Agilent NGS Fragment Kit (1 – 6000 bp)

Kit Guide

For Research Use Only.

Not for use in diagnostic procedures.

NGS Fragment kit (1 – 6000 bp), 500 Samples (Part # DNF-473-0500)

NGS Fragment kit (1 – 6000 bp), 1,000 Samples (Part # DNF-473-1000)

Notices

Manual Part Number

M5310-91473

Edition 12/2018

Copyright

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051
USA

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

1 Agilent NGS Fragment Kit 4

2 Additional Material and Equipment Required 7

Material and Equipment Required for Analysis with the Fragment Analyzer System 7

Additional Equipment/Reagents Required (Not Supplied) 8

3 Agilent NGS Fragment Kit Analysis Protocol 9

Gel Preparation 9

Inlet Buffer Preparation 12

Capillary Conditioning Solution Preparation 12

Instrument Preparation 13

Marker/Ladder/Sample Preparation 14

General Information 14

Sample Plate Preparation 14

Performing Experiments 16

Running an Experiment 16

Viewing and Editing Experimental Methods 20

Processing Experimental Data 23

Fragment Analyzer Shut Down/Storage 24

Instrument Shut Down/Storage 24

4 Checking Your Separation Results 25

DNA Ladder 25

DNA Smear Sample 26

5 Troubleshooting 27

1 Agilent NGS 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	25 minutes (22-47 Array)[3] 50 minutes (33-55 Array) 80 minutes (55-80 Array)

Table 2 Analytical Specifications

Type	Specifications
DNA Sizing Range	100 bp – 6,000 bp
DNA Sizing Precision[1]	2% CV
DNA Sizing Accuracy[1]	± 5% or better
Separation Resolution	25 bp - 100 bp ≤ 10% ; 100 bp – 2,000 bp ≤ 5% 2,000 bp – 5,000 bp ≤10%
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]	15 % 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	NGS Separation Gel	5x Capillary Conditioning Solution
NGS Diluent Marker (DM) Solution (1bp-6000bp)	5x 930 dsDNA Inlet Buffer	
NGS 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 NGS Diluent Marker (DM) Solution is now 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.

NGS Fragment kit (1 – 6,000 bp), 500 Samples (Part # DNF-473-0500)

Table 4 kit Components

Part Number	Name	Amount
DNF-240-0240	NGS Separation Gel	240 mL
DNF-600	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-374-0003	NGS Diluent Marker (DM) Solution (1 – 6,000 bp) • Lower Marker (Set to 1 bp) and 6,000 bp Upper Marker	2.4 mL x 5 vials
DNF-399-U100	NGS DNA Ladder • Fragments from 100 bp – 3,000 bp; 25 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

NGS Fragment kit (1 – 6,000 bp), 1,000 Samples (Part # DNF-473-1000)

Table 5 kit Components

Part Number	Name	Amount
DNF-240-0500	NGS Separation Gel	500 mL
DNF-600	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-374-0003	NGS Diluent Marker (DM) Solution (1 bp - 6,000 bp) • Lower Marker (Set to 1 bp) and 6,000 bp Upper Marker	2.4 mL x 10 vials
DNF-399-U100	NGS DNA Ladder • Fragments from 100 bp – 3,000 bp; 25 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

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 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 Separation 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 NGS Fragment kit Analysis Protocol

Gel Preparation

1. Store the NGS 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 NGS Separation Gel and Intercalating Dye to room temperature prior to mixing.
4. Mix appropriate volumes of Intercalating Dye and NGS 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 NGS 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 NGS Separation Gel required per run varies between 12-capillary, 48-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.

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.

