New Software Version

Refer to red highlighted text for additional updates

DNF-915 dsDNA Reagent Kit

User Guide

(DNF-915-K0500)

(DNF-915-K1000)

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

Fragment Analyzer™ Software Version 1.0.2

PROSize® 2.0 Software Version 1.3

Revised March 10th, 2014

NOTICE: The DNF-915 kit number has replaced the DNF-910/15L80M kit number. The kit components and performance characteristics have not changed. Future orders should reference the new DNF-915 kit number.

Advanced Analytical Technologies, Inc.
2711 South Loop Drive, Suite 4150
Ames, IA 50010
www.aati-us.com

Ph: 515-296-6600
Fax: 515-294-7141

DNF-915 dsDNA Reagent Kit, 35 bp – 5,000 bp (500 Samples)

Part # DNF-915-K0500

Kit Components

1. FA dsDNA Gel, 35 bp – 1,500 bp, 240 mL, Part # DNF-810-0240
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. 35 bp and 5,000 bp Markers, 3.2 mL, Part # FS-SMK480-0003
    a. 0.5 ng/µL concentration each in 1X TE buffer
6. 100bp Plus DNA Ladder, 1.0 mL, Part # FS-SLR915-0001
    a. 100 bp – 3,000 bp; 2.5 ng/µL total DNA concentration in 1X TE buffer
7. Mineral Oil, 15 mL, Part # FS-SMO15
8. Dilution Buffer 1X TE, 60 mL, Part # DNF-495-0060
9. Conical 250 mL Centrifuge Tube for prepared Separation Gel/Intercalating Dye mixture

DNF-915 dsDNA Reagent Kit, 35 bp – 5,000 bp (1000 Samples)

Part # DNF-915-K1000

Kit Components

1. FA dsDNA Gel, 35 bp – 1,500 bp, 500 mL, Part # DNF-810-0500
2. Intercalating Dye, 30 µL x 2, Part # DNF-600-U030
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. 35 bp and 5,000 bp Markers, 3.2 mL, Part # FS-SMK480-0003
    a. 0.5 ng/µL concentration each in 1X TE buffer
6. 100bp Plus DNA Ladder, 1.0 mL, Part # FS-SLR915-0001
    a. 100 bp – 3,000 bp; 2.5 ng/µL total DNA concentration in 1X TE buffer
7. Mineral Oil, 15 mL, Part # FS-SMO15
8. Dilution Buffer 1X TE, 125 mL, Part # DNF-495-0125
9. Conical 250 mL Centrifuge Tube for prepared Separation Gel/Intercalating Dye mixture

Application

The DNF-915 Reagent Kit from AATI is for the analysis of dsDNA fragments between 35 bp and 5,000 bp. Sizing and relative quantification between samples can be obtained using this kit. Example applications include general PCR fragment sizing and QC, and genotyping.

Specifications

Specifications	Description
Sample Volume Required	2 µL (adjustable depending upon sample concentration)
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	35 bp – 5,000 bp (defined by lower/upper marker)
Separation Resolution	35 bp - 100 bp ≤ 10% ; 100 bp – 2,000 bp ≤ 5% 2,000 bp – 5,000 bp ≤10%
DNA Sizing Accuracy[1]	± 5% or better
DNA Sizing Precision[1]	2% CV
DNA Fragment Concentration Range[1]	0.5 ng/µL – 50 ng/µL input DNA (adjustable by dilution of sample)

[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):
FA dsDNA Gel	Intercalating Dye	5X Capillary Conditioning Solution
5X 930 dsDNA Inlet Buffer	35 bp and 5,000 bp Markers	Mineral Oil
Dilution Buffer 1X TE	100bp Plus DNA Ladder	

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

Additional Material 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 dsDNA 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 dsDNA 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, it is recommended to use the Intercalating Dye at 2X normal concentration (1:10,000 dilution).

