

Variability in DNA Fragment Size and Distribution Analysis Across Various Fragment Analyzers

Introduction

This application note addresses the observed bias of the determination of DNA fragment size and distribution among popular analytical instruments (Fragment Analyzer, Bioanalyzer, and TapeStation) when Tris-EDTA (TE) is used as shearing buffer.

Materials and Methods

Materials

- Fragment Analysis Instruments and Kits:
 - 4200 TapeStation, Agilent
 - D1000 ScreenTape (PN 5067-5582), D1000 Reagents (PN 5067-5583), D1000 Ladder (PN 5067-5586)
 - 2100 Bioanalyzer, Agilent
 - High Sensitivity DNA Kit (PN 5067-4626)
 - DNA 12000 (12k) Kit (PN 5067-1508)
 - 5300 Fragment Analyzer, Agilent
 - HS (High Sensitivity) NGS Fragment Kit (1 to 6000 bp) (PN DNF-474-0500)
- Control Samples:
 - Mix Lambda DNA, New England BioLabs (PN N3011)

DNA SHEARING SETTINGS DEVELOPED BY COVARIS FOR TRUSIGHT TUMOR 170 LIBRARY CONSTRUCTION

- Please refer to the Covaris TST170 DNA Shearing Quick Guide for instrument settings ([PN 010515](#))

Methods

Lambda DNA (NEB) diluted in 1X Tris-EDTA (TE) buffer (30 ng/ μ L DNA concentration) was sheared on the LE220-plus using the TruSight Tumor 170 settings ([PN 010515](#)). Two microTUBE-50 Strip V2 consumables were used to emphasize differences (N = 16; N = 8 Lot 1, N = 8 Lot 2). Boxplots were created with Minitab 18 to visualize the data collected.

An input concentration of 30 ng/ μ L sheared DNA was used performing fragment analysis on the TapeStation (D1000 ScreenTape) (**Figure 1**). The same samples were diluted to 10 ng/ μ L with 1X Tris-EDTA buffer for Fragment Analyzer (Fragment Analyzer HS [High Sensitivity] NGS Fragment Kit) analysis

(**Figure 2**). An input concentration of 30 ng/ μ L sheared DNA was used to perform fragment analysis on the Bioanalyzer DNA 12000 kit (**Figure 3**). Samples diluted to 10 ng/ μ L were used for the Bioanalyzer High Sensitivity kit (**Figure 4**). Differences in fragment size between fragment analysis instruments and between kits is summarized in Figure 5.

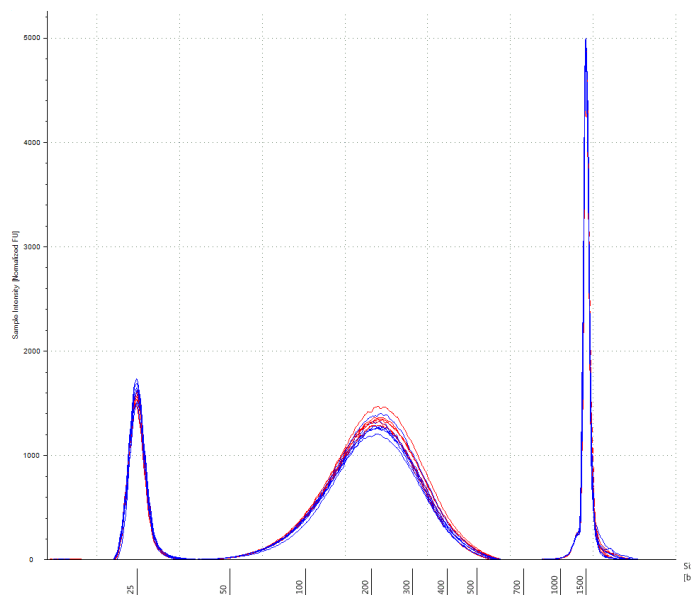


Figure 1: 4200 TapeStation-D1000 (D1000 ScreenTape): superposition of electropherograms of sheared DNA using two microTUBE-50 Strip V2 consumables (N = 8 Lot 1, N = 8 Lot 2) on an LE220-plus (30 ng/ μ L).

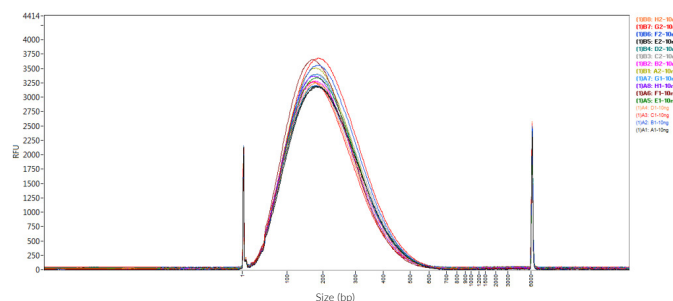


Figure 2: Fragment Analyzer HS (High Sensitivity) NGS Fragment Kit: superposition of electropherograms of sheared DNA using two microTUBE-50 Strip V2 consumables (N = 16; Lot 1 & 2) on an LE220-plus (diluted to 10 ng/ μ L).

