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

Genomic DNA 50Kb Analysis Kit

User Guide

(DNF-467-0500)

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

Fragment Analyzer™ Software Version 1.1

PROSize® 2.0 Software Version 2.0

Revised December 14, 2015

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Revision History

Revision Number	Date	Description of Change
DNF-467-2015DEC14	Dec 14, 2015	• New kit release

Kit Components

1. Genomic DNA Separation Gel, 240 mL, Part # DNF-270-0240
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 Genomic DNA Diluent Marker (DM) Solution, 120 mL, Part # DNF-375-0120
   a. Lower Marker in diluent solution (set to 1 bp)
6. Extended Genomic DNA Ladder, 50 µL, Part # DNF-367-U050
   a. Fragments from 75 bp –48,500 bp; 250 ng/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
9. Eppendorf LoBind® 0.5 mL tubes, package of 50

Kit Specifications

Specifications	Description
Sample Volume Required	1 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	50 minutes (33-55 Array)
DNA Sizing Range	75 bp – 48,500 bp
gDNA Concentration Range[1]	25 ng/µL - 250 ng/µL input gDNA (0.125 – 1.25 ng/ µL final concentration after dilution)
gDNA Quantification Accuracy[1]	± 30%
gDNA Quantification Precision[1]	25% CV
Maximum gDNA Concentration	250 ng/µL

[1]: Results using human blood genomic DNA in 1X TE buffer as sample.

Storage Conditions

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

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

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
   - 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.

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 – 200 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 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 930 Inlet Buffer and 5X Capillary Conditioning Solution)
10. Vortexer

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 Genomic DNA 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 Genomic DNA 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 Genomic DNA Separation Gel/Intercalating Dye mixture onto the instrument and insert into the desired gel fluid line (Gel 1 or Gel 2 pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.

7. When adding Separation Gel to the instrument, update the solution levels in the *Fragment Analyzer*™ 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).

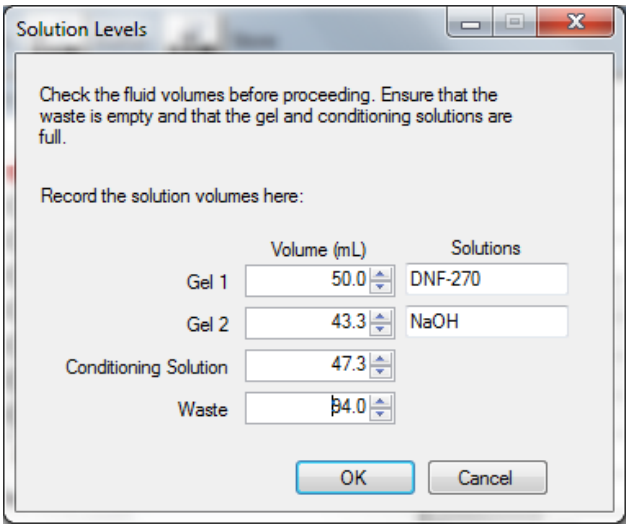

Figure 1. **Solution Levels** menu

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.

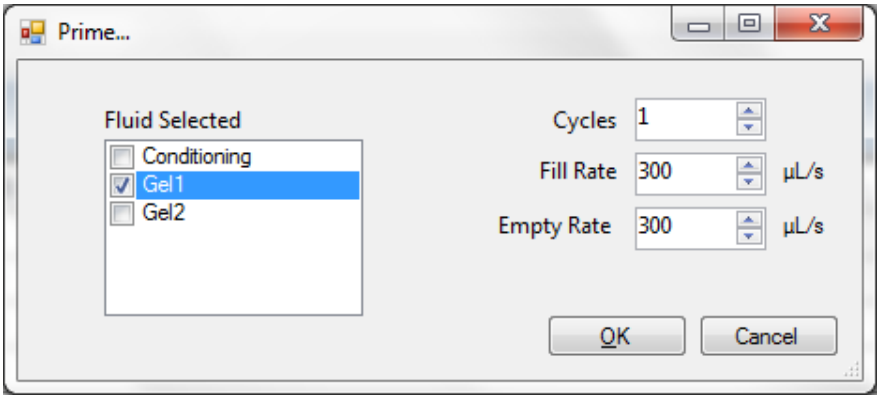

Figure 2. **Prime** menu

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 **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.

