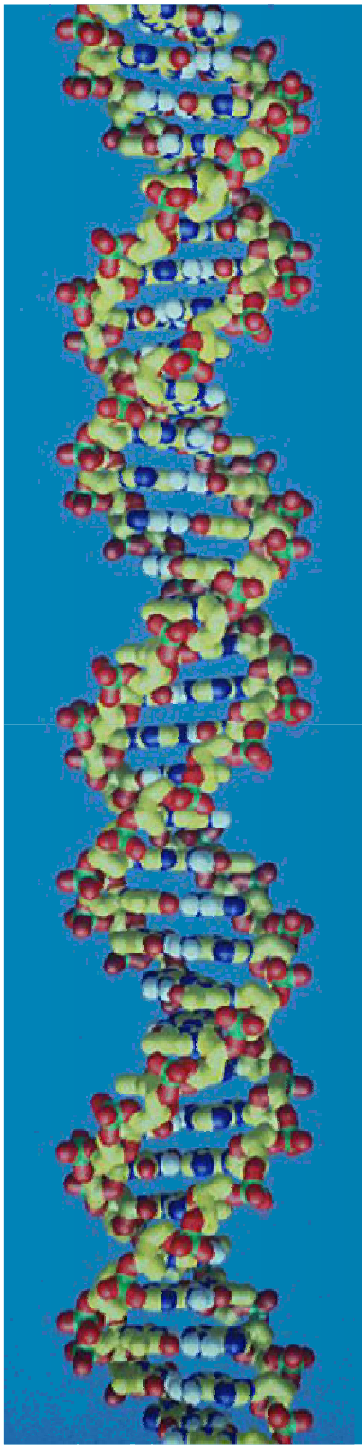


Preimplantation Genetic Diagnosis:
A Celebration of 20 Years
ESHRE Campus Symposium
Rome, July 1st, 2010

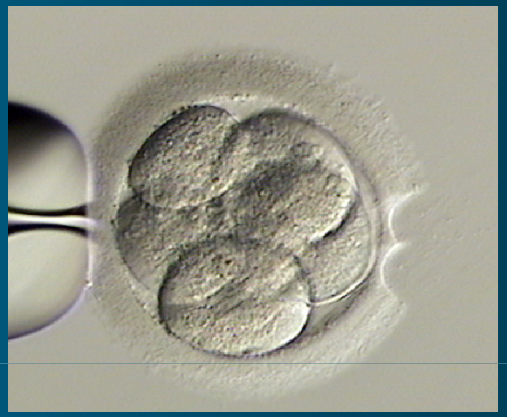
The evolution of technologies over 20 years

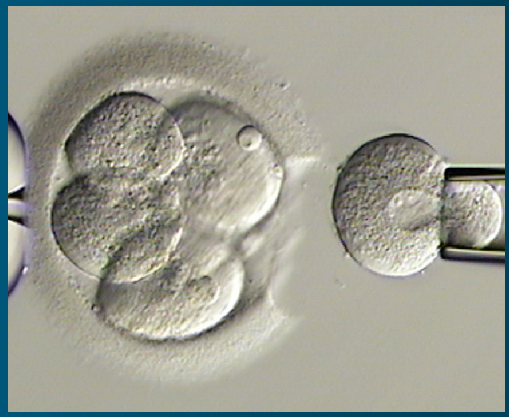
Alan H Handyside

London Bridge Fertility, Gynaecology
And Genetics Centre
London
United Kingdom

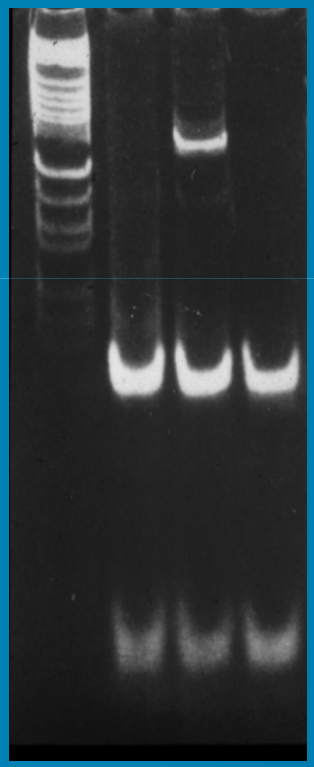
Preimplantation Genetic Diagnosis (PGD)

- In Vitro Fertilisation (IVF)
  - Downregulation of ovarian folliculogenesis
  - Superovulation and egg collection
  - Intracytoplasmic sperm microinjection (ICSI) and embryo culture
  - Cleavage stage biopsy by zona drilling and micromanipulation
- Single cell genetic analysis (12-72h)
- Selective transfer of unaffected embryos 3-5 days post fertilisation



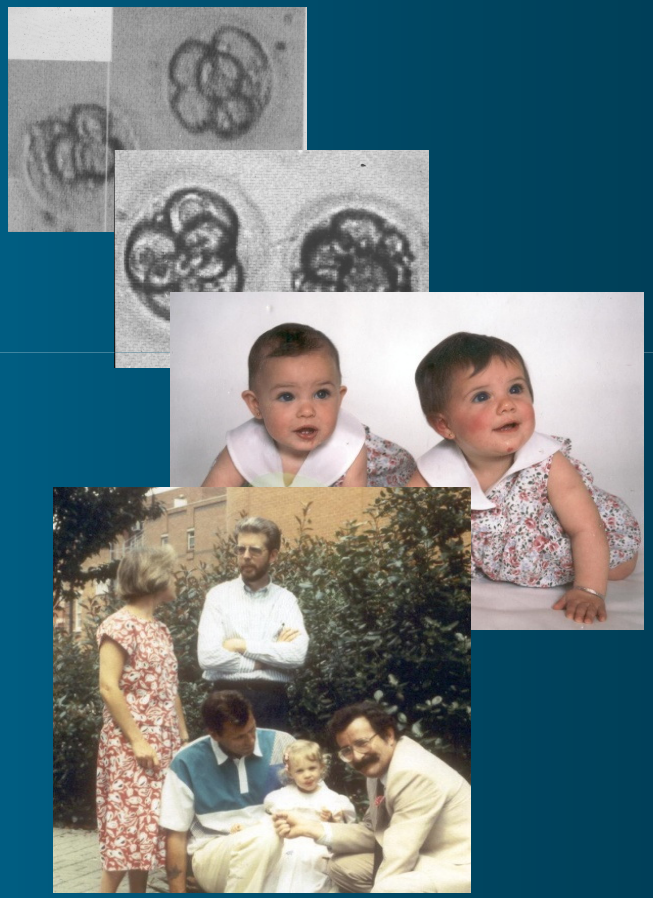


Pregnancies from biopsied human preimplantation embryos sexed by Y-specific amplification



Gender identification and selective transfer of unaffected female embryos in X-linked disease

Amplification of DYZ1 using 40 cycles of PCR from single cleavage stage blastomeres



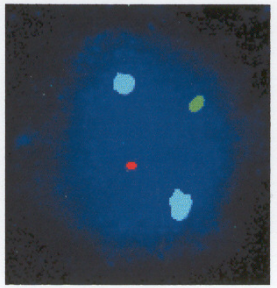
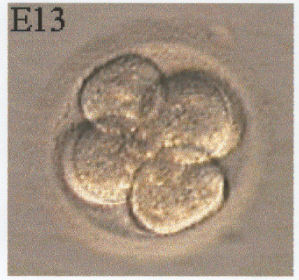
Handyside et al Nature (1990) 344, 768

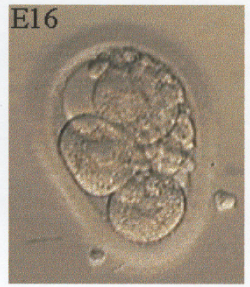
Born July, 1990

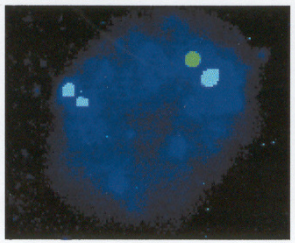
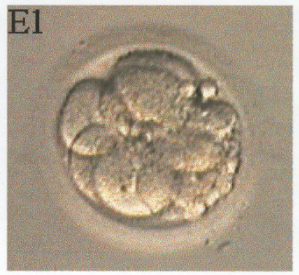
Identification of sex in X-linked disease

Biopsy FISH summary 2319C1

Biopsy Date 18/07/98 **Referral** X-LINKED ADRENOLEUKODYSTROPHY

		FISH Image	Embryo Image
Nucleus Ref	2319C1E13N1		 E13
Constitution	XY1818		
Classification	Diploid male		
Embryo Fate	Spread		

