PowerPlex® Fusion & Y23 – Capillary Electrophoresis on 3500*xL*

Follow all relevant processes in the General Guidelines for Forensic Biology and DNA Casework procedure.

Follow all relevant processes in the BEAST DNA Worksheet Setup Manual for creating and adding to worksheets and BEAST DNA Worksheet Processing Manual for how to record all relevant information while processing the worksheets.

1 Setting Up 3500xL Run

Important Reminder – At any time during set-up or if a plate is currently running, DO NOT open instrument doors if the autosampler is in motion.

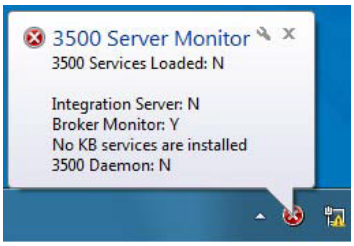
NOTE: Steps 1.1-1.5 should only be performed if instrument has been turned off.

1.1 Power on the computer attached to the instrument. Do not log in.

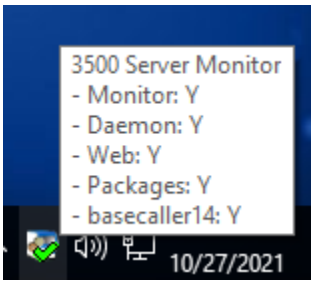
1.2 Power on the instrument and wait for green front panel indicator to stop blinking.

1.3 Log in to computer.

1.4 Look for a pop-up at the bottom right of the desktop.



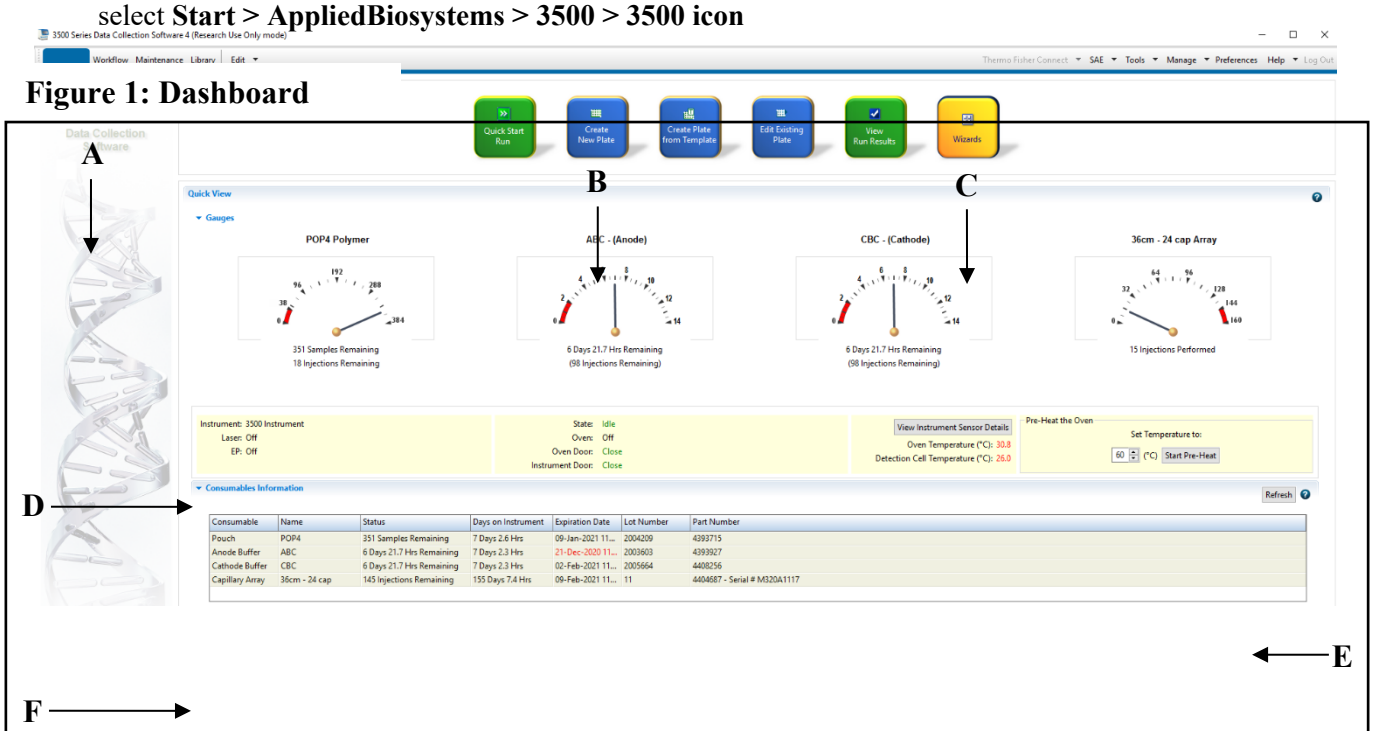
1.5 Wait for ~1-2 minutes for the Server Monitor icon to change from red X to green check mark.



NOTE: If the Server Monitor icon does not change to a green checkmark, you cannot start the software. See Section 6: Troubleshooting. Contact QA for assistance if issues arise.