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 Genomic DNA Diluent Marker (DM) Solution and Genomic DNA Ladder to warm to room temperature prior to use. Spin the Ladder tube to ensure liquid is at the bottom of the tube.

Important Genomic DNA Sampling Procedures

A. Before sampling, the sample stock genomic DNA must be acclimatized to room temperature for at least 30 minutes.

B. After the samples have been acclimatized to room temperature, mix the gDNA samples by vortexing or flicking the tube before sampling. This further ensures a more homogeneous sample.

1. **The total input genomic DNA sample concentration should be within a range of 25 ng/µL – 250 ng/µL for optimal sizing and quantification.** The above genomic 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.

2. Using a clean 96-well sample plate, pipette **199 µL of Genomic DNA Diluent Marker (DM) Solution to each well** in a row of the 96-well plate that is to contain sample. **Do not add any DM solution to the well reserved for the Genomic DNA Ladder** (Well 12 of each row to be analyzed for a 12-capillary system; Well D12 or H12 on a 48-capillary system; or Well H12 on a 96-capillary system). Fill any unused wells (no sample or Genomic DNA Ladder) within the row of the sample plate with 24 µL/well of BF-25 Blank Solution.

3. Pipette 1 µL of each genomic DNA sample into the 199 µL of Genomic DNA 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 as described below.

Important Sample Mixing Information:

When mixing sample with diluent marker solution, it is important to mix the contents of the wells thoroughly to achieve the most accurate quantification. After adding 1 µL of sample to the 199 µL of diluent marker, use a separate pipette tip with the pipettor set to ~100 µL volume, and pipette each well up/down about 10 times to further mix.

Ladder Preparation

Important information before handling the gDNA Ladder:

Do not manually mix the gDNA Ladder by repeated inverting of the tube or repeated pipetting up/down; doing so will result in the degradation of the lambda DNA fragment in the Ladder. **The ladder solution can only be vortexed by a vortex mixer.**

1. The Genomic DNA Ladder should be run in parallel with the samples for each experiment. To prepare the working Genomic DNA Ladder solution, add 199 µL of Genomic DNA Diluent Marker (DM) into a 0.5 mL Eppendorf DNA LoBind® tube. Gently vortex the vial containing the Genomic DNA Ladder, then pipette 1 µL of the Genomic DNA Ladder into the 199 µL of the DM Solution. This is now the working Genomic DNA Ladder solution. **Mix the working Genomic DNA Ladder solution only by vortexing in the vortex mixer.**

2. Pipette the entire 200 µL of the working Genomic DNA Ladder solution into **Well 12 of each row to be analyzed** (12-capillary system), **into well D12** when analyzing Row A to Row D, or **into well H12** when analyzing Row E to Row H (48-capillary system), or **Well H12** (96-capillary system) of the 96-well plate that already contains the gDNA samples, prepared as directed above.

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

IMPORTANT! Centrifugation should be done at a speed low enough to remove air bubbles as well as avoid settling of genomic DNA at the bottom of the sample well. High speed centrifugation can cause genomic DNA to settle at the bottom of the sample wells, leading to sampling errors and less accurate quantification. **A recommended relative centrifugal force (RCF) limit is 100 x g for less than 30 seconds.**

4. Run the sample plate immediately once prepared, or cover the sample plate with a cover film, and use as soon as possible. Alternatively, to prevent evaporation, place a mineral oil overlay on each sample (50 µL/well).

5. 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.

