Stem Cell Technology Center Genetica

Certificate of Analysis 2019

Invoice number: SCTC2017-00020

Name investigator: Anneke den Hollander
Cell line number: IPS17-00036
Project name: TWIN

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	HEP17-00069
Parental cell type	PBMCs
Diagnosis	AMD-CON
Number of clones	3
Passage (P) of iPSCs at submission	P10
Culture medium	Essential 8 Flex medium
Culture coating	Matrigel
Feeders during reprogramming	Mouse Embryonic Fibroblasts
Passage method	(MEFs) 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 *OCT4, SOX2, LIN28* compared with PBMCs	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	Pass
hPSC genetic analysis	qPCR	Detection of recurrent chromosomal abnormalities	See results in last page

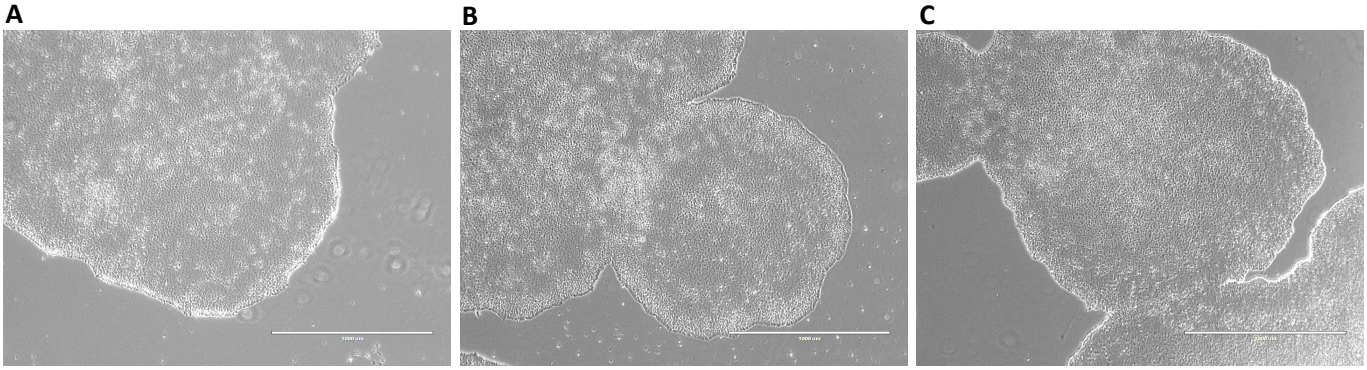


Figure 1: Cells prior to freezing. A - C, clone 1, clone 2 and clone 3, respectively at P10. Scale bar = 1000 µm.

Activation of stem cell markers

All clones were 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).

