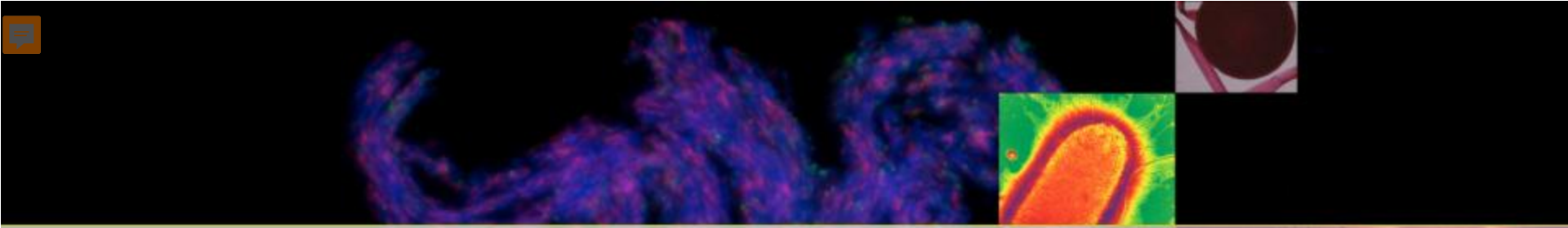
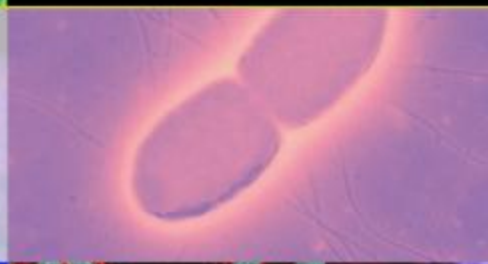
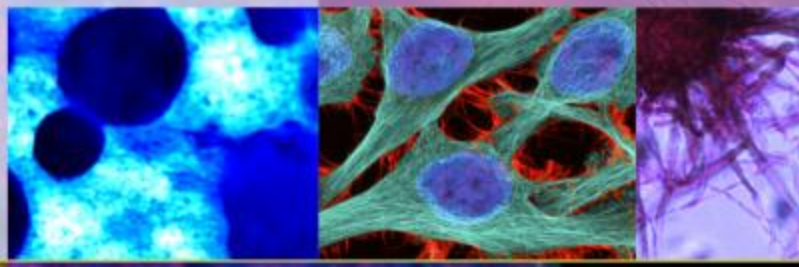
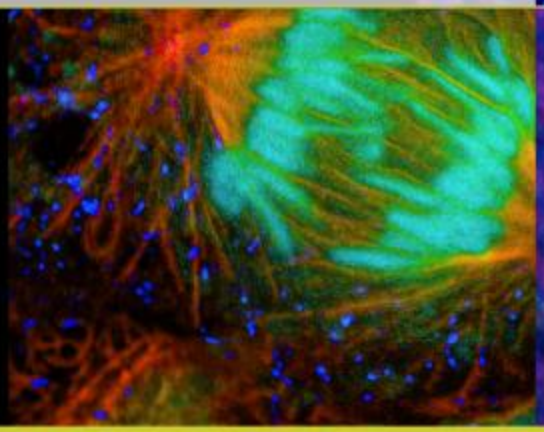
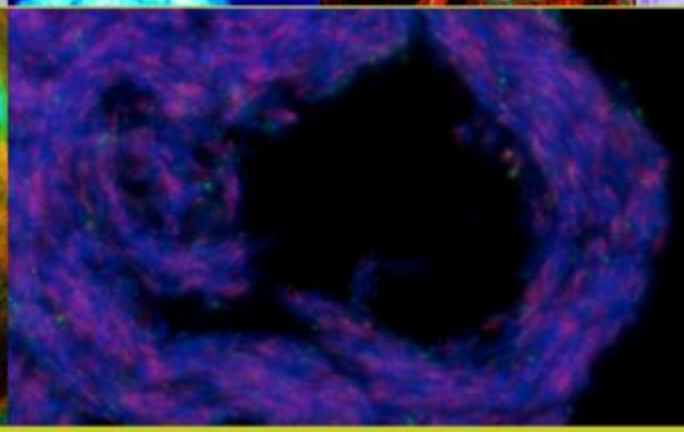
INDUCED PLURIPOTENT AND MESENCHYMAL STEM CELLS – CELLS WITH A LOT OF POTENTIAL

Chengkang Zhang, Ph.D.
Senior Scientist
ASCB Vendor Showcase
Dec. 15, 2013

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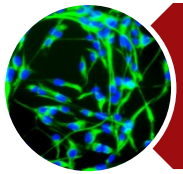
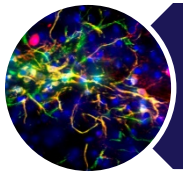
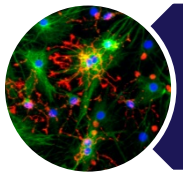
Outline



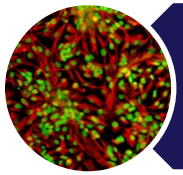
Human induced pluripotent stem cells (iPSCs)



ATCC iPSC quality standards



Characterization of Parkinson’s iPSC lines



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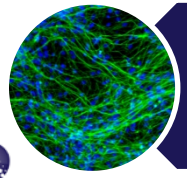

Human mesenchymal stem cells

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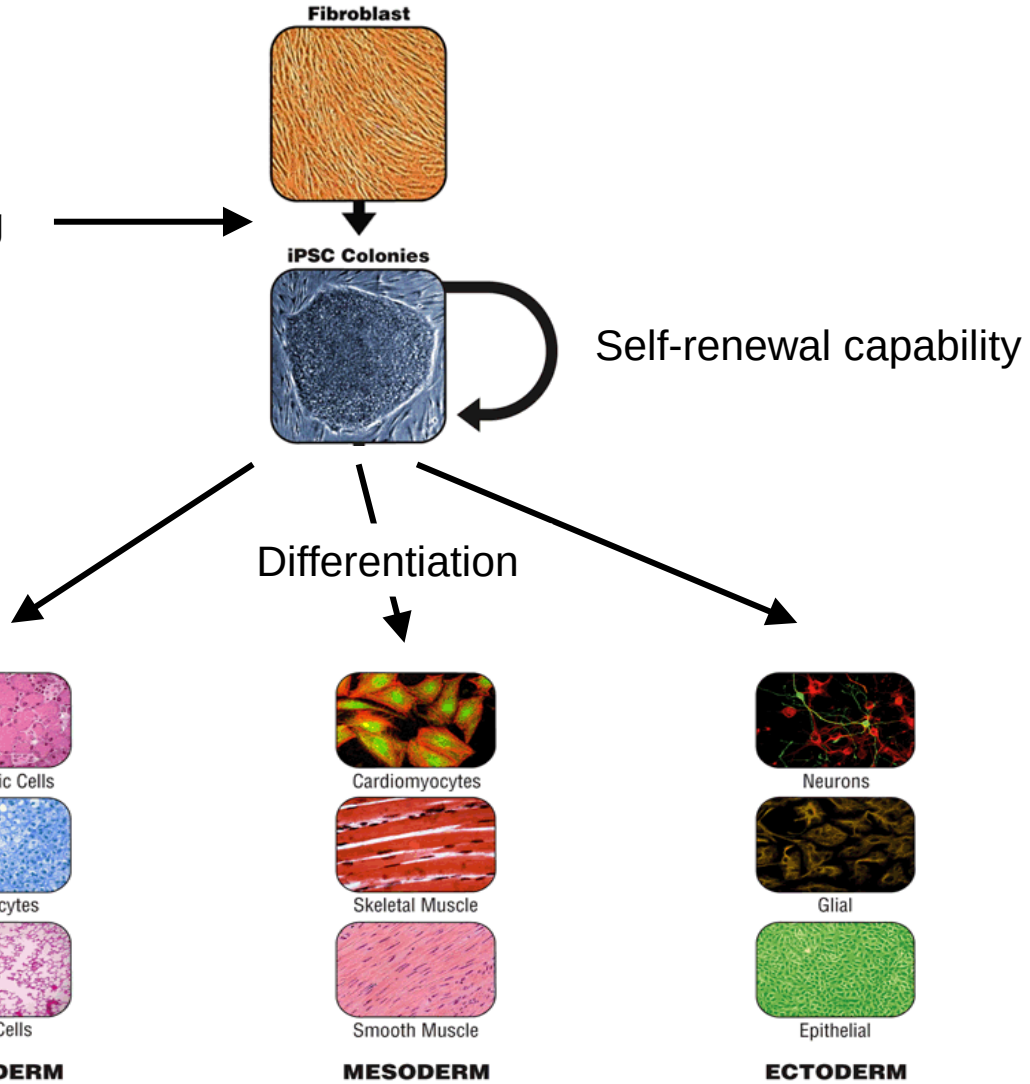
What are iPSC?

