

Materials List for

Analysis of Transgenerational Epigenetic Inheritance in *C. elegans* Using a Fluorescent Reporter and Chromatin Immunoprecipitation (ChIP)

Chengyin Li^{*1}, Phoebe A. W. Bhagoutie^{*1}, Victor Lao¹, Arneet L. Saltzman¹

¹Department of Cell and Systems Biology, University of Toronto

* These authors contributed equally

Corresponding Author

Arneet L. Saltzman

arneet.saltzman@utoronto.ca

Citation

Li, C., Bhagoutie, P.A.W., Lao, V., Saltzman, A.L. Analysis of Transgenerational Epigenetic Inheritance in *C. elegans* Using a Fluorescent Reporter and Chromatin Immunoprecipitation (ChIP). *J. Vis. Exp.* (195), e65285, doi:10.3791/65285 (2023).

Date Published

May 5, 2023

DOI

10.3791/65285

URL

jove.com/video/65285

Materials

Name	Company	Catalog Number	Comments
Agarose	Bioshop	AGA002	
Ampicillin	Bioshop	AMP201	Make a 100 mg/mL solution in ultrapure water. Filter-sterilize and store at -20 °C.
Anti-H3K9me3 Rabbit Polyclonal Antibody	Abcam	ab8898	Concentration is batch-dependent (0.9 - 1 mg/mL).
Anti-Histone H3 Rabbit Polyclonal Antibody	Abcam	ab1791	Concentration is batch-dependent (0.7 - 1 mg/mL).
Bleach (6% Sodium hypochlorite)	Lavo	02358107	
<i>C. elegans</i> strain with GFP RNAi Reporter	NA	SX1263	Sapetschnig et al. 2015 (ref. 7). A gift from E. Miska lab, University of Cambridge.
Carbenicillin	BioShop	CAR544	Make a 25 mg/mL solution in ultrapure water. Filter-sterilize and store at -20 °C.
Dynabeads Protein G Magnetic Beads	Invitrogen	10003D	
<i>E. coli</i> strain HT115(DE3)	Caenorhabditis Genetics Center (CGC)	HT115(DE3)	
<i>E. coli</i> strain OP50-1	Caenorhabditis Genetics Center (CGC)	OP50-1	
EDTA (0.5 M, pH 8.0)	Invitrogen	15575020	
Fluorescence Stereoscope	Zeiss	Axio Zoom.V16	
Formaldehyde (37%)	Sigma	F8775	
Glycine	Sigma	50046	Make a 1.25 M solution and store at 4 °C.
HEPES-KOH (1 M, pH 7.5)	Teknova	H1035	
Hydrophobic Printed Slides, 10 wells	VWR	100488-904	

