Stem Cell Technology Center Genetica

Certificate of Analysis 2021

Invoice number: SCTC2017-00044

Name investigator: Anneke den Hollander
Cell line number: IPS17-00056 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	HEP17-00094
Diagnosis	AMD
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) Mouse
Feeders during reprogramming	Embryonic Fibroblasts (MEFs) 0.5
Passage method	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	No upregulation of *NES*
hPSC genetic analysis	qPCR	Detection of recurrent karyotypic abnormalities	See results in last page

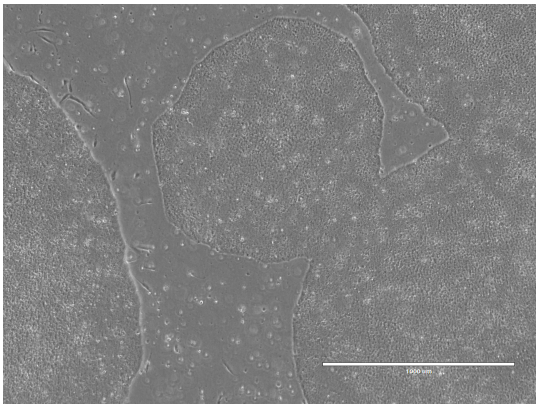

Figure 1: Cells prior to freezing. Scale bar = 1000 µm.

IPS17-00056 clone 1 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).

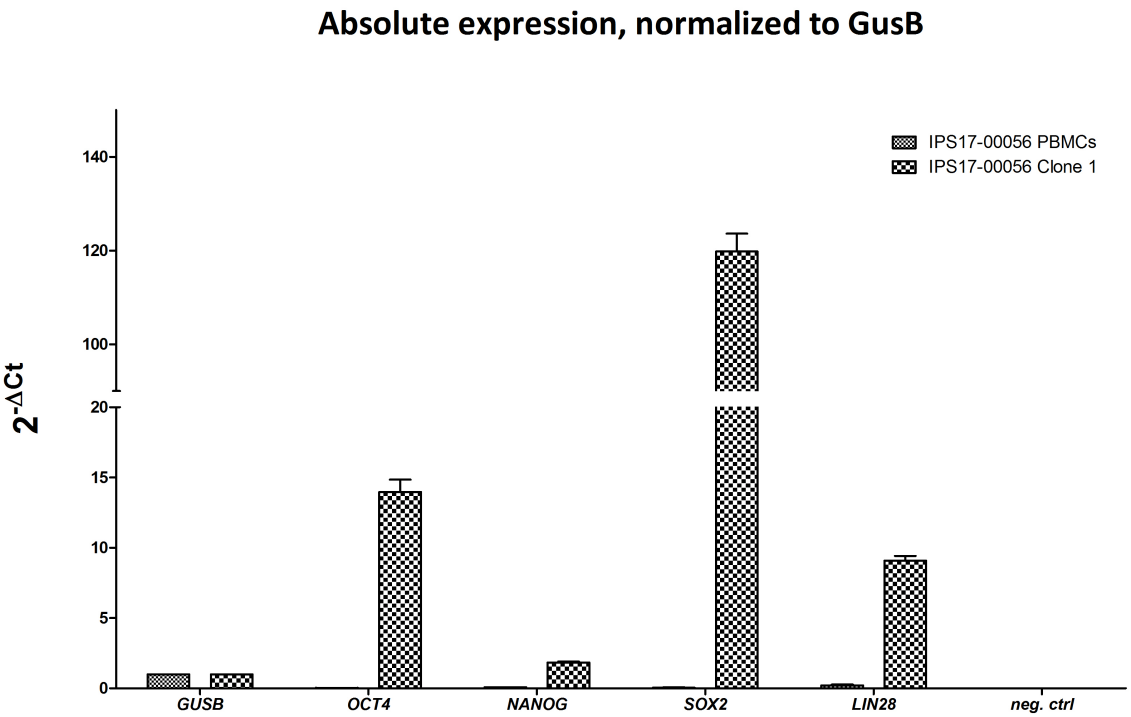

Figure 2: Gene expression of IPS17-00056 clone 1 (P6) compared with the parental PBMCs (ΔCt).

