

## Certificate of Analysis 2019

Invoice number: SCTC2019-00081

Name investigator: Hans van Bokhoven

Cell line number: IPS19-00072

Project name: DMI iNeurons

Table 1: Information on the reprogrammed cell line

Information cell line:	
Product description	Fibroblasts reprogrammed with viral vectors containing the genes OCT4, SOX2, KLF4, C-MYC
Parental cell line	CL17-00046
Parental cell type	Fibroblasts
Diagnosis	DM
Mutation	N/A*
Number of clones	1
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 <i>SOX2</i> , <i>LIN28</i> , <i>NANOG</i> , <i>DNMT3B</i> compared with fibroblasts	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

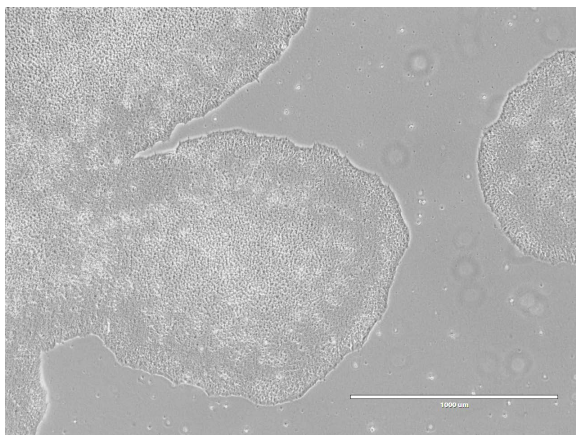


Figure 1: Cells prior to freezing at P6. Scale bar = 1000 μm.

## Activation of stem cell markers

The iPSC clone was 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).

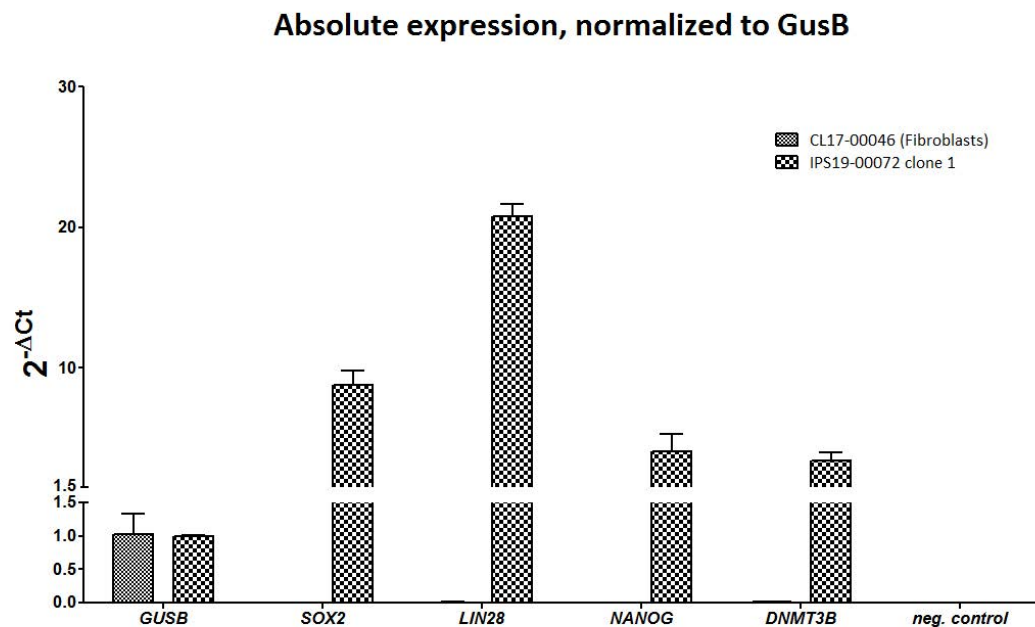


Figure 2: Gene expression of the iPSC clone compared with the parental fibroblasts ( $\Delta$ Ct).

