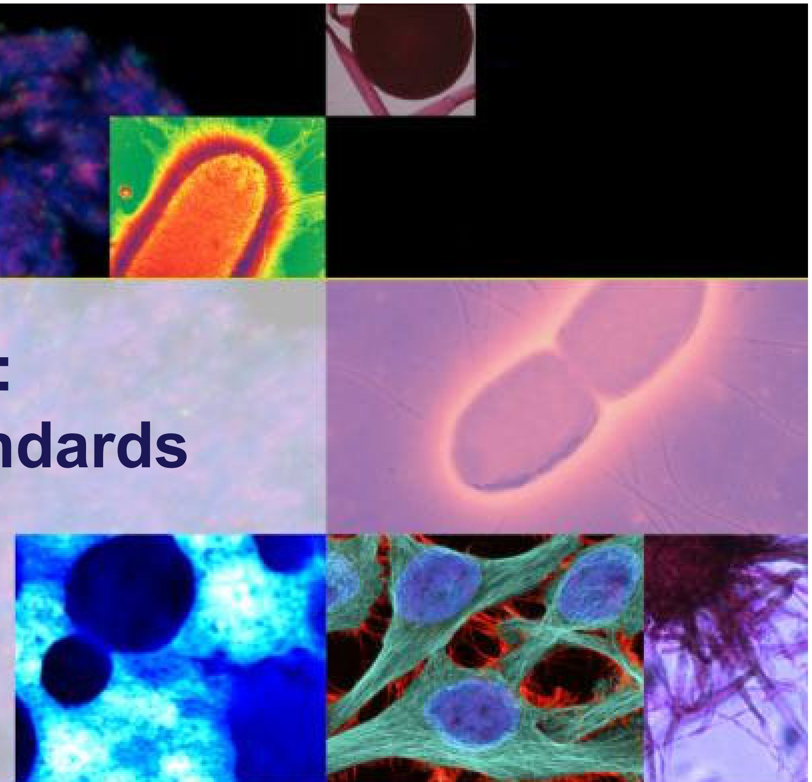
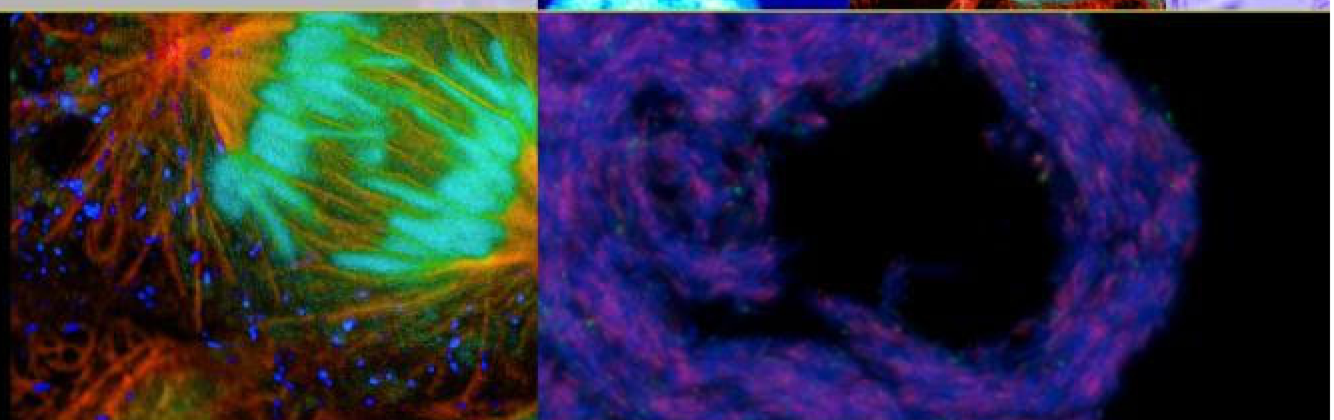


ATCC STEM CELLS SOLUTIONS:
Enabling Research Through Standards

Yukari Tokuyama, Ph.D.

Applications Scientist, ATCC

August 21, 2014



ATCC™ | THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

Outline

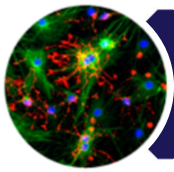
- Introduction to ATCC
- Human induced Pluripotent Stem Cells (iPSCs)
  - ATCC iPSC Collection
  - Quality Standards and Characterization
- Supporting Reagents and Products

ATCC®

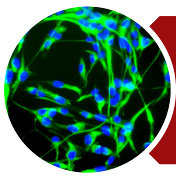
About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions
- Broad range of biomaterials
  - Cell lines
  - Microorganisms
  - Native & synthetic nucleic acids
  - Reagents

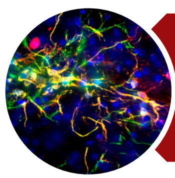
Outline



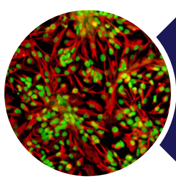
Introduction to ATCC



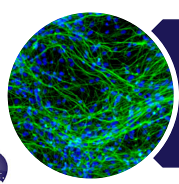
Human induced Pluripotent Stem Cells (iPSCs)



ATCC iPSC Collection



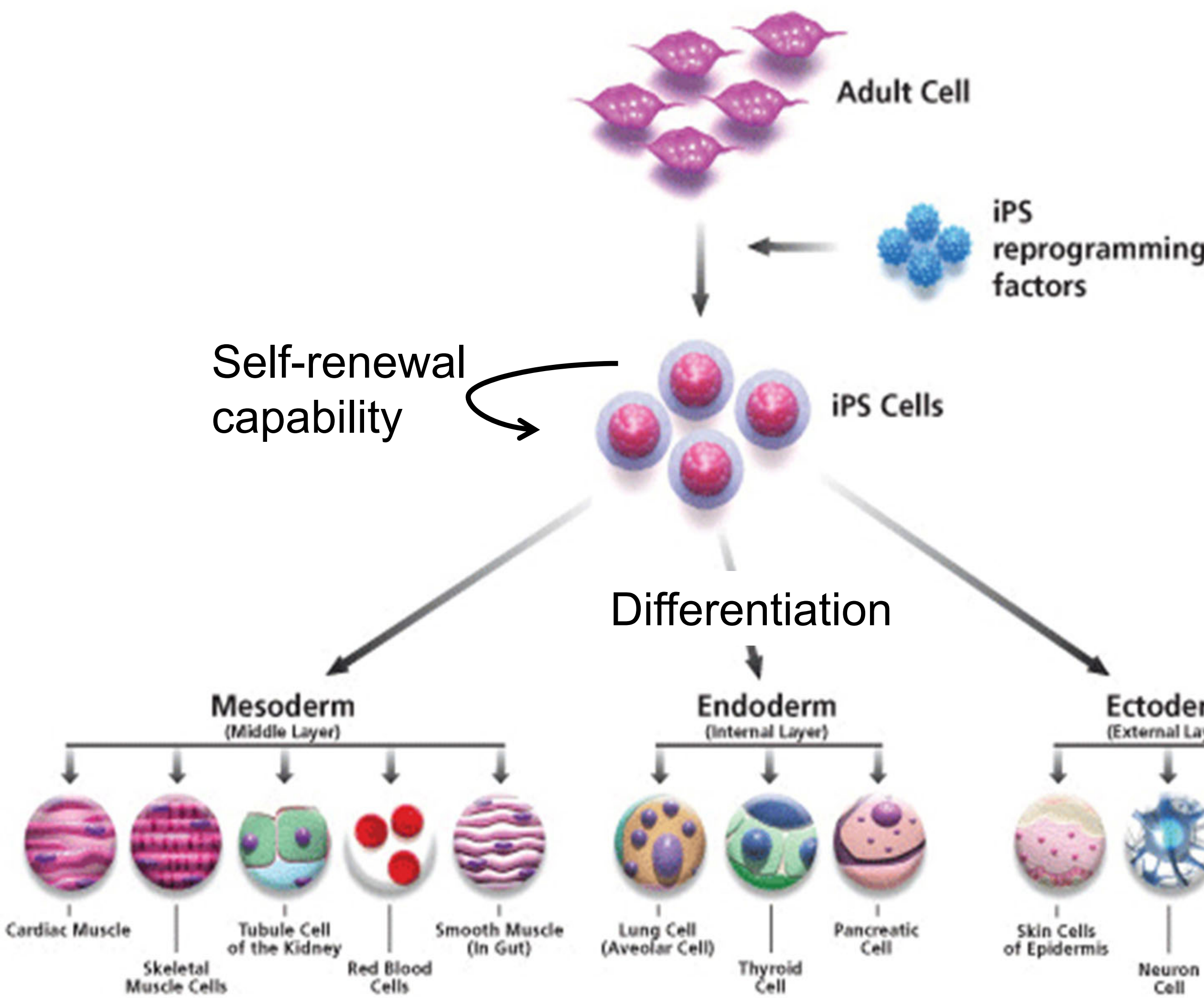
Quality Standards and Characterization



Supporting Reagents and Products

ATCC®

What are iPSCs?