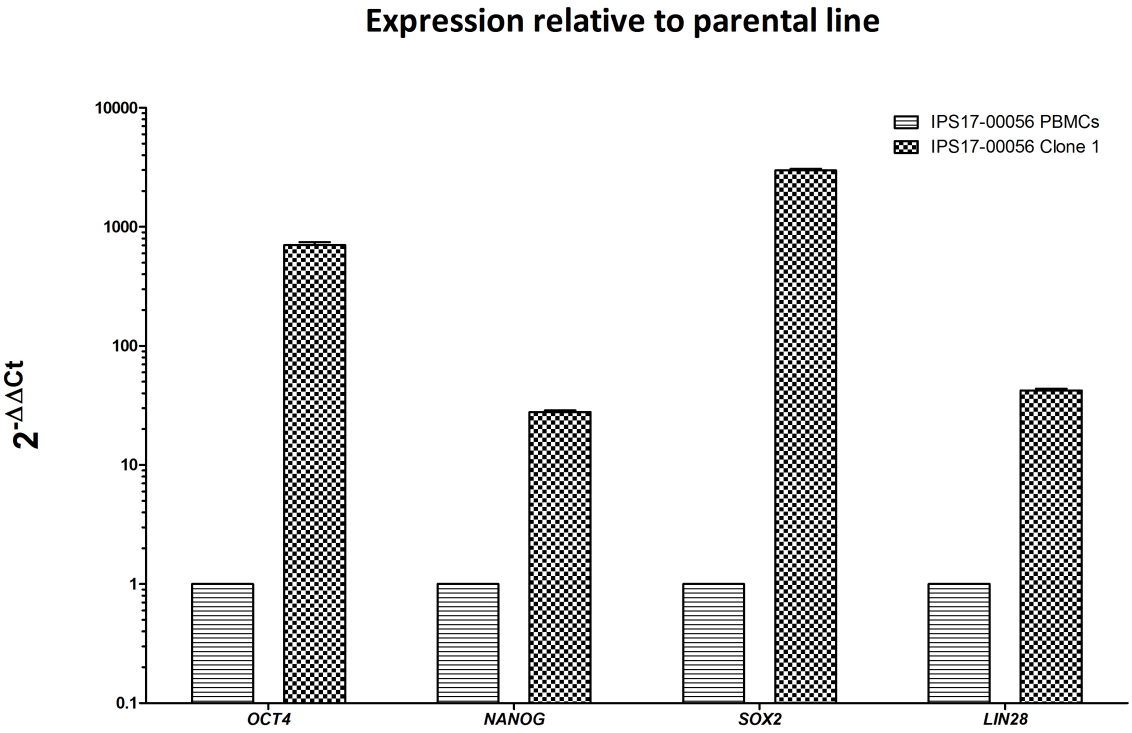

Figure 3: Pluripotency gene upregulation after reprogramming (ΔΔCt). The expression fold difference of IPS17-00056 clone 1 (P6) is relative to the parental PBMCs.

