

Certificate of Analysis 2020

Invoice number: SCTC2017-00029

Name investigator: Anneke den Hollander

Cell line number: IPS17-00041 clone 1

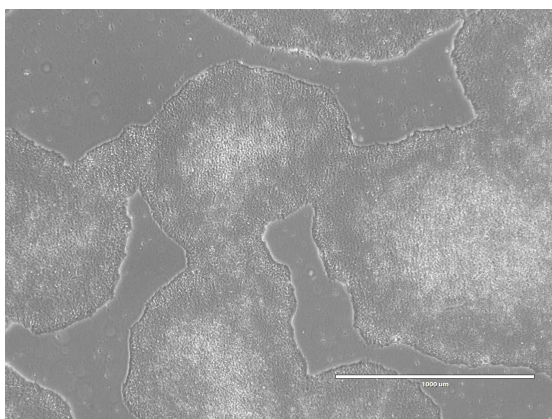
Project name: TWIN

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	HEP17-00082
Diagnosis	Control
Mutation	N/A
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 (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 <i>OCT4</i> , <i>SOX2</i> , <i>LIN28</i> , <i>NANOG</i> , <i>DNMT3B</i> 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 1 (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).

Absolute expression, normalized to Gusb

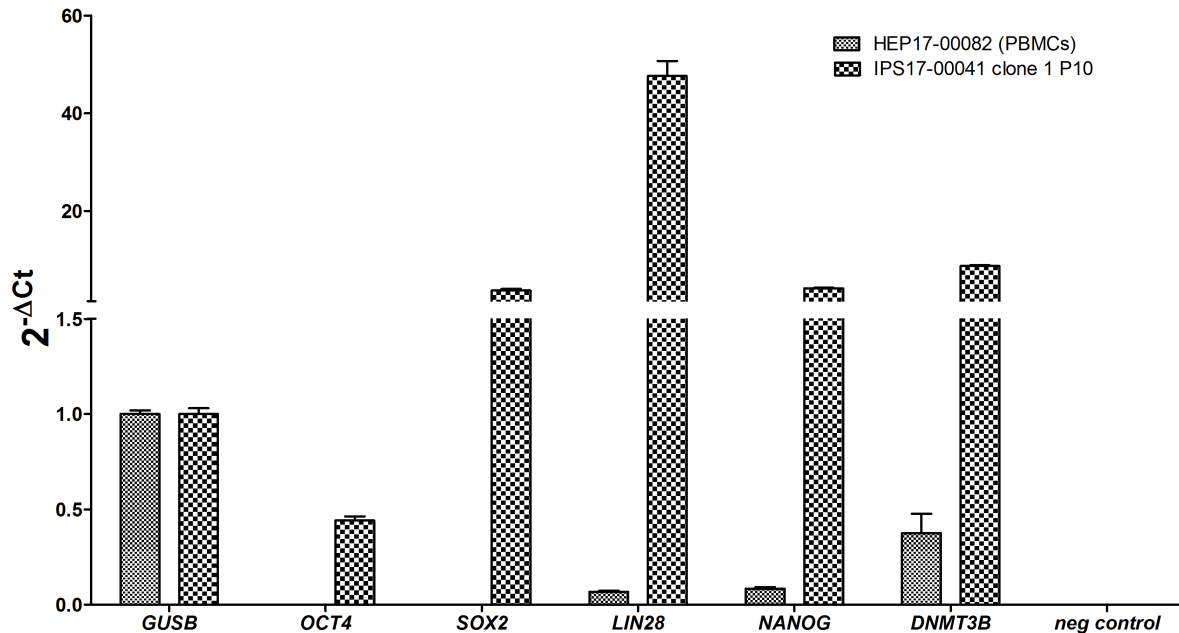


Figure 2: Gene expression of the iPSC clone compared with the parental PBMCs (Δ Ct).

