Certificate of Analysis

CELL LINE NAME	**BIHi291-A**	hPSCreg Link: *ttps://hpscreg.eu/cell-line/BIHi291-A*
DONOR GENDER/AGE:	☐ Male ☒ Female ☐ unknown Age: unknown	
TYPE OF DISEASE / GENETIC MODIFICATIONS	Breast cancer	
BANK	Master Bank, MB01, Passage 16, Freezing Date: 26.07.2023	
FREEZING METHOD	Bambanker	
CULTURE PLATFORM	Feeder Independent	
	Medium: E8	Coating: Geltrex
REPROGRAMMING	Sendai virus Vector details (e.g. Kit, Pub, AddgeneNr): CytoTune iPS 2.0	

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

Date 19.10.2023

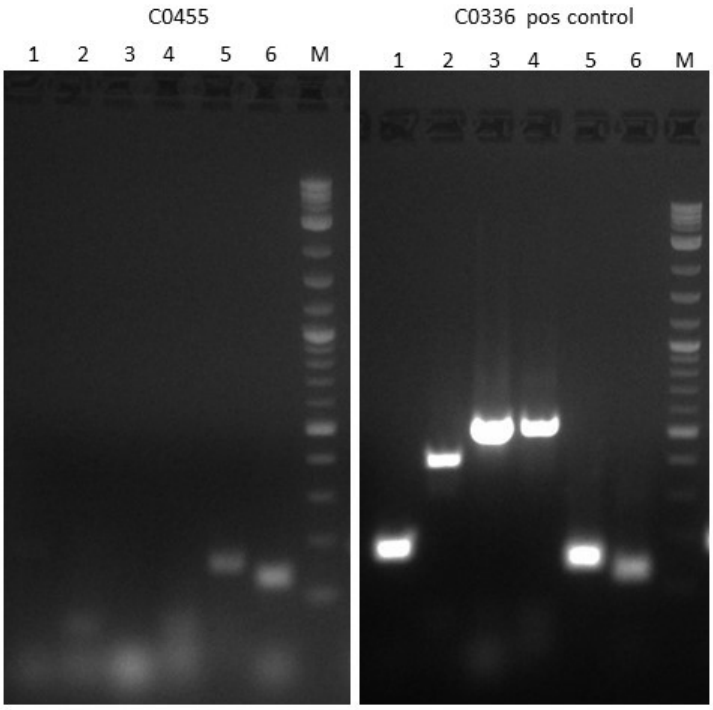
Stem Cell Core Unit

Report vector clearance of Sendai Virus

Cell line name	BIHi291
Bank / clone ID	Clone 4
Passage No.	13
Date of testing	21.07.2023
Protocol	8.4. Testing for remaining Sendai virus_CytoTune 2.0

Results

2 % standard agarose gel with DNA stain Ethidiumbromid 7µL/400 mL



cDNAs

cDNA sample	Clone name / passage
C0445	BIHi291-cl.4 p13
C0336	SenV pos ctr.

Primer

1	SeV OL0109/10	181 bp
2	SeV_Klf4 OL0111/2	410 bp
3	SeV_cMyc OL0113/4	532 bp
4	SeV_KOS OL0115/6	528 bp
5	Hu18sRNA OL0107/8	152 bp
6	beta-Actin OL0312/13	128 bp

PCR Results - Conclusion

The cell line is tested negative for Sendai virus.

Date 26.07.2023

Single Nucleotide Polymorphism (SNP)- Karyotype

	Reference			Engineered cell line		
Sample (cell type, ID)	PBMC	10-18B-V3		iPSC	BIHi291-A	
Passage No.	1			16		
Bank ID				MB01		
DNA sample ID	D0510			D0665		
Chip-ID and Position	206735420118, R09C01			207521920117, R10C02		
Date of testing	11.10.2023			10.08.2023		
Call Rate	0.991		√	0.991		√
Gender (provided/estimated from chip data)	Female	Female	√	Female	female	√

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.

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.

