Stem Cell Technology Center Genetica

Certificate of Analysis 2019

Invoice number: SCTC2019-00095

Name investigator: Sarah Weckhuysen
Cell line number: IPS19-00092
Project name: KCNQ2 encephalopathy - Antwerpen

Table 1: Information on the reprogrammed cell line

Information cell line:	
Product description	PBMCs nucleofected with episomal vectors containing the genes OCT3/4, SOX2, KLF4, L-MYC, LIN28
Parental cell line	PBM19-00024
Parental cell type	PBMCs
Diagnosis	N/A*
Mutation	N/A*
Number of clones	3
Passage (P) of iPSCs reported at submission	P6
Culture medium	Essential 8 Flex medium
Culture coating	Matrigel
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 *SOX2, LIN28, NANOG, DNMT3B* compared with PBMCs	Pass
Expression of stem cell markers	Immunocytochemistry	Expression of OCT4, NANOG, SSEA4, TRA-1-81	Pass
Mycoplasma test	PCR	Negative	Pass
Three lineage differentiation	Differentiation assay	Upregulation of germlayer-specific genes	N/A*

*N/A: Not Applicable

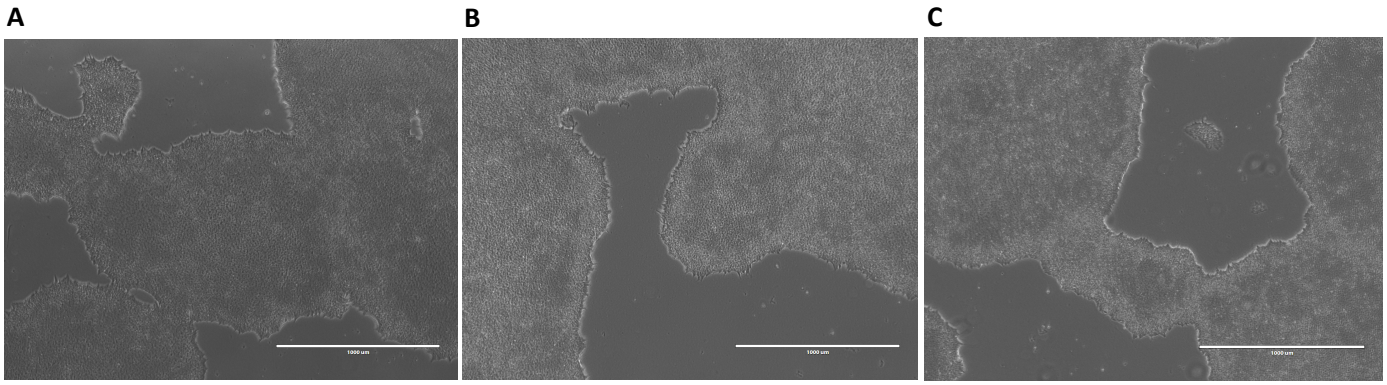


Figure 1: Cells prior to freezing. A - C, clone 1, clone 2 and clone 3, respectively at P6. Scale bar = 1000 µm.

