

ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR CT_PBiPS3_Sv4F_1* EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: CT_PBiPS3_Sv4F_1*

***(CTL3 in the publication)**

Annex 1: Morphology and AP staining

Annex 2: Pluripotency markers by immunofluorescence

Annex 3: *In vitro* differentiation markers by
Immunofluorescence

Annex 4: Karyotype

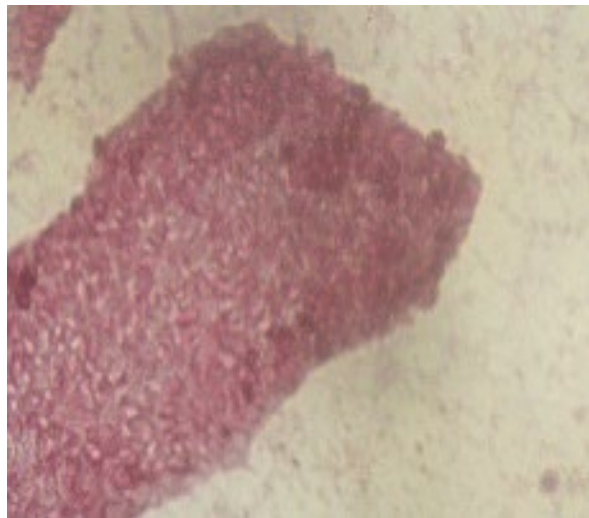
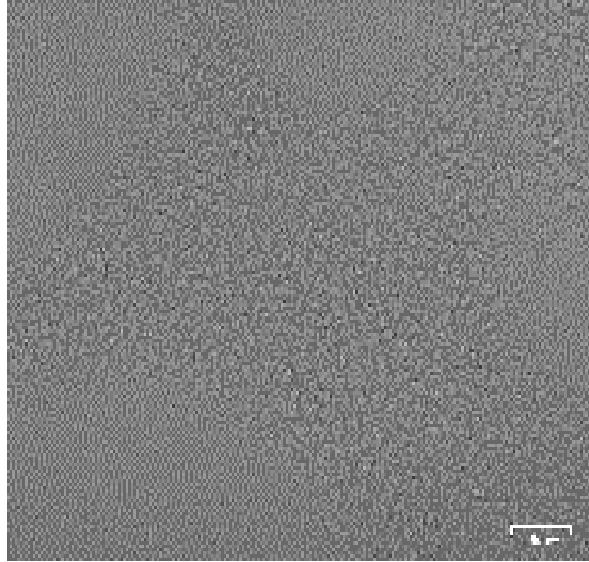
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Integration/silencing test

