



ANEXOS A LA SOLICITUD DE DEPÓSITO DE LA
LÍNEA CELULAR **SP11#1** EN EL BANCO
NACIONAL DE LÍNEAS CELULARES

ANEXOS

Anexo 1: Fenotipo. Marcadores de pluripotencia

Anexo 2: Diferenciación *in vitro*

Anexo 3: Diferenciación *in vivo*

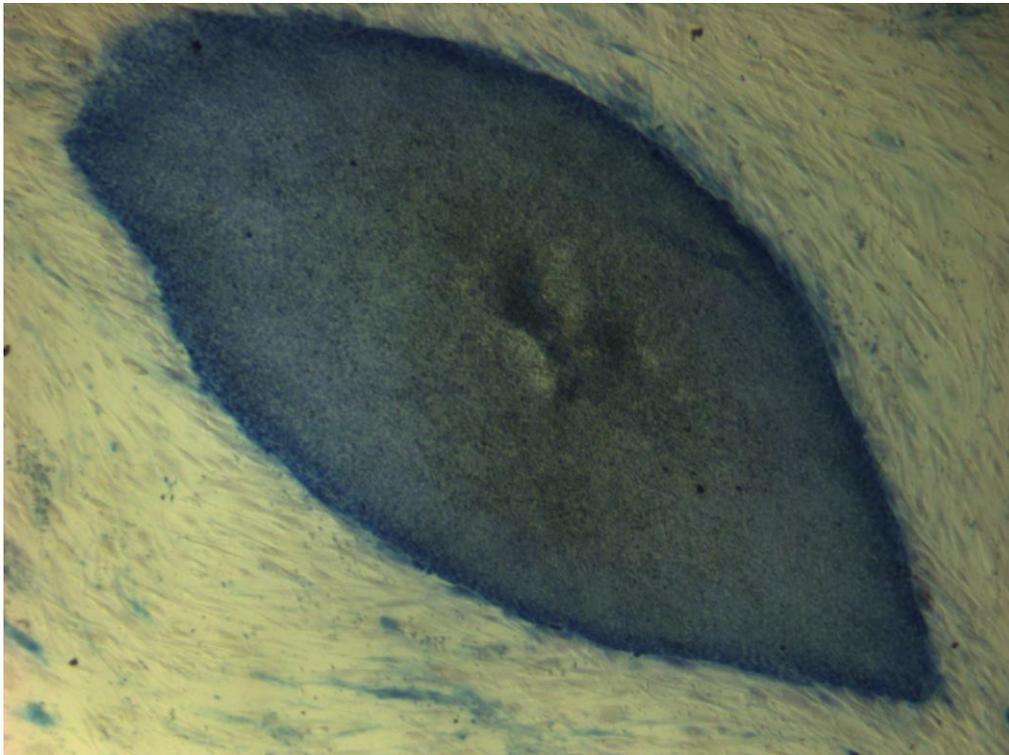
Anexo 4: Cariotipo

Anexo 5: Resultados microsatélites

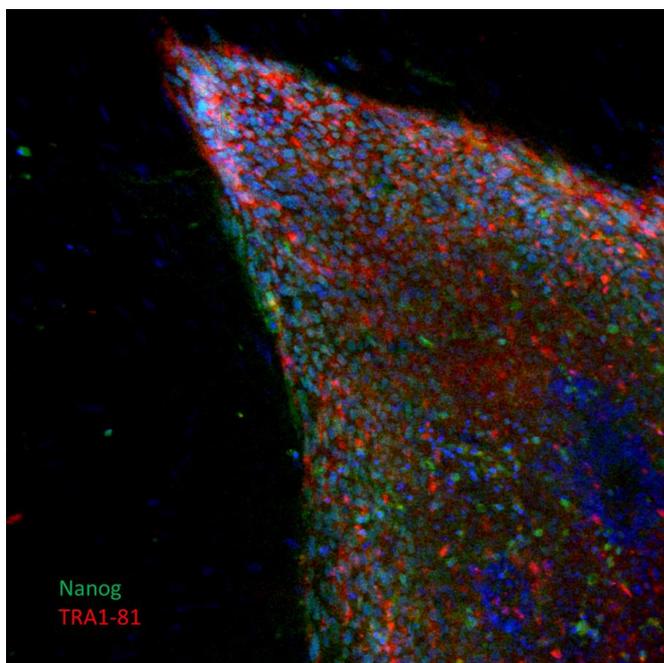
Anexo 6: Integración y silenciamiento de los transgenes de reprogramación

