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
What are iPSCs?

Yamanaka Factors
- Oct3/4<sup>1,2</sup>
- Sox2<sup>1,2</sup>
- Klf4<sup>1</sup>
- Myc<sup>1</sup>
- Nanog<sup>2</sup>
- Lin28<sup>2</sup>

The promise of iPSCs

Gene editing correcting inherited diseases

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® 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 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 Reference iPSC Collection

Creating Standards
- 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 Reference iPSC Collection

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Designation</th>
<th>Gender</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS-1024™</td>
<td>ATCC-BYS0110</td>
<td>Male</td>
<td>African America</td>
</tr>
<tr>
<td>ACS-1028™</td>
<td>ATCC-BYS0114</td>
<td>Female</td>
<td>African America</td>
</tr>
<tr>
<td>ACS-1025™</td>
<td>ATCC-BYS0111</td>
<td>Male</td>
<td>Hispanic</td>
</tr>
<tr>
<td>ACS-1029™</td>
<td>ATCC-BXS0115</td>
<td>Female</td>
<td>Hispanic</td>
</tr>
<tr>
<td>ACS-1026™</td>
<td>ATCC-BYS0112</td>
<td>Male</td>
<td>Caucasian</td>
</tr>
<tr>
<td>ACS-1030™</td>
<td>ATCC-BXS0116</td>
<td>Female</td>
<td>Caucasian</td>
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<tr>
<td>ACS-1027™</td>
<td>ATCC-BYS0113</td>
<td>Male</td>
<td>Asian</td>
</tr>
<tr>
<td>ACS-1031™</td>
<td>ATCC-BXS0117</td>
<td>Female</td>
<td>Asian</td>
</tr>
</tbody>
</table>

Coming soon!
Quality standards

Quality attribute

Authentication
- Identity
- Karyotype
- Sterility
- Mycoplasma
- Viral Panel

Preservation
- Post thaw viability
- Morphology
- Growth
- Purity

Characterization
- Pluripotency expression
- Germ layer differentiation
- Transcriptome analysis
ATCC iPSCs monitored for pluripotency

<table>
<thead>
<tr>
<th>Pluripotency Markers</th>
<th>Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanog</td>
<td>+</td>
</tr>
<tr>
<td>Tra 1-60</td>
<td>+</td>
</tr>
<tr>
<td>Tra 1-81</td>
<td>+</td>
</tr>
<tr>
<td>SSEA-4</td>
<td>+</td>
</tr>
<tr>
<td>SSEA-1</td>
<td>-</td>
</tr>
</tbody>
</table>

- **Tra 1-60**
- **Nanog**
- **Isotype control**
- **SSEA1**
- **SSEA4**
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.

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**Pluripotency Score**

Sample contains pluripotent signature

**Novelty Score**

Based on existing data from well-characterized PSC lines data

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

---

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

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

<table>
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<th>Reprogramming method</th>
</tr>
</thead>
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<tr>
<td>ACS-1012™</td>
<td>ATCC-DYR0530</td>
<td>Retrovirus</td>
</tr>
<tr>
<td>ACS-1013™</td>
<td>ATCC-DYS0530</td>
<td>Sendai virus</td>
</tr>
<tr>
<td>ACS-1014™</td>
<td>ATCC-DYP0530</td>
<td>Episomal</td>
</tr>
</tbody>
</table>

*Fonzo A.D. et al., Eur J Hum Genet. 2006; 14: 322-331*
Reprogramming methods do not affect differentiation potential

Normal

Parkinson’s/Retroviral

ACS-1007™

ACS-1012™

Parkinson’s/Sendai virus

Parkinson’s/Episomal

ACS-1013™

ACS-1014™

Nestin (red)
DAPI (blue)
**Identification of missense mutations in Parkinson’s hiPSC lines by exome sequencing**

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

*Venn diagram showing the number of shared mutations across different cell lines.*
Conserved gene mutations among Parkinson’s iPSC lines

