Agilent 165 kb BAC Kit Quick Guide for 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 165 kb BAC kit (275 samples) (Part # FP-1004-0275) is designed for the Pulse-Field CE separation and sizing of bacterial artificial chromosome (BAC) fragment up to 165 kB in length.

Specifications

Analytical specifications[1]	165 Kb BAC assay
Sizing Range	75 bp – 165 kb
Sizing Accuracy[1]	± 15% or better
Sizing Precision[1]	15% CV
DNA Fragment Concentration Range	
Single Fragment ≤ 48,500 bp	1.6 pg/µL – 25 pg/µL input DNA
Single Fragment ≥ 48,500 bp	3 pg/µL – 50 pg/µL input DNA
Multiple Fragments	12.5 pg/µL – 100 pg/µL input DNA

Physical Specifications	
Total electrophoresis run time	170 minutes
Samples per run	12-Capillary: 11 (+1 Ladder Well)
Sample volume required	2 µL
Kit stability	4 months

[1] Result based on sample BAC fragments.

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, 12 mL	1
	DNF-497-0060	0.25x TE Rinse Buffer, 60 mL	1
FP-1004-FR*		165 kb BAC, FR	
	FP-6001-U030	FP Intercalating Dye, 30 µL	1
	FP-8001-0003	FP gDNA Diluent Marker, 3 mL	3
	FP-7004-U035	FP 165 kb BAC Ladder, 35 µL	1
	FP-8004-U100	FP 165 kb Upper Marker, 100 µL	1
5191-6618*		BAC, RT	
	C27-130	Eppendorf LoBind 0.5 mLTubes (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 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 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 Inlet Buffer and 5x 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 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
Steps before sample preparation	• Allow Reagents to equilibrate at room temperature for 30 min prior to use • Store the gDNA Diluent Marker solution, 165 kb Upper Marker and 165 kb BAC Ladder at -20°C upon arrival.
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 • Use only wide bore pipette tips (provided) to aliquot and mix the 165 kb Ladder and 165 kb Upper Marker.

Marker/Ladder/Sample Preparation

165 kb Upper Marker Handling and Storage

Prior to first use, the 165 kb Upper Marker should be aliquoted to minimize the number of freeze/thaw cycles.

1. Equilibrate the 165 kb Upper Marker to room temperature for about 30 min and mix by gently pipetting up-and-down up to 3 times with wide-bore genomic pipette tips (provided with kit) and a pipettor set to a 15 µL volume. Do not vortex of flick the Marker tube. Only wide-bore genomic pipette tips should be used to mix the 165 kb Upper Marker solution.

2. Aliquot the 165 kb Upper Marker solution into 7 aliquots, 14 µL each, using the provided wide-bore genomic tips and the provided Eppendorf LoBind 0.5 mL tubes. Each aliquot is good for 4-times use (3 µL per use).

3. Store the 165 kb Upper Marker aliquots at -20°C. Store the in-use vial of the 165 kb Upper Marker at 2-8°C for up to 2 weeks. Avoid freeze-thawing of the 165 kb Upper Marker more than 4 times (more frequent freeze-thawing may result in degradation of the 165 kb Upper Marker).

165 kb Upper Marker Working Solution Preparation

1. Before use, equilibrate the 165 kb Upper Marker aliquot to room temperature for about 30 min.

2. In an Eppendorf LoBind 0.5 mL tube (provided), aliquot 297 µL of the DNF-498 0.25x TE Dilution Buffer.

3. Mix the 165 kb Upper Marker aliquot very slowly by pipetting 1-2 times with a wide-bore genomic pipette tip and a pipettor set to ~5 µL volume.

4. Using a regular pipette tip, immediately aliquot 3 µL of the mixed 165 kb Upper Marker into the tube containing 297 µL of the 0.25x TE Dilution Buffer from step 2. Do not Pipette up-and-down or vortex.

5. Using the wide-bore genomic pipette tip only and a pipettor set to a 24 µL volume slowly pipette the prepared 165 kb Upper Marker working solution up and down 5 times to mix. This is the **165 kb Upper Marker Working Solution**; use within one day of preparation.

