

ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR 5PNF_TDiPSsv_PM_6 EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: 5PNF_TDiPSsv_PM_6

Annex 1: Morphology and AP staining

Annex 2: Pluripotency markers by immunofluorescence

Annex 3: *In vitro* differentiation markers by
Immunofluorescence

Annex 4: Karyotype

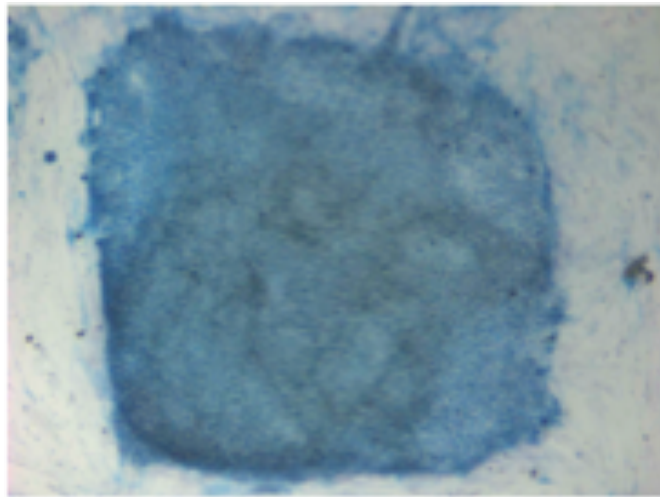
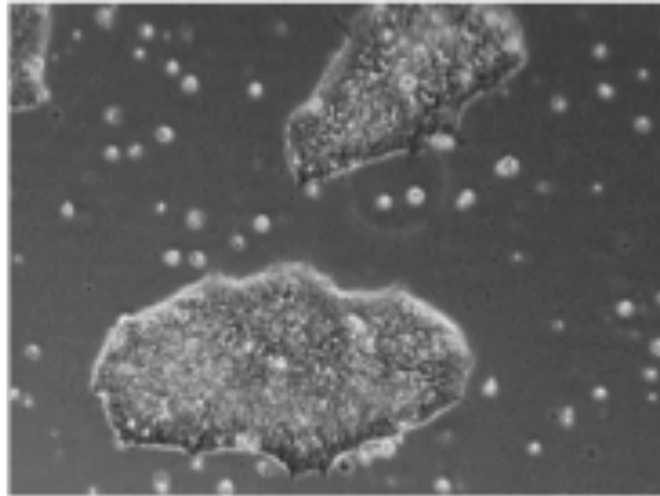
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Integration/silencing test

