Stem Cell Technology Center Genetica

Certificate of Analysis 2021

Invoice number: SCTC2021-00074

Name principal investigator: Frans Cremers
Cell line number: IPS15-00007
Project name: -

Table 1: Information on the reprogrammed cell line

Information cell line:	
Product description	Human fibroblasts reprogrammed with four factors (OCT4, SOX2, KLF4, C-MYC) by using lentiviral vectors
Parental cell line Parental cell type	CL14-00062 Fibroblasts
Diagnosis Mutation	STGD1 N/A*
Number of clones Passage (P) of iPSCs reported at delivery	3 P6
Culture medium Culture coating Feeders during reprogramming Passage method	Essential 8 Flex medium Vitronectin Mouse Embryonic Fibroblasts (MEFs) EDTA

*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 *OCT4, SOX2, LIN28, NANOG* in iPSCs compared with fibroblasts	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	Upregulaton of germlayer-specific markers	Pass

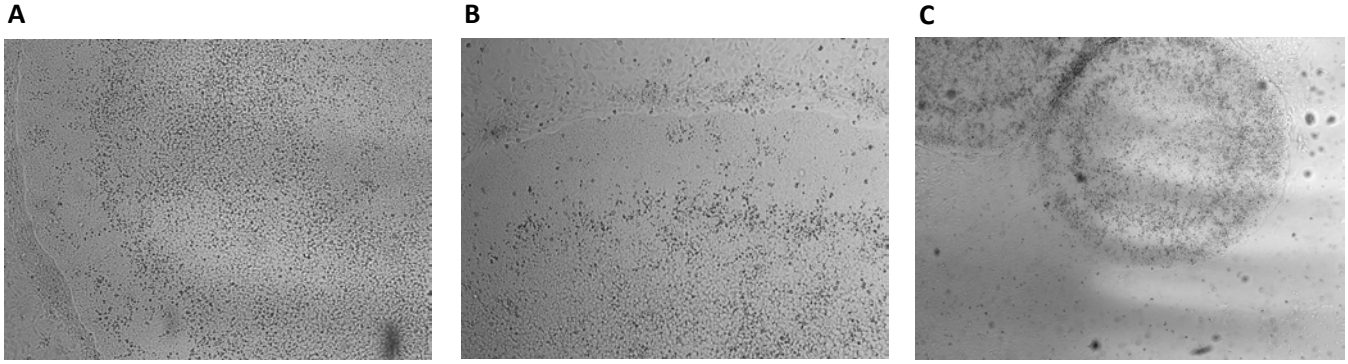


Figure 1: Cells prior to freezing. A - C, clones 1 - 3 at P6.

Activation of stem cell markers

The RNA of all clones was isolated before freezing and the gene expression was assessed by quantitative reverse transcription PCR (qRT-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 fibroblasts (ΔCt).

