ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR **5PNF_TDiPSsv_MM_4** EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: 5PNF_TDiPSsv_MM_4

Annex 1: Morphology and AP staining

Annex 2: Pluripotency markers by immunofluorescence

Annex 3: *In vitro* differentiation markers by Immunofluorescence

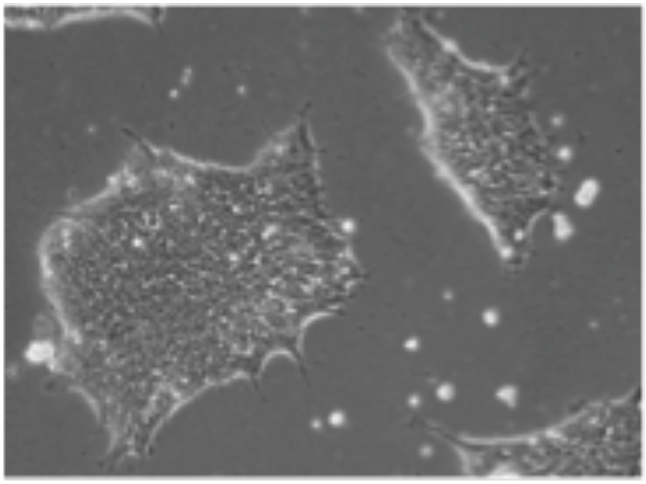
Annex 4: Karyotype

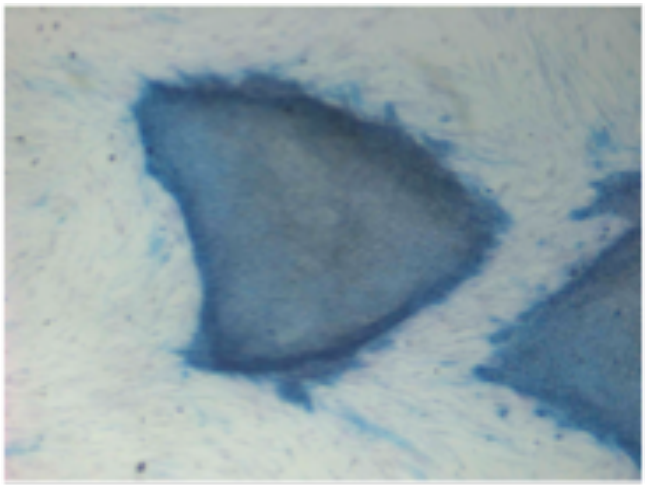
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Integration/silencing test

