

TECHNICAL NOTE

SPRIselect:DNA Ratios Affect the Size Range of Library Fragments – v1 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 (CG00022) 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 (CG00022) 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.

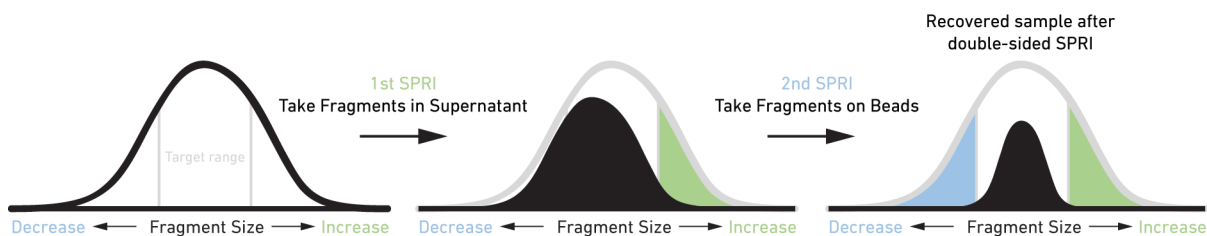


Fig. 1. Schematic overview of double-sided SPRI selection. After the first SPRI, supernatant is transferred for a second SPRI while larger fragments are discarded (green). After the second SPRI, fragments on beads are eluted and kept while smaller fragments are discarded (blue). Final sample has a tight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

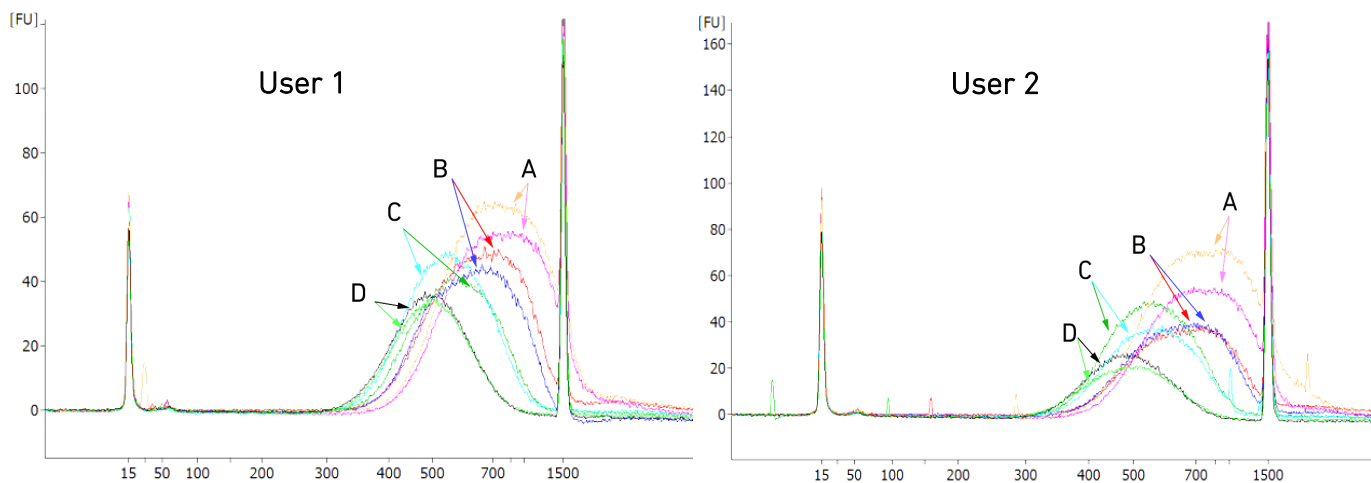


Fig. 2. Bioanalyzer traces obtained with Agilent Bioanalyzer DNA1000 assays after a double-sided SPRI size selection. NA12878 Genome Libraries were prepared by two different users with v1 reagents. Each double-sided size selection was performed in duplicate. DNA fragment size distributions are colored 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.

