



NGS High Sensitivity kit (DNF-474) _ Fragment Analyzer short protocol

This kit is used for DNA smears (e.g. NGS libraries) ranging from 50 pg/μL to 5 ng/μL, or DNA fragments from 5 pg/μL to 500 pg/μL. Sizing range is 25bp-5'000bp (above 6kb the migration is bad, therefore use the "Large Fragments" kit for these cases).

CAUTION: RNase-treated samples must have undergone a purification step before being run. Failure to do so will lead to failure of subsequent RNA runs. More generally, only purified samples should be run.

Preliminary step

1. Book the instrument. Billing is done according to this booking, so please book 1 hour per lane (1 lane = 11 samples + 1 ladder). Pricing is indicated on our web site.

30 minutes before samples/instrument preparation

2. Determine if a NGS gel needs to be prepared, by looking inside the instrument whether enough fresh NGS gel is present in one of the Falcon tubes (fresh = made within the last 6 days). You will need 10ml for 1 lane and 5 additional ml for each additional lane. You will also need the intercalating dye.

3. Collect the reagents as indicated below.

Take out of the fridge and put at room temperature (for approximately 30 minutes):

NGS Fragment separation gel (DNF-240)	Only if required (see above).
Only if you are the first user of the day:	
TE 0.25x buffer (DNF-497)	One 240ul strip.
1x dsDNA Inlet buffer (DNF-355)	

Take out of the -20° freezer and put at room temperature (for approximately 30 minutes):

HS NGS Fragment DNA Ladder (DNF-396)	
HS NGS Fragment Diluent Marker (DM) (DNF-373)	Take an aliquoted tube if available. Protect from light.
Intercalating dye (DNF-600)	Only if required (see above). Protect from light.

Instrument preparation (20 minutes before run)

4. Check volume of Capillary conditioning solution in the 250ml Falcon tube in the instrument (7ml per lane are needed). If not enough is left, please contact the GECF team. Check for absence of bubbles.

5. If required, prepare the gel in a *new* 50ml Falcon tube:

- For 1 lane, 10ml of NGS Separation Gel +1 ul of intercalating dye. Mix gently, AVOID BUBBLES.
- For each additional lane, add 5ml of gel + 0.5ul of intercalating dye.
- If the new gel will be added at a “gel position” in the instrument that contained another sort of gel, a priming will have to be performed (more details below), so prepare 3 additional ml.

→ Label the new Falcon tube with the date, and insert it in the instrument at either position “gel 1” or “gel 2” (if an older gel of same kind was already present in the instrument, use that position). Check the absence of bubbles. Update the gel name and volume in the software (main menu, Utilities → Solution Levels). If the new gel is added in a “gel position” that contained another sort of gel, prime the instrument with the newly prepared gel (main menu, Utilities → Prime → select your gel and press ok).

Note: when a new gel has to be made fresh because the one in the instrument is too old, please do not mix the new gel with the old leftover present the instrument (to avoid transferring putative bacterial contamination to the fresh gel). In case you prepare a fresh gel and would like to pool it with some leftover that is still fresh-enough, please indicate the date of the oldest gel on the tube.

6. Switch on the instrument and open the Fragment Analyzer instrument control software.

7. Press the *Park* button in the software to bring the buffer plate in its parking drawer “B”. Check the volume of Capillary Storage Solution in row H (blue solution). It must be at minimum two-thirds of the well height, if not contact the GECF team.

8. If you are the first user of the day: In the plate in “B” drawer, discard the old Inlet buffer in row A with a pipette and put 1ml/well of 1x Inlet buffer. Check for absence of bubbles.

9. If you are the first user of the day: Centrifuge briefly the new strip of 0.25x TE buffer and put it in the first row of the “M” drawer. Check for absence of bubbles.

10. Press the *Store* button to store back the plate in the instrument.

Sample Preparation

11. In a 96-well sample plate (twin-tec Eppendorf plate, provided by the GECF), pipette 22ul of the HS NGS Diluent Marker (DM) in each well that will be used.

12. Fill any unused wells within the row of the sample plate with 24 µL/well of HS diluent marker.

13. Add 2ul of samples or ladder (in last column) to the 22ul HS Diluent Marker. Do *not* put on ice. Mix well by pipetting up and down, then spin down (vertical plate centrifuge). MAKE SURE NO BUBBLE IS LEFT.