1.6 Open the 3500*xL* Data Collection v4 software by double clicking on the desktop Icon or select **Start > AppliedBiosystems > 3500 > 3500 icon**

Figure 1: Dashboard



A. Navigation Tabs – change between **Dashboard**, **Workflow**, and **Maintenance** views
B. Create New Plate – used to name and import plate information onto instrument
C. Wizards – contains wizards for changing POP4 and removing bubbles
D. Gauges – quick view of consumables and number of injections run on instrument
E. Start Pre-Heat – button to start oven pre-heating
F. Consumables Information Table – shows time consumables have been on the instrument

1.7 Click **Refresh** (on the right above the **Consumables Information** table) to update the consumables information and check the status of the consumables in the **Dashboard.**

1.8 Check the consumables. NOTE: QA team will routinely perform maintenance on the instruments and replace the consumables once they reach near expiration date. Notify QA if any issues arise. If consumables need to be replaced with no other maintenance needed, then a casework analyst may change the expired consumables and log them.

1.8.1 Capillary Array –

1.8.1.1 Proceed only if the total number of injections that have been run on the instrument plus the intended number of injections to be performed is ≤320. Notify QA if capillary injection number reaches close to 320 and needs to be replaced.

1.8.1.2 Check the number of injections on the capillary on the **Dashboard**

NOTE: The **Dashboard** image for the capillary will max out at 160 injections, check the number of injections as written under the graph.

1.8.2 POP4 Polymer –

1.8.2.1 Check the number of samples remaining and number of injections remaining. To ensure that the POP4 can accommodate your plate/samples, use the lower value.

For example, if the **Dashboard** shows 48 samples remaining and 10 injections remaining, the lower value would be 48 samples as that equates to 2 full injections, which is less than 10 injections. The number of injections remaining is more likely to be affected by multiple partial plates, i.e. the number of injections will lower at a faster rate than the number of samples remaining.

1.8.2.2 Check the Consumables Information Table (Fig1 F) under the 'Days on Instrument' column to ensure that the POP4 is ≤14 days old. The instrument will count down how long, to the hour and minute, the consumables will be usable.

1.8.2.2.1 One injection takes approximately 40 minutes. For example, 2 injections will take approximately 1 hour and 20 minutes. For a 2-injection run, all consumables must then have more than 1 hour 20 minutes remaining before starting the run.

1.8.2.3 If the POP4 needs to be changed, remove a polymer pouch from fridge and allow it to equilibrate to room temperature before use (~30 mins). Ensure there are no crystals before installing on the instrument. Click on the **Wizards** button (Fig1 C) and follow the **Replenish POP4** Wizard.

1.8.2.3.1 Each time the lever to fix the POP4 in place has been lowered, the bubble removal wizard should be performed, as the action may have introduced bubbles.

1.8.3 Anode Buffer Container (ABC) –

1.8.3.1 Check the time remaining and injections remaining to ensure that the days and hours are >0 and the consumables remaining can accommodate both your plate injections and run time (refer to step 1.8.2.2.1).

1.8.3.2 If the ABC needs to be changed, follow directions in Section 5.

1.8.3.3 Check the Anode Buffer level. If the level of buffer falls below the minimum line, notify QA.

1.8.4 Cathode Buffer Container (CBC) –

1.8.4.1 Check the time remaining and injections remaining to ensure that the days and hours are >0 and the consumables remaining can accommodate both your plate injections and run time (refer to step 1.8.2.2.1).

1.8.4.2 If the CBC needs to be changed, follow directions in Section 5.

2 Procedure

2.1 Retrieve amplified samples from the thermal cycler or refrigerator.

2.2 Turn on heat block(s) for the denature step. Ensure the heat blocks are set to 95°C.

2.3 Turn on the oven by clicking Start Pre-Heat on the 3500*xL* Dashboard and make sure that the temperature is set to 60°C . Pre-Heating the oven is recommended at least **30 minutes** before the run is started. The pre-heat button can be pressed right before a run; however, the instrument will not begin until the temperature has reached 60°C . Record the instrument.

2.4 Centrifuge samples at 1000 RPM for one minute.

2.5 Retrieve the following reagents for the relevant system and record the lot numbers. NOTE: HiDi Formamide must **NOT** be re-frozen.

Fusion 5C
PowerPlex Fusion® WEN ILS 500
PowerPlex Fusion® Allelic Ladder
HiDi Formamide

PPY 23
PowerPlex® WEN ILS 500 Y23
PowerPlex® Y23 Allelic Ladder Mix
HiDi Formamide

2.6 Prepare dilutions of amplified samples, if necessary. 0.1X TE^{-4} should be used to make dilutions. Pipette mix samples prior to aliquoting for dilution. Record the lot number for the TE^{-4}, if used.

2.7 Record lot numbers of Instrument reagents in LIMS.

2.7.1 The cathode and anode buffer, the POP4, and the capillary lot numbers must be recorded in LIMS for all 3500*xL* batches using the labels on the relevant reagent on the instrument.

2.8 Prepare master mix for all samples, negative and positive controls, and allelic ladders as specified below: (9.5µL of HiDi + 0.5µL of size standard per sample)

Fusion 5C & PPY23		
# Samples (+ 2)	**HiDi**	**ILS500**
24	247µL	13µL
48	475µL	25µL
72	703µL	37µL
96	931µL	49µL

2.9 Vortex the master mix for 10-15 seconds to mix.

2.10 Obtain a reaction plate and label the side with the plate ID (i.e. STR25-1234) and the run name – based on the instrument, date, and injection number(s) (i.e. Carmody121620 25). Record the Run name.

2.11 Aliquot 10µL of master mix to each well.

2.11.1 If an injection has less than 24 samples, add at least 10µL of either dH_2O, HiDi, buffer, or master mix to all unused wells within that injection.

