

Certificate of Analysis 2019

Invoice number: SCTC2018-00085

Name investigator: Nina Dirx (Nael Nadif Kasri).....

Cell line number: IPS18-00095.....

Project name: KCNQ2 (Antwerpen).....

Table 1: Information on the reprogrammed cell line

Information cell line:	
Product description	Fresh PBMCs nucleofected with episomal vectors containing the genes OCT4, SOX2, KLF4, L-MYC, LIN28
Parental cell line	HEP18-00198
Parental cell type	PBMC
Diagnosis	EPL
Mutation	KCNQ2
Number of clones	3
Passage (P) of iPS cells reported at submission	P6
Culture medium	Essential 8 Flex medium
Culture coating	Vitronectin
Feeders during reprogramming	Mouse Embryonic Fibroblasts (MEFs)
Passage method	0.5 mM EDTA
Protocols in Q-portal	046588; 046591

Table 2: Information on the characterization of the reprogrammed cell line

Test Description:	Test method:	Test specification:	Result:
Activation of stem cell markers	qPCR	Upregulation of <i>OCT4</i> , <i>SOX2</i> , <i>DNMT3B</i> , <i>REX1</i> compared with PBMCs	Pass
Expression of stem cell markers	Immunocytochemistry	Expression of OCT4, NANOG, SSEA-4, TRA-1-81	Pass
Mycoplasma	PCR	Negative	Pass
Pluripotency	Differentiation assay	Upregulation of germlayer specific genes	N/A*

*N/A: Not Applicable

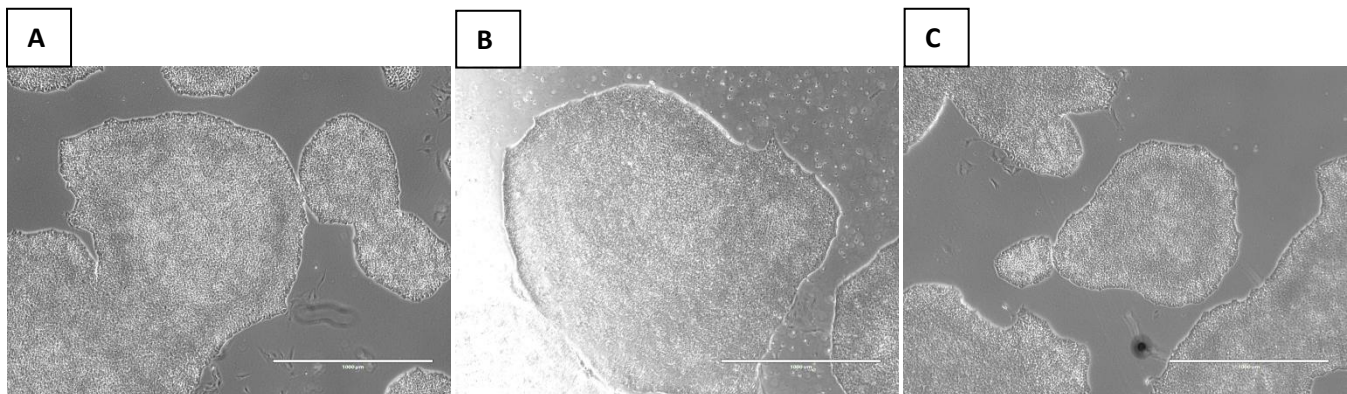


Figure 1: Cells prior to freezing. A - C, respectively clone 1, 2 and 3 at P6. Scale bar = 1000 µm.

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).

