**ABI 3500 Genetic Analyzer Procedure**

1. **PRINCIPLE:**

The Applied Biosystems 3500/3500xL Genetic Analyzer is an automated 8 and/or 24 capillary instrument designed for a wide range of sequencing and fragment analysis applications.

1. **PROCEDURE FOR OPERATION:**
   1. Starting the instrument:
      1. Verify that the instrument is connected to the appropriate power supply.
      2. Inspect the instrument interior. Ensure that the oven door is closed, and no objects are left inside the instrument.
      3. Close the instrument door.
      4. Turn on the instrument. Press the power on/off button on the front of the instrument and wait for the green status light to turn on.
         1. Press the Tray button on the outside of the instrument to bring the autosampler to the forward position. Wait until the autosampler stops at the forward position.
         2. **Note**: when the door is open, the yellow status light blinks while the instrument performs self-check and the autosampler adjusts.
   2. Starting the computer:
      1. Power on the computer.
      2. Power on the monitor.
      3. In the Log On to Windows dialog box: enter user name and password. Click OK. Wait until the computer finishes booting.
   3. Launching the application:
      1. Launch the Daemon: Start > Programs > Applied Biosystems > 3500 > Daemon.
      2. Launch the server monitor: Start > Programs > Applied Biosystems > 3500 > Server Monitor.
         1. This will take approximately 2 minutes.
         2. When Server Monitor setup is complete, the icon in the shape of an hourglass will disappear and a checkmark icon appears, indicating that the server monitor has started, and the services are loaded.
      3. Launch the 3500 application: Start > Programs > Applied Biosystems > 3500.
      4. After you launch the application, the 3500 Series Data Collection software splash screen appears. This will remain active for a few seconds until the 3500 Log in dialog box opens. After the splash screen disappears, one of the following occurs:
         1. The Dashboard is displayed.
         2. The Login dialog box is displayed.
   4. Logging in:
      1. Enter user name and password into the 3500 Log in Dialog box.
      2. Click OK. The splash screen will reappear before the software launches and the Dashboard appears.
   5. Restarting the instrument and computer:
      1. To Restart the instrument:
         1. Close out of the application.
         2. Power off the computer.
         3. Turn off the instrument. Press the power on/off button on the front of the instrument.
         4. Wait 2 minutes.
         5. Power on the computer, but do not log in.
         6. Turn on the instrument. Press the power on/off button on the front of the instrument and wait for the green status light to turn on.
         7. Log on to the computer and wait for the green check marks to appear signifying all the appropriate software checks have passed, including the 3500 Daemon.
      2. For Long-Term Shutdown:
         1. Use the instrument Shutdown wizard for short and long-term shutdown.
         2. From the Maintenance Wizards screen, click “Shutdown the Instrument”.
            1. **Note**: instrument shutdown wizard takes 60 minutes to complete.
         3. Follow the prompts in the instrument shutdown wizard window.
         4. Perform the appropriate shutdown procedure based on the table found on page 253 of the ABI 3500/3500xL Genetic Analyzer User Guide.
   6. Additional information from the ABI 3500/3500xL Genetic Analyzer User Guide:
      1. Instrument and software descriptions: refer to Chapter 1.
      2. Starting the system: refer to Chapter 2.
      3. Set up and running: refer to Chapter 3.
      4. Reviewing Results: refer to Chapter 4.
      5. Calibration and performance checks: refer to Chapter 5.
      6. Managing library resources: refer to Chapter 6.
      7. Using the security, audit, and E-sig functions (SAE module): refer to Chapter 7.
      8. Application reagents and run modules: refer to Appendix A.
      9. Sequencing analysis: refer to Appendix B.
      10. Fragment analysis: refer to Appendix C.
      11. Remote Auto-analysis setup: refer to Appendix D.
      12. Safety: refer to Appendix F.
2. **QC AND MAINTENANCE:**
   1. Before each run:
      1. Check consumables on the Dashboard.
      2. Visually inspect level of fluid inside the anode and cathode buffer containers.
      3. Ensure the plate assemblies are properly assembled.
      4. Ensure both the plate assemblies and the cathode buffer container are positioned on the plate deck correctly.
      5. Ensure the array locking lever on the capillary array is secured.
      6. Check for bubbles in the pump block and channels.
      7. Check the loading-end header to ensure that the capillary tips are not crushed or damaged.
      8. Ensure the pump block is in the pushed back position.
      9. Clean the instrument surfaces of dried residue, spilled buffer, or dirt.
      10. Check for leaks or dried residue around the Buffer-Pin Valve, check valve, and array locking lever.
   2. Weekly:
      1. Check the storage conditions of the used arrays to ensure the array tip is covered in the reservoir.
      2. Run the Wash Pump and Channels wizard.
      3. Use a lab wipe to clean the anode buffer container valve pin assembly on the polymer delivery pump.
      4. Restart the computer and the instrument. (See section II. E. above)
   3. Monthly:
      1. Flush the pump trap.
      2. Empty the condensation container and the water trap waste container.
      3. Replace cathode buffer container septa.
      4. Run a performance check for sequencing and fragment analysis.
      5. Clean the autosampler.
      6. Clean the drip tray.
      7. Check disk space on computer and defragment the hard drive, as needed.
   4. Annual: Annual planned maintenance tasks are performed by Applied Biosystems according to the service contract.
      1. The performance of all components is verified upon installation.
      2. Performance verification is performed by the vendor after maintenance at the contracted time.
   5. As-needed:
      1. Change the array.
      2. Spatial Calibration – must be completed when array is changed.
      3. Spectral Calibration – must be completed when array is changed for both sequencing and fragment analysis.
      4. Change the tray.
      5. Remove dried polymer from the capillary tips with a lint-free wipe moistened with deionized water.
      6. Archive and purge library objects.
   6. Additional information on maintaining the instrument can be found in chapter 8 of the ABI 3500/3500xL Genetic Analyzer User Guide.
3. **TROUBLESHOOTING:**
   1. For troubleshooting, refer to Appendix E of the ABI 3500/3500xL Genetic Analyzer User Guide and/or the Reference and Troubleshooting Guide.
4. **CONTACT INFORMATION:**
   1. Thermo Fisher Scientific

Technical Support US

Phone: 1-800-955-6288

Website: http://www.thermofisher.com/us/en/home/technical-resources/contact-us.html

1. **REFERENCES:**
   1. Applied Biosystems 3500/3500xL Genetic Analyzer User Guide.
   2. GeneMapper® Software Reference and Troubleshooting Guide Version 4.1.
   3. <https://www.appliedbiosystems.com>
2. **REVISIONS:**
   1. 4/13/2016 Edit of II.E Shutting down instrument to differentiate between Restarting the computer and Long-Term Shutdown.
   2. 1/15/2020: Updated footer to reflect new laboratory name.
   3. 11/18/2022: Clarified calibrations for both sequencing and fragment analysis.