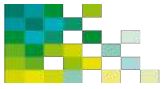


ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA LÍNEA
CELULAR **RP1_FiPS4F1.6** EN EL BANCO NACIONAL DE
LÍNEAS CELULARES.



Anexo 1. Test de pluripotencia

Annex 1. Pluripotency test

Anexo 2. Test de diferenciació *in vitro*

Annex 2. *In vitro* differentiation test

Anexo 3. Cariotipo

Annex 3. Karyotype

Anexo 4. Huella genética

Annex 4. DNA fingerprinting

Anexo 5. Genotipo

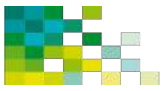
Annex 5. Genotype

Anexo 6. Test de micoplasma

Annex 6. Mycoplasma test

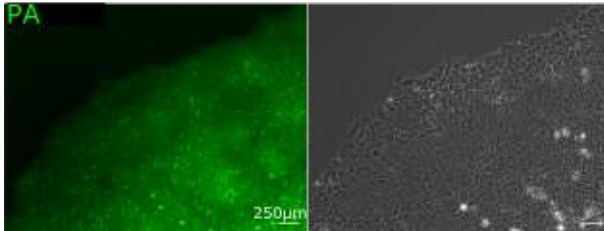
Anexo 7. Test de silenciament

Annex 7. Silencing Test



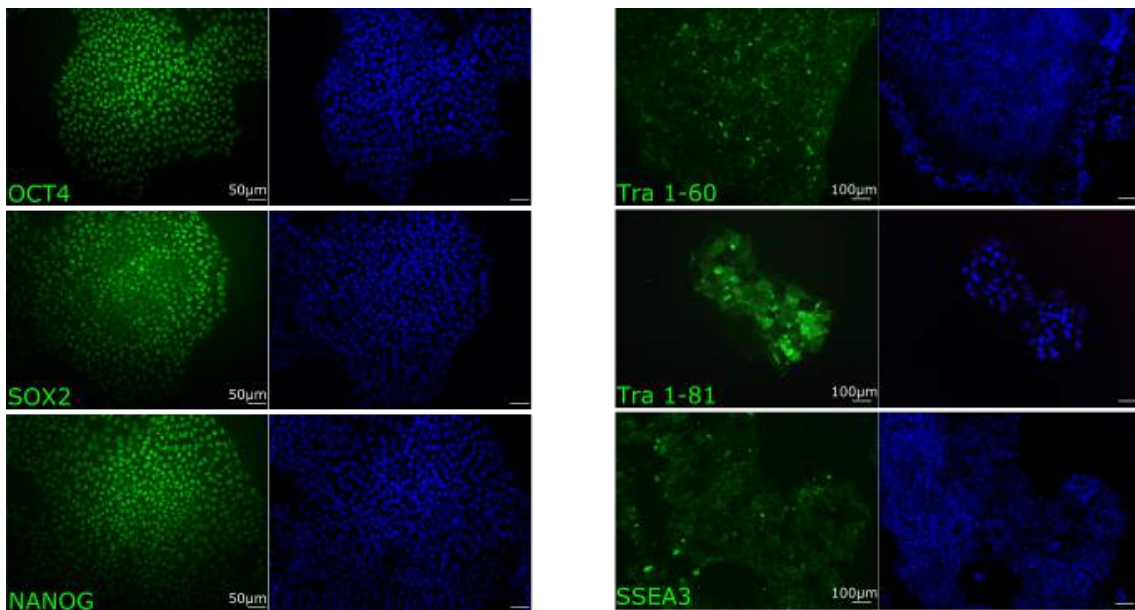
Anexo 1. Test de pluripotencia

Annex 1. Pluripotency test



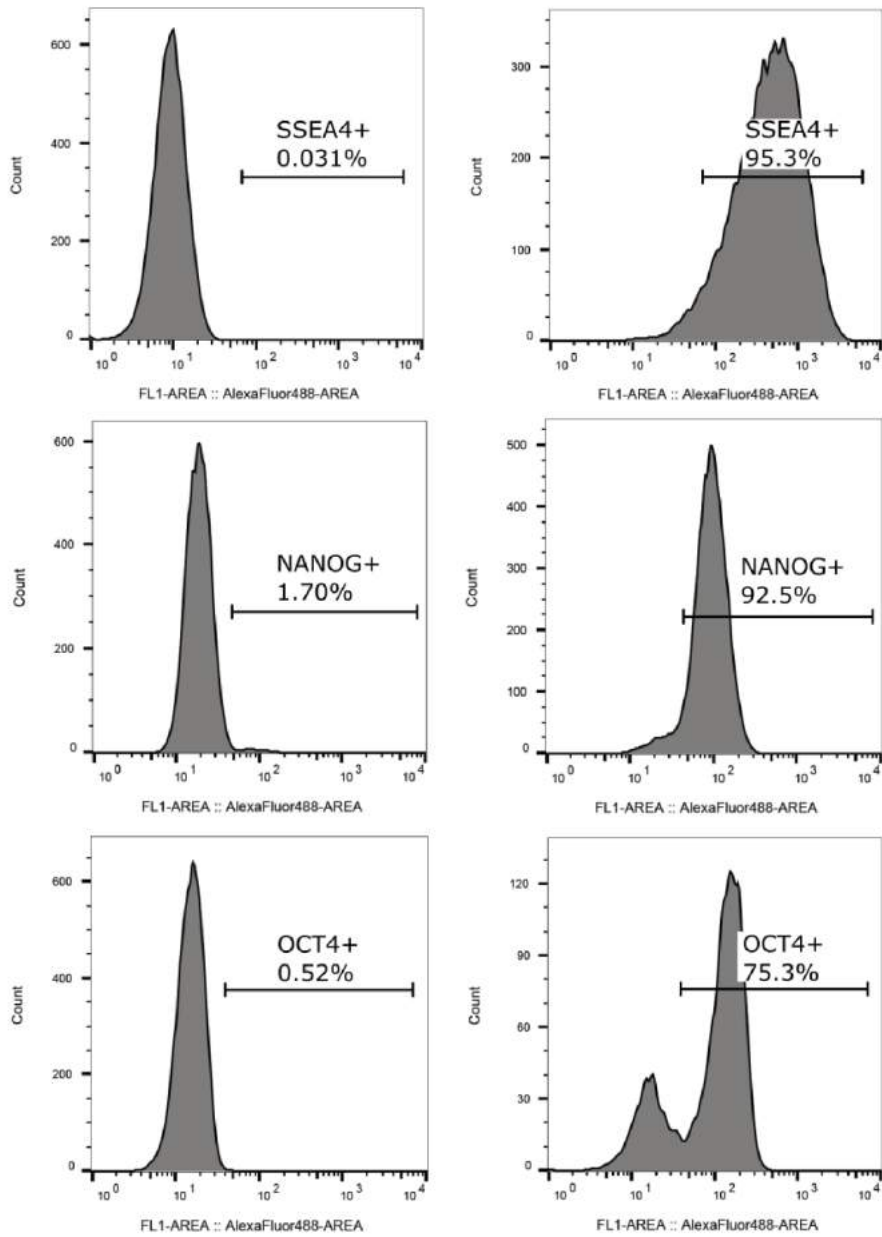
Test de fosfatasa alcalina en la línea RP1_FiPS4F1.6

Alkaline phosphatase test in RP1_FiPS4F1.6 cell line



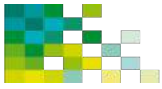
Detecció per immunofluorescència de los marcadors OCT4, SOX2, NANOG, Tra 1-60, Tra 1-81 y SSEA3 en la línia RP1_FiPS4F1.6

Immunofluorescence detection of OCT4, SOX2, NANOG, Tra 1-60, Tra 1-81 and SSEA3 in RP1_FiPS4F1.6 cell line



