ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR CT_FiPS3_Sv4F_1* EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: CT_FiPS3_Sv4F_1*

***(CTL4 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

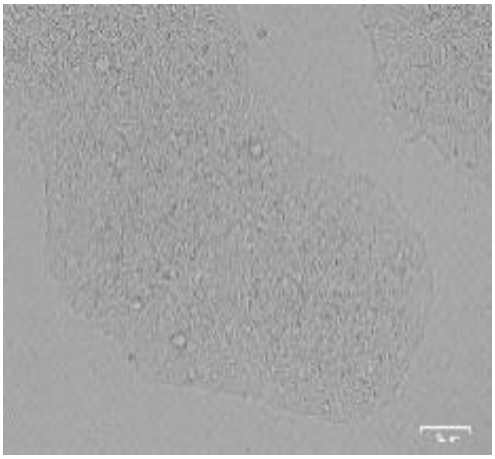
Annex 5: Authentication. Fingerprinting analysis

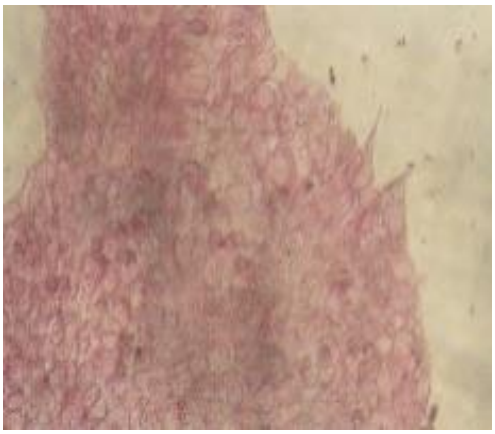
Annex 6: Integration/silencing test

Annex 7: Mycoplasma test

Annex 1

Morphology and Alkaline phosphatase staining

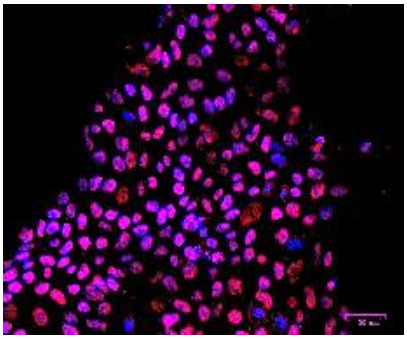




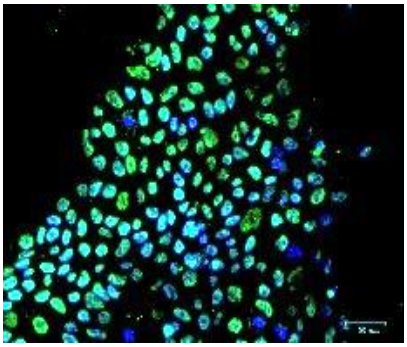
CT_FiPS3_Sv4F_1 Passage 1

Annex 2
Pluripotency markers

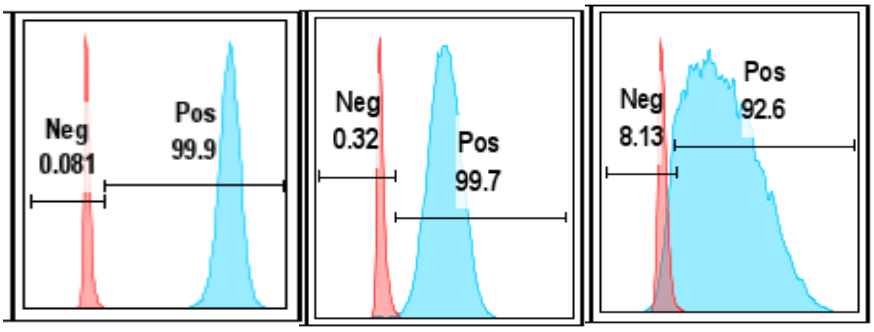
NANOG



OCT4



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



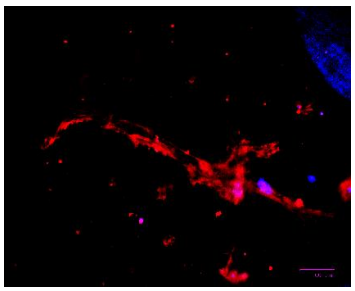
Immunofluorescence of pluripotency associated markers NANOG and OCT4, and fluorescence-activated cell sorting (FACS) of pluripotency markers SSEA4, TRA 1-60 and TRA 1-81 in CT_FiPS3_Sv4F_1 at passage 10.

