
Procedure for Use of the 3500xL Genetic Analyzer

1.0 Purpose – To specify the steps for preparing and running samples on the 3500xL Genetic Analyzers.

2.0 Scope – This procedure applies to DNA Database Section Forensic Scientists and trainees who perform DNA analysis using the 3500xL Genetic Analyzers.

3.0 Definitions

- **Run/Injection** – Each set of 24 samples that is injected and separated electrophoretically on a Capillary Electrophoresis Unit (3500xL Genetic Analyzer or equivalent).

4.0 Equipment, Materials, and Reagents

- Centrifuge
- Amplified DNA products
- 9700 ABI Thermal cycler
- Calibrated Pipettes
- ART Pipette Tips (or equivalent, various sizes)
- Anode Buffer Container (ABC)
- Cathode Buffer Container (CBC)
- POP-4
- Promega PowerPlex® Fusion Reagents
- Formamide
- 3500xL Genetic Analyzer
- 96 well plates with septa
- Plate retainer for 3500xL
- Ice plate from freezer (optional)
- Heat Block

5.0 Procedure

5.1 Logging In/Logging Out of the 3500xL Software

5.1.1 In the 3500 Login box, enter individualized user name and password. Performing maintenance procedures under another user's log-in name or under a generic log-in name is not permitted.

5.1.2 Click **OK**.

5.1.3 The 3500 Series Data Collection Software launches and the **Dashboard** appears.

5.1.4 To log out of the 3500 Series Data Collection Software, return to the **Dashboard** screen and click the **Log Out** button in the upper right corner.

5.2 Pre-Heating Oven Manually

5.2.1 A scheduled run will not start until the temperature is stabilized.

NOTE: Applied Biosystems recommends the oven be pre-heated at least 30 minutes prior to starting a run.

5.2.2 From the **Dashboard** screen, click the **Start Pre-Heat** button. The temperature shall be set to 60 °C.

5.2.3 The oven will remain at the desired temperature for 2 hours.

5.3 Checking Consumable Status and Replenishing Consumables

5.3.1 Click **Refresh** to update Consumables Information on the **Dashboard** screen. Check the Consumables Information for the number of injections, samples, or days remaining for a consumable. The Quality Control Officer (QCO) shall be notified and the instrument shall not be used if any consumables are expired, past the recommended use, or if any reagent volumes are too low for use.

5.3.2 Check to ensure that no weekly maintenance actions are pending in the Maintenance Notifications list. Notify the QCO if weekly maintenance is required or if the 3500xL needs additional polymer. The instrument shall not be used until the above maintenance is complete.

5.3.3 Ensure the fill levels of the buffers are at the top of the fill line and check that the septa are properly seated. Notify the QCO if buffer levels are low.

5.4 Labeling

5.4.1 The side of a 96 well plate used for capillary electrophoresis shall be labeled with the DNA Database Forensic Scientist's initials. The columns used for a particular run shall be labeled by writing the project name on the front of the plate and shall be aligned with the specific columns (e.g., analyst initials_CH_date of punching, QCB-2013-00001 or QCB-13-1, COS-2013-4). If subsequent runs are loaded in a capillary electrophoresis plate, a modified project name shall be used to designate the other additional runs (e.g., QCB-2013-1_replate).

5.5 Preparing Samples to Run on the 3500xL

5.5.1 Check the pump assembly for bubbles and run the Remove Bubbles wizard if needed. See the DNA Database Section Procedure for Instrument and Equipment Quality Control for further instruction.

5.5.2 The WEN ILS 500 shall be centrifuged briefly to bring contents to the bottom, then vortexed for 15 seconds. Pulse spin for 1-2 seconds in a microcentrifuge before use to ensure reagent is at the bottom of the tube. Do not centrifuge each tube longer than 1-2 seconds after vortexing as this may cause reagent to form a gradient.

