ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR IBM_FiPS2_Sv4F_1* EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: IBM_FiPS2_Sv4F_1*

***(IBM2 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

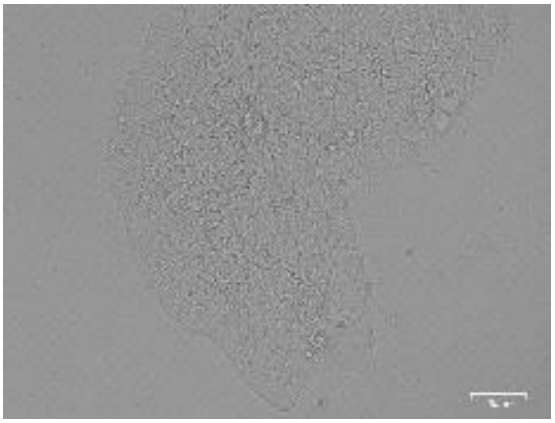
Annex 5: Authentication. Fingerprinting analysis

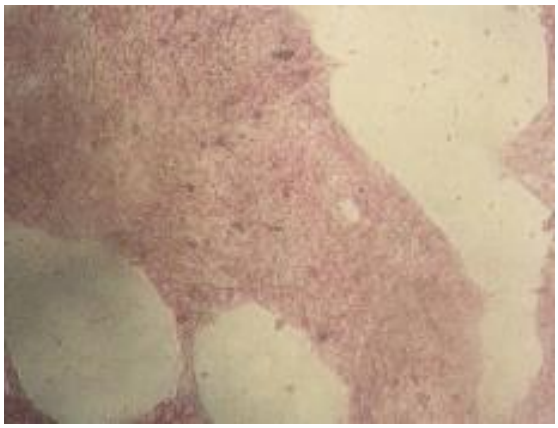
Annex 6: Integration/silencing test

Annex 7: Mycoplasma test

Annex 1

Morphology and Alkaline phosphatase staining

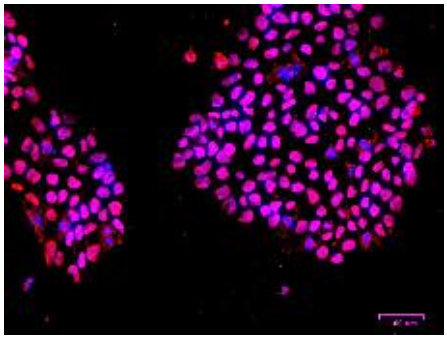




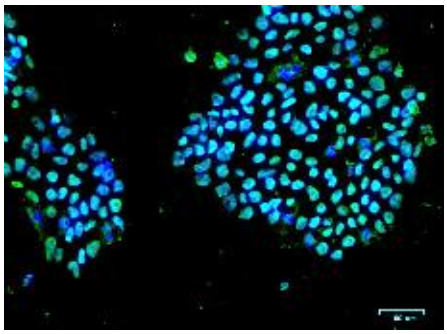
IBM_FiPS2_Sv4F_1 Passage 1

Annex 2
Pluripotency markers

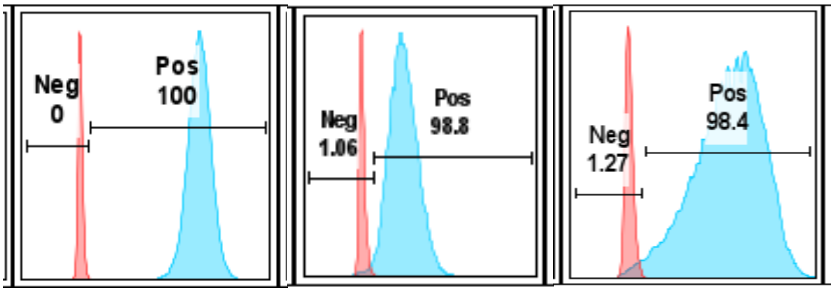
NANOG



OCT4



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



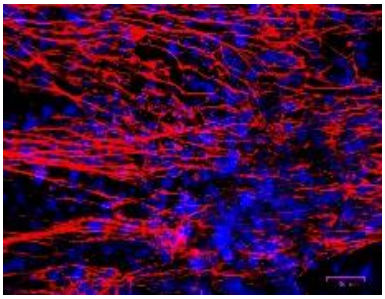
Immunofluorescence of pluripotency associated markers NANOG and OCT4, and fluorescence-activated cell sorting (FACS) of pluripotency markers SSEA4, TRA 1-60 and TRA 1-81 in IBM_FiPS2_Sv4F_1 at passage 10.