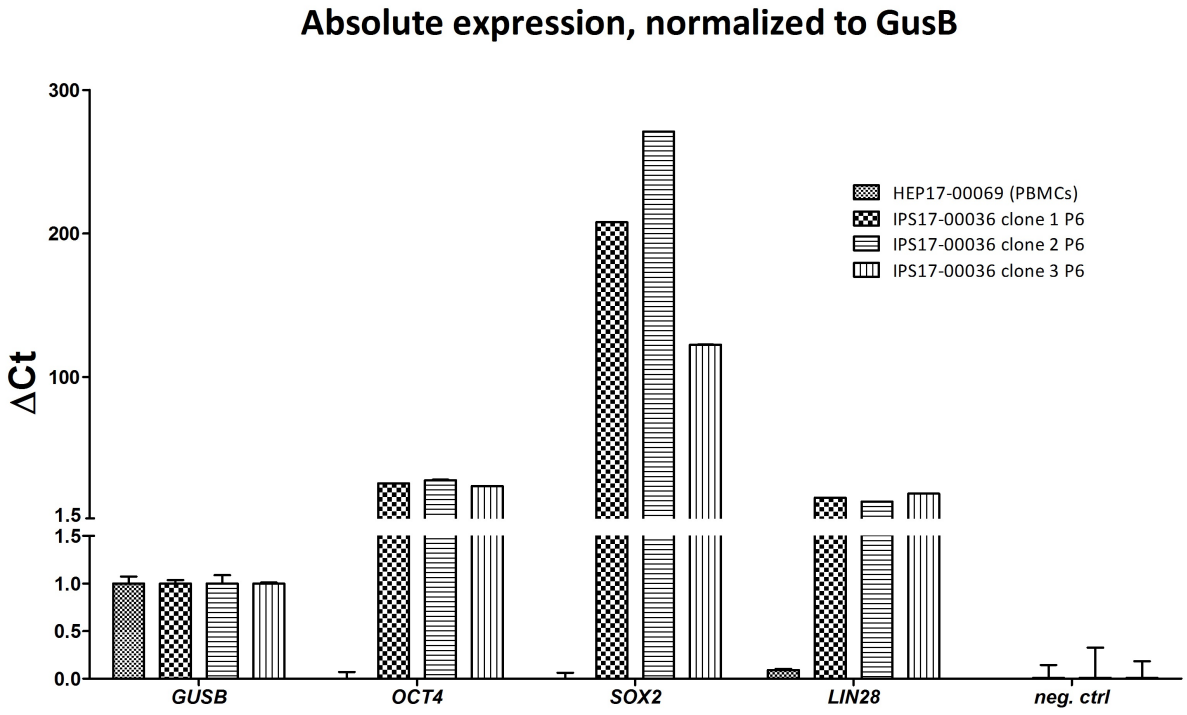


Figure 2: Gene expression of three iPSC clones compared with the parental PBMCs (ΔCt).

