

ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR 3PNF_FiPSsv_PM_2 EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: 3PNF_FiPSsv_PM_2

Annex 1: Morphology and AP staining

Annex 2: Pluripotency markers by immunofluorescence

Annex 3: *In vitro* differentiation markers by
Immunofluorescence

Annex 4: Karyotype

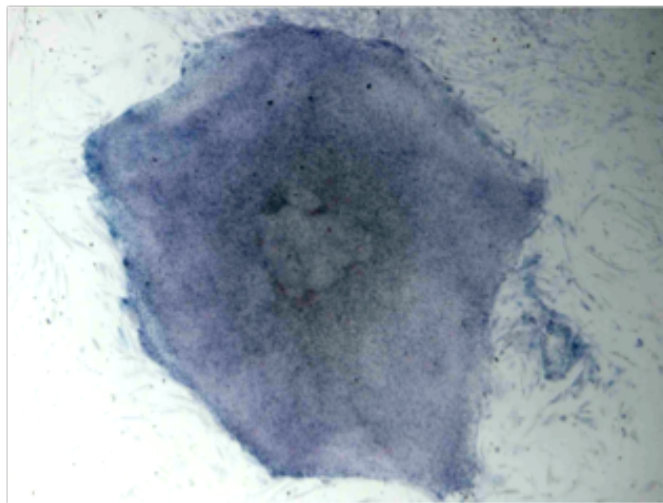
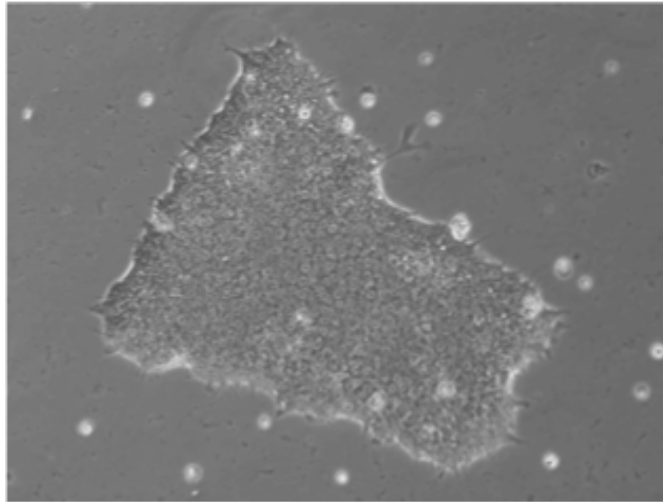
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Integration/silencing test