Annex 7: Mycoplasma test

Annex 1

Morphology and Alkaline phosphatase staining

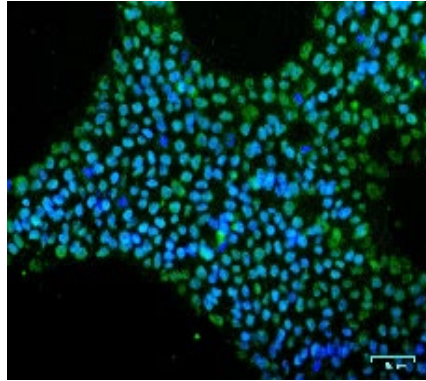


CT_PBiPS3_Sv4F_1 Passage 1

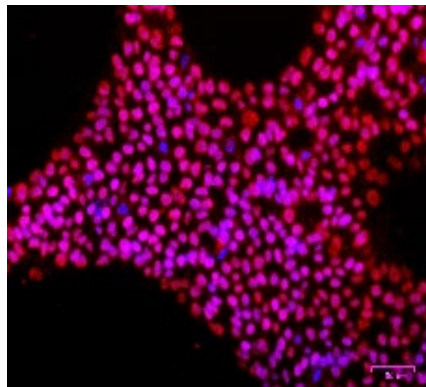
Annex 2

Pluripotency markers

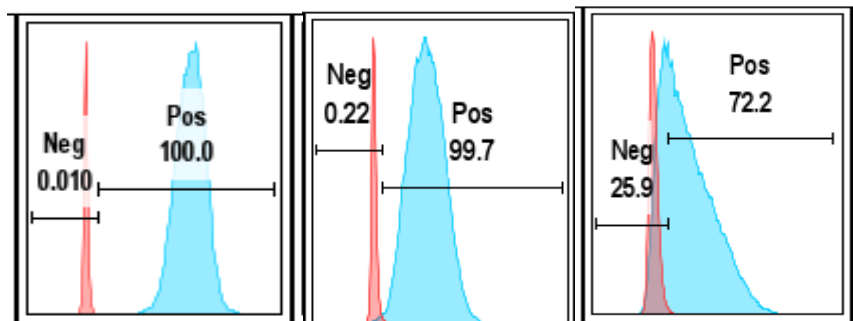
NANOG



OCT4



SSEA-4 / TRA 1-60 / TRA 1-81



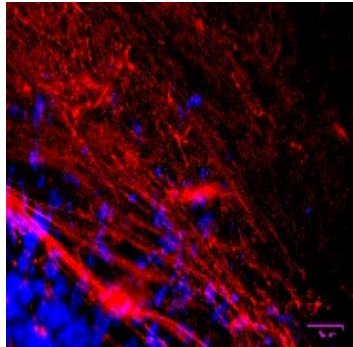
Immunofluorescence of pluripotency associated markers NANOG and OCT4, and fluorescence-activated cell sorting (FACS) of pluripotency markers SSEA4, TRA 1-60 and TRA 1-81 in CT_PBiPS3_Sv4F_1 at passage 10.

Annex 3

In vitro differentiation

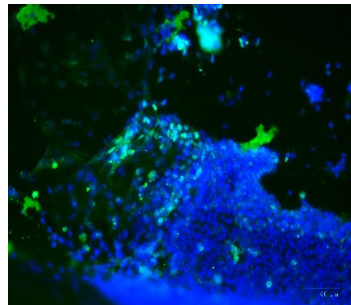
ECTODERM

TUJ1



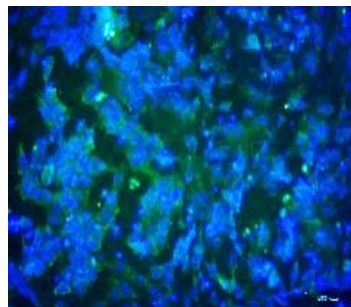
ENDODERM

SOX17



MESODERM

SMA

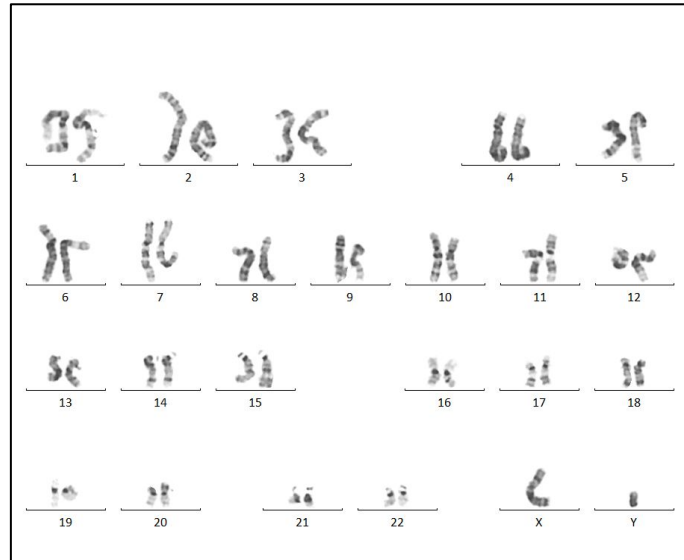


Immunofluorescence of differentiation associated markers TUJ1, for ectoderm; SOX17 for endoderm and SMA for mesoderm in CT_PBiPS3_Sv4F_1 at passage 12.

