Agilent Large Fragment Kit

Kit Guide

For Research Use Only.

Not for use in diagnostic procedures.

Large Fragment Kit, 500 Samples (Part # DNF-492-0500)

Large Fragment Kit, 1,000 Samples (Part # DNF-492-1000)

Notices

Manual Part Number

M5310-91492

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Contents

1 Agilent Large Fragment Kit

Table 1 Physical Specifications

Type	Specifications
Sample Volume Required	2 µL
Number of Samples per Run	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]
Total Electrophoresis Run Time	30 minutes (22-47 Array)[3] 50 minutes (33-55 Array) 80 minutes (55-80 Array)

Table 2 Analytical Specifications

Type	Specifications
DNA Sizing Range	50 bp – 20,000 bp
DNA Sizing Accuracy[1]	± 15% or better
DNA Fragment Concentration Range[1]	0.1 ng/µL - 10 ng/µL input DNA
DNA Smear Concentration Range[1]	5 ng/µL - 100 ng/µL input DNA
DNA Quantification Accuracy[1]	± 25 %
DNA Quantification Precision[1]	20 % CV
Maximum DNA Concentration	10 ng/µL per fragment; 100 ng/µL total

[1] Results using DNA Ladder or DNA Fragment standards initially prepared in 1x TE buffer.

[2] Results using DNA Fragment standards and DNA smears prepared from 1x TE buffer.

[3] The 22 cm effective, 47 cm total length capillary is only available for 12-capillary Fragment Analyzer instruments.

Table 3 Storage Conditions

Store at –20°C:	Store at 4°C (Do not freeze):	Store at Room Temperature (Do not freeze):
Intercalating Dye	dsDNA Gel	5x Capillary Conditioning Solution
Large Fragment Diluent Marker (DM) Solution*	5x 930 dsDNA Inlet Buffer	
Large Fragment DNA Ladder	BF-2000 Blank Solution	
	0.6x TE Rinse Buffer	

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

NOTE

*The Large Fragment Diluent Marker (DM) solution is provided in aliquots of 2.4 mL vials. To minimize the number of freeze/thaw cycles, it is highly recommended to work with only one aliquot of DM solution at a time.

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

Large Fragment kit, 500 Samples (Part # DNF-492-1000)

Table 4 kit Components

Part Number	Name	Amount
DNF-220-0240	Large Fragment Separation Gel	240 mL
DNF-600-U030	Intercalating Dye	30 µL
DNF-355-0125	5x 930 dsDNA Inlet Buffer	125 mL (dilute with sub-micron filtered water prior to use)
DNF-475-0050	5x Capillary Conditioning Solution	50 mL (dilute with sub-micron filtered water prior to use)
DNF-380-0003	Large Fragment Diluent Marker (DM) Solution • Lower Marker (Set to 1 bp) and Upper Marker (set to 100,000 bp)	2.4 mL x 5 vials
DNF-378-U100	Large Fragment DNA Ladder • Fragments from 75 bp – 20,000 bp; 50 ng/µL total DNA concentration	100 µL
DNF-496-0125	0.6x TE Rinse Buffer	125mL
DNF-302-0008	BF-2000 Blank Solution	8 mL

Large Fragment kit, 1,000 Samples (Part # DNF-492-1000)

Table 5 kit Components

Part Number	Name	Amount
DNF-220-0500	Large Fragment Separation Gel	500 mL
DNF-600-U030	Intercalating Dye	30 µL x 2
DNF-355-0300	5x 930 dsDNA Inlet Buffer	300 mL (dilute with sub-micron filtered water prior to use)
DNF-475-0100	5x Capillary Conditioning Solution	100 mL (dilute with sub-micron filtered water prior to use)
DNF-380-0003	Large Fragment Diluent Marker (DM) Solution • Lower Marker (Set to 1 bp) and Upper Marker (set to 100,000 bp)	2.4 mL x 10 vials
DNF-378-U100	Large Fragment DNA Ladder • Fragments from 75 bp – 20,000 bp; 50 ng/µL total DNA concentration	100 µL x 2
DNF-496-0125	0.6x TE Rinse Buffer	125mL
DNF-302-0008	BF-2000 Blank Solution	8 mL

NOTE

UPDATE: The separation gel part number and description has been changed to # DNF-220, Large Fragment Separation Gel. The formula has not been changed.

