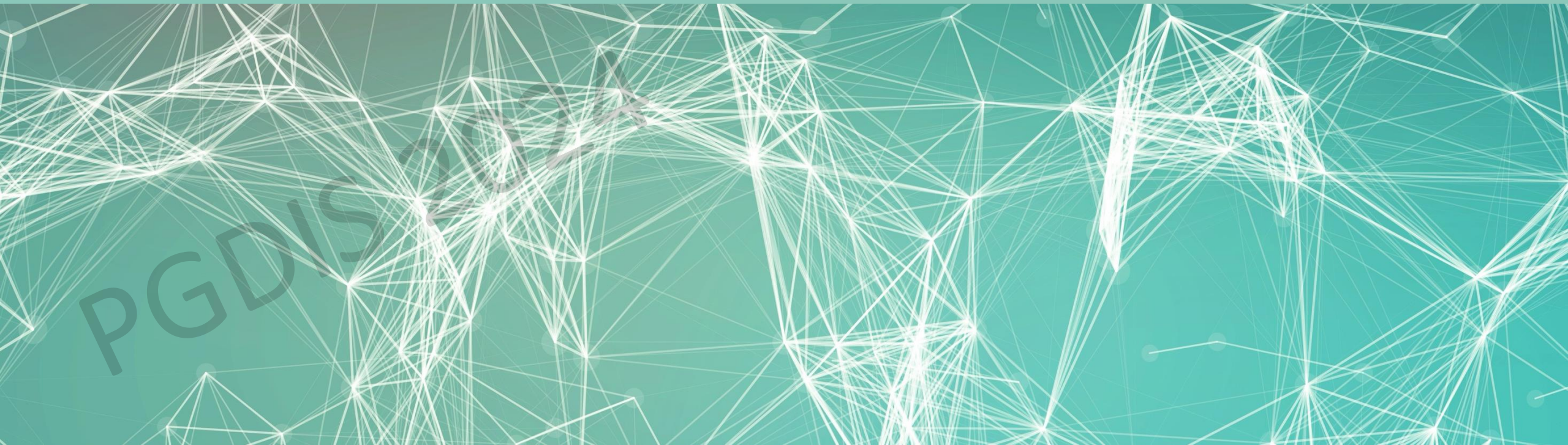
# Next Generation Sequencing

Prof. Dagan Wells
University of Oxford
Director, Juno Genetics

JUNO GENETICS



# Preimplantation genetic testing for aneuploidy – A 30 year old idea

Proposed that aneuploidy testing could assist embryo selection (Munne et al., 1993)


**Diagnosis of major chromosome aneuploidies in human preimplantation embryos**

Santiago Munné[1], Andrew Lee, Zev Rosenwaks, Jamie Grifo and Jacques Cohen

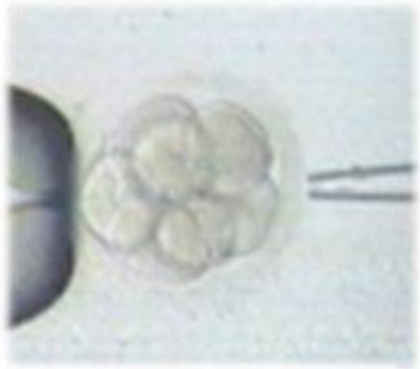

Preimplantation Diagnosis of Common Aneuploidies by the First- and Second-Polar Body FISH Analysis

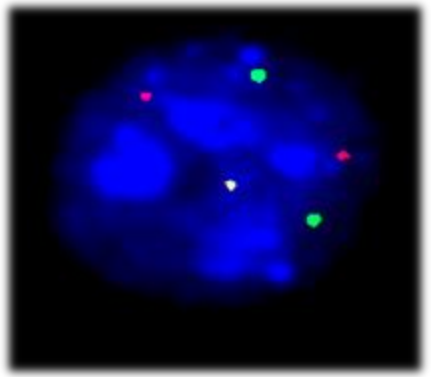
Y. VERLINSKY,[1,2] J. CIESLAK,[1] V. IVAKHNENKO,[1] S. EVSIKOV,[1] G. WOLF,[1] M. WHITE,[1] A. LIFCHEZ,[1] B. KAPLAN,[1] J. MOISE,[1] J. VALLE,[1] N. GINSBERG,[1] C. STROM,[1] and A. KULIEV[1]

# The evolution of PGT-A

Most early attempts utilised cleavage stage biopsy and fluorescence *in situ* hybridisation (FISH)

- Limited chromosomal analysis (5-9 chromosomes)
- Suboptimal accuracy and reproducibility
- Impact of day-3 biopsy on embryo viability





Clinical efficacy not supported by most randomised trials

By 2009 some PGT providers were testing as many as 12 chromosomes in each blastomere

# The evolution of PGT-A

Whole genome amplification (WGA) – First clinical application in PGT

Journal of Assisted Reproduction and Genetics

## Preimplantation Genetic Diagnosis of Inherited Cancer: Familial Adenomatous Polyposis Coli

ASANGLA AO,[1] DAGAN WELLS,[2] ALAN H. HANDYSIDE,[4] ROBERT M. L. WINSTON,[1] and JOY D. A. DELHANTY[2,3]

J Assist Reprod Genet. 1998 Mar;15(3):140-4. doi: 10.1023/a:1023008921386.

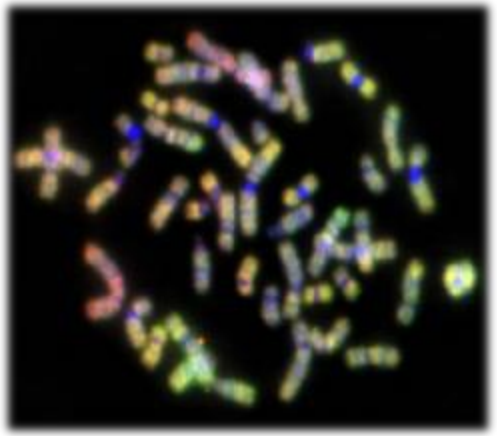
WGA can provide sufficient material for ‘DNA hungry’ methods

WGA + metaphase comparative genomic hybridization (CGH) (Wells et al 1999)

Nucleic Acids Research

## Detailed chromosomal and molecular genetic analysis of single cells by whole genome amplification and comparative genomic hybridisation

Dagan Wells*, Jon K. Sherlock, Alan H. Handyside[1] and Joy D. A. Delhanty



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JARG
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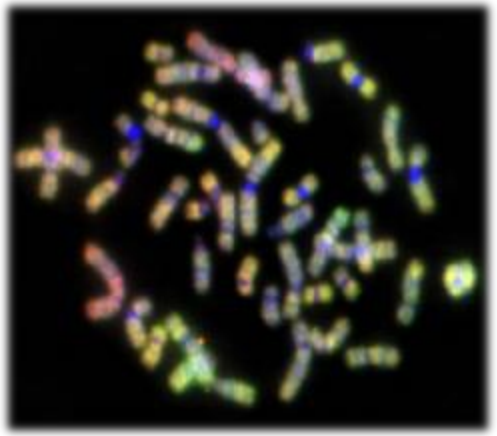
WGA can provide sufficient material for ‘DNA hungry’ methods

