Validation Package

Product Type	Cell Line
Name	PWS UPD1.2
Cell Type	Human induced pluripotent stem cell (iPSC)
Donor Gender	Female
Source Tissue	Fibroblasts
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	 Langouët M, Glatt-Deeley HR, Chung MS, Dupont-Thibert CM, Mathieux M, Banda EC, Stoddard CE, Crandall L, Lalande M; Zinc finger protein 274 regulates imprinted expression of transcripts in Prader-Willi syndrome neurons, *Human Molecular Genetics*, 2018;27(3):505-515
Biosafety Level	2
Thaw Recommendation	Thaw 1 vial into 1 well of a 6 well plate
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	28, these cells were cultured for 28 passages prior to freeze
Date Vialed	September 6, 2018
Cryopreservation	Bambanker (Wako Chemicals Cat. No, 302-14681) 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

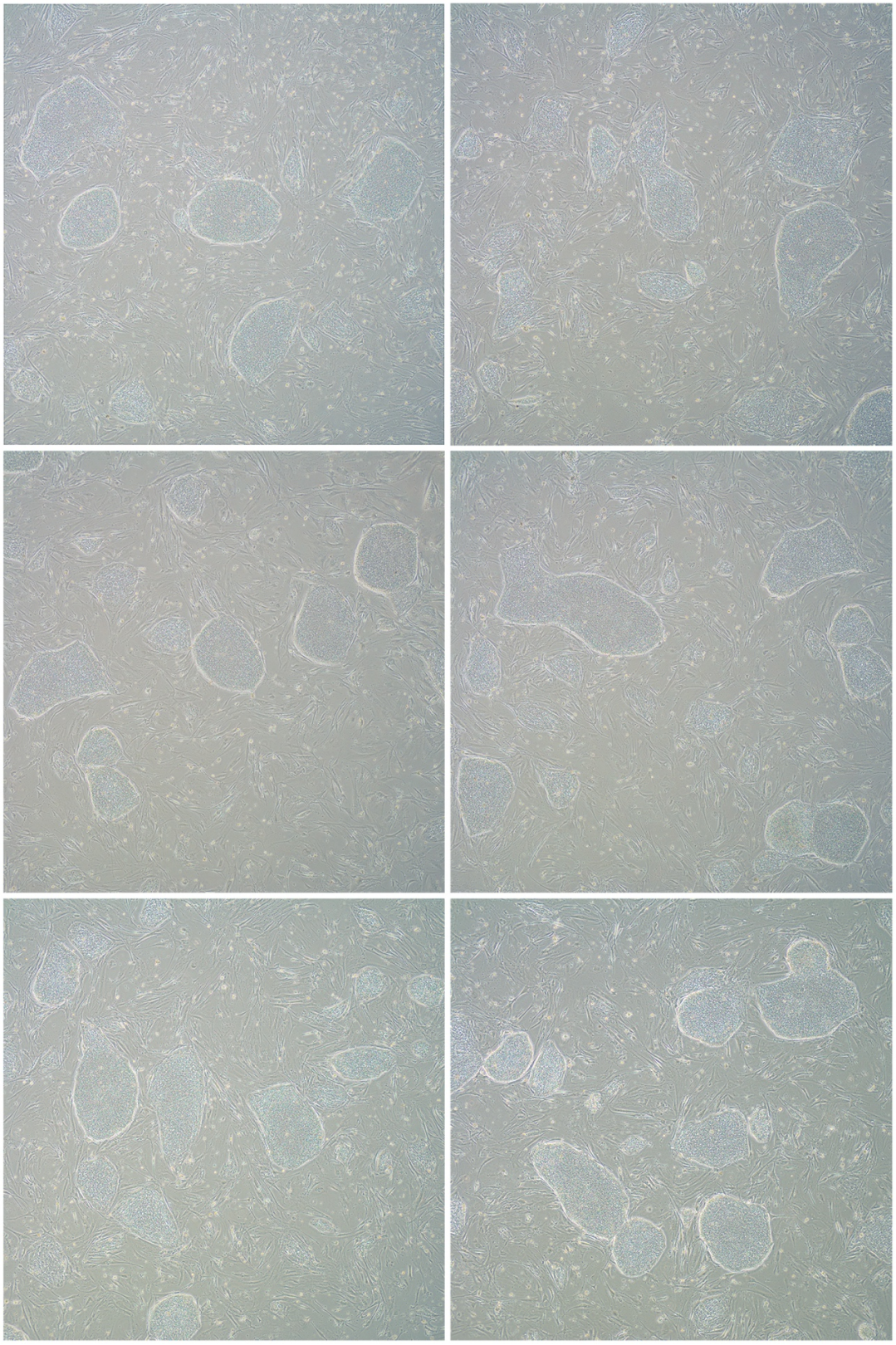
Culture Characteristics:

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.

