Agilent Genomic 55 kb BAC Kit Quick Guide for Femto Pulse Systems

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 55 kb BAC kit (275 Samples) (Part # FP-1003-0275) is designed for the Pulse-Field CE separation and sizing of bacterial artificial chromosome (BAC) fragments smaller than 55 kb in length.

Specifications

Analytical specifications[1]	55 Kb BAC assay	
Sizing Range	75 bp – 48,500 bp	
Sizing Accuracy[1]	± 15% or better	
Sizing Precision[1]	15% CV	
DNA Fragment Concentration Range	Single fragment at 48,500 bp[1]	3 pg/µL – 25 pg/µL input DNA
	Single fragment at 1,000 – 2,000 bp[2]	1pg/µL – 12.5 pg/µL input DNA
Maximum gDNA Concentration	25 pg/µL per fragment; 100 pg/µL total input DNA	
Physical Specifications		
Total electrophoresis run time	90 minutes	
Samples per run	12-Capillary: 11 (+1 Ladder Well)	
Sample volume required	2 µL	
Kit stability	4 months	

1 Lambda DNA sizing.

2 1kb and 1.5kb fragment sample sizing.

Kit Components – 275 Sample Kit

Kit Component Number	Part Number (Re-order Number)	Description	Quantity Per Kit
5191-6604*		Femto Pulse, 4°C	
	FP-5001-0250	FP Large DNA 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-1003-FR*		55 kb BAC, FR	
	FP-6001-U030	FP Intercalating Dye, 30 µL	1
	FP-8001-0003	FP gDNA Diluent Marker, 3 mL	3
	FP-7003-U035	FP 55 kb BAC Ladder, 35 µL	1
5191-6618*		BAC, RT	
	C27-130	Eppendorf LoBind 0.5 mL Tubes (Bag of 50)	1
	C280-101	Wide-Bore Genomic Pipette Tips, 1 Box	1
	DNF-425-0050	5x Conditioning Solution, 50 mL	1
	GP-435-0100	Storage Solution, 100 mL	1
	FS-SMO15	Mineral Oil Dropper Bottle, 15 mL	1

*Not Orderable.

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 System

- Femto Pulse systems 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 Part #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.
2. Store the gDNA Diluent Marker solution at -20ºC and the 55 kb BAC Ladder at 2-8ºC upon arrival.
3. Allow the gDNA Diluent marker and 55 kb ladder to warm up to room temperature prior to use. Briefly spin the tubes after thawing to ensure liquid is at the bottom of the tube.

   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.

55 kb BAC Ladder Handling and Storage

Prior to first use, the 55 kb BAC Ladder should be aliquoted to minimize the number of freeze/thaw cycles.

1. Equilibrate the 55 kb BAC Ladder to room temperature for about 30 min and mix briefly by vortexing. Aliquot 5.0 µL of the 55 kb BAC Ladder to 7 different Eppendorf LoBind tubes (provided with kit) using the provided wide-bore genomic pipette tips. Each aliquot is good for 4-times use (1 µL per use).
2. Store the 55 kb BAC Ladder aliquots at 2-8ºC; avoid freeze-thawing.

55 kb BAC Ladder Working Solution

1. Before use, equilibrate the 165 kb Ladder aliquot to room temperature for about 30 min.
2. In an Eppendorf LoBind 0.5 mL tube (provided), aliquot 9 µL of the DNF-498 0.25x TE Dilution Buffer.
3. To the same Eppendorf tube aliquot 90 µL of the FP-8001 gDNA Diluent Marker solution.
4. Aliquot 1 µL of the 55 kb BAC Ladder into the same Eppendorf tube containing 99 µL of the solutions from steps 2 and 3 above (FP-8001 Diluent Marker + 0.25x TE). Vortex to mix. This is the 55kbBAC Ladder Working Solution; use within one day of preparation.
5. Load 20 µL of the prepared 55 kb BAC Ladder Working Solution into Well 12 of a sample plate row that is to be analyzed.
6. The 55 kb BAC Ladder should be run in parallel with the samples for each experiment. It is not recommended to import a previously run 55 kb BAC Ladder.

