



TECHNICAL MANUAL

ProDye™ Terminator Sequencing System

Instructions for Use of Products
CR4324, CR4302 and CR4310

ProDye™ Terminator Sequencing System

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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1. Description

The ProDye™ Terminator Sequencing System provides users with an improved method of fluorescent Sanger sequencing. The ProDye™ Terminator Sequencing System is compatible with a variety of DNA templates and the sequencing products can be separated on the Spectrum Compact CE System as well as the Applied Biosystems® 3130, 3130xl, 3500, 3500xl, 3730, 3730xl and SeqStudio® Genetic Analyzers without changes to the capillary electrophoresis workflow or calibrations.

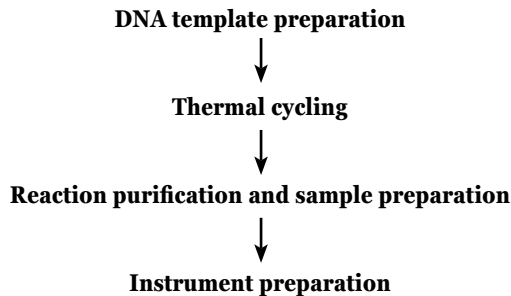


Figure 1. An overview of the ProDye™ Terminator Sequencing System protocol.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
ProDye™ Terminator Sequencing System	24 reactions	CR4324

For in vitro Research Use Only. Not for use in diagnostic procedures. Contains sufficient reagents for 24 reactions of 20µl each. Includes:

- 1 × 200µl ProDye™ 2.5X Master Mix
- 1 × 1,000µl ProDye™ 5X Sequencing Buffer
- 1 × 20µg pGEM®-3Zf(+) Vector
- 1 × 2µg pUC/M13 Forward Primer (24mer)
- 1 × 1,250µl Nuclease-Free Water

PRODUCT	SIZE	CAT. #
ProDye™ Terminator Sequencing System	200 reactions	CR4302

For in vitro Research Use Only. Not for use in diagnostic procedures. Contains sufficient reagents for 200 reactions of 20µl each. Includes:

- 2 × 800µl ProDye™ 2.5X Master Mix
- 3 × 1,000µl ProDye™ 5X Sequencing Buffer
- 1 × 20µg pGEM®-3Zf(+) Vector
- 1 × 2µg pUC/M13 Forward Primer (24mer)
- 5 × 1,250µl Nuclease-Free Water

PRODUCT	SIZE	CAT. #
ProDye™ Terminator Sequencing System	1,000 reactions	CR4310

For in vitro Research Use Only. Not for use in diagnostic procedures. Contains sufficient reagents for 1,000 reactions of 20µl each. Includes:

- 10 × 800µl ProDye™ 2.5X Master Mix
- 1 × 12ml ProDye™ 5X Sequencing Buffer
- 1 × 20µg pGEM®-3Zf(+) Vector
- 1 × 2µg pUC/M13 Forward Primer (24mer)
- 2 × 13ml Nuclease-Free Water

Storage Conditions: Store all components at –30°C to –10°C in a nonfrost-free freezer. After first use, store the ProDye™ 5X Sequencing Buffer at +2°C to +10°C for up to 6 months. The ProDye™ 2.5X Master Mix is light-sensitive and should be stored in a dark location. Avoid excessive (>10) freeze-thaw cycles. Dispense ProDye™ 2.5X Master Mix into smaller aliquots and freeze at –30°C to –10°C in a nonfrost-free freezer to avoid freeze-thaw cycles.

Available Separately

PRODUCT	SIZE	CAT. #
ProDye™ 4C Matrix Standard	5 preps	CR4500
ProDye™ Sanger Sequencing Standard	4 × 4 wells	CR4604
ProDye™ Sanger Sequencing Standard	2 × 96 wells	CR4696
ProDye™ 5X Sequencing Buffer	12ml	CR1011

We offer a sequence viewer, ProView™ Sequencing Software, free of charge. The software is available for download at: www.promega.com/ProviewDownload

3. Before You Begin

3.A. Precautions

The ProDye™ 2.5X Master Mix is light sensitive and should be stored in a location protected from light. All reactions should be assembled on ice.

The quality and quantity of template DNA in the sequencing reactions are critical factors for obtaining high-quality sequencing data. Extracted DNA should be free of inhibitory contaminants. PCR products should be purified to remove leftover primers and deoxynucleotides.



Some reagents used for analyzing sequencing reactions are potentially hazardous and should be handled accordingly. 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.B. Spectral Calibration

Prior to running completed reactions on the Spectrum Compact CE System or Applied Biosystems® Genetic Analyzers, a spectral calibration must be performed. This can be done using the ProDye™ 4C Matrix Standard (Cat.# CR4500) or ProDye™ Sanger Sequencing Standard (Cat.# CR4604, CR4696). Consult the instructions *ProDye™ 4C Matrix Standard Technical Manual #TM674* or *ProDye™ Sequencing Standard Quick Protocol #FB239* for further usage information. On Applied Biosystems® instruments, if a spectral calibration was previously performed for Dye Set Z-BigDyeV3 (3130/3730 instruments) or Dye Set Z (3500 instruments), the instrument is already calibrated for the ProDye™ Terminator Sequencing System. If the instrument was not calibrated previously or if you wish to rerun the calibration, we recommend using the matrix standards listed in Table 1.

Table 1. Spectral Calibration Options.

Instrument	Standard to Use for Calibration	Cat.#
Spectrum Compact CE System	ProDye™ 4C Matrix Standard	CR4500
Applied Biosystems® 3130/3130xl Genetic Analyzer*	ProDye™ 4C Matrix Standard	CR4500
Applied Biosystems® 3500/3500xl Genetic Analyzer*	ProDye™ 4C Matrix Standard	CR4500
Applied Biosystems® 3730/3730xl Genetic Analyzer	ProDye™ Sanger Sequencing Standard	CR4696

*ProDye™ Sanger Sequencing Standard (Cat # CR4604, CR4696) can also be used to calibrate these instruments although we recommend the ProDye™ 4C Matrix Standard.

3.C. Template Information

Control Template

This system includes the pGEM3Zf(+) Vector and pUC/M13 Forward Primer (24mer) to use as control DNA and primer for troubleshooting sequencing problems.

Template Quality

This system was developed to generate high-quality sequence data on a variety of templates, including plasmid DNA, PCR products, etc. Only use highly purified DNA that is devoid of contaminating DNA, RNA, protein, primers or amplification inhibitors.

Template Quantity

Template quantity and concentration are critical in obtaining high-quality sequence data. Measure DNA concentration using a spectrophotometer, or by comparing with a sample of known concentration during agarose gel electrophoresis. Table 2 lists recommended quantities of DNA, depending on the DNA size.

Table 2. Recommended DNA Quantities.

Template	Recommended Quantity per Reaction
PCR: 100–200bp	3ng
PCR: 200–500bp	10ng
PCR: 500–1,000bp	50ng
PCR: 1,000–2,000bp	75ng
PCR: >2,000bp	100ng
Single-stranded DNA	50ng
Double-stranded DNA	300ng

Note: Higher or lower amounts of template DNA may be used to optimize the reactions.

3.D. Primer Information

We recommend using a primer with an annealing temperature between 55–65°C, and adding 2.5–5.0pmol of purified primer into each reaction.

4. Protocols for Sequencing DNA using the ProDye™ Terminator Sequencing System

The ProDye™ Terminator Sequencing System is compatible with many currently available thermal cyclers such as the GeneAmp® 9700, Veriti® and ProFlex® 96-well thermal cyclers. Other thermal cyclers can be used, but you may need to optimize the thermal cycling protocol.

4.A. Reaction Setup

Materials to Be Supplied by the User

- MicroAmp® Optical 96-Well Reaction Plates (Applied Biosystems Cat.# N8010560) or 0.2ml MicroAmp® Optical 8-Tube Strips (Applied Biosystems Cat.# 4316567)
- strip caps or foil seals
- thermal cycler with heated lid
- silicone compression mat (for use with foil seals only)
- centrifuge compatible with 96-well plates or strip tubes and capable of $\geq 1,800 \times g$ (if using ethanol precipitation for cleanup; see Section 4.C)

4.A. Reaction Setup (continued)

Preparing the Reactions

Note: The ProDye™ 2.5X Master Mix is light-sensitive. We recommend minimizing exposure to light when setting up the reactions. All reactions should be assembled on ice.

1. At the first use, thaw the ProDye™ 2.5X Master Mix and ProDye™ 5X Sequencing Buffer completely. After the first use, store the ProDye™ 5X Sequencing Buffer at +2°C to +10°C. Store the ProDye™ 2.5X Master Mix at –30°C to –10°C protected from light. If necessary, dispense the ProDye™ 2.5X Master Mix into smaller aliquots to limit the number of freeze-thaw cycles.
2. Vortex each component for 5–10 seconds and then briefly spin in a benchtop microcentrifuge to ensure all materials are collected at the bottom of the tubes.
3. Add components as indicated in Table 3 to eight-well strip tubes or a 96-well plate:

Table 3. Sequencing Reaction Setup.

Component	Quantity (20µl reaction)	Quantity (10µl reaction)	pGEM®-3Zf(+) Control Reaction
ProDye™ 2.5X Master Mix	8µl	4µl	8µl
Template DNA	See Section 3.C.	See Section 3.C.	1µl*
Forward or Reverse primer	2.5–5pmol	2.5–5pmol	3µl
Nuclease-Free Water	to 20µl	to 10µl	8µl
Total reaction volume	20µl	10µl	20µl

*First dilute the pGEM®-3Zf(+) Vector by adding 2µl of vector to 8µl of Nuclease-Free Water.

In some cases, you may be able to optimize sequencing reactions using less ProDye™ 2.5X Master Mix and supplementing with ProDye™ 5X Sequencing Buffer as shown in Table 4.

Table 4. Alternate Reaction Setup.

Component	Quantity (20µl reaction)
ProDye™ 2.5X Master Mix	4µl
ProDye™ 5X Sequencing Buffer	2µl
Template DNA	See 3.C.
Forward or Reverse primer	2.5–5pmol
Nuclease-Free Water	to 20µl
Total reaction volume	20µl

4. Cap strip tubes or wells with compatible strip caps or a foil seal, vortex lightly to mix, and then centrifuge briefly to ensure all materials are collected at the bottom of the tubes or wells.

4.B. Thermal Cycling

1. Place strip tubes or plate in the thermal cycler.
2. Select and run the following protocol:

Note: If your thermal cycler offers sample volume adjustment, match the sample volume to the reaction size you used to set up your reactions.