Activation of stem cell markers

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

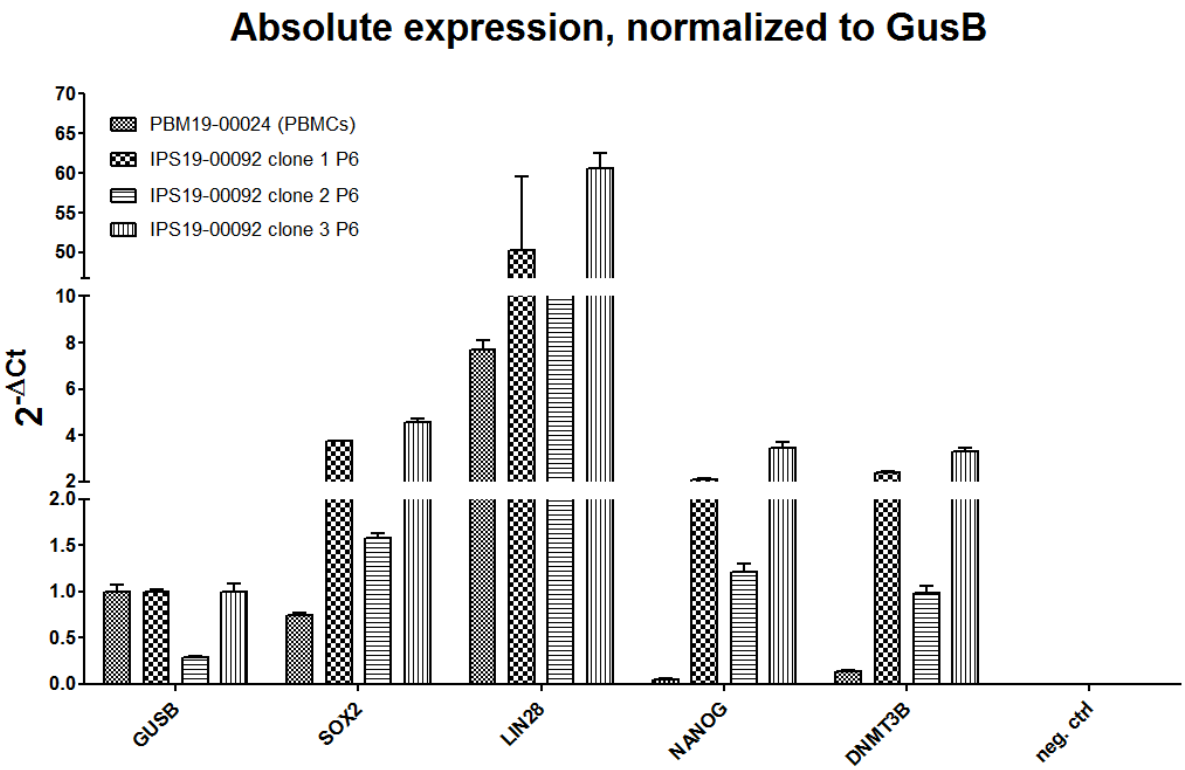


