Stem Cell Technology Center Genetica

Stem Cell Technology Center

Certificate of Analysis 2021

Invoice number: SCTC2021-00110

Name principal investigator: Hans van Bokhoven
Cell line number: IPS21-00178
Project name: FECD

Table 1: Information on the reprogrammed cell line

Information cell line:	
Product description	Erythroblasts nucleofected with episomal vectors containing the genes OCT3/4, SOX2, KLF4, L-MYC, LIN28
Parental cell line Parental cell type	PBM20-00032 Erythroblasts
Diagnosis Mutation	N/A* N/A*
Number of clones Passage (P) of iPSCs reported at delivery	1 P6
Culture medium Culture coating Feeders during reprogramming Passage method	Essential 8 Flex medium Matrigel Mouse Embryonic Fibroblasts (MEFs) EDTA

*N/A: Not Applicable

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* in iPSCs compared with erythoblasts	Pass
Expression of stem cell markers	Immunocytochemistry	Expression of OCT4, NANOG, SSEA4, TRA-1-81	Pass
Mycoplasma test	PCR	Negative	Pass

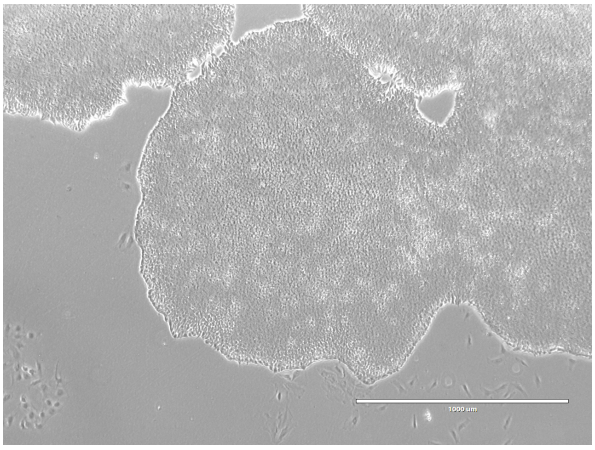

Figure 1: Cells prior to freezing. clone 1 at P6.

Activation of stem cell markers

The RNA of all clones was isolated before freezing and the gene expression was assessed by quantitative reverse transcription PCR (qRT-PCR). Ct values were normalized with the housekeeping gene GUSB, set at 1.

