Agilent FP-1003 55kb BAC

Quick Guide for the Femto Pulse

For Research Use Only. Not for use in diagnostic procedures

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%	
Sizing Precision[1]	15% CV	
DNA Fragment Concentration Range	Single fragment at 48,500 bp[1] Single fragment at 1,000 – 2,000 bp[2]	3 pg/µL – 25 pg/µL input DNA 1pg/µL – 12.5 pg/µL input DNA
Physical Specifications		
Total Electrophoresis Run Time	90 minutes	
Samples Per Run	12-Capillary: 11 (+1 Ladder Well)	
Sample Volume Required	2 µL	
Guaranteed Shelf Life	4 months	

1 Lambda DNA sizing.
2 1kb and 1.5kb fragment sample sizing

Kit Components – 275 Sample Kit – *Refer to product label for proper storage conditions*

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
	5460-0122 OR 5460-0121	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.

Altering any reagents and/or use of unapproved or non-recommended reagents may materially alter the performance of the instrument such that the instrument no longer performs to Agilent specifications. Any work performed by Agilent to bring the instrument back into compliance with Agilent specifications will be performed at the customer's expense.

WARNING

Working with Chemicals

- **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 personal protective equipment (PPE).**

Additional Material Required for Analysis with the Femto Pulse System

Instrument	Compatible Arrays	Part Number
Femto Pulse System	FP 12-Capillary Array, 22 cm	A1600-1250-2240

Software

- Agilent Femto Pulse controller software
- ProSize data analysis software

Reagents

- Capillary Storage Solution (GP-435-0100)

Additional equipment 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
- Adhesive PCR plate seal
- Wide-Bore Genomic pipette tips, USA Scientific Part #1011-8810 (as needed for pipetting gDNA samples)
    - Alternative Wide-Bore pipette tips, Midland Scientific Part # 30389188
- 96-well plate centrifuge (for spinning down bubbles from sample plates)
- Sub-micron filtered DI water system (for diluting the 5x 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

Essential Measurement Practices

Environmental conditions	• Ambient operating temperature: 19 – 25 °C (66 – 77 °F) • Keep reagents during sample preparation at room temperature
Sample Input Concentration	• Ensure sample input concentrations lie within kit specifications. • Sample signal should not exceed 60,000 RFU.
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 Plate Preparation	• The recommended 96-well sample plate for use with the Femto Pulse system is a semi-skirted PCR plate from Eppendorf (#951020303). • Store the gDNA Diluent Marker solution at -20ºC and the 55 kb BAC Ladder at 2-8ºC upon arrival. • 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.
Ladder Handling & Storage	• Prior to use, the 55kb BAC ladder should be aliquoted to minimize the number of freeze/thaw cycles. • 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). • Store the 55 kb BAC Ladder aliquots at 2-8ºC; avoid freeze-thawing.
55kb BAC ladder working solution	• Before use, equilibrate the 165 kb Ladder aliquot to room temperature for about 30 min. 1 In an Eppendorf LoBind 0.5 mL tube (provided), aliquot 9 µL of the DNF-498 0.25x TE Dilution Buffer. 2 To the same Eppendorf tube aliquot 90 µL of the FP-8001 gDNA Diluent Marker solution. 3 Aliquot 1 µL of the 55 kb BAC Ladder into the same Eppendorf tube containing 99 µL of the solutions from steps 1 and 2 above (FP-8001 Diluent Marker + 0.25x TE). Vortex to mix. This is the 55kb BAC Ladder Working Solution; use within one day of preparation. 4 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. • 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

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

1. 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.
2. 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.
3. Pipette 2 µL of each BAC sample into the 18 µL of DM in the respective wells of the Sample Plate.
4. 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).
5. 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.
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). The sample plate should be analyzed within a day after preparation.
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.

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.

Mixing and centrifugation recommendations

- 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 and down 2-3 times with a wide-bore genomic pipette tip and the pipettor set to ~18 µL volume.

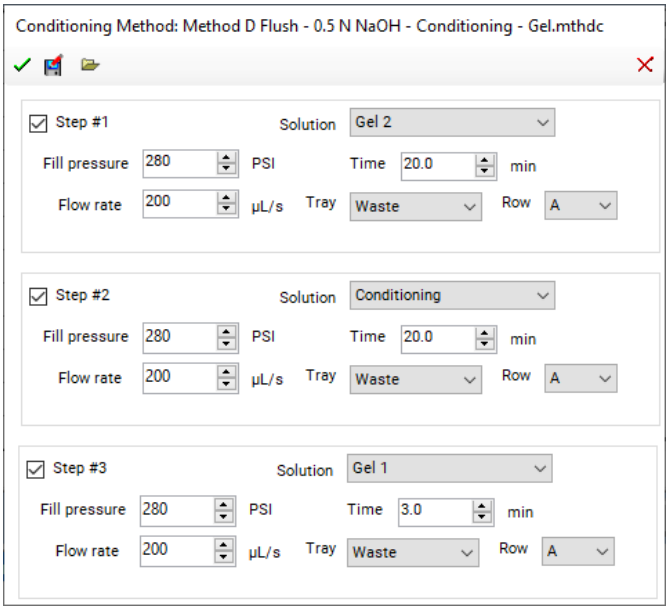
Daily Conditioning (Recommended)

