

ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR
BT2-UCiPS4F1
EN EL BANCO NACIONAL DE LÍNEAS CELULARES

ANNEXES TO THE iPSC LINE
BT2-UCiPS4F1

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6.1. Mycoplasma test - PCR

Annex 1

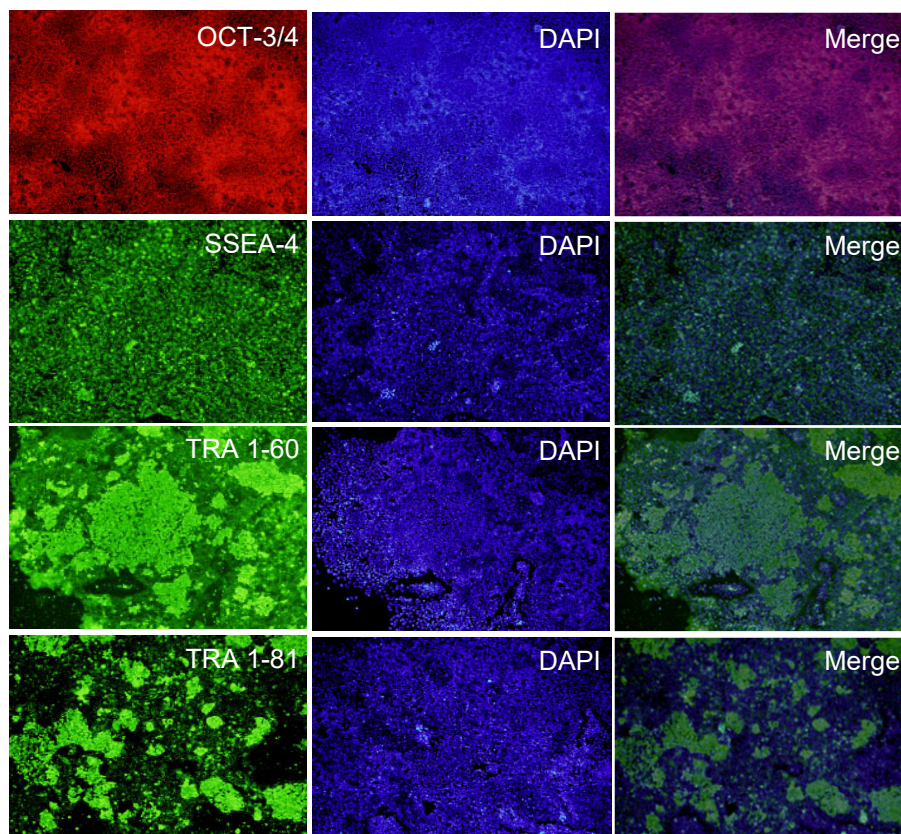
Authentication – Fingerprint analysis

| | Parental cells | iPSC generated |
|----------|---------------------------|----------------|
| | Urine Derived Cells (UDC) | BT2-UCiPS4F1 |
| STR loci | Alleles | |
| TH01 | 9, 9.3 | 9, 9.3 |
| D21S11 | 32.2, 33.2 | 32.2, 33.2 |
| D5S818 | 13 | 13 |
| D13S317 | 8 | 8 |
| D7S820 | 8, 13 | 8, 13 |
| D16S539 | 11, 12 | 11, 12 |
| CSF1PO | 9, 10 | 9, 10 |
| vWA | 15, 18 | 15, 18 |
| TPOX | 8 | 8 |
| AMEL | X | X |

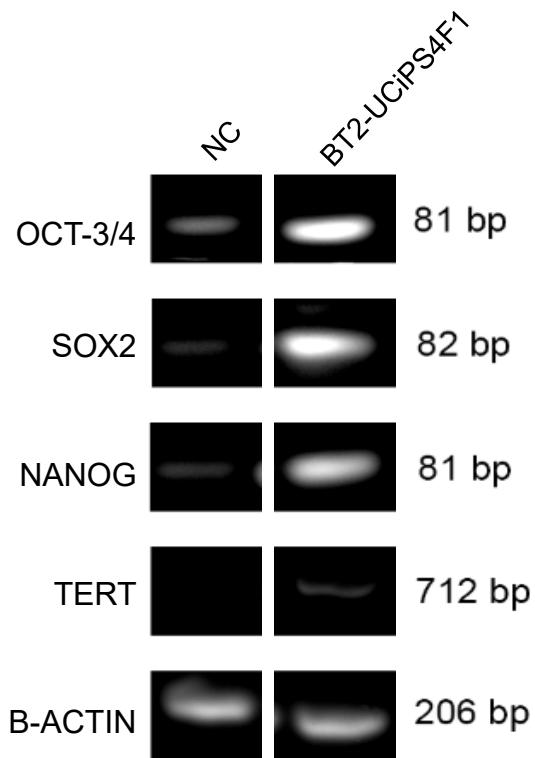
Annex 1.1. Fingerprint analysis. Autenticity of urine-derived parental cells and the generated iPSCs line. The DNA fingerprinting analysis shows that the allele pattern is 100% concordant between UDC and BT2-UCiPS4F1, and it is not concordant with any commercial cell line whose genotype is posted in public database.

