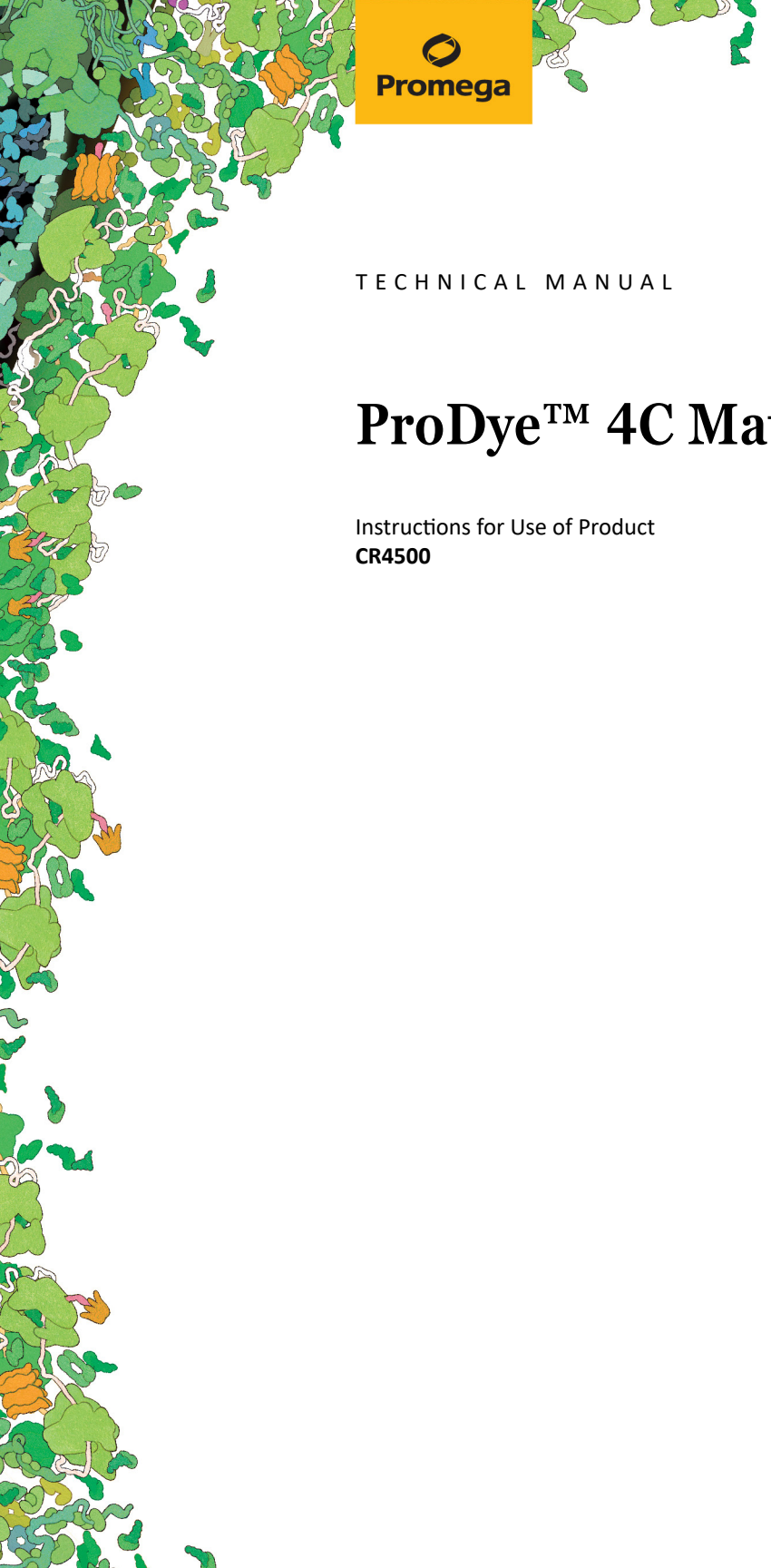

TECHNICAL MANUAL

ProDye™ 4C Matrix Standard

Instructions for Use of Product
CR4500

Revised 9/22
TM674

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

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Promega

1. Description

Properly generating a spectral calibration file is critical for evaluating multicolor systems using the Promega Spectrum Compact CE System, Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL and SeqStudio® Genetic Analyzers. The ProDye™ 4C Matrix Standard consists of DNA fragments labeled with four different fluorescent dyes (dTMR, dCXR, dRSixG and dROneTen) in one tube. These are the same dyes as those in BigDye™ Terminator V3.1 Cycle Sequencing Kit. The spectral calibration is performed using a specific dye set. Once generated, the spectral calibration is applied during sample detection to calculate the spectral overlap and separate the raw fluorescent signals into individual color signals.