14. Mark the used rows by a cross on the right, to allow for later use of the plate empty rows.

15. Put the sample strip/plate on the corresponding strip/plate holder in the drawer 1, 2 or 3. CAUTION: risk of damages to the instrument if the wrong holder is used.

Starting the Run

16. In the Fragment Analyzer control software, select the correct tray (drawer) and correct row, and enter the sample names (it is also possible to do it after the run). *Tip: indicate a putative sample dilution in the sample name as well.*

17. Choose “Add to queue” in the “Run Selected Group” section. The Separation Setup will open. Select the method (=kit name), the relevant gel position, and check that the relevant sample tray is mentioned. To copy the results to a user-defined location, check the Copy results box and select the desired Copy path (typically your user folder). Press OK.

18. Repeat these steps for any remaining sample rows to be analyzed.

19. Press the Play icon to start the run. It will take approximately 1 hour per row.

20. Put back the reagents to their respective storage places.

End of the run

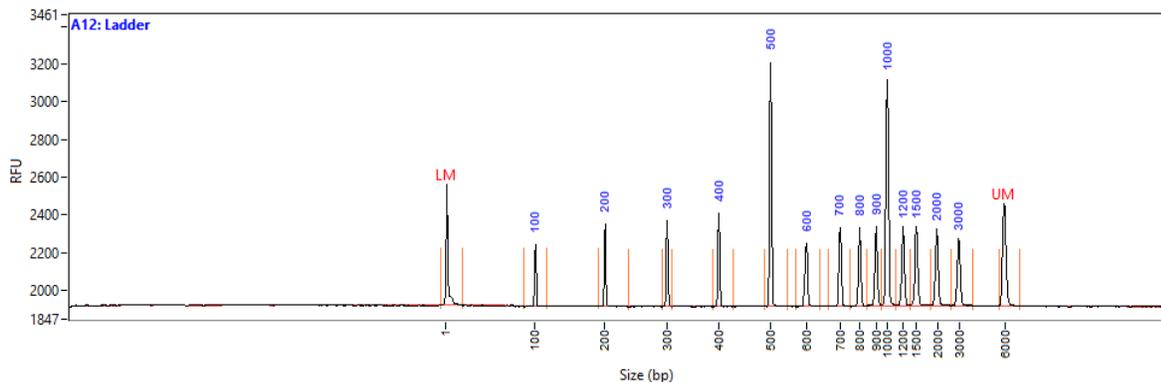
- At the end of the run, no end message appears, the queue simply disappears.
- No need to take out the gel Falcons from the instrument.
- Ideally, switch off the instrument.

Results analysis

- Open Prosize 3 software.
- Open your results file.

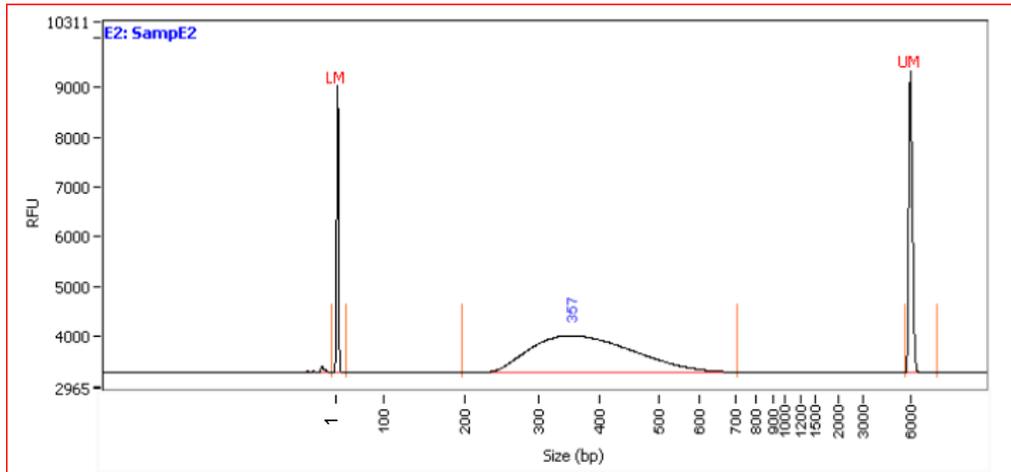
Sometimes a red warning message appears at the bottom of the window (“Mismatch between detected peaks and ladder assignment! No sizing calibration curve is established”). To correct this error, select “Show Size Calibration” to help the software detect/assign the ladder peaks. Right click on the undetected peak and select Add Peak. The software will automatically assign the right size to the newly detected peak. If many peaks failed to be detected, you may have to reduce the “peak width” setting in the little window on the right of the screen to 10 sec. Click Apply. If something does not work well, it may be that the wrong ladder is selected (right-click on the ladder numbers and select the correct one if required).

