Validation Package

Product Type	Cell Line
Name	PWS 2.9
Cell Type	Induced pluripotent stem cell (iPSC)
Source	Fibroblasts
Donor Gender	Male
Donor Age	19-24
Reprogramming Method	Lentivirus Method: Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, Mostoslavsky G. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem Cells*. 2009;27(3):543-9.
Publications	iPSCs reference: Martins-Taylor K, Hsiao JS, Chen PF, et al. Imprinted expression of UBE3A in non-neuronal cells from a Prader-Willi syndrome patient with an atypical deletion. *Hum Mol Genet*. 2013;23(9):2364-73. Source Fibroblast reference: De Smith AJ, Purmann C, Walters RG, et al. A deletion of the HBII-85 class of small nucleolar RNAs (snoRNAs) is associated with hyperphagia, obesity and hypogonadism. *Hum Mol Genet*. 2009;18(17):3257-65.
Biosafety Level	2
Thaw Recommendation	Thaw 1 vial into 1 well of a 6 well plate as per human PSC culture protocols
Growth Conditions	Feeder Dependent: irradiated MEF (Gibco A34181), hESC medium: DMEM/F12 (Gibco 11330-057) with 20% Knockout Serum Replacement (Invitrogen 10828-028), 1X Non-essential amino acids, 2mM L-glutamine, 0.1mM 2-Mercaptoethanol, 8ng/mL basic Fibroblast Growth Factor
Passage Number	14, these cells were cultured for 14 passages prior to freeze
Date Vialed	August 7, 2018
Cryopreservation	Bam Banker (Wako Chemicals USA, Inc, Part No 30214681) Serum-free cell freezing medium, containing 10% DMSO
Storage	Cryopreserved cells should be stored in liquid nitrogen Cells should be cultured at 37 °C upon arrival
Shipped	Frozen vials or ambient temperature as live cells in T25 flask
Banked by	Stem Cell Core, UConn Health

Contents

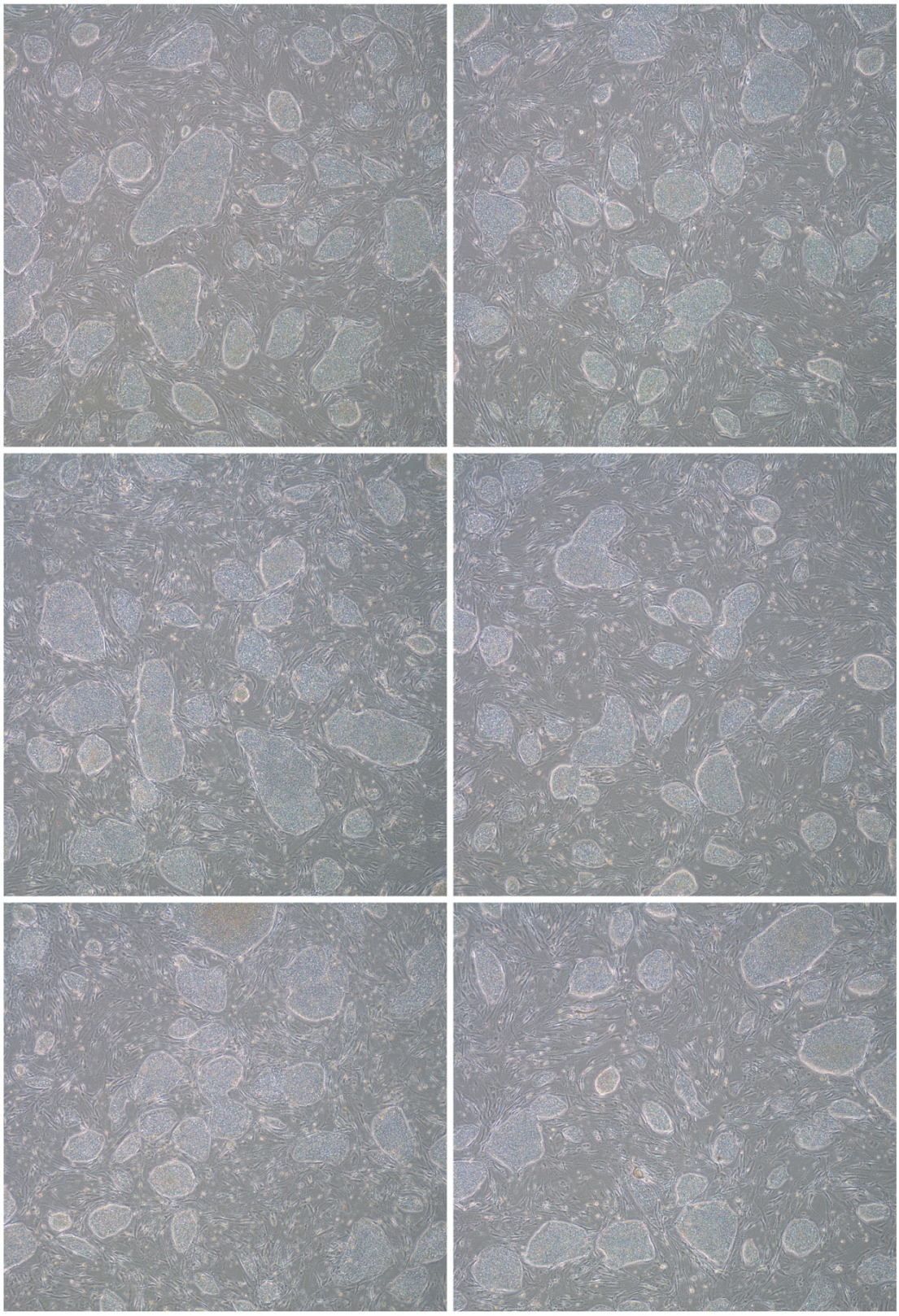
Culture Characteristics:

Cryopreservation: Aspirate culture medium from hPSC plate, wash once with PBS. Add 1 mL of 0.5uM EDTA (Invitrogen, 15575-038) dissociation solution, incubate 3-5 minutes at 37°C. Aspirate EDTA solution gently, add 1 ml of culture medium per well. Cut stem cell colonies using the StemPro EZPassage Disposable Stem Cell Passaging Tool (Invitrogen, 23181-010). Use a cell scraper to gently detach the cells off the surface of the culture plate. Transfer the medium containing colonies to a 15 ml tube and spin down at 1000 rpm (200 g) for 2 min. Aspirate the supernatant carefully to remove single cells or contaminating feeder cells (MEFs) from the population. Re-suspend colonies in Bambanker (Cat. No, 302-14681) serum-free cell freezing medium, containing 10% DMSO, and place the cells in cryogenic vials for freezing and preservation.