3. Bring the dsDNA Separation Gel and Intercalating Dye to room temperature prior to mixing.
4. Mix appropriate volumes of Intercalating Dye and Separation Gel necessary for less than two weeks of operation. Use the supplied 50 mL conical centrifuge tube to allow a small minimum working volume. For larger volumes, use a 250 mL conical centrifuge tube and remove the collar of the tube holder in the instrument reagent compartment. For maximum accuracy, it is recommended to dispense Separation Gel into a clean glass graduated cylinder for volume measurement and transfer to the working tube prior to adding Intercalating Dye.

   NOTE: Some loss of detection sensitivity will be observed over a two week period after the gel/dye mixture has been prepared. **For best results, it is recommended to prepare gel/dye mixture daily**. It is not recommended to use gel/dye mixture that is more than two weeks old.

5. The volume of Separation Gel required per run varies between 12-capillary and 96-capillary *Fragment Analyzer*™ systems. The volumes required are summarized below.

   For 12-capillary *Fragment Analyzer*™ systems:

# of samples to be analyzed	Volume of Intercalating dye	Volume of Separation Gel
12	1.0 µL	10 mL[1]
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]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 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 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).

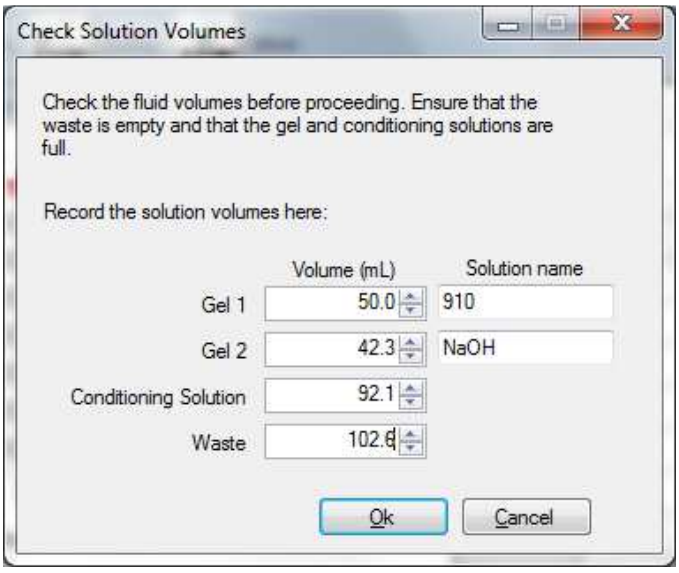

Figure 1. **Solution Levels** menu

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.

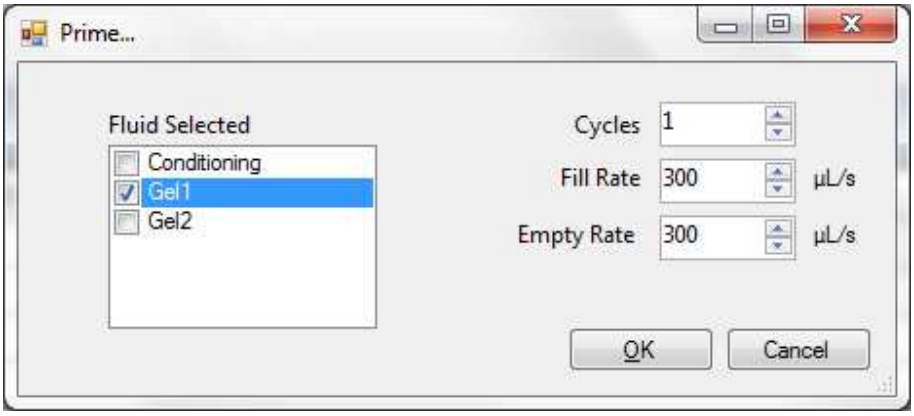

Figure 2. **Prime** menu

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 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.

Marker/Ladder/Sample Preparation

General Information

1. The recommended 96-well sample plate for use with the *Fragment Analyzer*™ system is a semi-skirted PCR plate from Eppendorf (#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.

35 bp/5,000 bp Marker Preparation

1. Store the 35 bp and 5,000 bp Marker solution at -20°C upon arrival.
2. Bring the 35 bp and 5,000 bp Marker solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.