Anexo 7: Resultado test de micoplasma

Anexo 1
Fenotipo. Marcadores de pluripotencia

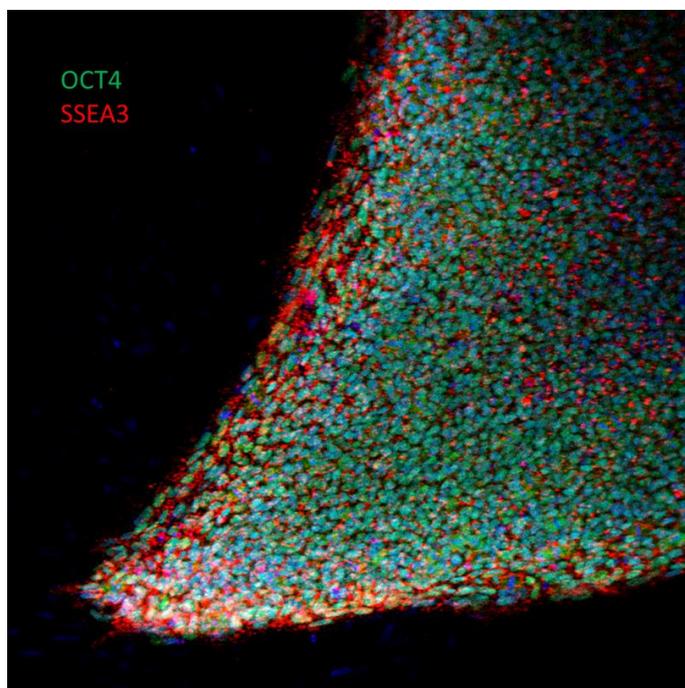


Actividad **fosfatasa alcalina** de la línea de células pluripotentes



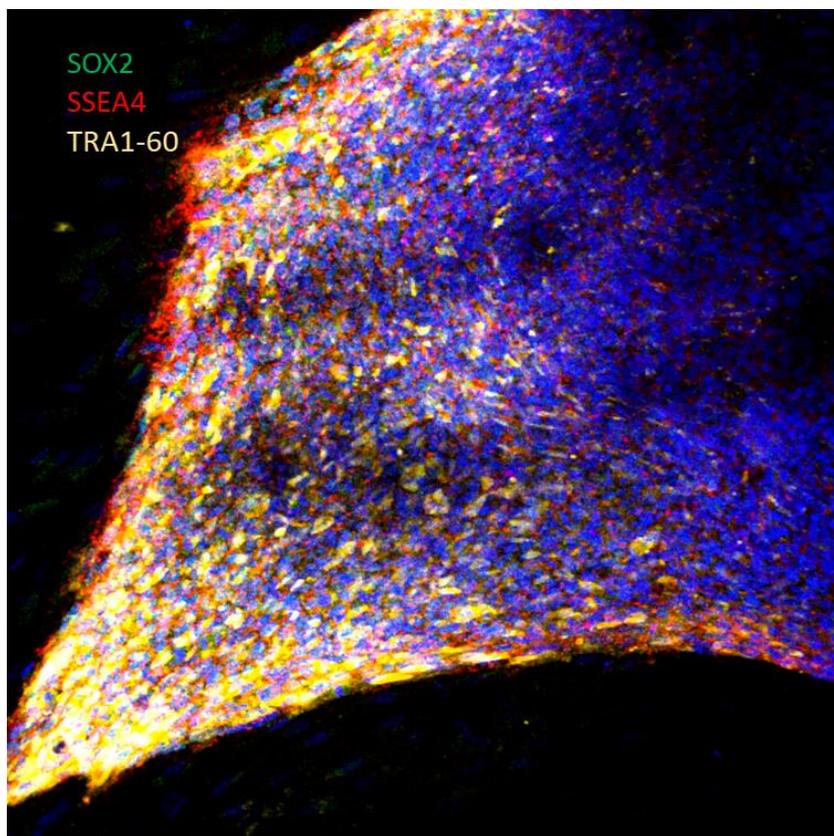
Resultado positivo por inmunocitoquímica para las proteínas de pluripotencia

Nanog y TRA1-81



Resultado positivo por inmunocitoquímica para las proteínas de pluripotencia **Oct-4** y

