New Software Version

Refer to red highlighted text for additional updates

High Sensitivity NGS Fragment Analysis Kit (1 bp – 6000 bp)

User Guide

(DNF-474-0500)

(DNF-474-1000)

For use with the *Fragment Analyzer*™ Automated CE System

Fragment Analyzer™ Software Version 1.0.2

PROSize® 2.0 Software Version 1.3

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High Sensitivity NGS Fragment Analysis Kit (1 bp – 6000 bp), 500 Samples

Part # DNF-474-0500

Kit Components

1. NGS Fragment Separation Gel, 240 mL, Part # DNF-240-0240
2. Intercalating Dye, 30 µL, Part # DNF-600
3. 5X 930 dsDNA Inlet Buffer, 125 mL, (dilute with sub-micron filtered water prior to use), Part # DNF-355-0125
4. 5X Capillary Conditioning Solution, 50 mL, (dilute with sub-micron filtered water prior to use), Part # DNF-475-0050
5. High Sensitivity NGS Fragment Diluent Marker (DM) Solution (1bp-6000bp), 2.4 mL x 5 vials, Part # DNF-373-0003
   a. Lower Marker (Set to 1 bp) and 6000 bp Upper Marker
6. High Sensitivity NGS Fragment DNA Ladder, 100 µL, Part # DNF-396-U100
   a. Fragments from 100 bp – 3000 bp; 1000 pg/µL total DNA concentration
7. 0.25X TE Rinse Buffer, 125 mL, Part #DNF-497-0125
8. BF-25 Blank Solution, 8 mL, Part# DNF-300-0008

High Sensitivity NGS Fragment Analysis Kit (1 bp – 6000 bp), 1000 samples

Part # DNF-474-1000

Kit Components

1. NGS Fragment Separation Gel, 500 mL, Part # DNF-240-0500
2. Intercalating Dye, 30 µL x 2, Part # DNF-600
3. 5X 930 dsDNA Inlet Buffer, 300 mL, (dilute with sub-micron filtered water prior to use), Part # DNF-355-0300
4. 5X Capillary Conditioning Solution, 100 mL, (dilute with sub-micron filtered water prior to use), Part # DNF-475-0100
5. High Sensitivity NGS Fragment Diluent Marker (DM) Solution (1bp-6000bp), 2.4 mL x 10 vials, Part # DNF-373-0003
   a. Lower Marker (Set to 1 bp) and 6000 bp Upper Marker
6. High Sensitivity NGS Fragment DNA Ladder, 100 µL x 2, Part # DNF-396-U100
   a. Fragments from 100 bp – 3000 bp; 1000 pg/µL total DNA concentration
7. 0.25X TE Rinse Buffer, 125 mL, Part #DNF-497-0125
8. BF-25 Blank Solution, 8 mL, Part# DNF-300-0008

Specifications

Specifications	Description
Sample Volume Required	2 µL
Number of Samples per Run	12-Capillary : 11 (+ 1 well DNA Ladder) 96-Capillary: 95 (+ 1 well DNA Ladder)
Total Electrophoresis Run Time	50 minutes (33-55 Array); 80 minutes (55-80 Array)
DNA Sizing Range	25 bp – 5,000 bp
Separation Resolution	25 bp – 100 bp ≤ 10% ; 100 bp – 2000 bp ≤ 5% 2000 bp – 5000 bp ≤10%
DNA Sizing Accuracy[1]	± 5% or better
DNA Sizing Precision[1]	2% CV
DNA Fragment Concentration Range[1]	5 pg/µL – 500 pg/µL input DNA
DNA Smear Concentration Range[1]	50 pg/µL – 5000 pg/µL input DNA
DNA Quantification Accuracy[1]	± 25 %
DNA Quantification Precision[1]	15 % CV
Maximum DNA Concentration	500 pg/µL per fragment; 5000 pg/µL total

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

Storage Conditions

Store at 4°C (DO NOT FREEZE):	Store at –20°C:	Store at Room Temperature (DO NOT FREEZE):
NGS Fragment Separation Gel	Intercalating Dye	5X Capillary Conditioning Solution
5X 930 dsDNA Inlet Buffer	NGS Fragment Diluent Marker (DM) Solution (1bp-6000bp)	
BF-25 Blank Solution	NGS Fragment DNA Ladder	
0.25X TE Rinse Buffer		

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

NOTE: The NGS Fragment 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.