3. The Marker solution is supplied as a **ready-to-use solution**, containing 0.5 ng/µL of each fragment in a 1X TE buffer solution. It is intended for use as an external standard marker plate.
4. Prepare the Marker solution plate by dispensing 30 µL/well into Row A only (12-Capillary) or every well (96-Capillary) of a separate sample plate. Cover the wells with 20 µL/well of the supplied mineral oil to allow reuse for at least 30+ injections.
5. The prepared Marker solution plate should be placed 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.

100bp Plus DNA Ladder Preparation

1. Store the 100bp Plus DNA Ladder solution at -20°C upon arrival.
2. Bring the 100bp Plus DNA Ladder solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.
3. The 100bp Plus DNA Ladder solution is supplied as a **ready-to-use** solution, containing approximately 2.5 ng/µL total DNA concentration in a 1X TE buffer solution. It is used for calibrating the size of analyzed DNA fragments, and is typically added to a well of the sample plate and run in parallel with the samples.

   12-Capillary Systems: Pipette 24 µL of 100bp Plus DNA Ladder solution **into well 12 of each row** of the sample plate.

   96-Capillary Systems: Pipette 24 µL of 100bp Plus DNA Ladder solution **into well H12** of the sample plate.

   Alternatively, once the 100bp Plus DNA Ladder has been run under the experimental method and additional samples are to be run under the same experimental conditions, the ladder can be imported in the *PROSize*® 2.0 software, enabling use of all 12 wells per row or all 96 wells of the sample plate.

Sample Plate Preparation

1. The protocol below assumes the sample is originally present in a typical PCR buffer matrix. Depending upon the concentration of product or sample matrix, it may be necessary to adjust the dilution and/or adjust the injection voltage and time to avoid overloading of the DNA sample.

2. Using a clean 96-well sample plate, pipette 22 µL of 1X TE dilution buffer (supplied with kit) to each well in a row that is to contain sample. If running the 100bp Plus DNA Ladder in parallel with the samples, pipette 24 µL of the Ladder solution directly (no dilution) into the specified well of the sample plate or row to be analyzed (see previous Section).

3. Pipette 2 µL of each DNA sample into the 22 µL of 1X TE dilution buffer 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 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 to the 22 µL of diluent, **swirl the pipette tip while pipetting** up/down to further mix.

B. After adding 2 µL of sample to the 22 µL of diluent, place a plate seal on the sample plate and **vortex the sample plate at 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** to the 22 µL of diluent, **use a separate pipette tip set to a larger 20 µL volume, and pipette each well up/down** to further mix.

D. Use an **electronic pipettor capable of mixing a 10 µL volume in the tip after dispensing the 2 µL sample volume**. Some models enable using the pipette tip for both adding and mixing.

4. After mixing the samples with 1X TE dilution buffer in each well, check the wells of the sample plate to ensure there are no air bubbles 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.

5. 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).

6. 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 edit the experimental method as described in the following sections.

7. The CCD detection system of the *Fragment Analyzer*™ system provides a high dynamic range for detection. An ideal injection range would yield peak heights from 100 – 20,000 RFUs. Overloading of sample can decrease separation resolution and saturate the detector, leading to mismatched lower/upper marker peak heights and poor results. It is important to optimize sample dilution and concentration, and use experimental parameters to work with within the specified RFU range.

8. **TIP:** If the above method (2 µL sample + 22 µL 1X TE diluent) yields peak heights consistently above 20,000 RFUs, decrease the marker/sample injection time or reduce the sample volume to 1 µL sample + 23 µL 1X TE.

   If low signals are encountered, increase the marker/sample injection time, or alternatively add 4 µL of sample + 20 µL of DI water in each well. When making adjustments to the sample dilution, the total volume should be maintained to at least 24 µL, and whenever possible the chloride (Cl^-) salt content of the final sample should be adjusted to

approximately 10 mM Cl^- to best match the 100bp Plus DNA Ladder and 35 bp/5,000 bp marker solution (both are in 1X TE buffer matrix) to maximize sizing accuracy.