Absolute expression, normalized to GusB

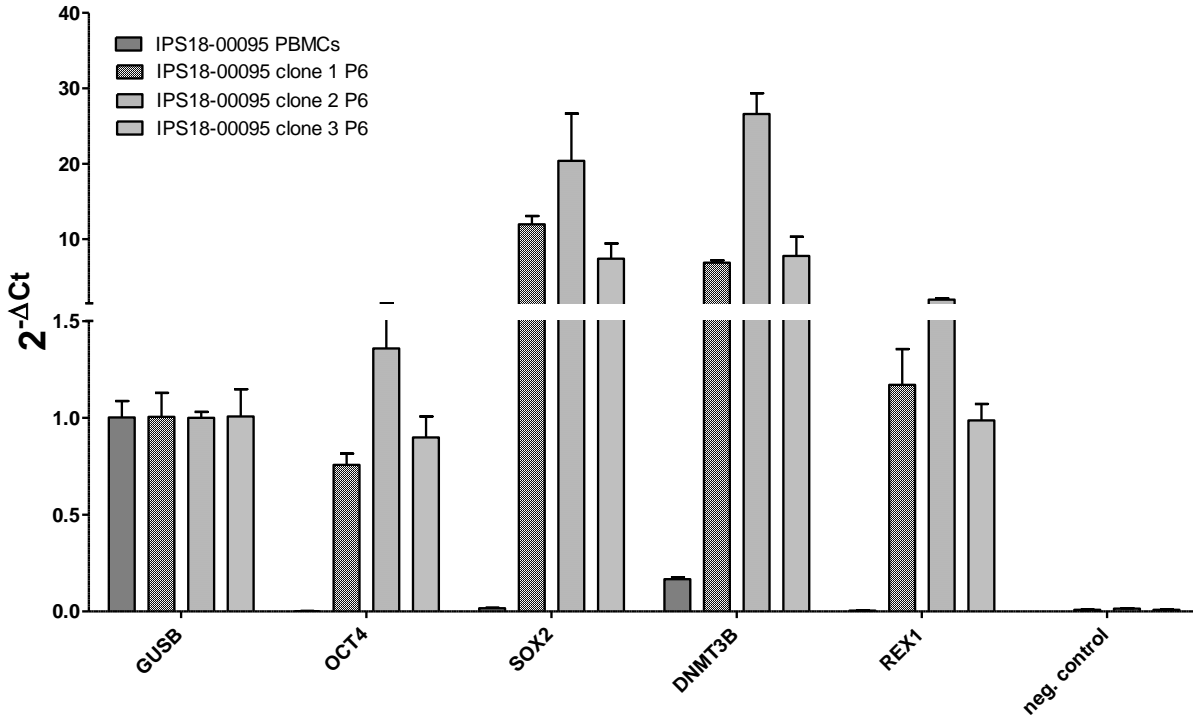


Figure 2: Gene expression of three iPS cell clones compared with