

Materials List for:

DamID-seq: Genome-wide Mapping of Protein-DNA Interactions by High Throughput Sequencing of Adenine-methylated DNA Fragments

Feinan Wu¹, Brennan G. Olson¹, Jie Yao¹

¹Department of Cell Biology, Yale University School of Medicine

Correspondence to: Jie Yao at yao.j@yale.edu

URL: <https://www.jove.com/video/53620>

DOI: [doi:10.3791/53620](https://doi.org/10.3791/53620)

Materials

Name	Company	Catalog Number	Comments
ViraPower Lentiviral Expression Systems	Life Technologies	K4950-00, K4960-00, K4970-00, K4975-00, K4980-00, K4985-00, K4990-00, K367-20, K370-20, and K371-20	
Gateway BP Clonase II Enzyme Mix	Life Technologies	11789-020	
Gateway LR Clonase II Enzyme Mix	Life Technologies	11791-020	
DNeasy Blood & Tissue Kit (250)	QIAGEN	69506 or 69504	
Gateway pDONR 201	Life Technologies	11798-014	
293T cells	American Type Culture Collection	CRL-11268	
Trypsin-EDTA (0.05%) , phenol red	Life Technologies	25300-054	
DMEM, high glucose, pyruvate	Life Technologies	11995-065	
Fetal Bovine Serum	Sigma	F4135	
Tris			brand not critical
EDTA			brand not critical
200 Proof EtOH			brand not critical
Isopropanol			brand not critical
Sodium Acetate			brand not critical
DpnI	New England Biolabs	R0176	supplied with buffer
DamID adaptors "AdRt" and "AdRb"	Integrated DNA Technologies		sequences available in ref. 24; no phosphorylation of the 5' or 3' end to prevent self-ligation.
T4 DNA Ligase	Roche Life Science	10481220001	supplied with buffer
DpnII	New England Biolabs	R0543	supplied with buffer
DamID PCR primer "AdR_PCR"	Integrated DNA Technologies		sequences available in ref. 24
Deoxynucleotide (dNTP) Solution Set	New England Biolabs	N0446	100 mM each of dATP, dCTP, dGTP and dTTP
Advantage 2 Polymerase Mix	Clontech	639201	supplied with buffer
1Kb Plus DNA Ladder	Life Technologies	10787018	1.0 µg/µl
QIAquick PCR Purification Kit	QIAGEN	28104 or 28106	
MinElute PCR Purification Kit	QIAGEN	28004 or 28006	for an elution volume of less than 30 µl
SPRI beads / Agencourt AMPure XP	Beckman Coulter	A63880	apply extra mixing and more elution time if less than 40 µl elution buffer is used
Buffer EB	QIAGEN	19086	
NEBNext dsDNA Fragmentase	New England Biolabs	M0348	supplied with buffer

T4 DNA Ligase Reaction Buffer	New England Biolabs	B0202	
T4 DNA Polymerase	New England Biolabs	M0203	
DNA Polymerase I, Large (Klenow) Fragment	New England Biolabs	M0210	
T4 Polynucleotide Kinase	New England Biolabs	M0201	
Klenow Fragment (3' → 5' exo-)	New England Biolabs	M0212	supplied with buffer
sequencing adaptors	Integrated DNA Technologies		sequences available in ref. 28
Quick Ligation Kit	New England Biolabs	M2200	used in 3.4.3; supplied with Quick Ligation Reaction Buffer and Quick T4 DNA Ligase
sequencing primer 1 and 2	Integrated DNA Technologies		sequences available in ref. 28
KAPA HiFi PCR Kit	Kapa Biosystems	KK2101 or KK2102	supplied with KAPA HiFi DNA Polymerase, 5x KAPA HiFi Fidelity Buffer and 10 mM dNTP mix
agarose	Sigma Aldrich	A4679	
ethidium bromide	Sigma Aldrich	E1510-10ML	10 mg/ml
QIAquick Gel Extraction Kit	QIAGEN	28704 or 28706	
iTaq Universal SYBR Green Supermix	Bio-Rad Laboratories	1725121 or 1725122	
Spectrophotometer			brand not critical
0.45 µm PVDF Filter			brand not critical
25 ml Syringe			brand not critical
10 cm Tissue Culture Plates			brand not critical
6-well Tissue Culture Plates			brand not critical
S1000 Thermal Cycler	Bio-Rad Laboratories		
C1000 Touch Thermal Cycler	Bio-Rad Laboratories		for qPCR
Vortex Mixer			brand not critical
Dry Block Heater or Thermomixer			brand not critical
Microcentrifuge			brand not critical
Gel electrophoresis system with power supply			brand not critical
Magnet stand			for purification of DNA with SPRI beads; should hold 1.5-2 ml tubes; brand not critical
UV transilluminator			brand not critical
E-gel electrophoresis system	Life Technologies	G6400, G6500, G6512ST	