DNA
Procedure for the Capillary Electrophoresis of Nuclear DNA

1 Scope

These procedures describe the separation by capillary electrophoresis (CE) of amplified nuclear DNA (nDNA) products from evidence and reference samples. The DNA Casework Unit (DCU) and Biometrics Analysis Unit (BAU) use Sample Tracking and Control Software (STACS) and robotic workstations to automate the set-up of the CE plates.

2 Equipment/Materials/Reagents

Equipment/Materials

- STACS, version 3.2.920 or higher
- Agilent Technologies "Bravo" Liquid Handler
  - VWorks Software, version 11.0.0.874 or higher
- Liquid handler tips, Agilent Technologies or equivalent
- Thermal Cycler, GeneAmp® PCR System 9700
- 3130xl Genetic Analyzer, Applied Biosystems
  - Data Collection Software, version 3.0 or higher
  - 16-Capillary (16-cap) Array, 36 cm
- 3500xL Genetic Analyzer, Applied Biosystems
  - Data Collection Software, version 3.1 or higher
  - 24-Capillary (24-cap) Array, 36 cm
- GeneMapperID-X, version 1.4 or higher
- General laboratory supplies (e.g., pipettes, tubes)
- 96-well plate, Applied Biosystems MicroAmp® optical or equivalent
- Plate septa, Applied Biosystems or equivalent
- Plate base and retainer, Applied Biosystems or equivalent

Reagents

- Hi-Di™ formamide, Applied Biosystems or equivalent
- GeneScan™-500 LIZ™ internal size standard, Applied Biosystems
- GeneScan™-600 LIZ™ Version 2.0 internal size standard, Applied Biosystems
- Ladder from the appropriate Amplification Kit, Applied Biosystems
  - Identifiler® Plus (ID+) Ladder
  - Yfiler™ (Y) Ladder
  - Globalfiler® (GF) Ladder

- 3130xl Instrument Reagents:
  - 1X Genetic analyzer buffer with EDTA, Applied Biosystems or equivalent
  - Performance Optimized Polymer 4 (POP-4™), Applied Biosystems
- 3500xL Instrument Reagents:
  - Anode Buffer Container (ABC) 3500 series, Applied Biosystems
  - Cathode Buffer Container (CBC) 3500 series, Appplied Biosystems
  - Performance Optimized Polymer 4 (POP-4) Pouch 3500 series, Applied Biosystems
  - Conditioning Reagent, Applied Biosystems
- DS-33 Matrix Standard Kit (Dye Set G5)[For ID+ and Y], Applied Biosystems
- DS-36 Matrix Standard Kit (Dye set J6) [For GF], Applied Biosystems
- Isopropyl alcohol, 70%
- Water, reagent grade or equivalent
- Purified Water or equivalent, available at laboratory sinks

3 Standards and Controls

The positive and negative amplification controls must be subjected to CE analysis in parallel with the associated set or batch of evidentiary samples. The positive must be included in any repreparation of samples for CE.

The amplified reagent blank (RB) from an extraction batch must be subjected to CE analysis using the same instrument model, same injection conditions, and most sensitive volume conditions as required by the sample(s) in the associated extraction batch.

Refer to the appropriate nuclear DNA interpretation procedure of the *DNA Procedures Manual* for interpretation of these controls.

4 Procedures

Refer to DNA Introduction Procedure (i.e., DNA QA 600) and follow applicable general precautions and cleaning instructions.

For water that will come into contact with the DNA samples (e.g., CE reservoirs), reagent grade, or equivalent, water will be used. The purified water, available via faucets (typically labeled DE) at the laboratory sinks, may be used for rinsing instrument components (e.g., Bravo reagent trough).

Ensure the appropriate fields (i.e., instruments, reagents) in STACS are completed from any network computer, as necessary.

4.1 Formamide: Internal Size Standard (ISS) Mixture (aka LIZ Formamide)

If needed, prepare the LIZ Formamide mix in the ratio listed below. Record the preparation in STACS. The LIZ Formamide mix may be stored refrigerated for up to one week.

