



Agilent DNF-940 Plasmid DNA Quick Guide for the Fragment Analyzer Systems

The Agilent Fragment Analyzer systems are automated capillary electrophoresis platforms for scalable, flexible, fast, and reliable electrophoresis of nucleic acids.

This Quick Guide is intended for use with the Agilent 5200, 5300, and 5400 Fragment Analyzer systems only. The DNF-940 Plasmid DNA kit from Agilent (500 Samples) (Part # DNF-940-K0500) was developed for the analysis of supercoiled plasmid DNA between 2,000 bp and 10,000 bp. Sizing of the supercoiled plasmid and relative quantification between samples can be obtained using this kit. The DNF-940 kit is capable of accurately sizing the supercoiled plasmid DNA form only. Linearized plasmid DNA can also be detected, but will migrate more slowly and not size accurately, as it is referenced to the supercoiled Plasmid DNA Ladder included with this kit. The nicked/open circular form of plasmid DNA cannot be detected or analyzed with the DNF-940 kit.

Specifications

Analytical specifications	Plasmid DNA assay
DNA Sizing Range	2,000 bp – 10,000 bp
DNA Sizing Accuracy ¹	± 10%
Recommended Concentration Range ¹	0.1 ng/μL – 1.0 ng/μL Final Concentration DNA in 1x TE Buffer
Physical Specifications	
Total electrophoresis run time	33cm: 30 minutes
Samples per run	12, 48 or 96; depending on the instrument type
Sample volume required	Variable (24 μL final sample volume required; optimal sample concentration range in 1x TE Buffer)
Kit stability	4 months

¹ Results using DNA Ladder of DNA Fragment standards initially prepared in 1x TE buffer.

Kit Components – 500 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6601*		Plasmid DNA, 500, 4°C	
	DNF-940-0240	Plasmid DNA Gel, 240 mL	1
	DNF-355-0125	5x 930 dsDNA Inlet Buffer, 125 mL <ul style="list-style-type: none"> Dilute with sub-micron filtered water prior to use 	1
	DNF-495-0060	Dilution Buffer 1X TE, 60mL	1
DNF-940-FR*		Plasmid DNA, FR	
	DNF-600-U030	Intercalating Dye, 30 µL	1
	FS-SLR940-0001	Plasmid DNA Ladder, 1.2 mL	1
	FS-SMK940-0003	Plasmid DNA Marker, 3.2 mL	2
5191-6614*		Qualitative DNA, 500, RT	
	FS-SM015	Mineral Oil Dropper Bottle, 15mL	1
	DNF-475-0050	5x Capillary Conditioning Soln, 50 mL	1

*not orderable

WARNING

- Refer to product safety data sheets for further information
- When working with the Fragment Analyzer kit components follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

Additional Material Required for Analysis with the Fragment Analyzer Systems

- Fragment Analyzer systems with LED fluorescence detection:
 - 5200 Fragment Analyzer system (p/n M5310AA)
 - FA 12-Capillary Array Ultrashort, 22 cm (p/n A2300-1250-2247) OR
 - FA 12-Capillary Array Short, 33 cm (p/n A2300-1250-3355) OR
 - FA 12-Capillary Array Long, 55 cm (p/n A2300-1250-5580)
 - 5300 Fragment Analyzer system (p/n M5311AA)
 - FA 48-Capillary Array Short, 33 cm (p/n A2300-4850-3355) OR
 - FA/ZAG 96-Capillary Array Short, 33 cm (p/n A2300-9650-3355) OR
 - FA/ZAG 96-Capillary Array Long, 55 cm (p/n A2300-9650-5580)
 - 5400 Fragment Analyzer system (p/n M5312AA)
 - FA 48-Capillary Array Short, 33 cm (p/n A2300-4850-3355) OR
 - FA/ZAG 96-Capillary Array Short, 33 cm (p/n A2300-9650-3355) OR
 - FA/ZAG 96-Capillary Array Long, 55 cm (p/n A2300-9650-5580):
 - Agilent Fragment Analyzer controller software (Version 1.1.0.11 or higher)
 - Agilent ProSize data analysis software (Version 2.0.0.61 or higher)

Additional equipment/reagents required (not supplied)

