

Materials List for:

A Method to Study *de novo* Formation of Chromatin Domains

Ozgur Oksuz^{1,2,3}, Danny Reinberg^{1,2}

¹Howard Hughes Medical Institute, New York University School of Medicine

²Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine

³Whitehead Institute for Biomedical Research

Correspondence to: Ozgur Oksuz at oksuzo@wi.mit.edu

URL: <https://www.jove.com/video/60039>

DOI: [doi:10.3791/60039](https://doi.org/10.3791/60039)

Materials

Name	Company	Catalog Number	Comments
(Z)-4-Hydroxytamoxifen (5 mg)	Sigma	H7904-5MG	For induction of EED expression
16% Paraformaldehyde aqueous solution (10x10 ml)	Electron Microscopy Sciences	15710	For immunofluorescence
2-mercaptoethanol	LifeTechnologies	21985-023	For mESCs culture
2% Gelatin Solution	Sigma	G1393-100ml	For mESCs culture
Accutase 500 ML	Innovative Cell Tech/FISHER	AT 104-500	For mESCs culture
Alexa Fluor 594 AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson immunoresaerch	711-585-152	For immunofluorescence
Aqua-Mount Mounting Medium	FISHER/VWR	41799-008	For immunofluorescence
CHAMBER SLD TC PRMA 8-CHM 16 PK	Fisher Sci	177445PK	For immunofluorescence
DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) - 10 mg	Life Tech	D1306	For immunofluorescence
ERK inhibitor, PD0325901	Stemgent	04-0006	For mESCs culture
ESGRO Recombinant Mouse LIF Protein	Millipore/Fisher	ESG1107	For mESCs culture
FBS Stem Cell Qualified	Atlanta	S10250	For mESCs culture
Gibson Assembly Master Mix	NEB	E2611L	For Donor template cloning
GSK3 inhibitor, CHIR99021	Stemgent	04-0004	For mESCs culture
H3K27me2 (D18C8) rabbit mAB	Cell Signaling	9728S	Antibody for ChIP-seq
H3K27me3	Cell Signaling	9733S	Antibody for ChIP-seq
Histone H2Av antibody (pAb)	Active motif	39715	Spike-in control for ChIP-seq
Knockout DMEM	Invitrogen	10829-018	For mESCs culture
L-glutamine	Sigma	G7513	For mESCs culture
Lipofectamine 2000	LifeTech	11668019	For transfection
MangoTaq DNA Polymerase	Bioline	BIO-21079	For Genotyping PCR
Normal donkey serum (10 mL)	Jackson ImmunoResearch	017-000-121	For immunofluorescence
Penicillin-Streptomycin	Sigma/Roche	P0781	For mESCs culture
pSpCas9(BB)-2A-GFP (PX458)	Addgene	48138	For gRNA cloning
QuickExtract DNA Extraction Solution	Lucigen	QE0905T	For Genotyping PCR
Triton X-100	Sigma	T8787-250ML	
Zero Blunt PCR Cloning Kit	Thermo Fisher	K270020	For Donor template cloning
Primers/gBlocks			

EED-KO-gRNA-1			Sequence: ctctggctactgtcaactag. gRNAs pairs to knockout EED in C57BL/6 ESCs for i-WT-r and i-MT-r systems.
EED-KO-gRNA-2			Sequence: TAGGCTATGACGCAGCTCAG. gRNAs pairs to knockout EED in C57BL/6 ESCs for i-WT-r and i-MT-r systems.
EED-gRNA-inducible			Sequence: atggcaccgccgaaattagaa. gRNA and Donor to generate i-WT-r system in the EED-KO background.
i-WT-r Donor			https://benchling.com/s/seq-l2LLIWNErLrGXcbdCxl . gRNA and Donor to generate i-WT-r system in the EED-KO background.
EED-gRNA-inducible			Sequence: atggcaccgccgaaattagaa. gRNA and Donor to generate i-WT-r system in the EED-KO background.
i-MT-r Donor			https://benchling.com/s/seq-n8eiZCB2XAkOuzzpv6qM . gRNA and Donor to generate i-MT-r system in the EED-KO background.
Genotyping Primers			
Gnt-EED-KO-FW-1			Sequence: ctgtaggctgccatctgtga. Wild type allele will produce a product of 1.9 kb. Knockout allele will produce a product of 200 bp.
Gnt-EED-KO-REV-1			Sequence: agccagggtacacagagaaa. Wild type allele will produce a product of 1.9 kb. Knockout allele will produce a product of 200 bp.
Inducible_Genotype-FW-1			Sequence: tgcagtgaacaaatttgaa. When the cassette is inserted, the primers will produce 1863 bp. The wild type allele will produce a product of ~200 bp.
Inducible_Genotype-REV-1			Sequence: gagaggggtggcactgtaa. When the cassette is inserted, the primers will produce 1863 bp. The wild type allele will produce a product of ~200 bp.
Inducible_Genotype-FW-2			Sequence: cccctctttctcctttct. When the cassette is inserted, the primers will produce 3200 bp. The wild type allele will produce a product of 1560 bp.
Inducible_Genotype-REV-2			Sequence: atgcctgggtgaatgaaaa. When the cassette is inserted, the primers will produce 3200 bp. The wild type allele will produce a product of 1560 bp.