Flexible, high performance methylation workflow for challenging samples

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Introduction

Epigenetic changes in global and focal methylation of CpG islands are widespread and have been shown to be important biomarkers of a variety of disease states, including cancer. In clinically relevant samples, which are often limited in quantity, purity, and quality; targeted epigenetic sequencing approaches coupled with highly efficient library preparation can provide a higher-resolution and cheaper alternative to whole genome methylation sequencing. Here, we present two future proof methylation workflows: (1) xGen™ Methyl-Seq DNA library prep leverages single stranded library preparation for highly efficient and template independent adapter attachment. This method overcomes both disadvantages of dsDNA methylation library prep: it reduces 3' methylation artefacts and maintains high library complexity from low input samples, regardless of conversion module. (2) xGen cfDNA & FFPE DNA methylation workflow is an enzymatic conversion compatible, double stranded library preparation strategy that incorporates UMIs for stand specific error correction. Both workflows are compatible with WGS and targeted enrichment on Illumina sequencing platforms.

We present whole genome methylation sequencing results that demonstrate greater mapping efficiency, CpG coverage and library complexity. To further reduce the cost of sequencing, a targeted custom methylation design strategy was employed to capture a broad range of methylation states with high efficiency.

Methods

Libraries were generated using the xGen Methyl-Seq DNA library prep kit (IDT), a methylation compatible version of the xGen cfDNA & FFPE DNA library prep kit (IDT), or NEBNext Enzymatic Methyl-Seq (EM-Seq), and target enriched using the xGen Hybridization Capture Core reagents (IDT). Conversion was completed using the Zymo EZ DNA Methylation Gold bisulfite conversion module or NEBNext® Methyl-Seq Conversion Module (enzymatic). Targeted Methyl-Seq was performed using the same workflow cited for WGS followed with enrichment using the xGen hybridization capture workflow (IDT). Sequencing was performed on an Illumina instrument, and alignment and methylation analyses were performed using Bismark (v0.22.3) and Picard (v2.18.9).

Dual approaches of library preparation for methylation sequencing

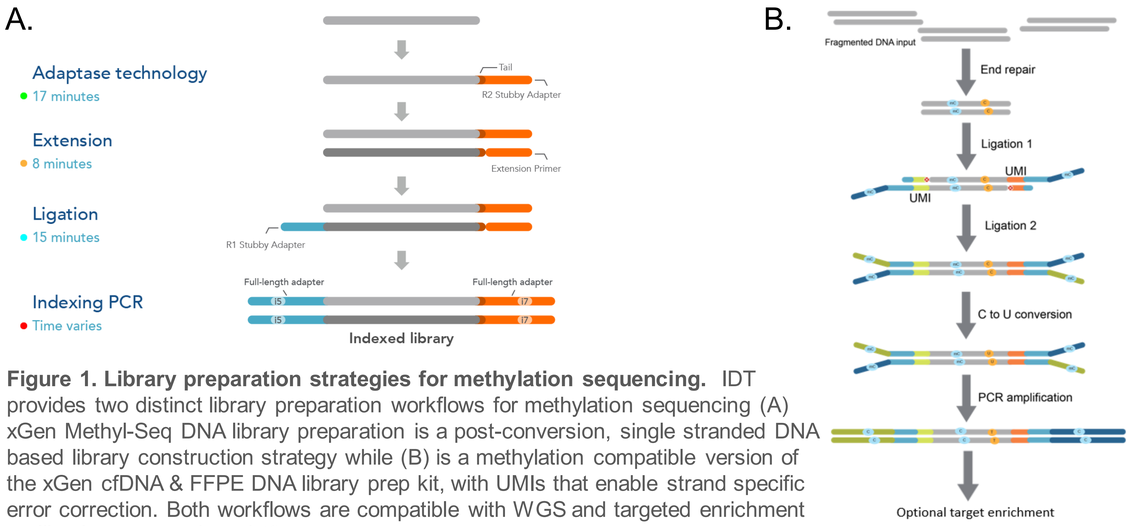

Figure 1. Library preparation strategies for methylation sequencing. IDT provides two distinct library preparation workflows for methylation sequencing (A) xGen Methyl-Seq DNA library preparation is a post-conversion, single stranded DNA based library construction strategy while (B) is a methylation compatible version of the xGen cfDNA & FFPE DNA library prep kit, with UMIs that enable strand specific error correction. Both workflows are compatible with WGS and targeted enrichment on Illumina sequencing platforms.

