

Materials List for:

Genetic Engineering of Primary Mouse Intestinal Organoids Using Magnetic Nanoparticle Transduction Viral Vectors for Frozen Sectioning

Lingling Xian¹, Lionel Chia^{1,5}, Dan Georgess², Li Luo¹, Shuai Shuai¹, Andrew J. Ewald^{3,4}, Linda M.S. Resar^{1,4,5,6}

¹Department of Medicine, Division of Hematology, Johns Hopkins University School of Medicine

²Department of Natural Sciences, School of Arts & Sciences, Lebanese American University

³Department of Cell Biology, Johns Hopkins University School of Medicine

⁴Department of Oncology, Johns Hopkins University School of Medicine

⁵Department of Pathology, Johns Hopkins University School of Medicine

⁶Institute for Cell Engineering, Johns Hopkins University School of Medicine

Correspondence to: Linda M.S. Resar at lresar@jhmi.edu

URL: <https://www.jove.com/video/57040>

DOI: [doi:10.3791/57040](https://doi.org/10.3791/57040)

Materials

Name	Company	Catalog Number	Comments
DMEM	Thermo Fisher Scientific	11965092	Base medium for 293T cells
DMEM/F12+	Thermo Fisher Scientific	12634010	Base medium for organoid culture medium and organoid digestion buffer
OPTI-MEM	Thermo Fisher Scientific	11058021	Virus plasmids transfection medium
Fetal Bovine Serum	Corning	35-011-CV	Component of virus collection medium and 293T medium
Pen/Strep	Thermo Fisher Scientific	15140122	Component of organoid culture medium and crypt dissociation buffer
PBS (without Ca ²⁺ , Mg ²⁺)	Thermo Fisher Scientific	10010049	A wash buffer and component of crypt dissociation buffer
Mem-NEAA	Thermo Fisher Scientific	11140050	Component of organoid culture medium
GlutamaxII	Thermo Fisher Scientific	35050061	Component of organoid culture medium
HEPES	Thermo Fisher Scientific	15630080	Component of organoid culture medium
EGF	Millipore Sigma	E9644	Component of organoid culture medium
Noggin	Peptotech	250-38 B	Component of organoid culture medium
R-spondin	R&D	7150-RS-025/CF	Component of organoid culture medium
Human recombinant insulin	Millipore Sigma	I9278-5ml	Component of organoid culture medium
Nicotinamide	Millipore Sigma	N3376-100G	Component of Transduction medium
Wnt3A	R&D	5036-WN-010	Component of Transduction medium
Y27632	Millipore Sigma	Y0503-1MG	Component of Transduction medium
0.5M EDTA	Thermo Fisher Scientific	15575020	Component of Crypts dissociation buffer

DTT (dithiothreitol)	Thermo Fisher Scientific	R0861	Component of Crypts dissociation buffer
Dispase I	Millipore Sigma	D4818-2MG	Component of organoid digestion buffer
DNase I	Millipore Sigma	11284932001	Component of organoid digestion buffer
matrigel(Growth factor reduced)	Corning	356231	Used as a matrix to embed organoids
Opti-MEM	Thermo Fisher Scientific	31985070	Medium for transfection in viral production
ViralMag R/L	Oz Biosciences	RL40200	Magnetic particles of viral transduction
Magnetic plate	Oz Biosciences	MF10000	Magnetic plate to facilitate viral transduction
Lipofectamine 2000	Thermo Fisher Scientific	11668019	Transfection agent in viral production
Poly-D-Lysine	Millipore Sigma	A-003-E	Coating for plates before seeding 293T cells
4% Formaldehyde Solution	Boster	AR1068	Solution to fix organoids
O.C.T embedding compound	Thermo Fisher Scientific	4583S	For embedding of the the organoids
5 mL Falcon polystyrene tubes	Corning	352054	
50 mL Falcon Tubes	Sarstedt	62.547.100	
Orbitron rotator II Rocker Shaker	Boekel Scientific	260250	
Olympus Inverted microscop CK30	Olympus	CK30	for scanning and counting crypts
Zeiss Axiovert 200 inverted fluorescence	Nikon	Axiovert 200	for viewing fluorescence in the crypts
Amicon Ultra-15 Centrifugal Filter unit with Ultracel-100 membrane	Millipore Sigma	UFC910024	For concentrating viruses
pluriStrainer 20 µm (Cell Strainer)	pluriSelect	SKU 43-50020	For preparing organoid fragments
Falcon Cell Strainer	Fisher Scientific	352340	For preparing cyrpts of similar size after crypt isolation
Greiner CELLSTAR multiwell culture plates 48 wells (TC treated with lid)	Millipore Sigma	M8937-100EA	ForD2:D37+D16:D37g organoid fragments
Animal strain: C57BL/6J	Jackson Lab	#000664	For organoid culture