Stem Cell Technology Center Genetica

Stem Cell Technology Center

Certificate of Analysis 2020

Invoice number: SCTC2018-00014

Name investigator: Anneke den Hollander
Cell line number: IPS17-00095 clone 1
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	PBMCs
Parental cell type	HEP17-00121
Diagnosis	Control
Mutation	
Number of clones	1
Passage (P) of iPS cells reported at submission	P10
Culture medium	Essential 8 Flex medium
Culture coating	Matrigel (Vitronectin until P6)
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, NANOG, DNMT3B* 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

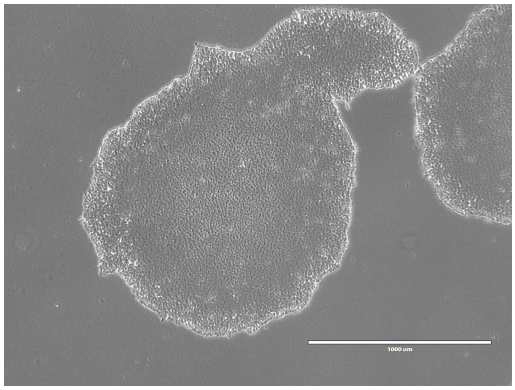

Figure 1: Cells prior to freezing.

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).

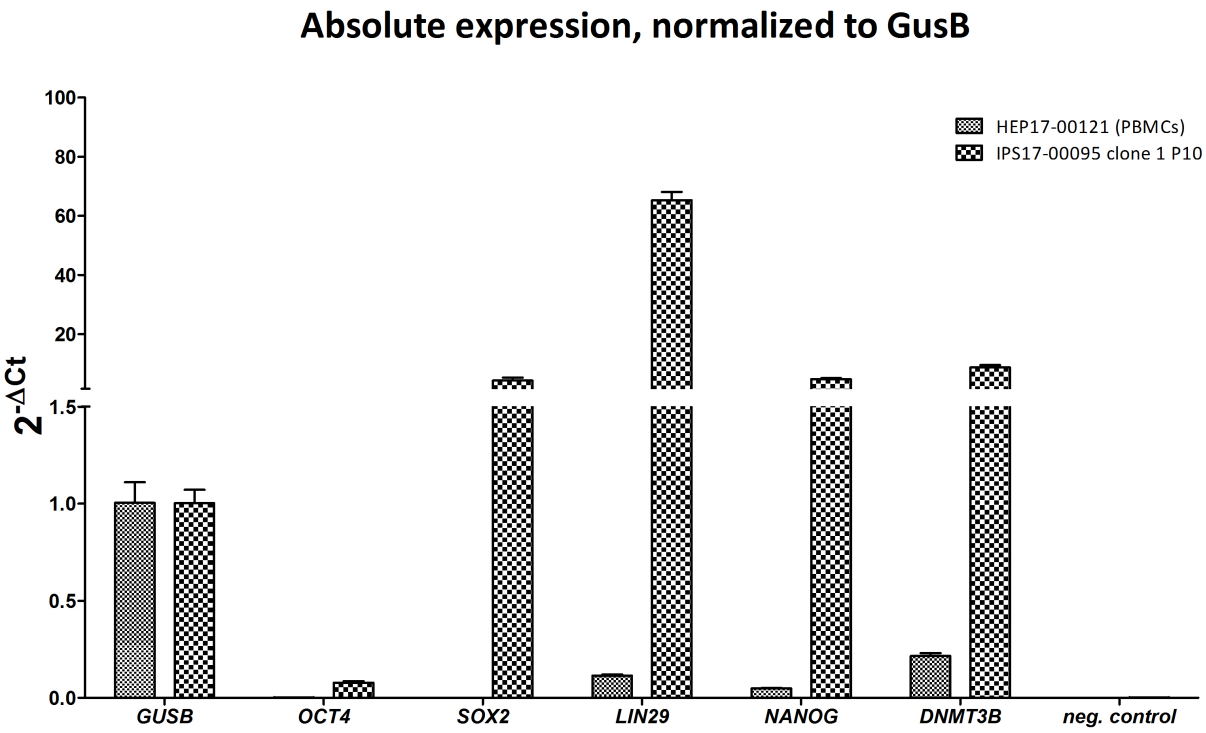

Figure 2: Gene expression of the iPSC clone compared with the parental PBMCs (ΔCt).

