Capillary Electrophoresis for DNA Databasing

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1 INTRODUCTION

These procedures are for preparing daughter plates of samples amplified with the GlobalFiler® Express (GFE) PCR Amplification Kit followed by the separation and detection by capillary electrophoresis (CE) with an Applied Biosystems (AB) 3500xL or 3730xL Series Genetic Analyzer in the Federal DNA Database Unit (FDDU).

2 SCOPE

These procedures apply to DNA personnel who prepare daughter plates of GFE amplified DNA database samples followed by the separation and detection by CE on the 3500xL or 3730xL.

3 EQUIPMENT

3.1 Equipment/Materials

- General Laboratory Supplies (e.g., pipettes, tubes)
- Barcode printer with appropriately sized labels (2.0" x 0.5" or equivalent)
- Barcode Scanner, Hand-held (Symbol LS4000i, 4008i, LS4071 or equivalent)
- STACS™ Database (STACS-DB) Software (Sample Tracking and Control Solutions [STACS DNA Inc.] part of InVita Healthcare Technologies), version 6.9 or above
- Robotic Workstation (Tecan EVO 150/200)
- Tecan EVOware Software, version 2.8 or higher (Tecan)
- 96-Well Sample (MicroAmp) Plates (Applied Biosystems or equivalent)
- 96-Well Plate Septa (Applied Biosystems or equivalent)
- Plate Sealer, microplate (Agilent Plate Loc or equivalent) with heat seal
- Thermal Cycler (Applied Biosystems GeneAmp® PCR System 9700 or ProFlex™ PCR System)
- 3500xL Genetic Analyzer (Applied Biosystems)
    - 3500 Series Data Collection Software, version 3.1 or higher (Applied Biosystems)
    - 96-Well Plate Base and Retainer (Applied Biosystems or equivalent)
    - 24-Capillary Array (3500xL), 36 cm (Applied Biosystems)
- 3730xL Genetic Analyzer (Applied Biosystems)
    - Data Collection software, version 5.0 or higher (Applied Biosystems)
    - 96-Well Plate Base and Retainer (Applied Biosystems or equivalent)
    - 48 – Capillary Array (3730xL), 36 cm (Applied Biosystems)

3.2 Reagents

- GlobalFiler® Express Allelic Ladder (Applied Biosystems)
- Hi-Di™ Formamide (Applied Biosystems or equivalent)
- GeneScan 600 LIZ ® Size Standard v2.0 (Applied Biosystems)
- 3500xL Anode Buffer Container (ABC), (Applied Biosystems)
- 3500xL Cathode Buffer Container (CBC), (Applied Biosystems)

- Performance Optimized Polymer, AB 3500xL POP-4™, 384 sample pouch or 960 sample pouch (Applied Biosystems) or Performance Optimized Polymer POP-7™
- Bleach, 3% (molecular grade or equivalent)
- Liquinox™ Critical Cleaning Liquid Detergent (Alconox or equivalent)
- DS-36 Matrix Standard Kit (Dye Set J6) (Applied Biosystems)
- 3500xL Conditioning Reagent, (Applied Biosystems)
- 10X 3730xL Running Buffer (Applied Biosystems)
- Reagent Grade Water (Corning or equivalent)

4 STANDARDS AND CONTROLS

The following controls are included on each amplification plate and will be transferred for capillary electrophoresis. These controls will be interpreted according to the criteria in the applicable FDDU Procedure (i.e., BIO-315).

GlobalFiler Express (GFE)
Negative (aka Combo)
Blood/Buccal Internal Standard (BIS)

5 PROCEDURE

Refer to the DNA Procedure Introduction (i.e., BIO-100) for applicable laboratory quality assurance and cleaning instructions.

When using a Robotic Workstation, ensure general instrument cleaning and maintenance is done prior to use, as needed. See the FDDU amplification procedure (i.e., BIO-305) Appendix A for additional guidance.

5.1 Post Amplification Incubation

A. Load each 96-Well amplification plate into a thermal cycler and close the thermal cycler.

B. Select the appropriate method on the thermal cycler and ensure that the method displayed on the instrument screen matches the method outlined below:
   - HOLD 60°C for 5 to 20 minutes, as appropriate.
   - HOLD 4°C Forever (∞)

C. Start the thermal cycler. Ensure that the reaction volume is 15 µl and that the correct ProFlex mode is selected.

D. Ensure the *Thermal Cycler Bar Code* and the *Plate Bar Code* for each plate to be incubated has been scanned into STACS-DB.

