

Agilent Fragment Analyzer Automated CE System

Standard Operating Protocol

I. Overview of Fragment Analyzer Automated CE System

- Maximum sample capacity: 191 samples and 1 ladder

	DNF474 kit	DNF930 kit
Detection range	1 – 6,000 bp	75 – 20,000 bp
Sizing range	50 – 5,000 pg/μl	0.5 ng/μL – 50 ng/μL
Time for run	~1 hour 20 mins for 96 samples, run 12 samples in parallel	~1 hour for 96 samples, run 12 samples in parallel

II. Reagents Preparation

Materials needed:

- High Sensitivity NGS Fragment Analysis Kit (DNF474) / dsDNA Reagent Kit (DNF930)
(Appendix: “Preparation guide for reagents”)

- Semi-skirted 96-well PCR plates
BioRad #HSS-9601
SSIBio # 3450-00
4titude Framestar #4ti-0900, -0950, -0770/C
Eppendorf #951020303
Greiner Bio-One #652280
Neptune #3742.X
MidSci #AVRT1
Scientific Specialty #3450-00
VWR #83007-374

Procedures:

1. Warm up all reagent to room temperature before use.

Storage Temperature	DNF474 kit		
-20°C	DNA Ladders	Diluent Markers	Intercalating Dye
4 °C	Blank Solutions	0.25x TE Rinse Buffers	Separation Gels

Storage Temperature	DNF930 kit		
-20°C	DNA Ladders	Internal Markers	Intercalating Dye
4 °C	Dilution Buffer 1X TE		Separation Gels

1x Inlet Buffer Solution (4°C) and **1x Capillary Conditioning** (4°C).
(Appendix: “Preparation guide for 1x IB and 1x CC loading”)

2. Prepare the **Sample plate** and **Rinse (DNF474)/ Marker (DNF930)** plate according to manufacturer's quick guides (Appendix).
 - *DNA Ladders should be prepared for at least one well per run.**
 - *Fill all empty wells in the rows of run with Blank Solutions (DNF474)/ 1X TE (DNF930).**
 - *Check the plate with CPOS staff before loading samples.**

III. Instrument Preparation

A. Materials needed:

- **Gel/Dye mix**
- **Sample plate(s)**
- **Marker (DNF930) / Rinse (DNF 474) plate**
- **1x Inlet Buffer Solution**
- **1x Capillary Conditioning Solution**

B. Reagent Preparation:

1. Gel/Dye mix

- Prepare the **Gel/Dye mix** by adding Intercalating Dye to Separation Gel (ratio of 1:10000) in a 50 mL tube according to the table below. Mark the date on the tube. Mix by inverting the tube. **Do not vortex.**

Number of run (row)	Volume of Separation Gel	Volume of Intercalating Dye
1	10 ml	1.0 µl
2	14 ml	1.4 µl
3	18 ml	1.8 µl
4	22 ml	2.2 µl
5	26 ml	2.6 µl
6	30 ml	3.0 µl
7	34 ml	3.4 µl
8	38 ml	3.8 µl

NOTE: 1) This volume includes 6 ml for priming. The remaining gel after run can be used for up to 2 weeks after preparation

- Remove the tube of MilliQ in Gel fluid line (1 or 2), clean the fluid line with Kimwipe only. Prime (Main Menu>Utilities>Prime> Gel 1 or 2) twice with air to remove remaining fluid in the line. Inserting Gel fluid line into the **Gel/Dye mix**. Turn the tube instead of cap to close the tube. Ensure the fluid line is positioned at the bottom of the conical tube and stand properly in the slot.
- Update the solution levels in the Fragment Analyzer instrument control software. Main Menu> Utilities> Solution Levels.

2. 1x Capillary Conditioning Solution

- Prepare the **1x Capillary Conditioning Solution** by diluting 5x Capillary Conditioning Solution with MilliQ in a 50 mL tube according to volume in the table below. Mark the date on the tube. Mix by inverting the tube.

Number of run (row)	Volume of 1x Capillary Conditioning Solution	Volume of 5x Capillary Conditioning Solution needed	MilliQ needed
1	14 ml	2.8 ml	11.2 ml
2	18 ml	3.6 ml	14.4 ml
3	22 ml	4.4 ml	17.6 ml
4	26 ml	5.2 ml	20.8 ml
5	30 ml	6.0 ml	24.0 ml
6	34 ml	6.8 ml	27.2 ml
7	38 ml	7.6 ml	30.4 ml
8	42 ml	8.4 ml	33.6 ml

NOTE: 1) This volume includes 4 ml for an additional conditioning step that **MUST BE** performed after every use of the instrument. 2) The remaining volume after run can be used for up to 2 weeks after preparation and 1 month if stored at +4°C

- Insert Conditioning Solution fluid line into the Conditioning Solution. Turn the tube instead of cap to close the tube. Ensure the fluid line is positioned at the bottom of the conical tube and stand properly in the slot.
- Update the solution levels in the Fragment Analyzer instrument control software. Main Menu> Utilities> Solution Levels.