Annex 7: Genotype

Annex 1

Morphology and Alkaline phosphatase staining

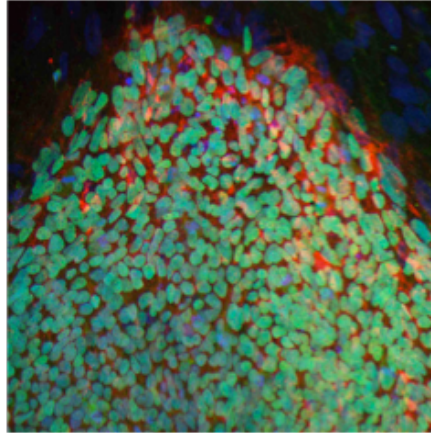


3PNF_FiPSsv_PM_2 Passage 1

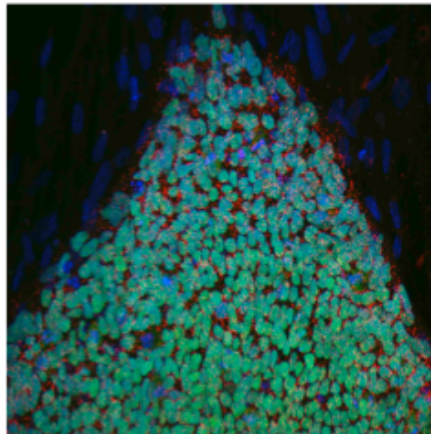
Annex 2

Pluripotency markers

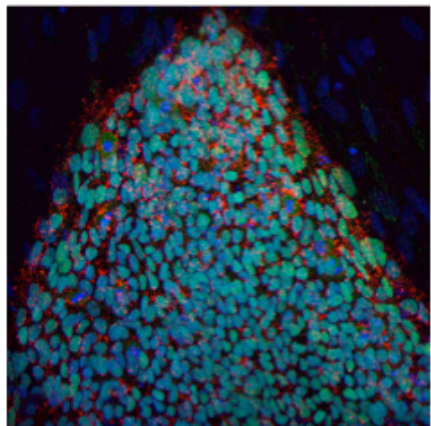
NANOG/TRA181



OCT4/SSEA3



SOX2/ SSEA4



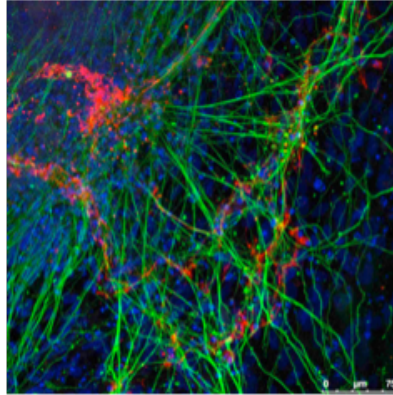
Immunofluorescence of pluripotency associated markers NANOG, TRA181, OCT4, SSEA3, SOX2 and SSEA4 in 3PNF_FiPSsv_PM_2 iPS at passage 10.

Annex 3

In vitro differentiation

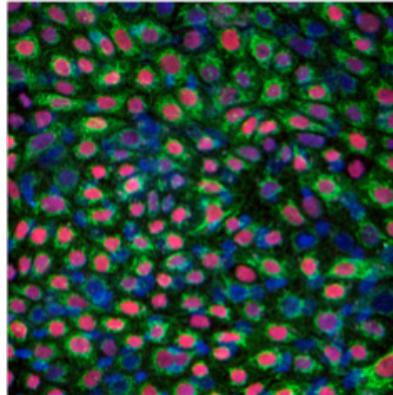
ECTODERM

TUJ1/TH



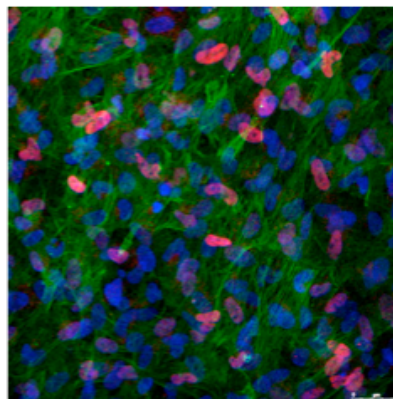
ENDODERM

aFETO/FOXA2



MESODERM

SMA/GATA4



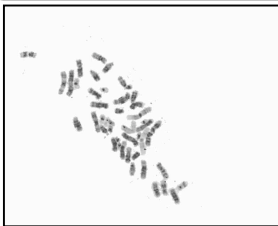
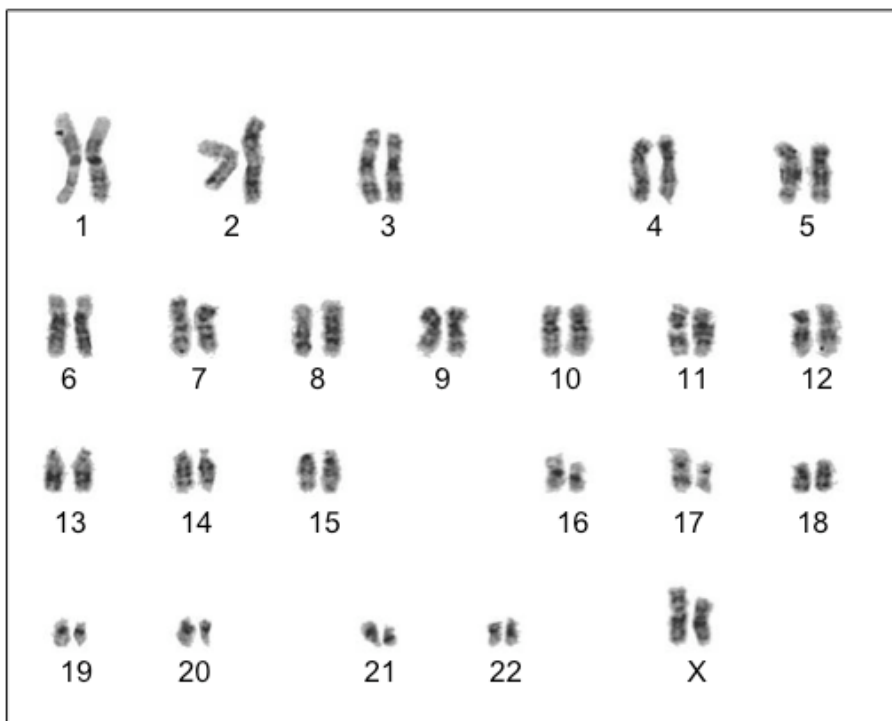
Immunofluorescence of differentiation associated markers TUJ1, TH for ectoderm; aFETO, FOXA2 for endoderm and SMA, GATA4 for mesoderm in 3PNF_FiPSsv_PM_2 iPS at passage 13.

