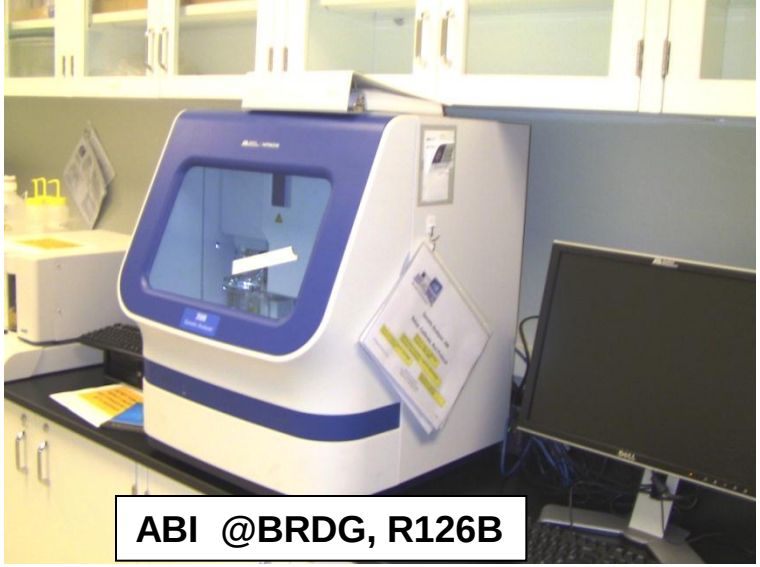


ABI 3500 Genetic Analyzer



ABI @BRDG, R126B



IKA MTS Vortex/Timed
Use with ABI protocols

Genetic Analyzer: ABI Setup and Run Protocol

Windows Logon ID: 3500-User
Password: 3500-USER

Delete LOGON boxes before publishing)

3500 Run/Data Acquisition Logon ID: Administrator
Password: Administrator1

Sequence Analysis Logon ID : SLCC3500
Password: password

Gene Mapping Logon ID:gm
Password: SLCC3500

Prepared by: Bob Morrison
STLCC-CPLS, Instrumentation Specialist
Initiated: Sep 10, Last Update Jan 2013

Genetic Analysis: ABI Videos; Basic System Overview, Big-Dye Sequencing, Fragment Analysis

ABI Big Dye Direct Sequencing Process
http://www.youtube.com/watch?v=cqFTGQ_JO1c&feature=relmfu

ABI 3500 System Overview for Sequencing and Fragment Analysis
http://www.youtube.com/watch?v=RY2Rn2ggjdA&feature=relmfu

ABI Fragment Analysis
http://www.youtube.com/watch?v=43-OQTLtrwQ

Genetic Analyzer: ABI, Prep Protocols, Manuals

Link to ABI 3500 BigDye Xterminator Purification Kit Protocol …. 2 pgs (pdf)

Link to ABI 3500 BigDye Terminator v3.1 Cycle Sequencing Kit Protocol .. 75 pgs (pdf)

Link to Summary SequencingUsing BigDye Terminator Mix (B.Boedeker) Protocol…. (pdf)

Link to Detailed Sequencing Using BigDye Terminator Mix (B.Boedeker) …(pdf)

Link to ABI BigDye Xterminator Purification Kit Protocol Summary Brochure (ABI) …(pdf)

Link to EDTA/Ethanol Precipitation of Cycle Sequenced Products (Amy Smith, B.Boedeker) …(pdf)

Link to Sequencing Tips and Troubleshooting (B.Boedeker) …(pdf)

Link to ABI 3500 User Manual… pdf (18mb)

Link to BioRad Complete Cloning and Sequencing Explorer Series Curriculum Manual.pdf (24mb)

Genetic Analyzer: ABI, Summary of Steps (recommended order)

- **Start instrument via Power on button:** wait for green light on front panel
- **Start computer**: Boot PC, Login Windows as 3500-USER, wait for Server Monitor green check mark in the bottom tray
- **Define Results Folder:** Locate RUNs folder on desktop, add/create a unique folder named for this run
- **Start 3500 Application:** wait for dashboard
- **Dashboard/Tray:** Install Cathode and Anode buffer containers, refresh RFID tags (Dashboard)
- **Maintenance Wizard**: Remove Conditioning pouch and Install Polymer pouch, Fill Array with fresh polymer
- **Check for Bubbles:** Inspect polymer lines, if necessary run Bubble Clean wizard
- **Tray:** Install Sample Plate
- **Dashboard:** Start pre-heat Oven
- **Dashboard:** Create Plate from templates, selecting for polymer, cap#, type of analysis
- **Create Plate:** Sweep active wells, then **c**heck boxes below for Assay, Filenames
- **Select Results Folder/Location**: From Dashboard, Select Preferences, select User, Run Setup , then browse to the D:/RUNS folder and the new folder established above for this run
- **Create Plate:** Link Plate to Run at bottom of screen
- **Start Run:**
- **Monitor Run:**
- **View Results:**

- **After Run: Be sure to remove the polymer pouch and replace with a Conditioning pouch unless other runs are imminent.**

Genetic Analyzer: ABI, Workflow Part I

(pages refer to User Manual)

Start the system:

1. Start the instrument (page 22).
2. Start the computer (page 24).
3. Check maintenance notifications in the Dashboard (page 28).
4. Check consumable status in the Dashboard (page 29).
5. Replenish consumables (page 31).

Set up and run:

1. Prepare the instrument (page 42).
2. Preheat the oven (page 42).
3. Check instrument status (page 53).

1. Create or import a plate (page 43).
2. Assign plate contents (page 46).
3. Print the plate layout (page 50).
4. Prepare and load sample plates (page 51).

Quick Start a run (page 55).

Genetic Analyzer: Workflow Part II (pages refer to User Manual)

1. Load plates for run and create the injection list (page 56).
2. Review and modify the injection list in Preview Run (page 59).
3. Start the run (page 61).
4. Monitor the run (page 61), check sequence or sample quality and specify re-injections (page 62).

Review sequencing results:

1. Review sequence quality (page 81).
2. Specify re-injections (page 95).
3. Review quality reports (page 85).
4. Export sequencing results (page 87).

Review fragment/HID results:

1. Review sample quality (page 89).
2. Specify re-injections (page 85).
3. Review quality reports (page 95).
4. Export sizing results (page 96).

(Optional) print or save (.pdf) calibration and performance check reports to save with results:

- Spatial calibration (page 99)
- Spectral calibration (page 103).
- Sequencing install standard performance check (page 122).
- Fragment or HID install standard performance check (page 132).

Genetic Analyzer: ABI 3500, Startup

1. Turn on the instrument and wait for a steady GREEN light. Then boot up the Computer and logon.

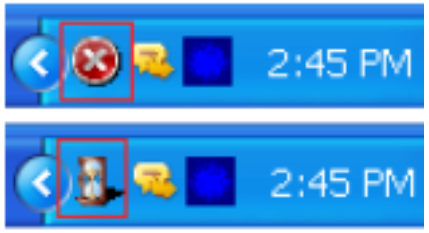
IMPORTANT! The status icon, on the right lower-corner of your screen, shows when the 3500 Server Monitor is active by displaying the icon shown here.



If the Server Monitor does not start automatically, launch the Server Monitor:
Start ▸ Programs ▸ Applied Biosystems ▸ 3500 ▸ Server Monitor



Note: It will take approximately 2 minutes for the Server Monitor to set up. During this time, you will see the status icon transition from a red circle, with an X in the middle (indicating that not all 3500 services are loaded) to the shape of an hour-glass on your desktop, next to the clock.



When Server Monitor set up is complete, the icon in the shape of an hour-glass will disappear and a checkmark icon appears indicating that the 3500 Server Monitor has started and all 3500 services loaded.



Genetic Analyzer: ABI; Check Consumables; Preheat

(references are pages in the user manual)

1. In the Dashboard, check consumable status (page 29). Ensure that:
   - Consumables are not expired
   - Adequate buffer levels are at the fill lines.

2. Set the oven temperature, then click **Start Pre-heat**:
   - 60 °C – POP-7™ and POP-4™ polymers
   - 50 °C – POP-6™ polymer

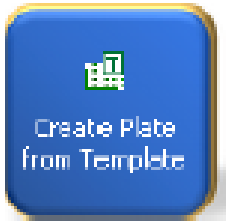
   Pre-heat the oven and detection cell while you prepare for a run (detection cell temperature is set by the software). Preheating helps mitigate subtle first-run migration rate effects. The preheat function automatically turns off after 2 hours of instrument inactivity.

