

ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR 7PNF_SiPSrv_PM_12 EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: 7PNF_SiPSrv_PM_12

Annex 1: Morphology and AP staining

Annex 2: Pluripotency markers by immunofluorescence

Annex 3: *In vitro* differentiation markers by
Immunofluorescence

Annex 4: Karyotype

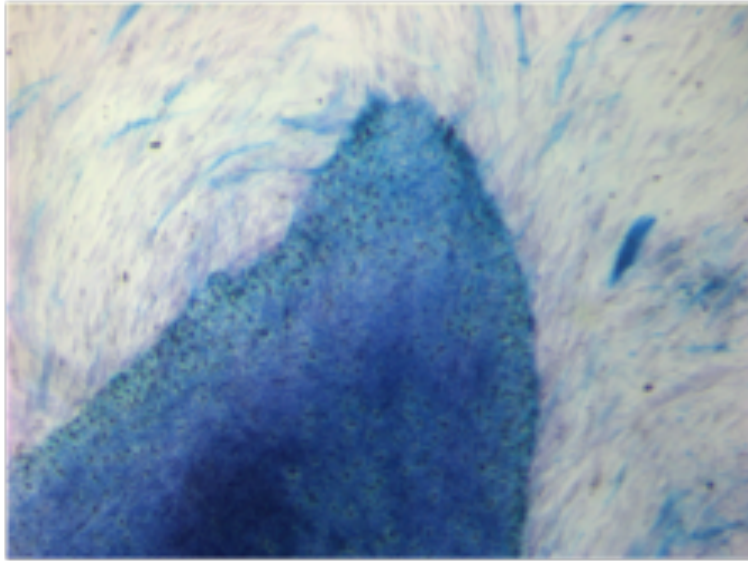
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Integration/silencing test

Annex 7: Genotype

Annex 1

Morphology and Alkaline phosphatase staining

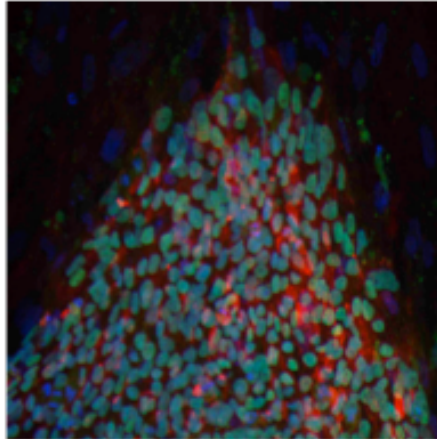


7PNF_SiPSrv_PM_12 passage 2

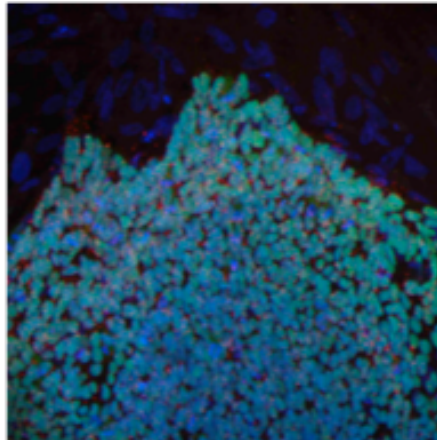
Annex 2

Pluripotency markers

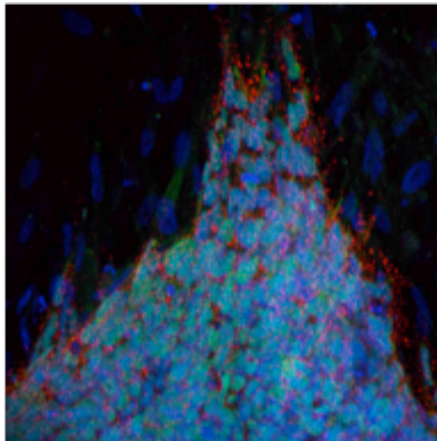
NANOG/TRA181



OCT4/SSEA3



SOX2/ SSEA4



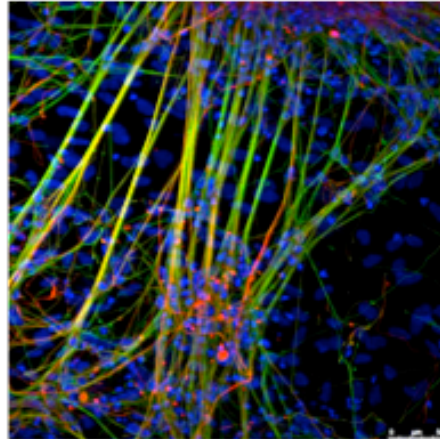
Immunofluorescence of pluripotency associated markers NANOG, TRA181, OCT4, SSEA3, SOX2 and SSEA4 in 7PNF_SiPSrv_PM_12 iPS at passage 10.

