
Procedure for Use of the 3130XL Genetic Analyzer for Casework

1.0 Purpose - This procedure specifies the steps for performing daily maintenance, preparing samples and running samples on the 3130XL Genetic Analyzer.

2.0 Scope - This procedure applies to casework analysts and trainees in the Forensic Biology Section who are qualified to use the 3130XL Genetic Analyzer.

3.0 Definitions

- **Run** – Each set of 16 samples that is injected and separated electrophoretically on the Capillary Electrophoresis Unit (ABI 3130XL Genetic Analyzer or equivalent)

4.0 Equipment, Materials and Reagents

- Centrifuge
- Amplified DNA products
- GeneAmp® PCR System 9700 Thermal Cycler
- Calibrated Pipets
- ART Pipet Tips (or equivalent, various sizes)
- 50 mL conical tube
- 10X Genetic Analyzer Buffer
- AmpFℓSTR® Identifiler® Plus Reagents, associated allelic ladder and the GeneScan™ 500 LIZ® size standard
- Formamide
- Applied Biosystems™ 3130XL
- QIAgility liquid handler
- 96 well plates with septa
- Holding tray for the 3130XL
- Ice plate from the freezer (optional)

5.0 Procedure

5.1 No more than 30 total items (including controls) per analyst may be processed simultaneously.

5.2 Pre-Heating Oven Manually

5.2.1 Pre-heating the oven shortens the overall run time for the plate.

5.2.2 Click Manual Control in the task pane to open the manual control window.

5.2.3 Choose Oven from the top drop down window.

5.2.4 In the Command Name drop down, choose Turn On/Off oven.

5.2.5 Choose On for the Value drop down and click Send Command.

5.2.6 In the Command Name drop down, choose Set oven temperature.

5.2.7 Change the Value from 50.0 to 60.0 and click Send Command.

5.3 Checking and Refilling Fluids

5.3.1 Visually determine if polymer needs to be added to the instrument before proceeding with instrument preparation. Ensure that weekly maintenance has been performed prior to use of the instrument. Notify the Quality Control Officer (QCO) or designee if the instrument needs additional polymer or if maintenance has not been performed. The instrument shall not be used until both conditions are met.

5.3.2 Before the first run of the day, replace the respective solutions in the anode buffer reservoir (i.e., shot glass), the cathode buffer reservoir and the water reservoirs.

5.3.3 Prepare 50 mL of 1X Genetic Analyzer Buffer: Add 5.0 mL of 10X Genetic Analyzer Buffer to a 50 mL conical tube, and then add 45 mL of purified water. Mix well by inversion several times. Label the tube with the reagent name, initials of preparer, date prepared and expiration date. The expiration date for 1X buffer is one week after preparation.

5.3.4 Press the tray button on the outside of the instrument to bring the autosampler to the forward position.

5.3.5 Wait until the autosampler has stopped moving before opening the instrument doors (the light on the front of the instrument is a solid green).

5.3.6 Remove the reservoirs from the instrument.

5.3.7 Dispose of the fluids and rinse the reservoirs with purified water.

5.3.8 Perform a second rinse using purified water on the water reservoirs and buffer on the cathode buffer reservoir.

5.3.9 Fill the cathode buffer and anode buffer reservoirs to the fill line with 1X Genetic Analyzer Buffer.

5.3.10 Fill the water reservoirs to the fill line with purified water.

5.3.11 Dry the outside of the reservoirs using a wipe. Discard the septa and replace with new strips. Ensure the septa strips fit snugly and flush on the tops of the reservoirs.

5.3.12 Place the reservoirs into the correct positions on the autosampler (the two water reservoirs in the back positions, the cathode buffer reservoir in the front left position and the front right position empty).

5.3.13 Put the anode buffer reservoir on the instrument (making sure the seal is tight and the O-ring is snug around all sides).

5.4 Preparing Samples to Run on the 3130XL (Manual Setup)

5.4.1 Prepare the master mix for a 16-well run by combining the following in a single microcentrifuge tube:

Reagent	Volume
HI-DI™ Formamide	Approx. 174 µL
GeneScan™-500 LIZ® Size Standard	6 µL for Identifiler® Plus

5.4.2 Vortex the tube containing the master mix, then pulse spin in a microcentrifuge.

5.4.3 In a 96-well reaction plate, dispense 9 µL of the formamide/size standard master mix into each well that is to contain a sample. Add 9 µL of the master mix to each blank well.

