FBS13 – Capillary Electrophoresis Using the 3130xl Genetic Analyzer

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1. Scope

1.1. This procedure is employed to detect amplified product by means of capillary electrophoresis (CE) using the Applied Biosystems 3130*xl* Genetic Analyzer.

2. Background

2.1. To establish the practices for documenting the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Science Laboratory (FSL) *Quality Assurance Manual*, the accreditation standards under ISO/IEC 17025:2005, and any supplemental standards.

2.2. The AB 3130*xl* Genetic Analyzer is a multicapillary electrophoresis instrument designed to convert amplified DNA into an interpretable, graphical display called an electropherogram. The data is utilized to facilitate the deduction of results to determine the potential contributor(s) of a DNA profile associated with an item of evidence.

2.3. The amplified DNA product is composed of a mixture of differently sized DNA fragments, each containing a fluorescent dye-labeled primer. These primers are specifically designed to outline and differentiate the assortment of amplified loci. As the DNA fragments migrate through the capillary via electrophoresis, a laser light excites the attached fluorescent dye generating an emission of light that is detected and converted to an electrical signal by a CCD camera. The intensity of the resulting signals are converted to relative fluorescence units (rfu) and plotted against the measured time span from a sample's injection to its detection. The

data collected corresponding to the amplified DNA fragments is ultimately represented by peaks on an electropherogram.

3. Safety

3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.

3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

4. Materials Required

4.1. 1X Genetic Analyzer Buffer (FBR41)
4.2. POP 4 Polymer
4.3. Hi-Di Formamide (FBR40)
  4.3.1. Note: Formamide aliquots are kept in the freezer at -20°C and are good for one year from the received date of the stock bottle of Formamide. Once thawed, the aliquots are stored at 4°C and are good for 5 days. Use only deionized formamide. Over time, formamide decomposes into formate. Formate ions are injected preferentially into the capillary, causing a loss in signal intensity.
4.4. Allelic Ladder
4.5. Size Standard
  4.5.1. Note: Keep the amplified product(s), ladder and size standard protected from direct exposure to light. Excessive exposure can affect fluorescent probes.

5. Standards and Controls

5.1. 9947A is a positive amplification control that is used to evaluate the performance of the amplification and subsequent typing procedures. This control must be included within each CE run conducted. See FBS16, Appendix I for the expected 9947A profile.

5.2. The negative amplification control is prepared and processed in parallel with each amplification sample set. This control contains all of the chemical components of the amplification reaction and detection procedure however it should not exhibit a profile. Unless the parameters of an associated sample are changed (higher preparation volume), a negative control does not need to be re-run once it has been confirmed negative. The preparation volume of a negative

amplification control is equal to the highest preparation volume of any sample in its associated sample set.

5.3. The ladders included in each run can also serve as positive run controls. They are used in the analysis of the generated profiles to assign allele calls to the sized peaks of each sample. Additionally, the ladders can be used to demonstrate that the detection equipment and computer software is operating properly by displaying the correct allele sets for each locus and exhibiting consistent peak heights throughout the call range. See FBS16 for a list of the designated alleles that should be detected in the ladder.

5.4. **To maintain a separation in time and space between questioned and known samples, it is preferential, but not necessary, to carry out electrophoresis of the questioned and known samples in separate plates and on separate instruments if and when possible. If the samples are on the same plate, all questioned samples must be injected separately from known samples.**

6. Calibration

6.1. Not applicable

7. Procedures

7.1. Preparing the Instrument:

7.1.1. Please refer to Chapter 1 *Preparing the Instrument* in the Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide to ensure the instrument is ready for use.

7.1.2. Starting the 3130*xl* Genetic Analyzer

7.1.2.1. The instrument and computer should already be turned on and the oven and instrument doors should be closed.

7.1.3. Data Collection Software

7.1.3.1. The Data Collection software should already be open. If it is not open, double click on the Data Collection software icon on the desktop. This will open the Service Console which will show red circles (off), then change to yellow triangles (activating) and finally green squares (on) as each application activates. The Foundation Data Collection window will then open automatically.

7.2. Set up the Instrument

7.2.1. Open the instrument doors to inspect the instrument and perform appropriate maintenance tasks. Please refer to FBQ29 and the 3130*xl* Instrument Maintenance Log for additional details on maintenance tasks and to determine which, if any tasks need to be performed.