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. IPS17-00056 clone 1 P6

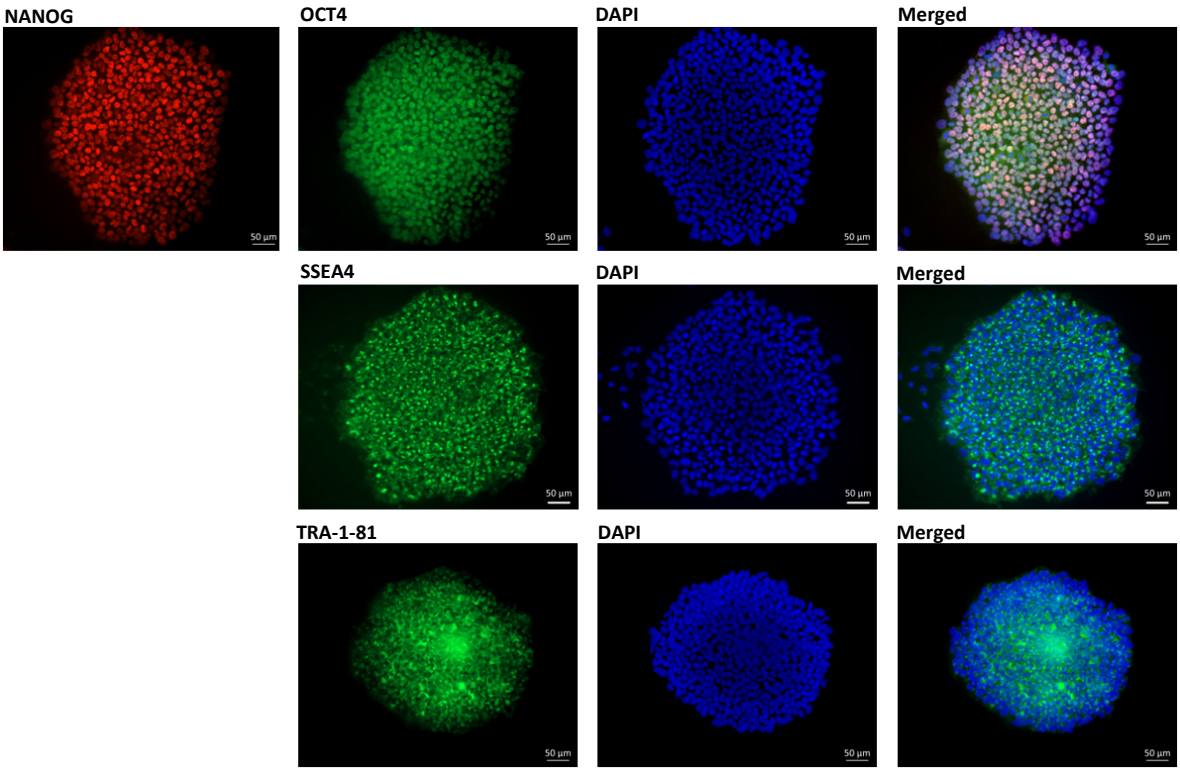

Figure 4: Immunofluorescence staining of the iPSC clones with pluripotency markers.