5.4.4 Load 1.0 µL of allelic ladder and 1.0 µL of amplified sample into the appropriate wells using the 3130 Setup Worksheet.

5.4.5 Cover the reaction plate with the 96-well septa. Label the tray with initials of Forensic Scientist and date of run.

5.4.6 Spin the reaction plate in a centrifuge to ensure the contents of each well are mixed and collected at the bottom.

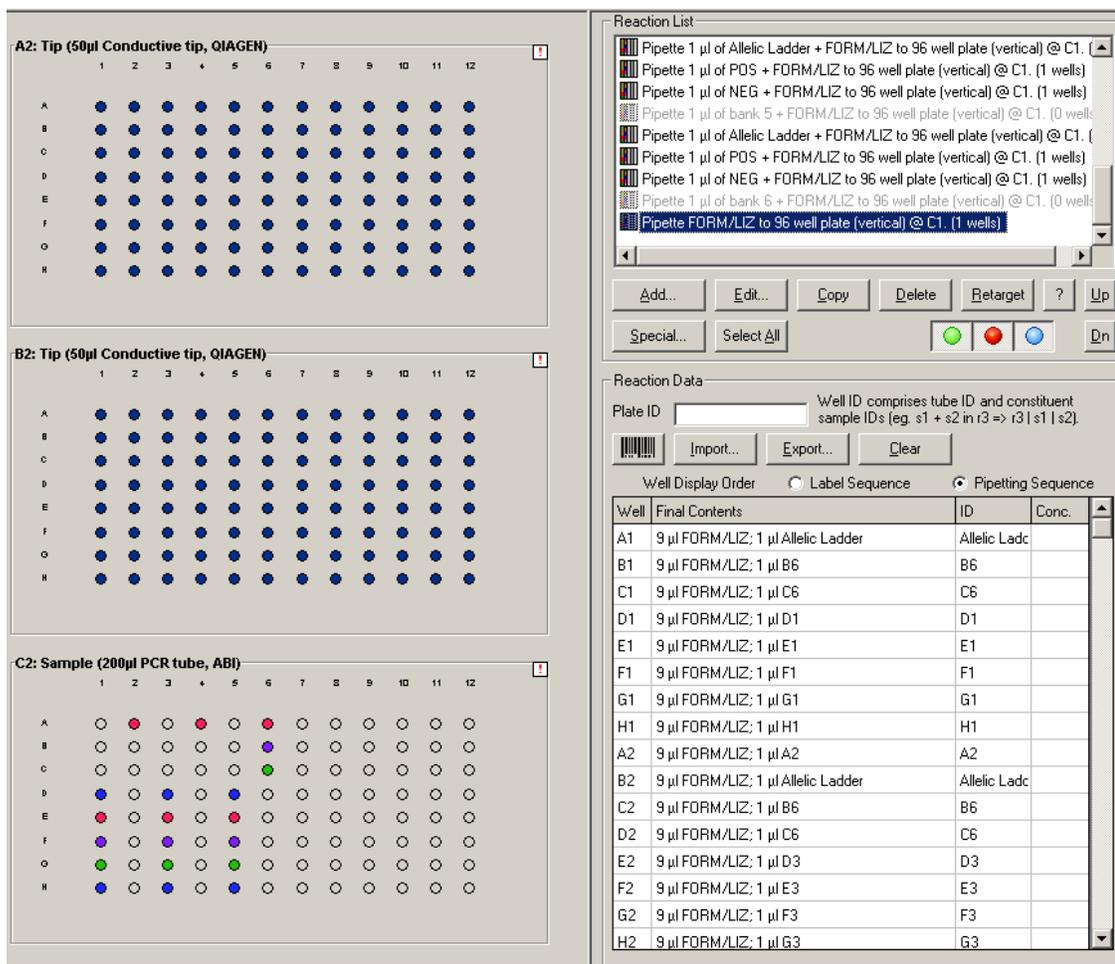
5.4.7 Using the Denature program on the 9700 thermal cycler, denature the reaction plate at 95 °C for 3 minutes.