	GlobalFiler on 3500xL	**Identifiler Plus or Yfiler on 3130xl**
Size Standard	GS-600 LIZv2	GS-500 LIZ
Hi-Di formamide: LIZ ratio	24:1	99:1

Example Calculations:

GS-600 LIZ Formamide (10 mL): 9.6 mL Hi-Di formamide + 400 µL GS-600 LIZv2

GS-500 LIZ Formamide (20 mL): 19.8 mL Hi-Di formamide + 200 µL GS-500 LIZ

4.2 CE Daughter Plate Preparation

If an Agilent Bravo Liquid Handler is unavailable, the CE daughter plate may be prepared manually.

For manual daughter plate prep:

4.2.1	For Identifiler Plus or Yfiler on 3130xl: • Add 24 µL GS-500 LIZ Formamide to each well (or set of 16 wells) in the CE daughter plate, • Add 1 µL of amplified product (excluding the ladder wells) from the amplification plate to the corresponding wells on the CE daughter plate. For GlobalFiler on 3500xL: • Add 10 µL GS-600 LIZ Formamide to each well (or set of 24 wells) in the CE daughter plate • Add 1 µL of amplified product (excluding the ladder wells) from the amplification plate to the corresponding wells on the CE daughter plate. *NOTE: Each set of wells for one injection (i.e., 16 wells on 3130xl, 24 wells on 3500xL) must be filled with LIZ Formamide even if not all wells in that set will be receiving samples.* Proceed to adding appropriate Ladder.	

When using the Agilent Bravo Liquid Handler, the work deck must be decontaminated with appropriate cleaner each workday before use and if it becomes visibly soiled. *Bleach should not be used.*

For robotic daughter plate prep:

4.2.2	Ensure the following items are provisioned to the robot and that the appropriate barcodes are entered as required by STACS (see Figure 1): • Position 1: A box of Agilent disposable tips. A full box or a half consumed box of tips may be used. • Position 2: The amplification plate, unsealed and in a plate holder. • Position 4: A tip box for used tips. This box must be empty or half empty. An empty box may only be used for 2 plates. • Position 5: A new 96-well plate with a CE daughter plate barcode on the right side. • Position 9: A reagent trough with wells containing a sufficient volume of the appropriate LIZ Formamide. - For full plate, enough to cover every well of the reagent trough. - For partial plate on a 3130xl, enough to cover each set of 16 wells (2 columns). - For partial plate on a 3500xL, enough to cover each set of 24 wells (3 columns). *NOTE: Each set of wells for one injection (i.e., 16 wells on 3130xl, 24 wells on 3500xL) must be filled with LIZ Formamide even if not all wells in that set will be receiving samples.*	

Ensure the robot is powered on, logon to the robot's computer, and launch and logon to the VWorks Software.

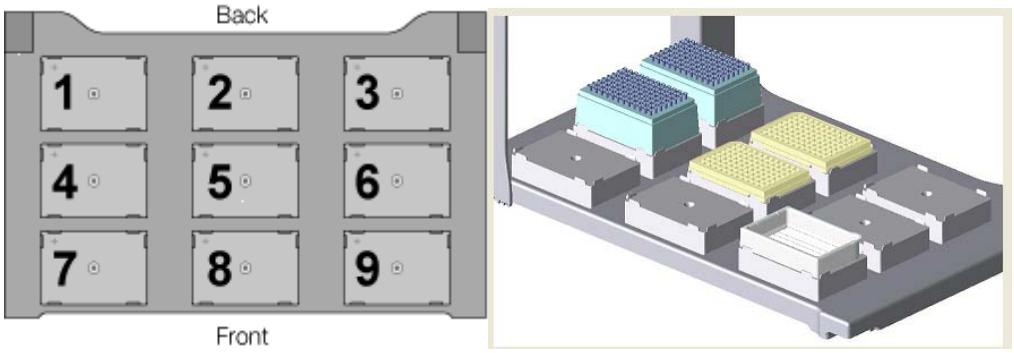

Figure 1 – Agilent Bravo Liquid Handler Deck Positions

4.2.2.1	Initiate the appropriate daughter plate protocol and follow the prompts. Ensure tip positioning for the new and used tip boxes is selected correctly.	

