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ARSM19061223
Certificate of analysis

Characterized clone :	ARSM19061223 clone 1
Description :	Human induced pluripotent stem cells
Legal status :	For research purposes only.
Researcher :	Dr François Gros-Louis
Institution :	CMDGT/LOEX
Parental cells and descripton :	ARSM19061223 ; PBMC
Pathology :	Ataxie récessive spastique de Charlevoix-Saguenay (ARSACS)
Donor information :	Male
Reprogramming method :	Sendai viral expression of Oct4, Sox2, Klf4 and c-Myc genes
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 : Geltrex™ LDEV-Free hESC-Qualified (ThermoFisher; A1413302) Passage : EDTA 0.5mM (Invitrogen; AM9260G) Environment : 37°C, 5% CO_2, >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.

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Table 1: ARSM19061223 clone 1 characterization

Test description	Method	Test specification	Results
Reprogramming efficiency	Alkaline Phosphatase (AP) assay	AP staining 21 days post-transduction with Sendai virus	**0.124% (Figure 1)**
Post-thaw cell viability	Microscopic observations	≥50% confluency 3-4 days post-thaw	**Passed (Figure 2)**
Stem cells protein markers expression	Immunofluorescence	Majority of cells expressing intracellular markers (SOX2 and OCT4) and surface markers (TRA-1-60 and SSEA4)	**Passed (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% OCT4 ≥ 70%	**Passed (Figure 4)**
Stem cells genes expression quantification	RT-qPCR	Positive expression of the following genes : *DNMT3B, TERT, ZFP42, TDGF1, UTF1, LIN28A, GDF3, DPP5A, FOXD3 and NANOG.*	**Passed (Figure 5)**
Three germ layers differentiation	Spontaneous differentiation (ScoreCard™ Panel[2])	Overexpression of genes associated with the three embryonic layers	**Passed (Figure 6)**
Mycoplasma[3]	MycoStrip™	Negative for mycoplasma	**Passed**
etection of Sendai virus genome and transgenes	RT-qPCR	Detection of SEV genome and transgenes by RT-qPCR	**Passed (Figure 7)**
Identity match	STR	Match parental cell line	**N.A. (Table 2)**
Karyotype	Karyostat+™	No chromosomal aberration was detected	**Normal (Figure 8)**
	iCS-digital™ PSC[4] (ddPCR)	No iPSC recurrent chromosomal aberration was detected	**Normal (ARSM19.pdf)**

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. 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)
3. MycoStrip™-Mycoplasma Detection Kit, Invivogen; cat# rep-mysnc-100.
4. Stem Genomics

