Certificate of Analysis 2018

Invoice number: SCTC2017-00057

Name investigator: Nael Nadif Kasri.....

Cell line number: IPS17-00063.....

Project name: Antwerpen.....

Table 1: Information reprogrammed cell line

Information cell line:	
Product description	Fresh PBMCs nucleofected with episomal vectors OCT3/4, SOX2, KLF4, L-MYC, LIN28
Parental cell line	HEP17-00110
Parental cell type	PBMC
Diagnosis	benign neonatal familial epilepsy
Mutation	N/A*
Number of clones	3
Passage (P) of iPS cells reported at submission	P10
Culture medium	Essential 8 Flex medium
Culture matrix	Matrigel
Feeder during reprogramming	Mouse Embryonic Fibroblasts (MEFs)
Passage method	0.5 mM EDTA
Protocols in Q-portal	046588; 046591

Tabel 2: Information characterization reprogrammed cell line

Test Description:	**Test method:**	**Test specification:**	**Result:**
Activation of stem cell markers	qPCR	Upregulation of *OCT4*, *SOX2*, *NANOG*, *LIN28* compared to PBMCs	Pass
Expression of stem cell markers	Immunocytochemistry	Expression of Oct4, Nanog, SSEA-4, Tra1-81	Pass
Mycoplasm	PCR	Negative	Pass
Pluripotency	Differentiation assay	Upregulation of germlayer specific genes	N/A*

*N/A: Not Applicable

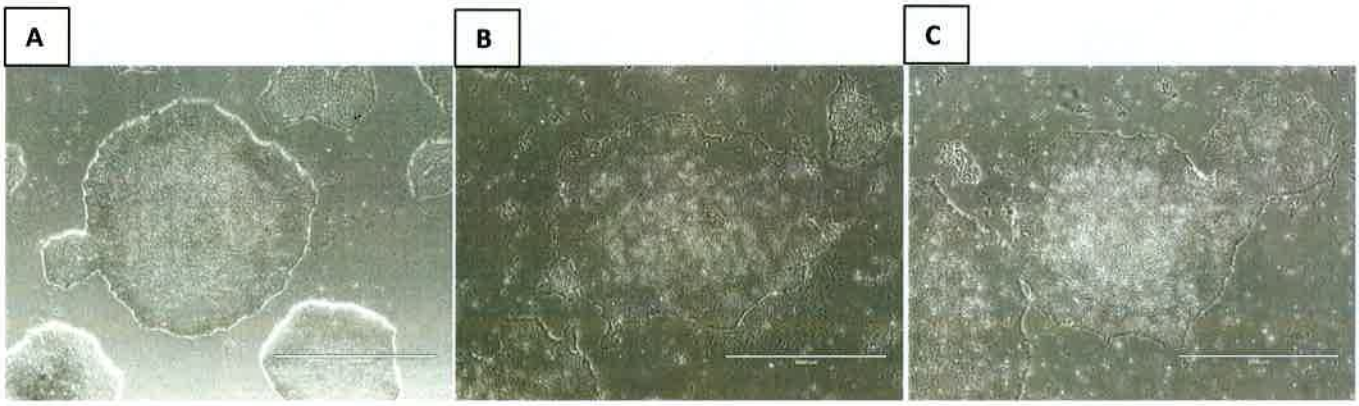


Figure 1: Cells prior to freezing. A - C, respectively clone 1, 2 and 3 at P10 Scale bar = 1000 µm.

Activation of stem cell markers

All clones are assessed for activation of stem cell markers before freezing. RNA is isolated and gene expression is assessed by quantitative reverse transcription PCR. Ct values are normalized with the housekeeping gene GUSB (set at 1).

