DNA: Typing with the 3500xL Genetic Analyzer

Principle   The PCR products from the amplification process are analyzed by electrophoresis to separate the STR alleles according to size. The alleles are detected using fluorescent dye labeling.

Promega's fluorescent multicolor dye technology allows multiple loci, including loci that have alleles with overlapping size ranges, to be analyzed in a single capillary injection. Alleles for overlapping loci are distinguished by labeling locus-specific primers with different color dyes.

Procedural safeguards   The following is a list of procedural safeguards for DNA typing.

- Access to the amplification/typing room is restricted to authorized personnel only.
- Gloves and a dedicated lab coat must be worn at all times.
- All tubes containing amplified DNA are to be pulse-centrifuged before being opened. Do not attempt to open tubes with just one hand.
- Pipette all amplified samples slowly to avoid creating an aerosol. Do not "blow out" the last bit of liquid in a pipette tip.
- All amplified DNA is to remain in the amplification/typing room.
- No equipment used in the amplification/typing room is to be removed from this area of the laboratory.
- Wash down all surfaces with ethanol followed by water.
- Wash the tube openers, thermal cycler, and pipette barrels with ethanol and dry with a clean lab wipe.

Continued on next page

Title:	DNA: Typing with the 3500xL Genetic Analyzer		
Approved by:	Laboratory Director	Qualtrax Revision No: 2	Page 1 of 15
Issue Date:	04/2017	Document Class: LFS Policy/Procedure	

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Equipment, reagent, and supplies

This procedure uses the following equipment:

- 3500xL Genetic Analyzer
- 3500xL capillary array, 36cm
- thermal cycler set to 95°C for denaturation
- freezer block
- centrifuge
- pipettes and barrier tips
- microcentrifuge tubes
- plate retainer & base set
- POP-4® polymer for the 3500xL Genetic Analyzer
- Anode Buffer Container (ABC) containing 1x running buffer
- Cathode Buffer Container (CBC) containing 1x running buffer
- Cathode Buffer Container septa
- Conditioning Reagent pouch
- Promega WEN Internal Lane Standard (ILS) 500, contained within PowerPlex® Fusion 6C kit
- Promega PowerPlex® Fusion 6C Ladder, contained within PowerPlex® Fusion 6C kit
- Promega PowerPlex® Fusion 6C Matrix Standard
- MicroAmp® Optical 96-well Reaction Plate and septa
- Hi-Di™ Formamide
- PCR product

WARNING – Formamide is an irritant and known teratogen. Avoid skin contact and inhalation; use in a well ventilated area. Wear lab coat, gloves and protective eyewear when handling.

3500xL parameters

The following parameters are used for samples amplified with the Promega PowerPlex® Fusion 6C PCR amplification kit:

4.13.2.5.2

Run Module	HID36_POP4XL
Dye Set	Promega J6
Injection Parameters	1.2 kV, 24 seconds
Run Parameters	13kV, 1500 seconds

Continued on next page

Title: DNA: Typing with the 3500xL Genetic Analyzer
Approved by: Laboratory Director
Issue Date: 04/2017

Qualtrax Revision No: 2
Document Class: LFS Policy/Procedure

Page 2 of 15

DNA: Typing with the 3500xL Genetic Analyzer, Continued

3500xL parameters (continued)

Notes on POP-4®:

- When starting a run, Data Collection will alert when there is insufficient polymer remaining for the number of injections designated for the run or if the polymer has been on the instrument for longer than 14 days.
- Polymer should not remain on the 3500xL for more than 14 days. Once this time has been reached, discard the polymer or mark it for training/validation. Perform the **Wash Pump and Channels** wizard to put fresh polymer on the instrument.
- If additional polymer is needed during the regular 14 day window of instrument use because an insufficient number of injections remain, the polymer can be replenished using the **Replenish Polymer** wizard. If the current Anode Buffer is still within its 14 day window, use an empty ABC for this wizard. Re-install the ABC after the wizard is complete.
- If an insufficient number of injections remain on the polymer pouch, it can be removed and stored in the refrigerator to be used for a smaller run before expiring. If storing polymer, indicate how many injections remain on the pouch.

