Procedure for Instrument and Equipment Quality Control

1.0 Purpose - To specify the required elements for the performance check, verification and/or maintenance of equipment used by the DNA Database Section as performed by the DNA Database Quality Control Officer or designee(s).

2.0 Scope – This procedure applies to equipment used by the DNA Database Section.

3.0 Definitions

- **ABI -** Applied Biosystems (Life Technologies).
- **Critical equipment –** Equipment that requires validation, performance check or verification. This is required prior to initial use by the DNA Database Section or as specified in this procedure. Critical equipment includes ABI 3500xL instruments, ABI 9700 thermal cyclers, bulb thermometers, balances, pipettes, temperature chart recorders/data loggers (or equivalent), biosafety cabinets, chemical fume hoods, laminar flow benches, Qiagen BioRobot®, heat blocks, and refrigerators/freezers that contain critical reagents.
- **FA -** Forensic Advantage.
- **Purified dH_2O -** Water that has been deionized and then filtered to the extent that no particle larger than a nanometer is present in the water.
- **NIST Traceable -** Sample, equipment or material(s) that has been verified against a National Institute of Standards and Technology certified sample, equipment or material(s).
- **QC Check -** Quality control assessment of materials or instrumentation prior to use within the DNA Database Section.
- **QCO -** Refers to the DNA Database Quality Control Officer or designee(s).
- **Water run** – A water run is a BioRobot run in which instrument components are activated and used and system liquid is run through the instrument.

4.0 Equipment, Materials and Reagents

- NIST traceable digital thermometer
- Ice Shaver/crusher
- Purified dH_2O
- dH_2O
- NIST traceable weight set
- Centrifuge
- Syringe
- Septa
- Conditioning Reagent
- POP-4
- Anode Buffer Container (ABC)
- Cathode Buffer Container (CBC)
- Wipes (delicate task wipes)
- 96 well reaction trays
- Pipettes
- Pipette tips

- Matrix standard set DG4850 to automatically analyze the five different colored fluorescent dye-labeled samples in a single capillary for the 3500xL
- PowerPlex® Fusion reagents
- Hi-Di Formamide
- Eutechnics 4500 probe (or equivalent)
- Microplate reader
- Disinfecting solutions including DeconQuat 100, RBS-35, Quaternary disinfectant cleaner, 10 % bleach, alcohol

5.0 Procedure

5.1 3500xL Genetic Analyzers

5.1.1 Maintenance to be Performed Prior to Each Run: Refer to the Checking Consumable Status and Replenishing Consumables Section of the DNA Database Section Procedure for Use of the 3500xL Genetic Analyzer.

5.1.2 Weekly Maintenance

5.1.2.1 Weekly Maintenance shall be performed by the QCO on the first day of the week that the instrument is used. Each item will be prompted in the **Maintenance Notifications** list in the **Dashboard**. As each item is completed, it shall be marked as complete by clicking on the green check mark that appears next to the notification. Documentation of such maintenance shall be retained as described in **5.1.5**.

5.1.2.2 At the 3500xL instrument, unlink any plates currently on the instrument. If plates need to be unlinked from the instrument, select **Library** from the menu bar. Select the yellow **Main Workflow** button on the left of the screen; select **Load Plates for Run** in the **Run Instrument** menu. Before selecting **Unlink** for a plate, ensure the plate is not currently in process. Select **Unlink** for each plate that is linked.

5.1.2.3 Restart the System

5.1.2.3.1 Close the 3500xL Data Collection Software.

5.1.2.3.2 Turn off the 3500xL.

5.1.2.3.3 Turn off the computer.

5.1.2.3.4 Turn on the computer. Log in to the Instr-User profile. A 3500 service monitor will appear in the lower right corner. Before proceeding to the next step, ensure each item in the service monitor is listed as Y and that the overall status is listed as "3500 Services Loaded: Y." When the associated icon has a green check mark, proceed to the next step.

5.1.2.3.5 Turn on the 3500xL. Do not proceed to the next step until the instrument's status light appears as a steady green.

5.1.2.3.6 Open the 3500xL Data Collection Software and log in with user name.

5.1.2.4 Water Wash

5.1.2.4.1 From the **Dashboard**, select **Maintain Instrument**.

5.1.2.4.2 Select the blue **Maintenance Wizards** button on the left of the screen.

5.1.2.4.3 From the **Maintenance Wizards** screen, select **Wash Pump Channels**.

5.1.2.4.4 Follow the Wash Wizard steps to complete a water wash.

5.1.2.4.5 When placing conditioning reagent on the instrument, check the expiration date on the label to make sure it is not expired prior to use.

NOTE: The RFID label must be facing the instrument to ensure that the RFID information is read accurately by the instrument.

5.1.2.4.6 A partially used polymer may be returned to the instrument after a water wash is complete if the polymer has not been on the instrument for 7 calendar days and has not reached its expiration date.

5.1.2.5 Change the Anode Buffer Container

5.1.2.5.1 The use of an Anode Buffer Container (ABC) shall not extend beyond 7 calendar days.

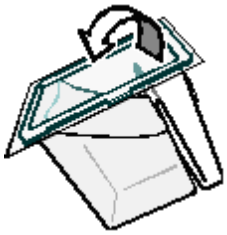
5.1.2.5.2 Remove an unopened ABC from storage.

5.1.2.5.3 Check the expiration date on the ABC label to make sure it is not expired prior to or during intended use.

5.1.2.5.4 Allow the refrigerated ABC to equilibrate to ambient temperature prior to first use. Do not remove the seal.

5.1.2.5.5 Verify that the seal is intact.

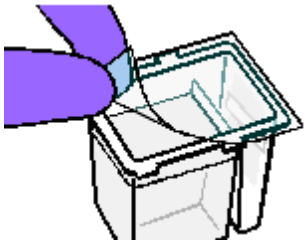
5.1.2.5.6 Tilt the ABC slightly (as shown in the figure below) to make sure most of 1X buffer is in the larger side of the container. There should be less than 1 mL of 1X buffer remaining in the smaller side of the container.



5.1.2.5.7 Verify that the buffer is at or above the fill line.

5.1.2.5.8 Remove the current ABC from the 3500xL. Pour contents of the reservoir down the sink. Discard the reservoir in the biohazard box.

5.1.2.5.9 Peel off the seal at the top of the ABC.



5.1.2.5.10 Place the ABC into the Anode end of the instrument, below the pump. Ensure the electrode is in the larger side of the container.

NOTE: The RFID label must be facing the instrument to ensure that the RFID information is read accurately by the instrument.

5.1.2.5.11 When all desired weekly maintenance is complete, close the instrument door to re-initialize.

5.1.2.5.12 Click **Refresh** from the **Dashboard** to update the screen.

5.1.2.5.13 Check the **Consumables Information** section of the **Dashboard** for updated consumable statuses.

5.1.2.6 Change the Cathode Buffer Container

5.1.2.6.1 The use of a Cathode Buffer Container (CBC) shall not extend beyond 7 calendar days.

5.1.2.6.2 Remove an unopened CBC from storage.

5.1.2.6.3 Check the expiration date on the CBC label to make sure it is not expired prior to or during intended use.

5.1.2.6.4 Allow the refrigerated CBC to equilibrate to ambient temperature before use.

5.1.2.6.5 Verify that the seal is intact.

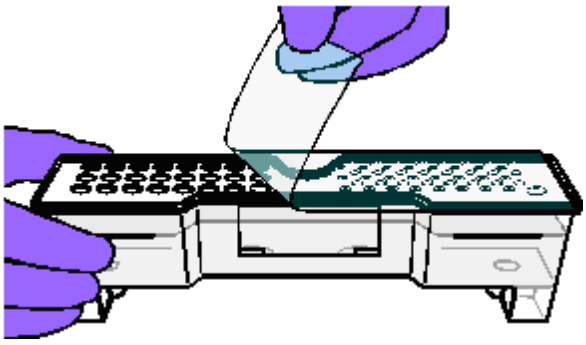
5.1.2.6.6 Tilt the CBC back and forth gently and carefully to ensure that the buffer is evenly distributed.

5.1.2.6.7 Verify that the buffer is at or above the fill line.

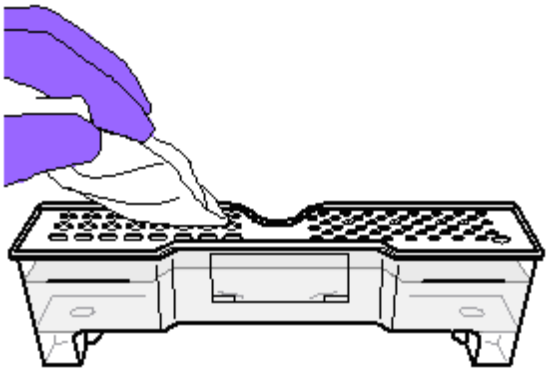
5.1.2.6.8 At the 3500xL, push the tray button and wait for the autosampler tray to come to the front of the instrument. After it comes to a complete stop, open the door.