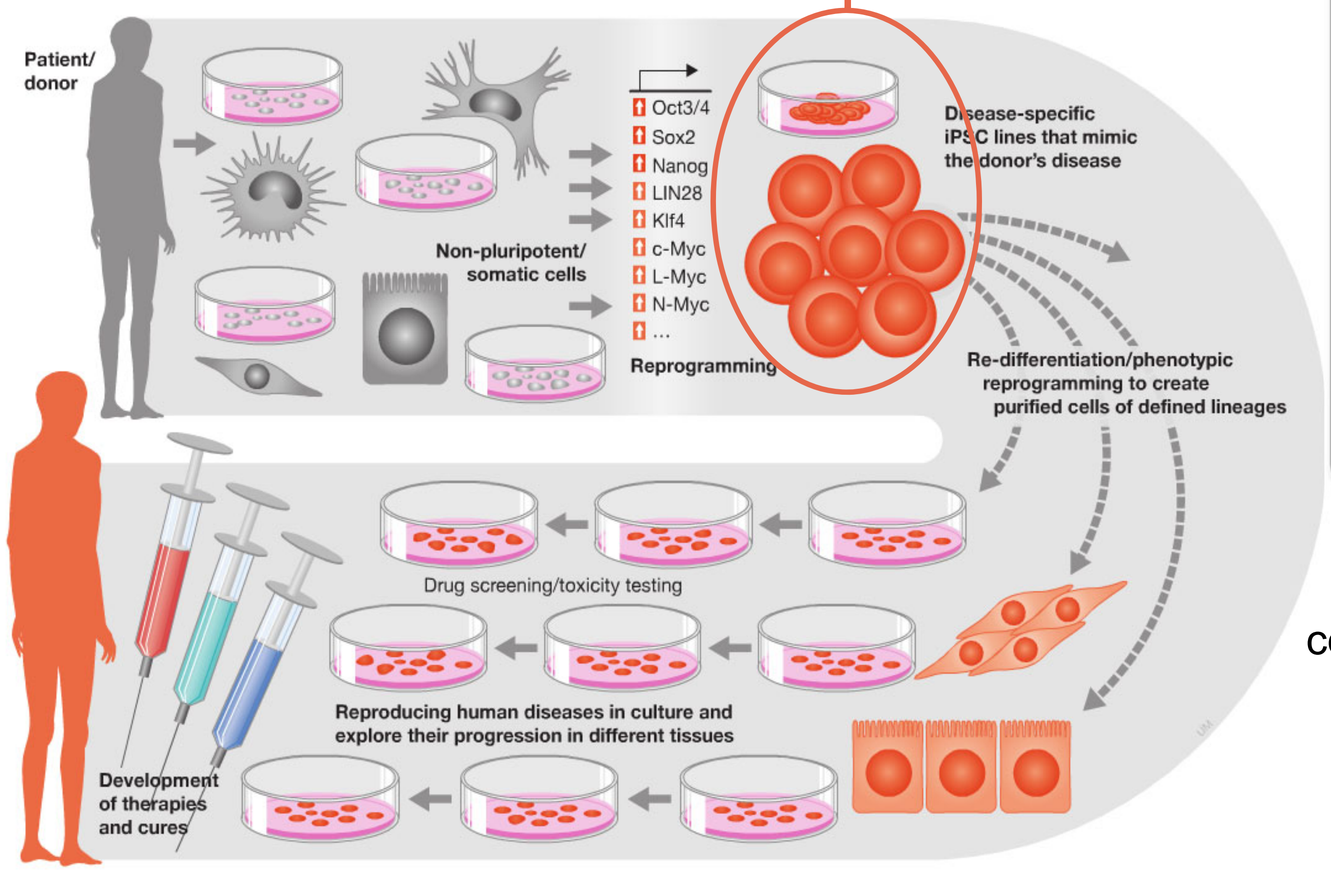
Yamanaka Factors

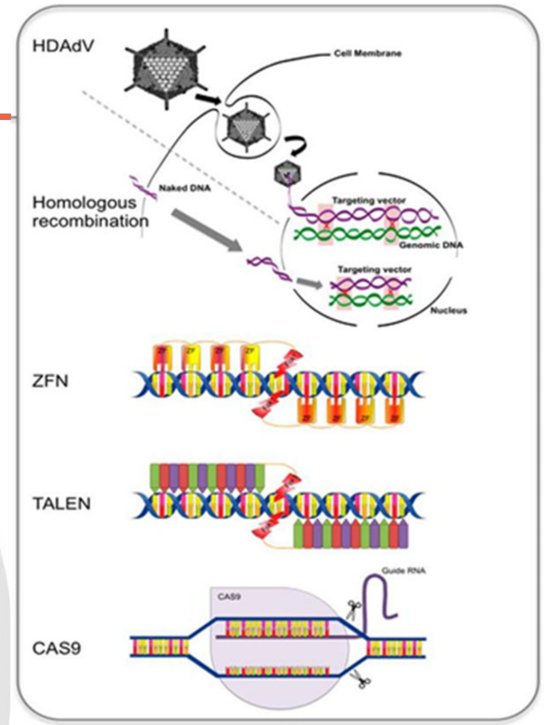
- Oct3/4[1,2]
- Sox2[1,2]
- Klf4[1]
- Myc[1]
- Nanog[2]
- Lin28[2]

1. Takahashi K. *Cell* 126: 663-76, 2006
2. Yu, J. *Science* 318: 1917-20, 2007

ATCC®

The promise of iPSCs





Gene editing
correcting inherited diseases

ATCC® Adapted from Li M et al. J. Biol. Chem. 289:4594-4599, 2014 and Deng W et al. EMBO Reports 11(3):161-5, 2010

ATCC iPSC Collection

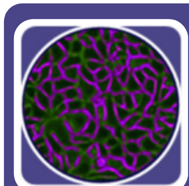
- Intellectual Property (IP) licenses
  - iPS Academia Japan (iAJ): iPSC-related technology
  - Sendai Virus Technology: Integration-free reprogramming delivery system
- ATCC iPSC lines
  - Normal: Foreskin, Hepatic and Cardiac fibroblasts
  - Diseased: Down Syndrome, Cystic Fibrosis, Parkinson’s Disease
  - Reference iPSCs: Ethnic diverse and gender
- Reprogramming methods used to introduce Yamanaka factors (Oct3/4, Sox2, Klf4 and Myc)
  - Integration: Retrovirus
  - Integration-free: Sendai virus, Episomal Plasmid

Normal human primary cells for reprogramming

- ATCC Primary Solutions provide complete culture reagents formulated for optimal cell growth, morphology, and functionality
- ATCC primary cells are provided at very low passage

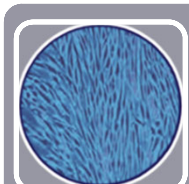
Melanocytes

- Adult
- Neonatal



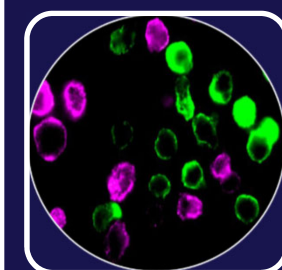
Keratinocytes

- Adult
- Neonatal



Dermal Fibroblasts

- Adult
- Neonatal
- Neonatal mitomycin C-treated



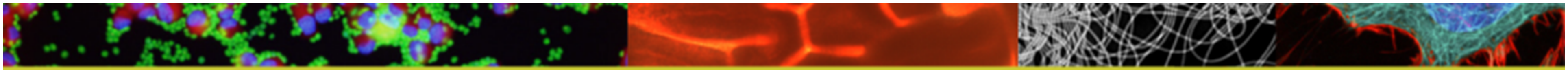
Hematopoietic cells

- CD 34+ cord blood
- CD 34+ bone mesenchymal
- Peripheral blood mononuclear
- Bone marrow mononuclear
- Peripheral CD14+ monocytes

