Agilent

Agilent Small RNA Kit

Kit Guide

For Research Use Only.

Not for use in diagnostic procedures.

Notices

Manual Part Number

M5310-91470

Edition 12/2018

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WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

1 Agilent Small RNA Kit

The Small RNA kit (275 Samples) (Part # DNF-470-0275) is designed for the sizing and quantification of Small RNA samples, and determination of microRNA region content. Synthetic RNA can also be analyzed within the defined sizing region with this kit.

Table 1 Physical Specifications

Type	Specifications
Sample Volume Required	2 µL
Number of Samples per Run	12-Capillary: 11 (+ 1 well DNA Ladder) or 12 (Imported DNA Ladder) [1] 48-Capillary: 47 (+ 1 well DNA Ladder) or 48 (Imported DNA Ladder) [1] 96-Capillary: 95 (+ 1 well DNA Ladder) or 96 (Imported DNA Ladder) [1]
Total Electrophoresis Run Time	18 minutes (22-47 Array)[2] 24 minutes (33-55 Array)

Table 2 Analytical Specifications

Type	Specifications
RNA Sizing Range	15 nt – 200 nt
Qualitative Range	25 pg/µL –2500 pg/µL (microRNA region)
Quantitative Range	50 pg/µL –2000 pg/µL (microRNA region)
Quantification Precision	25% CV (Small RNA Ladder)

Depending upon the type of sample being analyzed, the following initial sample concentration ranges are recommended for use in this kit:

Table 3 Recommended Sample Concentrations

Sample Type	Concentration
MicroRNA	50 pg/µL – 2000 pg/µL 10-40nt
Small RNA	1 ng/µL – 20 ng/µL 10-200nt
Total RNA	5 ng/µL – 100 ng/µL)

[1] Results using Total RNA, small RNA, and MicroRNA samples and fragments diluted in nuclease-free water.
[2] The 22 cm effective, 47 cm total length capillary is only available for 12-capillary Fragment Analyzer instruments.

Table 3 Storage Conditions

Store below –70°C:	Store at –20°C:	Store at 2-8°C (DO NOT FREEZE):	Store at Room Temperature (DO NOT FREEZE):
Small RNA Ladder	Intercalating Dye	Small RNA Separation Gel	5x Capillary Conditioning Solution
	Small RNA Diluent Marker	5x 930 dsDNA Inlet Buffer	
		BF-25 Blank Solution	
		0.25x TE Rinse Buffer	

NOTE

Always thaw Small RNA Ladder and Small RNA Diluent Marker on ice and keep it on ice. Ensure all other reagents are completely warmed to room temperature prior to use.

Table 5 kit Components

Part Number	Name	Amount
DNF-262-0250	Small RNA Separation Gel	250 mL
DNF-600-U030	Intercalating Dye	30 µL
DNF-355-0125	5x 930 dsDNA Inlet Buffer	125 mL, (dilute with sub-micron filtered water prior to use)
DNF-475-0050	5x Capillary Conditioning Solution	50 mL, (dilute with sub-micron filtered water prior to use)
DNF-368-0004	Small RNA Diluent Marker*	4 mL x 2,
DNF-361-U060	Small RNA Ladder*	60 µL
DNF-497-0125	0.25x TE Rinse Buffer	125 mL
DNF-300-0008	BF-25 Blank Solution	8 mL
	Eppendorf LoBind 0.5 mL Tubes	Package of 50

* **Danger:** Contains ≤ 50% Formamide. Refer to SDS for safety and handling information.

NOTE

RNA Handling

RNA samples and RNA Ladders are very sensitive to RNase contamination, which can lead to experimental failure. To minimize RNase contamination, wear gloves when working with RNA samples and reagents, and when handling accessories that will come in contact with the RNA sample. Use certified RNase-free plastics and disposable consumables. It is also recommended to work in a separate lab space if possible and decontaminate the pipettes and work surface to avoid cross contamination.

WARNING

Working with Formamide

The following kit component contains ≤ 50% Formamide and handling of the component might hold health risks:

Small RNA Diluent Marker, 4 mL x 2 (Part # DNF-368-0004)
Small RNA Ladder, 60 µL (Part # DNF-361-U060)

- **Refer to product material safety datasheets for further chemical and biological safety information.**
- **Follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.**

WARNING

Working with Chemicals

The handling of reagents and chemicals might hold health risks.

- **Refer to product material safety datasheets for further chemical and biological safety information.**
- **Follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.**

2 Additional Material and Equipment Required

Material and Equipment Required for Analysis with the Fragment Analyzer

Hardware:

- Fragment Analyzer with LED fluorescence detection:
  - 5200 Fragment Analyzer (Part # M5310AA)
  - 5300 Fragment Analyzer (Part # M5311AA)
  - 5400 Fragment Analyzer (Part # M5312AA)
- FA 12-Capillary Array Ultrashort, 22cm (Part # A2300-1250-2247) OR
- FA 12-Capillary Array Short, 33cm (Part # A2300-1250-3355) OR
- FA 12-Capillary Array Long, 55cm (Part # A2300-1250-5580) OR
- FA 48-Capillary Array Short, 33cm (Part # A2300-4850-3355) OR
- FA/ZAG 96-Capillary Array Short, 33cm (Part # A2300-9650-3355) OR
- FA/ZAG 96-Capillary Array Long, 55cm (Part # A2300-9650-5580)

Software:

- Fragment Analyzer control software (Version 1.1.0.11 or higher)
- ProSize data analysis software (Version 2.0.0.61 or higher)

Reagents:

- Capillary Storage Solution, 100 mL (Part #GP-440-0100)

Additional Equipment/Reagents Required (Not Supplied)

