

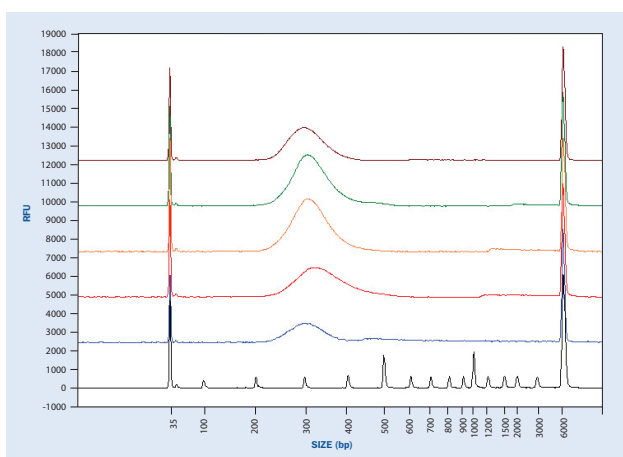
NGS ANALYSIS

Fragment Analyzer™ Automated CE System

Streamline workflow by rapidly quantifying and qualifying fragments for NGS runs.

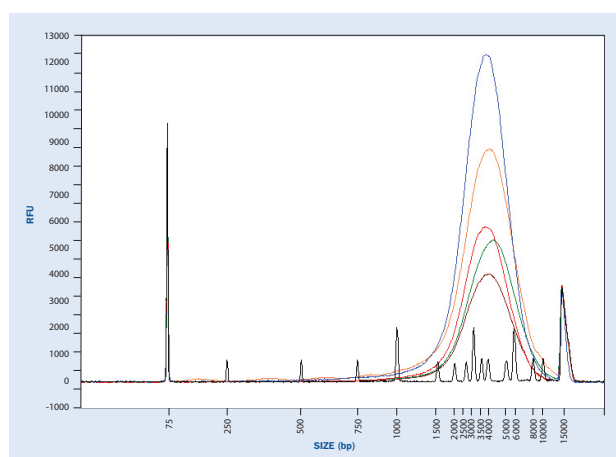
After dsDNA is fragmented in NGS library preparation, labs typically qualify and quantify the DNA prior to sequencing runs. Conventional fragment sizing with manual gels or lab-on-chip methods, however, often creates bottlenecks for NGS labs. Not anymore. The automated Fragment Analyzer™ allows NGS labs to accelerate throughput to 1,500 samples per day and can measure fragments as large as 15,000 base pairs, well beyond conventional methods.

Well	Sample ID	Range	ng/ul	% total	nmole/L	Avg. Size	%CV
C1	A1 (Blue)	200 bp to 1000 bp	2.47	92.6	10.67	381	39.8
C2	A2 (Red)	200 bp to 1000 bp	3.289	93.7	16.0054	338	10.8
C3	A3 (Orange)	200 bp to 1000 bp	4.613	96.1	24.2029	314	8.1
C4	A4 (Green)	200 bp to 1000 bp	4.531	97.1	23.2067	321	15
C5	A5 (Brown)	200 bp to 1000 bp	3.033	97.8	15.7404	317	24.4



Separation and quantification of NGS fragment libraries using the Fragment Analyzer™ system. Smear analysis was performed in the 200 bp to 1000 bp range. Concentration, % of total, molarity, average size, and %CV are reported for each sample across the user defined sizing range.

Well	Sample ID	Range	ng/ul	% total	nmole/L	Avg. Size	%CV
A1	1 (Blue)	1000 bp to 10,000 bp	25.249	96.7	11.0346	3767	39.1
A2	2 (Red)	1000 bp to 10,000 bp	9.592	98	4.1748	3782	39.5
A3	3 (Orange)	1000 bp to 10,000 bp	16.477	93.4	6.7782	4002	44.4
A4	4 (Green)	1000 bp to 10,000 bp	9.122	98.2	3.5506	4229	41.1
A5	5 (Brown)	1000 bp to 10,000 bp	6.84	96.9	2.8506	3950	40.4



Separation and quantification of large fragment size NGS libraries using the Fragment Analyzer™ system. Smear analysis was performed in the 1000 bp to 10,000 bp range. Concentration, % of total, molarity, average size, and %CV are reported for each sample across the user defined sizing range.

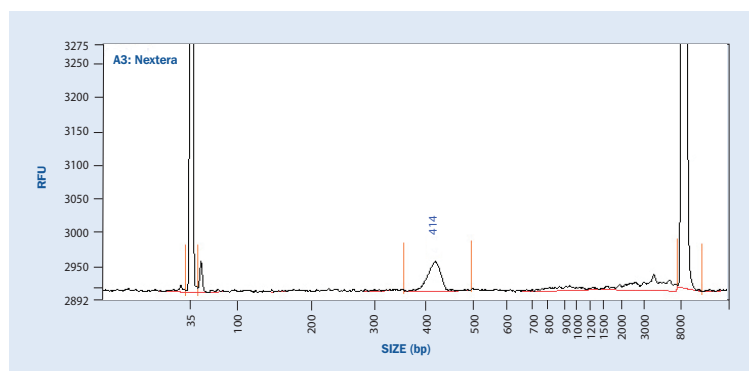
Compare Fragment Analyzer™ Automated CE Process Steps to the Slower Manual Method.

Manual Method

1. Prepare gel/dye prior to use, store in dark at 4°C
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 for exactly 60 seconds
6. Wait an additional 5 seconds
7. Remove chip priming station from chip
8. Pipette 9 µL of gel into 2 more wells on chip
9. Pipette 5 µL marker into each sample well being 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

Fragment Analyzer™ Automated CE System Method

1. Prepare gel-dye and store at room temperature
2. Prepare inlet buffer tray
3. Dilute samples with DM buffer into 96-well sample plate
4. Place sample plate onto instrument
5. Start method
6. Analyze results
7. Generate report



Detection of low concentration Nextera™ NGS DNA library using AAT's High Sensitivity NGS Fragment Analysis Kit. A sample concentration of 41.4 pg/µL was measured, using only 2 µL of input sample.

Features and Benefits

Automated Sample Handling:

No repetitive pipetting steps, simply load diluted samples in 96-well plate.

No Chip Loading:

Separation gel is automatically loaded into capillaries prior to each run.

High Sensitivity:

Detection limits as low as 5 pg/µL per fragment peak without desalting.

Short Run Times:

Analysis of 12 or 96 samples in as little as 45 or 55 minutes, respectively.

Suitable for All NGS Instruments:

Fragment sizing for each of the major NGS platforms, including up to 15,000 bp.

Powerful Data Analysis Software:

PRO Size™ 2.0 enables automated baseline selection, smear analysis and reporting of size distribution and concentration.

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