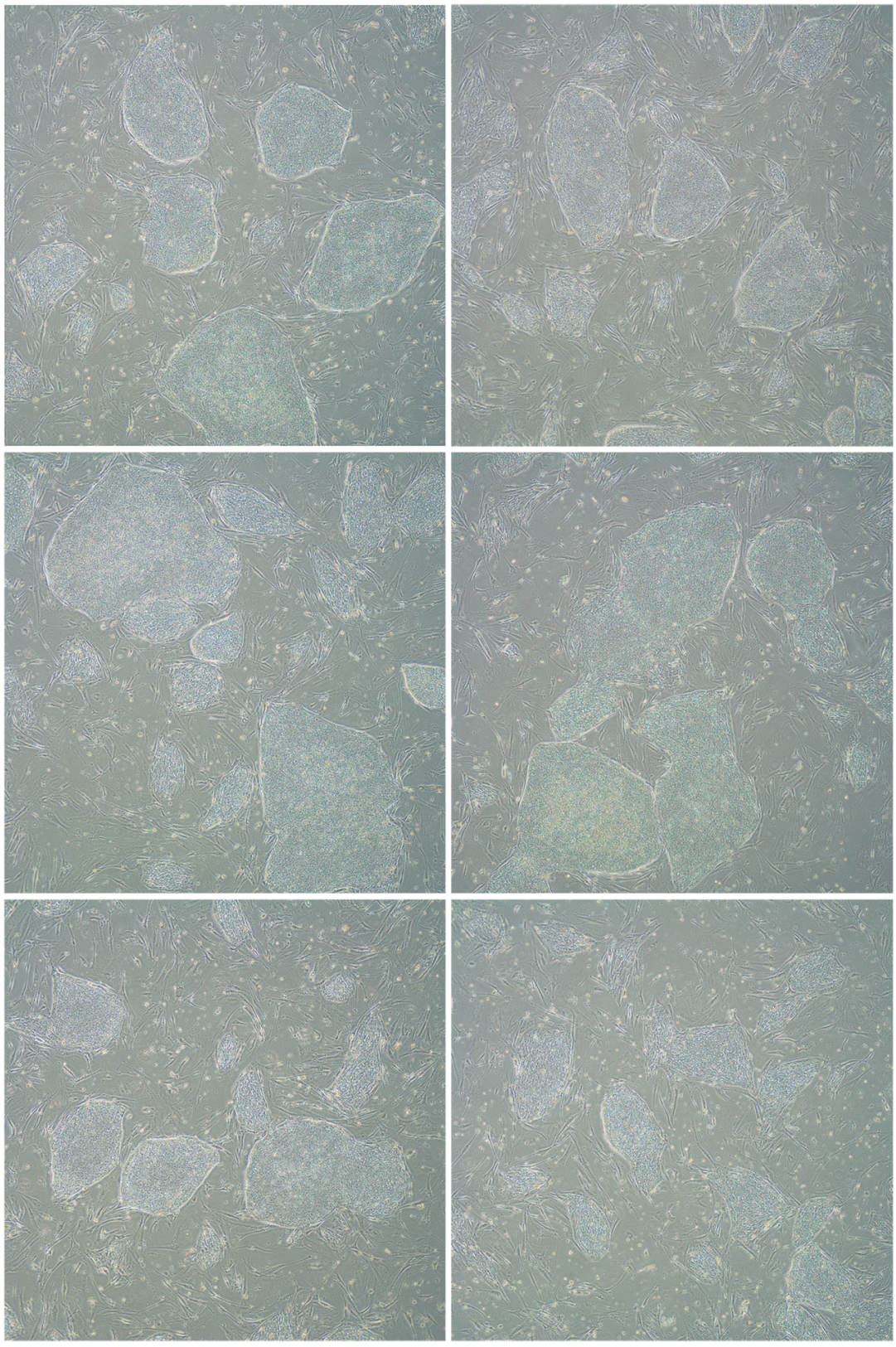
Recovery: Roll the vial between gloved hands for 3-5 seconds to remove the frost. Immerse the vial into a 37°C water bath. Swirl the vial gently and observe the progress of the thaw. When only a small ice crystal remains, wipe the outside of the vial with 70% ethanol. In a sterile biological safety cabinet, 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.

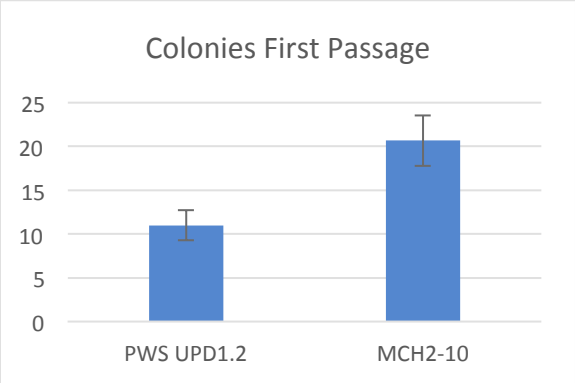
PWS-UPD1.2 before cryopreservation *Phase images*