CGH shed light on true aneuploidy rate (Wells et al 1999; Wells and Delhanty, 2000; Voullaire et al 2000)

molecular human reproduction

**Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization**

Dagan Wells[1] and Joy D.A.Delhanty



# The evolution of PGT-A

Clinical application of CGH in blastomeres (Wilton et al 2001)

Clinical application of CGH in polar bodies (Wells et al 2002)


**First clinical application of comparative genomic hybridization and polar body testing for preimplantation genetic diagnosis of aneuploidy**

*Dagan Wells, Ph.D.,[a,b] Tomas Escudero, B.Sc.,[b] Brynn Levy, Ph.D.,[c] Kurt Hirschhorn, M.D.,[c] Joy D. A. Delhanty, Ph.D.,[a] and Santiago Munné, Ph.D.[b]*

The NEW ENGLAND JOURNAL of MEDICINE

**BIRTH OF A HEALTHY INFANT AFTER PREIMPLANTATION CONFIRMATION OF EUPLOIDY BY COMPARATIVE GENOMIC HYBRIDIZATION**

LEEANDA WILTON, PH.D., ROBERT WILLIAMSON, PH.D., JOHN MCBAIN, M.B., B.S., DAVID EDGAR, PH.D., AND LUCILLE VOULLAIRE, M.SC.

# The evolution of PGT-A

Clinical application of CGH in blastomeres (Wilton et al 2001)

Clinical application of CGH in polar bodies (Wells et al 2002)

But…..

Highly skilled

Very labour intensive

Low throughput

High cost

It was not yet time for comprehensive chromosome screening, but all that was about to change…

Parallel development

blastocyst culture

vitrification

CGH


**Clinical application of comprehensive chromosomal screening at the blastocyst stage**

*William B. Schoolcraft, M.D.,[a] Elpida Fragouli, Ph.D.,[b,c] John Stevens, M.S.,[a] Santiago Munne, Ph.D.,[d] Mandy G. Katz-Jaffe, Ph.D.,[b] and Dagan Wells, Ph.D., F.R.C.Path.[b,c]*

First encouraging clinical data (Schoolcraft et al., 2010)

# The evolution of PGT-A

Microarray CGH (Alfarawati et al 2011; Gutiérrez-Mateo et al 2011; Le Caignec et al 2006; Vanneste et al 2011)

All chromosomes assessed

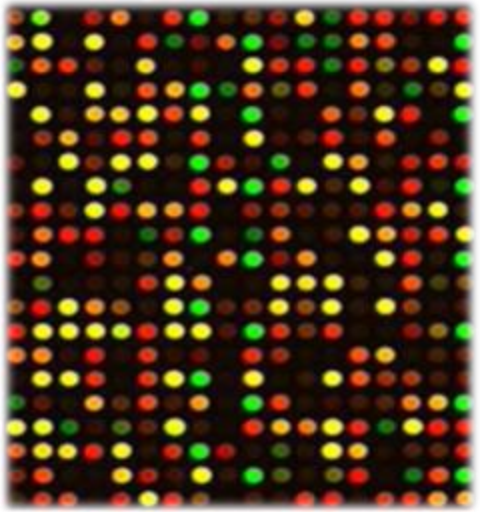
Higher throughput

Still relatively expensive

Fertility and Sterility®

**Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos**

*Cristina Gutiérrez-Mateo, Ph.D.,[a] Pere Colls, Ph.D.,[a] Jorge Sánchez-García, Ph.D.,[a] Tomas Escudero, B.Sc.,[a] Renata Prates, B.Sc.,[a] Kelly Ketterson, B.Sc.,[a] Dagan Wells, Ph.D.,[b] and Santiago Munné, Ph.D.[a]*



# The evolution of PGT-A

Next generation sequencing (Wells et al 2014; Tan et al 2014)

All chromosomes assessed

Allows for higher throughput

Journal of Medical Genetics

**Clinical utilisation of a rapid low-pass whole genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation**

Dagan Wells,[1] Kulvinder Kaur,[2] Jamie Grifo,[3] Michael Glassner,[4] Jenny C Taylor,[2] Elpida Fragouli,[5] Santiago Munne[6]

(GIGA)n SCIENCE

**Clinical outcome of preimplantation genetic diagnosis and screening using next generation sequencing**

Yueqiu Tan[1,5,6†], Xuyang Yin[3,4,7†], Shuoping Zhang[1,6,8], Hui Jiang[4,7,11], Ke Tan[1,5], Jian Li[2], Bo Xiong[6], Fei Gong[1,6], Chunlei Zhang[4,7], Xiaoyu Pan[4,7,14], Fang Chen[4,7,16], Shengpei Chen[4,7,15], Chun Gong[2], Changfu Lu[1,6], Keli Luo[1,6], Yifan Gu[1,6], Xiuqing Zhang[7], Wei Wang[3,4], Xun Xu[2], Gábor Vajta[2,9], Lars Bolund[2,10], Huanming Yang[2,12,13], Guangxiu Lu[1,5,6,8], Yutao Du[3*] and Ge Lin[1,5,6,8*]

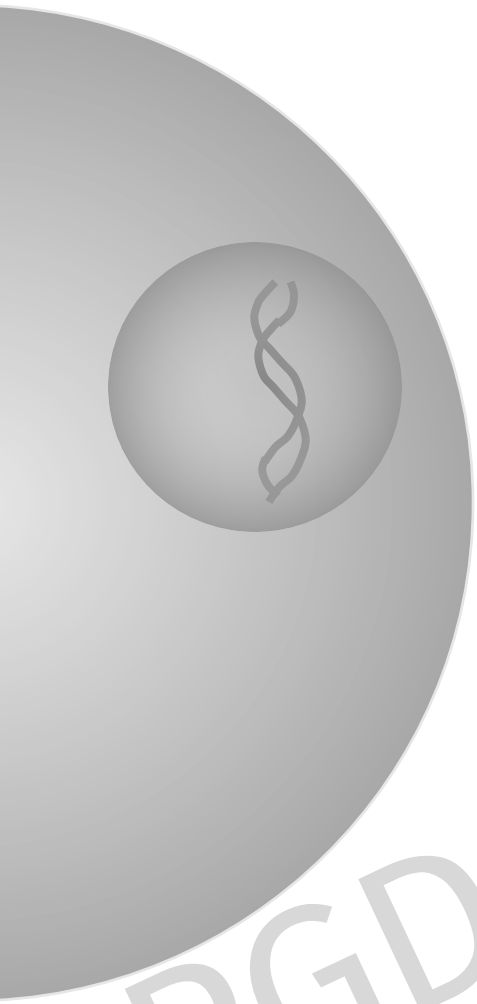
Sequence millions of DNA fragments from the biopsy specimen