Temperature	Time (minutes)	Cycles
96°C	1:00	1
96°C	0:10	
50°C	0:05	25
60°C	4:00	
4°C	Hold	

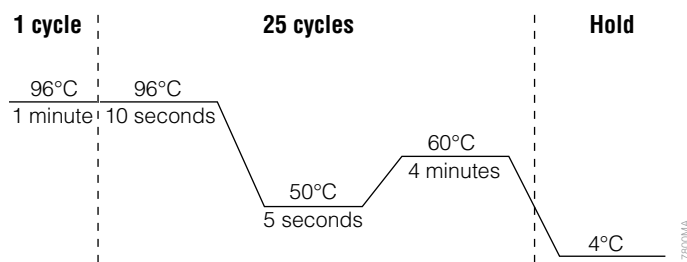


Figure 2. The thermal cycling protocol for the GeneAmp® PCR System 9700, Veriti® 96-Well Thermal Cycler and ProFlex® PCR System.

3. After completing the thermal cycling protocol, proceed with reaction purification or store reactions protected from light (or on the thermal cycler) at +4°C overnight, or at –20°C for longer-term storage.

4.C. Reaction Purification and Sample Preparation

Purifying the reactions, in particular removing the unincorporated dye-labeled terminators, is critical for obtaining accurate sequence information. We recommend using ethanol precipitation. Other methods that will remove the unincorporated dye-labeled terminators may work. However, not all purification methods have been tested and may require optimization by the user.

Materials to Be Supplied by the User

- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)
- 100% ethanol†
- 70% ethanol
- 125mM EDTA (pH 8.0)
- swinging bucket centrifuge with plate adapters
- 96-well plate base
- strip tube septa (Promega) or 96-well septa mats (Applied Biosystems)
- MicroAmp® 8-cap strips (Applied Biosystems) or foil plate seals

†Absolute (100%) ethanol is hygroscopic and will absorb moisture from the air. Use a fresh bottle and replace regularly.



The formamide quality is critical. Use only the recommended formamide. Freeze formamide in aliquots at -20°C . Multiple freeze-thaw cycles or long-term 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 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 warning label; take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Purifying Reactions in Strip Tubes

1. After thermal cycling is complete, briefly centrifuge the strip tubes to collect sample at the bottom of the tube.
2. Add 125mM EDTA (pH 8.0) and 100% ethanol to each well in the following amounts:

Table 5. Ethanol Precipitation Reagent Additions, 8-Well Strip Tube.

Reagent	20µl Reaction	10µl Reaction
125mM EDTA (pH 8.0)	5µl	2.5µl
100% ethanol	60µl	30µl

Note: Add EDTA to samples first and ensure that it mixes with the samples before adding ethanol. This will prevent the ethanol from precipitating the EDTA.

3. Cap tubes and mix by inversion or briefly vortexing.
4. Incubate at room temperature for 15 minutes, protected from light.
5. Insert strip tubes in a 96-well plate base, and place the base in a compatible swinging bucket centrifuge.

Note: Add several additional empty strip tubes throughout the plate base for support in future steps.

6. Centrifuge the samples at $1,800\text{--}2,000 \times g$ at 4°C for 45 minutes. Remove the plate base containing strip tubes after centrifugation is complete.
7. Place a paper towel on the swinging bucket plate adapter, remove the caps and invert the plate base with strip tubes onto the paper towel.
8. Centrifuge the plate base containing strip tubes at $180 \times g$ at 4°C for 1 minute.
9. Remove plate base with strip tubes from centrifuge and discard the paper towel.
10. Add $60\mu\text{l}$ of 70% ethanol to each sample well and cap the tubes.
11. Centrifuge the samples at $1,800\text{--}2,000 \times g$ at 4°C for 15 minutes. Remove the plate base containing strip tubes after centrifugation is complete.
12. Place a paper towel on the swinging bucket plate adapter, remove the caps and invert the plate base with strip tubes onto the paper towel.
13. Centrifuge the plate base containing strip tubes at $180 \times g$ at 4°C for 1 minute.
14. Remove plate base with strip tubes from centrifuge and discard paper towel.
15. Add $10\mu\text{l}$ of formamide to each sample well.
16. Insert a strip septa mat, lightly vortex and briefly centrifuge the strip tube to ensure contents are at the bottom. If you plan to run samples on an instrument that uses 96-well plates, transfer the samples to a 96-well plate. Briefly centrifuge the plate to ensure contents are at the bottom.

Purifying Reactions in 96-Well Plates

1. After thermal cycling is complete, briefly centrifuge the plate to collect sample at the bottom of the wells.
2. Add 125mM EDTA pH 8.0 and 100% ethanol to each well in the following amounts:

Table 6. Ethanol Precipitation Reagent Additions, 96-Well Plate.

Reagent	20 μl Reaction	10 μl Reaction
125mM EDTA (pH 8.0)	5 μl	2.5 μl
100% ethanol	60 μl	30 μl

Note: Add EDTA to samples first and ensure that it mixes with the samples before adding ethanol. This will prevent the ethanol from precipitating the EDTA.

3. Seal plate with an adhesive foil seal and mix by inversion or briefly vortexing.
4. Incubate at room temperature for 15 minutes, protected from light.
5. Insert plate in a 96-well plate base, and place the base in a swinging bucket centrifuge with plate adapters.
6. Spin the samples at $1,800\text{--}2,000 \times g$ at 4°C for 45 minutes. Remove the plate base after spin is complete.
7. Place a paper towel on the swinging bucket plate adapter, remove foil seal and invert the plate and plate base onto the paper towel.

4.C. Reaction Purification and Sample Preparation (continued)

8. Centrifuge the plate with plate base at $180 \times g$ at 4°C for 1 minute.
9. Remove plate and plate base from centrifuge and discard paper towel.
10. Add $60\mu\text{l}$ of 70% ethanol to each sample well and seal plate with an adhesive foil seal.
11. Spin the samples at $1,800\text{--}2,000 \times g$ at 4°C for 15 minutes. Remove the plate with plate base after spin is complete.
12. Place a paper towel on the swinging bucket plate adapter, remove foil seal and invert the plate with plate base onto the paper towel.
13. Centrifuge the plate and plate base at $180 \times g$ at 4°C for 1 minute.
14. Remove plate with plate base from centrifuge and discard paper towel.
15. Add $10\mu\text{l}$ of formamide to each well
16. Insert plate septa (or optional adhesive foil seal for the Applied Biosystems® 3730 and 3730xl Genetic Analyzers only), vortex briefly and centrifuge plate to ensure all contents are at the bottom of the wells. Alternatively, if you plan to run the samples on an instrument that uses 8-well strip tubes, transfer the samples into 8-well strip tubes and insert a strip septa mat. Briefly centrifuge strip tube(s) to ensure all contents are at the bottom of the wells.

5. Instrument Preparation

5.A. Capillary Electrophoresis on the Spectrum Compact CE System

Materials to Be Supplied by the User

- Spectrum Compact CE System (Cat.# CE1304)
- Spectrum Compact Capillary Cartridge, 4-Capillary 36cm (Cat.# CE2340)
- Spectrum Compact Polymer7 (Cat.# CE2307 or CE2407)
- 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)
- Strip Septa Mat, 8-Well (Cat.# CE2308)
- Centrifuge compatible with 8-tube strips
- MicroAmp® Optical 8-Tube Strip, 0.2ml (Applied Biosystems Cat.# 4316567)
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)



The quality of formamide is critical. Use only the recommended 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 may 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.

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. Instructions in Section 5 also apply to the HITACHI DS3000 Compact CE Sequencer.
4. Unused wells for any set of four wells being injected should contain formamide alone. Do not leave uninjected wells empty.
5. For polymer cartridge compatibility information, refer to the *Spectrum Compact CE System Operating Manual #TMD058*

Sample Layout

Samples prepared in 8-well strip tubes are assembled into the strip base and retainer (Figure 2) to form the sample cartridge (Figure 3), which can be loaded onto the Spectrum Compact CE System. Samples are injected in groups of four, such that one 8-well strip tube can be used for two injections. Each 8-well strip tube corresponds to one lane (A, B, C or D) of the sample cartridge. Samples are injected in groups of four across a lane (not by column). For example, samples in well positions A1 through A4 are injected together, followed by samples in well positions A5 through A8 for the second injection. This pattern is then repeated for samples in lanes B, C and D. Refer to the *Spectrum Compact CE System Operating Manual #TMD058* for information on changing the injection order during run setup.

		Wells							
		1	2	3	4	5	6	7	8
Lanes	A	Injection 1				Injection 2			
	B	Injection 3				Injection 4			
	C	Injection 5				Injection 6			
	D	Injection 7				Injection 8			

5.B. Assembling Sample Cartridge

1. Place the 8-well strip tube(s) with the Strip Septa Mat, 8-well, into the strip base (Figure 2). When using fewer than 4 strips in the run, you can place the strips in any lane.

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 when placing a sample 8-well strip tube into the strip base to make certain that the correct 8-well strip tube is in the correct lane.

Additionally, well numbers 1 to 8 are embossed on each 8-well strip tube. When placing a sample 8-well strip tube into the strip base, be sure that the well numbers on each sample 8-well strip tube match those on the strip base.

2. To complete the assembly, place the retainer over the strip(s) 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 3).



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Figure 2. Assembling the Spectrum Compact Strip Base and Retainer.



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Figure 3. Assembled Spectrum Compact Sample Cartridge.

5.C. Instrument Preparation

These instructions are intended as a guide for running samples on the Spectrum Compact CE System (after successful spatial and spectral calibration with the ProDye™ 4C Matrix Standard). 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 instrument maintenance, instructions for installing consumables, performing a spatial calibration and managing protocols (Assay, Instrument, and Basecalling protocols). Refer to the *ProDye™ 4C Matrix Standard Technical Manual #TM674* for instructions on performing a spectral calibration.

1. Before starting a run, ensure all consumables are installed and in sufficient supply. For best results, use unexpired reagents that are within the recommended use range (Figure 5 and Table 7). Refer to the ‘Consumables’ screen to determine if any consumables need to be replaced. To access the ‘Consumables’ screen, select the **Consumables** icon in the Header on the ‘Main Menu’ screen (Figure 4). The ‘Consumables’ screen displays information for the four consumables on the instrument: Polymer, Capillary Cartridge and Anode and Cathode Buffers.



Figure 4. Spectrum Compact CE System Software ‘Main Menu’ screen.

