GenePrint® Matrix Standards for Use on the Spectrum Compact CE System Technical Manual

Instructions for Use of Products B1930 and DG4800

TM594 | REVISED 12/21

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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 Manual.

E-mail Promega Technical Services if you have questions on use of this system: **genetic@promega.com**

Description 1

Proper generation of spatial and spectral calibration files is critical to evaluate multicolor STR systems with the Spectrum Compact CE System. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for the instrument maintenance schedule and instructions for installation of the capillary array, buffers and polymer cartridge and performing spatial calibration.

The *GenePrint*® 5C Matrix Standards[a–b] consist of DNA fragments labeled with different fluorescent dyes (fluorescein, JOE, TMR-ET, CXR-ET and WEN) in one tube, and the PowerPlex® 4C Matrix Standards consist of DNA fragments labeled with four different fluorescent dyes (Fluorescein, JOE, TMR and CXR) in one tube. Once generated, the spectral calibration file is applied during sample detection to calculate the spectral overlap and separate the raw fluorescent signals into individual color signals. A spectral calibration must be generated for each individual instrument, and must be performed after the installation of a capillary array following a spatial calibration. A spectral calibration should also be performed after any major maintenance on the system, such as changing the laser, replacing the camera or if a decrease in spectral separation is observed in the STR results. We also recommend generating a new matrix any time the instrument is moved to a new location.

Note: The instructions contained in this manual can be used with the HITACHI DS3000 Compact CE Sequencer.

GenePrint® 5C Spectral Calibration Using Polymer7

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2.1. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
GenePrint® 5C Matrix Standard	5 preps	B1930

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 the *GenePrint*® 5C Matrix Standard be stored with post-amplification reagents. The *GenePrint*® 5C Matrix Standard is light-sensitive; dilute the 5C Matrix Mix in the 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.

Materials to be Supplied by the User

- centrifuge compatible with 8-tube strips
- aerosol-resistant pipette tips
- Spectrum Compact Capillary Cartridge, 4-Capillary 36cm (Cat.# CE2340)
- Spectrum Compact Polymer7 (Cat.# CE2307)
- Spectrum Compact Buffer (Cat.# CE2300)
- Spectrum Compact Cathode Septa Mat (Cat.# CE2301)
- Spectrum Compact Cathode Retainer (Cat.# CE2302)
- Spectrum Compact Strip Base & Retainer, 32-Well (Cat.# CE2332)
- MicroAmp® Optical 8-Tube Strip, 0.2ml (Applied Biosystems, Cat.# 4316567)
- Strip Septa Mat, 8-Well (Cat.# CE2308)
- Hi-Di™ formamide (Applied Biosystems, Cat.# 4311320)

For additional information on performing spectral calibration, refer to the *Spectrum Compact CE System Operating Manual* #TMD058.

The quality of formamide is critical. Use Hi-Di™ formamide. Freeze the 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.

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.

Notes:

1. Only use MicroAmp® Optical 8-Tube Strips, 0.2ml (Applied Biosystems, Cat.# 4316567) as a source of 8-well strip tubes. Use of other 8-well strip tubes may affect performance or damage the Spectrum Compact CE System.
2. Wear gloves when handling consumables and sample cartridges.

2.2. 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 the 5C Matrix Mix to one tube of the Matrix Dilution Buffer. Vortex for 10–15 seconds. Label the tube with the date of dilution. The diluted 5C Matrix Mix can be stored for up to 1 week at 2–10°C.
3. Vortex the diluted 5C Matrix Mix prepared in Step 2 for 10–15 seconds, then add 10µl to 500µl of Hi-Di™ formamide.
4. Vortex the 5C Matrix Mix with formamide prepared in Step 3 for 10–15 seconds, then add 15µl to each of the first four wells of an 8-well strip tube. After placing the septa on the 8-well strip tube, briefly centrifuge the plate to remove bubbles. Do not heat denature.

		Wells							
		1	2	3	4	5	6	7	8
Lane	A	Matrix Mix							
	B								
	C								
	D								

2.3. Assembling Sample Cartridge

1. Place the 8-well strip tube into the strip base in Lane A with the samples in positions 1–4 (Figure 1).

   Note: Lane names A to D and well numbers 1 to 8 are embossed on the strip base. Be sure to check the lane name and well numbers when placing the 8-well strip tube into the base.

2. To complete the assembly, place the retainer over the strip in the strip base, aligning the lane names A to D and well numbers 1 to 8 on the retainer to those on the strip base and pressing until the retainer clicks into the strip base (Figure 2).

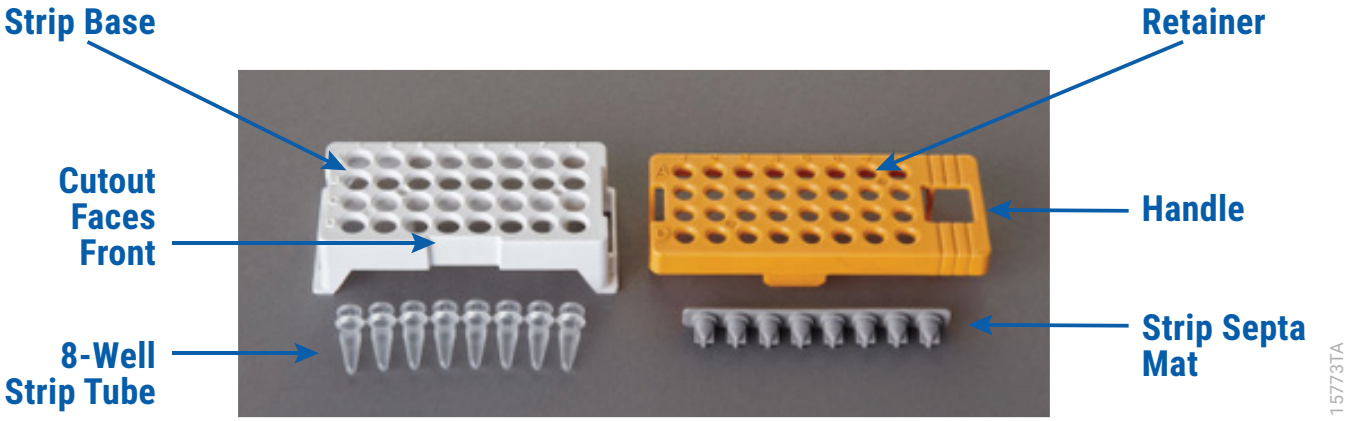

Figure 1. Assembling the Spectrum Compact Strip Base and Retainer.

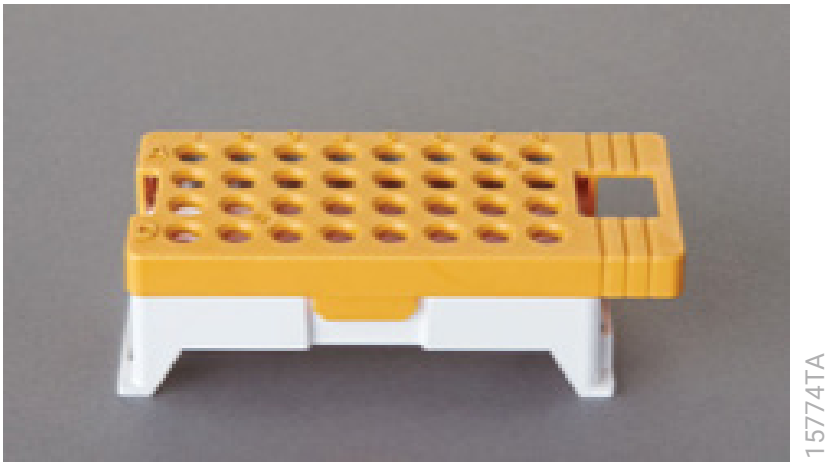

Figure 2. Assembled Spectrum Compact Sample Cartridge.

2.4. Instrument Preparation and Spectral Calibration

These instructions are intended as a guide for running *GenePrint*® 5C Matrix Standards on the Spectrum Compact CE System. They are not intended as comprehensive instructions for using the Spectrum Compact CE System. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for more details on performing spectral calibration.

