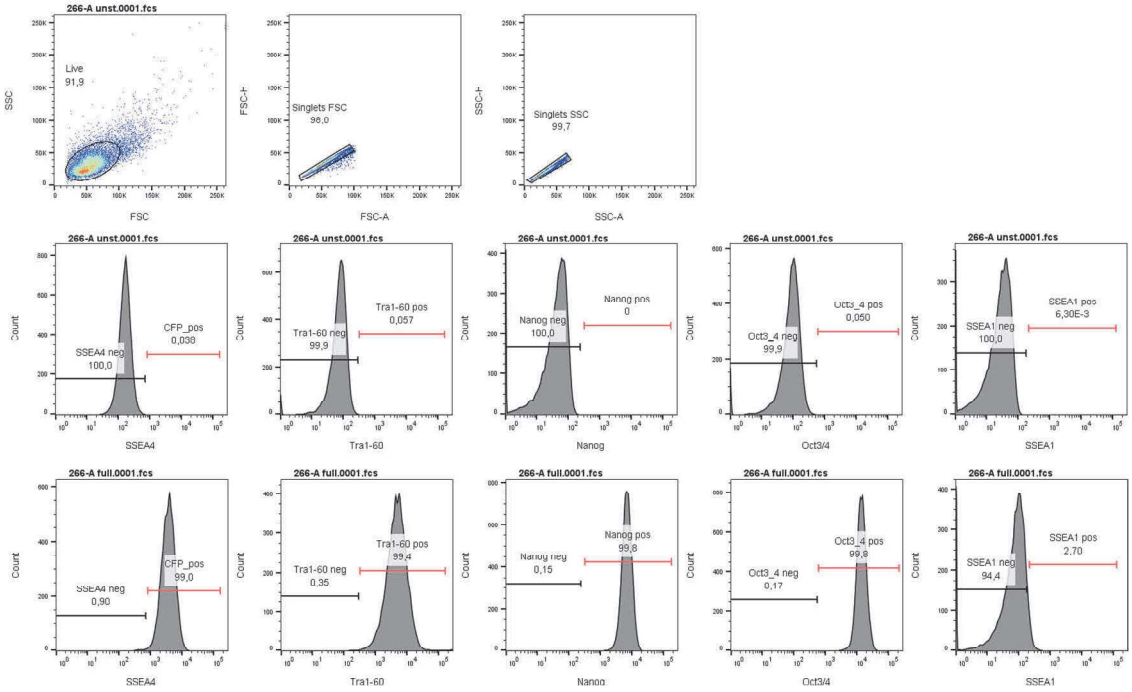
BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **FACS analysis of pluripotency markers**

Cell line name	BIHi266-A MB01
Passage No.	19
Name operator	Sandra Schommer
Date of testing	19. August 2020
Protocol	7.14 FACS analysis of pluripotency markers



Sample description	Unstained sample (%)	Sample (%)
Oct3/4	0,05	99,8
Tra1-60	0,06	99,4
Nanog	0	99,8
SSEA4	0,04	99,0
SSEA1	0	2,7

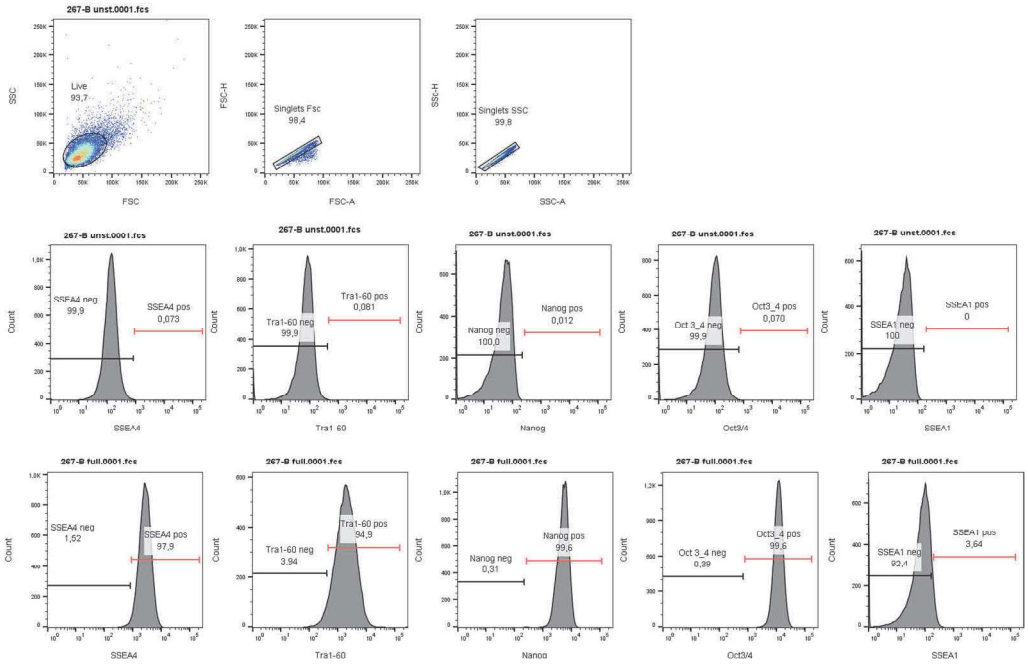
Conclusion

The cell line BIHi266-A MB01 at P19 shows positive FACS results (over 80% positive) for the tested pluripotency markers SSEA4, OCT3/4, NANOG and Tra1-60.

Responsible person / date: Sandra Schommer / 19.08.2020

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **FACS analysis of pluripotency markers**

Cell line name	BIHi267-B MB01
Passage No.	18
Name operator	Sandra Schommer
Date of testing	19. August 2020
Protocol	7.14 FACS analysis of pluripotency markers



Sample description	Unstained sample (%)	Sample (%)
Oct3/4	0,07	99,6
Tra1-60	0,08	94,9
Nanog	0,01	99,6
SSEA4	0,07	97,9
SSEA1	0	3,64

Conclusion

The cell line BIHi267-B MB01 at P18 shows positive FACS results (over 80% positive) for the tested pluripotency markers SSEA4, OCT3/4, NANOG and Tra1-60.

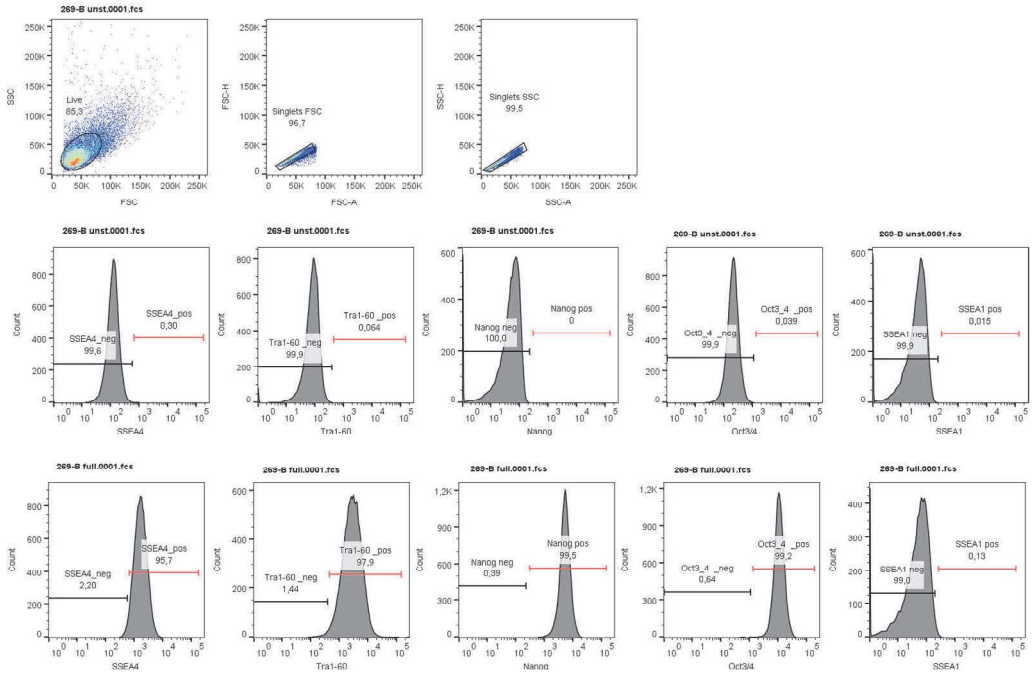
Responsible person / date: Sandra Schommer / 19.08.2020

BERLIN INSTITUTE OF HEALTH
Charité & Max Delbrück Center

Stem Cell Core Facility

FACS analysis of pluripotency markers

Cell line name	BIHi269-B MB 01
Passage No.	17
Name operator	Sandra Schommer
Date of testing	19.06.2020
Protocol	7.14 FACS analysis of pluripotency markers



Sample description	Unstained sample (%)	Sample (%)
Oct3/4-APC-A	0,04	99,2
Tra1-60-GFP/FITC-A	0,06	97,9
Nanog-PE-A	0	99,5
SSEA4 VioBlue	0,3	95,7

Conclusion

The Masterbank 01 of cell line BIHi269-B at P17 shows positive FACS results (over 80% positive) for the tested pluripotency markers SSEA4, OCT3/4, NANOG and Tra1-60.

