LeXPrep EZ DNA Library Preparation Kit v3

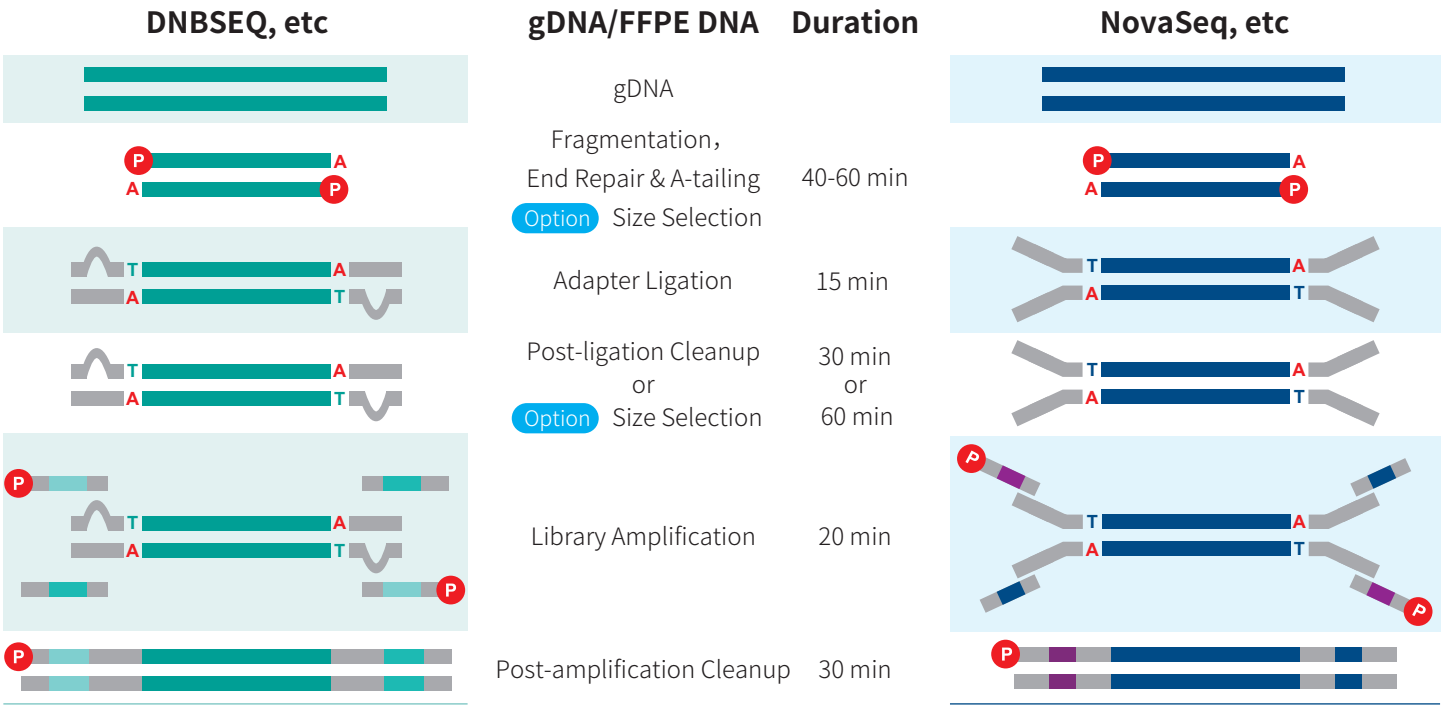
Background

DNA fragmentation is a critical step in NGS library preparation, primarily involving ultrasonic fragmentation and enzymatic digestion. Ultrasonic fragmentation relies on specialized equipment and requires repeated parameters optimization based on sample characteristics, involves complex procedures, and can lead to loss of ultra-low input samples, limiting its applicability. In contrast, enzymatic digestion uses enzymes to randomly shear DNA into fragments, offering clear advantages: 1) compatibility with automated workstations and simplified operation; 2) minimal DNA, particularly suited for ultra-low input samples; 3) rapid library preparation workflow, meeting the high time-sensitivity demands of applications such as pathogen detection. As the applications of enzymatic digestion continue to expand, the market is placing increasingly stringent requirements on the uniformity of fragment sizes, workflow efficiency, low-input compatibility, and the low background noise of low-quality samples, driving ongoing innovation and enhancement in this technology.

