Kit Guide

For Research Use Only.

Not for use in diagnostic procedures.
Notices

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Agilent Genomic DNA 165 kb Kit

The Genomic DNA 165 kb kit (275 Samples) (Part # FP-1002-0275) is designed for the pulsed-field CE separation, sizing and quantitation of high molecular weight, low concentration DNA smears and/or fragments from 1.3 kb though 165 kb.

### Table 1  Physical Specifications

<table>
<thead>
<tr>
<th>Type</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume Required</td>
<td>2 µL</td>
</tr>
<tr>
<td>Number of Samples per Run</td>
<td>12-Capillary: 11 (+ 1 Well Ladder)</td>
</tr>
<tr>
<td>Total Electrophoresis Run Time</td>
<td>70 min (22-40 Array; Fast Method) 180 min (22-40 Array; Extended Method)</td>
</tr>
</tbody>
</table>

### Table 2  Analytical Specifications

<table>
<thead>
<tr>
<th>Type</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Sizing Range</td>
<td>1.3 kb – 165 kb</td>
</tr>
<tr>
<td>Sizing Precision¹</td>
<td>20% CV</td>
</tr>
<tr>
<td>Sizing Accuracy¹</td>
<td>± 15% or better</td>
</tr>
<tr>
<td>DNA Fragment Concentration Range¹</td>
<td>0.3 pg/µL to 30 pg/µL input DNA</td>
</tr>
<tr>
<td>DNA Smear Concentration Range²</td>
<td>5 pg/µL – 500 pg/µL input DNA</td>
</tr>
<tr>
<td>Maximum gDNA Concentration</td>
<td>500 pg/µL</td>
</tr>
<tr>
<td>gDNA Quantification Precision²</td>
<td>25% CV</td>
</tr>
</tbody>
</table>

¹ Result based on Lambda DNA Fragment  
² Result based on Coriell gDNA sample (Cat #19238)

### Table 3  Storage Conditions

<table>
<thead>
<tr>
<th>Store at ~20°C: (DO NOT FREEZE):</th>
<th>Store at 2-8°C: (DO NOT FREEZE):</th>
<th>Store at Room Temperature: (DO NOT FREEZE):</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP gDNA Diluent Marker</td>
<td>FP Large DNA Separation Gel</td>
<td>5x Conditioning Solution</td>
</tr>
<tr>
<td>FP 165 kb Ladder</td>
<td>5x Inlet Buffer</td>
<td>Storage Solution</td>
</tr>
<tr>
<td>FP Intercalating Dye</td>
<td>0.25x TE Rinse Buffer</td>
<td>Dilution Buffer 0.25x TE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BF-P25 Blank Solution</td>
</tr>
</tbody>
</table>

Ensure all reagents are completely warmed to room temperature prior to use.
## Table 4  Kit Components

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Name</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP-5001-0250</td>
<td>FP Large DNA Separation Gel</td>
<td>250 mL</td>
</tr>
<tr>
<td>FP-6001-U030</td>
<td>FP Intercalating Dye</td>
<td>30 µL</td>
</tr>
<tr>
<td>DNF-325-0075</td>
<td>5x Inlet Buffer</td>
<td>75 mL</td>
</tr>
<tr>
<td>DNF-425-0050</td>
<td>5x Conditioning Solution</td>
<td>50 mL</td>
</tr>
<tr>
<td>FP-8001-0003</td>
<td>FP gDNA Diluent Marker</td>
<td>3 mL, x 3 vials</td>
</tr>
<tr>
<td>FP-8004-U100</td>
<td>FP 165 kb Upper Marker</td>
<td>100 µL</td>
</tr>
<tr>
<td>FP-7002-U035</td>
<td>FP 165 kb Ladder* Fragments from 1.3 kb to 165 kb</td>
<td>35 µL, 2 ng/µL total DNA Concentration</td>
</tr>
<tr>
<td>DNF-498-0012</td>
<td>Dilution Buffer 0.25x TE</td>
<td>12 mL</td>
</tr>
<tr>
<td>DNF-497-0060</td>
<td>0.25x TE Rinse Buffer</td>
<td>60 mL</td>
</tr>
<tr>
<td>DNF-306-0005</td>
<td>BF-P25 Blank Solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>GP-435-0100</td>
<td>Storage Solution</td>
<td>100 mL</td>
</tr>
<tr>
<td></td>
<td>Eppendorf LoBind 0.5 mL Tubes</td>
<td>Package of 50</td>
</tr>
<tr>
<td>21-402-157</td>
<td>Wide-Bore Genomic pipette tips, Thermo Scientific</td>
<td>1 box</td>
</tr>
</tbody>
</table>