Whenever making adjustments to the sample dilution, ensure the **Dilution Factor** of the *PROSize*® 2.0 software is adjusted accordingly when processing the data.

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.

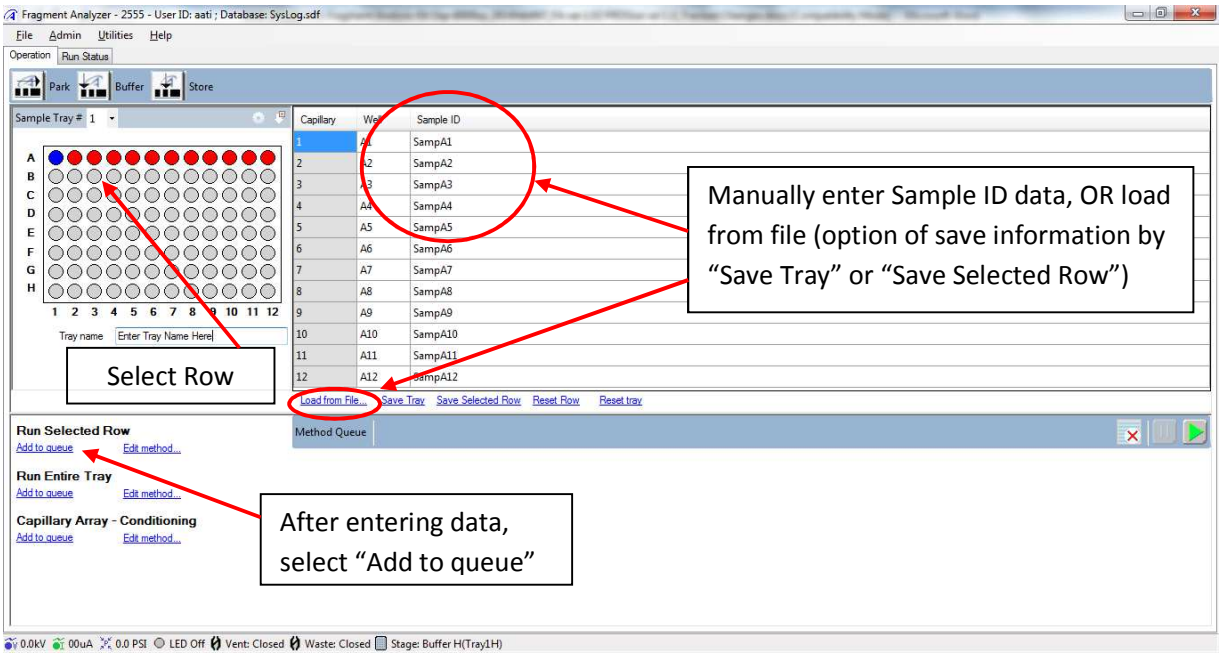

Figure 3. Main Screen showing selection of sample row and entering sample information

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).

