

Materials List for:

Selection-dependent and Independent Generation of CRISPR/Cas9-mediated Gene Knockouts in Mammalian Cells

Erin L. Sternburg¹, Kristen C. Dias¹, Fedor V. Karginov¹

¹Cell Biology and Neuroscience, University of California, Riverside

Correspondence to: Fedor V. Karginov at fedor.karginov@ucr.edu

URL: <https://www.jove.com/video/55903>

DOI: [doi:10.3791/55903](https://doi.org/10.3791/55903)

Materials

Name	Company	Catalog Number	Comments
Competent <i>E. coli</i> cells			
plasmid prep kit			
pSpCas9(BB) plasmid	Addgene	42230	Ran <i>et al.</i> 2013, cloning sgRNAs
BbsI enzyme	ThermoFisher	FD1014	Ran <i>et al.</i> 2013, cloning sgRNAs
T7 DNA ligase	NEB	M0318L	Ran <i>et al.</i> 2013, cloning sgRNAs
Tango Buffer	ThermoFisher	BY5	Ran <i>et al.</i> 2013, cloning sgRNAs
PlasmidSafe exonuclease	Epicentre	E3105K	Ran <i>et al.</i> 2013, cloning sgRNAs
Q5 hot start high fidelity polymerase	NEB	M0494A	HA amplification
pUC19-BsaI			Modified pUC19 plasmid, mutated existing BsaI site and inserted two outward facing BsaI sites after BamHI/EcoRI digestion
pGolden-Neo	Addgene	51422	Resistance cassette
pGolden-Hygro	Addgene	51423	Resistance cassette
BsaI enzyme	NEB	R3535S	Homology arm construction
T7 DNA ligase	NEB	M0318L	
PlasmidSafe exonuclease	Epicentre	E3105K	
Toothpicks			bacterial PCR
Taq polymerase			bacterial colony PCR
HEK293 human cells			
DMEM	Corning	10-013-CV	
FBS	Corning	MT35010CV	
Penicillin-Strep (opt.)	Gibco	15140-122	
6 well plates	BioLite	12556004	
TransIT-LTI Transfection Reagent	Mirus	MIR2300	for lipofection only
Opti-MEM	ThermoFisher	31985062	for lipofection only
G418 (Neomycin)	Sigma Aldrich	A1720-5G	
Hygromycin	Sigma Aldrich	H3274-250MG	
96 well plates	ThermoFisher	12556008	
Passive Lysis Buffer, 5x	Promega		
1x SDS loading buffer			recipe described in protocol
Nitrocellulose Membrane	Bio-Rad	162-0115	
TBST			recipe described in protocol
Dehydrated milk			

SuperSignal West Dura Extended Duration Substrate	ThermoFisher	34075	for HRP-conjugated secondary antibodies
Extracta DNA prep for PCR	Quantabio	95091-025	
KOD polymerase	Novagen	71316	