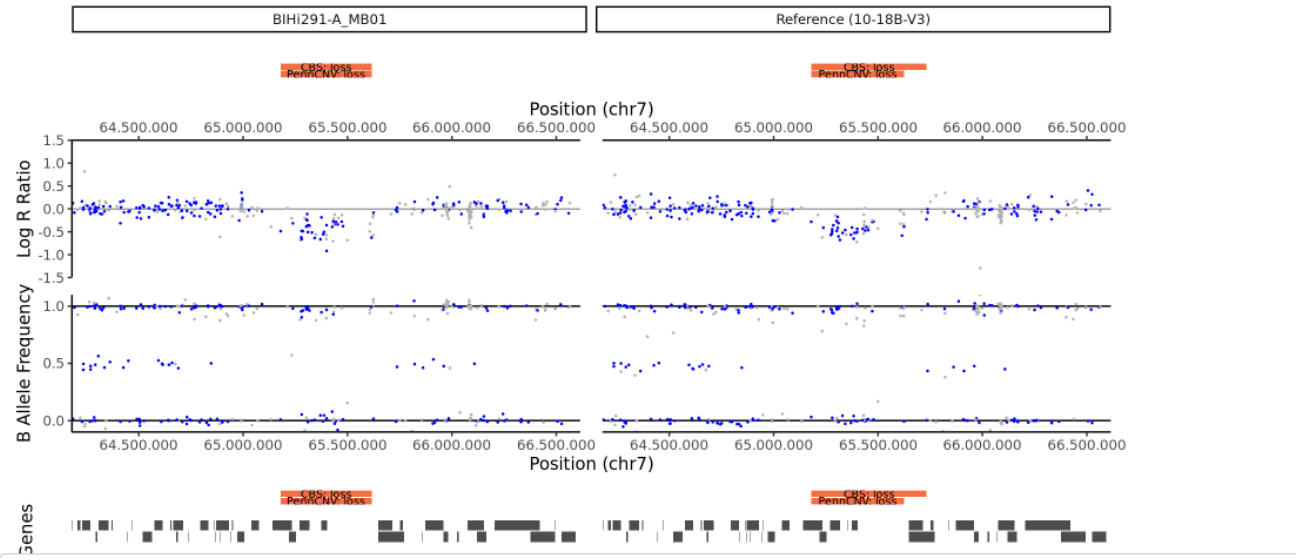
Single Nucleotide Polymorphism (SNP)- Karyotype

	BIHi291-A_MB01	Reference (10-18B-V3)
call_rate	0.991	0.991
computed_gender	F	F
SNPs_post_filter	74.10 %	74.15 %
SNP.distance.to.ref	1	-
loss.gain_log2ratio	-1.61	-0.58
total_calls_CNV	18	10
total_calls_LOH	16	13
reportable_new_calls_CNV	0	1
reportable_new_calls_LOH	0	0
critical_new_calls_CNV	0	0
critical_new_calls_LOH	0	0

Chr	Start	End	Size	reportable	CNV.state	ID	call.in.reference	coverage.by.ref
	A	A		All	All	All	All	All
chr7	65.179.960	65.614.864	434.905	yes, in ref.	loss	combined_loss_chr7_65179960_65614864	TRUE	100

Showing 1 to 1 of 1 entries

loss (in ref.): chr7 - 0.4Mb



- There was **1** reportable copy number change identified in the iPSC line BIHi291-A and the primary cells.
  - > A 0.435 Mb loss on chromosome 7 was observed. Genes in this area can be found in the html report.

The CNV analysis result suggests that the iPSC line 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

