

ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR
Ctrl1-UCiPS4F1
EN EL BANCO NACIONAL DE LÍNEAS CELULARES

ANNEXES TO THE iPSC LINE
Ctrl1-UCiPS4F1

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Annex 6: Mycoplasma test

6.1. Mycoplasma test - PCR

Annex 1

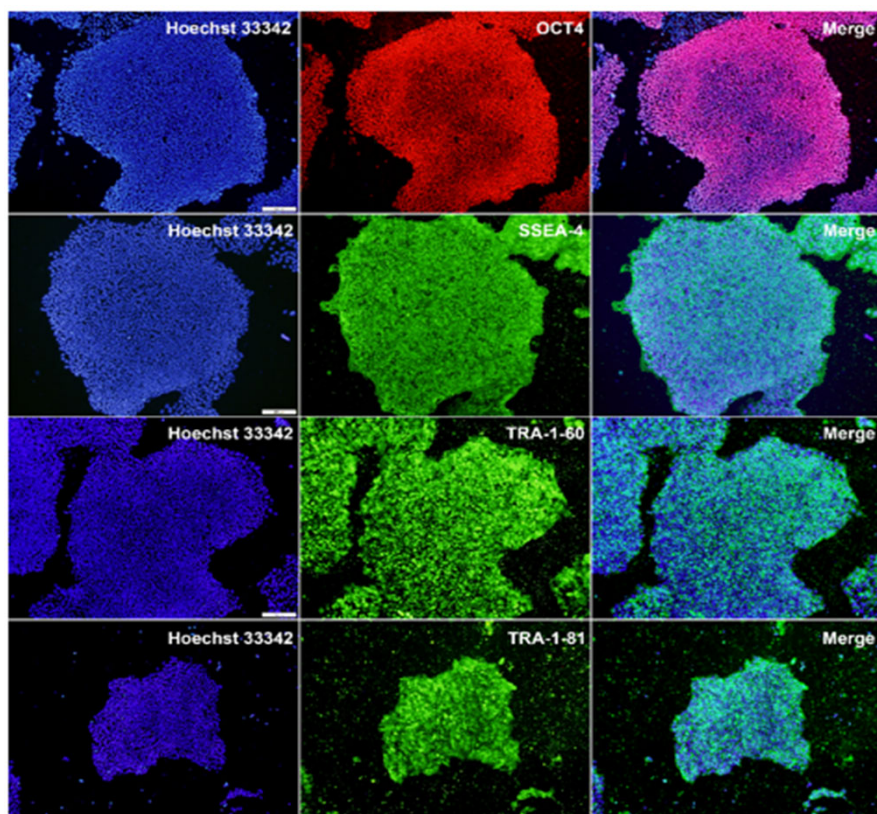
Authentication – Fingerprint analysis

	Parental cells	iPSC generated
	Urine Derived Cells (UDC)	Ctrl1-UCiPS4F1
STR loci	Alleles	
TH01	7, 8	7-8
D21S11	31.2, 32.2	31.2, 32.2
D5S818	11, 12	11, 12
D13S317	8, 11	8, 11
D7S820	8, 9	8, 9
D16S539	10, 13	10, 13
CSF1PO	10, 11	10, 11
vWA	14, 19	14, 19
TPOX	8, 11	8, 11
AMEL	XY	XY

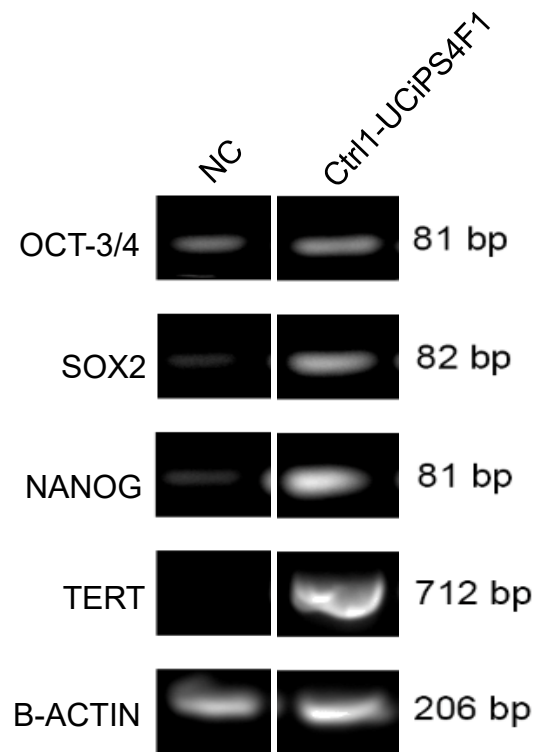
Annex 1.1. Fingerprint analysis. Autenticity of urine-derived parental cells and the generated iPSCs line. The DNA fingerprinting analysis shows that the allele patern is 100% concordant between UDC and Ctrl1-UCiPS4F1, and it is not concordant with any commercial cell line whose genotype is posted in public database.