2.12 **Tube Setup WITNESS.** Have another analyst verify the tube setup by comparing the tube labels and sample positions indicated on the Worksheet. The entire amp tube label must be read for each sample.

2.12.1 Record the 'Tube Setup Witness'.

2.12.2 For samples being aliquotted from a Fusion Direct amplification plate:

2.12.2.1 Generate the Fusion Direct Amplification Crystal Report via the [Print] button from the Fusion Direct worksheet. The witness must verify that each individual sample well is in the same order by comparing the FDAMP Crystal Report against the 3500 Plate Worksheet screen.

2.12.2.2 The analyst must state the plate ID on the amplification plate; the witness must verify this information in the FDAMP crystal report in LIMS.

2.12.2.3 Controls - verify that the positive control ID from the amplification worksheet is the same as the positive control ID on the 3500 Plate worksheet.

2.12.2.4 Sample wells - analyst setting up the plate must state which wells hold amplified product and are being transferred from the amplification plate. Analyst must also state the wells being loaded on the STR plate. Witness must verify that the wells are correct in the FDAMP Crystal Report and the 3500 Plate worksheet.

2.13 Aliquot 1µL of allelic ladder, positive/negative control, and sample into their appropriate wells.

2.13.1 Sample order is as follows: A1, B1, C1… A2, B2, C2, etc.

2.13.2 Samples may be loaded from a PowerPlex Fusion Direct amplification plate into the CE plate by using a multichannel pipette. 1µL of allelic ladder must be loaded separately into the appropriate well(s).

2.14 Once all samples have been added to the plate, place a new 96-well septa over the reaction plate and firmly press the septa into place.

2.15 Centrifuge the plate at 1000 RPM for one minute.

2.16 Denature plate for 3 minutes with the heat block set to 95°C (+/- 3 °C).

2.17 Immediately chill plate for 3 minutes in the cold block in the refrigerator.

2.18 Centrifuge the plate at 1000 RPM for one minute again.

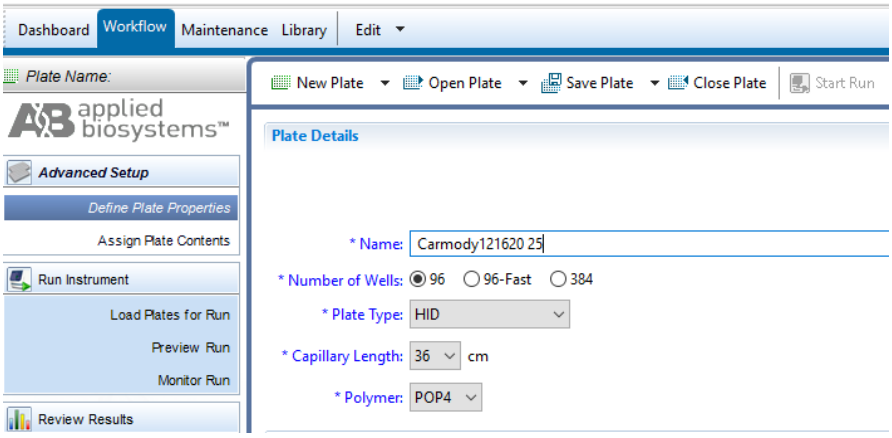
2.19 Record the 'Plate Setup By' and 'Run By' review task.

2.20 While plates are denature/chilling, set up the plates in the 3500*xL* data collection software.

2.21 Export the plate record from the worksheet. Transfer the file to the instrument computer.

2.21.1 Copy the plate record from the LIMS file share to the Plate Records folder on the instrument computer.

2.22 Click **Create New Plate** button on the **Dashboard** and ensure the following screen is visible:



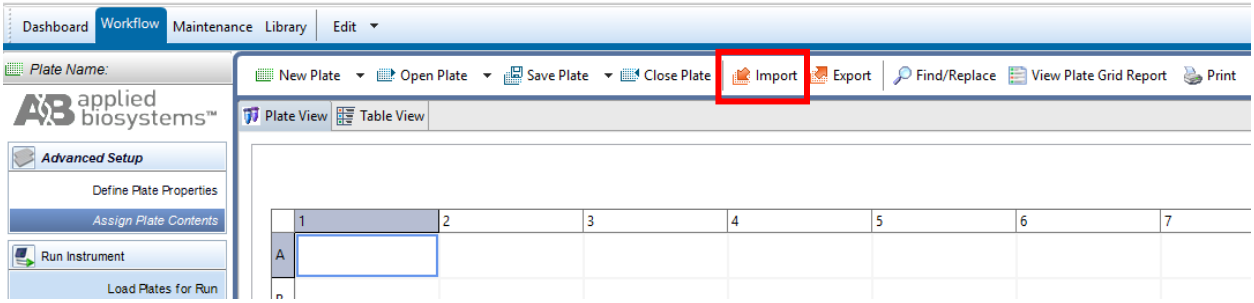
2.22.1 Name your plate using the naming system based on the instrument, date, and injection number(s) (i.e. Carmody121620 25).

