

# **ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR IBM\_FiPS3\_Sv4F\_1\* EN EL BANCO NACIONAL DE LÍNEAS CELULARES**

## **Annexes iPSC line: IBM\_FiPS3\_Sv4F\_1\***

**\*(IBM3 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

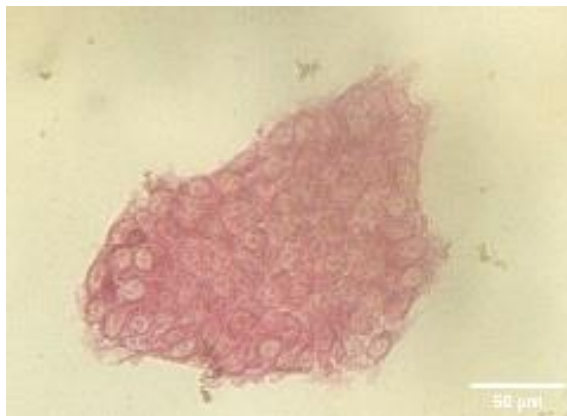
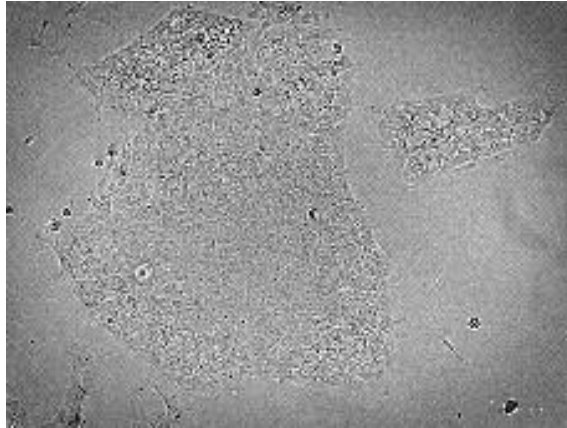
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Integration/silencing test