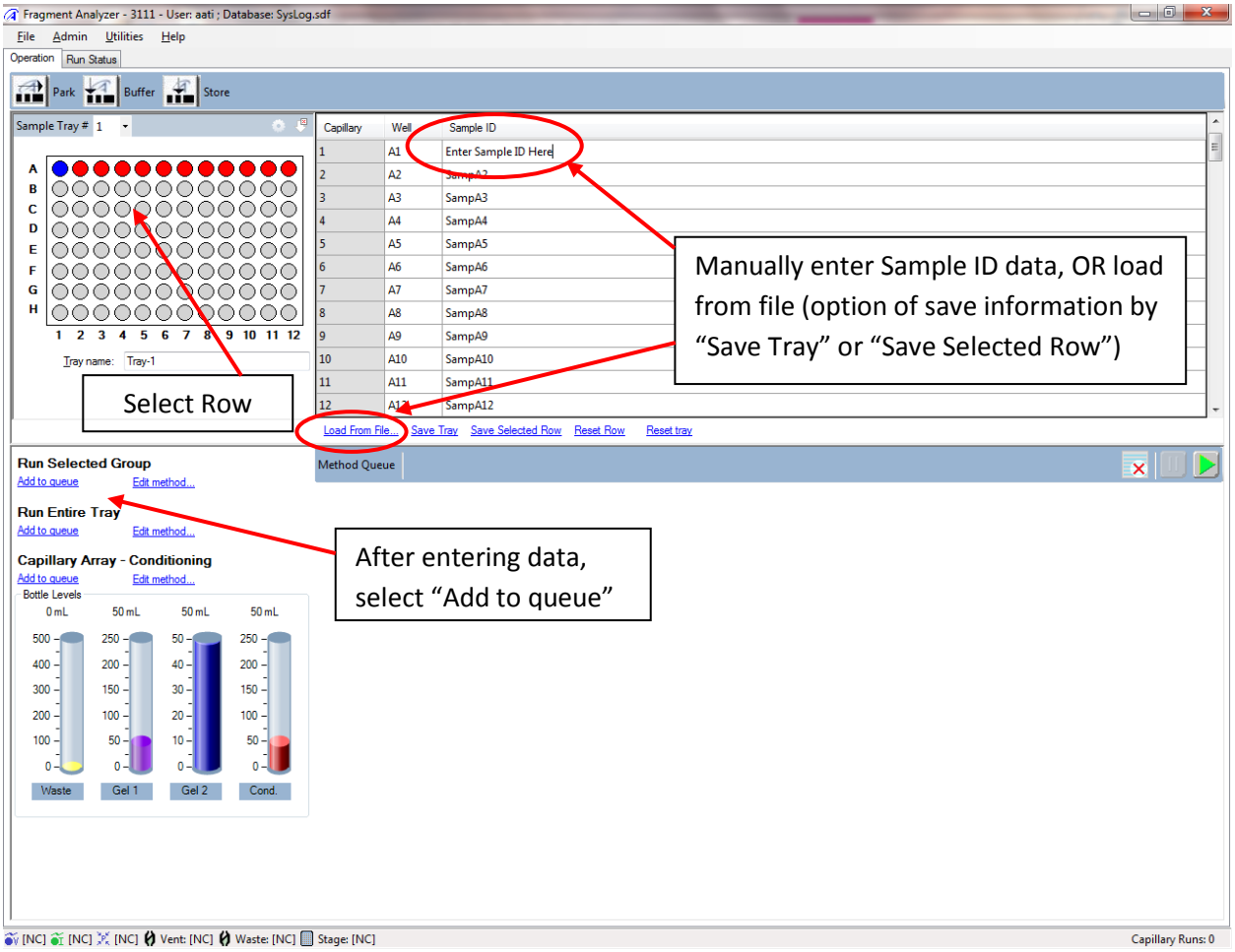

Figure 3. Main Screen showing selection of sample row and entering sample information

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).

