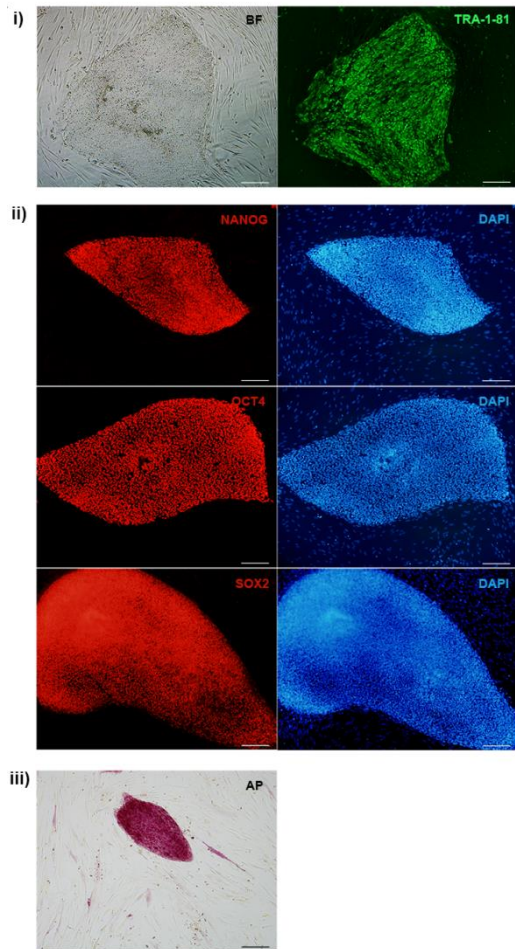


Anexo a la solicitud de depósito de la línea celular

Annexes to the deposited cell line

RP1-FiPS4F1

Marcadores de pluripotencia/ Pluripotency markers

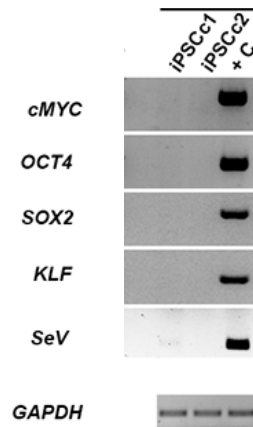


i) *In situ* staining for TRA-1-81 (bright field BF and fluorescence image).

ii) Immunocytochemistry of pluripotency markers (NANOG, OCT4, SOX2).

iii) Alkaline phosphatase assay. Scale bar 200 μ m.

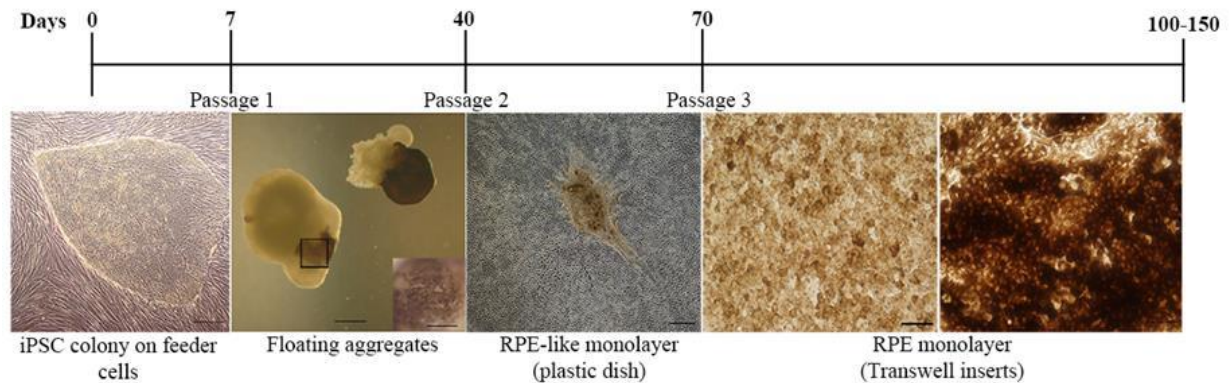
Detección del genoma de SeV y los transgenes (Klf-4, Oct-4 Sox2, c-Myc) en células reprogramadas mediante virus Sendai. Detection of SeV genome and transgenes in cells reprogrammed using Sendai reprogramming vectors.



