MethylC-seq using EZ DNA Methylation-Gold Bisulfite and Accel-NGS DNA Library Kits

Diep Ganguly,Steven Eichten

Abstract

Protocol to use ACCEL-NGS® METHYL-SEQ DNA LIBRARY KIT (NGS Prep with Bisulfite-Converted DNA Capability; Cat. No. DL-ILMMS-12/48) to perform MethylC-sequencing in Arabidopsis.

Code used to analyse raw sequencing data can be found: Please see https://github.com/dtrain16/NGS-scripts/tree/master/MethylC.

Citation: Diep Ganguly,Steven Eichten MethylC-seq using EZ DNA Methylation-Gold Bisulfite and Accel-NGS DNA Library Kits. **protocols.io**

dx.doi.org/10.17504/protocols.io.p88drzw

Published: 25 Jul 2018

Before start

Make sure you have fresh nuclease-free water (or low EDTA TE) and 80% ethanol. You will also need working magnetic beads stocks (e.g. AMPure, SPRIselect) and a magnetic stand that holds plates or eppendorf tubes (depending on preference).

Extract high-quaity gDNA using method of choice, our lab relies largely on the DNeasy Plant Mini Kit (Qiagen). However, yields from this are quite low despite good quality gDNA.

For all resuspension steps, use low EDTA (0.1 mM) Tris (10mM, pH 8). Alternatively, we also use the elution buffer provided in the DNeasy Plant Mini Kit (Qiagen) - which is probably the same.

Materials

100ml TE Buffer [1X], pH 8.0, Low EDTA (Tris-EDTA; 10mM Tris base, 0.1mM EDTA) 786-150 by G-Biosciences

Qubit by Invitrogen - Thermo Fisher

Homemade SPRI beads solution for DNA clean-up by Contributed by users

Agencourt AMPure XP SPRI beads A63881 by Beckman Coulter

Covaris S2/S220 Focused-ultrasonicators View by Covaris

EZ DNA Methylation-Gold Kit D5005 by Zymo Research

Accel-NGS® Methyl-Seq DNA Library Kit 30024 30096 by Contributed by users

Methyl-Seq Set A Indexing Kit (12 indices, 24 rxns) 36024 by Contributed by users

DNeasy Plant Mini Kit 69104/69106 by Qiagen

LabChip GX/GXII View by Perkin Elmer

Qubit DS HS assay Q32854 by Thermo Fisher Scientific

Protocol

Extract and QC gDNA

Step 1.

1. Perform gDNA extraction using method of choice. For example, CTAB can give decent quality gDNA. The DNeasy Plant Mini Kit from Qiagen gives really high-quality plant gDNA, however, yields can be quite low.
2. Run gDNA on a 1% agarose gel to make sure there are no contaminants (e.g. CTAB extraction can leave a large amount of sheared nucleic material) nor degradation.

Normalize and shear gDNA

Step 2.

1. Normalize all gDNA samples to amount of choice (typically 0.5 - 1 µg).
2. Shear aliquoted gDNA with desired input (e.g. 1 µg) and shear using the Covaris ultrasonicator (optimized for obtaining a smear from 200bp - 500bp; peak 300bp).
    - e.g. covaris settings: 1 x 60 sec cycle; Duty 10%, intensity 5, cycles/burst 200
3. Run on agarose gel (or on LabChip GX) or QC the obtained smear.
4. Use SPRI select beads to clean smaller fragments (Optional - generally not needed). E.g. 0.8X ratio SPRI : sample to remove anything <110bp. Or can also do a right-side cleanup for larger fragments.
 - More info available at:

https://research.fhcrc.org/content/dam/stripe/hahn/methods/mol_biol/SPRIselect%20User%20Guide.pdf
5. Quantify sheared gDNA samples using Qubit.

Bisulfite conversion

Step 3.

1. Conversion is performed using EZ DNA Methylation-Gold Kit (D5005/D5006) as per their manual (see attached PDF).
2. We perform this on an aliquot (20ul) of the sheared gDNA sample (in case anything goes wrong).
3. Make sure to use fresh CT conversion reagent (can be stored at -20C for 1 month).

Generate sequencing library using Accel-NGS Kit

Step 4.

1. Bisulfite converted DNA is used to prepare a next-generation sequencing library as per the Accel-NGS Manual (see PDF attachment) using a normalized input (e.g. 100 - 200 ng converted DNA).
2. The protocol is quite straightforward and reliable. I have had no issues with it. If you are new to library preparations, make sure to read the tips on p. 8.
3. Make sure to plan indexing combinations ahead of time (Methyl-Seq Set A Indexing Kit contains Illumina TruSeq indexing adapters) - prior to PCR amplifcation (see attachment of low plex pooling) - to facilitate optimal demultiplexing of your samples after the sequencing run. Particularly important when doing a small number of samples (see PDF attachments).
4. It has been tested that relibaly sequence-able libraries have been made using 1/2 reaction volumes from the manual - however, so far I have used full volumes due to a limited number of samples being generated.
5. Depending on input, we try to limit PCR cycling to 6 - 8 cycles, aiming to get a final library molarity of 8nM. Performing a test run on a pooled sample library is definitely worthwhile to optimize the minimal number of samples required to generate a reasonable quantity of library to minimize PCR duplicates.

Pool high-quality libraries equal molar for sequencing

Step 5.

1. Final libraries should undergo appropriate quality control. Namely, using a bioanalyser or LabChip GX/GXII to check for fragment size distribution and quantification (we rely on Qubit DS HiSense for quantification, and use the most abundant molecule size to determine library molarity (with the assumption that the average molecular weight of a double-stranded DNA base-pair = 650 g/mol) . This has worked well for us and led to reliable equal molar pooling of samples.
2. Once all high-quality libraries that are required have been generated, pool all libraries together into an equal-molar sample (make sure to pay attention to molarity and volume required by your sequencing facility, e.g. we aim for 2nM in 15ul using Tris/TE pH 8-8.5). Prepare using aliquots of each individual library, but retain each individual library until data has been generated, screened, and backed-up (consider NCBI or EBI databases which allow for private uploads - ie. data is not accessible publicly).