Alkaline Phosphatase

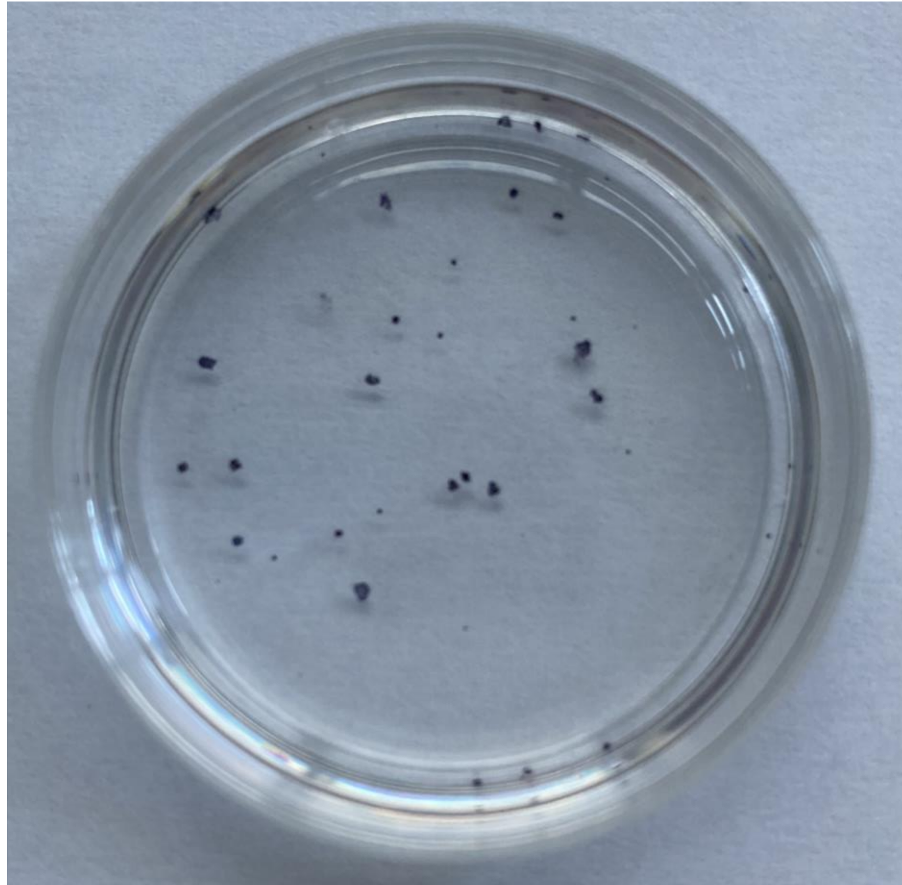

Figure 1 : Alkaline phosphatase staining assay of iPSC clone, obtained following Sendai virus infection, to determine the reprogramming efficiency. A total of 25 000 transduced cells were seeded in a 35mm Petri dish. Alkaline phophatase positive clones were counted with ImageJ.

The reprogramming efficiency (0.124%) was defined as the ratio of the number of colonies positive for alkaline phosphatase activity to the total number of cells used in the reprogramming experiment. A reprogramming efficiency between 0.1–1% is considered normal following somatic cell reprogrammation with the Sendai virus vector. Note that the reprogramming efficiency is not a maker of iPSC-line quality since only one high-quality iPSC clone per patient is tested. Additional marker evaluation is necessary to identify and qualify bona fide iPSCs.

