Premplantation genetic testing

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Outline

- Definitions and background
- Clinical cycle and embryo biopsy
- PGT-M: indications
- PGT-M: methods

History of PGT

- 1990: Handyside et al.:
first PGT for X-linked disease

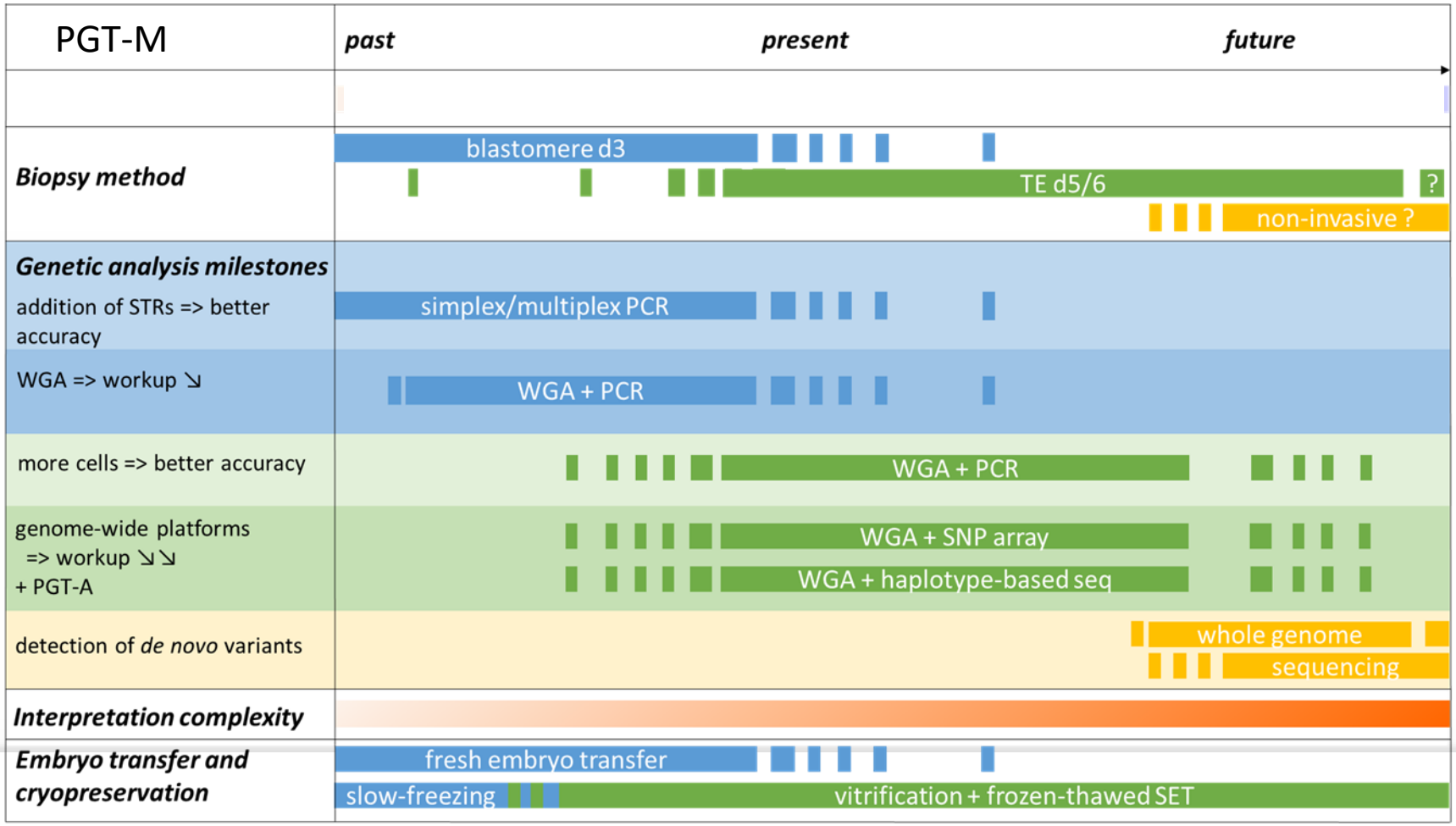
- 1992: Handyside et al.:
baby after PGT for Cystic Fibrosis

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

A. H. Handyside, E. H. Kontogianni, K. Hardy & R. M. L. Winston
Institute of Obstetrics and Gynaecology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK

OVER 200 recessive X chromosome-linked diseases, typically affecting only hemizygous males, have been identified. In many of these, prenatal diagnosis is possible by chorion villus sampling (CVS) or amniocentesis, followed by cytogenetic, biochemical or molecular analysis of the cells recovered from the conceptus. In others, the only alternative is to determine the sex of the fetus. If the fetus is affected by the defect or is male, abortion can be offered. Diagnosis of genetic defects in preimplantation embryos would allow those unaffected to be identified and transferred to the uterus[1]. Here we report the first established pregnancies using this procedure, in two couples known to be at risk of transmitting adrenoleukodystrophy and X-linked mental retardation. Two female embryos were transferred after *in vitro* fertilization (IVF), biopsy of a single cell at the six- to eight-cell stage, and sexing by DNA amplification of a Y chromosome-specific repeat sequence. Both women are confirmed as carrying normal female twins.