		FISH Image	Embryo Image
Nucleus Ref	2319C1E16N1		 E16
Constitution	XY1818		
Classification	Diploid male		
Embryo Fate	Spread		

		FISH Image	Embryo Image
Nucleus Ref	2319C1E1N1		 E1
Constitution	X1818		
	Aneuploid female		
	Spread		

Griffin et al (1991) Hum Reprod 6, 101
Griffin et al (1993) BMJ 306, 1382

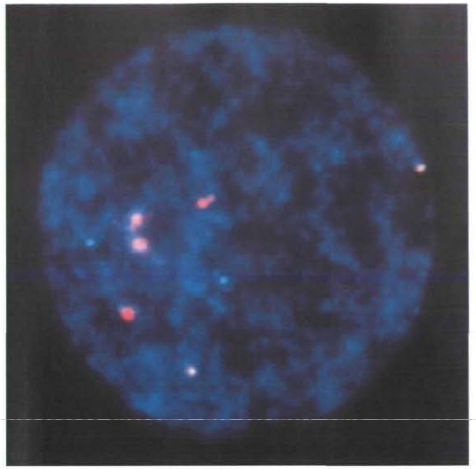
Human Reproduction vol.8 no.12 pp.2185–2191, 1993

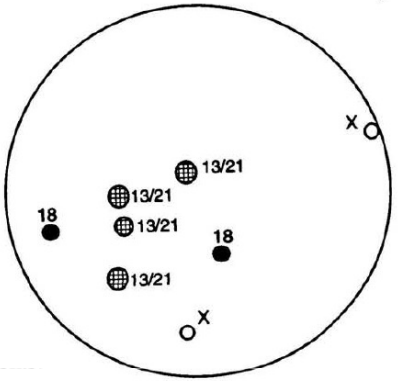
Diagnosis of major chromosome aneuploidies in human preimplantation embryos

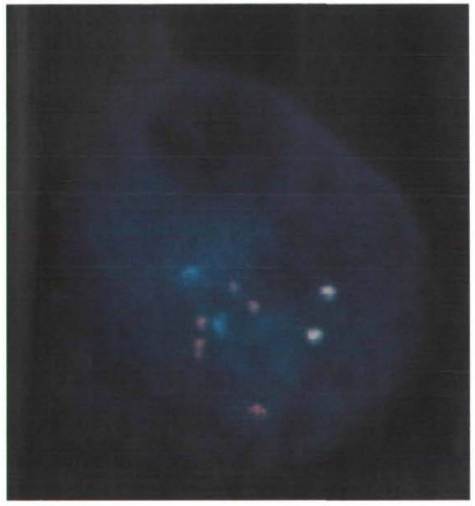
Santiago Munné[1], Andrew Lee, Zev Rosenwaks, Jamie Grifo and Jacques Cohen

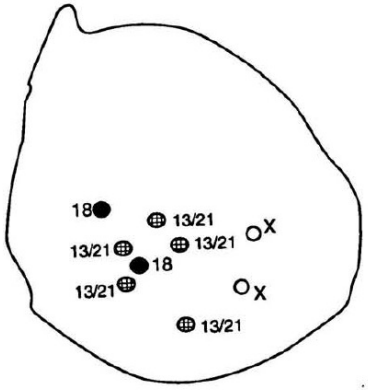
Center for Reproductive Medicine and Infertility, Department of Obstetrics and Gynecology, New York Hospital–Cornell University Medical Center, PO Box 30, 1300 York Avenue, New York, NY 10021, USA

[1]To whom correspondence should be addressed









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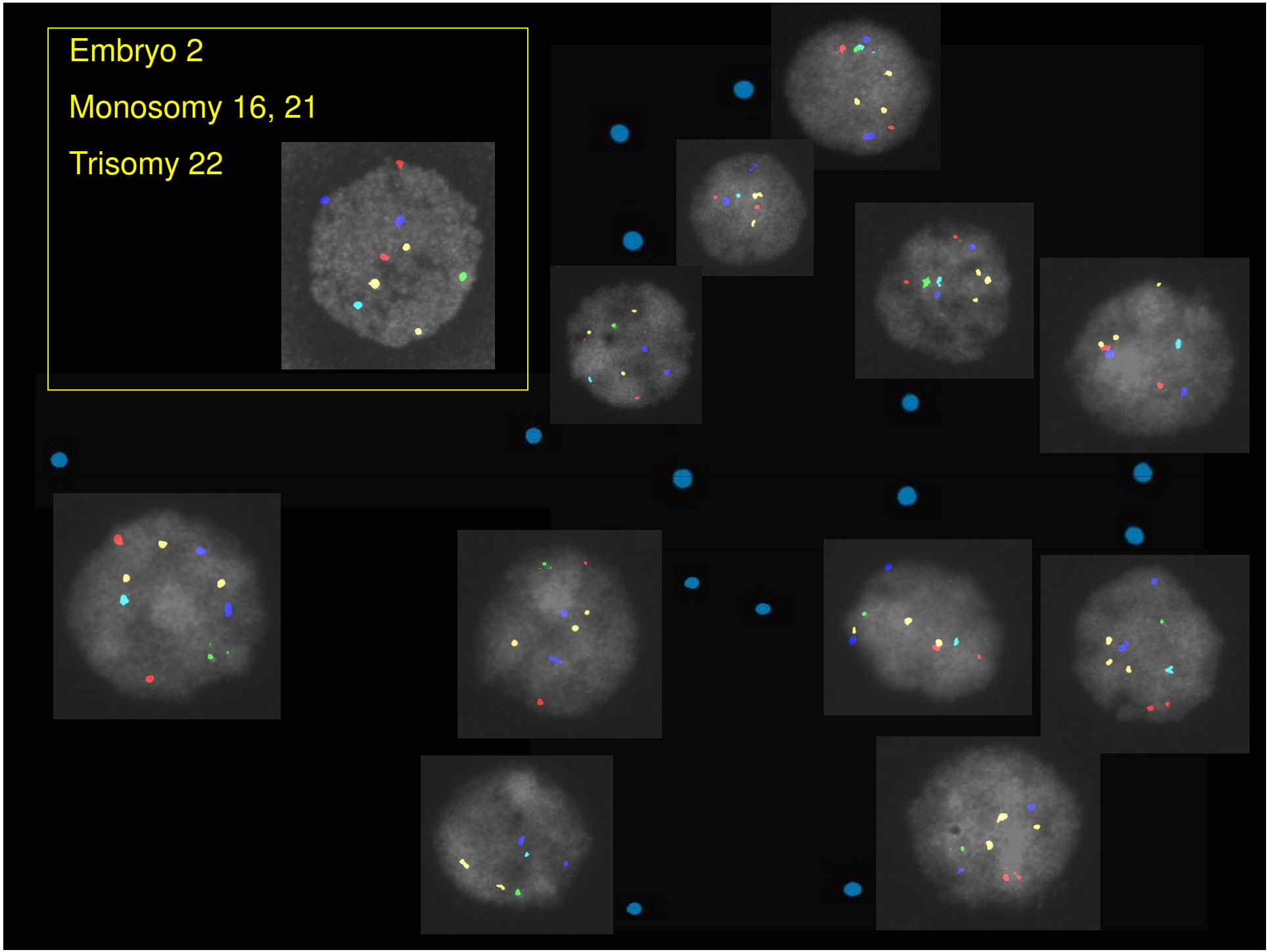
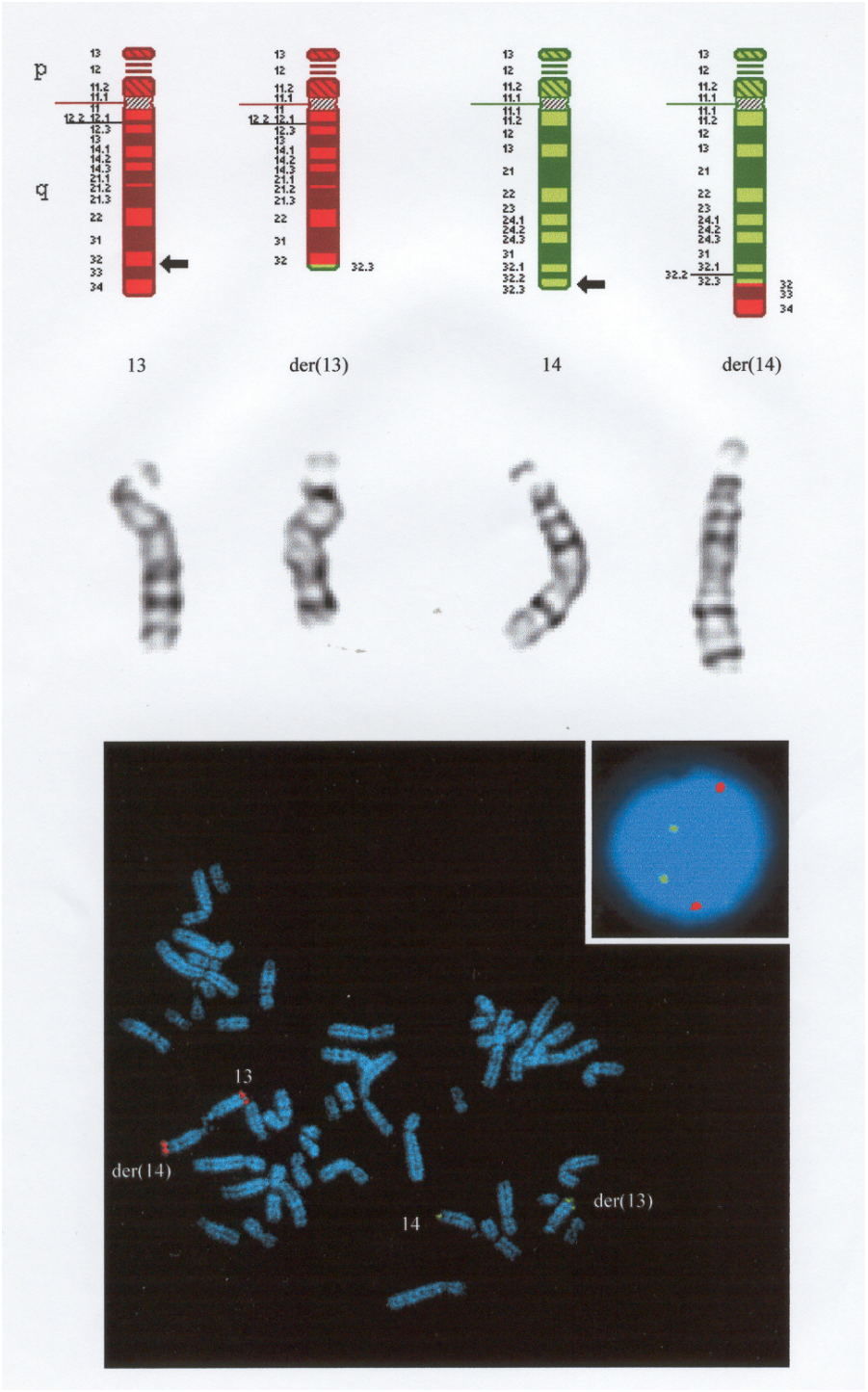
Preimplantation genetic diagnosis increases the implantation rate in human in vitro fertilization by avoiding the transfer of chromosomally abnormal embryos