Microscopic observation

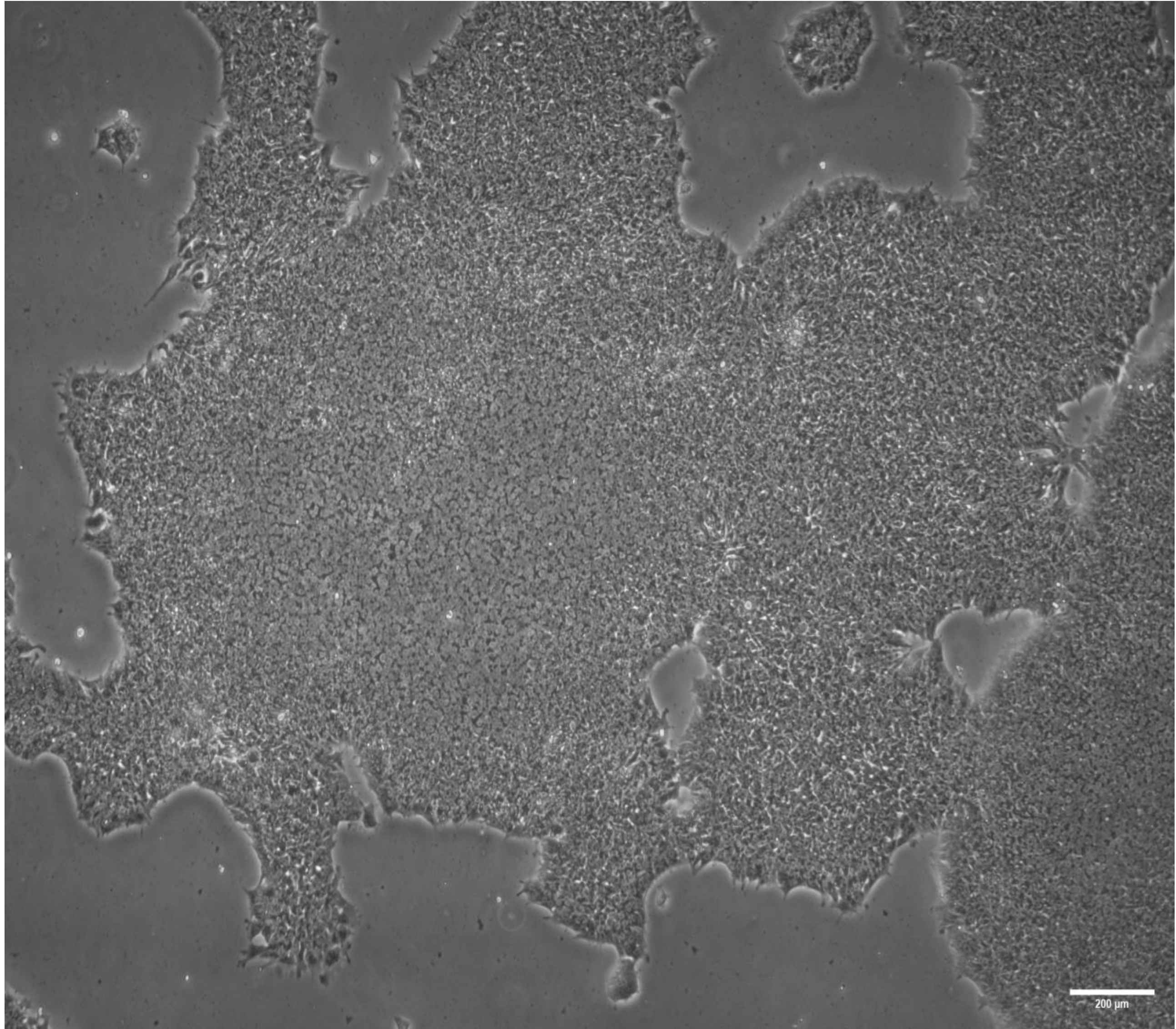

Figure 2 : iPSC ARSM19061223 clone 1 morphology, 3 days post-thawing.