* Refer to the 165 kb Ladder Handling and Storage on page 15 of this manual for critical Ladder handling and storage information.

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**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.
2 Additional Material and Equipment Required

Material and Equipment Required for Analysis with the Femto Pulse System

Hardware
- Femto Pulse system (Part # M5330AA)
  - Femto Pulse 12-Capillary Array (Part # A1600-1250-2240)

Software:
- Femto Pulse control software (Version 1.0 or higher)
- ProSize data analysis software (Version 3.0 or higher)
Additional Equipment/Reagents Required (Not Supplied)

- 96-well PCR sample plates. Please refer to Appendix C – Femto Pulse Compatible Plates and Tubes in the Femto Pulse 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 for preparing Inlet Buffer and rinse plates
- 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 5x Inlet Buffer and 5x Conditioning Solution)
- Fisherbrand 96 DeepWell 1mL Plate, Polypropylene, Fisher Scientific Part #12-566-120 (Inlet Buffer and 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 Gel and/or 1x Conditioning Solution:
  - 50 mL (for smaller volumes): BD Falcon #352070, available from Fisher Scientific #14-432-22 or VWR #21008-940
  - 250 mL (for larger volumes): Corning #430776, available from Fisher Scientific #05-538-53 or VWR #21008-771
3 Agilent gDNA 165 kb Kit Protocol

Gel Preparation

1 Store the Large DNA Separation 5001 Gel at 2-8°C upon arrival.
2 The Femto Pulse Intercalating Dye should be stored at -20°C.
3 Bring the Large DNA Separation 5001 Gel and Femto Pulse Intercalating Dye to room temperature prior to use.
4 Mix appropriate volume of Femto Pulse Intercalating Dye and Large DNA Separation 5001 Gel necessary for one day of operation. Use a 50 mL conical centrifuge tube to allow a small minimum working volume. For larger volumes, use a 250 mL conical centrifuge tube and remove the collar of the tube holder in the instrument reagent compartment.
5 The volume of gel required per run is summarized below. For best performance, prepare the gel/dye mixture daily and use within a day.

<table>
<thead>
<tr>
<th># of Samples to be Analyzed</th>
<th>Volume of Femto Pulse Intercalating Dye</th>
<th>Volume of Gel</th>
<th>Volume of 1x Conditioning Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1.0 µL</td>
<td>10 mL</td>
<td>10 mL</td>
</tr>
<tr>
<td>24</td>
<td>2.0 µL</td>
<td>20 mL</td>
<td>20 mL</td>
</tr>
<tr>
<td>36</td>
<td>3.0 µL</td>
<td>30 mL</td>
<td>30 mL</td>
</tr>
<tr>
<td>48</td>
<td>4.0 µL</td>
<td>40 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>96</td>
<td>8.0 µL</td>
<td>80 mL</td>
<td>80 mL</td>
</tr>
</tbody>
</table>

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

6 Place the prepared gel/dye mixture onto the instrument and insert into the desired gel fluid line (Gel 1 or Gel 2 pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.

