Single Nucleotide Polymorphism (SNP)- Karyotype

Cell line name	11692 Fibroblast, **MDCi238-A**
Gender	Female
Passage No.	5, 14
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)

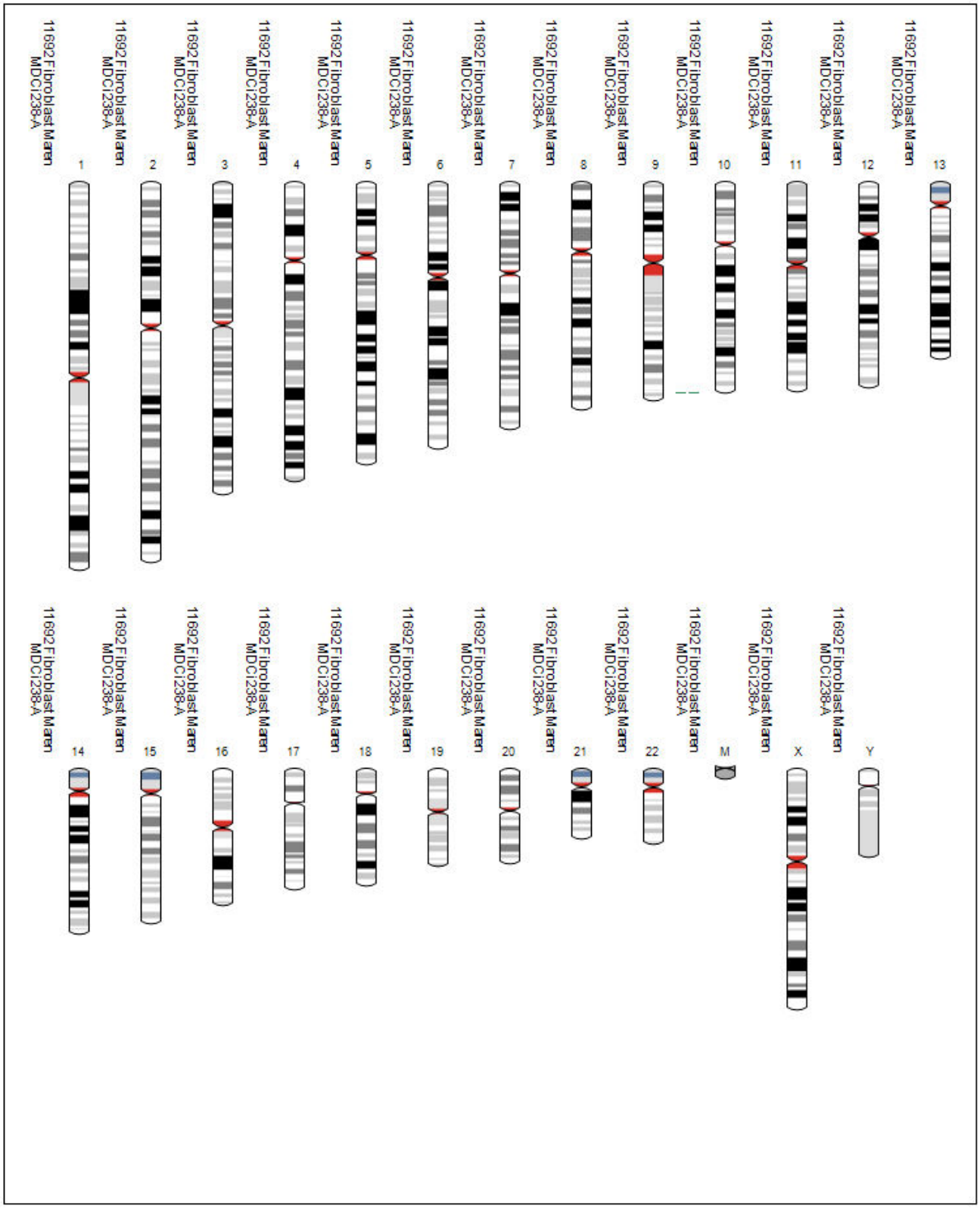
<u>Limitations:</u>
Other aberrations like the once listed below can´t be detected using this array.

