Agilent DNF-467 Genomic DNA 50 kb Kit 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 Genomic DNA 50 kb kit (p/n DNF-467-0500) is designed for assessing the integrity, approximate size and quantitation of genomic DNA.

Specifications

Analytical specifications[1,2]	Genomic DNA 50 Kb assay
Sizing Range	75 bp – 60,000 bp
gDNA Concentration Range[2]	25 ng/µL – 250 ng/µL input gDNA
gDNA Quantification Precision[2]	25% CV
gDNA Quantification Accuracy[2]	± 30%
Maximum gDNA Concentration	250 ng/µL
Physical Specifications[3]	
Total electrophoresis run time	22cm[1]: 50 minutes, 33cm: 60 minutes
Samples per run	12, 48 or 96; depending on the instrument type
Sample volume required	1 µL
Kit stability	4 months

1 The FA 12-Capillary Array Ultrashort, 22 cm is only available for 5200 Fragment Analyzer system.
2 Results using gDNA sample in 1x TE buffer.

Kit Components – 500 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6569*		Genomic DNA 50kb, 500, 4°C	
	DNF-270-0240	Genomic DNA Separation Gel, 240 mL	1
	DNF-300-0008	BF-25 Blank Solution, 8mL	1
	DNF-355-0125	5x 930 dsDNA Inlet Buffer, 125 mL • Dilute with sub-micron filtered water prior to use	1
	DNF-367-U050	Extended Genomic DNA Ladder, 50 µL	1
	DNF-375-0120	HS Genomic DNA Diluent Marker, 120 mL	1
	DNF-497-0125	0.25x TE Rinse Buffer, 125 mL	1
5191-6612*		Quantitative DNA, RT	
	DNF-475-0050	5x Capillary Conditioning Soln, 50 mL	1
	C275-130	Eppendorf LoBind 0.5 mL tubes (bag of 50)	1
DNF-600-U030	DNF-600-U030	Intercalating Dye, 30 µL	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.

NOTE: The Lambda DNA fragment (48,500 bp) in the Extended Genomic DNA Ladder is sensitive to degradation. The ladder should be kept at 2–8°C. Do not subject the ladder to repetitive freeze-thaw cycles. Do not pipette the ladder up and down; vortex with care.

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 µL volumes (sample plates) and 1,000 µL 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 1mL 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	• Ambient operating temperature: 19 – 25 °C (66 – 77 °F) • Keep reagents during sample preparation at room temperature
Steps before sample preparation	• Allow reagents to equilibrate at room temperature for 30 min prior to use
Pipetting practice	• 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	• Apply a new seal to 96-well sample plate prior to mixing and centrifugation
	• When mixing sample with Diluent Marker (DM), it is important to mix the contents of the well thoroughly to achieve the most accurate quantification. The following mixing procedure should be performed to ensure complete mixing. After mixing, briefly centrifuge and visually confirm that all liquid is collected at the bottom of the 96-well sample plate or tube strips and any air bubble is removed
	  • When mixing sample with diluent marker solution, it is important to miz the contents of the wells thoroughly to achieve the most accurate quantification. After adding 1 µL of sample to the 199 µL of DM, use a separate pipette tip with the pipettor set to ~100 µL volume, and pipette each well up/down about 10 times to further mix.
	  • 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

Important Information Before Handling the Ladder

Do not manually mix the Extended Genomic DNA Ladder by repeated inverting of the tube or repeated pipetting up/down; doing so will result in the degradation of the Lambda DNA fragment in the ladder. The ladder solution can only be vortexed by a vortex mixer.

Centrifugation should be done at a speed low enough to remove air bubbles as well as avoid settling of genomic DNA at the bottom of the sample well. High speed centrifugation can cause genomic DNA to settle at the bottom of the sample wells, leading to sampling errors and less accurate quantification. A recommended relative centrifugal force (RCF) limit is 100 x g for less than 30 seconds.

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

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

[1] One sample well per separation is dedicated to the ladder.
[2] A 5 mL minimum volume in the tube is included.

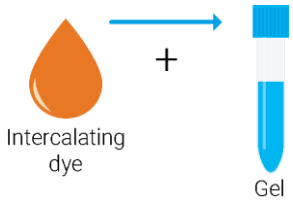
5300 and 5400 Fragment Analyzer systems volume specifications with 96-capillary arrays

# 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 Genomic DNA 50kb DNF-467 assay operating procedure

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



2. Place a fresh 1x 930 dsDNA Inlet Buffer in drawer 'B' on the system, 1.0 mL/well. Replace daily.

    2.1. 5200 system; Fill row A of buffer plate

    2.2. 5300 system - 48 capillary; Fill rows A-D of buffer plate

    2.3. 5300/5400 system - 96 capillary; Fill all rows of buffer plate

3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.