ATCC iPSC Normal Collection

- iPSC lines derived from apparent normal donors
- Fully consent and licensed for research use
- All ATCC iPSC lines are pre-adapted to an optimized serum-free, xeno-free, feeder-free cell culture environment

ATCC® No.	Designation	Reprogramming Method	Tissue of Origin	Disease
ACS-1019™	ATCC-DYS0100	Sendai Virus	Foreskin Fibroblast	Normal
ACS-1020™	ATCC-HYS0103	Sendai Virus	Liver Fibroblast	Normal
ACS-1021™	ATCC-CYS0105	Sendai Virus	Heart Fibroblast	Normal
ACS-1007™	ATCC-HYR0103	Retrovirus	Liver Fibroblast	Normal
ACS-1011™	ATCC-DYR0100	Retrovirus	Foreskin Fibroblast	Normal



ATCC Reference iPSC Collection

Expand CD34+ cells

Introduce reprogramming factors

Pick, expand, & QC iPS cell clones

<u>Creating Standards</u>

- Control lines for better comparison of data
- Develop lines with gender and ethnic diversity
- Fully characterized differentiation potential
- Reprogrammed from bone marrow CD34+
- Footprint free, sendai virus reprogrammed

ATCC®

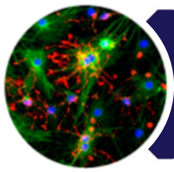
ATCC Reference iPSC Collection

Coming soon!

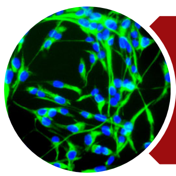
ATCC® No.	Designation	Gender	Ethnicity
ACS-1024™	ATCC-BYS0110	Male	African America
ACS-1028™	ATCC-BYS0114	Female	African America
ACS-1025™	ATCC-BYS0111	Male	Hispanic
ACS-1029™	ATCC-BXS0115	Female	Hispanic
ACS-1026™	ATCC-BYS0112	Male	Caucasian
ACS-1030™	ATCC-BXS0116	Female	Caucasian
ACS-1027™	ATCC-BYS0113	Male	Asian
ACS-1031™	ATCC-BXS0117	Female	Asian

ATCC®

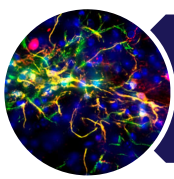
Outline



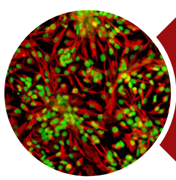
Introduction to ATCC



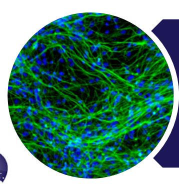
Human induced Pluripotent Stem Cells (iPSCs)



ATCC iPSC Collection



Quality Standards and Characterization



Supporting Reagents and Products

ATCC®

Quality standards

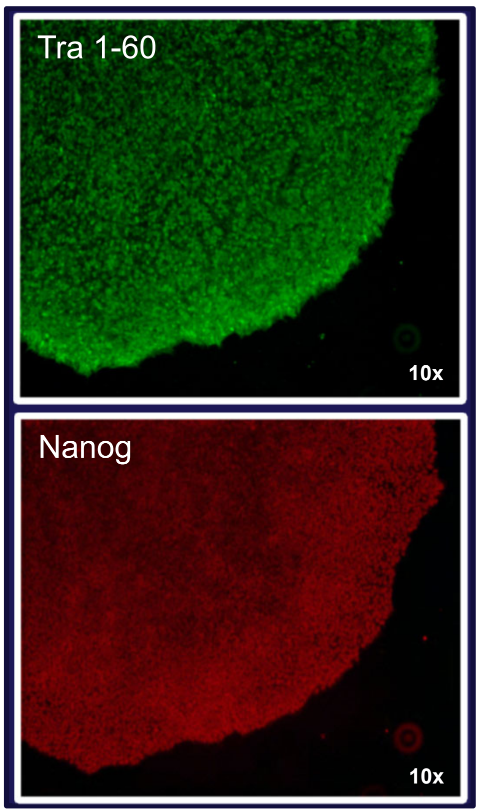
Quality attribute

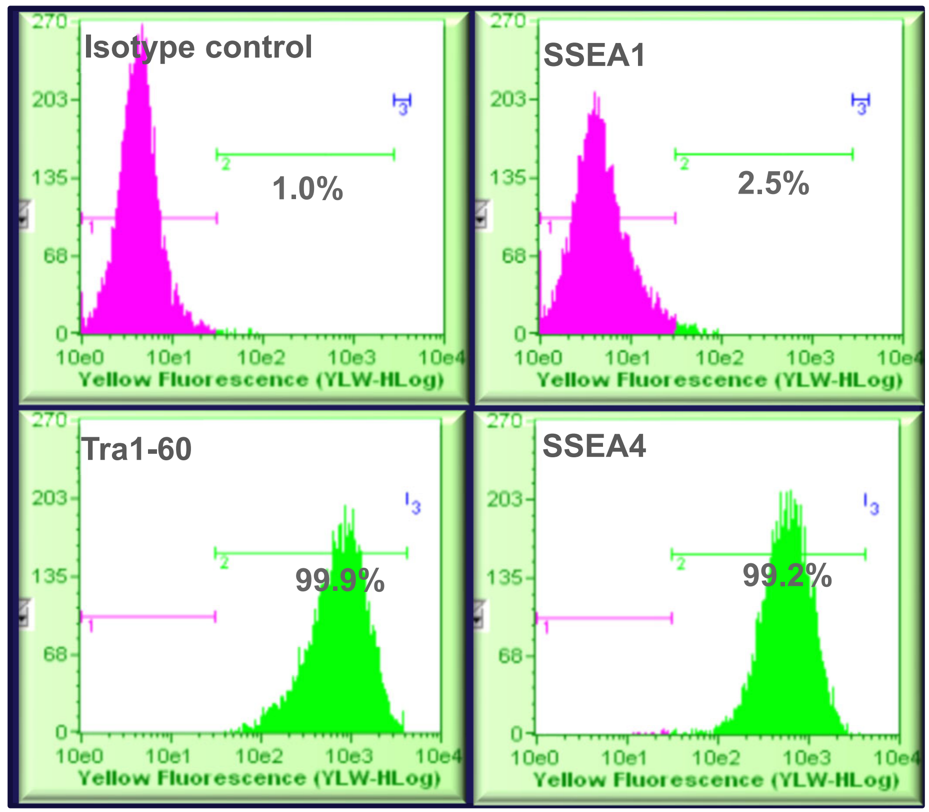
Authentication	Preservation	Characterization
• Identity • Karyotype • Sterility • Mycoplasma • Viral Panel	• Post thaw viability • Morphology • Growth • Purity	• Pluripotency expression • Germ layer differentiation • Transcriptome analysis

