Agilent Ultra Sensitivity NGS Kit Quick Guide for the Femto Pulse System

The Agilent Femto Pulse system is an automated capillary electrophoresis platform for scalable, flexible, fast, and reliable electrophoresis of nucleic acids.

This Quick Guide is intended for use with the Agilent Femto Pulse system only. The Ultra Sensitivity NGS kit (275 Samples) (Part # FP-1101-0275) is designed for the sizing and quantitation of NGS libraries at low sample concentrations ranges.

Specifications

Analytical specifications[1]	Ultra Sensitivity NGS assay
Sizing Range	100 bp – 6,000 bp
Sizing Accuracy	± 5% or better
Sizing Precision	2% CV
DNA Fragment Detection Range[1] (S/N >5)	0.05 pg/µL - 5 pg/µL input DNA
DNA Smear Detection Range[2] (S/N >5)	5 pg/µL - 250 pg/µL input DNA
DNA Fragment Detection Range[1] (S/N > 10)	0.1 pg/µL - 5 pg/µL input DNA
DNA Smear Detection Range[2] (S/N > 10)	25 pg/µL - 250 pg/µL input DNA
DNA Quantification Accuracy[1,2]	± 25%
DNA Quantification Precision[1,2]	15% CV
Maximum gDNA Concentration	5 pg/µL per DNA fragment; 250 pg/µL total
Physical Specifications	
Total electrophoresis run time	50 minutes
Samples per run	12-Capillary: 11 (+1 Ladder Well)
Sample volume required	2 µL
Kit stability	4 months

1 Results using DNA Fragment standards initially prepared in 0.25x TE buffer
2 Results using NGS Library sample with smear range from 100 bp to 1,200 bp in 0.25x TE buffer

Kit Components – 275 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6605*		Ultra Sensitivity NGS, 275, 4C	
	FP-5101-0250	FP NGS Separation Gel, 250 mL	1
	DNF-306-0005	BF-P25 Blank Solution, 5 mL	1
	DNF-325-0075	5x Inlet Buffer, 75 mL	1
	DNF-498-0012	Dilution Buffer 0.25x TE, 12mL	1
	DNF-497-0060	0.25x TE Rinse Buffer, 60mL	1
FP-1101-FR*		Ultra Sensitivity NGS, FR	
	FP-6001-U030	FP Intercalating Dye, 30 µL	1
	FP-8101-U025	FP US NGS Diluent Marker, 25 µL	1
	FP-7101-U060	FP US NGS Ladder, 60 µL	1
5191-6619*		Femto Pulse, RT	
	C27-130	Eppendorf LoBind 0.5 mL Tubes (Bag of 50)	1
	DNF-425-0050	5x Conditioning Solution, 50 mL	1
	GP-435-0100	Storage Solution, 100 mL	1

*Not Orderable.

** It is highly recommended to aliquot the Ultra Sensitivity NGS Ladder prior to first use, to minimize the number of freeze/thaw cycles. Using the provided Eppendorf LoBind 0.5 mL tubes, aliquot 12 µL of Ultra Sensitivity NGS Ladder per tube into 5 tubes and store the aliquots at -20°C. Each aliquot is good for 5 freeze/thaw cycles.

WARNING

- Refer to product safety data sheets for further information
- When working with the Femto Pulse assay follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

Additional Material Required for Analysis with the Femto Pulse Systems

- Femto Pulse system with LED fluorescence detection:
- Femto Pulse system (p/n M5330AA)
    - FP 12-Capillary Array, 22 cm (p/n A1600-1250-2240)
- Agilent Femto Pulse controller software (Version 1.0 or higher)
- Agilent ProSize Data Analysis software (Version 3.0 or higher)

Additional equipment/reagents required (not supplied)

- 96-well PCR sample plates. Please refer to Appendix – Femto Pulse Compatible Plates and Tubes in the Femto Pulse 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
- Wide-Bore Genomic pipette tips, Thermo Scientific #21-402-157 (as needed for pipetting gDNA samples)
- 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 (Femto Pulse system): BD Falcon #352070, available from Fisher Scientific #14-432-22 or VWR #21008-940
    - 250 mL (Femto Pulse system or larger volumes): Corning #430776, available from Fisher Scientific #05-538-53 or VWR #21008-771
- 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

Marker/Ladder/Sample Preparation

General Information

1. The recommended 96-well sample plate for use with the Femto Pulse system is a semi-skirted PCR plate from Eppendorf (#951020303). Please refer to the Appendix – Femto Pulse Compatible Plates and Tubes in the Femto Pulse 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.

