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4.5 Maintenance of the Applied Biosystems 3500/3500xl Genetic Analyzer

The Applied Biosystems 3500/3500xl Genetic Analyzer with 3500 Series Data Collection Software is fluorescence-based DNA analysis instrument using capillary electrophoresis technology with 8 or 24 capillaries.

The Applied Biosystem 3500/3500xl Genetic Analyzer is identified as a critical instrument requiring a maintenance schedule to ensure proper operation of the instrument. Regular weekly, monthly, and semiannual maintenance is described in the [User's Guide](#).

Planned Maintenance shall be performed by an authorized Applied Biosystems Technician once per calendar year.

The maintenance shall be documented using STACS-CW. All accompanying results, data and documents shall be stored in STACS-CW. Refer to [Technician Instrument Maintenance](#).

4.5.1 3500/3500xl Genetic Analyzer Wizards

Maintenance tasks may be conducted with the use of wizards. To access the wizards, select the Maintenance Instrument icon in the dashboard of the Data Collection software. The available wizards are:

- Install Capillary Array Wizard
- Replenish Polymer Wizard
- Remove Bubbles Wizard
- Wash Pump and Channels Wizard
- Shut Down the Instrument Wizard


4.5.2 Maintenance Schedule

Weekly maintenance:

- Ensure array tip is covered in the reservoir.
- Shutdown and restart the computer and instrument. (4.5.13)
- Clean the surface of the 3500 instrument with deionized water and a lint free cloth.
- Flushing and Filling the Water Trap (4.5.3)
- Empty the oven condensation reservoir.
- Run the Wash Pump and Channels wizard, manual capillary wash, Replenish Polymer wizard (4.5.4 – 4.5.6)
- Replace the polymer
- Refresh Dashboard and check off maintenance schedule tasks (4.5.7)

Biweekly maintenance:

- Replace the cathode and anode buffers (4.5.8)
- Refresh Dashboard and check off scheduled maintenance tasks

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Monthly Maintenance:

- Check Disk Space (4.5.9)
- Defragment the hard drive
- Check off maintenance schedule tasks

As needed or indicated:

- Capillary Replacement (4.5.10)
- Spatial Calibration (4.5.11)
- Spectral Calibration (4.5.12)
- Shut Down the Instrument/Reactivate Instrument (4.5.13)
- Audit Summary File (4.5.14)

Annual Planned Maintenance:

- Performed each calendar year by an authorized Applied Biosystems Technician

Performance Verification: (4.5.15)

- Following an annual planned maintenance or repair.

Signage on the instrument shall indicate the instrument is unavailable for use.


Shutdown and Restart the computer and instrument:

- Close out of all computer windows and 3500 Data Collection Software
- Turn off the instrument, wait 30 seconds
- Turn on the computer and log into Windows Vista OS
- Turn on the instrument
- Wait until the green light on the instrument appears
- When the 3500 Data Collection Software icon on the bottom right icon shows a green check mark, open the desktop 3500 Data Collection Software icon.

4.5.3 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. **Note:** DO NOT USE EXCESSIVE FORCE when you push the syringe plunger as this may damage the trap seals. Take ~30 seconds to flush.
- Remove the syringe and tighten the front facing luer by turning clockwise
- Empty the reservoir as needed.

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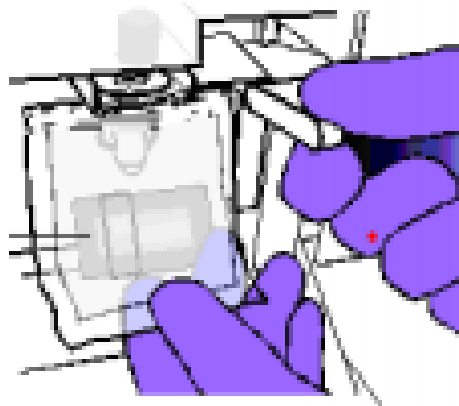
4.5.4 Running Pump Chamber and Channels Wash