CAUTION: The instrument is equipped with a safety light curtain. If the operator reaches in during a run, the pipette head motors are disabled and the operation stops.

4.2.2.2	When the run is complete, ensure all appropriate wells contain LIZ Formamide.	

For the 3130xl: Each set of 16 wells (2 columns of 8) that contain any sample(s), must be filled with liquid to prevent introducing bubbles into the capillaries on the CE instrument.

For the 3500xL: Each set of 24 wells (3 columns of 8) that contain any sample(s), must be filled with liquid to prevent introducing bubbles into the capillaries on the CE instrument.

4.2.3	Add 1 µL of the appropriate allelic ladder to the designated CE daughter plate well(s).	

4.2.4	Seal the CE daughter plate with septa. 3500xL plates require a full septa. Vortex and quick spin the CE daughter plate.	

4.2.5	Denature the CE daughter plate in a thermal cycler by using the appropriate method (i.e., Denature).	

Leave the lid of the thermal cycler unlocked to avoid the septa adhering to it.

Each thermal cycler is programmed with the following method for denaturing:

HOLD 95°C 3 minutes
HOLD 4°C 3 minutes
HOLD 4°C ∞

Ensure the amplification plate is resealed and stored at 4°C until it is appropriate to discard.

The remaining LIZ Formamide may be recovered from the reagent trough and appropriately stored for future use. The reagent trough should be rinsed twice (collect the first rinsate in appropriate waste container), dried, and reused.

4.3 Setting Up the 3130xl

Log on to the computer workstation, ensure the CE is on, and launch the Data Collection software. If the instrument is off, ensure the attached computer is turned on prior to turning on the instrument.

The 1X buffer and reagent grade water in the reservoirs and the POP-4 are generally replaced weekly. If necessary, replenish the reservoirs with 1X buffer and/or reagent grade water, and fill the capillary array with POP-4.

The CE oven temperature may be set to 60°C to allow the CE to warm up and expedite the start of the run.

4.3.1	*At any point prior to scheduling the run:* Import the plate record created in STACS into the Data Collection software. Verify that all fields are filled in correctly.	

If necessary, the plate record may be manually created in the Data Collection software.

4.3.2	After denaturation of the CE daughter plate (i.e., anytime during the final 4°C hold), place the plate into a plate base and secure with a plate retainer. Place the CE daughter plate assembly on the CE autosampler with the notched end of the plate assembly facing the right front of the instrument. One or two plates may be loaded on the CE.	

Ensure the plate retainer seats directly over the septa to avoid damage to the capillary array.

4.3.3	Use the Run Scheduler in the Data Collection software to link the appropriate plate record(s) with the CE daughter plate(s).	

To confirm that a run has been properly scheduled, the run view can be selected.

4.3.4	Ensure the instrument doors are closed and start the run.	

Run status, instrument status, event log, raw data, and capillary/array can be monitored during the course of a run.

4.4 Setting Up the 3500xL

Ensure that the oven and all instrument doors are shut and power on the computer, but do not log on. Press the power button on the front of the analyzer to start the instrument. Ensure that the green status light is on before proceeding.

Log onto the workstation and then launch the 3500 Series Data Collection Software application only AFTER the 3500xL Server Monitor has fully initialized.

Check consumable status in the dashboard. Replenish the consumables (POP-4, Anode Buffer Container, Cathode Buffer Container, or Array), if necessary.

Caution: *To avoid electrical arcing, all surfaces and containers must be clean and dry.*

The CE oven temperature may be set to 60°C to allow the CE to warm up and expedite the start of the run. Ensure the oven is set to 60°C and select the "Start Pre-Heat" button. The preheat function turns off after 2 hours of instrument inactivity.