Three germ layer differentiation

iPS17-00056 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, NES

Table 4: ICC markers for three lineage differentiation

Lineage	Marker
Endoderm	SOX17
Mesoderm	DESMIN
Ectoderm	NESTIN

Activation of germlayer-specific markers

Endoderm

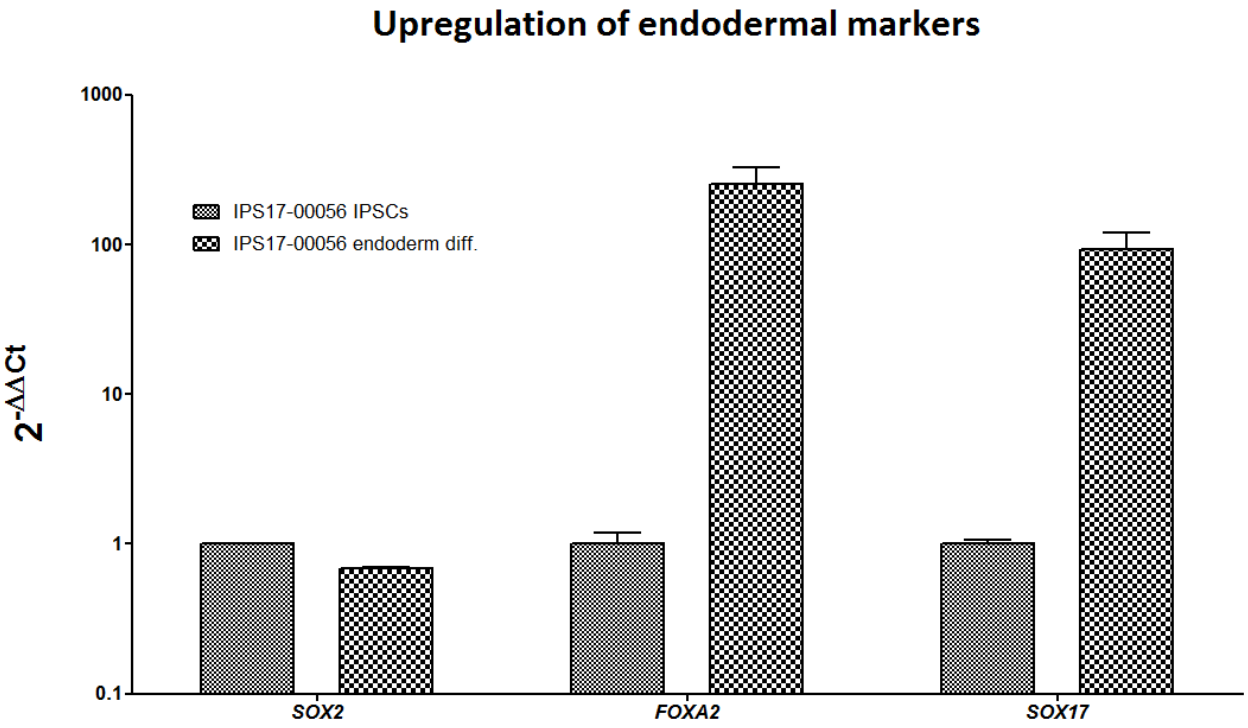

Figure 5: Expression fold difference of endoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *SOX2* was used as a reference for pluripotency.

