Certificate of Analysis

CELL LINE NAME	**BIHi005-A-87**	hPSCreg Link: https://hpscreg.eu/cell-line/BIHi005-A-87
DONOR GENDER/AGE:	☒ Male ☐ Female ☐ unknown Age:	
TYPE OF DISEASE / GENETIC MODIFICATIONS	KIT D816V (c.2447A>T) base editing BIHi005-A-KIT D816V_cl.G2 PAM (HO-mut) KIT (HE)	
BANK	Master Bank, MB01, Passage 27, Freezing Date: 05.03.2023	
FREEZING METHOD	Bambanker	
CULTURE PLATFORM	Feeder Independent	
	Medium: E8	Coating: Geltrex
REPROGRAMMING	self-replicating RNA Vector details (e.g. Kit, Pub, AddgeneNr): ReproRNA-OKSGM Kit	

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)	Select method	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	not done
PLURIPOTENT DIFFERENTIATION POTENTIAL	directed differentiation	Successful differentiation to cells of all three germ layers	not done
CONFIRMATION OF DISEASE GENOTYPE / EDITING	Sequencing of mutated site	Sequencing shows mutation	Pass

Date 11.05.2023

Single Nucleotide Polymorphism (SNP)- Karyotype

	Reference			Engineered cell line		
Sample (cell type, ID)	iPSC	BIHi005-A		iPSC	BIHi005-A-87	
Passage No.	14			27		
Bank ID	MB02			MB01		
DNA sample ID	D0313			D0581		
Chip-ID and Position	204362030005, R07C01			207228530124 R03C01		
Date of testing	13.03.2020			23.03.2023		
Gender (provided/estimated from chip data)	Male	Male	√	Male	Male	√

Genetic Modification BIHi005-A-87

Modification type: SNP
Modification Name (include protein position for KI): KIT_D816V_het
Gene (NCBI ID): KIT (3815)
Assembly: GRCh38.p14
Chromosome: 4
Chr. Gene location: NC_000004.12 (54657957..54740715)