5.1.2.6.9 Remove the current CBC. Squeeze along the front tab of the CBC to allow the container to release from the autosampler tray. Remove septa. Pour contents of the reservoir down the sink. Discard the reservoir and septa in the biohazard box.

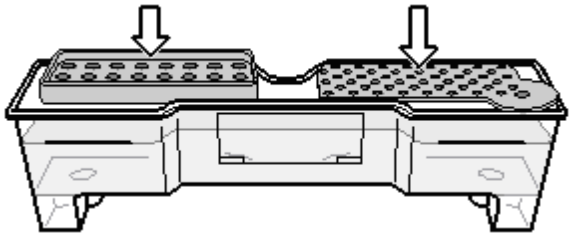
5.1.2.6.10 When ready to install the CBC, place the container on a flat surface and peel off the seal.



5.1.2.6.11 Wipe off any buffer on the top of the CBC with a lint-free cloth. Ensure that the top of the container is dry.



5.1.2.6.12 Place the appropriate septa on both sides of the CBC.

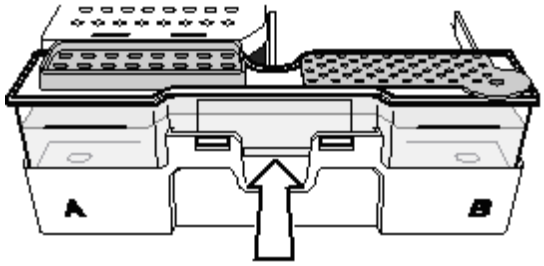


5.1.2.6.12.1 Align the buffer septa (the part that is symmetrical) over the 24 holes of the CBC.

5.1.2.6.12.2 Push the septa lightly into the holes to start and then push firmly to seat the septa.

5.1.2.6.13 Install the CBC on the autosampler.

NOTE: When properly installed, it will click on the autosampler as the tabs are snapped in place.



5.1.2.6.14 When all desired weekly maintenance is complete, close the instrument door to re-initialize.

5.1.2.6.15 Click **Refresh** from the **Dashboard** to update the screen.

5.1.2.6.16 Check the **Consumables Information** section of the **Dashboard** for updated consumable statuses.

5.1.2.7 Clean Anode Buffer Cup Pin-Valve Assembly on Polymer Delivery Pump

5.1.2.7.1 Wipe off the anode buffer cup pin-valve assembly using a lint-free tissue that is dampened with water.

5.1.3 Monthly Maintenance

5.1.3.1 By the end of the first Monday (or first day of the first full week) of each month, monthly maintenance shall be performed by the QCO. Each item will be prompted in the **Maintenance Notifications** list in the **Dashboard**. As each item is completed, it shall be marked as complete by clicking on the green check mark that appears next to the notification. Documentation of such maintenance shall be retained as described in **5.1.5**.

5.1.3.2 Disk Space

5.1.3.2.1 To ensure available disk space on the 3500xL, run data shall be removed from the 3500xL computers at least quarterly.

5.1.3.2.2 Plate maps from the previous month shall be cleared from each 3500xL computer. From the **Dashboard**, select **Library**. Select **Plates** on the left of the screen. Double click the Run Date header to sort the plates by run date. Highlight the plates from the previous month. Right click and select **Delete**. When the dialog box pops up asking if you are sure you want to delete your selection, select **Yes**.

5.1.3.3 Defragment the Computer Hard Drive

5.1.3.3.1 Purpose: The fragmentation of files decreases the performance of both the Data Collection software and the computer operating system. Programs take a longer time to access files by performing multiple search operations of the fragments.

5.1.3.3.2 Go to the computer's Start menu.

5.1.3.3.3 Select **Programs**.

5.1.3.3.4 Select **Accessories**.

5.1.3.3.5 Select **System Tools**.

5.1.3.3.6 Select **Disk Defragmenter** and follow the prompts.

5.1.3.4 Flush the Water Trap (Pump Trap)

5.1.3.4.1 Fill the supplied 20 mL, plastic Luer lock syringe with distilled or deionized water. Expel any bubbles from the syringe.

5.1.3.4.2 Open the Luer fitting by grasping the body of the fitting and turning it counterclockwise approximately one half turn to loosen. Hold the fitting with one hand while threading the syringe onto the fitting in a clockwise direction with the other hand.

5.1.3.4.3 Take approximately 30 seconds to flush 5 mL of either distilled or deionized water through the trap.

5.1.3.4.4 Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

5.1.3.4.5 Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.

5.1.3.4.6 Empty the waste trap container and the condensation container. The waste trap container is to the right of the pump block.

5.1.3.5 Clean Autosampler

5.1.3.5.1 Press the Tray button on the front of the instrument to move the autosampler to the forward position.

5.1.3.5.2 Wipe off any liquid on or around the autosampler using a lint-free tissue.

5.1.3.6 Clean Drip Tray

5.1.3.6.1 Clean out the drip tray with deionized water (or ethanol (absolute)) and lint-free tissue.

NOTE: The drip tray can be removed.

5.1.4 As Needed Maintenance

5.1.4.1 Replenish Polymer

5.1.4.1.1 The use of a POP-4 pouch shall not extend beyond 7 calendar days. When placing POP-4 on the instrument, check the expiration date on the label to ensure it has not expired prior to or during intended use.

5.1.4.1.2 From the **Dashboard**, select **Maintain Instrument**.

5.1.4.1.3 Select the blue **Maintenance Wizards** button on the left of the screen.

5.1.4.1.4 From the **Maintenance Wizards** screen, click **Replenish Polymer**.

5.1.4.1.5 Follow the Replenish Polymer Wizard steps to replenish the polymer.

5.1.4.1.6 Refresh the **Consumables Information** section of the **Dashboard** for an updated status of the polymer.

5.1.4.1.7 Add the POP-4 pouch change to the schedule.

5.1.4.1.7.1 From the **Dashboard**, select **Maintain Instrument**.

5.1.4.1.7.2 Select **Schedule** on the left of the screen.

5.1.4.1.7.3 Select **Create** from the menu bar at the top of the screen.

5.1.4.1.7.4 Fill the fields as follows.

5.1.4.1.7.4.1 **Title**: POP-4 Change

5.1.4.1.7.4.2 **Schedule Starts On**: Date POP-4 change occurred

5.1.4.1.7.4.3 **Priority**: High

5.1.4.1.7.4.4 **Repeat**: Never

5.1.4.1.7.4.5 In the **Description** field, list the date the POP-4 was changed and the lot number of the POP-4 along with the date and initials of the analyst who completed the task. If any other notes are needed, they may also be added to the **Description** field.

5.1.4.1.7.5 Select **OK**.

5.1.4.1.8 The item will add to the **Maintenance Notifications** list in the **Dashboard**. When the notification appears, it shall be marked as complete by clicking on the green check mark that appears next to the notification. Documentation of such maintenance shall be retained as described in **5.1.5**.

5.1.4.2 Remove Bubbles from the Polymer Pump

5.1.4.2.1 From the **Dashboard**, select **Maintain Instrument**.

5.1.4.2.2 Select the blue **Maintenance Wizards** button on the left of the screen.

5.1.4.2.3 From the **Maintenance Wizards** screen, click **Remove Bubbles**.

5.1.4.2.4 Follow the Bubble Remove Wizard steps to remove bubbles from the polymer pump fluid path.

5.1.4.2.5 Refresh the **Consumables Information** section of the **Dashboard** for an updated status of the polymer.

5.1.4.3 Shutdown the Instrument – This wizard prepares the instrument for an extensive period of disuse (greater than 2 weeks). In this procedure, the capillary array is removed and a conditioning reagent pouch is placed on the instrument.

5.1.4.3.1 From the **Dashboard**, select **Maintain Instrument**.

5.1.4.3.2 Select the blue **Maintenance Wizards** button on the left of the screen.

5.1.4.3.3 From the **Maintenance Wizards** screen, click **Shutdown the Instrument**.

5.1.4.3.4 Follow the Instrument Shutdown Wizard steps.

5.1.4.3.5 Ensure that the Data Collection Software, computer, and instrument are all off.

5.1.5 Documentation of Weekly Maintenance, Monthly Maintenance, and POP-4 Changes

5.1.5.1 All maintenance actions are recorded in the **Notification Log**. For each month, a record of weekly maintenance, monthly maintenance, and as needed POP-4 changes shall be listed in a monthly **Notification Report** and maintained in the DNA Database Section. From the **Dashboard**, click the yellow **Maintain Instrument** button. Select **Notifications Log** in the **Planned Maintenance** menu on the left of the screen. Select **View Notification Log Report**. Enter the desired date range the report should cover. Select **OK**. At the top of the screen, select **Print** and then the **Print Report** option. In the printer dialog box, select **CutePDF Writer** and print the report as a .pdf file.