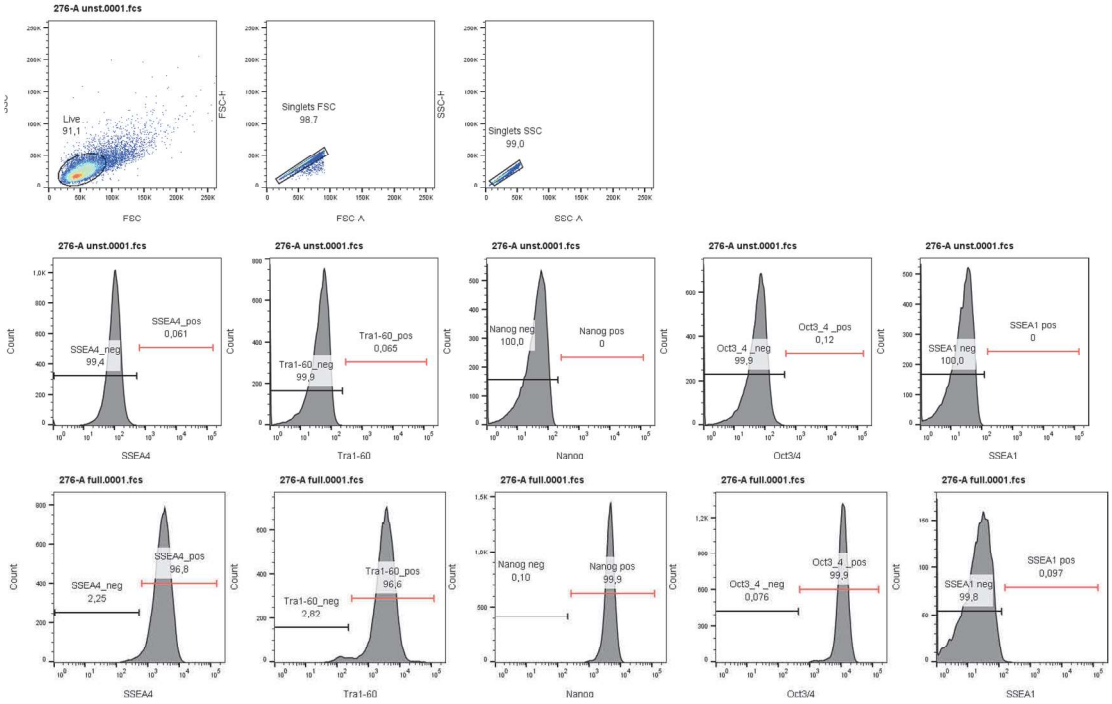
Responsible person / date: Sandra Schommer / 22.06.2020

BERLIN INSTITUTE OF HEALTH
Charité & Max Delbrück Center

Stem Cell Core Facility

FACS analysis of pluripotency markers

Cell line name	BIHi276-A MB 01
Passage No.	15
Name operator	Sandra Schommer
Date of testing	06.11.2020
Protocol	7.14 FACS analysis of pluripotency markers



Sample description	Unstained sample (%)	Sample (%)
Oct3/4-APC-A	0,12	99,9
Tra1-60-GFP/FITC-A	0,07	96,6
Nanog-PE-A	0	99,9
SSEA4 VioBlue	0,06	96,8

Conclusion

The Masterbank 01 of cell line BIHi276-A at p15 shows positive FACS results (over 80% positive) for the tested pluripotency markers SSEA4, OCT3/4, NANOG and Tra1-60.

Responsible person / date: Sandra Schommer / 09.11.2020

Single Nucleotide Polymorphism (SNP)- Karyotype

Cell line name	BIHi267-B MB01, BIHi269-B MB
Gender	Male, Female
Passage No.	P16, P16
Name operator	Sebastian Diecke, Gabi Born
Date of testing	22.07.2020, 14.07.2020

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	7	50
Inside	Loss	75000	7	50
Inside	CNLOH	3000000	20	50
Outside	Gain	200000	7	50
Outside	Loss	150000	7	50
Outside	CNLOH	8000000	20	50

This method can detect the following aberrations:

- 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)

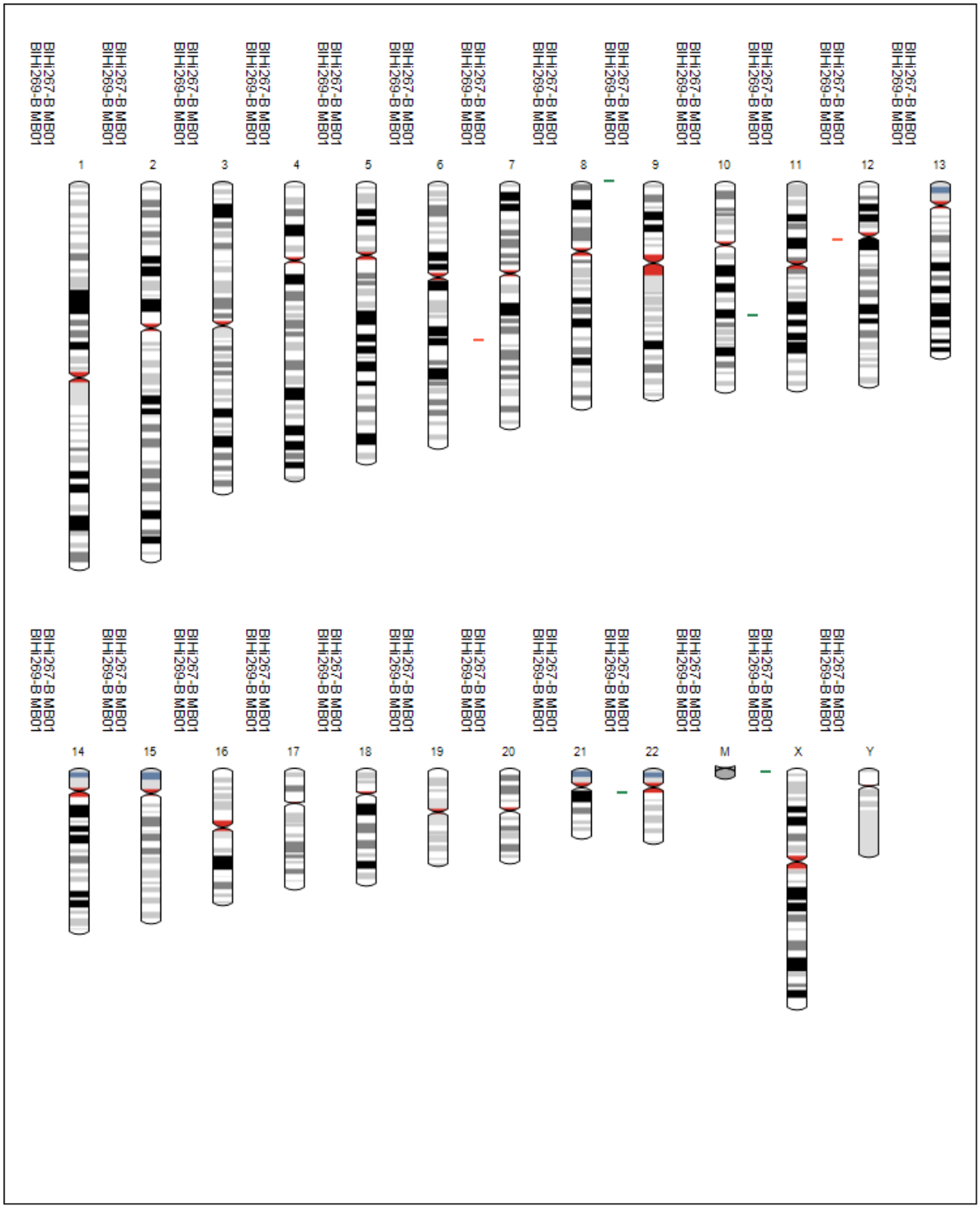
Limitations:
Other aberrations like the once listed below can´t be detected using this array.

  - 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
BIHi267-B	22	16114244	17456773	1342529	3
BIHi269-B	11	85220773	85787824	567051	3
BIHi267-B	12	37986081	38432009	445928	1

Interpretations:

- The cell lines BIHi267-B MB01 and BIHi269-B MB has a deletion of the entire x-chromosome.
- There was 1 additional reportable copy number change within the tested clone.
    - 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.