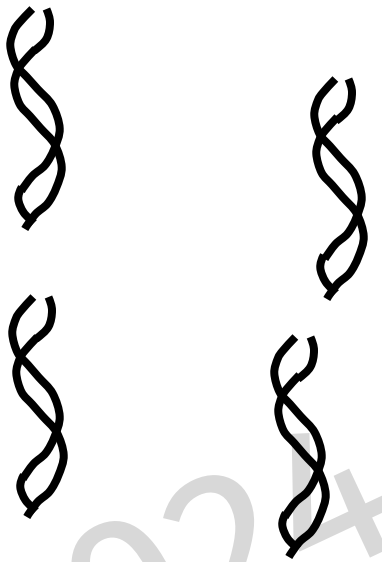
Measure proportion of fragments from each part of the genome

Potential to examine DNA sequence as well as detect aneuploidy

Provides PGT-A at lower cost than other methods

# Next generation sequencing (NGS)

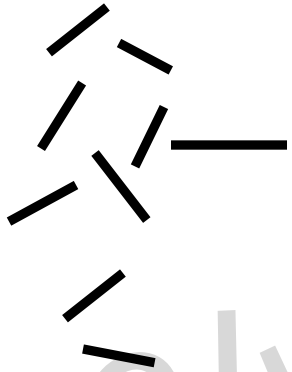




Whole genome amplification

# Next generation sequencing (NGS)

Fragmentation



Sequencing of multiple small pieces of DNA from across the genome

ATTAGACTTAGCCTAGATTCCAATGACTG

Millions of short sequence ‘reads’ produced

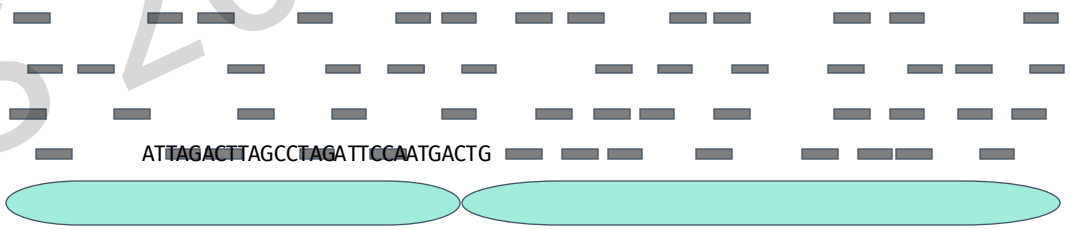
# Next generation sequencing (NGS)