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 48-Capillary Array Short, 33cm (Part # A2300-4850-3355) 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 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 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 930 dsDNA Inlet Buffer and 5x Capillary Conditioning Solutions)
- 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 dsDNA 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 dsDNA Gel volume and dilution of 5x dsDNA Inlet Buffer and 5x Capillary Conditioning Solution

3 Agilent Large Fragment kit Protocol

Gel Preparation

1. Store the RNA Separation 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, the Intercalating Dye should be used at 2x normal concentration (1:10,000 dilution).

3. Bring the Separation Gel and Intercalating Dye to room temperature prior to mixing.
4. Mix appropriate volumes of Intercalating Dye and Separation Gel necessary for less than two weeks of operation. Use the supplied 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 Separation 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 6 Volume Specifications for 12-Capillary Fragment Analyzer Systems

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of separation gel	Volume of 1x Conditioning Solution
12	1.0 µL	10 mL	10 mL
24	1.5 µL	15 mL	15 mL
36	2.0 µL	20 mL	20 mL
48	2.5 µL	25 mL	25 mL
96	4.5 µL	45 mL	45 mL

[1] A 5 mL minimum volume should be initially added to the tube. One sample well per separation is dedicated to the ladder.

Table 7 Volume Specifications for 48-Capillary Fragment Analyzer Systems

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of separation gel	Volume of 1x Conditioning Solution
48	2.5 µL	25 mL	25 mL
96	4.0 µL	40 mL	40 mL
144	5.5 µL	55 mL	55 mL
192	7.0 µL	70 mL	70 mL
240	8.5 µL	85 mL	85 mL
288	10.0 µL	100 mL	100 mL

[1] One sample well per separation is dedicated to the ladder.

Table 8 Volume Specifications for 96-Capillary Fragment Analyzer Systems

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of separation gel	Volume of 1x Conditioning Solution
96	4.0 µL	40 mL	40 mL
192	8.0 µL	80 mL	80 mL
288	12.0 µL	120 mL	120 mL
384	16.0 µL	160 mL	160 mL
480	20.0 µL	200 mL	200 mL

[1] One sample well per separation is dedicated to the ladder.

6 Place the prepared separation 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 separation 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).

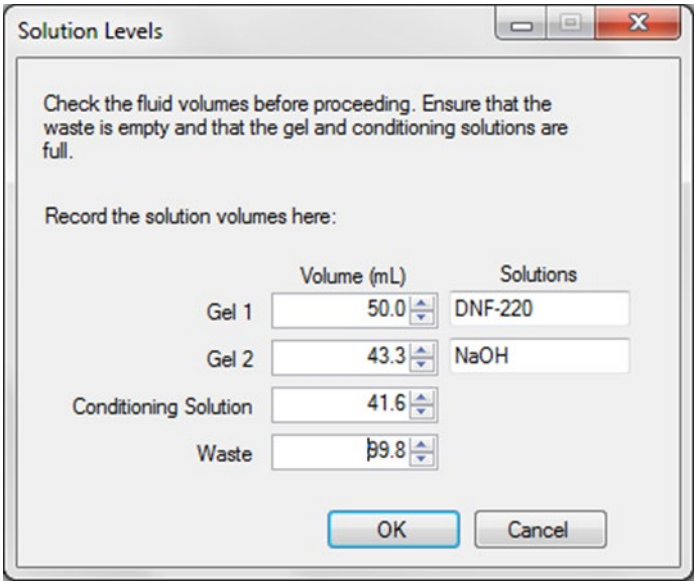


Figure 1 Solution Levels menu

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 control software, select **Utilities > Prime...** Select the desired fluid line(s) (Conditioning, Gel 1, or Gel 2) and press **OK** to prime the fluid line with fresh gel.