- RNase-free 96-well PCR sample plates. Please refer to Appendix 3 – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer User Manual for a complete approved sample plate list.
- RNase-free water (for diluting sample)
- Sub-micron filtered DI water system (for dilution of 5x 930 Inlet Buffer and 5x Capillary Conditioning Solutions)
- RNaseZap, Ambion, Part # AM9782 or equivalent product
- Multichannel pipettor(s) and/or liquid handling device capable of dispensing 1 – 100 µL volumes (sample plates) and 1,000 µL volumes (Inlet Buffer plate) with RNase-free pipette tips
- Thermal cycler (for sample denaturing)
- Eppendorf DNA LoBind Safe-Lock Tubes, 0.5 mL (Eppendorf Part # 022431005; as needed)
- Fisherbrand 96 DeepWell 1mL Plate, Polypropylene, Fisher Scientific Part #12-566-120 (Inlet Buffer and waste plate)
- Reagent Reservoir, 50 mL (VWR Part # 89094-680 or similar) (for use in pipetting Inlet Buffer plates)
- Conical centrifuge tubes for prepared Separation Gel/Dye mixture and/or 1x Capillary Conditioning Solution
  - 50 mL (for smaller volumes): BD Falcon Part # 352070, available from Fisher Scientific Part # 14-432-22 or VWR Part # 21008-940
  - 250 mL (for larger volumes): Corning Part # 430776, available from Fisher Scientific Part # 05-538-53 or VWR Part # 21008-771
- 96-well plate centrifuge (for spinning down bubbles from sample plates)
- Vortexer

3 Agilent Small RNA kit Protocol

Gel Preparation

1. Store the Small RNA Separation Gel at 2-8°C upon arrival.
2. The Intercalating Dye should be stored at -20°C.
3. Bring the Small RNA Separation Gel and Intercalating Dye to room temperature prior to mixing.

NOTE

Slight phase separation of the Small RNA Separation Gel may occur upon storage. Gently invert gel bottle five times prior to pouring to mix. Take care to avoid bubble formation in the gel while mixing.

4. Mix appropriate volumes of Intercalating Dye and Small RNA Separation Gel necessary for one day of operation. Use a 50 mL conical centrifuge tube to allow a small minimum working volume. For larger volumes, use a 250 mL conical centrifuge tube and remove the collar of the tube holder in the instrument reagent compartment.
5. The volume of Small RNA Separation Gel required per run in a 12-capillary Fragment Analyzer system is summarized below

Table 5 Volume Specifications for 12-Capillary Fragment Analyzer Systems

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of separation gel	Volume of 1x Conditioning Solution
12	1.0 µL	10 mL	10 mL
24	1.5 µL	15 mL	15 mL
36	2.0 µL	20 mL	20 mL
48	2.5 µL	25 mL	25 mL
96	4.5 µL	45 mL	45 mL

[1] A 5 mL minimum volume should be initially added to the tube. One sample well per separation is dedicated to the ladder.

Table 6 Volume Specifications for 48-Capillary Fragment Analyzer Systems

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of separation gel	Volume of 1x Conditioning Solution
48	2.5 µL	25 mL	25 mL
96	4.0 µL	40 mL	40 mL
144	5.5 µL	55 mL	55 mL
192	7.0 µL	70 mL	70 mL
240	8.5 µL	85 mL	85 mL
288	10.0 µL	100 mL	100 mL

[1] One sample well per separation is dedicated to the ladder.

Table 7 Volume Specifications for 96-Capillary Fragment Analyzer Systems

# of Samples to be Analyzed[1]	Volume of Intercalating Dye	Volume of separation gel	Volume of 1x Conditioning Solution
96	4.0 µL	40 mL	40 mL
192	8.0 µL	80 mL	80 mL
288	12.0 µL	120 mL	120 mL
384	16.0 µL	160 mL	160 mL
480	20.0 µL	200 mL	200 mL

[1] One sample well per separation is dedicated to the ladder.

5. Place the prepared separation gel/Intercalating Dye mixture onto the instrument and insert into the desired gel fluid line (Gel 1 or Gel 2 pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.
6. When adding separation gel to the instrument, update the solution levels in the Fragment Analyzer control software. From the main screen, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1).

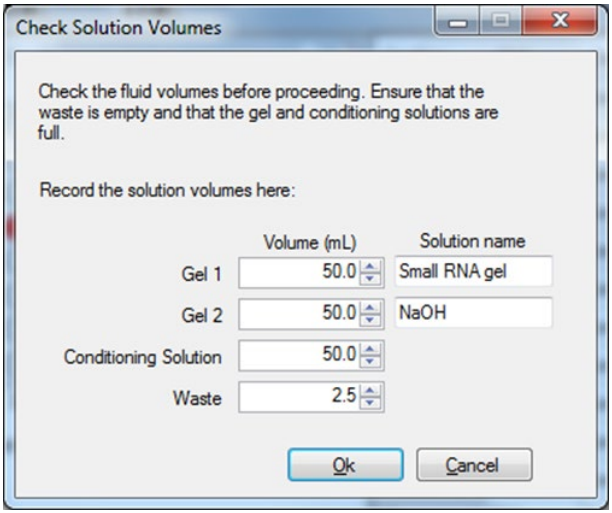

Figure 1 Solution Levels menu

7 When switching applications (e.g., between kits), prime the appropriate gel fluid line after loading fresh Gel solution. From the main screen of the Fragment Analyzer control software, select **Utilities > Prime.** Select the desired fluid line(s) (Conditioning, Gel 1, or Gel 2) and press **OK** to purge the fluid line with fresh gel (Figure 2).