Immunofluorescence

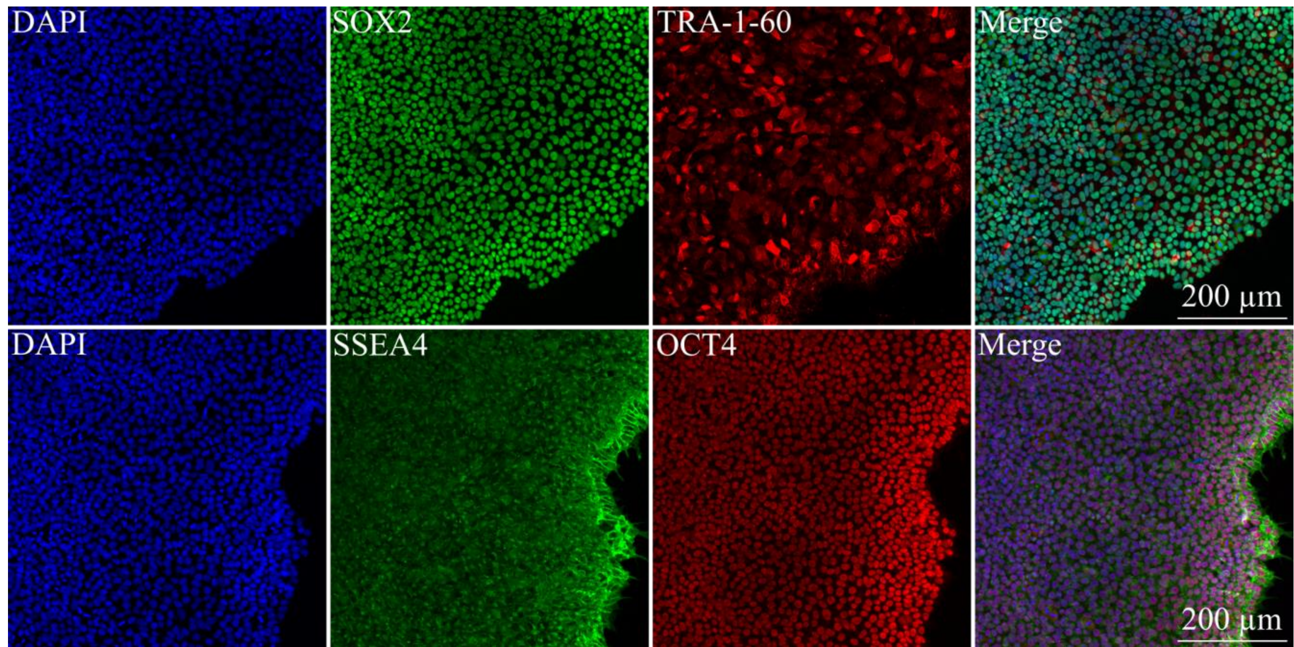

Figure 3: Immunofluorescence with four self-renewal markers (green and red). The nuclei were stained with DAPI (blue). Majority of cells expressing intracellular markers (SOX2 and OCT4), and surface markers (TRA-1-60 and SSEA4).

