Fast and Efficient Post-Bisulfite-Seq Library Construction with QIAseq Ultralow Input DNA Library Protocol

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Introduction

Epigenetic changes play a crucial role in the regulation of important cellular processes such as gene expression and cellular differentiation, and are also identified as key factors in many diseases.

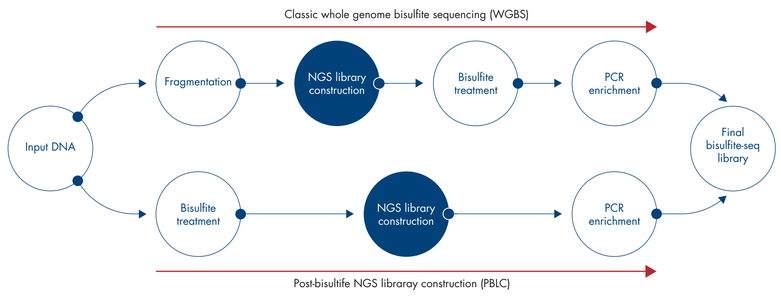
The methylation of cytosines in the genome, an important epigenetic regulatory mechanism, reduces the transcriptional activity of adjacent genes. Whole genome bisulfite sequencing (WGBS) – combining bisulfite-mediated conversion of unmethylated cytosines to uracil – and next-generation sequencing (NGS), allow the genome-wide detection of 5-methyl-cytosine residues at unprecedented single-base resolution. It also enables the connection between gene activity and the precise localization of a DNA methylation mark. However, the commonly used approach to treat samples after NGS library preparation with bisulfite to convert unmethylated cytosines to uracils reveals major challenges, such as significant bisulfite-induced sample loss due to DNA degradation. Therefore, the traditional library prep method for bisulfite sequencing demands very high DNA input amounts or requires a large number of PCR cycles during NGS library construction.

Here, we present a fast and streamlined workflow for bisulfite treatment, double-strand synthesis and NGS library construction, which overcomes these traditional challenges. We demonstate that this post-bisulfite NGS library construction (PBLC) approach creates significantly higher library yields than the classic WGBS approach – without any compromise in data quality. Another advantage is that DNA fragmentation through bisulfite means that no further DNA fragmentation is needed prior to library prep. This reduces the overall workflow time.

Comparison of WGBS and PBLC Workflow

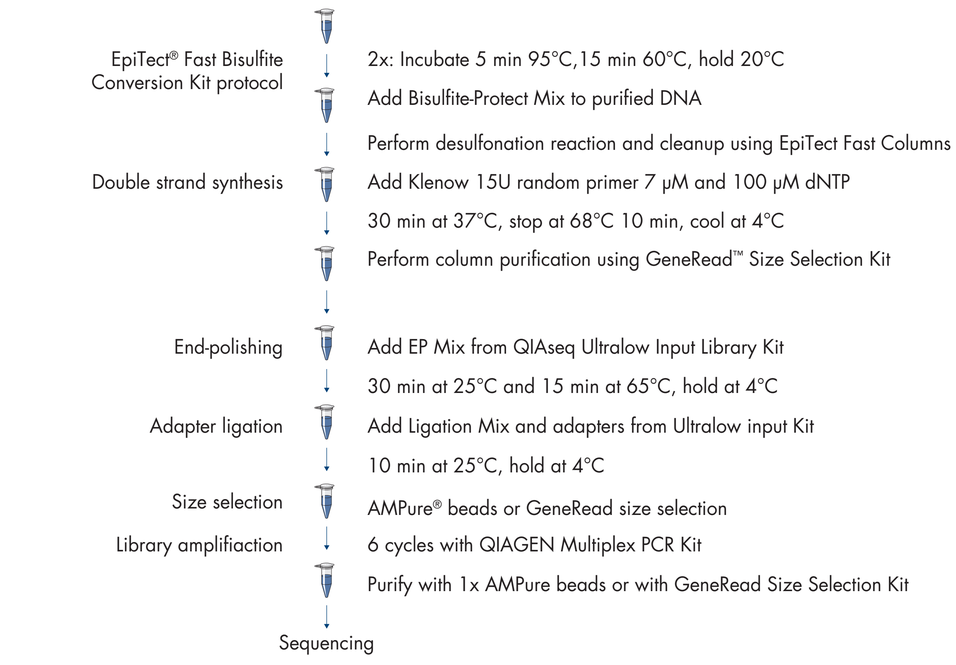
The WGBS method is the treatment of DNA using bisulfite to determine DNA methylation patterns. Treating DNA with bisulfite leaves 5-methylcytosine residues intact and converts unmethylated cystosine residues to uracil. These specific DNA sequence changes provide information about the methylation status of a DNA segment. The WGBS method is important for the detection of epigenetic modifications that impact transcriptional processes. However, this poses some challenges; importantly, the necessity of larger (microgram) quantities of DNA samples, since WGBS bisulfite treatment of NGS libraries leads to a degradation of DNA and results in significant sample loss.

Therefore, a new and more sensitive method of DNA methylation is used for smaller DNA sample sizes: post-bisulfite library construction (PBLC). In the PBLC method, the bisulfite treatment precedes NGS library prep. Then, a subsequent DNA repair step using random primers allows the generation of NGS libraries from reduced DNA input amounts. During bisulfite treatment, DNA is fragmented so that no additional DNA fragmentation is required. PBLC enables epigenomic applications that are not possible with WGBS, especially for a limited amount of starting material.



