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| | Document Manager: Kristin Schelling | Approved By: Jeffrey Nye |

4.3 Operation and Maintenance of ABI 3500/3500xL Genetic Analyzer

4.3.1 Starting the Instrument

Turn instrument on and wait for green status light

Start computer and log into Windows Vista OS

Status icon shows when the 3500 **Server Monitor** is active by displaying the icon with a green check mark. Do not close or shutdown the **Service Monitor** icon.

Launch the 3500 **Data Collection Software**

4.3.2 Performing a Spatial Calibration

It is necessary to perform a spatial calibration under the following circumstances:

- Removing or replacing a capillary array
- Opening the detector door or moving the detection cell
- Moving the instrument

In the dashboard of the **Data Collection** software, select the **Maintenance** icon and **Calibrate>Spatial** from the left task pane.

Select **No-Fill** from the options if the capillaries contain fresh polymer or select **Fill** from the options if the capillaries do not contain fresh polymer. Check **Perform QC Checks**. Select **Start Calibration**.

Evaluating the **Spatial Calibration** includes:

- One sharp peak for each capillary (small shoulders are acceptable)
- One mark  at the apex of each peak
- An even peak profile with similar peak heights
- Spacing should be between 13 and 16

If the spatial calibration results meet the above criteria, click **Accept Results**.

Click **View Spatial Calibration Report**. Select **Print** and print a PDF of the calibration report. Save the PDF files in the designated location for the instrument.

If the spatial calibration does not meet the criteria listed above, click **Reject** results and attempt again.

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4.3.3 Performing a Spectral Calibration (For use with CC5 dye only)

Note: A run cannot start unless a calibration file that matches the dye set and capillary array length is active. Every new spectral calibration is automatically the active one for that dye set.

It is necessary to perform a spectral calibration under the following circumstances:

- When a new dye set is being used on the instrument
- Service on the optics (realigning or replacing the laser, CCD camera, or mirrors)
- Presence of pull-up/pull-down in raw or analyzed data
- Capillary array is replaced on the instrument

Use PowerPlex Matrix standards 3500 5-Dye for PowerPlex Fusion. Thaw matrix standards and mix thoroughly. Spin briefly in a micro centrifuge.

Combine 665 uL of Hi-Di Formamide with 30 uL of nuclease free water in a micro centrifuge tube. Add 1 uL of each dye to tube.

Vortex thoroughly and briefly centrifuge the mixture.

Dispense 25 uL of the matrix standard mix into the first row (wells A1-H1) of a 96-well reaction plate for a 3500 or the first three rows (A1-H1, A2-H2 and A3-H3) of a 96-well reaction plate for a 3500xL. Seal the plate with the 96-well septa mat.

Briefly centrifuge the plate with matrix standards and ensure that any bubbles are removed from the wells. Repeat if bubbles are still present.

Prepare the plate assembly by placing the plate on the base and snapping the plate retainer onto the plate and base. Verify that the holes of the plate retainer and septa are aligned and the sides are snapped properly.

Preheat oven/detection cell to 60C from the dashboard. With instrument doors closed, press the tray button. Wait for the autosampler to stop in the forward position. Open the front door and place the plate assembly onto the instrument in Position A. Close the instrument door.

In the dashboard of the **Data Collection** software, select the **Maintenance** icon and **Calibrate>Spectral** from the left task pane. Select **Matrix Standard** in the **Chemistry Standard** drop down box and Promega 5 Dye Matrix in the Dye Set drop down box. Verify that 96-well plate and Plate A position is selected and that **Allow Borrowing** is deselected. Select **Start Run**.

Evaluating the Spectral Calibration includes:

- Raw data profile from left to right-orange, red, yellow, green, blue
- No extraneous peaks in the raw data profile
- No gross dips, overlaps, or other irregular morphology

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- Spectral profile peaks are separate and distinct

Note: The Q-Value and Condition number will appear for each capillary and the plate diagram box will indicate a green box if the capillary passed or a red box if the capillary failed.

If the spectral calibration results meet the above listed criteria, click **Accept Results**. Click **View Spectral Report**. Select **Print** and print a PDF of the calibration Report. Save the PDF files in the designated location for the instrument.

If the spectral calibration does not meet the criteria, click **Reject Results** and attempt again.

4.3.4 Performing a Spectral Calibration (For use with WEN dye)

Note: A run cannot start unless a calibration file that matches the dye set and capillary array length is active. Every new spectral calibration is automatically the active one for that dye set.

