Agilent Plasmid DNA Kit

Kit Guide
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Notices

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Agilent Plasmid DNA Kit

The DNF-940 Plasmid DNA kit from Agilent (500 Samples) (Part # DNF-940-K0500) was developed for the analysis of supercoiled plasmid DNA fragments between 2,000 bp and 10,000 bp. Sizing of the supercoiled plasmid form and relative quantification between samples can be obtained using this kit.

The DNF-940 kit is capable of accurately sizing the supercoiled plasmid DNA form only. Linearized plasmid DNA can also be detected, but will migrate more slowly and not size accurately, as it is referenced to the supercoiled Plasmid DNA Ladder included with this kit. The nicked/open circular form of plasmid DNA cannot be detected or analyzed with the DNF-940 kit.

Example applications include supercoiled plasmid quality control and/or sizing.

Table 1 Physical Specifications

<table>
<thead>
<tr>
<th>Type</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume Required</td>
<td>Variable (24 µL final sample volume required; optimal sample concentration range in 1x TE specified below)</td>
</tr>
<tr>
<td>Number of Samples per Run</td>
<td>12-Capillary: 11 (+ 1 well DNA Ladder) or 12 (Imported DNA Ladder)² 48-Capillary: 47 (+ 1 well DNA Ladder) or 48 (Imported DNA Ladder)² 96-Capillary: 95 (+ 1 well DNA Ladder) or 96 (Imported DNA Ladder)²</td>
</tr>
<tr>
<td>Total Electrophoresis Run Time</td>
<td>30 min (33-55 Array)</td>
</tr>
</tbody>
</table>

Table 2 Analytical Specifications

<table>
<thead>
<tr>
<th>Type</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid DNA Sizing Range</td>
<td>2,000 bp – 10,000 bp</td>
</tr>
<tr>
<td>DNA Sizing Accuracy¹</td>
<td>± 10 % or better</td>
</tr>
<tr>
<td>Recommended Concentration Range¹</td>
<td>0.1 ng/µL – 1.0 ng/µL Final Concentration DNA in 1x TE Buffer</td>
</tr>
</tbody>
</table>

¹ Results obtained using Plasmid DNA Ladder or supercoiled DNA sample prepared in 1x TE buffer.
² Refer to Marker/Ladder/Sample Preparation section for information on using an imported DNA Ladder.
Table 3  Storage Conditions

<table>
<thead>
<tr>
<th>Store at –20°C:</th>
<th>Store at 4°C (DO NOT FREEZE):</th>
<th>Store at Room Temperature (DO NOT FREEZE):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid DNA Marker Solution*</td>
<td>Plasmid DNA Gel</td>
<td>5x Capillary Conditioning Solution</td>
</tr>
<tr>
<td>Plasmid DNA Ladder</td>
<td>5x 930 dsDNA Inlet Buffer</td>
<td>Mineral Oil</td>
</tr>
<tr>
<td>Intercalating Dye</td>
<td>Dilution Buffer 1x TE</td>
<td></td>
</tr>
</tbody>
</table>

Ensure all reagents are completely warmed to room temperature prior to use.

NOTE

The Plasmid DNA Marker (PDM) Solution is provided in aliquots of 3.2 mL vials.

*The Plasmid DNA Marker Solution is light and temperature sensitive. For maximum performance, the PDM solution should be kept frozen at –20°C and protected from light when not in use. The PDM solution should NOT be left at room temperature longer than 1 h at a time for sample preparation.

