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This document contains references to "Advanced Analytical" or "AATI." Please note that Advanced Analytical was purchased by Agilent in June 2018. For more information, contact Agilent via: [www.agilent.com/chem/contactus](http://www.agilent.com/chem/contactus)



# NGS Analysis

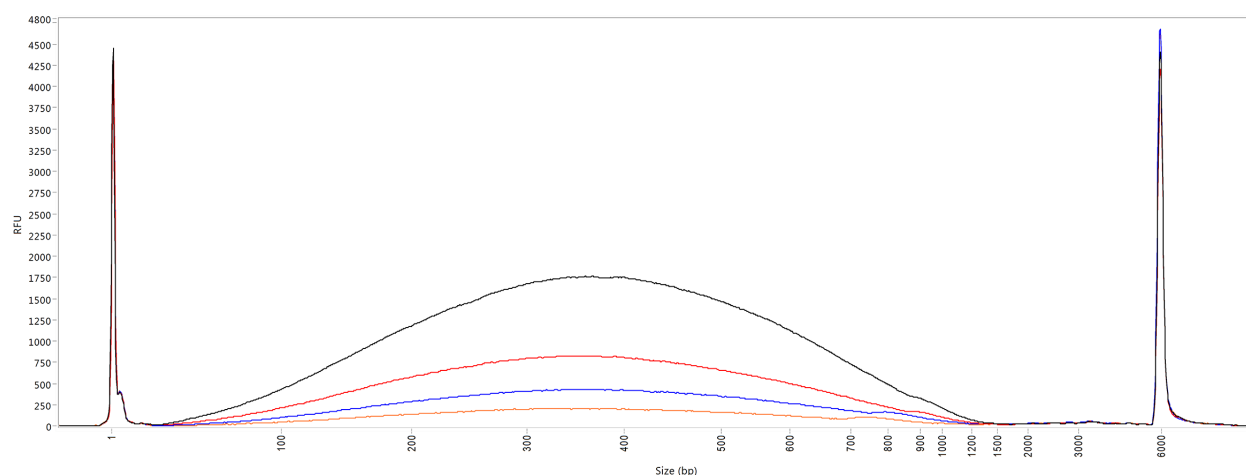
## Fragment Analyzer Automated CE System

### 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.

Sample ID	Trace	Range	ng/ $\mu$ L	% Total	nmole/L	Avg. Size	% CV
NGS 1	Black	30 – 1,500 bp	3.8759	99.6	17.6542	361	50.54
NGS 2	Red	30 – 1,500 bp	1.7835	99.2	357	51.42	
NGS 3	Blue	30 – 1,500 bp	0.9293	98.1	4.1529	368	51.64
NGS 4	Orange	30 – 1,500 bp	0.4368	96.2	1.9389	371	52.34



*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.*



# Process Comparison Methods

## Fragment Analyzer Method

1. Prepare gel-dye and store at room temperature
2. Prepare inlet buffer tray
3. Prepare rinse buffer tray
4. Dilute 2  $\mu\text{L}$  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



## Lab-on-Chip 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  $\mu\text{L}$  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  $\mu\text{L}$  of gel into 2 more wells on chip
9. Pipette 5  $\mu\text{L}$  marker into each sample well used
10. Pipette 1  $\mu\text{L}$  of ladder into one well
11. Pipette 1  $\mu\text{L}$  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/ $\mu\text{L}$ .
- 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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 Published in the USA, November 5, 2018  
 5994-0420EN

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