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

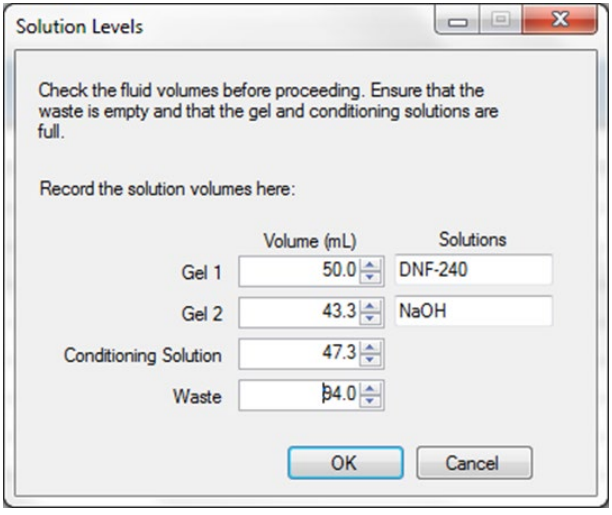


Figure 1 Solution Levels menu

7 When switching applications (e.g., between kits), prime the appropriate gel fluid line after loading fresh gel/dye mixture. From the main screen 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 purge the fluid line with fresh gel (Figure 2).

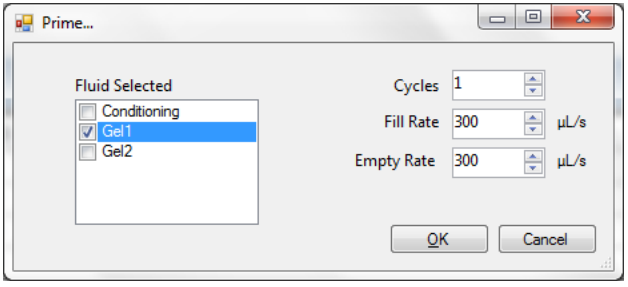


Figure 2. Prime menu

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 screen, 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 Sample 3 (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 C – 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 NGS Diluent Marker (DM) solution and NGS 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.

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 DNA smears should lie in an optimal range between 20 – 2000 RFUs. The peak heights for individual DNA fragments should lie in an optimal range between 100 – 20,000 RFUs.

3 Using a clean 96-well sample plate, pipette 22 µL of NGS 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 DNA Ladder: It is highly recommended to run NGS DNA Ladder in parallel with the samples.

   a Pipette 2 µL of NGS DNA Ladder into the 22 µL of NGS Diluent Marker (DM) Solution in Well 12 of each row to be analyzed (12-capillary system) or Well H12 (96-capillary system).

   b Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

NOTE

If you use an imported NGS DNA Ladder, the imported ladder must be prepared using the same lot # of NGS Diluent Marker Solution as the samples, to ensure proper quantification.

5 Pipette 2 µL of each DNA sample into the 22 µL of NGS Diluent Marker (DM) Solution in the respective wells of the Sample Plate; 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 screen 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.

