

## Certificate of Analysis 2020

Invoice number: SCTC2019-00041

Name investigator: Anneke den Hollander

Cell line number: IPS19-00027 clone 1

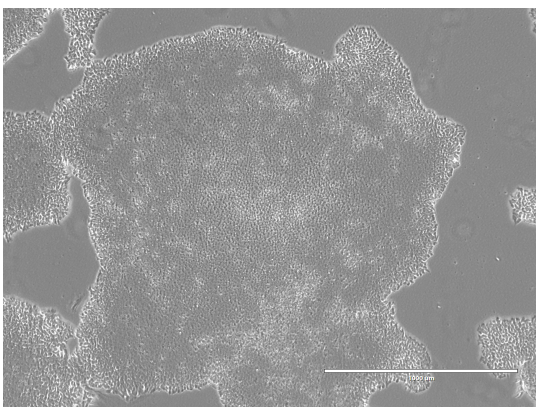
Project name: VICI

**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                          | HEP19-00066  |
| Parental cell type                          | PBMCs  |
| Diagnosis                                   | AMD  |
| Mutation                                    | N/A*   |
| Number of clones                            | 1  |
| Passage (P) of iPSCs reported at submission | P10  |
| Culture medium                              | Essential 8 Flex medium  |
| Culture coating                             | Matrigel   |
| 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>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.**

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

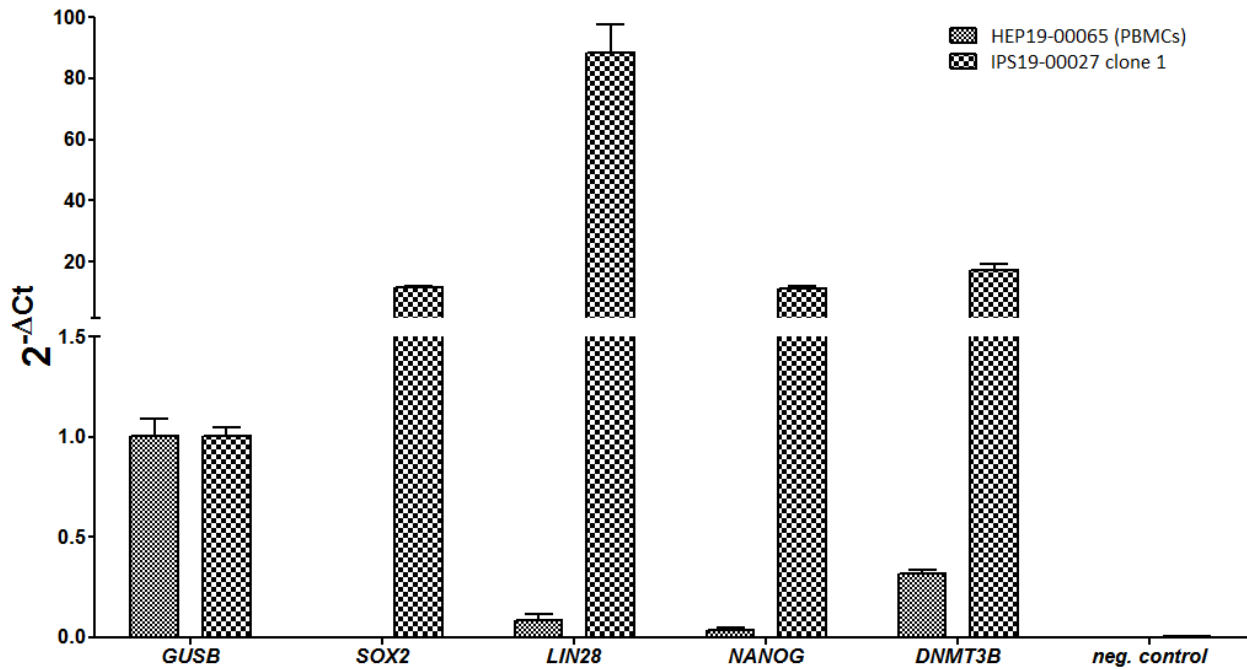


Figure 2: Gene expression of the iPSC clone compared with the parental PBMCs ( $\Delta$ Ct).

