Stem Cell Technology Center Genetica

Certificate of Analysis 2021

Invoice number: SCTC2021-00004

Name principal investigator: Anneke den Hollander
Cell line number: IPS19-00051 CRISPR clone H6
Project name: VICI

Table 1: Information on the reprogrammed cell line

Information cell line:	
Product description	Trilineage differentiation
Parental cell line Parental cell type	IPS19-00051 N/A*
Diagnosis Mutation	N/A* N/A*
Number of clones Passage (P) of iPSCs reported at delivery	1 P25

*N/A: Not Applicable

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 *SOX2*, *LIN28*, *NANOG*, *OCT4* in iPSCs compared with PBMCs	N/A*
Expression of stem cell markers	Immunocytochemistry	Expression of OCT4, NANOG, SSEA4, TRA-1-81	N/A*
Mycoplasma test	PCR	Negative	N/A*
Three lineage differentiation	Differentiation assay	Upregulaton of germlayer-specific markers	No upregulation of *HAND1*

Three germ layer differentiation

IPS19-00051 CRISPR clone H6 was differentiated into the endodermal, mesodermal and ectodermal germ layers. The RNA was isolated and the 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	DESMIN
Ectoderm	NESTIN

Endoderm

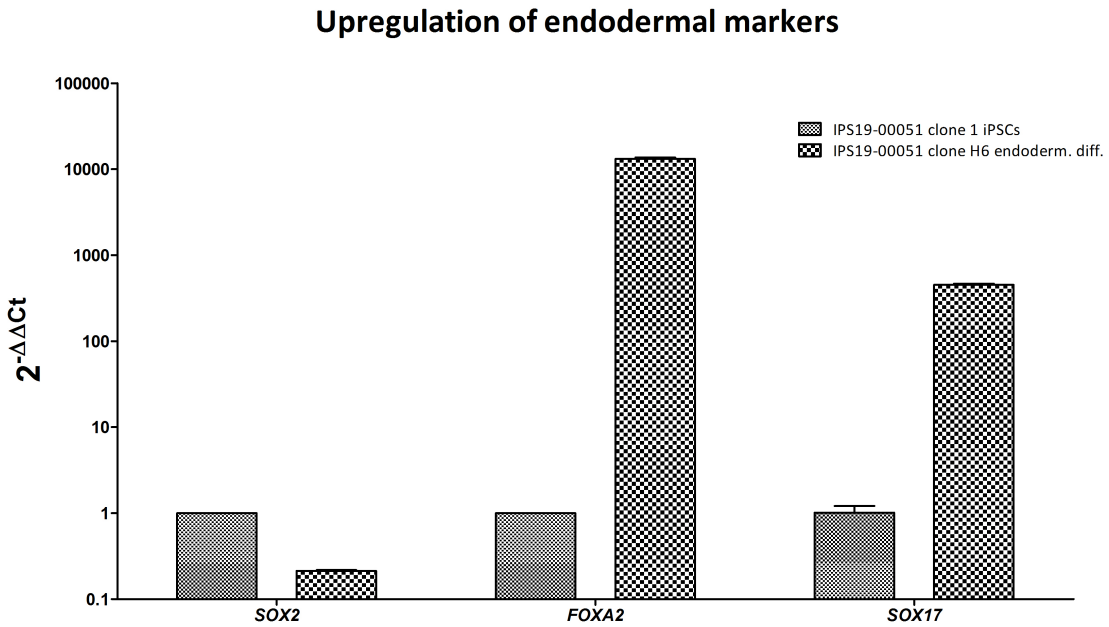

Figure 1: Expression fold difference of endoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *SOX2* was used as a reference for pluripotency.