History of PGT



What is PGT?

Preimplantation genetic testing (PGT):

- a genetic test on samples from *in vitro* fertilised embryos (d3 or d5/6/7) for determination of single gene disorders, chromosomal numerical or structural aberrations (or for HLA typing)

  => selection and transfer of embryos unaffected for the condition under study

- alternative to prenatal diagnosis and possible termination of pregnancy

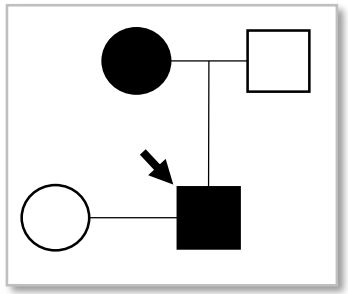
- close collaboration between genetics and IVF department

PGT indications

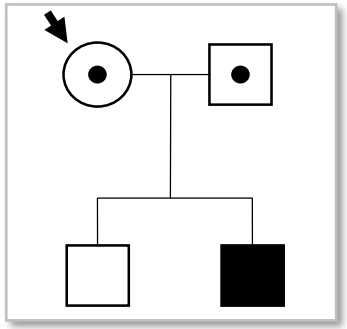
Monogenic disorder

PGT-M workup

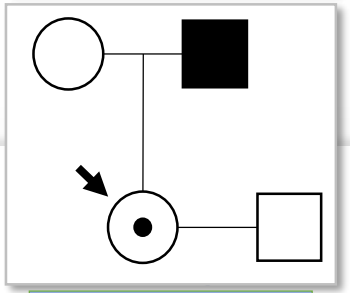
AD



AR



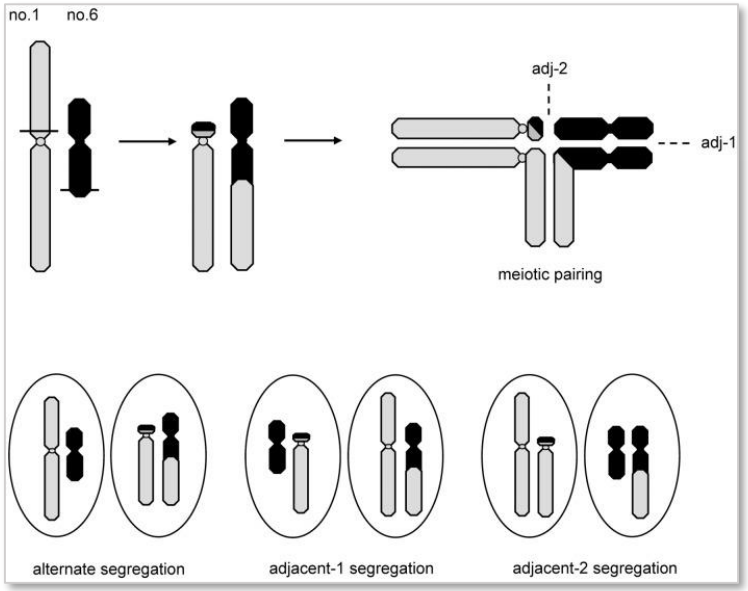
XL



diagnostic test

Structural rearrangements

PGT-SR No workup

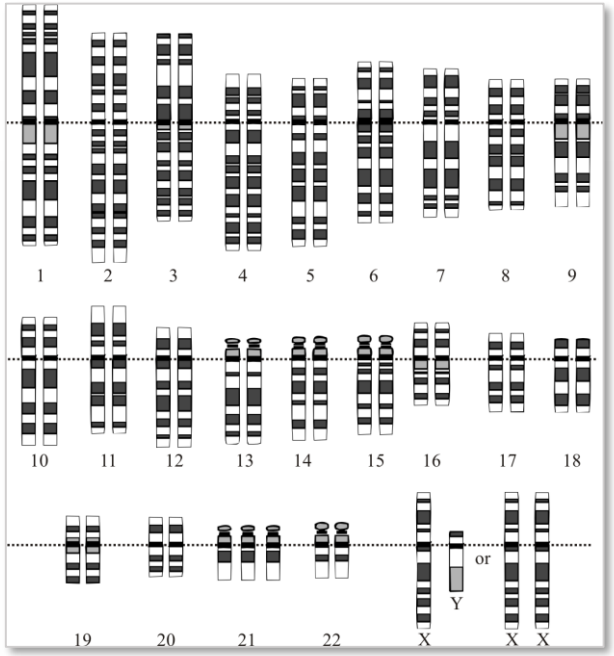


- Reciprocal translocation
- Robertsonian translocations
- Inversions
- Deletions
- Duplications