Annex 7: Genotype

Annex 1

Morphology and Alkaline phosphatase staining

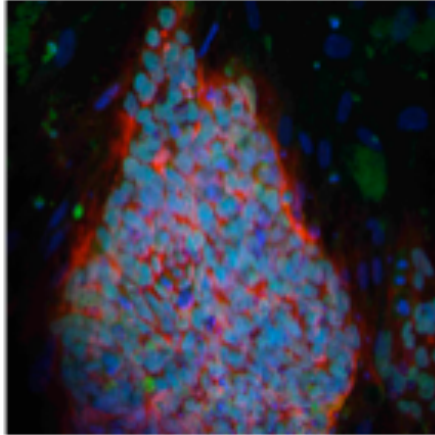


5PNF_TDiPSsv_PM_6 Passage 1

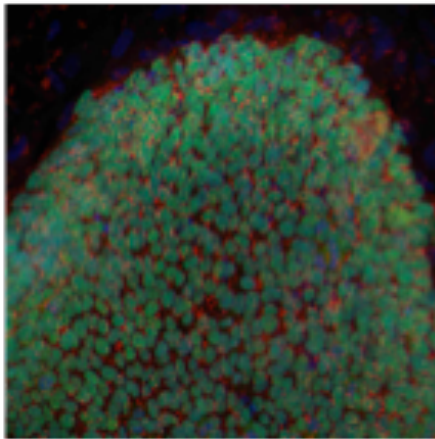
Annex 2

Pluripotency markers

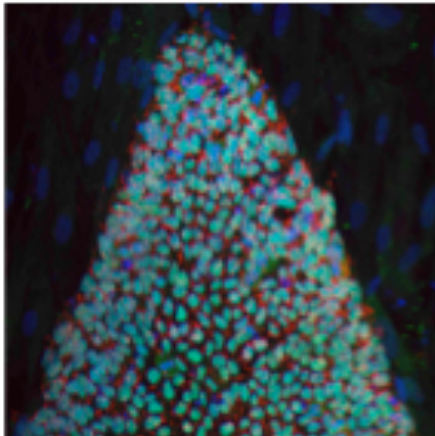
NANOG/TRA181



OCT4/SSEA3



SOX2/ SSEA4



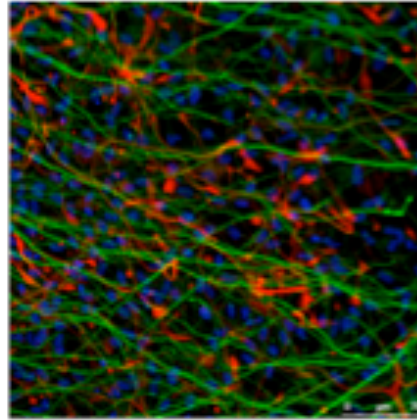
Immunofluorescence of pluripotency associated markers NANOG, TRA181, OCT4, SSEA3, SOX2 and SSEA4 in 5PNF_TDiPSsv_PM_6 iPS at passage 9.

Annex 3

In vitro differentiation

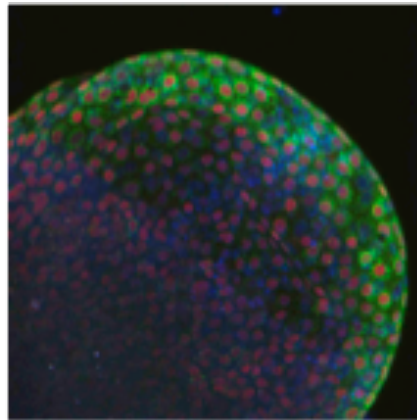
ECTODERM

TUJ1/GFAP



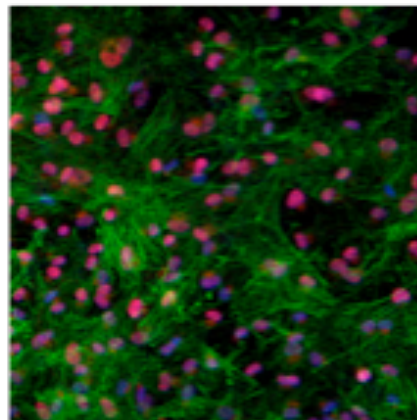
ENDODERM

aFETO/FoxA2



MESODERM

SMA/GATA4



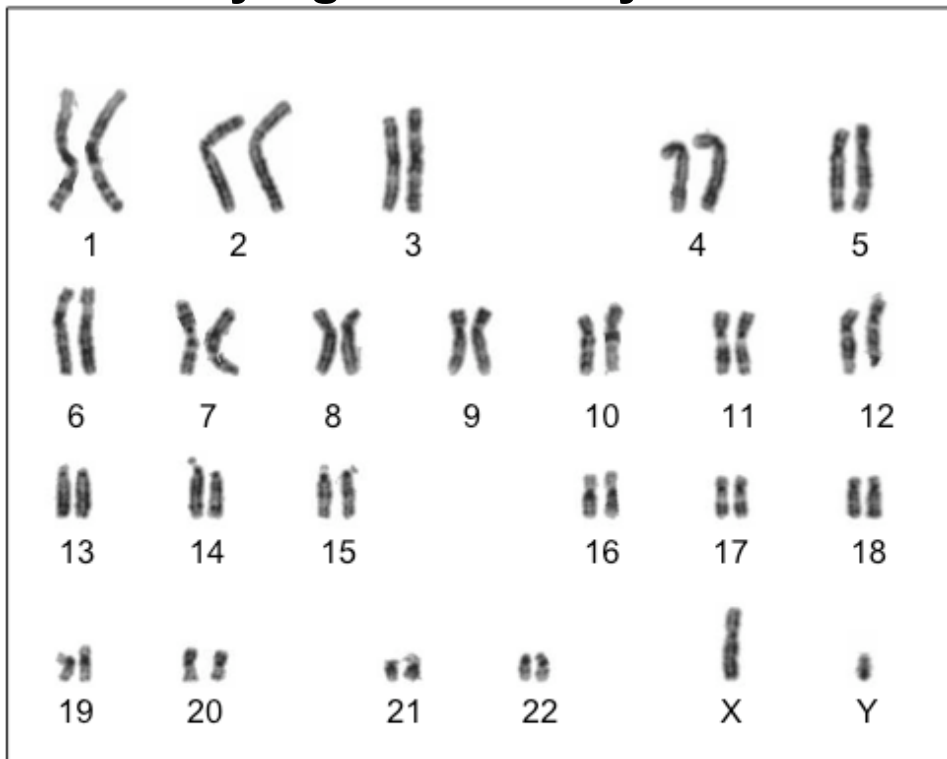
Immunofluorescence of differentiation associated markers TUJ1, GFAP for ectoderm; aFeta, FoxA2 for endoderm and aSMA, Gata4 for mesoderm in 5PNF_TDiPSsv_PM_6 iPS at passage 11.