Additional Materials and Equipment Required

Hardware, Software, and Reagents available from AATI:

1. Hardware
   - *Fragment Analyzer*™ 12-capillary or 96-capillary CE system with LED fluorescence detection
   - 12-Capillary Array Cartridge (Fluorescence), 33 cm effective/55 cm total length, 50 µm ID (part # A2300-1250-3355) OR
   - 12-Capillary Array Cartridge (Fluorescence), 55 cm effective/80 cm total length, 50 µm ID (part # A2300-1250-5580) OR
   - 96-Capillary Array Cartridge (Fluorescence), 33 cm effective/55 cm total length, 50 µm ID (part # A2300-9650-3355) OR
   - 96-Capillary Array Cartridge (Fluorescence), 55 cm effective/80 cm total length, 50 µm ID (part # A2300-9650-5580)
2. Software
   - *Fragment Analyzer*™ instrument control software **(Version 1.0.2 or higher)**
   - *PROSize*® 2.0 data analysis software **(Version 1.3 or higher)**
3. Reagents
   - Capillary Storage Solution, 100 mL (AATI #GP-440-0100)

Equipment/Reagents to Be Supplied by User

1. 96-well PCR sample plates. Please refer to **Appendix C – Fragment Analyzer™ Compatible Plates and Tubes** in the *Fragment Analyzer*™ User Manual for a complete approved sample plate list.
2. Multichannel pipettor(s) and/or liquid handling device capable of dispensing 1 – 100 µL volumes (sample plates) and 1000 µL volumes (Inlet Buffer plate)
3. Pipette tips
4. 96-well plate centrifuge (for spinning down bubbles from sample plates)
5. Sub-micron filtered DI water system (for diluting the 5X 930 dsDNA Inlet Buffer and 5X Capillary Conditioning Solutions)
6. Fisherbrand 96 DeepWell 1mL Plate, Natural Polypropylene, part # 12-566-120 (Inlet Buffer and Waste plate)
7. Reagent Reservoir, 50 mL (VWR 82026-355 or similar) (for use in pipetting Inlet Buffer plates/sample trays)
8. Conical centrifuge tubes for prepared Separation Gel/Dye mixture and/or 1X Capillary Conditioning Solution
   a. 250 mL (for 96-Capillary instruments or larger volumes): Corning #430776, available from Fisher #05-538-53 or VWR #21008-771
   b. 50 mL (for 12-Capillary instruments or 50 mL volumes): BD Falcon™ #352070, available from Fisher #14-432-22 or VWR #21008-940
9. Clean graduated cylinder (for measurement of dsDNA Gel volume and dilution of 5X 930 dsDNA Inlet Buffer and 5X Capillary Conditioning Solution)

Safety

When working with chemicals, always follow standard safety guidelines such as wearing a suitable lab coat, disposable gloves, and protective eyewear. For more information about the specific reagents, please refer to the appropriate material safety data sheets (MSDSs) that can be obtained from the product supplier.

Fragment Analyzer™ Start Up / Instrument Preparation

Gel Preparation

1. Store the NGS Fragment 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 Fragment Separation Gel and Intercalating Dye to room temperature prior to mixing.
4. Mix appropriate volumes of Intercalating Dye and NGS Fragment Separation 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 NGS Fragment 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 maximum detection sensitivity applications, 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 Fragment Separation Gel required per run varies between 12-capillary and 96-capillary *Fragment Analyzer*™ systems. The volumes required are summarized below.

   For 12-capillary *Fragment Analyzer*™ systems:

# of samples to be analyzed	Volume of Intercalating dye	Volume of NGS Fragment Separation Gel
12	1.0 µL	10 mL[1]
24	1.50 µL	15 mL
36	2.0 µL	20 mL
48	2.50 µL	25 mL
96	4.50 µL	45 mL

[1]A 5 mL minimum volume should be initially added to the tube.

For 96-capillary *Fragment Analyzer*™ systems:

# of samples to be analyzed	Volume of Intercalating dye	Volume of NGS Fragment Separation Gel
96	4.0 µL	40 mL
192	8.0 µL	80 mL
288	12.0 µL	120 mL
384	16.0 µL	160 mL
480	20.0 µL	200 mL

6. Place the prepared 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*™ 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).