Yamanaka Factors

- Oct3/4[1,2]
- Sox2[1,2]
- Klf4[1]
- Myc[1]
- Nanog[2]
- Lin28[2]

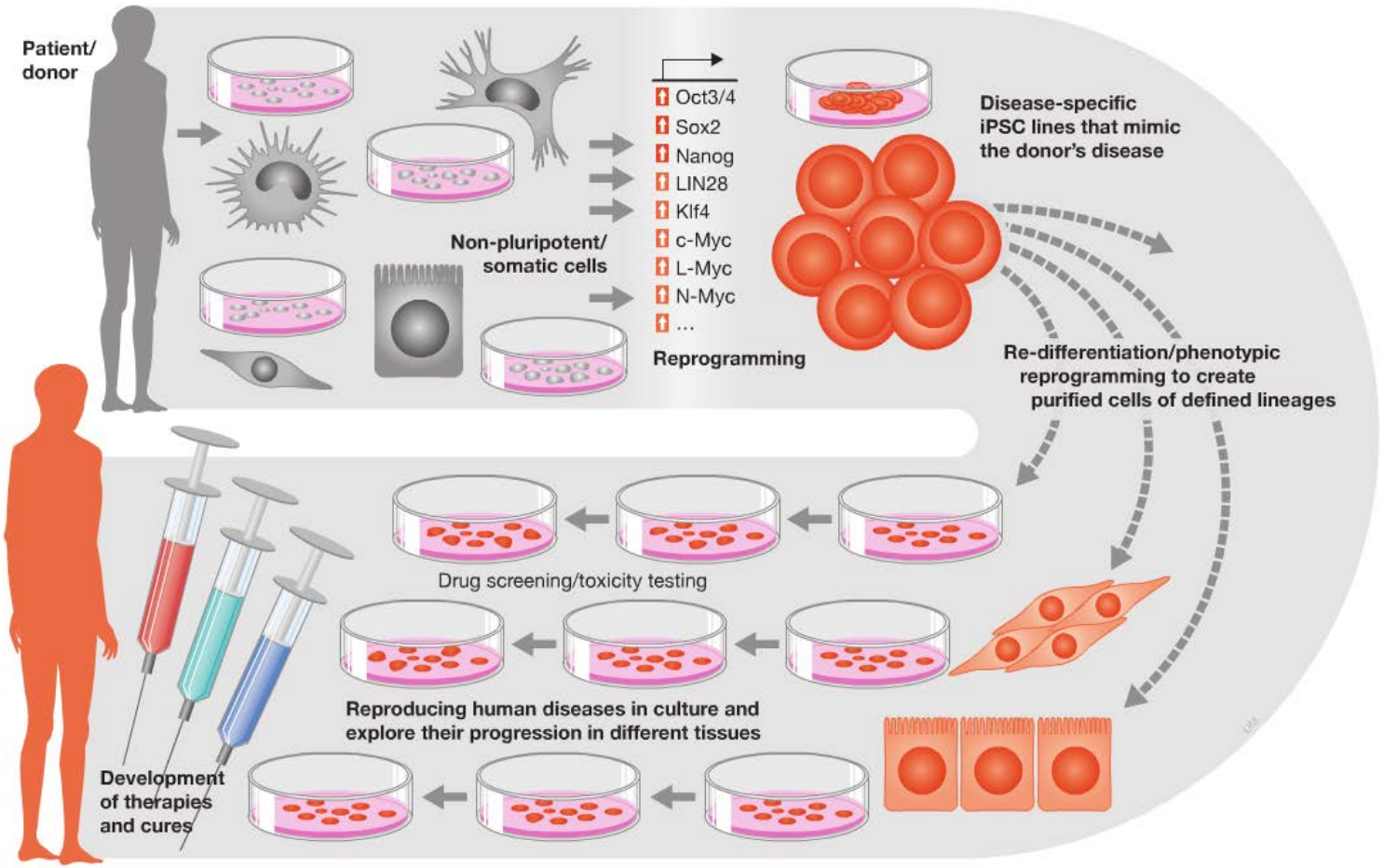
iPS reprogramming factors



Takahashi K. *et al*. *Cell* 126(4): 663-76, 2006.
Yu J. *et al*. *Science* 318(5858): 1917-20, 2007.

www.sabiosciences.com

The promise of iPSCs



Deng, W. *EMBO Reports* 11(3): 161-5, 2010

Overview of ATCC iPSC lines collection

iPSC lines derived from apparent normal donors

ATCC® No.	Designation	Reprogramming System	Tissue and Cells Origin	Disease
NEW ACS-1019™	ATCC-DYS0100	OSKM / Sendai Virus	Foreskin, Fibroblast	Normal
NEW ACS-1020™	ATCC-HYS0103	OSKM / Sendai Virus	Liver, Fibroblast	Normal
NEW ACS-1021™	ATCC-CYS0105	OSKM / Sendai Virus	Heart, Fibroblast	Normal
ACS-1007™	ATCC-HYR0103	OSKM / Retrovirus	Liver, Fibroblast	Normal
ACS-1011™	ATCC-DYR0100	OSKM / Retrovirus	Foreskin, Fibroblast	Normal

iPSC lines derived from donors with diseases

ATCC® No.	Designation	Reprogramming System	Tissue and Cells Origin	Disease
ACS-1012™	ATCC-DYR0530	OSKM / Retrovirus	Skin, Fibroblast	Parkinson's Disease, Asthma, Depression
ACS-1013™	ATCC-DYS0530	OSKM / Sendai virus	Skin, Fibroblast	Parkinson's Disease, Asthma, Depression
ACS-1014™	ATCC-DYP0530	OSKM / Episomal	Skin, Fibroblast	Parkinson's Disease, Asthma, Depression
ACS-1003™	ATCC-DYP0730	OSKM / Episomal	Foreskin, Fibroblast	Down syndrome
ACS-1004™	ATCC-DYP0250	OSKM / Episomal	Skin, Fibroblast	Cystic fibrosis: Homozygous CFTRΔ508

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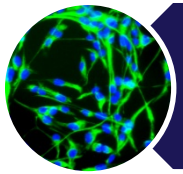
Outline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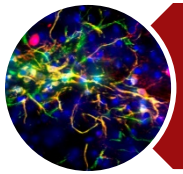
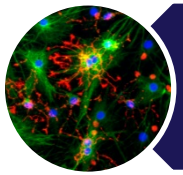

