Department of Forensic Science

FORENSIC BIOLOGY PROCEDURES MANUAL

CE FOR POWERPLEX® FUSION

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1 CAPILLARY ELECTROPHORESIS

All sample preparation work surfaces will be cleaned with 10% bleach (or a solution that will degrade DNA) followed by cleaning with isopropyl alcohol and/or ethanol before sample preparation.

For the purpose of this manual, the following definitions apply:

Injection - all samples that were separated via CE at a single time. Thus, for one injection on the 3500*xl*, up to 24 samples can be separated at a single time.

Run - all samples that were defined on a single plate and loaded onto the instrument at a single time for separation, regardless of the number of injections utilized to separate each sample contained on the plate. Thus, a run could contain up to 96 samples for separation on a single plate.

A minimum of 2 ladder samples are required per original plate set up/run. For reinjections, the data from a passing ladder from the original run may be used or a ladder may be reinjected, at the examiner's discretion.

1.1   Equipment

- Applied Biosystems 3500*xl* Genetic Analyzer
- Heat block, capable of 95 °C
- 96-well plate centrifuge
- Pipettes – 2 µL, 10 µL, 20 µL 100 µL, 200 µL, and 1000 µL
- 8- Channel Pipette – Range 0.5 µL to 10 µL
- Repeater pipette

1.2   Materials

- 36-cm capillary array (Applied Biosystems, Inc. P/N 4404687 (24-capillary))
- MicroAmp® Optical 96-Well Reaction Plate (Applied Biosystems, Inc. P/N N801-0560)
- 96-well plate septum (Applied Biosystems, Inc. P/N 4315933)
- 96-well plate retainer and base (Applied Biosystems, Inc. P/N 4409530)
- Freezer block
- Sterile ART tips for pipettes – 10 µL, 20 µL, 100 µL, 200 µL, and 1000 µL
- Microcentrifuge tubes
- Cathode buffer septa (Applied Biosystems, Inc. P/N 4410715)

1.3   Reagents

- PowerPlex® 5 Matrix Standard (Promega Corp. P/N DG4850)
- Hi-Di Formamide (Applied Biosystems, Inc. P/N 4311320)
- PowerPlex® Fusion Allelic Ladder (component of Fusion kit)
- WEN Internal Lane Standard (ILS) 500 Size Standard (component of Fusion kit)
  - May also be ordered independently of Fusion kit (Promega Corp. P/N DG5001)
- POP-4 Polymer (Applied Biosystems, Inc. P/N A26070 (96 samples), P/N 4393715 (384 samples) or P/N 4393710 (960 samples))
- Anode buffer container (Applied Biosystems, Inc. P/N 4393925)
- Cathode buffer container (Applied Biosystems, Inc. P/N 4408256)

1.4   Safety Considerations

The Applied Biosystems 3500*xl* Genetic Analyzer uses a single-line 505nm solid-state laser. All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating, (during service with safety interlocks disabled) you may be exposed to laser emissions in excess of the Class 3B rating. Do not remove safety labels or disable safety interlocks.

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Performance Optimized Polymer (POP) is an irritant and a neurotoxin. Always wear gloves when handling or cleaning liquid or dried polymer. It may cause skin, eye, and respiratory tract irritation.

Exposure to Hi-Di formamide causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.

1.5   References

- PowerPlex® Fusion System Technical Manual, Promega, rev. 10/12.
- Applied Biosystems 3500 and 3500*xl* Genetic Analyzer with 3500 Series Data Collection Software 3.1 User Guide (or other association User Guide/User Bulletin produced by Applied Biosystems/ThermoFisher Scientific.

1.6   Instrument Preparation

1.6.1   With the 3500*xl* turned off, turn on the computer, but do NOT log on.

1.6.2   Power on the 3500*xl* instrument. Wait for the solid green light indicating the initialization process is complete.

1.6.3   Log onto the computer - the Server Monitor/Daemon will launch automatically. Do NOT launch the 3500 Series Data Collection Software until the "3500 Services Loaded" at the lower right corner of the screen is completely loaded and indicated with the green check mark.

1.6.4   Double-click on the 3500 instrument icon to launch the Data Collection Software, which will launch the Dashboard screen.

1.6.5   Select "Start Pre-Heat" at least 30 minutes prior to the first injection of the day to preheat the oven to 60°C (this is the default temperature).

1.6.6   Click "Refresh" in the "Consumables Information" window. The readings/gauges will be updated once the Radio Frequency Identification (RFID) tags are read.