5.C. Instrument Preparation (continued)

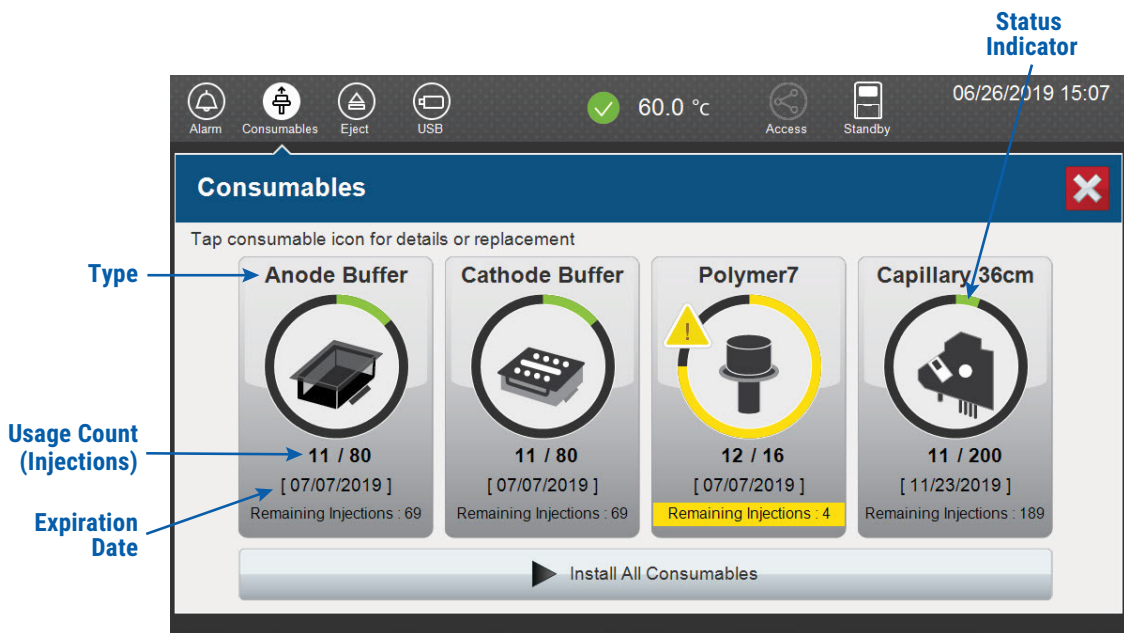





Figure 5. Spectrum Compact CE System ‘Consumables’ screen. Usage count (number of injections), on-instrument expiration date and remaining injections are displayed on the ‘Consumables’ screen for each consumable, as well as polymer type.

A consumable status indicator will appear on the icons of consumables that need attention. There are three indicators (Table 7).

Table 7. Consumable Warning Symbols.

Symbol	Description
	Reaching consumable expiration date, on-instrument expiration date or injection limit for consumable.
	Consumable expiration date, on-instrument expiration date or injection limit for consumable has passed.
	Cannot perform a run because maximum injection count reached for this consumable.

2. Select the **Oven Temperature** icon in the Header on the ‘Main Menu’ screen as shown in Figure 6 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.

Oven Temperature Icon

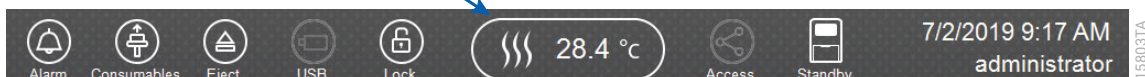


Figure 6. Preheating oven.

3. Select **Sequencing Analysis** on the ‘Main Menu’ screen (Figure 7)

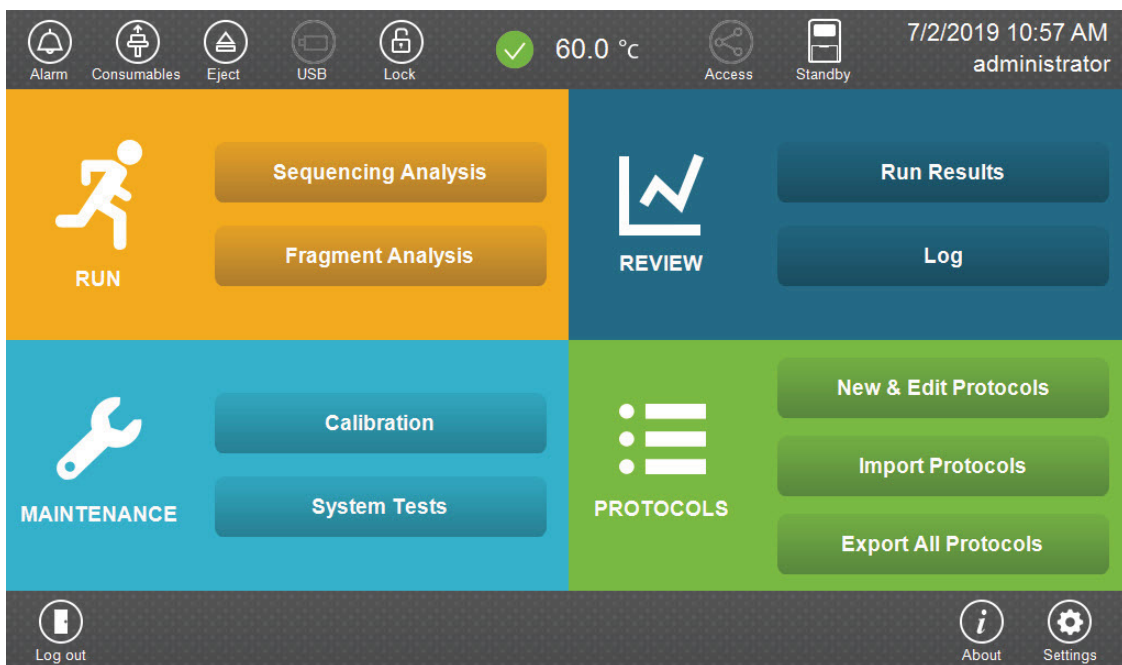


Figure 7. Spectrum Compact CE System Software ‘Main Menu’ screen.

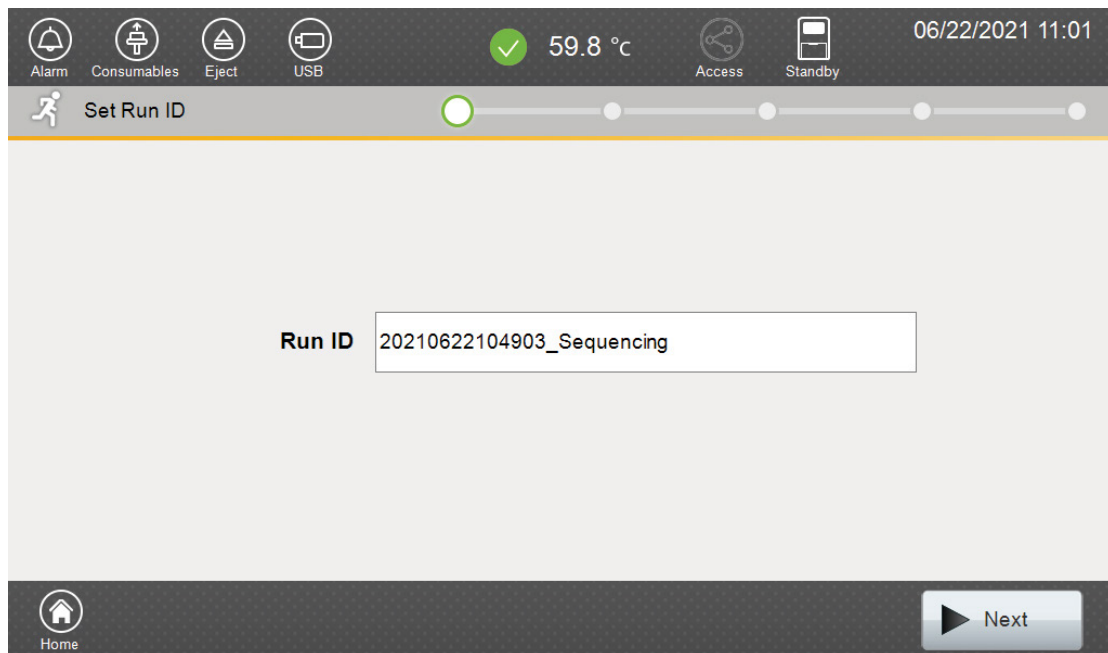
5.C. Instrument Preparation (continued)

- Use the default name that includes date, time and “Sequencing” or enter a Run ID on the ‘Set Run ID’ screen (Figure 8). Select the Run ID box to open the ‘Run ID’ window and a keypad will become active on the touch screen. Alternatively, the Run ID can be entered using a traditional keyboard if one is connected to the Spectrum Compact CE System.

Notes:

- The Run ID will be included in the file names for the samples.
- The following table lists rules for characters that can be used for a Run ID, Strip ID and sample name.

Acceptable Characters	1 to 30 characters for Strip IDs
	1 to 50 characters for Run IDs and sample names
	Upper and lowercase alphabetic characters
	Numbers
	Symbols unless listed below
Unacceptable Characters	# % & { } \ < > * ? / \$! “ ” : @ + ‘ ’ = and spaces



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Figure 8. ‘Set Run ID’ screen.

5. Select **Next** to proceed to the message screen for placement of strips into the sample cartridge (Figure 9).
6. Follow the message screen for placement of strips into the sample cartridge (Figure 9).
Note: Ensure that the correct strip tube is placed into the correct lane (A through D) on the strip base and that wells 1 to 8 of the strip tube are correctly aligned with well positions 1 to 8 on the strip base.

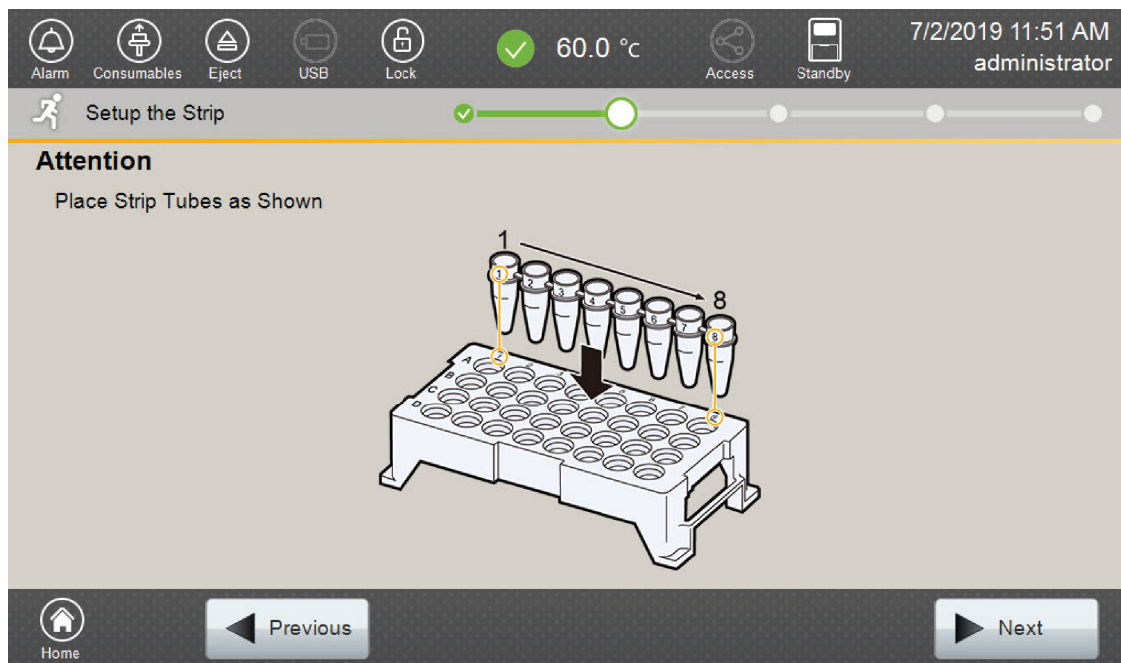


Figure 9. 'Setup the Strip' screen.