Annex 7: Genotype

Annex 1
Morphology and Alkaline phosphatase staining

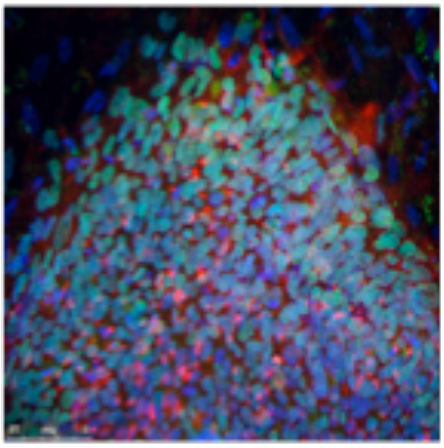




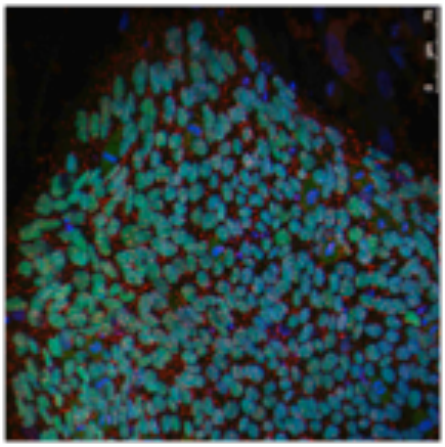
5PNF_TDiPSsv_MM_4 Passage 1

Annex 2
Pluripotency markers

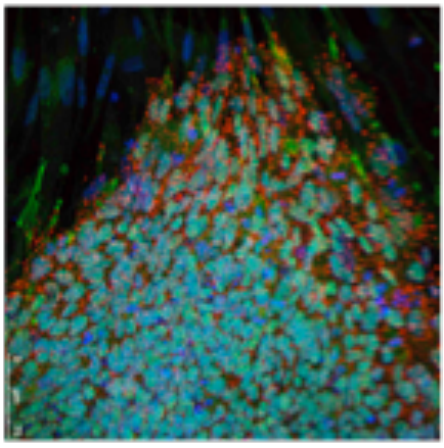
NANOG/TRA181



OCT4/SSEA3



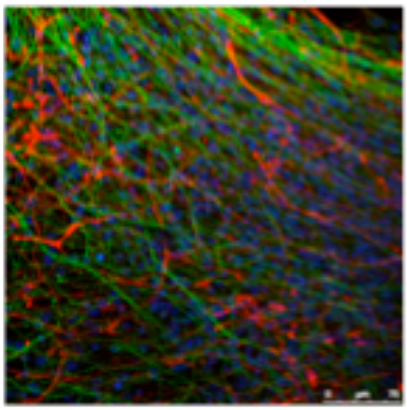
SOX2/ SSEA4



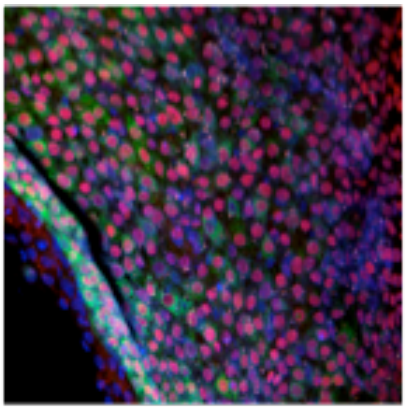
Immunofluorescence of pluripotency associated markers NANOG, TRA181, OCT4, SSEA3, SOX2 and SSEA4 in 5PNF_TDiPSsv_MM_4 iPS at passage 11.

