

## Certificate of Analysis 2021

Invoice number: SCTC2021-00085

Name principal investigator: Hans van Bokhoven

Cell line number: IPS21-00148

Project name: DM1 stem cells

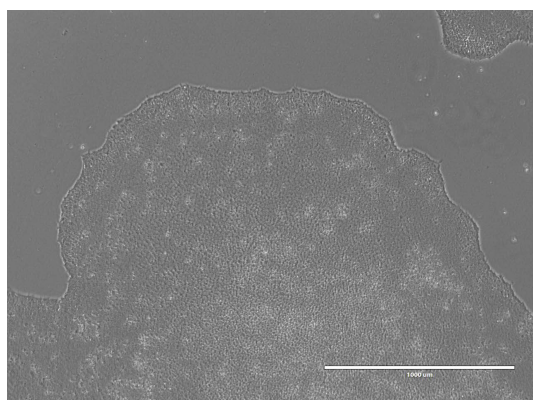
**Table 1: Information on the reprogrammed cell line**

Information cell line:	
Product description	Human fibroblasts reprogrammed with four factors (Oct4, Sox2, Klf4, cMyc) by using lentiviral vectors
Parental cell line Parental cell type	CL18-00018 Fibroblasts
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 <i>SOX2</i> , <i>LIN28</i> , <i>NANOG</i> , <i>DNMT3B</i> in iPSCs compared with fibroblasts	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.

### Absolute expression, normalized to GusB

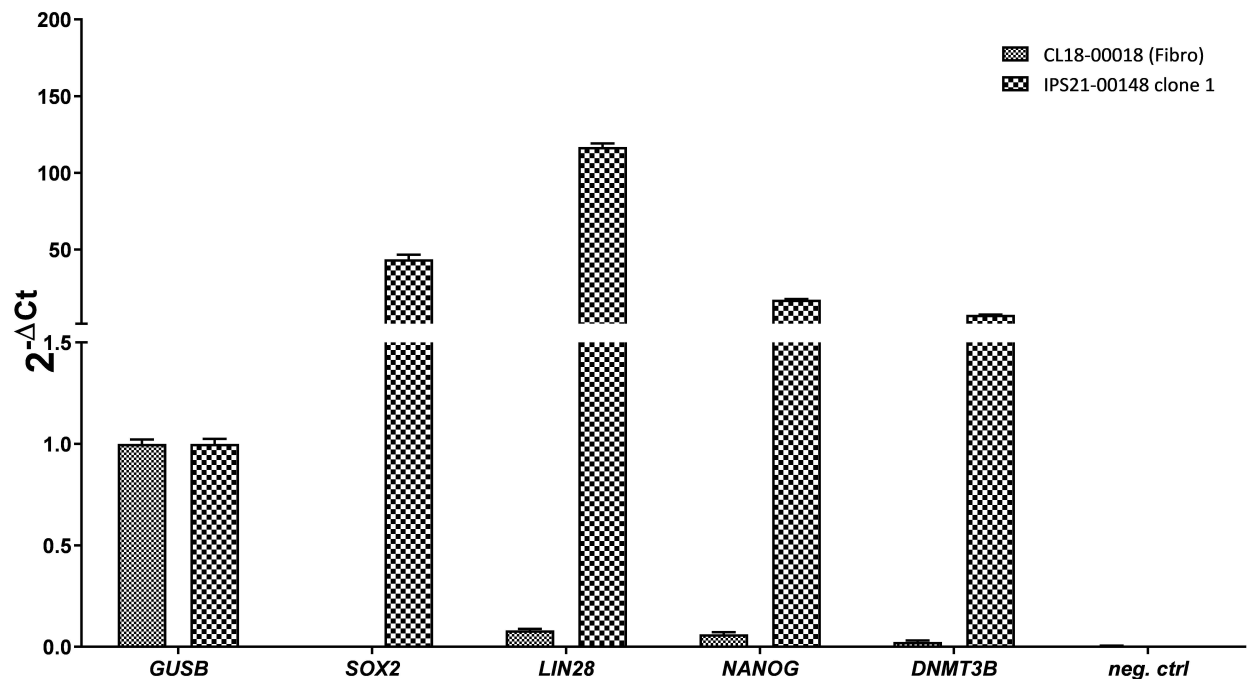


Figure 2: Gene expression of the iPSC clone compared with the parental fibroblasts ( $\Delta Ct$ ).