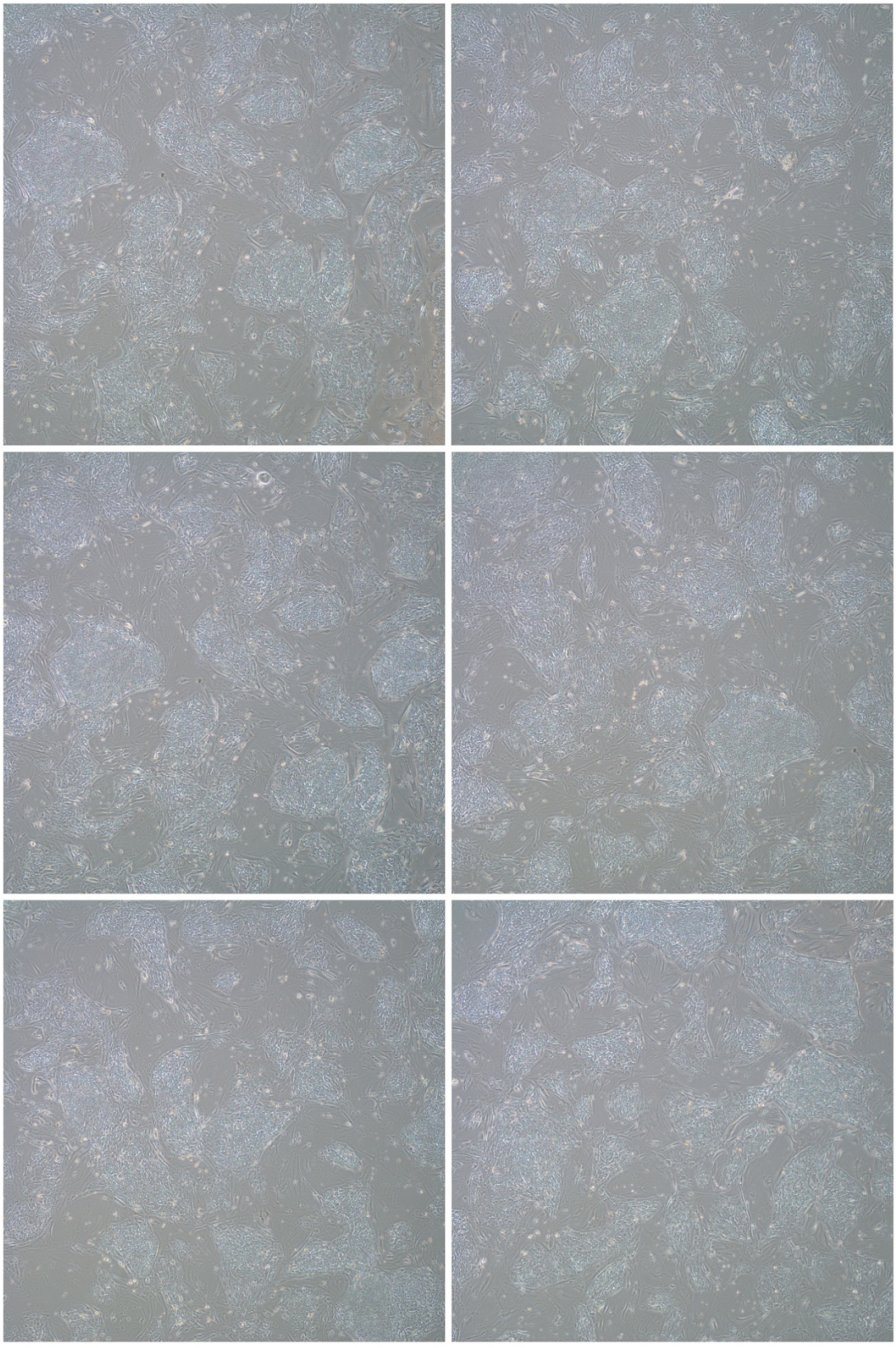
Recovery: Roll the vial between gloved hands for 3-5 seconds to remove the frost. Immerse the vial into a 37°C water bath. Swirl the vial gently and observe the progress of the thaw. When only a small ice crystal remains, wipe the outside of the vial with 70% ethanol. In a sterile biological safety cabinet, transfer the contents of the cryogenic vial directly to the bottom of a 15 mL conical tube. Slowly add 4 mL of hESC medium to the tube. Centrifuge the cells for 5 minutes at 200 x g. Gently resuspend the cells in hESC medium. Aspirate the PBS from the MEF feeder well and slowly add the cell suspension to the prepared well of the 6-well plate.

Growth Curve: Cells from hPSC were passaged using Accutase (EMD Millipore, SCR005) for 8 minutes, and then mechanically dissociated into single cells using pipette 1000ul tips. Centrifuge the cells at 200 x g for 5 minutes. 1500 cells per well of a 6-well plate were plated on MEF using hESC medium. MCH2-10 (generated from an unaffected donor) served as a control. Cells from three separate wells were harvested every passage, accutased and counted.