7 Update the solution levels in the Femto Pulse control software. From the main screen, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1). When loading gel, the user will be prompted to scan the associated barcode from the gel bottle label. Scan the barcode and select OK.
When switching applications (e.g., between kits), prime the appropriate gel fluid line after loading fresh gel solution. From the main screen of the Femto Pulse control software, select **Utilities > Prime**. Select the desired fluid line(s) (Conditioning, Gel 1, or Gel 2) and press **OK** to purge the fluid line with fresh gel.
Agilent gDNA 165 kb Kit Protocol

Inlet Buffer Preparation

1. Store the 5x Inlet Buffer at 2-8°C upon arrival. Do not freeze.
2. Bring the 5x Inlet Buffer to room temperature prior to mixing and use.
3. In a clean container, add 10 mL of the 5x Inlet Buffer per 40 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at 2-8°C if desired.

Conditioning Solution Preparation

1. Store the 5x Conditioning Solution at room temperature upon arrival. Do not freeze.
2. In a clean container (e.g. 50 mL or 250 mL conical centrifuge tube), add 10 mL of the 5x Conditioning Solution per 40 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at room temperature if desired.
3. Once mixed, place the 1x Conditioning Solution onto the instrument and insert the conditioning fluid line (conditioning solution pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.
4. The 1x Conditioning Solution should be added to the system as use demands. Refer to Table 5 for volume specifications.
5. When adding fresh 1x Conditioning Solution to the instrument, update the solution levels in the Femto Pulse control software. From the main screen, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1).
Agilent gDNA 165 kb Kit Protocol

Instrument Preparation

1. Check the fluid level of the waste bottle and waste tray daily and empty as needed.

2. Prepare a fresh 96 DeepWell 1mL Plate filled with 1.0 mL/well of 1x Inlet Buffer daily in Row A only. Do not overfill the wells of the inlet buffer plate.

3. In Row H of the same prepared buffer plate, place 1.0 mL/well of Storage Solution. Row H of the buffer plate is used for the Store location, and the array moves to this position at the end of the experimental sequence. The Storage Solution should be replaced weekly, as the solution will gradually thicken following exposure to the open air via evaporation.

   **NOTE**

   Ensure Row H of the buffer tray is always filled with the Storage Solution, and the capillary array is placed against the Storage Solution when not in use, to prevent the capillary tips from drying out and potentially plugging.

   Replace Storage Solution weekly for best array performance.

4. Place the prepared inlet buffer plate into Drawer “B” (top drawer) of the Femto Pulse. Ensure that the plate is loaded with well A1 toward the back left on the tray.

5. Place an empty 96 DeepWell 1mL Plate into Drawer “W” (second from top) of the Femto Pulse. This plate serves as the capillary waste tray and should be emptied daily. Alternatively, the supplied open reservoir waste plate may be used.

6. Prepare a fresh sample plate with Row A filled with 200 µL/well of 0.25x TE Rinse Buffer daily.

7. Place the prepared 0.25x TE Rinse Buffer plate 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.
Daily Conditioning (Recommended)

For optimal array performance when running the FP-1002 Genomic DNA 165 kb 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 (Figure 3). 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 (Figure 4).

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 (Figure 5).

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.

Figure 3. Main Screen showing selection of conditioning method
Agilent gDNA 165 kb Kit Protocol

Figure 4. Select Conditioning Method form to select conditioning method

Figure 5. 20 min Conditioning method
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 Appendix C – 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.

2. Store the gDNA Diluent Marker solution and the 165 kb Ladder at -20°C upon arrival.

3. Allow the marker and ladder solution 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.

165 kb Ladder Handling and Storage

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

1. Equilibrate the 165 kb 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 or flick the Ladder tube. Only wide-bore genomic pipette tips should be used to mix the 165 kb Ladder solution.