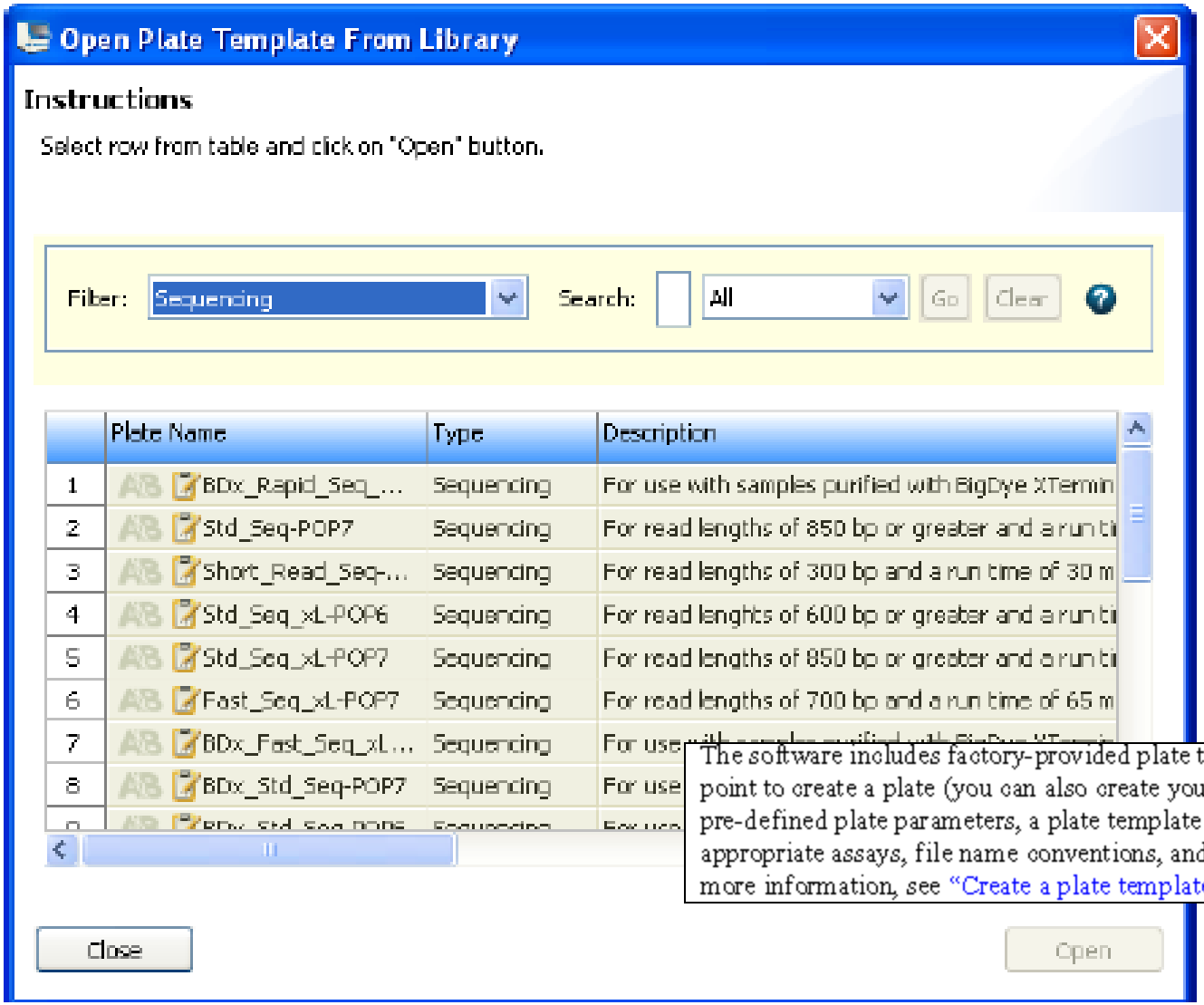
   Applied Biosystems recommends that you pre-heat the oven for at least 30 minutes before you start a run if the instrument is cold.

3. Check the pump assembly for bubbles and run the Remove Bubble wizard if needed (see page 251).

Genetic Analyzer: ABI; Setup, Create a Plate

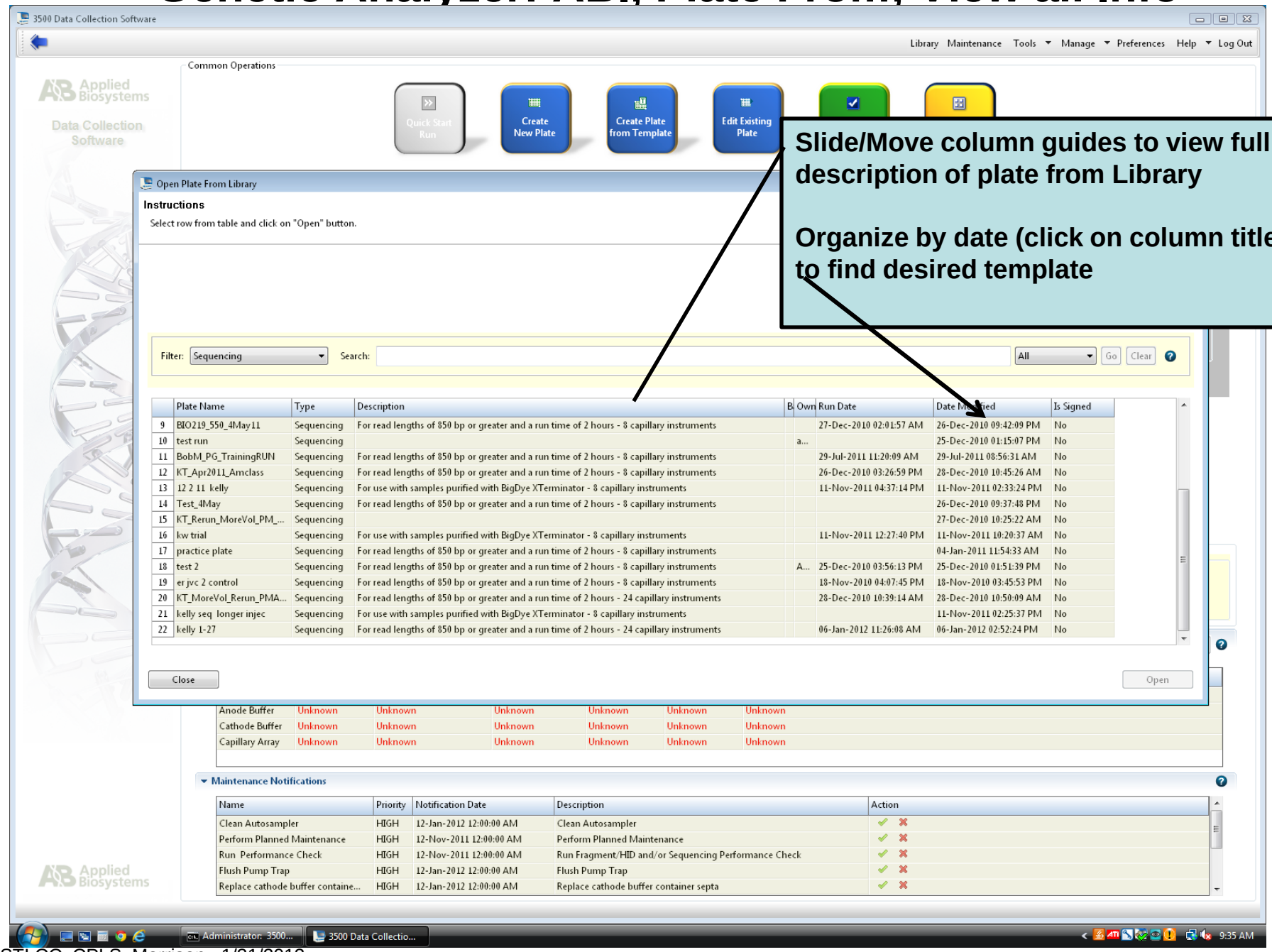
1. In the Dashboard, click **Create Plate From Template** to display the Open Plate Template from Library dialog box.



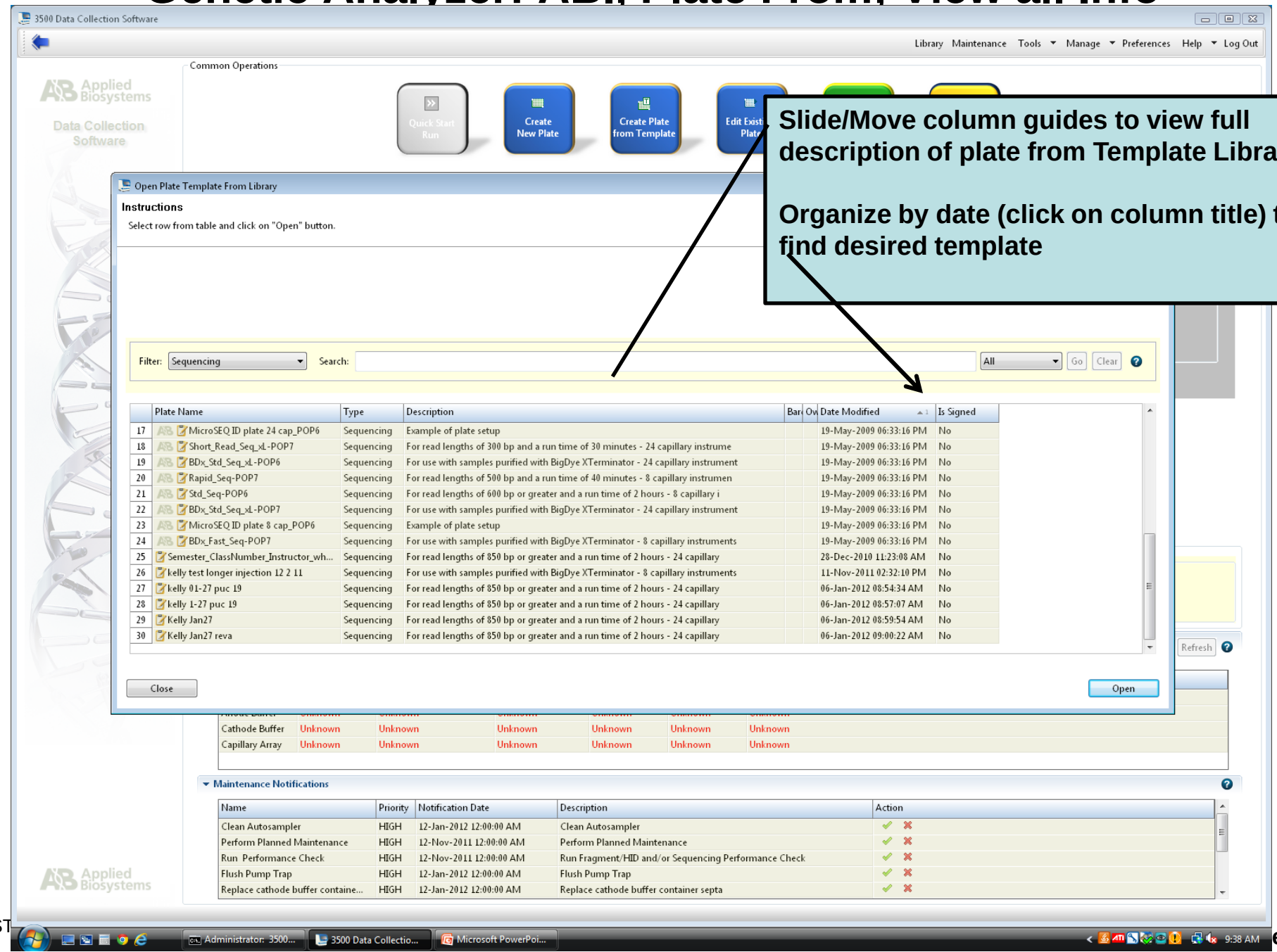


The software includes factory-provided plate templates that you can use as a starting point to create a plate (you can also create your own plate templates). In addition to pre-defined plate parameters, a plate template can also contain a list of the appropriate assays, file name conventions, and results groups for an application. For more information, see "Create a plate template" on page 75.

