ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR
BT1-UCiPS4F1
EN EL BANCO NACIONAL DE LÍNEAS CELULARES

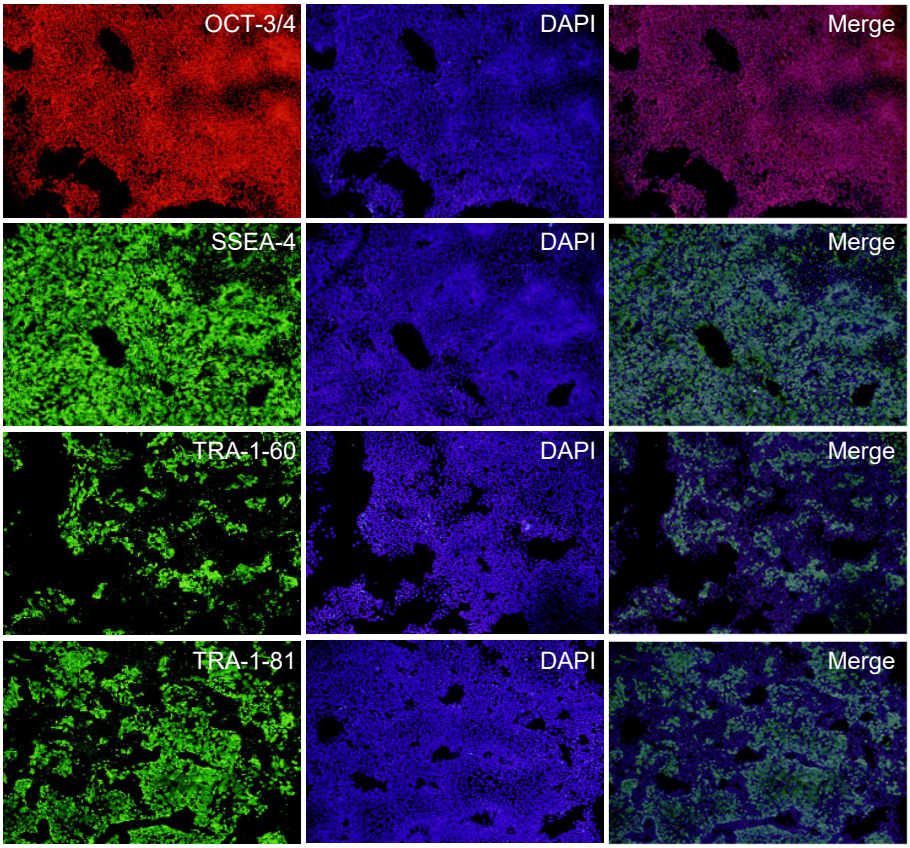
ANNEXES TO THE iPSC LINE
BT1-UCiPS4F1

Annex 1
Authentication – Fingerprint analysis

	Parental cells	iPSC generated
	Urine Derived Cells (UDC)	**BT1-UCiPS4F1**
STR loci	**Alleles**	
TH01	6	6
D21S11	31	31
D5S818	13	13
D13S317	11, 13	11, 13
D7S820	9, 11	9, 11
D16S539	11	11
CSF1PO	10, 12	10, 12
vWA	16, 17	16, 17
TPOX	8	8
AMEL	X	X

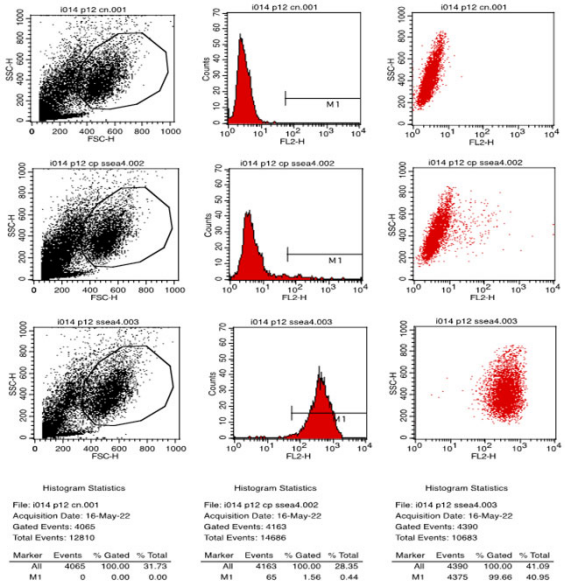
Annex 1.1. Fingerprint analysis. Autenticity of urine-derived parental cells and the generated iPSCs line. The DNA fingerprinting analysis shows that the allele patern is 100% concordant between UDC and BT1-UCiPS4F1, and it is not concordant with any commercial cell line whose genotype is posted in public database.