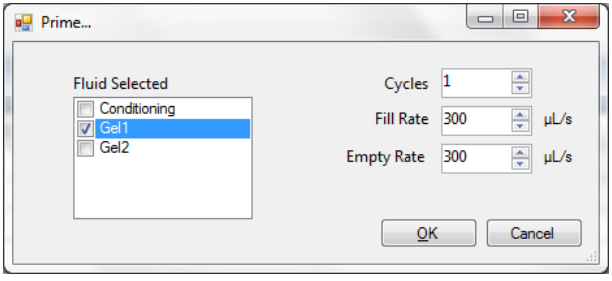

Figure 2. Prime menu

Inlet Buffer Preparation

1. Store the 5x 930 dsDNA Inlet Buffer at 2-8°C upon arrival. Do Not Freeze.
2. Bring the 5x 930 dsDNA Inlet Buffer to room temperature prior to mixing and use.
3. In a clean container, add 10 mL of the 5x 930 dsDNA Inlet Buffer per 40 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at 2-8°C if desired.

Capillary Conditioning Solution Preparation

1. Store the 5x Capillary Conditioning Solution at room temperature upon arrival. Do not freeze.
2. In a clean container (e.g. 50 mL or 250 mL conical centrifuge tube), add 10 mL of the 5x Capillary Conditioning Solution per 40 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at room temperature if desired.
3. Once mixed, place the 1x Capillary Conditioning Solution onto the instrument and insert the conditioning fluid line (Conditioning Solution pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.
4. The 1x Capillary Conditioning Solution should be added to the system as use demands. Tables 5-7 show the volume specifications for the conditioning solution.
5. When adding fresh 1x Capillary Conditioning Solution to the instrument, update the solution levels in the Fragment Analyzer control software. From the main screen, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1).

Instrument Preparation

1. Check the fluid level of the waste bottle and waste tray daily and empty as needed.
2. Prepare a fresh 96 DeepWell 1mL Plate filled with 1.0 mL/well of 1x 930 dsDNA Inlet Buffer daily in Row A only. Do not overfill the wells of the inlet buffer plate.
3. In Row H of the same prepared buffer plate, place 1.0 mL/well of Capillary Storage Solution. Row H of the buffer plate is used for the Store location, and the array moves to this position at the end of the experimental sequence.

NOTE

Ensure Row H of the buffer tray is always filled with Capillary Storage Solution, and the capillary array is placed in the Storage Solution when not in use, to prevent the capillary tips from drying out and potentially plugging.

The Capillary Storage Solution should be replaced every 2-4 weeks, as the solution will gradually thicken following exposure to the open air via evaporation. More frequent replacement may be required in low humidity or warm lab environments.

4. Place the prepared inlet buffer plate into Drawer “B” (top drawer) of the Fragment Analyzer. Ensure that the plate is loaded with well A1 toward the back left on the tray.
5. Place an empty 96 DeepWell 1mL Plate into Drawer “W” (second from top) of the Fragment Analyzer. This plate serves as the capillary waste tray and should be emptied daily. Alternatively, the supplied open reservoir waste plate may be used.
6. Prepare a fresh sample plate with Row A filled with 200 µL/well of 0.25x TE Rinse Buffer daily.
7. Place the prepared 0.25x TE Rinse Buffer plate into Drawer “M” (third from top) of the Fragment Analyzer. Ensure that the plate is loaded with well A1 toward the back left on the tray.

Ladder and Sample Preparation

General Information

The recommended 96-well sample plate for use with the Fragment Analyzer system is a semi-skirted PCR plate from Eppendorf (Part #951020303). Please refer to Appendix 3 – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer User Manual for a complete approved sample plate list. The system has been designed to operate using these dimensions/styles of PCR plates.

NOTE

The use of PCR plates with different dimensions to the above recommended plate could lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible.

Small RNA Diluent Marker Preparation

1 Remove the Small RNA Diluent Marker from -20°C and keep it on ice before use. Vortex the tube briefly to mix the content. Spin the tube after mixing to ensure liquid is at the bottom of the tube.

Small RNA Ladder Preparation

1 Prior to first use, the Small RNA Ladder solution should be aliquoted to minimize the number of freeze/thaw cycles. Using the provided Eppendorf LoBind 0.5 mL tubes, aliquot 12 µL of Small RNA Ladder per tube into 5 tubes and store the aliquots at less than -70°C. Each aliquot is good for 5 freeze/thaw cycles.

2 Thaw a Small RNA Ladder aliquot on ice.

3 Transfer a volume of the Small RNA Ladder for one day of use to an RNase-free PCR tube. Heat-denature the Small RNA Ladder at 70°C for 10 min using a thermal cycler, immediately cool to 4°C and keep on ice before use.

RNA Sample Preparation

1 It is recommended to heat-denature all RNA samples at 70°C for 10 min and immediately cool to 4°C and keep on ice before use.

2 Depending upon the sample type, for optimal assay results, the following input sample concentration range is recommended:

Table 4 Recommended Sample Concentrations

Sample Type	Concentration
MicroRNA	50 pg/µL – 2000 pg/µL
Small RNA	1 ng/µL – 20 ng/µL
Total RNA	5 ng/µL – 100 ng/µL)

3 If the concentration of the input sample is above the recommended range, dilute the sample with RNase-free water.

Sample Plate Preparation

1 Using a fresh RNase-free 96-well sample plate, pipette 18 µL of the Small RNA Diluent Marker (DM) Solution to each well in a row that is to contain sample or Small RNA Ladder. Fill any unused wells within the row of the sample plate with 20 µL/well of BF-25 Blank Solution.