the parental PBMCs (ΔCt).

Expression relative to parental line

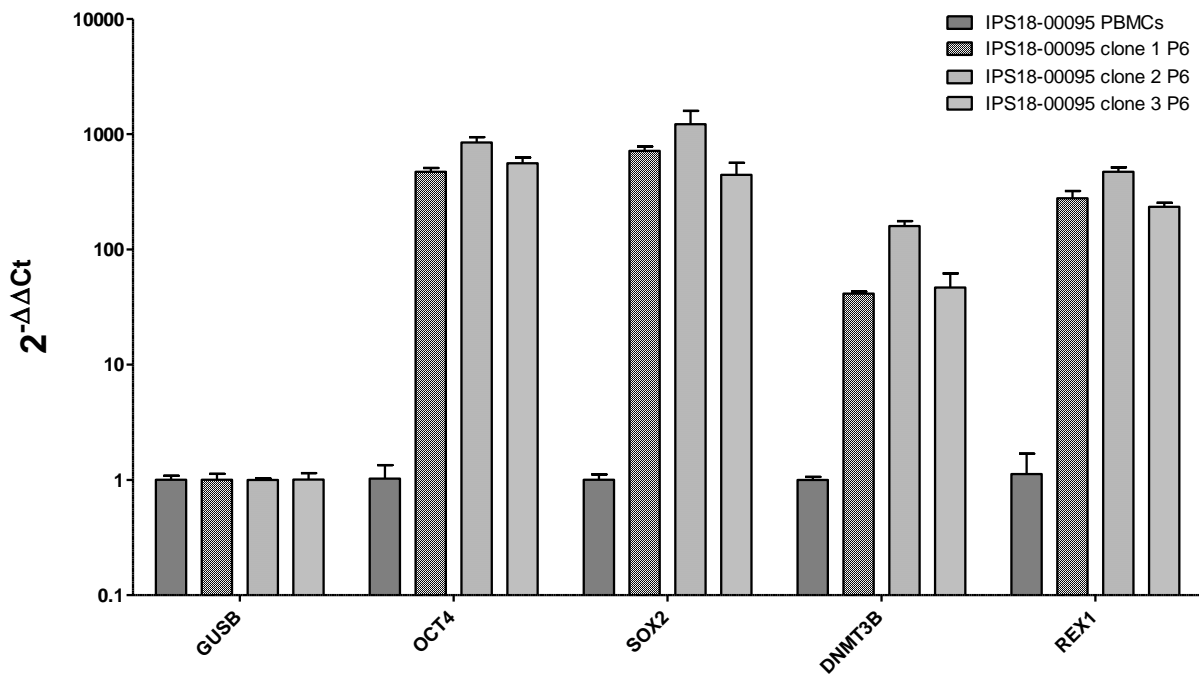
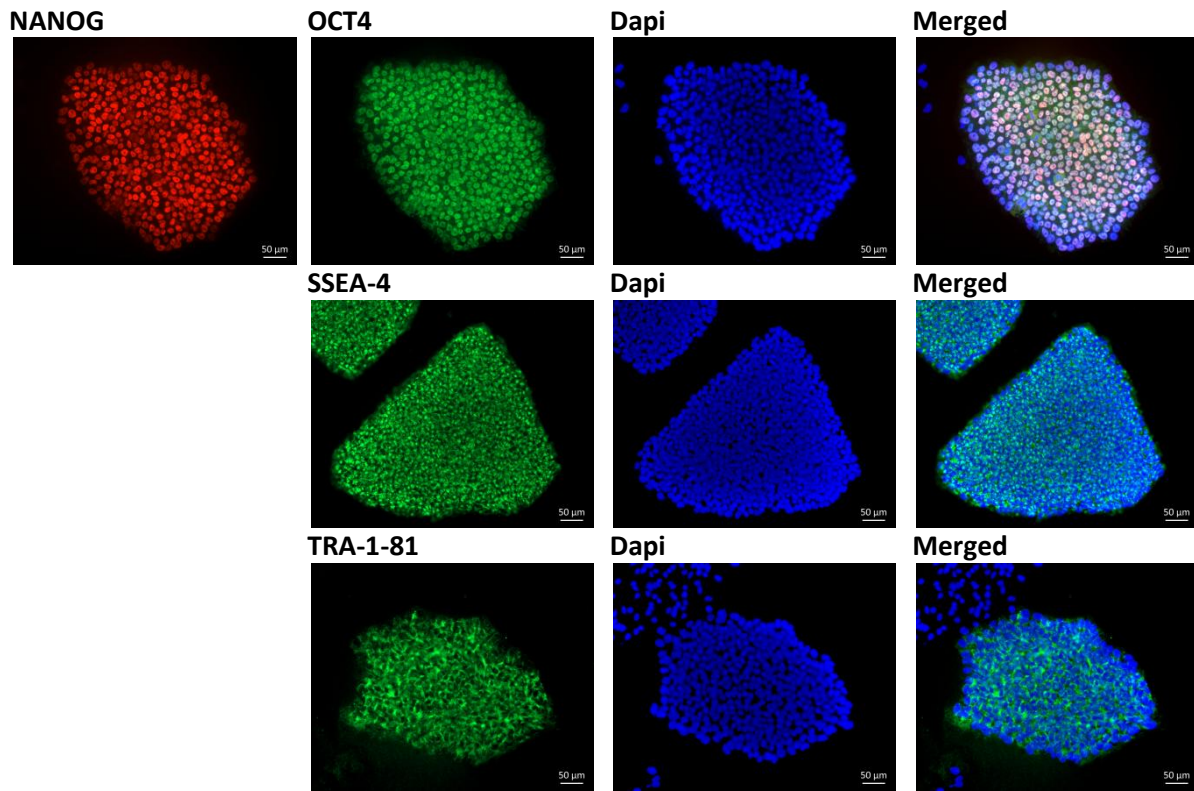


