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Single cell testing for embryos

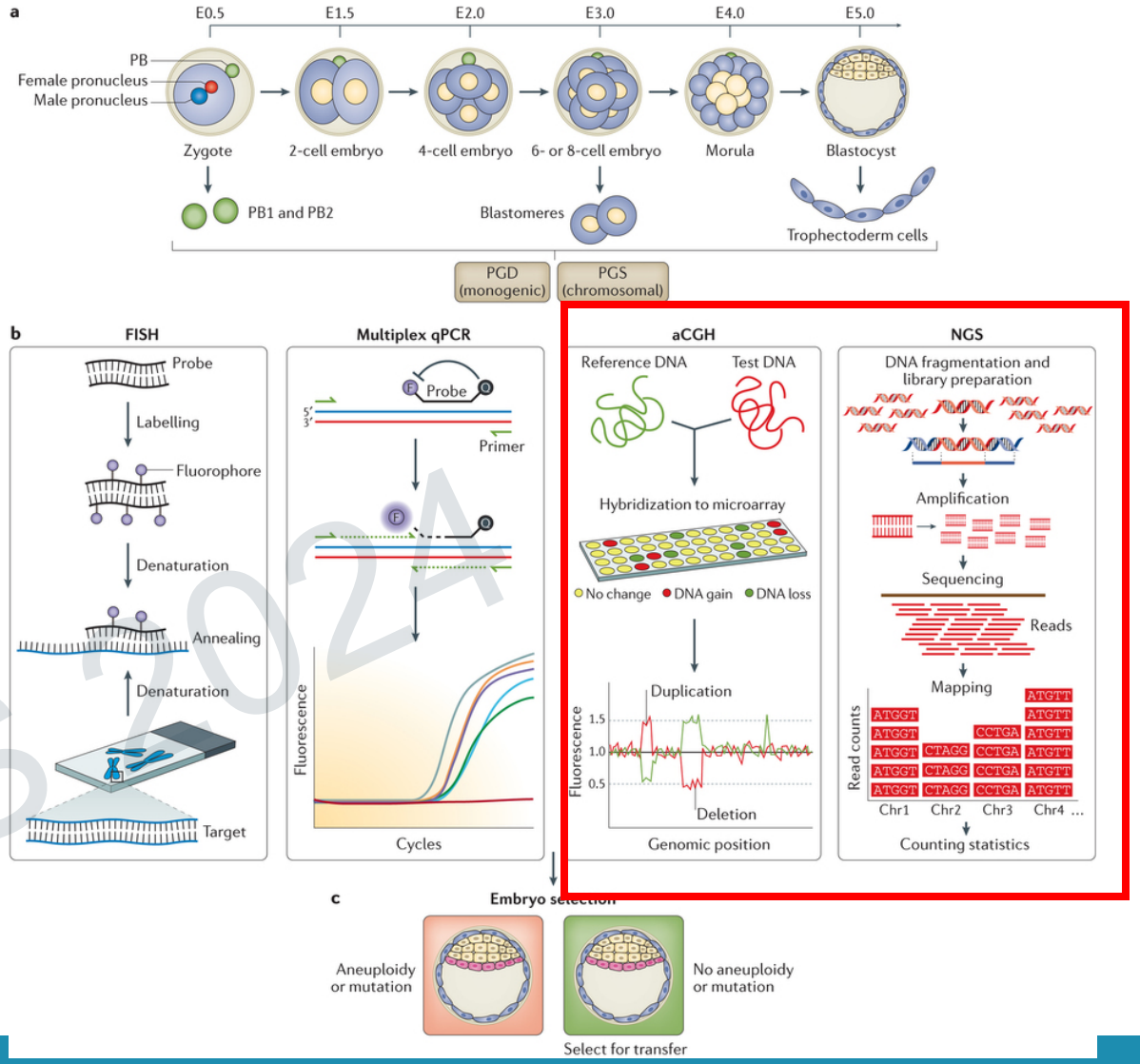
Joris Vermeesch

PGDIS

April 18, 2023

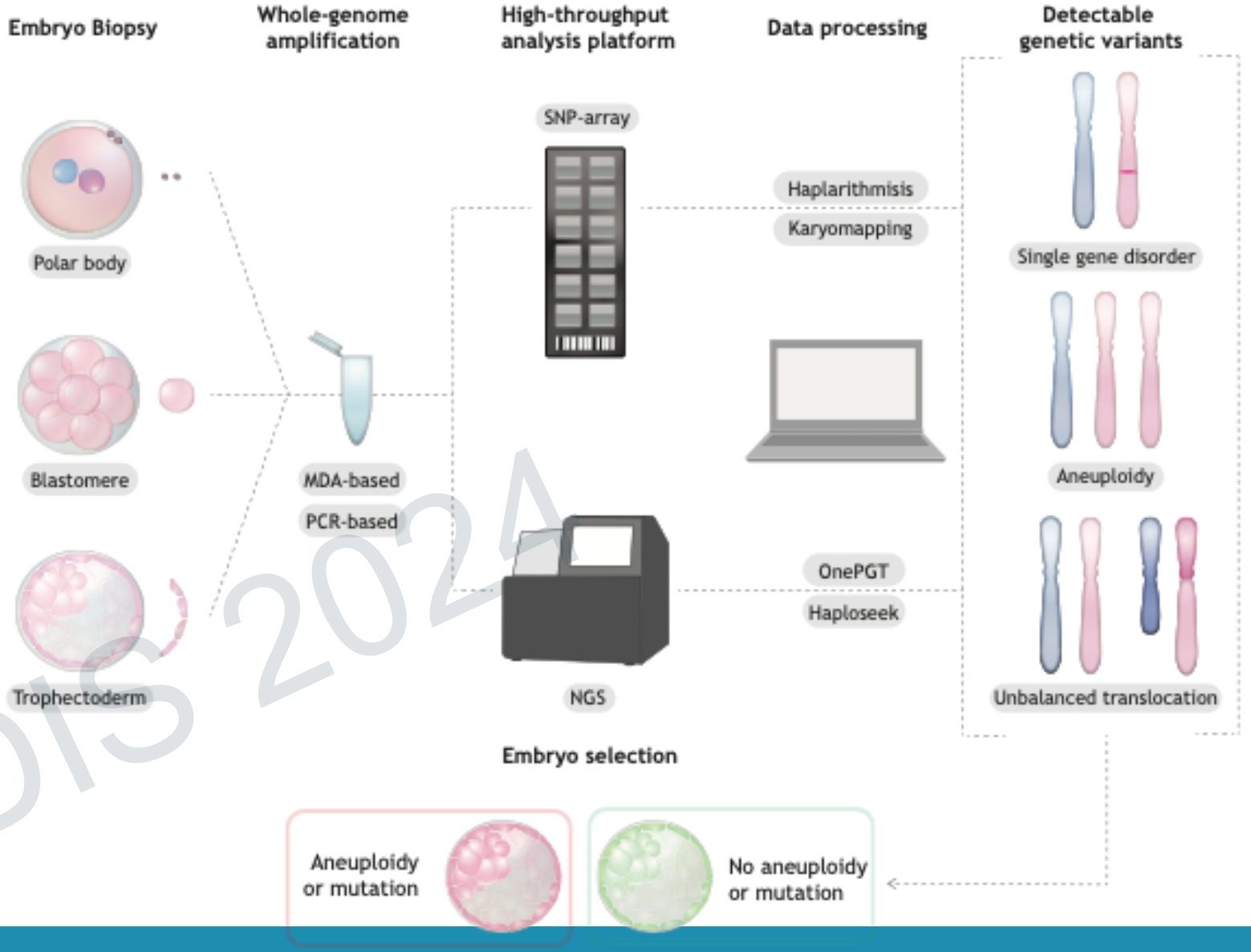
Paris

Preimplantation genetic testing



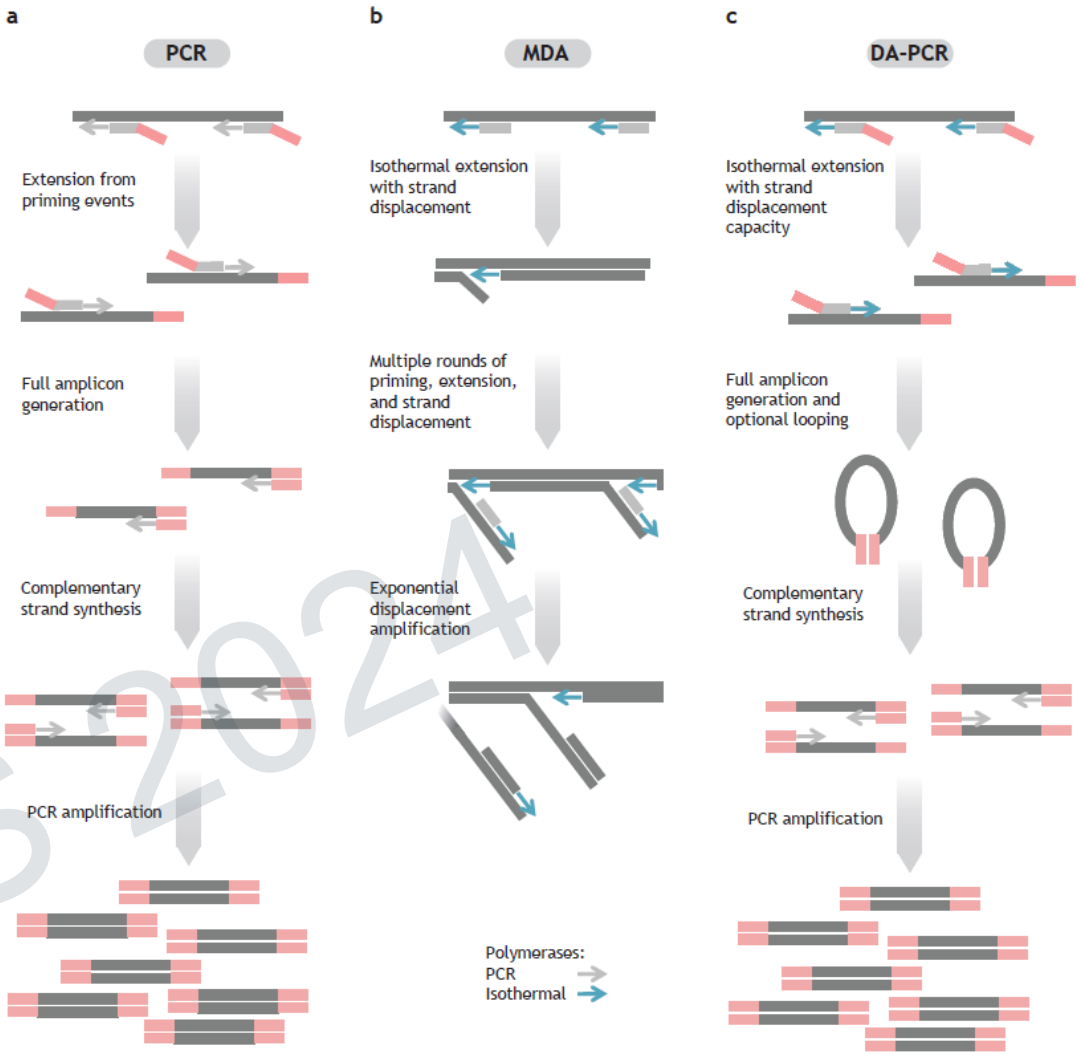
Vermeesch JR., Voet T., Devriendt K. Nat Rev Genet. 2016.

Single cell omics technologies for PGT



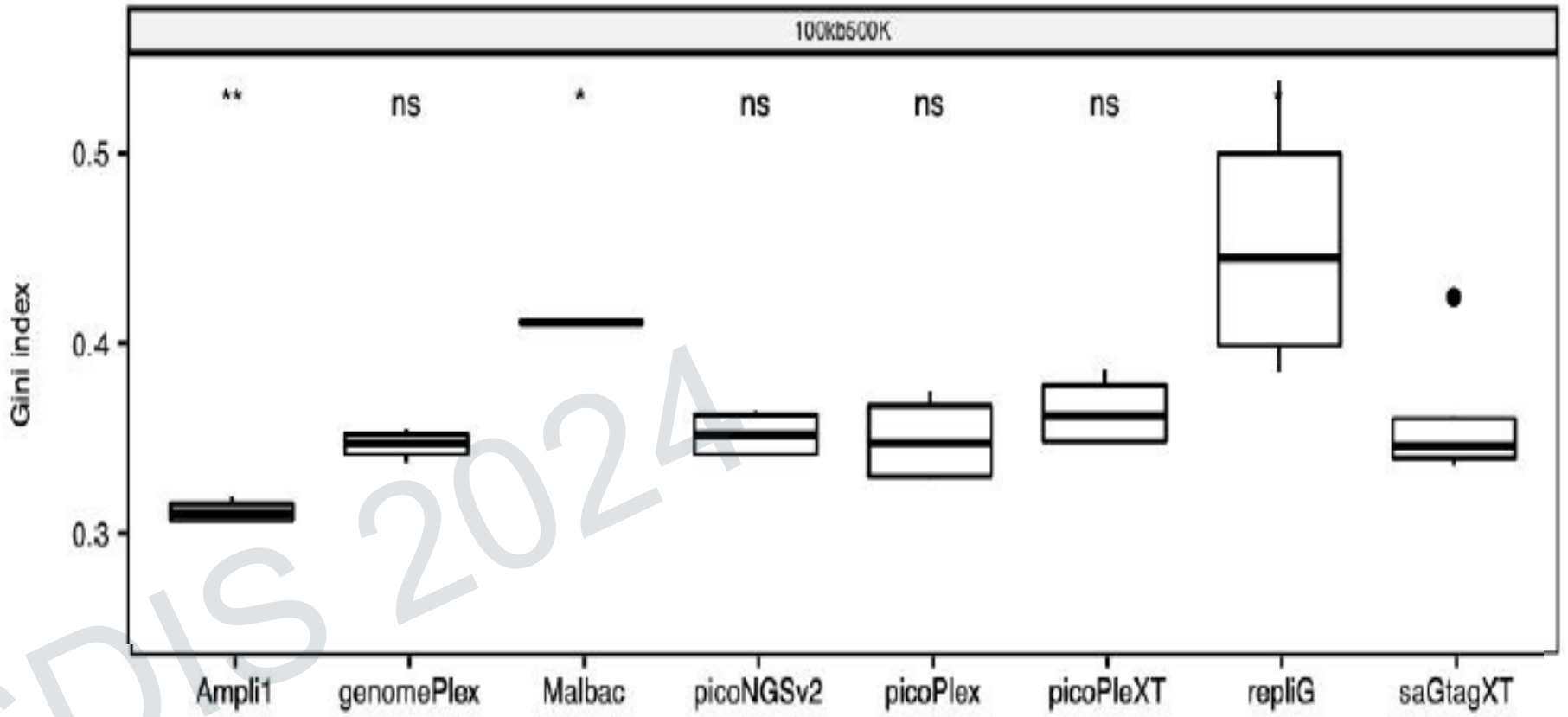
Tšuiko and Fernandez Gallardo et al., 2020 Reproduction

