xGen™ cfDNA & FFPE DNA Library Prep v2 MC Kit

High library complexity from low quality samples

Get higher conversion rates compared to TA-ligation-based methods

Identify variants at less than or equal to 1% variant allele frequency (VAF)

Get data from even the most degraded samples

Eliminate sequence errors with UMIs

Prepare next generation sequencing (NGS) libraries from degraded samples with the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit (Figure 1). The kit enables reliable variant identification in samples such as cell-free DNA (cfDNA) or DNA derived from formalin-fixed, paraffin-embedded (FFPE) tissue. Increase 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:

1. **End repair.** cfDNA or sheared, input DNA is prepared for ligation by conversion into blunt-ended DNA with the End Repair Enzyme Mix.
2. **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 and therefore decreases the formation of chimeras. The 3′ end of the Ligation 1 Adapter also contains a blocking group to prevent adapter dimer formation.
3. **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.
4. **PCR amplification.** xGen 2x HiFi PCR Mix is included to perform indexing PCR (primers sold separately), for Illumina® sequencing.

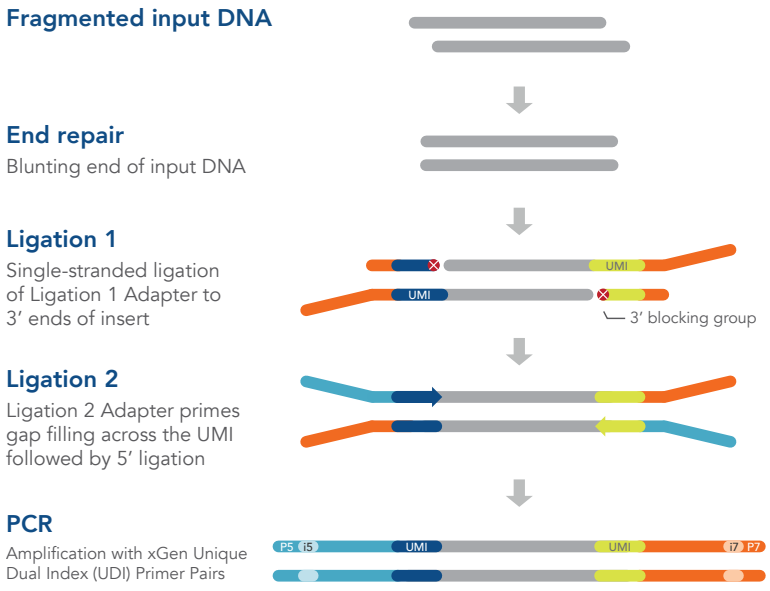

Figure 1. Overview of the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit Workflow.

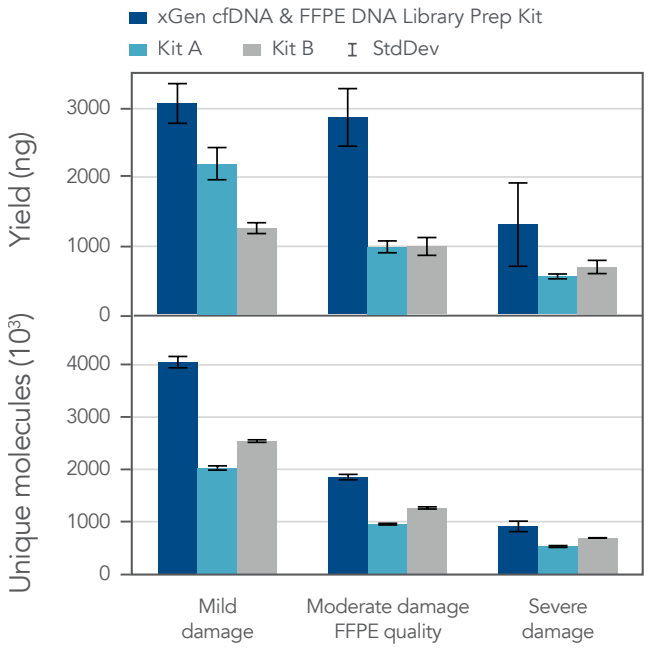

Figure 2. Library yield and complexity from varying qualities of formalin compromised DNA (fcDNA) reference standards. Libraries (N=4 each) were prepared in triplicate by three commercial library prep kits following each of the manufacturer's protocols with 25 ng total input. Following 10 cycles of PCR, libraries were quantified using a Qubit™ dsDNA HS Kit (Thermo Fisher). 500 ng of each library was than captured using a custom 180 kb (target space) xGen Hyb Panel with the xGen Hybridization and Wash v2 Reagents and xGen Hybridization and Wash v2 Beads, following the xGen Hybridization and Wash Kit protocol with an overnight hybridization and 13 cycles of PCR. The captured libraries were then pooled to be equimolar and sequenced on an NextSeq™ 500 (Illumina), using a high-output 300 cycle kit, following the manufacturer's protocol. Samples were subsampled to 8 million paired-end reads, and the number of unique molecules (HS library size) was determined with Picard.