Figure 2: Gene expression of three iPSC clones compared with the parental PBMCs (ΔCt).

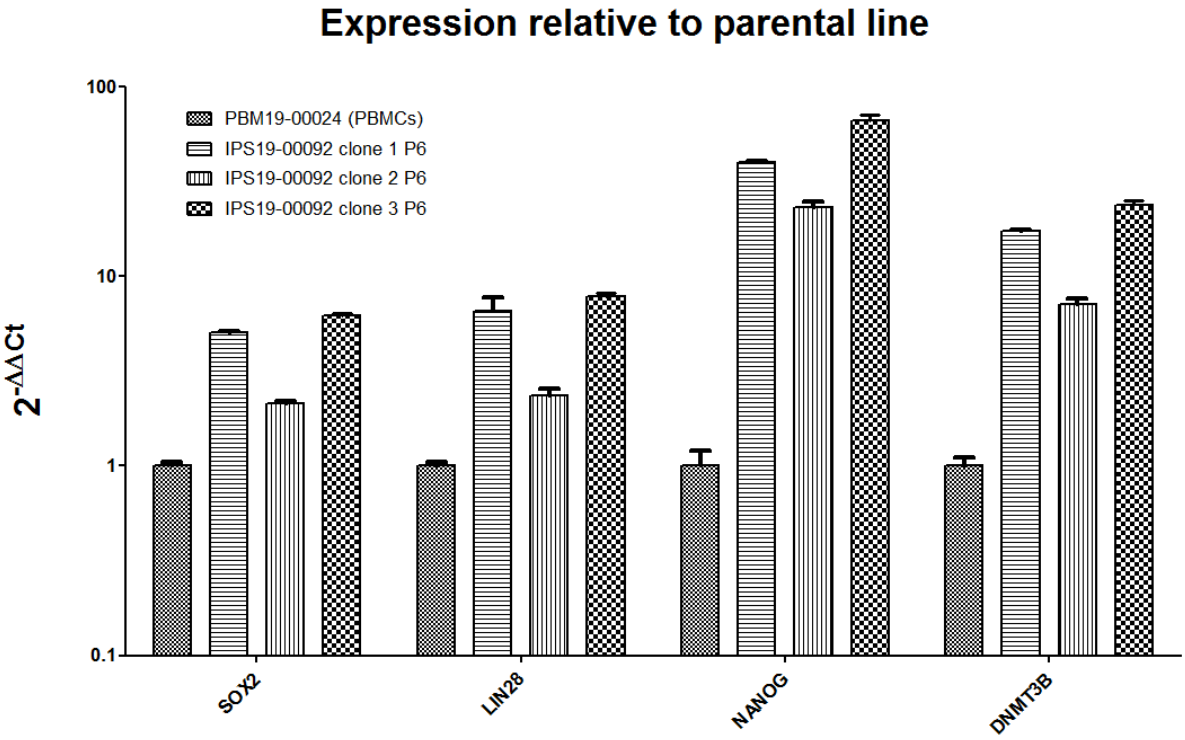
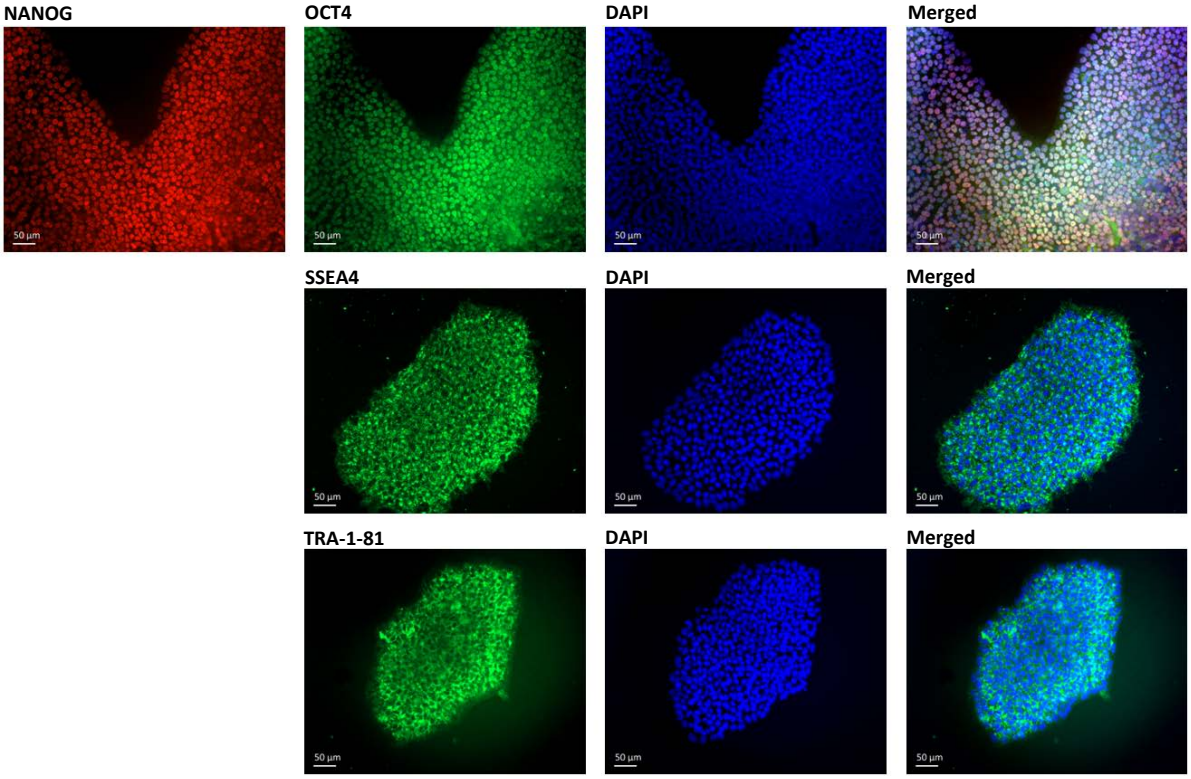


