MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE IN THE HELMHOLTZ ASSOCIATION

Certificate of Analysis

CELL LINE NAME	**MDCi239-A**	hPSCreg Link: *https://hpscreg.eu/user/cellline/edit/MDCi239-A*
DONOR GENDER/AGE:	☐ Male ☒ Female ☐ unknown Age:	
DISEASE PHENOTYPE / GENETIC VARIANT		
BANK	MB01 , ID MDCi239-A , Passage 12, Freezing Date: 23.03.2022	
FREEZING METHOD	Single cells in Bambanker	
CULTURE PLATFORM	Feeder Independent	
	Medium: E8life	Coating: Geltrex
REPROGRAMMING	Sendai virus (CytoTune 2.0) Vector details (e.g. Kit, Pub, AddgeneNr):	
GENETIC MODIFICATION	☐ yes ☒ no Targeting Vector: ☐TALEN, ☐CRISPR, ☐ZNF, Addgene: ☐ Isogenic control/SNP ☐ Gene knock-out ☐ Transgene knock-in	
	Parental/isogenic cell line	#11398 Fibroblasts
	Target gene/Transgene/Locus (het/hom?)	
	Validation (e.g. PCR, sequencing)	

TEST DESCRIPTION	Test Method	Test Specification	Result
STERILITY (viral pathogens)	☐ Blood screening Donor ☐ PCR (primary cells) ☐ PCR (iPS clone/subclone)	HBV, HCV, HIV negative	Pass
STERILITY (mycoplasma)	Test Method	No contamination detected	Pass
STERILITY (bacteria/ yeast/ fungi)	Culture for 7 days in antibiotic free medium	No contamination detected	Pass
REPROGRAMMING VECTORE CLEARANCE	☒ PCR ☐ AB staining ☐ Confirmed in parental line	Vector not present	Pass
VIABILITY / MORPHOLOGY	Phase contrast microscopy of cells at 24, 48, and 72 hrs	Growth rate and confluency typical of hPSCs	Pass
UNDIFFERENTIATED PHENOTYPE	Markers for undifferentiated hPSCs ☐ IF-Staining ☒FACS ☐other	Expression of at least three pluripotency markers detected	Pass
	☐ Pluritest	Pluripotency and Novelty Scores above threshold	not done
PLURIPOTENT DIFFERENTIATION POTENTIAL	3-germ layer differentiation: ☐ spontaneous (e.g. EB formation)	Detection of markers for cells from the three germ layers	not done
	☐ directed differentiation	Successful differentiation to cells of all three germ layers	not done
	☐ Teratoma formation	Observation of tissues derived from the three germ layers	not done

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KARYOTYPE	PerkinElmer KaryoLite BoBs™	Karyotype matches Donor	not done
	Virtual karyotyping using Illumina OMNI-EXPRESS-8v1.6 Chip	No significant changes compared to the primary cells detected	Pass
	G-Banding	Karyotype matches Donor	not done
IDENTITY (STR ANALYSIS)	Promega GenePrint® 10 System	Identical to profile of primary cells	Pass

date / signature: 03.06.2022 /Sandra Schommer

Single Nucleotide Polymorphism (SNP)- Karyotype

Cell line name	11398 Fibroblast, **MDCi239-A**
Gender	Female
Passage No.	5
Name operator	Sebastian Diecke, Gabi Born
Date of testing	19.04.2022

Specifications:
iPSCs where karyotyped using the ISCAN machine and the Illumina platform OMNI-EXPRESS-8v1.6 Chip (Marker coverage 958,497 spanning whole human genome). The analysis was performed by using Karyostudio 1.3 software based on the information of GRCh36/hg18 dataset.

The analysis software stringency settings used to identify aberrant regions are listed below. Reportable copy number changes are gains and losses greater than 0,4 Mb and regions of LOH (loss of heterozygosity) above 3 Mb (in accordance with WiCell criteria (service provider pluripotent stem cell banking and characterization).

