Agilent Genomic DNA 50 kb Kit

Kit Guide

For Research Use Only.
Not for use in diagnostic procedures.
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Agilent Genomic DNA 50 kb Kit

The Genomic DNA 50 kb kit is designed for assessing the integrity, approximate size and quantitation of genomic DNA.

Table 1  Physical Specifications

<table>
<thead>
<tr>
<th>Type</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume Required</td>
<td>1 µL</td>
</tr>
</tbody>
</table>
| Number of Samples per Run         | 12-Capillary: 11 (+ 1 well DNA Ladder) or 12 (Imported DNA Ladder)
                                           48-Capillary: 47 (+ 1 well DNA Ladder) or 48 (Imported DNA Ladder)
                                           96-Capillary: 95 (+ 1 well DNA Ladder) or 96 (Imported DNA Ladder) |
| Total Electrophoresis Run Time    | 50 minutes (22-47 Array)                           |
|                                   | 60 minutes (33-55 Array)                           |

Table 2  Analytical Specifications

<table>
<thead>
<tr>
<th>Type</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Sizing Range</td>
<td>75 bp – 60,000 bp</td>
</tr>
<tr>
<td>gDNA Quantification Precision¹</td>
<td>25% CV</td>
</tr>
<tr>
<td>gDNA Quantification Accuracy¹</td>
<td>± 30%</td>
</tr>
<tr>
<td>Maximum gDNA Concentration</td>
<td>250 ng/µL</td>
</tr>
</tbody>
</table>
| gDNA Concentration Range¹         | 25 ng/µL - 250 ng/µL input gDNA
                                           (0.125 – 1.25 ng/µL final concentration after dilution) |

¹ Results using DNA Fragment standards at 600 pg/µL and DNA smears at 1 ng/µL prepared from 1x TE buffer.

² Results using DNA Fragment standards and DNA smears prepared from 1x TE buffer.

³ The 22 cm effective, 47 cm total length capillary is only available for 12-capillary Fragment Analyzer instruments.
### Table 3  Storage Conditions

<table>
<thead>
<tr>
<th>Store at –20°C:</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>Intercalating Dye</td>
<td>Genomic DNA separation gel</td>
<td>5x Capillary Conditioning Solution</td>
</tr>
<tr>
<td></td>
<td>5x 930 dsDNA Inlet Buffer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BF-25 Blank Solution</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25x TE Rinse Buffer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HS Genomic DNA Diluent Marker (DM) Solution</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extended Genomic DNA Ladder*</td>
<td></td>
</tr>
</tbody>
</table>

Ensure all reagents are completely warmed to room temperature prior to use.

**NOTE**

* The Lambda DNA fragment (48,500 bp) in the Extended Genomic DNA Ladder is sensitive to degradation. The Ladder should be kept at 2-8°C. Do not subject the ladder to repetitive freeze/thaw cycles. Do not pipette the ladder up and down; vortex with care.
Agilent Genomic DNA 50 kb Kit

Table 4  Kit Components

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Name</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNF-270-0240</td>
<td>Genomic DNA separation gel</td>
<td>240 mL</td>
</tr>
<tr>
<td>DNF-600-U030</td>
<td>Intercalating Dye</td>
<td>30 μL</td>
</tr>
<tr>
<td>DNF-355-0125</td>
<td>5x 930 dsDNA Inlet Buffer</td>
<td>125 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
<tr>
<td>DNF-475-0050</td>
<td>5x Capillary Conditioning Solution</td>
<td>50 mL (dilute with sub-micron filtered water prior to use)</td>
</tr>
<tr>
<td>DNF-375-0120</td>
<td>HS Genomic DNA Diluent Marker (DM)</td>
<td>120 mL</td>
</tr>
<tr>
<td></td>
<td>• Lower Marker in diluent solution (set to 1 bp)</td>
<td></td>
</tr>
<tr>
<td>DNF-367-U050</td>
<td>Extended Genomic DNA Ladder</td>
<td>50 μL</td>
</tr>
<tr>
<td></td>
<td>• Fragments from 75 bp – 48,500 bp; 250 ng/μL total DNA concentration</td>
<td></td>
</tr>
<tr>
<td>DNF-497-0125</td>
<td>0.25x TE Rinse Buffer</td>
<td>125 mL</td>
</tr>
<tr>
<td>DNF-300-0008</td>
<td>BF-25 Blank Solution</td>
<td>8 mL</td>
</tr>
<tr>
<td></td>
<td>Eppendorf LoBind 0.5 mL tubes</td>
<td>Package of 50</td>
</tr>
</tbody>
</table>