Annex 2

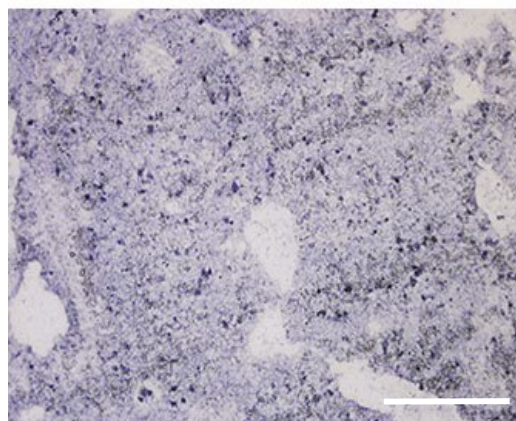
Phenotype – Pluripotency markers



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, TERT evaluated by PCR. Negative control (NC): Urine derived cells.

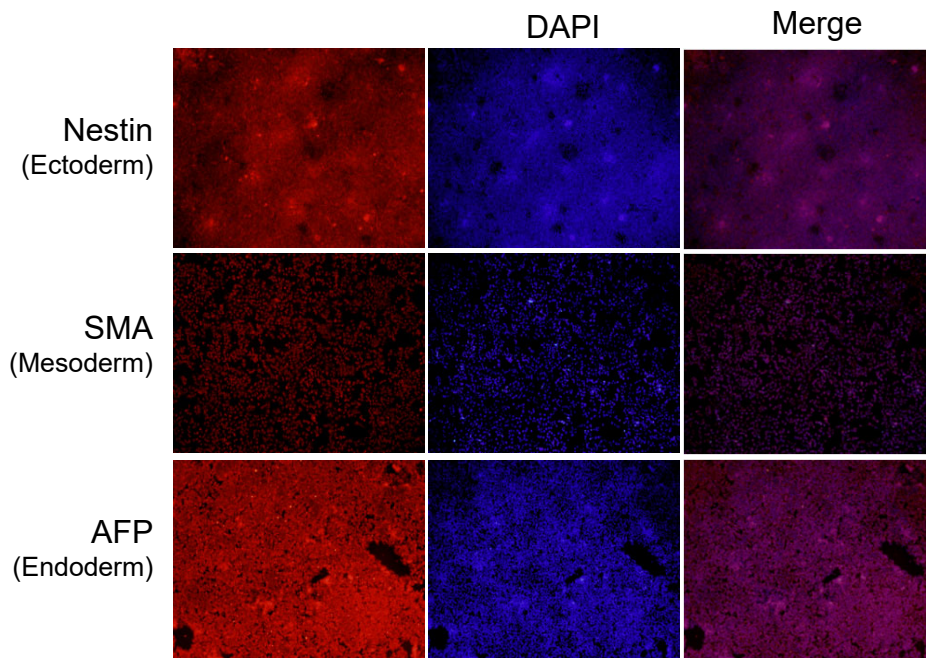


Annex 2.3. Positive Alkaline Phosphatase staining of iPSC colonies. 10X magnification, scale bar: 200 μ m.

BT2-UCiPS4F1

Annex 3

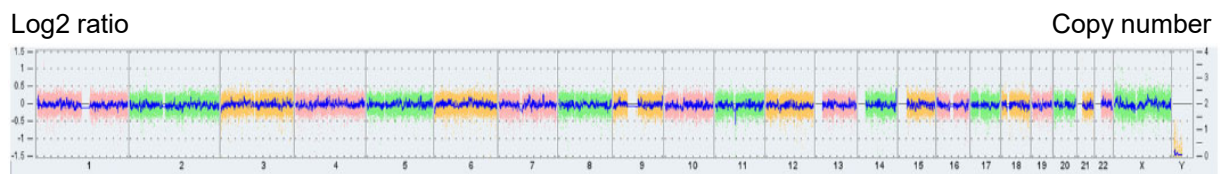
In vitro differentiation



Annex 3.1. Immunocytochemistry analysis showing BT2-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 BT2-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.