To avoid damage to the pump assembly. The polymer delivery pump can be irreversibly damaged if:

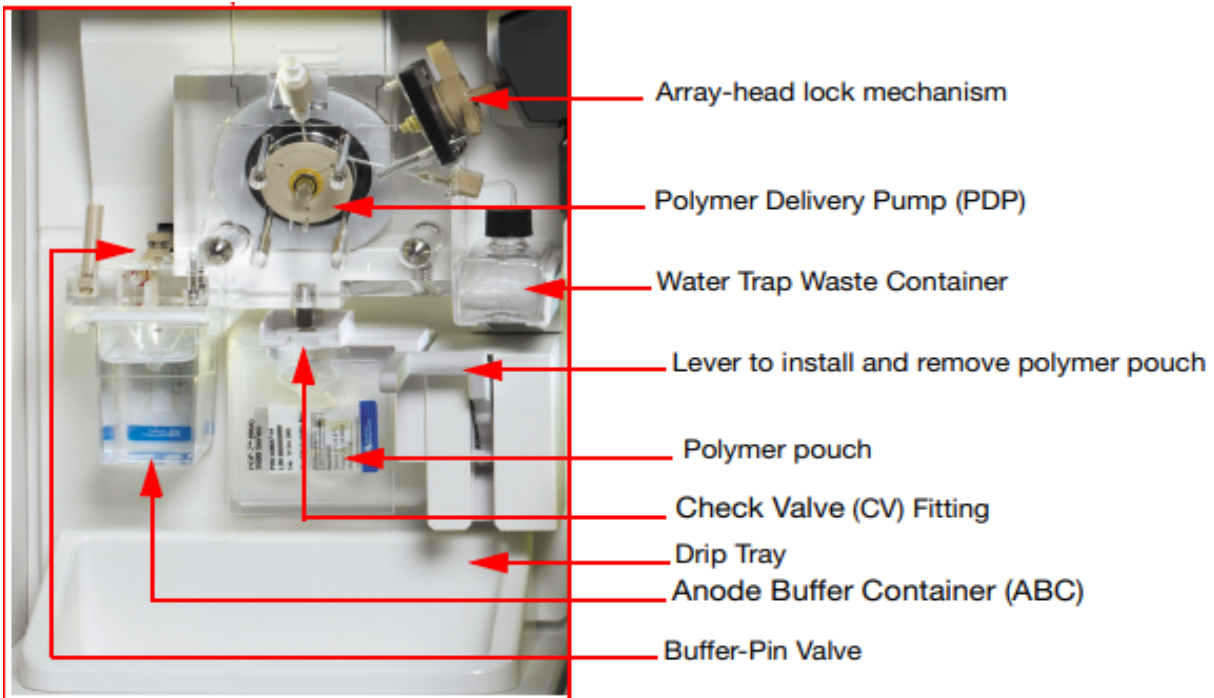
- Polymer dries in the polymer channels of the pump assembly, which can scratch the channels in the pump and can cause blockage.
- The pump assembly is exposed to organic solvent (alcohol) which can cause cracking and clouding of the acrylic pump material.
- The pump assembly is exposed to temperatures greater than 40°C which can damage the pump components.
- There is arcing in the pump assembly, which can damage the acrylic pump material.

Possible sources of contaminants may be introduced when installing or removing a polymer pouch or conditioning reagent at the check valve fitting.

As the lever mechanism is moved up or down, with your free hand assist the manual lever by pushing up or pulling down on the “shelf” area (highlighted yellow) where the collar of the pouch is inserted.



