



Certificate of Analysis

NIGMS Human Genetic Cell Repository

Human induced Pluripotent Stem Cell (iPSC) Line: **GM25256*G**

| | |
|---|---|
| Diagnosis | Apparently Healthy Control |
| Parental cell line mutation | |
| Parental cell type, cell line ID | Fibroblast, N/A |
| Sex | Male |
| Reprogramming method | Episomal vectors containing OCT3/4 with shp53, SOX2, KLF4, LMYC, and LIN28A |
| Passage number at freeze | P39 |
| Culture media | mTesR1™ |
| Feeder or Matrix substrate | Matrigel® |
| Recommended passage method and split ratio | Versene; 1:9 every 5-7 days |
| iPSC line establishment publication(s) | Kreitzer; PMID:23862100 |

The following testing specifications have been met for this product lot:

| Test Description | Test Method | Test Specification | Result |
|--|---|---|--------|
| Post-Thaw Cell Viability | Colony doubling | Colony formation and diameter doubling within 5 days | Pass |
| Sterility | Growth on agar and broth | Negative | Pass |
| Mycoplasma | qRT-PCR | Negative | Pass |
| Alkaline Phosphatase Staining | Cell staining | >80% cells with positive staining | Pass |
| Identity Match | STR (THO-1, D22S417, D10S526, vWA31, D5S592, and FES/FPS) | Match parental cell line | Pass |
| Genomic Integration of Episomal Plasmid | Genomic PCR using plasmid specific primers and endogenous FBXO1 control | No plasmid specific sequence amplified using 100 ng gDNA template | N/A |
| Detection of Sendai Virus Genome and Transgene | qRT-PCR using SEV specific primers | No detection of SEV genome or transgenes | N/A |
| Surface Antigen Expression of Stem Cell Markers | Immunostaining and flow cytometric detection | >80% expression of SSEA4 | Pass |
| Differentiation Potential | Embryoid body (EB) formation and gene expression | Minimum of 1 gene per germ layer expressed 2 fold or higher | Pass |
| Cytogenomics | G-banding | 46,XY[19] | Pass |

*Note:

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Technician, Stem Cell Laboratory Date

Christine Grandizio 07/17/2020
.....
Manager, Stem Cell Laboratory Date

Disclaimer: iPSC lines distributed by Coriell Institute for Medical Research may differ from one passage or expansion to another.

Form 1701-07 Rev Q-050720: NIGMS HGCR Certificate of Analysis GM25256*G

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Post-Thaw Cell Viability

One distribution lot vial of the cell line was thawed and placed in culture. Cultures were observed daily. Colonies were photographed upon first appearance, then 2 days later. Colonies must double in diameter within 5 days. The area for 5 colonies was measured using CellSens software on the Olympus IX50 microscope at 40x magnification. The average area is reported here.

| Day | Average area (μm^2) |
|-----|----------------------------------|
| 1 | 38,644 |
| 3 | 304,137 |

Colony area increased by 8 fold.

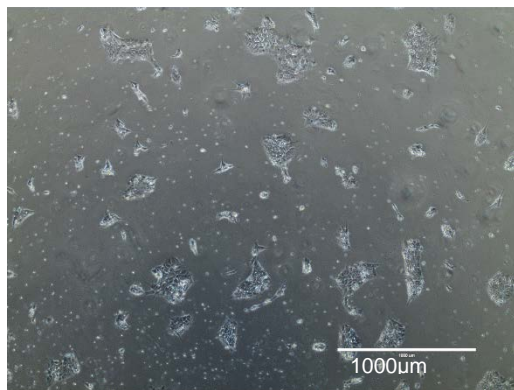


Figure 1A. Colonies post thaw (Day 1)

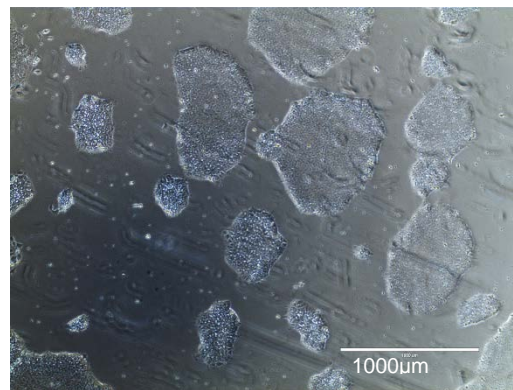


Figure 1B. Colonies 2 days after first observation (Day 3)

Alkaline Phosphatase Staining

Cells were stained using the StemTAG™ Alkaline Phosphatase Staining Kit from CellBiolabs, Inc.