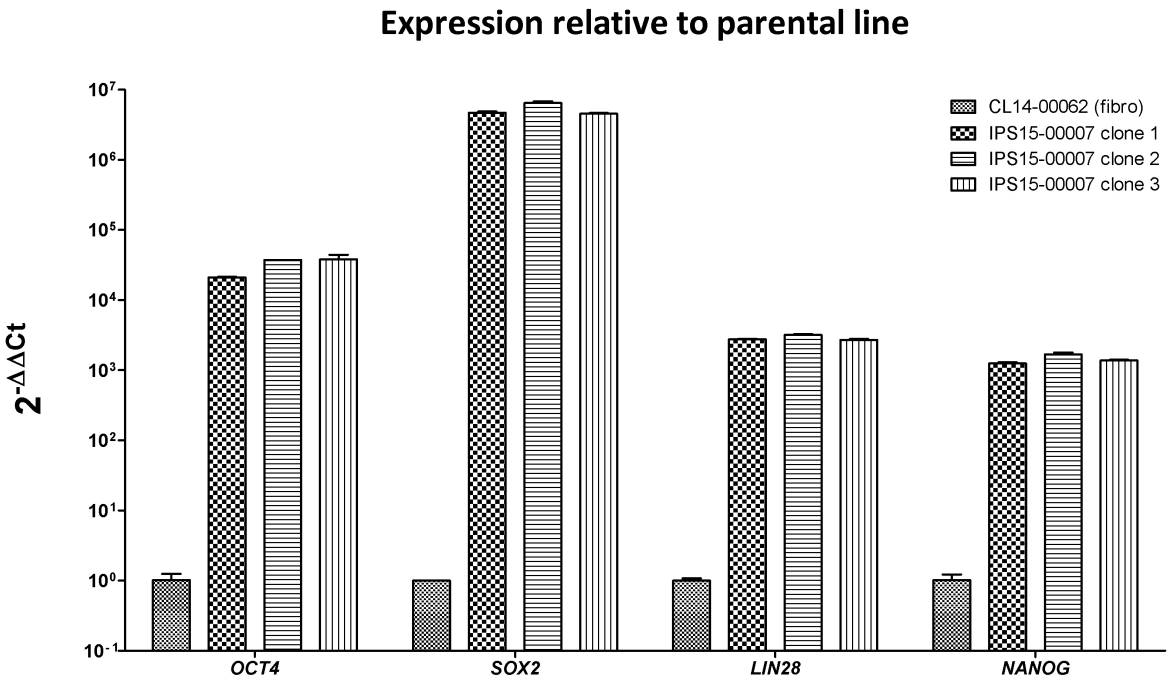
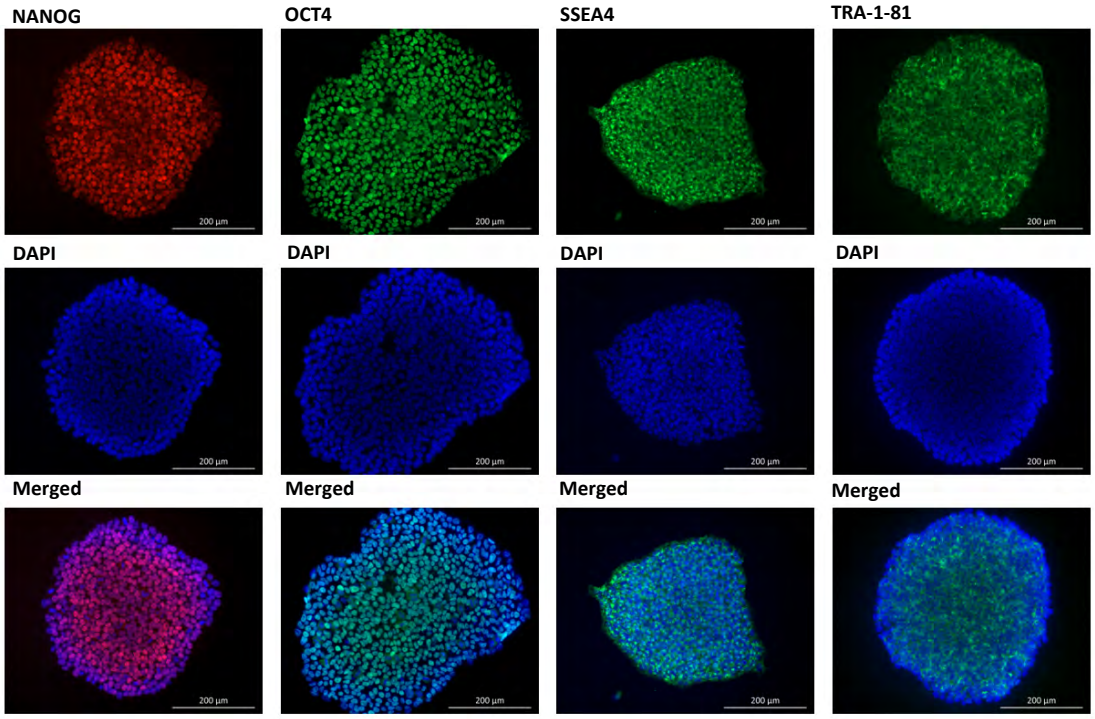


Figure 3: Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of the iPSCs is relative to the parental fibroblasts.