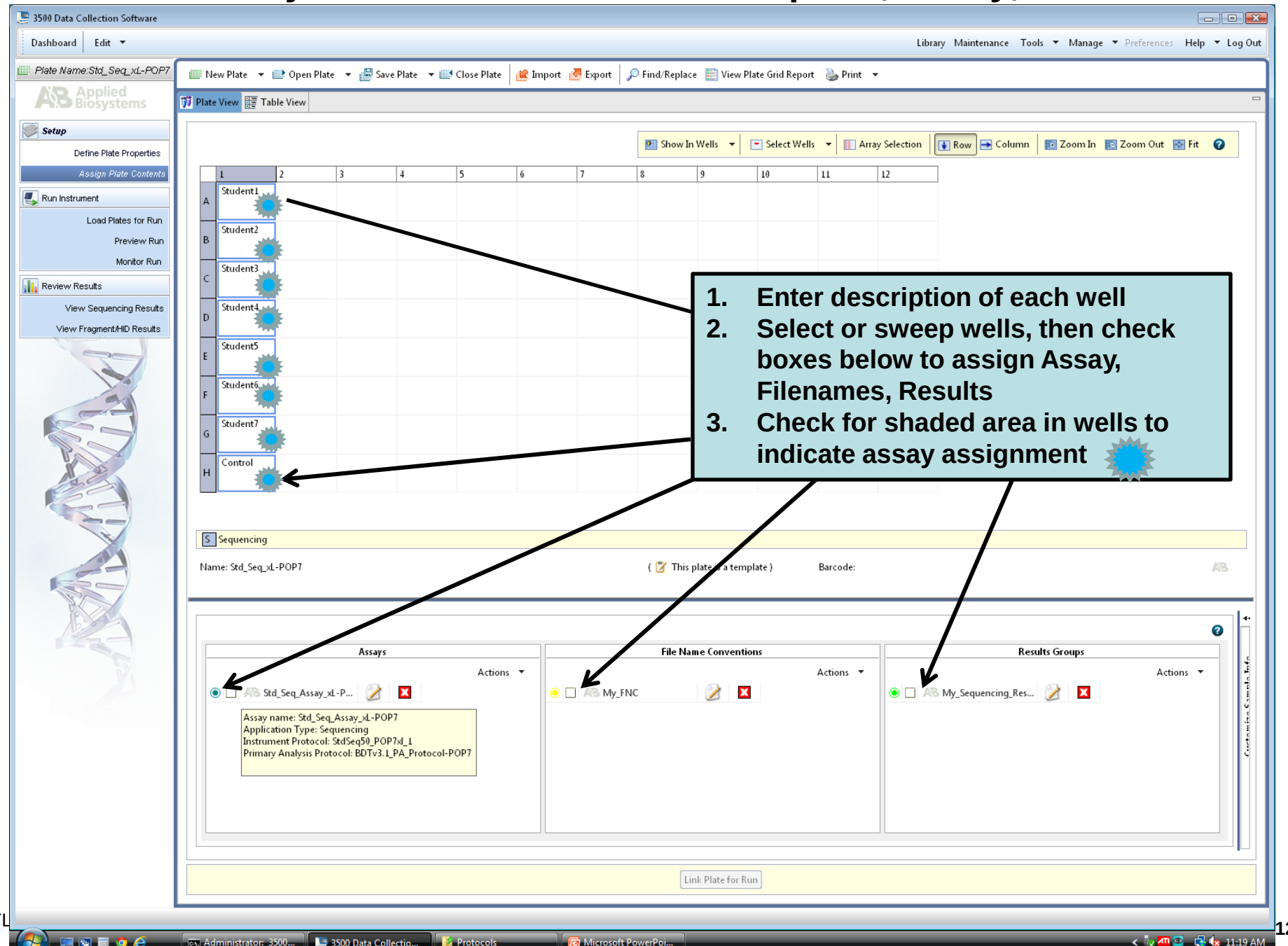
Genetic Analyzer: ABI, Plate From; View all Info



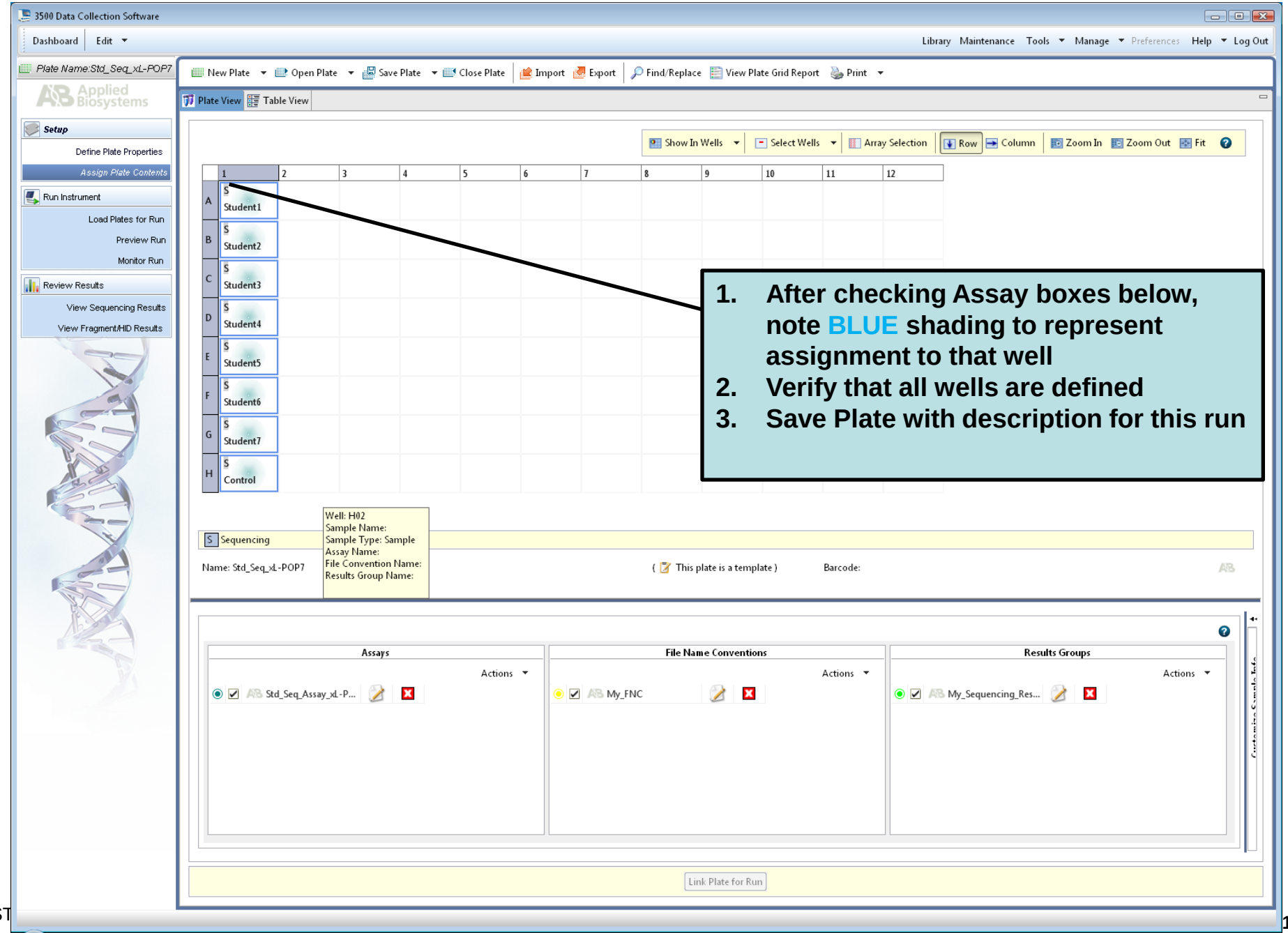
Genetic Analyzer: ABI, Plate From: View all Info



