



50Kb Large Fragment High Sensitivity kit (DNF-464) _ FA short protocol

This kit is used for DNA smears (e.g. NGS libraries) ranging from 50 pg/μL to 5 ng/μL (optimal conc. 1 ng/ul), or DNA fragments from 5 pg/μL to 600 pg/μL per fragment (optimal conc. 500pg/ul per fragment). If your samples are more concentrated, dilute them with the 1x TE buffer (4°). Sizing range is 75bp-48'000bp.

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 gel needs to be prepared. This is most likely the case as this Large Fragment gel is only stable for 1 day at RT°. 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):

50kb Large Fragment separation gel (DNF-220)	
HS Large Fragment extended DNA Ladder (DNF-365)	Take an aliquot if available (not single use). Do not pipet up and down. Vortex with care (sensitive 48Kb fragment).
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 50kb Large Fragment Fragment Diluent Marker (DM) (DNF-381)	Take an aliquoted tube if available. Protect from light. Do not let at RT° for > 1 hour.
Intercalating dye (DNF-600)	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. Prepare the gel in a *new* 50ml Falcon tube:
 - For 1 lane, 10ml of Large Fragment 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).
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

Note: If a lane has to wait in the instrument, increase DM and sample volumes: by 20% if 3rd to 6th in the queue, and 30% if 7th or 8th. Especially important when weather is dry.

11. In a 96-well sample plate (twin-tec Eppendorf plate, provided by the GECF), pipette 22ul of the HS 50kb 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 50Kb diluent marker.
13. Add 2ul of samples or ladder (in last column) to the 22ul HS 50Kb Diluent Marker. Do *not* put on ice. Cover with an adhesive foil. Vortex for 2 minutes on GECF blue IKA vortexer (make sure the plate does not fly), 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 the relevant sample tray. 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

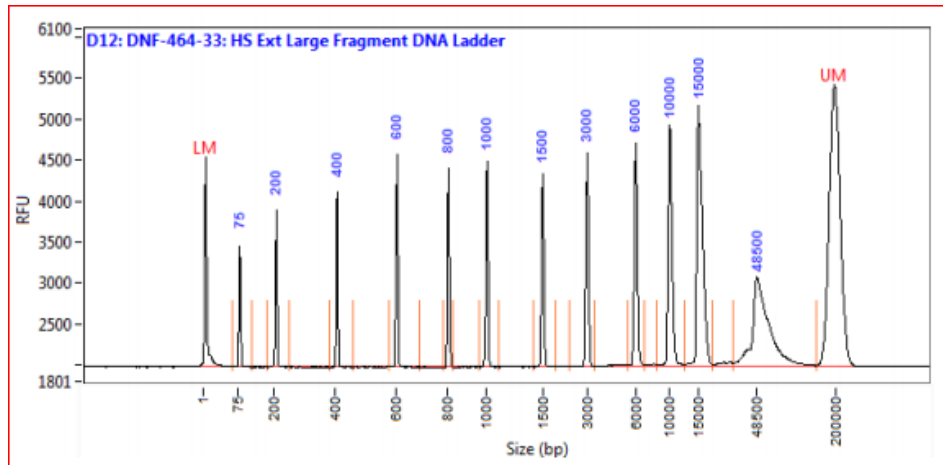
- At the end of the run, no end message appears, the queue simply disappears.
- No need to take out the gel Falcons from the instrument.
- If Friday end of afternoon/evening, switch off the instrument. Otherwise, it is not mandatory.

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 values must be 75-200-400-600-800-1000-1500-3'000-6'000-10'000-15'000-48'500bp.
- The Lower Marker (LM) must be at 1nt, and the Upper Marker (UP) at 200'000bp (arbitrary values). They should be present in all samples, unless in the empty wells (where the BF-25 marker has an intermediate size). 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 0.125 ng/ul) and dilution 12x. If no ladder was used, you can also use “LM” or “UM” quantification (contact us if needed).

- 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

- 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 sample 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 (migration) of the ongoing 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

- v1.01: initial version.
- v2.12: Clarified the timing of run preparation. Clarified that samples in high salt buffers may migrate aberrantly. Clarified that only purified samples should be run.
- v2.13: Modified Prosize 2 → Prosize 3. Defined where to find lab coats and safety glasses. Clarified how queuing samples when a run is ongoing.
- v2.14: Clarified when to switch off instrument (only before the weekend). Mentioned to increase volume if plate will wait more than 2 hours in the instrument. Clarified how to add samples to the queue of ongoing runs.