1.6.7   If the capillary array needs to be changed, follow the directions given in the Install a Capillary Array wizard. The wizard should take approximately 15-45 minutes. Otherwise, continue to 1.6.8.

1.6.8   If the anode buffer container (ABC) and cathode buffer container (CBC) have been on the instrument longer than 14 days, they will need to be changed. Otherwise, continue to 1.6.9.

    1.6.8.1   Changing the ABC:

        1.6.8.1.1   Verify that the buffer level is at or above the fill line and check that the seal is intact.

        1.6.8.1.2   Invert the ABC, then tilt it slightly to make sure most of the buffer is in the larger side of the container. There should be less than 1 mL of the buffer remaining in the smaller side of the container.

        1.6.8.1.3   Peel off the seal at the top of the ABC and place the ABC into the anode end of the instrument.

        1.6.8.1.4   Click the "Refresh" on the dashboard to update the screen.

    1.6.8.2   Changing the CBC:

        1.6.8.2.1   Press the TRAY button on the instrument to bring the old CBC forward.

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1.6.8.2.2   Discard the container and septa.

1.6.8.2.3   Verify that the buffer level is at or above the fill line and check that the seal is intact. Tilt the CBC back and forth gently to ensure that the buffer is evenly distributed across the top of the baffles.

1.6.8.2.4   Peel off the seal at the top of the CBC. Wipe off any buffer on the top of the CBC to make sure the container is dry.

1.6.8.2.5   Align and place the appropriate septa on both sides. Push the septa lightly into the holes to start and then push firmly to seat the septa.

1.6.8.2.6   Install the CBC on the autosampler.

1.6.8.2.7   Close the instrument door and wait for the green light.

1.6.8.2.8   Click "Refresh" on the dashboard to update the screen.

1.6.9   Verify that enough polymer remains in the pouch (# of samples remaining/#injections remaining). This information is available in the dashboard window. If the polymer has been on the instrument longer than 14 days and/or it has expired, and/or the amount is insufficient for the run, the polymer pouch will need to be changed. Otherwise, continue to 1.6.10.

1.6.9.1   Changing the polymer pouch:

1.6.9.1.1   Verify that the new pouch to be installed has not expired.

1.6.9.1.2   Allow the new pouch to equilibrate to room temperature.

1.6.9.1.3   Select the Replenish Polymer Wizard. Follow the directions given. The wizard will take 10 to 20 minutes to complete.

1.6.9.1.4   Click "Refresh" on the dashboard to update the screen.

1.6.10   Daily or before each run, whichever comes last do the following:

1.6.10.1   Visually inspect the level of fluid inside the ABC and CBC. The fluid must line up with the fill line.

1.6.10.2   Ensure the CBC septa are positioned on the plate deck properly and securely.

1.6.10.3   Check for bubbles in the pump block and channels.

1.6.10.3.1   If bubbles are observed, use the Remove Bubble wizard.

1.6.10.4   Check the loading-end header to ensure that the capillary tips are not crushed or damaged. If crushed or damaged, the array will need to be changed.

1.6.10.5   Check for leaks and dried residue around the buffer-pin valve, check valve and array locking lever. If leaks persist, the manufacturer will need to be contacted.

1.7   Sample Preparation

When loaded onto the same plate, all evidence samples pertaining to a case must be loaded on to the plate prior to loading reference samples for the same case.

Samples may not be added to empty wells previously subjected to injection.

1.7.1   To prepare a sample plate, prepare a master mix of formamide and size standard as follows:

1.7.1.1   Thaw the Hi-Di formamide to room temperature and vortex to mix.

1.7.1.2   Vortex the WEN ILS to mix (stored at 2-10°C after first use).

1.7.1.3   Prepare a loading cocktail master mix by combining and mixing as follows:

[(0.5 µL WEN ILS) x (# samples)] + [(9.5 µL Hi-Di formamide) x (# samples)]

NOTE: When calculating, include an extra number of samples to account for pipetting variation. The number of extra samples needed will be dependent upon the total number of actual samples being loaded.

1.7.1.4   Vortex the master mix.

1.7.1.5   Pipet 10 µL of the master mix into each well to be used for an injection of sample or ladder in the 96-well plate.

1.7.1.6   Pipet 10 µL of extra master mix or Hi-Di formamide into any unused wells of the plate that are in a set of three columns in which samples/ladders will be injected to prevent the injection of air by the instrument.