2 Pipette 2 µL of each denatured RNA sample into the respective wells of the sample plate; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

3 Small RNA Ladder: The Small RNA Ladder must be run in parallel with the samples for each experiment to ensure for accurate quantification. Pipette 2 µL of denatured Small RNA Ladder into the 18 µL of Diluent Marker (DM) Solution in Well 12 of each row to be analyzed. Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.

NOTE

Important Sample Mixing Information:

When mixing sample with diluent marker, it is important to mix the contents of the well thoroughly to achieve the most accurate quantification. It is highly suggested to perform one of the following methods to ensure complete mixing:

- When adding 2 µL of sample or ladder to 18 µL of diluent marker, swirl the pipette tip while pipetting up/down to further mix. OR
- After adding 2 µL of sample or ladder to 18 µ µL of diluent marker, place a plate seal on the sample plate and vortex the sample plate at 3,000 rpm for 2 min. Any suitable benchtop plate vortexer can be used. Ensure that there is no well-to-well transfer of samples when vortexing. The plate should be spun via a centrifuge after vortexing to ensure there are no trapped air bubbles in the wells. OR
- When adding 2 µL of sample or ladder to 18 µL of diluent marker, use a separate pipette tip set to a larger 18 µL volume, and pipette each well up/down to further mix. OR
- Use an electronic pipettor capable of mixing a 10 µL volume in the tip after dispensing the 2 µL sample volume. Some models enable using the pipette tip for both adding and mixing.

4 After mixing sample or Small RNA Ladder with Small RNA Diluent Marker Solution in each well, centrifuge the plate to remove any air bubbles. Check the wells of the sample plate to ensure there are no air bubbles trapped in the bottom of the wells. The presence of trapped air bubbles can lead to injection failures.

5 For best results, run the plate as soon as possible. If the sample plate will not be used immediately, cover the sample plate with RNase-free cover film, store at 2-8°C and use within the same day. Spin the plate again if any bubbles developed in the sample wells. Be sure to remove the cover film before placing the plate into the instrument.

6 To run the samples, place the plate in one of the three sample plate trays (Drawers 4-6 from the top) of the Fragment Analyzer instrument. Load or create the experimental method as described in the following sections.

Performing Experiments

Running an Experiment

1 To set up an experiment, from the main screen of the Fragment Analyzer control software, select the **Operation** tab (Figure 3). Select the sample tray location to be analyzed (1, 2, or 3) by left clicking the **Sample Tray #** dropdown or by clicking the appropriate sample plate tab (alternate plate view) and choosing the appropriate location.

2 Left click a well of the desired sample plate row with the mouse. The selected row will be highlighted in the plate map (e.g., Row A in Figure 3). Enter the sample name if desired into the respective **Sample ID** cell by left clicking the cell and typing in the name. Alternatively, sample information can be imported from .txt or .csv file by selecting the **Load from File...** option.

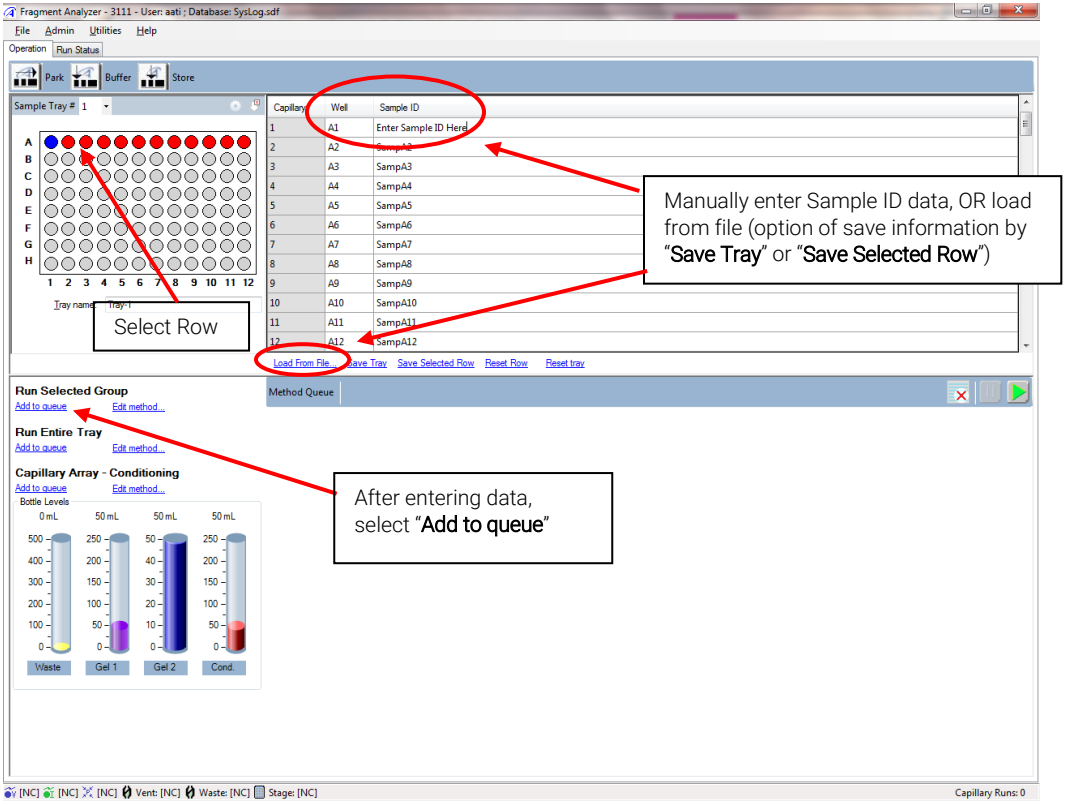

Figure 3. Main screen showing selection of sample row and entering sample information

3 After sample information for the row or plate has been entered, under the **Run Selected Group** field press **Add to queue**. The **Separation Setup** form will be displayed enabling the user to select the experimental method and enter additional information (Figure 4).