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 Fragment Analyzer

Hardware:
- Fragment Analyzer with LED fluorescence detection:
  - 5200 Fragment Analyzer (Part # M5310AA)
  - 5300 Fragment Analyzer (Part # M5311AA)
  - 5400 Fragment Analyzer (Part # M5312AA)
- FA 12-Capillary Array Ultrashort, 22cm (Part # A2300-1250-2247) OR
- FA 12-Capillary Array Short, 33cm (Part # A2300-1250-3355) OR
- FA 12-Capillary Array Long, 55cm (Part # A2300-1250-5580) OR
- FA 48-Capillary Array Short, 33cm (Part # A2300-4850-3355) OR
- FA/ZAG 96-Capillary Array Short, 33cm (Part # A2300-9650-3355) OR
- FA/ZAG 96-Capillary Array Long, 55cm (Part # A2300-9650-5580)

Software:
- Fragment Analyzer control software (Version 1.1.0.11 or higher)
- ProSize data analysis software (Version 2.0.0.61 or higher)

Reagents:
- Capillary Storage Solution, 100 mL (Part #GP-440-0100)
Additional Equipment/Reagents Required (Not Supplied)

- 96-well PCR sample plates. Please refer to Appendix 3 – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer 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
- 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 Solutions)
- Fisherbrand 96 DeepWell 1mL Plate, Natural Polypropylene, part # 12-566-120 (Inlet Buffer and waste plate)
- Reagent Reservoir, 50 mL (VWR 82026-355 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 (for 12-Capillary instruments or 50 mL volumes): BD Falcon #352070, available from Fisher #14-432-22 or VWR #21008-940
  - 250 mL (for 96-Capillary instruments or larger volumes): Corning #430776, available from Fisher #05-538-53 or VWR #21008-771
- Vortexer
3 Agilent Genomic DNA 50 kb kit Protocol

Gel Preparation

1. Store the Genomic DNA separation gel at 2-8°C upon arrival.
2. The Intercalating Dye should be stored at -20°C.
3. Bring the Genomic DNA separation gel and Intercalating Dye to room temperature prior to mixing.
4. Mix appropriate volumes of Intercalating Dye and separation 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 separation gel required per run varies between 12-capillary, 48-capillary and 96-capillary Fragment Analyzer systems. The volumes required are summarized below.
### Table 5 Volume Specifications for 12-Capillary Fragment Analyzer Systems

<table>
<thead>
<tr>
<th># of Samples to be Analyzed</th>
<th>Volume of Intercalating Dye</th>
<th>Volume of separation 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>1.5 µL</td>
<td>15 mL</td>
<td>15 mL</td>
</tr>
<tr>
<td>36</td>
<td>2.0 µL</td>
<td>20 mL</td>
<td>20 mL</td>
</tr>
<tr>
<td>48</td>
<td>2.5 µL</td>
<td>25 mL</td>
<td>25 mL</td>
</tr>
<tr>
<td>96</td>
<td>4.5 µL</td>
<td>45 mL</td>
<td>45 mL</td>
</tr>
</tbody>
</table>

1 A 5 mL minimum volume should be initially added to the tube. One sample well per separation is dedicated to the ladder.