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Performing the Pump Chamber and Channels Wash:


- Remove the Anode Buffer cup, set the Anode Buffer aside if reinstalling, and replace with empty container below the valve.
- In the Dashboard, click Wizards
- In the Maintenance wizards screen, click Wash Pump and Channels
- Follow the prompts in the Wash wizard window
- When the water (conditioning reagent) wash portion of the wizard is completed and **before choosing next** continue to Manual Capillary Wash instructions.
- The wizard takes >40 minutes to complete

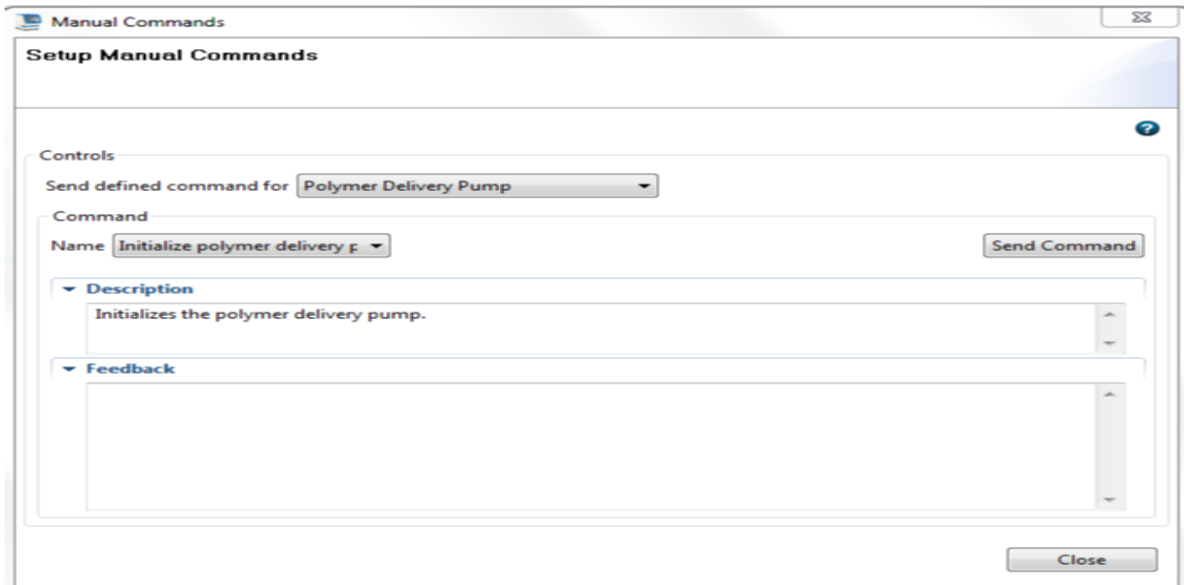
4.5.5 Performing the Manual Capillary Wash

To avoid polymer build up within the capillaries and to flush any contaminants.

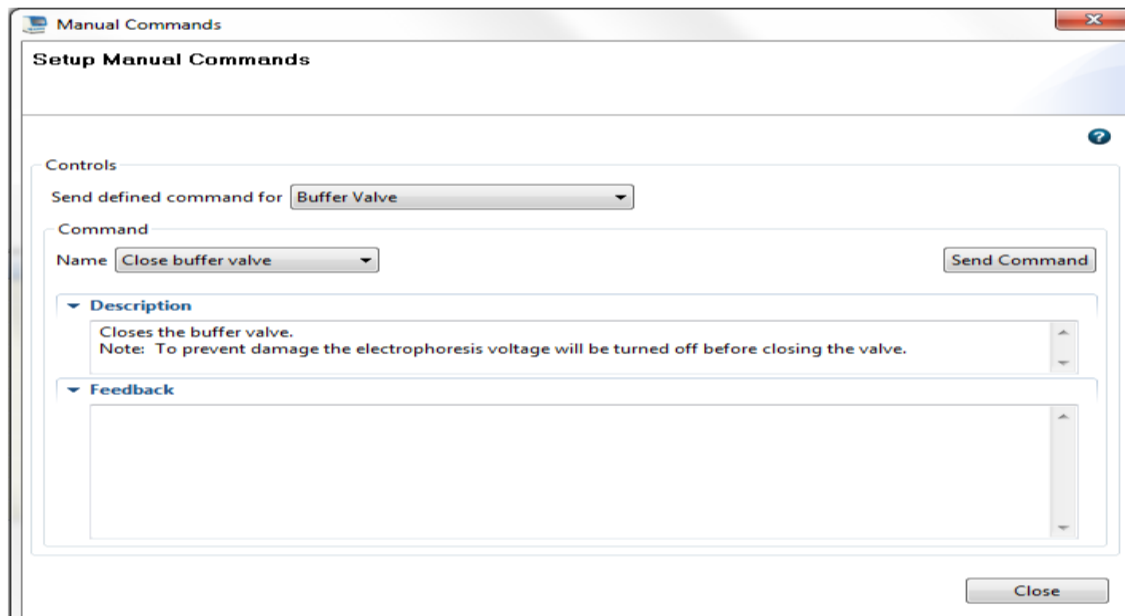
The instrument door should be open and the tray forward before while performing the following steps.