It is necessary to perform a spectral calibration under the following circumstances:

- When a new dye set is being used on the instrument
- Service on the optics (realignment or replacing the laser, CCD camera, or mirrors)
- Presence of pull-up/pull-down in raw or analyzed data
- Capillary array is replaced on the instrument

Storage Conditions:

Upon receipt, store all components at -30°C to -10°C in a non-frost-free freezer, protected from light. Do not store reagents in the freezer door, where the temperature can fluctuate. After the first use, store the PowerPlex® 5C Matrix Standard components at $2-10^{\circ}\text{C}$, protected from light. It is strongly recommend that the PowerPlex® 5C Matrix Standard be stored with the post-amplification reagents. The PowerPlex® 5C Matrix Standard is light-sensitive; dilute the 5C Matrix Mix in Matrix Dilution Buffer in the provided amber tube. Store the diluted 5C Matrix Mix at $2-10^{\circ}\text{C}$ for up to 1 week.

Do not refreeze the PowerPlex® 5C Matrix Standards components.

Matrix Sample Preparation

1. At the first use, thaw the 5C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at $2-10^{\circ}\text{C}$, protected from light.

2. Vortex the 5C Matrix Mix for 10–15 seconds prior to use. Add 10 μl of 5C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds. Note the date of dilution on the tube.

Note: The diluted 5C Matrix Mix can be stored for up to 1 week at $2-10^{\circ}\text{C}$.

3. Add 10 μl of the diluted 5C Matrix Mix prepared in Step 2 to 500 μl of Hi-Di™ formamide. Vortex for 10–15 seconds.

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4. For the Applied Biosystems® 3500xL Genetic Analyzer, 24 wells are used for spectral calibration on 24 capillaries (wells A1 through H3 of a 96-well plate). Add 15µl of 5C Matrix Mix with formamide prepared in Step 3 to each of the 24 wells. After placing the septa on the plate, briefly centrifuge the plate to remove any air bubbles. *Do not heat denature.*

For the Applied Biosystems® 3500 Genetic Analyzer, 8 wells are used for spectral calibration on 8 capillaries (wells A1 through H1 of a 96-well plate). Add 15µl of 5C Matrix Mix with formamide prepared in Step 3 to each of the eight wells. After placing the septa on the plate, briefly centrifuge the plate to remove any air bubbles. *Do not heat denature.*

5. Place the plate in the 3500 series 96-well standard plate base, and cover with the plate retainer. Do not start the spectral calibration run until the oven is preheated to 60°C.

Instrument Preparation

Use of fresh polymer and a new capillary array results in an optimal spectral calibration.