Expression relative to parental line

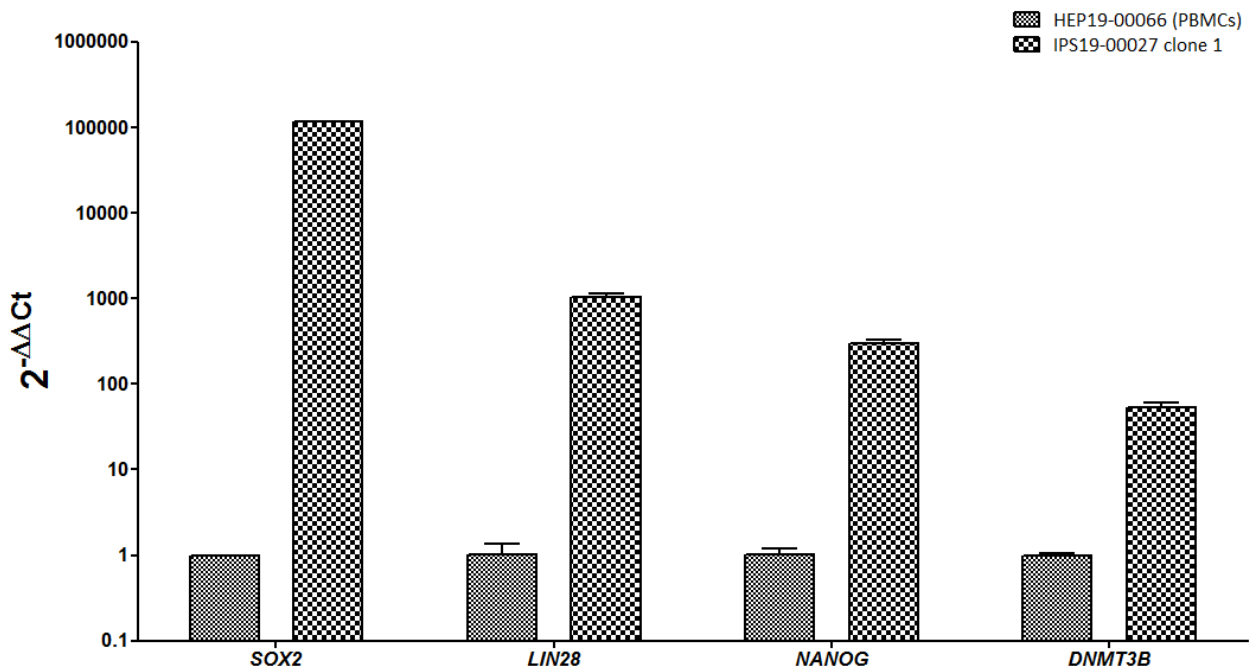


Figure 3: Pluripotency gene upregulation after reprogramming ( $\Delta\Delta$ Ct). The expression fold difference of the iPSC clone is relative to the parental PBMCs.

