Capillary Electrophoresis of Nuclear DNA

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1 INTRODUCTION

These procedures describe the separation by capillary electrophoresis (CE) of amplified nuclear DNA (nDNA) products from evidence and reference samples.

2 SCOPE

These procedures apply to DNA personnel who prepare CE daughter plates, run plates on a genetic analyzer, evaluate data, and/or perform the related quality control (QC) procedures.

3 EQUIPMENT

3.1 Equipment/Materials

- STACS™ Casework Software (STACS), Sample Tracking and Control Solutions [STACS DNA Inc.] part of InVita Healthcare Technologies, version 5.0 or higher
- Agilent Technologies "Bravo" Liquid Handler
    - VWorks Software, version 13 or higher
- Liquid handler tips, Agilent Technologies or equivalent
- Thermal Cycler, ProFlex™ PCR System
- 3500xL Genetic Analyzer, Applied Biosystems
    - Data Collection Software, version 4.0 or higher
    - 24-Capillary (24-cap) Array, 36 cm
- GeneMapperID-X, version 1.6 or higher
- General laboratory supplies (e.g., pipettes, tubes, vortex, centrifuge)
- 96-well plate, Applied Biosystems MicroAmp® optical or equivalent
- Reagent troughs/reservoirs
- Clear and foil plate seals
- Thermal Microplate Sealer
- Plate septa, Applied Biosystems or equivalent
- Plate base and retainer, Applied Biosystems or equivalent

3.2 Reagents

- Hi-Di™ Formamide, Applied Biosystems or equivalent
- For the 3500xL Instrument:
    - Anode Buffer Container (ABC) 3500 series, Applied Biosystems
    - Cathode Buffer Container (CBC) 3500 series, Applied Biosystems
    - Performance Optimized Polymer 4 (POP-4) Pouch 3500 series, Applied Biosystems
    - Conditioning Reagent, Applied Biosystems
- For Globalfiler®:
    - Globalfiler® (GF) Ladder
    - GeneScan™-600 LIZ™ Version 2.0 internal size standard, Applied Biosystems
- For Y23:
    - PowerPlex® Y23 Allelic Ladder Mix

    - WEN Internal Lane Standard (ILS) 500 Y23
- For QC:
    - For GF: DS-36 Matrix Standard Kit (Dye Set J6), Applied Biosystems
    - For Y23: PowerPlex® 5C Matrix Standard (5C Matrix Mix and Matrix Dilution Buffer), Promega
- Water, molecular grade or equivalent
- Purified Water or equivalent, available at laboratory sinks

4 STANDARDS AND CONTROLS

The positive and negative amplification controls must be subjected to CE analysis with the associated batch of amplified samples. See section 5.4 for guidance for reinjections and repreparations of the CE daughter plate.

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

Refer to the appropriate nuclear DNA interpretation procedure (i.e., BIO-570 or BIO-572) for interpretation of these controls.

5 PROCEDURE

Refer to DNA Introduction Procedure (i.e., BIO-100) and follow applicable general precautions and cleaning instructions.

For water that will come into contact with the DNA samples (e.g., CE reservoirs), molecular 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, as necessary.

5.1 Internal Size Standard (ISS) Mixture

If needed, prepare the ISS Mixture (i.e., LIZ Formamide or WEN Formamide) in the ratio listed below. Record the preparation in STACS. The ISS mixture should be used within one day of preparation.

	GlobalFiler	**Y23**
Size Standard	GS-600 LIZv2	WEN ILS 500
Hi-Di Formamide:ISS ratio	24:1	19:1

Example Volume Calculations:

LIZ Formamide (for 5 mL) = 4.8 mL Hi-Di Formamide + 200 µL GS-600 LIZv2

WEN Formamide (per well) = 9.5 µL Hi-DI Formamide + 0.5 µL WEN

5.2 CE Daughter Plate Preparation

The amplification plate should be quick spun prior to removing the seal. Ensure the ISS mixture is vortexed prior to use.

To prevent introducing bubbles into the capillaries on the CE instrument, each set of wells for one injection (i.e., 24 wells on 3500xL) must be filled even if not all wells in that set will be receiving samples. Wells not receiving sample can be filled with Formamide without LIZ or WEN.