The ProDye™ 4C Matrix Standard was developed for use with our ProDye™ Terminator Sequencing System as well as other existing dye-terminator chemistries and is compatible with the Promega Spectrum Compact CE System, Applied Biosystems® 3500 and 3500xL Genetic Analyzers as well as Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers with Sequence Analysis Software (Life Technologies) and SeqStudio® Genetic Analyzer. A protocol to operate the Applied Biosystems® 3130, 3130*xl*, 3500, 3500xL or SeqStudio® Genetic Analyzer should be obtained from the manufacturer.

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 generating a new matrix. Individual labs should determine the frequency of matrix generation.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ProDye™ 4C Matrix Standard	**5 preps**	**CR4500**

For in vitro Research Use Only. Includes:

- 1 × 150µl ProDye™ 4C Matrix Mix
- 5 × 200µl Matrix Dilution Buffer

Storage Conditions: Store all components at –30°C to –10°C in a nonfrost-free freezer. After first use, store the ProDye™ 4C Matrix Standard components at +2°C to +10°C. The ProDye™ 4C Matrix Mix is light sensitive; dilute the ProDye™ 4C Matrix Mix with the Matrix Dilution Buffer in the provided amber tube. Store the diluted ProDye™ 4C Matrix Mix at +2°C to +10°C for up to 1 week.

3. Instrument Preparation and Spectral Calibration Using the Promega Spectrum Compact CE System

Materials to Be Supplied by the User

- centrifuge compatible with 8-well strip tubes
- aerosol-resistant pipette tips
- Spectrum Compact Capillary Cartridge, 4-Capillary 36cm (Cat.# CE2340 or CE2407)
- Spectrum Polymer7 Cartridge (Cat.# CE2307)
- Spectrum Compact Buffer (Cat.# CE2300)
- 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 or for polymer cartridge compatibility information, refer to the *Spectrum Compact CE System Operating Manual* #TMD058.

3.A. Matrix Sample Preparation

1. At the first use, thaw the ProDye™ 4C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at +2°C to +10°C.
2. Vortex the ProDye™ 4C Matrix Mix for 10–15 seconds prior to use. Add 10µl of the ProDye™ 4C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds. Label the tube with the dilution date. The diluted ProDye™ 4C Matrix Mix can be stored for up to 1 week at +2°C to +10°C.
3. Add 10µl of the diluted ProDye™ 4C Matrix Mix prepared in Step 2 to 500µl of Hi-Di™ formamide. Vortex for 10–15 seconds.
4. For the Spectrum Compact CE System, the first four wells of a strip tube are used for spectral calibration. Add 15µl of the ProDye™ 4C Matrix Mix with formamide prepared in Step 3 to each of the four wells. After placing the septa on the strip, briefly centrifuge the strip to remove bubbles. Do not heat denature.
5. Place the prepared strip 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 tube base. Be sure to check the lane name and well numbers when placing the 8-well strip tube into the base.
6. 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). Do not start the spectral calibration run until the oven is preheated to 60°C.

3.A. Matrix Sample Preparation (continued)

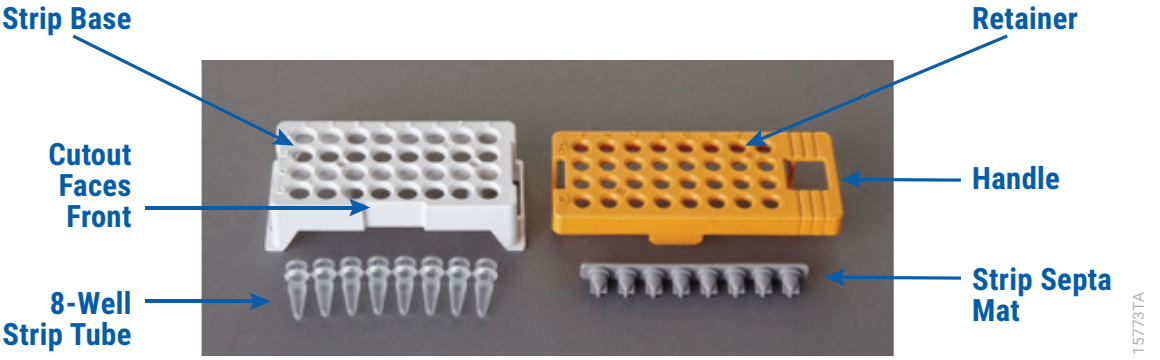

Figure 1. Assembling the Spectrum Compact Strip Base and Retainer.

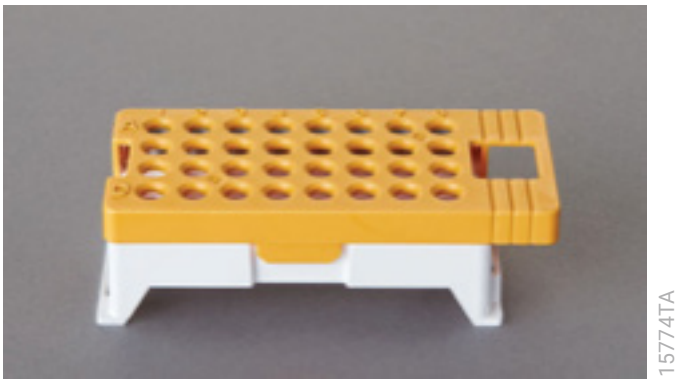

Figure 2. Assembled Spectrum Compact Sample Cartridge.