6. Using a wide bore tip load 24 µL of the **165 kb Upper Marker Working Solution** into row A of a 96-well sample plate. Overlay each well with a drop of mineral oil (provided) to prevent evaporation. The prepared **165 kb Upper Marker Working Solution** can be used for multiple injections within one day of preparation.

7. The 96-well sample plate containing the **165 kb Upper Marker Working Solution** should be placed into Drawer "M" (third from top) of the Femto Pulse. Ensure that the plate is loaded with well A1 toward the back left on the tray.

165 kb BAC Ladder Handling and Storage

Prior to first use, the 165 kb BAC Ladder should be aliquoted to minimize the number of freeze/thaw cycles.

1. Equilibrate the 165 kb BAC Ladder to room temperature for about 30 min and mix by gently pipetting up-and-down up to 3 times with wide-bore genomic pipette tips (provided with kit) and a pipettor set to a 15 µL volume.
   Do not vortex of flick the Ladder tube. Only wide-bore genomic pipette tips should be used to mix the 165 kb BAC Ladder solution.

2. Aliquot the 165 kb BAC Ladder solution into 7 aliquots, 5 µL each, using the provided wide-bore genomic tips and the provided and the provided Eppendorf LoBind 0.5 mL tubes. Each aliquot is good for 4-times use (1 µL per use).

3. Store the 165 kb BAC Ladder aliquots at -20°C. Store the in-use vial of the 165 kb BAC Ladder at 2-8°C for up to 2 weeks. Avoid freeze-thawing of the 165 kb BAC Ladder more than 4 times (more frequent freeze-thawing may result in degradation of the large size fragments in the 165 kb BAC Ladder).

165 kb Ladder Working Solution Preparation

1. Before use, equilibrate the 165 kb BAC 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. Mix the contents of the tube by vortexing.

4. Mix the 165 kb BAC Ladder aliquot very slowly by pipetting 1-2 times with a wide-bore genomic pipette tip and a pipettor set to ~5 µL volume.

5. Using a regular pipette tip, immediately aliquot 1 µL of the mixed 165 kb BAC Ladder into the tube containing the 0.25x TE Dilution Buffer and gDNA Diluent Marker from steps 2-3 above. Do not Pipette up-and-down or vortex.

6. Using the wide-bore genomic pipette tip only and a pipettor set to a 20 µL volume slowly pipette the prepared 165 kb BAC Ladder working solution up and down 5 times to mix. This is the **165 kb BAC Ladder Working Solution**; use within one day of preparation.

7. Load 20 µL of the **165 kb BAC Ladder Working Solution** into Well 12 of a sample plate row that is to be analyzed.

8. The 165 kb BAC Ladder should be run in parallel with the samples for each experiment. It is not recommended to import a previously run 165 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 Appendix: Preparation of 165 kb BAC Ladder Working Solution for instructions on preparing a 165 kb BAC Ladder Working Solution that contains sample matrix.

Sample Plate Preparation

NOTE: Important BAC fragments Sampling Procedures.

- Before sampling, the sample stock BAC fragments must be acclimatized to room temperature for at least 30 minutes.
- When mixing BAC samples containing large fragments, slowly pipette up-and-down with wide-bore genomic pipette tips.
- 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.
- 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.
- The sample plate should be analyzed within a day after preparation.

1. If the starting material is higher than the specified concentration, pre-dilute the sample to the specified concentration range with DNF-498 Dilution Buffer 0.25x TE.
2. 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.
3. If the sample predilution is less than 160x or not required due to a low initial sample concentration, refer to Appendix: Preparation of 165 kb BAC Ladder Working Solution for special instructions 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. 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.
6. Load 20 µL of the 165 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 165 kb BAC Ladder Working Ladder 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).
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.

Gel preparation

Prepare the 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.

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.

