CORIELL INSTITUTE
FOR MEDICAL RESEARCH

Certificate of Analysis **_NIGMS Human Genetic Cell Repository_**

Human induced Pluripotent Stem Cell (iPSC) Line: **GM27629*A**

Diagnosis	Rett Syndrome, Congenital Variant
Parental cell line mutation	FOXG1; c.685A>G; (p.Asn232Ser)
Parental cell type, cell line ID	Fibroblast, GM27616
Sex	Male
Reprogramming method	Episomal vectors containing OCT3/4 with shp53, SOX2, KLF4, LMYC, and LIN28A
Passage number at freeze	P13
Culture media	mTeSR1
Feeder or Matrix substrate	Matrigel
Recommended passage method and split ratio	Versene; 1:6 every 5 days
iPSC line establishment publication(s)	

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, Affymetrix Human SNP Array 6.0	46,XY[24].arr[hg19] 6p25.1(5,200,030-5,604,647)x1	Pass*

*Note: Microarray analysis has identified a copy number loss of uncertain clinical significant at 6p25.1 containing autosomal recessive gene (FARS2). The FARS2 gene is located on chromosome 6, unrelated to the FOXG1 gene on chromosome 14.

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Manager, Stem Cell Laboratory Date

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

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	6,636
3	71,016

Colony area increased by 11 fold.

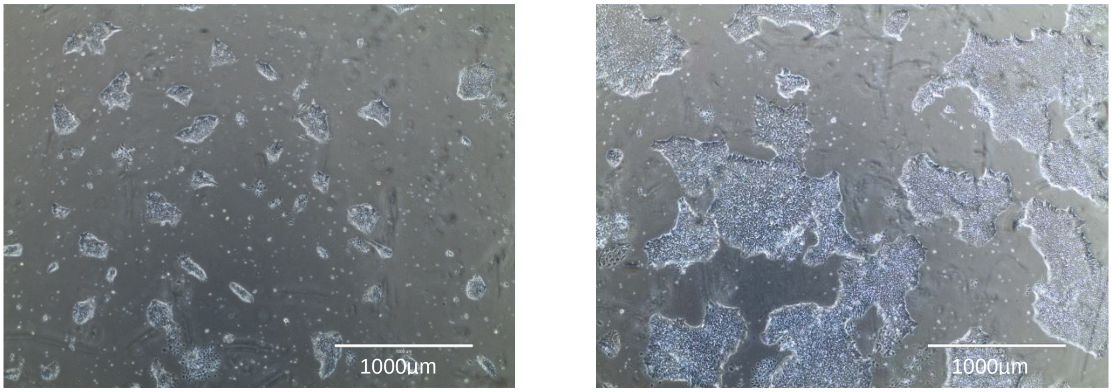


Figure 1A. Colonies post thaw (Day 1)

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.

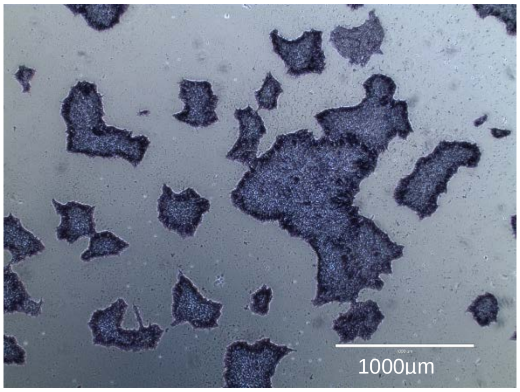


Figure 2. iPSC colonies showing alkaline phosphatase activity

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 Miltyeni Biotec. More than 80% of cells should stain with antibodies specific for SSEA4.

