# ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR CT_PBiPS2_Sv4F_1* EN EL BANCO NACIONAL DE LÍNEAS CELULARES

## Annexes iPSC line: CT_PBiPS2_Sv4F_1*

***(CTL3 in the publication)**

Annex 1: Morphology and AP staining

Annex 2: Pluripotency markers by immunofluorescence

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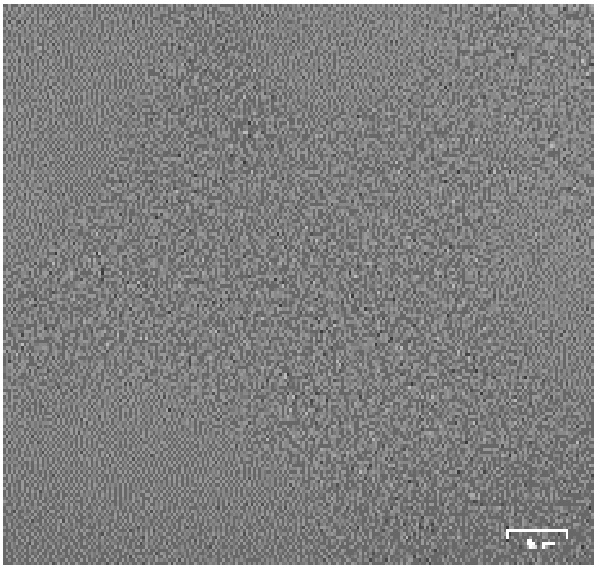
# Annex 1

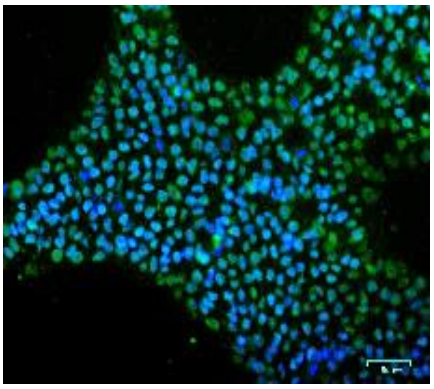
# Morphology and Alkaline phosphatase staining



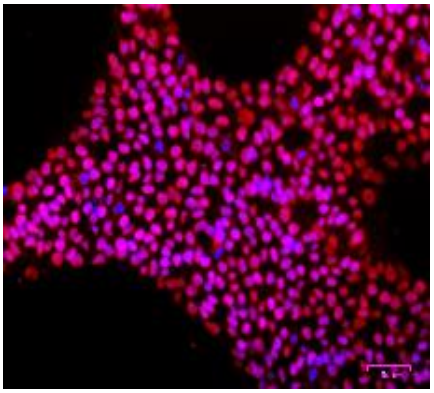
CT_PBiPS2_Sv4F_1 Passage 1

# Annex 2
# Pluripotency markers

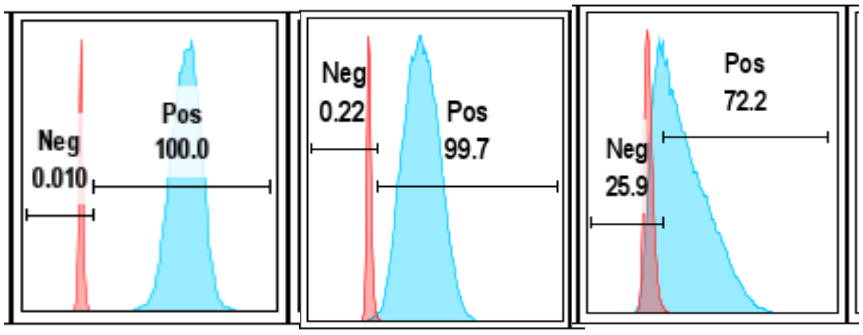
NANOG



OCT4



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



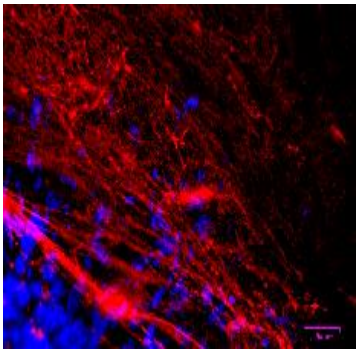

Immunofluorescence of pluripotency associated markers NANOG and OCT4, and fluorescence-activated cell sorting (FACS) of pluripotency markers SSEA4, TRA 1-60 and TRA 1-81 in CT_PBiPS2_Sv4F_1 at passage 10.