xGen Methyl-Seq DNA lib prep provides high coverage at low inputs

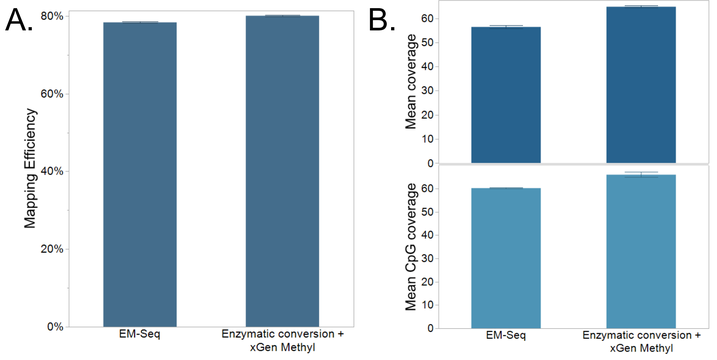

Figure 2. High coverage WGS using a single stranded DNA library preparation strategy. xGen Methyl-Seq DNA library preparation with 25 ng A. thaliana gDNA (n=3) enables (A) comparable mapping efficiency and (B) higher coverage compared to NEB EM-seq using the same enzymatic conversion module. (C) Comparison of NGS metrics obtained using xGen Methyl-Seq DNA libraries (1-100 ng) to traditional or random-primed methylation workflows. All workflows used bisulfite conversion.

C.

Input	Sample	% Reads aligned	Genome coverage	% Duplicate reads	Est. library size (Millions)	% CpX missing	% CpX covered ≥ 10X
100 ng *Arabidopsis*	xGen Methyl-Seq	89.6	22.0X	1.9	714	0.56	92.2
	Traditional	80.2	21.0X	2.7	604	0.57	88.1
	Random Primer	71.4	16.0X	22.1	48	7.70	39.4
10 ng *Arabidopsis*	xGen Methyl-Seq	87.8	22.0X	2.7	406	0.58	90.4
	Traditional	76.7	19.0X	11.9	70	0.57	83.9
	Random Primer	71.9	16.0X	22.2	45	5.2	45.2
1 ng *Arabidopsis*	xGen Methyl-Seq	83.3	18.0X	18.2	38	0.59	77.1
	Traditional	80.7	10.0X	62.3	6	2.00	17.0
	Random Primer	73.4	12.0X	46.1	12	6.60	31.3

Targeted xGen Methyl-Seq DNA lib prep provides higher mapping efficiency and minimal methylation bias

