Combined NGS-based copy number and genome-wide SNP analysis for the screening of abnormal ploidy and maternal contamination for PGT-A

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Motivation and background

- Going beyond PGT-A limitations → detection of triploid embryos 69,XXX
- Provide accurate and reliable PGT-A for embryos with abnormal PN (0PN, 1PN, 3PN)

NGS-based PGT-A evolution 2014 - 2024

NGS-based PGT-A has become very fast and cost effective over the last decade

VeriSeq PGS Kit (2014), Illumina

- ✓ WGA (3 hours)
- ✓ Library preparation (5-6 hours)
- ✓ Sequencing of 24 samples on MiSeq
- ✓ 1000k of 36 bp reads / embryo
- ✓ Mosaic detection 20-80%

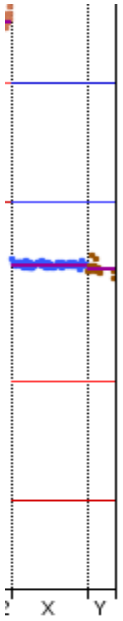
- ➢ Mosaics (intermediate CNVs) → not clinically significant
- ➢ Resolution of NGS is scalable
  1000k → 500k → 250k →100k reads / embryo
- ➢ 100k reads per embryo is sufficient to detect whole chromosomal monosomies and trisomies

PG-Seq Rapid v2 Kit (2024), Revvity

- ✓ Library prep is a part of WGA
- ✓ Individual sample normalization not needed
- ✓ Ready-to-go sequencing of 96 samples in 4 hours
- ✓ Hands-on time <1hour
- ✓ No automation needed
- ✓ Sequencing fully scalable
- ✓ MiSeq, NextSeq, NovaSeq
- ✓ 100k → 5900k → ?

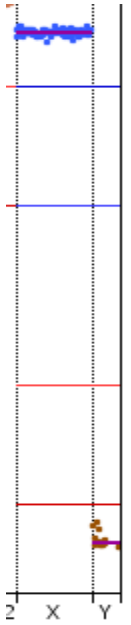
NGS-based PGT-A limitations and options

Ploidy detection is limited using NGS-based CNV analysis



normal male 46,XY

triploid 69,XXY



normal female 46,XX
haploid 23,X ?
triploid 69,XXX ?

Options for genome-wide SNP analysis:

1. SNP array – laborious and involves higher financial costs
2. SNP targeted amplification and NGS – not commercially available for our lab
3. SNP enrichment and NGS following WGA – not commercially available for our lab
4. **Increase number of reads per embryo → explore combination of CNV and SNP-based PloidyAnalysis using our current PGT-A platform PG-Seq Rapid v2 (Revvity)**

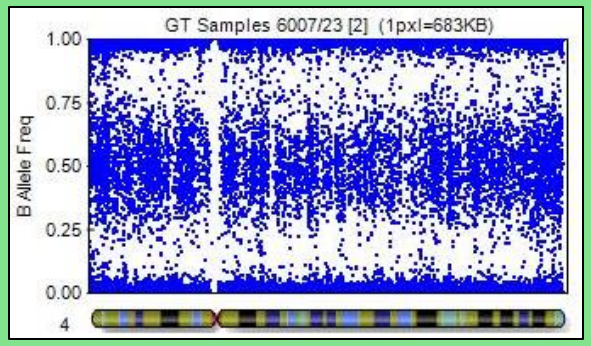
Pilot study on PloidyAnalysis

- PGT-M cases (SNP array) → 4 diploid, 2 haploid and 4 triploid embryos
- 5,9 mil reads per embryo → SNP identification (depth >10x) → allele ratio analysis

		B-freq	
B	A	0.50	HET
B	B	1.00	HOM
A	A	0.00	HOM
B	A	0.50	HET
A	B	0.50	HET
A	A	0.00	HOM
B	B	1.00	HOM

Ploidy Result	Diploid (2n)	
Total SNPs	250	%
DIV	70	**28%**
HET	48	**19%**
HOM	132	**53%**

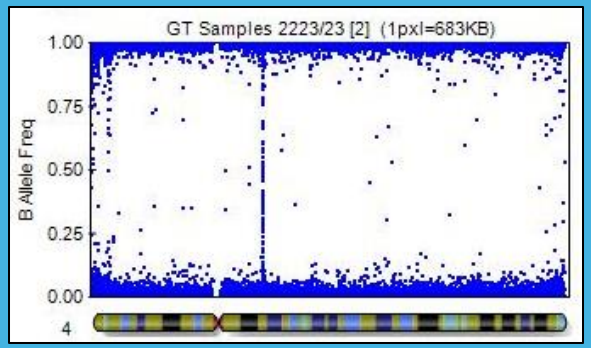
diploid sample (2n)



	B-freq	
A	0.00	HOM
B	1.00	HOM
A	0.00	HOM
A	0.00	HOM
B	1.00	HOM
A	0.00	HOM
B	1.00	HOM

Ploidy Result	Haploid (1n)	
Total SNPs	250	%
DIV	0	**0%**
HET	0	**0%**
HOM	250	**100%**

