SHP-2
Certificate of analysis

Characterized clone :	SHP-2 clone 5
Description :	Human induced pluripotent stem cells
Legal status :	For research purposes only.
Researcher :	Dr Yvan Torrente
Institution :	Università degli Studi di Milano
Parental cells and descripton :	SHP β sarco mut esone 3 SANCHEZ C ; PBMC
Pathology :	Autosomal recessive limb-girdle muscular dystrophy-4
Donor information :	Male
Reprogramming method :	Sendai viral expression of Oct4, Sox2, Klf4 and c-Myc genes
Reprogramming efficiency :	0.55 % (figure 1)
Thawing recommendation	One cryovial in two 35mm petri dishes + 10µM Rock Inhibitor Y-27632 or CEPT[1]
Culture conditions :	Media : mTeSR™ Plus (StemCell Technologies; 05826) Matrix : Matrigel™ hESC-Qualified Matrix (Corning; 08-774-552) Passage : EDTA 0.5mM (Invitrogen; AM9260G) Environment : 37°C, 5% CO2, >95% RH

1. Chen Y, Tristan CA, Chen L, Jovanovic VM, Malley C, Chu PH, et al. A versatile polypharmacology platform promotes cytoprotection and viability of human pluripotent and differentiated cells. Nat Methods. 2021.

Table 1: SHP-2 clone 5 characterization

Test description	Method	Test specification	Results
Post-thaw cell viability	Microscopic observations	≥50% confluency 3-4 days post-thaw	Pass (Figure 2)
Stem cells protein markers expression	Immunofluorescence	Majority of cells expressing intracellular markers (NANOG, POU5F1) and surface markers (TRA-1-81, SSEA4)	Pass (Figure 3)
Stem cells protein markers quantification[1]	Flow cytometry	Surface markers SSEA4 ≥ 70% TRA-1-60 ≥ 70% TRA-1-81 ≥ 70% Intracellular markers NANOG ≥ 70% SOX2 ≥ 70% POU5F1 ≥ 70%	Pass (Figure 4)
Stem cells genes expression quantification	RT-qPCR	Positive expression of the following genes : *DNMT3B*, *TERT*, *ZFP42*, *TDGF1*, *UTF1*, *LIN28A*, *GDF3*, *DPP5A* et *FOXD3*.	Pass (Figure 5)
Three germ layers differentiation	Directed differentiation[2] (Flow cytometry)	Overexpression of proteins associated with the three embryonic layers	Pass (Figure 6)
	Spontaneous differentiation (ScoreCard™ Panel[3])	Overexpression of genes associated with the three embryonic layers	Réussi (Figure 7)
Mycoplasma[4]	MycoStrip™	Negative for mycoplasma	Pass
Detection of Sendai virus genome and transgene	qRT-PCR	No detection of SEV genome or transgene	Pass*
Identity match	STR	Match parental cell line	Pass (Table 2)
Karyotype	Karyostat+™	No gain or loss	Normal (Figure 8)

1. Baghbaderani, BA, *et al*., Detailed Characterization of Human Induced Pluripotent Stem Cells Manufactured for Therapeutic Applications, Stem Cell Rev and Rep, (2016), 12:394–420.
2. StemMACS™ Trilineage Differentiation kit, Miltenyi Biotec; cat# 130-115-660.
3. Tsanlov, Am, *et al*., A qPCR scorecard quantifies the differentiation potential of human pluripotent stem cells, Nat Biotechnol, (2015), 33:1182-92. (TaqMan ScoreCard™ Panel, ThermoFisher, cat# A15870)
4. Venor®GeM Mycoplasma OneStep, Cedarlane, cat# 11-8100