- Once the water (conditioning reagent) wash portion of the Wash Pump and Channels wizard is completed and before choosing NEXT to the polymer step, choose CANCEL.
- A pop-up window warning will appear, choose YES.
- The conditioning reagent should remain on the instrument.
- From the top right tool bar choose TOOLS then Manual Command.


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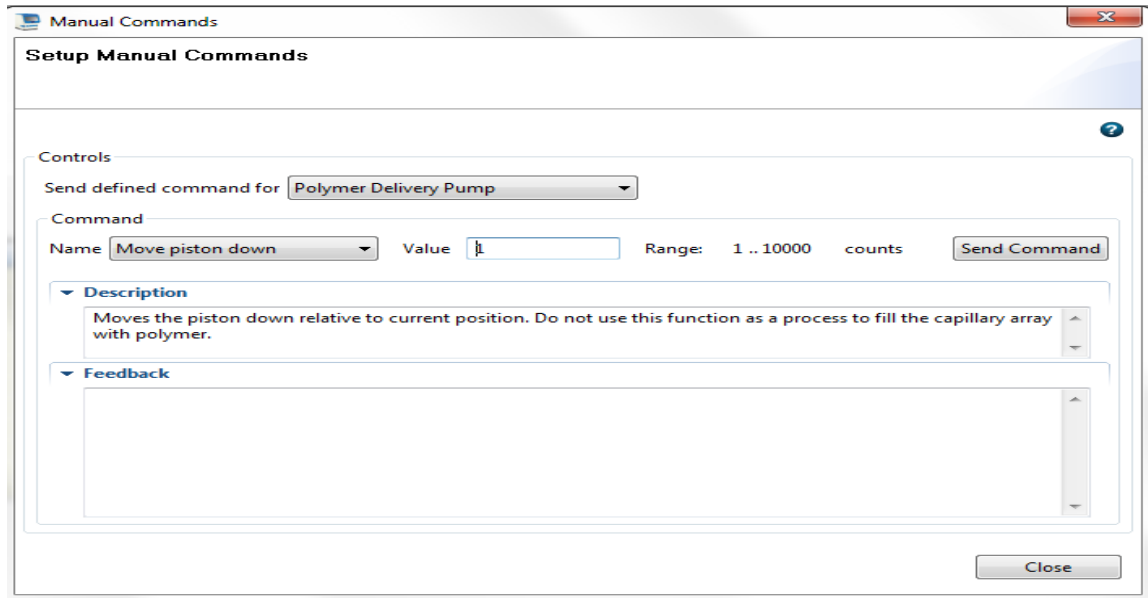
- Under the Controls heading, choose Polymer Delivery Pump from the Send defined command drop-down
- Under the Command heading, choose Initialize Polymer Delivery Pump from the Name drop-down
- Click the Send Command button. The pump chamber will fill with conditioning reagent.