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

Regeneron 165 kb Ladder Working Solution Preparation

1. Before use, equilibrate the Regeneron 165 kb Ladder aliquot to room temperature for about 30 min.
Agilent gDNA 165 kb Kit Protocol

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 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 Ladder into the tube containing 99 µL of the solution from steps 2 and 3 above (DNF-498 0.25x TE Dilution Buffer + FP-8001 gDNA Diluent Marker). 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 Ladder working solution up and down 5 times to mix. This is the 165 kb Ladder Working Solution; use within one day of preparation.

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

8 The 165 kb Ladder should be run in parallel with the samples for each experiment. It is not recommended to import a previously run 165 kb Ladder.

Sample Plate Preparation

NOTE

Important BAC fragments Sampling Procedures:

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

When mixing large gDNA samples, slowly pipette up-and-down with wide-bore genomic pipette tips.

1 The total input genomic DNA sample concentration should be within a range of 5 pg/µL – 500 pg/µL. If the starting material is higher than 500 pg/µL total concentration, pre-dilute the sample to the specified concentration range with 1x TE buffer. The above genomic DNA sample concentrations assume a starting sample matrix of 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA). If the chloride salt concentration is greater than 10 mM, some loss of sensitivity may be observed and slight adjustments may need to be made to the sample injection conditions.
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.

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 gDNA 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.

4 Load 20 µL of the 165 kb 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 165 kb 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.

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.
Performing Experiments

Running an Experiment

1. To set up an experiment, from the main screen of the Femto Pulse control software, select the Operation tab (Figure 6). Select the sample tray location to be analyzed (1, 2, or 3) by left clicking the Sample Tray # dropdown or by clicking the appropriate sample plate tab (alternate plate view) and choosing the appropriate location.

2. Left click a well of the desired sample plate row with the mouse. The selected row will be highlighted in the plate map (e.g., Row A in Figure 6). Enter the sample name if desired into the respective Sample ID cell by left clicking the cell and typing in the name. Alternatively, sample information can be imported from .txt or .csv file by selecting the Load from File... option.

Figure 6. Main screen showing selection of sample row and entering sample information
3 After sample information for the row or plate has been entered, under the Run Selected Group field press Add to queue. The Separation Setup form will be displayed enabling the user to select the experimental method and enter additional information (Figure 7).

![Figure 7. Separation Setup form to select experimental Method and enter additional information](image)

4 In the Separation Setup pop-up form, left click the dropdown and select the appropriate preloaded experimental Method file. The available methods are sorted by kit number and are linked to the directory containing methods for the currently installed capillary array length.

**NOTE**

Methods for gDNA sample analysis:

The FP-1002 165 kb gDNA Analysis Kit offers two methods for gDNA sample analysis:

- **FP-1002-22 - gDNA 165kb.mthds** is a 70 min CE pulsed-field separation method that is best suited for resolution and sizing of gDNA smears ≤ 80 kb.
- **FP-1002E22 - Extended gDNA 165kb.mthds** is a 3 h CE pulsed-field separation method that provides enhanced resolution and sizing for gDNA smears ≥ 80 kb.

5 From the dropdown menu select the method **FP-1002-22 – gDNA 165kb.mthds** or **FP-1002E22 - Extended gDNA 165kb.mthds**.

6 Select the appropriate Gel line being used for the experiment (Gel 1 or Gel 2) using the dropdown.
7 The **Tray Name** can be entered to identify the sample plate. The **Folder Prefix** if entered will amend the folder name (normally a time stamp of HH-MM-SS from the start of the CE run).

8 To copy the experimental results to another directory location in addition to the default save directory, check the **Copy results** box and select the desired **Copy path**: directory by clicking the ... button and navigating to the desired save directory.

9 Any **Notes** can be entered regarding the experiment; they will be saved and displayed in the final PDF report generated by the ProSize software.

10 Once all information has been entered, press **OK** to add the method to the instrument queue (press **Cancel** to abort adding the method).

11 Repeat Steps 1-10 for any remaining sample rows to be analyzed.

12 If the entire 96-well sample tray is to be run using the same experimental method, under the **Run Entire Tray** field press **Add to queue**. A form like Figure 4 will be displayed for entering information and adding the run to the instrument queue for the entire 96-well sample tray.