### 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. *IPS19-00027 clone 1 P10*

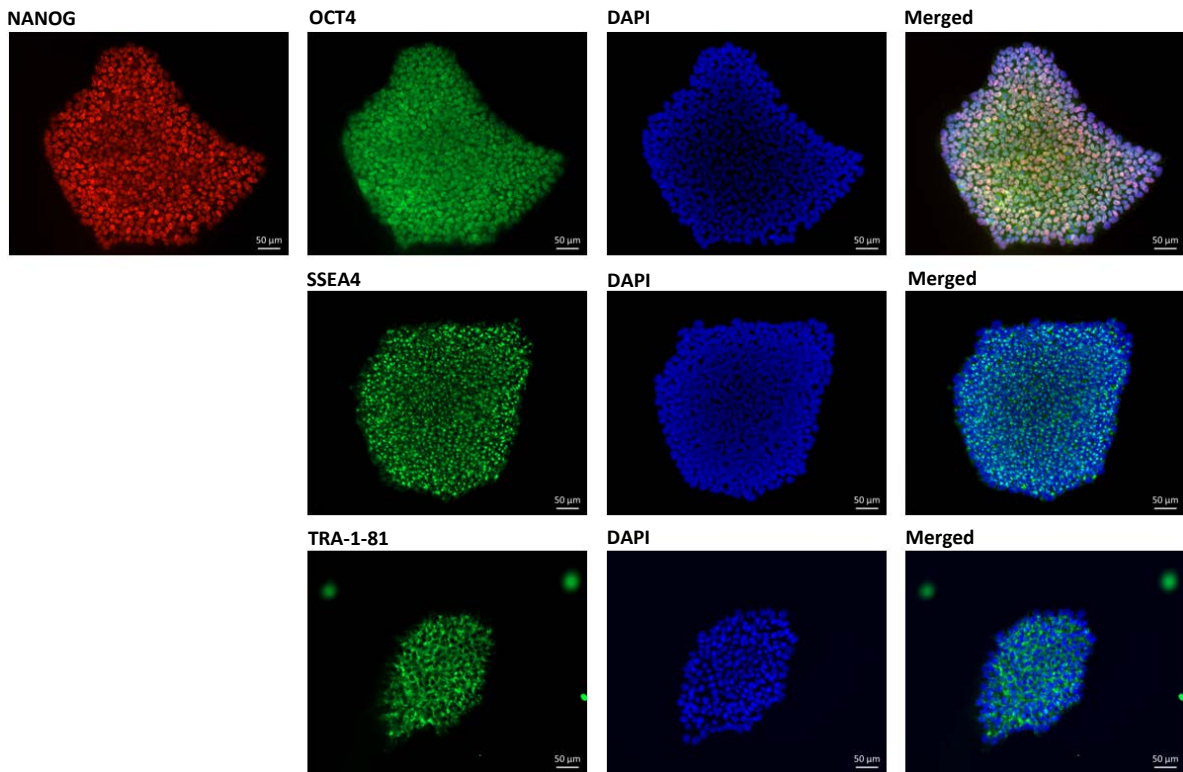


Figure 4: Immunofluorescence staining of the iPSC clone with pluripotency markers.

### Three germ layer differentiation

IPS19-00027 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**

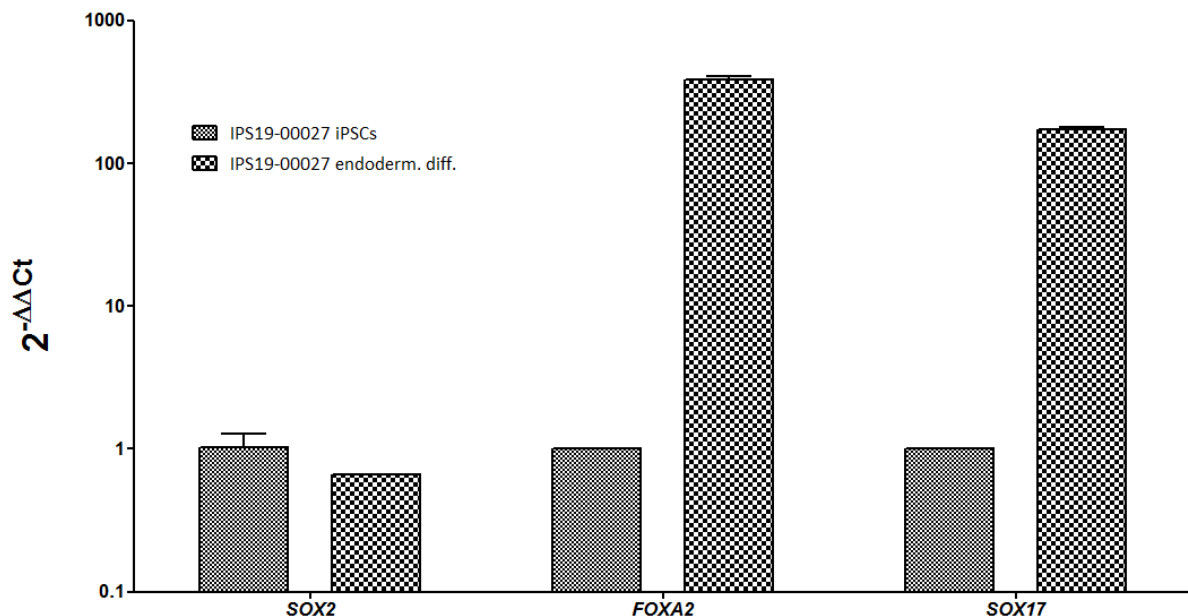
| Lineage  | Marker           |
|----------|------------------|
| Endoderm | FOXA2, SOX17     |
| Mesoderm | Brachyury, HAND1 |
| Ectoderm | PAX6, NCAM1      |

