NGS

Use of MagMAX Pure Bind beads for size selection in Illumina NGS workflows

Summary

- MagMAX Pure Bind beads exhibit performance equivalent to AMPure XP beads
- An affordable, user-friendly alternative for cleanup and size selection
- Reduced carbon emissions with room temperature shipping and storage

Introduction

Paramagnetic beads are commonly used post–library preparation for size selection and cleanup during next-generation sequencing (NGS) workflows. Altering the ratio of beads to libraries helps to eliminate unwanted sizes, partial amplicon fragments, or unincorporated oligos that may interfere with sequencing. Ensuring a clean library without losing the target size product is a critical step in obtaining high-quality sequencing data. For this study, Applied Biosystems™ MagMAX™ Pure Bind beads were used as size selection beads to clean up libraries for Illumina™ systems. These beads are optimized to be stored at room temperature, making the workflow user-friendly and efficient. Additionally, MagMAX Pure Bind beads are used at the same volume as AMPure™ XP Beads (Beckman Coulter, Inc.), allowing for a direct substitution. Here we evaluate MagMAX Pure Bind and AMPure XP beads and compare performance characteristics with next-generation sequencing data.

Materials and methods

DNA isolation

DNA for library preparation was isolated from formalin-fixed, paraffin-embedded (FFPE) bladder and cervical tissue, using the DNA-only protocol for the Applied Biosystems™ MagMAX™ FFPE DNA/RNA Ultra Kit on the Thermo Scientific™ KingFisher™ Duo Prime Purification System with Applied Biosystems™ AutoLys tubes for deparaffinization. Duplicate 10 µm curls were cut from the FFPE blocks of both tissues, and processed in AutoLys tubes.

The recommended volume of protease solution was added to each curl in an AutoLys tube and incubated at 60°C for 1 hour, and then at 90°C for 1 hour to fully deparaffinize the samples. The samples were then processed on the KingFisher Duo Prime system following procedural guidelines. The purified DNA was quantified using the Invitrogen™ Qubit™ dsDNA High Sensitivity Kit, and replicates were pooled to reduce variability during library preparation and sequencing.

Additionally, two immortal human cell lines were selected for the studies—Jurkat and HCT116. DNA was extracted from the cell lines using the Invitrogen™ PureLink™ Genomic DNA Mini Kit, with an input of 2 x 10^6 cells in 200 µL PBS. Samples were isolated in triplicate and then pooled after quantifying with the Applied Biosystems™ NanoDrop™ Eight Spectrophotometer. Structural Multiplex Reference Standard (gDNA) (Horizon Discovery Ltd., Cat. No. HD753) was used as a control with a 10 ng input for the library preparation workflow.

Library preparation

Library preparation and size selection were done manually by one user. Pooled FFPE bladder tissue replicates, pooled FFPE cervical tissue replicates, pooled cell line replicates, and control DNA were separated into duplicates for the library preparation workflow. The AmpliSeq Cancer Hotspot Panel v2 for Illumina™ systems (Illumina, Cat. No. 20019161), AmpliSeq Library PLUS for Illumina™ systems (24 reactions) (Illumina, Cat. No. 20019101), and AmpliSeq™ UD Indexes for Illumina™ systems (24 indexes) (Illumina, Cat. No. 20019104) were selected for library preparation from FFPE tissue and DNA samples; the recommended protocols in the user guides were followed.

All samples had a 10 ng input of DNA. For the target amplification stage, control and cell samples were cycled 27 times but FFPE samples were cycled 30 times (per the user guide) because FFPE DNA is considered low-quality. Both MagMAX Pure Bind and AMPure XP beads can be used at the same volume; therefore, the initial clean-up of the libraries was done using 30 µL of MagMAX Pure Bind and AMPure XP beads at a bead:library volume ratio of 1:1. After amplification of the libraries, two clean-up steps were done: first with a bead:library ratio of 1:2 (25 µL beads, 50 µL library) to remove high molecular weight DNA, and the second at a ratio of 4:5 (60 µL beads, 75 µL supernatant) to remove primers. The primers remained in the supernatant, and the libraries were eluted off the beads. The same volume of MagMAX Pure Bind and AMPure XP beads was used to perform size selection to obtain the library size of 200–320 bp. The volumes of washes and elution also remained the same across all samples processed with both bead types. The samples were assigned unique dual barcodes to make pooling easier and to enable all samples to be processed together on one flow cell. Libraries for all sample types were successfully prepared in duplicate and quantified using the Qubit DNA High-Sensitivity Kit. Final libraries were pooled by a third-party service provider, and the normalized pool was run on the Illumina™ HiSeq X™ system. Raw BCL files generated from the HiSeq X system were converted to FASTQ format for each sample. Sequencing adapters and low-quality bases in raw reads were trimmed using Trimmomatic version 0.39 software. Cleaned reads were then aligned to the *Homo sapiens* GRCh37 reference genome using Sentieon version 202112.01 software. Alignments were then sorted, and PCR/optical duplicates were marked.