Amplification methods



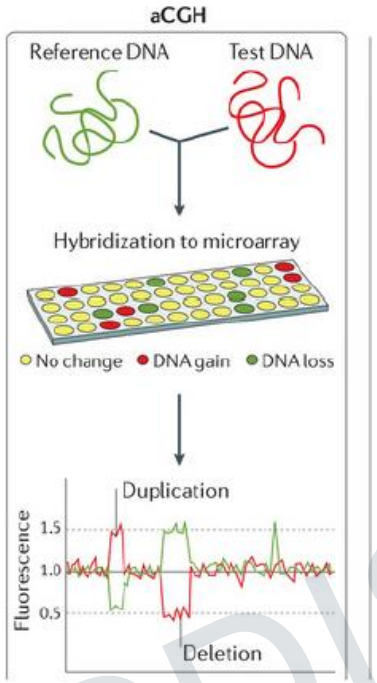
Tšuiko and Fernandez Gallardo et al., 2020 Reproduction

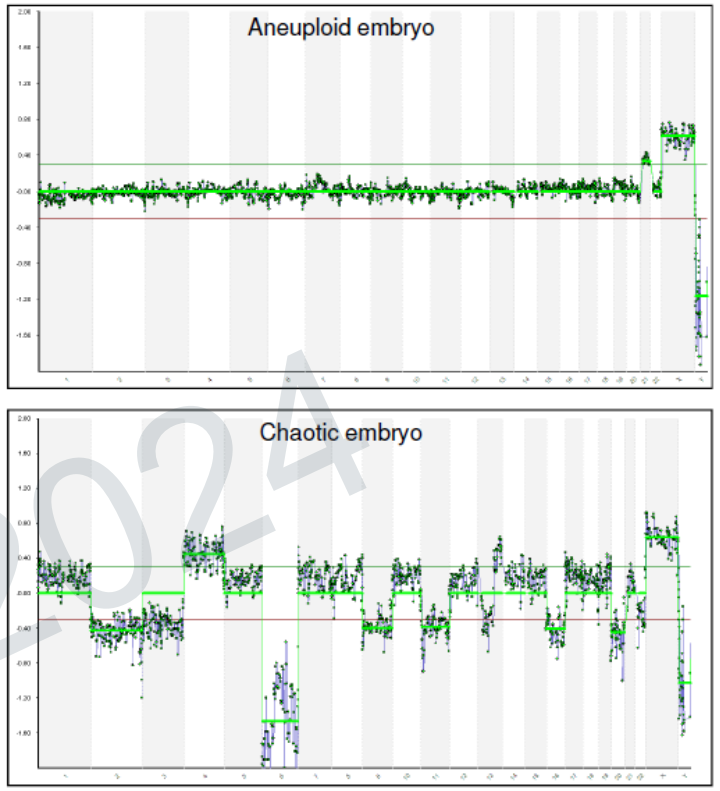
Quality of amplification depends on methodology



Theunis et al. unpublished

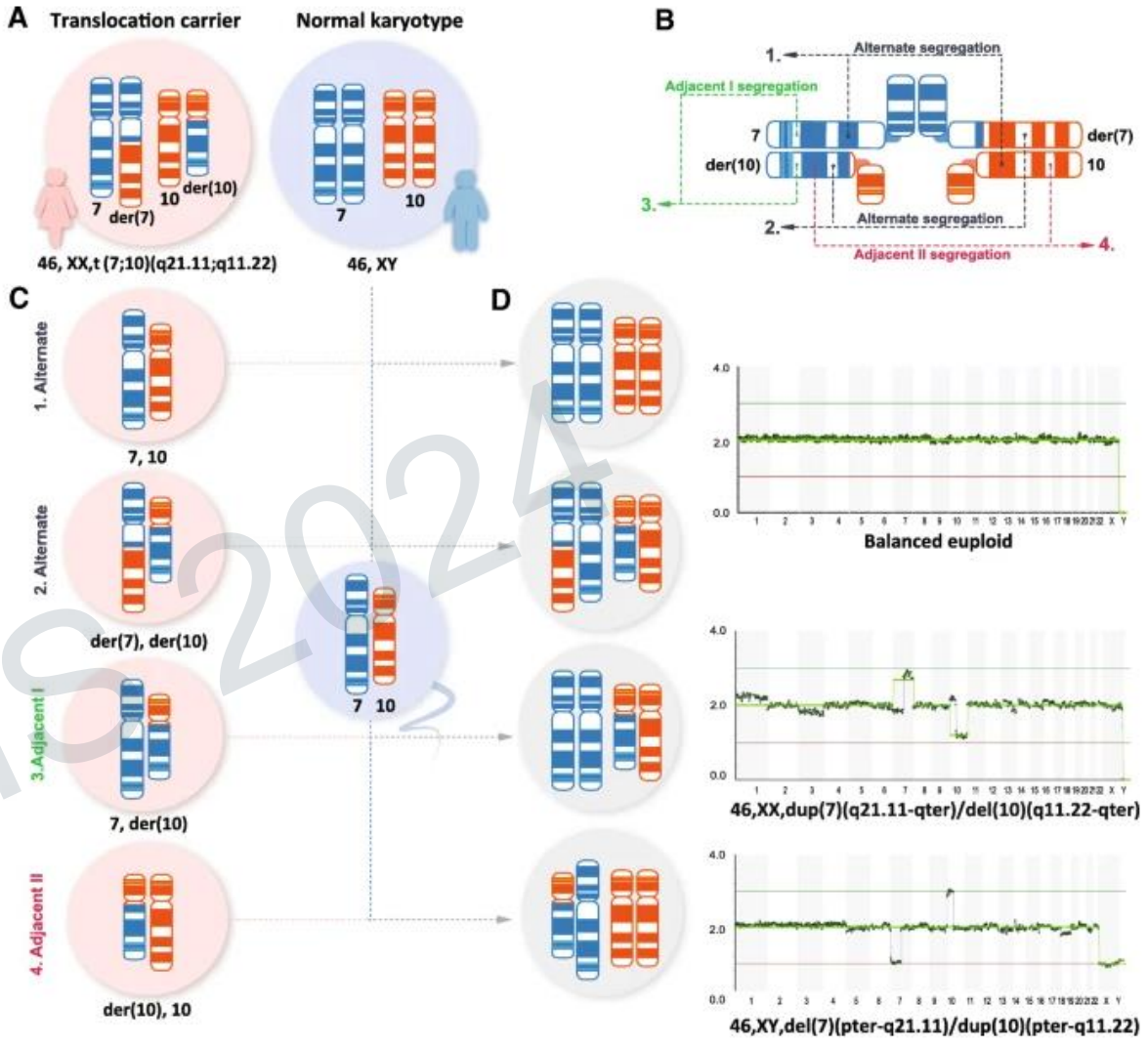
Single cell aneuploidy detection by *low pass sequencing and aCGH (PGT-A)*





Le Caignec et al. Nucleic Acid Research, 2006
Voet et al., Nucleic Acid Research, 2013

Preimplantation genetic testing for segmental rearrangements (PGT-SR)

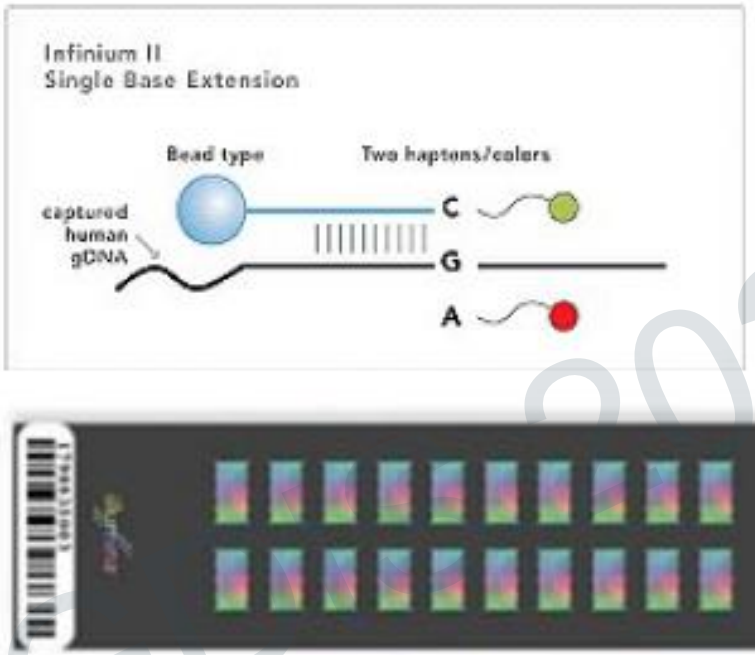


Limitations

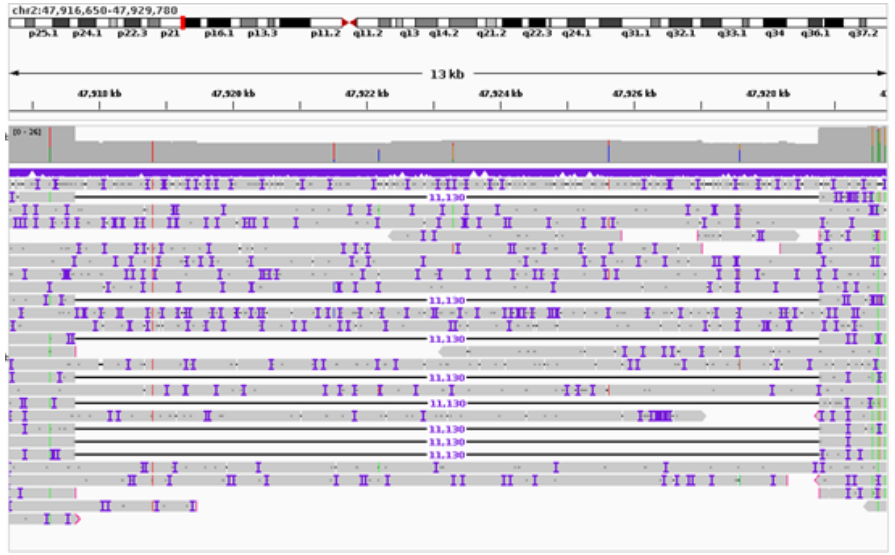
- Cannot be used for PGT-M
- No information on the origins of the aneuploidy: meiotic or mitotic?
- No information about ploidy aberations: haploid, uniparental diploid and triploidy

