Refer to **Revision History** for important updates or information

High Sensitivity Large Fragment 50Kb Analysis Kit

User Guide

(DNF-464-0500)

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

Fragment Analyzer™ Software Version 1.1

PROSize® 2.0 Software Version 2.0

Revised January 25, 2016

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Revision History

Revision Number	Date	Description of Change
DNF-464-2016JAN25	Jan 25, 2016	• Added new kit component: Dilution Buffer 1X TE, 60 mL (Part # DNF-495-0060). The 1X TE buffer is to be used when pre-diluting sample to the target concentration to maximize sizing accuracy, as described in Appendix A. • Added 22cm 12-capillary array method conditions. • Appendix A: Added optimal target concentration of 500 – 600 pg/µL, for pre-dilution of dsDNA fragment samples to maximize sizing accuracy.
DNF-464-2015NOV03	Nov 03, 2015	• New kit release

High Sensitivity Large Fragment 50Kb Analysis Kit (500 Samples)

Part # DNF-464-0500

Kit Components

1. Large Fragment Separation Gel, 240 mL, Part # DNF-220-0240
2. Intercalating Dye, 30 µL, Part # DNF-600-U030
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 Large Fragment Diluent Marker (DM), 2.4 mL x 5 vials, Part # DNF-381-0003
   a. Lower Marker (Set to 1 bp) and Upper Marker (set to 200,000 bp)
6. High Sensitivity Extended Large Fragment DNA Ladder, 125 µL, Part # DNF-365-U125
   a. Fragments from 75 bp – 48,500 bp; 1.5 ng/µL total DNA concentration (aliquot prior to use)
7. 0.25X TE Rinse Buffer, 125 mL, Part #DNF-497-0125
8. Dilution Buffer 1X TE, 60 mL, Part # DNF-495-0060
9. BF-25 Blank Solution, 8 mL, Part # DNF-300-0008
10. Eppendorf LoBind® 0.5 mL Tubes, Package of 50

Application

The High Sensitivity Large Fragment 50Kb Analysis Kit is designed for the sizing and quantification of medium to high molecular weight dsDNA smears/fragments. Example applications include quality control of long read Next Generation Sequencing (NGS) libraries (e.g., PacBio).

Kit Specifications

Specifications	Description
Sample Volume Required	2 µL
Number of Samples per Run	12-Capillary: 11 (+ 1 well DNA Ladder) 48-Capillary: 47 (+ 1 well DNA Ladder) 96-Capillary: 95 (+ 1 well DNA Ladder)
Total Electrophoresis Run Time	35 min (22-47 Array)[3]; 55 min (33-55 Array)
DNA Sizing Range	75 bp – 48,500 bp
DNA Sizing Accuracy[1]	± 15% or better
DNA Fragment Concentration Range[2]	5 pg/µL – 600 pg/µL input DNA (optimal concentration 500-600 pg/µL) per fragment.
DNA Smear Concentration Range[2]	50 pg/µL - 5 ng/µL input DNA (optimal concentration of 1 ng/µL)
DNA Quantification Accuracy[2]	± 25 %
DNA Quantification Precision[2]	20 % CV
Maximum DNA Concentration	600 pg/µL per fragment; 5 ng/µL per total sample

[1] Results using DNA Fragment standards at about 600 pg/µL and DNA smears at about 1 ng/µL initially prepared in 1X TE buffer.

[2] Results using DNA Fragment standards and DNA smears prepared in 1X TE buffer.

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

Storage Conditions

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

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

IMPORTANT:

***The Lambda DNA fragment (48,500 bp) in the High Sensitivity Extended Large Fragment DNA Ladder is sensitive to degradation.** The Ladder should be kept at 4°C. Do not pipette the ladder up and down; vortex with care. Before using the kit, please read and follow the ladder handling instructions below (page 9).

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.

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), 22 cm effective/47 cm total length, 50 µm ID (part # A2300-1250-2247) OR
   - 12-Capillary Array Cartridge (Fluorescence), 33 cm effective/55 cm total length, 50 µm ID (part # A2300-1250-3355) OR
   - 48-Capillary Array Cartridge* (Fluorescence), 33 cm effective/55 cm total length, 50 µm ID (part # A2300-4850-3355) OR
   - 96-Capillary Array Cartridge (Fluorescence), 33 cm effective/55 cm total length, 50 µm ID (part # A2300-9650-3355)
2. Software
   - *Fragment Analyzer*™ instrument control software **(Version 1.0.2.9 or higher)***
   - *PROSize*® 2.0 data analysis software **(Version 1.3.1.1 or higher)***
3. Reagents
   - Capillary Storage Solution, 100 mL (AATI #GP-440-0100)

* 48-Capillary Array Cartridge requires *Fragment Analyzer*™ instrument control software Version 1.1.0.7 or higher and *PROSize*® 2.0 data analysis software Version 2.0.0.40 or higher. Contact AATI Technical Support for further information.