3.B. Instrument Preparation and Spectral Calibration

These instructions are intended as a guide for running the ProDye™ 4C 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 a spectral calibration.

Notes:

1. We have found that using fresh polymer and a 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 consumable installation, instrument maintenance and spatial calibration.

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

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

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 that 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.

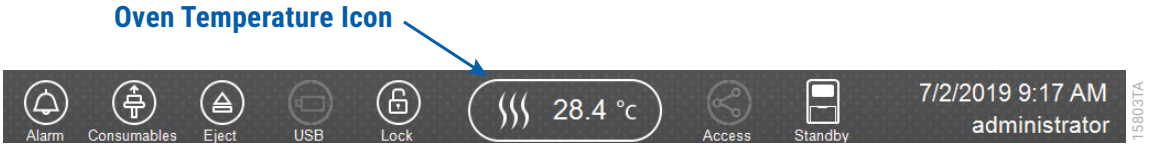

Figure 4. Preheating oven.

3.B. Instrument Preparation and Spectral Calibration (continued)

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

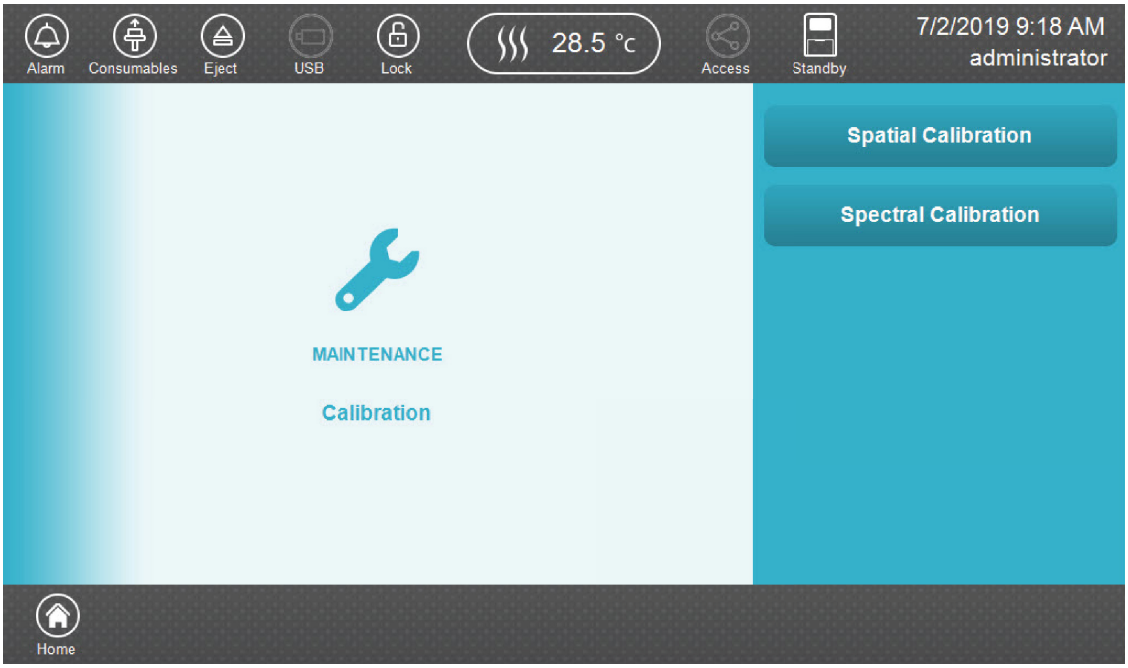

Figure 5. 'Maintenance Calibration' screen.

4. Use the scroll arrows on the right 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 ProDye™ 4C Matrix Standard on Polymer7, select **Promega 4-dye Sequencing** with "Sequencing" and "Polymer7" as application and polymer types, respectively, then select **Calibration**. The 'Assemble the Cartridge' screen will open (Figure 7).

