**iPS-00028** 13-09-2017

**Certificate of Analysis**

| | |
|---|---|
| Product description | Fresh PBMCs nucleofected with episomal vectors OCT3/4, SOX2, KLF4, L-MYC, LIN28 |
| Parent cell line | HEP17-00064 |
| Parent cell type | PBMC |
| Diagnosis | ID |
| Mutation | NA |
| Number of clones | 3 |
| Passage (P) of iPS cells reported at submission | P6 |
| Medium | Essential 8 Flex medium (cat. no. A2858501, Life Technologies) |
| Feeder during reprogramming | Mouse Embryonic Fibroblasts (MEFs) |
| Passage method | 0.5 mM EDTA |
| Protocols in Q-portal | 046588; 046591 |

The three individual clones were analyzed for the following specifications

| Test Description | Test method | Test specification | Result |
|---|---|---|---|
| Activation of stem cell markers | qPCR | Upregulation of *OCT4*, *SOX2*, *NANOG*, *LIN28* compared with PBMCs | Pass |
| Expression of stem cell markers | Immunocytochemistry | Expression of Oct4, Nanog, SSEA-4, Tra1-81 | Pass |
| Mycoplasm | PCR | Negative | Pass |
| Pluripotency | Differentiation assay | Upregulation of germlayer specific genes | N/A* |

*N/A: Not Applicable

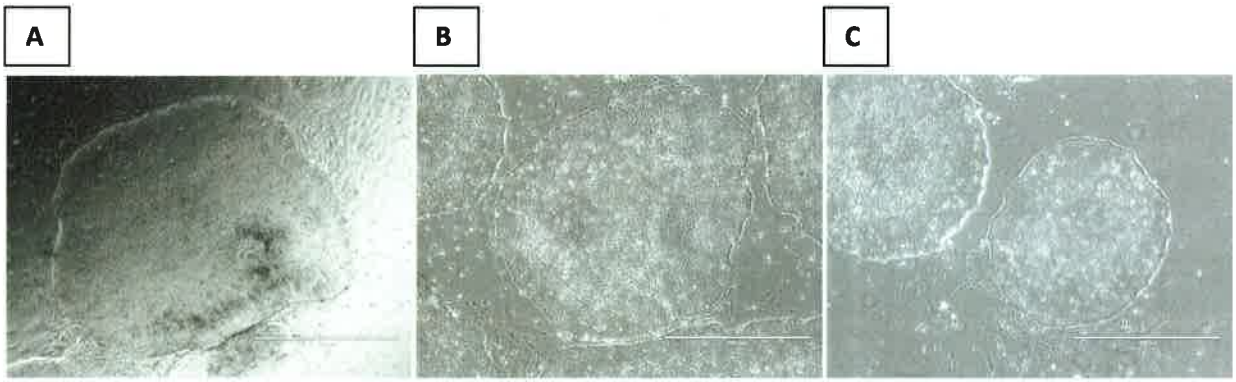



Figure 1: Cells prior freezing. A - C, respectively clone 1, 2 and 3 at P6. Scale bar = 1000 µm.