- 5.5.3** Prepare the master mix for a 24-well run by combining the following in a single microcentrifuge tube.

| Reagent | Volume |
|------------------|----------------|
| Hi-Di™ Formamide | Approx. 247 µL |
| WEN ILS 500 | 13 µL |

- 5.5.4** Prepare the master mix for a 96-well run by combining the following in a single microcentrifuge tube.

| Reagent | Volume |
|------------------|----------------|
| Hi-Di™ Formamide | Approx. 988 µL |
| WEN ILS 500 | 52 µL |

- 5.5.5** Vortex the tube containing the master mix for 10-15 seconds, then spin in a microcentrifuge.
- 5.5.6** In a 96-well reaction plate, dispense 10 µL of the formamide/size standard master mix into each well of the run.
- 5.5.7** The allelic ladder shall be centrifuged briefly to bring contents to the bottom, then vortexed for 15 seconds. Pulse spin for 1-2 seconds in a microcentrifuge before use to ensure reagent is at the bottom of the tube. Do not centrifuge each tube longer than 1-2 seconds after vortexing as this may cause reagent to form a gradient.
- 5.5.8** Spin the amplification plate in a centrifuge for 1 minute at 2000 rpm to ensure all punches and reagents are at the bottom of the wells and no bubbles are present.
- 5.5.9** Load 1.0 µL of allelic ladder and 1.0 µL of amplified sample into the appropriate wells of the 96-well reaction plate as specified by the 3500xL plate map worksheet.
- 5.5.10** Cover the reaction plate with the 96-well septa. Ensure the the tray is labeled according to the Labeling section of this procedure.
- 5.5.11** Spin the reaction plate in a centrifuge for 1 minute at 2000 rpm to ensure the contents of each well are mixed and collected at the bottom.
- 5.5.12** Using a heat block or thermal cycler, denature the reaction plate at 95 °C for 3 minutes.
- 5.5.13** Place the reaction plate immediately on an ice block for approximately 3 minutes, or allow the thermal cycler to ramp down to 4 °C and let the plate remain on the thermal cycler at 4 °C for at least 3 minutes.

5.6 Creating/Importing a Plate Record on the 3500xL

- 5.6.1** Samples run shall have the following run parameters:

5.6.1.1 Assay:

- 5.6.1.1.1** The standard injection for both buccal and blood is Promega G5 18s WEN.

5.6.1.2 The File Name Convention shall be set to HID_Validation.

5.6.1.3 The Results Group shall be set to HID Validation.

5.6.2 Sample Types shall be assigned as follows:

- Ladder: Allelic Ladder
- Reagent Blank: Negative Control
- Negative Amplification Control: Negative Control
- 2800M (Positive Amplification Control): Positive Control
- Sample: Sample

5.6.3 To create a new plate record:

5.6.3.1 Click on the **Create New Plate** icon on the **Dashboard**.

5.6.3.2 Fill out the **Plate Details** screen.

5.6.3.2.1 Enter the name of the plate.

5.6.3.2.2 The Number of Wells shall be set to 96.

5.6.3.2.3 The Plate Type shall be HID.

5.6.3.2.4 The Capillary Length shall be 36 cm.

5.6.3.2.5 The Polymer Type shall be POP-4.

5.6.3.2.6 The owner shall be the initials of the individual running the plate.

5.6.3.3 Click **Assign Plate Contents**. The screen will update to the **Assign Plate Contents** view of the **Setup** menu.

5.6.3.4 Plate Contents may be entered in **Plate View** or **Table View**.

5.6.3.4.1 Plate View: At the bottom of the screen, add the appropriate Assay, File Name Convention, and Results Group from the Library. Manually type the sample names in the corresponding wells. Apply the run parameters to the plate by highlighting the cells with sample names and clicking the check mark box next to the desired Assay, File Name Convention, and Results Group. Ensure blank wells are not selected when applying the run parameters.

To assign sample types, expand the **Customize Sample Info** menu from the bottom right of the screen. Select the well(s) of interest and add the appropriate Sample Type from the drop down menu.

5.6.3.4.2 Table View: At the bottom of the screen, add the appropriate Assay, File Name Convention, and Results Group from the Library. Manually type the Sample Names by the corresponding wells. Once the sample names are entered, complete each row by adding the desired Assay, File Name Convention, Results Group, and Sample Type.

NOTE: When completing a column, fill down by highlighting the desired cells and selecting “Ctrl” + “D” on the keyboard.

5.6.3.5 When the plate layout is complete, the plate may be immediately run or saved to run at a later time.

5.6.3.5.1 To immediately link the reaction plate, maintain the current screen and follow the instructions for Linking the Reaction Plate.

