

Materials List for:

Mapping RNA-RNA Interactions Globally Using Biotinylated Psoralen

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Materials

Name	Company	Catalog Number	Comments
1 kb Plus DNA Ladder	Life Technologies Holdings Pte Ltd	10787026	DNA ladder
10 bp DNA ladder	Life Technologies Holdings Pte Ltd	10821-015	DNA ladder
20% SDS solution	First BASE	BUF-2052-1L	
20x SSC	First BASE	BUF-3050-20X1L	
3.0 M Sodium Acetate Solution	First BASE	BUF-1151-1L-pH5.2	Required for nucleic acid precipitation
40% Acrylamide/Bis Solution, 19:1	Bio-Rad	1610145	TBE Urea gel component
Ambion Buffer Kit	Life Technologies Holdings Pte Ltd	AM9010	
Ammonium Persulfate, Molecular Grade	Promega	V3131	TBE Urea gel component
Bromophenol Blue	Sigma-Aldrich	B0126-25G	
Chloroform	Merck	1.02445.1000	RNA extraction
Single strand DNA ligase	Epicentre	CL9025K	CirLigase II ssDNA Ligase
Centrifuge tube filters	Sigma-Aldrich	CLS8160-96EA	Costar Spin-X centrifuge tube filters
D5628-1G DIGITONIN CRYSTALLINE	Sigma-Aldrich	D5628-1G	For cell treatment
Dark Reader Transilluminator	Clare Chemical Research	Dark Reader DR89X Transilluminator	Blue light transilluminator
DNA Gel Loading Dye (6x)	Life Technologies Holdings Pte Ltd	R0611	Required for agarose gel electrophoresis
Dulbecco's Modified Eagle Medium	Pan BioTech	P04-03500	For HeLa cell culture
Streptavidin magnetic beads	Life Technologies Holdings Pte Ltd	65002	Dynabeads MyOne Streptavidin C1
Magnetic stand for 15 mL tubes	Life Technologies Holdings Pte Ltd	12301D	DynaMag-15
Magnetic stand for 15 mL tubes	Life Technologies Holdings Pte Ltd	12321D	DynaMag-2
ThermoMixer	Eppendorf	5382 000.015	Eppendorf ThermoMixer C
Biotinylated psoralen	Life Technologies Holdings Pte Ltd	29986	EZ-Link Psoralen-PEG3-Biotin
F8T5/BL	Hitachi	F8T5/BL	365 nm UV bulb
Fetal Bovine Serum	Life Technologies Holdings Pte Ltd	10270106	Components of HeLa medium
Formamide	Promega	H5052	Component in hybridization buffer
G8T5	Sankyo-Denki	G8T5	254 nm UV bulb
Glycogen	Life Technologies Holdings Pte Ltd	10814010	Required for nucleic acid precipitation
Nanodrop	Life Technologies Holdings Pte Ltd	Nanodrop 2000	Spectrophotometer for nucleic acid quantification
Nuclease free water	First BASE	BUF-1180-500ml	

Penicillin Streptomycin	Life Technologies Holdings Pte Ltd	15140122	Components of Hela medium
High-Fidelity DNA polymerase (2x)	Life Technologies Holdings Pte Ltd	F531L	Phusion High-Fidelity PCR Master Mix with HF Buffer (2x)
Primers Set 1	New England Biolabs	E7335L	PCR primers
DNA Gel Extraction Kit	QIAgen	28106	QIAquick Gel Extraction Kit
Fluorometric Quantification kit	Life Technologies Holdings Pte Ltd	Q32854	Qubit dsDNA HS Assay Kit
RNA clean up kit	Qiagen	74106	RNeasy Mini Kit Silica-membrane column
Rnase Inhibitor	Life Technologies Holdings Pte Ltd	AM2696	SUPERase In
Reverse transcriptase	Life Technologies Holdings Pte Ltd	18080400	SuperScript III First-Strand Synthesis SuperMix
Nucleic Acid Gel Stain	Life Technologies Holdings Pte Ltd	S11494	SYBR GOLD NUCLEIC ACID 500 UL
T4 Polynucleotide Kinase	New England Biolabs	M0201L	End repair enzyme
T4 RNA Ligase 1	New England Biolabs	M0204L	Enzyme for proximity ligation
T4 RNA Ligase 2, truncated KQ	New England Biolabs	M0373L	Enzyme for adaptor ligation
Temed	Bio-Rad	1610801	TBE Urea gel component
Guanidinium thiocyanate-phenol-chloroform	Life Technologies Holdings Pte Ltd	15596018	TRIzol® Reagent for RNA extraction
Urea	First BASE	BIO-2070-5kg	
UV crosslinker	Stratagene	400072	UV Stratalinker 1800
UV Transilluminator	UVP	95-0417-01	For visualizing of bands
Xylene Cyanol FF	Sigma-Aldrich	X4126-10G	
DNA cleanup it	Zymo Research	D4004	Zymo DNA concentrator-5
List of software required			
FastQC software	0.11.4	http://www.bioinformatics.babraham.ac.uk/projects/fastqc/	
SeqPrep software	1.0.7	https://github.com/jstjohn/SeqPrep	
BWA software	0.7.12	http://bio-bwa.sourceforge.net/	
SAMTOOLS software	1.2.1	http://www.htslib.org/	
PULLSEQ software	1.0.2	https://github.com/bcthomas/pullseq	
STAR software	2.5.0c	https://github.com/alexdobin/STAR	
rem_dups.py PYTHON script	PYTHON 2.7.11	https://github.com/CSB5/splash/tree/master/src	
find_chimeras.py PYTHON script	PYTHON 2.7.11	https://github.com/CSB5/splash/tree/master/src	
pickJunctionReads.awk AWK script	AWK GNU 4.1.2	https://github.com/CSB5/splash/tree/master/src	
Buffer composition			
Elution buffer			
0.3 M sodium acetate			
Lysis Buffer			
50 mM Tris-Cl pH 7.0			
10 mM EDTA			
1% SDS			
Always add Superase-in fresh before use except when washing beads			

Proteinase K Buffer			
100 mM NaCl			
10 mM Tris-Cl pH 7.0			
1 mM EDTA			
0.5% SDS			
Hybridization Buffer			
750 mM NaCl			
1% SDS			
50 mM Tris-Cl pH 7.0			
1 mM EDTA			
15% formamide (store in the dark at 4 °C)			
Always add Superase-in fresh before use			
2x SSC Wash Buffer			
2x NaCl and Sodium citrate (SSC) (diluted from 20x SSC Invitrogen stock)			
0.5% SDS			
2x RNA fragmentation buffer			
18 mM MgCl ₂			
450 mM KCl			
300 mM Tris-Cl pH 8.3			
2x RNA loading Dye			
95% Formamide			
0.02% SDS			
0.02% bromophenol blue			
0.01% Xylene Cyanol			
1 mM EDTA			
3' RNA adapter sequence: 5rAppCTGTAGGCACCATCAAT/3ddC			
RT primer sequence: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGC/iSp18/CACTCA/iSp18/ TTCAGACGTGTGCTCTTCCGATCTATTGATGGTGCCTACAG			