In Known Regions	Type of CNV	Size Threshold	Markers Threshold	CNV Confidence Threshold
Inside	Gain	100000	15	100
Inside	Loss	75000	15	100
Inside	CNLOH	3000000	30	100
Outside	Gain	200000	15	100
Outside	Loss	150000	15	100
Outside	CNLOH	8000000	30	100

<u>This method can detect the following aberrations:</u>

- Genomic gains and losses
  - Copy number variants (CNVs)
  - Duplications/deletions
  - Unbalanced translocations
  - Aneuploidies

- Copy neutral aberrations Loss of heterozygosity (LOH) / Absence of heterozygosity (AOH)
- >20% mosaicism (for example: cultures where >1 of 5 cells is trisomy 12)

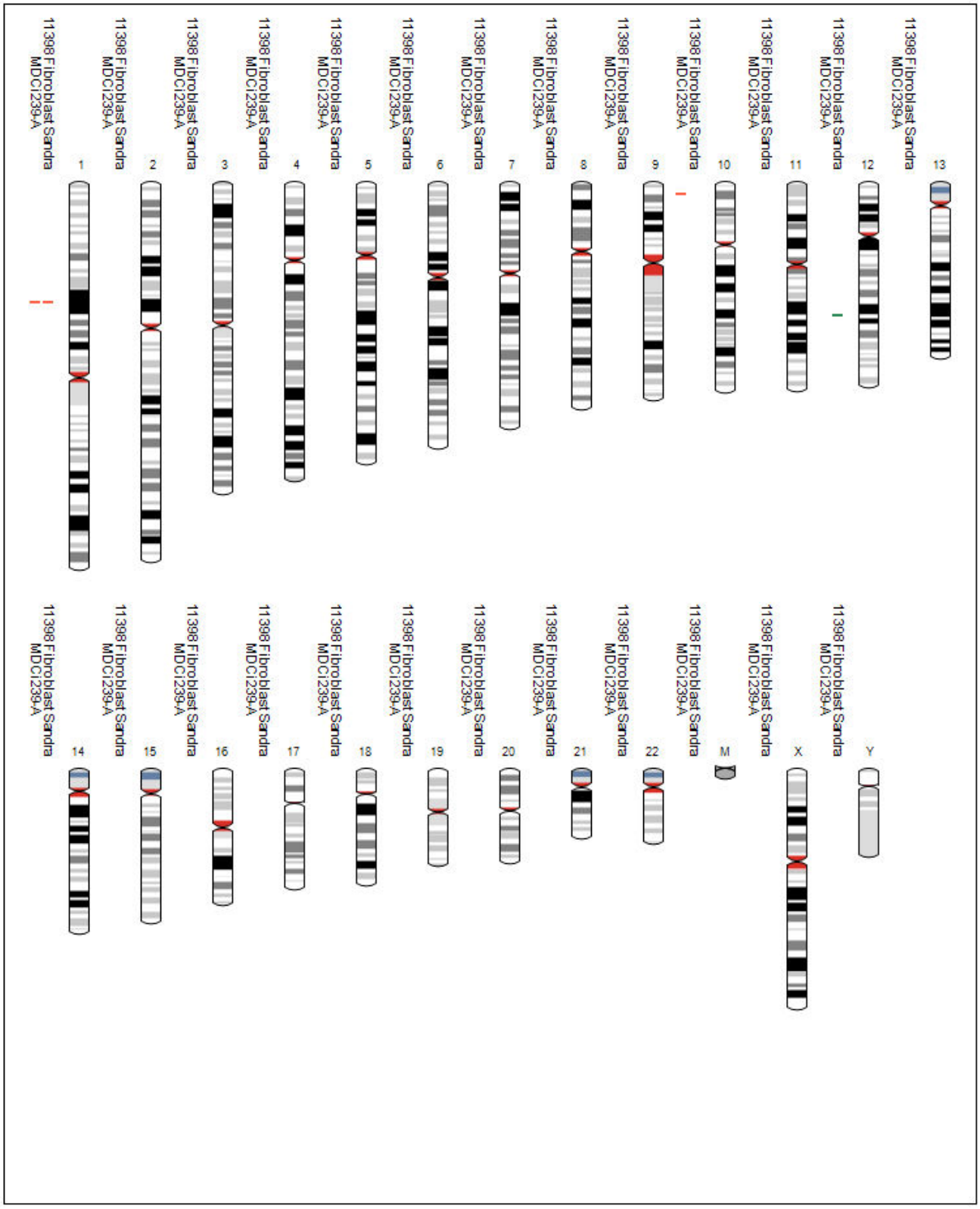
<u>Limitations:</u>
Other aberrations like the once listed below can´t be detected using this array.