2.22.2 Ensure the settings match the following:

- Number of Wells: **96**
- Plate Type: **HID**
- Capillary Length: **36cm**
- Polymer: **POP4**

2.22.3 Click **Assign Plate Contents** at the bottom of the screen

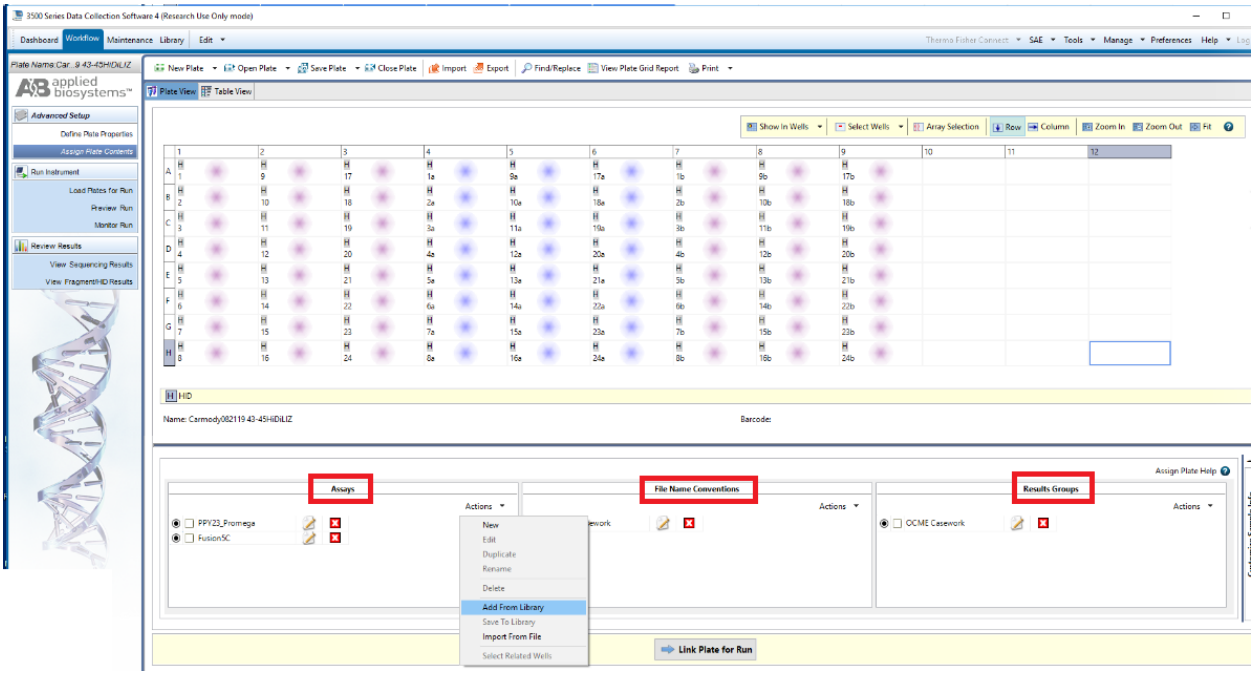
2.22.4 Click on the **Import** button at the top of the screen to import your plate record file.



2.22.5 Ensure that all 3 settings at the bottom of the screen (Assay, File Name Convention, and Results Group) have check marks and have the correct settings from the library, and check that all used wells are the correct samples and are highlighted as shown below.

2.22.5.1 Clicking the square in upper left corner in plate view will **highlight** the entire plate. If plate view appears compressed, click **Fit** on upper right corner.

Settings	Fusion	PPY 23
Assay	Fusion5C	PPY23_Promega
File Name Convention	OCME Casework	OCME Casework
Results Group	OCME Casework	OCME Casework



2.23 Check the Plate View to ensure the correct number of samples and injections are being run.

2.24 Different assays can run together on a single plate if they are in different injections. For example, injection one (first 24 wells) can be assigned to the Fusion assay and injection two can be assigned to the PPY23 assay.

2.24.1 PPY wells are colored **blue** and Fusion wells are colored **magenta.**

2.24.2 If any of the library settings are missing, select actions dropdown/Add from Library to add necessary settings.

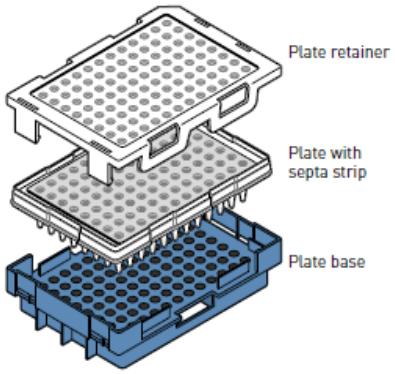
2.25 Prepare the plate assembly on a level surface. Place the plate with the septa into the plate base. Securely snap the plate retainer cover onto the plate, septa, and plate base. Check both sides of the plate to ensure the retainer is securely fastened to the base.

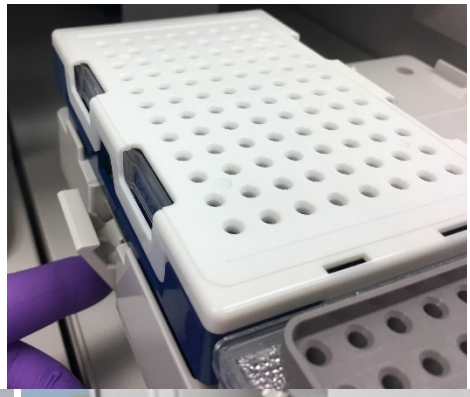
2.26 Load plate on instrument.

