

Materials List for:

# Multiplexed Single Cell mRNA Sequencing Analysis of Mouse Embryonic Cells

Wei Feng<sup>1</sup>, Andrew Przysinda<sup>1</sup>, Guang Li<sup>1</sup>

<sup>1</sup>Department of Developmental Biology, University of Pittsburgh School of Medicine

Correspondence to: Guang Li at [guangli@pitt.edu](mailto:guangli@pitt.edu)

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## Materials

Name	Company	Catalog Number	Comments
10% Tween-20	Bio-Rad	1610781	
10x Chip Holder	10x Genomics	120252 330019	
10x Chromium Controller	10x Genomics	120223	
10x Magnetic Separator	10x Genomics	120250 230003	
10x Vortex Adapter	10x Genomics	330002, 120251	
10x Vortex Clip	10x Genomics	120253 230002	
4200 TapeStation System	Agilent	G2991AA	
Agilent High Sensitivity DNA Kit	Agilent	5067-4626	University of Pittsburgh Health Sciences Sequencing Core
Barcode Oligo	Integrated DNA Technologies	Single-stranded DNA	25 nmol
Buffer EB	Qiagen	19086	
CD1 mice	Chales River	Strain Code 022	ordered pregnant mice
Centrifuge 5424R	Appendorf	2231000214	
Chromium Chip B Single Cell Kit, 48 rxns	10x Genomics	1000073	Store at ambient temperature
Chromium i7 Multiplex Kit, 96 rxns	10x Genomics	120262	Store at -20 °C
Chromium Single Cell 3' GEM Kit v3,4 rxns	10x Genomics	1000094	Store at -20 °C
Chromium Single Cell 3' Library Kit v3	10x Genomics	1000095	Store at -20 °C
Chromium Single Cell 3' v3 Gel Beads	10x Genomics	2000059	Store at -80 °C
Collagenase A	Sigma/Millipore	10103578001	Store powder at 4 °C, store at -20 °C after it dissolves
Collagenase B	Sigma/Millipore	11088807001	Store powder at 4 °C, store at -20 °C after it dissolves
D1000 ScreenTape	Agilent	5067-5582	University of Pittsburgh Health Sciences Sequencing Core
DNA LoBind Tube Microcentrifuge Tube, 1.5 mL	Eppendorf	022431021	
DNA LoBind Tube Microcentrifuge Tube, 2.0 mL	Eppendorf	022431048	
Dynabeads MyOne SILANE	10x Genomics	2000048	Store at 4 °C, used in Beads Cleanup Mix (Table 1)
DynaMag-2 Magnet	Thermo Scientific	12321D	
Ethanol, Pure (200 Proof, anhydrous)	Sigma	E7023-500mL	
Falcon 15mL High Clarity PP Centrifuge Tube	Corning Cellgro	14-959-70C	

Falcon 50mL High Clarity PP Centrifuge Tube	Corning Cellgro	14-959-49A	
Fetal Bovine Serum, qualified, United States	Fisher Scientific	26140079	Store at -20 °C
Finnpipette F1 Multichannel Pipettes, 10-100µl	Thermo Scientific	4661020N	
Finnpipette F1 Multichannel Pipettes, 1-10µl	Thermo Scientific	4661000N	
Flowmi Cell Strainer	Sigma	BAH136800040	Porosity 40 µm, for 1000 uL Pipette Tips, pack of 50 each
Glycerin (Glycerol), 50% (v/v)	Ricca Chemical Company	3290-32	
HBSS, no calcium, no magnesium	Thermo Fisher Scientific	14170112	
Human TruStain FcX (Fc Receptor Blocking Solution)	BioLegend	422301	Add 5 µl of Human TruStain FcX per million cells in 100 µl staining volume
Isopropanol (IPA)	Fisher Scientific	A464-4	
Kapa HiFi HotStart ReadyMix (2X)	Fisher Scientific	NC0295239	Store at -20 °C, used in Lipid-tagged barcode library mix (Table 1)
Lipid Barcode Primer (Multi-seq Primer)	Integrated DNA Technologies	Single-stranded DNA	100 nmol
Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	Thermo Fisher Scientific	12090-015	
MasterCycler Pro	Eppendorf	950W	
Nuclease-Free Water (Ambion)	Thermo Fisher Scientific	AM9937	
PCR Tubes 0.2 ml 8-tube strips	Eppendorf	951010022	
Phosphate-Buffered Saline (PBS) 1X without calcium & magnesium	Corning Cellgro	21-040-CV	
Phosphate-Buffered Saline (PBS) with 10% Bovine Albumin (alternative to Thermo Fisher product)	Sigma-Aldrich	SRE0036	
Pipet 4-pack (0.1–2.5µL, 0.5-10µL, 10–100µL, 100–1,000µL variable-volume pipettes)	Fisher Scientific	05-403-151	
Selection reagent (SPRIselect Reagent Kit)	Beckman Coulter	B23318 (60ml)	
Template Switch Oligo	10x Genomics	3000228	Store at -20 °C, used in Master Mix (Table 1)
The antibody based barcoding strategy is also known as Cell Hashing			
The cell browser is Loup Cell Browser	10x Genomics		<a href="https://support.10xgenomics.com/single-cell-gene-expression/software/visualization/latest/what-is-loupe-cell-browser">https://support.10xgenomics.com/single-cell-gene-expression/software/visualization/latest/what-is-loupe-cell-browser</a>
The commercial available analysis pipeline in step 8.1 is Cell Ranger	10x Genomics		<a href="https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger">https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger</a>
The lipid based barcoding strategy is also known as MULTI-seq			
The well maintained R platform is Seurat V3	satijalab		<a href="https://satijalab.org/seurat/">https://satijalab.org/seurat/</a>
TipOne RPT 0.1-10/20 ul XL ultra low retention filter pipet tip	USA Scientific	1180-3710	

TipOne RPT 1000 ul XL ultra low retention filter pipet tip	USA Scientific	1182-1730	
TipOne RPT 200 ul ultra low retention filter pipet tip	USA Scientific	1180-8710	
TotalSeq-A0301 anti-mouse Hashtag 1 Antibody	BioLegend	155801	0.1 - 1.0 µg of antibody in 100 µl of staining buffer for every 1 million cells
TotalSeq-A0302 anti-mouse Hashtag 2 Antibody	BioLegend	155803	0.1 - 1.0 µg of antibody in 100 µl of staining buffer for every 1 million cells
TotalSeq-A0302 anti-mouse Hashtag 3 Antibody	BioLegend	155805	0.1 - 1.0 µg of antibody in 100 µl of staining buffer for every 1 million cells
TrueSeq RPI primer	Integrated DNA Technologies	Single-stranded DNA	100 nmol, used in Lipid-tagged barcode library mix (Table 1)
Trypan Blue Solution, 0.4%	Fisher Scientific	15250061	
Trypsin-EDTA (0.25%), phenol red	Fisher Scientific	25200-056	
Universal I5	Integrated DNA Technologies	Single-stranded DNA	100 nmol