10x GENOMICS

TECHNICAL NOTE

SPRIselect:DNA Ratios Affect the Size Range of Library Fragments – v2 Reagents

INTRODUCTION

Solid Phase Reversible Immobilization (SPRI) beads selectively bind DNA fragments according to the ratio of SPRI beads to DNA solution. The steps for Library Preparation listed in the Genome Reagent Kits User Guide (CG00043) utilize SPRIselect beads to enrich for DNA fragments with a specific size range that is essential to achieve high quality sequencing data for Genome libraries. This Technical Note describes how SPRIselect beads work and illustrates how changes in the size distribution of the library fragments can occur when the SPRIselect:DNA ratios are changed. The 10x Genomics protocols have been optimized for product performance and the discussion here gives additional recommendations for best practices in library preparation to maintain consistency and accuracy in technique. Following these guidelines will enable the user to produce the correct SPRIselect:DNA ratio during the workflow and result in libraries with the desired insert fragment size. Bioanalyzer traces are used to illustrate the impact that altered ratios can have on the fragment size distributions.

METHOD

We prepared a Chromium™ Genome library with 1 ng of NA12878 DNA following the Genome Reagent Kits User Guide (CG00043) through Step 4.5 – Sample Index PCR. One double-sided size selection was performed in two steps which are briefly outlined here and presented as a schematic overview in Figure 1.

Step 1 - A SPRIselect:DNA ratio of 0.5X removes most library fragments >1000bp. During this step fragments and beads are mixed together and incubated. The fragments larger than the desired library insert size bind to the SPRIselect beads and are discarded. The supernatant is saved.

Step 2 - The supernatant from Step 1 is mixed with a fresh volume of SPRIselect beads to make a SPRIselect:DNA ratio of 0.7X. This second incubation preferentially immobilizes DNA fragments >400bp on the beads which are then washed and fragments eluted for sequencing.

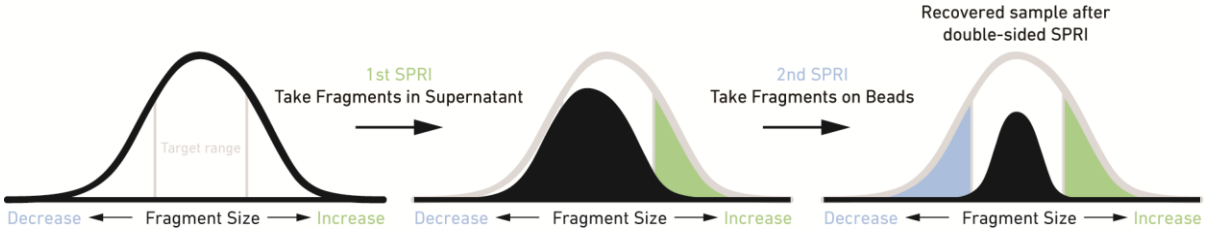

Fig. 1. Schematic overview of double-sided SPRI selection. After the first SPRI, supernatant is transferred for a second SPRI while larger fragments are discarded (green). After the second SPRI, fragments on beads are eluted and kept while smaller fragments are discarded (blue). Final sample has a tight fragment size distribution with reduced overall amount (black).

Two technicians participated in this study to highlight user-to-user variability. Double-sided size selection for protocol C and D was performed in duplicate. Two Agilent Bioanalyzer chips (DNA1000 and D7500) were used

to analyze resulting fragment distributions and to highlight how they differ in their appearance between both chips (Figure 2 and 3). Table 1 outlines in detail how different SPRIselect:DNA ratios altered the length of fragments that are bound to the beads or left in solution.