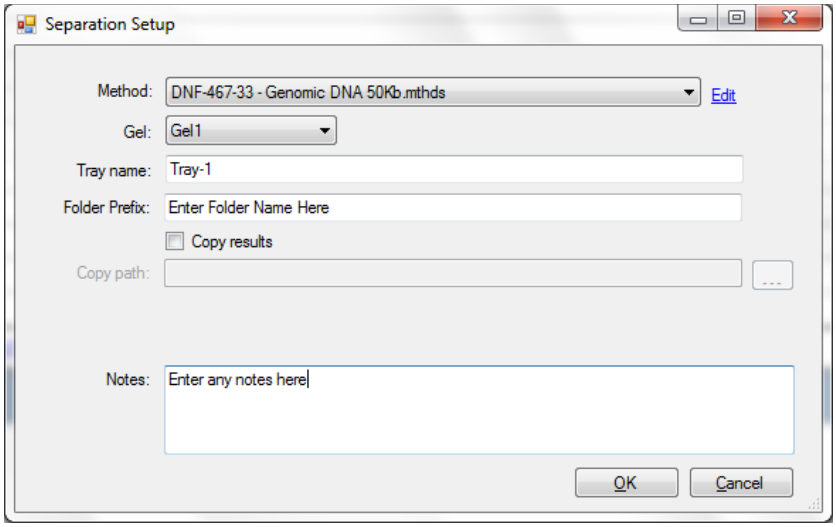

Figure 4. **Separation Setup** form to select experimental Method and enter tray/folder information

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., 33cm). Select the following method:

    a. **DNF-467-33 –Genomic DNA 50Kb.mthds** for the 33 cm effective, 55 cm total "short" capillary array.

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

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

7. To copy the experimental results to another directory location in addition to the default save directory (**`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.