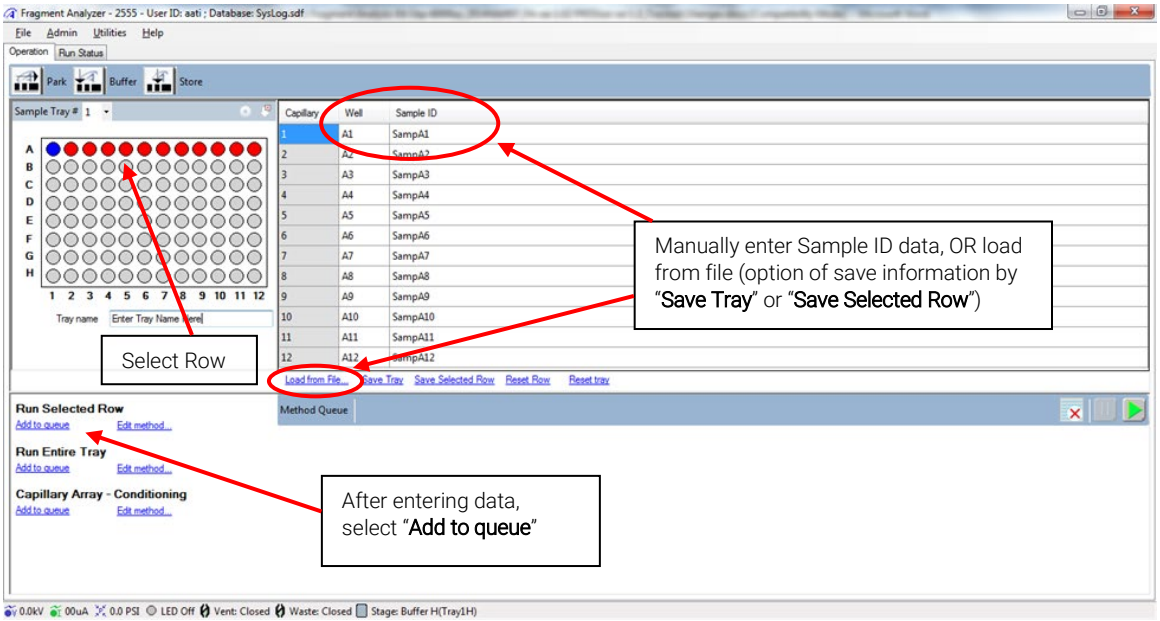


Figure 3. Main screen showing selection of sample row and entering sample information

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

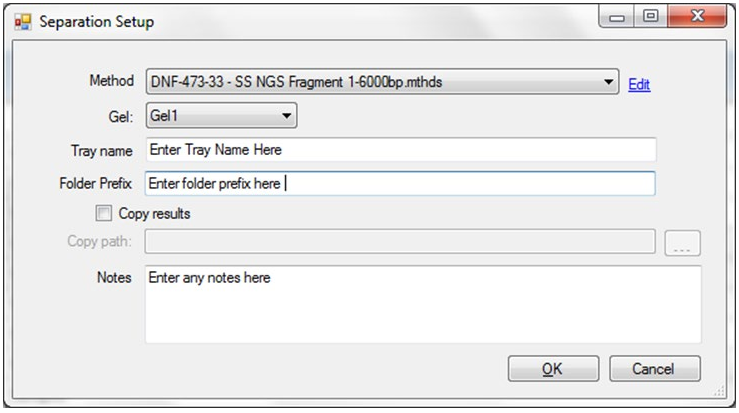


Figure 4. Separation Setup form to select experimental Method and enter tray/folder information

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-473-33 - SS NGS Fragment 1-6000bp.mthds when the 33 cm effective, 55 cm total "short" capillary array is installed.
   - Select DNF-473-55 - SS NGS Fragment 1-6000bp.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 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.

