
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 casework analysts and trainees in the Forensic Biology Section who are qualified to use the 3500xl Genetic Analyzers.
- 3.0 Definitions** – See Section Definition list.

4.0 Equipment, Materials, and Reagents

- Centrifuge
- Amplified DNA products
- 9700 ABI Thermal cycler (or equivalent)
- Calibrated Pipettes
- ART Pipette Tips (or equivalent, various sizes)
- Anode Buffer Container (ABC)
- Cathode Buffer Container (CBC)
- POP-4
- Promega PowerPlex® Fusion 6C or Y23 Reagents
- Formamide
- QIAgility liquid handler
- 3500xL Genetic Analyzer
- 96 well plates with septa
- Plate retainer for 3500xL
- Ice plate from freezer (optional)
- Heat Block
- CSV file (optional)

5.0 Procedure

5.1 Logging In/Logging Out of the 3500xL Software

- 5.1.1** If necessary, in the 3500 Login box, enter the user name xAdministrator and password Administrator and click OK.
- 5.1.2** The 3500 Series Data Collection Software launches and the **Dashboard** appears.

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 or designee (QCO) shall be notified if consumables are past the recommended used, expired, or low volumes. The instrument shall not be used if the buffers are expired 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 - The side of a 96 well plate used for capillary electrophoresis shall be labeled with the Forensic Scientist's initials and date. The plate shall be assigned a plate name.

5.5 Preparing Samples to Run on the 3500xL

5.5.1 Samples shall be setup using the QIAgility instrument. Manual set up is allowed if the QIAgility is unavailable (e.g. due to maintenance), or if multiple analysts are setting up on the same plate, or multiple amplification plates are being combined, or if written supervisory approval is obtained.

5.5.2 Check the pump assembly for bubbles and run the Remove Bubbles wizard if needed. See the Forensic Biology Procedure for Calibration and Instrument Maintenance for further instruction.

5.5.3 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.**

Note: Separate WEN ILS is contained in the Y23 and Fusion 6C kits. The scientist shall ensure the proper WEN for the amplification kit is used.

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

Reagent	Volume
Hi-Di™ Formamide	Approx. 265µL
WEN ILS 500	14 µL


- 5.5.5** Vortex the tube containing the master mix for 10-15 seconds, then briefly spin in a microcentrifuge.