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Alkaline Phospatase

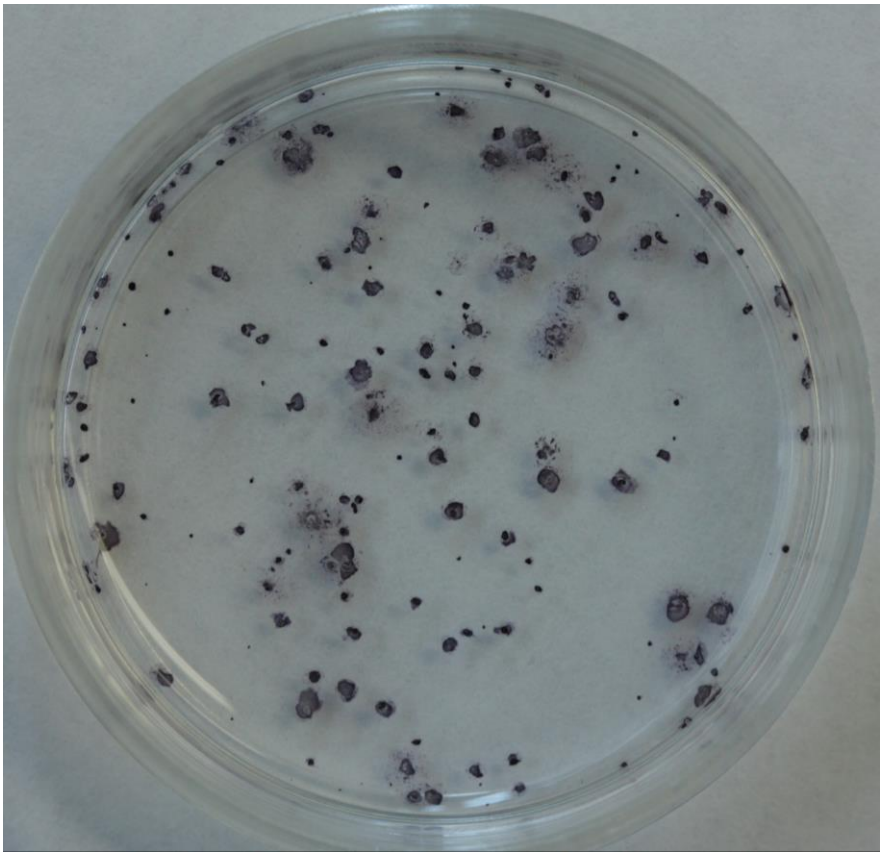


Figure 1 : Fixation and alkaline phosphatase staining of iPSC clones obtained during Sendai virus reprogramming. A total of 30 000 transduced cells were seeded in a 35mm. Alkaline phophatase positive clones were counted with ImageJ.