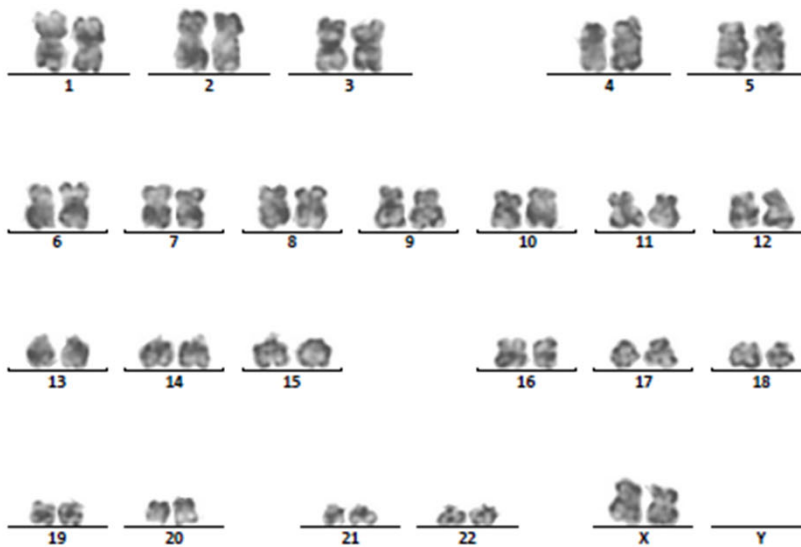
G-Banded Karyotyping



Código de Biobanco: INVN02526A157CROMA003
Código de origen: i019 p35
Petición de servicio: S2500472

Fecha de entrada: 28/01/26
Tipo de muestra: iPSC
Técnica: Bandas G

RESULTADOS ANÁLISIS CITOGENÉTICO



Cariotipo: 46,XX

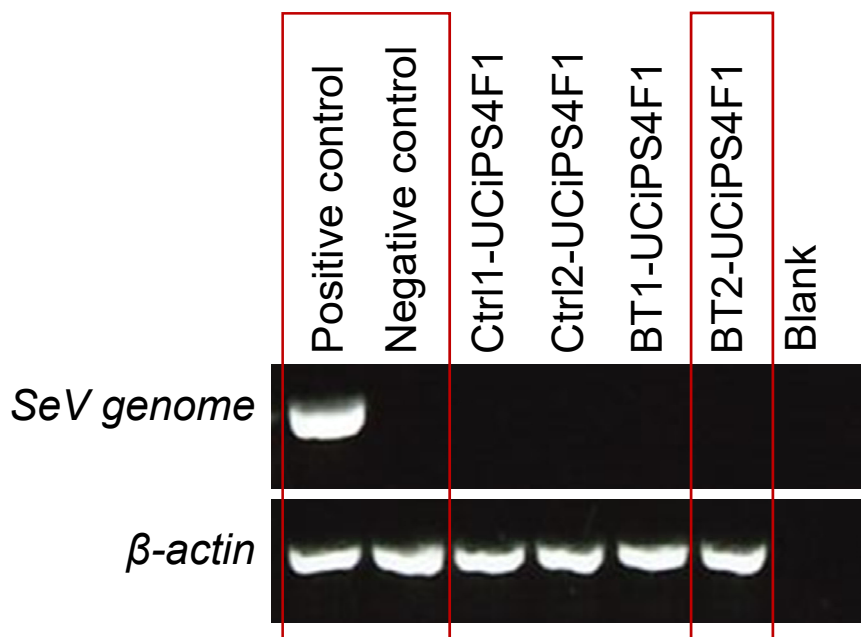
Diagnóstico citogenético: Línea celular compatible con cariotipo femenino sin alteraciones cromosómicas observables mediante Bando-G

Annex 4.2. G-banded karyotyping. The BT2-UCiPS4F1 cell line is compatible with a female karyotype without visible alterations. The result is limited by the sensitivity of the technique.

BT2-UCiPS4F1

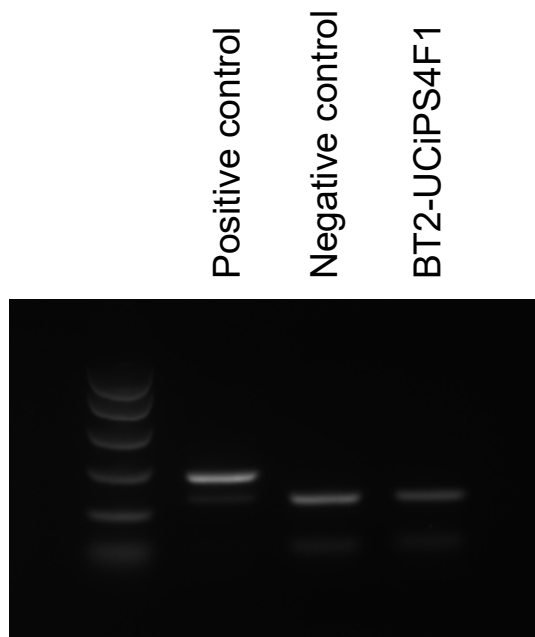
Annex 5

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 the BT2-UCiPS4F1 cell line.

Annex 6 Mycoplasma test



Annex 6.1. Mycoplasma test. Negative result for the presence of mycoplasma in the BT2-UCiPS4F1 cells. The evaluation was performed using the conventional Venor®GeM Classic kit for the detection of mycoplasma using PCR. Negative control: water.