Certificate of Analysis

CELL LINE NAME	**BIHi001-B-13**	hPSCreg Link: *https://hpscreg.eu/cell-line/BIHi001-B-13*
DONOR GENDER/AGE:	☒ Male ☐ Female ☐ unknown Age: neonate	
TYPE OF DISEASE / GENETIC MODIFICATIONS	Knock out gene B2M (MHCI, ID: 567) and CIITA (MHCII, ID: 4261)	
BANK	Master Bank, MB01, Passage 21, Freezing Date: 19.08.2023	
FREEZING METHOD	Bambanker	
CULTURE PLATFORM	Feeder Independent	
	Medium: E8	Coating: Geltrex
REPROGRAMMING	self-replicating RNA Vector details (e.g. Kit, Pub, AddgeneNr): N/A	

TEST DESCRIPTION	Test Method	Test Specification	Result
STERILITY (viral pathogens)	☐ donor tested ☒ primary cells tested ☐ iPS clone tested	HBV, HCV, HIV negative	Pass
REPROGRAMMING VECTOR CLEARENCE	☒ parental cells tested ☐ antibody staining ☐ PCR	Vector not present	Pass
KARYOTYPE	CNV using SNP arrays	Result matches QC criteria	Pass
	G-Banding	Result matches expected karyotype	Pass
IDENTITY	STR Analysis	Identical to cells of origin	Pass
VIABILITY	Images of cells immediately post-thaw, at 48 hrs and at confluence	Growth to confluency typical of hPSCs	Pass
MORPHOLOGY	Light microscopy of cells	Typical morphology of undifferentiated hPSCs	Pass
STERILITY (mycoplasma)	Minerva Venor®GeM qOneStep	No contamination detected	Pass
STERILITY (bacteria/ yeast/ fungi)	Culture for 7 days in antibiotic free medium	No contamination detected	Pass
UNDIFFERENTIATED PHENOTYPE	Markers for undifferentiated hPSCs ☒ IF-Staining ☒FACS	Expression of at least three pluripotency markers detected	Pass
PLURIPOTENT DIFFERENTIATION POTENTIAL	directed differentiation	Successful differentiation to cells of all three germ layers	Pass
CONFIRMATION OF DISEASE GENOTYPE / EDITING	Sequencing of mutated site	Sequencing shows mutation	Pass

Date 28.12.2023

Stem Cell Core Facility

Single Nucleotide Polymorphism (SNP)- Karyotype

	Reference			Engineered cell line		
Sample (cell type, ID)	iPSC	BIHi001-B		iPSC	BIHi001-B-13	
Passage No.	5			21		
Bank ID	WB02			MB01		
DNA sample ID	D0422			D0685		
Chip-ID and Position	205955840044, R06C01			207762960094, R08C01		
Date of testing	03.03.2023			10.10.2023		
Gender (provided/estimated from chip data)	Male	Male	√	Male	Male	√

Technology: Illumina BeadArray
Product: Illumina Infinium Global Screening Array-24 BeadChip
Manifest: GSAMD-24v3-0-EA_20034606_A1
Clusterfile: GSA-24v3-0_A1_ClusterFile

CNV Analysis
Algorithm: CNV-Partition
Version: 3.2.0

Parameters are set to detect copy number variations (CNVs) ≥ 45 kb and loss of heterozygosity (LOH) regions > 1 Mb with a confidence value > 35. Balanced translocations and inversions cannot be detected with this method. Aberrant copy number regions are identified by log R ratio and B allele frequency. Copy number changes (gains and losses) greater than **0.4 Mb** and regions of LOH above **5 Mb** are considered reportable and taken into account for interpretation. Genomic positions are based on genome build GRCh37/hg19.

If in the tested cell line (compared to the reference) new CNVs greater than **2 Mb** and/or LOH greater than **5 Mb** are detected the CNV QC test has "failed" regarding the internal QC criteria of CUSCO. We recommend not to use a "failed" cell line for further research or only after careful consideration.

Single Nucleotide Polymorphism (SNP)- Karyotype

	BIHi001-B-13_MB01	Reference (BIHi001-B_WB02)
call_rate	0.991	0.997
computed_gender	M	M
SNPs_post_filter	73.13 %	74.61 %
SNP.distance.to.ref	0	-
loss.gain_log2ratio	-1.64	0.77
total_calls_CNV	37	27
total_calls_LOH	25	13
reportable_new_calls_CNV	1	0
reportable_new_calls_LOH	0	0
critical_new_calls_CNV	0	0
critical_new_calls_LOH	0	0

Interpretation:

The CNV analysis result suggests that the iPSC line contains neither CNVs > 2 Mb nor regions of LOH > 5 Mb.

More information can be found in the attached html report.

Further information about genes in the detected regions and linked known diseases may be provided by the UCSC Genome Browser (https://genome.ucsc.edu) and Decipher (https://decipher.sanger.ac.uk/search).

References:

1. LaFramboise, T. (1 July 2009). "Single nucleotide polymorphism arrays: a decade of biological, computational and technological advances". Nucleic Acids Research. 37 (13): 4181–4193.

2. Arsham, M. S., Barch, M. J., & Lawce, H. J. (Eds.) (2017). The AGT Cytogenetics Laboratory Manual (4th Ed.). Hoboken, NJ: John Wiley & Sons, Inc.

3. Haraksingh RR, Abyzov A, Urban AE. Comprehensive performance comparison of high-resolution array platforms for genome-wide Copy Number Variation (CNV) analysis in humans. BMC Genomics. 2017 Apr 24;18(1):321. doi: 10.1186/s12864-017-3658-x.