diagnostic test

Aneuploidy

PGT-A No workup



- Trisomy antecedents
- Recurrent miscarriage
- Recurrent implantation failure
- Advanced maternal age

screening test

PGT can be

Diagnostic test

- involves genetic testing of cells biopsied from *in vitro* obtained oocytes and/or *in vitro* fertilised embryos and selective transfer of unaffected embryos
- for couples at *high* risk of transmitting a genetic condition to their children
- PGT-M, PGT-SR and high risk PGT-A (ex mosaic 45,X/46,XX)
- (not necessarily infertile, but undergo IVF as part of PGT treatment)

PGT can be

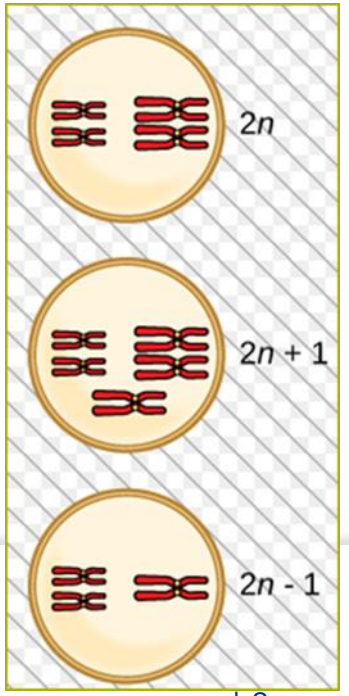
Screening test

- PGT-A
- involves genetic testing of cells biopsied from *in vitro* obtained oocytes and/or *in vitro* fertilised embryos and selective transfer of euploid embryos

- for couples to improve IVF results and reduce miscarriage rates

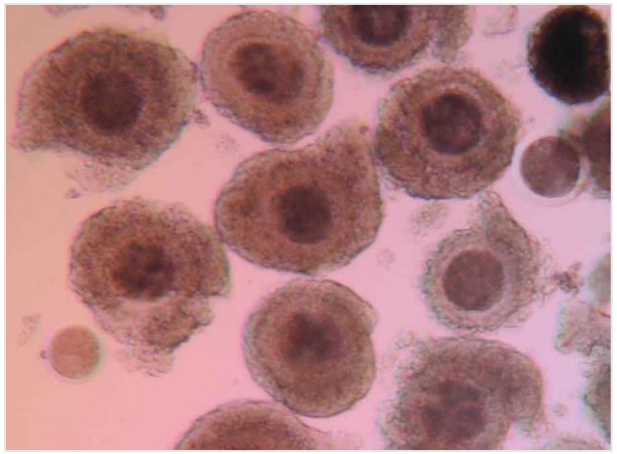
  for specific IVF patient groups at *low* risk

  (advanced maternal age, recurrent IVF failure or repeated miscarriages)