5.D. Assigning Run Information to Samples

There are four methods to assign run information to samples:

1. Creating new strip information
2. Reusing run information from a list of completed runs
3. Loading saved strip information
4. Importing strip information

The following instructions are for creating new strip information only. For details on the remaining three methods, please see the *Spectrum Compact CE System Operating Manual #TMD058*.

Note: The Spectrum Compact CE System includes software for remote access to the instrument. This software allows a user to create and setup sample strips by remotely accessing the Spectrum Compact CE System software using a PC connected directly or via a lab network to the instrument. This software also allows a user to edit protocols and assays, monitor runs and export data. Refer to the *Spectrum Compact CE System Remote Access Software Manual #TMD064*.

1. Select **Next** on the ‘Setup the Strip’ screen shown in Figure 9. This will take you to the ‘Setup Strip Information’ screen shown in Figure 10.

Note: The ‘Setup Strip Information’ screen is divided into two sections: Sample Information and Strip ID.

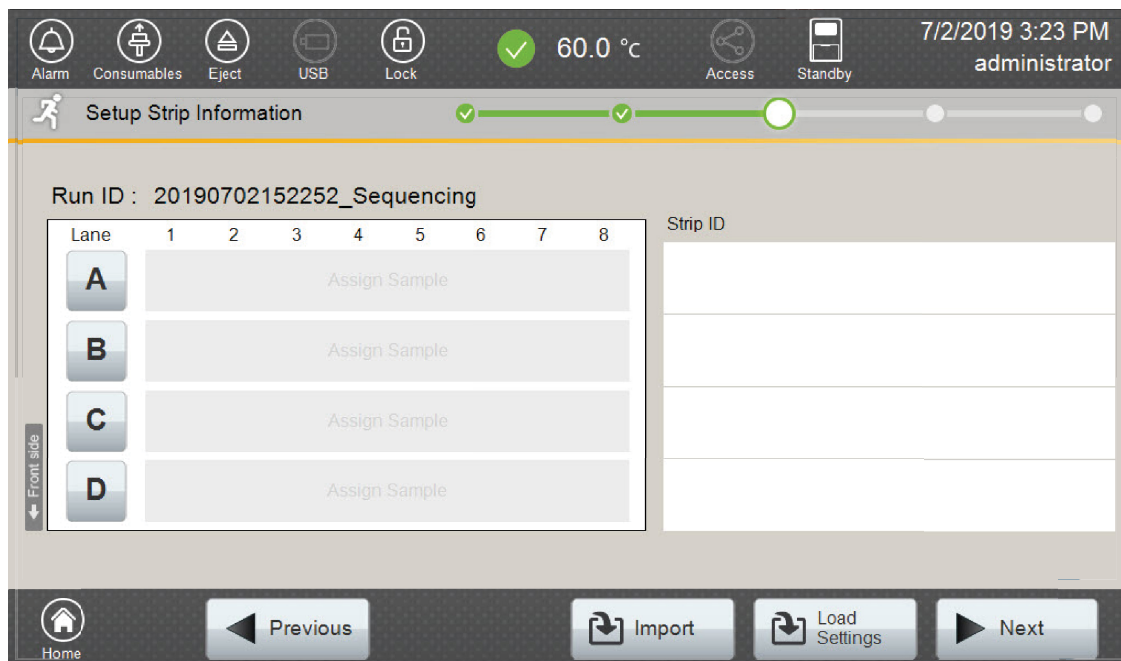


Figure 10. ‘Setup Strip Information’ screen.

2. Select a lane icon (A, B, C or D) to enter information for that 8-well strip tube. This opens the ‘Edit Strip Information’ screen for that lane (Figure 11). This screen displays fields for defining the Strip ID, run assay, sample name and sample type for that lane. Each well in the strip is represented along the left side of the screen. The first injection set of the strip (wells 1–4) is displayed on the screen. You can use the arrows on the right side of the screen to scroll to the second injection set (wells 5–8).

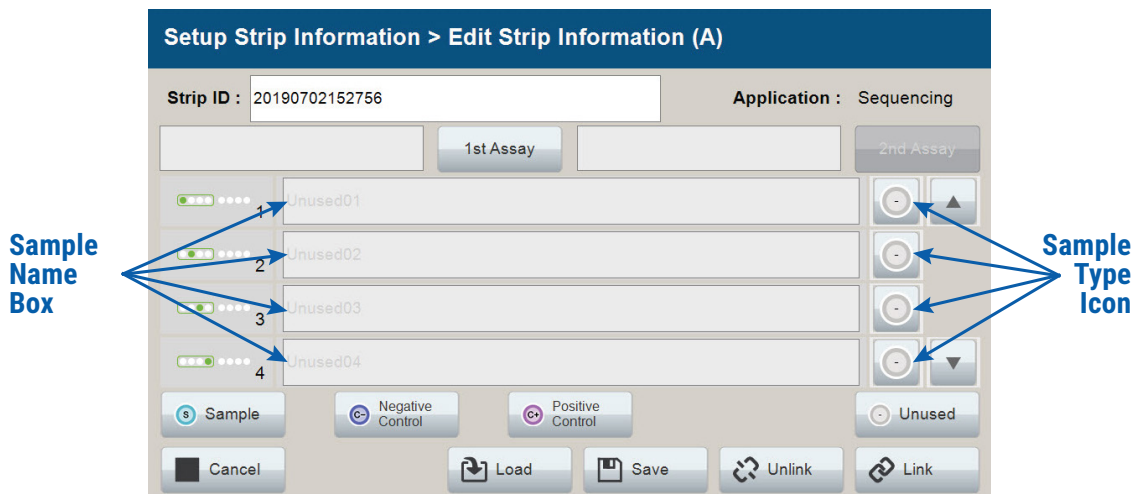


Figure 11. ‘Edit Strip Information’ screen.

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5.D. Assigning Run Information to Samples (continued)

3. The default Strip ID is date and time. It can be modified by selecting the Strip ID field. This opens the 'Set Strip ID' window and a keypad will become active on the touch screen (Figure 12). Alternatively, the Strip ID can be entered using a traditional keyboard if one is connected to the Spectrum Compact CE System. Enter the appropriate Strip ID, and then select **OK** to exit and return to the 'Edit Strip Information' screen.

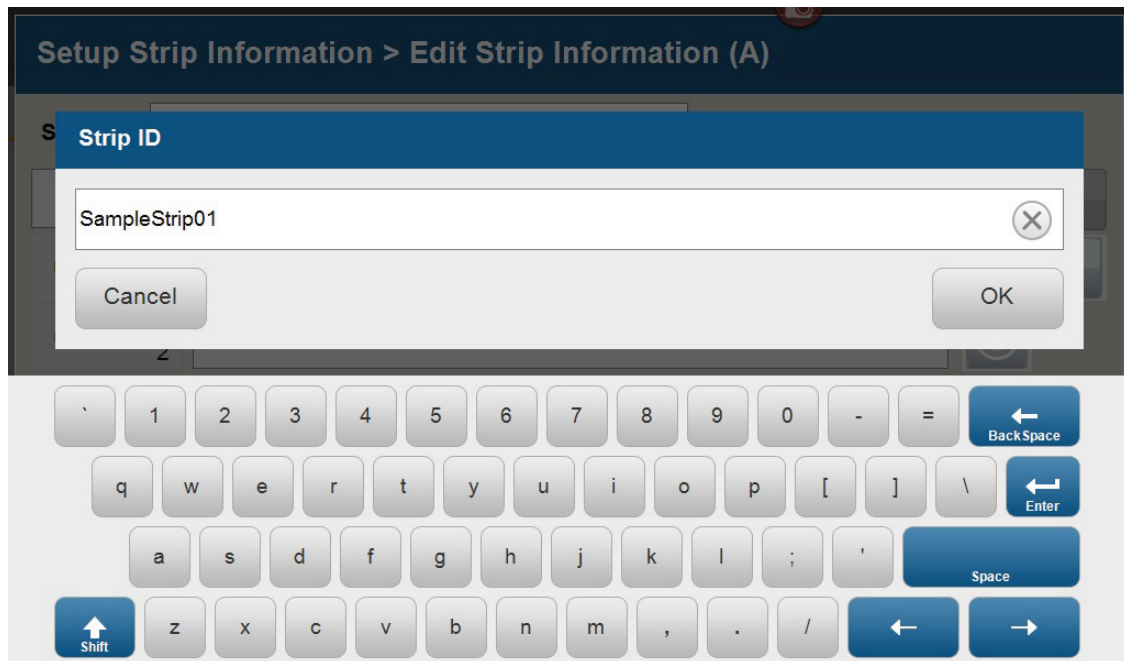






Figure 12. 'Set Strip ID' window.

- Select a sample type on the 'Edit Strip Information' screen (Figure 13). Sample types must be selected for each well position before the Sample Name box becomes active for entry of sample name. Sample types are as follows:

Symbol	Sample Type
	Sample
	Negative Control
	Positive Control
	Unused

- To assign a sample type to a well, select the appropriate sample type along the bottom of the 'Edit Strip Information' screen (Figure 13), and then select the **Sample Type** icon to the right of the sample name field for the desired well. This icon will then display the sample type selected for that well (Figure 14).

Note: A sample type other than “Unused” must be assigned to at least one well in each injection set. If all of the four wells in an injection set are assigned as “Unused”, the injection set will not be run. If all eight wells in a strip are assigned as “Unused”, a warning message will be displayed, and no strip information will be assigned. Unused wells for any set of four wells being injected should contain formamide alone. Do not leave uninjected wells empty.

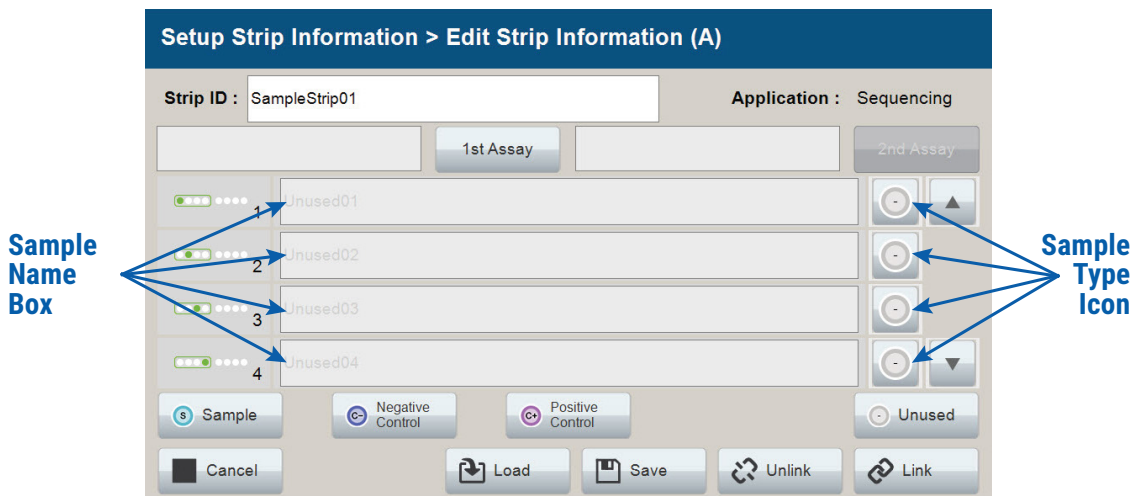


Figure 13. Sample Type and Sample Name on 'Edit Strip Information' screen.