Two technicians participated in this study to highlight user-to-user variability. Each double-sided size selection was performed in duplicate. An Agilent Bioanalyzer DNA1000 chip was used to analyze resulting fragment distributions (Figure 2). 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.

Protocol	PCR MIX	Keep Supernatant/ Discard Beads				Keep Beads/ Discard Supernatant				Average Size		
		1st SPRI	Ratio	Fragments on Beads	Fragments in Supernatant	Take Supernatant	2nd SPRI	Ratio	Fragments on Beads		Fragments in Supernatant	Fragments eluted
A	96 µl	44 µl	0.46X	> 1500bp	< 1500bp	135 µl	18 µl	0.65X	> 400bp	< 400bp	400bp - 1500bp	782bp
B	96 µl	48 µl	0.50X	> 1000bp	< 1000bp	135 µl	18 µl	0.70X	> 400bp	< 400bp	400bp - 1000bp	702bp
C	96 µl	53 µl	0.55X	> 850bp	< 850bp	140 µl	18 µl	0.75X	> 350bp	< 350bp	350bp - 850bp	584bp
D	96 µl	57 µl	0.59X	> 700bp	< 700bp	140 µl	18 µl	0.80X	> 350bp	< 350bp	350bp - 700bp	512bp

Table. 1. Workflow of double-sided size selection with 4 different protocols (A.-D.). Protocol B (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 immobilized 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 Figure 2. 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. Both Fig. 2 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 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

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

We look for Bioanalyzer traces showing fragments that range in size between 400 – 1000bp with a significant amount of inserts that are 400 – 600bp in length (see Fig. 2, trace B). 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 traces in Fig. 2 the user is **encouraged to sequence the library**.

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 to detect the distribution of fragment sizes prior to sequencing.

REFERENCES

- Chromium™ Genome Reagent Kits User Guide (CG00022)
- 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

CG00060 Rev A *Technical Note*

Legal Notices

© 2016 10x Genomics, Inc. All rights reserved. Duplication and/or reproduction of all or any portion of this document without the express written consent of 10x Genomics, Inc., is strictly forbidden. Nothing contained herein shall constitute any warranty, express or implied, as to the performance of any products described herein. Any and all warranties applicable to any products are set forth in the applicable terms and conditions of sale accompanying the purchase of such product. 10x Genomics provides no warranty and hereby disclaims any and all warranties as to the use of any third party products or protocols described herein. The use of products described herein is subject to certain restrictions as set forth in the applicable terms and conditions of sale accompanying the purchase of such product. "10x", "10x Genomics", "Changing the Definition of Sequencing", "Chromium", "GemCode", "Loupe", "Long Ranger", "Cell Ranger" and "Supernova" are trademarks of 10x Genomics, Inc. All other trademarks are the property of their respective owners. All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

The use of 10x Product(s) in practicing the methods set forth herein has not been validated by 10x, and such non-validated use is NOT COVERED BY 10X STANDARD WARRANTY, AND 10X HEREBY DISCLAIMS ANY AND ALL WARRANTIES FOR SUCH USE.

Nothing in this document should be construed as altering, waiving or amending in any manner 10x Genomics, Inc., terms and conditions of sale for the Chromium™ Controller, consumables or software, including without limitation such terms and conditions relating to certain use restrictions, limited license, warranty and limitation of liability, and nothing in this document shall be deemed to be Documentation, as that term is set forth in such terms and conditions of sale. Nothing in this document shall be construed as any representation by 10x Genomics, Inc that it currently or will at any time in the future offer or in any way support any application set forth herein.

Customer Information and Feedback

For technical information or advice, please contact our Customer Technical Support Division online at any time.

Email: support@10xgenomics.com

10x Genomics 7068 Koll Center Parkway

Suite 401

Pleasanton, CA 94566 USA