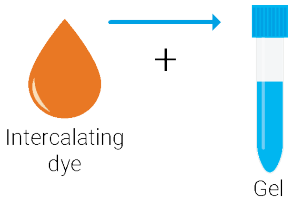
Daily Conditioning (Recommended)

For optimal array performance when running the FP-1004-0275 165 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.
    a Ensure there is 50 mL Conditioning Solution loaded on the system.
    b Ensure 15 mL PF-50001 FP Large DNA Separation Gel is loaded on the system.
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-1004 165 kb BAC assay operating procedure

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



2. Place a fresh 1x 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 Storage Solution plate. Replace every 2 weeks for optimal results.

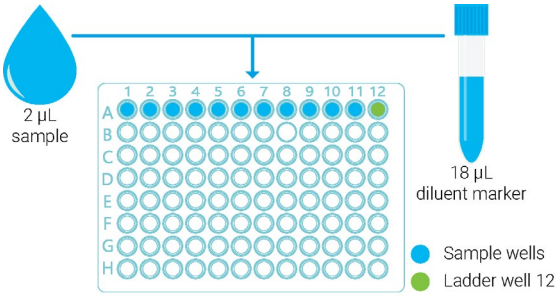
   3.1. Femto Pulse system; Fill row H of buffer plate with 1.0mL/well, place in drawer "B "

4. Load 165 kb Upper Marker Working Solution plate (prepared above) in drawer "M".
5. Load sample/ladder plate (prepared above) in one of the three sample plate trays (Drawers 4-6 from the top) of the Femto Pulse instrument.

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.



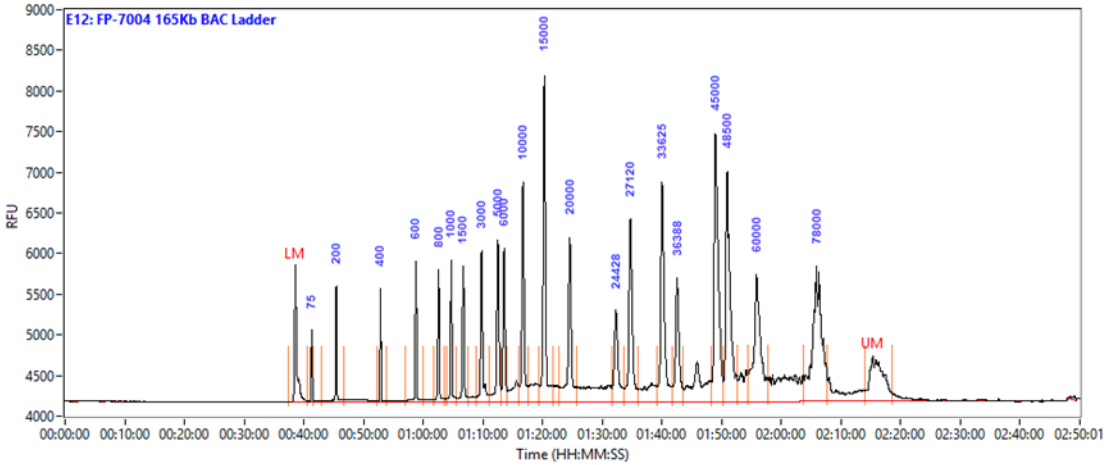
Femto Pulse system; Working Ladder Solution – well 12, depending on which row is chosen

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-1004-22 – 165kb 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.

165 kb BAC Ladder Result



165 kb BAC Ladder result, using the Femto Pulse System with the FP-1004 165 kb BAC kit. Peaks are annotated by size (bp). Method: **FP-1004-22 – 165kb BAC**

Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the FP-1004 165 kb BAC kit and suggested remedies. Contact Agilent local technical support if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
The 165 kb BAC Upper Marker or Ladder peaks are low, split, or not detected.	1 The 165 kb BAC Upper Marker or Ladder has degraded, or handling instructions have not been followed. 2 The 165 kb BAC Upper Marker or Ladder was vortexed or pipetted with regular pipette tips (not wide-bore). 3 The Femto Pulse capillary array requires a daily 20 min conditioning and Method D flush. 4 The 165 kb BAC Ladder solution is too old.	1 Start with a new aliquot of the 165 kb BAC Upper Marker or Ladder. Prepare and handle the ladder as directed in the manual. 2 Handle the Upper Marker or ladder as directed in the manual. 3 Perform the Method D Flush – 0.5 N NaOH – Conditioning Gel conditioning method. Perform 20 min conditioning daily. 4 Prepare a fresh 165 kb BAC Ladder solution. Ensure that a new 165 kb BAC Ladder Working Solution is prepared daily.
The peaks of the BAC sample appear spiky or split.	1 Input sample concentration is too high.	1 Further dilute input sample with DNF-498 Dilution Buffer 0.25x TE and repeat the experiment.