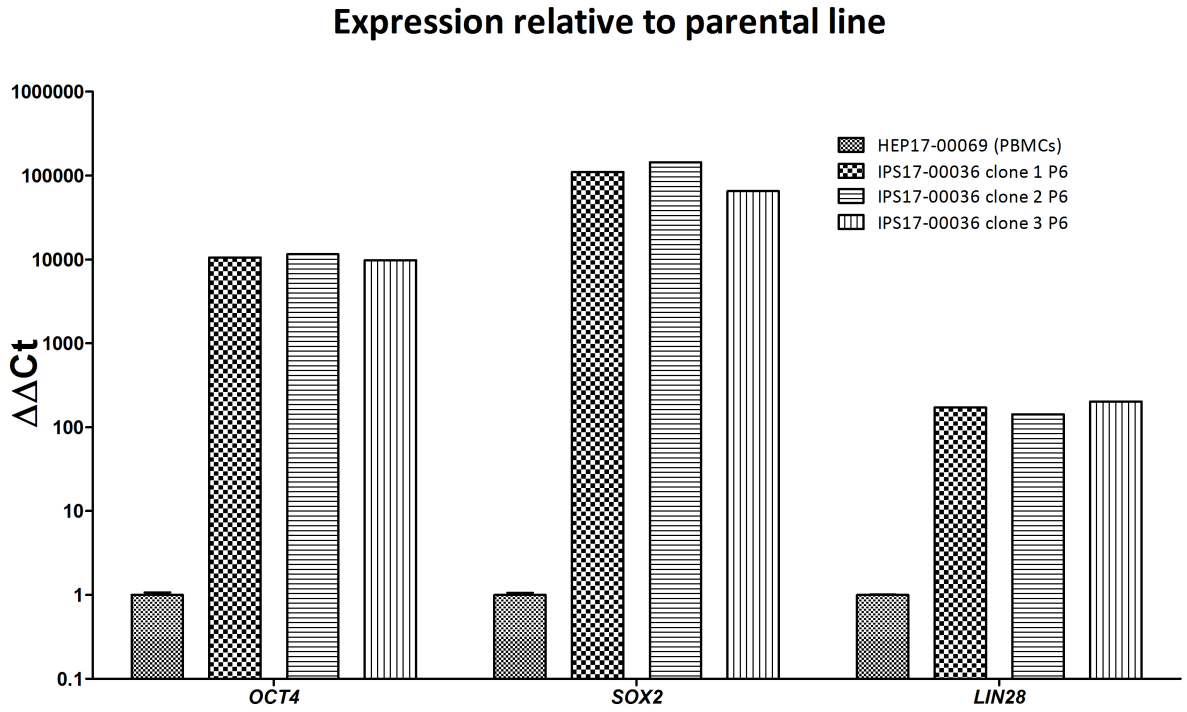
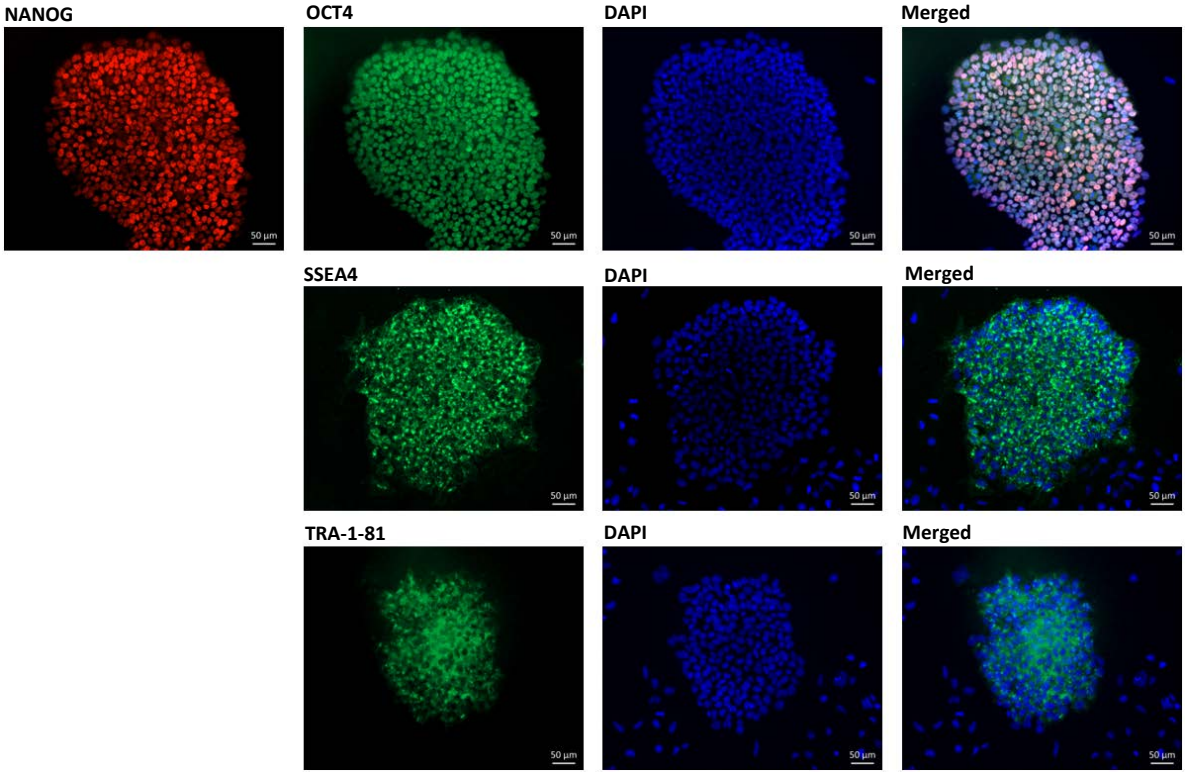


Figure 3: Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPSCs is relative to the parental PBMCs.