**Activation of stem cell markers**

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

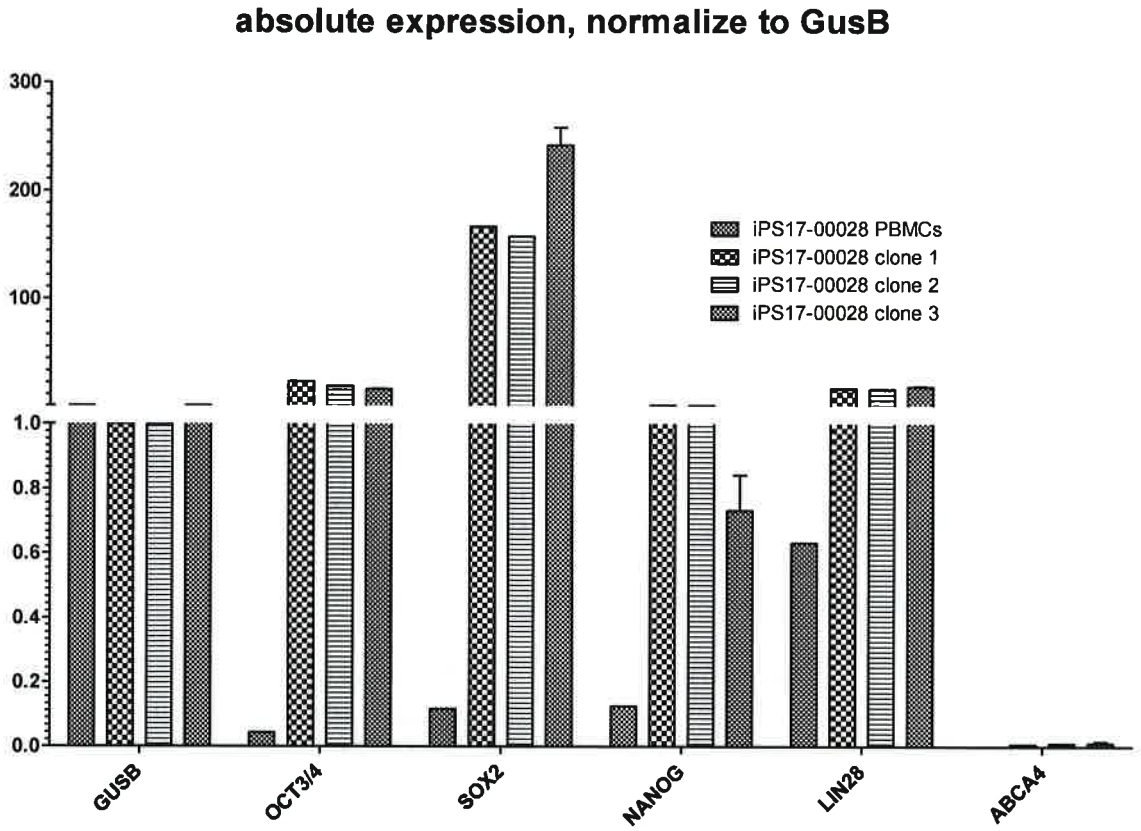



Figure 2. Gene expression of three iPS cell clones compared with the parental PBMCs (ΔCt).