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

Genotype Analysis

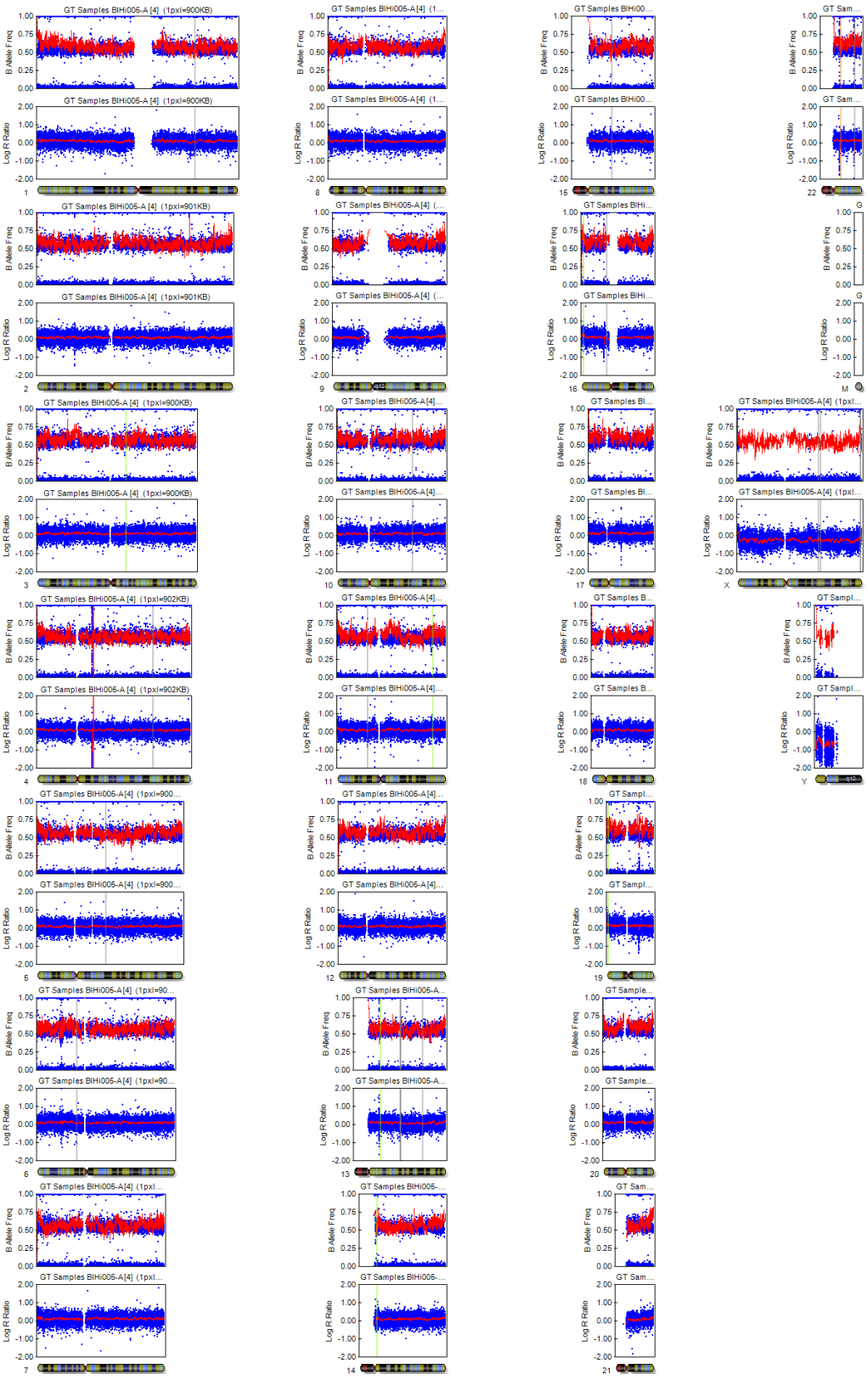
GenomeStudio: GenomeStudio V2.0.5
Genotyping Module: V2.0.5