**Table 4: ICC markers for three lineage differentiation**

| Lineage  | Marker |
|----------|--------|
| Endoderm | SOX17  |
| Mesoderm | NCAM1  |
| Ectoderm | NESTIN |

## Endoderm

### Upregulation of endodermal markers



**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

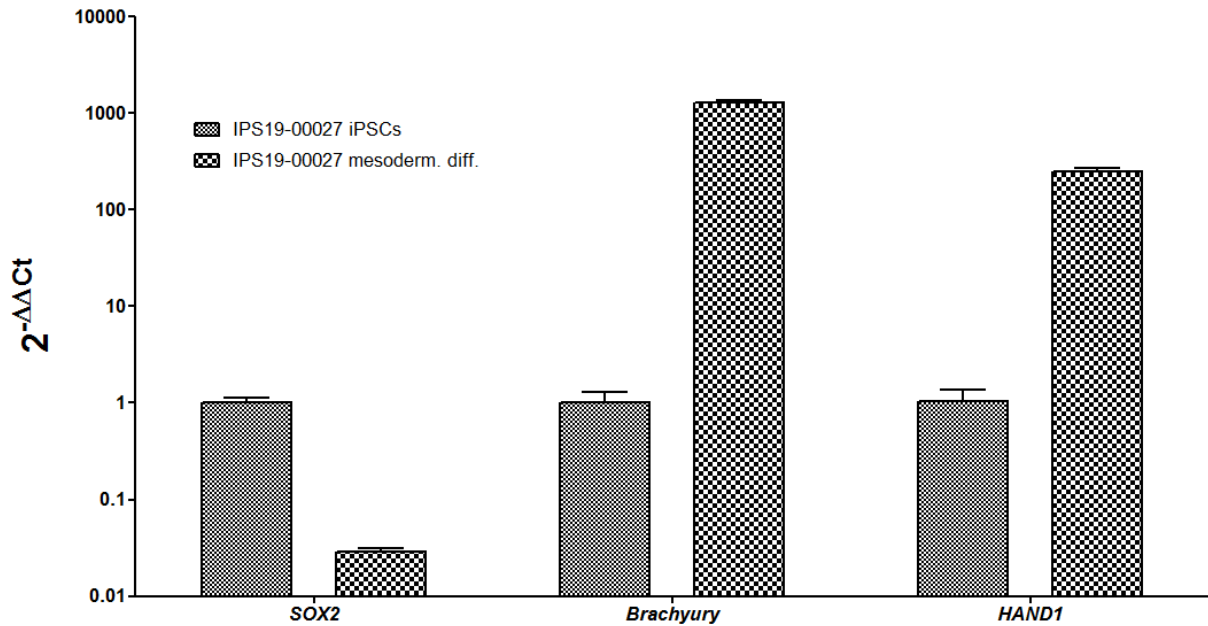


Figure 6: Expression fold difference of mesoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone. *SOX2* was used as a reference for pluripotency.