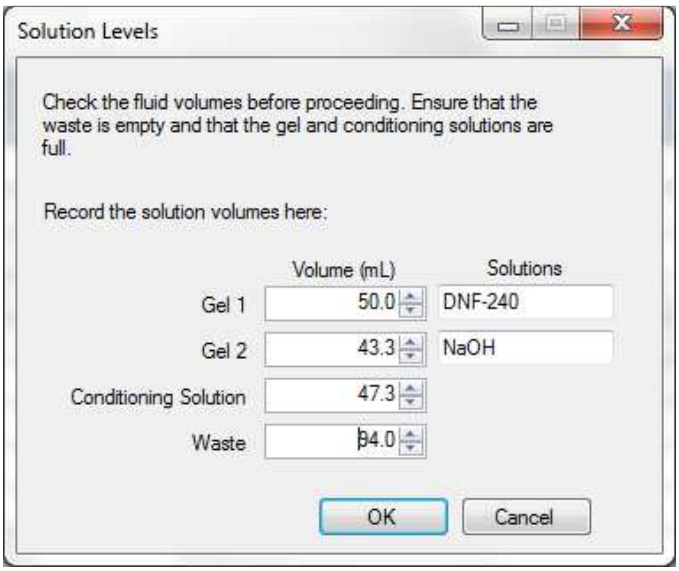


Figure 1. **Solution Levels** menu

8. When switching applications (e.g., between gel 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.

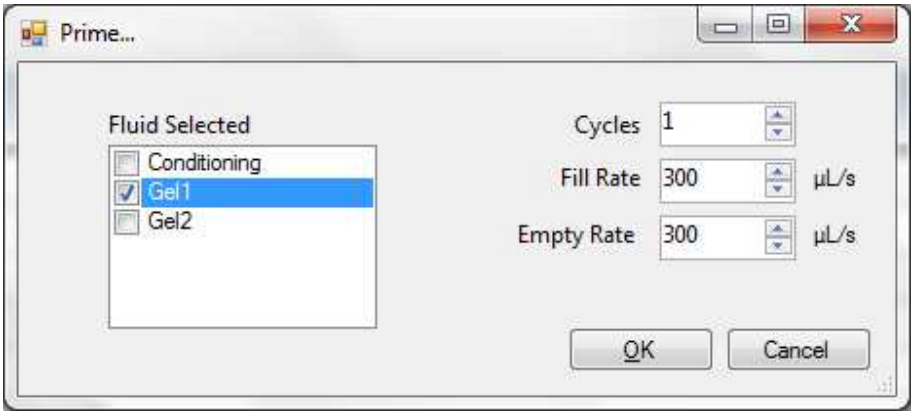


Figure 2. **Prime** menu

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. A typical 12-capillary experiment cycle consumes less than 4 mL; a typical 96-capillary experiment consumes less than 35 mL.
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.
3. 12-Capillary Systems: In Row H of the same prepared buffer plate, place 1.1 mL/well of Capillary Storage Solution (AATI # 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.
4. 96-Capillary Systems: In the Sample 3 drawer, place a sample plate filled with 100 µL/well of Capillary Storage Solution (AATI # 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.

IMPORTANT! 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.

5. 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.
6. 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.
7. Prepare a fresh sample plate filled with 100 µL/well of 0.25X TE Rinse Buffer daily. (12-Capillary System: Row A only; 96-Capillary System: All Rows).
8. Place the prepared 0.25X 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/Sample/Ladder 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 (#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. Plates with similar dimensions may be used, but note that capillary damage may occur with the use of poor quality PCR plates.

IMPORTANT! Contact AATI if a different vendor or style of PCR plate is to be used in order to verify compatibility. The use of PCR plates with different dimensions to the above recommended plate could possibly damage the tips of the capillary array cartridge.

2. Allow the NGS Fragment Diluent Marker (DM) solution and NGS 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.

Sample Plate Preparation

1. The total input DNA sample concentration **MUST be within a range of 5 pg/µL to 500 pg/µL (DNA fragment) or 50 pg/µL to 5000 pg/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.

IMPORTANT! 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 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-25 Blank Solution.

4. DNA Ladder: It is highly recommended to run NGS Fragment DNA Ladder in parallel with the samples. Pipette 2 µL of NGS Fragment DNA Ladder into the 22 µL of NGS Fragment Diluent Marker (DM) Solution in **Well 12 of each row to be analyzed** (12-capillary system) or **Well H12** (96-capillary system). Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

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

5. Pipette 2 µL of each DNA sample into the 22 µL of NGS Fragment 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.

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:

A. When adding 2 µL of sample or ladder to the 22 µL of diluent marker, **swirl the pipette tip** while pipetting up/down to further mix.