CNV Analysis

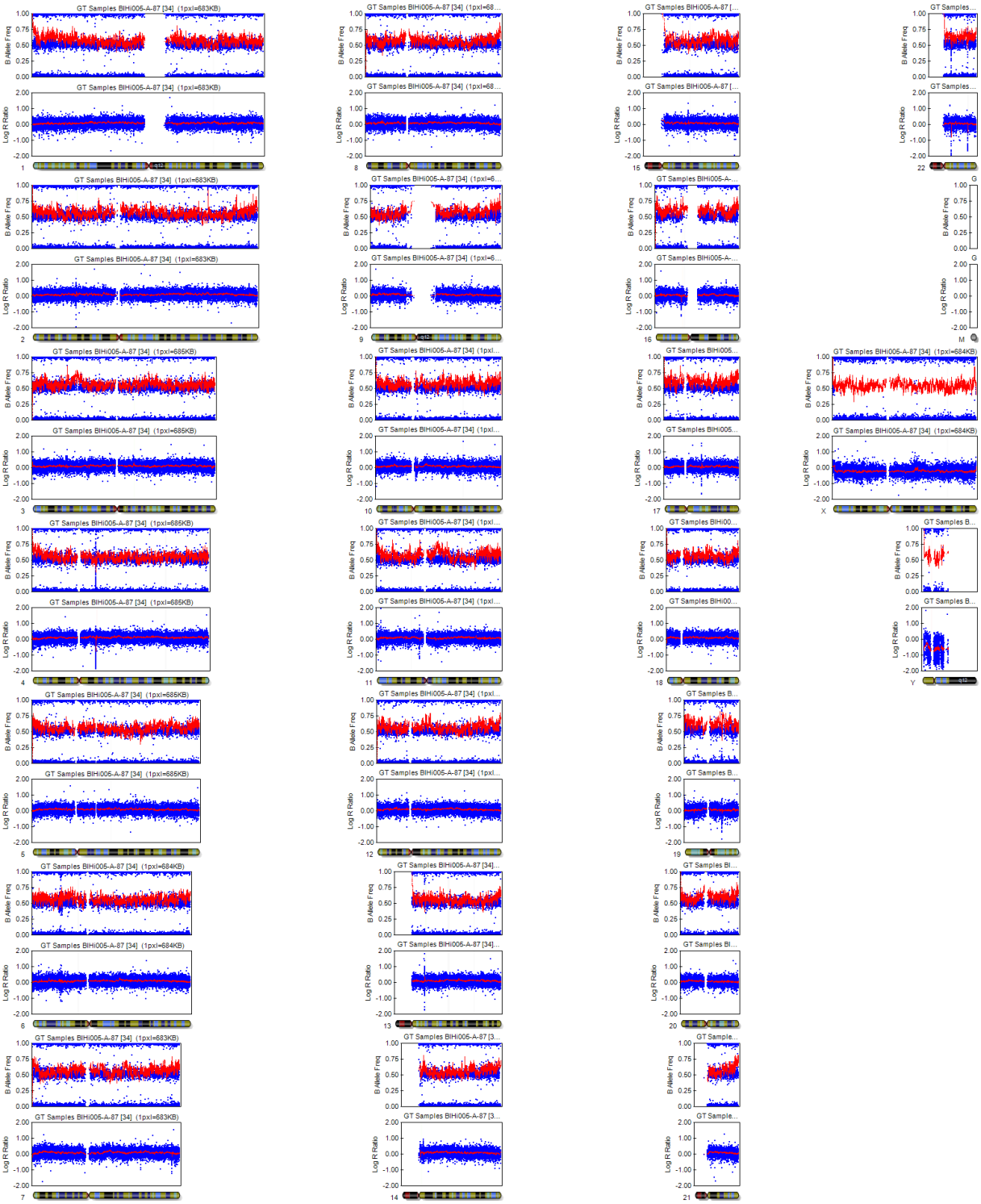
Algorithm: CNV-Partition
Version: 3.2.0

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.

Virtual Karyotype: *BIHi005-A MB02*



Virtual Karyotype: *BIHi005-A-87*



Call Tables

CNV regions common to BIHi005-A and BIHi005-A-87

Chr	Start	End	Size (bp)	CNV Value	Variant Type	Number of Genes*
1	194,908,124	195,991,301	1,083,177	2	LOH	
3	**109,741,385**	**110,232,519**	**491,134**	**3**	**Gain**	**0**
4	143,355,234	144,927,540	1,572,306	2	LOH	
5	84,160,853	85,194,705	1,033,852	2	LOH	
6	48,357,970	49,854,988	1,497,018	2	LOH	
10	93,442,365	94,642,848	1,200,483	2	LOH	
11	37,092,098	38,181,000	1,088,902	2	LOH	
13	57,031,979	59,166,750	2,134,771	2	LOH	
13	85,023,668	86,248,320	1,224,652	2	LOH	
15	48,949,078	50,042,635	1,093,557	2	LOH	
16	30,515,893	31,670,496	1,154,603	2	LOH	
22	41,054,954	42,433,887	1,378,933	2	LOH	

CNV regions only found in BIHi005-A

Chr	Start	End	Size (bp)	CNV Value	Variant Type	Number of Genes*
11	117,957,750	118,031,986	74,236	3	Gain	
14	20,471,786	20,677,723	205,937	3	gain	

CNV regions only found in BIHi005-A-87

Chr	Start	End	Size (bp)	CNV Value	Variant Type	Number of Genes*
4	143,355,234	144,922,413	1,567,179	2	LOH	
14	20,471,786	20,677,591	205,805	3	gain	
17	41,246,812	41,435,270	188,458	3	gain	

*Number of genes in CNV/LOH regions given only for **reportable** calls (see Appendix for details on genes in reported regions).