Mesoderm

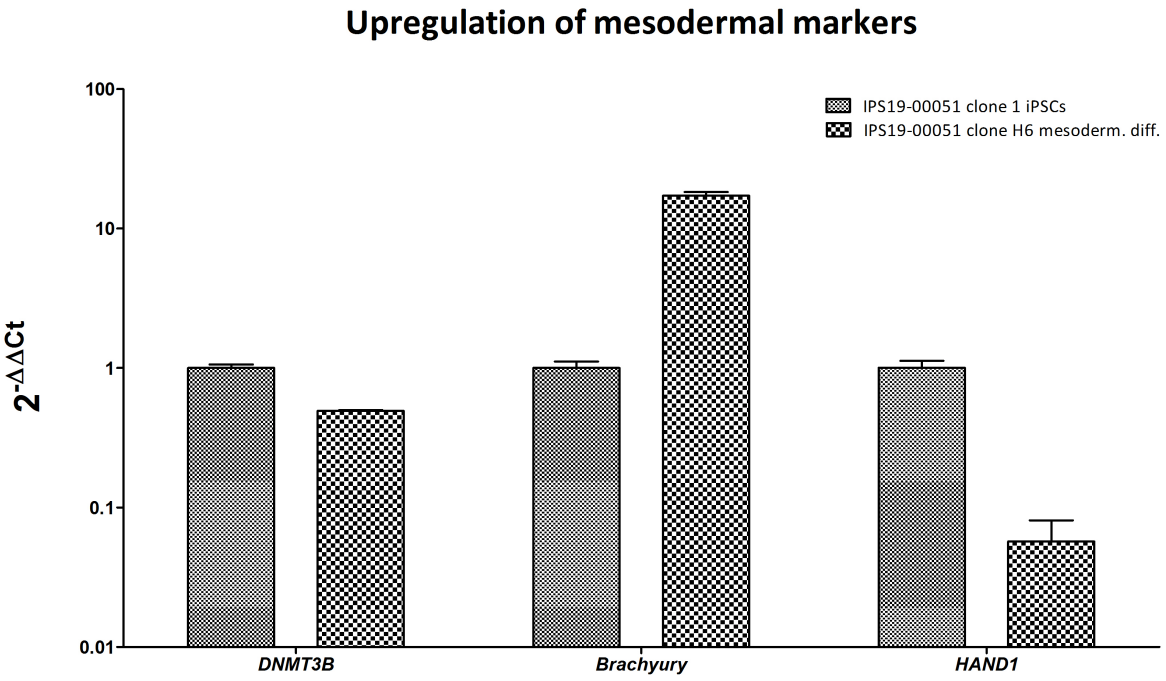

Figure 2: Expression fold difference of mesoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *DNMT3B* was used as a reference for pluripotency.

Ectoderm

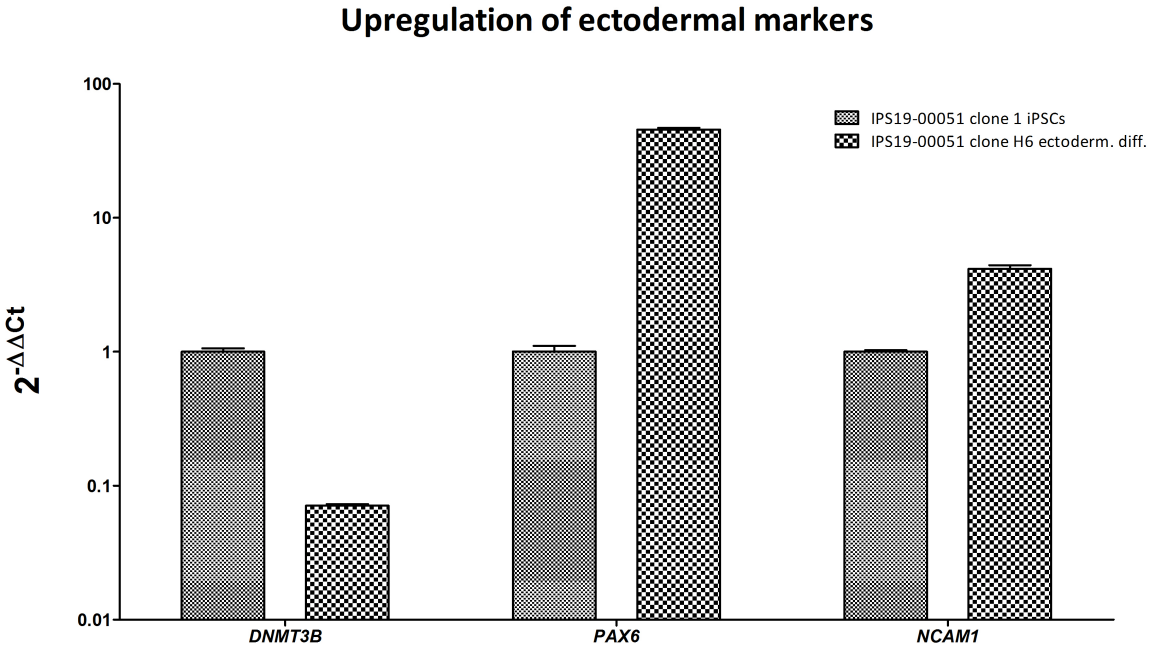

Figure 3: Expression fold difference of ectoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *DNMT3B* was used as a reference for pluripotency.