B. After adding 2 µL of sample or ladder to the 22 µL of diluent marker, place a plate seal on the sample plate and **vortex the sample plate at 3000 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.

C. **After adding 2 µL of sample or ladder** to the 22 µL of diluent marker, **use a separate pipette tip set to a larger 20 µL volume, and pipette each well up/down** to further mix.

D. Use an **electronic pipettor capable of mixing a 10 µL volume in the tip after dispensing the 2 µL sample or ladder volume**. Some models enable using the pipette tip for both adding/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*™ 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. 96-Capillary Systems: Note that 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.

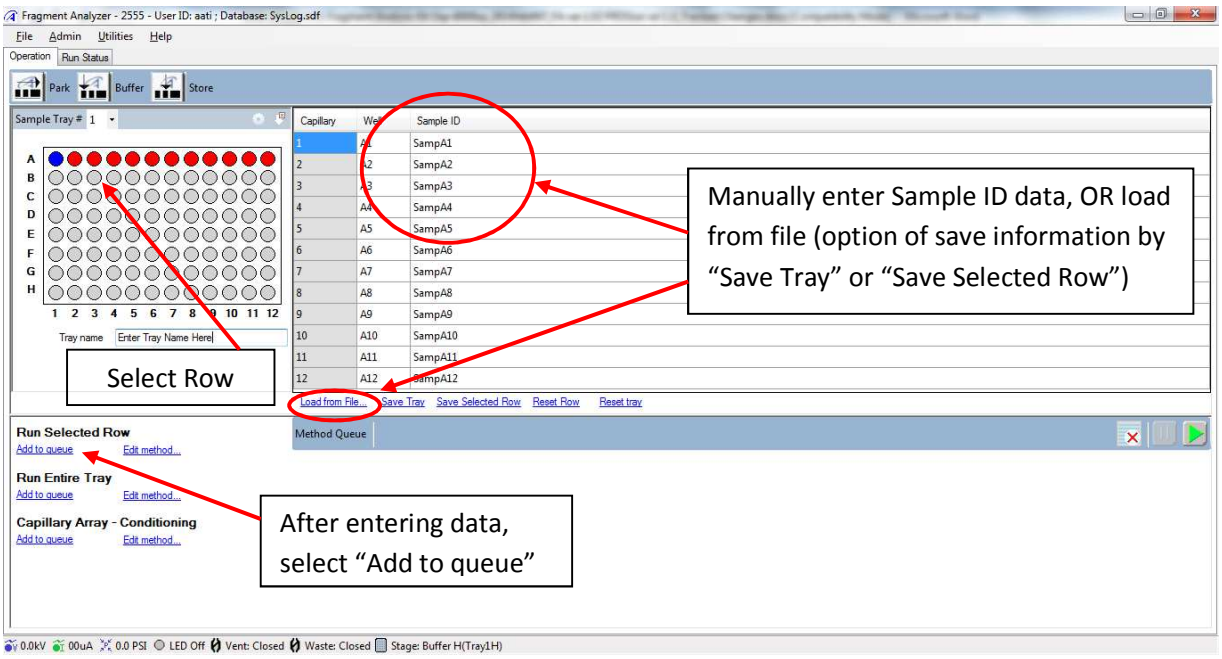


Figure 3. Main Screen showing selection of sample row and entering sample information

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).