Annex 3

In vitro differentiation

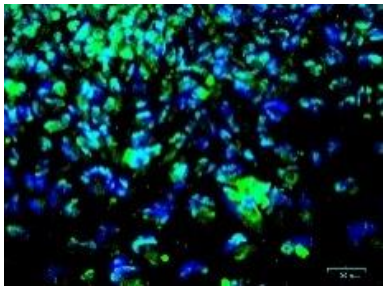
ECTODERM

TUJ1



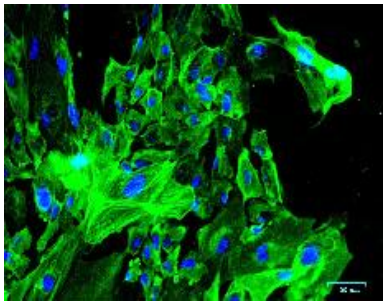
ENDODERM

SOX17



MESODERM

SMA

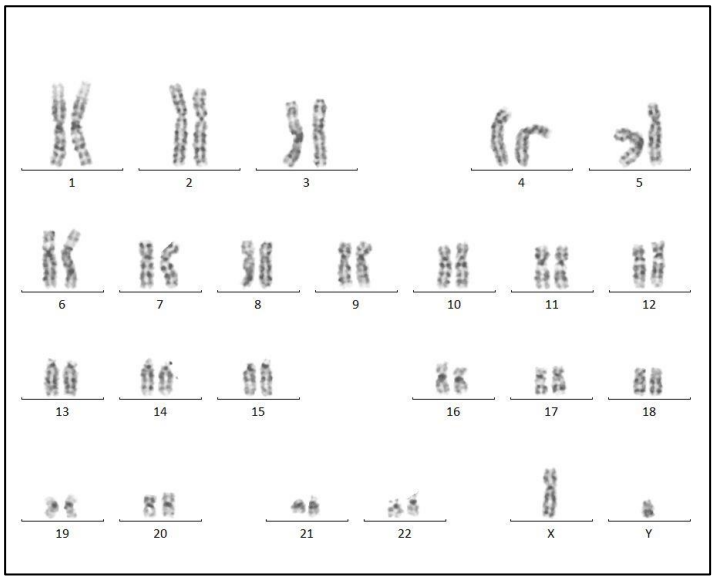


Immunofluorescence of differentiation associated markers TUJ1, for ectoderm; SOX17 for endoderm and SMA for mesoderm in IBM_FiPS2_Sv4F_1 at passage 12.

Annex 4

Karyotype

Cytogenetic analysis



Patient name: IBM_FiPS2_Sv4F_1 passage 14

Result: 46, XY

Specimen type: iPSC

Annex 5

Authentication

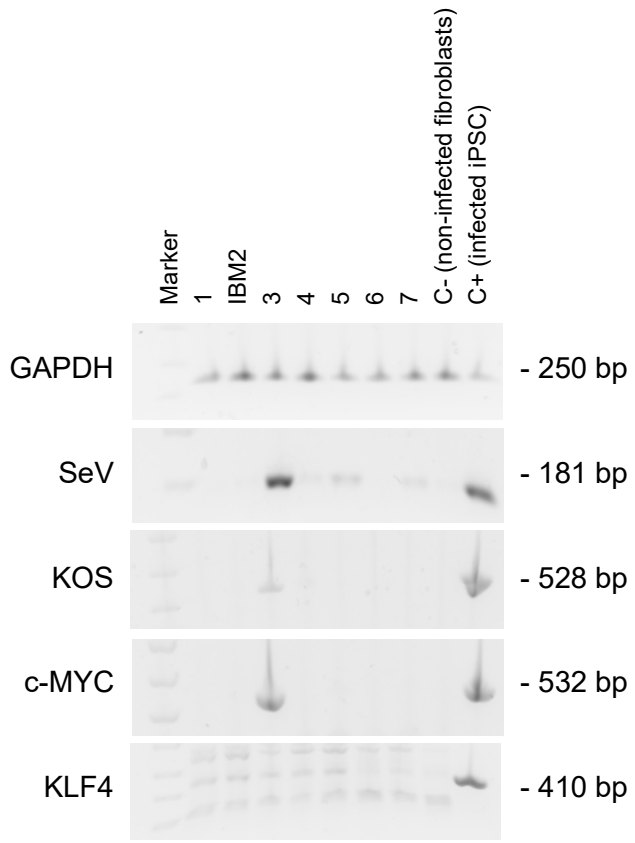
AmpFlSTR Identifier Loci	IBM2 fibroblasts	IBM2 iPSC
CSF1PO	10,11	10,11
D2S1338	20	20
D3S1358	16	16
D5S818	10,12	10,12
D7S820	9,12	9,12
D8S1179	15	15
D13S317	9	9
D16S539	12,13	12,13
D18S51	12,14	12,14
D19S433	11,14	11,14
D21S11	29,3	29,3
FGA	20,23	20,23
TH01	9.3	9.3
TPOX	11	11
vWA	14,16	14,16
Amelogenin (gender)	X, Y	X, Y

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

Parental reprogrammed fibroblasts cells: IBM2 fibroblasts
iPS generated: IBM2 iPSC (IBM_FiPS2_Sv4F_1)

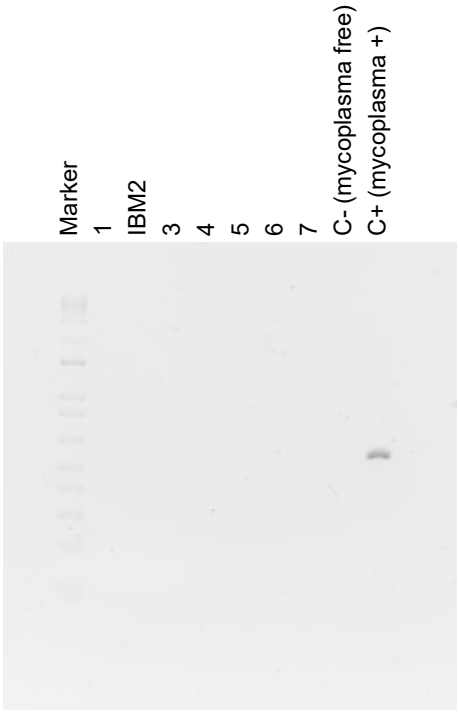
Annex 6

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_FiPS2_Sv4F_1 (IBM2) iPS line.

Annex 7
Mycoplasma test



PCR analysis showing the absence of mycoplasma in the IBM_FiPS2_Sv4F_1 (IBM2) iPS line.