

Materials List for:

Identification of Protein Complexes in *Escherichia coli* using Sequential Peptide Affinity Purification in Combination with Tandem Mass Spectrometry

Mohan Babu^{1,2}, Olga Kagan¹, Hongbo Guo¹, Jack Greenblatt^{1,3}, Andrew Emili^{1,3}

¹Banting and Best Department of Medical Research, Donnelly Centre, University of Toronto

²Department of Biochemistry, Research and Innovation Centre, University of Regina

³Department of Medical Genetics and Microbiology, University of Toronto

Correspondence to: Andrew Emili at andrew.emili@utoronto.ca

URL: <https://www.jove.com/video/4057>

DOI: [doi:10.3791/4057](https://doi.org/10.3791/4057)

Materials

Name	Company	Catalog Number	Comments
I. Antibiotics			
Kanamycin	Bioshop	#KAN201	
Ampicillin	Bioshop	#AMP201	
2. Terrific-Broth medium			
Bio-Tryptone	Bioshop	#TRP 402	
Yeast extract	Bioshop	#YEX 555	
Glycerol	Bioshop	#GLY 002	
K ₂ HPO ₄	Bioshop	#PPM 302	
KH ₂ PO ₄	Bioshop	#PPM 303	
3. Bacterial Strain and Plasmid			
DY330		Yu <i>et al.</i> (2000) ¹⁰	
pJL148		Zeghouf <i>et al.</i> (2004) ⁷	
4. PCR and Electrophoresis Reagents			
Taq DNA polymerase	Fermentas	# EP0281	
10 X PCR buffer	Fermentas	# EP0281	
10 mM dNTPs	Fermentas	# EP0281	
25 mM MgCl ₂	Fermentas	# EP0281	
Agarose	Bioshop	# AGA002	
Loading dye	NEB	#B7021S	
Ethidium bromide	Bioshop	# ETB444	
10X TBE buffer	Thermo Scientific	#28355	
Tris Base	Bioshop	#TRS001	
Boric acid	Bioshop	#BOR001	
0.5 M EDTA (pH 8.0)	Sigma	# E6768	
DNA ladder	NEB	#N3232L	
5. Plasmid isolation and Clean-up Kits			
Plasmid Midi kit	Qiagen	#12143	
QIAquick PCR purification kit	Qiagen	#28104	
6. PCR and Transformation Equipments			
Thermal cycler	BioRad	iCycler	
Agarose gel electrophoresis	BioRad		
Electroporator	Bio-Rad	GenePulser II	

0.2 cm electroporation cuvette	Bio-Rad		
42 °C water bath shaker		Innova 3100	
Beckman Coulter TJ-25 centrifuge	Beckman Coulter	TS-5.1-500	
32 °C Shaker	New Brunswick Scientific, USA		
32 °C large Shaker	New Brunswick Scientific, USA		
32 °C plate incubator	Fisher Scientific		
7. Electrophoresis and Western blotting			
Acrylamide monomer, N,N'-methylenebis-acrylamide	Bio-Rad	#161-0125	
Ammonium persulfate	Bioshop	# AMP001	
n-butanol	Sigma	# B7906	
TEMED	Bioshop	#TEM001	
Whatman No. 1 filter paper	Fischer Scientific	#09-806A	
Mini protean 3 cell	Bio-Rad	#165-3301	
iBlot gel transfer device	Invitrogen	#IB1001	
Nitrocellulose membranes	Bio-Rad	#162-0115	
Monoclonal Anti-Flag M2 antibody	Sigma	#F3165	
Horseradish peroxidase	Amersham	#NA931V	
Pre-stained protein molecular weight standards	Bio-Rad	#161-0363	
Chemiluminescence reagent	PIERCE	#1856136	
Autoradiography film	Clonex Corp	#CLEC810	
Quick Draw blotting paper	Sigma	#P7796	
C2 platform rocking shaker	New Brunswick Scientific, USA		
8. Sonication Equipment and Reagents			
Sonicator	Branson Ultrasonic	#23395	
NaCl	Bioshop	#SOD001	
Protease inhibitors	Roche	#800-363-5887	
0.5 mM TCEP-HCl	Thermo Scientific	#20490	
9. Affinity Purification Reagents and Equipment			
0.8 x 4 cm Bio-Rad polypropylene column	Bio-Rad	#732-6008	
Benzonase nuclease	Novagen	#70746	
Anti-FLAG M2 agarose beads	Sigma	#A2220	
Calmodulin-sepharose beads	GE Healthcare	#17-0529-01	
TEV protease	Invitrogen	#12575-015	
Triton X-100	Sigma	#T9284	
CaCl ₂	Sigma	#C2661	
EGTA	Sigma	#E3889	
LabQuake Shaker	Thermolyne	#59558	
10. Silver Staining Reagents			
Methanol	Bioshop	#MET302	
Acetic acid	Bioshop	#ACE222	
Sodium-thiosulfate	Sigma	#S-7143	
Silver nitrate	Fischer Scientific	#S181-100	
Formaldehyde	Bioshop	#FOR201	