Responsible person / date: Sebastian Diecke/ 02/09/2022

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

Single Nucleotide Polymorphism (SNP)- Karyotype

Cell line name	BIHi266-A MB01, BIHi276-A MB01
Gender	Female, Male
Passage No.	P16, P18
Name operator	Sebastian Diecke, Gabi Born
Date of testing	22.07.2020, 22.03.2021

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	7	50
Inside	Loss	75000	7	50
Inside	CNLOH	3000000	20	50
Outside	Gain	200000	7	50
Outside	Loss	150000	7	50
Outside	CNLOH	8000000	20	50

<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)

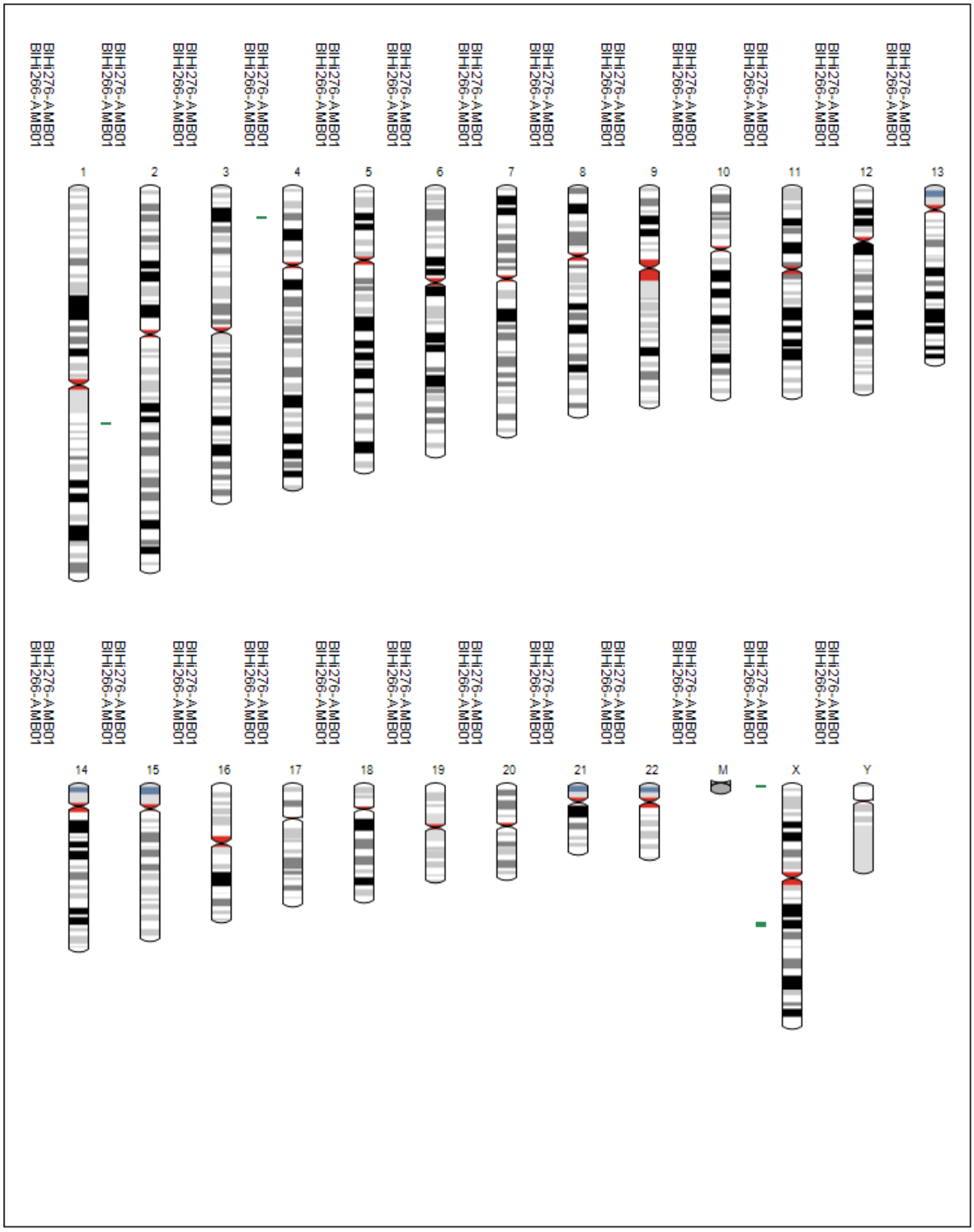
<u>Limitations:</u>
Other aberrations like the once listed below can´t be detected using this array.

  - 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
BIHi276-A	X	88365553	92181907	3816354	2
BIHi276-A	X	2700157	3540527	840370	2
BIHi276-A	4	20727464	21244175	516711	3
BIHi266-A	2	149192933	149511650	318717	3

Interpretations:

- The cell lines BIHi266-A MB01 and BIHi276-A MB01 has a deletion of the entire x-chromosome.
- There was 1 additional reportable copy number change within the tested clone.
    - 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.

Responsible person / date: Sebastian Diecke/ 02/09/2022

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

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Single Nucleotide Polymorphism (SNP)- Karyotype**

Cell line name	BIHi266-A MB, female
Passage No.	16
Name operator	Sebastian Diecke, Claudia Schaar
Date of testing	22.07.2020

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)

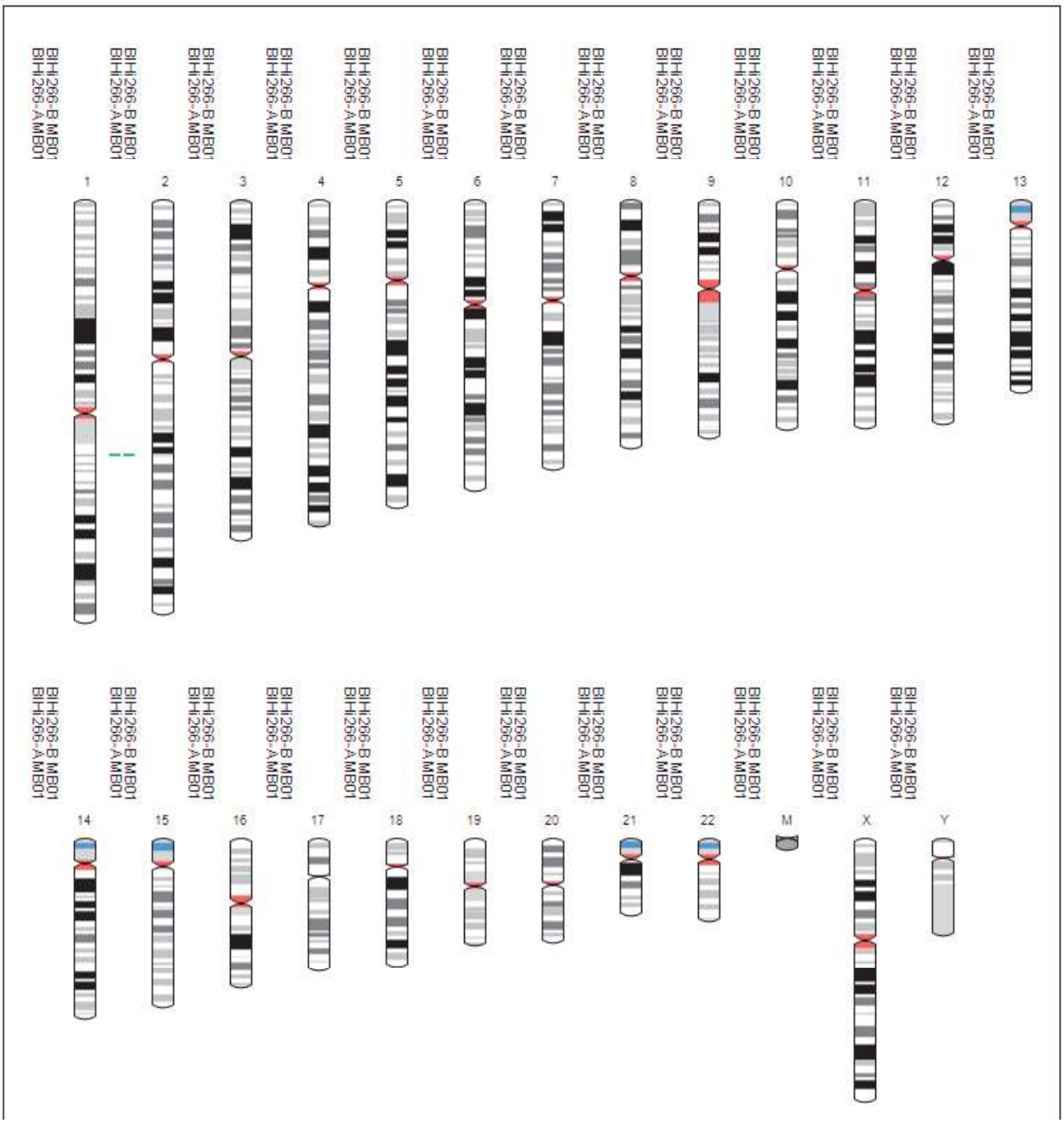
<u>Limitations:</u>
Other aberrations like the once listed below can´t be detected using this array.

- 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)



Interpretations:

- The cell line BIHi266-A has a normal karyotype showing no larger areas of deletions or insertions (above 2 MB).