5.4.8 Place the reaction plate immediately on an ice block for approximately 3 minutes or allow the thermal cycler to ramp down to 4 °C and let the plate remain on the thermal cycler at 4 °C for at least 3 minutes.

5.5 Operation of the QIAgility to Prepare Samples

5.5.1 Prepare the master mix as described in **5.4.1**. Combine the microcentrifuge tubes for each run into a single tube. Vortex the tube containing the combined master mix, then pulse spin in a microcentrifuge.

- 5.5.2 Turn on the QIAgility instrument. The switch is located on the lower back left corner of the instrument. Forensic Scientists shall open the QIAgility software by double clicking the “Shortcut to CE Setup.QAS” Protocol located on the computer desktop.
- 5.5.3 The worktable for CE (capillary electrophoresis) setup shall be set up as indicated on the screen. Holding the cursor over any colored well will give information about the contents of that well.
- 5.5.4 Within the CE Setup Protocol, left-click on Sample block C2. The right hand side of the screen will populate with information for that section. Click the Import button. The “Import Well Data” dialog box will appear.
- 5.5.5 Under Import File in the top left hand corner, click the browse  button. Locate the .CSV file and click open.
- 5.5.6 Click the Import button at the bottom of the screen to import the samples and then click “Finish.”
- 5.5.7 Left-click on Reaction block C1. In the Reaction List on the upper right hand side of the screen, highlight the “Pipette FORM/LIZ to 96 well plate” line located at the end of the list. For each run that contains blank wells, click “Copy.” If you only have one run, do not click Copy.



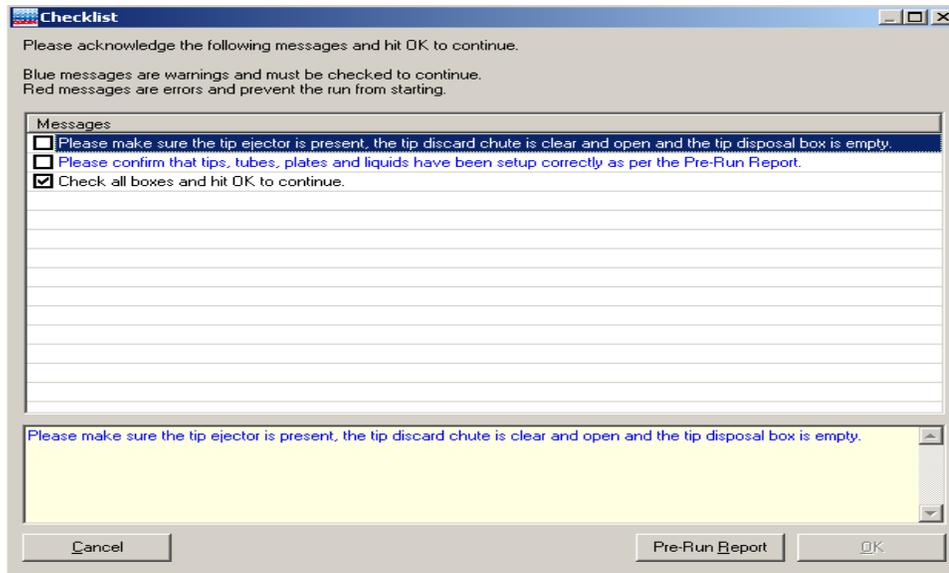
The screenshot displays the QIAgility software interface. On the left, there are three reaction blocks: A2 (Tip), B2 (Tip), and C2 (Sample). Each block shows a 96-well plate grid with colored dots indicating pipetting actions. On the right, the 'Reaction List' panel is active, showing a list of pipetting instructions. The last instruction, 'Pipette FORM/LIZ to 96 well plate (vertical) @ C1. (1 wells)', is highlighted. Below the list are buttons for 'Add...', 'Edit...', 'Copy', 'Delete', 'Retarget', '?', 'Up', 'Special...', and 'Select All'. At the bottom right, the 'Reaction Data' panel shows a table of well contents.

