

TECHNICAL MANUAL

# *GenePrint® 5C Matrix Standard*

Instructions for Use of Product  
**B1930**



# GenePrint® 5C Matrix Standard

All technical literature is available at: [www.promega.com/protocols/](http://www.promega.com/protocols/)  
Visit the web site to verify that you are using the most current version of this Technical Manual.  
E-mail Promega Technical Services if you have questions on use of this system: [genetic@promega.com](mailto: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 *GenePrint® 5C Matrix Standard*<sup>(a)</sup> consists of DNA fragments labeled with five different fluorescent dyes (fluorescein, JOE, TMR-ET, CXR-ET and WEN) in one tube. The spectral calibration is performed using the G5 dye set. Once generated, the spectral calibration file is applied during sample detection to calculate the spectral overlap and separate the raw fluorescent signals into individual dye signals. The *GenePrint® 5C Matrix Standard* can be used with any of the 5-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.#
<b><i>GenePrint® 5C Matrix Standard</i></b>	<b>5 preps</b>	<b>B1930</b>

This product is intended for research use only and is not intended for use in diagnostic, forensic or paternity procedures. Includes:

- 150µl 5C 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 *GenePrint® 5C Matrix Standard* components at 2–10°C, protected from light. We strongly recommend that you store the *GenePrint® 5C Matrix Standard* with the post-amplification reagents. The *GenePrint® 5C Matrix Standard* is light-sensitive; dilute the 5C Matrix Mix in Matrix Dilution Buffer in the provided amber tube. Store the diluted 5C Matrix Mix at 2–10°C for up to 1 week.



Do not refreeze the *GenePrint® 5C Matrix Standard* components.

### 3. Instrument Preparation and Spectral Calibration Using POP-7™ Polymer in 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, 50cm
- performance optimized polymer 7 (POP-7™) for the 3500 or 3500xL
- anode buffer container with 1X running buffer
- cathode buffer container with 1X running buffer
- MicroAmp® optical 96-well plate
- 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 5C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at 2–10°C, protected from light.
2. Vortex the 5C Matrix Mix for 10–15 seconds prior to use. Add 10µl of 5C 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 5C Matrix Mix can be stored for up to 1 week at 2–10°C.
3. Add 10µl of the diluted 5C 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 5C 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 5C 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.

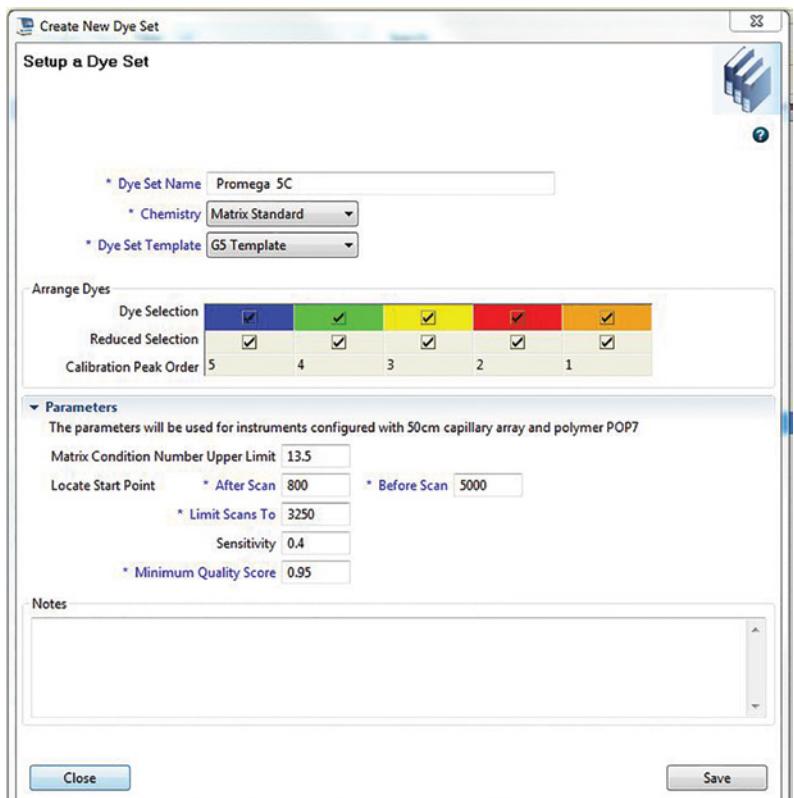
**!** Do not heat denature.