Microscopic observation

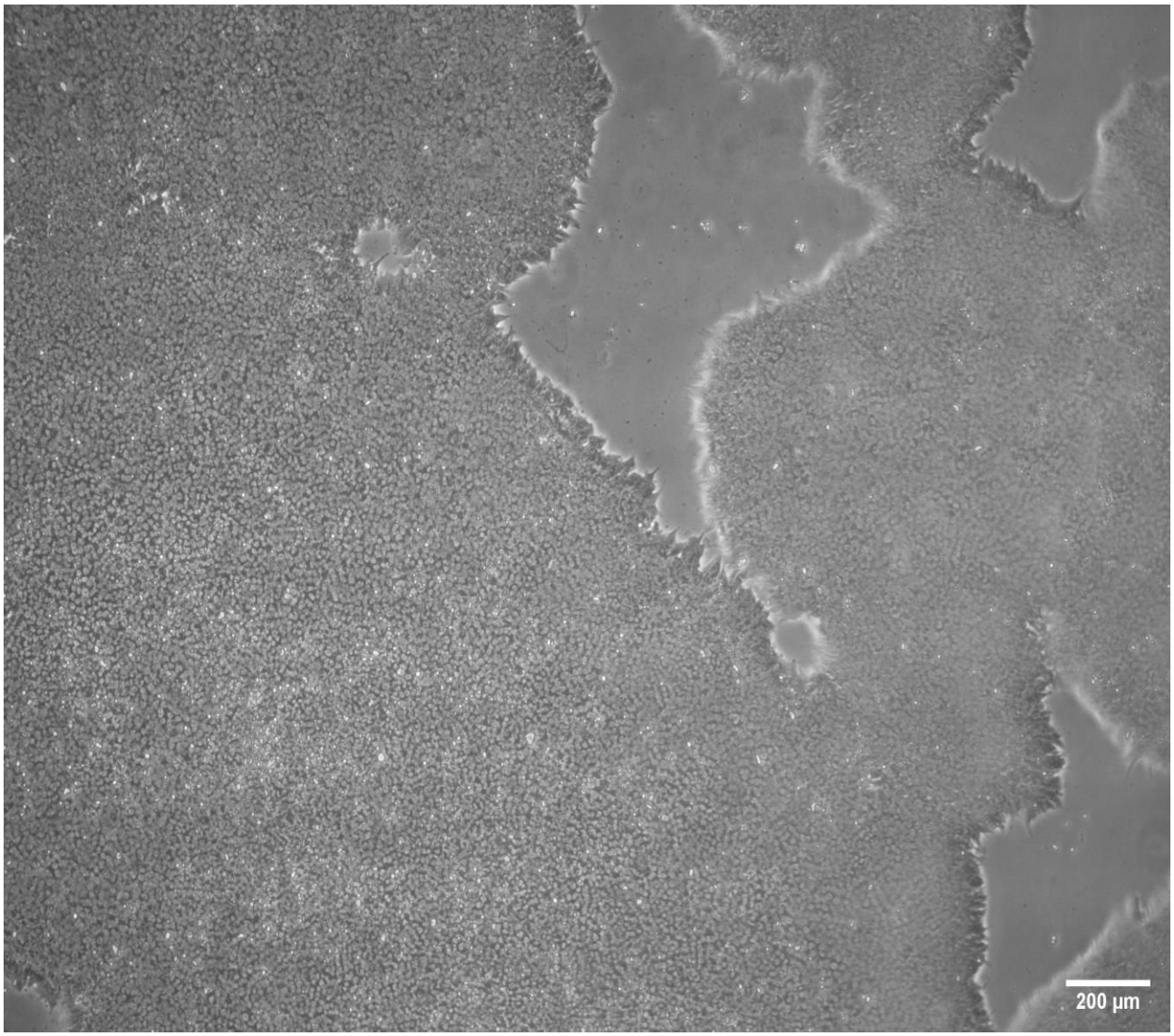
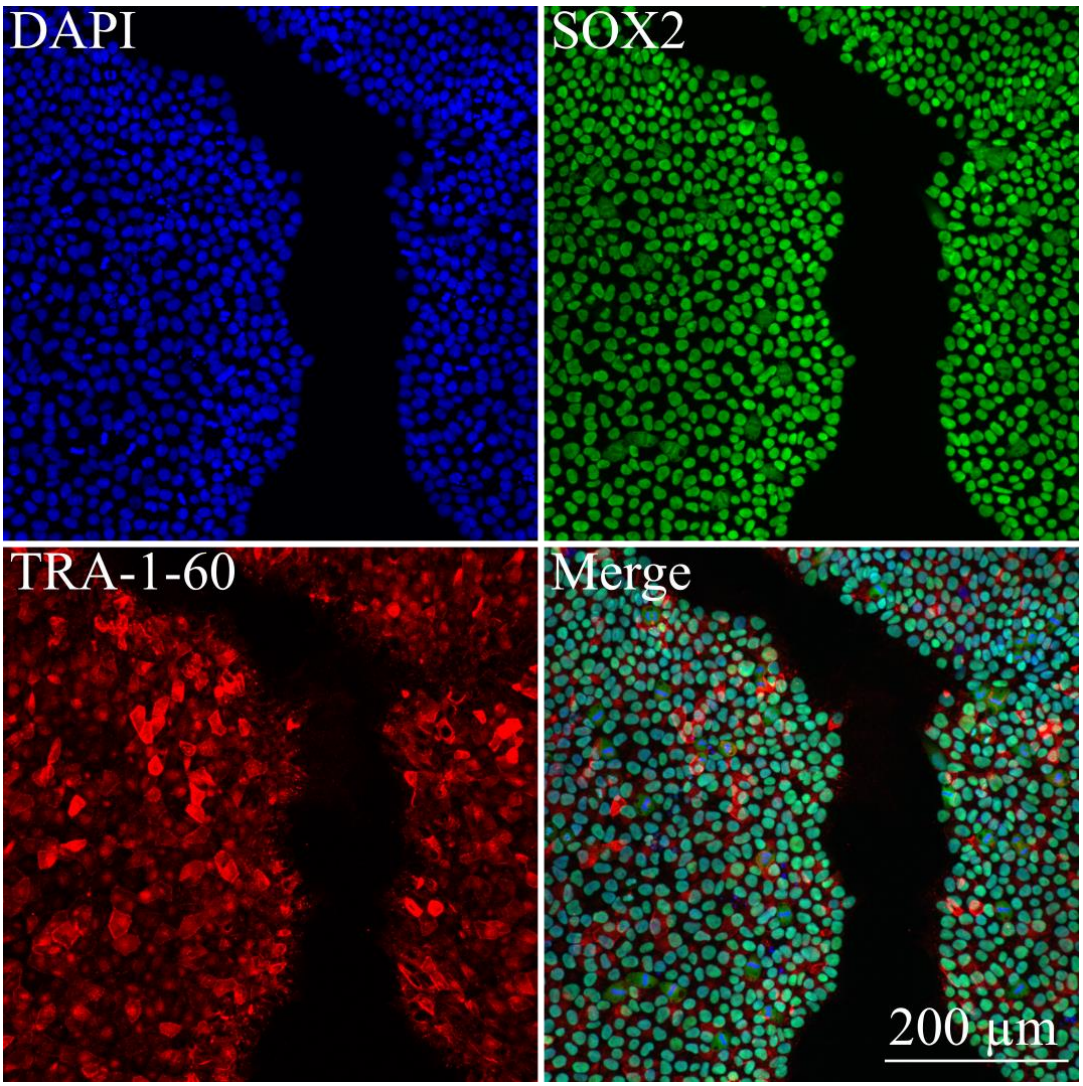