## Ectoderm

### Upregulation of ectodermal markers

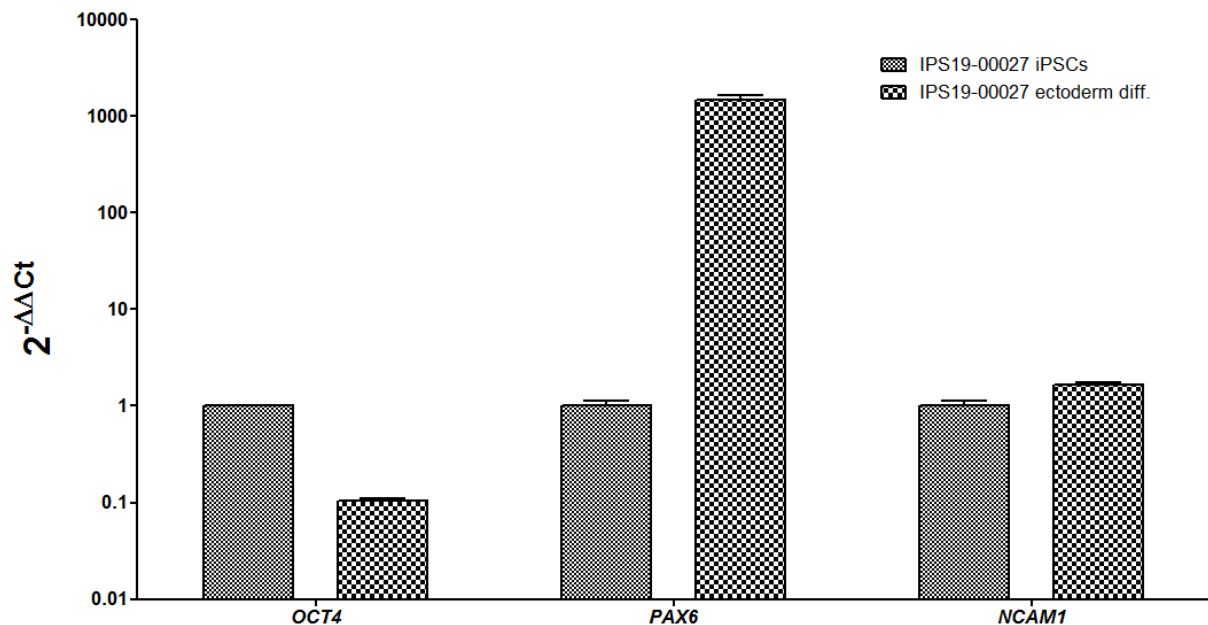


Figure 7: Expression fold difference of ectoderm-specific genes in differentiated cells, compared with the undifferentiated iPSC clone. *OCT4* was used as a reference for pluripotency.

