ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR 6PNF_SiPSrv_PM_2 EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: 6PNF_SiPSrv_PM_2

Annex 1: Morphology and AP staining

Annex 2: Pluripotency markers by immunofluorescence

Annex 3: *In vitro* differentiation markers by Immunofluorescence

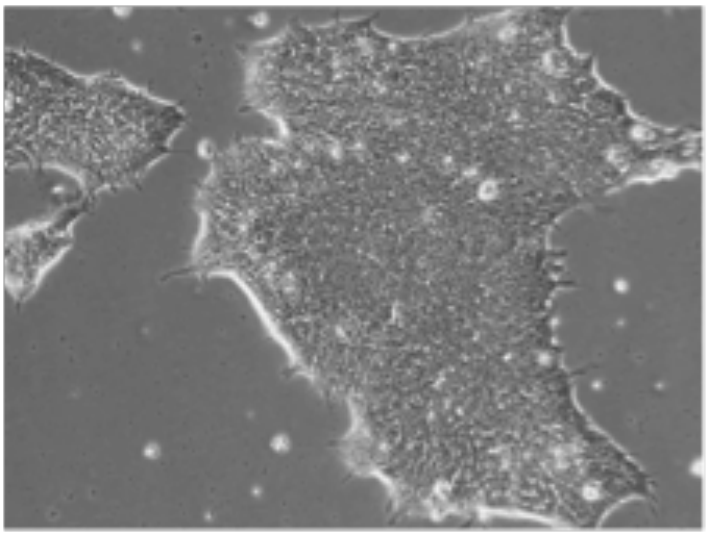
Annex 4: Karyotype

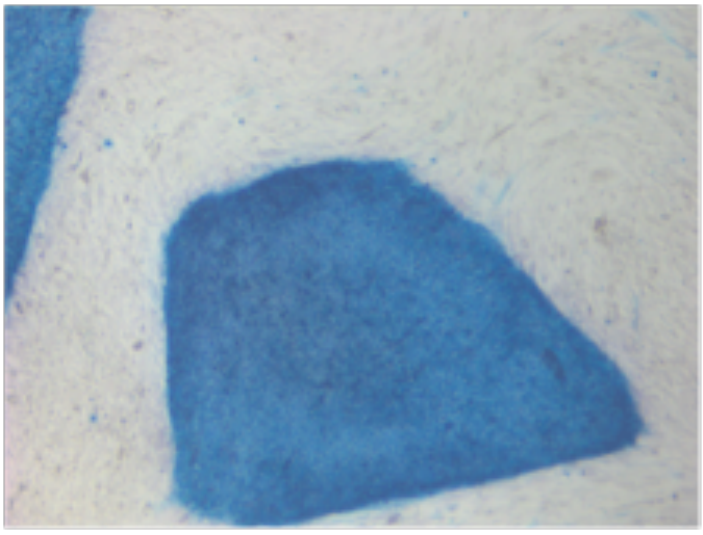
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Integration/silencing test

