Efficient Generation of iPSCs Derived From Parkinson's Disease (PD) Study Patient Fibroblasts Using CytoTune®-iPS 2.0 Sendai Reprogramming Kit in the Essential 8® Feeder-Free Media System

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ABSTRACT

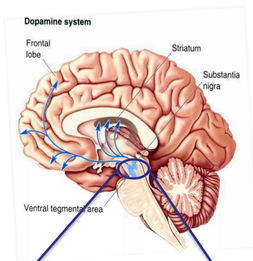
The absence of cellular models for Parkinson's Disease (PD) represents a major bottleneck and unmet need in PD research. Patient-derived induced pluripotent stem cells (iPSCs) offer exciting potential in cell therapy and *in vitro* disease modeling. Efficient reprogramming of patient somatic cells to iPSCs in feeder-free conditions plays a key role in realizing this potential. Many reprogramming methods have been optimized for use with numerous cell lines, but lead to technical challenges for researchers in converting adult or disease somatic cells to iPSCs consistently and efficiently. The CytoTune®-iPS 2.0 Sendai Reprogramming Kit uses Sendai virus and polycistronic vectors to reprogram somatic cells into induced pluripotent stem cells (iPSCs) which provides a more robust reprogramming efficiency, lower cytotoxicity, and faster viral clearance to generate integration -free iPSCs in feeder-free conditions. In this study, fibroblasts from a skin biopsy of a Parkinson's disease (PD) study donor were reprogrammed in feeder-free conditions to iPSCs using Life Technologies™ CytoTune®-iPS 2.0 Sendai Reprogramming Kit. These iPSCs are transgene-free and karyotypically normal, express known pluripotency markers and are able to differentiate into embryoid bodies that present the three germ layer lineages: ectoderm, mesoderm, and endoderm. Given the efficiency, speed and ease of reprogramming of these adult, disease fibroblasts in feeder-free conditions, the CytoTune®-iPS 2.0 Sendai Reprogramming Kit can be applied to large scale reprogramming of multiple disease lines in an automated fashion to provide significant impact for researchers worldwide.

INTRODUCTION

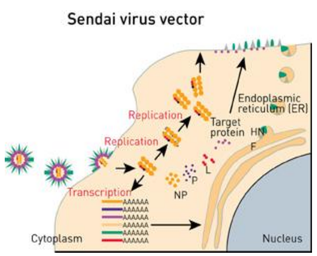
Parkinson's disease (PD)

A progressive neurodegenerative disorder that affects 1% of people over age 60 and more than 5 million people worldwide.

PD results primarily from the selective loss of dopaminergic neurons in the substantia nigra. The absence of physiologically relevant cellular models for PD represents a major bottleneck for PD research. Novel models are urgently needed to accelerate the discovery of disease mechanisms and drug targets as well as for screening purposes.



Generation of iPSCs using Sendai reprogramming vectors



- **RNA virus**. No DNA during replication, and replicates exclusively in the cytoplasm. No possibility of integration into host genome
- **Safe**. CytoTune® 2.0 has Fusion (F) gene deleted and cannot produce infectious particles. Non-pathogenic to humans
- **Broad tropism**. Capable of transducing a wide range of cells with a short contact time.

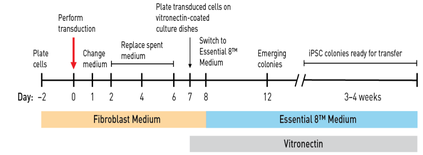
MATERIALS AND METHODS

Cell Culture

All cell culture media, components, growth factors pluripotency characterization antibodies are Life Technologies, part of Thermo Fisher Scientific.

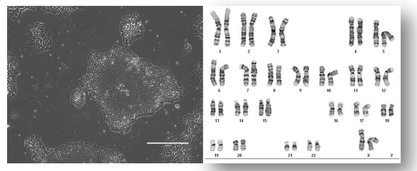
iPSC generation and live-staining

Reprogramming of fibroblasts to generate iPSCs and live-staining was performed according to the User Guide of the CytoTune® -iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific).



