



TECHNICAL MANUAL

PowerPlex[®] 4C Matrix Standard

Instructions for Use of Product
DG4800

PowerPlex® 4C Matrix Standard

All technical literature is available at: www.promega.com/protocols/
 Visit the web site to verify that you are using the most current version of this Technical Bulletin.
 E-mail Promega Technical Services if you have questions on use of this system: genetic@promega.com

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1. Description

Proper generation of a spectral calibration file is critical to evaluate multicolor STR data on multicapillary electrophoresis instruments. The PowerPlex® 4C Matrix Standard consists of DNA fragments labeled with four different fluorescent dyes (fluorescein, JOE, TMR and CXR) in one tube. The spectral calibration is performed using the F dye set. Once generated, the spectral calibration file is applied during sample detection to calculate the spectral overlap between the four different dye colors and separate the raw fluorescent signals into individual dye signals. The PowerPlex® 4C Matrix Standard can be used with any of the 4-dye Promega STR amplification systems.

A spectral calibration must be generated for each individual instrument. A new matrix should be run after major maintenance on the system, such as changing the laser, calibrating or replacing the CCD camera or changing the polymer type or capillary array. We also recommend that you generate a new matrix after the instrument is moved to a new location. In some instances, a software upgrade may necessitate generation of a new matrix. Individual laboratories should determine the frequency of matrix generation.

Protocols to operate the fluorescence-detection instruments should be obtained from the manufacturer.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PowerPlex® 4C Matrix Standard	5 preps	DG4800

Not For Medical Diagnostic Use. Includes:

- 150µl 4C Matrix Mix
- 5 × 200µl Matrix Dilution Buffer


Storage Conditions: Upon receipt, store all components at –30°C to –10°C in a nonfrost-free freezer, protected from light. Do not store reagents in the freezer door, where the temperature can fluctuate. After the first use, store the PowerPlex® 4C Matrix Standard components at 2–10°C, protected from light. Do not refreeze the PowerPlex® 4C Matrix Standard components. We strongly recommend that you store the PowerPlex® 4C Matrix Standard with the post-amplification reagents. The PowerPlex® 4C Matrix Standard is light-sensitive; dilute the 4C Matrix Mix in the Matrix Dilution Buffer in the provided amber tube. Store the diluted 4C Matrix Mix at 2–10°C for up to 1 week.


3. Instrument Preparation and Spectral Calibration Using the Applied Biosystems® 3500 and 3500xL Genetic Analyzers

Materials to Be Supplied by the User

- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3500/3500xL capillary array, 36cm
- performance optimized polymer 4 (POP-4®) for the 3500 or 3500xL
- anode buffer container with 1X running buffer
- cathode buffer container with 1X running buffer
- MicroAmp® optical 96-well plate and septa
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

For additional information on performing spectral calibration, refer to the *Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide*.

 The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C can cause breakdown of formamide. Poor-quality formamide can contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

 Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

3.A. Matrix Sample Preparation

1. At the first use, thaw the 4C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at 2–10°C, protected from light.
2. Vortex the 4C Matrix Mix for 10–15 seconds prior to use. Add 10µl of 4C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds. Note the date of dilution on the tube.
Note: The diluted 4C Matrix Mix can be stored for up to 1 week at 2–10°C.
3. Add 10µl of the diluted 4C Matrix Mix prepared in Step 2 to 500µl of Hi-Di™ formamide. Vortex for 10–15 seconds.
4. For the Applied Biosystems® 3500xL Genetic Analyzer, 24 wells are used for spectral calibration on 24 capillaries (wells A1 through H3 of a 96-well plate). Add 15µl of 4C Matrix Mix with formamide prepared in Step 3 to each of the 24 wells. After placing the septa on the plate, briefly centrifuge the plate to remove any air bubbles.



Do not heat denature.

For the Applied Biosystems® 3500 Genetic Analyzer, 8 wells are used for spectral calibration on 8 capillaries (wells A1 through H1 of a 96-well plate). Add 15µl of 4C Matrix Mix with formamide prepared in Step 3 to each of the eight wells. After placing the septa on the plate, briefly centrifuge the plate to remove any air bubbles.



Do not heat denature.

5. Place the plate in the 3500 series 96-well standard plate base, and cover with the plate retainer. Do not start the spectral calibration run until the oven is preheated to 60°C.