E. Indicate the result of the process in STACS-DB as successful, failed or aborted. Comments and observations must be entered for plates with process failed results.

5.2 CE Plate Setup Daughter Plate Creation

A. Create the daughter plate in STACS-DB.

B. Upon completion of the daughter plate creation, STACS-DB prints out plate barcodes with a daughter plate designation. Place each barcode accordingly, on the CE Plate Setup MicroAmp plate and support base.

C. Ensure the barcodes affixed to both the CE Plate Setup MicroAmp plate and support base has been scanned into STACS-DB.

D. Repeat for each plate that is being processed at CE Plate Setup.

5.3 GS-600 [LIZ] v2.0 Formamide Preparation

A. Prepare the GS-600 [LIZ] v2.0 formamide. The solution is prepared by combining Hi-Di formamide with GS-600 [LIZ] size standard v2.0 in a 19:1 ratio. Extra wells should be included in the calculation for overage.

GS-600 [LIZ] v2.0 Formamide (per 96-Well daughter plate)	
Hi-Di™ Formamide (9.5µl per reaction)	950 µl
GS-600 [LIZ] size standard v2.0 (0.5µl per reaction)	50 µl

B. Ensure the preparation has been recorded in the *Chemical Preparation* module of STACS-DB.

C. Store the GS-600 [LIZ] v2.0 formamide solution refrigerated.

5.4 CE Plate Setup

The CE Plate Setup procedure can be performed either manually or by the robotic workstation.

A. Quick-spin the 96-Well Amplification plate(s) for approximately 30 seconds.

B. Within STACS-DB, select the daughter plate(s) to be processed and select the appropriate scenario.

1. Additionally, for automated processing only:
    - Scan the instrument barcode on the Tecan EVO Robotic Workstation.
    - Ensure the Robot Maintenance Checks have been performed.
    - Indicate whether each check passed.

C. Scan the barcode on each of the 96-Well Amplification plate(s), the CE Plate Setup Daughter plate(s) and each reagent required for the selected scenario.

D. Select "Process" and proceed with the CE Plate Setup procedure.

    1. For manual processing, remove the seal cover from the 96-Well Amplification plate.

    2. Additionally, for automated processing only:
        - STACS-DB launches the robotic software for the Tecan EVO Robotic Workstation
        - If necessary, enter the appropriate user name and password at the robotic software log-in screen.
        - Verify that the appropriate script has been opened.
        - Remove any plastic cover(s) on the 96-Well Amplification plate(s), if necessary.
        - Ensure the selected 96-Well Amplification plate(s), the corresponding CE Plate Setup Daughter plate(s) and the required reagents have been loaded on to the instrument.
        - Ensure the instrument has been properly flushed and no air bubbles are visible in the tubing or syringes.
        - Start the CE Plate Setup script.
        - Indicate the number of plates to be processed.

    The following CE Plate Setup procedure will be performed manually or by the Robotic Workstation:

E. Aliquot 10 µl of the GS-600 [LIZ] v2.0 formamide solution into each well of the CE Plate Setup Daughter plate(s).

F. Add 1 µl of PCR product from the 96-Well Amplification plate(s) to its corresponding sample well in the CE Plate Setup Daughter plate(s) and 1 µl of the appropriate allelic ladder to the designated well(s) in the CE Plate Setup Daughter plate(s).

G. Ensure the 96-Well amplification plate(s) are sealed with a plastic cover.

H. Visually inspect the CE Plate Setup Daughter Plate(s).

I. Indicate the result in STACS-DB as successful, failed or aborted. Comments and observations must be entered for plates with process failed results. If the plate(s) were processed on the Tecan EVO Robotic Workstation, indicate in STACS-DB whether the bleach process was performed.

J. Ensure the CE Plate Setup Daughter plate(s) are covered with septa.

K. Ensure the CE Plate Setup Daughter Plate(s) has been vortexed (approximately 2 seconds) and quick-spin (approximately 30 seconds).

L. Return the 96-Well Amplification plate(s) to refrigerated storage in the post-amplification laboratory. Plate(s) can be discarded as needed.

