

Cell line name	11624 Fibroblast, MDCi237-A
Gender	Male
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

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)

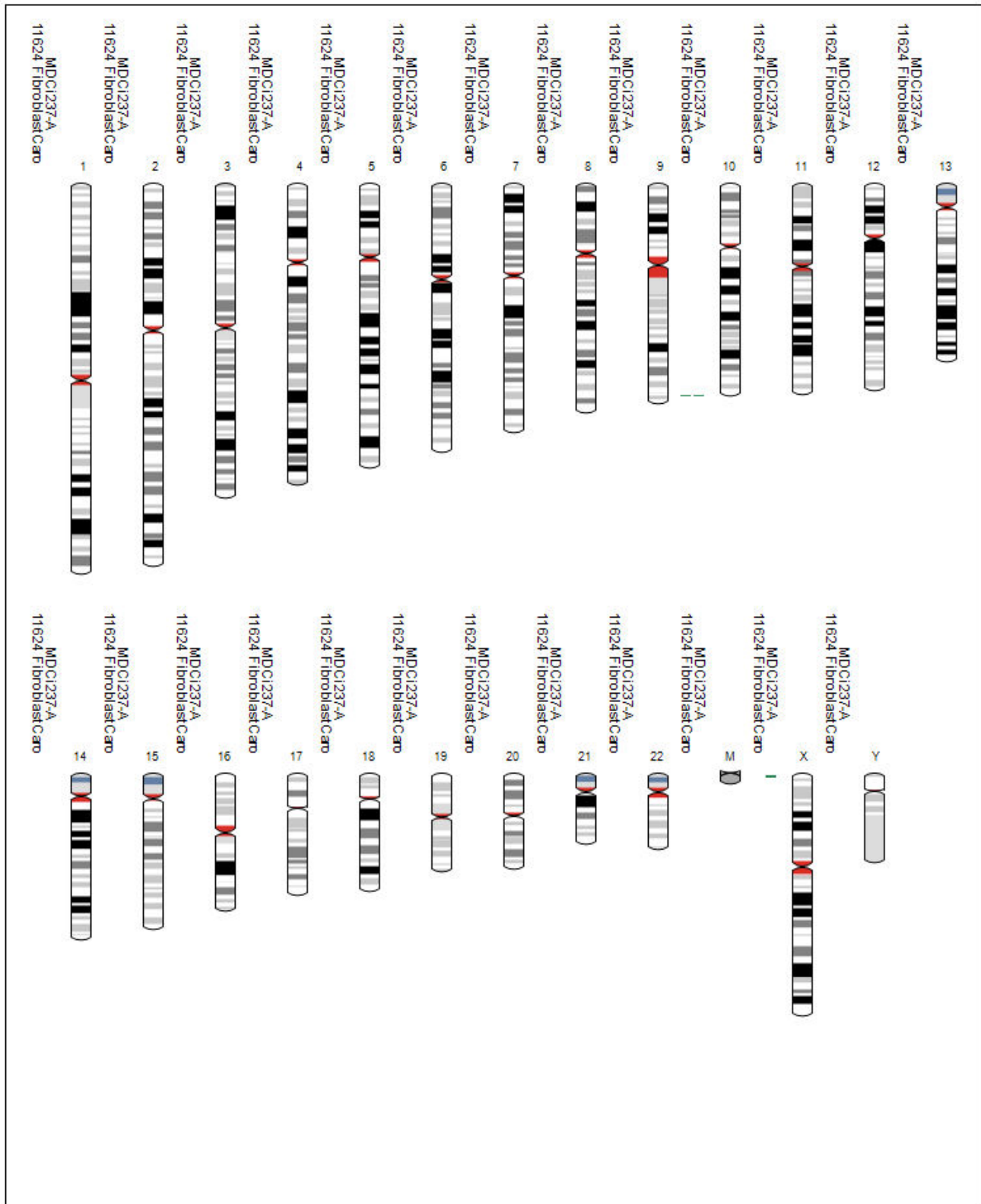
Limitations:

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:

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
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Interpretations:

- The cell line MDCi237-A has an inconspicuous karyotype.
- There were some reportable copy number changes (CNV's) within the tested clone but below the threshold values mentioned above.
 - 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: 2022.05.17 08:50:15 +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-microarray-.cmsx>

Attachments:

Cytogenetics Report
Table of affected genes
Karyogram only

Cell line name	11624 Fibroblast, MDCi237-B
Gender	Male
Passage No.	5, 15
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

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)