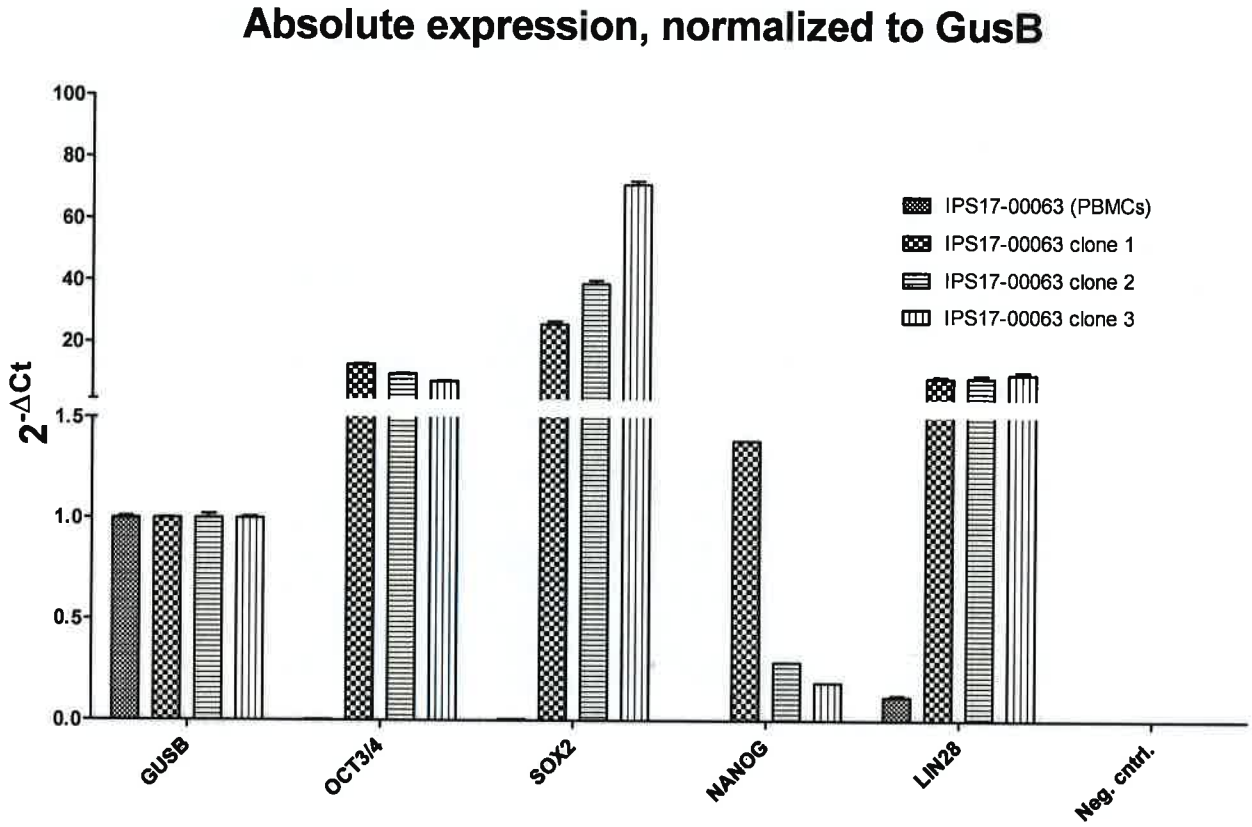


Figure 2: Gene expression of three iPS cell clones compared to the parental PBMCs (ΔCt).