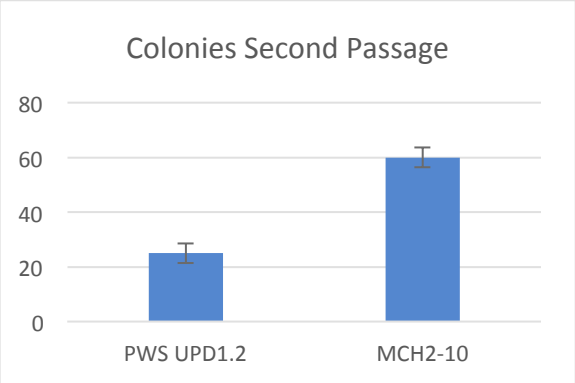


PWS-UPD1.2 Day 5 after recovery from cryopreservation *Phase images*

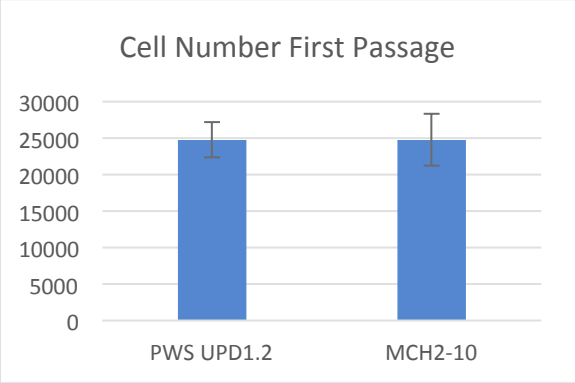


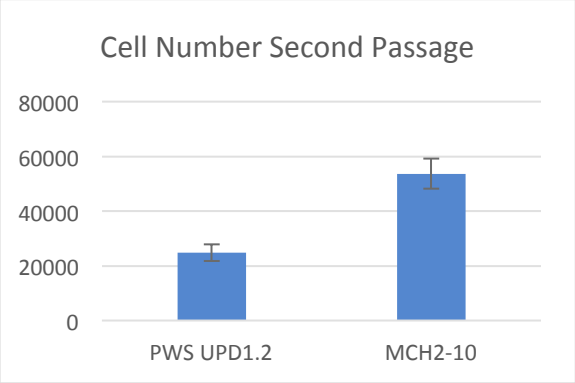
Growth Curve: colonies in one well of a 6 well plate in triplicate wells, 1500 cells plated to each for both test and control. MCH2-10 was used as a control.



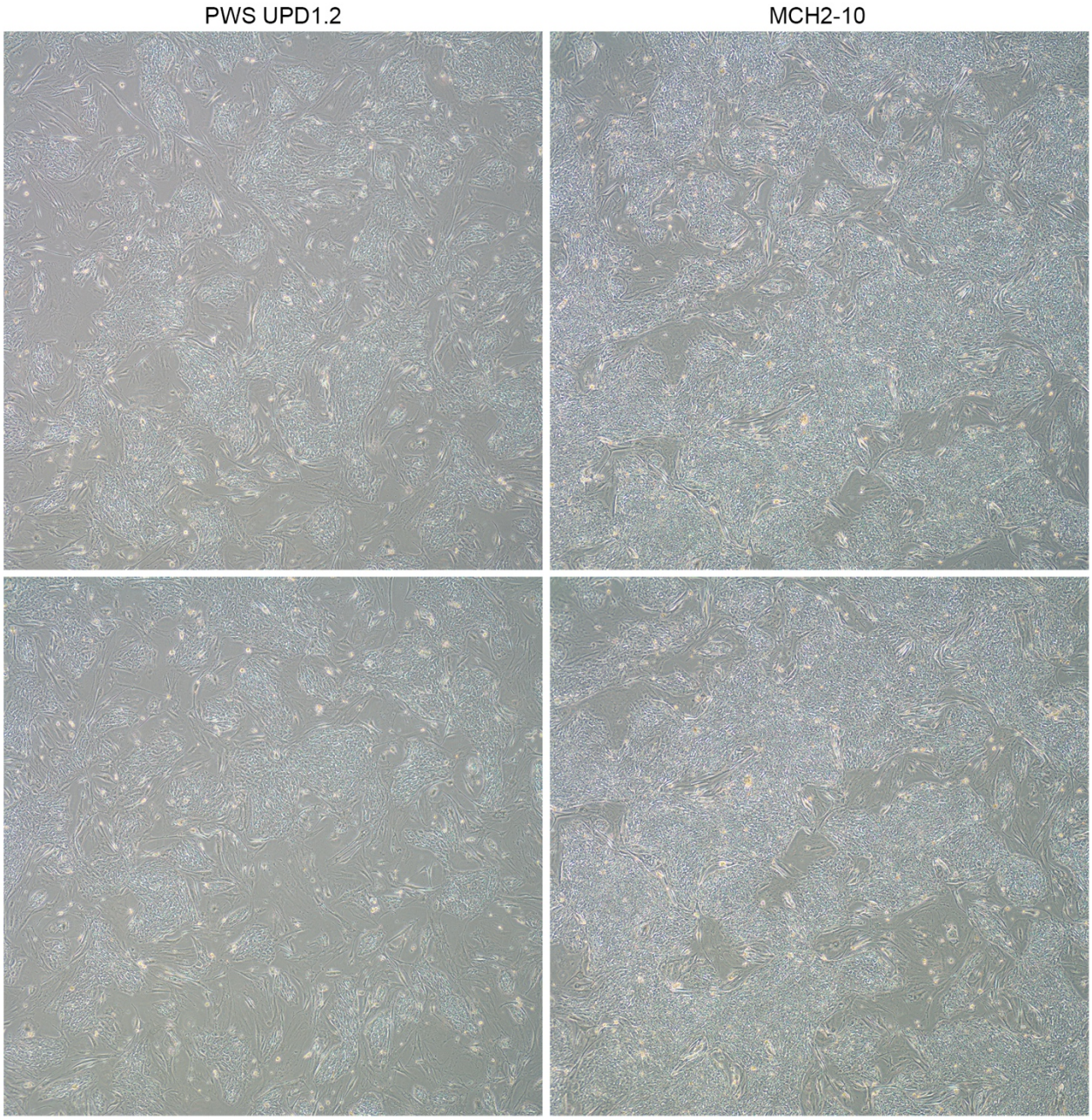


Culture Characteristics - Growth Curve: cell number in one well of a 6 well plate