5.1.6 Changing the Capillary Array – When a capillary has repeated ILS (i.e., size standard) failure, the bases of the alleles in samples broaden (monitor closely once the array usage approaches 160 injections), the background noise in the electropherograms becomes repeated and excessive (based upon the training and experience of the DNA Database Forensic Scientists), the array has reached its maximum of 160 runs, or the array has reached its expiration date, the array shall be replaced. DNA Database Forensic Scientists shall notify the QCO and Technical Leader if they observe any of the above-mentioned scenarios.

5.1.6.1 From the **Dashboard**, select **Maintain Instrument**.

5.1.6.2 Select the blue **Maintenance Wizards** button on the left of the screen.

5.1.6.3 From the **Maintenance Wizards** screen, click **Install Capillary Array**.

5.1.6.4 Follow the Install Capillary Array Wizard steps to install a capillary.

5.1.6.5 Refresh the **Consumables Information** section of the **Dashboard** for an updated status of the capillary array.

5.1.6.6 Perform both a Spatial and Spectral Calibration (see **5.1.10** and **5.1.11)**.

5.1.7 Service and/or Repair

5.1.7.1 Repair: If a 3500xL becomes inoperable due to a need for repair by the manufacturer, the QCO shall notify the Section via email as well as by placing a notice on the specific instrument that it is not available for use. The QCO shall also notify the Technical Leader and the manufacturer that repair is needed.

Performance QC Check: If a 3500xL instrument is removed from use due to repair, a post maintenance QC check on the instrument shall be performed by the QCO prior to its return to use in the Section.

5.1.7.2 Annual Preventative Maintenance: The 3500xL Genetic Analyzers shall have preventative maintenance performed annually by the manufacturer.

5.1.7.2.1 Refer to the maintenance reports provided by the vendor for specific calibrations, verifications, and tests performed during the annual preventative maintenance.

5.1.7.2.2 Performance QC Check: After preventative maintenance, each 3500xL shall have a post maintenance QC check performed by the QCO.

5.1.7.3 Solid State Laser Failure: the solid state laser inside the 3500xL instrument excites the dyes attached to the DNA fragments in the capillaries. When the laser fails, no fluorescent data is generated across all color channels.

5.1.7.3.1 If the solid state laser fails on a 3500xL instrument, the QCO shall proceed as described in **5.1.7.1**. Only the manufacturer (via field engineer) may replace the laser.

5.1.7.3.2 Once the laser has been replaced, the QCO shall perform both spatial and spectral calibrations (see **5.1.10** and **5.1.11**) if not already performed by the manufacturer during laser replacement.

5.1.7.3.3 The QCO shall then perform a Post Maintenance Performance QC Check on the instrument (see **5.1.8**).

5.1.7.3.4 Additionally, a sensitivity study shall be performed on the instrument by the QCO at the direction of the Technical Leader.

5.1.7.3.5 After all conditions set in **5.1.7.3.1** through **5.1.7.3.4** are satisfied, the Technical Leader shall release the instrument for use in the Database Section. The QCO shall notify the Section by email and by placing a notice on the specific instrument that it is again available for use.

5.1.7.3.6 All documentation pertaining to a laser failure shall be retained as described in **5.1.7.4**.

5.1.7.4 Documentation of any repair or annual preventative maintenance, as well as subsequent QC checks shall be retained in the Section.

5.1.8 Post Maintenance Performance QC Check: Before any validated 3500xL shall be used by DNA Database Forensic Scientists in the DNA Database Section after repair or maintenance, a Performance QC check shall be performed by the QCO. Additionally, this check shall be performed after the instrument has been taken offline due to temperature fluctuations in the room. This QC check shall be performed as follows:

5.1.8.1 A NIST-Traceable Standard (NIST-TS) and associated Reagent Blank (see DNA Database Section Procedure for DNA Reagent Quality Control) shall be amplified using the current amplification kit along with the appropriate amplification controls.

5.1.8.2 Items listed in **5.1.8.1** shall be electrophoresed on the 3500xL at the 18 second injection protocol.

5.1.8.3 The NIST-TS, positive amplification control(s), and allelic ladder must provide the expected allele calls at all the loci tested.

5.1.8.4 All testing negatives (Reagent Blank(s), amplification negative control(s)) must be free of any peaks above the analytical threshold used in the Section.

5.1.8.5 If either **5.1.8.3** or **5.1.8.4** are not satisfied (for reasons other than instrument failure, known artifacts), then the QCO may retest (reelectrophorese or reamplify) the samples one more time. If **5.1.8.3** or **5.1.8.4** are not satisfied by the retest, the DNA TL shall be notified and shall determine the appropriate course of action.

5.1.8.6 The QCO shall notify the Section via email, as well as by placing a notice on the specific instrument, that it is available for use once the QC check is completed.

5.1.8.7 The QCO shall document the testing performed and retain such information in the Section.

5.1.9 Yearly QC Requirement

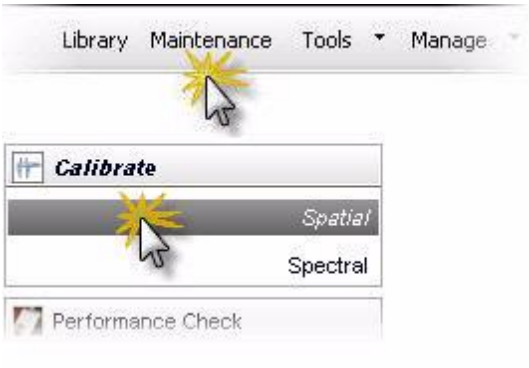
5.1.9.1 NIST SRM/NIST Traceable Standard: A NIST SRM, or NIST traceable standard shall be run on each 3500xL annually. The amplifications shall be set up manually and shall be electrophoresed at the 18 second injection time. See the DNA Database Section Procedure for DNA Reagent Quality Control.

5.1.10 Spatial Calibrations

5.1.10.1 Purpose: Establish a relationship between the signal emitted by each capillary and the position where that signal falls and is detected by the CCD camera.

5.1.10.2 A spatial calibration shall be performed when the capillary array is removed or replaced, the detector cell door is opened or the detection cell is moved, or the instrument is moved. The spatial shall be performed by the QCO.

5.1.10.3 Access the **Spatial Calibration** screen. From the **Dashboard**, select **Maintenance** and then select **Spatial Calibration** in the navigation pane.



5.1.10.4 Select **No Fill**, or select **Fill** to fill the array with polymer before starting the calibration.

5.1.10.5 Select **Perform QC Checks** for the system to check each capillary against the specified range for spacing and intensity. During the calibration, the software calculates:

Attribute	Calculation	Threshold
Average peak height	$\frac{\text{Sum of all peak heights}}{\text{Number of peaks}}$	24-cap: 3000 RFU
Uniformity (peak height similarity)	$\frac{\text{Standard deviation}}{\text{Average peak height}}$	0.2
Capillary spacing	Max spacing – Min spacing	2 pixels

5.1.10.6 Select **Start Calibration**. The display updates as the run progresses. If the average of any of the QC values exceeds the threshold, a Spatial QC Check error message is displayed. Click **OK** and rerun the spatial calibration.

5.1.10.7 Evaluate the spatial calibration profile to ensure that you see:

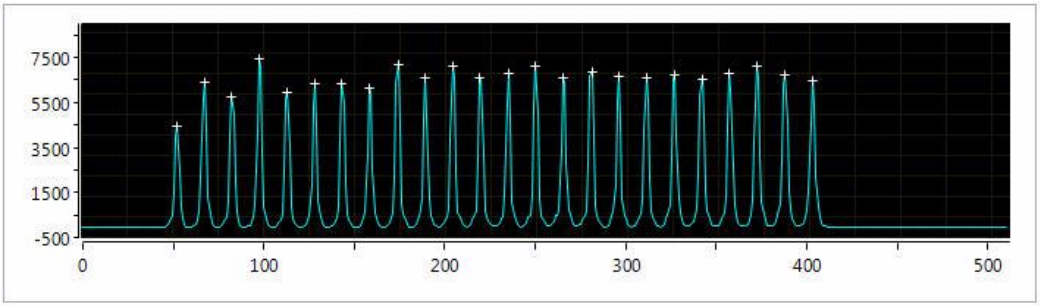
5.1.10.7.1 One sharp peak for each capillary. Small shoulders are acceptable.

5.1.10.7.2 One marker (+) at the apex of every peak. No off-apex markers.

5.1.10.7.3 An even peak profile (all peaks about the same height).

5.1.10.7.4 Spacing should be between 13 and 16.

NOTE: If any peaks are lower than usual for the instrument or the heights drastically slope up or down, repeat the calibration with a fill.