3. **1x Inlet Buffer**

- Mix and use Inlet Buffer at Room Temperature only.
- **2.6 ml of 5x Inlet Buffer and 10.4 ml of MilliQ water** to prepare 13 ml of **1x Inlet Buffer**.
- Mix by gently inverting the tube.
- Discard any liquid in row A in the 96-well Deep Well Plate in the Buffer drawer (B). Reuse the plate and added 1ml of 1x Inlet Buffer prior to your run.

4. **0.25X Rinse Buffer (DNF474)**

- Discard any liquid in the 96 well PCR Plate in the Buffer drawer (M). Reuse the plate and add 100 µl of **0.25X Rinse Buffer** to the indicated current using row.
- Spin the plate shortly to remove air bubbles.

5. **Sample Plate (DNF474)**

- Use 96-well PCR plates in approved list in P.2 only for loading sample. Plates not in the list will **DAMAGE THE CAPILLARIES. Check your plate with CPOS staff** before loading samples.
- Add 22 µl of the **Diluent Marker** to each well to be analyzed.
- Add 2 µl of Ladder in one of the well that containing 22 µl **Diluent Marker**.
- Add 2 µl of samples in other wells that containing 22 µl **Diluent Marker**. Mix the solution by swirling with the pipette tip while pipetting up/down.
- You may change the ratio of the sample / ladder to **Diluent Marker** if necessary. Update the dilution factor in the software for proper analysis.
- Fill any unused wells within the row with 24 µl/well of Blank Solution.
- Remove the bubbles by spinning the plate shortly.
- Load the Sample plate in sample drawer 1 or 2 and perform the separation immediately. Otherwise store the plate at +4 °C until use.

C. Procedure:

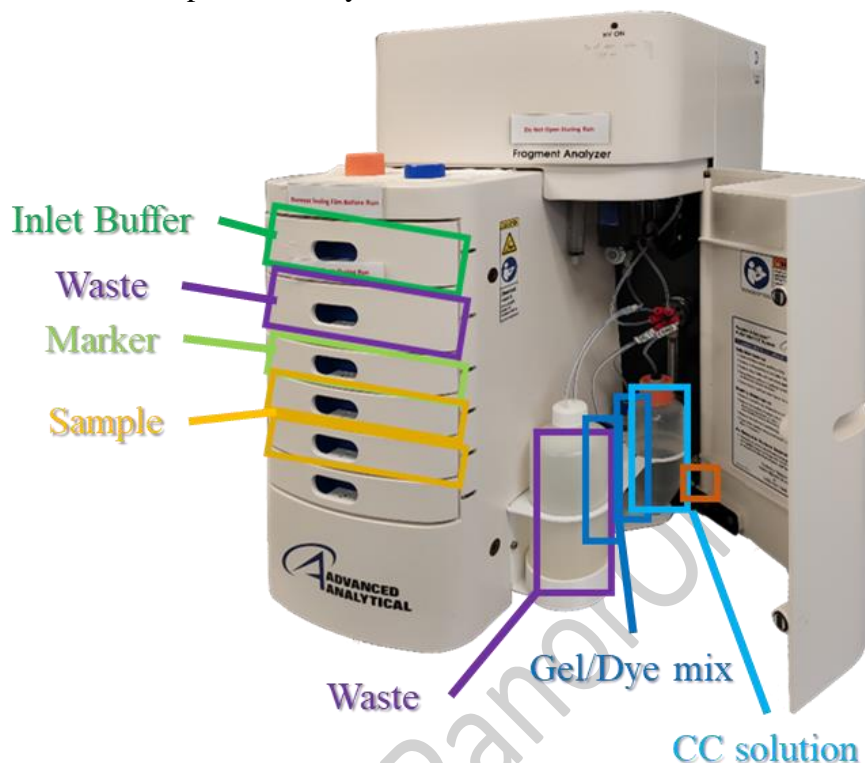
1. Sign on the Log Book before start.
2. Turn on the **Fragment Analyzer's Power Switch** and computer.
3. Login to computer user account with the password in the lower left corner.
4. Open Fragment Analyzer Instrument control Software.

User ID: User

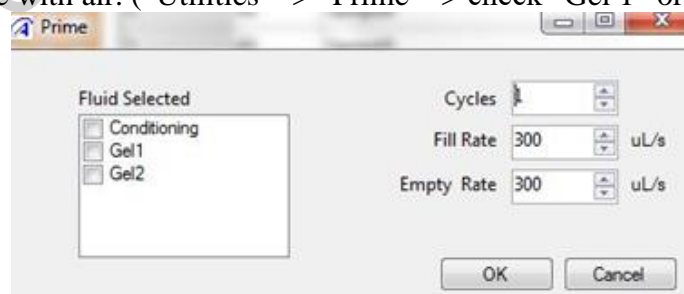
Password: 1234

Acknowledge the maintenance reminder.

