



Small RNA kit (DNF-470) _ Fragment Analyzer short protocol

This kit is used for profiling RNAs from 15 nt to 200 nt. Starting concentrations are 0.05 - 2 ng/μL for purified MicroRNAs (<40nt), 1 - 20 ng/μL for purified small RNAs (<200nt), or 5 – 100 ng/μL for total RNA. Only clean samples should be run (without leftover from enzymatic reactions or other proteins/buffers).

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) for the actual run + 0.5 hour during the night to account for higher price of small RNA reagents. Pricing is indicated on our web site.

30 minutes before samples/instrument preparation

2. Determine if *small RNA* gel needs to be prepared, by looking inside the instrument whether enough fresh *small RNA* gel is present in one of the Falcon tubes (fresh = made within the last 72h). 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):

Small RNA gel (DNF-262)	Only if required (see above). Take an aliquoted tube if available. Invert the bottle 2-3 times.
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)	The “dsDNA Inlet buffer” is used also for RNA gels.

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

Intercalating dye (DNF-600)	Only if required (see above). Protect from light.
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Take out of the -20° freezer and keep on ice:

Small RNA Diluent Marker (DNF-368)	Take an aliquoted tube if available. Protect from light. Keep on ice.
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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 RNA Separation Gel +1 ul of intercalating dye. Mix gently, AVOID/REMOVE 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 kind 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

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. Get one aliquot of Small RNA ladder per lane (DNF-361 in single-use PCR tubes, located at -80°, in a bag labelled “small RNA ladder”). Keep on ice.

12. If you have an unused well, consider asking a positive control sample to GECF.

13. Heat-denature 2.5ul-5ul of each sample in 0.2ml PCR tubes (or ABgene PCR strip), 10min at 70° and immediately put on ice (do not allow the PCR block to go down from 70°). Do the same in parallel for the ladder PCR tube.

14. In a 12-wells PCR strip (recommended for RNA!) or a 96-well sample plate (in case several lanes are planned; only use our Eppendorf *twin-tec* plates), pipette 18ul of the Small RNA Diluent Marker (DM) in each well that will be used.

15. Fill any unused wells within the row of the sample plate with 20 µL/well of diluent marker.

16. Add 2ul of denatured samples or ladder (in last column) to the 18ul Small RNA 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.

17. If using a plate (not recommended for RNA), mark the used rows by a cross on the right, to allow for later use of the plate empty rows.

18. 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

19. 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.*

20. Choose “Add to queue” in the “Run Selected Group” section. The Separation Setup will open. Select the method (=kit name, DNF-470-33 – Small RNA.mthds). Select 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.

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

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

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

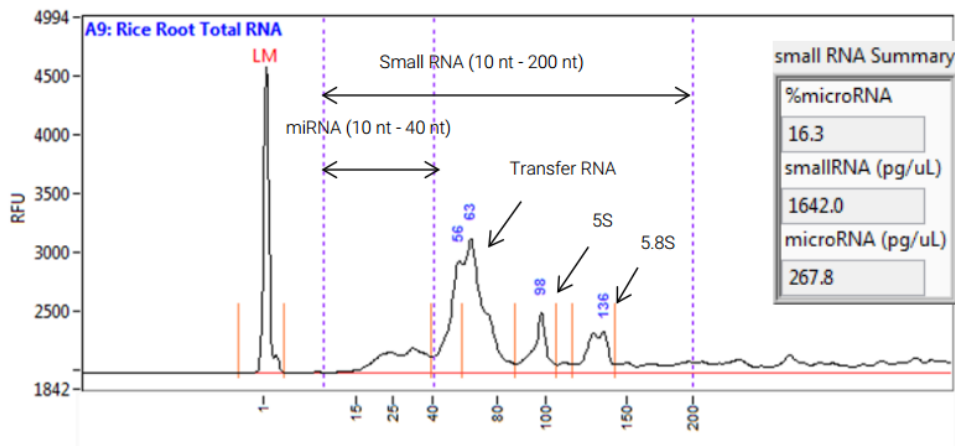
End of the run

- 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 15-25-40-80-100-150-200bp.
- The Lower Marker (LM) must be at “1nt”, and should be present in all samples, unless in the empty wells (where the marker has a bigger size). If the lower marker is not correctly assigned, right click on the marker peak and select “Set as lower marker”.

- In 2019, the LM is typically around XXX in RFU (when looked with “baseline shown”).
- Check *marker analysis* tab: *Marker analysis* setting must be set to “lower marker only”.
- Check *quantification* tab: Standard values are “use Ladder” (with 0.4ng/ul) and dilution 10x. It is not offered as a standard possibility to use “LM” for RNA quantification.
- In the RNA Property Summary, you can get:
 - the RNA Concentration.
 - the concentration and ratio of MicroRNAs and small RNAs.
- The baseline should be flat below the smears/peaks. If not, play with the *Manual Baseline Setpoints* settings in the *Peak Analysis* tab.
- A pdf report can be generated (select the pdf icon).
- Typical result:



Troubleshooting and notes

- 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, your 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

- The intercalating dye is a putative carcinogen, the RNA Diluent Marker contains the carcinogen formamide, and the RNA gel contains ethylene glycol, which is toxic when ingested. Therefore, appropriate handling procedures must be applied. In addition, wastes must be poured in the special container located near the instrument.
- Lab coats are available near lab doors, and safety glasses are in the drawers below the benches.
- 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.

Versions log

- v1.0: First version of the protocol.
- v1.01: minor edits
- v1.02: Corrected a typo in number of hours to book per run.
- v1.03: Clarified when to switch off instrument (only before the weekend). Clarified to use only ABgene PCR strips. 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.