Annex 4

Karyotype



Cytogenetic analysis



Case name: A157472

Patient name: 3PNF_FiPSsv_PM_2 passage 16

Result: 46,XX

Specimen type: stem cells

Annex 5

Authentication

AmpFISTR Identifier loci	3PNF	3PNF-F	3PNF_FiPSsv_PM_2
CSF1PO	11,14	11,14	11,14
D2S1338	17,24	17,24	17,24
D3S1358	15,16	15,16	15,16
D5S818	10,11	10,11	10,11
D7S820	10	10	10
D8S1179	10,14	10,14	10,14
D13S317	8,13	8,13	8,13
D16S539	9,12	9,12	9,12
D18S51	13,15	13,15	13,15
D19S433	12,14.2	12,14.2	12,14.2
D21S11	30,32.2	30,32.2	30,32.2
FGA	21,24	21,24	21,24
THO1	8,9.3	8,9.3	8,9.3
TPOX	8	8	8
vWA	16	16	16
Amelogenin (gender)	X	X	X

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

Tumor: 3PNF

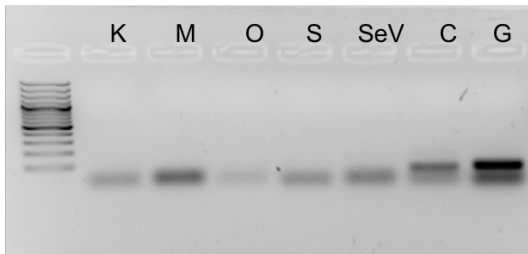
Parental reprogrammed fibroblasts cells: 3PNF-F

iPS generated: 3PNF_FiPSsv_PM_2

Annex 6

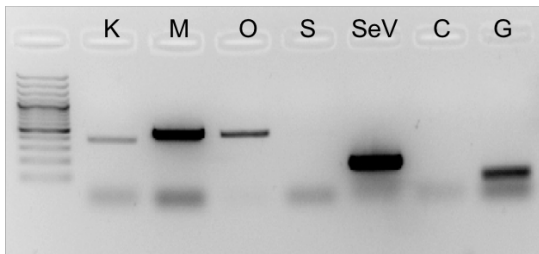
Integration/silencing test

3PNF_FiPSsv_PM_2

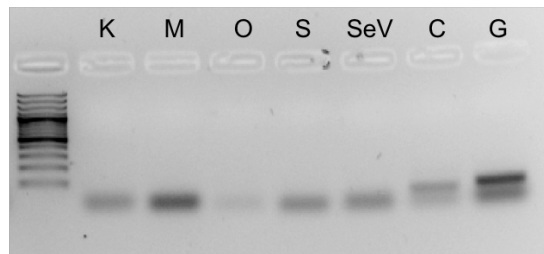


K: KLF4 (Transgene)
M: c-MYC (Transgene)
O: OCT4 (Transgene)
S: SOX2 (Transgene)
SeV: Sendai virus
C: CRIPTO
G: GAPDH

Infected fibroblasts (positive control)



Non-infected iPS (negative control)

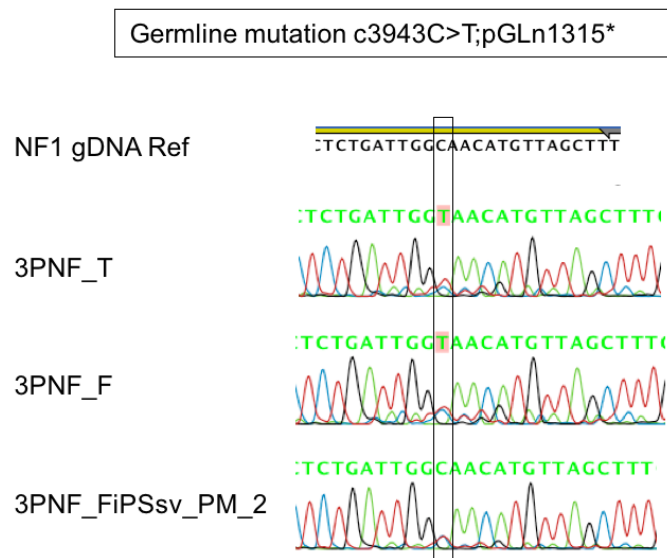


RT-PCR analysis showing silencing of the transgenes KLF4, c-MYC, OCT4 and Sox2 and the absence of Sendai virus in the 3PNF_FiPSsv_PM_2 iPS line.

Annex 7

Genotype

Germline mutation c3943C>T;pGLn1315*



Sanger sequencing showing the germline mutation c3943C>T;pGLn1315* in the NF1 gene. The germline mutation was found in the tumor (3PNF_T), in the reprogrammed primary fibroblasts (3PNF_F) and in the iPS line generated 3PNF_FiPSsv_PM_2.