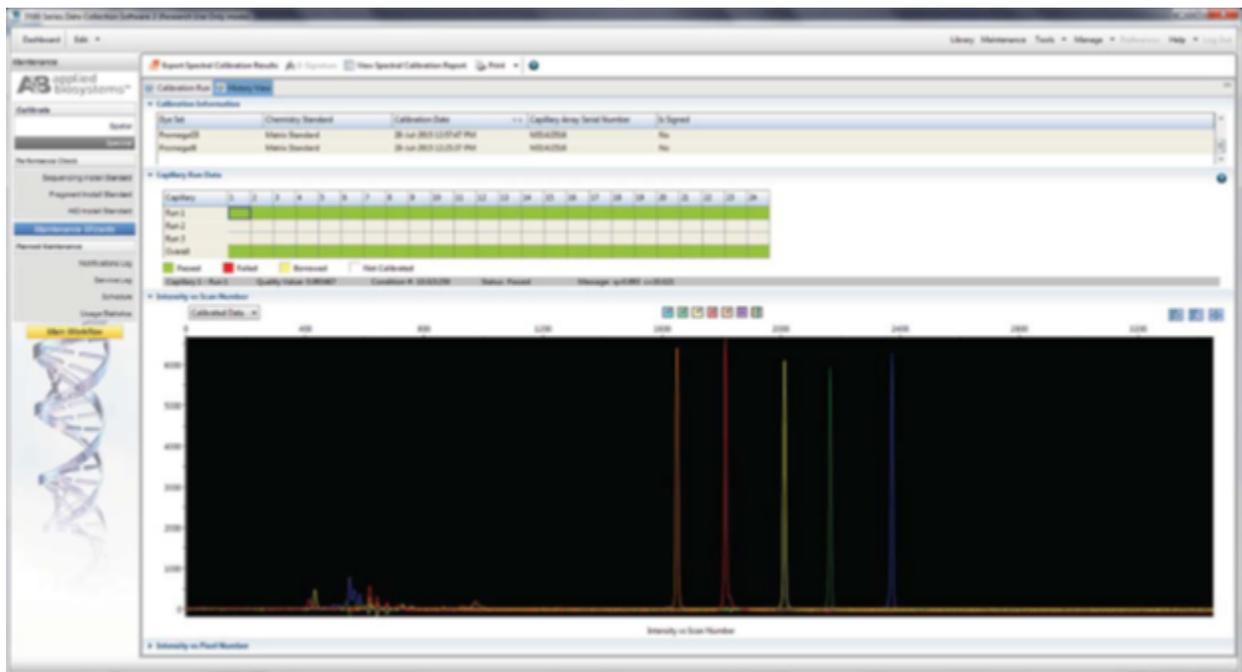


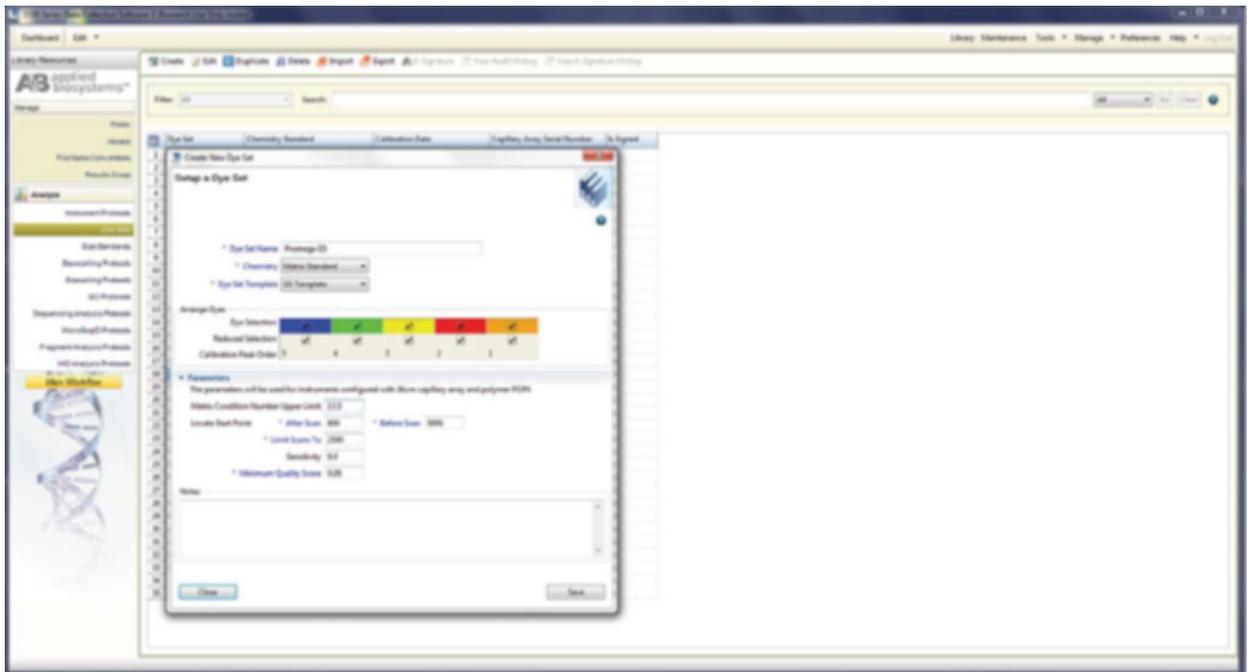
Figure 1. Representative data for the PowerPlex® 5C Matrix Standard on the Applied Biosystems®

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1. Set the oven temperature to 60°C, and then select the Start Pre-Heat icon at least 30 minutes prior to the first injection to preheat the oven.

2. To perform a spectral calibration for the Promega 5-dye STR amplification systems, a new dye set should be created. If a new dye set was created previously, proceed to Step 2.c.

- a. To create the new dye set, navigate to the Library, highlight “Dye Sets” and select “Create”.
- b. The Create New Dye Set window will appear (Figure 2). Name the Dye Set with a unique name for the 5 dye system, select “Matrix Standard” for the Chemistry and select “G5 Template” for the Dye Set Template. Under Parameters, change the After Scan number to 800 from the default number of 500. Select “Save”.



c. To perform the spectral calibration, go to the Maintenance tab, select “Spectral” and, under the Calibration Run tab, choose the appropriate fields: Choose “Matrix Standard” from the Chemistry Standard drop-down menu and the new Promega 5C dye set (e.g., Promega G5) created in Step 2.b from the Dye Set drop-down menu. Verify that 96-well plate and Plate A position is selected and that Allow Borrowing is deselected. Select Start Run.

d. Select “Start Run”.