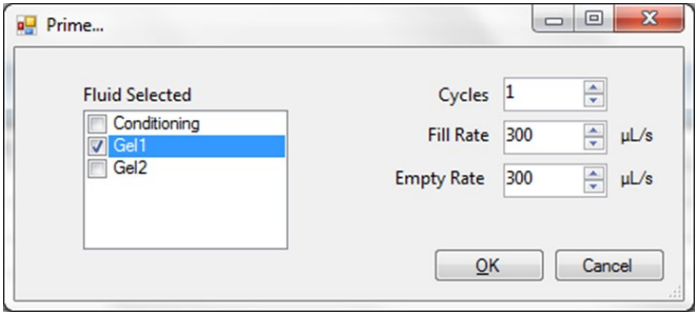


Figure 2. Prime menu

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 5x 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 show 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 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 tray (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.

5 Prepare a fresh sample plate filled with 100 µL/well of 0.6x TE Rinse Buffer daily. (12-Capillary System: Row A only; 96-Capillary System: All Rows).

6 Place the prepared 0.6x TE Rinse Buffer plate 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.

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 Large Fragment Diluent Marker (DM) solution and Large Fragment DNA Ladder solution to warm to room temperature prior to use. Spin the tube after thawing to ensure liquid is at the bottom of the tube.

NOTE

Do not leave the Large Fragment DM at room temperature for any longer than necessary when preparing samples.

Sample Plate Preparation

1 The total input DNA sample concentration MUST be within a range of 0.1 ng/µL to 10 ng/µL (DNA fragment) or 5 ng/µL to 100 ng/µL (DNA smear) for optimal assay results. If the concentration of the sample is above this range, pre-dilute the sample with 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 the smears should lie in an optimal range between 20 – 2000 RFUs. The peak heights for individual fragments should lie in an optimal range between 100 – 20,000 RFUs.

3 Using a clean 96-well sample plate, pipette 22 µL of Large Fragment Diluent Marker (DM) Solution to each well in a row that is to contain sample or DNA Ladder. Fill any unused wells within the row of the sample plate with 24 µL/well of BF-2000 Blank Solution.

4 Pipette 2 µL of each DNA sample into the respective wells of the sample; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

5 DNA Ladder: The Large Fragment DNA Ladder should be run in parallel with the samples during each experiment for best results. It is not recommended to import a previously run sizing ladder.

12-Capillary Systems:

a Pipette 2 µL of Large Fragment DNA Ladder into well 12 of each row of the sample plate containing 22 µL of the Large Fragment Diluent Marker (DM) Solution. Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

96-Capillary Systems:

b Pipette 2 µL of Large Fragment DNA Ladder into well H12 of the sample plate containing 22 µL of the Large Fragment Diluent Marker (DM) Solution. Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

NOTE

Important Sample Mixing Information

When mixing sample with diluent marker solution, it is important to mix the contents of the well thoroughly to achieve the most accurate quantification. It is highly suggested to perform one of the following methods to ensure complete mixing:

- When adding 2 µL of sample to the 22 µL of diluent, swirl the pipette tip while pipetting up/down to further mix.
- After adding 2 µL of sample to the 22 µL of diluent, place a plate seal on the sample plate and vortex the sample plate at 3,000 rpm for 2 min. Any suitable benchtop plate vortexer can be used. Ensure that there is no well-to-well transfer of samples when vortexing. The plate should be spun via a centrifuge after vortexing to ensure there are no trapped air bubbles in the wells.
- After adding 2 µL of sample to the 22 µL of diluent, use a separate pipette tip set to a larger 20 µL volume, and pipette each well up/down to further mix.
- Use an electronic pipettor capable of mixing a 10 µL volume in the tip after dispensing the 2 µL sample volume. Some models enable using the pipette tip for both adding and mixing.

6 After mixing sample/DNA Ladder and Diluent Marker Solution in each well, centrifuge the plate to remove any air bubbles. Check the wells of the sample plate to ensure there are no air bubbles trapped in the bottom of the wells. 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

Performing Experiments

Running an Experiment

1 To set up an experiment, from the Main Menu of the Fragment Analyzer 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.