ATCC iPSC quality standards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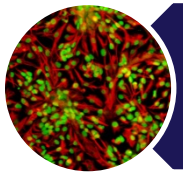
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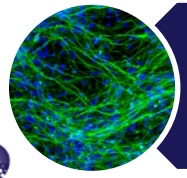
Human mesenchymal stem cells

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iPSC quality control

Description	QC Methods	QC Specification
Post-Thaw Viable Cell Recovery	hiPSC culture	≥ 30 colonies in 5 days
Expression of Stem Cell Markers	Immunocytochemistry	Tra1-60, Tra1-81, SSEA-4, Nanog
Surface Antigen Expression of Stem Cell Markers	Flow Cytometry	Pluripotency (SSEA4, Tra-1-60) >85% Differentiation (SSEA1) <15%
Karyotype	G banding	Normal karyotype, 46,XY or 46,XX
Germ Layer Differentiation *	qRT-PCR analysis of EBs	Gene expression relative to pluripotent cells (endoderm, mesoderm and ectoderm layers)
PluriTest (Transcriptome analysis)	Illumina Human HT-12v4 Expression Beadchip	Pluripotency and Novelty Scores
Sterility (Bacterial and Fungal Testing)	Growth on agar	No bacterial growth after 21 days
Mycoplasma	Direct culture and Hoechst DNA staining	None detected
Identity	STR	Consistent with donor sample
Viral Panel Testing	PCR	None detected for CMV, EBV, HBV, HIV1 and HPV

EB differentiation is being replaced by transcriptome-based pluripotency analysis

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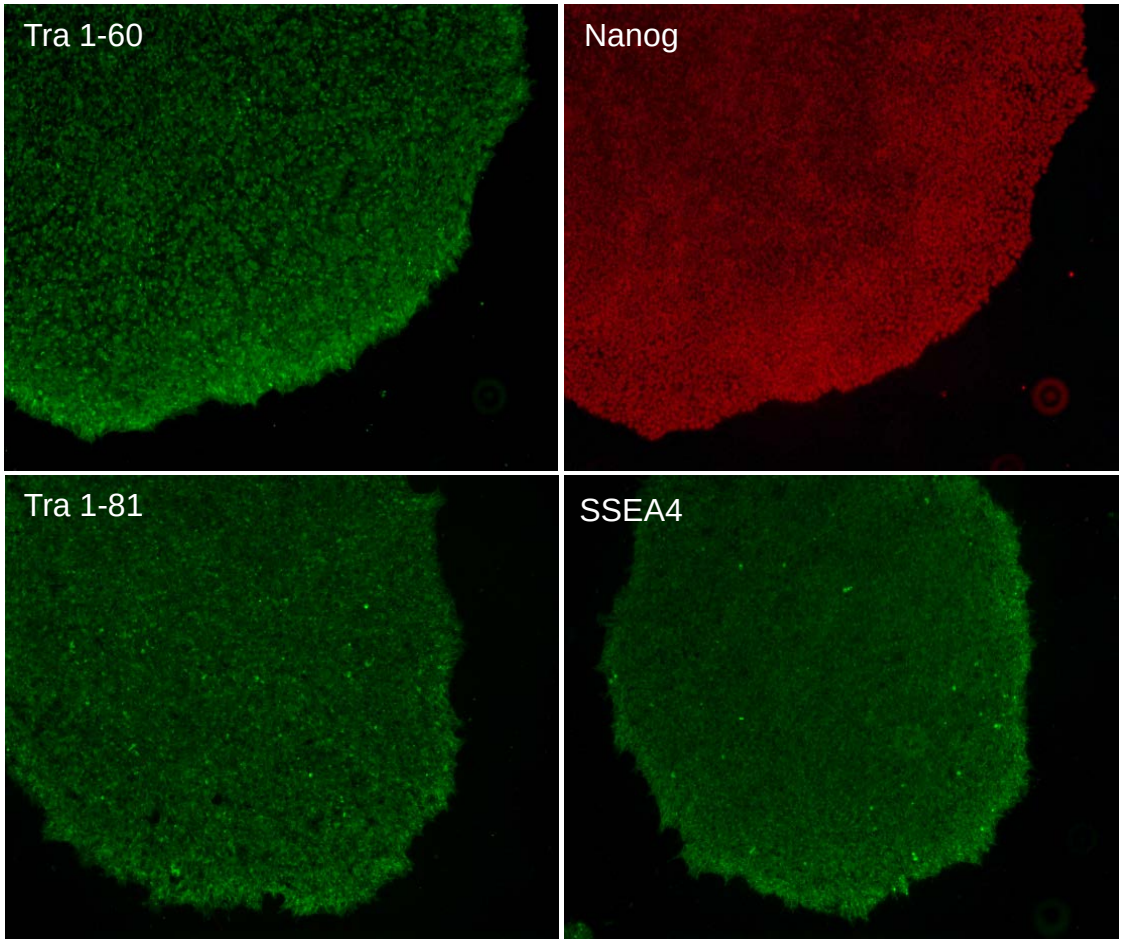
ATCC iPSCs are monitored for pluripotency