1.7.1.7   Add 1 µL of allelic ladder or sample to the appropriate wells containing the master mix. Alternatively, 0.5 µL or 2.0 µL of sample may be loaded at the analyst's discretion. If a sample is over-amplified, a dilution of the amp product with Type I water or formamide is also acceptable.

1.7.1.8   Place the plate septum on top of the sample wells and press it fully into the plate.

1.7.1.9   If needed, centrifuge the plate briefly.

1.7.1.10   Denature the samples at 95°C for 3 minutes, then immediately chill in a freezer block for 2-3 minutes.

1.7.1.11   Place the denatured and chilled plate into a blue plate base and snap the white plate retainer over the top, taking care to align the tabs on both ends. (See below):
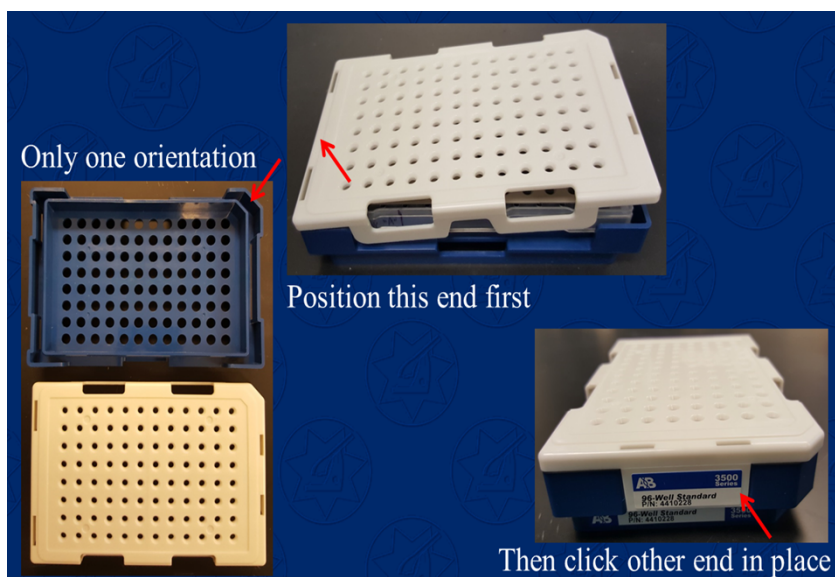
1.7.1.12 Press the TRAY button on the 3500*xl* to bring the autosampler tray forward.

1.7.1.13 Open the instrument door and place the plate assembly into an open position (A or B) with the labeled end of the white retainer facing the analyst. The plate can only be placed in one orientation.

1.7.1.14 Close the instrument door.

1.8 Manual Creation of an Injection Plate File

1.8.1 In the dashboard, click on "Create Plate from Template".

1.8.2 Select "PPFusion Template" and enter the plate name. Entering the Owner as DFS is optional.

    1.8.2.1 The following parameters are provided in this template:

- Number of wells: 96 (default)
- Plate Type: HID
- Capillary Length: 36 cm (default)
- Polymer: POP4 (default)
- Three Assays: PPFusion_12s, PPFusion_24s and PPFusion_24s_12s
- File Name Conventions: Casework
  - Sample File Name format is: Well_SampleName_InjectionTime_PlateName
- Results Groups: Select Casework or other predesignated Results Group

1.8.3 Click on "Assign Plate Contents".

1.8.4 Using either the Plate View or the Table View, enter the sample names in the appropriate wells.

1.8.5 Under "Assays", select one of the three assays for each assigned well.

    1.8.5.1 If the pre-designated Assays, File Name Conventions and/or Results Groups are not available for selection, click on "Actions" for each and click on "Add from Library."

1.8.6 Under "File Name Conventions", select Casework for all assigned wells.

1.8.7 Under "Results Groups", select Casework or another pre-designated Results Group from the list provided.

1.8.8 Using the drop down menu under "Sample Type", designate the ladder(s). If desired, the positive and negative controls may also be designated.

1.8.9 Review the parameters for each well and change, if necessary.

1.8.10 Save the plate file and proceed to 1.10.

1.9 Importing an Injection Plate File

1.9.1 If the plate file is not first imported into the Library:

    1.9.1.1 Under Workflow, select "New Plate" and then select "Create new plate from a standard format file."

    1.9.1.2 Click Import. Use the drop down menu to select "*.xls", browse to find the appropriate run file and double click to import the file. Click OK after verifying the correct file was imported.