Annex 2

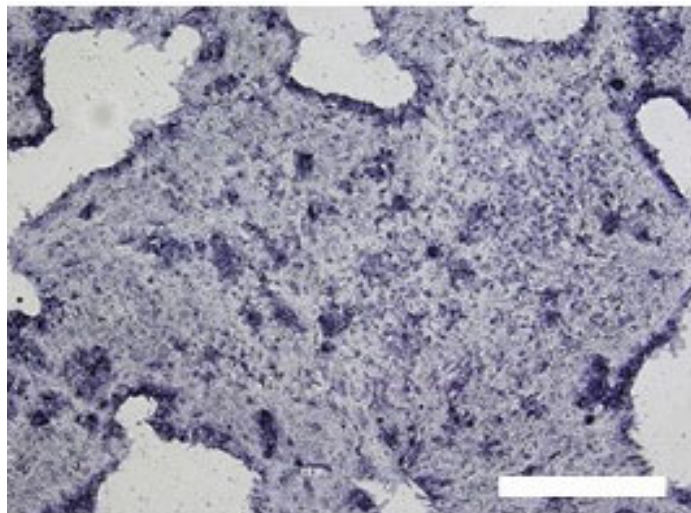
Phenotype – Pluripotency markers



Annex 2.1. Positive staining for the pluripotency markers OCT-3/4, SSEA-4, TRA-1-60 and TRA-1-81 evaluated by immunocytochemistry.



Annex 2.2. Positive mRNA expression of the pluripotency markers OCT-3/4, SOX2, NANOG and TERT evaluated by PCR. Negative control (NC): Urine derived cells.