4. Wicell: https://www.wicell.org/home/characterization/cytogenetics/snp-microarray/single-nucleotide-polymorphism-snp-mircroarray-.cmsx

Stem Cell Core Facility

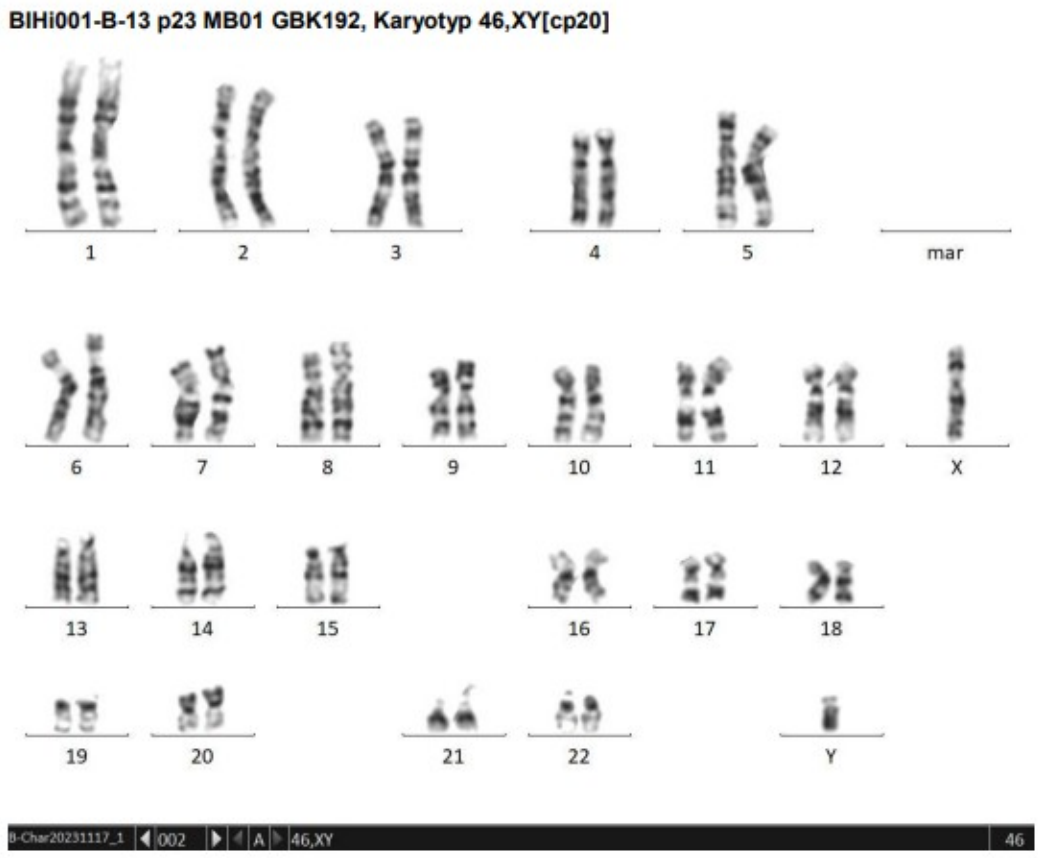
G-Banding - Karyotype

Cell line name	BIHi001-B-13
Bank ID	MB01
Passage No.	23
Date of testing	10.11.2023
Protocol	7.7 G-banded karyotyping

The sample preparation was carried out at BIH Stem Cell Core Facility and sent for G-banded-karyotyping to the “Institut für Humangenetik, Universitätsklinikum Jena”.

General comments: Karyotyping is performed using GTG stained metaphase chromosomes. With an average resolution of at least 200 bands per haploid chromosome set. Sub-microscopic changes (microdeletions/duplications) and changes <10Mb cannot be excluded by this method. Mosaics in the form of clonal changes are reported when the same change or chromosome gain occurs more than twice, and chromosome losses occur more than 3 times. A composite karyotype (cp) from 20 metaphase plates in the currently valid ISCN nomenclature is reported and a representative karyogram is provided

Results



Conclusion:

A *normal male karyotype 46XY* was detected for the examined sample.

Date: 08.12.2023

BIH Berlin Institute of Health @Charité	Core Unit pluripotent Stem Cells and Organoids (CUSCO) **Cell Line Identity (STR Analysis)**

Cell line name	BIHi001-B-13
Bank ID	MB01
Passage No.	21
Date of testing	23.10.2023
Protocol	8.05. STR DNA Profiling Analysis

The GenePrint® 10 System (Promega Corporation) allows co-amplification and three-color detection of nine human loci, including the ASN-0002 loci (TH01, TPOX, vWA, Amelogenin, CSF1PO, D16S539, D7S820, D13S317 and D5S818) as well as D21S11. These loci collectively provide a genetic profile with a random match probability of 1 in 2.92 × 109.

Results

	TH01		**D21S11**		**D5S818**		**D13S317**		**D7S820**		**D16S539**		**CSF1PO**		**AMEL**		**vWA**		**TPOX**	
BIHi001-B-13	7	9.3	29	29	11	12	12	12	8	10	11	12	10	10	X	Y	16	18	11	11
BIHi001-B MB01	7	9.3	29	29	11	12	12	12	8	10	11	12	10	10	X	Y	16	18	11	11

The Alleles of the cell line BIHi001-B-13 and cell line BIHi001-B MB01 at the 10 STR Loci are identically.

