

Detection range Sizing range: 1 – 6000 bp; 50 – 5000 pg/μl

1. Getting Ready for Fragment Analysis

Get **DNA Ladders**, **Diluent Markers** and **Intercalating Dye** from -20°C storage and **0.25x TE Rinse Buffers**, **Separation Gels** and **Blank Solutions** from +4 °C storage. **Warm up all reagent to room temperature before use.**

1x Inlet Buffer Solution and **1x Capillary Conditioning** should be prepared and loaded by Core Facility staffs just before your booked sessions.

Prepare the sample plate with **Sample, Diluent Markers and Ladders** (at least one well of Ladder per run)

Use specified semi-skirted 96-well PCR plates only in approved list below for loading sample. Plates not in the list will **DAMAGE THE CAPILLARIES. Check the plate with administrator before loading samples.**

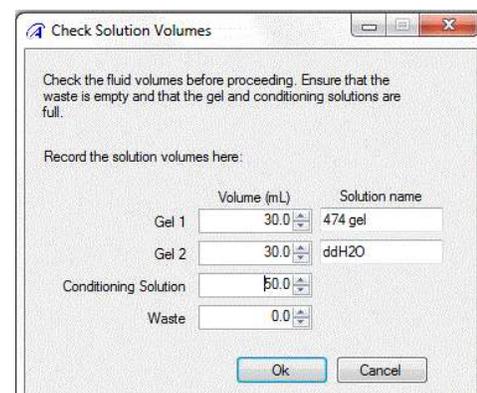
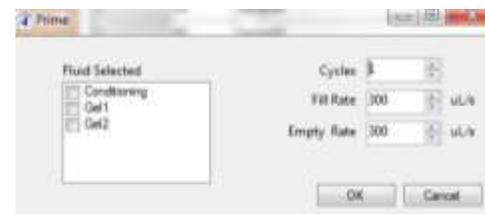
VWR #83007-374, #89049-178; Eppendorf #951020303; MidSci Pryme #AVRT1; BioRad #HSS-9601; Greiner Bio-One #652280; 4titude Framestar #4ti-0900,-0950,-0770/C; Scientific Specialty #3450-00; Neptune #3742.X;

2. Setting up Fragment Analyzer

1. Sign on the Log Book before start.
2. Turn on the **Fragment Analyzer's Power Switch** and computer.
3. Open Fragment Analyzer Instrument control Software (User ID: User, password: 1234). Acknowledge the maintenance reminder. Maintenance is performed by Core Facility's Staff.
4. Check the **Waste Bottle and the Waste plate** at the Waste drawer (W) and empty if needed. Update the solution level in the software (Utilities – Solution levels).
5. Get the **Gel fluid line 1 or 2** from the storage MilliQ tube and **prime** with the tube with air. (Utilities – Prime, select Gel 1 or Gel 2, click OK). Clean the outside surface of the tube with Kimwipe.
6. Put the **Gel/Dye mix** solution to the Gel line and update the solution level to the software (Utilities – Solution levels).
7. Check the level of **1x Capillary Conditioning Solution** and add or replace if necessary and update the solution level in the software (Utilities – Solution levels).
8. Check the **1x Inlet Buffer** in the 96-well Deepwell plate in the Buffer drawer (B).
9. Replace the **0.25X Rinse Buffer** in 96 well PCR plate at designated row in drawer M.
10. Load the sample plate with **Sample, Diluent Markers and Ladders** (at least one well of Ladder per run) into **put into Sample Drawer I or II.**

REMOVE any PLATE SEAL before loading the plate into drawer. The seal would DAMAGE THE CAPILLARIES!!!

Load all plate into drawer with A1 IN INNER LEFT CORNER.