- For optimal array performance when running FP-1003 55kb kit, it is recommended to perform an additional daily conditioning of the capillary array for 20 minutes.
    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.

Method D Flush

- Occasionally when preforming 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.

1. From the main screen of the Femto Pulse controller software, select the **Operations** 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.

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.

FP-1003 55kb BAC Reagent Kit Quick Guide for the Femto Pulse System

Gel Preparation

Centrifuge dye prior to opening the vial to reduce risk of leaking. Ensure the gel + dye is mixed without generating bubbles, gently invert tube 5-10 times.

Number of Samples	Intercalating Dye Volume (µL)	Separation Gel Volume (mL)
12	1.0	10
24	2.0	20
36	3.0	30
48	4.0	40
96	8.0	80

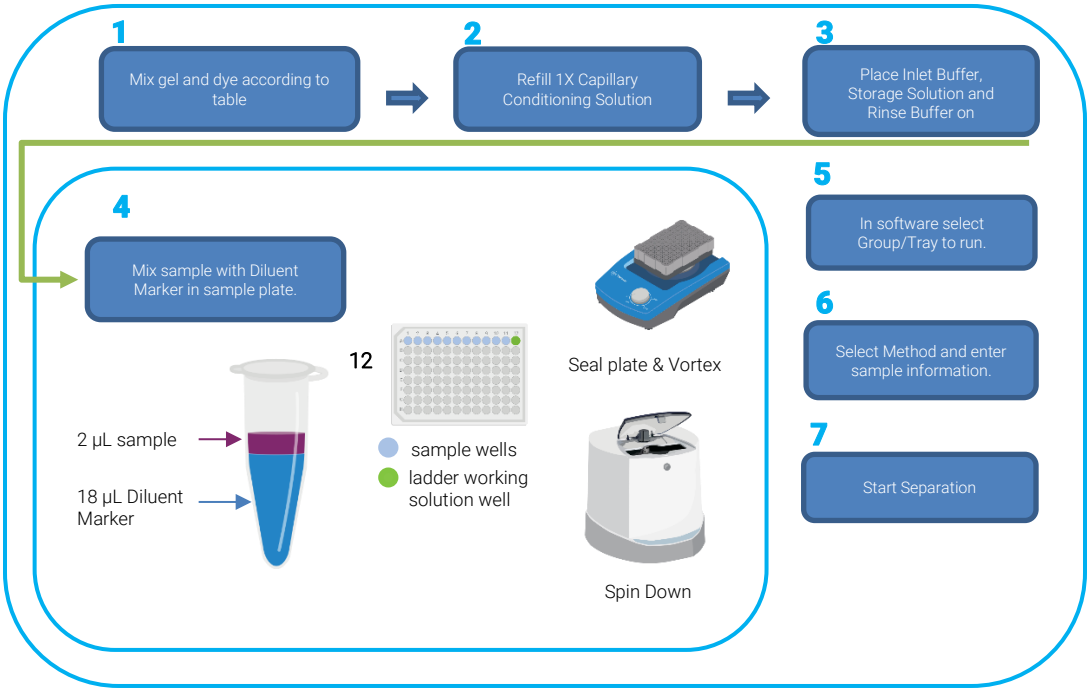
Conditioning Solution

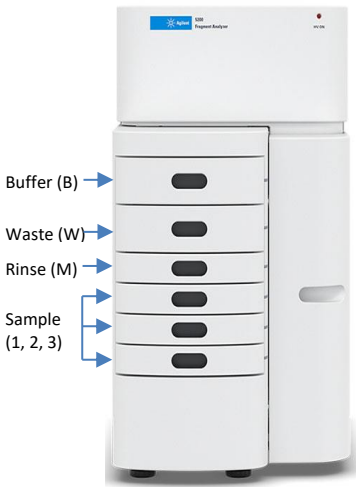
The provided 5X Conditioning Solution must be diluted to 1X using submicron DI water prior to use. Invert to mix.

