ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR IBM_FiPS1_Sv4F_1* EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: IBM_FiPS1_Sv4F_1*

***(IBM1 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

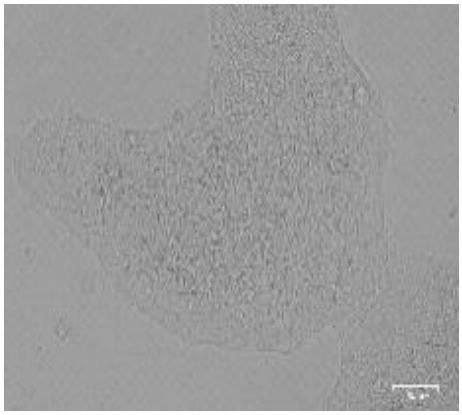
Annex 5: Authentication. Fingerprinting analysis

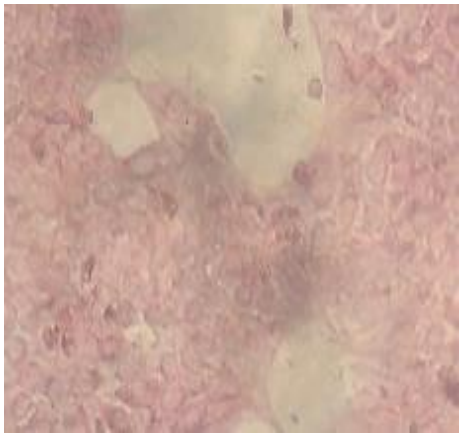
Annex 6: Integration/silencing test

Annex 7: Mycoplasma test

Annex 1

Morphology and Alkaline phosphatase staining

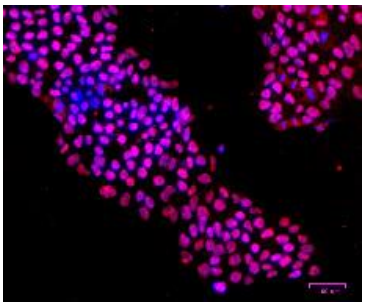




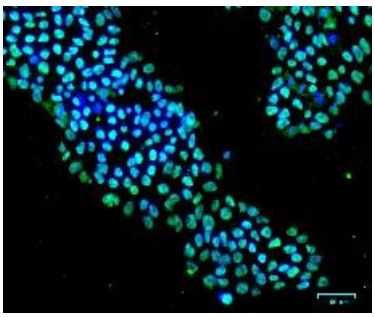
IBM_FiPS1_Sv4F_1 Passage 1

Annex 2
Pluripotency markers

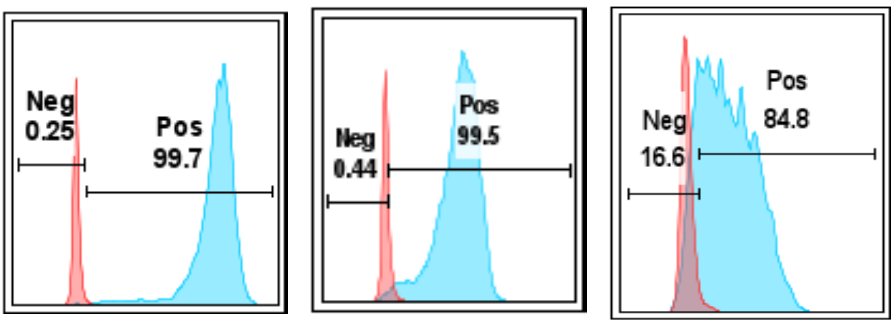
NANOG



OCT4



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



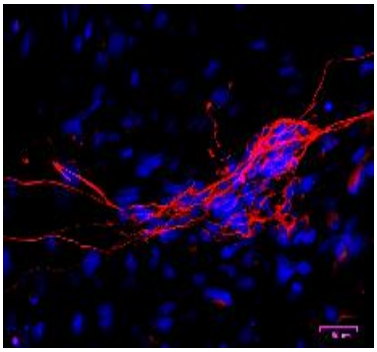
Immunofluorescence of pluripotency associated markers NANOG and OCT4, and fluorescence-activated cell sorting (FACS) of pluripotency markers SSEA4, TRA 1-60 and TRA 1-81 in IBM_FiPS1_Sv4F_1 at passage 10.