Immunocytochemistry: Tra 1-60, Tra 1-81, SSEA-4 and Nanog

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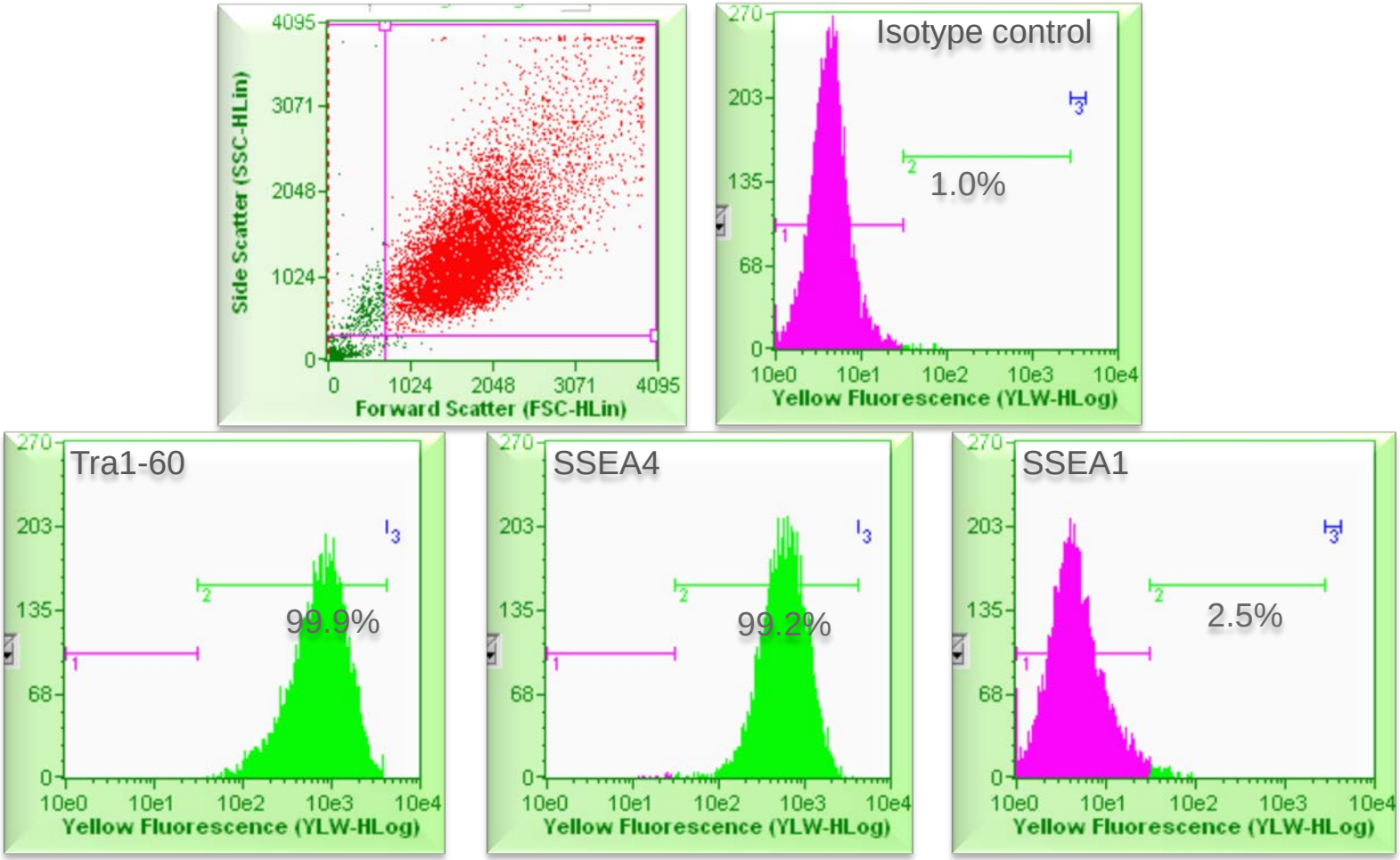
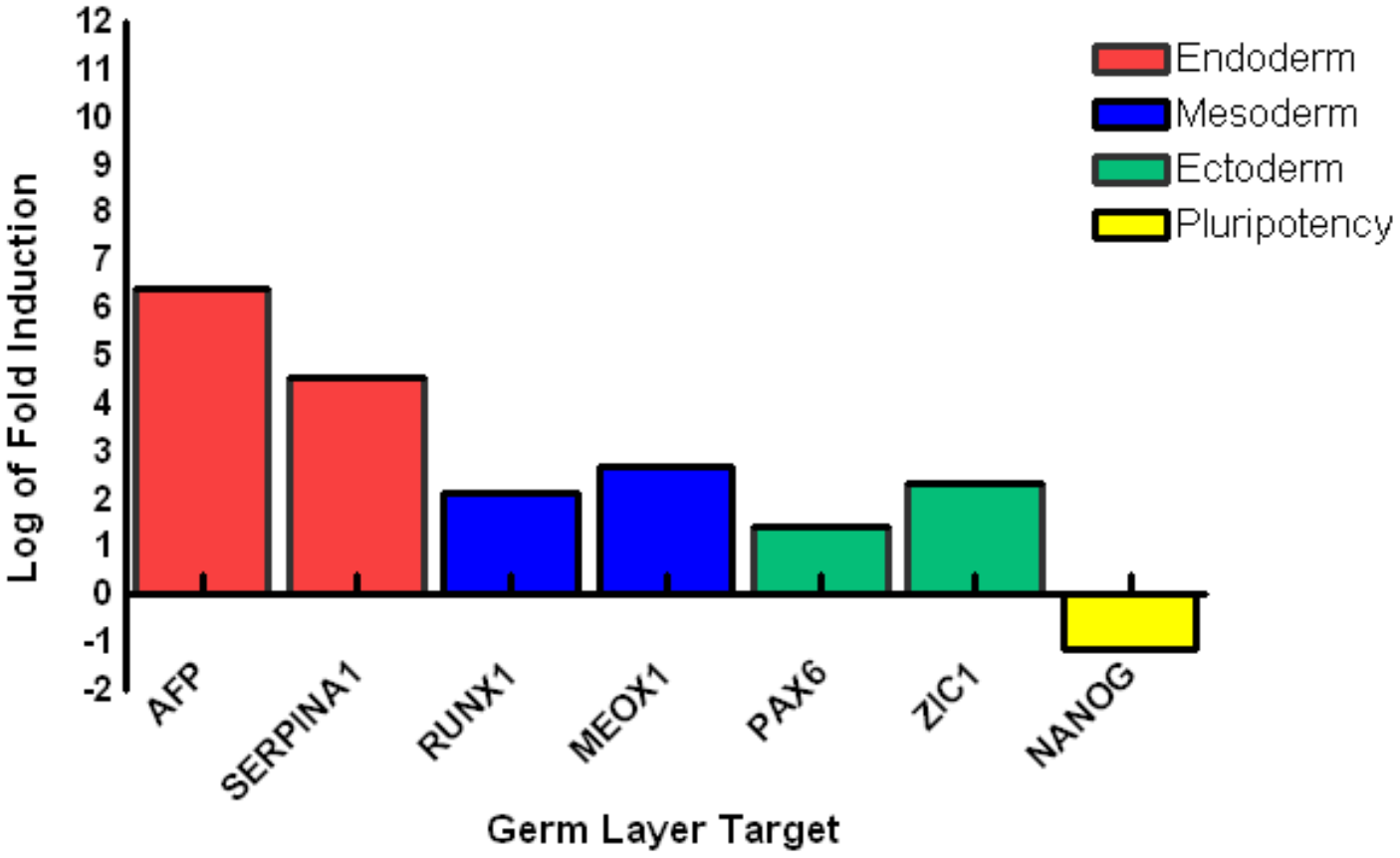


Flow Cytometry: SSEA-4, Tra 1-60 and SSEA-1

ATCC iPSCs are tested for their capacity to differentiate into the three germ layers

HFF iPS cell line (ATCC® ACS-1011™)



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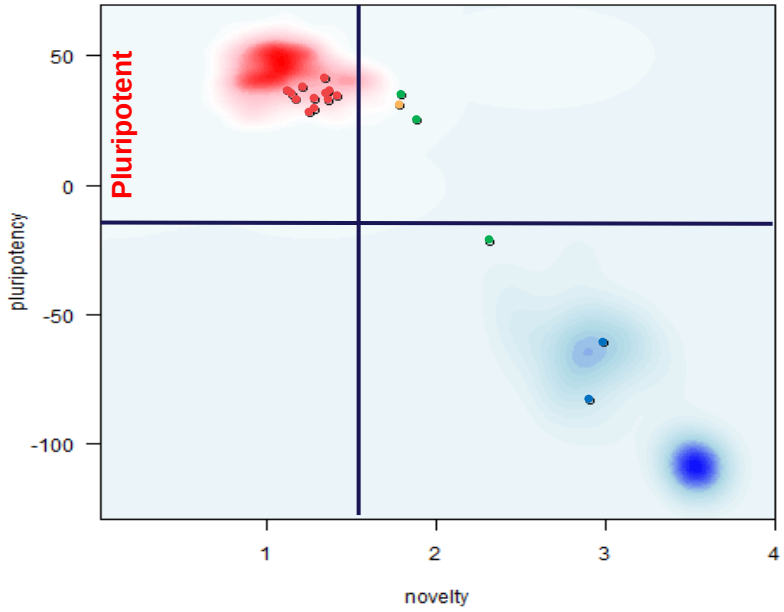
Transcriptome-based pluripotency: PluriTest

- Assesses pluripotency and differentiation based on a comparison of gene expression profiles from a large database of known pluripotent cell samples
  - Pluripotent stem cells (223 hESCs, 41 hiPSCs)
  - Differentiated cell types, developing and adult tissues (204 somatic cells)
- Pluripotency is based on empirically determined thresholds
  - Pluripotency Score: indication of a sample containing a pluripotent signature
  - Novelty Score: based on existing data from other well-characterized PSC lines



- ATCC iPSC lines
- Somatic cells used for reprogramming
- EBs collected at 2, 3 and 4 weeks
- BG01V hESC