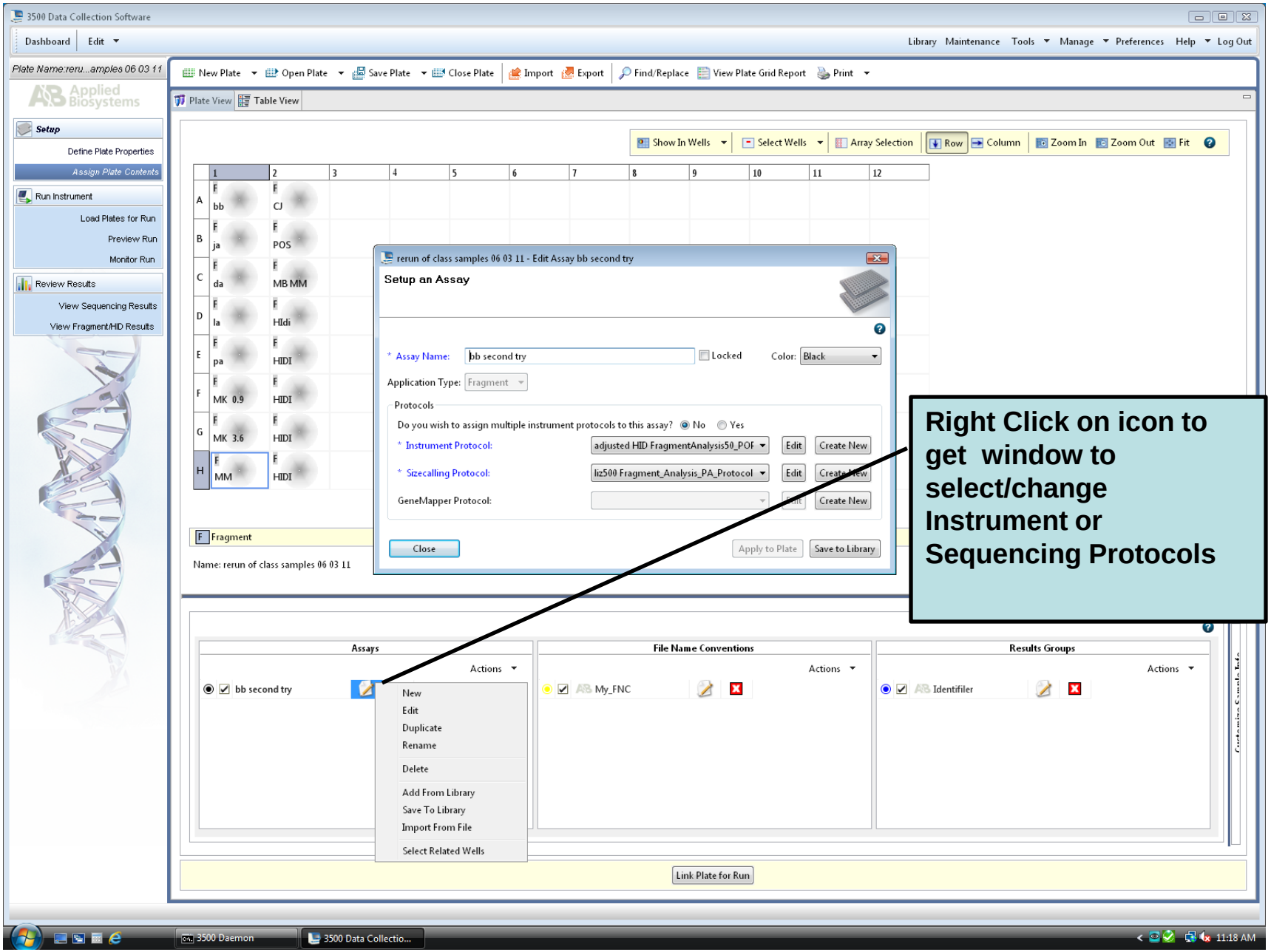
Genetic Analyzer: ABI: Enter Plate Description, Assay, Filenames



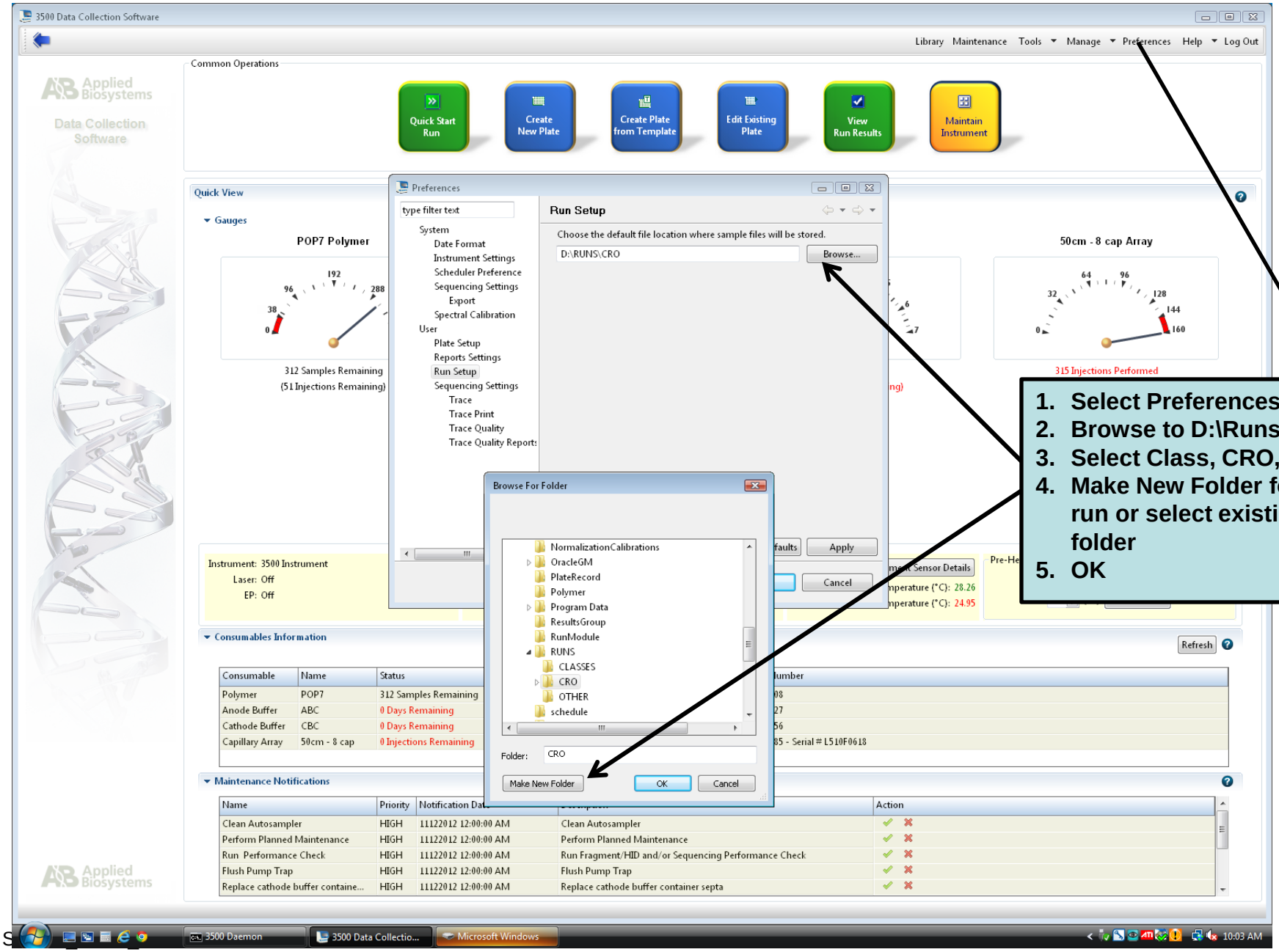
Genetic Analyzer: ABI: Enter Plate Description, Assay, Filenames



Genetic Analyzer: ABI, Setup, Change Assay Instrument Protocols



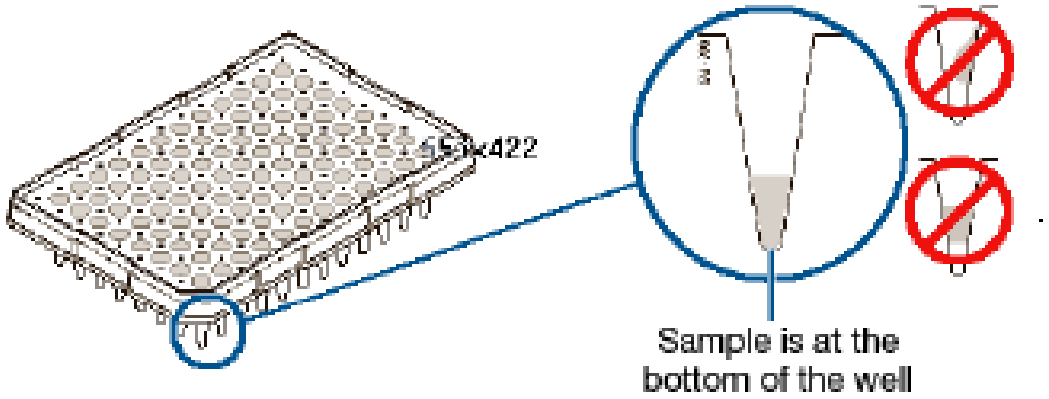
Genetic Analyzer: ABI, Setup, Preferences, Set Results Folder



1. Select Preferences
2. Browse to D:\Runs
3. Select Class, CRO, Other
4. Make New Folder for this run or select existing folder
5. OK

Genetic Analyzer: Prepare a Plate

1. Pipette samples into the plate according to the plate layout (see "Print the plate layout" on page 50).
2. Briefly centrifuge the plate.
3. Verify that each sample is positioned correctly in the bottom of its well.



IMPORTANT! If the reagents of any well contain bubbles or are not located at the bottom of the well, briefly centrifuge the plate, remove the plate from the centrifuge, and verify that each sample is positioned correctly in the bottom of its well.