3.B. Instrument Preparation

We have found that the use of fresh polymer and a new capillary array results in an optimal spectral calibration.

Representative data are shown in Figure 1.

3.B. Instrument Preparation (continued)

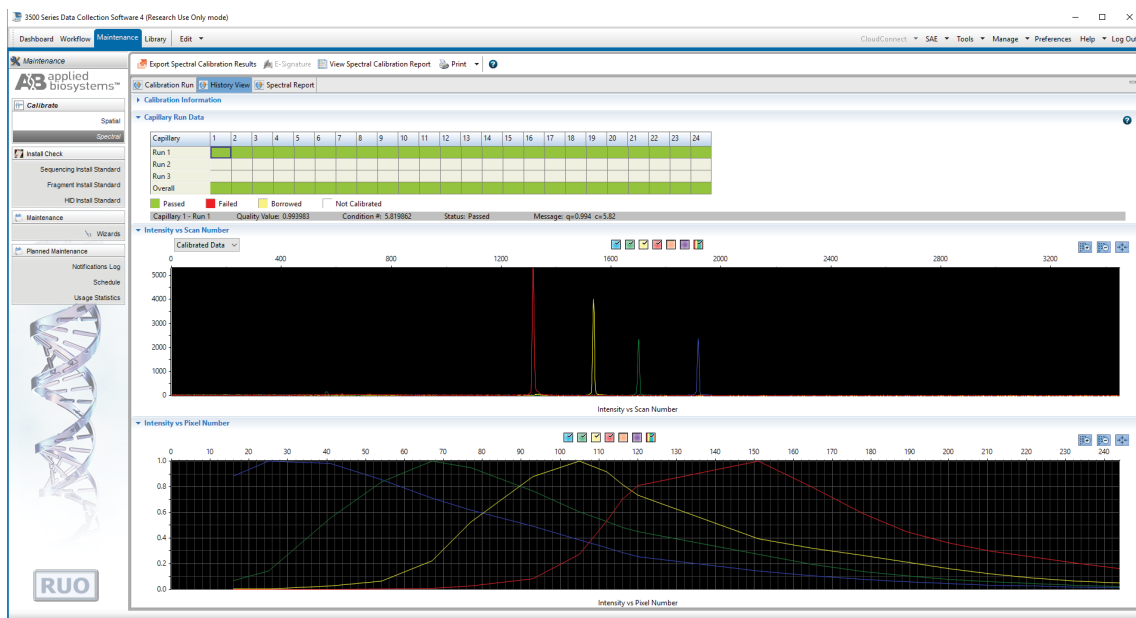


Figure 1. Representative data for the PowerPlex® 4C Matrix Standard on the Applied Biosystems 3500xL Genetic Analyzer using POP-4® polymer and Data Collection Software Version 4.0.

1. Set the oven temperature to 60°C, and then select the **Start Pre-Heat** icon at least 30 minutes prior to the first injection to preheat the oven.
2. To perform a spectral calibration for the Promega 4-dye STR amplification systems, a new dye set should be created. If a new dye set was created previously, proceed to Step 2.c.
 - a. To create this new dye set, navigate to the Library, highlight “Dye Sets” and select **Create**.

- b. The 'Create New Dye' Set window will appear (Figure 2). Name the Dye Set (e.g., Promega F), select **Matrix Standard** for the Chemistry and select **F Template** for the Dye Set Template. Select **Save**.