Flow cytometry

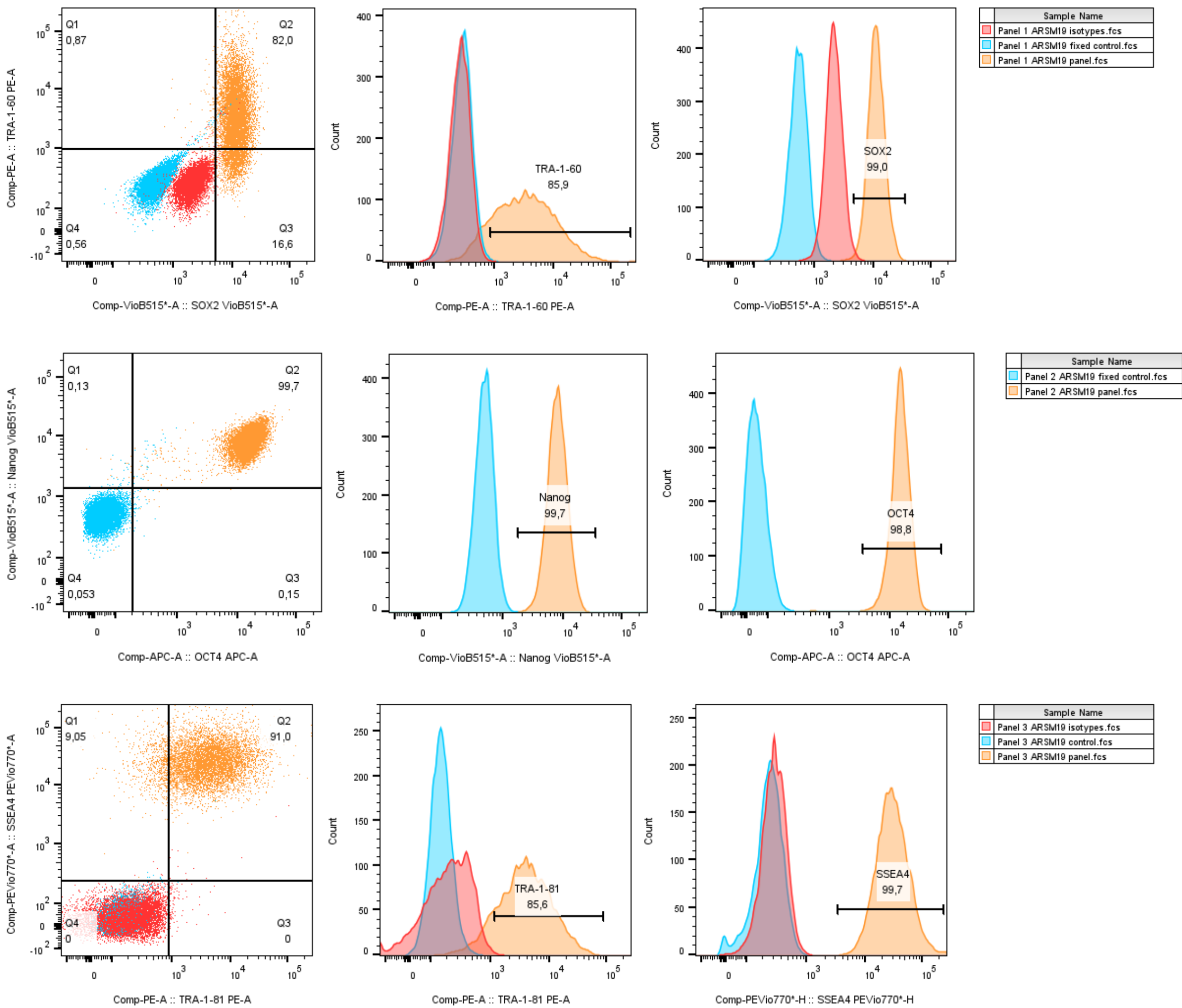

Figure 4: Expression analysis of iPSC self-renewal markers for ARSM19061223 clone 1, quantified by flow cytometry, revealed high expression of pluripotency markers (TRA-1-60 ≥ 95.9%, SOX2 ≥ 99.0%, NANOG ≥ 99.7%, OCT4 ≥ 98.8%, TRA-1-81 ≥ 85.6%, and SSEA4 ≥ 99.7%).

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RT-qPCR

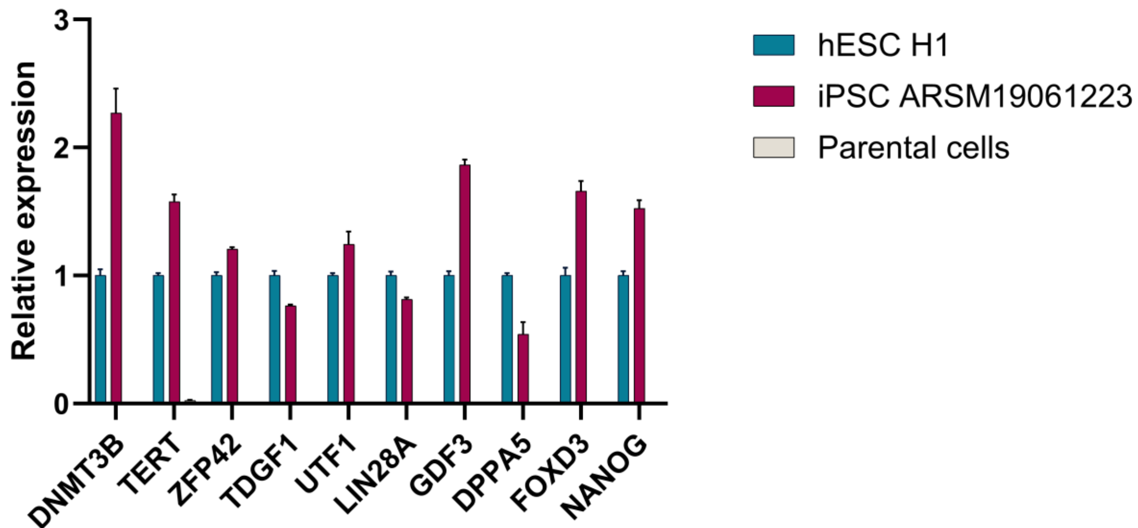
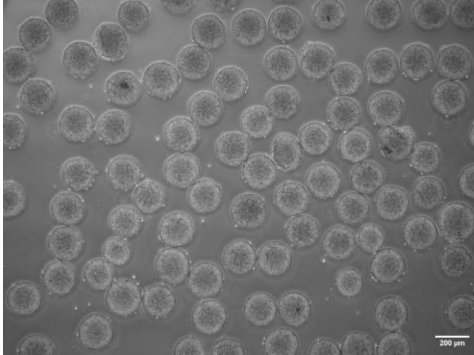


