

Materials List for:

Generation of In-Frame Gene Deletion Mutants in *Pseudomonas aeruginosa* and Testing for Virulence Attenuation in a Simple Mouse Model of Infection

Meagan E. Valentine¹, Brandon D. Kirby¹, Hongwei D. Yu^{1,2}

¹Progenesis Technologies, LLC

²Department of Biomedical Sciences, Pediatrics, Joan C. Edwards School of Medicine at Marshall University

Correspondence to: Hongwei D. Yu at yuh@marshall.edu

URL: <https://www.jove.com/video/60297>

DOI: [doi:10.3791/60297](https://doi.org/10.3791/60297)

Materials

Name	Company	Catalog Number	Comments
0.2 mL tubes with flat caps	ThermoScientific	AB-0620	via Fisher Scientific
1 mL Syringe	BD	22-253-260	via Fisher Scientific
1.5 mL disposable polystyrene cuvette	Fisher Scientific	14955127	
1.5 mL Microcentrifuge Tubes	Fisher Scientific	05-408-129	
2.0 mL Cryogenic Vials	Corning	430659	via Fisher Scientific
27G needle	BD	14-821-13B	via Fisher Scientific
50 mL tubes	Fisher Scientific	05-539-13	via Fisher Scientific
Accu block Digital Dry Bath	Labnet	NC0205808	via Fisher Scientific
Benchtop Centrifuge 5804R	Eppendorf	04-987-372	via Fisher Scientific
Benchtop Microcentrifuge	Sorvall	75-003-287	via Fisher Scientific
Cabinet Incubator	VWR	1540	
Carbenicillin disodium salt	Fisher Scientific	BP2648250	
Culture Test Tube, Polystyrene	Fisher Scientific	14-956-6D	via Fisher Scientific
Diposable Inoculation Loops	Fisher Scientific	22-363-597	
Dneasy UltraClean Microbial Kit (50)	Qiagen	12224-50	or preferred method/vendor
E.Z.N.A. Cycle Pure Kit (50)	Omega bio-tek	D6493-01	or preferred method/vendor
EcoRI-HF, restriction endonuclease	New England BioLabs	R3101L	
Electroporation Cuvettes	Bulldog Bio	NC0492929	via Fisher Scientific
FastLink II DNA Ligation Kit	Epicentre Technologies	LK6201H	via Fisher Scientific
Gentamycin Sulfate	Fisher Scientific	BP918-1	
Glycerol	Fisher Scientific	BP229-4	
GoTaq G2 Colorless Master Mix	Promega	M7833	via Fisher Scientific
Isothesia Isoflurane	Henry Schein Animal Health	29405	
IVIS Lumina XRMS Series III In Vivo Imaging System	Perkins and Elmer	CLS136340	
Kanamycin monosulfate	Fisher Scientific	BP906-5	
LE agarose	Genemate	3120-500	via Fisher Scientific
Luria Broth	Difco	240230	via Fisher Scientific
MicroPulser Electroporator	BioRad	1652100	
Noble agar, ultrapure	Affymetris/USB	AAJ10907A1	via Fisher Scientific
NotI-HF, restriction endonuclease	New England BioLabs	R3189	

One Shot TOP10 Electrocomp <i>E. coli</i>	Invitrogen	C404052	via Fisher Scientific
Phosphate buffered saline powder	Sigma	P3813-10PAK	Sigma-Aldrich
Prism 7	GraphPad		https://www.graphpad.com/scientific-software/prism/
<i>Pseudomonas</i> isolation agar	Difco	292710	via Fisher Scientific
<i>Pseudomonas</i> isolation broth	Alpha Biosciences	P16-115	Custom made batch
QIAprep Spin Miniprep Kit (250)	Qiagen	27106	or preferred method/vendor
Shaking Incubator	New Brunswick Scientific	Innova 4080	shake at 200 rpm
SimpliAmp Thermal Cycler	Applied Biosystems	A24811	
Skim Milk	Difco	DF0032-17-3	via Fisher Scientific
Small Plates (100 O.D. x 10 mm)	Fisher Scientific	FB0875713	
SmartSpec Plus Spectrophotometer	Bio-Rad	170-2525	or preferred method/vendor
Sucrose	Fisher Scientific	S5-500	
Toothpicks, round	Diamond		Any brand of toothpicks, autoclaved
TOPO TA Cloning Kit, for sequencing	Invitrogen	45-0030	
XAF-8 Anesthesia System Filters	Perkins and Elmer	118999	
XGI 8 Gas Anesthesia System	Caliper Life Sciences/Xenogen		