10x GENOMICS®

TECHNICAL NOTE

QC of Chromium™ Genome Libraries: Qualitative Evaluation Using Agilent Bioanalyzer

INTRODUCTION

Preparation of Chromium™ Genome 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 some of the QC procedures used in library preparation with a focused discussion on evaluation of insert sizes as a QC metric.

DISCUSSION

The current method for qualitative evaluation of Chromium™ Genome libraries is through analysis with the Agilent Bioanalyzer DNA1000 chip and quantification with qPCR. To QC the final library, 1 µl of the Genome library is run on the Bioanalyzer. The trace in Fig. 1 is from Section 4.8 of the Genome Reagent Kit User Guide. In Fig. 2, we provide additional Bioanalyzer traces of libraries that were successfully sequenced. These traces are typical of Chromium™ Genome libraries and were all generated using Human gDNA purified with the QIAGEN® MagAttract® HMW Kit per the Chromium™ Genome User Guide.

Note the different peak values and curve shapes for each library indicating different means and distributions of size for the library fragments. Importantly, all of these libraries generated successful sequencing results. Therefore, the shape and bp size of the peak in the trace are not correlated with sequencing success and overall application performance. We look for traces with a significant amount of inserts of 400 bp – 600 bp in length, similar to what is seen in the traces in Fig. 1 and Fig. 2. Inserts in this size range are optimal for cluster formation in Illumina® flowcells. If the peak of the fragment distribution curve on the Bioanalyzer trace is within a 400 bp to 1000 bp range and the curve distribution indicates the presence of inserts from 400 bp to 600 bp **we encourage customers to sequence the sample and review the application results**. The presence of larger fragments does not affect application performance.

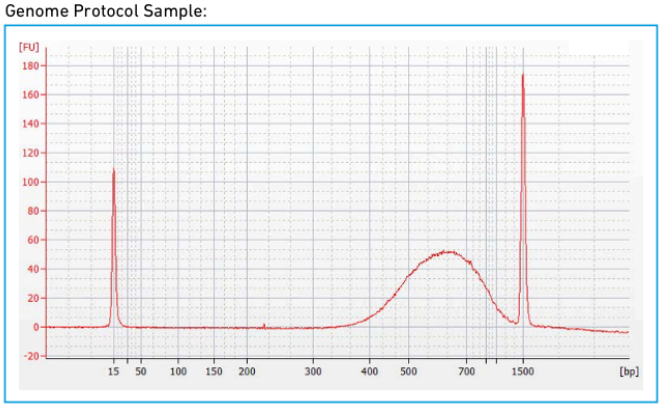

Fig. 1. Bioanalyzer trace for 1µl of genomic library sample from the Chromium™ Genome User Guide

a b

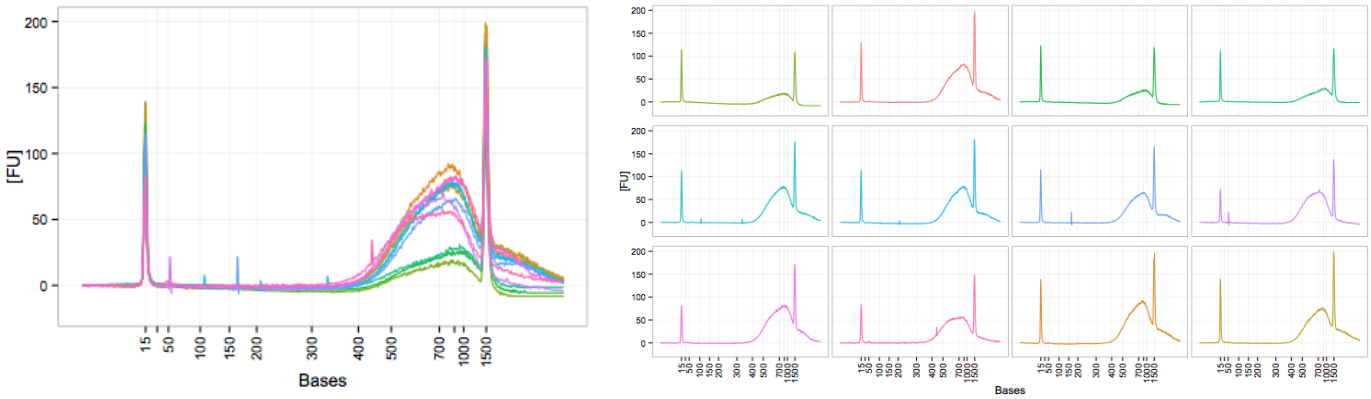

Fig. 2. Bioanalyzer traces for different Chromium™ Genome libraries that have yielded successful sequencing results are presented in a) composite and b) individual graphs.

CONCLUSION

This discussion looks at insert size as one metric to predict success in library sequencing. The Bioanalyzer traces are to be used as a QC guide for fragment/ insert size. While peak shapes may vary it is most important that the peak of the curve is in the range of 400 bp – 1000 bp and that there be evidence of inserts in the range of 400 bp to 600 bp in length. Other parameters that must be monitored carefully include accurate library quantitation by qPCR and correct amount of library loaded onto the sequencer. Libraries prepared and sequenced according to guidelines discussed here and outlined in the Genome Reagent Kit User Guide can deliver high quality sequencing data for genomic libraries.

Notices

Document Number

CG00047 Rev A *Technical Note*

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