  - Balanced translocations
    - Robertsonian
  - Balanced insertions
  - Inversions
  - <20% culture mosaicism (for example: cultures where 1 of 5 cells is trisomy 12
  - Chromosomal position of genomic gains

Single Nucleotide Polymorphism (SNP)- Karyotype

Virtual Karyotype:

Gain (Area marked in green), Loss (Area marked in red), Loss of heterozygosity (Area marked in gray)



Results:

Estimate of the physical copy number of a detected region:

- 0 indicates a homozygous deletion (loss of both copies)
- 1 indicates a hemizygous deletion (loss of one copy)
- 2 indicates a copy-neutral loss of heterozygosity (e.g., Uniparental disomy (UPD or autozygosity)
- 3 indicates a duplication (gain of one copy)
- 4 indicates a copy number of 4 or above

Sample ID	Chr	Start	Stop	Length	Value

Interpretations:

- The cell line MDCi239-A has a normal karyotype.
- We detected 1 copy number change within the parental cell line and the tested clone which was below our detection criteria.
    - Refer to the data section and the excel table “table of affected genes” and see above
- Besides the information listed in the cytogenetic report about known diseases linked to the reported aberrations the UCSC Genome Browser (https://genome.ucsc.edu) and Decipher (https://decipher.sanger.ac.uk/search) may provide additional information on the detected regions.

Sebastian Diecke Digitally signed by Sebastian Diecke
Date: 2022.05.05 13:57:25 +02'00'

Responsible person / date: Sebastian Diecke/ 26/04/2022

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

Attachments:

Cytogenetics Report
Table of affected genes
Karyogram only

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Technology Platform Pluripotent Stem Cells

Cell Line Identity (STR Analysis)

Cell line name / Passage No.	**MDCi239-A**, MDCi239-B **,**11398
Bank	**MB01,**MB01 **,**Fibros
Name operator	Gabi Born
Date of testing	24.05.2022
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.

Date	Passage	Cell line	TH01	D21S11	D5S818	D13S317	D7S820	D16S539	CSF1PO	AMEL	vWA	TPOX
24.05. 22	**12**	**MDCi239-A**	**8 9**	**29 29**	**12 12**	**12 13**	**10 11**	**9 10**	**10 12**	**X X**	**17 17**	**8 10**
24.05. 22	12	MDCi239-B	8 9	29 29	12 12	12 13	10 11	9 10	10 12	X X	17 17	8 10
24.05. 22	5	11398	8 9	29 29	12 12	12 13	10 11	9 10	10 12	X X	17 17	8 10

Results

The Alleles of the cell lines MDCi239-A, MDCi239-B and the 11398 the 10 STR Loci are identically.

Conclusion

All samples tested are from the same donor.