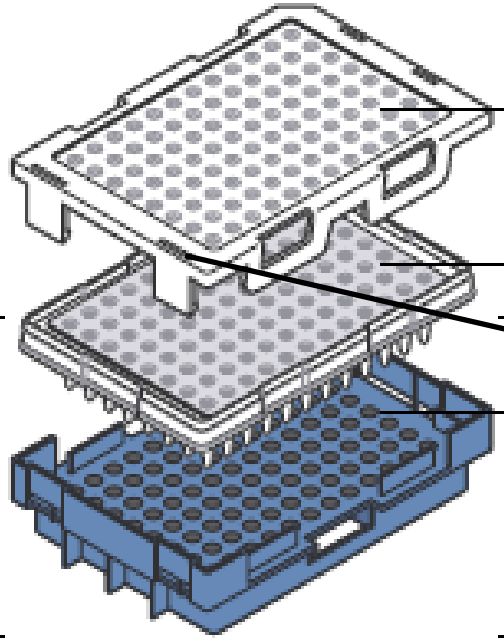
4. Store the plate on ice until you prepare the plate assembly and load the plate in the instrument.

Be sure to label the SAMPLE plate on the plate assembly before loading the centrifuge….

Genetic Analyzer: Prep & Load Plate Assembly

1. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.
2. Place the sample plate into the plate base.

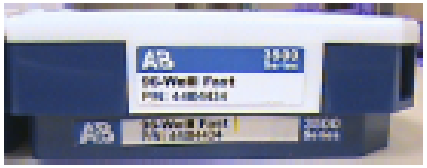
IMPORTANT! Make sure to use the correct plate base for standard plates versus 8-tube strips and fast plates. Using the wrong plate base may affect performance.



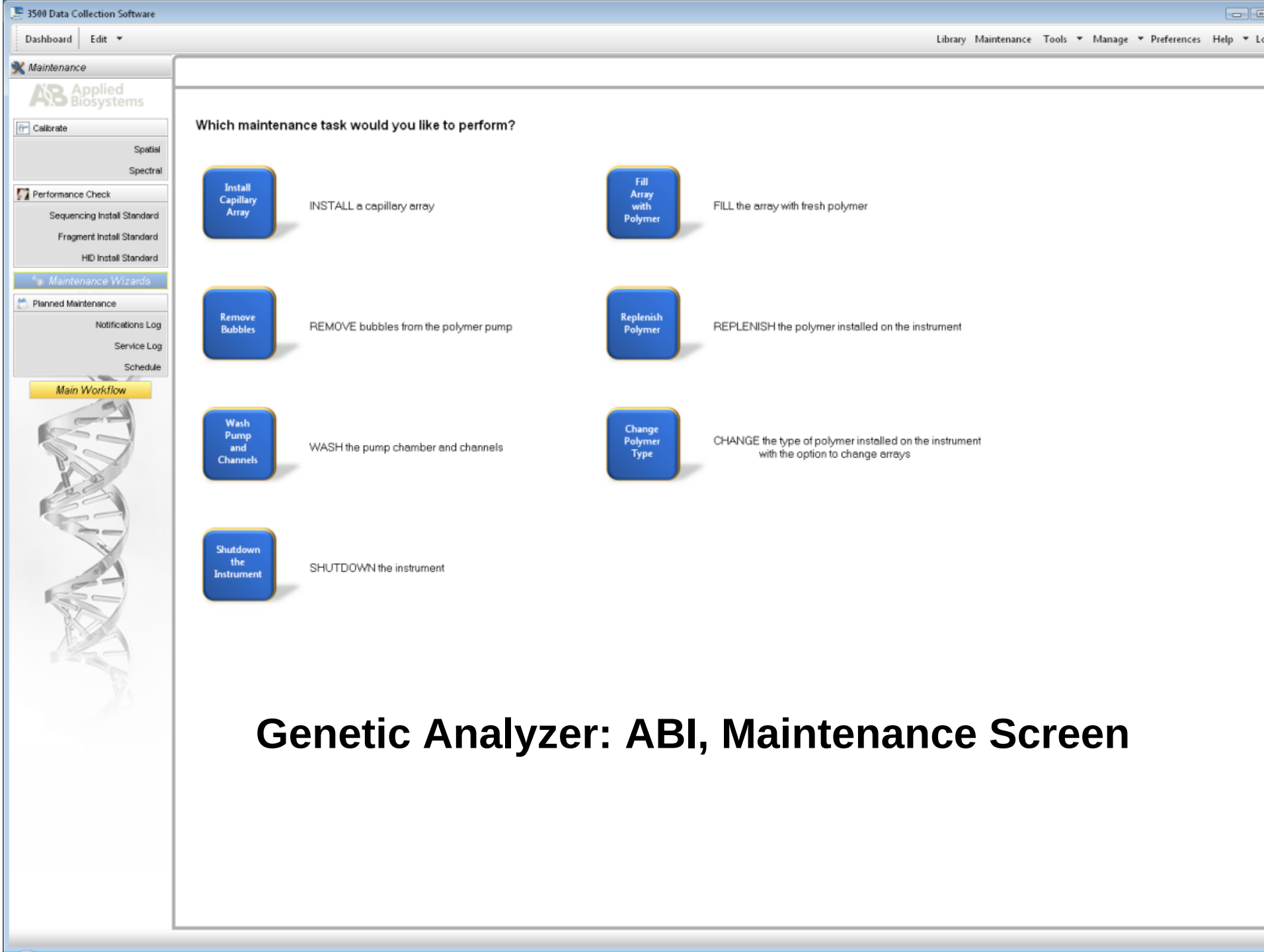
3. Snap the plate retainer (cover) onto the plate, septa, and plate base.
4. Verify that the holes of the plate retainer and the septa strip are aligned. If not aligned, re-assemble and then assemble the plate assembly.

IMPORTANT! The array tips will be damaged if the plate retainer and septa strip holes do not align correctly.

1. Place the plate in the autosampler with the labels facing you (or the instrument door) and the notched corner of the plate in the notched corner of the autosampler.



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



Genetic Analyzer: ABI, Maintenance Screen

Genetic Analyzer: Change the Anode Buffer Container (ABC)

1. Remove the ABC from storage.
2. Check for expiration date on the ABC label to make sure it is not expired prior to or during intended use.
3. Allow refrigerated ABC to equilibrate to ambient temperature prior to first use. Do not remove the seal until you have completed step 5, below.

IMPORTANT! Ensure that all the buffer is moved to the larger side of the ABC prior to removing the seal.

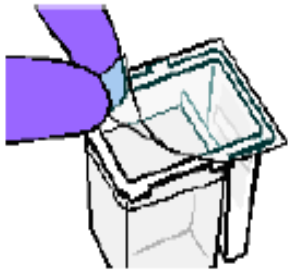
4. Verify that buffer level is at or above the fill line and check that seal is intact.

IMPORTANT! Do not use if buffer level is too low or seal has been compromised. A fill tolerance of ± 1 mm is acceptable.

5. 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.



6. Verify that the buffer is at the fill line.
7. Peel off the seal at the top of the ABC.



1. To removed used ABC, pull back and downward to release clips
2. Install fresh ABC from below then push into guide slots

8. Place the ABC into the Anode end of the instrument, below the pump.

IMPORTANT! The RFID label must be facing the instrument (and not you) to ensure that the RFID information is read accurately by the instrument.



9. Close the instrument door to re-initialize.

Note: If you do not close the instrument door to re-initialize, you need to click **Refresh** from the Dashboard.

10. Click **Refresh** from the Dashboard to update the screen.
11. Check the Quick View section of the Dashboard for updated status after changing the ABC.

After replacing ABC, Select REFRESH on the main dashboard to force the system to read the RFID tags and update usage parameters.

Genetic Analyzer: Change Cathode Buffer Container (CBC)

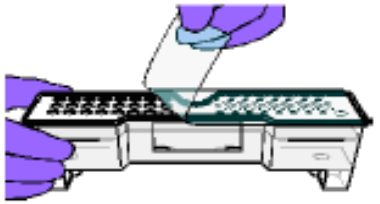
1. Remove the CBC from storage.
2. Check for expiration date on the CBC label to make sure it is not expired prior to or during intended use.
3. Allow refrigerated CBC to equilibrate to ambient temperature before use.
4. Wipe away condensation on the CBC exterior with a lint-free lab cloth.
5. Verify that buffer level is at or above the fill line and check that seal is intact.

---

6. Tilt the CBC back and forth gently and carefully to ensure that the buffer is evenly distributed across the top of the baffles.

Note: If you do not tilt the CBC back and forth, the buffer sticks to the baffles, due to surface tension.

7. Verify that the buffer is at or above the fill line.
8. When ready to install CBC, place the container on a flat surface (such as a lab bench) and peel off the seal.



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

IMPORTANT! Failure to perform this action may result in an arcing event and termination of the run.

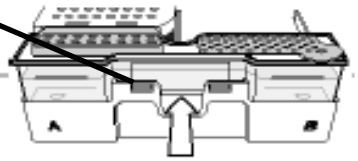


10. Place the appropriate septa on both sides of the CBC.
    a. Align the buffer septa (the part that is symmetrical) over the 24 holes of the CBC.
    b. Push the septa lightly into the holes to start and then push firmly to seat the septa.



11. Install the CBC on the autosampler.

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



12. Close the instrument door to re-initialize.
13. Click **Refresh** from the Dashboard to update the screen.

1. To remove CBC, push in on center tab to release clips, then pull forward.
2. To install fresh CBS, push into position until tabs click into slots.

After replacing CBC, Select REFRESH on the main dashboard to force the system to read the RFID tags and update usage parameters.

