Forensic Biology Section

Operation of the 3500 Genetic Analyzer

1. Scope

1.1. The 3500 Genetic Analyzer (3500) is an automated capillary electrophoresis instrument that detects the size and quantity of DNA STR profiles. The system consists of a 3500 and a computer.

1.2. Amplified DNA samples with up to five different fluorescent dyes are placed in the autosampler and are electrophoresed through a polymer-filled eight-capillary array, the DNA fragments are separated by size as they migrate, and laser-induced fluorescence detects the blue, green, yellow, red and orange peaks.

1.3. DNA Analysis software analyzes the peaks as alleles and internal lane size standards.

2. Safety

2.1. Chemical Hazard: Formamide is a teratogen and is harmful by inhalation, skin contact and ingestion. Use in well-ventilated area. Use chemical resistant gloves when handling.

3. Reagents

- Hi-Di Formamide (Life Technologies)
- Cathode Buffer Container (Life Technologies)
- Anode Buffer Container (Life Technologies)
- Performance Optimized Polymer 3500 Series (POP-4, 384) (Life Technologies)
- 3500 Capillary Array, 36-cm (Life Technologies)
- 5C Matrix (Promega)
- Distilled, molecular biology grade water

4. Instrumentation

- 3500 Genetic Analyzer (Life Technologies)

5. Quality Assurance

5.1. Replace polymer if it has been in the instrument more than 14 days.

5.2. Change the Anode and Cathode Buffer containers every 14 days or as needed.

5.3. The capillary array does not expire with the Manufacturer's expiration date. It will typically last between 150 to 250 injections. An array can be used beyond the expiration date if the ILS and sample peaks have appropriate peak morphology, but it should be replaced when the quality of the peaks begin to degrade (e.g., peaks become broad or there are missing peaks).

5.4. Hi-Di does not expire. Ladder and Pos Control are indicators of effective denaturation.

5.5. Perform a spatial calibration (alignment of capillaries) if the array or detection block have been moved.

5.6. Perform a spectral calibration ("matrix") each time the capillary array is replaced or if pull-up or unexplained artifacts appears in numerous runs.

5.7. Wipe the electrodes with a Kimwipe if dried gel and/or salt build up on them.

5.8. Use a syringe to flush the Polymer Delivery Pump and fill the Water Trap Waste Container once a month or as needed (see "Flushing and Filling the Water Trap" steps).

5.9. Run the Wash Pump and Channels Wizard using Conditioning Reagent (warmed to approximately 30 – 35 degrees but not be over 40 degrees) when the array is changed or as needed (e.g., when double primer dimer artifact appears very high in raw data).

6. Procedural Notes

6.1. Applied Biosystems recommends that the oven be pre-heated for at least 30 minutes before starting a run. Pre-heating helps mitigate subtle first-run migration rate effects.

6.2. A sample plate may be re-injected within 24 hours using the same plate saved with a new name.

6.2.1. In Workflow, choose "Open Plate", "Edit Existing Plate".

6.2.2. Select "Plate Name", click "Open" and then "Unlink and Edit".

6.2.3. Select "Save Plate" and "Save as". Enter a new plate name.

7. Turning On the 3500 and DataCollection

7.1. Power on the computer but do NOT log in yet.

7.2. Power on the 3500 and wait for a steady green light on the front of the instrument.

- A steady yellow light means the computer and instrument are not communicating with each other (software could be corrupted and may need to be reinstalled).
- A steady red light means the instrument cannot initialize.

7.3. Log into the computer as INSTR-ADMIN (the password is posted on the computer).

7.4. Wait for the GREEN CHECKMARK to appear in the 3500 SERVER MONITOR ICON in the lower-right corner of the computer screen. This usually takes 2-3 minutes.

7.5. Launch Data Collection Software by clicking on the 3500 icon on the desktop (username and password are posted on instrument).

8. Performing Spatial Calibration (alignment of capillaries)

8.1. Select "Maintenance" and then "Spatial Calibration" in the navigation pane.

8.2. Select "No Fill", or "Fill" to choose if the array gets filled with polymer. Select "Perform QC Checks" if you want the system to check each capillary against the specified range for spacing and intensity.

