Targeted Bisulfite Sequencing: An Efficient, Streamlined Approach to Maximize Throughput and Minimize Cost

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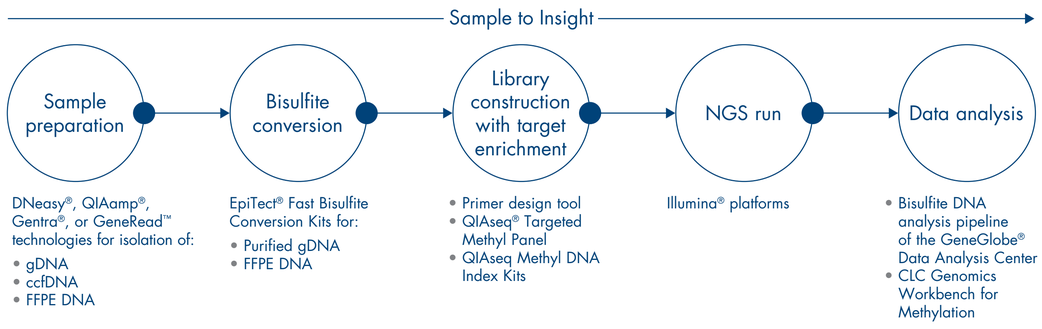
Introduction

Epigenetic changes have a significant impact on human health and disease susceptibility since they play a crucial role in the regulation of important cellular processes. In recent years, significant progress has been made in this rapidly advancing field.

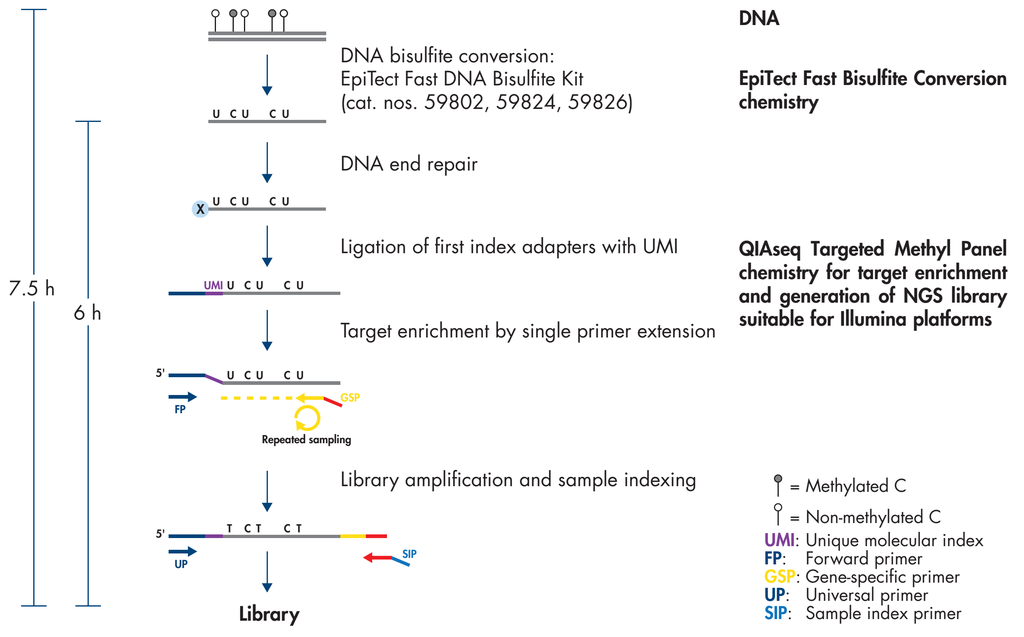
One of the most stable forms of epigenetic regulation is DNA methylation. Bisulfite sequencing (BS) is considered the best method for detecting DNA methylation with single nucleotide resolution. However, the high cost of whole genome BS and the low throughput of amplicon BS are challenges that must be overcome.