Annex 7: Mycoplasma test

# Annex 1

## Morphology and Alkaline phosphatase staining

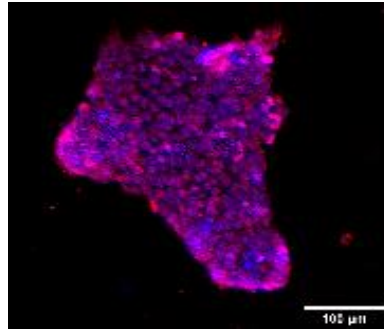


IBM\_FiPS3\_Sv4F\_1 Passage 1

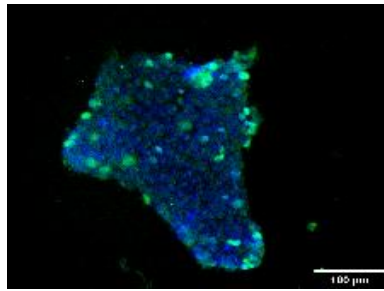
# Annex 2

## Pluripotency markers

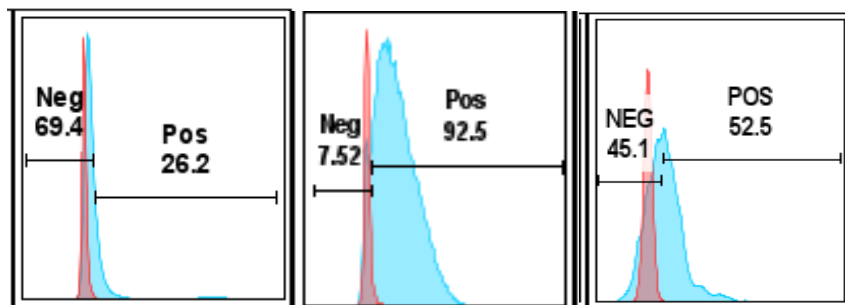
NANOG



OCT4



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



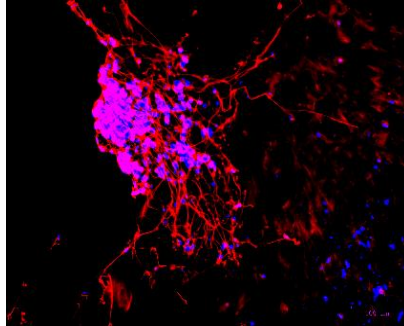
Immunofluorescence of pluripotency associated markers NANOG and OCT4, and fluorescence-activated cell sorting (FACS) of pluripotency markers SSEA4, TRA 1-60 and TRA 1-81 in IBM\_FiPS3\_Sv4F\_1 at passage 10.

## Annex 3

### *In vitro* differentiation

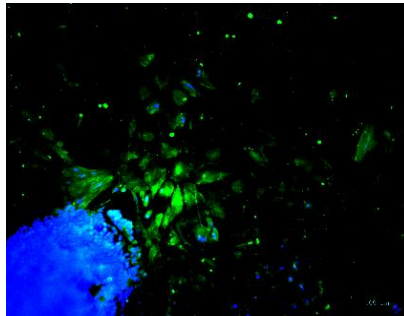
ECTODERM

TUJ1



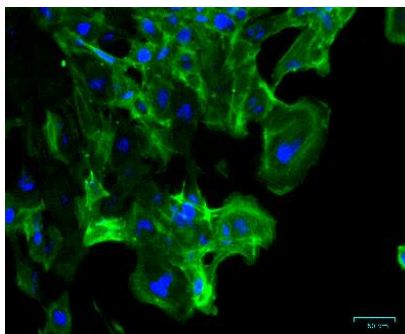
ENDODERM

SOX17



MESODERM

SMA

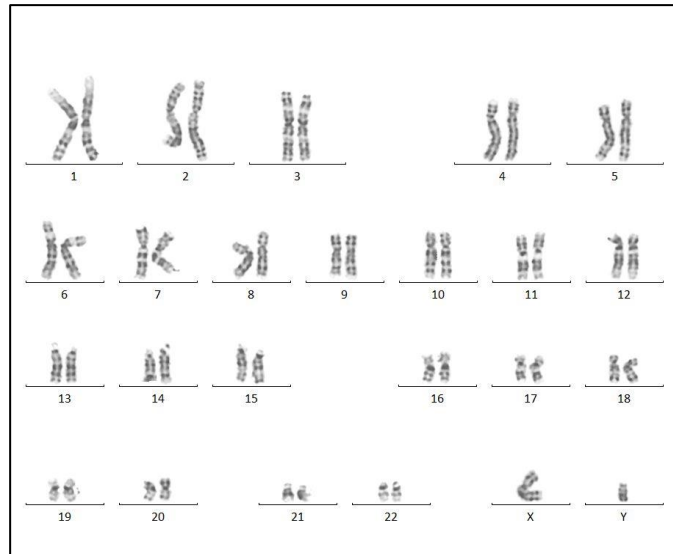


Immunofluorescence of differentiation associated markers TUJ1, for ectoderm; SOX17 for endoderm and SMA for mesoderm in IBM\_FiPS3\_Sv4F\_1 at passage 12.