### Expression relative to parental line

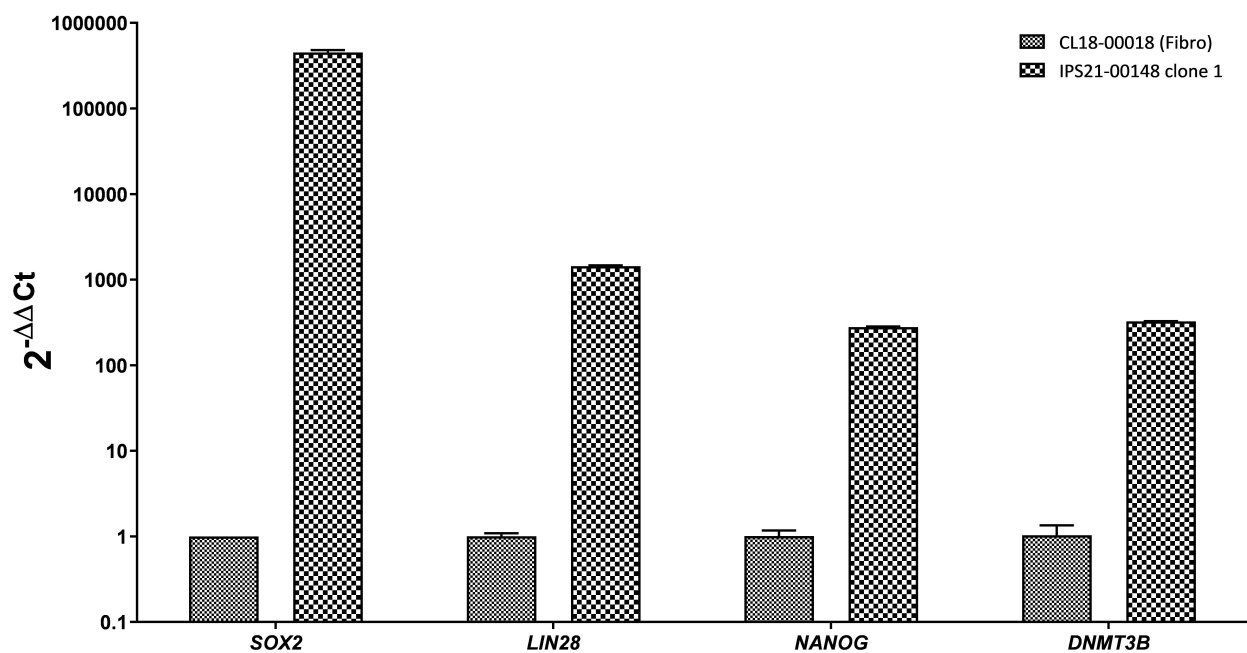


Figure 3: Pluripotency gene upregulation after reprogramming ( $\Delta\Delta Ct$ ). The expression fold difference of the iPSC clone is relative to the parental Fibroblasts.

## 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. IPS21-00148 clone 1

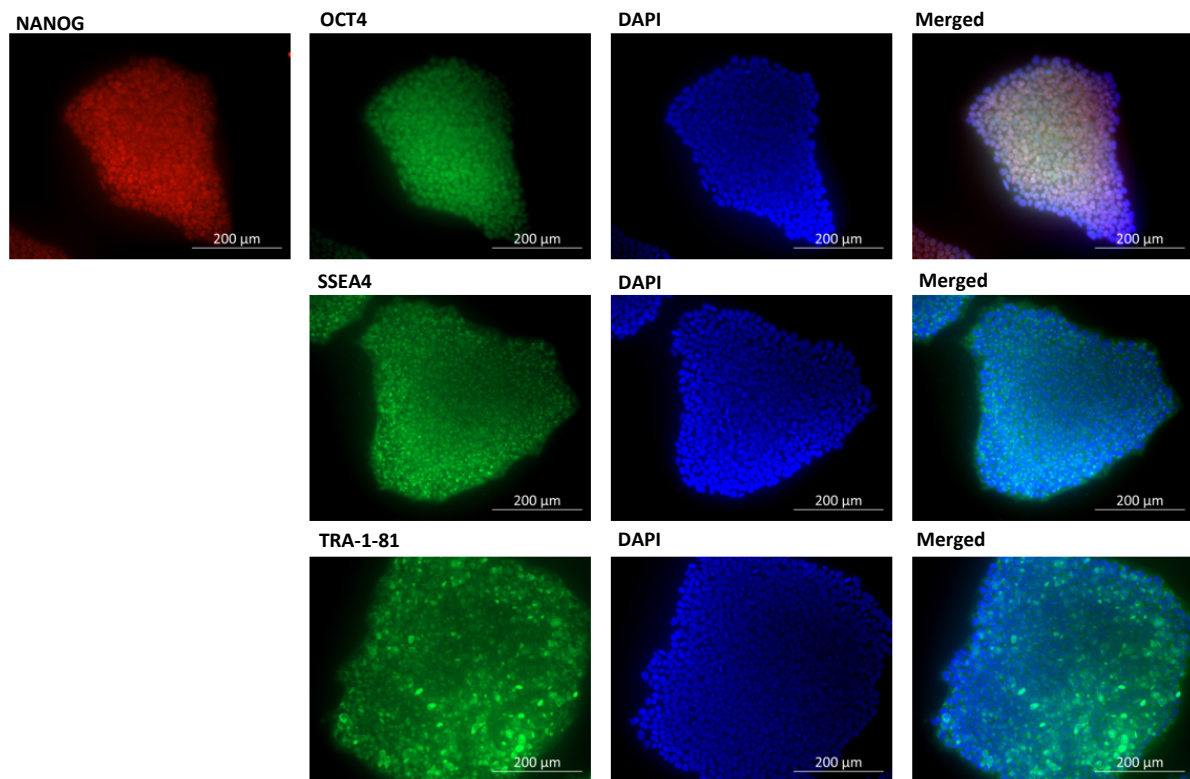


Figure 4: Immunofluorescence staining of the iPSC clone with pluripotency markers.

Pass

Fail

Other:

*Silvia Albert*

**Silvia Albert, PhD**

Manager, Radboud Stem Cell Technology Center

Date