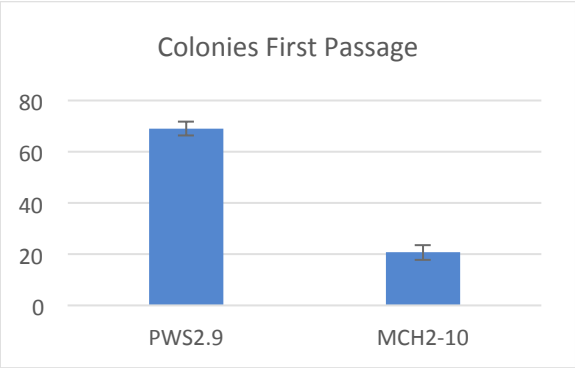
PWS2.9 images taken day of cryopreservation, passage 14

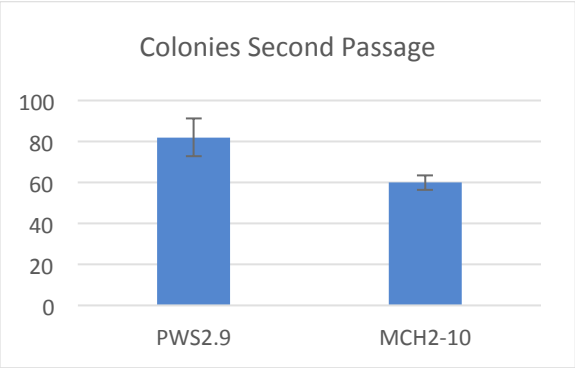


PWS2.9 recovery at day 3, one vial thawed cells to one well of a 6-well plate, passage 15

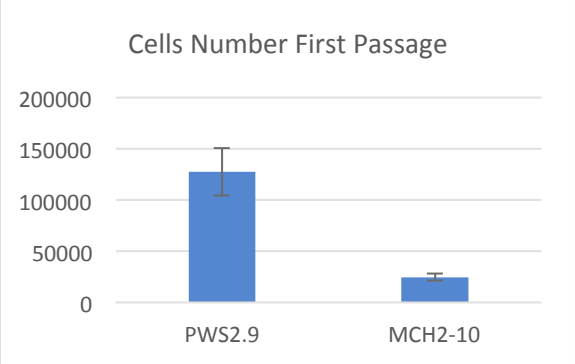


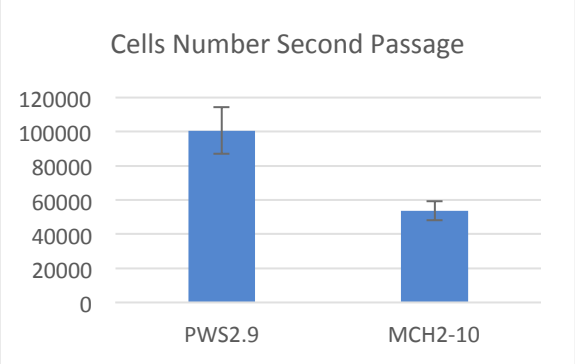
Growth Curve
Colonies in one well of a 6 well plate in triplicate wells, 1500 cells plated to each for both test (PWS2.9) and control (MCH2-10 iPSC, generated from an unaffected donor).



