

Standard Operating Procedure Approval Page:

Date Implemented: 01 April 2006

Updated: Ashima Amin 16 June 2020
Name Date

Supersedes: Malania Wilson 03 April 2013
Name Date

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Changes made:

061620: Review

030813- Updated format. Updated center name and director. Renumber SOPs.
Previously CGSR-009 (archived) and BPC.009 (archived).

Note: *No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.*

Instrument Principles: The ABI Prism 3130xl Genetic Analyzer is an automated capillary electrophoresis system that can separate, detect, and analyze up to 16 capillaries of fluorescently labeled DNA fragments in one run.

Specimen Types: Cycle sequenced and cleaned products or PCR products for fragment analysis.

Required Reagents:

- | | |
|--|---------------------------|
| • MicroAmp Optical Plates (96 or 384-well) | Fisher 4314320 or 4343370 |
| • Septa (96 or 384-well) | Fisher 4315933 or 4315934 |
| • HiDi Formamide | Fisher 4311320 |
| • 10x Running Buffer | Fisher 402824 |
| • ILS Markers | |
| ○ 400HD | Fisher 402985 |
| ○ 500 | Fisher 401734 |
| ○ MapMarker1000 | BioVentures MM1000-Rox |

Quality Control: An internal size standard is run with every genotyping/fragment analysis sample to ensure that the instrument is functioning properly.

Safety: All employees are required to follow universal safety protocols when working with potentially infectious biohazardous materials. Safety procedures are outlined in the Emory University Bloodborne Pathogen Exposure Control Plan (see Employee Safety Notebook). Acrylamide is used to separate DNA fragments on the capillary sequencer and is a known neurotoxin. Care must be exercised when using the capillary sequencer.

Procedural Steps:

The amount of sample loaded on the ABI 3130xL for Sequencing or Fragment Analysis protocols will vary with each assay – see specific protocols for amount of sample to be analyzed.

Sample Preparation:

1. Prepare a loading cocktail by combining and mixing the Internal Lane Standard and Hi-Di™ formamide as follows:

$$[(0.5\mu\text{l ILS 600}) \times (\# \text{ samples})] + [(9.5\mu\text{l Hi-Di}^{\text{TM}} \text{ formamide}) \times (\# \text{ samples})]$$

Size Standard	0.5 μl per sample
Hi-Di Formamide	9.5 μl per sample
	10.0 μl per sample master mix

2. Vortex briefly to mix.
3. Add 10 μl of formamide/internal lane standard mix into each well.

Note: 3130xl runs in 16 well format (A1-H2, A3-H4, A5-H6, A7-H8, A9-H10, A11-H12). Partial plates should be plated in columns. Each well in each set of 16 should be filled. Hi-Di Formamide can be used in empty wells to complete a set of 16.

4. Add 1 μl of amplified sample to MicroAmp Optical 96-well or 384-well Reaction Plate. Cover with septa.

- Centrifuge plate briefly to remove air bubbles from the wells.
- Denature samples at 95°C for 5 minutes, then immediately chill on cold plate block, crushed ice, or in an ice-water bath for at least 5 minutes.

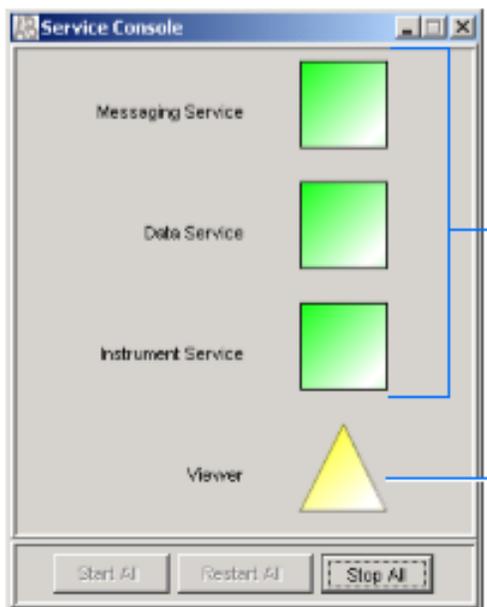
Set-up for Fragment Analysis:

If instrument is off:

- Turn computer on.
- Logon by control, alt, delete. Enter login and password.
- Wait for 1-2 minutes before using the power button (front left corner) to turn on the 3130xl.
- Wait until the green light on the 3130xl. If the green light does not come on after a few minutes and the yellow light remains on, reboot the computer, and repeat the startup process.

