SCIEX
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DNA 20 kb Plasmid and Linear Kit

For the BioPhase 8800 System

Application Guide

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The DNA 20 kb Plasmid and Linear kit supplies the reagents for sample preparation and the methods to separate a wide range of plasmids and linearized dsDNA by size.

This document gives instructions for sample preparation with the DNA 20 kb Plasmid and Linear kit. It also gives instructions for data acquisition with the BioPhase software and the Waters Empower™ software with the BioPhase 8800 driver for Empower™. This document also gives instructions for data analysis of data acquired with BioPhase software. The analysis of data acquired with the Waters Empower™ software must be done with the Waters Empower™ software.

Use the information in this application guide as a place to start. If required, change the injection time, voltage, injection type, or other parameters to find the best conditions for the requirements.

Note: For instructions about how to use the system safely, refer to the document: *Operator Guide*.

Safety

Refer to the safety data sheets (SDSs), which are available at sciex.com/tech-regulatory, for information about the correct handling of materials and reagents. Always follow standard laboratory safety guidelines. For information about hazardous substances, refer to the section: Hazardous Substance Information.

Intended Use

The DNA 20 kb Plasmid and Linear kit is for laboratory use only.

Introduction

The DNA 20 kb Plasmid and Linear kit is used to do isoform and purity analysis of plasmids and size and purity analysis of linearized dsDNA. This kit supplies high analytical resolution and can analyze a wide range of double-stranded nucleic acid molecules. Plasmid isoforms from 2,000 bp to 19,000 bp can be separated, including super coiled, linear, and open circular species. The kit can do size estimation and purity analysis for linear dsDNA samples from 100 bp to 20,000 bp.

Before the sample is injected, the conditioning method coats the capillaries. A pressure injection sends the DNA sample into the capillary. The different species in the sample are separated by size in a bare fused-silica (BFS) capillary filled with a replaceable polymer gel that supplies sieving selectivity. The fluorescent labeling dye SYBR™ Gold Nucleic Acid gel stain[1] is added to

the polymer gel matrix during reagent preparation. The dye binds to DNA molecules so that the DNA can be detected by laser-induced fluorescence (LIF) at 520 nm during the separation.

The separation can be done with a capillary cartridge with 30 cm bare fused-silica capillaries for both plasmid isoform and linearized dsDNA samples. To increase peak efficiencies for linear dsDNA fragments, use a capillary cartridge with 50 cm bare fused-silica capillaries.

Workflow

The workflow has the following steps:

1. Identify the number of samples to be analyzed and the number of replicates.
2. For BioPhase software users:
   a. Create or change the methods. Refer to the section: Methods.
   b. Create the sequence and the sample and reagent plate layouts. Refer to the section: Create the Sequence.
3. For Waters Empower™ software users:
   a. Import the BioPhase software methods. Refer to the section: Import the BioPhase Software Methods to Create Instrument Methods.
   b. Create the sample set methods and the sample and reagent plate layouts. Refer to the section: Create the Sample Set Method.
4. Prepare the samples. Refer to the section: Prepare the Samples.
5. Use the sample and reagent plate layouts to prepare the plates. Refer to the section: Plate Layouts.
6. Put the plates in the BioPhase 8800 system. Refer to the sections: Load the Sample Inlet and Outlet Plates and Load the Reagent Inlet and Outlet Plates.
7. Start data acquisition.
   - BioPhase software: Start the sequence from the front panel. Refer to the section: Run the Samples.
   - Waters Empower™ software: Start the sample set method from the Waters Empower™ software with the BioPhase 8800 driver for Empower™. Refer to the section: Start the Sample Set Method.
8. After the run, stow the cartridge. Refer to the section: Stow the Cartridge After the Run
9. Analyze the data.
   - BioPhase software: When the sequence is complete, use the BioPhase Analysis software to analyze the data. Refer to the section: Analyze the Data.

[1] SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Gold Nucleic Acid gel stain is not available for resale.

- Waters Empower™ software: When the sample set method is complete, use the Waters Empower™ software to analyze the data. For data analysis instructions, refer to the Waters Empower™ software guides and help file.

Required Equipment and Materials 2

Note: For items with a reorder part number, sometimes the reorder quantity is different than the quantity in the kit.

Table 2-1 DNA 20 kb Plasmid and Linear Kit (PN 5311708)

Component	Quantity	Reorder Part Number
Acid wash/regenerating solution (100 mL)	1	5312349
CE Grade water (140 mL)	2	C48034
DNA 20 kb Plasmid and Linear conditioning solution (135 mL)	1	5312283[2]
DNA 20 kb Plasmid and Linear gel (135 mL)	1	5312283[2]
DNA 20 kb Plasmid and Linear sample buffer (60 mL)	1	5312283[2]
DNA 20 kb Plasmid test mix (8 ng in 20 µL)	6	N/A
SYBR™ Gold Nucleic Acid gel stain[3] [4](120 µL)	6	N/A

Table 2-2 Other Supplies from SCIEX

Component	Quantity	Part Number
Capillary cartridge coolant (450 mL)	1	359976
BioPhase 8800 bare fused-silica capillary cartridge (50 µm i.d. × 30 cm capillaries)	1	5080121
BioPhase 8800 bare fused-silica capillary cartridge (50 µm i.d. × 50 cm capillaries)	1	5080123
BioPhase 8800 outlet plates	20	5080315
BioPhase 8800 reagent plates	20	5080314

[2] The DNA 20 kb Plasmid and Linear separation pack (PN 5312283) contains DNA 20 kb Plasmid and Linear conditioning solution, DNA 20 kb Plasmid and Linear gel, and DNA 20 kb Plasmid and Linear sample buffer. None of the parts can be ordered separately.

[3] SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Gold Nucleic Acid gel stain is not available for resale.

[4] The concentration of the dye is diluted approximately 100 × from the concentration of the dye available from Thermo Fisher Scientific.

Table 2-2 Other Supplies from SCIEX (continued)

Component	Quantity	Part Number
BioPhase 8800 sample plates	20	5080313
BioPhase 8800 Starter Plate Pack (4 sample plates, 4 reagent plates, 8 outlet plates)	1	5080311

Table 2-3 Other Required Reagents or Supplies

Description	Vendor	Part Number
(Optional) 1 Kb Plus DNA Ladder (250 µg)	ThermoFisher Scientific	10787018
X-Pierce Film	USA Scientific	2997-0100

Storage Conditions

Note: For storage conditions for prepared reagents, refer to the preparation instructions.

- Upon receipt, keep the following at 15 °C to 30 °C:
  - Acid wash/regenerating solution
  - CE Grade water
  - DNA 20 kb Plasmid and Linear sample buffer
- Upon receipt, keep the following at 2 °C to 8 °C:
  - DNA 20 kb Plasmid and Linear conditioning solution
  - DNA 20 kb Plasmid and Linear gel
- Upon receipt, keep the DNA 20 kb Plasmid test mix and the SYBR™ Gold Nucleic Acid gel stain[5] at –35 °C to –15 °C.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Protective eyewear
- Laboratory coat
- Analytical balance

[5] SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Gold Nucleic Acid gel stain is not available for resale.

- Centrifuge with swinging-bucket rotor to hold plates
- Disposable plastic conical tubes, 50 mL (or equivalent)
- Microcentrifuge, or equivalent, and nuclease-free microcentrifuge tubes (USA Scientific PN 1615-5510)
- PCR tubes, 0.2 mL flat-cap nuclease-free (VWR USA PN 20170-012 or VWR EUR PN 732-0548)
- Pipettes and applicable tips

  Note: For reagent plate preparation a repeater pipette or equivalent is recommended.

- Vortex mixer

Required Detector

An LIF detector with a 488 nm excitation source and a 520 nm emission filter is required.

Required Cartridge

CAUTION: Potential Wrong Result. If a cartridge is used with the DNA 20 kb Plasmid and Linear kit, then do not use the same cartridge for another application. If the same cartridge with different buffers and sample types is used, then sample carryover, nonspecific binding, and poor separation can occur.

A BioPhase 8800 bare-fused silica capillary cartridge with 50 µm inner diameter (i.d.) × 30 cm capillaries is required.

(Optional) To increase the resolution for linear dsDNA samples, a BioPhase 8800 bare-fused silica capillary cartridge with 50 µm inner diameter (i.d.) × 50 cm capillaries can be used.

Refer to the table: Table 2-2.

Condition the Capillaries

CAUTION: Potential Wrong Result. Do not use a basic solution to clean the capillary. The solution might negatively ionize the capillary wall and result in electro-osmotic flow detrimental to the separation of nucleic acids. These interactions might cause poor separation.

- Before a new cartridge is used, condition the capillaries with DNA 20 kb Plasmid and Linear conditioning solution and either the `30cm-DNA 20kb Conditioning` or the `50cm-DNA 20kb Conditioning` method.

Note: Do not use the conditioning method more frequently than every 30 to 50 injections. Examine run-to-run separation metrics such as **Res.** (peak resolution) and **N** (theoretical plates) before conditioning. If the metrics are acceptable, then conditioning is not required.

Methods and Sequences 3

Methods and Sequences for the BioPhase Software

Note: If the validated methods and sequences are not included with the software, then they are available for download from the SCIEX website. Refer to the section: Download and Configure the Required Files. The methods can also be created manually with the BioPhase software. Refer to the section: Methods.

The following methods and sequence are required.

- For a cartridge with 30 cm capillaries:
  - `30cm-DNA 20kb Conditioning`: Conditions the capillaries as part of a sequence, if required.
  - `30cm-DNA 20kb Linear Separation`: Does the separation, best for linear dsDNA samples and plasmids larger than 4 kb.
  - `30cm-DNA 20kb Plasmid Separation`: Does the separation, best for plasmid samples.
  - `30cm-DNA 20kb Shutdown`: Cleans the capillaries at the end of a sequence, rinses the capillaries for storage, and then turns off the lamp.
  - `30cm-DNA 20kb Rinse`: Rinses the capillary between sequences, if required.
- For a cartridge with 50 cm capillaries:
  - `50cm-DNA 20kb Conditioning`: Conditions the capillaries as part of a sequence, if required.
  - `50cm-DNA 20kb Linear Separation`: Does the separation, best for linear dsDNA samples that require maximum resolution.
  - `50cm-DNA 20kb Shutdown`: Cleans the capillaries at the end of a sequence, rinses the capillaries for storage, and then turns off the lamp.
  - `50cm-DNA 20kb Rinse`: Rinses the capillary between sequences, if required.
- Sequence templates:
  - `30cm-DNA 20kb Plasmid Test Mix- 6 runs with conditioning`: A sequence template for a cartridge with 30 cm capillaries.

- `50cm-DNA 20kb DNA ladder- 6 runs with conditioning`: A sequence template for a cartridge with 50 cm capillaries.

Methods for the Waters Empower™ Software

To create the required instrument methods, import the BioPhase software methods.

Note: If the methods are not included with the software, then they are available for download from the SCIEX website. Refer to the section: Download and Configure the Required Files (Waters Empower™ Software). The methods can also be created manually with the Method Editors for BioPhase System software. Refer to the section: Methods.

Create the Sequence

Note: If the Waters Empower™ software will be used to acquire the data, then this procedure is not applicable. Refer to the section: Create the Sample Set Method.

Note: This procedure is for users who know how to use the BioPhase software. For detailed instructions, refer to the document: *Software Help System*.

This procedure gives instructions to create a sequence with a template that is supplied with the BioPhase software.

Two templates are supplied: one for a cartridge with 30 cm capillaries and one for a cartridge with 50 cm capillaries.

Sequences can also be created without a template. In most cases, a sequence should start with a conditioning method, include separation methods, and then end with a shutdown method. Usually, the shutdown method should be identified as the error recovery method. Refer to the document: *Software Help System*.

Note: If the sequence includes replicate samples, then make that sure the replicates are in the same row of the sample plate to decrease any capillary-to-capillary variation.

1. On the Home page of the BioPhase software, click **Sequence Editor**.
2. Click **Open Sequence**.
   The Open a Sequence dialog opens.
3. Search for, and then select a sequence:
   a. (Optional) Type a **Start Date** and **End Date** for the search, or click the calendar icons, select the dates, and then click **Search**.
      The available project folders are shown in the Folder Name pane.
   b. Click the **DNA 20kb** project folder.
      The available sequences in the project are shown in the table to the right.

c. In the table, click the sequence template, and then click **Open**.

- For 30 cm capillaries, click **`30cm-DNA 20kb Plasmid Test Mix- 6 runs with conditioning`**.
- For 50 cm capillaries, click **`50cm-DNA 20kb DNA ladder- 6 runs with conditioning`**.

The Open a Sequence dialog closes, and then the Sequence Summary tab opens.

4. Above the Sequence Summary pane, click **Edit**.
The Sample Plate Setup tab opens.
5. If required, then do any of the following actions:

- Add or remove samples.
- Clear a method that is assigned to a sample well.
- Assign a different method to a sample well.
- In the Sequence Summary table, assign the recovery method to a method in the sequence. Usually, the shutdown method should be identified as the recovery method.

For detailed instructions, refer to the document: *Software Help System*.

6. If required, edit the information in the Sequence Summary table.

Note: The method names are different for each sequence.

Figure 3-1 Sequence Summary Table: `30cm-DNA 20kb Plasmid Test Mix- 6 runs with conditioning`

Run #	Column	Method Name	Rep. #	Error Recovery
1	0	30cm - DNA 20kb - Conditioning	1	☐
⊞2	1	30cm - DNA 20kb - Separation	1	☐
⊞3	1	30cm - DNA 20kb - Separation	2	☐
⊞4	1	30cm - DNA 20kb - Separation	3	☐
⊞5	1	30cm - DNA 20kb - Separation	4	☐
⊞6	1	30cm - DNA 20kb - Separation	5	☐
⊞7	1	30cm - DNA 20kb - Separation	6	☐
8	0	30cm - DNA 20kb - Shutdown	1	☑

7. To see details of a run, click the **+** in the row with the run.

Figure 3-2 Sequence Summary Table Expanded

	Run #	Column	Method Name	Rep. #	Error Recovery
	1	0	30cm - DNA 20kb - Conditioning	1	☐
⊟	2	3	30cm - DNA 20kb - 20kV - Plasmid Separation	1	☐

Well	Sample Id	Run Type	Data File
A03	<WP>_DNA 20kb Plasmid Test Mix	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
B03	<WP>_DNA 20kb Plasmid Test Mix	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
C03	<WP>_DNA 20kb Plasmid Test Mix	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
D03	<WP>_DNA 20kb Plasmid Test Mix	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
E03	<WP>_DNA 20kb Plasmid Test Mix	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
F03	<WP>_DNA 20kb Plasmid Test Mix	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
G03	<WP>_DNA 20kb Plasmid Test Mix	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
H03	<WP>_DNA 20kb Plasmid Test Mix	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>

⊞	3	3	30cm - DNA 20kb - 20kV - Plasmid Separation	2	☐
⊞	4	3	30cm - DNA 20kb - 20kV - Plasmid Separation	3	☐
⊞	5	3	30cm - DNA 20kb - 20kV - Plasmid Separation	4	☐
⊞	6	3	30cm - DNA 20kb - 20kV - Plasmid Separation	5	☐
⊞	7	3	30cm - DNA 20kb - 20kV - Plasmid Separation	6	☐
	8	0	30cm - DNA 20kb - Shutdown	1	☑

8. If required, change the information in the **Sample Id** and **Data File** columns.
9. To see the sample plate and reagent plate layouts, open the Plates Layout tab. If required, change the reagent locations in the Reagent Plate pane.
10. To save the sequence, click **SAVE**, and then add the required information.

Note: If errors occurred, then the **SAVE** button is not available. Correct all of the errors in the Validation pane, and then click **SAVE**.

11. (Optional) To print the sample plate and reagent plate layouts, do the following:
    a. Click **PRINT**.
    The Print Preview dialog opens.
    b. Click **Plate Layout Report**, and then click **Print**.
    c. Select the printing options, and then click **OK**.
    The report is printed.
    d. Close the Print Preview dialog.

Prepare the Samples 4

Best Practices for Work with DNA

Note: CE Grade water is not certified as nuclease-free. DNA 20 kb Plasmid and Linear sample buffer is made from nuclease-free components but is not certified as nuclease-free.

Tips for DNA Samples

To avoid contamination and degradation, handle DNA samples with care.

- Use ice to keep the sample cool.
- Work in a nuclease-free environment.
- Wear gloves at all times.
- Use sterile, filtered pipette tips.
- Avoid repeated freeze-thaw cycles.
- Lower the speed of the vortex mixer.
- Set the centrifuge to use gentle acceleration and braking profiles.

Tips to Optimize the Signal

DNA concentration is most important parameter to optimize for accurate quantification. For plasmid analysis, the optimal range for DNA concentration depends on the quantity of the open circular isoforms in the sample. Use the lowest concentration range where all isoforms of interest have a signal-to-noise ratio >10. In this assay, open circular isoforms are detected at a concentration of approximately 0.30 ng/µL. For example, if the expected open circular isoform percent corrected peak area (% CPA) is approximately 30%, then the total plasmid DNA concentration should be no more than 1.0 ng/µL to keep the signal of all isoforms in the linear range.

Linearized and relaxed open circular (nicked) DNA isoforms have a higher affinity for dye than the supercoiled isoform. If the supercoiled isoform is expected, then start with a 250× dilution of SYBR™ Gold Nucleic Acid gel stain in the gel buffer. This is the dye concentration used by the procedure given in this guide. To optimize signal between all isoforms, more titration of the dye concentration may be required.

For samples that contain only linearized dsDNA, the recommended concentration range is greater than the recommended concentration range for plasmid DNA isoforms. To detect all peaks of interest, adjust the sample concentration to make sure that all linearized peaks have concentrations greater than the limit of quantitation (LOQ).

The gel stain concentration has a large effect on peak migration time. A higher concentration of dye increases migration time and a lower concentration decreases migration time. The volume specified in this application guide is suitable for most samples.

Prepare the DNA Ladder

The 1 Kb Plus DNA Ladder[6] can be used as a test sample for the analysis of linearized double-stranded DNA (dsDNA). It can also be used to create a calibration curve for analysis of linearized dsDNA.

Note: For a test sample for plasmid isoform analysis, use the DNA 20 kb Plasmid test mix. Refer to the section: Prepare a Test Sample for Plasmid Isoform Analysis.

1. Thaw the 1 Kb Plus DNA Ladder[6] on ice.
2. To make enough for eight samples (one column in the sample tray), add 5 µL of 1 Kb Plus DNA Ladder[6] (500 ng/µL) to 800 µL of DNA 20 kb Plasmid and Linear sample buffer, and then mix with a vortex mixer.
3. Add between 95 µL and 100 µL to the sample plate well.
4. Make sure that, for any column on the sample plate with sample, the related column on the sample outlet plate has 1.5 mL of CE Grade water.

Prepare a Test Sample for Plasmid Isoform Analysis

To help identify issues with the BioPhase 8800 system or reagent preparation, run the DNA 20 kb Plasmid test mix before doing the analysis of new samples. The test mix is made from DNA that is 5.4 kb in length and contains the three main plasmid isoforms: supercoiled, linear, and open circular forms.

Note: For a test sample for linearized DNA, use the 1 Kb Plus DNA Ladder[6]. Refer to the section: Prepare the DNA Ladder.

1. Thaw a vial of DNA 20 kb Plasmid test mix on ice.
2. To make enough for eight samples (one column in the sample tray), add 800 µL of DNA 20 kb Plasmid and Linear sample buffer to the vial of DNA 20 kb Plasmid test mix, and then mix with a vortex mixer.

   After dilution, the final DNA concentration of the DNA 20 kb Plasmid test mix is approximately 1 ng/µL.
3. Add between 95 µL and 100 µL to the sample plate well.

[6] 1 Kb Plus DNA Ladder (Invitrogen™) is available for purchase from ThermoFisherScientific.

4. Make sure that, for any column on the sample plate with sample, the related column on the sample outlet plate has 1.5 mL of CE Grade water.
5. Keep the solution that is not used at –35 °C to –15 °C for a maximum of 3 months.
6. Put the sample plate in the system.
7. Make sure that the plate compartment temperature is set to 10 °C.

Prepare a Plasmid DNA Sample

1. Thaw the DNA on ice.
2. Measure the total DNA concentration of sample using UV absorbance or fuorescence spectrometer.
3. Prepare the sample in DNA 20 kb Plasmid and Linear sample buffer at a concentration between 0.5 ng/µL and 5 ng/µL, depending on the abundance of open circular isoform in the sample.

