

Materials List for:

A Protocol for Multiple Gene Knockout in Mouse Small Intestinal Organoids Using a CRISPR-concatemer

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Materials

Name	Company	Catalog Number	Comments
Optimized CRISPR Design Tool	Feng Zhang group		CRISPR gRNA design tool; http://crispr.mit.edu/
Webcutter 2.0			restriction mapping tool; http://rna.lundberg.gu.se/cutter2/
T4 PNK (Polynucleotide Kinase)	New England Biolabs	M0201L	
T4 DNA ligase buffer	New England Biolabs	M0202S	
T7 DNA Ligase	New England Biolabs	M0318L	
DTT (dithiothreitol)	Promega	P1171	
ATP (adenosine triphosphate)	New England Biolabs	P0756S	
FastDigest BbsI (Bpil)	Thermo Fisher	FD1014	
Tango buffer (BSA-containing restriction enzyme buffer)	Thermo Fisher	BY5	
BglII	New England Biolabs	R0144	
EcoRI	New England Biolabs	R0101	
Plasmid-safe exonuclease	Cambio	E3101K	
Thermal cycler	Applied biosystems	4359659	
10G competent E. coli bacteria	Cambridge Bioscience	60108-1	
Plasmid mini kit	Qiagen	12125	
Table top microcentrifuge	Eppendorf	UY-02580-01	
Inoculating loops	Microspec	PLS5	
Bacteria incubator	Sanyo	MIR-262	
Luria-Bertani broth (LB)	Sigma-Aldrich	L3522	
Agarose	Sigma-Aldrich	A4718	
Agarose gel electrophoresis apparatus	Bioneer	A-7020	
Advanced DMEM/F12(cell culture medium)	Invitrogen	12634-034	
Glutamax (L-Glutamine) 100x	Invitrogen	35050-068	
HEPES 1 M (buffering agent)	Invitrogen	15630-056	
Penicillin-streptomycin 100x	Invitrogen	15140-122	
B27 supplement (Neuronal cell serum-free supplement) 50x	Invitrogen	17504-044	
N2 supplement (Neuronal cell serum-free supplement) 100x	Invitrogen	17502-048	
n-Acetylcysteine 500 mM	Sigma-Aldrich	A9165-5G	

Mouse EGF 500 µg/mL	Invitrogen Biosource	PMG8043	
Mouse Noggin 100 µg/mL	Peprotech	250-38	
Nicotinamide 1 M	Sigma	N0636	
R-Spondin conditioned medium	n.a.	n.a.	Produced in house from HEK293 cells, for details see Sato and Clevers 2013
Wnt conditioned medium	n.a.	n.a.	Produced in house from HEK293 cells, for details see Sato and Clevers 2013
Y-27632 10 µM	Sigma-Aldrich	Y0503-1MG	
Standard BD Matrigel matrix	BD Biosciences	356231	
48-well Plate	Greiner Bio One	677980	
CHIR99021	Sigma-Aldrich	A3734-1MG	
IWP-2	Cell Guidance Systems	SM39-10	
TrypLE (recombinant protease)	Invitrogen	12605-010	
Opti-MEM (reduced serum medium)	Life technologies	51985-034	
Electroporation Cuvettes 2 mm gap	NepaGene	EC-002S	
Low binding 15 mL tubes	Sigma-Aldrich	CLS430791	
Bürker's chamber	Sigma-Aldrich	BR719520-1EA	
NEPA21 Super Electroporator	NepaGene	contact supplier	
Protein LoBind tubes low binding	Thermo Fisher	10708704	
BTXpress electroporation buffer	Harvard Apparatus	45-0805	
DMSO (Dimethyl sulfoxide)	AppliChem	A3672	