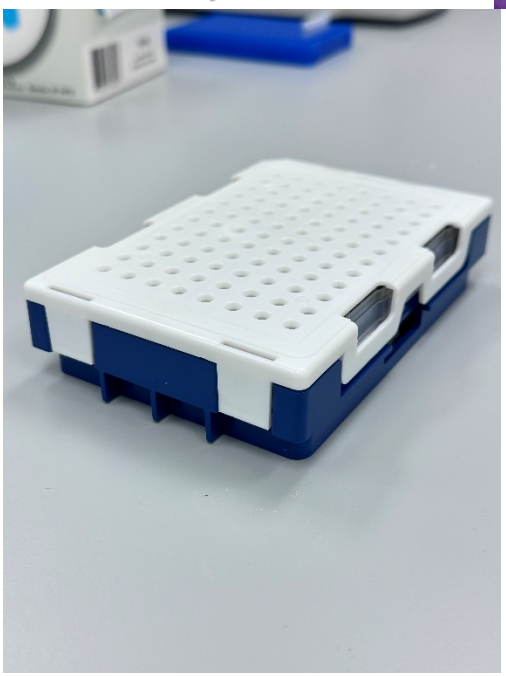
2.26.1 Push the tray button on the bottom left of the machine and wait for the autosampler to move forward and stop at the forward position before opening the door.

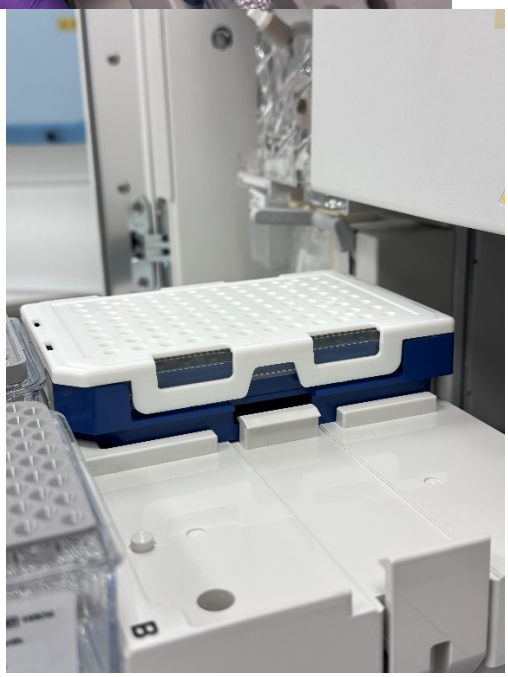
2.26.2 Open the door and place the tray onto the autosampler in the correct tray position, A or B. There is only one orientation for the plate. (The barcode on the plate base and retainer should be facing outwards, towards the user.)

- NOTE: Push and open the clip on the side of the autosampler, place plate on instrument and release clip so that the plate is secure.









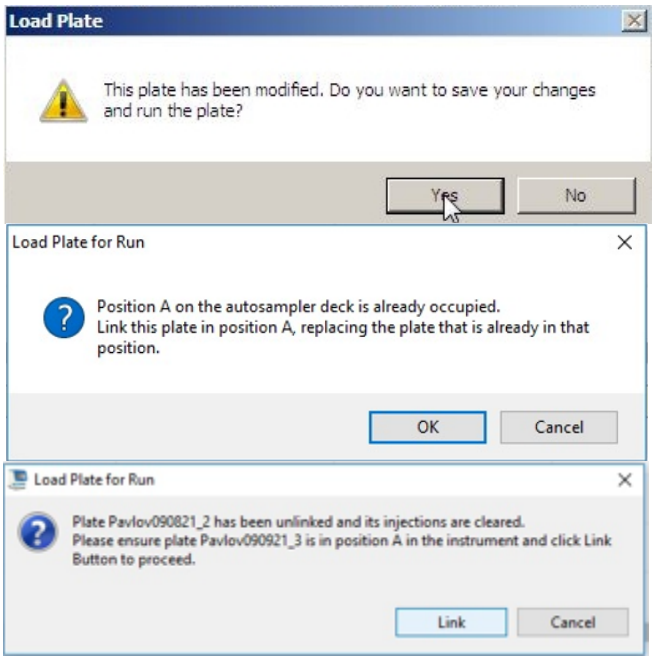
2.26.3 Ensure the plate assembly fits flat in the autosampler.

2.26.4 Close the instrument door and allow the autosampler to move back to the home position. It takes approximately 10 seconds for the instrument to initialize after the instrument door is closed.

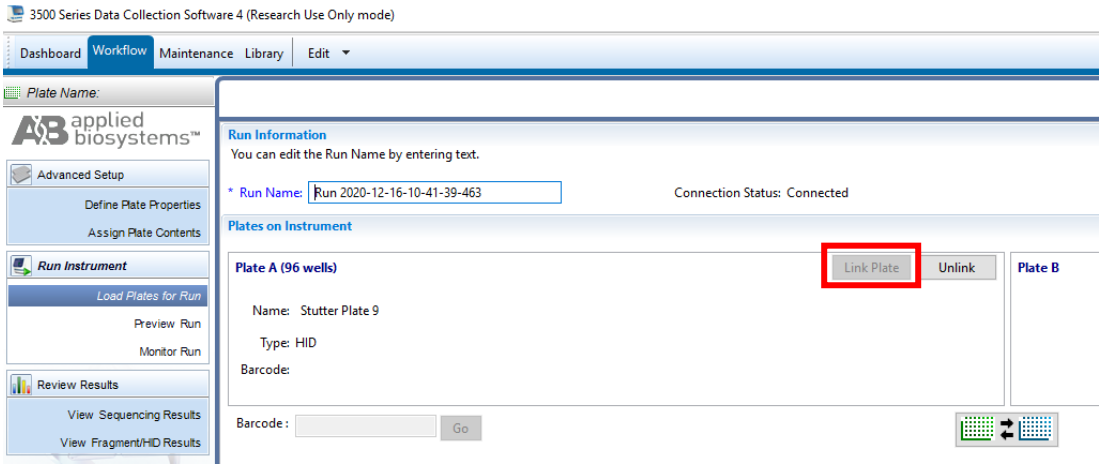
2.26.5 Wait for the green light on the front panel before linking the plate.