Figure 2 : iPSC SHP-2 clone 5 morphology, 4 days post-thaw.

Immunofluorescence



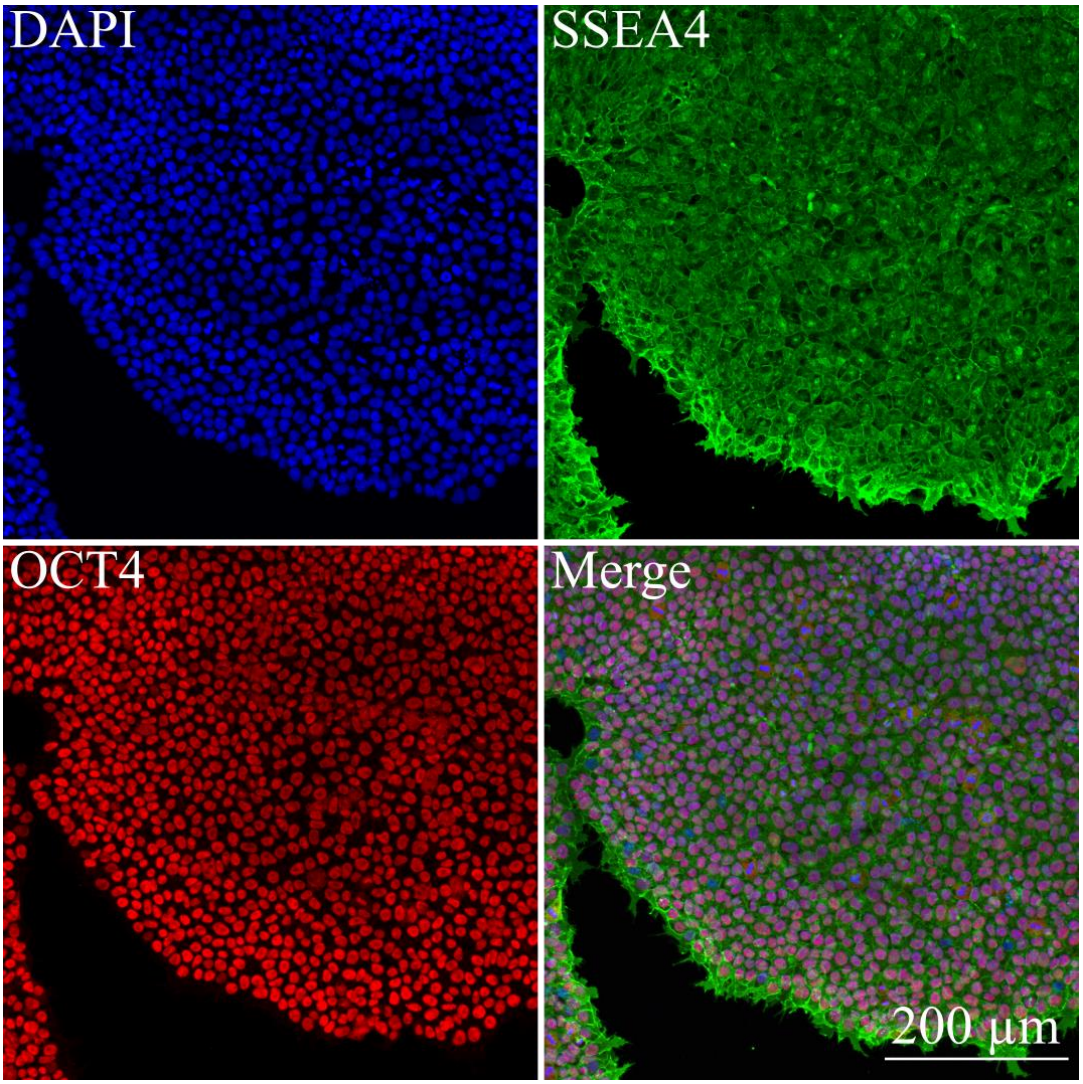


Figure 3 : Immunofluorescence with four self-renewal markers (green and