Once prepared and covered with mineral oil, the PDM solution may be used for up to one week, and stored in the dark when not in use.
### Agilent Plasmid DNA Kit

#### Table 4  kit Components

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Name</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNF-940-0240</td>
<td>Plasmid DNA Gel</td>
<td>240 mL</td>
</tr>
<tr>
<td>DNF-600-U030</td>
<td>Intercalating Dye</td>
<td>30 µL</td>
</tr>
<tr>
<td>DNF-355-0125</td>
<td>5x 930 dsDNA Inlet Buffer</td>
<td>125 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
<tr>
<td>DNF-475-0050</td>
<td>5x Capillary Conditioning Solution</td>
<td>50 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
<tr>
<td>FS-SLR940-0001</td>
<td>Plasmid DNA Ladder</td>
<td>1.2 mL</td>
</tr>
<tr>
<td></td>
<td>• 2,000 bp – 10,000 bp supercoiled plasmid DNA; 1.0 ng/µL total DNA concentration in 1x TE buffer</td>
<td></td>
</tr>
<tr>
<td>FS-SMK940-0003</td>
<td>Plasmid DNA Marker (PDM) Solution</td>
<td>3.2 mL x 2 vials</td>
</tr>
<tr>
<td></td>
<td>• Lower Marker (set to 1 bp) and Upper Marker (set to 100,000 bp)</td>
<td></td>
</tr>
<tr>
<td>FS-SMO15</td>
<td>Mineral Oil Dropper Bottle</td>
<td>15 mL</td>
</tr>
<tr>
<td>DNF-495-0060</td>
<td>Dilution Buffer 1x TE</td>
<td>60 mL</td>
</tr>
</tbody>
</table>

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**WARNING**

Working with Chemicals

The handling of reagents and chemicals might hold health risks.

- Refer to product material safety datasheets for further chemical and biological safety information.
- Follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.
2 Additional Material and Equipment Required

Material and Equipment Required for Analysis with the Fragment Analyzer

Hardware:

- Fragment Analyzer with LED fluorescence detection:
  - 5200 Fragment Analyzer (Part # M5310AA)
  - 5300 Fragment Analyzer (Part # M5311AA)
  - 5400 Fragment Analyzer (Part # M5312AA)
- FA 12-Capillary Array Ultrashort, 22cm (Part # A2300-1250-2247), OR
- FA 12-Capillary Array Short, 33cm (Part # A2300-1250-3355), OR
- FA 12-Capillary Array Long, 55cm (Part # A2300-1250-5580), OR
- FA/ZAG 96-Capillary Array Short, 33cm (Part # A2300-9650-3355), OR
- FA/ZAG 96-Capillary Array Long, 55cm (Part # A2300-9650-5580)

Software:

- Fragment Analyzer instrument control software (Version 1.1.0.11 or higher)
- ProSize data analysis software (Version 2.0.0.61 or higher)

Reagents:

- Capillary Storage Solution, 100 mL (Part #GP-440-0100)
Additional Material and Equipment Required

**Additional Equipment/Reagents Required (Not Supplied)**

- 96-well PCR sample plates. Please refer to Appendix 3 – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer User Manual for a complete approved sample plate list
- Multichannel pipettor(s) and/or liquid handling device capable of dispensing 1 – 100 µL volumes (sample plates) and 1,000 µL volumes (Inlet Buffer plate)
- Pipette tips
- 96-well plate centrifuge (for spinning down bubbles from sample plates)
- Sub-micron filtered DI water system (for diluting the 5x dsDNA Inlet Buffer and 5x Capillary Conditioning Solutions, and for preparing optional Water Dip plate)
- Fisherbrand 96 DeepWell 1mL Plate, Natural Polypropylene, part # 12-566-120 (Inlet Buffer and waste plate)
- Reagent Reservoir, 50 mL (VWR 82026-355 or similar) (for use in pipetting Inlet Buffer plates/sample trays)
- Conical centrifuge tubes for prepared Seperation Gel/Dye mixture and/or 1x Capillary Conditioning Solution
  - 250 mL (for 96-Capillary instruments or larger volumes): Corning #430776, available from Fisher #05-538-53 or VWR #21008-771
  - 50 mL (for 12-Capillary instruments or 50 mL volumes): BD Falcon #352070, available from Fisher #14-432-22 or VWR #21008-940
- Clean graduated cylinder (for measurement of Separation Gel volume and dilution of 5x 930 dsDNA Inlet Buffer and 5x Capillary Conditioning Solution)
3 Agilent Plasmid DNA Kit Protocol

Gel Preparation

1. Store the Plasmid DNA Gel at 4°C upon arrival.
2. The Intercalating Dye is supplied as a 20,000x concentrate in DMSO and should be stored at -20°C.