Conclusion

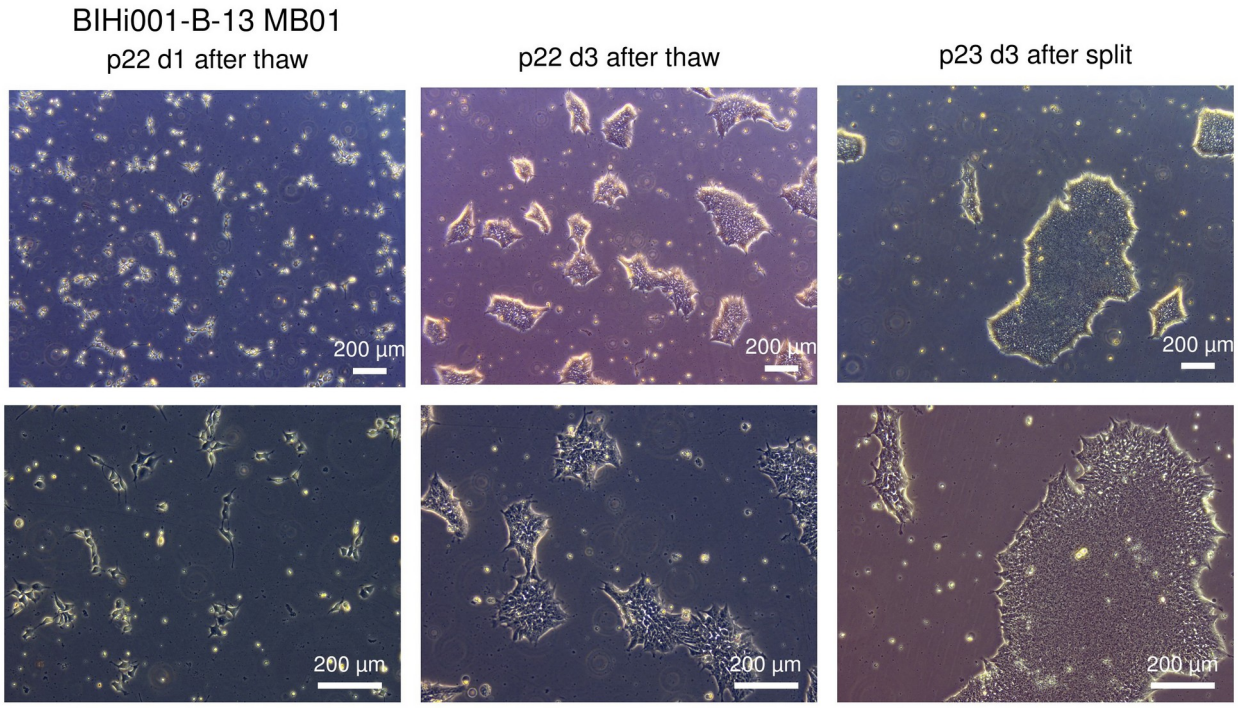
Both samples tested are from the same donor.

BIH Berlin Institute of Health @Charité	Core Unit Pluripotent Stem Cell and Organoids (CUSCO) **Morphology and Viability**

Cell line name	BIHi001-B-13
Bank ID	MB01
Passage No.	22
Date of testing	19.09.2023
Coating / Medium	Geltrex / E8

One vial of the cell bank was thawed and monitored during antibiotics-free cultivation. ROCK Inhibitor was used during the first 24 hours only. Cultures were evaluated regarding their morphology and viability.

Images:



Conclusion:

Cells show a good post-bank recovery after thawing and form colonies exhibiting typical morphology of undifferentiated hPSCs.

Date: 24.10.2023

BIH Berlin Institute of Health @Charité	Stem Cell Core Facility **Sterility (Mycoplasma, Bacteria/Yeast/Fungi)**

Cell line name	BIHi001-B-13
Bank ID	MB01
Passage No.	23
Test date	28.09.2023
Protocol	8.1.3 Mycoplasma testing_qPCR Minerva
Samples	1: Negative Control (culture medium of Cell Line tested) 2: Positive Control (Mycoplasma DNA from *Venor® GeM qOneStep Kit*) 3: Cell culture supernatant from cell line

Bacteria/Yeast/Fungi

Test

Cells were cultured without the addition of antibiotics over a period of 7 days. Cultures were checked daily for growth of bacteria, yeast and fungi by microscopy.

Results

No turbidity of the cell culture medium or microbial colonies were detected.

Mycoplasma

Test

Cells were cultured without the addition of antibiotics to a confluency of 80-90%. Mycoplasma contamination was tested by the qPCR-based *Venor®GeM qOneStep Kit*. Mycoplasma are detected at 520 nm by amplifying the 16S rRNA coding region in the mycoplasma genome. False-negative results caused by PCR inhibition are identified by the internal amplification control, detected at 560 nm.

Mycoplasma 520 nm	Internal amplification control 560 nm	Interpretation
Ct<40	Irrelevant	Sample is Mycoplasma contaminated
Ct≥40	Ct≥40	qPCR inhibition
Ct≥40	Ct<40	Sample is Mycoplasma free

Results

Sample	Ct of Mycoplasma DNA	Ct of Internal amplification DNA	Result
1 (neg. control)	>45	28,665	*Passed*
2 (pos. control)	26,354	28,696	*Passed*
3	>45	28,460	***Negative***

Conclusion

The cell line was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Date: 10.10.2023