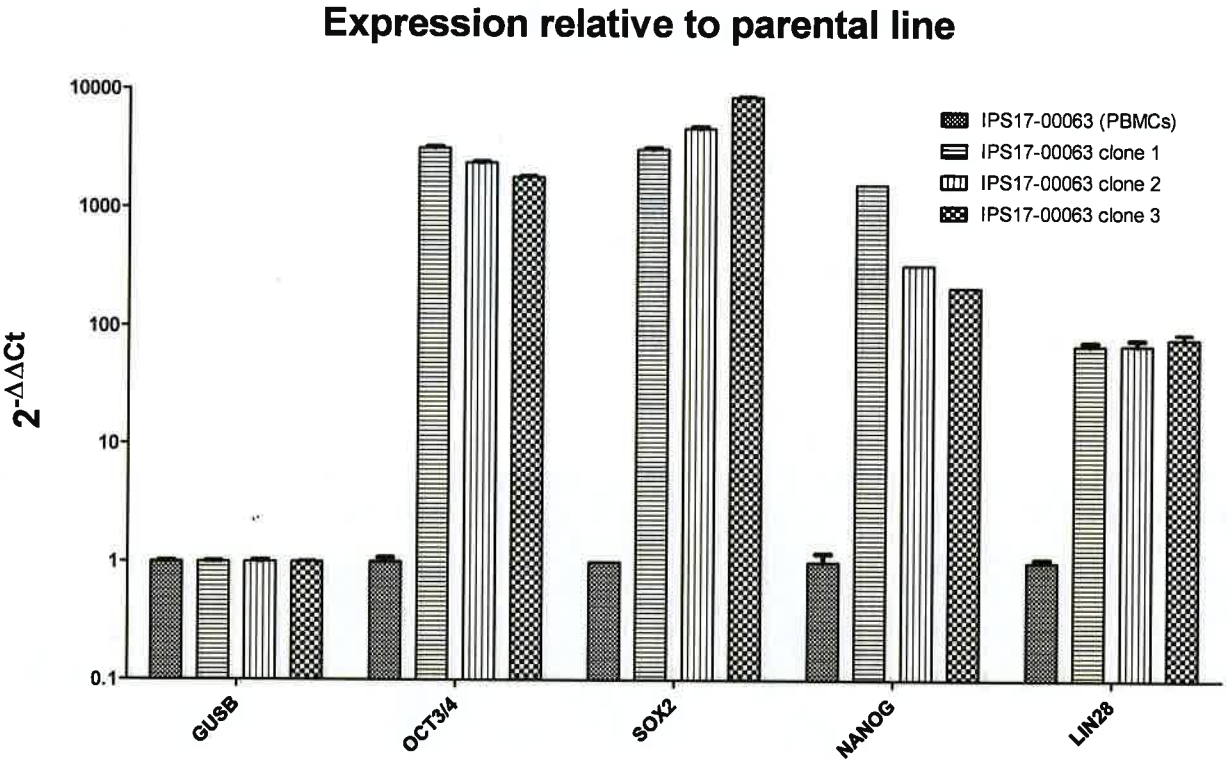
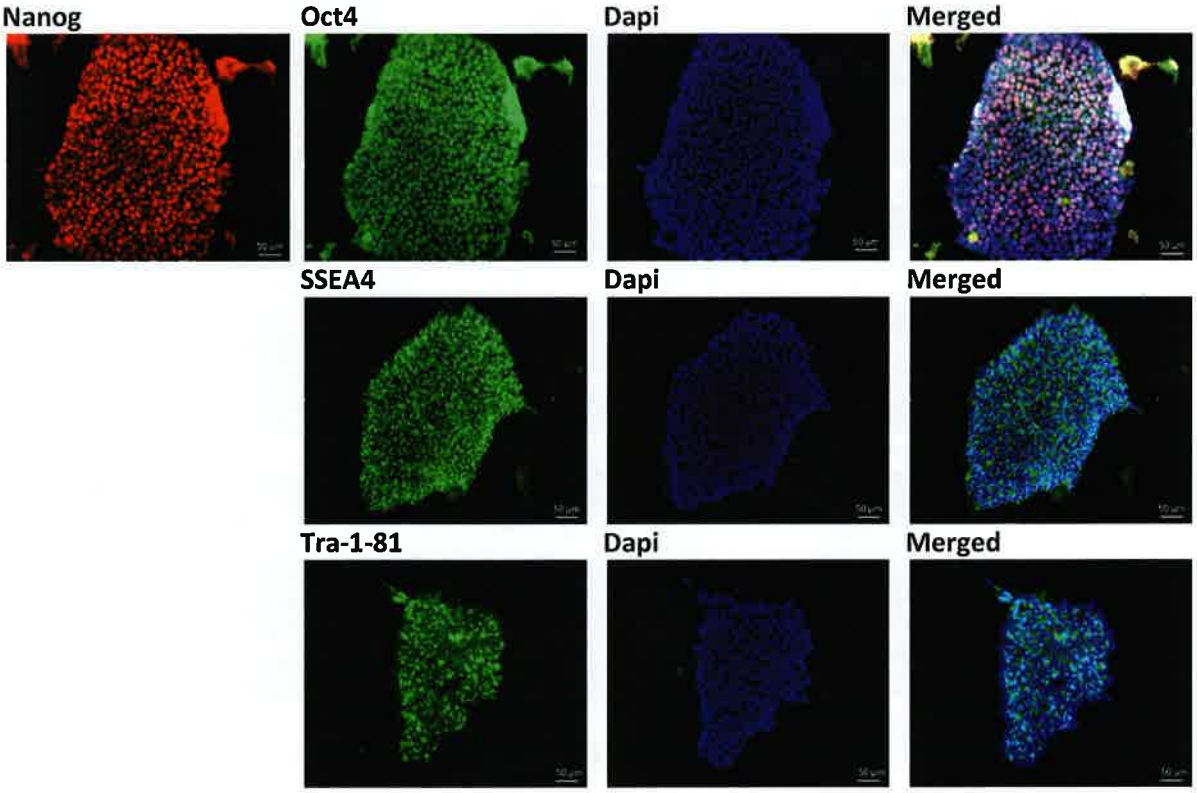