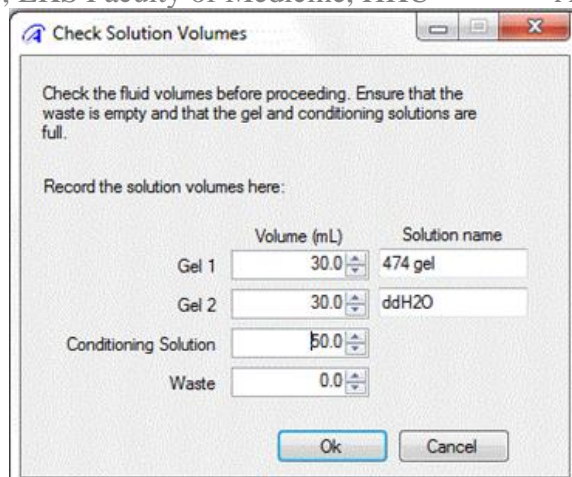
Maintenance is performed by CPOS Staff.



5. Check the **Waste Bottle and the Waste plate** at the Waste Tray (W) and empty if needed.
6. Remove any solution from Buffer Tray (B).
7. Load 1 mL **1x Inlet Buffer Solution** per well.
8. Load **1x Capillary Conditioning Solution**.
9. Take out the **Gel fluid line 1 or 2** from the storage MilliQ tube
10. **Prime** with the tube with air. ("Utilities" -> "Prime" -> check "Gel 1" or "Gel 2" -> "OK").



11. Clean the outside surface of the tube with Kimwipe.
12. Put the **Gel/Dye mix** solution to the Gel line.
13. Update the solution levels to the software ("Utilities" -> "Solution levels").



Check the fluid volumes before proceeding. Ensure that the waste is empty and that the gel and conditioning solutions are full.

Record the solution volumes here:

	Volume (mL)	Solution name
Gel 1	30.0	474 gel
Gel 2	30.0	ddH2O
Conditioning Solution	50.0	
Waste	0.0	

Ok Cancel

14. Load **Marker (DNF930) / Rinse (DNF 474) plate into Marker tray (M).**

15. Load **Sample plate(s) into put into Sample Tray I (and II).**

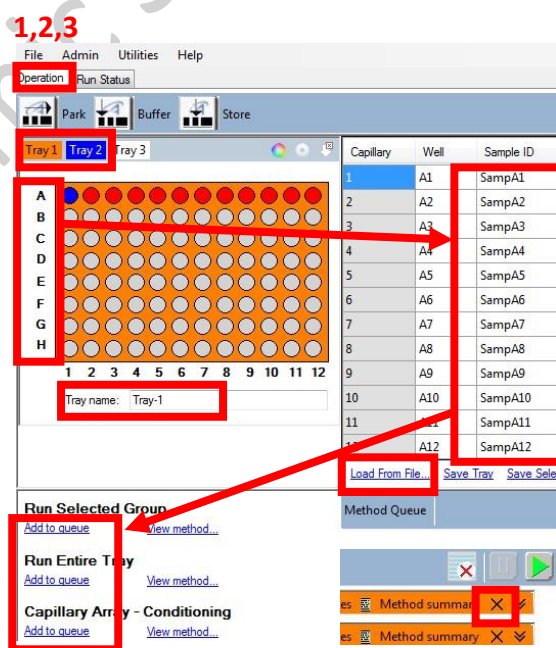
***REMOVE any PLATE SEAL before loading the plate into tray.**

!!!The seal would DAMAGE THE CAPILLARIES!!!

***Load all plate into tray with A1 IN INNER LEFT CORNER.**

IV. Software Settings

- In Operation tab, the Tray (1 or 2) that loaded with sample plates.
- Select the sample row that loaded with sample. Sample name can be input in "Sample ID" column or import from .csv or .txt file. Enter Tray name with prefix of "Supervisor Name_User Name" and followed by the experiment name
- For analyzing single row of samples, click "Add to queue" under "Run Selected Group". For analyzing more than 1 row of samples, click "Add to queue" under "Run Entire Tray". Remove extra queue with "X" next to "Method summary".
- In Separation Setup window, Select or input:
 - Method: Name of Analysis Kit Used
DNF-474-33 – HS NGS Fragment 1-6000bp methods/
DNF-930-33 – DNA 75-20000bp methods
 - In "Edit", select the row of marker or rinse buffer loaded
 - Gel: Position of the gel loaded (Gel 1 or Gel 2)
 - Tray Name: Same as name in Step 2.
 - Folder Prefix: Enter in form of "Supervisor Name_User Name"
 - Any Notes: Enter if necessary
 - OK: to add the method to queue.
- Select other row or plates in the Tray window and repeat steps 3-4



1,2,3

File Admin Utilities Help

Operation Run Status

Park Buffer Store

Tray 1 Tray 2 Tray 3

Capillary	Well	Sample ID
1	A1	SampA1
2	A2	SampA2
3	A3	SampA3
4	A4	SampA4
5	A5	SampA5
6	A6	SampA6
7	A7	SampA7
8	A8	SampA8
9	A9	SampA9
10	A10	SampA10
11	A11	SampA11
12	A12	SampA12

Tray name: Tray-1

Run Selected Group

Add to queue View method...