5.1.10.8 If the results meet the criteria above, click **Accept Results**. If the results do not meet the criteria above, click **Reject Results** and run a new spatial calibration or refer to the Applied Biosystems 3500/3500xL Genetic Analyzer User Guide for troubleshooting information.

5.1.10.9 View and print a spatial calibration report.

5.1.10.9.1 Click **View Spatial Calibration Report**.

5.1.10.9.2 To print the report, click **Print**; then select **Print Report**.

5.1.10.9.3 In the printer dialog box, select **CutePDF Writer** and print the report as a .pdf file.

NOTE: After performing a calibration, the calibration report can be saved electronically for record keeping. The software does not save historical calibration results. Only the most recent spatial calibration is maintained in the software.

5.1.10.9.4 Close the report.

5.1.10.9.5 Spatial Calibration Reports shall be maintained in the Section.

5.1.11 Spectral Calibrations

5.1.11.1 Spectral calibration creates a matrix that corrects for the overlapping fluorescence emission spectra of the dyes. Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes. The goal of multicomponent analysis is to correct for spectral overlap and minimize the presence of artifacts, such as spectral pull-up, in the data.

5.1.11.2 A spectral calibration shall be performed if any of the following conditions occur: the capillary array is changed, the instrument is moved, the laser or CCD camera has been realigned/replaced by a service engineer, an increase in pull-up and/or pull-down peaks is observed, or the capillary array length or polymer type is changed.

5.1.11.3 Prepare the instrument.

5.1.11.3.1 If you have not already done so, perform a spatial calibration.

5.1.11.3.2 In the **Dashboard**, check the **Consumables Information**. Ensure that consumables have not expired and that adequate injections remain for consumables.

5.1.11.3.3 Ensure that the buffer levels are at the fill lines.

5.1.11.3.4 Set the oven temperature to 60 °C; then select **Start Pre-Heat**.

5.1.11.3.5 Check the pump assembly for bubbles and run the Remove Bubble wizard if needed.

5.1.11.4 Remove the 5C matrix mix from the freezer (-30 °C to -10 °C) and allow to thaw for the first use. If previously opened, remove the 5C matrix mix from the refrigerator (2°C to 10 °C). Remove one tube of Matrix Dilution Buffer from the freezer (-30 °C to -10 °C) and allow to thaw.

5.1.11.5 Remove at least 500 µL of Hi-Di Formamide from the freezer (-20 ˚C) and allow to thaw.

5.1.11.6 Vortex the 5C Matrix Mix for 10-15 seconds prior to use. Add 10 µL of the 5C Matrix Mix to one tube of the Matrix Dilution Buffer. Vortex for 10-15 seconds. Note the date of dilution on the tube. The diluted 5C Matrix Mix may be stored for up to one week at 2°C to 10 °C.

5.1.11.7 Add 10 µL of the 5C Matrix Mix/Matrix Dilution Buffer mixture to 500 µL of Hi-Di formamide. Vortex for 10-15 seconds.

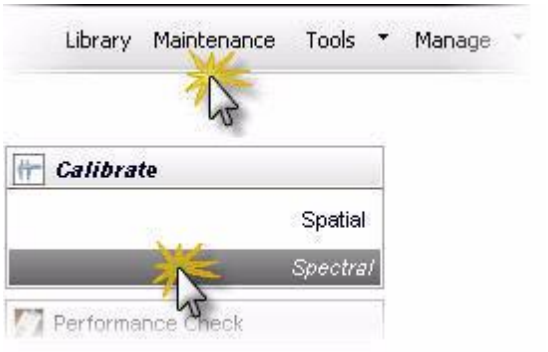
5.1.11.8 Using a 96-well reaction plate in columns 1-3 and rows A-H, add 15 µL of the matrix/formamide mixture to each well.

5.1.11.9 Cover plate with a 96-well plate septa and briefly centrifuge the plate to remove bubbles. Do not heat denature.

5.1.11.10 Place the plate in the 3500 series 96-well standard plate base and cover with the plate retainer. Place the plate assembly in Position A on the autosampler, positioned correctly with the notch in the lower right corner.

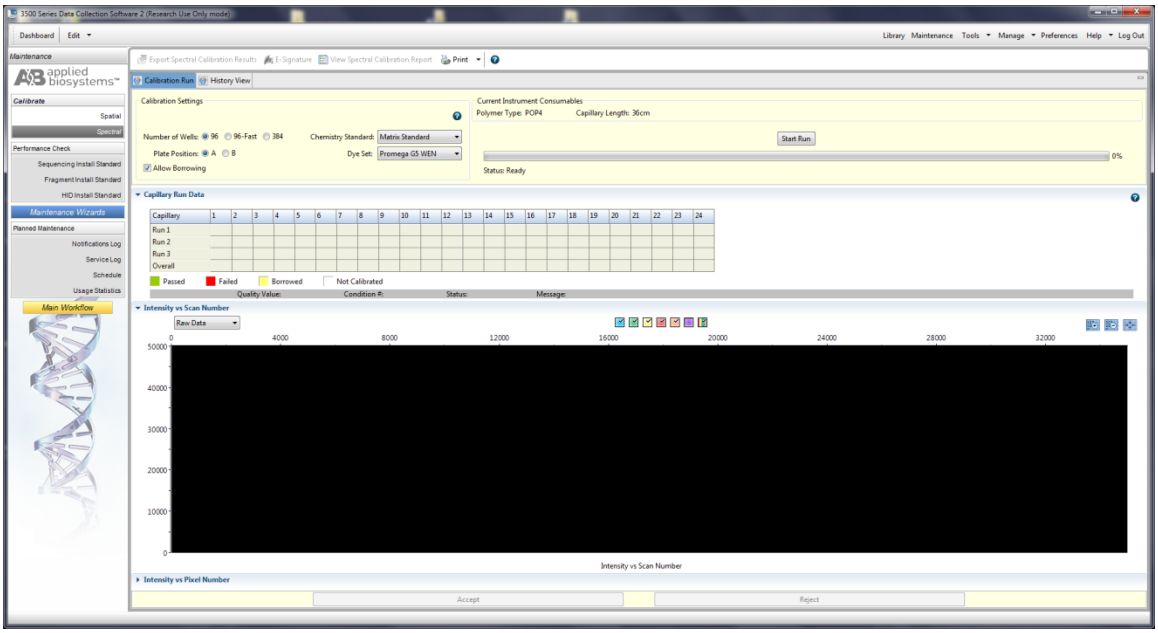
5.1.11.11 Close the instrument door to re-initialize the instrument.

5.1.11.12 Once the temperature of the oven has stabilized at 60°C, access the **Spectral Calibration** screen. From the **Dashboard**, select **Maintenance** and then select **Spectral Calibration** in the navigation pane.



5.1.11.13 Choose “96” for number of wells and specify the plate location on the instrument.

5.1.11.14 Choose **Matrix Standard** from the **Chemistry Standard** drop-down menu and **Promega G5 WEN** from the **Dye Set** drop-down menu.



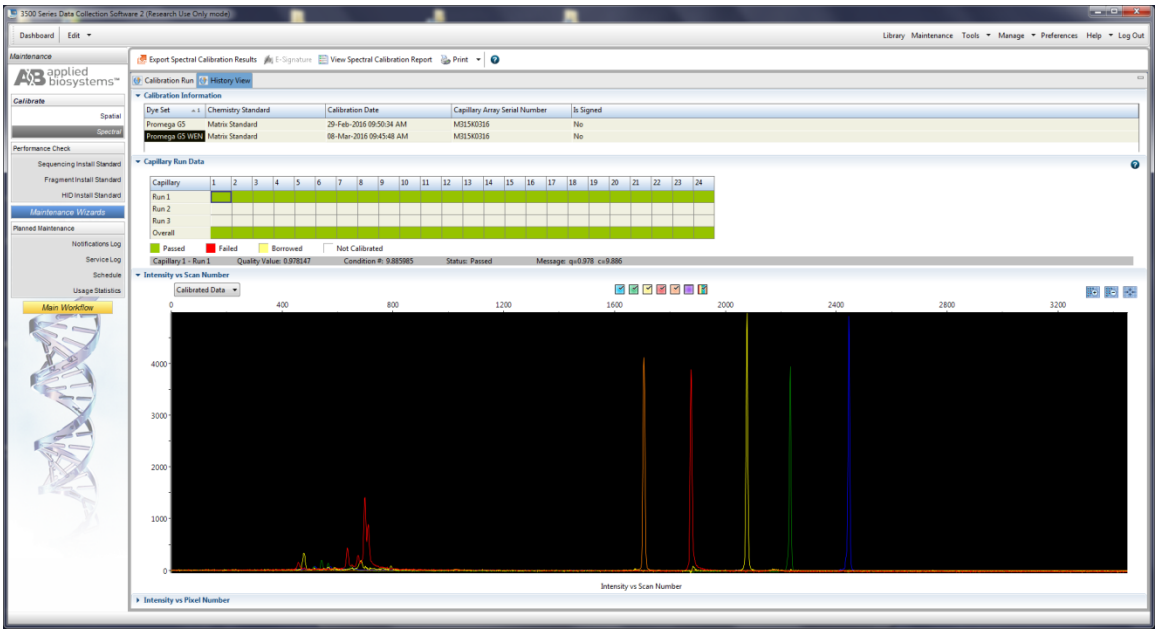
5.1.11.15 Select **Allow Borrowing**. Selecting this option instructs the software to automatically replace information from failed capillaries with information from an adjacent passing capillary with the highest Quality value.