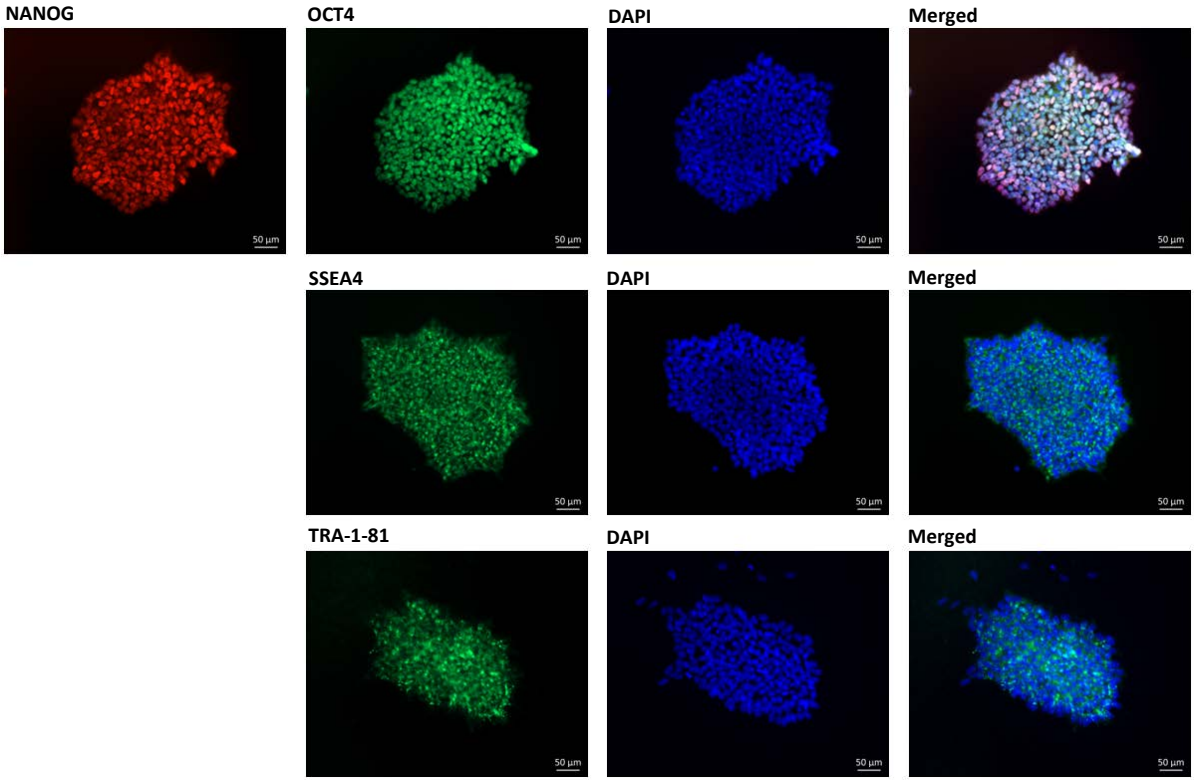
Expression of stem cell markers

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. IPS17-00036 clone 1 P6



B. IPS17-00036 clone 2 P6



C. IPS17-00036 clone 3 P6

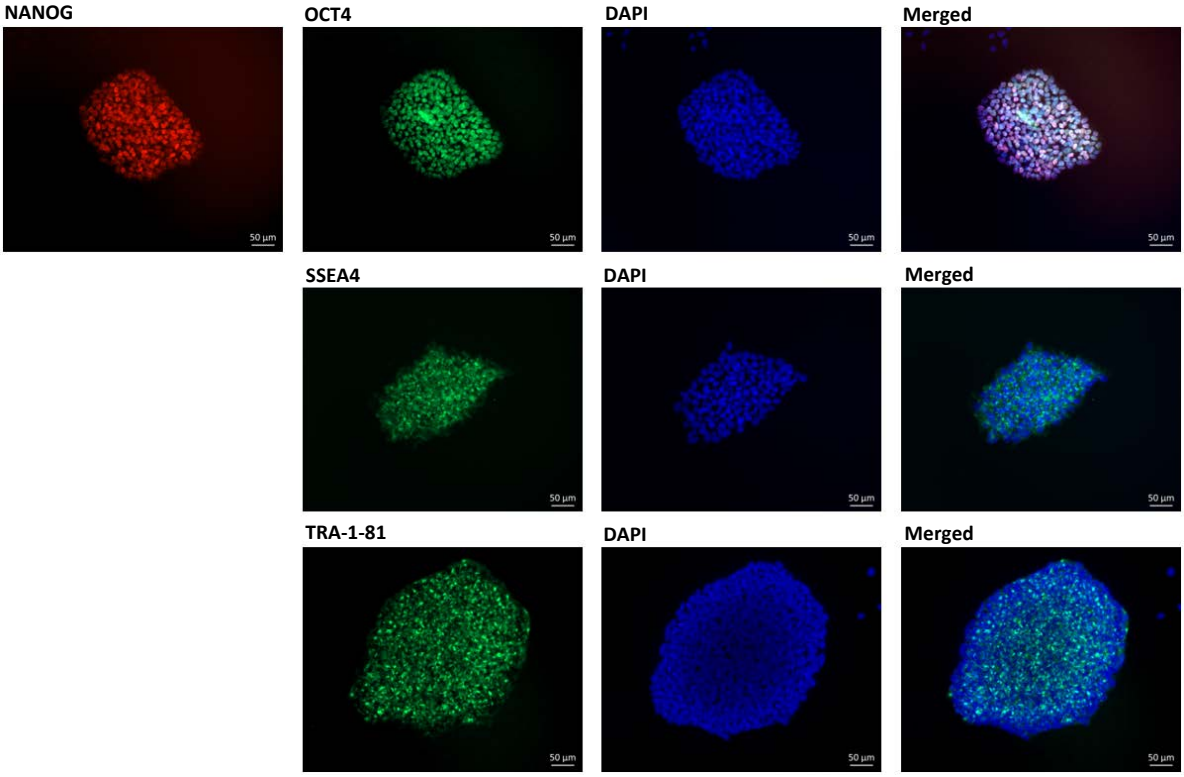


Figure 4: Immunofluorescence staining of the iPSC clones with pluripotency markers.

Three germ layer differentiation

iPSC clones were differentiated into the endodermal, mesodermal and ectodermal germ layers. RNA was isolated and gene expression was checked by qPCR. Ct values are normalized with the housekeeping gene GUSB (set at 1). For