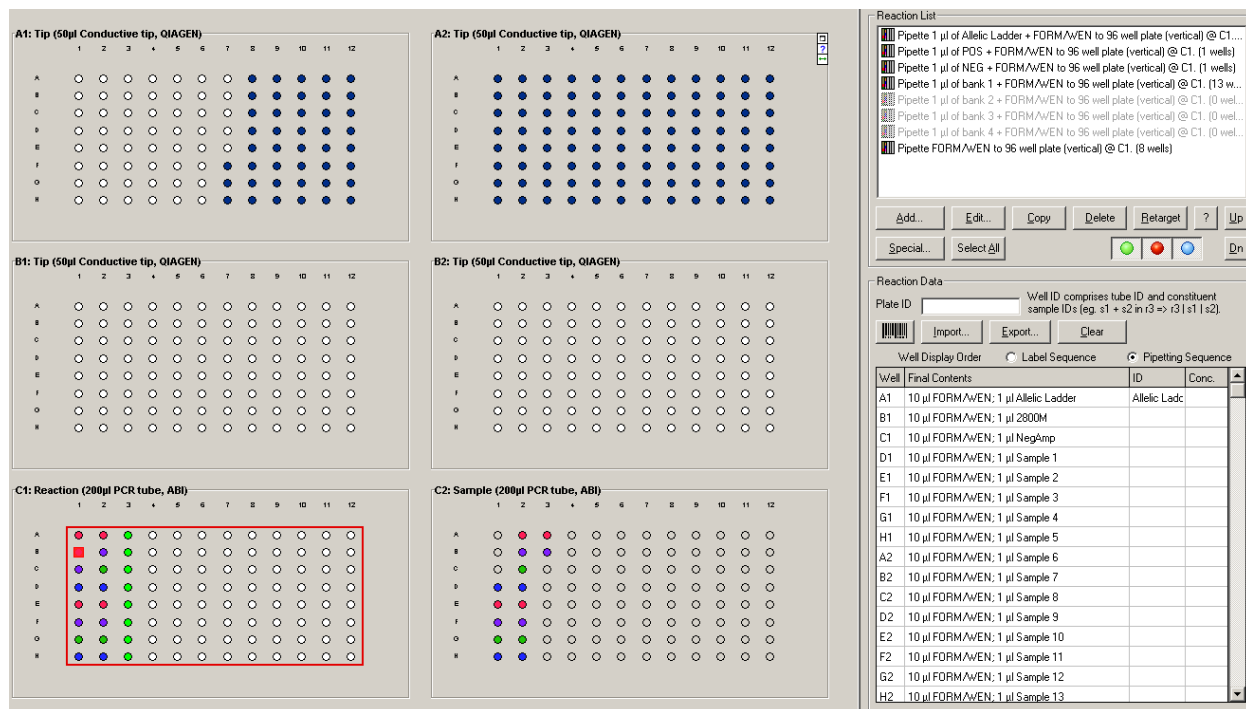
5.6 Manual Setup

- 5.6.1** In a 96-well reaction plate, dispense 10 µL of the formamide/size standard master mix into each well of the run.
- 5.6.2** 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.**
- 5.6.3** Spin the amplification plate in a centrifuge for 1 minute at 2000 rpm to ensure all reagents are at the bottom of the wells and no bubbles are present.
- 5.6.4** 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. Only one set of strip caps shall be removed at a time from the amplified product reaction plate when preparing the plate.
- 5.6.5** Cover the reaction plate with the 96-well septa. Ensure the tray is labeled according to the Labeling section of this procedure.
- 5.6.6** 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.
- 5.6.7** Using a heat block or thermal cycler, denature the reaction plate at 95 °C for 3 minutes.
- 5.6.8** 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.

5.7 Operation of the QIAgility to Prepare Samples

- 5.7.1** Prepare the master mix as described in **5.5.4**. Combine the microcentrifuge tubes for each run into a single tube. Vortex the tube containing the combined master mix, then pulse spin in a microcentrifuge.
- 5.7.2** Ensure the QIAgility instrument is on. The switch is located on the lower back left corner of the instrument. Forensic Scientists shall open the QIAgility software by double clicking the “Shortcut to CE Setup3500.QAS” protocol located on the computer desktop.
- 5.7.3** The worktable for CE (capillary electrophoresis) setup shall be set up as indicated on the screen. Holding the cursor over any colored well will give information about the contents of that well.


- 5.7.4 Within the CE Setup Protocol, left-click on Sample block C2. The right hand side of the screen will populate with information for that section. Click the Import button. The “Import Well Data” dialog box will appear.
- 5.7.5 Under Import File in the top left hand corner, click the browse  button. Locate the CSV file and click open.
- 5.7.6 Click the Import button at the bottom of the screen to import the samples and then click “Finish.”
- 5.7.7 Left-click on Reaction block C1. In the Reaction List on the upper right hand side of the screen, highlight all entries that do not correspond with the runs being processed and click “Delete.” The message “This will delete multiple reactions from the reaction list. Continue?” will appear. Click “Yes.”