Responsible person / date: Gabi Born / 25.05.2022

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Stem Cell Core Facility

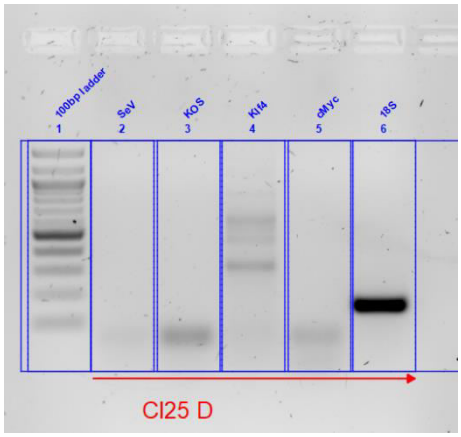
Report Sendai Virus Testing

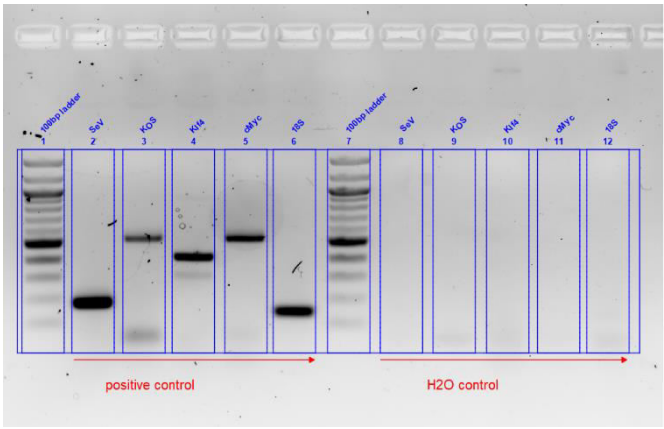
Cell line name	MDCi239-A (#11398 Cl25D)
Passage No.	8
Name operator	Sandra Schommer
Date of testing	26.01.2021
Protocol	8.4. Testing for remaining Sendai virus_CytoTune 2.0
Sample	Cl25D: MDCi239-A +: positive control -: water

Results

2 % standard agarose gel with DNA stain RotiSafe 5µL/100 mL

PCR picture:





PCR Results - Conclusion

The cell line MDCi239-A is tested negative for Sendai virus.

Responsible person / date: Sandra Schommer / 20.04.2022

MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE IN THE HELMHOLTZ ASSOCIATION	Stem Cell Core Facility **Sterility (Mycoplasma, Bacteria/Yeast/Fungi)**

Cell line / Passage No.	MDCi239-A / p15
Cell bank	MB01
Operator name	Norman Krüger
Test date	05.05.2022
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	28,1	Passed
2 (pos. control)	25,6	28,3	Passed
3	>45	29,0	**Negative**

Conclusion

The cell line MDCi239-A MB01 p15 was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Responsible person / date: Norman Krüger / 05.05.2022

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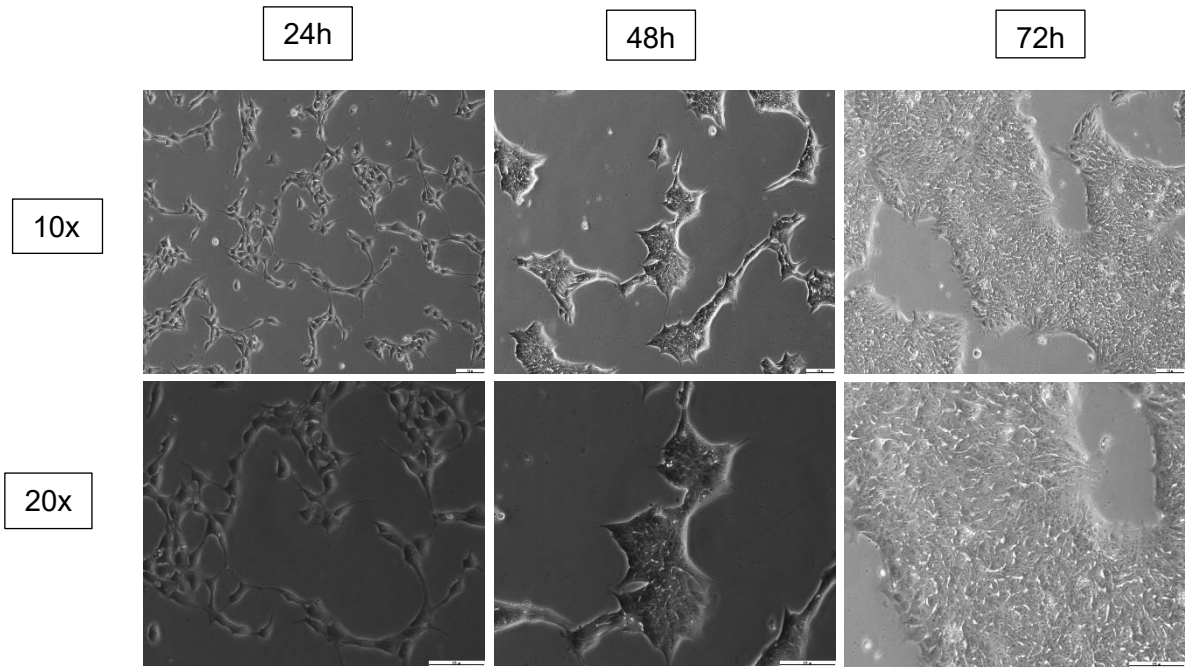
Stem Cell Core Facility

Morphology and Viability

Cell line name	MDCi239-A
Passage No.	12
Bank	Master bank 01
Name operator	Sandra Schommer
Date of testing	06.-08.04.2022

An aliquot of the master cell bank was thawed and monitored during antibiotics-free cultivation. ROCK inhibitor was used only during the first 24 hours.