WGBS and PBLC workflows.

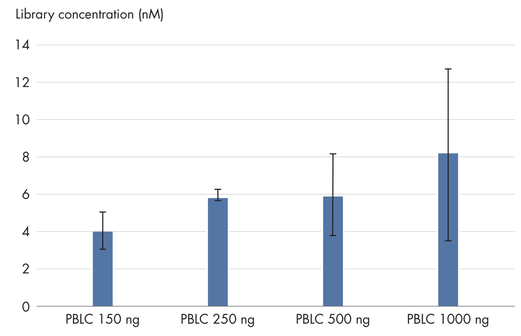
Detailed Description of PBLC Workflow



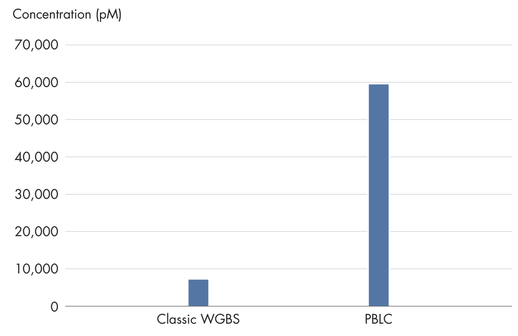
PBLC workflow.

PBLC Delivers Higher Yields of NGS Libraries

Human leucocyte DNA was used to generate libraries using the PBLC workflow. DNA was bisulfite treated using the EpiTect Fast Bisulfite Conversion Kit. Then, double-strand synthesis was performed using the Klenow fragment (3'→5' exo-) and random octamers. Using the double-stranded fragments, libraries were generated using the QIAseq Ultralow Input Library Kit. Library amplification was performed with the QIAGEN Multiplex PCR Kit using either 6 or 10 cycles of amplification. The PBLC workflow can create a significantly higher amount of an NGS library than WGBS, independent of the DNA input. For WGBS, the minimum concentration needed for an Illumina® MiSeq® run is 2 nM, but this can be achieved from only 150 ng of PBLC input DNA.



Generated library yields. Final NGS library concentrations and molarities are shown, as calculated from Agilent Bioanalyzer traces. PBLC DNA input 150–1000 ng; 6x amplification cycles.



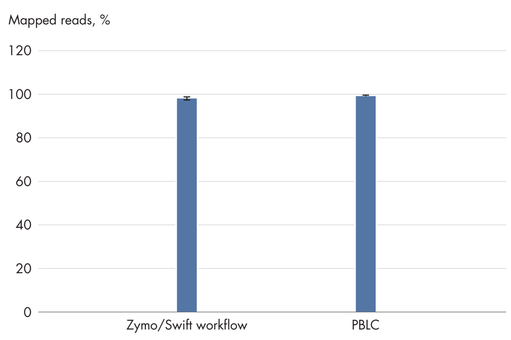
Higher yields with PBLC. 250 ng DNA was used to generate bisulfite-treated libraries using classic WGBS and PBLC workflows. Higher amounts were obtained with PBLC, as bisulfite treatment after library construction led to high losses of the generated DNA library.

Comparable Results to Existing WGBS Workflows

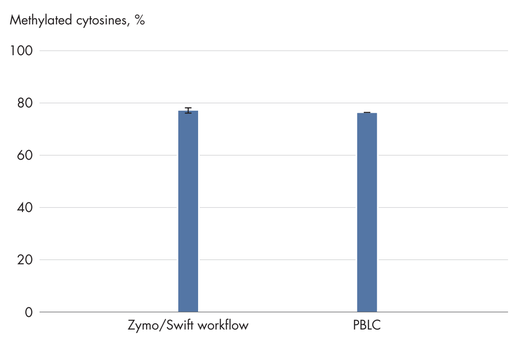
Bisulfite-treated libraries were generated from:

- 250 ng human leucocyte DNA using the PBLC workflow (<4.5 h).
- 2 µg human leucocyte DNA using the WGBS workflow: fragmentation using Adaptive Focused Acoustics™ (AFA) technology (Covaris), EZ DNA Methylation™ Kit (Zymo) and the Accel-NGS® Methyl-Seq DNA Library Kit (Swift) (5 h).
- Libraries were sequenced at depths of 0.3–0.4X and analyzed with the QIAGEN® CLC Genomics Workbench (v8.01).

Two replicate libraries generated using each method were sequenced. Sequencing of the PBLC workflow resulted in a high percentage of mapped reads and comparable methylation degree to the WGBS workflow.



Percentage of mapped reads to the reference genome hg19.



Percentage of methylated cytosines in CpG sites.

Conclusions

The combination of highly specific bisulfite conversion reagents of the EpiTect Fast Bisulfite Conversion Kit and the ultra-efficient end-polishing and adapter ligation chemistries of the QIAseq Ultralow Input Library Kit allow fast and efficient bisulfite conversion and NGS library preparation to accurately interrogate the methylome. Efficient amplification of library is performed using the QIAGEN Multiplex PCR Kit.

The PBLC method enables:

- Epigenetic studies even from low DNA input amounts
- A low number of cycles for library amplification, reducing the number of PCR duplicates
- A very fast protocol
- No extra fragmentation of DNA
- The use of non-methylated Illumina adapters that are commonly used in library construction for NGS libraries.

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Sample to Insight