Notes:

1. We have found that the use of fresh polymer and new capillary array results in an optimal spectral calibration.
2. We do not recommend performing spectral calibration with expired reagents. Expired reagents should be replaced before performing a spectral calibration.
3. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for more details on installation of consumables, instrument maintenance and spatial calibration.

Figure 3. Spectrum Compact CE System Software 'Main Menu' screen.

1. Select the **Consumables** icon in the Header on the 'Main Menu' screen (Figure 3). Ensure that the consumables are not expired and that adequate injections remain for consumables installed.

2. Select the **Oven Temperature** icon in the Header on the 'Main Menu' screen as shown in Figure 4 to start preheating the oven temperature to 60°C. The temperature displayed will change as the temperature of the oven increases. When 60°C is reached, a check mark will appear adjacent to the temperature.

   Note: We recommend you preheat the oven for at least 30 minutes prior to starting a run. The oven will automatically turn off after 2 hours if a run is not started.

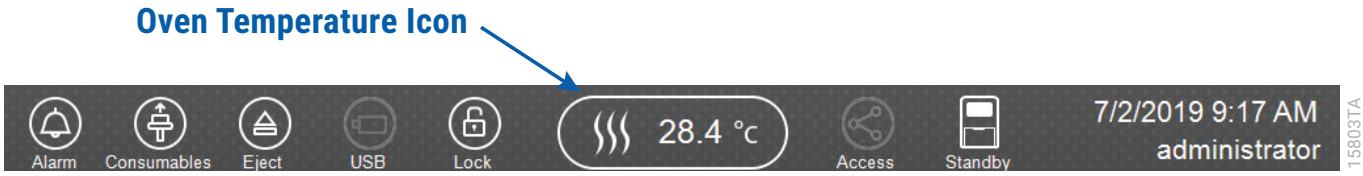

Figure 4. Preheating Oven.

3. Select **Calibration** on the maintenance portion of the 'Main Menu' screen (Figure 3) then select **Spectral Calibration** on the 'Maintenance Calibration' screen (Figure 5).

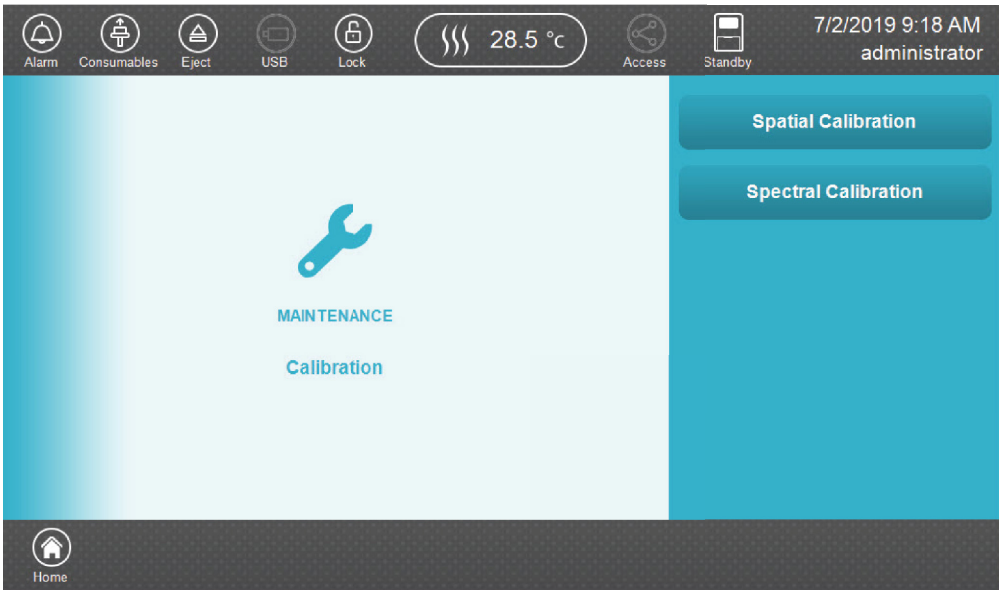

Figure 5. 'Maintenance Calibration' screen.

4. Use the scroll arrows on the right-hand side of the 'Dye Set List' screen (Figure 6) to find the correct Dye Set/Application Type/Polymer combination from the displayed list. To perform a spectral calibration using the *GenePrint*® 5C Matrix Standard on Polymer7, select **Promega 5-dye** with "Fragment" and "Polymer7" as application and polymer types, respectively, then select **Calibration**. The 'Assemble the Cartridge' screen will open (Figure 7).

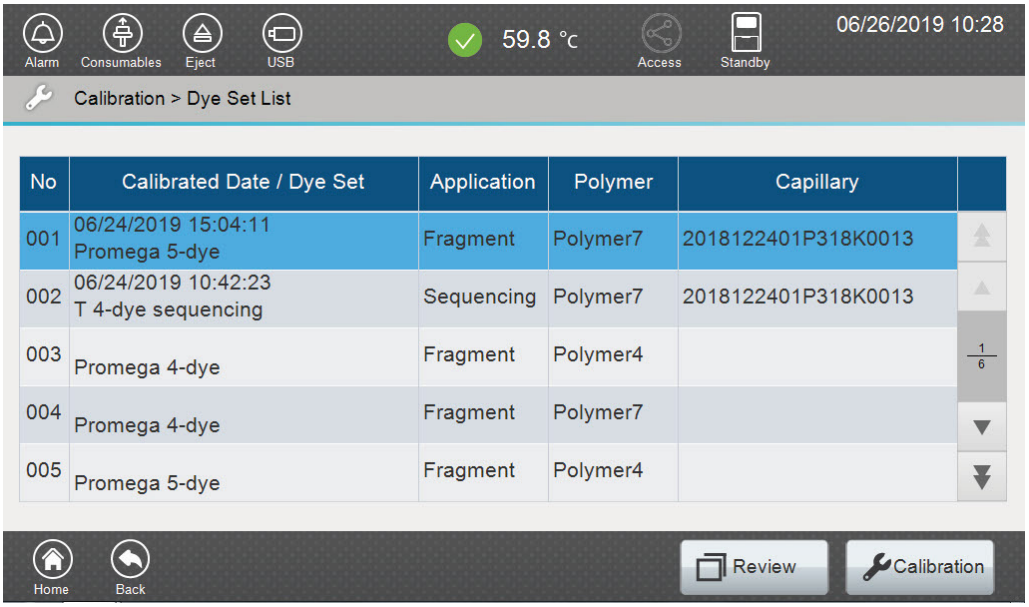

Figure 6. 'Dye Set List' screen.

5. Select **Next** on the 'Assemble the Cartridge' screen (Figure 7). A message window will open indicating that the autosampler is moving and telling the user to not open the door. In addition, the status indicator flashes green while the autosampler is moving. After autosampler movement is complete, the message window closes and the status indicator returns to a steady green.

   Note: Do not open the front door of the Spectrum Compact CE System while the autosampler is in motion.

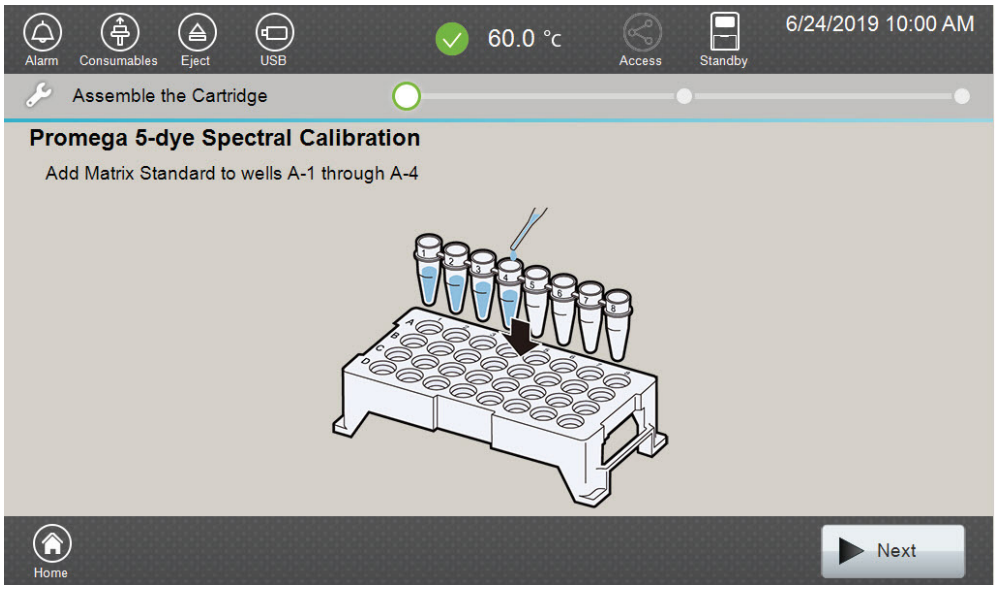

Figure 7. 'Assemble the Cartridge' screen.