Growth Curve: Phase images from day 3 after passage; third passage of assay.

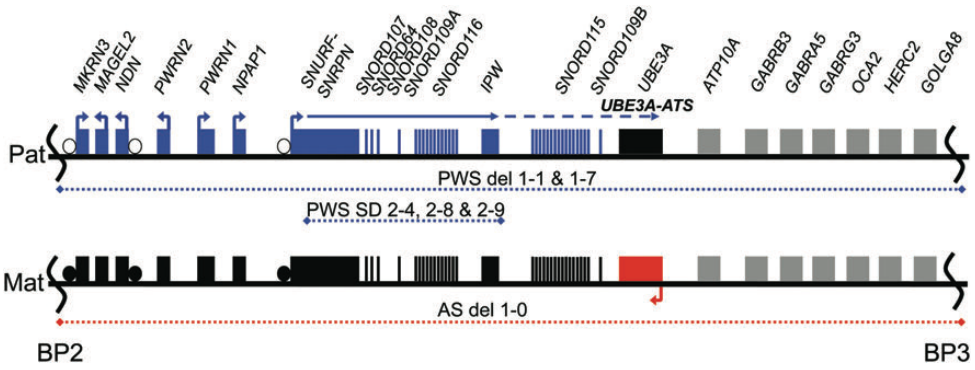


Gene Expression - PWS chromosome 15q11 – q13 region genes (qRT-PCR)

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.

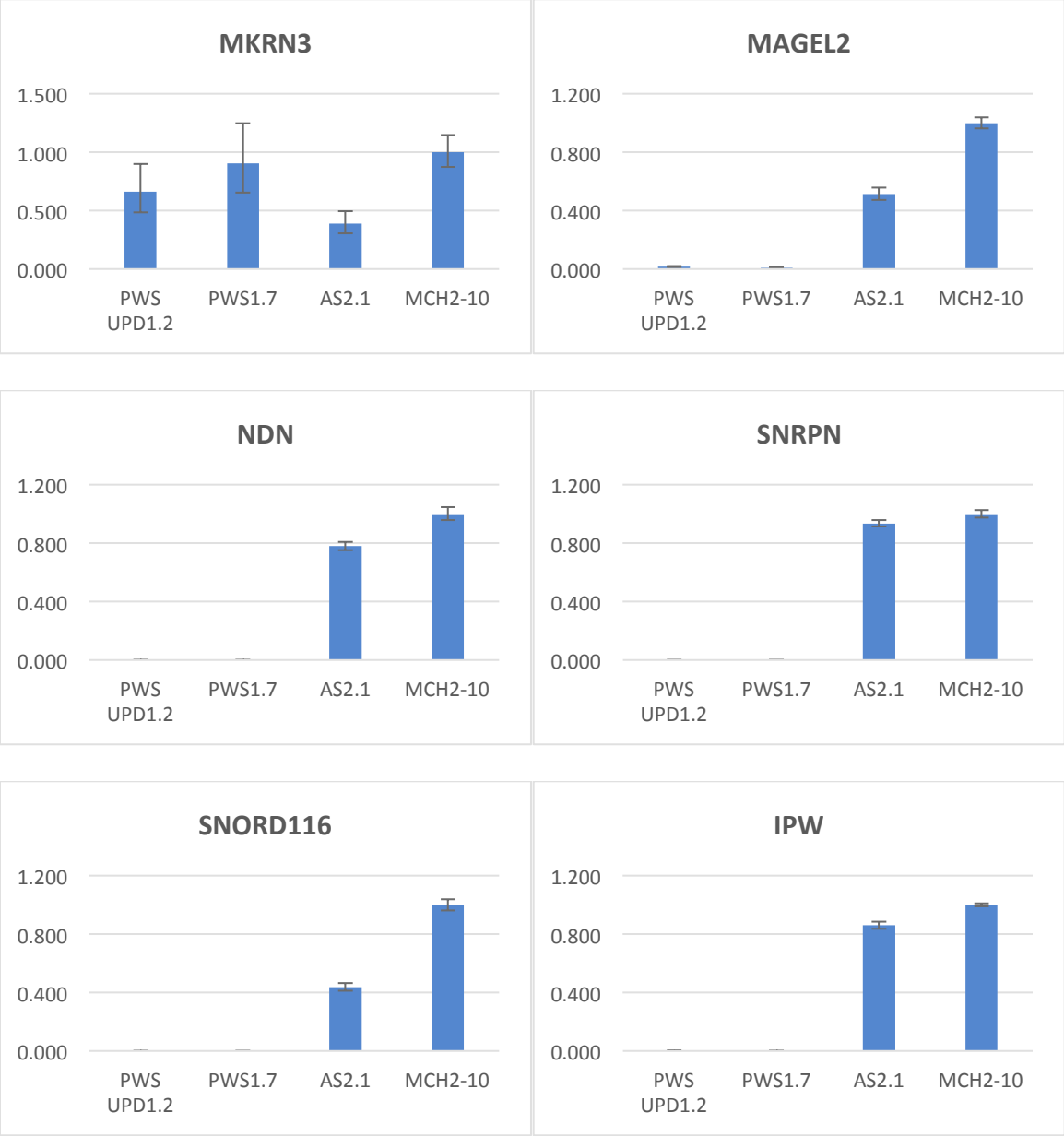
TaqMan Gene Expression assays are used for quantitative real-time PCR analysis of gene expression and consist of a pair of unlabeled PCR primers and a TaqMan probe with a dye label (FAM) on the 5' end and a minor groove binder (MGB) and non-fluorescent quencher (NFQ) on the 3' end.