ATCC®

ATCC iPSCs monitored for pluripotency

Pluripotency Markers	Reactivity
Nanog	+
Tra 1-60	+
Tra 1-81	+
SSEA-4	+
SSEA-1	-

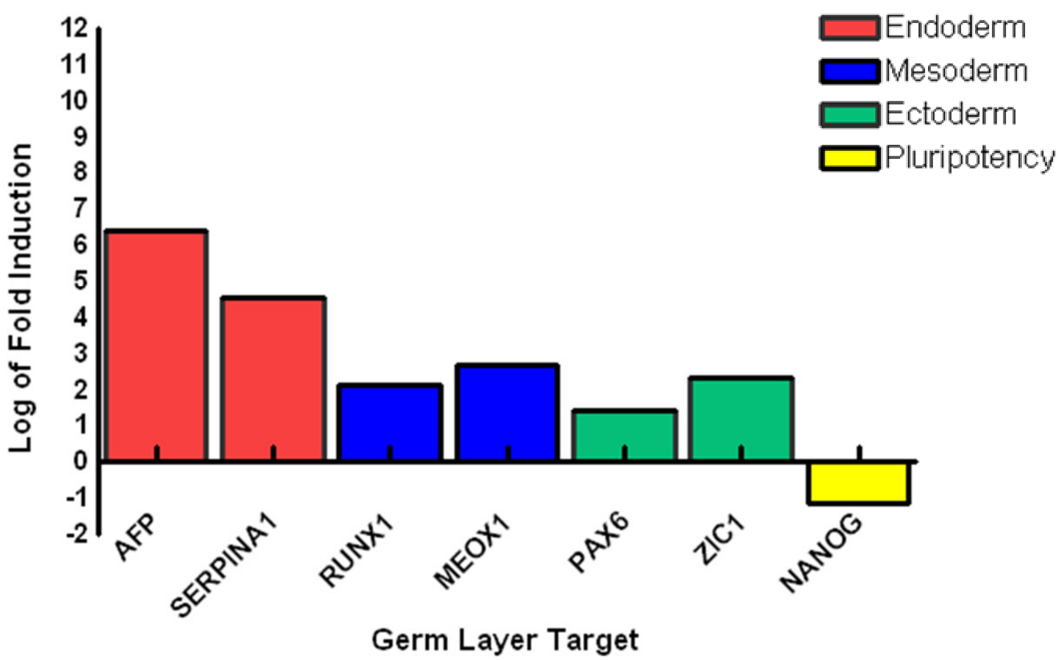


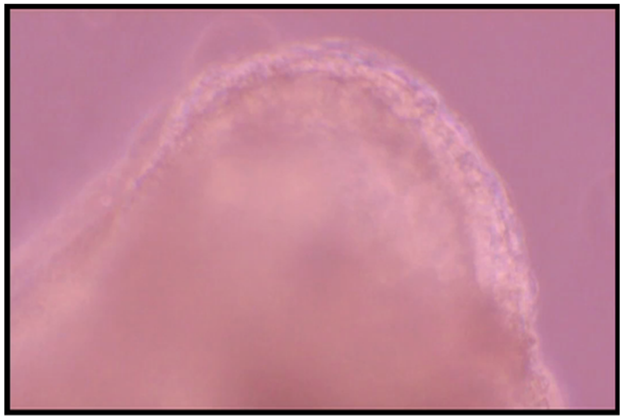


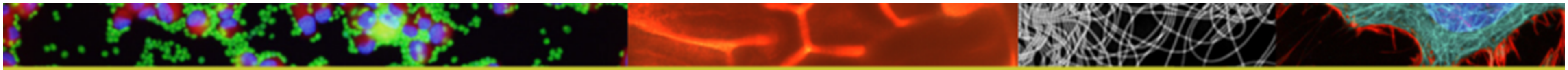
ATCC®

ATCC iPSCs maintain differentiation potential

Pluripotency:
Three germ layer differentiation by EB formation

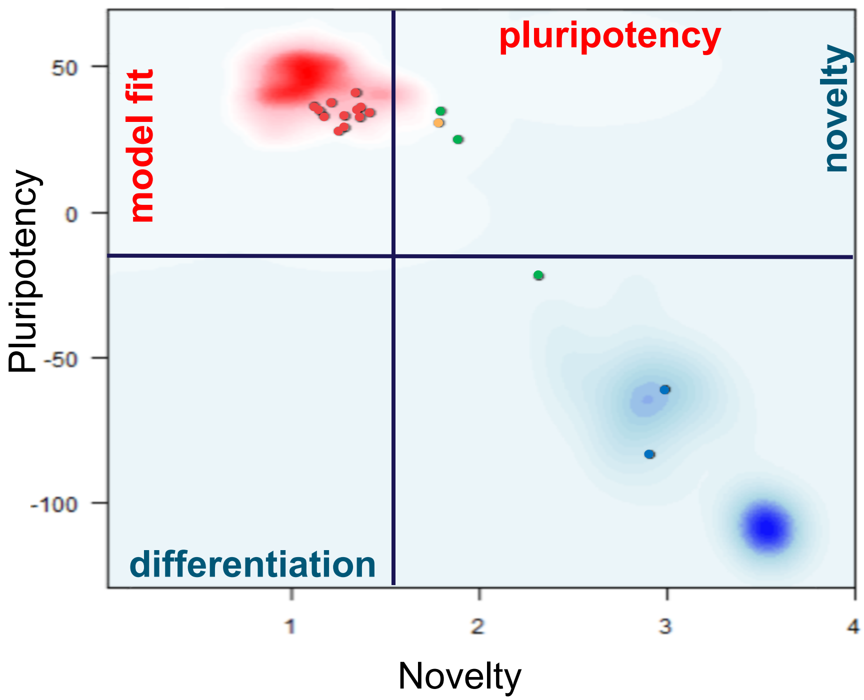






PluriTest™: ATCC iPSCs deemed pluripotent

Bioinformatic Analysis: Assesses pluripotency and differentiation based on a comparison of gene expression profiles from a large database of known samples



Pluripotency Score

Sample contains pluripotent signature

Novelty Score

Based on existing data from well-characterized PSC lines data

- ATCC iPSC lines
- Somatic cells used for reprogramming (HFF, CD34+)
- EBs 2, 3 and 4 weeks
- BG01V hESC (karyotypically abnormal)

ATCC

http://www.pluritest.org

[1]Müller *Nat Methods* 8:315-317, 2011

ATCC Disease iPSC Collection

- iPSC lines derived from donors with diseases
- Fully consent and licensed for research use
- All ATCC iPSC lines are pre-adapted to an optimized serum-free, xeno-free, feeder-free cell culture environment

ATCC® No.	Designation	Reprogramming Method	Tissue of Origin	Disease
ACS-1012™	ATCC-DYR0530	Retrovirus	Skin	Parkinson's Disease, Asthma, Depression
ACS-1013™	ATCC-DYS0530	Sendai viral	Skin	Parkinson's Disease, Asthma, Depression
ACS-1014™	ATCC-DYP0530	Episomal	Skin	Parkinson's Disease, Asthma, Depression
ACS-1003™	ATCC-DYP0730	Episomal	Foreskin	Down syndrome
ACS-1004™	ATCC-DYP0250	Episomal	Skin	Cystic fibrosis: homozygous for CFTR Δ508

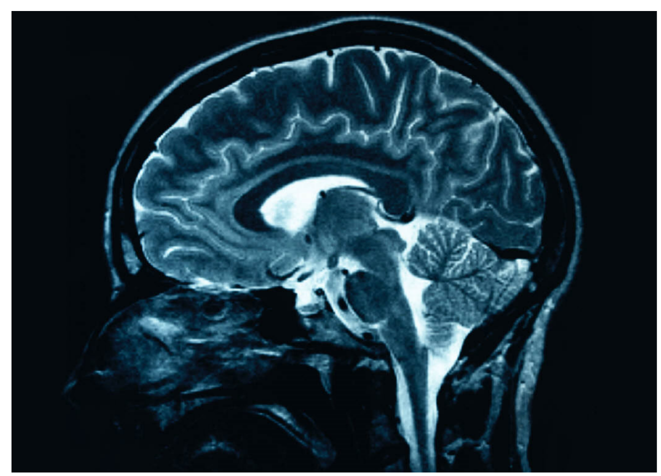
ATCC®

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
  - Selective degeneration of dopaminergic neurons in the substantia nigra
- **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