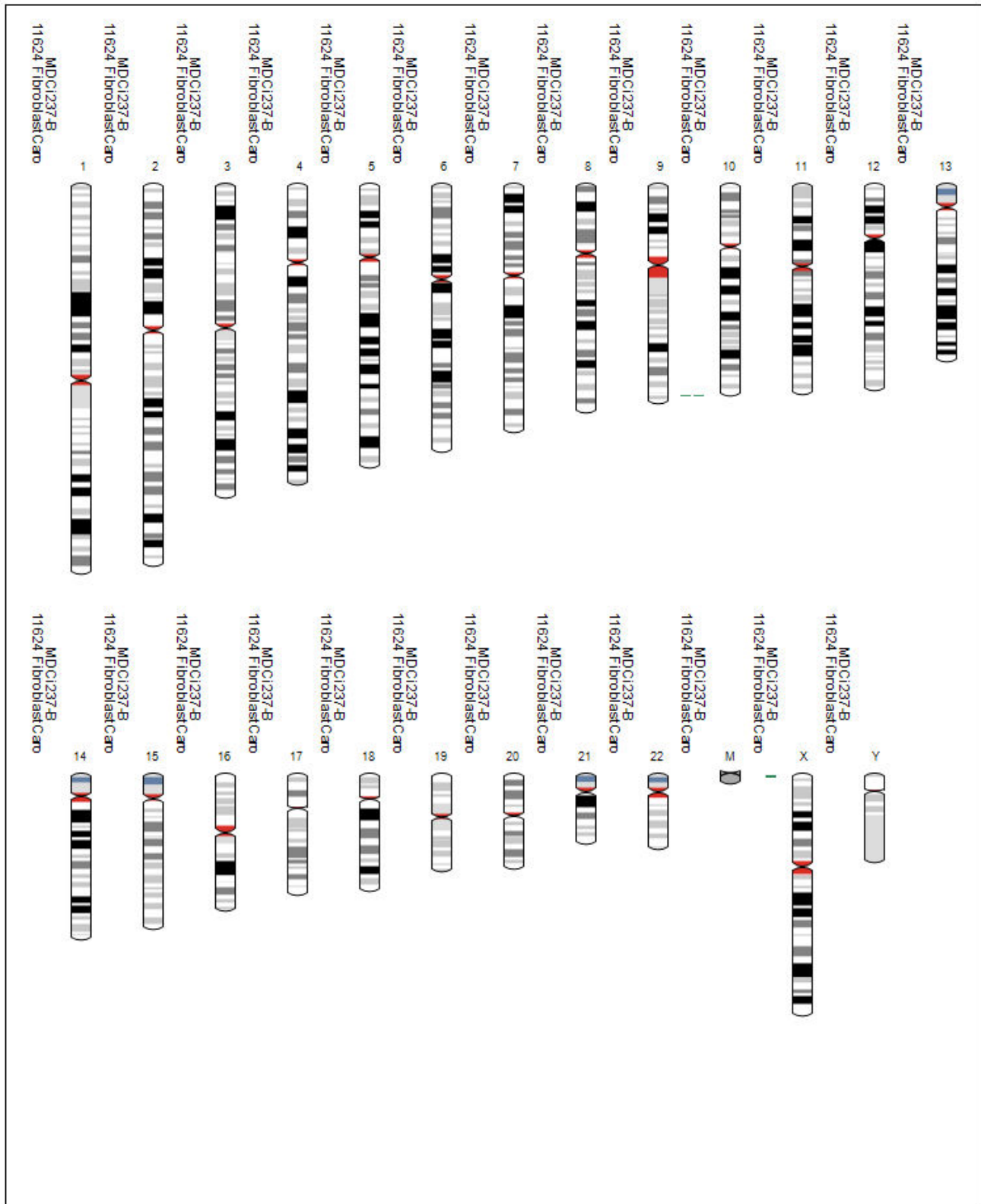
Limitations:

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:

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
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Interpretations:

- The cell line MDCi237-B has an inconspicuous karyotype.
- There were some reportable copy number changes (CNV's) within the tested clone but below the threshold values mentioned above.
 - 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: 2022.05.17 08:49:43 +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-microarray-.cmsx>

Attachments:

Cytogenetics Report
Table of affected genes
Karyogram only

	cell line		
Sample (cell type, ID)	iPSC	CRMi004-A	
Passage No.	25		
Bank ID	MB01		
DNA sample ID	D0527		
Chip-ID and Position	26676550040, R08C01		
Date of testing	09.11.2022		
Call Rate	0.9926064		✓
Gender (provided/estimated from chip data)	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