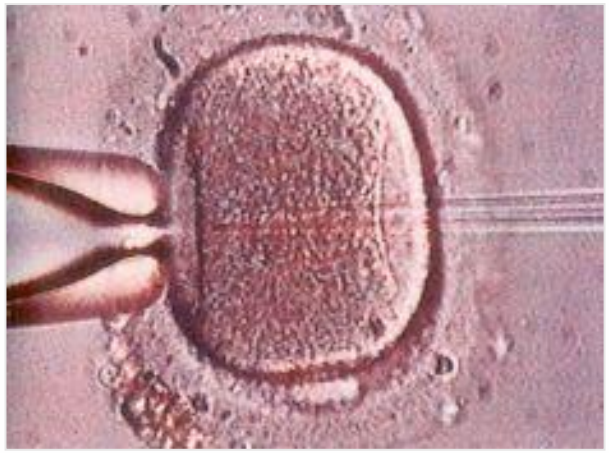


PGT clinical cycle

oocyte collection after hormonal stimulation

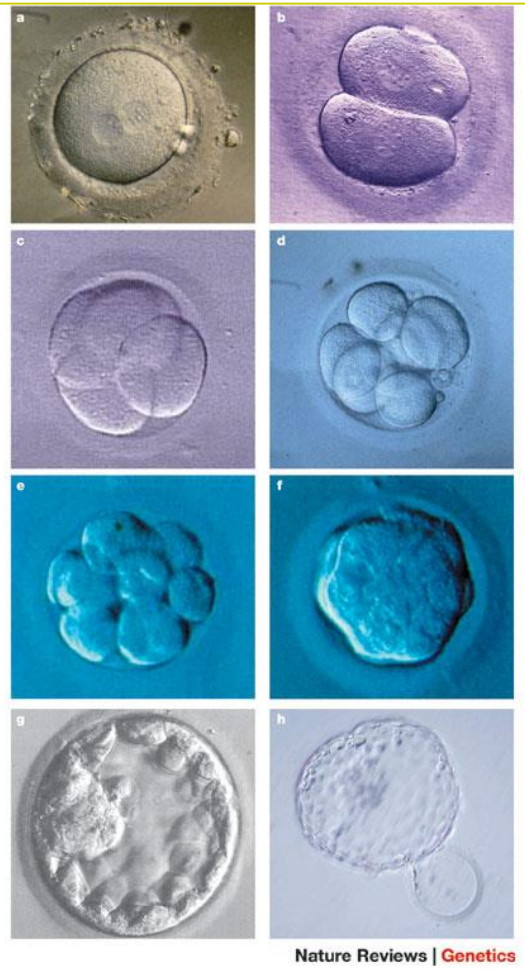


IVF with Intracytoplasmic sperm injection



Day 0

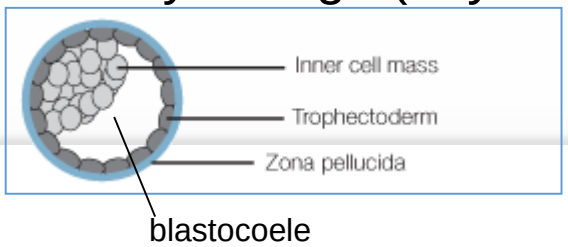
PGT clinical cycle



cleavage stage (day 3)

morula stage (day 4)

blastocyst stage (day 5/6/7)



in vitro embryo culture

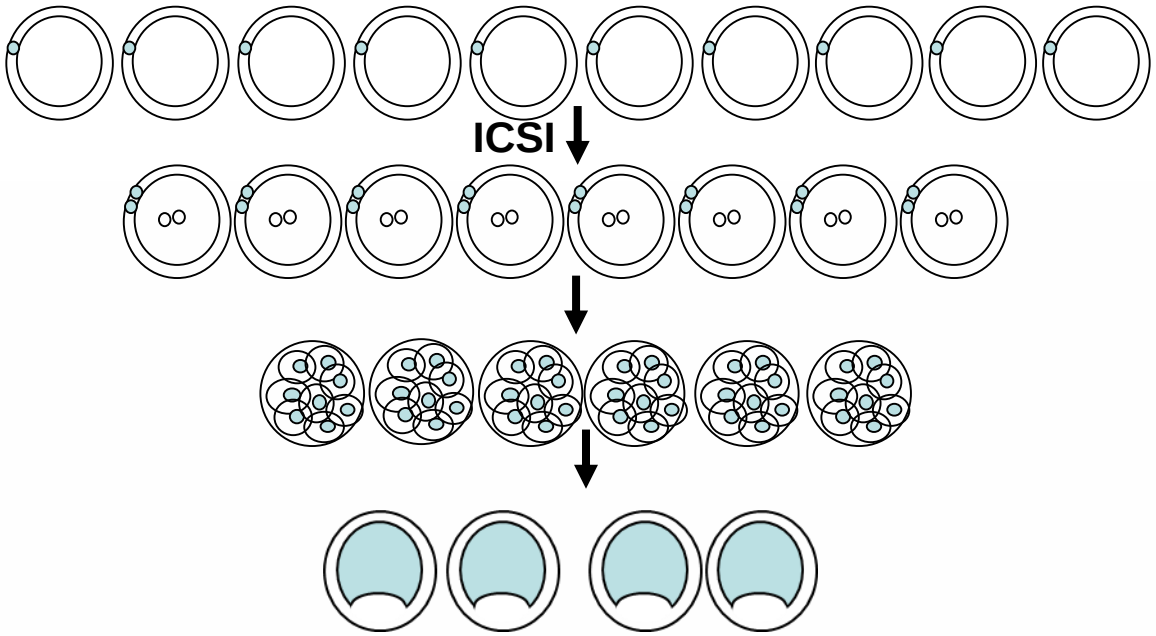
PGT clinical cycle

10 oocytes
day 0

8 normally fertilised oocytes
day 1

6 embryos for biopsy
day 3

4 embryos for biopsy
day 5/6/7



PGT clinical cycle: biopsy

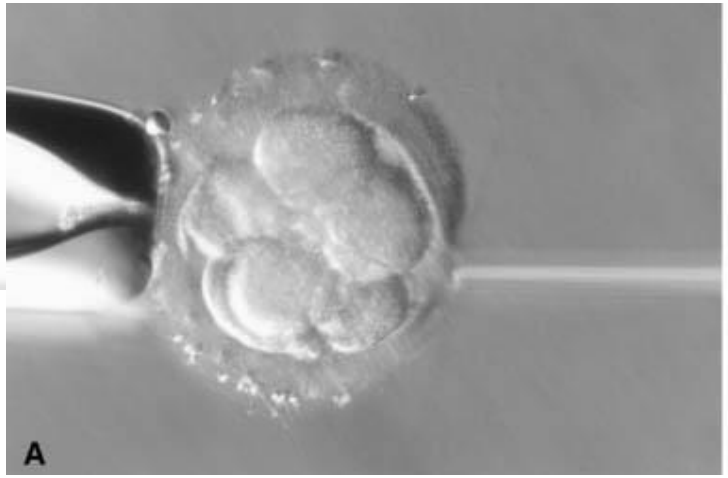
biopsy is a two step process:

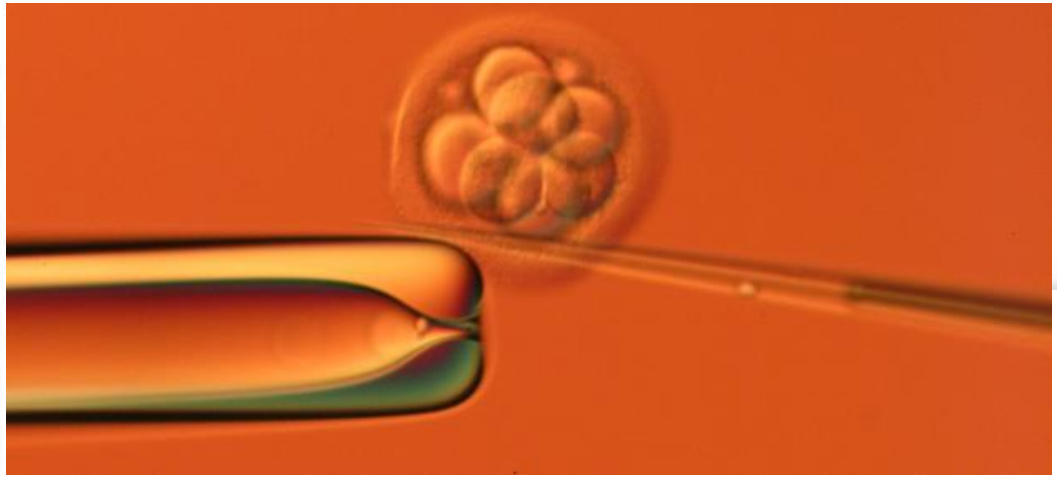
1) punction or removal of part of the ZP
   - mechanical opening (microneedle) (a)
   - chemical opening (Acidic Tyrode) (b)
   - laser pulses (most common) (c)

2) removal of nucleated cell(s)

c

b





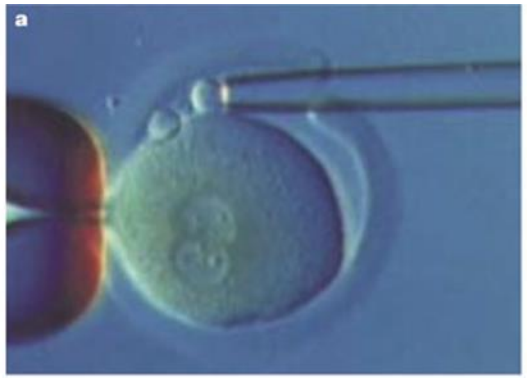
a

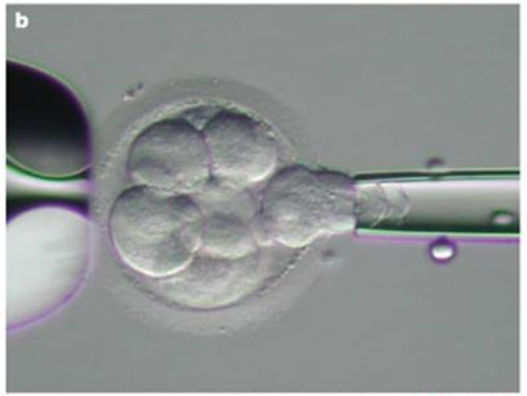
PGT clinical cycle: biopsy

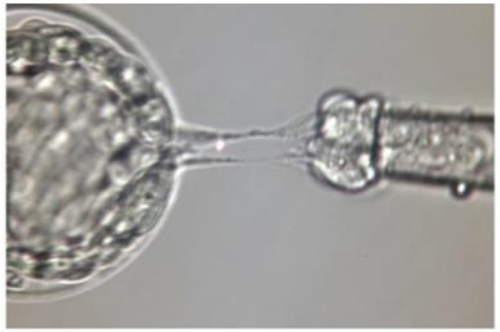
1 or 2 polar bodies
from oocytes
day 0/1

