Interpretation of Tapestation Traces

Genomics Research Center

MEDICINE of
THE HIGHEST ORDER



Introduction to Tapestation Assays

An Automated Electrophoresis Solution that Delivers DNA and RNA Sample QC

The Agilent TapeStation system is an automated electrophoresis solution for the sample quality control of DNA and RNA samples. The system integrates an instrument, data processing software, reagents, and ScreenTape devices specific for DNA and RNA. It is suitable to analyze size, quantity, and integrity of your samples. It fits for example in a next-generation sequencing (NGS) or biobanking workflow with low to high throughput delivering highly precise analytical evaluation.

Advantages of automated electrophoresis over traditional gel electrophoresis include dramatically reduced samples (1-2 µL) and reagent consumption, significantly faster analysis time (1-2 minutes per sample) and data analysis. Additionally, the easy to use ScreenTape technology enables less hands-on activities during sample preparation. Each ScreenTape device has 16 lanes so that each sample is analyzed in an individual lane, completely eliminating contamination and carryover. The unique benefit of the TapeStation system is the flexibility to run any sample number at a constant cost per sample.

Agilent Tapestation Instrumentation



Available Tapestation Assays







	D1000	High Sensitivity D1000	D5000	High Sensitivity D5000	Genomic DNA
Volume	1 uL	2 uL	1 uL	2 uL	1 uL
Size Range	35 - 1,000 bp	35 - 1,000 bp	100 - 5,000 bp	100 - 5,000 bp	200 to >60,000 bp
Concentration	0.1 - 50 ng	0.01 - 1 ng	0.1 - 50 ng	0.01 - 1 ng	10 - 100 ng

The GRC will determine the most appropriate Tapestation assay for your sample type based on:

Expected Size (D1000 vs D5000)

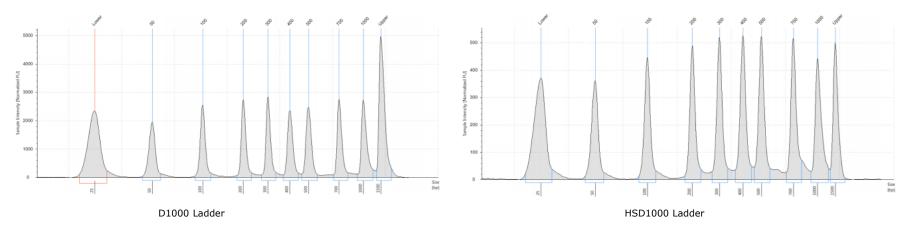
Concentration (Regular vs High Sensitivity – HS)



DNA - D1000 + HS D1000

	D1000	High Sensitivity D1000
Volume	1 uL	2 uL
Size Range	35 - 1,000 bp	35 - 1,000 bp
Concentration	5 - 50 ng	0.01 - 1 ng

Ladder – A ladder is included with each run. The ladder contains two internal standards (Lower and Upper Markers) to align the ladder data with samples to determine sizing. The concentration of the upper marker is known and used to help determine sample concentration*.

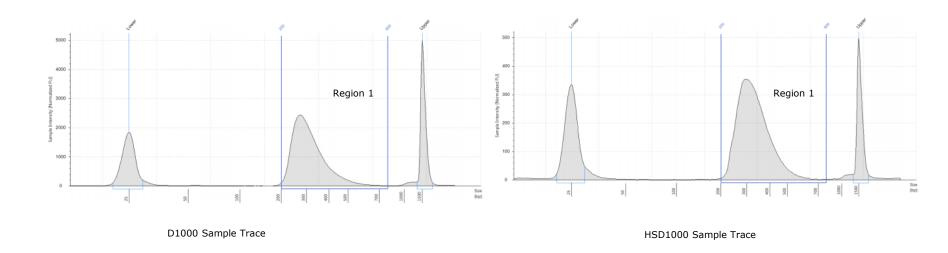


*The GRC does not use Tapestation to determine concentration. Tapestation is used for quality assessment and library size for normalization prior to sequencing.



DNA - D1000 + HS D1000

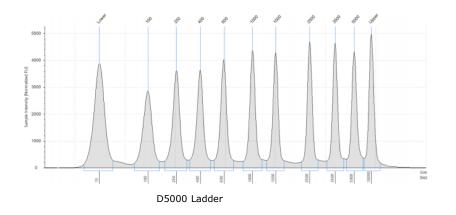
Example Sample Trace – Lower and upper markers are included with each sample and are used to align the sample with the ladder. A region (Region 1) has been set and is indicated by the vertical blue lines on either side of the library trace. This is manually applied to each sample based on the library profile. This calculates the size of the library within this region and is used for normalization prior to sequencing.