Genetic Analyzer: Install Polymer

1. Remove the polymer from storage 4 °C.
2. Allow refrigerated polymer to equilibrate to ambient temperature before use.
3. Check for expiration date on the pouch label to make sure it is not expired prior to use.

IMPORTANT! Do not use if the pouch and/or the label is damaged or the top seal is missing.

---

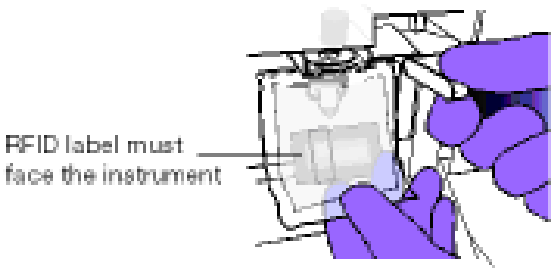
4. Peel off seal at the top of the pouch fitment.

Note: You may occasionally notice a tiny droplet of polymer inside the fitment (residual from the pouch filling process). This is **not** expected to cause any performance issues.

---

5. Slide the pouch fitment on to the slot of the lever assembly. Push the lever up to snap the pouch into the connector end of the instrument pump.

Note: The RFID label must be facing the instrument (and not you) to ensure that the RFID information is read accurately by the instrument.



6. If a partially used pouch is removed for later use, use the suggested cap to plug the fitment opening and store the pouch under recommended storage conditions.

Genetic Analyzer: Fill Cap Array with fresh Polymer

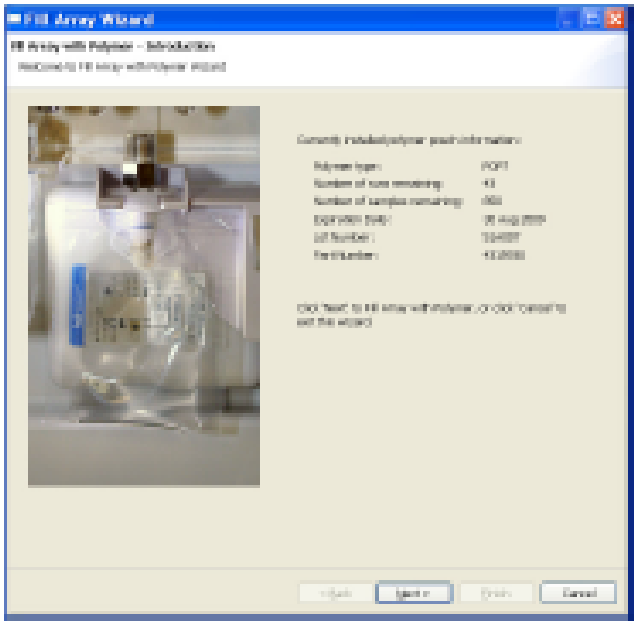
1. To fill capillary array with fresh polymer (same type of polymer), click **Fill the Array with fresh Polymer**.



2. Follow the prompts in the Fill Array Wizard window.

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

4. Check the Quick View section of the Dashboard for updated status after filling of the Capillary Array with fresh polymer.



from the polymer pump

Remove bubbles from the polymer pump fluid path before each run. See "Daily instrument maintenance tasks" on page 230 for more information.

IMPORTANT! Wear gloves while handling polymer, the capillary array, septa, or CBC.

Genetic Analyzer: ABI Fill Array with Polymer Screen

Fill Array Wizard

Fill Array with Polymer

Fill the array with polymer (optional)



To fill the array with polymer:

1. Close the instrument door and wait for the green status light to illuminate.
2. Click 'Fill Array'. [Fill Array]

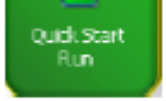
   Wait approximately 2 minutes for the Fill Array procedure to complete.
3. Click 'Finish'.

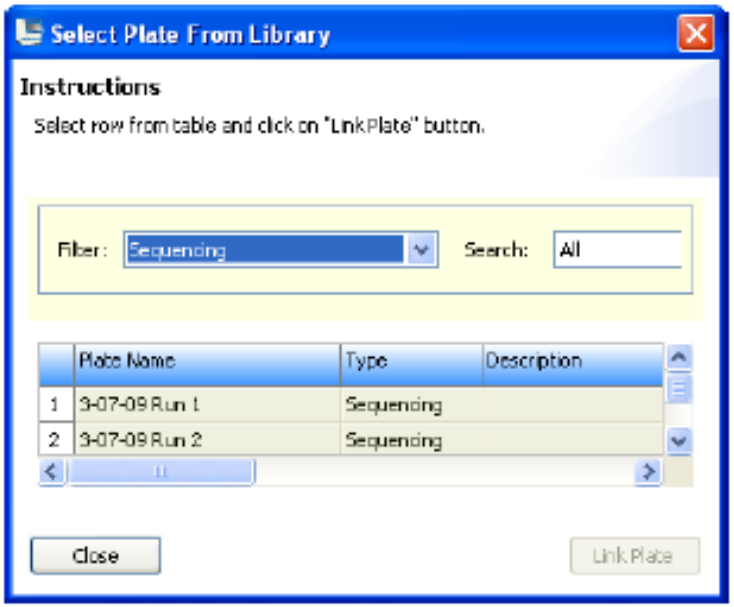
   To skip this step, click 'Finish'.

< Back | Next > | Finish | Cancel

Genetic Analyzer: ABI; Quick Start Run

1. In the Dashboard, click **Quick Start Run** to display the Select Plate from Library dialog box.

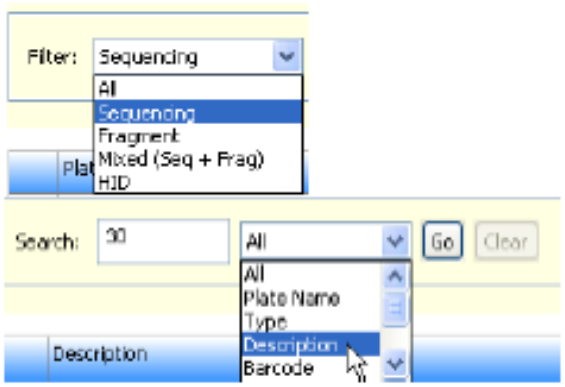




2. (Optional) Filter the plates listed:

   a. Select a plate type (you can set the default plate type in Preferences, *see* "Specify the default plate type for the Open Plate dialog box" on page 76).

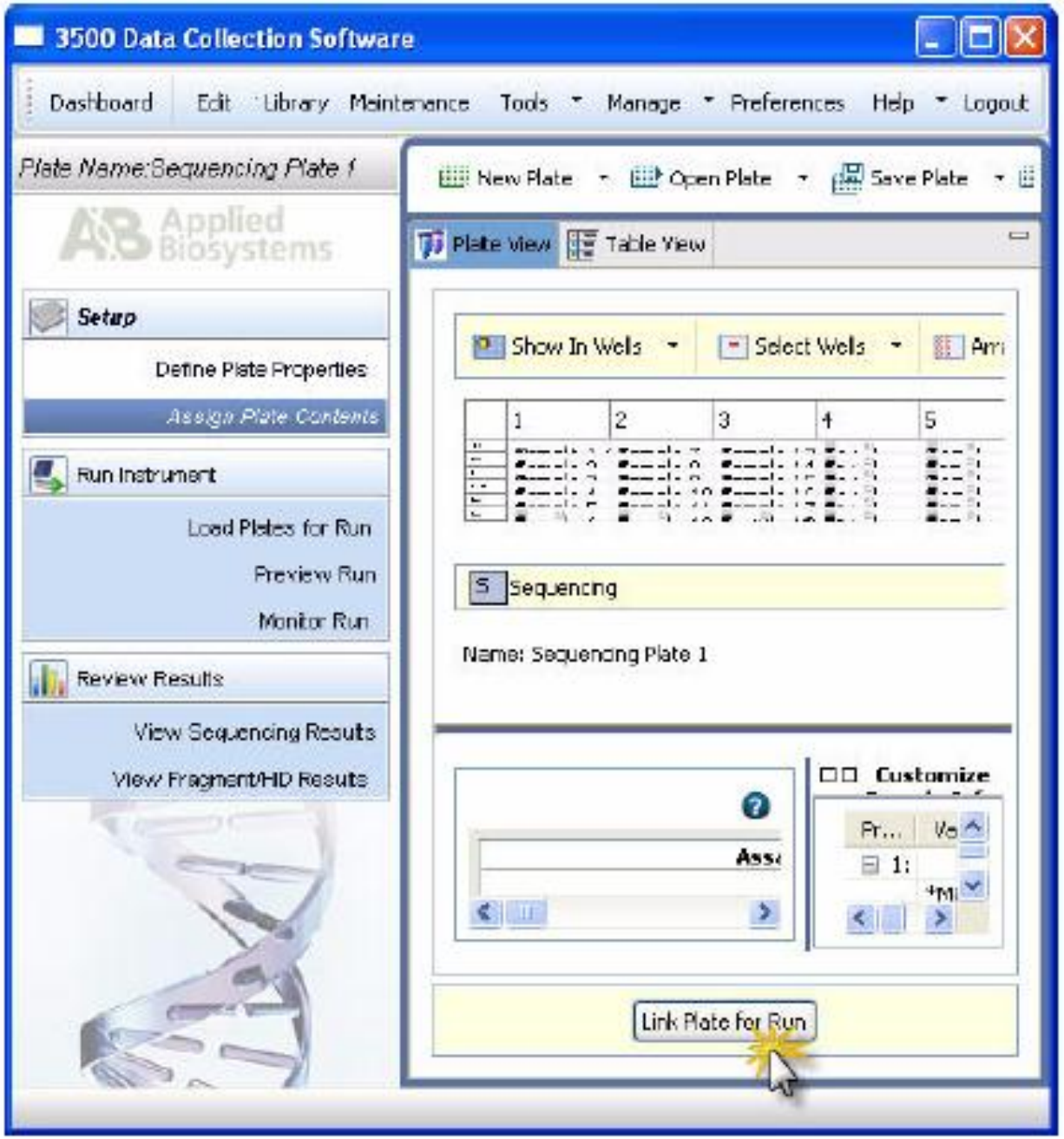
   b. Find a plate based on an attribute by selecting an attribute, entering the text to search for, then clicking **Go**. (Click **Clear** to clear the field and enter different search criteria).



