

Standard Operating Procedure Approval Page:

Date Implemented: 16 June 2020

Updated:	Ashima Amin	16 June 2020
	Name	Date

Supersedes:	n/a	n/a
	Name	Date

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Changes Made:

061620: New protocol

Changes to previous procedures:

N/A

Agilent Fragment Analyzer Usage, Troubleshooting, and Maintenance

Note: *No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.*

Instrument Principles

The Fragment Analyzer systems utilize automated parallel capillary electrophoresis to provide reliable quality control (QC) for nucleic acids. With its unique design and intuitive features, common QC bottlenecks are resolved by the automation of key steps such as gel loading and sample injection increasing lab efficiency. A broad range of kits are available allowing you to easily qualify and quantify DNA and RNA samples.

Specimen Type

DNA

Quality Control

A DNA ladder is run as a control to determine if the fluorescence is appropriate, and if the gel resolution is adequate.

Safety

Powder-free gloves should be worn.

Kit	Agilent Catalog Number	DNA Fragment Range	Fragment Concentration Range	Smear Concentration Range
High Sensitivity NGS Fragment Analysis Kit	DNF-474-0500, DNF-474-1000	100-6000 bp	5-500 pg/ μ L	50-5000 pg/ μ L
High Sensitivity Large Fragment 50 kb Kit	DNF-464-0500	75-48,500 bp	5-600 pg/ μ L	50-5000 pg/ μ L

Prepare Reagents, Samples, and File

1. **Thaw reagents and/or equilibrate to room temperature for ~1 hour.**
 - a. NGS Separation Gel (4°C)
 - i. High Sensitivity NGS Fragment Separation Gel
OR
 - ii. High Sensitivity Large Fragment Separation Gel
 - b. 5X930 dsDNA Inlet buffer (4°C)
 - c. BF-25 Blank Solution (brown bag) (4°C)
 - d. 0.25X TE Rinse Buffer (4°C)
 - e. Intercalating Dye (-20°C)
 - f. NGS Fragment Diluent Marker Solution (-20°C)
 - i. HS NGS Diluent Marker
OR
 - ii. HS Large Fragment Diluent Marker
 - g. Ladder
 - i. HS NGS Fragment DNA Ladder (-20°C)
OR
 - ii. HS Large Fragment 50 kb DNA Ladder (2-8°C)
 - h. Note: Intercalating Dye and Blank Solutions are light sensitive. During thawing, keep them in the brown bags.
2. **Thaw the samples to room temperature and select the proper kit. Ensure that the DNA is within the correct concentration range for the kit and sample type. If the samples are more concentrated than the recommendation, dilute them with 1X TE (not water).**
3. **Enter the sample names into the Sample ID column in the FA sample sheet template .csv file, which can be found on the desktop of the FA computer.**
 - a. Open the sample manifest (from PPMS) on the FA computer.
 - b. Open the FA sample sheet template Excel file on the desktop. Do not save over the template.
 - c. Copy the sample names from the manifest into the designated column only (Column C).
 - d. Save the new file to the PPMS order folder with the project ID and order number in the filename.
 - e. This file should be uploaded onto the FA computer before starting a run. See Step 9 below.
4. **Turn on the instrument and log into the data collection software.**
 - a. Username: Administrator; Password: leave the field blank
5. **Prepare Fragment Separation Gel solution using the following table.**

For 96-capillary *Fragment Analyzer*TM systems:

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

6. Prepare Inlet Buffer

- a. Mix 20 mL of 5X 930 dsDNA Inlet Buffer and 80 mL of deionized water.
- b. This 1X solution can be stored at 4° C until needed.

7. Prepare Capillary Conditioning Solution

- a. Mix 20 mL of the 5X Capillary Conditioning Solution and 80 mL deionized water.
- b. This 1X solution can be stored at RT until needed.
- c. A typical 96-capillary experiment consumes less than 35 mL of this solution.
- d. If the Conditioning Solution is not completely used within a month, change out for fresh solution.