4.4.1	*At any point prior to scheduling the run:* Import the plate record created in STACS into the Data Collection software. Verify that all fields are filled in correctly and that the correct Assay has been added to the plate along with the File Name Convention and Results Group	

If necessary, the plate record may be manually created in the Data Collection software.

4.4.2	After denaturation of the CE daughter plate (i.e., anytime during the final 4°C hold), place the plate into a plate base and secure with a plate retainer. Place the CE daughter plate assembly on the CE autosampler with the notched end of the plate assembly facing the right front of the instrument. One or two plates may be loaded on the CE.	

Ensure the plate retainer seats directly over the septa to avoid damage to the capillary array.

4.4.3	Click *Link Plate for Run* or *Load Plates for Run* in the navigation panel to assign the plate(s) and specify the position of the plate(s) in the autosampler (A and/or B).	

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 or choose Preview Run on the left navigation panel before starting the run.

4.4.4	Ensure the instrument doors are closed and start the run.	

Run status, instrument status, event log, raw data, and capillary/array can be monitored during the course of a run by selecting *Monitor Run* from the navigation panel.

4.5 Data Review

4.5.1	After the CE run, move the data generated by the CE (i.e., .fsa files, .hid files) to the appropriate network folder. Use GMIDX to screen the data for samples that need reinjection or repreparation.	

The entire plate does not need to be run to screen sample data. Sample data may be viewed after each injection is completed.

The plate record may be edited to add injections, as needed, after the completion of the run. When reinjecting samples after the run has ended, a ladder(s) should also be reinjected.

An examiner will review the CE data for compliance with the requirements in the nDNA interpretation procedure of the *DNA Procedures Manual* and queue any samples that need to be rerun (aka reprep).

For samples that require a new CE daughter plate set up (aka reprep), repeat this procedure for at

least the applicable samples. At minimum, the positive amplification control and ladder(s) need to be included and injected. A new plate record is generated for the reprep.

5 Calculations

Not applicable.

6 Sample Selection

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

Not applicable.

9 Safety

9.1 Refer to the "Safe Work Practices and Procedures," "Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)," "Personal Protective Equipment Policy," and "Chemical Hygiene Plan" sections of the *FBI Laboratory Safety Manual* for important personal safety information to conducting these procedures.

9.2 Refer to the "Hazardous Waste Disposal" section of 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.

9.3 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. Any pregnant Biologist should advise a supervisor so that arrangements can be made to have an alternate individual(s) perform all formamide handling procedures.
- Performance Optimized Polymer (POP-4) is caustic. Avoid inhalation, skin contact, or ingestion. Use gloves when handling. Dispose of unused portions in appropriate hazardous waste containers.

10 References

FBI Laboratory Quality Assurance Manual (QAM)

FBI Laboratory Safety Manual

DNA Procedures Manual

Applied Biosystems. *GeneAmp® PCR System 9700 User's Manual Set.* 1997.

Applied Biosystems. *3130/3130xl Genetic Analyzers Getting Started Guide*. 2004.

Applied Biosystems. *3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide.* 2004.

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

Applied Biosystems. *Multi-Capillary DS-33 (Dye Set G5) Matrix Standard Product Insert.* 2004.

Applied Biosystems. *Multi-Capillary DS-36 (Dye Set J6) Matrix Standard Product Insert.*

Applied Biosystems. *GlobalFiler® PCR Amplification Kit User Guide*. Applied Biosystems, Foster City, CA.

Applied Biosystems. *AmpFlSTR® Identifiler® Plus PCR Amplification Kit User's Guide*, P/N 4402743, 2008.

Applied Biosystems. *AmpFlSTR® Yfiler™ PCR Amplification Kit User's Manual*, P/N 4358101 Rev. A 4305246, Foster City, CA.

