Certificate of Analysis

CELL LINE NAME	**BIHi005-A-95**	hPSCreg Link: *https://hpscreg.eu/cell-line/BIHi005-A-95*
DONOR GENDER/AGE:	☒ Male ☐ Female ☐ unknown Age: 25-29	
TYPE OF DISEASE / GENETIC MODIFICATIONS	SLC26A1-KO homozygous	
BANK	Master Bank, MB01, Passage 35, Freezing Date: 25.04.2024	
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
CONFIRMATION OF DISEASE GENOTYPE / EDITING	Sequencing of mutated site	Sequencing shows mutation	Pass

Date 23.08.2024

Stem Cell Core Facility

Single Nucleotide Polymorphism (SNP)- Karyotype

	Reference			Engineered cell line		
Sample (cell type, ID)	iPSC	BIHi005-A		iPSC	BIHi005-A-95	
Passage No.	17			34		
Bank ID	WB04			MB01		
DNA sample ID	D0548			D0822		
Chip-ID and Position	207521920117, R02C01			208305080104, R11C02		
Date of testing	10.08.2023			16.05.2024		
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

	BIHi005-A-95	Reference (BIHi005-A_WB04)
call_rate	0.997	0.996
computed_gender	M	M
SNPs_post_filter	74.65 %	74.35 %
SNP.distance.to.ref	6	-
loss.gain_log2ratio	1.28	-0.17
total_calls_CNV	24	17
total_calls_LOH	22	40
reportable_new_calls_CNV	0	1
reportable_new_calls_LOH	0	1
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

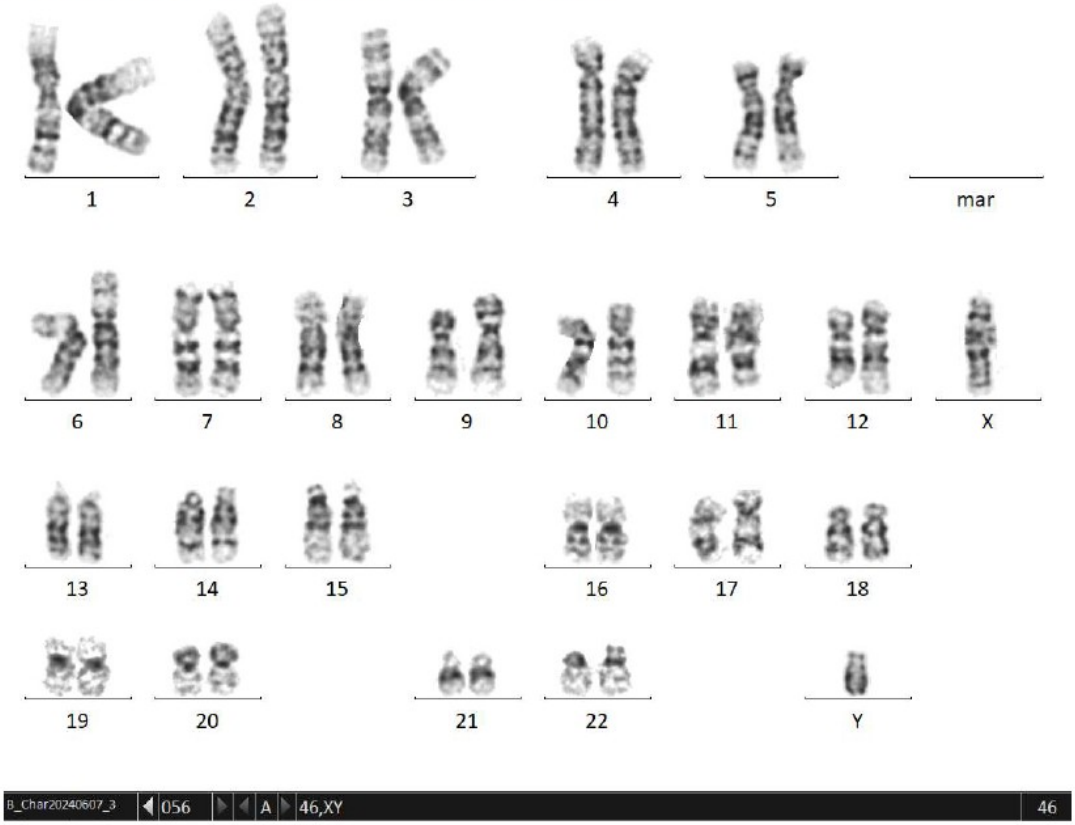
Cell line name	BIHi005-A-95
Bank ID	MB01
Passage No.	35
Date of testing	05.06.2024
Protocol	7.7 G-banded karyotyping

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

BIHi005-A-95 p35 MB01 GBK211, Karyotyp 46,XY[cp20]



Conclusion:

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

Date: 25.06.2024

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

Cell line name	BIHi005-A-95
Bank ID	MB01
Passage No.	34
Date of testing	04.07.2024
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**	
BIHi005-A-95	6	9	29	33.2	10	12	8	12	12	13	8	9	9	12	X	Y	17	18	8	11
BIHi005-A WB02	6	9	29	33.2	10	12	8	12	12	13	8	9	9	12	X	Y	17	18	8	11

The Alleles of the cell line BIHi005-A-95 and cell line BIHi005-A WB02 at the 10 STR Loci are identically.