SNP information

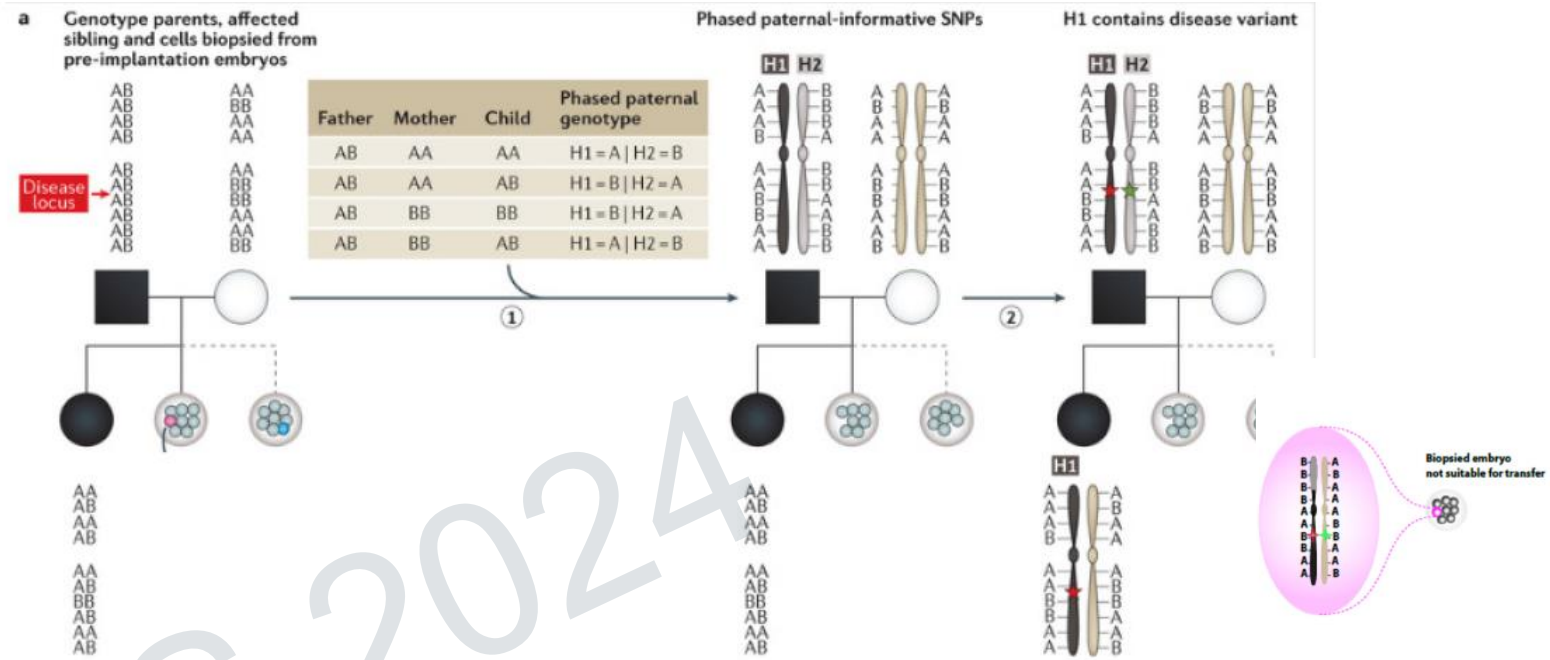
SNP Array



Sequencing based SNP typing



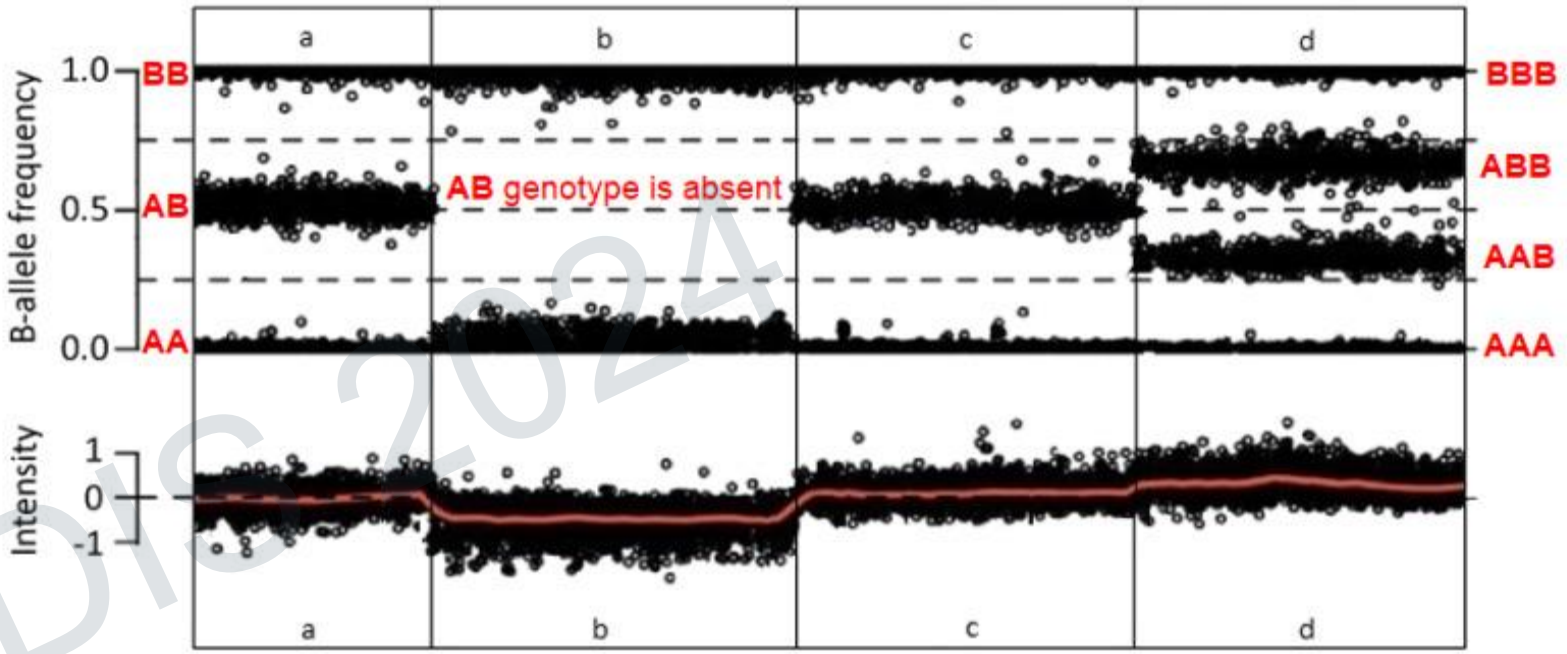
Generic PGT-M
Genotyping and haplotyping single cells



Vermeesch JR., Voet T., Devriendt K. Nat Rev Genet. 2016.
Handyside HA et al., J. Med.Gen. 2010
Natesan et al. Genet. Med. 2014

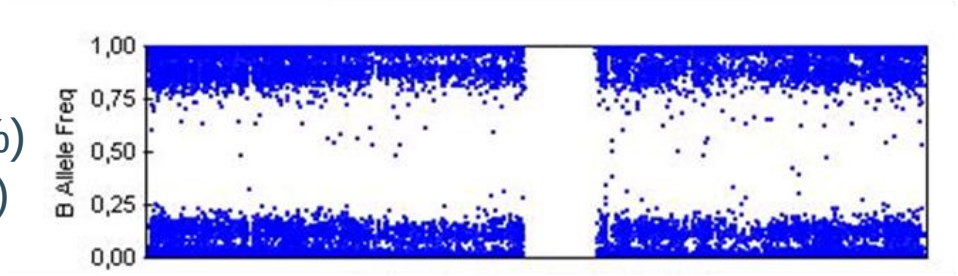
Copy number profiling using B-allele frequencies

B-allele frequency (BAF) = $\frac{\#\,B-alleles}{\#\,(A+B-alleles)}$

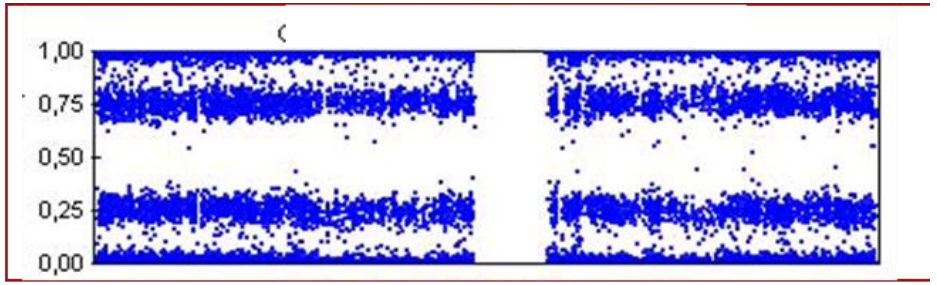


B-allele frequency

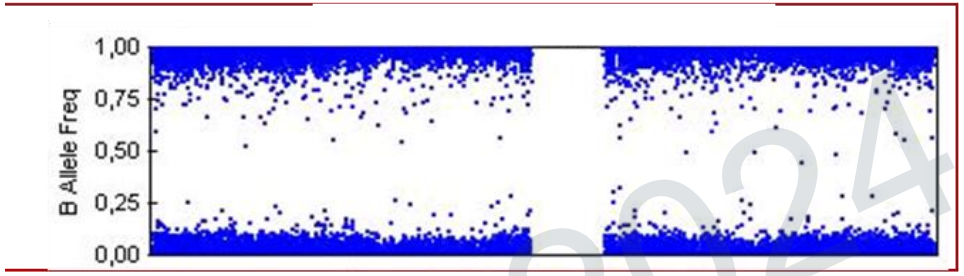
Haploid(80%)
Diploid(20%)



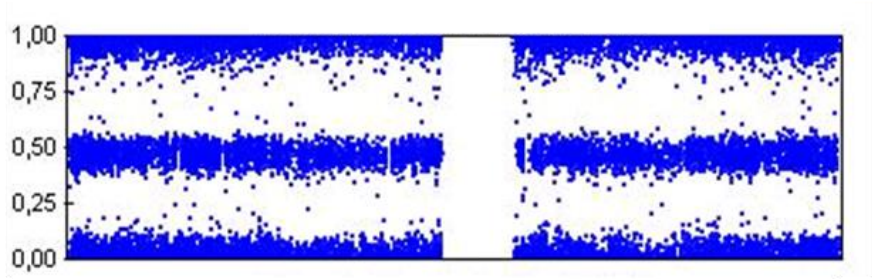
Triploid



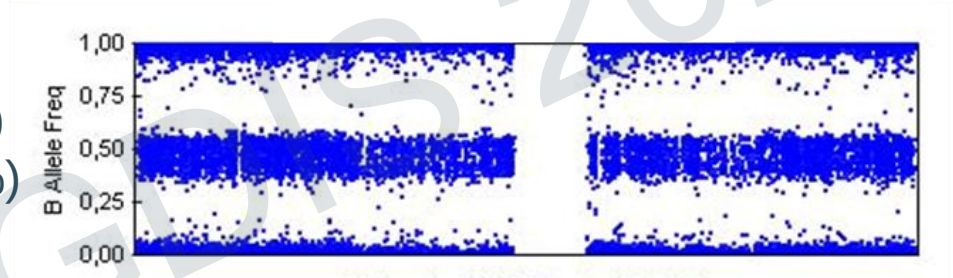
Haploid



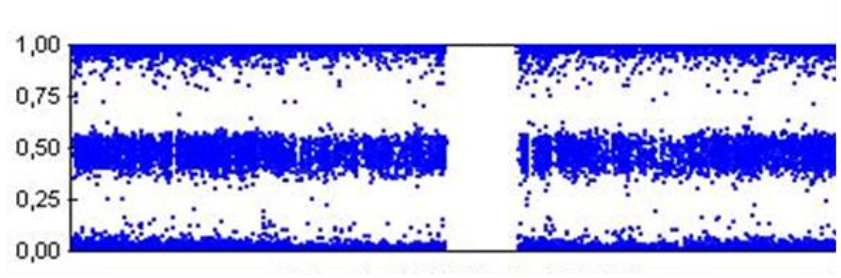
Diploid



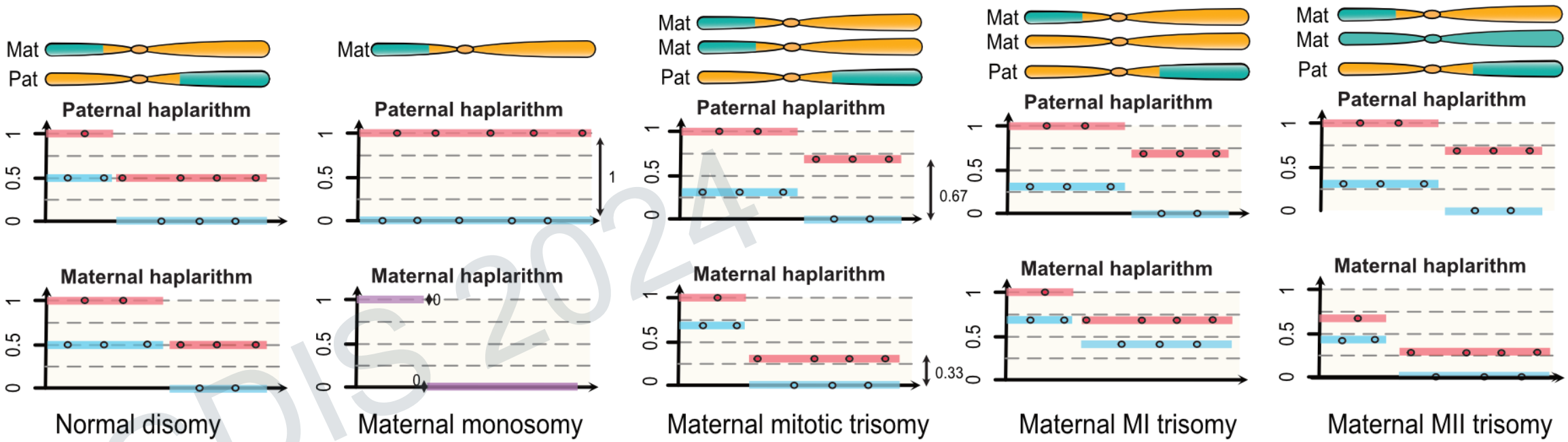
Diploid(90%)
Haploid(10%)



Diploid(90%)
Haploid(10%)



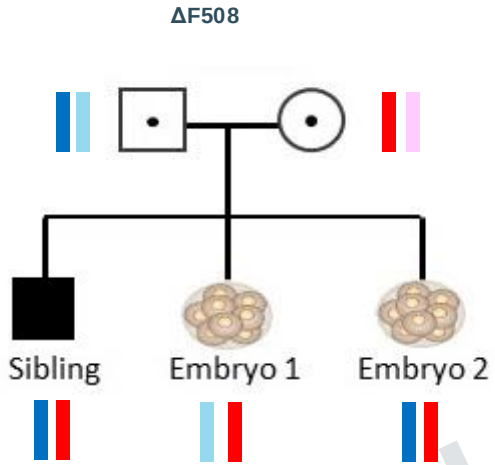
Concurrent haplotyping and copy number profiling (haplarithmisis: haplotype aware plotting of B-allele frequencies enables the mapping of cross-overs)