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5.D. Assigning Run Information to Samples (continued)

- Enter a sample name for each well position by selecting the Sample Name box adjacent to the well number on the 'Edit Strip Information' screen (Figure 14). This opens the 'Set Sample Name' window and a keypad will become active on the touch screen (Figure 15). Alternatively, the sample name can be entered using a traditional keyboard if one is connected to the Spectrum Compact CE System. Enter the appropriate sample name, and then select **OK** to exit and return to the 'Edit Strip Information' screen.

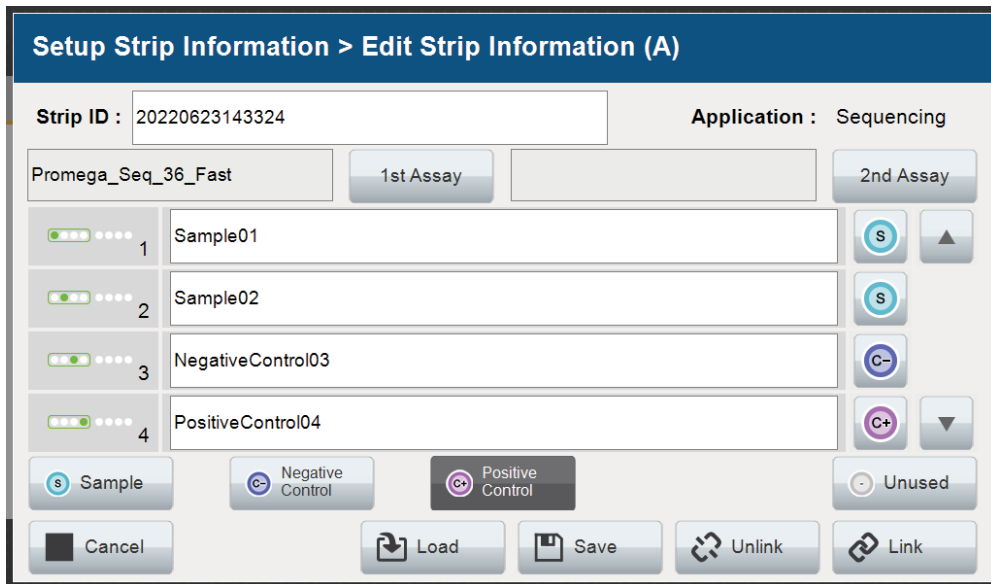


Figure 14. Sample Name Entry on 'Edit Strip Information' screen.

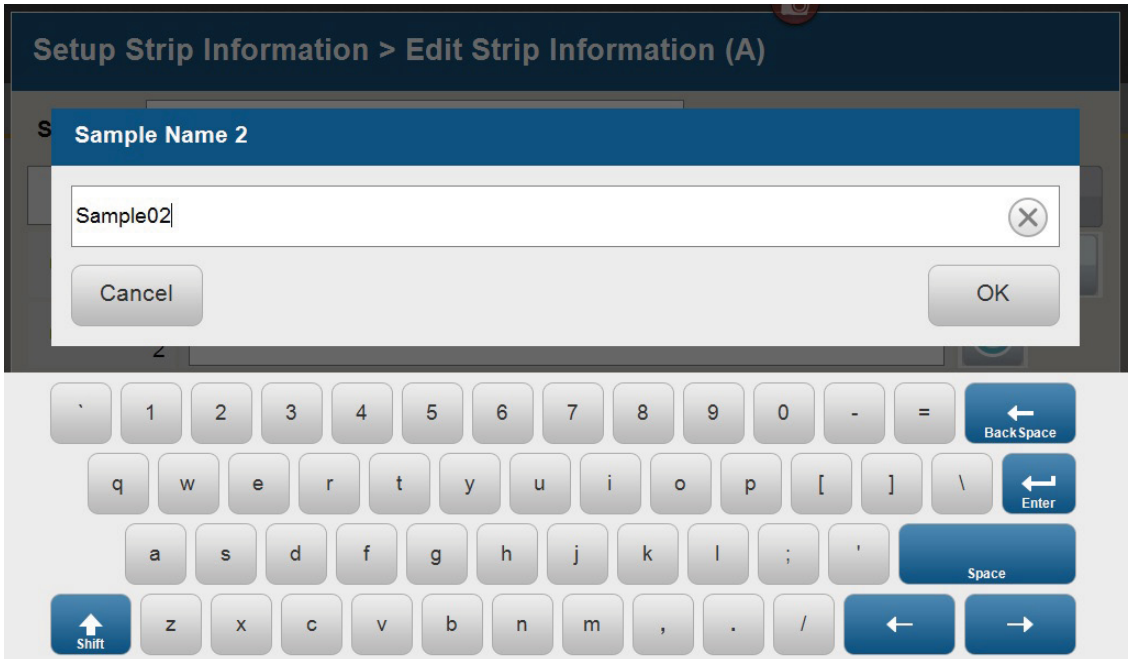


Figure 15. 'Set Sample Name' window.

5.D. Assigning Run Information to Samples (continued)

- The Spectrum Compact Control Software includes preloaded run assays for use with chemistries available from Promega and other vendors. Refer to the *Spectrum Compact CE System Operating Manual #TMD058* for information on creating or modifying assays. To assign a run assay to an injection set, select the **1st Assay** button on the right side of the 1st Assay field (Figure 14). This opens the ‘Select Assay’ window (Figure 16). Select the appropriate assay from the drop-down list (Figure 16) and then select **Apply**.

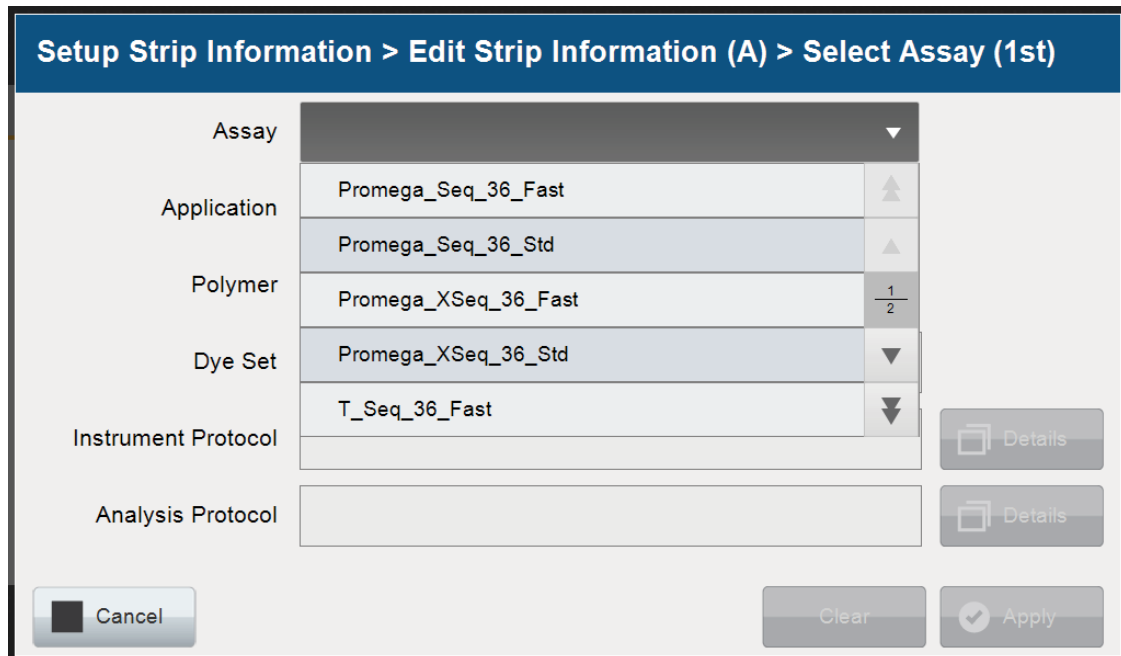
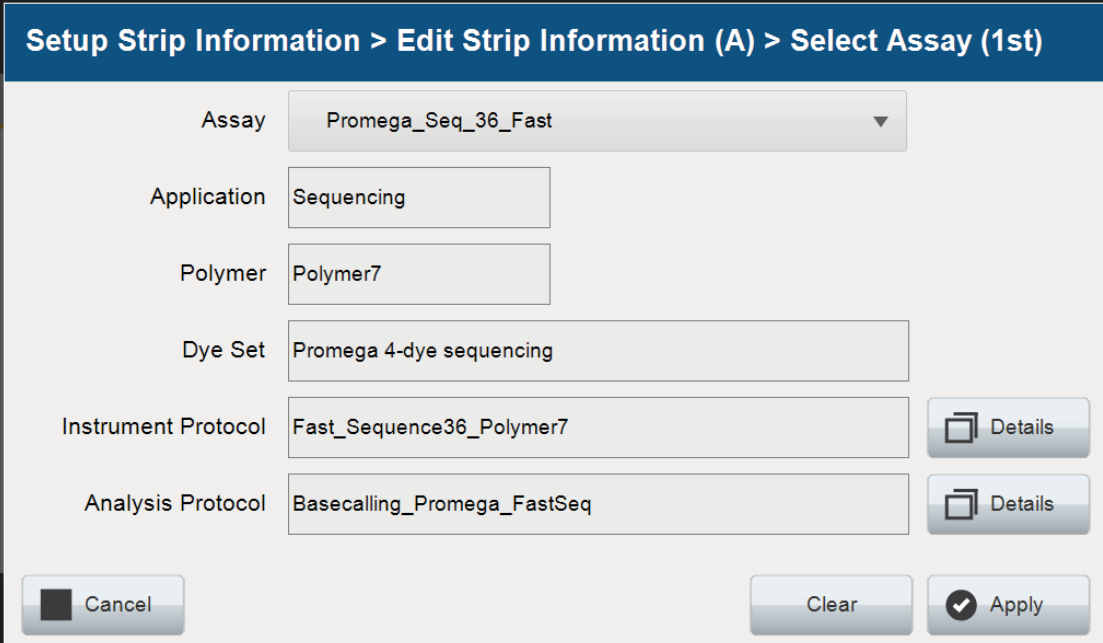


Figure 16. ‘Select Assay’ window.

- You can verify the settings of the Instrument Protocol and Analysis Protocol associated with the assay chosen by selecting **Details** next to these fields (Figure 17). This will display a window showing the settings in these protocols but will not allow you to edit these settings. Refer to the *Spectrum Compact CE System Operating Manual #TMD058* to edit the Instrument Protocol or Analysis Protocol. When the assay information is confirmed, select **Apply** to return to the ‘Edit Strip Information’ screen.