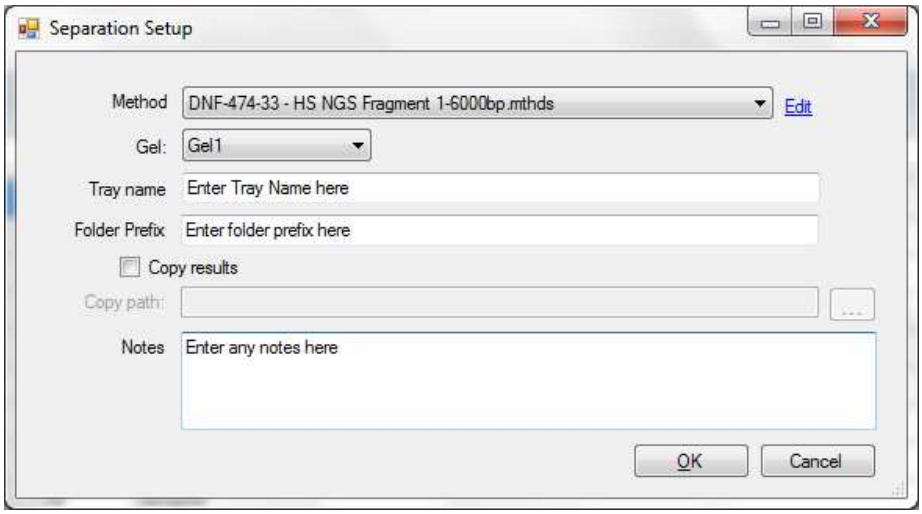


Figure 4. **Separation Setup** form to select experimental Method and enter tray/folder information

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:

    a. Select **DNF-474-33 - HS NGS Fragment 1-6000bp.mthds** when the 33 cm effective, 55 cm total "short" capillary array is installed;

    b. Select **DNF-474-55 - HS 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 (**`C:\AATI\Data`**), check the **Copy results** box and select the desired **Copy path:** directory by clicking the **…** button and navigating 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 3-9 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, rinse, waste, 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 runs press the  button.

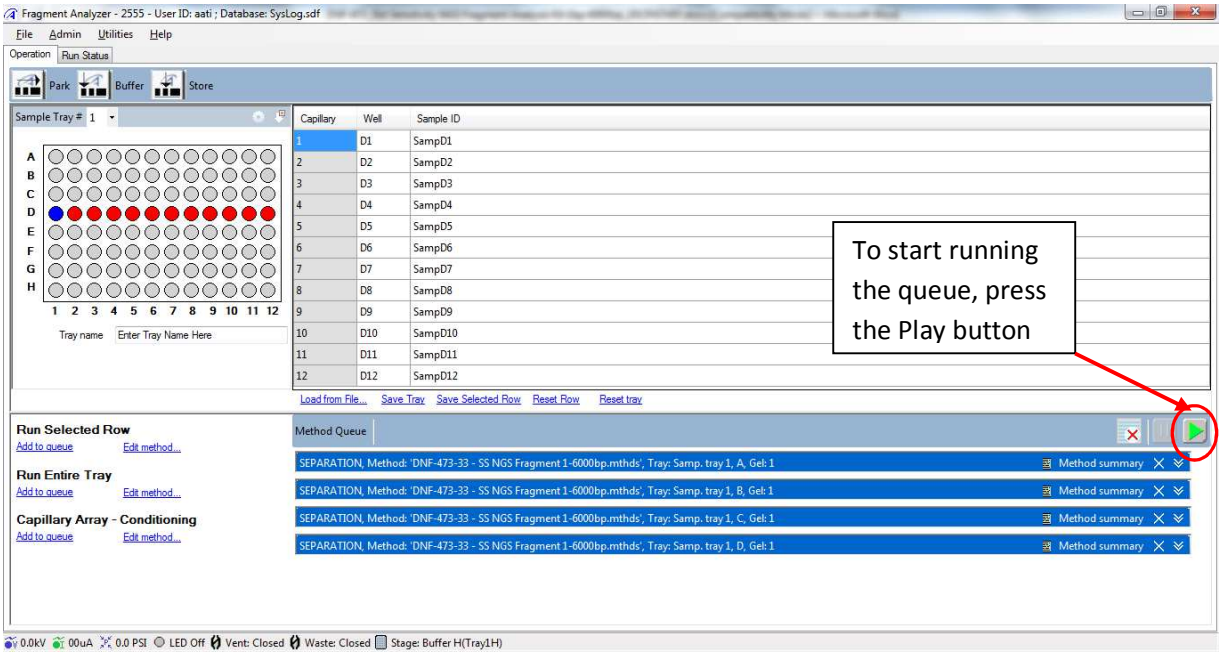


Figure 5. Main Screen after selection of samples to the run queue.

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-474-33** (Figure 6) and **DNF-474-55** (Figure 7) methods are shown below. The general steps of the method are as follows:

    1) **Full Condition** flushing method (Automatically enabled). Default Gel Selection: Gel 1.
    2) Perform Prerun (ENABLED) (6 kV, 30 sec)
    3) Rinse (DISABLED)
    4) Marker Injection (DISABLED)
    5) Rinse (ENABLED; Tray = Marker; Row = A; # Dips = 1). This step moves to the Marker tray and rinses the capillary tips with 0.25X TE Rinse Buffer.
    6) Sample Injection (ENABLED) Voltage Injection (5 kV, 30 sec). This step injects the prepared sample plate.
    7) Separation (ENABLED) Voltage (6 kV, 50 min). This step performs the CE Separation.