each lineage two genes were assessed (Table 3). The differentiated cells were also stained for lineage-specific markers (Table 4).

Table 3: qPCR markers for three lineage differentiation

Lineage	Marker
Endoderm	FOXA2, SOX17
Mesoderm	Brachyury, HAND1
Ectoderm	PAX6, NCAM1

Table 4: ICC markers for three lineage differentiation

Lineage	Marker
Endoderm	SOX17
Mesoderm	NCAM1
Ectoderm	NESTIN

Endoderm

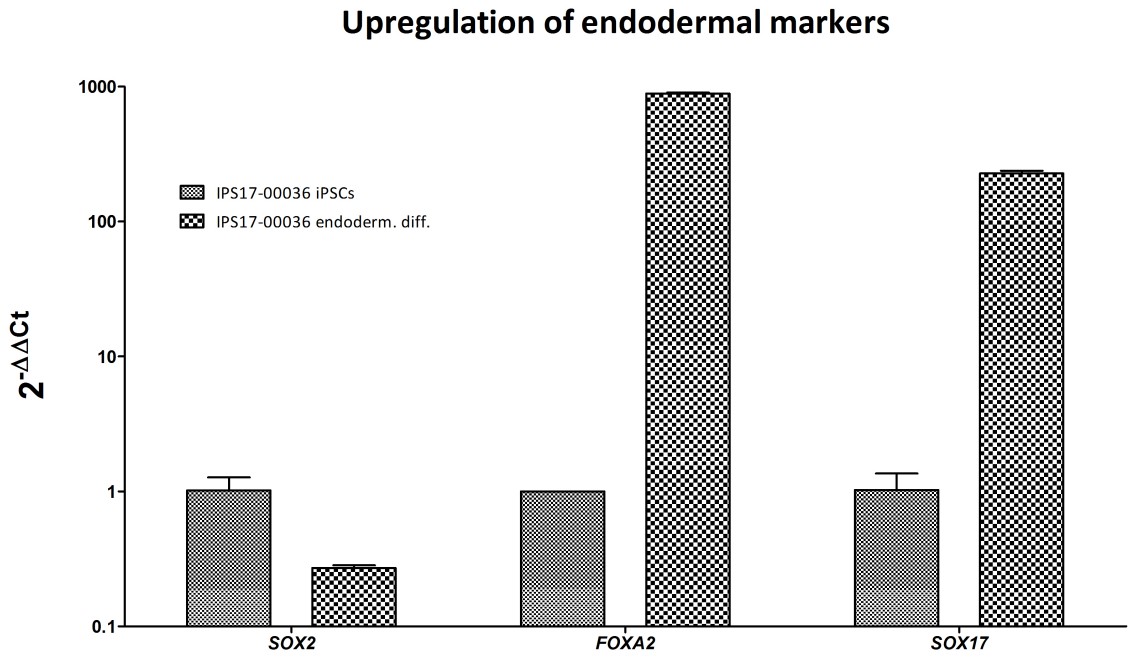


Figure 5: Expression fold difference of endoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *SOX2* was used as a reference for pluripotency.