Well	Final Contents	ID	Conc.
A1	9 µl FORM/LIZ; 1 µl Allelic Ladder	Allelic Ladc	
B1	9 µl FORM/LIZ; 1 µl B6	B6	
C1	9 µl FORM/LIZ; 1 µl C6	C6	
D1	9 µl FORM/LIZ; 1 µl D1	D1	
E1	9 µl FORM/LIZ; 1 µl E1	E1	
F1	9 µl FORM/LIZ; 1 µl F1	F1	
G1	9 µl FORM/LIZ; 1 µl G1	G1	
H1	9 µl FORM/LIZ; 1 µl H1	H1	
A2	9 µl FORM/LIZ; 1 µl A2	A2	
B2	9 µl FORM/LIZ; 1 µl Allelic Ladder	Allelic Ladc	
C2	9 µl FORM/LIZ; 1 µl B6	B6	
D2	9 µl FORM/LIZ; 1 µl C6	C6	
E2	9 µl FORM/LIZ; 1 µl D3	D3	
F2	9 µl FORM/LIZ; 1 µl E3	E3	
G2	9 µl FORM/LIZ; 1 µl F3	F3	
H2	9 µl FORM/LIZ; 1 µl G3	G3	

- 5.5.8** Highlight a “Pipette FORM/LIZ to 96 well plate” line and click Up until it is below the first “bank” of samples that contains empty wells.
- 5.5.9** Click “Edit” and change the Number of Wells to correspond with the number of blank wells at the end of the corresponding run. Click “OK.”
- 5.5.10** Repeat steps **5.5.8** to **5.5.9** for each run with blank wells.
- 5.5.11** Highlight all entries that do not correspond with the runs being processed and click “Delete.” The message “This will delete multiple reactions from the reaction list. Continue?” will appear. Click “Yes.”
- 5.5.12** Grasp the handle and pull up to open the Instrument lid. Inspect the worktable to ensure that the tip holders and plate holders are in the position which appears on the screen.
- 5.5.13** Place the following materials on the worktable:

<u>Position</u>	<u>Reagent/Item</u>
N of Reagent block R1 (green well in software)	1.5 mL tube containing formamide/LIZ
D of Reagent block R1 (red well in software)	2 mL tube containing Ladder
Reaction block C1	Empty 96 well optical plate
Sample block C2	96 well optical plate containing amplified DNA samples

- 5.5.14** Check to ensure there are enough tips to process the run. To do this, left-click on either Tip Holder B1 or B2. On the right hand side of the screen it will indicate if there are enough tips. If there are not enough tips, insert a new set of tips into Tip Holder B1 or B2 (whichever is empty). Right click on the Tip holder that was just filled and choose “Set all tips on current plate to Available.” Close the instrument lid.
- 5.5.15** Click the green arrow  to start the run. The “Save as” dialog box will appear. Save the run file to the Forensic Scientist’s folder under C:\Program Files\QIAGEN\Agility\Data. The file shall be named in the following format: CE Setup_MMDDYYY_ABC (CE Setup_full date_Forensic Scientist initials). This file shall also be imported into the Forensic Scientist’s case record repository.
- 5.5.16** After the Forensic Scientist clicks “Save,” the pre-run Checklist screen will appear. If the run has been set up correctly, the checklist will not list any warnings or errors other than the default messages listed below. If other messages are listed, user intervention is required before the run can be started. After completing the listed tasks, select the boxes next to the warnings to continue. Click “OK.”