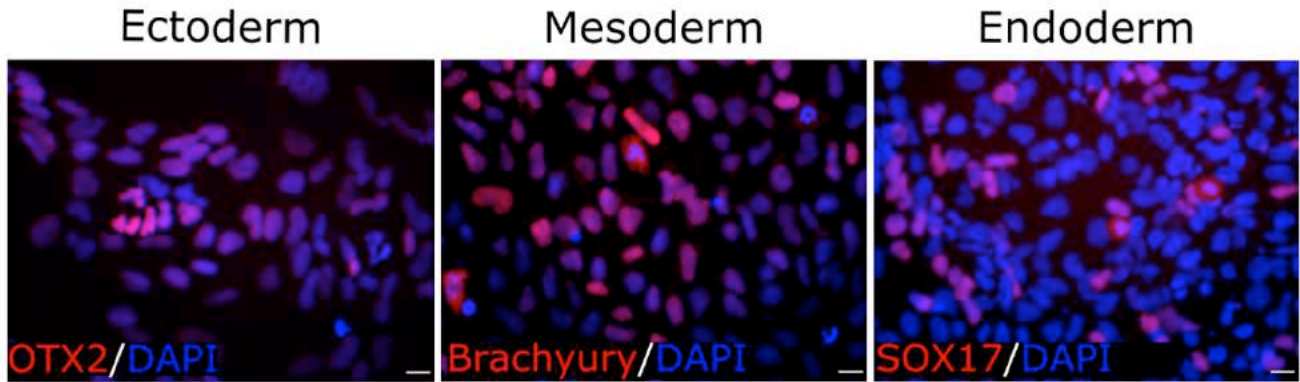
Detecció per citometria de fluxe de los marcadors SSEA4, NANOG y OCT4 en la línia RP1_FiPS4F1.6

Flow cytometry detection of SSEA4, NANOG and OCT4 in RP1_FiPS4F1.6 cell line



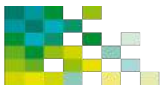
Anexo 2. Test de diferenciació *in vitro*

Annex 2. *In vitro* differentiation test



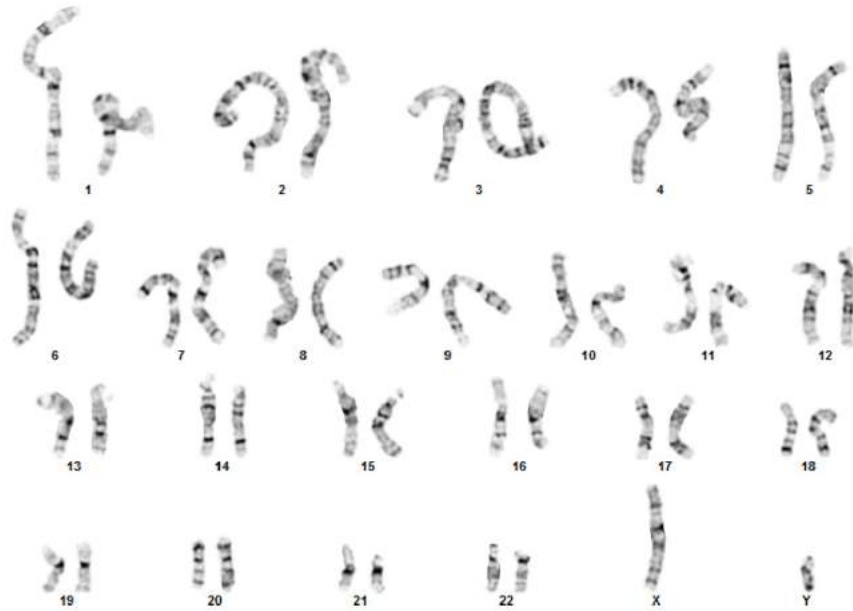
Diferenciació *in vitro* a ectodermo, mesodermo y endodermo de la línia RP1_FiPS4F1.6

In vitro differentiation into ectoderm, mesoderm and endoderm of RP1_FiPS4F1.6 line

