

FBS13 – Capillary Electrophoresis Using the 3130x/ Genetic Analyzer

Table of Contents

1. Scope
2. Background
3. Safety
4. Materials Required
5. Standards and Controls
6. Calibration
7. Procedures
8. Sampling
9. Calculations
10. Uncertainty of Measurement
11. Limitations
12. Documentation
13. References

1. Scope

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

2. Background

- 2.1. The AB 3130x/ Genetic Analyzer is a multicapillary electrophoresis instrument designed to detect amplified DNA and convert data into an interpretable, graphical display called an electropherogram.
- 2.2. 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 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.
- 4.6. Amplified DNA product

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 plate. A positive control does not need to be re-run once it has been confirmed positive. See FBS21 Identifiler® Plus Interpretation (Document Control Number: 2519) Standards and Controls section for the expected 9947A profile.

- 5.2. The negative amplification control is prepared and processed in parallel with each amplification sample set. A negative control does not need to be re-run once it has been confirmed negative.
- 5.3. The ladder included in each run can also serve as a positive run control. It is 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 FBS21 Identifiler[®] Plus Interpretation (Document Control Number: 2519) Standards and Controls section for a list of the designated alleles that should be detected in the ladder.

6. Calibration

- 6.1. Not applicable

7. Procedures

- 7.1. Preparing the Instrument:

- 7.1.1. Starting the 3130x/ Genetic Analyzer

- 7.1.1.1. The instrument and computer should already be turned on and instrument doors closed.

- 7.1.2. Data Collection Software

- 7.1.2.1. 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. Refer to FBQ29 and the 3130x/ 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 will 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 will be inspected for fluid level and length of time on the instrument. If the polymer level is sufficient (each 3130x/ run uses approximately 50 to 80 μ l of polymer) and the polymer bottle has been on the instrument for less than one week, the polymer does not need to be changed. Change the polymer if fluid levels are low or the bottle has been on the instrument for one week or longer. 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 will be replaced daily before a run. The reservoir septa will 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 diH₂O. If reservoir septa do not need to be replaced, they will be kept dry and retained (septas 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 diH₂O 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 3130x/. 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 3130x/ Setup Sheet and enter the sample names into the Sample Entry tab for placement orientation. A ladder will be present in each injection of the run. The plate name will be automatically generated when the analyst enters his/her initials and date into the worksheet.

7.3.1.2. The amount of each component needed in the Formamide/LIZ mixture will be calculated upon entry of the number of reactions into the Hi-Di Formamide and LIZ rows on the Plate Setup tab. The worksheet will calculate the appropriate volume of each component.

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 be in multiples of 16. 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. Tab to Sheet 3 of the 3130x/ Setup Sheet.

7.3.2.2. Delete out any blank rows.

7.3.2.3. Copy and paste the entire excel worksheet into a new text document.

7.3.2.4. Save the text document onto the network and transfer to 3130x/ computer.

7.3.2.5. Select plate manager.

7.3.2.6. Select import and find text document.

7.3.2.7. Select ok when finished importing.

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.

7.3.3. Pre-heating the Oven

7.3.3.1. This is an optional but recommended 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 and expiration dates on the 3130x/ 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, 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 name, 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 3130x/ 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 at 3000 rpm for approximately 30 seconds to 1 minute to ensure all liquid is concentrated in the bottom of each well.
- 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:

<i>HOLD</i>	<i>95°C</i>	<i>3:00 MIN</i>
<i>HOLD</i>	<i>4°C</i>	<i>3:00 MIN</i>
<i>HOLD</i>	<i>4°C</i>	<i>FOREVER</i>
- 7.3.4.13. When denaturation is complete, the plate will be left at 4 °C in the thermal cycler (or placed in the fridge) until it is ready for assembly onto the autosampler.
- 7.3.4.14. 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.

7.4. Performing the Run

7.4.1. 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. After the instrument doors are closed, 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 before continuing to the next step.
- 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 or type in plate name and click search.

- 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, the instrument status can be checked to monitor the temperature, voltage, current, and laser power. The data can also be viewed 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. Once the run has been completed select “My Computer” on the desktop > “E Drive” > “Applied Biosystems” > “UDC” > “DATA COLLECTION” > “DATA”. Alternatively, click on the data collection shortcut.
- 7.5.2. Locate the project folder, copy and paste the project to the designated storage location (i.e. share drive or thumb drive).

7.6. Storing the amplified DNA product:

- 7.6.1. 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.

8. Sampling

- 8.1. Not applicable

9. Calculations

- 9.1. Not applicable

10. Uncertainty of Measurement

10.1. Not applicable

11. Limitations

11.1. Not applicable

12. Documentation

12.1. FBU 3130x/ Setup Sheet (Document Control Number: 1590)

13. References

13.1. Applied Biosystems 3130/3130x/ 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. FBR41 - 1X Genetic Analyzer Buffer (Current Version)

13.4. FBR40 - Hi-Di Formamide (Current Version)

13.5. FBS12 - PCR Amplification Using the AmpF ℓ STR $\text{\textcircled{R}}$ Identifiler + TM Kit (Current Version)

13.6. FBS21 - Identifiler $\text{\textcircled{R}}$ Plus Interpretation (Current Version)

13.7. FBQ26 - Quality Control of AmpF ℓ STR $\text{\textcircled{R}}$ Identifiler + TM PCR Amplification Kits (Current Version)

13.8. FBQ29 - Maintenance of the AB 3130x/ Genetic Analyzer (Current Version)

13.9. Forensic Science Laboratory Quality Assurance Manual (Current Version)

13.10. DFS Departmental Operations Manuals (Current Versions)

13.11. FSL Laboratory Operations Manuals (Current Versions)

13.12. Forensic Biology Unit Quality Assurance Manual (Current Revision)