8.3. Review the peak shapes and spacing. The following criteria should be met:

- Similar heights for all eight peaks.
- One orange cross marking the top of every peak. No misplaced crosses.
- Single sharp peak for each capillary. Small shoulders are acceptable.

8.4. Click "Accept Results" if all criteria are met. Otherwise, click "Reject Results", reinstall the array and re-run the spatial calibration (see the "Spatial calibration troubleshooting" section on page 300 of the ABI 3500 Genetic Analyzer User Guide if necessary).

9. Performing Spectral Calibration (dye matrix)

9.1. Set the oven temperature to 60 °C and click "Start Pre-heat". Pre-heating helps to mitigate subtle first-run migration rate effects.

9.2. If frozen, thaw the 5C Matrix Mix and Matrix Dilution Buffer completely. Do NOT refreeze the PowerPlex 5C Matrix Standards components. Store the Matrix Mix and Matrix Dilution at 2-10 °C, protected from light.

9.3. Thaw the Hi-Di Formamide (Hi-Di).

9.4. Vortex the 5C Matrix Mix for 10-15 seconds. Add 10 µl of 5C Matrix Mix to a tube of Matrix Dilution Buffer and vortex for 10-15 seconds (diluted 5C Matrix Mix can be stored up to 1 week at 2-10 °C).

9.5. Add 10 µl of the diluted 5C Matrix Mix to 500 µl of Hi-Di and vortex for 10-15 seconds.

9.6. Add 15 µl of 5C Matrix Mix/Hi-Di cocktail to wells A1 thru H1. Cover plate with septa and remove any air bubbles present in wells. DO NOT HEAT DENATURE.

9.7. Place the plate in a 3500 series 96-well plate base and cover with a plate retainer. Mount the plate on the 3500 autosampler.

9.8. Click the "Maintenance" tab and select "Spectral" under the Calibration Run Tab on the left side of the screen.

- 9.8.1. Choose "Matrix Standard" from the Chemistry Standard drop-down menu and "Promega G5" from the Dye Set drop-down menu.
- 9.8.2. The "starting well" should be set to "A01". IMPORTANT: you do not create a plate for the calibration (the software uses predetermined positions for the calibration), and you cannot specify standard location on the plate.
- 9.8.3. The number of wells should be set at 96.
- 9.8.4. Choose the appropriate plate position (A or B).
- 9.8.5. Be sure the "Allow Borrowing" option is NOT check marked.
- 9.8.6. Click "start run." (the Spectral Run takes approximately 30 minutes).

9.9. When the run has completed, the Spectral Quality Value (SQV) and Condition Number are displayed.

- 9.9.1. The SQV needs to be 0.95 or greater. An SQV of 1.0 is ideal.
- 9.9.2. The Condition Number needs to be less than 13.5. The Condition Number indicates the amount of overlap between the dye peaks in the fluorescence emission spectra of the dyes in the dye set.
- 9.9.3. When a spectral calibration completes successfully, the "Overall" row displays green. Click a capillary to display the spectral and raw data for a capillary. The order of the peaks for the G5 spectral is orange-red-yellow-green-blue. The peaks should be separate and distinct with no gross overlaps, dips, or other irregularities.
- 9.9.4. Note: The G5 profile may not be as smooth as the profiles for other dye sets due to the effect of variable binning, which reduces signal variation between dyes of different fluorescent efficiencies. A spectral calibration automatically sets up three injections. The number of injections performed depends on the number of capillaries that pass or fail during an injection, and whether you select the "Allow Borrowing" option. When borrowing is not allowed, all eight capillaries must pass calibration.

9.10. Click "Accept Results" if all criteria above are met. If any capillary data does not meet the criteria above, click "Reject Results" and see the "Spectral calibration troubleshooting" section on page 301 of the ABI 3500 Genetic Analyzer User Guide.

10. Run Setup

10.1. Changing Polymer:

10.1.1. In the dashboard, click "Refresh" to update the status of the consumables. (i.e. polymer, buffers) NOTE: All consumables have an RFID label. The RFID label must be facing the instrument (and not you) to ensure that the RFID information is read accurately by the instrument.

10.1.2. Allow the polymer pouch to equilibrate to room temperature on the countertop.

10.1.3. In the Maintenance Wizards screen, click "Replenish Polymer".

10.1.4. Follow the prompts in the Replenish Polymer Wizard window. When completed, click "Refresh" from the Dashboard to update the screen.