Setup Strip Information > Edit Strip Information (A) > Select Assay (1st)

Assay: Promega_Seq_36_Fast

Application: Sequencing

Polymer: Polymer7

Dye Set: Promega 4-dye sequencing

Instrument Protocol: Fast_Sequence36_Polymer7 **Details**

Analysis Protocol: Basecalling_Promega_FastSeq **Details**

Cancel **Clear** **Apply**

Figure 17. Accessing Instrument and Analysis Protocols on ‘Select Assay’ window.

- Repeat these steps for the 2nd Assay field if a second assay will be run for the strip. A second assay might include a longer or shorter injection time.
- Use the down arrow on the right side of the ‘Edit Strip Information’ screen to enter sample information and assign an assay for samples in wells 5–8 of the strip.

5.D. Assigning Run Information to Samples (continued)

11. When all information is entered and verified for the strip, select **Link** on the lower right corner of the ‘Edit Strip Information’ screen (Figure 18). This will link the strip to the run. If you want to save the strip information to use in future runs, save the information by selecting **Save** at the bottom of the ‘Edit Strip Information’ screen (Figure 18). This will save the strip information so that it can be loaded later into another run as well as save the strip to the run. Refer to the *Spectrum Compact CE System Operating Manual #TMD058* for use of saved strip information.

Notes:

1. If you select **Unlink** without having previously selected **Save** at the bottom of the ‘Edit Strip Information’ screen, you will lose the strip information.
2. If you forget to assign an assay in the ‘Edit Strip Information’ screen, a warning window stating “Invalid Data Entered” will appear. Close this window and assign an assay on the ‘Edit Strip Information’ screen before continuing.

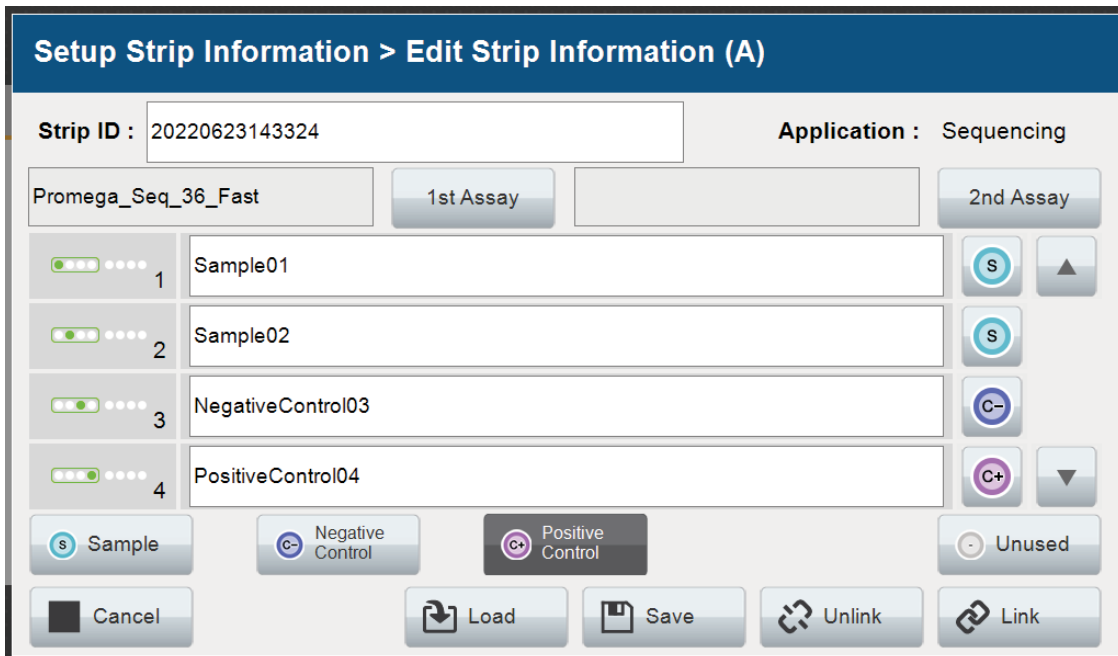


Figure 18. Completed ‘Edit Strip Information’ screen.

12. Repeat Steps 2–11 for additional lanes as necessary.
13. After all the required lanes have been set up, select **Next** on the ‘Setup Strip Information’ screen to load the sample cartridge (Figure 19).

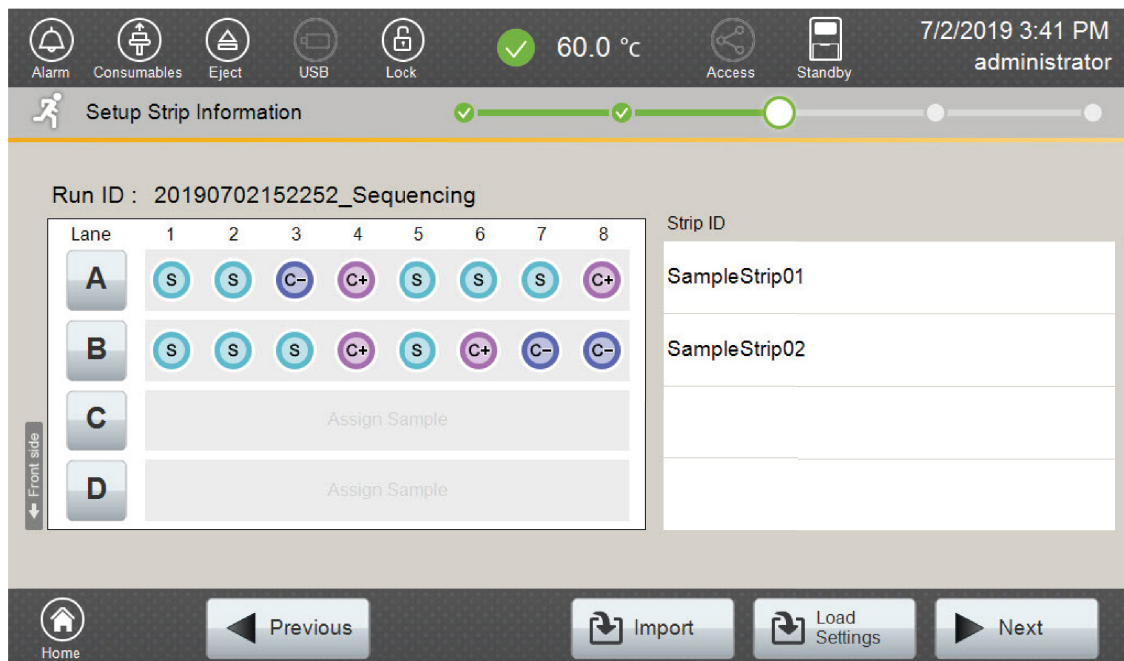


Figure 19. Completed ‘Setup Strip Information’ screen.

5.E. Loading the Sample Cartridge

1. After selecting **Next** on the 'Setup Strip Information' screen, 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 door while the autosampler is in motion.

2. Open the instrument door, and then place the sample cartridge on the autosampler, following the instructions displayed on the 'Install the Cartridge' screen (Figure 20). Press down on the yellow tab on the autosampler deck that locks the sample cartridge in place before placing sample cartridge into position. Release the tab to lock the sample cartridge in place on the autosampler deck.

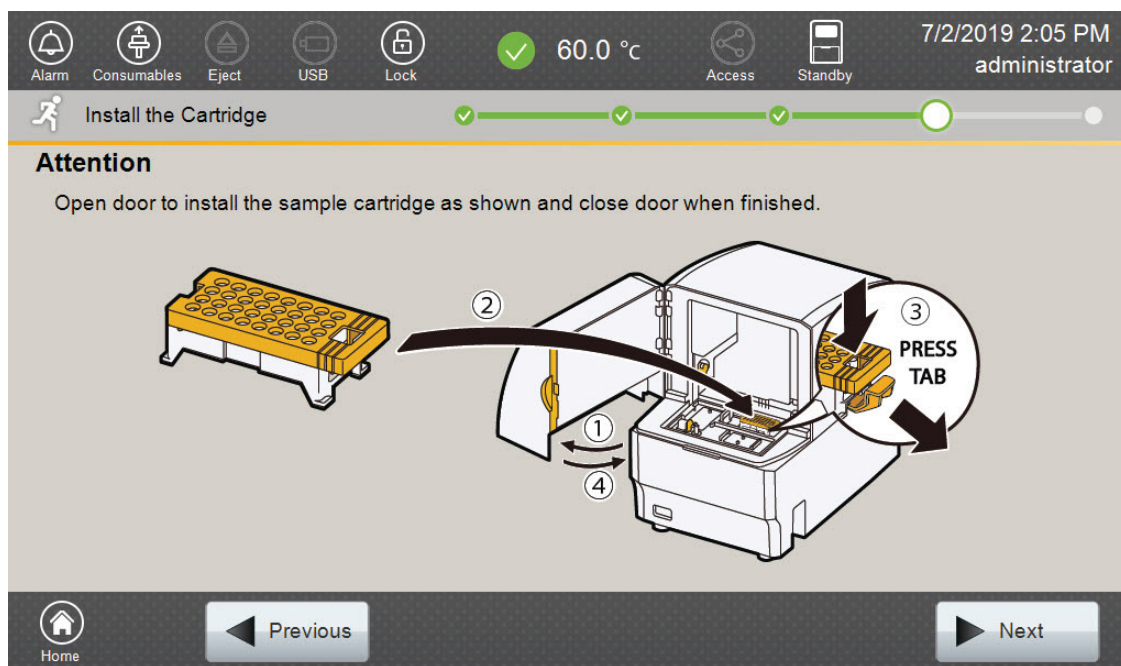
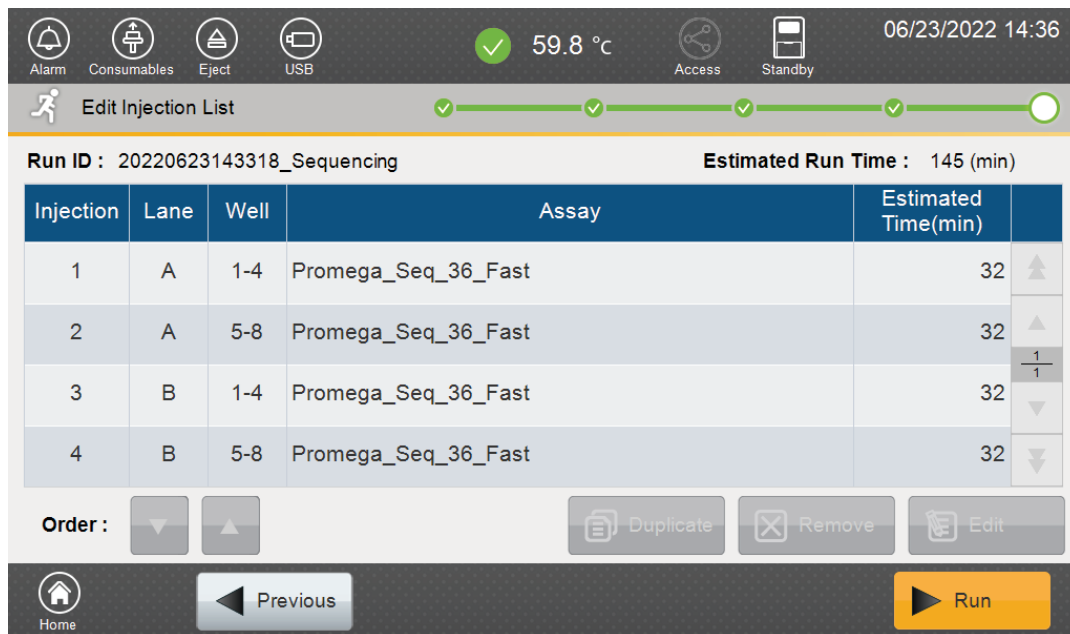


Figure 20. 'Install the Cartridge' screen.