The peak signal is >>5,000 RFU.	1 Input sample concentration too high.	1 Further dilute input sample concentration with 0.25x TE 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 limit. 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.
BAC fragment sizes differ by greater than 15% from expected sizes.	1 Possible sample matrix effect on sample sizing, particularly if sample dilution is less than 160x. 2 Input sample concentration is too high.	1 For important instructions, refer to the Preparation 165 kb BAC Ladder working solution. 2 Further dilute input sample with DNF-498 Dilution Buffer 0.25x TE and repeat the experiment.
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 20 µL is required. 3 Capillary is plugged.	1 Check sample/marker plate wells for trapped air bubbles. Centrifuge 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 outline in the Appendix – Capillary Array Cleaning of the Femto Pulse User Manual for unclogging a capillary array.

Appendix A: Preparation of 165 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 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 pre-dilutions above 160x, it is generally not necessary to add sample matrix to the 165 kb BAC Ladder working solution, as any sample matrix effects are eliminated by dilution.

165 kb BAC Ladder working solution preparation using the sample matrix

1. Dilute the restriction digestion buffer used for BAC samples to a 1x concentration using nuclease free water. This is the **1x Sample Matrix Solution (1x SMS)**.
2. Dilute the 1x SMS, 10x using Dilution Buffer 0.25x TE (e.g., add 2 µL of the 1x SMS to 18 µL of Dilution Buffer 0.25x TE). This is now the **0.1x Sample Matrix Solution (0.1x SMS)**.
3. Refer to **Table A1** below for example sample dilutions and corresponding volumes of components to be added to make the 165 kb BAC Ladder working solution.

Table A1 Preparation of 165 kb BAC Ladder working solution with sample matrix.

Sample Predilution	FP-8001 DM, µL	Sample Matrix Solution (SMS), µL	DNF-498 Dilution Buffer 0.25x TE, µL	165 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 pre-dilutions other than those listed in the Table A1 are used, refer to the general formulas below to calculate the volumes of FP-8001 DM solution, SMS and Dilution Buffer 0.25x TE to be added to make the ladder working solution.

For sample pre-dilutions less than 10x:

a (10/sample pre-dilution) = Volume (µL) 1x SMS

b 100 – 1x SMS Volume (µL) – 90.0 µL (DM solution) – 1 µL (165 kb BAC Ladder Volume) = Volume (µL) of 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 DM solution

iii _____ µL Dilution Buffer 0.25x TE (from calculation B)

iv 1 µL 165 kb BAC Ladder

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

For sample pre-dilutions greater than 10x:

a (100/sample pre-dilution) = Volume (µL) 0.1x SMS

b 100 – 0.1x SMS Volume (µL) – 90.0 µL (DM solution) – 1 µL (165 kb BAC Ladder Volume) = Volume (µL) of 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 DM solution

iii _____ µL Dilution Buffer 0.25x TE (from calculation B)

iv 1 µL 165 kb BAC Ladder

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

5. Following preparation of the 165 kb BAC Ladder working solution with sample matrix, load 20 µL of the prepared 165 kb BAC Ladder working solution into well 12 of a sample plate row that is to be analyzed.
6. The 165 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.

Appendix B: DNA fragment analysis without using the Upper Marker in the sample wells

The FP-8004 165 kb Upper Marker (UM) can co-migrate with DNA fragments above 100 kb interfering with fragment sizing. Instructions for preparation of the Upper Marker row when the 165 kb UM is NOT desired in the samples are outlined below.