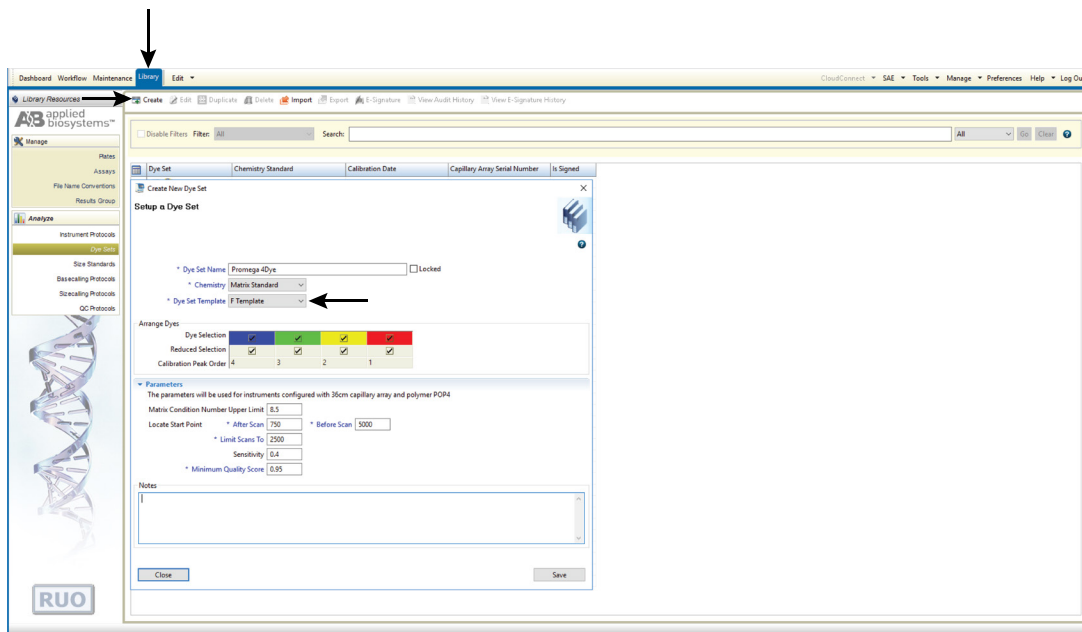


Figure 2. The 'Create New Dye Set' window.

3.B. Instrument Preparation (continued)

- c. To perform the spectral calibration, go to the 'Maintenance' tab, select **Spectral**, and under the 'Calibration Run' tab, choose the appropriate fields: Choose **Matrix Standard** from the Chemistry Standard drop-down menu and the new Promega 4C dye set (e.g., Promega F) created in Step 2.b from the 'Dye Set' drop-down menu (Figure 3).
- d. Select **Start Run**.

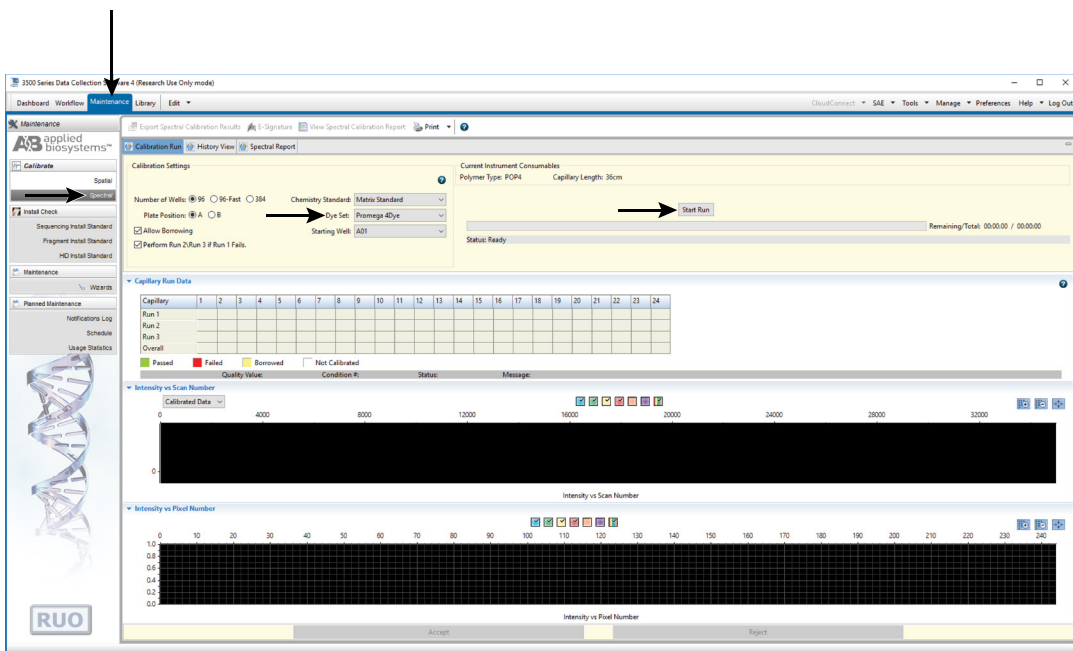


Figure 3. Calibration Run.

- If fewer than the recommended number of capillaries pass, the spectral calibration run may be repeated automatically up to three times. Upon completion of the spectral calibration, check the quality of the spectral in the Capillary Run Data display (Figure 4), and choose either **Accept** or **Reject** (not shown).