   To get the most reproducible results, the higher the concentration of open circular isoform in the sample, the lower the total DNA concentration should be. Refer to the section:Tips to Optimize the Signal.
4. Add between 95 µL and 100 µL to the sample plate well.
5. Make sure that, for any column on the sample plate with sample, the related column on the sample outlet plate has 1.5 mL of CE Grade water.
6. Put the sample plate in the system.
7. Make sure that the plate compartment temperature is set to 10 °C.

Prepare a Linearized dsDNA Sample

If the sample contains only of linearized dsDNA, then the recommended concentration range is wider than when analyzing plasmid DNA isoforms. It is crucial to use a sample concentration in which all linear peaks of interest meet the limit of quantitation (LOQ).

1. Thaw the DNA on ice.

   Keep the sample cool to help prevent degradation of the DNA.
2. Use the table below to find the recommended DNA concentration, and then prepare the DNA sample in DNA 20 kb Plasmid and Linear sample buffer.

Table 4-1 Recommended DNA Concentrations

DNA Sample	Recommended Concentration (ng/µL)
Purified single-peak DNA	0.1
DNA ladder	0.5 to 1.0

Table 4-1 Recommended DNA Concentrations (continued)

DNA Sample	Recommended Concentration (ng/µL)
Linearized DNA digest	0.5 to 1.0

3. Add between 95 µL and 100 µL to the sample plate well.
4. Make sure that, for any column on the sample plate with sample, the related column on the sample outlet plate has 1.5 mL of CE Grade water.
5. Put the sample plate in the system.
6. Make sure that the plate compartment temperature is set to 10 °C.

Prepare the BioPhase 8800 System 5

Use the procedures in this section to prepare the BioPhase 8800 system to acquire data.

Tip! To save time, turn on the light source 30 minutes before the start of the run to let it become warm.

Load the Reagent Inlet and Outlet Plates

Note: The gel buffer and dye mixture is referred to as *gel buffer* in this document.

CAUTION: Potential Wrong Result. Do not prepare the gel buffer before it is required. Degradation of the SYBR™ Gold Nucleic Acid gel stain in the gel buffer might occur during storage and cause peaks with decreased intensity.

Note: To prevent air bubbles, do not shake or vigorously mix the gel buffer. Air bubbles might cause issues with the separation.

Note: Only X-Pierce film from USA Scientific has been validated for use. If a different film is used, then test it before use.

CAUTION: Potential System Damage. Do not use a heated plate sealer to attach the film cover. If the heat causes damage to the surface of the plates, then issues with the pressure system might occur.

CAUTION: Potential Wrong Result. Before the plates are loaded in the system, spin them to remove air bubbles. Air bubbles might an unsatisfactory separation.

CAUTION: Potential System Damage. Before the plates are loaded in the system, remove the film cover. Do not load plates in the system before the film cover is removed. If the film cover is not removed, then damage to the capillary tips might occur.

1. Add the following reagents to a conical tube.

   Note: For replicate injections, make sure to adjust the volume accordingly.

Table 5-1 Gel Buffer (DNA 20 kb Plasmid and Linear gel with SYBR™ Gold Nucleic Acid gel stain)

Reagent	For 1 to 96 Samples or 12 Injections
DNA 20 kb Plasmid and Linear gel	20 mL
SYBR™ Gold Nucleic Acid gel stain[7]	80 µL[8]

Tip! Wrap the tube that contains the prepared gel buffer in aluminum foil to decrease photobleaching of the SYBR™ Gold Nucleic Acid gel stain.

2. Carefully invert the tube a minimum of 20 times.

   Make sure that air bubbles are not created when the tube is inverted.

3. Use the reagent plate layout to add the following reagents to the reagent inlet and outlet plates. Refer to the figure: Figure C-4.

Table 5-2 Reagents for the Reagent Inlet and Outlet Plates

Plate	Reagent
Inlet plate	800 µL per well
Outlet plate	• 2.8 mL of reagent per well for separation or wait positions • 1.5 mL of CE Grade water per well for waste positions

Note: For the outlet plate, make sure that the chamfered corner is on the top right, and then fill only the wells on the left side of the plate. The wells on the right side are for overflow and must be empty.

4. Put a film cover on the plates.
5. Put the plates in a swinging-bucket rotor, and then spin them for 4 minutes at 30 × *g*. Make sure that the buckets are balanced.
6. Make sure that there are no air bubbles in the plates. To remove air bubbles, then spin the plates again at a higher relative centrifugal force (RCF).

   For the reagent plate, the maximum RCF is 1,000 × *g*. For the sample plate, the maximum RCF is 375 × *g*.
7. On the front panel, touch **Eject Reagent**.

[7] SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Gold Nucleic Acid gel stain is not available for resale.

[8] The structure of the DNA has an effect on the volume of dye required. Refer to the section: Best Practices for Work with DNA.

Figure 5-1 Eject Reagent Button

The plate compartment opens.

8. Remove the film cover from the plates.
9. If the plate compartment contains reagent plates, then remove them.
10. Align the notch in the reagent inlet plate with the tab, and then put the plate in the plate carrier.
11. Make sure that the chamfered corner of the reagent outlet plate is in the top left, and then put the plate in the back of the plate carrier.
12. Touch **Load Reagent**.

Figure 5-2 Load Reagent Button

The plate compartment closes.

Load the Sample Inlet and Outlet Plates

CAUTION: Potential System Damage. Do not use a heated plate sealer to attach the film cover. If the heat causes damage to the surface of the plates, then issues with the pressure system might occur.

Note: Only X-Pierce film from USA Scientific has been validated for use. If a different film is used, then test it before use.

1. Use the sample plate layout to add the samples to the sample inlet plate. Refer to the figure: Figure C-3.

   The recommended sample volume is 100 µL. The minimum sample volume is 50 µL. The maximum sample volume is 200 µL.

2. Use the sample plate layout to add the following reagents to the sample outlet plate. Refer to the figure: Figure C-3.

   The maximum volume is 2.0 mL.

Note: For the outlet plate, make sure that the chamfered corner is on the top right, and then fill only the wells on the left side of the plate. The wells on the right side are for overflow and must be empty.

Table 5-3 Reagents for the Sample Outlet Plate

Plate	Reagent
Outlet plate	• 1.5 mL of CE Grade water per well

3. Make sure that, for any column on the sample plate with sample, the related column on the outlet plate has 1.5 mL of CE Grade water.
4. Put a film cover on the plates.
5. Put the plates in a swinging-bucket rotor, and then spin them for 4 minutes at 30 × *g*. Make sure that the buckets are balanced.
6. Make sure that there are no air bubbles in the plates. To remove air bubbles, then spin the plates again at a higher relative centrifugal force (RCF).

   For the reagent plate, the maximum RCF is 1,000 × *g*. For the sample plate, the maximum RCF is 375 × *g*.
7. On the front panel, touch **Eject Sample**.

Figure 5-3 Eject Sample Button

The plate compartment opens.

8. Remove the film cover from the plates.
9. If the plate compartment already contains sample plates, then remove the sample plates.
10. Align the notch in the sample plate with the tab, and then put the plate in the plate carrier.
11. Make sure that the chamfered corner of the sample outlet plate is in the top left, and then put the plate in the back of the plate carrier.
12. Touch **Load Sample**.

Figure 5-4 Load Sample Button

The plate compartment closes.

Examine the Capillary Cartridge

WARNING! Puncture Hazard. Touch the cartridge carefully. The capillary tips are extremely sharp.

CAUTION: Potential System Damage. Do not let the gel buffer or other reagents crystallize on the electrodes, capillary tips, cartridge seals, or cartridge body. Electrolyte salt crystals or precipitate can cause blocked capillaries, improper pressure seals, errors when samples are injected, arcing, or current leakage.

CAUTION: Potential System Damage. Do not use organic solvents, such as methanol or acetone, to clean the capillary window. Organic solvents can dissolve the adhesives and make a residue on the capillary window that causes interference with the detector.

1. Examine the electrodes, capillary tips, cartridge seals, and cartridge body before use.
2. If gel or liquid has collected on the outside of the cartridge, then clean the cartridge with a moist lint-free wipe. After cleaning, make sure to dry the cartridge.

   Note: Do not use soap or detergent to clean the cartridge.

3. If the capillary tips are blocked, then do this:
   a. Use CE Grade water to clean the outside surface of the capillary inlets.
   b. Use a lint-free wipe to clean the capillary inlets carefully in an outward direction.
4. Use a magnifying glass to examine both sides of the capillary window. If lint or other particles have collected, then use short bursts of electronics-grade compressed air to remove them. Do not use water or other liquids to clean the capillary window.
5. Dampen a lint-free wipe or cotton swab with ethanol or isopropanol, and then clean the surface of the ID chip. Let the ID chip air-dry before the cartridge is installed.

Install the Capillary Cartridge

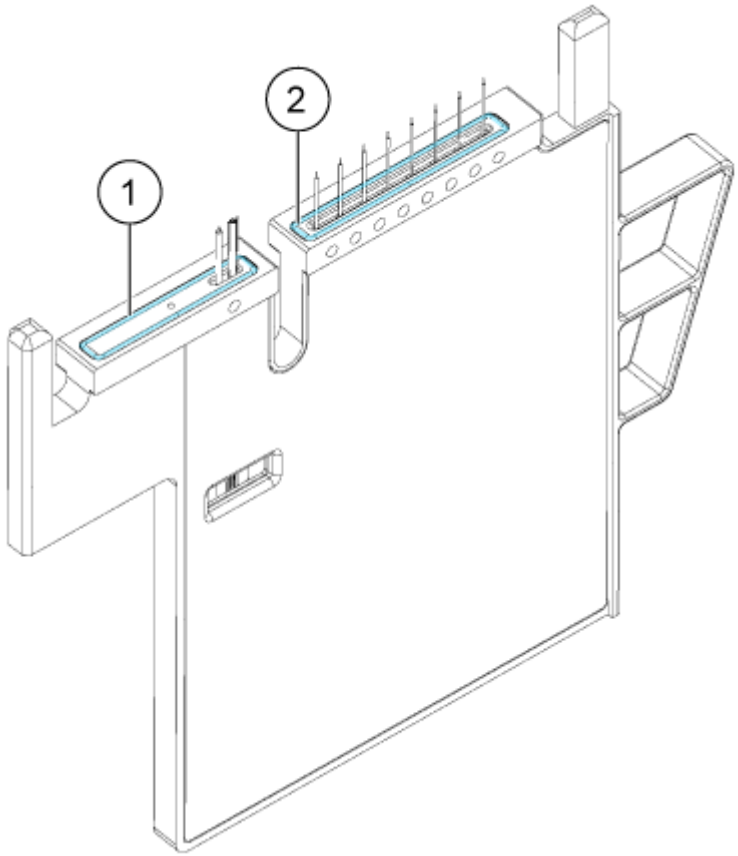
WARNING! Puncture Hazard. Touch the cartridge carefully. The capillary tips are extremely sharp.

WARNING! Pinching Hazard. When the front panel is opened, do not put fingers to the left of the front panel.

CAUTION: Potential System Damage. Make sure that the reagent plates are installed in the system before the cartridge is installed. Otherwise, damage to the cartridge might occur.

1. If the cartridge was kept in the refrigerator, then let the cartridge equilibrate to ambient temperature for approximately 30 minutes to prevent condensation in the system.
2. Remove the cartridge from the wetting tray.
3. To prevent arcing, use a disposable laboratory wipe to dry the cartridge body to prevent arcing.
4. Turn the bottom of the cartridge up.
5. Use a disposable laboratory wipe to very carefully dry the area where the capillaries and electrodes come out of the cartridge. Do not touch or move the seals.

Figure 5-5 Bottom of the Cartridge



Item	Description
1	Outlet plate seal
2	Inlet plate seal

6. If the reagent plates are not installed in the system, then install them. Refer to the section: Load the Reagent Inlet and Outlet Plates.
7. Open the front panel, and then put the cartridge in the system.
8. Close the front panel, and then touch **EJECTED** to lock the cartridge.

Figure 5-6 EJECTED Button

The system moves the reagent plate until the capillaries are in position over column 1, and then lifts the plate until the capillary tips are immersed in CE Grade water.

9. Examine the coolant level on the front panel. If required, add coolant to the system.
   Refer to the section: "Add Capillary Cartridge Coolant" in the document: *Operator Guide*.

Run the Samples 6

Tips for Best Results

Use the Rinse Method

A rinse method is supplied with the other methods for the kit.

After the capillary has been conditioned, use the applicable rinse method as an alternative to the conditioning method. If the cartridge has done more than 50 injections since it was conditioned, then examine run-to-run separation metrics such as **Res.** (peak resolution) and **N** (theoretical plates). If the metrics are acceptable, then conditioning is not required. Use the rinse method in the sequence. If the quality of the separation has decreased, then use the conditioning method in the sequence.

Start the Sequence from the Front Panel

To use the Waters Empower™ software, refer to the section: Start the Sample Set Method.

1. If required, load the cartridge, reagent plates, and sample plates.
2. On the front panel, touch **RUN SEQUENCE**.

Figure 6-1 RUN SEQUENCE Button

3. In the Projects pane, touch **DNA 20kb**.
4. In the Available Sequences pane, touch the applicable sequence in the list.
   - For a cartridge with 30 cm capillaries, click **`30cm-DNA 20kb Plasmid Test Mix- 6 runs with conditioning`**
   - For a cartridge with 50 cm capillaries, click **`50cm-DNA 20kb DNA ladder- 6 runs with conditioning`**
5. (Optional) To see the details of the method, sample plates, or reagent plates, touch anywhere in the **Method** column.
   To hide the details, touch the column or box again.

6. Touch **Run Sequence**.

Figure 6-2 Run Sequence Button

If the sequence contains a method that is incompatible with the system configuration, then **Run Sequence** is not available.

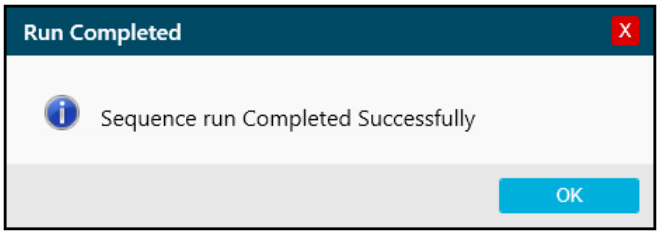
The data files are saved at the location specified in the sequence. If this location has a data file with the same name, then, to prevent the older data file from being overwritten, a date stamp is automatically added to the file name.

If an error occurs during a run and an error recovery method is included in the sequence, then the BioPhase 8800 system starts the error recovery method.

During the run, various actions are available. Refer to the section: Monitor the Run on the BioPhase 8800 Front Panel.

When the run is complete, the Run Completed dialog opens.

Figure 6-3 Run Completed Dialog



7. To close the Run Completed dialog, touch **OK**.
8. If required, stow the cartridge. Refer to the section: Stow the Cartridge After the Run.

Monitor the Run on the BioPhase 8800 Front Panel

CAUTION: Potential System Damage. If the run is stopped, then sample or reagent might be lost and damage to the cartridge might occur.

CAUTION: Potential System Damage. If the run is stopped and will not be started again, then use the shutdown method to rinse the capillaries before the cartridge is stowed. If the capillaries are not rinsed, then electrolyte salt crystals or precipitate can collect and might cause blocked capillaries, incorrect pressure seals, errors when the samples are injected, arcing, or current leakage.

CAUTION: Potential System Damage. Before the run is started again, make sure to empty or replace the outlet plate to prevent the overflow of reagent and possible damage to the instrument.

CAUTION: Potential Wrong Result. Before the run is started again, prepare new reagent plates. If the run has been stopped, then there might not be sufficient reagents available to complete the run.

CAUTION: Potential Wrong Result. If the samples have been inside of the system for more than 24 hours, sample degradation might have occurred. Discard the samples before the run is started again.

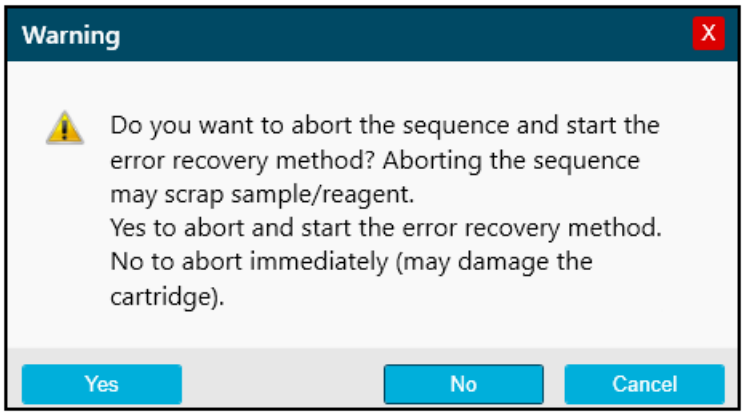
Use this procedure to monitor the sequence progress, and then, if required, pause or stop the sequence.

To use the Waters Empower™ software, refer to the section: Monitor the Run in the Waters Empower™ Software.

Note: The sequence shown in the following figures is for the purpose of illustration. It does not show a sequence for the DNA 20 kb Plasmid and Linear kit.

1. Monitor the traces for the detector and the current to make sure that the sequence is in progress.
2. To stop the run, touch  .

Figure 6-4 Warning Dialog

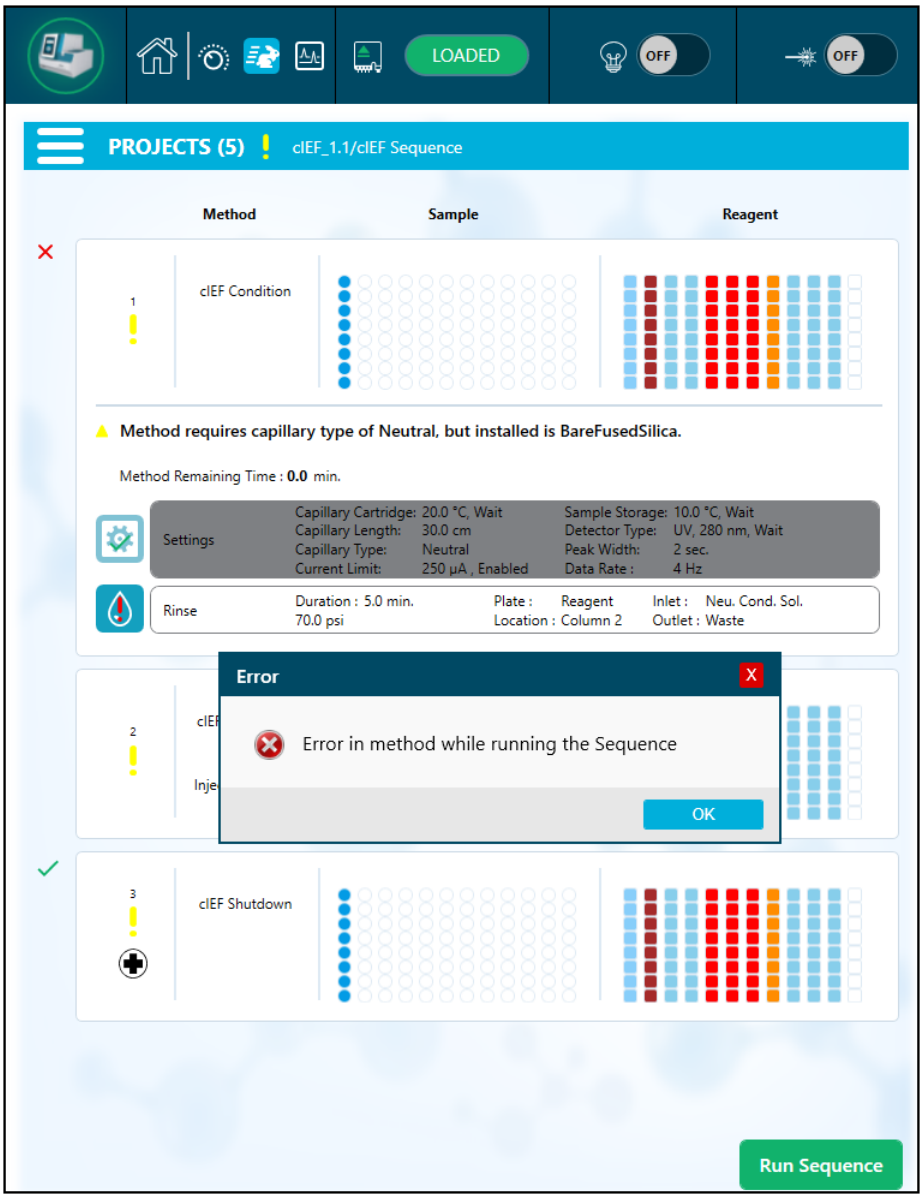


3. Touch one of the following options:
   - **Yes**: To start the error recovery method, if one is assigned.
   - **No**: If an error recovery method is not assigned.