Note: If yellow light appears a second time or red light appears, shut instrument down and repeat one more time. If it still does not turn green, shut instrument down and contact supervisor.

- When the green light is on, double click on 3130xl Data Collection. This will prompt the viewer's screen and launch data collection to run the machine with.

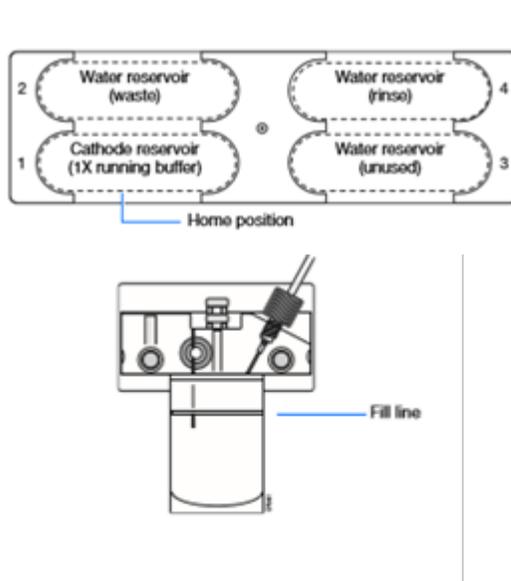


- Create a Sample Sheet. Remember to fill out the Project name and order in the top fields. Delete rows on the plate not being used. The only characters allowed besides numbers and letters are dashes, underscores, and periods. Please keep sample names less than 10 characters. Save as txt file. Templates for the various size standards can be found in EIGC.013_Appendixes.

7. Upload sample sheet to machine.

Starting the Run:

1. Press the "Tray" button on the front of the 3130xl machine.
2. DO NOT open the machine until the tray has stopped moving.
3. Change buffer (1x) and water in reservoir chambers and fill to fill line. Replace septa making sure it is flat. Change the buffer in the waste reservoir. These all should be done each day the machine is being run.



4. Make sure there is enough polymer for your samples. Each run of 16 uses about 100 μ l of polymer.
5. Assemble the plate and holder and load onto the empty plate location on the platform. When the plate is loaded, the plate location on the Data Collection screen will show a yellow plate in that location. Link your sample sheet to the plate location.

Note: Take care not to push on the machine too hard when placing assembly on machine.

6. Close the instrument door.
7. Press the green arrow when available to start the run. The machine will start blinking green.
8. Each run of 16 samples (except for those using MM1000) take about 45 min per run of 16 samples. MM1000 takes about 75 min per run of 16 samples.
9. When run is finished, the machine will come to a solid green light.
10. Data can be found in the following location on the E Drive.

E:\AppliedBiosystems\UDC\DataCollection\Data

11. FastQ files must be transferred using a windows computer.

Troubleshooting:

- For mechanical or software problems with the ABI 3130xl, refer to the Troubleshooting Guide in the ABI Prism® 3130xl Genetics Analyzer User's Manual.
See EIGC.013 Appendixes

Maintenance:

- Instrument maintenance
Daily Maintenance
 - Ensure the reservoir septa are firmly sealed and flat and that the plate assemblies are put together properly.
 - Change the buffer and water daily or before a new run.
 - Check the levels of polymer. 100µl is needed per run.
 - Check for bubbles in the polymer block and flush bubbles out of needed. – Refer to the User's Manual for instructions.

Monthly/As Needed Maintenance

- Clean syringes. – Refer to the User's Manual for instructions.
- Clean water and buffer reservoirs with warm water
- Clean upper and lower polymer blocks
- Backup data
- Database Cleanup

As needed Maintenance

- Change array – Refer to the User's Manual for instructions.
- Change polymer- Refer to the User's Manual for instructions.