    3.1. 5200 system; Fill row H of buffer plate with 1.0mL/well, place in drawer "B "

    3.2. 5300 system - 48 capillary; Fill rows A-D of a sample plate with 100 µL/well, place in drawer '3'

    3.3. 5300/5400 system - 96 capillary; Fill all rows of a sample plate with 100 µL/well, place in drawer '3'

        3.3.1. 5400 system; place in drawer "S"

4. Place 0.25x TE Rinse Buffer plate in drawer 'M' on the system, 200 µL/well. Replace daily.

    4.1. 5200 system; Fill row A of sample plate

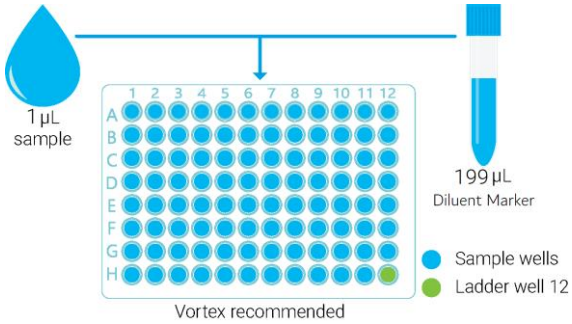
    4.2. 5300 system - 48 capillary; Fill rows A-D of sample plate

    4.3. 5300/5400 system - 96 capillary; Fill all rows of sample plate

5. Mix samples with Diluent Marker in sample plate, add 24 µL of BF-25 Blank Solution to unused wells. Place ladder in corresponding well dependent on the capillary size.
6. Ladder preparation:

    6.1. Add 199 uL of Genomic DNA DM into a 0.5 mL Eppendorf DNA LoBind tube. Gently vortex the vial containing the Genomic DNA Ladder, then pipette 1 µL of the Genomic DNA Ladder into the 199 uL of the DM Solution. This is now the working Genomic DNA Ladder solution.

    6.2. Mix the working Genomic DNA Ladder solution only by vortexing in the vortex mixer. Pipette the entire 200 µL of the working Genomic DNA Ladder solution into the designated ladder wells for each capillary array system as noted below.



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

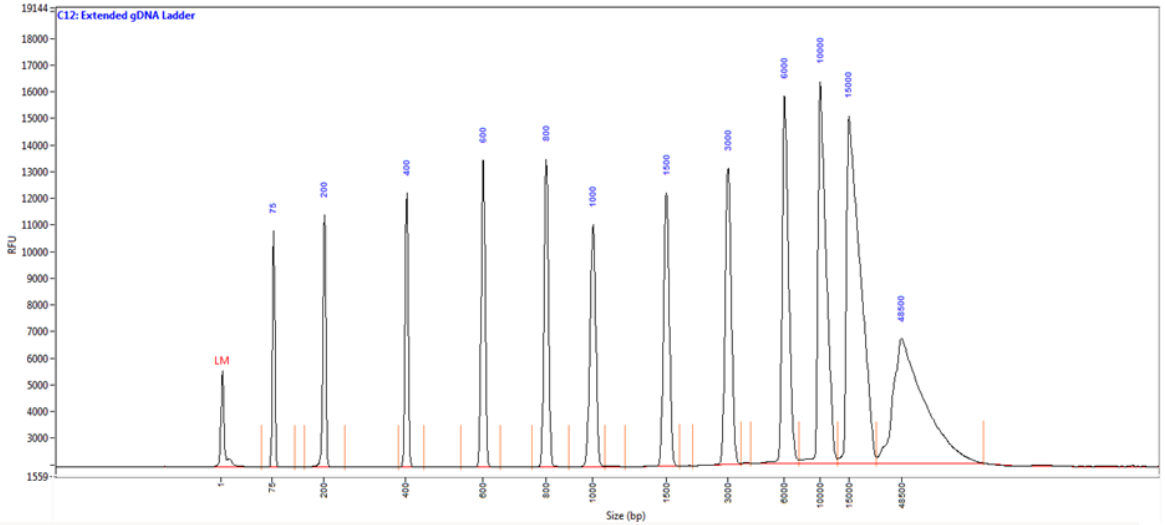
WARNING Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- 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-467-22 – Genomic DNA 50kb
   3.2 DNF-467-33 – Genomic DNA 50kb
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select to start the separation.

Extended Genomic DNA Ladder result



Representative Extended Genomic DNA Ladder result using the Fragment Analyzer systems with the DNF-467 Genomic DNA 50 kb kit. The peaks are annotated by size (bp). Method: DNF-467-33 (short capillary array).

Troubleshooting

The following table lists several potential assay-specific issues which may be encountered when using the DNF-467 Genomic DNA 50 kb kit and suggested remedies. Contact Agilent Technical Support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
No peak observed for sample when expected. Lower marker peak observed.	1 Sample concentration too low and out of range.	1 Prepare more concentrated sample and repeat experiment; OR prepare sample and analyze with HS Genomic DNA 50 kb kit.
	2 Sample not homogenously mixed before sampling.	2 Make sure the sample is equilibrated to room temperature for at least 30 min before use, vortex the sample or pipette up-down to mix before sampling.
	3 Sample not added to DM.	3 Verify sample was correctly added.
	4 Sample highly degraded; no dye intercalates.	4 Sample not suitable for use
Much lower concentration obtained for gDNA sample than expected.	1 Sample contains very high molecular weight (HMW), aggregated genomic DNA (>>60kb).	1 The analysis of HMW, aggregated genomic DNA can result in lower than expected concentration values due to the nature of sample aggregation, which can inhibit sample injection. Analysis of these types of samples at lower concentrations with this kit or with the HS Genomic DNA 50 kb kit may improve the quantitation.
Extra peaks/smear near lower marker	1 Genomic DNA possibly contaminated with	1 Remove RNA contaminants from the

observed (10-1000bp).	RNA.	genomic DNA sample and reanalyze; OR exclude the extra peaks in data processing for better quantitation and sizing accuracy.
Degradation of the 48,500 bp Lambda DNA fragment in the ladder.	1 Ladder solution was manually mixed by repeated inverting of the tube or repeated pipetting up/down, or ladder solution was exposed to freeze/thaw cycles.	1 Prepare fresh ladder solution. Mix the ladder solution only by vortexing and avoid freeze/thaw cycles.
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of the sample plate well, or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20µL 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 System 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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