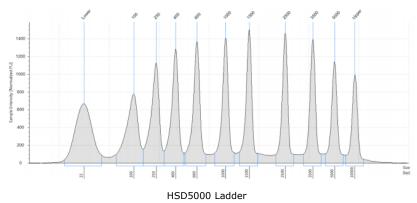


DNA - D5000 + HS D5000

	D5000	High Sensitivity D5000
Volume	1 uL	2 uL
Size Range	100 - 5,000 bp	100 - 5,000 bp
Concentration	5 - 50 ng	0.01 - 1 ng

Ladder – A ladder is included with each run. The ladder contains two internal standards (Lower and Upper Markers) to align the ladder data with samples to determine sizing. The concentration of the upper marker is known and used to help determine sample concentration*.

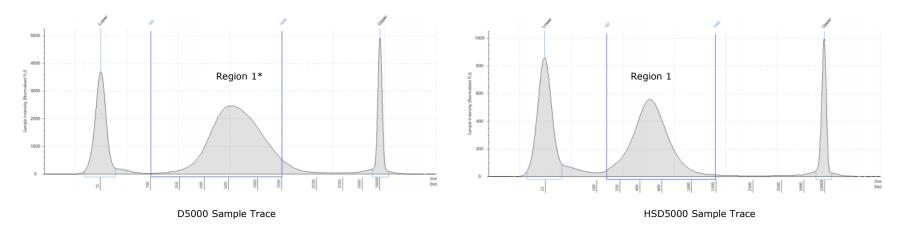




*The GRC does not use Tapestation to determine concentration. Tapestation is used for quality assessment and library size for normalization prior to sequencing.

DNA - D5000 + HS D5000

Example Sample Trace – Lower and upper markers are included with each sample and are used to align the sample with the ladder. A region (Region 1) has been set and is indicated by the vertical blue lines on either side of the library trace. This is manually applied to each sample based on the library profile. This calculates the size of the library within this region and is used for normalization prior to sequencing.



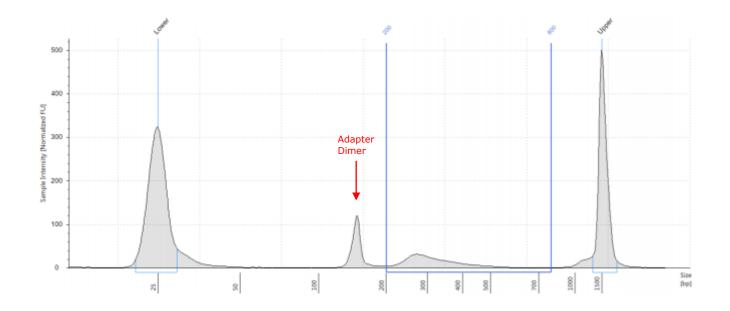
^{*}Region 1 is often placed up to 1500 bp even if the library trace extends beyond this limit. In our experience, library fragments larger than 1500 bp do not cluster well and are not considered when determining library size for sequencing normalization.



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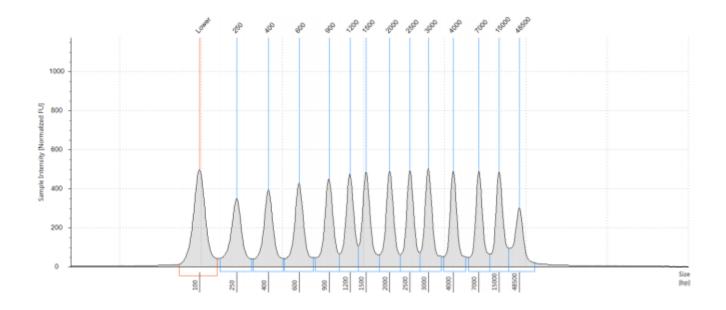
Unexpected Library Peaks

If peaks are present outside the expected library range, it could be due to primer dimers or adapter dimers. Further purification should be performed to remove these peaks prior to next generation sequencing.



High Sensitivity Genomic DNA

Ladder – A ladder is included with each run. The ladder contains a lower marker (LM) to align the ladder data with samples to determine sizing, concentration, and DIN (DNA Integrity Number)

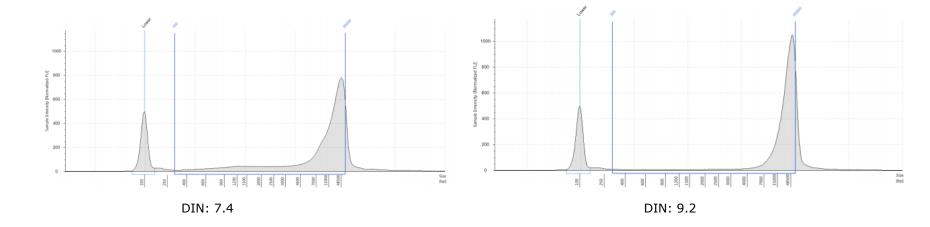


High Sensitivity Genomic DNA

Example Sample Trace – Below is an example of a genomic DNA sample. A Lower Marker (LM) is included with each sample to align to the ladder data.

DIN: DNA Integrity Number: Used to assess genomic DNA degradation.

DIN is on a scale of 1-10 with 1 indicating severe degradation.





^{*}Tapestation software does not allow a region beyond 60,000 bp. The Tapestation is not used for quantification and only quality assessment.

Contact Us



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