ARTEL. *MVS Multichannel Verification System User Guide.* 2006

Rev. #	Issue Date	History
7	05/25/16	Complete revision for simplification of procedure. Changed from nDNAU to DCU throughout. Changed from nDNAU LIMS to STACS thoughout and made necessary adjustments for STACS. Moved QC procedures to Appendix.
8	12/30/16	Revised to add Globalfiler and the 3500xL. Made manual CE Plate Prep a more clear option. Incorporated BAU for when Huntsville lab is authorized to perform procedure on casework.

Approval

Redacted - Signatures on File

Appendix A: Quality Control Procedures

1. Critical Reagents

Refer to the DNA procedure for reagent purchasing, preparation and records (i.e., DNA QA 609) for additional requirements.

The Amplification Kit Ladders are evaluated with the associated amplification kit. Refer to the nuclear DNA amplification procedure (i.e., DNA 213) for instructions.

2. Instruments

Refer to the DNA procedure for instrument calibration and maintenance (i.e., DNA QA 608) for minimum frequency and additional requirements.

A. Performance Verification (PV) of the Agilent Bravo Liquid Handler

1. An Artel MVS Multichannel Verification System and NIST traceable standards will be used to test the accuracy and precision of the liquid handling by the Agilent Bravo Liquid Handler. Refer to the *Artel MVS Multichannel Verification System User Guide* for operation of the Artel MVS.

2. The Agilent Bravo Liquid Handler workstations are configured with a ninety six (96) barrel pipette head and multiple volumes aliquoted during each procedure. A minimum of three repetitions (i.e., three plates) must be performed by the head for each volume to assess the accuracy and precision of the pipette head.

3. The results must be within the tolerance limits set by DCU for each volume. At times, it may be necessary to modify/optimize the Liquid Handler Liquid Class parameters (e.g., polynomial coefficient or pipette volume offset).

4. If the performance verification of the Agilent Bravo Liquid Handler does not meet the above listed criteria, the performance verification will be repeated. If the results are still deemed unsuitable, then the Technical Leader will be consulted.

B. General Maintenance of the 3130xl Genetic Analyzer

The following recommended intervals and instructions provide guidance for the general maintenance of the 3130xl Genetic Analyzer and 3500xL Genetic Analyzer to include instruction for changing the capillary array and performing a spatial calibration or a spectral calibration. The Applied Biosystems 3130/3130xL Genetic Analyzers *Getting Started Guide*s and *Maintenance, Troubleshooting, and Reference Guide*s *and 3500/3500xL Genetic Analyzer User Guide* may be referenced for additional guidance.

General Maintenance	3130xl Recommended Interval	3500xL Recommended Interval
Replace Polymer	Weekly	14 days (or as required by usage)
Replace 1x Buffer/Water/ Waste	Weekly	
Replace Buffer (Anode and Cathode)		14 days (or as required by usage)
Install New Array	As needed	As needed
Water Wash	Weekly	
Flush Water Trap	Weekly	
Flush pump chamber and channels		Weekly
Flush pump trap		Monthly
Database Cleanup	Quarterly	Quarterly
Data Backup	Quarterly	Quarterly
Disk Defragmentation	Quarterly	Quarterly
Spatial Calibration	With array change or as needed	With array change, if detection cell window is opened, or as needed
Spectral Calibration	As needed or Quarterly	With array change or as needed
GS 600 LIZ v2 Sensitivity Evaluation		Semiannually or after optical adjustment

I. General Maintenance of the Applied Biosystems 3130xl

1. Prepare a new bottle of POP-4
    a. Loosen the POP-4 bottle cap and allow it to sit on the bench top for approximately 15 minutes to degas.
2. Flush the polymer delivery pump (PDP)
    a. Run the **water wash wizard** and use reagent grade water to flush the PDP. Note: For a warm water wash, heat water to < 60°C.
    b. Follow wizard prompts.
3. Flush the PDP water trap
    a. Use a 20 mL Luer lock syringe filled with reagent grade water.
    b. Attach the syringe to the forward facing Luer fitting at the top of the pump block, open the Luer approximately one-half turn counter clockwise.
    c. Open the exit fitting at the top left side of the pump block approximately one-half turn counter clockwise.
    d. Flush the water trap with approximately 5 mL of water
    e. Close both fittings by turning them clock wise until finger-tight, do not over tighten.