- **Cancel**: To continue the run.

4. If an error occurs, then touch **OK** in the error dialog that opens.

Figure 6-5 Run Sequence Error Dialog



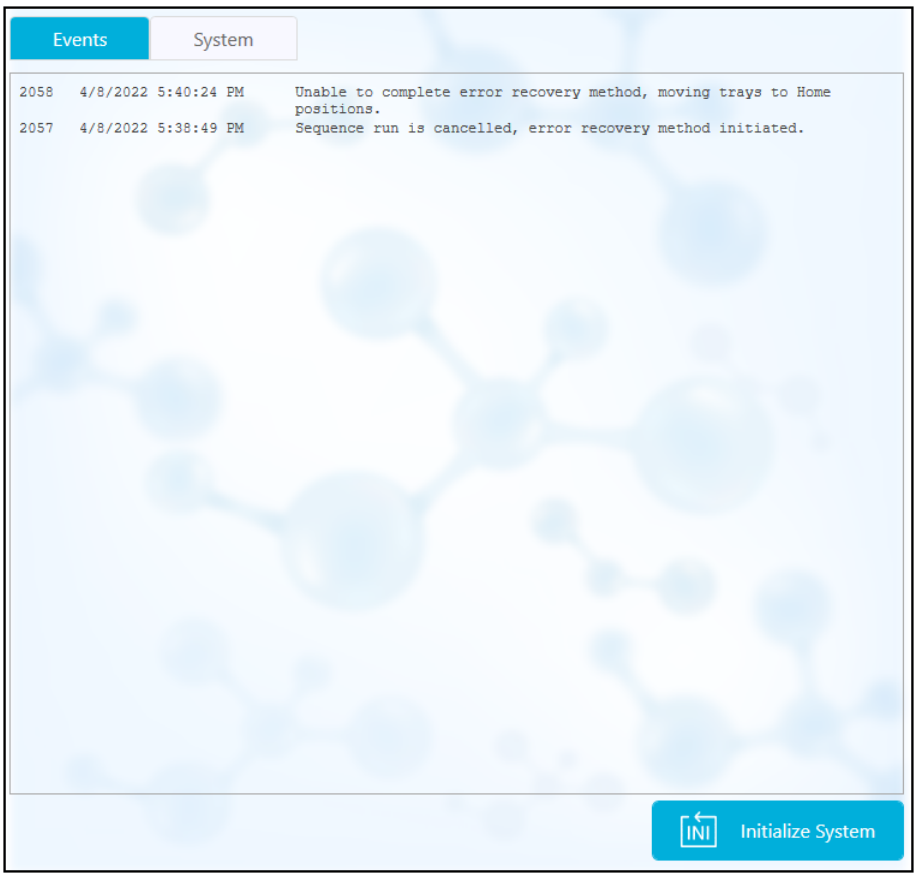
Note: The shows an error at the **Rinse** action. The grey shading in the row above the **Rinse** action shows that the action is in progress or completed.

5. Review the error:

a. On the **Events** tab of the front panel log, touch 

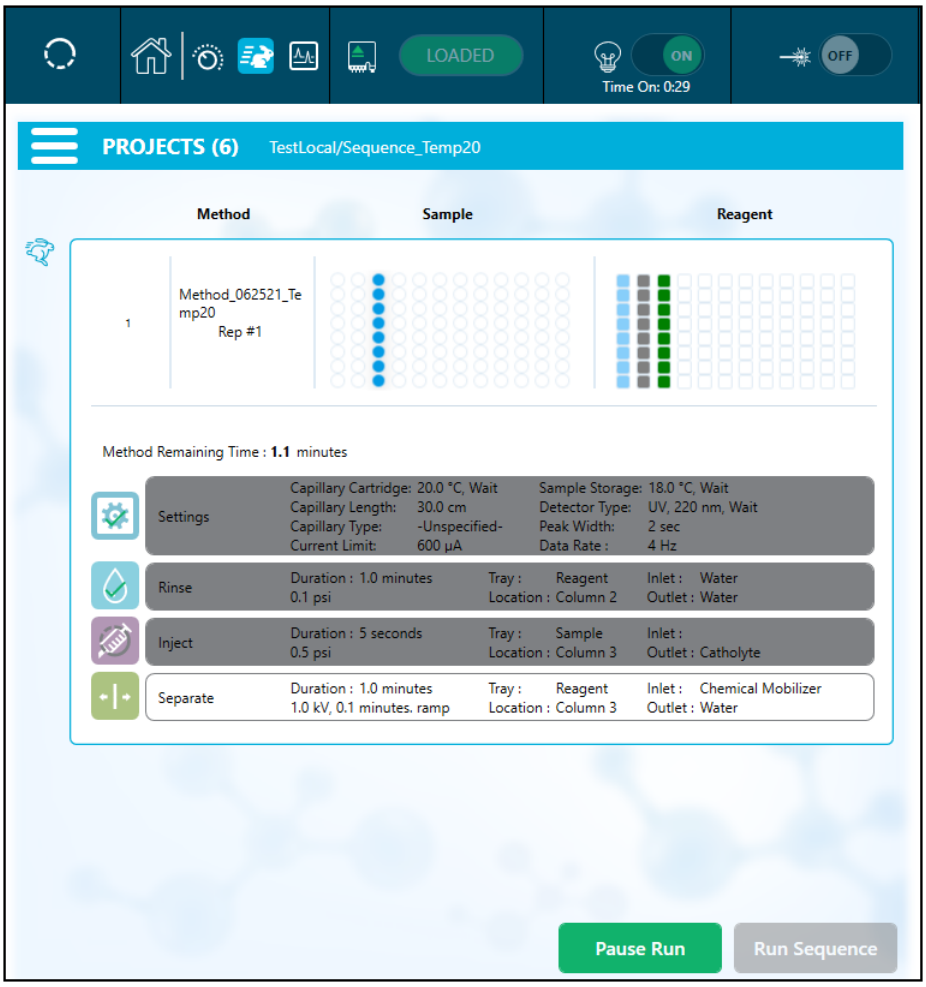
b. Touch **Initialize System** to initialize the system and change the system status to idle.

Figure 6-6 Sequence Error Events Log



6. If required, then touch **Pause Run**.

Figure 6-7 Run Sequence in Progress



To continue the run, touch **Cancel Pause**.

Figure 6-8 Run Sequence Paused

7. To see the data during acquisition, touch  in the ribbon.

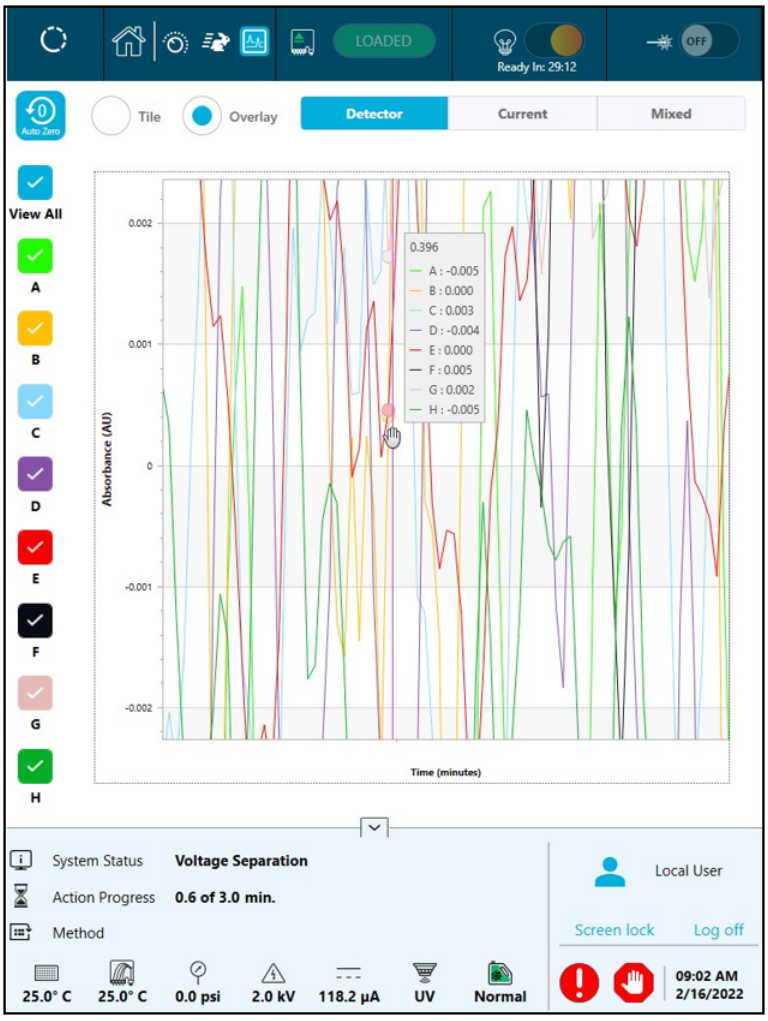
Note: The data in the following figure is for illustration only. It does not show results for samples prepared with the DNA 20 kb Plasmid and Linear kit.

Figure 6-9 Capillary View

8. (Optional) To zoom in on the data, do the following:
   a. Touch **Overlay**.
   b. Use two fingers to zoom in or out to see the electropherogram.
   c. Use the hand icon to move the electropherogram.

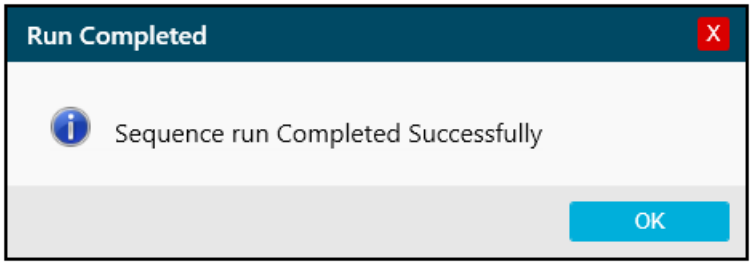
> **Note:** The zoom feature can only be used with the Overlay view for **Detector** and **Current**.

Figure 6-10 Zoom In or Out



9. Make sure that the message `Sequence run Completed Successfully` is shown when the run completes. In the dialog, touch **OK**.

Figure 6-11 Run Completed Dialog



If the system has been idle for 24 hours, then the cooler for the sample storage compartment turns off.

Waste Disposal

WARNING! Biohazard or Toxic Chemical Hazard. Obey local directives to discard chemicals, cartridges, reagent plates, sample plates, and the remains of the prepared samples. They might contain regulated compounds and biohazardous agents.

Stow the Cartridge After the Run

WARNING! Puncture Hazard. Touch the cartridge carefully. The capillary tips are extremely sharp.

Stow the Cartridge for Less than 24 Hours

1. If the sequence or sample set method does not include the shutdown method, then use the shutdown method to clean the capillaries.
   The shutdown method fills the capillaries with CE Grade water and decreases the cartridge temperature to 20 °C.
2. Keep the cartridge in the system for as long as 24 hours with the capillary tips immersed in CE Grade water.

Note: If the cartridge has not been used for 3 hours or longer, then run the conditioning method before a separation is done.

Stow the Cartridge for More than 24 Hours

1. If the sequence or sample set method does not include the shutdown method, then use the shutdown method to clean the capillaries.
   The shutdown method fills the capillaries with CE Grade water and decreases the cartridge temperature to 20 °C.
2. On the ribbon on the BioPhase 8800 system front panel, touch 

(**Loaded**), and then wait for approximately 1 minute to let the coolant go back to the coolant reservoir before the cartridge is removed.
3. Remove the cartridge from the system. Stow the cartridge upright in the cartridge box at 2 °C to 8 °C with the capillary tips immersed in CE Grade water.

Note: Replace the CE Grade water in the tray regularly to prevent microbial growth in the tray.

Note: Make sure that there is sufficient CE Grade water in the tray to immerse the capillary tips.

Prepare the Cartridge After Storage

- If the cartridge has not been used for more than a day, and has done fewer than 50 injections since it was conditioned, then use the applicable rinse method to rinse the capillaries.
- If the cartridge has not been used for more than a day, and has done more than 50 injections since it was conditioned, then examine run-to-run separation metrics such as **Res.** (peak resolution) and **N** (theoretical plates). If the metrics are acceptable, then conditioning is not required. Use the rinse method to prepare the cartridge. If the quality of the separation has decreased, then prepare the cartridge with the applicable conditioning method.

Note: To prevent arcing, and before the cartridge is installed in the system, carefully remove any water from around the electrodes and cartridge body.

Analyze the Data 7

Use an Analysis Parameters File to Analyze the Data

Note: If the Waters Empower™ software will be used to acquire the data, then this procedure is not applicable.

An analysis parameters file contains all of the information required to integrate the data and then identify peaks.

Note: This procedure is for users who know how to use the BioPhase software. For detailed instructions, refer to the document: *Software Help System*.

Note: The analysis parameters file in this procedure is an example. The parameters might not be optimal for all data files.

1. On the Home page of the BioPhase software, click **Data Analysis**.
   The BioPhase Analysis software main window opens.
2. Click **File** > **Open**, select the data files to analyze, and then click **Open**.
3. On the **Project** toolbar, click  , browse to the analysis parameters files, and then click **Open**.

   Three analysis parameters files are available. Click the applicable file:

   - **`30 cm_Plasmid test mix analysis example`**: Analyze the plasmid test mix for data acquired with the 30 cm capillaries.
   - **`30 cm_1kb plus ladder integration example`**: Analyze the 1 Kb Plus DNA Ladder for data acquired with the 30 cm capillaries.
   - **`50cm_1kb plus ladder integration example`**: Analyze the 1 Kb Plus DNA Ladder for data acquired with the 50 cm capillaries.
4. Right-click  , and then select **Apply & Analyze (all)**.

   The software applies all of the parameters on the Integration, Library, and Post Analysis tabs to all of the data files, and then shows the results.

   In the Files pane, the file name is red to show that the data has been analyzed. The number of peaks identified is shown in the **Peaks** column.

In the Data pane, the analysis results are shown in the table below the graph. At the top of the table, **RMS Noise**, **P-P Noise**, and **Drift** are shown. In the graph, the baseline is red and the threshold is a grey horizontal line. Any peaks identified in the analysis have a blue marker at the peak start, a red marker at the peal apex, and a green marker at the peak end.

Peaks in the graph have the following shading:

- Green: The peak is identified as a peak in the Marker Table on the Library tab.
- Blue: The peak is identified as a peak in the Peak Table on the Library tab.
- Red: The peak is not a named peak.

Refer to the section: Examine the Results.

5. Show the peak names on the graph:

   a. Right-click  .
   The Information Setup dialog opens.

   b. Select **Name** and any other information to include on the graph, such as **MT**, and then click **OK**.

   c. Click  .

   The peak names are shown on the graph. Refer to the section: Examine the Results.

   The names are part of the analysis parameters file. To use different names, refer to the section "Identify the Peaks" in the document: *Software Help System*.

6. Click in the Files pane below the list of files, and then press the **Up** and **Down** arrow keys to see the data for each file in the Data pane.
   If required, drag over a region on the graph to zoom in to see the details of the identified peaks in that region.

   Tip! To use the same zoom settings to all of the data files, click  .

7. Make sure that the integration is satisfactory. If required, then adjust the integration parameters, and then analyze the data again.

8. Examine the electropherogram for the peaks in the Marker Table and Peak Table.

   a. For each peak in the Marker Table and Peak Table, make sure that the correct peak has a label in the graph.

   b. If required, adjust **MT** in the Marker Table and **MT** (or **Cal MT**) in the Peak Table.

   c. If required, adjust **Tol** and **Crit**, and then click  .

- **Tol** is the tolerance used to find a match between a peak in the graph and a peak in the Marker Table or the Peak Table. To use the tolerance as a percentage, type % after the value.
- **Crit** is the peak characteristic used to find a match.
  - **Ctr**: The peak closest to the center of the range is used to find a match.
  - **Ht**: The tallest peak in the range is used to find a match.
  - **Area**: The largest peak in the range is used to find a match.

d. When the peak assignments are satisfactory, right-click  , and then select **Apply & Analyze (all)**.

The software applies the changes to all of the data files.

9. (Optional) On the **Project** toolbar, click  , type a name, select a location, and then click **OK**.
   The analysis parameters are saved to a file for later use.

10. (Optional) On the **File** toolbar, right-click  , and then select **Print (all)**.
    The contents of the Data pane are printed in the active report template. For instructions to create a report template, refer to the section "Configure a Report" in the document: *Operator Guide*.

11. On the **File** toolbar, right-click  , and then select **Save (all)**.
    All changes to the results are saved to the data files. The analysis parameters are included.