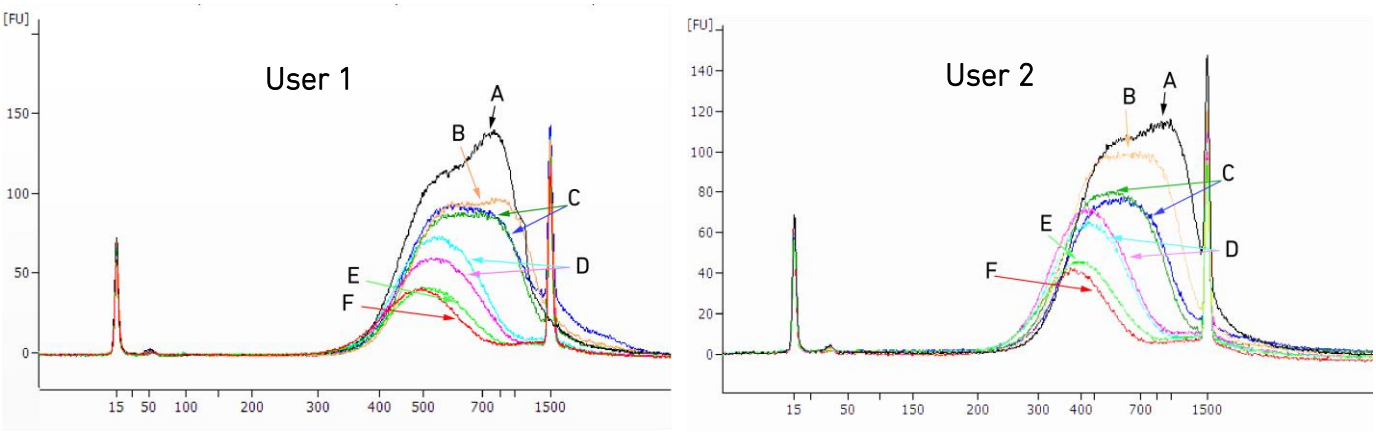

Fig. 2. Bioanalyzer traces obtained with Agilent Bioanalyzer DNA1000 assays after a double-sided SPRI size selection. NA12878 Genome Libraries were prepared by two different users with v2 reagents. Double-sided size selection for protocol C and D was performed in duplicate. DNA fragment size distributions are colored and labeled according to Table 1. Biological replicates demonstrate reproducibility of double-sided size selection.

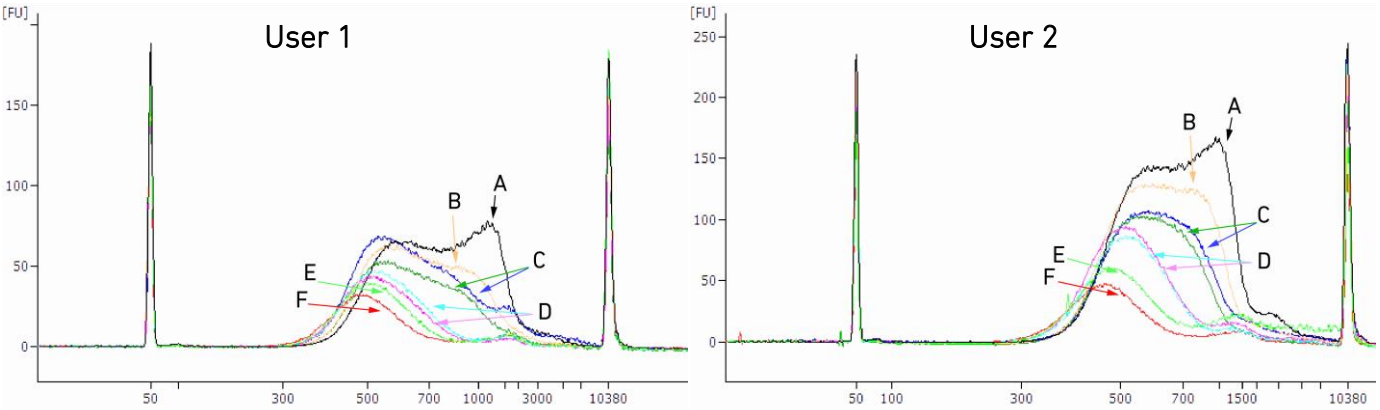

Fig. 3. Bioanalyzer traces obtained with Agilent Bioanalyzer DNA7500 assays after a double-sided SPRI size selection. NA12878 Genome Libraries were prepared by two different users with v2 reagents. Double-sided size selection for protocol C and D was performed in duplicate. DNA fragment size distributions are colored and labeled according to Table 1. Biological replicates demonstrate reproducibility of double-sided size selection. Biological duplicates highlight variability in quantity across the same library that went through a double sided SPRI.