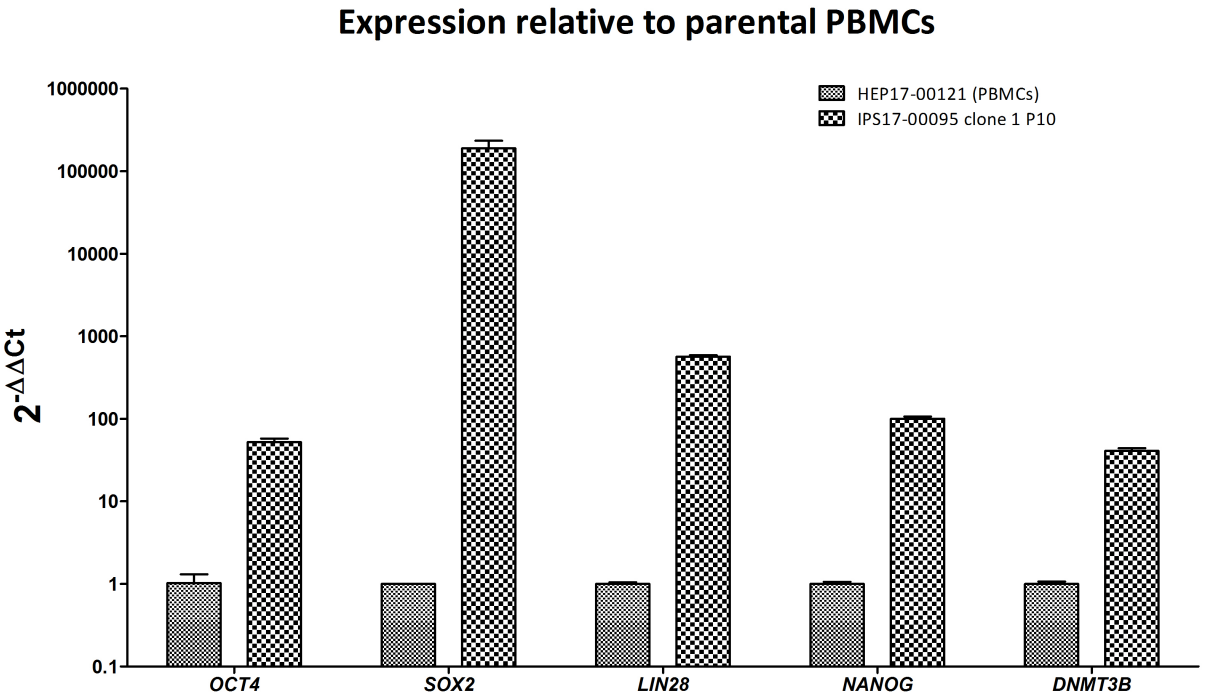

Figure 3: Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPSC clone is relative to the parental PBMCs.

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.