12. On the **File** toolbar, right-click  , and then select **Close (all)**.
    All of the data files close.

Examine the Results

Figure 7-1 DNA 20 kb Plasmid test mix with 30 cm Capillaries

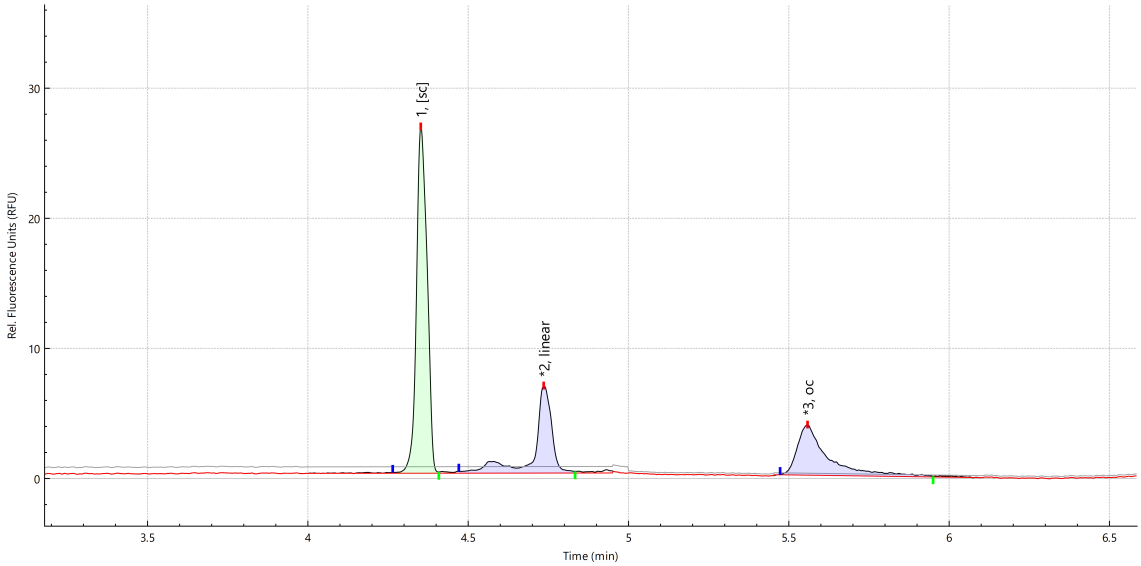


Figure 7-2 1 Kb Plus DNA Ladder with 30 cm Capillaries

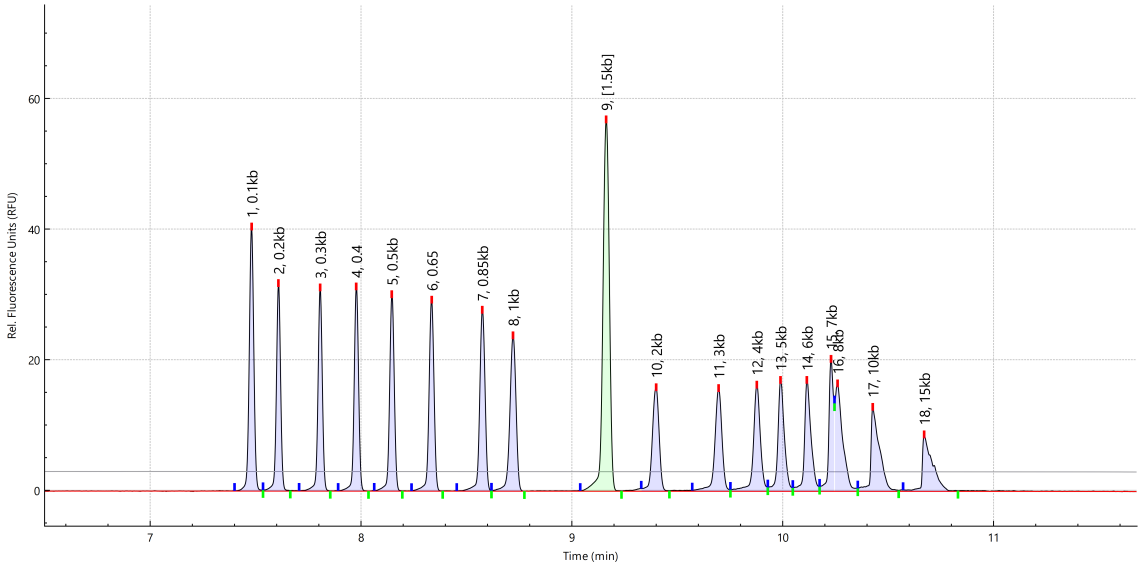
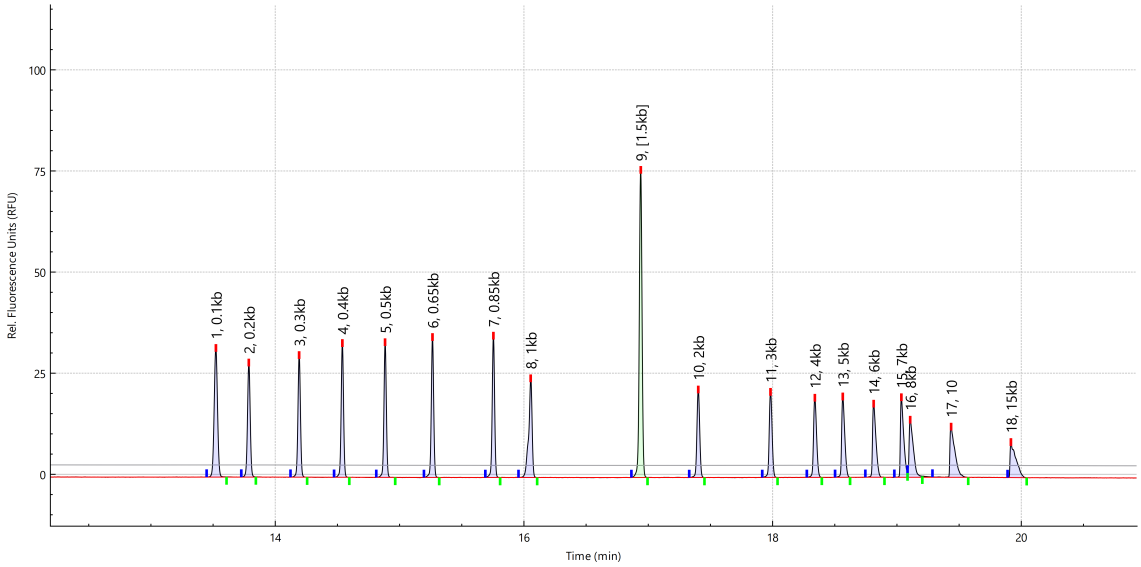


Figure 7-3 1 Kb Plus DNA Ladder with 50 cm Capillaries



Review the Results on the Overlay Tab

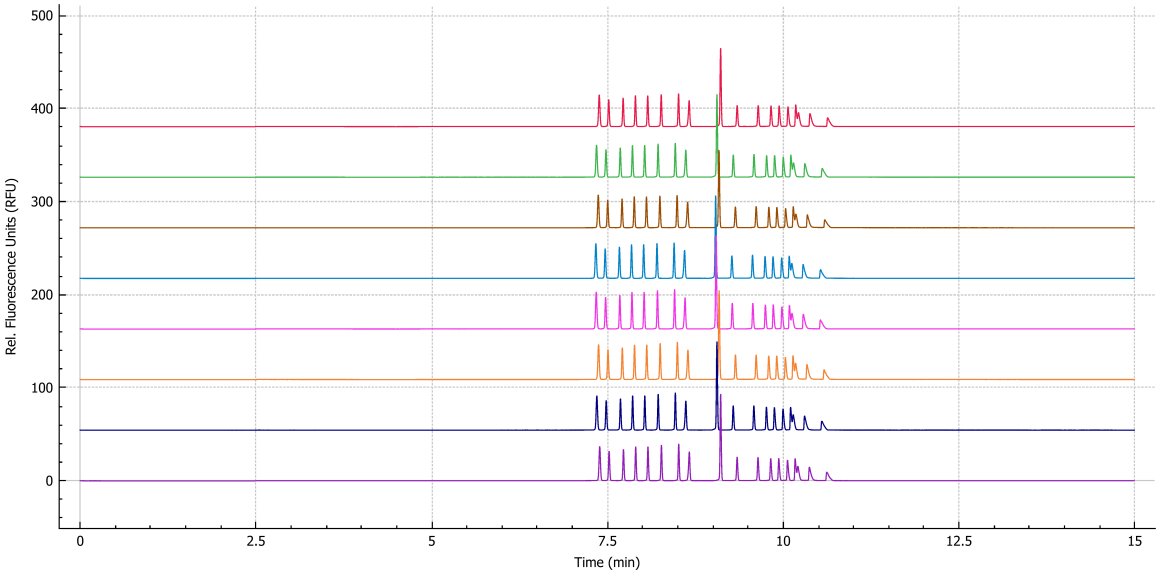
The Overlay tab shows the graphs for the selected data files. This tab has statistics for the selected data files, as well as the system suitability report.

Note: This section does not describe the system suitability function. For information about system suitability, refer to the document: *Operator Guide*.

1. Open a set of data files and the analysis parameters file, and then analyze the data. If required, adjust the analysis parameters until the results are satisfactory.

2. In the Files pane, click  , and then open the Overlay tab.

Figure 7-4 Overlay Tab



The color of the trace in the graph corresponds to the color in the circle next to the file name in the Files pane.

The thicker line is the trace for the file selected in the Files pane.

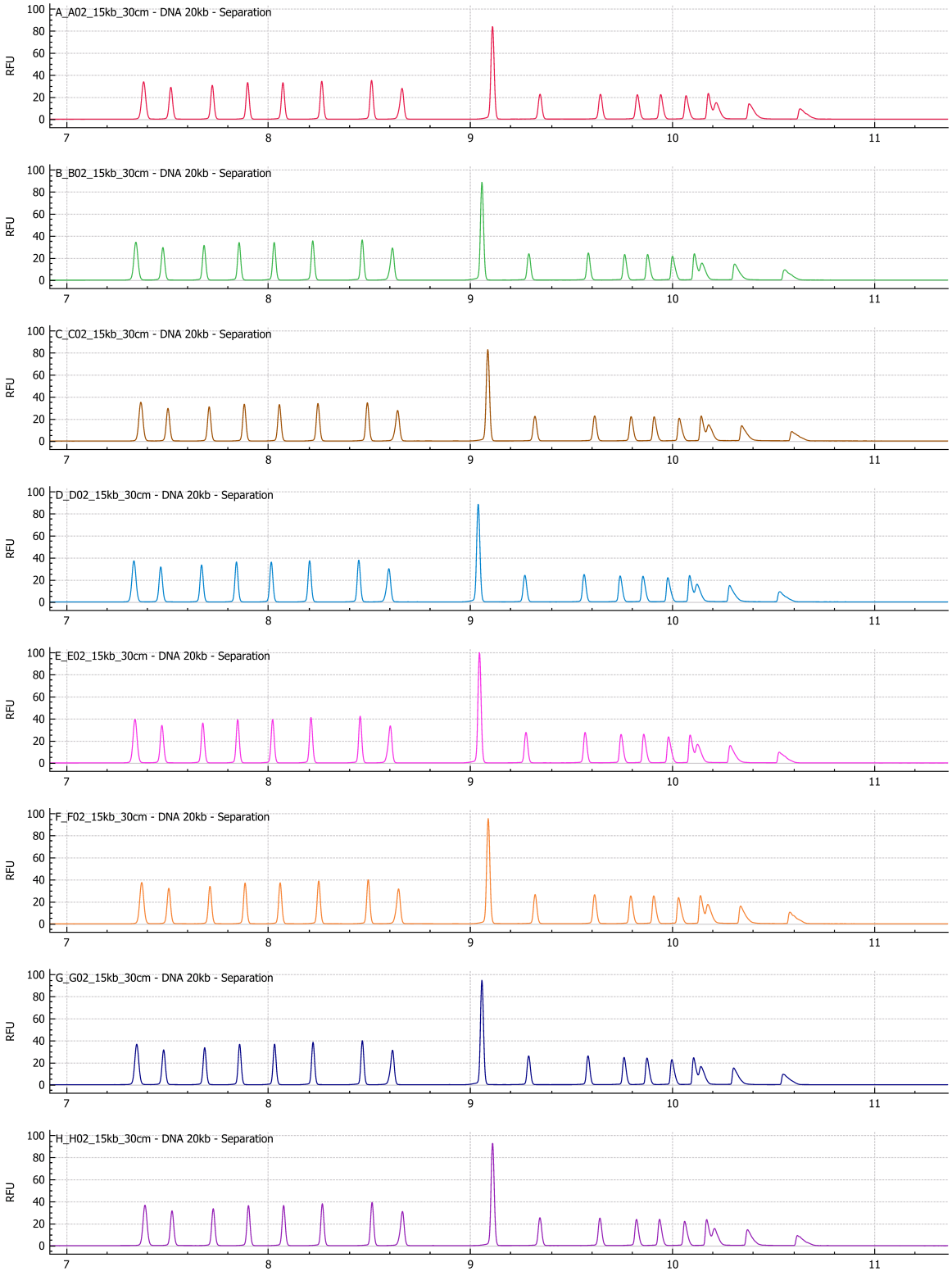
3. To change the order of the files in the overlay, in the Files pane, click the file to be moved, and then drag it up or down the list of files.

Note: The order of the colors assigned to the files does not change after a file is moved.

4. To adjust the distance between the traces, move the slider on the right side of the graph up or down.

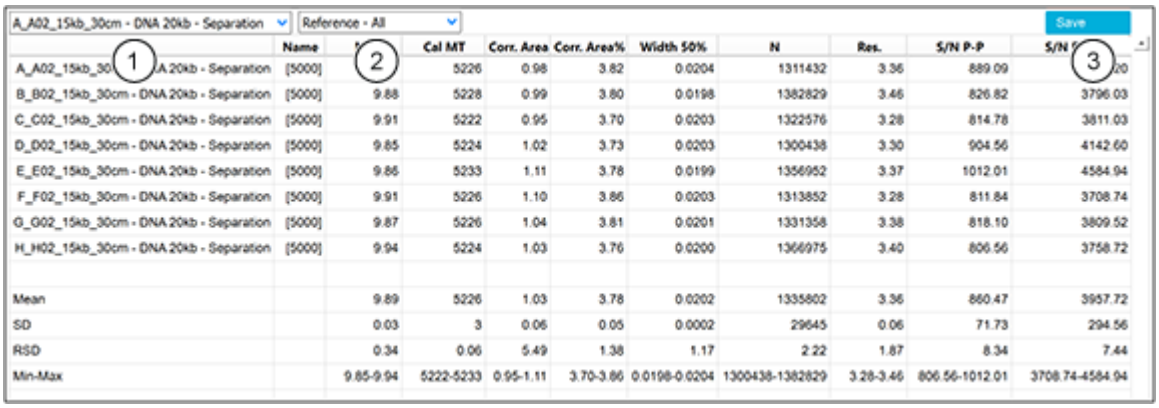
Note: To see the traces as a series of tiled graphs, move the slider all of the way to the top.

Figure 7-5 Tiled Results



5. Calculate the results for all of the files on the Overlay tab.

Figure 7-6 Results Table



Name	Cal MT	Corr. Area	Corr. Area%	Width 50%	N	Res.	S/N P-P	S/N
A_A02_15kb_30 A 20kb - Separation [5000]	5226	0.98	3.82	0.0204	1311432	3.36	889.09	20
B_B02_15kb_30cm - DNA 20kb - Separation [5000]	9.88	5228	0.99	3.80	0.0198	1382829	3.46	826.82	3796.03
C_C02_15kb_30cm - DNA 20kb - Separation [5000]	9.91	5222	0.95	3.70	0.0203	1322576	3.28	814.78	3811.03
D_D02_15kb_30cm - DNA 20kb - Separation [5000]	9.85	5224	1.02	3.73	0.0203	1300438	3.30	904.56	4142.60
E_E02_15kb_30cm - DNA 20kb - Separation [5000]	9.86	5233	1.11	3.78	0.0199	1356952	3.37	1012.01	4584.94
F_F02_15kb_30cm - DNA 20kb - Separation [5000]	9.91	5226	1.10	3.86	0.0203	1313852	3.28	811.84	3708.74
G_G02_15kb_30cm - DNA 20kb - Separation [5000]	9.87	5226	1.04	3.81	0.0201	1331358	3.38	818.10	3809.52
H_H02_15kb_30cm - DNA 20kb - Separation [5000]	9.94	5224	1.03	3.76	0.0200	1366975	3.40	806.56	3758.72
Mean	9.89	5226	1.03	3.78	0.0202	1335802	3.36	860.47	3957.72
SD	0.03	3	0.06	0.05	0.0002	29645	0.06	71.73	294.56
RSD	0.34	0.06	5.49	1.38	1.17	2.22	1.87	8.34	7.44
Min-Max	9.85-9.94	5222-5233	0.95-1.11	3.70-3.86	0.0198-0.0204	1300438-1382829	3.28-3.46	806.56-1012.01	3708.74-4584.94

Item	Description
1	Reference file
2	Type of analysis
3	Save the results to a comma-separated file

a. To select the type of analysis, click the list on the right side of the Results Table header.

   These options are available:

   - **Reference - All**: In the Results Table, show statistics for every peak in the reference file that is in all of the other data files.
   - **Reference - Peak Table**: In the Results Table, show statistics for every named peak in the reference file that is in all of the other data files.
   - **Named Peaks**: In the Results Table, show statistics for all of the named peaks in the data files.
   - **All Data (not displayed)**: Calculate but do not show statistics for all of the peaks in all of the data files.
   - **System Suitability**: System suitability testing is different than the other reports. For more information, refer to the section "System Suitability Testing" in the documents: *Operator Guide* or *Software Help System*.

   If the migration times of the peak apexes in the data file are within 5% of the peak apexes in the reference file, then a match is made.

b. To select the reference file, click the list on the left.

   The reference file is the file to which all of the other files are compared.

Only the **Reference - All** and **Reference - Peak Table** analyses use a reference file.

The Results Table changes to show the selected analysis or the system suitability report.

If **All Data (not displayed)** is selected, then the Results Table is empty. To see the results, click **Save** to save the results to a comma-separated file, and then use another program to open the file.

6. (Optional) To use a different reference file or see a different type of analysis, do step 5 again.
7. (Optional) Click **Save**.
   The Results Table is saved to a comma-separated text file. Only the columns that are shown in the table are saved.

   Note: To save the system suitability results, click **File** > **Save Report**. The results are saved as a PDF.
8. (Optional) Click **File** > **Print**.
   The contents of the Overlay tab are printed in the current report template.
9. (Optional) On the **File** toolbar, right-click  , and then select **Save (all)**.
   All changes to the results and the analysis parameters are saved to the data files.
10. On the **File** toolbar, right-click  , and then select **Close (all)**.
    All of the data files close.

Guidance for Acceptance Criteria

To create acceptance criteria for use with this kit for SOPs or other purposes, use parameters that are inherent to the quality of the separation and attributes that show critical sample qualities. Differences between gel and capillary lots and different systems might cause variations in absolute migration times.

For plasmid isoform analysis, the ratio of the migration time of the linear isoform to the migration time of the supercoiled isoform can be used a system suitability criterion. The resolution between the peaks for the supercoiled and linear isoforms can also be used. Do not use percent corrected peak area because it can vary from lot to lot of the DNA 20 kb Plasmid test mix.

For linearized dsDNA analysis, if the 1kb plus DNA ladder is present, then the migration time ratio between 1.5 kb and 0.1 kb peaks (or any combination of spaced peaks) for relative migration time. Resolution between resolved peaks can also be used.

SCIEX strongly discourages the use of absolute migration time as an acceptance criterion for either type of analysis.

Run the Samples with the Waters Empower™ Software 8

This section gives instructions for the use of the Waters Empower™ software and the BioPhase 8800 driver for Empower™ with the BioPhase 8800 system.

Import the BioPhase Software Methods to Create Instrument Methods

Note: Methods created with the BioPhase software are supplied with the BioPhase 8800 driver for Empower™. The methods are also available for download from the SCIEX website. Refer to the section: Download and Configure the Required Files (Waters Empower™ Software).

Instrument methods can also be created in the Method Editors for BioPhase System software. Refer to the documents: *Operator Guide* and *Software Help System*.

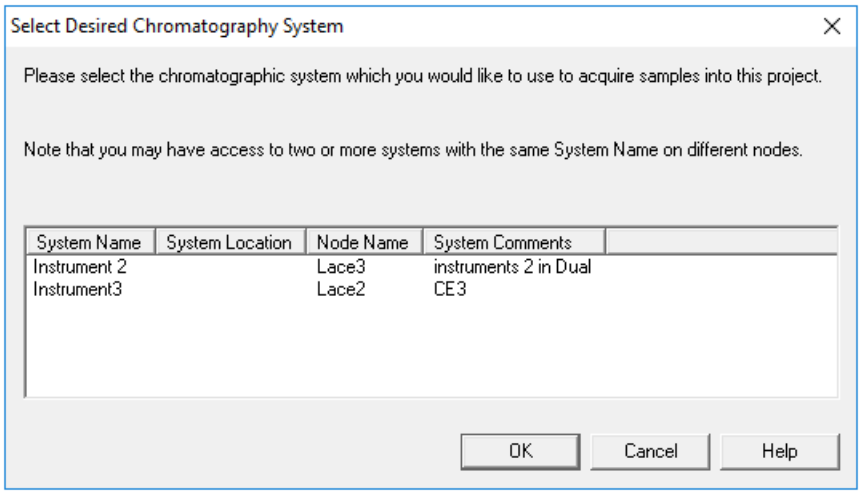
Typically, three types of methods are required: a conditioning method, separation method, and shutdown method. For some workflows, there are more methods.

The following methods are available.

- For 30 cm capillaries:
  - `30cm-DNA 20kb Conditioning`: Conditions the capillaries before first use.
  - `30cm-DNA 20kb Linear Separation`: Does the separation, best for linear dsDNA samples and plasmids larger than 4 kb.
  - `30cm-DNA 20kb Plasmid Separation`: Does the separation, best for plasmid samples.
  - `30cm-DNA 20kb Shutdown`: Cleans the capillaries at the end of a sequence, rinses the capillaries for storage, and then turns off the lamp.
  - `30cm-DNA 20kb Rinse`: Rinses the capillary between sequences, if required.
- For 50 cm capillaries:
  - `50cm-DNA 20kb Conditioning`: Conditions the capillaries before first use.
  - `50cm-DNA 20kb Linear Separation`: Does the separation with more resolution.
  - `50cm-DNA 20kb Shutdown`: Cleans the capillaries at the end of a sequence, rinses the capillaries for storage, and then turns off the lamp.
  - `50cm-DNA 20kb Rinse`: Rinses the capillary between sequences, if required.