Annex 7: Genotype

Annex 1
Morphology and Alkaline phosphatase staining



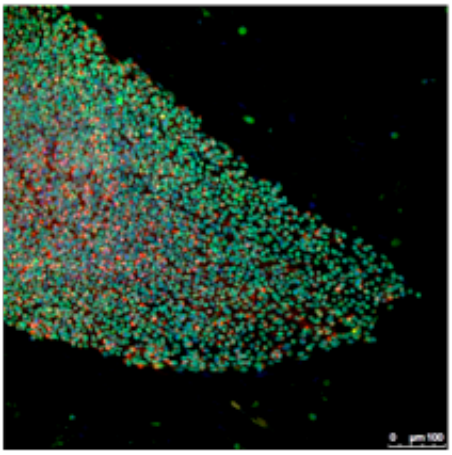


6PNF_SiPSrv_PM_2 passage 2

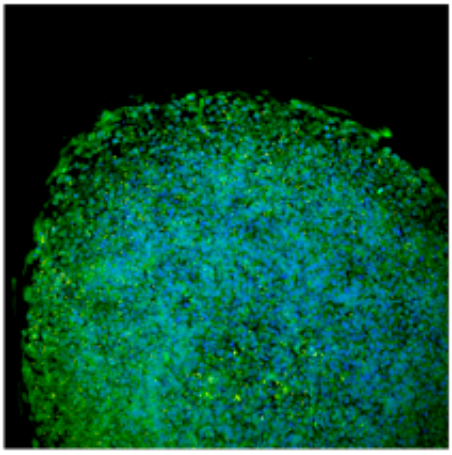
Annex 2

Pluripotency markers

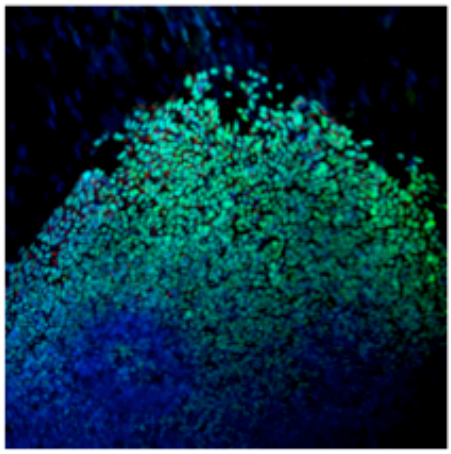
NANOG/TRA181



OCT4/SSEA3



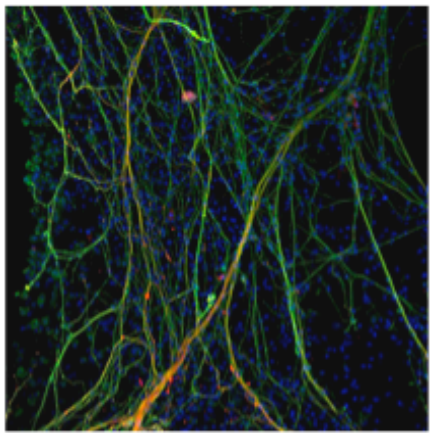
SOX2/ SSEA4



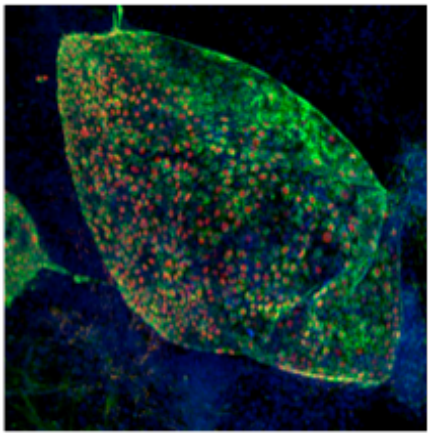
Immunofluorescence of pluripotency associated markers NANOG, TRA181, OCT4, SSEA3, SOX2 and SSEA4 in 6PNF_SiPSrv_PM_2 iPS at passage 9.