- 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

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Datum: 2020.11.24 10:07:19 +01'00'

Responsible person / date: Sebastian Diecke/ 17/09/2020

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

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Single Nucleotide Polymorphism (SNP)- Karyotype**

Cell line name	BIHi267-B MB, Male
Passage No.	16
Name operator	Sebastian Diecke, Claudia Schaar
Date of testing	22.07.2020

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)

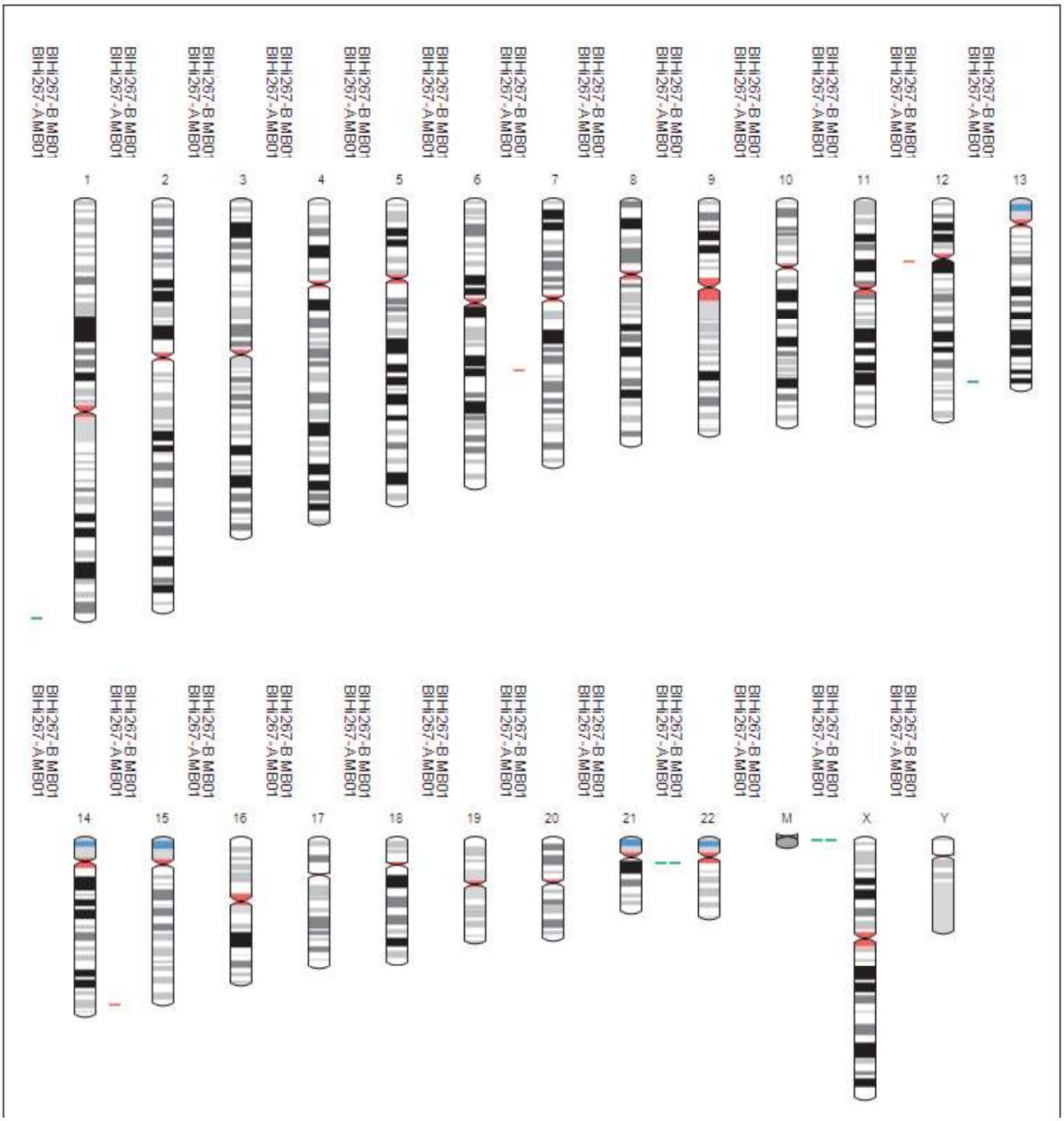
<u>Limitations:</u>
Other aberrations like the once listed below can´t be detected using this array.

  - 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)



Interpretations:

- The cell line BIHi267-B has a normal karyotype showing no larger areas of deletions or insertions (above 2 MB).
- A 0,446 Mb gain region was observed on chromosome 12.
- A 1,342 Mb gain region was observed on chromosome 22. The region is comparable with the 1,331 Mb gain region at BIHi267-A.

- 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

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Datum: 2020.12.19 15:10:14 +01'00'

Responsible person / date: Sebastian Diecke/ 17/09/2020

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

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Single Nucleotide Polymorphism (SNP)- Karyotype**

Cell line name	BIHi269-B_MB, iPSC, female
Passage No.	16
Name operator	Sebastian Diecke, Claudia Schaar
Date of testing	14.07.2020

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)

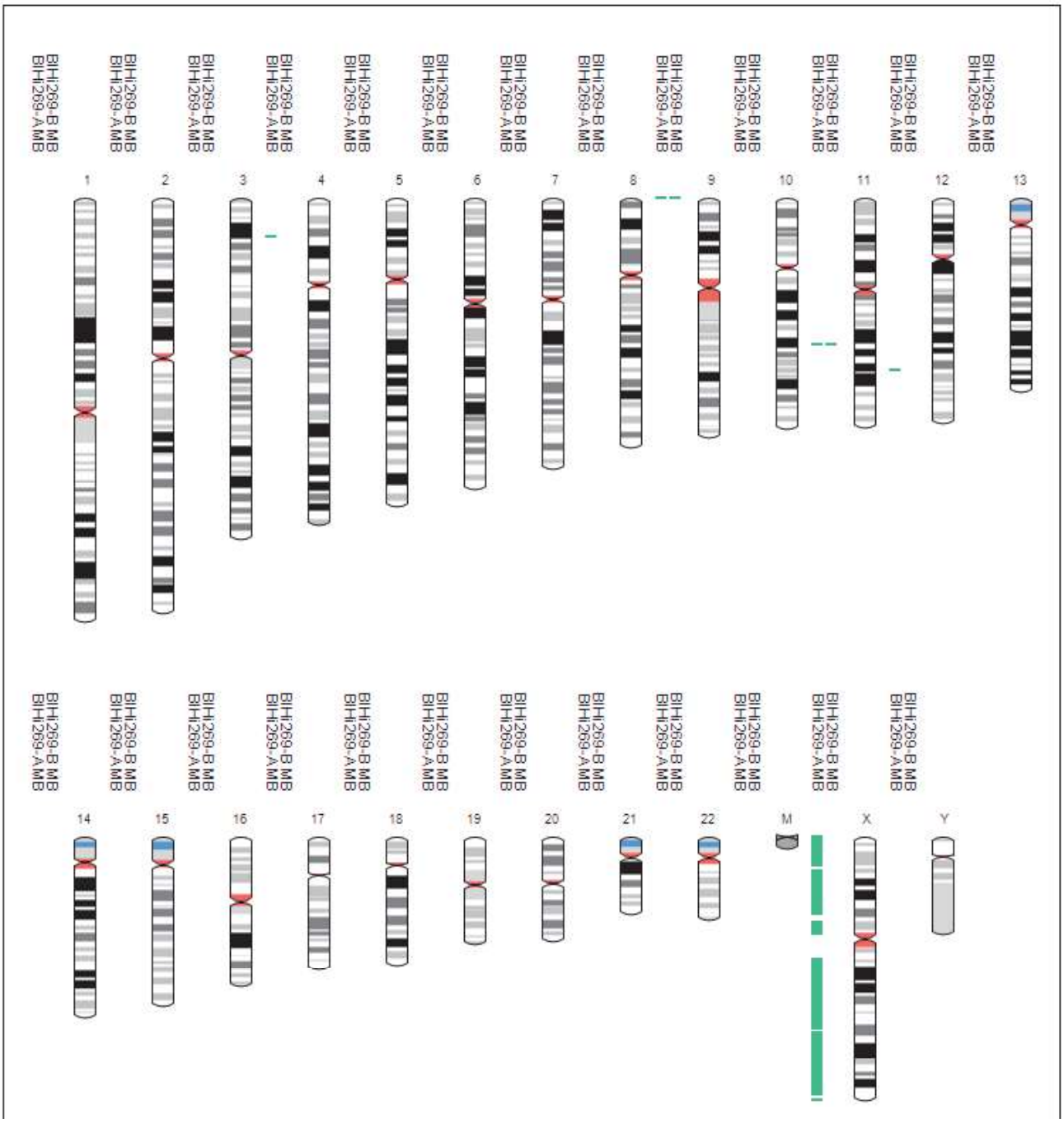
<u>Limitations:</u>
Other aberrations like the once listed below can´t be detected using this array.