6. Open the front door of the Spectrum Compact CE System and mount the sample cartridge on the autosampler following the instructions displayed on the 'Install the Cartridge' screen (Figure 8).

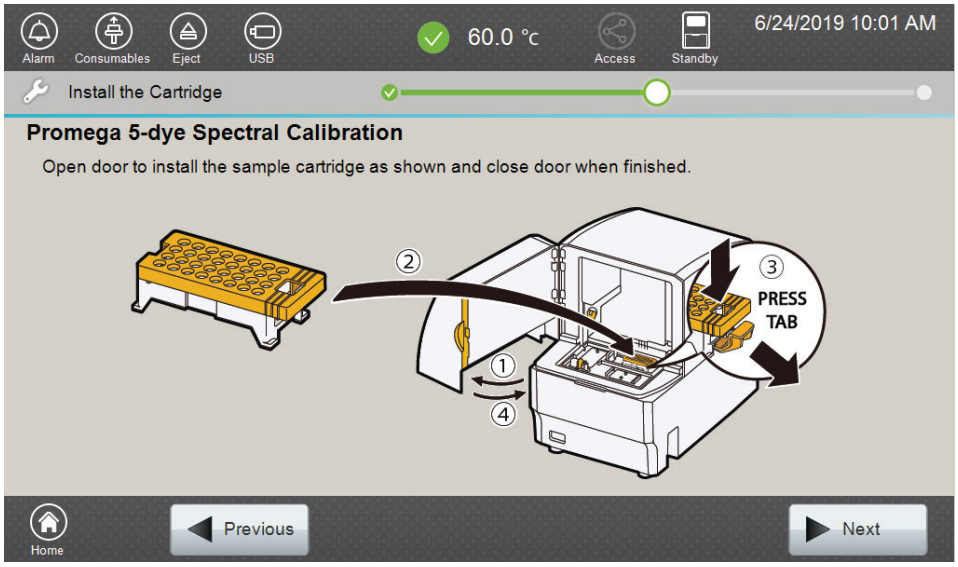

Figure 8. 'Install the Cartridge' screen.

7. After mounting the sample cartridge on the autosampler, close the front door of the Spectrum Compact CE System and wait for the status indicator to stop flashing amber and turn steady green.

   Note: Do not open the front door of the Spectrum Compact CE System while the autosampler is in motion.

8. After the autosampler has returned to its home position, the 'Spectral Calibration' screen will automatically be displayed (Figure 9). Select **Run** to start the spectral calibration.

   Note: The 'Raw Data' tab can be used to monitor the run

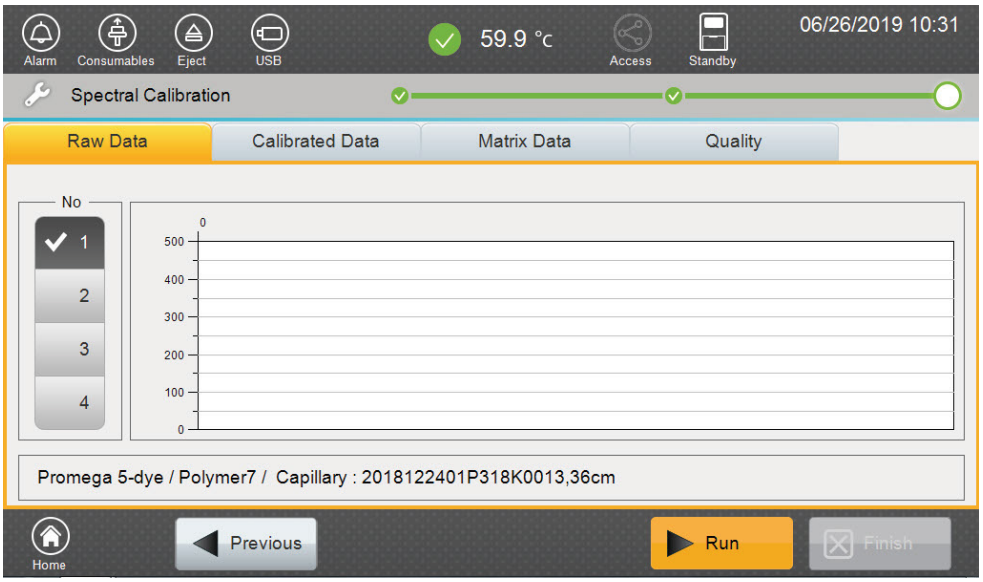

Figure 9. 'Spectral Calibration' screen.

2.5. Results

1. Following the run, the ‘Raw Data’ tab (Figure 10) will be displayed. The minimum peak height for spectral calibration is 500 relative fluorescent units (RFU) and the maximum peak height is 32,767RFU.

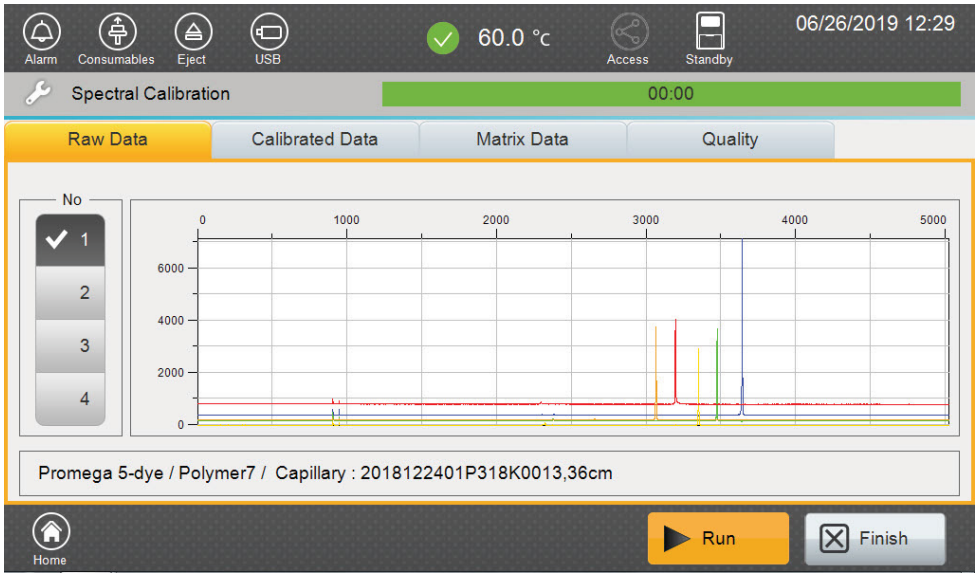

Figure 10. Spectral Calibration ‘Raw Data’ tab screen.

2. The ‘Calibrated Data’ tab can be used to view the matrix peaks with both baseline and spectral applied for each capillary (Figure 11).