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

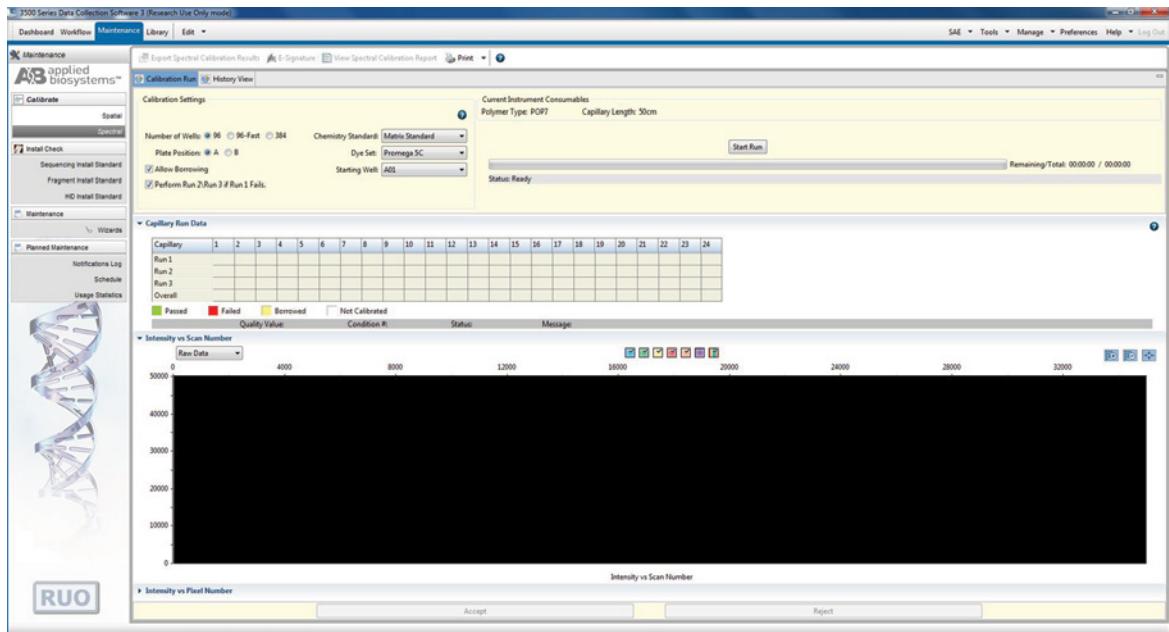
We have found that the use of fresh polymer and a new capillary array results in an optimal spectral calibration.

1. Preheat the oven to 60°C at least 30 minutes prior to the first injection.
2. To create a new dye set, navigate to the Library, highlight “Dye Sets” and select “Create”. The Setup a Dye Set tab will appear (Figure 1). Name the Dye Set (e.g., Promega 5C), select “Matrix Standard” for the Chemistry and select “G5 Template” for the Dye Set Template. Under Parameters, change the After Scan number to 800 from the default number of 500. Select “Save”. Select the “Edit Parameters” option, and make sure the minimum Q value is set to 0.95.



**Figure 1. The Setup a Dye Set tab.**

3. 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 drop-down menu, and then choose the Promega 5C dye set created in Step 3. See Figure 2.