Notes on the buffers:

- When installing ABC, tip the container sideways to transfer any buffer in the small chamber to the larger chamber and then remove the adhesive seal.
- When installing the CBC, remove the adhesive seal and wipe away any excess liquid on the lid. Install the two appropriate reservoir septa. Check that the septa are fully in contact with the container lid.
- As prompted by the software, this buffer is replaced if it has been on the instrument more than 14 days.
- As long as a run is started prior to the fourteenth day expiration time, the buffer may be used and Data Collection will not prevent completion of the run. However, once started, Data Collection will not allow additional injections to be added to the run.
- Try to synchronize changing buffers with changing polymer. Perform the **Wash Pump and Channels** wizard PRIOR to changing the ABC and CBC. (Alternatively, the ABC can be set aside during the wizard and put back on the instrument after the wizard is complete.)

Continued on next page

Title:	DNA: Typing with the 3500xL Genetic Analyzer			
Approved by:	Laboratory Director	Qualtrax Revision No:	2	Page 3 of 15
Issue Date:	04/2017	Document Class:	LFS Policy/Procedure	

DNA: Typing with the 3500xL Genetic Analyzer, Continued

3500xL parameters (continued)

Notes on capillary arrays:

- Arrays may be used past the marked expiration date and suggested injection number as long as the array is performing as expected.
- When the array is replaced, new spatial and spectral calibrations must be performed.
- Spatial calibration should also be performed:
  - If the laser, optics, or CCD camera have been realigned or replaced.
  - If data appears to have excessive pull-up/pull-down peaks.
  - If the instrument has been moved to a different location.
  - The detector window door is opened.
- Spectral calibration should also be performed:
  - If the laser, optics, or CCD camera have been realigned or replaced.
  - If data appears to have excessive pull-up/pull-down peaks.
  - If the instrument has been moved to a different location.
- To access Spatial or Spectral calibration screens, go to the **Maintenance** tab and select the appropriate function under the Calibrate window.

Title:	DNA: Typing with the 3500xL Genetic Analyzer		
Approved by:	Laboratory Director	Qualtrax Revision No: 2	Page 4 of 15
Issue Date:	04/2017	Document Class: LFS Policy/Procedure	

DNA: Typing with the 3500xL Genetic Analyzer, Continued

3500xL instrument preparation

Before you start a run:

Step	Action
1	In the Dashboard View, go to the **Consumables Information** pane and click the **Refresh** button to update the status of consumables. Review gauges for Polymer, Anode Buffer, Cathode Buffer, and Array to determine if any consumables need replacement or replenishment.
2	As needed, click the **Maintain Instrument** (yellow) button. Select the appropriate Wizard(s) to prepare the instrument: <table><tr><td>**If ...**</td><td>**Then ...**</td></tr><tr><td>A new array needs to be installed</td><td>Start the **Install Capillary Array** wizard.</td></tr><tr><td>Fresh polymer is needed</td><td>Start the **Wash Pump and Channels** wizard.</td></tr><tr><td>There are bubbles in the channel</td><td>Start the **Remove Bubbles** wizard.</td></tr></table>
3	Follow the prompts to complete each task.
4	If fresh anode buffer is needed, open the door and replace the ABC. Click **Refresh**.
5	If fresh cathode buffer is needed, press the tray button. Open the door and replace the CBC. Click **Refresh**.
6	In the Dashboard view, set the oven to 60°C. Click the **Start Pre-heat** button to warm the oven and detection cell. NOTE: Applied Biosystems recommends that the instrument remain at the selected temperature for at least 30 minutes before the start of a run. The pre-heat function automatically turns off after two hours.

Continued on next page

Title: DNA: Typing with the 3500xL Genetic Analyzer
Approved by: Laboratory Director
Issue Date: 04/2017

Qualtrax Revision No: 2
Document Class: LFS Policy/Procedure

Page 5 of 15

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Creating or importing a plate

The following is the procedure for entering plate information. Plate information can be entered manually or imported.