### Table 6 Volume Specifications for 48-Capillary Fragment Analyzer Systems

<table>
<thead>
<tr>
<th># of Samples to be Analyzed</th>
<th>Volume of Intercalating Dye</th>
<th>Volume of separation gel</th>
<th>Volume of 1x Conditioning Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>2.5 µL</td>
<td>25 mL</td>
<td>25 mL</td>
</tr>
<tr>
<td>96</td>
<td>4.0 µL</td>
<td>40 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>144</td>
<td>5.5 µL</td>
<td>55 mL</td>
<td>55 mL</td>
</tr>
<tr>
<td>192</td>
<td>7.0 µL</td>
<td>70 mL</td>
<td>70 mL</td>
</tr>
<tr>
<td>240</td>
<td>8.5 µL</td>
<td>85 mL</td>
<td>85 mL</td>
</tr>
<tr>
<td>288</td>
<td>10.0 µL</td>
<td>100 mL</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

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

### Table 7 Volume Specifications for 96-Capillary Fragment Analyzer Systems

<table>
<thead>
<tr>
<th># of Samples to be Analyzed</th>
<th>Volume of Intercalating Dye</th>
<th>Volume of separation gel</th>
<th>Volume of 1x Conditioning Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>4.0 µL</td>
<td>40 mL</td>
<td>40 mL</td>
</tr>
<tr>
<td>192</td>
<td>8.0 µL</td>
<td>80 mL</td>
<td>80 mL</td>
</tr>
<tr>
<td>288</td>
<td>12.0 µL</td>
<td>120 mL</td>
<td>120 mL</td>
</tr>
<tr>
<td>384</td>
<td>16.0 µL</td>
<td>160 mL</td>
<td>160 mL</td>
</tr>
<tr>
<td>480</td>
<td>20.0 µL</td>
<td>200 mL</td>
<td>200 mL</td>
</tr>
</tbody>
</table>

1 One sample well per separation is dedicated to the ladder.
6 Place the prepared separation gel/Intercalating 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 When adding separation gel to the instrument, update the solution levels in the Fragment Analyzer control software. From the main screen, select **Utilities > Solution Levels**. A menu will be displayed to enter in the updated fluid levels (Figure 1).

![Figure 1 Solution Levels menu](image1.png)

8 When switching applications (e.g., between kits), prime the appropriate gel fluid line after loading fresh Gel solution. From the main screen of the Fragment Analyzer 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 (Figure 2).

![Figure 2. Prime menu](image2.png)
Inlet Buffer Preparation

1. Store the 5x 930 dsDNA Inlet Buffer at 4°C upon arrival. Do not freeze.
2. Bring the 5x 930 dsDNA Inlet Buffer to room temperature prior to mixing and use.
3. In a clean container, add 10 mL of the 5x 930 dsDNA 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.

Capillary Conditioning Solution Preparation

1. Store the 5x Capillary 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 Capillary 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 Capillary 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 Capillary Conditioning Solution should be added to the system as use demands. Tables 6-8 illustrate the volume specifications for the conditioning solution.
5. When adding fresh 1x Capillary Conditioning Solution to the instrument, update the solution levels in the Fragment Analyzer control software. From the main screen, select Utilities > Solution Levels. A menu will be displayed to enter in the updated fluid levels (Figure 1).
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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 930 dsDNA Inlet Buffer daily.
   - 12-Capillary System: Row A only
   - 48-Capillary System: Row A to Row D
   - 96-Capillary System: All Rows
     Do not overfill the wells of the inlet buffer plate.

12-Capillary Systems:
   a In Row H of the same prepared buffer plate, place 1.0 mL/well of Capillary Storage Solution (Part # GP-440-0100).
      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.

48-Capillary Systems:
   a In the Sample 3 drawer, place a sample plate filled with 100 μL/well of Capillary Storage Solution (Part # GP-440-0100) in Row A to Row D.
      Row A to Row D of the Sample 3 is used for the Store location, and the array moves to this position at the end of the experimental sequence.