- Return to Controls heading, choose Buffer Valve from the Send Defined Command drop-down

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- Command heading, choose Close Buffer Valve from the Name drop-down
- Click the Send Command button. There will be an audible sound when the buffer valve closes.



- Return to the Controls heading, choose Polymer Delivery Pump from the Send Defined Command drop-down
- Command heading, choose Move Piston Down from the Name drop-down
- Enter 100 in the Range Value box
- Click Send Command


This step will begin to push conditioning reagent through the capillary flushing the polymer from the capillary. The polymer is viscous, it's important to flush slowly during the initial 5-6 commands to avoid undue pressure. Place a kimwipe under the capillaries to absorb any water that may drop from the capillaries as they are flushed.

Repeat the above steps 5-6 times with a 2-5 second delay between each command.

CAUTION: The enter button on the keyboard also acts as Send Command. Do not press enter multiple times when moving the piston down.

Once the polymer has been cleared from the capillaries you may flush at a greater rate.

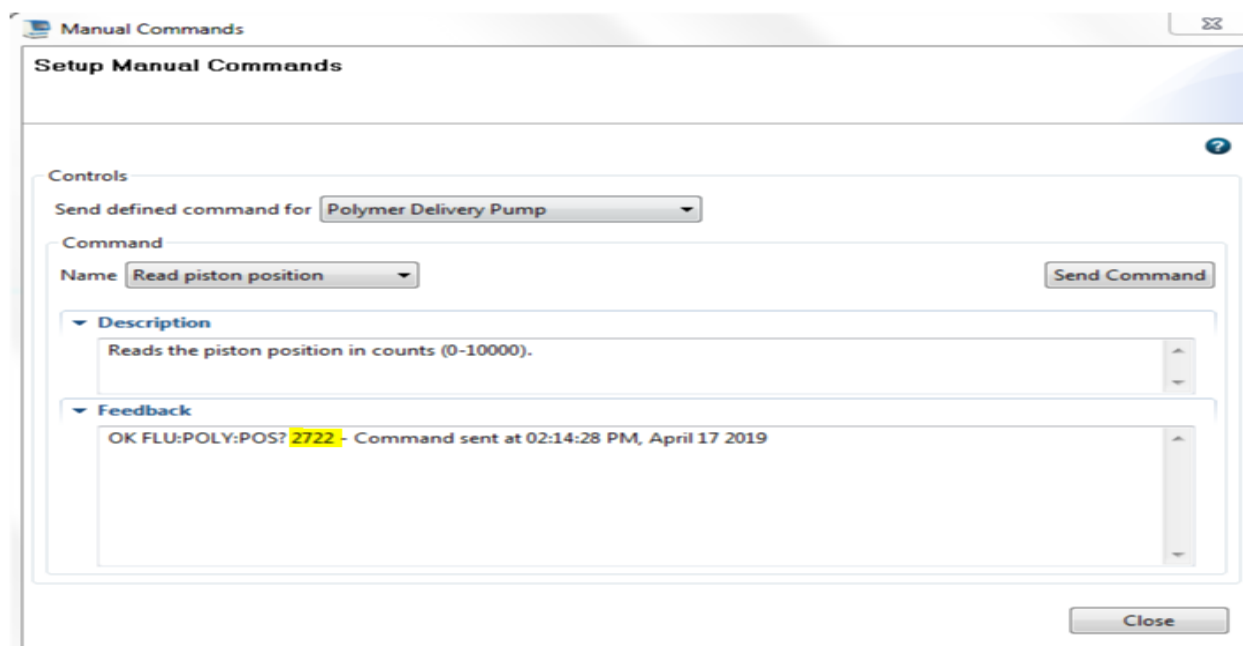
- Enter 1,000 in the Range Value box
- Click Send Command

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CAUTION: Be aware of the piston location within the pump chamber. The Range Value shows as 1 – 10,000. The actual Range is 0 - 5,000. 0 represents the chamber is completely full and 5,000 is empty and the piston is fully forward in the chamber. DO NOT push the piston down beyond a value of 5,000. You may determine the exact location of the piston value by performing the following:

- Control heading command should be Polymer Delivery Pump
- Command heading choose, Read Piston Position from the Name drop-down
- Click Send Command

Below the piston is at a value of 2722




If you near the maximum piston position of 5,000 the pump piston should be re-initialized to fill the pump chamber with conditioning reagent.