Müller *Nat Methods* 8:315-317, 2011, **http://www.pluritest.org**

Easy to use, all-in-one culture system

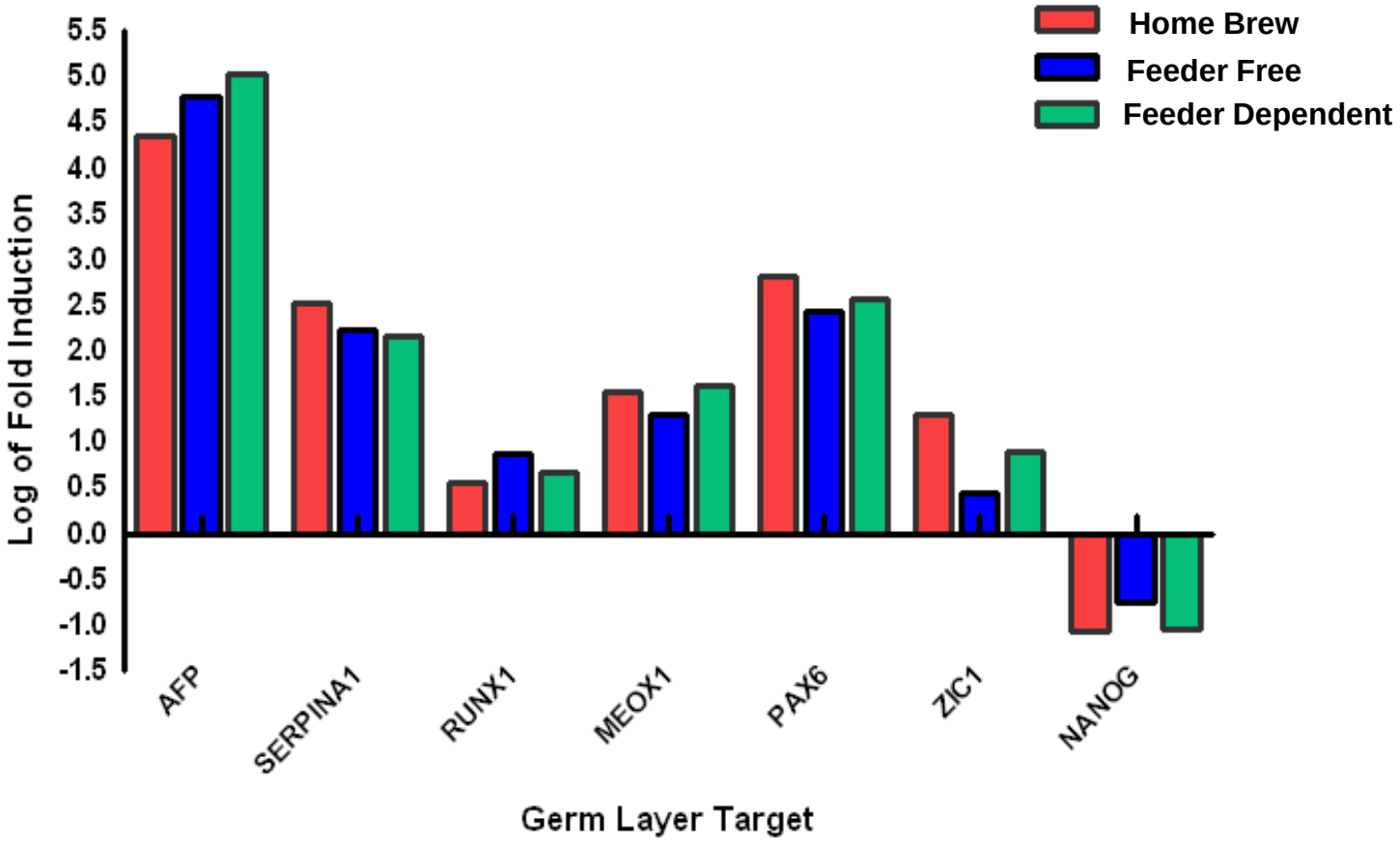
	Feeder-Dependent	Feeder-Free	Conventional
Media	Pluripotent Stem Cell SFM XF (Serum Free, Xeno Free)	Pluripotent Stem Cell SFM XF/FF (Serum Free, Xeno Free)	DMEM:F12 ES Qualified FBS
Substrate	MEF/HFF Mitomycin C treated; γ-irradiated	Cell Basement Membrane Gel	MEF/HFF Mitomycin C treated; γ-irradiated
Passaging	Dissociation Reagent	Dissociation Reagent	Dissociation Reagent
Cryopreservation	Stem Cell Freezing Media	Stem Cell Freezing Media	Stem Cell Freezing Media
Supporting Reagent	ROCK inhibitor	ROCK inhibitor	ROCK inhibitor
Growth Factor	N/A	N/A	bFGF

No adaptation necessary, all reagents are formulated to work together!

Visit www.atcc.org for a complete list of feeder cells

ATCC iPSC cultured in different media system retain consistent differentiation capabilities

HFF iPS cell line (ATCC® ACS-1011™)



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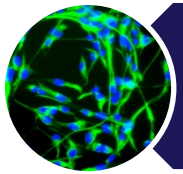
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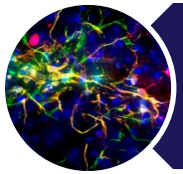
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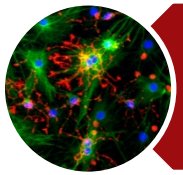
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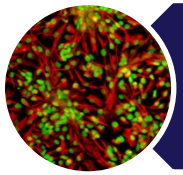
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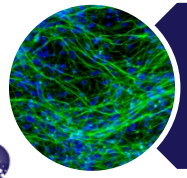
Human mesenchymal stem cells

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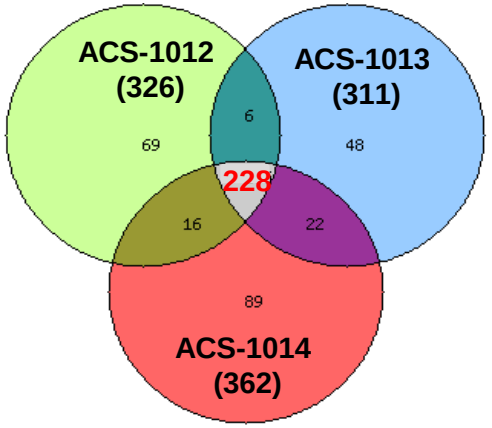
ATCC Parkinson's iPSC lines

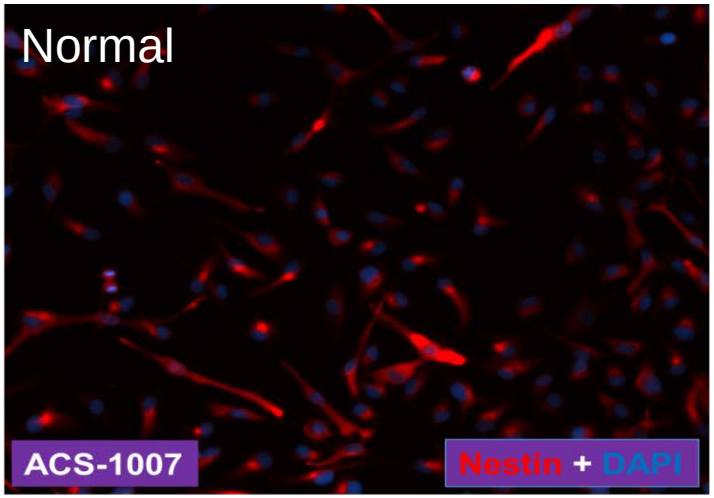
- Patient-specific iPSCs provide an opportunity to model human disease in culture – 'Disease-in-a-dish'
- **Parkinson's Disease** – Second most common neurodegenerative disorder
- **Donor information**: 63 years old Caucasian male diagnosed with Parkinson's disease, asthma, and depression
- Exome sequencing identified multiple missense mutations in Leucine-Rich Repeat Kinase 2 (LRRK2) gene: R50H, I1723V, M2397T