Introduction

LeXPrep EZ DNA Library Preparation Kit v3 is designed for preparation of high-quality sequencing libraries from double-stranded DNA (dsDNA) on mainstream sequencing platforms. This kit optimizes the enzymatic fragmentation system to increase library yield while maintaining low background noise and significantly enhancing library complexity. This kit is suitable for multiple types of samples, including gDNA and FFPE DNA, and has been optimized for ultra-low-quality FFPE samples to ensure stable library yield. To simplify the experimental process, multiple processes were applied in one single step, including the fragmentation, end repair and A-tailing. This A-T ligation-based kit applies to the whole genome sequencing with DNA input ranging from 5 to 500 ng, and is compatible with hybridization capture based targeted sequencing.

Workflow



Performance

Stable Increase in Library Yield

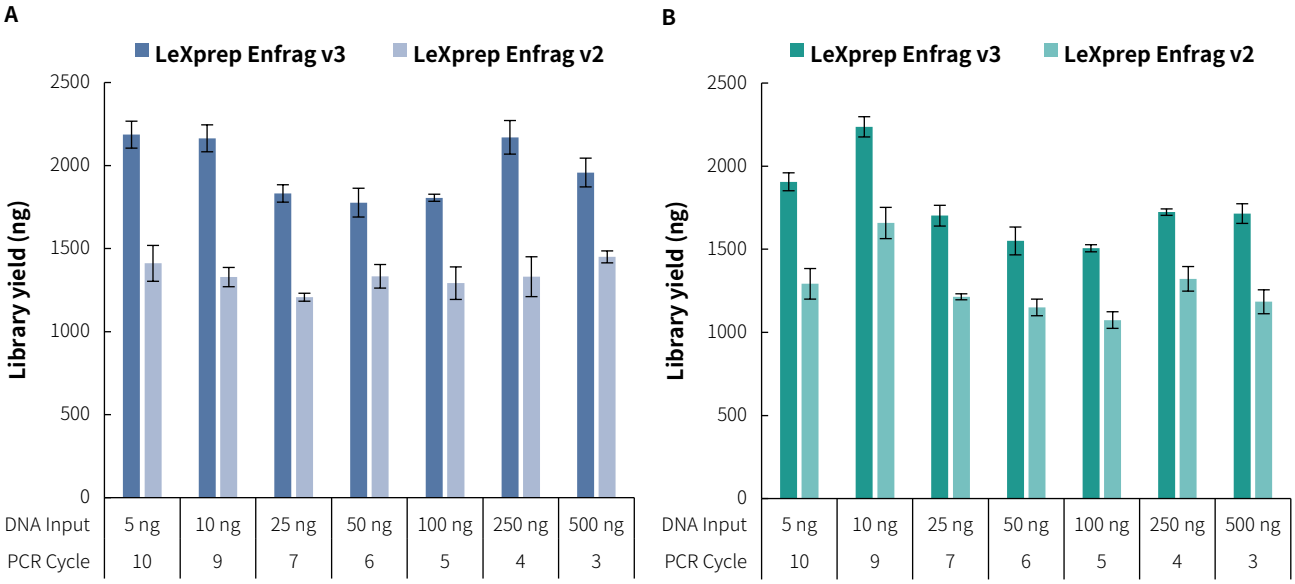


Figure 1. Library yield of LeXprep EZ DNA Library Preparation Kit v3 and v2 for gDNA samples with different input amounts. The libraries were prepared by using LeXprep EZ DNA Library Preparation Module v3 (LeXprep Enfrag v3) and v2 (LeXprep Enfrag v2) coupled with **A.** LeXprep Universal Stubby Adapter (UDI) Module and **B.** LeXprep Universal Adapter (MDI) Module (for MGI) respectively, with 20 min of enzymatic digestion and recommended PCR cycles.

Note: The samples are human genome DNA standard (Promega, G1471).

