

# Gene Knock-in by CRISPR/Cas9 and Cell Sorting in Macrophage and T Cell Lines

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## Citation

 Zhang, L., Huang, R., Lu, L., Fu, R., Guo, G., Gu, Y., Liu, Z., He, L., Malissen, M., Liang, Y. Gene Knock-in by CRISPR/Cas9 and Cell Sorting in Macrophage and T Cell Lines. *J. Vis. Exp.* (177), e62328, doi:10.3791/62328 (2021).

## Date Published

November 13, 2021

## DOI

10.3791/62328

## URL

jove.com/video/62328

## Materials

Name	Company	Catalog Number	Comments
Amersham Imager 600	Ge Healthcare		imaging of chemiluminescence
Ampicillin, sodium salt	MP Biomedicals	194526	
Anti-rabbit IgG, HRP-linked Antibody	Cell Signaling Technology	7074	at 1/5000 dilution
Anti-RasGRP1 antibody, clone 10.1	Merck	MABS146	1.0 µg/mL of working concentration
Ascl	New England BioLabs	R0558S	
β-Actin (D6A8) Rabbit mAb	Cell Signaling Technology	8457	at 1/1000 dilution
BamHI-HF	New England BioLabs	R3136S	
BbsI-HF	New England BioLabs	R3539S	
Cellometer Mini Automated Cell Counter	Nexcelom Bioscience		
<i>E. coli</i> DH5α Competent Cells	Takara	9057	
DMSO (Dimethyl Sulfoxide)	MP Biomedicals	196055	
DNeasy Blood & Tissue Kits	Qiagen	69506	cell culture reagent
DPBS (10X), no calcium, no magnesium	ThermoFisher Scientific	14200075	
Dulbecco's Modified Eagle Medium (DMEM) with high glucose	HyClone	SH30022.01	
EcoRI-HF	New England BioLabs	R3101S	
FACSAria™ Fusion	BD Biosciences		equipped with biosafety cabinet
FACS Canto flow cytometer	BD Biosciences		
Falcon 5 ml polystyrene round bottom test tube	BD Biosciences	352003	
Fetal bovine serum (FBS)	ThermoFisher Scientific	10099141	
FlowJo version 10.7	BD Biosciences		
GAPDH (D16H11) XP Rabbit mAb	Cell Signaling Technology	5174	at 1/1000 dilution
Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP	ThermoFisher Scientific	31430	at 1/5000 dilution
Immobilon ECL Ultra Western HRP Substrate	Millipore	WBKLS0500	

Immobilon-PSQ PVDF Membrane	Millipore	ISEQ00010	
Jurkat	ATCC	TIB-152	<a href="https://www.atcc.org/">https://www.atcc.org/</a>
Kanamycin sulfate	MP Biomedicals	194531	
LB agar powder	ThermoFisher Scientific	22700041	
Multi-channel Pipette (30-300 µL)	Eppendorf, or similar		
Neon Transfection System	ThermoFisher Scientific	MPK5000	
Neon Transfection System, 10 µL kit	ThermoFisher Scientific	MPK1096	
Nunc 15 mL Conical Sterile Centrifuge Tubes	ThermoFisher Scientific	339651	
OneTaq® Hot Start Quick-Load® 2X Master Mix	New England BioLabs	(M0489)	for high GC% template
PageRuler Prestained Protein Ladder, 10 to 180 kDa	ThermoFisher Scientific	26616	
Pipette tip 0.1-20µl	Eppendorf, or similar	0030 075.005	
Pipette tip 2-200µl	Eppendorf, or similar	0030 075.021	
Pipette tip 50-1000µl	Eppendorf, or similar	0030 075.064	
Plasmid Maxi Kit	Qiagen	12163	
pX458-DsRed2	Addgene	112219	
QIAquick PCR Purification Kit	Qiagen	28104	purify plasmid from restriction digestion
Q5 Hot Start High-Fidelity 2X Master Mix	New England BioLabs	M0494S	
RAW264.7	ATCC	TIB-71	<a href="https://www.atcc.org/">https://www.atcc.org/</a>
Recombinant Anti-ACE2 antibody [EPR4435(2)]	Abcam	ab108252	at 1/1000 dilution
RPMI 1640 Medium	HyClone	SH30027.01	
Strep-Tactin Sepharose beads	IBA Lifesciences	2-1201-010	
Penicillin-Streptomycin	ThermoFisher Scientific	15140122	
SYTOX™ Red Dead Cell Stain, for 633 or 635 nm excitation	ThermoFisher Scientific	S34859	
T4 DNA ligase	New England BioLabs	M0202S	
T4 Polynucleotide Kinase	New England BioLabs	M0201S	
Trypan Blue Solution, 0.4%	ThermoFisher Scientific	15250061	
Trypsin-EDTA solution (0.25%), with phenol red	ThermoFisher Scientific	25200056	
ZOE Fluorescent Cell Imager	Bio-Rad		
1.5 mL microtubes, PCR-clean	Eppendorf, or similar	0030 125.215	
24-well Clear TC-treated Multiple Well Plates	Corning	3524	
96-well Clear Flat Bottom Polystyrene TC-treated Microplates	Corning	3599	
96-well Clear Round Bottom TC-treated Microplate	Corning	3799	