- 96-well PCR sample plates. Please refer to Appendix – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer System User Manual for a complete approved sample plate list
- Multichannel pipettor(s) and/or liquid handling device capable of dispensing 1 – 100 μ L volumes (sample plates) and 1,000 μ L volumes (inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge (for spinning down bubbles from sample plates)
- Sub-micron filtered DI water system (for diluting the 5x 930 dsDNA Inlet Buffer and 5x Capillary Conditioning Solution)
- 96-deepwell 1 mL plate: Fisher Scientific #12-566-120 (inlet buffer and/or waste plate)
- Reagent reservoir, 50 mL (VWR #89094-680 or similar) (for use in pipetting inlet buffer plates/sample trays)
- Conical centrifuge tubes for prepared separation gel/dye mixture and/or 1x Capillary Conditioning Solution
 - 50 mL (for 5200 Fragment Analyzer system or 50 mL volumes): BD Falcon #352070, available from Fisher Scientific #14-432-22 or VWR #21008-940
- 250 mL (for 5300 and 5400 Fragment Analyzer systems or larger volumes): Corning #430776, available from Fisher Scientific #05-538-53 or VWR #21008-771
- Vortexer (for mixing of samples, ladders, and/or markers in tubes and/or plates)
- Capillary Storage Solution (p/n GP-440-0100)

Essential Measurement Practices

Environmental conditions	<ul style="list-style-type: none"> Ambient operating temperature: 19 – 25 °C (66 – 77 °F) Keep reagents during sample preparation at room temperature
Steps before sample preparation	<ul style="list-style-type: none"> Allow reagents to equilibrate at room temperature for 30 min prior to use
Pipetting practice	<ul style="list-style-type: none"> Pipette reagents carefully against the side of the 96-well sample plate or sample tube Ensure that no sample or Diluent Marker remains within or on the outside of the tip
Mixing and centrifugation recommendations	<ul style="list-style-type: none"> Apply a new seal to 96-well plate prior to mixing and centrifugation When mixing sample with diluent buffer, it is important to mix the contents of the well thoroughly to achieve the best results. It is highly suggested to perform one of the following methods to ensure complete mixing. Apply a new seal to 96-well plate prior to mixing and centrifugation. 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. After adding 2 µL of sample or ladder to the 22 µL of DM, use a separate pipette tip set to a larger 20 µL volume, and pipette each well up/down to further mix. Use an electronic pipettor capable of mixing a 10 µL volume in the tip after dispensing the 2 µL sample or ladder volume. Some models enable using the pipette tip for both adding and mixing. Run samples immediately after preparation, or within a day with oil overlay. If not using right away, cover and keep at 4°C, warm to RT and centrifuge before running plate

Plasmid DNA Marker Preparation

1. Store the Plasmid DNA Marker (PDM) solution at -20°C upon arrival.
2. Bring the PDM solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.
3. The PDM solution is supplied as a ready-to-use solution, containing a lower and upper marker fragment in a 1x TE buffer solution. It is intended for use as an external standard marker plate.
4. Prepare the PDM solution plate by dispensing 30 µL/well into Row A only (12-Capillary) or every well (96-Capillary) of a separate sample plate. Cover the wells with 20 µL/well of the supplied mineral oil.
5. The PDM solution is light and temperature sensitive. Once prepared and covered with mineral oil, the PDM solution may be used for up to one week. When not stored in the instrument, the prepared PDM solution plate should be stored in the dark.
6. The prepared PDM solution plate should be placed 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.

Plasmid DNA Ladder Preparation

1. Store the Plasmid DNA Ladder solution at -20°C upon arrival.
2. Bring the Plasmid DNA Ladder solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.
3. The Plasmid DNA Ladder solution is supplied as a ready-to-use solution, containing approximately 1.0 ng/μL total DNA concentration in a 1x TE buffer solution. It is used for calibrating the size of analyzed DNA fragments, as well as the approximate sample concentration. The Plasmid DNA Ladder should be added to a well of the sample plate and run in parallel with the samples for optimal results.
 - 12-Capillary Systems – Pipette 24 μL of Plasmid DNA Ladder solution into well 12 of each row of the sample plate.
 - 96-Capillary Systems – Pipette 24 μL of Plasmid DNA Ladder solution into well H12 of the sample plate.
4. Alternatively, once the Plasmid DNA Ladder has been run under the experimental method and additional samples are to be run under the same experimental conditions, the ladder can be imported in the ProSize software, enabling use of all 12 wells per row or all 96 wells of the sample plate. However, note that the sizing and quantification accuracy will be reduced when using an imported ladder.