Compatible with Different Types of Samples

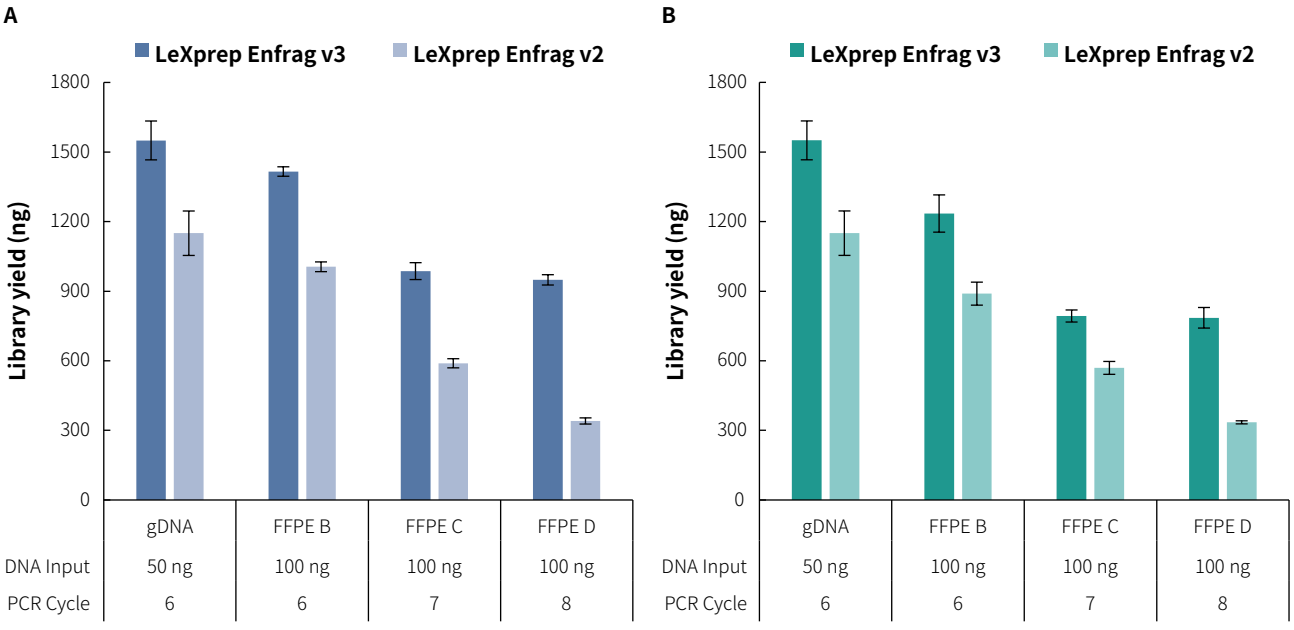


Figure 2. Library yield of LeXprep EZ DNA Library Preparation Kit v3 and v2 for different types of samples.

Note: The grading standards by electrophoresis are as follows: gDNA: one bright and non-trailing stripe with about 15 kb in size. FFPE B: one indistinct stripe with about 15 kb in size, with medium diffusion. FFPE C: multiple stripes ranging from 200 bp to 2,500 bp, with severe diffusion. FFPE D: multiple stripes ranging from 250 bp to 1,000 bp, with severe diffusion.

Low Background Noise Introduced

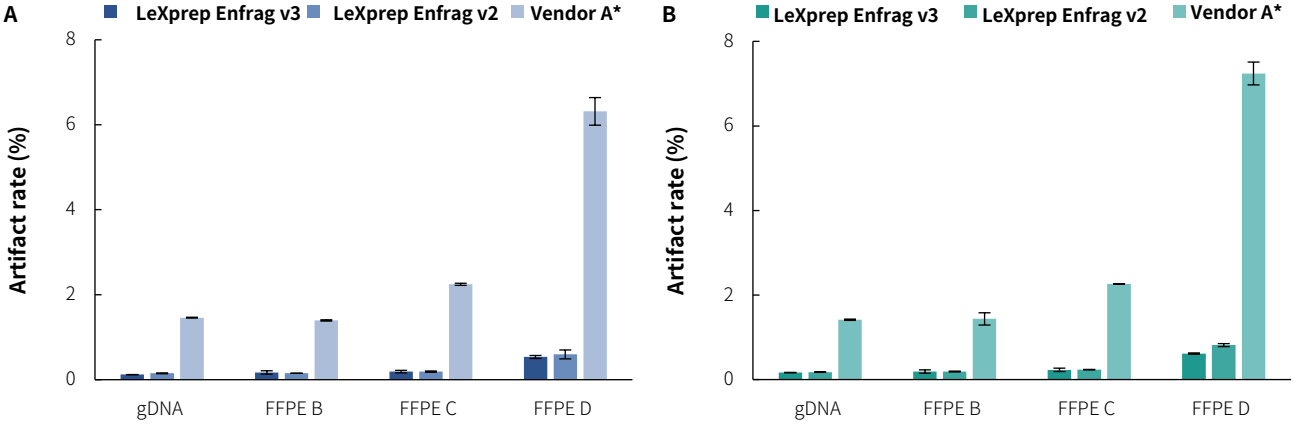


Figure 3. The abnormal reads ratio of libraries prepared with different types of samples by using different library preparation kits. The libraries were prepared with gDNA and different grades of FFPE DNA samples (50 ng each) with different library preparation kits including LeXprep Enfrag v3, LeXprep Enfrag v2 and Vendor A*. The multi-hybridization reaction was initiated with 500 ng of each library with the same library preparation scheme. The capture was performed by using LeXOnco Plus Panel v3.0 and LeXprep Hybrid Capture Reagents, with calculation of total reads. Sequencing was performed on **(A)** NovaSeq 6000, PE150 and **(B)** DNBSEQ-T7, PE150.