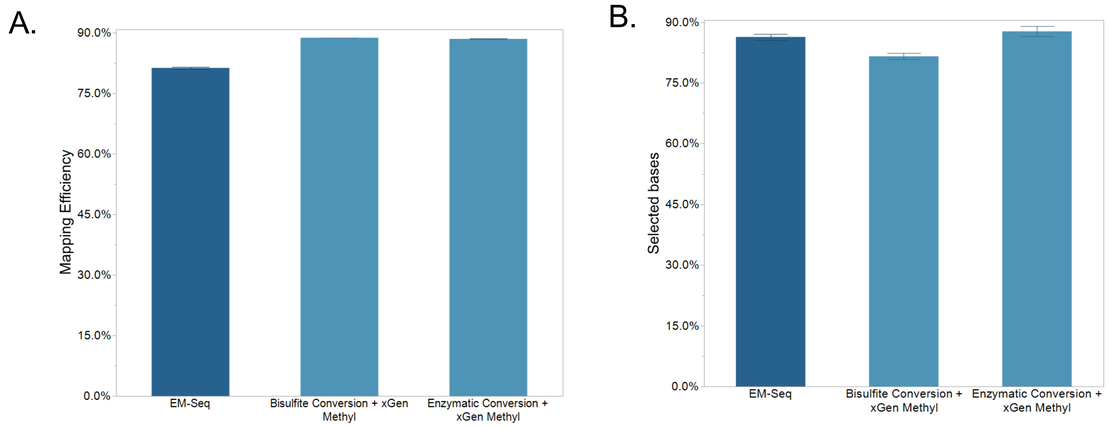

Figure 3. Targeted methyl-seq using a single stranded DNA library preparation strategy. xGen Methyl-Seq DNA library preparation with 25 ng NA12878 (n=3) enables (A) higher mapping efficiency and (B) comparable on-target rates compared to NEB EM-seq using either bisulfite or enzymatic conversion (C) minimal methylation bias detected in read 2 m-bias plots unlike the NEB double stranded library preparations, due to end polishing.

xGen cfDNA & FFPE DNA methylation workflow enables accurate methylation calling and high performance with challenging samples

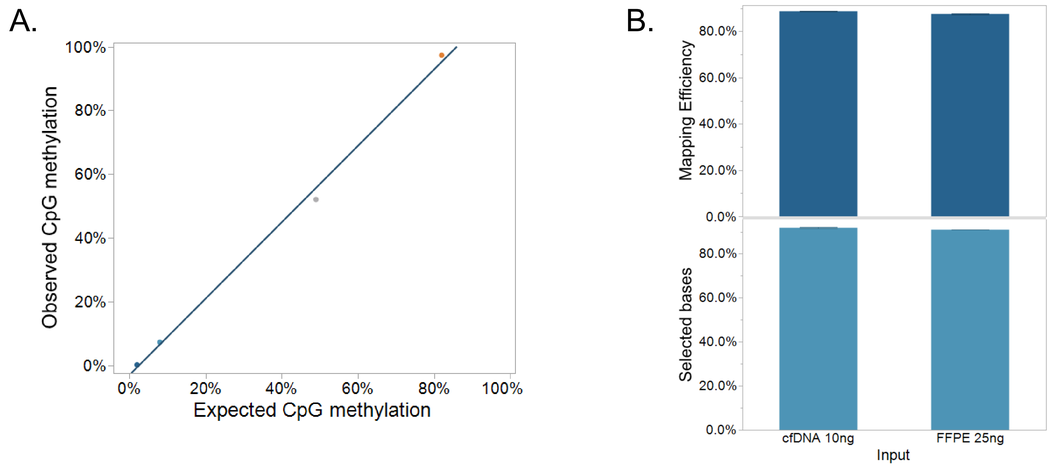

Figure 4. xGen cfDNA & FFPE methylation workflow enables (A) accurate methylation level detection with a high correlation (R^2=0.997) between expected and observed methylation levels using 100 ng EpiGenDx control samples by whole genome methylation sequencing and (B) high performance with cfDNA (Horizon) and FFPE DNA (SeraSeq) in targeted sequencing workflows using a custom design strategy.

Conclusions

- We present two high performance methylation workflows that provide flexibility on conversion strategy (enzymatic or bisulfite) and workflow (WGS or targeted sequencing). The single stranded library preparation reduces 3' methylation artefacts and maintains high library complexity from low input samples, regardless of conversion module. The double stranded library preparation strategy incorporates UMIs for strand specific error correction.
- xGen Methyl-Seq DNA lib prep provides high coverage down to 1 ng using whole genome bisulfite sequencing.
- Targeted xGen Methyl-Seq DNA lib prep provides higher mapping efficiency and less-biased methylation levels when compared to NEB EM-seq.
- The xGen cfDNA & FFPE DNA library prep methylation workflow accurately identifies methylation levels in control samples in whole genome methylation sequencing.
- A combination of the methylation compatible version of the xGen cfDNA & FFPE DNA library prep kit and xGen hybridization enrichment can be used to generate high-quality sequencing data for targeted Methyl-Seq applications with low input cfDNA and FFPE samples.