Luca Gianaroli, M.D.
M. Cristina Magli, M.Sc.
Anna Pia Ferraretti, Ph.D.
Agnese Fiorentino, B.Sc.
John Garrisi, Ph.D.
Santiago Munné, Ph.D.

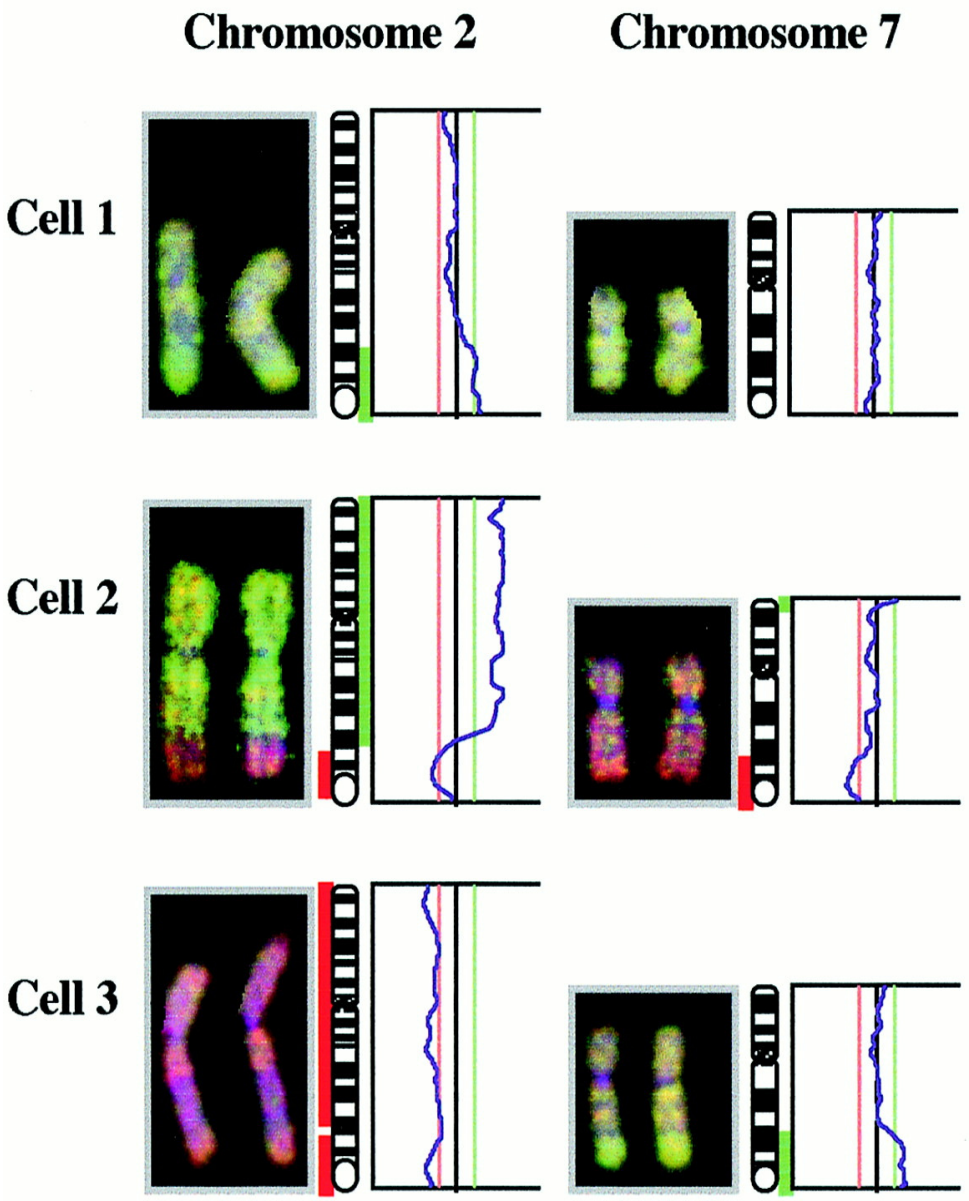
Società Italiana Studi Medicina della Riproduzione, Bologna, Italy; and The Institute for Reproductive Medicine and Science of St. Barnabas Medical Center, Livingston, New Jersey


