SRSLY STRINGENT SIZE SELECTION WITH CLAREFY DNA PURIFICATION BEADS

INTRODUCTION

Size-selection of library molecules is a vital part of next-generation sequencing (NGS) library preparation. Adapter dimers (~150 bp) can cluster more efficiently on a sequencing flow-cell due to their smaller size and reduce the amount of high-quality, mappable reads generated. Therefore, removal of adapter and primer dimer molecules is necessary for improved sequencing performance.

Additionally, fragments of specific size ranges may be of interest in certain applications. Efficient cluster amplication on Illumina sequencers (the more commonly used NGS technology) also requires library molecules to be less than 600 bp. Traditional library preparation methods incorporate steps that fragment input DNA molecules to a normal distribution of ~200 to 400 bp and perform stringent size selection for library molecules between ~300 to 500 bp (after adapter ligation), which also guarantees removal of adapter and primer dimers. Conversely, forensic and ancient DNA libraries, oligonucleotides and small RNA libraries generally have a size range <100 nt and require size-selection strategies that capture short molecules. Due to their small insert size, the library generated with these molecules are only slightly larger than adapter dimers and require additional calibration of library size-selection. Other applications may require a wider size-selection. In cell-free DNA (cfDNA) applications, fragments that are ~100-320 bp largely represent DNA derived from nucleosomes, whereas shorter fragments (between 30-100 bp) are enriched for DNA derived from transcription factors, microbial DNA, and tumor derived DNA[1]. While most library preparation methods are either unable to convert shorter molecules to sequence-ready library or lose these molecules due to their size-selection stringency, **Claret Bioscience's SRSLY™ NGS library preparation** method captures these fragments. Appropriate size-selection strategies that effectively remove adapter dimers, while retaining short fragments are integral to the optimum performance of the SRSLY library preparation protocol and subsequent sequencing.

SRSLY, like other library preparation methods relies on the Solid Phase Reversible Immobilization (SPRI), ferromagnetic bead-based method for stringent selection of DNA molecules of a desired size range[2]. The Clarefy DNA purification beads, formulated for use with SRSLY have been further optimized for efficient size-selection of the uniquely short-sized fragments that SRSLY generates, regardless of input type. The **"minimum short fragment retention (Min-retention)"** protocol is the default protocol reserved for libraries from high molecular weight sources such as gDNA and cDNA (RNA-Seq). However, there are more stringent protocols for cfDNA, forensic, oligonucleotide SRSLY libraries. The **"moderate short fragment retention (Mod-retention)"** protocol efficiently captures DNA greater than 85 bp in length whereas the **"maximum short fragment retention (Max-retention)"** protocol captures extremely short fragments (down to 10 nt in length), while minimizing adapter dimer formation.

In this application note we provide an overview of the molecular and sequencing differences between cfDNA SRSLY libraries generated with both Mod- and Max-retention strategies. We also optimize and compared the performance of DNA purification protocols for other commercially available DNA purification beads (Zymo Research Select-A-Size DNA Clean & Concentrator MagBead Kit – Zymo and the Beckman Coulter AMPure DNA purification beads – AMPure) for moderate and maximum short fragment retention.

EXPERIMENTAL DESIGN

Library preparation

SRSLY library preparation workflow. All SRSLY libraries were prepared with 1 ng of pooled plasma-derived cfDNA from healthy donors using the SRSLY PicoPlus Library Preparation Kit. The protocol includes two DNA purification steps: DNA purification I occurs after SRSLY adapter ligation, to remove adapter dimers and to select library molecules of desired size range, and DNA purification II after index PCR, to remove primer dimers. DNA purification and size-selection was performed with both Mod- and Max-retention protocols using the Clarefy, AMPure and Zymo beads, with specific parameters provided in the table below.