5.5 CE Plate Denature

A. Load the CE Plate Setup Daughter Plate(s) into the thermal cycler(s). Close the thermal cycler.

B. Select the appropriate method on the thermal cycler and verify that the method displayed on the instrument screen matches the method outlined below:
   - HOLD 95°C 3 minutes
   - HOLD 4°C 3 minutes
   - HOLD 4°C Forever (∞)

C. Start the thermal cycler. Ensure that the reaction volume is 11 µl and that the correct ProFlex mode is selected.

D. Ensure the *Thermal Cycler Bar Code* and *CE Plate Setup Daughter Plate Bar Code* for each plate to be denatured has been scanned into STACS-DB.

E. Indicate the result of the process in STACS-DB as successful, failed or aborted. Comments and observations must be entered for plates with process failed results.

5.6 Setting Up the 3500xL or 3730xL Genetic Analyzer (Sequencer)

NOTE: If sequencer general maintenance is required, refer to Appendix A for guidance.

A. Log onto the workstation and then launch the 3500 Series or 3730xL Data Collection Software application.

B. Check consumable status in the dashboard or visually inspect the consumables. Replenish the consumables (e.g., POP, Buffers, or Array), if necessary. ***Caution:*** *To avoid electrical arcing, all surfaces of the containers must be clean and dry.*

	Consumable		**Recommended Frequency** (whichever comes first)
3500xL	POP-4 (24-cap)	960 sample pouch	14 days, 960 samples, or 50 injections
		384 sample pouch	14 days, 384 samples, or 20 injections
	ABC and CBC	24-cap	14 days or 100 injections
	Capillary Array	24-cap	As needed

	Consumable		Recommended Frequency (whichever comes first)
3730xL	POP-7	28 ml bottle	7 days
	1X Run Buffer*	Reservoir volume	48 hrs
	Capillary Array	48-cap	As needed

*To prepare the 1X Run Buffer, dilute the 10X 3730xL Running Buffer with water and record in the appropriate module in STaCS.

C. The oven may be turned on and the temperature set in advance to shorten the interval between run activation and execution.

D. Ensure the chemicals/reagents/array required for the Genetic Analyzer have been defined and/or verified in STACS-DB using the *CE Preparation* module.

E. If necessary, ensure any changes to chemicals/reagents/array performed on the Genetic Analyzer have been recorded in STACS-DB using the *Instrument Maintenance* module.

5.7 CE Analysis and Sample Sheet Creation

In the *CE Analysis* module, the type of Genetic Analyzer (*Sequencer*) to be used for capillary electrophoresis is selected, the reagents assigned to the sequencer are recorded and a sample sheet(s) generated.

A. Within STACS-DB, scan the *Sequencer Bar Code* of the selected instrument that will be utilized to perform capillary electrophoresis.

B. Scan the barcode on the CE Plate Setup daughter plate(s) to be processed.

C. If necessary, specify the *Destination Directory* for the sample sheet(s) in STACS-DB.

D. Create the sample sheets. STACS-DB creates a sample sheet file with the same name as its corresponding CE Plate Setup daughter plate barcode.

NOTE: If multiple injections of any FDDU samples and/or controls are required, the user may later add the injections within the Data Collection software of the sequencer.

E. Indicate the result of the process in STACS-DB as successful, failed or aborted. Comments and observations must be entered for plates with process failed results.

5.8 Initiating Capillary Electrophoresis

5.8.1 3500xL Genetic Analyzer

A. Define the Plate Properties for the CE Plate Setup daughter Plate to be run.

B. Review the plate contents for each imported plate in the Table View tab and bottom of the Assign Plate Contents screen. Save the plate and any changes to the Library. If necessary, repeat for 2nd plate to be run on the Genetic Analyzer.
   - Additional Assays may be created (saved in the Library) from the Workflow - Assign Plate Contents screen that include multiple injections of all samples and/or controls the assay is applied to. Choose "Add From Library".

C. Load Plates for the Run
   - Ensure each AB 3500xL plate assembly has been securely prepared with the CE Plate Setup Daughter Plate and the plate assemblies are properly seated in the autosampler with the instrument door closed.
   - Specify the position of the plate(s) in the autosampler (A and/or B).

D. Confirm that the linked plate(s) are in the correct position of the autosampler. Click Create Injection List to review the injection list and/or make any changes. Ensure there are no bubbles in the polymer delivery pump. The Data Collection Wizard for bubble removal may be run, if necessary.

