

Materials List for:

Mapping Bacterial Functional Networks and Pathways in *Escherichia Coli* using Synthetic Genetic Arrays

Alla Gagarinova^{*1}, Mohan Babu^{*2,3}, Jack Greenblatt^{1,2}, Andrew Emili^{1,2}

¹Department of Molecular Genetics, University of Toronto

²Banting and Best Department of Medical Research, Donnelly Centre, University of Toronto

³Department of Biochemistry, Research and Innovation Centre, University of Regina

*These authors contributed equally

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

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Materials

Name	Company	Catalog Number	Comments
I. Antibiotics			
Chloramphenicol	Bioshop	#CLR201	
Kanamycin		#KAN201	
Ampicillin		# AMP201	
2. Luria-Bertani medium			
LB powder	Bioshop	#LBL405	
Agar	Bioshop	#AGR003	
3. Bacterial Strains and Plasmids			
Hfr Cavalli strain λ_{red} system (JL238)	Babu <i>et al.</i> ¹⁴		
pKD3	<i>E. coli</i> Genetic Stock Centre, Yale		
Keio <i>E. coli</i> F- recipient collection	National BioResource Project (NBRP) of Japan ¹¹		
Hypomorphic <i>E. coli</i> F- SPA-tag strains	Open biosystems; Babu <i>et al.</i> ¹⁴		
4. Primers			
pKD3-based desalted constant primers			F1: 5'-GGCTGACATGGGAATTAGC-3' R1: 5'-AGATTGCAGCATTACACGTCTT-3'
Desalted custom primers			Cm-R: 5'-TTATACGCAAGGCGACAAGG-3' Cm-F: 5'-GATCTTCCGTACAGGTAGG-3'
Desalted custom primers			F2 and R2: 20 nt constant regions based on pKD3 sequence and 45 nt custom homology regions F2 constant region: 5'-CATATGAATATCCTCCTTA-3' R2 constant region: 5'-TGTGTAGGCTGGAGCTGCTTC-3' S1 and S2: 27 nt constant regions for priming the amplification of the SPA-Cm cassette and 45 nt custom homology regions S1 constant region: 5'AGCTGGAGGATCCATGGAAAAGAGAAG-3'

			<p>S2 constant region: 5'- GGCCCCATATGAATATCCTCCTTAGTT -3' KOCO-F and KOCO-C: 20 nt primers 200 bp away from the non- essential gene deletion site or the essential gene SPA-tag insertion site</p>
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5. PCR and Electrophoresis Reagents

Taq DNA polymerase	Fermentas	# EP0281	
10X 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	Bioshop	# ETB444.10	
Tris Base	Bioshop	# TRS001	
Boric acid	Sigma	# T1503-1KG	
0.5 M EDTA (pH 8.0)	Sigma	# B6768-500G	
DNA ladder	NEB	#N3232L	

6. DNA isolation and Clean-up Kits

Genomic DNA isolation and purification kit	Promega	#A1120	
Plasmid Midi kit	Qiagen	# 12143	
QIAquick PCR purification kit	Qiagen	#28104	

7. Equipment for PCR, Transformation and Replica-pinning

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		
32 °C shaker	New Brunswick Scientific, USA		
32 °C plate incubator	Fisher Scientific		
RoToR-HDA benchtop robot	Singer Instruments		
96, 384 and 1,536 pin density pads	Singer Instruments		
96 or 384 long pins	Singer Instruments		

8. Imaging Equipments

Camera stand	Kaiser		
Digital camera, 10 megapixel	Any Vendor		
Light boxes, Testrite 16" x 24" units	Testrite		

9. Pads or Plates Recycling

10% bleach	Any Vendor		
70% ethanol	Any Vendor		
Sterile distilled water	Any Vendor		
Flow hood	Any Vendor		
Ultraviolet lamp	Any Vendor		

10. Labware			
50 ml polypropylene tubes	Any Vendor		
1.5 ml micro-centrifuge tubes	Any Vendor		
250 ml conical flasks	VWR	# 29140-045	
15 ml sterile culture tubes	Thermo Scientific	# 366052	
Cryogenic vials	VWR	# 479-3221	
Rectangular Plates	Singer Instruments		
96-well and 384-well microtitre plates	Singer Instruments	Nunc	
Plate roller for sealing multi-well plates	Sigma	#R1275	
	ABgene	# AB-0580	
Adhesive plate seals	Fisher Scientific	# 13-990-14	
-80 °C freezer	Any Vendor		