**NOTE**
For this assay, it is recommended to use the Intercalating Dye at 2x normal concentration (1:10,000 dilution).

3. Bring the Plasmid DNA Gel and Intercalating Dye to room temperature prior to mixing.

4. Mix appropriate volumes of Intercalating Dye and Plasmid DNA Gel necessary for less than two weeks of operation. Use a 50 mL conical centrifuge tube to allow a small minimum working volume. For larger volumes, use a 250 mL conical centrifuge tube and remove the collar of the tube holder in the instrument reagent compartment. For maximum accuracy, it is recommended to dispense Plasmid DNA Gel into a clean glass graduated cylinder for volume measurement and transfer to the working tube prior to adding Intercalating Dye.

**NOTE**
Some loss of detection sensitivity will be observed over a two-week period after the gel/dye mixture has been prepared. For best results, it is recommended to prepare gel/dye mixture daily. It is not recommended to use gel/dye mixture that is more than two weeks old.

5. The volume of Separation Gel required per run varies between 12-capillary and 96-capillary Fragment Analyzer systems. The volumes required are summarized below.
### Table 5  Volume Specifications for 12-Capillary Fragment Analyzer Systems

<table>
<thead>
<tr>
<th># of Samples to be Analyzed(^1)</th>
<th>Volume of Intercalating Dye</th>
<th>Volume of Separation Gel</th>
<th>Volume of 1x Conditioning Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1.0 µL</td>
<td>10 mL</td>
<td>10 mL</td>
</tr>
<tr>
<td>24</td>
<td>1.5 µL</td>
<td>15 mL</td>
<td>15 mL</td>
</tr>
<tr>
<td>36</td>
<td>2.0 µL</td>
<td>20 mL</td>
<td>20 mL</td>
</tr>
<tr>
<td>48</td>
<td>2.5 µL</td>
<td>25 mL</td>
<td>25 mL</td>
</tr>
<tr>
<td>96</td>
<td>4.5 µL</td>
<td>45 mL</td>
<td>45 mL</td>
</tr>
</tbody>
</table>

\(^1\) A 5 mL minimum volume should be initially added to the tube. One sample well per separation is dedicated to the ladder.

### Table 6  Volume Specifications for 48-Capillary Fragment Analyzer Systems

<table>
<thead>
<tr>
<th># of Samples to be Analyzed(^1)</th>
<th>Volume of Intercalating Dye</th>
<th>Volume of Separation Gel</th>
<th>Volume of 1x Conditioning Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>2.5 µL</td>
<td>25 mL</td>
<td>25 mL</td>
</tr>
<tr>
<td>96</td>
<td>4.0 µL</td>
<td>40 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>144</td>
<td>5.5 µL</td>
<td>55 mL</td>
<td>55 mL</td>
</tr>
<tr>
<td>192</td>
<td>7.0 µL</td>
<td>70 mL</td>
<td>70 mL</td>
</tr>
<tr>
<td>240</td>
<td>8.5 µL</td>
<td>85 mL</td>
<td>85 mL</td>
</tr>
<tr>
<td>288</td>
<td>10.0 µL</td>
<td>100 mL</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

\(^1\) One sample well per separation is dedicated to the ladder.

### Table 7  Volume Specifications for 96-Capillary Fragment Analyzer Systems

<table>
<thead>
<tr>
<th># of Samples to be Analyzed(^1)</th>
<th>Volume of Intercalating Dye</th>
<th>Volume of Separation Gel</th>
<th>Volume of 1x Conditioning Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>4.0 µL</td>
<td>40 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>192</td>
<td>8.0 µL</td>
<td>80 mL</td>
<td>80 mL</td>
</tr>
<tr>
<td>288</td>
<td>12.0 µL</td>
<td>120 mL</td>
<td>120 mL</td>
</tr>
<tr>
<td>384</td>
<td>16.0 µL</td>
<td>160 mL</td>
<td>160 mL</td>
</tr>
<tr>
<td>480</td>
<td>20.0 µL</td>
<td>200 mL</td>
<td>200 mL</td>
</tr>
</tbody>
</table>

\(^1\) One sample well per separation is dedicated to the ladder.
6 Place the Plasmid DNA Gel/Intercalating Dye mixture onto the instrument and insert into the desired gel fluid line (Gel 1 or Gel 2 pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.