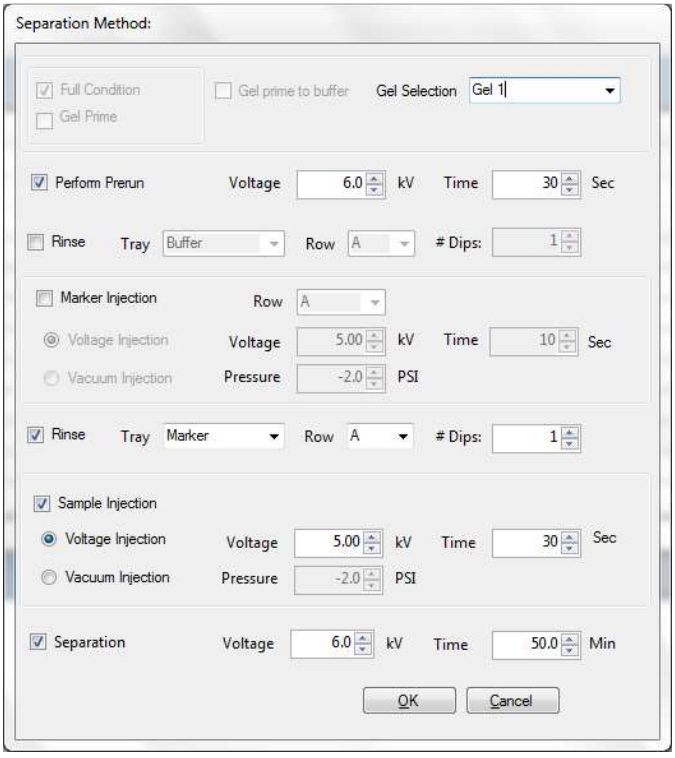


Figure 6. **DNF-474-33** High Sensitivity NGS Fragment Analysis Kit (1bp – 6000 bp) method

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 7.5 kV, and the **Separation** time to 80 min.

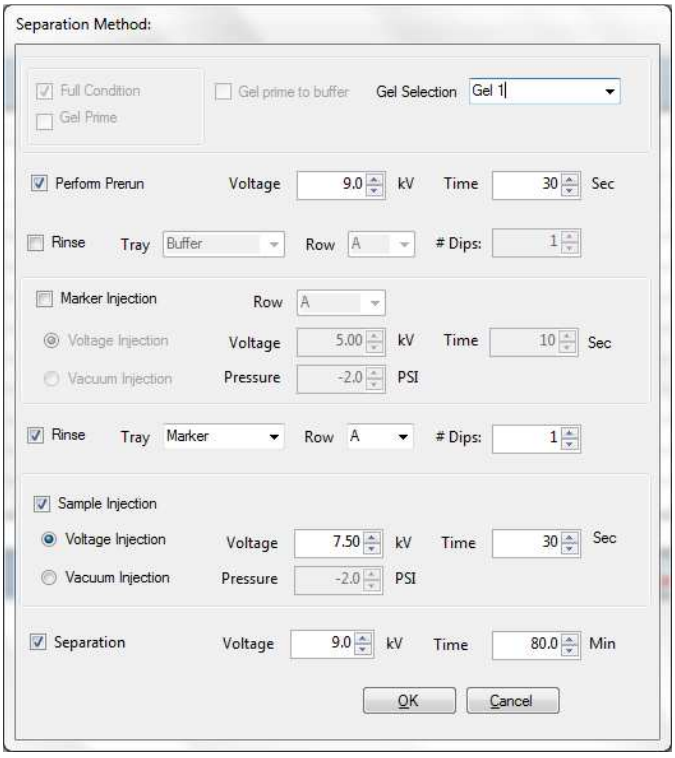


Figure 7. **DNF-474-55** High Sensitivity NGS Fragment Analysis Kit (1bp – 6000bp) method

5. An Administrator level user has the option to adjust the **Gel Selection**; **Prerun** settings; **Rinse** settings including **Tray**, **Row** and **# Dips**; **Sample Injection** settings; and the **Separation** settings. For example, if the rinse buffer 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.