5.6.3.5.2 If the plate will be run at a later time, save and close the plate.

5.6.4 To create a plate from a template:

5.6.4.1 Click on the **Create Plate from Template** icon on the **Dashboard**.

5.6.4.2 A pop-up will appear. Choose **PPF Plate Template** and click **Open**.

5.6.4.3 Fill out the **Plate Details** screen as follows:

5.6.4.3.1 Enter the name of the plate.

5.6.4.3.2 The Number of Wells shall be set to 96.

5.6.4.3.3 The Plate Type shall be HID.

5.6.4.3.4 The Capillary Length shall be 36 cm.

5.6.4.3.5 The Polymer Type shall be POP-4.

5.6.4.3.6 The owner shall be the initials of the individual running the plate.

5.6.4.4 Click **Assign Plate Contents**. The screen will update to the **Assign Plate Contents** view of the **Setup** menu.

5.6.4.4.1 Enter Plate Contents

5.6.4.4.1.1 Plate View: Manually type the sample names in the corresponding wells. Apply the run parameters to the plate by highlighting the cells with sample names and clicking the check mark box next to the desired Assay, File Name Convention, and Results Group. Ensure blank wells are not selected when applying the run parameters.

To assign sample types, expand the **Customize Sample Info** menu from the bottom right of the screen. Select the well(s) of interest and add the appropriate Sample Type from the drop down menu.

5.6.4.4.1.2 Table View: Manually type the Sample Names by the corresponding wells. Once the sample names are entered, complete each row by adding the desired Assay, File Name Convention, Results Group, and Sample Type.

NOTE: When completing a column, fill down by highlighting the desired cells and selecting “Ctrl” + “D” on the keyboard.

5.6.4.5 When the plate layout is complete, the plate may be immediately run or saved to run at a later time.

5.6.4.5.1 To immediately link the reaction plate, maintain the current screen and follow the instructions for Linking the Reaction Plate.

5.6.4.5.2 If the plate will be run at a later time, save and close the plate.

5.6.5 To import the plate setup text file:

5.6.5.1 Using the 3500xL plate map worksheet, copy the information from the 3500xL Import Sheet tab.

5.6.5.2 Paste the information into a notepad document and save the resulting .txt file. Do not copy/paste empty horizontal wells as this prevents the file from importing. Be sure to hit enter after the last sample entry so the cursor is below the last sample for the run.

5.6.5.3 To import the file, click on **Library** in the menu bar. Select **Import** toward the top of the screen. Select the .txt file corresponding to the plate to be run and click **Open**. A pop-up will give notification of the import status. Click **OK** after successful import.

5.7 Linking the Reaction Plate

5.7.1 If plates need to be unlinked from the instrument, select **Library** from the menu bar. Select the yellow **Main Workflow** button on the left of the screen; select **Load Plates for Run** in the **Run Instrument** menu. Before selecting **Unlink** for a plate, ensure the plate is not currently in process. Select **Unlink** for each plate that is linked.

5.7.2 If the 3500xL is not currently processing any plates, press the Tray button on the front of the instrument. This will bring the autosampler to the front. When the autosampler stops, open the instrument door and remove processed plates from the instrument. Remove each processed plate from the retainer assembly and store appropriately.