Femto Pulse Upper Marker Plate Preparation

1. Before use, equilibrate the 165kb Upper Marker aliquot to room temperature for about 30 min.
2. In an Eppendorf LoBind® 0.5 mL tube (provided), aliquot 99 µL of the DNF-498 0.25X TE Dilution Buffer.
3. Mix the 165 kb Upper Marker aliquot very slowly by pipetting 1-2 times with a wide-bore genomic pipette tip and a pipettor set to ~5 µL volume.
4. Using a regular pipette tip, immediately aliquot 1 µL of the mixed 165 kb Upper Marker into the tube containing 99 µL of the 0.25X TE Dilution Buffer from step 2. **DO NOT pipette up-and-down or vortex.**
5. Using the wide-bore genomic pipette tip only and a pipettor set to a 24 µL volume slowly pipette the prepared 165 kb Upper Marker working solution up and down 5 times to mix. This is the **165kb Upper Marker Working Solution**; use within one day of preparation.
6. Using a wide bore tip load 24 µL of the **165 kb Upper Marker Working Solution** into ONLY well 12 of row A (the Upper Marker row) of a 96-well sample plate.
7. Using a regular pipette tip pipette 24 µL of the DNF-498 0.25X TE Dilution buffer in wells 1-11 of row A (the Upper Marker Row).
8. Overlay each well with a drop of mineral oil (provided) to prevent evaporation. The prepared **165 kb Upper Marker Working Solution** can be used for multiple injections within one day of preparation.
9. The 96-well sample plate containing the **165 kb Upper Marker Working Solution** should be placed into Drawer "M" (third from top) of the Femto Pulse. Ensure that the plate is loaded with well A1 toward the back left on the tray.

ProSize Data Analysis Settings

1. In the ProSize data analysis software select the **Marker Analysis** settings (Figure B1).
2. Click on the drop-down menu as shown in Figure B1 and select "Use Lower Marker Only".
3. Click "Apply to All".
4. The Upper Marker will be displayed as a 165 kb peak in the ladder, and there will be no Upper Marker designation in the sample wells.
5. Without Upper Marker, sizing accuracy can exceed the specification of ±15%.