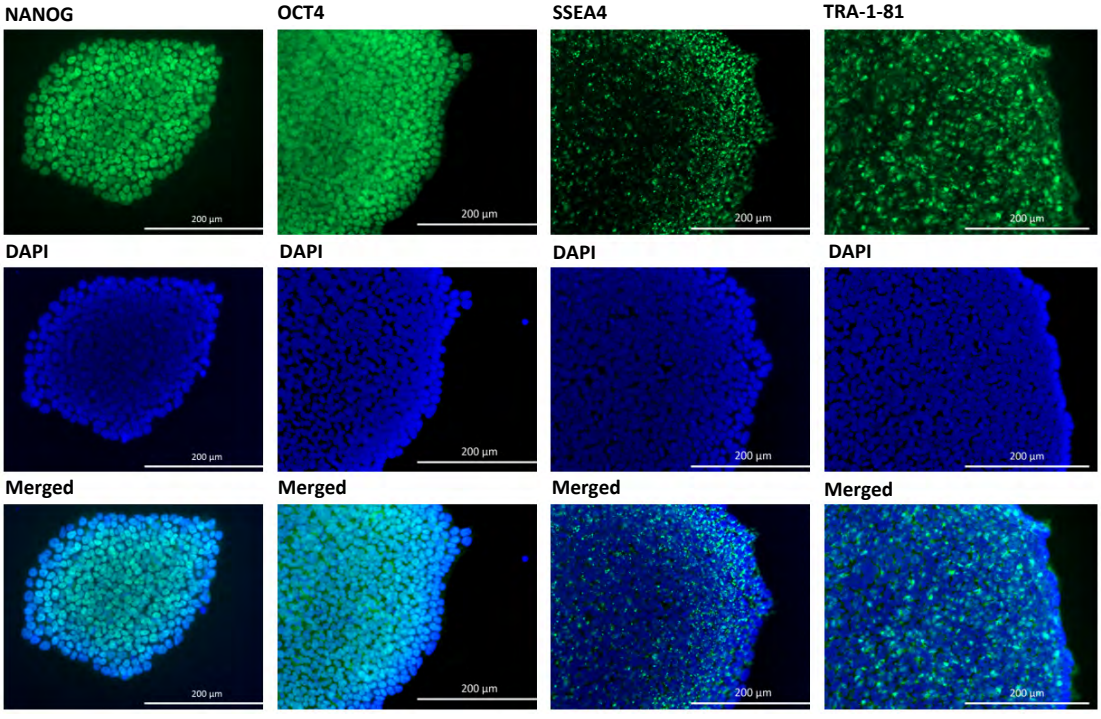
Expression of stem cell markers

The 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. IPS15-00007 clone 1



B. IPS15-00007 clone 2



C. IPS15-00007 clone 3

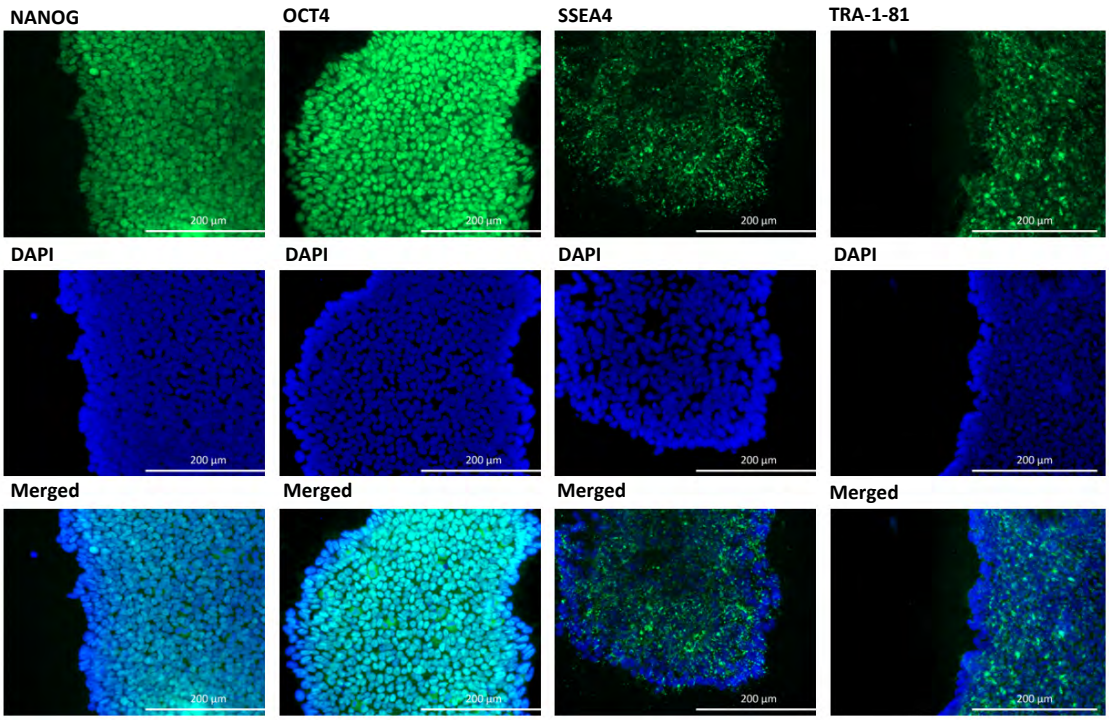


Figure 4: Immunofluorescence staining of the iPSC clones with pluripotency markers.