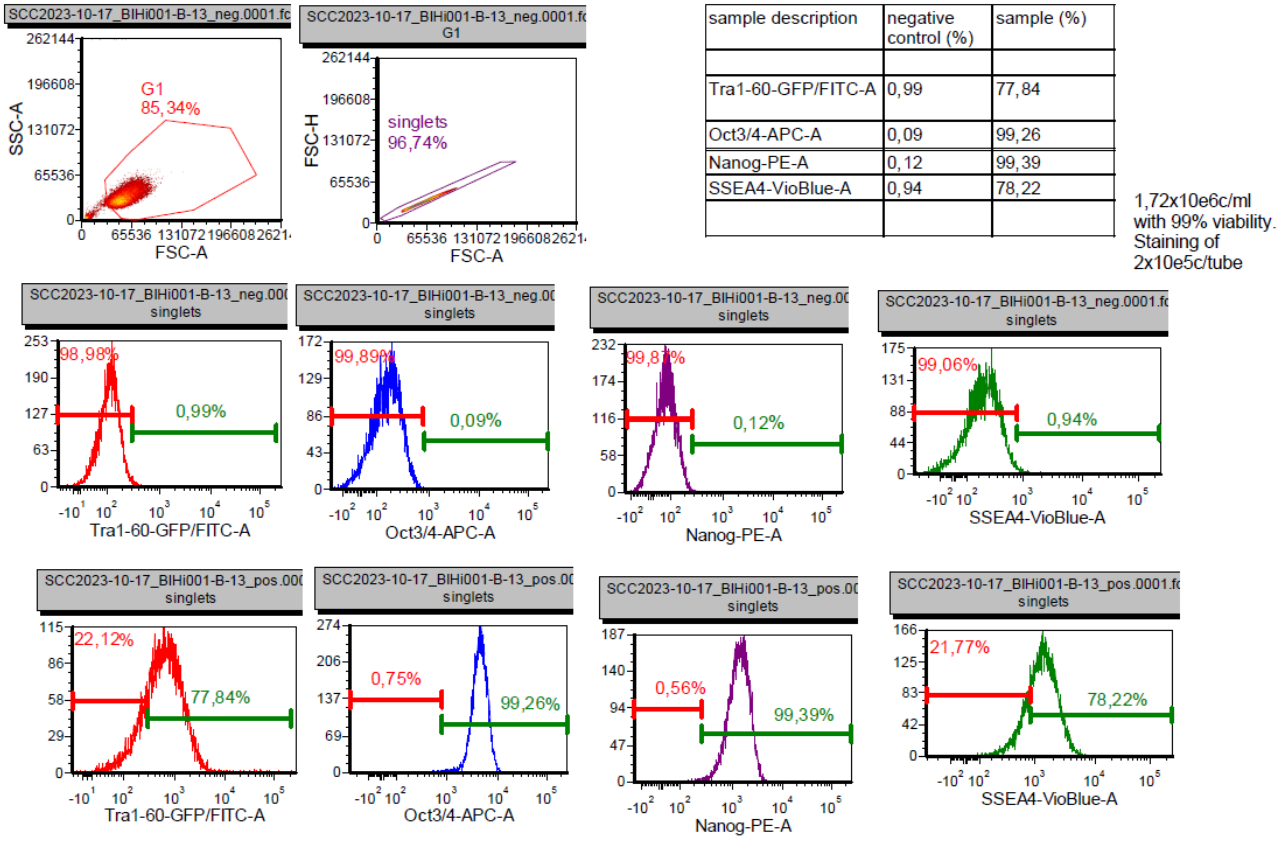
Stem Cell Core Unit

FACS analysis of markers in undifferentiated hPSCs

Cell line name	BIHi001-B-13
Bank ID	MB01
Passage No.	23
Date of testing	17.10.2023
Protocol	7.14 FACS analysis of pluripotency markers

Results

20231017_FACS analysis of markers of undifferentiated BIHi001-B-13 MB01 p23 in E8 medium_one sample stained with all antibodies

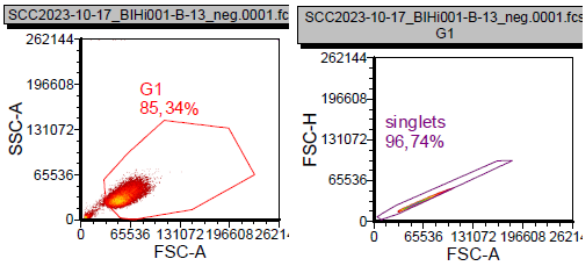


sample description	negative control (%)	sample (%)
Tra1-60-GFP/FITC-A	0,99	77,84
Oct3/4-APC-A	0,09	99,26
Nanog-PE-A	0,12	99,39
SSEA4-VioBlue-A	0,94	78,22

1,72x10e6c/ml with 99% viability. Staining of 2x10e5c/tube

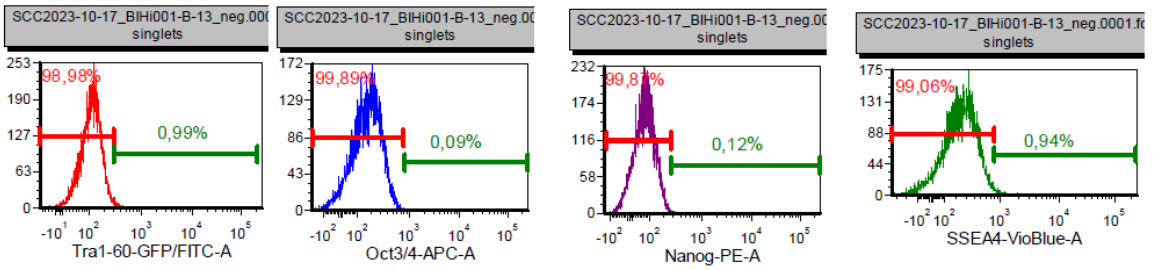
FACS analysis of markers in undifferentiated hPSCs

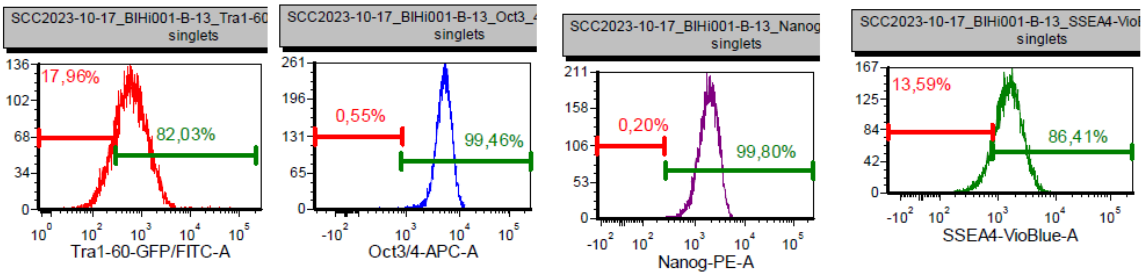
20231017_FACS analysis of markers of undifferentiated BIHi001-B-13 MB01 p23 in E8 medium_single stained samples



sample description	negative control (%)	sample (%)
Tra1-60-GFP/FITC-A	0,99	82,03
Oct3/4-APC-A	0,09	99,46
Nanog-PE-A	0,12	99,80
SSEA4-VioBlue-A	0,94	86,41