Annex 3

In vitro differentiation

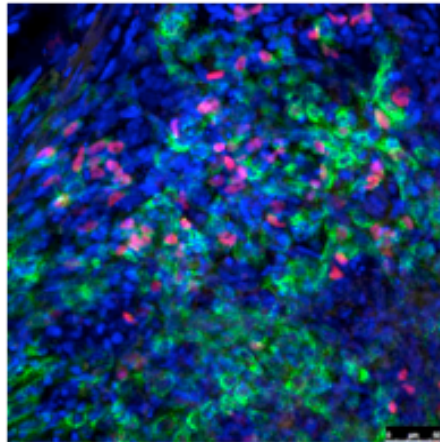
ECTODERM

TUJ1/GFAP



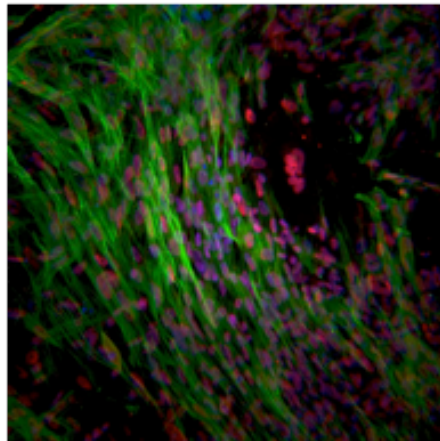
ENDODERM

a-FETO/FOXA2



MESODERM

SMA/GATA4



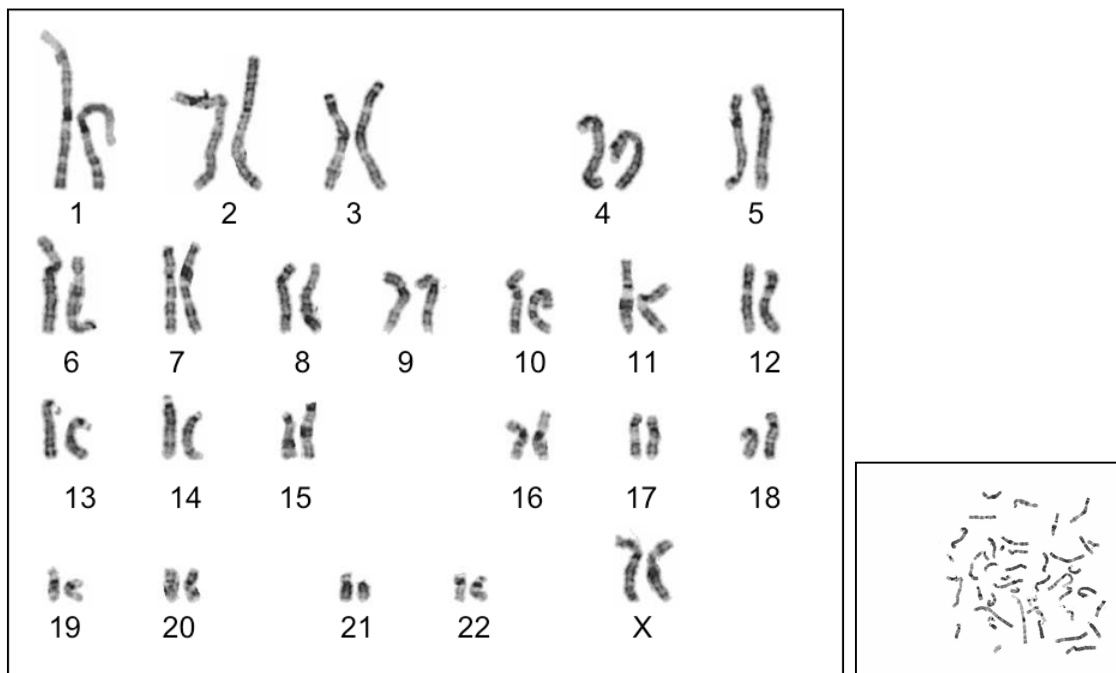
Immunofluorescence of differentiation associated markers TUJ1, GFAP for ectoderm; a-FETO, FOXA2 for endoderm and SMA, GATA4 for mesoderm in 7PNF_SiPSrv_PM_12 iPS at passage 12.

Annex 4

Karyotype



Cytogenetic analysis



Case name: A158000

Patient name: 7PNF_SiPSsv_PM_12 passage 9

Result: 46,XX

Specimen type: stem cells

Annex 5

Authentication

AmpFISTR Identifier loci	7PNF	7PNF-F	7PNF_SiPSrv_PM_12
CSF1PO	11,13	11,13	11,13
D2S1338	24	24	24
D3S1358	15,17	15,17	15,17
D5S818	10,12	10,12	10,12
D7S820	8,12	8,12	8,12
D8S1179	13,14	13,14	13,14
D13S317	8,13	8,13	8,13
D16S539	11,12	11,12	11,12
D18S51	12,15	12,15	12,15
D19S433	13,15	13,15	13,15
D21S11	29,30	29,30	29,30
FGA	20,25	20,25	20,25
THO1	6,9.3	6,9.3	6,9.3
TPOX	10,11	10,11	10,11
vWA	15,20	15,20	15,20
Amelogenin (gender)	X	X	X