Three germ layer differentiation

Clone 1 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

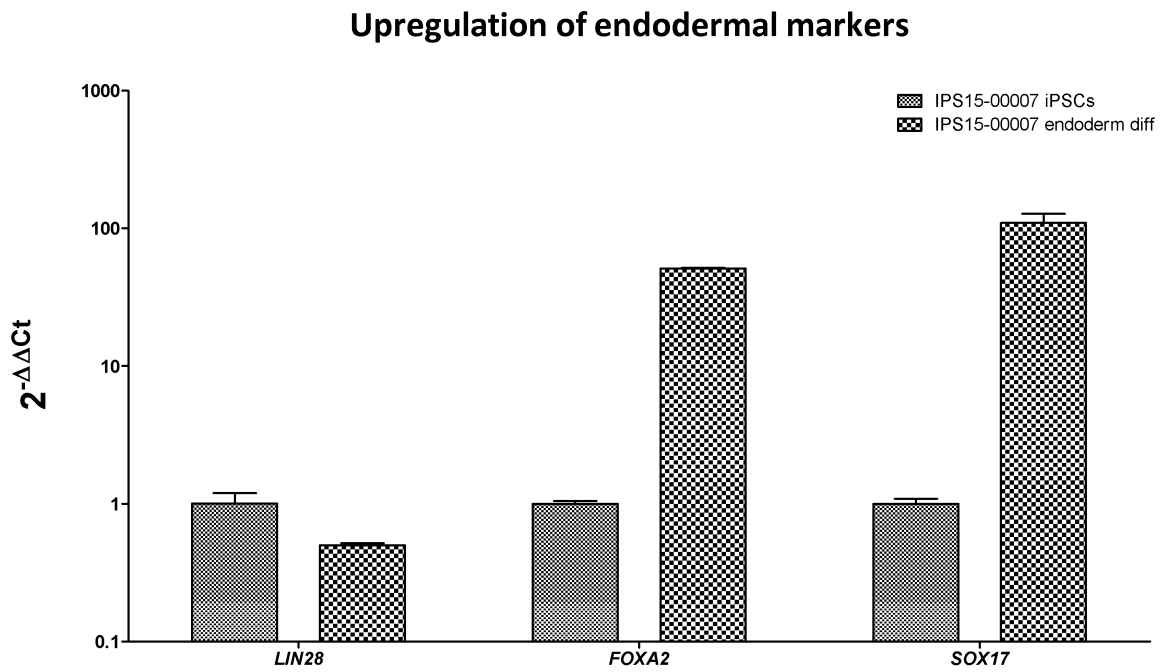


Figure 5: Expression fold difference of endoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *LIN28* was used as a reference for pluripotency.