Step	Action
1	In the Dashboard view, click **Create New Plate** button.
2	Under the side menu, **Define Plate Properties** is highlighted. In the **Plate Details** pane, enter a run name and verify the following settings: • Number of Wells: 96 • Plate type: HID • Capillary Length: 36 cm • Polymer: POP4
3	Click **Assign Plate Contents** either in the Setup menu or at the bottom of the Plate Details pane.
4	To import the plate setup: <table><tr><th>Step</th><th>Action</th></tr><tr><td>1</td><td>In the Plate view, click **Import** button.</td></tr><tr><td>2</td><td>Select the desired text file and open it.</td></tr><tr><td>3</td><td>After importing the plate setup, click on the **Table View**.</td></tr><tr><td>4</td><td>Check that the Assays, File Name Conventions, and Results Groups are correct.</td></tr><tr><td>5</td><td>Assign the Sample Type for the positive and negative amplification controls and the reagent blanks. All other sample types import.</td></tr><tr><td>6</td><td>Save the changes made by selecting **Save Plate**.</td></tr></table>

Table continued on next page

Title:	DNA: Typing with the 3500xL Genetic Analyzer		
Approved by:	Laboratory Director	Qualtrax Revision No: 2	Page 6 of 15
Issue Date:	04/2017	Document Class: LFS Policy/Procedure	

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Creating or importing a plate
(continued)

Step	Action
5	To enter the plate information manually:

Step	Action
1	At the bottom of the **Assign Plate Contents** pane, add Assay, Filename Convention and Results Group parameters from libraries. • Select the appropriate **Assay** (Fusion 6C) and Add to Plate. • Select the appropriate **File Name Convention** (Fusion6C) and Add to Plate. • Select the appropriate **Results Group** (Fusion 6C) and Add to Plate. Alternatively, **Create Plate from Template** (Fusion 6C template) will pre-fill the parameters for you.
2	Under the **Plate or Table View** tab, enter sample names for the run (remember one injection is three full columns).
3	On the **Table View** tab, assign the Assay/File Name Convention/Results Group/and Sample Type (Sample/Positive Control/Negative Control/Allelic Ladder) for each sample.
4	To use Fill Down for these selections, highlight the wells you want to fill and enter Ctrl D, do not Fill Down by selecting the top of the column. This will add these parameters to the full plate instead of just your intended injections and the full plate will inject.
5	Save the changes made by selecting **Save Plate**.

Continued on next page

Title:	DNA: Typing with the 3500xL Genetic Analyzer		
Approved by:	Laboratory Director	Qualtrax Revision No: 2	Page 7 of 15
Issue Date:	04/2017	Document Class: LFS Policy/Procedure	

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Sample preparation   This is the procedure for preparing samples on the 3500xL genetic analyzers.

Step	Action
1	Thaw the Hi-Di™ Formamide. Prepare a mixture of WEN ILS 500 size standard/formamide. • 0.5 µl WEN ILS 500 X (# samples + 4) • 9.5 µl Hi-Di™ Formamide X (# samples + 4) Vortex for 10-15 seconds to mix.
2	Using the plate layout, pipet 10 µl of the WEN ILS 500 standard/formamide mixture into all wells that will contain sample or Allelic Ladder. Ensure that at least one Allelic Ladder well is included within each 24-sample injection. DNA Manager will automatically designate a ladder and blank per injection.
3	Add 1 µl of amplified sample or 1µl of PowerPlex® Fusion 6C Allelic Ladder to each well.
4	Into each Blank well, pipet 10 µl of formamide and 1 µl of WEN ILS 500 standard/formamide mixture. Ensure that there is at least one Blank well per plate.
5	Cover the plate with a plate septa.
6	Centrifuge the plate briefly (~ 20 seconds).
7	Denature the plate at 95°C for 3 minutes. Snap cool the plate for 3 minutes.
8	Place the plate into a plate base. Snap the retainer over the plate and into the base.

Title:	DNA: Typing with the 3500xL Genetic Analyzer		
Approved by:	Laboratory Director	Qualtrax Revision No: 2	Page 8 of 15
Issue Date:	04/2017	Document Class: LFS Policy/Procedure	

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Starting the run

This is the procedure for starting a run on the 3500xL genetic analyzers.

Step	Action
1	Press the **Tray** button. When the tray has reached the front, open the instrument doors and load the plate in the autosampler with the notched corners aligned.
2	Close the instrument doors and wait for the instrument light to turn green.
3	Click **Load Plates for Run** within the navigation pane to assign the plate and specify the position of the plate in the autosampler.
4	Unlink any previously linked plates. This is very important because the instrument does not automatically unlink plates after a run is complete and will rerun any currently linked plates when **Start Run** is clicked.
5	Click **Link Plate** for desired position and chose plate from the list.
6	You can use the **Link Plate for Run** at the bottom of the **Assign Plate Contents** screen, but the instrument will link the plate to any position where a plate is loaded on the instrument. This defaults to position A if both plate positions are loaded. If the plate is linked this way, double check that the plate is linked to the desired position and previously run plates are unlinked.
7	Ignore the Run Name field at the top of this view. That is an instrument created run name, not the name the plate will be saved as.
8	Click **Create Injection List** and verify the correct injections and plates will run.
9	Click the **Start Run** button.
10	Verify that the run begins. NOTE: Warning pop-ups may need to be accepted if the array is outside of its marked expiration date or suggested injection number.
11	The instrument will pause after the last injection to allow for the immediate addition of re-injections. To complete the run, click **Resume Run**. To re-inject, see *Monitoring the run*.