Note: Only FFPE tissue and control libraries were sequenced and in duplicate.

Results

Library quantitation

The reference guide for the AmpliSeq Cancer HotSpot Panel v2 for Illumina systems recommends that the quality of the libraries be assessed using an Agilent™ 2100 Bioanalyzer™ system with the Agilent™ DNA 1000 Kit, and the size distribution range be set between 200 bp and 320 bp to quantify the average size of the library.

The individual library sizes are listed in Table 1, and electrophero-grams from the Agilent™ 4200 TapeStation™ System are shown in Figure 1. The electropherogram traces of all samples were comparable within a sample type, regardless of the beads used. However, some variability between replicates was observed, and the variability can be attributed to the differences in overall efficiency of PCR or the variability introduced from manual size selection [1].

Table 1. Average library size from the 2100 Bioanalyzer system. Sizes are within the suggested size distribution of 200–320 bp, suggesting that the size selection was successful, with larger and smaller sizes eliminated effectively. Replicate libraries are shown.

Sample	MagMAX Pure Bind beads	AMPure XP beads
FFPE, bladder	262	271
	263	271
FFPE, cervix	274	268
	271	268
Jurkat cells	275	273
	278	277
HCT116 cells	270	278
	276	274
Control	276	278
	283	279

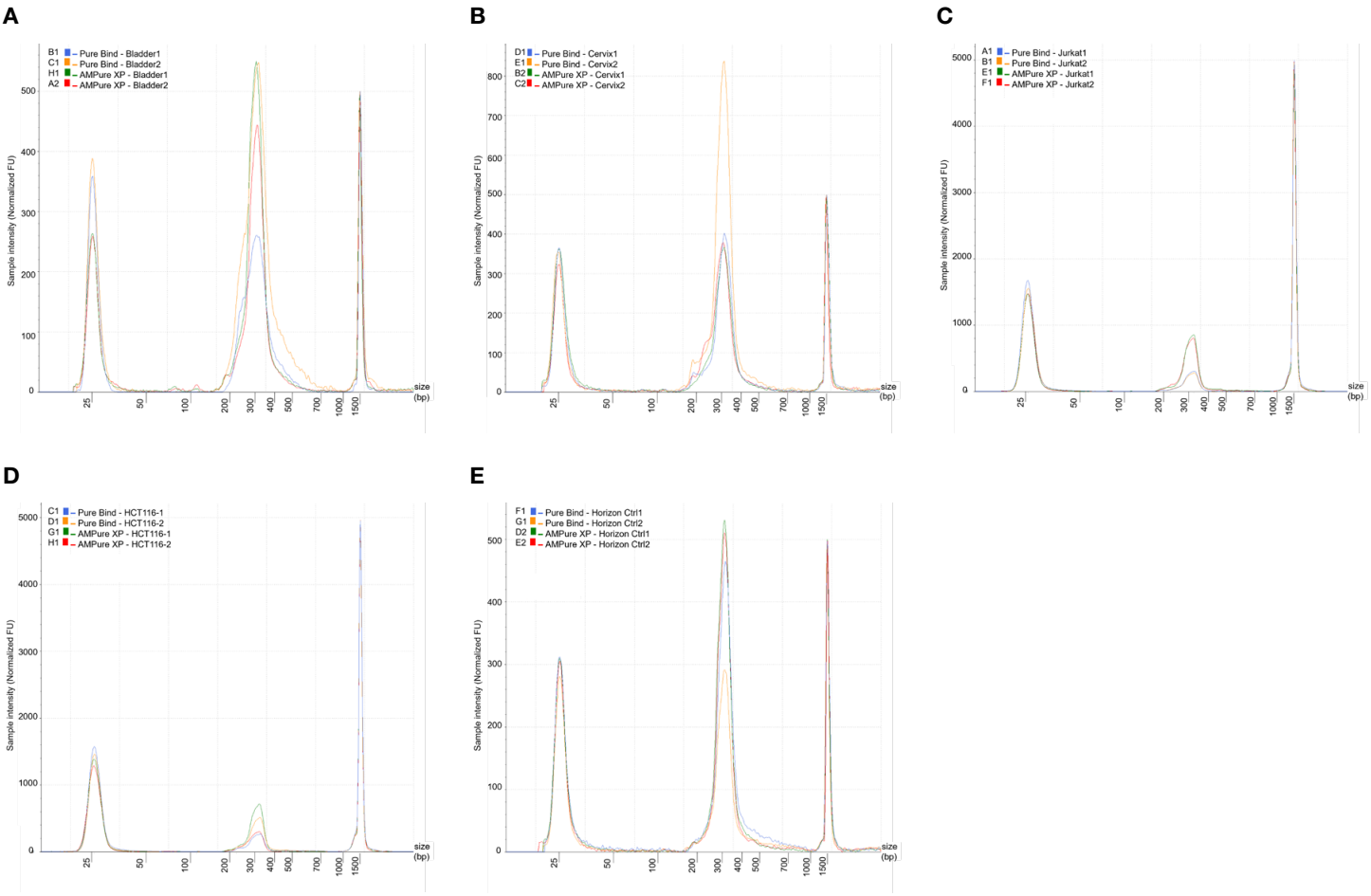

Figure 1. Electropherograms from the 4200 TapeStation System. Traces from **(A)** bladder, **(B)** cervix, **(C)** Jurkat cells, **(D)** HCT116 cells, and **(E)** control samples are shown. The traces from all samples were comparable within a sample type, regardless of the beads used. Replicate libraries are numbered 1 and 2.

Sequencing results