IMPORTANT! 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*® 2.0 software (Version 1.3 and higher) will automatically recognize the separation method performed and apply the appropriate matching configuration file from the **C:\PROSize 2.0\Configurations** directory:

   a. The **DNF-474-33** separation method will be processed using the **DNF-474-33 - HS NGS Fragment 1-6000bp** configuration file;

   b. The **DNF-474-55** separation method will be processed using the **DNF-474-55 - HS NGS Fragment 1-6000bp** configuration file.

   NOTE: If the preloaded *PROSize*® 2.0 software configuration files "**DNF-474-33 - HS NGS Fragment 1-6000bp** " and "**DNF-474-55 - HS NGS Fragment 1-6000bp**" are not located in the **C:\PROSize 2.0\Configurations** directory, contact AATI 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 Fragment 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 Fragment DNA Ladder using the **DNF-474-33** separation method. A total of 16 peaks should be observed.

3. The *PROSize*® 2.0 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 **0.083** 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.

4. For full information on processing data, refer to the *PROSize*® 2.0 User Manual.

Fragment Analyzer™ Shut Down/Storage

Instrument Shut Down/Storage

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) after each experiment; no further action is required.

If the instrument is to be idle for more than one day, turn off power to the system to preserve lamp lifetime.

Typical Separation Results

DNA Ladder

1. Figure 8 shows the typical expected results for the High Sensitivity NGS Fragment DNA Ladder, provided at an initial total DNA concentration of 1000 pg/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 resolved.

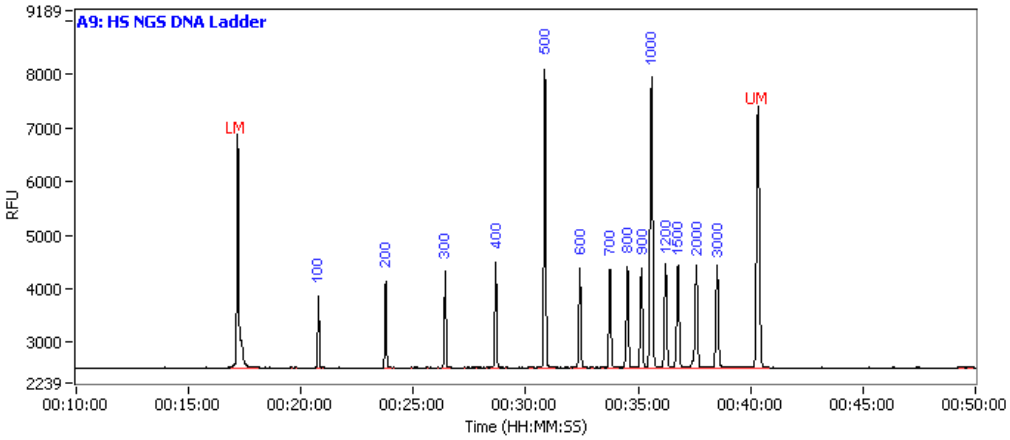
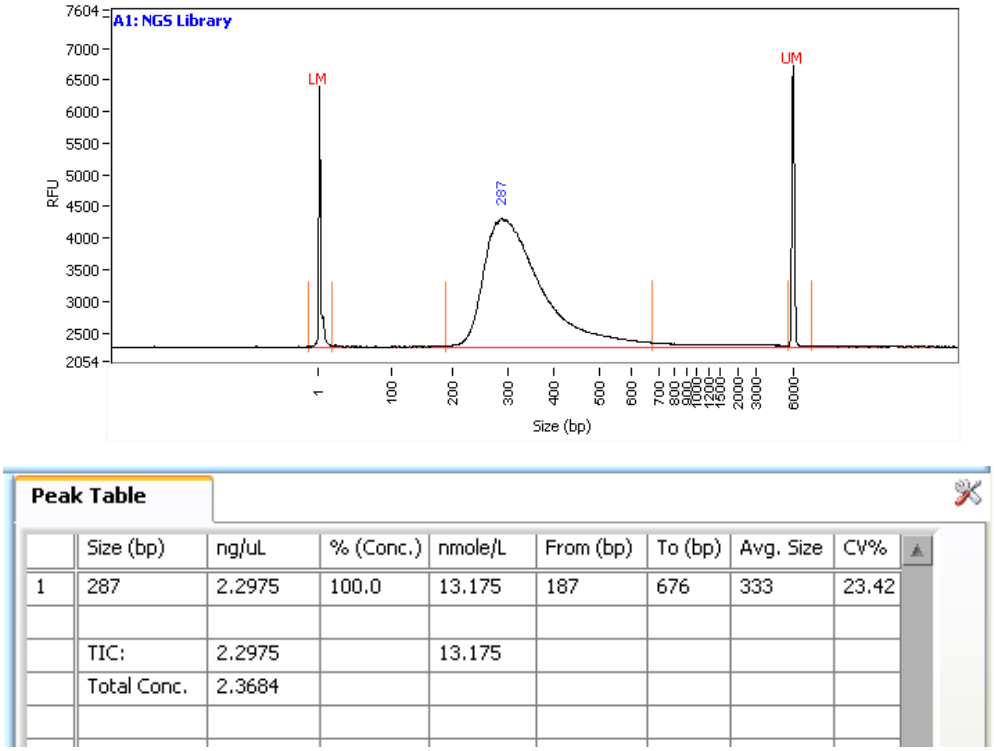