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

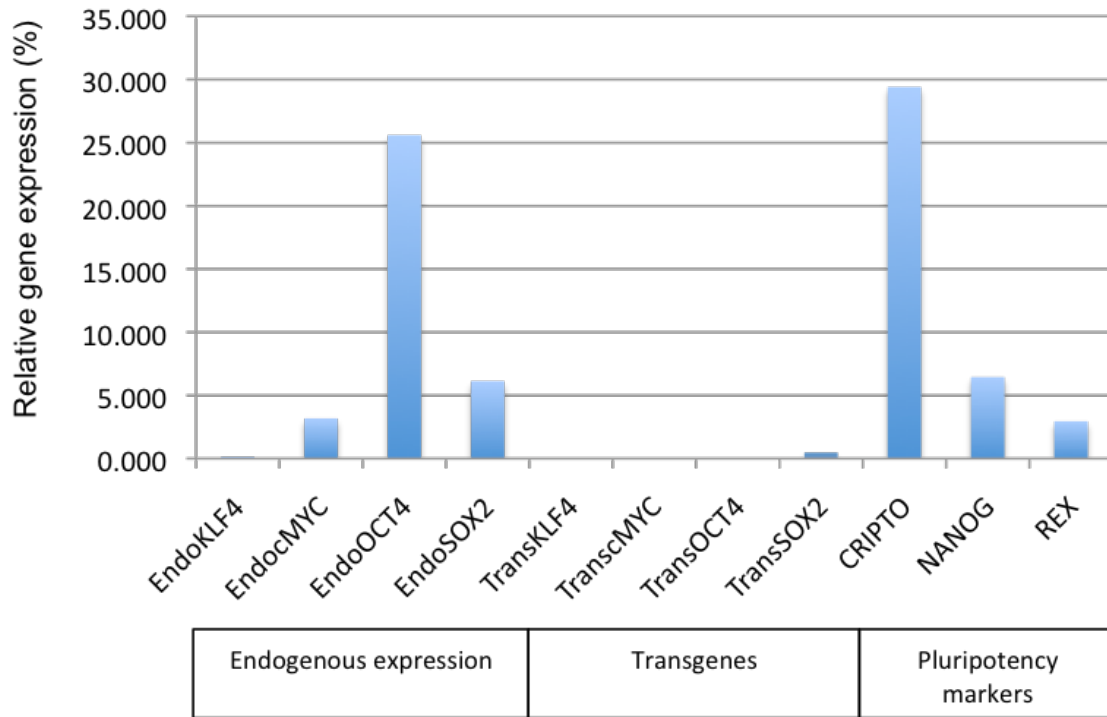
Tumor: 7PNF

Tumor fibroblast cells: 7PNF-F

iPS generated: 7PNF_SiPSrv_PM_12

Annex 6

Integration/silencing test

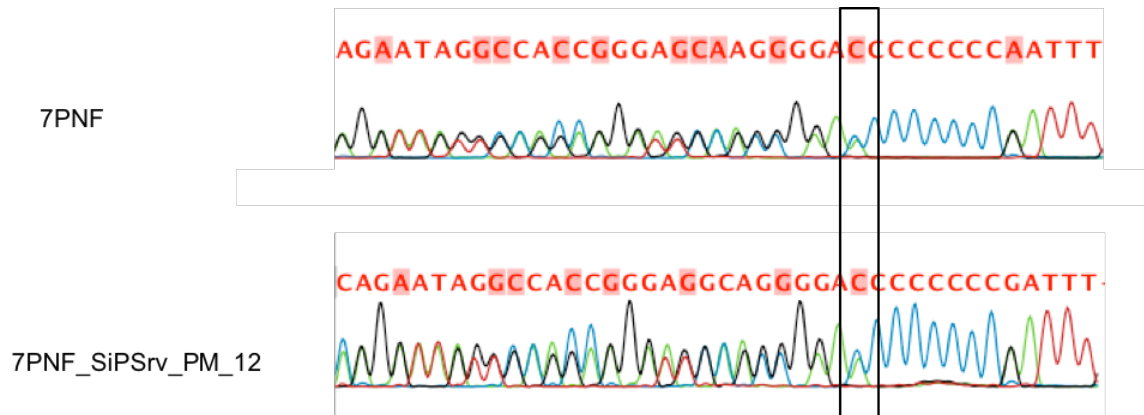


RT-PCR analysis showing endogenous expression of the 4 genes KLF4, c-MYC, OCT4 and Sox2 and the silencing of the transgenes in the 7PNF_SiPSrv_PM_12 iPS line. CRIPTO, NANOG and REX are use as pluripotency marker controls.

Annex 7

Genotype

Germline mutation:



Sanger sequencing showing the germline mutation present in the tumor, and iPS, 7PNF_SiPSrv_PM_12