3. When the sample cartridge is locked into place on the autosampler, close the instrument door and wait for the status indicator to stop flashing amber and turn steady green.

Note: Do not open the door while the autosampler is in motion. Follow the instructions displayed on the screen.

- After the autosampler has returned to its home position, the 'Edit Injection List' screen will be displayed (Figure 21).
- Select **Run** to start the run.



Injection	Lane	Well	Assay	Estimated Time(min)
1	A	1-4	Promega_Seq_36_Fast	32
2	A	5-8	Promega_Seq_36_Fast	32
3	B	1-4	Promega_Seq_36_Fast	32
4	B	5-8	Promega_Seq_36_Fast	32

Figure 21. 'Edit Injection List' screen.

5.F. Viewing and Exporting Data

You can view data on the Spectrum Compact CE System, and you can also export the data for viewing using a variety of sequence viewers.

- On the 'Home' screen, touch **Run Results** in the Review panel.
- Select the Run ID on the left panel. To export the results, insert a USB drive in the front USB port and select **Export**. To view results on the instrument, touch **View**.
- Touch a sample name and then touch **View**. You can also export single files in this menu.

5.G. Capillary Electrophoresis on Applied Biosystems 3500 or 3500xL Genetic Analyzer

Materials to Be Supplied by the User

- 3500/3500xL capillary array, 50cm or 36cm
- plate retainer and base set (standard)
- POP-7® Polymer for the Applied Biosystems® 3500 or 3500xL Genetic Analyzer
- anode buffer container
- cathode buffer container

Refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* for the instrument maintenance schedule and instructions to install the capillary array, buffers and polymer pouch and perform a spatial calibration. Samples may be analyzed as described in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide*. Ensure that the instrument has undergone a spectral calibration for Dye Set Z. If a calibration has not been previously performed, consult the *ProDye™ 4C Matrix Standard Technical Manual #TM674* or *ProDye™ Sequencing Standard Quick Protocol #FB239* for instructions on how to perform a spectral calibration.

1. Open the 3500 Data Collection Software. The Dashboard screen will launch (Figure 22). To ensure that you are viewing the most up-to-date information, press the **Refresh** button. Ensure that the Consumables Information and Maintenance Notifications are acceptable.

Set the oven temperature to 60°C, and then select **Start Pre-Heat**.

Note: The instrument manufacturer recommends that you preheat the oven for at least 30 minutes before you start a run if the instrument is at ambient temperature.

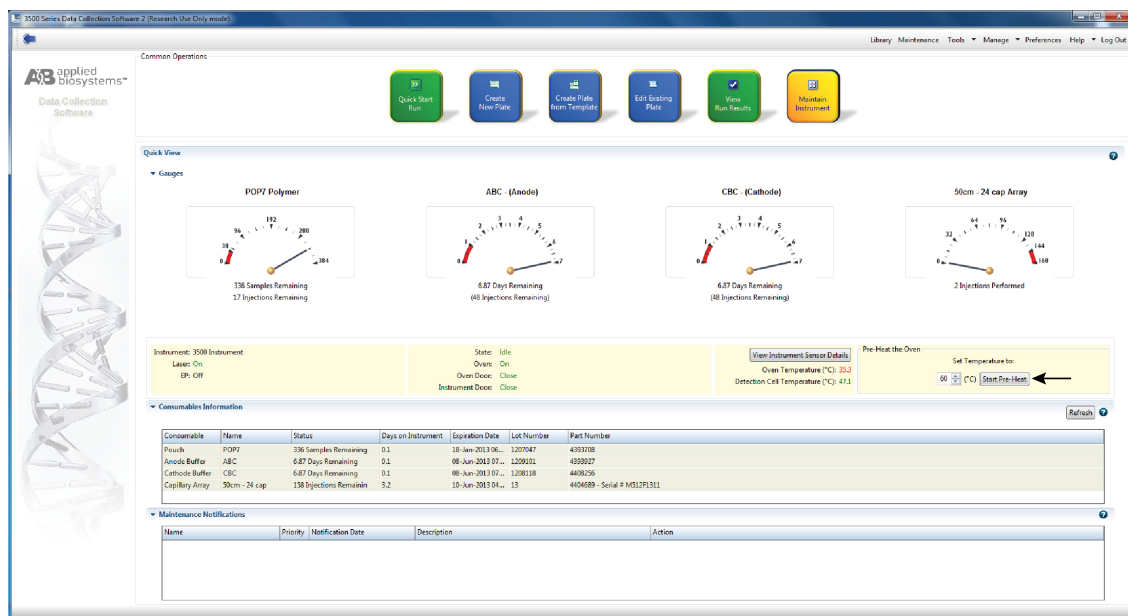
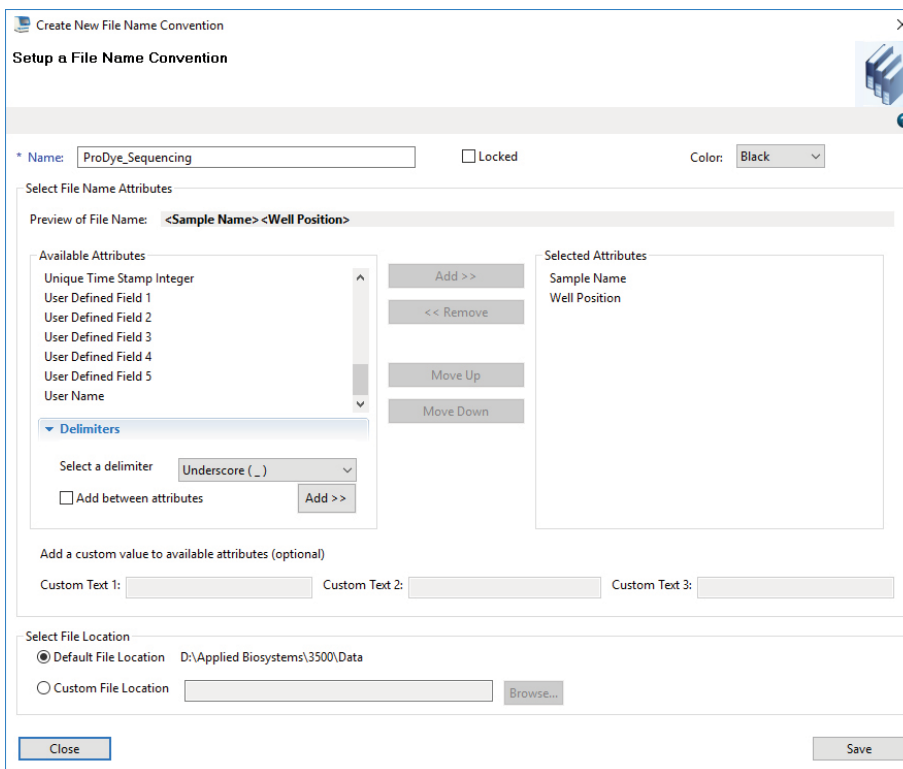


Figure 22. The Dashboard.

Prior to the first time using ProDye™ Terminator Sequencing System on the instrument, a File Name Convention and Results Group must be created. Alternatively, an existing File Name Convention and Results Group can be used.

2. Create a new File Name Convention. To create a new File Name Convention (Figure 23), navigate to the Library. Select **File Name Conventions**, and then select **Create**. Alternatively, a previously created File Name Convention can be used.

Select the File Name Attributes according to your laboratory practices, and save the File Name Convention with a descriptive name.



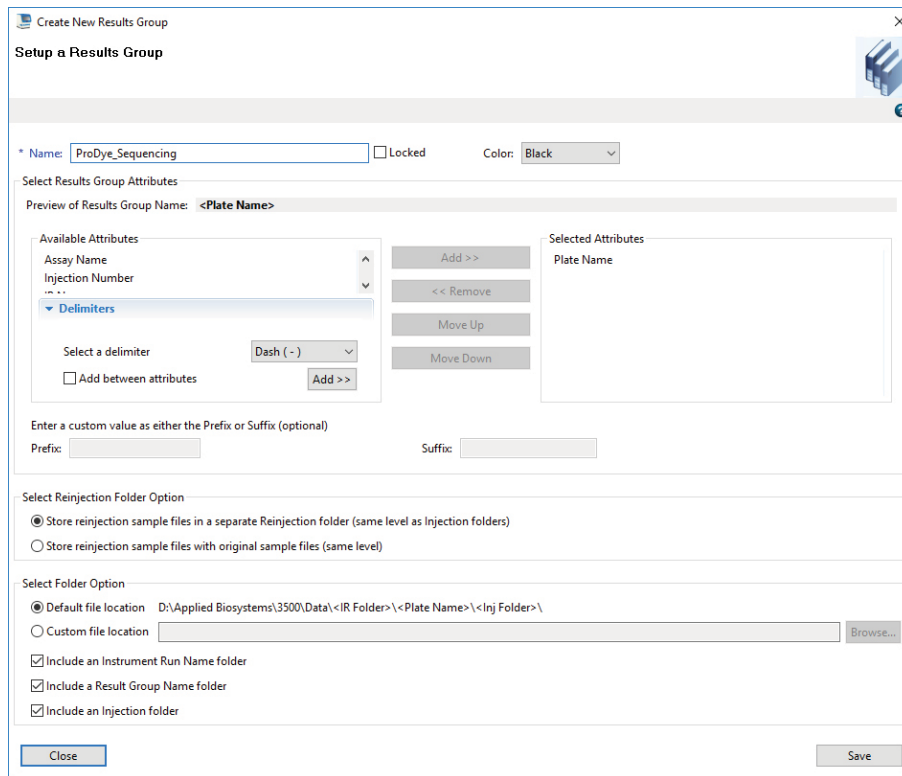
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Figure 23. The ‘Setup a File Name Convention’ window.

5.G. Capillary Electrophoresis on Applied Biosystems 3500 or 3500xL Genetic Analyzer (continued)

3. Create a new Results Group. To create a new Results Group (Figure 24), navigate to the Library. Select **Results Group**, and then select **Create**. Alternatively, a previously created Results Group can be used.

Select the Results Group Attributes according to your laboratory practices. Save the Results Group with a descriptive name.