- Controls heading, choose Polymer Delivery Pump from the Send defined command drop-down
- Under the Command heading, choose Initialize Polymer Delivery Pump from the Name drop-down
- Click the Send Command button. The pump chamber will fill with conditioning reagent.

The buffer valve must be closed again before proceeding.

Return to Controls heading, choose Buffer Valve from the Send Defined Command drop-down
 Command heading, choose Close Buffer Valve from the Name drop-down
 Click the Send Command button. There will be an audible sound when the buffer valve closes.

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Continue to flush the capillaries.

- Return to the Controls heading, choose Polymer Delivery Pump from the Send Defined Command drop-down
- Enter 1,000 in the Range Value box
- Click Send Command
- Repeat 3-4 times

The capillary wash is completed, Manual Commands window may now be closed.

4.5.6 Replenish Polymer

- Remove the conditioning reagent
- Wipe the pin valve with a kimwipe
- Install the new appropriately sized polymer pouch

Performing Replenish Polymer

- In Dashboard, click Wizards
- Click Replenish Polymer
- Follow the prompts of the wizard
- You will get a warning pop-up you have not changed the polymer choose yes you would like to proceed.
- Remove any bubbles from the polymer pouch when prompted by the wizard, repeat as many times as necessary.

4.5.7 Refresh Dashboard Tasks


Calendar Reminders Pane on the Dashboard list the tasks and dates maintenance is due. Tasks which are completed are logged in the Notification Log.

When you complete a task, click the green check mark
If a task is not to be completed, click the red x.

4.5.8 Installation of Buffers

The Cathode Buffer provides a rinse position to rinse the capillary tips and a waste position for expelled polymer. The Anode Buffer is in the ground position of the instrument.

- Allow buffers to reach ambient temperature.
- Ensure the Anode buffer is in the larger side of the container before removing the seal. The narrow side should remain empty to collect waste.
- Remove the seal from the Cathode Buffer Container (CBC). With a kimwipe, remove any residual liquid from the top of the container and is completely dry. Moisture can cause arcing and termination of a run.
- Place appropriate septum on each side of the CBC, ensuring the septa are firmly seated on the container and no gaps are visible.
- Install buffers on the instrument.

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4.5.9 Performing the monthly disk cleanup and defragmentation

Use the defragment icons on the computer desktop to perform this task

- Click Analyze to see if you should defragment
- Choose Defragment if indicated

When disk space is within 20% of maximum capacity, transfer the older data to a backup storage device.

- Go to My Computer, right-click the D: drive, then select Properties>General
- When the hard disk is 70-75% full, the software will not run. Move the sample file data to a designated hard drive location.

4.5.10 Installing a Capillary Array (8cap and 24cap)

A new capillary array is indicated when the expiration date of the capillary has been reached or if there is an ongoing loss of resolution in the capillary electrophoresis data. The number of injections at which the resolution degrades varies from array to array. Applied Biosystems warranties the capillary array up to 160 injections.

Possible contamination may occur when installing the capillary array. This may occur by overtightening the array knob which may cause the capillaries to break. It can also occur if the array knob is too loose which may result in an insufficient seal between the array port and polymer block causing leakage of the polymer. An insufficient seal could also cause an improper polymer fill of the capillary array. Dried polymer on the threads inside the array port is an indication of an improper seal.

