iPS17-00042 2-2-2018

Certificate of Analysis

Product description	Fresh PBMCs nucleofected with episomal vectors OCT3/4, SOX2, KLF4, L-MYC, LIN28
Parent cell line	HEP17-00088
Parent cell type	PBMC
Diagnosis	Epileptic encephalopathy
Mutation	NA
Number of clones	3
Passage (P) of iPS cells reported at submission	P10
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 to 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

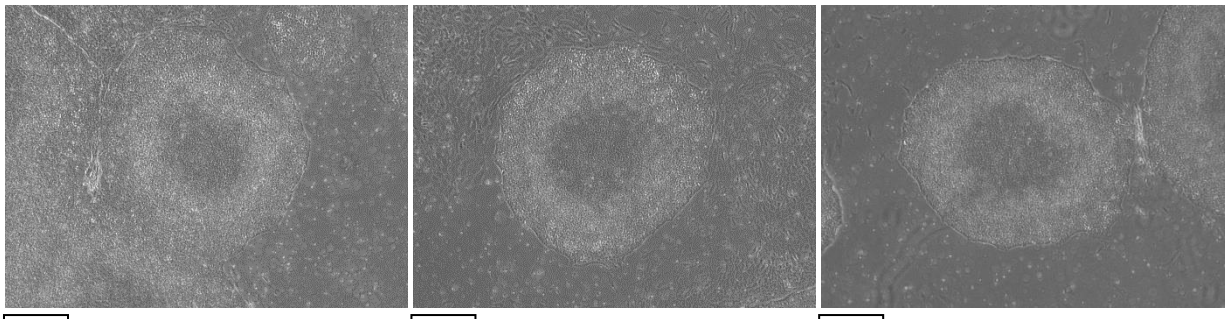


Figure 1: Cells prior freezing. A - C, respectively clone 1, 2 and 3 at P10. 4x magnification

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).

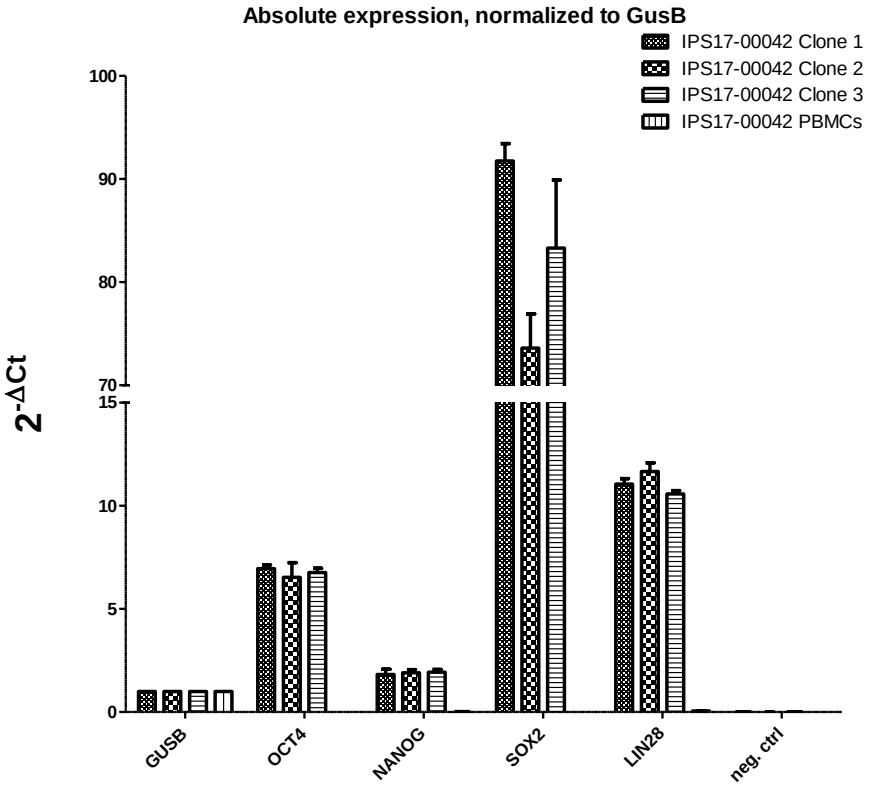


Figure 2. Gene expression of three iPS cell clones compared to the parental PBMCs (ΔCt).