II. General Maintenance of the Applied Biosystems 3500xL

1. Flush the pump chamber and channels
    a. In the Maintenance Wizards screen, select **Wash Pump and Channels** and follow prompts to include the replacement of the polymer (POP-4) pouch.
2. Replace polymer (POP-4) pouch.
    a. In Maintenance Wizards screen, select **Replenish Polymer** and follow prompts.
3. Replace Anode Buffer Container (ABC) and Cathode Buffer Container (CBC)
    a. Allow refrigerated buffers to equilibrate to room temperature prior to first use. Do not remove seal.
    b. 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.
    c. Verify that the buffer is at the fill line, remove seal and place the ABC into the Anode end of the instrument.
    d. Tilt the CBC back and forth gently to ensure that the buffer is distributed evenly across the container and that the buffer is at or above the fill line.
    e. Remove the seal from the CBC, wipe off any excess buffer and place appropriate septa on both sides of the CBC.
    f. Install the CBC on the autosampler. The CBC will click into the autosampler as the tabs are snapped into place.
    g. Close the instrument door and click Refresh on the Dashboard to update status after changing the buffers.

III. Data Maintenance for the 3130xl and 3500xL

3130xl	**3500xL**
1. Maintenance of the storage databases used by the Data Collection software. a. Open the appropriate results group folder and create a new backup folder using the naming convention, CE #XX_Backup_MMDDYY. b. Move all plate folders into the newly created backup folder and then copy it to the appropriate CE Backup folder on the network.	1. Maintenance of the storage databases used by the Data Collection software. a. Open the appropriate results group folder and create a new backup folder using the naming convention, CE #XX_Backup_MMDDYY. b. Move all plate folders into the newly created backup folder and then copy it to the appropriate CE Backup folder on the network.
2. Delete records from the database a. From the navigation pane, select **Database Manager** and **Cleanup Processed Plates**. b. Allow the software the appropriate amount of time to delete the associated records and close the dialog box once	2. Archive records from the database a. From the navigation pane, select **Archive** b. When prompted, enter the desired date range to be archived c. Specify the storage location to save the archive file d. Move the archive folder to the appropriate

complete. 3. Defragment the data storage hard drive using the disk defragmenter in Windows system tools to defragment the (E:) drive.	CE Backup folder on the network 3. Purge the previously archived records a. From the navigation pane, select Purge b. When prompted, enter the same date range as the Archive 4. Defragment the data storage hard drive using the disk defragmenter in Windows system tools to defragment the (E:) drive.

IV. 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. Generally, the array on the 3500xl should be changed after 160 injections. Be careful not to leave fingerprints on the array detection window.

3130xl	**3500xL**
1. From the toolbar select the **Install Array Wizard**. 2. Install the array as instructed by the wizard. a. Ensure the proper type (16-capillary) and length (36 cm) is entered in the array information fields. 3. 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.	1. From the Maintenance Wizards screen, click **Install Capillary Array** 2. Install the array as instructed by the wizard. a. Ensure the proper type (24-capillary) and length (36 cm) is entered in the array information fields. 3. 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.

A spatial calibration must be performed whenever a new array is installed. For a 3500xl, spatial calibration must be performed whenever the detection cell window is opened.