96-Capillary Systems:
   a In the Sample 3 drawer, place a sample plate filled with 100 μL/well of Capillary Storage Solution (Part # GP-440-0100).
      Sample 3 is used for the Store location, and the array moves to this position at the end of the experimental sequence.

NOTE
Ensure Row H of the buffer tray (12-capillary systems) or the Sample 3 drawer (48-Capillary and 96-capillary systems) is always filled with Capillary Storage Solution, and the capillary array is placed against Storage Solution when not in use, to prevent the capillary tips from drying out and potentially plugging.

The Capillary Storage Solution should be replaced every 2-4 weeks, as the solution will gradually thicken following exposure to the open air via evaporation. More frequent replacement may be required in low humidity or warm lab environments.

3 Place the prepared inlet buffer plate into Drawer “B” (top drawer) of the Fragment Analyzer. Ensure that the plate is loaded with well A1 toward the back left on the tray.
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4 Place an empty 96 DeepWell 1mL Plate into Drawer "W" (second from top) of the Fragment Analyzer. This plate serves as the capillary waste tray and should be emptied daily. Alternatively, the supplied open reservoir waste plate may be used.

5 Prepare a fresh sample plate filled with 200 µL/well of 0.25x TE Rinse Buffer daily.
   - 12-Capillary System: Row A only
   - 48-Capillary System: Row A to Row D
   - 96-Capillary System: All Rows

6 Place the prepared 0.25x TE Rinse Buffer plate into Drawer "M" (third from top) of the Fragment Analyzer. Ensure that the plate is loaded with well A1 toward the back left on the tray.

Marker/Ladder/Sample Preparation

General Information

1 The recommended 96-well sample plate for use with the Fragment Analyzer system is a semi-skirted PCR plate from Eppendorf (Part #951020303). Please refer to Appendix 3 – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer 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 Allow the High Sensitivity Genomic DNA Diluent Marker (DM) Solution and Extended Genomic DNA Ladder to warm to room temperature prior to use. Spin the Ladder tube to ensure liquid is at the bottom of the tube.
Sample Plate Preparation

**Important Genomic DNA Sampling Procedures**

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

2. After the samples have been acclimatized to room temperature, mix the gDNA samples by vortexing before sampling. This further ensures a more homogeneous sample.

3. The total input genomic DNA sample concentration should be within a range of 25 ng/µL – 250 ng/µL for optimal sizing and quantification. 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.

4. Using a clean 96-well sample plate, pipette 199 µL of High Sensitivity Genomic DNA Diluent Marker (DM) Solution to each well in a row of the 96-well plate that is to contain sample. Do not add any DM solution to the well reserved for the Extended Genomic DNA Ladder, that is:
   - Well 12 of each row to be analyzed for a 12-capillary system
   - Well D12 or H12 on a 48-capillary system
   - Well H12 on a 96-capillary system

5. Fill any unused wells (no sample or Genomic DNA Ladder) within the row of the sample plate with 24 µL/well of BF-25 Blank Solution.

6. Pipette 1 µL of each genomic DNA sample into the 199 µL of High Sensitivity Genomic DNA Diluent Marker (DM) Solution in the respective wells of the Sample Plate; mix the contents of the well using the pipette by aspiration/expulsion in the pipette tip as described below.

**Important Sample Mixing Information**

When mixing sample with diluent marker solution, it is important to mix the contents of the wells thoroughly to achieve the most accurate quantification. After adding 1 µL of sample to the 199 µL of diluent marker, use a separate pipette tip with the pipettor set to ~100 µL volume, and pipette each well up/down about 10 times to further mix.
Ladder Preparation

**Important information before handling the Extended Genomic DNA Ladder:**
Do not manually mix the Extended Genomic DNA Ladder by repeated inverting of the tube or repeated pipetting up/down; doing so will result in the degradation of the Lambda DNA fragment in the Ladder. The Ladder solution can only be vortexed by a vortex mixer.

