

Materials List for

Engineering Oncogenic Heterozygous Gain-of-Function Mutations in Human Hematopoietic Stem and Progenitor Cells

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Materials

Name	Company	Catalog Number	Comments
175 cm ² Cell Culture Flask, Vent Cap, TC-treated	Corning	431080	
150 mm x 25 mm dishes	Corning	430599	
293T	DSMZ	ACC 635	https://www.dsmz.de/collection/catalogue/details/culture/ACC-635
4D Nucleofector Core Unit	Lonza	-	For nucleofection of human HSPCs use the DZ-100 program.
4D Nucleofector X Unit	Lonza	-	
500 ml Centrifuge Tube	Corning	431123	
7-AAD	BD Biosciences	559925	
AAVpro Purification Kit	Takara	6666	
Alt-R S.p. Cas9 Nuclease V3	Integrated DNA Technologies (IDT)	1081058	
Avanti JXN-30 Ultracentrifuge	Beckman Coulter	-	
Benchling sgRNA design tool			Online tool for sgRNA design: http://www.benchling.com/crispr
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A7906-100G	
C1000 Touch Thermal Cycler	Bio-Rad	-	
Chemically modified synthetic sgRNA	Synthego		Website: https://www.synthego.com/products/crispr-kits/synthetic-sgrna Sequence for the sgRNA targeting intron 7 of CALR: 5'-CGCCTGTAATCCTCGCCCAG-3' An 80 nucleotide SpCas9 scaffold is added to the 20 nucleotide RNA sequence to complete the sgRNA. Chemical modifications of 2'-O-Methyl are added to the first and last 3 bases and 3' phosphorothioate bonds are added in the first 3 and last 2 bases. *Alternatively

			chemically modified synthetic sgRNAs can be acquired from IDT (https://eu.idtdna.com/site/order/oligoentry/index/crispr) and Trilink (https://www.trilinkbiotech.com/custom-oligos)
CHOPCHOP sgRNA design tool			Online tool for sgRNA design: http://chopchop.cbu.uib.no
Costar 24-well Clear TC-treated Multiple Well Plates	Corning	3526	
CRISPick sgRNA design tool			Online tool for sgRNA design: https://portals.broadinstitute.org/gppx/crispick/public
CRISPOR sgRNA design tool			Online tool for sgRNA design: http://crispor.tefor.net
ddPCR 96-Well Plates	Bio-Rad	12001925	
ddPCR Supermix for Probes (no dUTP)	Bio-Rad	1863024	
DG8 Cartridges for QX200/QX100 Droplet Generator	Bio-Rad	1864008	
DG8 Gaskets for QX200/QX100 Droplet Generator	Bio-Rad	1863009	
DreamTaq Green PCR Master Mix (2X)	Thermo Scientific	K1081	
Droplet Generation Oil for Probes	Bio-Rad	1863005	
Dulbecco's Modified Eagle Medium (DMEM) with high glucose	Sigma-Aldrich	D6429-6X500ML	
Dulbecco's Phosphate Buffered Saline (DPBS)	Sigma-Aldrich	D8537-500ML	
FACSAria Fusion	BD Biosciences	-	
Falcon 5 mL Round Bottom	Corning	352054	
Fetal Bovine Serum (FBS) Good Forte (heat inactivated), 500 ml	Pan Biotech	P40-47500	
FlowJo 10.8.0	BD Biosciences	-	
GenAgarose L.E.	Inno-train	GX04090	
GeneRuler 100 bp Plus DNA Ladder	Thermo Scientific	SM0321	
Gibson Assembly Master Mix	New England Biolabs Inc. (NEB)	E2611L	
HEK293T			
HEPES solution	Sigma-Aldrich	H0887-100ML	
ICE	Synthego		https://ice.synthego.com
IDT codon optimization tool	IDT		https://www.idtdna.com/pages/tools/codon-optimization-tool
IDT sgRNA design tool			Online tool for sgRNA design: https://www.idtdna.com/site/order/designtool/index/CRISPR_CUSTOM
LB Broth (Lennox) EZMix powder microbial growth medium	Sigma-Aldrich	L7658-1KG	
LB Broth with agar (Lennox) EZMix powder microbial growth medium	Sigma-Aldrich	L7533-1KG	
Midori Green Advance	Nippon Genetics	MG04	
Monarch Plasmid Miniprep Kit	NEB	T1010L	
Monarch DNA Gel Extraction Kit	NEB	T1020L	
NEB 5-alpha Competent E. coli (High Efficiency)	NEB	C2987U	
Nuclease-Free Water, 5X100 ml	Ambion	AM9939	

