TECHNICAL NOTE

Chromium™ Genome v2 Libraries – Gel Bead Oligo Primer Contamination Affects Sequencing & Application Performance

INTRODUCTION

The Chromium™ Genome v2 Protocol (found in *Chromium™ Genome Reagents Kits v2 User Guide*, CG00043) produces Chromium™ Genome v2 libraries ready for Illumina® sequencing. Throughout library preparation, SPRIselect beads remove excess amounts of unused Gel Bead oligo primers. Inefficient SPRIselect cleanup can result in residual Gel Bead oligo primers in the final library (Fig. 1) which may impact sequencing results and overall application performance. This Technical Note compares Chromium™ Genome v2 final libraries, which contain varying amounts of Gel Bead oligo primers, and describes differences seen in sequencing and application performance metrics. This document is intended to provide general guidance for additional SPRIselect cleanups when needed to remove excess Gel Bead oligo primers from Chromium™ Genome v2 libraries prior to sequencing.

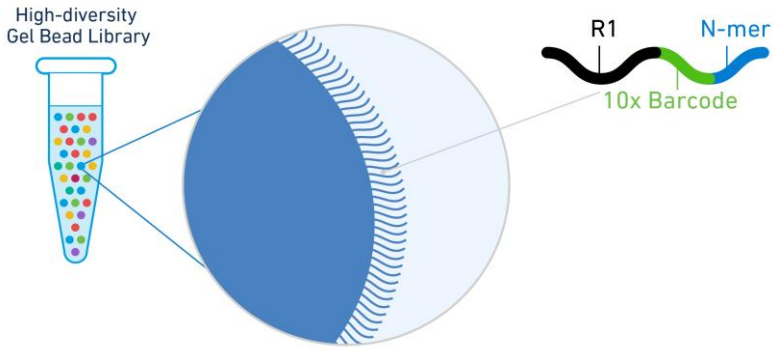

Figure 1. Gel Bead Oligo Primer used for the Chromium™ Genome v2 Protocol. The length of each Gel Bead oligo primer is 44 nucleotides (nt) (22 nt partial R1 sequence + 16 nt 10x™ Barcode sequence + 6 nt N-mer sequence).

METHOD

Sequencing and application performance metrics were compared across three different Chromium™ Genome v2 libraries that were prepared according to the *Chromium™ Genome Reagent Kits v2 User Guide* (CG00043) with the following modifications where applicable. Note that the oligos spiked into the reactions were free in solution and not bound to Gel Beads:

A. Control Chromium™ Genome v2 library (human, NA12878) (no modification from the protocol)
B. Chromium™ v2 library + oligo primer spike-in. Oligo primers were spiked into the Elution Solution II during *Post GEM Incubation Cleanup –SPRIselect* (Step 3.2 of the User Guide).
C. Same library as B, but a second SPRIselect cleanup step was performed to remove Gel Bead oligo primer spike-in. Bioanalyzer traces were run before and after the additional SPRIselect cleanup.

We have qualitatively assessed all three libraries with the Agilent Bioanalyzer DNA1000 chip. Fig. 2 shows the Bioanalyzer traces for the isothermally amplified post-GEM products and the traces for the final library. For Library B, the low molecular weight peak (at ~44 bp) in the Bioanalyzer trace indicates the presence of the oligo primer spike-in (Fig. 2B). After performing a second SPRIselect cleanup and rerunning the Bioanalyzer assay, the absence of the low molecular weight peak indicates the successful removal of residual oligo primers (library C, Fig. 2C). Note that the overall fragment yield may decrease by as much as ~40-50% when the additional cleanup is performed.

All three final libraries were quantified with the Kapa DNA Quantification Kit. Once quantified, libraries were denatured and diluted as recommended for loading onto the Illumina® HiSeq® 4000 flow cell. Refer to Illumina protocol documentation for denaturing and diluting libraries. Libraries were sequenced to 15Gb depth to assess quality control specifications.