1. The Extended Genomic DNA Ladder should be run in parallel with the samples for each experiment. To prepare the working Extended Genomic DNA Ladder solution:
   a. Add 199 µL of Genomic DNA Diluent Marker (DM) into a separate 0.5 mL Eppendorf DNA LoBind tube.
   b. Gently vortex the vial containing the Extended Genomic DNA Ladder, then pipette 1 µL of the Extended Genomic DNA Ladder into the 199 µL of the DM Solution. Mix the Working Genomic DNA Ladder solution only by vortexing in the vortex mixer.

2. Pipette the entire 200 µL of the working Extended Genomic DNA Ladder solution:
   **For 12-Capillary Systems:**
   a. into well 12 of each row of the sample plate.
   **For 48-Capillary Systems:**
   a. into well D12 when analyzing Row A to Row D of the sample plate, or
   b. into well H12 when analyzing Row E to Row H of the sample plate.
   **For 96-Capillary Systems:**
   a. into well H12 of the sample plate.

3. Check the wells of the sample plate/row to ensure no air bubbles are trapped in the bottom of the wells. If necessary, centrifuge the plate to remove any air bubbles. The presence of trapped air bubbles can lead to injection failures.

**NOTE**
Centrifugation should be done at a speed low enough to remove air bubbles as well as avoid settling of genomic DNA at the bottom of the sample well. High speed centrifugation can cause genomic DNA to settle at the bottom of the sample wells, leading to sampling errors and less accurate quantification. A recommended relative centrifugal force (RCF) limit is 100 x g for less than 30 seconds.
4 Run the sample plate immediately once prepared, or cover the sample plate with a cover film, and use as soon as possible. Alternatively, to prevent evaporation, place a mineral oil overlay on each sample (50 µL/well).

5 To run the samples, place the plate in one of the three sample plate trays (Drawers 4-6 from the top) of the Fragment Analyzer instrument. Load the experimental method as described in the following sections. 48-Capillary or 96-Capillary Systems: Note that Sample 3 is typically assigned to the Capillary Storage Solution.

Performing Experiments

Running an Experiment

1 To set up an experiment, from the main screen of the Fragment Analyzer control software, select the Operation tab (Figure 3). 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.

**NOTE**

For 48-Capillary or 96-Capillary Systems: Sample 3 is typically assigned to the Capillary Storage Solution.

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 3). 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.
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 4).

**Figure 3.** Main screen showing selection of sample row and entering sample information
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 (e.g., 22 cm or 33 cm). Select the following method:

- Select **DNF-467-22 – Genomic DNA 50kb.mthds** when the 22 cm effective, 47 cm total length “ultra-short” capillary array is installed.
- Select **DNF-467-33 – Genomic DNA 50kb.mthds** when the 33 cm effective, 55 cm total length “short” capillary array is installed.

5. Select the appropriate **Gel** line being used for the experiment (Gel 1 or Gel 2) using the dropdown.

6. 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).

7. 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.

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

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

10. Repeat Steps 1-9 for any remaining sample rows to be analyzed.

11. On 96-capillary systems, or in 12-capillary or 48-capillary systems 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 similar to Figure 4 will be
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displayed for entering information and adding the run to the instrument queue for the entire 96-well sample tray.

12 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 5).

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

14 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.

Figure 5. Main screen after selection of samples to the run queue

15 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. 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.
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16 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.

17 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 Fragment Analyzer User Manual.

18 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 stage will automatically move to the Store location (12-Capillary Systems: Row H of the inlet buffer tray containing the Capillary Storage Solution; 48-Capillary and 96-Capillary Systems: Sample 3 location).

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. 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 4). The method editor screen is displayed, showing the steps of the method (Figure 6).

