

# ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA CELULAR **C1(RP25)\_UiPSC\_mR5F\_7** EN EL BANCO NACIONAL DE LÍNEAS CELULARES

**Annexes iPSC line: C1(RP25)\_UiPSC\_mR5F\_7**

Annex 1: Morphology & exponential growth

Annex 2: Pluripotency markers by immunofluorescence and PCR

Annex 3: *In vitro* differentiation markers by Immunofluorescence

Annex 4: Karyotype

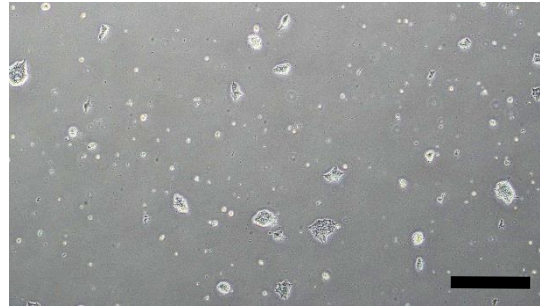
Annex 5: Authentication. Fingerprinting analysis

Annex 6: Mycoplasma test

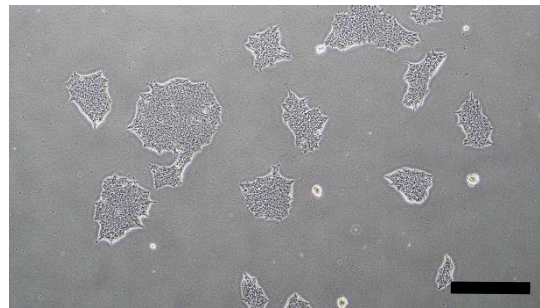
# Annex 1

## Morphology & exponential growth

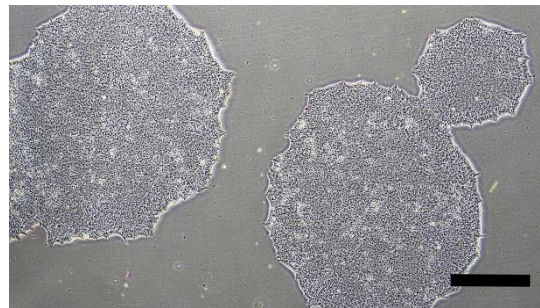
