Stem Cell Technology Center Genetica

# Certificate of Analysis 2021

Invoice number: -

Name principal investigator: SCTC
Cell line number: IPS21-00075 (control line)

**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<br>Parental cell type | PBM21-00011<br>PBMCs |
| Diagnosis<br>Mutation | N/A*<br>N/A* |
| Number of clones<br>Passage (P) of iPSCs reported at delivery | 3<br>P6 |
| Culture medium<br>Culture coating<br>Feeders during reprogramming<br>Passage method | Essential 8 Flex medium<br>Matrigel<br>Mouse Embryonic Fibroblasts (MEFs)<br>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 PBMCs | Pass |
| Expression of stem cell markers | Immunocytochemistry | Expression of OCT4, NANOG, SSEA4, TRA-1-81 | Pass |
| Mycoplasma test | PCR | Negative | Pass |

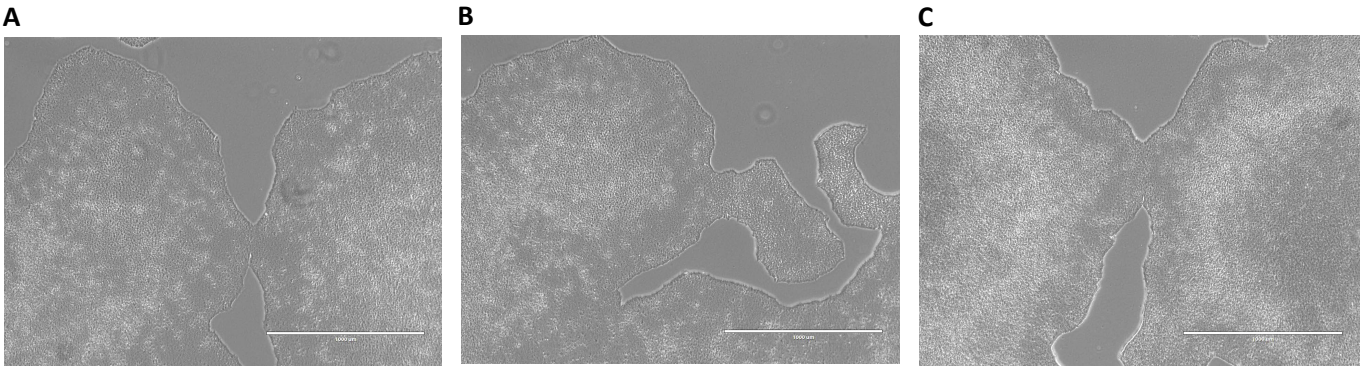