1. In the Waters Empower™ Software Project window, click **File** > **New Method** > **Instrument Method**.

Figure 8-1 Select Desired Chromatography System Dialog



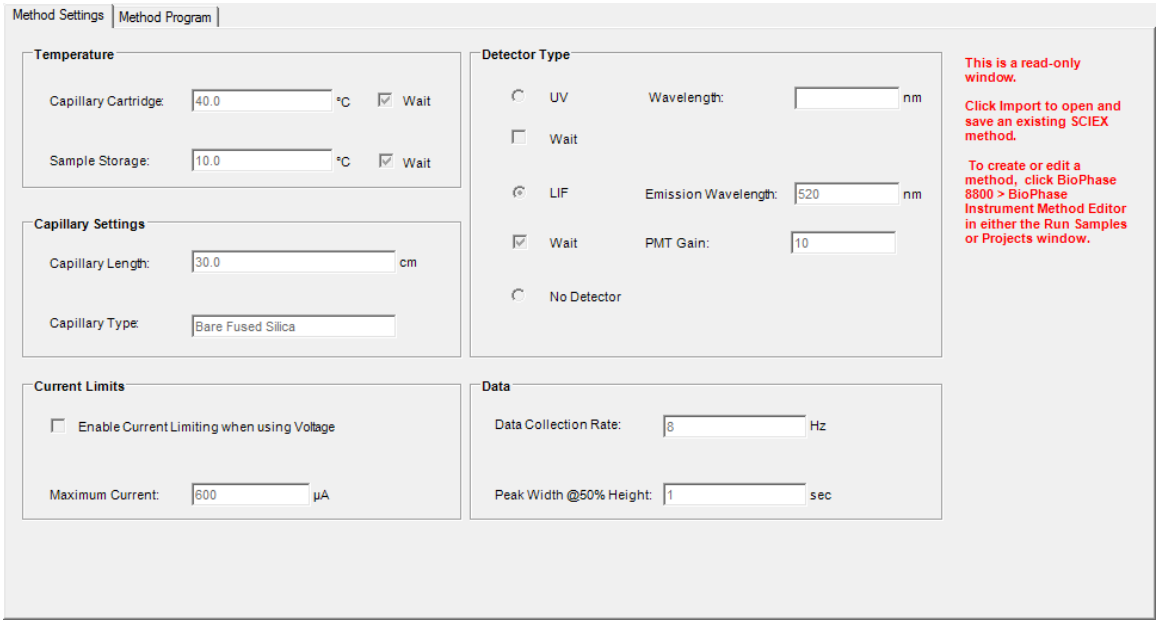
2. Click the name of system to be used, and then click **OK**.

   The Instrument Method Editor opens.

3. Click **Import**, and then browse to the conditioning method.
   The method opens in the Instrument Method Editor with the Method Settings tab in front.

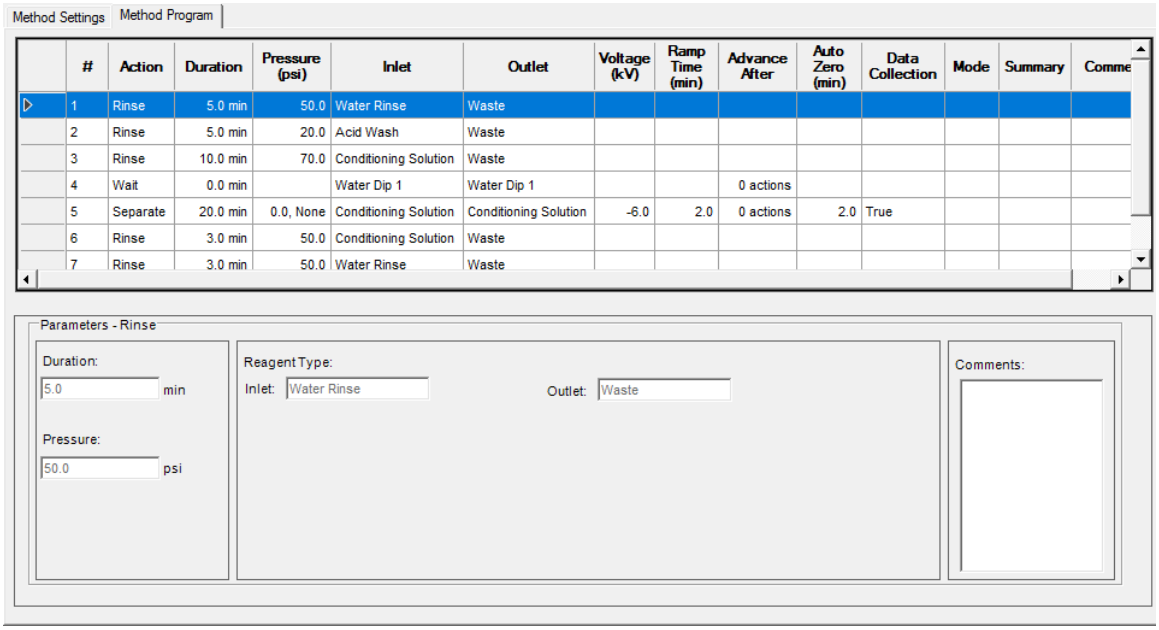
Note: The Instrument Method Editor window is read-only. If changes to the method are required, then save the instrument method, and then edit the method in the Method Editors for BioPhase System software. Refer to the section: "Edit an Existing Instrument Method" in the document: *Software Help*.

Figure 8-2 Instrument Method Editor: Method Settings



4. (Optional) To see the actions, open the Method Program tab.
5. To see the parameters for an action, click the row in the table.
   The Parameters pane changes to show the parameters.

Figure 8-3 Instrument Method Editor: Method Program



6. Save the conditioning instrument method:
   a. Click **File** > **Save with Method Set**.
      The Save current Instrument Method dialog opens.
   b. In the **Name** field, type a name.

      Note: The name must be less than 30 characters and can contain alphanumeric characters, spaces, and the special characters @, _, and %. Some versions of the Waters Empower™ software accept more than 30 characters and other special characters. If the method is edited in the Method Editors for BioPhase System software, then those characters might cause issues.

   c. (Optional) In the **Method Comments** field, type the information.
   d. If a prompt is shown, then in the **Password** field, type the Waters Empower™ software password for the current user, and then click **Save**.

   The instrument method and method set are saved to the current project.
7. Click **File** > **Exit**.

   Note: After a method has been imported, the **Import** button in the Instrument Method Editor is not available unless the window is closed and then opened again.
8. To create the other instrument methods and method sets, repeat steps 3 through 7.

Create the Sample Set Method

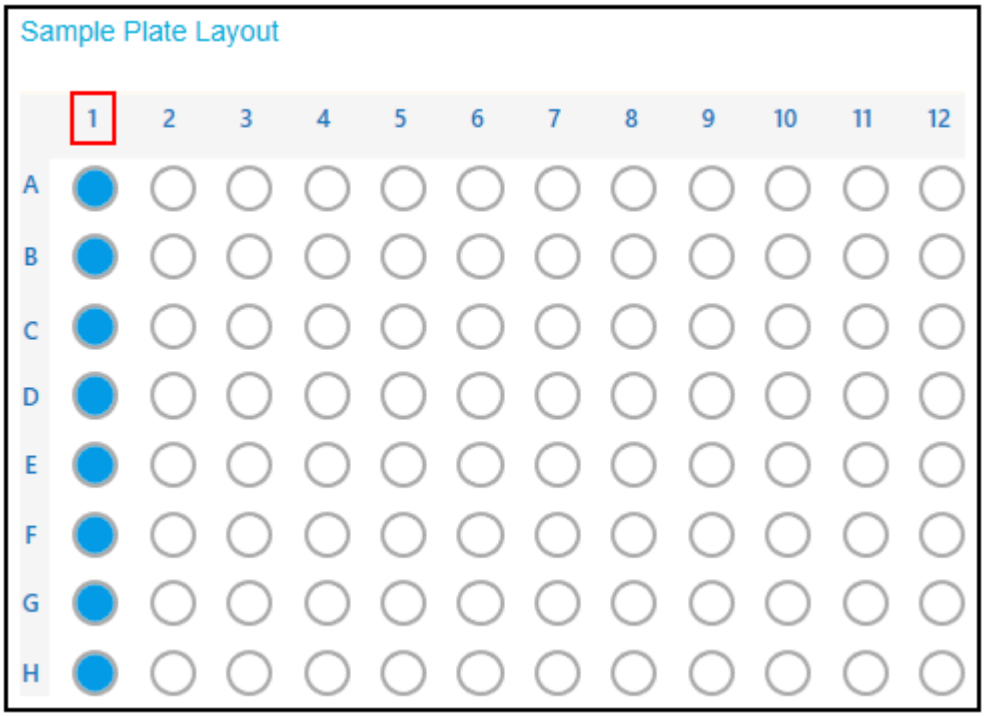
Note: A sample set method requires method sets. Make sure that any required instrument method is part of a method set.

The following instructions create a sample set method for eight samples (the number of wells in one column in a sample plate).

1. In the Waters Empower™ Software Run Samples window, click **BioPhase 8800** > **BioPhase Sample Set Editor**.

   The Method Editors for BioPhase System software opens, to the Sample Set Method Editor workspace.
2. Click **New Sample Set Method**.
   The Sample Set Method Editor opens to the Sample Plate Setup tab.
3. In first row of the Sample Set Summary table, click the **Method Set Name** cell, and then select the applicable conditioning method set.
4. In the Sample Plate Layout pane, click **1**.
   The first column in the sample plate is selected and the Sample Set Summary table changes to show the selected wells.

Figure 8-4 Sample Plate Layout Pane



5. In the Sample Set Summary table add the required sample information. In row 2 through row 9, do the following:
   a. In the **Sample Name** cell, type a name.
   b. Click the **Method Set Name** cell, and then select the applicable separation method set.

   Tip! After the method set is selected for row 2, right-click and select **Apply method to all samples in column** to assign the method set to all of the samples.

6. In the last row, click the **Method Set Name** cell, and then select the applicable shutdown method set.

Figure 8-5 Sample Set Summary Table

Sequence Summary

	Run #	Column	Method Name	Rep. #	Error Recovery
	1	0	30cm - DNA 20kb - Conditioning	1	☐
⊞	2	3	30cm - DNA 20kb - 20kV - Plasmid Separation	1	☐
⊞	3	3	30cm - DNA 20kb - 20kV - Plasmid Separation	2	☐
⊞	4	3	30cm - DNA 20kb - 20kV - Plasmid Separation	3	☐
⊞	5	3	30cm - DNA 20kb - 20kV - Plasmid Separation	4	☐
⊞	6	3	30cm - DNA 20kb - 20kV - Plasmid Separation	5	☐
⊞	7	3	30cm - DNA 20kb - 20kV - Plasmid Separation	6	☐
	8	0	30cm - DNA 20kb - Shutdown	1	☑

7. If the Validation pane is shown, then click the pane to see the errors. Click an error to identify where it occurs, and then make the required change.

   If there are no errors, then the Validation pane is not shown.

8. Save the sample set method:

   a. Click **SAVE AS**.

   Note: If errors occurred, then the **SAVE AS** button is not available. Correct all of the errors in the Validation pane, and then click **SAVE AS**.

   The Save Sample Set dialog opens.

   b. In the **Sample Set Name** field, type a name.

   Note: The name must be less than 30 characters and can contain alphanumeric characters, spaces, and the special characters @, _, and %. Some versions of the Waters Empower™ software accept more than 30 characters and other special characters. If the method is edited in the Method Editors for BioPhase System software, then those characters might cause issues.

   c. (Optional) In the **Description** field, type information.

   d. Click **Save**.

   e. To acknowledge the saved method, click **OK**.

   The sample set method is saved to the Waters Empower™ software database.

9. To see, save, or print the plate layouts:

   a. Open the Plate Layouts tab.

b. (Optional) Click **PRINT**.
The Print Preview window opens.

c. If required, click the applicable button to print or save the plate layouts.
Refer to the section: "Print Preview Dialog" in the document: *Software Help System*.

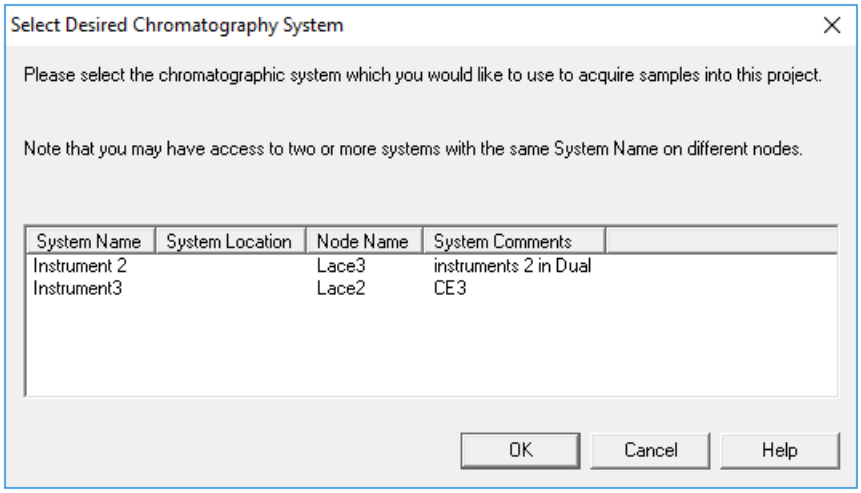
d. Close the Print Preview dialog.

10. Close the Method Editors for BioPhase System software.
The Run Samples window is shown.

Start the Sample Set Method

1. Load the cartridge and plates. Refer to the section: Prepare the BioPhase 8800 System.
2. In the Waters Empower™ software Project window, click **Tools** > **Run Samples**.

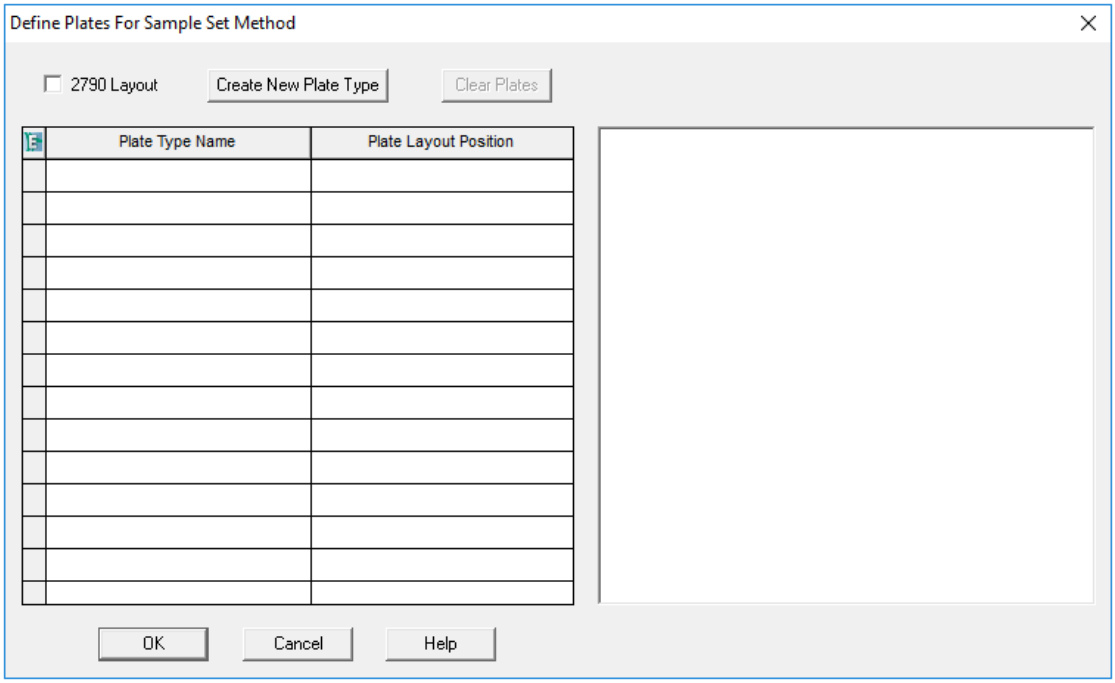
Figure 8-6 Select Desired Chromatography System Dialog



3. Click the name of the system to be used, and then click **OK**.
The Run Samples window opens.
4. Configure the plate type:

a. Click **Edit** > **Plates**.

Figure 8-7 Define Plates for Sample Set Method Dialog



Note: If the dialog does not look like the preceding figure, then clear the **2790 Layout** check box.

b. Click the **Plate Type Name** cell, and then select **ANSI-96well2mL**.
The dialog changes with an image of the plate and buttons for the plate sequencing mode.

c. Click the **Plate Layout Position** cell, and then type `1`.

d. To set the order of access for the wells during the run, click  .

e. To save the changes, click **OK**.

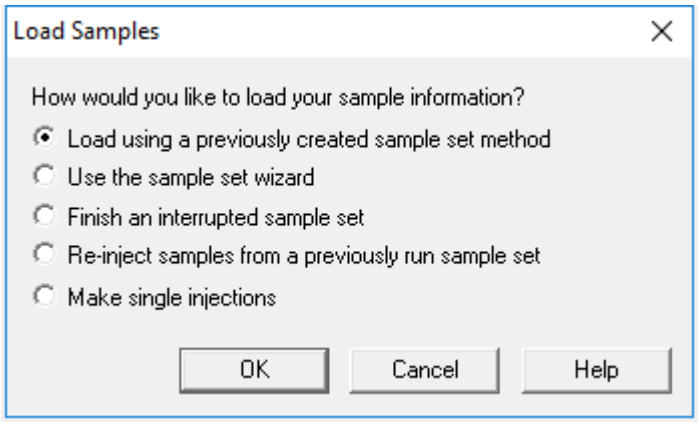
f. Close the dialog.

Tip! To permanently configure the plate type, click **Customize** > **Defaults**, click **Plates**. In the Define Plates For Sample Set Method dialog, do steps 4.b through 4.e, and then, in the Run Samples Defaults dialog, click **OK**.

In the Sample Set Method table, the heading for the **Vials** column changes to **Plate/Well**.

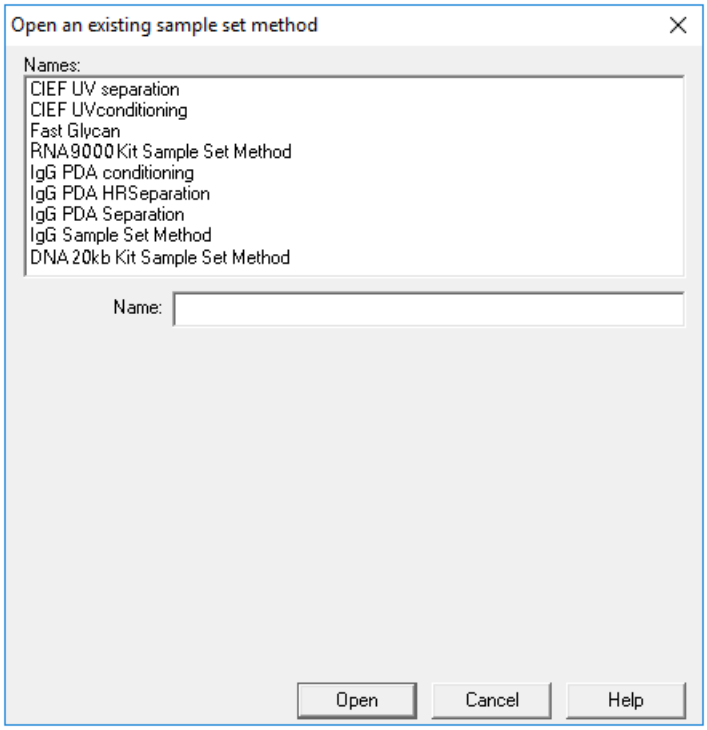
5. Click  (**Load Sample Set**).

Figure 8-8 Load Samples Dialog



6. Click **Load using a previously created sample set method**, and then click **OK**.

Figure 8-9 Open an existing sample set method Dialog



7. Click **DNA 20kb Kit Sample Set Method**, and then click **Open**.
   The sample set method opens in the Samples tab.
8. (Optional) Configure the table to show only the columns that are relevant for the BioPhase 8800 system:

a. Right-click, and then select **Table Properties**.
The Table Properties dialog opens.

b. Click **Hide All**, and then clear the **Plate/Well**, **# of Injs**, **SampleName**, **Function**, and **Method Set / Report or Export Method** check boxes.

c. Click **OK**.

The table changes to show the selected columns.