Sequences are aligned to the human genome

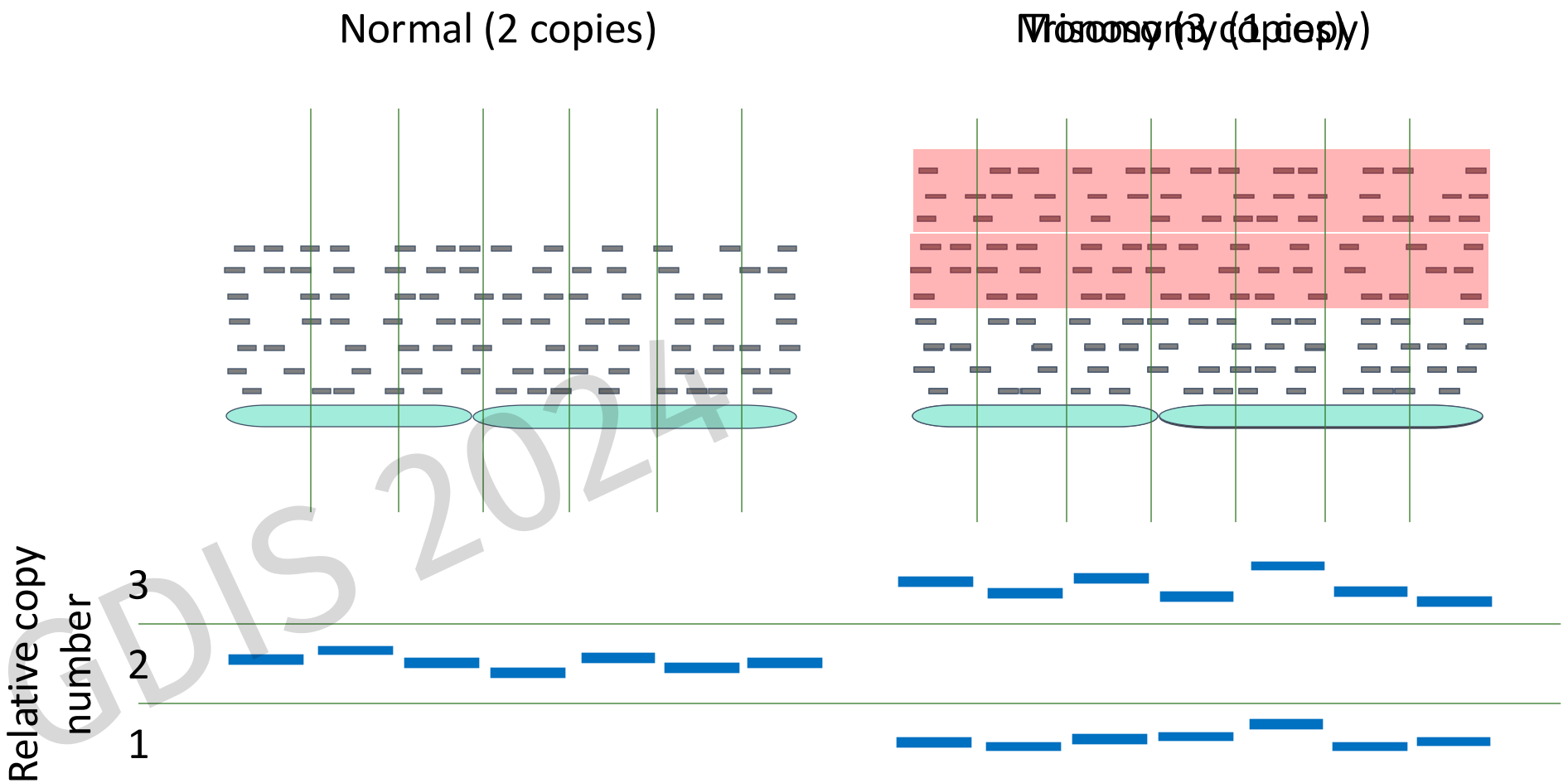
ATTAGACTTAGCCTAGATTCCAATGACTG

# Next generation sequencing (NGS)

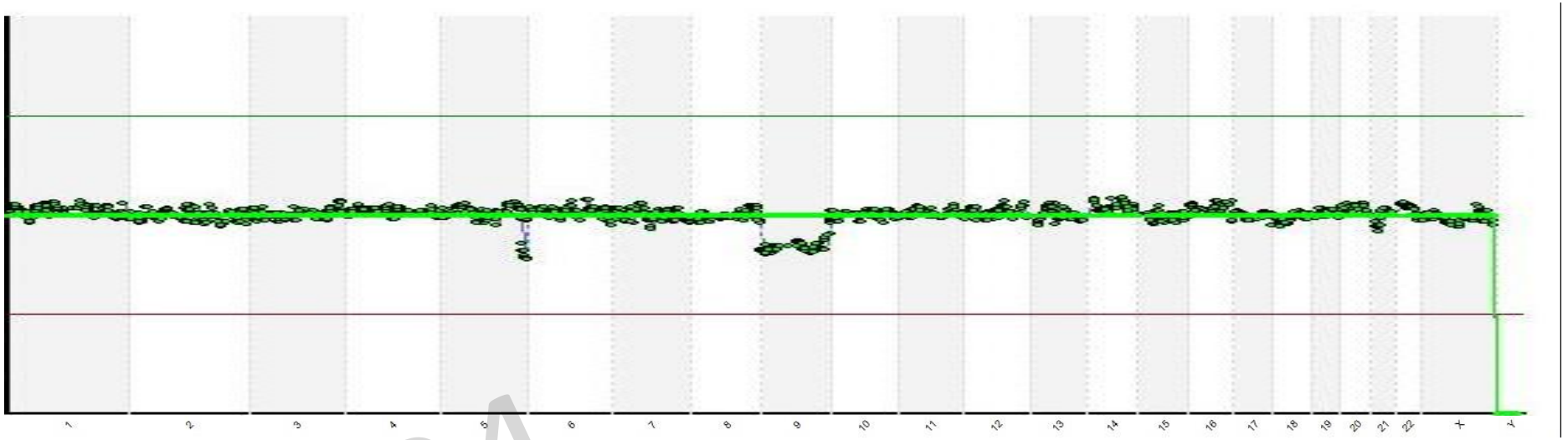
Thousands of DNA fragments from each chromosome are identified (but <0.1% of genome sequenced)



# Next generation sequencing (NGS)



# Additional information from NGS: Mosaicism



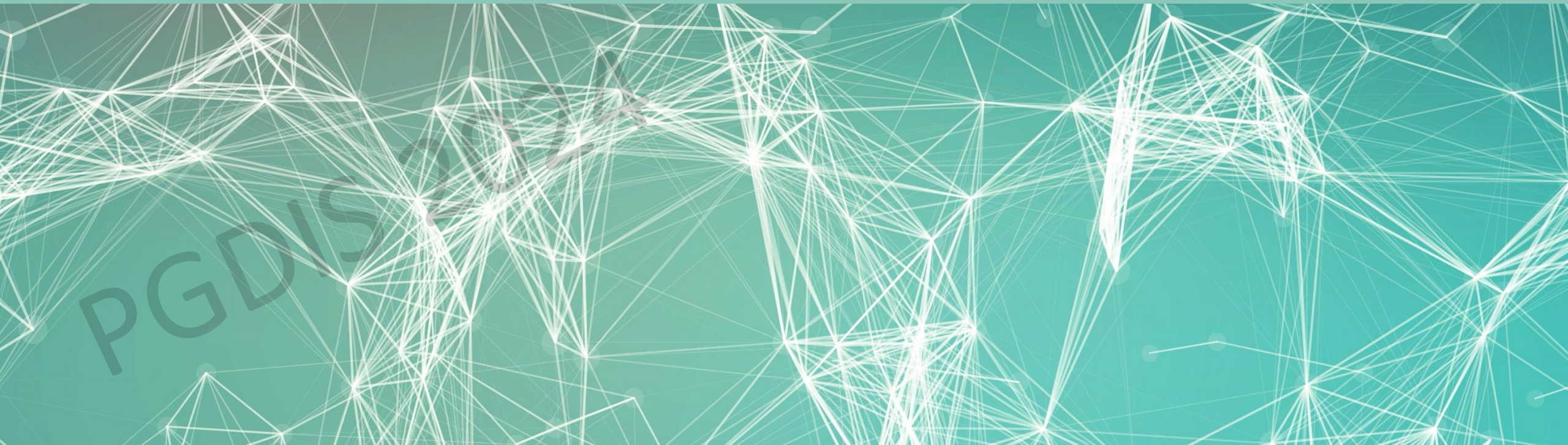
NGS - Normal (possible loss of chromosome 9, also mosaic segmental abnormality on chromosome 5

aCGH - definite loss of chromosome 9, also mosaic segmental abnormality on chromosome 5

NGS is the most effective method for detecting mosaic trophectoderm biopsies

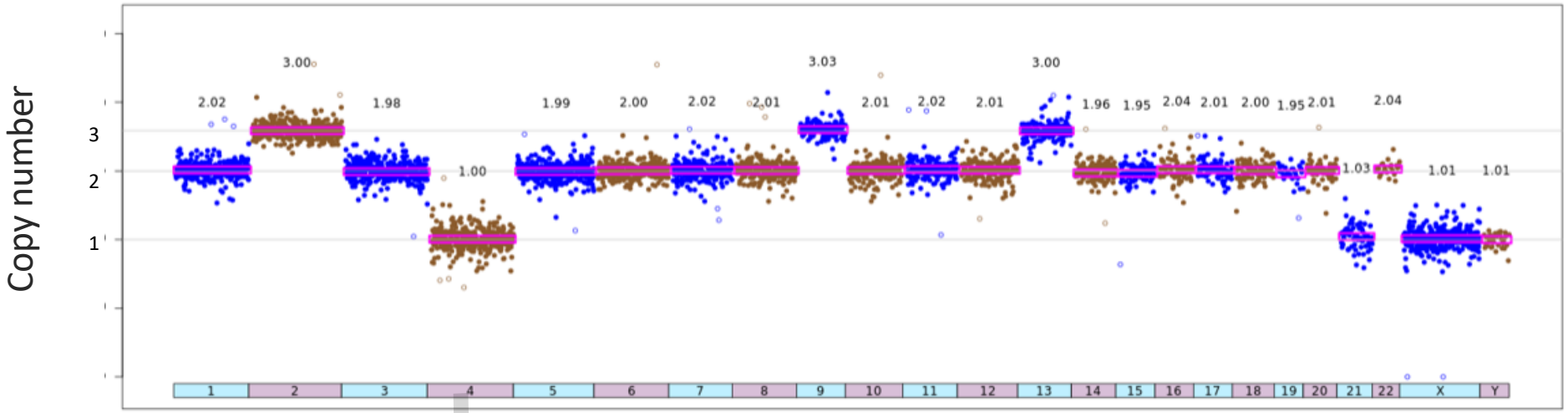
# Latest developments:

# Achieving maximum PGT-A accuracy by extracting more information from NGS

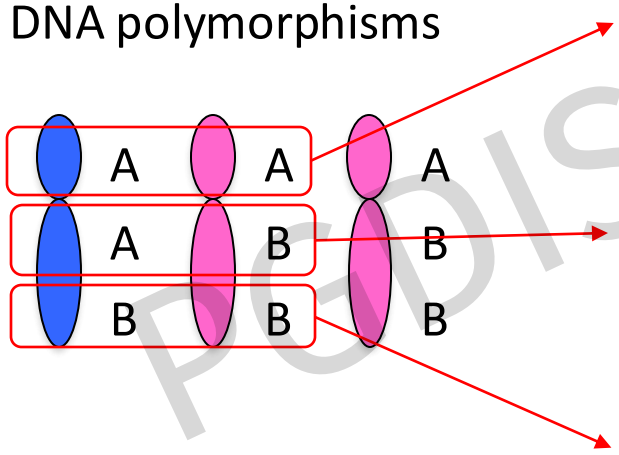


# NGS-based PGT-A: Latest developments

Extracting more information from next generation sequencing (PGTseq)