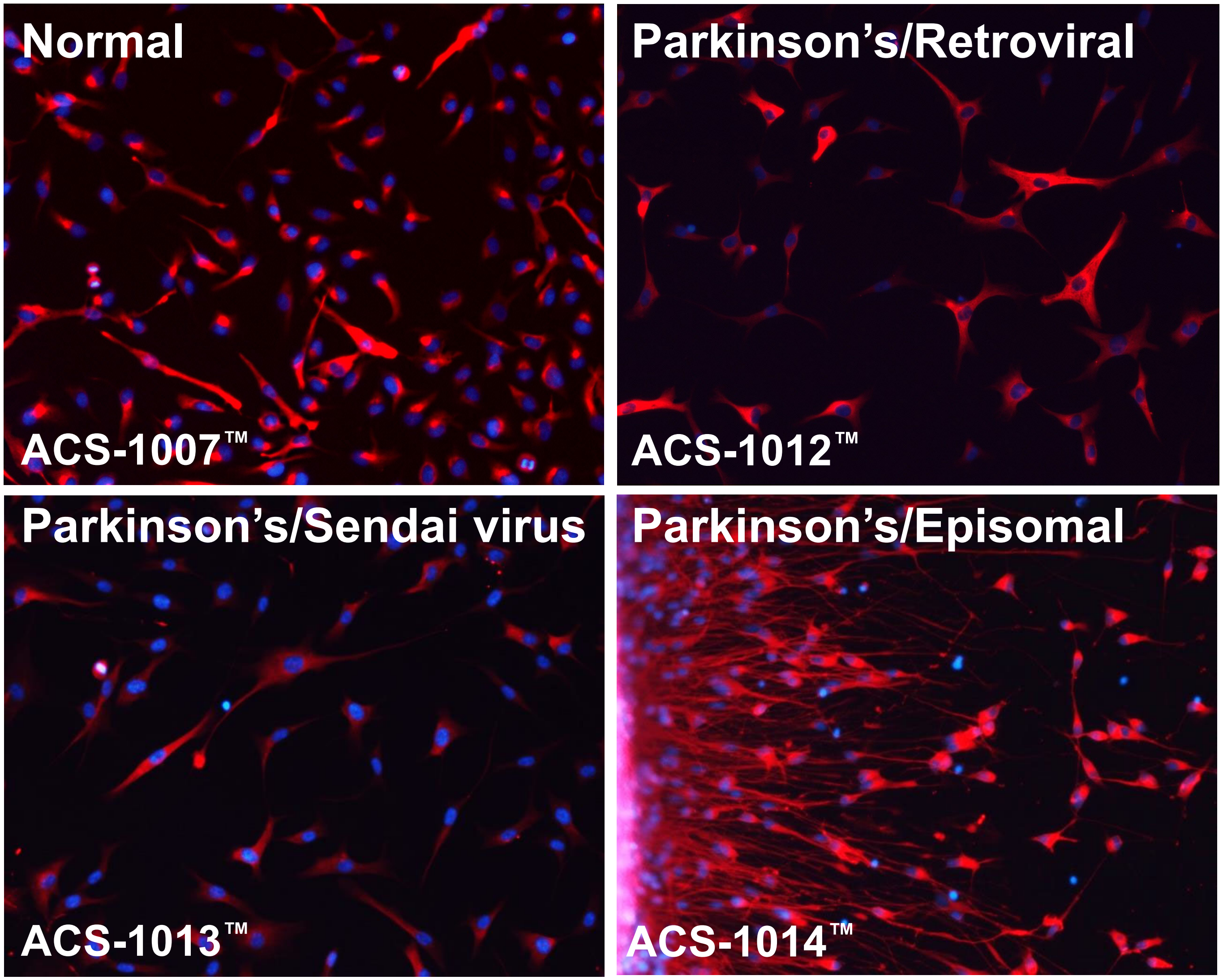
ATCC® No.	Designation	Reprogramming method
ACS-1012™	ATCC-DYR0530	Retrovirus
ACS-1013™	ATCC-DYS0530	Sendai virus
ACS-1014™	ATCC-DYP0530	Episomal



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

ATCC®

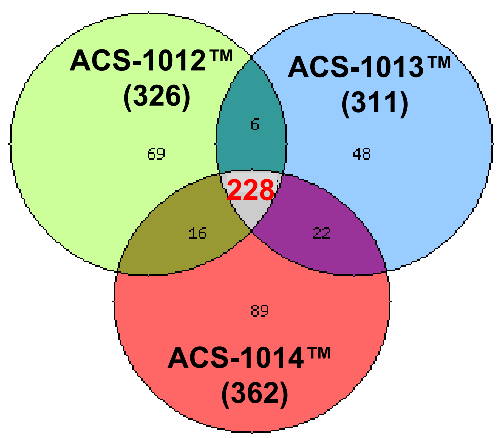
Reprogramming methods do not affect differentiation potential



Nestin (red)
DAPI (blue)

Identification of missense mutations in Parkinson's hiPSC lines by exome sequencing

Cell line	Reprograming method	Passage #	Mutation #	Shared mutations with fibroblast	Shared mutations with ACS-1012™	Shared mutations with ACS-1013™	Shared mutations with ACS-1014™
Parental Fibroblasts	N/A	P5	319	N/A	77%	78%	80%
ACS-1012™	Retroviral	P13	325	75%	N/A	74%	77%
ACS-1013™	Sendai viral	P12	310	81%	77%	N/A	83%
ACS-1014™	Episomal	P16	362	71%	69%	71%	N/A



Conserved gene mutations among Parkinson's iPSC lines

Gene symbol									
ABCA7	C3orf20	DHRS2	GCC1	L3MBTL4	NARG2	PLCB4	SDK2	SPTBN4	UBR4
ACCN5	CACNA1B	DISP2	GLIS3	LACTB	NASP	PLEKHG2	SEC24C	SRC	UGT1A4
ACER1	CCM2	DNAH10	GRB10	LAMA5	NCAPH2	PMEPA1	SEMA3G	SSPO	UMODL1
ADNP2	CD28	DNAH2	HCLS1	LAMC3	NDUFAF1	PNCK	SEMA4A	STAT2	UNC13A
AHDC1	CDC45	DNAH3	IAH1	LCP1	NGF	PNPLA2	SERPINA1	STX8	UPK3B
ALDH1L1	CENPF	DNAH7	IER5	LGR5	NLRP8	POLDIP3	SERTAD4	SYDE2	USP53
ALKBH8	CHAF1A	DNAI1	IMPG1	LRP2	NLRX1	POLQ	SHISA7	SYNE1	VGLL3
ALMS1	CHD6	DNAJC11	INHBE	LRRC66	NPC1	POLR2F	SIGLEC1	TADA2A	VSIG10
AMDHD2	CLEC3B	DOCK2	INPP5D	LRWD1	NWD1	PRRC2B	SIVA1	TBC1D30	VTA1
AMPH	CLIC6	DYRK1B	IPO13	MAPK12	OAZ1	PRSS1	SLC26A2	TBL3	VWA5B1
ANO7	CMA1	EHD2	JMJD7	MBTPS1	OGFR	PRSS2	SLC45A4	TC2N	WBSCR16
AP3B2	CNNM3	ERVMER34-1	JMJD7-PLA2G4B	MDGA2	OR4C15	PRSS3	SLC5A1	TDRD12	WDFY1
ARHGAP4	CNTNAP4	F5	KCNAB1	MDN1	OR4L1	PRX	SLC7A2	TEP1	WDR65
ARHGEF19	COBLL1	FAM120A	KCNK10	MEF2A	OSBPL2	PTPRN2	SMCR8	TEX15	ZNF250
ATP1A4	COL14A1	FAM178A	KIAA0232	MILR1	PABPC1	RAB11FIP4	SNPH	TIAM2	ZNF469
ATP9B	COL27A1	FAM186A	KIAA1274	MKNK2	PCDHGA3	RANGAP1	SOLH	TM9SF1	ZNF507
BSND	CRLS1	FAM208B	KIF17	MPG	PDE4C	RCOR2	SPAG1	TMEM2	ZNF629
C10orf27	CSF1	FAM63A	KIF20B	MPPE1	PIAS4	REN	SPEF1	TMEM60	ZNF679
C14orf49	CSMD1	FAM71D	KIF7	MSLN	PILRA	RFXANK	SPEM1	TNFRSF1B	ZNF717
C18orf8	DBT	FAM91A1	KITLG	MUC16	PKMYT1	RIF1	SPEN	TPRG1	ZNF783
C1QA	DCAKD	FBXO44	KRTAP9-1	MUC17	PKNOX2	RNF219	SPTB	TRANK1	ZNF804B
C1orf127		FCGBP		MUC6		RNLS		TRIM59	ZNF880
C20orf132		FITM1		MYL6		RP1L1		TRMT2A	ZSWIM2
C2CD4B		FN3K		N4BP2L2		SDCCAG3		TSPYL4	LRRK2

ATCC®

Disease-related mutations in Parkinson's iPSCs

Gene symbol	Protein function	Gene mutation-associated disease
NPC1	Maintaining the structural and functional integrity of nerve terminals	Autosomal recessive neurodegenerative disorder
NGF	Neural plasticity and apoptosis of neurons	Hereditary sensory and autonomic neuropathy
LRP2	HDL endocytosis	Donai-Barrow syndrome
MEF2A	Neural differentiation and survival	Autosomal dominant coronary artery disease 1
LRP2	Regulation of HDL endocytosis	Doonai-Barrow Syndrome
MEF2A	Neural differentiation and survival	Autosomal dominant coronary artery disease 1
DNAI1	Regulation of dynein activity	Primary ciliary dyskinesia and kartagener syndrome
SLC5A1	Sodium/glucose cotransporter	Glucose-galactose malabsorption
RP1L1	Differentiation of photoreceptor cells	Macular dystrophy
PNPLA2	Hydrolysis of triglycerides	Neutral lipid storage disease with myopathy
DBT	Amino acid metabolism	Maple syrup urine disease
BSND	Chloride reabsorption	Bartter syndrome
ZNF469	Regulator of collagen fibers	Cornea syndrome