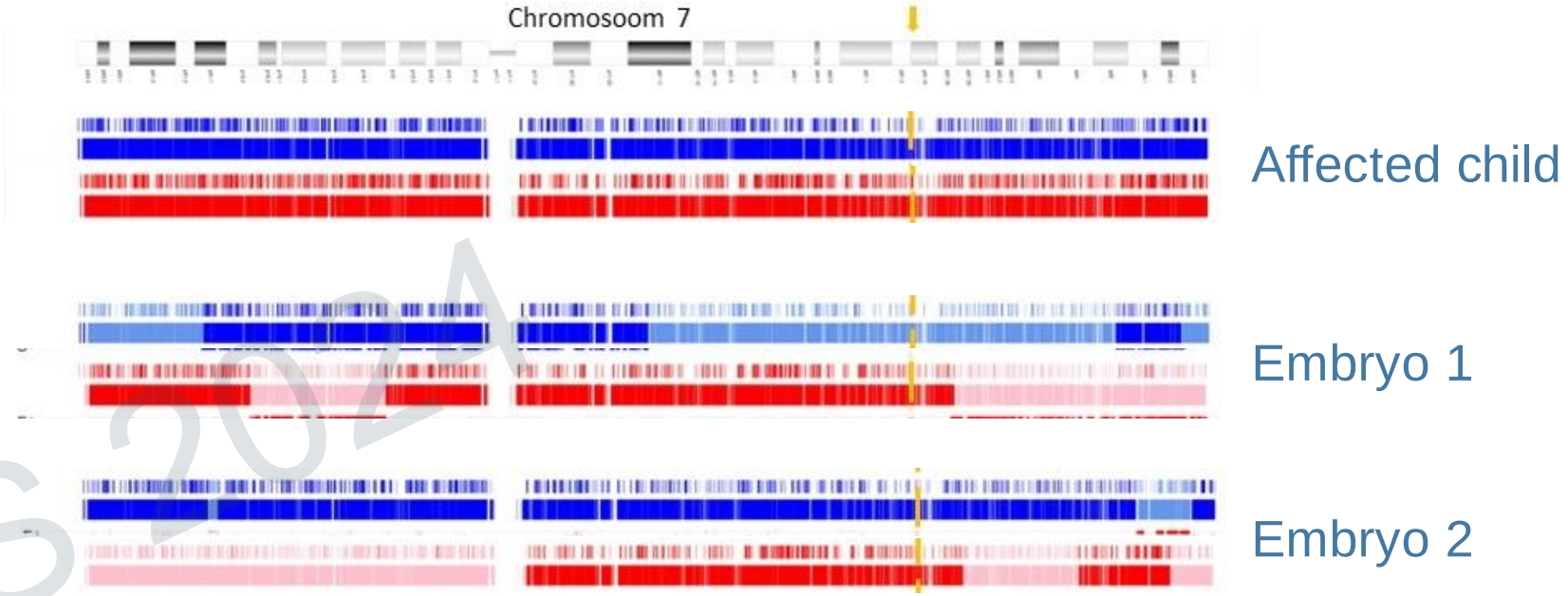


Zamani et al., American Journal of Human Genetics, 2015

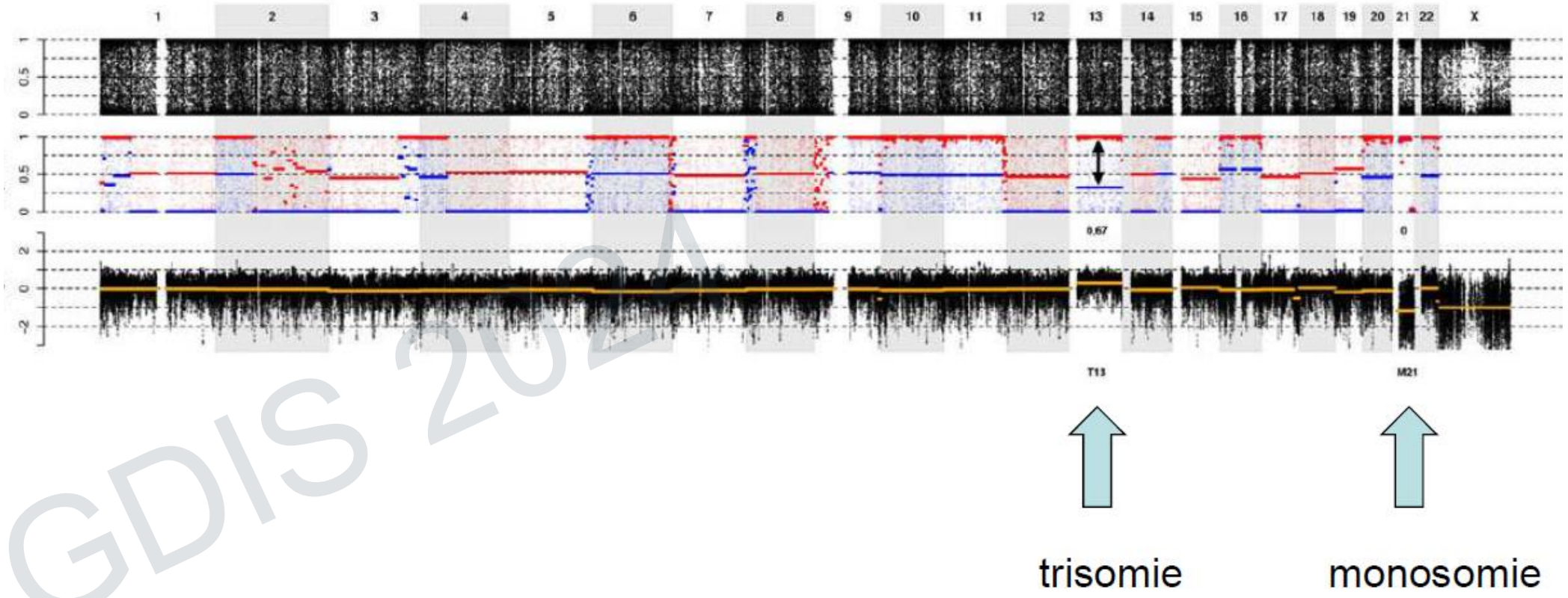
Comprehensive PGT-M

CFTR

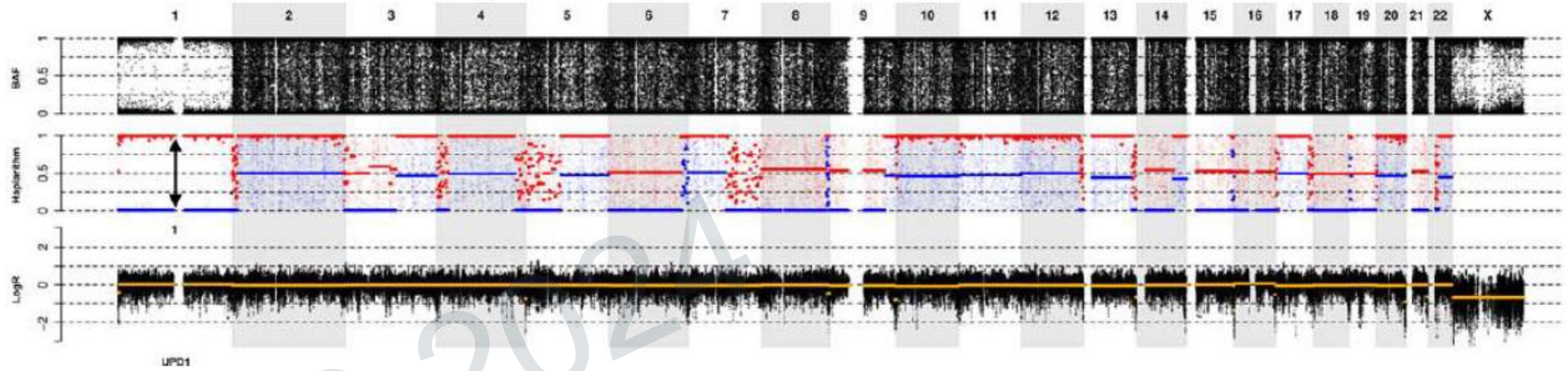




Genome wide analysis allows the detection of aneuploidies and their origin

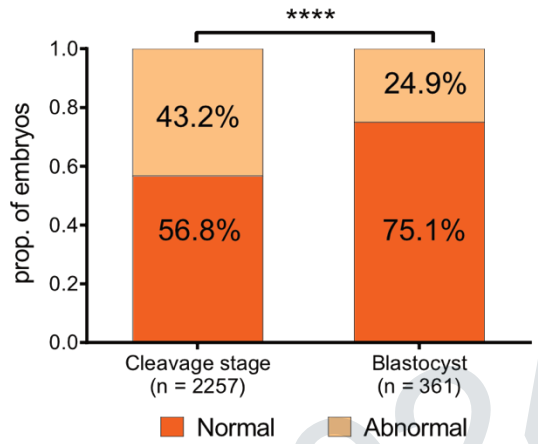


Genome wide analysis allows the detection of uniparental disomy



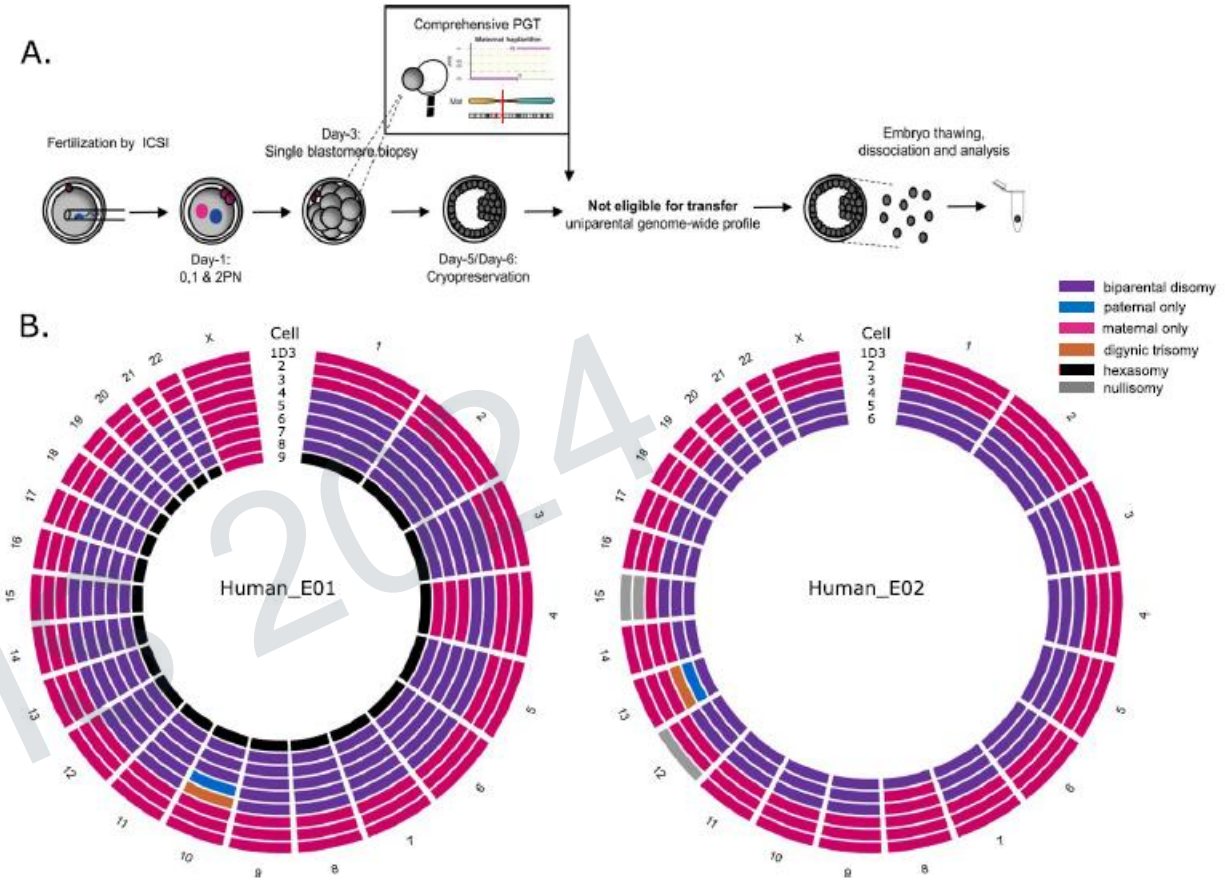
Uniparentele disomie

Genomic landscapes of PGT embryo's



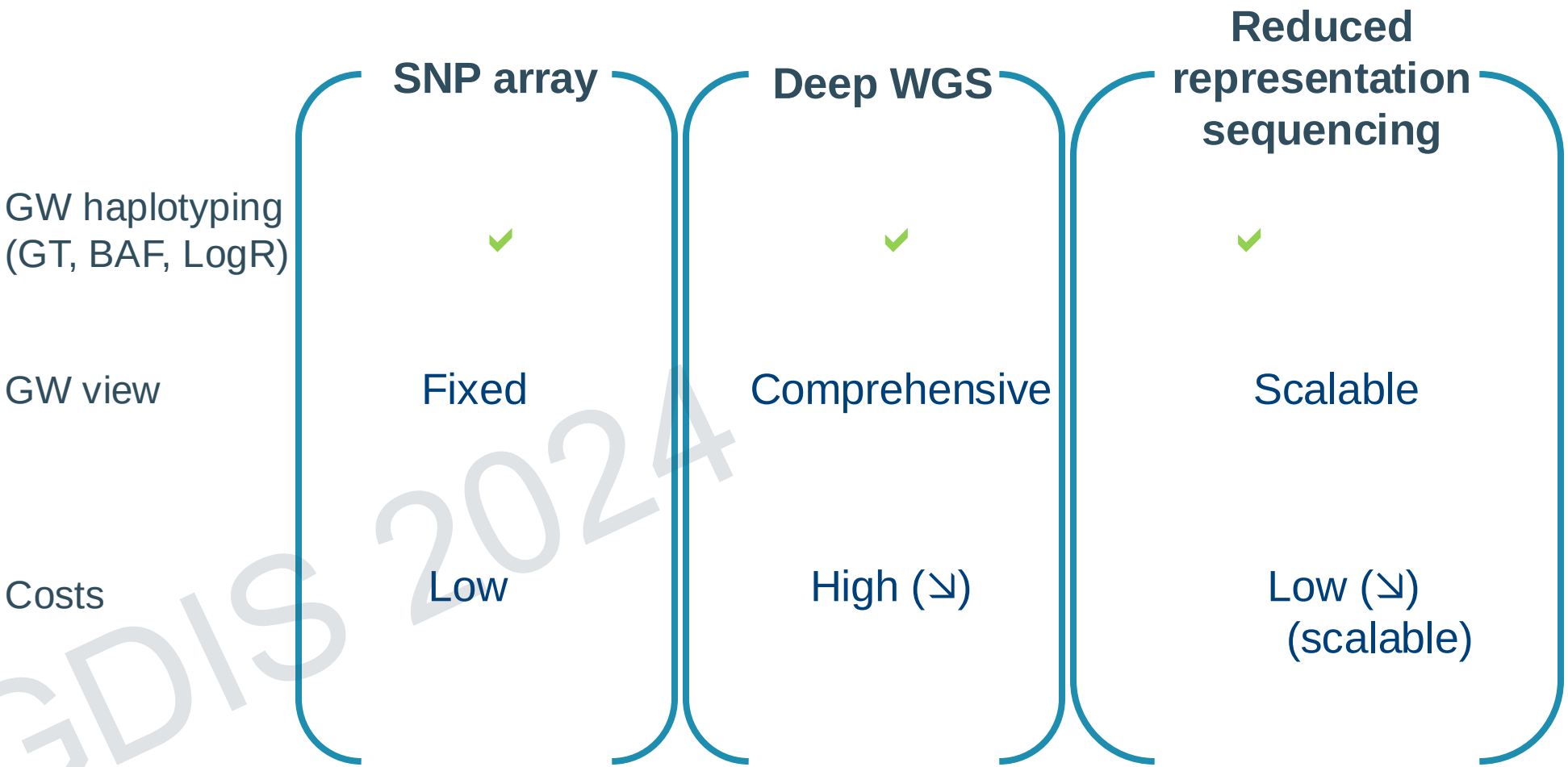
Tsuiko et al., Genom.Med., 2021

Mixoploidy:
The presence of haploid & diploid cells in one embryo

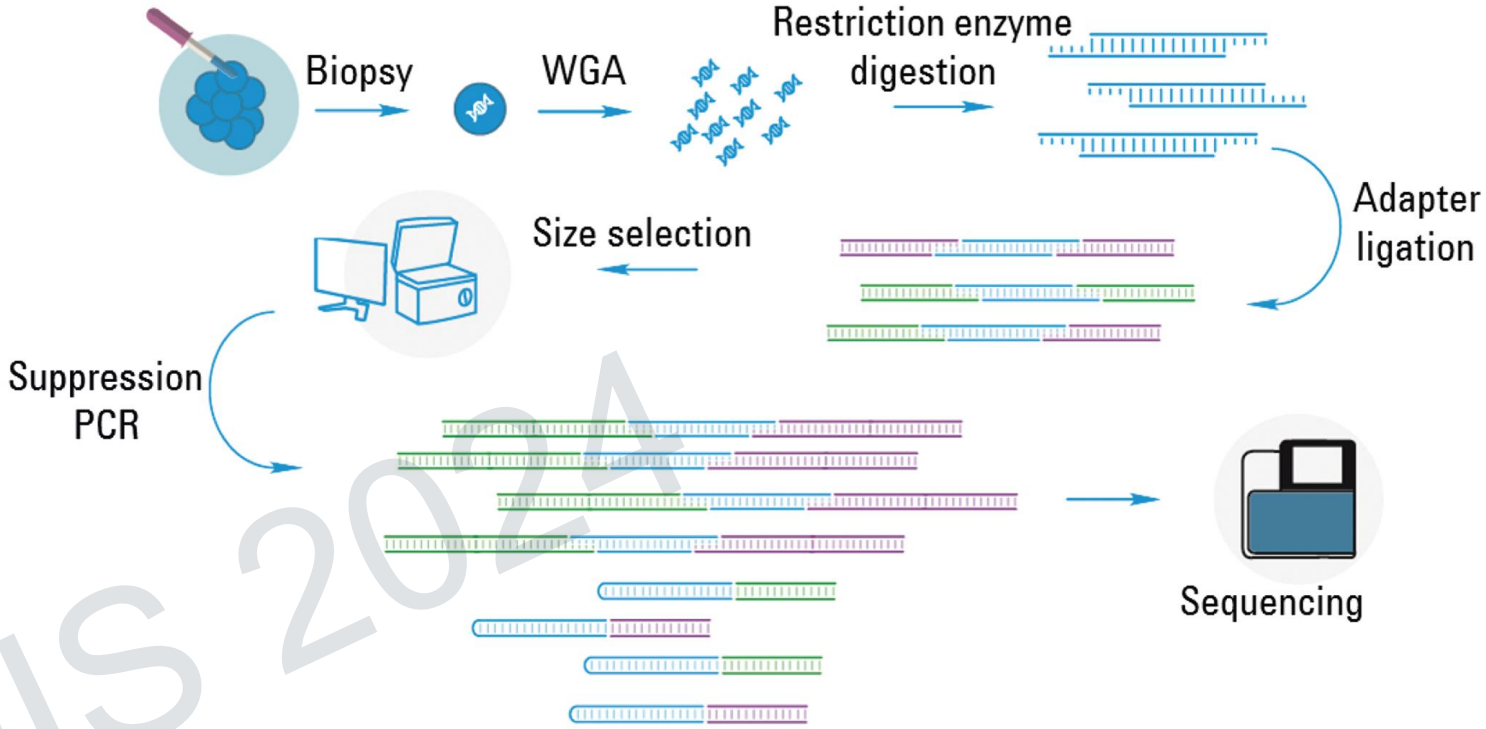


De Coster et al., Genome Biology, 2023

The era of MPS and high-throughput

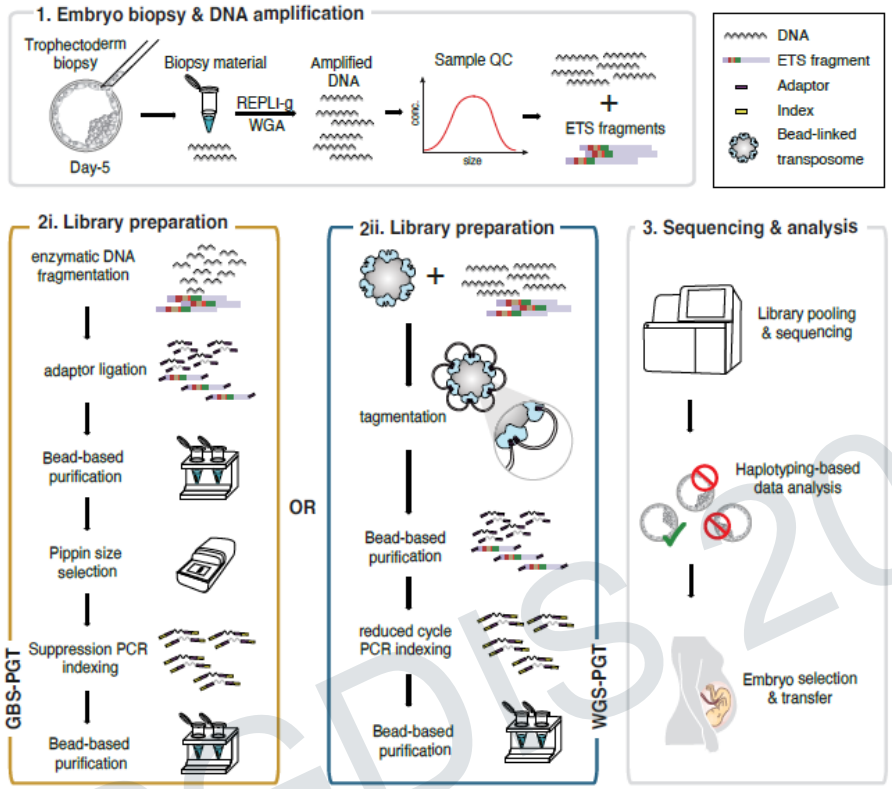


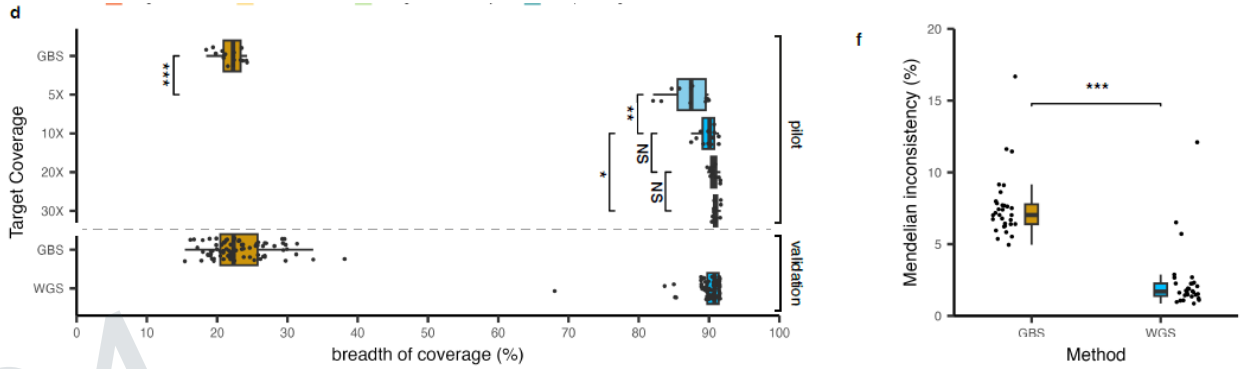
Genotyping-by-Sequencing (GBS) – based PGT



Elshire et al. 2011 PLoS One.
Masset et al. 2019 Hum Reprod
Masset et al. 2022 Nucl.Acids Research

Clinical-grade whole genome sequencing-based haplarithmisis





Clinical-grade whole genome sequencing-based haplarithmisis enables all forms of preimplantation genetic testing

Authors:

Anouk E.J. Janssen[1,2,*], Rebekka M. Koeck[1,2,*], Rick Essers[1,2,*], Wanwisa van Dijk[1], Marion Drüsedau[1], Jeroen Meekels[1], Burcu Yaldiz[1], Maartje van de Vorst[1], Ping Cao[1,2], Bart de Koning[1], Debby M.E.I. Hellebrekers[1], Servi J.C. Stevens[1], Su Ming Sun[1], Malou Heijligers[1], Sonja A. de Munnik[1], Chris M.J. van Uum[1], Jelle Achten[1], Lars Hamers[1], Marjan Naghdi[1,2,3], Lisenka E.L.M. Vissers[4], Ron J.T. van Golde[5], Guido de Wert[6], Jos C.F.M. Dreesen[1], Christine de Die-Smulders[1,2], Edith Coonen[1,5], Han G. Brunner[1,2,4], Arthur van den Wijngaard[1], Aimee D.C. Paulussen[1,2], Masoud Zamani Esteki[1,2,7]

*Joint first-author

Janssen et al., 2023, MedRxiv

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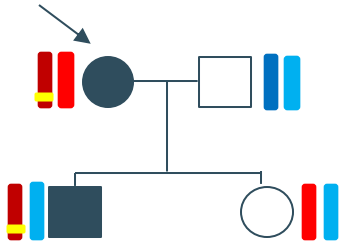
Advantages of comprehensive PGT

- Genome wide: all chromosomal aneuploidies can be detected
- Meiotic aneuploidies and ploidy anomalies can be detected

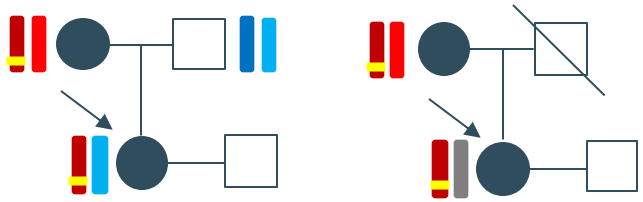
Limitations of genome wide PGT

- DNA of family members is required for PGT-M
- Only detection of inherited variants

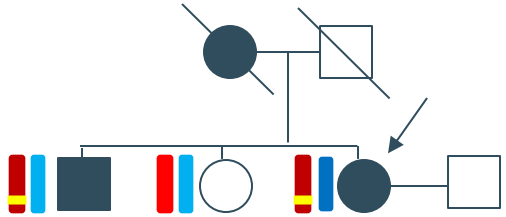
Phasing is not possible when there is no family member available



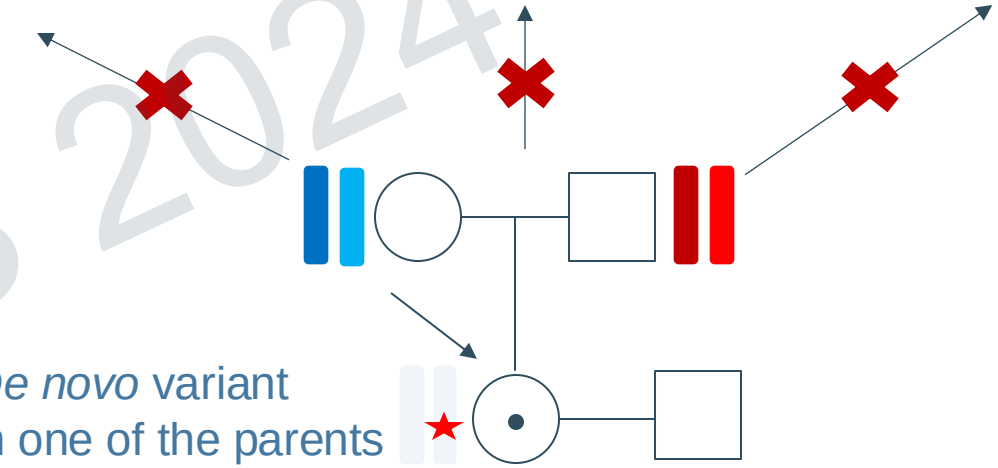
Phasing option 1:
Affected/unaffected child



Phasing option 2:
Grandparent/single grandparent



Phasing option 3:
Parental sibling (=aunt/uncle)