8. Load buffers into the instrument

- a. NGS Fragment Separation Gel solution
 - i. Fill the Gel Solution bottle within the instrument and label the bottle with "Gel Solution – MM-DD-YY" and your initials. Change the bottle once per month (Corning #430776).
 - ii. New gel can be added to the old in the bottle if not older than a week.
 - iii. Update the amount of solution that is in the bottle in the Utilities -> Solution Levels menu on the software.
 - iv. If the gel type ever needs to be changed to another, the new gel solution must be primed to flush the capillaries and fill them with the new solution (Utilities -> Prime).
- b. Capillary Conditioning Solution (into the labeled bottle)
 - i. Fill the Capillary Conditioning Solution bottle within the instrument and label with "Capillary Conditioning Solution – MM-DD-YY" and your initials. Change the bottle once per month.
 - ii. New conditioning solution can be added to the old in the bottle if not older than two weeks.
 - iii. Update the amount of solution that is in the bottle in the Utilities -> Solution Levels menu on the software.
- c. Inlet Buffer
 - i. Prepare a 96 well, deep well plate (Fisher Scientific (Part # 12-566-120) 31 mm height) filled with 1 mL of 1X 930 dsDNA Inlet Buffer per well.
 - ii. Place plate into drawer B ensuring the plate is oriented with A1 at the back-left corner of the tray.
 - iii. A new plate with fresh inlet buffer should be prepared for each sample run.
- d. Capillary Storage Solution
 - i. Prepare a 96 well, semi-skirted plate (MidSci Pryme PCR Ergonomic Plates, 96x0.2mL, Semi-Skirted, Natural, 25/pk cat. AVRT1) or (Eppendorf, cat. # 951020303) filled with 100 µL of Capillary Storage Solution per well.
 - ii. Seal and centrifuge the plate at 3000 RPM for 2 min to ensure solution is at the bottom of wells. Inspect for the bubbles.
 - iii. Remove seal and place plate into sample drawer 3 (6th plate from the top of the instrument).
- e. 0.25X TE Rinse Buffer
 - i. Prepare a 96 well, semi-skirted plate filled with 200 µL of 0.25X TE Rinse Buffer
 - ii. Place plate into drawer M.
- f. Waste
 - i. Place the reservoir waste plate (Fisher Scientific 31 mm plate, Part #12-566-120) into drawer W. Empty the waste plate if it contains any liquid.
 - ii. Empty the large waste bottle if it is full and update the solution level using the software, Utilities -> Solution Levels.

Perform Experiment**9. Prepare the sample plate**

- a. Use a 96 well, semi-skirted plate.
- b. Pipette 22 μ L of NGS Fragment Diluent Marker Solution into each well of the plate which will hold a DNA sample.
- c. Pipette 22 μ L of NGS Fragment Diluent Marker Solution into well H12, where the ladder will be loaded.
- d. Pipette 24 μ L of BF-25 Blank Solution into any well which will not hold a DNA sample.
- e. Pipette 2 μ L of sample DNA into the appropriate well of the plate according to the Sample Sheet template.
- f. Because the ladder must always be run, each plate can hold a maximum of 95 samples.
- g. Pipette 2 μ L of DNA Ladder into well H12.
- h. Seal the plate and vortex for 10 sec.
- i. Briefly centrifuge the plate to collect the liquid at the bottom of the wells.
- j. 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.

10. Carefully remove the plate seal.**11. Place the sample plate in sample drawer 1. If you have two plates, place the sample plates into sample drawers 1 and 2. Be sure to log the correct plates to the correct tray locations in the software.****12. Enter the sample plates and sample information into the software.**

- a. From the Operation tab, select the sample tray by left clicking the Sample Tray number dropdown.
- b. Use the Excel file created in step 3 to upload the sample information.
- c. Upload the sample name file to the software.
- d. Click on "Load from File".
- e. Select "all file types".
- f. Select the file.
- g. The sample names will populate in the Sample ID column.
- h. Select the option save a copy of the data file and navigate to the PPMS order folder for the save location.