1,72x10e6c/ml with 99% viability. Staining of 2x10e5c/tube





Conclusion

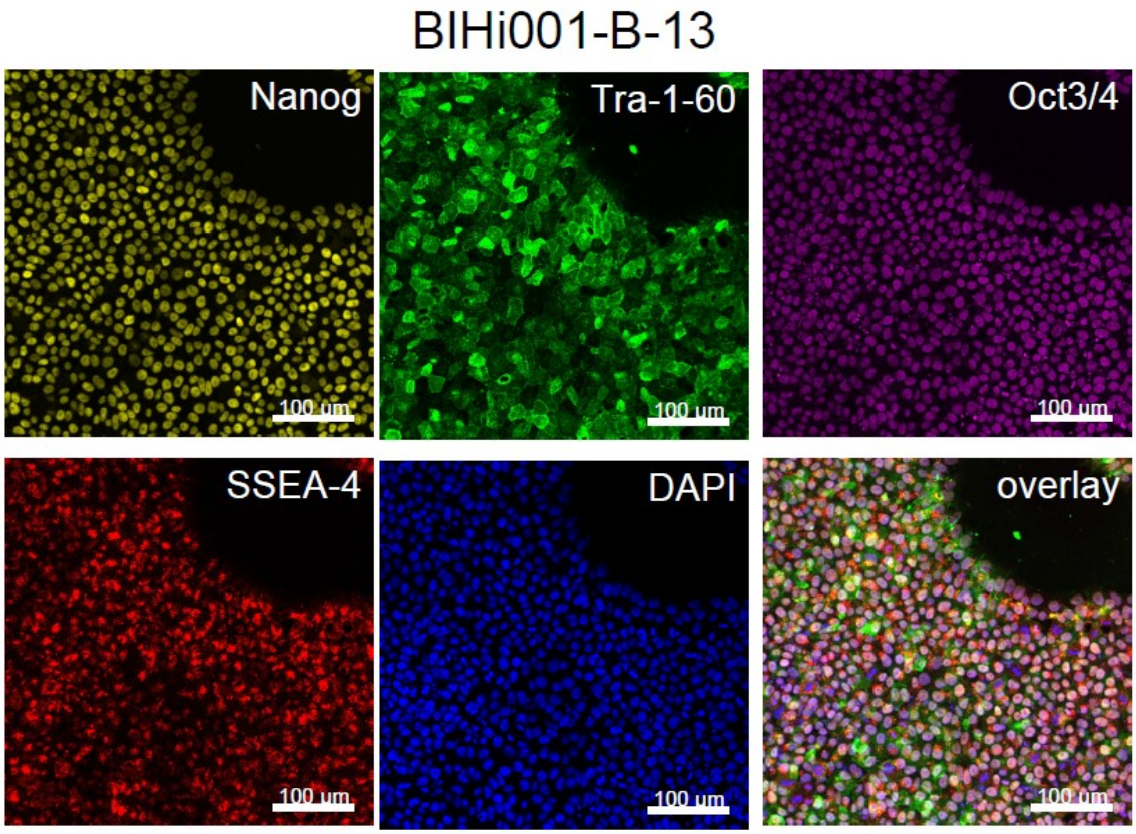
The cell line shows positive FACS results (over 80% positive) for the tested undifferentiated stem cell markers Tra1-60, OCT3/4, NANOG and SSEA-4.

Date: 17.10.2023

Immunofluorescence staining of markers for undifferentiated hPSCs

Cell line name	BIHi001-B-13
Bank ID	MB01
Passage No.	23
Date of testing	10.11.2023
Protocol	7.1 Immunofluorescence staining of markers for undifferentiated cells

Results:



Conclusion:

The cell line shows positive staining results for the tested undifferentiated stem cell markers Nanog, OCT3/4, Tra-1-60 and SSEA4.

