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NGS-based deep bisulfite sequencing

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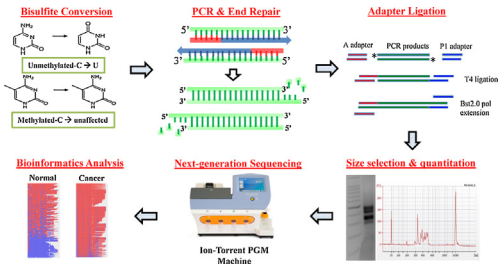
GRAPHICAL ABSTRACT



ABSTRACT

We have developed an NGS-based deep bisulfite sequencing protocol for the DNA methylation analysis of genomes. This approach allows the rapid and efficient construction of NGS-ready libraries with a large number of PCR products that have been individually amplified from bisulfite-converted DNA. This approach also employs a bioinformatics strategy to sort the raw sequence reads generated from NGS platforms and subsequently to derive DNA methylation levels for individual loci. The results demonstrated that this NGS-based deep bisulfite sequencing approach provide not only DNA methylation levels but also informative DNA methylation patterns that have not been seen through other existing methods.

- This protocol provides an efficient method generating NGS-ready libraries from individually amplified PCR products.
- This protocol provides a bioinformatics strategy sorting NGS-derived raw sequence reads.
- This protocol provides deep bisulfite sequencing results that can measure DNA methylation levels and patterns of individual loci.

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Abbreviations: NGS, Next-Generation-Sequencing; PCR, polymerase chain reaction.

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Method details

Overview

DNA methylation on CpG dinucleotides is as an epigenetic modification indicating the functional status of a given locus in a cell type [1]. This epigenetic mark can be determined through the bisulfite sequencing method, which involves a series of chemical reactions and subsequent sequencing of a genomic region [2]. The bisulfite sequencing method has been traditionally used for measuring the methylation levels of a small number of genomic regions in a targeted fashion. In recent years, this bisulfite sequencing method has been adopted as a genome-wide approach owing to the advancement of Next-Generation-Sequencing (NGS) platforms [3,4]. The NGS-driven bisulfite sequencing is useful for providing a global view of epigenomes and also for identifying genomic loci with different levels of DNA methylation. For a given locus, however, the majority of the NGS-driven data are not sufficiently deep enough to report the reliable and comprehensive measurement of its DNA methylation levels due to the relatively low levels of sequencing depth. This is particularly problematic in a situation where a testing DNA is derived from a mixture of different types of cells and/or functional status.

To solve this problem, we have developed a protocol in the current study (Fig. 1A). The main objective of this protocol is NGS-based deep bisulfite sequencing of a relatively small number of

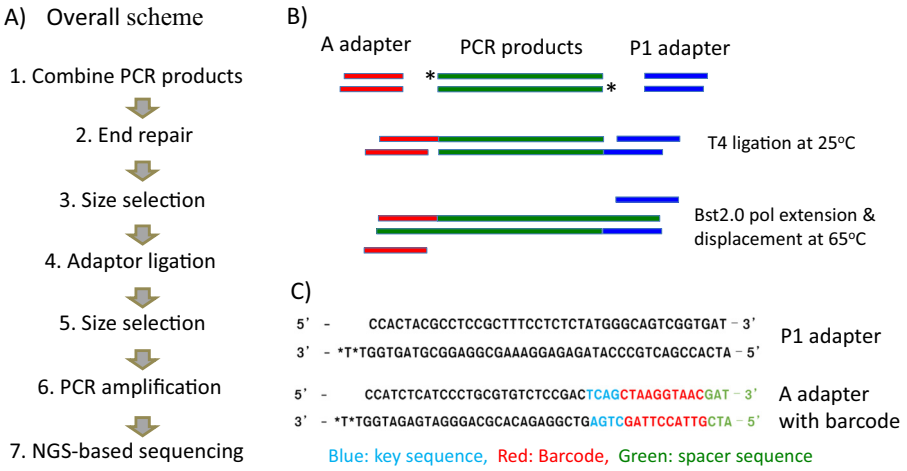

Fig. 1. Overall scheme for NGS-based deep bisulfite sequencing. (A) The entire procedure of the NGS-based deep bisulfite sequencing protocol is shown as a flow chart. (B) The adaptor ligation step for the current protocol has adopted one strategy, in which the added PCR products and two adaptors are ligated through two stepwise incubations. Since both adaptors lack the phosphate group at their 5′-ends, the ligation reaction by T4 at 25 °C occurs between only one strand of the adaptors and the PCR products. In this case, the phosphate groups are derived from the 5′-end of the PCR products. At 65 °C, the activated Bst2.0 WarmStart polymerase extends and displaces the other unligated strand from the partially joined products. The * symbol indicates the phosphate group at the 5′-end of the end-repaired PCR products. (C) The sequences of Ion Torrent P1 and A adaptors are shown with different colors to indicate the key, barcode and spacer regions. The * symbol indicates a phosphothiate bonding between two nucleotides, which protects the duplex adaptor from being digested by the exonuclease activity of DNA polymerases.

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