Note: Artifact rate: the ratio of abnormal sequence introduced by enzymatic digestion by-products. The initial input amounts of gDNA and FFPE DNA were 50 ng and 100 ng respectively.

Higher Library Complexity

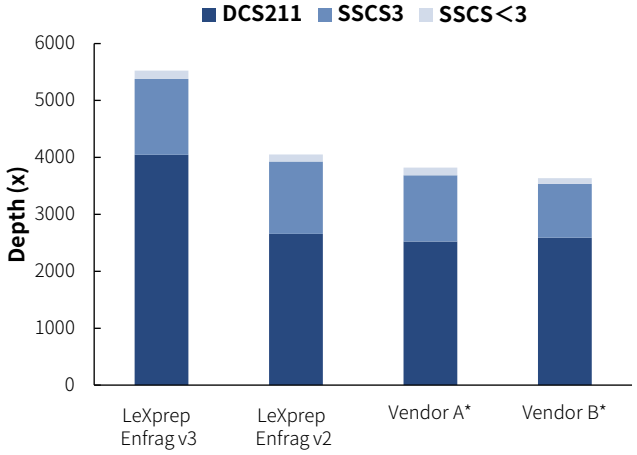


Figure 4. Library complexity of LeXprep EZ DNA Library Preparation Kit v3 and v2. Libraries were prepared by LeXprep Enfrag v3, LeXprep Enfrag v2, VendorA* and Vendor B*. The multi-hybridization reaction was initiated with 500 ng of each library with the same library preparation scheme. The capture was performed by using LeXso Full Screen LF Custom Panel (custom probes designed for 15 mutations in GW-OGTM800 reference standard) and LeXso Hybrid Capture Reagents v2. For each sample, 2 M read pairs were randomly selected for data analysis, with analytical filtering criteria set to Duplex Consensus Sequences (DCS211) and Single-Stranded Consensus Sequences (SSCS).

Note: The sample was Pancancer Light 800 gDNA Reference Standard (GeneWell, GW-OGTM800) with an initial input of 50 ng.

Assessment of Variants in Wild-type SNV gDNA Samples

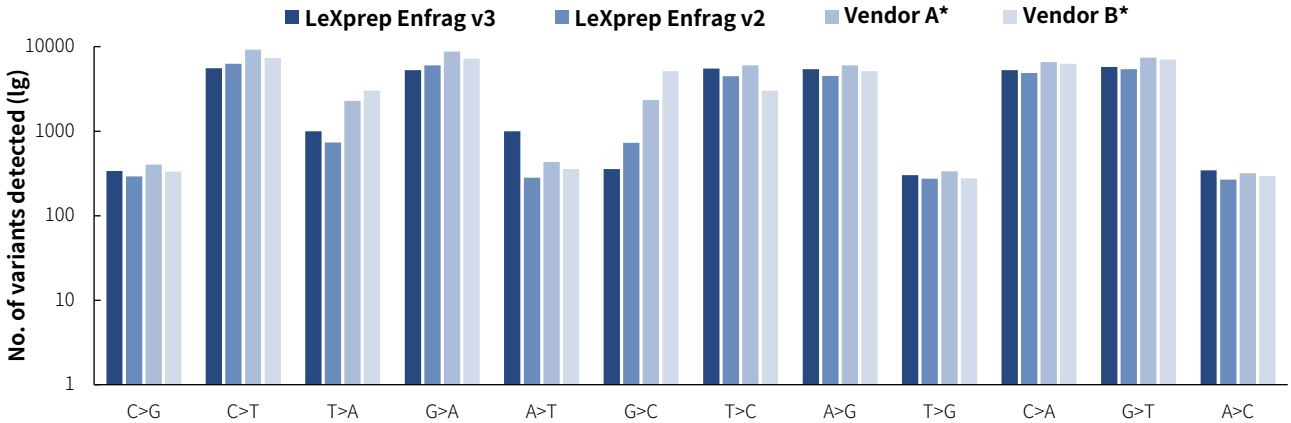


Figure 5. Variant statistics from the capture data of Onco Wildtype gDNA Reference Standard using different library preparation kits. The capture was performed using LeXOnco Plus Panel v3.0 and LeXprep Hybrid Capture Reagents. For each sample, 10 M read pairs were randomly selected and deduplicated for data analysis. Variants were called with a background noise frequency ranging from 0 to 0.005 (at least 3 reads per site).