During a capillary removal, note how tightly closed the knob is before loosening. Re-tighten the knob to finger tight, then slowly tighten to gauge how much further the knob will tighten. Once satisfied, remove the old capillary and inspect the array port threads for evidence of dried polymer. If there is polymer present, dampen an Applied Biosystems foam swab with sterile water to clean the port and threads. Use a second foam swab to dry the interior of the port.

Take into consideration how tight the array knob of the old capillary had been and if there was polymer present in the port when tightening the array knob of the newly installed capillary.

During the life of the capillary, visually inspect around the array knob for signs of polymer leakage. Do not loosen the array knob to inspect.

In the Dashboard, click Wizards

In the Maintenance wizards screen, click Install Capillary Array.


Follow the prompts in the Install Capillary Array wizard window.

Check the Quick View section of the Dashboard for updated status of the capillary array.

4.5.11 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

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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.

Complete the maintenance in STACS-CW. Save the Spatial Report to the files pane.

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

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

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.

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.


4.5.12.1 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°C, protected from light.

2. Vortex the 5C Matrix Mix for 10–15 seconds prior to use. Add 10µl 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°C.

3. Add 10µl of the diluted 5C Matrix Mix prepared in Step 2 to 500µl 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.

4.5.12.2 Instrument Preparation

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

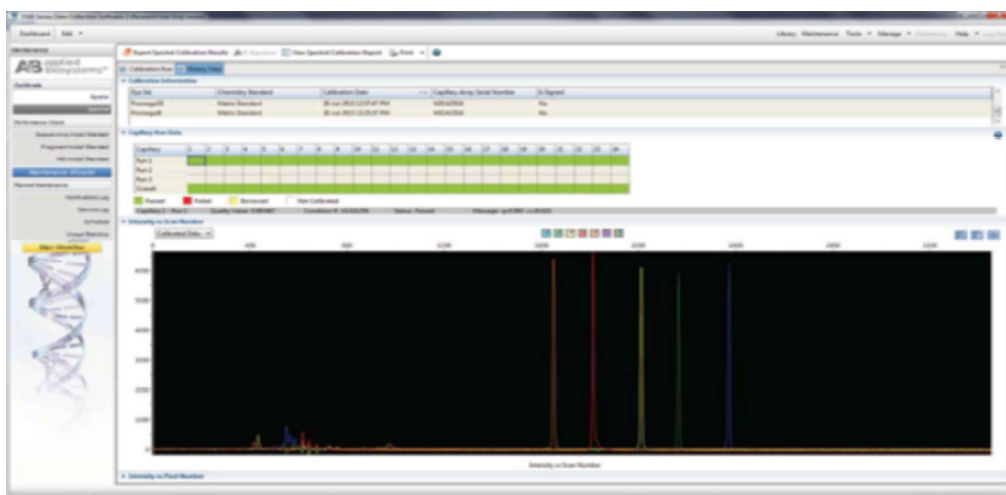



Figure 1. Representative data for the PowerPlex® 5C Matrix Standard on the Applied Biosystems®

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".

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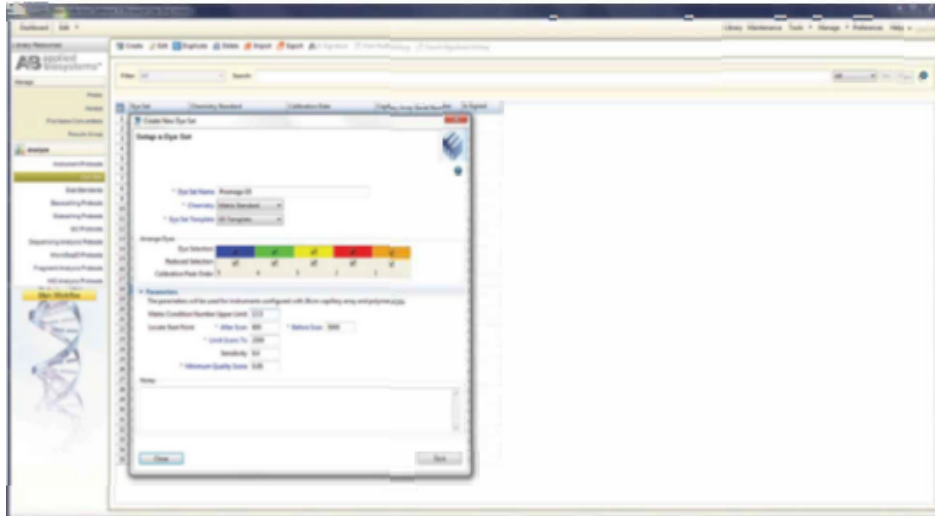