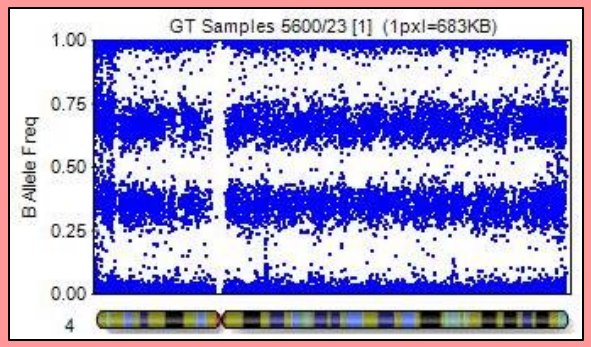
haploid sample (1n)



			B-freq	
B	A	A	0.33	DIV
B	B	B	1.00	HOM
A	A	A	0.00	HOM
B	A	A	0.33	DIV
A	B	B	0.66	DIV
A	A	A	0.00	HOM
B	B	B	1.00	HOM

Ploidy Result	Triploid (3n)	
Total SNPs	250	%
DIV	118	**47%**
HET	35	**14%**
HOM	97	**39%**

triploid sample (3n)



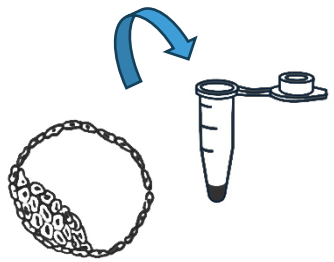
Validation study on combined NGS-based copy number and genome-wide SNP analysis

- Designed as a prospective study in PGT-A for 2023
- 5.9 mio reads followed by CNV and PloidyAnalysis for every embryo sample
- Embryos with divergent allele ratio subjected to rebiopsy and reanalysis using SNP array

PGT-A in 2023

In 2023 - trophectoderm cells from **9789** embryos were examined using NGS

TE biopsy → **amplification** → **NGS** → **analysis**



PG-Seq™ Rapid Kit v2
(Revvity)

High Output Reagent Kit v2.5
75 cycles - single end
NextSeq 550 (Illumina)

5.9 mio reads / embryo

PG-Find 2.0
(Revvity)

+

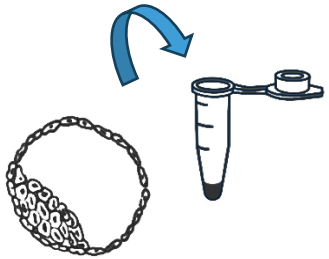
SNP-based
PloidyAnalysis
(in house pipeline)

62 embryos (0.63%)
rebiopsy and reanalysis

PloidyAnalysis validation

62 samples with divergent allele ratio subjected to rebiopsy and reanalysis

rebiopsy → **MDA amplification** → **SNP array** → **data analysis**



REPLI-g Advanced
DNA Single Cell Kit
(Qiagen)

Infinium Global Screening
Array-24+ v3.0 Kit
iScan
(Illumina)

GenomeStudio 2.0 Software
(Illumina)

+

Omnia AneuScan™

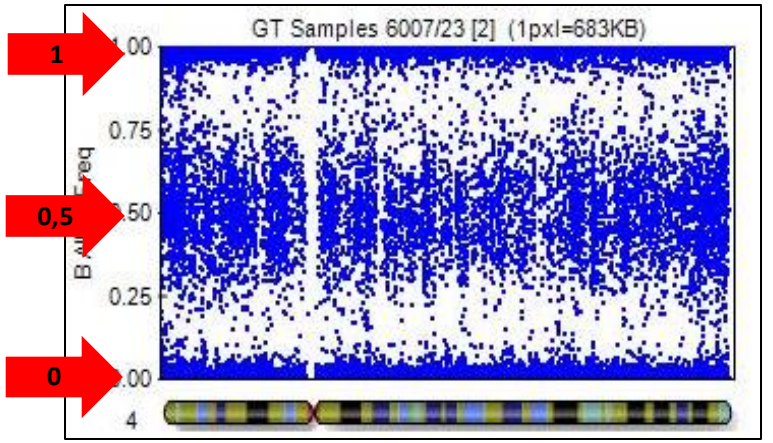
(ExOvo Genomics, UK, **A. Handyside**)

Sample verification

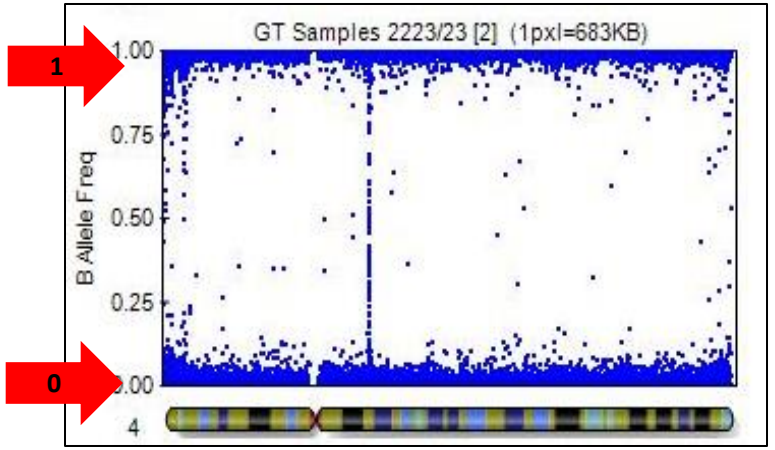
data analysis

GenomeStudio 2.0 Software
(Illumina)