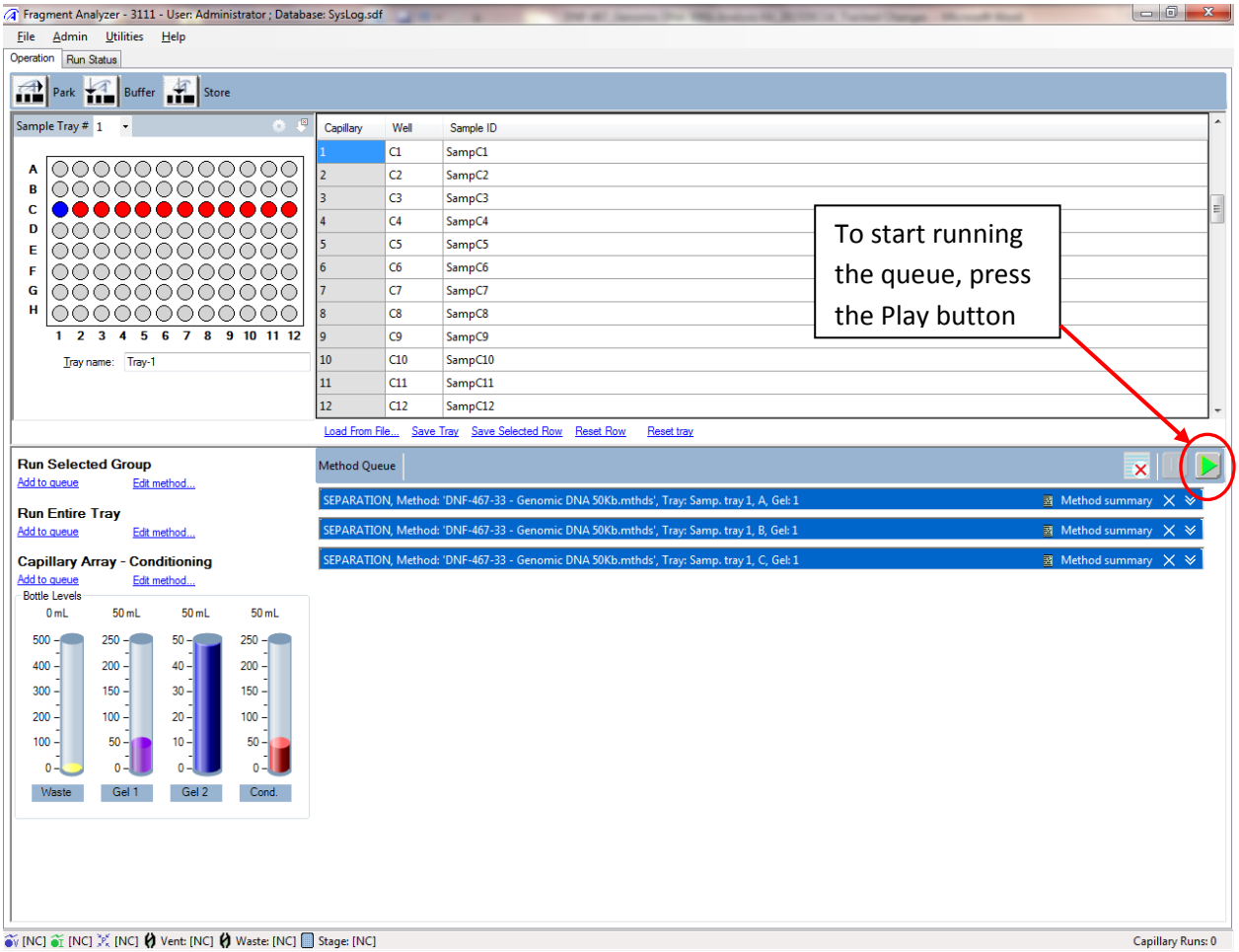

Figure 5. Main Screen after selection of samples to the run queue.

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 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 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-467-33** method (Figure 6) 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, 15 sec). This step injects the prepared sample plate.
    7) Separation (ENABLED) Voltage (6 kV, 50 min). This step performs the CE Separation.

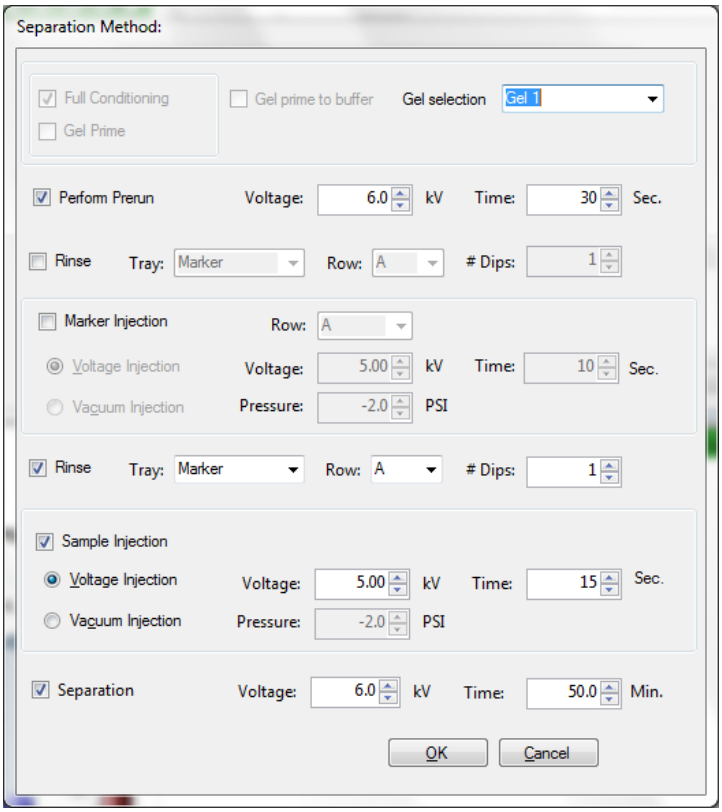

Figure 6. **DNF-467-33** Genomic DNA 50Kb method

4. 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.

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

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:

   The **DNF-467-33** separation method will be processed using the **DNF-467-33 - Genomic DNA 50Kb** configuration file.

