xGen™ Methyl-Seq DNA Library Preparation Kit

Comprehensive methylome coverage from low-input research samples

- Comprehensive sample representation
- High multiplex capability
- Low bias library preparation from low input amounts

xGen Methyl-Seq Library Prep Kit utilizes Adaptase™ technology that efficiently captures bisulfite-converted ssDNA molecules into library molecules for epigenetic research studies. The resultant libraries represent uniform and comprehensive genome coverage.

Applications:

- Whole genome bisulfite sequencing (WGBS)
- Targeted sequencing with hybridization capture enrichment and reduced-representative bisulfite sequencing (RRBS)
- Genome-wide methylation identification using cfDNA
- Bisulfite-converted DNA enriched by methylated DNA immunoprecipitation (MeDIP), chromatin immunoprecipitation (ChIP), etc.

Sample types:

- Genomic DNA
- Formalin-fixed, paraffin-embedded (FFPE)
- Circulating, cell-free DNA (cfDNA)
- Fresh and frozen tissue

2-hour workflow for bisulfite-converted samples

The xGen Methyl-Seq DNA Library Prep Kit workflow maximizes DNA recovery through a post-bisulfite library preparation, utilizing an efficient adapter attachment that is compatible with single-stranded, bisulfite-converted DNA (**Figure 1**). Library complexity from this kit is significantly greater than those from methods that bisulfite convert after library construction. Additionally, the template-independent adapter attachment chemistry of the xGen Methyl-Seq DNA Library Prep Kit provides a more complete, less biased library as observed from comprehensive methylome coverage by WGBS.

Adaptase technology
- 17 minutes

Extension
- 8 minutes

Ligation
- 15 minutes

Indexing PCR
- Time varies

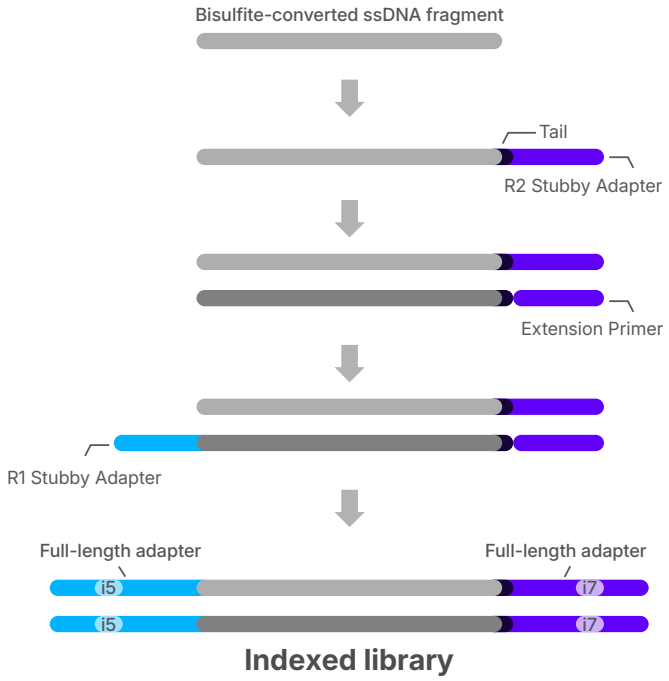
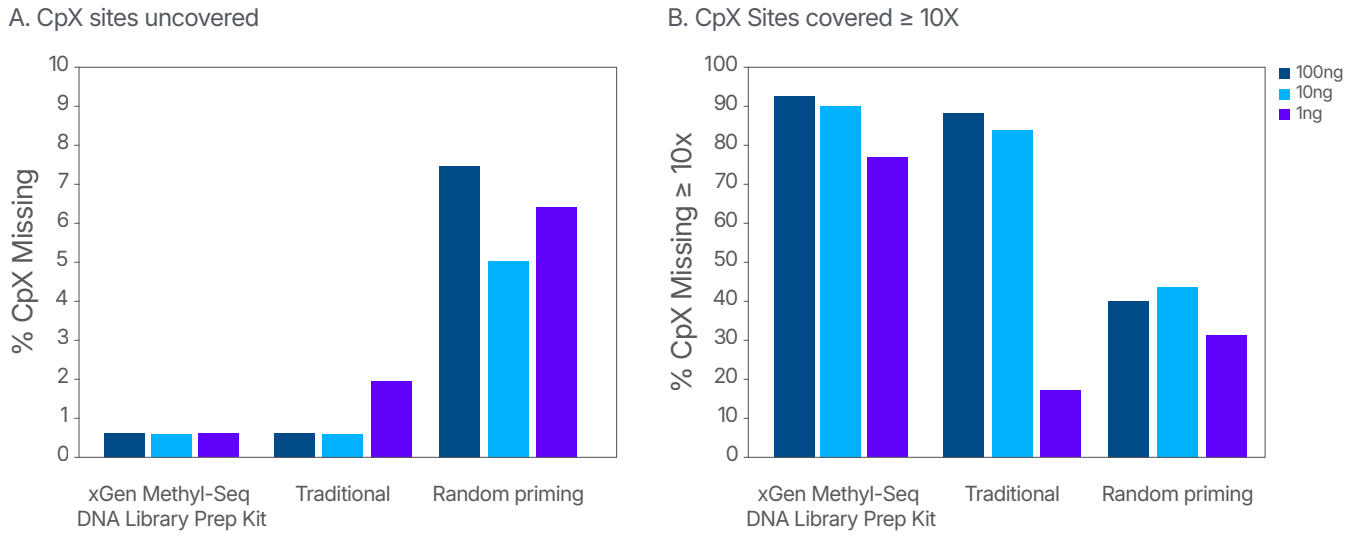


Figure 1. The xGen Methyl-Seq DNA Library Prep Kit workflow constructs libraries from single-stranded, bisulfite-converted DNA fragments. The Adaptase step simultaneously performs end repair, tailing, and ligation of the R2 Stubby Adapter to the 3′ end of each fragment. The extension step produces a uracil-free strand and the ligation step adds R1 Stubby Adapter to the uracil-free strand. Indexing PCR increases library yield and incorporates full-length adapters with sample-specific index sequences.

Comprehensive coverage from 1 ng of input DNA



(**A**) At all three sample inputs (100 ng, 10 ng, and 1 ng) (*n* = 2), the xGen Methyl-Seq Library Prep Kit and the traditional bisulfite library prep method leave a minimal amount of CpX (CpG + CpH) sites uncovered. However, the random priming method exhibits at least 4% of CpX sites missing at every input amount tested. (**B**) The percentage of CpX sites covered at least 10X is greater than 90% for the xGen Methyl-Seq DNA Library Prep Kit at 100 ng but decreases to approximately 75% at 1 ng. The traditional bisulfite library prep method covers greater than 80% of CpX sites at least 10X for 100 ng and 10 ng inputs, but this coverage diminishes greatly at 1 ng. The random priming method exhibits poor coverage of CpX sites at each input, with the percentage of CpX sites covered at least 10X at about 40% for all inputs.

For more information, visit **www.idtdna.com/methyl-seq**