Stem Cell Technology Center Genetica

Certificate of Analysis 2020

Invoice number: SCTC2018-00016

Name investigator: Anneke den Hollander
Cell line number: IPS17-00097 clone 2
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	HEP17-00123
Parental cell type	PBMCs
Diagnosis	AMD-con
Mutation	N/A*
Number of clones	1
Passage (P) of iPSCs reported at submission	P10
Culture medium	Essential 8 Flex medium
Culture coating	Matrigel
Feeders during reprogramming	Mouse Embryonic Fibroblasts (MEFs)
Passage method	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	PCR	Negative	Pass
Three lineage differentiation	Differentiation assay	Upregulation of germlayer-specific genes	Pass
Copynumbervariation (CNV) analysis	Array	Comparing the genetic profile with the donor DNA	Pass

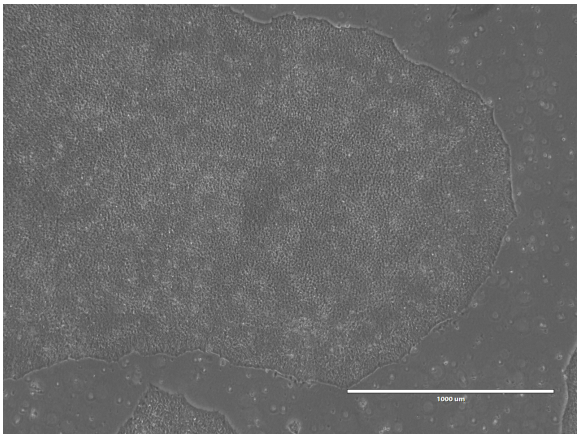

Figure 1: Cells prior to freezing, clone 2 (p10).

Activation of stem cell markers

The clone was 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).

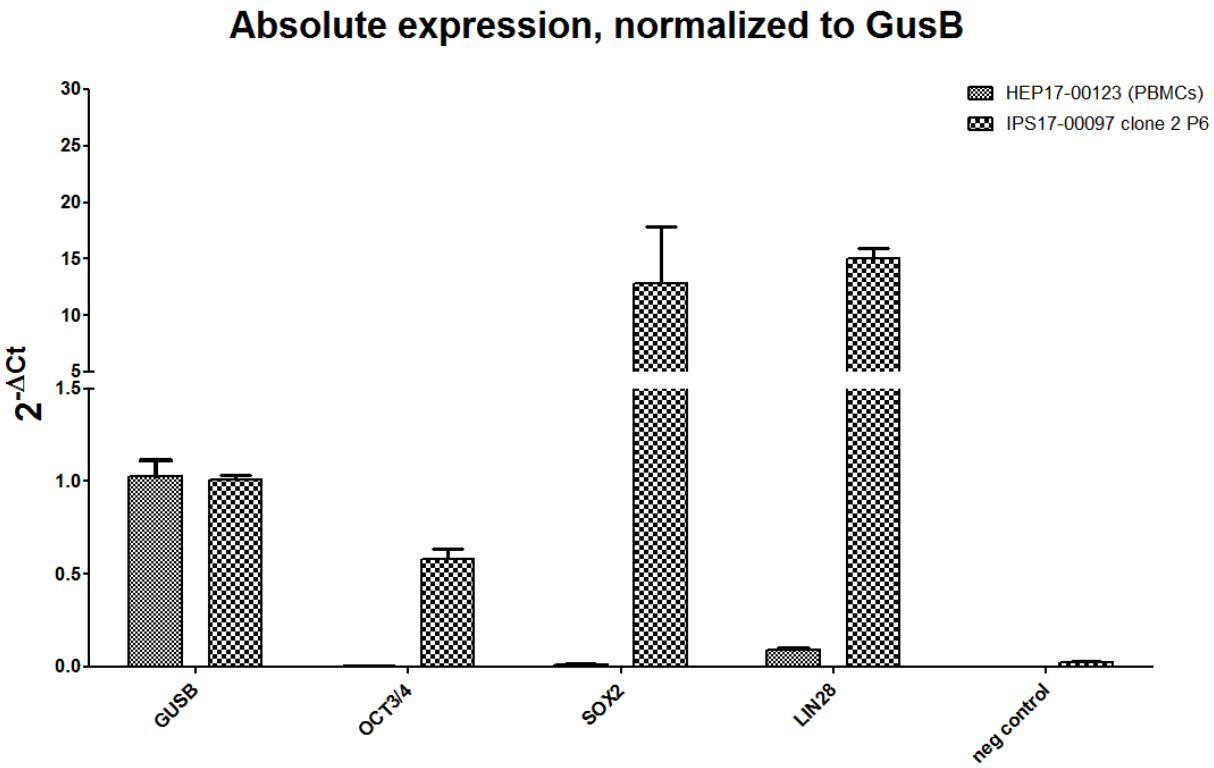

Figure 2: Gene expression of the iPSC clone compared with the parental PBMCs (ΔCt).