10.2. Installing a New Array:

10.2.1. If a new capillary array needs to be installed, click "Install Capillary Array" from the Maintenance Wizards screen and follow the prompts.

10.2.2. Check the Quick View section of the Dashboard for updated status of the capillary array.

10.3. Changing the anode buffer container (ABC):

10.3.1. Allow refrigerated ABC to equilibrate to room temperature prior to use. Be sure the seal on the top of the container is intact.

10.3.2. Tilt the ABC slightly towards the large side of the container to make sure that most of the buffer is in the larger side and less than 1 ml of buffer in the smaller side of the container.

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

10.3.4. Place the ABC into the Anode end of the instrument, below the pump. The RFID label must be facing the instrument to ensure that the RFID information is read accurately by the instrument.

10.3.5. Close the instrument door to re-initialize.

10.3.6. Click "Refresh" from the Dashboard to update the dashboard.

10.4. Changing the Cathode Buffer Container (CBC):

10.4.1. Allow refrigerated CBC to equilibrate to room temperature prior to use. Be sure the seal on the top of the container is intact and the buffer level is at or above the fill line.

10.4.2. Wipe away condensation on the CBC exterior using a Kaydry/Kimwipe.

10.4.3. Tilt the CBC back and forth gently to ensure the buffer is evenly distributed across the baffles.

10.4.4. Place the container on a flat surface and peel off the seal. Wipe off any buffer on top of the CBC with a Kimwipe to ensure that the top of the container is dry. NOTE: failure to dry the CBC may result in arcing and termination of the run.

10.4.5. Place the appropriate septa on both sides of the CBC. Push the septa lightly into the holes to start and then push firmly to seat the septa.

10.4.6. Install the CBC on the autosampler. When properly installed, it will click into the autosampler as the tabs snap into place.

10.4.7. Close the instrument door to re-initialize.

10.4.8. Click "Refresh" from the dashboard to update the dashboard.

11. Preparing a Sample Run

11.1. In the dashboard, click "Refresh" and check consumables status to ensure the consumables are not expired and that buffer levels are adequate (i.e. at or near the fill lines).

11.2. Set the oven temperature to 60.0 ˚C and then click "Start Pre-heat". Pre-heating the oven for at least 30 minutes before starting a run helps mitigate subtle first-run migration rate effects.

11.3. Create a new plate record by clicking on "Create Plate from Template".

11.4. Select the plate template "HID_36_POP4", then click "Open".

11.5. In the Define Plate Properties screen, enter the Plate Name to include the run date, the kit name and your initials (e.g. 030220_Fusion_tc). The next 4 plate details should self-populate as follows:

- Number of wells: 96
- Plate Type: HID
- Capillary Length: 36
- Polymer: POP4

11.6. Click "Assign Plate Contents".

11.7. Note: "Plate View" is automatically displayed. "Table View" is also an option. Using "Plate View", enter the sample names in the appropriate well positions.

11.8. At the bottom right of the "Assign Plate Contents" screen, expand the Customize Sample Info pane. In the plate view, click on a well of interest to assign a Sample Type to that well. For example, click on the Allelic Ladder sample well and select "Allelic ladder" from the drop-down menu. Repeat this for the positive and negative controls.

11.9. Right click in "Assays" box and choose an Assay from the library, click "Add to Plate", then "close".

- For Fusion, choose "FUSION_HID_POP4_10sec".
- For Y23 choose "Y23_HID_POP4".

11.10. Repeat this process for "File Name Conventions" and "Results Group".

11.11. Highlight the associated sample well positions and click on the chosen Assay, File Name Convention and Results Group.

11.12. Save the plate.

12. Preparing Samples and Allelic Ladders

12.1. Prepare Hi-Di formamide and Internal Lane Size Standard (ILS):

- Aliquot (10.0 µl) x (# injections, plus extra) of Hi-Di into a tube.
- Add (0.5 µl) x (# injections, plus extra) of WEN ILS or Y23 WEN ILS to the Hi-Di.
- Add (0.5 µl) x (# injections, plus extra) of Stabilizer Reagent FOR Y23 SET-UP ONLY.
- Vortex the Hi-Di/WEN-ILS mixture, then gently tap tube on bench top.
- Record lot numbers on the 3500 Injection worksheet.