Annex 4

Karyotype



Cytogenetic analysis



Case name: A157998

Patient name: 5PNF_TDiPSsv_PM_6 passage 8

Result: 46,XY

Specimen type: stem cells

Annex 5

Authentication

AmpFISTR Identifier loci	5PNF	5PNF-F	5PNF_TDiPSsv_PM_#6
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 Identifier Plus PCR Amplification Kit (Life Technologies, cat #: 4427368, lot #: 1212014).

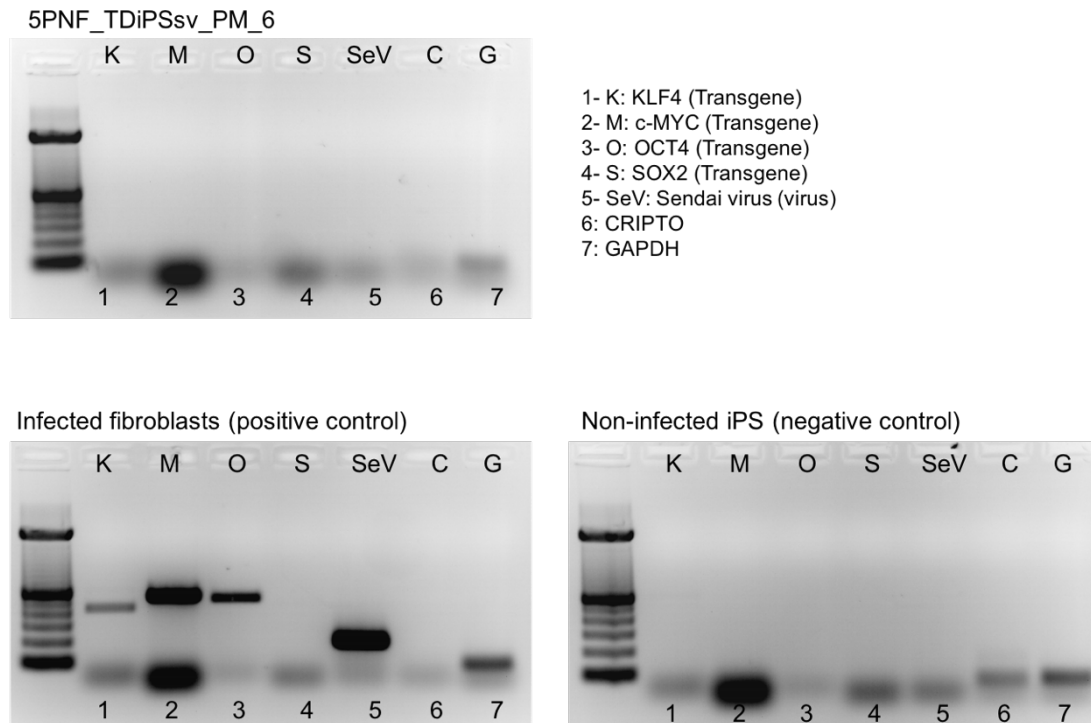
Tumor: 5PNF

Tumor fibroblast cells: 5PNF_F

iPS generated: 5PNF_TDiPSsv_PM_6

Annex 6

Integration/silencing test



RT-PCR analysis showing silencing of the transgenes KLF4, c-MYC, OCT4 and Sox2 and the absence of Sendai virus in the 5PNF_TDiPSsv_PM_6 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). The deletion is detected in fibroblasts from the patient and in the iPS line generated 5PNF_TDiPSsv_PM_6