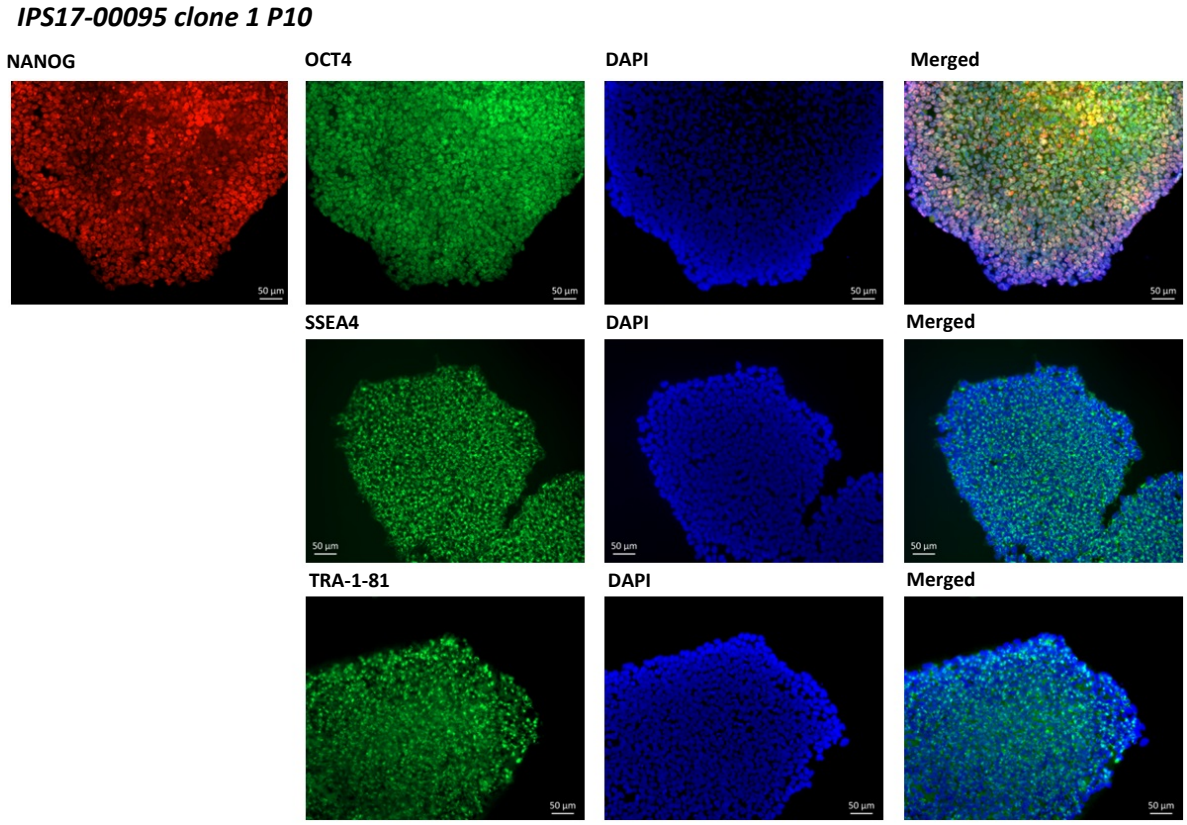

Figure 4: Immunofluorescence staining of the iPSC clone with pluripotency markers.