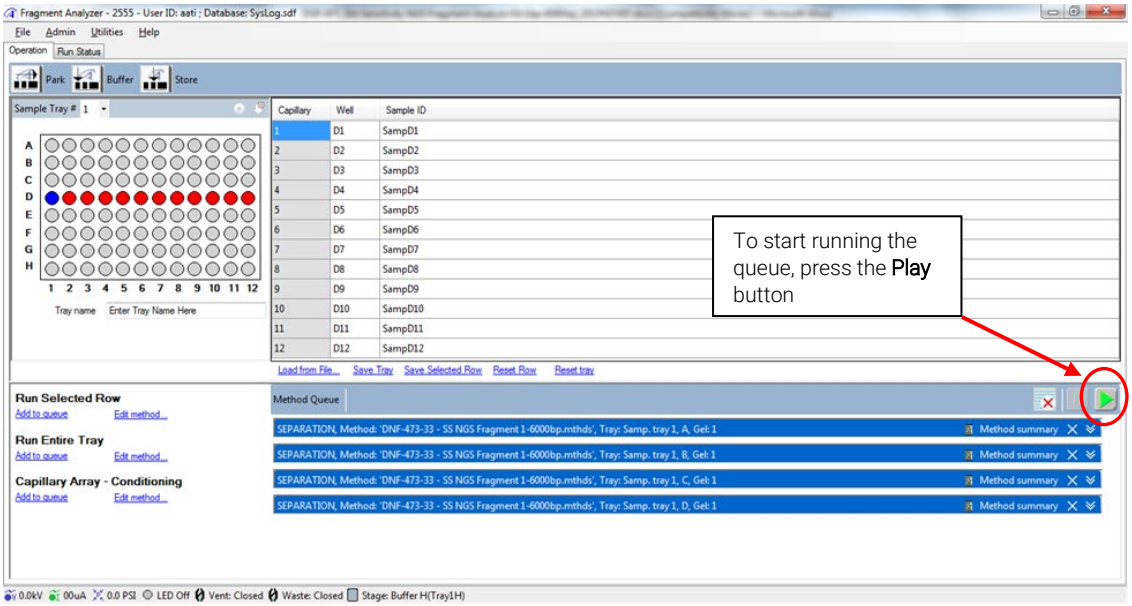


Figure 5. Main screen after selection of samples to the run queue

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-473-33 (Figure 6) and DNF-473-55 (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.

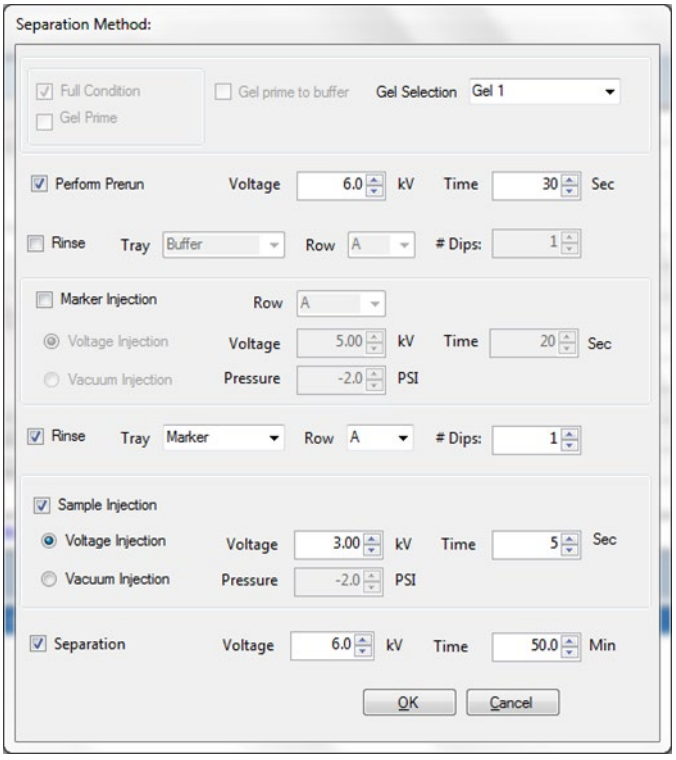


Figure 6. DNF-473-33 NGS Fragment kit (1bp – 6000 bp) method

4 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.