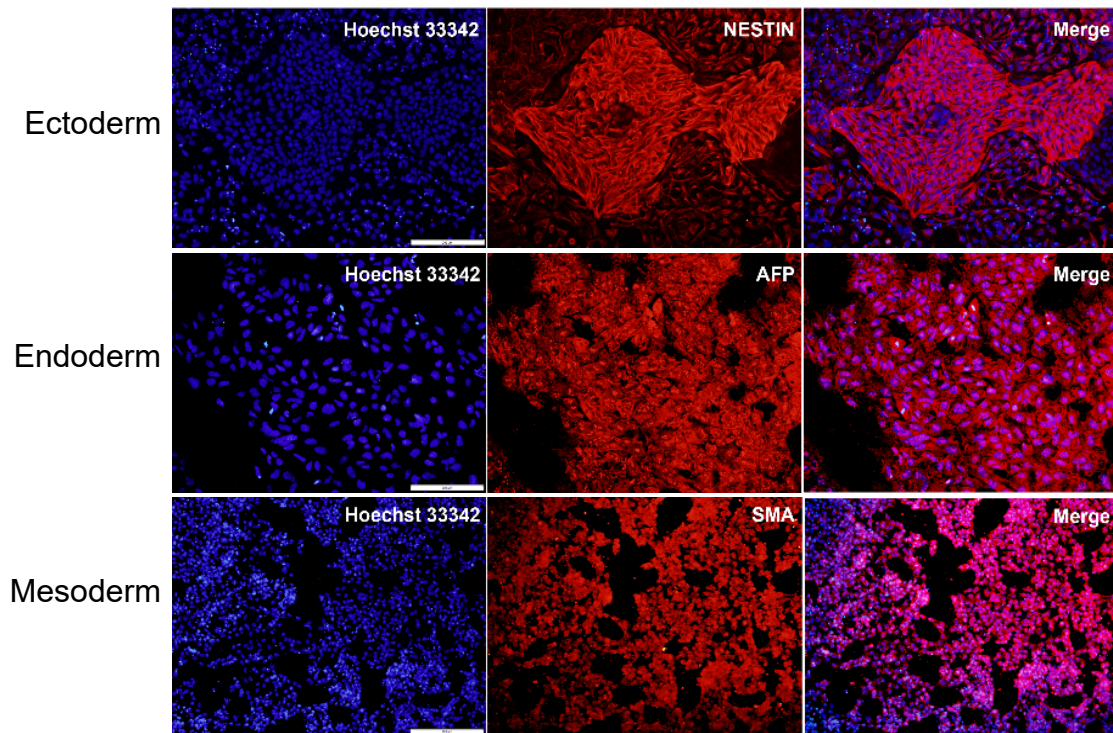


Annex 2.3. Positive Alkaline Phosphatase staining of iPSC colonies. 10X magnification, scale bar: 200 μ m.

Ctrl1-UCiPS4F1

Annex 3

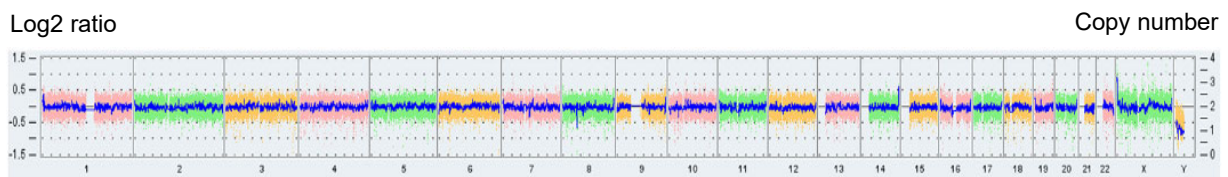
In vitro differentiation



Annex 3.1. Immunocytochemistry analysis showing Ctrl1-UCiPS4F1 cells with positive staining for the differentiation markers: Nestin, AFP and SMA, corresponding respectively, to the differentiated germ layers: ectoderm, endoderm, and mesoderm.

Annex 4

Molecular karyotyping by SNP array



47, XXY

Annex 4.1. Molecular karyotyping by SNP array. The frame shows a whole view of the Ctrl1-UCiPS4F1 cells genome displaying all somatic and sex chromosomes. The smooth signal plot (right y-axis) is the smoothing of the Log₂ ratios (left y-axis), which depict the signal intensities of probes on the microarray, and represents the number of copies of each chromosome. The pink, green and yellow colors represent the raw signal for each individual chromosome probe, and the blue signal represents the normalized probe signal, used to identify the copy number and any aberration.

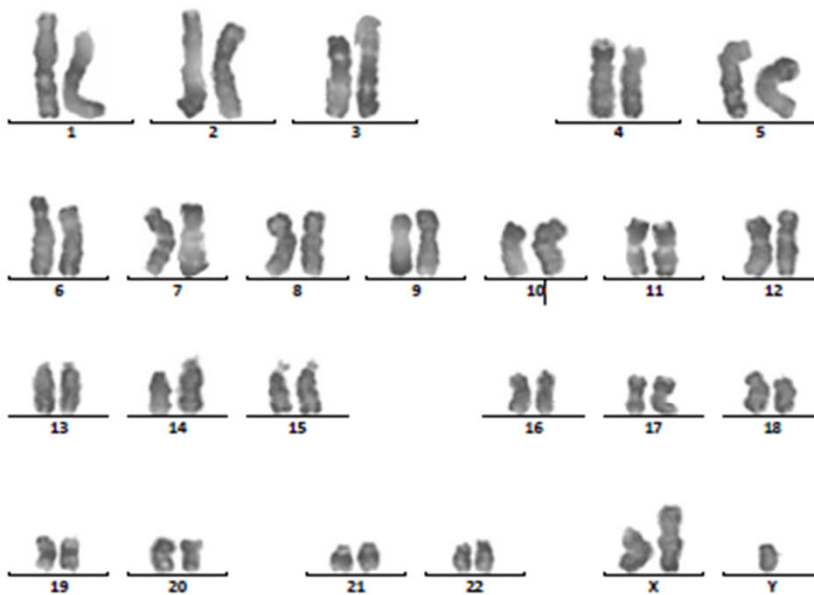
G-Banded Karyotyping



Código de Biobanco: INVN02526A314CROMA003
Código de origen: iPSC i015 P29
Petición de servicio: S2500472