Annex 3

In vitro differentiation

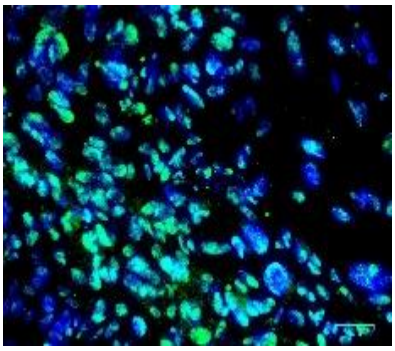
ECTODERM

TUJ1



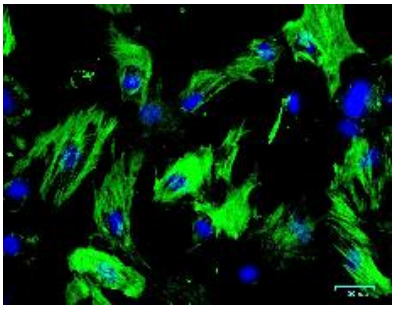
ENDODERM

SOX17



MESODERM

SMA

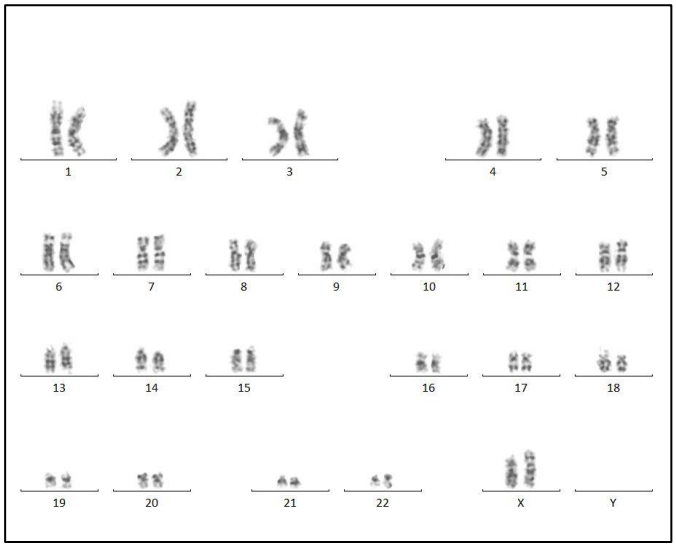


Immunofluorescence of differentiation associated markers TUJ1, for ectoderm; SOX17 for endoderm and SMA for mesoderm in IBM_FiPS1_Sv4F_1 at passage 12.

Annex 4

Karyotype

Cytogenetic analysis



Patient name: IBM_FiPS1_Sv4F_1 passage 8

Result: 46, XX

Specimen type: iPSC

Annex 5

Authentication

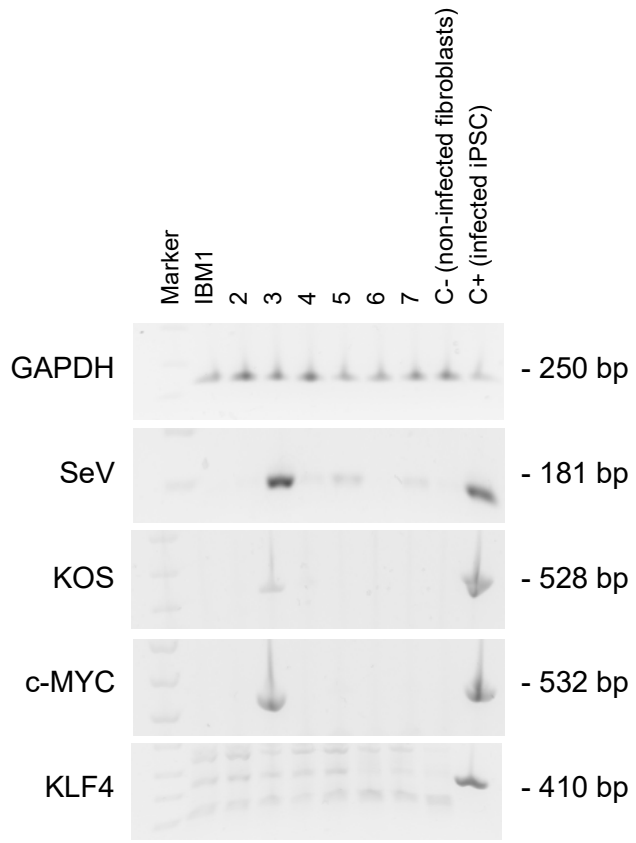
AmpFlSTR Identifier Loci	IBM1 fibroblasts	IBM1 iPSC
CSF1PO	10,11	10,11
D2S1338	17,2	17,2
D3S1358	17,19	17,19
D5S818	10,12	10,12
D7S820	11,12	11,12
D8S1179	14,15	14,15
D13S317	8,9	8,9
D16S539	12,13	12,13
D18S51	16,18	16,18
D19S433	13	13
D21S11	30,31.2	30,31.2
FGA	21	21
TH01	6,7	6,7
TPOX	8,9	8,9
vWA	14,18	14,18
Amelogenin (gender)	X	X

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

Parental reprogrammed fibroblasts cells: IBM1 fibroblasts
iPS generated: IBM1 iPSC (IBM_FiPS1_Sv4F_1)

Annex 6

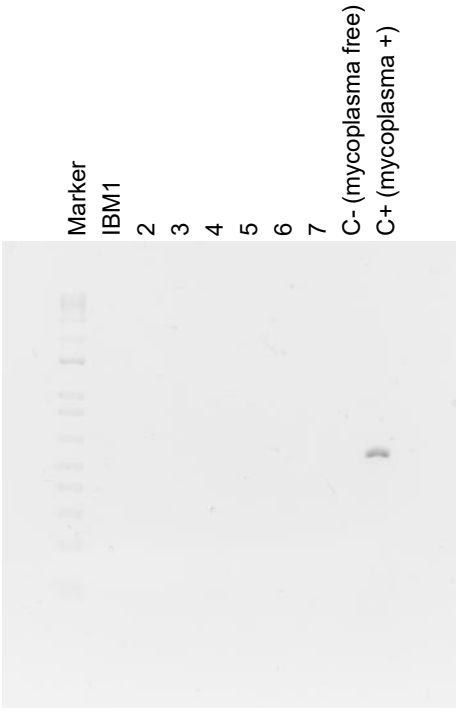
Integration / silencing test



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 IBM_FiPS1_Sv4F_1 (IBM1) iPS line.

Annex 7

Mycoplasma test



PCR analysis showing the absence of mycoplasma in the IBM_FiPS1_Sv4F_1 (IBM1) iPS line.