Whole Genome Bisulfite Sequencing (WGBS)
Myers Lab, HudsonAlpha Institute for Biotechnology
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This protocol is currently used by the Myers Lab at the HudsonAlpha Institute for Biotechnology to generate whole genome bisulfite sequencing (WGBS) libraries compatible with the Illumina X DNA sequencing platform.

Reagents
Qiagen Dneasy Blood and Tissue Kit (Qiagen 69581)
MasturePure DNA Purification Kit (Epicentre MC89010)
Lambda DNA (Promega D1521)
NEBNext end repair module (E6050S)
NEBNext dA tailing module (E6053S)
Lucigen 2X Rapid Ligation Kit (30243-1)
ilAdap Methyl PE1 (IDT: ACACTCTTTCCCTACACGACGCTCTTCCGATC*T; all C's are methylated, *=phosphorothioate bond)
ilAdap Methyl PE2 (IDT: GATCGGAAGAGCGGTTCAGCAGGAATGCCGA*G; all C's are methylated, 5' phosphate, *=phosphorothioate bond)
100 bp DNA Ladder (NEB N3231L) EZ DNA Methylation Gold Kit (ZymoResearch D5005)
iPCR PE1 (IDT: AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC* T; *=phosphorothioate bond)
iPCR PE2 (IDT: CAA GCA GAA GAC GGC ATA CGA GAT CGG TCT CGG CAT TCC TGC TGA ACC GCT CTT CCG ATC* T; *=phosphorothioate bond)
Platinum Taq DNA Polymerase 5U/ml (Invitrogen 10966-026; sold with 10X PCR Buffer and 50 mM magnesium chloride)
5M Betaine (Sigma B0300)
Agencourt AMPure XP – PCR Purification (BeckmanCoulter A63881)

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Equipment

LE220 Covaris Focused-ultrasonicator Rack 96 Place microTUBE (Covaris 500282)
microTUBE AFA Fiber Crimp-Cap 6x16mm (Covaris 520052)

Protocol

1. Isolate genomic DNA (gDNA)

- Isolate genomic DNA from **cell lines** with the DNeasy Blood and Tissue Kit following the manufacturer's instructions (5 million cells per column)
- Elute genomic DNA in 100-200 µl Buffer AE, and measure concentration with a Qubit fluorometer
- Isolate genomic DNA from **tissues** with the MasturePure DNA Purification Kit following the manufacturer's instructions (5 mg tissues per tube).
- Resuspend DNA in 40 ul Buffer AE and measure concentration with a Qubit fluorometer
- Store at -30°C or proceed to next step.

2. Add Lambda DNA to gDNA

- Add 0.5% (w/w) unmethylated lambda DNA to 2 µg genomic DNA (for 2 µg genomic DNA, add 10 ng lambda DNA). If necessary, dilute lambda DNA in Buffer EB
- Bring up solution to 50 µl using Buffer EB

3. Sonicate gDNA + lambda DNA using Covaris LE220 sonicator

- Place 50 µl genomic + lambda DNA mixture into a microTUBE AFA Fiber Crimp-Cap (6x16mm)
- Make sure there are no air bubbles on the bottom of the tube
- Add samples to entire column (need to fill up entire column) on metallic Covaris rack (pn 500282)
- Sonicate gDNA on Covaris machine using 200 bp target size (450W, Duty cycle 15%, Cycles per burst 200, number of cycles 3.)

4. Purify gDNA

- Follow the Agencourt AMPure XP manual instructions with the following specifications:
- Use 90 µl of beads for 50 µl gDNA (1.8X volume gDNA)
- Resuspend DNA in 45 µl H_2O at the final step

5. End repair gDNA

Mix in a PCR tube on ice (a master mix can be prepared when processing more than one sample):
NEBNext End Repair Module

- 42.5 µl recovered DNA fragments
  5 µl NEBNext End Repair Buffer (10X)
  2.5 µl NEBNext End Repair Enzyme Mix

  Total volume = 50 µl

- Spin down briefly and incubate at 20°C in a PCR machine for 30 minutes.
- Clean up DNA using the Agencourt AMPure XP manual instructions with the following specifications:
- Use 90 µl of beads for 50 µl reaction (1.8X volume)
- Resuspend DNA in 45 µl H_2O at the final step

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6. Add dATP to gDNA

- Mix in a PCR tube on ice:
  - 42 µl end-repaired DNA fragments
  - 5 µl NEBNExt dA Tailing Reaction Buffer (10X)
  - 3 µl NEBNExt Klenow Fragment (3'-> 5' exo-)

  Total volume = 50 µl

- Spin down briefly and incubate at 37°C in a PCR machine for 30 minutes.
- Clean up DNA using the Agencourt AMPure XP manual instructions with the following specifications:
- Use 90 µl of beads for 50 µl reaction (1.8X volume)
- Resuspend DNA in 15 µl H_2O at the final step

7. Ligate methylated Illumina PE Adaptors

a. Set up the following reaction in a PCR tube:
- 25 µl 2X Lucigen Rapid Ligation Buffer
- 10 µl 40µM annealed Methylated Adapters
- 2 µl Lucigen High Concentration T4 Ligase
- 13 µl DNA from prior step

Total volume = 50 µl

b. Incubate in a thermal cycler with the following program:
- 20 min at 20°C
- Hold at 4°C

8. Size select gDNA with a double bead selection

- Dilute the sample above to 100ul with water.
- Clean up DNA using the Agencourt AMPure XP manual instructions with the following specifications:
- Use 180 µl of beads for 100 µl reaction (1.8X volume)
- Resuspend DNA in 100 µl H_2O at the final step
- Clean up DNA using the Agencourt AMPure XP manual instructions with the following specifications:
- Use 100 µl of beads for 100 µl DNA (1X volume)
- Resuspend DNA in 22 ul water

9. Bisulfite convert gDNA

- Use 20 µl gDNA sample from above
- Perform EZ DNA Methylation-Gold Kit as shown in manual
- Elute in 30 µl M-Elution buffer

10. Amplify BS converted gDNA

- Mix in a PCR tube on ice:
  30 µl BS converted DNA
  5 µl 10x PCR buffer (no MgCl2)
  2 µl MgCl2 (50mM)
  2 µl NextFlex Primer Mis (12.5 uM)
  2 µl dNTPs (12.5µM each)
  3 µl dH2O
  5 µl Betaine (5M)

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1 µl Platinum Taq DNA polymerase
Total volume = 50 µl

- Amplify as follows:

1 min 98°C
30 sec 95°C
3 min 62°C; 18 cycles (of steps 2-3)
Hold at 4°C

11. Final clean-up of BS converted gDNA

- Follow the Agencourt AMPure XP manual instructions with the following specifications:
- Use 90 µl of beads for 50 µl reaction (1.8X volume)
- Resuspend DNA in 22 µl water at the final step

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