Ultra Sensitivity NGS Diluent Marker Preparation

1. Store the Ultra Sensitivity NGS Diluent Marker (DM) at -20ºC upon arrival.
2. Bring the DM to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to diluting.
3. The DM is provided as a stock solution. Dilute 400x with DNF-498 Dilution Buffer 0.25x TE before use (1 µL DM + 399 µL DNF-498). Agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing. For best results, use the diluted DM solution within one day and discard the unused portion.

Ultra Sensitivity NGS Ladder Preparation

Prior to first use, the Ultra Sensitivity NGS Ladder solution should be aliquoted to minimize the number of freeze/thaw cycles.

1. Using the provided Eppendorf LoBind 0.5 mL tubes, aliquot 12 µL of Ultra Sensitivity NGS Ladder per tube into 5 tubes and store the aliquots at -20°C. Each aliquot is good for 5 freeze/thaw cycles.
2. Bring the Ultra Sensitivity NGS Ladder to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to diluting.
3. The Ultra Sensitivity NGS Ladder is provided as a 1.0 ng/µL stock solution. Dilute 20x to a final concentration of 50 pg/µL with DNF-498 Dilution Buffer 0.25x TE before use (2 µL Ladder + 38 µL DNF-498). For best results, use the diluted Ladder solution within one day and discard the unused portion.

Sample Plate Preparation

1. The total input DNA sample concentration must be within a range of 0.1 pg/µL to 5 pg/µL (DNA fragment) or 5 pg/µL to 250 pg/µL (DNA smear) for optimal assay results. If the concentration of the sample is above this range, pre-dilute the sample with 0.25x TE buffer prior to performing the assay. Do not pre-dilute samples with DI water.
2. Using a clean 96-well sample plate, pipette 18 µL of diluted DM Solution to each well in a row that is to contain sample or DNA Ladder. Fill any unused wells within the row of the sample plate with 20 µL/well of BF-P25 Blank Solution.
3. DNA Ladder: It is highly recommended to run NGS Ladder in parallel with the samples.
   a) Pipette 2 µL of diluted NGS Ladder into the 18 µL of diluted DM Solution in Well 12 of each row to be analyzed.
   b) Mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.
4. Samples: Pipette 2 µL of each DNA sample into the 18 µL of diluted DM Solution in the respective wells of the Sample Plate; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip.
5. After mixing sample/DNA Ladder and diluted DM 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.

6. Run the sample plate immediately once prepared, or cover the sample plate with a cover film, store at 2-8°C, and use as soon as possible. Alternatively, to prevent evaporation, place a mineral oil overlay on each sample (20 µL/well).
7. To run the samples, place the plate in one of the three sample plate trays (Drawers 4-6 from the top) of the Femto Pulse instrument. Load the experimental method as described in the following sections.

NOTES:

1. Avoid total DNA input sample concentrations above the specified limits. Overloading of DNA sample can result in saturation of the CCD detector and poor results. The peak heights for individual DNA fragments should lie in an optimal range between 20 –3,000 RFUs.
2. If you use an imported NGS Ladder, the imported ladder must be prepared using the same diluted DM as the samples, to ensure proper quantification.
3. The sample plate should be analyzed within a day after preparation.

Important Sample Mixing Information

When mixing sample with the diluted DM Solution, 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 the 18 µL of diluted DM Solution, swirl the pipette tip while pipetting up/down to further mix. OR
- After adding 2 µL of sample or ladder to the 18 µL of diluted DM Solution, 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
- After adding 2 µL of sample or ladder to the 18 µL of diluted DM Solution, use a separate pipette tip set to a larger 16 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 or ladder into the 18 µL of diluted DM Solution. Some models enable using the pipette tip for both adding/mixing.

Gel preparation

Prepare gel/dye mixture for Femto Pulse System. 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, when possible.

Femto Pulse system volume specifications

# 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	2.0 µL	20 mL	20 mL
36	3.0 µL	30 mL	30 mL
48	4.0 µL	40 mL	40 mL
96	8.0 µL	80 mL	80 mL

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

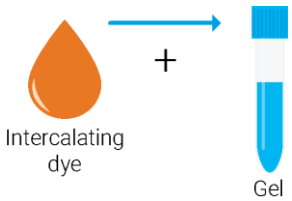
Daily Conditioning (Recommended)

For optimal array performance when running the FP-1101 Ultra Sensitivity NGS kit, it is recommended to perform an additional daily conditioning of the capillary array for 20 min.

1. From the main screen of the Femto Pulse control software, select the Operation tab. Under the Capillary Array > Conditioning field press **Add to queue**. The Select Conditioning Method form will be displayed, enabling the user to select the conditioning method from the dropdown menu.
2. Select the "20 min Conditioning" method from the dropdown menu. This method performs a 20 min conditioning solution flush followed by a 3 min Gel fill.
3. Press **OK** to add the method to the instrument queue (press **Cancel** to abort adding the method).
4. Press the Play icon to start the sequence loaded into the queue.