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

Gene expression (Prader Willi) analysis of MKRN3, MAGEL2, NDN, SNRPN, SNORD116, IPW. GADPH was used as an endogenous control and data were normalized to MCH2-10.



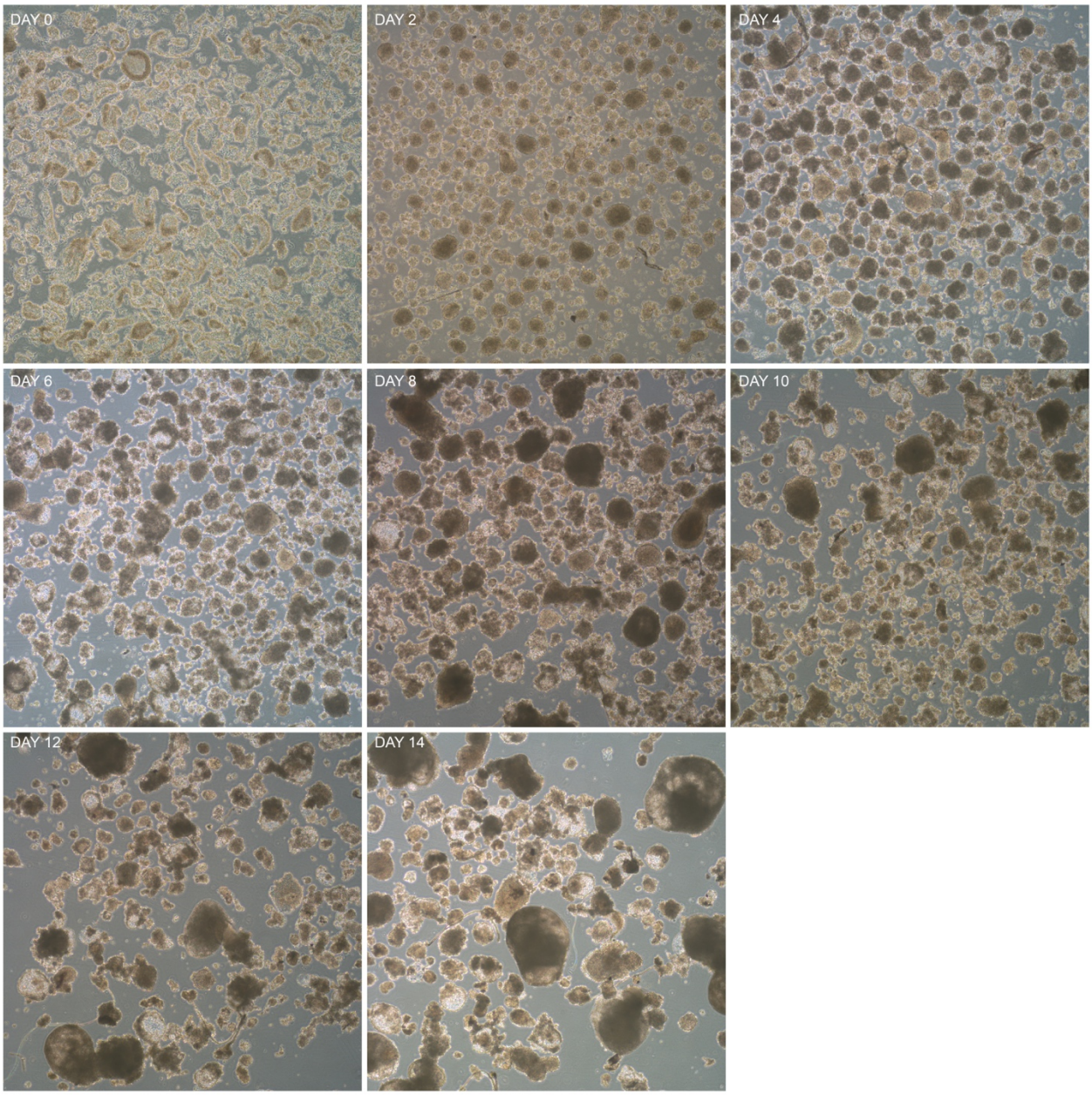
AS2.1 is an iPSC line generated from a donor with Angelman Syndrome*
PWS1.7 is an iPSC line generated from a donor with Prader Willi Syndrome (Large Deletion)*
MCH2-10 is an iPSC generated from an unaffected donor*

*Chamberlain SJ, Chen PF, Ng KY, et al. Induced pluripotent stem cell models of the genomic imprinting disorders Angelman and Prader-Willi syndromes. *Proc Natl Acad Sci U S A*. 2010;107(41):17668-73.