Embryo 2
Monosomy 16, 21
Trisomy 22

PGD of reciprocal translocations using subtelomeric probes

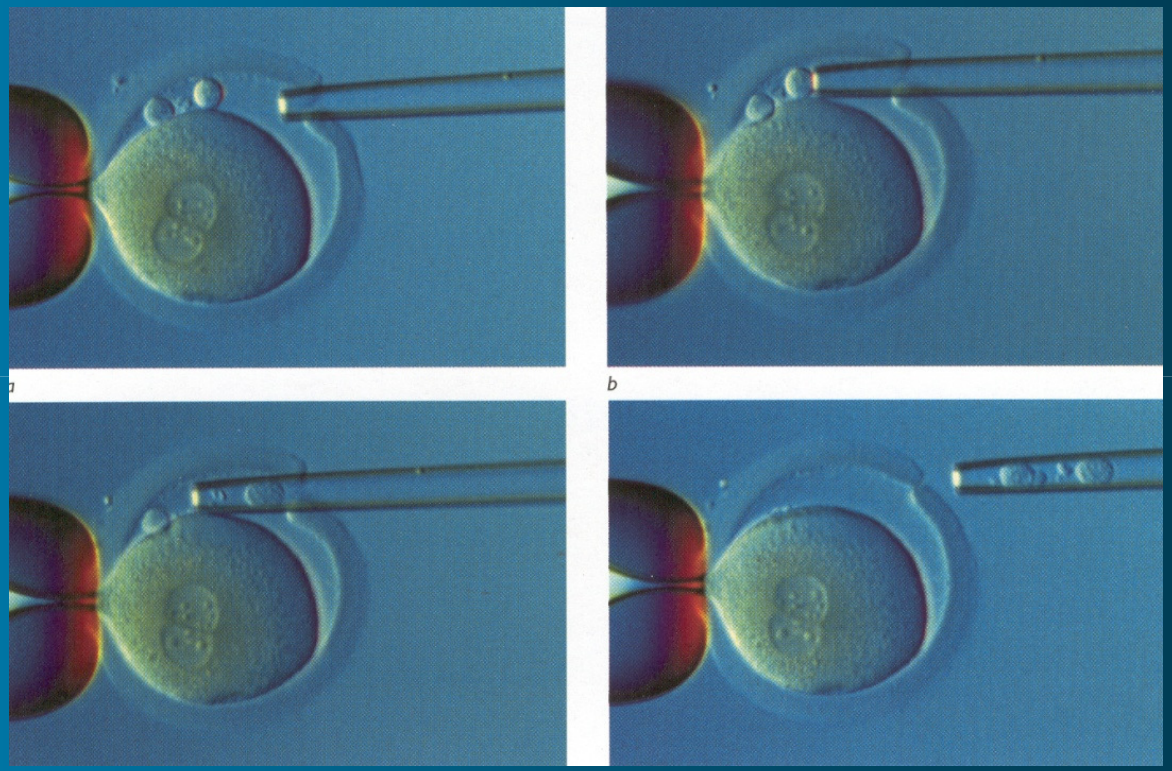


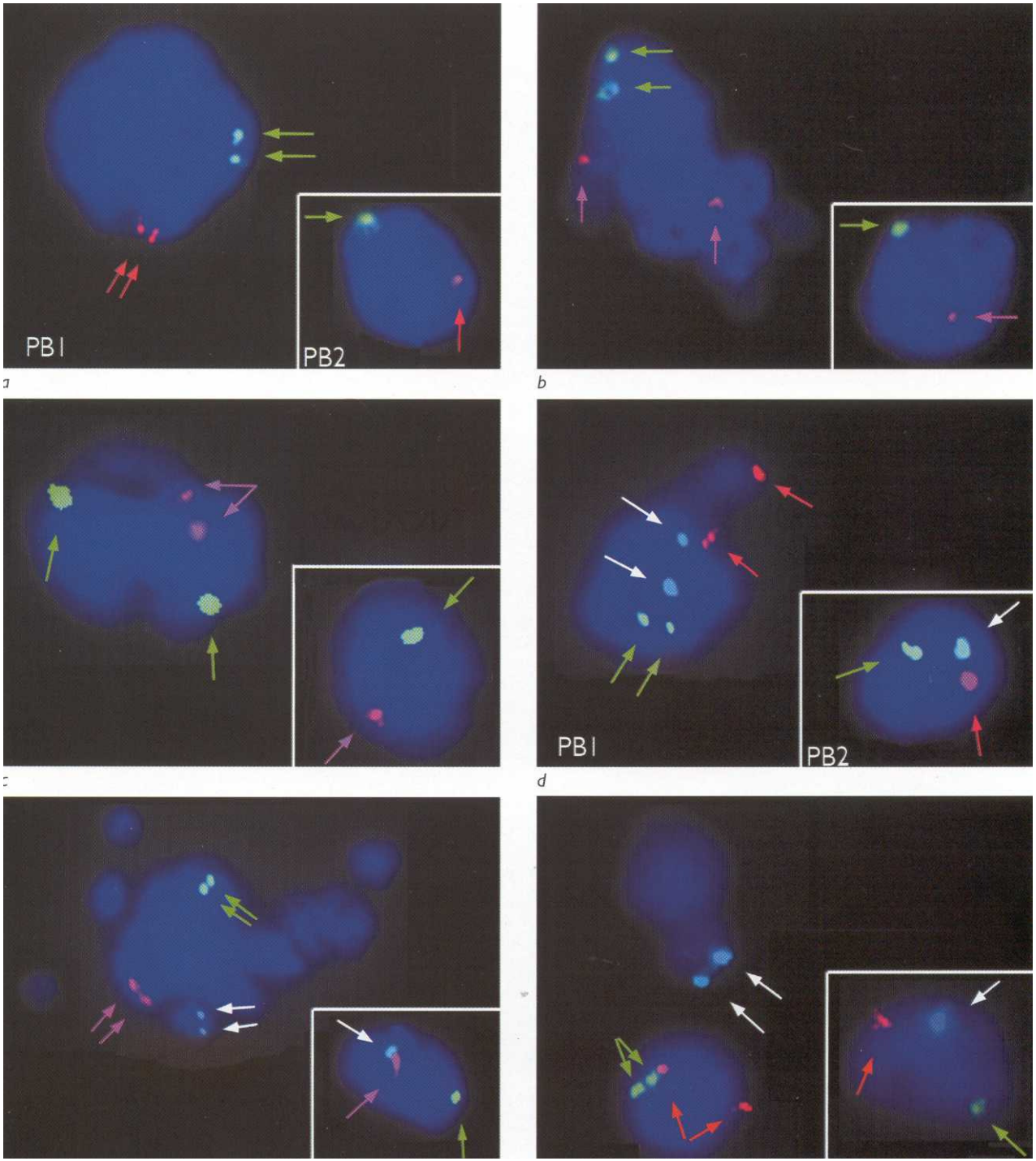
Scriven, Handyside and Ogilvie (1998) Prenatal Diagn 18, 1437