Annex 2
Phenotype – Pluripotency markers

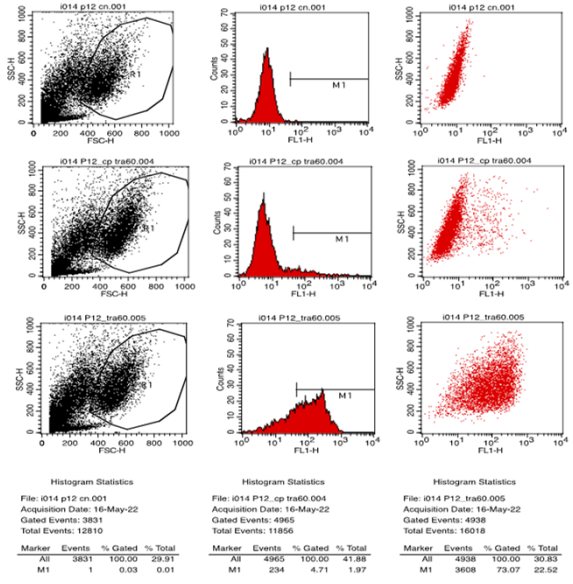


Annex 2.1. Positive staining for the pluripotency markers OCT-3/4, SSEA-4, TRA-1-60 and TRA-1-81 evaluated by immunocitochemistry.

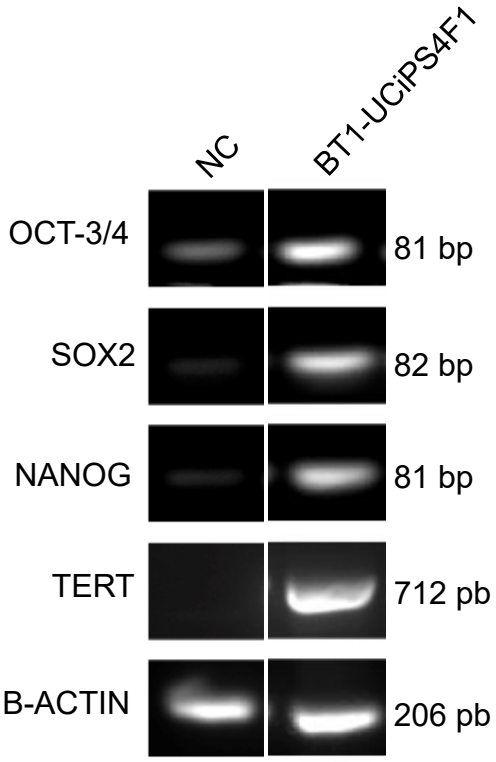
SSEA-4



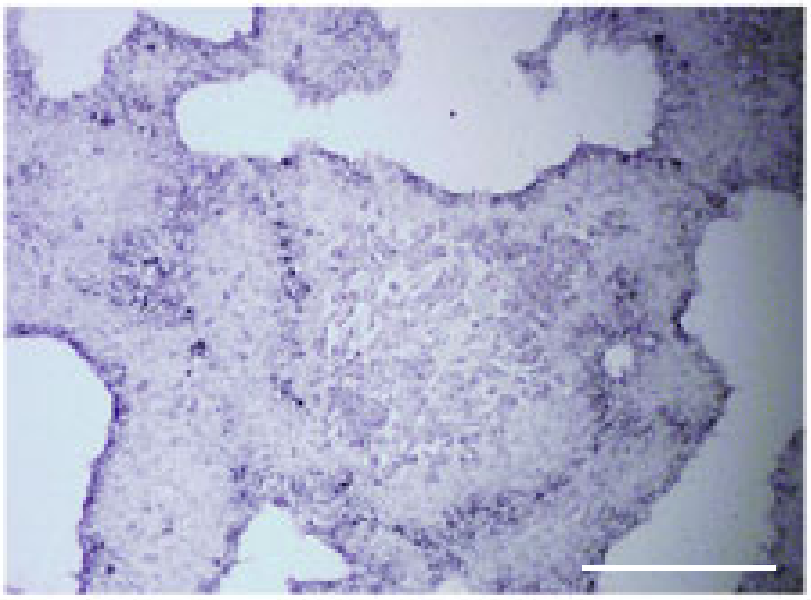
TRA-1-60



Annex 2.2. The BT1-UCiPS4F1 cells were positive for the expression of the pluripotency markers SSEA-4 and TRA-1-60 evaluated by flow cytometry.