Continued on next page

Title: DNA: Typing with the 3500xL Genetic Analyzer
Approved by: Laboratory Director   Qualtrax Revision No: 2   Page 9 of 15
Issue Date: 04/2017   Document Class: LFS Policy/Procedure

All printed copies are uncontrolled.

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Monitoring the run

Samples can be monitored while running on the instrument under the **Monitor Run** tab. Select a sample from the plate view and view raw data in the bottom pane.

More detailed results for <u>completed</u> injections can be viewed under the **View Fragment HID Results** tab. Viewing the quality of the results here will help determine if re-injections are needed. The results of a run that is still "paused after last injection" can be viewed here prior to resuming the run.

Step	Action
1	Determine the samples that need to be re-injected. <table><tr><td>**To ...**</td><td>**Then ...**</td></tr><tr><td>Re-inject an entire injection</td><td>Select that injection and click **Duplicate**.</td></tr><tr><td>Re-inject specific samples</td><td>Select those samples using the Ctrl key and click the **Re-Inject** button on the right above the raw data pane. Include the ladder, if necessary.</td></tr></table> NOTE: If additional samples need to be added to the re-injection, delete that injection from the injection list and select ALL samples to be re-injected. Otherwise, a third injection will be added.
2	If a ladder was not selected, the instrument will prompt whether you want to include a ladder in the re-injection. Selecting a ladder here will add an additional separate injection for the ladder.
3	The instrument defaults to: • Reuse existing protocol • Following all injections. This may be changed to "After original injection" if needed.

Table continued on next page

Title: DNA: Typing with the 3500xL Genetic Analyzer
Approved by: Laboratory Director   Qualtrax Revision No: 2   Page 10 of 15
Issue Date: 04/2017   Document Class: LFS Policy/Procedure

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Monitoring the run (continued)

Step	Action
4	To re-inject samples after the run has completed:

Step	Action
1	Select **Edit Existing Plate**. Select the plate to be re-injected. If the plate is still linked, select **Unlink** and edit.
2	Clear the Assay, File Name Convention, and Results Group for all samples by clicking the three red X boxes in the bottom pane.
3	Add the Assay, File Name Convention, and Results Group from the Library to the plate as described in Step 1 of manually entering plate information.
4	For the samples needing re-injection, assign the Assay/File Convention/Results Group in the **Table View**. Leave all other samples blank for these three selections.
5	Save the changes made by selecting **Save Plate**.
6	**Link** and **Run** the plate as described previously.

Continued on next page

Title: DNA: Typing with the 3500xL Genetic Analyzer
Approved by: Laboratory Director
Issue Date: 04/2017

Qualtrax Revision No: 2
Document Class: LFS Policy/Procedure

Page 11 of 15

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Maintaining the instrument

See the *3500/3500xL Genetic Analyzer User Guide* for more detailed instructions on performing maintenance tasks.

Daily or before each run:

- Check the status of the consumables in the Dashboard by viewing the status of the anode buffer, cathode buffer, and polymer.
- Ensure that all the buffer is in the non-waste section of the anode buffer container.
- Check for bubbles in the pump block and channels. If bubbles are present, use the Remove Bubble wizard.
- Check for leaks and dried residue around the buffer pin, check valve, and array locking lever.
- Ensure that the plates are properly assembled.
- Ensure that the plate assemblies and the cathode buffer container are positioned on the plate deck properly. They should sit securely on the deck.
- Ensure the reservoir septa are seated firmly on the cathode buffer container.

Other periodic maintenance tasks:

- Restart the computer and instrument:

Step	Action
1	Exit Data Collection software application.
2	Restart the computer by selecting **Shutdown** from the **Start** menu.
3	Press the 3500xL power button to turn off the instrument.
4	Turn on the computer, but do not login.
5	Turn on the 3500xL and wait for the solid green light to appear.
6	Login to the computer.
7	Wait for indicator in bottom right corner of screen to turn to a green checkmark.
8	Launch Data Collection and login.