Figure 3: Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPS clones is relative to the parental PBMCs.

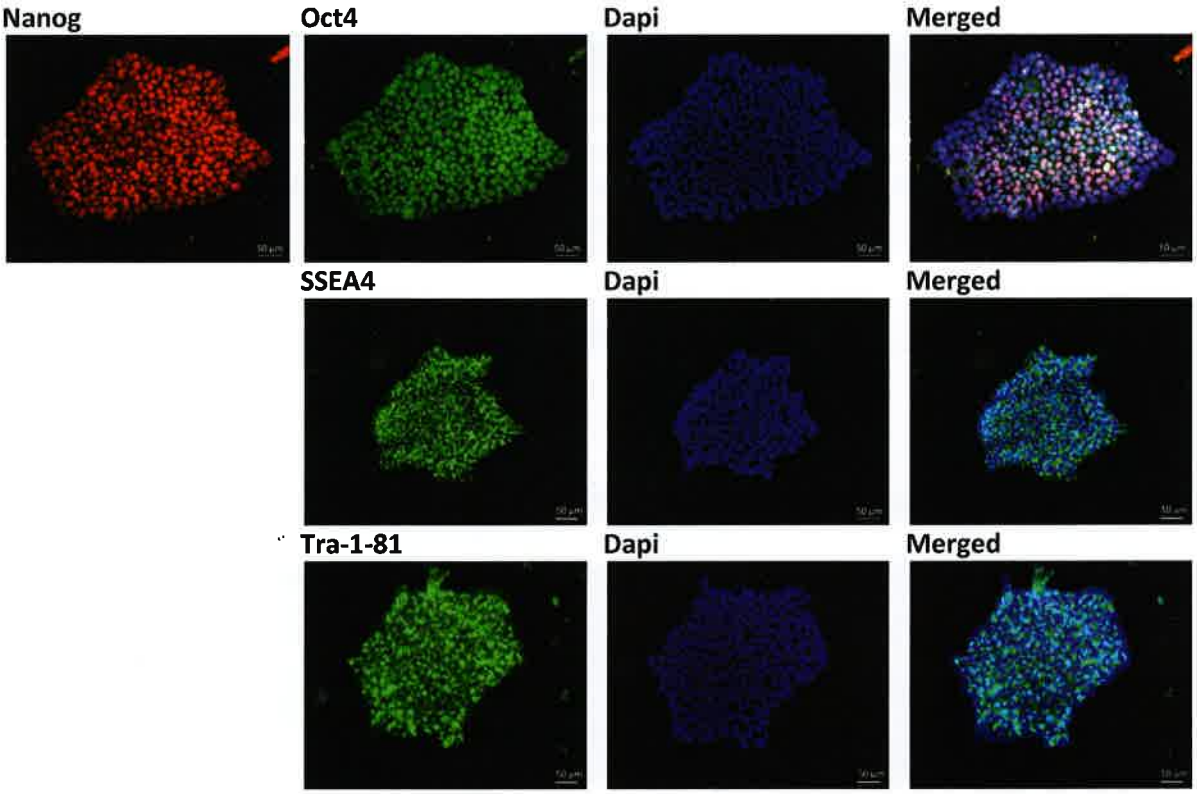
Expression of stem cell markers

Undifferentiated iPS cell clones are stained for the nuclear markers NANOG and OCT4 and surface antigens SSEA4 and TRA1-81. All markers are expressed in pluripotent human stem cells.