Sample Plate Preparation

1. The recommended total input DNA sample concentration for this method is a range of 0.1 ng/μL to 1 ng/μL in 1xTE buffer (DNA fragments) for optimal assay results. If the concentration of the sample is above this range, pre-dilute the sample with the supplied 1x TE buffer prior to performing the assay. Do not pre-dilute samples with DI water.
2. The above DNA sample concentrations assume a starting sample matrix of 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA). If the chloride salt concentration is greater than 10 mM, some loss of sensitivity may be observed, and slight adjustments may need to be made to the sample injection conditions.
3. Ensure that samples are well mixed (vortexed) before loading into the sample plate. Using a clean 96-well sample plate, into each sample well pipette 24 μL of the samples diluted to the concentration range specified in Step 1 above.
4. If running the Plasmid DNA Ladder in parallel with the samples, pipette 24 μL of the Ladder solution directly (no dilution) into Well 12 of each row to be analyzed (12-capillary systems) or Well H12 of the sample plate (96-capillary system).
5. Fill any unused wells within the sample row or plate with 24 μL of 1x TE dilution buffer.
6. Check the wells of the sample plate to ensure there are no air bubbles trapped in the bottom of the wells. If necessary, centrifuge the plate to remove any air bubbles. The presence of trapped air bubbles can lead to injection failures.
7. Run the sample plate immediately once prepared, or cover the sample plate with a cover film, store at 4°C, and use as soon as possible. Alternatively, to prevent evaporation, place a mineral oil overlay on each sample (20 μL/well).
8. 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.
9. The CCD detection system of the Fragment Analyzer system provides a high dynamic range for detection. An ideal injection range would yield peak heights from 100 – 10,000 RFUs. Overloading of sample can decrease separation resolution and saturate the detector, leading to mismatched lower/upper marker peak heights and poor results. It is important to optimize sample dilution and concentration and use experimental parameters to work with within the specified RFU range.

Gel preparation

Prepare gel/dye mixture for 5200, 5300, and 5400 Fragment Analyzer Systems. To ensure the gel/dye mixture is mixed homogeneously without generating bubbles, gently invert the centrifuge tube 5 to 10 times, depending on the volume of the mixture. **NOTE:** Centrifuge dye prior to opening the vial to reduce risk of leaking.

5200 Fragment Analyzer system volume specifications

# of Samples to be Analyzed ¹	Volume of Intercalating Dye	Volume of Separation Gel ²	Volume of 1x Conditioning Solution ²
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

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

²A 5 mL minimum volume in the tube is included.

5300 Fragment Analyzer system volume specifications with 48-capillary array

# of Samples to be Analyzed ¹	Volume of Intercalating Dye	Volume of Separation Gel ²	Volume of 1x Conditioning Solution ²
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

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

²A 5 mL minimum volume in the tube is included.

5300 and 5400 Fragment Analyzer systems volume specifications with 96-capillary arrays

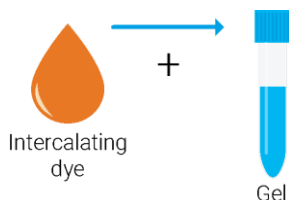
# of Samples to be Analyzed ¹	Volume of Intercalating Dye	Volume of Separation Gel ²	Volume of 1x Conditioning Solution ²
96	4.0 µL	40 mL	40 mL
192	8.0 µL	80 mL	80 mL
288	12.0 µL	120 mL	120 mL
384	16.0 µL	160 mL	160 mL
480	20.0 µL	200 mL	200 mL

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

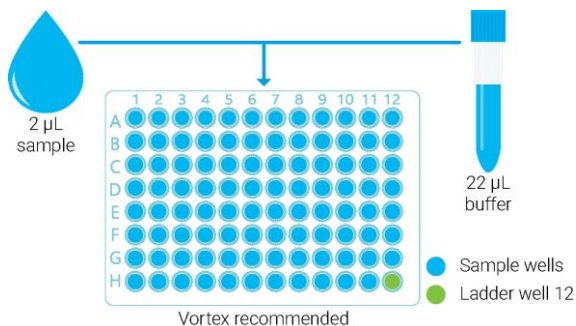
² A 5 mL minimum volume in the tube is included.

Agilent DNF-940 assay operating procedure

- Mix fresh gel and dye according to the volumes in the Gel preparation tables. Refill 1x Capillary Conditioning Solution as needed.