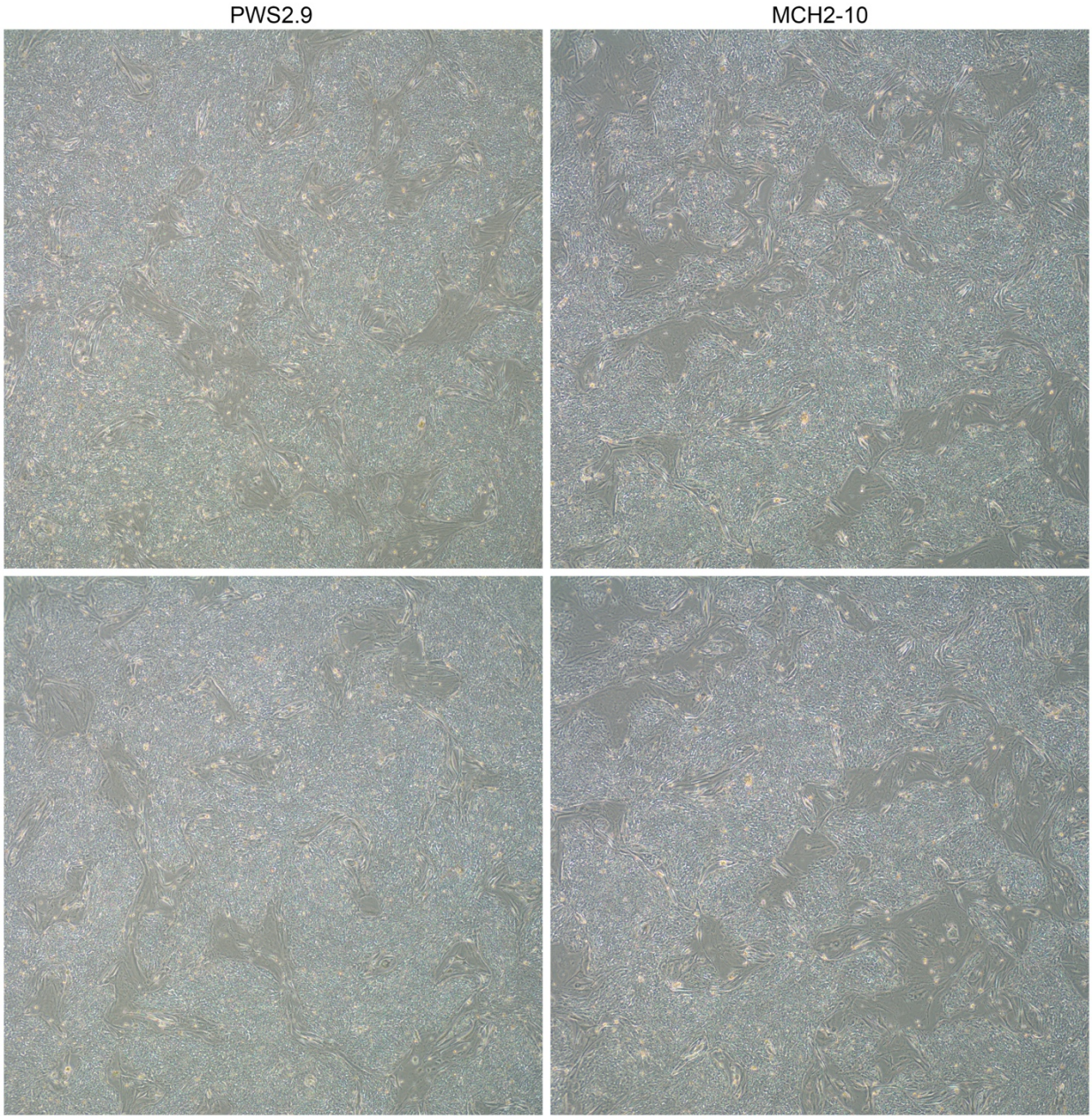


Growth Curve
Cell number in one well of a 6 well plate





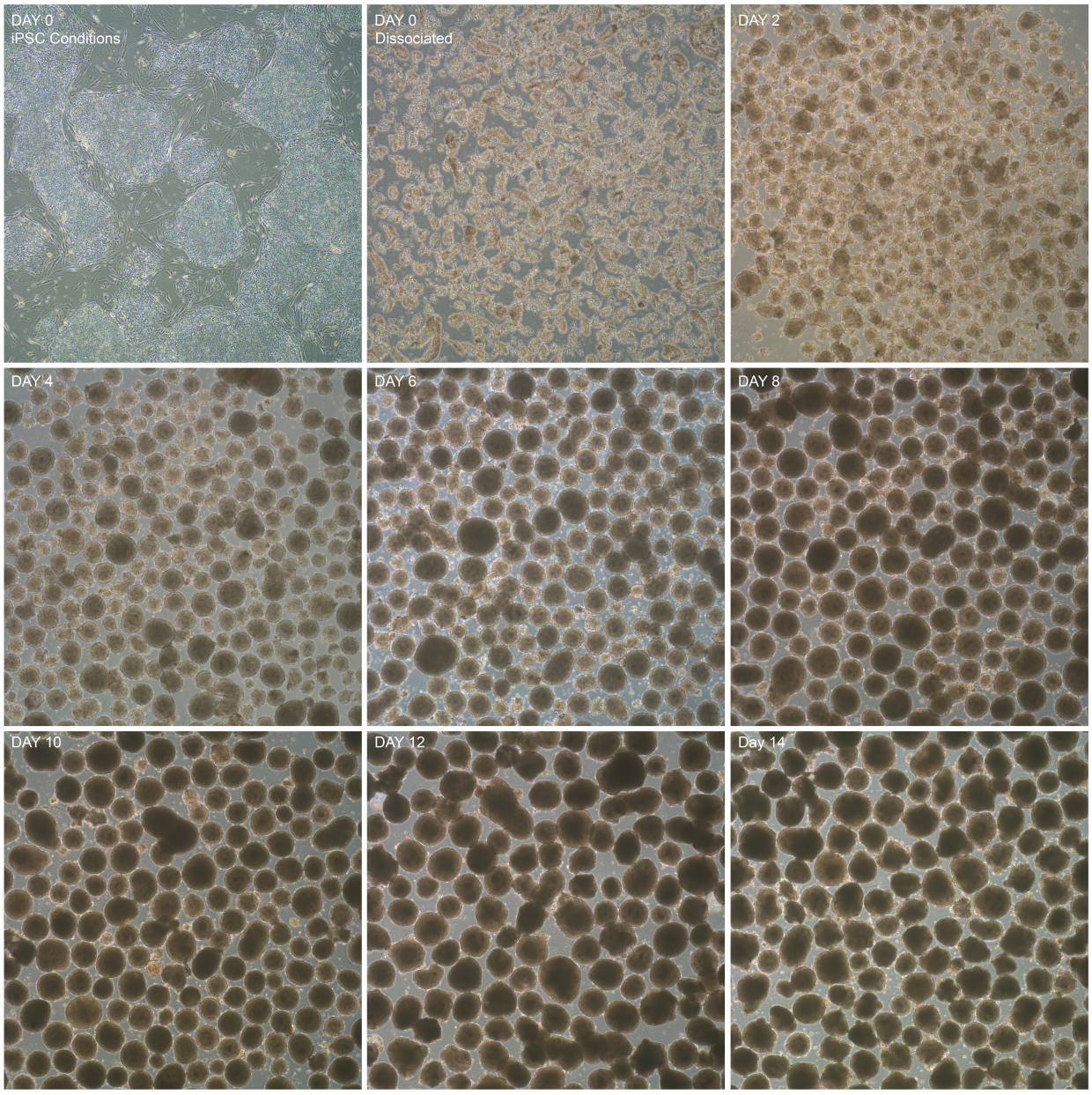
Growth Curve: At the second passage, cells were accutased and counted. And then cells from three wells were combined and seeded in one well of a 6 well plate. Images taken at day 3.



Embryoid bodies (EB) are the three-dimensional aggregates formed in suspension by the iPSCs. Embryoid Body culture is used to examine the differentiation potential of the iPSCs.

Growth and differentiation of embryoid bodies: aspirate off the culture medium from the culture plates, and then add 1 mL pre-warmed EB medium (hESC medium lacking basic fibroblast growth factor) to each well of 6-well plate. Cut stem cell colonies using the StemPro EZPassage disposable stem cell passaging tool (Invitrogen, 23181-010). Use a cell scraper to gently detach the cells off the surface of the culture plate. Gently transfer the cell clumps into a 15-mL conical tube. Allow the cells to gravity sediment for approximately 5 minutes. Aspirate the supernatant, and then gently tap the tube to loosen the cell pellet. Transfer the cell clumps to a corning ultra-Low attachment cell culture flask (Sigma, CLS3815) in a total of 10 mL of EB medium. Replaced medium and took image every other day. RNA was collected at day 14 for tri-Lineage differentiation assay.

Phase images: PWS2.9 embryoid body formation from day 0 to day 14



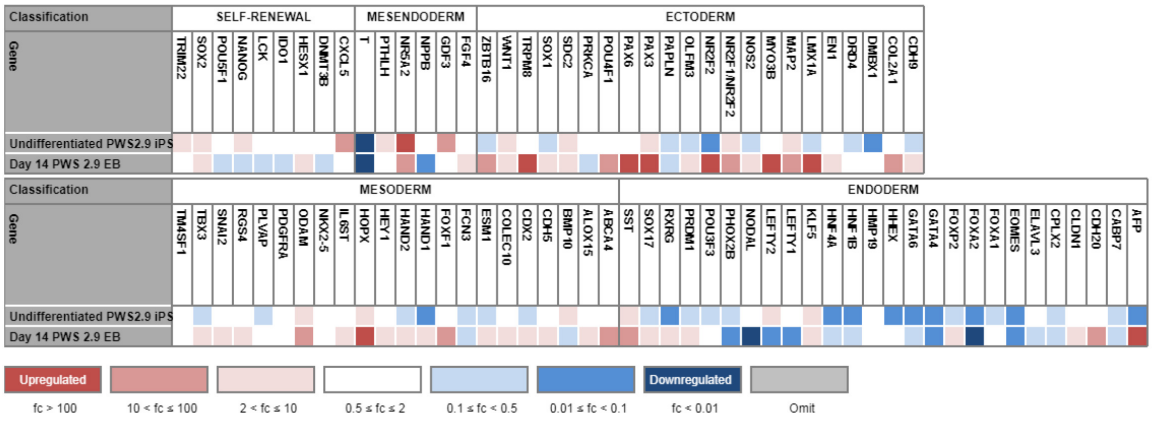
Gene Expression: Pluripotency and Tri-Lineage Differentiation Assay

