

Materials List for:

Enhanced Reduced Representation Bisulfite Sequencing for Assessment of DNA Methylation at Base Pair Resolution

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Materials

Name	Company	Catalog Number	Comments
MspI	New England Biolabs	R0106M	100,000 units/ml
NEBuffer 2	New England Biolabs	B7002S	Reaction buffer for MspI enzyme; protocol step 1.2
Phenol solution	Sigma-Aldrich	P4557	Equilibrated with 10 mM Tris HCl, pH 8.0; see safety and handling instructions at http://www.sigmaaldrich.com/catalog/product/sigma/p4557
Chloroform	Sigma-Aldrich	C2432	See safety and handling instructions at http://www.sigmaaldrich.com/catalog/product/sial/c2432
Glycogen	Sigma-Aldrich	G1767	19-22 mg/ml
NaOAc	Sigma-Aldrich	S7899	3 M, pH 5.2
Ethanol	Sigma-Aldrich	E7023	200 proof, for molecular biology
Buffer EB	Qiagen	19086	10 mM Tris-Cl, pH 8.5
tris(hydroxymethyl)aminomethane (Tris)	Sigma-Aldrich	T1503	prepare a 1 M, pH 8.5 solution
Tris- Ethylenediaminetetraacetic acid (TE)	Sigma-Aldrich	T9285	Dilute to 1x buffer solution per manufacturer's recommendations
T4 DNA Ligase Reaction Buffer	New England Biolabs	B0202S	10x concentration
Deoxynucleotide triphosphate (dNTP) Solution Mix	New England Biolabs	N0447L	10 mM each nucleotide
T4 DNA Polymerase	New England Biolabs	M0203L	3,000 units/ml
DNA Polymerase I, Large (Klenow) Fragment	New England Biolabs	M0210L	5,000 units/ml
T4 Polynucleotide Kinase	New England Biolabs	M0201L	10,000 units/ml
QIAquick PCR Purification Kit	Qiagen	28104	Used for DNA product purification in protocol step 2.3
2'-deoxyadenosine 5'-triphosphate (dATP)	Promega	U1201	100 mM
Klenow Fragment (3'→5' exo-)	New England Biolabs	M0212L	5,000 units/ml
MinElute PCR Purification Kit	Qiagen	28004	Used for DNA product purification in protocol step 3.3
T4 DNA Ligase	New England Biolabs	M0202M	2,000,000 units/ml

Methylation Adapter Oligo Kit	Illumina	ME-100-0010	
Agencourt AMPure XP	Beckman Coulter	A63881	Used in protocol sections that implement magnetic bead purification steps (steps 4.3 and 8.2). Equilibrate to room temperature before use.
Pippin Prep Gel Cassettes, 2% Agarose, dye-free	Sage Science	CDF2010	with internal standards
Certified Low Range Ultra Agarose	Bio-Rad	161-3106	
Tris-Borate-EDTA (TBE) buffer	Sigma-Aldrich	T4415	
Ethidium bromide solution	Sigma-Aldrich	E1510	10 mg/ml
50 bp DNA Ladder	NEB	N3236S	
100 bp DNA Ladder	NEB	N3231S	
Gel Loading Dye, Orange (6x)	NEB	B7022S	
Scapel Blade No. 11	Fisher Scientific	3120030	
QIAquick Gel Extraction Kit	Qiagen	28704	
EZ DNA Methylation Kit	Zymo Research	D5001	Used in protocol step 6.2
EZ DNA Methylation-Lightning Kit	Zymo Research	D5030	Alternative for step 6.2
Universal Methylated Human DNA Standard	Zymo Research	D5011	Used as bisulfite conversion control
FastStart High Fidelity PCR System	Roche	03553426001	
Qubit dsDNA High Sensitivity Assay Kit	Life Technologies	Q32854	A fluorescence-based DNA quantitation assay; used in protocol steps 1.1, 9.1 and 10.1
DynaMag-2 Magnet	Life Technologies	12321D	
High Sensitivity DNA Kit	Agilent Technologies	5067-4626	
2100 Bioanalyzer	Agilent Technologies		
PhiX Control v3	Illumina	FC-110-3001	
HiSeq 2500	Illumina		
Pippin Prep	Sage Science		
Qubit 2.0 Fluorometer	Life Technologies	Q32872	
TruSeq SR Cluster Kit v3-cBot-HS	Illumina	GD-401-3001	
TruSeq SBS Kit v3-HS	Illumina	FC-401-3002	
TruSeq RNA Sample prep	Illumina	RS-122-2001	Barcoded adapters used for multiplexing libraries; See Supplemental file for multiplexing protocol.
Microcentrifuge			
Vortex Mixer			
Dry Block Heater			
Thermal Cycler			
Water Bath			
Gel electrophoresis system			
Electrophoresis power supply			
Gel doc			
UV or blue light transilluminator			