    1.9.1.3 Follow steps 1.8.5 through 1.8.10 above, as applicable.

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1.9.2   If the plate file is first imported into the Library:

    1.9.2.1   In the navigation pane, click Library→Plates.

    1.9.2.2   Click Import. Use the drop down menu to select "*.xls", browse to find the appropriate run file and double click to import the file. Click OK after verifying the correct file was imported.

    1.9.2.3   Select the plate from the list and select "edit."

    1.9.2.4   Follow steps 1.8.5 through 1.8.10 above, as applicable.

1.10   Linking a Plate File for a Run

NOTE: If two plates are to be loaded to be run, both plates must be linked prior to starting the run. If plates A and B are loaded onto the instrument, but only plate A is linked prior to starting the run, only plate A will be injected. Plate B cannot be linked until the instrument run for plate A is complete or aborted and terminated.

1.10.1   In the navigation pane, click Load Plates for Run.

1.10.2   Plate(s) must be loaded onto the autosampler tray before linking for a run.

1.10.3   Click Link Plate in the appropriate position (A or B) and browse in the pop-up window to find the appropriate plate file. Double click on the plate file to select it and then close the window.

1.10.4   Click on the Create Injection List and refer to the Injection List Details to review the selected injection parameters and/or to move selected injections up or down to change the order in which they will occur. Once the order is as desired, click Start Run.

    NOTE: The order of any remaining injections may also be adjusted during a run.

1.10.5   Once Start Run has been clicked, the instrument will notify the user if there are any instrument or reagent issues with the run. If an issue is detected, the run will not begin until the issue is resolved. Expired reagents may not be used for casework. However, a capillary array may be used past the manufacturer's recommended use as long as the quality of the data obtained does not decline. If this is the reason for the notification, click on "Use" to continue.

    NOTE: Once the run has ended, the instrument will "pause" to allow for reinjections (see 1.12). If no reinjections are necessary, terminate the run to officially complete the run for the software.

1.11   Pausing or Aborting a Run

1.11.1   To pause during a run, click Pause and the run will pause after the current injection is completed.

    1.11.1.1   To terminate the instrument run at this point, click Terminate Injection List.

    1.11.1.2   To resume the run instead, click Resume.

1.11.2   To abort a run, click Abort.

    1.11.2.1   Verify that the current injection is to be aborted by clicking "yes" or "no" in the comment box.

        NOTES: Choosing "yes" will terminate the current injection. The option to resume at this point will resume at the NEXT injection. The option to terminate injection list will abort all remaining injections, including the current one.

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Choosing "no" will cause the instrument to pause. The option to resume at this point will resume the CURRENT injection. The option to terminate injection list will abort all remaining injections, including the current one.

1.11.2.2   To terminate the instrument run at this point, click Terminate Injection List.

1.11.2.3   To resume the run instead, click Resume.

1.12   Creating Reinjections

Reinjections may be created either the same day as the plate set up or on the day following the plate set up.

1.12.1   Creating reinjections within the same plate file when the "pause" at the end of the run has been enabled

Following the last injection of an instrument run, the instrument will "pause" if that feature is enabled. The oven will stay heated for two hours following the end of the run as well.

During this time, reinjections can be created either within the same plate file or as a separate plate file.

If not created during this pause time, reinjections will have to be created as a separate plate file – see 1.12.2.

1.12.1.1   Click on "Monitor Run" and highlight the well(s) or injection to be reinjected.

1.12.1.2   If the sample(s) or injection is to be reinjected at the same parameters, click "Duplicate" (at the top of the screen).

1.12.1.3   If the sample(s) or injection is to be reinjected under different parameters, click "Re-inject".

1.12.1.3.1   In the "Re-injection" dialog box, click on "Reuse a protocol in the library" and select the desired Instrument Protocol from the drop down menu. Also select "Following all injections" or "After original injection" and click OK.

1.12.1.3.2   In the "Setup and Instrument Protocol" dialog box, click "Apply to Assay" at the bottom, but DO NOT click on "Save to Library".

NOTE: On the "Monitor Run" screen, the selected reinjection(s) will reflect the original selected Assay name with a suffix of "_#"; however, the correct reinjection time will be reflected under the column "Instrument Protocol". The sample file name(s) for the reinjected sample(s) will reflect the correct reinjection time.

1.12.1.4   If a sample selected for reinjection includes an allelic ladder in its results group, but the ladder has not been selected for reinjection, the software prompts the user to select one or more allelic ladders to reinject.

