# NINDS HUMAN GENETICS DNA AND CELL LINE REPOSITORY

NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

CORIELL INSTITUTE FOR MEDICAL RESEARCH

## ND34391*H

Certificate of Analysis

| | | |
|---|---|---|
| Product description | Human fibroblast reprogrammed with four factors (OCT4, SOX2, KLF4, CMYC) using retroviral vectors | |
| Publication(s) describing iPSC establishment | | |
| Parent cell line and cell type | ND27760 | Fibroblast |
| Diagnosis | Parkinson Disease | |
| Mutation | SNCA triplication | |
| Passage of iPSC reported at submission | 20 | |
| Number of passages at Coriell | 25 | |
| Media | DMEM/F12 + 20% KOSR +10 ng/ml bFGF + Penicillin/Streptomycin | |
| Feeder or matrix substrate | CF1 MEFs on 0.1% Gelatin seeded at 2 x $10^5$ per well | |
| Passage method | TrypLE + 10uM Y-27632 in passage media | |
| Split ratio | 1:3 for first three passages post thaw, then can increase to 1:4 or 1:5; every 6-7 days | |

The following testing specifications have been met for the specified product lot:

| Test Description | Test Method | Test Specification | Result |
|---|---|---|---|
| Post-Thaw Viable Cell Recovery | Colony Doubling | Colony formation and diameter doubling within 5 days of observation | Pass |
| Sterility | Growth on agar | Negative | Pass |
| Mycoplasma | PCR | Negative | Pass |
| Karyotype | G-banding | 46,XX | Pass |
| Identity Match | STR (THO-1, D22S417, D10S526, vWA31, D5S592, and FES/FPS) | Match parent fibroblast line | Pass |
| Surface Antigen Expression of Stem Cell Markers | Immunostaining | > 80% expression of SSEA-4 | Pass |
| Pluripotency | Embryoid Body Assay | Upregulation of genes associated with each of the three germ layers | Pass |

## Post-Thaw Viability

One vial of distribution lot was thawed. Cultures were observed daily. Colonies were photographed when they first appeared, then 4 days later (Colonies must double in diameter within 5 days).

| | |
|---|---|
| Day 6 | 235 µm |
| Day 10 | 621 µm |

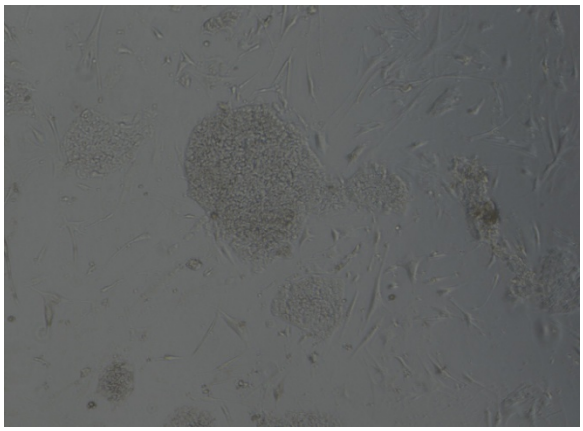

Figure 1A: Colony observed post thaw

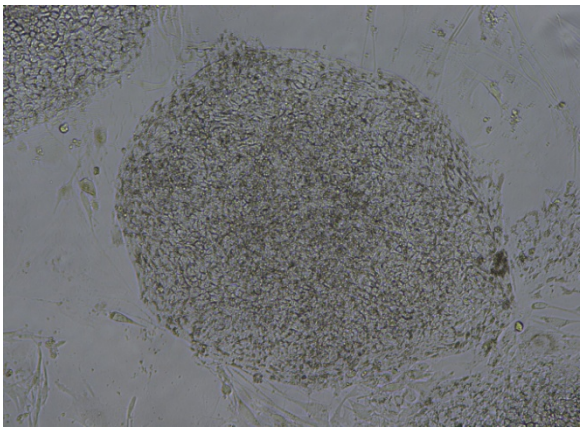

Figure 1B: Colony 4 days after first observation

## Karyotype Analysis


Figure 2: G-banded karyotype showing 46,XX

## Surface Antigen Expression of Stem Cell Markers

Undifferentiated cells are stained for the surface antigens SSEA4. SSEA4 (stage specific embryonic antigen 4) is expressed on undifferentiated human stem cells.

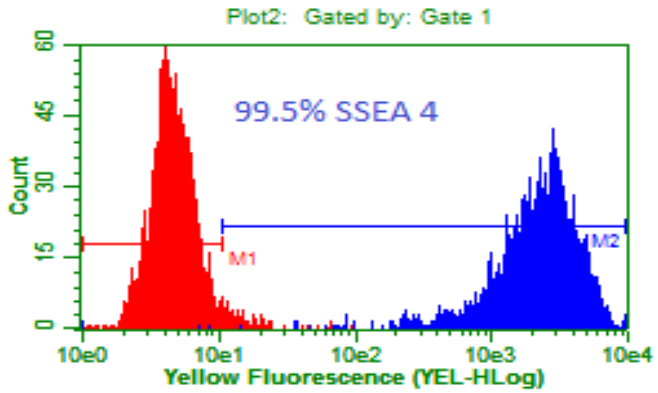


Figure 3: Representative histogram of SSEA-4 positive population. Histogram is an overlay of negative control (red) and SSEA-4 positive population (blue).