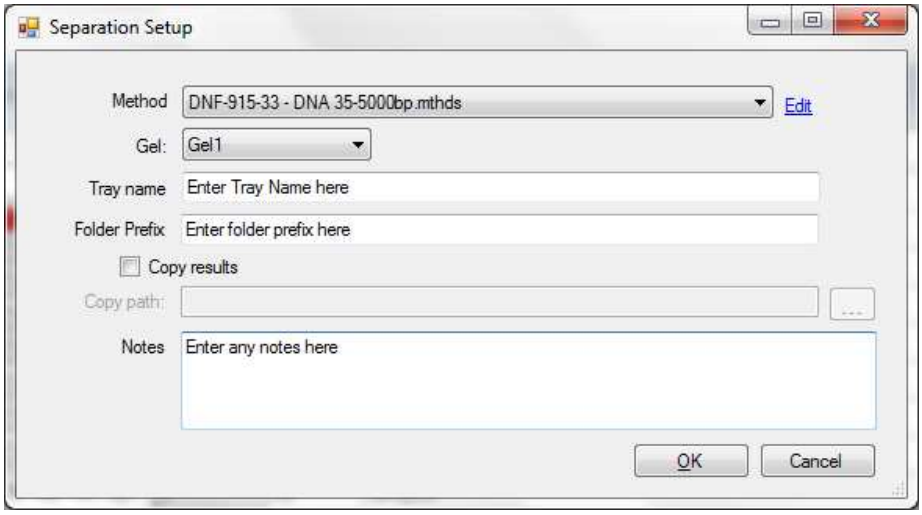

Figure 4. **Separation Setup** form to select experimental Method and enter tray/folder information

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-915-33 – DNA 35-5000bp.mthds** when the 33 cm effective, 55 cm total "short" capillary array is installed;

    b. Select **DNF-915-55 – DNA 35-5000bp.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, waste, marker, sample, etc.) have been loaded into their respective drawer locations.

14. Press the **Play** icon ( ) to start the sequence loaded into the queue. To **Pause** the queue after the currently running experiment is completed, press the button. To **Clear** the run queue of all loaded runs press the button.

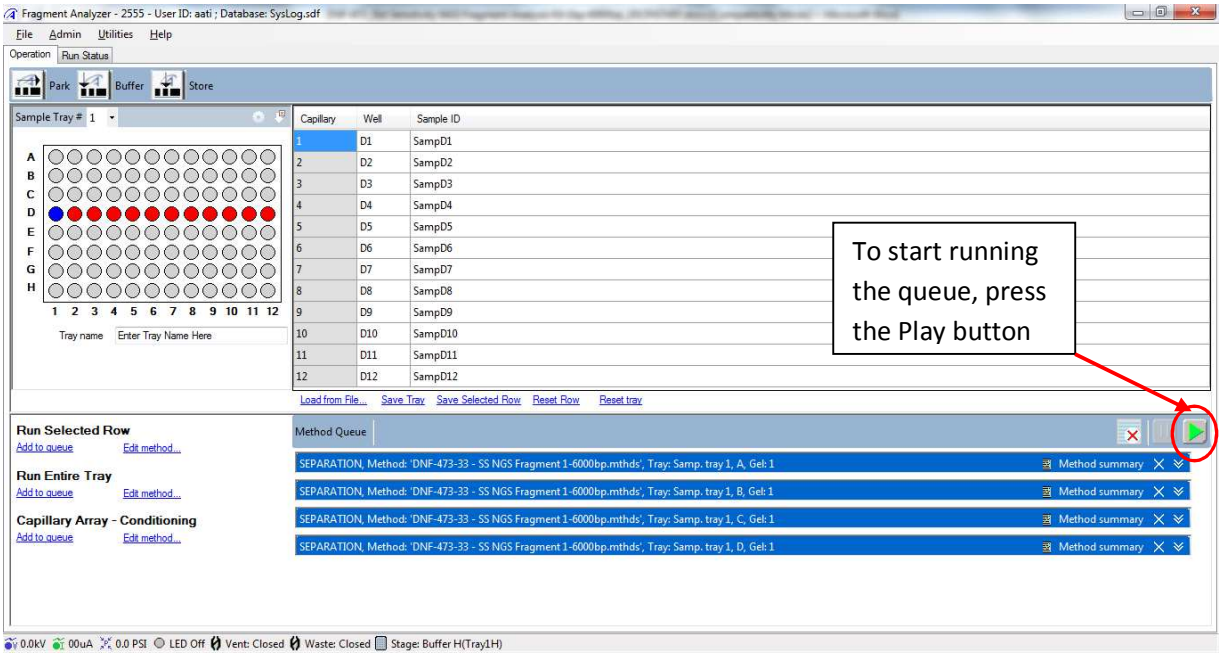

Figure 5. Main Screen after selection of samples to the run queue.