5.2.1 Robotic Globalfiler daughter plate prep

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.*

1.	Ensure the following items are provisioned to the robot (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 side. • Position 9: A reagent trough with wells containing sufficient volume of LIZ Formamide.

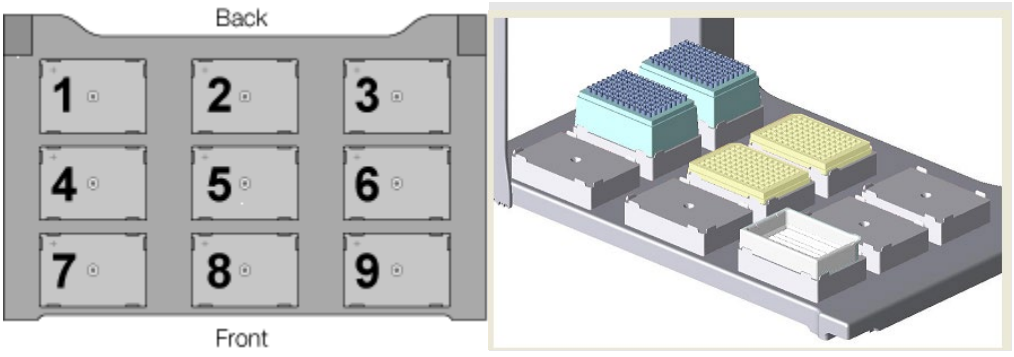

Figure 1 – Agilent Bravo Liquid Handler Deck Positions

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.

1A.	Initiate the appropriate daughter plate protocol in the VWorks Software and follow the prompts. Ensure tip positioning for the new and used tip boxes is selected correctly.

1B.	When the run is complete, ensure all appropriate wells contain LIZ Formamide.

Continue at section 5.2.4.

5.2.2 Semi-automated Y23 daughter plate prep

1.	Manually add to the CE daughter plate: • 10 µL WEN Formamide to each well that will contain sample, ladder or control. • 10 µL Formamide to any remaining empty wells of an injection set (i.e., 24 wells on 3500xL).

2.	Complete the CE daughter plate prep on the Bravo. Ensure the following items are provisioned to the robot (see Figure 1): • Position 1: A box of Agilent disposable tips. At least one set of 96 tips must be present in the box. • Position 2: The amplification plate, unsealed and in a plate holder. • Position 4: A tip box for used tips. This box must have at least one empty quadrant. • Position 5: The CE daughter plate with WEN Formamide and/or Formamide and a CE daughter plate barcode on the side, also in a plate holder.

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

Continue at section 5.2.4.

5.2.3 Manual daughter plate prep

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

1.	**For GlobalFiler on 3500xL:** • Add 10 µL LIZ Formamide to each well in the CE daughter plate that will contain sample, ladder or control. • Add 10 µL LIZ Formamide (or Formamide) to any remaining empty wells of an injection set (i.e., 24 wells on 3500xL). • Add 1 µL of amplified product from the amplification plate to the corresponding wells on the CE daughter plate.	**For Y23 on 3500xL:** • Add 10 µL WEN Formamide to each well in the CE daughter plate that will contain sample, ladder or control. • Add 10 µL Formamide to any remaining empty wells of an injection set (i.e., 24 wells on 3500xL). • Add 1 µL of amplified product from the amplification plate to the corresponding wells on the CE daughter plate.

5.2.4 Completing CE Daughter Plate Prep

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

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

3.	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 refrigerated (GF) or frozen (Y23) until it is appropriate to discard. The seal can be applied with the Thermal Microplate Sealer or, if needed, manually. Ensure that the edges of each well are sealed.

Remaining LIZ Formamide can 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.

5.3 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.

1.	*At any point prior to scheduling the run:* • Import the sample sheet generated in STACS into the Data Collection software to create the plate record. • Ensure 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.

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 in the correct orientation. 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.

3.	*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.	Ensure the instrument doors are closed and start the run.

The 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.

5.4 Data Review

1.	• After the CE run, move the data generated by the CE (i.e., .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, a ladder(s) should also be reinjected. A reduced injection time may be used if data is oversaturated or if excessive artifacts could interfere with interpretation.