2.27 Link plate to instrument.

2.27.1 Click on Link Plate for Run at the bottom of the screen. If you see the following pop-up messages, →Click Yes/OK/Link and ensure correct plate is on the autosampler.



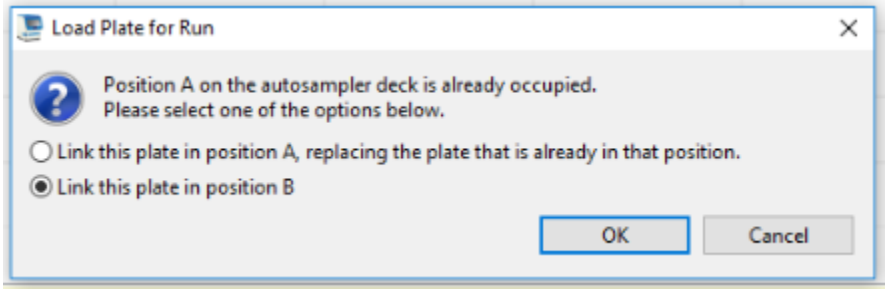
2.27.2 The following screen should then appear:



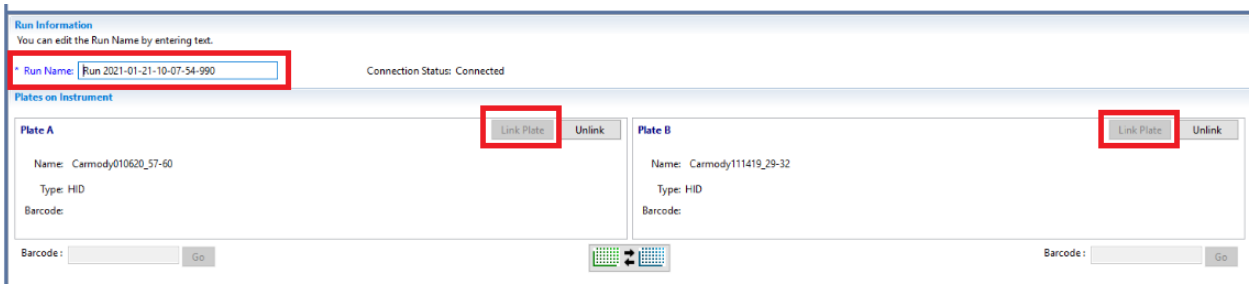
2.28 If two plates are to be loaded, navigate back to Define Plate Properties from the left tabs after setting up plate A to create and set up the second plate.

2.28.1 Repeat steps 2.21 to 2.25 for plate B.

2.28.2 Upon linking plate B, the following message will show up. Select **Link this plate in position B**.



2.28.3 The following screen should appear:



2.29 Change the Run Name to reflect all names of the plates that are linked to the instrument.

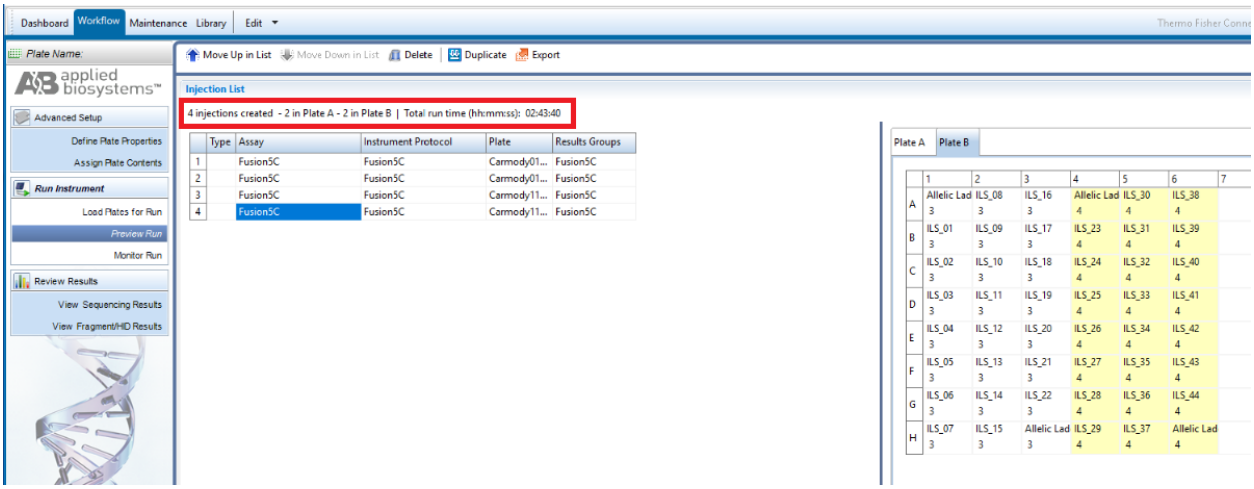
2.29.1 If only a single plate is being run, fill in the run name as is - i.e. Carmody121620 25.