Mesoderm

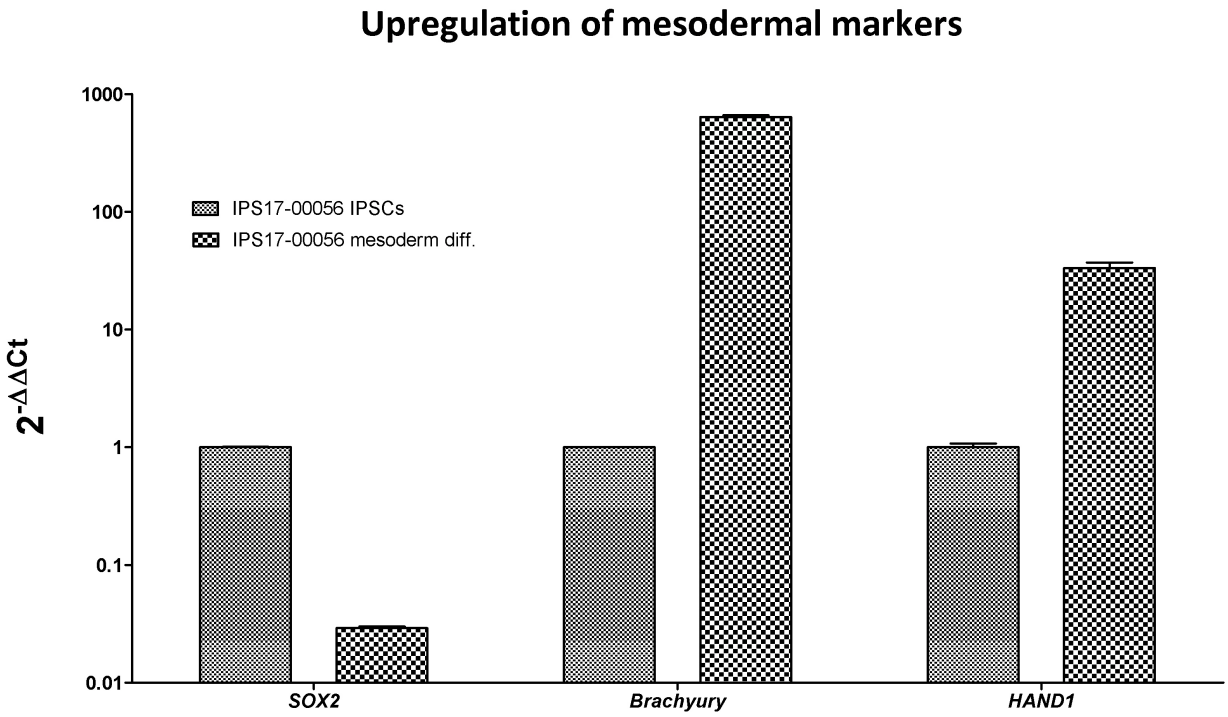

Figure 6: Expression fold difference of mesoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *SOX2* was used as a reference for pluripotency.

Ectoderm

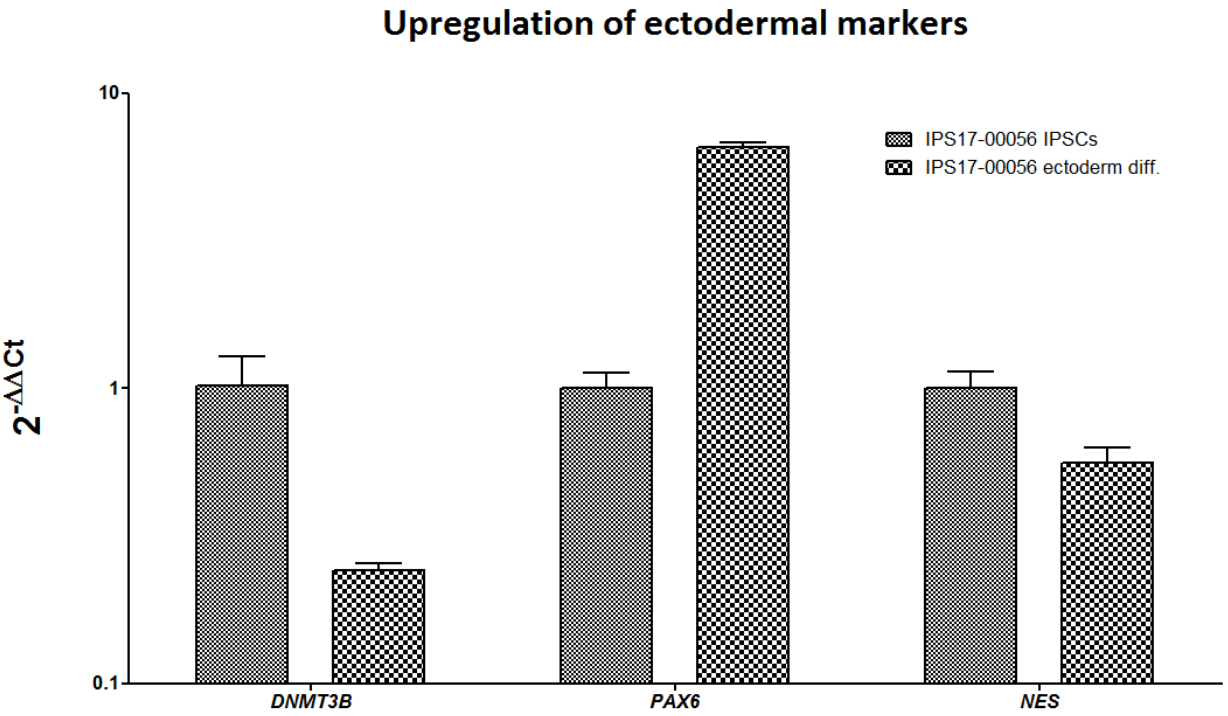

Figure 7: Expression fold difference of ectoderm-specific genes in differentiated cells, compared with undifferentiated iPSCs. *DNMT3B* was used as a reference for pluripotency.