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 Separation Gel volume and dilution of 5X dsDNA Inlet Buffer and 5X Capillary Conditioning Solution)

Safety

When working with chemicals, always follow usual 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 Safety Data Sheets (SDSs) that can be obtained from the product supplier.

SDS information for AATI products can be found online at: http://www.aati-us.com/sds-sheets

Fragment Analyzer™ Start Up / Instrument Preparation

Gel Preparation

1. Store the 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 **one day** 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.
5. The volume of Separation Gel required per run varies between 12-capillary, 48-capillary and 96-capillary *Fragment Analyzer*™ systems. The volumes required are summarized below.

For 12-capillary *Fragment Analyzer*™ systems:

# of samples to be analyzed[1]	Volume of Intercalating dye	Volume of Separation Gel
12	1.0 µL	10 mL
24	1.5 µL	15 mL
36	2.0 µL	20 mL
48	2.5 µL	25 mL
96	4.5 µL	45 mL

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

For 48-capillary *Fragment Analyzer*™ systems:

# of samples to be analyzed[1]	Volume of Intercalating dye	Volume of Separation Gel
48	2.5 µL	25 mL
96	4.0 µL	40 mL
144	5.5 µL	55 mL
192	7.0 µL	70 mL
240	8.5 µL	85 mL
288	10.0 µL	100 mL

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

For 96-capillary *Fragment Analyzer*™ systems:

# of samples to be analyzed[1]	Volume of Intercalating dye	Volume of 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

[1] Typically 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 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).

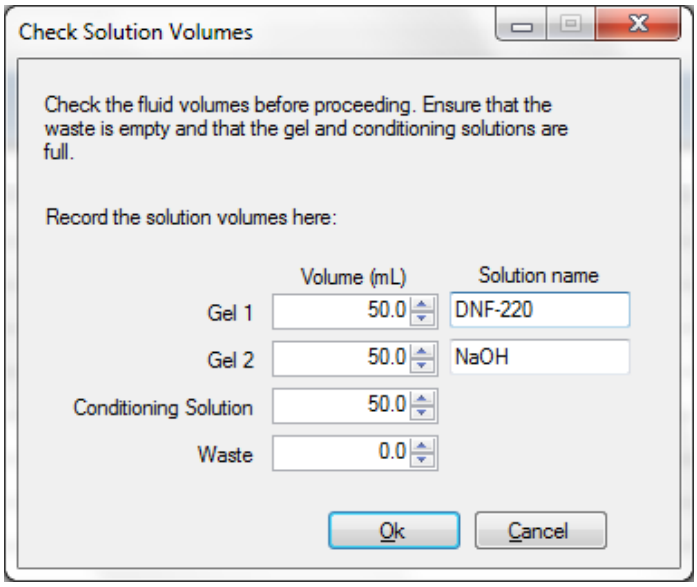


Figure 1. **Solution Levels** menu

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

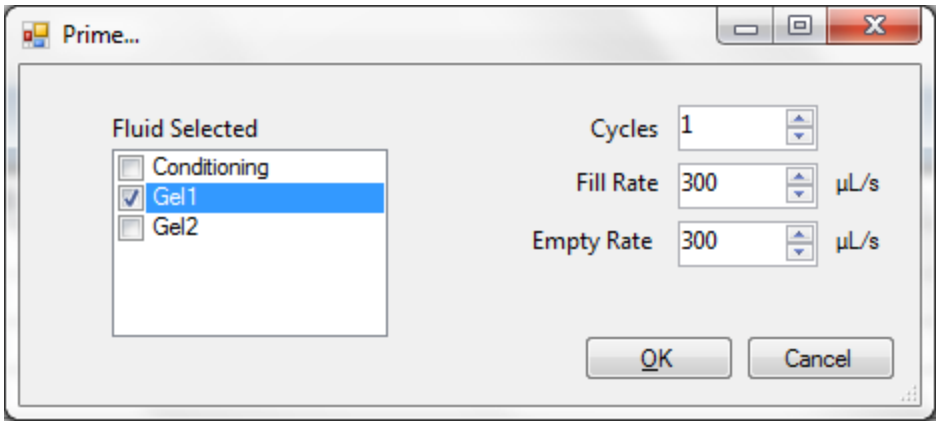


Figure 2. **Prime** menu

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 about 4 mL; a typical 48-capillary experiment consumes about 15 mL; and a typical 96-capillary experiment consumes about 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; 48-Capillary System: Row A to Row D; 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 **1.0 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. 48-Capillary System: In the Sample 3 drawer, place a sample plate filled with **100 µL/well** of Capillary Storage Solution (AATI # GP-440-0100) in Row A to Row D. Row A to Row D of the Sample 3 is used for the **Store** location, and the array moves to this position at the end of the experimental sequence.
5. 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 (48-Capillary and 96-Capillary Systems) is always filled with Capillary Storage Solution, and the capillary array is placed against the Storage Solution when not in use, to prevent the capillary tips from drying out and potentially plugging.