Figure 3: Pluripotency gene upregulation after reprogramming ($\Delta\Delta Ct$). The expression fold difference of the iPS clones is relative to the parental PBMCs.

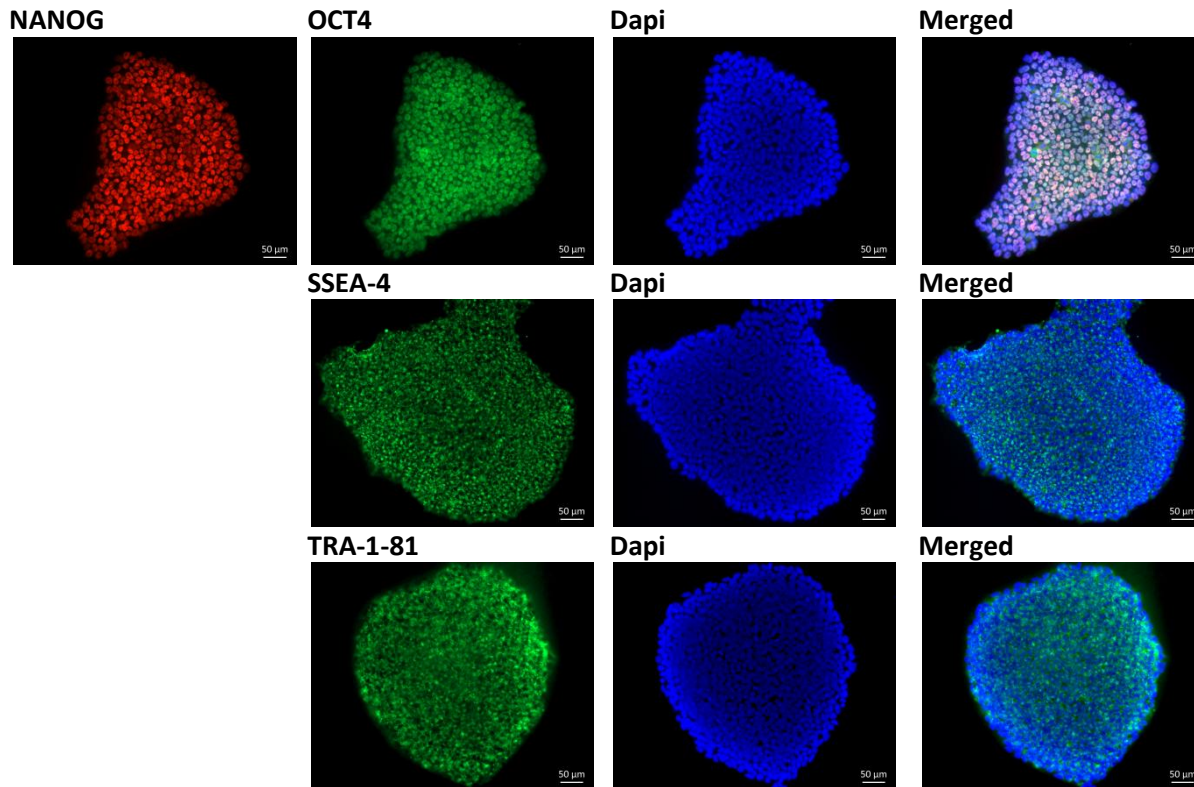
Expression of stem cell markers

Undifferentiated iPS cell clones are stained for the nuclear markers NANOG and OCT4 and surface antigens SSEA-4 and TRA-1-81. All markers are expressed in human pluripotent stem cells.

A. IPS18-00095 clone 1 P6



B. IPS18-00095 clone 2 P6



C. IPS18-00095 clone 3 P6

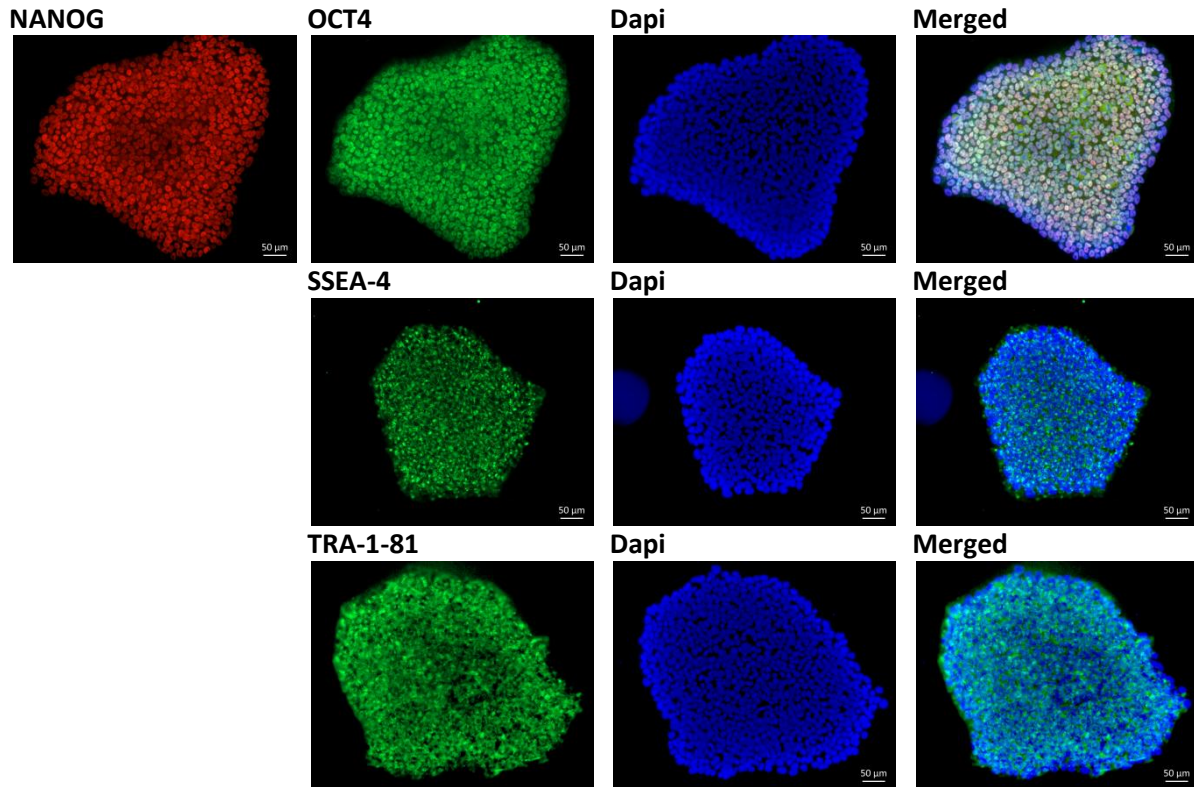


Figure 4. Immunofluorescence staining of the iPS cell clones with pluripotency markers.

- Pass
- Fail
- Other:

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