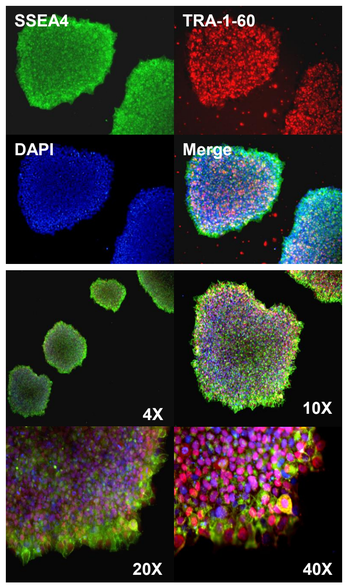
RESULTS

Figure 1. Patient-derived iPSCs and normal karyotype data



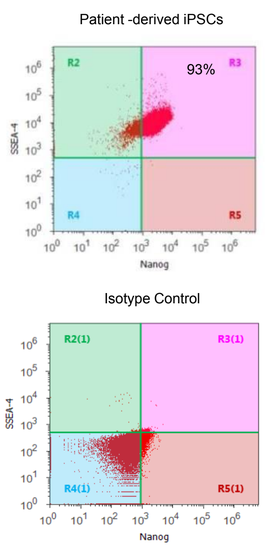
Cytogenetic analysis of the iPSCs
The iPSC line was cultured in Essential 8® Medium on 6-well plates for 4 days. Cells were harvested for G-banded karyotypic analysis. The resulting cell line tested was karyotypically normal at passage 15. No clonal abnormality was detected at the G-banded level of resolution.

Figure 2. iPSC Characterization



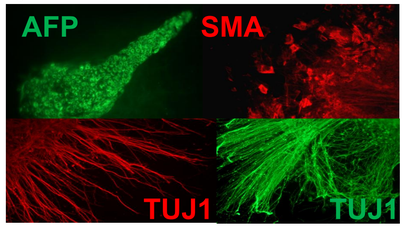
Immunocytochemistry (ICC) demonstrated that the iPSCs express pluripotency markers Oct4, TRA-1-60 and SSEA4. ICC was performed using the Pluripotent Stem Cell 4-Marker ICC Kit (Cat# A24881). Images were captured using the Floid® Cell Imaging Station and the EVOS® FL Cell Imaging System.

Figure 3. Flow Cytometry Analysis



Immunostaining and flow cytometry analysis demonstrated that the iPSCs express pluripotency markers Nanog and SSEA4. iPSCs were cultured in Essential 8® medium on vitronectin for 4 days. Cells were harvested, fixed, permeabilized and co-stained with isotype control antibodies (bottom) and antibodies (Top) for Nanog and SSEA4. FACS analysis was performed using the Attune® Acoustic Focusing Cytometer.

Figure 4. Embryoid Body Formation and Three Lineage Markers Expression Analysis



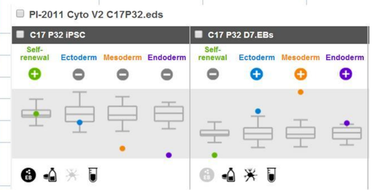
iPSC-derived embryoid bodies were cultured on Geltrex® (Cat# A1569601) and allowed to randomly differentiate for 17 – 20 days in DMEM/F12 medium with KnockOut™ Serum Replacement (Cat# 10828028). The cells were stained using the 3-Germ Layer Immunocytochemistry Kit (Cat# A25538). Images were acquired on a Zeiss® Axiovert® 25 CFL microscope.

Figure 5. Analysis using TaqMan® hPSC Scorecard™ Panel

Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
C17 P32 iPSC	0.01	-0.42	-1.73	-2.06
C17 P32 D7.EBs	-1.40	1.42	2.66	0.66

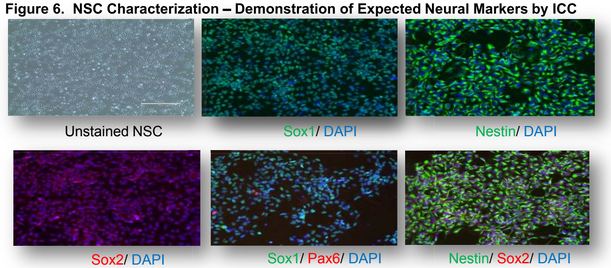
Gene expression relative to the reference standard

x>1.5	Upregulated
1.0<x<=1.5	
0.5<x<=1.0	
-0.5<=x<=0.5	Comparable
-1.0<=x<-0.5	
-1.5<=x<-1.0	
x<-1.5	Downregulated