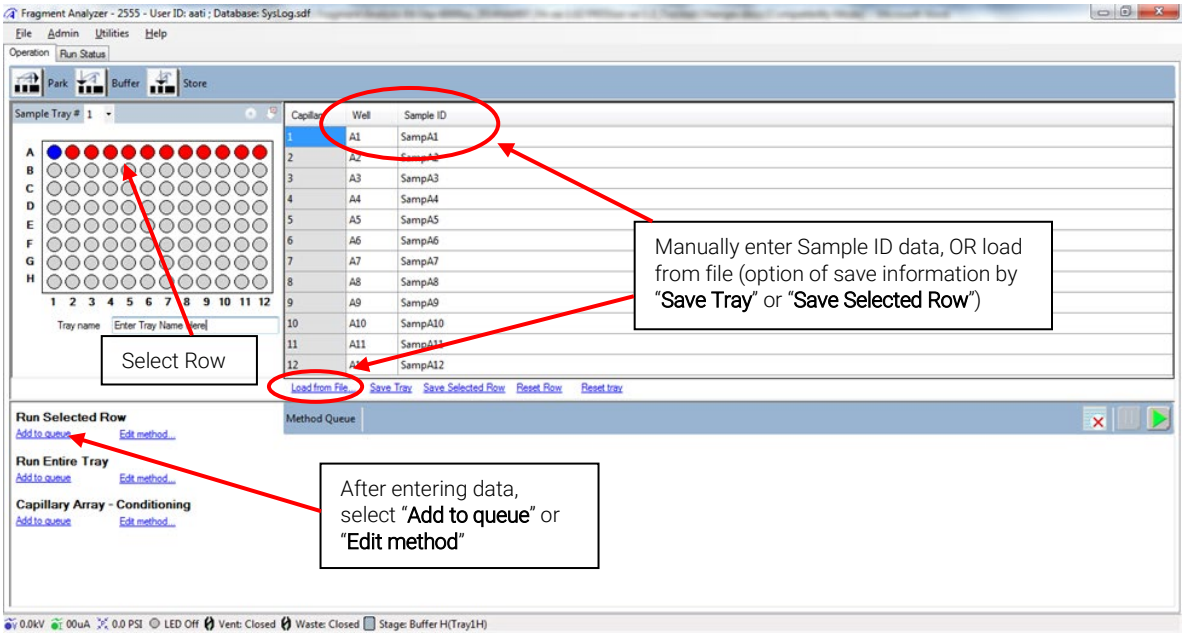


Figure 3. Main screen showing selection of sample row and entering sample information

3 After sample information for the row or plate has been entered, under the **Run Selected Row** 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).

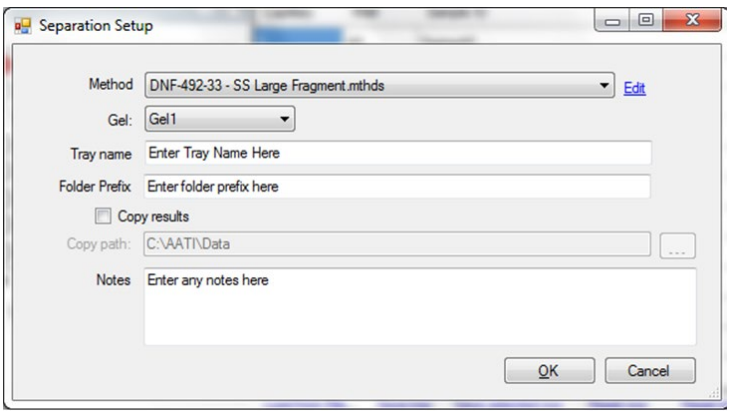


Figure 4. Separation Setup form to select experimental Method and enter tray/folder information

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 or 55cm). Select the following method:
   - Select DNF-492-33 – SS Large Fragment.mthds when the 33 cm effective, 55 cm total "short" capillary array is installed.
   - Select DNF-492-55 – SS Genomic DNA.mthds when the 55 cm effective, 80 cm total "long" capillary array is installed.