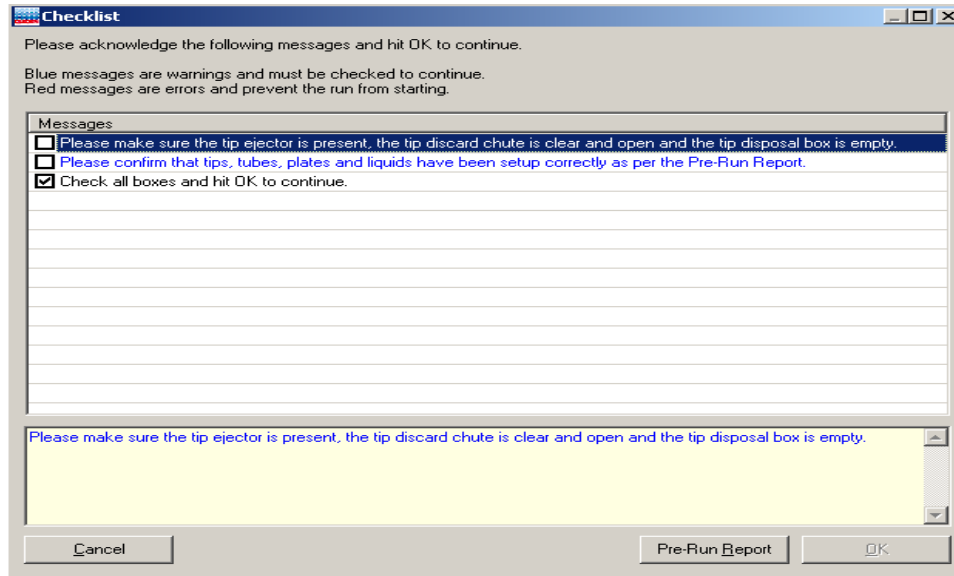



- 5.7.8 Highlight the “Pipette FORM/WEN to 96 well plate” line located at the end of the list. For each run that contains blank wells, click “Copy.” (If you have only one run, do not click Copy.) Highlight a “Pipette FORM/WEN to 96 well plate” line and click up until it is below the first “bank” of samples that contains empty wells.
- 5.7.9 Click “Edit” and change the “Number of Wells” to correspond with the number of blank wells at the end of the corresponding run. Click “OK.”
- 5.7.10 Repeat steps 5.6.8 to 5.6.9 for each run with blank wells.

- 5.7.11** Highlight all entries that do not correspond with the runs being processed and click “Delete.” The message “This will delete multiple reactions from the reaction list. Continue?” will appear. Click “Yes.”
- 5.7.12** Grasp the handle and pull up to open the Instrument lid. Inspect the worktable to ensure that the tip holders and plate holders are in the position which appears on the screen.
- 5.7.13** Place the following materials on the worktable:

Position	Reagent/Item
N of Reagent block R1 (green well in software)	1.5 mL tube containing formamide/WEN
I of Reagent block R1 (red well in software)	1.5 mL tube containing Ladder
Reaction block C1	Empty 96 well optical plate
Sample block C2	96 well optical plate containing amplified DNA samples

- 5.7.14** Check to ensure there are enough tips to process the run. To do this, left-click on either Tip Holder B1 or B2. On the right hand side of the screen it will indicate if there are enough tips. If there are not enough tips, insert a new set of tips into Tip Holder B1 or B2 (whichever is empty). Right click on the Tip holder that was just filled and choose “Set all tips on current plate to Available.” Close the instrument lid.
- 5.7.15** Click  to start the run. The “Save as” dialog box will appear. Save the run file to the Forensic Scientist’s folder under C:\Program Files\QIAgility\Data. The file shall be named to include at least the Forensic Scientist’s initials. This file shall also be imported into the Forensic Scientist’s case record repository.
- 5.7.16** After the Forensic Scientist clicks “Save,” the pre-run Checklist screen will appear. If the run has been set up correctly, the checklist will not list any warnings or errors other than the default messages listed below. If other messages are listed, user intervention is required before the run can be started. After completing the listed tasks, select the boxes next to the warnings to continue. Click “OK.”



- 5.7.17** A run can be paused and aborted at any time by clicking . Pauses will be logged in the post-run report. If the run is aborted, the instrument will discard the tip and return to its resting position. **Note:** A run will also be paused when the instrument lid is opened.
- 5.7.17.1** The instrument will complete the current operation and will then pause. A dialog box will appear indicating that the run was paused and whether the run will be continued or aborted. **Note:** The instrument does not stop immediately when the lid is opened. Therefore, wait until the Y-arm has stopped moving and the warning screen is displayed before continuing.
- 5.7.17.2** The instrument lid must be closed to abort the run. **Note:** An aborted run cannot be restarted from the point at which it was aborted.
- 5.7.18** If an error occurs or a warning is detected during a run, a dialog box which contains information about the error will appear. The Forensic Scientist will have 4 options: “Abort run,” “Retry operation,” “Ignore error,” and “Skip step.” Determine the error and the appropriate option.
- 5.7.19** Upon completion of each run, a support package is constructed and saved automatically within the program. This package contains the log file for the run and is used when reporting a problem to QIAGEN Technical Services. Click “OK.” **Note:** The Forensic Scientist will see a message concerning the support package only if an exception occurred during the run.
- 5.7.20** Upon completion of each run a Post-Run Report will also be displayed. This report shall be saved within the Forensic Scientist’s folder on the C drive (C:\ Program Files\QIAgility\Data\) on the computer associated with the QIAgility. Forensic Scientists

shall also print a copy of this report using the print to .pdf function to add to the case record repository.