Wells et al (1999) NAR 27, 1214-1218

1st September 1943 -16th July 2009

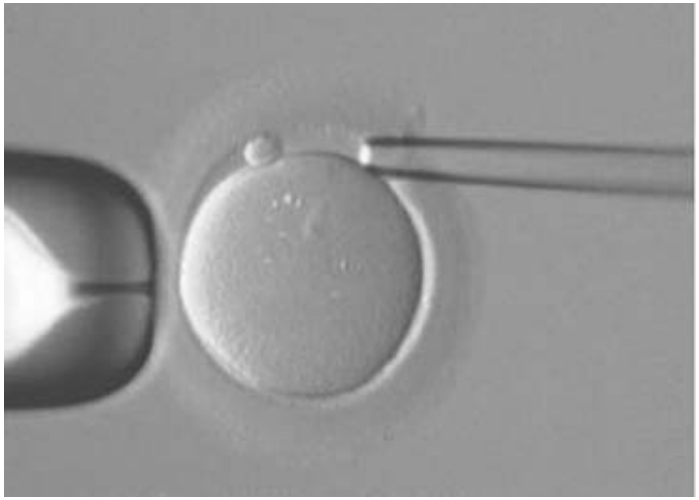




a-c mFISH with probes to 18 and 21

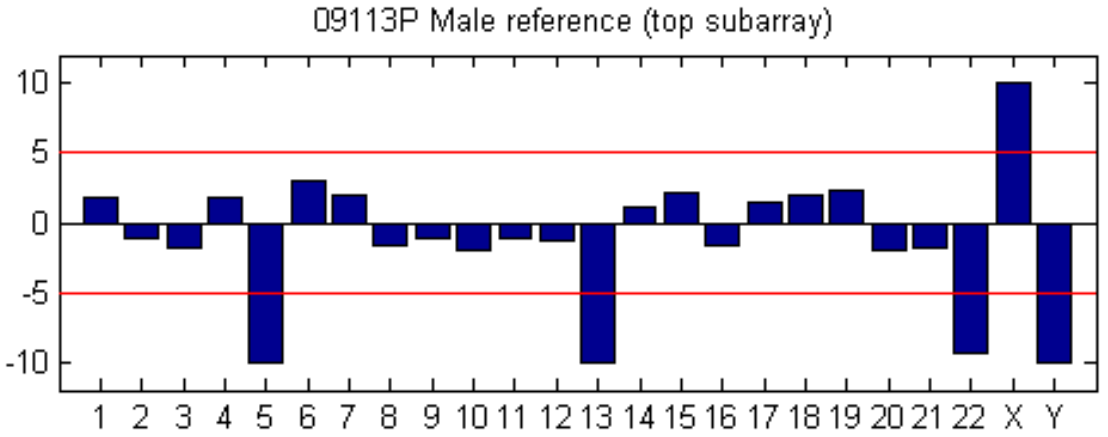
Normal signals in PB1 and PB2

d-f mFISH with probes to 13, 18 and 21

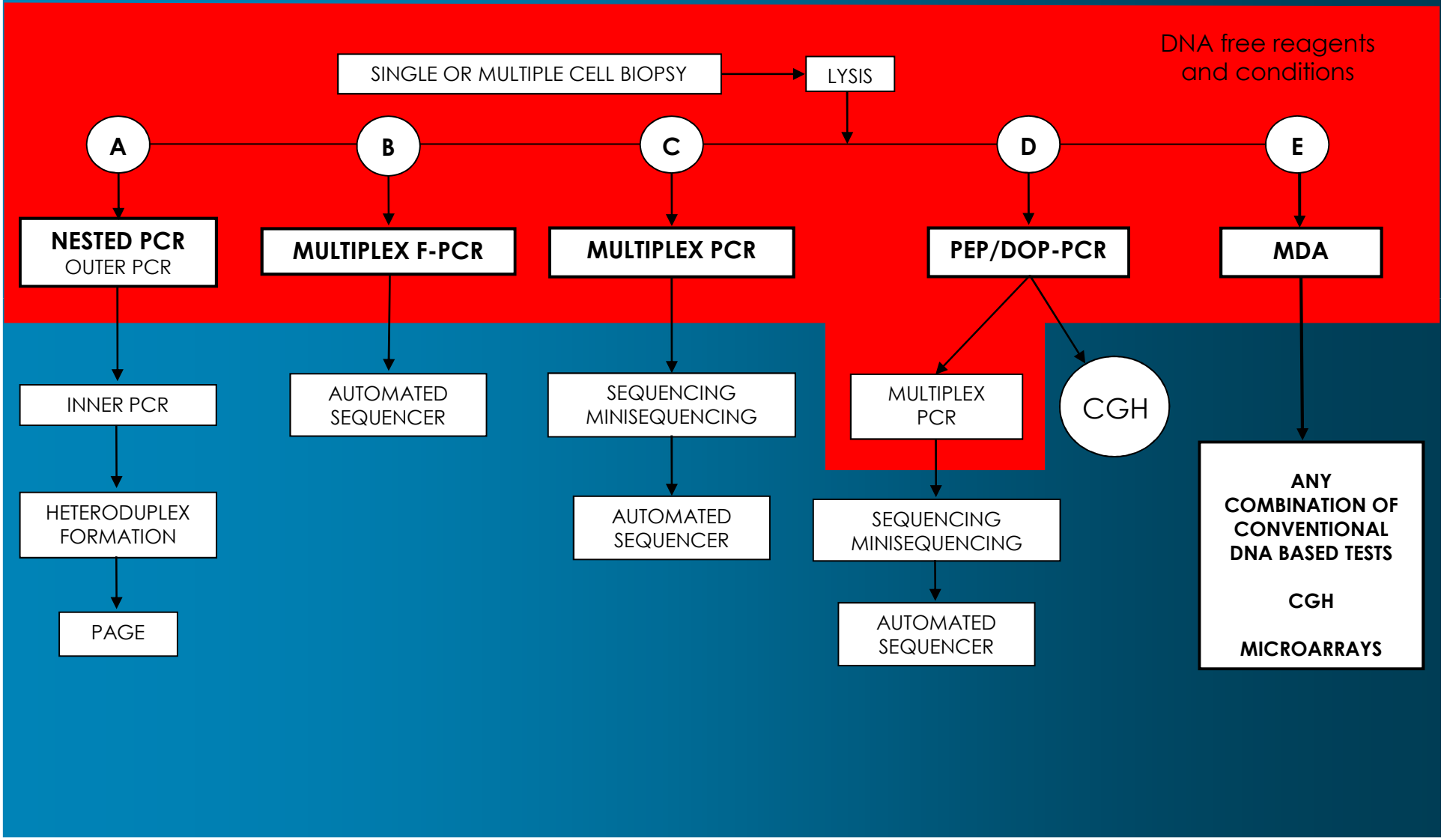


First or first and second polar body biopsy on day 0 or day 1

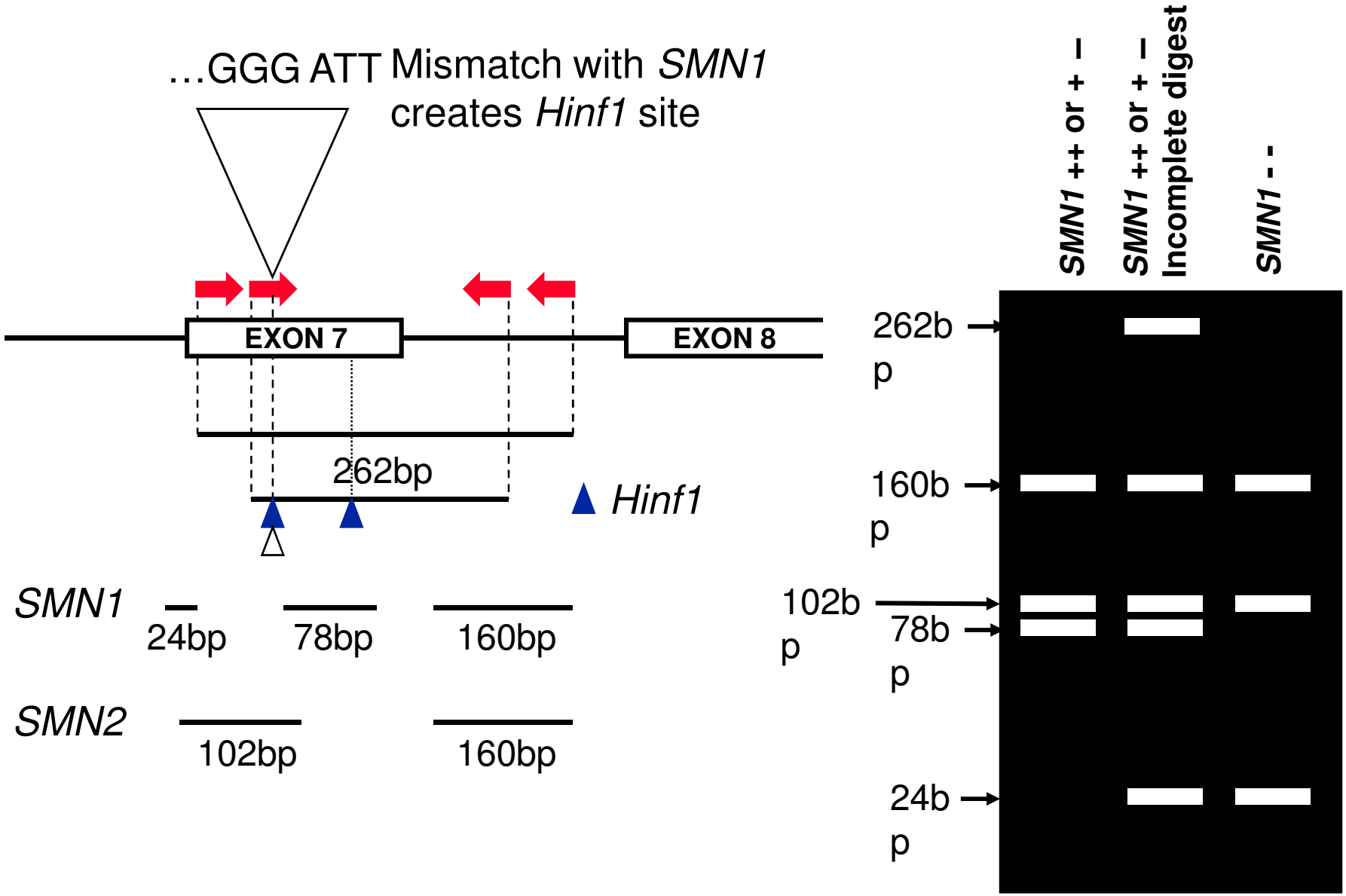
Array comparative genomic hybridisation (CGH) following whole genome amplification