7 When adding Plasmid DNA Gel to the instrument, update the solution levels in the Fragment Analyzer control software. From the main menu, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1).

8 When switching applications (e.g., between kits), prime the appropriate gel fluid line after loading fresh gel/dye mixture. From the main menu of the Fragment Analyzer instrument control software, select Utilities > Prime... Select the desired fluid line(s) (Conditioning, Gel 1, or Gel 2) and press OK to purge the fluid line with fresh gel (Figure 2).
Inlet Buffer Preparation

1. Store the 5x 930 dsDNA Inlet Buffer at 4°C upon arrival. Do not freeze.
2. Bring the 5x 930 dsDNA Inlet Buffer to room temperature prior to mixing and use.
3. In a clean container, add 20 mL of the 5x 930 dsDNA Inlet Buffer per 80 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at 4°C if desired.

Capillary Conditioning Solution Preparation

1. Store the 5x Capillary Conditioning Solution at room temperature upon arrival. Do not freeze.
2. In a clean container (e.g. 50 mL or 250 mL conical centrifuge tube), add 20 mL of the Capillary Conditioning Solution per 80 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at room temperature if desired.
3. Once mixed, place the 1x Capillary Conditioning Solution onto the instrument and insert the conditioning fluid line (Conditioning Solution pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.
4. The 1x Capillary Conditioning Solution should be added to the system as use demands. Tables 6-8 illustrate the volume specifications for the conditioning solution.
5. When adding fresh 1x Capillary Conditioning Solution to the instrument, update the solution levels in the Fragment Analyzer instrument control software. From the main menu, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1).
Instrument Preparation

1  Check the fluid level of the waste bottle and waste tray daily and empty as needed.
2  Prepare a fresh 96 DeepWell 1mL Plate filled with 1.0 mL/well of 1x 930 dsDNA Inlet Buffer daily.
   • 12-Capillary System: Row A only
   • 96-Capillary System: All Rows
     Do not overfill the wells of the inlet buffer plate.

12-Capillary Systems:
   a  In Row H of the same prepared buffer plate, place 1.1 mL/well of Capillary Storage Solution (Part # GP-440-0100).
      Row H of the buffer plate is used for the Store location, and the array moves to this position at the end of the experimental sequence.

96-Capillary Systems:
   a  In the Sample 3 drawer, place a sample plate filled with 100 µL/well of Capillary Storage Solution (Part # GP-440-0100).
      Sample 3 is used for the Store location, and the array moves to this position at the end of the experimental sequence.

NOTE  Ensure Row H of the buffer tray (12-capillary systems) or the Sample 3 drawer (96-capillary systems) is always filled with Capillary Storage Solution, and the capillary array is placed against Storage Solution when not in use, to prevent the capillary tips from drying out and potentially plugging.

3  Place the prepared inlet buffer plate into Drawer “B” (top drawer) of the Fragment Analyzer. Ensure that the plate is loaded with well A1 toward the back left on the tray.
4  Place an empty 96 DeepWell 1mL Plate into Drawer “W” (second from top) of the Fragment Analyzer. This plate serves as the capillary waste tray and should be emptied daily. Alternatively, the supplied open reservoir waste plate may be used.
Marker/Ladder/Sample Preparation

General Information

1 The recommended 96-well sample plate for use with the Fragment Analyzer system is a semi-skirted PCR plate from Eppendorf (Part #951020303). Please refer to Appendix 3 – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer User Manual for a complete approved sample plate list. The system has been designed to operate using these dimensions/styles of PCR plates.