Annex 3
In vitro differentiation

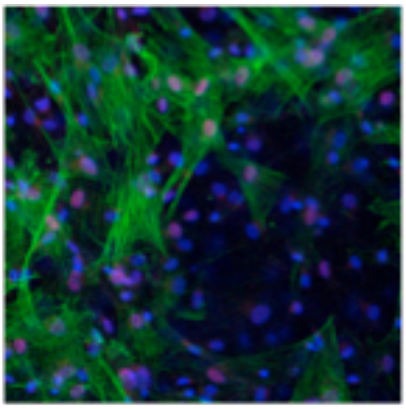
ECTODERM TUJ1/GFAP



ENDODERM a-FETO/FOXA2



MESODERM SMA/GATA4

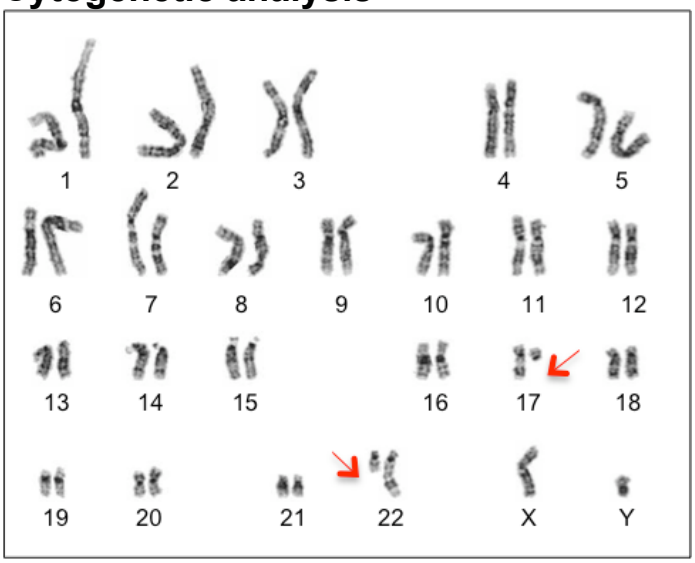


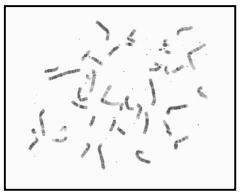
Immunofluorescence of differentiation associated markers TUJ1, GFAP for ectoderm; a-FETO, FOXA2 for endoderm and SMA, GATA4 for mesoderm in 5PNF_TDiPSsv_MM_4 iPS at passage 12.

Annex 4
Karyotype

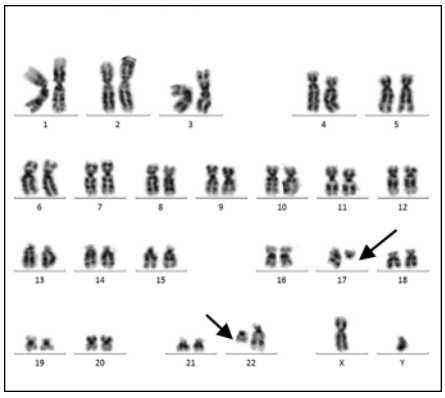
Ambar
ANÀLISIS MÈDIQUES
BARCELONA

Cytogenetic analysis





Case name: A157475
Patient name: 5PNF_TDiPSsv_MM_4 passage 8
Result: 46,XY,t(17;22)(q10;q13.3)
Specimen type: stem cells
*Note that primary Schwann cells derived form this tumor (5PNF SC) also carry the translocation found in 5PNF_TDiPSsv_MM_4 iPS line. This means that this alteration is already present in the reprogrammed cells and is not due to the reprogramming process.



5PNF SC passage 3, 46,XY,t(17;22)(q10;q13.3)

Annex 5
Authentication

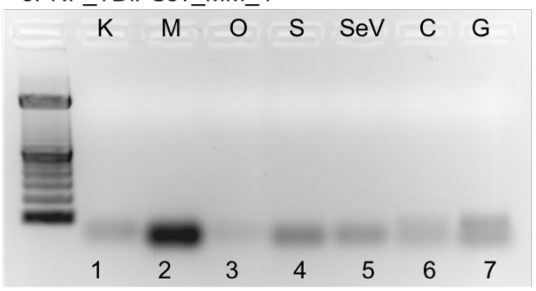
AmpFISTR Identifiler loci	5PNF	5PNF-F	5PNF_TDiPSsv_MM_#4
CSF1PO	10	10	10
D2S1338	17,19	17,19	17,19
D3S1358	15,16	15,16	15,16
D5S818	12,13	12,13	12,13
D7S820	10,13	10,13	10,13
D8S1179	8,13	8,13	8,13
D13S317	8,12	8,12	8,12
D16S539	12,13	12,13	12,13
D18S51	15,16	15,16	15,16
D19S433	14,15	14,15	14,15
D21S11	29	29	29
FGA	21,23	21,23	21,23
THO1	9,3	9,3	9,3
TPOX	10,11	10,11	10,11
vWA	18,19	18,19	18,19
Amelogenin (gender)	XY	XY	XY

Microsatellite analysis results. Method used: AmpFISTR Identifiler Plus PCR Amplification Kit (Life Technologies, cat #: 4427368, lot #: 1212014).

