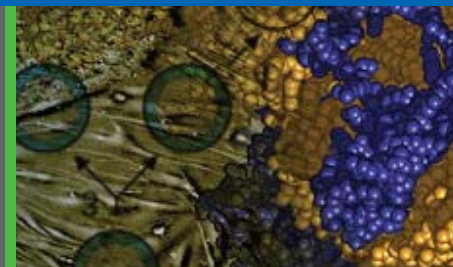


Disease-specific iPS Cells

Create Ideal Screening Cell Lines for Accelerated Drug Discoveries



Disease-specific iPS Cell Lines

SBI and DV Biologics have partnered to develop novel Human iPS cell lines from patient-derived sources. Utilizing iPS cell lines from these disorders represents an opportunity to recapitulate both normal and pathological tissue formation *in vitro* for the ideal drug development screening cell line to facilitate new therapeutic discovery and disease modeling.

Metabolism and Autoimmune: Type I Diabetes iPS cell line

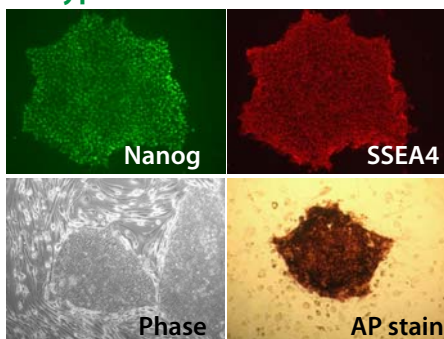
- Twenty-nine year old female Hispanic-Caucasian
- Diagnosed with Type I Diabetes at age 12
- Dermal fibroblasts taken in August 2009
- Single donor for clear genetic background



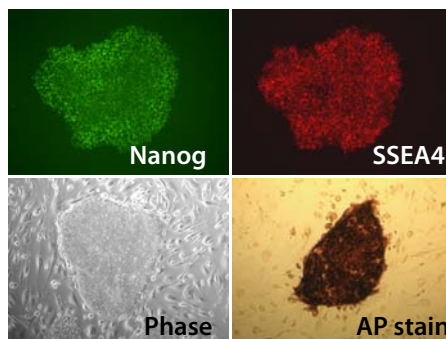
The human patient fibroblasts are derived from cultured skin explants from a single donor. These cells enable researchers to study skin diseases such as dermatitis, wound healing and they may be used to study the development of skin and pancreatic cells to study disease models. Diabetes Type I is an autoimmune disease that results in destruction of insulin-producing beta cells of the pancreas. The etiology of Diabetes Type I remains unknown.

All cell lines are certified for pluripotency and karyotyped for authenticity

Type I Diabetes iPS cell line



MLD iPS cell line



Highlights

- Disease and patient-specific source fibroblast cells
- Reprogrammed using standard retroviral factors OSKM
- Certified pluripotent through stem cell marker immunostaining
- Grown with standard stem cell media conditions
- Study the disease process more accurately with better models

Type I Diabetes: Autoimmune Model

- Juvenile onset Diabetic model
- Pancreatic beta cells destroyed
- Diabetes-related autoantibodies
- Polygenic, developmental disease
- Chronic insulin therapy required

MLD: Polyneuropathy Model

- Juvenile onset Neuropathy
- Weakness: Proximal & Distal
- Hypotonia
- Tendon reflexes: Decreased or absent
- Sensory loss: Mild; Distal

Neurodegeneration: Metachromatic Leukodystrophy (MLD) iPS cell line

Metachromatic leukodystrophy, or MLD, is a group of disorders marked by storage buildup in the white matter of the central nervous system and in the peripheral nerves and to some extent in the kidneys. MLD affects the myelin that covers and protects the nerves. This autosomal recessive disorder is caused by a deficiency of the enzyme Arylsulfatase A. Both males and females are affected by this disorder with juvenile onset and death generally occurs within 6 to 14 years after onset of symptoms.

Differentiate the disease-specific iPS cells into any lineage to screen for drugs that can alleviate the disease phenotype.

Growth Conditions for Human iPS Cells

Human iPS Cells should be grown on mitomycin C-treated MEF cells

MEFs are available from Applied Stemcell, Inc (<http://www.appliedstemcell.com/>).

For a full protocol of how to grow MEF or human iPS cells, please refer to the user manual. MEF cells must already be growing before thawing and plating iPS cells. http://www.systembio.com/downloads/Manual_iPSCellLines.pdf

Thawing Human iPS Cells

To initially plate human iPS cells, remove the vial from liquid nitrogen and thaw quickly in a 37°C water bath. Remove the vial from the water bath as soon as the cells are half-thawed. Spray the vial with 70% ethanol. Transfer the cells with 10 ml Human iPS medium to a 15-ml conical tube and centrifuge at 200g for 5 min. While centrifuging, remove the MEF medium from a 6-well plate of MEFs, and wash twice with 1 ml of DMEM/F12. Add 1 ml of human iPS cell media containing 10 µM ROCK inhibitor. Discard the supernatant and resuspend with 5 ml fresh Human iPS media with ROCK inhibitor. Plate cells 1 ml per well in a 6-well plate. Incubate at 37°C with 5% CO₂. Change the medium every day or when the pH decreases. ROCK inhibitor may be removed from the media after 1 day.

MEF Media

Component	Cat. #	Final concentration	Source
FBS	16000077	10%	Invitrogen
Glutamax-1	35050061	2 mM	Invitrogen
penicillin and streptomycin	15140122	50 U and 50 µg /ml	Invitrogen
DMEM	11995065		Invitrogen

Human iPS cell media

Component	Cat. #	Final concentration	Source
Knockout serum replacement	10828028	20%	Invitrogen
Glutamax-1	35050061	2 mM	Invitrogen
Nonessential amino acid	11140050	1 x 10 ⁻⁴ M	Invitrogen
2-mercaptoethanol	M7522	1 x 10 ⁻⁴ M	Sigma
penicillin and streptomycin	15140122	50 U and 50 µg /ml	Invitrogen
bFGF	233-FB-025	10 ng/ml	R&D
KO DMEM/F12	12660012		Invitrogen

Component	Cat. #	Final concentration	Source
Rock Inhibitor Y-27632	Y0503	10 mM/ml	Sigma
0.1% (w/v) Gelatin	G1890	Dissolve 0.5 g of gelatin from porcine skin in 500 ml DPBS and autoclave. Stable for 1 yr at room temperature	Sigma
Accutase	SCR005	1:1 dilution into DPBS, aliquot in 10 ml, store -20°C	Millipore

For freezing, use
90% FBS 10% DMSO