TaqMan hPSC Scorecard Panel 384-well (Applied Biosystems, A15870) enables verification of pluripotency and determination of lineage bias for iPSC cell line. The 384-well plate contains four sets of 94 predefined TaqMan Gene Expression assays (including endogenous controls) dried-down in the wells. The Scorecard run on the 7900HT Real-Time PCR System. The data were analyzed using Applied Biosystems hPSC Scorecard analysis software.

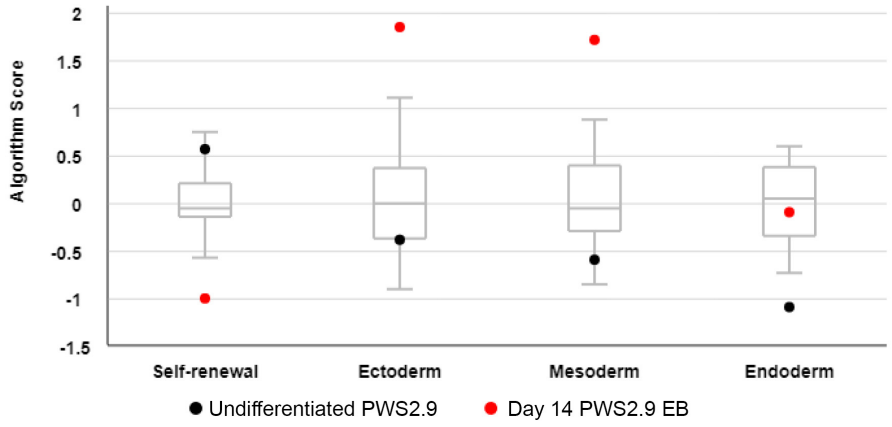
Scorecard: A simple-to-interpret summary of gene expression level data that confirms pluripotency or indicate germ layer bias of your sample.
Heat Map: Colors indicate the fold change in expression relative to the undifferentiated reference set for each gene.
Scores Box & Whisker Plot: View samples scores (color) in relation to the range of scores for the undifferentiated reference set (gray).
Correlation Plot: See how gene expression levels correlate between samples.
Assay QC: Perform a quick quality control check to make sure the sample amplified as expected.

Scorecard Results: A simple-to-interpret summary of gene expression level data that confirms pluripotency or indicate germ layer bias of the sample.

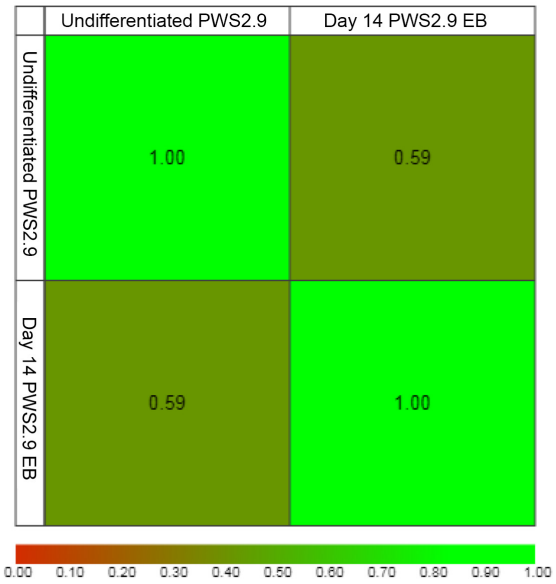
Expression Plot: Colors correlate to the fold change in expression of the indicated gene relative to the undifferentiated reference set.



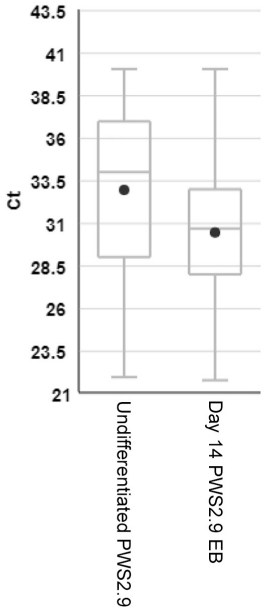
Scores Box Plot: Sample scores are plotted in color. The range of scores for the undifferentiated reference set is indicated by the gray box plot.



Correlation Plot: Pair-wise comparison of the 96 Ct or delta Ct values for all selected samples in the project. Scatter plots are shown in the upper right half of the matrix whereas corresponding correlation coefficients (r2 values) are shown in the lower right half of the matrix.



Assay QC Plot: The box plot shows the range of Ct values or dealt Ct values for all 96 genes in the hPSC Scorecard Panel.

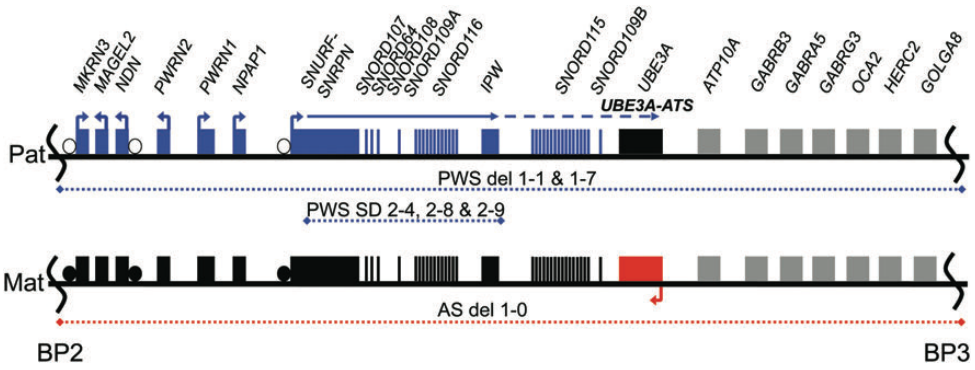


Gene Expression (qRT-PCR)

RNA was isolated from iPSC cells using Quick-DNA/RNA Miniprep Kit (ZYMO Research, D7001). cDNA was synthesized using SuperScript II Reverse Transcriptase (Invitrogen, 18064-022). Gene expression was analyzed using TaqMan Gene Expression Assays, and the GAPDH was used as an endogenous control. The data were analyzed using Bio-Rad CFX Manager 3.1 software, normalized to MCH2-10 (iPSC generated from unaffected donor). The Taqman FAM-MGB qRT-PCR primers used to examine the gene expression of MKRN3, MAGEL2, NDN, SNRPN, SNORD116 and IPW.