NOTE: If the preloaded *PROSize*® 2.0 software configuration file "**DNF-467-33 – Genomic DNA 50Kb**" is not located in the **`C:\PROSize 2.0\Configurations`** directory, contact AATI Technical Support to obtain this file.

2. The data is normalized to the lower marker (set to 1 bp) and calibrated to the Extended Genomic DNA Ladder run in parallel to the samples. Figure 7 shows an example of the 1 bp marker injected with the Extended Genomic DNA Ladder using the **DNF-467-33** separation method. A total of 13 peaks should be observed.

3. The *PROSize*® 2.0 software is set to the **gDNA** mode in the **Advanced Settings**. The **Quantification** settings are set to **Use Ladder** for quantification with a **Conc. (ng/uL)** of **1.25** and a **Dilution Factor** of **200** (1 µL sample + 199 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; 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

Genomic DNA Ladder

1. Figure 7 shows the typical expected results for the Extended Genomic DNA Ladder, provided at an **input total DNA concentration of 250 ng/µL in 1X TE buffer** (1:200 dilution, 1 µL Ladder + 199 µL DM solution). A total of 13 peaks should be observed, with the sizes annotated as in Figure 7. Fragments up to 6000 bp in the ladder should be baseline resolved. Fragments from 6000 bp to 48500 bp should be well separated. The Genomic DNA Ladder should be fitted with a point-to-point curve fitting algorithm in the *PROSize*® 2.0 software.

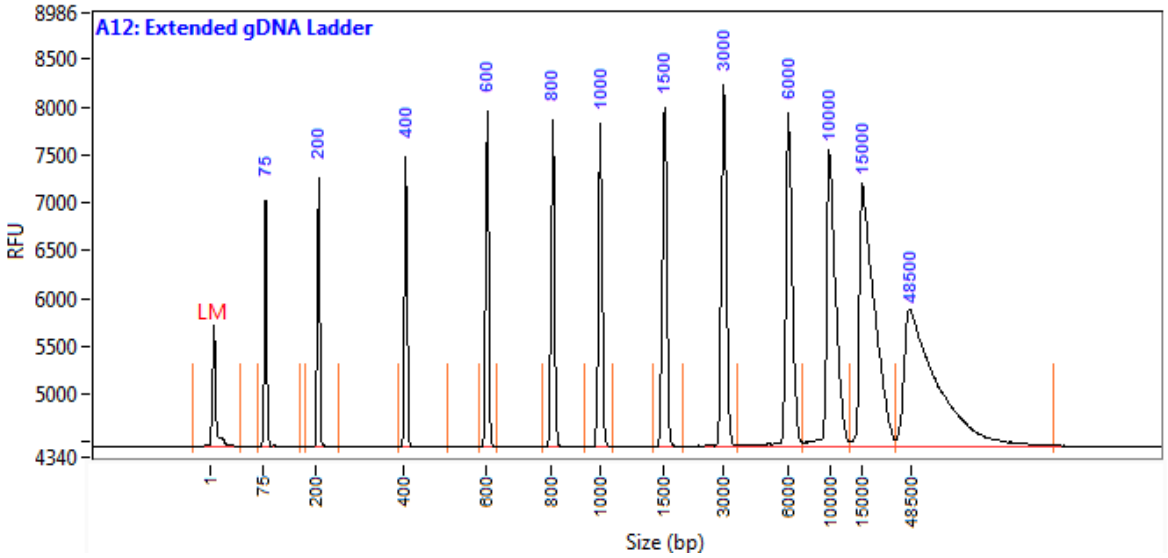

Figure 7. Representative Extended Genomic DNA Ladder result using the *Fragment Analyzer*™ system with the Genomic DNA 50Kb Analysis Kit. Method: **DNF-467-33** (33cm array).