Three germ layer differentiation

IPS17-00095 clone 1 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

Activation of germlayer-specific markers

Endoderm

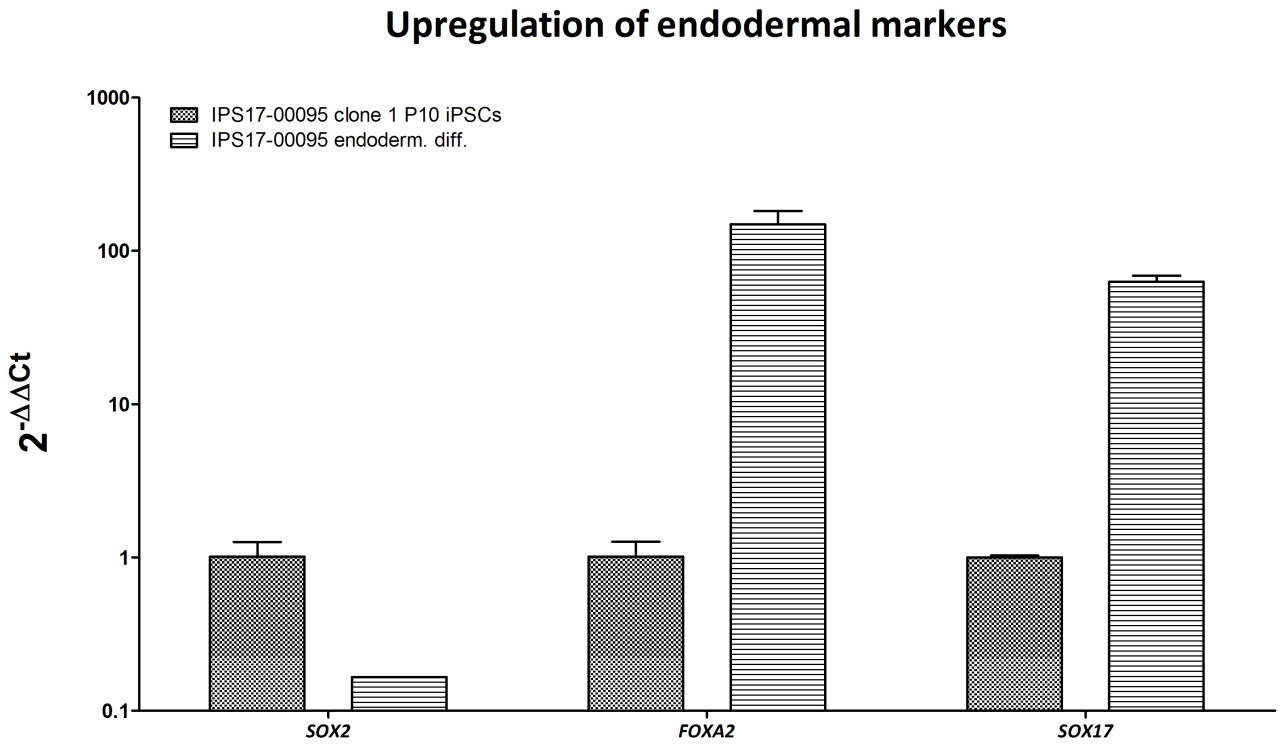

Figure 5: Expression fold difference of endoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone. *SOX2* was used as a reference for pluripotency.

Mesoderm

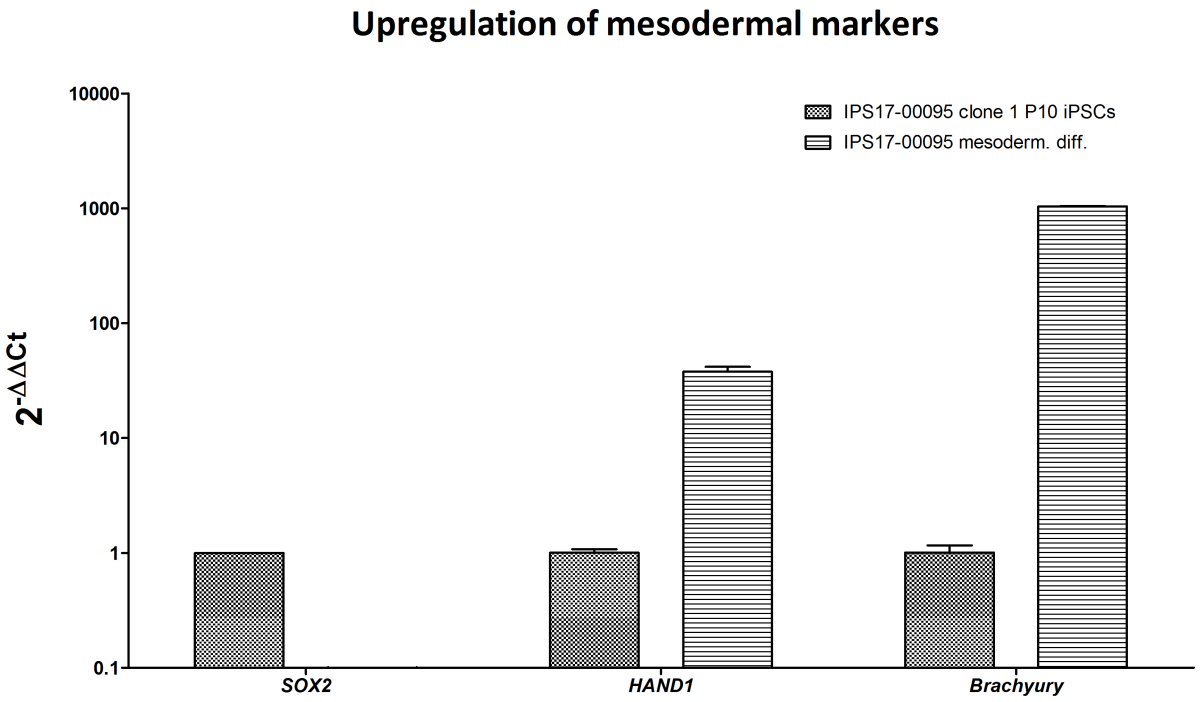

Figure 6: Expression fold difference of mesoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone. SOX2 was used as a reference for pluripotency.