Run Entire Tray

Add to queue View method...

Capillary Array - Conditioning

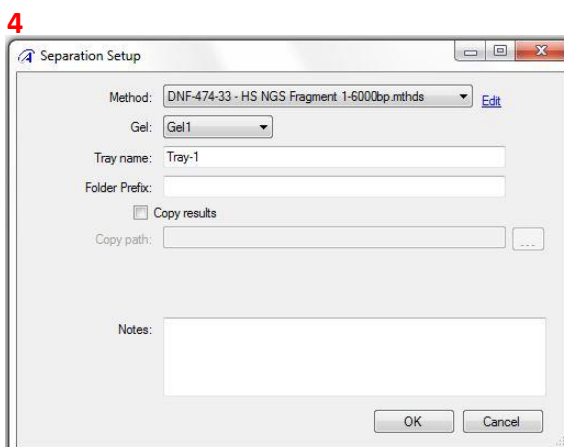
Add to queue View method...

Load From File... Save Tray Save Selections

Method Queue

Method summary X

Method summary X



4

Separation Setup

Method: DNF-474-33 - HS NGS Fragment 1-6000bp.mthds Edit

Gel: Gel1

Tray name: Tray-1

Folder Prefix:

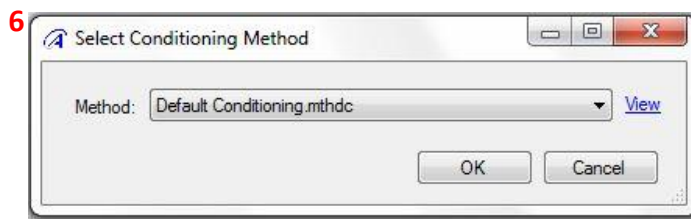
☐ Copy results

Copy path:

Notes:

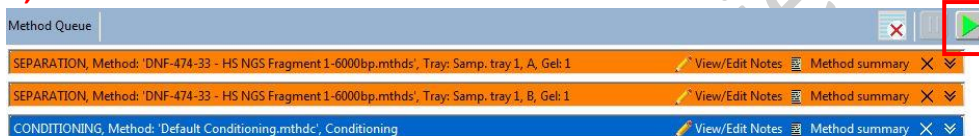
OK Cancel

6. Click “Add to queue” under “Capillary Array – Conditioning” and select “Default conditioning” to remove gel in the capillaries after your experiment.
7. Verify all method in queue and trays (Inlet buffer, Rinse buffer/Marker, Sample tray I or II, Gel I or II, Conditioning Solution) are loaded with appropriate container/plate with sufficient reagents.
8. Click start to initiate the run.
9. Separation status can be observed in “Run Status” tap. Queue of Separation can be modified or added in the Operation tap. Separation method should be always followed by “Default conditioning”.



DO NOT Open Top Compartment or Drawer B and W. The high voltage would be stopped when any of these drawers is opened.