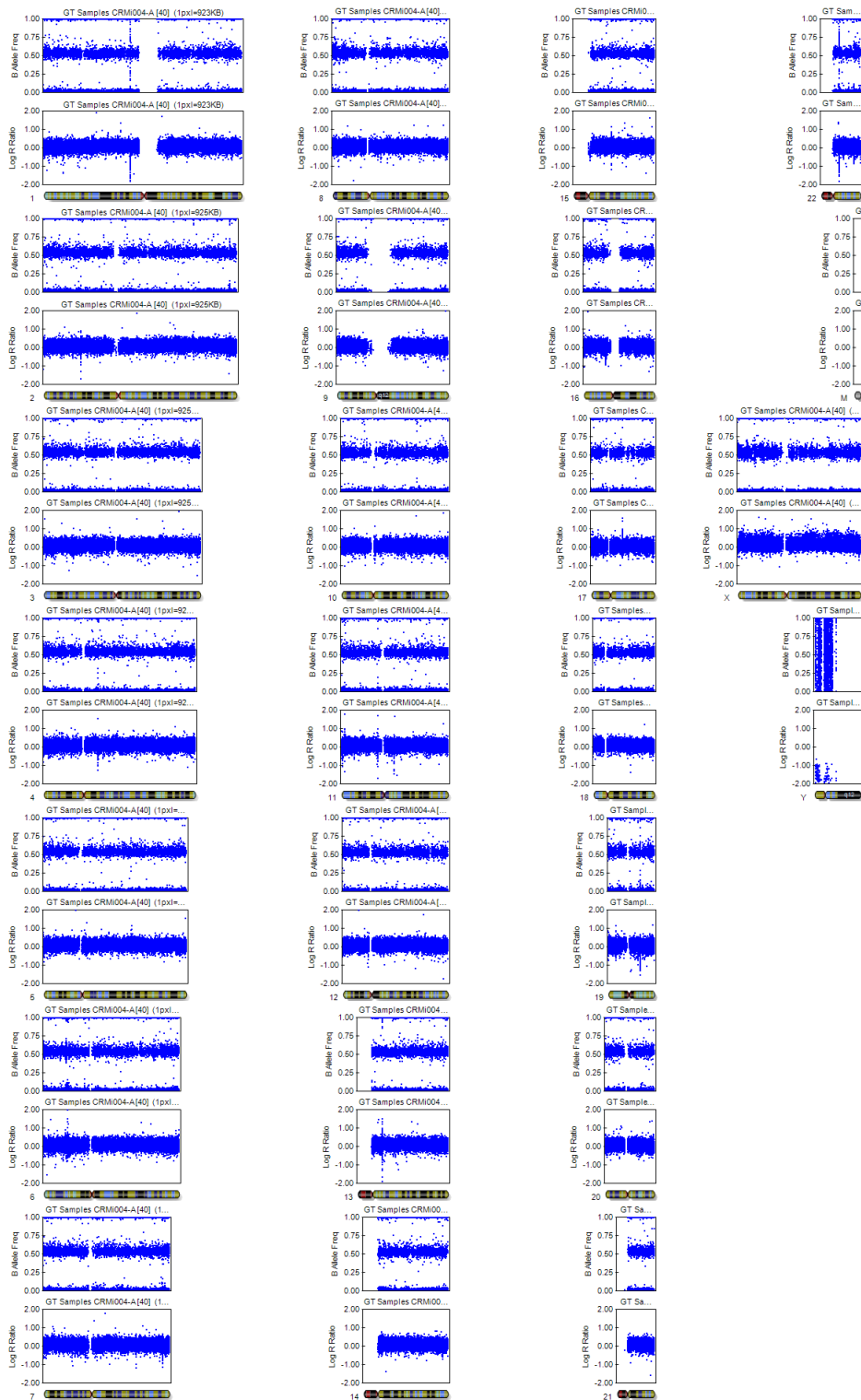
Genotype Analysis
GenomeStudio: GenomeStudio V2.0.5
Genotyping Module: V2.0.5

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.

Virtual Karyotype: CRMi004-A



Call Tables

CNV regions found in **CRMi004-A**

Chr	Start	End	Size (bp)	CNV Value	Variant Type	Number of Genes*
1	185394834	187057847	1663013	2	LOH	
4	132731954	133740742	1008788	2	LOH	
5	143042579	144369950	1327371	2	LOH	
6	31605167	32798548	1193381	2	LOH	
12	78643490	80341397	1697907	2	LOH	
12	111226383	113032772	1806389	2	LOH	
17	49886915	51308172	1421257	2	LOH	
17	43487968	46020698	2532730	2	LOH	

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

Interpretation

No reportable genomic abnormalities were detected in the CRMi004-A iPSC line at the stated level of resolution.

Information about genes in the non-reportable 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>). The cell line (add name) passes molecular karyotyping.

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-microarray-.cmsx>