Ectoderm

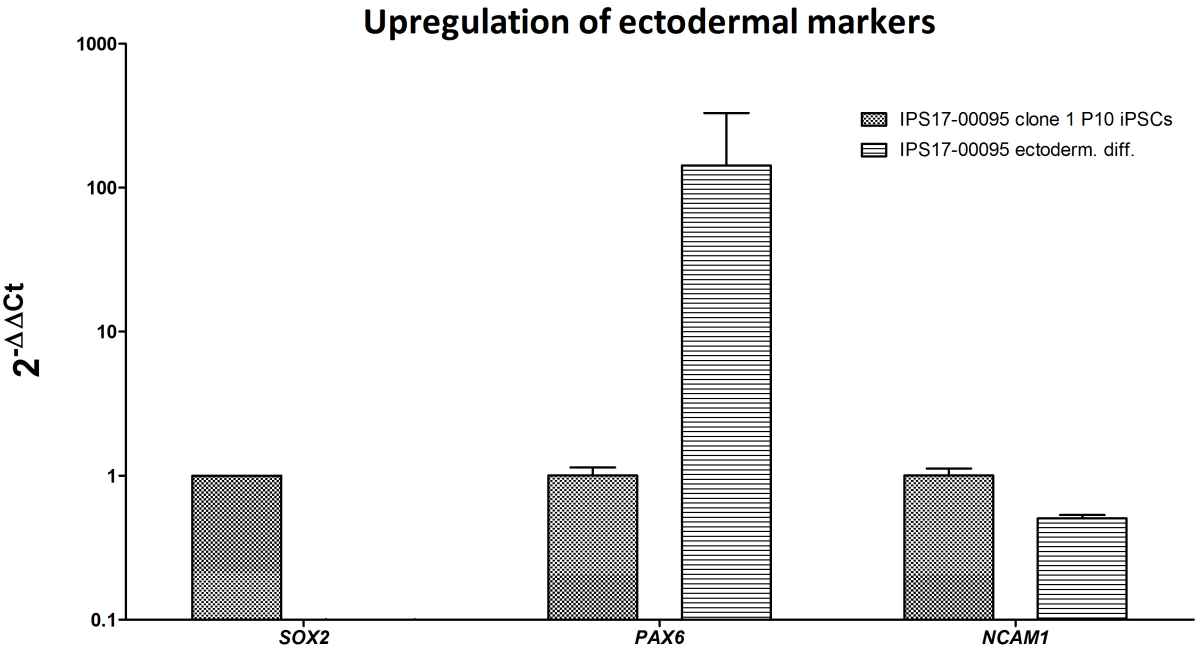

Figure 7: Expression fold difference of ectoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone. SOX2 was used as a reference for pluripotency.