Expression relative to parental line

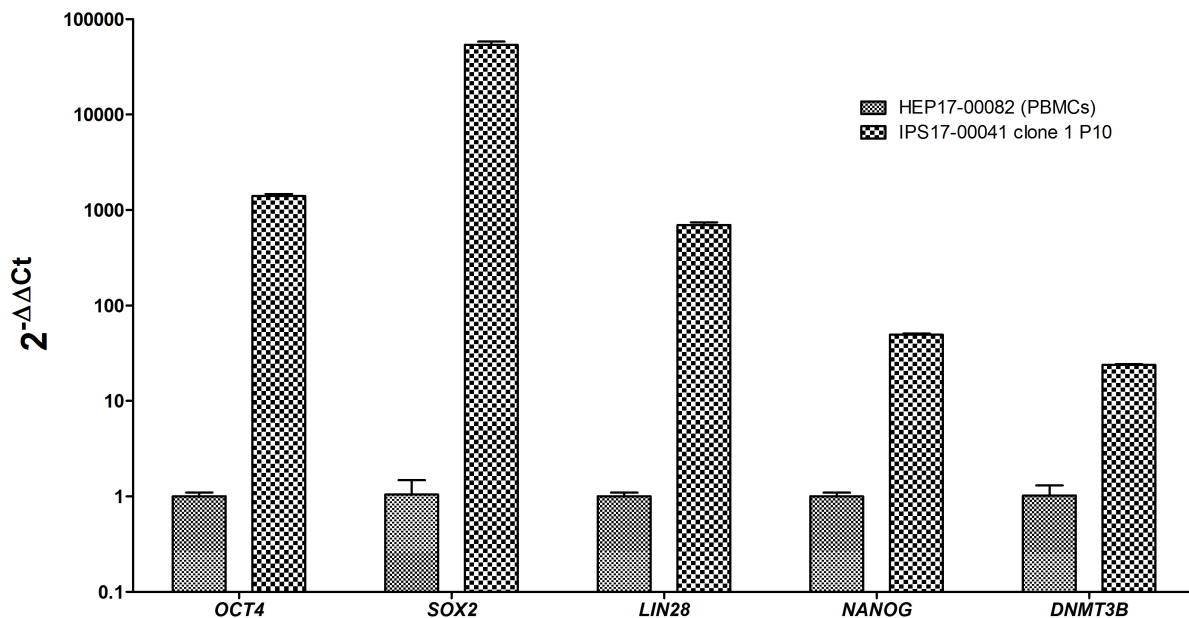


Figure 3: Pluripotency gene upregulation after reprogramming ($\Delta\Delta$ Ct). The expression fold difference of the iPSC clone is relative to the parental PBMCs.

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.