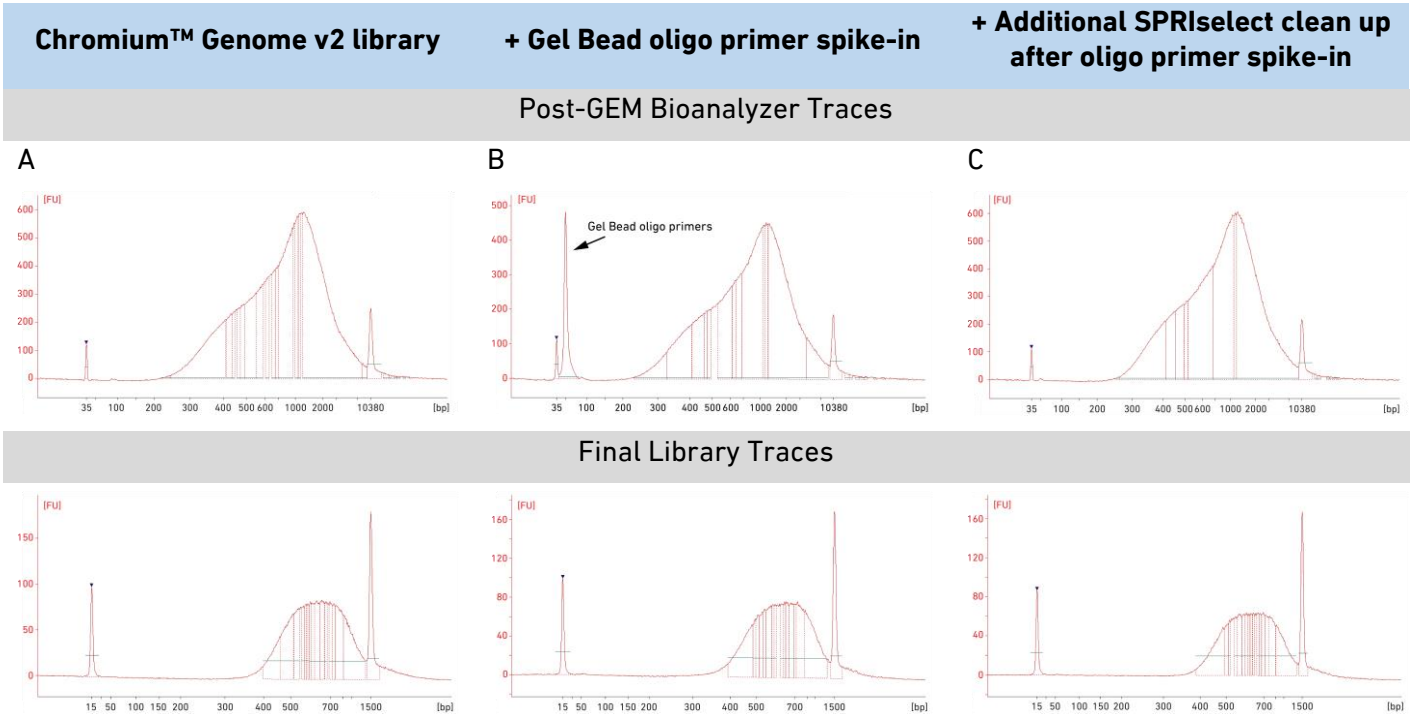

Figure 2. Bioanalyzer traces for post-GEM samples and final libraries. Shown are (A) traces for control library, (B) library + oligo primer spike-in, (C) library with oligo primer spike-in that was cleaned with an additional SPRIselect. Spike-in post-GEM traces show a peak at ~44 bp which indicates the presence of excess oligo primers.

RESULTS AND DISCUSSION

Sequencing and application performance metrics (*e.g.* Cluster %PF, Q30 metrics, Unmapped Read Fraction) were calculated for the three library runs and assembled in Table 1. Overall, the libraries gave high quality data and performance metrics when run on the Illumina® HiSeq® 4000. Each sequencing and application performance metric was compared to the quality control specifications (QC Specifications). Metrics that meet the QC Specifications are labeled in green and those that fall outside of the specifications are labeled in orange. In general, **the presence of the oligo primers in the final Chromium™ Genome v2 libraries does affect sequencing and application performance metrics**. Specifically, the percentage of clusters passing filter (Cluster %PF) drops substantially (56.3 %) compared to libraries that are not contaminated with the oligo primers. Moreover, the fraction of unmapped reads and consequently the fraction of wasted data increased for library B. Note that the oligo primers in library B appear to be absent in the final library trace (see Figure 2B), despite their clear presence in the post-GEM product. Library trace B resembles those traces that do not contain any Gel Bead oligo primers (library A and C). The decrease in sequencing and application performance for

library B indicates that excess oligo primers (or their conversion products) are still present in the final library, even though the oligo primers are not seen on the Bioanalyzer traces.

If sequencing and/or application performance metrics are lower than expected (e.g. %PF < 60%), we recommend to re-evaluate the library trace that was generated at Step 3.3 of the User Guide (*Post GEM QC*) and check for excess amounts of oligo primers (peak at ~44 bp). If residual oligo primers are present in the trace, we recommend a second SPRIselect cleanup to remove the oligo primers. Please follow Step 3.2 of the User Guide (*Post GEM incubation Cleanup –SPRIselect*) for specific instructions. For additional details on SPRIselect cleanup beads please refer to the Technical Note *SPRIselect:DNA Ratios Affect the Size Range of Library Fragments – v2 Reagents* (CG000061).

QC Metric	QC Specifications	Library A: Chromium Genome v2 Library	Library B: + Gel Bead oligo primer spike-in	Library C: + Additional double-sided SPRIselect
Cluster %PF	> 70.0	74.8	56.3	72.8
Q30 Read 1	> 0.79	0.88	0.87	0.89
Q30 Read 2	> 0.65	0.72	0.73	0.71
Q30 Sample Index	> 0.90	0.95	0.95	0.95
Unmapped Read Fraction	< 0.032	0.029	0.042	0.032

Table 1. Sequencing and application performance metrics for Chromium™ Genome v2 libraries with varying degrees of Gel Bead oligo primers present in final library. Metrics highlighted in green meet QC Specifications. Metrics highlighted in orange do not meet QC Specifications.

CONCLUSION

The protocol in the *Chromium™ Genome Reagent Kits v2 User Guide* (CG00043) produces high quality Chromium™ Genome v2 libraries ready for sequencing on Illumina® sequencers. In this study we have seen differences in sequencing and application performance depending on the degree of residual Gel Bead oligo primers that may be present in the final library and the result of incomplete SPRIselect cleanup. We outline steps on how to remediate excess Gel Bead oligo primers. Following these recommendations should allow for successful sequencing and improved application performance for Chromium™ Genome v2 libraries.

REFERENCES

- *Chromium™ Genome Reagent Kits v2 User Guide* (CG00043)
- *SPRIselect:DNA Ratios Affect the Size Range of Library Fragments – v2 Reagents* (CG000061)

Notices

Document Number

CG000104 Rev A *Technical Note*

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