- 5.5.17** A run can be paused and aborted at any time by clicking the red X . Pauses will be logged in the post-run report. If the run is aborted, the instrument will discard the tip and return to its resting position. **Note:** A run will also be paused when the instrument lid is opened.
- 5.5.18** The instrument will complete the current operation and will then pause. A dialog box will appear indicating that the run was paused and whether the run will be continued or aborted. **Note:** The instrument does not stop immediately when the lid is opened. Therefore, wait until the Y-arm has stopped moving and the warning screen is displayed before continuing.
- 5.5.19** The instrument lid must be closed to abort the run. **Note:** An aborted run cannot be restarted from the point at which it was aborted.
- 5.5.20** If an error occurs or a warning is detected during a run, a dialog box which contains information about the error will appear. The Forensic Scientist will have 4 options: “Abort run,” “Retry operation,” “Ignore error,” and “Skip step.” Determine the error and the appropriate option.
- 5.5.21** Upon completion of each run, a support package is constructed and saved automatically within the program. This package contains the log file for the run and is used when reporting a problem to QIAGEN Technical Services. Click “OK.” **Note:** The Forensic Scientist will see a message concerning the support package only if an exception occurred during the run.
- 5.5.22** Upon completion of each run a Post-Run Report will also be displayed. This report shall be saved within the Forensic Scientist’s folder on the C drive (C:\Program Files\QIagility\Data) on the computer associated with the QIagility. Forensic Scientists shall also print a copy of this report using the print to pdf function to add to the case record repository.
- 5.5.23** Open the instrument lid and remove the optical plate from position C1. Cover this plate with the 96-well septa. Label the tray with the initials of the Forensic Scientist and date of run. Follow steps **5.4.6** through **5.4.8** above.

5.5.24 Remove the Ladder and Formamide/LIZ tubes from the worktable. Remove and re-cap the optical plate from position C2. Empty the biohazard box.

5.5.25 Close the software by clicking the X in the top right corner. Follow the prompts to shutdown the instrument. Click “Yes” to quit QIAgility. Click “Move to Safety Position (recommended).” Click “OK.”

5.5.26 Turn off the instrument.

5.6 Creating/Importing a Plate Record on the 3130XL

5.6.1 To create a plate record:

5.6.1.1 Open the 3130XL Data Collection Software.

5.6.1.2 In the task pane expand the GA Instruments folder.

5.6.1.3 In the task pane expand the ga3130xl folder.

5.6.1.4 Click Plate Manager in the task pane to open the plate manager window.

5.6.1.5 Click New to open the New Plate Dialog box.

5.6.1.6 Type the plate name.

5.6.1.7 Optional – Add a description for the plate.

5.6.1.8 Choose GeneMapper-Generic from the Application drop down menu.

5.6.1.9 Choose 96-Well from the Plate Type drop down menu.

5.6.1.10 Type SBI in the Owner Name box.

5.6.1.11 Type the initials of the Forensic Scientist in the Operator Name box.

5.6.1.12 Click OK to open the GeneMapper Plate Editor spreadsheet.

5.6.1.13 Plate Editor spreadsheet: Complete the plate Editor spreadsheet for the wells that are to be run.

5.6.1.14 In the Sample Name column, type a name for each of the samples. The value 100 is automatically displayed in the Priority column.

5.6.1.15 In the Sample Type column, type the sample type (Ladder, Positive Control, Negative Control or leave blank).

5.6.1.16 In the Results Group 1 column, select Basic_Fragment_Analysis.

5.6.1.17 In the Instrument Protocol 1 column, select HID_POP4_G5_CW.

5.6.1.18 Double check all entries and click OK.

5.6.2 To import a case record:

5.6.2.1 Using the 3130 Setup Worksheet, copy the information from the 3130XL set-up tab.

5.6.2.2 Paste the information into a notepad document and save the resulting .txt file into the plate setup folder for the appropriate instrument. Do not copy/paste empty horizontal wells as this prevents the file from importing.

5.6.2.3 In the Data collection window of the 3130XL, click on the Plate Manager.

5.6.2.4 Click import and select the previously saved file.

5.7 Linking the Reaction Plate

5.7.1 Place the reaction tray containing the denatured DNA onto the 3130XL, positioned correctly with the notch in the lower right corner.

5.7.2 In the task pane, expand the 3130XL folder.

5.7.3 In the task pane, expand the Run Scheduler folder.

5.7.4 Click Plate View in the task pane.

5.7.5 For Type of Search select Advanced from the drop down menu.

5.7.6 Enter the search criteria by choosing an option from the condition column and entering information describing the plate in the value columns. (For example: Status=Pending, Date Last Modified-Condition: =; Value 1:2008-01-08). Descriptive information may be entered into as few or as many categories as the user determines necessary to narrow the search for his/her plate.

5.7.7 Click Search.

5.7.8 The plate records that fit the search criteria appear in the window at the bottom of the screen. Highlight the plate record for the plate being linked by clicking on it.