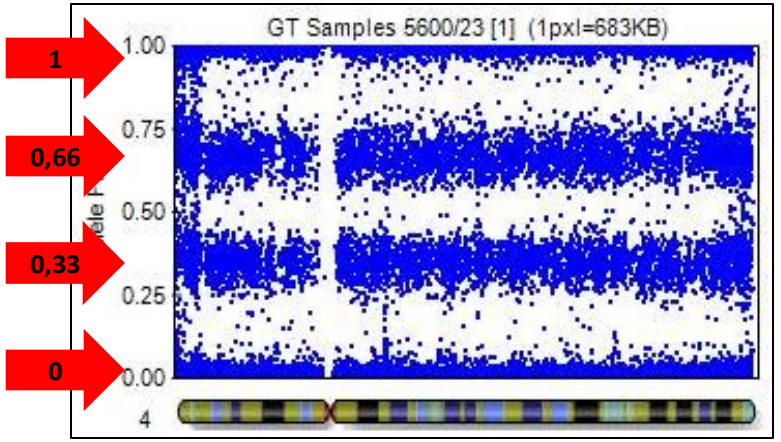
diploid sample



haploid sample

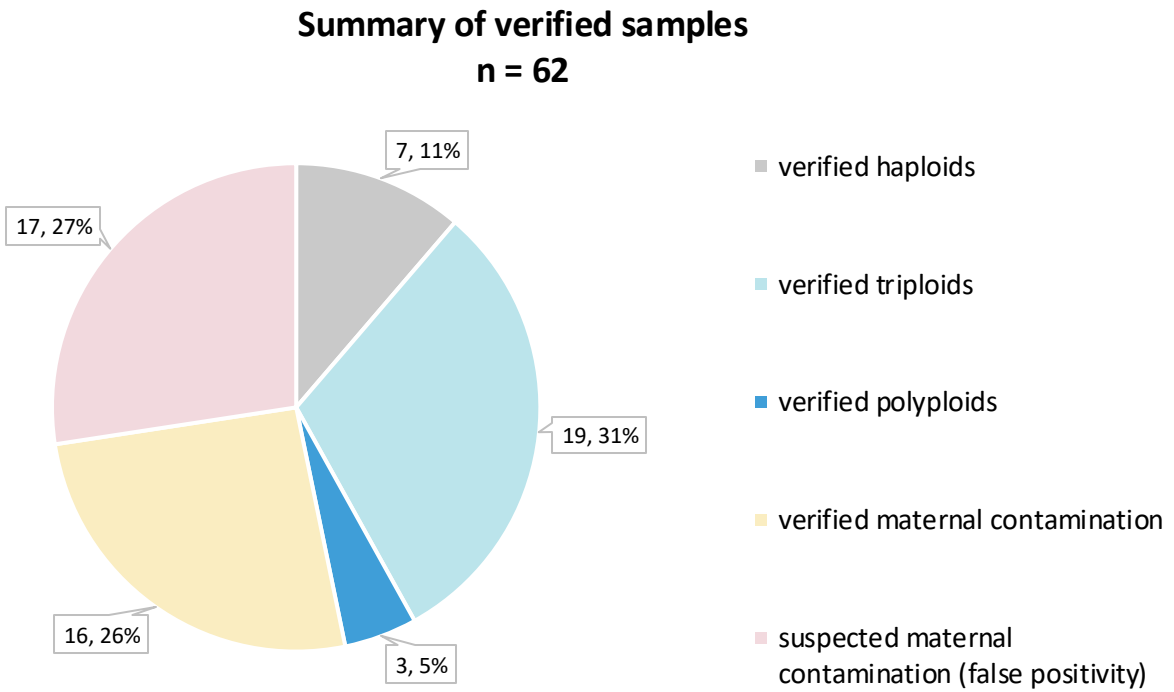


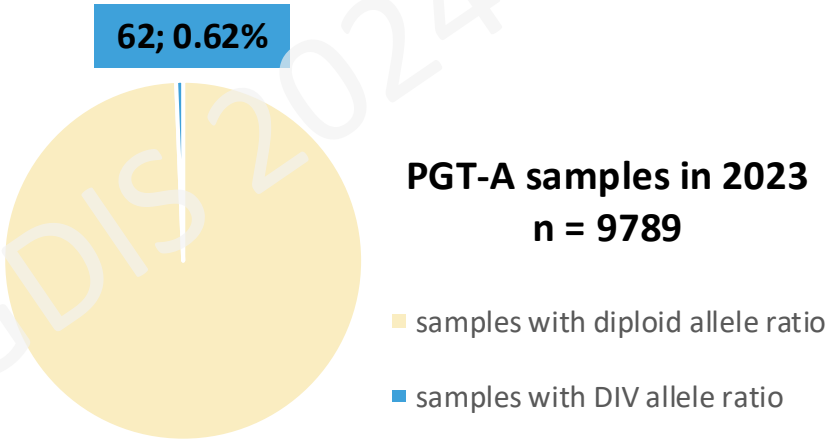
triploid sample



Validation results

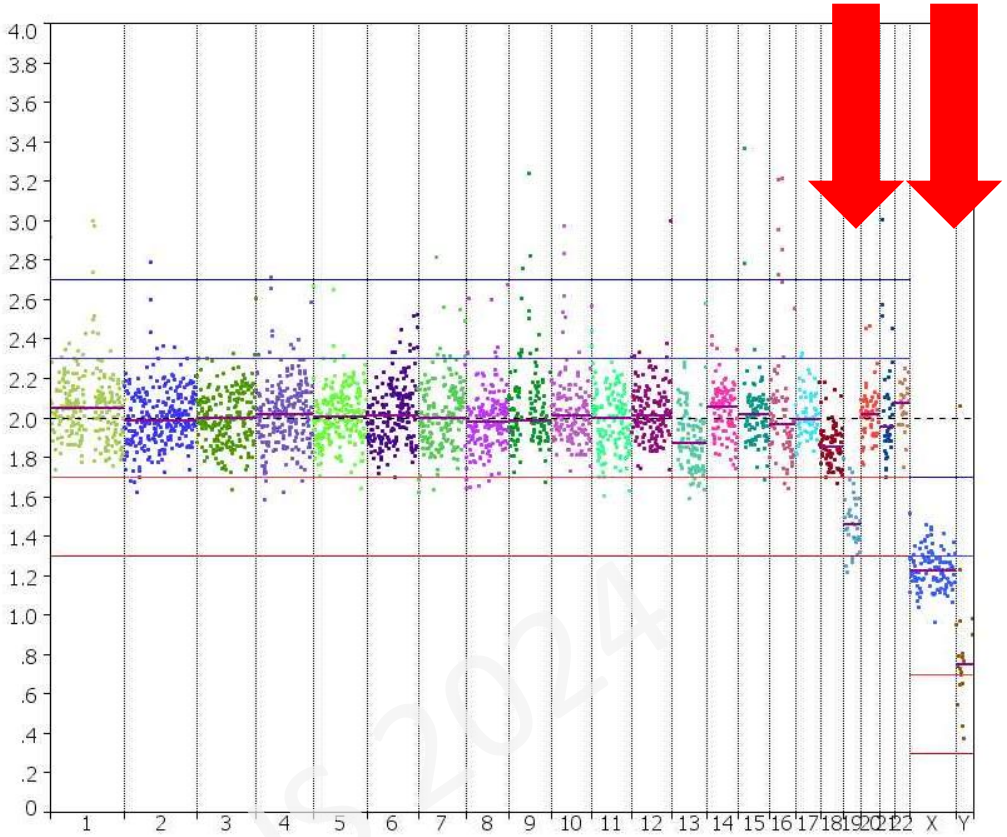
Summary of verified samples			
total samples included (post PGT-A)	62		
verified haploids (1n)	7		11.29%
verified triploids (3n)	19	9x XXX 10x XXY	30.65%
verified polyploids	3		4.84%
verified maternal contamination	16		25.81%
suspected maternal contamination (FP)	17		27.42%