SSEA-3

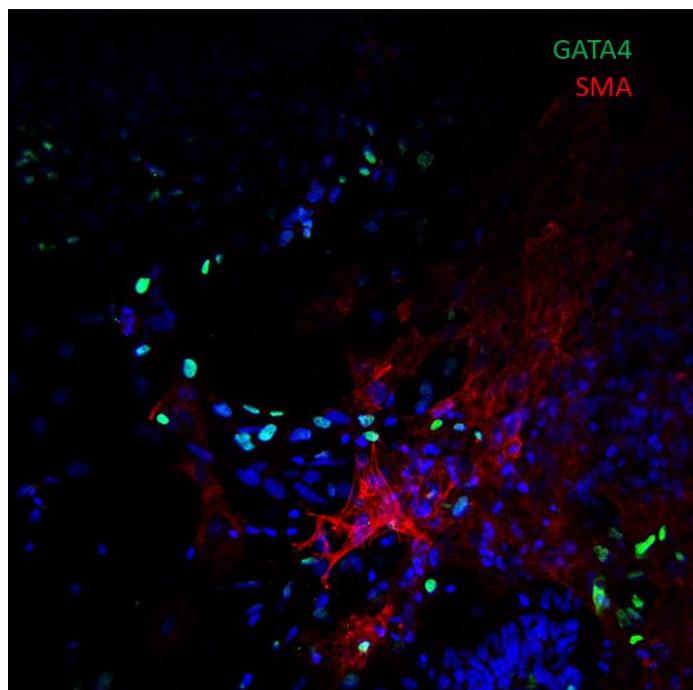


Resultado positivo por inmunocitoquímica para las proteínas de pluripotencia

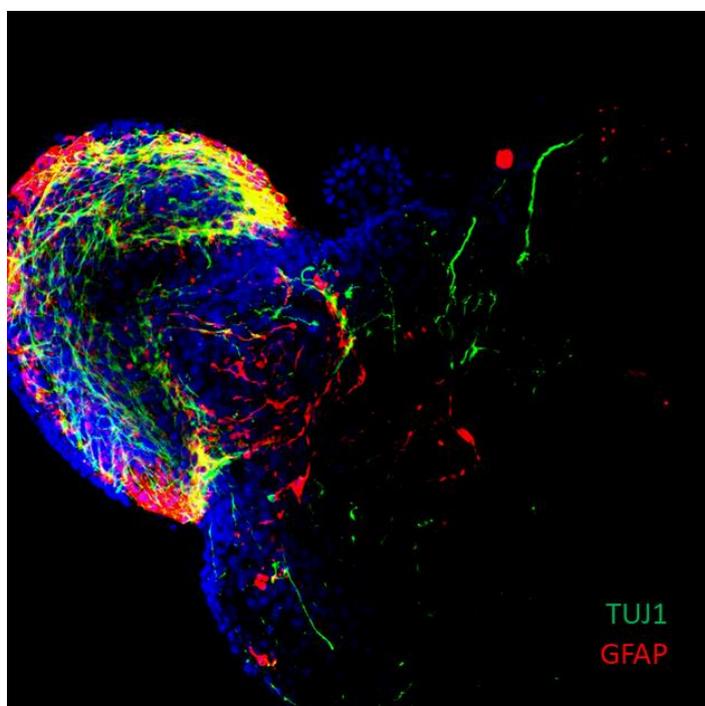
Sox-2, SSEA-4 y TRA1-60



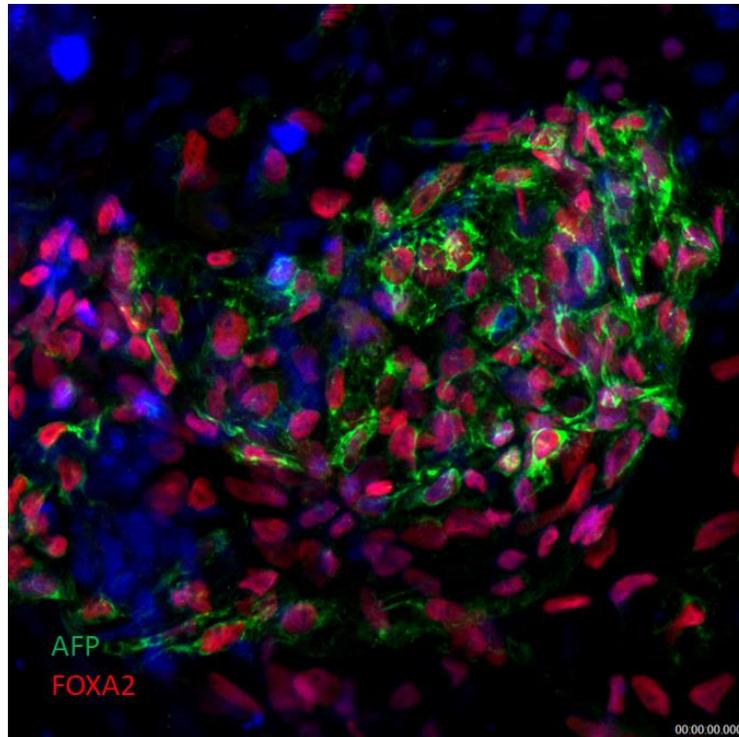
Anexo 2
Diferenciación *in vitro*