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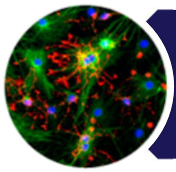
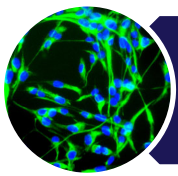
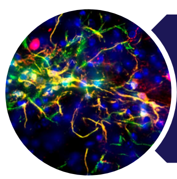
Outline



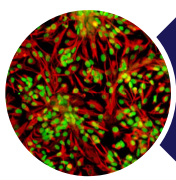
Introduction to ATCC



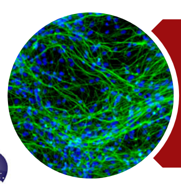
Human induced Pluripotent Stem Cells (iPSCs)



ATCC iPSC Collection



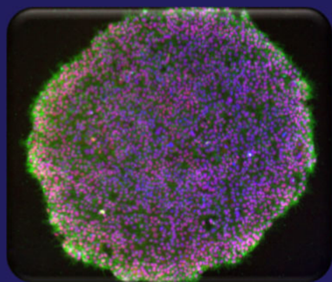
Quality Standards and Characterization



Supporting Reagents and Products

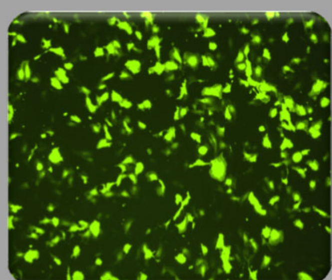
ATCC®

Supporting reagents and products



Complete Culture Systems

- Feeder-Free: serum-free, xeno-free
- Feeder-Dependent: serum-free, xeno-free
- Conventional: DMEM/F12, ES qualified FBS
- Antibiotics-Free



Transfection Reagents

- GeneX*Plus*: Xeno-free
- TransfeX™: Xeno-free, hard-to-transfect cell lines
- Low cytotoxicity: balanced cytotoxicity and potency
- Performance tested

CoolCell®

- Alcohol-free cell freezing container
- Standardized controlled rate -1°C/minute
- High post-thaw viability and proliferation
- 4 hours at -80°C before transfer to liquid nitrogen

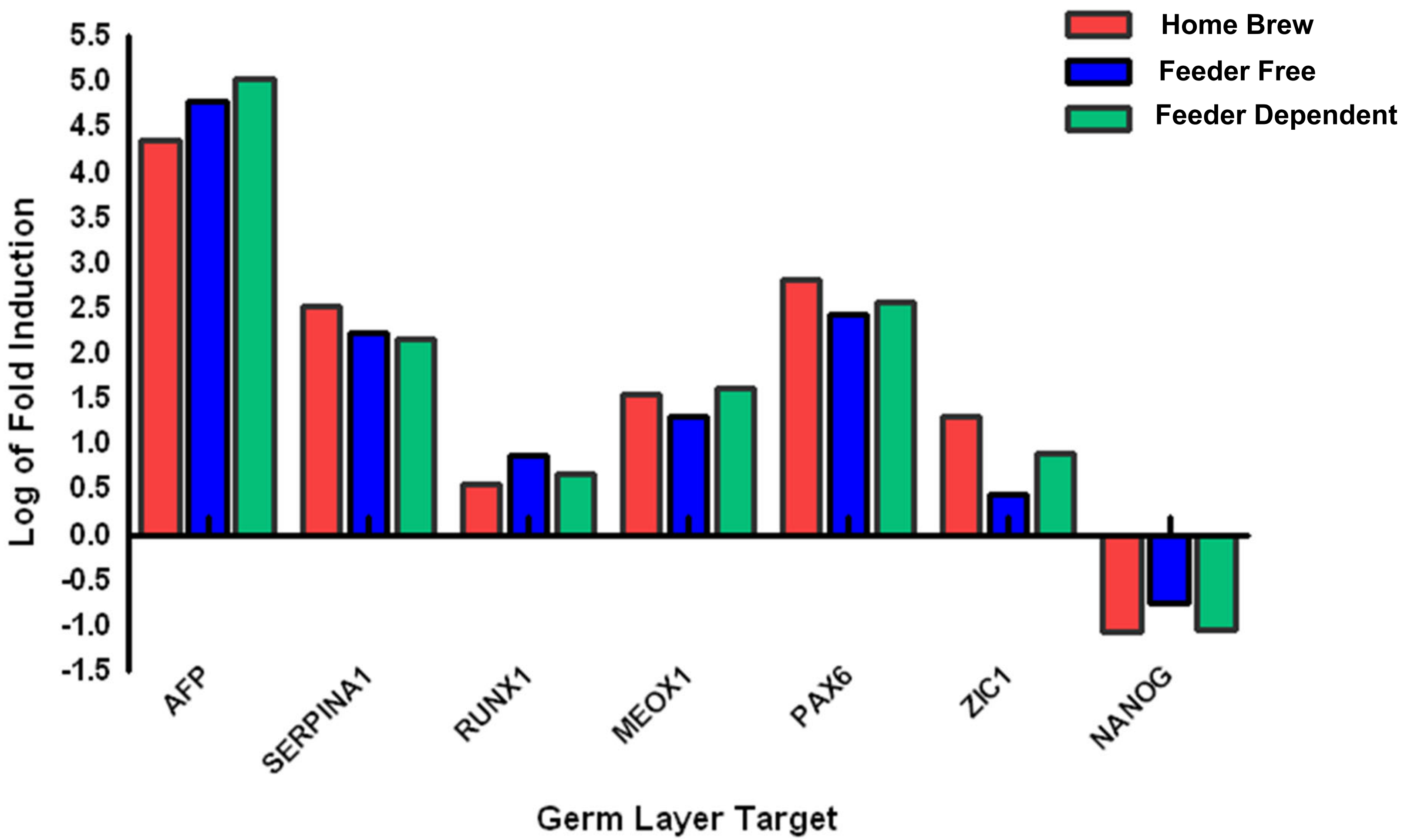
Supporting reagents and products

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

	Feeder-Free	Feeder-Dependent	Conventional
Media	Pluripotent Stem Cell SFM XF/FF (Serum Free, Xeno Free)	Pluripotent Stem Cell SFM XF (Serum Free, Xeno Free)	Home Brew DMEM:F12 ES Qualified FBS
Substrate	Cell Basement Membrane	MEF/HFF Mitomycin C treated; γ-irradiated	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

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

ATCC Media system is reliable and consistent



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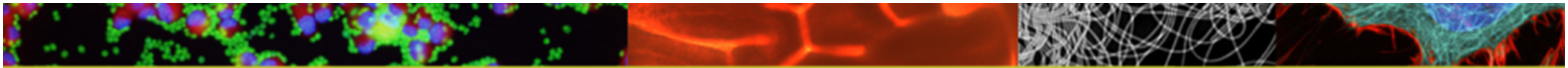
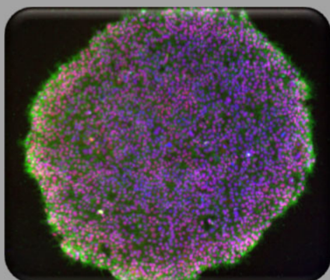


Supporting reagents and products



Complete Culture Systems

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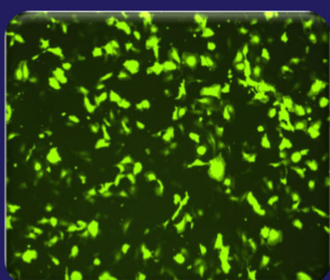
- Conventional: DMEM/F12, ES qualified FBS
- Antibiotics-Free



Transfection Reagents

- GeneX*Plus*: Xeno-free
- TransfeX: Xeno-free, hard-to-transfect cell lines
- Low cytotoxicity: balanced cytotoxicity and potency
- Performance tested