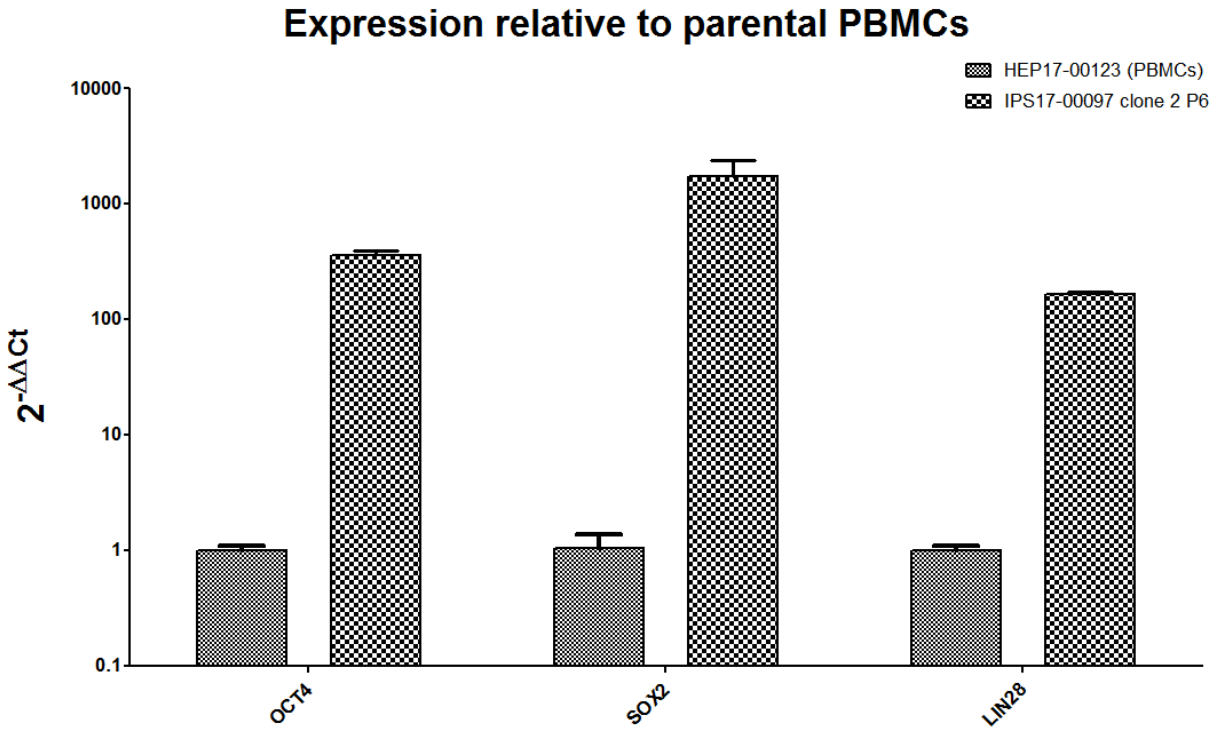

Figure 3: Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPSC clone is relative to the parental PBMCs.

Expression of stem cell markers

The 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. IPS17-00097 clone 2 P6