Diferenciación *in vitro* a mesodermo: Células positivas para **GATA4** y **SMA**



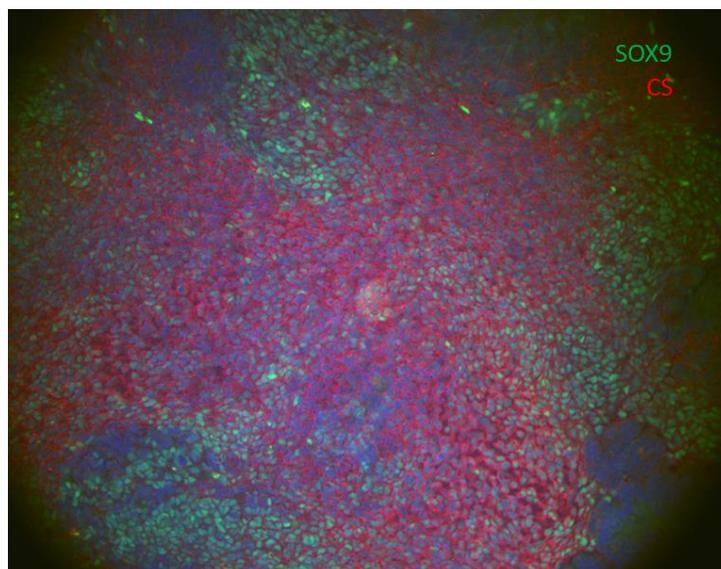
Diferenciación *in vitro* a ectodermo: Células positivas para **Tuj1** y **GFAP**



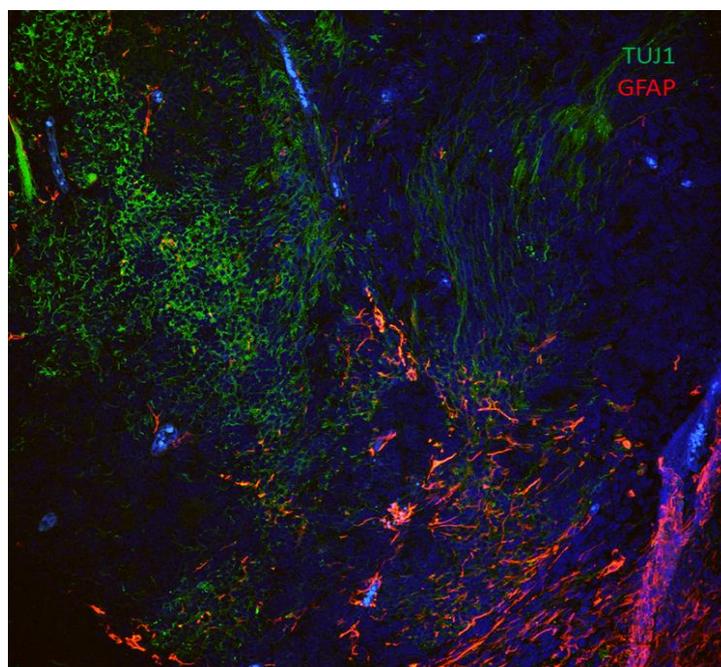
Diferenciación *in vitro* a endodermo: Células positivas para **AFP y FOXA2**