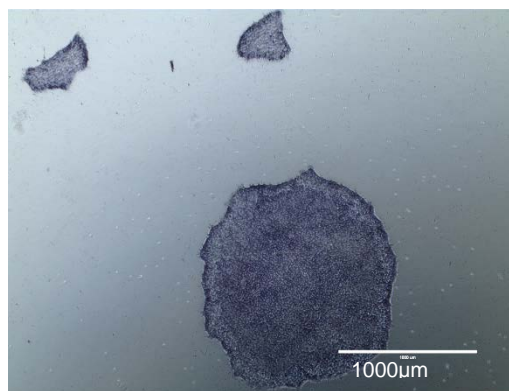


Figure 2. iPSC colonies showing alkaline phosphatase activity



Surface Antigen Expression of Stem Cell Markers

Undifferentiated cells are stained for stage specific embryonic antigen 4 (SSEA4) which is expressed on the surface of undifferentiated human pluripotent stem cells. Cells were analyzed using the MACSQuant Flow Cytometer by Miltenyi Biotec. More than 80% of cells should stain with antibodies specific for SSEA4.

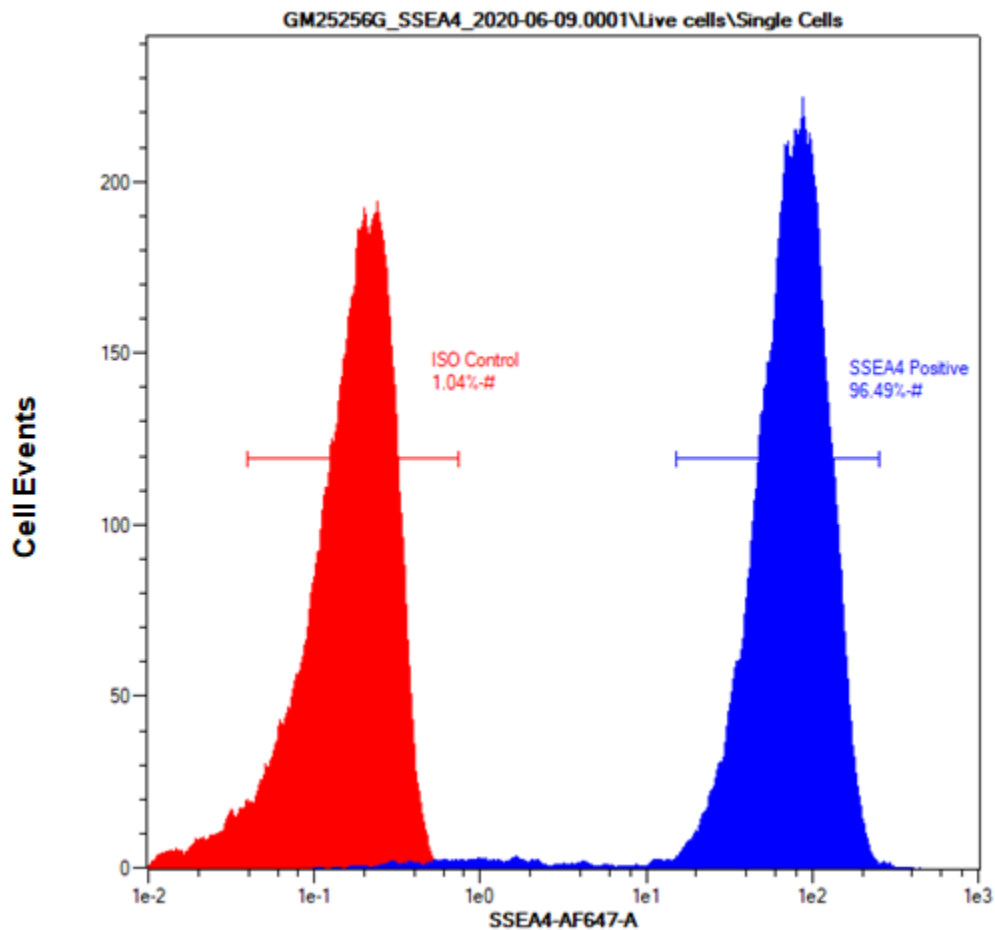
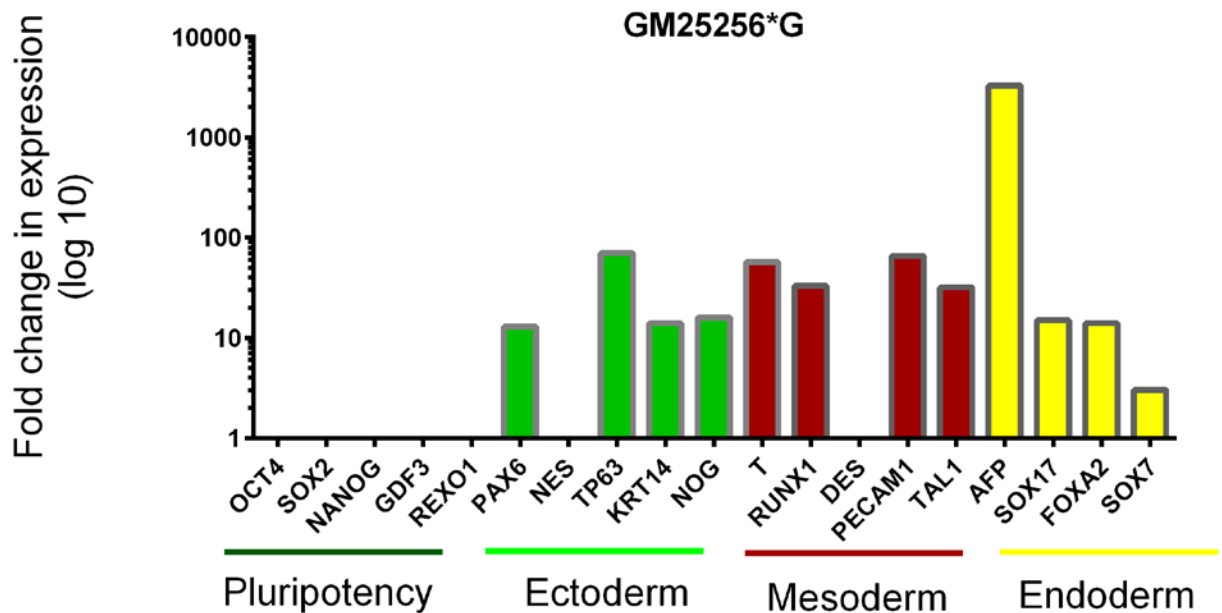


