

Materials List for

Generating Transposon Insertion Libraries in Gram-Negative Bacteria for High-Throughput Sequencing

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Materials

Name	Company	Catalog Number	Comments
10 mM ddCTP, 2',3'-Dideoxycytidine-5'-Triphosphate	Affymetrix	77112	
100 mM dCTP 2'-Deoxycytidine-5'-Triphosphate	Invitrogen	10217-016	
100bp DNA Ladder Molecular Weight Marker	Promega	PR-G2101	
100mm x 15mm Petri Dishes	Corning	351029	
150mm x 15mm Petri Dishes	Corning	351058	
1X B&W	N/A	N/A	Dilute 2X B&W by half to get 1X B&W.
2,6-Diaminopimelic acid	Alfa Aesar	B2239103	used at 600 µM
2X B&W	N/A	N/A	Add 2 M NaCl, 10 mM Tris-HCl, 1 mM EDTA (pH 7.5) in water. Used with Streptavidin beads. Solutions keep at room temperature.
50mL Conical Sterile Polypropylene Centrifuge Tubes	Fisher Scientific	12-565-271	
9.5 mM dCTP/0.5 mM ddCTP	N/A	N/A	9.5 ml 100 mM dCTP; 5 ml 10 mM ddCTP; 85.5 ml water. Store at -20°C.
AccuPrime™ Pfx DNA Polymerase	Invitrogen	12344	
Acinetobacter baumannii ATCC 17978	ATCC	N/A	Amp ^S , Kan ^S
Ampicillin (100 mg/L)	Fisher Scientific	BP1760	used at 100 mg/L
AMPure XP PCR purification system	BECKMAN COULTER	A63881	
BioAnalyzer	Agilent	G2939B	
Bioanalyzer High Sensitivity DNA Analysis	Agilent	5067-4626	
Deoxynucleotide Solution Mix (dNTP)	New England Biolabs (NEB)	N0447L	
DynaMag-2 Magnetic rack	Invitrogen	12321D	
E.coli MFD Dap-	N/A	N/A	DAP Auxotroph, requires 600 mM exogenously added DAP to grow.

			Contains RP4 machinery for plasmid transfer. Carrier for JNW68 (36).
Ethanol	Fisher Scientific	A4094	
Externally Threaded Cryogenic Vials	Corning	09-761-71	
Glass beads	Corning	72684	
Glycerol	Fisher Scientific	G33	
Inoculating loops	Fisher Scientific	22-363-602	Scraping tool
Kanamycin	Fisher Scientific	BP906	used at 25 mg/L
LB agar, Miller	Fisher Scientific	BP1425	
LB broth, Miller	Fisher Scientific	BP1426	
LoTE	N/A	N/A	Add 3 mM Tris-HCl, 0.2 mM EDTA (pH 7.5) in water. Used with Streptavidin beads. Solutions keep at room temperature.
Lysis buffer	N/A	N/A	9.34 mL TE buffer; 600 ml of 10% SDS; 60 ml of proteinase K (20 mg/mL)
Phenol/Chloroform/Isoamyl Alcohol (25:24:1 Mixture, pH 6.7/8.0, Liq.)	Fisher Scientific	BP1752I	
Phosphate Buffered Saline, 10X Solution	Fisher Scientific	BP39920	Diluted to 1X
Qubit 4 Fluorometer	Thermo Fisher	Q33238	
Qubit Assay Tubes	Thermo Fisher	Q32856	
Qubit dsDNA HS Assay Kit	Thermo Fisher	Q32851	
Sonicator with refridgerated waterbath	Qsonica Sonicators	Q2000FCE	
Streptavidin Magnetic Beads	New England Biolabs (NEB)	S1420S	
TE buffer	N/A	N/A	10 mM Tris-HCl (pH 8.0); 1 mM EDTA (pH 8.0)
Terminal Deoxynucleotidyl Transferase (rTdt)	Promega	PR-M1875	