Annex 4

Karyotype

Cytogenetic analysis



Patient name: CT_PBiPS3_Sv4F_1 passage 15

Result: 46, XY

Specimen type: iPSC

Annex 5

Authentication

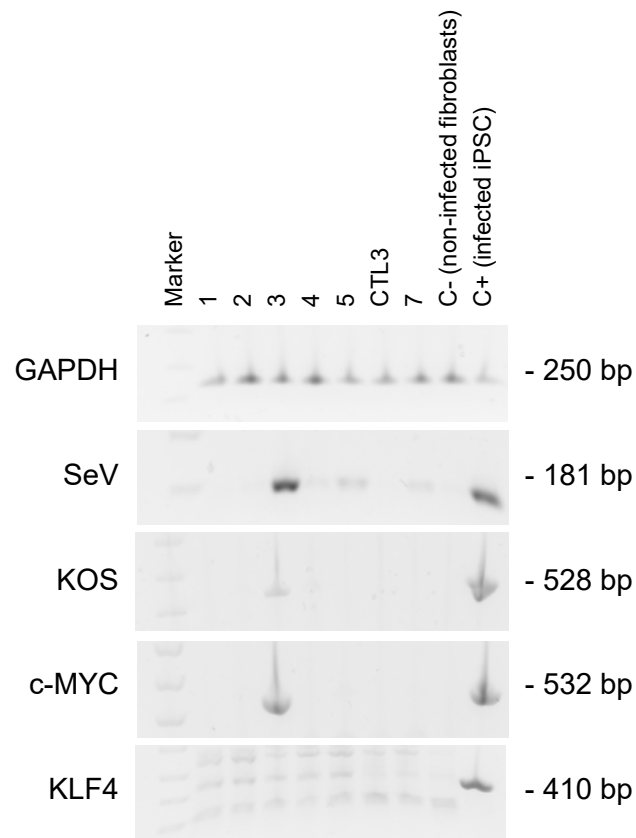
AmpFISTR Identifier Loci	CTL3 PBMC	CTL3 iPSC
CSF1PO	11,12	11,12
D2S1338	20,23	20,23
D3S1358	15,16	15,16
D5S818	7,12	7,12
D7S820	10,11	10,11
D8S1179	11,15	11,15
D13S317	10,13	10,13
D16S539	11	11
D18S51	15,17	15,17
D19S433	12.2,14.2	12.2,14.2
D21S11	28,3	28,3
FGA	21	21
TH01	6,7	6,7
TPOX	8,12	8,12
vWA	16	16
Amelogenin (gender)	X,Y	X,Y

Microsatellite analysis results. Method used: AmpFISTR® Identifier® Plus PCR Amplification Kit (Applied Biosystems, cat #: 4427368).

Parental reprogrammed PBMC cells: CTL3 PBMC
iPS generated: CTL3 iPSC (CT_PBiPS3_Sv4F_1)

Annex 6

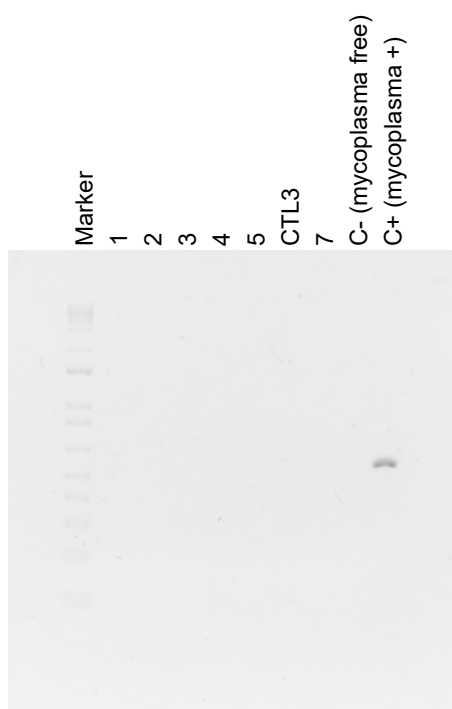
Integration / silencing test



RT-PCR analysis showing the absence of Sendai virus and the silencing of the transgenes KOS (KLF4, OCT4 and Sox2), c-MYC, KLF4 and in the CT_PBiPS3_Sv4F_1 (CTL3) iPS line.

Annex 7

Mycoplasma test



PCR analysis showing the absence of mycoplasma in the CT_PBiPS3_Sv4F_1 (CTL3) iPS line.