3 The preloaded, optimized steps for the DNF-467-22 (Figure 6) and DNF-467-33 (Figure 7) methods are shown below. The DNF-467-22 method steps are:

   a Full Conditioning flushing method (Automatically enabled).
      Default Gel Selection: Gel 1.
   b Perform Prerun (enabled) (4 kV, 30 sec)
   c Rinse (disabled)
   d Marker Injection (disabled)
   e Rinse (enabled; Tray = Marker; Row = A; # Dips = 1). This step moves to the Marker tray and rinses the capillary tips with 0.25x TE Rinse Buffer.
   f Sample Injection (enabled) Voltage Injection (2 kV, 10 sec). This step injects the prepared sample plate.
   g Separation (enabled) Voltage (4 kV, 50 min). This step performs the CE Separation.
Figure 6. DNF-467-22 - Genomic DNA 50 kb method
Agilent Genomic DNA 50 kb kit Protocol

4 Figure 7 shows the preloaded method for the 33 cm effective, 55 cm total length “short” array. The prerun and separation voltage is set to 5 kV, the injection voltage to 5 kV and time to 10 sec, and the Separation time to 60 min.

![Separation Method](image)

Figure 7. DNF-467-33 - Genomic DNA 50 kb method

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

6 To apply any adjustments to the method being placed on the experimental queue, press the OK button. To exit the editor screen without applying any changes press the Cancel 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.
Agilent Genomic DNA 50 kb kit Protocol

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 2.0\Configurations or C:\PROSize 3.0\Configurations directory, depending upon the version of ProSize software used.
   - The DNF-467-22 separation method will be processed using the DNF-467-22 - Genomic DNA 50kb configuration file.
   - The DNF-467-33 separation method will be processed using the DNF-467-33 - Genomic DNA 50kb configuration file.

NOTE
If the preloaded ProSize software configuration files are not located in the C:\PROSize 2.0\Configurations or C:\PROSize 3.0\Configurations directory, contact Agilent Technical Support to obtain the files.

2. The data is normalized to the lower marker (set to 1 bp) and calibrated to the Extended Genomic DNA Ladder run in parallel to the samples. Figure 8 shows an example of the 1 bp marker injected with the Extended Genomic DNA Ladder using the DNF-467-33 separation method. A total of 13 peaks should be observed.

3. For the Genomic DNA 50 kb kit, ProSize is set to the gDNA mode in the Advanced Settings. The Quantification settings are set to Use Ladder for quantification with a Conc. (ng/µL) of 1.25 and a Dilution Factor of 200 (1 µL sample + 199 µL Diluent Marker). Note that if a pre-dilution was performed prior to the experiment, the 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.
Fragment Analyzer Shut Down/Storage

Instrument Shut Down/Storage

After each experiment, the instrument automatically places the capillary array in the **Store** position against Capillary Storage Solution:

- 12-Capillary Systems: Row H of the buffer tray
- 48-Capillary Systems: Sample 3
- 96-Capillary Systems: Sample 3

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.
Extended Genomic DNA Ladder

Figure 8 shows the typical expected results for the Extended Genomic DNA Ladder, provided at an input total DNA concentration of 250 ng/µL in 1x TE buffer (1:200 dilution, 1 µL Ladder + 199 µL DM solution). A total of 13 peaks should be observed, with the sizes annotated as in Figure 8. The first peak corresponds to the 1 bp lower marker (LM) peak.

Figure 8. Representative Extended Genomic DNA Ladder result using the Fragment Analyzer system with the DNT-467 Genomic DNA 50 kb kit. Peaks are annotated by size (bp). Method: DNF-467-33 (33cm “short” array).
Genomic DNA Sample

Figure 9 shows the result for a genomic DNA sample. In this example, a human blood genomic DNA sample was analyzed.