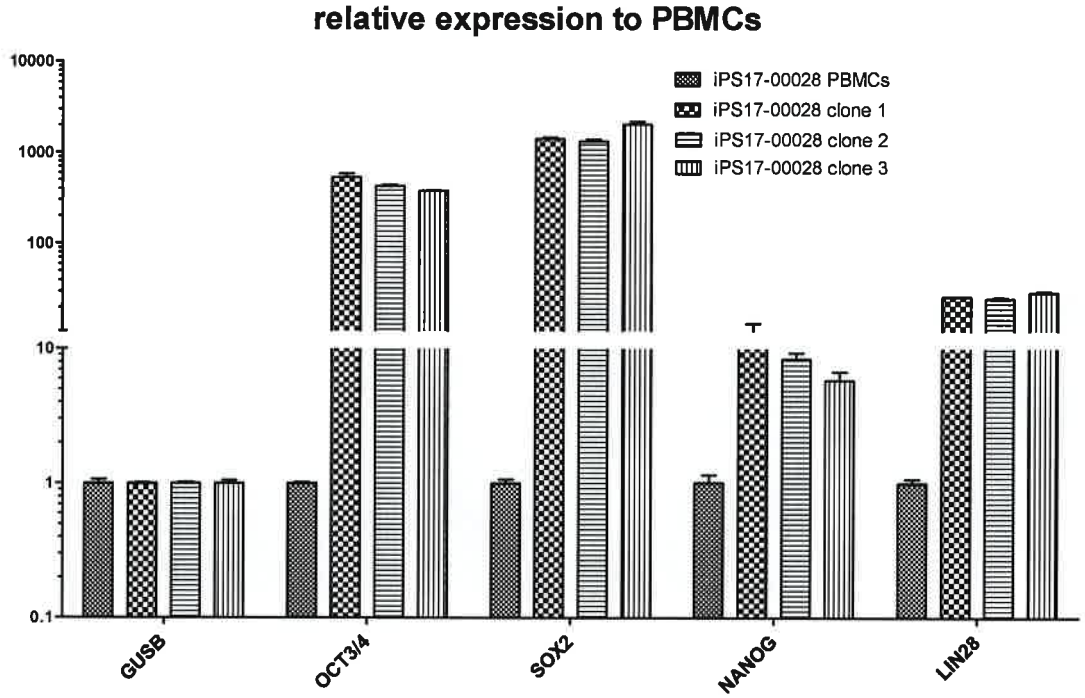
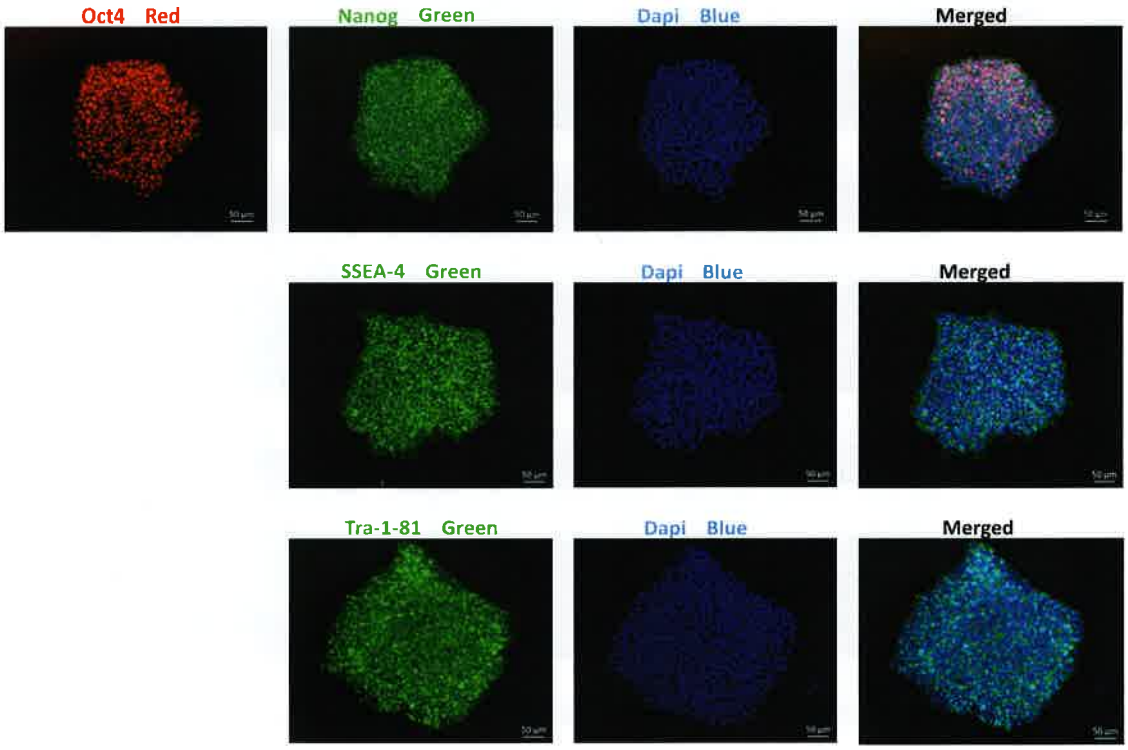



Figure 3. Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPS clones is relative to the parental PBMCs.