3130xl	**3500xL**
1. Select **Spatial Run Scheduler** in the navigation pane. 2. Select **SpatialFill_1**. **SpatialNoFill_1** can be selected if there is no need to fill the array with fresh polymer. 3. Click the **Start** button to initiate the spatial calibration. 4. Select **Accept** to accept the spatial calibration if the following criteria are met: a. Peaks of the spatial calibration are approximately the same height. b. An orange cross appears at the top (apex)	1. Select **Maintenance** in the navigation pane then select **Spatial Calibration** 2. Select **Fill** to fill the array with polymer before starting the calibration 3. Select **Perform QC Checks** 4. Click **Start Calibration** button to initiate the spatial calibration. 5. Select **Accept Results** to accept the spatial calibration if the following criteria are met: a. Peaks of the spatial calibration are approximately the same height.

of each peak in the profile. c. No irregular peaks are contained in the profile. d. RFU values for the peaks are greater than 2,000. e. The values for the Left Spacing and Right Spacing columns are 13-16 pixels. f. 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. The spatial calibration may be repeated as necessary.	b. One marker(a cross) appears at the top (apex) of each peak in the profile. c. No irregular peaks are contained in the profile d. RFU values for the peaks are greater than 3000 for a 24-capillary 3500xl array. e. Uniformity or peak height similarity values are 0.2 f. The values for the Capillary spacing are 2 pixels

V. Spectral Calibration

A spectral plate may be reinjected or used for multiple instruments, of the same type, within a 24 hour period. A spectral calibration is generally run as needed for a 3500xL (e.g., decrease in spectral separation, new dye set, optical adjustment). A spectral calibration is required after changing the capillary array on a 3500xl.

3130xl	**3500xL**
1. Combine 195 µL of formamide with 5 µL of DS-33 Matrix Standard. 2. Dispense 10 µL of solution into the first two columns (wells A1-H1 and A2-H2). 3. Spin down and denature plate on thermal cycler then place on instrument. 4. Click **Plate Manager** in the navigation pane. Note: A previously created spectral plate can be duplicated by highlighting a plate in the Plate Manager window and clicking "Duplicate". This method will only require a new plate name be entered while retaining all the previously entered information. 5. Select **New** and the **New Plate Dialog** dialog box will open, fill out fields: a. Name: Use the naming convention CE#XX_Spectral_MMDDYY. b. Select **Spectral Calibration** from the **Application** drop down menu. c. Complete the remaining fields and select **OK**. This will open the "Spectral Calibration Plate Editor" window.	1. Combine 294 µL of formamide with 6 µL of DS-36 Matrix Standard (J6 Dye Set). 2. Dispense 10 µL of solution into the first three columns (wells A1-H1, A2-H2, & A3-H3) 3. Spin down and denature plate on thermal cycler then place on instrument 4. Access the Spectral Calibration screen, Select **Maintenance,** then select **Spectral Calibration** in the Navigation pane 5. Select number of wells on the plate (e.g., 96 well plate) and specify plate position on instrument 6. Select the chemistry standard and dye set for the calibration plate 7. Select **Allow Borrowing.** 8. 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.

6. Create sample sheet. a. Fill out the **Sample Name** fields to mirror the plate layout. b. Select **Spectral_G5** from the drop down in the **Instrument Protocol 1** field. c. Press **OK** to save plate sample sheet. 7. Select **Run Scheduler** from the navigation pane a. Search the plate name or select find all and click on the plate to be run in order to highlight it within the list. b. Click **Link** to associate the sample sheet to the plates on the instrument. 8. Click the green arrow to start processing the spectral plate. 9. The Data Collection software indicates the pass/fail status of each capillary. Review the spectral profile and raw data of each passing capillary. It is recommended that < 3 capillaries fail and no more than 2 in a row. The spectral plate may be reinjected if necessary. The Data Collection software automatically applies a saved spectral and no further action is required by the user.	1. All capillaries have to meet the spectral Quality Value and Condition Number limits. 2. The passing Quality Value for J6 Dye Set is a minimum of 0.95. 3. The passing Condition Number value is a maximum of 8.0 for J6 Dye Set. 4. ≤ 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.**

C. Performance Verification of the 3130xl Genetic Analyzer and the 3500xL Genetic Analyzer

The performance verification of the 3130xl should be assessed with positive control DNA 9947A (0.10 ng/µL) amplified with Identifiler® Plus. The performance verification of the 3500xL should be assessed with positive control DNA 007 (0.10 ng/µL) amplified with Globalfiler.