**Figure 9.** Representative genomic DNA sample result (Human blood gDNA) using the Fragment Analyzer system with the DNF-467 Genomic DNA 50 kb kit. The peak is annotated by size in bp. Method: DNF-467-33 (33cm "short" array). Any fragment above 60,000 bp cannot be sized rationally and will be displayed as ‘>60000’.
5 Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the Genomic DNA 50 kb kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

<table>
<thead>
<tr>
<th>Issue</th>
<th>Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peak observed for sample. Lower Marker peak observed.</td>
<td>1 Sample concentration too low and out of range.</td>
<td>1 Prepare more concentrated sample and repeat experiment; OR</td>
</tr>
<tr>
<td></td>
<td>2 Sample not homogenously mixed before sampling.</td>
<td>Prepare sample and analyze with DNF-468 HS gDNA 50 kb kit.</td>
</tr>
<tr>
<td></td>
<td>3 Sample highly degraded; no dye intercalates.</td>
<td>2 Make sure the sample is equilibrated to room temperature for at least 30 min</td>
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<td></td>
<td></td>
<td>before use, vortex the sample or pipette up-down to mix before sampling.</td>
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<tr>
<td></td>
<td></td>
<td>3 Sample not suitable for use.</td>
</tr>
<tr>
<td>Much lower concentration obtained for gDNA sample than expected.</td>
<td>1 Sample contains very high molecular weight (HMW), aggregated genomic DNA (&gt;&gt;60 kbp).</td>
<td>1 The analysis of HMW, aggregated genomic DNA can result in lower than expected</td>
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<tr>
<td></td>
<td></td>
<td>concentration values due to the nature of sample aggregation, which can inhibit</td>
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<td></td>
<td></td>
<td>sample injection. Analysis of these types of samples at lower concentrations</td>
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<tr>
<td></td>
<td></td>
<td>with the DNF-467 kit, or with the DNF-468 HS gDNA 50 kb kit, may improve the</td>
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<tr>
<td></td>
<td></td>
<td>quantitation.</td>
</tr>
<tr>
<td>Extra peaks/smear near Lower Marker observed (10-1000 bp).</td>
<td>1 Genomic DNA possibly contaminated with RNA.</td>
<td>1 Remove RNA contaminants from the genomic DNA sample and reanalyze; OR</td>
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<tr>
<td></td>
<td></td>
<td>Exclude the extra peaks in data processing for better quantitation and sizing</td>
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<tr>
<td></td>
<td></td>
<td>accuracy.</td>
</tr>
<tr>
<td>Degradation of the 48,500 bp Lambda DNA fragment in the ladder.</td>
<td>1 Ladder solution was manually mixed by repeated inverting of the tube</td>
<td>1 Prepare fresh ladder solution. Mix the ladder solution only by vortexing and</td>
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<td></td>
<td>or repeated pipetting up/down, or ladder solution was exposed to</td>
<td>avoid freeze/thaw cycles.).</td>
</tr>
<tr>
<td></td>
<td>freeze/thaw cycles.</td>
<td></td>
</tr>
<tr>
<td>No sample peak or Lower Marker peak observed for individual sample.</td>
<td>1 Air trapped at the bottom of sample plate well, or bubbles present</td>
<td>1 Check sample plate wells for trapped air bubbles. Centrifuge plate.</td>
</tr>
<tr>
<td></td>
<td>in sample well. A minimum of 20 µL is required.</td>
<td>2 Verify proper volume of solution was added to sample well.</td>
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<td></td>
<td>3 Capillary is plugged.</td>
<td>3 Check waste plate for liquid in the capillary well. If no liquid is observed,</td>
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<tr>
<td></td>
<td></td>
<td>follow the steps outlined in Appendix 7 – Capillary Array Cleaning of the Fragment</td>
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<tr>
<td></td>
<td></td>
<td>Analyzer User Manual for unclogging a capillary array.</td>
</tr>
</tbody>
</table>
In This Book

This kit Guide describes the following:

- Agilent Genomic DNA 50 kb Analysis Kit
- Additional Material and Equipment Required
- Agilent Genomic DNA 50 kb Kit Protocol
- Checking Your Separation Results
- Troubleshooting