- Run the Wash Pump and Channels wizard.
- Flush the water seal trap.
- Clean the anode buffer container pin-valve assembly on the polymer delivery pump.

Continued on next page

Title:	DNA: Typing with the 3500xL Genetic Analyzer		
Approved by:	Laboratory Director	Qualtrax Revision No: 2	Page 12 of 15
Issue Date:	04/2017	Document Class: LFS Policy/Procedure	

All printed copies are uncontrolled.

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Instrument shutdown

The 3500xL may be shut down as needed or during an extended period of no use.

Step	Action
1	Start the **Shutdown the Instrument** wizard from the **Maintenance Wizards** screen. NOTE: This wizard takes 60 minutes to complete.
2	Remove the polymer bag and install a conditioning reagent pouch on the instrument.
3	If the polymer pouch will be saved for a later use, place a pouch cap securely on the polymer bag and store it in the refrigerator. Otherwise, discard the polymer pouch.
4	Exit Data Collection software application.
5	Turn off the computer by selecting **Shutdown** from the **Start** menu.
6	Press the 3500xL power button to shut down the instrument.

Continued on next page

Title: DNA: Typing with the 3500xL Genetic Analyzer
Approved by: Laboratory Director   Qualtrax Revision No: 2   Page 13 of 15
Issue Date: 04/2017   Document Class: LFS Policy/Procedure

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Spectral calibration

This is the procedure for performing a spectral calibration on the 3500 genetic analyzers.

Step	Action
1	Pre-heat the oven to 60° C. Thaw Hi-Di™ Formamide.
2	At first use, thaw the 6C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at 2-10° C.
3	Vortex 6C Matrix Mix. Add 10 µL of the 6C Matrix Mix to one tube of Matrix Dilution Buffer and vortex. NOTE: The diluted 6C Matrix Mix can be stored for up to one week at 2-10° C.
4	Add 10 µL of the diluted 6C Matrix Mix to 500 µL of Hi-Di™ Formamide and vortex.
5	Wells A1 through H3 of the 96-well plate are used for the spectral calibration. Add 15 µL of the diluted 6C Matrix Mix/ formamide prepared in Step 4 to each of the 24 wells.
6	Place the septa on the plate. Briefly centrifuge the plate to remove bubbles. Do **NOT** denature the plate.
7	Place the plate into a plate base. Snap the retainer over the plate and into the base. Place plate onto instrument and close the door.
8	Go to the **Maintenance** tab and select **Spectral**. Under the Calibration Run tab, choose the Plate Position (**A** or **B**), **Matrix Standard** from the Chemistry Standard drop-down menu, and **Promega J6** from the drop-down dye set menu.
9	Click **Start Run**. NOTE: Do not start the spectral calibration run until the oven is fully pre-heated.
10	After the run, review the results and check that the Quality Value is > .95 and that the Condition Number is < 8.0. If a capillary does not meet these criteria, the software can use information from an adjacent capillary that meets the acceptance criteria. Up to three adjacent capillary-borrowing events are allowed for a 3500xL genetic analyzer.

Continued on next page

Title: DNA: Typing with the 3500xL Genetic Analyzer
Approved by: Laboratory Director
Issue Date: 04/2017

Qualtrax Revision No: 2
Document Class: LFS Policy/Procedure

Page 14 of 15

DNA: Typing with the 3500xL Genetic Analyzer, Continued

Spatial calibration

This is the procedure to perform a spatial calibration on the 3500 genetic analyzers.

Step	Action
1	Go to the **Maintenance** tab and select **Spatial**.
2	Select Fill or No Fill. Click Start Calibration.
3	After the plate runs, evaluate Spectral data and check that there is • One sharp peak for each capillary. Small shoulder peaks are acceptable. • One (+) marker at the apex of every peak. No off apex markers. • An even peak profile (all peaks about the same height).
4	Accept or Reject Results. See the *User Guide* for troubleshooting if results are not passing.

Title:	DNA: Typing with the 3500xL Genetic Analyzer		
Approved by:	Laboratory Director	Qualtrax Revision No: 2	Page 15 of 15
Issue Date:	04/2017	Document Class: LFS Policy/Procedure	