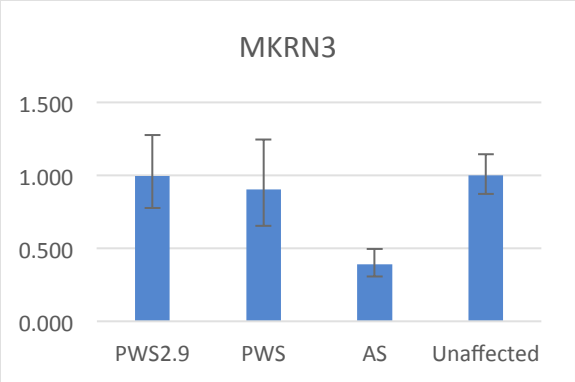
TaqMan Gene Expression assays are used for quantitative real-time PCR analysis of gene expression and consist of a pair of unlabeled PCR primers and a TaqMan probe with a dye label (FAM) on the 5' end and a minor groove binder (MGB) and non-fluorescent quencher (NFQ) on the 3' end.

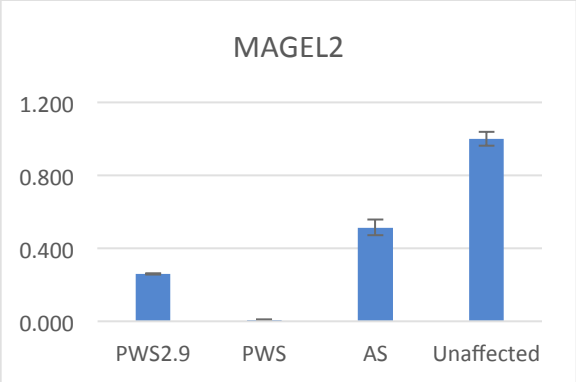
Map of chromosome 15q11 – q13 region:

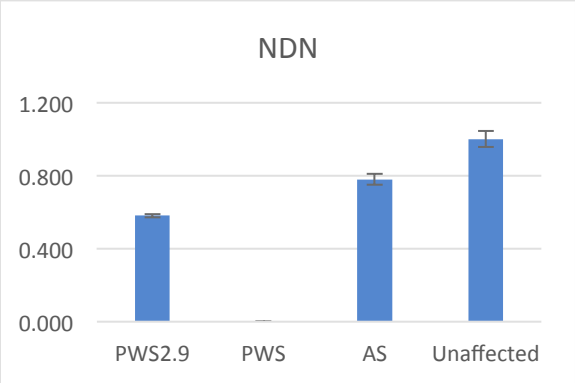


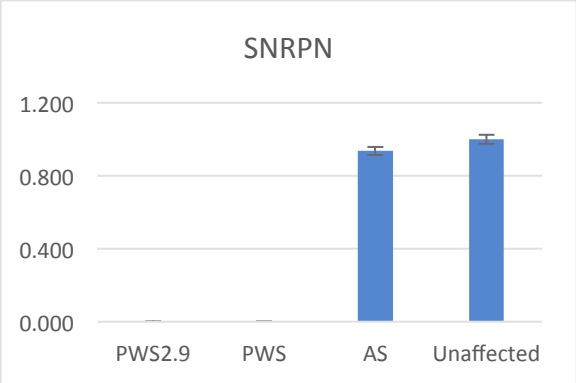
Gene Symbol	TaqMan Assay ID
MKRN3	Hs00271653_s1
MAGEL2	Hs00255922_s1
NDN	Hs00267349_s1
SNRPN	Hs00243205_m1
SNORD116	Hs03454084_m1
IPW	Hs03455409_s1
GAPDH	Hs99999905_m1

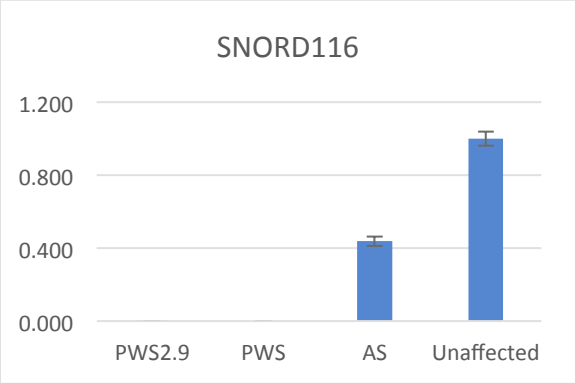
PWS = Prader Willi Syndrome iPSC PWS1.7
AS = Angelman Syndrome iPSC AS2.1
Unaffected = iPSC MCH2.10