An examiner will review the CE data for compliance with the requirements in the applicable DNA interpretation procedure and queue any samples that need to be rerun (aka reprep) or reamped. The *GlobalFiler Re-Amp Form* (BIO-002), the *Y Amp/Re-Amp Form* (BIO-003), or the *Re-Prep Form* (BIO-004), as applicable, will be used to capture notes in addition to those captured in STACS pertaining to the reamp or reprep of a sample(s).

For samples that require a new CE daughter plate set up (aka reprep), repeat this procedure for at least the applicable samples. A new sample sheet is generated from STACS for the reprep. At minimum, the positive amplification control and ladder(s) need to be included and injected. The negative amplification control and RBs are generally not included unless they require re-prep or the entire plate requires re-prep.

6 LIMITATIONS

Not applicable.

7 SAFETY

- All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material.
- Refer to the FBI Laboratory Safety Manual for information on personal protection, the 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. 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.

8 REFERENCES

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

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.

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

Promega. PowerPlex® Y23 System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual

9 REVISION HISTORY

Revision	Issued	Changes
00	09/01/2022	Reformatted DNA 214-8 into new template and assigned new Doc ID. Removed Identifiler Plus from procedure with exception of QC use. Added parameters for ID+ amplification to QC Appendix.
01	09/04/2024	Revised to add Y23 on the 3500 procedures and remove Yfiler and 3130 requirements. Added reference to new Forms BIO-002,3&4.

10 APPENDIX A: QUALITY CONTROL PROCEDURES

10.1 Critical Reagents

Refer to the DNA procedure for reagent purchasing, preparation and records (i.e., BIO-103) for additional requirements.

The amplification kit ladders are evaluated with the associated amplification kit. Refer to the nuclear DNA amplification procedure (i.e., BIO-530) for instructions.

10.2 Instruments

Refer to the DNA procedure for instrument calibration and maintenance (i.e., BIO-104) for minimum frequency and additional requirements.

Refer to the nuclear DNA amplification procedure (i.e., BIO-530) for QC procedures for the thermal cyclers.

10.2.1 Performance Verification (PV) of the Agilent Bravo Liquid Handler

A. 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.

B. 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.

C. The results must be within the tolerance limits set 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).

D. 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.

10.2.2 General Maintenance of the Genetic Analyzers

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

General Maintenance	3500xL Recommended Interval
Replace Polymer	14 days (or as needed)
Replace Buffer (Anode and Cathode)	14 days (or as needed)
Install New Array	As needed
Flush pump chamber and channels	Weekly
Flush pump trap	Monthly
Spatial Calibration	With array change, if detection cell window is opened, or as needed
Spectral Calibration	With array change or as needed and Quarterly for Y23
CE Sensitivity Evaluation	Semiannually or after optical adjustment

10.2.2.1 General Maintenance of the 3500xL Genetic Analyzer

A. Flush the pump chamber and channels
    1. In Maintenance Wizards screen, run the appropriate wizard (i.e., Wash Pump and Channel or Change Polymer Type) and follow prompts.
B. Replace Anode Buffer Container (ABC) and Cathode Buffer Container (CBC)
    1. Allow refrigerated buffers to equilibrate to room temperature prior to first use. Do not remove seal.
    2. 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.
    3. Verify that the buffer is at the fill line, remove seal and place the ABC into the Anode end of the instrument.
    4. 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.
    5. Remove the seal from the CBC, wipe off any excess buffer and place appropriate septa on both sides of the CBC.
    6. Install the CBC on the autosampler. The CBC will click into the autosampler as the tabs are snapped into place.
    7. Close the instrument door and click Refresh on the Dashboard to update status after changing the buffers.

10.2.2.2 Array Change

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. Be careful not to leave fingerprints on the array detection window.

1. From the Maintenance Wizards screen, click **Install Capillary Array**
2. Install the array as instructed by the wizard.

i. 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. A spectral calibration is required after changing the capillary array on a 3500xL.

10.2.2.3 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.