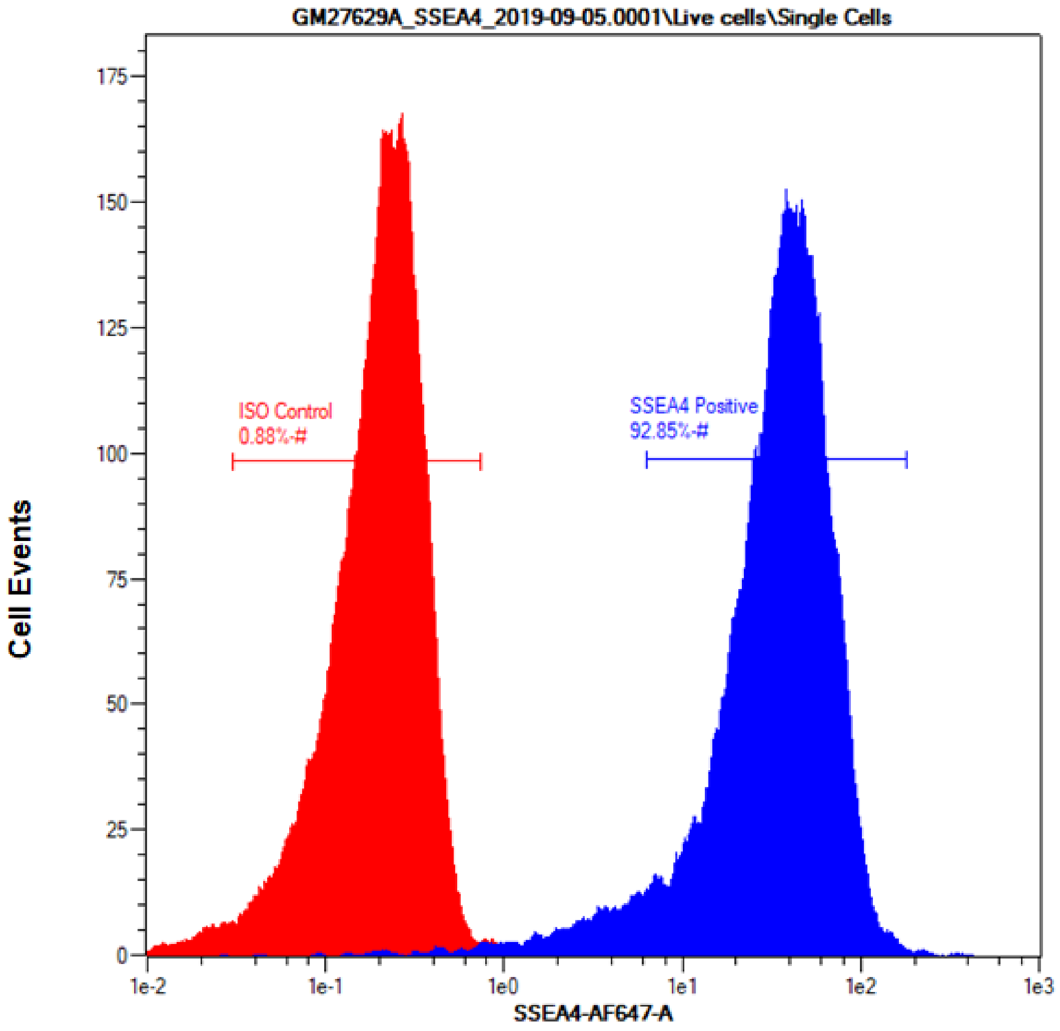
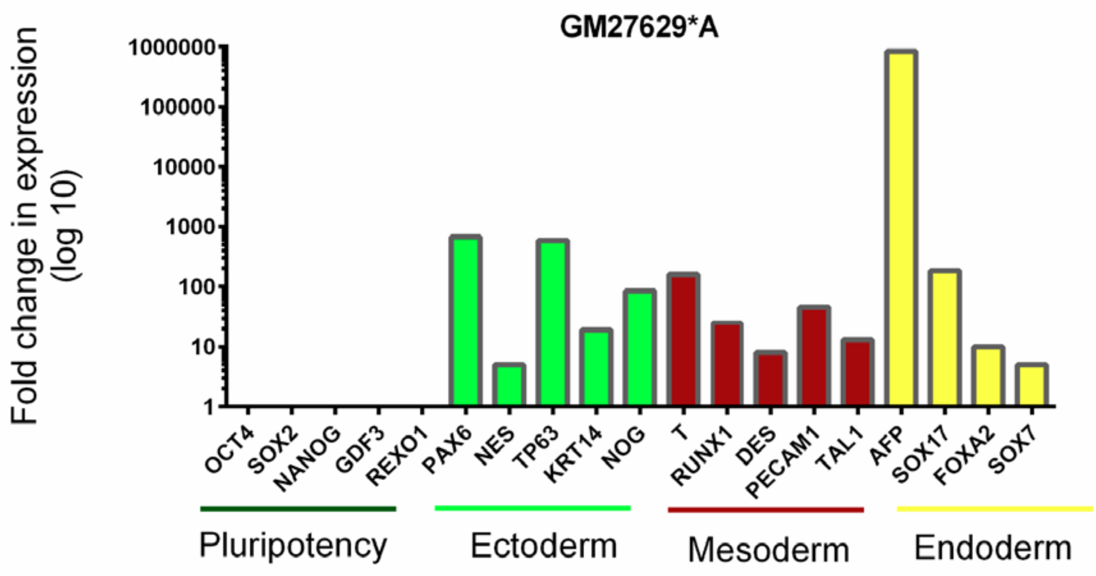