red). The nucleus were stained with DAPI (blue).

Flow cytometry

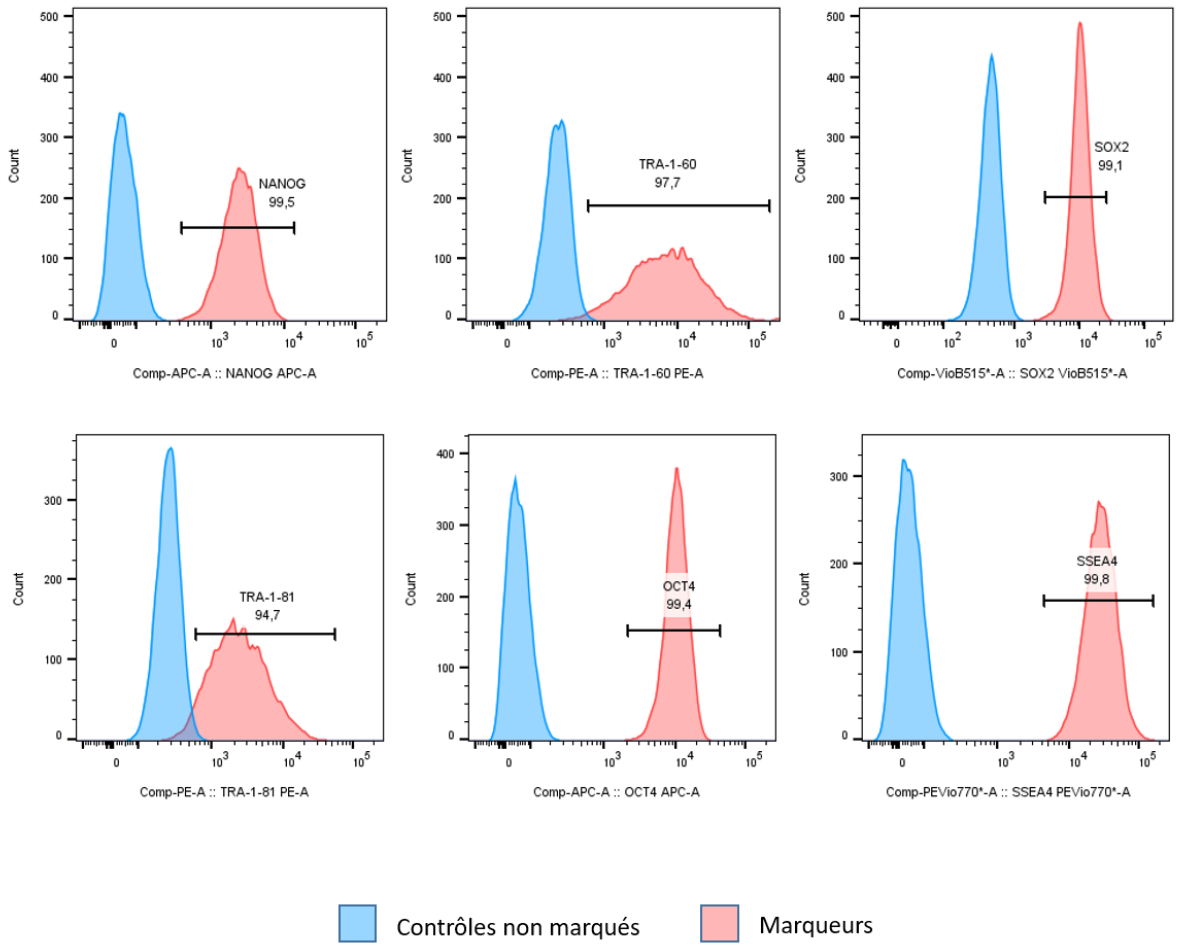


Figure 4 : Expression of self-renewal markers of iPSC SHP-2 clone 5, by flox cytometry.