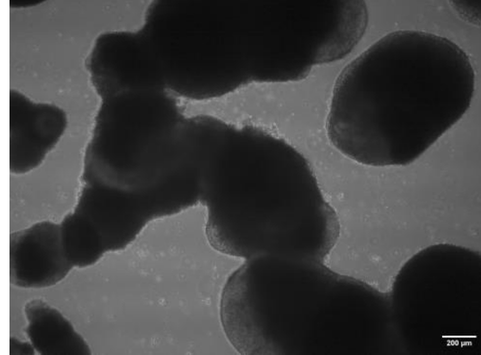
Figure 5 : Relative expression of stem cells specific genes based on H1 embryonic stem cells. The results were normalized with three reference genes : *GAPDH, YWHAZ* et *C1orf43*. RT-qPCR analysis revealed positive expression of the following genes: DNMT3B, TERT, ZFP42, TDGF1, UTF1, LIN28A, GDF3, DPP5A, FOXD3 and NANOG. These genes are associated with pluripotency and embryonic development. Pluripotency refers to the ability of cells to differentiate into various cell types. These genes are therefore involved in the regulation and maintenance of pluripotent stem cells. hESC = H1 embryonic stem cell and acts as a positive control, while CTRL = parental somatic cells acting as a negative control.

hPSC Scorecard™ Panel

a)

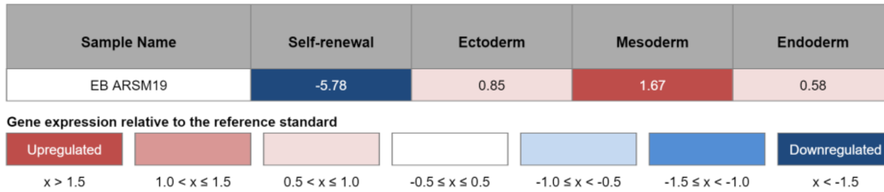


b)



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 ARSM19	-5.78	0.85	1.67	0.58

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.