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 2.0 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).

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.

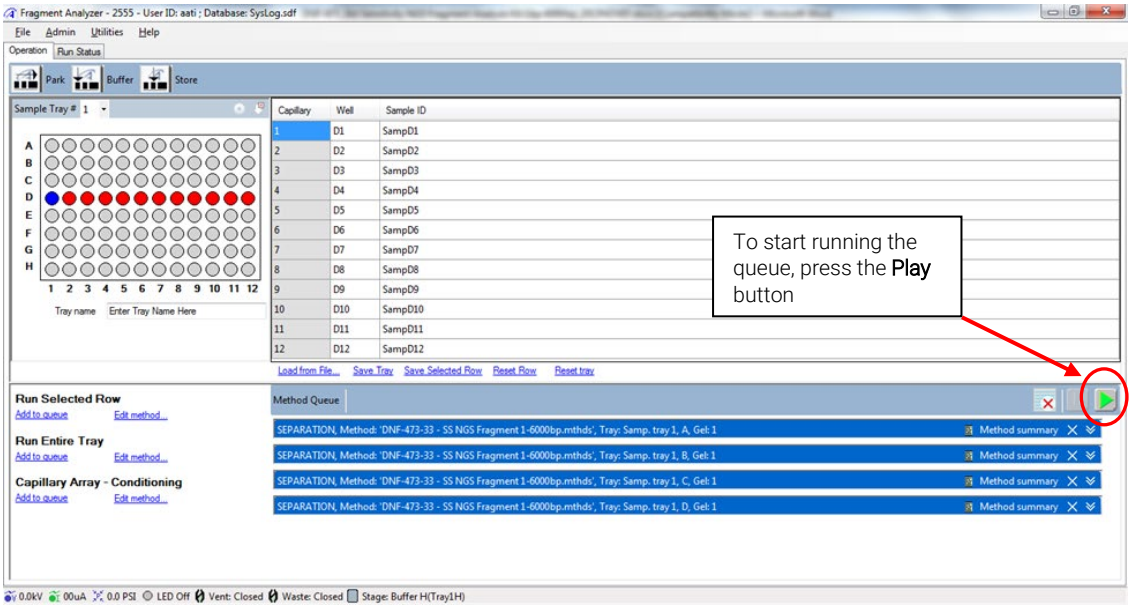


Figure 5. Main screen after selection of samples to the run queue

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.

18 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 containing the Capillary Storage Solution; 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-492-33 (Figure 6) and DNF-492-55 (Figure 7) methods are shown below. The general steps of the method are as follows:
   - **a** Full Condition flushing method (Automatically enabled). Default Gel Selection: Gel 1.
   - **b** Perform Prerun (enabled) (6 kV, 30 sec)
   - **c** Rinse (disabled)
   - **d** Marker Injection (disabled)
   - **e** Rinse (enabled; Tray = Marker; Row = A; # Dips = 1). This step moves to the Marker tray and rinses the capillary tips with 0.6x TE Rinse Buffer.
   - **f** Sample Injection (enabled) Voltage Injection (3 kV, 5 sec). This step injects the prepared sample plate.
   - **g** Separation (enabled) Voltage (6 kV, 50 min). This step performs the CE Separation.