E. Start the Run.

   NOTE: The electrophoresis run can be monitored by selecting Monitor Run from the navigation panel.

5.8.2 3730xL Genetic Analyzer

A. Turn on the oven, buffer heater and set the oven temperature.

B. Import the STaCS generated sample sheets.

C. Review the plate record for a minimum of one plate in each run. Use the pull down menus to make any necessary changes to the spreadsheet.

D. Load Plates for the Run
   - Ensure each AB 3730xL plate assembly has been prepared with the EPP Daughter Plates and that the plate assemblies are properly seated in the In Stacker tower on the plate stacker.
   - Verify that the EPP daughter plate barcode for each plate is clearly visible when the door to the In Stacker tower is open.
   - Ensure that the Out Stacker tower has sufficient space to process all plates to be run. Close both tower doors and close the stacker drawer.

E. Ensure the instrument Run Mode is on Auto.

F. Ensure the Output Stack portion of the Run Scheduler window has sufficient space to process all plates being run, that there are no bubbles in the tubing, array port or pump blocks.

G. Start the Run.

NOTE: The electrophoresis run can be monitored by selecting Monitor Run from the navigation panel.

6 LIMITATIONS

The appropriate processing methods are selected for a plate based on the sample type added to the plate and the amplification kit to be used. Based on internal studies, only the combinations of processes listed below are approved for use.

Sample Type	Amp Kit	CE Plate Setup	Genetic Analyzer
Blood [FTA] or Buccal [FTA and Non-FTA]	GlobalFiler Express (GFE)	Manual or Automated using a Tecan EVO Robotic Workstation	AB 3500xL or 3730xL

7 SAFETY

- All FDDU samples that contain blood are considered potentially infectious regardless of the perceived status of the source individual or the age of the material. All FDDU personnel who work with such material will refer to the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated.

- Procedural Specific Chemical Hazards:

    - Formamide is a teratogen. Avoid inhalation, skin contact, or ingestion. Use nitrile gloves when handling. Dispose of unused portions in appropriate hazardous waste containers. Pregnant women must not handle formamide.

    - Performance Optimized Polymer is caustic. Avoid inhalation, skin contact, or ingestion. Use gloves when handling. Dispose of unused portions in appropriate hazardous waste containers.

8 REFERENCES

Federal Bureau of Investigation Quality Assurance Standards for DNA Databasing Laboratories, current version.

Sample Tracking and Control Solutions [STACS DNA Inc.] part of InVita Healthcare Technologies. *STACS™ Database (STACS-DB) User's Guide.*

Applied Biosystems. *GeneAmp® PCR System 9700 User's Manual Set*. Applied Biosystem, Foster City, CA. 1997.

Applied Biosystems. *GlobalFiler® Express PCR Amplification Kit User Guide*.

Applied Biosystems, Foster City, CA. Applied Biosystems. 3500/3500xL Genetic Analyzer User Guide, Foster City, CA.

Applied Biosystems. 3730xl DNA Analyzer User Guide. Publication Number 1000077621, Revision C (2020).

Applied Biosystems. *Proflex™ PCR System User Guide*. Life Technologies Corporation, Carlsbad, CA. 2016.

9 REVISION HISTORY

Revision	Issued	Changes
00	02/04/2022	Reformatted DNA 316-2 into new template and assigned new Doc ID. Changed from EPP to CE Plate Setup throughout.
01	11/30/2023	Added procedures for 3730. Removed IT maintenance topics from Appendix A.
02	01/16/2024	Corrected number of 3730xl capillaries from 96 to 48. Added guidance for the preparation of 3730 1X buffer.

10 APPENDIX A: CE MAINTENANCE AND PERFORMANCE VERIFICATION PROCEDURES

General maintenance and performance verification procedures are performed in accordance with the level 1 documents and the DNA procedure for equipment calibration and maintenance (i.e., BIO-104). General maintenance is generally performed at the intervals listed below. Performance verification of the genetic analyzer instruments will be conducted at the minimum frequency described in the DNA procedure for equipment calibration and maintenance.