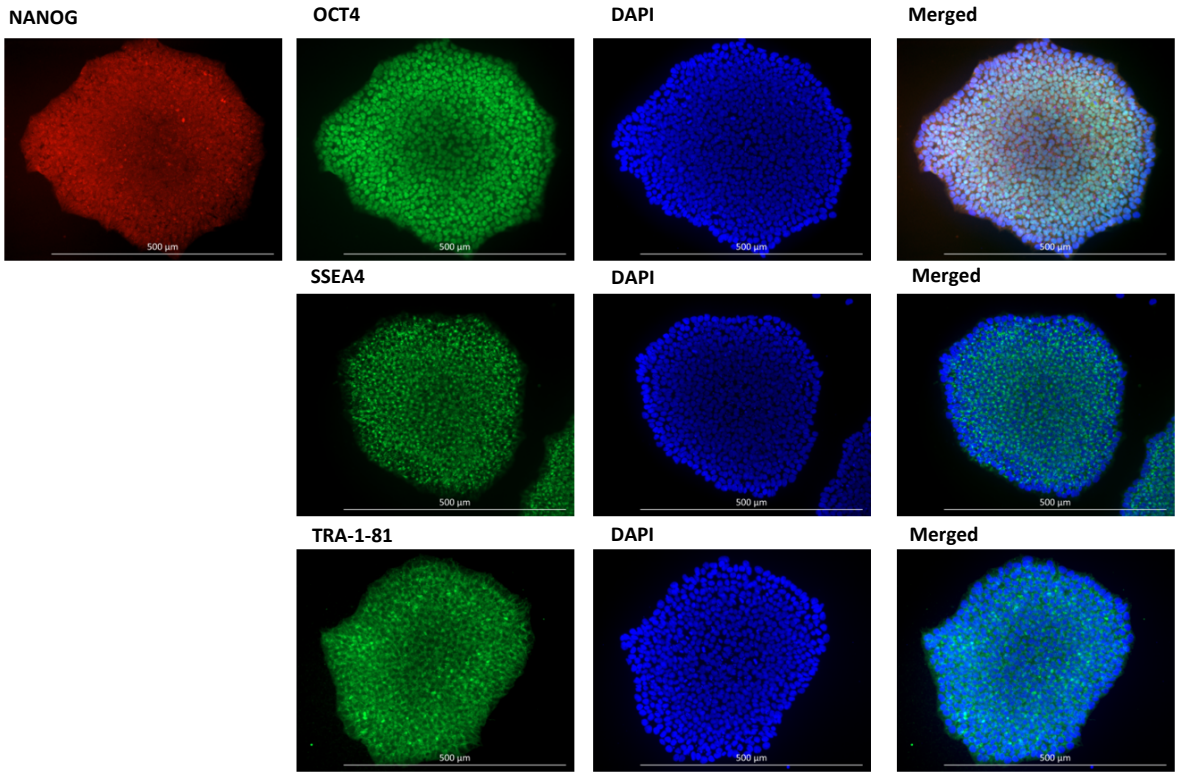

Figure 4: Immunofluorescence staining of the iPSC clone with pluripotency markers.