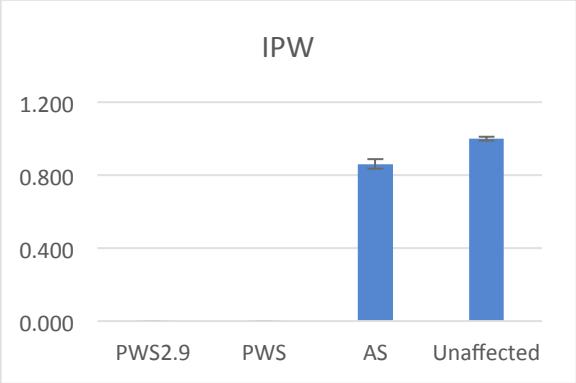

MKRN3
1.500
1.000
0.500
0.000
PWS2.9
PWS
AS
Unaffected


MAGEL2
1.200
0.800
0.400
0.000
PWS2.9
PWS
AS
Unaffected


NDN
1.200
0.800
0.400
0.000
PWS2.9
PWS
AS
Unaffected


SNRPN
1.200
0.800
0.400
0.000
PWS2.9
PWS
AS
Unaffected

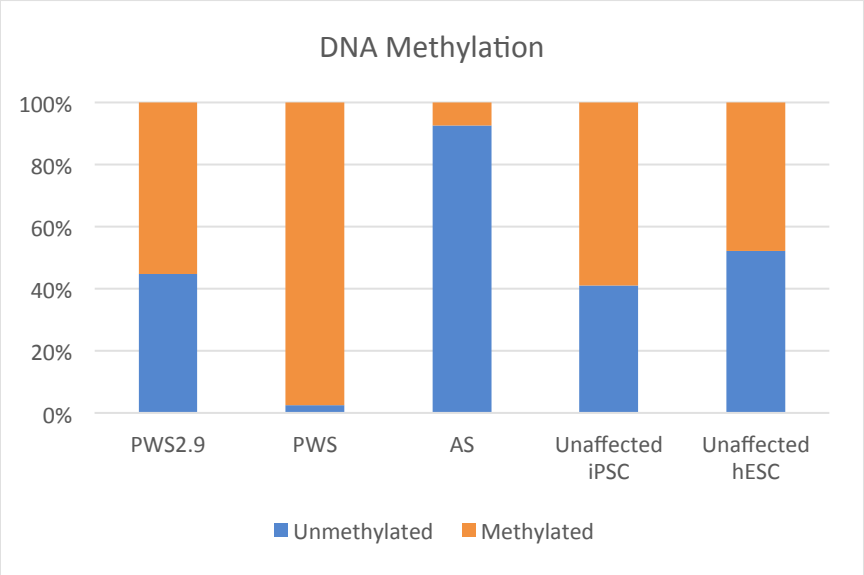

SNORD116
1.200
0.800
0.400
0.000
PWS2.9
PWS
AS
Unaffected


IPW
1.200
0.800
0.400
0.000
PWS2.9
PWS
AS
Unaffected

DNA Methylation analysis of PWS-IC using a methylation-sensitive restriction endonuclease quantitative PCR assay. The EpiTect II DNA Methylation Enzyme Kit (Qiagen, 335452) prepares genomic DNA samples for DNA methylation analysis using EpiTect Methyl II PCR Assays for individual and predicted methylated CpG islands. Using the enzymes and buffer provided in the kit, 4 digests are performed to detect different methylated DNA fractions. The product of a mock digest (Mo) contains all of the input genomic DNA. The product of the methylation-sensitive restriction enzyme mixture (Enzyme A) digest (Ms) contains methylated DNA sequences, while the product of the methylation-dependent restriction enzyme mixture (Enzyme B) digest (Md) contains unmethylated DNA sequences. The product of a double digest (Msd) measures the background and the success of both enzymatic digestions.

PWS = Prader Willi Syndrome iPSC PWS1.7
AS = Angelman Syndrome iPSC AS2.1
Unaffected iPSC = iPSC MCH2.10
Unaffected hESC = H9 PWS

Cell Line	Unmethylated	Methylated
PWS2.9	44.85%	55.15%
PWS	2.39%	97.61%
AS	92.61%	7.39%
Unaffected iPSC	40.96%	59.04%
Unaffected hESC	52.11%	47.89%



Cyto-SNP

The Affymetrix CytoScan HD Array includes 750,000 SNPs and 2.6 million copy number markers to enable detection of accurate breakpoint assignment and high-resolution (~25kb resolution) detection of copy number variation (CNV), loss of heterozygosity (LOH), uniparental disomy (important for imprinting syndrome studies) and low-level mosaicism in cell lines.

- To identify chromosome abnormalities at less than 5MB resolution
- To confirm G-band and FISH findings
- To define specific breakpoints and/or gene insertions
- When LOH and/or CNV analyses are needed
- To identify amplifications or deletions for genes of interest
- When whole genome genotyping is needed
- To derive genomic information on subtelomeric and pericentromeric regions

Genomic microarray analysis and G-banded karyotyping are complementary and provide a comprehensive panel of genome integrity assessment.

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CENTER FOR GENOME INNOVATION

Chromosome Core

Case Report

Sample ID:	**CC18-31**
Sample Name:	**PWS_2.9**
Experiment date:	November 5, 2018
Report date:	December 6, 2018
Microarray type:	Illumina CytoSNP-850K v1.2
Microarray Barcode:	202917700009
SNP manifest file:	CytoSNP-850Kv1-2_NS550_B3.bpm
Annotation DB:	BG_Annotation_Ens74_20180801.db
SNP cluster file:	CytoSNP-850Kv1-2_NS550_B1_ClusterFile.egt
Genome build name: GRCh37	Ensembl version: 74
GTC file:	202917700009_R02C01.gtc
Algorithm:	BeadArray v2 - Standard
Smoothing:	Backbone = 9
CGH Reporting:	Minimum Del and Dup Size = 600 Kb Minimum LOH Region Size (Mb) = 3.0
Significant Clones:	CGH Region = 10 LOH Region = 500
QC Measures:	Pass
Median Log R Deviation: Ratio Intensity Signal (<0.2)	0.13
Median Call Rate: genotype calling performance estimate (>0.98)	1
Sample sex:	Male

ISCN	Type	Chromosome	Start	End
5q13.2-5q15	**Gain**	5	71,156,895	95,207,168
Copy number gain on chromosome 5 of 22.15 Mb; overlaps 176 HGNC and 67 OMIM gene(s).				
15q11.2-15q11.2	**Loss**	15	25,207,273	25,391,924
Copy number loss of 185Kb (<1Mb); overlaps 34 HGNC and 5 OMIM gene(s).				

The significance of the Illumina molecular karyotyping findings should be interpreted by the principle investigator for research purposes only and include consideration of cell origin, culture conditions and experimental questions.*LogR changes and B-allele frequencies were manually scanned across all chromosomes. Gains and losses at 400 Kb or larger and LOH at 5 Mb are reported.*

Array processing:	Lisa LaBelle, MS, MB (ASCP)
Data analysis and sign out:	Judy Brown, PhD, CG, MB (ASCP)

Warning: The results reported herein are for research use only and not to be used for patient diagnosis or treatment.