  - 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)



Interpretations:

- The cell line BIHi269-B has a normal karyotype showing no larger areas of deletions or insertions (above 2 MB).
- A 0,567 Mb gain region was observed on chromosome 11.
- 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

Digital unterschrieben von Sebastian Diecke
DN: c=DE, st=Berlin, l=Berlin, o=Max-Delbrueck-Centrum fuer Molekulare Medizin (MDC), ou=Sebastian Diecke, cn=Sebastian Diecke, email=sebastian.diecke@mdc-berlin.de
Datum: 2020.10.20 12:06:09 +02'00'

Responsible person / date: Sebastian Diecke/ 17/09/2020

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

Single Nucleotide Polymorphism (SNP)- Karyotype

Cell line name	BIHi276-A
Passage No.	P18
Name operator	Sebastian Diecke, Gabi Born
Date of testing	27.05.2021

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

This method can detect the following aberrations:

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

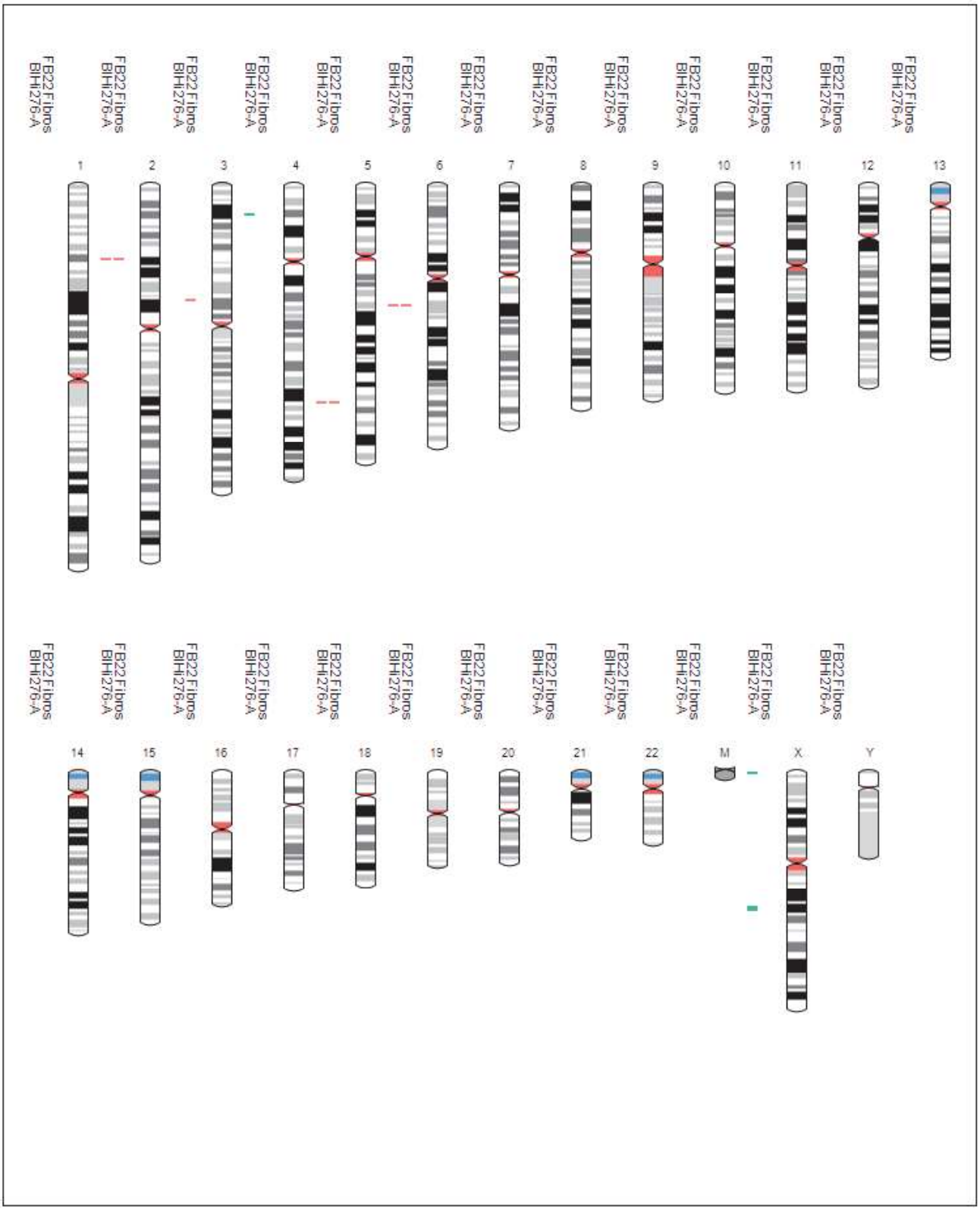
- Copy neutral aberrations Loss of heterozygosity (LOH) / Absence of heterozygosity (AOH)
- >20% mosaicism (for example: cultures where >1 of 5 cells is trisomy 12)

Limitations:
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

Virtual Karyotype:

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



Results:

Sample ID	Chr	Start	Stop	Length
BIHi276-A	X	88365553	92181907	3816354
BIHi276-A	X	2700157	3540527	840370
BIHi276-A	4	20727464	21244175	516711
FB22 Fibros	3	75453100	75636449	183349
BIHi276-A	2	49069969	49147027	77058
FB22 Fibros	2	49069969	49147027	77058
BIHi276-A	6	78975090	79019754	44664
FB22 Fibros	6	78975090	79019754	44664
FB22 Fibros	5	139931607	139931740	133
BIHi276-A	5	139931607	139931661	54

Interpretations:

- The cell line BIHi276-A has 3 reportable copy number change which are not present in the parental cell line (FB22).
    - Detected aberrations
        - 3,8 MB X-Chromosome
        - 0,8 MB X-Chromosome
        - 0,5 Mb Chromosome 4
    - Refer to “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

Digital unterschrieben von Sebastian Diecke
DN: c=DE, st=Berlin, l=Berlin, o=Max-Delbrueck-Centrum fuer Molekulare Medizin (MDC), ou=Sebastian Diecke, cn=Sebastian Diecke, email=sebastian.diecke@mdc-berlin.de
Datum: 2021.05.27 11:50:06 +02'00'

Responsible person / date: Sebastian Diecke/ 27/05/2021

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Sterility (Mycoplasma, Bacteria/Yeast/Fungi)**

Cell line / Passage No.	BIHi266-A / p19
Cell bank	MB01
Operator name	Norman Krüger
Test date	25.08.2020
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	28,8	Passed
2 (pos. control)	26,4	28,1	Passed
3	>45	26,5	**Negative**

Conclusion

The cell line BIHi266-A MB01 p19 was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Responsible person / date: Norman Krüger / 31.08.2020

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Sterility (Mycoplasma, Bacteria/Yeast/Fungi)**

Cell line / Passage No.	BIHi267-B / p19
Cell bank	MB01
Operator name	Norman Krüger
Test date	25.08.2020
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	28,8	Passed
2 (pos. control)	26,4	28,1	Passed
3	>45	26,6	**Negative**

Conclusion

The cell line BIHi267-B MB01 p19 was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Responsible person / date: Norman Krüger / 31.08.2020

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Sterility (Mycoplasma, Bacteria/Yeast/Fungi)**

Cell line / Passage No.	BIHi269-B / p19
Cell bank	MB01
Operator name	Norman Krüger
Test date	03.07.2020
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	29,3	Passed
2 (pos. control)	26,4	28,3	Passed
3	>45	27,7	**Negative**

Conclusion

The cell line BIHi269-B MB01 p19 was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Responsible person / date: Norman Krüger / 03.07.2020

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Sterility (Mycoplasma, Bacteria/Yeast/Fungi)**

Cell line / Passage No.	BIHi276-A / p18
Cell bank	MB01
Operator name	Norman Krüger
Test date	13.01.2021
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	28,7	Passed
2 (pos. control)	26,6	28,2	Passed
3	>45	28,3	**Negative**

Conclusion

The cell line BIHi276-A MB01 p18 was tested negative for Mycoplasma and Bacteria/Yeast/Fungi.