General Maintenance	Recommended Interval	
	3500xL	**3730xL**
Replace Polymer	14 days (or as required in procedure)	Weekly (or as required in procedure)
Replace buffers, water and waste.	14 days (or as required in procedure)	Weekly (or as required in procedure)
Install New Array	As needed	As needed
Flush pump chamber and channels	Weekly	Weekly
Flush pump or water trap	Weekly	Weekly
Database Cleanup	As needed	As needed
Data Backup	As needed	As needed
Disk Defragmentation	As needed	As needed
Spatial Calibration	With array change or as needed	With array change or as needed
Spectral Calibration	With array change or as needed	With array change or as needed
GS600 LIZ Sensitivity Evaluation	Semiannually or after optical adjustment	Quarterly or after optical adjustment

10.1 General Maintenance

10.1.1 Applied Biosystems 3500xL

A. Flush the pump chamber and channels
    1. From the Maintenance Wizards screen, click **Wash Pump and Channels**.
    2. Follow the prompts in the Wash Wizard window. The Wash Pump and Channels wizard takes approximately 40 minutes to complete.
B. Replace polymer (POP-4) pouch. Allow refrigerated polymer to equilibrate to room temperature prior to first use. Follow prompts in Maintenance Wizards screen, select Replenish Polymer.
C. Replace Anode Buffer Container (ABC)
    1. Allow refrigerated ABC to equilibrate to room temperature prior to first use. Do not remove seal.
    2. Verify that buffer level is at or above the fill line and check that seal is intact

3. Invert the ABC, then tilt slightly to make sure most of the buffer is in the larger side of the container. There should be less than 1mL of the buffer remaining in the smaller side of container.
4. Verify that the buffer is at the fill line.
5. Peel off the seal at the top of the ABC. Place the ABC into the Anode end of the instrument, below the pump.
6. Close the instrument door and click Refresh on the Dashboard to update status after changing the ABC.

D. Replace the Cathode Buffer Container (CBC)
1. Allow refrigerated CBC to equilibrate to ambient temperature prior to first use.
2. Wipe away condensation on the CBC exterior with lint-free lab cloth.
3. Verify that buffer level is at or above the fill line and check that seal is intact.
4. Tilt the CBC back and forth gently to ensure that the buffer is distributed evenly across the container.
5. Verify that the buffer is at or above the fill line.
6. Place the container on a flat surface and peel off the seal.
7. Wipe off any excess buffer on top of the CBC and ensure that the top of the container is dry.
8. Place the appropriate septa on both sides of the CBC.
9. Click the Tray button on the front panel to move the autosampler to the front position.
10. Install the CBC on the autosampler. The CBC will click into the autosampler as the tabs are snapped into place.
11. Close the instrument doors. Click Refresh from the Dashboard to update status after changing the CBC.

10.1.2 Applied Biosystems 3730xL

A. Replace Polymer (POP-7). Loosen the bottle cap and allow it to degas for approximately 15 min.
B. Flush the polymer delivery pump (PDP)
1. Run the "water wash wizard" and use reagent grade water to flush the PDP.
2. Follow wizard prompts.

C. Flush the PDP water trap
1. Use a 20 mL Luer lock syringe filled with reagent grade water.
2. Attach the syringe to the forward facing Luer fitting at the top of the pump block, open the Luer approximately one-half turn counterclockwise.
3. Open the exit fitting at the top left side of the pump block approximately one-half turn counterclockwise.
4. Flush the water trap with approximately 5 mL of water.
5. Close both fittings by turning them clockwise until finger-tight, do not over tighten.

10.2 Array Change and Spatial Calibration

The capillary array will be changed as needed. The determination to change the array will be based upon a review of the quality of the data generated by the instrument.

10.2.1 Applied Biosystems 3500xL

A. From the Maintenance Wizards screen, click Install Capillary Array
B. Install the array as instructed by the wizard being careful not to leave fingerprints on the detection window.
    1. Ensure the proper type (24-cap) and length (36 cm) is entered in the array information fields.
C. In the final step of the wizard, you can choose to fill the array with polymer or click "finish" if the array will be filled during the spatial calibration.

10.2.1.1 Spatial Calibration

A spatial calibration must be performed whenever a new array is installed or every time the detection cell window is opened.