A. iPS17-00063 clone 1 P10



B. iPS17-00063 clone 2 P10



C. iPS17-00063 clone 3 P10

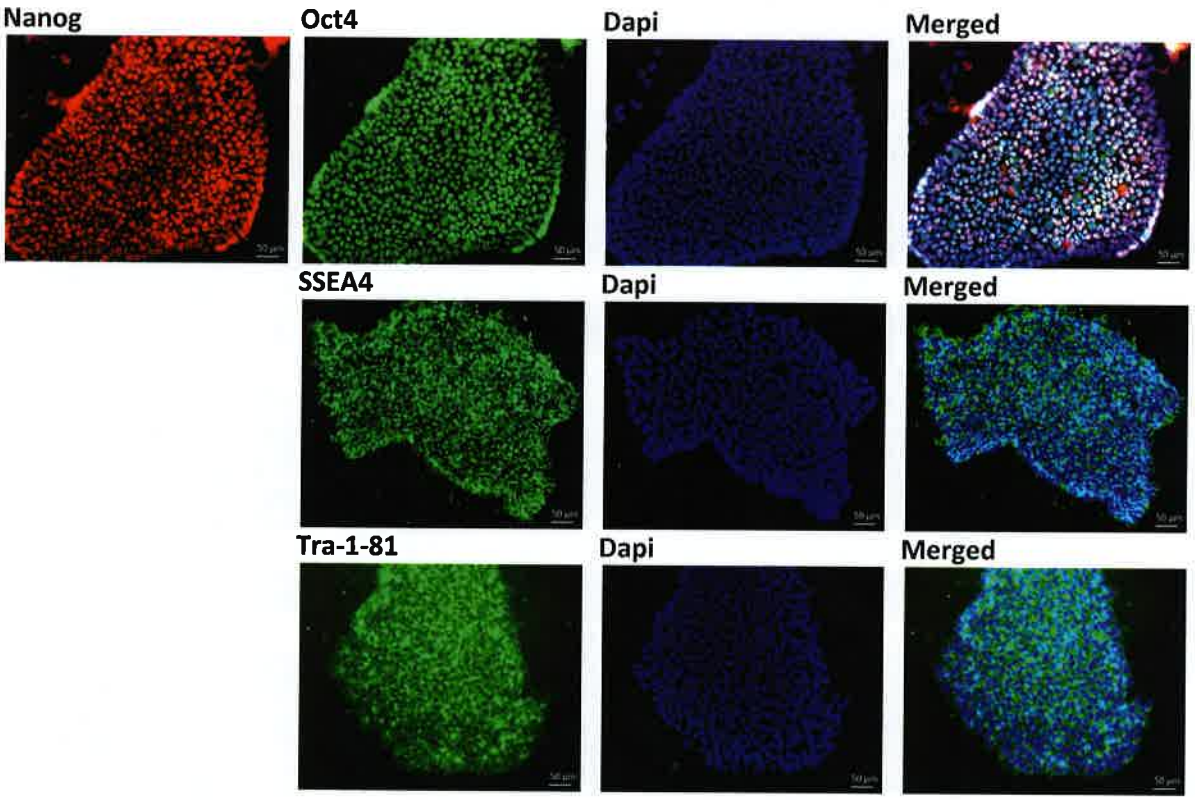


Figure 4. Immunofluorescence staining of the iPS cell clones with pluripotency markers.

- ✓ Pass
- ○ Fail
- ○ Other:

Silvia Albert

Silvia Albert, PhD
Manager, Radboud Stem Cell Technology Center
Date