Figure 3: Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPSCs is relative to the parental PBMCs.

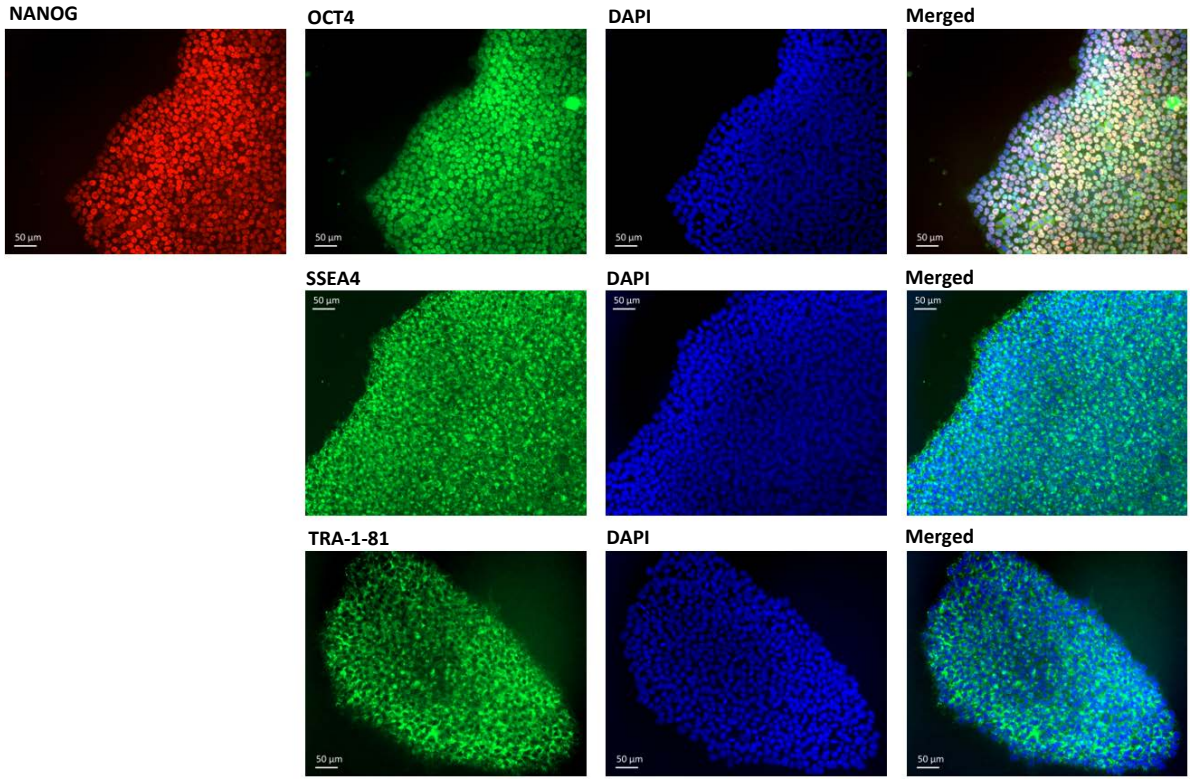
Expression of stem cell markers

Undifferentiated iPSC clones were stained for the nuclear markers NANOG and OCT4 and surface antigens SSEA4 and TRA-1-81. All markers are expressed in human pluripotent stem cells.

A. IPS19-00092 clone 1



B. IPS19-00092 clone 2



C. IPS19-00092 clone 3

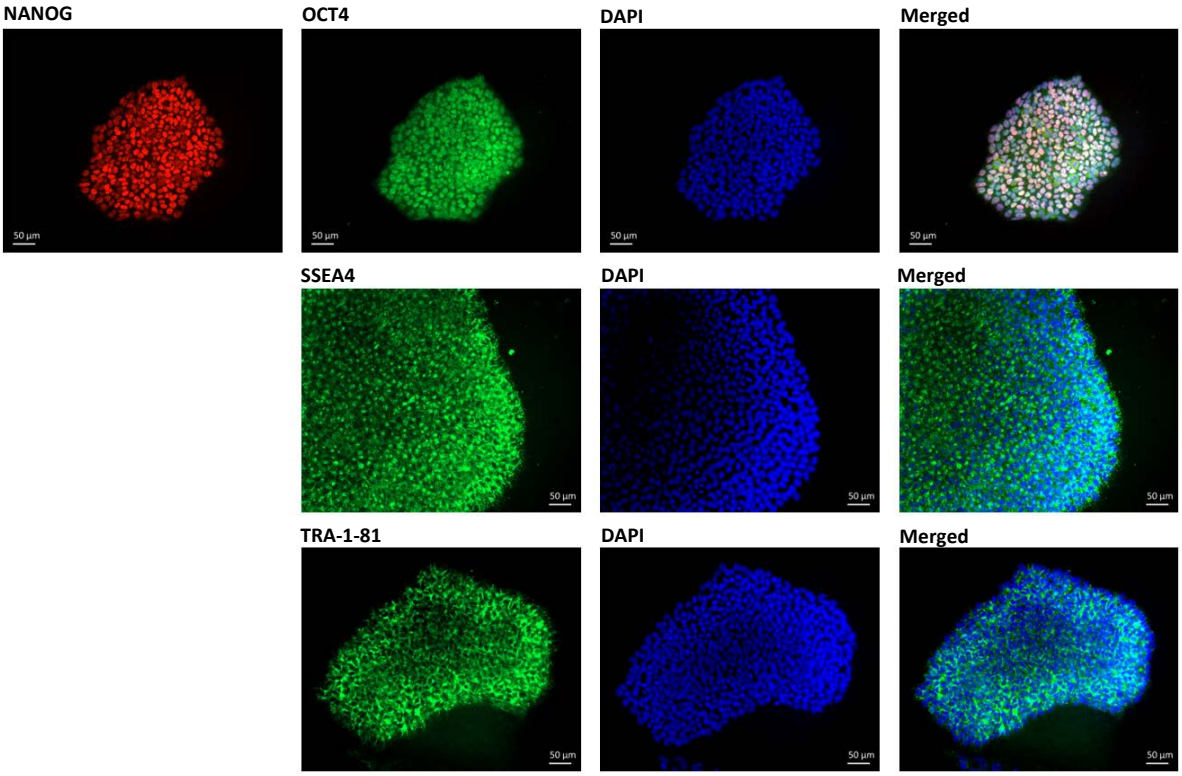


Figure 4: Immunofluorescence staining of the iPSC clones with pluripotency markers.

(●) Pass
( ) Fail
( ) Other:

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Date 23-12-2019