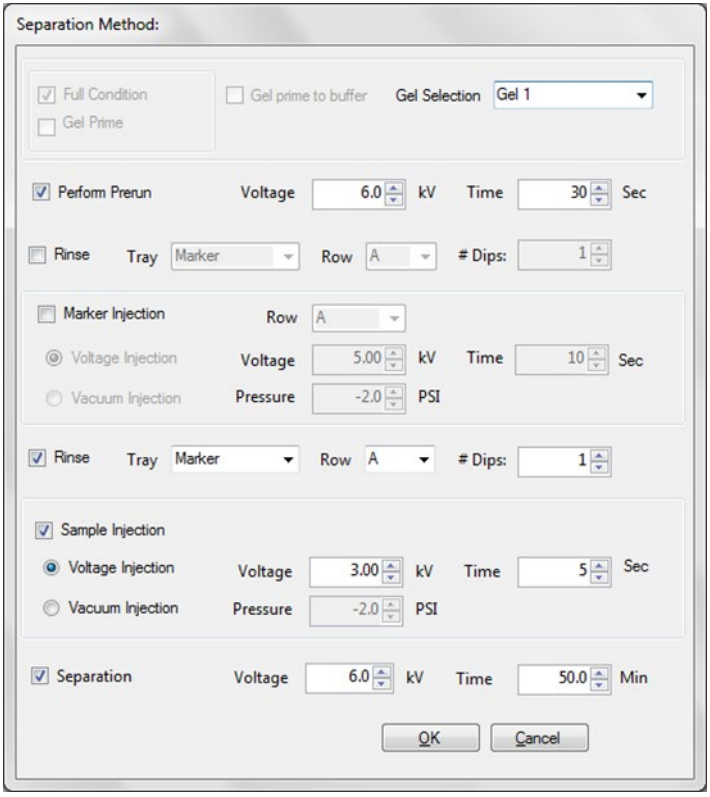


Figure 6. DNF-492-33 Large Fragment kit method

Figure 7 shows the preloaded method for the 55 cm effective, 80 cm total length "long" array. The **Prerun** and **Separation** voltage is set to 9 kV, the **Injection** voltage to 5 kV, and the **Separation** time to 80 min.

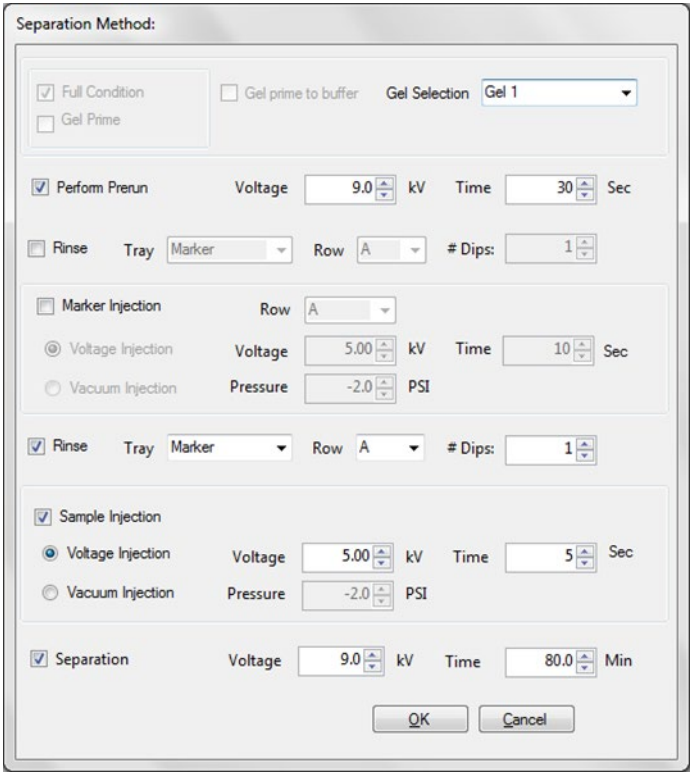


Figure 7. DNF-492-55. Large Fragment kit method

4 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 this can be adjusted prior to or while the method is loaded on the experimental queue.

5 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.

NOTE

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-492-33 separation method will be processed using the DNF-492-33 - SS Large Fragment configuration file.
- The DNF-492-55 separation method will be processed using the DNF-492-55 - SS Large Fragment configuration file.

NOTE

If the preloaded ProSize software configuration files "DNF-492-33 – SS Large Fragment" and "DNF-492-55 – SS Large Fragment " are 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 Large Fragment DNA Ladder run in parallel to the samples.