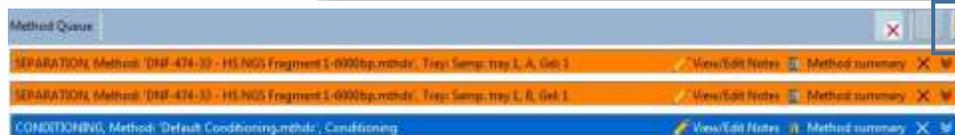
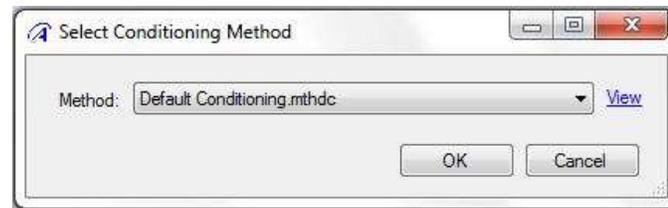
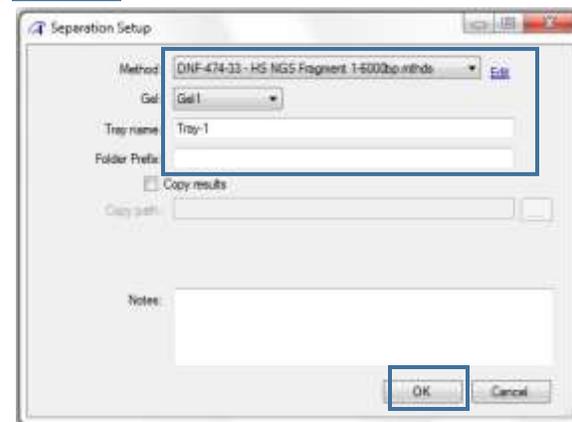
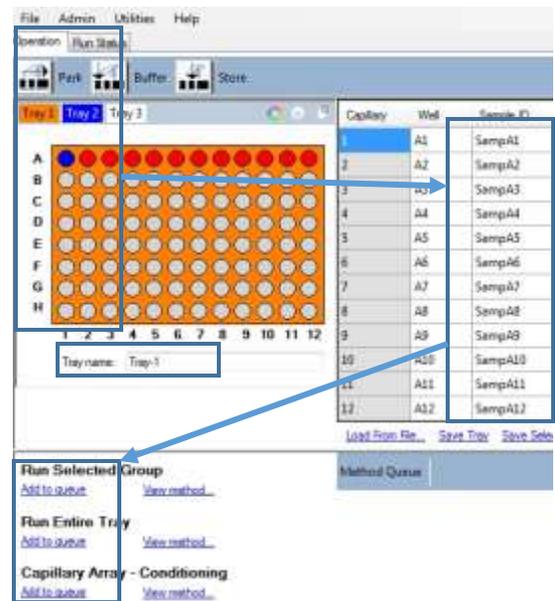
3. Setting in the Software

1. In Operation tab, the Tray (1 or 2) that loaded with sample plates.
2. 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
3. For analyzing 1-7 rows of samples, click "Add to queue" under "Run Selected Group". For analyzing entire plate of samples, click "Add to queue" under "Run Entire Tray".

In Separation Setup window, Select or input:

- Method: Name of Analysis Kit Used
 - In Edit, select the row of marker loaded 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.
4. Select other row or plates in the Tray window and repeat steps 3
 5. After adding analysis Method for your sample click "Add to queue" under "Capillary Array –Conditioning" and select "Default conditioning" to for remove gel in the capillaries after your experiment.
 6. Verify all method in queue and trays (Buffer, Rinse, Sample I or II, Gel I or II, Conditioning Solution) are loaded with appropriate container/plate with sufficient reagent.
 7. Click start to initiate the run.
 8. 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.