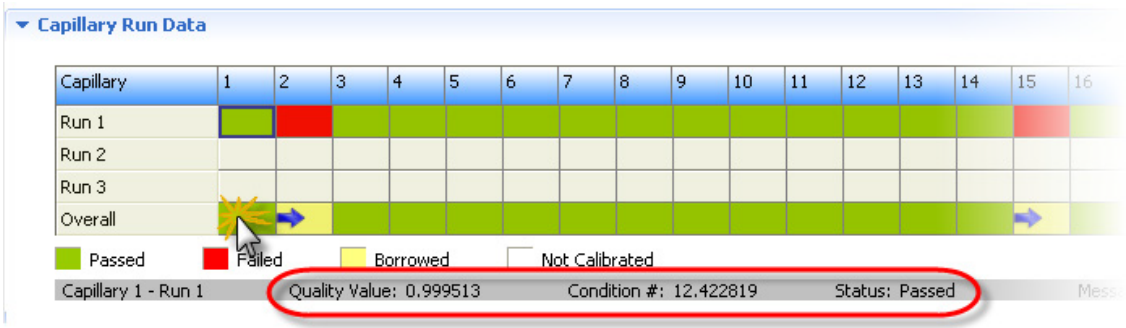
5.1.11.16 Select **Start Run**.

5.1.11.17 If fewer than the recommended number of capillaries pass, the spectral calibration run will be repeated automatically up to three times. When borrowing is enabled, all capillaries must pass within the borrowing limits.

5.1.11.18 Upon completion of the spectral calibration, check the quality in the Capillary Run Data display. Passing and failing capillaries are shown in green and red respectively. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed. To display the results for each capillary (spectral data, Quality Value, and Condition Number) below the run results table, click a capillary in the table. The ranges that the software uses to determine if a capillary passes or fails with a G5 dye set are a Quality Value minimum of 0.95 and a Condition Number maximum of 13.5.

NOTE: The results displayed when you click a borrowed capillary are the passing results borrowed from the adjacent capillary. To determine the reason that a capillary fails, view the spectral calibration report.

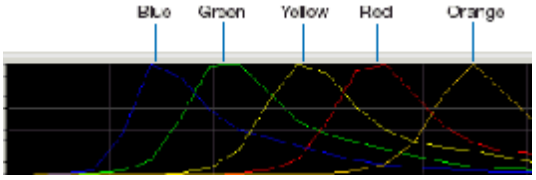




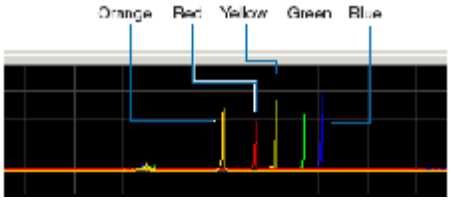
5.1.11.19 Viewing the pass/fail status after the run:

5.1.11.19.1 View the status of each capillary. Each capillary should have a Q-value above 0.95 (if spectral calibration failed, see the troubleshooting and reference guide in the 3500/3500xL Genetic Analyzer User Guide).

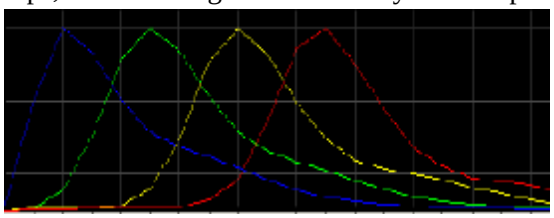
5.1.11.19.2 Evaluate the spectral profile and raw data for each capillary. Verify that the order of the peaks in the spectral profile for Intensity vs. Pixel Number (from left to right) is blue, green, yellow, and red followed by orange for 5-dye chemistry.



5.1.11.19.3 Verify that the order of the peaks in the raw data profile for Intensity vs. Scan Number (from left to right) is orange, red, yellow, green, and blue.



5.1.11.19.4 Verify that the peaks in the spectral profile do not contain gross overlaps, dips, or other irregularities. Verify that the peaks are separate and distinct.



5.1.11.20 If the spectral calibration is acceptable, then click **Accept Results**. If the spectral calibration is not acceptable, click **Reject Results** and run a new spectral calibration or refer to the Applied Biosystems 3500/3500xL Genetic Analyzer User Guide for troubleshooting information.

5.1.11.21 View and print a spectral calibration report.

5.1.11.21.1 Click **View Spectral Calibration Report**.

5.1.11.21.2 To print the report, click **Print**; then select **Print Report**.

5.1.11.21.3 In the printer dialog box, select **CutePDF Writer** and print the report as a .pdf file.

NOTE: After performing a calibration, the calibration report can be saved electronically for record keeping. The software does not save historical calibration results. Only the most recent spectral calibration is maintained in the software.

5.1.11.21.4 Close the report.

5.1.11.21.5 Spectral Calibration Reports shall be maintained in the Section.

5.1.12 Errors

5.1.12.1 Error messages in the 3500 Series Data Collection Software include a **Details** button. Click **Details** to display more information about an error message.

5.1.12.2 For error help, see the Troubleshoot section of the 3500/3500xL Genetic Analyzer User Guide.

5.2 ABI 9700 Thermal Cyclers

5.2.1 Internal Quarterly Verification: All thermal cyclers currently in service within the Section shall be subjected to a series of temperature verifications on a quarterly basis by the QCO. Gloves, masks and lab coats shall be worn at all times. Caution shall be exercised at all times as the thermal cyclers can reach temperatures in excess of 100 °C. Documentation of all verifications shall be noted on the Thermal Cycler Verification Record by the QCO performing the verification for each thermal cycler. This documentation shall be retained indefinitely by the QCO.

5.2.1.1 Temperature Uniformity: A set of twelve wells on each thermal cycler shall be tested for two temperature groups: 95 °C and 40 °C. For each temperature group, the range between the highest and lowest values shall not exceed +/- 1 °C. Additionally, each individual well, for each individual temperature, shall not deviate +/- 1 °C from the set temperature.

5.2.1.1.1 Turn on the thermal cycler, select "run" and "TNU" (or "Temp Uniformity"). Select a 25 µL reaction volume. Select "start."

5.2.1.1.2 When the thermal cycler reaches 95 °C as indicated on the display, select the "pause" button. Insert the digital Eutechnics 4500 probe (or equivalent) into the appropriate well (listed below) and shut the lid. Do not pinch the cord. Tested wells are as follows: A1, A6, A12, C4, C9, D7, E3, F2, F11, H1, H7, and H12.

5.2.1.1.3 Allow the probe to stabilize (may take a few minutes). Record the temperature to the nearest tenth of a degree for that well as indicated by the probe. Proceed to the next well until all twelve wells have been recorded on the Thermal Cycler Verification Record (TCVR) for the 95 °C temperature range. NOTE: QCO may have to continue selecting the "pause" mode to complete this step so as to keep the thermal cycler at 95 °C.

5.2.1.1.4 Select the "resume" button on the display or allow the "pause" mode to time out. The thermal cycler begins to cool down to 40 °C. Using the same wells as listed in **5.2.1.1.2**, test and record the 40 °C temperature results as described in **5.2.1.1.2** and **5.2.1.1.3**. Record the temperature for each well on the TCVR.

5.2.1.1.5 Record temperatures from **5.2.1.1.3** to **5.2.1.1.4**. These values shall not exceed +/- 1 °C from the set temperature and from each other (compare highest recorded temperature for each range to the lowest recorded temperature for each range). Record the results on the TCVR.

5.2.1.1.6 If the recorded temperatures exceed the criteria described in **5.2.1.1**, the QCO shall notify the Technical Leader and the thermal cycler in question shall be removed from service. The QCO shall notify the Section via email, as well as by placing an "Out of Service" sticker on the thermal cycler.