7.2.2. Installing or Replacing the Capillary Array

7.2.2.1. The capillary array should be changed after approximately 150 runs. See FBQ29 for capillary array replacement instructions.

7.2.3. When to Replenish or Change Polymer

7.2.3.1. Polymer should be inspected for fluid level and length of time on the instrument. If the polymer level is sufficient (each 3130*xl* run uses approximately 50 to 80 µl of polymer) and the polymer bottle has been on the instrument for less than one week, you do not need to change the polymer. If fluid levels are low or the bottle has been on the instrument for one week or longer you must change the polymer. If polymer must be changed, use the Replenish Polymer Wizard in the Wizard menu.

7.2.3.2. Before using the Polymer:

7.2.3.3. Remove the polymer from the refrigerator

7.2.3.4. Loosen the cap and bring the polymer to room temperature

7.2.3.5. To dissolve deposits, tighten the cap and gently swirl the polymer

7.2.4. Preparing Buffer and Filling Reservoirs

7.2.4.1. At a minimum, the buffer should be replaced daily. The reservoir septa should be replaced once per week during cleaning (see FBQ29).

7.2.4.2. Prepare a 1X Solution of Genetic Analyzer Buffer (GAB). Refer to FBR41.

7.2.4.3. Remove the Anode Buffer Jar, Buffer and Water Reservoirs. Pour out the old buffer and water and thoroughly rinse with diH_2O. If septa do not need to be replaced, they should be kept dry and retained (septa are replaced during weekly cleaning tasks).

7.2.4.4. Completely dry the Reservoirs and the Anode Buffer Jar with a lint free laboratory wipe. This step is critical because any excess moisture found in the instrument can interfere with the run. DO NOT dry with canned air.

7.2.4.5. Fill the Anode Buffer Jar and Buffer Reservoir with 1X GAB to the fill line. Fill the Water Reservoirs with diH_2O to the fill line. Seal the Buffer and Water Reservoirs with septa.

7.2.4.6. Place the Anode Buffer Jar and all Reservoirs back onto the 3130xl. Ensure that the Anode Buffer Jar is flush with the Lower Pump Block and that the septa are properly aligned.

7.3. Preparing a Run:

7.3.1. Creating a Setup Sheet

7.3.1.1. Open the 3130xl Setup Sheet and type the sample names into the Sample Entry tab for placement orientation. Indicate plate name on the worksheet, the plate name will typically consist of the date and initials of the analyst.

7.3.1.2. Calculate the amount of each component needed in the Formamide/LIZ mixture. Type in the number of reactions in the # of Reactions column in the Plate Setup tab. The worksheet will calculate the appropriate volume of each component for you.

# of Samples x 8.7 µl Hi-Di Formamide

# of Samples x 0.3 µl GS500 LIZ Standard

7.3.1.3. Note: The number of reactions should include enough of the Formamide/LIZ mixture to complete an injection/run (16 wells). An empty well may cause damage to the capillary.

7.3.1.4. Note: Extra reactions or an additional percentage of the number of reactions can be added to the calculations in order to account for any volume lost during pipetting.

7.3.1.5. Print the worksheet.

7.3.2. Creating a Data Collection Software Plate Record

7.3.2.1. Please refer to Chapter 6 *Autoanalysis and Fragment Analysis* in the Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide or use the following steps as a guide.

7.3.2.2. From the left pane in the software menu, select Plate Manager.

7.3.2.3. Click “New.”

7.3.2.4. Fill in the blanks with the following information:

7.3.2.5. Enter a name for the plate (this typically includes the date and initials of the analyst).

7.3.2.6. A description of the plate record (optional).

7.3.2.7. In the Application drop down list, choose GeneMapper Generic.

7.3.2.8. In the plate-type drop down list, choose 96-well.

7.3.2.9. Enter “DFS” for the owner.

7.3.2.10. Enter your initials for the operator.

7.3.2.11. Click “OK.”

7.3.2.12. The GeneMapper Plate Editor window will now open. For each sample well, enter information about the sample and how it will be analyzed.

7.3.2.13. **Required** - Fill in the sample name column.