CoolCell®

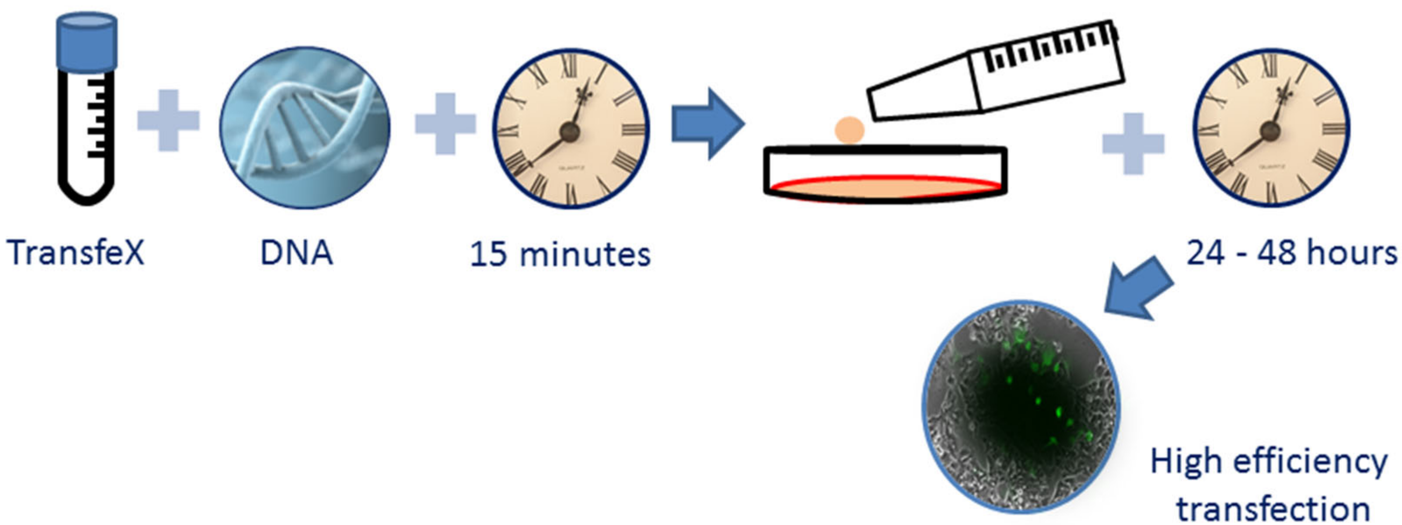
- Alcohol-free cell freezing container
- Standardized controlled rate -1°C/minute
- High post-thaw viability and proliferation
- 4 hours at -80°C before transfer to liquid nitrogen

ATCC®

ATCC TransfeX transfection reagent (ACS-4005)

TransfeX Reagent is:

- Designed for hard-to-transfect cell lines
- Validated in many iPSCs, adult stem cells, primary cells, immortalized cell lines, and continuous cell lines
- Free from animal components
- Performance tested



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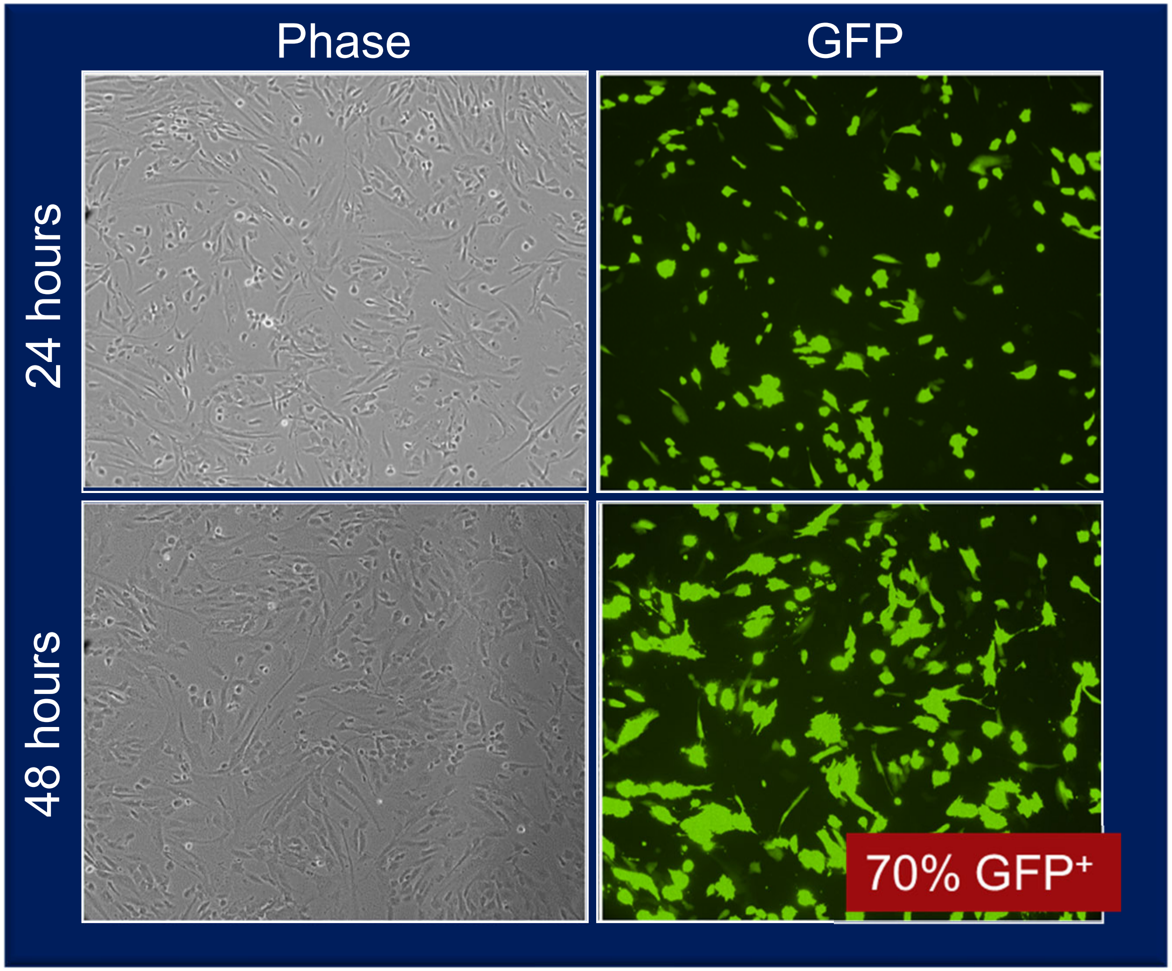
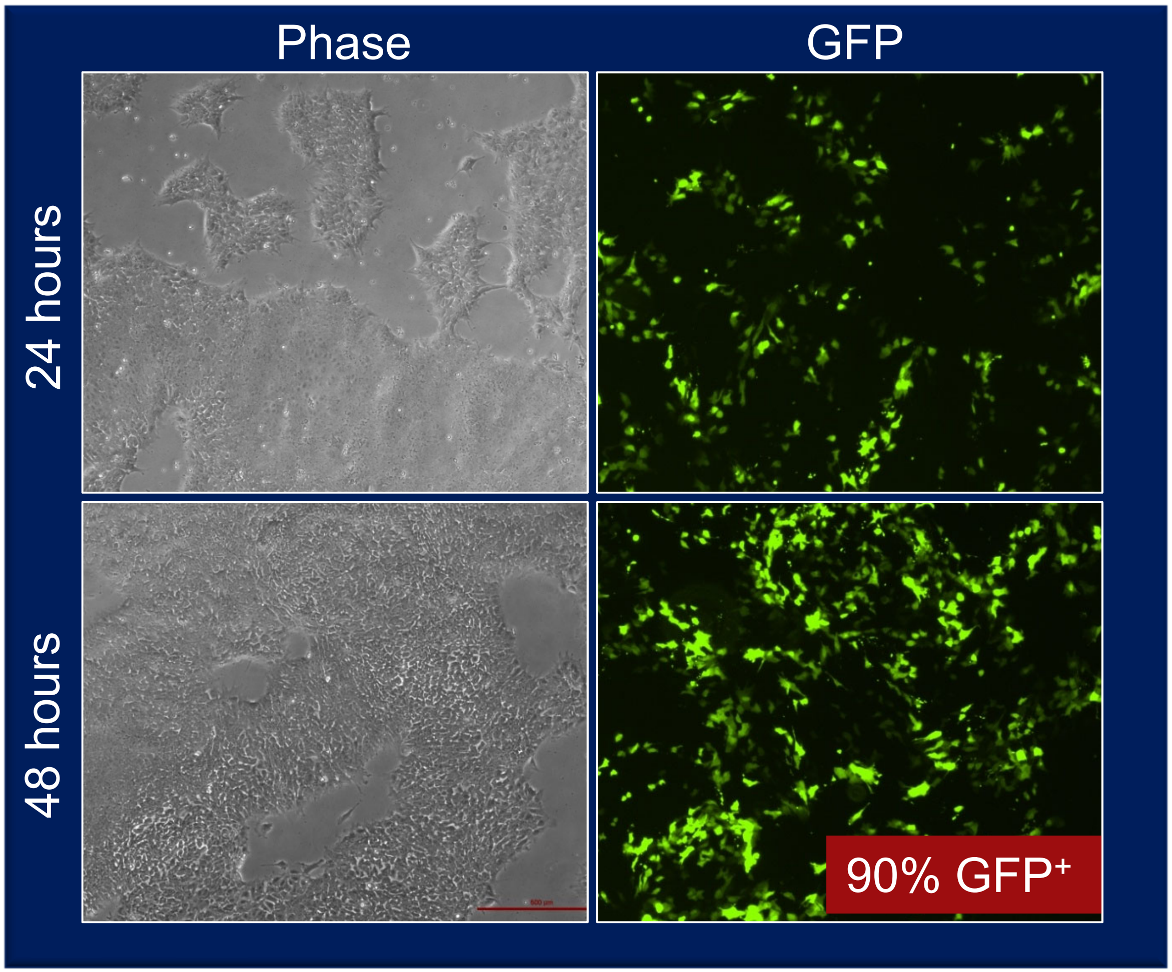
Transfection of dermal fibroblasts with TransfeX