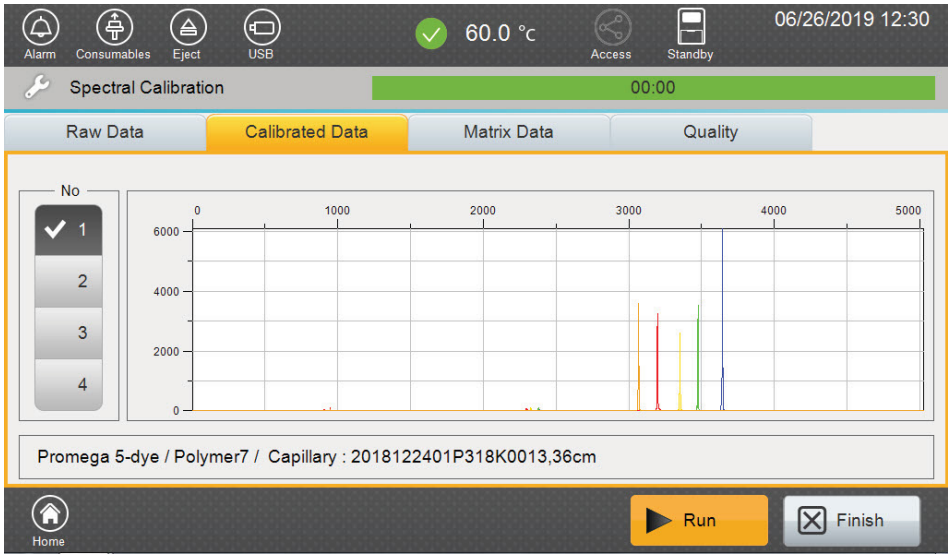

Figure 11. Spectral Calibration ‘Calibrated Data’ tab screen.

3. The ‘Matrix Data’ tab can be used to view emisson spectra for each capillary (Figure 12).

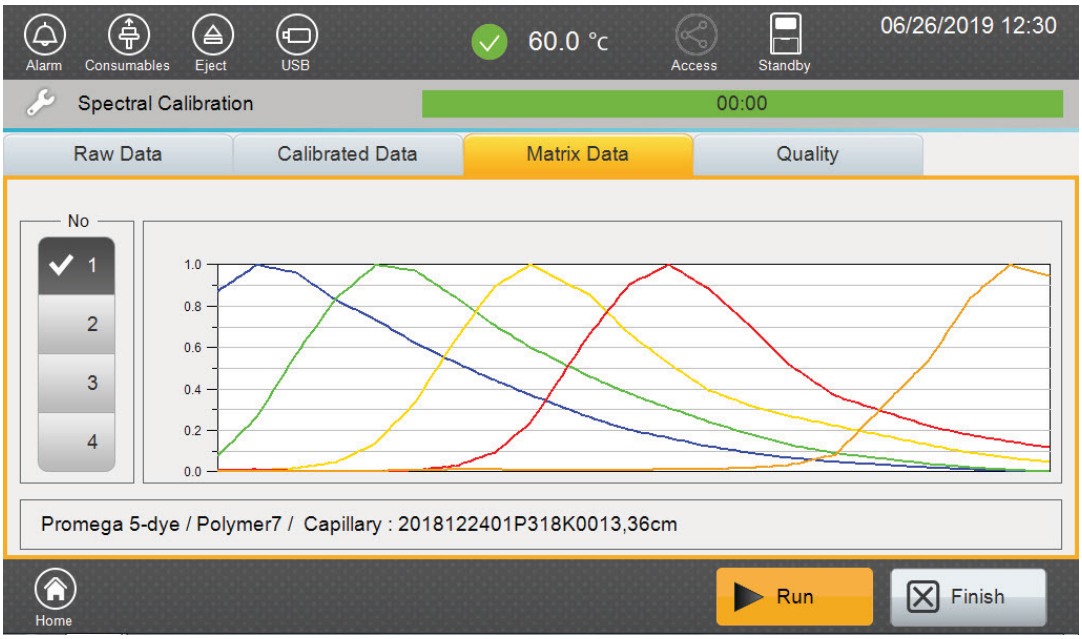

Figure 12. Spectral Calibration ‘Matrix Data’ tab screen.

4. Review the quality of the spectral calibration by selecting the ‘Quality’ tab (Figure 13).

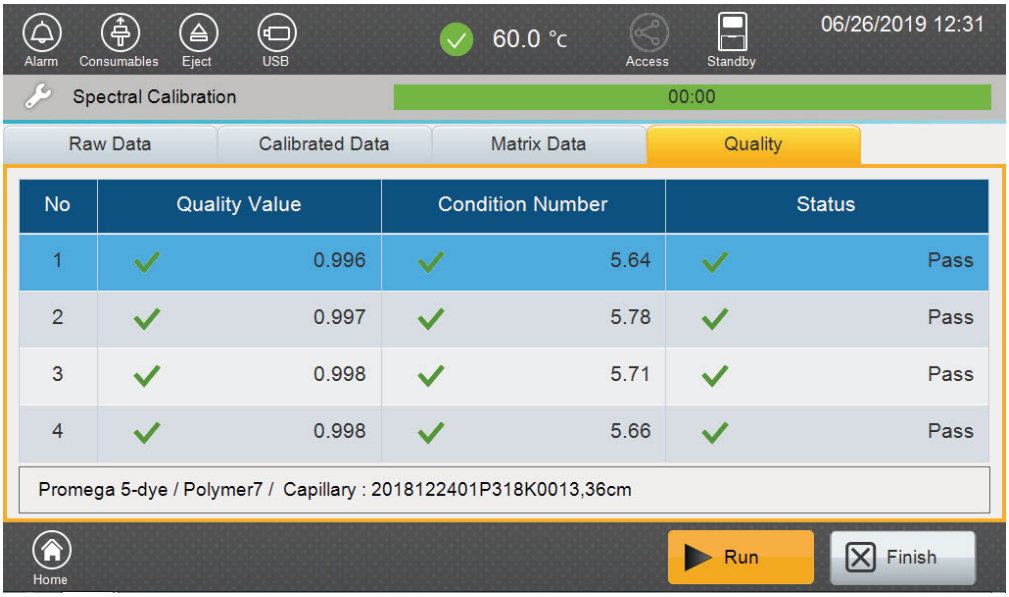

Figure 13. Spectral Calibration ‘Quality’ tab screen.

5. Each capillary must meet the passing criteria of ≥0.95 for the Quality Value and <13.5 for the Condition Number.

6. If one capillary fails to meet the criteria, it is possible to borrow spectral data from an adjacent capillary. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for details. If more than one capillary fails, the spectral must be rerun.

   Notes:

   1. Selecting **Run** will rerun the spectral calibration.
   2. Refer to Section 4 for troubleshooting if more than one capillary fails to meet the criteria.

7. After reviewing the results, select **Finish**. This will open a confirmation window. Select **Yes** to apply the spectral calibration results (Figure 14). The spectral calibration results will not be saved unless you select **Yes** on this window.

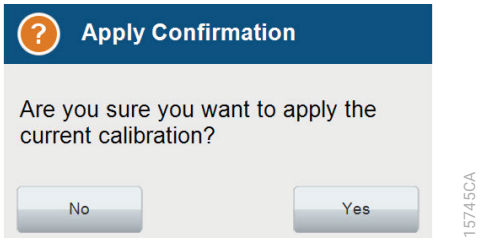

Figure 14. Apply spectral calibration confirmation window.

PowerPlex® 4C Spectral Calibration Using Polymer4

3

3.1. 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 the PowerPlex® 4C Matrix Standard be stored 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.

Do not refreeze the PowerPlex® 4C Matrix Standard components.

Materials to be Supplied by the User

- centrifuge compatible with 8-tube strips
- aerosol-resistant pipette tips
- Spectrum Compact Capillary Cartridge, 4-Capillary 36cm (Cat.# CE2340)
- Spectrum Compact Polymer4 (Cat.# CE2304)
- Spectrum Compact Buffer (Cat.# CE2300)
- Spectrum Compact Cathode Septa Mat (Cat.# CE2301)
- Spectrum Compact Cathode Retainer (Cat.# CE2302)
- Spectrum Compact Strip Base & Retainer, 32-Well (Cat.# CE2332)
- MicroAmp® Optical 8-Tube Strip, 0.2ml (Applied Biosystems, Cat.# 4316567)
- Strip Septa Mat, 8-Well (Cat.# CE2308)
- Hi-Di™ formamide (Applied Biosystems, Cat.# 4311320)

For additional information on performing spectral calibration, refer to the *Spectrum CE System Operating Manual* #TMD058.

The quality of formamide is critical. Use Hi-Di™ formamide. Freeze the 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.

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.

Notes:

1. Only use MicroAmp® Optical 8-Tube Strips, 0.2ml (Applied Biosystems, Cat.# 4316567) as a source of 8-well strip tubes. Use of other 8-well strip tubes may affect performance or damage the Spectrum Compact CE System.
2. Wear gloves when handling consumables and sample cartridges.