7.3.2.14. No information is required in the comment column.

7.3.2.15. Optional - The priority column is used to direct the instrument to which samples should be processed first. If all of the samples have a 100 in the priority column, the plate will be processed in order from left to right.

7.3.2.16. Optional - In the Sample Type column choose Sample, Positive (+) Control, Negative (-) Control, or Allelic Ladder. This indication will assist with additional analysis. This step is optional as these designations may be selected in GeneMapper® ID-X during data analysis.

7.3.2.17. Optional - Choose the appropriate Size Standard for each sample.

7.3.2.18. Optional - Choose the appropriate Panel for each sample.

7.3.2.19. Optional - Choose the appropriate Analysis Method for each sample.

7.3.2.20. The SNP Set column should be blank.

7.3.2.21. Optional - The columns labeled User-Defined 1 and User-Defined 2 are not used.

7.3.2.22. **Required** - Choose the appropriate setting under Results Group 1 for each sample. Each sample must have a Results Group.

7.3.2.23. **Required** - Choose the appropriate setting under Instrument Protocol 1 for each sample. Each sample must have an Instrument Protocol.

7.3.2.24. Click “OK” to save. The Plate Record will save in the Run Scheduler and will show a status as “pending.”

7.3.2.25. Note: If a sample is to be injected more than once, an additional Results Group and Instrument Protocol may be selected by clicking Edit > Add Sample Run. If the parameters for this injection are changed, remember to also inject that sample’s associated control(s). Optional – Plate Records may also be imported.

7.3.3. Pre-heating the Oven

7.3.3.1. Please refer to the *Using Manual Control* section of Chapter 1 *Maintenance* in the Applied Biosystems 3130/3130*xl* Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide. This is an optional step. If this step is not done, the instrument will not begin until the oven is 60°C.

7.3.3.2. In the left pane of the software menu, open the Manual Control.

7.3.3.3. Use the Send Defined Command for drop down menu to choose Oven. For the Command Name use the drop down menu to choose Set Temperature. In the Value menu type 60. Then click Send Command.

7.3.3.4. Use the Send Defined Command for drop down menu to choose Oven. For the Command Name use the drop down menu to choose Turn On/Off Oven. In the Value menu select On. Then click Send Command.

7.3.4. Sample Preparation

7.3.4.1. Retrieve Hi-Di Formamide, Size Standard and Allelic Ladder.

7.3.4.2. Vortex and pulse spin all of the reagents.

7.3.4.3. Retrieve a 1.5ml or 2.0ml tube and label. (If running a large quantity of samples, the Formamide/Size Standard mixture can be prepared in a V-bottom basin.)

7.3.4.4. Add the required amount of each component to the tube (or V-bottom basin). Record the appropriate lot numbers on the 3130xl Setup Worksheet.

7.3.4.5. Vortex and pulse spin the Formamide/Size Standard mixture if prepared in a tube. If prepared in a V-bottom basin, tip from side to side to thoroughly mix.

7.3.4.6. Allow the amplified product to equilibrate to room temperature. Spin all of the tubes/plates to ensure amplified product is concentrated in the bottom of each tube/well.

7.3.4.7. Obtain a 96-well plate and properly label it with the plate or case number, date, and initials. Additional markings can be made on the plate to indicate rows and columns.

7.3.4.8. Aliquot 9 µl of the Formamide/Size Standard mixture into each sample well. Be sure to fill ALL of the wells associated with the injection/run (16 wells) with Formamide/Size Standard mixture. Capillaries should not attempt to inject empty wells.

7.3.4.9. Following the 3130xl Setup Worksheet, aliquot 1µl of Allelic Ladder or 1µl amplified product to the appropriate wells.

7.3.4.10. Obtain a 96-well septum and check to be sure all holes are open. Seal the plate by laying the septum flat on the plate, aligning the wells, and pressing down. Be certain that the septum fits securely and completely on the plate.

7.3.4.11. Centrifuge the plate.

7.3.4.12. Turn on the thermal cycler (thermal cycler may be pre-heated). Place the plate in the thermal cycler. Do not close the lid because the septa may melt to the plate. Select and start the appropriate thermal cycling program in order to denature the plate. The method on the screen should correspond to the following:

HOLD	*95^{o}C*	*3:00 MIN*
HOLD	*4 ^{o}C*	*3:00 MIN*
HOLD	*4 ^{o}C*	*FOREVER*

7.3.4.13. When denaturation is complete, the plate should be left at 4 ^{o}C in the thermal cycler (or placed in the freezer) until it is ready for assembly onto the autosampler.