0 DIV



2 DIV



4 DIV



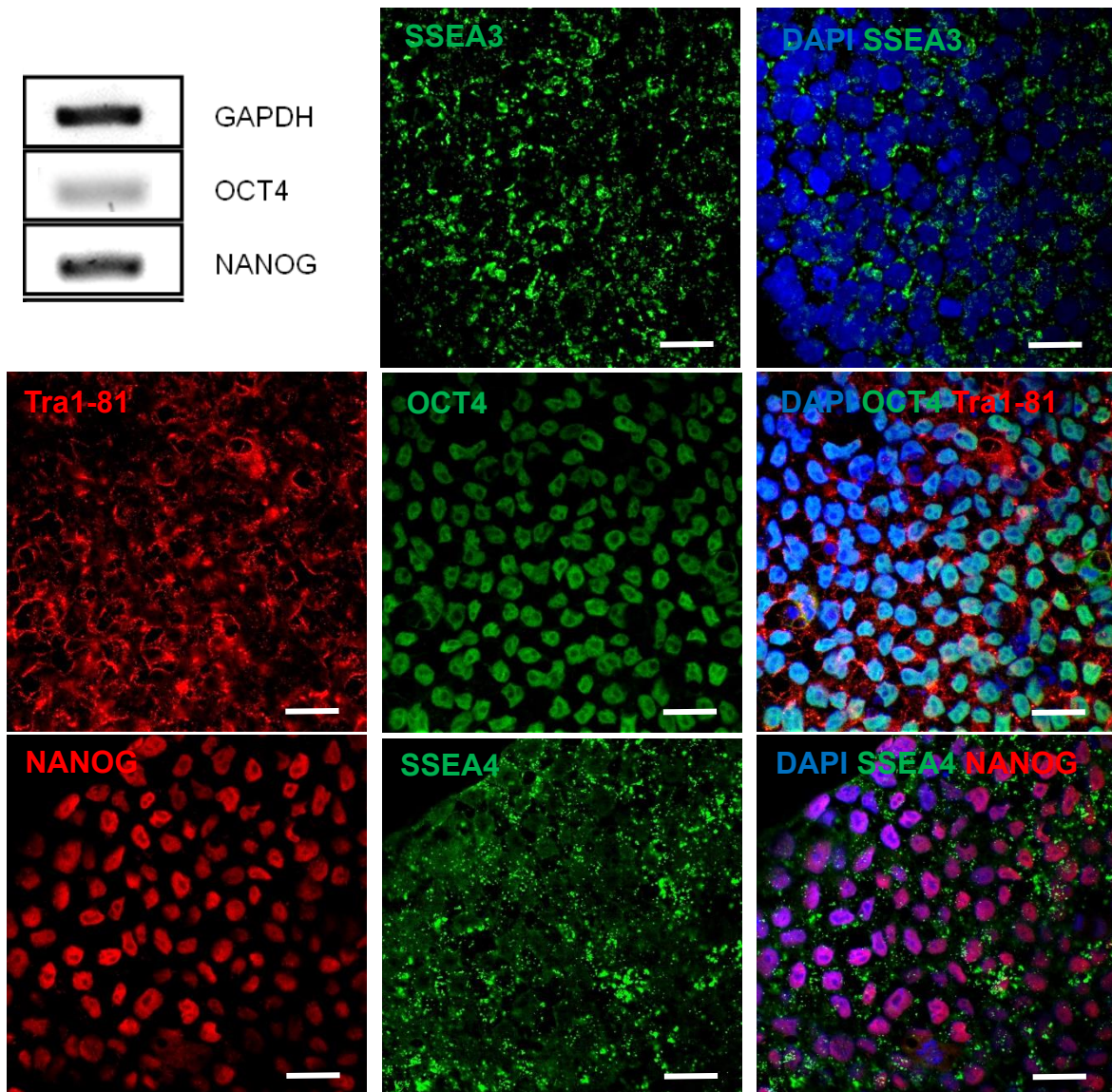
6 DIV



C1(RP25)\_UiPSC\_mR5F\_7 Passage 7 Scale bars: 500µm

## Annex 2

### Pluripotency markers



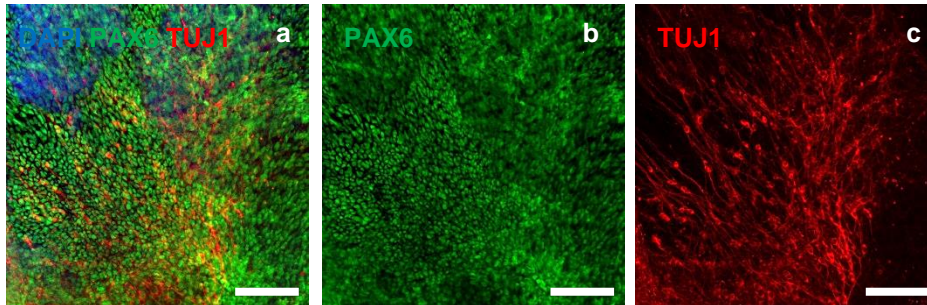
Pluripotency associated markers OCT4, NANOG, and GAPDH (as a Housekeeping gene) by PCR and SSEA3, OCT4, TRA181, SSEA4 and NANOG by immunofluorescence in C1(RP25)\_UiPSC\_mR5F\_7 at passage 5. Scale bars: 50µm.

## Annex 3

### In vitro differentiation

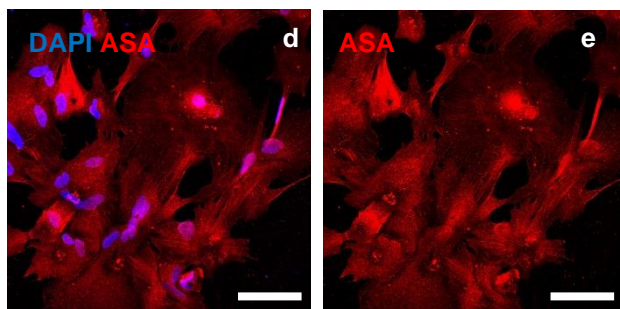
#### ECTODERM

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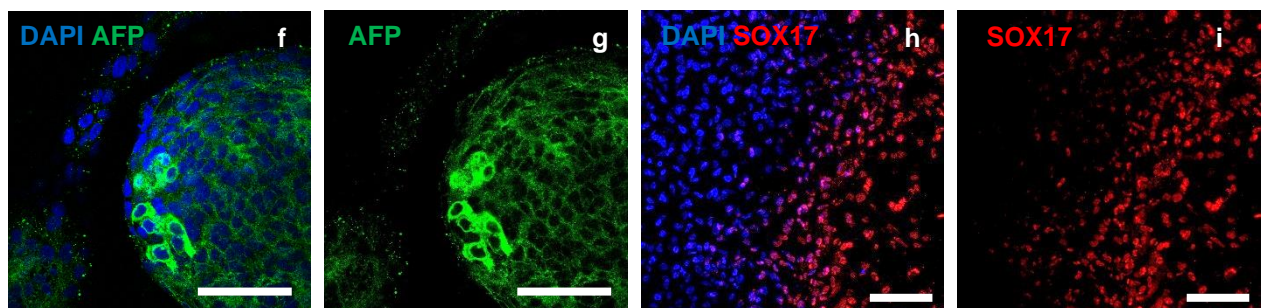
#### MESODERM