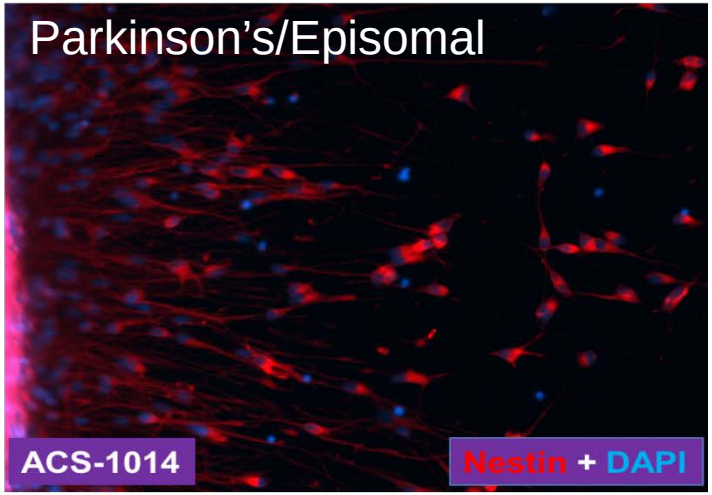
ATCC® No.	Designation	Reprogramming Method
ACS-1012™	ATCC-DYR0530	Retrovirus
ACS-1013™	ATCC-DYS0530	Sendai virus
ACS-1014™	ATCC-DYP0530	Episomal



Fonzo A.D. et al., Eur J Hum Genet. 2006; 14: 322-331

Reprogramming methods do not affect differentiation potential





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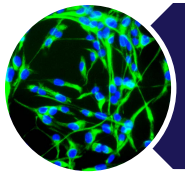
Outline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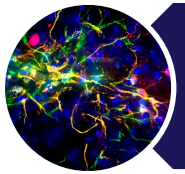
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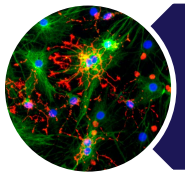
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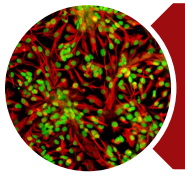
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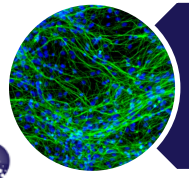
Human mesenchymal stem cells

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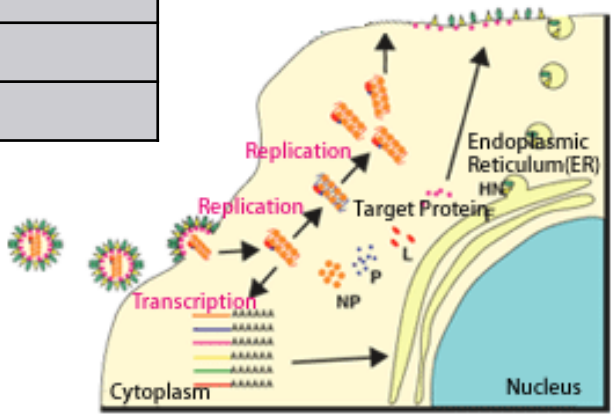
Reference iPSC collection

Coming Soon!

- Derivation criteria

Somatic cell of origin	CD34+ cells
Disease state	Clinically normal
Reprogramming method	Sendai virus (Foot-print free)
Gender	Male
	Female
Age	Adult, 25-60
Ethnicity	African American
	Hispanic
	Caucasian
	Asian
Somatic cell of origin	CD34+ cells

- In-depth characterization
  - Differentiation potential
  - Stability over long-term in *vitro* culture
  - Whole exome sequencing



http://www.lifetechnologies.com

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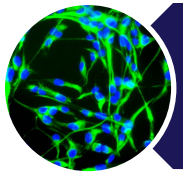
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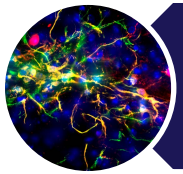
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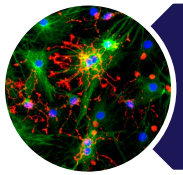
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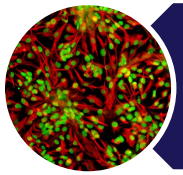
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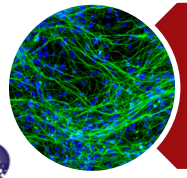
Human mesenchymal stem cells

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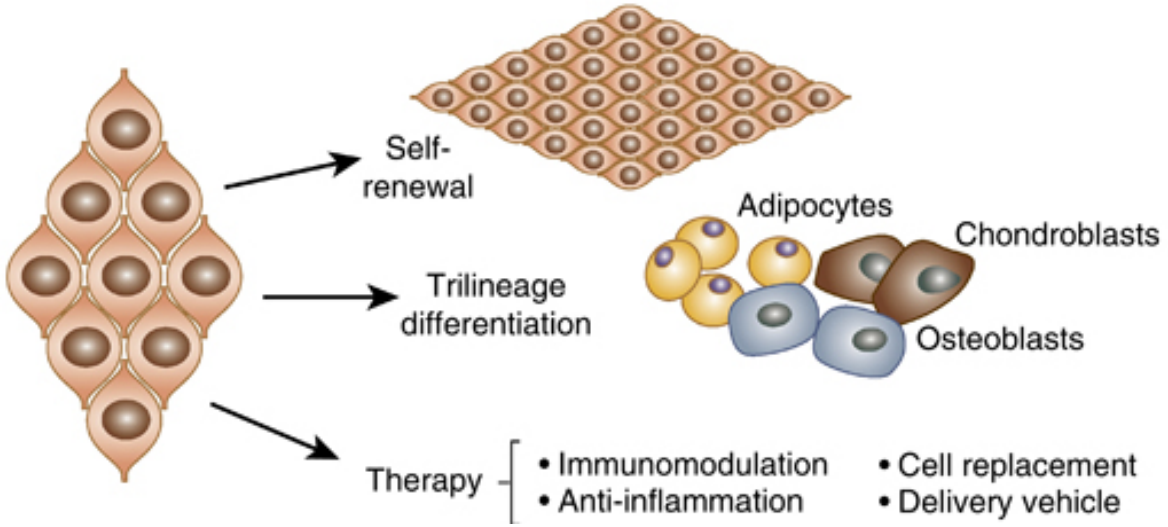
Mesenchymal stem cells



Mesenchymal stem cells, or MSCs, are multipotent stromal cells that can differentiate into a variety of cell types, including: osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells).

The cells could potentially be used to treat diseases by providing immunomodulation, anti-inflammatory actions, and cell replacement as well as delivering therapeutic agents.

ATCC

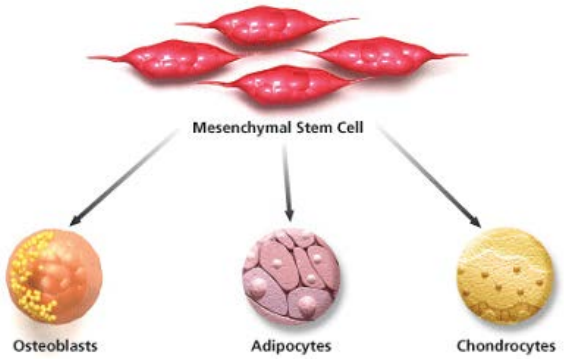
Lin F. *Kidney International* 82: 731–733, 2012.