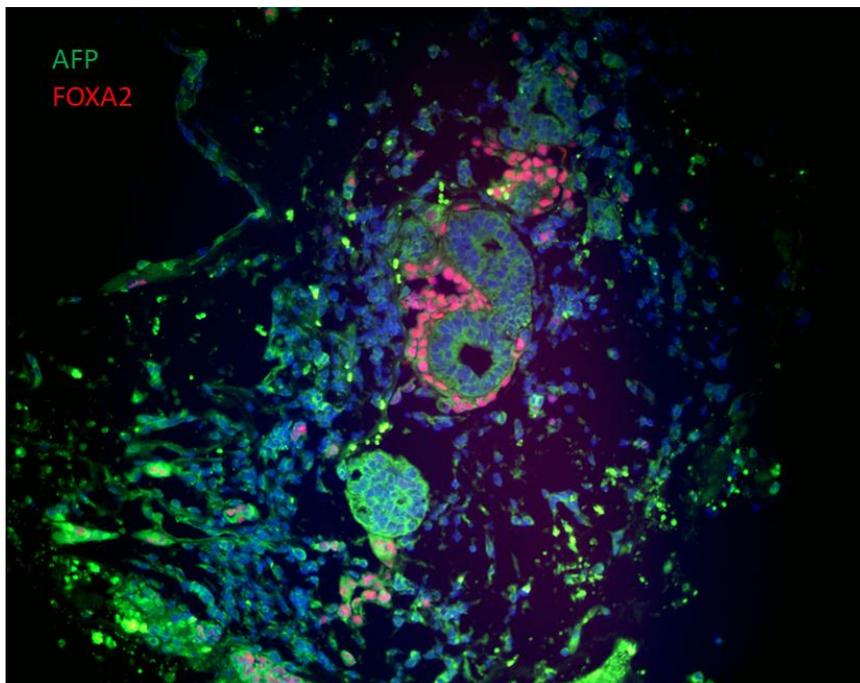
Anexo 3
Diferenciación *in vivo*



Diferenciación *in vivo* a mesodermo: Células positivas para **SOX9** y **CS**



Diferenciación *in vivo* a ectodermo: Células positivas para **TUJ1** y **GFAP**

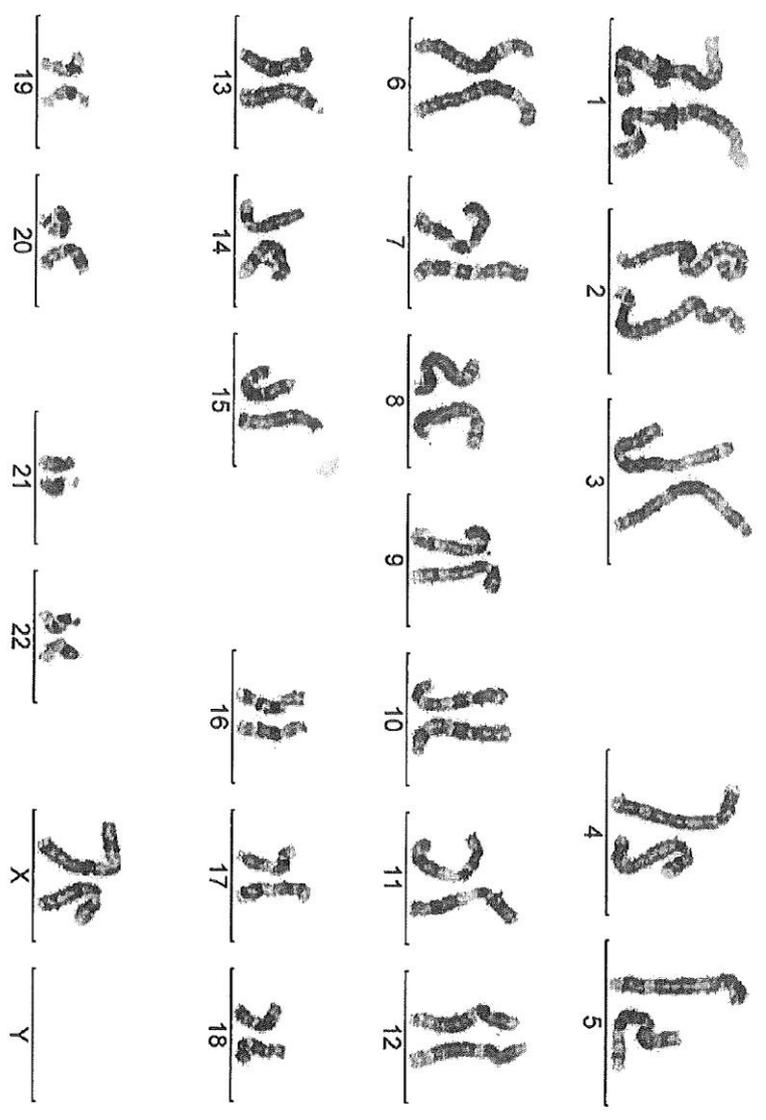


Diferenciación in vivo a endodermo: Células positivas para **AFP y FOXA2**



Anexo 4

Cariotipo

| | | | | | |
|---|--|---|--|-------------------------|--|
| <p>Nom del cas: 11cm0514 Imatge: 006</p> | | <p>Pacient: SP-11 Carlotip: 46,XX</p> | | <p>Data: 04/05/2011</p> | |
|  | | | | | |



Anexo 5

Resultado microsatélites

Cell line authentication service

Request Information

Petitioner: Antonella Consiglio
Center: Universitat de Barcelona
Department: - Patologia i Terapèutica Experimental

Sample Information

External ID: SP11_Kerato
Internal ID: qG20056240
Description:

Results

Sample Quality: Correct

| Sample | TH01 | D21S11 | D5S818 | D13S317 | D7S820 | D16S539 | CSF1PO | AMEL | vWA | TPOX |
|-------------|-------|--------|--------|---------|--------|---------|--------|------|-----|-------|
| SP11_Kerato | 9-9.3 | 28-30 | 12 | 11-13 | 8-11 | 11-12 | 11-12 | X | 16 | 10-11 |

Known cell line match: No **Specify:**

Contamination with other human cell lines: Not detected

Comments: The analyzed sample shows a 100 % concordance in allele composition with the cell line SP11 iPSC (qG20056239).

Signature:
Manel Garcia

Date: 28/05/2020