Expression of germlayer-specific markers

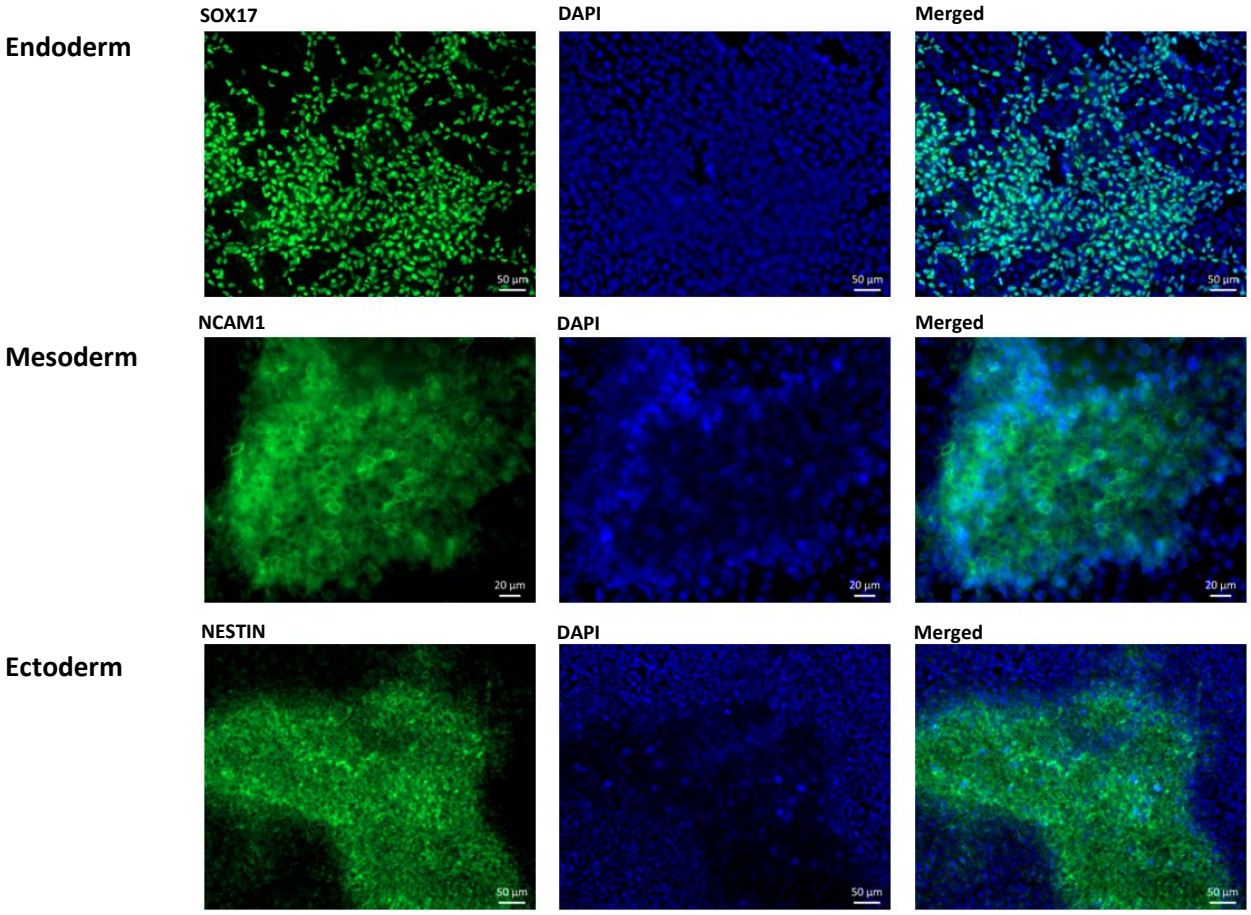

Figure 8: Immunofluorescence staining of differentiated cells showing positive signals of germ layer-specific markers.

CNV analysis

The DNA was isolated from the iPSC clone (IPS17-00095) and the donor's blood (HEP17-00121) 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

IPS17-00095	HEP17-00121
chr1: 179,602,103 - 179,622,544	chr1: 179,602,444 - 179,622,544
chr3: 85,586,457 - 85,605,860	chr3: 85,586,457 - 85,605,860
chr5: 90,683,702 - 90,696,946	chr14: 80,999,776 - 81,011,937
chr6: 78,737,388 - 78,768,251	chr22: 18,560,247 - 18,564,772
chr7: 27,223,610 - 27,235,942	chrX: 2,449,603 - 2,464,967
chr7: 46,415,321 - 46,442,319	chrX: 48,329,405 - 48,338,177
chr9: 2,011,339 - 2,018,826	chrX: 100,529,163 - 100,541,551
chr11: 103,191,837 - 103,204,922	chrX: 102,993,211 - 103,007,059
chr15: 54,059,344 - 54,075,547	chrX: 111,719,243 - 111,762,215
chr15: 85,052,858 - 85,105,201	chrY: 2,915,751 - 2,964,008
chr16: 15,879,441 - 15,889,908	chrY: 3,802,528 - 3,837,156
chr17: 39,195,864 - 39,214,298	chrY: 4,213,243 - 4,245,405
chrX: 377,909 - 395,647	chrY: 5,365,041 - 5,417,674
chrX: 29,336,997 - 29,347,246	chrY: 27,636,037 - 27,656,896
chrX: 46,415,611 - 46,427,421	chrY: 28,665,594 - 28,695,314
chrX: 48,329,405 - 48,338,177	chrY: 2,650,141 - 28,799,938
chrX: 83,781,889 - 83,793,258	
chrX: 100,529,163 - 100,541,551	
chrX: 102,993,211 - 103,007,059	
chrX: 119,697,813 - 119,714,082	
chrX: 148,490,769 - 148,501,356	
chrY: 27,636,037 - 27,656,896	
chrY: 2,650,141 - 28,799,938	

Conclusion:

All of the differences shown in the iPSC column in Table 5 are due to noise.

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- (●) Pass
- ( ) Fail
- ( ) Other:

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