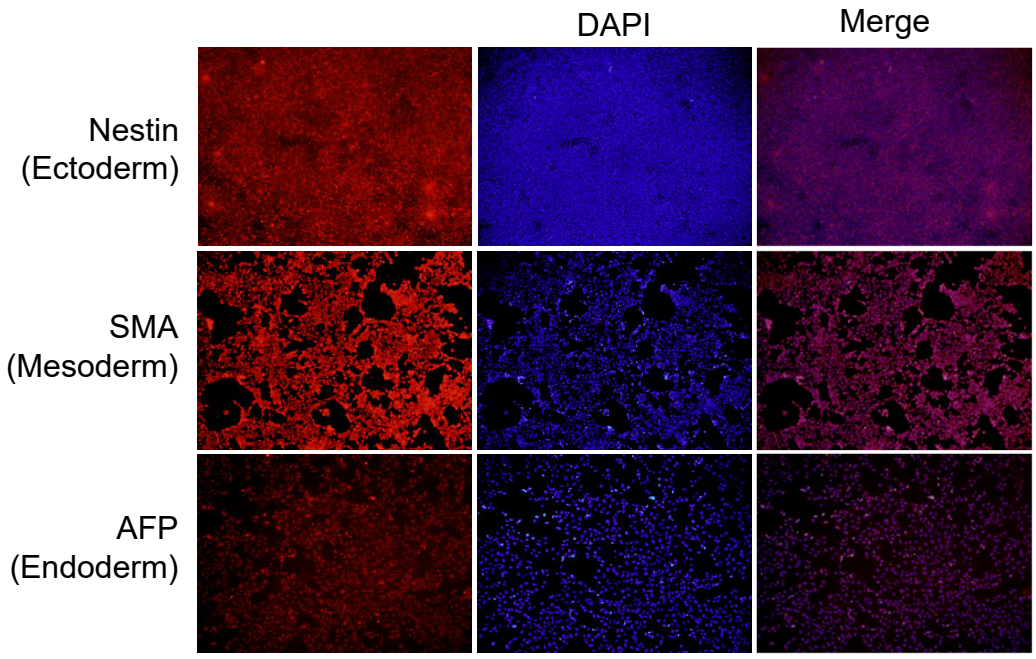
Annex 2.3. Positive mRNA expression of the pluripotency markers OCT-3/4, SOX2, NANOG and TERT evaluated by PCR. Negative control (NC): Urine derived cells.



Annex 2.4. Positive Alkaline Phosphatase staining of iPSC colonies. 10X magnification, scale bar: 200 μm.

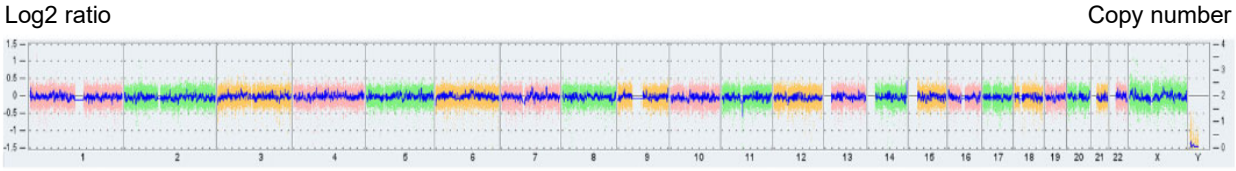
BT1-UCiPS4F1

Annex 3
In vitro differentiation



Annex 3.1. Immunocitochemistry analysis showing BT1-UCiPS4F1 cells with positive staining for the differentiation markers: Nestin, SMA and AFP, corresponding respectively, to the differentiated germ layers: ectoderm, mesoderm, and endoderm.

Annex 4
Molecular karyotyping by SNP array



46, XX

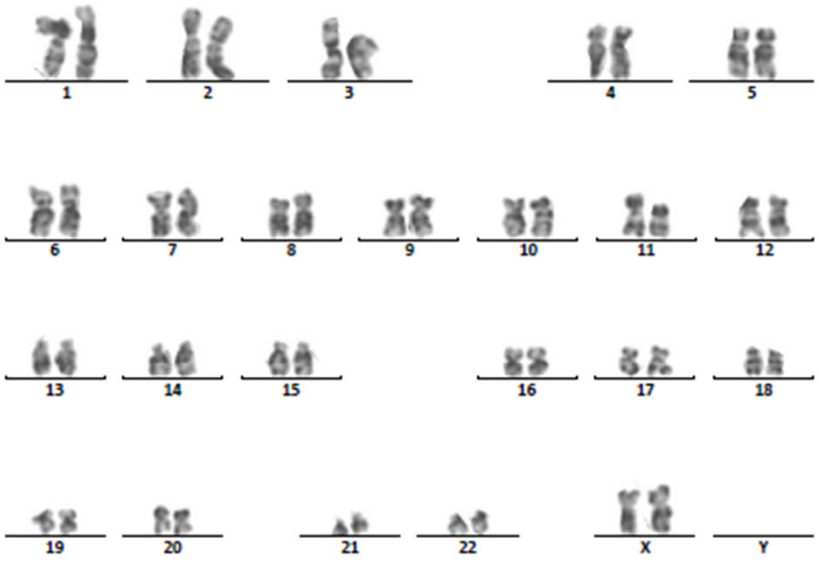
Annex 4.1. Molecular karyotyping by SNP array. The frame shows a whole view of the BT1-UCiPS4F1 cells genome displaying all somatic and sex chromosomes. The smooth signal plot (right y-axis) is the smoothing of the Log2 ratios (left y-axis), which depict the signal intensities of probes on the microarray, 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