13 After a row or tray has been added to the queue, the method(s) will be listed on the main screen under the **Method Queue** field (Figure 8).

14 Prior to starting the experiment, verify all trays (buffer/storage, waste, rinse, sample, etc.) have been loaded into their respective drawer locations.

15 Press the **Play** icon ( ) to start the sequence loaded into the queue. To **Pause** the queue after the currently running experiment is completed, press the button. To **Clear** the run queue of all loaded experiments, press the button.
Once an experiment has been loaded onto the queue, the user can view or edit the method (Administrator level only can edit a method) by pressing the Method Summary field. The user can also view or edit the Notes field by selecting the View/Edit Notes field. To remove the method from the queue, press the “x” button; to view the stepwise details of the method press the double down arrow icon.

The user may add a Pause or Prime step into the queue by right clicking the mouse while over the queue and selecting Insert Pause or Insert Prime.

The order of the experimental queue can be rearranged by dragging down individual entries. Further information regarding the Method Queue operation is provided in the Femto Pulse User Manual.

Once started, the instrument will perform all the programmed experiments in the Method Queue uninterrupted unless a pause step is present. Note that additional experiments can be programmed and added to the Method Queue at any time while the instrument is running if desired. After completion of the last queued experiment, the instrument stage will automatically move to the Store location (Row H of the inlet buffer tray containing the Storage Solution).

Figure 8. Main Screen after selection of samples to the run queue
Viewing and Editing Experimental Methods

1. A User level operator can View the steps of the experimental method by pressing the View link on the Separation Setup screen, or by pressing the Method Summary option once a method has been loaded onto the experimental queue. A User level operator can also edit or add notes to an experiment on the queue by selecting the View/Edit Notes field. User level operators cannot edit any steps of a queued separation method.

2. Administrator level operators can Edit certain steps of the experimental method. To open the method editor screen, press the Edit link from the Separation Setup screen (Figure 7). The method editor screen is displayed, showing the steps of the method (Figure 9).

3. The preloaded, optimized steps for the FP-1002-22 - gDNA 165kb.mthds (Figure 9) and FP-1002E22 - Extended gDNA 165kb.mthds methods (Figure 10) are shown below. The method steps are:
   a. Full Conditioning flushing method (Automatically enabled).
      Gel Selection: Gel 1.
   b. Perform Prerun (enabled) (5 kV, 60 sec)
   c. Rinse (disabled)
   d. Marker Injection (disabled).
   e. Rinse (enabled: Tray = Marker; Row = A). This step moves to the Marker tray and rinses the capillary tips with 0.25x TE Rinse Buffer.
   f. Sample Injection (enabled) Voltage Injection (5 kV, 30 sec). This step injects the prepared sample plate.
   g. Separation (enabled) pulse field CE separation, automatically enabled. This step performs the pulsed-field CE Separation. Note that the two analysis methods differ in the CE pulsed-field separation settings optimized for the specific resolution.
Figure 9. FP-1002-22 – gDNA 165kb method
Figure 10. FP-1002E22 – Extended gDNA 165kb method

4 An Administrator level user has the option to adjust the Gel Selection; Prerun settings; Rinse settings including Tray and Row; and the Sample Injection settings. For example, if the rinse solution is loaded into a row other than Row A on a 12-capillary instrument, this can be adjusted prior to or while the method is loaded on the experimental queue.

5 To apply any adjustments to the method being placed on the experimental queue, press the button. To exit the editor screen without applying any changes press the button.

NOTE Any edits made to the experimental method from the Separation Setup or Method Summary screen will only apply to the currently loaded experiment in the queue. No changes are made to the original separation method file.
Processing Experimental Data

1 When processing data, the ProSize software will automatically recognize the separation method performed and apply the appropriate matching configuration file from the C:\PROSize 3.0\Configurations directory:
   - The FP-1002-22 - gDNA 165kb separation method will be processed using the FP-1002-22 – gDNA 165kb configuration file.
   - The FP-1002E22 – Extended gDNA 165kb separation method will be processed using the FP-1002E22 – Extended gDNA 165kb configuration file.