5.7.9 Click the plate position indicator that corresponds to the plate to be linked.

5.7.10 Verify the plate has been linked.

5.7.10.1 The green run button on the toolbar is enabled, meaning the instrument is ready to run.

5.7.10.2 The plate position indicator for the linked plate changes from yellow to green.

5.7.10.3 The plate position (A or B) appears in the Link column.

5.7.11 Repeat steps **5.6.1** through **5.6.10** above to link a second plate, if applicable.

5.7.12 Click Run View in the task pane to view the run schedule.

5.8 Starting the Run

5.8.1 Click the green run instrument button to begin the scheduled runs.

5.8.2 A dialog box stating “You are about to start processing plates...” appears. Click “OK.”

5.8.2.1 The green arrow is not enabled until the plate is linked and back in the home position.
Leave the INSTRUMENT STATUS screen selected while the instrument is running.

5.9 Sequence for Restarting the Instrument

5.9.1 Close the 3130XL Data Collection Software by clicking Stop All on the Service Console.

5.9.2 Turn off the 3130XL.

5.9.3 Restart the computer (or shut down and restart).

5.9.4 Turn on the 3130XL. Do NOT proceed to the next step until the instrument’s status light appears as a steady green.

5.9.5 Open the 3130XL Data Collection Software.

5.10 Performing a Subsequent Run Using the Same Plate Record

5.10.1 The plate records may be reused by highlighting the plate in the Plate Manager window and clicking Edit. In the Plate Editor spreadsheet, select Edit, then choose Add Sample Run (Alt + A).

5.10.2 Select the samples that need to be injected again.

5.10.2.1 For a re-injection with the same plate, only the allelic ladder is required to be re-injected along with the sample(s).

5.10.2.2 For a plate re-setup (i.e., preparing samples on another plate or another portion of the same plate with a new aliquot of formamide and LIZ), only the allelic ladder is required to be re-injected along with the sample(s).

5.10.3 In the Results Group 2 column, select Basic_Fragment_Analysis.

5.10.4 In the Instrument Protocol 2 column, select HID_POP4_G5_CW.

5.10.5 Double check all entries and click OK. Note: The 3130XL software does not allow for the selection of a sample for re-injection if it was not run in the previous run.

5.10.6 Link the plate and start the run as described in **5.6** and **5.7**.

5.11 Maintenance of the QIAgility

5.11.1 Refer to the Forensic Biology Section Procedure for Calibration and Equipment Maintenance.

6.0 Limitations - Once a plate has been set up, it may be used for up to, but shall not exceed, 72 hours. Plates are stored at room temperature. After this time, the samples must be set up again either on another plate or in different wells if another injection is to be performed.

Temperature: The results from the 3130XL instrumentation can be affected by temperature changes. If the temperature in the room where the instrument is located is outside the range of 60 °C to 85 °C, the results may be affected and should be taken into account during analysis. If the results are affected (i.e., the data is incomplete), the DNA QC officer (or designee) shall take the affected instrument(s) off-line until the temperature is within range and the instrument has passed a QC check.

7.0 Safety

7.1 Formamide – consult MSDS sheet prior to use.

7.2 Heat – the thermal cycler is hot during the denaturation process; care should be used when removing the plate from the thermal cycler to place it in the ice block.

7.3 Pregnancy – Forensic Scientists who are currently pregnant shall avoid using this procedure.

8.0 References

Applied Biosystems™ 3130/3130XL Genetic Analyzers. User Bulletin. 2005 Applied Biosystems™. Part Number 4363787. Rev A. (or most recent revision)

Forensic Biology Section Procedure for DNA Casework Training

Forensic Biology Section Procedure for Calibration and Equipment Maintenance

9.0 Records

- Temperature chart recordings

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
12/31/2012	1	Original Document
02/15/2013	2	6.0 – added plate storage at room temperature
07/31/2013	3	5.1 - changed 4 case batch limit to sample limit of 30 samples at a time
09/25/2013	4	Header – added issuing authority titles; 4.0 – added QIAgility to equipment list; 5.4 – added manual to section heading; 5.5 – inserted new section for operation of QIAgility; 5.11 – added QIAgility maintenance