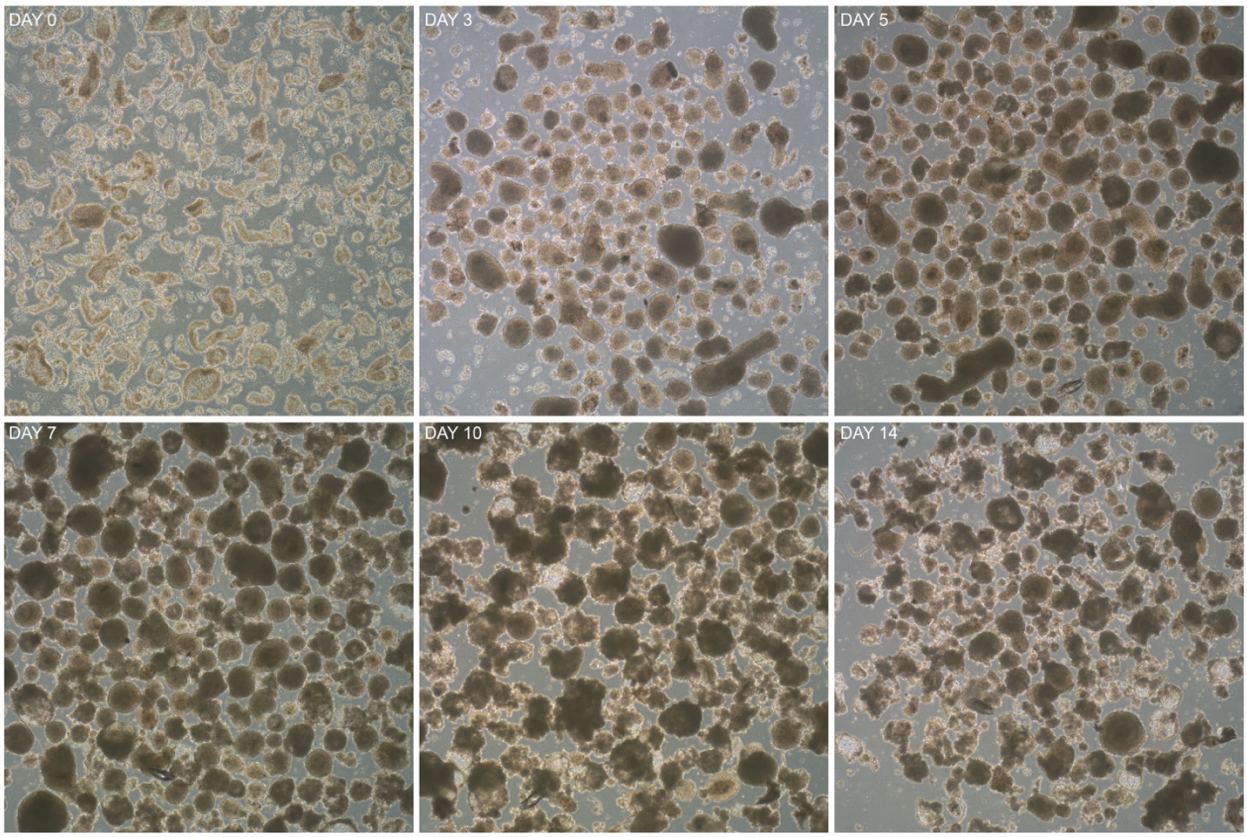
Embryoid bodies (EB) are the three-dimensional aggregates formed in suspension by the iPSCs. Embryoid Body culture is used to examine the differentiation potential of the iPSCs.

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.