Expresión de los transgenes del virus SeV en dos líneas de células iPS derivadas del mismo paciente (RT-PCR). Las líneas se analizaron en pases 7-10. Los fibroblastos recogidos el día 7 tras la trasducción representan el control positivo (Lukovic et al., 2015).

Expression of viral transgenes in derived iPSC lines by RT-PCR. Two iPSC cell lines from the same patient are analyzed at passages 7-10. The fibroblasts at day 7 after viral transduction are taken as positive control (Lukovic et al., 2015).

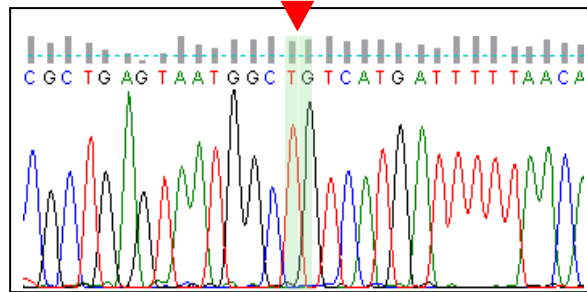
Diferenciación *in vitro* hacia epitelio pigmentario de la retina (EPR) mediante los cuerpos embrionarios (EBs) / *In vitro* differentiation toward retinal pigment epithelial (RPE) cells *via* embryoid bodies (EBs) formation



Diferenciación de las células iPS hacia el epitelio pigmentario de la retina (EPR). Una vez crecidas hasta la confluencia las células se cultivan en condiciones no adherentes hasta la formación de parches pigmentados. Las zonas pigmentadas se microdisecionan, tripsinizan y siembran en plástico recubierto con Matrigel. Esta moncapa se tripsiniza, pasa por el filtro y siembra en insertos transwell hasta pigmentarse.

Differentiation of iPS cells into retinal pigment epithelial (RPE) cells. iPS cells are grown to confluence and cultured in low-adhesion plates until the dark patches are formed. The dark patches are microdissected, trypsinized and plated in Matrigel- coated plastic plates. The RPE cell monolayer is trypsinized, passed through a strainer and plated on transwell inserts. The cells are cultured until they reach high pigmentation. Scale bars: 200µm (iPSC colony, floating aggregates, RPE-like monolayer) and 50µm (RPE monolayer).

Secuenciación dirigida del exón 7 (*MERTK*)/Directed sequencing of exon 7 (*MERTK*)



El cromatograma de secuenciación alrededor de la mutación. Sequencing chromatogram around the mutation site.

MERTK (NM_006343.2) c.992_993delCA

The sequence of amplified region:

```
cgaagaggttctaagagagagagaggctttatTTTTatagtataaatgaattctaataatcacaattattt
gctccgttacctgccacagtgagaattgtaattggtatTTtaggcgttttcctcaggtgttcagttatt
gtccgagggcatgagtaacacacagtgatgctagcggtaaaatgtgtgtgtgcccagaaaggaagcaggt
ccttccccttagcgtagaggaaggccacgtgcaccgaatgcacacaggccccgtgctgacattccac
caccttactaatgcccgtcctcatgtttactcttcgttttagGTC AAGGAAGCTGATCCGCTGAGTAATG
GTC CA GTCATGATTTTTAAACACCTCTGCCTTACCACATCTGTACCAAATCAAGCAGCTGCAAGCCCTGGC
TAATTACAGCATTGGTGTTCCTGCATGAATGAAATAGGCTGGTCTGCAGTGAGCCCTTGGATTCTAGCC
AGCACGACTGAAGGAGgtaattcctggggttcagaatgtatattgccccaatgacatgtgattcaaaa
acccttcccagtgccctgactgagagttgaaactttgctttgtttggactttgtctctggaggagaaaat
tattgagggcactgatgaaaatgg
```

EXON7

The **CA** is absent in the patient's genome.