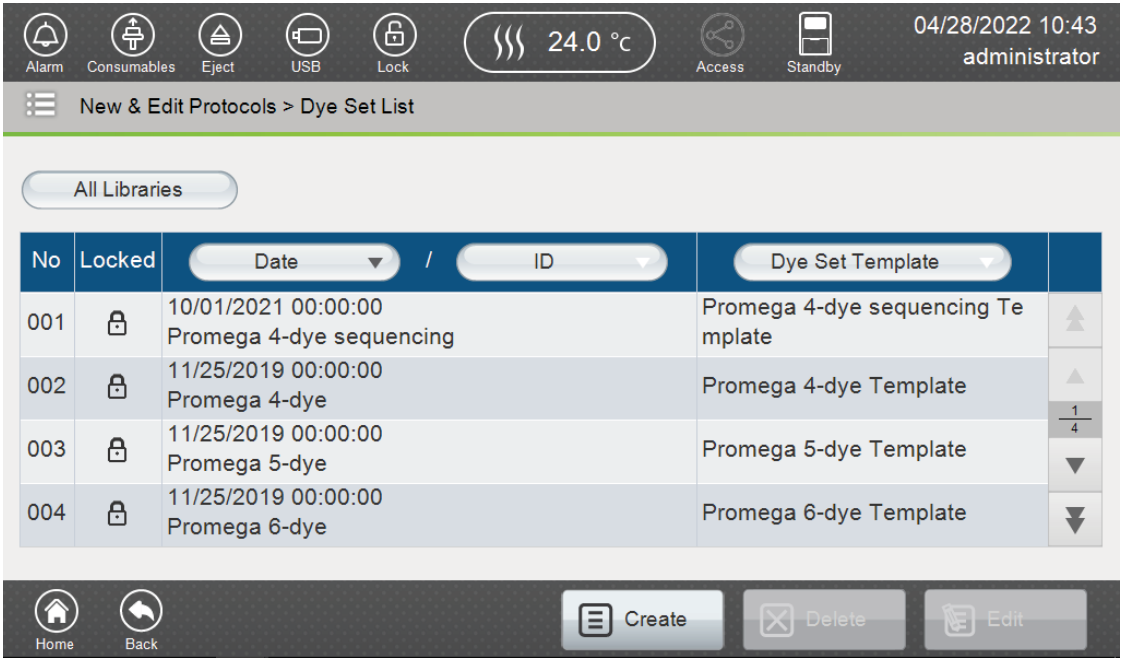

Figure 6. 'Dye Set List' screen.

3.B. Instrument Preparation and Spectral Calibration (continued)

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.

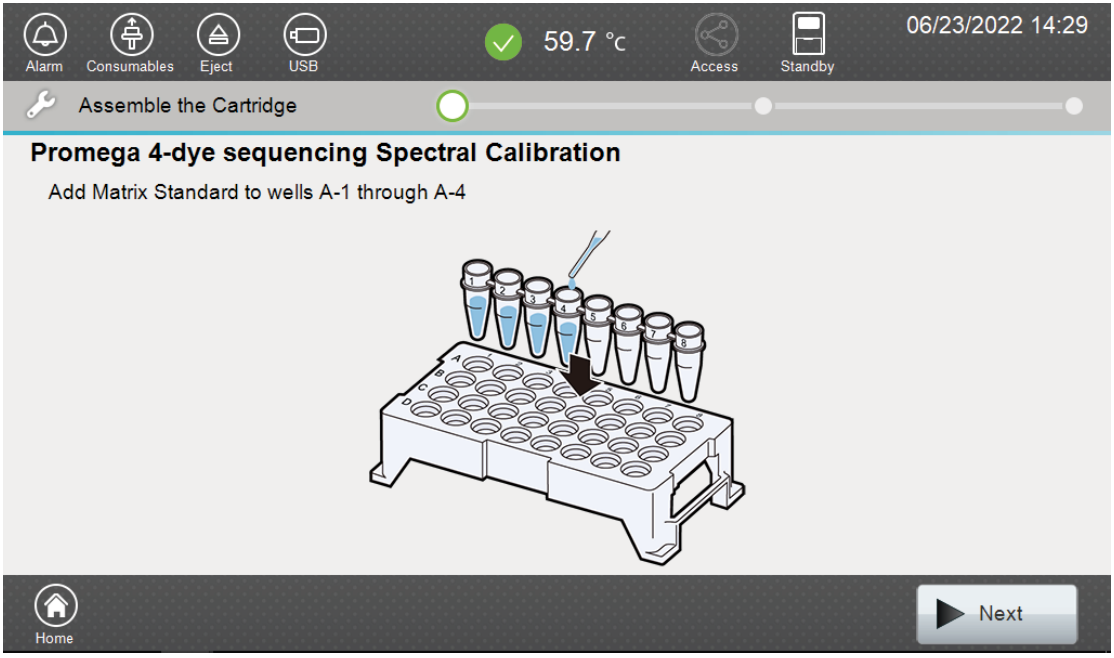

Figure 7. 'Assemble the Cartridge' screen.

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).