Here, we present a streamlined workflow combining optimal bisulfite treatment and targeted bisulfite sequencing. This method utilizes single-primer extension (SPE) and unique molecular identifier (UMI) barcoding technology. This enables highly efficient, flexible and scalable targeted analysis of DNA methylation from genomic DNA (gDNA), formalin-fixed paraffin-embedded (FFPE) and circulating cell-free DNA (ccfDNA) samples.



Streamlined workflow for DNA methylation analysis.

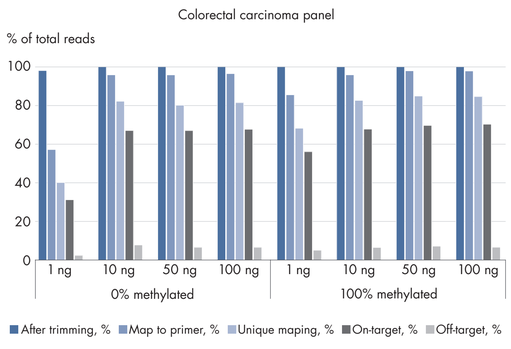
QIAseq Targeted Methyl Panel Workflow



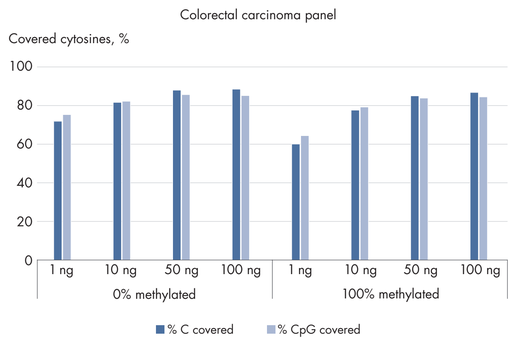
QIAseq Targeted Methyl Panel workflow.

Performance: gDNA

Control gDNA (0% and 100% methylated, with inputs of 1 ng, 10 ng, 50 ng and 100 ng) was bisulfite treated using the EpiTect Fast DNA Bisulfite Kit and libraries were generated using the using the Colorectal Cancer Panel (MHS-002Z) and QIAseq Targeted Methyl Panel chemistry. The libraries were sequenced using a MiSeq® instrument with sequencing depth of approx. 150x. Data were analyzed using the Bisulfite DNA analysis pipeline of the GeneGlobe Data Analysis Center. The results show high library quality, a high mapping rate – even starting from 10 ng input gDNA and high cytosine (C) coverage.



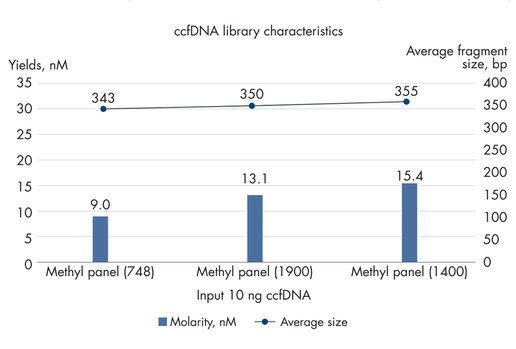
Read statistics as percentage of all reads obtained. A high unique mapping and on target rate can be achieved even from 10 ng gDNA input in the complete workflow.



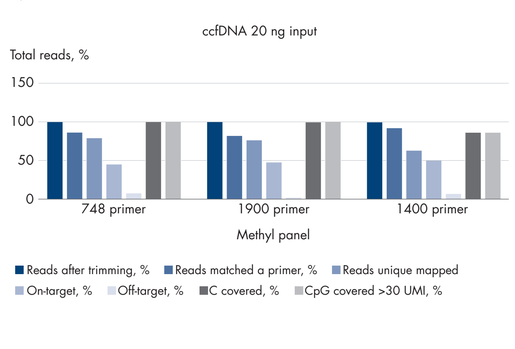
Cytosine coverage. Total cytosine coverage and coverage of cytosines in CpG content as percentage of the theoretical C expected to be covered by the corresponding panel.