Sodium carbonate	Bioshop	#SOC512	
11. Reagents and Equipment for Protein Identification			
Trypsin Gold, Mass Spectrometry Grade	Promega	# V5280	
50 mM NH ₄ HCO ₃	Bioshop	#AMC555	
1 mM CaCl ₂	Bioshop	#CCL302	
Acetonitrile	Sigma	#A998-4	
Formic acid	Sigma	#F0507	
HPLC grade water	Sigma	#95304	
Iodoacetamide	Sigma	#16125	
Millipore Zip-Tip	Millipore	# ZTC18M960	
~10 cm of 3 μm Luna-C18 resin	Phenomenex		
Proxeon nano HPLC pump	Thermo Fisher Scientific		
LTQ Orbitrap Velos mass spectrometer		Thermo Fisher Scientific	
12. Labware			
4 liter conical flasks	VWR	#89000-372	
50 ml polypropylene falcon tubes	Any Vendor		
1.5 ml micro-centrifuge tubes	Any Vendor		
250 ml conical flaks	VWR	#29140-045	
15 ml sterile culture tubes	Thermo Scientific	#366052	
Cryogenic vials	VWR	#479-3221	
-80 °C freezer	Fisher Scientific	#13-990-14	
Speed vacuum system	Thermo Scientific		
Buffers and Solutions			
1. 1 liter Terrific Broth (TB) media			
<p>11 g Bio-Tryptone 22 g Yeast Extract 2% Glycerol 50 ml potassium salt stock solution</p>			
2. Potassium Salt Stock Solution			
<p>1.5 M K₂HPO₄ 0.35 M KH₂PO₄</p>			
3. Sonication Buffer			
<p>20 mM Tris-HCl (pH 7.9) 150 mM NaCl 0.2 mM EDTA 10% Glycerol Before use add protease inhibitor (PI) and 0.1-0.5 mM TCEP</p>			
4. AFC buffer			
<p>30 mM Tris-HCl (pH 7.9) 150 mM NaCl 0.1% detergent Before use add PI and 0.1-0.5 mM TCEP</p>			
5. TEV cleavage buffer			
<p>30 mM Tris-HCl (pH 7.9) 150 mM NaCl 0.2 mM EDTA 0.1% detergent</p>			

Before use add PI and 0.1-0.5 mM TCEP

6. Calmodulin binding buffer

30 mM Tris-HCl (pH 7.9)
150 mM NaCl
2 mM CaCl₂
0.1% detergent
Before use add PI and 0.1-0.5 mM TCEP

7. Calmodulin wash buffer

30 mM Tris-HCl pH 7.9
150 mM NaCl
2 mM CaCl₂
0.1-0.5 mM TCEP

8. Calmodulin elution buffer

30 mM Tris-HCl (pH 7.9)
100 mM NaCl
10 mM EGTA
0.1-0.5 mM TCEP

9. Developing solution (1L)

37% Formaldehyde
30 g sodium carbonate
1000 ml distilled water

10. Digestion buffer

50 mM NH₄HCO₃
1 mM CaCl₂

11. Wetting and Equilibration solution

70% acetonitrile (ACN) in 0.1% formic acid

12. Washing solution

100% H₂O in 0.1% formic acid