De novo variant
in one of the parents

Current ESHRE PGT consortium guidelines

PGT-M

- Male partner is affected: Analysis of DNA from blood and single sperm cells is performed to determine healthy and mutant haplotype, and thus establish a phase
- Female partner is affected: Analysis of DNA from blood and multiple polar bodies/oocytes is performed
- If the origin of *de novo* variant is unknown: STR analysis coupled with direct mutation detection can be performed during the PGT cycle

- Recommendations:
  - Multiple single sperm cells and polar bodies should be analyzed to exclude germline mosaicism
  - Multiple embryos should be analyzed for direct mutation detection to determine one affected and one unaffected embryo (can require multiple PGT cycles)

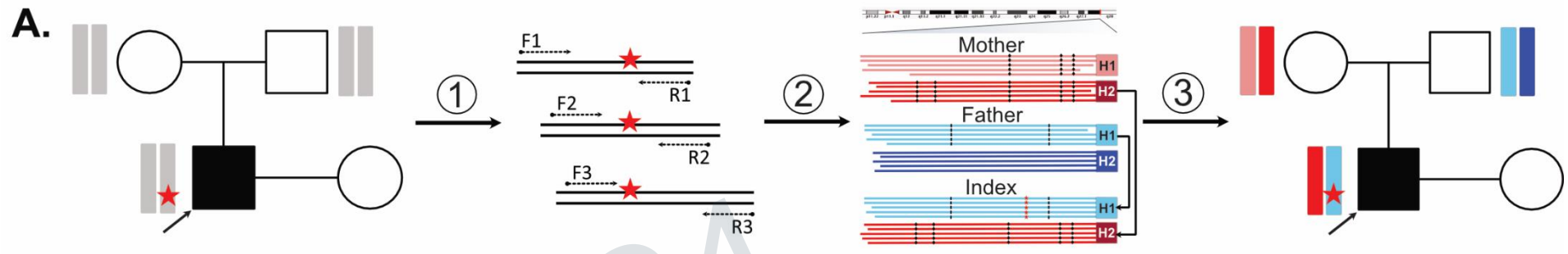
ESHRE PGT-M Working Group et al., 2020

Using long-read amplicon sequencing to determine parental origin of mutated allele

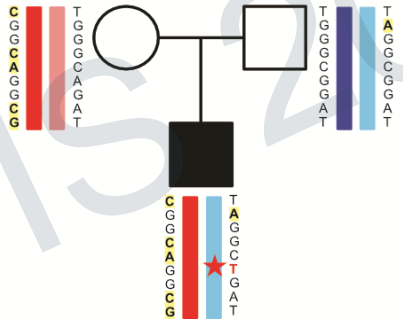
LR-PCR (ca. 10Kb)

Long-read sequencing

Variant calling & linkage of de novo variant with parental allele



B.



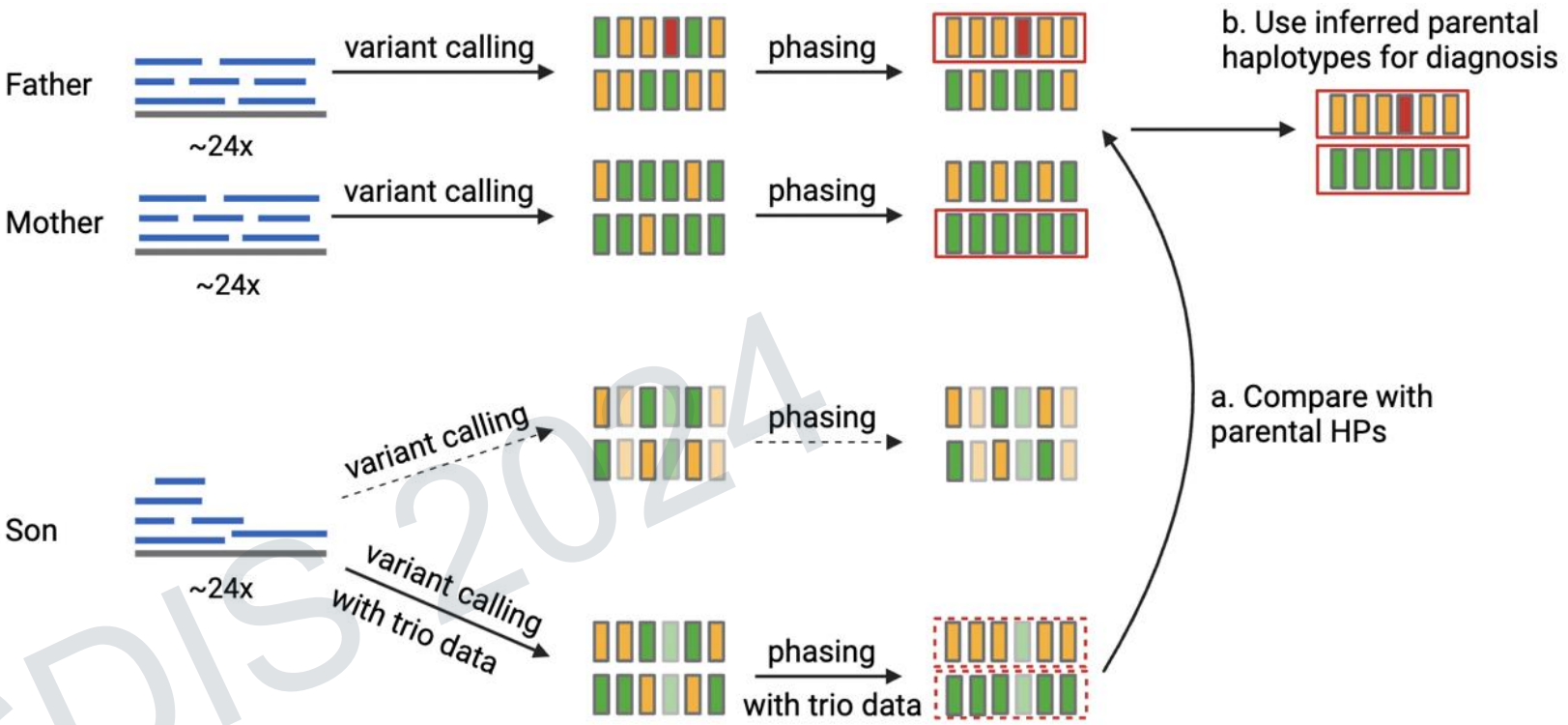
CHR	Position	REF	ALT	Father	Mother	Index	Phasing	Index H1	Index H2
17	31231064	T	C	0/0	1|0	0|1	Maternal	T	**C**
17	31231212	G	A	0|1	0/0	1|0	Paternal	**A**	G
17	31231275	T	G	1/1	1/1	1/1	-	G	G
17	31232570	G	C	0/0	1|0	0|1	Maternal	G	**C**
17	31232914	C	A	0/0	1|0	0|1	Maternal	C	**A**
17	31233160	G	T	0/0	0/0	**1|0**	*De novo*	**T**	G
17	31233515	G	A	0/0	0|1	0/0	-	G	G
17	31233660	A	C	0/0	1|0	0|1	Maternal	A	**C**
17	31233759	T	G	0/0	1|0	0|1	Maternal	T	**G**

Tsuiko et al., Human Reproduction, 2023

Limitations of genome wide PGT

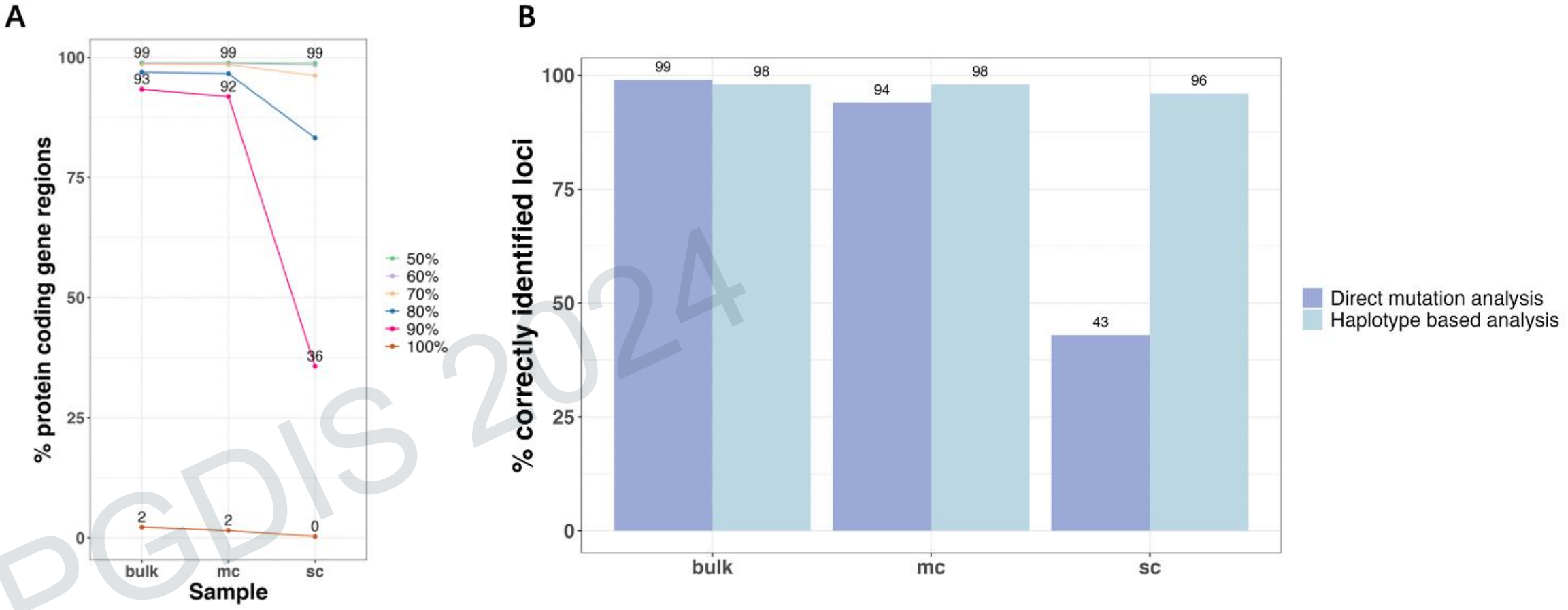
- DNA of family members is required for PGT-M
- Only detection of inherited variants, whereas the majority of severe developmental disorders is caused by de novo mutations!