Notes:

- Refer to the *3500 Series Data Collection Software HID User Manual* for the criteria recommended when accepting or rejecting a spectral calibration.
- Some Applied Biosystems® 3500 and 3500xL Genetic Analyzers show imbalance in peak heights (e.g., peaks in the red and yellow dye channels are higher than those in the blue and green dye channels). This imbalance should not affect kit performance.

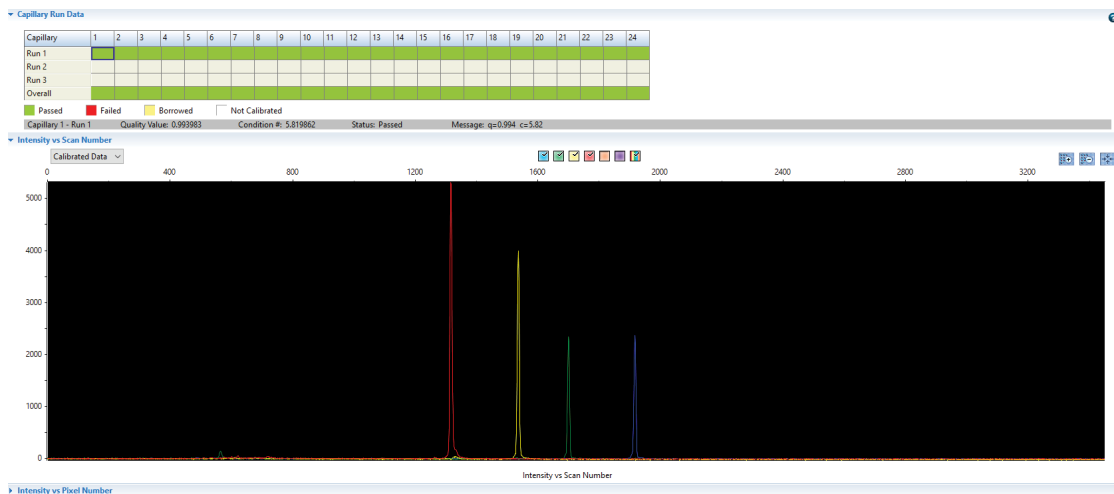



Figure 4. The Capillary Run Data display.


4. Instrument Preparation and Spectral Calibration Using the Applied Biosystems® 3130 and 3130xl Genetic Analyzers with Data Collection Software Version 3.0 and Higher

Materials to Be Supplied by the User

- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3130 capillary array, 36cm
- performance optimized polymer 4 (POP-4® polymer) for the 3130
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate and septa
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)


For additional information on performing spectral calibration, refer to the *Applied Biosystems® 3130/3130xl Genetic Analyzer User Guide*.

 The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C can cause breakdown of formamide. Poor-quality formamide can contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.


 Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

4.A. Matrix Sample Preparation

1. At the first use, thaw the 4C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at 2–10°C, protected from light.
2. Vortex the 4C Matrix Mix for 10–15 seconds prior to use. Add 10µl of 4C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds. Note the date of dilution on the tube.
Note: The diluted 4C Matrix Mix can be stored for up to 1 week at 2–10°C.
3. Add 10µl of the diluted 4C Matrix Mix prepared in Step 2 to 500µl of Hi-Di™ formamide. Vortex for 10–15 seconds.
4. For the Applied Biosystems® 3130xl Genetic Analyzer, 16 wells are used for spectral calibration on 16 capillaries (wells A1 through H2 of a 96-well plate). Add 15µl of 4C Matrix Mix with formamide prepared in Step 3 to each of the 16 wells. After placing the septa on the plate, briefly centrifuge the plate to remove any air bubbles.

 Do not heat denature.

For the Applied Biosystems® 3130 Genetic Analyzer, four wells are used for spectral calibration on four capillaries (wells A1 through D1 of a 96-well plate). Add 15µl of 4C Matrix Mix with formamide prepared in Step 3 to each of the four wells. After placing the septa on the plate, briefly centrifuge the plate to remove any air bubbles.

 Do not heat denature.

5. Place the plate in the 3130 series 96-well standard plate base, and cover with the plate retainer. Do not start the spectral calibration run until the oven is preheated to 60°C.

4.B. Instrument Preparation

We have found that the use of fresh polymer and a new capillary array results in an optimal spectral calibration. Representative data are shown in Figure 5.