13. Press the green arrow to start running the instrument.**14. Return all reagents to proper storage conditions. Clean up once run is complete (can be left to run overnight).**

After the run

15. Once the run has completed, empty the reservoir waste plate in Drawer W, close the software, and turn off the machine.
16. After the run is completed, data is saved in the default location: C:\AATI\AdvanCE-FS\Data, as well as copied to the location selected in step 9.
17. The only file necessary to post to the client data folder along with the PDF, created later, is found in the data folder (16-50-02 in the first image below) and ends with extension “.raw” (2020 02 20 16H 50M.raw in the second image below).

This PC > eigc_ppms (\\170.140.138.170) (Z:) > Projects > JBO11358 > JBO11358-59540

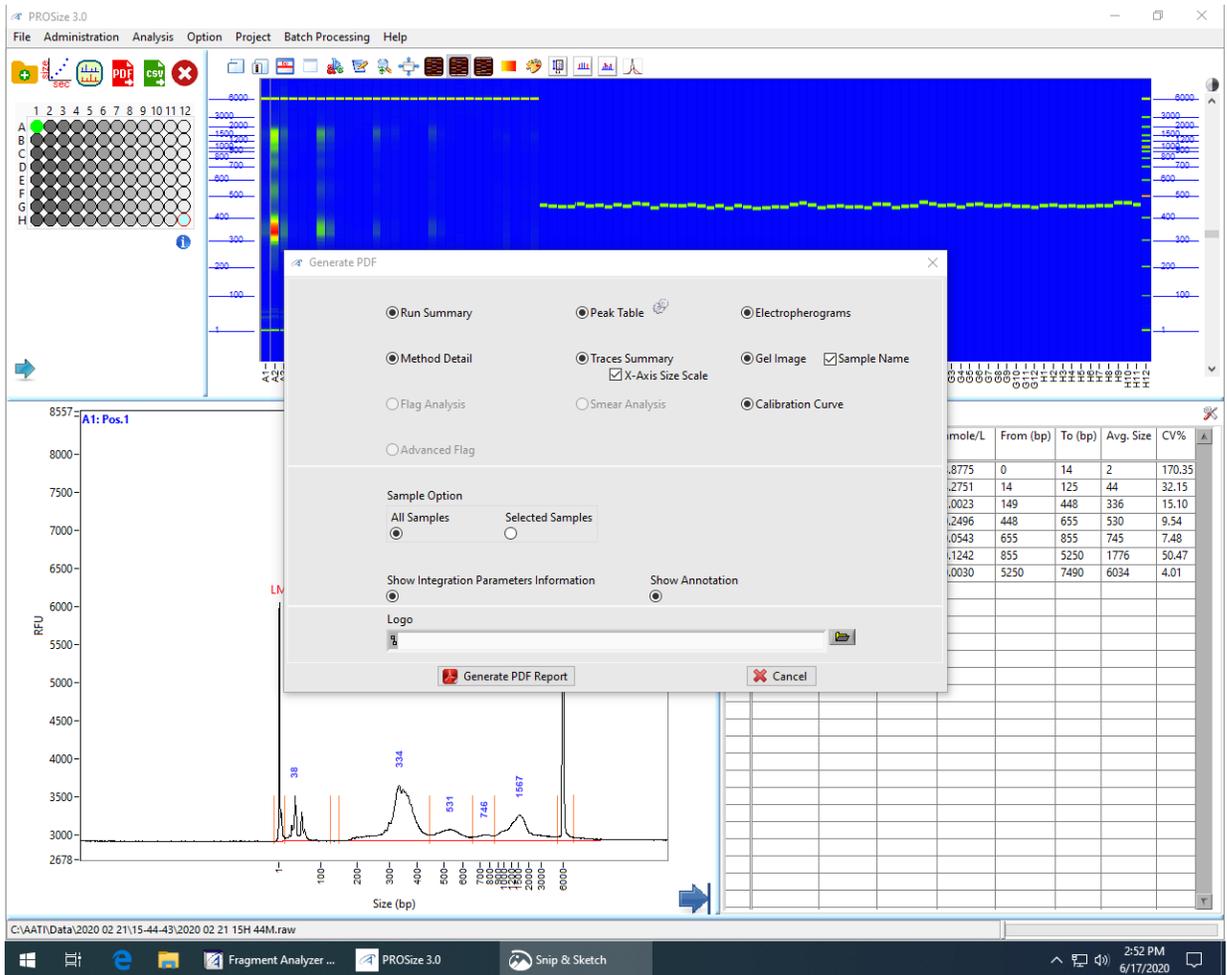
Name	Date modified	Type	Size
16-50-02	2/21/2020 9:36 AM	File folder	
JBO11358-59540_FA_022120.pdf	2/21/2020 9:27 AM	Adobe Acrobat Docu...	3,865 KB
manifest-2413.csv	2/18/2020 4:59 PM	Microsoft Excel Com...	2 KB
manifest-2414.csv	2/18/2020 4:59 PM	Microsoft Excel Com...	6 KB