A. Select Maintenance in the navigation pane then select Spatial Calibration
B. Select Fill to fill the array with polymer before starting the calibration
C. Select Perform QC Checks
D. Click Start Calibration button to initiate the spatial calibration.
E. Select Accept Results to accept the spatial calibration if the following criteria are met:
    1. Peaks of the spatial calibration are approximately the same height.
    2. One marker (a cross) appears at the top (apex) of each peak in the profile.
    3. No irregular peaks are contained in the profile.
    4. RFU (relative fluorescence unit) values for the peaks are greater than 3000 for a 24-cap 3500xL array.
    5. Uniformity or peak height similarity values are 0.2
    6. The values for the Capillary spacing are ≤2 pixels

10.2.2 Applied Biosystems 3730xL

A. From the toolbar select the "Install Array Wizard".
B. Install the array as instructed by the wizard.
    1. Ensure the proper type (48-cap) and length (36 cm) is entered in the array information fields.
C. In the final step of the wizard you can choose to fill the array with polymer or click "finish" if the array will be filled during the spatial calibration.

10.2.2.1 Spatial Calibration

A spatial calibration must be performed whenever a new array is installed, every time the detection cell window is opened, or each week for a 3730.

A. Select "Spatial Run Scheduler" in the navigation pane.
B. Select "SpatialFill_1"

C. Click the “Start” button to initiate the spatial calibration.
Note: “SpatialNoFill_1” can be selected if there is no need to fill the array with fresh polymer.
D. Select “Accept” to accept the spatial calibration if the following criteria are met:
    1. Peaks of the spatial calibration are approximately the same height.
    2. An orange cross appears at the top (apex) of each peak in the profile.
    3. No irregular peaks are contained in the profile
    4. RFU values for the peaks are greater than 1,000 fora 3730 array
    5. The values for the Left Spacing and Right Spacing columns for a 3730 array are 9-11 pixels. (A spatial calibration can be accepted if one or more of the spacing values lie outside of this range, but it is preferable to have all the values within this specification.)

10.3 Spectral Calibration

A spectral calibration must be performed whenever a new array is installed or as needed (e.g., decrease in spectral separation, new dye set, or optical adjustment). A spectral plate may be injected several times within a 24-hour period; a fresh spectral plate should be used for each instrument being calibrated.

10.3.1 Applied Biosystems 3500xL

A. Combine 294 µl of formamide with 6 µl of DS-36 Matrix Standard for the J6 dye set.
B. Dispense 10 µl of solution into the first three columns (A1-H1, A2-H2, and A3-H3).
C. Spin down and denature plate on thermal cycler then place on instrument.
D. Access the Spectral Calibration screen: Select Maintenance, then select Spectral Calibration in the Navigation pane.
E. Select number of wells on the plate (e.g., 96 well plate) and specify plate position on instrument.
F. Select the chemistry standard and dye set for the calibration plate.
G. Select Allow Borrowing.
H. Click Start Run.

- **Pass Criteria:** The data collection software indicates the pass/fail status of each capillary. The spectral calibration is acceptable if the following criteria are met, and there is proper separation between the color channels.
    - All capillaries have to meet the spectral Quality Value and Condition Number limits.
    - The passing Quality Value for J6 Dye Set is a minimum of 0.95.
    - The passing Condition Number value is a maximum of 8.0 for J6 Dye Set.
    - ≤ 3 adjacent-capillary borrowing events allowed

  The software gives a pass/fail status to each capillary. The user must evaluate the spectral profile traces and **Accept Results** or **Reject Results.**

10.3.2 Applied Biosystems 3730xL

A. Combine 490 uL of formamide with 10 uL of DS-36 Matrix Standard

B. Dispense 10 uL of solution into wells A1-H1, A3-H3, A5-H5, A7-H7, A9-H9, and A11-H11
C. Spin down and denature plate on thermal cycler then place on instrument.
D. Click "Plate Manager" in the navigation pane.
E. Select "New" and the "New Plate Dialog" dialog box will open, fill out fields as follows
    1. Name: Use the naming convention CE#XX_Spectral_MMDDYY.
        i. Enter the same information in the "ID (Barcode)" field if running in manual mode, otherwise enter the plates barcode ID in this field.
    2. Select "Spectral Calibration" from the "Application" drop down menu.
    3. Select "Septa" from the "Plate Sealing" drop down menu
    4. Complete the remaining fields and select "OK". This will open the "Spectral Calibration Plate Editor" window.
F. Create sample sheet.
    1. Fill out the "Sample Name" fields to mirror the plate layout.
    2. Select "Spectral_G6" from the drop down in the "Instrument Protocol 1" field.
    3. Press "OK" to save plate sample sheet.
G. Select "Run Scheduler" from the navigation pane
    1. Search the plate name or select find all and click on the plate to be run in order to highlight it within the list.
    2. Click "Add" to add the plate to the input stack.
H. Click the green arrow to start processing the spectral plate.