**Figure 1: Cells prior to freezing. A - C, clones 1 - 3, respectively at P6. Scale bar = 1000 µm.**

**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.

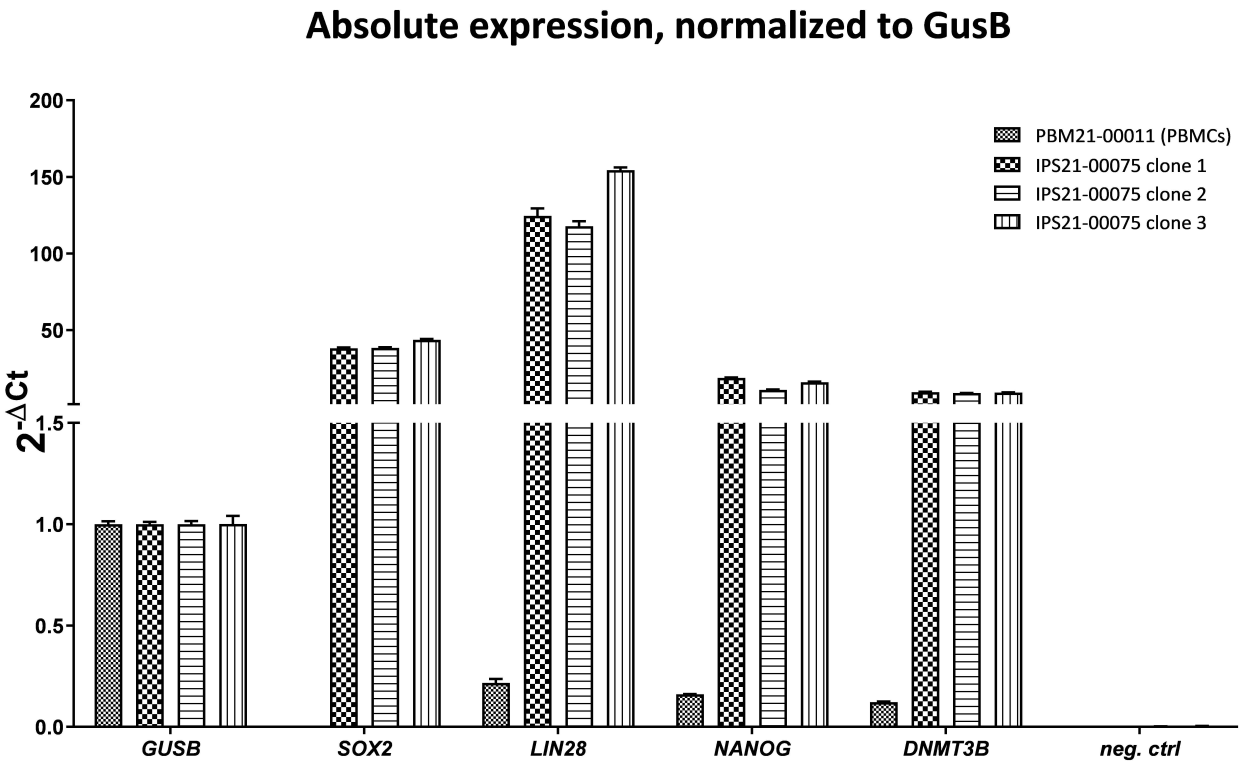



**Figure 2: Gene expression of three iPSC clones compared with the parental PBMCs (ΔCt).**

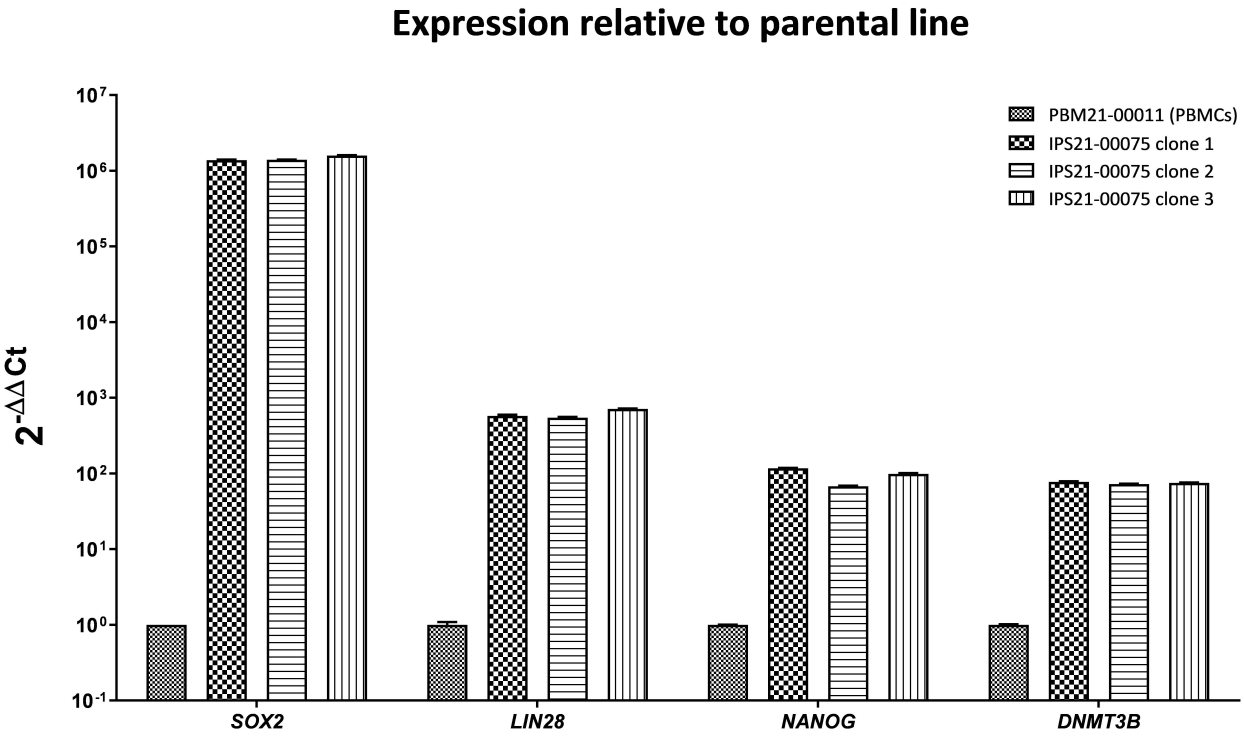
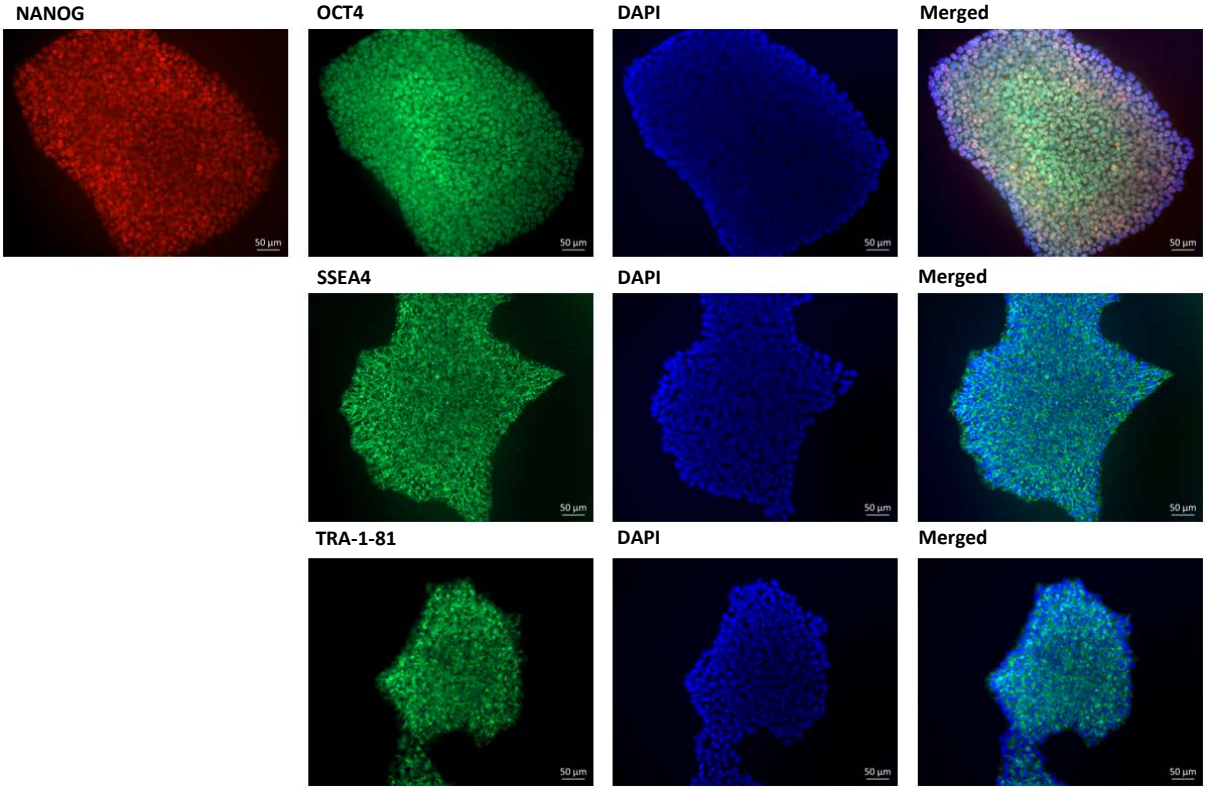