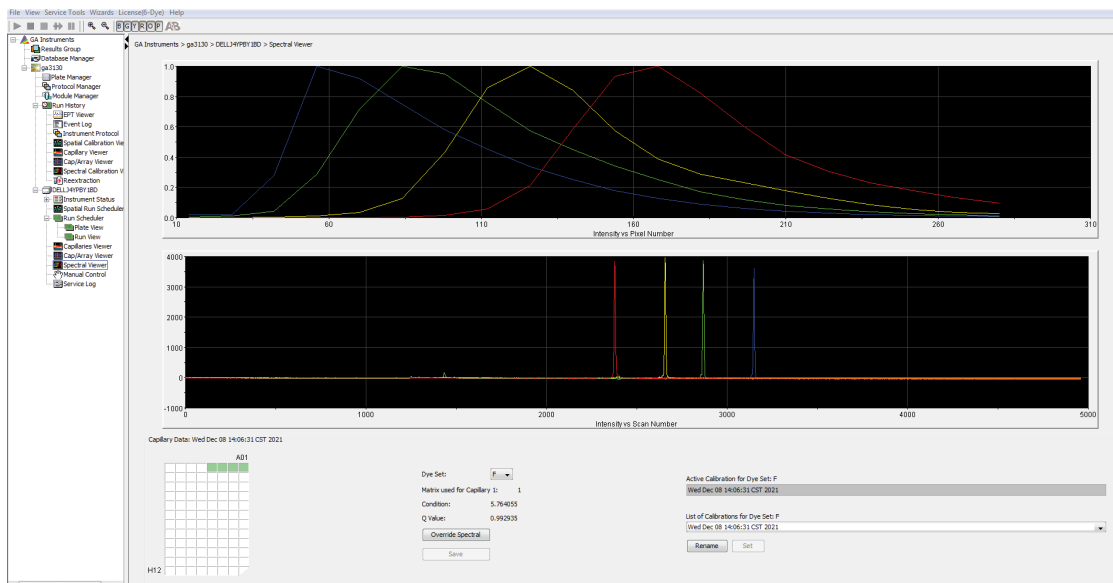


Figure 5. Representative data for the PowerPlex® 4C Matrix Standard on the Applied Biosystems® 3130 Genetic Analyzer using POP-4® polymer and Data Collection Software Version 4.0.

1. Set the oven temperature to 60°C, and preheat the oven for at least 15 minutes prior to the first injection.
2. To perform a spectral calibration for Promega 4-dye STR amplification systems, create a new Run Module and Protocol. If a new Run Module and Protocol were created previously, proceed to Step 3.
 - a. In the Module Manager, select **New**. Select **Spectral** in the “Type” drop-down list, and select **Spect36_POP4** in the Template drop-down list. Confirm or change the following settings:

Injection Voltage: 2kV
 Injection Time: 10 seconds
 Data Delay Time: 100 seconds
 Run Time: 800 seconds

Note: There can be instrument-to-instrument variation in the sensitivity of detection. You may need to adjust injection time or voltage to achieve a passing spectral calibration. Peak heights in the range of 1,000–4,000RFU are ideal. Peak heights above 750RFU and below the saturation point of the instrument are required.

4.B. Instrument Preparation (continued)

- b. Name the Run Module (e.g., Promega F), and select **OK**.
- c. In the Protocol Manager, under Instrument Protocols, select **New**. Type a name for your protocol (e.g., Promega F).
- d. Make the following selections in the Protocol Editor:
 - **Spectral** in the ‘Type’ drop-down list
 - **F** in the ‘Dye Set’ drop-down list
 - **POP4** for the polymer
 - **36** in the ‘Array Length’ drop-down list
 - **Matrix Standard** in the ‘Chemistry’ drop-down list
 - Select the spectral module you created in the previous step in the ‘Run Module’ drop-down list.

Finally, select **Edit Parameters**, and make the following modifications:

- Change the lower condition bound to 4.0, and change the upper condition bound to 12.0.
- Confirm that the Minimum Quality Score (“Q value”) is 0.95.

Select **OK** in the Edit Parameters window, and select **OK** in the Protocol Editor.

Note: The condition number (“C value”) obtained when generating a spectral calibration will vary with the instrument. After obtaining a spectral calibration that performs acceptably, the condition bounds range in the previous step can be narrowed to more critically evaluate C values for subsequent spectral calibrations.