Stem Cell Core Facility

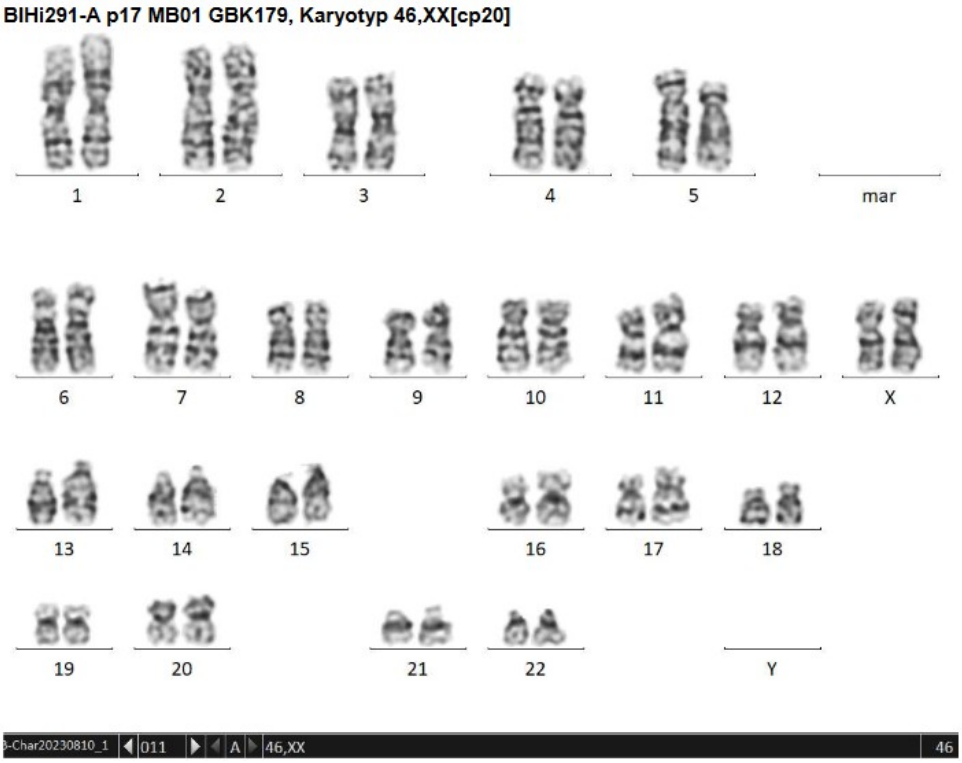
G-Banding - Karyotype

Cell line name	BIHi291-A
Bank ID	MB01
Passage No.	17
Date of testing	04.09.2023
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 female karyotype 46; XX was detected for the examined sample.

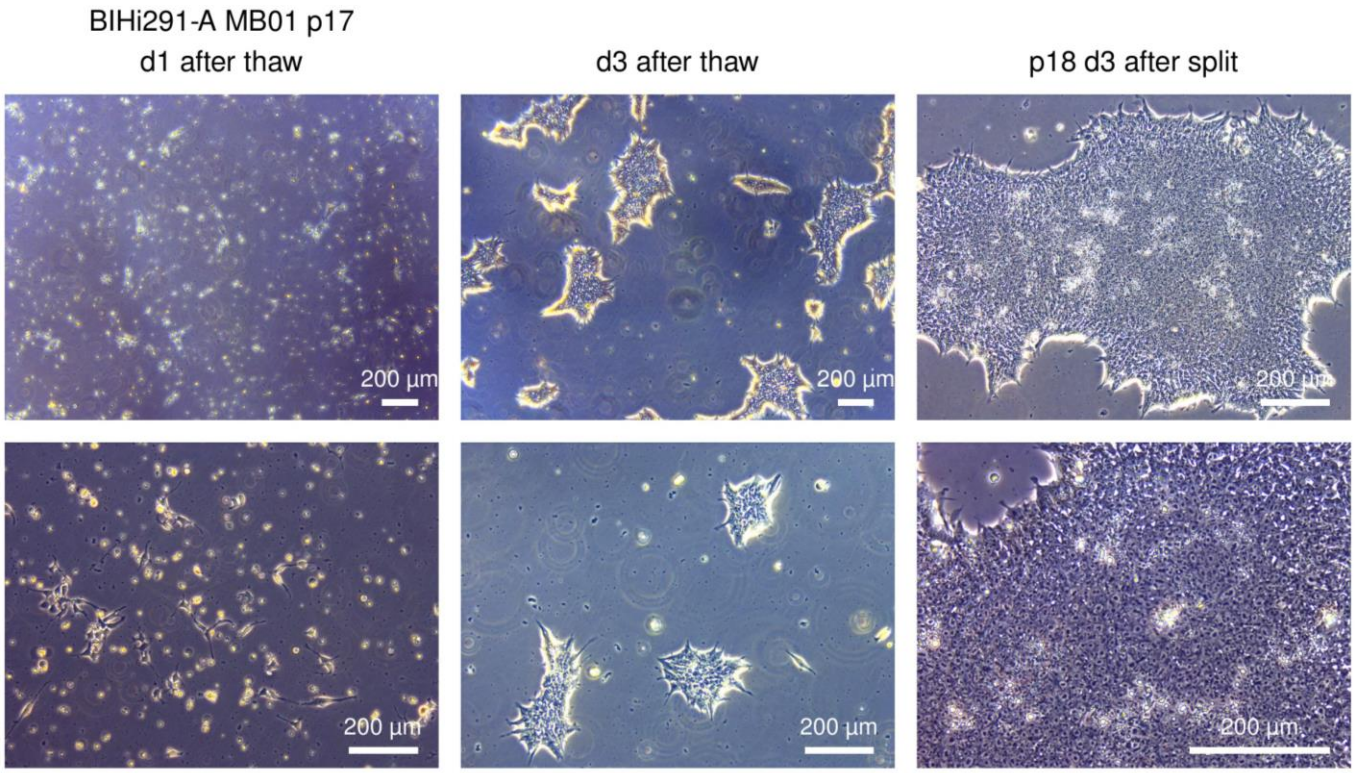
Date: 11.09.2023

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

Cell line name	BIHi291-A
Bank ID	MB01
Passage No.	17
Date of testing	31.07.2023
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: 11.08.2023

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

Cell line name	BIHi291-A
Bank ID	MB01
Passage No.	18
Test date	15.08.2023
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	28,865	*Passed*
2 (pos. control)	25,501	28,95	*Passed*
3	>45	28,846	***Negative***

Conclusion

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

Date: 15.08.2023