2.29.2 If two plates are being run, separate the injection numbers by an underscore - i.e. Carmody121620 25_26-27.

2.30 If the **Link Plate** button associated with your plate(s) is not clicked, click it now.

2.30.1 Both plates **must** be linked prior to starting the run.

2.31 Click on Create Injection List at the bottom of the screen. The following screen will appear.



2.32 The injection list screen shows total number of injections created, number of injections per plate, and the total estimated run time.

2.32.1 One injection takes approximately ~ 40 minutes

2.33 Ensure correct library settings (left side) are selected for each injection created (right side).

2.33.1 Clicking each injection will highlight the corresponding wells on the plate.

2.34 Ensure the instrument is ready to go by checking reservoirs are filled properly, plate is linked correctly, and no bubbles are present in the polymer block.

2.34.1 If bubbles are present, click on **Wizards→Remove Bubbles Wizard**

2.35 The run will not start until all indicators shown in the dashboard are green.

2.35.1 Navigate back to dashboard by clicking Dashboard p of the screen to ensure all indicators are green

2.35.2 Return to the injection list by clicking Workflow on top of the screen and then Preview Run on the left side of the screen.

2.36 Click on Start Run at the bottom center of the screen.

2.37 Store the Amplification tubes in the fridge at approximately 4°C-8°C, filed by instrument and run name. Fusion Direct amplification plates should be discarded.

2.37.1 Amplification tubes should be stored with their most recent 3500 run. For example, a sample initially run on Carmody is rerun on Avogadro. That tube should now be stored with the samples on the Avogadro run. Do not return the tube to its initial run storage box.

2.37.2 If a positive control is being pulled for use from a previous run, the tube should be returned to its original run box after use to remain associated with its original amp set.

2.38 The following are the instrument parameters for each assay:

	Fusion	**PPY 23**
Oven Temp	60°C	60°C
Pre-Run Voltage	15.0 kV	15.0 kV
Pre-Run Time	180 sec	180 sec
Injection Voltage	1.2 kV	1.2 kV
Injection Time	24 sec	24 sec
Run Voltage	13 kV	15 kV
Run Time	1500 sec	1500 sec

3 Collecting Data

3.1 When a run is complete, it will automatically be placed in D:/AppliedBio/Current Runs folder and labeled with either the *plate name* (e.g. Carmody102320 12-14) or *run name* (e.g. Carmody102320 12-14_15-16).

3.1.1 In these folders, additional inner folders with the HID files, separated per run, are labeled with date and *plate ID (*e.g. 10-27-2025-STR25-1234). Transfer these inner folders to the FBIO network folder.

3.1.2 Proceed to Analysis section of the GeneMarker manual.

4 Re-injections

4.1 Plates should be re-injected as soon as possible.

4.1.1 Create a 3500 Plate reinjection and plate record. See BEAST Manual for DNA WORKSHEET SET UP.

4.1.2 Ensure Instrument is set up.

4.1.3 Follow steps in section 2, starting with steps 2.15, re-denature/chill the plate.

5 Changing Buffers

5.1 Change buffer on instrument. Buffer only needs to be changed once every 14 days.

5.1.1 Anode Buffer Container (ABC)

5.1.1.1 Remove ABC from fridge and allow to equilibrate to room temperature prior to use. Do not remove the seal until you have completed step 5.1.1.4

5.1.1.2 Verify that the seal is intact. Do not use if buffer level is too low or seal has been compromised. A fill tolerance of ±1 mm is acceptable.

5.1.1.3 Invert the ABC, then tilt it slightly to move most of the buffer to the larger side of the container. The smaller side of the container should contain <1 mL of the buffer.

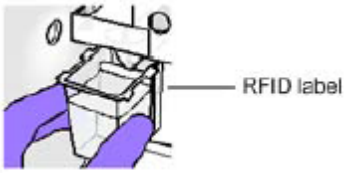
5.1.1.3.1 NOTE: If you have already removed the seal and there is >1 mL of buffer on the smaller side, use a pipette to transfer buffer to larger side of the container.

5.1.1.4 Verify that the buffer is at the fill line.

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

5.1.1.6 Remove old ABC from the instrument and discard.

5.1.1.7 With the RFID label toward the instrument, place the ABC into the anode-end of the instrument, below the pump. Position the anode in the large chamber of the ABC, then push the ABC up and back to install.



5.1.1.8 Close the instrument door to re-initialize.

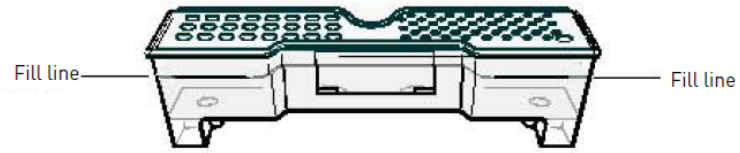
5.1.1.9 In the **Dashboard**, click **Refresh**, then check the Quick View section for updated status.

5.1.2 Cathode Buffer Container (CBC)

5.1.2.1 Remove CBC from fridge and allow to equilibrate to room temperature prior to use. Do not remove the seal until you have completed step 5.1.2.5

5.1.2.2 Wipe away condensation on the CBC exterior with a lint-free tissue.

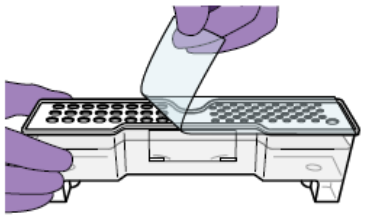
5.1.2.3 Check that the seal is intact. Do not use if buffer level is too low or seal has been compromised. A fill tolerance of ±0.5mm is acceptable.