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Conserved gene mutations among Parkinson’s iPSC lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA7</td>
<td>C3orf20</td>
</tr>
<tr>
<td>ACCN5</td>
<td>CACNA1B</td>
</tr>
<tr>
<td>ACER1</td>
<td>CCM2</td>
</tr>
<tr>
<td>ADNP2</td>
<td>CD28</td>
</tr>
<tr>
<td>AHDC1</td>
<td>CDC45</td>
</tr>
<tr>
<td>ALDH1L1</td>
<td>CENPF</td>
</tr>
<tr>
<td>ALKBH8</td>
<td>CHAF1A</td>
</tr>
<tr>
<td>ALMS1</td>
<td>CHD6</td>
</tr>
<tr>
<td>AMDHD2</td>
<td>CLEC3B</td>
</tr>
<tr>
<td>AMPH</td>
<td>CLIC6</td>
</tr>
<tr>
<td>ANO7</td>
<td>CMA1</td>
</tr>
<tr>
<td>AP3B2</td>
<td>CNNM3</td>
</tr>
<tr>
<td>ARHGAP4</td>
<td>CNTNAP4</td>
</tr>
<tr>
<td>ARHGEF19</td>
<td>COBLL1</td>
</tr>
<tr>
<td>ATP1A4</td>
<td>COL14A1</td>
</tr>
<tr>
<td>ATP9B</td>
<td>COL27A1</td>
</tr>
<tr>
<td>BSND</td>
<td>CRLS1</td>
</tr>
<tr>
<td>C10orf27</td>
<td>CSF1</td>
</tr>
<tr>
<td>C14orf49</td>
<td>CSMD1</td>
</tr>
<tr>
<td>C18orf8</td>
<td>DBT</td>
</tr>
<tr>
<td>C1QA</td>
<td>DCAKD</td>
</tr>
<tr>
<td>C1orf127</td>
<td>FCBP</td>
</tr>
<tr>
<td>C2orf132</td>
<td>FITM1</td>
</tr>
<tr>
<td>C2CD4B</td>
<td>FN3K</td>
</tr>
<tr>
<td>C2CD4B</td>
<td>FN3K</td>
</tr>
</tbody>
</table>
## Disease-related mutations in Parkinson’s iPSCs

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

1. Introduction to ATCC
2. Human induced Pluripotent Stem Cells (iPSCs)
3. ATCC iPSC Collection
4. Quality Standards and Characterization
5. Supporting Reagents and Products
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
- GeneXPlus: 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!

<table>
<thead>
<tr>
<th></th>
<th>Feeder-Free</th>
<th>Feeder-Dependent</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Media</strong></td>
<td>Pluripotent Stem Cell SFM XF/FF (Serum Free, Xeno Free)</td>
<td>Pluripotent Stem Cell SFM XF (Serum Free, Xeno Free)</td>
<td>Home Brew DMEM:F12 ES Qualified FBS</td>
</tr>
<tr>
<td><strong>Substrate</strong></td>
<td>Cell Basement Membrane</td>
<td>MEF/HFF Mitomycin C treated; γ-irradiated</td>
<td>MEF/HFF Mitomycin C treated; γ-irradiated</td>
</tr>
<tr>
<td><strong>Passaging</strong></td>
<td>Dissociation Reagent</td>
<td>Dissociation Reagent</td>
<td>Dissociation Reagent</td>
</tr>
<tr>
<td><strong>Cryopreservation</strong></td>
<td>Stem Cell Freezing Media</td>
<td>Stem Cell Freezing Media</td>
<td>Stem Cell Freezing Media</td>
</tr>
<tr>
<td><strong>Supporting Reagent</strong></td>
<td>ROCK inhibitor</td>
<td>ROCK inhibitor</td>
<td>ROCK inhibitor</td>
</tr>
</tbody>
</table>

Visit [www.atcc.org](http://www.atcc.org) for a complete list of feeder cells
ATCC Media system is reliable and consistent

![Bar chart showing log of fold induction for different germ layer targets with legend for Home Brew, Feeder Free, and Feeder Dependent.]
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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**CoolCell®**
- Alcohol-free cell freezing container
- Standardized controlled rate -1°C/minute
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- 4 hours at -80°C before transfer to liquid nitrogen
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
Transfection of dermal fibroblasts with TransfeX

Transfected with EF1α-GFP vector

<table>
<thead>
<tr>
<th>Phase</th>
<th>GFP</th>
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</thead>
<tbody>
<tr>
<td>24 hours</td>
<td><img src="image" alt="Phase 24 hours" /></td>
</tr>
<tr>
<td>48 hours</td>
<td><img src="image" alt="Phase 48 hours" /></td>
</tr>
</tbody>
</table>

70% GFP+
Transfection of hiPSCs with TransfeX

Transfected with EF1α-GFP vector

<table>
<thead>
<tr>
<th>Phase</th>
<th>GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td><img src="image" alt="24 hours Phase" /> <img src="image" alt="24 hours GFP" /></td>
</tr>
<tr>
<td>48 hours</td>
<td><img src="image" alt="48 hours Phase" /> <img src="image" alt="48 hours GFP" /></td>
</tr>
</tbody>
</table>

90% GFP+
ATCC TransfeX transfection guide

Protocols for using TransfeX to transfect . . .

Continuous
- MDA-MB-231
- HepG2
- Caco-2
- C2C12
- 3T3-L1
- NuLi-1
- TIME1
- 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
Supporting Reagents and Products

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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 overnight 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
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
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 high throughput screening using Corning® Epic™ technology.

**October 16, 2014**
10:00 AM, 3:00 PM ET
Dr. Tigwa H. Davis will 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