PWS UPD1.2 embryoid body formation (1 of 2)
Phase Images Day 0 to day 14



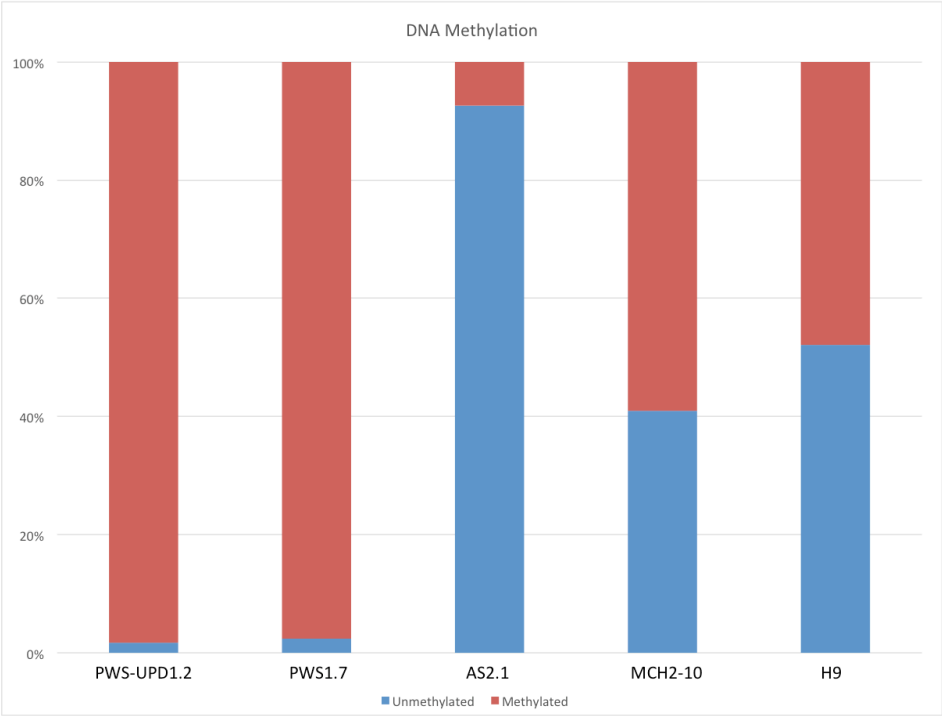
PWS UPD1.2 embryoid body formation (2 of 2)
Phase Images Day 0 to day 14



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.

DNA Methylation analysis of PWS-IC using a methylation-sensitive restriction endonuclease quantitative PCR assay.

Cell Line	Unmethylated	Methylated
PWS UPD1.2	1.71%	98.29%
PWS1.7	2.39%	97.61%
AS2.1	92.61%	7.39%
MCH2-10	40.96%	59.04%
H9	52.11%	47.89%



AS2.1 is an iPSC line generated from a donor with Angelman Syndrome*
PWS1.7 is an iPSC line generated from a donor with Prader Willi Syndrome (Large Deletion)*
MCH2-10 is an iPSC generated from an unaffected donor*
H9 hESC is from WiCell Research Institute, Madison, WI
*Chamberlain SJ, Chen PF, Ng KY, et al. Induced pluripotent stem cell models of the genomic imprinting disorders Angelman and Prader-Willi syndromes. *Proc Natl Acad Sci U S A*. 2010;107(41):17668-73.

Scorecard
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.

applied biosystems
by Thermo Fisher Scientific

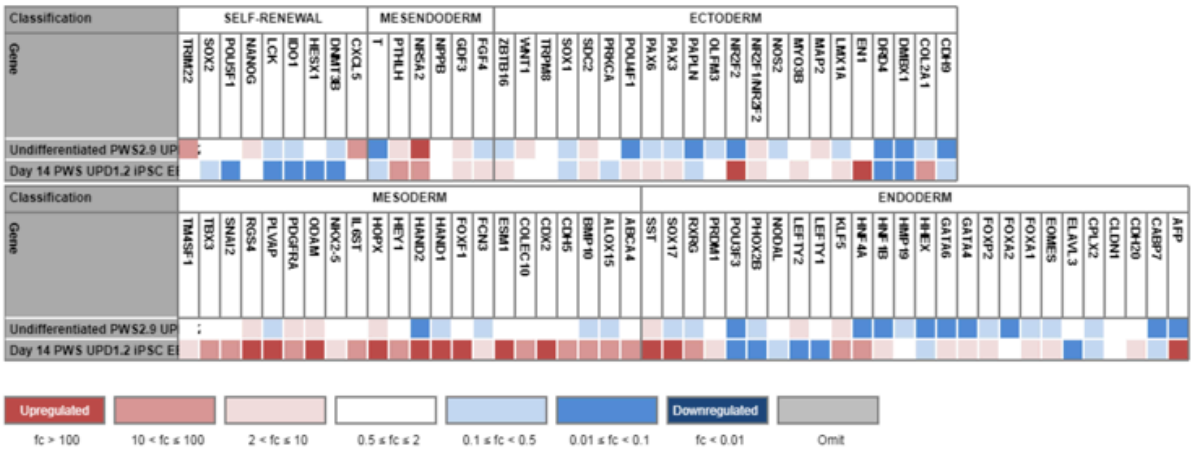
hPSC Scorecard™ Data Analysis Report

Scorecard Results

PWS UPD1.2							
Undifferentiated PWS UPD1.2				Day 14 PWS UPD1.2 EB			
Self-renew	Ecto	Meso	Endo	Self-renew	Ecto	Meso	Endo
⊕	⊖	⊖	⊖	⊖	⊖	⊕	⊕

Expression Plot

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



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www.thermofisher.com/scorecard