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- 5.7.3 Place the 96-well plate that is to be processed onto the plate base and snap the plate retainer over the top. Ensure the holes of the plate retainer and the septa are aligned.
 - 5.7.4 Place the plate onto the 3500xL autosampler, positioned correctly with the notch in the lower right corner. Close the instrument door.
 - 5.7.5 If a plate record is open in the **Assign Plate Contents** screen, click **Link Plate for Run**.
 - 5.7.6 When opening a previously saved or imported plate, select **Library** from the menu bar and then select the yellow **Main Workflow** button on the left of the screen. Select **Open Plate** and choose the option to **Edit Existing Plate**. Select the appropriate plate from the list and choose **Open**. After verifying the plate setup run and parameters, select **Link Plate for Run**.
 - 5.7.7 A pop-up will give notification of the loaded plate's status. Click **OK**.
 - 5.7.8 The Screen will update to the **Load Plates for Run** view of the **Run Instrument** menu.
 - 5.7.9 Repeat steps 5.7.6 through 5.7.8 above to link a second plate if applicable.
 - 5.7.10 Verify plates are correctly linked. Click **Create Injection List** to view the run schedule. The screen will update to the **Preview Run** screen of the **Run Instrument** menu. Verify the plate setup and run parameters.
- 5.8 **How to Quick Start a run**
- 5.8.1 From the **Dashboard**, select **Quick Start Run**.
 - 5.8.2 Select the appropriate plate from the Library; click **Load Plate**.
 - 5.8.3 The screen will update to the **Load Plates for Run** view of the **Run Instrument** menu.
 - 5.8.4 Verify plates are correctly linked. Click **Create Injection List** to view the run schedule. The screen will update to the **Preview Run** screen of the **Run Instrument** menu. Verify the plate setup and run parameters.
- 5.9 **Starting the Run**
- 5.9.1 Click **Start Run**.
 - 5.9.2 A dialog box appears. Do not leave run unattended until the box has disappeared and the run has begun. The dialog box may bring up actions to complete or questions to answer. When a run is in progress, the injection will be listed as active and the corresponding plate map will have that injection highlighted in green.
- 5.10 **Performing a Subsequent Run Using the Same Plate Record**
- 5.10.1 From the **Dashboard** screen, select **Edit Existing Plate**. Choose the desired plate from the list and select **Open**. Click the samples which do not need to be re-run and deselect the Assay.

5.10.2 Select the samples that need to be injected again. Select the desired Assay and ensure it is assigned to the samples for re-injection. It may be necessary to add an additional assay from the Library.

5.10.2.1 For re-injection, only the allelic ladder is required to be re-injected along with the sample(s).

5.10.2.2 For plate re-setup (i.e., preparing samples on another plate or another portion of the same plate with a new aliquot of formamide and WEN ILS 500), only the allelic ladder shall be re-injected along with the sample(s).

5.10.3 Double check all entries, link plate, and start run as above in Linking the Reaction Plate and Starting the Run.

6.0 Limitations - Once a run on a plate has been set up, the run can be used for up to 72 hours. If another injection is to be performed after 72 hours, the samples shall be set up either on another plate or in different wells.

Temperature: The results from the 3500xL instrumentation can be affected by temperature changes. If the temperature in the room where the instrument is located is outside of the range of 60 °F to 85 °F, this shall be taken into account during analysis. If the results are affected, then the QCO (or designee) shall take the affected instrument(s) offline until the temperature is within range and the instrument has passed a QC check.

7.0 Safety

7.1 Formamide – consult MSDS sheet prior to use.

7.2 Heat – the thermal cycler is hot during the denaturation process. Use caution when removing a plate from the thermal cycler to place in the ice block.

7.3 Pregnancy – DNA Database Forensic Scientists and DNA Database Trainees who are pregnant shall not use this procedure.

8.0 References

Applied Biosystems 3500/3500xL Genetic Analyzer. User Bulletin. 2011 Life Technologies Corporation. Part Number 4469192 Rev. A.

Applied Biosystems 3500/3500xL Genetic Analyzer User Guide. 2010 Life Technologies Corporation. Part Number 4401661 Rev. C.

DNA Database Section Procedure for DNA Database Training for PowerPlex® Fusion

DNA Database Section Procedure for Instrument and Equipment Quality Control

DNA Database Section Procedure for Sample Processing Quality Control

PowerPlex Fusion System: Instructions for Use of Product DC402 and DC2408. 2012 Promega Corporation. Part Number TMD039 Rev. 10/12. (or most recent revision)

9.0 Records – N/A

10.0 Attachments - N/A

| Revision History | | |
|-------------------------|----------------|---|
| Effective Date | Version Number | Reason |
| 08/03/2015 | 1 | Original Document |
| 12/21/2015 | 2 | 4.0 added heat block; 5.5.11 added heat block/thermal cycler; 5.5.8 added centrifuge time/rpm requirement; 5.5.9 to 5.5.13 renumber |
| 4/18/2016 | 3 | Remove 5.6.1.1.2, 5.10.2.2, and 5.10.2.3 due to removal of 24s injection; 5.5.2 - remove option to tap tubes, must briefly pulse tubes; 5.5 and 5.6.1 - updated CC5 to WEN. |