DNA purification bead parameters

Bead	cfDNA Fragment Retention	DNA Purification I			DNA Purification II
		Volume of 10mM Tris or water To Add (µl)	Volume of 100% Isopropanol To Add (µl)	Volume of DNA Purification Beads To Add (µl)	Volume of DNA Purification Beads To Add (µl)
Clarefy	Maximum	48	12	65.2	75
	Moderate	60	0	65.2	60
AMPure	Maximum	48.4	11.6	59.4	60
	Moderate	60	0	59.4	50
Zymo	Maximum	48.4	11.6	59.4	60
	Moderate	60	0	59.4	50

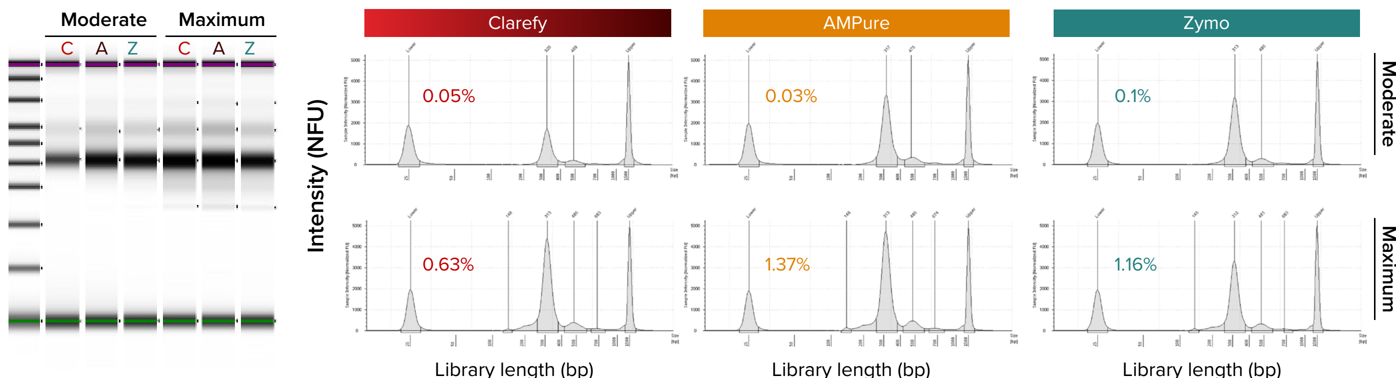
Beads volume and buffer composition for DNA purification with Clarefy and other commercial beads. Prior to using commercial DNA purification beads, the bead volumes and buffer compositions were optimized for both Mod- and Max-retention protocols using a molecular weight ladder (data not shown). The table provides bead volumes and buffer modification for each purification step and bead type.

Sequencing and analysis:

All libraries were sequenced on the Illumina MiSeq at a read length of 2x151 bp following manufacturer's instructions to a depth of ~300,000 reads. The reads with filtered to remove adapter dimer reads and other low-quality reads using SepPrep2 and mapped to the human genome using the Burrow Wheeler Aligner (BWA). Insert size distribution metrics were extracted from mapped reads and plotted using custom R pipelines. Duplication rate was calculating using PicardTools.

RESULTS

Library Size Distribution



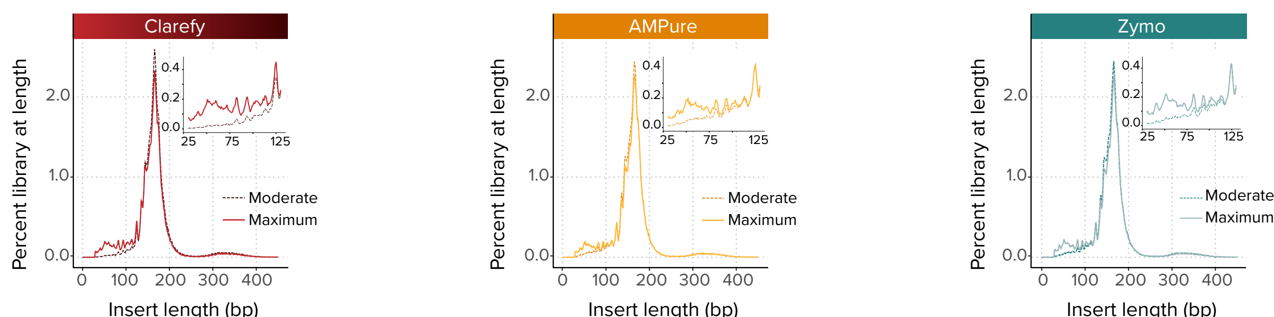
Library size distribution. The final libraries were run on an Agilent Tapestation 4200 using a D1000 screen tape. Percentage of the library that comprises molecules that are most likely adapter or primer dimers are provided for each library and fragment retention protocol. The mock-gel, the image and electropherogram showed equivalent fragment retention for all three beads tested for Mod-retention. Max-retention protocol retained higher amount of short fragments in comparison to the Mod-retention protocol. However, more adapter dimers were seen with the commercial beads for the Max-retention protocol.