3. Select a plate, then click **Load Plate**.

4. Click **Start Run** from the Load Plates on Run Screen.

1. In the Assign Plates for Run screen, click **Link Plate for Run**.

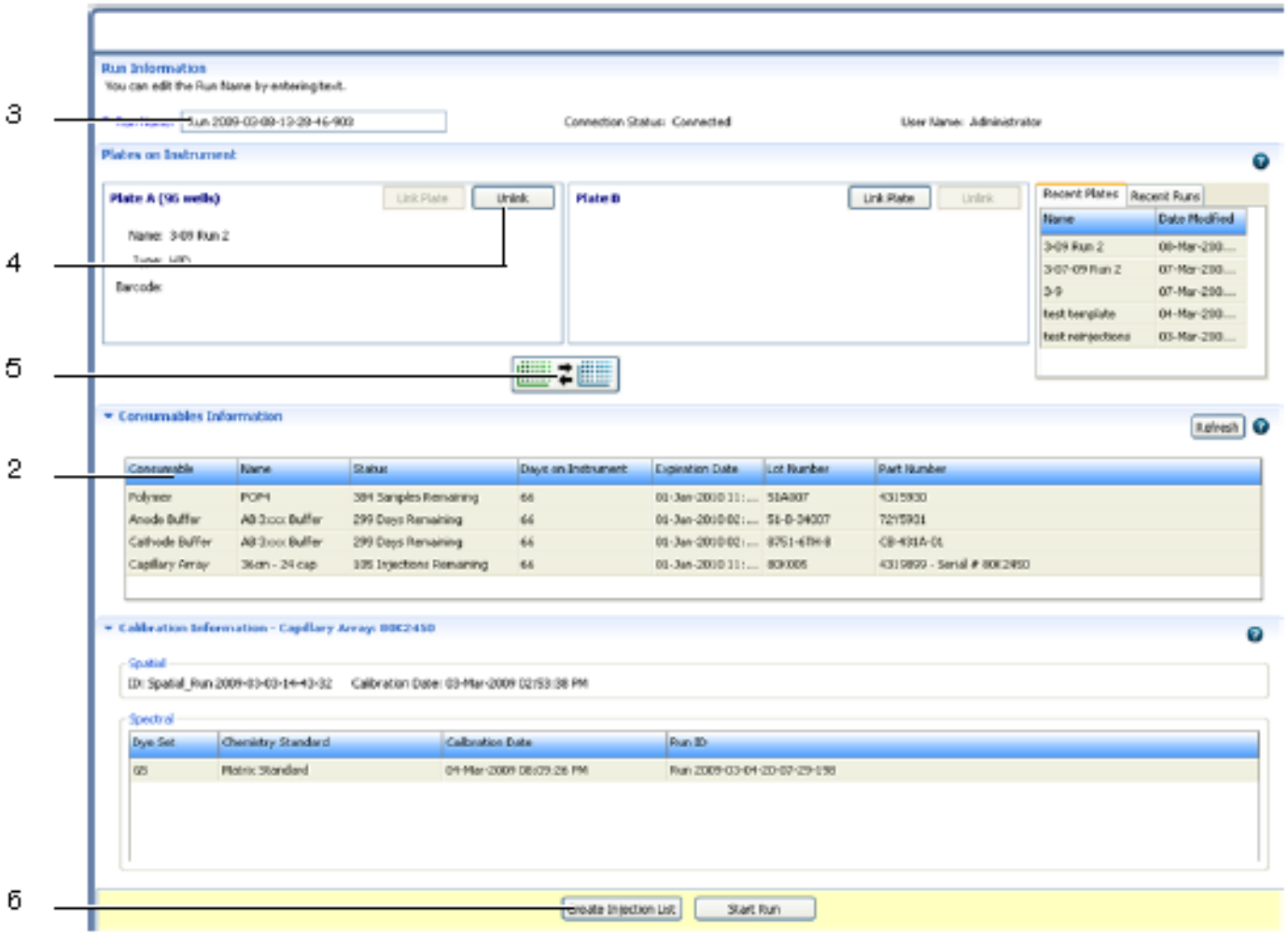


Genetic Analyzer: ABI; Link Plate for a Run

Genetic Analyzer: ABI; Load Plates for a Run

1. Access the Load Plates for Run screen (Figure 6 on page 56) from:
   - The **Assign Plate Contents** screen by clicking **Link Plate for Run**.
   - The navigation pane by selecting **Load Plates for Run** in the navigation pane.
   - The Dashboard by clicking the **Main workflow arrow** , then selecting **Load Plates for Run** in the navigation pane.





Genetic Analyzer: ABI, Run Checklist

- **Is the Server Monitor (bottom screen bar) marked with Green checkmark?**
- **Has oven been pre-heated to required temperatures?**
- **Have anode and cathode buffer and polymer pouch been replaced (if expired)?**
- **Are there any bubbles in the pump assembly area?**
- **Was the cap array filled with fresh polymer?**
- **Was the correct template used for plate definition (matches prep requirements)?**
- **Do wells in the plate have appropriate volume?**
- **Was the plate centrifuged prior to installation?**
- **Were all wells of the plate tagged/shaded with the appropriate Assay and file/folder definitions?**
- **Has the appropriate plate definition been "linked" to this run setup?**
- **Has "In-Use" signage been prepared to tag ABI and PC with instructions on how to label and store plate if necessary?**
- **After run, has the polymer pouch been removed and replaced with a Conditioning pouch?**

Genetic Analzyer; ABI, Start and Monitor a Run

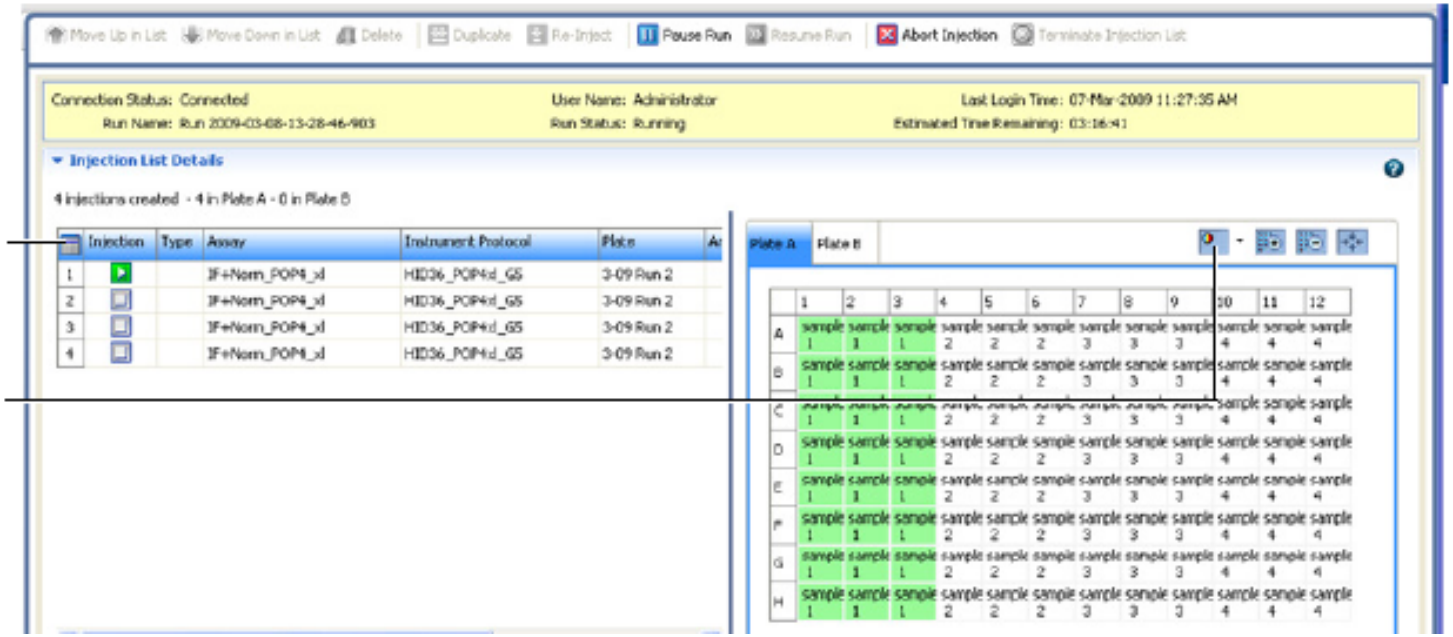
When the injection list is configured, click **Start Run**. The Monitor Run screen is automatically displayed.

IMPORTANT! You must specify re-injections before the run completes.