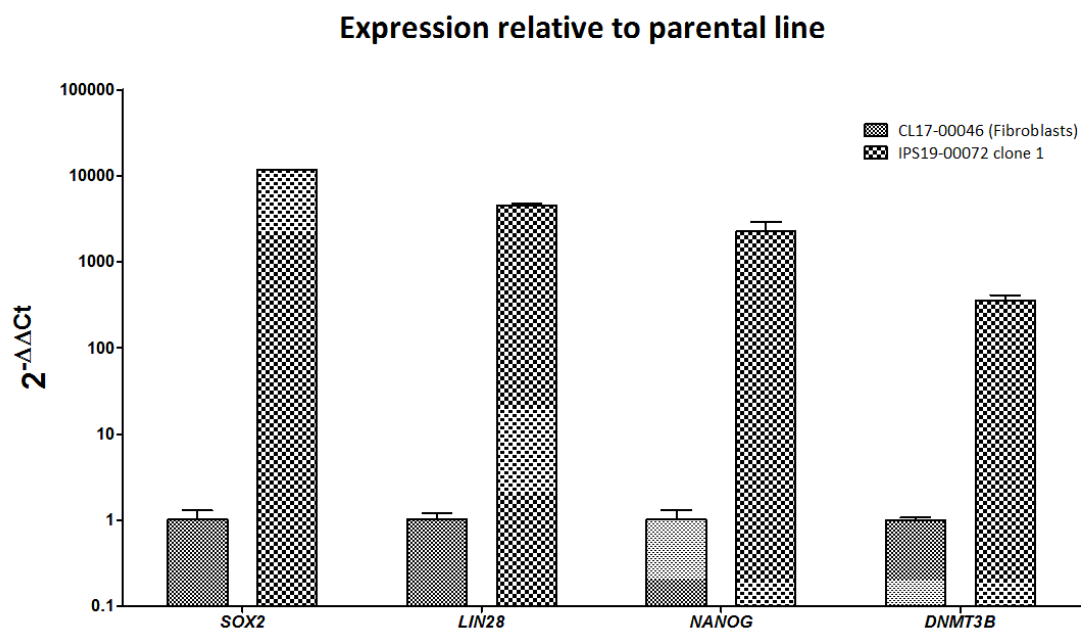


Figure 3: Pluripotency gene upregulation after reprogramming ( $\Delta\Delta$ Ct). The expression fold difference of the iPSCs is relative to the parental fibroblasts.

## 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. IPS19-00072 clone 1

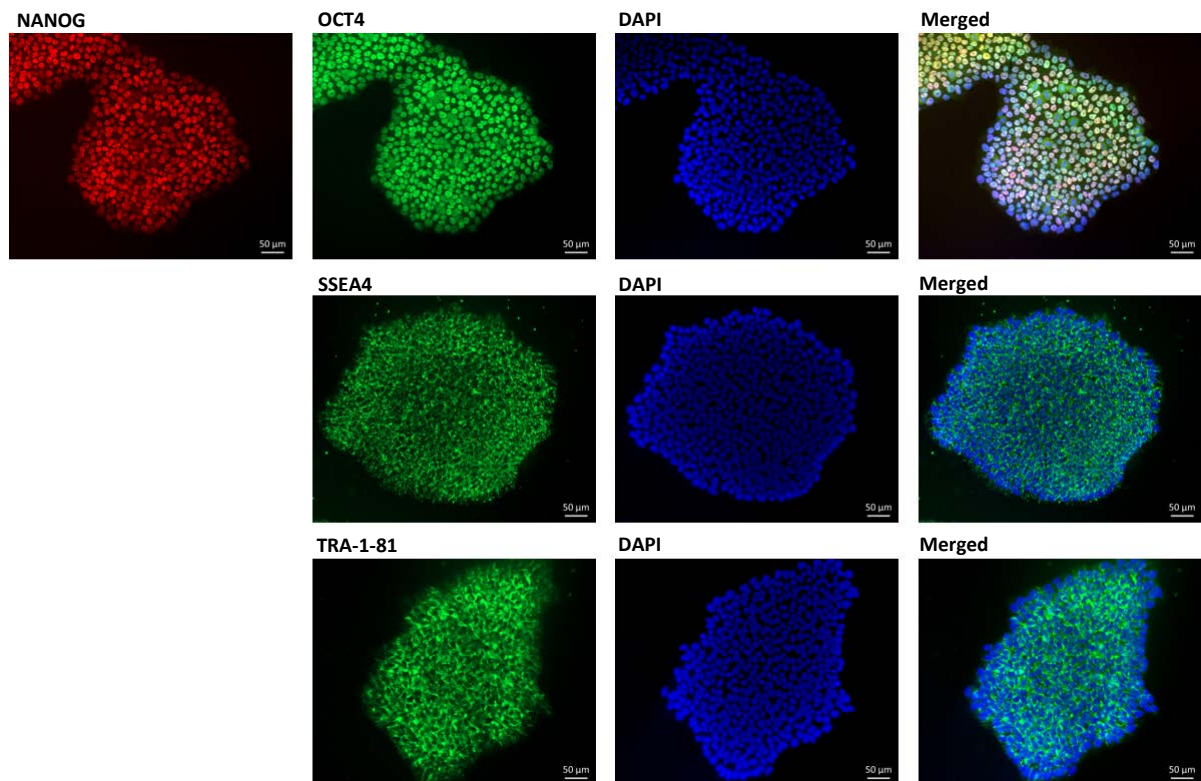


Figure 4: Immunofluorescence staining of the iPSC clone with pluripotency markers.

Pass

Fail

Other:

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Date