NOTE: For samples that do not require predilution or the predilution is less than 160x, the sample matrix is expected to influence sample sizing. Please refer to Preparation of 55 kb BAC Ladder Working Solution for instructions on preparing a 55 kb BAC Ladder Working Solution that contains the sample matrix.

Sample Plate Preparation

Important gDNA Sampling Procedures:

Before sampling, the sample stock of BAC fragments must be acclimatized to room temperature for at least 30 minutes.

1. The total input BAC sample concentration should be no higher than 100 pg/µL. As a general note, smaller BAC fragments should be run at a lower limit of the concentration range; larger BAC fragments should be run at a higher limit of the concentration range.

2. If the starting material is at a higher than 100 pg/µL concentration, pre-dilute the sample to the specified concentration range with DNF-498 Dilution Buffer 0.25x TE.
3. The sample matrix, i.e. salt concentration in the restriction digestion buffers, can affect the mobility of the DNA fragments and therefore the sample sizing accuracy. Pre-diluting the BAC samples 160-times or more usually nullifies the sample matrix effect on sample sizing. If the sample pre-dilution is less than 160x or not required due to a low initial sample concentration, refer to the Appendix - Preparation of 55 kb BAC Ladder Working Solution of this manual for special instructions on the preparation of the 55 kb BAC Ladder Working Solution to compensate for the matrix effect on the sample migration.
4. Using a clean 96-well sample plate, pipette 18 µL of FP-8001 gDNA Diluent Marker Solution (DM) to each well of the 96-well plate to contain a sample. Fill any unused wells within the row of the sample plate with 20 µL/well of BF-P25 Blank Solution.
5. Pipette 2 µL of each BAC sample into the 18 µL of DM in the respective wells of the Sample Plate.
6. Load 20 µL of the 55 kb BAC Ladder Working Solution into Well 12 of a sample plate row that is to be analyzed (See previous Section).
7. After loading the samples and 55 kb BAC Ladder Working Solution in each well, check the wells of the sample plate to ensure there are no air bubbles trapped in the bottom of the wells. Centrifuge the plate to remove any trapped air bubbles. The presence of trapped air bubbles can lead to injection failures.
8. 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). The sample plate should be analyzed within a day after preparation.
9. 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.

NOTE: 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 100–5,000 RFUs.

NOTE: The sample matrix, i.e. salt concentration in the restriction digestion buffers, can affect the mobility of the DNA fragments and therefore the sample sizing accuracy. Pre-diluting the BAC samples 160-times or more usually nullifies the sample matrix effect on sample sizing. If the sample pre-dilution is less than 160x or not required due to a low initial sample concentration, refer to Appendix A - Preparation of 55 kb BAC Ladder Working Solution of this manual for special instructions on the preparation of the 55 KB BAC Ladder Working Solution to compensate for the matrix effect on the sample migration.

Important Sample Mixing Information

For best results, it is important to mix the contents of the sample wells thoroughly. It is highly suggested to perform one of the following methods to ensure complete mixing:

- After adding 2 µL of sample to the 18 µL of diluent marker, place a plate seal on the sample plate and vortex the sample plate at 3000 rpm for 2 min (two vortexing pulses, 1 min each are recommended). 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.
- Mix the sample wells by pipetting up/down 2-3 times with a wide-bore genomic pipette tip and the pipettor set to ~18 µL volume.

Gel preparation

Prepare gel/dye mixture for Femto Pulse 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.

5200 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.

