

Materials List for:

Derivation and Characterization of a Transgene-free Human Induced Pluripotent Stem Cell Line and Conversion into Defined Clinical-grade Conditions

Jason P. Awe¹, Agustin Vega-Crespo¹, James A. Byrne^{1,2}

¹Department of Molecular and Medical Pharmacology, University of California, Los Angeles (UCLA)

²Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles (UCLA)

Correspondence to: James A. Byrne at jbyrne@mednet.ucla.edu

URL: <https://www.jove.com/video/52158>

DOI: [doi:10.3791/52158](https://doi.org/10.3791/52158)

Materials

Name	Company	Catalog Number	Comments
Media and reagents			
DMEM/F12 (basal media)	Invitrogen (Carlsbad, CA, USA)	11330057	
Fetal bovine serum	Invitrogen	16000044	
Minimum essential medium (MEM) non-essential amino acids (NEAA), 100x	Invitrogen	11140050	
Glutamax, 100x	Invitrogen	35050-061	
PenStrep, penicillin-streptomycin, 100x	Invitrogen	15140-122	Thaw at 4 °C, aliquot and store at -20 °C
Knockout serum replacement	Invitrogen	10828028	Thaw at 4 °C, aliquot and store at -20 °C
Trypsin/EDTA, 0.5%	Invitrogen	15400-054	Dilute stock out to 0.05% in 1x PBS
Basic fibroblast growth factor	GlobalStem (Rockville, MD, USA)	GSR-2001	Reconstitute to 10 µg/ml stock in 0.1% bovine serum albumin dissolved in 1x PBS and store at -80 °C
β-mercaptoethanol	Millipore (Billerica, MA, USA)	ES-007-E	
Matrigel (basement membrane matrix)	BD Biosciences (San Jose, CA, USA)	356231	Dilute stock Matrigel vial with 10 ml of DMEM/F12 while on ice for a 1:2 dilution. Aliquot and store at -20 °C.
CELLstart (Synthetic Substrate)	Invitrogen	A1014201	
Stem molecule Y27632	Stemgent (Cambridge, MA, USA)	04-0012-02	
Puromycin	Invitrogen	A1113802	
LightCycler 480 Probes Master	Roche (Basel, Switzerland)	4707494001	
ProFreeze-CDM Medium/freezing medium	Lonza (Basel, Switzerland)	12-769E	
Dimethyl sulfoxide	Sigma-Aldrich (St. Louis, MO, USA)	D8418	
PBS	Invitrogen	14190-250	
100 BP DNA Ladder	Invitrogen	15628019	
SYBR Safe DNA Gel Stain 10,000x	Invitrogen	S33102	
Agarose	Bio-Rad Laboratories, Inc. (Hercules, CA, USA)	161-3101	

Gelatin, from porcine skin	Sigma-Aldrich	G1890-100G	Make stock at 0.2% in PBS, autoclave and store at room temperature.
mTeSR1	StemCell Technologies (Vancouver, BC, Canada)	5850	Combine Supplement 5x with the basic medium, aliquot and store at 4 °C for up to 2 weeks.
Stemedia NutriStem XF/FF Culture Medium	Stemgent	05-100-1A	Thaw at 4 °C O/N, aliquot and store at 4 °C for up to 2 weeks.
Primocin	InvivoGen (San Diego, CA, USA)	ant-pm-1	
Accutase (Dissociation Reagent)	Invitrogen	A1110501	
Donkey anti-Chicken IgG AlexaFluor 488	Jackson ImmunoResearch (West Grove, PA, USA)	703-546-155	
Polybrene/transfection agent	Millipore	TR-1003-G	
Plasticware			
12-well plates	VWR (West Chester, PA, USA)	29442-038	
6-well plates	VWR	29442-042	
10-cm plates	Sigma-Aldrich	Z688819	
18-gauge needle	Fisher Scientific (Pittsburgh, PA, USA)	148265D	
21-gauge needle	Fisher Scientific	14-829-10D	
Equipment			
BD LSRII Flow Cytometer	KSystem by Nikon (Tokyo, Japan)		
BD FACSDiva Version 6.1.3 Software	BD Biosciences		
Kits			
PureLink Genomic DNA Mini Kit	Invitrogen	K182000	
KAPA HiFi Hotstart ReadyMix PCR Kit	KAPA Biosystems (Wilmington, MA, USA)	KK2601	
High Pure RNA Isolation Kit	Roche	11828665001	
Transcriptor First Strand cDNA Synthesis Kit	Roche	4379012001	
Sialix anti-Neu5Gc Basic Pack Kit	Sialix (Newton, MA, USA)	Basic Pack	
Media			
Combined media 1	StemCell Technologies and Stemgent		Consists of equal parts mTeSR1 and Nutristem
Combined media 2	StemCell Technologies and Stemgent		Consists of equal parts TeSR2 and Nutristem
HUF Media			Dulbecco's modified Eagle's medium/F12 [DMEM/F12] supplemented with 10% fetal bovine serum, 1x non-essential amino acids, 1x Glutamax, and 1x Primocin
Human Pluripotent Stem Cell Media			DMEM/F12 supplemented with 20% knockout serum replacement, 1x Glutamax, 1x non-essential amino acids, 1x Primocin, 1x β -mercaptoethanol, and 10 ng/ml basic fibroblast growth factor.
DMEM/F12, Dulbecco's modified Eagle's medium/F12; PBS, phosphate-buffered saline.			