

Materials List for:

Enhanced Crosslinking Immunoprecipitation (eCLIP) Method for Efficient Identification of Protein-bound RNA in Mouse Testis

Qiushi Xu¹, Caifeng Wang¹, Li Ling¹, Qiuling Yue¹, Mengrou Liu¹, Shuya Zhang¹, Kaiqiang Fu¹, Lan Ye¹, Ke Zheng¹

¹State Key Laboratory of Reproductive Medicine, Nanjing Medical University

*These authors contributed equally

Correspondence to: Ke Zheng at kezheng@njmu.edu.cn

URL: <https://www.jove.com/video/59681>

DOI: [doi:10.3791/59681](https://doi.org/10.3791/59681)

Materials

Name	Company	Catalog Number	Comments
Antibodies			
Anti-mouse MOV10 antibody	Proteintech, China	10370-1-AP	
Anti-mouse MOV10L1 antibody	Zheng et al.2010 ⁹	polyclonal antisera UP2175	provided by P. Jeremy Wang lab, University of Pennsylvania
HRP Goat Anti-Rabbit IgG	ABclonal	AS014	
Rabbit IgG	Beyotime, China	A7016	
Equipment			
Centrifuge	Eppendorf, Hamburg, Germany	5242R	
Digital sonifier	BRANSON, USA	BBV12081048A	450 Watts; 50/60 Hz
DynaMag-2 Magnet	Invitrogen, USA	12321D	
Mini Blot Module	Invitrogen, USA	B1000	
Mini Gel Tank	Invitrogen, USA	A25977	
Shaking incubator	Eppendorf, Hamburg, Germany	Thermomixer comfort	
Tissue Grinder, Dounce	PYREX, USA	1234F35	only the "loose" pestle is used in this protocol
TProfessional standard 96 Gradient	Biometra, Germany	serial no.: 2604323	
Tube Revolver	Crystal, USA	serial no.: 3406051	
UV-light cross-linker	UVP, USA	CL-1000	
Materials			
TC-treated Culture Dish	Corning, USA	430167	100 mm
Tubes	Corning, USA	430791	15 mL
Microtubes tubes	AXYGEN , USA	MCT-150-C	1.5 mL
Reagents			
Acid phenol/chloroform/isoamyl alcohol	Solarbio, China	P1011	25:24:01
AffinityScript Enzyme	Agilent, USA	600107	
Antioxidant	Invitrogen, USA	NP0005	
DH5α competent bacteria	Thermo Scientific, USA	18265017	these economical cells yield >1 x 10 ⁶ transformants/μg control DNA per 50 μL reaction.
DMSO	Sigma-Aldrich, USA	D8418	
DNA Ladder	Invitrogen, USA	10416014	
dNTP	Sigma-Aldrich, USA	DNTP100-1KT	
Dynabeads Protein A	Invitrogen, USA	10002D	

ECL reagent	Vazyme, China	E411-04	
EDTA	Invitrogen, USA	AM9260G	
EDTA free protease inhibitor cocktail	Roche, USA	04693132001	add fresh
Exo-SAP-IT	Affymetrix, USA	78201	PCR Product Cleanup Reagent
FastAP enzyme	Thermo Scientific, USA	EF0652	
LDS Sample Buffer	Thermo Scientific, USA	NP0007	
MetaPhor Agarose	lonza, Switzerland	50180	
MgCl ₂	Invitrogen, USA	AM9530G	
MiniElute gel Extraction	QIAGEN, Germany	28604	column store at 4 °C; buffer QG=gel dissolving buffer; buffer PE= wash buffer(for step 14)
MyONE Silane beads	Thermo Scientific, USA	37002D	nucleic acids extraction magnetic beads
NaCl	Invitrogen, USA	AM9759	
NP-40	Amresco, USA	M158-500ML	
NuPAGE Bis-Tris Protein Gels	Invitrogen, USA	NP0336BOX	4%–12%, 1.5 mm, 15-well
NuPAGE MOPS SDS Buffer Kit	Invitrogen, USA	NP0050	
PBS	Gibco, USA	10010023	
Phase-Locked Gel (PLG) heavy tube	TIANGEN, China	WM5-2302831	
PowerUp SYBR Green Master Mix	Applied Biosystems, USA	A25742	
Proteinase K	NEB, New England	P8107S	
Q5 PCR master mix	NEB, New England	M0492L	
RLT buffer	QIAGEN, Germany	79216	RNA purification lysis buffer
RNA Clean & Concentrator-5 columns	ZYMO RESEARCH, USA	R1016	RNA purification and concentration columns
RNase I	Invitrogen, USA	AM2295	
RNase Inhibitor	Promega, USA	N251B	
RQ1 DNase	Promega, USA	M610A	
Sample Reducing Agent	Invitrogen, USA	NP0009	
SDS Solution	Invitrogen, USA	15553027	10%
Sodium deoxycholate	Sigma-Aldrich, USA	30970	protect from light
T4 PNK enzyme	NEB, New England	M0201L	
T4 RNA ligase 1 high conc	NEB, New England	M0437M	
TA/Blunt-Zero Cloning Mix	Vazyme, China	C601-01	
TBE	Invitrogen, USA	AM9863	
Tris-HCl Buffer	Invitrogen, USA	15567027	
Triton X-100	Sangon Biotech, China	A600198	
Tween-20	Sangon Biotech, China	A600560	
Urea	Sigma-Aldrich, USA	U5378	
X-ray Films	Carestream, Canada	6535876	