6. 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.
7. 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.
8. Prepare a fresh sample plate filled with **200 µL/well** of 0.25X TE Rinse Buffer **daily**. (12-Capillary System: Row A only; 48-Capillary System: Row A to Row D; 96-Capillary System: All Rows).
9. 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 High Sensitivity Large Fragment Diluent Marker (DM) solution and Extended High Sensitivity 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 High Sensitivity Large Fragment DM at room temperature for any longer than necessary when preparing samples.

Ladder Handling and Storage

1. Before using the kit, aliquot the High Sensitivity Extended Large Fragment DNA Ladder into 10 µL aliquots (12 tubes), using the Eppendorf LoBind® 0.5 mL tubes provided in the kit.
2. Label the aliquots and **store at 4°C**. Do not store the Ladder frozen.
3. Before use, equilibrate the ladder aliquots to room temperature for about 30 min. Do not pipette the ladder aliquots up and down, nor flick the tube to mix, as this may induce degradation of lambda DNA ladder fragment.
4. Gently vortex the aliquot and spin the ladder tube prior to use. Each 10 µL aliquot is good for 4-times use (2 µL per use).

Sample Plate Preparation

1. The total input DNA sample concentration **MUST be within a range of 5 pg/µL to 600 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 the provided 1X TE buffer (Part # DNF-495) 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 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. For maximum sizing accuracy and sizing reproducibility of DNA smears, it is highly recommended to first normalize sample concentration to a total concentration of 1 ng/µL prior to analysis, using the supplied 1X TE Buffer (Part # DNF-495). Refer to **Appendix A – Maximum Sizing Accuracy Alternate Workflow** for further sample preparation information.

4. 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-25 Blank Solution.

5. 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.

6. DNA Ladder: The Extended 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**. Refer to the Ladder Handling and Storage section above before working with the High Sensitivity Extended Large Fragment DNA Ladder.

   12-Capillary Systems: Pipette 2 µL of Extended Large Fragment DNA Ladder **into well 12 of each row** of the sample plate containing 22 µL of Large Fragment Diluent Marker (DM) solution.

   48-Capillary Systems: Pipette 2 µL of Extended Large Fragment DNA Ladder **into well D12** when analyzing Row A to Row D, or **into well H12** when analyzing Row E to Row H of the sample plate containing 22 µL of Large Fragment Diluent Marker (DM) solution.

   96-Capillary Systems: Pipette 2 µL of Extended Large Fragment DNA Ladder **into well H12** of the sample plate containing 22 µL of Large Fragment Diluent Marker (DM) solution.

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.

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 (we recommend two vortexing pulses, 1 min each)**. 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.

7. 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.

8. 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).

9. 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. 48-Capillary or 96-Capillary Systems: Note that Sample 3 is typically assigned to the Capillary Storage Solution.

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. 48-Capillary or 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.

