

Materials List for:

mRNA Interactome Capture from Plant Protoplasts

Zhicheng Zhang¹, Kurt Boonen¹, Meixia Li¹, Koen Geuten¹

¹Department of Biology, KU Leuven

Correspondence to: Koen Geuten at koen.geuten@kuleuven.be

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Materials

Name	Company	Catalog Number	Comments
REAGENTS			
0.8 M Mannitol	Sigma	M1902-500G	Primary isotonic Enzyme solution & MMg solution
2 M KCl	MERCK	Art. 4935	Primary isotonic Enzyme solution & W5 buffer
0.2 M MES (pH 5.7) (4-morpholineethanesulfonic acid)	Sigma-aldrich	M2933	Primary isotonic Enzyme solution, W5 buffer & MMg solution, Filtration sterilization
Cellulase R10	Yakult Pharmaceutical Industry Co., Ltd.	CELLULASE "ONOZUKA" R-10, 10 g	Final isotonic enzyme solution
Macerozyme R10	Yakult Pharmaceutical Industry Co., Ltd.	MACEROZYME R-10, 10g	Final isotonic enzyme solution
10% (w/v) BSA (Bovine Serum Albumin)	Sigma-aldrich	A7906-100G	Final isotonic enzyme solution & Filtration sterilization
1 M CaCl ₂	Chem-Lab NV	CL00.0317.1000	Final isotonic enzyme solution, W5 buffer & Digestion buffer
1 M NaCl	Fisher Chemical	S/3160/60	W5 buffer
2 M MgCl ₂	Sigma	M8266-100G	MMg solution
1 M LiCl (Lithium Chloride)	Acros	199885000	Lysis/binding buffer, Wash buffer 1, Wash buffer 2 & Low salt buffer
5% (w/v) LiDS (Lithium Dodecyl Sulphate)	Sigma-aldrich	L4632-25G	Lysis/binding buffer, Wash buffer 1 & Filtration sterilization
1 M DTT (Dithiothreitol)	Thermo Fisher Scientific	307866	Lysis/binding buffer, Wash buffer 1 & Wash buffer 2
1 M Tris-HCl (pH 7.5) (Tris(hydroxymethyl)aminomethane, Hydrochloric acid S.G. (HCl))	Acros & Fisher Chemical	167620010 & H/1200/PB15	Lysis/binding buffer, Wash buffer 1, Wash buffer 2, Low salt buffer & Elution buffer
0.5 M EDTA (pH 8.0) (Ethylenediaminetetraacetic acid)	Sigma-aldrich	ED-500G	Lysis/binding buffer, Wash buffer 1, Wash buffer 2, Low salt buffer & Elution buffer
Tween 20	MERCK	8.22184.0500	Regeneration of oligo-d(T) ₂₅ beads
0.1 M NaOH	VWR PROLABO CHEMICALS	28244.295	Regeneration of oligo-d(T) ₂₅ beads
1x PBS (pH 7.4)	Fisher Chemical, MERCK,	S/3160/60,	Regeneration of oligo-d(T) ₂₅ beads