UConn Chromosome Core
(860) 486-3618 https://cgi.uconn.edu/

Judith D Brown

Human Pathogen Testing
Microbiology (Bacterial and Fungal)
Mycoplasma Testing

IDEXX BioAnalytics

Certificate of Analysis

IDEXX BioAnalytics Case #: 29919-2018
Case Official: Berg, Heath

ID: 1
Date Completed: 10/26/2018

Specimen Description

ID	Client ID	Cell Line	Species	ATCC #	Other1
1	PWS2.9	PWS2.9 iPSC	Human	fibroblast PW	human iPSC

PCR Evaluation

cells	1
HCMV	-
Hepatitis A	-
Hepatitis B	-
Hepatitis C	-
HIV1	-
HIV2	-
HTLV 1	-
HTLV 2	-
LCMV	-
Mycoplasma sp.	-
Treponema pallidum	-

Legend: + = positive - = negative id:id = pooled sample range id+id+id = non-range pooled sample NT or blank = no test performed wps = weak positive

Microbiology

cell line	1
Bacterial growth	n
Fungal growth	n

Legend: + = agent recovered - = agent not recovered blank = test not performed n = no growth X = Preliminary

DNA Profile
PWS2.9

CellCheck is a comprehensive cell line authentication service that combines interspecies and intraspecies testing to verify the identity of a human cell line.

Human 9 species-specific STR marker profile. Testing for intraspecies contamination and/or misidentification of a human cell line is performed by short tandem repeat (STR) analysis using the Promega CELL IDTM System (8 STR markers + amelogenin) and is used to verify that the genetic profile of the sample matches the known profile of the cell line.

Testing for interspecies contamination is performed by multiplex PCR and identifies DNA from the species of cell lines that are most commonly used in biomedical research. Interspecies contamination check for human, mouse, rat, African green monkey, and Chinese hamster cells.

Comparative analysis (Identity Match): Once the STR genetic profile testing is completed for the sample, this profile is then compared to the publicly available reference profile of the cell line to determine if the sample profile is consistent with the reference profile. If a reference profile has not been established for a cell line, the sample profile is compared to the profiles found in the DSMZ online STR matching analysis database to determine if the sample has a unique profile or is a match to an established profile.

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Certificate of Analysis

IDEXX BioAnalytics Case #: 29919-2018
Case Official: Berg, Heath

ID: 1
Date Completed: 10/26/2018

Specimen Description

ID	Client ID	Cell Line	Species	ATCC #	Other1
1	PWS2.9	PWS2.9 iPSC	Human	fibroblast PW	human iPSC

CellCheck

Species-specific PCR Evaluation

Species	1
mouse	+
rat	-
human	+
Chinese hamster	-
African green monkey	-

Marker Analysis

Marker Name	1	
	Sample Results	PWS2.9
AMEL	X, Y	NA
CSF1PO	11	NA
D13S317	11, 13	NA
D16S539	10, 12	NA
D5S818	12, 13	NA
D7S820	8	NA
TH01	9, 9.3	NA
TPOX	8	NA
vWA	17, 18	NA
Identity Match	N/A, see comments	

Sample ID	Remarks
1	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. However, the genetic profiles for samples 1 (Client ID PWS2.9) and sample 4 (Client ID SCC156.1) are identical confirming these two samples were derived from a common donor source. Without a sample representing the original source material the samples were derived from, it is not possible to make any interpretations in terms of authentication of the samples other than they have the same genetic profile and this profile is a unique profile not found in the current public databases. If these samples were derived from different donors, then the common profile is indicative of cross contamination. However if the samples were derived from the same donor tumor, then the common profile is to be expected and the genetic profile established for these samples should be published in subsequent manuscripts and can be used for future comparisons for these cell lines. This sample also tested positive for mouse origin DNA indicating contamination with mouse origin material. If the sample was propagated using mouse origin materials (feeder cell line, mouse serum, etc.), then this material is the source of the mouse origin DNA. However, if no mouse origin materials were used in the propagation of this sample, then the positive test result may be indicative of contamination with a mouse origin cell line.

IDEXX BioAnalytics

Certificate of Analysis

IDEXX BioAnalytics Case #: 29919-2018
Case Official: Berg, Heath

ID: 4
Date Completed: 10/26/2018

Specimen Description

ID	Client ID	Cell Line	Species	ATCC #	Other1
4	SCC156.1	SCC156.1 iPSC	Human	fibroblast PW	human iPSC

CellCheck

Species-specific PCR Evaluation

Species	4
mouse	+
rat	-
human	+
Chinese hamster	-
African green monkey	-

Marker Analysis

Marker Name	4	
	Sample Results	SCC156.1
AMEL	X, Y	NA
CSF1PO	11	NA
D13S317	11, 13	NA
D16S539	10, 12	NA
D5S818	12, 13	NA
D7S820	8	NA
TH01	9, 9.3	NA
TPOX	8	NA
vWA	17, 18	NA
Identity Match	N/A, see comments	

Sample ID	Remarks
4	NA in the table indicates profile data is not available for comparison purposes for this sample. The genetic profile for the sample was compared to the cell line genetic profiles available in the DSMZ STR database and did not match any other reported profiles in the DSMZ database. However, the genetic profiles for samples 1 (Client ID PWS2.9) and sample 4 (Client ID SCC156.1) are identical confirming these two samples were derived from a common donor source. Without a sample representing the original source material the samples were derived from, it is not possible to make any interpretations in terms of authentication of the samples other than they have the same genetic profile and this profile is a unique profile not found in the current public databases. If these samples were derived from different donors, then the common profile is indicative of cross contamination. However if the samples were derived from the same donor tumor, then the common profile is to be expected and the genetic profile established for these samples should be published in subsequent manuscripts and can be used for future comparisons for these cell lines. This sample also tested positive for mouse origin DNA indicating contamination with mouse origin material. If the sample was propagated using mouse origin materials (feeder cell line, mouse serum, etc.), then this material is the source of the mouse origin DNA. However, if no mouse origin materials were used in the propagation of this sample, then the positive test result may be indicative of contamination with a mouse origin cell line.

UConn Stem Cell Core
Email: ucscicore@uchc.edu
Website: www.health.uconn.edu/stem-cell-core