5.2.1.2 Heat and Cool Rate Test: The ability of the thermal cycler to heat and cool the block quickly is determined by the following steps:

5.2.1.2.1 Turn on the thermal cycler, select "Utilities," "Diag," "System," and "Rate."

5.2.1.2.2 The thermal cycler displays a warning. At this time, place an empty 96-well plate, with septa, onto the thermal cycler and close the lid. Select "Cont."

5.2.1.2.3 The thermal cycler runs the program. When the program is completed, the display indicates "pass" or "fail." It also provides the rate at which the thermal cycler both heats and cools. Rates, as well as "pass" or "fail," shall be recorded on the TCVR.

5.2.1.2.4 If a "fail" result is obtained, the QCO may retest the thermal cycler once more. If the particular thermal cycler indicates a second "fail", the QCO shall notify the Technical Leader and the thermal cycler in question shall be removed from service. The QCO shall notify the Section via email, as well as by placing an "Out of Service" sticker on the thermal cycler.

5.2.1.3 Temperature Verification: The digital probe is used to verify that the thermal cycler is producing a temperature within +/- 1 °C of the set temperature.

5.2.1.3.1 In the display window, select "Utilities," "Diag," "TempVer."

5.2.1.3.2 Place the digital probe into well A6 and close the lid. Do not pinch the cord. Select "Run."

5.2.1.3.3 The thermal cycler ramps up to 85 °C and prompts the user when complete. At this point, the QCO records the temperature on the digital probe on the TVCR and enters that value as prompted into the thermal cycler to the nearest tenth of a degree.

5.2.1.3.4 Continue the test by allowing the thermal cycler to ramp down to 45 °C ("stabilizing at setpoint") and record the resulting information as described in **5.2.1.3.3**. Select "accept" once both the 85 °C and 45 °C values are entered into the display window.

5.2.2 Notification for Use: If any thermal cycler fails any of the three tests, the QCO shall immediately notify the Technical Leader and the Section via email, and an "Out of Use" sticker shall be placed on the affected instrument.

5.2.3 Performance QC Check: If a thermal cycler requires a QC check after repair, for validation, or before a new instrument is put online, a QC check shall be performed by the QCO, in addition to the three tests as described in **5.2.1**.

5.2.3.1 This QC check shall consist of the amplification of the following as a set:

5.2.3.1.1 Positive amplification control (9947A) and negative amplification control (Neg Amp), using the current amplification kit.

5.2.3.1.2 NIST-TS and associated Reagent Blank.

5.2.3.2 Five total sets shall be amplified at the following well locations and electrophoresed and analyzed per DNA procedures:

5.2.3.2.1 E1-H1, C4-F4, B7-E7, E10-H10, A12-D12

5.2.3.3 The expected results for the NIST-TS, positive amplification controls, and allelic ladders shall be obtained for all loci and the alleles shall be balanced within and between loci and peak heights generally between 1000 and 6000 RFU's. All Reagent Blanks and negative amplification controls shall be free of any peaks or activity. If any of these conditions are not met (for reasons other than instrument failure, known artifacts), then the QCO may retest the affected wells in the thermal cyclers once. If the conditions are not met this second time, the QCO shall keep the thermal cycler offline and notify the Technical Leader and manufacturer. If the thermal cycler is under a manufacturer warranty, the manufacturer shall be contacted for repair. If the thermal cycler is no longer under any warranty, it shall be placed in storage for surplus.

5.2.4 External Calibrations/Verification: If the thermal cyclers are verified by an external vendor, the results shall be documented. The thermal cyclers that are passed by the external vendor shall be accepted as calibrated/verified and noted as such until the next quarterly verification is due. This documentation shall be retained indefinitely by the QCO.

5.3 Digital Probes

5.3.1 Annual External Calibration: The digital probes (Eutechnics 4500 or equivalent) shall be calibrated annually by a contract vendor against an appropriate NIST traceable standard.

5.4 Bulb Thermometers

5.4.1 Purpose/Use: Used to measure temperatures in heat blocks, incubators and select refrigeration storage units. Surplus calibrated bulb thermometers shall be retained by the QCO, unless broken, and they shall be disposed of in accordance with the DNA Database Administrative Policy and Procedure for Safety and Hazardous Waste Disposal.

5.4.2 Annual Internal Performance Check: All bulb thermometers in use within the DNA Database Section shall be checked on an annual basis internally against a NIST traceable thermometer (i.e., the "NIST lollipop") in an ice bath.

5.4.2.1 Freeze several trays of dH_2O into ice cubes; once frozen, grind or crush them in an ice shaver (or equivalent). Mix the ice shavings with dH_2O and place into an insulated container deep enough (thermos or equivalent) to contain the metal probe portion of the NIST Traceable Thermometer.

5.4.2.2 The QCO shall wipe down each bulb thermometer with fresh 10 % bleach followed by an ethanol rinse and allow it to dry (either through evaporation or wiping with a wipe) before inserting it into the ice bath.

5.4.2.3 Using clamps and foam (or equivalent) to hold both the NIST traceable thermometer and the bulb thermometer to be calibrated within an inch of each other in the ice bath, wait for the NIST traceable thermometer to register 0.0 °C. Align the bulb thermometer such that the bulb portion is submerged in the ice bath, but that the area marked for 0.0 °C can be visualized by the QCO.

5.4.2.4 Once the NIST traceable thermometer reads 0.0 °C, record the temperature to the nearest tenth of a degree of the bulb thermometer. If the bulb thermometer is greater than +/- 1 °C

from the NIST traceable thermometer, it shall be destroyed and replaced with a calibrated bulb thermometer.

5.4.2.5 The QCO shall record both the NIST traceable thermometer and calibrated bulb thermometer readings on the Bulb Thermometer Temperature Performance Check Form. The QCO shall also create and place a sticker on each calibrated bulb thermometer that indicates the specific bulb thermometer number, the date the next performance check is due, the initials of the QCO performing the check, and whether the user of the bulb thermometer shall add or subtract tenths of a degree to the reading of that bulb thermometer to bring it to specifications as indicated by the NIST traceable thermometer (i.e., if the bulb thermometer reads 0.5 °C higher than the NIST traceable thermometer, the Forensic Scientist shall subtract 0.5 °C from the bulb thermometer reading before recording a temperature).

5.4.2.6 This process shall be completed for all bulb thermometers, including those set aside for storage or future use (i.e., replacement).

5.4.2.7 Documentation of the performance checks shall be retained indefinitely by the QCO in the Section.

5.5 Digital Thermometers: Purchased from external vendor; shall be NIST traceable and replaced when NIST traceability expires. Digital thermometers shall be used to monitor temperatures in freezers and refrigerators in the Section as needed. Surplus digital thermometers shall be retained by the QCO.

5.6 NIST Traceable Thermometer (i.e., the "NIST lollipop"): Has an elongated metal probe which is used for testing against bulb thermometers purchased from an external vendor; shall be NIST traceable and replaced when NIST traceability expires.

5.7 Balances

5.7.1 Monthly Verification Check: Using a NIST traceable weight set, all balances in the DNA Database Section shall be verified monthly by the QCO with the following weights and limits:

5.7.1.1 Weight #1: 1 gram; Limit: 0.90 gram to 1.10 grams.

5.7.1.2 Weight #2: 25 grams; Limit: 24.90 to 25.10 grams.

5.7.1.3 Weight #3: 100 grams; Limit: 99.90 to 100.10 grams.

5.7.1.4 If any tested weight falls above or below the established limit criteria listed above, the QCO shall immediately notify the Technical Leader and the balance shall be removed from service until or unless repaired and calibrated by an external vendor.

5.7.1.5 The performance checks shall be recorded to the nearest hundredth of a gram by the QCO on the Monthly Balance Verification Form.

5.7.2 Biannual External Calibrations: All balances in the DNA Database Section shall be calibrated twice a year by a contract vendor.

5.8 Pipettors

5.8.1 Biannual External Calibrations: All pipettors in the DNA Database Section shall be calibrated biannually by a contract vendor.

5.8.2 Repair: If a pipettor breaks, or a DNA Database Forensic Scientist based on their training and experience believes that the pipettor does not work properly, it shall be given to the QCO for storage until an external calibration vendor can repair and calibrate it. If the pipettor is not repairable, it shall be removed from the Section.

5.9 Temperature Chart Recorders/Data Loggers

5.9.1 Temperature Chart Recorders or Data Loggers may be used to monitor temperature in post amplification rooms where 3500xLs (or equivalent) are currently in use.

5.9.2 Temperature Chart Recorders:

5.9.2.1 Biannual External Calibrations: All temperature chart recorders in the DNA Database Section shall be calibrated biannually by a contract vendor.

5.9.2.2 Retention of data: Paper temperature discs shall be changed weekly when in use by the QCO. Any circular discs shall be scanned into digital images. Both the original disc and the digital image shall be retained indefinitely by the QCO in the Section.