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-915-33** (Figure 6) and **DNF-915-55** (Figure 7) methods are shown below. The general steps of the method are as follows:

    1) **Full Condition** flushing method (Automatically enabled). Gel Selection: Gel 1.
    2) Perform Prerun (ENABLED) (6 kV, 30 sec)
    3) Rinse (DISABLED)
    4) Marker Injection (ENABLED) Voltage Injection (5 kV, 10 sec). This step injects the 35 bp/5,000 bp marker plate.
    5) Rinse (DISABLED)
    6) Sample Injection (ENABLED) Voltage Injection (5 kV, 10 sec). This step injects the prepared sample plate.
    7) Separation (ENABLED) Voltage (6 kV, 50 min). This step performs the CE Separation.

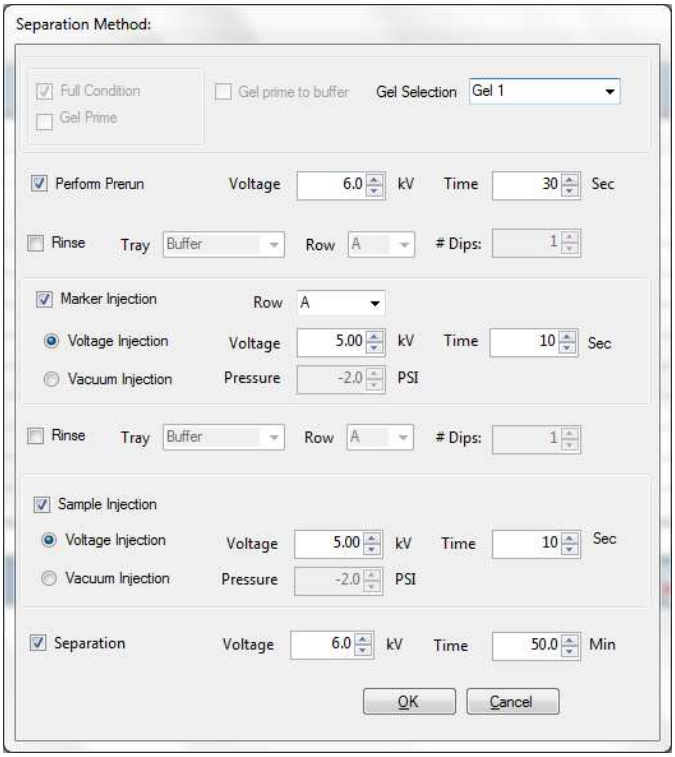

Figure 6. **DNF-915-33** dsDNA Reagent Kit (35bp – 5000 bp) method

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.