Mesoderm

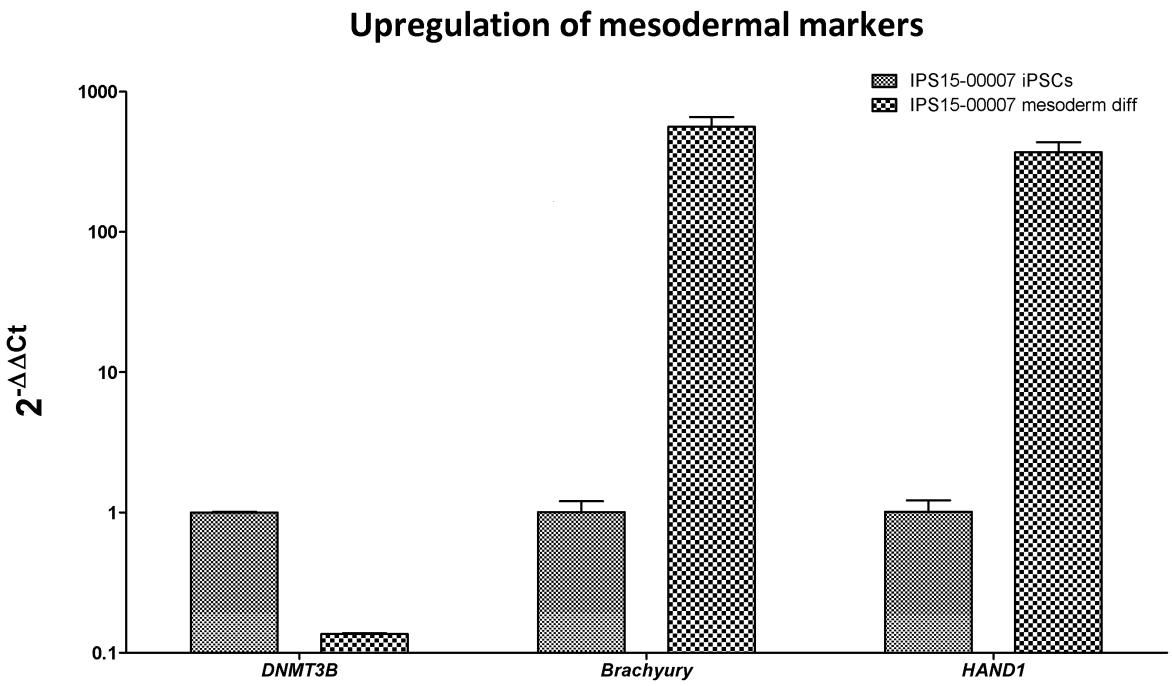


Figure 6: Expression fold difference of mesoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *DNMT3B* was used as a reference for pluripotency.

Ectoderm

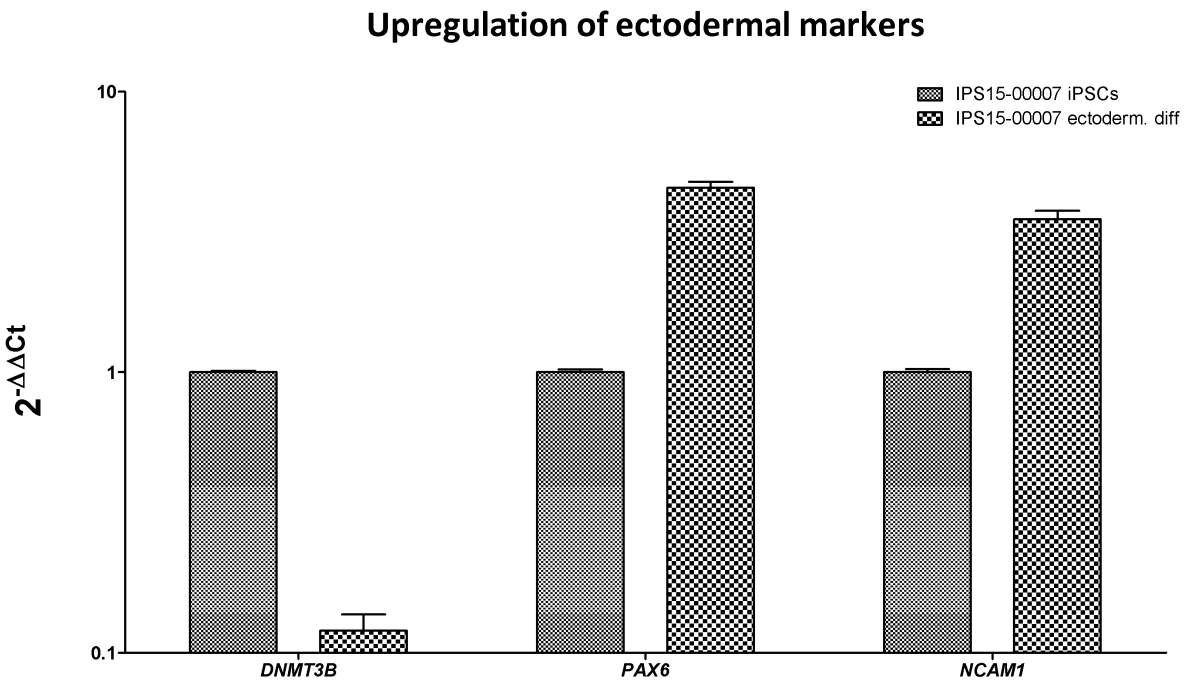


Figure 7: Expression fold difference of ectoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *DNMT3B* was used as a reference for pluripotency.