Mesoderm

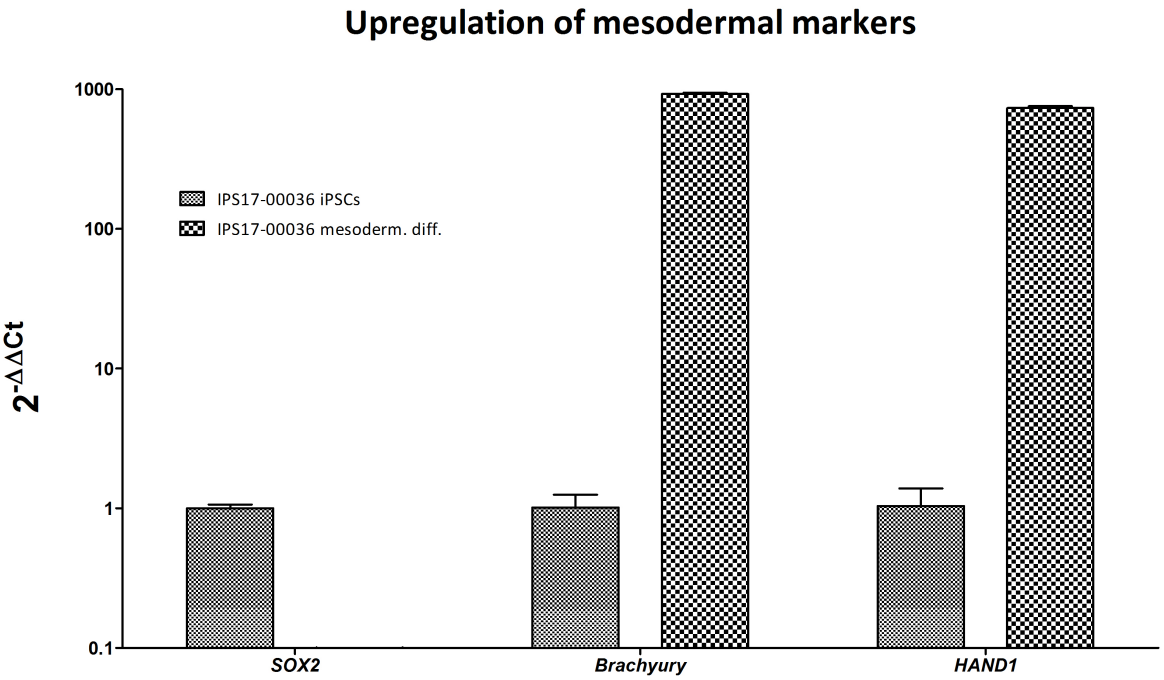


Figure 6: Expression fold difference of mesoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *SOX2* was used as a reference for pluripotency.