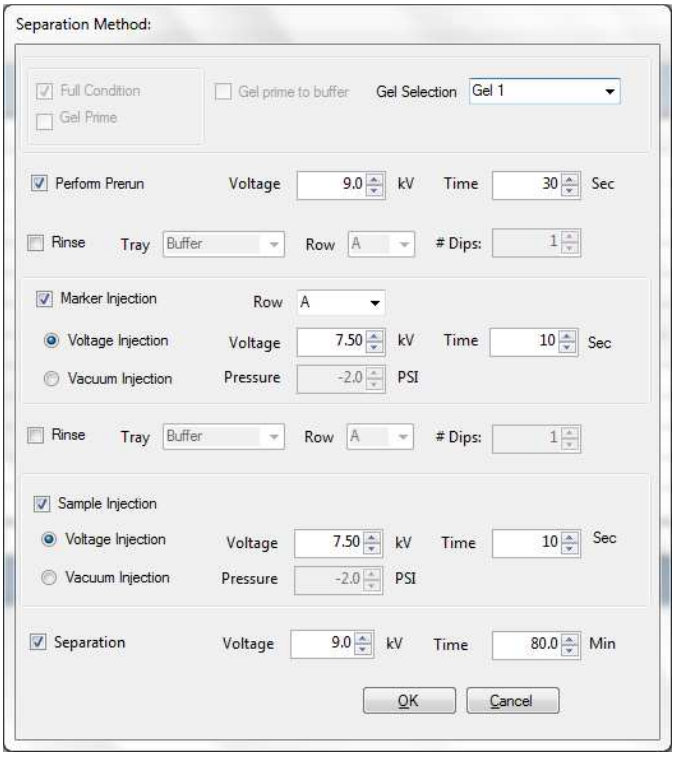

Figure 7. **DNF-915-55** dsDNA Reagent Kit (35bp – 5000 bp) method

5. An Administrator level user has the option to adjust the **Gel Selection**; **Prerun** settings; **Rinse** settings including **Tray**, **Row** and **# Dips**; **Marker Injection** settings including **Row**; **Sample Injection** settings; and the **Separation** settings. For example, if the marker solution is loaded into a row other than Row A on a 12-capillary instrument, 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-915-33** separation method will be processed using the **DNF-915-33 – DNA 35-5000bp** configuration file;

   b. The **DNF-915-55** separation method will be processed using the **DNF-915-55 – DNA 35-5000bp** configuration file.

   NOTE: If the preloaded *PROSize*® 2.0 software configuration files "**DNF-915-33 – DNA 35-5000bp**" and "**DNF-915-55 – DNA 35-5000bp**" 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 35 bp) and upper marker (set to 5,000 bp), and calibrated to the 100bp Plus DNA Ladder run in parallel to the samples. Figure 8 shows an example of the 35 bp and 5,000 bp markers injected with the 100bp Plus DNA Ladder. A total of 16 peaks should be observed.

3. When processing data, the *PROSize*® 2.0 software is set to the **DNA** mode in the **Advanced Settings**. The **Quantification** settings are set to **Use Upper Marker** for quantification with a **Conc. (ng/uL)** of 0.5 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 reflect the estimated 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

100 bp Plus DNA Ladder

1. Figure 8 shows the typical expected results for the 100 bp Plus DNA Ladder provided at an input total DNA concentration of 2.5 ng/µL in 1X TE buffer, co-injected with the 35 bp lower marker and 5,000 bp upper marker. A total of 16 peaks should be observed, with the sizes annotated as in Figure 8. All fragments in the ladder should be resolved.

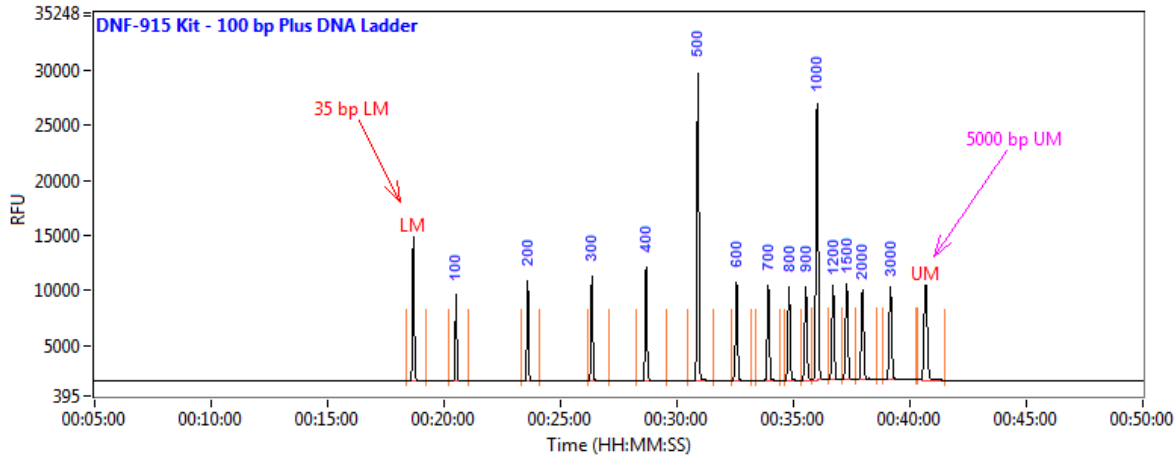

Figure 8. Representative 100 bp Plus DNA Ladder result injected with 35 bp lower marker and 5,000 bp upper marker, using the *Fragment Analyzer*™ system with the DNF-915 reagent kit. Method: **DNF-915-33** (short array).