Images:



Conclusion:

Cells from the cell bank show a good post-bank recovery after thawing and getting confluent within one week.

The cell line MDCi239-A MB01 shows typical morphology of undifferentiated hiPSC.

Responsible person: Sandra Schommer / date: 20.04.2022

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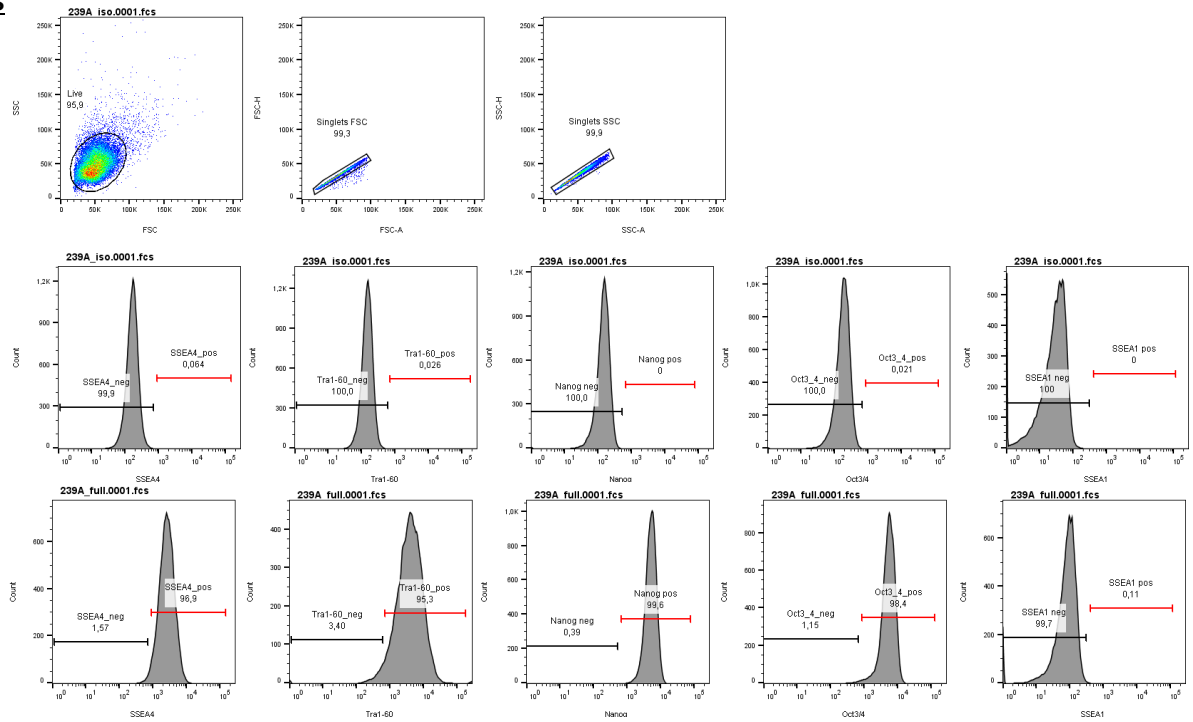
Stem Cell Core Facility

FACS analysis of markers in undifferentiated hPSCs

Cell line name	MDCi239-A
Passage No.	15
Bank	Masterbank 01
Name operator	Sandra Schommer
Date of testing	13.04.2022
Protocol	7.14 FACS analysis of pluripotency markers

Results



	SSEA4	Tra1-60	Nanog	Oct3/4	SSEA1
isotype	**0,06%**	**0,03%**	**0%**	**0,02%**	**0%**
positive	**96,9%**	**95,3%**	**99,6%**	**98,4%**	**0,11%**

Conclusion

The Masterbank of cell line MDCi239-A at p15 shows positive FACS results (over 80% positive) for the tested undifferentiated stem cell markers Tra1-60, OCT3/4, NANOG and SSEA-4. Additionally it shows negative FACS results (lower than 20% positive) for the differentiation marker SSEA-1.

Responsible person Sandra Schommer / date: 19.04.2022