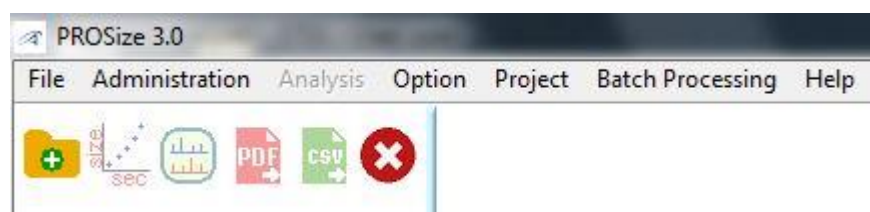
7, 8



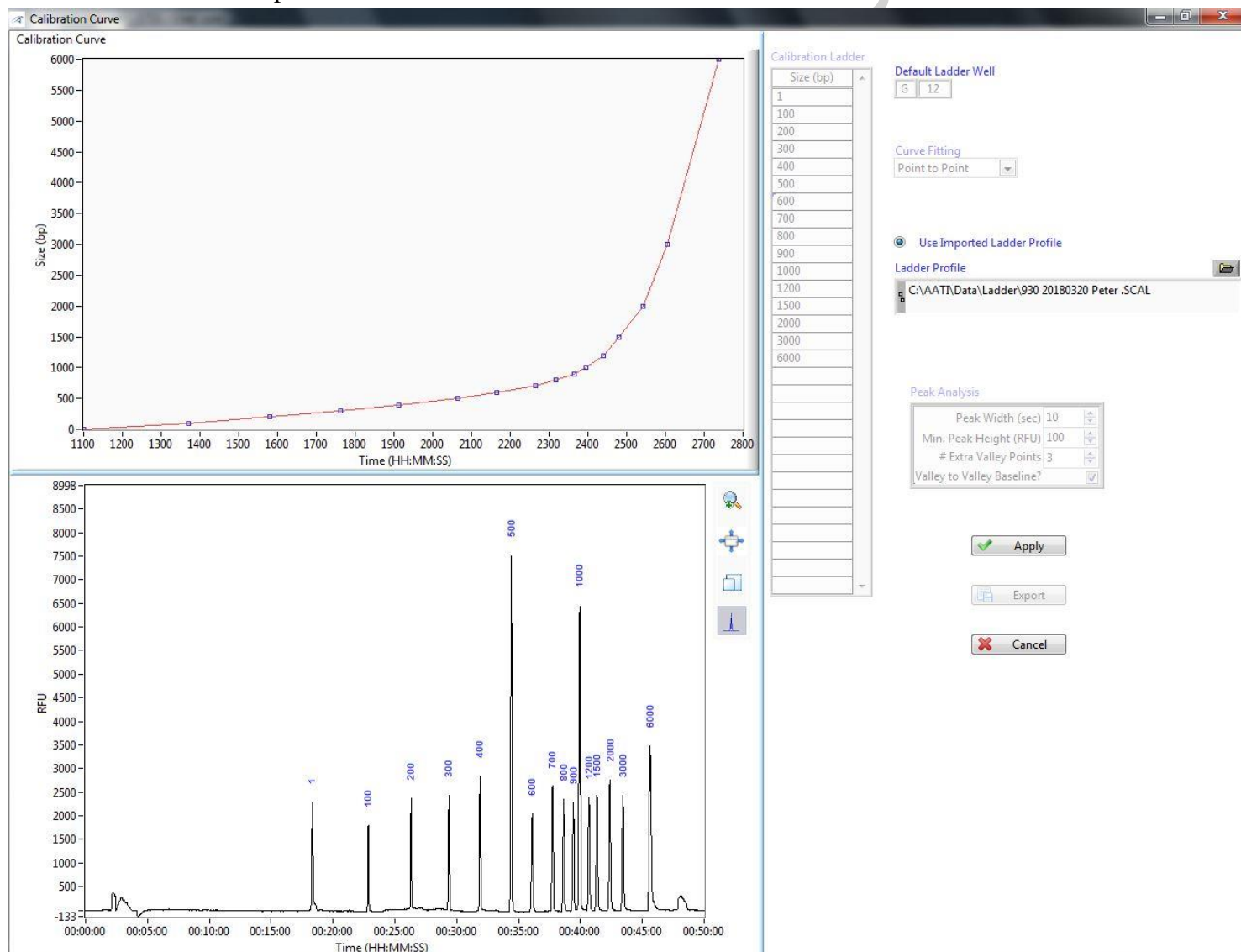
V. Data Analysis

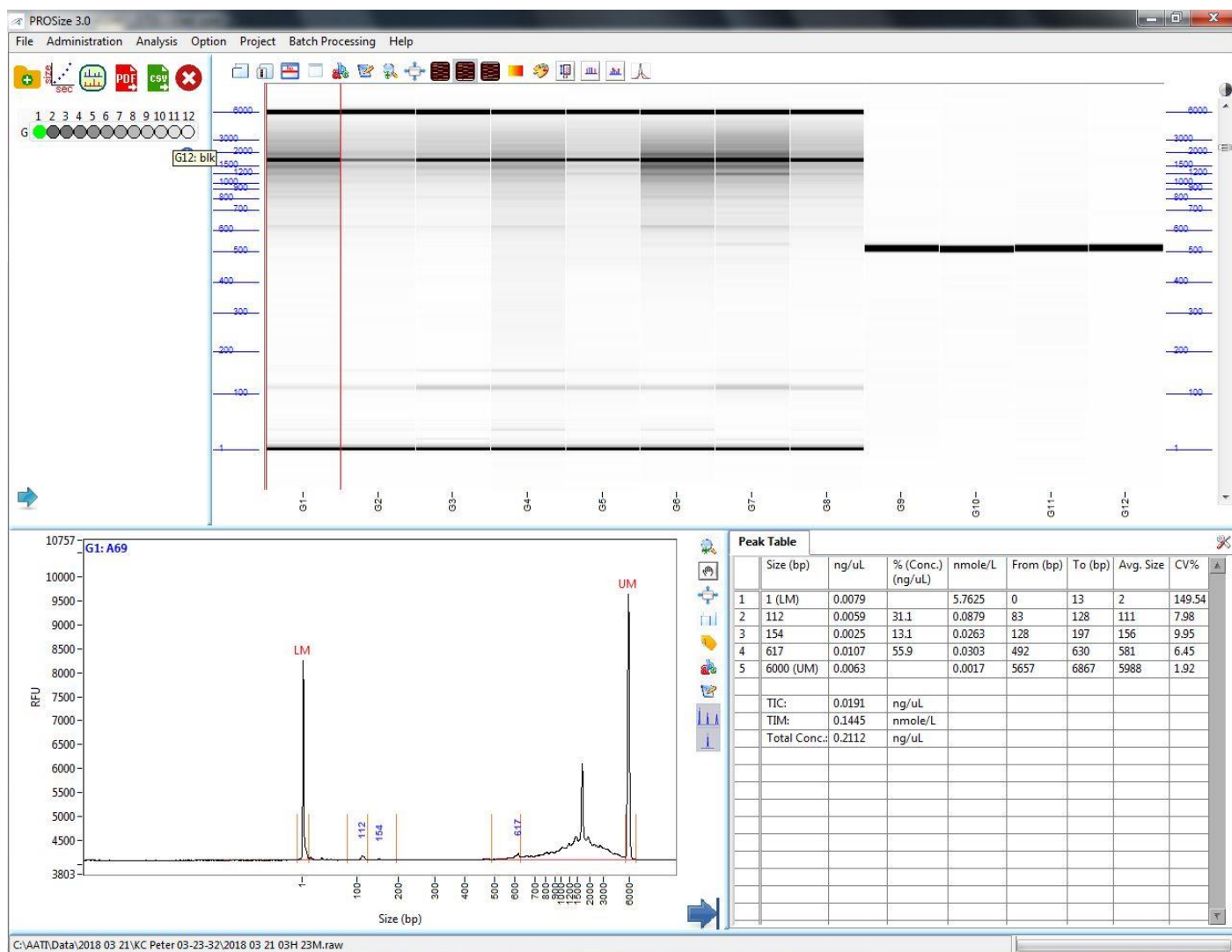
You can install **PROSize 3.0** on your own computer to analyze the data.