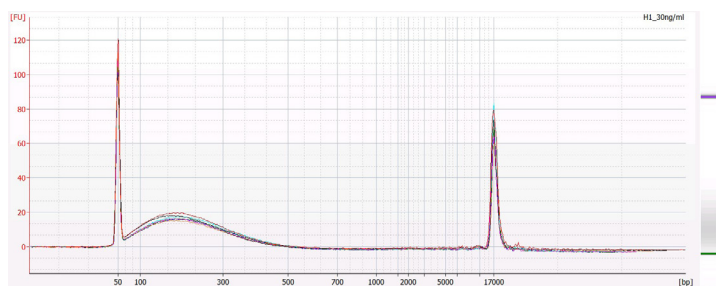


Figure 3: Bioanalyzer DNA 12000 Kit: superposition of electropherograms of sheared DNA using two microTUBE-50 Strip V2 consumables (N = 8, Lot 1) on an LE220-plus (30 ng/ μ L).

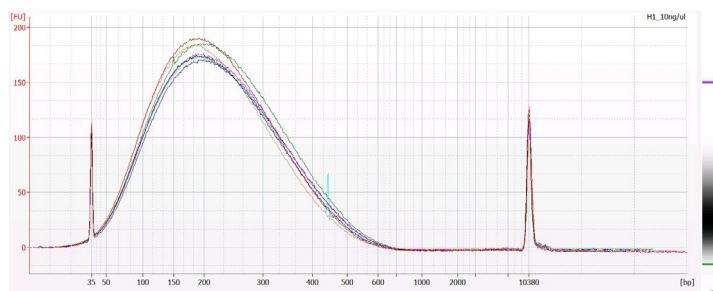


Figure 4: Bioanalyzer High Sensitivity Kit: superposition of electropherograms of sheared DNA using two microTUBE-50 Strip V2 consumables (N = 8, Lot 1) on an LE220-plus (diluted to 10 ng/ μ L).

Analyzer	Strip	N	Buffer	DNA Conc. (ng/ μ L)	Avg. Mode (bp)	St. Dev.	% CV	Target (bp)	% Target
TS-D1000	1	8	1X TE	30	218	4.2	1.9	187.5	116
	2	8	1X TE		216	2.7	1.2	187.5	115
FA-NGS	1	8	1X TE	10	176	7.1	4	187.5	94
	2	8	1X TE		174	4.1	2.4	187.5	93
BA-12k	1	8	1X TE	30	185	12.7	6.9	187.5	99
	2	8	1X TE		188	8.8	4.7	187.5	100
BA-HS	1	8	1X TE	10	192	3.4	1.8	187.5	102
	2	8	1X TE		187	10.2	5.5	187.5	100

Table 1: Bias between TapeStation (TS-D1000) and Fragment Analyzer (FA) is shown to be between 40 and 50 bp. Bias between Bioanalyzer (BA) and Fragment Analyzer is shown to be between 10 to 20 bp. Bias between TapeStation and Bioanalyzer is approximately 20 bp. As the fragment length increases, bias has been observed to shift.

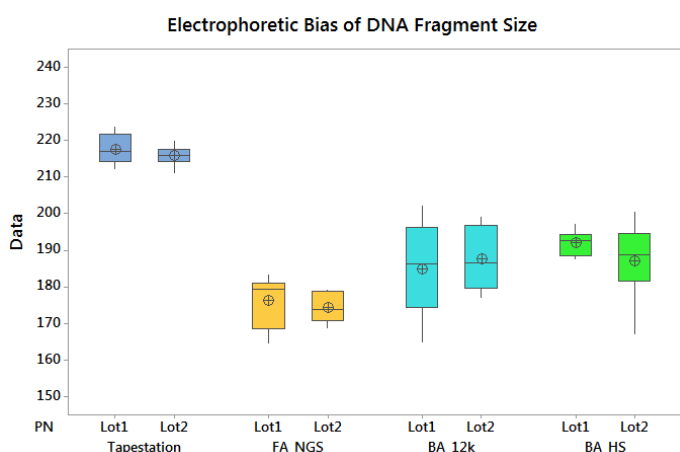


Figure 5: TS-D1000 = TapeStation D1000 ScreenTape, FA-NGS = Fragment Analyzer HS (High Sensitivity) NGS Fragment Kit, BA-12k= Bioanalyzer DNA 12000 Kit, BA-HS= Bioanalyzer High Sensitivity DNA Kit; Box plot depicts the varying DNA fragment size results across the different DNA fragment analyzer platforms from the same sheared DNA material.

Results & Discussion

When performing optimization for library construction, it is important to acknowledge the differences in fragment analysis methodologies. The fragment analysis instrument and assay used contribute to the peak size determination. As noted in this document, gel-based electrophoresis systems, such as the TapeStation, are less sensitive at detecting smaller fragment sizes than their capillary-electrophoresis counterparts, such as the Bioanalyzer and the Fragment Analyzer. Therefore, when performing gel-based analysis specifically for the TST170 protocol, fragment sizes are skewed to > 200 bp. It is always best to optimize DNA shearing based on sequencing results, not solely on post-shearing fragment analysis. Covaris recommends the pulsing protocols provided in this Application Note for TST-170 DNA shearing ([PN 010515](#)) and it is critical to note these fragment sizing differences listed in Table 1.

Conclusions

When performing Covaris DNA shearing protocols, specifically for the TruSight Tumor workflow, it is crucial to acknowledge that:

- There is ~40 to 50 base pair bias observed between the TapeStation D1000 and the Fragment Analyzer.
- There is ~40 base pair bias observed between the TapeStation D1000 and the Bioanalyzer.
- There is ~10 to 20 base pair bias observed between the Fragment Analyzer and the Bioanalyzer for fragment size in the range 150 to 200 bp.