NOTE

The use of PCR plates with different dimensions to the above recommended plate could lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible

2 Allow the Plasmid DNA Marker (PDM) solution and Plasmid DNA Ladder solutions to warm to room temperature prior to use. Spin the tubes after thawing to ensure liquid is at the bottom of the tube.

NOTE

Do not leave the PDM solution at room temperature for any longer than necessary when preparing samples.

Plasmid DNA Marker Preparation

1 Store the Plasmid DNA Marker (PDM) solution at -20°C upon arrival.

2 Bring the PDM solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.

3 The PDM solution is supplied as a ready-to-use solution, containing a lower and upper marker fragment in a 1x TE buffer solution. It is intended for use as an external standard marker plate.

4 Prepare the PDM solution plate by dispensing 30 µL/well into Row A only (12-Capillary) or every well (96-Capillary) of a separate sample plate. Cover the wells with 20 µL/well of the supplied mineral oil.

5 The PDM solution is light and temperature sensitive. Once prepared and covered with mineral oil, the PDM solution may be used for up to one week. When not stored in the instrument, the prepared PDM solution plate should be stored in the dark.

6 The prepared PDM solution plate should be placed into Drawer “M” (third from top) of the Fragment Analyzer. Ensure that the plate is loaded with well A1 toward the back left on the tray.
Plasmid DNA Ladder Preparation

1. Store the Plasmid DNA Ladder solution at -20°C upon arrival.

2. Bring the Plasmid DNA Ladder solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.

3. The Plasmid DNA Ladder solution is supplied as a ready-to-use solution, containing approximately 1.0 ng/µL total DNA concentration in a 1x TE buffer solution. It is used for calibrating the size of analyzed DNA fragments, as well as the approximate sample concentration. The Plasmid DNA Ladder should be added to a well of the sample plate and run in parallel with the samples for optimal results.

12-Capillary Systems:
   a. Pipette 24 µL of Plasmid DNA Ladder solution into well 12 of each row of the sample plate.

96-Capillary Systems:
   a. Pipette 24 µL of Plasmid DNA Ladder solution into well H12 of the sample plate.

4. Alternatively, once the Plasmid DNA Ladder has been run under the experimental method and additional samples are to be run under the same experimental conditions, the ladder can be imported in the ProSize software, enabling use of all 12 wells per row or all 96 wells of the sample plate. However, note that the sizing and quantification accuracy will be reduced when using an imported ladder.
Sample Plate Preparation

1. The recommended total input DNA sample concentration for this method is a range of 0.1 ng/µL to 1 ng/µL in 1x TE buffer (DNA fragments) for optimal assay results. If the concentration of the sample is above this range, pre-dilute the sample with the supplied 1x TE buffer prior to performing the assay. Do not pre-dilute samples with DI water.

2. The above DNA sample concentrations assume a starting sample matrix of 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA). If the chloride salt concentration is greater than 10 mM, some loss of sensitivity may be observed and slight adjustments may need to be made to the sample injection conditions.

**NOTE**
Avoid total DNA input sample concentrations above the specified limits. Overloading of DNA sample can result in saturation of the CCD detector and poor results. The peak heights for individual fragments should lie in an optimal range between 100 – 10,000 RFUs.

3. Ensure that samples are well mixed (vortexed) before loading into the sample plate. Using a clean 96-well sample plate, into each sample well pipette 24 µL of the samples diluted to the concentration range specified in Step 1 above.

4. If running the Plasmid DNA Ladder in parallel with the samples, pipette 24 µL of the Ladder solution directly (no dilution) into the Well 12 of each row to be analyzed (12-capillary systems) or Well H12 of the sample plate (96-capillary system).

5. Fill any unused wells within the sample row or plate with 24 µL of 1x TE dilution buffer.

6. Check the wells of the sample plate to ensure there are no air bubbles trapped in the bottom of the wells. If necessary, centrifuge the plate to remove any air bubbles. The presence of trapped air bubbles can lead to injection failures.