1. Open your run by clicking “File” – “Open File”. Select the correct file and click “Open”.
The data is saved in “:C:\AATI\Data\” by default according to date.

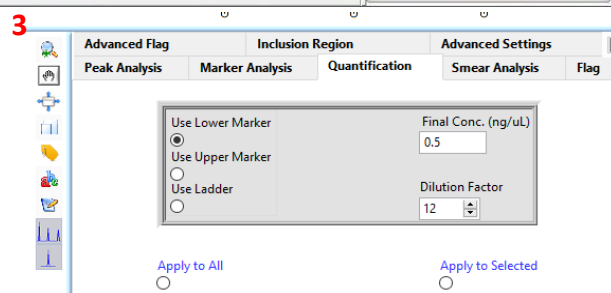


2. Select “Analysis” – “Show size calibration”. Adjust the peak Width and Height so that all the points fit the red line. Right click on the peak to add or remove single peak. If the ladder is not ok, you can import ladder from a previous successful run.

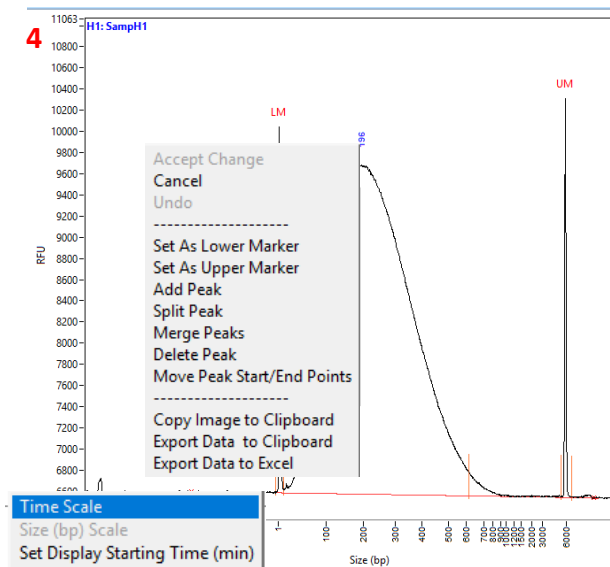
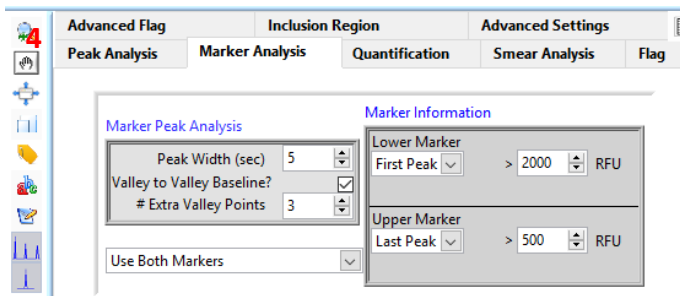




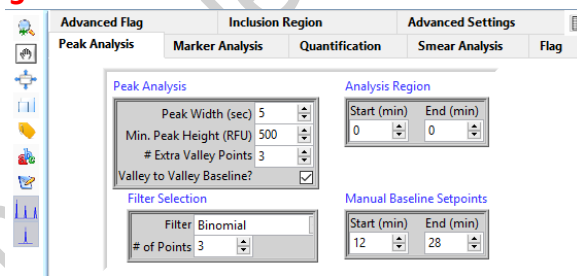
- Change the dilution factor if your dilution is not 12 (2 μ l sample + 22 μ l Diluent). It can be changed by clicking on  icon on the upper right corner of a peak table. Select "Quantification", change the dilution factor and press "Apply to selected" or "Apply to all".