12.2. Aliquot **10 µl of Hi-Di/ILS** mix into a 96-well plate, filling one well for each sample and ladder, including any blank wells within the sets of injections.

12.3. Transfer approximately **1.0 µl of each sample** or **allelic ladder** into corresponding wells in the plate (can be mixed by pipetting up and down).

12.4. NOTE: if pull-up or spectral overlap is seen in the ladders, using less volume of ladder (such as 0.5 µl) may alleviate the problem.

12.5. NOTE: if the ILS is too low, a larger volume of ILS (such as 1.0 µl) can be added to the Hi-Di/ILS mix.

12.6. Seal the plate with a 96-well plate septum (may be cut to size for small runs) and heat the plate in a thermal cycler or heat block at 95 °C for 3 minutes. ***Do NOT close the thermal cycler lid or the septum may melt and/or adhere to the heated lid***.

12.7. Transfer the plate to a frozen metal plate holder and chill for at least 3 minutes.

12.8. Briefly centrifuge the plate and verify that no bubbles are present in the sample wells.

13. Loading the Samples

13.1. Place the 96-well plate into the base. Snap a plate retainer over the plate and base (should be an audible "click"). Verify the holes in the plate retainer and septum line up.

13.2. Press the tray button so the autosampler moves to the front, open the instrument door, place the assembled plate on the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler, and close the instrument door.

13.3. Check the instrument status in the Dashboard. Temperatures are displayed in red as they warm to the set-points. When temperatures are at the set point, they are displayed in green.

13.4. Link the plate by clicking on "Link Plate for Run". Verify the Run Information.

13.5. Click on "Start Run". The Monitor Run Screen is automatically displayed. (Note: do not attempt to start a run until the instrument status light is green).

13.6. Check that the dates and lot numbers are entered on the 3500 Use and Maintenance Log on the front of the instrument.

13.7. When the run has completed, copy the run folder to H:\Crimelab\DNA\3500 runs\ and the relevant user's data folder for analysis and archiving.

14. Flushing and Filling the Water Trap

14.1. The Polymer Delivery Pump (PDP) water trap should be flushed with molecular biology grade water approximately once per month to wash out any diluted polymer, to prolong the life of the pump, and to prevent algae growth.

14.2. Fill the 20 ml luer Lock plastic syringe with molecular biology grade water. Expel any bubbles from the syringe. Note: use a syringe 20 ml or larger. Smaller syringes may generate excessive pressure within the trap.

14.3. Attach the syringe to the forward-facing luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.

14.4. Open the luer fitting by grasping the body of the fitting and turn the fitting and attached syringe one-half turn counterclockwise to open the fitting.

14.5. Take approximately 30 seconds to flush 5 ml of distilled (or MB Grade) water through the trap. Excess water will flow into the overflow container. Do not use excessive force when pushing the syringe plunger as this may damage the trap seals. Note: the water trap volume is approximately 325 ul, so a relatively small volume of water is adequate for complete flushing.

14.6. Remove the syringe from the luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

14.7. Close the luer fitting by lightly turning clockwise until the fitting seals against the block.

15. Short-Term and Long-Term Storage and Cleaning (for more than two weeks)

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

15.2. From the Maintenance Wizards screen, click "Shutdown the Instrument". Note: the Instrument Shutdown Wizard takes 60 minutes to complete.

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

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

16. Instrument and Computer Maintenance

16.1. If there is a service contract on a Genetic Analyzer, the annual performance check will consist of the vendor performing the annual Performance Maintenance check on the instrument and computer during the contracted annual preventative maintenance service period.

16.2. If there is no service contract on a Genetic Analyzer, the annual performance check will consist of running the NIST Standard Reference Materials (SRM) on the instrument and verifying the correct NIST-traceable profiles are obtained.

16.3. If a Genetic Analyzer's computer reacts slowly or gives error messages, restart the computer and the 3500. See the "Turning On the 3500 and DataCollection" section of this protocol.

16.4. If restarting does not work, see the "Computer maintenance" section on page 254 of the ABI 3500 Genetic Analyzer User Guide for troubleshooting.