3. In the Plate Manager, create a new plate record as described in the instrument user’s manual. In the dialog box that appears, select **Spectral Calibration** in the Application drop-down list, and select **96-well** as the plate type. Add entries in the owner and operator windows, name the plate and select **OK**.
4. In the Spectral Calibration Plate Editor dialog box, record sample names in the appropriate cells.
5. In the Instrument Protocol column, select the protocol you created in Step 2. Ensure that this information is present for each row that contains a sample name. Select **OK**.
6. Run your plate as described in the instrument user’s manual.
7. Upon completion of the run, check the status of the spectral calibration in the Event Log window. For the Applied Biosystems® 3130xl Genetic Analyzer, we recommend that a minimum of 12 of 16 capillaries pass calibration. For the Applied Biosystems® 3130 Genetic Analyzer, we recommend that a minimum of three of four capillaries pass calibration. If fewer than the recommended numbers of capillaries pass, repeat the spectral calibration.

Note: The same plate of matrix standards can be re-injected up to four times. To re-inject the same matrix standards plate, add an injection by selecting **Plate Manager** and then **Edit**. Select **Edit** again in the top left corner of the window, and then select **Add sample run**.

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. E-mail: genetic@promega.com

5.A. Applied Biosystems® 3500 and 3500xL Genetic Analyzers

Symptoms

Fewer than the recommended number of capillaries passed the spectral calibration

Causes and Comments

Matrix standard was too dilute. Matrix standard that is too dilute will result in low spectral calibration peak heights, which can result in spectral calibration failure. Increase the volume of diluted 4C Matrix Mix added to the Hi-Di™ formamide during sample preparation.

Matrix standard was too concentrated. Matrix standard that is too concentrated can result in spectral calibration failure due to saturated peaks, bleedthrough or oversubtraction in other dye colors. Decrease the volume of diluted 4C Matrix Mix added to the Hi-Di™ formamide during matrix sample preparation.

Poor-quality formamide was used. The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or storage at 4°C can cause breakdown of formamide. Poor-quality formamide can contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity.

Reboot the CE instrument and the instrument's computer.
Repeat the spectral calibration.

Ensure that the oven is preheated to 60°C prior to spectral calibration.

For best spectral calibration results, use fresh polymer, fresh buffer and water, and a capillary array with fewer than 100 injections.

5.B. Applied Biosystems® 3130 and 3130xl Genetic Analyzers

Symptoms

Fewer than the recommended number of capillaries passed spectral calibration

Causes and Comments

Peak heights for the matrix standard were too low. Matrix peak heights must be a minimum of 750RFU to pass spectral calibration. Increase the injection time or voltage. Alternatively, increase the volume of diluted 4C Matrix Mix added to the Hi-Di™ formamide during matrix sample preparation.

Peak heights for the matrix standard were too high (>6,000RFU). Decrease the injection time or voltage. Alternatively, decrease the volume of diluted 4C Matrix Mix added to the Hi-Di™ formamide during matrix sample preparation.

Peaks in multiple dye channels were detected prior to the red peak. Clean the instrument, and use fresh polymer, fresh buffer and water, and new septa.

Poor-quality formamide was used. The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or storage at 4°C can cause breakdown of formamide. Poor-quality formamide can contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity.

For best spectral calibration results, use a fresh bottle of polymer, fresh buffer and water, new septa and a capillary array with fewer than 100 injections.

Check the Event Log in the Instrument Status screen for a reason for the failure (e.g., bad dye order or insufficient number of dye spectra). Check the raw data view of the failed capillaries in the Spectral Viewer. Look for signs of low signal, high signal or baseline noise before the matrix peaks. Adjust the run conditions as described above, and re-inject the matrix standard.

If the cause for failure is unclear after viewing the Event Log and Spectral Viewer, monitoring fragment migration in the Capillaries Viewer during spectral calibration run can provide information that will be useful for troubleshooting purposes. Re-inject the matrix standard, and monitor the Capillaries Viewer during the run. Note any unusual peak formations or extremely low or high peak heights. Based on the information obtained while watching the Capillaries Viewer, you may need to adjust the run conditions.

6. Summary of Changes

The following changes were made to the 12/21 revision of this document:

1. Updated Sections 3.B, 4 and 5.B.
2. Removed Related Products section.
3. Updated cover page.

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