1 or 2 blastomeres from
cleavage stage embryos
day 3

5-8 trophectoderm cells
from blastocysts
day 5/6/7







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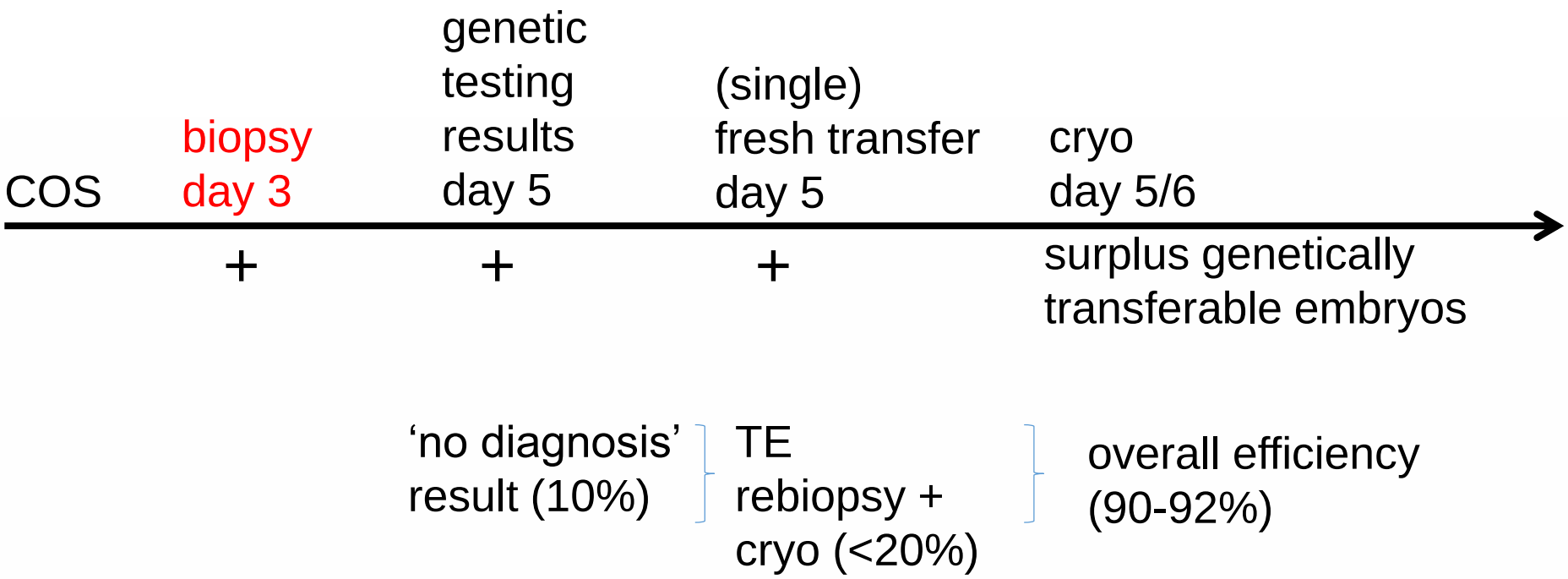
PGT cycle: day 3 biopsy

cleavage stage embryo biopsy: at day 3: removal of 1 or 2 cells

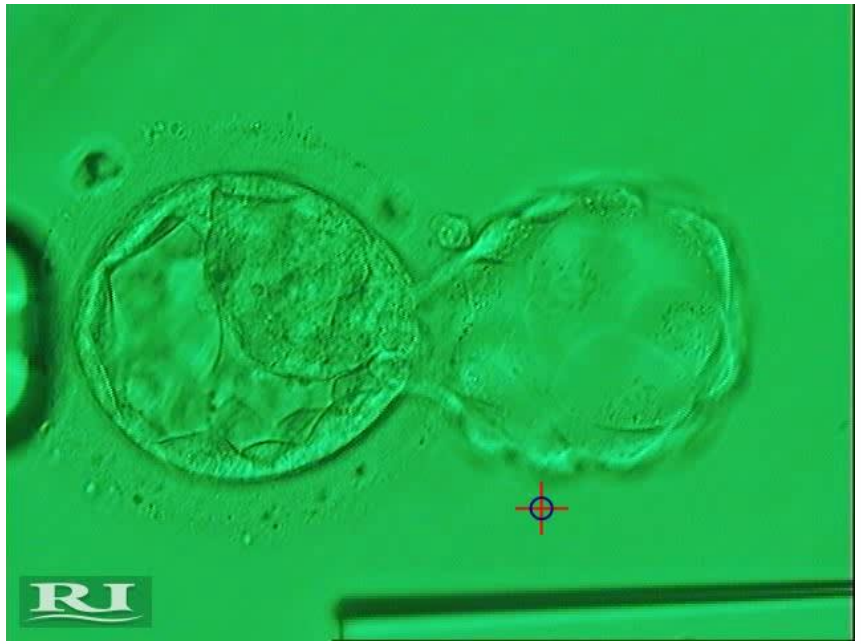
- still used in many PGT centres
- embryo incubation in Ca/Mg-free medium to disassemble cellular junctions
- for maternally and paternally inherited disorders
- gender determination possible
- impact of 1 or 2 cell removal on embryonic development/implantation
- fresh ET at day 5 possible