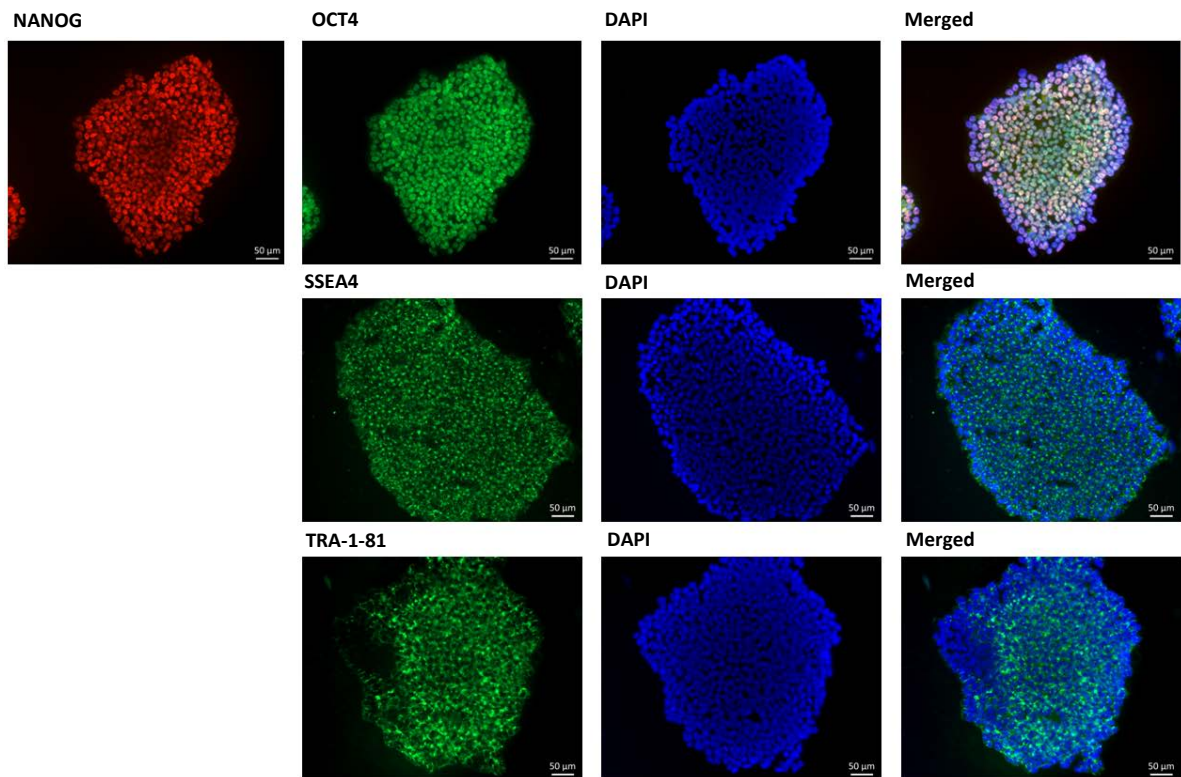
A. IPS17-00041 clone 1 P10

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

Three germ layer differentiation

The iPSC clone 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

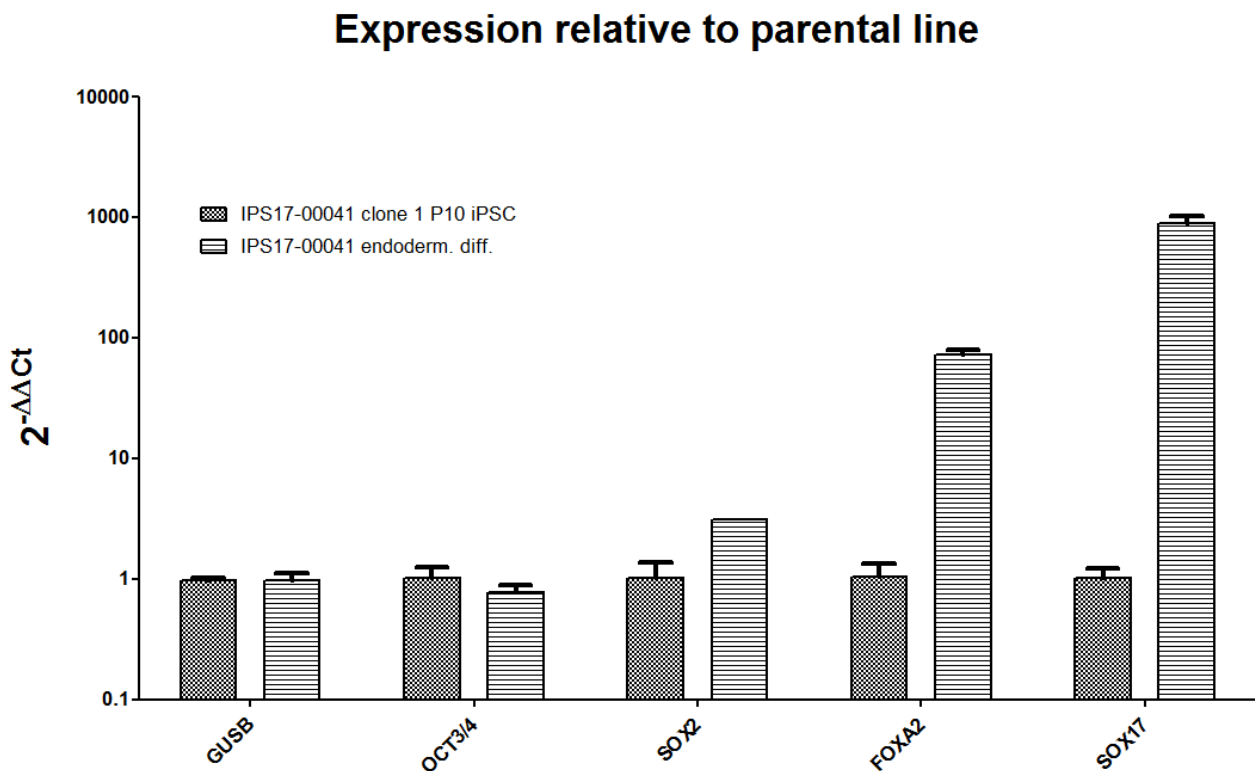


Figure 5: Expression fold difference of endoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone. SOX2 was used as a reference for pluripotency.

Mesoderm

Upregulation of mesodermal markers

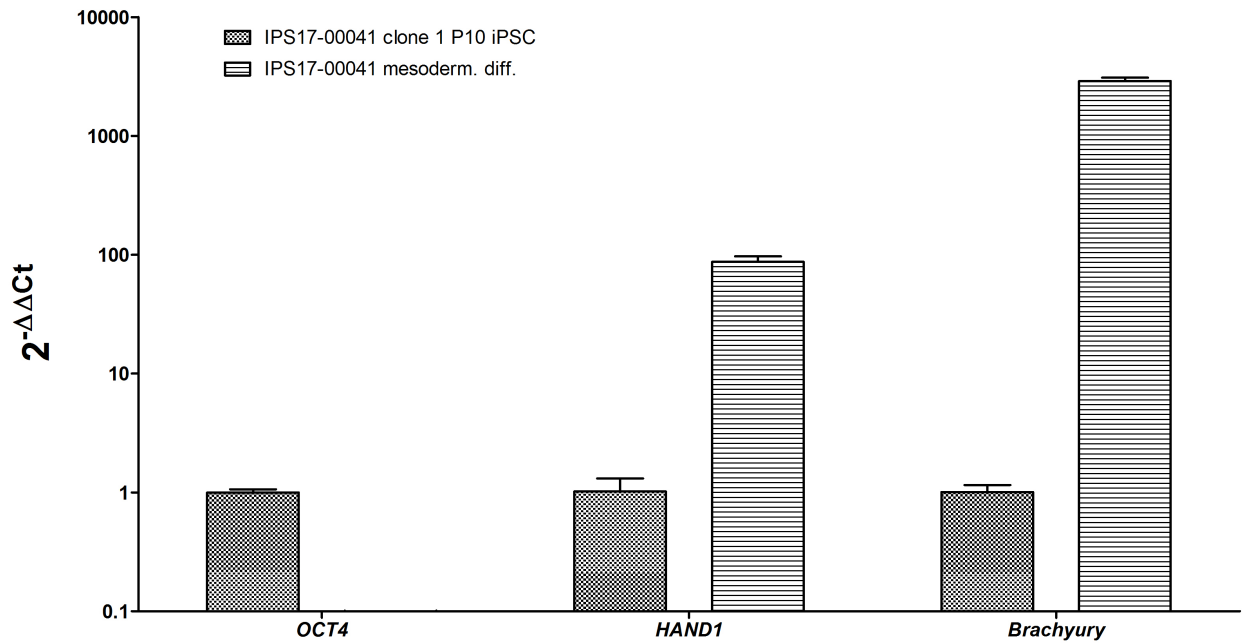


Figure 6: Expression fold difference of mesoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone. SOX2 was used as a reference for pluripotency.

