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TECHNICAL NOTE

Comparison between Bioanalyzer and TapeStation traces for QC of Chromium™ Genome Libraries

INTRODUCTION

Preparation of Chromium™ Genome v2 libraries follows a protocol that includes DNA extraction/ quantitation, GEM preparation, amplification and size selection followed by the actual library construction. Within the workflow are points where the success of the procedure may be monitored. This Technical Note highlights the differences and similarities between Bioanalyzer and TapeStation traces that are used as a form of QC in library preparation with a focused discussion on evaluation of insert sizes.

DISCUSSION

The current method for qualitative evaluation of Chromium™ Genome libraries is through 1) qualitative analysis with either the Agilent Bioanalyzer DNA1000 chip or the Agilent TapeStation D1000 ScreenTape chip and 2) quantification by qPCR. For QC of the final Genome library, 1 µl of the library is run on either the Bioanalyzer or TapeStation. The traces in Fig. 1 are from eight different libraries, each analyzed on both a Bioanalyzer and TapeStation and illustrate the differences in fragment size distribution for each instrument. The kits and protocols (Bioanalyzer and TapeStation) have been optimized for separation of fragments in a certain size range which is achieved with different properties of the gel matrix. The shape of the curves in the electropherograms may differ between instruments, even when the same library is loaded for QC. Differences in DNA fragment migration result in different shapes of the curves on the electropherograms with respect to peak value, curve shape, fluorescent units on the y-axis, and overall distribution of fragments. For instance, Library #2 indicates a peak ~650bp on the Bioanalyzer while the same library peaks at ~530bp on the TapeStation. Generally, the peak of TapeStation library traces appear to be skewed toward smaller fragments (left) compared to Bioanalyzer traces.

Importantly, all of these 8 libraries generated successful sequencing results. Therefore, the shape and absolute bp size of the peak in the trace are not correlated with sequencing success and overall application performance. We look for traces that have a significant proportion of inserts in the 400 bp – 600 bp range, similar to what is seen in the traces in Fig. 1. Inserts in this size range are optimal for cluster formation in Illumina® flowcells. If the peak of the fragment distribution curve on the Bioanalyzer or TapeStation trace is between 400 bp to 1000 bp and the curve distribution indicates the presence of inserts from 400 bp to 600 bp in size, **we encourage customers to sequence the sample and evaluate the results**. The presence of larger fragments does not affect application performance.

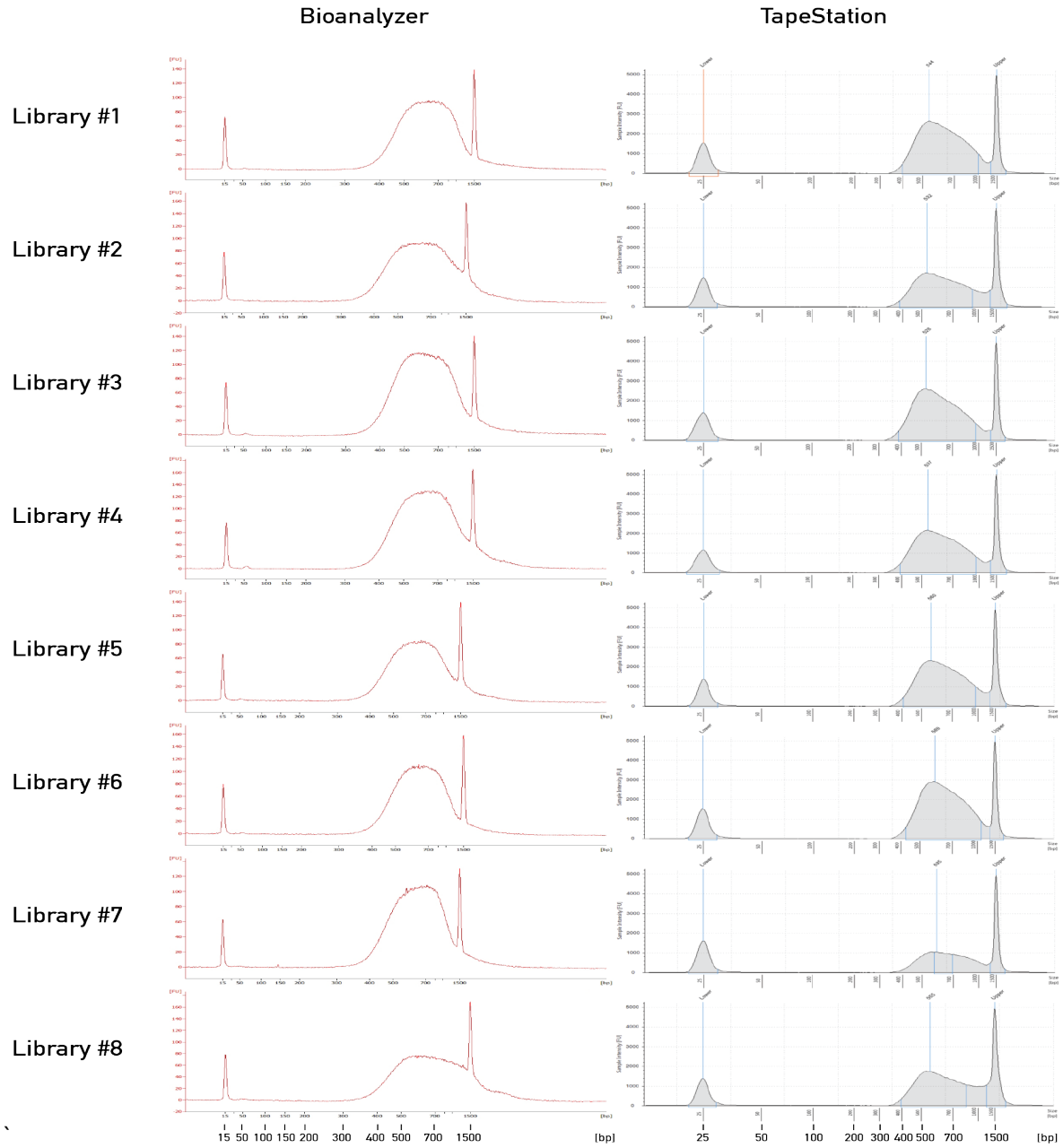

Fig. 1. Eight Chromium™ Genome v2 libraries run on an Agilent Bioanalyzer DNA1000 chip and an Agilent TapeStation D1000 ScreenTape chip for direct comparison. Library #5 was generated manually with the HT protocol. Library #7 was prepared automatically with the HT protocol.

CONCLUSION

This Technical Note highlights the differences of fragment size distribution lengths seen when identical libraries are run on both the BioAnalyzer and TapeStation. All traces in this Technical Note are typical of Chromium™ Genome v2 libraries and were generated using Human gDNA purified with the QIAGEN® MagAttract® HMW Kit per the protocol in the Chromium™ Reagent Kits v2 User Guide (CG00043). Curve shapes and peak values of library traces are to be used as a quality measure prior to library quantitation and sequencing. The examples of eight individual libraries presented here serve as a general guideline to compare with other libraries generated with the Chromium™ Reagent Kits v2.

REFERENCES

- Chromium™ Genome Reagent Kits v2 User Guide (CG00043)

Notices

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