Annex 3
In vitro differentiation

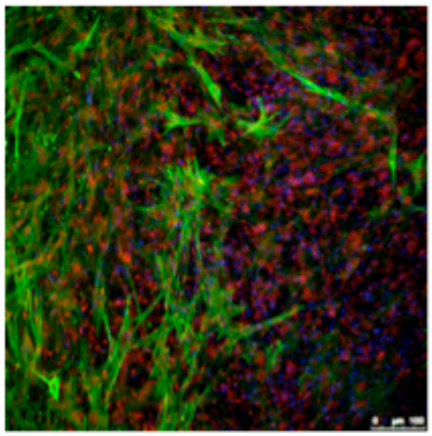
ECTODERM TUJ1/GFAP



ENDODERM a-FETO FOXA2



MESODERM SMA/GATA4

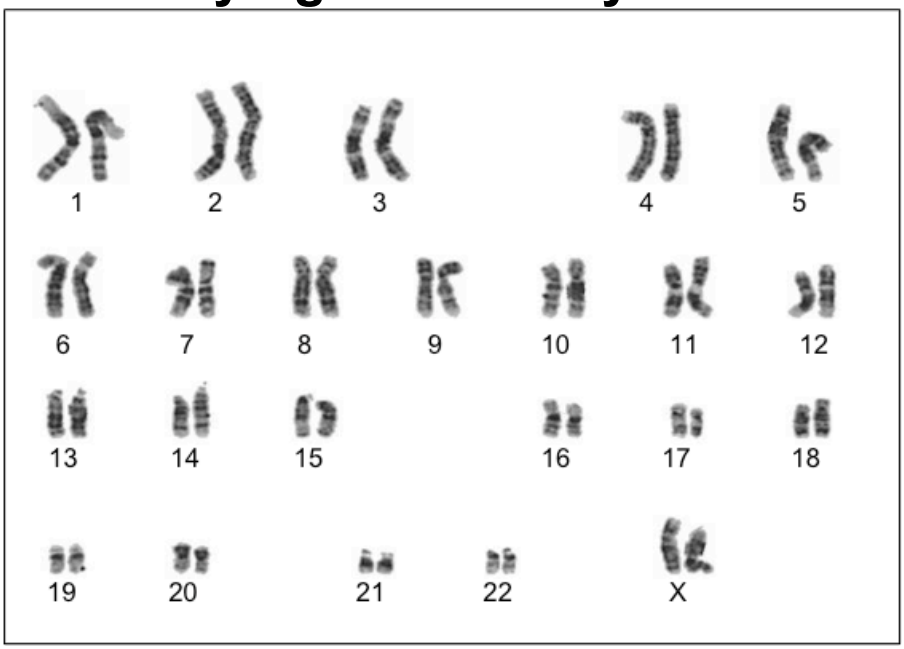


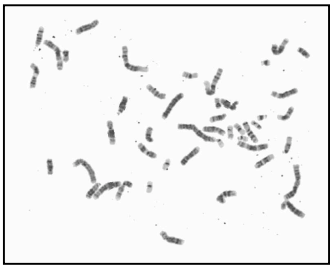
Immunofluorescence of differentiation associated markers TUJ1, GFAP for ectoderm; a-FETO, FOXA2 for endoderm and SMA, GATA4 for mesoderm in 6PNF_SiPSrv_PM_2 iPS at passage 12.

Annex 4
Karyotype

Ambar
ANÀLISIS MÈDIQUES
BARCELONA

Cytogenetic analysis





Case name: A159016
Patient name: 6PNF_SiPSsv_PM_2 passage 4
Result: 46,XX
Specimen type: stem cells