5.9.3 Data Loggers (USB)

5.9.3.1 Annual External Calibrations: All data loggers in the DNA Database Section shall be calibrated annually by a contract vendor.

5.9.3.2 Retention of data: The data loggers shall be set to record data every five minutes. The data shall be printed as a .pdf file by the QCO every month and retained indefinitely by the QCO in the Section. The data shall include the date range captured by the logger as well as the serial number of the logger. Once monthly data is captured and retained, the data logger shall be cleared to record data for the next month by the QCO.

5.10 Centrifuges

5.10.1 Annual Preventative Maintenance: The Beckman-Coulter Allegra X-12R centrifuges shall have annual preventative maintenance performed by the manufacturer. The manufacturer shall place a maintenance sticker on the centrifuge documenting that the service was performed.

5.10.2 Repair: If repairs are necessary, the manufacturer shall be notified by the QCO and an "Out of Use" sticker placed on the affected centrifuge notifying the Section of its unavailability. Once the affected centrifuge is repaired, the QCO shall remove the "out of use" sticker.

5.11 Biosafety Cabinets/Chemical Fume Hoods/Laminar Flow Clean Air Benches

5.11.1 Annual External Calibrations: All Nuaire Biological Safety Cabinets, Chemical Fume Hoods, and Laminar Flow Clean Air Benches (amplification hoods) in the Section shall be calibrated annually by a contract vendor.

5.11.2 Any hood listed in **5.11.1** that does not pass certification shall not be used.

5.12 Qiagen BioRobot® Universal System

5.12.1 Maintenance

5.12.1.1 Prior to operating the Qiagen BioRobot®, perform any maintenance required. If the run button is yellow, any needed maintenance is listed in the maintenance environment.

5.12.1.2 The Qiagen BioRobot® and its components shall be cleaned with ~1 % DeconQuat or ethanol based disinfectant when specified.

5.12.1.3 Water Run: To perform a water run, go to Environment > Execute. The P90X Module Workout2 protocol is used for water runs and is listed in the Applications menu under the Service section. Select the protocol and follow the prompts on the screen to complete a water run. It shall be the responsibility of the DNA Database Quality Control Officer to ensure water runs are completed when required. When the robot is online, a water run shall be performed weekly. When the robot is offline, a water run shall be performed at a minimum of every 10 working days unless the robot is inoperable.

5.12.1.4 Daily: Daily maintenance procedures include emptying, rinsing, and refilling the system liquid containers followed by a system flush. It also includes emptying the water container and vacuum trap, cleaning the tip disposal station, and cleaning the worktable. Daily maintenance procedures shall be conducted each day the robot is in use by the Forensic Scientist operating the robot.

5.12.1.5 Conditional: Conditional maintenance procedures shall be done between each molecular biology application. Instructions to do these tasks occur at the beginning and/or end of the protocol.

5.12.1.6 Weekly: Weekly maintenance procedures include cleaning the barcode reader windows and the reagent carousel. Follow the prompts on the screen or see 5.3 of the Qiagen BioRobot 8000 User Manual. Weekly maintenance is not required when the robot is offline.

5.12.1.7 Monthly: Monthly maintenance procedures include cleaning the system liquid container, worktable and robotic handling system, as well as running the high-speed dispensing system and liquid detectors. Follow the prompts on the screen or see 5.4 of the Qiagen BioRobot 8000 User Manual. Per Qiagen recommendation, tap water may be used in lieu of the salt solution for the high-speed dispensing system and liquid detectors. Monthly maintenance is not required when the robot is offline.

NOTE: When cleaning the system liquid container, the detergent RBS-35 is added to the water in the container for cleaning. Prior to disposal, the water and RBS-35 solution shall be pH tested. The acceptable pH range for disposal is between 3 and 12. To note compliance with the testing and that the solution is within the accepted range, use a pH strip to test the pH and record the pH in the box labeled “pH range 3-12” on the robot maintenance log located beside the instrument.

5.12.1.8 Biannual: The biannual maintenance is a pipette calibration of the robot utilizing a plate reader and balance.

5.12.1.9 Annual Preventative: The annual preventative maintenance is performed by a Qiagen representative and is followed with a performance check. The Forensic Scientist

Manager or designee shall be responsible for scheduling annual preventative maintenance with the manufacturer.

5.12.1.10 NIST SRM/NIST Traceable Standard: A NIST SRM, or NIST traceable standard shall be run on the Qiagen BioRobot® annually. It shall be electrophoresed on a 3500xL at the 18 secondinjection time. See the DNA Database Section Procedure for DNA Reagent Quality Control.

5.12.1.11 It is recommended that the main power switch to the robot be turned off when the robot is not in use for an extended period.

5.12.2 Repair, Service, and Calibration Performed by Manufacturer – After any repair, service, or calibration performed by Qiagen on the Qiagen BioRobot®, the following shall occur:

5.12.2.1 The robotic platform and any worktable components used during the procedure shall be cleaned per instructions in the monthly maintenance procedure.

5.12.2.2 A performance check shall be completed as outlined in section **5.12.3**.

5.13 Performance Checks – A performance check shall be conducted (at a minimum) after repair, service, and calibration.

5.13.1 A NIST-TS must be run as part of a performance check.

5.13.2 Using the DNA Database Section Procedure for Qiagen BioRobot® Universal Using PowerPlex® Fusion, amplify, run, and analyze at least two known DNA samples. This step may use the same known sample, processed in duplicate.

5.13.3 Criteria for Success

5.13.3.1 Negative controls must not produce detectable alleles. All positive amplification controls must be void of extraneous, detectable alleles. At least one positive amplification control must produce a full, expected profile.

5.13.3.2 At least one NIST positive control shall be run and produce a full profile. If multiple NIST positive controls are run, only one must produce a full profile.

5.13.3.3 If the Qiagen BioRobot® does not pass the performance check, the root cause shall be determined. The Qiagen BioRobot® shall be posted as not in use until such time as the root cause is determined and the Qiagen BioRobot® passes a performance check.

5.13.4 Performance Check/QC Worksheet

5.13.4.1 A final performance check/QC worksheet shall be generated and shall be approved by the Technical Leader prior to analysis/acceptance of any database sample profiles.

5.13.4.2 Standard documentation shall be included (e.g., worksheets) with the Performance Check/QC worksheet. The robot batch number for all additional documentation is noted on the Performance Check/QC worksheet for reference.

5.13.4.3 Electropherograms of the appropriate ladders and controls shall be included with the Performance Check/QC worksheet.

5.13.4.4 All documentation shall be maintained in either hard copy or electronic format as authorized by the record retention schedule as set forth by the North Carolina Department of Cultural Resources.

5.14 Heat Blocks

5.14.1 Heat blocks shall have stickers placed on them to indicate their specific purpose and associated temperature:

5.14.1.1 Knowns: 70 °C

5.14.2 The heat block temperatures shall be monitored by a calibrated bulb thermometer (see **5.4**).

5.14.3 If a DNA Database Forensic Scientist uses a designated heat block, the temperature shall be recorded on the Temperature Record Form (TRF) associated with that specific heat block on the day(s) that it is used.

5.14.3.1 If the heat block is not used, the DNA Database Forensic Scientist shall strike through the box which corresponds to the day(s) not in use.

5.14.3.2 The DNA Database Forensic Scientist shall fill out all required information regarding equipment name and serial number, the location of the equipment, the set temperature of the equipment, and the associated bulb thermometer number.

5.14.3.3 If at any point during the calendar year a new bulb thermometer is needed, the DNA Database Forensic Scientist shall write at the bottom of the TRF the date on which a new thermometer was used and the number for the new thermometer.

5.14.4 If a heat block deviates more than +/- 5°C from the set temperature for more than five consecutive readings, the DNA Database Forensic Scientist shall use the temperature knob controls on the heat block to readjust the temperature back into range (this may take several attempts). If all efforts with the temperature knobs fail, the DNA Database Forensic Scientist shall request a new bulb thermometer from the QCO to determine if the temperature issue is due to the heat block or the bulb thermometer. During this period of adjustment, the heat block shall not be used by the DNA Database Forensic Scientist. If after both temperature knob adjustments and a new bulb thermometer are unsuccessful, the DNA Database Forensic Scientist shall notify the QCO immediately and that particular heat block shall be removed from use. The DNA Database Forensic Scientist shall note on the bottom of the TRF for that particular heat block the date it ceased to be in use.

5.15 Freezers/Refrigerators

5.15.1 Recording Temperatures: The QCO shall make every effort to record temperatures for all common area refrigerators/freezers in the Section at the beginning of every business day; however, if the QCO has not yet recorded the temperature and a DNA Database Forensic Scientist uses a common area refrigerator/freezer, the DNA Database Forensic Scientist shall record the temperatures prior to opening the door(s). Refrigerators/freezers which are in limited access areas (such as between suites) shall have their temperatures recorded weekly by the DNA Database Forensic Scientist who has access to such refrigerators/freezers.