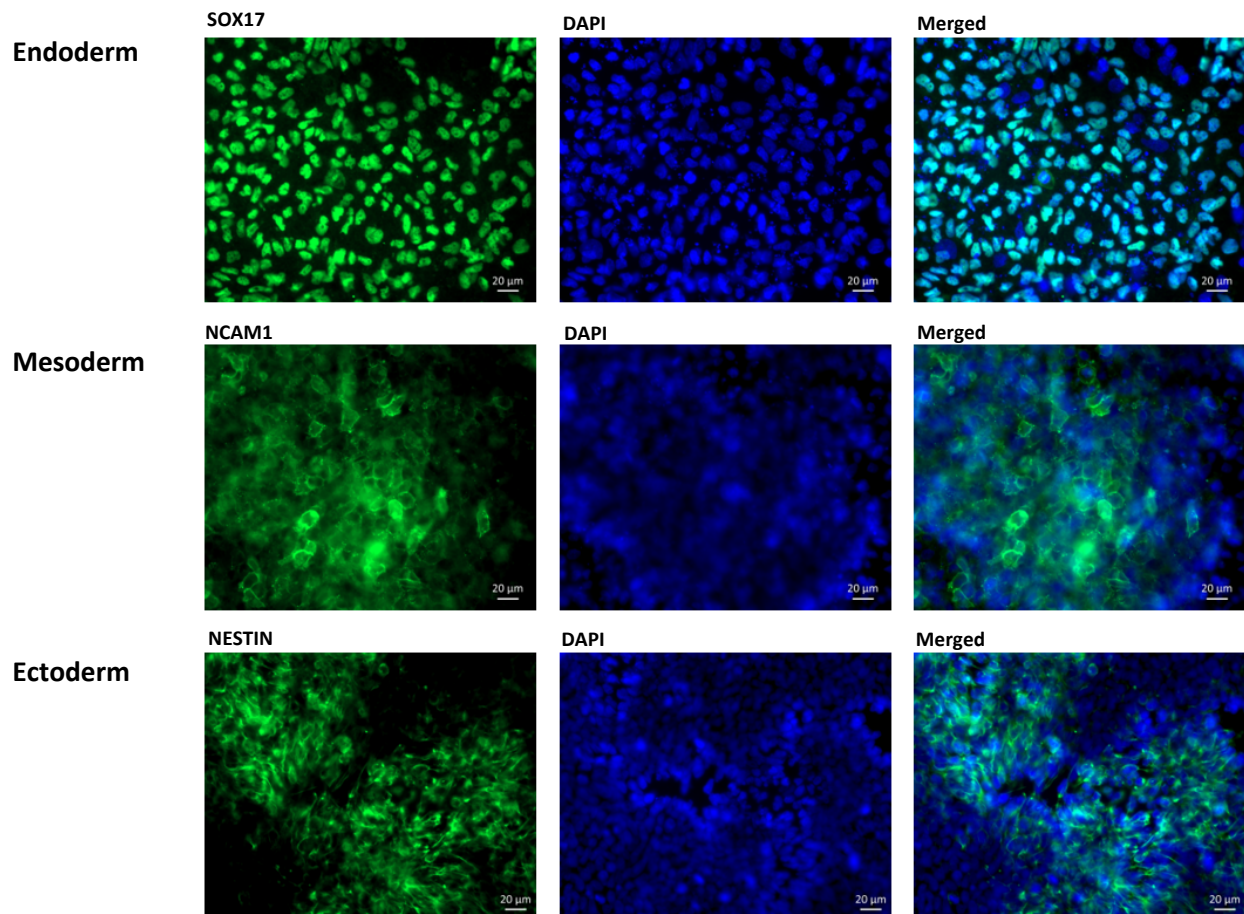


Figure 8: Immunofluorescence staining of differentiated cells showing positive signal of germlayer-specific markers.



### CNV analysis

The DNA was isolated from the iPSC clone (IPS19-00027) and the donor's blood (HEP19-00066) 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**

| IPS19-00027                      | HEP19-00066                      |
|----------------------------------|----------------------------------|
| chr2: 34,701,643 - 34,737,164    | chr3: 53,893,709 - 53,917,625    |
| chr2: 122,468,922 - 122,479,675  | chr4: 79,314,557 - 79,326,032    |
| chr4: 79,314,557 - 79,326,032    | chr4: 88,899,305 - 88,992,581    |
| chr4: 88,899,305 - 88,989,106    | chr6: 169,509,080 - 169,539,122  |
| chr4: 93,020,720 - 93,413,065    | chr7: 33,130,674 - 33,189,479    |
| chr6: 7,717,073 - 7,742,347      | chr8: 51,022,541 - 51,066,443    |
| chr6: 169,509,080 - 169,539,122  | chr9: 740,711 - 747,279          |
| chr7: 27,223,610 - 27,235,942    | chr9: 7,504,540 - 7,519,065      |
| chr7: 33,130,674 - 33,189,479    | chr11: 103,191,837 - 103,204,922 |
| chr7: 57,291,122 - 57,347,352    | chr14: 23,988,477 - 24,009,828   |
| chr7: 82,931,652 - 83,024,797    | chr15: 54,059,344 - 54,083,137   |
| chr8: 51,022,541 - 51,066,443    | chrY: 15,221,775 - 15,238,207    |
| chr8: 123,448,950 - 123,462,067  | chrX: 168,547 - 155,233,732      |
| chr9: 740,711 - 747,279          | chrY: 2,650,141 - 28,799,938     |
| chr9: 2,011,339 - 2,018,826      |                                  |
| chr11: 103,191,837 - 103,204,922 |                                  |
| chr12: 93,762,028 - 93,772,632   |                                  |
| chr14: 22,746,792 - 22,944,508   |                                  |
| chr14: 88,402,944 - 88,414,876   |                                  |
| chr15: 54,059,344 - 54,083,137   |                                  |
| chr20: 58,907,693 - 58,918,595   |                                  |
| chr22: 51,183,855 - 51,197,839   |                                  |
| chr8: 134,196,909 - 140,534,064  |                                  |
| chrX: 2,777,846 - 155,233,732    |                                  |
| 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:

---

**Silvia Albert, PhD**

Manager, Radboud Stem Cell Technology Center

Date