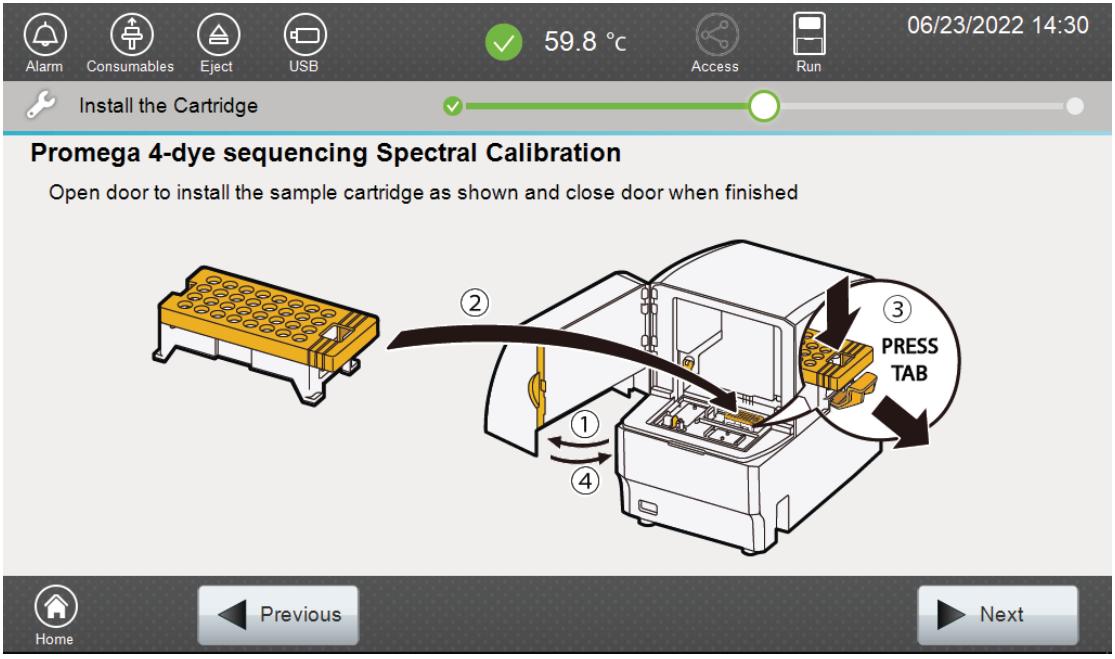

Figure 8. 'Install the Cartridge' screen.

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.

3.B. Instrument Preparation and Spectral Calibration (continued)

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.

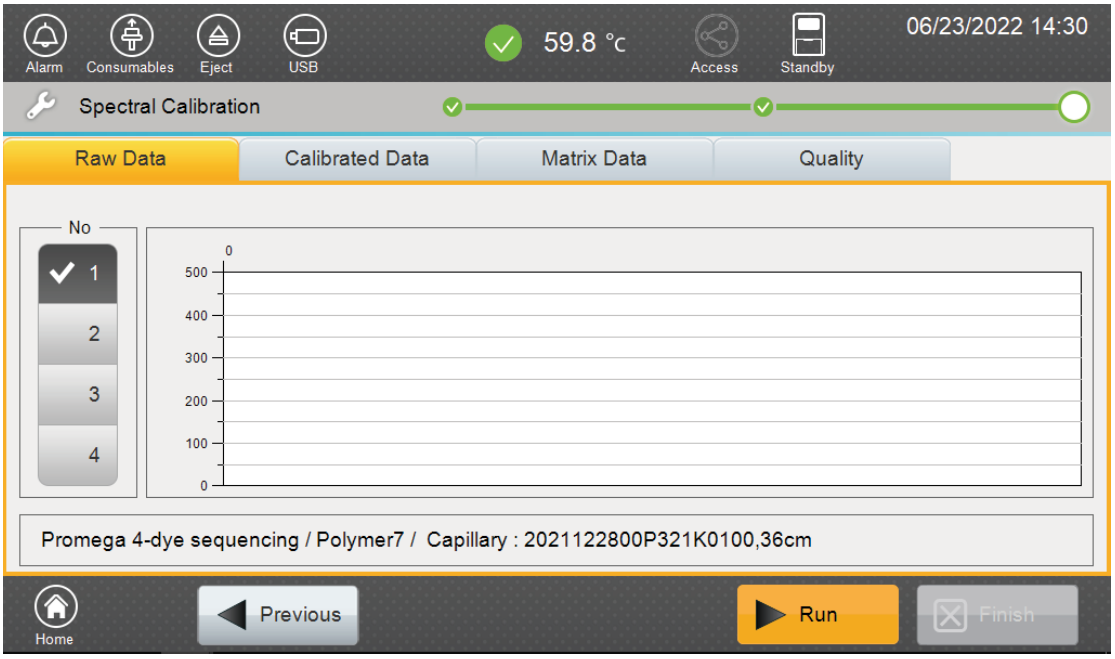

Figure 9. 'Spectral Calibration' screen.

3.C. Reviewing 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.