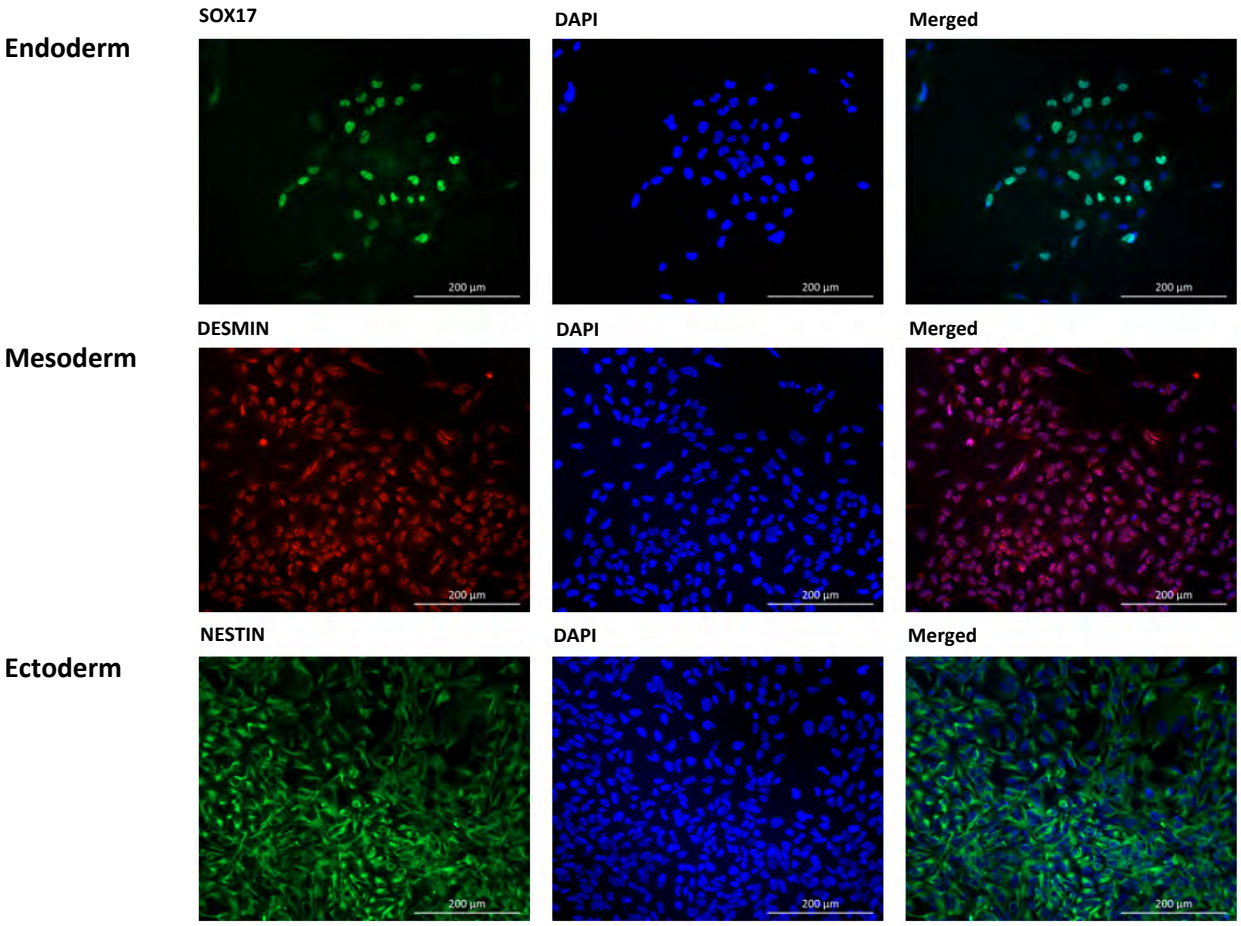


Figure 8: Immunofluorescence staining of differentiated cells showing a positive signal of germlayer-specific markers.

(●) Pass

( ) Fail

( ) Other:

Silvia Albert

Silvia Albert, PhD
Manager, Radboud Stem Cell Technology Center
Date 23.11.2021