Expression of germlayer-specific markers

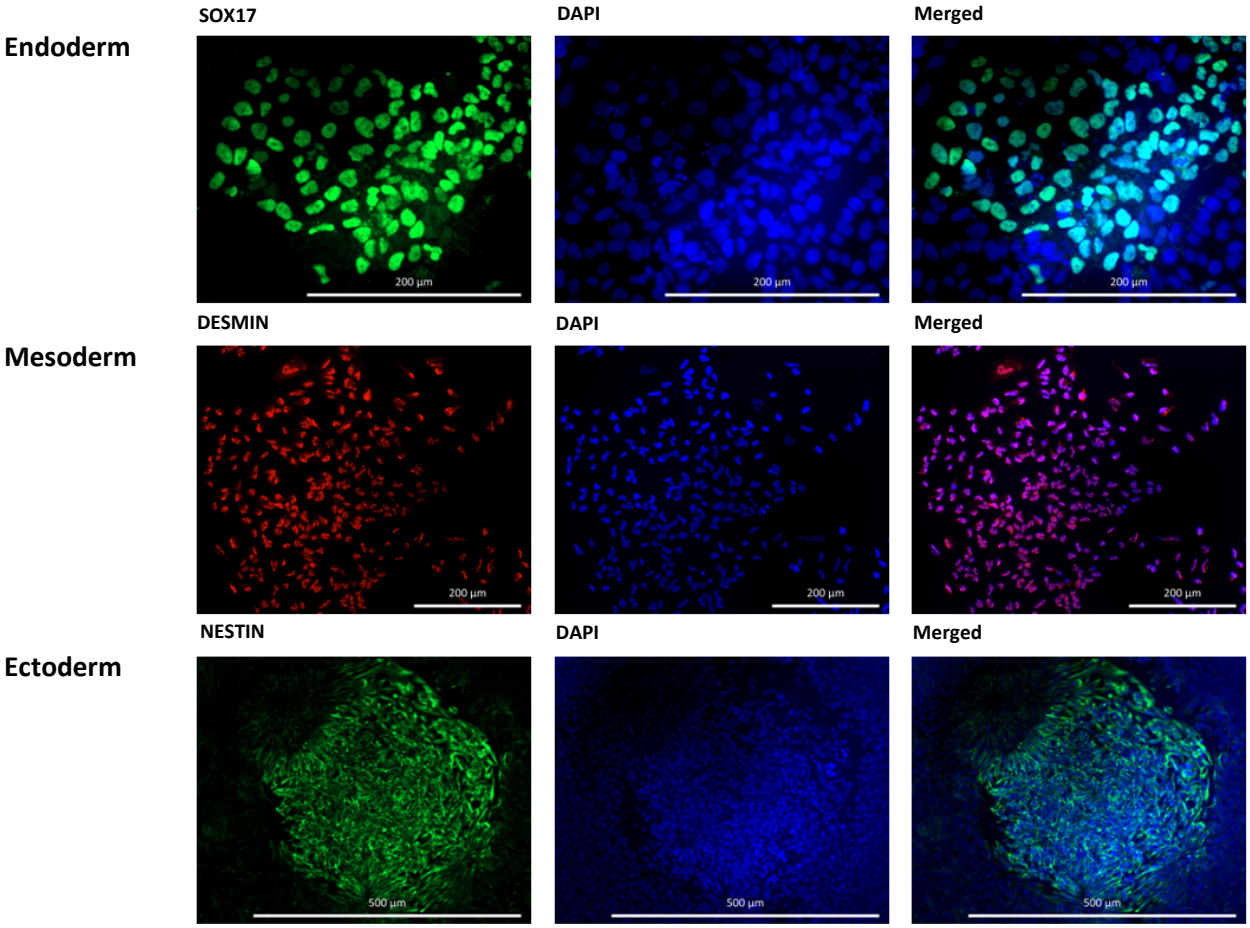

Figure 8: Immunofluorescence staining of differentiated cells showing positive signals of germ layer-specific markers.