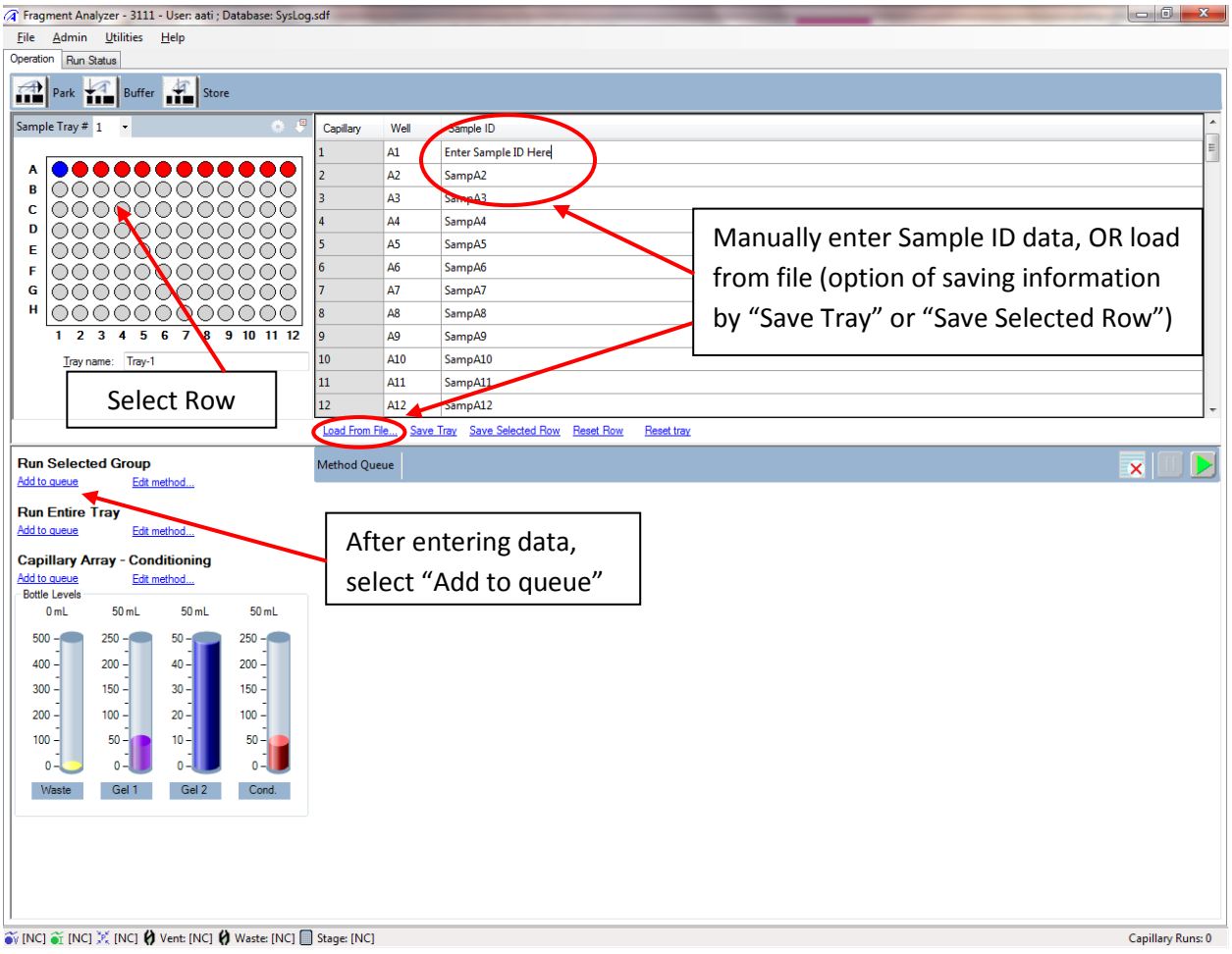


Figure 3. Main Screen showing selection of sample row and entering sample information

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

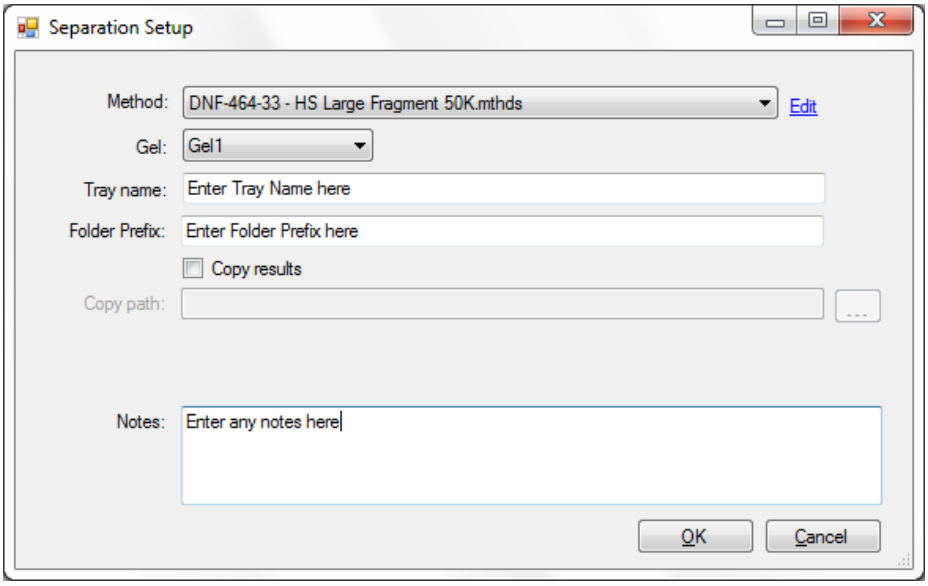


Figure 4. **Separation Setup** form to select experimental Method and enter tray/folder information

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., 22cm or 33cm). Select the following method:

    a. Select **DNF-464-22 – HS Large Fragment 50Kb.mthds** when the 22 cm effective, 47 cm total length "ultra-short" capillary array is installed;

    b. Select **DNF-464-33 – HS Large Fragment 50Kb.mthds** when the 33 cm effective, 55 cm total length "short" 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 1-9 for any remaining sample rows to be analyzed.