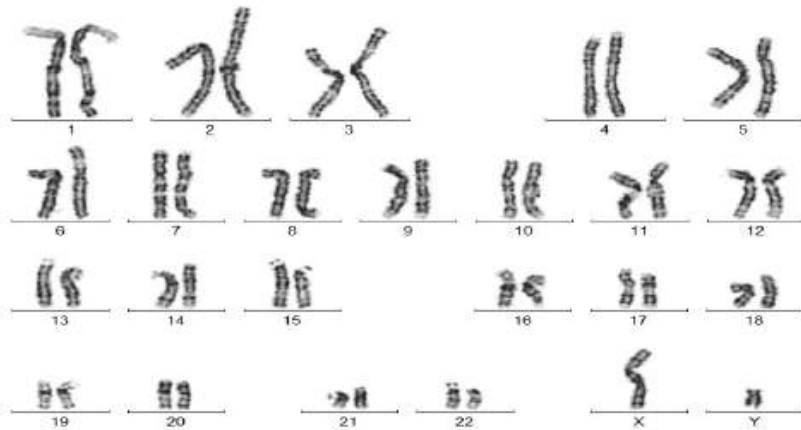


Anexo 3. Cariotipo

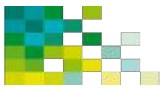
Annex 3. Karyotype



46, XY p6



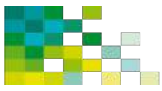
46, XY p13



Anexo 4. Huella genética

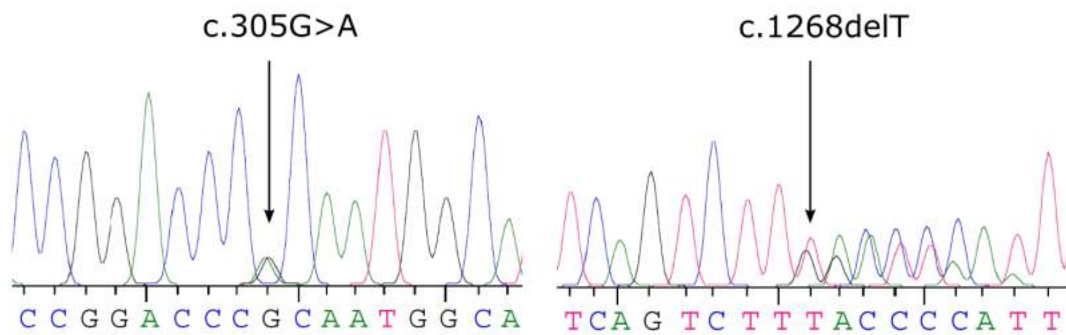
Annex 4. DNA fingerprinting

| STR | Fibroblasts | iPSC |
|------------|--------------------|-------------|
| AMEL | X, Y | X, Y |
| vWA | 15, 18 | 15, 18 |
| D8S1179 | 11 | 11 |
| TPOX | 8, 10 | 8, 10 |
| FGA | 20, 21 | 20, 21 |
| D3S1358 | 14, 17 | 14, 17 |
| THO1 | 6, 9.3 | 6, 9.3 |
| D21S11 | 32.2, 33.2 | 32.2, 33.2 |
| D18S51 | 14, 17 | 14, 17 |
| Penta E | 8, 11 | 8, 11 |
| D5S818 | 11, 13 | 11, 13 |
| D13S317 | 8, 12 | 8, 12 |
| D7S820 | 8, 11 | 8, 11 |
| D16S539 | 12, 13 | 12, 13 |
| CSF1PO | 10, 12 | 10, 12 |
| Penta D | 11, 12 | 11, 12 |



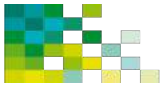
Anexo 5. Genotipo

Annex 5. Genotype



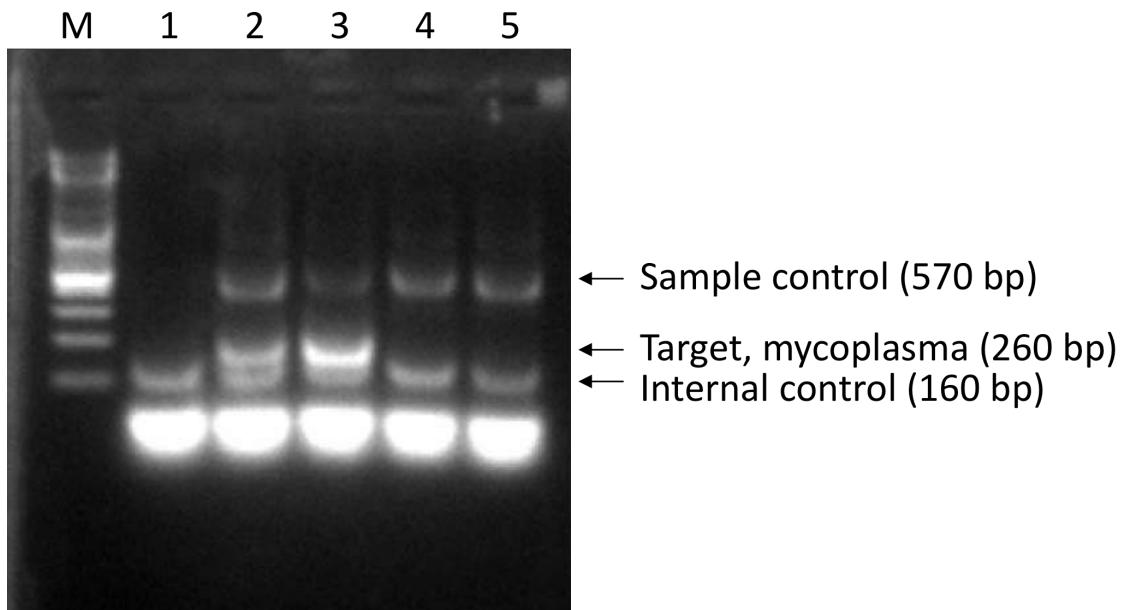
Confirmación del diagnóstico genotípico de la línea RP1_FiPS4F1.6 por secuenciación Sanger