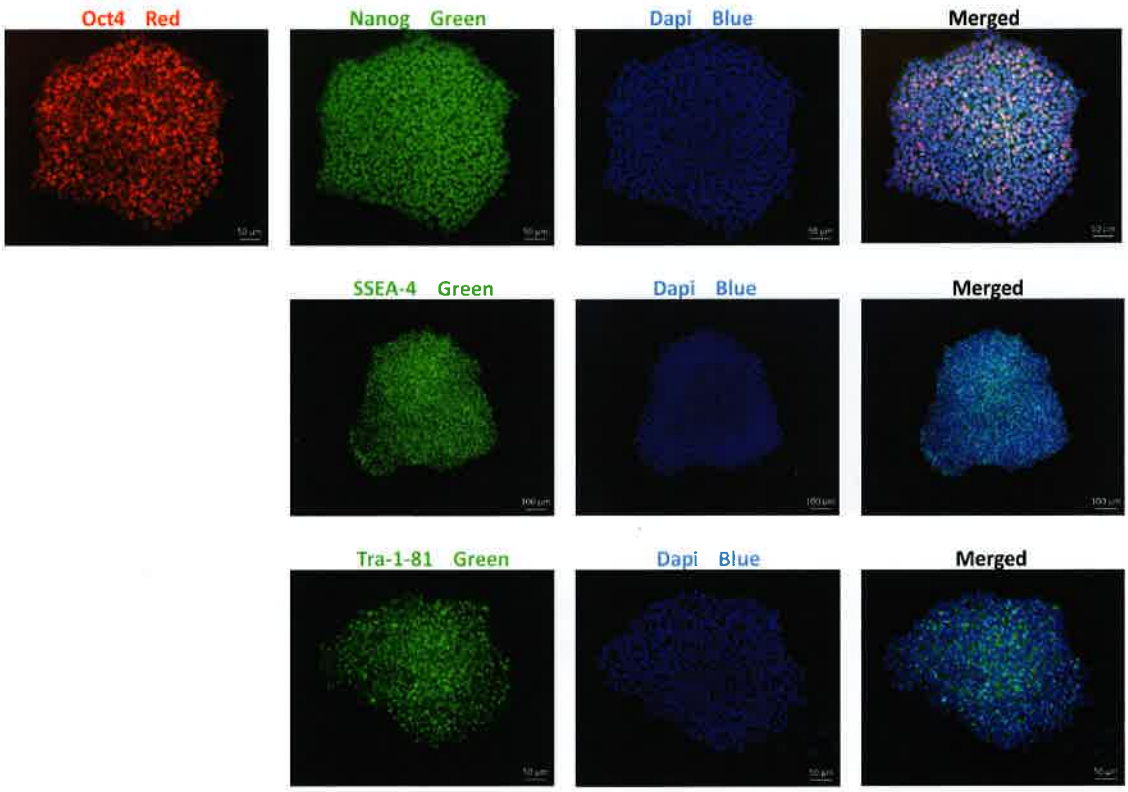
**Expression of stem cell markers**

Undifferentiated iPS cell clones are stained for the nuclear markers NANOG and OCT4 and surface antigens SSEA4 and TRA1-81. All markers are expressed in pluripotent human stem cells.