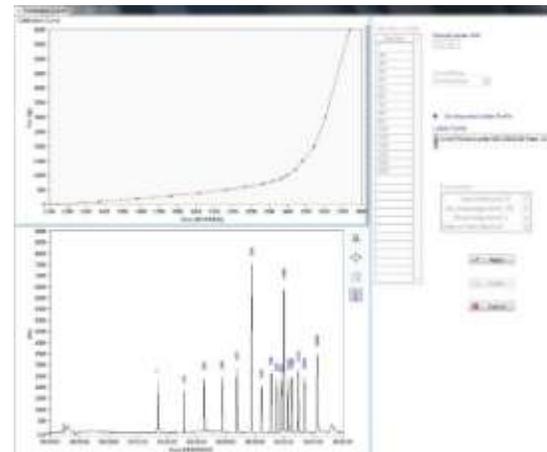
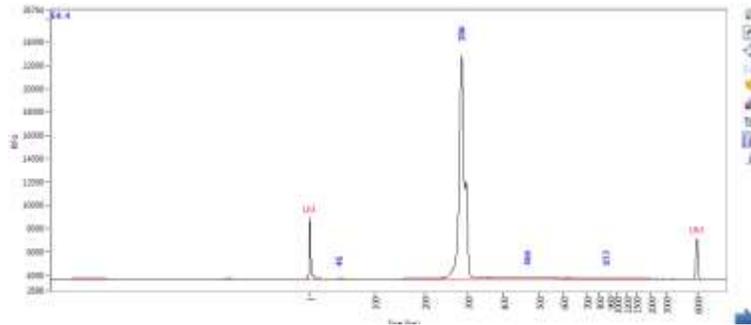
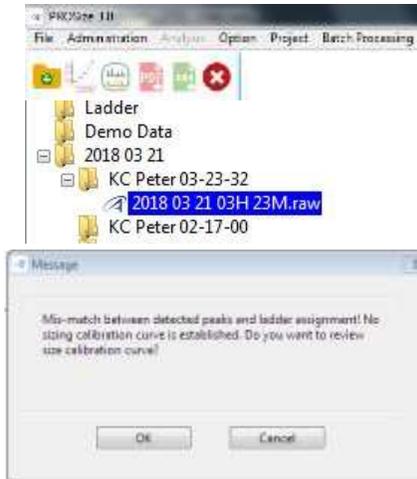
4. Turning Off the System

1. Remove the sample plate or store it for future use.
2. Remove the Separation Gel. Clean the outside surface of the tube with Kimwipe. Prime the gel line 1 and/or 2 with MilliQ in 50ml centrifuge tube. Store the Gel line in MilliQ. Gel can be reused within two weeks from preparation date.
3. Discard Liquid and rinse in **Waste Bottle and the Waste plate** at the Waste drawer (W). Update the solution level to the software (Utilities – Solution levels).
4. Discard the solution in Inlet buffer plate and Rinse/Marker plate. Return the plate to the drawer B and M. !!!Do not throw away the plate.
5. Exit the software, analyze data with ProSize 3.0 if necessary, and turn off the computer and **Fragment Analyzer's Power Switch**
6. Sign off in Log Book before leave

5. Data Analysis

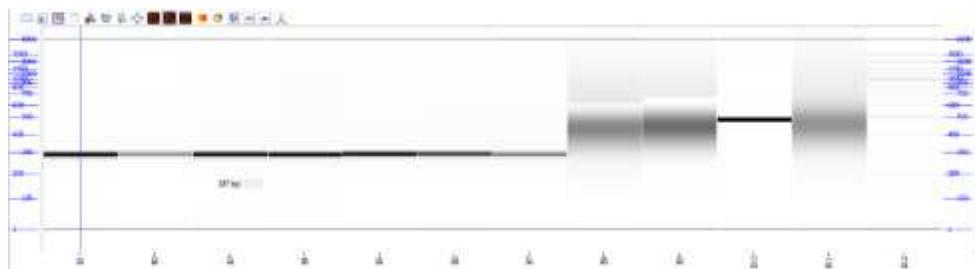
PROSize 3.0 for Fragment Analyzer's data analysis can be downloaded from manufacturer's website. (<https://www.aati-us.com/support/software/>) on your own computer.