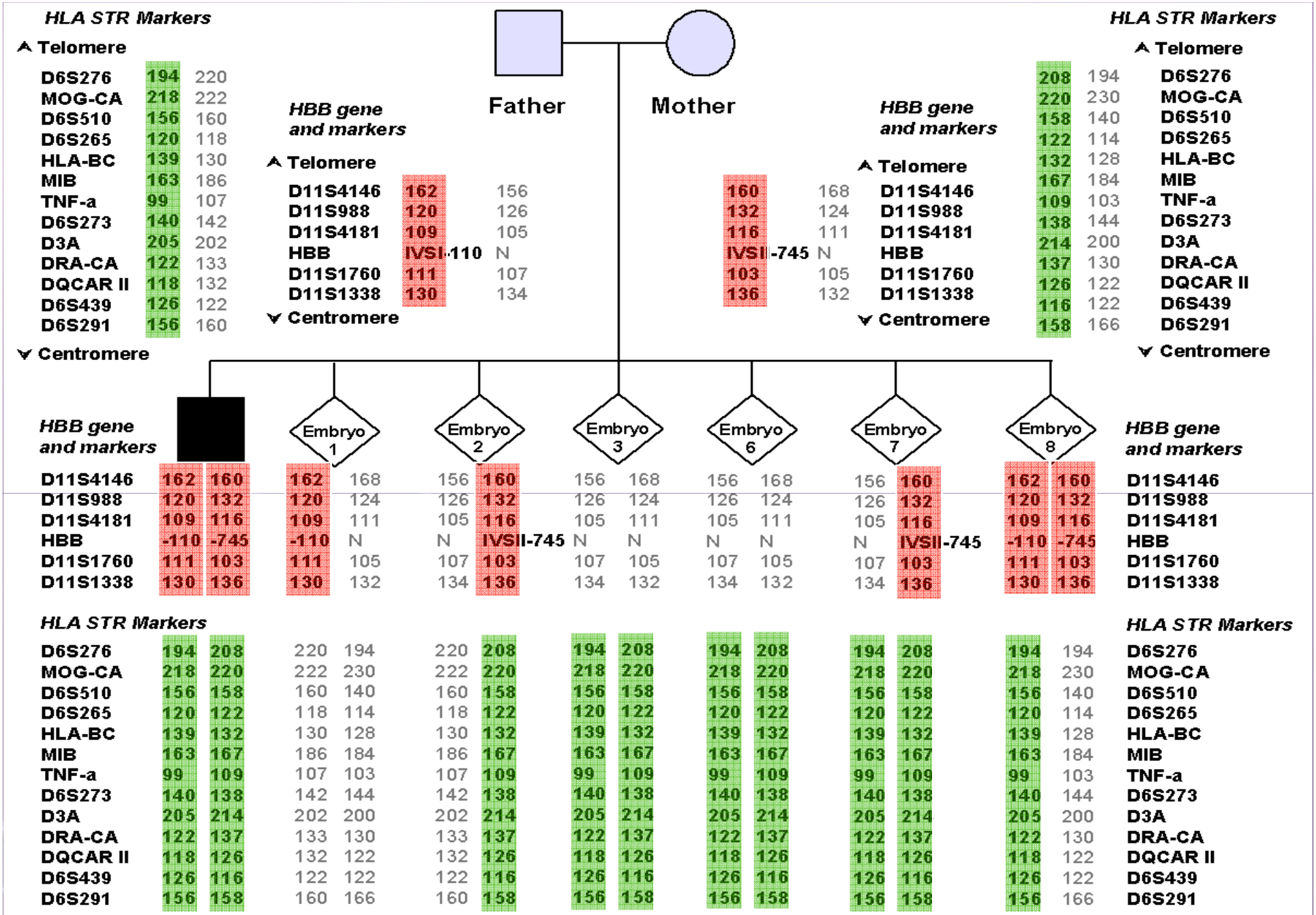
Strategies for single cell genetic analysis



Primer mismatch strategy for detecting *SMN1* and *SMN2*

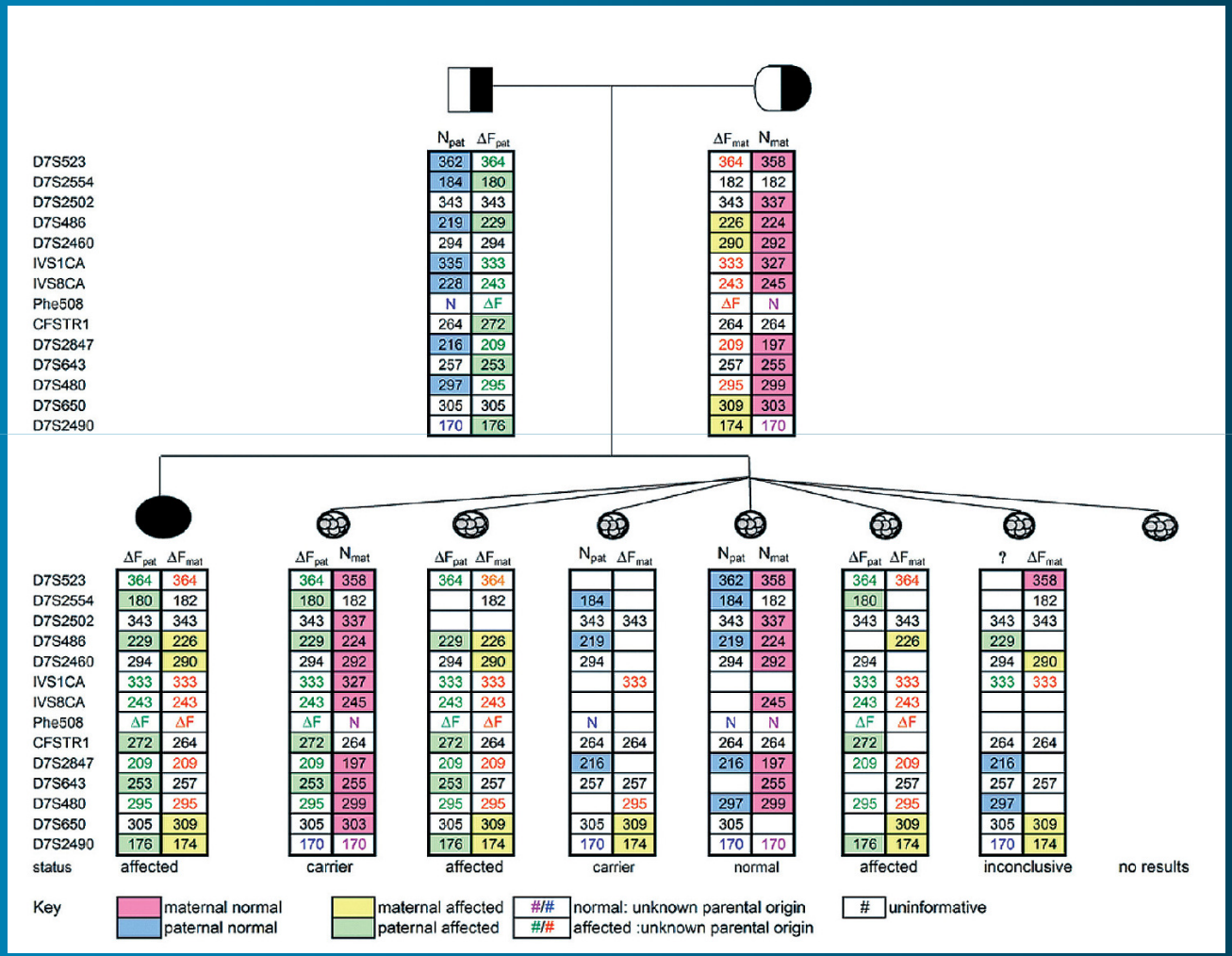


Daniels et al (2001) Mol Hum Rep



Fiorentino et al (2005) Eur J Hum Genet 13, 953

Preimplantation genetic haplotyping following MDA from single cells



Renwick et al (2006) Reprod Biomed Online 13, 110

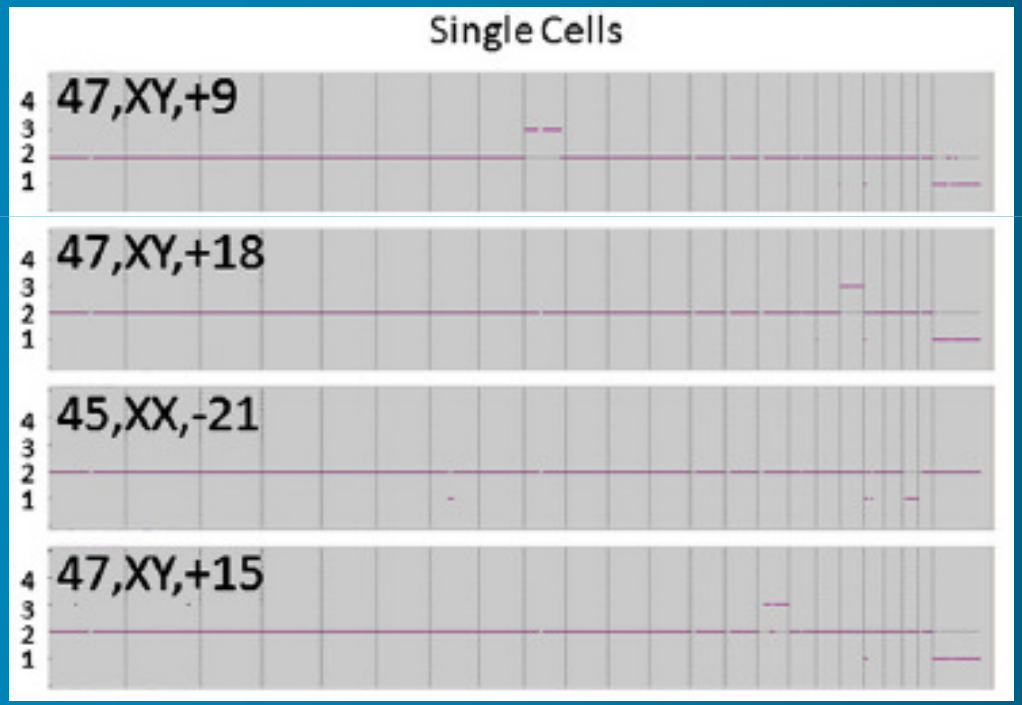
Single nucleotide polymorphisms (SNPs)