Three germ layer differentiation

IPS17-00097 clone 2 was 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

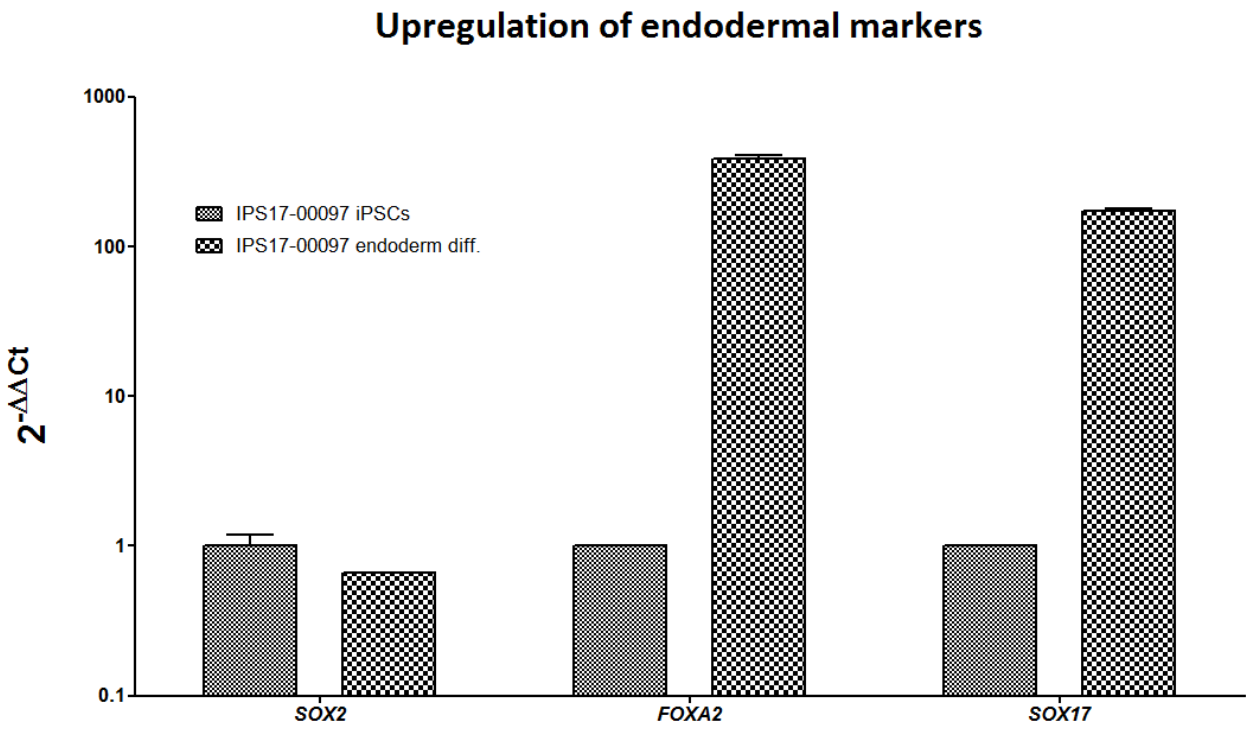

Figure 5: Expression fold difference of endoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone at P10. *SOX2* was used as a reference for pluripotency.

Mesoderm

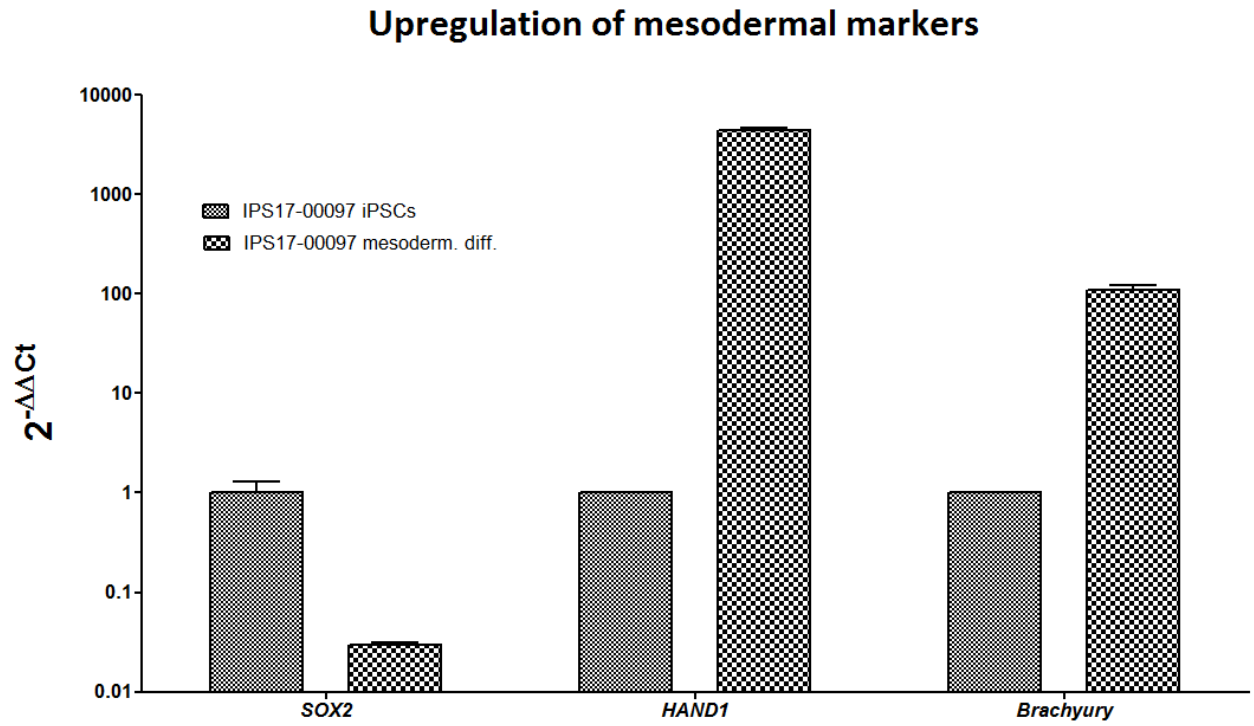

Figure 6: Expression fold difference of mesoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone at P10. *SOX2* was used as a reference for pluripotency.