**Figure 3: Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPSCs is relative to the parental PBMCs.**

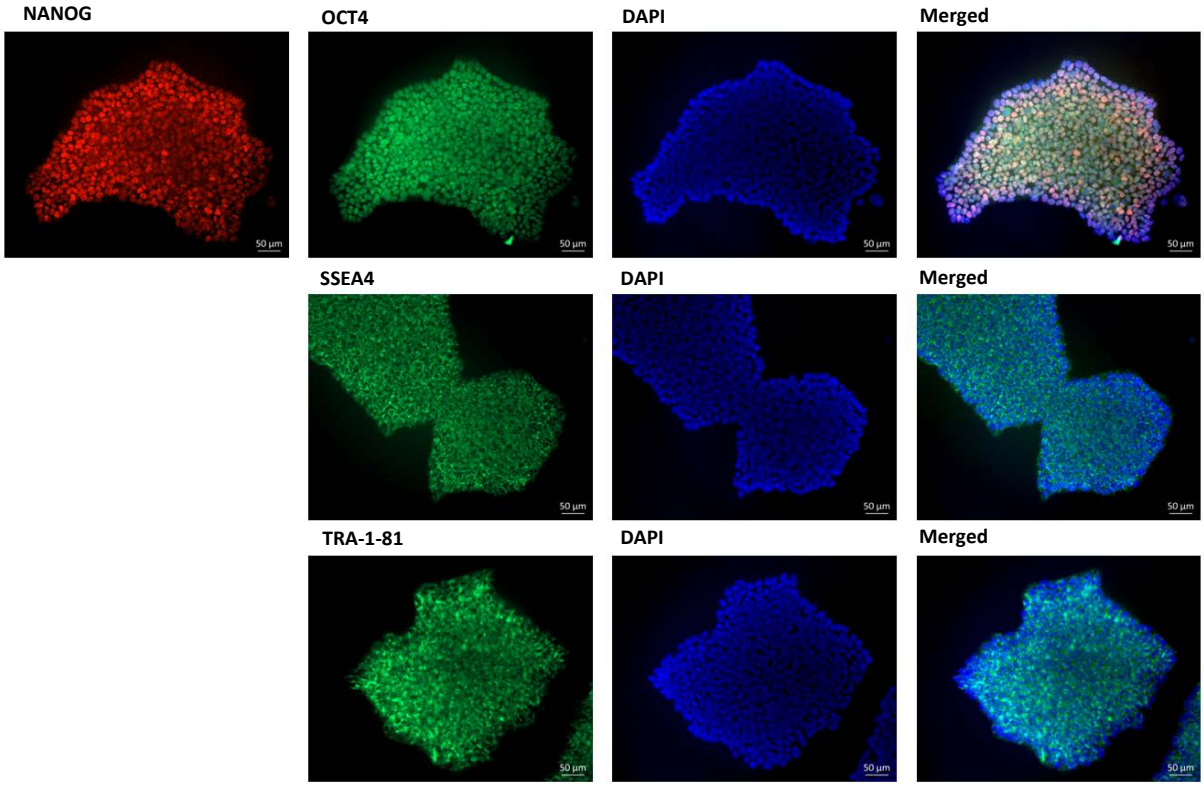
**Expression of stem cell markers**

The 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. IPS21-00075 clone 1*




### *B. IPS21-00075 clone 2*



### *C. IPS21-00075 clone 3*

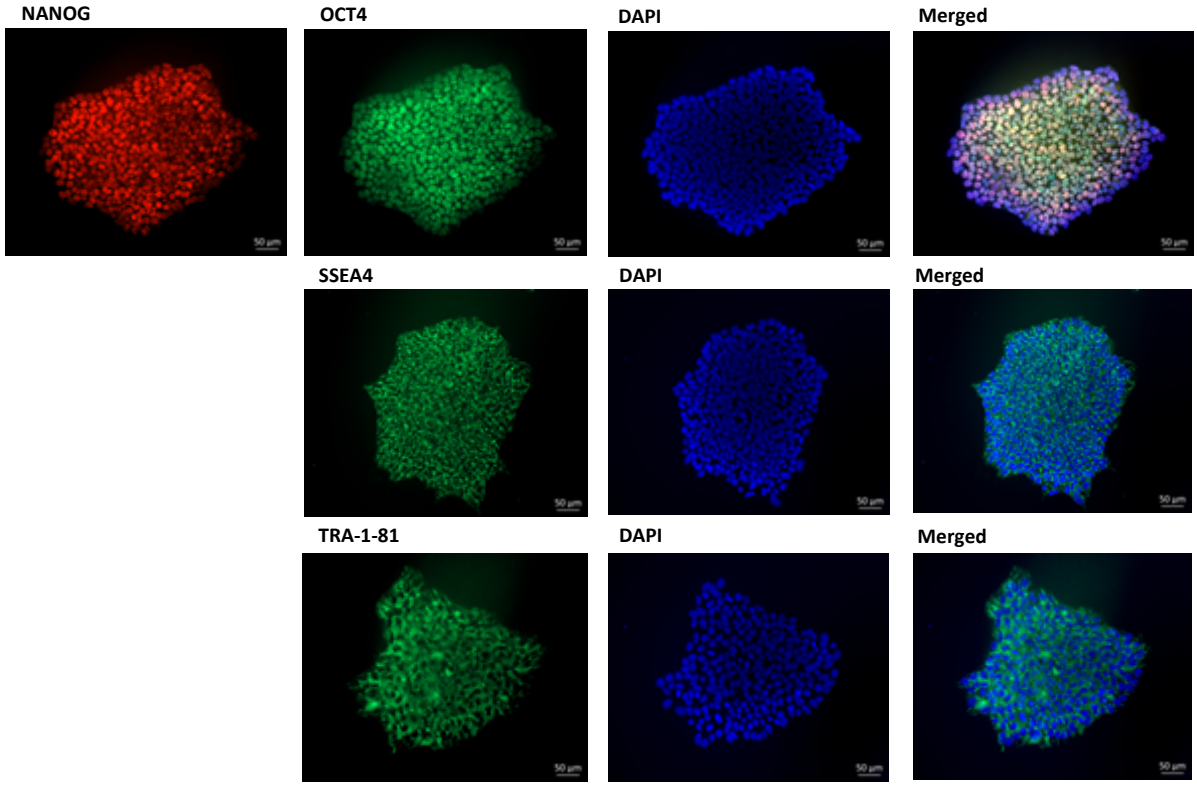



**Figure 4: Immunofluorescence staining of the iPSC clones with pluripotency markers. Scale bar = 200 µm.**

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◉ Pass
○ Fail
○ Other:

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