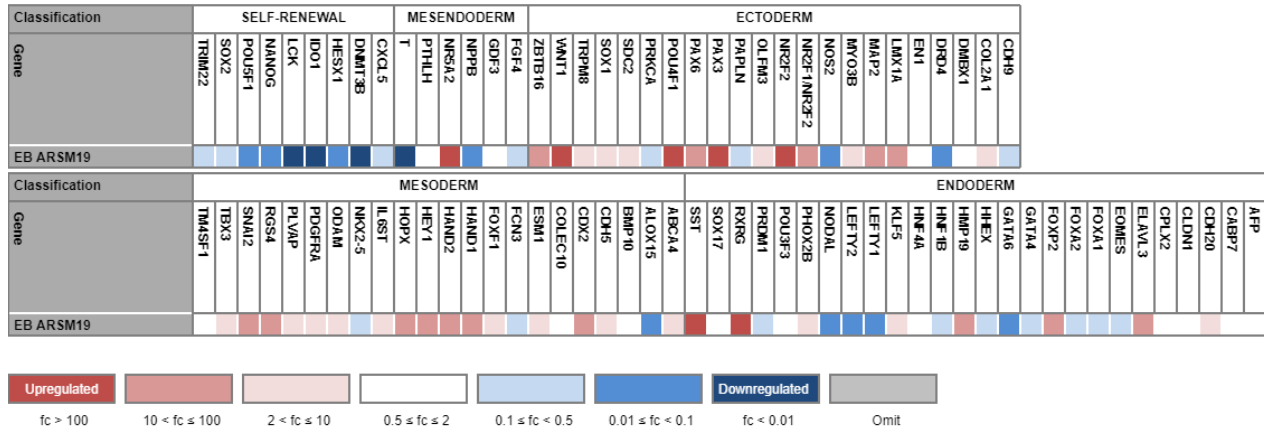


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Figure 6: Embryonic bodies at 2 days (a) and 21 days (b) of spontaneous differentiation in free floating. Scores (c) and gene expression (d) of genes from the three embryonic layers according to a group of 23 undifferentiated pluripotent stem cell lines. The ScoreCard panel is based on quantitative reverse transcription PCR (RT-qPCR) measurements of 96 genes can be used to detect early germ-layer-specific differentiation. It is therefore expected that the overall relative gene expression score, calculated over the reference standard, may varied. As long as some of the genes are upregulated in all the embryonic germ layers, even if the overall score is downregulated, the test is considered positive. Note that the expression of iPSCs self-renewal pluripotent makers should be turned down. Note as well that the reduced global trilineage differentiation potency may have been impacted by the persistence of Sendai virus.

Sendai virus genome and transgenes detection

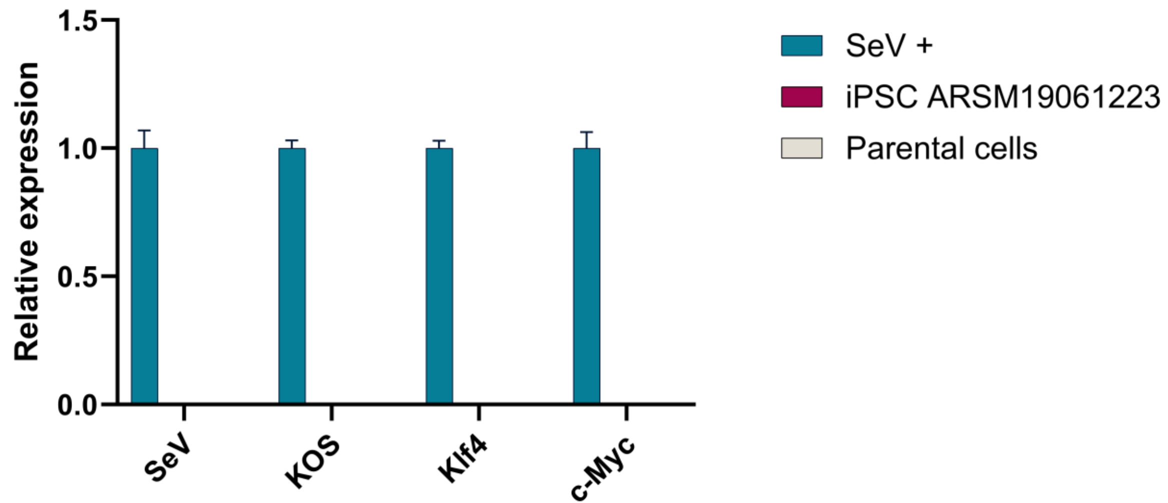

Figure 7: Relative expression of Sendai genome and transgenes based on cells freshly infected as positive control. The results were normalized with three reference genes : *GAPDH*, *YWHAZ* et *C1orf43*. CTRL = parental somatic cells acting as a negative control.

Short Tandem Repeat (STR) analysis

Table 2 : STR analysis provided by Genome Quebec revealed that the iPSC ARSM19061223 match parental cell line.