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:
    i. Peaks of the spatial calibration are approximately the same height.
    ii. One marker (a cross) appears at the top (apex) of each peak in the profile.
    iii. No irregular peaks are contained in the profile
    iv. RFU values for the peaks are greater than 3000 for a 24-capillary 3500xL array.
    v. Uniformity or peak height similarity values are 0.2
    vi. The values for the Capillary spacing are 2 pixels

10.2.2.4 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). Each spectral calibration is required after changing the capillary array on a 3500xL. A spectral calibration is required for Y23 quarterly.

Globalfiler	**Y23**
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 the plate on a thermal cycler, then place on instrument 4. Access the Spectral Calibration screen, select	1. Add 10 µL of 5C Matrix Mix (G5 Dye Set) to one tube of Matrix Dilution Buffer [This diluted 5C Matrix Standard can be stored refrigerated and used for up to one week.] 2. Combine 10 µL of diluted 5C Matrix Standard with 500 µL of formamide. 3. Dispense 15 µL of solution into the first three columns (wells A1-H1, A2-H2, & A3-H3).

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 "Matrix" chemistry standard and "Any6Dye VB" 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. 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.**	4. Spin down plate and place on instrument. **Do not** denature. C. 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 "Matrix" chemistry standard and "Promega G5" 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. 1. All capillaries have to meet the spectral Quality Value and Condition Number limits. 2. The passing Quality Value for G5 Dye Set is a minimum of 0.99. 3. The passing Condition Number value is a maximum of 13.5 for G5 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.**

10.2.3 Performance Verification (PV) of the Genetic Analyzers

The PV of the 3500xL is assessed with positive control DNA 007 (0.10 ng/µL) amplified with Globalfiler and 2800M (at the appropriate concentration determined during kit QC) amplified with Y23 and the corresponding ladders for each kit.

A. Prepare a CE daughter plate with a single (24 capillary) injection of samples for each kit. A single plate may be prepared with both sets of samples and the plate(s) can be used to evaluate multiple 3500xL instruments.
    1. For Globalfiler: LIZ Formamide with 21 replicates of amplified 007 and 3 allelic ladders.
    2. For Y23: WEN Formamide with 8 replicates of amplified 2800M (at the appropriate concentration for the kit lot) and 16 allelic ladders.
B. Run the plate(s) on the 3500xL instrument(s) and evaluate the data.
C. A genetic analyzer is deemed suitable for casework analysis if:
    1. Correct and interpretable typing results are obtained for the positive control DNA or ladder in at least one injection for each capillary.
    2. No allelic peaks, other than those attributable to the positive amplification control, are detected
    3. An appropriate sensitivity of detection is achieved. The sensitivity of detection is generally acceptable when:
        i. the average peak heights of each locus of the 007 samples are between 1900-6800 RFU using data analyzed with normalization. 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 average peak height for the 11 GS600 LIZ peaks used for normalization (see 10.3) is between 1700-5100 RFU for all the successful injections using data analyzed without normalization
        iii. the 2800M samples produce an average peak height of ~9000 RFU.
D. 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.

10.2.4 <u>CE Sensitivity Evaluation</u>

A. Prepare a CE daughter plate for the 3500xL using the instructions in section 10.2.3. 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 10.2.3 may also be used for this evaluation.
B. Analyze the data without normalization.
C. The sensitivity of each instrument will be accepted if:
    1. For Globalfiler: the average peak heights for the 11 GS600 LIZ peaks used for normalization (see 10.3) is between 1700-5100 RFU for all the successful injections.
    2. For Y23: the 2800M samples (at the appropriate concentration determined during kit QC) produce an average peak height of ~9000 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.

10.3 GS600 LIZ used for normalization

The following are the 11 GS600 LIZ peaks used for normalization:

200	220	240	260	280	300	314	320	340	360	400

10.3.1 Qualification of GS600 LIZ v2 Internal Size Standard

WEN for Y23 kit is evaluated during the amp kit QC.

A. Prepare a CE daughter plate for the 3500xL using the instructions in section 10.2.3 and 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 allelic peak heights for the 11 GS600 LIZ peaks used for normalization is between 1700-5100 RFU for all the successful injections (with a minimum of 16 of the 21 wells).
    1. 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.