All samples' quality scores (percentage of bases with Q score ≥30) were over 80%, with the lowest value being 87.7%, indicating high-quality sequencing data [2]. Mean target coverage depth, shown in Figure 2, shows all samples having similar coverage with the two library preparation options. All sequenced libraries had 100% of target regions ≥20X, indicating that all target regions were read or covered. The read length for all libraries was 150 bp; the median insert sizes, shown in Figure 3, are comparable across all libraries. Quality and sequencing metrics are comparable across libraries prepared using MagMAX Pure Bind and AMPure XP beads.

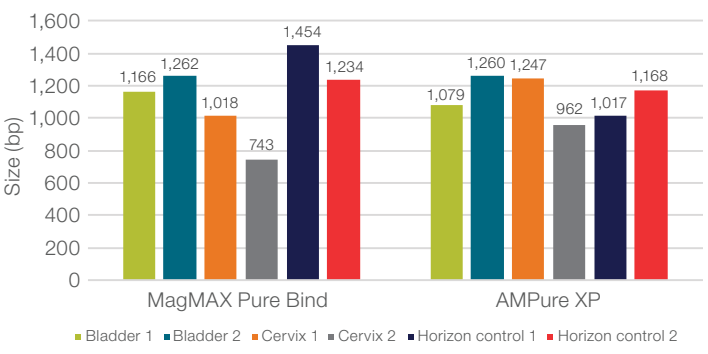

Figure 2. Mean target coverage depth. Replicate libraries are numbered 1 and 2.

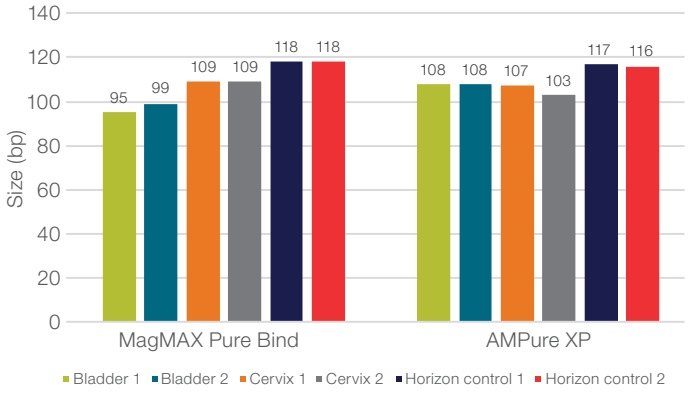

Figure 3. Median insert size. Replicate libraries are numbered 1 and 2.

Conclusions

MagMAX Pure Bind beads exhibit performance comparable to AMPure XP Beads, with no substantial difference observed in average library size, mean target coverage depth, and median insert size. MagMAX Pure Bind beads allow users to seamlessly integrate this technology into established workflows without compromising performance or time. MagMAX Pure Bind beads are an affordable, user- and eco-friendly alternative to AMPure XP Beads.

Authors

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Ordering information

Product	Quantity	Cat. No.
MagMAX Pure Bind Beads	5 mL	A58521
	50 mL	A58522
	250 mL	A58523
MagMAX FFPE DNA/RNA Ultra Kit	1 kit	A31881
AutoLys M Tubes and Caps	25 each	A38738
KingFisher Duo Prime Purification System	1 system	5400110
PBS, pH 7.4	500 mL	10010023
Qubit dsDNA High Sensitivity Kit	100 assays	Q32851
PureLink Genomic DNA Mini Kit	250 preps	K182002

References

1. Head SR, Komori HK, LaMere SA et al. (2014) Library construction for next-generation sequencing: overviews and challenges. *Biotechniques* 56(2):61-passim. doi:10.2144/000114133
2. Illumina (2011) Technical note: Quality scores for next-generation sequencing. illumina.com/Documents/products/technotes/technote_Q-Scores.pdf

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