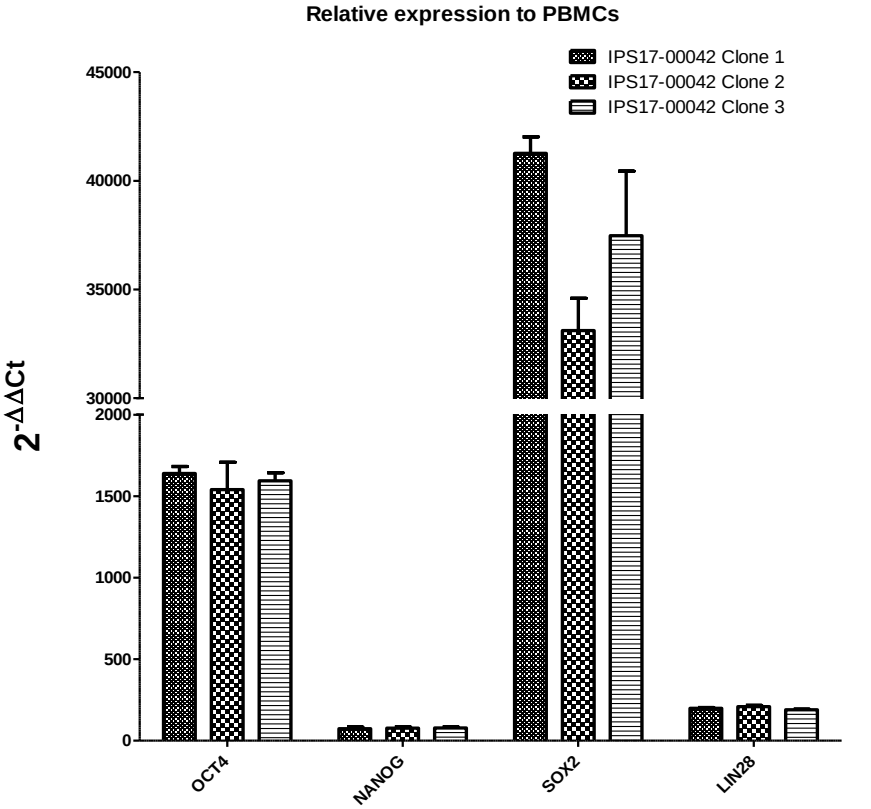
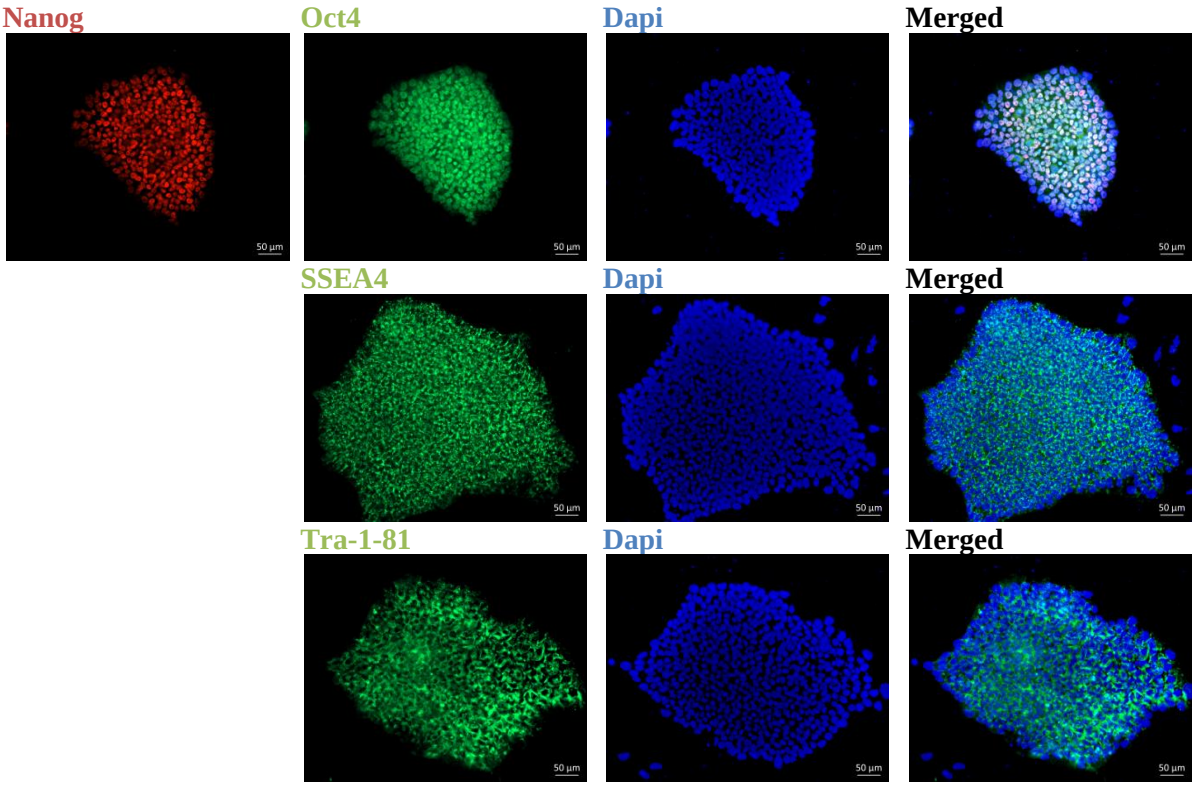