11. On 96-capillary systems, or in 12-capillary or 48-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.

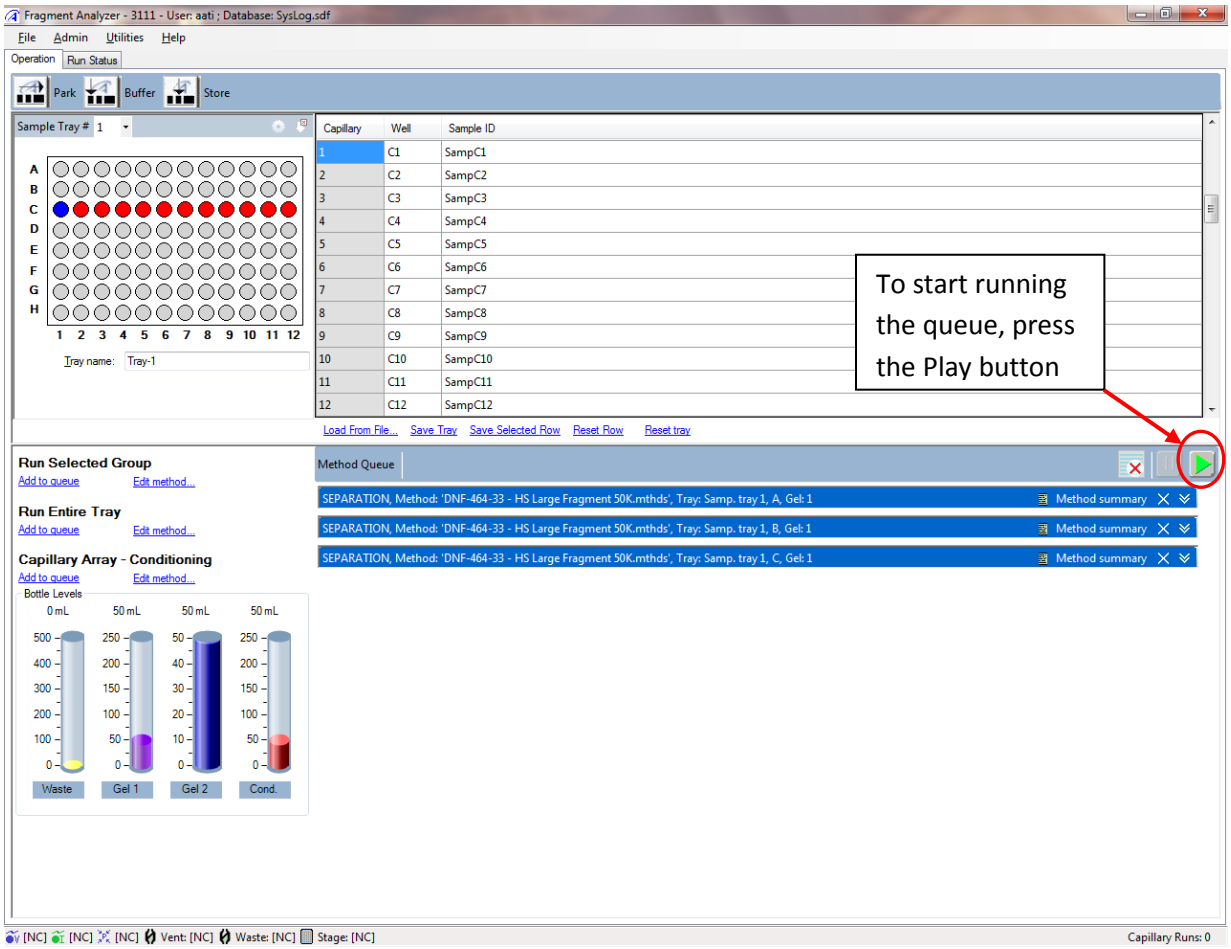


Figure 5. Main Screen after selection of samples to the run queue.

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 performs all 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 stage will automatically move to the **Store** location (12-Capillary Systems: Row H of the inlet buffer tray containing the Capillary Storage Solution; 48-Capillary and 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-464-22** (Figure 6) and **DNF-464-33** (Figure 7) methods are shown below. The **DNF-464-22** method steps are:

    1) **Full Condition** flushing method (Automatically enabled). Default Gel Selection: Gel 1.
    2) Perform Prerun (ENABLED) (5 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 (4.5 kV, 30 sec). This step injects the prepared sample plate.
    7) Separation (ENABLED) Voltage (5 kV, 35 min). This step performs the CE Separation