NucleoBond Xtra Midi	Macherey-Nagel	740410	
Opti-MEM, Reduced Serum Medium, 500 ml	Gibco	31985070	
P3 Primary Cell 4D-Nucleofector X Kit L	Lonza	V4XP-3024	The Lonza Primary P3 solution is supplied as a 2.25 mL P3 Primary Cell Nucleofector Solution and 0.5 mL Supplement 1. To reconstitute, add the Supplement 1 to the P3 Primary Cell Nucleofector Solution and mix.
pAAV-MCS2	Addgene	46954	
PCR Plate Heat Seal Foil, pierceable	Bio-Rad	1814040	
pDGM6	Addgene	110660	
Penicillin-Streptomycin (P/S)	Gibco	15140122	
Polyethylenimine (PEI)	Polysciences	23966	Add 50 mL of PBS 4.5 pH (made with HCl) to 50 mg of PEI in a tube. Dissolve by placing the tube in a 70°C water bath and vortexing every 10 minutes until the solution is dissolved. After the solution has reached RT, filter sterilize through a 0.22 µm filter, make 1120 µL aliquots, and store at -80°C.
Polystyrene Test Tube, with Snap Cap			
Primers	Eurofins	-	Primers were ordered from Eurofins (eurofinsgenomics.eu) as unmodified salt free custom oligos. The primers were designed by using PRIMER-Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) Primer 1 Fwd: AAGTGATCCGTTCCGCATGAC; Primer 2 Rev CALR WT specific: ACGTCCTCTTCCTCGTCCTC; Primer 2 Rev CALR DEL specific: CCAACCCTGGAGACACGCTTC
PrimeTime qPCR Primer Assay	IDT	-	PrimeTime qPCR Probe Assays (1 probe/2 primers) that can be ordered from IDT (https://eu.idtdna.com/site/order/qpcr/assayentry). Scale: Std - qPCR Assay 500 reactions; Primer 1 Forward (5'-3') : GGAACCCCTAGTGATGGAGTT; Primer 2 Reverse (5'-3'): CGGCCTCAGTGAGCGA; Probe (5'-3'): CACTCCCTCTGCGCGCTCG; 5' Dye/3' Quencher: FAM/ZEN/IBFQ; Primer to probe ratio: 3.6
PX1 PCR Plate Sealer	Bio-Rad	1814000	
QuantaSoft Software	Bio-Rad		
Quick Extract DNA Extraction Solution	Lucigen	QE0905T	
QX200 Droplet Generator	Bio-Rad	1864002	
QX200 Droplet Reader	Bio-Rad		
Recombinant human Flt3-ligand	Peprtech	300-19	
Recombinant human IL-6	Peprtech	200-06	
Recombinant Human SCF	Peprtech	300-07	
Recombinant Human TPO	Peprtech	300-18	

RPMI 1640	Sigma-Aldrich	R8758-6X500ML	
SnapGene	Dotmatics		Molecular cloning software https://www.snapgene.com *Alternatively also Benchling (https://www.benchling.com) and Geneious (https://www.geneious.com) can be used.
Soc outgrowth medium	NEB	B9020S	
Sodium-butyrate	Sigma-Aldrich	B5887-1G	
Stem Regenin 1 (SR1)	Biogems	1224999	
StemSpan SFEM II	STEMCELL Technologies	9655	
TAE Buffer (Tris-acetate-EDTA) 50X	Thermo Scientific	B49	
TIDE			http://shinyapps.datacurators.nl/tide/
Trypan blue 0.4%	Sigma-Aldrich	T8154-100ML	
TrypLE (with phenol red), 500 ml	Thermo Scientific	16605-028	
UltraPure 0.5: EDTA, pH 8.0, 100 ml	Thermo Scientific	15575-038	
UM171	STEMCELL Technologies	72914	
Vector Builder codon optimization tool	Vector Builder		https://en.vectorbuilder.com/tool/codon-optimization.html