- The ladder bp values must be 1 (LM), 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 2000, 3000, 6000 (UM). The profile should look like this:



- The Lower Marker (LM) must be at 1nt, and the Upper Marker (UP) at 6000bp. If the markers are not correctly assigned, right click on the marker peak and select “Set as lower/upper marker”.
- Check quantification tab: Standard values are “use Ladder” (with 0.083ng/ul) and dilution 12x. If no ladder was used, you can also use “LM” or “UM” quantification (with 0.05 ng/ul final, and 12x dilution).

- A typical NGS library will look like that:



- The baseline should be flat below the smears/peaks. If not select another values in the window on the right.

- A pdf report can be generated (select the pdf icon).

Troubleshooting

- If “Monument Valley”-like pattern appears: Warn GECF staff, which will change all buffers & tubes/plates and perform a NaOH wash.

For advanced users only

- Run parameters can be edited in some situation. Please make sure you discuss with the GECF staff about these modifications, to prevent any damage to the instrument. Here is how to edit a method:

- In the Fragment Analyzer software, log out.
- Log in back as Administrator (no password).
- In the main interface, in the “Run selected row” section, select “Add method to queue”.
- In the small window that opens, select your method, click “edit” and change the parameters as required. Click OK.
- This way, you run will be performed with the new parameters, but the next user will find again the unmodified parameters.

CAUTION: Please do not edit the method by directly selecting “Edit method” in the “Run selected row” section of the main interface. By doing so, you would save the modification and would replace the original default method.

Notes

- Single-stranded DNA (ssDNA) will also be detected with this kit, albeit with less sensitivity (around 3-4x less according to our rough internal testing).

- If your samples contain a high salt buffer, migration may be affected.
- The intercalating dye is a putative carcinogen so appropriate handling procedures must be applied. In addition, wastes must be poured in the special container located near the instrument.
- If a run is already ongoing and you would like to add your samples to the running queue, use one of the drawers/trays that are not used. Do not open the drawers B, M or W during a run! If more gel or conditioning buffer is needed, only add them during the core of the run, not during the initial 10 minutes when all the various pumping processes occur. Then simply “add to queue” in the software.
- Lab coats are available near lab doors, and safety glasses are in the drawers below the benches.

Versions log

- v2.05: mentions the risk of damage to the instrument if wrong plate/strip holder is used. Small corrections.
- v2.06: mentions to bring intercalating dye at room temperature before use.
- v2.07: mentions to select relevant sample tray.
- v2.08: clarifies where to select relevant sample tray.
- v2.09: clarifies how to prepare fresh gel.
- v2.10: clarified that RNase-treated samples must have undergone a purification step before being run. Clarifies that bubbles are to be avoided in all buffers. Added a note regarding ssDNA. Added a note regarding biosafety and wastes handling.
- v2.11: Diluent marker is now recommended instead of BF-25 solution for empty wells. Removed the erroneous information that the green light means that a tray can be opened. Clarified the procedure to follow when a run is already ongoing when user adds his/her samples. Clarified that drawers B, M or W should not be opened during a run.
- v2.12: Commented on the fact that the migration is poor above 6 Kb. Clarified that samples in high salt buffers may migrate aberrantly. Clarified the timing of run preparation. Clarified that only purified samples should be run.
- v2.13: Modified the definition of a fresh gel from 7 to 6 days. Added an image of the ladder profile.
- v2.14: Modified Prosize 2 → Prosize 3. Defined where to find lab coats and safety glasses. Clarified how queuing samples when a run is ongoing.