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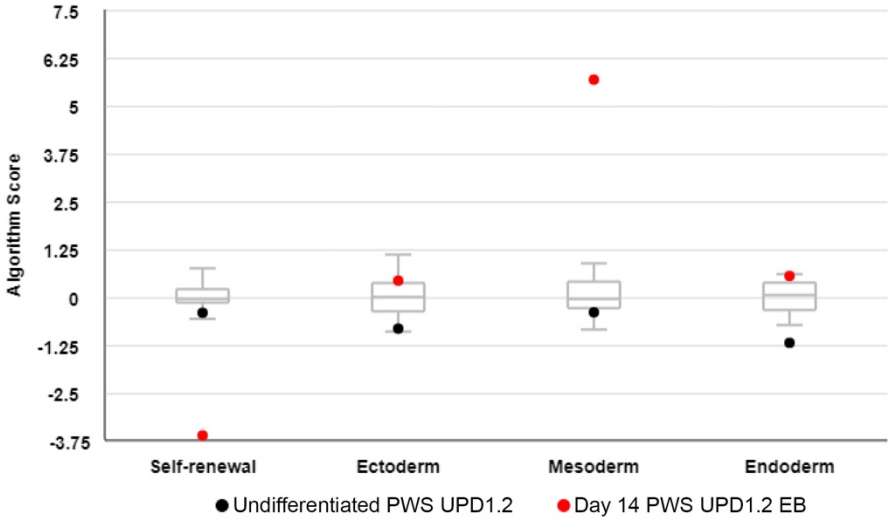
hPSC Scorecard™ Data Analysis Report

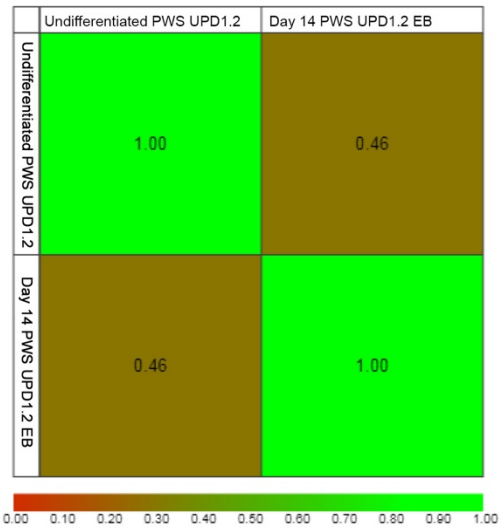
Scores Box Plot

Sample scores are plotted in color. The range of scores for the undifferentiated reference set is indicated by the gray box plc



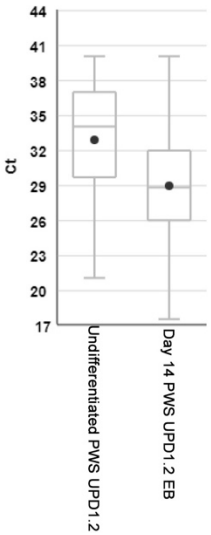
Correlation Plot

Pair-wise comparison of the 96 Ct or delta Ct values for all selected samples in the project



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



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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Chromosome Core
Case Report

Sample ID:	**CC18-30**
Sample Name:	**PWS_UPD 1.2**
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-
Genome build name: GRCh37	Ensembl version: 74
GTC file:	202917700009_R01C01.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)	0.18
Median Call Rate: genotype calling performance estimate (>0.98)	1
Sample sex:	Female

ISCN	Type	Chromosome	End
15q21.1-15q21.1	**LOH**	15	48,957,597
Loss of heterozygosity on long arm chromosome 15 of 0.58 Mb (<1 Mb) ; overlaps 6 HGNC and 4 OMIM gene(s).			
15q26.2-15q26.3	**LOH**	15	102,376,655
Loss of heterozygosity on long arm chromosome 15 of 6.99 Mb ; overlaps 54 HGNC and 16 OMIM gene(s).			
17q21.31-17q25.3	**LOH**	17	81,060,040
Loss of heterozygosity on long arm chromosome 17 of 39.614896 Mb ; overlaps 698 HGNC and 383 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* . Chromosome 15 was evaluated separately. ***Please note:*** loss of heterozygosity was NOT identified for the entire length of the long arm of chromosome 15. The PWS critical region (15q11.2-q13) is reported as heterozygous. No deletions or duplications were detected.

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.

Judith D Brown

Specimen Description

ID	Client ID	Cell Line	Species	cells	Other1
2	PWS UPD1.2	PWS UPD1.2 iPSC	Human	PWS UPD U	human iPSC

Date Completed: 10/26/2018

IDEXX BioAnalytics Case #: 29919-2018

IDEXX BioAnalytics

IDEXX

Certificate of Analysis

PCR Evaluation

cells	2
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

Marker Analysis

Marker Name	2 PWS UPD1.2
AMEL	X
CSF1PO	12
D13S317	9, 13
D16S539	10, 12
D5S818	11, 12
D7S820	8, 9
TH01	8
TPOX	8, 9
vWA	16, 18

Specimen Description

ID	Client ID	Cell Line	Species	ATCC #	Other1
3	PWS UPD U	PWS UPD U of Flo	Human	PWS UPD U	human derm

Microbiology

cell line	2
Bacterial growth	n
Fungal growth	n

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

Marker Analysis

Marker Name	3 PWS UPD
AMEL	X
CSF1PO	12
D13S317	9, 13
D16S539	10, 12
D5S818	11, 12
D7S820	8, 9
TH01	8
TPOX	8, 9
vWA	16, 18

CellCheck

Species-specific PCR Evaluation

Species	2
mouse	+
rat	-
human	+
Chinese hamster	-
African green monkey	-

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