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Sample preparation for genome wide DNA methylation analysis

COMMENTS 0

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ABSTRACT

Background: DNA methylation, the most common epigenetic modification, is defined as the removal or addition of methyl groups to cytosine bases. Studying DNA methylation provides insight into the regulation of gene expression, transposon mobility, genomic stability, and genomic imprinting. Whole-genome DNA methylation profiling (WGDM) is a powerful tool to find DNA methylation. This technique combines standard whole-genome sequencing methodology (*e.g.*, Illumina high-throughput sequencing) with additional steps where unmethylated cytosine is converted to uracil. However, factors such as low cytosine conversion efficiency and inadequate DNA recovery during sample preparation oftentimes render poor-quality data. It is therefore imperative to benchmark sample preparation protocols to increase sequencing data quality and reduce false positives in methylation detection.

Methods: A survey analysis was performed to investigate the efficiency of the following commercially available cytosine conversion kits when coupled with the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB): Zymo Research EZ DNA Methylation™ kit (hereafter known as Zymo Conversion kit), QIAGEN EpiTect Bisulfite kit (hereafter known as QIAGEN Conversion kit), and NEBNext® Enzymatic Methyl-seq Conversion Module(hereafter known as NEB EM-seq kit). Input DNA was derived from soybean (*Glycine max* [L.] Merrill) leaf tissue.

Results: Of those tested, the QIAGEN Conversion kit provided the best sample recovery and the highest number of sequencing reads, whereas the Zymo Conversion kit had the best cytosine conversion efficiency and the least duplication. The sequence library obtained with the NEB EM-seq kit had the highest mapping efficiency (percentage of reads mapped to the genome). The data quality (defined by Phred score) and methylated cytosine call were similar between kits.

Conclusions: This study offers the groundwork for selecting an effective DNA methylation detection kit for crop genome research.

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Plant growth and DNA isolation

1 a) Grow seeds of soybean genotype Williams 82 in a Miracle-Gro Moisture Control potting medium at 25°°C, 16 h d−1 light at 230 to 365 µM m−2 s−1, and 90% relative humidity in a growth chamber.

2 b) Collect a trifoliate leaf at the V2 developmental stage for DNA isolation.

3 c) Isolate genomic DNA (gDNA) using the Zymo Research Quick-DNA Plant/Seed Miniprep kit (Cat #D6020; Irvine, CA, USA).

4 d) Mesure DNA Concentration using a Qubit fluorometer (Thermo Fisher Scientific; Waltham, MA, USA) coupled with a ThermoFisher Scientific dsDNA High Sensitivity Assay kit (Cat #Q32851).

5 e) Assess DNA purity from 260/230 and 260/280 nm absorbance ratios using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific).

6 f) Determine the quality and size of obtained DNA using gel electrophoresis (1% agarose gel with 1X Tris acetate-EDTA buffer).

7 g) Prepare a non-methylated control by spiking the soybean gDNA with Escherichia coli gDNA (Zymo Research, Cat #D5016) representing 1% of the total gDNA input.

h) Separate the spiked sample into three aliquots (each 65 µL), and shear the samples using Qsonica

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8 sonicators (Newtown, CT, USA).

9 i) Use sonication settings as 15 sec on/90 sec off for 8 cycles at 20 kHz frequency, resulting in DNA fragments of approximately 350 bp.

10 j) Follow library preparation as described below with the sheared aliquots.

2) Whole-genome Illumina sequencing library preparation

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12 a) Prepare sequencing libraries using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs; Cat #E7645S; Ipswich, MA, USA) following manufacturer's instruction.

13 b) Use a total of 200 ng (for NEB EM-seq kit) or 1 µg (Zymo and QIAGEN Conversion kits) of starting DNA based on the manufacturer's instructions.

14 c) Use NEBNext® Multiplex Oligos for Illumina (NEB Cat #E7535S/L) to repair the ends of sheared DNA and then to ligate Illumina Methylated Adaptors.

15 d) Now, purify the adapter-ligated fragments using Solid Phase Reversible Immobilization (SPRI) magnetic beads (Beckman Coulter Inc.; Cat #B23317; Brea, CA, USA).

16 e) For the cytosine conversion of adapter-ligated purified DNA samples, prepare samples following manufacturer's instructions using Zymo Conversion kit (EZ DNA Methylation™ Kit, Zymo Research; Cat #D5001), QIAGEN Conversion kit (EpiTect Bisulfite Kit, QIAGEN; Cat #59104), or NEB EM-seq kit (NEBNext® Enzymatic Methyl-seq Conversion Module, New England Biolab; Cat #E7125S/L).

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17 f) For the Zymo Conversion kit (EZ DNA Methylation™ Kit, Zymo Research; Cat #D5001), incubate the DNA sample with CT Conversion Reagent (Zymo Research; Cat #D5001-1) in a thermocycler for approximately 16 hrs using following PCR settings.

18 g) Treat the resulting sample with M-Desulphonation Buffer from the kit for desulphonation (Zymo Research; Cat #D5001-5).

19 h) For the QIAGEN Conversion kit (EpiTect Bisulfite Kit, QIAGEN; Cat #59104), incubate the sample with bisulfite mix and the DNA protection buffer provided in the kit in the thermocycler for approximately 5 hrs using the following PCR settings.

20 i) Treat the converted DNA samples with Buffer BD from the kit for desulphonation.

21 j) For the NEB EM-seq kit (NEBNext® Enzymatic Methyl-seq Conversion Module, New England Biolab; Cat #E7125S/L), follow the manufacturer's instructions using TET2 (NEB Cat #E7130AVIAL) and APOBEC (NEB Cat #E7133AVIAL) enzymes in a series of steps, which ultimately converts cytosines to uracil, allowing for the detection of 5 mC and 5 hMC in the DNA sample. Use the following PCR settings.

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22 k) Purify the cytosine converted DNA samples using Solid Phase Reversible Immobilization (SPRI) magnetic beads (Beckman Coulter Inc.; Cat #B23317; Brea, CA, USA).

23 l) Now, use the purified-cytosine converted samples to prepare illumine sequencing samples with NEBNext® Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1) (NEB Cat #E7535S/L) using EpiMark® Hot Start Taq DNA Polymerase (NEB Cat # M0490S) following manufacturer instructions with following PCR settings.

3) Quality control and sequencing of WGBS and EM-seq libraries

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25 a) Purify the illumine sequencing libraries using Solid Phase Reversible Immobilization (SPRI) magnetic beads (Beckman Coulter Inc.; Cat #B23317; Brea, CA, USA).

26 b) Measure the concentration and quality of the libraries using Thermo-Invitrogen Qubit Fluorometer, NanoDrop ND-1000 spectrophotometer, Agilent Bioanalyzer (Agilent Technologies; Santa Clara, CA, USA).

27 c) Sequence the libraries using an Illumina Hi-Seq sequencer to obtain 150 bp paired-end reads.

4) Data analysis

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28 Analysis code available from: https://github.com/ajwije/DNA_methylation_analysis.git

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