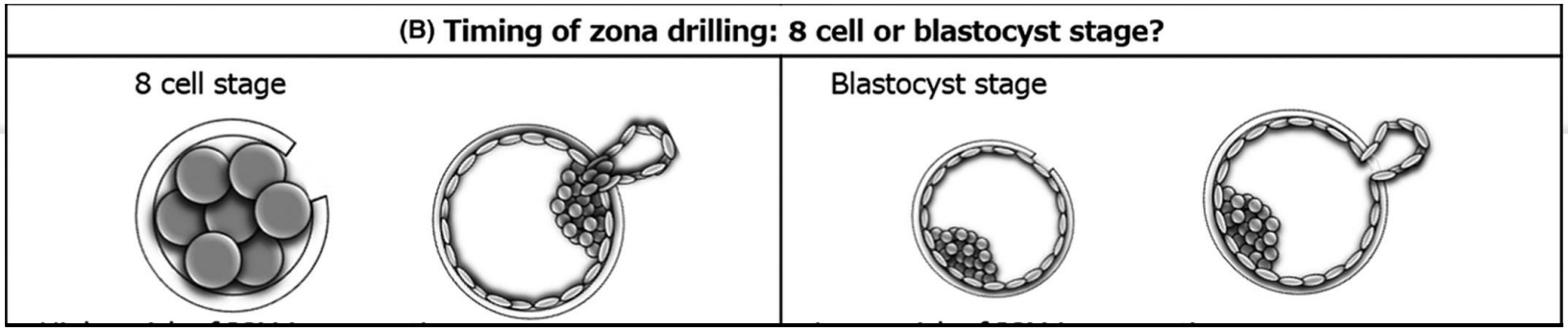
PGT cycle: 'fresh embryo transfer'

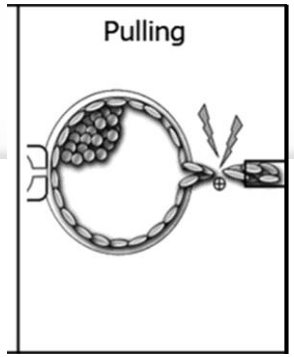


PGT cycle: TE biopsy

5-8 TE cells from blastocyst embryos (day 5/6/7):

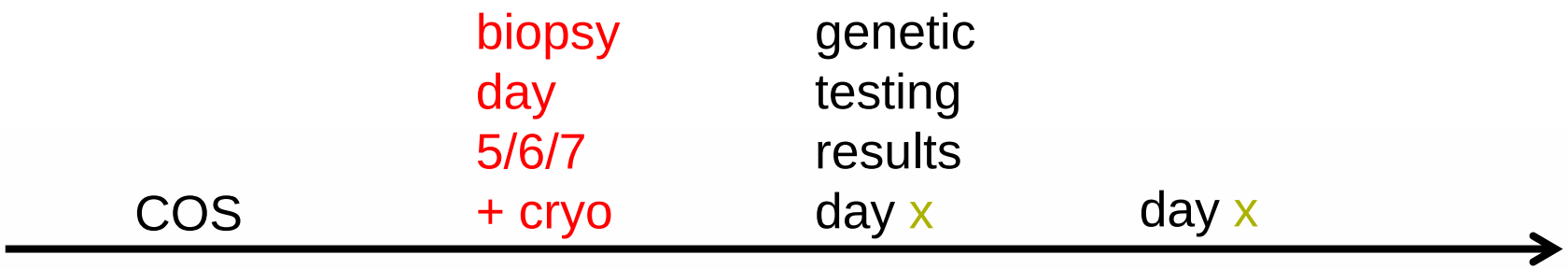






Reproductive Medicine and Biology, First published: 26 January 2020, DOI: (10.1002/rmb2.12318)

PGT cycle: TE biopsy



‘no diagnosis’ result (5%)

warm embryo TE rebiopsy + cryo (80%)

overall efficiency (99%)

PGT cycle: TE biopsy

5-8 TE cells from blastocyst embryos (day 5/6/7):

- TE cells = extraembryonic cells / safer option
- multiple cells / higher diagnostic accuracy
- no negative impact on implantation
- less samples for testing
- need for optimal blastocyst culture systems
- need for optimal vitrification/warming procedures