Interpretation

- There was **1** reportable reportable copy number change identified in die BIHi005-A MB and the engineered cell line BIHi005-A-87.
  - A 0.491 Mb gain on chromosome 3 was observed. No genes were found in this genomic region.

The CNV analysis result suggests that the engineered iPSC line BIHi005-A-87 contains neither CNVs > 2 Mb nor regions of LOH > 5 Mb.

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

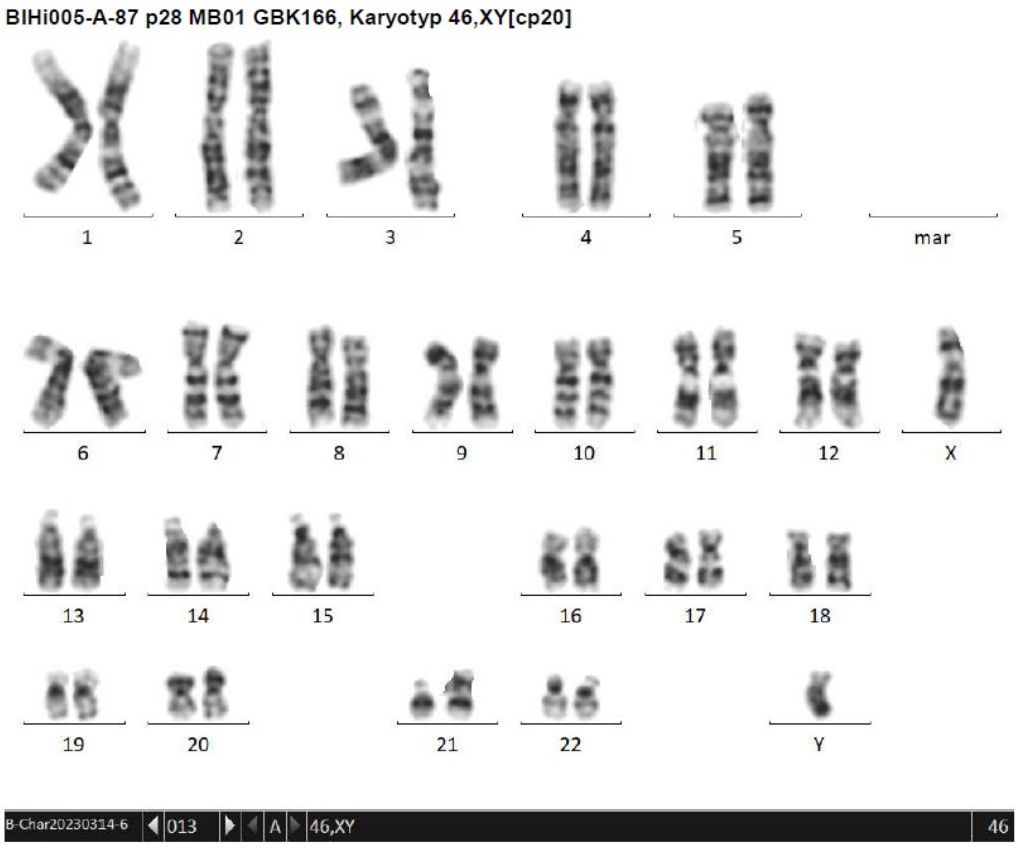
BIH Berlin Institute of Health @Charité	Stem Cell Core Facility **G-Banding - Karyotype**

Cell line name	BIHi005-A-87
Bank ID	MB01
Passage No.	28
Date of testing	09.03.2023
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 46; XY was detected for the examined sample.

Date: 29.03.2023

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

Cell line name	BIHi005-A-87
Bank ID	MB01
Passage No.	27
Date of testing	16.03.2023
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	
BIHi005-A-87	6	9	29	33.2	10	12	8	12	12	13	8	9	9	12	X	Y	17	18	8	11
BIHi005-A MB01	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-87 and cell line BIHi005-A MB01 at the 10 STR Loci are identically.