Brief method description

The analysis was performed by genotyping the following human-specific STR markers (microsatellites): *TH01*, *D21S11*, *D5S828*, *D13S317*, *D7S820*, *D16S539*, *CSF1PO*, *vWA* and *TPOX*. The combination of these nine genetic markers results in allele profile with a probability of 1 in 2.9×10^9 of random matching. A gender-specific marker is also analysed (AMEL) to establish the presence of sex chromosomes, as well as a specific marker to detect the presence of murine sequences, that would derive from feeder cells commonly used in cell culture.

This method is not able to distinguish the underlying genomic structure of the observed results. For instance, it cannot tell if an homozygous sample has two equal alleles or if it is the combination of a certain allele plus a deletion in the other one.

Cell line authentication service

Request Information

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Sample Information

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Internal ID: qG20056239
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Results

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| SP11_iPSC | 9-9.3 | 28-30 | 12 | 11-13 | 8-11 | 11-12 | 11-12 | X | 16 | 10-11 |

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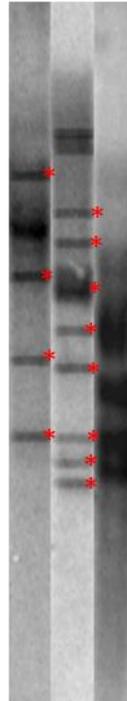
Análisis de microsatélites en la línea de hiPSC y en los queratinocitos de los que procede.



