

SUMMARY

[0007] In a first aspect, the present disclosure refers to a method of detecting methylated DNA pattern in DNA in a biological sample, comprising:

(a) converting un-methylated cytosine of the DNA to uracil by deamination to thereby generate converted DNA;

(b) purifying the converted DNA from step (a);

(c) tagging a barcode sequence on the converted DNA, by performing a first PCR amplification using a plurality of forward and reverse primer pairs specific to the converted sequence of target regions, wherein each forward primer of the plurality of forward and reverse primer pairs comprises a barcode sequence on its 5'end, wherein the barcode sequence of each forward primer is different from each other, wherein the target regions comprise one or more CpG sites;

(d) subjecting the tagged converted DNA from step (c) to a second PCR amplification with universal indexed primers to thereby create a sequencing library with components required for multiplex sequencing;

(e) subjecting the sequencing library to multiplex sequencing on a next-generation sequencing platform;

(f) detecting the presence of the barcode sequence using Bioinformatics methods to count and assign each DNA sequence from the next-generation sequencing to an original parental DNA molecule carrying the same barcode sequence, comprising:

(i) performing cluster reassignment of sequencing reads with the same barcode sequence to thereby generate barcode clusters wherein each barcode cluster contains reads from the same amplicon and with the same barcode sequence; and

(ii) performing consensus calling for each barcode cluster to thereby obtain consensus reads;

(g) reconstructing the methylated DNA pattern of the DNA by

(I) comparing the DNA sequence to a reference genome using a sequence alignment tool; and

(II) conducting variant analysis of the DNA sequence by comparing the consensus reads to the reference genome to detect the variations;

to thereby assess 1) the conversion efficiency of non-CpG cytosines to thymine as quality control, and 2) the frequency of methylation at each CpG cytosine across all