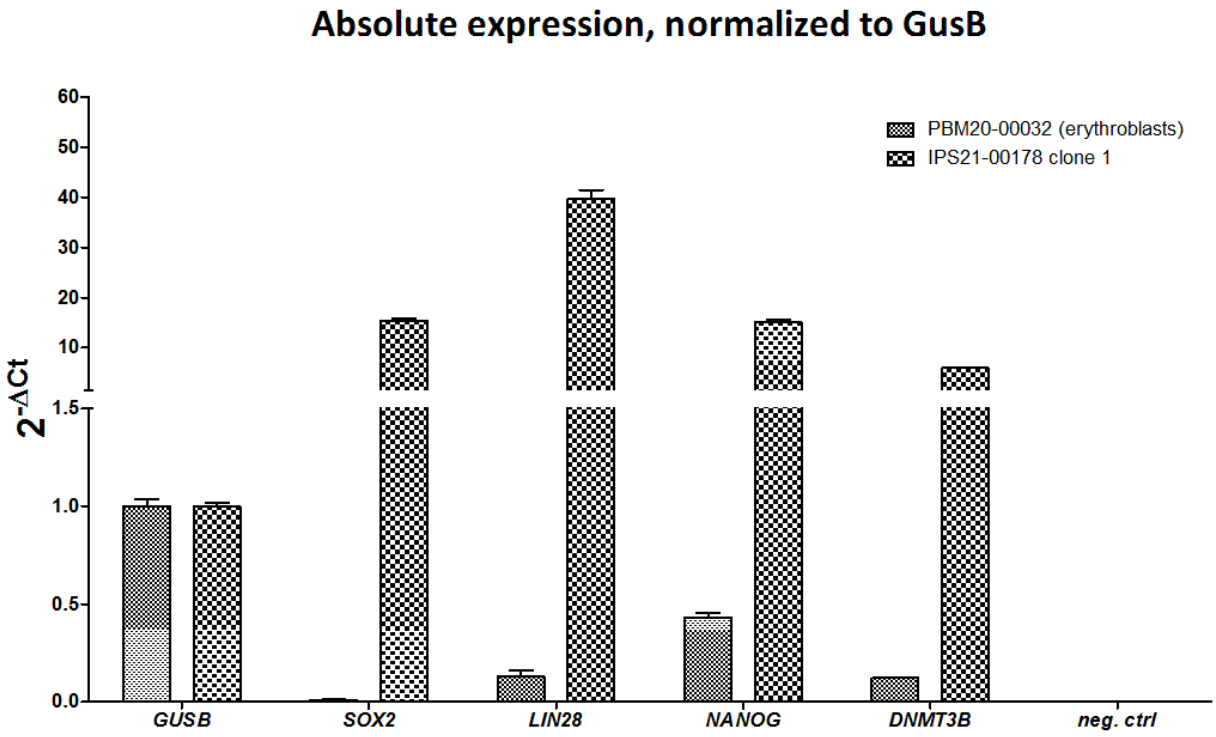

Figure 2: Gene expression of the iPSC clone compared with the parental erythroblasts (ΔCt).