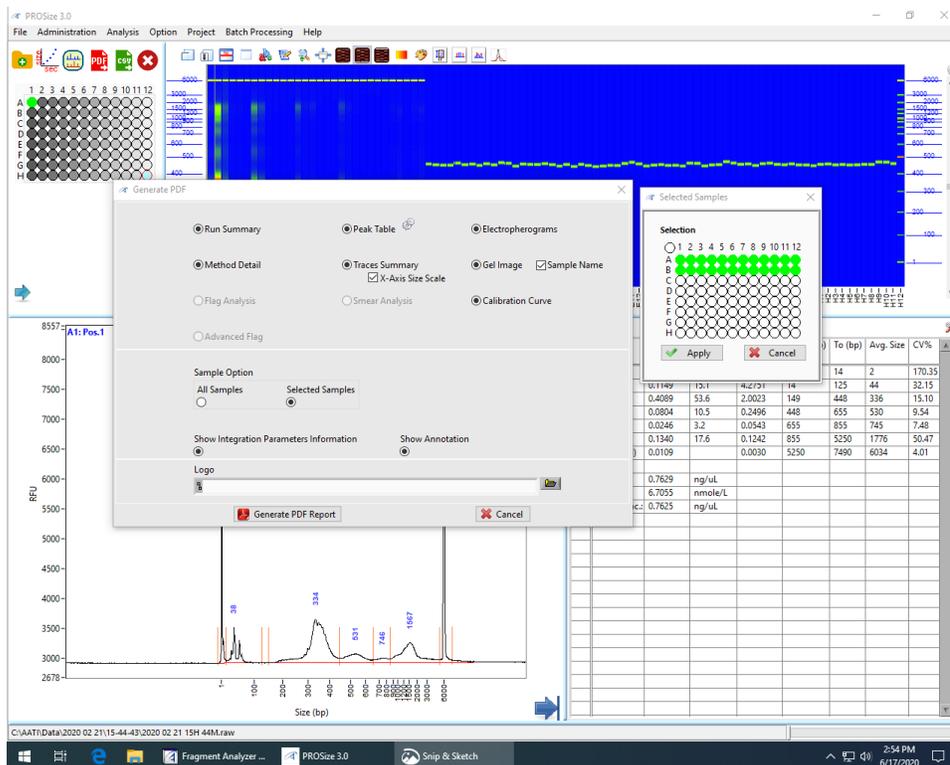
PC > eigc_ppms (\\170.140.138.170) (Z:) > Projects > JBO11358 > JBO11358-59540 > 16-50-02