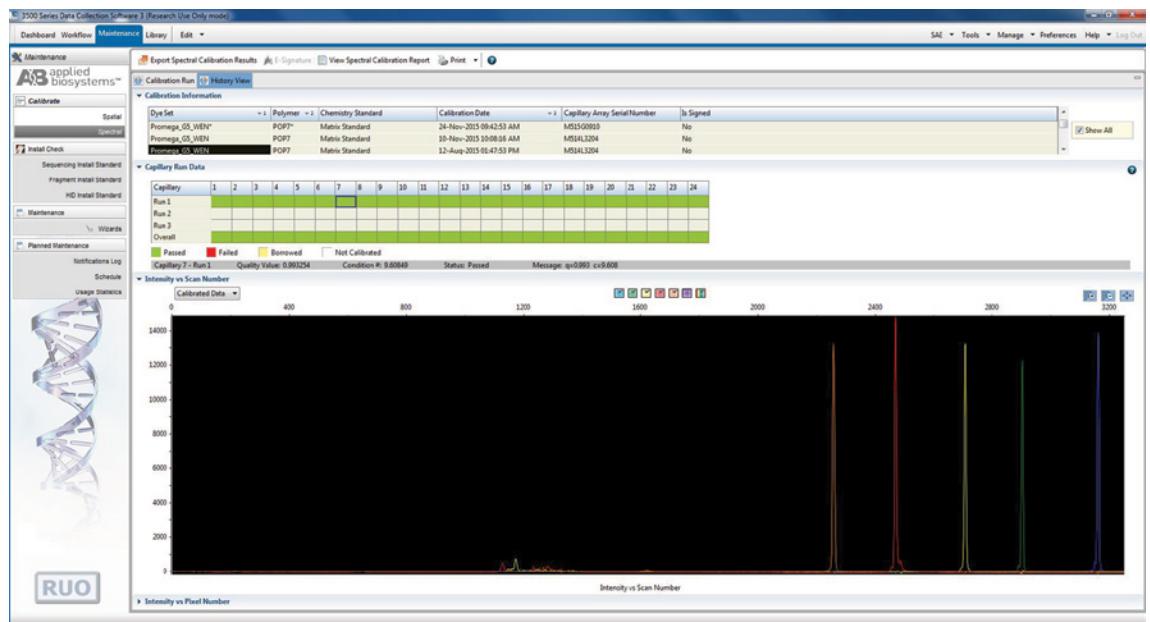


**Figure 2. Setting up the Promega G5 calibration run.**

4. Select “Start Run”.
5. 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, and choose either “Accept” or “Reject”.

### 3.B. Instrument Preparation (continued)

An example of a successful spectral calibration is shown in Figure 3.



**Figure 3. Example data for the GenePrint® 5C Matrix Standard on the Applied Biosystems® 3500xL Genetic Analyzer.**

## 4. Instrument Preparation and Spectral Calibration Using POP-7™ Polymer in the Applied Biosystems® 3130 or 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 or 3130xl capillary array, 50cm
- performance optimized polymer 7 (POP-7™) for the 3130 or 3130xl
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate
- 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 5C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at 2–10°C, protected from light.
2. Vortex the 5C Matrix Mix for 10–15 seconds prior to use. Add 10µl of 5C 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 5C Matrix Mix can be stored for up to 1 week at 2–10°C.
3. Add 10µl of the diluted 5C 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 5C 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 in a 96-well plate). Add 15µl of 5C 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.

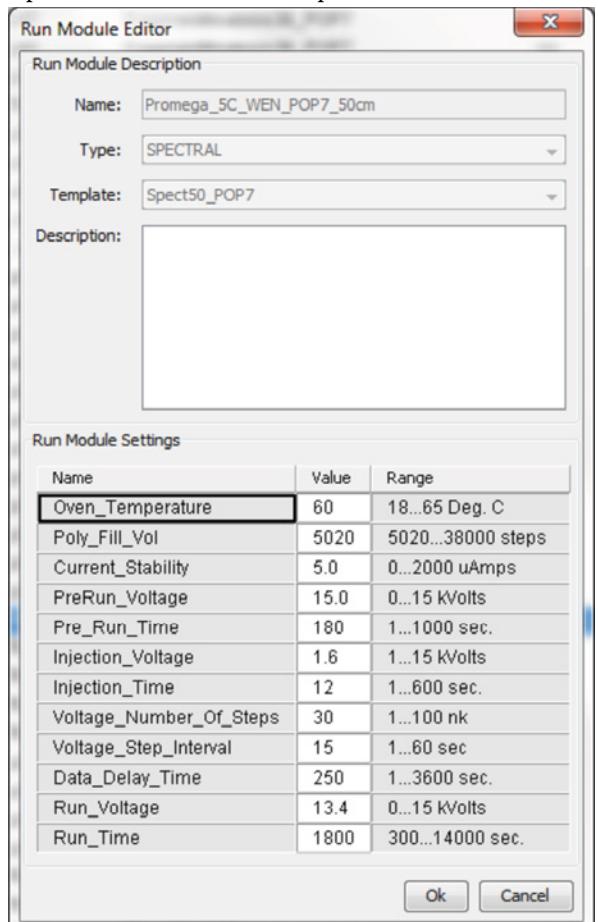
5. Place the plate in the 3130 series 96-well standard plate base and cover with the plate retainer. Do not start the spectral calibration run until the oven is preheated to 60°C.

#### 4.B. Instrument Preparation

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

1. Preheat the oven to 60°C at least 15 minutes prior to the first injection.
2. To perform a spectral calibration for Promega 5-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 “Spect50\_POP7” in the Template drop-down list. Confirm the settings match those in Figure 4.

**Note:** Differences in instrument sensitivity can result in peak imbalance or reduced peak height of the matrix standards. 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.