RT-qPCR

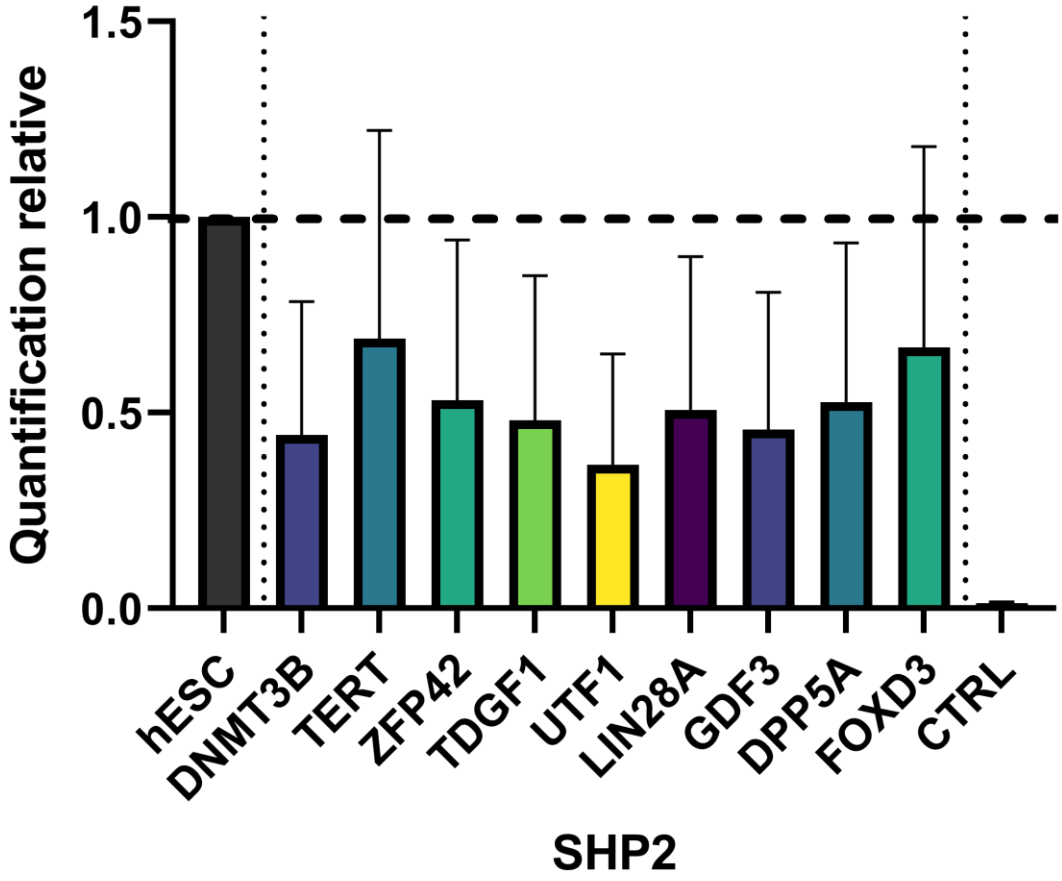


Figure 5 : Relative expression of stem cells specific genes based on H1 embryonic stem cells. The results were normalized with three reference genes : *GAPDH, YWHAZ* et *C1orf43*. CTRL=parental cells.