Number of Samples	Volume of 1X Conditioning Solution (mL)
12	10
24	20
36	30
48	40
96	80

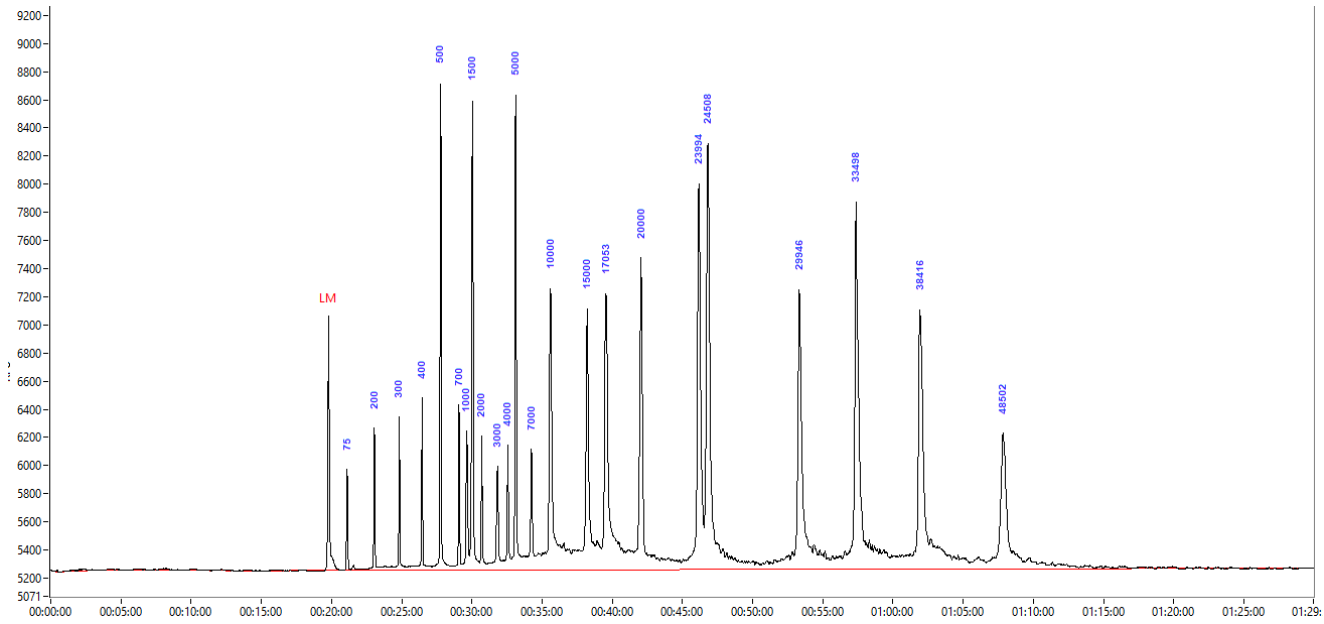
Agilent FP-1003 55 kb BAC assay operating procedure

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 Inlet Buffer in drawer 'B' on the system, 1.0 mL/well. Replace daily.
3. Femto Pulse system; Fill row A of buffer plate
4. Prepare Capillary Storage Solution plate. Replace every 2-4 weeks for optimal results.
5. Femto Pulse system; Fill row H of buffer plate with 1.0mL/well, place in drawer "B "
6. Place 0.25x TE Rinse Buffer plate in drawer 'M' on the system, 200 µL/well. Replace daily.
7. Femto Pulse system; Fill row A of sample plate
8. 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.
   - 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 (see sample plate image below).
9. Select Row/Group/Tray to run. Enter sample ID and Tray ID, if desired.
10. Add to queue, from the dropdown select the corresponding method based on the sample type and capillary length;
    - FP-1003-22 – 55kb BAC
11. Add method to the queue by selecting "OK", press play 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.	2	The BAC sample is too large for the resolution using the FP-1003 55 kb BAC kit and method.	2	Analyze the BAC sample using the FP-1004 method 165kb BAC Analysis kit.
The peak signal is >60,000 RFU.	3	Input sample concentration too high.	3	Further dilute input sample concentration with 1xTE buffer and repeat the experiment
No expected DNA fragment peak(s) observed. Lower Marker observed.	4	Sample concentration is below detection.	4	Prepare more concentrated sample and repeat the experiment.
	5	Sample was not added to a sample plate, or wrong sample row was selected for analysis.	5	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.	6	Air trapped at the bottom of sample plate and/or marker plate well or bubbles present in well.	6	Check sample/marker plate wells for trapped air bubbles. Centrifuge the plate.
	7	Insufficient sample volume. A minimum of 20µL is required.	7	Verify proper volume of solution was added to sample well and marker well.
	8	Capillary is plugged.	8	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.

