Time	2000 sec

3.22 Monitor instrument until event log shows the instrument as begun Pre-Run.

3.23 To check the progress of a run, click on the **Capillary Viewer** or **Cap/Array Viewer** in the tree pane of the Foundation Data Collection software. The **Capillary Viewer** will show you the raw data of the capillaries you select to view whereas the **Cap/Array Viewer** will show the raw data of all 16 capillaries at once.

3.23.1 IMPORTANT: Always exit from the **Capillary Viewer** and **Cap/Array Viewer** windows. During a run, do not leave these pages open for extended periods. Leave the **Instrument Status** window open.

3.24 Enter a LIMS usage log for current run, recording lot numbers of buffer and POP4 in the usage log if they were changed during for run.

3.24.1 Amplification tubes should be stored at 4^{O}C-8^{O}C, filed by instrument and run name.

3.24.2 Amplification tubes should be stored with their most recent 3130 run. For example, a sample initially run on Newton is rerun on Meyer. That tube should now be stored with the samples on the Meyer run. Do not return the tube to its initial run storage box.

3.24.3 If a positive control is being pulled for use from a previous run, the tube should be returned to its original run box after use to remain associated with its original amp set.

4 Collecting Data

4.1 When a run is complete, it will automatically be placed in D:/AppliedBio/Current Runs folder, labeled with either the *plate name-date* (e.g. Einstein19-025ID-015PPY-2019-03-11) or *instrument name, date and run folder ID* (e.g. Run_Athena_2019-07-13_0018). Proceed to Analysis section of the GeneMarker manual.

4.1.1 One injection takes approximately 45 minutes.

5 Re-injecting Plates

5.1 Plates should be re-injected as soon as possible.

5.1.1 Create a new test batch and plate record See LIMS Manual for STR Reinjections.

5.1.2 Ensure Instrument is Set up (section 1)

5.1.3 Follow steps in section 3, starting with steps 3.2 and 3.13, Re-denature/chill the plate (if needed).

5.1.3.1 If a plate is being reinjected the same day on which it was originally run, it does not require an additional denature/chill step before being rerun. If denature/chill is not required, start with step 3.14.

6 Water Wash and POP Change

6.1 See Water Wash & POP Change in the QC 135-ABI 3130xl Maintenance Procedure