Assessment of Large DNA Fragments Size, Quality, and Quantity

Reliable quality assessment of large DNA fragments/smears is essential in a number of downstream applications, such as long-read next generation sequencing, FFPE DNA analysis, and DNA restriction analysis. Traditionally, quality analysis of large size DNA fragments is performed by agarose gel electrophoresis or pulsed-field gel electrophoresis, both of which are time and labor consuming. The DNF-464 High Sensitivity Large Fragment 50 Kb Analysis Kit provides researchers a fast and accurate method to assess DNA fragments and smears up to 48.5 Kb.

**DNF-365 Extended High Sensitivity Large Fragment DNA Ladder allows for DNA sizing through 48.5 Kb.**

**Sheared and un-sheared E. coli gDNA samples sized by DNF-464 High Sensitivity Large Fragment 50 Kb Analysis Kit.**
Provides Improved Large DNA Sample Analysis at Different Stages of DNA Shearing

Electropherogram of large DNA samples sheared to different sizes. The figures depict a standard run.

Large DNA Fragment Sizing and Quantification Data

From three representatives samples, averaged from 11 runs on the Fragment Analyzer.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Expected Size, Traditional Method</th>
<th>Average Concentration by Fluorometric Instrument</th>
<th>Fragment Analyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Size bp, n=11 Runs</td>
<td>%CV</td>
<td>%Difference from Expected Size</td>
</tr>
<tr>
<td>2</td>
<td>55 Kb</td>
<td>12.3</td>
<td>5.2%</td>
</tr>
<tr>
<td>3</td>
<td>50 Kb</td>
<td>12.6</td>
<td>3.5%</td>
</tr>
<tr>
<td>5</td>
<td>25 Kb</td>
<td>12.4</td>
<td>8.8%</td>
</tr>
</tbody>
</table>

The Fragment Analyzer estimated sample size was compared to the expected size generated from pulsed-field gel electrophoresis. The sizing accuracy and precision was <10%. The concentration of the samples estimated by the Fragment Analyzer was compared to the concentration values estimated by a fluorometric instrument. The quantification accuracy was <15% and precision was <5%.
Genomic DNA samples were sheared by sonication according to manufacturer specifications to produce a 10 Kb smear. Two-fold serial dilutions of the sheared DNA were prepared and analyzed by the Fragment Analyzer.

The concentration of the DNA dilutions was measured by a fluorometric method and compared to the Fragment Analyzer sample concentration. The results showed a linear relationship between this method and the Fragment Analyzer values. The quantification accuracy was <20% and precision was <10%.

**Sheared gDNA Concentration Comparison Between Fluorometry and Fragment Analyzer**

\[ y = 0.8642x + 0.0367 \]
\[ R^2 = 0.9987 \]

A near one-to-one linear relationship of sample concentration measured on the Fragment Analyzer and a fluorometric instrument over a dilution series.
Analysis of DNA shearing and integrity are important for many downstream applications that require fragmentation of the gDNA. At a normalized concentration of 1 ng/µL, the size difference between sheared and unsheared DNA samples can be visualized by the Fragment Analyzer. The size discrimination between sheared and unsheared E. coli gDNA has been reproducibly demonstrated (7% CV) over multiple sample preparations and Fragment Analyzer runs in the 12-capillary short array.

**Features and Benefits**

- **Simple and Convenient Sample Handling**
  Requires 2 µL of 50 pg/µL - 5 ng/µL of DNA smear sample for analysis. The samples are loaded into a convenient 96 or 12-well format, in a single row or entire 96-well plate or strip tubes.

- **Accurately Size Large DNA Fragments and Smears**
  The kit provides accurate sizing over a wide range (75 bp – 48.5 Kb) of normalized large DNA fragments/smears.

- **Extended DNA Sizing**
  Separate and size DNA fragments and smears.

- **Size Comparison Between Sheared and Unsheared Large DNA Smears**
  Normalized large fragment DNA smears can be evaluated for shearing, an essential step in preparation of long-read sequencing libraries.

- **Improved DNA Sizing Accuracy**
  Achieved through the use of the lower and upper markers.

- **Short Run Times**
  12, 48, or 96 samples can all be analyzed in about 55 minutes of total electrophoresis times.