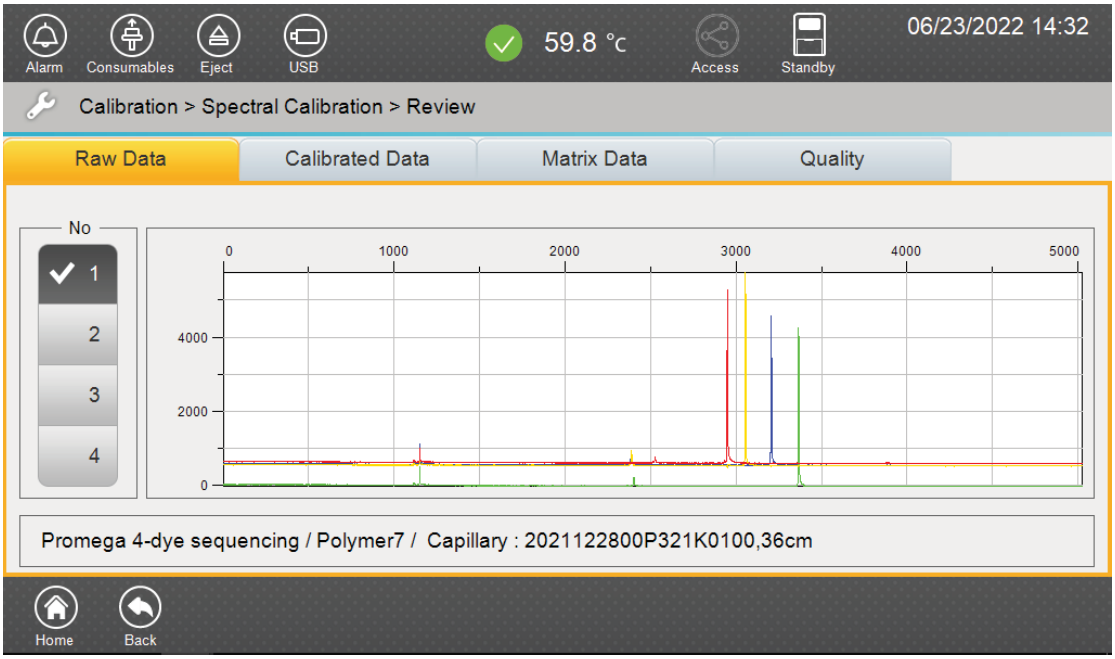

Figure 10. 'Spectral Calibration' screen, 'Raw Data' tab.

3.C. Reviewing Results (continued)

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

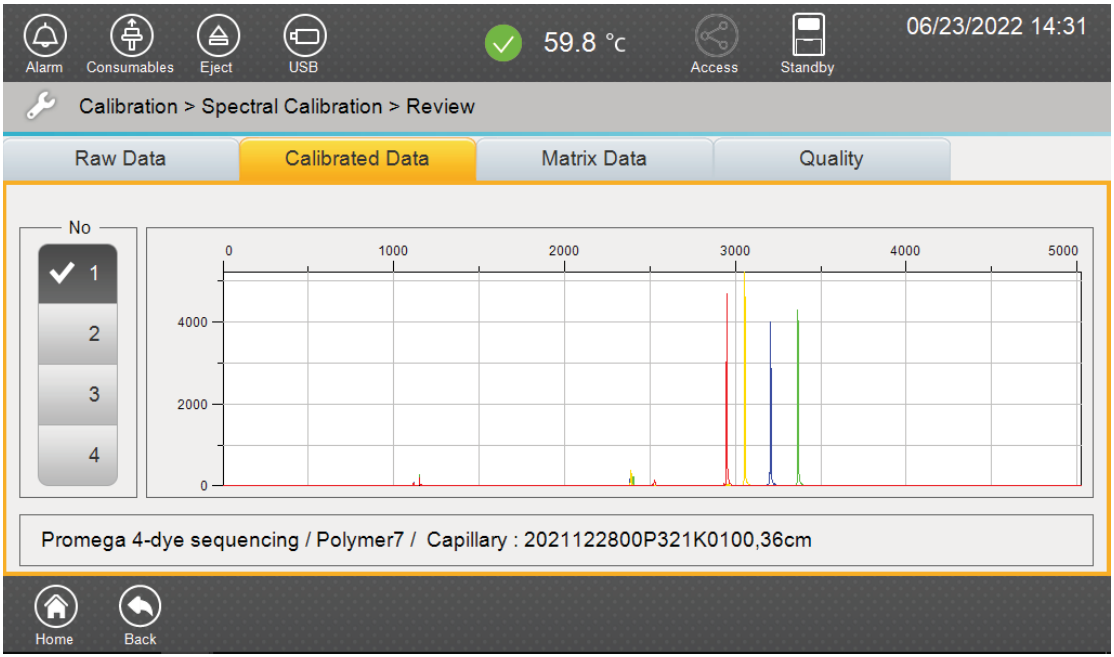

Figure 11. 'Spectral Calibration' screen, 'Calibrated Data' tab.