# Annex 4

## Karyotype

### Cytogenetic analysis



Patient name: IBM\_FiPS3\_Sv4F\_1 passage 15

Result: 46, XY

Specimen type: iPSC

# Annex 5

## Authentication

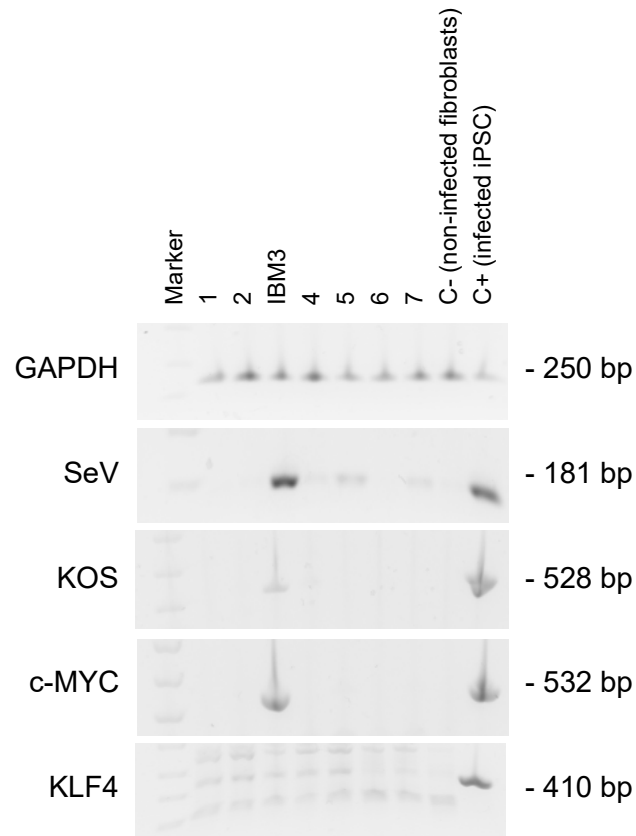
<b>AmpFISTR Identifier Loci</b>	<b>IBM3 fibroblasts</b>	<b>IBM3 iPSC</b>
<b>CSF1PO</b>	12,13	12,13
<b>D2S1338</b>	20	20
<b>D3S1358</b>	16,18	16,18
<b>D5S818</b>	9	9
<b>D7S820</b>	10	10
<b>D8S1179</b>	11,12	11,12
<b>D13S317</b>	12	12
<b>D16S539</b>	9,11	9,11
<b>D18S51</b>	15,16	15,16
<b>D19S433</b>	14,15	14,15
<b>D21S11</b>	29,31	29,31
<b>FGA</b>	22	22
<b>TH01</b>	8,9	8,9
<b>TPOX</b>	8	8
<b>vWA</b>	15,16	15,16
<b>Amelogenin (gender)</b>	X,Y	X,Y

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

Parental reprogrammed fibroblasts cells: IBM3 fibroblasts  
 iPS generated: IBM3 iPSC (IBM\_FiPS3\_Sv4F\_1)

# Annex 6

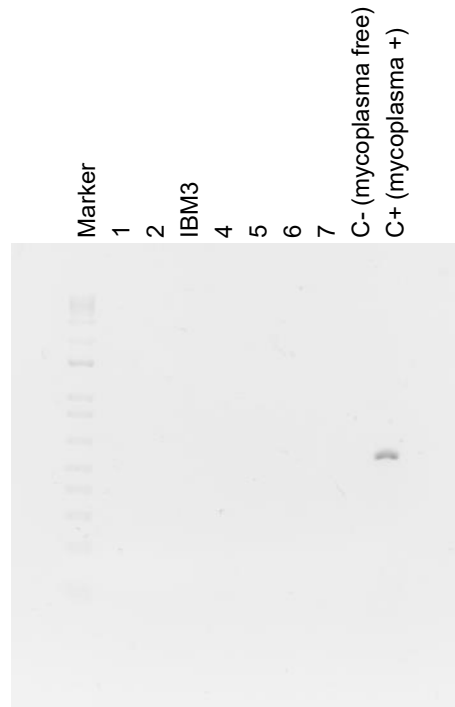
## Integration / silencing test



RT-PCR analysis showed that IBM\_FiPS3\_Sv4F\_1 (IBM3) iPS line expressed the Sendai virus and slightly the transgenes KOS (KLF4, OCT4 and Sox2) and c-MYC after multiple passages. KLF4 transgene was silenced.

# Annex 7

## Mycoplasma test



PCR analysis showing the absence of mycoplasma in the IBM\_FiPS3\_Sv4F\_1 (IBM3) iPS line.