- **Pass Criteria:** The data collection software indicates the pass/fail status of each capillary. The spectral calibration is acceptable if the following criteria are met, and there is proper separation between the color channels.
    - The number of failing wells ≤ 5 capillaries, no more than 2 in a row
    - The minimum peak height is 500 RFUs

  The Data Collection software automatically applies a passing spectral. No further action is required by the user.

10.4 Performance Verification

Performance verification (PV) of the genetic analyzer instruments should be conducted at the minimum frequency described in the DNA procedure for equipment calibration and maintenance.

10.4.1 Applied Biosystems 3500xL

A. Using 007 amplified with the GlobalFiler Express amplification kit for 26 cycles.
B. Prepare a stock solution in a 10:1 ratio of GS600LIZ/Formamide to 007 amplicon.
C. Add 11 µl of the stock solution to 21 wells in the first 3 columns of a plate.
D. Add 10 µl of GS600LIZ/Formamide and 1 µl of Globalfiler Express ladder to the remaining 3 wells in the first 3 columns of the plate.
E. Inject the samples two times at the instrument's current injection setting

- **Pass Criteria:** A genetic analyzer will be deemed suitable for FDDU analysis if:
    - All peak heights of all alleles in the 007 positive control are greater than 175 RFU when analyzing the data with normalization.
    - Correct typing results obtained for 007.
    - The average peak heights of the 007 samples for the 11 GS600 LIZ peaks used for normalization is between 1675 and 5025 RFU when analyzing the data without normalization.

- The following are the 11 peaks used for normalization:

200	220	240	260	280	300	314	320	340	360	400

10.4.2 Applied Biosystems 3730xL

A. Amplify 007 with the Globalfiler Express amplification kit for 26 cycles.
B. Prepare a stock solution in a 10:1 ratio of GS600LIZ/Formamide to amplicon.
C. Add 10 uL of stock solution to wells A1- G1, A3-H3, A5-G5, A7-H7, A9-H9, A11-G11.
D. Add 1 uL of Globalfiler Express ladder and 10 uL of GS600LIZ/Formamide solution to wells H1, H5, and H11.
E. Inject the samples two times at the instrument's current injection setting.
F. Inject the samples two times, at various voltage settings above and/or below the previously determined injection voltage.

- **Pass Criteria:** A genetic analyzer will be deemed suitable for FDDU analysis if:
    - All peak heights of all alleles in the 007 positive control are greater than 150 RFU when analyzing the data with normalization.
    - Correct typing results obtained for 007.

10.5 Quality Control of GS600 LIZ v2 Internal Size Standard (for 3500xL only)

A. Prepare a plate using the instructions in section 10.4.1 using the new lot of GS600 LIZ.
B. Run the plate on an appropriate 3500xL instrument.
C. Analyze the data without normalization.
D. The sensitivity of the new lot will be accepted if the average peak heights of the 007 samples for the 11 GS600 LIZ peaks used for normalization (listed above) is between 1675 and 5025 RFU.
E. If the lot of GS600 does not meet sensitivity expectations, the assessment will be repeated. If the results are still deemed unsuitable, the Technical Leader will be consulted.

10.6 Sensitivity Evaluation using GS600 LIZ v2 (for 3500xL only)

A. Prepare a plate(s) using the instructions in section 10.4.1. A single plate or multiple plates prepared with the stock solution may be used to evaluate multiple 3500xL instruments. Run the plate(s) on the appropriate 3500xL instrument(s). A plate

prepared for or data generated from section 10.4.1 may also be used for this evaluation.

B. Analyze the data without normalization.
C. The sensitivity of each instrument will be accepted if the average peak heights of the 007 samples for the 11 GS600 LIZ peaks used for normalization (listed above) is between 1675 and 5025 RFU.
D. If one or more instruments do not meet sensitivity expectations, the evaluation for that instrument will be repeated. If the results are still deemed unsuitable, the Technical Leader will be consulted.