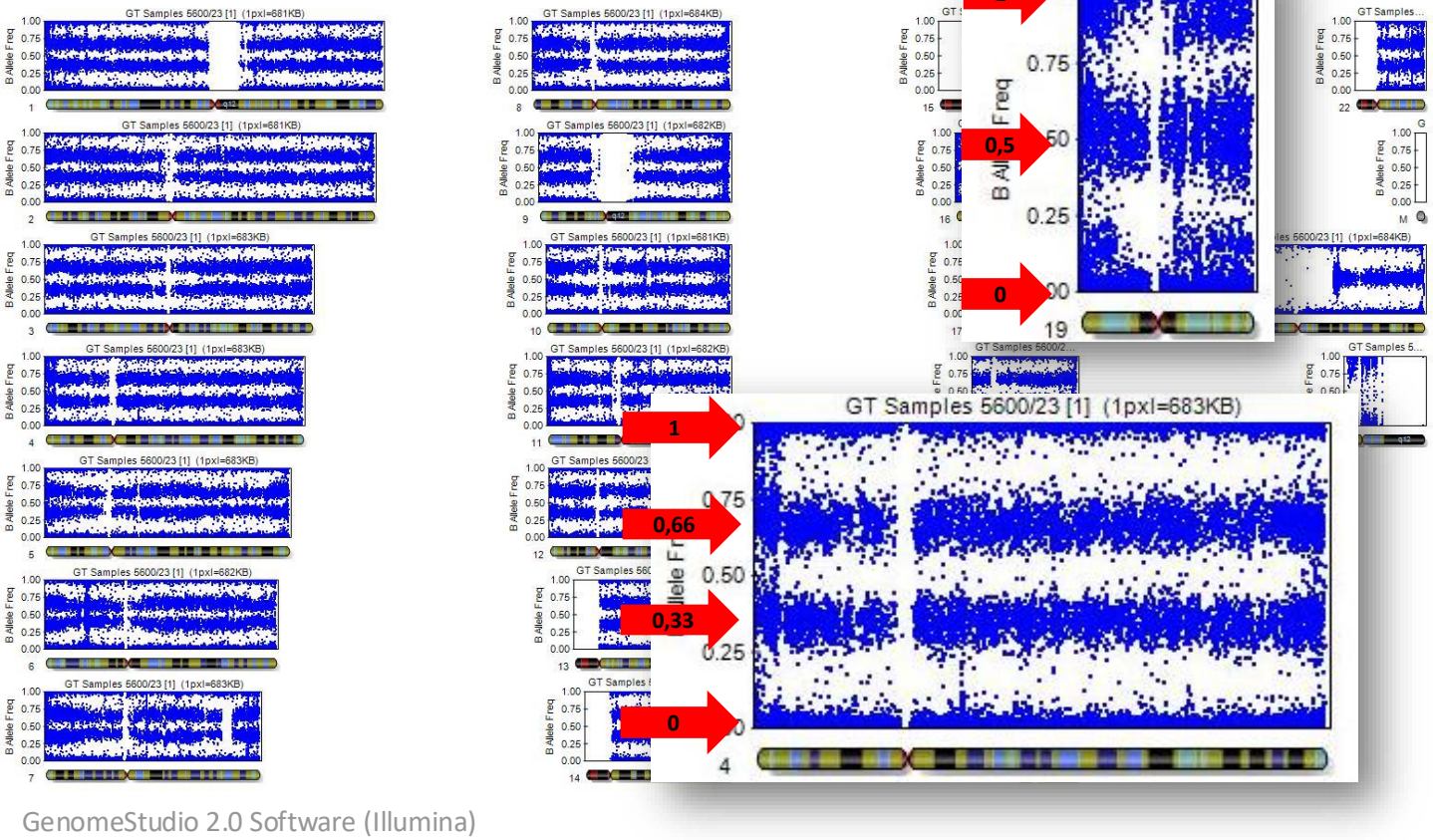


Triploid sample

PloidyAnalysis		
total amount of SNPs	191	
DIV	90	47.1 %
HET	27	14.1 %
HOM	74	38.7 %



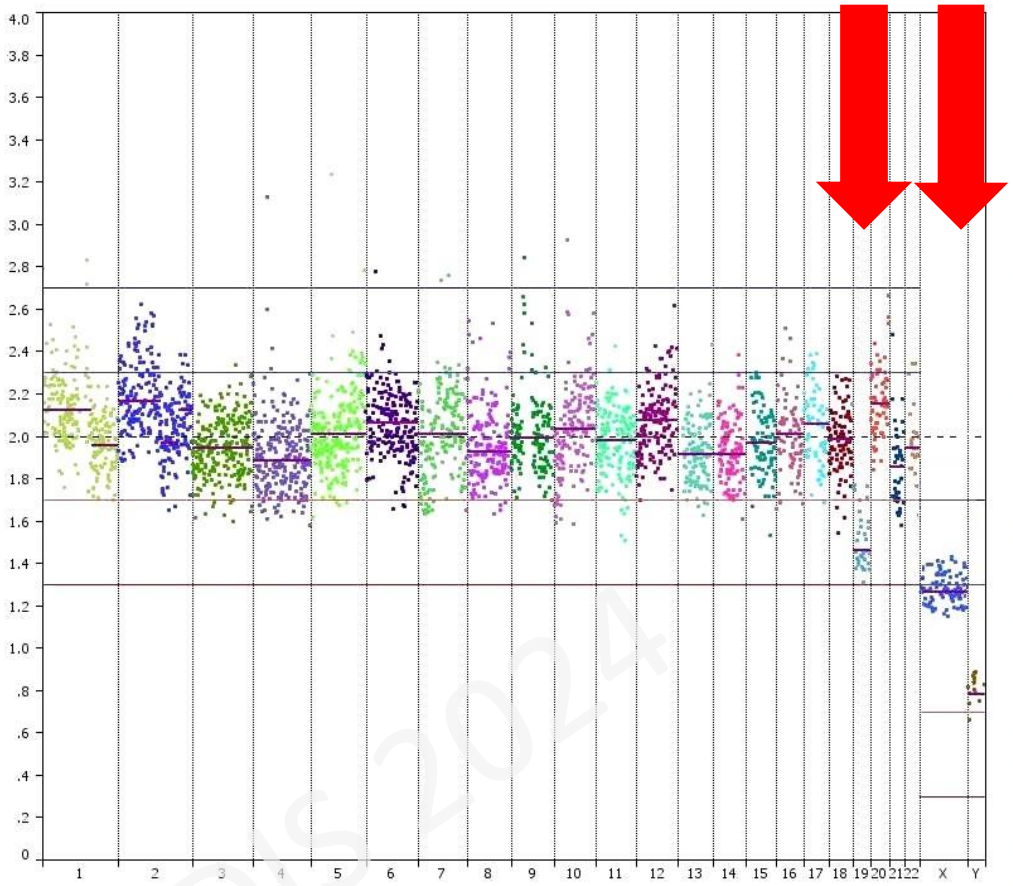
PG-Find 2.0 (Revvity)



