General Questions

1. Will we be able to download the presentation?
   This presentation will be available to watch on demand on the ATCC website, or click here.

2. Episomal and Sendai virus (SeV) methods of reprogramming are both footprint-free; why did you choose to mainly use the SeV method?
   SeV is much more efficient at reprogramming somatic cells with pluripotency genes than episomal plasmids.

3. Is the Parkinson’s whole-exome sequencing data available? If so, where can I find the information?
   Yes, the whole-exome sequencing data is available for ATCC® ACS-1012™, ACS-1013™, and ACS-1014™ inducible pluripotent stem cell (iPSC) lines, which are derived from Parkinson’s disease donors. The raw data may be found on the NCBI BioProject website (Accession: PRJNA226266, ID: 226266). You can also contact ATCC Technical Support by email at tech@atcc.org, or by phone at 1-(800)-638-6597.

4. What is the function of ROCK Inhibitor (ATCC® ACS-3030) in iPSC cultures?
   ROCK Inhibitor Y27632 (ATCC® ACS-3030) should be added to iPSC cultures to increase the viability and attachment of human iPSCs during thawing and subcultivation. The ROCK inhibitor should be supplemented into the culture medium at a final concentration of 10 µM.

5. How can one determine pluripotency?
   One can either use a bioinformatics approach by using the Pluritest™ assay, or assess for pluripotency gene expression using traditional laboratory methods such as immunocytochemistry staining, qPCR, or flow cytometry.

6. Have you used your transfection reagent to reprogram cells to become iPSCs?
   No. ATCC has not tried the Transfex™ (ATCC® ACS-4005) reagent to reprogram cells to become iPSCs. However, due to the highly efficient optimized protocol (90% transfection efficiency) we have developed for iPSCs, the Transfex™ (ATCC® ACS-4005) reagent could be used to reprogram iPSCs using episomal plasmids.
7. How many passages will the iPSCs remain undifferentiated when cultured in ATCC® Pluripotent Stem Cell SFM XF (ATCC® ACS-3001) or ATCC® Pluripotent Stem Cell SFM XF/FF (ATCC® ACS-3002) media?

ATCC has tested iPSCs up to 30 passages using the ATCC culture systems without observing differentiation.

8. How can I be sure I have stem cell colonies?

Stem cell colonies exhibit a round morphology with well-defined sharp edges, and contain tightly packed cells. Individual cells within the colony exhibit prominent nucleoli with a high ratio of nucleus to cytoplasm volume.

9. How often do I need to karyotype my iPSC cultures?

Human embryonic stem cells and iPSCs may exhibit karyotypic instability. Therefore, it is good practice to periodically verify that the karyotype of your culture has not changed. We recommend karyotyping iPSC cells every 20 passages.

10. My iPSC are not recovering after passage. What are some possible causes?

Multiple factors may affect recovery of your iPSC following passage.

   a. Make sure to remove the Stem Cell Dissociation Reagent (ATCC® ACS-3010) and rinse the culture once with DMEM: F-12 Medium (ATCC® 30-2006) to remove any remaining traces of the dissociation reagent.

   b. We recommend the addition of 10 µM ROCK Inhibitor Y27632 (ATCC® ACS-3030) to the medium to aid the recovery of pluripotent stem cells.

   c. Prior to passaging, make sure the culture is approximately 80% confluent and use a split ratio of 1:4 to sub-culture the cells. Do not over pipette when passaging; pluripotent stem cells do not survive as a single cell suspension.

   d. If the iPSC culture has been growing for 6 days and is not yet 80% confluent, we recommend that you passage the culture. Adjust the split ratio if necessary.

   e. If the iPSC colonies were seeded at a low density when passaged, the cells will take longer to recover. If the colonies are seeded too sparsely, the cells will begin to differentiate.

11. What GMO status are the SeV reprogrammed cells?

The SeV-reprogrammed iPSCs are considered to be genetically modified organisms (GMO) since they are generated by artificially reprogramming somatic cells with integration-free SeV vectors. The GMO classification group will have to be determined by the end-user after an appropriate risk assessment is performed. SeV-reprogrammed iPSCs are safe to use since the virus has been modified to be non-integrating, and after many passages the virus dilutes out from the cell population and cannot be detected in the iPSCs. ATCC recommends that an appropriate GMO classification group be assigned after the biosafety level (BSL) of the parental cells used to generate the iPSC line has been taken into consideration.
12. Why not use mRNA reprogramming for creating iPSC?

mRNA reprogramming is a valid, integration-free reprogramming method; however, we found that the SeV reprogramming method is easy to use with high reprogramming efficiency.

13. Does ATCC carry antibodies specific for the cell surface markers mentioned in the webinar?

ATCC does not carry antibodies specific for the cell surface markers mentioned in the webinar. The following table contains the antibodies, suppliers, and methods that are used to identify the cell surface markers on ATCC iPSCs.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Supplier</th>
<th>Catalog number</th>
<th>Method</th>
<th>Specification</th>
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<tr>
<td>PE-isotype control</td>
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<td>60069PE</td>
<td>Flow cytometry</td>
<td>Nonspecific binding assessment</td>
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<td>PE-Tra-1-60</td>
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