# Annex 3

# *In vitro* differentiation

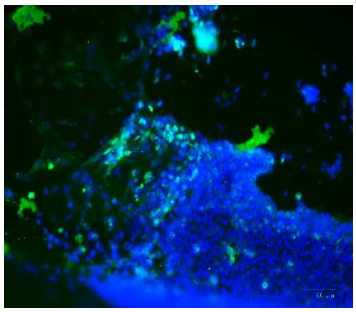
ECTODERM

**TUJ1**



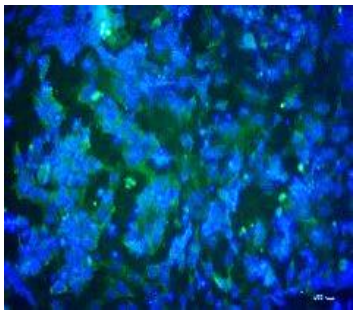
ENDODERM

**SOX17**



MESODERM

**SMA**

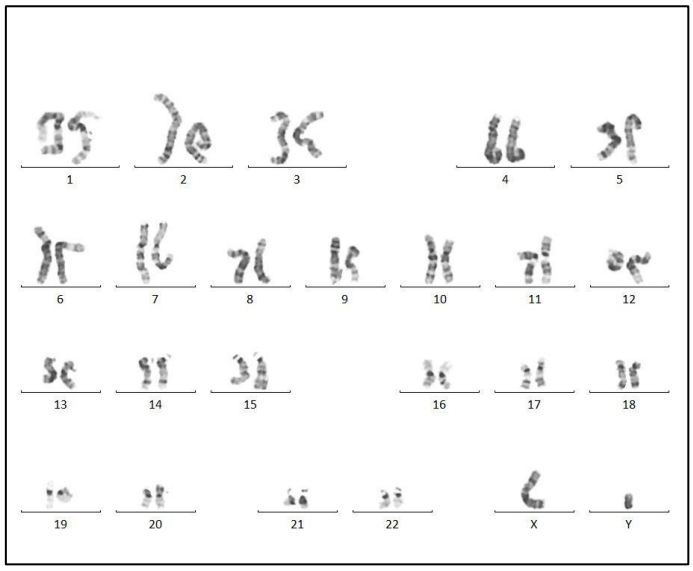


Immunofluorescence of differentiation associated markers TUJ1, for ectoderm; SOX17 for endoderm and SMA for mesoderm in CT_PBiPS2_Sv4F_1 at passage 12.

# Annex 4

# Karyotype

## Cytogenetic analysis




Patient name: CT_PBiPS2_Sv4F_1 passage 15

Result: 46, XY

Specimen type: iPSC

# Annex 5

# Authentication

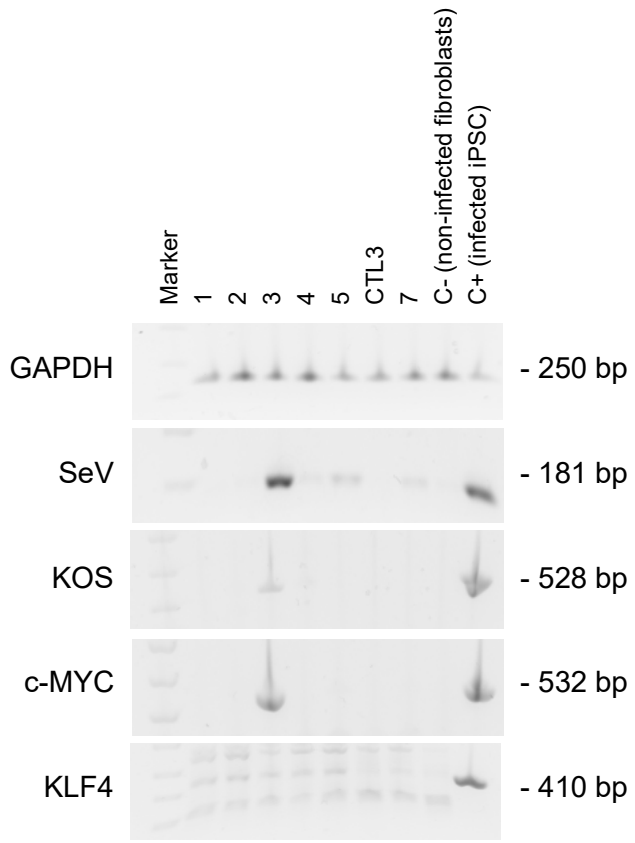
| AmpFlSTR Identifier Loci | CTL3 PBMC | CTL3 iPSC |
|---|---|---|
| **CSF1PO** | 11,12 | 11,12 |
| **D2S1338** | 20,23 | 20,23 |
| **D3S1358** | 15,16 | 15,16 |
| **D5S818** | 7,12 | 7,12 |
| **D7S820** | 10,11 | 10,11 |
| **D8S1179** | 11,15 | 11,15 |
| **D13S317** | 10,13 | 10,13 |
| **D16S539** | 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: AmpFlSTR® Identifiler® Plus PCR Amplification Kit (Applied Biosystems, cat #: 4427368).

Parental reprogrammed PBMC cells: CTL3 PBMC
iPS generated: CTL3 iPSC (CT_PBiPS2_Sv4F_1)

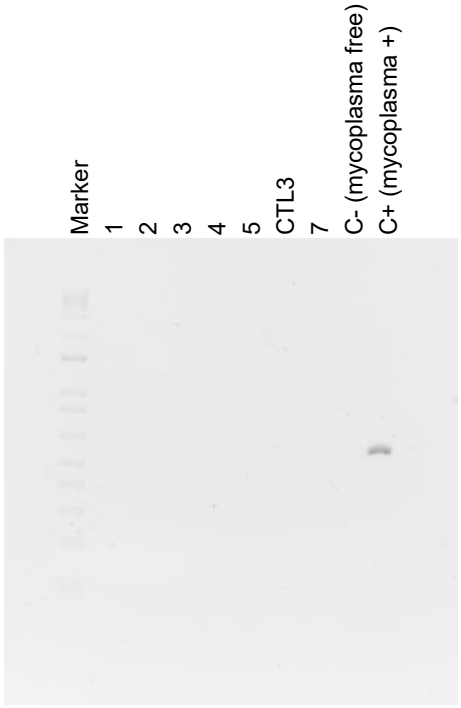
# Annex 6

# 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_PBiPS2_Sv4F_1 (CTL3) iPS line.

# Annex 7
# Mycoplasma test




PCR analysis showing the absence of mycoplasma in the CT_PBiPS2_Sv4F_1 (CTL3) iPS line.