5.15.2 -20 °C Freezers: These freezers shall not vary more than +5 °C from the set temperature. The temperature for these freezers shall be recorded by personnel using the TRF as described in **5.16.1**.

5.15.2.1 The QCO shall fill out all required information regarding freezer serial number, the location of the freezer, the set temperature of the freezer, and the associated digital thermometer serial number at the beginning of every calendar year on a TRF for each common area -20 °C freezer.

5.15.2.2 If at any point during the calendar year a new digital thermometer is needed, the QCO or designee shall write at the bottom of the TRF the date on which a new thermometer was used and the serial number for the new thermometer.

5.15.2.3 If a -20 °C freezer must be thawed, the contents shall immediately be moved to another -20 °C freezer that is within range and the QCO shall note this, as well as the affected dates, on the TRF. The contents shall not be returned to the original -20 °C until the temperature is within range.

5.15.2.4 If the temperature for a -20 °C freezer exceeds the +5 °C range for more than 5 consecutive business days, QCO shall immediately move the contents to another -20 °C freezer that is within range and note this, as well as the affected dates, on the TRF. The contents shall not be returned to the original -20 °C freezer until the temperature is within range.

5.15.3 -10 °C/4 °C Freezer/Refrigerator Units: These units shall not vary more than +5 °C from the set temperature(s) for the freezer portion; the refrigerator portion shall not fall below 0 °C or exceed 9 °C. The temperature for these freezers shall be recorded using the TRF by personnel as described in **5.15.3.1**, **5.15.3.2, 5.15.3.3,** and **5.15.3.4**.

5.15.3.1 The QCO shall fill out all required information for common area -10 °C/4 °C freezer/refrigerator units regarding the unit serial number, location, set temperatures, and the associated digital thermometer serial number on the TRF. For limited access -10 °C/4 °C freezer/refrigerator units, the DNA Database Forensic Scientist(s) that have access to such units shall fill out the information on a TRF.

5.15.3.2 If a DNA Database Forensic Scientist is out of the office unexpectedly (e.g., sick day), the Manager or designee for that DNA Database Forensic Scientist shall record the temperature for that day. If a DNA Database Forensic Scientist has planned days out of the office (e.g., court or vacation), it is the responsibility of the DNA Database Forensic Scientist to arrange for a suitemate or Manager/designee to perform temperature recordings.

5.15.3.3 If at any point during the calendar year a new digital thermometer is needed, the QCO shall be notified and the new thermometer serial number shall be recorded on the TRF associated with the refrigerator/freezer.

5.15.3.4 If the QCO or DNA Database Forensic Scientist observes temperatures out of the range specified in **5.15.3** for more than five consecutive business days, then the QCO (for common area units) or the DNA Database Forensic Scientist (limited access units), shall

attempt to adjust the temperature back in range using the thermostat for the unit. If the temperature does not come within range within an 24 hour period, the QCO (or DNA Database Forensic Scientist) shall transfer the contents of the unit to another unit with the same temperature parameters and note on the TRF the unit to which the contents were transferred and the date of transfer. If additional adjustments of the thermostat are unsuccessful, the unit shall be removed from service and clearly marked as being out of service. If additional adjustments are successful at restoring the unit to the temperatures specified in **5.15.3**, then the contents may be returned to the unit.

5.16 Incubators: Temperatures shall be recorded on the day(s) the incubator is in use. If the incubator is in a common area, the QCO shall record the temperature. If the incubator is in a shared suite, the DNA Database Forensic Scientist shall record the temperature. Temperatures shall be recorded on a TRF specific for the incubator.

5.16.1 The QCO or DNA Database Forensic Scientist shall fill out all required information regarding the unit serial number, location, set temperatures, and the associated bulb thermometer number on the TRF.

5.16.2 If the incubator is not used, the QCO or DNA Database Forensic Scientist shall strike through the box which corresponds to the day(s) not in use.

5.16.3 The incubators shall be +/- 5 °C degrees within the set temperature. If an incubator deviates more than this over a period of five consecutive readings, then the QCO or DNA Database Forensic Scientist shall attempt to adjust the temperature back into the acceptable range over a period of 24 hours. If all attempts at obtaining a set temperature within range fail, the QCO shall be notified and the incubator removed from service and marked as such.

5.17 All verification, calibration, maintenance, and QC documentation shall be retained within the Forensic Biology and DNA Database Sections.

5.18 When any of the following instruments/equipment need repair and are taken out of use from the Section, the QCO shall notify the Technical Leader, and if necessary, the manufacturer. The QCO shall also notify the Technical Leader when the instruments/equipment are suitable for use by the Section.

- 3500xL, ABI 9700, BioRobot, Centrifuges, Hoods, Freezers/Refrigerators, Balances.

6.0 Limitations - Once a 3500xL 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 performed.

Temperature: The results from the 3500xL instrumentation can be affected by temperature changes. If the temperature in the room where the instrument is located is outside of the range of 60 °F to 85 °F, this shall be taken into account during analysis. If the results are affected, then the QCO (or designee) shall take the affected instrument(s) offline until the temperature is within range and the instrument has passed a QC check.

7.0 Safety

7.1 Thermal cyclers can exceed temperatures of 100 °C; use with caution to avoid burns.

7.2 Gloves, masks, and lab coats shall be worn when performing any maintenance, verifications, calibrations, or QC checks described in section 5.

7.3 If the ice shaver (or equivalent) used as described in **5.4.2.1** is not self-contained, safety glasses shall be worn during operation.

7.4 Formamide is a known chemical hazard; causes eye, skin and respiratory tract irritation. It is also a possible teratogen. Wear appropriate eyewear, masks, gloves and clothing when using.

7.5 DeconQuat 100 is corrosive.

7.6 RBS-35, used for cleaning the BioRobot system liquid containers, is corrosive. Use gloves, lab coat, and protective eyewear during use.

7.7 Bleach shall not be used to clean or disinfect the Qiagen® BioRobot.

7.8 While emptying the vacuum trap on the Qiagen® BioRobot, the sink shall be flushed with tap water. After emptying the vacuum trap, clean the container thoroughly with liquid disinfectant before reconnecting the container to the BioRobot.

8.0 References

Applied Biosystems 3500/3500xL Genetic Analyzer. User Bulletin. 2011 Life Technologies Corporation. Part Number 4469192 Rev. A. (or most recent revision)

Applied Biosystems 3500/3500xL Genetic Analyzer User Guide. 2010 Life Technologies Corporation. Part Number 4401661 Rev. C. (or most recent revision)

DNA Database Section Administrative Policy and Procedure

DNA Database Section Administrative Policy and Procedure for Safety and Hazardous Waste Disposal

DNA Database Section Procedure for DNA Database Analysis and Technical Review of Database Samples Amplified with PowerPlex® Fusion

DNA Database Section Procedure for DNA Database Operations

DNA Database Section Procedure for DNA Database Training for PowerPlex® Fusion

DNA Database Section Procedure for DNA Reagent Quality Control

DNA Database Section Procedure for GeneMapper ID-X

DNA Database Section Procedure for PCR Amplification with PowerPlex® Fusion

DNA Database Section Procedure for Sample Processing Quality Control

DNA Database Section Procedure for Use of the 3500xL Genetic Analyzer

DNA Database Section Procedure for Qiagen BioRobot® Universal Using PowerPlex® Fusion

Instrument manuals

NIST Special Publication 819

North Carolina Department of Cultural Resources Record Retention Schedule

PowerPlex® 5C Matrix Standard: Instructions for Use of Product DG4850. 2015 Promega Corporation. Part Number TMD049 Rev. 10/15.

QIAsoft 5 Operating System User Manual

State Crime Laboratory Safety Manual

3500xL Data Collection Software

9.0 Records

- Temperature logs for freezers, refrigerators, heat blocks (Daily and Weekly).
- Thermal Cycler Temperature Performance Check Forms.
- Bulb Thermometer Calibration Forms.
- Biosafety Cabinets/Chemical Fume Hoods/Laminar Flow Clean Air Benches Certificates.
- Certificates of Calibration for NIST Traceable Digital Thermometer, Digital Thermometers, Balances, Pipettes, Digital Probes, Data Loggers, and Temperature Chart Recorders.
- Manufacturer documentation of preventative maintenance and/or repair for 3500xLs and centrifuges.
- BioRobot Performance Check QC Documentation Notebooks
- BioRobot Log Notebooks

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
08/03/2015	1	Original Document
04/18/2016	2	Removed water run requirements from definition, Removed reference to 24 sec injection throughout, 5.1.3.2.1-updated to reflect 3500 data may be removed from 3500xL (data saved elsewhere); updated throughout for CC5 to WEN size standard change; 5.11 updated for new matrix