NGS Analysis

Fragment Analyzer™ Automated CE System

Streamline Workflows by Rapidly Quantifying and Qualifying Fragments for NGS

During NGS library preparation, initial DNA samples may be fragmented/sheared, tailed, ligated, size selected, captured, released, and/or amplified. Accurate quantification and qualification of DNA at crucial quality control checkpoints during NGS library construction ensures successful sequencing. Conventional fragment analysis with lab-on-chip methods generates bottlenecks for high-throughput NGS environments.

Hundreds of NGS laboratories have adopted a better quality control strategy. The Fragment Analyzer provides NGS labs with variable throughput, enabling dozens to thousands of samples to be analyzed per day. The Fragment Analyzer, used in conjunction with the DNF-473 Standard Sensitivity NGS Fragment Analysis Kit and the DNF-474 High Sensitivity NGS Fragment Analysis Kit, provides confident sizing and quantification data for all short-read sequencing NGS libraries.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Trace</th>
<th>Range</th>
<th>ng/µL</th>
<th>% Total</th>
<th>n mole/L</th>
<th>Avg. Size</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGS 1</td>
<td>Black</td>
<td>30 – 1,500 bp</td>
<td>3.8759</td>
<td>99.6</td>
<td>17.6542</td>
<td>361</td>
<td>50.54</td>
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<tr>
<td>NGS 2</td>
<td>Red</td>
<td>30 – 1,500 bp</td>
<td>1.7835</td>
<td>99.2</td>
<td>8.2248</td>
<td>357</td>
<td>51.42</td>
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<tr>
<td>NGS 3</td>
<td>Blue</td>
<td>30 – 1,500 bp</td>
<td>0.9293</td>
<td>98.1</td>
<td>4.1529</td>
<td>368</td>
<td>51.64</td>
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<tr>
<td>NGS 4</td>
<td>Orange</td>
<td>30 – 1,500 bp</td>
<td>0.4368</td>
<td>96.2</td>
<td>1.9389</td>
<td>371</td>
<td>52.34</td>
</tr>
</tbody>
</table>

Separation and quantification of NGS libraries using the DNF-474 High Sensitivity NGS Fragment Analysis Kit. Capillary electrophoresis was performed on a Fragment Analyzer equipped with a Short Capillary Array (33-55). Post-electrophoretic analysis was completed with the proprietary PROSize® Data Analysis Software. Smear Analysis was performed with a smear range of 30 bp to 1,500 bp, reporting: concentration, % of total, molarity, average size, and % CV.
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Process Comparison Methods

**Fragment Analyzer Method**

1. Prepare gel-dye and store at room temperature
2. Prepare gel-dye prior to use, store in dark at 4°C
3. Prepare rinse buffer tray
4. Dilute 2 µL samples and ladder with diluent marker into 96-well sample plate
5. Place sample plate onto instrument
6. Start method
7. Analyze results
8. Generate report
9. Assemble chip priming station and depress syringe plunger, wait exactly 60 seconds
10. Wait an additional 5 seconds
11. Remove chip priming station
12. Pipette 9 µL of gel onto chip
13. Pipette 9 µL of gel into 2 more wells on chip
14. Pipette 5 µL marker into each sample well used
15. Pipette 1 µL of ladder into one well
16. Pipette 1 µL of sample into each well on the chip
17. Place loaded chip onto vortexer
18. Vortex for 60 seconds
19. Load chip and begin run within 5 minutes
20. Start method
21. Analyze results
22. Generate report

**Lab-on-Chip Method**

1. Prepare gel-dye and store at room temperature
2. Equilibrate gel to room temperature for 30 minutes
3. Remove chip from bag
4. Pipette 9 µL of gel onto chip
5. Assemble chip priming station and depress syringe plunger, wait exactly 60 seconds
6. Wait an additional 5 seconds
7. Remove chip priming station
8. Pipette 9 µL of gel into 2 more wells on chip
9. Pipette 5 µL marker into each sample well used
10. Pipette 1 µL of ladder into one well
11. Pipette 1 µL of sample into each well on the chip
12. Place loaded chip onto vortexer
13. Vortex for 60 seconds
14. Load chip and begin run within 5 minutes
15. Start method
16. Analyze results
17. Generate report

**Features and Benefits**

- **Simplified Sample Handling**
  Requires a single dilution step into a 96-well plate.

- **No Chip Loading**
  Separation gel is automatically loaded into capillaries prior to each run.

- **High Sensitivity**
  Quantify library smears as low as 50 pg/µL.

- **Short Run Times**
  Complete analysis as quickly as 40 minutes.

- **Multi-Plate Capacity**
  Holds up to 288 samples in three, 96-well plates. Sample rows/plates can be analyzed in user-defined order.

- **Long Reagent Shelf-Life**
  Hold reagents at room temperature for extended periods, allows for quick access and reduced instrument preparation time.

- **Suitable for All NGS Platforms**
  Provides quality and quantity analysis for the major NGS platforms.

- **Powerful Data Analysis Software**
  PROSize enables automated baseline selection, Smear Analysis, “Apply to All” feature, and customized report formatting for size distribution and concentration measurements.

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