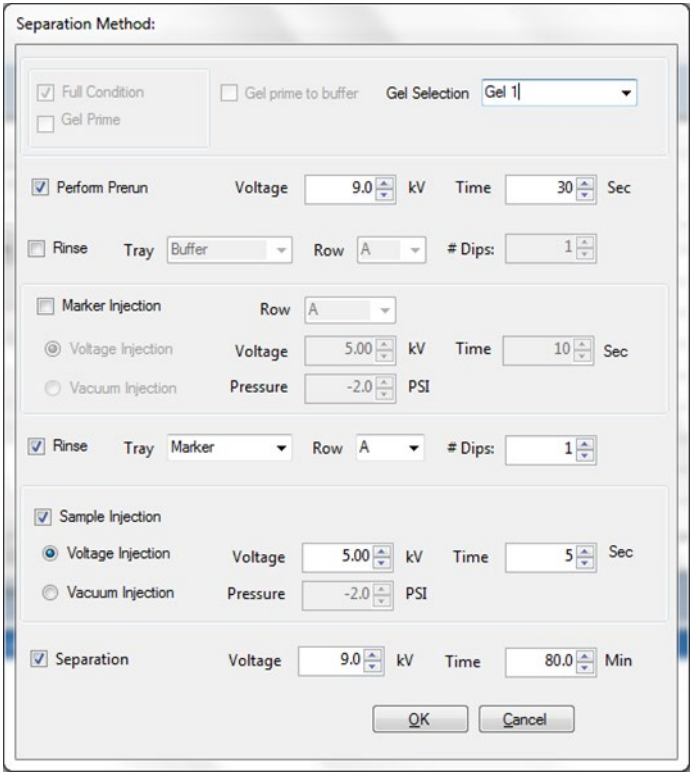


Figure 7. DNF-473-55 NGS Fragment kit (1bp – 6000bp) method

5 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.

6 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-473-33 separation method will be processed using the DNF-473-33 - SS NGS Fragment 1-6000bp configuration file.
- The DNF-473-55 separation method will be processed using the DNF-473-55 - SS NGS Fragment 1-6000bp configuration file.

NOTE

If the preloaded ProSize software configuration files "DNF-473-33 - SS NGS Fragment 1-6000bp " and "DNF-473-55 - SS NGS Fragment 1-6000bp" 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 6,000 bp), and calibrated to the NGS DNA Ladder run in parallel to the samples. Figure 8 shows an example of the 1 bp and 6,000 bp markers injected with the NGS DNA Ladder using the DNF-473-33 separation method. A total of 16 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 2.083 and a Dilution Factor of 12 (2 µL sample + 22 µL Diluent Marker). Note that if a pre-dilution was performed prior to the experiment, the Dilution Factor setting should be changed to accurately reflect the final sample concentration.

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 NGS DNA Ladder, provided at an initial total DNA concentration of 25 ng/µL in 1x TE buffer (2 µL + 22 µL DM solution; 1:12 dilution). A total of 16 peaks should be observed, with the sizes annotated as in Figure 8. All fragments in the ladder should be baseline resolved.

