

ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR **C2(RP25)_UiPSC_mR5F_1** EN EL BANCO NACIONAL DE LÍNEAS CELULARES

Annexes iPSC line: C2(RP25)_UiPSC_mR5F_1

Annex 1: Morphology & exponential growth

Annex 2: Pluripotency markers by immunofluorescence and PCR

Annex 3: *In vitro* differentiation markers by Immunofluorescence

Annex 4: Karyotype

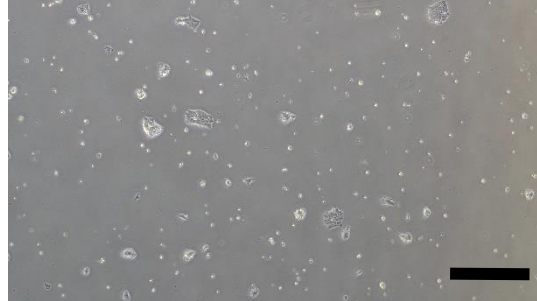
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Mycoplasma test

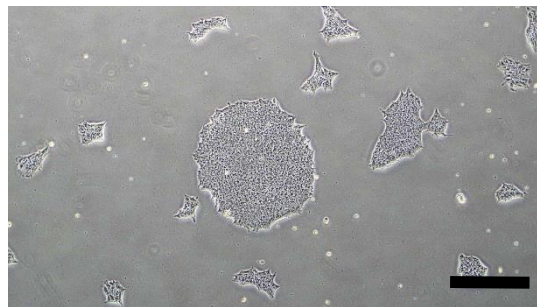