Figure 3. Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPS clones is relative to the parental PBMCs.

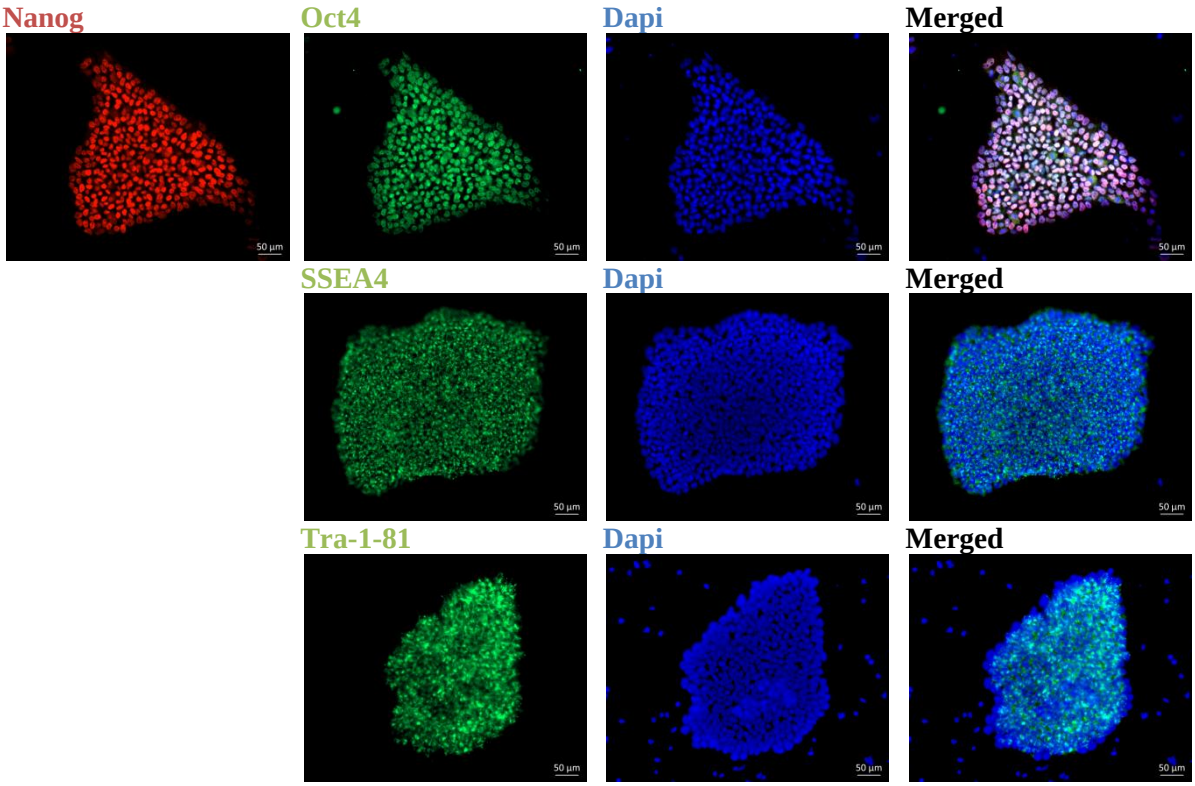
Expression of stem cell markers

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. iPS17-00042 clone 1 P10