Responsible person / date: Norman Krüger / 13.01.2021

BERLIN INSTITUTE OF HEALTH
Charité & Max Delbrück Center

Stem Cell Core Facility

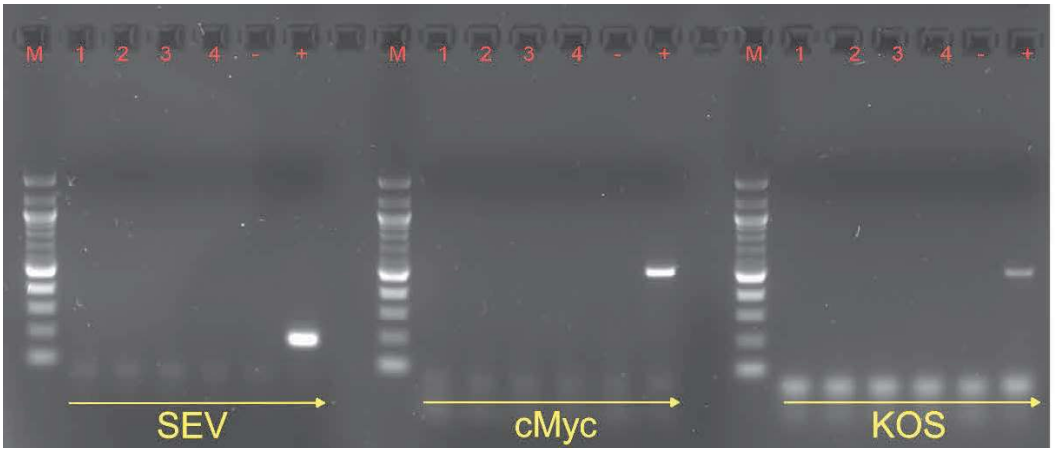
Report Sendai Virus Testing

Cell line name	BIHi266-A
Passage No.	13
Name operator	Norman Krüger
Date of testing	23.09.2020
Protocol	8.4. Testing for remaining Sendai virus_CytoTune 2.0
Sample	1: BIHi266-A +: positive control -: water

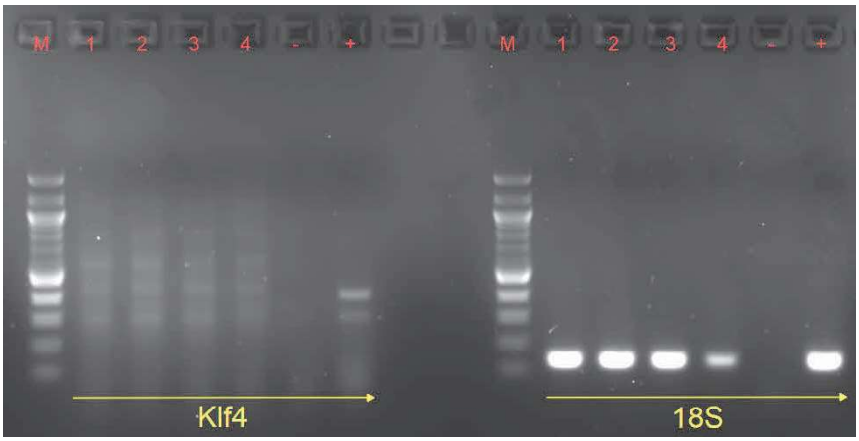
Results

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

PCR picture:



M = 100bp
1 = BIHi266-A
- = neg. ctrl.
+ = pos. ctrl.



PCR Results - Conclusion

The cell line BIHi266-A is tested negative for Sendai virus.

Responsible person / date: Norman Krüger / 23.09.2020

BERLIN INSTITUTE OF HEALTH
Charité & Max Delbrück Center

Stem Cell Core Facility

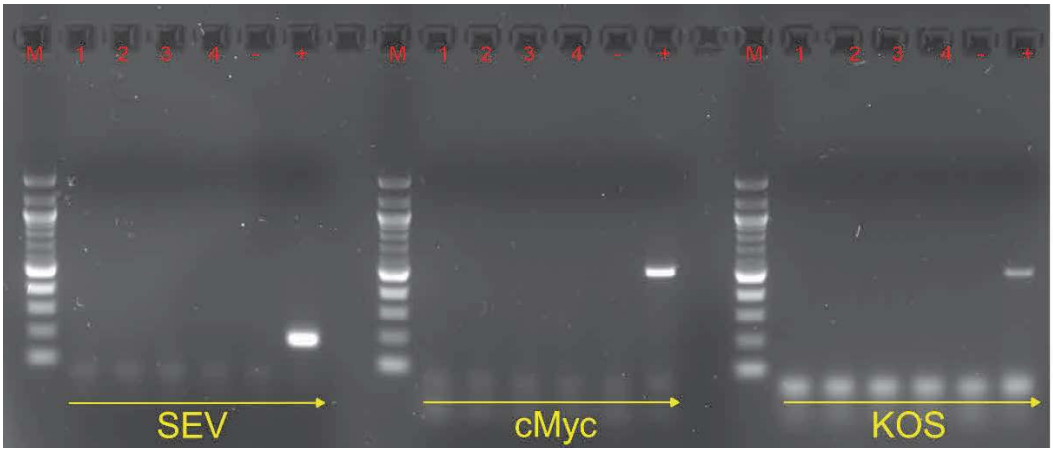
Report Sendai Virus Testing

Cell line name	BIHi267-B
Passage No.	13
Name operator	Norman Krüger
Date of testing	23.09.2020
Protocol	8.4. Testing for remaining Sendai virus_CytoTune 2.0
Sample	4: BIHi267-B +: positive control -: water

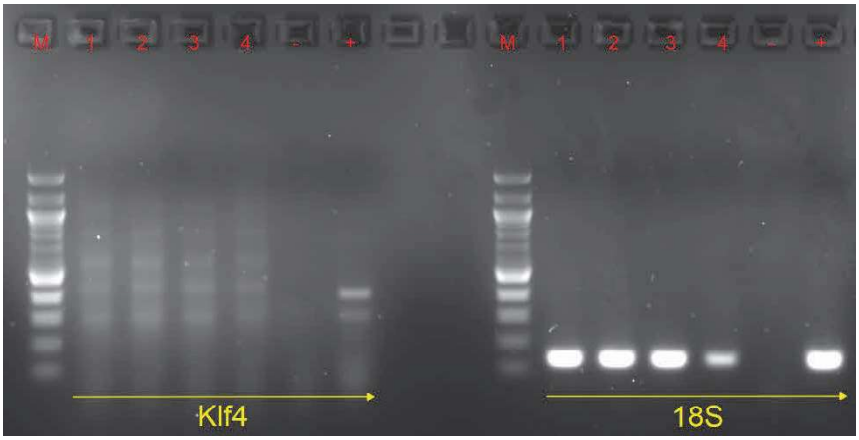
Results

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

PCR picture:



M = 100bp
4 = BIHi267-B
- = neg. ctrl.
+ = pos. ctrl.



Report Sendai Virus Testing

PCR Results - Conclusion

The cell line BIHi267-B is tested negative for Sendai virus.

Responsible person / date: Norman Krüger / 23.09.2020

BERLIN INSTITUTE OF HEALTH Charité & Max Delbrück Center	Stem Cell Core Facility **Report Sendai Virus Testing**

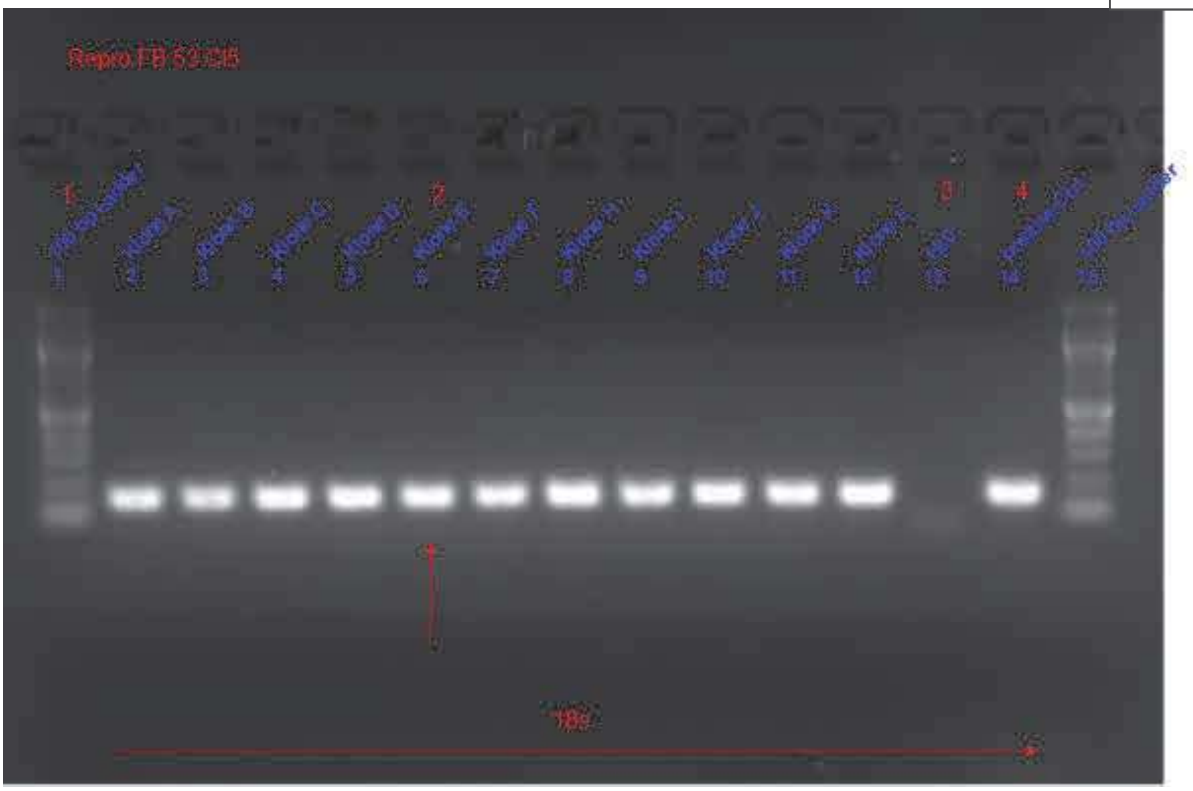
Cell line name	BIHi269-B
Passage No.	12
Name operator	Maren Wendt
Date of testing	03.06.2020
Protocol	8.4. Testing for remaining Sendai virus_CytoTune 2.0
Sample	5E: BIHi269-B +1: positive control H2O : water