- 5.7.21 Open the instrument lid and remove the optical plate from position C1. Cover this plate with the 96-well septa. Label the tray with the initials of the Forensic Scientist and date of run.
- 5.7.22 Remove the Ladder and Formamide/WEN tubes from the worktable. Remove and re-seal the optical plate from position C2. Empty the biohazard box.
- 5.7.23 Close the software by clicking the X in the top right corner. Follow the prompts to shut down the instrument. Click “Yes” to quit QIAgility. Click “Move to Safety Position (recommended).” Click “OK.”

5.8 Creating/Importing a Plate Record on the 3500xL

- 5.8.1 Samples run shall have the following run parameters (automatically added on import from workbook):

- 5.8.1.1 Assay: The standard injection is Fusion 6C (Promega G5 Y23 24s).

- 5.8.1.2 The File Name Convention shall be set to PowerPlex6C (Promega Y23).

- 5.8.1.3 The Results Group shall be set to PowerPlex 6C (PowerPlex Y23).

- 5.8.2 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

- 5.8.3 To create a new plate record:

- 5.8.3.1 Click on the **Create New Plate** icon on the **Dashboard**.

- 5.8.3.2 Fill out the **Plate Details** screen.

- 5.8.3.2.1 Enter the name of the plate.

- 5.8.3.2.2 The Number of Wells shall be set to 96.

- 5.8.3.2.3 The Plate Type shall be HID.

- 5.8.3.2.4 The Capillary Length shall be 36 cm.

- 5.8.3.2.5 The Polymer Type shall be POP-4.

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

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

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

5.8.3.4.1 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.

5.8.3.4.2 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.

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

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

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

5.8.4 To create a plate from a template:

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

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

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

- 5.8.4.3.1 Enter the name of the plate.
- 5.8.4.3.2 The Number of Wells shall be set to 96.
- 5.8.4.3.3 The Plate Type shall be HID.
- 5.8.4.3.4 The Capillary Length shall be 36 cm.
- 5.8.4.3.5 The Polymer Type shall be POP-4.
- 5.8.4.3.6 The owner shall be the initials of the individual running the plate.

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

5.8.4.4.1 Enter Plate Contents

5.8.4.4.1.1 **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.

5.8.4.4.1.2 **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.

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

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

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

5.8.5 To import the plate setup text file:

5.8.5.1 Using the 3500xL plate map worksheet, copy the information from the 3500xL Import Sheet tab. Save this tab as a .txt file.

5.8.5.2 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.

5.9 Linking the Reaction Plate

5.9.1 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.

5.9.2 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.

5.9.3 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.

5.9.4 Place the plate onto the 3500xL autosampler, positioned correctly with the notch in the lower right corner. Close the instrument door.

5.9.5 If a plate record is open in the **Assign Plate Contents** screen, click **Link Plate for Run**.

5.9.6 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**.

5.9.7 A pop-up will give notification of the loaded plate's status. Click **OK**.

5.9.8 The Screen will update to the **Load Plates for Run** view of the **Run Instrument** menu.

5.9.9 Repeat steps **5.8.6** through **5.8.8** above to link a second plate if applicable.

5.9.10 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.

5.10 How to Quick Start a run

5.10.1 From the **Dashboard**, select **Quick Start Run**.

5.10.2 Select the appropriate plate from the Library; click **Load Plate**.

5.10.3 The screen will update to the **Load Plates for Run** view of the **Run Instrument** menu.

5.10.4 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.

5.11 Starting the Run

5.11.1 Click **Start Run**.

5.11.2 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.

5.12 Performing a Subsequent Run

5.12.1 Reinjections shall be performed only if the entire run needs to be re-injected. Without supervisory approval, individual samples shall be re-setup only when a sufficient number of samples are available to ensure full injections are being performed.

5.12.2 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.

5.12.3 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.

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

5.12.3.2 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 required for injection along with the sample(s).

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

5.13 3500 instrument files shall be saved as a .zip file. Each case shall have a separate file, containing only samples and controls from that case. This file shall be added to the case object repository.

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 –Forensic Scientists and Trainees who are pregnant should 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.

Forensic Biology Procedure for Instrument and Equipment Quality Control

PowerPlex Fusion and/or Y23 System

9.0 Records – N/A

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
12/20/2016	1	Original Document