Ectoderm

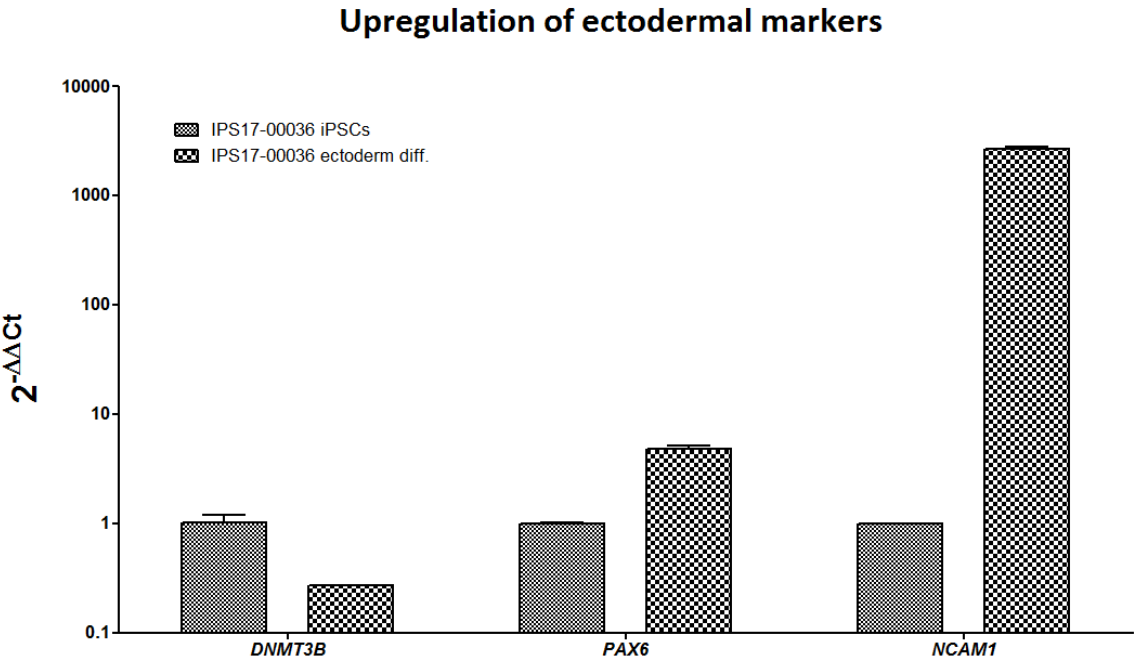


Figure 7: Expression fold difference of ectoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *DNMT3B* was used as a reference for pluripotency.

