Materials List for:

## RNA-based Reprogramming of Human Primary Fibroblasts into Induced Pluripotent Stem Cells

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## **Materials**

Name	Company	Catalog Number	Comments
Plasmid templates for PCR			
pcDNA3.3_KLF4	Addgene	26815	
pcDNA3.3_SOX2	Addgene	26817	
pcDNA3.3_c-MYC	Addgene	26818	
pcDNA3.3_LIN28A	Addgene	26819	
pCR-Blunt_hM3O	Addgene	112638	
pCR-Blunt_hNANOG	Addgene	112639	
pCR-Blunt_mWasabi	Addgene	112640	
Modified mRNA <i>in vitro</i> transcription and miRNA mimics			
Forward Primer	Integrated DNA Technologies	TTGGACCCTCGTACAGAAGC TAATACG	
Reverse Primer (Ordered as ultramer, 4nmol scale)	Integrated DNA Technologies	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	
(ARCA Cap) 3´-0-Me- m7G(5')ppp(5')G	New England Biolabs	S1411S	
Pfu Ultra II Hotstart 2x Master Mix	Agilent	600850-51	
5-Methylcytidine-5'-Triphosphate	Trilink Biotechnologies	N-1014	
Antarctic Phosphatase	New England Biolabs	M0289L	
DNase I	NEB	M0303S	
MEGAscript T7 Transcription Kit	ThermoFisher Scientific	AM1334	
Pseudouridine-5'-Triphosphate	Trilink Biotechnologies	N-1019	
Riboguard RNase Inhibitor	Lucigen	RG90910K	
RNA Clean & Concentrator	ZymoResearch	R1019	
Syn-hsa-miR-302a-3p miScript miRNA Mimic	Qiagen	MSY0000684	
Syn-hsa-miR-302b-3p miScript miRNA Mimic	Qiagen	MSY0000715	
Syn-hsa-miR-302c-3p miScript miRNA Mimic	Qiagen	MSY0000717	
Syn-hsa-miR-302d-3p miScript miRNA Mimic	Qiagen	MSY0000718	

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Syn-hsa-miR-367-3p miScript miRNA Mimic	Qiagen	MSY0000719	
Water (Nuclease free, Not DEPC-treated)	Fisher	AM9937	Use to dilute modified mRNAs and miRNA mimics
Fibroblast culture and reprogramming			
0.1% Gelatin in H <sub>2</sub> O	StemCell technologies	#07903	
Stericup-GV Sterile Vacuum Filtration System	EMD Millipore	SCGVU05RE	Use to sterilize the transfection buffer and 0.5 mM EDTA in DPBS
2-Mercaptoethanol	ThermoFisher Scientific	21985023	
6-well plates (tissue culture treated)	Corning	3516	
DMEM/F12	ThermoFisher Scientific	11320033	
Fetal Bovine Serum	Fisher	26-140-079	
FGF Basic	ThermoFisher Scientific	phg0263	
GlutaMax Supplement	ThermoFisher Scientific	35050061	Glutamine supplement used for the prepration of media
Heat Inactivated FBS	Gibco Technologies	10438026	
KnockOut Serum Replacement	ThermoFisher Scientific	10828010	
Lipofectamine RNAiMAX Transfection Reagent	ThermoFisher Scientific	13778500	The protocol is optimized for the Lipofectamine RNAiMax transfection reagent
MEM	ThermoFisher Scientific	11095080	
MEM Non-essential amino acids	ThermoFisher Scientific	11140050	
Opti-MEM I Reduced Serum Medium	ThermoFisher Scientific	31985070	Reduced-serum medium, use to make the transfection buffer by adjusting the pH to 8.2, 500 mL
Opti-MEM I Reduced Serum Medium	ThermoFisher Scientific	31985062	Reduced-serum medium, use to make the transfection buffer by adjusting the pH to 8.2, 100 mL
10 M NaOH	Sigma-Aldrich	72068	Make a 1 M solution by diluting in nuclease free water and use for pH adjustment
Water (Nuclease free, Not DEPC-treated)	Fisher	AM9932	Use to dilute NaOH and wash a pH meter
Pen/Strep/Fungizone	GE Healthcare	SV30079.01	
rhLaminin-521	ThermoFisher Scientific	A29248	Supplied at a concentration of 100 µg/mL, use as a matrix for the reprogramming procedure
Dulbecco's phosphate-buffered saline (DPBS)	Life Technologies	14190144	
Trypsin-EDTA 0.25% Phenol Red	Life Technologies	2520056	
Vaccinia Virus B18R (CF)	ThermoFisher Scientific	34-8185-86	
iPSC culture			
Corning Matrigel hESC-Qualified Matrix	Corning	354277	Extracellular matrix (ECM) for culturing iPSCs
EDTA, 0.5 M stock solution	K&D Medical	RGF-3130	Dilute to 0.5 mM in DPBS, filter sterilize and use for iPSC passaging
mTeSR1	StemCell Technologies	85850	Pluripotent stem cell (PSC) medium, provides growth advantage to iPSCs over fibroblasts
Antibodies and Detection			

Rabbit anti Mouse (HRP conjugated)	Abcam	ab97046	
Tra-1-60 (mouse anti human)	Stemgent	09-0010	
Hydrogen Peroxide (30%)	LabChem	LC154301	Dilute to 3% with PBS
Bovine Serum Albumin (BSA)	Fisher	BP9703100	
Phosphate-buffered salin (PBS)	Hyclone	SH30258.02	Supplied as 10x, dilute to 1x
VECTOR NovaRED Peroxidase Substrate Kit	Vector Laboratories	SK-4800	
Special Equipment			
Description			Notes
Biological safety cabinet			
Regular humidified tissue culture incubator			Calibrate CO <sub>2</sub> using digital meter, fyrite, or equivalent. Equilibrate incubator to 5% CO <sub>2</sub> , 37 °C.
Tri-gas humidified tissue culture incubator			Calibrate CO <sub>2</sub> using digital meter, fyrite, or equivalent. Equilibrate incubator to 5% CO <sub>2</sub> , 5% O <sub>2</sub> , 37 °C. Use for the reprogramming procedure.
pH Meter			Must have resolution to two decimal places. Designate to RNA work if possible.
Inverted microscope			Microscope configured to visualize EGFP for monitoring transfection efficiency.