

Materials List for:

Monitoring Protein-RNA Interaction Dynamics in vivo at High Temporal Resolution using χ CRAC

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Materials

Name	Company	Catalog Number	Comments
1,4-dithioereitol	Merck	10708984001	Buffer component in mammalian cell lysis
1.5 mL tubes	Eppendorf	0030 120.086	General reaction tube
2 mL tubes	Eppendorf	0030 123.344	For holding columns and collection of waste
32P- γ ATP	Perkin Elmer	NEG502Z-250	For radiolabelling the 5' end of the RNA
4-12% Bis-Tris gel	Invitrogen	NP0321BOX	SDS-PAGE gel
4X loading buffer	Novex	NP0008	Protein loading dye concentrate
50 bp ladder	New England Biolabs	N3236	Reference ladder for excising region of interest from the amplified cDNA library
50% PEG	NEB	B100045	For the L5 linker ligation
6% TBE gel	Invitrogen	EC6265BOX	For separation and purification of the cDNA library
Acetone	ACROS Organics	423245000	Washing of TCA-precipitated proteins
anti-FLAG beads	Sigma Aldrich	M8823-1ML	For purification of FLAG-tagged RBPs
ATP (100 mM)	Thermo Fisher Scientific	R0441	For ligation of the L5 linker onto the 5' end of captured RNAs
Beta-mercaptoethanol	Sigma Aldrich	M3148-100ML	Buffer component
Biomax MS intensifying screen	Sigma Aldrich	Z363162-1EA	For intensifying the autoradiogram signal
Chloroform	Thermo Fisher Scientific	1010219	For phenol-chloroform extraction following RNA purification
cOmplete EDTA-free protease inhibitor cocktail	Roche	11873580001	For inhibition of cellular proteases after lysis
Complete supplement mixture - TRP	Formedium	DCS0149	For preparation of synthetic defined medium
Costar Spin-X 0.22 μ m filters	Sigma Aldrich	CLS8160	For isolating the excised cDNAs following gel extraction
DNase RQ1	Promega	M6101	For DNA digest following cell lysis
dNTPs (10 mM)	Sigma Aldrich	4638956001	For reverse transcription and PCR

Ethanol	Thermo Fisher Scientific	10041814	For phenol-chloroform extraction following RNA purification and DNA precipitation
Ethylenediaminetetraacetic acid	Invitrogen	AM9261	For protease K buffer
Exonuclease I	New England Biolabs	M0293	For degradation of primers following PCR
Glass microfiber filters	Whatman	1823-010	For isolating the excised cDNAs following gel extraction
Glucose	Formedium	GLU03	For preparation of glucose-containing, synthetic defined medium
Glycogen (20 mg/mL)	Roche	10901393001	Precipitation of proteins, RNA and DNA
GST-TEV	Homemade		Construct and purification protocol is available upon request
Guanidium hydrochloride	Thermo Fisher Scientific	10071503	Required for pulldown denaturing conditions and washing buffer
IgG beads	GE Healthcare	17-0969-01	For purification of protein A-tagged RBPs
Imidazole	Sigma Aldrich	I2399-100G	For elution of captured proteins from Nickel beads
Isoamyl alcohol	Thermo Fisher Scientific	A393-500	For phenol-chloroform extraction following RNA purification
Luna Universal One-Step RT-qPCR	NEB	E3005S	For qPCR of the cDNA in order to calculate required number of PCR cycles
Magnesium chloride	Fluka Analytical	63020-1L	For PNK buffer
Membrane filters	Millipore	AAWP09000 for yeast or HAWP09000 for bacteria	For vacuum filtration of cells
Micro bio-spin columns	Biorad	732-6204	For collecting eluate after gel extraction
Ni-NTA beads	Qiagen	30210	For secondary protein capture
NP-40	Sigma Aldrich	I8896-100ML	Buffer component
Pfu polymerase	Promega	M7741	For amplification of the cDNA library
Phenol	Sigma Aldrich	P4682-400ML	For phenol-chloroform extraction following RNA purification
Pierce spin columns	Thermo Fisher Scientific	69725	For on-column enzymatic reactions
Protease K	Roche	3115887001	For degradation of the RBP following gel extraction
Quartz Petri dish	UVO3	N/A	For cross-linking of adherent cells. Available from https://www.vari-x-link.com for 400 GBP
Radiography films	Amersham	28906843	For autoradiography visualisation
RNAClean XP beads	Beckmann	A63987	SPRI beads for clean up of RNAs and cDNAs
RNase H	New England Biolabs	M0297	For degradation of RNAs following reverse transcription
RNase-It	Agilent	400720	For RNA digestion
rRNasin	Promega	N2511	For inhibition of any contaminating RNases during enzymatic reaction
Sodium acetate	Sigma Aldrich	S2889-1KG	For phenol-chloroform extraction following RNA purification and DNA precipitation

Sodium chloride	Thermo Fisher Scientific	7647-14-5	Buffer component
Sodium deoxycholate	Sigma Aldrich	D6750-100G	Buffer component in mammalian cell lysis
Sodium dodecylsulfate	Sigma Aldrich	L3771-1KG	For protease K buffer
SUPERase-In	Invitrogen	AM2694	For inhibition of cellular RNases after lysis
SuperScript IV	Thermo Fisher Scientific	18090010	For reverse transcription
T4 PNK	New England Biolabs	M0201	For radiolabelling the 5' end of the RNA
T4 RNA ligase 1	New England Biolabs	M0204	For ligation of the L5 adaptor onto the RNA 5' end
T4 RNase ligase 2, truncated K222Q	NEB	M0351S	For ligation of the App_PE linker onto the 3' end of captured RNAs
TBE buffer (10X)	Invitrogen	15581-028	For running TBE gels
TEV protease	Homemade		For eluting captured proteins following FLAG capture
Thermosensitive alkaline phosphatase	Promega	M9910	For 5' and 3' dephosphorylation of RNAs
Trichloroacetic acid (100%)	Sigma Aldrich	T0699-100ML	For precipitation of RBP-RNA complexes
Tris hydrochloride	Invitrogen	15504-020	Buffer component
Triton X-100	Sigma Aldrich	T8787-100ML	Buffer component in mammalian cell lysis
Vari Filter	UVO3	N/A	Device for vacuum harvesting cells. Available from https://www.vari-x-link.com for 100 GBP
Vari-X-Linker	UVO3	N/A	Cross-linker for cross-linking cells. Available from https://www.vari-x-link.com for 16,000 GBP
Yeast nitrogen base	Formedium	CYN0410	For preparation of synthetic defined medium
Zirconia beads	Thistle	11079105Z for yeast or 11079101Z for bacteria	For cell lysis via bead beating