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Tunable insert sizes with Illumina DNA PCR-Free Prep, Tagmentation

Adjust the median insert size of library fragments with slight workflow modifications.

Introduction

Next-generation sequencing (NGS) read length is the number of base pairs sequenced from a DNA fragment. After sequencing, the regions of overlap between reads are used to assemble and align the reads to a reference genome, reconstructing the full sequence. Choosing the right sequencing read length depends on several factors, including sample type, application, and coverage requirements. Because long reads allow for more sequence overlap, they are useful for *de novo* assembly and resolving repetitive areas of the genome. For other applications, such as expression profiling or counting studies, shorter reads are sufficient and more cost-effective than longer ones. Sequencing read length determines the library insert size (referring to the library portion between the adapter sequences, which have a constant length).

Illumina DNA PCR-Free Prep, Tagmentation (Illumina DNA PCR-Free) enables customers to select and tune the median insert size 100 bp up or down from the standard size (450 bp), with slight modifications to the library preparation workflow.[1] The broader range of insert sizes provides more flexibility in the types of sequencing applications possible with Illumina DNA PCR-Free. This technical note provides an overview of the workflow changes needed when generating libraries with different insert sizes and the resulting data.

Insert size selection during library preparation

During library preparation, different ratios of sample purification beads are used in two rounds of purification to select the appropriate library size (Figure 1). In the first round, larger fragments are removed, and in the second round, smaller fragments are removed. The range of fragment sizes between the two cut-offs is captured for clean-up and sequencing. The median fragment (insert) size can be selected by adjusting the amounts of sample purification beads used in each round.

Figure 1: Illumina DNA PCR-Free Library Prep—Slight modifications to the standard workflow (steps noted with an asterisk) enable users to adjust the library insert size.

Modifications to Illumina DNA PCR-Free workflow

To demonstrate the slight workflow modifications required to adjust insert size, the first and second sample purification bead additions were changed to capture various median fragment sizes during Illumina DNA PCR-Free library preparation. Based on previous data, median insert sizes of 350 bp and 550 bp were targeted, in addition to the standard insert size of 450 bp. Different proportions of sample purification beads were used to calculate the volumes of beads to be added in each round (Table 1).

Table 1: Recommended conditions for sample purification bead addition and insert size selection

Volume into first SPRI	Fold first SPRI	Volume first SPRI	Total volume	Volume transferred into second SPRI	Fold second SPRI	Volume second SPRI	Mean insert size	Mean yield	Target median insert size
45 µl	1	45 µl	90 µl	85 µl	2.5	63.8 µl	339 bp	14.2 nM	350 bp
45 µl	0.8	36 µl	81 µl	76 µl	1.8	42.2 µl	457 bp	7.4 nM	450 bp
45 µl	1.4	63 µl	108 µl	—	—	—	551 bp	5.6 nM	550 bp

Abbreviations: SPRI, solid-phase reversible immobilization

Results

Results show that the workflow modifications described in this technical note successfully captured library insert sizes of 350 bp and 550 bp (Figure 2, green dashed lines). Although adjusting the median insert size affects the amount of total library that is captured, library yields for each insert size condition are sufficient for sequencing (Figure 2). Users should note that the different fragment sizes will need to be factored into library quantification.

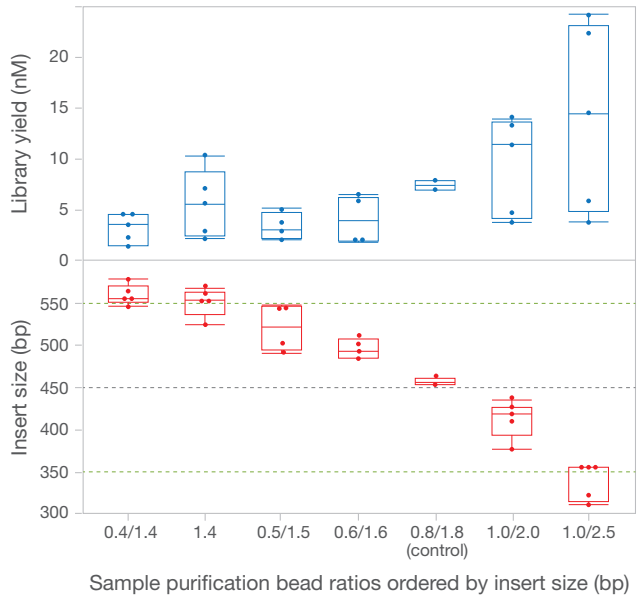

Figure 2: Library insert sizes and yields generated with workflow modifications —Different proportions of sample purification beads used in the two rounds of size selection are plotted against insert size (red boxes) and library yield (blue boxes). Target insert sizes of 450 bp (gray dashed line), 350 bp, and 550 bp (green dashed lines) are shown. LIbrary yields for all conditions tested are sufficient for sequencing.

As with any library preparation method, fragment size is not monolithic. Rather, a distribution of fragments of different lengths centered around the target insert size is obtained. Likewise, the changes to the Illumina DNA PCR-Free workflow presented in this technical note resulted in libraries with fragment sizes distributed around the targeted median insert sizes of 350 bp and 550 bp (Figure 3).

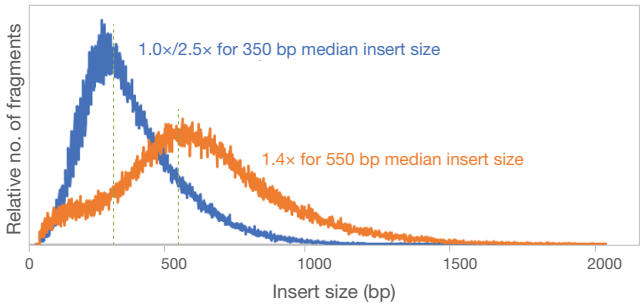

Figure 3: Distribution of fragment sizes—Prepared libraries show the expected distribution of fragment sizes around the median insert sizes (green dashed lines).

Summary

This technical note presents slight modifications to the standard workflow required to target insert sizes of 350 bp and 550 bp. The ability to adjust the target insert size during library preparation provides more flexibility in the types of sequencing applications possible with Illumina DNA PCR-Free.

Learn more

To learn more about Illumina DNA PCR-Free, visit www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/dna-pcr-free-prep.html

References

1. Illumina (2020) Illumina DNA PCR-Free, Tagmentation Reference Guide. Accessed March 26, 2020.

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