

Materials List for:

Enhanced Genome Editing with Cas9 Ribonucleoprotein in Diverse Cells and Organisms

Behnom Farboud^{*1,2}, Erin Jarvis^{*1}, Theodore L. Roth^{*3,4,5,6}, Jiyung Shin^{*1,3}, Jacob E. Corn^{1,3}, Alexander Marson^{3,5,6,7,8,9}, Barbara J. Meyer^{1,2}, Nipam H. Patel^{1,10}, Megan L. Hochstrasser³

¹Department of Molecular Cell Biology, University of California, Berkeley

²Howard Hughes Medical Institute, University of California, Berkeley

³Innovative Genomics Institute, University of California, Berkeley

⁴Biomedical Sciences Graduate Program, University of California, San Francisco

⁵Department of Microbiology and Immunology, University of California, San Francisco

⁶Diabetes Center, University of California, San Francisco

⁷Chan Zuckerberg Biohub

⁸Department of Medicine, University of California, San Francisco

⁹UCSF Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco

¹⁰Department of Integrative Biology, University of California, Berkeley

*These authors contributed equally

Correspondence to: Megan L. Hochstrasser at megan.hochstrasser@berkeley.edu

URL: <https://www.jove.com/video/57350>

DOI: [doi:10.3791/57350](https://doi.org/10.3791/57350)

Materials

Name	Company	Catalog Number	Comments
Reagents/Materials			
DNA oligonucleotides	Integrated DNA Technologies	-	IDT will provide custom DNA sequences, including those in Table 1
Guide RNAs	Synthego	-	Synthego will provide high-quality sgRNAs for <i>S. pyogenes</i> Cas9, including custom sgRNAs containing the targeting sequences included in Table 1
Purified Cas9 protein (EnGen Cas9 NLS, <i>S. pyogenes</i>)	New England Biosciences	M0646T	If possible, purifying Cas9 in-house or purchasing from local core facilities is a less expensive option
Normal peripheral blood CD34+ stem/progenitor cells	AllCells	PB032-2	
StemSpan SFEM	StemCell Technologies	09650	
StemSpan CC110	StemCell Technologies	02697	
P3 Primary Cell 4D-Nucleofector X Kit	Lonza	V4XP-3032	
RPML-1640 Medium, With sodium bicarbonate, without L-glutamine, liquid	Sigma	R0883-6X500ML	
EasySep™ Human T Cell Isolation Kit	Stemcell	17951	
cell culture plate, 96 wells, round	Fisher Scientific	3799	
CTS (Cell Therapy Systems) Dynabeads CD3/CD28	Life Tech	40203D	
Reombinant Human IL-2	UCSF Pharmacy	NA	
SepMate-50 500-pack IVD	Stemcell Technologies	85460	
OP50 <i>Escherichia coli</i>	Caenorhabditis Genetics Center	OP-50	https://cgc.umn.edu/

Nematode Growth Media agar in petri dishes	-	-	See Stiernagle, T (ref. 59)
Standard borosilicate glass capillaries with filament: 4 in (100 mm), 1/0.58 OD/ID	World Precision Instruments	1B100F-4	
Single-barrel standard borosilicate glass capillaries: 6 in (152 mm), 2/1.12 OD/ID	World Precision Instruments	1B200-6	
Cover glass; 24 × 50 mm	Thermo Fisher Scientific	12-544E	
Cover glass; 22 × 22 mm	Thermo Fisher Scientific	12-518-105K	
Apex LE agarose	Genesee Scientific	20-102	
Halocarbon oil 700	Sigma-Aldrich	H8898-100ML	
pCFJ90 plasmid	Addgene	19327	
Compressed nitrogen	-		
60 mM culture dishes	BD		
Capillary tubes with filament: 4 in (1.0 mm)	World Precision Instruments	T2100F-4	
Sylgard 184	Dow Corning		
Petri dishes (100 × 15 mm)	-		
Tungsten wire (0.005 in. diameter)	Ted Pella		
Perfluoroalkoxy alkane (PFA)	-		
Marine salt	-		
9" pasteur pipettes	-		
Phenol red	-		
Nuclease-free water	-		
Equipment			
4D Nucleofector	Lonza	AAF-1002X	
MZ75 Stereomicroscope	Leica	Out-of-production. Current model is the M80 Stereomicroscope	
Axio Vert35 inverted phase contrast fluorescent microscope	Zeiss	Out-of-production. Current model is the Axio VertA.1	
Laser-based micropipette puller (for <i>C. elegans</i> protocol)	Sutter Instrument	FG-P2000	
Picoliter Microinjector (for <i>C. elegans</i> protocol)	Warner Instruments	PLI-100A	
Three-axis Joystick oil hydraulic micromanipulator	Narishige International	MO-202U	
Coarse manipulator	Narishige International	MMN-1	
Micropipette puller (for <i>P. hawaiiensis</i> protocol)	Sutter Instrument	P-80/PC	
Microinjector (for <i>P. hawaiiensis</i> protocol)	Narishige	IM300	
Microloader pipette tips	Eppendorf	5242956003	
NG-agar			