Some sequenced fragments contain DNA polymorphisms



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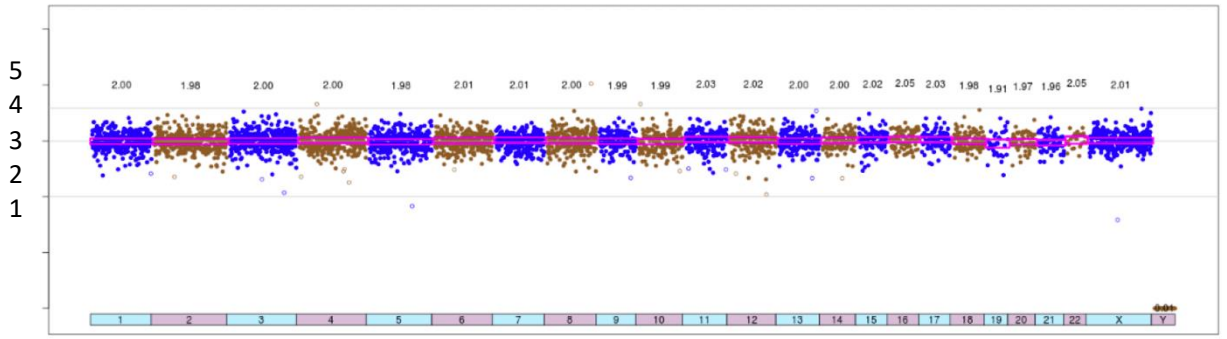



All autosomes have the same copy number

X-chromosome also has the same copy number

No Y-chromosome

Therefore, normal female?



Only methods that include polymorphism analysis can detect XXX triploidy

# Validated PGT-A methods can provide a molecular fertilization/ploidy check

**Correct assignment of ploidy (haploidy/diploidy/triploidy) is important**

1PNs – 63% that produce blastocysts turn out to be diploid

3PNs- Almost half that form blastocysts turn out to be diploid

Traditionally, these potentially viable embryos have been wrongly discarded

They can be considered for transfer and may produce healthy babies

2PNs – Approximately 1% are triploid or haploid

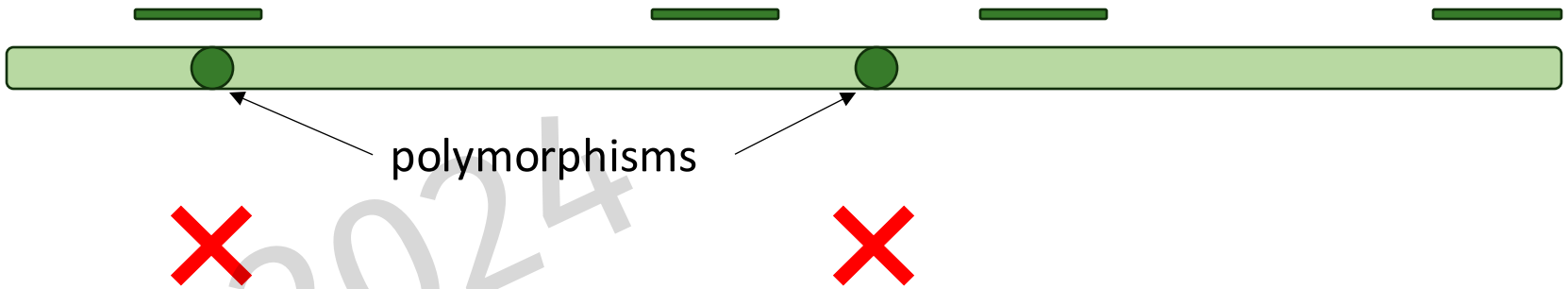
PGTseq molecular fertilization check that has close to 100% accuracy

# Analysis of polymorphisms using NGS

Most methods involve WGA

Low pass genome sequence provides information on the amount of DNA from each chromosome

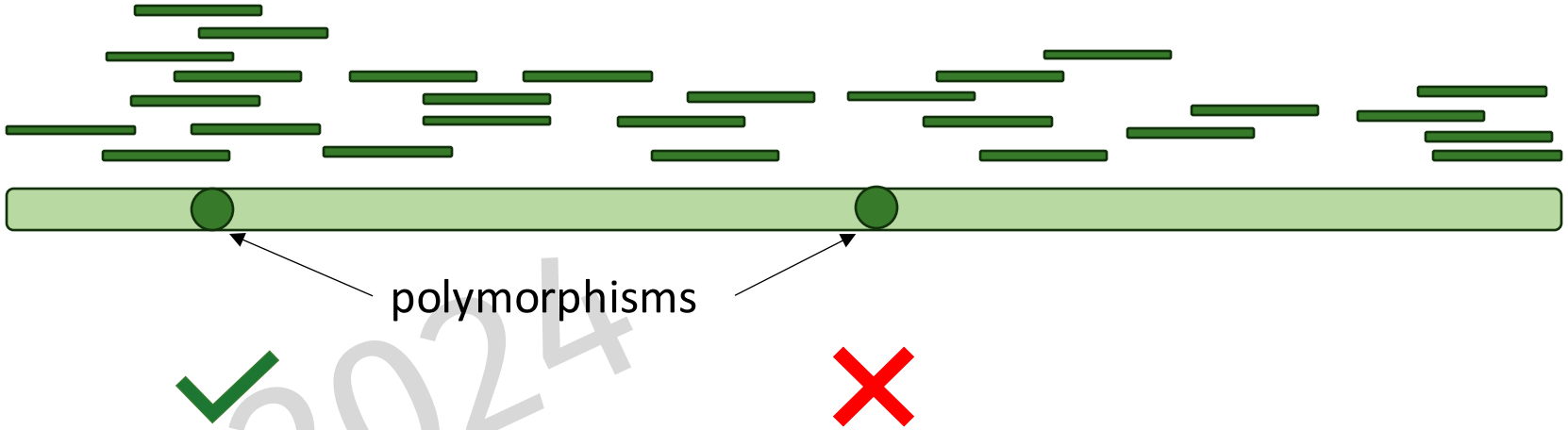
Polymorphic sites are sequenced at insufficient depth for genotyping



# Analysis of polymorphisms using NGS

Most methods involve WGA

Deep sequencing of each sample – Aim is to provide sufficient coverage depth for accurate genotyping of multiple polymorphisms on each chromosome