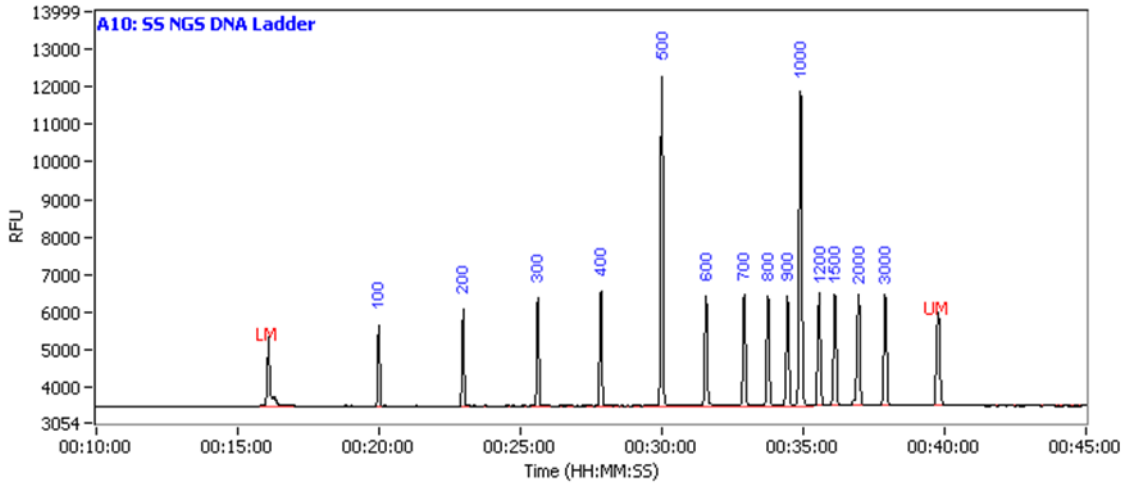
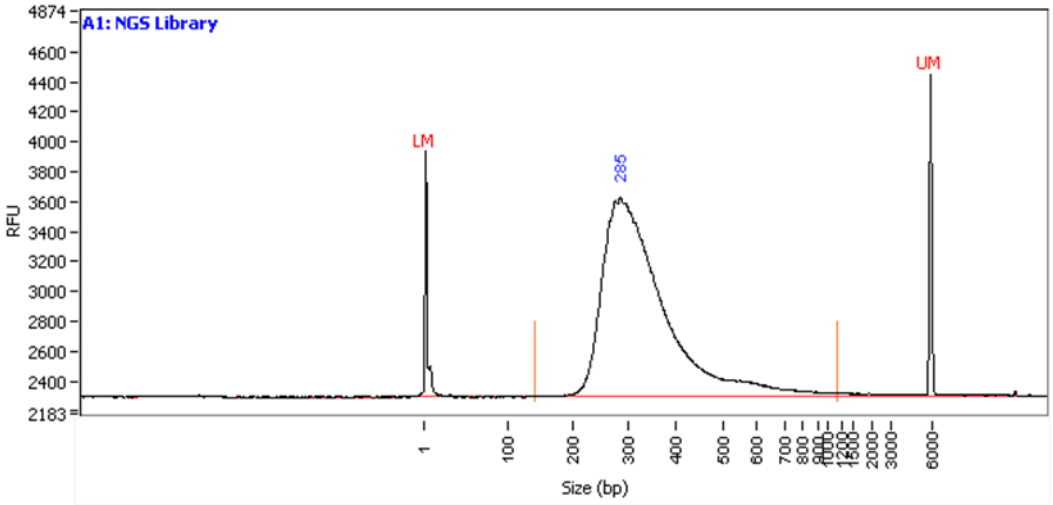


Figure 8. Representative NGS DNA Ladder result using the Fragment Analyzer system with the DNF-473 NGS Fragment kit (1bp – 6,000bp). Method: DNF-473-33 (short array). Peaks annotated by size (bp).

DNA Smear Sample

Figure 9 shows a typical result for a DNA smear sample. In this example, a next generation sequencing (NGS) DNA library sample was analyzed.



Peak Table

	Size (bp)	ng/uL	% (Conc.)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	285	35.3413	100.0	204.066	141	1149	343	31.24
	TIC:	35.3413		204.066				
	Total Conc.	36.2858						

Figure 9. Representative NGS DNA library sample result using the Fragment Analyzer system with the DNF-473 NGS Fragment kit (1bp – 6,000bp); peak annotated by size in bp. The measured concentration of this sample was 33.2 ng/µL via fluorometry; the measured concentration via the Fragment Analyzer was 36.2 ng/µL (< 10% difference) as shown in the Peak Table. The quantification setting was set to **Use Ladder** with a ladder **Total Conc. (ng/uL)** of 2.083 and **Dilution Factor** of 12.

5 Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the NGS Fragment kit (1 bp – 6,000 bp) 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 voltage (e.g., 2 kV).
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 Prepare fresh sample using Large Fragment kit (50bp – 20,000bp), (Part # DNF-492). 2 Dilute input DNA sample concentration with 1x TE buffer and repeat experiment.
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 HS NGS Fragment kit (1bp – 6,000bp), (Part # DNF-474). 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 NGS Fragment Kit
- Additional Material and Equipment Required
- Agilent NGS Fragment Kit Protocol
- Checking Your Separation Results
- Troubleshooting

www.agilent.com

Edition 12/2018

M5310-91473

Agilent