Long read WGS based PGT

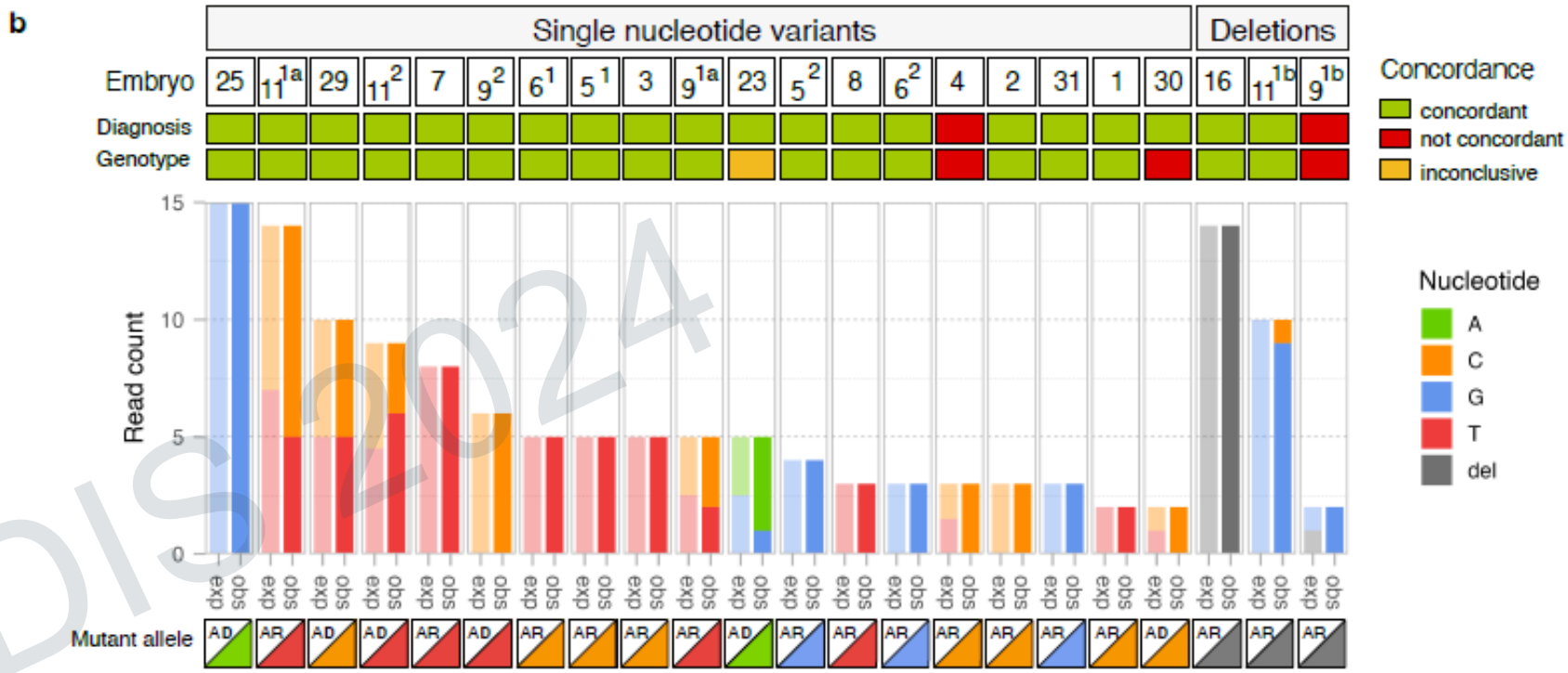


Zhao et al., unpublished

Long read WGS based PGT shows accurate haplotyping



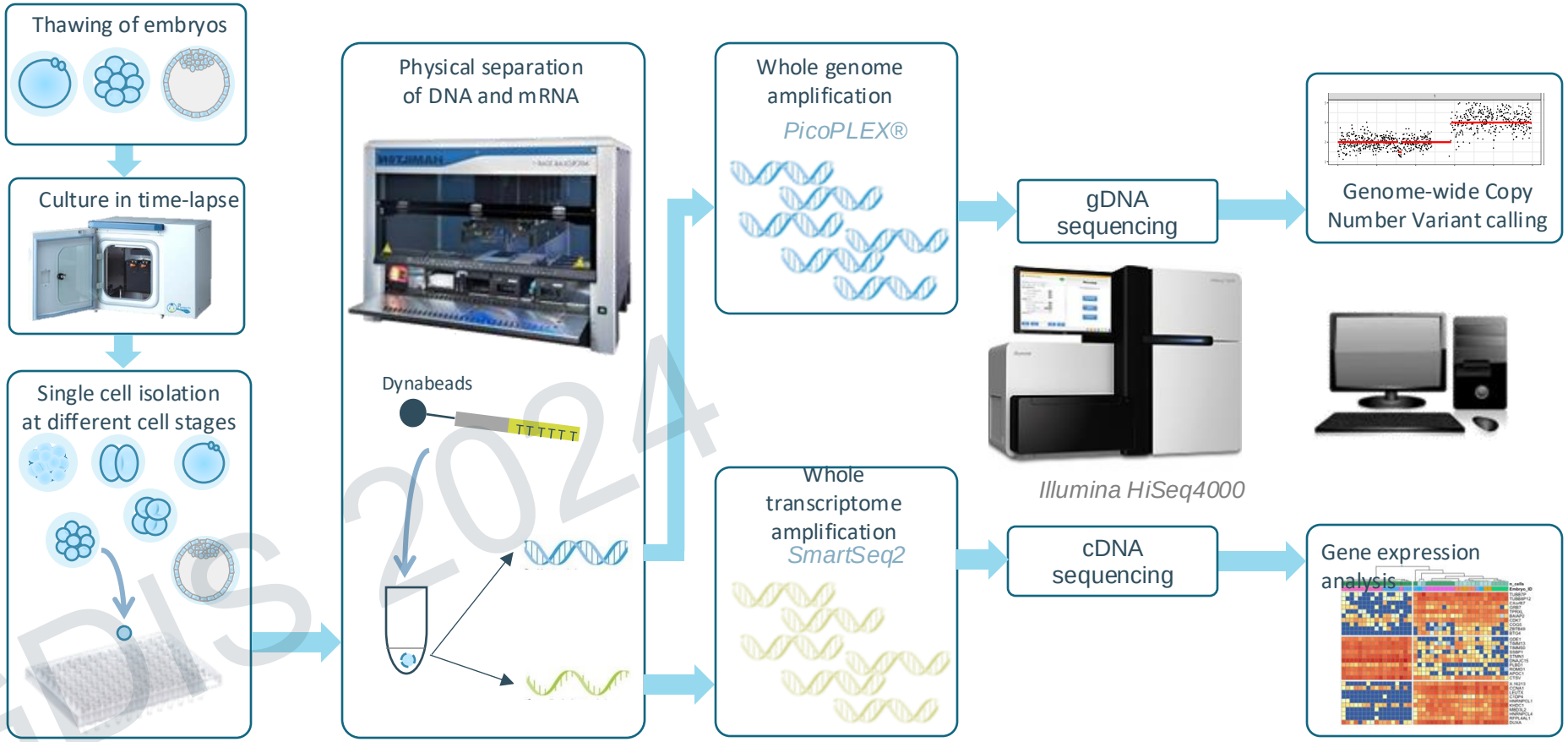
Can identify mutations directly & potentially map de novo variants



Janssens et al., MedRxiv, 2023

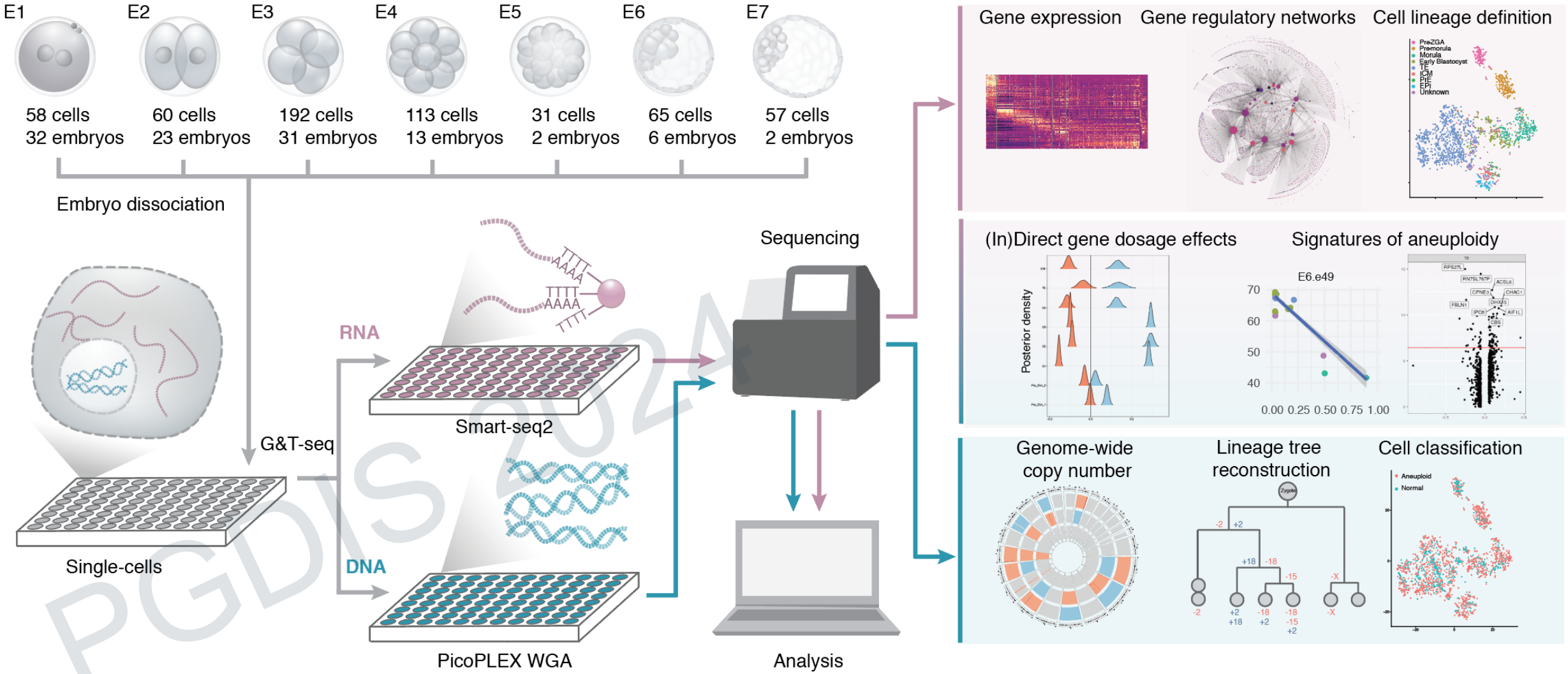
Beyond the genome

Genome & transcriptome analysis



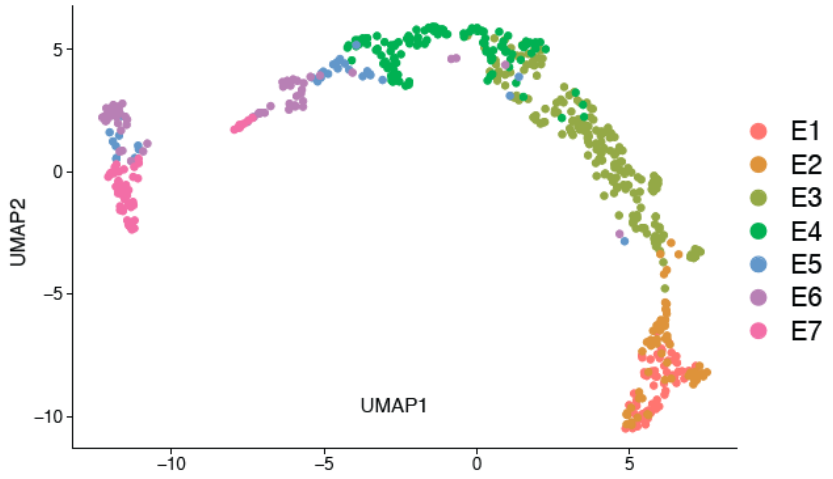
Macaulay et al., 2015, Nature

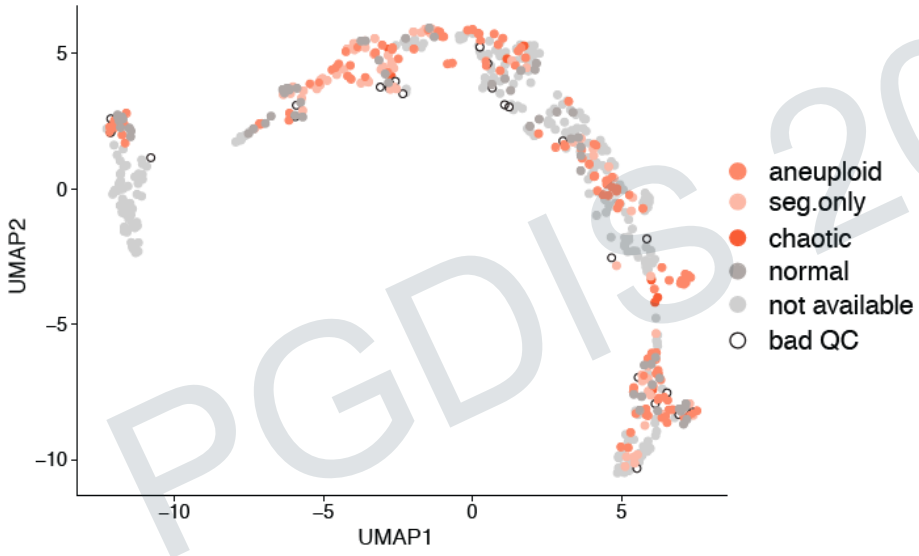
G&Tseq allows mapping both aneuploidy and transcriptome profiles

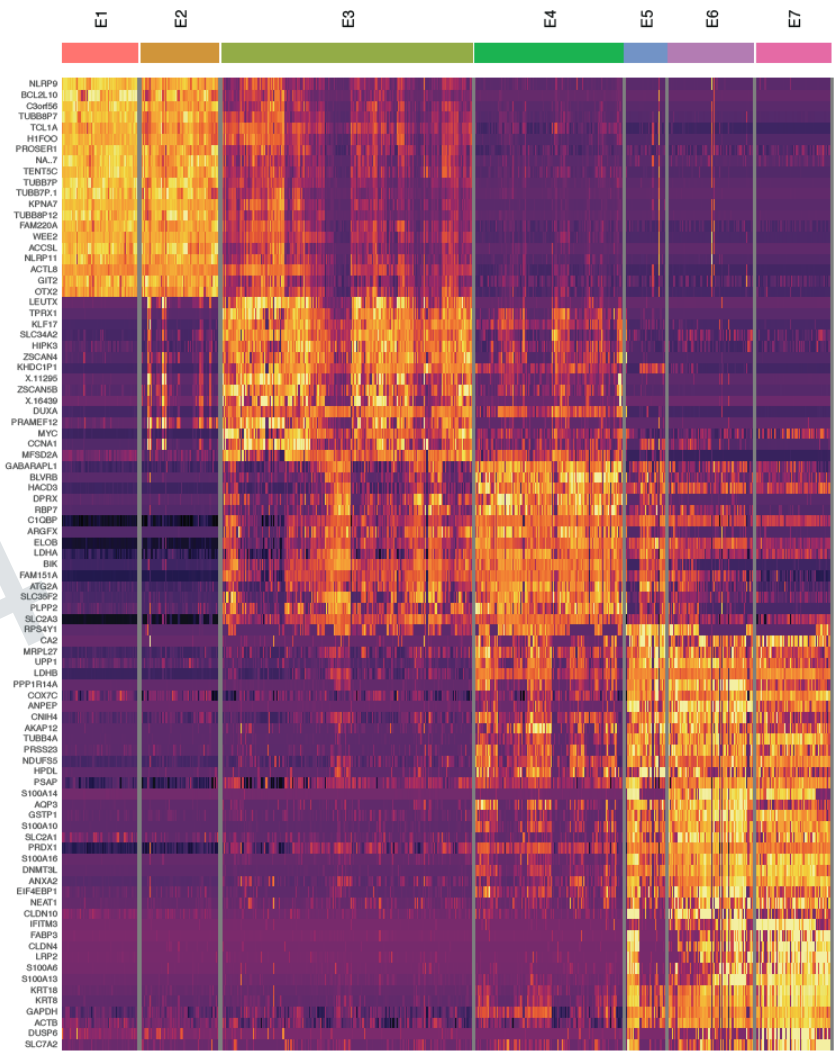


Macaulay et al., 2015 Nature Methods

Embryonic development drives expression program







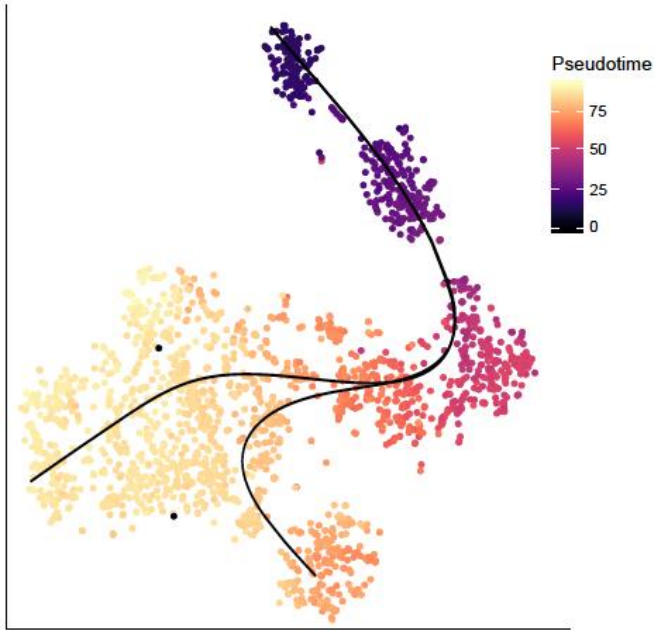
E1-2: oocyte expressed genes
(e.g. BCL2L10, HF1OO, WEE2)