Trilineage directed differentiation

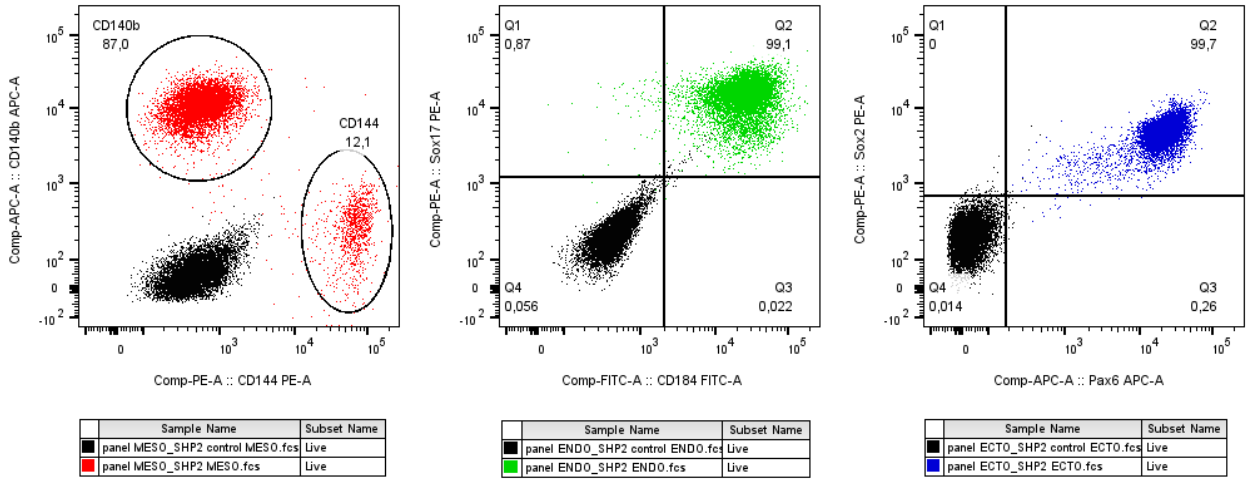
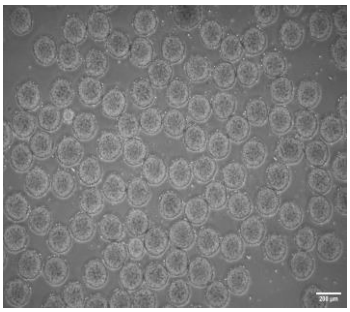


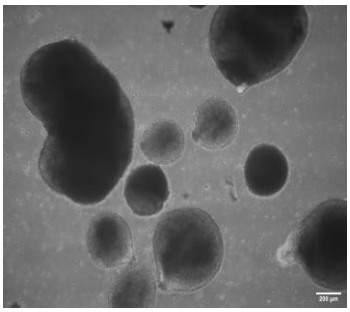
Figure 6 : Flow cytometry of iPSC SHP-2 clone 5 after 7 days of 2D directed trilineage differentiation

hPSC Scorecard™ Panel

a)



b)



c)

Scores are a statistical comparison of the expression profile of the sample to that of the undifferentiated reference set

Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
EB SHP-2	-5.92	1.43	0.91	0.37

Gene expression relative to the reference standard

Upregulated						Downregulated
x > 1.5	1.0 < x ≤ 1.5	0.5 < x ≤ 1.0	-0.5 ≤ x ≤ 0.5	-1.0 ≤ x < -0.5	-1.5 ≤ x < -1.0	x < -1.5

d)

