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TECHNICAL NOTE

Comparison between Bioanalyzer and Fragment Analyzer Traces for QC of Chromium™ Genome v2 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 usage of Bioanalyzer and Fragment Analyzer traces as a form of QC after library preparation with a focused discussion on evaluation of insert sizes.

METHODS AND RESULTS

The current method for qualitative evaluation of Chromium Genome libraries is through 1) qualitative analysis with either the Agilent Bioanalyzer DNA1000 chip, the Agilent TapeStation D1000 ScreenTape chip, or the Advanced Analytical Fragment Analyzer DNF-474 HS NGS kit and 2) quantification by qPCR. Four Chromium Genome v2 libraries were generated using human gDNA purified with the QIAGEN® MagAttract® HMW Kit per the protocol in the *Chromium™ Genome Reagent Kits v2 User Guide* – CG00043. For QC of the final Genome v2 library, 1 µl of each of the four libraries is used on either the Bioanalyzer or Fragment Analyzer. The traces in Figure 1 are from the four different libraries, each analyzed on both the Bioanalyzer and the Fragment Analyzer and illustrate the differences in fragment size distribution for each instrument. The Bioanalyzer and the Fragment Analyzer use different gel matrices and physical methods of separation that influence both resolution and sensitivity throughout the dynamic range of analysis. As a result, the shape of eletropherogram curves, peak maxima, peak number, fluorescent units on the y-axis, and average size will differ slightly between the two instruments. For instance, Library #2 indicates a peak ~650bp on the Bioanalyzer while the same library peaks at ~700bp on the Fragment Analyzer.

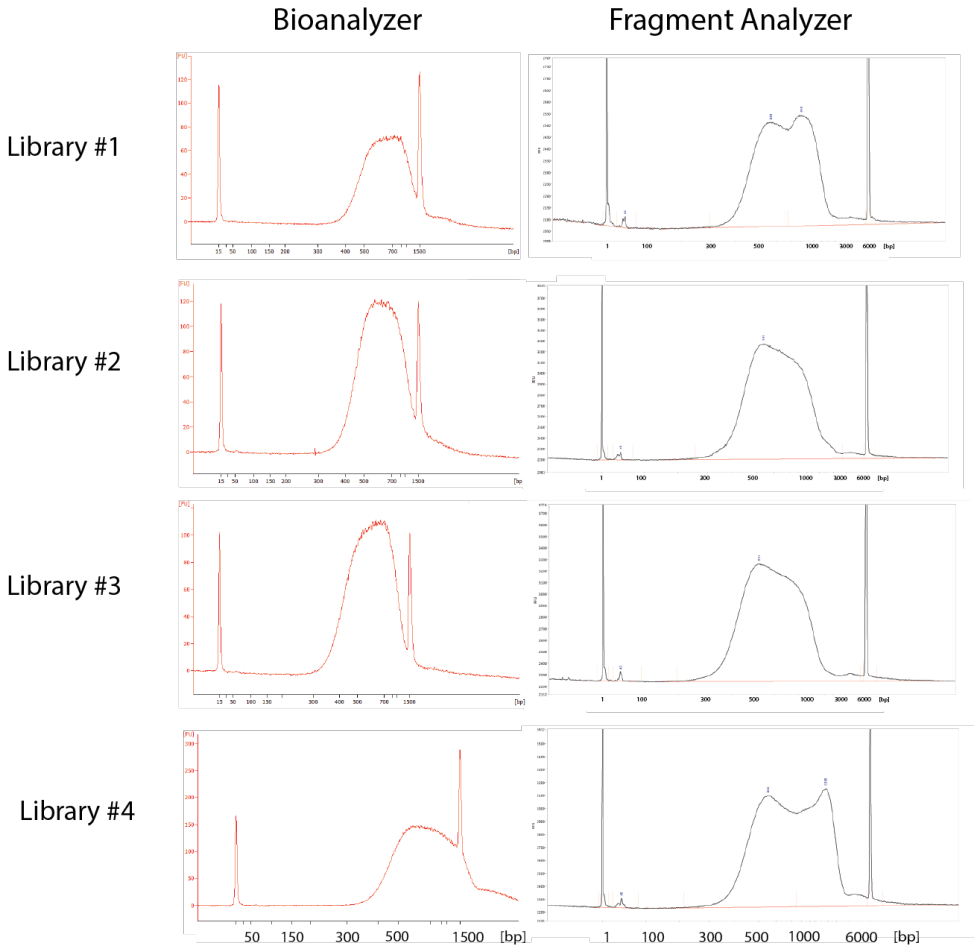

Fig. 1. Chromium™ Genome v2 libraries run on an Agilent Bioanalyzer DNA1000 chip (left) and a Fragment Analyzer DNF-474 High Sensitivity NGS Analysis kit (right) for direct comparison.

All traces in this Technical Note are typical of Chromium Genome v2 libraries and were generated using the protocol in the *Chromium™ Genome Reagent Kits v2 User Guide* – CG00043. Importantly, all of these libraries generated successful sequencing results. Therefore, the shape and absolute bp size of the peaks in the traces are not correlated with sequencing success and overall application performance. In general, 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 examples in Figure 1. Inserts in this size range are optimal for cluster formation on Illumina® flow cells. If the peak of the fragment distribution curve on the Bioanalyzer or Fragment Analyzer electropherogram 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 the User to sequence the sample. The presence of larger fragments does not affect application performance.

DISCUSSION

This Technical Note highlights the differences in fragment size distribution lengths observed when the same libraries are run on both the Bioanalyzer and the Fragment Analyzer. 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 four 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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