3.2. 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 the 4C Matrix Mix to one tube of the Matrix Dilution Buffer. Vortex for 10–15 seconds. Label the tube with the date of dilution. The diluted 4C Matrix Mix can be stored for up to 1 week at 2–10°C.
3. Vortex the diluted 4C Matrix Mix prepared in Step 2 for 10–15 seconds, then add 10µl to 500µl of Hi-Di™ formamide.
4. Vortex the 4C Matrix Mix with formamide prepared in Step 3 for 10–15 seconds, then add 15µl to each of the first four wells of an 8-well strip tube. After placing the septa on the 8-well strip tube, briefly centrifuge the plate to remove bubbles. Do not heat denature.

		Wells							
		1	2	3	4	5	6	7	8
Lane	A	Matrix Mix							
	B								
	C								
	D								

3.3. Assembling Sample Cartridge

1. Place the 8-well strip tube into the strip base in Lane A with the samples in positions 1–4 (Figure 15).

   Note: Lane names A to D and well numbers 1 to 8 are embossed on the strip base. Be sure to check the lane name and well numbers when placing the 8-well strip tube into the base.

2. To complete the assembly, place the retainer over the strip in the strip base, aligning the lane names A to D and well numbers 1 to 8 on the retainer to those on the strip base and pressing until the retainer clicks into the strip base (Figure 16).

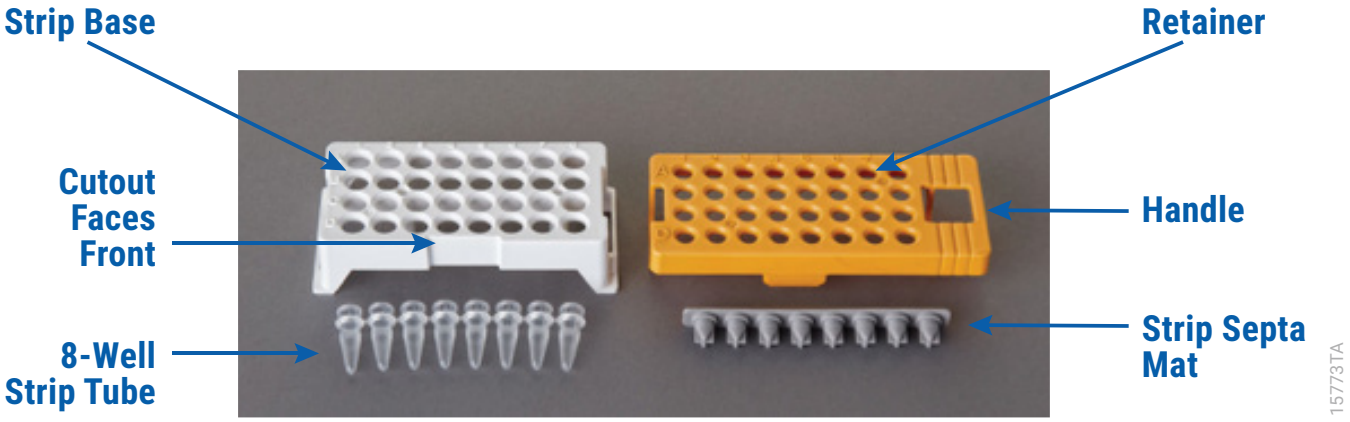

Figure 15. Assembling the Spectrum Compact Strip Base and Retainer.

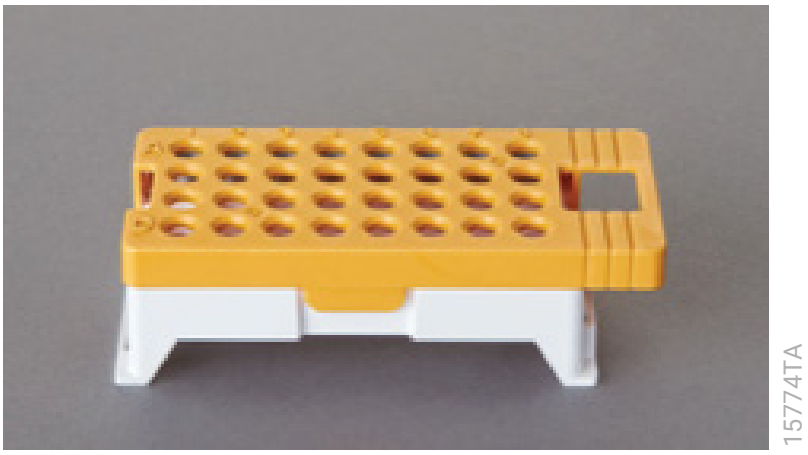

Figure 16. Assembled Spectrum Compact Sample Cartridge.

3.4. Instrument Preparation and Spectral Calibration

These instructions are intended as a guide for running PowerPlex® Matrix Standards on the Spectrum Compact CE System. They are not intended as comprehensive instructions for using the Spectrum Compact CE System. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for more details on performing spectral calibration.

Notes:

1. We have found that the use of fresh polymer and new capillary array results in an optimal spectral calibration.
2. We do not recommend performing spectral calibration with expired reagents. Expired reagents should be replaced before performing a spectral calibration.
3. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for more details on installation of consumables, instrument maintenance and spatial calibration.

Figure 17. Spectrum Compact CE System Software 'Main Menu' screen.

1. Select the **Consumables** icon in the Header on the 'Main Menu' screen (Figure 17). Ensure that the consumables are not expired and that adequate injections remain for consumables installed.

2. Select the **Oven Temperature** icon in the Header on the 'Main Menu' screen, as shown in Figure 18, to start preheating the oven temperature to 60°C. The temperature displayed will change as the temperature of the oven increases. When 60°C is reached, a check mark will appear adjacent to the temperature.

   Note: We recommend you preheat the oven for at least 30 minutes prior to starting a run. The oven will automatically turn off after 2 hours if a run is not started.

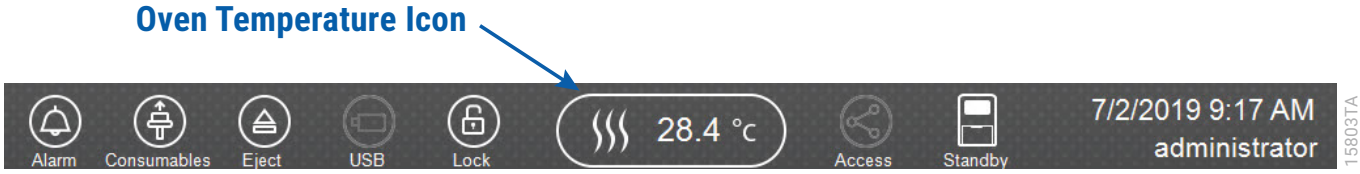

Figure 18. Preheating Oven.

3. Select **Calibration** on the maintenance portion of the 'Main Menu' screen (Figure 17) then select **Spectral Calibration** on the 'Maintenance Calibration' screen (Figure 19).