Figure 9 shows the same separation performed on a 55 cm effective, 80 cm total length capillary array.

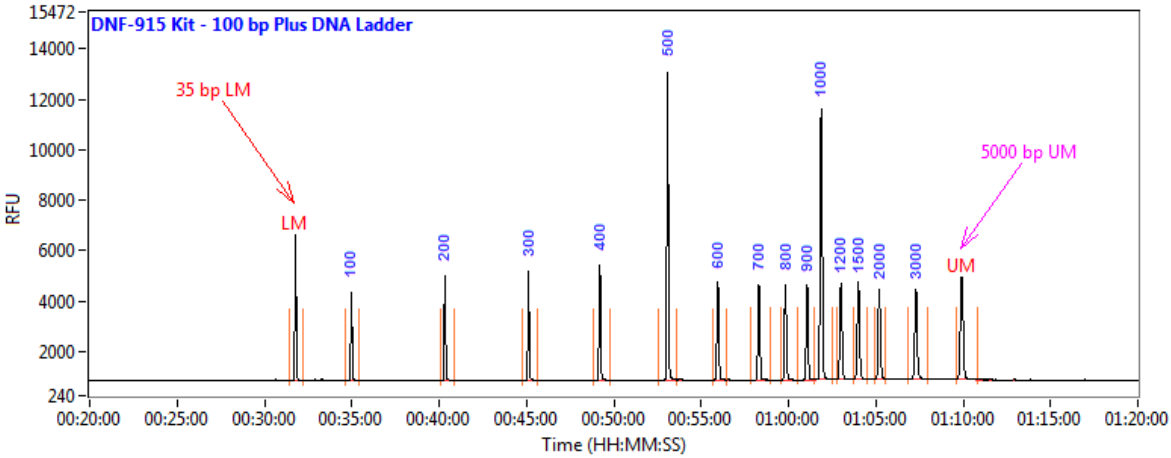

Figure 9. Representative 100 bp Plus DNA Ladder result injected with 35 bp lower marker and 5,000 bp upper marker, using the *Fragment Analyzer*™ system with the DNF-915 reagent kit. Method: **DNF-915-55** (long array).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-915 Reagent Kit and suggested remedies. For a full list of instrument specific troubleshooting information, refer to the **Troubleshooting and Maintenance Guide** for the *Fragment Analyzer*™ system.

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.	1. Further dilute input DNA sample concentration with 1X TE buffer and repeat experiment. 2. Reduce injection time and/or injection voltage, and repeat experiment. Use the same injection voltage/time settings for the Marker Plate and Sample Plate to maximize quantification accuracy.

B. No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.	1. Sample concentration too low and out of range. 2. Sample was not added to 1X TE diluent or not mixed well.	1. Prepare more concentrated sample and repeat experiment (e.g. 4 uL sample + 20 uL DI water); OR Repeat experiment using increased injection time and/or injection voltage for Marker Plate and Sample Plate. 2. Verify sample was correctly added and mixed to sample well.
C. Sample peak(s) migrate before or co-migrate with 35 bp Lower Marker.	1. Excess primer-dimer species in sample.	1. Further dilute input DNA sample concentration with 1X TE buffer to minimize primer-dimer interference, and repeat experiment.
D. Sample peak(s) migrate after or co-migrate with 5,000 bp Upper Marker.	1. DNA sample size out of range of assay.	1. Analyze samples with DNF-920 (75 bp – 15,000 bp) or DNF-930 (75 bp – 20,000 bp) reagent kit.
E. No sample peak or marker peak observed for individual sample.	1. Air trapped at the bottom of sample plate and/or marker plate well, or bubbles present in well. 2. Insufficient sample volume. A minimum of 20 µL is required. 3. Capillary is plugged.	1. Check sample/marker 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

Technical Support and Contact Information

1. For questions with *Fragment Analyzer*™ operation or about the DNF-915 Reagent Kit, contact AATI Technical Support by phone at (515)-296-6600 or by email at support@aati-us.com.

Notes