Annex 1

Morphology & exponential growth

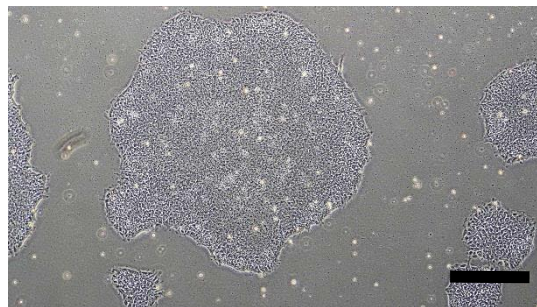
0 DIV



2 DIV



4 DIV



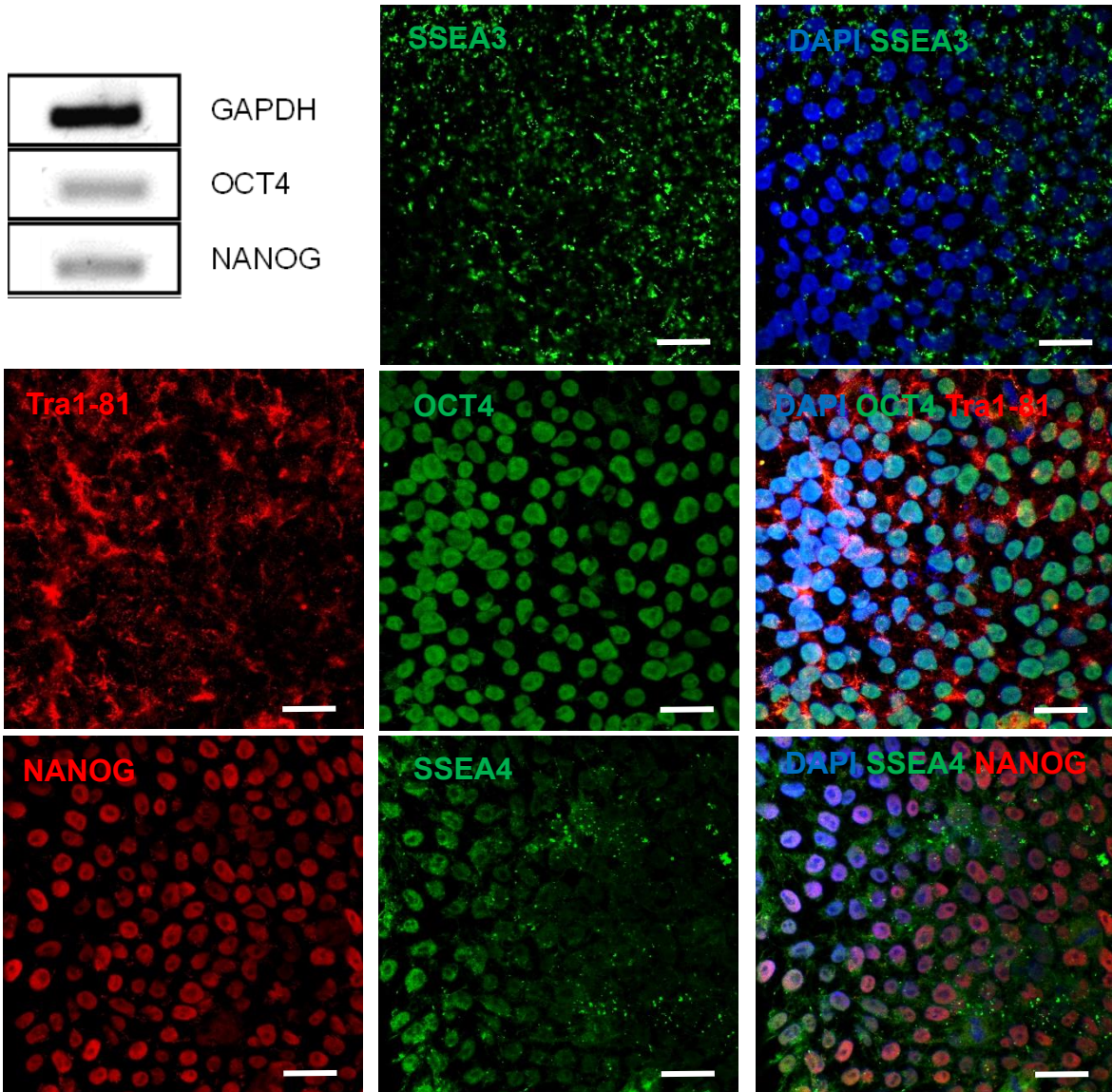
6 DIV



C2(RP25)_UiPSC_mR5F_1 Passage 7 Scale bars: 500 μ

Annex 2

Pluripotency markers

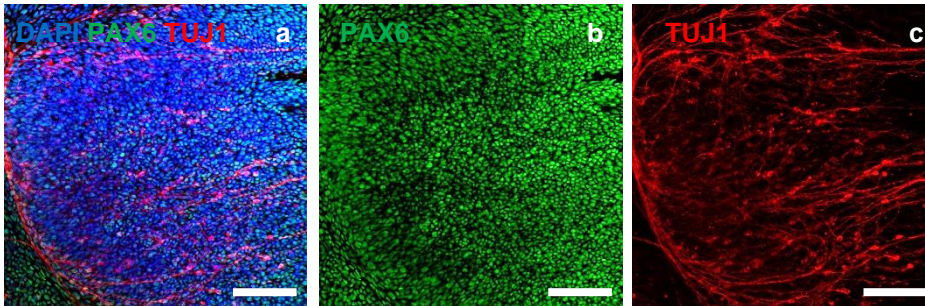


Pluripotency associated markers OCT4, NANOG, and GAPDH (as a housekeeping gene) by PCR and SSEA3, OCT4, TRA181, SSEA4 and NANOG by immunofluorescence in C2(RP25)_UiPSC_mR5F_1 at passage 5. Scale bars: 50μm.