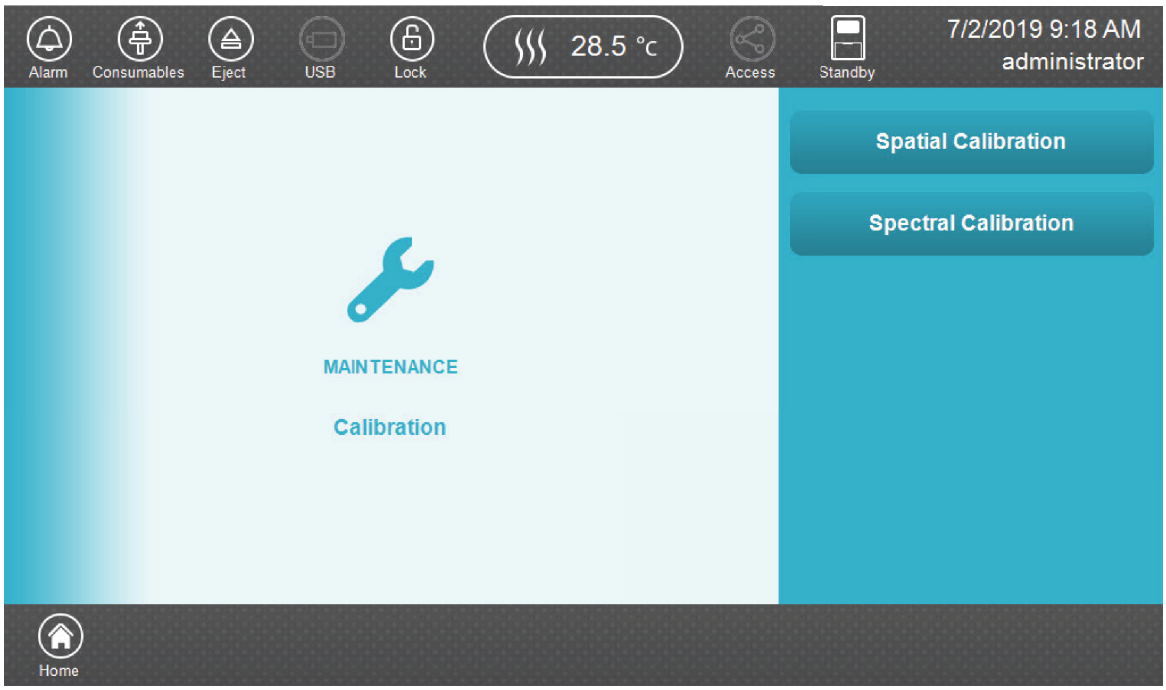

Figure 19. 'Maintenance Calibration' screen.

4. Use the scroll arrows on the right-hand side of the 'Dye Set List' screen (Figure 20) to find the correct Dye Set/Application Type/Polymer combination from the displayed list. To perform a spectral calibration using the PowerPlex® 4C Matrix Standard on Polymer4, select **Promega 4-dye** with "Fragment" and "Polymer4" as application and polymer types, respectively, then select **Calibration**. The 'Assemble the Cartridge' screen will open (Figure 21).

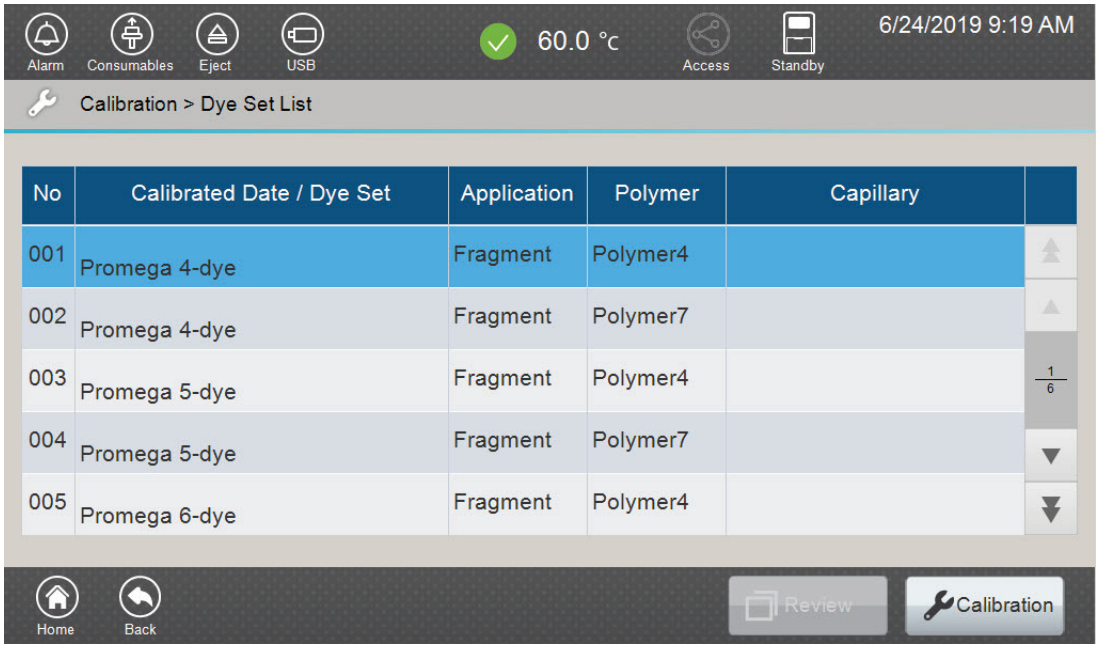

Figure 20. 'Dye Set List' screen.

5. Select **Next** on the 'Assemble the Cartridge' screen (Figure 21). A message window will open indicating that the autosampler is moving and telling the user to not open the door. In addition, the status indicator flashes green while the autosampler is moving. After autosampler movement is complete, the message window closes and the status indicator returns to a steady green.

   Note: Do not open the front door of the Spectrum Compact CE System while the autosampler is in motion.

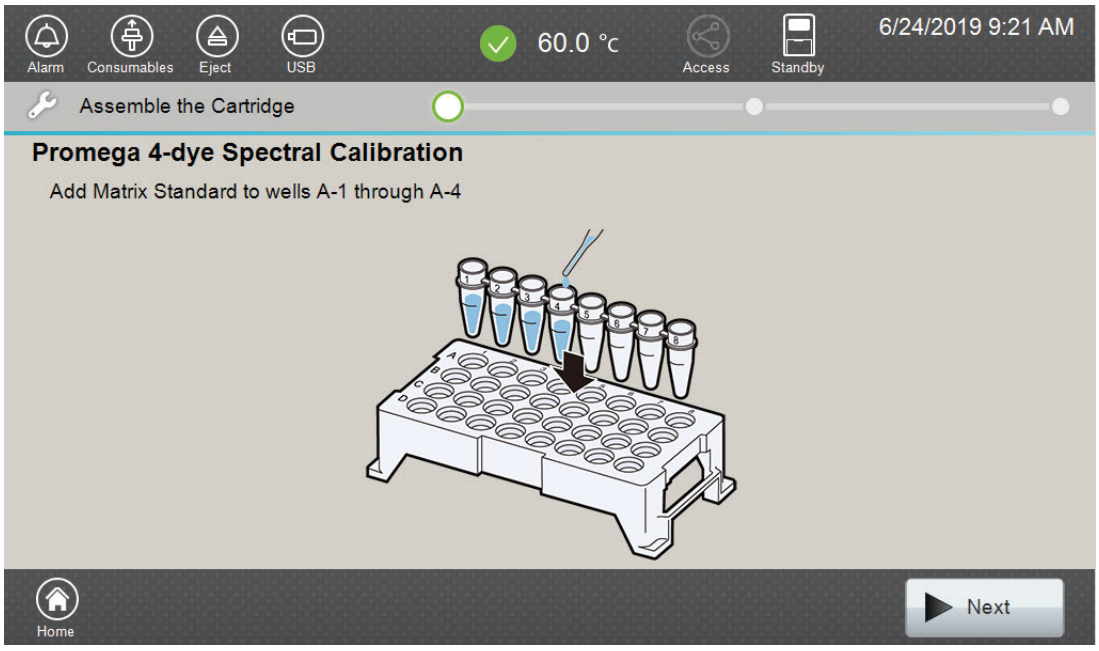

Figure 21. 'Assemble the Cartridge' screen.

6. Open the front door of the Spectrum Compact CE System and mount the sample cartridge on the autosampler by following the instructions displayed on the 'Install the Cartridge' screen (Figure 22).