GenomeStudio 2.0 Software (Illumina)

Confirmed contamination

PloidyAnalysis		
total amount of SNPs	250	
DIV	105	42 %
HET	40	16 %
HOM	105	42 %



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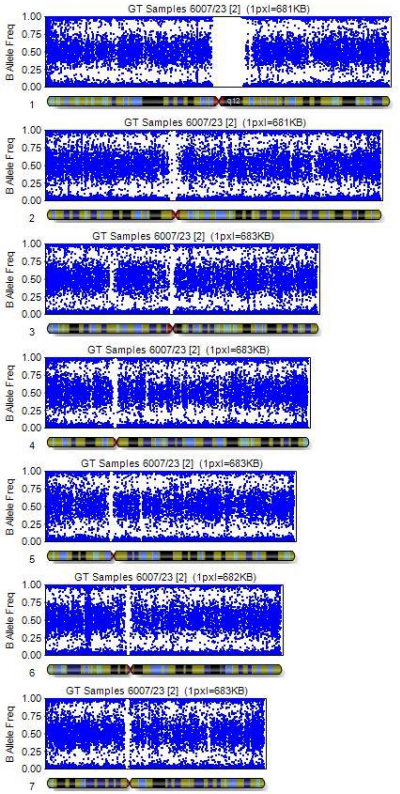
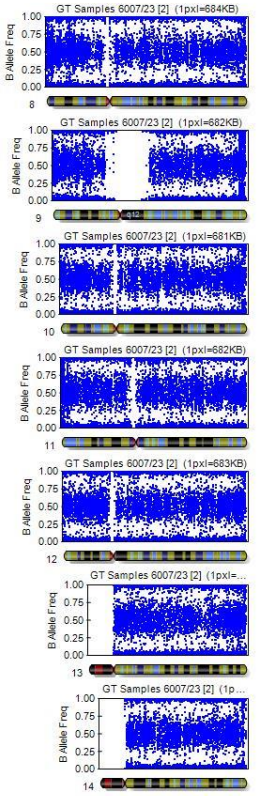
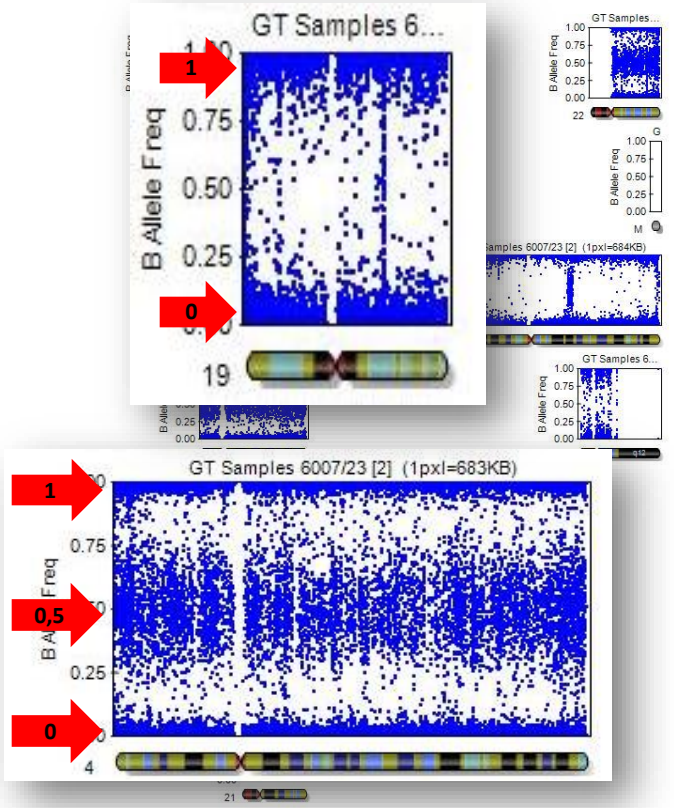