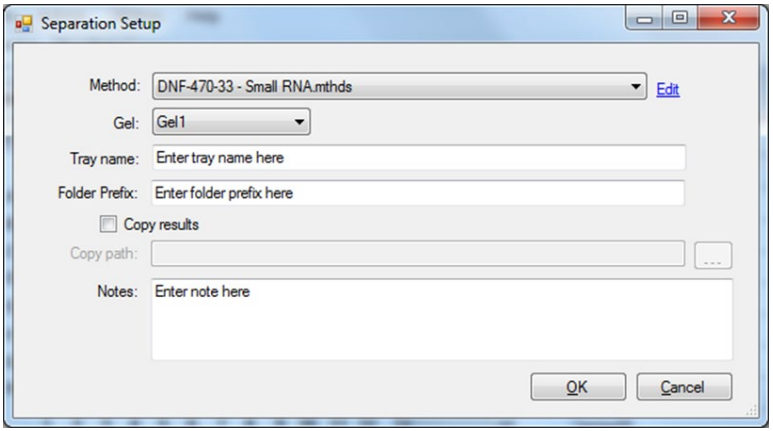

Figure 4. Separation Setup form to select experimental Method and enter tray/folder information

4 In the Separation Setup pop-up form, left click the dropdown and select the appropriate preloaded experimental Method file. The available methods are sorted by kit number and are linked to the directory containing methods for the currently installed capillary array effective length (e.g., 22 cm or 33 cm). Select the following method:

- **DNF-470-22 – Small RNA.mthds** for the 22 cm effective, 47 cm total "ultra-short" capillary array.
- **DNF-470-33 – Small RNA.mthds** for the 33 cm effective, 55 cm total "short" capillary array.

5 Select the appropriate **Gel** line being used for the experiment (Gel 1 or Gel 2) using the dropdown.

6 The **Tray Name** can be entered to identify the sample plate. The **Folder Prefix** if entered will amend the folder name (normally a time stamp of HH-MM-SS from the start of the CE run).

7 To copy the experimental results to another directory location in addition to the default save directory, check the **Copy results** box and select the desired **Copy path:** directory by clicking the **…** button and navigating to the desired save directory.

8 Any **Notes** can be entered regarding the experiment; they will be saved and displayed in the final PDF report generated by the ProSize software.

9 Once all information has been entered, press **OK** to add the method to the instrument queue (press **Cancel** to abort adding the method).

10 Repeat Steps 1-9 for any remaining sample rows to be analyzed.

11 On 12-capillary systems if the entire 96-well sample tray is to be run using the same experimental method, under the **Run Entire Tray** field press **Add to queue**. A form similar to Figure 4 will be displayed for entering information and adding the run to the instrument queue for the entire 96-well sample tray.

12 After a row or tray has been added to the queue, the method(s) will be listed on the main screen under the **Method Queue** field (Figure 5).

13 Prior to starting the experiment, verify all trays (buffer/storage, waste, marker, sample, etc.) have been loaded into their respective drawer locations.

14 Press the **Play** icon (▶) to start the sequence loaded into the queue. To **Pause** the queue after the currently running experiment is completed, press the ⏸ button. To **Clear** the run queue of all loaded experiments, press the ✕ button.

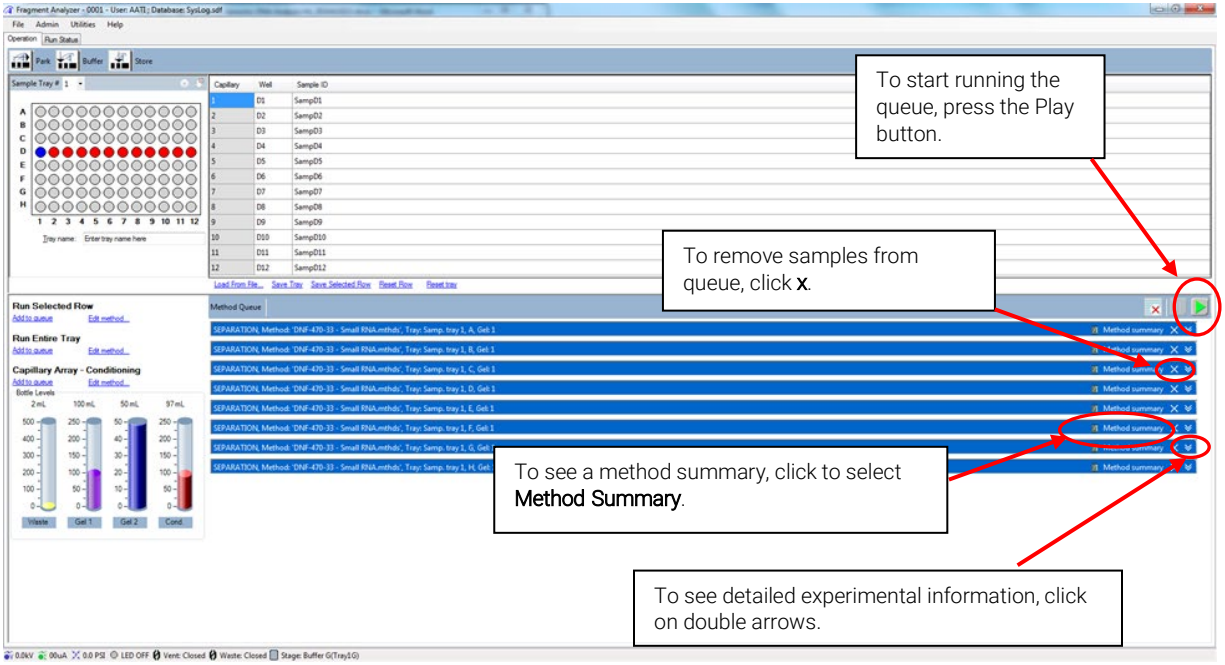

Figure 5. Main screen after selection of samples to the run queue

15 Once an experiment has been loaded onto the queue, the user can view or edit the method (Administrator level only can edit a method) by pressing the Method Summary field. To remove the method from the queue, press the "x" button; to view the stepwise details of the method press the double down arrow icon.