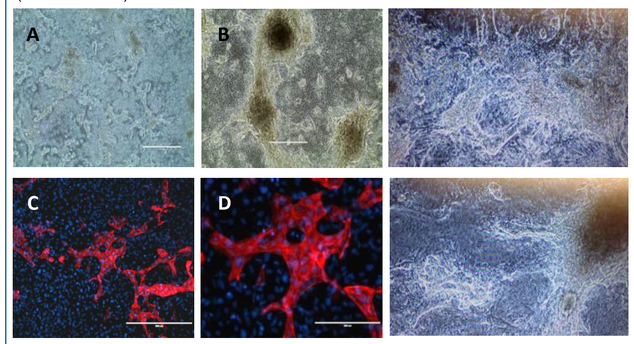
Embryoid bodies were generated by transferring 90% confluent iPSCs to a non-tissue culture treated flask using a StemPro® EZPassage™ (Cat# 23181010) tool and cell scraper in EB medium without FGF. Cells were grown in suspension for 4 days then transferred to Geltrex™ coated dishes and cultured to day 14. Samples were lysed directly on tissue culture plates by adding TRI Reagent® (Cat# AM9738) and incubating 5min at room temp. RNA was isolated from the lysate using a RiboPure™ Kit (Cat# AM1924). RNA was reverse transcribed into cDNA using SuperScript® VILO™ MasterMix (Cat# 11755250). cDNA samples were combined with TaqMan® Gene Expression Master Mix (Cat# 4369016) and Nuclease-Free Water (Cat# AM9937) before adding to TaqMan® hPSC Scorecard™ Panel (Cat#AM15870) and run on a QuantStudio™ 12K Flex Real-Time System (Cat# 4471134).

Figure 6. NSC Characterization – Demonstration of Expected Neural Markers by ICC



iPSC-derived NSC line stained positive for known neural markers Sox1, Sox2, and Nestin. In addition, NSCs stained positive for Pax6, a known regulator in neurogenesis and molecular regulation of the central nervous system. ICC samples were analyzed on a FLoid® Cell Imaging Station. NSCs were generated with PSC Neural Induction Medium (Cat# A1647801) and immunocytochemistry was carried out using the Human Neural Stem Cell Immunocytochemistry Kit (Cat# A24354).

Figure 7. Generation of Cardiomyocytes Using PSC Cardiomyocyte Differentiation Kit (Cat# A25042SA)



A) Phase contrast image of Parkinson's Disease iPSC line PI-2011 clone 17 on vitronectin-coated surface at Day 10. B) Phase contrast image of Parkinson's Disease iPSC PI-2011 line clone 17 on Geltrex-coated surface at Day 14. (Far right images are 10x) C) 10X fluor escent image of TNNT2+ GIBCO episomal-derived iPSC (red) on vitronectin-coated surface at Day 15 with DAPI counterstain (blue). D) Paired 20X fluorescent image of Figure C.

CONCLUSIONS

Previously we have successfully generated iPSCs from the dermal fibroblasts of 3 Parkinson's disease donors, one MSA donor, and 2 age-matched control individuals using an earlier version of the CytoTune® -iPS Sendai Reprogramming Kit. Here we demonstrate the use of CytoTune® -iPS 2.0 Sendai Reprogramming Kit.

- CytoTune®-iPS 2.0 Sendai reprogramming kit is easy to use with high efficiency;
- The resulting iPSCs express the expected pluripotency markers and are karyotypically normal.

Given the efficiency, speed, and ease with which we were able to reprogram adult disease fibroblasts, it is clear that the CytoTune® -iPS 2.0 Sendai Reprogramming Kit can be applied to large-scale reprogramming of multiple disease lines in an automated fashion.

The establishment of these fully characterized iPSC lines, from patients of known clinical histories, now sets the stage for further disease-relevant studies. Here we demonstrate the robust generation of an MSA patient -derived iPSC line and show application of different characterization tools and differentiation to NSCs and cardiomyocytes available from the Life Science Solutions Group at Thermo Fisher Scientific. In addition it will be possible to generate other neural cell types, such as DA neurons and glia/astrocytes (these studies are currently underway). With the generation of these fully characterized Parkinson's disease patient -derived NSCs we can look at downstream gene editing methodologies to better understand the nature of Parkinson's and other neurodegenerative diseases. In addition we can utilize these different cell types (NSCs, and cardiomyocytes) for disease modeling.

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TRADEMARKS/LICENSING

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