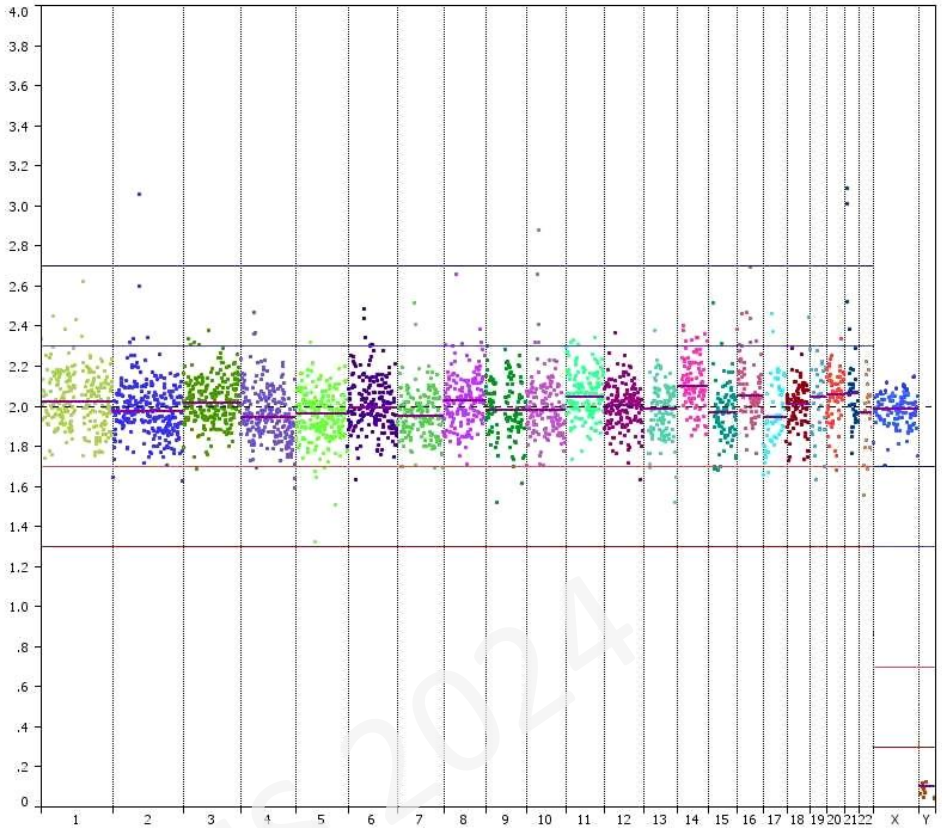




GenomeStudio 2.0 Software (Illumina)

Haploid sample

PloidyAnalysis		
total amount of SNPs	**83**	
DIV	1	1.2 %
HET	0	1.1 %
HOM	82	98.8 %