Method D Flush

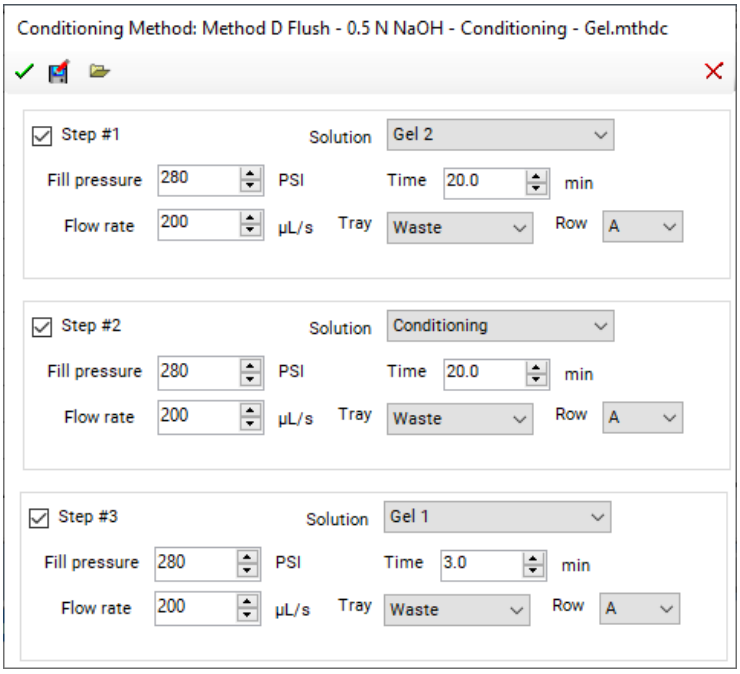
Occasionally when performing separations of high molecular weight (HMW) DNA > 100kb, loss of HMW DNA peak shape or peak signal can occur. In such cases, it is recommended to perform an additional cleaning of the capillary array with 0.5 N NaOH solution, conditioning solution, and gel using the Method D Flush to restore separation performance. Instructions for performing this protocol are outlined below.

WARNING 0.5 N NaOH is corrosive

- Use extreme caution when handling, as exposure can cause severe eye and skin burns. Avoid contact with eyes, skin, or clothing. Wear eye protection and impervious gloves. Clearly label containers to avoid accidental exposure.
- Refer to the product material safety datasheets for all warnings and precautions before proceeding.

1 From the main screen of the Femto Pulse controller software, select the **Operation** tab. Under the **Capillary Array > Conditioning** field click **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 **Method D Flush – 0.5 N NaOH – Conditioning – Gel** from the method dropdown menu. This method will perform a 20 min 0.5 N NaOH solution flush from the Gel2 fluid line, a 20 min conditioning solution flush from the Conditioning line, and a 3 min gel flush from the Gel1 line (figure below).



3 Click **OK** to add the method to the instrument queue (click **Cancel** to abort adding the method).

4 Open the Femto Pulse system side compartment and replace the **Gel2** bottle with a bottle containing a minimum of 25 mL of 0.5N NaOH solution.

5 Ensure there is a minimum of 25 mL of 1x Conditioning Solution in the **Conditioning** bottle, and a minimum of 10 mL of FP Large DNA Separation Gel in the **Gel1** bottle.

6 Close the door to the system side compartment and click the **Play** icon to start the selected capillary conditioning method.

7 Once the capillary conditioning method is complete, open the waste drawer and remove the 96-deepwell 1mL plate. Empty the waste plate contents in the proper waste disposal area and return the empty plate to the waste drawer.

8 The Femto Pulse system is now ready to run additional samples or can be stored until next use.

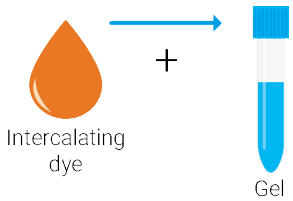
Daily Conditioning (Recommended)

For optimal array performance when running the FP-1003 55 kb BAC 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 controller 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-1003 55 kb BAC 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

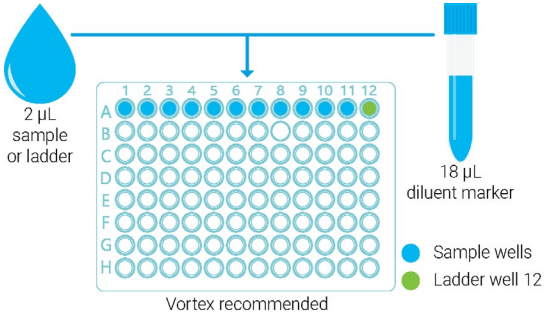
3. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.