Figure 3. Representative histogram of SSEA4 positive population showing an overlay of isotype stained control (red) and SSEA4 positive population (blue)

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**	676	**T**	159	**AFP**	845478
SOX2	1	**NES**	5	**RUNX1**	25	**SOX17**	184
NANOG	0	**TP63**	591	**DES**	8	**FOXA2**	10
GDF3	1	**KRT14**	19	**PECAM1**	46	**SOX7**	5
REXO1	0	**NOG**	86	**TAL1**	13		

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

Microarray	Affymetrix Human SNP Array 6.0
Cytogenetic Banding Technique	G-banding
Passage at Analysis	P15
Metaphase Cells Counted	25
Metaphase Cells Analyzed	25
Metaphase Cells Karyotyped	5
Short ISCN	46, XY[24].arr[hg19] 6p25.1(5,200,030-5,604,647)x1

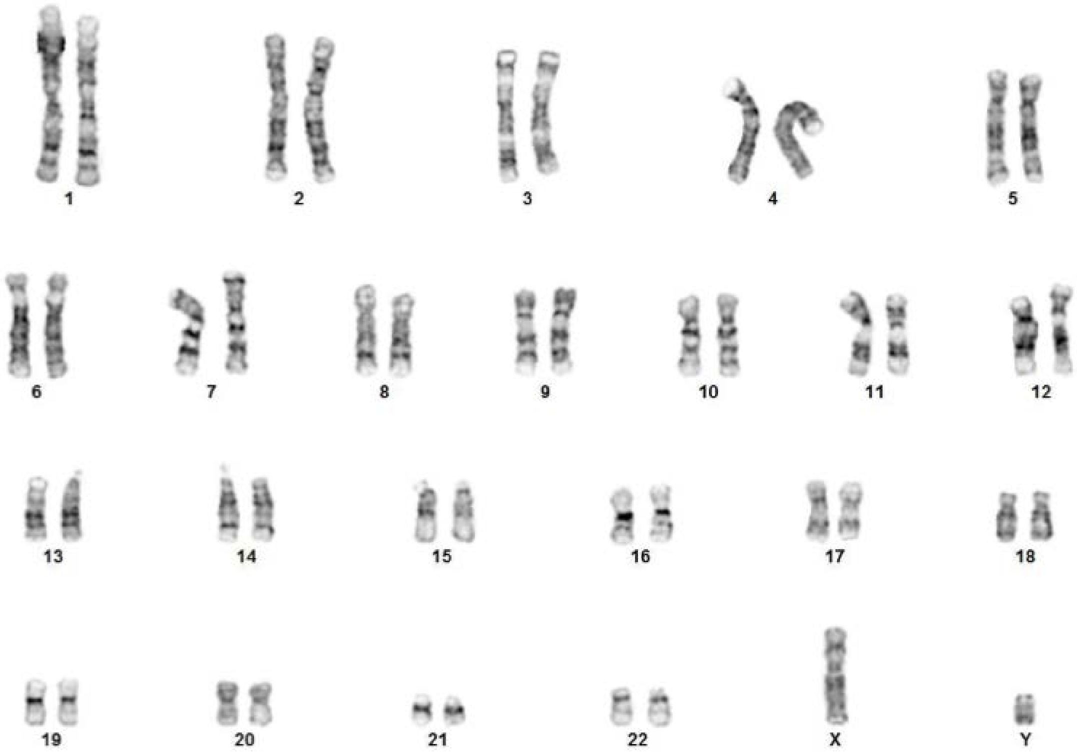


Figure 5. G-banding karyogram