7.4. Performing the Run

7.4.1. Please refer to Chapter 7 *Running the Instrument* in the Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide or use the following steps as a guide.

7.4.1.1. Construct the plate assembly by placing the sample plate in the plate base and snapping on the retainer. Ensure that the

notches line up and that the retainer holes are properly aligned with the septum holes.

7.4.1.2. Press the tray button on the front of the 3130xl. Wait for the autosampler to finish moving to the front and then open the instrument doors.

7.4.1.3. Place the plate assembly on the autosampler. The notched area on the base will be toward the back of the instrument. Press gently but firmly to be certain the tray is flat and properly placed on the autosampler.

7.4.1.4. Check the septa on the buffer and water reservoirs to be certain that they are flush.

7.4.1.5. Close the doors and allow the autosampler to completely move into the home position.

7.4.1.6. Select the Run Scheduler on the left pane of the software menu.

7.4.1.7. Select Find All to locate the appropriate plate record and click to highlight.

7.4.1.8. Link the plate by clicking on the yellow area of the autosampler that the plate has been loaded onto (plate position A or B). The yellow area will now turn green. If loading two plates on the instrument at once, be certain the correct plate is linked to the correct plate record.

7.4.1.9. Review the run schedule before starting the run by clicking the Run View tab.

7.4.1.10. Once the plate has been linked, the green RUN arrow in the left corner will be enabled. Click the arrow to begin the run.

7.4.1.11. During the run, you can check the instrument status to monitor the temperature, voltage, current, and laser power. You can also view the data using the Capillaries Viewer and the Cap/Array Viewer pages. However, it is important not to leave these windows open for extended periods of time while the instrument is running.

7.5. Exporting Data:

7.5.1. 1. Once the run has been completed select “My Computer” on the desktop > “E Drive” > “Applied Biosystems” > “UDC” > “DATA COLLECTION” > “DATA”

7.5.2. 2. Locate your project folder, copy and paste the project to the designated storage location (i.e. share drive or thumb drive).

8. Sampling

8.1. Not applicable

9. Calculations

9.1. Not applicable

10. Uncertainty of Measurement

10.1. When quantitative results are obtained, and the significance of the value may impact the report, the uncertainty of measurement must be determined. The method used to determine the estimation of uncertainty can be found in the *FSL Quality Assurance Manual – Estimation of Uncertainty of Measurement (Section 5.4.6).*

11. Limitations

11.1. Once a case is complete, preserve the amplified DNA product for those samples where the DNA extract and/or stain material was consumed during analysis. If sufficient stain material and/or extract remains for additional testing, the amplified product can be discarded.

11.2. The fluorescent dyes attached to the primers are light-sensitive. Be sure to minimize their exposure time by storing all samples, allelic ladder and LIZ size standard away from light.

12. Documentation

12.1. FBU 3130xl Setup Sheet

13. References

13.1. Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide. Applied Biosystems, Foster City, CA, 2004.

13.2. Applied Biosystems Maintenance, Troubleshooting, and Reference Guide. Applied Biosystems, Foster City, CA 2004.

13.3. *Forensic Science Laboratory Quality Assurance Manual* (Current Version)

13.4. *FSL Departmental Operations Manuals* (Current Versions)

13.5. *FSL Laboratory Operations Manuals* (Current Versions)

13.6. *FBR41 - 1X Genetic Analyzer Buffer* (Current Version)

13.7. *FBR40 - Hi-Di Formamide* (Current Version)

13.8. *FBS12 - PCR Amplification Using the AmpFℓSTR® Identifiler +™ Kit* (Current Version)

13.9. *FBS15 - DNA Results Interpretation Guidelines (Identifiler +)* (Current Version)

13.10. *FBQ26 - Quality Control of AmpFℓSTR® Identifiler +™ PCR Amplification Kits* (Current Version)

13.11. *FBQ29 - Maintenance of the AB 3130xl Genetic Analyzer* (Current Version)