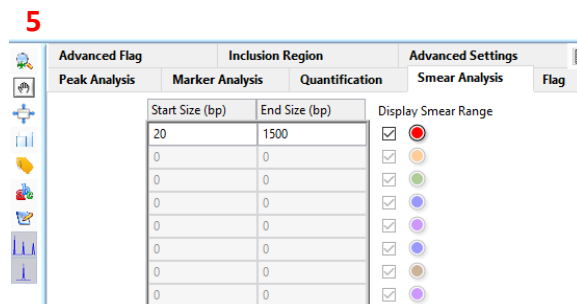
- Select “Marker Analysis”, change the Marker Information and press “Apply to selected” or “Apply to all”. If the software hasn’t placed the lower (LM) and upper marker(UM) correctly for a certain sample, right-click on the correct peak and choose “Set as lower marker” or “Set as upper marker”.



- Change other parameters including the baseline (“Peak Analysis” – “Manual Baseline Setpoints” – “Start (min)” and “End (min)”); 12-48 for DNF474; 12-28 for DNF930), peak analysis and smear analysis.



- Data can be exported in .csv and .pdf format. The data is given in three different concentrations: TIC = total integrated concentration in ng/μL, TIM = total integrated molarity in nmole/L and total concentration in ng/μL. Be aware that based on your application, you may want to use for example TIC rather than the total concentration.



- Exit the program. Your changes are saved automatically. In case you want to return to the original raw data without any changes made by you, go to the corresponding folder to delete .ANAI and .PKS files.



Fragment Analyzer™ Automated CE System

Quick Start Guide - 12 Capillary

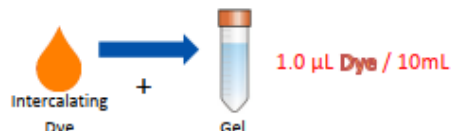
High Sensitivity NGS Analysis Kit,

1 bp - 6,000 bp

DNF-474

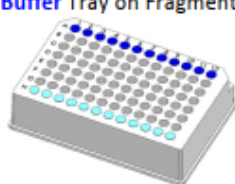
Preparation:

1. Mix fresh Gel and **Dye**. Refill 1X **Conditioning Solution** as needed.



2. Place a fresh **1X Inlet Buffer** Tray on Fragment Analyzer.

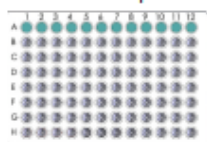
Replace Capillary Storage Solution every 2-4 weeks
1.0 mL/well



Replace Inlet Buffer Daily
1.0 mL/well

- = Inlet Buffer - Row A Only
- = Storage Solution - Row H only

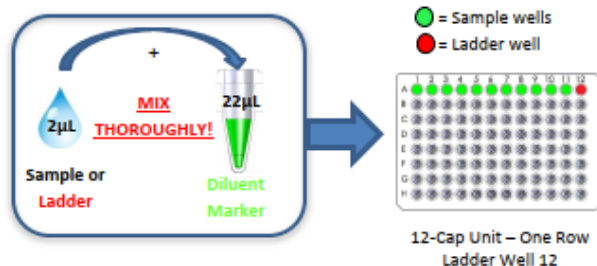
3. Place **Rinse Buffer** plate in Marker Drawer location.



Replace Rinse Buffer Daily
200 µL/well

- = Rinse Buffer - Fill Row A Only

4. Mix **Samples** or **Ladder** with **Diluent Marker** in Sample Plate. Add 24 µL of **Blank Solution** to any unused wells of the row to be analyzed.



Software:

1. Select Tray and Row to run for 12-Cap.
2. Enter Sample ID and Tray ID (optional).
3. Select "Add to Queue", select the DNF-474-(22, 33 or 55) HS NGS Fragment 1-6000bp method from the Dropdown menu.
4. Enter Tray Name, Folder Prefix, and Notes (optional), Select OK to add Method to the Queue.
5. Select to Start the Separation.

*Please refer to the Kit User Manual for additional details.

Reagents:

- NGS Fragment Separation Gel, part # DNF-240
- Intercalating Dye, part # DNF-600-U030
- 5X 930 dsDNA Inlet Buffer, part # DNF-355 (Dilute to 1X)
- 5X Capillary Conditioning Solution, part # DNF-475 (Dilute to 1X)
- 0.25X TE Rinse Buffer, part # DNF-497
- HS NGS Diluent Marker 1bp-6000bp, part # DNF-373
- HS NGS DNA Ladder, part # DNF-396
- BF-25 Blank Solution, part # DNF-300

- Capillary Storage Solution, part # GP-440-0100 (sold separately)

*Note: Color codes of Guide may not correlate with color codes of actual reagent component.

