xGEN PRISM DNA LIBRARY PREP KIT

High library complexity from low quality samples

- Get higher conversion rates compared to TA-ligation-based methods
- Detect variants at ≤1% variant allele frequency (VAF)
- Get data from even the most degraded samples
- Eliminate sequence errors with UMIs

Prepare high-quality next generation sequencing libraries from degraded samples with the xGen Prism DNA Library Prep Kit (Figure 1). The kit enables sensitive and accurate variant detection in samples such as cell-free DNA (cfDNA) or DNA derived from formalin-fixed, paraffin-embedded (FFPE) tissue (Figures 2, 3, 4). Maximize conversion and suppress adapter dimer formation with the kit's proprietary ligation strategy. The unique molecular identifier (UMI) sequences incorporated during single-stranded ligation enable a variety of deduplication and error correction strategies. The kit generates libraries in 4 steps:

- **End repair.** cfDNA or sheared, input DNA is prepared for ligation by conversion into blunt-ended DNA with the End Repair Enzyme Mix.
- **Ligation 1.** The Ligation 1 Enzyme catalyzes the single stranded addition of the Ligation 1 Adapter to only the 3′ end of the insert. This novel enzyme is unable to ligate inserts together, which minimizes the formation of chimeras. The 3′ end of the Ligation 1 Adapter also contains a blocking group to prevent adapter dimer formation.
- **Ligation 2.** The Ligation 2 Adapter acts as a primer to gap-fill the bases complementary to the UMI, followed by ligation to the 5′ end of the DNA insert to create a double-stranded product.
- **PCR amplification.** Sample index sequences needed for Illumina instruments are incorporated via PCR.

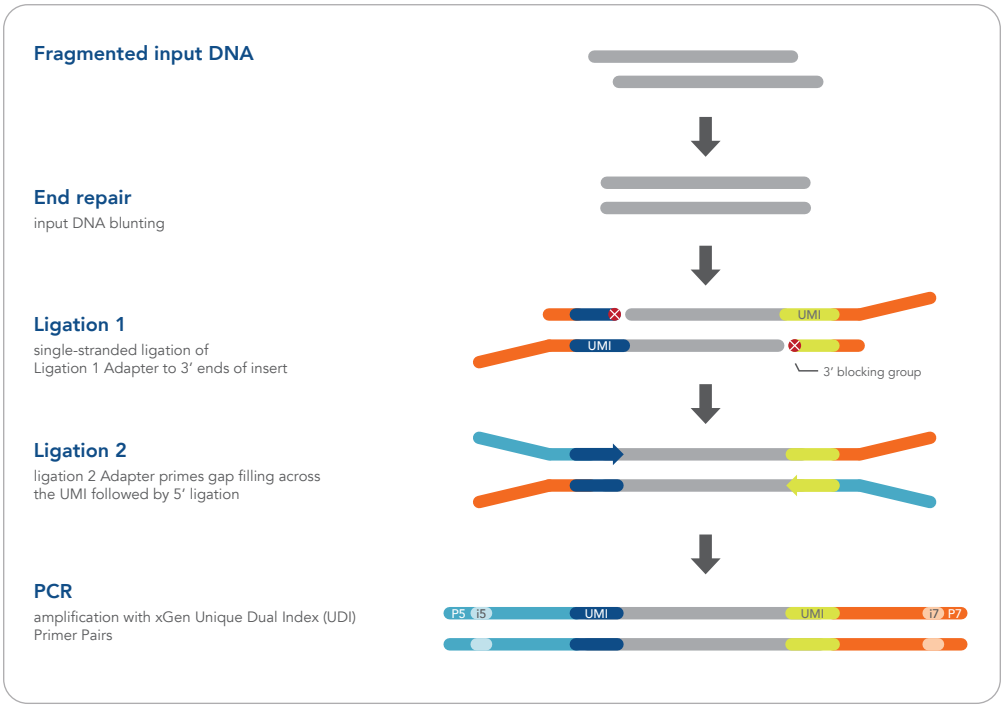

Figure 1. Overview of the xGen Prism DNA Library Prep Kit process.

