TECHNICAL NOTE

Optimal variant calling with Illumina DNA PCR-Free Prep on the NovaSeq™ X Series

A simple library prep protocol modification increases insert sizes to optimize variant calling performance

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Introduction

Illumina DNA PCR-Free Prep, Tagmentation offers a fast, flexible, and optimized library prep solution for various whole-genome sequencing (WGS) applications. Based on bead-linked transposome chemistry, Illumina DNA PCR-Free Prep provides even coverage across the genome and supports easy volume-based library pooling.[1,2]

The NovaSeq X Series is powered by XLEAP-SBS™ chemistry—a faster, higher fidelity, and more robust advancement to proven Illumina sequencing by synthesis (SBS) chemistry. XLEAP-SBS reagents are optimized for performance and speed to maximize throughput and deliver high-quality data.

Illumina DNA PCR-Free Prep can combine with the unprecedented power of the NovaSeq X Series as part of an integrated, scalable, and rapid WGS workflow (Figure 1). However, Illumina DNA PCR-Free Prep libraries sequenced on the NovaSeq X System may yield shorter insert sizes, which can result in slight reductions in variant calling for single nucleotide variants (SNVs) and insertions/deletions (indels). This technical note highlights a simple modification to the Illumina DNA PCR-Free Prep protocol to optimize sequencing library insert size and subsequent variant calling with the NovaSeq X Series.

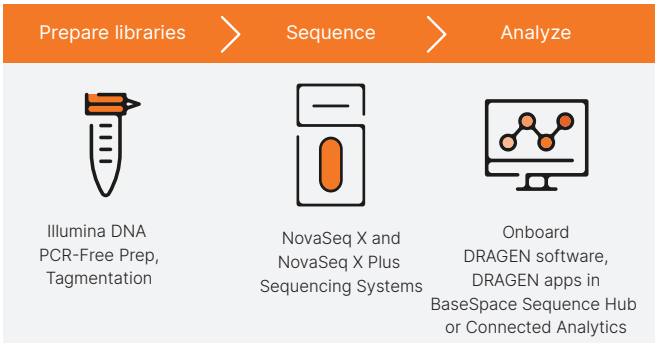

Figure 1: Integrated and scalable WGS workflow with Illumina DNA PCR-Free Prep and the NovaSeq X Series.

Methods

Library preparation

Library preparation was performed using 300 ng human reference DNA (Coriell, Catalog no. NA12878) and the Illumina DNA PCR-Free Prep, Tagmentation (Illumina, Catalog no. 20041794) workflow. The protocol is modified such that an additional bead purification step is performed before final elution (Figure 2). Refer to the Appendix for details.

Sequencing

Samples were quantified by qPCR, using 450-bp insert size, and loaded at 180 pM on NovaSeq X 10B flow cells following the 'Denature and Dilute Libraries' protocol described in the NovaSeq X System user guide (Document # 200027529 v05). Note: the loading concentration recommended for this modified protocol is slightly higher than the concentration stated in the user guide for a standard Illumina DNA PCR-Free library. Sequencing was performed using the NovaSeq X Series 10B Reagent Kit (300 cycles) (Illumina, Catalog no. 20085594) and the 2 × 151 bp run configuration.

Data analysis

Sequencing data were streamed directly from the instrument into the cloud ecosystem for analysis using DRAGEN™ apps available through BaseSpace™ Sequence Hub. The DRAGEN Germline app v4.1.23 was used for alignment against the hg38 reference genome assembly and variant calling. Data was downsampled to 30× coverage and F1 scores were calculated using variant calling assessment tool (VCAT) 4.1.0.

1. **Tagment gDNA**
   DNA is tagmented using bead-linked transposomes
2. **Post-tagmentation clean-up**
   Tagmented DNA fragments are purified
3. **Ligate indexes**
   Index adapters are ligated to DNA fragments
4. **Clean up libraries**
   Purification beads are added to select library size
4b. **Additional purification**
   Purification beads are added to remove short library fragments
5. **Quantify and pool libraries**
   Libraries are pooled and quantified to ensure optimal cluster density
6. **Dilute to starting concentration**
   Libraries are diluted and ready for denaturation
7. **Sequencing setup**
   Libraries are ready for sequencing

Figure 2: Modifying the Illumina DNA PCR-Free Prep protocol—An additional round of bead purification (step 4b) added to the protocol optimizes insert sizes and variant calling performance on the NovaSeq X Series.

Results

Increased insert sizes with additional bead purification

Modifying the Illumina DNA PCR-Free Prep protocol to include an additional round of bead purification results in increased insert sizes, as compared to the unmodified protocol (Figure 3).

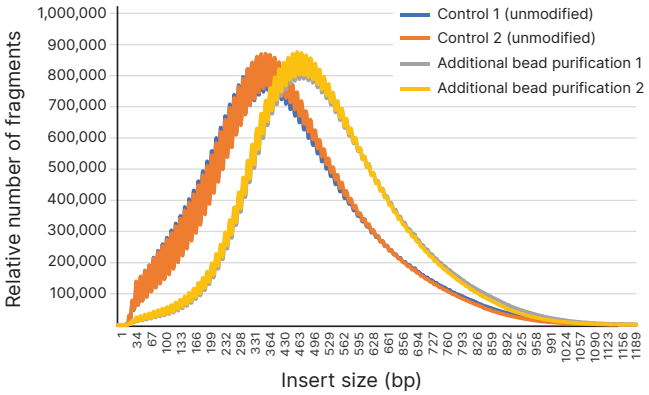

Figure 3: Increased insert size with additional bead purification—An additional round of bead purification results in increased insert sizes (gray and yellow lines), as compared to the unmodified protocol (blue and orange lines).