Figure 8. Representative NGS Fragment DNA Ladder result using the *Fragment Analyzer*™ system with the DNF-474 High Sensitivity NGS Fragment Analysis kit (1bp – 6000bp). Method: **DNF-474-33** (short array). Peaks annotated by size (bp).

DNA Smear Sample

1. Figure 9 shows a typical result for a DNA smear sample. In this example, a next generation sequencing (NGS) DNA library sample was analyzed. Note the similar intensity of the lower marker peak to the upper marker; this indicates that the input DNA concentration is within the specified input DNA concentration range.



Peak Table

	Size (bp)	ng/uL	% (Conc.)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	287	2.2975	100.0	13.175	187	676	333	23.42
	TIC:	2.2975		13.175				
	Total Conc.	2.3684						

Figure 9. Representative NGS DNA library sample result using the *Fragment Analyzer*™ system with the DNF-474 High Sensitivity NGS Fragment Analysis kit (1-6000bp); peak annotated by size in bp). The quantification setting was set to **Use Ladder** with a ladder **Total Conc. (ng/uL)** of 0.083 and **Dilution Factor** of 12.

Troubleshooting

The following section lists several potential assay specific issues which may be encountered when using the DNF-474 High Sensitivity NGS Fragment Analysis Kit (1bp – 6000bp) and suggested remedies. For a full list of instrument specific troubleshooting information, refer to the **Troubleshooting and Maintenance Guide** for the *Fragment Analyzer*™ system.

IMPORTANT NOTE: The DNF-474 High Sensitivity NGS Fragment Analysis Kit (1bp – 6000bp) is designed to work with low concentration DNA smears with **input concentrations from 50 pg/µL to 5 ng/µL**. If the input sample concentration is too high and out of range of this assay, the upper marker peak may be reduced in signal compared to the lower marker peak. If this is observed, it is recommended to either reduce the input sample concentration to within the kit range, or reduce the sample injection time.

Table 1. Troubleshooting

Issue	Cause	Corrective Action
A. The peak signal is >> 20,000 RFU; upper marker peak is low or not detected relative to lower marker.	1. Input DNA sample concentration is too high. Ensure total signal height does not exceed 20,000 RFU, or total input DNA concentration does not far exceed 5000 pg/µL.	Dilute input DNA sample concentration with 1X TE buffer and repeat experiment; OR Repeat experiment using decreased injection time (e.g., 10 sec); OR Prepare fresh sample using Standard Sensitivity NGS Fragment Analysis Kit (1bp – 6000bp), AATI # DNF-473, which covers input DNA range from 5 – 100 ng/µL.
B. 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 High Sensitivity Large Fragment Analysis Kit (50bp – 20,000bp), AATI #DNF-493. 2. Dilute input DNA sample concentration with 1X TE buffer and repeat experiment.
C. 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. 2. Verify sample was correctly added and mixed to sample well.

D. 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 G – Capillary Array Cleaning** of the *Fragment Analyzer*™ User Manual for unclogging a capillary array.

Technical Support

For questions with the *Fragment Analyzer*™ instrument operation or about the DNF-474 High Sensitivity NGS Fragment Analysis Kit (1bp – 6000bp), contact AATI Technical Support by phone at (515)-296-6600 or by email at support@aati-us.com.