16 The user may add a pause or prime step into the queue by right clicking the mouse while over the queue and selecting Insert Pause or Insert Prime.

17 The order of the experimental queue can be rearranged by dragging down individual entries. Further information regarding the Method Queue operation is provided in the Fragment Analyzer User Manual.

18 Once started, the instrument will perform all the programmed experiments in the Method Queue uninterrupted unless a pause step is present. Note that additional experiments can be programmed and added to the Method Queue at any time while the instrument is running if desired. After completion of the last queued experiment, the instrument stage will automatically move to the Store location (Row H of the inlet buffer tray containing the Capillary Storage Solution for 12-Capillary Systems).

Viewing and Editing Experimental Methods

1 A user level operator can View the steps of the experimental method by pressing the View link on the Separation Setup screen, or by pressing the Method Summary option once a method has been loaded onto the experimental queue. User level operators cannot edit any steps of a queued separation method.

2 Administrator level operators can Edit certain steps of the experimental method. To open the method editor screen, press the Edit link from the Separation Setup screen (Figure 4). The method editor screen is displayed, showing the steps of the method (Figure 6).

3 The preloaded, optimized steps for the DNF-470-22 (Figure 6) and DNF-470-33 (Figure 7) methods are shown below. The DNF-470-22 method steps are:

- **a** Full Condition flushing method (Automatically enabled). Default Gel Selection: Gel 1. Gel prime to buffer (Automatically enabled). This step moves the inlet buffer tray to the capillary array during the gel flushing step, to submerge the capillary inlets into the inlet buffer while flushing gel through the array.
- **b** Perform Prerun (enabled) (7 kV, 30 sec)
- **c** Rinse (enabled; Tray = Marker; Row = A; # Dips = 2). This step moves to the Marker tray and rinses the capillary tips twice with 0.25x TE Rinse Buffer.
- **d** Marker Injection (disabled)
- **e** Rinse (disabled)
- **f** Sample Injection (enabled) Voltage Injection (6 kV, 50 sec). This step injects the prepared sample plate.
- **g** Separation (enabled) Voltage (7 kV, 18 min). This step performs the CE Separation.

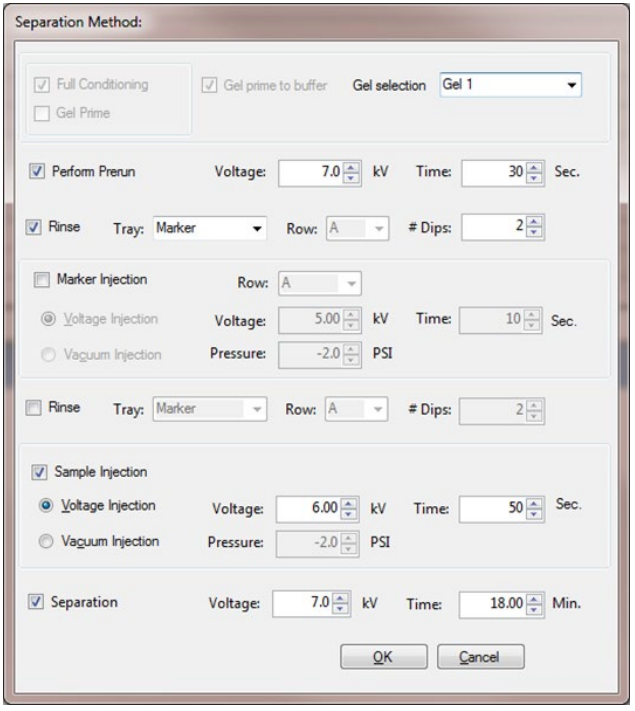

Figure 6. DNF-470-22 – Small RNA method

4 Figure 7 shows the preloaded method for the 33 cm effective, 55 cm total length "short" array. The prerun and separation voltage is set to 8 kV, the injection voltage to 7 kV 50 sec, and the Separation time to 24 min.

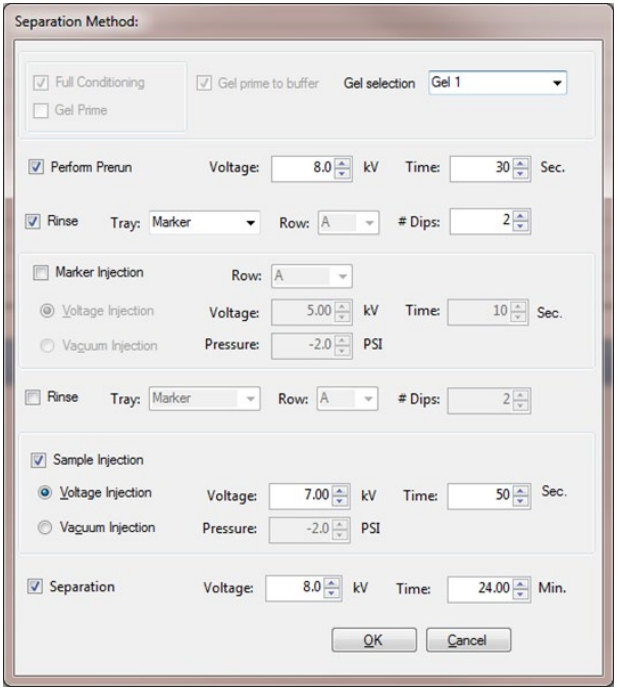

Figure 7. DNF-470-33 – Small RNA method

5 An Administrator level user has the option to adjust the **Gel Selection**; **Prerun** settings; **Rinse** settings including **Tray**, **Row** and **# Dips**; **Marker Injection** settings including **Row; Sample Injection** settings; and the **Separation** settings. For example, if the rinse buffer is loaded into a row other than Row A this can be adjusted prior to or while the method is loaded on the experimental queue.