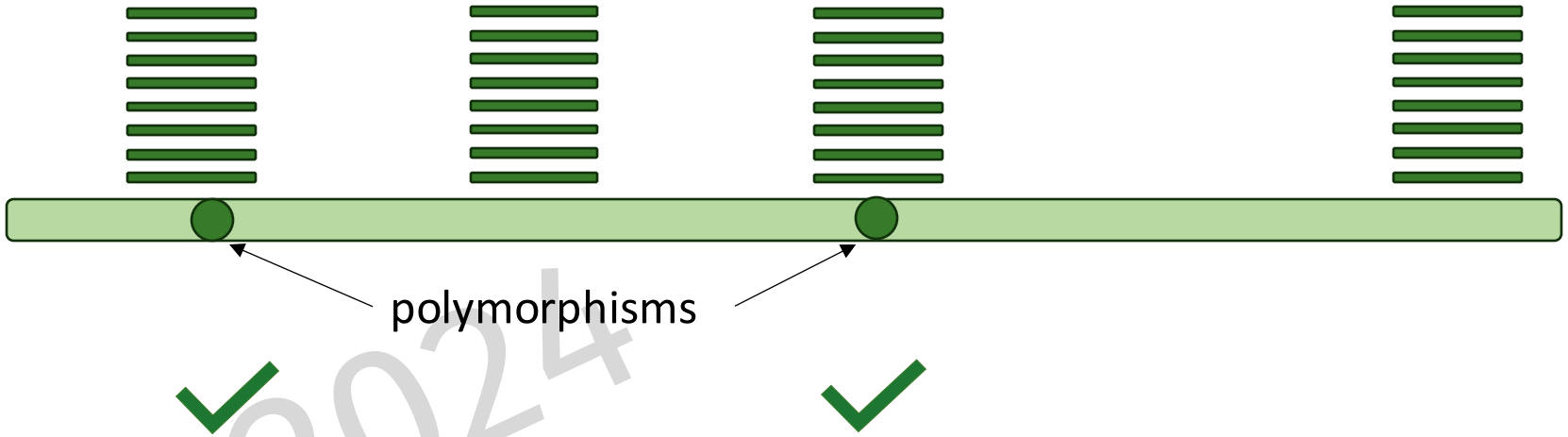



Alternatively, targeted PCR amplification of polymorphisms from additional aliquot of WGA product

# Analysis of polymorphisms using NGS

Instead of WGA, can perform PGT-A via massive targeted amplification

Thousands of defined sites containing polymorphisms reproducibly amplified - Guarantees accurate genotyping with less sequencing



## Advantages of NGS-based PGT including polymorphism analysis

Increased accuracy of aneuploidy detection

Detection of 23,X and 69,XXX (molecular fertilization check, >99% accurate)

Detection of some forms of uniparental disomy

# How do we know a PGT method is accurate?

## PGTseq method (Juno Genetics)

Kim JG et al., 2021

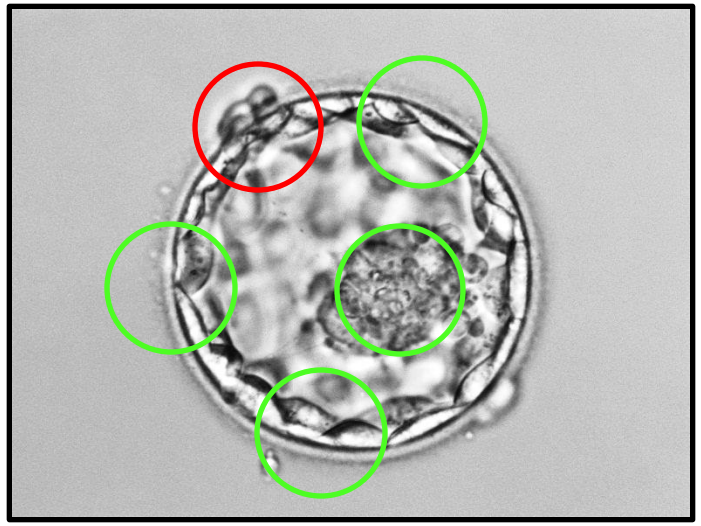
Blastocysts with a routine PGT-A result

↓

Donated for research

↓

Biopsied an additional 4 times



| | Original PGT-A result Euploid |
|---|---|
| PGT-A result confirmed in ≥1 other biopsy | 137/137 (100%) |
| PGT-A result confirmed in all other biopsies | 135/137 (98.5%) |

## Advantages of NGS-based PGT including polymorphism analysis

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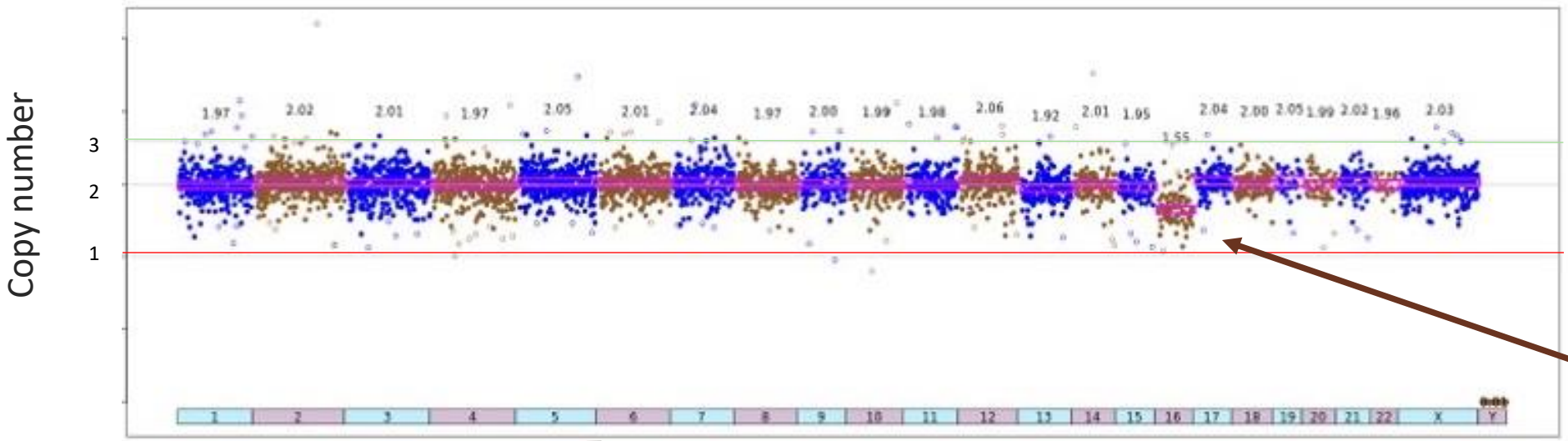
Detection of some forms of uniparental disomy