Junta de Andalucía
Consejería de Salud y Familias
Biobanco del Sistema Sanitario Público de Andalucía

Código de Biobanco: INVN02526A155CROMA003 **Fecha de entrada:** 28/01/26
Código de origen: i014 p31 **Tipo de muestra:** iPSC
Petición de servicio: S2500472 **Técnica:** Bandas G

RESULTADOS ANÁLISIS CITOGENÉTICO



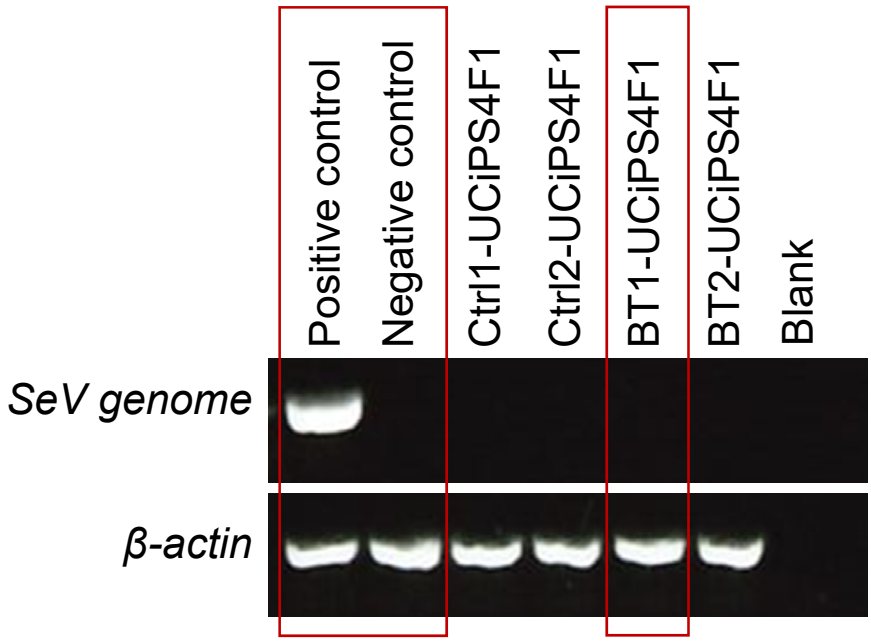
Cariotipo: 46,XX

Diagnóstico citogenético: Linea celular compatible con cariotipo femenino sin alteraciones observables mediante Bandeo_G

Annex 4.2. G-banded karyotyping. The BT1-UCiPS4F1 cell line is compatible with a female karyotype without visible alterations. The result is limited by the sensitivity of the technique.

BT1-UCiPS4F1

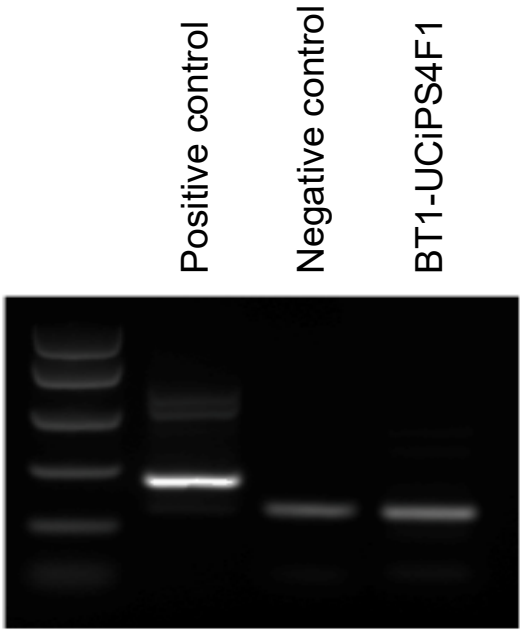
Annex 5
Clearance of the Sendai virus vector



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 results for controls and the BT1-UCiPS4F1 cell line.

BT1-UCiPS4F1

Annex 6
Mycoplasm test



Annex 6.1. Mycoplasm test. Negative result for the presence of mycoplasm in the BT1-UCiPS4F1 cells. The evaluation was performed using the conventional Venor®GeM Classic kit for the detection of mycoplasm using PCR. Negative control: water.