Performance: ccfDNA

ccfDNA was purified using QIAamp chemistry and subsequently bisulfite treated using EpiTect Fast chemistry. 10 ng ccfDNA was processed using three different QIAseq Targeted Methyl Panels. Library amplification with 19 cycles resulted in high yields of the generated library. The expected 350 bp library size represents the combination of 170 bp (typical size of ccfDNA with 180 bp adapters). This shows that no DNA degradation occurred during bisulfite treatment with EpiTect Fast chemistry. The libraries where sequenced on MiSeq 2x150 bp paired end run and a sequencing depth of approx. 200x. The high-quality libraries resulted in high number of unique mapped reads which led to a high cytosine coverage to allow accurate methylation calls even from low input of the strong fragmented ccfDNA.



Yields and mean size of generated libraries using three different panels consisting of different primer numbers.



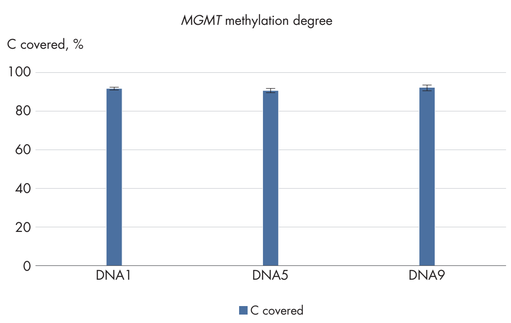
Quality of generated libraries. Libraries and panels used as a percentage of the reads after trimming; reads matched to primer sequence; unique mapped reads on target and off-target reads to total number of reads. The figure also shows the high coverage of cytosines and cytosines in CpG content as percentage of the theoretical C expected to be covered by the corresponding panels.

Performance: FFPE DNA

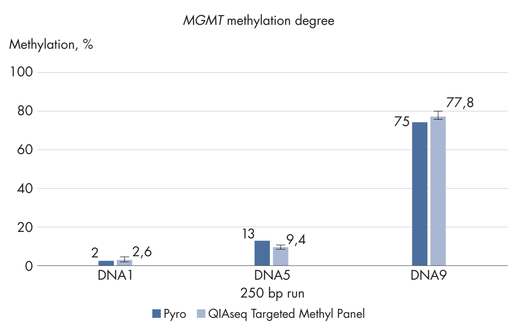
A QIAseq Targeted Methyl Panel consisting of 93 primers was used to generated libraries with a starting input of 40 ng FFPE DNA. The DNA was first bisulfite converted using EpiTect Fast chemistry. The generated libraries were sequenced on a MiSeq instrument with a sequencing depth of approx. 200x, generating a mean of 65 UMI per cytosine.

The Methylation degree of 7 CpG sites of the *MGMT* gene was previously validated with Pyrosequencing®. The evaluated methylation degree of the *MGMT* gene using the QIAseq Targeted Methyl Panel highly correlate with Pyrosequencing data.

Comparable high C coverage was obtained with all FFPE DNA having different methylation status.



Cytosine coverage obtained in the region of interest. Comparable high C coverage was obtained with all FFPE DNAs having different methylation status.



Comparison of evaluated methylation degree with QIAseq Targeted Methyl Panel and Pyrosequencing. The evaluated methylation degree of the *MGMT* gene highly correlates with the methylation degree of the 7 CpG sites previously validated with Pyrosequencing.

Conclusions

We present a workflow and chemistry that enables methylation sequencing in a targeted mode, which in turn, allows testing of multiple samples and regions of interest in a cost-efficient manner.

Our method:

- Combines highly efficient EpiTect Fast bisulfite conversion chemistry and the QIAseq Methyl Targeted Panels to reduce turnaround time over probe-capture-based enrichment
- Shows high enrichment specificity with low bias for bisulfite-converted DNA, using an optimized SPE methodology including UMI
- Allows accurate detection of CpG methylation at different levels
- Is compatible with gDNA, FFPE DNA and ccfDNA
- Allows lower DNA input
- Increases flexibility of methylation analysis through highly efficient panel design to enrich only the regions of interest
- Integrates analysis tools to standardize the method of methylation status evaluation

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