A. iPS17-00028 clone 1 P6




B. iPS17-00028 clone 2 P6



C. iPS17-00028 clone 3 P6

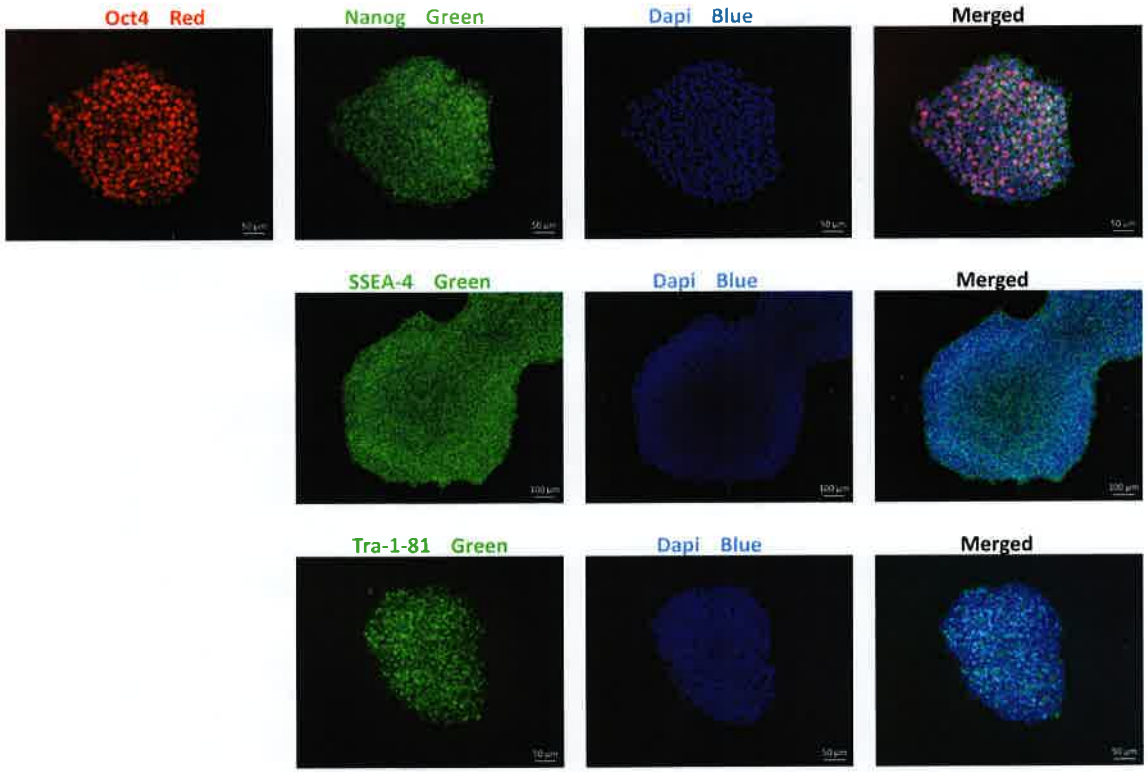



Figure 4. Immunofluorescence staining of the iPS cell clones with pluripotency markers.

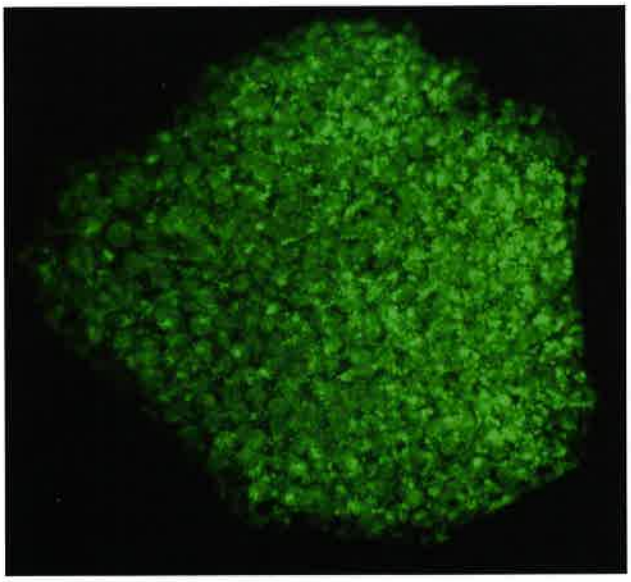


Figure 5. Magnification of iPS17-00028 clone 3 Nanog: there were some issues with the staining of this protein. In the right part of the colony, the TRA-1 antibody was mixed in. But on left there is a clear staining of Nanog. This also applies to clone 1, but this is not clear in the pictures.

- [x] Pass
- [ ] Fail
- [ ] Other:

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21. 9. 2017