   **NOTE** If the above preloaded ProSize software configuration file is not located in the C:\PROSize 3.0\Configurations directory, contact your local Agilent Technical Support to obtain the file.

2 The data is normalized to the lower marker (set to 1 bp) and 165 kb Upper Marker and calibrated to the Regeneron 165 kb Ladder run in parallel to the samples. Figure 10 shows an example of the markers injected with the Regeneron 165 kb Ladder using the FP-1004-22 - 165kb BAC separation method. A total of 23 peaks should be observed.

3 The FP-1002-22 - gDNA 165kb and the FP-1002E22 – Extended gDNA 165kb configuration are set to the gDNA mode in the Advanced Settings. The Marker Analysis settings are set to Use Lower Marker Only. The quantification setting is set to Use Ladder for quantification with a Conc. (ng/µL) of 0.02 and a Dilution Factor of 10 (2 µL of sample + 18 µL of Diluent Marker).

   **NOTE** If a pre-dilution step was performed prior to the experiment, the sample dilution factor setting should be changed to accurately reflect the final sample concentration.

4 For full information on processing data, refer to the ProSize User Manual.
Femto Pulse Shut Down/Storage

Instrument Shut Down/Storage
The instrument automatically places the capillary array in the Store position against the Storage Solution (Row H of the buffer tray) after each experiment; no further action is required.

If the instrument is to be idle for more than one day, it is recommended to turn off the power to the system.
4 Checking Your Separation Results

165 kb Ladder

The typical expected results for the 165 kb Ladder, provided at an initial total DNA concentration of 2 ng/µL in 1x TE buffer and prepared at a final dilution of 1:100 with the gDNA Diluent Marker solution are shown the Figures 11 and 12 below. A total of 9 predominant peaks should be observed, annotated by size in Figures 11 and 12.

**NOTE** Occasionally several smaller impurity peaks may be observed in the 165 kb Ladder, as shown in Figures 11 and 12. The presence of these impurity peaks will not affect the sample sizing or quantification if the correct peaks are assigned for the ladder.

- In Figure 11 the Ladder is resolved using the **FP-1002-22 – gDNA 165kb** method.
- In Figure 12 the Ladder is resolved using the **FP-1002E22 – Extended gDNA 165kb** method.

![Figure 11](image-url)  
*Figure 11. Expected 165 kb Ladder result, using the Femto Pulse System with the FP-1002 Genomic DNA 165 kb kit. Peaks are annotated by size (bp). Method: FP-1002-22 – gDNA 165kb.*
Checking Your Separation Results

Figure 12. Expected 165 kb Ladder result, using the Femto Pulse System with the FP-1002 Genomic DNA 165 kb kit. Peaks are annotated by size (bp). Method: FP-1002E22 - Extended gDNA 165kb

Figure 13. Representative Coriell gDNA (Cat #19238) sample result using the Femto Pulse System with the FP-1002 gDNA 165 kb method; the average smear size (printed in red) and selected peak sizes are annotated in bp

gDNA Smear Sample

Figure 13 shows a result for the pulsed-field CE separation of a commercially available gDNA smear sample using the FP-1002-22 - gDNA 165kb method.

Different gDNA samples, depending on size, integrity, and method of extraction, possess slightly different types of smear distributions.
Checking Your Separation Results

Figure 14 and 15 show the electropherograms of high integrity gDNA samples separated using the FP-1002 gDNA 165kb method (Figure 14) versus the FP-1002E22 – Extended gDNA 165kb method (Figure 15).

The large gDNA smear is further resolved into a smear of high integrity gDNA using the extended gDNA 165 kb method.