(Phosphate Buffered Saline) containing (NaCl; KCl; Na ₂ HPO ₄ ; KH ₂ PO ₄)	Sigma-aldrich & SAFC	Art. 4935, 71640-250G & 60230	
Proteinase K solution (2 µg/µL)	Thermo Fisher Scientific	11789020	Protein digestion
Loading dye	Invitrogen	LC5925	SDS-PAGE
qPCR master mix	Promega	A6001	qRT-PCR assay
RNase Cocktail	Thermo Fisher Scientific	AM2286	RNA digestion
Methanol	Sigma-aldrich	322415	Gel fixation and gel destaining
Acetic acid	Sigma-aldrich	537020	Gel fixation and gel destaining
Coomassie Brilliant Blue R-250	Thermo Fisher Scientific	20278	Gel staining
1 M NH ₄ HCO ₃ (Ammonium bicarbonate)	Sigma-aldrich	09830-500G	Gel hydration & Digestion buffer
CH ₃ CN (Acetonitrile)	Sigma-aldrich	34851-100ML	Gel dehydration & Peptide dissolving solution
IAA (Iodoacetic acid)	Sigma-aldrich	I4386-10G	Alkylating agent
TFA (Trifluoroacetic acid)	Sigma-aldrich	302031-10X1ML	Peptide dissolving solution
FA (Formic acid)	Sigma-aldrich	06554-5G	Peptide extraction
Trypsin solution (6 ng/µL)	Promega	V5280	Digestion buffer
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EQUIPMENT			
Soil	Peltracom	LP2D	Plant growth
Vermiculite 3	Sibli AS	05VERMICULIET	Plant growth
Petri dish (150 x 20 mm)	Sarstedt	82.1184.500	Carrier for protoplast suspension
0.22 µm filter	Millipore	SE2M229104	Homogenization of final isotonic enzyme solution
Razorblade	Agar Scientific	T585	Rosette leaf strips
35-75 µm nylon mesh	SEFAR NITEX	74010	Protoplast suspension filtration
50 mL round bottom tubes	Sigma-aldrich	T1918-10EA	Carrier for protoplast suspension
Hemocytometer (Bürker hemocytometer)	MARIENFELD	650030	Protoplast cell counting
UV crosslinking apparatus (HL-2000 HybriLinker)	UVP, LLC	UVP95003101	<i>in vivo</i> UV crosslinking
UV lamp (Sankyo-Denki G8T5)	SANKYO DENKI	SD G8T5	<i>in vivo</i> UV crosslinking
50 mL glass syringe	FORTUNA Optima	Z314560	Homogenization of protoplast lysate
Narrow needle (0.9 x 25 mm)	Becton Dickinson microlance 3	2021-04	Homogenization of protoplast lysate
Rotator Model L26	Labinco BV	26110912	Sample incubation by rotating
Oligo-d(T) ₂₅ magnetic beads (5 mg/mL)	New England BioLabs	S1419S	mRNPs and mRNAs binding and pull-down
Magnetic rack	Invitrogen	CS15000	mRNPs and mRNAs binding and pull-down
Centrifugal filter units (Amicon Ultra-4 centrifugal filter units)	EMD Millipore	UFC800308	mRBP concentration
Pierce Silver Stain Kit	Thermo Fisher Scientific	24612	Silver-staining assay
RNA purification kit	STRATEC Molecular	1064100300	RNA purification

(InviTrap Spin Plant RNA Mini Kit)			
Spectrophotometer device (NanoDrop 1000 Spectrophotometer)	Thermo Fisher Scientific	ND-1000	RNA quality and quantity
Real-Time PCR cycler (StepOne Real-Time PCR cycler)	Thermo Fisher Scientific	4376600	cDNA quantification
μ-C18 columns (Millipore Zip Tip μ-C18 columns)	Sigma-aldrich	720046-960EA	Peptide purification
Mass spectrometer (Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer)	Thermo Fisher Scientific	IQLAAEGAAPFALGMAZR	Mass spectrometry-based proteomics
Liquid chromatography instrument (Ultimate 3000 ultra-high performance liquid chromatography (UHPLC) instrument)	Thermo Fisher Scientific	ULTIM3000RSLCNANO	Mass spectrometry-based proteomics
C18 column (Easy Spray Pepmap RSLC C18 column)	Thermo Fisher Scientific	ES800	Mass spectrometry-based proteomics
C18 precolumn (Acclaim Pepmap 100 C18 precolumn)	Thermo Fisher Scientific	160321	Mass spectrometry-based proteomics
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Primers for qRT-PCR assay	Sequences		
<i>UBQ10</i> mRNA (Li <i>et al.</i> , 2014)	Fw: AACTTTGGTGGTTTGTGTTTTGG Rv: TCGACTTGTCATTAGAAAGAAAGAGATAA		
18S rRNA (Durut <i>et al.</i> , 2014)	Fw: CGTAGTTGAACCTTGGGATG Rv: CACGACCCGGCCAATTA		