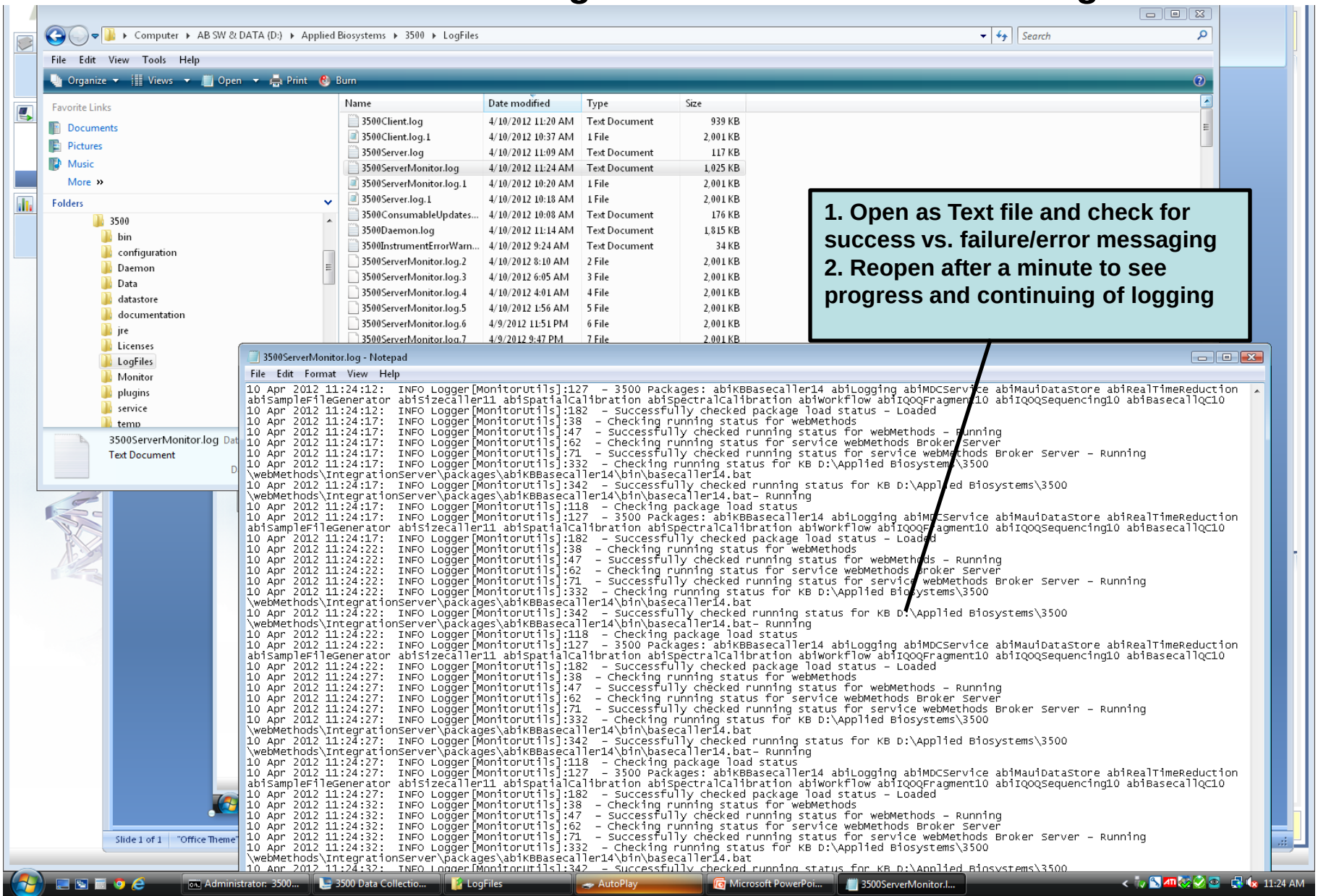
Note: It takes, approximately, 10 seconds for the instrument to initialize after the instrument door is closed. Do not start a run until the instrument status light is green.

Monitor the run

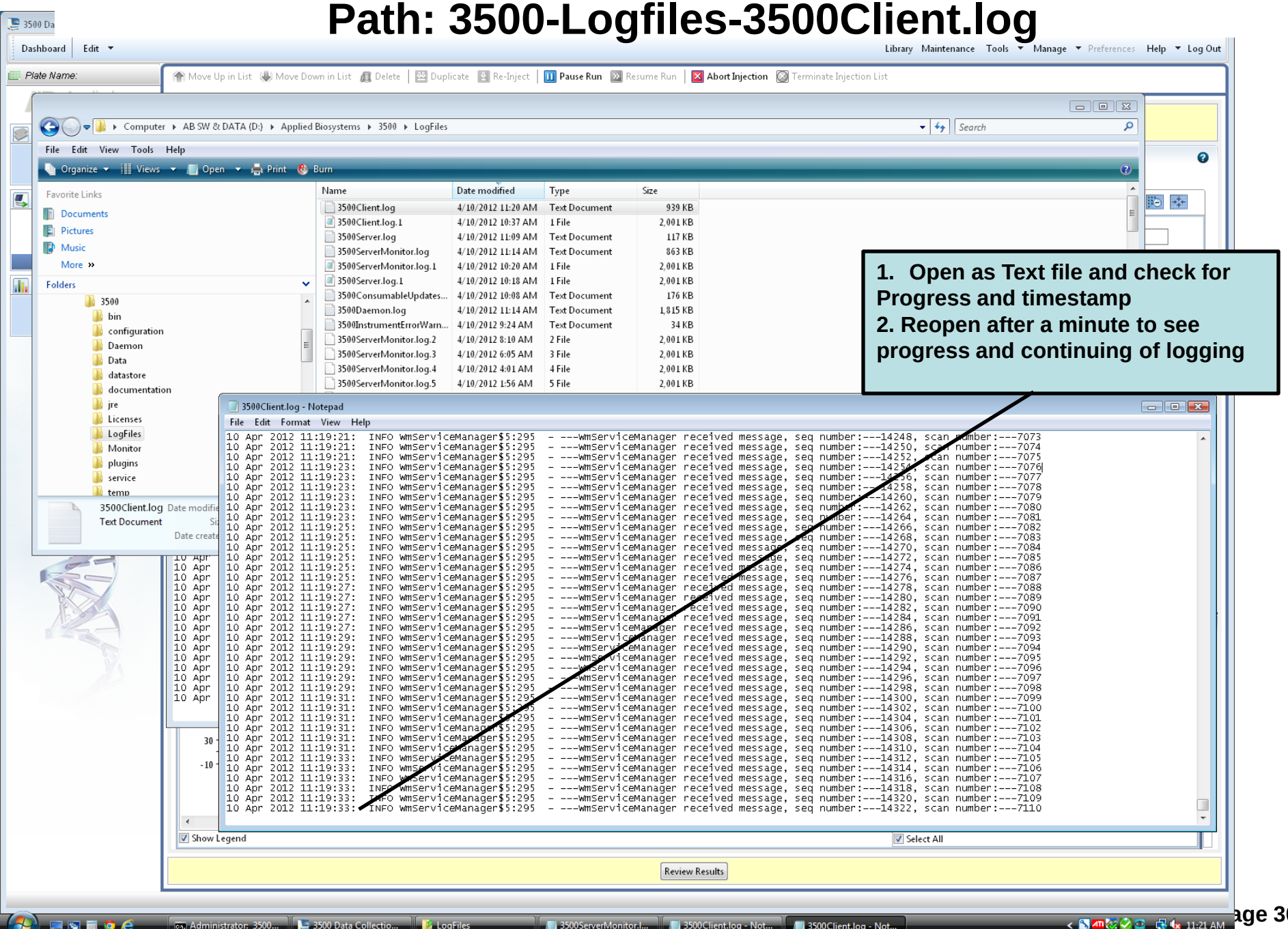
The Monitor Run screen (Figure 8 on page 61) is automatically displayed when you click Start Run in the Load Plates for Run screen or the Preview Run screen. The current injection is highlighted in green in the plate view. The injection list is linked to the plate view. Click an injection to select the associated wells in the plate view. A selected injection is highlighted in yellow in the plate view.



Genetic Analyzer: ABI,Setup/Run,ServerMonitor Log File Path: 3500-Logfiles-3500Servermonitor.log



Genetic Analyzer: ABI,Setup/Run,ServerMonitor Log File Path: 3500-Logfiles-3500Client.log



Genetic Analyzer: ABI, Setup-Run, Shutdown Procedures

Remove polymer and put on Conditioning Pouch for shutdown

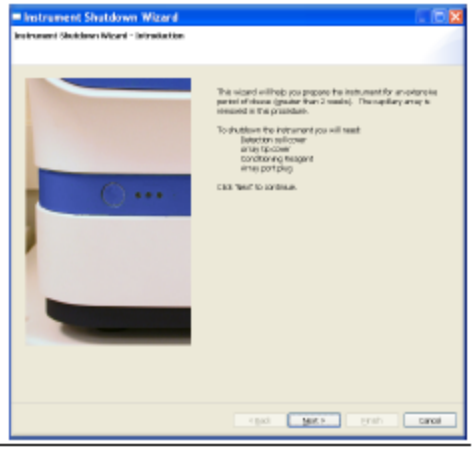
Use the Instrument Shutdown Wizard for short- and long-term shutdown.

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



Note: The Instrument Shutdown Wizard takes 60 minutes to complete.

2. Follow the prompts in the Instrument Shutdown Wizard window.



Perform the appropriate shutdown procedure based on the information in the following table:

IMPORTANT! Place a conditioning reagent pouch onto the instrument when performing instrument shutdown.

If the instrument will be unattended for ...	Perform this shutdown procedure ...
no more than 1 week	No action is required.
1 to 2 weeks	IMPORTANT! Keep the load-end of the capillary array in 1X buffer to prevent the polymer from drying in the capillaries. If fluid level is low, add DI water to buffer solution. Install the new CBC when ready to resume runs.
for more than 2 weeks	Long-term. See below for long-term instrument shutdown.