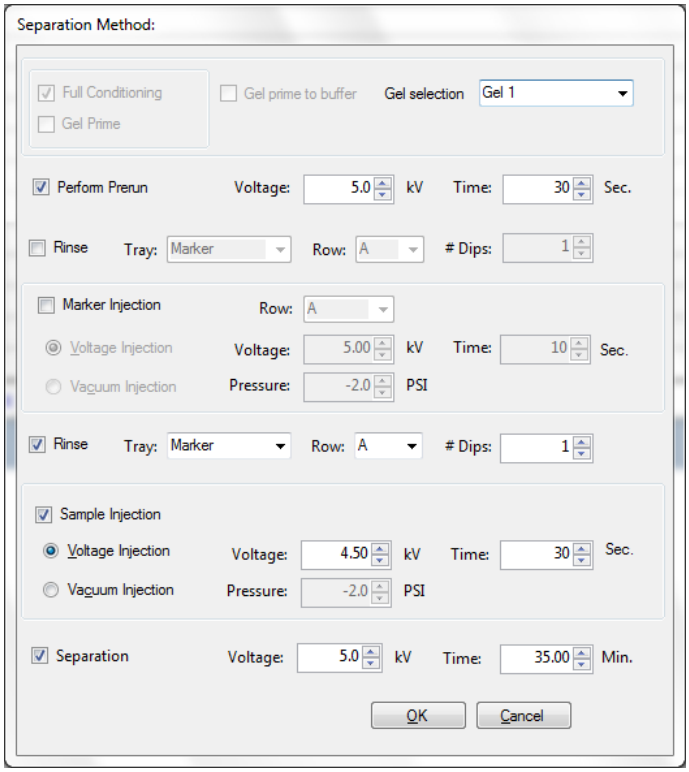


Figure 6. **DNF-464-22** High Sensitivity Large Fragment 50Kb Analysis Kit method

4. Figure 7 shows the preloaded method for the 33 cm effective, 55 cm total length "Short" array. The **Prerun** and **Separation** voltage is set to 5 kV, the **Injection** voltage to 5 kV, and the **Separation** time to 55 min.

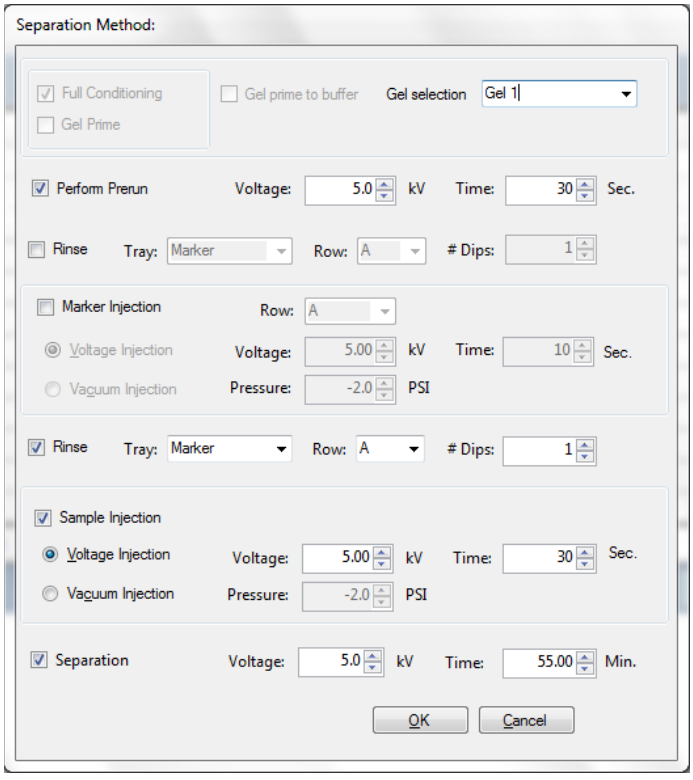


Figure 7. **DNF-464-33** High Sensitivity Large Fragment 50Kb Analysis Kit method

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-464-22** separation method will be processed using the **DNF-464-22 – HS Large Fragment 50Kb** configuration file.

   b. The **DNF-464-33** separation method will be processed using the **DNF-464-33 – HS Large Fragment 50Kb** configuration file.

   NOTE: If the preloaded *PROSize*® 2.0 software configuration files "**DNF-464-22 – HS Large Fragment 50Kb**" and "**DNF-464-33 – HS Large Fragment 50Kb**" are not located in the **`C:\PROSize 2.0\Configurations`** directory, contact AATI Technical Support to obtain these files.

7. The data is normalized to the lower marker (set to 1 bp) and upper marker (arbitrarily set to 200,000 bp), and calibrated to the High Sensitivity Extended Large Fragment DNA Ladder run in parallel to the samples. Figure 8 shows an example of the 1 bp and 200,000 bp markers injected with the Extended Large Fragment Ladder using the **DNF-464-33** separation method. A total of 14 peaks should be observed.