Name	Date modified	Type	Size
2020 02 20 16H 50M.ANAI	2/21/2020 8:30 AM	ANAI File	90 KB
2020 02 20 16H 50M.current	2/20/2020 6:09 PM	CURRENT File	54 KB
2020 02 20 16H 50M.PKS	2/21/2020 9:24 AM	PKS File	38 KB
2020 02 20 16H 50M.raw	2/21/2020 8:30 AM	RAW File	4,396 KB
2020 02 20 16H 50M.raw2D	2/20/2020 5:19 PM	RAW2D File	23 KB
2020 02 20 16H 50M.txt	2/20/2020 6:09 PM	TXT File	7 KB
CameraImage.bmp	2/20/2020 5:19 PM	BMP File	38 KB
ExpTime.txt	2/20/2020 6:09 PM	TXT File	446 KB
method.mthd	2/20/2020 6:09 PM	MTHD File	2 KB
Timing.txt	2/20/2020 6:09 PM	TXT File	222 KB

18. Generate the PDF Report.

- a. Open ProSize software to analyze the data from the Fragment Analyzer.
- b. Ensure all fragments in the ladder are automatically registered by the software. Ensure all sample traces contain the upper and lower marker.
- c. Generate a PDF Report for the run and save the PDF to the PPMS order folder. If the run has multiple orders, the PDF Report should be generated separately for each order (choose the option “Generate PDF Report for Selected Samples”).





d. Create a zip file containing the raw data file and PDF and post to the PPMS client data folder.

Maintenance

- **Weekly maintenance**
 - a. On Mondays, the storage solution plate should be changed.
 - b. On Fridays, if no plate was run during the week, a validation plate containing known DNA fragments should be run.
- **Monthly maintenance**
 - a. Replace the buffer and waste plates with new ones. Date and initial the plates when changed.
 - b. Inspect the capillary array vent valve for dried gel and clean if necessary.
 - c. Perform a Method 2 hot water wash. See manual Chapter 9, page 10 for details.
- **As needed maintenance**
 - a. If any capillaries show signs of clogging, a 0.5% NaOH wash should be performed. See manual Chapter 9, page 14 for details.
 - b. Do not use aromatic or hydrocarbon-based solvents, chlorinated solvents, or alcohol-based fluids for instrument cleaning.
 - c. Capillary arrays will need to be replaced. Agilent states that an array will typically last for approximately 1 year (or 1000 runs, whatever comes first) if properly maintained. Instructions for removing, installing, and storing arrays can be found in the manual, Chapter 8. Following installation, an alignment of the array may be necessary. Instructions for alignment can be found in Chapter 7 of the manual. We perform the alignment from a file.
 - d. Z error fix

- i. First perform a power cycle to reconnect all boards and ensure that the cables do not have any interruptions in communication.
 - ii. Turn off the instrument and then the computer.
 - iii. Unplug the two USB cables from the back of the computer and wait approximately 10 minutes before reconnecting.
 - iv. Reconnect the USB cables to two, different, unused ports with one port space empty between cables.
 - v. Turn on the computer, allowing it to fully boot to the desktop screen.
 - vi. Turn on the instrument and wait at least 10 seconds before opening the data collection software.
- e. If this does not correct the error, perform a stage reset.
- i. First, ensure the stage is not currently holding a plate by opening each drawer and making sure all the white plate carriers are in their respective drawers. If a carrier is missing, it is being held by the stage.
 - ii. Select Utilities -> Prime and select the Hardware I/O option.
 - iii. Locate the stage section of the new window and select the Reset Stage button. Ensure that the holding number directly above this button goes to zero.
 - iv. Once finished, click the store button on the main screen to ensure the capillaries are immersed in the storage solution again.
 - v. The light next to the Drawer 3 (6th drawer from the top of the instrument) should be off when the plate with the storage solution is on the capillary.

Troubleshooting

See user's manual.