1. Open your data by selecting the appropriate .raw file. and click "Open".
2. The data would be automatically normalized if Ladder is recognized by the software. Results of other well then can be analyzed directly. Otherwise click on "OK" or the Mis-match message Or click "Ladder calibration" to adjust ladder calibration or import Ladder from other run. Add or deleted peaks in the retention graph so that the elution time of identifies peaks fit into the size calibration curve. (Ladder information can be checked from the kit manual or APP "MyFrag". Calibrated Ladder profile from other run can be load by selecting "Use Imported Ladder Profile"
3. To ensure sample size is correctly calibrate for each sample, select the well and check each electropherogram that "Lower Marker" and "Upper Marker" at appropriate retention time are recognized and marked as "LM" and "UM". (Marker's information can be obtained from kit manual or "MyFrag"). Right click on the appropriate peak and select "Set as Lower/Upper Marker" if the marker are not correctly recognized by the software. To review retention time in raw data, select "Quick Display"



"Analysis menu.

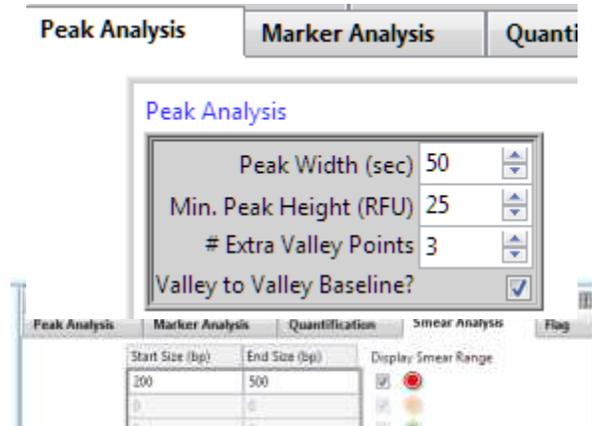
4. With proper size calibration, the Digital Gel Image of the row can be reviewed or copies in the top right windows. Contrast of the Gel Image can be adjust in the right slider. Psuedo color can be changed in the tools bar.



5. To compare electropherogram or Digital Gel picture from different runs, use "Sample Overlay" in Analysis menu.
6. Details of the Size and concentration can be reviewed in the Peak Table. To change the threshold for peak detection, in "Set Individual Parameters", go to "Peak Analysis" tape, change "", Click "Show Results" and find the updated result in the Peak Table. Or in the electropherogram, right click on the desired peak and "Add Peak" and "Accept Change"

Peak Table							
	Size (bp)	ng/uL	% (Conc. (ng/uL))	nmole/L	From (bp)	To (bp)	Avg. Size
1	1 (LM)	0.0725		94.6760	0	12	182.83
2	46	0.0426	0.5	1.3422	30	78	18.66
3	266	7.0603	93.3	45.6740	224	361	4.88
4	466	0.3286	3.8	1.1804	394	543	8.75
5	873	0.2000	2.4	0.2860	741	1354	33.43
6	6000 (UM)	0.0281		0.0077	5676	6583	1.62
TIC:		8.5413	ng/uL				
TBM:		48.4826	nmole/L				
Total Conc:		8.0833	ng/uL				

7. For Smear Analysis, in "Set Individual Parameters", go to "Smear Analysis" tape, input Smear range. "Apply to All" or "Apply to Selected if necessary. Click "Show Results" and go to "Smear Analysis" tab to review smears' data.
8. If marker and sample ratio is different from default of the kit, in "Set Individual Parameters", go to "Quantification" tape, and change "Dilution Factor" to appropriate number. "Apply to All" or "Apply to Selected if necessary. Click "Show Results" and find the updated result in the Peak Table.



6. Appendix

1. Sample Plate

- Use 96-well PCR plates in approved list in P.1 only for loading sample. Plates not in the list will **DAMAGE THE CAPILLARIES. Check your plate with administrator** 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

2. Gel/Dye mix

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

- 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.**
NOTE: 1) This volume include 6 ml for priming. The remaining gel after run can 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>Pri me> 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.

3. 0.25X Rinse Buffer

- 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.

4. 1x Capillary Conditioning Solution (Prepared by Core Facility)

- 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 include 4 ml for an additional conditioning step that **MUST BE** performed after every use of the instrusment. 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.

5. 1x Inlet Buffer (Prepared by Core Facility)

- 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.