Fecha de entrada: 20/02/26
Tipo de muestra:
Técnica: Bandas G

RESULTADOS ANÁLISIS CITOGENÉTICO



Cariotipo: 47, XXY

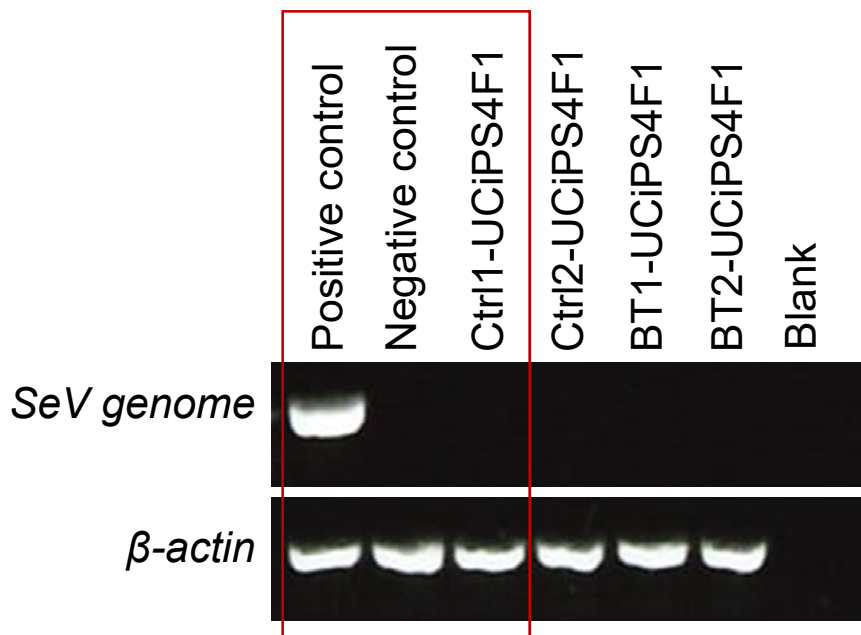
Diagnóstico citogenético: Línea celular con aneuploidía de cromosomas sexuales compatible con síndrome de Klinefelter

Annex 4.2. G-banded karyotyping. The Ctrl1-UCiPS4F1 cell line exhibits sex chromosome aneuploidy consistent with Klinefelter syndrome. The result is limited by the sensitivity of the technique.

Ctrl1-UCiPS4F1

Annex 5

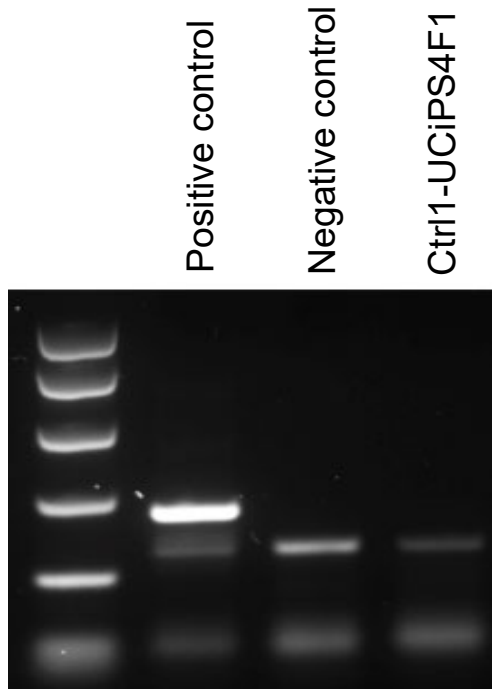
Clearance of the Sendai virus vector



Annex 5.1. Silencing test. PCR analysis showing the clearance of the Sendai virus (SeV) genome in the established iPSC lines. UDCs were used as negative control and recent transfected iPSC as positive control. Red lines indicate the result for controls and the Ctrl1-UCiPS4F1 cell line.

Ctrl1-UCiPS4F1

Annex 6 Mycoplasma test



Annex 6.1. Mycoplasma test. Negative result for the presence of mycoplasma in the Ctrl1-UCiPS4F1 cells. The evaluation was performed using the conventional Venor®GeM Classic kit for the detection of mycoplasma using PCR. Negative control: water.

Ctrl1-UCiPS4F1