- Place a fresh 1x 930 dsDNA Inlet Buffer in drawer 'B' on the system, 1.0 mL/well. Replace daily.
 - 5200 system; Fill row A of buffer plate
 - 5300 system - 48 capillary; Fill rows A-D of buffer plate
 - 5300/5400 system - 96 capillary; Fill all rows of buffer plate
- Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.
 - 5200 system; Fill row H of buffer plate with 1.0mL/well, place in drawer "B"
 - 5300 system - 48 capillary; Fill rows A-D of a sample plate with 100 μ L/well, place in drawer '3'
 - 5300/5400 system - 96 capillary; Fill all rows of a sample plate with 100 μ L/well, place in drawer '3'
 - 5400 system; place in drawer "S"
- Prepare Marker plate and place in drawer 'M' on the system, 30 μ L/well. Add 1 drop or \sim 30 μ L of mineral oil to each well. The marker plate should last for 30+ injections or \sim 1 month.
 - 5200 system; Fill row A of sample plate
 - 5300 system - 48 capillary; Fill rows A-D of sample plate
 - 5300/5400 system - 96 capillary; Fill all rows of sample plate
- Mix samples with Diluent Buffer 1x TE in sample plate. Add ready to use ladder in corresponding well, dependent on the capillary size.



5200 system; Ladder – well 12, depending on which row is chosen

5300 system - 48 capillary; Ladder – well D12 or H12, depending on which group is chosen

5300/5400 system - 96 capillary; Ladder – well H12


WARNING

Working with Chemicals
The handling of reagents and chemicals might hold health risks.

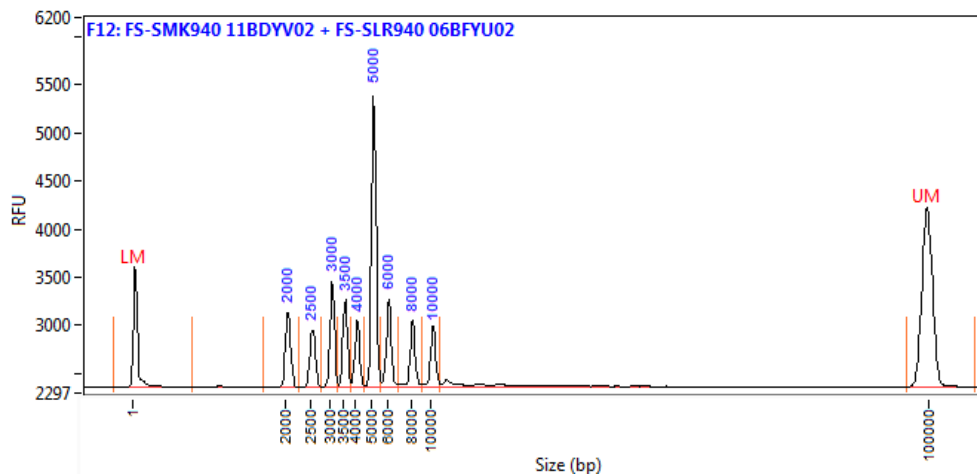
- Refer to product material safety datasheets for further chemical and biological safety information.

- Follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

Agilent Fragment Analyzer software operating procedure

1. Select Row, Group or Tray to run.
2. Enter **sample ID** and **Tray ID**(optional).
3. Select **Add to Queue**, from the dropdown menus select the corresponding method based on your capillary length;
 - 3.1 DNF-940-33 – Plasmid DNA
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

Plasmid DNA Ladder result



Plasmid DNA Ladder result using the Fragment Analyzer system with the Plasmid DNA Analysis kit. Peaks annotated by size (bp).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-940 Plasmid DNA Analysis kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
The peak signal is >> 20,000 RFU; upper marker peak is low or not detected relative to lower marker.	1 Input DNA sample concentration is too high. Ensure peak height does not exceed 10,000 RFU (fragment) or total input concentration does not exceed recommended limits.	1 Further dilute input DNA sample concentration with 1x TE buffer and repeat experiment. 2 Reduce injection time and/or injection voltage and repeat experiment. Use the same injection voltage/time settings for the Marker Plate and Sample Plate to maximize quantification accuracy.
No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.	1 Sample concentration too low and out of range. 2 Sample was not added to 1x TE diluent or not mixed well.	1 Prepare more concentrated sample and repeat experiment (e.g. 4 μ L sample + 20 μ L DI water); OR Repeat experiment using increased injection time and/or injection voltage for Marker Plate and Sample Plate. 2 Verify sample was correctly added and mixed to sample well.
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 μ L 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 the Appendix – Capillary Array Cleaning of the Fragment Analyzer User Manual for unclogging a capillary array.

For Research Use Only

Not for use in Diagnostic Procedures.

Technical Support and Further Information

For technical support, please visit www.agilent.com. It offers useful information and support about the products and technology.

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