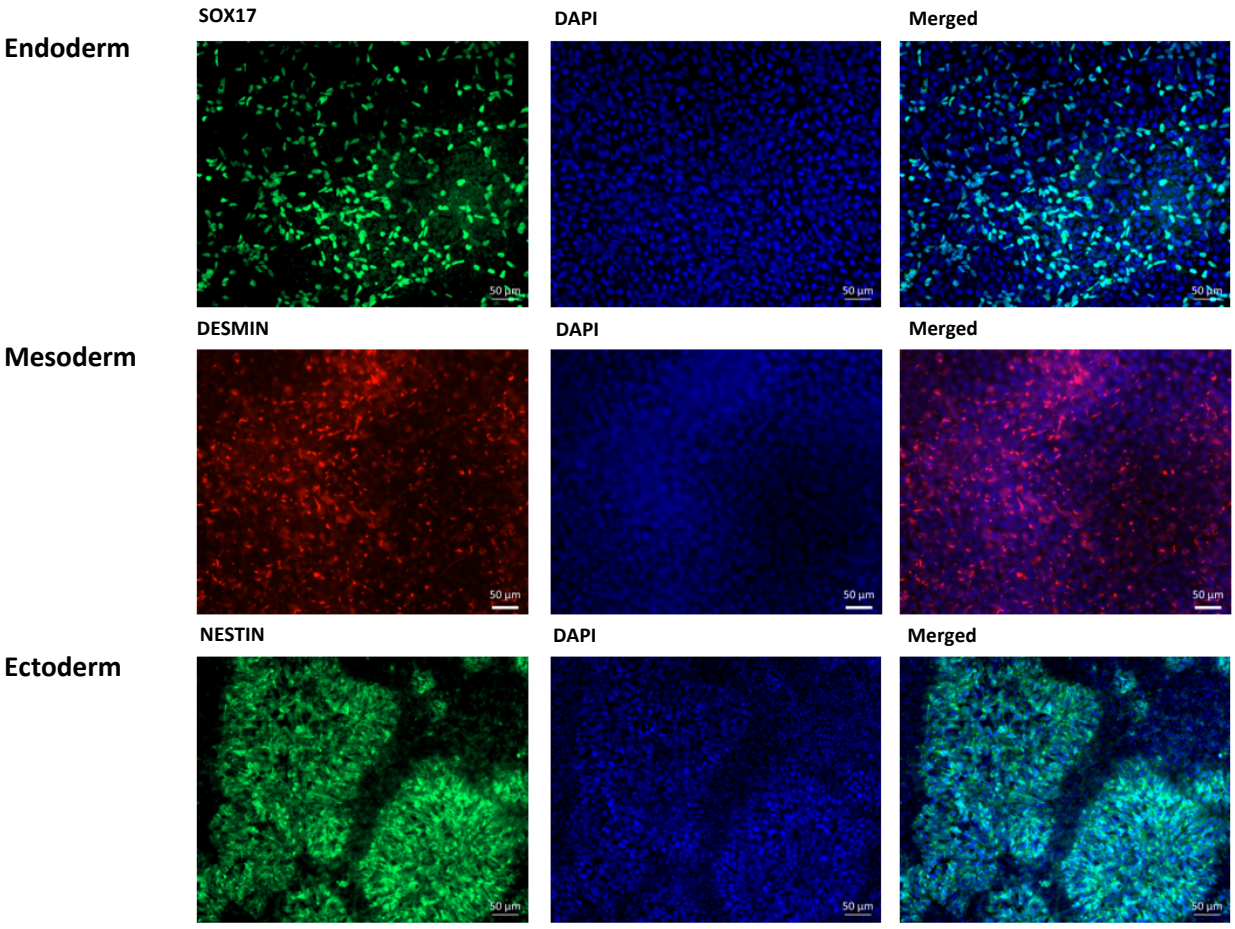

Figure 4: Immunofluorescence staining of differentiated cells showing a positive signal of germlayer-specific markers.

- ◯ Pass
- ◯ Fail
- ◯ Other:

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Silvia Albert, PhD
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Date