Results

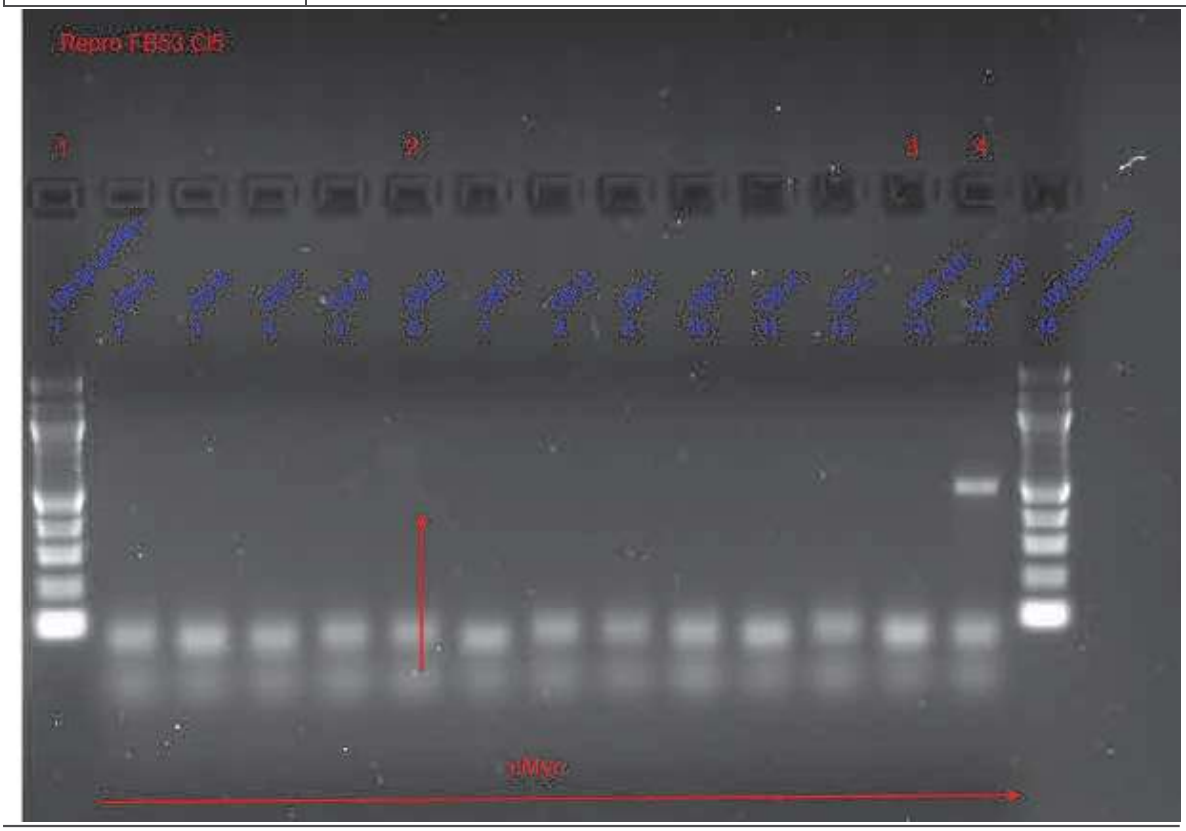
2 % standard agarose gel with DNA stain RotiSafe 5µL/100 mL

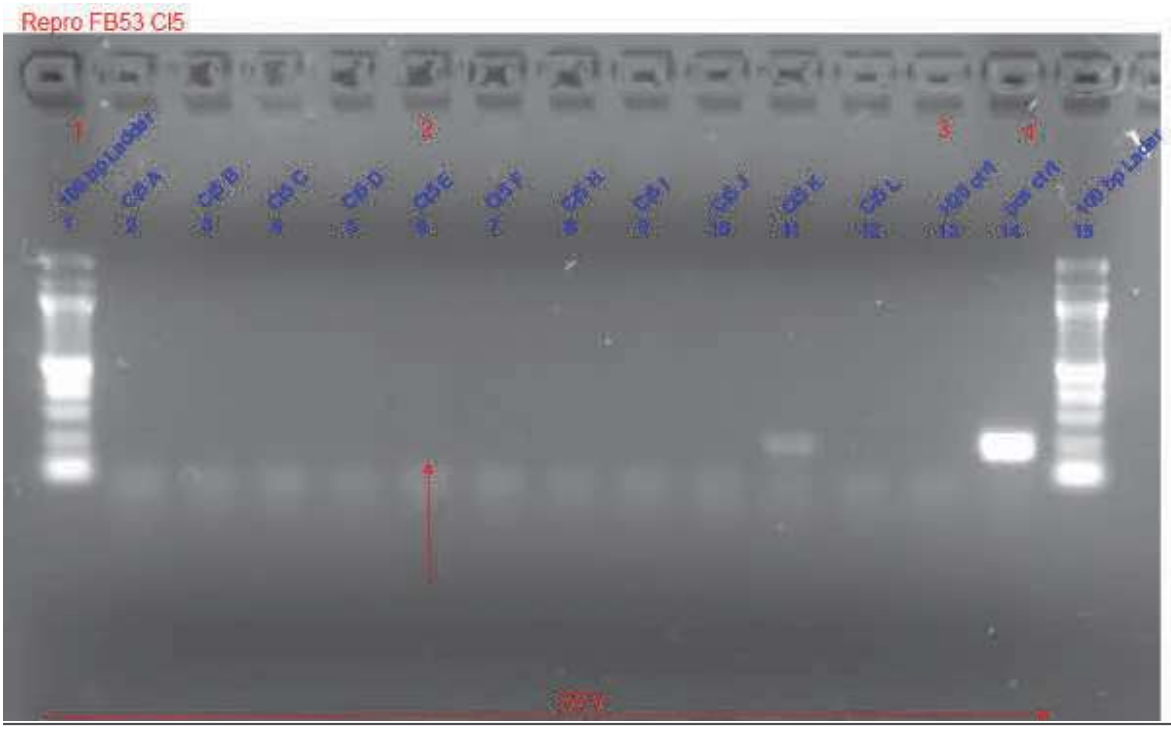
1 = 100bp
2 = BIHi269-B
3 = neg. ctrl.
4 = pos. ctrl.

PCR picture:

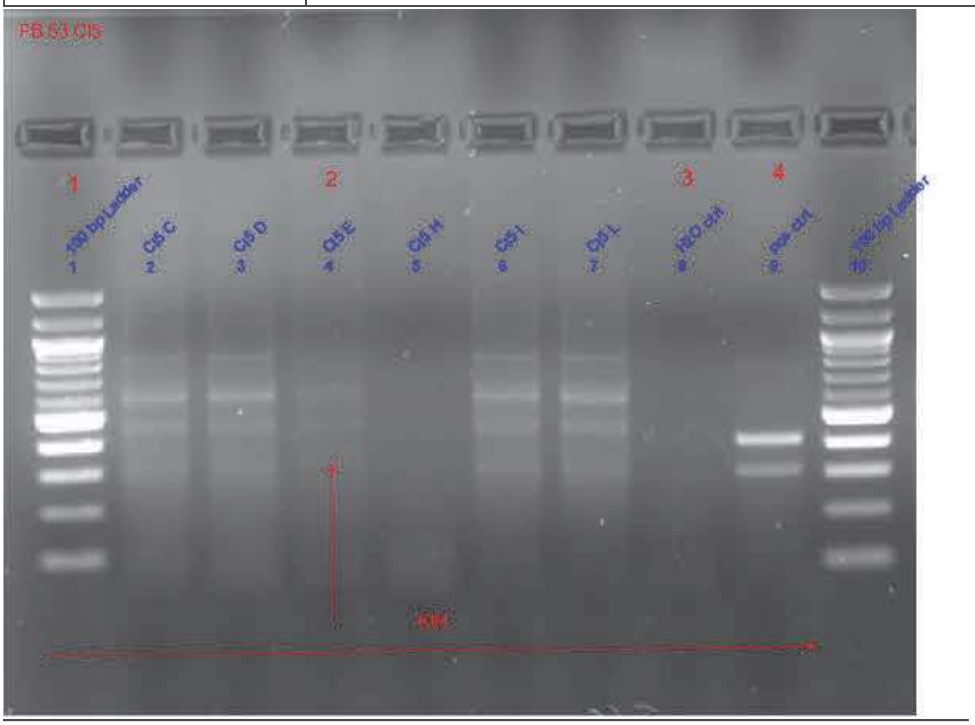


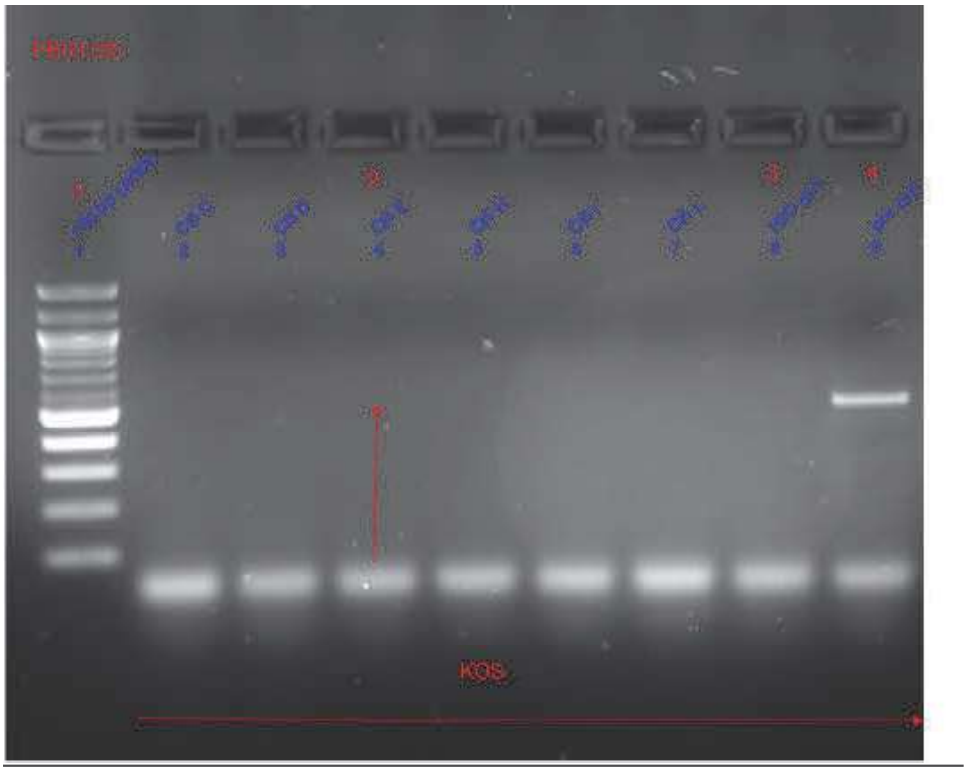
Report Sendai Virus Testing





Report Sendai Virus Testing





PCR Results - Conclusion

The cell line BIHi269-B is tested negative for Sendai virus.

Responsible person / date: Maren Wendt / 01.10.2020

BERLIN INSTITUTE OF HEALTH
Charité & Max Delbrück Center

Stem Cell Core Facility

Report Sendai Virus Testing

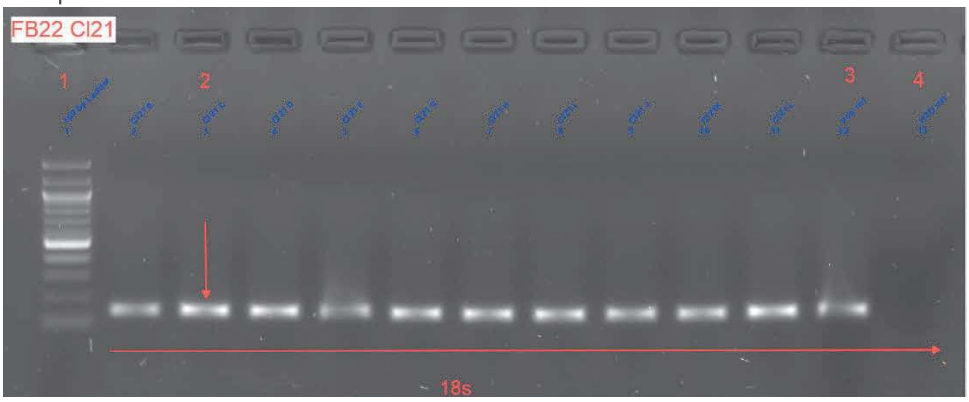
Cell line name	BIHi276-A
Passage No.	10
Name operator	Maren Wendt
Date of testing	10.09.2020
Protocol	8.4. Testing for remaining Sendai virus_CytoTune 2.0
Sample	21-C: BIHi276-A +1: positive control H2O : water

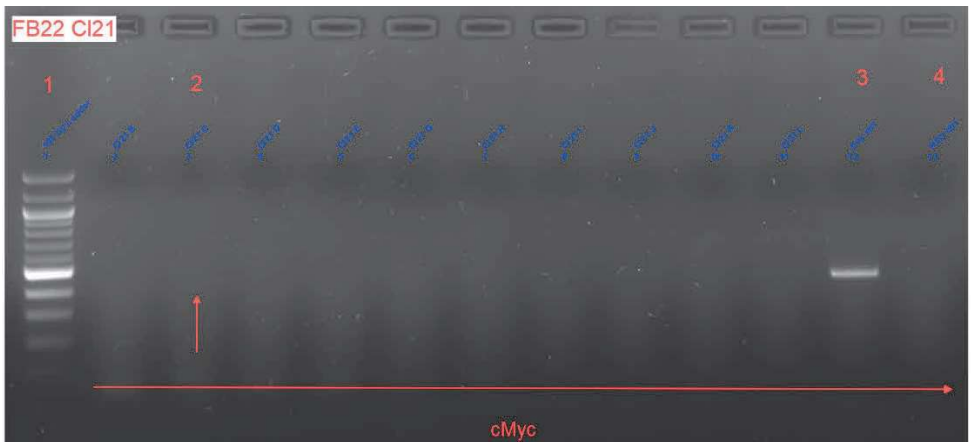
Results

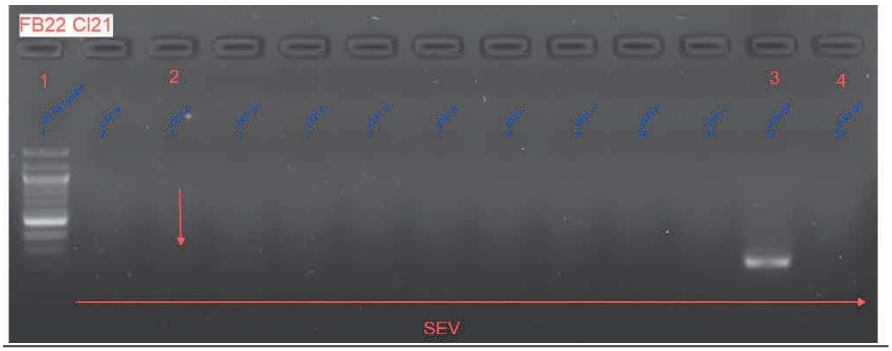
2 % standard agarose gel with DNA stain RotiSafe 5µL/100 mL

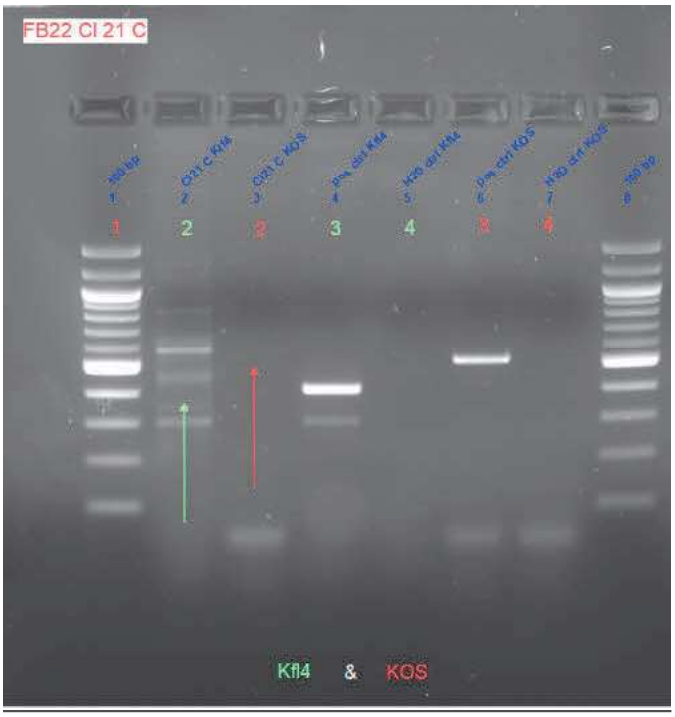
1 = 100bp
2 = BIHi276-A
3 = pos. ctrl.
4 = neg. ctrl.

PCR picture:









PCR Results - Conclusion

The cell line BIHi276-A is tested negative for Sendai virus.

Responsible person / date: Maren Wendt / 06.10.2020