Sequencing Performance

Bead	Short Fragment Retention	Reads	Merged	Discarded	Pass Filter %	Mapped Reads	Mapped (duplicates removed)	Mapped/ Total Reads	Mapped/ Pass Filter Reads	Percent Duplicates	30-100 bp Mapped
Clarefy	Moderate	411911	373448	1795	99.6%	368511	367599	89.2%	89.6%	0.28%	1.3
Ampure		401734	365985	3601	99.1%	357072	356265	88.7%	89.5%	0.26%	3.9
Zymo		400838	365342	2960	99.3%	357370	356597	89.0%	89.6%	0.25%	3.8
Clarefy	Maximum	372556	333976	10940	97.1%	323099	322522.5	86.6%	89.2%	0.21%	9.0
Ampure		288460	256314	8377	97.1%	249277	248946	86.3%	88.9%	0.15%	7.8
Zymo		351818	305883	15048	95.7%	297900	297424	84.5%	88.3%	0.18%	8.7

Sequencing metrics for SRSLY libraries. All libraries were sequenced on a MiSeq to a depth of ~300,000 reads and mapping metrics were obtained as described in the Sequencing and analysis section. For the Mod-retention protocol all beads tested showed equivalent mapping metrics, but slightly higher amount of discarded reads and consequently higher reads in the 30-100bp bin were observed with commercial beads. The Max-retention protocol, resulted in higher retention of fragments in the 30-100 bp bin irrespective of the beads used, however the Zymo beads showed overall lower mapping performance.

Insert Size Distribution



Insert size distribution Following removal of adapter sequences and mapping, the cfDNA size distribution was evaluated. Profiles were characteristic of cfDNA libraries with SRSLY. Inset shows difference in the retention of the short fragments (30-100 bp) between the two protocols. Libraries generated with the Max-retention protocol captured higher percent of short molecules (reads below 30 bp are filtered as a part of the analytical pipeline) regardless of the beads used. All three beads showed similar size distribution for each retention methods, however, the Clarefy beads showed clearer distinction between the Mod- and Max-retention methods in the capture of short fragments (inset).

CONCLUSION

This application notes showcases the performance of the Clarefy DNA purification beads in generating high quality next-generating sequencing libraries with minimal adapter dimers. These results show that the bead formulation used in the Clarefy DNA purification bead provides the optimum DNA size-selection and purification for standard cfDNA applications and for the retention of short fragments. We also demonstrate the feasibility of commercial DNA purification protocols albeit with modifications for use with the SRSLY library preparation kit.

Write to us at technicalsupport@claretbio.com for detailed DNA purification protocols

CLARETBIO PRODUCT INFORMATION:

To order the Clarefy Purification beads email at orders@claretbio.com

Product	SKU	Amount	Use with
Clarefy DNA Purification beads (24 reaction)	CBS-BD-24	7 ml	SRSLY PicoPlus and NanoPlus 24 reaction kit with and without UM-24
Clarefy DNA Purification beads (96 reaction)	CBS-BD-96	28 ml	SRSLY PicoPlus and NanoPlus 96 reaction kit with and without UM-96
Clarefy DNA Purification beads (bulk)	Available upon request		

REFERENCES

1 Mouliere, F. et al. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci Transl Med 10, doi:10.1126/scitranslmed.aat4921 (2018).
2 DeAngelis, M. M., Wang, D. G. & Hawkins, T. L. Solid-phase reversible immobilization for the isolation of PCR products. Nucleic Acids Res 23, 4742-4743, doi:10.1093/nar/23.22.4742 (1995).

P.O. Box 3052 | Santa Cruz, CA 95063 | +1.833.425.2738 | www.ClaretBio.com | info@ClaretBio.com

Copyright © 2020 Claret Bioscience. All rights reserved. 08072020