## Assessment of Pluripotency of a Cell Line

Cells are directed to differentiate to assess the pluripotency of the cell line. RNA is harvested and gene expression is analyzed by real-time PCR. Ct values are normalized for loading using a housekeeping gene. Gene expression is shown as fold difference to undifferentiated cells.

## Embryoid Body (EB) Formation Assay

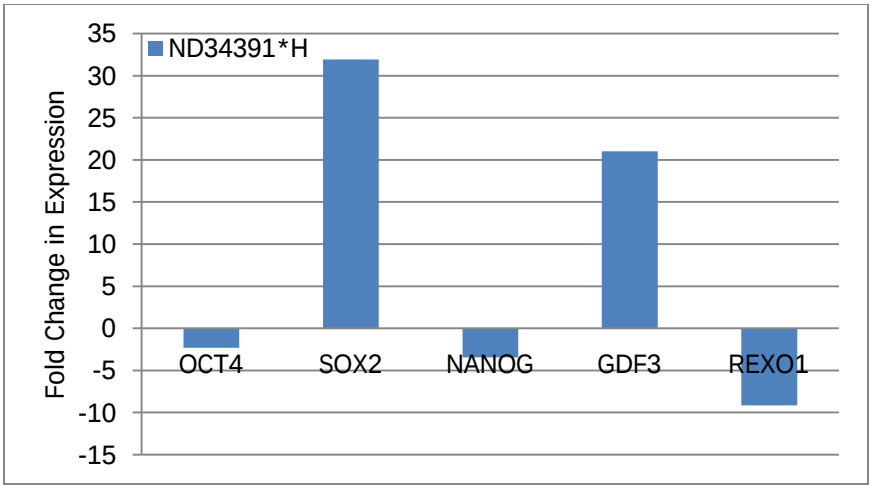


Figure 4A. Pluripotency gene silencing following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

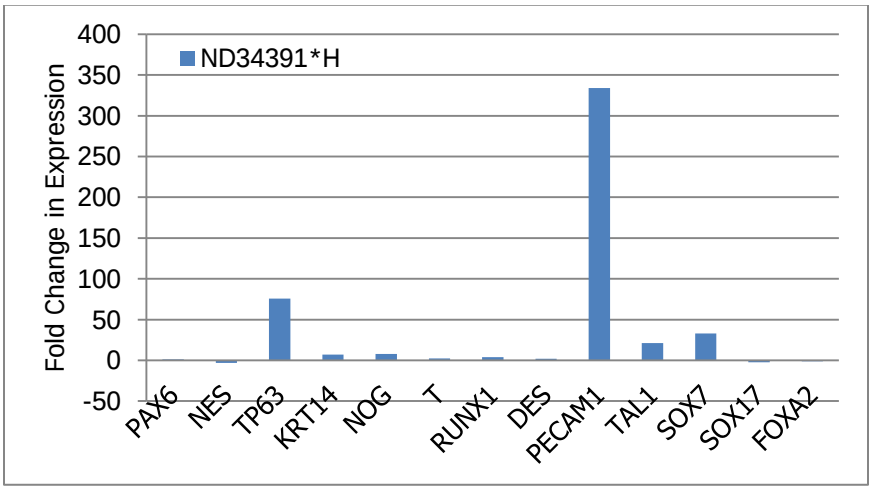


Figure 4B. Lineage specific gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

**Pluripotency Markers**

| | OCT4 | SOX2 | NANOG | GDF3 | REXO1 |
|---|---|---|---|---|---|
| ND34391*H | -2 | 32 | -3 | 21 | -9 |

**Ectoderm**

| | PAX6 | NES | TP63 | KRT14 | NOG |
|---|---|---|---|---|---|
| ND34391*H | 1 | -3 | 76 | 7 | 8 |

**Mesoderm**

| | T | RUNX1 | DES | PECAM1 | TAL1 |
|---|---|---|---|---|---|
| ND34391*H | 2 | 4 | 2 | 334 | 21 |

**Endoderm**

| | SOX17 | FOXA2 | SOX7 | AFP |
|---|---|---|---|---|
| ND34391*H | -2 | -1 | 33 | 21861 |

Table 1. Fold difference values of gene expression of EB. Fold difference is shown relative to undifferentiated iPS cell line. Ct values are normalized to that of GAPDH.

Notes:

☑ Pass
☐ Fail
☐ Other: _____

Shilpa Gandre-Babbe, PhD
Group Leader, Stem Cell Biobank
Date 1.22.14