Conclusion

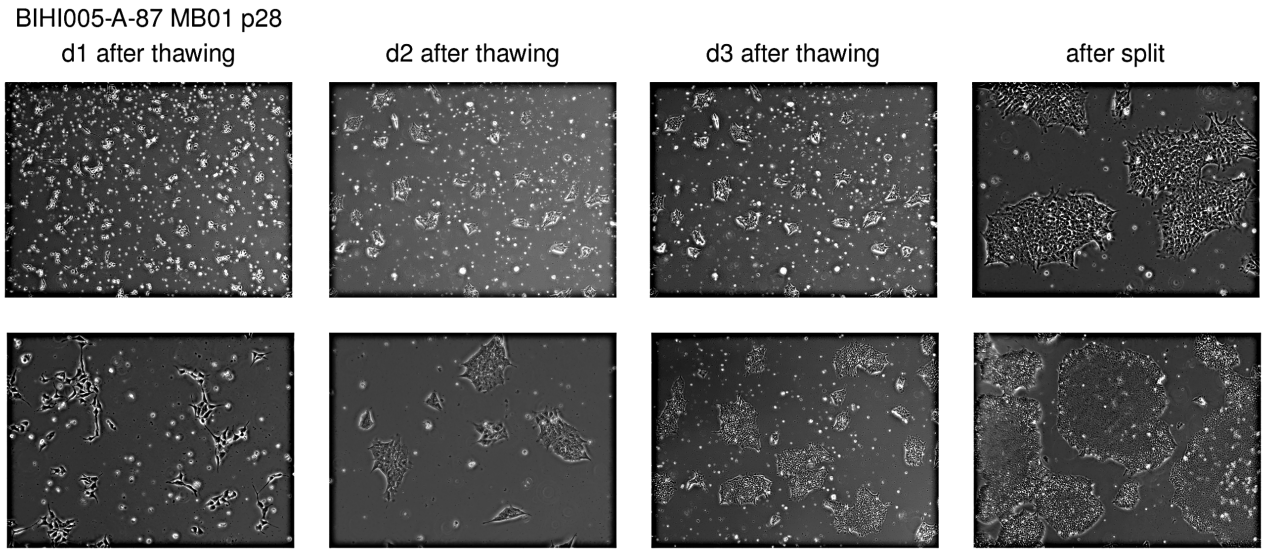
Both samples tested are from the same donor.

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

Cell line name	BIHi005-A-87
Bank ID	MB01
Passage No.	28
Date of testing	06.03.2023
Coating / Medium	Geltrex/ Homemade 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: 30.03.2023

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

Cell line name	BIHi005-A-87
Bank ID	MB01
Passage No.	29
Test date	17.03.2023
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	26,909	*Passed*
2 (pos. control)	25,586	27,180	*Passed*
3	>45	27,112	***Negative***

Conclusion

The cell line BIHi005-A-87 was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Date: 21.03.2023

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

Cell line name	*BIH005-A-87*
Parental cell line name:	*BIH005-A*
Genetic modification	Base editing of KIT D816V (c.2447A>T) (GeneID:3815); PAM (HO) KIT (HE)
Bank ID	MB01
Passage No.	27
Date of testing	19.04.2023

Gene edited: KIT D816V (c.2447A>T); PAM (HO-mut) KIT (HE)

Unedited sequence (+20bp upstream and downstream of the first and last edited bp):
BIHi005-A: gatttgtgattttggtctag**cCa**ga**gAc**atcaagaatgattctaattatgtgg
Expected edited sequence (+20bp upstream and downstream of the first and last edited bp):
BIHi005-A-87:gatttgtgattttggtctag**cTa**ga**gTc**atcaagaatgattctaattatgtgg
Primers used for generation of sequenced fragment:

FWD: TGAATGAAAGCAGTCCTGAGAA (OL0387)
REV: TCCTGCTGTGACCTTCAATG (OL0388)

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).