  - 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 parental cell line 11692 Fibroblast and the established iPSC line MDCi238-A has a normal Karyotype.
- There was 1 copy number change within the parental line and the tested clone which was below our internal cutoff 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: 2022.04.29 09:51:51 +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

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
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- Genomic gains and losses
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- Copy neutral aberrations Loss of heterozygosity (LOH) / Absence of heterozygosity (AOH)
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<u>Limitations:</u>
Other aberrations like the once listed below can´t be detected using this array.

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

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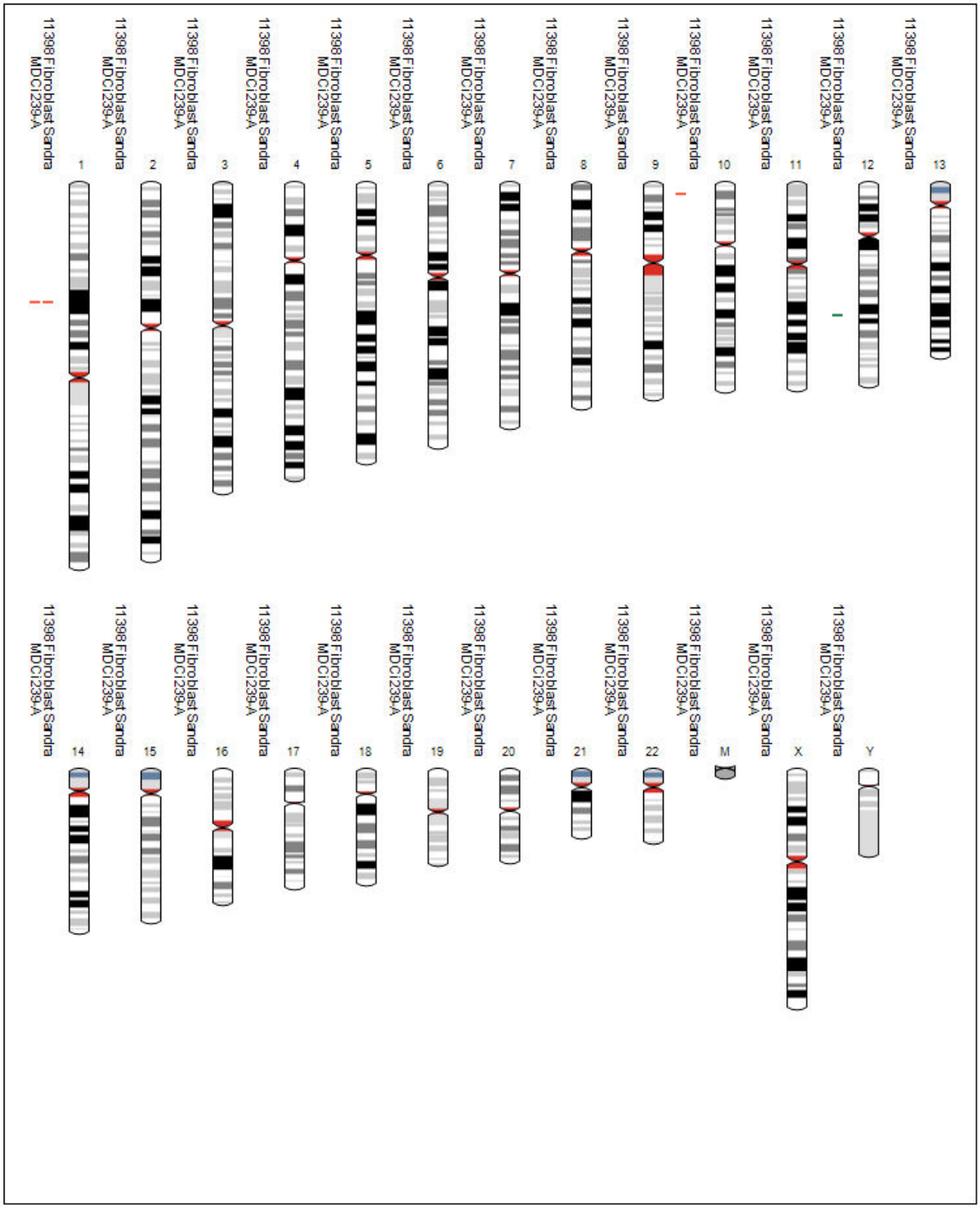
Virtual Karyotype:

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



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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.

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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.

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