Tumor: 5PNF
Tumor fibroblast cells: 5PNF-F
iPS generated: 5PNF_TDiPSsv_MM_4

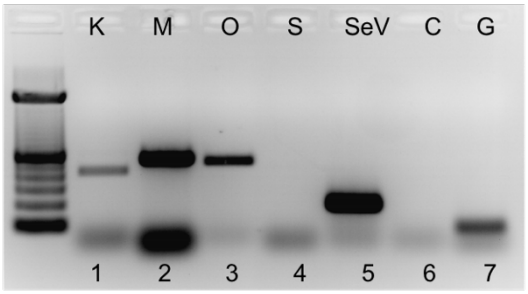
Annex 6
Integration/silencing test

5PNF_TDiPSsv_MM_4

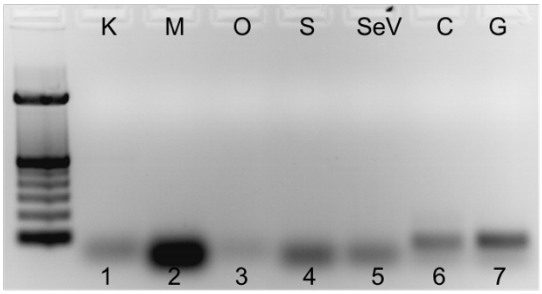


1- K: KLF4 (Transgene)
2- M: c-MYC (Transgene)
3- O: OCT4 (Transgene)
4- S: SOX2 (Transgene)
5- SeV: Sendai virus (virus)
6: CRIPTO
7: GAPDH

Infected fibroblasts (positive control)



Non-infected iPS (negative control)

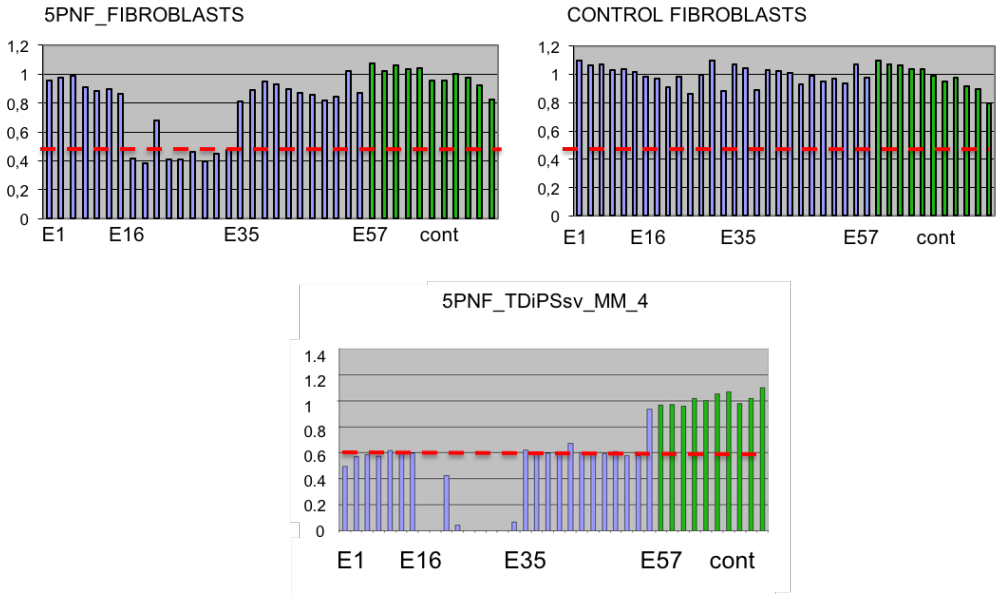


RT-PCR analysis showing silencing of the transgenes KLF4, c-MYC, OCT4 and Sox2 and the absence of Sendai virus in the 5PNF_TDiPSsv_MM_4 iPS line.