Figure 8-10 Samples Tab

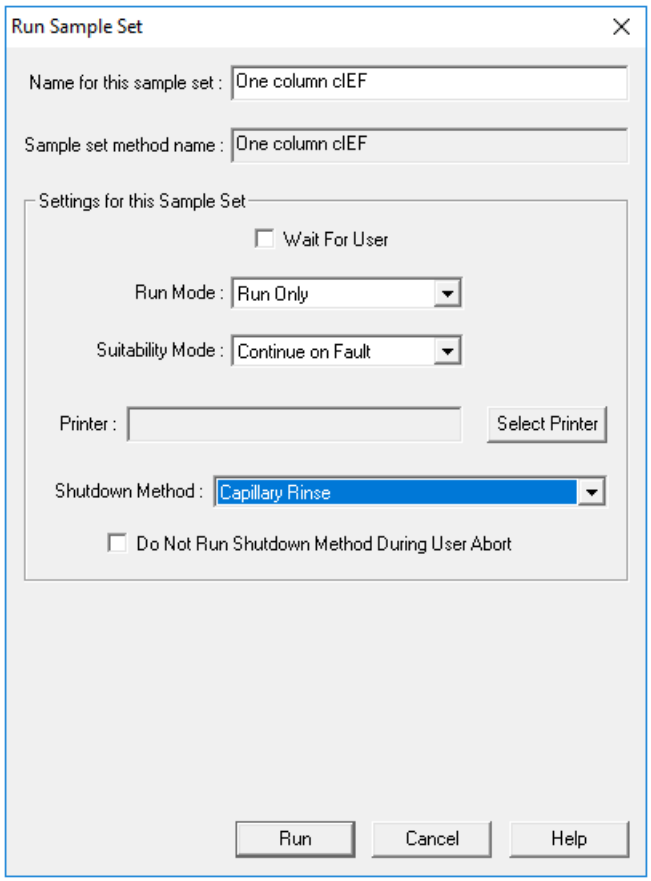
Sample Set Method: DNA 20kb Kit Sample Set Method

	Plate/Well	# of Injs	SampleName	Function	Method Set / Report or Export Method
1				Condition Column	30cm DNA 20 kb Conditioning
2	1:A,1	1	Washington	Inject Samples	30cm 20kb 9kV Linear
3	1:B,1	1	Hoover	Inject Samples	30cm 20kb 9kV Linear
4	1:C,1	1	Polk	Inject Samples	30cm 20kb 9kV Linear
5	1:D,1	1	Coolidge	Inject Samples	30cm 20kb 9kV Linear
6	1:E,1	1	Jackson	Inject Samples	30cm 20kb 9kV Linear
7	1:F,1	1	Eisenhower	Inject Samples	30cm 20kb 9kV Linear
8	1:G,1	1	Kennedy	Inject Samples	30cm 20kb 9kV Linear
9	1:H,1	1	Truman	Inject Samples	30cm 20kb 9kV Linear
10				Condition Column	30cm DNA 20kb Shutdown

9. Review the sample set method. Make sure that the correct reagent plate layout was used. If changes are required, then edit the method in the Method Editors for BioPhase System software. Changes to the instrument methods or method sets are automatically transmitted to the sample set method.

10. In the Waters Empower™ Software Project window, click  (**Start**).

Figure 8-11 Run Sample Set Dialog



11. If required, edit the information:
    a. If required, edit the **Name for this sample set** field.
    b. (Optional) Click **Shutdown Method**, and then select the rinse instrument method that was created previously.

       If the system encounters an error during a run, then it executes this instrument method, and then stops the run.
    c. If required, select **Do Not Run Shutdown Method During User Abort**.
    d. Click **Run**.

    The run starts. During the run, in the Sample Set Method window, the text in the row for the sample being acquired is red.

CAUTION: Potential Data Loss. During a run, do not start any actions from the BioPhase 8800 driver for Empower™ Direct Control pane, even if the system status is idle. Any actions that are started might cause interference with data acquisition.

Monitor the Run in the Waters Empower™ Software

CAUTION: Potential Data Loss. Do not stop the run until all of the data is saved. The data is saved when the sample set method is on the next row.

CAUTION: Potential System Damage. If the run is stopped and will not be started again, then use the shutdown method to rinse the capillaries before the cartridge is stowed. If the capillaries are not rinsed, then electrolyte salt crystals or precipitate can collect and might cause blocked capillaries, incorrect pressure seals, errors when the samples are injected, arcing, or current leakage.

CAUTION: Potential System Damage. Before the run is started again, make sure to empty or replace the outlet plate to prevent the overflow of reagent and possible damage to the instrument.

CAUTION: Potential Wrong Result. Before the run is started again, prepare new reagent plates. If the run has been stopped, then there might not be sufficient reagents available to complete the run.

CAUTION: Potential Wrong Result. If the samples have been inside of the system for more than 24 hours, sample degradation might have occurred. Discard the samples before the run is started again.

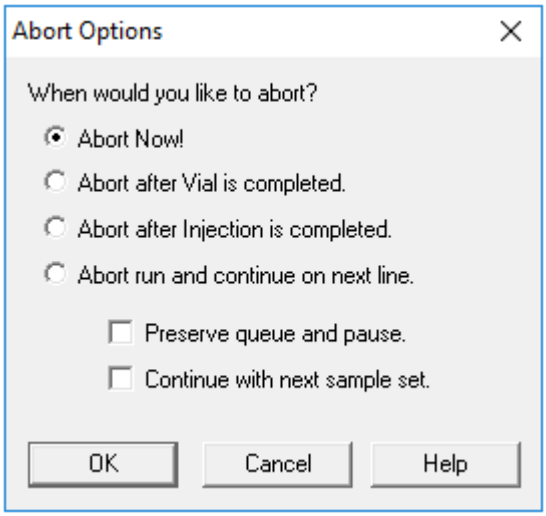
Use this procedure to monitor the progress of the sample set method, and then, if required, stop or pause the sample set method.

Note: Most of the panes in the Waters Empower™ software are made for chromatography. Use the following steps to monitor the progress of the capillary electrophoresis separation and ignore the information in the Time Remaining and Solvent Required panes.

1. To stop the run, click  (**Abort**).

   Note: Do not use the **Stop** button in the Direct Control pane. That button is only applicable to functions that are started from the Direct Control pane.

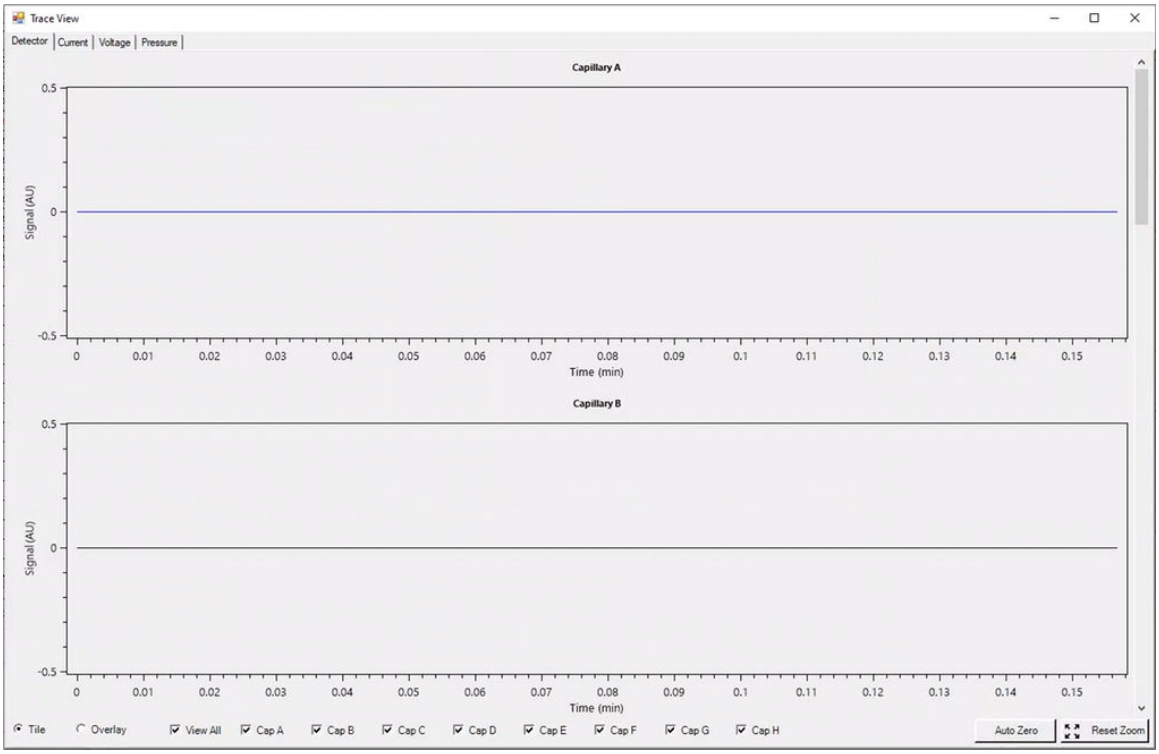
Figure 8-12 Abort Options Dialog



When the run ends, the text in all of the rows in the Sample Set Method window is red.

2. To see the data during acquisition, in the Direct Control pane, click  (**Monitor**).

Figure 8-13 Trace View Window



3. If required, do any of the following:
   - To see current, voltage, or pressure, open the applicable tab in the top left.
   - To see one graph with the data for all of the capillaries, in the bottom left, click **Overlay**.
   - To see data for some of the capillaries, select or clear the check boxes at the bottom of the window for the capillaries of interest.
   - To see the time and detector values for any point on a trace, click the trace at the position of interest.
   - To zoom in on the data, make sure that **Overlay** is selected, and then drag to select the area to zoom. The mouse scroll wheel can also be used to zoom.
   - To go back to the original data dimensions, in the bottom right, click **Reset Zoom**.
   - To see a different area of a zoomed plot, right-click the X- or Y-axis, and then drag.
4. If required, at the bottom right, right-click **Auto Zero**.
   The detector signal is set to zero.
5. Wait until  (**Abort**) changes to  (**Start**).
   A delay between data acquisition and when all of the data is saved might occur. The green button identifies that all of the data is saved.
6. If required, discard the samples and reagents. Refer to the section: Waste Disposal.
7. If required, stow the cartridge. Refer to the section: Stow the Cartridge After the Run.

Troubleshooting 9

After each corrective action is complete, we recommend that the analysis be done again to make sure that the symptom has been corrected.

Symptom	Possible Cause	Corrective Action
Cartridge not detected error	1. The ID chip on the cartridge is not clean. 2. The contact pins on the system are not clean. 3. The BioPhase 8800 system firmware is not up to date.	1. Moisten a lint-free laboratory wipe or cotton swab with ethanol or isopropanol, and then clean the surface of the ID chip. Let the ID chip air-dry before the cartridge is installed. 2. Moisten a lint-free laboratory wipe or cotton swab with ethanol or isopropanol, and then clean the contact pins. Let the pins air-dry before the cartridge is installed. 3. Do the following: a. On the BioPhase 8800 system front panel, touch the icon in the top left corner. b. Record the firmware version. c. Contact sciex.com/request-support.
Error found at start of run	1. When the optics door was opened and closed, a sensor error occurred.	1. Turn off, and then turn on the BioPhase 8800 system. To prevent this from occurring again, always use the applicable procedure to change the filters. Refer to the sections: "Install a UV Filter" and "Install the Filters for the LIF Detector" in the document: *Operator Guide*.

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution	1. The capillary tip is damaged. 2. The sample concentration is too high. 3. The capillary is blocked. 4. The internal surface of the capillary is contaminated. 5. The capillary coating is damaged.	1. Do the following actions: • Use a magnifying lens to examine the capillary tip. • Use a lint-free wipe to clean the capillary inlets carefully in an outward direction. • Make sure that the capillary tip extends approximately 2 mm from the gold cannula electrode. • Make sure that the capillary tip has a straight cut and is not broken. If the capillary cannot be used, then refer to the section: Options for a Blocked or Damaged Capillary. 2. Do one or all of the following: • Decrease the **Duration** in the **Inject** action in the separation method to inject less sample. If the results are not satisfactory, then decrease the **Pressure** or **Voltage**. • Dilute the sample again with the sample diluent. 3. Refer to the section: Options for a Blocked or Damaged Capillary. 4. Edit the sequence to omit the contaminated capillary or replace the cartridge. 5. Condition the capillary.

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution (continued)	1. The cartridge is no longer serviceable. 2. The capillary cartridge was kept at ambient temperature for more than a week.	1. Do a test separation of a linearized dsDNA ladder. If the peak widths are consistently wider than previous runs, then replace the cartridge. 2. Replace the cartridge.
Carryover	1. The sample concentration is too high. 2. The reagent plate is contaminated with sample.	1. Do one or all of the following: • Decrease the **Duration** in the **Inject** action in the separation method to inject less sample. If the results are not satisfactory, then decrease the **Pressure** or **Voltage**. • Dilute the sample again with the sample diluent. 2. In the separation method, add one or more water dip steps after sample injection. With the edited method, do the following steps: a. Create a new sequence that uses this separation method. b. Prepare new reagent plates for the new sequence. Refer to the section: Load the Reagent Inlet and Outlet Plates.

Symptom	Possible Cause	Corrective Action
Extra peaks	1. Non-nucleic acid components of the sample interacted with the dye. 2. The plasticware used during sample preparation or the sample plate is contaminated with materials that interact with the dye. 3. Light scattering caused by particulates larger than 1 µm in the gel buffer occurred.	1. Prepare the sample again. Make sure that it is pure. 2. Use clean plasticware and a clean sample plate to prepare the sample again. 3. Before the gel buffer is added to the reagent plates, put the sample through a syringe filter with a 0.45 µm pore-size membrane.
High current	1. The gel buffer is contaminated. 2. The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence.	1. Prepare the inlet and outlet reagent plates again to replace the gel buffer. 2. Make sure that the positions of the reagents in the reagent plates agree with the plate layouts. If the positions are not correct, then use the plate layout to prepare the plates again. Refer to the section: Plate Layouts.

Symptom	Possible Cause	Corrective Action
Low signal	1. The capillary tip is dirty or plugged. 2. The sample volume is too low. 3. The initial nucleic acid concentration is too low. 4. Degradation of the nucleic acids in the sample occurred because of sample preparation or exposure to nucleases.	1. Rinse the capillary. Refer to the section: Remove Blockage from a Capillary. Do a blank separation run to equilibrate the capillary surface. 2. Make sure that there is a minimum of 50 µL of sample in the sample well. 3. Do one or all of the following: • Increase the **Duration** up to 15 seconds in the **Inject** action in the separation method to inject more sample. If the results are not satisfactory, then increase the **Pressure** or **Voltage**. • Prepare the sample again, with the recommended concentration. The recommended concentration is 50 ng/mL to 50 µg/mL. 4. Prepare the sample again. Make sure that the sample is not exposed to nucleases.
Low current	1. The capillary is blocked. 2. The position of the gel buffer in the reagent plate does not agree with the sequence.	1. Refer to the section: Remove Blockage from a Capillary. 2. Make sure that the position of the gel buffer during the voltage separation step is correct. If the positions are not correct, then use the plate layout to prepare the plates again. Refer to the section: Load the Reagent Inlet and Outlet Plates.

Symptom	Possible Cause	Corrective Action
Low or unsteady current	1. The capillary tip is blocked or contaminated. 2. The gel buffer has air bubbles.	1. Refer to the section: Remove Blockage from a Capillary. If the current is low or unsteady, then replace the cartridge. 2. Do one or all of the following: • Use a centrifuge to spin the plate at 30 × *g* for 5 minutes to remove air bubbles. • De-gas the gel buffer with 5 inches Hg to 15 inches Hg vacuum for 5 minutes.

Symptom	Possible Cause	Corrective Action
No electrical current during separation	1. The capillary is damaged. 2. The electrode is broken or bent. 3. The capillary tip is blocked or contaminated. 4. The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence. 5. A capillary is filled with air bubbles.	1. Refer to the section: Options for a Blocked or Damaged Capillary. 2. Replace the cartridge. 3. Refer to the section: Remove Blockage from a Capillary. 4. Make sure that the positions of the samples and reagents in the plates agree with the plate layouts. If the positions are not correct, then use the plate layout to prepare the plates again. Refer to the section: Plate Layouts. 5. Do one or all of the following: • Make sure that the wells of the sample and reagent plates contain sufficient solution. • Make sure that the positions of the reagents in the reagent plates agree with the plate layouts. If the positions are not correct, then use the plate layout to prepare the plates again. Refer to the section: Plate Layouts. • Use a centrifuge to spin the plate at 30 × *g* for 5 minutes to remove air bubbles.
Slower migration time, with or without concurrent low current	1. The capillary tip is blocked or contaminated.	1. Refer to the section: Remove Blockage from a Capillary.

Symptom	Possible Cause	Corrective Action
Unstable baseline	1. The concentration of dye in the inlet reagent plate is not the same as the concentration in the outlet reagent plate.	1. Prepare sufficient gel buffer for both the inlet and outlet reagent plates.
Corr. Area% for the open circular isoform is lower than expected	1. The concentration of the open circular isoform relative to the total plasmid DNA concentration is too high and the signal is saturated.	1. Do a serial dilution of the sample to find a concentration range which provides stable isoform ratios (SC:L:OC) and a linearly decreasing open circular corrected peak area relative to the amount of sample dilution.
Peaks for the supercoiled plasmid isoform are split	1. For shorter plasmids (< 3 kb), interactions between the plasmid, the labeling dye, and the gel buffer result in split peaks for the supercoiled isoform.	1. Use the `30cm-DNA 20kb Plasmid Separation` method which has a 20 kV injection. If the results are not satisfactory, then increase the injection voltage to a maximum of 30 kV.
Low signal-to-noise ratio	1. The sample concentration is too low.	1. Do the following: a. Increase the sample concentration but not so much that the signal of the open circular isoform is saturated. b. If the results are not satisfactory, then increase **PMT Gain** from 10 to 100.
Supercoiled and linear isoforms are more abundant than expected relative to the open circular isoform	1. The total DNA concentration is too high and the signal for the open circular isoform is saturated.	1. Dilute the DNA sample so that the concentration of the open circular isoform is 0.30 ng/µL or less.
For linear double-stranded DNA the peak resolution is less than expected	1. The linearized dsDNA species are too similar in size to be resolved with the 30 cm cartridge.	1. Use the 50 cm cartridge and the applicable methods.

Symptom	Possible Cause	Corrective Action
Poor resolution	1. The tips of the capillary are dry. 2. The internal surface of the capillary is contaminated. 3. The capillary coating is damaged.	1. To restore the capillary surface, use the `30cm-DNA 20kb Rinse` method or the `30cm-DNA 20kb Conditioning` method. 2. To restore the capillary surface, use the `30cm-DNA 20kb Rinse` method or the `30cm-DNA 20kb Conditioning` method. 3. Condition the capillary.

Remove Blockage from a Capillary

1. Rinse the capillary with CE Grade water at 75 psi for 10 minutes.
2. Use CE Grade water to clean the outside surface of the capillary inlets.
3. Use a lint-free wipe to clean the capillary inlets carefully in an outward direction.
4. If the blockage cannot be removed, then edit the sequence to ignore the damaged capillary, or replace the cartridge.

Options for a Blocked or Damaged Capillary

If blockage in a capillary cannot be removed or a capillary is damaged, then edit the sequence to omit the blocked or damaged capillary. Refer to the section: Create the Sequence.

Hazardous Substance Information A

The following information must be noted and the related safety measures must be obeyed. For more information, refer to the related safety data sheets. The safety data sheets are available on request or can be downloaded from our website, at sciex.com/tech-regulatory.

Hazard classification according to HCS 2012.

Acid wash/regenerating solution

DANGER! Causes severe skin burns and eye damage.

DNA 20 kb Plasmid and Linear conditioning solution

DANGER! May damage fertility or the unborn child.

Other Reagents

- CE Grade water
- DNA 20 kb Plasmid and Linear gel
- DNA 20 kb Plasmid and Linear sample buffer
- DNA 20 kb Plasmid test mix
- SYBR™ Gold Nucleic Acid gel stain[9]

For reagents from other vendors, read the safety data sheet from the vendor before use.

[9] SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Gold Nucleic Acid gel stain is not available for resale.

Download Required Files B

Do the procedure for the software in use:

- BioPhase software: Refer to the section: Download and Configure the Required Files.
- Waters Empower™ software: Refer to the section: Download and Configure the Required Files (Waters Empower™ Software).

Download and Configure the Required Files

Files with methods, sequences, reagents, and analysis parameters for the DNA 20 kb Plasmid and Linear kit are available at sciex.com. Use the following instructions to download, extract, and then copy the files to the applicable location.

Note: The following procedure is only required when BioPhase software 1.1 is used. The required files for the DNA 20 kb Plasmid and Linear kit are included as part of the BioPhase software 1.2 or later.