	PBMC ARSM19061223		iPSC ARSM19061223 clone 1	
AMEL	N.A.	N.A.	X	Y
CSF1PO	N.A.	N.A.	8	12
D13S317	N.A.	N.A.	12	13
D16S539	N.A.	N.A.	9	12
D21S11	N.A.	N.A.	31.2	33.2
D5S818	N.A.	N.A.	12	
D7S820	N.A.	N.A.	8	12
TH01	N.A.	N.A.	6	8
TPOX	N.A.	N.A.	8	11
vWA	N.A.	N.A.	15	18

Karyostat+

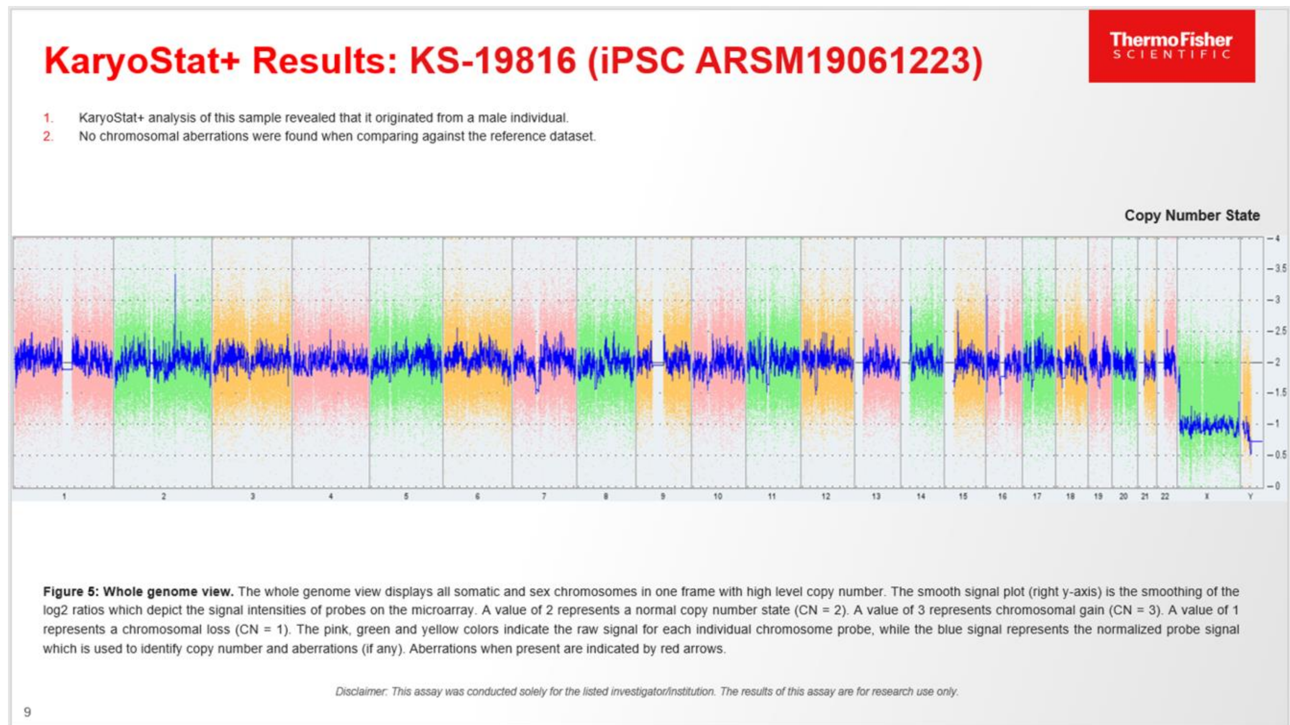

Figure 8 : Karyotype of iPSC ARSM19061223 clone 1 at passage 5. Resolution > 1Mb done by ThermoFisher service.

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Notification:

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Approved by :

17-07-2024

___________________________________ ________________________

Laurie Martineau, MSc
Plaform manager

Date

17-07-2024

___________________________________ ________________________

François Gros-Louis, Ph.D.
Director

Date