Figure 2

- c. To perform the spectral calibration, 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 **de-selected**.
- d. Select "Start Run"

If fewer than the recommended number of capillaries pass, the spectral calibration run will 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:


- 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.

Complete the maintenance in STACS-CW, save the Spectral Report to the files pane. Note the number of injections on the current capillary array in the comment pane. Or if in the case of a capillary installation, the number of injections of the prior array in the comment pane.

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

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4.5.13 Shut Down the Instrument/Reactivate Instrument

This procedure is used if an instrument will be idle for 1-2 weeks. Long term shutdown is used when the instrument will be idle for longer than two weeks or the instrument will be moved.

Short term shutdown:

- Install a conditioning reagent pouch before performing instrument shutdown.
- Keep the load-end of the capillary array in buffer to prevent the polymer from drying in the capillaries. If fluid level is low, add DI water to buffer solution.
- Install the new Cathode Buffer Container when ready to resume runs.

Long term shutdown:

- Install a conditioning reagent pouch before performing instrument shutdown.
- In the Dashboard, click Wizards
- Run the Install Capillary Wizard and store the capillary array.
- Clean any spills or residual polymer.
- Run the Shutdown the Instrument wizard.
- Unplug the instrument

Reactivate the Instrument: (if long term shutdown was performed)

In the Dashboard, click Wizards.

In the Maintenance wizards screen, click Reactivate the Instrument

Follow the prompts in the Instrument Reactivation wizard window.

4.5.14 Audit Summary File

The error 'Javaheap' may occur when attempting to save a plate on the 3500 instrument. The error occurs when the Audit Summary File reaches the maximum data capacity.


- Close the 3500 Software
- Navigate to D: drive>Applied Biosystems>3500>Datastore>Security
- Locate the "AuditSummary.aud" file
- Rename the file as "AuditSummary1.aud" If a file exists with the same name or there are multiple numbered files, use the next sequential number
- Reopen the software
- The software will automatically create another "AuditSummary.aud" file to store data

4.5.15 Performance Verification

Evaluate the performance of the instruments following repair, service, calibration or annual Planned Maintenance conducted by an authorized service technician.

A PM certificate and/or a Field Service Report shall be provided by an authorized service technician following periodic service calls resulting from instrument malfunction or scheduled maintenance. The documentation shall be maintained in STACS-CW. Refer to Technician Instrument Maintenance and specifically [Planned Maintenance/Performance Verifications](#).

Conducting a Performance Verification

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All Performance Verifications must include an allelic ladder, a 2800M positive control and amplification negative for each amplification kit being utilized on the instrument. These may include PowerPlex® Fusion, PowerPlex® Y-23 and Promega Direct Amplification kit.

If the service included adjustments to the optics (realignment, replacing the laser, CCD Camera or mirrors) a spectral calibration shall be performed.

Prior to the instrument re-activation for casework samples, the base pair sizes, allele calls and relative peak heights of the 2800M positive control shall be compared to the recorded positive control evaluated prior to the repair service or calibration. Supervisor or their designee approval shall be noted.

If a spectral was generated as part of the Performance Verification, the effectiveness of the spectral shall be evaluated according to 4.5.12. Supervisor or their designee approval shall be noted.