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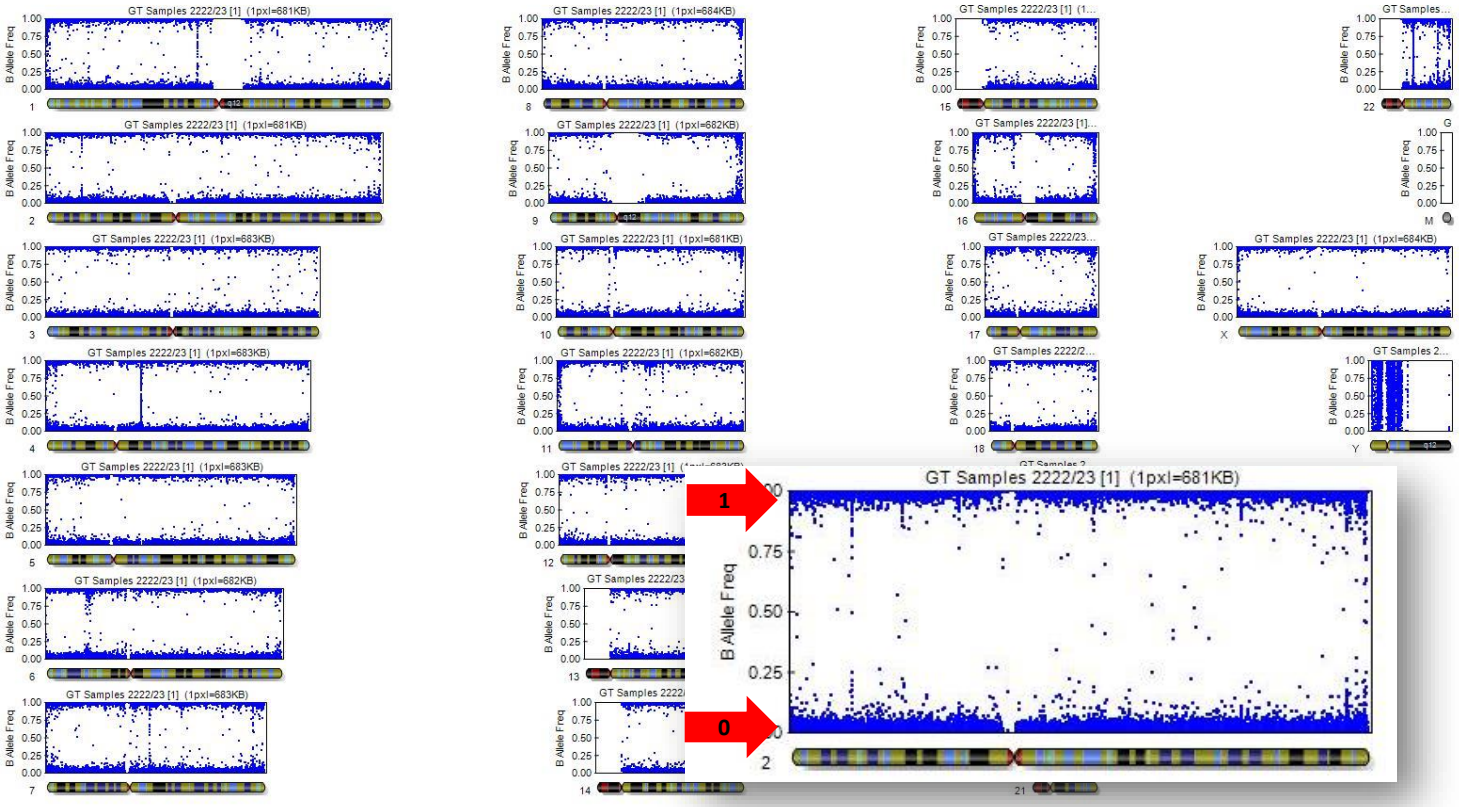
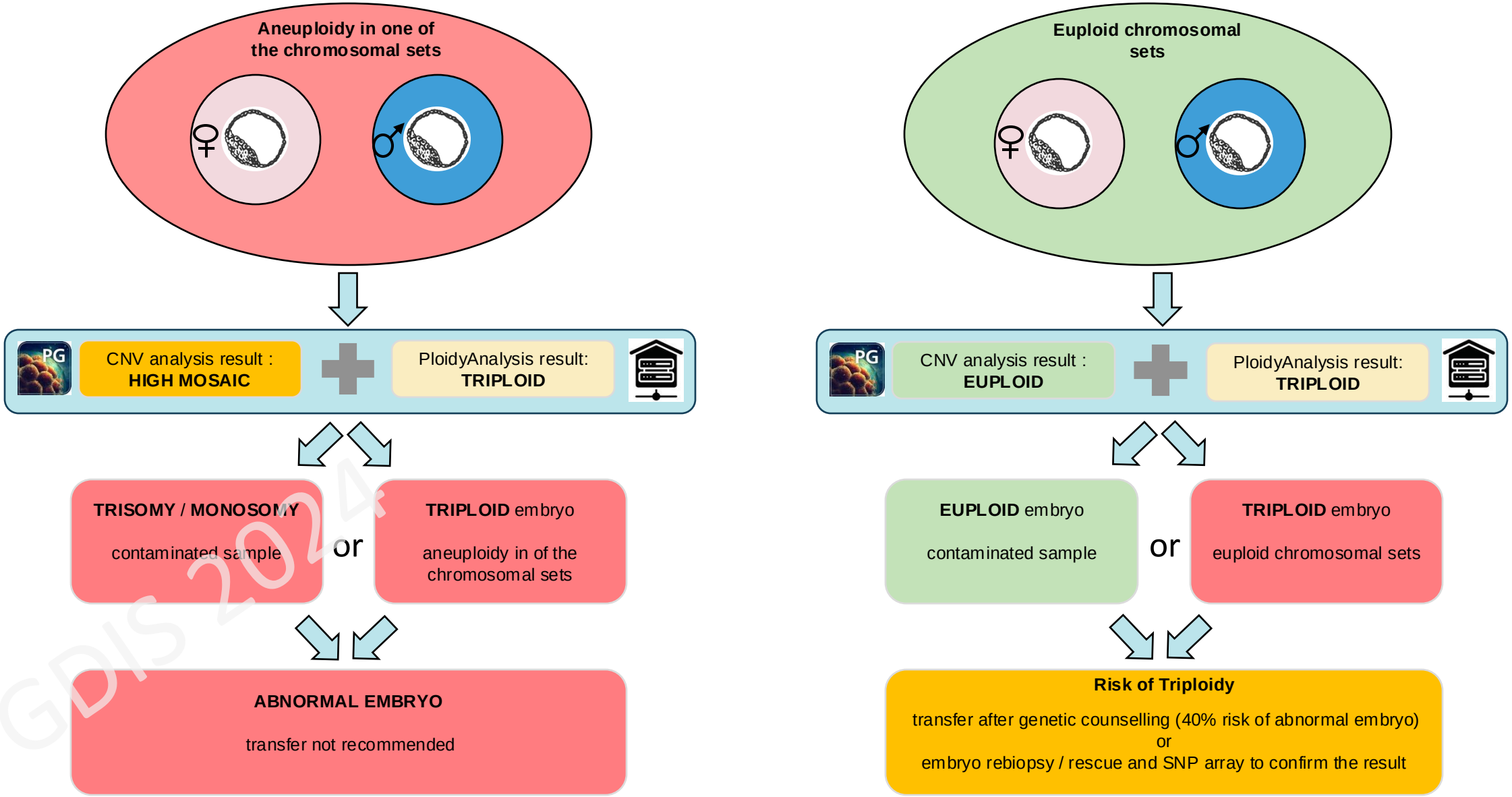


GenomeStudio 2.0 Software (Illumina)

Conclusions

- Maternal DNA contamination mimics triploidy in the sample and occurs with similiar frequency in PGT-A embryos (40% triploids, 60% contaminated)
- Contaminated aneuploid embryos could be false negatively reported mosaics which could lead to aneuploid embryo transfer
- Contaminated euploid embryos could be false positively reported triploid which could lead to discarding the viable embryo from transfer → rescue biopsy and testing is an option to avoid the 40% risk of triploid embryo transfer
- Embryos with abnormal PN could be accepted for PGT-A if genome-wide SNP analysis (PloidyAnalysis) is performed in parallel with CNV analysis of NGS data

Categorization of embryos with divergent SNP allele ratio in 2024



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Omnia AneuScan™ (ExOvo Genomics Ltd.)