Agilent FP-1101 Ultra Sensitivity NGS 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. Femto Pulse system; Fill row A of buffer plate

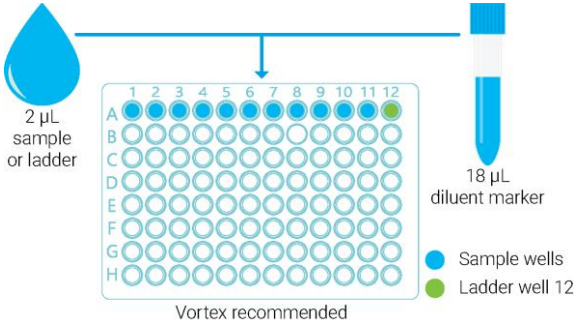
3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.

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

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

    4.1. Femto Pulse system; Fill row A of sample plate

5. Mix samples or Ladder with Diluent Marker in sample plate, add 20 µL of BF-P25 Blank Solution to unused wells. Place ladder in corresponding well dependent on the capillary size.



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

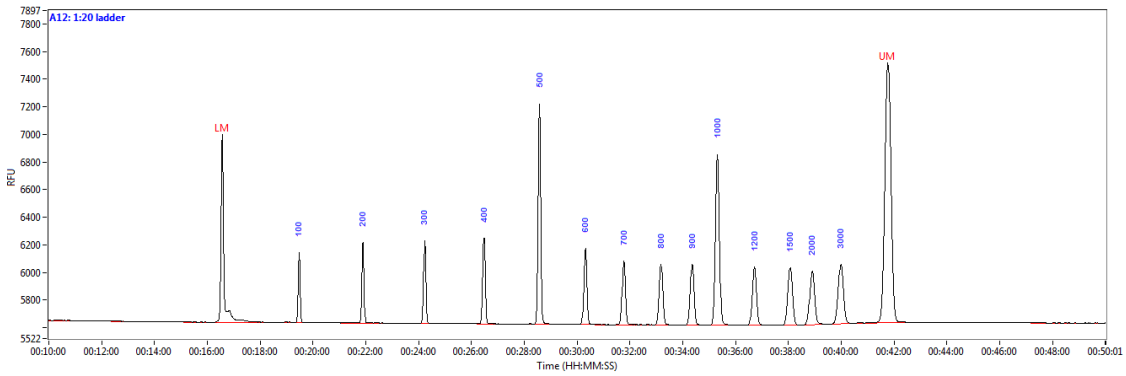
WARNING Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- 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 Femto Pulse 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 FP-1101-22 – US NGS
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select ▶ to start the separation.

Ultra Sensitivity NGS Ladder result



Representative Ultra Sensitivity NGS Ladder result using the Femto Pulse system with the FP-1101 Ultra Sensitivity NGS kit (1bp-6000 bp). Method: FP-1101-22 –US NGS. Peaks annotated by size (bp).

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the Ultra Sensitivity NGS 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 >> 3,000 RFU; upper marker peak is low or not detected relative to lower marker.	1 Input sample concentration is too high. Ensure total signal height does not exceed 3,000 RFU, or total input DNA concentration does not exceed 250 pg/µL.	1 Further dilute input sample concentration with 0.25x TE buffer and repeat experiment OR reduce injection time and/or injection voltage, and repeat experiment.
DNA sample smear overlaps with Lower/Upper Marker peak.	1 Input DNA sample size distribution outside of assay range.	1 Perform further size selection of sample to narrow DNA size distribution and repeat experiment.
	2 Input DNA sample concentration too high.	2 Dilute input DNA sample concentration with 0.25x TE buffer and repeat experiment.
No peak observed for DNA sample when expected. Lower/Upper Marker peak observed.	1 Sample concentration too low and out of range.	1 Prepare more concentrated sample and repeat experiment OR repeat experiment using increased injection time and/or injection voltage for sample plate.
	2 Sample was not added to Diluent Marker solution, or not mixed well.	2 Verify sample was correctly added and mixed in the sample well.
No sample peak or marker peak observed for individual sample.	1 Air trapped at the bottom of sample plate and/or marker plate well or bubbles present in well. 2 Insufficient sample volume. A minimum of 20µL is required. 3 Capillary is plugged.	1 Check sample/marker plate wells for trapped air bubbles. Centrifuge the plate. 2 Verify proper volume of solution was added to sample well and marker 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 Femto Pulse 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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Edition 02/22

SD-AT000143