Increased insert sizes improve variant calling

Sequencing of Illumina DNA PCR-Free Prep libraries prepared with the modified protocol on the NovaSeq X System results in improved variant calling, as compared to the unmodified protocol (Figure 4). The additional bead purification slightly reduces library yield (data not shown), but due to low input DNA requirements on NovaSeq X System, sufficient library is still generated for multiple runs.

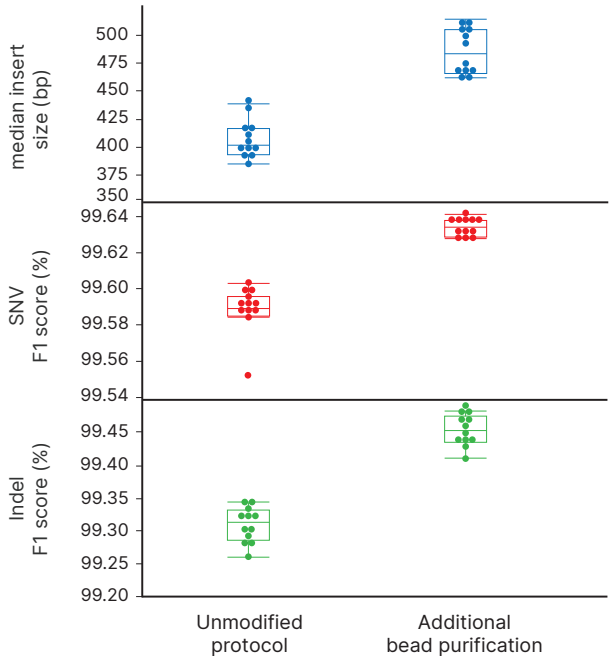

Figure 4: Improved variant calling with increased insert sizes—Sequencing of libraries generated with the modified protocol (with increased insert sizes) on the NovaSeq X System results in improved variant calling for SNVs and indels using Genome in a Bottle (GIAB) version v4.2.1.

Summary

Illumina DNA PCR-Free Prep combined with the unprecedented power of the NovaSeq X Series delivers an integrated, scalable, and rapid WGS workflow. By modifying the Illumina DNA PCR-Free Prep protocol to include an additional bead purification step, library insert sizes are increased and subsequent variant calling with the NovaSeq X Series is optimized.

Learn More

Illumina DNA PCR-Free Prep, Tagmentation

NovaSeq X and NovaSeq X Plus Sequencing Systems

Data sets available on BaseSpace Sequence Hub

References

1. Illumina. High-performance whole-genome sequencing with Illumina DNA PCR-Free Prep, Tagmentation. illumina.com/content/dam/illumina-marketing/documents/products/appnotes/illumina-dna-pcr-free-wgs-app-note-770-2020-006.pdf. Published 2020. Accessed December 1, 2023.
2. Bruinsma S, Burgess J, Schlingman D, et al. Bead-linked transposomes enable a normalization-free workflow for NGS library preparation. *BMC Genomics*. 2018;19(1):722. Published 2018 Oct 1. doi:10.1186/s12864-018-5096-9.

Appendix

Additional bead purification protocol

Follow the standard input Illumina DNA PCR-Free Prep, Tagmentation protocol (Document no. 1000000086922 v03, Feb 2021) up until the 'Clean-Up Libraries' step. Within the Clean-Up Libraries section, deviate from the protocol at elution and add 52 µl RSB to the beads instead of the noted 22 µl. Continue with the Clean-Up Libraries protocol. Once complete, transfer 50 µl of the supernatant into a new plate (LP3) and proceed with the additional purification protocol below.

1. Vortex Illumina Purification Beads (IPB).
2. Add 52.5 µl IPB to each well containing a sample.
3. Pipette 10 times to mix.
4. Incubate at room temperature for 2 minutes.
5. Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
6. Without disturbing the bead pellet, remove and discard all supernatant from each well.
7. Wash beads as follows:
   a. Keep on the magnetic stand and add 180 µl fresh 80% ethanol to each well.
   b. Wait 30 seconds.
   c. Remove and discard all supernatant from each well.
8. Wash beads a second time.
9. Remove residual ethanol from each well.
10. Air-dry on the magnetic stand (~2 minutes).
11. Remove from the magnetic stand.
12. Add 22 µl resuspension buffer (RSB) onto the beads in each well.
13. Pipette to mix until beads are fully resuspended and not on the side of the well.
14. Incubate at room temperature for 2 minutes.
15. Centrifuge 280 × g for 10 seconds.
16. Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
17. Label a new PCR plate "FLP".
18. Transfer 20 µl supernatant from each well of "LP3" to the corresponding well of "FLP".
19. Proceed with the "Quantify and Pool Libraries" portion of the protocol in the reference guide.

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