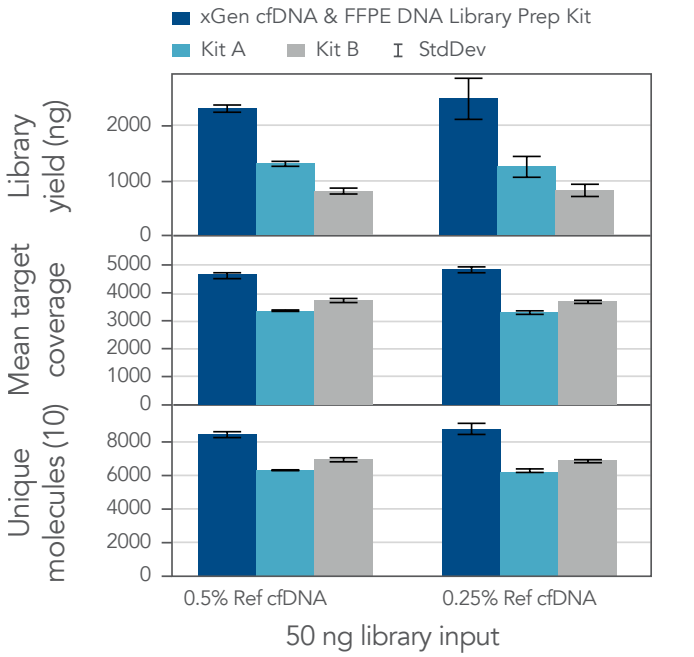

Figure 3. The xGen cfDNA & FFPE DNA Library Prep v2 Kit delivers higher yields, complexity, and coverage. Libraries (N=3) were prepared in triplicate according to the manufacturer's instructions with 50 ng of Horizon cfDNA reference standards and 7 cycles of PCR. Following quantification, libraries were captured with a custom 61 kb (target space) xGen Hyb Panel using the xGen Hybridization and Wash kit. Captured libraries were pooled and sequenced on an NextSeq™ 500 (Illumina) instrument using a high output 300 cycle kit, following the manufacturer's protocol. After subsampling to 85M total reads, coverage and complexity were calculated.

Table 1. The xGen cfDNA & FFPE DNA Library Prep v2 MC Kit identifies ultra-low frequency variants in NGS reference samples

Mutation	Expected VAF	cfDNA & FFPE DNA Prep v2 MC Kit	Library Kit A	Library Kit B
EGFR:L858R	0.25	0.13 (3/3)	0.21 (3/3)	0.21 (3/3)
EGFR:E746–A750	0.25	0.11 (3/3)	0.19 (3/3)	0.12 (3/3)
EGFR:T790M	0.25	0.29 (3/3)	0.36 (3/3)	0.12 (3/3)
KRAS:G12D	0.32	0.33 (3/3)	0.36 (3/3)	0.33 (3/3)
NRAS:Q61K	0.32	0.23 (3/3)	0.31 (2/3)	0.22 (3/3)
NRAS:A59T	0.32	0.17 (3/3)	0.43 (2/3)	0.22 (3/3)
PIK3CA:E545K	0.32	0.16 (3/3)	0.11 (3/3)	0.36 (3/3)

Table 1. Libraries (N=3) were prepared in triplicate from 50 ng input Horizon cfDNA reference standards using the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit in addition to two other commercially available library prep kits. Libraries were then captured with a custom 180 kb (target space) xGen Hyb Panel targeting seven confirmed SNPs using the using the xGen Hybridization and Wash v2 Reagents and xGen Hybridization and Wash v2 Beads. Captured libraries were pooled and sequenced on an NextSeq™ 500 (Illumina) instrument, using a high-output 300 cycle kit. After subsampling to 85M total reads, the average variant allele frequency for each of the targeted mutations was calculated for each library prep kit using VarDict.

ORDERING INFORMATION

Product	Size	Catalog#
xGen cfDNA & FFPE DNA Library Prep v2 MC Kit	16 rxn	10010206
	96 rxn	10010207
IDT sample indexing products	**Size**	**Catalog#**
xGen™ UDI Primers 16 rxn	Index 1–16	10005975
xGen™ UDI Primer Plate 1, 8 nt	Index 1–96	10005922
Custom Indexing Options	Various	applicationsupport@idtdna.com

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