Genomic DNA Sample

1. Figure 8 shows the result for a genomic DNA sample. In this example, a human blood genomic DNA sample was analyzed.

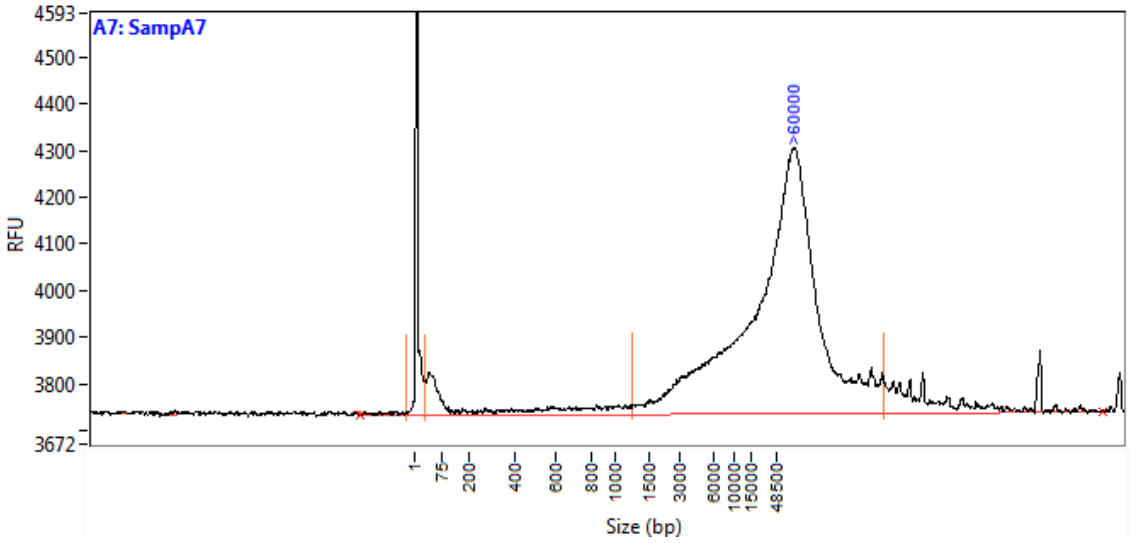

Figure 8. Representative genomic DNA sample result (Human blood gDNA) using the *Fragment Analyzer*™ system with the DNF-467 Genomic DNA 50Kb Analysis Kit. The peak is annotated by size in bp. Any fragment above 60,000 bp cannot be sized rationally and will be displayed as '>60000'.

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-467 Genomic DNA 50Kb Analysis Kit and suggested remedies. For a full list of instrument specific troubleshooting information, refer to **Troubleshooting and Maintenance Guide** for the *Fragment Analyzer*™ system.

Issue	Cause	Corrective Action
A. No peak observed for gDNA sample when expected. Lower Marker peak observed.	1. Sample highly degraded; no dye intercalates. 2. Sample not homogenously mixed before sampling. 3. Sample concentration too low and out of range.	1. Sample not suitable for use. 2. Make sure the sample is equilibrated to room temperature for at least 30 min. before use, vortex the sample or pipette up-down to mix before sampling. 3. Prepare more concentrated sample and repeat experiment.
B. Much lower concentration obtained for gDNA sample than expected.	1. Sample contains very large size, aggregated genomic DNA (>>60 kbp)	1. The analysis of the very large sized, aggregated genomic DNA can result in lower than expected concentration values due to the nature of sample aggregation, which can inhibit sample injection. Analysis of these types of samples at lower concentrations in the DNF-467 kit may improve the quantitation.
C. Extra peaks/smear near Lower Marker observed (10-1000 bp).	1. Genomic DNA possibly contaminated with RNA.	1. Remove RNA contamination from the genomic DNA sample and reanalyze, or perform selective peak/smear integration above 1000 bp.
D. Degradation of the Lambda DNA fragment in the ladder.	1. Ladder solution was manually mixed by repeated inverting of the tube or repeated pipetting up/down.	1. Mix the ladder solution by vortexing.

E. No sample peak or Lower 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 *Fragment Analyzer*™ operation or about the DNF-467 Genomic DNA 50Kb Analysis Kit, contact AATI Technical Support by phone at (515)-964-8500 or by email at support@aati-us.com.