**Figure 4. Run Module Editor Settings.**

- b. Name the Run Module (e.g., Promega G5), and select “OK”.
- c. In the Protocol Manager, under Instrument Protocols, select “New”. Type a name for your protocol (e.g., Promega G5).
- d. Make the following selections in the Protocol Editor:
  - “Spectral” in the Type drop-down list
  - “G5” in the Dye Set drop-down list
  - “POP7” for the polymer
  - “50” 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 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.

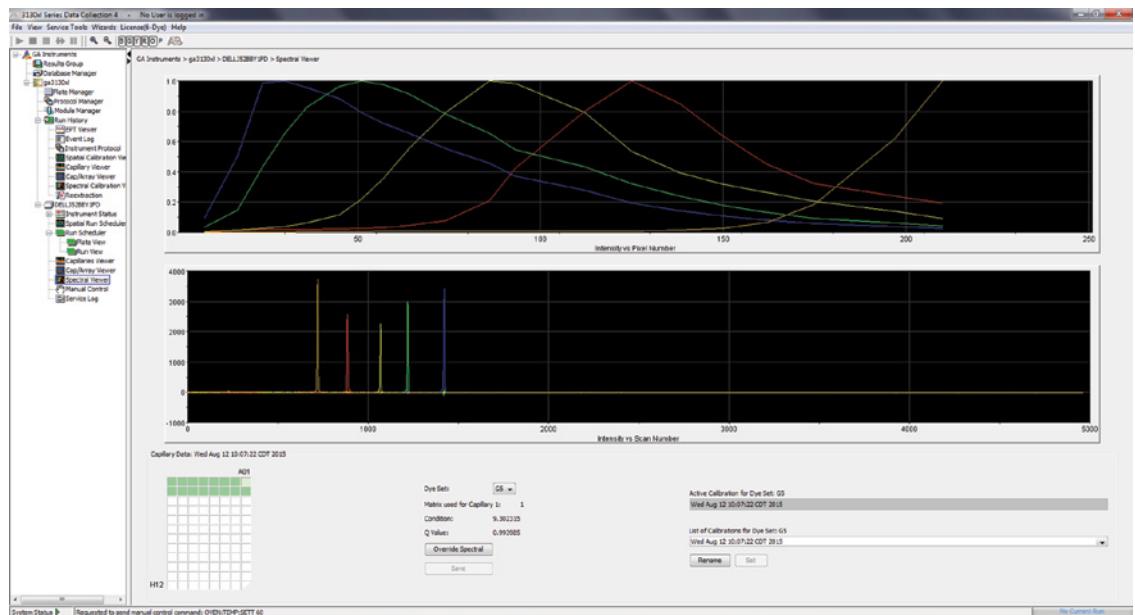
3. In the Plate Manager, create a new plate record as described in the instrument user’s manual. In the dialog box that appears, select “Spectral Calibration” in the Application drop-down list, and select “96-well” as the plate type. Add entries in the owner and operator windows, name the plate and select “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 Analyzers, we recommend that a minimum of 12 of 16 capillaries pass calibration. For the Applied Biosystems® 3130 Genetic Analyzers, 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.

**Notes:**

1. Some Applied Biosystems® 3130 and 3130xl Genetic Analyzers show imbalance in peak heights (e.g., peaks in the red and yellow dye channels are lower than those in the orange, blue and green dye channels). This imbalance should not affect kit performance.
2. 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”.

#### 4.B. Instrument Preparation (continued)

An example of a successful spectral calibration is shown in Figure 5.



**Figure 5. Representative data for the *GenePrint® 5C Matrix Standard* on the Applied Biosystems® 3130xl Genetic Analyzer using POP-7™ polymer and Data Collection Software, Version 4.0.**

## 5. Troubleshooting

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

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

Symptoms	Causes and Comments
Fewer than the recommended number of capillaries passed the spectral calibration	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 5C 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 5C 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. <u>Repeat the spectral calibration.</u>
	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. ABI PRISM® 3100 and 3100-Avant and 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 5C 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 5C Matrix Mix added to the Hi-Di™ formamide during matrix sample preparation.

Peaks in multiple dye channels were detected prior to the orange peak. Increase the data delay time to exclude those peaks, and re-inject the matrix standard.

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. Related Products

Product	Size	Cat.#
<i>GenePrint®</i> 24 System	100 reactions	B1870

This product is intended for research use only and is not intended for use in diagnostic, forensic or paternity procedures.

U.S. Pat. No. 9,139,868 and other patents pending.

<sup>(a)</sup>TMR-ET, CXR-ET and WEN dyes are proprietary.

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Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.