Stemcell Karyotype report

DNA was isolated from three iPSC clones at P6 and the majority of recurrent chromosomal abnormalities reported in human embryonic stem cells and induced pluripotent stem cells was analysed.

Sample	Sex	Status
IPS17-00056 cl1 P7	Male	Normal (Sex Difference)
IPS17-00056 cl2 P7	Male	Normal (Sex Difference)
IPS17-00056 cl3 P7	Male	Normal (Sex Difference)

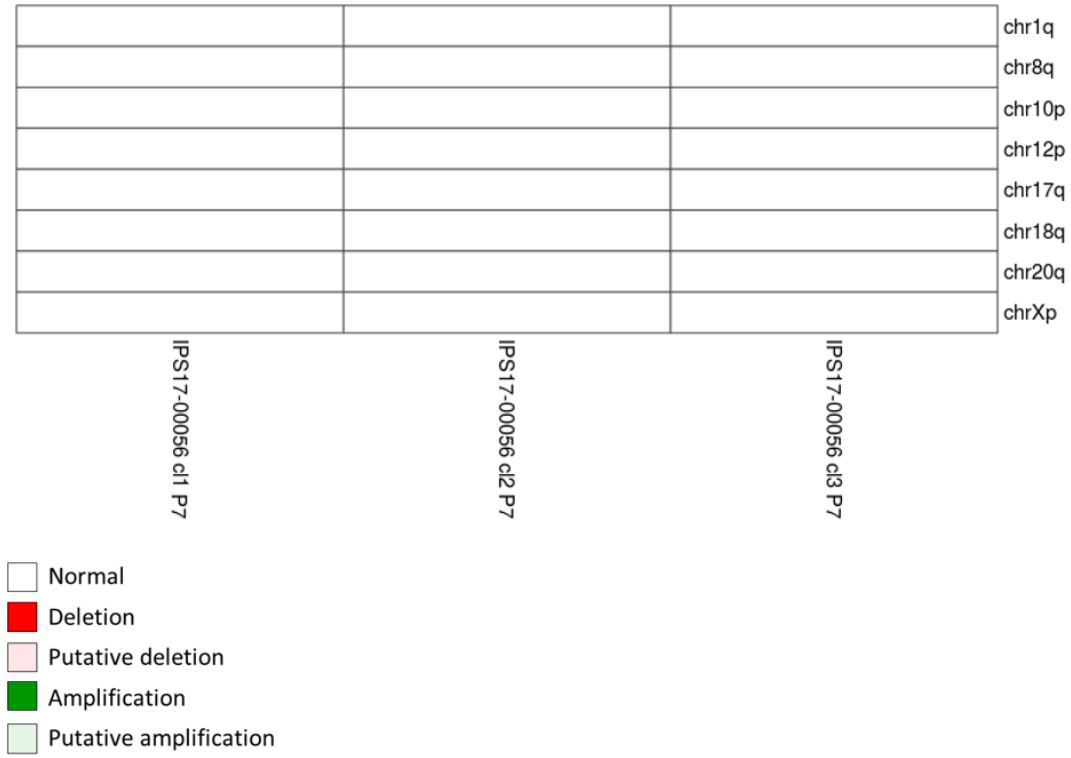

Figure 9: Summary of the genetic analysis

Conclusion:

For further experiments it is suggested to use IPS17-00056 clone 1, 2 or 3. No aberrations were found in all three clones.

More detailed results are on request.

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- (●) Pass
- ( ) Fail
- ( ) Other:

Silvia Albert

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Date 22.11.2021