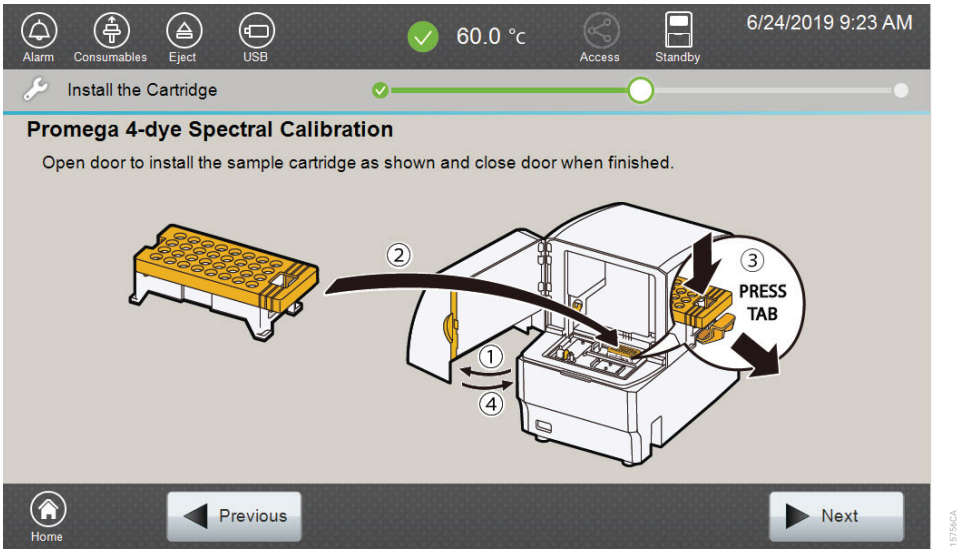

Figure 22. 'Install the Cartridge' screen.

7. After mounting the sample cartridge on the autosampler, close the front door of the Spectrum Compact CE System and wait for the status indicator to stop flashing amber and turn steady green.

   Note: Do not open the front door of the Spectrum Compact CE System while the autosampler is in motion.

8. After the autosampler has returned to its home position, the 'Spectral Calibration' screen will automatically be displayed (Figure 23). Select **Run** to start the spectral calibration.

   Note: The 'Raw Data' tab can be used to monitor the run.

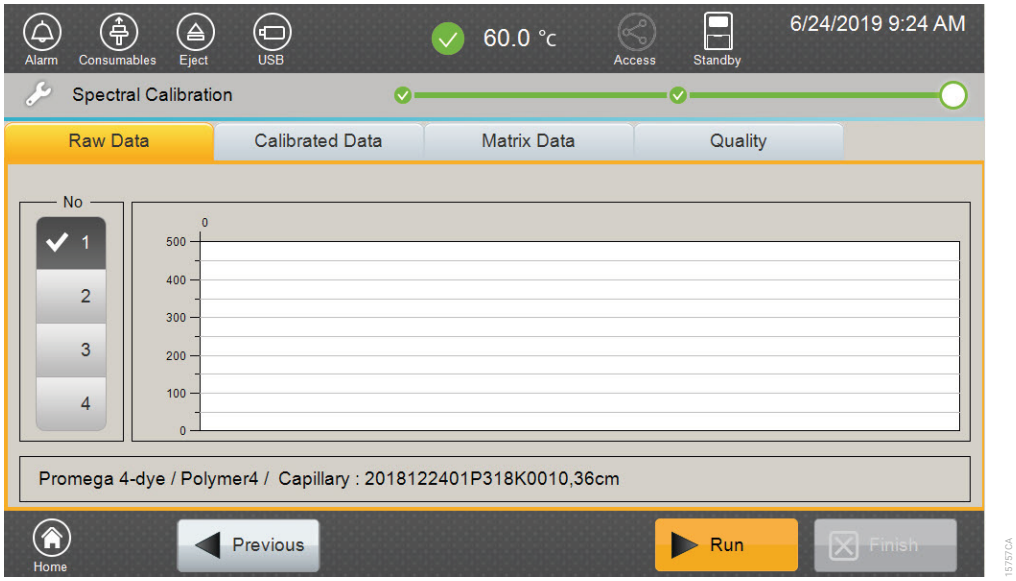

Figure 23. 'Spectral Calibration' screen.

3.5. Results

1. Following the run, the 'Raw Data' tab (Figure 24) will be displayed. The minimum peak height for spectral calibration is 500 relative fluorescent units (RFU) and the maximum peak height is 32,767RFU.

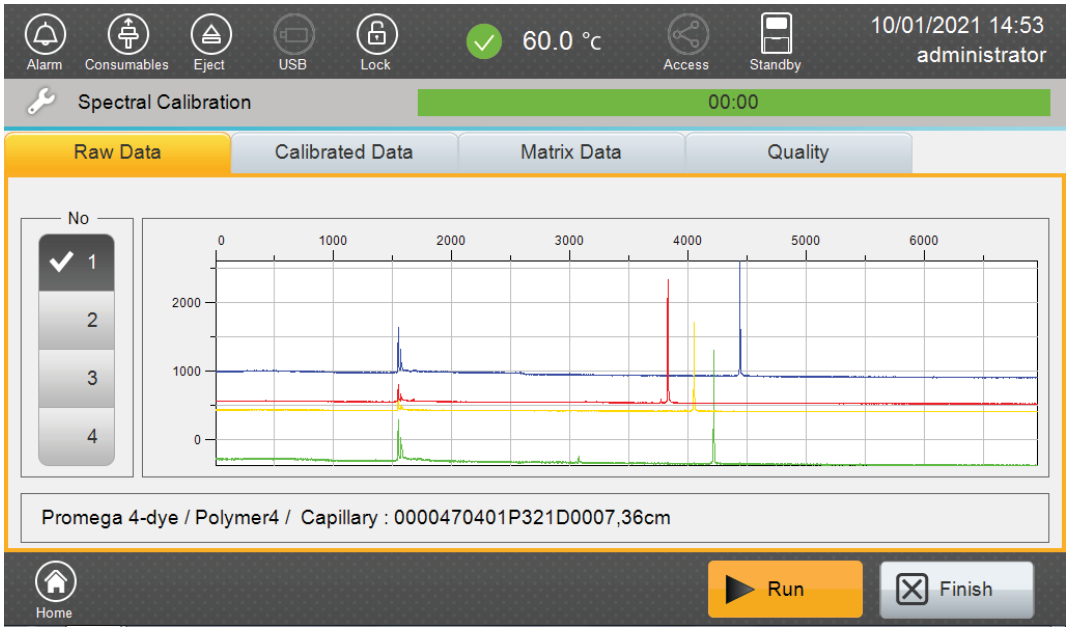

Figure 24. Spectral Calibration 'Raw Data' tab screen.

2. The 'Calibrated Data' tab can be used to view the matrix peaks with both baseline and spectral applied for each capillary (Figure 25).

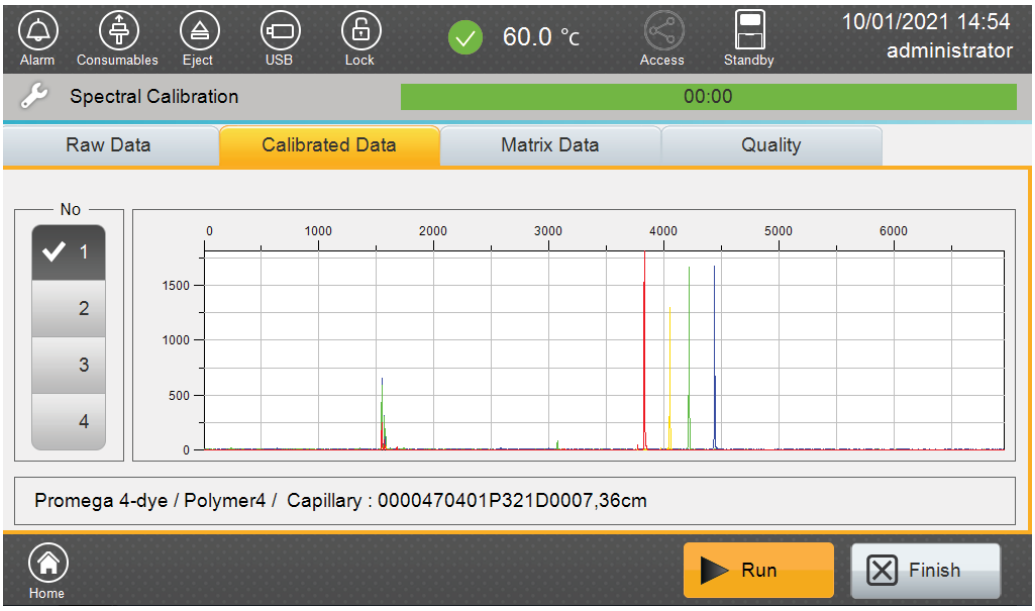

Figure 25. Spectral Calibration 'Calibrated Data' tab screen.