1.12.1.4.1   In the "Add Allelic Ladder to Re-injection" screen, select one or more ladder samples, if desired, and then select whether to collect data for the remaining samples in the allelic ladder reinjection.

1.12.1.5   The injection parameters will be designated for selected wells of the plate. Review and change if necessary.

1.12.1.6   If reinjections are created during the instrument pause, click "Resume" to continue the instrument run with the newly created reinjections.

1.12.2   Creating reinjections as a separate plate file

1.12.2.1   If the reinjections are created as a separate plate file, but during the instrument pause, click "Terminate Injection List" to terminate the pause. Otherwise, proceed to 1.12.2.2.

1.12.2.2   Under "New Plate", click on "Create New Plate from Existing Plate", select the original plate file and name the new plate file.

1.12.2.3   Deselect and unassign all samples with previously designated injection parameters that were initially run. Highlight the desired well(s) or injection and select the desired Instrument Protocol.

1.12.2.4   Link and run the newly created reinjection plate file.

APPENDIX A - REAGENTS

ANODE BUFFER CONTAINER

Expiration date: Manufacturer's expiration date
Purchased from Applied Biosystems, Foster City, CA. P/N 4393925.

CATHODE BUFFER CONTAINER

Expiration date: Manufacturer's expiration date
Purchased from Applied Biosystems, Foster City, CA. P/N 4408256.

HI-DI™ FORMAMIDE

Expiration date: Manufacturer's expiration date
Purchased from Applied Biosystems, Inc., Foster City, CA, PN 4311230, 25 mL each.

Aliquot into 250 µL, 500 µL, 1000 µL or other suitable volume aliquots and store at -20ºC.

PERFORMANCE OPTIMIZED POLYMER – 4 (POP-4)

Expiration date: Manufacturer's expiration date
Purchased from Applied Biosystems, Foster City, CA. P/N A26070 (96 samples), P/N 4393715 (384 samples) or P/N 4393710 (960 samples)

POWERPLEX® FUSION SYSTEM KIT

Expiration date: Manufacturer's expiration date
Purchased from Promega, Madison, WI., Catalog Number DC2402 for 200 reaction kit or DC2408 for 800 reaction kit.

Kit components included (expiration date same as kit unless specified):

- POWERPLEX® FUSION 5X MASTER MIX
- POWERPLEX® FUSION 5X PRIMER PAIR MIX
- WATER, AMPLIFICATION GRADE
- 2800M CONTROL DNA, 10 ng/µL
- POWERPLEX® FUSION ALLELIC LADDER MIX
- WEN INTERNAL LANE STANDARD 500

NOTE: Only the Allelic Ladder Mix and Internal Lane Standard (WEN ILS 500) are stored and used in the post-amplification room for Capillary Electrophoretic Detection.

WEN INTERNAL LANE STANDARD 500

Expiration date: Manufacturer's expiration date
Purchased from Promega, Madison, WI., Catalog Number DG5001.

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APPENDIX B – INITIAL SETUP OF THE 3500*xl* AND DATA COLLECTION SOFTWARE

After installation of the 3500 series Data Collection Software for a new 3500*xl* instrument, a number of customizations must be made. Displayed below are screen captures for settings in use by the 3500*xl* instruments. These settings may also be exported/imported from an established instrument to a new instrument.

For step by step directions for set up, the user guides (Applied Biosystem's 3500*xl* Genetic Analyzer User Guide and PowerPlex® Fusion System Technical Manual) should be consulted

1   The figure below shows settings used in creating a dye set for the PowerPlex® Fusion System:

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2   The figure below shows the Library Resources window of the Data Collection software to access resources to manage settings:

3   Creating Instrument Protocols

3.1   The figure below shows settings used in creating the "PPF24s" Injection Protocol (a 24 second injection protocol).

3.2   The figure below shows settings used in creating the "PPF12s" Injection Protocol (a 12 second injection protocol).

4   Creating Assays

4.1   The figure below shows the settings used in creating "PPFusion_24s" (24 second assay).

4.2   The figure below shows settings used in creating "PPFusion_12s" (12 second assay).

4.3   The figure below shows settings used in creating an optional "PPFusion_24s_12s" (the 24s followed by the 12s assay).

5   Casework Results Group Setting

   5.1   The following figures show settings used in creating the Casework Results Group.

            NOTE: Individual Results Groups may be created similarly.

5.2   The figure below shows the Casework file name convention settings:

5.3   The following figure shows the "PPFusion" plate template.

5.4   The figure below shows the WEN ILS settings.

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