Conclusion

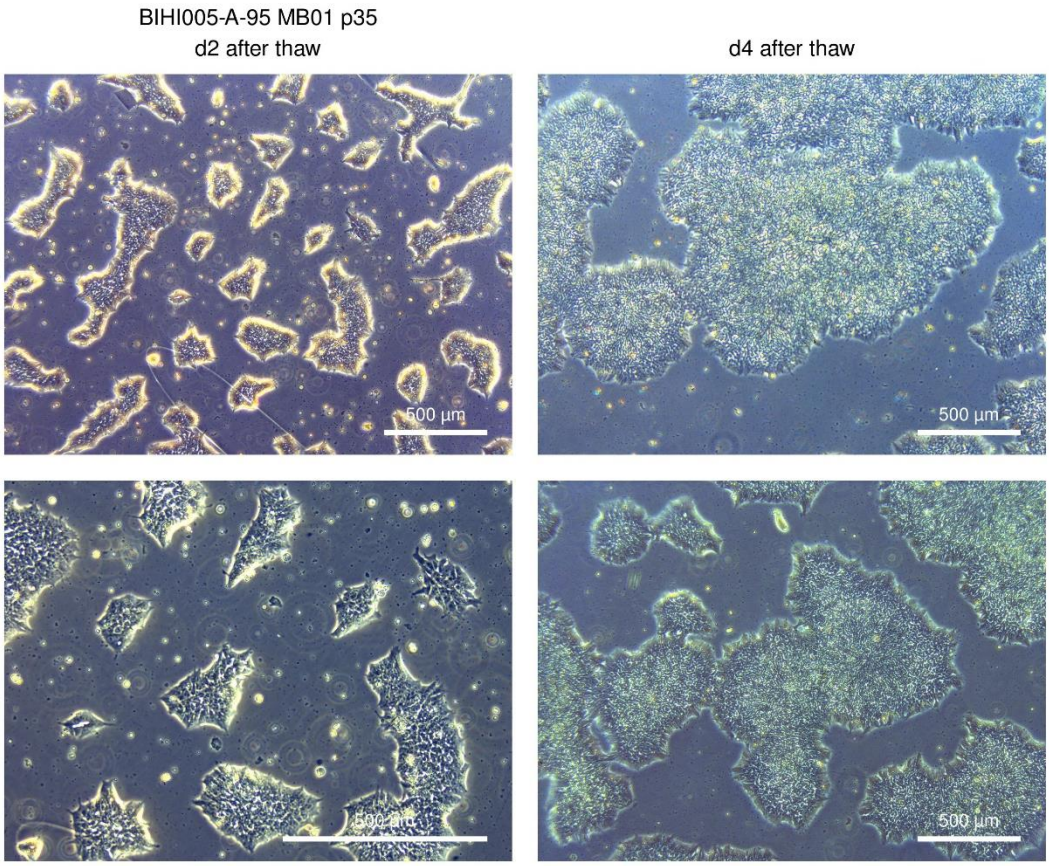
Both samples tested are from the same donor.

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

Cell line name	BIHi005-A-95
Bank ID	MB01
Passage No.	35
Date of testing	08.05.2024
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: 12.07.2024

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

Cell line name	BIHi005-A-95
Bank ID	MB01
Passage No.	36
Test date	23.07.2024
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	26,400	*Passed*
2 (pos. control)	24,231	27,381	*Passed*
3	>45	26,313	***Negative***

Conclusion

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

Date: 21.08.2024

Stem Cell Core Unit

CRISPR editing sequence validation

Cell line name	*BIHi005-A-95*
Parental cell line name:	*BIHi005-A*
Genetic modification	Knock out of SLC26A1 gene (GeneID: 10861)
Bank ID	MB01
Passage No.	34
Date of testing	28.06.2024

Gene edited: SLC26A1
Unedited sequence (+20bp upstream and downstream of the first and last edited bp):
ccacgggacctgacgcaaca**ggatggacgagtcccctgagcctctgcagcagggcagagggccggtgccggtccgacggcagcgcccagcaccccgggg**tctgcgtgagatgctgaag
Expected edited sequence (+20bp upstream and downstream of the first and last edited bp):
ccacgggacctgacgcaacaggtctgcgtgagatgctgaag