Figure 3. Representative histogram of SSEA4 positive population showing an overlay of isotype stained control (red) and SSEA4 positive population (blue)

Differentiation Potential

Cells are differentiated by embryoid body (EB) formation to assess pluripotency. RNA is extracted and gene expression is measured by quantitative RT-PCR. Ct values are adjusted to the endogenous housekeeping gene GAPDH. Relative gene expression is shown as the fold difference in expression compared to undifferentiated cells. Expression of at least one gene per germ layer should increase by 2 fold or higher.



| Gene | Fold change | Gene | Fold change | Gene | Fold change | Gene | Fold change |
|-------|-------------|-------|-------------|--------|-------------|-------|-------------|
| OCT4 | 0 | PAX6 | 13 | T | 57 | AFP | 3293 |
| SOX2 | 0 | NES | 0 | RUNX1 | 33 | SOX17 | 15 |
| NANOG | 0 | TP63 | 70 | DES | 0 | FOXA2 | 14 |
| GDF3 | 0 | KRT14 | 14 | PECAM1 | 66 | SOX7 | 3 |
| REXO1 | 0 | NOG | 16 | TAL1 | 32 | | |

Figure 4. Fold change in expression of pluripotency genes and tri-lineage specific genes

Note: Negative values are set as 0. Calculations are performed using the $2^{-\Delta\Delta CT}$ method. (Livak KJ, Schmittgen TD. *Methods*. 2001 Dec;25(4):402-8.PMID:11846609)

Cytogenomics

| Cytogenetic Banding Technique | G-banding |
|-------------------------------|-------------|
| Passage at Analysis | P40 |
| Metaphase Cells Counted | 20 |
| Metaphase Cells Analyzed | 8 |
| Metaphase Cells Karyotyped | 6 |
| Short ISCN | 46, XY [19] |

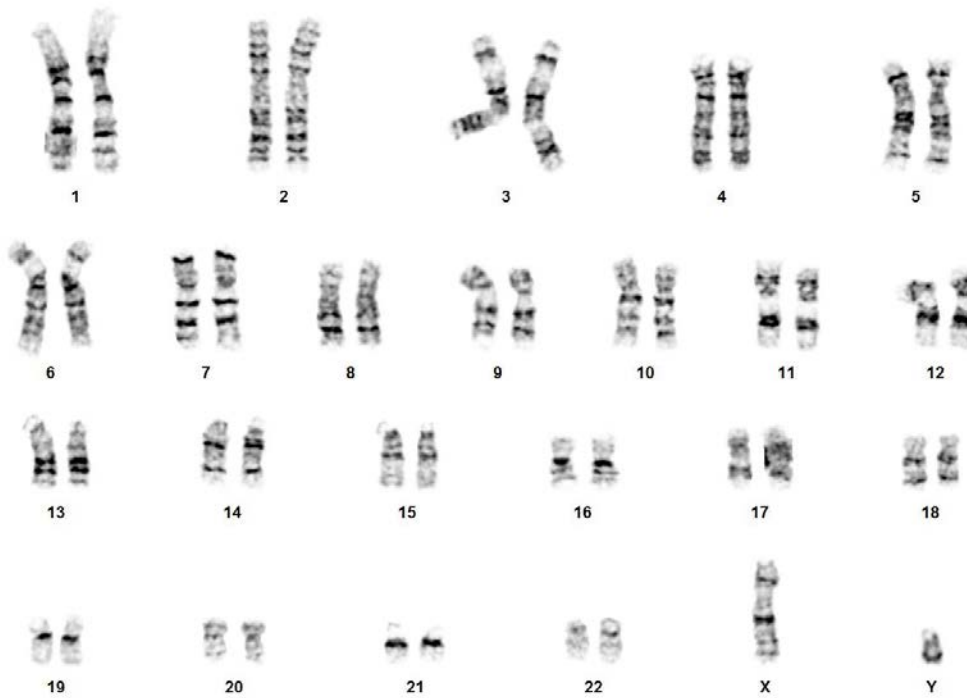


Figure 5. G-banding karyogram