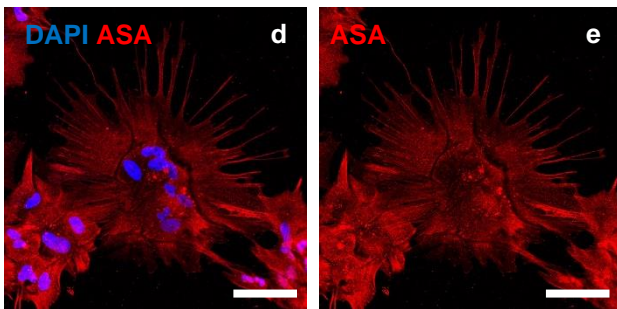
Annex 3

In vitro differentiation

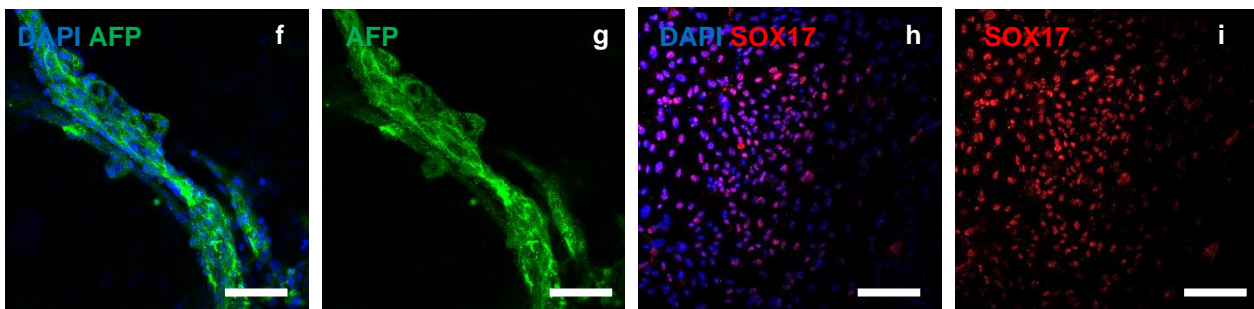
ECTODERM



MESODERM



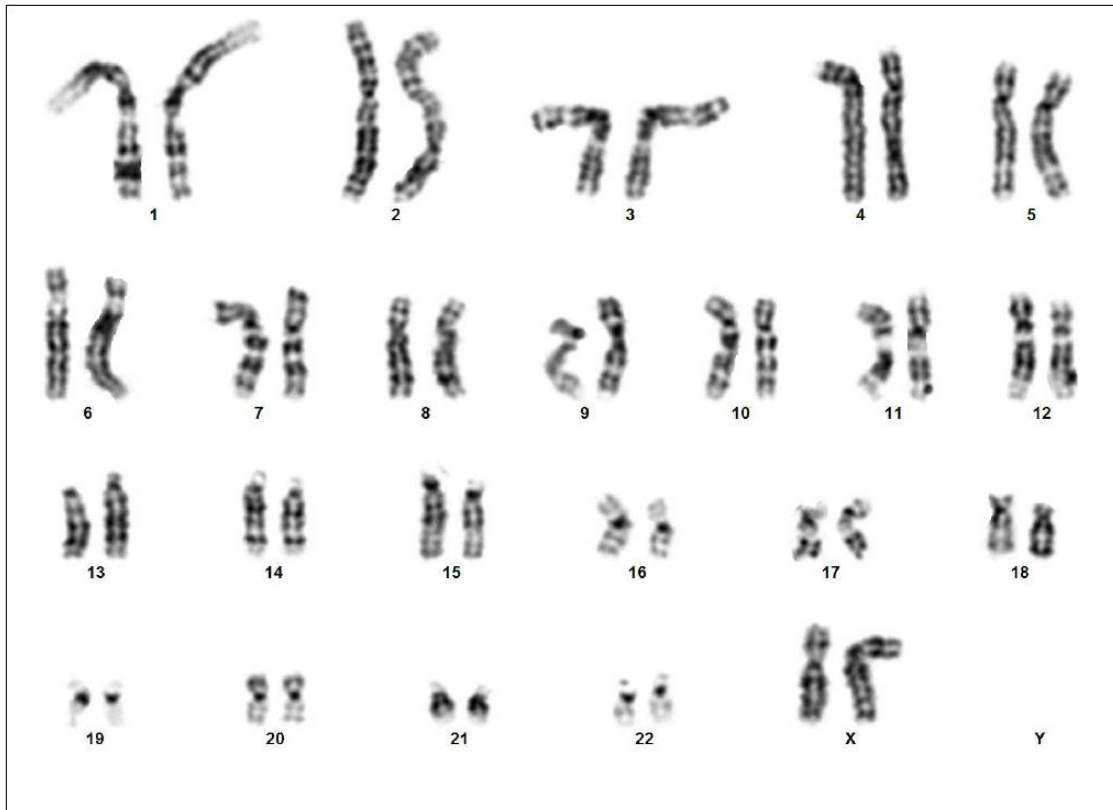
ENDODERM



In vitro differentiation of embryoid bodies (EB) towards three germ layers and confocal images of immunofluorescence using specific antibodies against the ectodermal markers β III-tubulin (TUJ1) and paired box protein 6 (PAX6), mesodermal marker α -sarcomeric actin (ASA) and endodermal markers α -fetoprotein (AFP) and SRY-box transcription factor 17 (SOX17), in C2(RP25)_UiPSC_mR5F_1 at passage 13. Nuclei stained with DAPI. Scale bars: 100 μ m in a-c, d-e, h-i; 50 μ m f-g.

Annex 4 Karyotype

 **SJD** Sant Joan de Déu
Barcelona · Hospital



Case name: 21950503

NHC: CT0457

Name: C2 (RP25) p4

Date: 7/18/2022

Result: 46,XY

Annex 5

Authentication (microsatellites)

Línea celular	Loci STRs analizados									
	TH01	D21S11	D5S818	D13S317	D7S820	D16S539	CSF1PO	AMEL	vWA	TPOX
C2 urine cells p2	6; 9.3	26; 32.2	12	10; 12	11; 12	9; 12	10; 13	X	16; 17	8; 11

Línea celular	Loci STRs analizados									
	TH01	D21S11	D5S818	D13S317	D7S820	D16S539	CSF1PO	AMEL	vWA	TPOX
C2 (CRP25)-UiPSC-mR5F-1 p5	6; 9.3	26; 32.2	12	10; 12	11; 12	9; 12	10; 13	X	16; 17	8; 11

Microsatellite analysis authentication
 Parental urine cells: C2 urine cells p2
 hiPS generated: C2(RP25)_UiPSC_mR5F_1 p5

Annex 7 Mycoplasma test