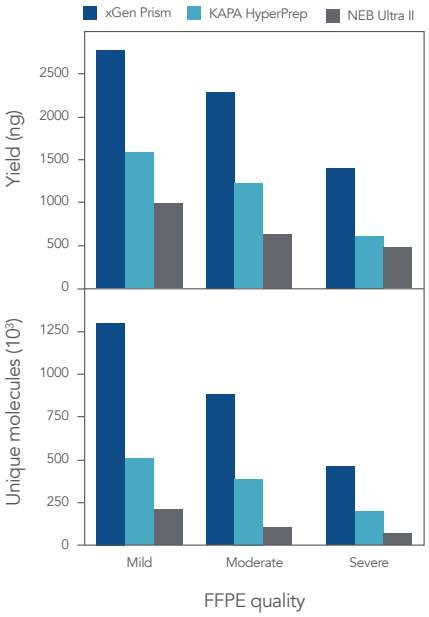

Figure 2. The xGen Prism DNA Library Prep Kit delivers higher library yield and complexity from FFPE samples across a range of sample qualities. Libraries from 25 ng of FFPE reference standards were captured with a custom 61 kb xGen Lockdown Panel and sequenced on an Illumina NextSeq® 500 instrument. Reads were mapped using BWA (0.7.15), and the number of unique molecules (HS library size) were calculated using Picard (2.18.9).

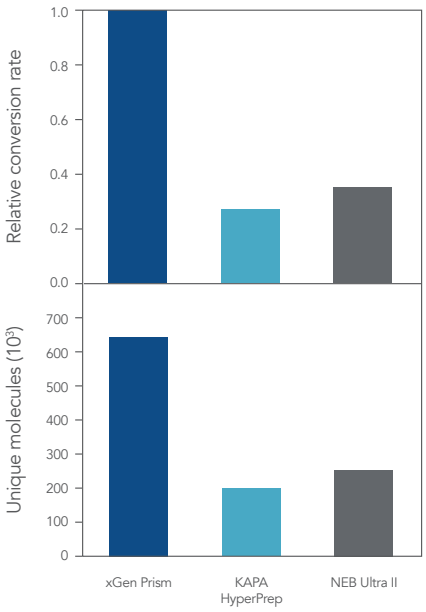

Figure 3. The xGen Prism DNA Library Prep Kit delivers higher conversion rates and complexity. Libraries were generated according to the manufacturer's instructions with 10 ng of cfDNA reference standards, then captured with a custom 61 kb xGen Lockdown Panel. Libraries were pooled and sequenced on an Illumina NextSeq 500 instrument. Reads were mapped using BWA (0.7.15). Complexity (estimated unique molecules; HS library size) was calculated using Picard (2.18.9). Relative conversion rates were calculated from mean target coverage at very high duplication rates.

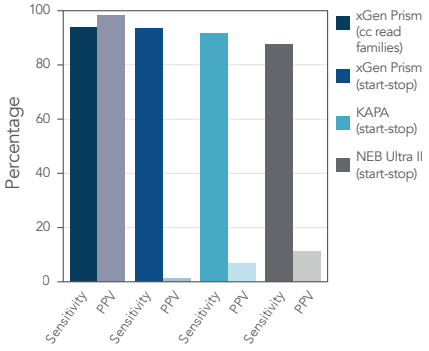

Figure 4. The xGen Prism DNA Library Prep Kit enables higher sensitivity and specificity for ultra-low variant detection. Libraries were generated according to the manufacturer's instructions using 25 ng of a cfDNA reference standard with an allele frequency of 0.25%. Libraries were captured with a custom 61 kb xGen Lockdown Probe Panel. After sequencing, reads were mapped using BWA (0.7.15). Start-stop deduplication or cc (collapsed combined) read families error correction was performed as described in the xGen Prism Analysis Guide. Finally, variants were called using VarDict (1.5.8); only variants with PASS/f0.02 filters were used for start-stop deduplicated data, while no filters were applied for cc read families error corrected xGen Prism data. PPV = positive predictive value.

ORDERING INFORMATION

Product	Size	Catalog #
xGen Prism DNA Library Prep Kit	16 rxn	10006202
	96 rxn	10006203
IDT sample indexing primer pairs	**Size**	**Catalog #**
xGen UDI Primer Pairs	Index 1-16	10005975
	Index 1-96	10005922

> FOR MORE INFORMATION, VISIT **WWW.IDTDNA.COM/PRISM**.

For Research Use Only. Not for use in diagnostic procedures.

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