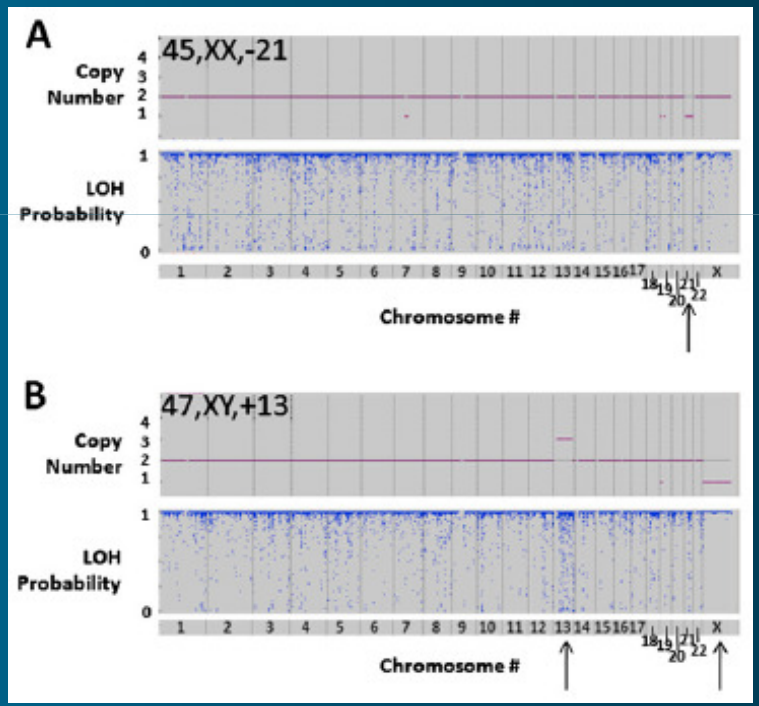
- 10 million SNPs across human genome
- Many biallelic (AA, AB, BB)
- Major contribution to genetic diversity, inherited disease and variants associated with common multifactorial conditions

Accurate single cell 24 chromosome aneuploidy screening using whole genome amplification and single nucleotide polymorphism microarrays

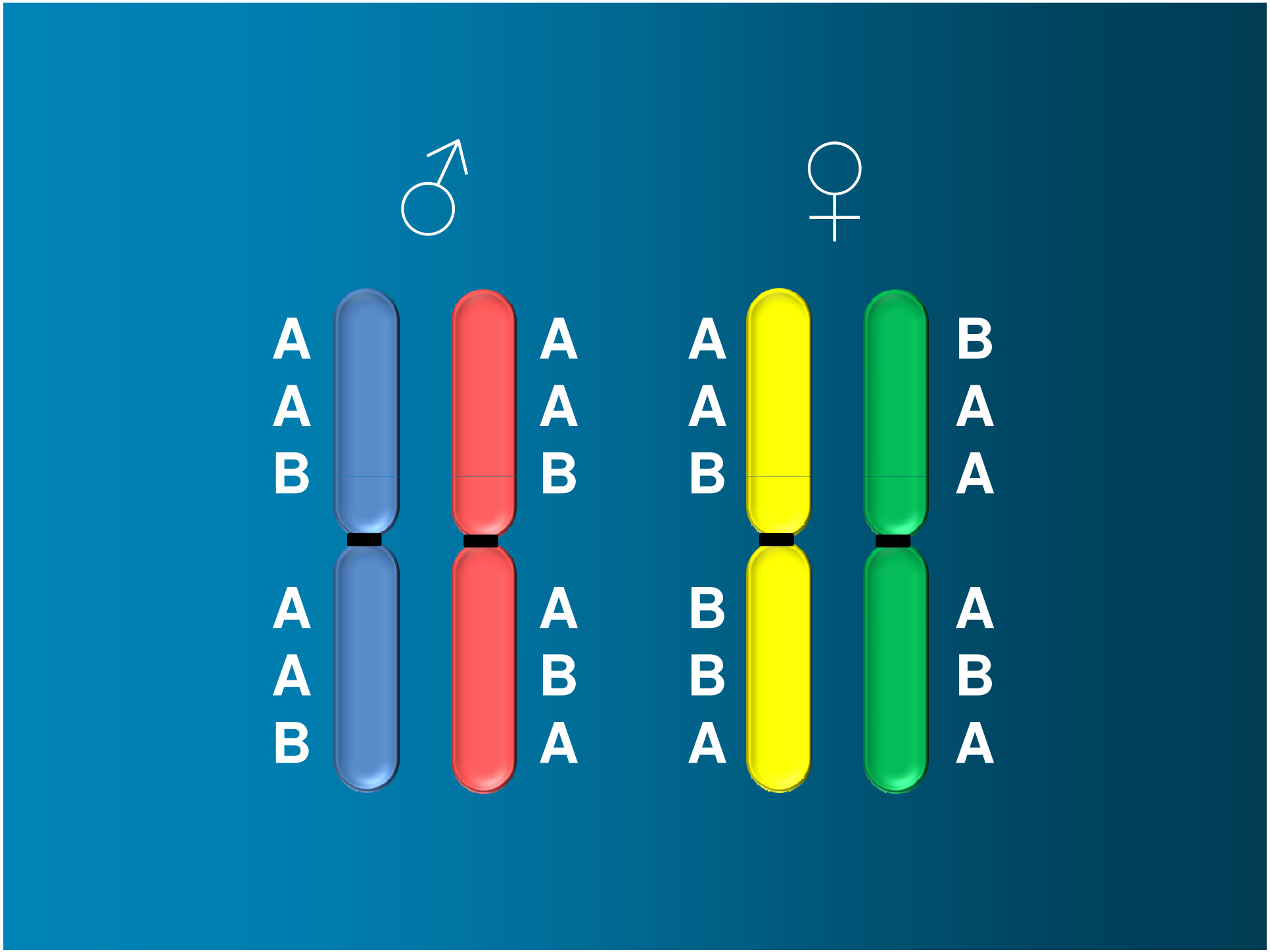
Nathan R. Treff, Ph.D.,[a,b] *Jing Su, M.Sc.,*[a] *Xin Tao, M.Sc.,*[a] *Brynn Levy, Ph.D.,*[a,c] *and Richard T. Scott, Jr., M.D.*[a,b]

[a] Reproductive Medicine Associates of New Jersey, Morristown; [b] Division of Reproductive Endocrinology, Department of Obstetrics Gynecology and Reproductive Science, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey; and [c] Department of Pathology, College of Physicians and Surgeons of Columbia University, New York, New York