5.1.2.4 Tilt the CBC back and forth gently and carefully to ensure that the buffer is evenly distributed across the top of the container. If you do not tilt the CBC back and forth, the buffer can stick to the top because of surface tension.

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

5.1.2.6 Place new CBC container on a flat surface and peel off the seal.



5.1.2.7 Wipe off any buffer on top of the CBC with a lint-free tissue. Ensure that the top of the container is dry. Moisture can cause termination of a run.

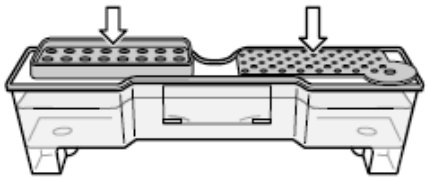
5.1.2.8 Place the appropriate septum on each side of the CBC:

5.1.2.8.1 Align the buffer septum (the part that is symmetrical) over the 24 holes of the CBC.

5.1.2.8.2 Push the septum lightly into the holes to start and then push firmly to seal it.

5.1.2.8.3 Align the capillary washing septum over the other chamber of the CBC.

5.1.2.8.4 Push the septum lightly into the holes to start and then push firmly to seal it.

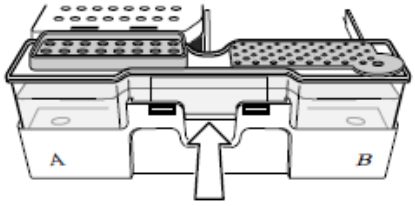


NOTE: Look at the CBC from the side and ensure there is no gap between the container and lip of the septum.

5.1.2.9 Click the Tray button on the front panel of the 3500*xL* instrument to move the autosampler to the front position.

5.1.2.10 After the autosampler has completed moving, open the door and carefully remove the old CBC from instrument and discard.

5.1.2.11 With the tab facing you and the RFID tag to the right, install the CBC on the autosampler. When properly installed, the CBC tabs will click as you snap them into place on the autosampler.

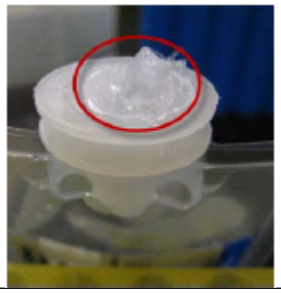


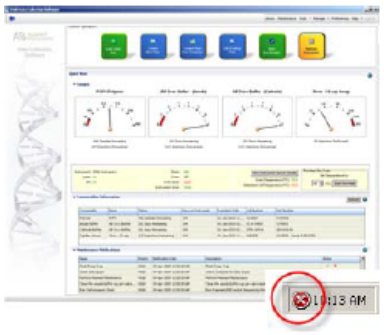
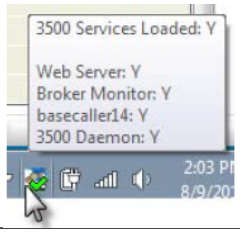
5.1.2.12 Click the Tray button to retract the autosampler, then close the instrument door to initialize.

5.1.2.13 The cathode buffer lot number information will be entered into the individual 3500xL worksheet.

5.2 In the **Dashboard**, click **Refresh**, then check the Quick View section for updated status.

6 Troubleshooting

Instrument		
Symptom	**Possible Cause**	**Action**
Autosampler does not move the plate to a higher position	The plate base is not sitting properly on the autosampler.	The plate base should sit flat on the autosampler. When placing the plate on the autosampler, ensure that the pins in the autosampler are properly aligned with the holes at the bottom of the plate base, and that the left and right sides are latched.
	The plate retainer is lifted off the plate base by array.	Securely clip the plate retainer and plate base together.
	The septum is lifted off the CBC.	Ensure that the septum is completely inserted into position. Listen for the light clicking sound that occurs when the septum is pressed down firmly into position.
When you remove the heat seal from a new pouch, some residual seal remains on top of the pouch. 	The top seal of the pouch has become delaminated and left the polyethylene behind on the pouch cap. Pouch did not equilibrate to room temperature.	Use a pipette tip to remove the entire seal from the pouch cap before installing on the instrument.
Error Messages		
Symptom	**Possible Cause**	**Action**
'Bubble' error	Bubbles present	Run the Remove Bubbles wizard.

Dashboard		
Symptom	**Possible Cause**	**Action**
Consumables status in the Dashboard is not updated.	Dashboard does not update automatically.	Click **Refresh**.
After installing new CBC or ABC, the consumables status in the Dashboard is not updated automatically.	Dashboard does not update automatically.	Click **Refresh** after changing or installing consumables.
Software		
Symptom	**Possible Cause**	**Action**
Status icon is instead of . 	One or more of the services are stopped.	Hover the mouse pointer over the status icon. If any item does not display a checkmark, select Programs Applied Biosystems 3500 Server Monitor. Right-click the status icon, then select **Services**. If any item does not display a checkmark, click the item to start the service. 
Create Injection List and Start Run buttons dimmed	The Pause After Last Injection preference is set, and the instrument is paused.	Go to Monitor Run and resume the run. When the run is complete, Create Injection List and Start Run buttons are active.