6 To apply any adjustments to the method being placed on the experimental queue, press the **OK** button. To exit the editor screen without applying any changes press the **Cancel** button.

NOTE

Any edits made to the experimental method from the **Separation Setup** or **Method Summary** screen will only apply to the currently loaded experiment in the queue. No changes are made to the original separation method file.

Processing Experimental Data

1 When processing data, the ProSize data analysis software will automatically recognize the separation method performed and apply the appropriate matching configuration file from the C:\ProSize 3.0\Configurations directory:

- The DNF-470-22 separation method will be processed using the DNF-470-22 - Small RNA configuration file.
- The DNF-470-33 separation method will be processed using the DNF-470-33 - Small RNA configuration file.

NOTE

If the preloaded ProSize configuration files "DNF-470-22 - Small RNA" or "DNF-470-33 - Small RNA" are not located in the C:\ProSize 3.0\Configurations directory, contact Agilent Technical Support to obtain the files.

2 The data is normalized to the lower marker (set to 1 nt) and calibrated to the Small RNA Ladder run in parallel to the samples. Figure 8 shows an example of the 1 nt lower marker injected with the Small RNA Ladder using the DNF-470-33 separation method.

3 For the Small RNA Analysis kit, ProSize is set to the Small RNA mode in the Advanced Settings. The Quantification settings are set to Use Ladder for quantification with a Conc. (ng/uL) of 0.4 and a Dilution Factor of 10 (2 µL sample + 18 µL Diluent Marker). Note that if a pre-dilution was performed prior to the experiment, the Dilution Factor setting should be changed to accurately reflect the final sample concentration.

4 Start with the preloaded Global Configuration initially applied to the opened data file in ProSize, and modify the parameters as needed to fit the data during data processing

5 For full information on processing data, refer to the ProSize User Manual.

Fragment Analyzer Shut Down/Storage

Instrument Shut Down/Storage

The instrument automatically places the capillary array in the Store position against Capillary Storage Solution (Row H of the buffer tray) after each experiment; no further action is required.

If the instrument is to be idle for more than one day, it is recommended to turn off the power to the system.

4 Checking Your Separation Results

Small RNA Ladder

Figure 8 shows the typical result for the Small RNA Ladder when running the Small RNA kit method. The initial concentration of the ladder was 4 ng/µL (final concentration of the ladder after mixing with Diluent Marker was 0.4 ng/µL). A total of 8 major peaks should be observed with the sizes annotated as in Figure 8. The first peak corresponds to the 1 nt lower marker (LM) peak.

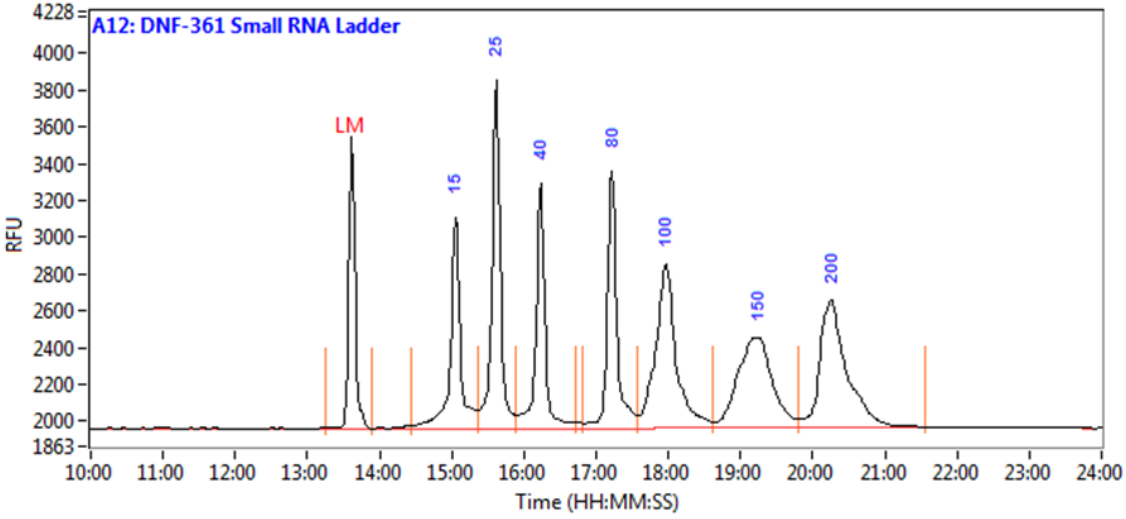

Figure 8. Representative Small RNA Ladder result using Fragment Analyzer system with the DNF-470 Small RNA Analysis kit. Method: DNF-470-33. Peaks annotated by size (nt).