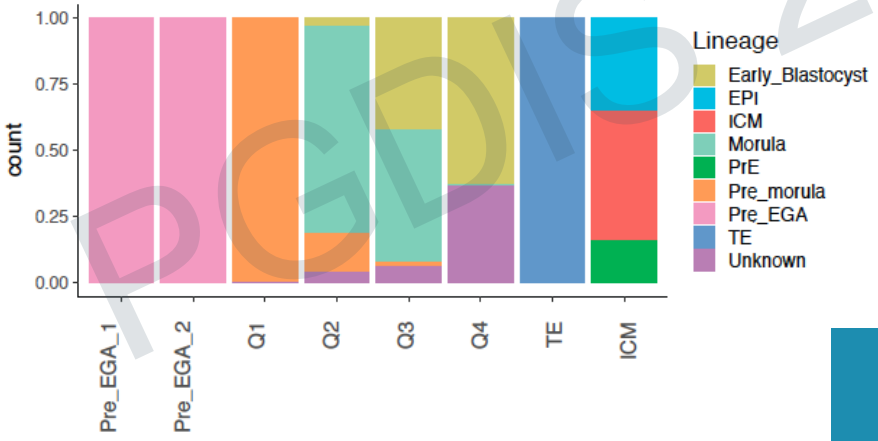
E3: embryonic genome activation
(e.g. DUXA, ZSCAN4, KLF17)

E4-7:
Respiration (e.g. LDHB, GAPDH, COX7C, NDUFS5)
Cytoskeleton (TUBB4A, KRT)
Regulation of cell growth and cell cycle progression (e.g. AKAP12, S100, DUSP6)
Methylation (DMT3L)
Cell junctions (CLDN)
Translation regulation (RPS4Y1, NEAT1, EIF4EBP1)
Blastocoel formation (AQP3)
Hatching (PRSS23)

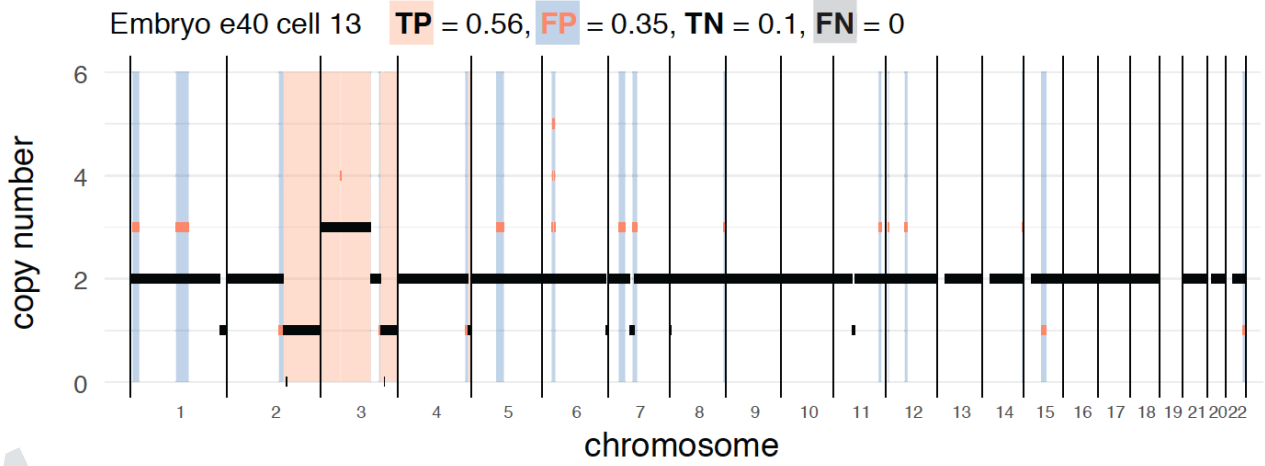
Inferring aneuploidy using inferCNV

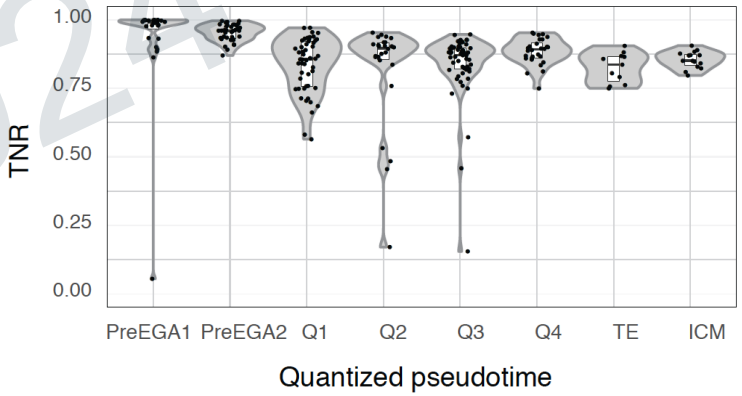
Transcriptionally similar cells by quantized pseudotime

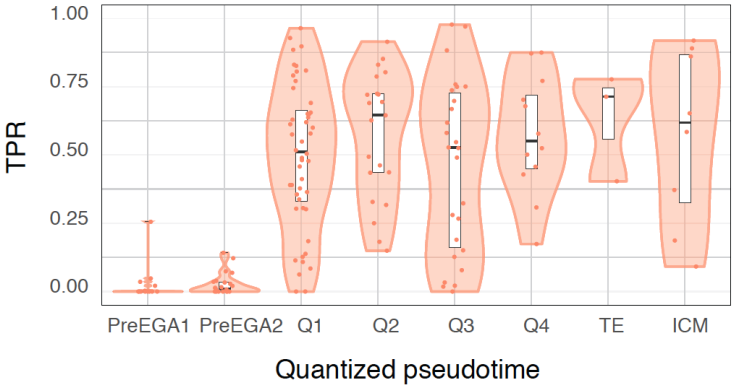




InferCNV benchmark with G&T-seq data (254 cells)

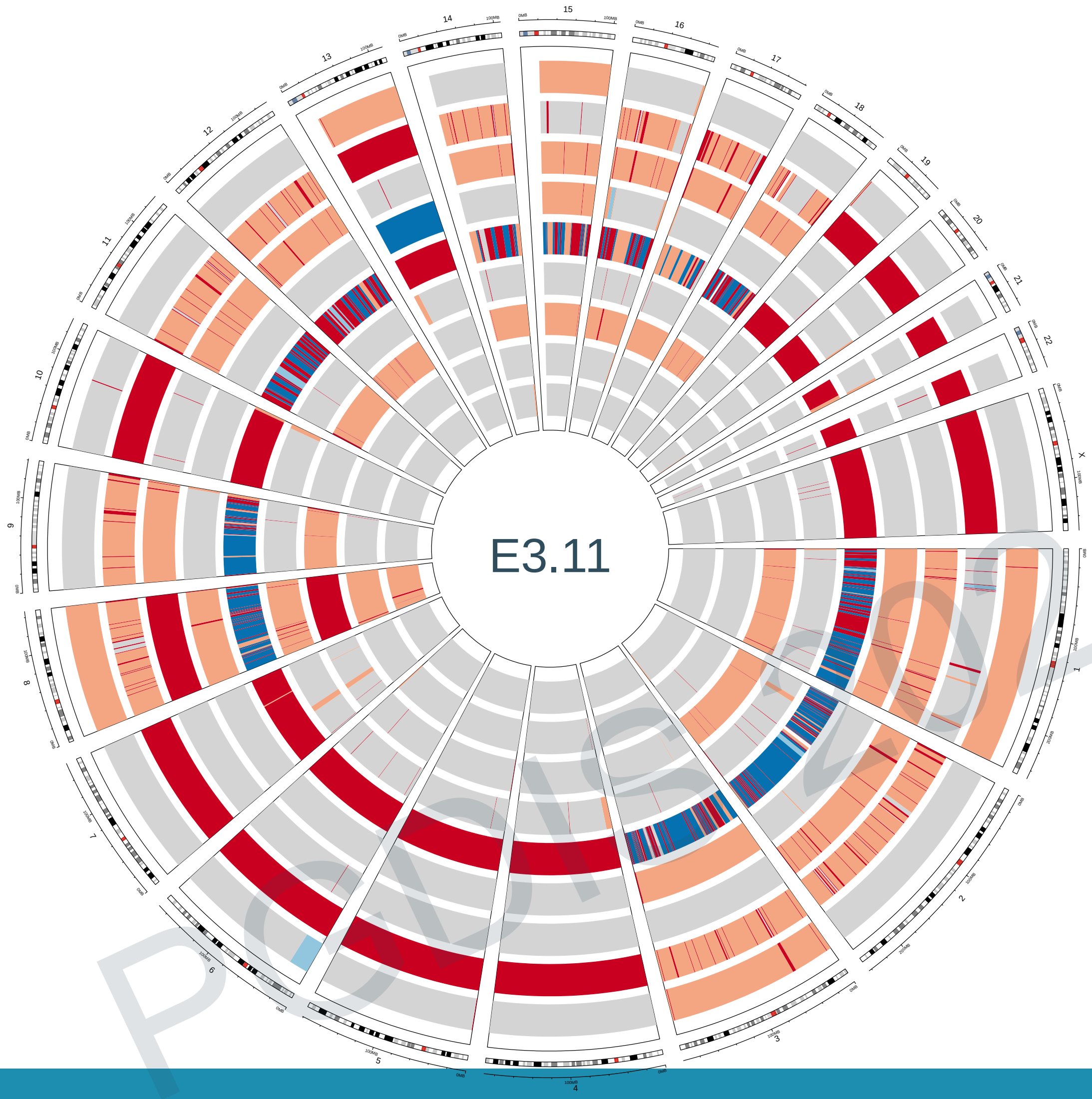


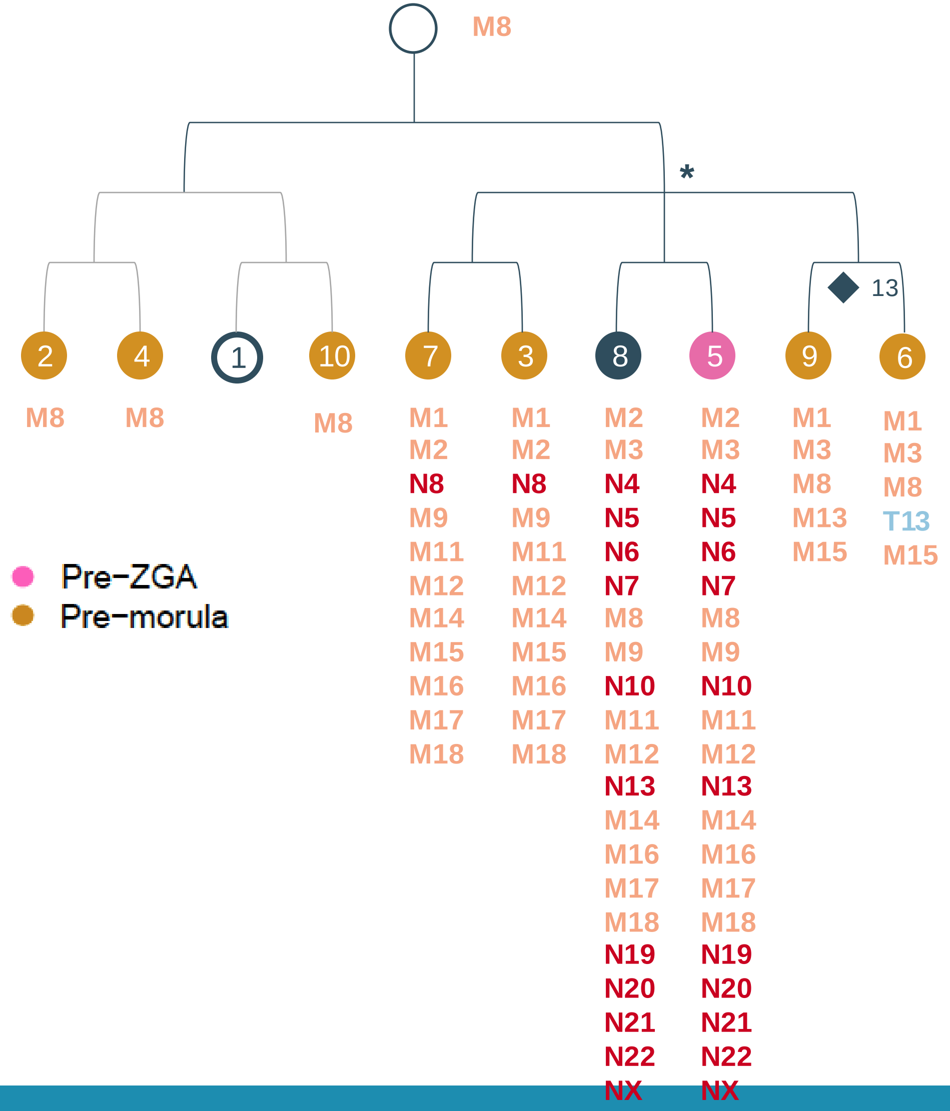




Genomic regions: After EGA has occurred, inferCNV is able to capture about **50% of DNA-seq detected CNVs** and about 90% of euploid genomic regions.

Cell lineage trees indicate different lineages within the same embryo





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