1. Go to sciex.com/software-downloads. In the More software downloads section, click **BioPhase Resources**.
2. Click **BioPhase Project Files 1.4**.
3. In File Explorer, right-click the `BioPhase 1.X.zip` file, and then click **Extract All**.
4. Browse to a location, click **Select Folder**, and then click **Extract**.
   The extracted files are copied to the selected file path.
5. Move the extracted files to the correct locations:

   Note: The following instructions use the default location `C:\Biophase` for the BioPhase software files. If the project folders are in a different location, then put the extracted files in that location.

   a. Drag the `BioPhase 1.X\Projects\DNA 20kb` folder to the `C:\BioPhase\Projects` folder.

   b. Drag the `BioPhase 1.X\Reagents\DNA 20kb` folder to the `C:\BioPhase\Reagents` folder.

   c. Drag the `BioPhase 1.X\Data Analysis\DNA 20kb` folder to the `C:\BioPhase\Data Analysis` folder.

Download and Configure the Required Files (Waters Empower™ Software)

Files for the DNA 20 kb Plasmid and Linear kit are available on sciex.com. Use the following instructions to download, extract, and then copy the files to the applicable location.

1. Go to sciex.com/software-downloads. In the More software downloads section, click **BioPhase Driver Resources**.
2. Click **BioPhase Method Files 1.4**.
3. In File Explorer, right-click the `BioPhase-Empower-Method-Files-1.4.zip` file, and then click **Extract All**.
4. Browse to the location to save the method files, click **Select Folder**, and then click **Extract**. The files are extracted and then copied to the specified location.

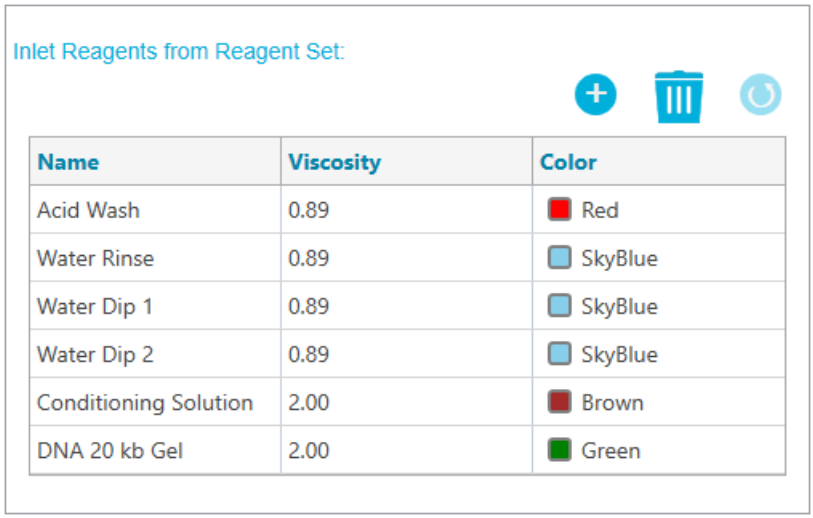
Reagents, Plate Layouts, and Methods

C

Reagent Set

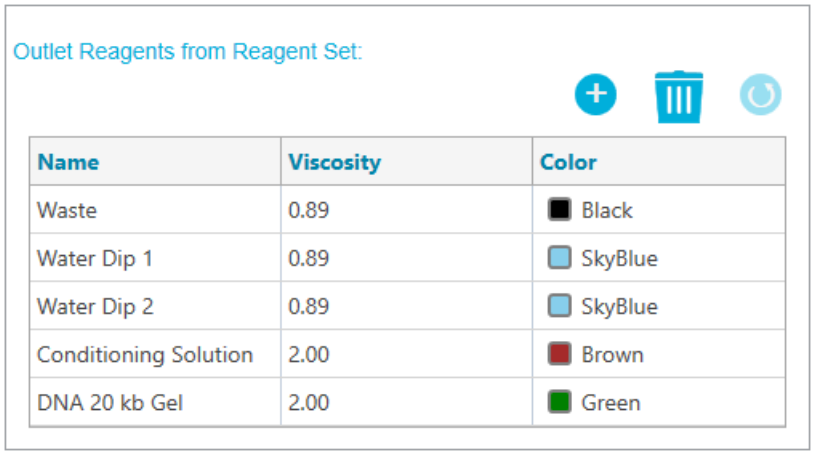
If the reagents are not available, then use the following figures to create a new reagent set.

Figure C-1 DNA 20 kb Plasmid and Linear Kit Inlet Reagents



Name	Viscosity	Color
Acid Wash	0.89	Red
Water Rinse	0.89	SkyBlue
Water Dip 1	0.89	SkyBlue
Water Dip 2	0.89	SkyBlue
Conditioning Solution	2.00	Brown
DNA 20 kb Gel	2.00	Green

Figure C-2 DNA 20 kb Plasmid and Linear Kit Outlet Reagents



Name	Viscosity	Color
Waste	0.89	Black
Water Dip 1	0.89	SkyBlue
Water Dip 2	0.89	SkyBlue
Conditioning Solution	2.00	Brown
DNA 20 kb Gel	2.00	Green

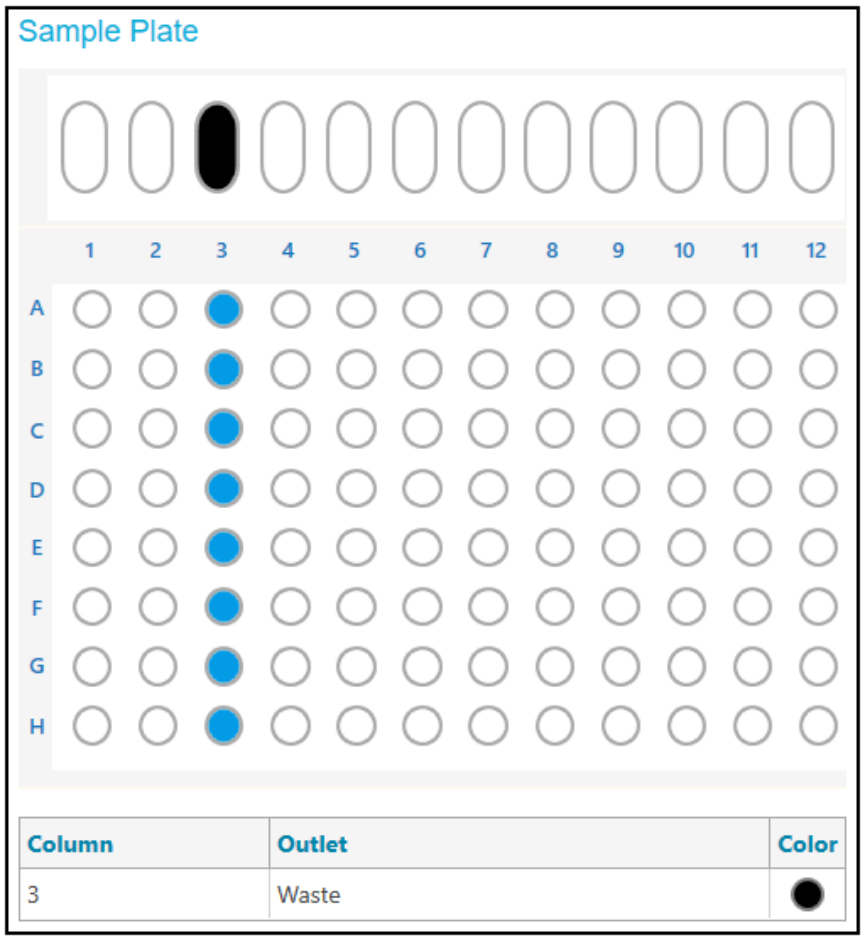
Plate Layouts

Note: The following figures show the plate layouts for the sequence supplied with the software. If more samples have been added or the reagent positions have been changed, then the following layouts are not correct.

Sample Plates

Note: The top row shows the layout for the sample outlet plate. The bottom section shows the layout for the sample inlet plate.

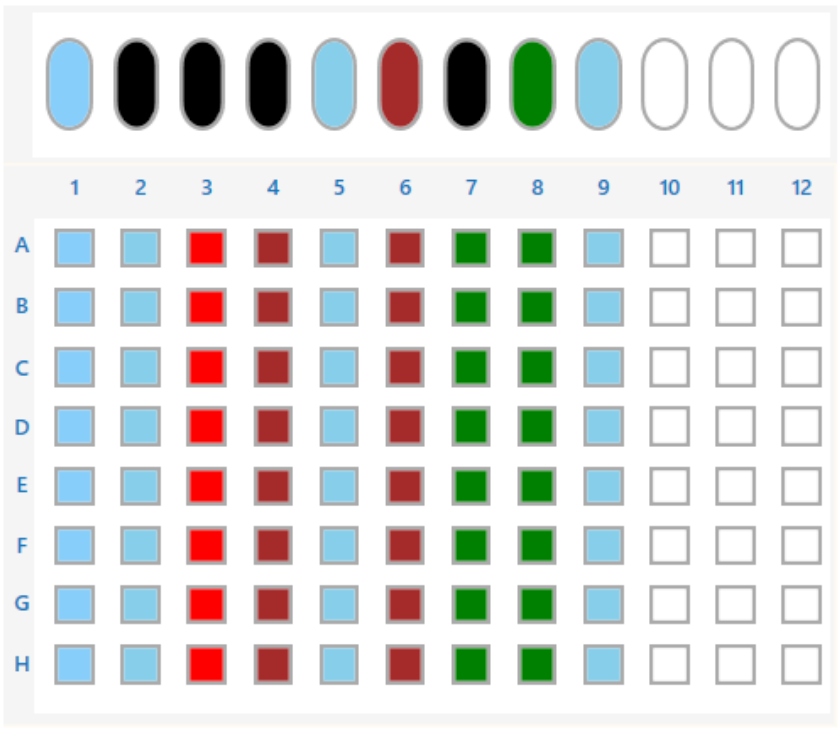
Figure C-3 Layouts for the Sample Inlet Plate and Sample Outlet Plate



Reagent Plates

Note: The top row shows the layout for the reagent outlet plate. The bottom section shows the layout for the reagent inlet plate.

Figure C-4 Layouts for the Reagent Inlet Plate and Reagent Outlet Plate



Column	Inlet	Color	Outlet	Color
1	Capillary Protect		Capillary Protect	
2	Water Rinse		Waste	
3	Acid Wash		Waste	
4	Conditioning Solution		Waste	
5	Water Dip 1		Water Dip 1	
6	Conditioning Solution		Conditioning Solution	
7	DNA 20 kb Gel		Waste	
8	DNA 20 kb Gel		DNA 20 kb Gel	
9	Water Dip 2		Water Dip 2	

Methods

For instructions to create a method, refer to the document: *Software Help System*.

Conditioning Method for 30 cm Capillaries

Figure C-5 Method Settings for the Conditioning Method for 30 cm Capillaries

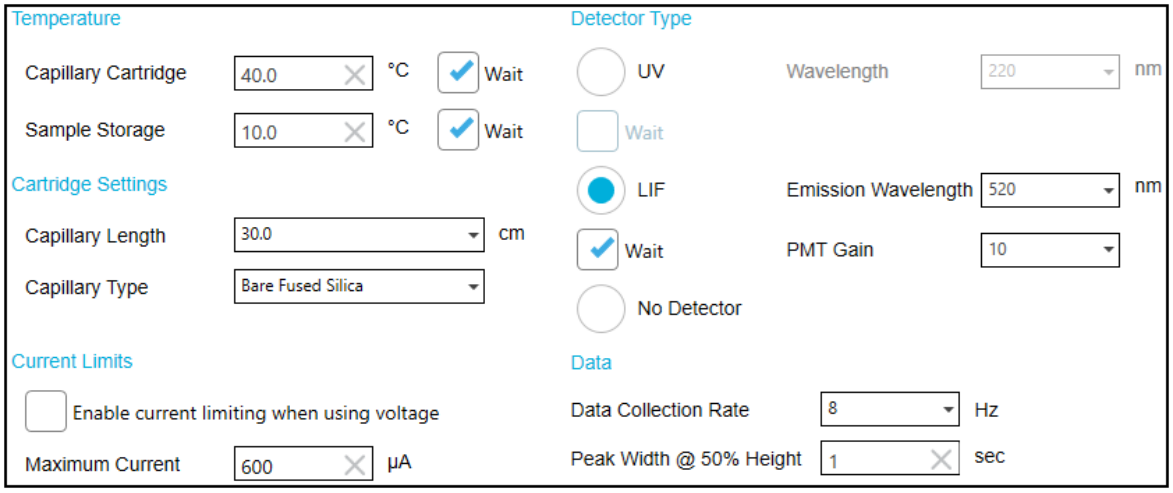


Figure C-6 Actions in the Program Pane for the Conditioning Method for 30 cm Capillaries

Figure C-7 Summary of Actions in the Conditioning Method for 30 cm Capillaries

Settings	Capillary Cartridge: 40.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 10 Peak Width: 1 sec. Data Rate: 8 Hz
Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Water Rinse Outlet: Waste
Rinse	Duration: 5.0 min. 20.0 psi	Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 10.0 min. 70.0 psi	Inlet: Conditioning Solution Outlet: Waste
Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1
Separate	Duration: 20.0 min. -6.0 kV Ramp Time: 2.0 min. Autozero: 2.0 min.	Inlet: Conditioning Solution Outlet: Conditioning Solution
Rinse	Duration: 3.0 min. 50.0 psi	Inlet: Conditioning Solution Outlet: Waste
Rinse	Duration: 3.0 min. 50.0 psi	Inlet: Water Rinse Outlet: Waste
Wait	Duration: 30.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1

Linear dsDNA Separation Method for 30 cm Capillaries

Figure C-8 Method Settings for the Linear dsDNA Separation Method for 30 cm Capillaries

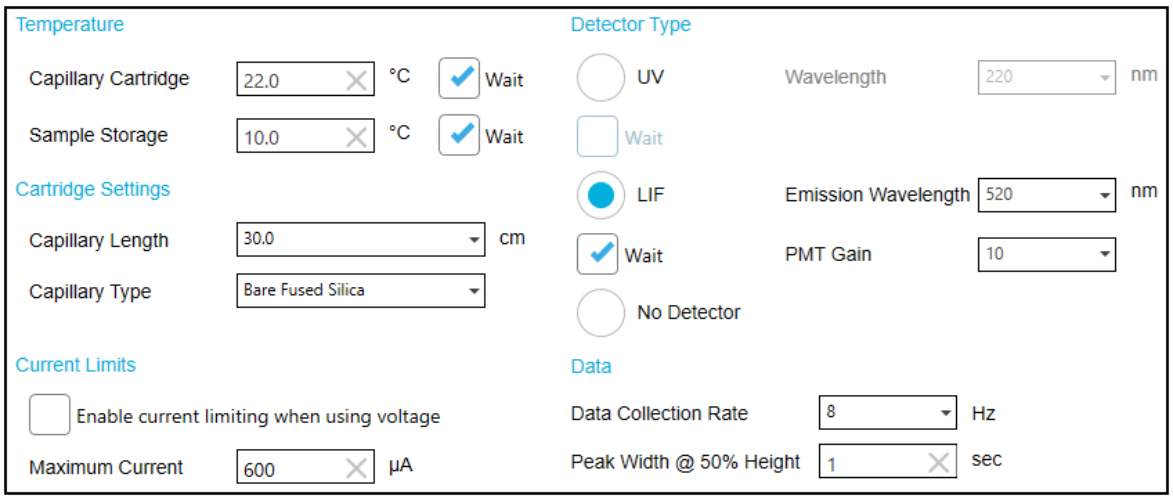


Figure C-9 Actions in the Program Pane for the Linear dsDNA Separation Method for 30 cm Capillaries

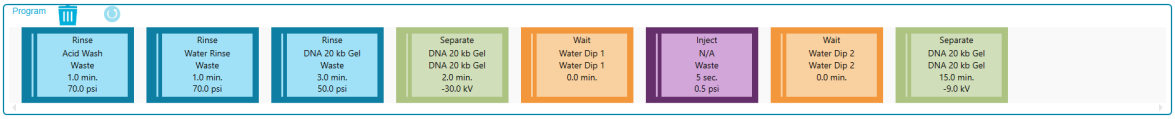


Figure C-10 Summary of Actions in the Linear dsDNA Separation Method for 30 cm Capillaries

Settings	Capillary Cartridge: 22.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA		Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 10 Peak Width: 1 sec. Data Rate: 8 Hz
Rinse	Duration: 1.0 min. 70.0 psi		Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 1.0 min. 70.0 psi		Inlet: Water Rinse Outlet: Waste
Rinse	Duration: 3.0 min. 50.0 psi		Inlet: DNA 20 kb Gel Outlet: Waste
Separate	Duration: 2.0 min. -30.0 kV Ramp Time: 0.2 min. Disable Data Collection, Advance after: 24 actions		Inlet: DNA 20 kb Gel Outlet: DNA 20 kb Gel
Wait	Duration: 0.0 min.		Inlet: Water Dip 1 Outlet: Water Dip 1
Inject	Duration: 5 sec. 0.5 psi	Plate: Sample	Outlet: Waste
Wait	Duration: 0.0 min.		Inlet: Water Dip 2 Outlet: Water Dip 2
Separate	Duration: 15.0 min. -9.0 kV Ramp Time: 2.0 min. Autozero: 2.5 min., Advance after: 24 actions		Inlet: DNA 20 kb Gel Outlet: DNA 20 kb Gel

Plasmid DNA Separation Method for 30 cm Capillaries

Figure C-11 Method Settings for the Plasmid DNA Separation Method for 30 cm Capillaries

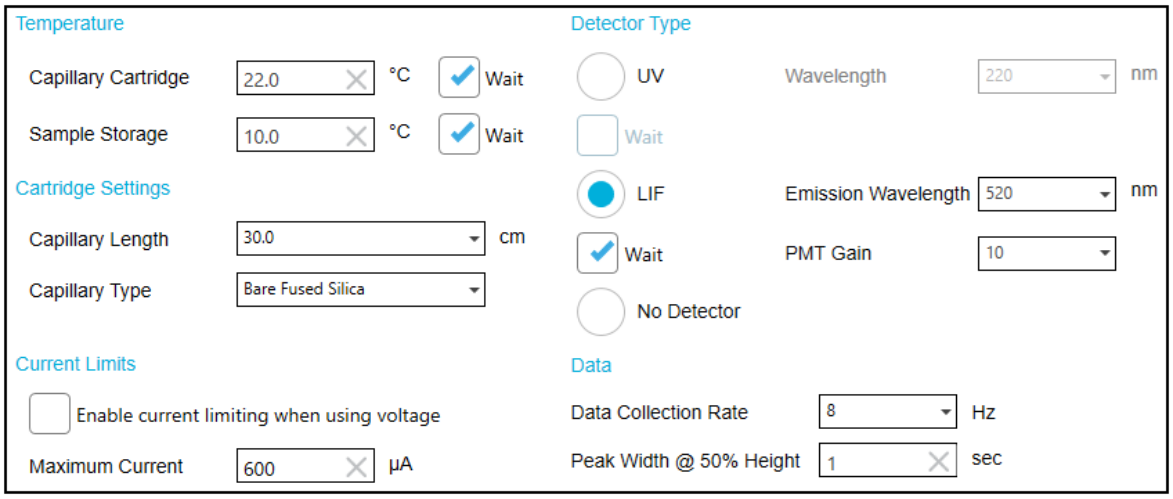


Figure C-12 Actions in the Program Pane for the Plasmid DNA Separation Method for 30 cm Capillaries