Small RNA Sample

Figure 9 shows the typical results for an RNA sample with peaks annotated by size in nt. The data was normalized to the lower marker and the size was calibrated to the Small RNA Ladder run in parallel to the sample. The micro RNA (miRNA) size region and the small RNA size region are set to default values of 10 nt – 40 nt and 10 nt – 200 nt, respectively, and marked with vertical dashed cursors. These regions can be adjusted in ProSize by the user if desired. The smallRNA (pg/µL) concentration, microRNA (pg/µL) concentration, and %microRNA values (percentage miRNA in the small RNA region) are reported for each sample.

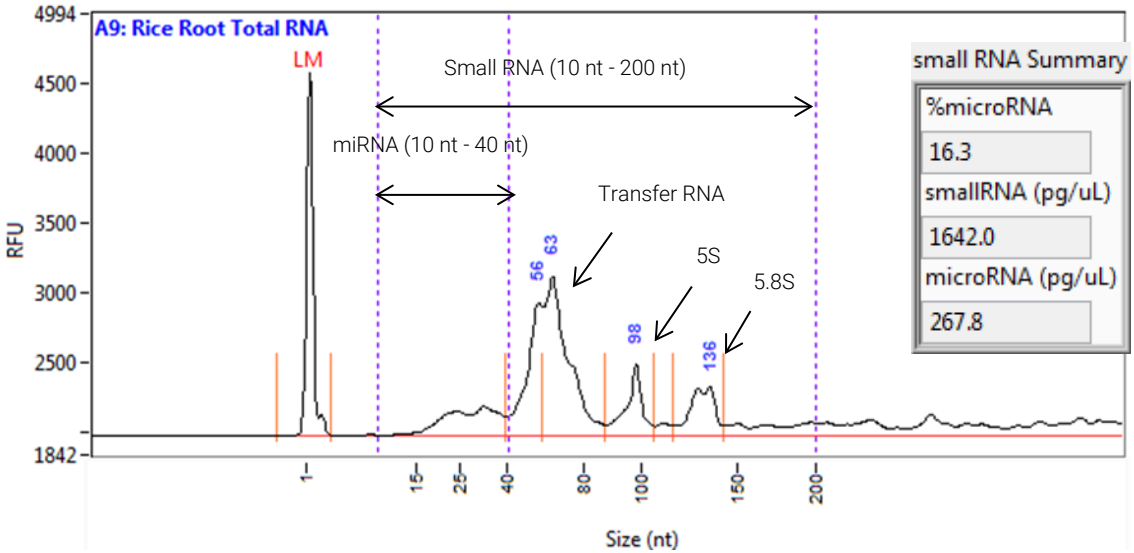

Figure 9. Rice root RNA sample result using the Fragment Analyzer system with the DNF-470 Small RNA Analysis kit. The miRNA and small RNA sizing ranges are adjustable by user in ProSize. Method: DNF-470-33. Peaks annotated by size (nt).

5 Troubleshooting

The following table lists several potential assay specific issues which may be encountered on rare occasion when using the Small RNA Analysis kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Table 5 Troubleshooting actions for assay specific issues

Issue	Cause	Corrective Action
Sample and/or ladder signal too weak or degraded.	1 Sample and/or ladder degraded. 2 Sample, ladder and/or diluent marker are contaminated. 3 Sample concentration is too low and out of range. 4 Sample not added to Diluent Marker solution or not mixed well. 5 Rinse buffer is not fresh or a wrong rinse buffer is used. 6 Array was contaminated.	1 Use fresh sample and/or ladder. 2 Clean working area and equipment with RNaseZap. Always wear gloves when preparing sample/ladder. Use new sample, ladder aliquot, and diluent marker. 3 Verify sample was within concentration range specified for the Small RNA Analysis kit and prepare sample at higher concentration; OR Repeat experiment using increased injection time and/or injection voltage. 4 Verify sample was correctly added and mixed to sample well. 5 Prepare a new rinse buffer plate with 200 µL/well 0.25x TE Rinse Buffer. 6 Follow the Method C outlined in Appendix 7 – Capillary Array Cleaning of the Fragment Analyzer User Manual to decontaminate and clean the capillary array.
Sample signal drops abruptly at the end of separation.	1 Sample concentration too high and out of range.	1 Verify sample was within concentration range specified for the Small RNA Analysis kit.
Missing LM signal or noisy baseline.	1 Expired Diluent Marker solution. 2 Dirty array inlet. 3 Aging array.	1 Use a fresh Diluent Marker solution. 2 Follow Method C outlined in Appendix 7 – Capillary Array Cleaning of the Fragment Analyzer User Manual to clean the array. 3 Replace the array with a new array. If issue persists, contact Agilent Technical Support.

Issue	Cause	Corrective Action
Peak too broad, signal too low and/or migration time too long.	1 Capillary array needs to be reconditioned. 2 Capillary array vent valve is clogged.	1 Follow Method C outlined in Appendix 7 – Capillary Array Cleaning of the Fragment Analyzer User Manual to clean the array. 2 Flush the vent valve with deionized water using the [Utilities-Clean Reservoir Vent Valve] function as outlined in Appendix 8 - Reservoir Vent Valve Cleaning of the Fragment Analyzer User Manual.
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of sample plate well, or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20 µL is required. 3 Capillary is plugged.	1 Check sample plate wells for trapped air bubbles. Centrifuge plate. 2 Verify proper volume of solution was added to sample well. 3 Check waste plate for liquid in the capillary well. If no liquid is observed, follow the steps outlined in Appendix 7 – Capillary Array Cleaning of the Fragment Analyzer User Manual for unclogging a capillary array.

In This Book

This kit Guide describes the following:

- Agilent Small RNA Kit
- Additional Material and Equipment Required
- Agilent Small RNA Kit Protocol
- Checking Your Separation Results
- Troubleshooting

www.agilent.com

Edition 12/2018

M5310-91470

Agilent