7. Run the sample plate immediately once prepared, or cover the sample plate with a cover film, store at 4°C, and use as soon as possible. Alternatively, to prevent evaporation, place a mineral oil overlay on each sample (20 µL/well).

8. To run the samples, place the plate in one of the three sample plate trays (Drawers 4-6 from the top) of the Fragment Analyzer instrument. Load or create the experimental method as described in the following sections.

9. The CCD detection system of the Fragment Analyzer system provides a high dynamic range for detection. An ideal injection range would yield peak heights from 100 – 10,000 RFUs. Overloading of sample can decrease separation resolution and saturate the detector, leading to mismatched lower/upper marker peak heights and poor results. It is important to optimize sample dilution and concentration, and use experimental parameters to work with within the specified RFU range.
Performing Experiments

Running an Experiment

1. To set up an experiment, from the main menu of the Fragment Analyzer instrument control software, select the Operation tab (Figure 3). Select the sample tray location to be analyzed (1, 2, or 3) by left clicking the Sample Tray # dropdown or by clicking the appropriate sample plate tab (alternate plate view) and choosing the appropriate location.

   **NOTE**
   For 96-Capillary Systems: Sample 3 is typically assigned to the Capillary Storage Solution.

2. Left click a well of the desired sample plate row with the mouse. The selected row will be highlighted in the plate map (e.g., Row A in Figure 3). Enter the sample name if desired into the respective Sample ID cell by left clicking the cell and typing in the name. Alternatively, sample information can be imported from .txt or .csv file by selecting the Load from File... option.

3. After sample information for the row or plate has been entered, under the Run Selected Group field press Add to queue. The Separation Setup form will be displayed enabling the user to select the experimental method and enter additional information (Figure 4).
4 In the **Separation Setup** pop-up form, left click the dropdown and select the appropriate preloaded experimental **Method** file. The available methods are sorted by kit number and are linked to the directory containing methods for the currently installed capillary array length (e.g., 33cm). Select the following method:

- Select **DNF-940-33 – Plasmid DNA.mthds** when the 33 cm effective, 55 cm total "short" capillary array is installed.
- The Plasmid DNA kit is not currently supported on the 55 cm effective, 80 cm total length "long" capillary array or the 22 cm effective, 47 cm total “ultrashort” capillary array.

5 Select the appropriate **Gel** line being used for the experiment (Gel 1 or Gel 2) using the dropdown.

6 The **Tray Name** can be entered to identify the sample plate. The **Folder Prefix** if entered will amend the folder name (normally a time stamp of HH-MM-SS from the start of the CE run).

7 To copy the experimental results to another directory location in addition to the default save directory, check the **Copy results** box and select the desired **Copy path:** directory by clicking the … button and navigating to the desired save directory.

8 Any **Notes** can be entered regarding the experiment; they will be saved and displayed in the final PDF report generated by the ProSize software.

9 Once all information has been entered, press **OK** to add the method to the instrument queue (press **Cancel** to abort adding the method).
Agilent Plasmid DNA Kit Protocol

10 Repeat Steps 1-9 for any remaining sample rows to be analyzed.

11 On 96-capillary systems, or in 12-capillary systems if the entire 96-well sample tray is to be run using the same experimental method, under the Run Entire Tray field press Add to queue. A form similar to Figure 4 will be displayed for entering information and adding the run to the instrument queue for the entire 96-well sample tray.

12 After a row or tray has been added to the queue, the method(s) will be listed on the main screen under the Method Queue field (Figure 5).

13 Prior to starting the experiment, verify all trays (buffer/storage, waste, marker, sample, etc.) have been loaded into their respective drawer locations.

14 Press the Play icon ( ) to start the sequence loaded into the queue. To Pause the queue after the currently running experiment is completed, press the button. To Clear the run queue of all loaded experiments, press the button.

15 Once an experiment has been loaded onto the queue, the user can view or edit the method (administrator level only can edit a method) by pressing the Method Summary field. To remove the method from the queue, press the “X” button; to view the stepwise details of the method press the double down arrow icon.