Detection of contamination

# Contamination detection



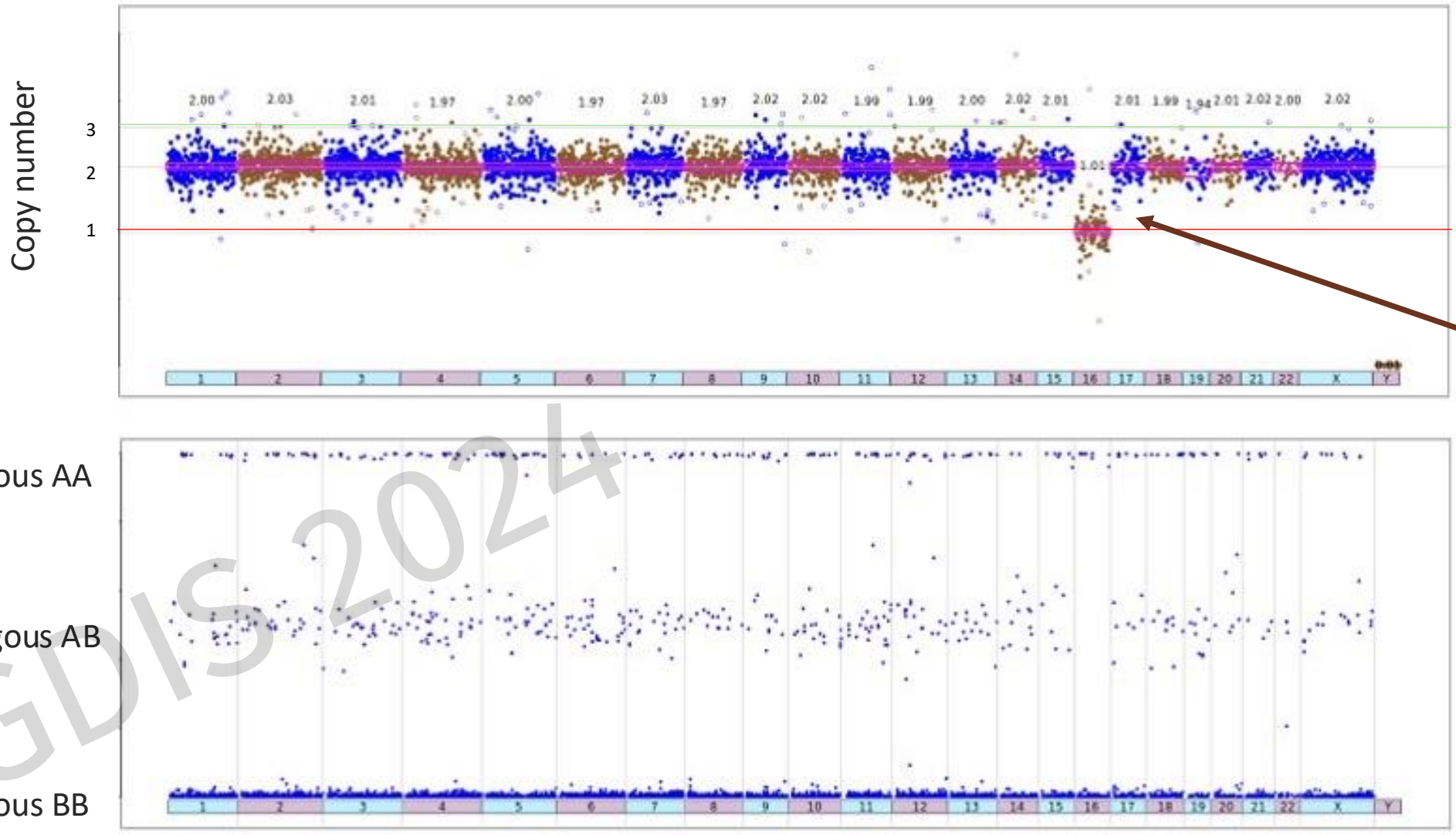

Mosaic?



Biopsy sample is contaminated

# Contamination detection

Re-biopsy result



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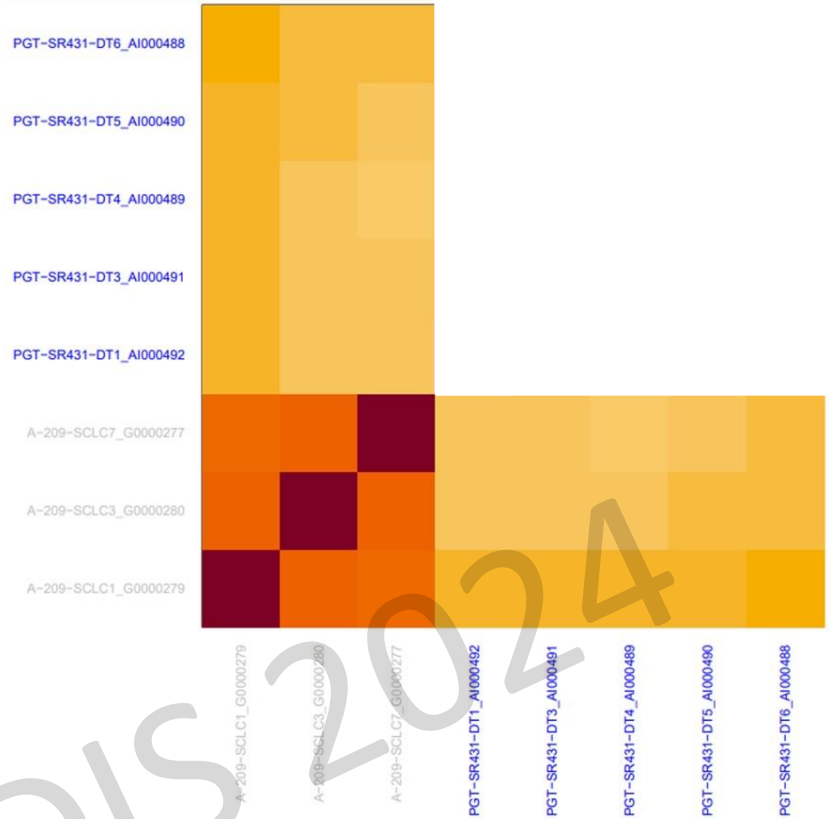
Detection of contamination