16.5. It may be necessary to place a service call with a vendor.

17. Diagram of 3500 Instrument Interior

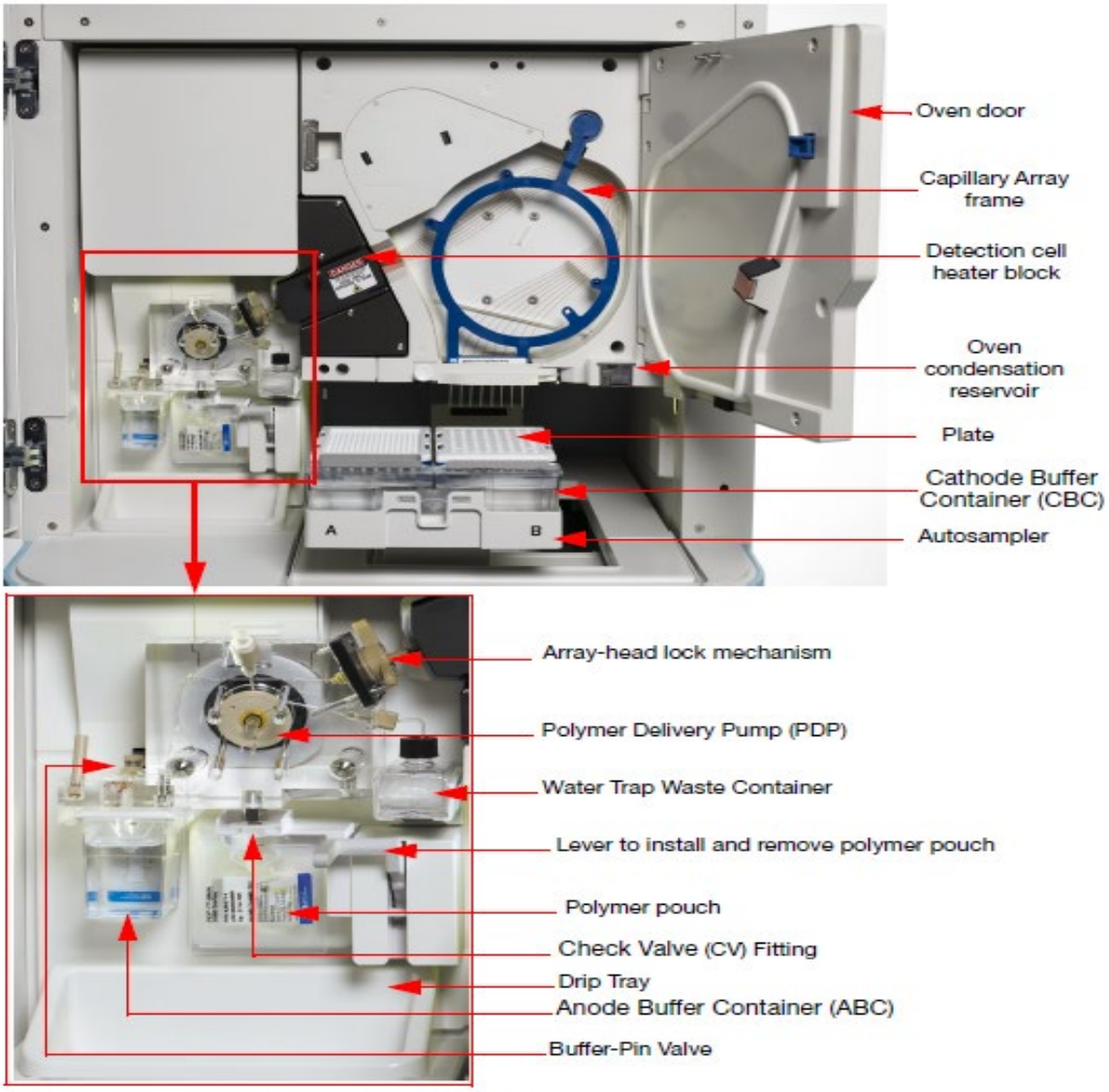

Figure 1 Instrument interior components

18. References

18.1. Applied Biosystems 3500/3500xL Genetic Analyzer User Guide.