Identificación celular mediante la huella genética por análisis de microsatelites STR de la línea celular / Cell identity: genetic fingerprinting by microsatellite analysis/STR of the iPS cell line

MARKER	STR Size
D19S572	130/130
D2S2159	174/182
D17S1533	217/217
D14S972	197/197
D8S601	226/234
D9S1853	252/256
D8S1179	181/189
D18S51	277/293
D2S1338	181/181
TH01	250/254
D16S539	147/164
D19S433	199/199
vWA	148/148
D21S11	222/227

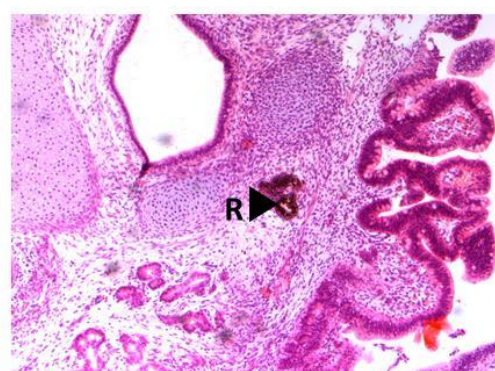
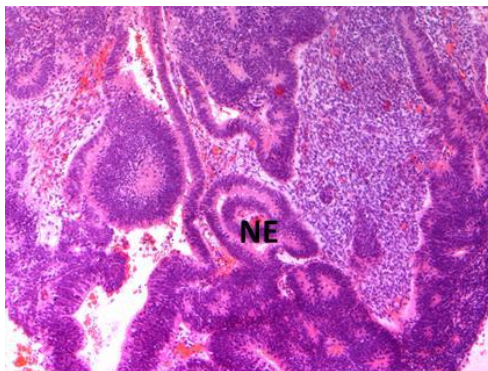
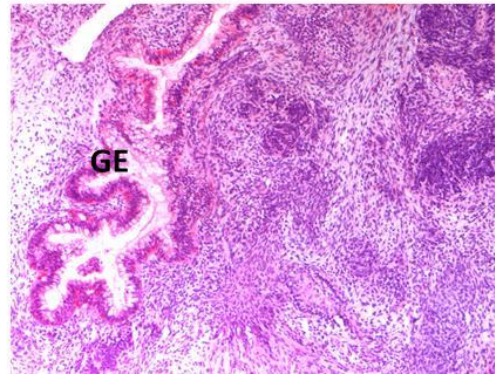
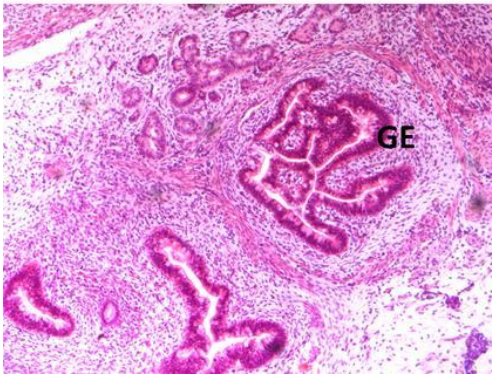
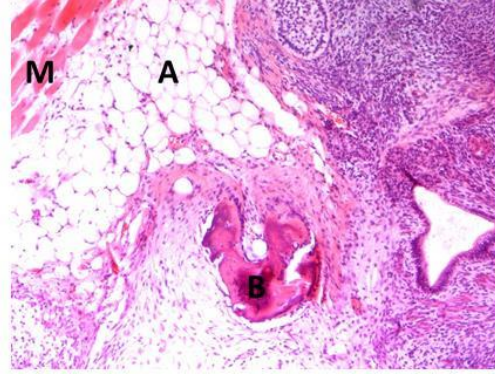
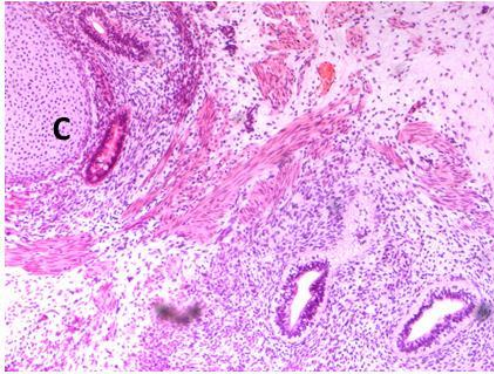
Test de *Mycoplasma*/*Mycoplasma* detection

Mycoplasma enzymes activity test.

Result: negative.

Ensayo de formación teratoma: análisis histológico (tinción Hematoxilina Eosina).

Teratoma formation assay: histological analysis (Hematoxylin Eosin staining)



Ectodermo: retina (R), neuroepitelio (NE). Endodermo: epitelio de intestino (GE). Mesodermo: musculo (M), hueso (B), cartílago (C), adipocitos (A).

Ectoderm: retina (R), neuroepithelium (NE). Endoderm: gut epithelium (GE). Mesoderm: muscle (M), bone (B), cartilage (C), adipocytes (A).