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

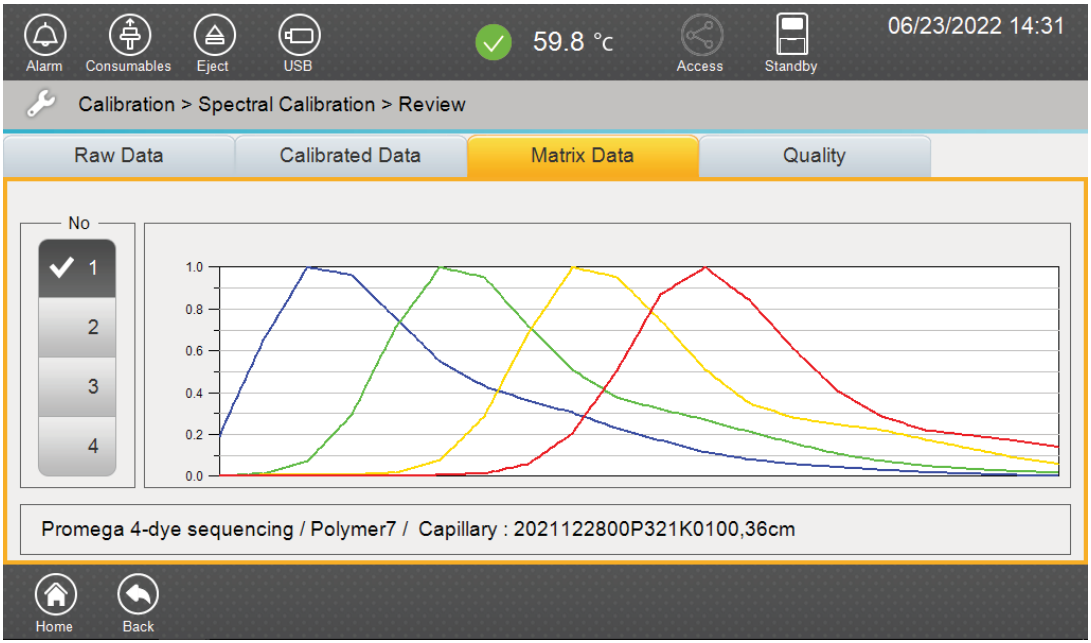

Figure 12. ‘Spectral Calibration’ screen, ‘Matrix Data’ tab.

3.C. Reviewing Results (continued)

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

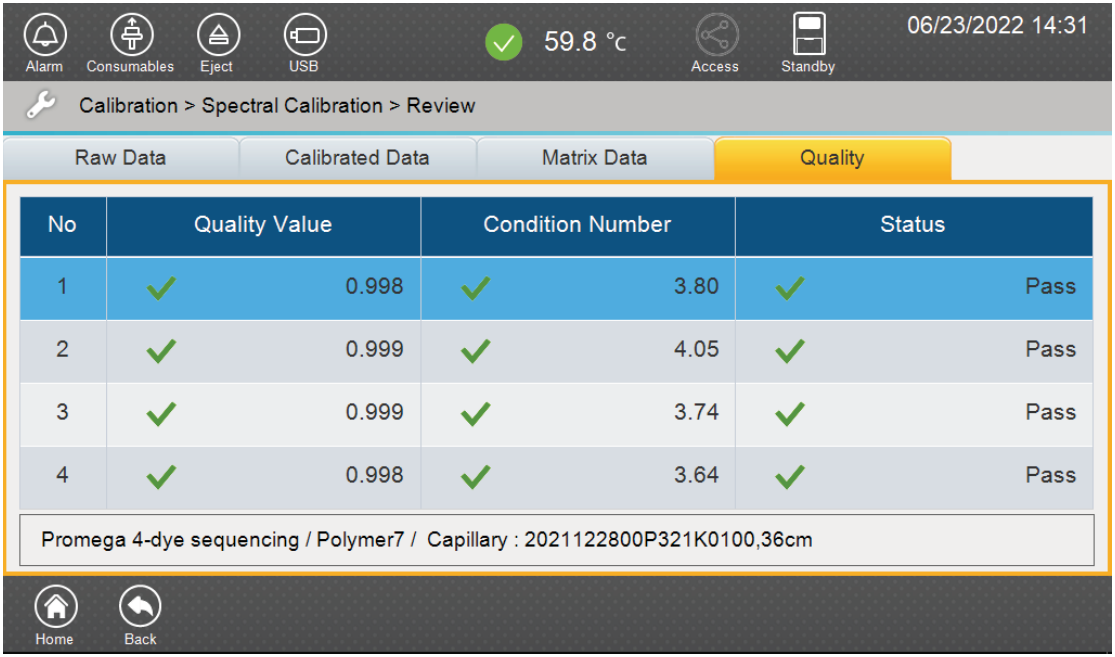

Figure 13. 'Spectral Calibration' screen, 'Quality' tab.

5. Each capillary must meet the passing criteria of ≥0.95 for the Quality Value and ≤5.5 for the Condition Number.

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

   Notes:

   1. Selecting **Run** will rerun the spectral calibration.
   2. Refer to Section 5 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.