Date: 21/11/2023

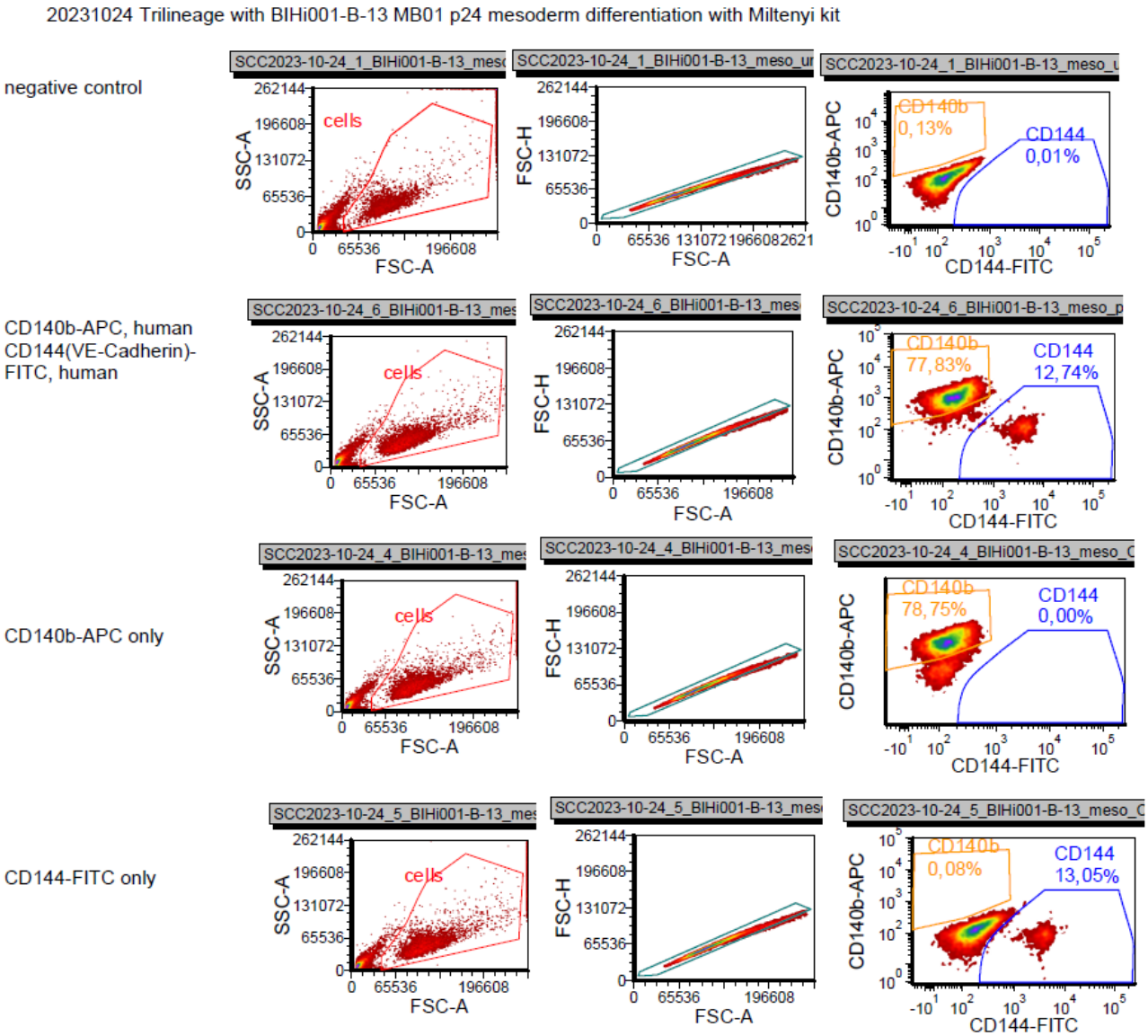
BIH Berlin Institute of Health @Charité	Stem Cell Core Facility **Validation of pluripotent differentiation potential**

Cell line name	BIHi001-B-13
Bank ID	MB01
Passage No.	24
Date of testing	24.10.2023
Protocol	7.19 Validation of pluripotent differentiation potential with Trilineage

Method

Test was performed regarding the StemMACS Trilineage Differentiation Kit, human (MACS Miltenyi Biotec, Cat-No. 130-115-660). The 7-day assay enables direct differentiation of pluripotent stem cells into ecto-, meso and endoderm. The resulting cell population was measured by FACS analysis.

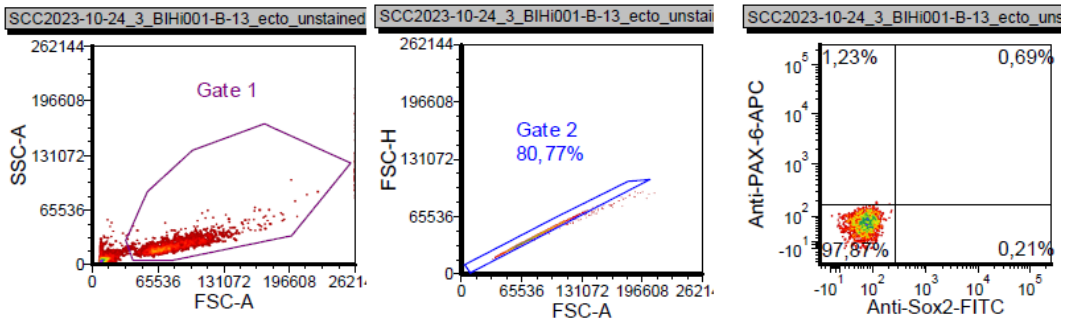
Result



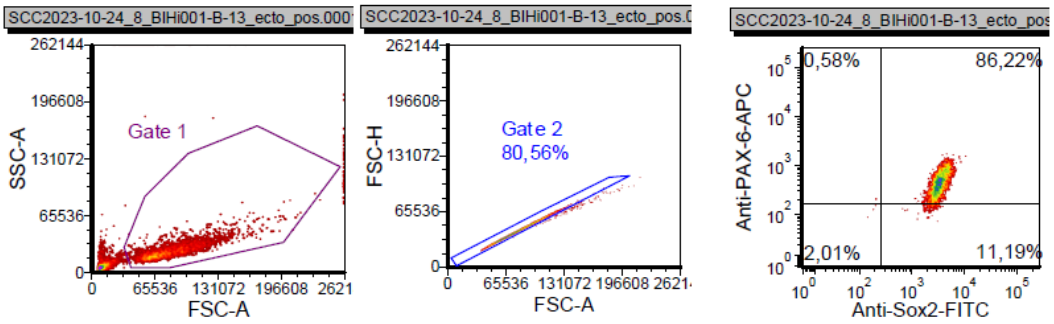
Validation of pluripotent differentiation potential