		Keep Supernatant/ Discard Beads					**Keep Beads/ Discard Supernatant**				
Protocol	**PCR MIX**	**1st SPRI**	**Ratio**	**Fragments on Beads**	**Fragments in Supernatant**	**Take Supernatant**	**2nd SPRI**	**Ratio**	**Fragments on Beads**	**Fragments in Supernatant**	**Fragments eluded**
A	96 µl	44 µl	0.46X	> 1700bp	< 1700bp	135 µl	18 µl	0.65X	> 400bp	< 400bp	~ 400bp - 1700bp
B	96 µl	46 µl	0.48X	> 1500bp	< 1500bp	135 µl	18 µl	0.68X	> 400bp	< 400bp	~ 400bp - 1500bp
C	96 µl	48 µl	0.50X	> 1000bp	< 1000bp	135 µl	18 µl	0.70X	> 400bp	< 400bp	~ 400bp - 1000bp
D	96 µl	53 µl	0.55X	> 700bp	< 700bp	140 µl	18 µl	0.75X	> 350bp	< 350bp	~ 350bp - 700bp
E	96 µl	55 µl	0.57X	> 600bp	< 600bp	140 µl	18 µl	0.77X	> 300bp	< 300bp	~ 300bp - 600bp
F	96 µl	57 µl	0.59X	> 550bp	< 550bp	140 µl	18 µl	0.80X	> 300bp	< 300bp	~ 300bp - 550bp

Table. 1. Workflow of double-sided size selection with 6 different protocols (A.-F.). Protocol C (framed in red) achieves a defined target fragment size range between 400 – 1000bp that is optimal for 10x Chromium™ Genome libraries as defined in the User Guide. DNA fragments bound to SPRIselect beads are discarded in the first size selection and supernatant is saved for the second size selection. In the second SPRI, DNA fragments of the desired target size range are immobolized on SPRIselect beads and subsequently eluted to give the final fragment library that is ready for downstream sequencing. The resulting fragment distributions are shown in Figures 2 and 3. Results are averages between two users.

DISCUSSION

The current method used for qualitative evaluation of Chromium™ Genome libraries is analysis with the Agilent Bioanalyzer DNA1000 chip. Fig. 2, Fig. 3 and Table 1 illustrate how different SPRIselect:DNA ratios during double-sided size selection impact the size range of target fragments. **Note that with each SPRIselect:DNA step the overall yield of the library is decreased.**

Because of the sensitivity of the size selection to the SPRIselect:DNA ratio minor differences in SPRIselect bead volume can shift the peak of the size distribution trace either to smaller or larger fragment sizes. Therefore, consistent technique is essential and the following guidelines are suggested to maintain uniform fragment size distributions across different library preps:

- Use well calibrated pipettes
- Use appropriate pipettes for SPRIselect handling to increase pipetting accuracy (e.g. P200 for volumes of ~30µl – 200 µl)
- Equilibrate SPRIselect beads and sample to room temperature before use
- Vortex SPRIselect beads before each transfer to sample
- Visually inspect levels of solutions in tips during transfers
- Be aware of and avoid transfer of excess SPRIselect bead volume that may have formed on the outside of the pipette tip or as a drop hanging from the tip
- Mix SPRI beads thoroughly into solutions to create a uniform mixture.
- Use fixed volumes for the final double-sided SPRI to improve reproducibility

Adherence to these practices will be critical to reproducibly generate a uniform distribution of fragments around a targeted average size range. Additional guidelines are reviewed in Technical Notes CG00047 and CG00048.

We look for traces that range between 400 – 1000bp with a significant amount of inserts that are 400 – 600bp in length (see Fig. 2 and Fig. 3, trace C). 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 resembles the traces in Fig. 2 or Fig. 3 the user is **encouraged to sequence the library and review the results.**

Libraries over the entire range of tested SPRIselect:DNA conditions were sequenced and passed quality metrics. Read 2 quality may be slightly impacted with libraries that contain larger inserts. In contrast, libraries with smaller fragments may have slightly reduced coverage evenness and slightly increased duplication rates.

The presence of fragments larger than 600bp does not affect sequencing performance as these fragments have low efficiencies in binding to the flowcells to form clusters. Particularly, patterned flowcells preferentially cluster smaller fragments. Should fragment sizes be significantly outside the recommended range (e.g. peak < 300bp or > 1000bp), we recommend to remake the sample. We do not recommend a second double-sided SPRI to correct the fragment size range as it will decrease the overall fragment yield by as much as 50% and affect library diversity.

CONCLUSION

This Technical Note highlights the different fragment distribution lengths that may be obtained from the Library construction as a result of varying SPRIselect bead: DNA library ratios. Traces are to be used as a QC guide for to detect the distribution of fragment/ insert size prior to sequencing.

REFERENCES

- Chromium™ Genome Reagent Kits v2 User Guide (CG00043)
- Technical Note – QC of Chromium™ Genome Libraries: Qualitative Evaluation Using Agilent Bioanalyzer (CG00047)
- Technical Note – QC of Chromium™ Genome Libraries: Quantitative Evaluation Using qPCR (CG00048)

Notices

Document Number

CG00061 Rev A *Technical Note*

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