If fewer than the recommended number of capillaries pass, the spectral calibration run may be repeated automatically up to three times. Upon completion of the spectral calibration, check the quality of the spectral in the Capillary Run Data display, and choose either “Accept” or “Reject”.

Evaluating the Spectral Calibration includes:

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- Raw data profile from left to right-orange, red, yellow, green, blue
- No extraneous peaks in the raw data profile
- No gross dips, overlaps, or other irregular morphology
- Spectral profile peaks are separate and distinct

Note: The Q-Value and Condition number will appear for each capillary and the plate diagram box will indicate a green box if the capillary passed or a red box if the capillary failed.

If the spectral calibration results meet the above listed criteria, click Accept Results. Click View Spectral Report. Select Print and print a PDF of the calibration Report. Save the PDF files in the designated location for the instrument.

If the spectral calibration does not meet the criteria, click Reject Results and attempt again.

4.3.5 3500/3500xL Genetic Analyzer Wizards

To access the wizards on the 3500/3500xL Genetic Analyzers, select the **Maintenance Instrument** icon in the dashboard of the **Data Collection** software.

Install Capillary Array Wizard

Use this wizard when installing a new capillary array on the instrument, installing a capillary array to reactivate an instrument that has been shut down, or installing a capillary array to replace a previously installed array. Select the **Install Capillary Array** wizard and follow the prompts. This wizard will include a water wash, polymer replenish, and bubble remove (if necessary).

Replenish Polymer Wizard

Use this wizard when replenishing the polymer supply, replacing the polymer in the polymer delivery pump with polymer of the same or different lot. Bring the polymer to room temperature before putting the pouch on the instrument. This wizard will include a bubble remove if necessary.

Remove Bubbles Wizard

Use this wizard to remove bubbles from the polymer delivery pump chamber, channels, and tubing. Select the **Remove Bubbles** wizard and follow prompts.

Wash Pump and Channels Wizard

Use this wizard to wash the polymer deliver pump, channels, and tubing with conditioning reagent. (Note: The software will not allow you to load a previously installed Conditioning Pouch). This wizard may also be used to remove suspected contaminants and/or persistent bubbles, to replace old polymer in the polymer deliver pump. Select **Wash Pump and Channels** wizard and follow the prompts. Use this wizard as weekly routine maintenance.

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Shut Down the Instrument Wizard

Use this wizard for short (1-2 weeks) and long term shutdown (more than two weeks). Select the **Shut Down the Instrument** wizard and follow the prompts. Check fluid levels on instrument and of stored capillary arrays periodically while in shutdown.

4.3.6 Additional Routine Maintenance

Replace the Anode Buffer Chamber and the Cathode Buffer Chamber. Click the **Refresh** button in the **Consumables Information** section of the dashboard.

Flushing and Filling the Water Trap

Flush the polymer delivery pump water trap with the nuclease free water at least once per week to wash out any diluted polymer and to clear bubbles. Leave the trap filled with nuclease free water.

Fill the supplied 25 ml, all-plastic luer lock syringe with nuclease free water. Expel any bubbles from the syringe. Do not use a syringe smaller than 25 ml to prevent excessive pressure within the trap.

Attach the syringe to the forward facing luer fitting on the pump block.

Open the luer fitting approximately one half turn counterclockwise. Flush the trap with approximately 5 ml of nuclease free water.

Remove the syringe and tighten the front facing luer by turning clockwise.

4.3.7 Computer Maintenance on the 3500/3500xL Genetic Analyzer

Prior to running samples on the 3500/3500xL genetic analyzer, the database space should be checked to ensure that there is sufficient space. To check the available space on Drive D, go to **My Computer**, select drive, right click with the mouse, and select **Properties**. At 78% full, the software will not start a run.

If there is insufficient space, archive the sample files and delete the samples from Drive D and empty the contents of the Recycle Bin. It is recommended by the manufacturer to restart the computer and instrument weekly.

It is recommended by the manufacturer to defragment the computer hard drive at least once every month and/or before fragmentation reaches 10% to optimize performance of Data Collection software and the computer operating system.

Backup the Run Folder, Spectral Calibration Report, and Spatial Calibration reports quarterly from each 3500 and/or 3500xL to a designated archive location. After archiving, remove Run folders that are older than 1 year from the instrument.