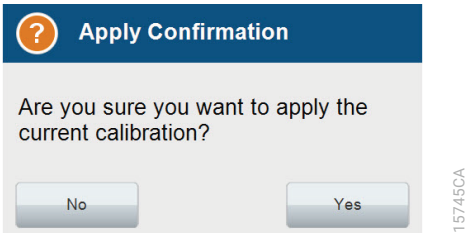

Figure 14. 'Apply Spectral Calibration' confirmation window.

4. 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
- POP-7® polymer for the 3500 or 3500xL Genetic Analyzers
- anode buffer container with 1X buffer
- cathode buffer container with 1X buffer
- MicroAmp® Optical 96-Well Plate (Applied Biosystems Cat.# N8010560) and Septa (Applied Biosystems Cat.# 4412614)
- 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*.

4.A. Matrix Sample Preparation

1. At the first use, thaw the ProDye™ 4C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents at +2°C to +10°C.
2. Vortex the ProDye™ 4C Matrix Mix for 10–15 seconds prior to use. Add 10µl of the ProDye™ 4C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds. Label the tube with the dilution date. The diluted ProDye™ 4C Matrix Mix can be stored for up to 1 week at +2°C to +10°C.
3. Add 10µl of the diluted ProDye™ 4C Matrix Mix prepared in Step 2 to 500µl of Hi-Di™ formamide. Vortex for 10–15 seconds.

4.A. Matrix Sample Preparation (continued)

4. For the Applied Biosystems® 3500xL Genetic Analyzer, wells A1 through H3 of the 96-well plate are used for spectral calibration. Add 15µl of the ProDye™ 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 strip to remove bubbles. Do not heat denature.

   For the Applied Biosystems® 3500 Genetic Analyzer, wells A1 through H1 of the 96-well plate are used for spectral calibration. Add 15µl of the ProDye™ 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 strip to remove 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.

4.B. Instrument Preparation and Spectral Calibration

These instructions are intended as a guide for running the ProDye™ 4C Matrix Standards on the Applied Biosystems® 3500 and 3500xL Genetic Analyzers. They are not intended as comprehensive instructions for using the Applied Biosystems® 3500 and 3500xL Genetic Analyzers. Refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* for more details on performing spectral calibration.

Notes:

1. We have found that using fresh polymer and a 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 *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* for more details on consumable installation, instrument maintenance and spatial calibration.

1. Set the oven temperature to 60°C, and then select **Start Pre-Heat** icon to preheat the oven for at least 30 minutes before the first injection.
2. To perform the spectral calibration, go to the 'Maintenance' tab, select **Spectral**, and under the 'Calibration Run' tab, choose the appropriate fields: "Matrix Standard" from the Chemistry Standard drop-down menu and "Dye Set Z" from the Dye Set drop-down menu.
3. Select **Start Run**.
4. If fewer than the recommended number of capillaries pass, the spectral calibration run will be repeated automatically up to three times. When the spectral calibration is complete, check the quality of the spectral calibration in the Capillary Run Data display and choose either **Accept** or **Reject**.

   Note: Refer to the *3500 Series Data Collection Software User Manual* for the criteria recommended when accepting or rejecting a spectral calibration.

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**

Symptoms	Causes and Comments
Fewer than the recommended number of capillaries passed the spectral calibration	Poor-quality formamide was used. The formamide quality is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or storage at 4°C may cause formamide breakdown. Poor-quality formamide can contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity.
	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 ProDye™ 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 ProDye™ 4C Matrix Mix added to the Hi-Di™ formamide during matrix sample preparation.
	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.
All capillaries failed spectral calibration	For best spectral calibration results, use fresh polymer, fresh buffer and water, and a capillary cartridge/array with fewer than 100 injections.

6. Related Products

Product	Size	Cat. #
ProDye™ Terminator Sequencing System	24 reactions	CR4324
	200 reactions	CR4302
	1,000 reactions	CR4310
ProDye™ Sanger Sequencing Standard	4 × 4 wells	CR4604
	2 × 96 wells	CR4696
ProDye™ 5X Sequencing Buffer	12ml	CR1011

Not for Medical Diagnostic Use.

Spectrum Compact CE System Accessories and Consumables

Product	Size	Cat.#
Spectrum Compact Capillary Cartridge, 4-Capillary, 36cm	1 each	CE2340
Spectrum Compact Polymer7	4 × 64 wells	CE2307
Spectrum Compact Buffer	2 pairs	CE2300
Spectrum Compact Cathode Septa Mat	10 each	CE2301
Spectrum Compact Cathode Retainer	4 each	CE2302
Spectrum Compact Strip Base & Retainer, 32-Well	4 each	CE2332
Strip Septa Mat, 8-Well	24 each	CE2308

Not for Medical Diagnostic Use.

7. Summary of Changes

The following changes were made to the 9/22 revision of this document:

1. Updated Materials to Be Supplied by the User in Section 3.
2. Corrected calibration method name in Section 3.B, Step 4.
3. Replaced Figures 6–13 in Section 3.