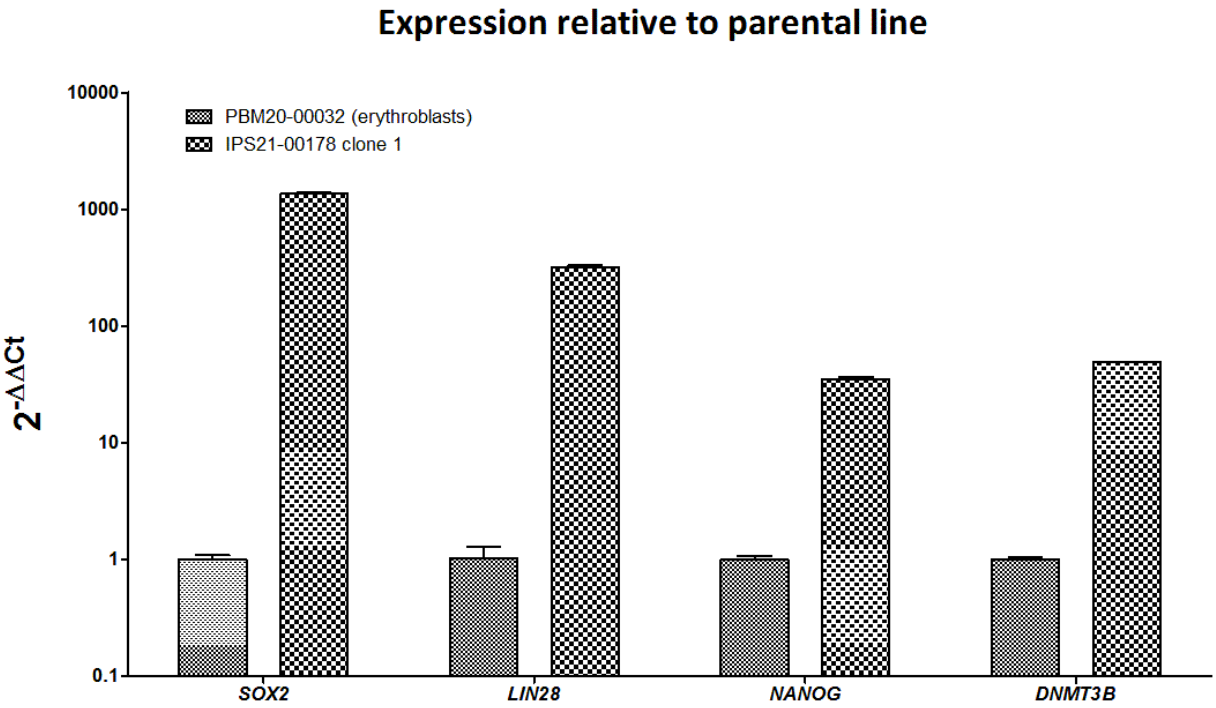

Figure 3: Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPSC clone is relative to the parental erythroblasts.

Expression of stem cell markers

One undifferentiated iPSC clone was 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. IPS21-00178 clone 1

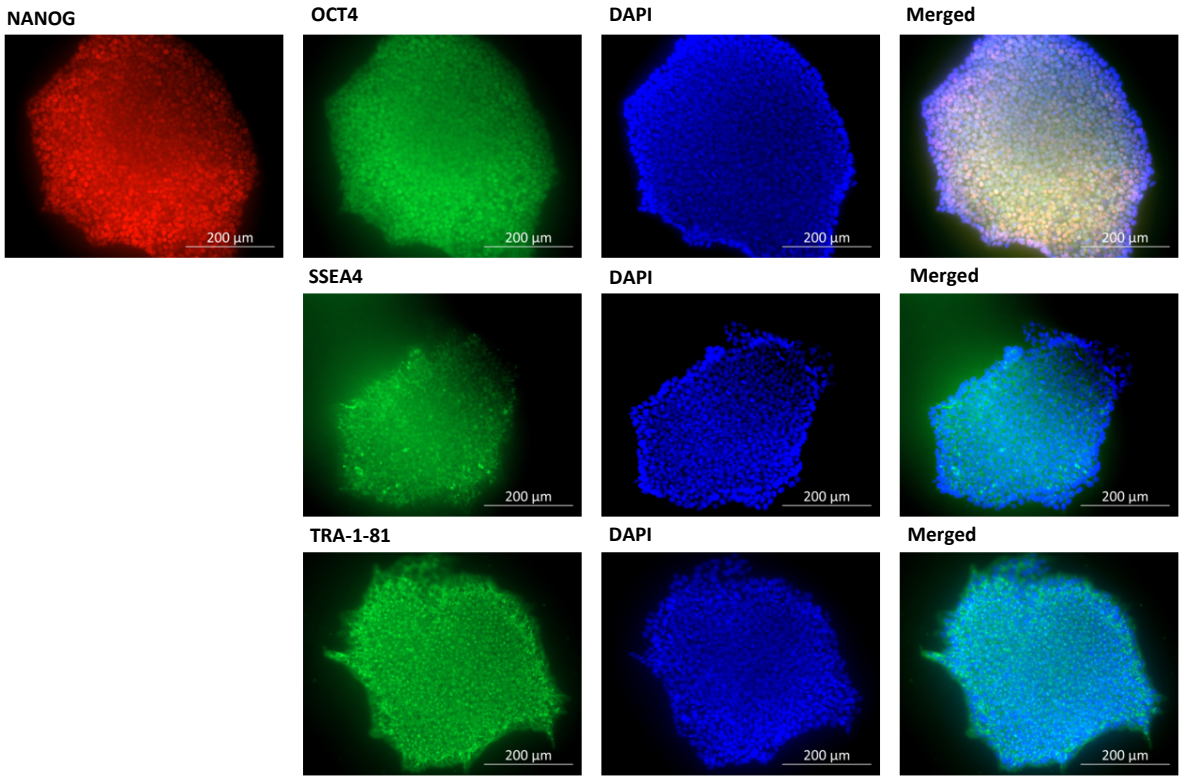

Figure 4: Immunofluorescence staining of the iPSC clone with pluripotency markers.

(●) Pass
( ) Fail
( ) Other:

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