Note: The samples are Onco Wildtype gDNA Reference Standard (GeneWell, GW-OGTM005), with an initial input of 50 ng.

Excellent Capture Performance

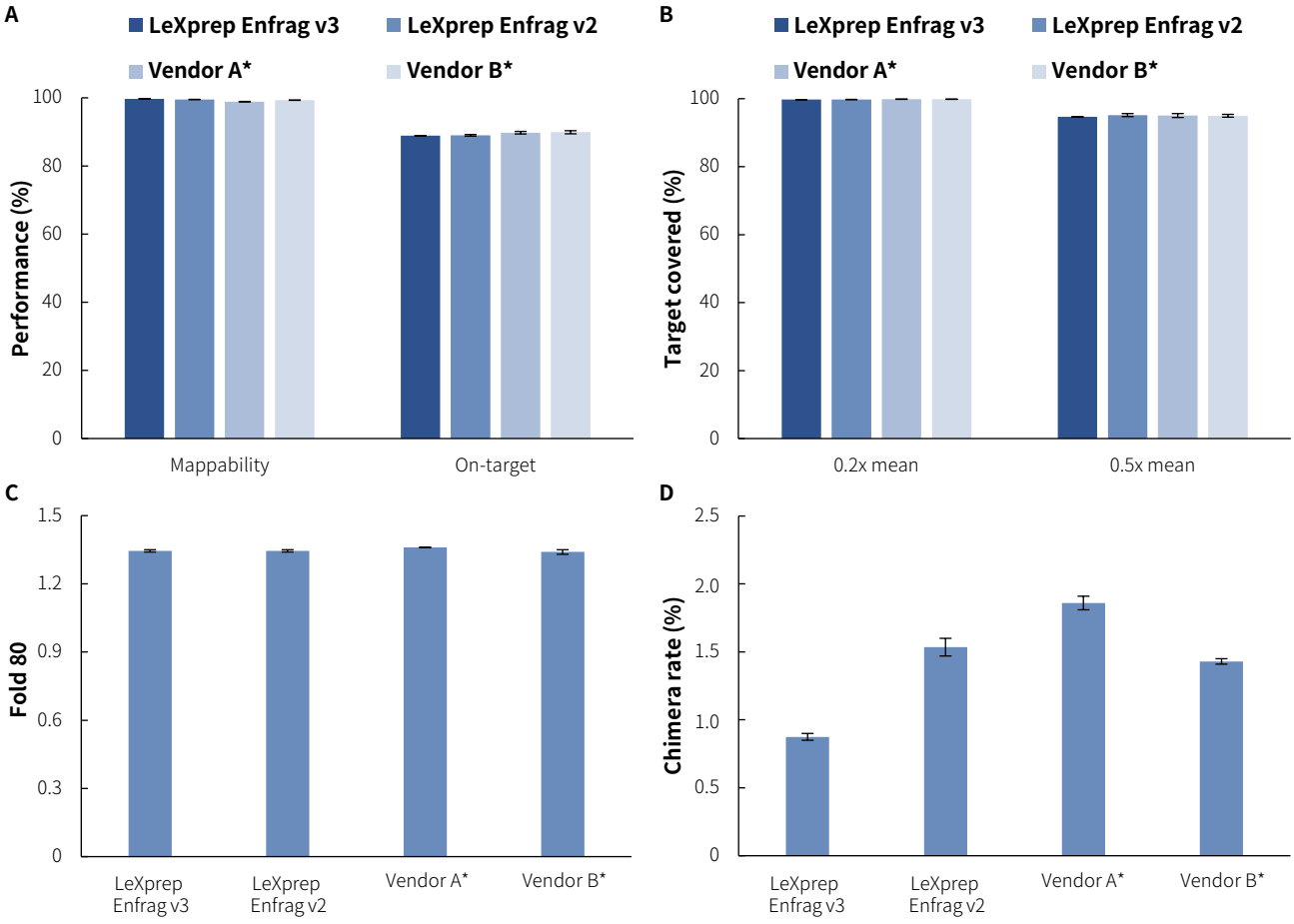


Figure 6. The capture performance of pre-libraries prepared by LeXprep EZ DNA Library Preparation Kit v3. For each sample, 2 M read pairs were randomly selected for data analysis, with calculation of total reads. **A.** Mappability and On-target rate; **B.** Target covered; **C.** Fold 80 base penalty; **D.** Chimera rate (chimeric reads/total reads).

Ordering Information

Product	Scale	Catalog#
LeXprep EZ DNA Library Preparation Module v3, 24 rxn	24 rxn	LX02611
LeXprep EZ DNA Library Preparation Module v3, 96 rxn	96 rxn	LX02612

Statement

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