More accurate detection of mosaicism

Confirmation of siblingship between embryos

# Confirmation of relationships




Important part of routine QC using PGTseq

Compare relatedness of all samples on a PGT-A run

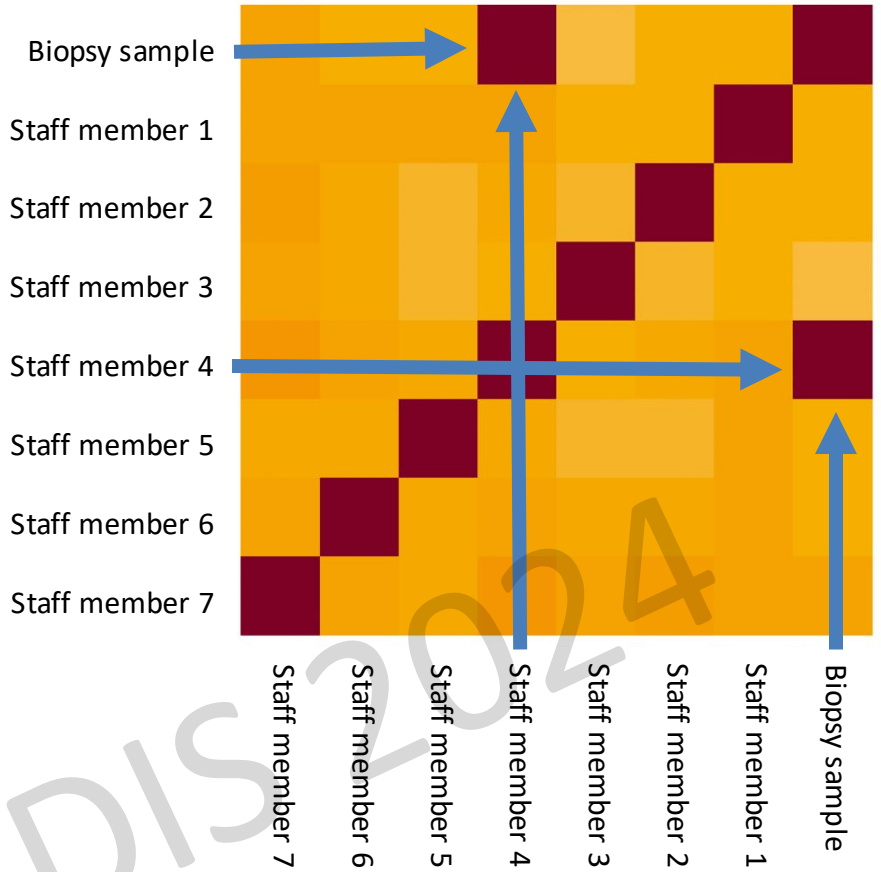
Does not require parental DNA

Genetically identical

Sibling

Unrelated

# Confirmation of relationships

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Increased accuracy of aneuploidy detection

Detection of 23,X and 69,XXX

Detection of some forms of uniparental disomy

Detection of contamination

More accurate detection of mosaicism

Confirmation of siblingship between embryos

Can reveal parental origin of meiotic aneuploidies

# NGS approaches based upon targeted amplification can permit high accuracy PGT-M in combination with PGT-A

WGA methods increase the rate of allele dropout (although less so for some modern WGA methods)

PNAS
Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

## Accurate genomic variant detection in single cells with primary template-directed amplification

Veronica Gonzalez-Pena[a,b,1], Sivaraman Natarajan[b,c,1], Yuntao Xia[a], David Klein[a], Robert Carter[a], Yakun Pang[a,b], Bridget Shaner[c], Kavya Annu[b], Daniel Putnam[c], Wenan Chen[c], Jon Connelly[d], Shondra Pruett-Miller[d], Xiang Chen[c], John Easton[c], and Charles Gawad[a,b,c,e,2]

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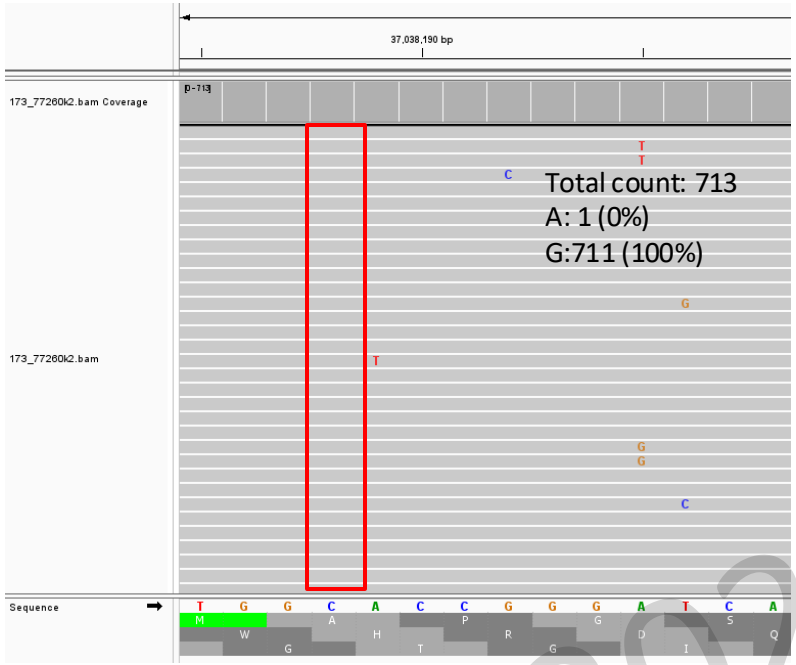
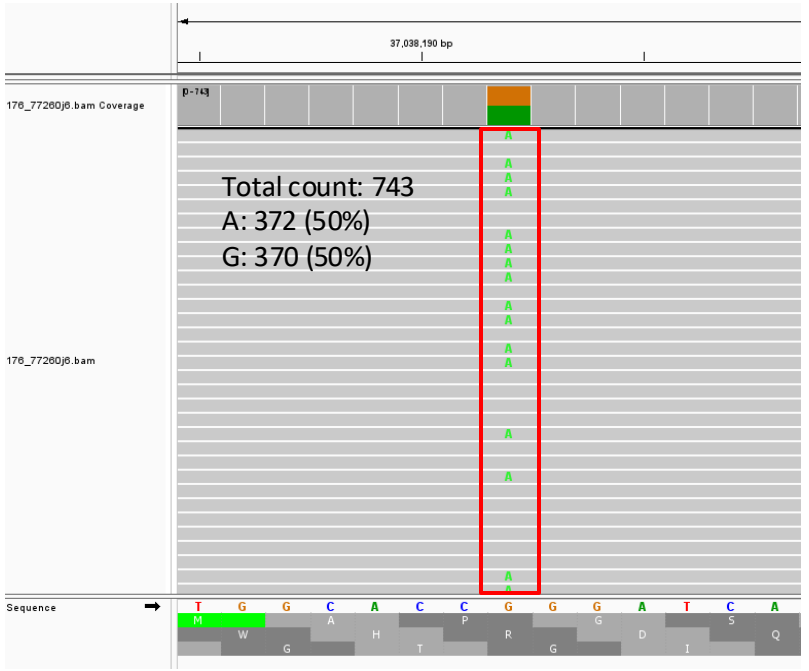
NGS allows sequencing of mutations at the same time as performing PGT-A

# NGS approaches based upon targeted amplification can permit high accuracy PGT-M in combination with PGT-A

Using a targeted amplification method, applied at the blastocyst stage, ADO is extremely rare (0.17%)




Analysis of the mutation site alone provides high accuracy PGT-M

without needing a reference individual (linked markers become less important)

without expensive microarrays or large panels of polymorphisms

## Conclusions

Next generation sequencing has ushered in a new age in preimplantation genetic testing

PGT-A using NGS, including analysis of polymorphisms, provides the highest accuracy yet achieved

haploidy, triploidy, some UPD, contamination, sample identity, molecular fertilization check

However, levels of validation still require improvement

NGS provides highly accurate PGT-M, especially when combined with targeted amplification methods

With continually improving WGA methods and falling costs of sequencing, genome sequencing of embryos is becoming a realistic possibility.

Will that be clinically useful?

Is it ethically acceptable?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