Transfected with EF1α-GFP vector



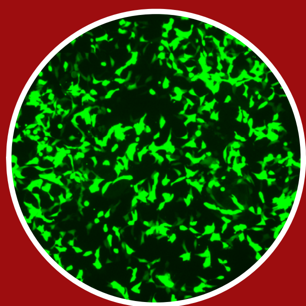
Transfection of hiPSCs with TransfeX

Transfected with EF1α-GFP vector



ATCC TransfeX transfection guide

Protocols for using TransfeX to transfect . . .



Continuous

MDA-MB-231

HepG2 ▪ LNCap

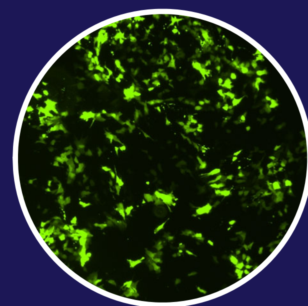
Caco-2 ▪ C2C12

3T3-L1

NuLi-1 ▪ TIME

RPTEC-hTERT

hTERT-HME

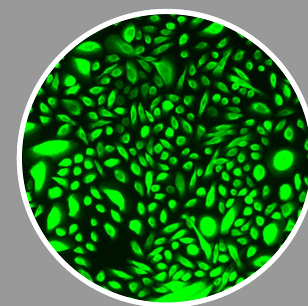


Stem

Bone marrow-derived MSCs

hiPSCs

BT-142



Primary

Dermal Fibroblasts

Dermal Microvascular Endothelial

HUVECs

RPTECs

Large Airway Epithelial

hMECs

Download this and our other free culture guides at www.atcc.org.

Contact Technical Service at tech@atcc.org

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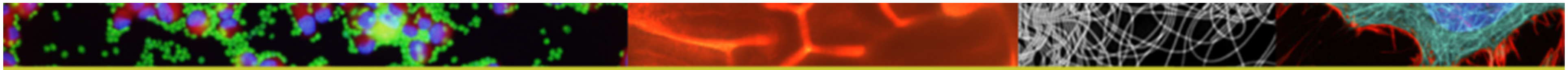
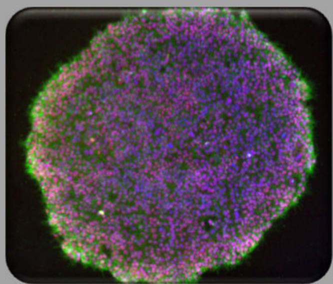
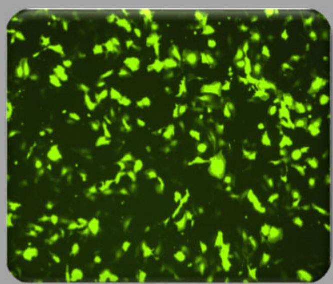


Supporting Reagents and Products



Complete Culture Systems

- Feeder-Free: serum-free, xeno-free
- Feeder-Dependent: serum-free, xeno-free
- Conventional: DMEM/F12, ES qualified FBS
- Antibiotics-Free



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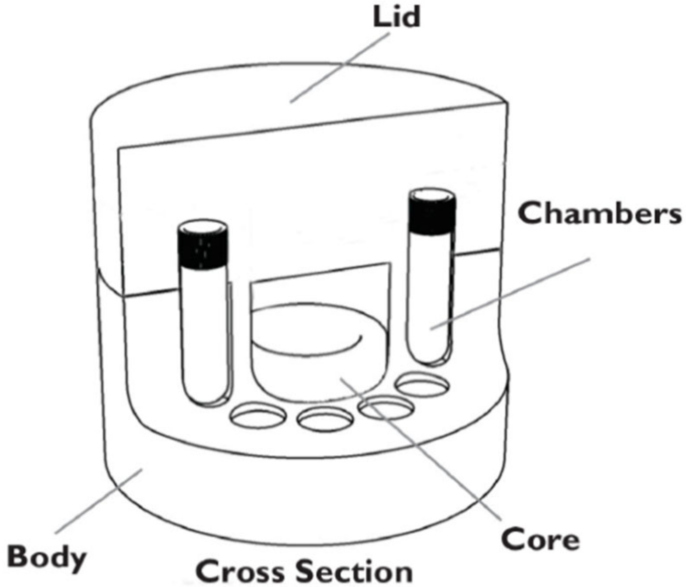
- 4 hours at -80°C before transfer to liquid nitrogen

CoolCell® LX - ATCC® ACS-6000

- Alcohol-free cell freezing container
  - Insulation foam
  - Radial symmetry
  - Heat transfer core to regulate heat loss
  - Freezing at rate of 1°C per/minute in -80°C freezer
- Cells ready for LN storage after 4 hours (compared to over night with Mr. Frosty)
- Ready after 15 minutes for re-use, Mr. Frosty can take hours to come back to room temp for re-use



ATCC – Your trusted source

- Human induced pluripotent stem cells collection
  - Normal, Diseased, Reference iPSC Collection
  - Quality Standards and Characterization
  - Complete Culture Systems
    - Feeder-Dependent culture system
    - Feeder-Free culture system
    - Conventional culture system
  - In-depth Characterization

- TransfeX
  - Universal transfection reagent that can be used to transfect difficult-to-transfect cells like stem and primary cells
  - High efficiency and low cytotoxicity
  - Cost effective and scalable

ATCC cell lines are authenticated, and quality control tested in media formulations which support optimal growth characteristics

Resources for iPSC culture

Never cultured stem cells before?

View the ATCC Excellence in Research Series "on demand"

Stem Cell Solutions, presented by John Pulliam, Ph.D.

This webinar demonstrates helpful tips and solutions, including:

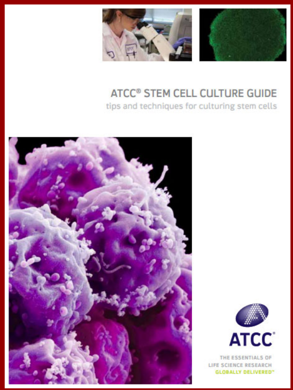
- Thawing
- Passaging
- Cryopreservation of stem cells

ATCC® Stem Cell Culture Guide – All the tips and techniques you'll need to successfully culture any stem cell

You'll find information for:

Characterization ▪ Cryopreservation ▪ Culturing ▪ Applications

Download this and our other free culture guides at www.atcc.org/guides



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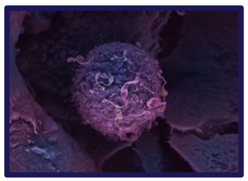
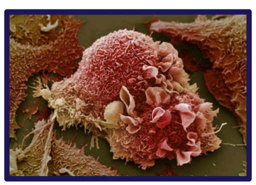
Thank you!

Register for more webinars in the ATCC® "*Excellence in Research*" webinar series at www.atcc.org/webinars.



September 11, 2014
10:00 AM, 3:00 PM ET
Dr. Shamaila Ashraf will discuss using ATCC® influenza research materials in the development and validation of novel preventative and therapeutic techniques

September 18, 2014
10:00 AM, 3:00 PM ET
Dr. Fang Tian and Dr. David H. Randall will talk about ATCC® Genetic Alterations Panels and how they can be effective tools in highput screening using Corning® Epic™ technology.



October 16, 2014
10:00 AM, 3:00 PM ET
Dr. Tigwa H. Davis with discuss using LUHMES cells as a model system to study dopaminergic neuron cell biology.

Thank you for joining today!
Please send additional questions to tech@atcc.org

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