Ectoderm

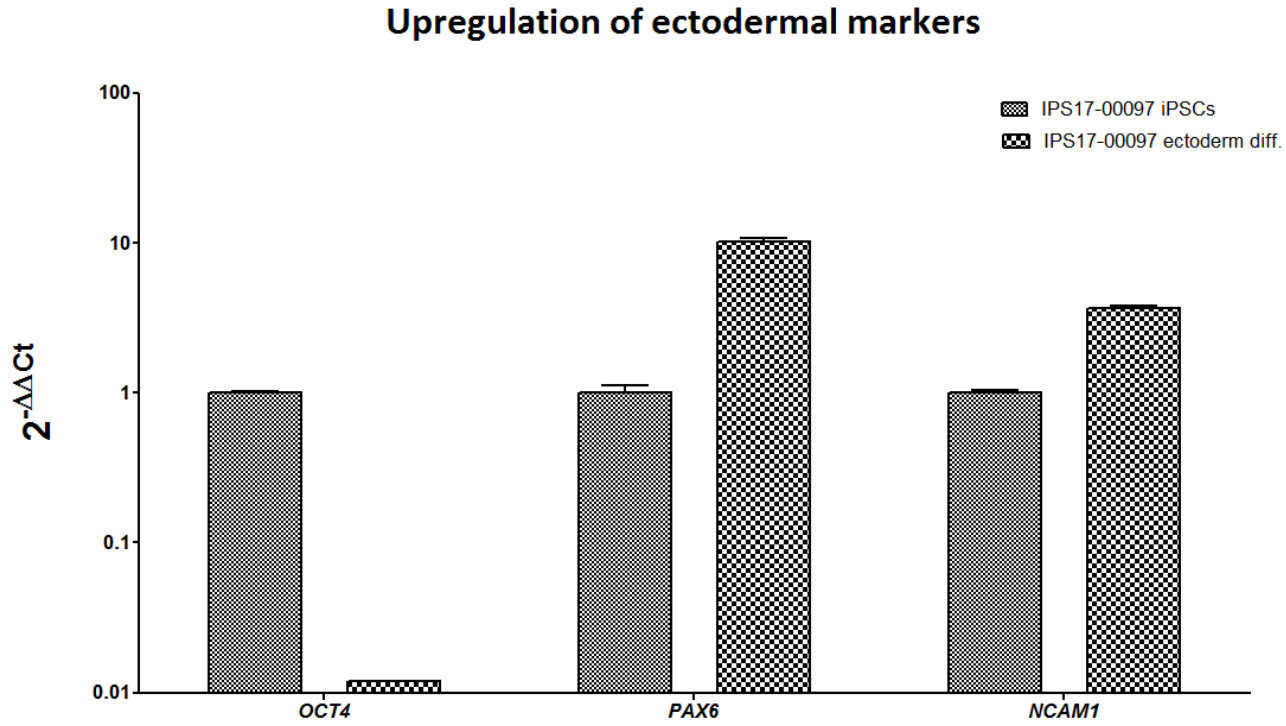

Figure 7: Expression fold difference of ectoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone at P10. *OCT4* was used as a reference for pluripotency.