Figure 8 shows an example of the 1 bp and 100,000 bp markers injected with the Large Fragment Ladder using the DNF-492-33 separation method. A total of 17 peaks should be observed

3 The ProSize software is set to the NGS mode in the Advanced Settings. The Quantification settings are set to Use Ladder for quantification with a Conc. (ng/uL) of 4.167 and a Dilution Factor of 12 (2 µL sample + 22 µL Diluent Marker)

NOTE

If a pre-dilution was performed prior to the experiment, the Dilution Factor setting should be changed to reflect the estimated final sample concentration.

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

DNA Ladder

Figure 8 shows the typical expected results for the Large Fragment DNA Ladder, provided at an initial total DNA concentration of 50 ng/µL in 1x TE buffer (2 µL + 22 µL DM solution; 1:12 dilution). A total of 17 peaks should be observed, with the sizes annotated as in Figure 8. All fragments in the ladder should be well resolved.

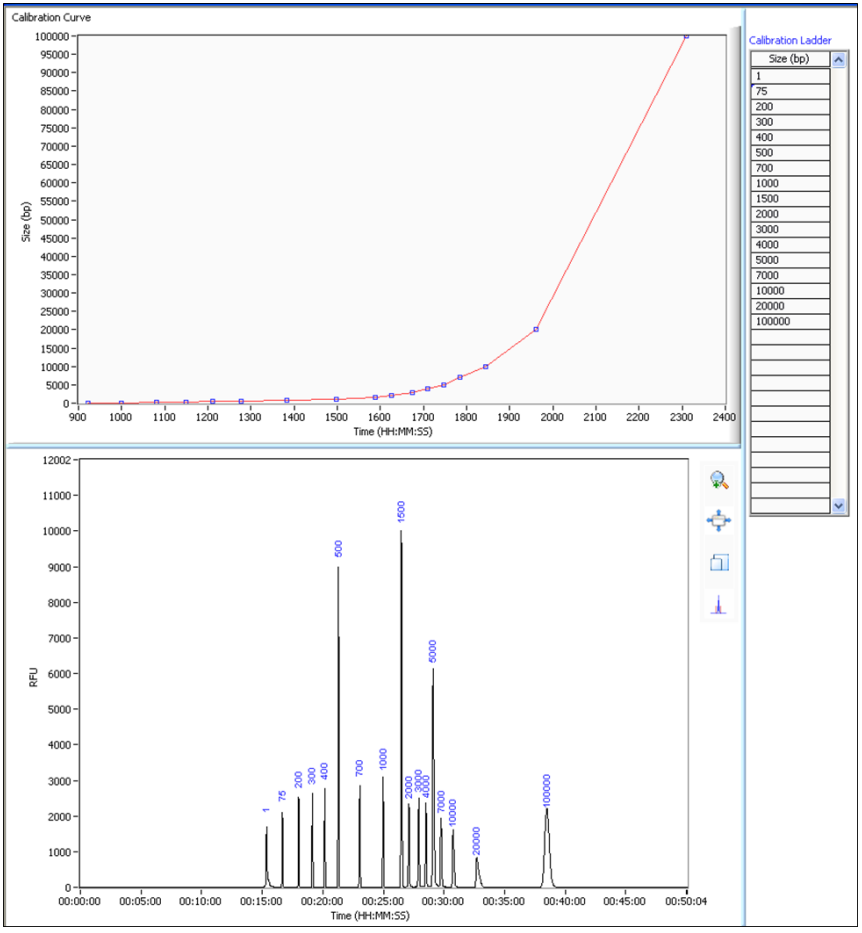
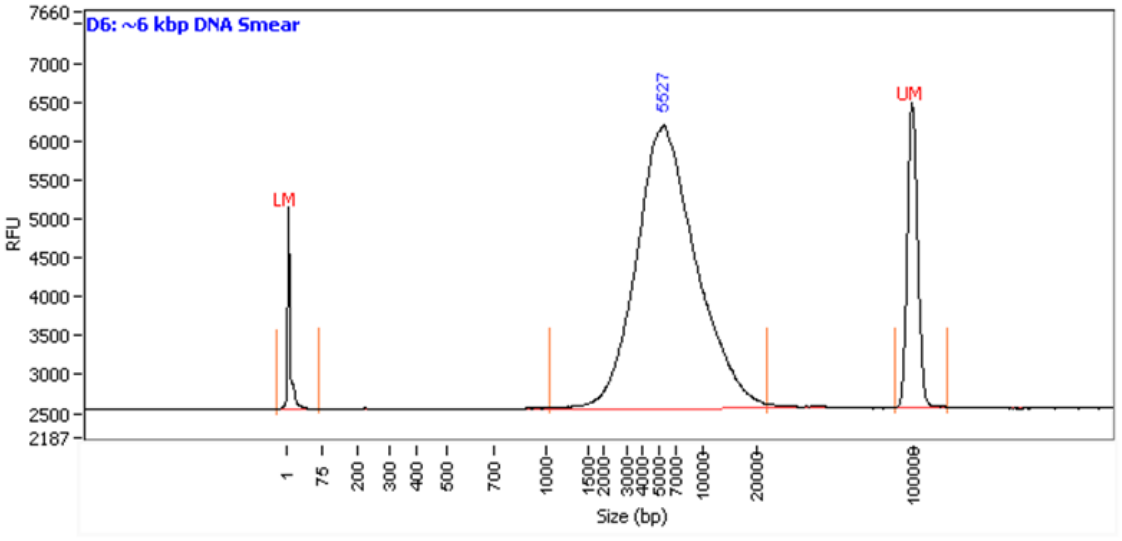