3130xl	**3500xl**
1. Prepare a CE daughter plate for a single (16-capillary) injection typically including 14 replicates of amplified 9947A sample and 2 allelic ladders. A single plate may be used to evaluate multiple 3130xl instruments. 2. Run the plate on the appropriate 3130xl instrument(s) and evaluate the data. 3. A genetic analyzer is deemed suitable for casework analysis if:	1. Prepare a CE daughter plate for a single (24-capillary) injection typically including 21 replicates of amplified 007 sample and 3 allelic ladders. A single plate may be used to evaluate multiple 3500xL instruments. 2. Run the plate on the appropriate 3500xL instrument(s) and evaluate the data. 3. A genetic analyzer is deemed suitable for casework analysis if:

a. Correct and interpretable typing results are obtained for all successful injections of the positive amplification control DNA
b. No allelic peaks, other than those attributable to the positive amplification control, are detected
c. An appropriate sensitivity of detection is achieved.
   i. The sensitivity of detection is generally acceptable when the average peak height of each locus is between 850-1800 RFU. These values represent the lowest (Amelogenin) and highest (TH01) average values observed at a locus during the establishment of the 3130xl sensitivity of detection relative to the Match Interpretation Threshold as part of the Identifiler® Plus Amplification Kit at 27 cycles validation. Minor differences in average RFU values relative to the targeted sensitivity may be acceptable.
   ii. At the direction of the Technical Leader, the injection voltage of an individual 3130xl may be adjusted to maintain the instrument's sensitivity of detection. Generally, replicates of amplified control DNA 9947A are injected at the voltage that is currently in use, as well as higher and lower voltages, to determine the average sensitivity (per locus) under each injection condition. These averages are compared to the targeted RFU values.

4. If the performance verification of the 3130xl does not meet the above criteria, the performance verification will be repeated. If the results are still deemed unsuitable, the Technical Leader will be consulted.

a. Correct and interpretable typing results are obtained for the positive amplification control DNA or ladder in at least one injection for each capillary.
b. No allelic peaks, other than those attributable to the positive amplification control, are detected
c. An appropriate sensitivity of detection is achieved.
   i. The sensitivity of detection is generally acceptable when the average peak height of each locus is between 1900-6800 RFU. These values represent the lowest (Th01) and highest (D8S1179) average values observed at a locus during the establishment of the positive control QC parameters during validation. Minor differences in average RFU values relative to the targeted sensitivity may be acceptable.
   ii. The sensitivity of detection is generally acceptable when the average peak height for the 11 GS600 LIZ peaks used for normalization is between 1700-5100 RFU for all the successful injections.

4. If the performance verification of the 3500xL does not meet the above criteria, the performance verification will be repeated. If the results are still deemed unsuitable, the Technical Leader will be consulted.

The following are the 11 peaks used for normalization:

200	220	240	260	280	300	314	320	340	360	400

D. Qualification of GS600 LIZ v2 Internal Size Standard

1. Prepare a CE daughter plate for the 3500xL using the instructions in section C and the new lot of GS600 LIZ.
2. Run the plate on an appropriate 3500xL instrument.
3. Analyze the data without normalization.
4. The sensitivity of the new lot will be accepted if the average allelic peak heights for the 11 GS600 LIZ peaks used for normalization (listed above) is between 1700-5100 RFU for all the successful injections (with a minimum of 16 of the 21 wells).
5. 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.

E. GS600 LIZ v2 Sensitivity Evaluation

1. Prepare a CE daughter plate for the 3500xL using the instructions in section C. A single plate or multiple plates 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 C may also be used for this evaluation.
2. Analyze the data without normalization.
3. The sensitivity of each instrument will be accepted if the average peak heights for the 11 GS600 LIZ peaks used for normalization (listed above) is between is between 1700-5100 RFU for all the successful injections.
4. 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.