Anexo 6
**Integración y silenciamiento de los transgenes de
reprogramación**

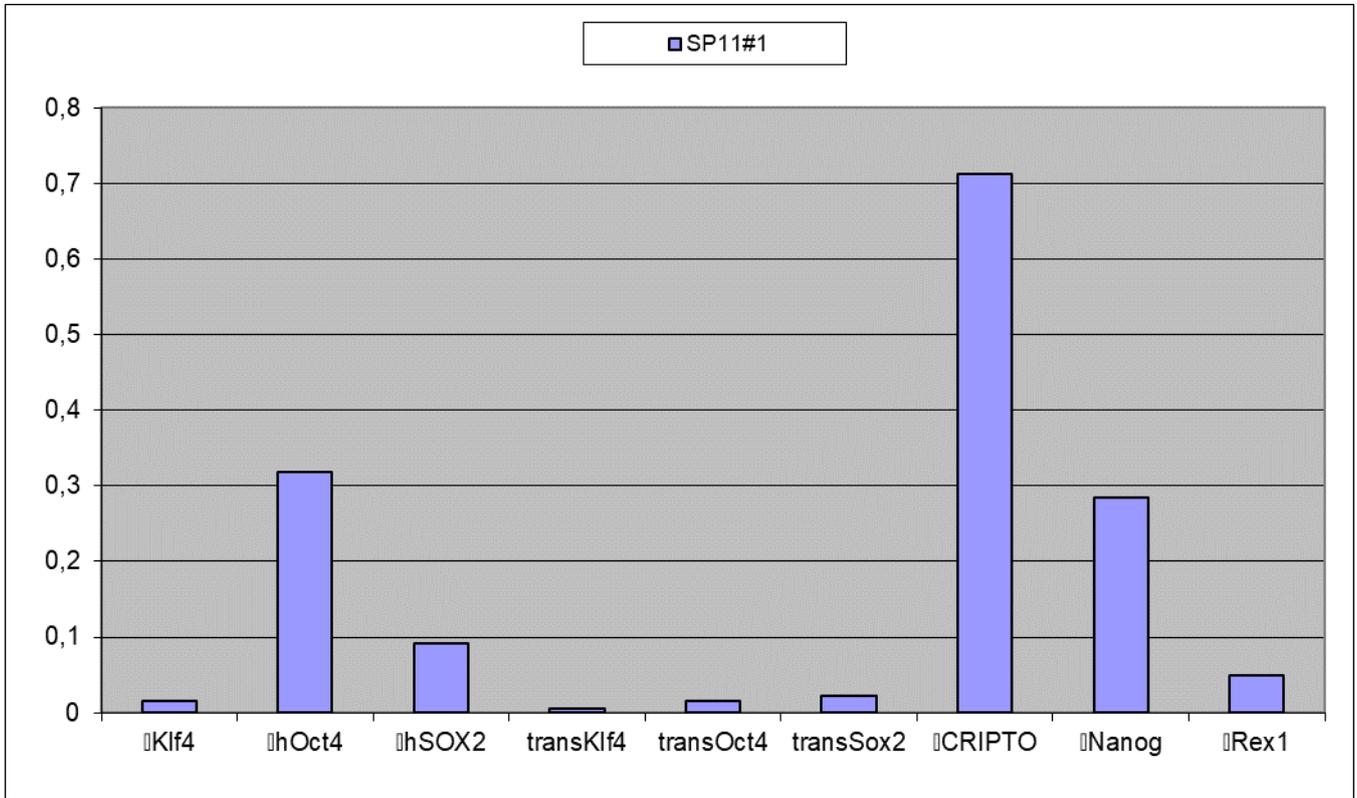
SP 11

SOX2
OCT4
KLF4



4 8 3

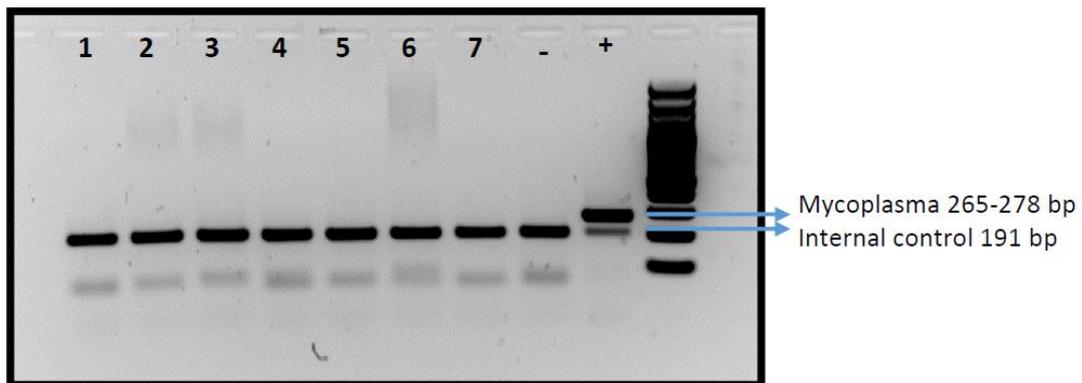
Southern blot mostrando las integraciones genómicas de los genes SOX2, OCT4 y KLF4 utilizados para generar la línea.



Silenciamiento de los transgenes de reprogramación
Análisis QRT-PCR que muestra los niveles de expresión de mRNA de los marcadores de pluripotencia endógenos



Anexo 7
Resultado test de micoplasma



Nº 6. SP11#1 p15