Confirmation of genotypic diagnosis of the line RP1_FiPS4F1.6 by Sanger sequencing



Anexo 6. Test de micoplasma

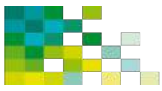
Annex 6. Mycoplasma test



1: negative control

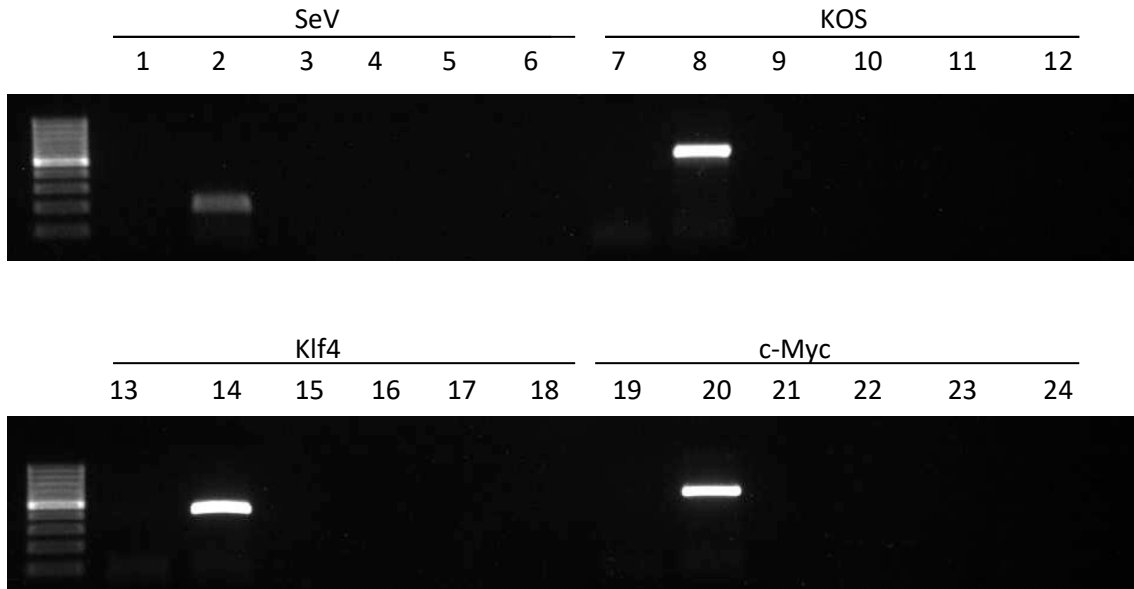
2 and 3: positive control

4: RP1_FiPS4F1.6 cells



Anexo 7. Test de silenciament

Annex 7. Silencing Test



1, 7, 13 and 19: negative control
2, 8, 14 and 20: positive control
3, 9, 15 and 21: RP1_FiPS4F1.6 cells

| Detection | Target | Forward/Reverse primer (5'-3') |
|------------------------|--------------|---|
| Sendai virus detection | <i>SeV</i> | GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC |
| Transgene detection | <i>KOS</i> | ATGCACCGCTACGACGTGAGCGC ACCTTGACAATCCTGATGTGG |
| Transgene detection | <i>Klf4</i> | TTCCTGCATGCCAGAGGAGCCC AATGTATCGAAGGTGCTCAA |
| Transgene detection | <i>c-Myc</i> | TAACTGACTAGCAGGCTTGTCG TCCACATACAGTCCTGGATGATGATG |