Gel Guide:

For 12-capillary Fragment Analyzer™ systems:

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

Specifications:

Specifications	Description
DNA Sizing Range	100 bp - 6,000 bp
Separation Resolution	100 bp - 2000 bp ≤ 5%; 2000 bp - 6000 bp ≤ 10%
DNA Sizing Accuracy ¹	± 5% or better
DNA Sizing Precision ¹	2% CV
DNA Fragment Concentration Range ¹	5 pg/µL to 500 pg/µL input DNA
DNA Smear Concentration Range ¹	50 pg/µL to 5,000 pg/µL input DNA
DNA Quantification Accuracy ¹	± 25%
DNA Quantification Precision ¹	15% CV
Maximum DNA Concentration	500 pg/µL per fragment; 5 ng/µL total

¹: Using DNA Fragment standards and DNA smears prepared in 1X TE buffer



Fragment Analyzer™ Automated CE System

Quick Start Guide - 12 Capillary

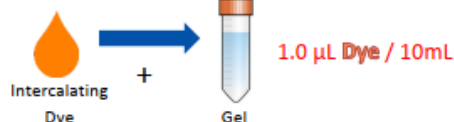
dsDNA 930 Reagent Kit,

75 bp - 20,000 bp

DNF-930

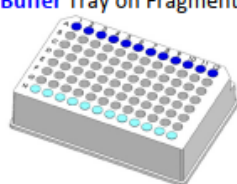
Preparation:

1. Mix fresh Gel and Dye. Refill 1X Conditioning Solution as needed.



2. Place a fresh 1X Inlet Buffer Tray on Fragment Analyzer

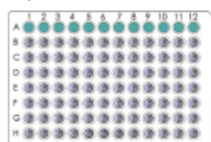
Replace Capillary Storage Solution every 2-4 weeks
1.0 mL/well



Replace Inlet Buffer Daily
1.0 mL/well

- = Inlet Buffer - Row A Only
- = Storage Solution - Row H only

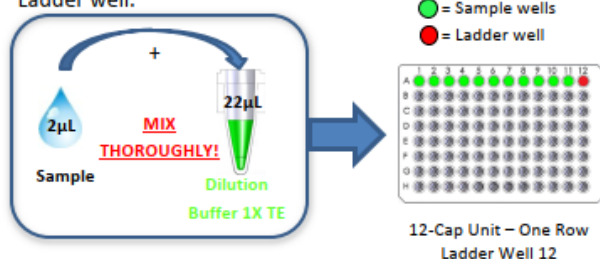
3. Place Marker plate in Marker Drawer location.



30 µL/well +
Mineral Oil
Overlay

- = Marker - Fill Row A Only

4. Dilute Ladder (50 ng/µL) to desired working concentration:
 - a. Dilute 12X (2 µL Ladder per 22 µL 1X TE Buffer) for samples > 10 ng/µL
 - b. Dilute 50X (1 µL Ladder per 49 µL 0.1X TE Buffer) for samples < 10 ng/µL
5. Mix Samples with 1X TE Dilution Buffer or 0.1X TE Buffer depending upon concentration.* Place prepared ladder in Ladder well.



- = Sample wells
- = Ladder well

Software:

1. Select Tray and Row to run for 12-Cap.
2. Enter Sample ID and Tray ID (optional).
3. Select "Add to Queue", select the DNF-930-(22, 33 or 55) - DNA 75-20,000bp method from the Dropdown menu.
4. Enter Tray Name, Folder Prefix, and Notes (optional), Select OK to add Method to the Queue.
5. Select ▶ to Start the Separation.

*Please refer to the Kit User Manual for additional details.

Reagents:

- dsDNA 930 Separation Gel, part # DNF-930
- Intercalating Dye, part # DNF-600-U030
- 5X 930 dsDNA Inlet Buffer, part # DNF-355 (Dilute to 1X)
- 5X Capillary Conditioning Solution, part # DNF-475 (Dilute to 1X)
- Dilution Buffer 1X TE, part # DNF-495
- 75 bp and 20,000 bp Markers, part # FS-SMK930
- 1,000 bp Plus DNA Ladder, part # FS-SLR930
- Mineral Oil, part # FS-SMO15

- Capillary Storage Solution, part # GP-440-0100 (sold separately)

*Note: Color codes of Guide may not correlate with color codes of actual reagent component.

Gel Guide:

For 12-capillary Fragment Analyzer™ systems:

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

Specifications:

Specifications	Description
DNA Sizing Range	75 bp - 20,000 bp
Separation Resolution	75 bp - 1,500 bp ≤ 10%; 1,500 bp - 20,000 bp ≤ 15%
DNA Sizing Accuracy ¹	± 10% or better
DNA Sizing Precision ¹	5% CV
DNA Fragment Concentration Range	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 with 1X TE buffer