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#### ENDODERM

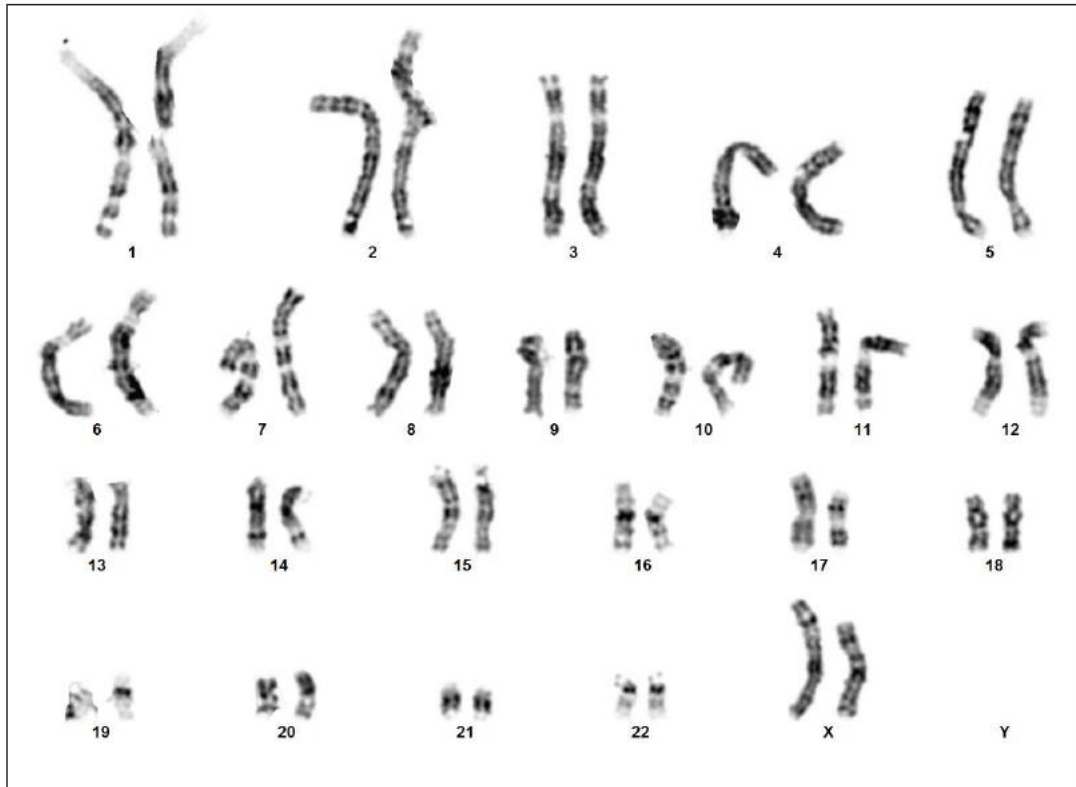
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*In vitro* differentiation of embryoid bodies (EB) towards three germ layers and confocal images of immunofluorescence using specific antibodies against the ectodermal markers  $\beta$ III-tubulin (TUJ1) and paired box protein 6 (PAX6), mesodermal marker  $\alpha$ -sarcomeric actin (ASA) and endodermal markers  $\alpha$ -fetoprotein (AFP) and SRY-box transcription factor 17 (SOX17), in C1(RP25)\_UiPSC\_mR5F\_7 iPS at passage 13. Nuclei stained with DAPI. Scale bars: 100  $\mu$ m in a-c, d-e, h-i; 50  $\mu$ m f-g.

# Annex 4 Karyotype

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



Case name: 21950501

NHC: CT0456

Name: C1 (RP25) p6

Date: 7/18/2022

Result: 46,XX

# Annex 5

## Authentication (microsatellites)

Línea celular	Loci STRs analizados									
	TH01	D21S11	D5S818	D13S317	D7S820	D16S539	CSF1PO	AMEL	vWA	TPOX
C1 urine cells p2	6; 9.3	26; 28	12; 13	11; 12	11; 12	9; 15	10; 11	X	17; 18	11

Línea celular	Loci STRs analizados									
	TH01	D21S11	D5S818	D13S317	D7S820	D16S539	CSF1PO	AMEL	vWA	TPOX
C1 (CRP25)-UiPSC-mR5F-7 p5	6; 9.3	26; 28	12; 13	11; 12	11; 12	9; 15	10; 11	X	17; 18	11

Microsatellite analysis authentication  
 Parental urine cells: C1 urine cells p2  
 hiPS generated: C1(RP25)\_UiPSC\_mR5F\_7 p5

## Annex 6 Mycoplasma test