Ectoderm

Upregulation of ectodermal markers

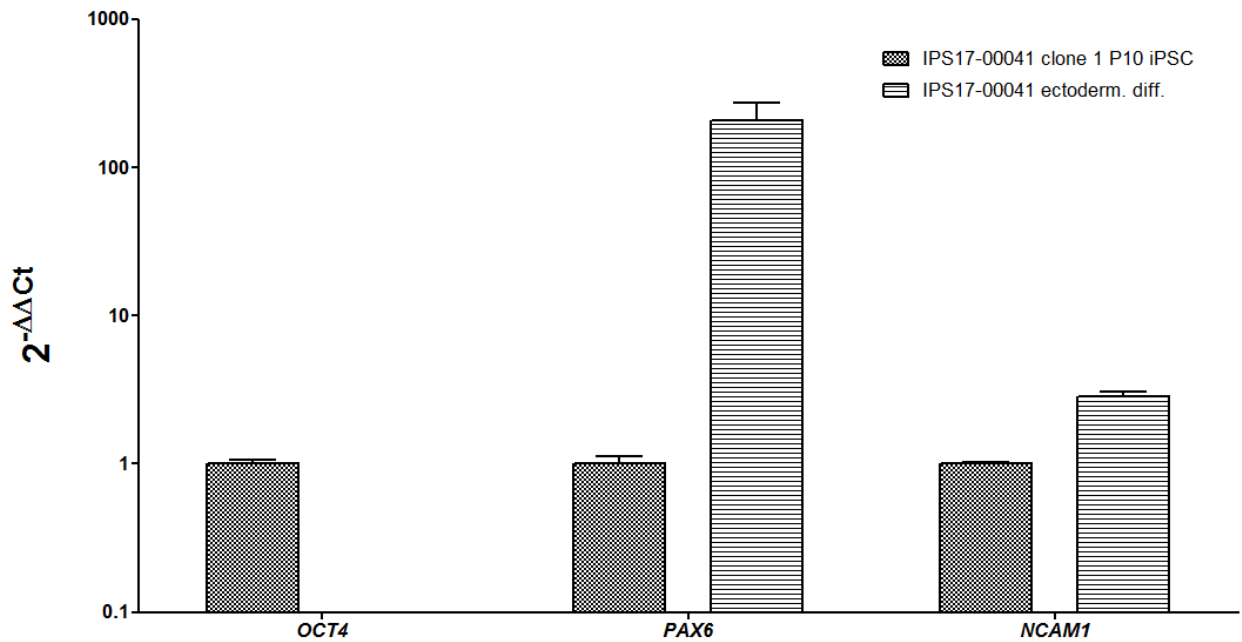


Figure 7: Expression fold difference of ectoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone. SOX2 was used as a reference for pluripotency.