Figure 8. Large Fragment DNA Ladder result using the Fragment Analyzer system with the Large Fragment kit. Peaks annotated by size (bp).

DNA Smear Sample

Figure 9 shows a result for a DNA smear sample. In this example, a large size DNA smear sample was analyzed.



Peak Table

	Size (bp)	ng/uL	% (Conc.)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	5527	53.5050	100.0	15.937	1029	26012	6658	50.50
	TIC:	53.5050		15.937				
	Total Conc.	53.9142						

Figure 9. Representative large size DNA smear sample result using the Fragment Analyzer system with the Large Fragment kit. The measured concentration of this sample was 53.9 ng/µL via fluorometry; the measured concentration via the Fragment Analyzer was 53.5 ng/µL as shown in the TIC of the Peak Table.

5 Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-492 Large Fragment kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Table 9 Troubleshooting actions for assay specific issues

Issue	Cause	Corrective Action
The peak signal is >> 20,000 RFU; upper marker peak is low or not detected relative to lower marker.	1 Input DNA sample concentration too high. Ensure peak height does not exceed 2,000 RFU (smear) or 20,000 RFU (fragment), or total input concentration does not exceed recommended limits.	1 Dilute input DNA sample concentration with 1x TE buffer and repeat experiment; OR Repeat experiment using decreased injection time or voltage.
DNA sample smear overlaps with Lower/Upper Marker peak.	1 Input DNA sample size distribution outside of assay range. 2 Input DNA sample concentration too high.	1 Perform further size selection of sample to narrow DNA size distribution and repeat experiment; OR 2 Repeat experiment using DNF-487 Standard Sensitivity gDNA Analysis kit (uses lower marker only).
No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.	1 Sample concentration too low and out of range. 2 Sample not added to Diluent Marker solution or not mixed well.	1 Prepare more concentrated sample and repeat experiment; OR Repeat experiment using increased injection time and/or injection voltage; OR Prepare fresh sample and analyze with High Sensitivity Large Fragment Analysis kit (# DNF-493). 2 Verify sample was correctly added and mixed to sample well.
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of sample plate well, or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20 µL is required. 3 Capillary is plugged.	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 7 — Capillary Array Cleaning of the Fragment Analyzer User Manual for unclogging a capillary array.

In This Book

This Kit Guide describes the following:

- Agilent Large Fragment Kit
- Additional Material and Equipment Required
- Agilent Large Fragment Kit Protocol
- Checking Your Separation Results
- Troubleshooting

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