Easy to use MSC Culture System from ATCC

- Mesenchymal Stem Cells derived from various tissue sources

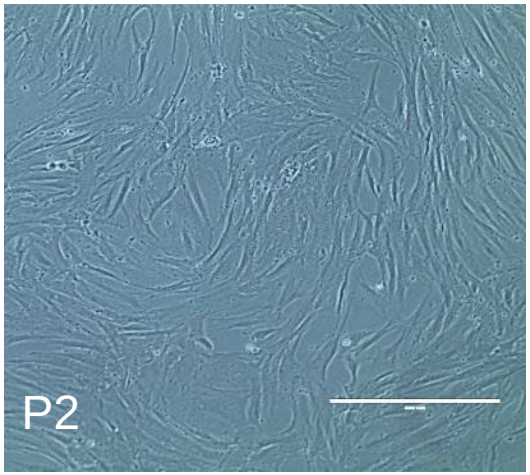
ATCC® No.	Name	Source	Age	Passage #
PCS-500-010	Umbilical Cord-Derived MSCs; Normal, Human	Umbilical cord matrix	Neonatal	2
PCS-500-011	Adipose-Derived MSCs; Normal, Human	Lipo-Aspirate	Adult	2
PCS-500-012	Bone Marrow-Derived MSCs; Normal, Human	Bone Marrow	Adult	2

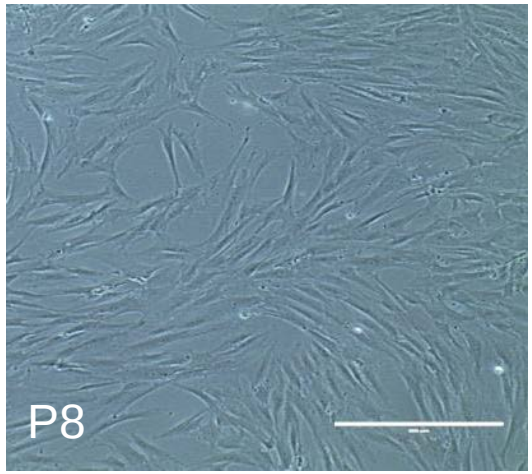
- Optimized Mesenchymal Stem Cell Culture Medium to ensure:
  - Functional expression of MSC markers
  - Growth and proliferation
  - Multi-lineage differentiation capability
- Multi-lineage Differentiation Kit
  - Adipocyte Differentiation Toolkit
  - Chondrocyte Differentiation Toolkit
  - Osteocyte Differentiation Toolkit

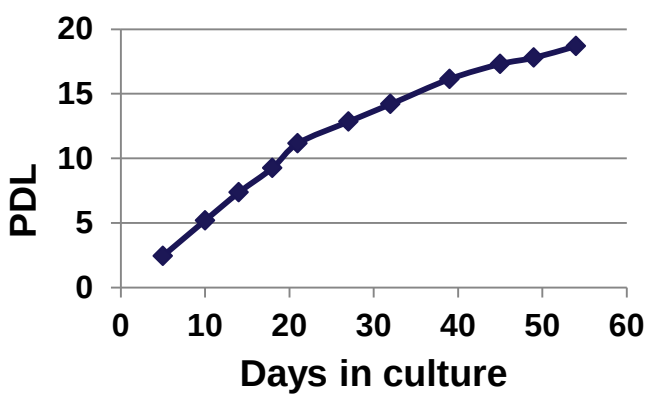


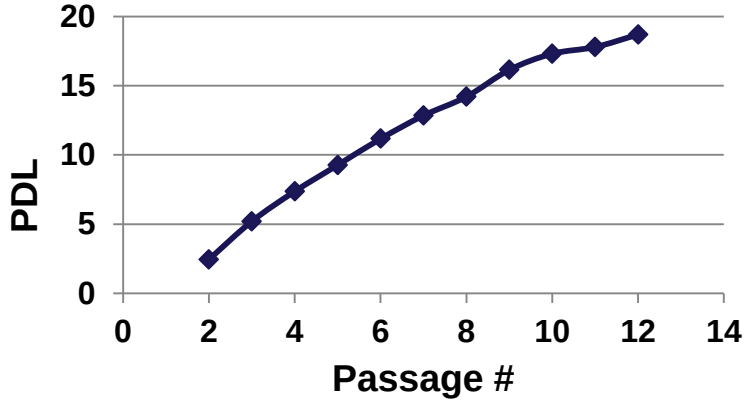
http://pharmapeek.com

Characterization of BM-MSCs





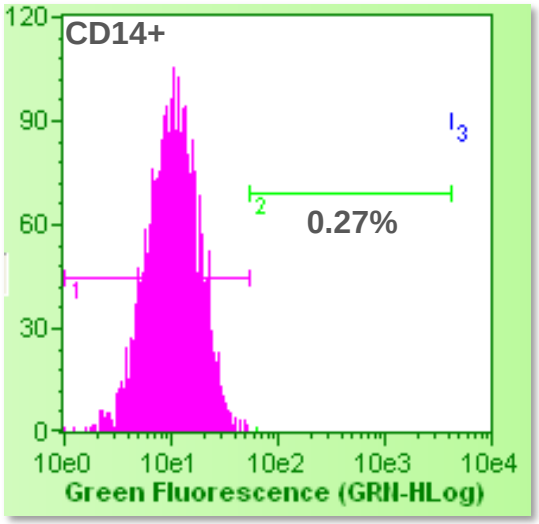


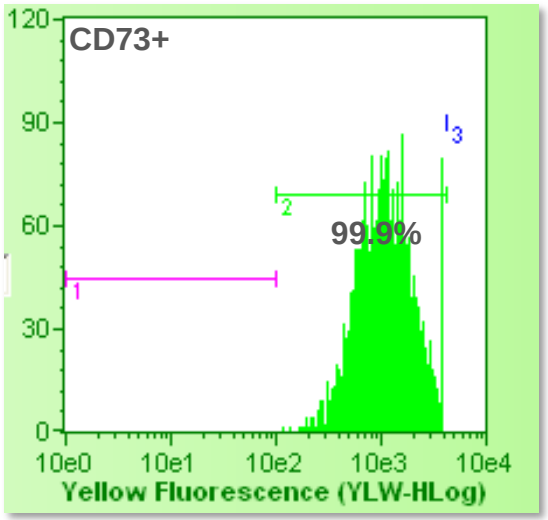


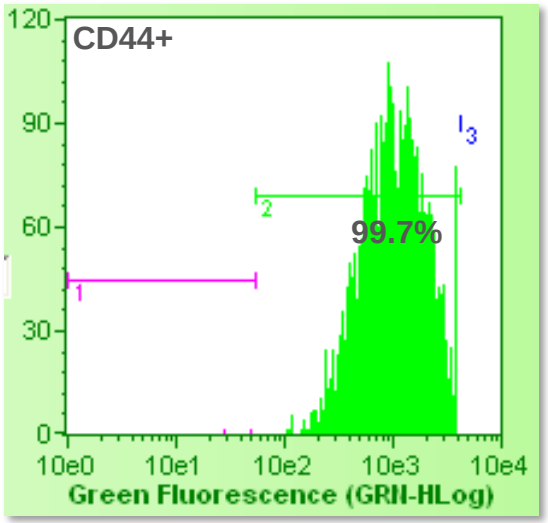
Post-thaw recovery and growth curve of BM-MSCs

Characterization of BM-MSCs

Flow analysis of a panel of MSC-specific CD markers recommended by ISCT (International Society for Cellular Therapy) in BM-MSCs after 3 or 6 passages of *in vitro* culture.