16 The user may add a Pause or Prime step into the queue by right clicking the mouse while over the queue and selecting Insert Pause or Insert Prime.

17 The order of the experimental queue can be rearranged by dragging down individual entries. Further information regarding the Method Queue operation is provided in the Fragment Analyzer User Manual.
Once started, the instrument will perform all the programmed experiments in the Method Queue uninterrupted unless a pause step is present. Note that additional experiments can be programmed and added to the Method Queue at any time while the instrument is running if desired. After completion of the last queued experiment, the instrument stage will automatically move to the Store location (12-Capillary Systems: Row H of the inlet buffer tray; 96-Capillary Systems: Sample 3 location).

Viewing and Editing Experimental Methods

1. A user level operator can View the steps of the experimental method by pressing the View link on the Separation Setup screen, or by pressing the Method Summary option once a method has been loaded onto the experimental queue. User level operators cannot edit any steps of a queued separation method.

2. Administrator level operators can Edit certain steps of the experimental method. To open the method editor screen, press the Edit link from the Separation Setup screen (Figure 4). The method editor screen is displayed, showing the steps of the method (Figure 6).

3. The preloaded, optimized steps for the DNF-940-33 method (Figure 6) are shown below. The general steps of this method are as follows:
   a. Full Conditioning flushing method (Automatically enabled).
      Default Gel Selection: Gel 1.
   b. Perform Prerun (enabled) (10 kV, 30 sec)
   c. Rinse (disabled)
   d. Marker Injection (enabled) Voltage Injection (5 kV, 15 sec). This step injects the prepared Plasmid DNA Marker plate.
   e. Rinse (disabled)
   f. Sample Injection (enabled) Voltage Injection (5 kV, 15 sec). This step injects the prepared sample plate.
   g. Separation (enabled) Voltage (10 kV, 30 min). This step performs the CE Separation.
An administrator level user has the option to adjust the Gel Selection; Prerun settings; Rinse settings including Tray, Row and # Dips; Marker Injection settings including Row; Sample Injection settings; and the Separation settings. For example, if the marker solution is loaded into a row other than Row A on a 12-capillary instrument, this can be adjusted prior to or while the method is loaded on the experimental queue.

To apply any adjustments to the method being placed on the experimental queue, press the OK button. To exit the editor screen without applying any changes press the Cancel button.

Any edits made to the experimental method from the Separation Setup or Method Summary screen will only apply to the currently loaded experiment in the queue. No changes are made to the original separation method file.
Processing Experimental Data

1. When processing data, the ProSize software will automatically recognize the separation method performed and apply the appropriate matching configuration file from the C:\ProSize 3.0\Configurations directory:
   - The DNF-940-33 separation method will be processed using the DNF-940-33 – Plasmid DNA configuration file.

   **NOTE**

   If the preloaded ProSize software configuration file DNF-940-33 – Plasmid DNA is not located in the C:\ProSize 3.0\Configurations directory, contact Agilent Technical Support to obtain these files.

2. The data is normalized to the lower marker (set to 1 bp) and upper marker (set to 100,000 bp), and calibrated to the Plasmid DNA Ladder run in parallel to the samples. Figure 7 shows an example of the 1 bp and 100,000 bp markers of the Plasmid DNA Marker solution co-injected with the Plasmid DNA Ladder. A total of 11 peaks should be observed.

3. The ProSize configuration is set to the DNA mode in the Advanced Settings. The Quantification settings are set to Use Ladder for quantification with a Final Conc. (ng/µL) of 1.0 and a Dilution Factor of 1 (24 µL Plasmid DNA Ladder loaded at 1.0 ng/µL concentration).

   For samples not pre-diluted in 1x TE buffer, the Dilution Factor setting should be set to 1. If the samples were diluted in 1x TE buffer then the Dilution Factor setting should be set accordingly (e.g., 2 µL sample + 22 µL 1x TE should be set to 12).