Treff et al (2009) Fertil Steril in press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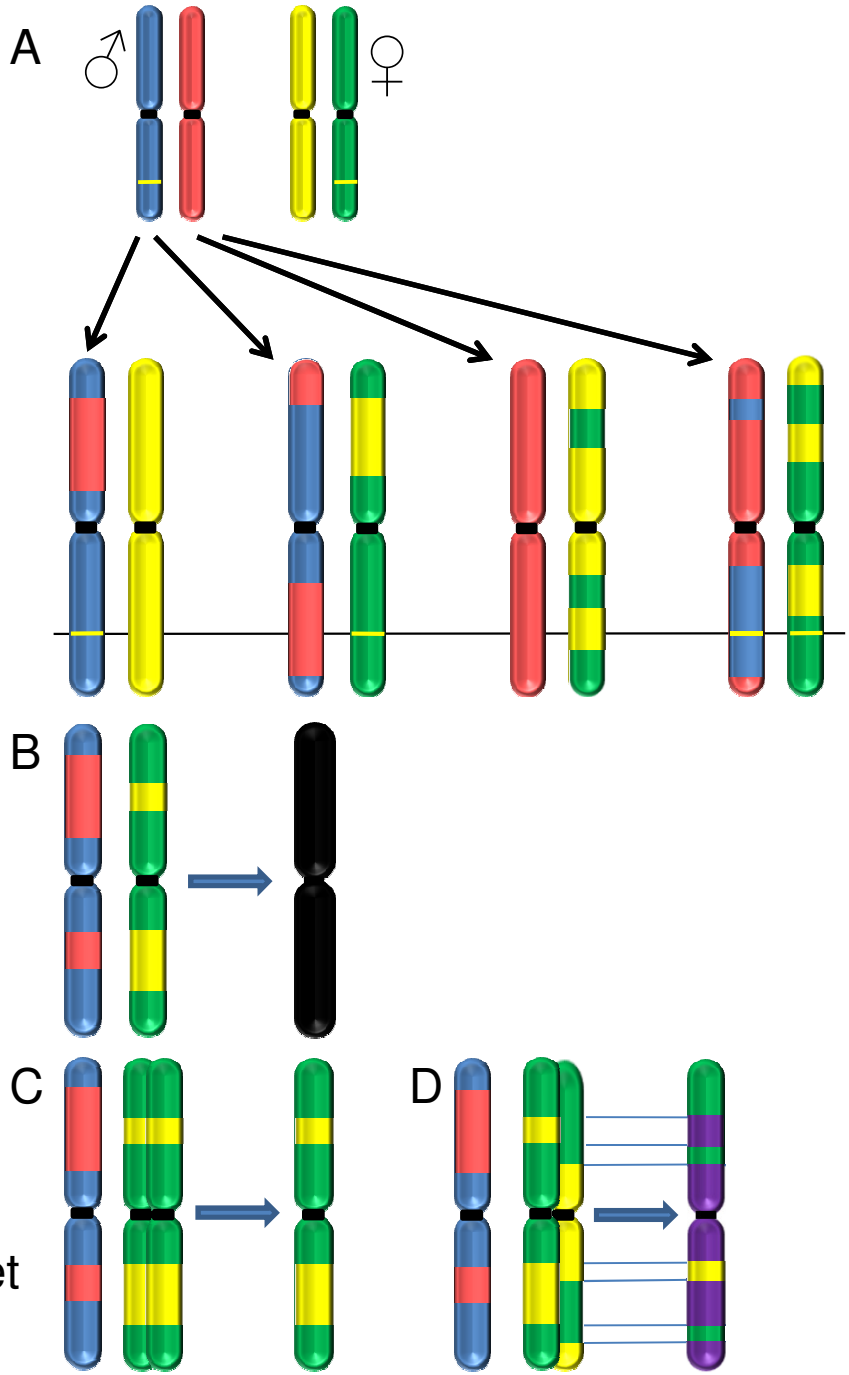
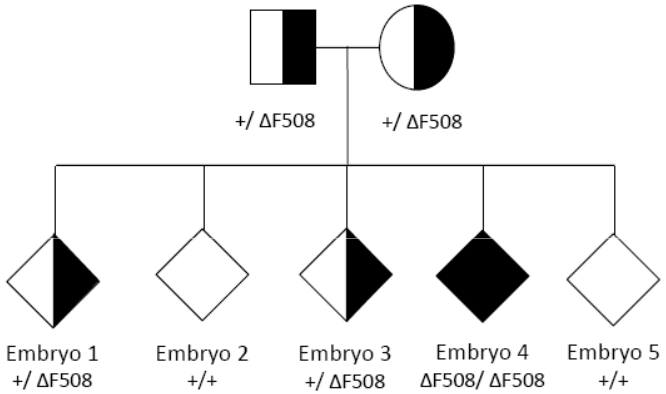
Karyomapping combines genome wide linkage based detection of single gene defects (A) with chromosomal aneuploidy including monosomy/deletions (B) and trisomies involving inheritance of two different meiotic chromosomes from one parent (D). Chromosome duplication is not detected (C).

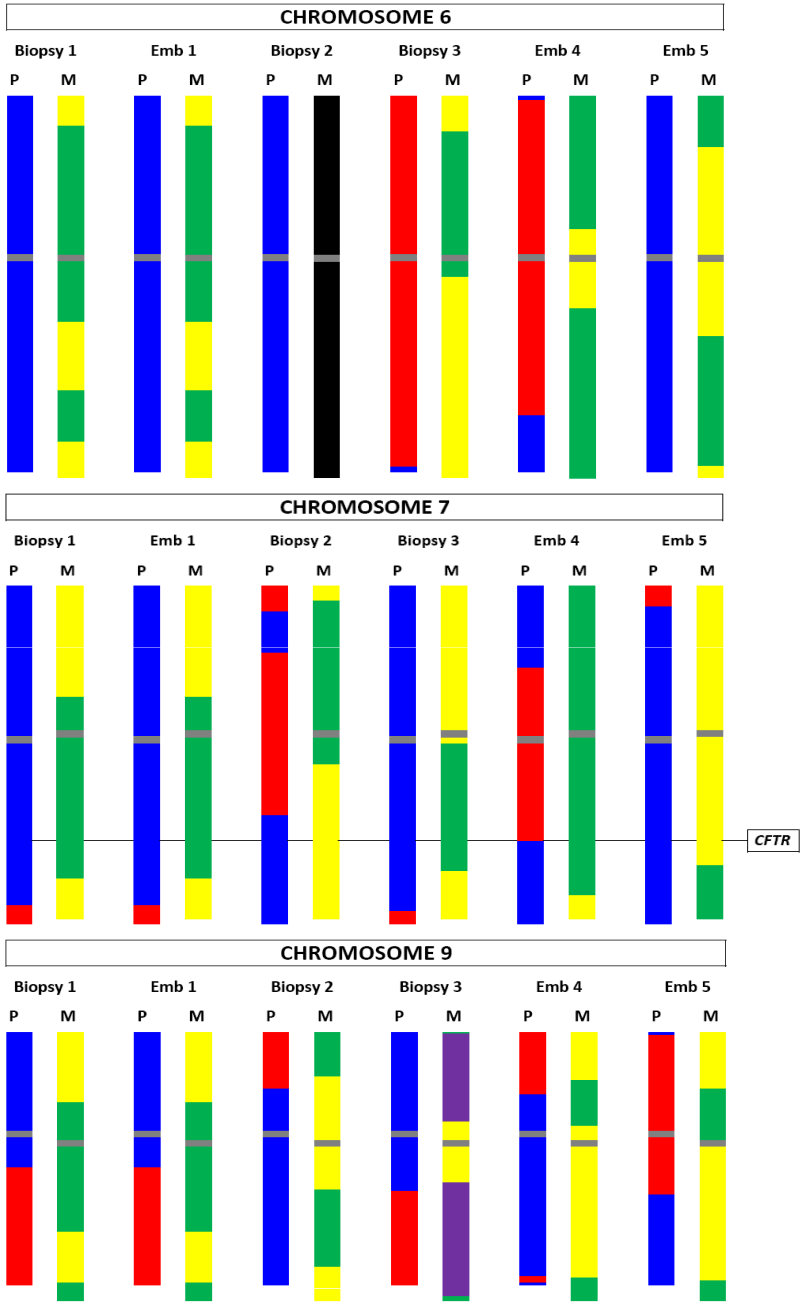


Handyside et al (2009) J Med Genet

Family 2
Preimplantation genetic diagnosis for cystic fibrosis



Whole genome amplification by isothermal multiple displacement amplification of 2-10 cells in each biopsy/embryo



	Mutation detection	Multiplex PCR and minisequencing	Preimplantation genetic haplotyping	Fluorescence in situ hybridisation	Array CGH	Quantitative SNP array analysis and Karyomapping
Single gene defects						
Single or combination	✓	Any	✓			Any
Exclusion	✗	✓	✓			✓
SGD + HLA typing	✗	✓	✗			✓
Chromosome screening						
Aneuploidy			✗	✓ (5-12 chr)	✓ (24 chr)	✓ (24 chr)
Meiotic aneuploidy				✓ (PB only)	✓ (PB only)	✓ Trisomy only
With parental origin		✓		✗	✗	✓
With meiotic origin		✗		✗	✗	✓
Mosaicism				✓	✓✗	✓✗
Duplications/deletions				✗	✓✗	✓✗
Uniparental disomy				✗	✗	✓
Translocation chromosome imbalance						
Reciprocal/Robertsonian		✓		✓	✗	✓
Normal vs balanced		✗		✗	✗	✓
With 24 chr aneuploidy		✗		✗	✗	✓
Other						
Multifactorial recurrence risk						✓
Copy Number Variants						✓
Errors						
Allele dropout		✓	✓			✓
Contamination		✓	✓			✓