20231024 Trilineage with BIHi001-B-13 MB01 p24 ectoderm differentiation with Miltenyi kit

negative control

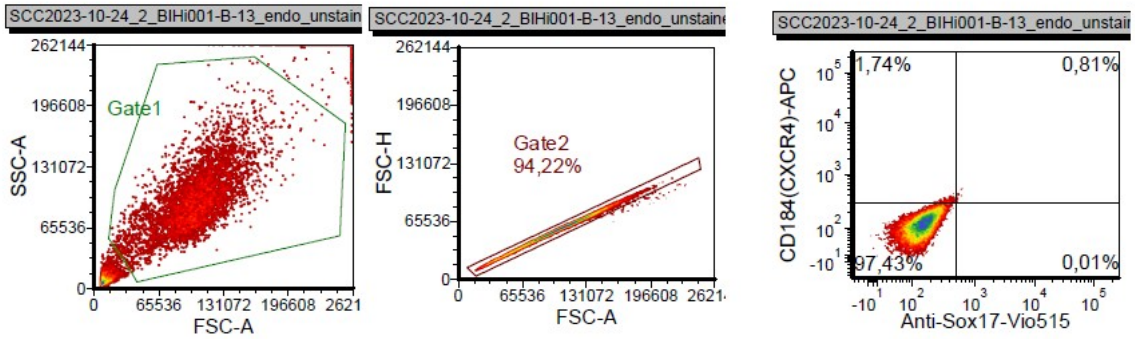


Anti-PAX-6-APC, human
Anti-Sox2-FITC, human

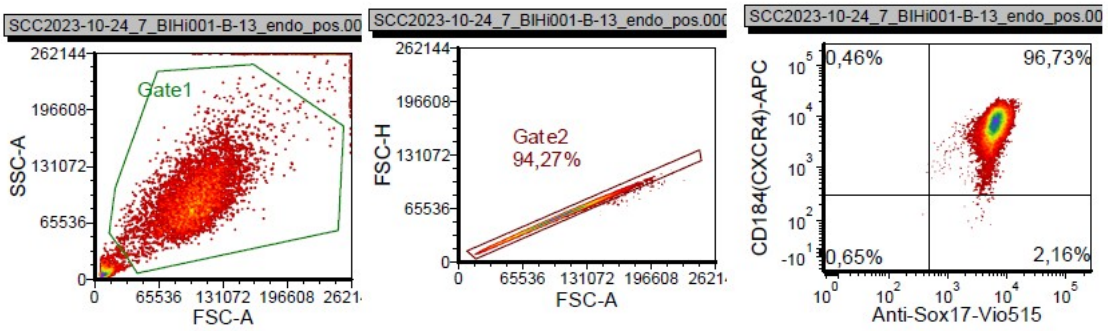


20231024 Trilineage with BIHi001-B-13 MB01 p24 endoderm differentiation with Miltenyi kit

negative control



CD184 (CXCR4)-APC Anti-Sox17-Vio515, human



Conclusion

The cell line shows potency to differentiate into mesoderm, ectoderm and endoderm lineages. The lineage markers CD140b, CD144 (Mesoderm), Sox2, Pax6 (Ectoderm) and Sox17, CD184 (Endoderm) showed positive FACS results.

Date: 24.10.2023

BIH Berlin Institute of Health @Charité	Stem Cell Core Unit **CRISPR editing sequence validation**

Cell line name	*BIHi001-B-13*
Parental cell line name:	*BIHi001-B*
Genetic modification	Knock out gene B2M (MHCI, ID: 567) and CIITA (MHCII, ID: 4261)
Bank ID	MB01
Passage No.	21
Date of testing	03/11/23

Gene edited: **B2M** – target exon 1 for gene disruption and KO generation
Unedited sequence (+20bp upstream and downstream sgRNA):
gctgacagcattcgggccgagatgtctcgctccgtggccttagctgtgctcgcgctac

Primers used for generation of sequenced fragment:
FOR= AGACAGCAAACTCACCCAGTC
REV= TGACGCTTATCGACGCCCTA
Product Lengh= 591 bp

Results:
Chromatogram of Sanger sequencing of the genomic fragment amplified from gDNA of the parental (unedited) BIHi001-B and edited line BIHi001-B-13 shows 1 bp insertion at ATG codon (AT(T)G). This insertion produces a frame-shift and consequent gene knock-out. Confirmed by online tool Mutation Taster (data not shown).

Figure 1 Sanger Sequencing results showing alignment of parental line BIHi001-B (lower sequence) and edited line BIHi001-B-13 (upper sequence). Red box indicates position of insertion.

Gene edited: **CIITA** – target exon 1 for gene disruption and KO generation
Unedited sequence (+20bp upstream and downstream sgRNA):
GACATGGAAGGTGATGAAGAGACCAGGGAGGCTTATGCCAATATCGGTGAGGAAGCACCTGAGCCCAGAAAAG

Primers used for generation of sequenced fragment:
Seq_FOR (OL0472)= GGTCTCTTCCGGTATCCCCC
Seq_REV (OL0473)= AGTGAAGGGGCCTATTTCCC
Product Lengh= 526 bp

Results:
Chromatogram of Sanger sequencing of the genomic fragment amplified from gDNA of the parental (unedited) BIHi001-B and edited line BIHi001-B-13 shows INDELS. Complete sequence disruption and consequent gene knock-out. Confirmed by online tool Mutation Taster (data not shown).