    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. Dilute the FP 55 kb BAC Ladder solution 100x to make the ladder working solution (9 µL Dilution Buffer 0.25x TE + 90 µL FP gDNA Diluent Marker + 1 µL FP 55 kb BAC Ladder solution). Mix by vortexing. Prepare daily and use within a day.
6. Mix samples with Diluent Marker in sample plate, add 20 µL of BF-P25 Blank Solution to unused wells. Place ladder in corresponding well 12.



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

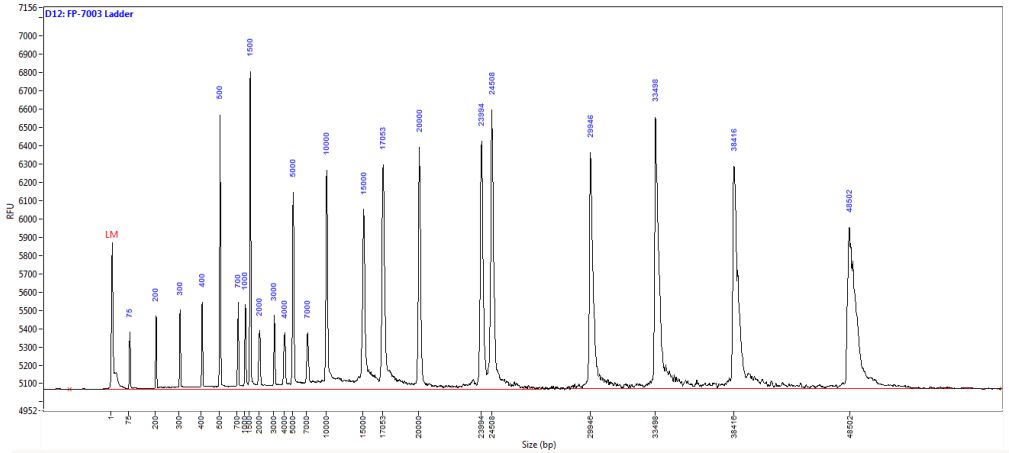
WARNING Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- 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-1003-22 – 55kb BAC
4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

55 kb BAC Ladder result



Expected 55 kb BAC Ladder result, using the Femto Pulse System with the FP-1003 55 kb BAC kit. Peaks are annotated by size (bp). Method: FP-1003-22 –55kbBAC

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the FP-1003 55 kb BAC kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
55 kb BAC Ladder peaks are missing, and/or48,500bp ladder peak is degraded.	1 The 55 kb BAC Ladder has degraded or handling instructions have not been followed.	1 Start with a new aliquot of the 55 kb BAC Ladder. Prepare and handle the ladder as directed in the manual.
Large size compressed peak at the end of the BAC sample.	1 The BAC sample is too large for the resolution using the FP-1003 55 kb BAC kit and method.	1 Analyze the BAC sample using the FP-1004 method 165kb BAC Analysis kit.
The peak signal is >>5,000 RFU.	1 Input sample concentration too high.	1 Further dilute input sample concentration with 1xTE buffer and repeat the experiment OR Reduce injection time and/or injection voltage and repeat the experiment.
No expected DNA fragment peak(s) observed. Lower Marker observed.	1 Sample concentration is below detection. 2 Sample was not added to a sample plate, or wrong sample row was selected for analysis.	1 Prepare more concentrated sample and repeat the experiment. 2 Verify sample was correctly added or the correct sample row was selected for the analysis.
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.

Technical Support and Further Information

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

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