3. The ‘Matrix Data’ tab can be used to view emission spectra for each capillary (Figure 26).

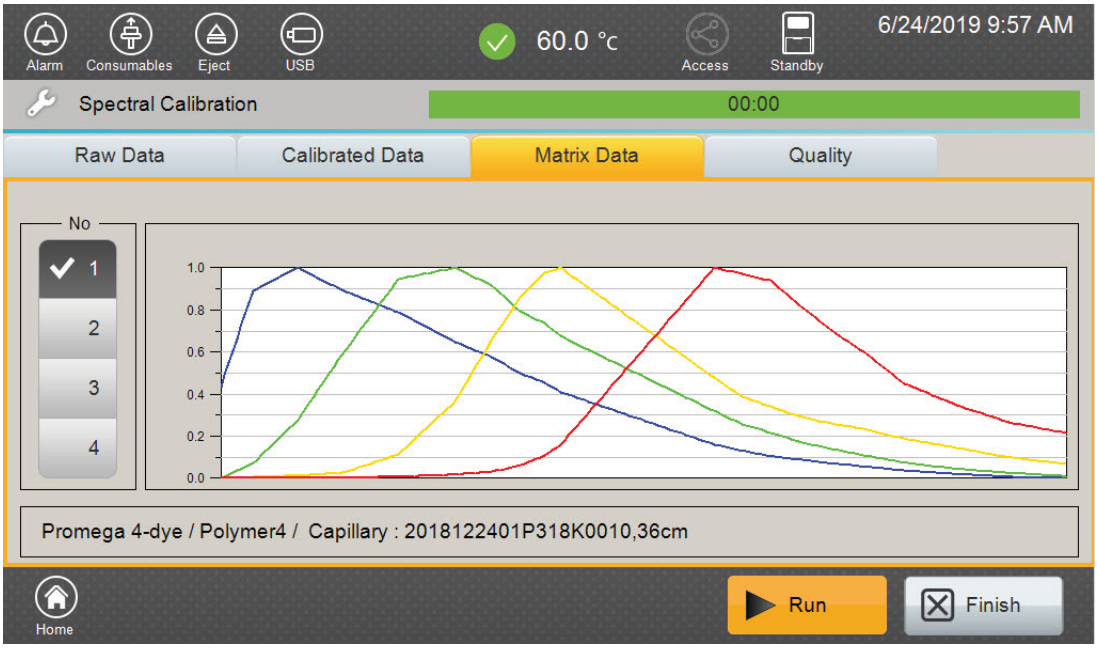

Figure 26. Spectral Calibration ‘Matrix Data’ tab screen.

4. Review the quality of the spectral calibration by selecting the ‘Quality’ tab (Figure 27).

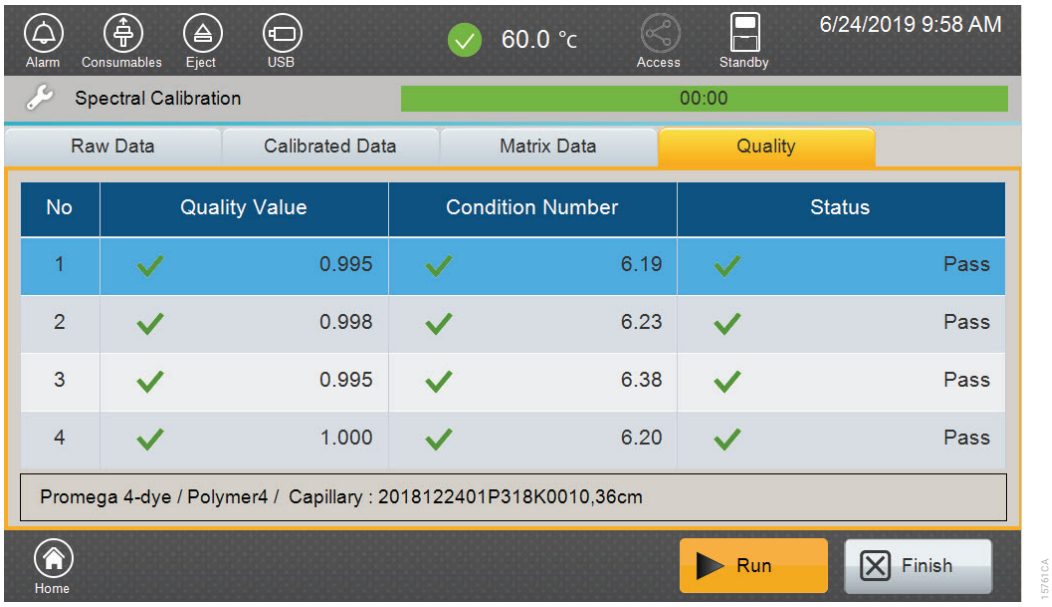

Figure 27. Spectral Calibration ‘Quality’ tab screen.

5. Each capillary must meet the passing criteria of ≥0.95 for the Quality Value and <8.5 for the Condition Number.

6. If one capillary fails to meet the criteria, it is possible to borrow spectral data from an adjacent capillary. Refer to the *Spectrum Compact CE System Operating Manual* #TMD058 for details. If more than one capillary fails, the spectral must be rerun.

   Notes:

   1. Selecting **Run** will rerun the spectral calibration.
   2. Refer to Section 4 for troubleshooting if more than one capillary fails to meet the criteria.

7. After reviewing the results, select **Finish**. This will open a confirmation window. Select **Yes** to apply the spectral calibration results (Figure 28). The spectral calibration result will not be saved unless you select **Yes** on this window.

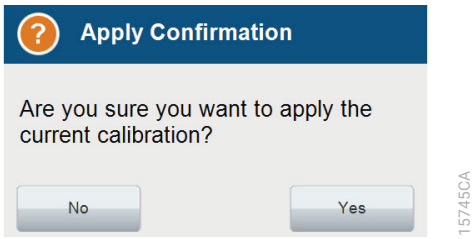

Figure 28. Apply spectral calibration confirmation window.

Troubleshooting 4

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**

Symptoms	Causes and Comments
Fewer than the recommended number of capillaries passed the spectral calibration	Poor-quality formamide was used. The quality of formamide is critical. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights.
	Matrix Mix was too dilute. Matrix Mix that is too dilute will result in low spectral calibration peak heights (<500RFU), which may result in spectral calibration failure. Increase the volume of diluted Matrix Mix added to the formamide during sample preparation.
	Diluted Matrix Mix stored longer than one week at 4°C or at the incorrect temperature. Prepare a fresh dilution.
	Matrix Mix was too concentrated. Matrix Mix that is too concentrated may result in spectral calibration failure due to saturated peaks, bleed-through or over-subtraction in other dye colors. Decrease the volume of diluted Matrix Mix added to the formamide during matrix sample preparation.
	Carryover from previous injection detected as matrix peak. Replace cathode buffer cartridge septa. Use fresh reagents.
	For best spectral calibration results, use fresh polymer and fresh buffer.
	Use an array with fewer than 200 injections.
	Poor quality 8-well strip tubes. For best results, use MicroAmp® Optical 8-Tube Strips, 0.2ml (Applied Biosystems, Cat.# 4316567).
Elevated spectral bleedthrough in one or more capillaries	If elevated spectral bleedthrough is observed in one or more capillaries after installing a new capillary cartridge, reinstall the capillary cartridge. Completely remove the capillary cartridge from the oven and reinstall as indicated in Section 3.2 of the *Spectrum Compact CE System Operating Manual* #TMD058. Simply lift the capillary cartridge completely out of the oven by its yellow knob and reinstall immediately back in the oven. Repositioning the detection unit of the capillary cartridge into the detection window of the oven during reinstallation can improve spectral performance. **Note:** Perform a new spatial and spectral calibration after uninstalling and reinstalling the capillary cartridge.

Summary of Changes 5

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

1. Updated Figures 24 and 25.
2. Updated disclaimers.

[a]U.S. Pat. No. 9,139,868, European Pat. No. 2972229, Japanese Pat. No. 6367307 and other patents pending.

[b]TMR-ET, CXR-ET and WEN dyes are proprietary.

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