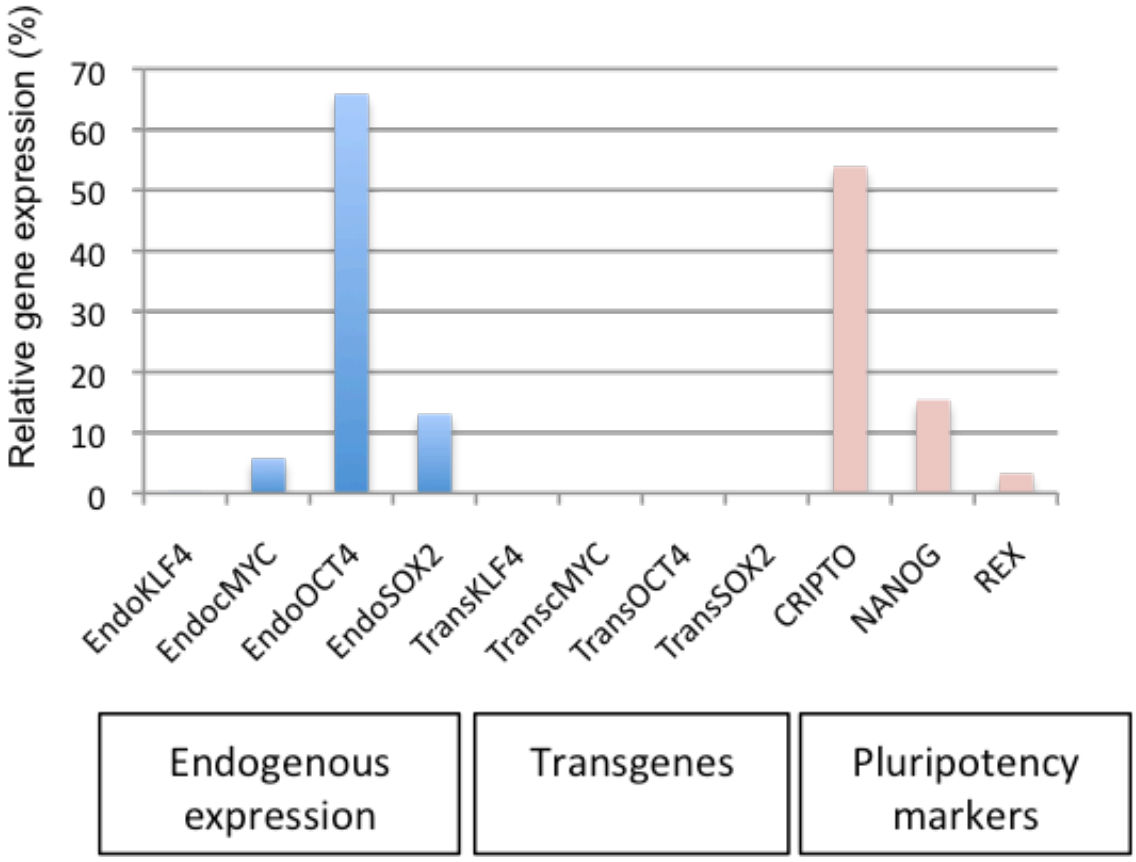
Annex 5
Authentication

AmpFISTR Identifiler loci	6PNF	6PNF-F	6PNF_SiPSrv_PM_2
CSF1PO	10,12	10,12	10,12
D2S1338	17,23	17,23	17,23
D3S1358	16,18	16,18	16,18
D5S818	11,13	11,13	11,13
D7S820	11	11	11
D8S1179	10,12	10,12	10,12
D13S317	11,14	11,14	11,14
D16S539	9,13	9,13	9,13
D18S51	11,13	11,13	11,13
D19S433	13,14	13,14	13,14
D21S11	29,30.2	29,30.2	29,30.2
FGA	21,27	21,27	21,27
THO1	7,9.3	7,9.3	7,9.3
TPOX	9,11	9,11	9,11
vWA	17	17	17
Amelogenin (gender)	X	X	X

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

Tumor: 6PNF
Tumor fibroblast cells: 6PNF-F
iPS generated: 6PNF_SiPSrv_PM_2

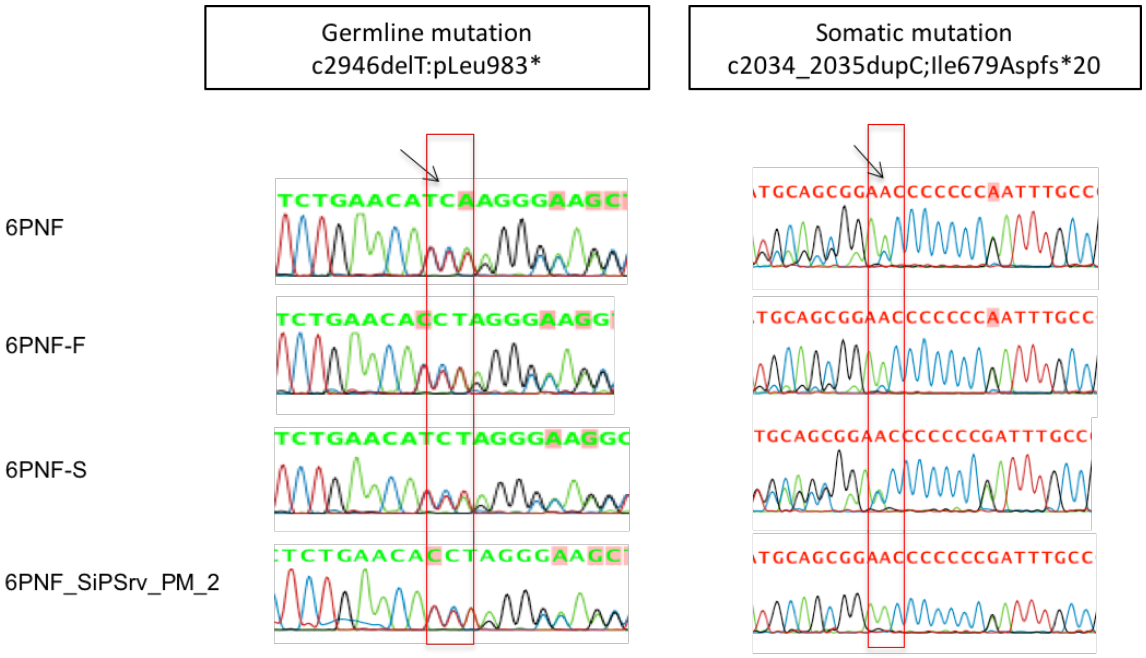
Annex 6
Integration/silencing test



RT-PCR analysis showing endogenus expression of the 4 genes KLF4, c-MYC, OCT4 and Sox2 and the silencing of the transgenesin the 6PNF_SiPSrv_PM_2 iPS line. CRIPTO, NANOG and REX are use as pluripotency mrker controls.

Annex 7
Genotype

Germline mutation: c2946delT;pLeu983*
Somatic tumor mutation: c2034_2035dupC;Ile679Aspsf*20



Sanger sequencing showing the germline mutation present in the tumor (6PNF), fibroblasts (6PNF-F) and iPS, and the tumor somatic mutation only present in the Schwann cells (6PNF-S), and not in the fibroblasts nor in iPS line 6PNF_SiPSrv_PM_2.