8. 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 **0.125** 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.

9. 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; 48-Capillary and 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 Extended Large Fragment DNA Ladder, provided at an initial total DNA concentration of 1.5 ng/µL in 1X TE buffer (2 µL + 22 µL DM solution; 1:12 dilution). A total of 14 peaks should be observed, with the sizes annotated as in Figure 8. All fragments in the ladder should be well resolved.

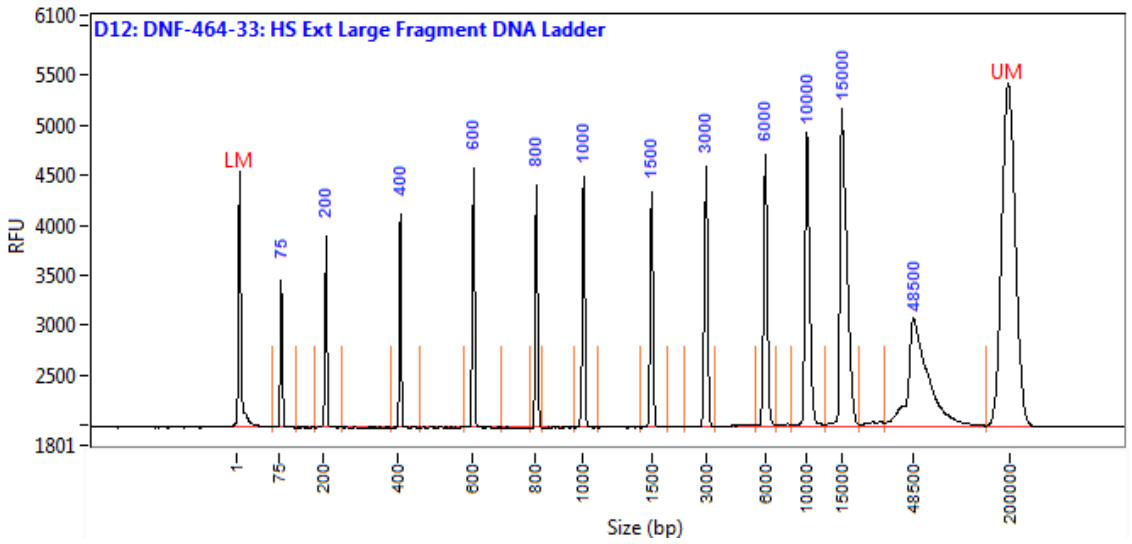
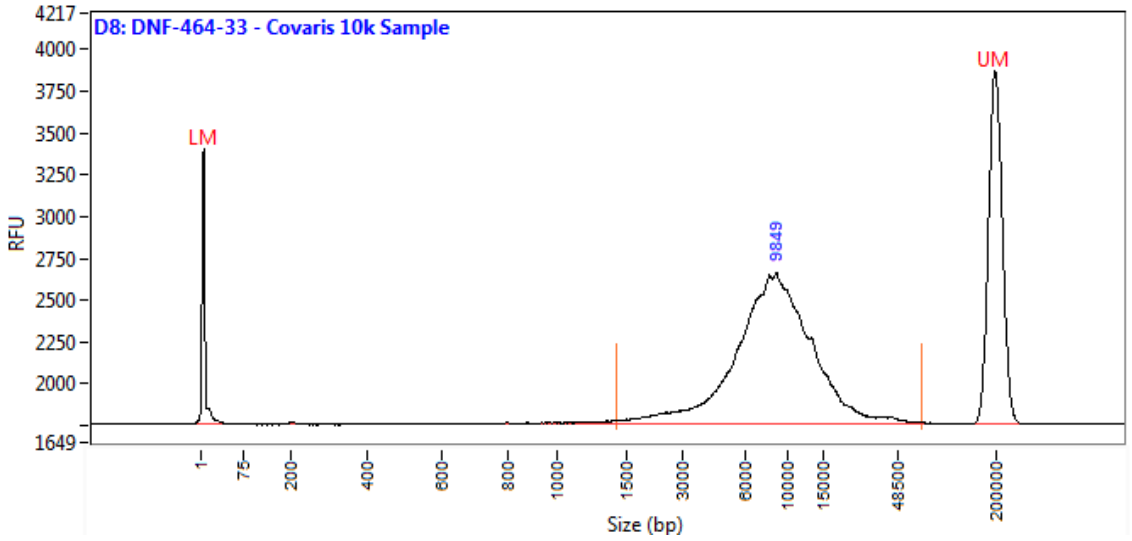


Figure 8. High Sensitivity Extended Large Fragment DNA Ladder result, using the *Fragment Analyzer*™ system with the DNF-464 High Sensitivity Large Fragment 50Kb Analysis kit. Peaks are annotated by size (bp). Method: **DNF-464-33** (33cm "short" array).

