

Materials List for:

# CRISPR/Cas9 Gene Editing to Make Conditional Mutants of Human Malaria Parasite *P. falciparum*

Heather M. Kudyba<sup>\*1,2</sup>, David W. Cobb<sup>\*1</sup>, Anat Florentin<sup>1,2</sup>, Michelle Krakowiak<sup>1</sup>, Vasant Muralidharan<sup>1,2</sup>

<sup>1</sup>Department of Cellular Biology, University of Georgia

<sup>2</sup>Center for Tropical and Emerging Global Diseases, University of Georgia

\*These authors contributed equally

Correspondence to: Vasant Muralidharan at [vasant@uga.edu](mailto:vasant@uga.edu)

URL: <https://www.jove.com/video/57747>

DOI: [doi:10.3791/57747](https://doi.org/10.3791/57747)

## Materials

Name	Company	Catalog Number	Comments
Gene Pulser Xcell Electroporator	Bio-Rad	1652660	
Gene Pulser Xcell Electroporator	Bio-Rad	165-2086	We buy the ones that are individually wrapped
Sodium Acetate	Sigma-Aldrich	S2889-250g	
DSM1	Gift from Akhil Vaidya lab	Ganesan <i>et al.</i> Mol. Biochem. Parasitol. 2011 177:29-34	
TPP Tissue Culture 6 Well Plates	MIDSCI	TP92006	
TPP 100 mm Tissue Culture Dishes (12 mL Plate)	MIDSCI	TP93100	
TPP Tissue Culture 96 Well Plates	MIDSCI	TP92096	
TPP Tissue Culture 24 Well Plates	MIDSCI	TP92024	
NEBuffer 2	New England Biolabs	#B7002S	
NEBuffer 2.1	New England Biolabs	#B7202S	
BtgZI	New England Biolabs	#R0703L	
SacII	New England Biolabs	#R0157L	
HindIII-HF	New England Biolabs	#R3104S	
Afe1	New England Biolabs	#R0652S	
Nhe1-HF	New England Biolabs	#R3131L	
T4 DNA Polymerase	New England Biolabs	#M0203S	
500 mL Steritop bottle top filter unit	Millipore	SCGPU10RE	You can use any size that fits your needs
EGTA	Sigma	E4378-100G	
KCl	Sigma-Aldrich	P9333-500g	
CaCl <sub>2</sub>	Sigma-Aldrich	C7902-500g	
MgCl <sub>2</sub>	Sigma-Aldrich	M8266-100g	
K <sub>2</sub> HPO <sub>4</sub>	Fisher	P288-500	
HEPES	Sigma-Aldrich	H4034-500g	
pMK-U6	Generated by the Muralidharan Lab	n/a	
pHA-glmS	Generated by the Muralidharan Lab	n/a	
pUF1-Cas9	Gift from the Jose-Juan Lopez-Rubio Lab	Ghorbal <i>et al.</i> Nature Biotech 2014	
Glucose	Sigma-Aldrich	G7021-1KG	

Sodium bicarbonate	Sigma-Aldrich	S5761-500G	
Sodium pyruvate	Sigma-Aldrich	P5280-100G	
Hypoxanthine	Sigma-Aldrich	H9636-25g	
Gentamicin Reagent	Gibco	15710-064	
Thymidine	Sigma-Aldrich	T1895-1G	
PL6-eGFP BSD	Generated by the Muralidharan Lab		
Puf1-cas9 eGFP gRNA	Generated by the Muralidharan Lab		
NucleoSpin Gel and PCR Clean-up	Macherey-Nagel	740609.250	
Albumax I	Life Technologies	N/A	You will want to try a few batches to find out what the parasites will grow in best
Human Red Blood Cells	Interstate Blood Bank, Inc	Email or call them directly for ordering	We typically use O+ blood
3D7 parasite line	Available upon request	N/A	
Lysogeny Broth (LB)	Fisher	BP1426-2	You can make your own, it is not necessary to use exactly this
Ampicilin	Fisher	BP1760-25	We make a 1000X stock at 100mg/ml in water and store in the -20C
Ampicilin	Clontech	R050A	
Anti-EF1alpha	Dr. Daniel Goldberg's Lab	Washington University in St. Louis	You can use your preferred loading control for western blots. This is just the one we use in our laboratory
Rat Anti-HA Clone 3F10, monoclonal	Made by Roche, sold by Sigma	11867423001	You can use your preferred anti-HA antibody
0.6 mL tubes	Fisher	AB0350	
Fisher HealthCare* PROTOCOL* Hema 3* Manual Staining System (Fixative+Solution I and II)	Fisher	22-122-911	You can also use giemsa stain
Fisherfines Premium Frosted Microscope Slides - Size: 3 x 1 in.	Fisher	12-544-3	