Passage #	CD14+	CD19+	CD34+	CD45+	CD29+	CD44+	CD73+	CD90+	CD105+	CD166+
P5	0.27%	0.1%	2.90%	0.15%	99.5%	99.7%	99.9%	98.0%	99.7%	98.4%
P8	1.5%	0.2%	3.1%	1.8%	99.7%	99.8%	99.8%	99.5%	99.3%	97.2%

BM-MSCs exhibit consistent immunophenotype over extended culture

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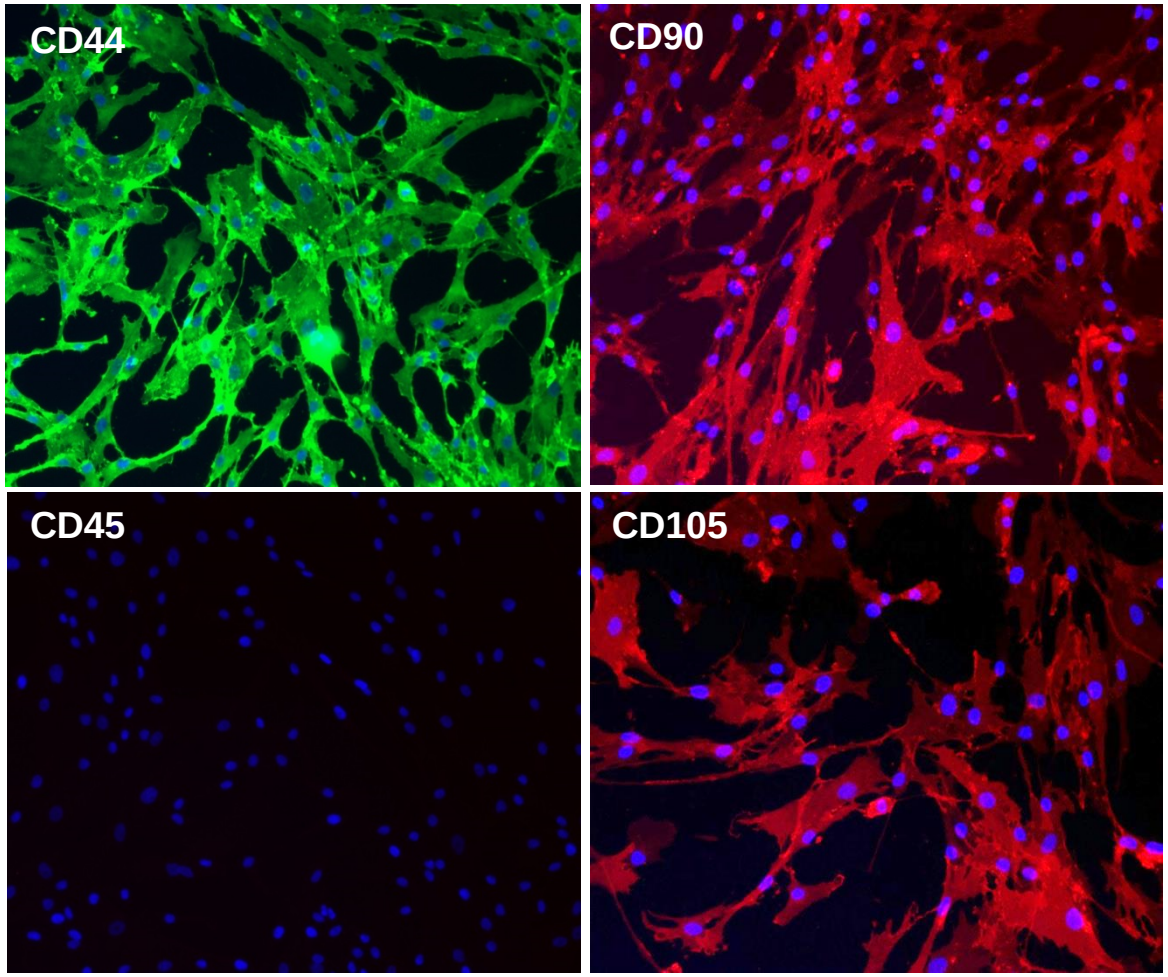
Dominici M. *Cytotherapy*. 8(4): 315-317, 2006

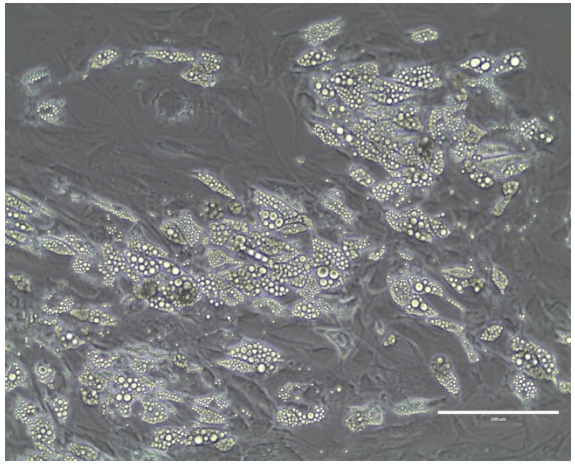
Characterization of BM-MSCs

Immunocytochemistry analysis of MSC markers expression

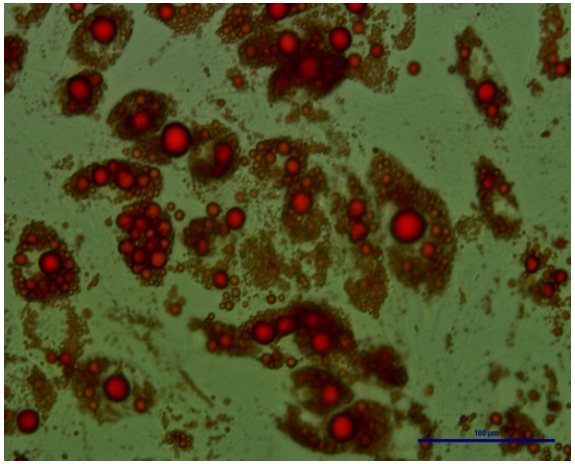


Characterization of BM-MSCs

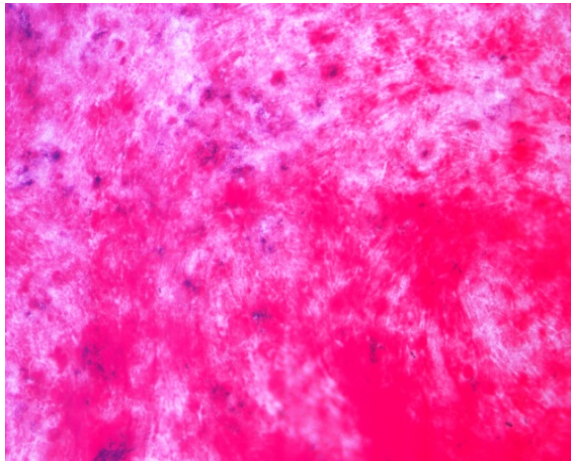
Adipogenic, osteogenic, and chondrocyte differentiation of BM-MSCs



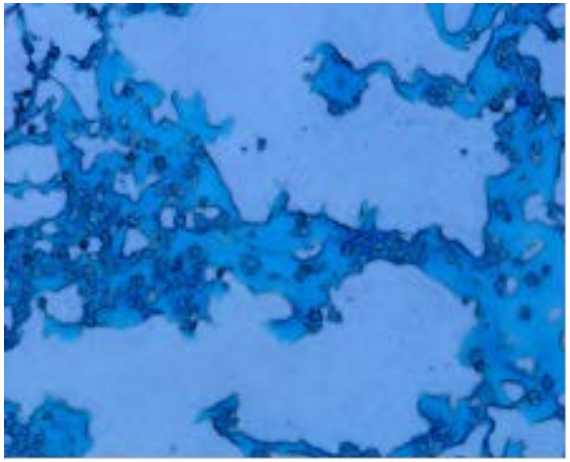
Adipocytes



Adipocytes stained with Oil Red O



Osteoblasts stained with Alizarin Red



Chondrocytes stained with Alcian Blue