B. iPS17-00042 clone 2 P10



C. iPS17-00042 clone 3 P10

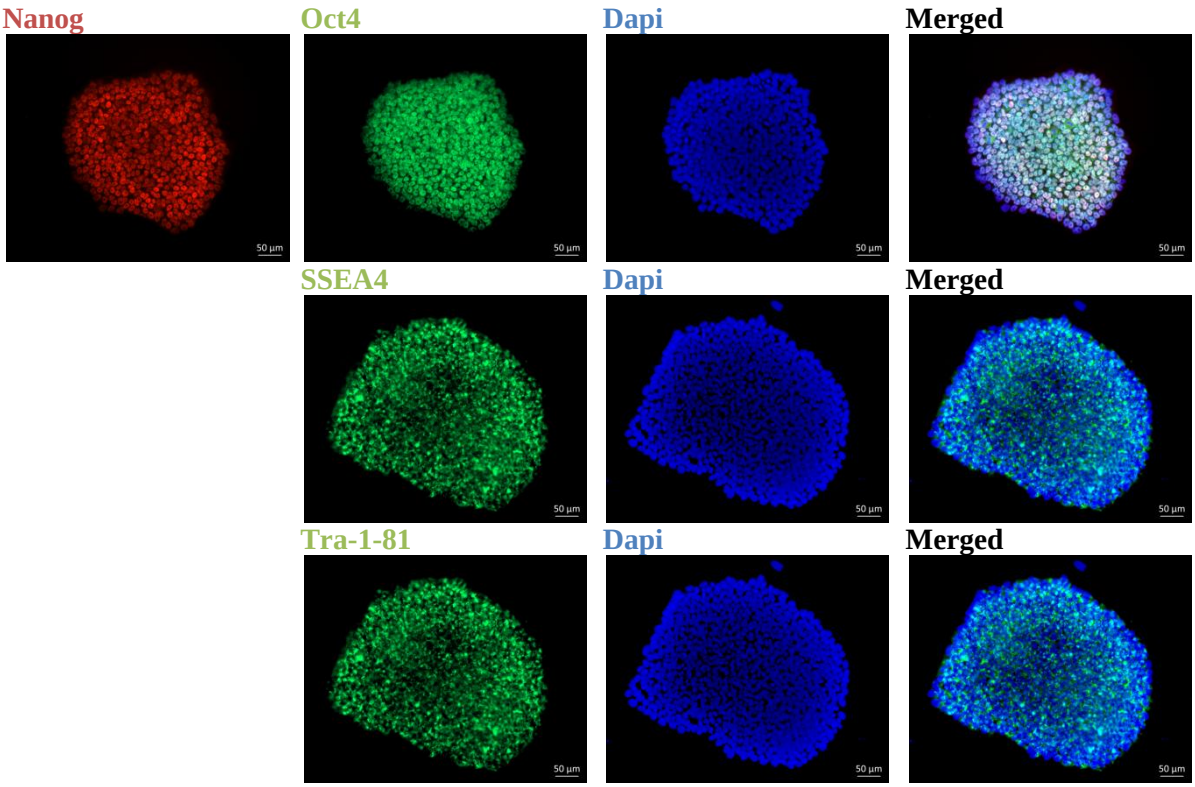


Figure 4. Immunofluorescence staining of the iPS cell clones with pluripotency markers.

- X Pass
- o Fail
- o Other:

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Date
8-2-2018