Appendix A: Preparation of 55 kb BAC Ladder Working Solution by Adding Sample Matrix for Optimal Sample Sizing

The presence of salts in BAC samples digested with restriction enzymes can affect the migration of the fragments relative to the sizing Ladder, and thus impact the sizing accuracy of the samples. To account for sample matrix (salt concentration) effects on the mobility of DNA fragments, it is recommended to add the sample matrix to the sizing Ladder.

The ideal sample buffer/ matrix is the same restriction enzyme buffer that was used to digest the BAC Clone samples. The sample matrix should be added to the Ladder at the same final concentration as is present in the prepared samples.

NOTE When performing sample predilutions above 160x, it is generally not necessary to add sample matrix to the 55 kb BAC Ladder Working Solution, as any sample matrix effects are eliminated by dilution.

Steps for 55 kb BAC Ladder Working Solution Preparation Using the Sample Matrix

1. Dilute the restriction digestion buffer used for BAC samples to the 1x concentration using nuclease free water. This is the **1x Sample Matrix Solution (1x SMS)**.
2. Dilute the 1x Sample Matrix Solution (1x SMS) 10x using DNF-498 Dilution Buffer 0.25x TE (e.g., add 2 µL of the 1x SMS to 18 µL of DNF-498). This is now the **0.1x Sample Matrix Solution (0.1x SMS)**.
3. Refer to the **Table 1** below for example sample dilutions and corresponding volumes of components to be added to make the 55 kb BAC Ladder Working Solution.

Table 1 Preparation of the 55 kb BAC Ladder Working Solution with Sample Matrix.

Sample Predilution	FP-8001 DM, µL	Sample Matrix Solution (SMS), µL	DNF-498 Dilution Buffer or 0.25x TE, µL	55 kb BAC Ladder, µL	Total Volume of Ladder Working Solution, µL
None	89.0	10.0 (1x SMS)	None	1	100
10x	89.0	10.0 (0.1x SMS)	None	1	100
20x	90.0	5.0 (0.1x SMS)	4.0	1	100
40x	90.0	2.5 (0.1x SMS)	6.5	1	100
60x	90.0	1.67 (0.1x SMS)	7.33	1	100
80x	90.0	1.25 (0.1x SMS)	7.75	1	100
100x	90.0	1.0 (0.1x SMS)	8.0	1	100
120x	90.0	0.83 (0.1x SMS)	8.17	1	100
140x	90.0	0.71 (0.1x SMS)	8.29	1	100
160x	90.0	0.63 (0.1x SMS)	8.37	1	100

4. If sample predilutions other than those listed in the **Table 1** are used, refer to the general formulas below to calculate the volumes of FP-8001 DM, sample matrix solution and DNF-498 Dilution Buffer 0.25x TE to be added to make the ladder working solution.

For Sample Predilutions Less than 10x:

a (10/Sample Predilution) = Volume (µL) 1x Sample Matrix Solution (1x SMS)

b 100 – 1x SMS Volume (µL) – 90.0 µL (FP-8001 DM) – 1 µL (55 kb BAC Ladder Volume) = Volume (µL) of DNF-498 Dilution Buffer 0.25x TE

c Combine the components using the calculated volumes (µL) from A and B above:

i _____ µL 1x SMS (from calculation A)

ii 90.0 µL FP-8001 DM

iii ___ µL DNF-498 Dilution Buffer 0.25x TE (from calculation B)

iv __1__ µL 55 kb BAC Ladder

d The total volume of the Ladder working solution should equal 100 µL.

For Sample Predilutions Greater than 10x:

a (100/Sample Predilution) = Volume (µL) 0.1x Sample Matrix Solution (0.1x SMS)

b 100 – 0.1x SMS Volume (µL) – 90.0 µL (FP-8001 DM) – 1 µL (55 kb BAC Ladder Volume) = Volume (µL) of DNF-498 Dilution Buffer 0.25x TE.

c Combine the components using the calculated volumes (µL) from A and B above:

i _____ µL 0.1x SMS (from calculation A)

ii 90.0 µL FP-8001 DM

iii ___ µL DNF-498 Dilution Buffer 0.25x TE (from calculation B)

iv __1__ µL 55 kb BAC Ladder

d The total volume of the ladder working solution should equal 100 µL.

5 Following preparation of the 55 kb BAC Ladder Working Solution with sample matrix, load 20 µL of the prepared 55 kb BAC 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 sizing Ladder.

Technical Support and Further Information
For technical support please visit www.agilent.com which offers useful information and support regarding the products and technology.

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