Annex 3

In vitro differentiation

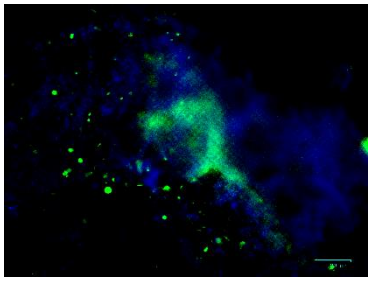
ECTODERM

TUJ1



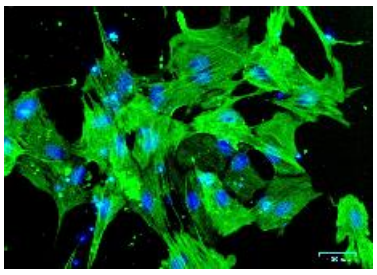
ENDODERM

SOX17



MESODERM

SMA

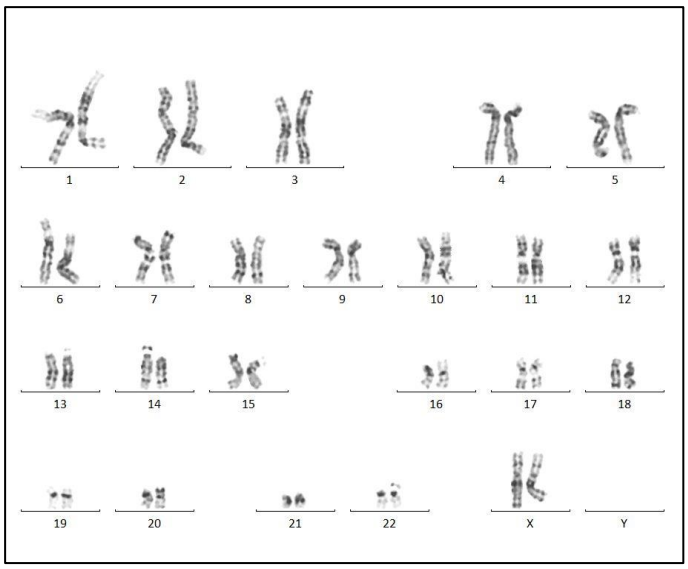


Immunofluorescence of differentiation associated markers TUJ1, for ectoderm; SOX17 for endoderm and SMA for mesoderm in CT_FiPS3_Sv4F_1 at passage 12.

Annex 4

Karyotype

Cytogenetic analysis



Patient name: CT_FiPS3_Sv4F_1 passage 14

Result: 46, XX

Specimen type: iPSC

Annex 5

Authentication

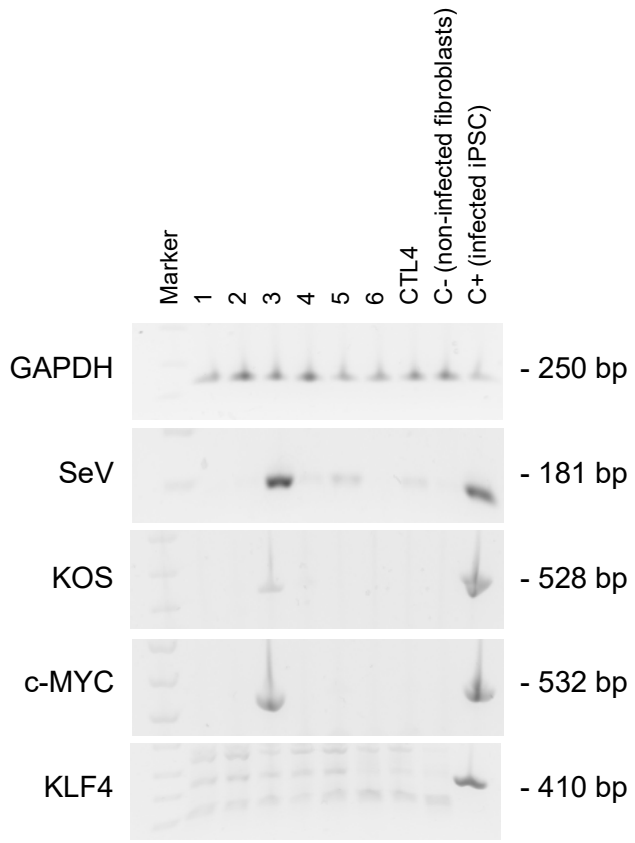
AmpFlSTR Identifier Loci	CTL4 fibroblasts	CTL4 iPSC
CSF1PO	10,12	10,12
D2S1338	17,2	17,2
D3S1358	16,17	16,17
D5S818	12	12
D7S820	9,1	9,1
D8S1179	8,14	8,14
D13S317	11,13	11,13
D16S539	9,11	9,11
D18S51	13,18	13,18
D19S433	13,16	13,16
D21S11	29,32.2	29,32.2
FGA	21,22	21,22
TH01	9.3	9.3
TPOX	8	8
vWA	14,16	14,16
Amelogenin (gender)	X	X

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

Parental reprogrammed fibroblasts cells: CTL4 fibroblasts
iPS generated: CTL4 iPSC (CT_FiPS3_Sv4F_1)

Annex 6

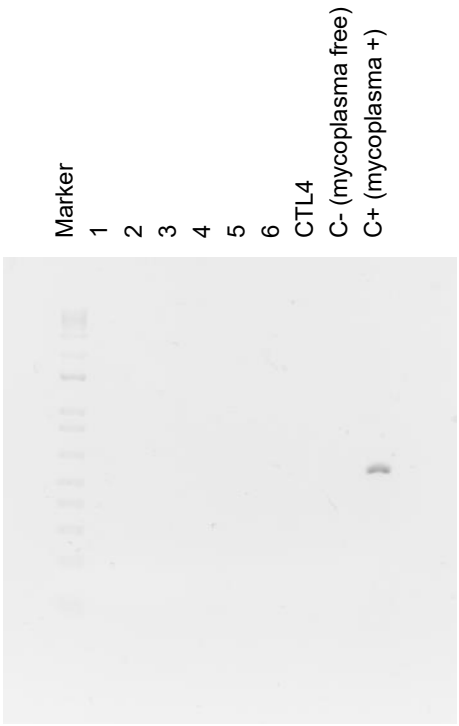
Integration / silencing test



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_FiPS3_Sv4F_1 (CTL4) iPS line.

Annex 7

Mycoplasma test



PCR analysis showing the absence of mycoplasma in the CT_FiPS3_Sv4F_1 (CTL4) iPS line.