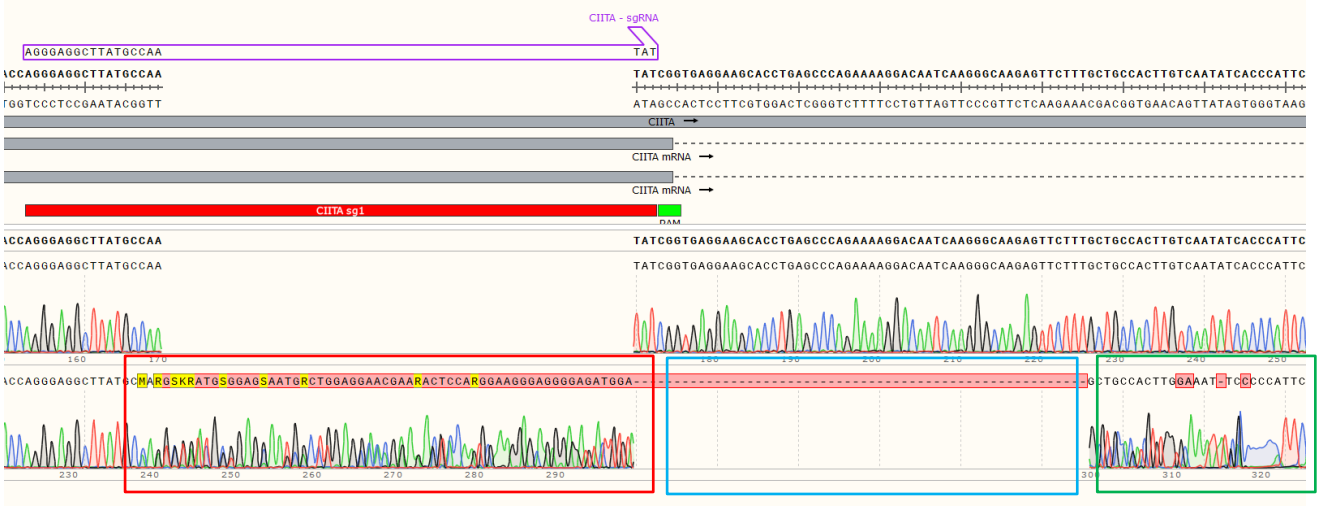


Figure 2 Sanger Sequencing results showing alignment of parental line BIHi001-B (upper sequence) and edited line BIHi001-B-13 (lower sequence). Red box indicates position of insertions. Blue box indicates position of deletions. After that (blue box) sequence becomes very noisy.

Conclusion

Sample tested contains genetic modifications for genes B2M and CIITA which derive in a double KO hiPSC line.

Date: 22.12.2023

Core Unit Pluripotent Stem Cell and Organoids (CUSCO)

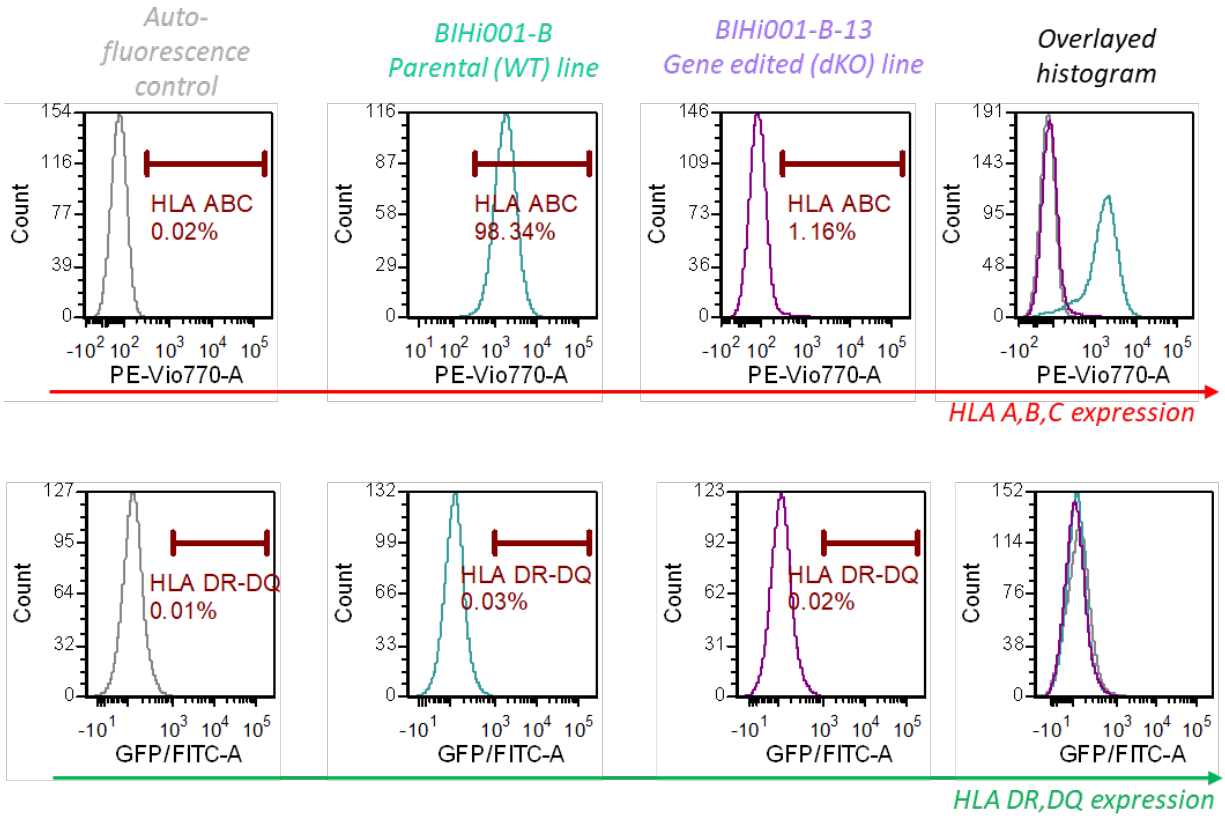
KO validation by absence of surface protein expression in genetic modified cell lines

Cell line name	BIHi001-B-13
Parental cell line name:	BIHi001-B
Genetic modification	Knock out gene B2M (MHCI, ID: 567) and CIITA (MHCII, ID: 4261)
Bank ID	MB01
Passage No.	33
Date of testing	03/11/23

FACS results:

B2M gene validated by absence of HLA A, B, C expression
CIITA gene validated by absence of HLA DR,DQ expression

Positive control (WT) = BIHi001-B_WB05_p9



Conclusion

Genetic engineered line BIHi001-B-13 show absence of HLA A, B, C expression, validating B2M KO generation at the protein level.
CIITA KO can not be validated using surface protein expression since HLA DR,DQ expression is negative in hiPSC (see parental line BIHi001-B expression). Therefore, CRISPR editing success has been validated by Sanger sequencing. See report “CRISPR editing sequence validation”.

Date: 22/12/23