Expression of germ layer specific markers

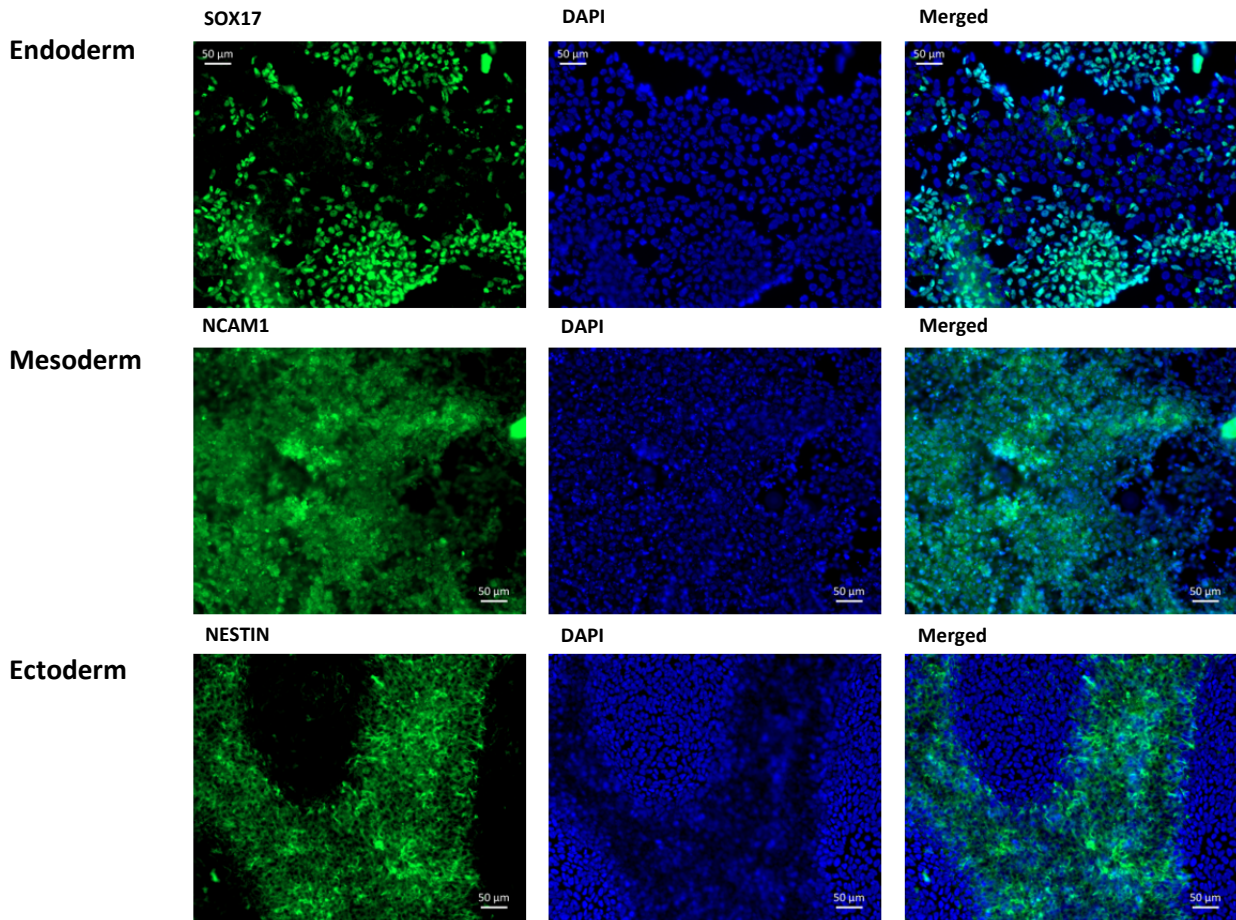


Figure 8: Immunofluorescence staining of differentiated cells showing positive signal of germ layer-specific markers.

CNV analysis

The DNA was isolated from the iPSC clone (IPS17-0041) and the donor's blood (HEP17-00082) 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

IPS17-00041	HEP17-00082
chr2: 34,701,643 - 34,737,164	chr11: 75,972,511 - 76,005,007
chr2: 117,324,030 - 117,340,323	chr17: 44,195,807 - 44,784,640
chr4: 35,145,836 - 35,156,807	chr18: 63,902,250 - 63,918,694
chr6: 7,717,073 - 7,742,347	chr20: 30,040,497 - 30,052,658
chr6: 162,933,636 - 162,946,824	chrX: 25,720,331 - 25,747,428
chr7: 27,223,610 - 27,235,942	chrY: 2,650,141 - 28,799,938
chr7: 57,300,956 - 57,347,352	
chr7: 67,647,248 - 67,657,295	
chr7: 122,154,166 - 122,226,267	
chr8: 20,807,694 - 20,819,543	
chr8: 36,882,518 - 36,898,253	
chr9: 2,011,339 - 2,018,826	
chr10: 34,654,092 - 34,670,663	
chr10: 120,619,740 - 120,630,746	
chr14: 22,692,124 - 22,944,508	
chr17: 44,212,824 - 44,784,640	
chrX: 1,024,440 - 1,039,250	
chrX: 1,042,598 - 1,101,194	
chrX: 25,720,331 - 25,747,428	
chrX: 46,415,642 - 46,427,421	
chrX: 48,329,405 - 48,338,177	
chrX: 49,173,938 - 49,301,920	
chr8: 134,229,535 - 139,691,617	
chrX: 132,271,839 - 133,559,879	
chrY: 2,650,141 - 28,799,938	

Conclusion:

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

Pass

Fail

Other:

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Manager, Radboud Stem Cell Technology Center

Date