Figure 14. High integrity gDNA sample result using the fast FP-1002-22 - gDNA 165 kb method. The average smear size (printed in red) and selected peak sizes are annotated in bp

Figure 15. High integrity gDNA sample result using the FP-1002E22 – Extended gDNA 165 kb method. The average smear size (printed in red) and selected peak sizes are annotated in bp
Checking Your Separation Results

Figures 16 and 17 show another example of a gDNA smear that contains a wide size distribution, analyzed using the **FP-1002 gDNA 165kb** method (Figure 16) versus the **FP-1002E22 – Extended gDNA 165kb** method (Figure 17).

This gDNA sample is predominantly composed of fragments from 20-60 kb (average smear size ~30 kb). The DNA fragment sizes above 60 kb are resolved into a wider smear using the Extended gDNA 165 kb method.

**Figure 16.** Example result from a gDNA sample containing a wide side distribution, using the **FP-1002-22 - gDNA 165 kb** method

**Figure 17.** Example result from a gDNA sample containing a wide side distribution, using the **FP-1002E22 – Extended gDNA 165 kb** method
## 5 Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the FP-1002 Genomic DNA 165 kb kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

<table>
<thead>
<tr>
<th>Table 6</th>
<th>Troubleshooting actions for assay specific issues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Issue</strong></td>
<td><strong>Cause</strong></td>
</tr>
<tr>
<td>The 165 kb Ladder peak is lower than 1,000 RFUs or non-present.</td>
<td>1 The Femto Pulse capillary array may require a NaOH conditioning flush. If the Method D Flush is not successful, proceed to Steps 2 and 3. 2 The 165 kb Ladder has degraded, or handling instructions have not been followed. 3 The 165 kb Ladder was vortexed or pipetted with regular pipette tips (not wide-bore).</td>
</tr>
<tr>
<td>Large size tight peak or a compression peak at the end of the gDNA smear.</td>
<td>1 The gDNA sample is too large for resolution using the fast FP-1002-22 - gDNA 165kb method.</td>
</tr>
<tr>
<td>The peak signal is &gt;&gt; 5,000 RFU.</td>
<td>1 Input sample concentration is too high.</td>
</tr>
<tr>
<td>No expected gDNA smear or DNA fragment peak observed. Lower Marker peak observed.</td>
<td>1 Sample concentration is below detection. Sample was not added to a sample plate, or wrong sample row was selected for analysis. 2</td>
</tr>
<tr>
<td>No sample peak or marker peak observed for individual sample.</td>
<td>1 Air trapped at the bottom of sample plate and/or marker plate well, or bubbles present in well. Insufficient sample volume. A minimum of 20 µL is required. 2 Capillary is plugged.</td>
</tr>
</tbody>
</table>
Appendix A: Method D Flush - Capillary Array Conditioning to Clean the Capillary Tips, Electrodes and Capillary Walls

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.

0.5 N NaOH is corrosive

- Refer to the product material safety datasheets for all warnings and precautions before proceeding.
- 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.

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 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 A1).
Method D Flush - Capillary Array Conditioning to Clean the Capillary Tips, Electrodes and Capillary Walls

3 Press **OK** to add the method to the instrument queue (press **Cancel** to abort adding the method).

4 Ensure an open waste plate is located in the waste drawer.

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

6 Ensure there is a minimum of **25 mL of 1x DNF-425 Conditioning Solution** in the **Conditioning** bottle, and a minimum of **10 mL of FP-5001 Large DNA Separation 5001 Gel** in the **Gel1** bottle.

7 Close the door to the instrument side compartment and press the **Play** icon ( ) to start the selected capillary conditioning method.

8 The Femto Pulse instrument is now ready to run additional samples or can be stored until next use.
In This Book

This Kit Guide describes the following:

- Agilent 165 kb BAC Analysis Kit
- Additional Material and Equipment Required
- Agilent 165 kb BAC Kit Protocol
- Checking your Separation Results
- Troubleshooting