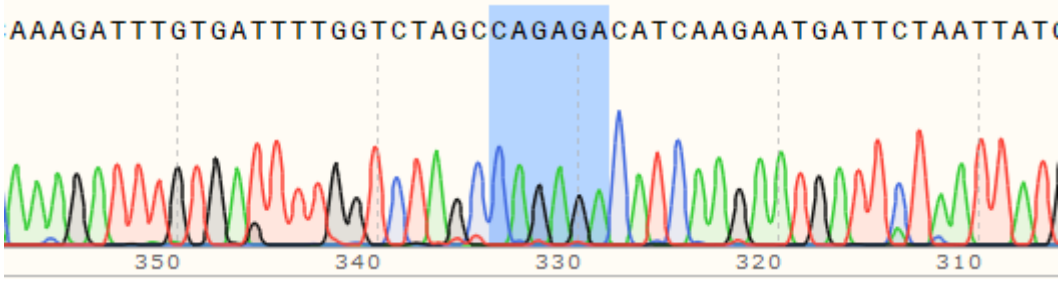


Figure 1 BIH005-A Sanger sequencing results

Chromatogram of Sanger sequencing of the genomic fragment amplified from gDNA of the edited BIHi005-A-87 (+20bp upstream and downstream of the first and last edited bp).

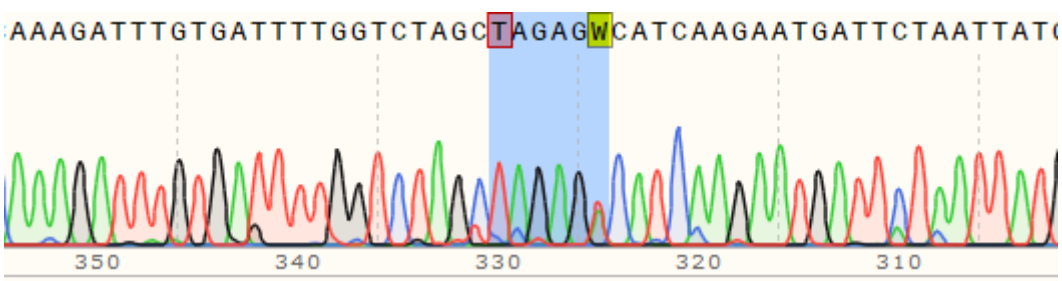
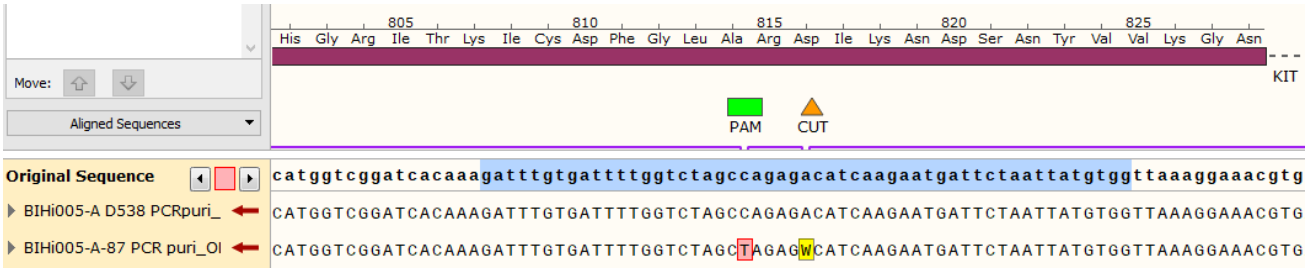


Figure 2 BIH005-A-86 Sanger sequencing results

Alignment of the sequences from the parental BIHi005-A and edited BIHi005-A-86 with edited sequences highlighted.



Conclusion

Sample tested contains designed genetic modification.

Date: 19.04.2023