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Figure 24. The ‘Setup a Results Group’ window.

4. Create a new Plate and begin the run.
 - a. To create a New Plate, navigate to the Library, and from the Manage menu, select **Plates** and then **Create**.
 - b. Assign a descriptive plate name. Select the plate type **Sequencing** from the drop-down menu (Figure 25).

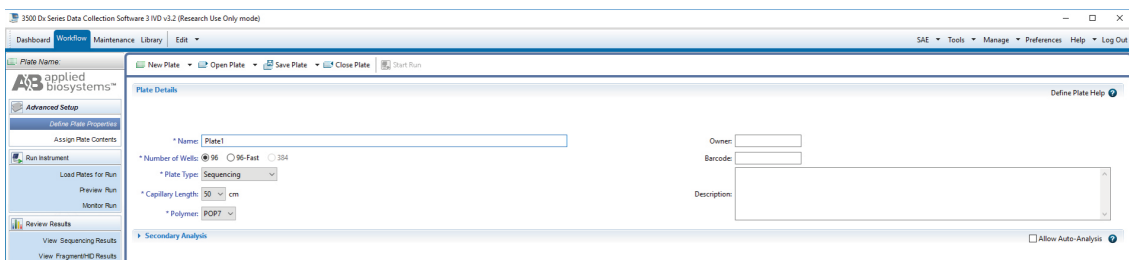


Figure 25. Defining plate properties.

- c. Select **Assign Plate Contents**.
- d. Assign sample names to wells.
- e. In the lower left portion of the screen (Figure 26), under Assays, use the Add from Library option to choose the desired Assay. Select the **Add to Plate** button, and close the window.

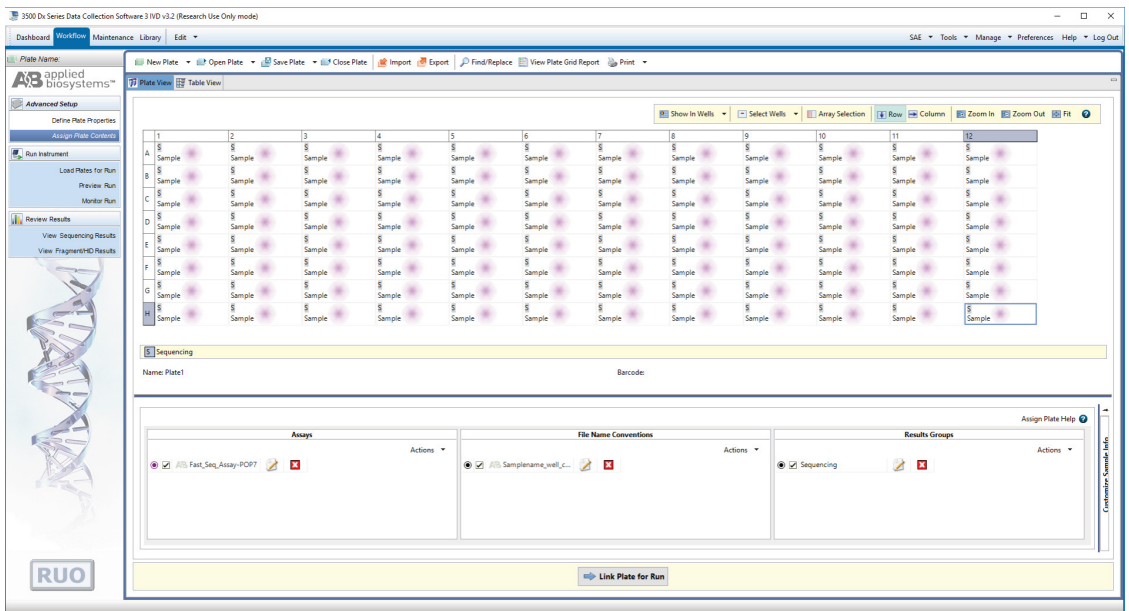


Figure 26. Assigning plate contents.

5.G. Capillary Electrophoresis on Applied Biosystems 3500 or 3500xL Genetic Analyzer (continued)

5. Under File Name Conventions, use the 'Add from Library' option to choose the File Name Convention created in Step 1 or one previously created. Select the **Add to Plate** button, and close the window.
6. Under Results Groups, use the 'Add from Library' option to choose the Results Group created in Step 2 or one previously created. Select the **Add to Plate** button, and close the window.
7. Highlight the sample wells, and then select the boxes in the Assays, File Name Conventions and Results Groups that pertain to those samples.
8. Select **Link Plate for Run**.
9. The 'Load Plate' window will appear. Select **Yes**.
10. In the 'Run Information' window (Figure 27), assign a Run Name. Select **Start Run** (not shown).

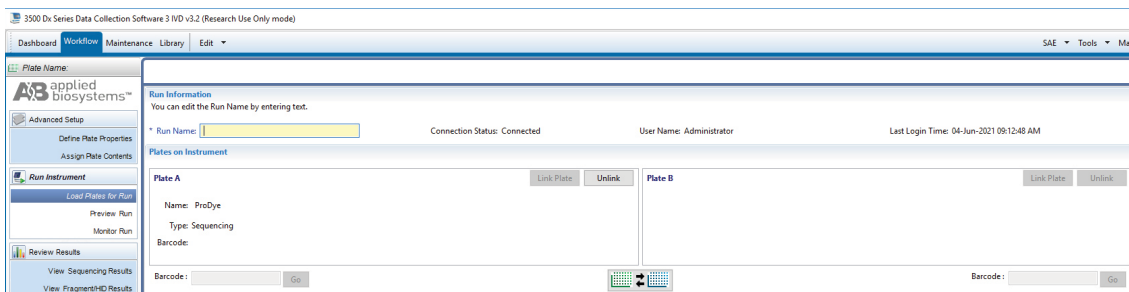


Figure 27. Assigning a run name.

Viewing and Exporting Data

Once the run is complete, you can select **Review Results** to view the results on the instrument. Consult the instrument manual for information on how to transfer the run files to another computer for analysis.

5.H Capillary Electrophoresis on Applied Biosystems 3130, 3130xL, 3730 or 3730xl Genetic Analyzer

Consult the *Applied Biosystems® 3130/3130xl Genetic Analyzers Getting Started Guide* or *Applied Biosystems 3730/3730xl DNA Analyzers User Guide* for detailed instructions on how to perform sequencing runs on these instruments.

6. 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

No signal or low signal

Causes and Comments

Insufficient template or template omitted.

- Confirm the template DNA concentration.
- Increase the amount of template present in the reaction.

Template not of sufficient purity. Repurify the sample.

Insufficient primer or primer omitted. Confirm concentration of the primer and increase the amount of primer, if necessary.

Incorrect primer used (no annealing site). Use correct primer.

Missing, inactive or expired reagent. Repeat reactions, including a control reaction using pGEM-3Zf(+) Vector. Verify expiration dates, try fresh reagents or do both.

Incorrect thermal cycling performed.

- Verify that the thermal cycling protocol was correct.
- Confirm that the correct plate or tubes were used.

Extension products lost during purification.

- Confirm that centrifuge speeds are correct.
- Use fresh ethanol stocks.
- Try an alternative purification method.

Electrokinetic injection failure.

- Confirm that the minimum volume is present in the wells.
- Confirm there are no bubbles at the bottom of the wells; centrifuge briefly to remove bubbles if necessary.
- Run the ProDye™ Sanger Sequencing Standard to check instrument performance.
- Ensure the correct Assay was used.

Mixed signal throughout trace

Multiple primers present.

- Confirm only a single primer was added to reactions.
- If sequencing PCR products, repurify product to remove excess amplification primers.

Multiple priming sites in template. Check for multiple primer binding sites in template; if needed, redesign primer.

Multiple templates present. Run template on an agarose gel and confirm a single band is present. If not, gel-purify the correct band.

Primer degradation. Use new HPLC- or PAGE-purified primer.

6. Troubleshooting (continued)

Symptoms

Causes and Comments

Mixed signal throughout trace (continued)

Incorrect Assay. Reinject with correct Assay.

Incorrect spectral matrix selected. Select correct spectral matrix and reinject.

Mixed signal over part of trace

Multiple templates present. Run template on an agarose gel and confirm a single band is present; if not, gel-purify correct band.

Primer-dimer present in reaction.

- Optimize PCR thermal cycling.
- Redesign primers to remove complementarity.
- Use a hot start for thermal cycling.

Heterozygous insertion or deletion mutation. Re-amplify, repurify template DNA or both.

Dye blobs present

(Dye blobs are caused by excess unincorporated dye-labeled terminators and appear as large, broad peaks in the early part of a sequencing trace (generally between 50–150 bases). These generally appear as C or T signals and can obscure the sequence information in that region of the trace.)

Incorrect ethanol concentration during ethanol precipitation. Repeat with correct ethanol concentration. Use fresh ethanol.

Alternate purification method was used. Repeat using ethanol precipitation or consult troubleshooting information for the purification method used.

Insufficient template DNA was used. Repeat reactions with additional template, confirm DNA concentration and/or use an alternative method for DNA quantitation

Saturated signal present

(Saturated signal occurs when the fluorescent signal saturates the detector on the instrument. Saturation occurs at different RFU values on different instruments. Consult the operating manual for the instrument you are using for these values. Signal saturation causes spectral pull-up, which can cause mixed bases to be called.)

Too much template was added to the sequencing reaction.

- If the sample is fresh (on instrument <24 hours), re-inject sample with a reduced injection time.
- Repeat reaction using less template DNA.

Water was used in place of Hi-Di™ formamide. Use Hi-Di™ formamide for injecting samples

7. Control Template Sequence Information

The control template pGEM™-3Zf(+) partial sequence for control reactions (GenBank® Accession Number X65306):
GTTGTAAAACGACGGCCAGTGAATTGTAATACGACTACTATAGGGCGAATTCGAGCTCGGTACCCGGGGATCCTC-
TAGAGTCGACCTGCAGGCATGCAAGCTTGAGTATTCTATAGTGTACCTAAATAGCTTGGCGTAATCATGGT-
CATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTA-
AAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCAGTC-
GGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGGGGAGAGGCGGTTTTCGTATTGGGC-
GCTCTCCGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTTCGTTTCGGCTGCGGCGAGCGGTATCAGCTCACT-
CAAAGGCGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAG-
CAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCA-
CAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCTG-
GAAGCTCCCTCGTGCGCTCCTCTGTTCCGACCCTGCGCTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGC-
GTGGCGTTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTG-
CACGAACCCCGTTCAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACAC-
GACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTA-
CAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGC-
CAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTT-
GTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGAC-
GCTCAGTGAACGAAAACCTCACGTTAAGGG

This sequence can also be downloaded from www.promega.com/resources/vector-sequences/

8. Related Products

Product	Size	Cat. #
ProDye™ 4C Matrix Standard	5 preps	CR4500
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 Polymer4	4 × 64 wells	CE2304
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.

9. Summary of Changes

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

1. Updated Sections 5.A and 5.C.
2. Replaced some images in Sections 5.D and 5.E.

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