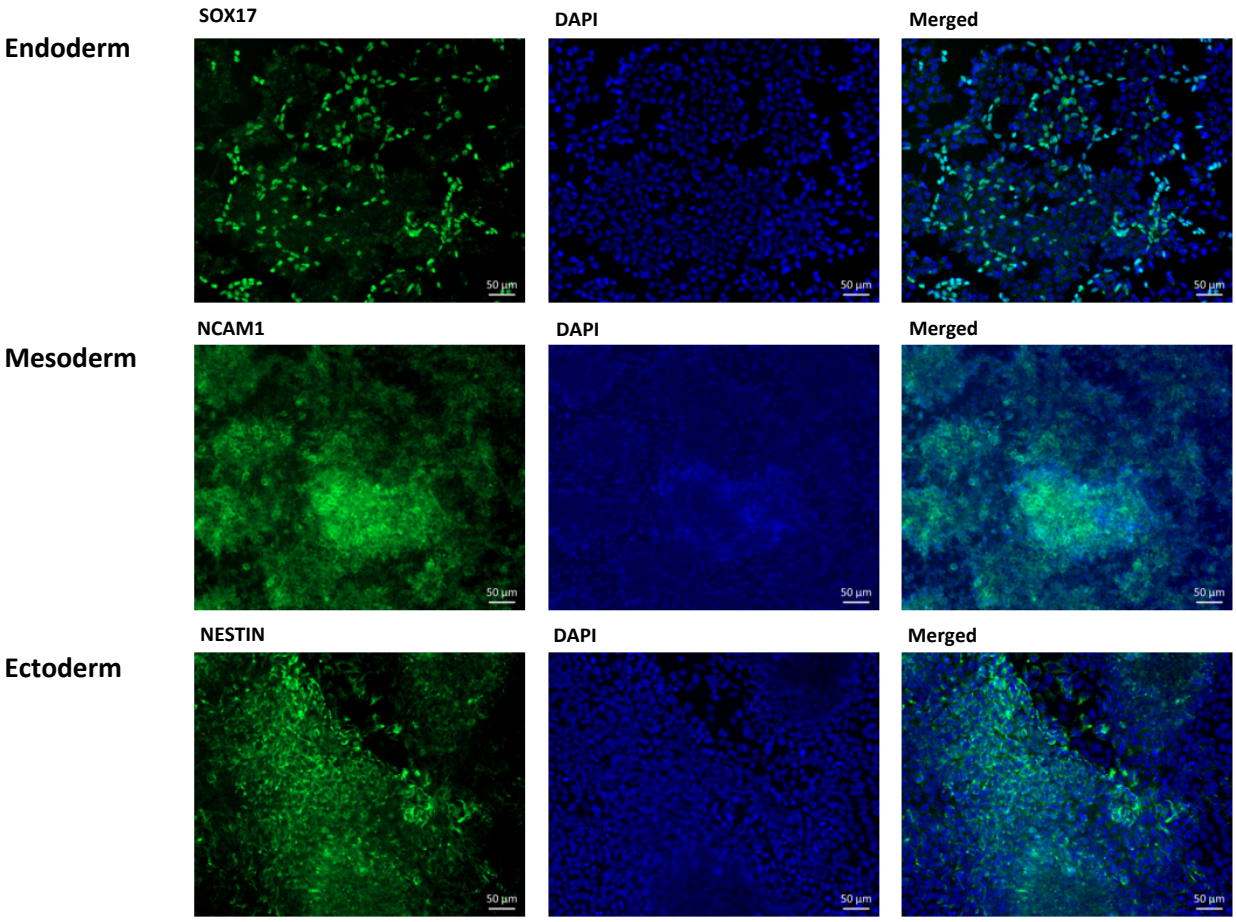


Figure 8: Immunofluorescence staining of differentiated cells showing positive signal of germlayer-specific markers.

Genetic analysis

DNA was isolated from three iPSC clones and the majority of recurrent chromosomal abnormalities reported in human embryonic stem cells and iPSCs was analysed.

Sample	Sex	Status
IPS17-00036 clone 1	Female	Normal
IPS17-00036 clone 2	Female	Possibly Abnormal
IPS17-00036 clone 3	Female	Possibly Abnormal

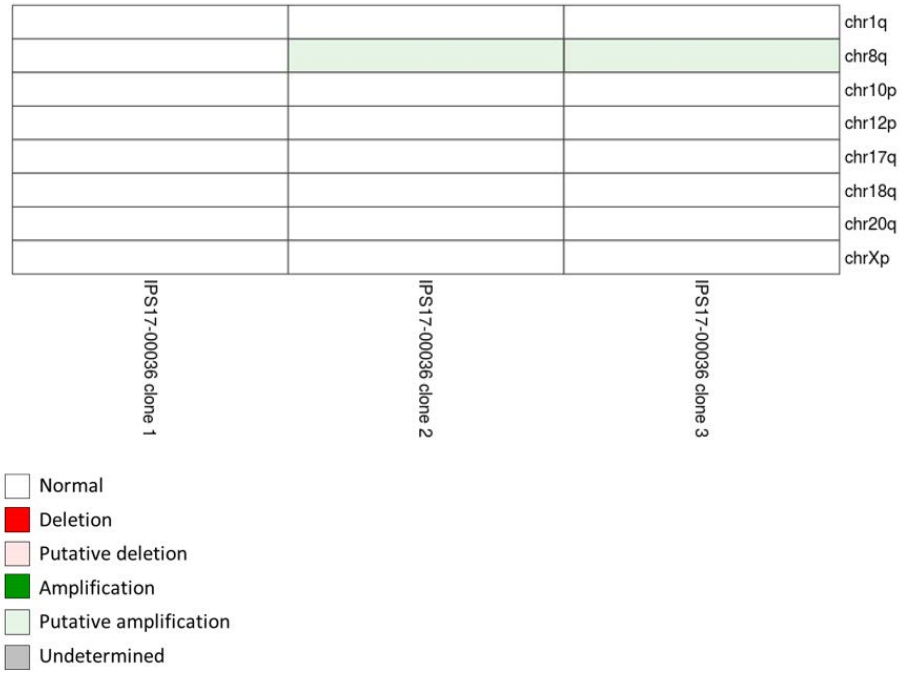


Figure 9: Summary of the genetic analysis

For further experiments it is suggested to use IPS17-00036 clone 1. It is suggested to check IPS17-00036 clone 2 and 3 at a later passage to assess whether there is indeed a mutant clone in the culture that expands over time.

More detailed results are on request.

- (●) Pass
- ( ) Fail
- ( ) Other:

Silvia Albert, PhD
Manager, Radboud Stem Cell Technology Center
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