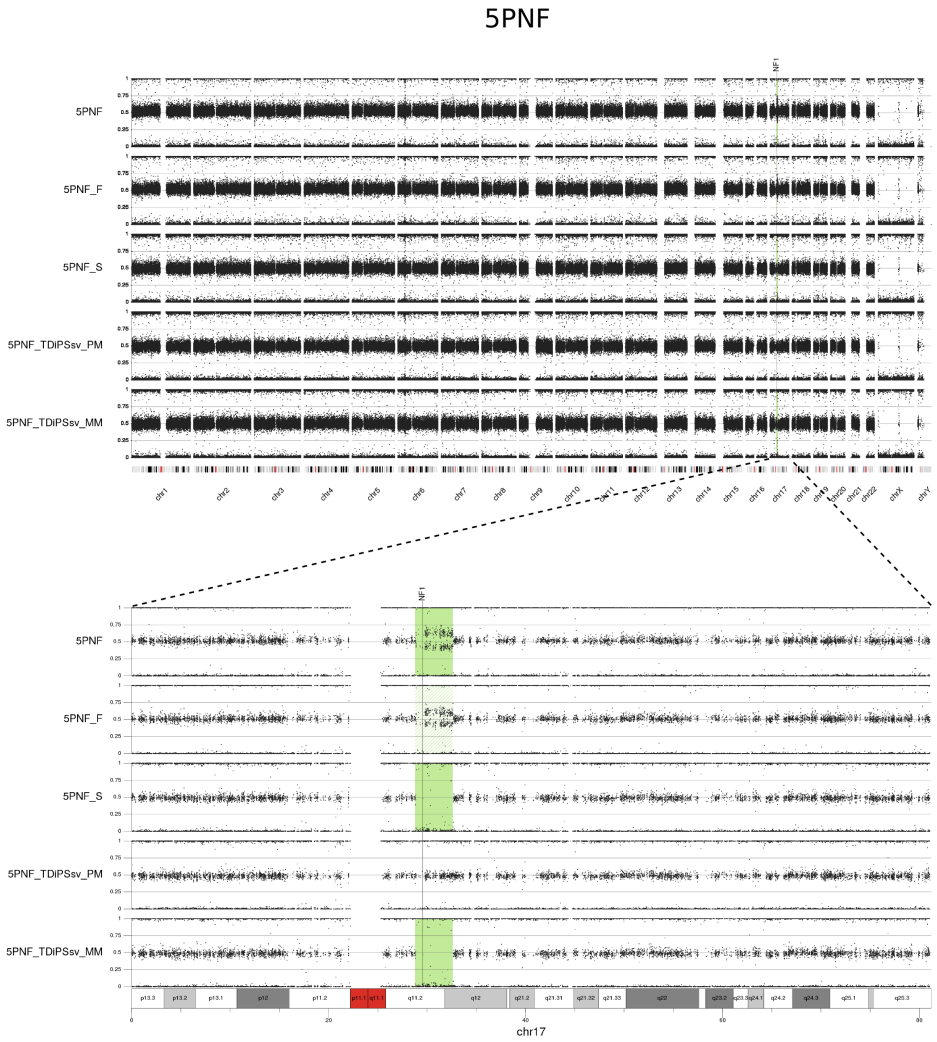
Annex 7
Genotype

Germline mutation: Intragenic deletion from E16-E57

MLPA analysis showing an intragenic deletion in the NF1 gene, from exon 16 (E16) to exon 57 (E57) as a germline mutation (fibroblasts from the patient also bear it).



Somatic mutation: LOH



Upper panel shows the B-allele frequency (BAF) data from SNP-array analysis showing the somatic mutation in the tumor. 5PNF: primary tumor; 5PNF_F: primary fibroblasts; 5PNF_S: primary Schwann cells; 5PNF_TDiPSsv_PM: *NF1* (+/-) iPSC generated; 5PNF_TDiPSsv_MM: *NF1* (-/-) iPSC generated. The genome of all samples is mostly 2n, denoted by a BAF signal around 0.5.

Lower panel, a detailed view of BAF for chromosome 17. Somatic *NF1* inactivation is produced by a deletion generating CN-LOH in 17q and the reduction to homozygosity for the constitutional *NF1* mutation. LOH is observed in 5PNF and in 100% of cells in 5PNF-derived primary SCs (5PNF_S) and in the generated *NF1*(-/-) iPSCs (5PNF_TDiPSCsv_MM), but not in 5PNF_TDiPSsv_PM. Fibroblast primary culture (5PNF_F) is an early passage and still exhibit a residual LOH due to the presence of tumor SCs.