IGEPAL CA-630 (Octylphenol ethoxylate)	BioShop	NON999	Make a 10% (v/v) solution in ultrapure water and store at room temperature.
IPTG (Isopropyl- β -D-thiogalactoside)	BioShop	IPT001	Make a 0.2 g/mL solution in ultrapure water. Filter-sterilize and store at -20 °C.
iTaq Universal SYBR Green Supermix	Bio-Rad	1725122	
LB Agar Plates supplemented with 100 μ g/mL Ampicillin	NA	NA	Standard lab recipe.
Levamisole (Tetramisole hydrochloride)	Sigma	L9756	Make a 200 mM solution in ultrapure water. Store at -20 °C.
LiCl (8 M)	Sigma	L7026	
M9 Buffer	NA	NA	22 mM KH ₂ PO ₄ , 42 mM Na ₂ HPO ₄ , 86 mM NaCl, 1 mM MgSO ₄ .
Magnetic Separator (1.5 mL tubes)	Applied Biosystems	A13346	
Magnetic Separator (0.2 mL tubes)	Permagen	MSR812	
Microscope Cover Glass	Fisher Scientific	12541B	
Microscope Slide	Technologist Choice	LAB-037	
NaCl (5 M)	Promega	V4221	For ChIP buffers.
NaOH	Sigma	S5881	Make a 10 M solution and store at room temperature.
NGM Plates	NA	NA	1.7% (w/v) agar, 0.3% (w/v) NaCl, 0.25% (w/v) peptone, 1 mM CaCl ₂ , 5 μ g/mL cholesterol, 25 mM Potassium phosphate pH 6.0, 1 mM MgSO ₄ , 50 μ g/mL streptomycin.
Normal Rabbit IgG (1 mg/mL)	Cell Signaling Technology	2729	
Petri Dishes (35 mm x 10 mm)	Sarstedt	82.1135.500	
Phosphate Buffered Saline (10X)	Fisher BioReagents	BP3991	
Plasmid - Control RNAi	Addgene	L4440 (Plasmid #1654)	
Plasmid - GFP-targetting RNAi	Addgene	L4417 (Plasmid #1649)	Note, alternative L4440-derived plasmids targeting GFP can be used.
Primer pair [-3.3 kb upstream of gfp]	Integrated DNA Technologies	NA	F: AAACCAAAGGACGAGAGATTCA, R: GGCTCGATCAAGTAAAATTTTCG
Primer pair [+1.3 kb downstream of gfp]	Integrated DNA Technologies	NA	F: TCGACCAGTTCTAAAGTCACCG, R: ACGTGCGGGATCATTCTTACT
Primer pair [clec-18]	Integrated DNA Technologies	NA	F: TGCTCCATGACCTCAACAACA, R: AGTACAGTTCACCGATCCAGA
Primer pair [gfp exon 1]	Integrated DNA Technologies	NA	F: CTGGAGTTGTCCCAATTCTTGT, R: GGGTAAGTTTTCCGTATGTTGC
Primer pair [gfp exon 4]	Integrated DNA Technologies	NA	F: GATGGCCCTGTCCTTTTACCA, R: ATGCCATGTGAATCCAGCA
Primer pair [hrp-2]	Integrated DNA Technologies	NA	F: CGTCAACAGGGAGCAGCTG, R: CCTCCGAACCTTTCTCTGTCCA
Protease Inhibitor Cocktail Tablet	Roche	11836170001	
Proteinase K	Bioline	BIO-37084	
QIAquick PCR Purification Kit	Qiagen	28104	
Real-Time PCR Detection System	Bio-Rad	CFX96	
RNAi-NGM plates	NA	NA	1.7% (w/v) agar, 0.3% (w/v) NaCl, 0.25% (w/v) peptone, 1 mM CaCl ₂ , 5 μ g/mL cholesterol, 25 mM Potassium phosphate buffer pH 6.0, 1 mM MgSO ₄ , 25 μ g/mL carbenicillin and 5 mM IPTG.

RNase A	Sigma	R4642	
Sarkosyl (N-Lauroylsarcosine sodium salt)	Sigma	L5777	Make a 10% (w/v) solution and store at room temperature protected from light for a maximum of 1 month.
SDS	Sigma	74255	Make a 10% (w/v) solution and store at room temperature.
Sodium deoxycholate	Sigma	30970	Make a 5% (w/v) solution and store at room temperature protected from light for a maximum of 1 month.
Sonication Tube	Evergreen	214-3721-010	
Sonication Tube Cap	Evergreen	300-2911-020	
Sonicator	Qsonica	Q800R3-110	
Streptomycin sulfate	Bioshop	STP101	Make a 50 mg/mL solution in ultrapure water. Filter-sterilize and store at -20 °C.
TAE buffer (1X)	NA	NA	40 mM Tris, 20 mM acetate, 1 mM EDTA
Tally counter clicker	Uline	H-7350	
Tetracycline	Bioshop	TET701	Make a 5 mg/mL solution in ethanol and store at -20 °C.
Thermomixer	Eppendorf	05-400-205	
Tris-HCl (1 M, pH 8.0)	Invitrogen	15568025	
Triton X-100	Sigma	T8787	Make a 10% (v/v) solution in ultrapure water and store at room temperature.