4. For full information on processing data, refer to the ProSize User Manual.
Fragment Analyzer Shut Down/Storage

Instrument Shut Down/Storage

After each experiment, the instrument automatically places the capillary array in the Store position against Capillary Storage Solution:

- 12-Capillary Systems: Row H of the buffer tray
- 96-Capillary Systems: Sample 3

No further action is required.

If the instrument is to be idle for more than one day, it is recommended to turn off the power to the system.
4 Checking Your Separation Results

Plasmid DNA Ladder

Figure 7 shows the typical expected results for the Plasmid DNA Ladder, injected at a total DNA concentration of 1.0 ng/µL in 1x TE buffer. A total of 11 peaks should be observed, with the sizes annotated as in Figure 7. All fragments in the ladder should be resolved. The Plasmid DNA Ladder should be fitted with a point-to-point curve fitting algorithm in the ProSize software.

Figure 7. Plasmid DNA Ladder result using the Fragment Analyzer system with the Plasmid DNA Analysis kit. Peaks annotated by size (bp).
Checking Your Separation Results

Plasmid DNA Sample

Figure 8 shows a result for a Plasmid DNA sample. In this example, a supercoiled plasmid DNA sample was analyzed.

![Graph showing DNA analysis results](image)

**Figure 8.** Supercoiled plasmid DNA sample result using the Fragment Analyzer system with the Plasmid DNA Analysis kit. The expected size of this sample was 6000 bp and the approximate expected concentration was 0.25 ng/µL. The peaks are annotated by size; the second peak at 13209 bp is presumed to be dimerized species.

<table>
<thead>
<tr>
<th>Size (bp)</th>
<th>ng/µL</th>
<th>% (Conc.)</th>
<th>nmole/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (LM)</td>
<td>0.0941</td>
<td>123.008</td>
<td></td>
</tr>
<tr>
<td>6001</td>
<td>0.2517</td>
<td>95.5</td>
<td>0.059</td>
</tr>
<tr>
<td>13209</td>
<td>0.0120</td>
<td>4.5</td>
<td>0.001</td>
</tr>
<tr>
<td>100000 (UM)</td>
<td>0.1164</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

TIC: 0.2537 ng/µL
TIM: 0.071 nmole/L
Total Conc.: 0.2941 ng/µL
5 Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-940 Plasmid DNA Analysis kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

<table>
<thead>
<tr>
<th>Issue</th>
<th>Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>The peak signal is &gt;&gt; 20,000 RFU; upper marker peak is low or not detected relative to lower marker.</td>
<td>1 Input DNA sample concentration too high. Ensure peak height does not exceed 10,000 RFU (fragment), or total input concentration does not exceed recommended limits.</td>
<td>1 Further dilute input DNA sample concentration with 1x TE buffer and repeat experiment. 2 Reduce injection time and/or injection voltage, and repeat experiment. Use the same injection voltage/time settings for the Marker Plate and Sample Plate to maximize quantification accuracy.</td>
</tr>
<tr>
<td>No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.</td>
<td>1 Sample concentration too low and out of range. 2 Sample was not added or not mixed well.</td>
<td>1 Prepare more concentrated sample and repeat experiment, OR Repeat experiment using increased injection time and/or injection voltage for Marker Plate and Sample Plate. 2 Verify sample was correctly added and mixed to sample well.</td>
</tr>
<tr>
<td>No sample peak or marker peak observed for individual sample.</td>
<td>1 Air trapped at the bottom of sample plate well, or bubbles present in sample well. Insufficient sample volume. A minimum of 20 µL is required. 2 Capillary is plugged</td>
<td>1 Check sample plate wells for trapped air bubbles. Centrifuge plate. 2 Verify proper volume of solution was added to sample well. 3 Check waste plate for liquid in the capillary well. If no liquid is observed, follow the steps outlined in Appendix G – Capillary Array Cleaning of the Fragment Analyzer User Manual for unclogging a capillary array.</td>
</tr>
</tbody>
</table>
In This Book

This Kit Guide describes the following:

- Agilent Plasmid DNA Kit
- Additional Material and Equipment Required
- Agilent Plasmid DNA Kit Protocol
- Checking Your Separation Results
- Troubleshooting