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

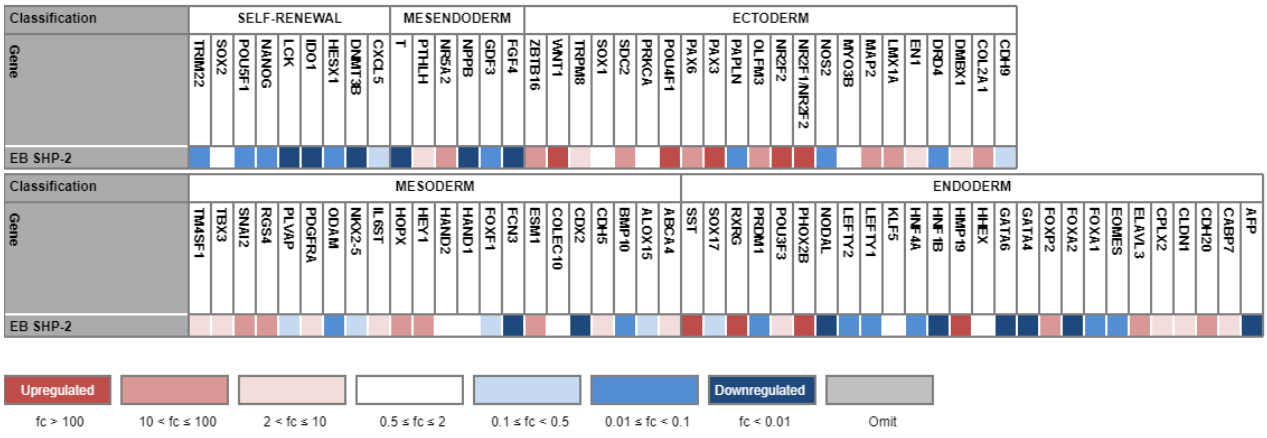


Figure 7 : Embryonic bodies at 2 days (a) and 21 days (b) of spontaneous differentiation in suspension. Score (c) and gene expression (d) of genes from the three embryonic layers according to a group of 23 undifferentiated pluripotent stem cell lines.

Short Tandem Repeat (STR) analysis

Table 2 : STR analysis provided by Genome Quebec.

	PBMC SHP-2		**iPSC SHP-2 clone 5**	
	X	Y	X	Y
	11	12	11	12
	11	13	11	13
	11	12	11	12
	28		28	
	11	12	11	12
	7	11	7	11
	7		7	
	8	9	8	9
	15	16	15	16

Karyostat+

KaryoStat+ Results: KS-18089 (SHP-2)

1. KaryoStat+ analysis of this sample revealed that it originated from a male individual.
2. No chromosomal aberrations were found when comparing against the reference dataset.

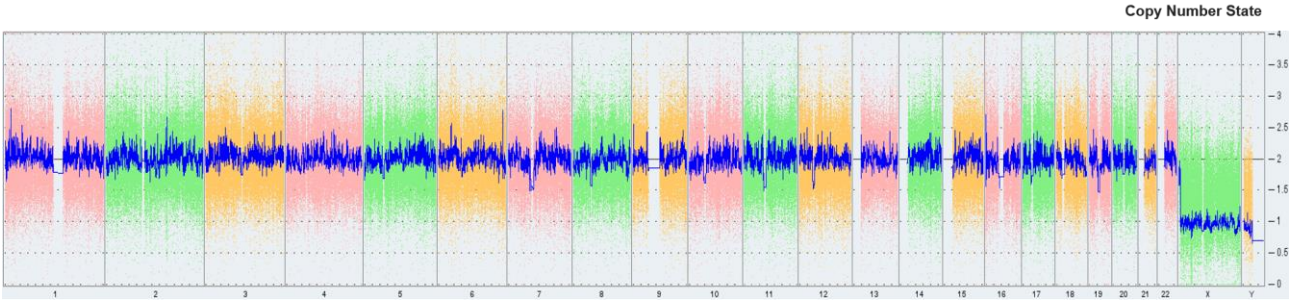


Figure 1: Whole genome view. The whole genome view displays all somatic and sex chromosomes in one frame with high level copy number. The smooth signal plot (right y-axis) is the smoothing of the log2 ratios which depict the signal intensities of probes on the microarray. A value of 2 represents a normal copy number state (CN = 2). A value of 3 represents chromosomal gain (CN = 3). A value of 1 represents a chromosomal loss (CN = 1). The pink, green and yellow colors indicate the raw signal for each individual chromosome probe, while the blue signal represents the normalized probe signal which is used to identify copy number and aberrations (if any). Aberrations when present are indicated by red arrows.

Disclaimer: This assay was conducted solely for the listed investigator/institution. The results of this assay are for research use only.

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Figure 8 : Karyotype of iPSC SHP-2 clone 5 at passage 15. Resolution > 1Mb.

Notification:

Cells distributed by the *Plateforme de production de cellules souches du CRCHU de Québec* are destined to fundamental research use only. They are not destined for use on human.

Adequate safety measure must be followed to work with iPSC. The end user is solely responsible for ensuring that these cells are handled and store appropriately. The *Plateforme de production de cellules souches du CRCHU de Québec* is not responsible of the damages or injuries that could result from the use of these cells.

Approved by :

Laurie Martineau, MSc
Plaform manager

2023-11-28
Date

François Gros-Louis, Ph.D.
Director

2023-11-28
Date