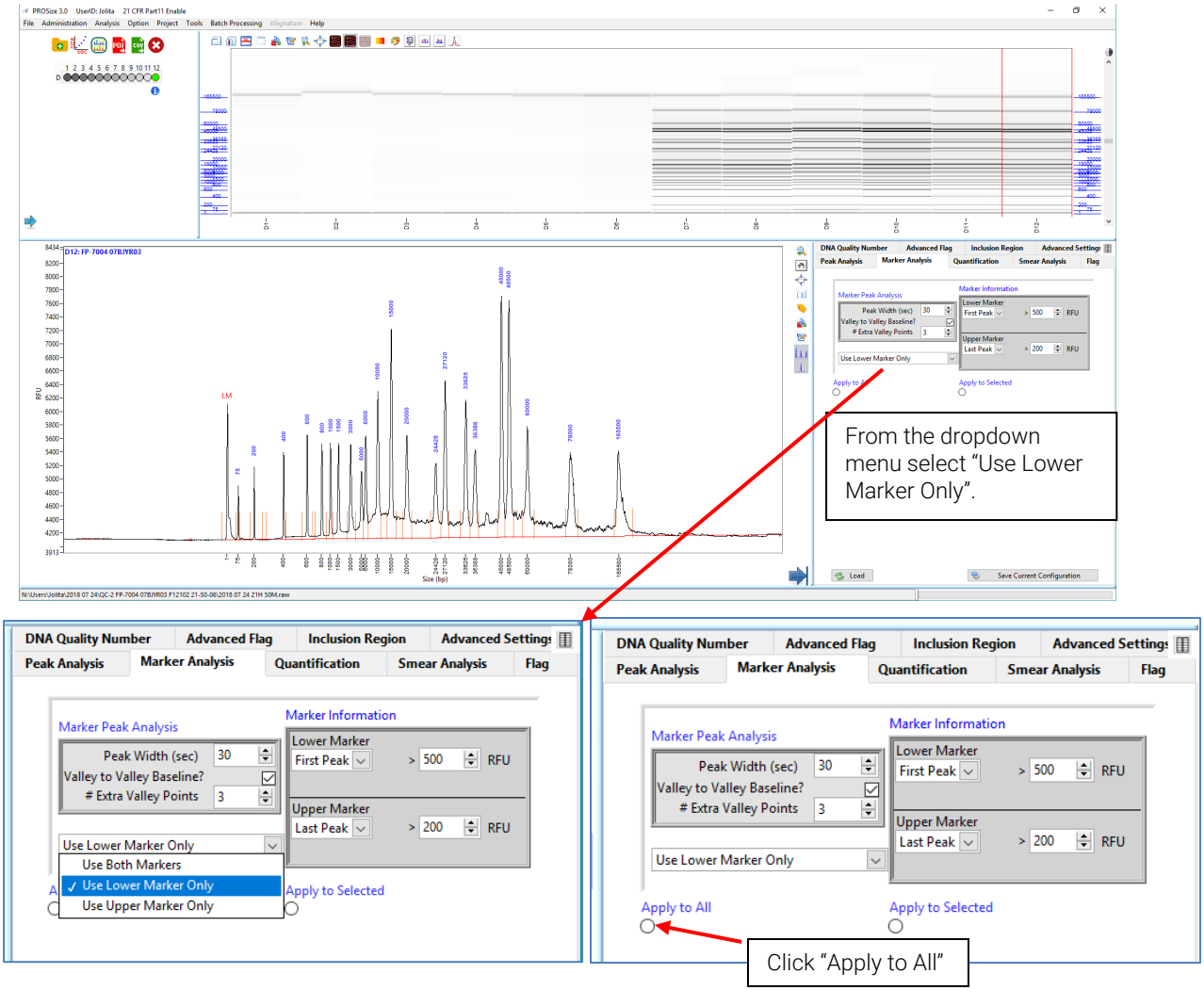


Figure B1. ProSize data analysis software window for selecting the Lower Marker Only analysis option.

Appendix C: Method D Flush

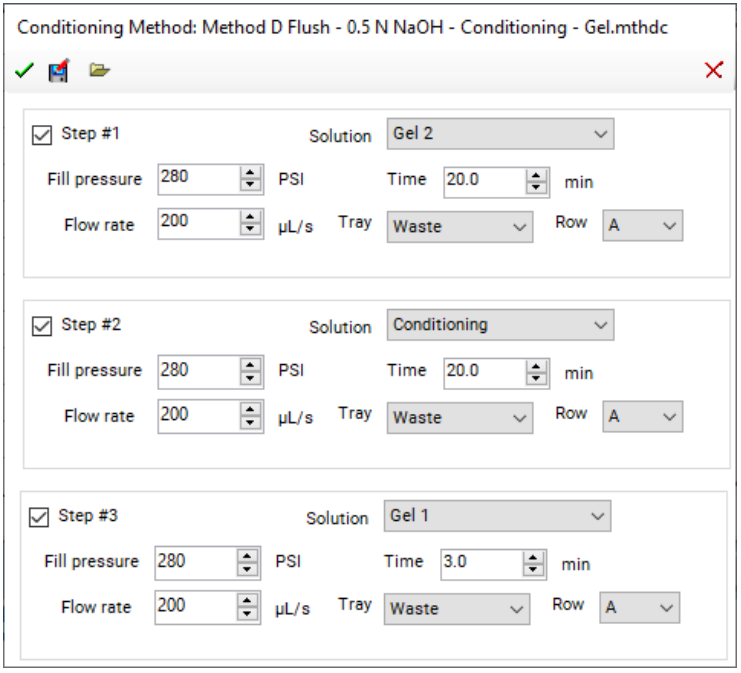
Occasionally when performing separations of high molecular weight (HMW) DNA > 100kb, loss of HMW DNA peak shape or peak signal can occur. In such cases, it is recommended to perform an additional cleaning of the capillary array with 0.5 N NaOH solution, conditioning solution, and gel using the Method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

Technical Support and Further Information

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

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