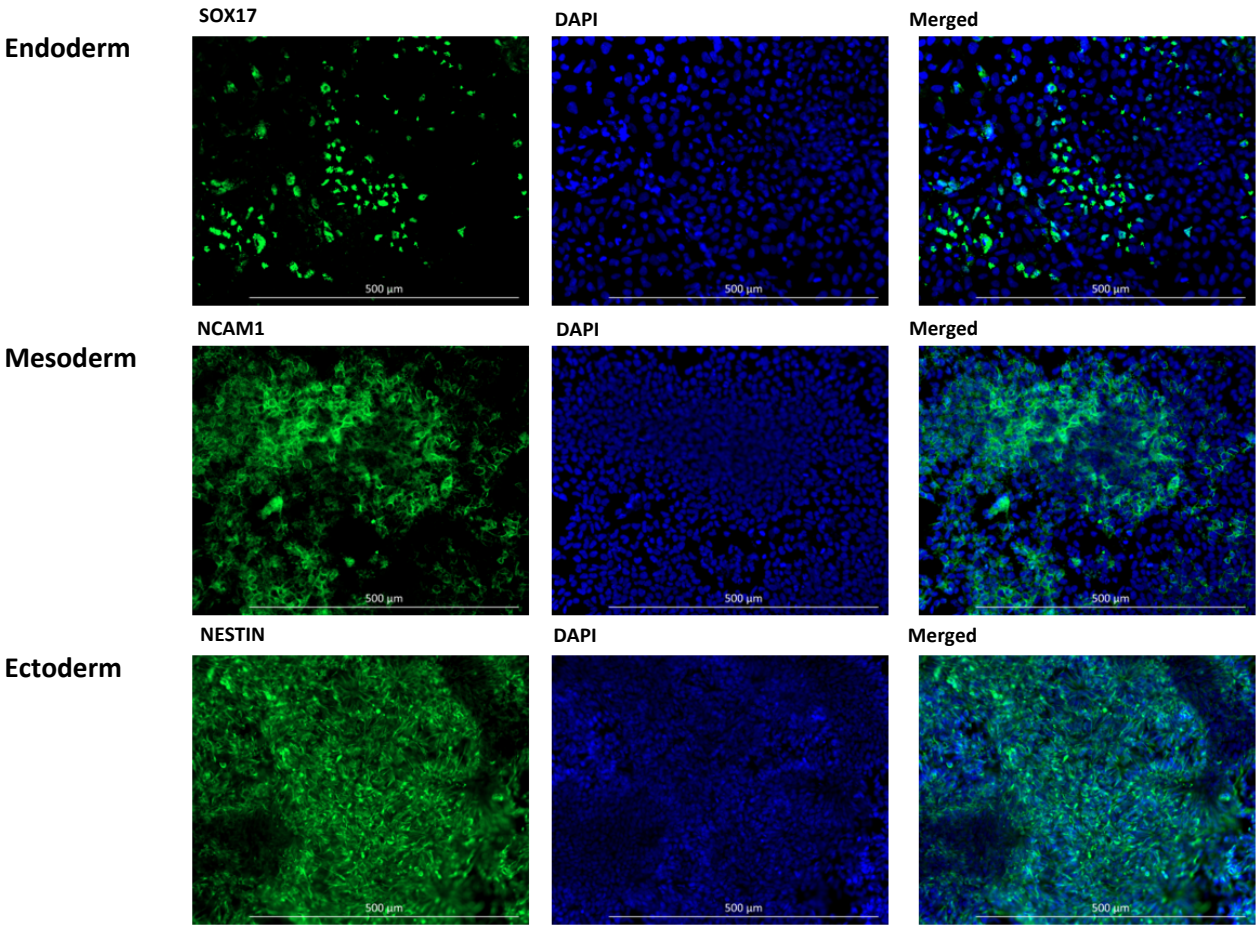

Figure 8: Immunofluorescence staining of differentiated cells showing positive signal of germlayer-specific markers.

CNV analysis

The DNA was isolated from the iPSC clone (IPS17-00097) and the donor's blood (HEP17-00123) before performing the CNV analysis, to determine abnormalities caused by reprogramming and passaging of the iPSC clone.

Table 5: The CNVs found in the iPSC clone DNA and the donor DNA

IPS17-00097	HEP17-00123
chr1: 2,619,888 - 2,701,631	chr5: 120,426,675 - 120,478,976
chr1: 189,810,172 - 189,835,703	chr6: 110,394,605 - 110,415,416
chr2: 211,963,828 - 211,982,808	chr15: 52,667,421 - 52,667,898
chr3: 21,617,615 - 21,628,131	chr16: 78,371,498 - 78,384,989
chr4: 176,627,750 - 176,646,379	chr17: 44,194,152 - 44,784,640
chr5: 120,408,473 - 120,467,653	chrX: 18,223,926 - 18,243,304
chr5: 151,777,901 - 151,796,850	chrX: 41,604,610 - 41,609,536
chr6: 110,394,605 - 110,415,233	chrX: 83,781,889 - 83,793,258
chr7: 27,223,610 - 27,235,942	chrY: 2,915,751 - 2,944,244
chr7: 38,290,714 - 38,356,733	chrY: 2,650,141 - 28,799,938
chr7: 57,291,122 - 57,347,352	
chr7: 122,016,870 - 122,107,629	
chr7: 122,163,550 - 122,206,045	
chr9: 2,011,339 - 2,018,826	
chr9: 68,665,171 - 69,002,884	
chr10: 63,588,246 - 63,599,601	
chr14: 22,635,581 - 22,944,508	
chr15: 52,667,421 - 52,667,898	
chr17: 44,196,447 - 44,784,640	
chr22: 51,106,758 - 51,121,296	
chrX: 29,336,997 - 29,347,246	
chrX: 46,415,611 - 46,427,421	
chrX: 83,781,889 - 83,793,258	
chrY: 2,650,141 - 28,799,938	

Conclusion:

All of the differences shown in the iPSC collumn in table 5 are due to noise.

- (●) Pass
- ( ) Fail
- ( ) Other:

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Date 11.11.2020