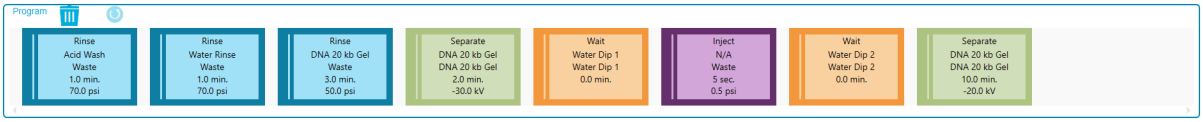
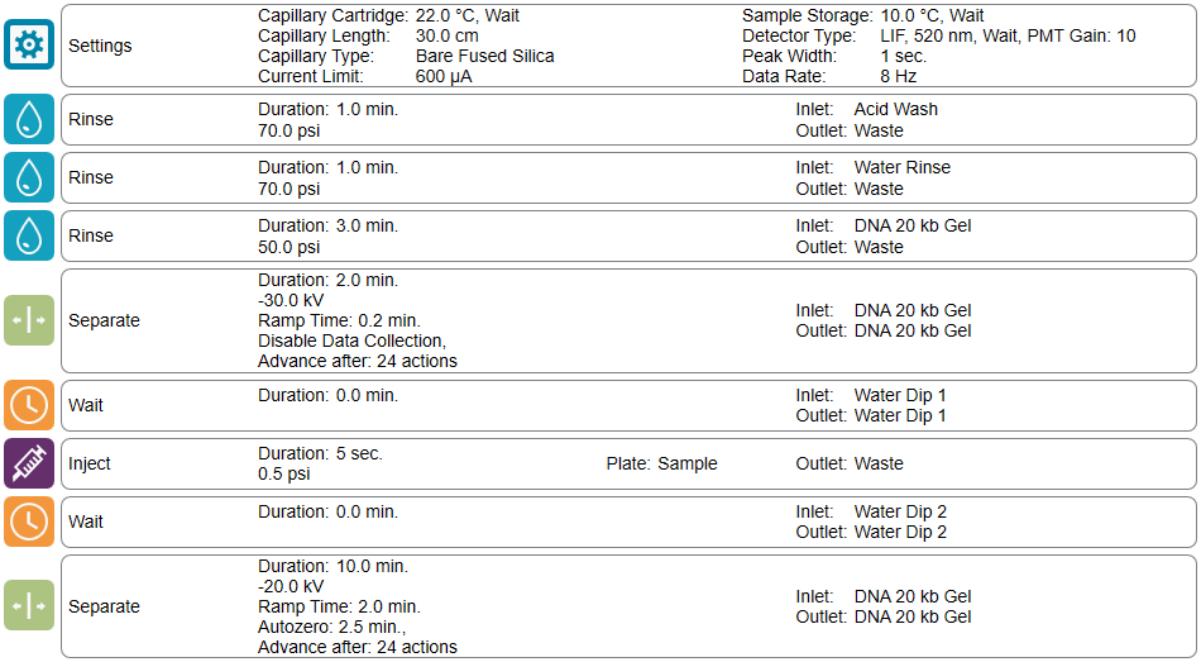


Figure C-13 Summary of Actions in the Plasmid DNA Separation Method for 30 cm Capillaries



Shutdown Method for 30 cm Capillaries

Figure C-14 Method Settings for the Shutdown Method for 30 cm Capillaries

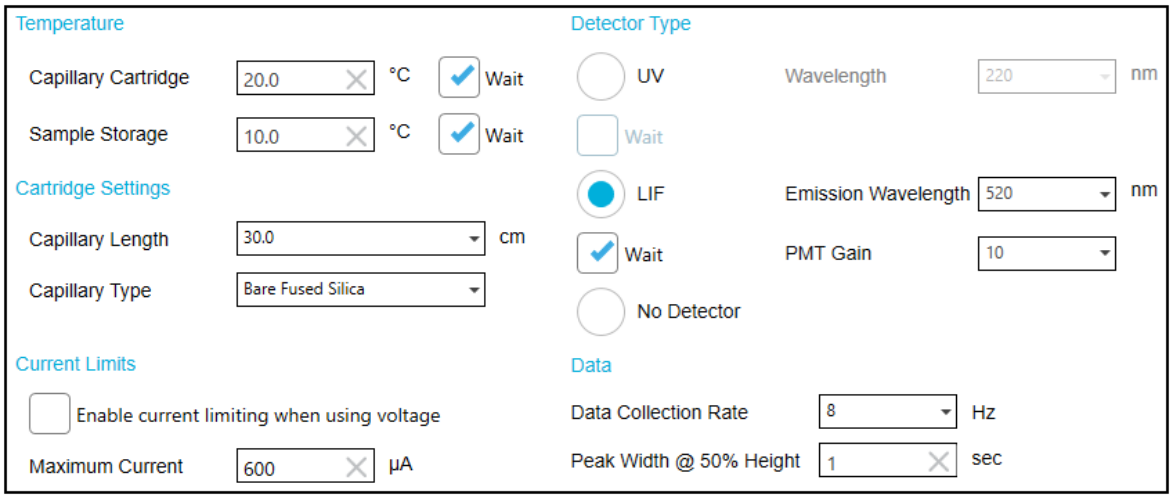


Figure C-15 Actions in the Program Pane for the Shutdown Method for 30 cm Capillaries

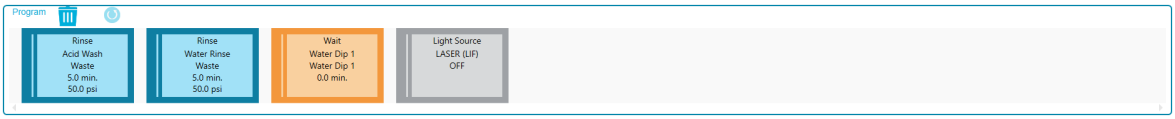


Figure C-16 Summary of Actions in the Shutdown Method for 30 cm Capillaries

Settings	Capillary Cartridge: 20.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 10 Peak Width: 1 sec. Data Rate: 8 Hz
Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Water Rinse Outlet: Waste
Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1
LIF Laser	OFF	

Capillary Rinse Method for 30 cm Capillaries

Figure C-17 Method Settings for the Capillary Rinse Method for 30 cm Capillaries

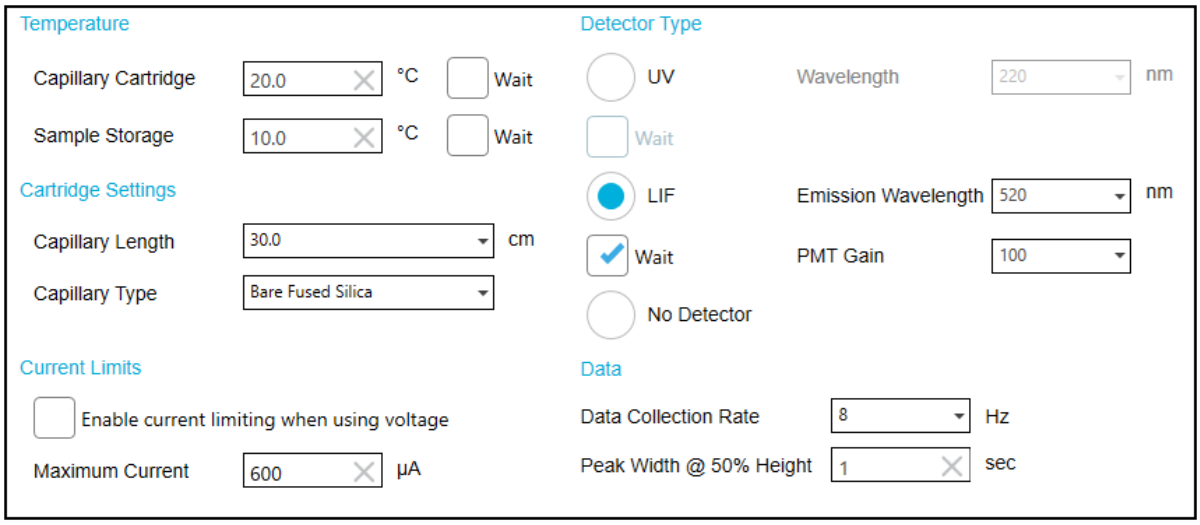


Figure C-18 Actions in the Capillary Rinse Method for 30 cm Capillaries

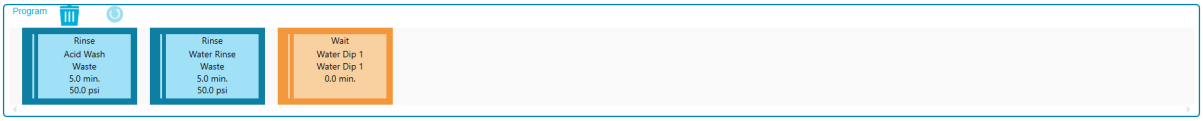


Figure C-19 Summary of Actions in the Capillary Rinse Method for 30 cm Capillaries

Settings	Capillary Cartridge: 20.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 10 Peak Width: 1 sec. Data Rate: 8 Hz
Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Water Rinse Outlet: Waste
Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1

Conditioning Method for 50 cm Capillaries

Figure C-20 Method Settings for the Conditioning Method for 50 cm Capillaries

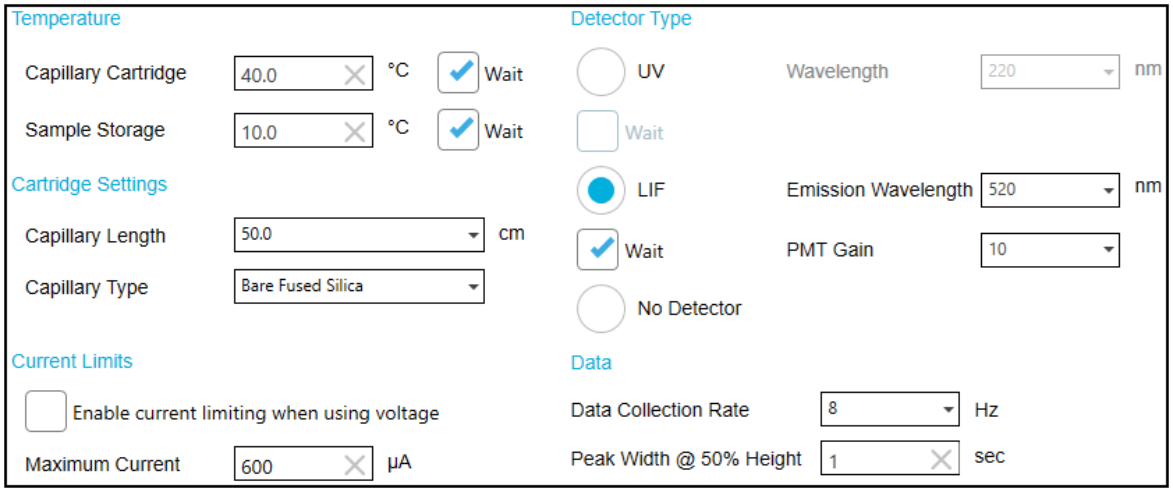


Figure C-21 Actions in the Program Pane for the Conditioning Method for 50 cm Capillaries

Figure C-22 Summary of Actions in the Conditioning Method for 50 cm Capillaries

Settings	Capillary Cartridge: 40.0 °C, Wait Capillary Length: 50.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 10 Peak Width: 1 sec. Data Rate: 8 Hz	
Rinse	Duration: 8.5 min. 50.0 psi	Inlet: Water Rinse Outlet: Waste	
Rinse	Duration: 8.5 min. 20.0 psi	Inlet: Acid Wash Outlet: Waste	
Rinse	Duration: 17.0 min. 70.0 psi	Inlet: Conditioning Solution Outlet: Waste	
Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1	
Separate	Duration: 20.0 min. -10.0 kV Ramp Time: 2.0 min. Autozero: 2.0 min.	Inlet: Conditioning Solution Outlet: Conditioning Solution	
Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Conditioning Solution Outlet: Waste	
Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Water Rinse Outlet: Waste	
Wait	Duration: 30.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1	

Linear dsDNA Separation Method for 50 cm Capillaries

Figure C-23 Method Settings for the Linear dsDNA Separation Method for 50 cm Capillaries

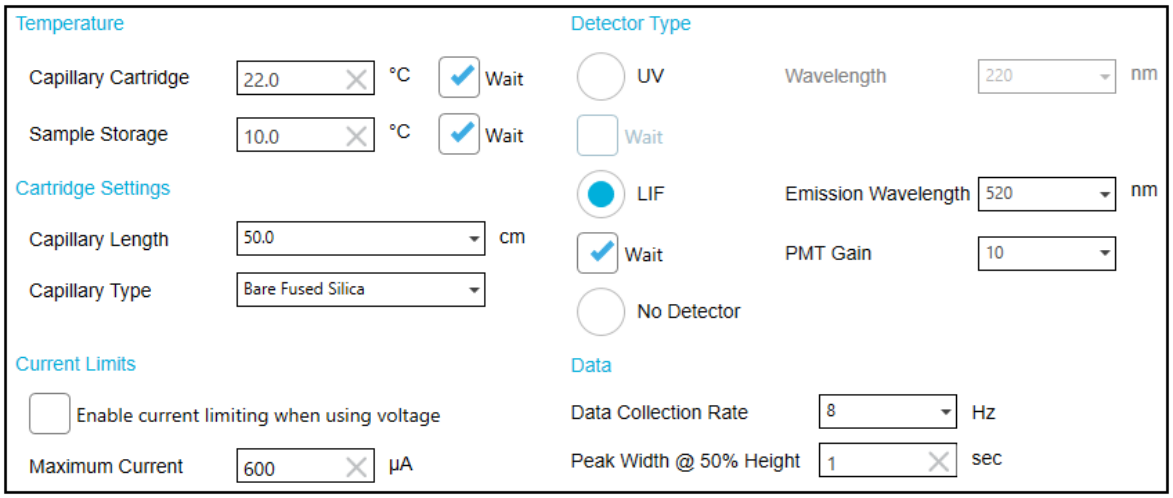


Figure C-24 Actions in the Program Pane for the Linear dsDNA Separation Method for 50 cm Capillaries

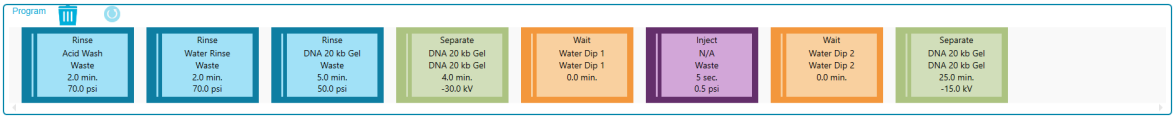


Figure C-25 Summary of Actions in the Linear dsDNA Separation Method for 50 cm Capillaries

Method Duration: 38.0 min. Number of Actions: 8

Settings	Capillary Cartridge: 22.0 °C, Wait Capillary Length: 50.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA		Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 10 Peak Width: 1 sec. Data Rate: 8 Hz
Rinse	Duration: 2.0 min. 70.0 psi		Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 2.0 min. 70.0 psi		Inlet: Water Rinse Outlet: Waste
Rinse	Duration: 5.0 min. 50.0 psi		Inlet: DNA 20 kb Gel Outlet: Waste
Separate	Duration: 4.0 min. -30.0 kV Ramp Time: 0.2 min. Disable Data Collection, Advance after: 24 actions		Inlet: DNA 20 kb Gel Outlet: DNA 20 kb Gel
Wait	Duration: 0.0 min.		Inlet: Water Dip 1 Outlet: Water Dip 1
Inject	Duration: 5 sec. 0.5 psi	Plate: Sample	Outlet: Waste
Wait	Duration: 0.0 min.		Inlet: Water Dip 2 Outlet: Water Dip 2
Separate	Duration: 25.0 min. -15.0 kV Ramp Time: 2.0 min. Autozero: 3.0 min., Advance after: 24 actions		Inlet: DNA 20 kb Gel Outlet: DNA 20 kb Gel

Shutdown Method for 50 cm Capillaries

Figure C-26 Method Settings for the Shutdown Method for 50 cm Capillaries

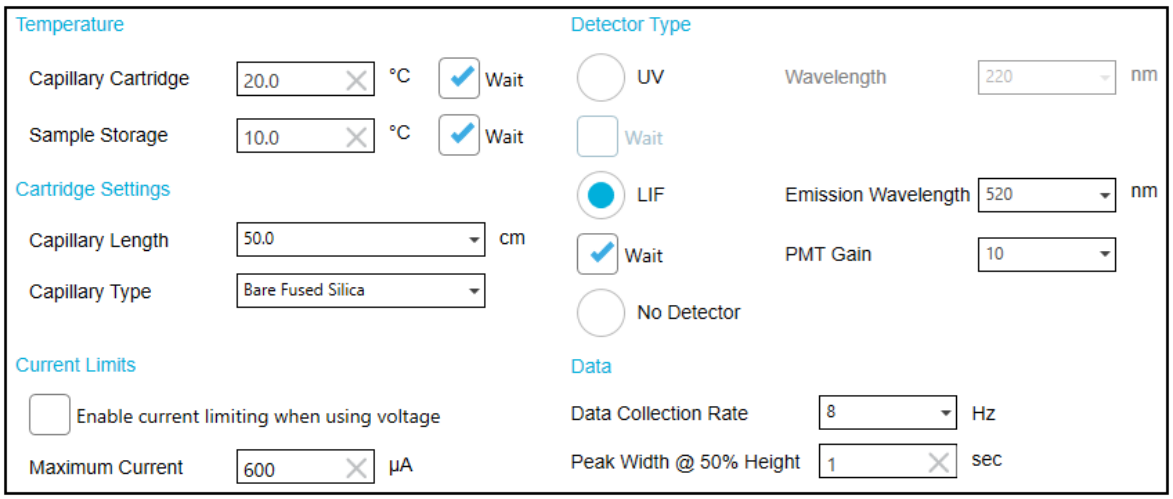


Figure C-27 Actions in the Program Pane for the Shutdown Method for 50 cm Capillaries

Figure C-28 Summary of Actions in the Shutdown Method for 50 cm Capillaries

Settings	Capillary Cartridge: 20.0 °C, Wait Capillary Length: 50.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 10 Peak Width: 1 sec. Data Rate: 8 Hz
Rinse	Duration: 5.0 min. 80.0 psi	Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 5.0 min. 80.0 psi	Inlet: Water Rinse Outlet: Waste
Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1
LIF Laser	OFF	

Capillary Rinse Method for 50 cm Capillaries

Figure C-29 Method Settings for the Capillary Rinse Method for 50 cm Capillaries

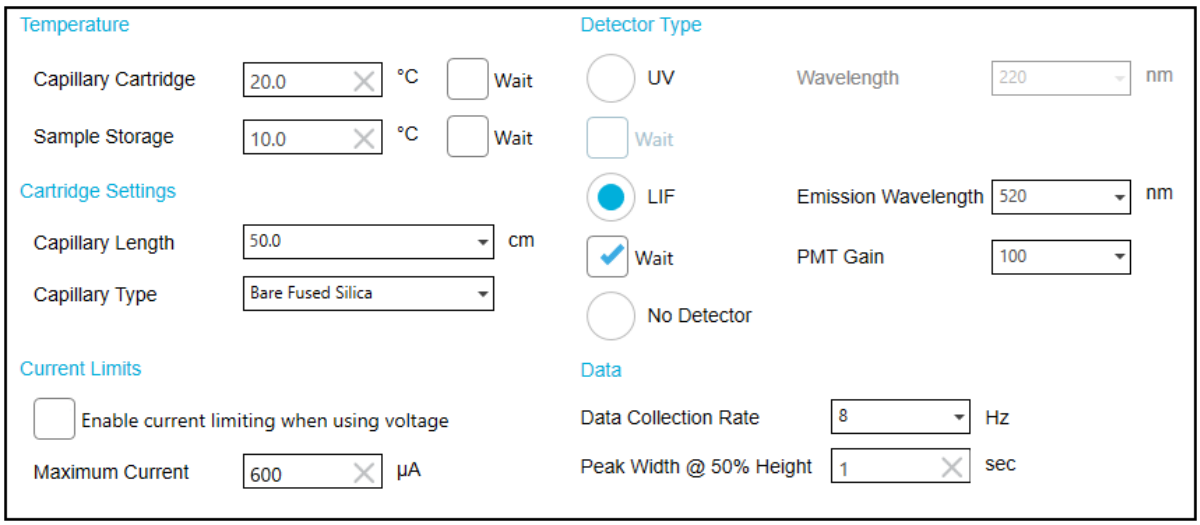


Figure C-30 Actions in the Program Pane for the Capillary Rinse Method for 50 cm Capillaries

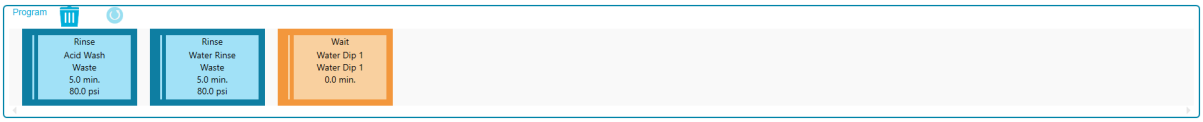


Figure C-31 Summary of Actions in the Capillary Rinse Method for 50 cm Capillaries

Settings	Capillary Cartridge: 20.0 °C, Wait Capillary Length: 50.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 10 Peak Width: 1 sec. Data Rate: 8 Hz
Rinse	Duration: 5.0 min. 80.0 psi	Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 5.0 min. 80.0 psi	Inlet: Water Rinse Outlet: Waste
Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1

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Documentation

This version of the document supersedes all of the previous versions of this document.

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