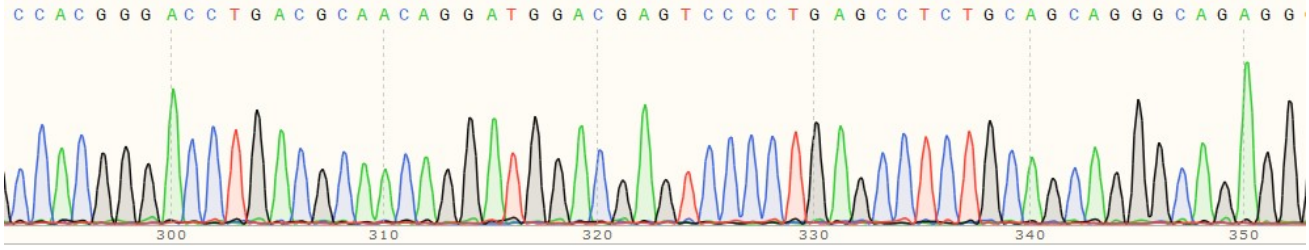
Primers used for generation of sequenced fragment:
FWD: CAGGAACCGCAGAGCCAATA
REV: TGAGGTTGGCGAAGAAGGAC

PCR conditions:

Component	25ul rxn
H2O	9,00
2x KAPA2G mix	12,50
Primer F (10uM)	1,25
Primer R (10uM)	1,25
Template (~ 10 ng)	1,00

Step	Temp.[°C]	Duration	Cycle #
Initial denaturation	95	3min	1
Denaturation	95	10sec	35
Annealing	60	10sec	
Extension	72	10sec	
Final extension	72	1min	1
Storage	6	inf	1

Results:
Chromatogram of Sanger sequencing of the genomic fragment amplified from gDNA of the parental (unedited) BIHi005-A (+20bp upstream and downstream of the first and last edited bp).



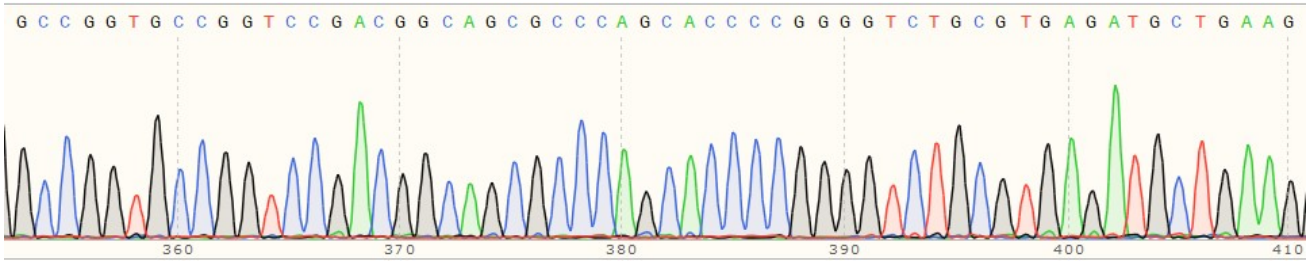


Figure 1 BIHi005-A sequencing results

Chromatogram of Sanger sequencing of the genomic fragment amplified from gDNA of the edited BIHi005-A-95. (+20bp upstream and downstream of the KO).

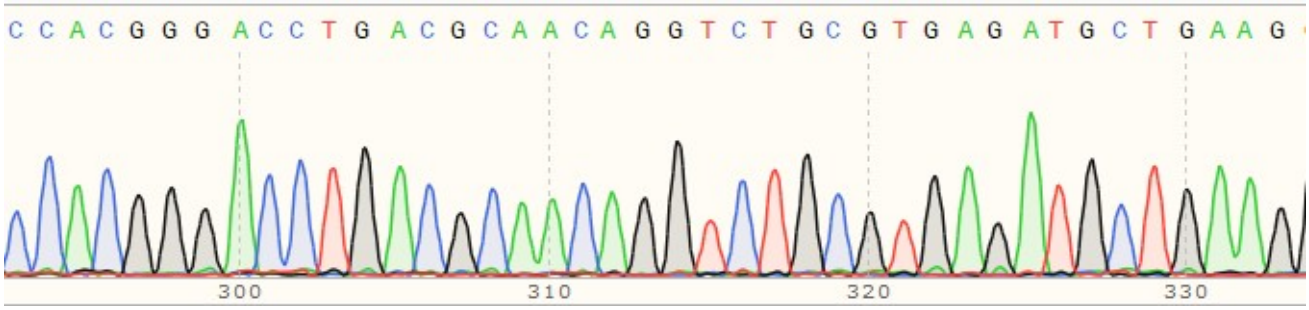


Figure 2 BIHi005-A-95 sequencing results

Alignment of the sequences from the parental BIHi005-A and edited BIHi005-A-95 with edited sequences highlighted.

Conclusion

Sample tested contains designed genetic modification.

Date: 18.07.2024