1. Figure 9 shows a result for a DNA smear sample analyzed by the DNF-464 High Sensitivity Large Fragment 50Kb Analysis Kit. In this example, a large size DNA smear sample, sheared to approximately 10 kb, was analyzed. The sample concentration was initially normalized to approximately 1 ng/µL concentration, following the protocol outlined in **Appendix A – Maximum Sizing Accuracy Alternate Workflow** (1.05 ng/µL as measured by Qubit post dilution).

   The measured concentration by the *Fragment Analyzer*™ was 1.14 ng/µL, as shown in the **Total Conc.** field of the Peak Table result. The **Average Size** was calculated to be 9849 bp, in good agreement with the expected average sheared size.



Peak Table

	Size (bp)	ng/uL	% (Conc.)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	8957	1.1256	100.0	0.207	1424	87805	9849	69.44
	TIC:	1.1256	ng/uL					
	TIM:	0.207	nmole/L					
	Total Conc.:	1.1405	ng/uL					

Figure 9. Representative large size DNA smear sample result using the *Fragment Analyzer*™ system with the DNF-464 High Sensitivity Large Fragment 50Kb Analysis kit. The sample was diluted to approximately 1 ng/µL concentration (1.05 ng/µL verified by Qubit). The measured concentration by the *Fragment Analyzer*™ was 1.14 ng/µL, as shown in the **Total Conc.** field of the Peak Table. The sample is annotated by **Average Size;** the Average Size for the smear was calculated to be 9849 bp, in good agreement with the expected average sheared size. Method: **DNF-464-33** (33cm "short" array).

Appendix A: Maximum Sizing Accuracy Alternative Workflow

The sizing of dsDNA fragments during electrophoresis can be sensitive to sample concentration, with higher concentration samples generally running faster than lower concentration samples. This phenomena is more pronounced for high molecular weight dsDNA fragments or smears, such as genomic DNA and large fragment NGS libraries used for long read sequencing applications.

To maximize the sizing accuracy and reproducibility of large molecular weight dsDNA samples, and better enable sample to sample sizing comparisons, it is highly recommended to first normalize the sample concentration prior to performing the analysis.

The High Sensitivity Extended Large Fragment DNA Ladder concentration and the method employed in the DNF-464 High Sensitivity Large Fragment 50Kb Analysis Kit has been optimized to provide high sizing accuracy for **dsDNA smears** when the total sample concentration is normalized to a target concentration of **1 ng/µL** prior to analysis.

For **dsDNA fragments**, a target concentration of **500 – 600 pg/µL** is recommended to provide maximum sizing accuracy.

Essential Steps for Sample Preparation

1. Measure the total concentration of the DNA sample using a fluorometric, dye-based detection method, such as the Qubit® fluorometer.

2. Dilute the concentration of the **DNA smear to a target concentration of 1 ng/µL**, using the supplied Dilution Buffer 1X TE (Part # DNF-495). Do not pre-dilute samples with DI water.

3. Dilute the concentration of a **DNA fragment sample to a target concentration of 500 - 600 pg/µL**, using the supplied Dilution Buffer 1X TE (Part # DNF-495). Do not pre-dilute samples with DI water.

4. Following normalization of the DNA sample concentration, follow the standard sample plate preparation procedures outlined in the **Sample Plate Preparation** section of this User Manual (2 µL sample added to 22 µL of High Sensitivity Large Fragment Diluent Marker).

Troubleshooting

Issue	Cause	Corrective Action
A. 48,500 bp Lambda DNA fragment in the Ladder is degraded or split.	1. The Ladder was pipetted up and down excessively. 2. The Ladder was stored inappropriately. The Ladder should be stored at 4ºC and freeze-thaw cycles avoided.	1. Use a new Ladder aliquot, and avoid pipetting the Ladder up and down excessively. 2. Store and handle the Ladder as directed in this User Manual.
B. 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 supplied Dilution Buffer 1X TE (DNF-495) and repeat experiment; OR Repeat experiment using decreased injection time or voltage.
C. DNA sample smear overlaps with Lower/Upper Marker peak.	1. Input DNA sample size distribution outside of assay range.	1. Perform further size selection of sample to narrow DNA size distribution and repeat experiment; OR Repeat experiment using DNF-488 High Sensitivity gDNA Analysis Kit (uses lower marker only).
D. 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.
E. 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 High Sensitivity Large Fragment 50Kb Analysis Kit, contact AATI Technical Support by phone at (515)-964-8500 or by email at support@aati-us.com.

Notes

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