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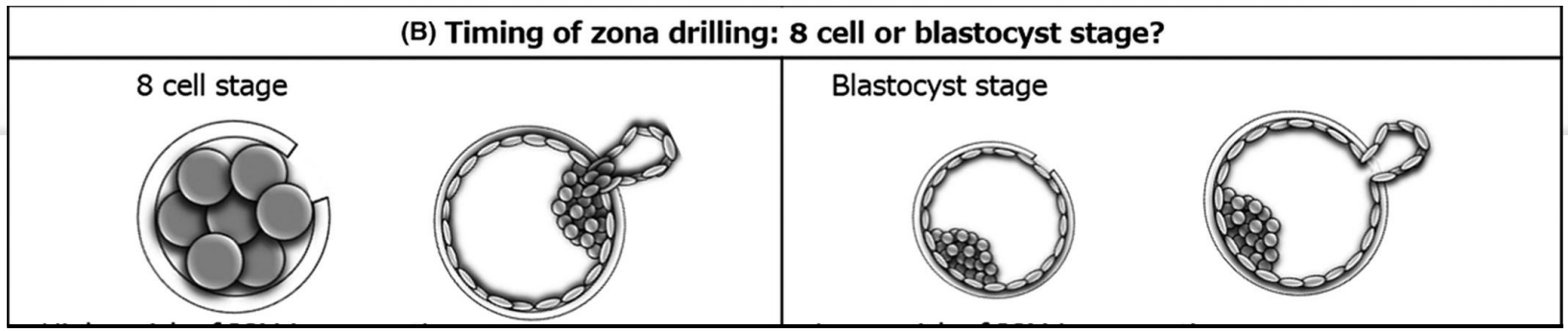
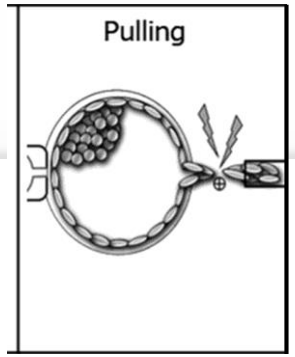
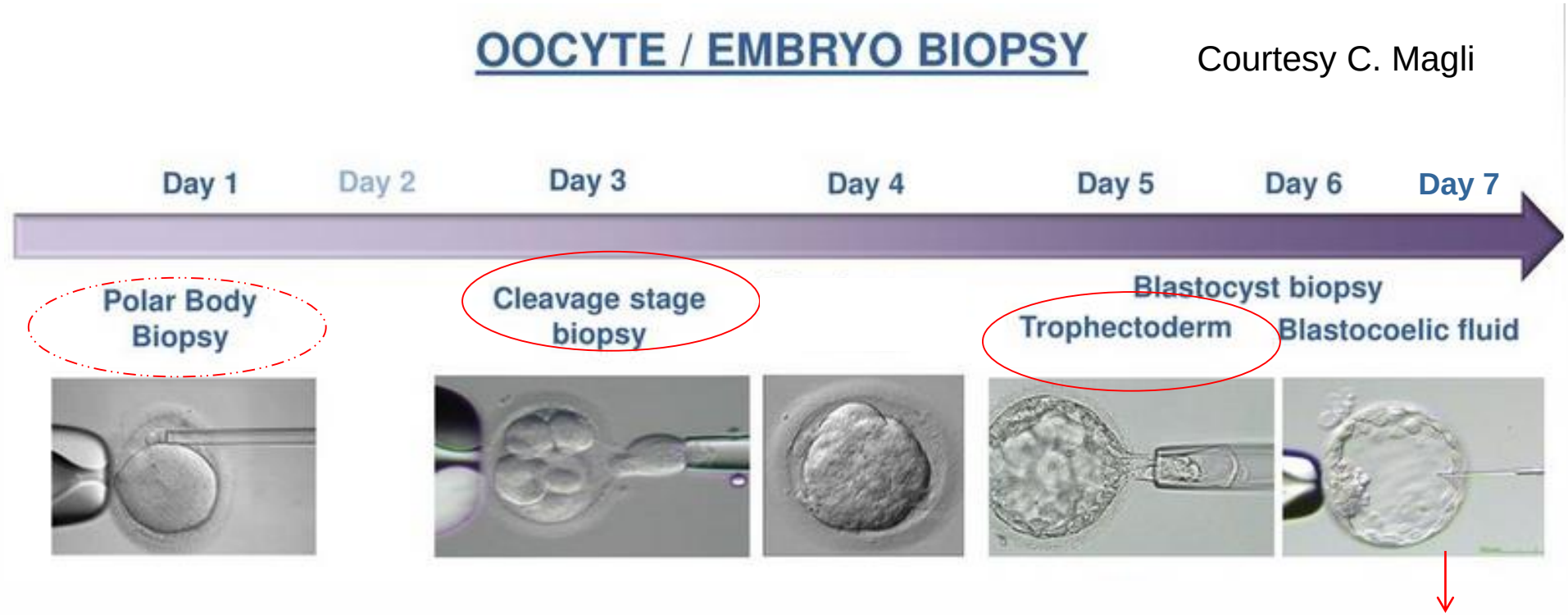




PGT clinical cycle: biopsy

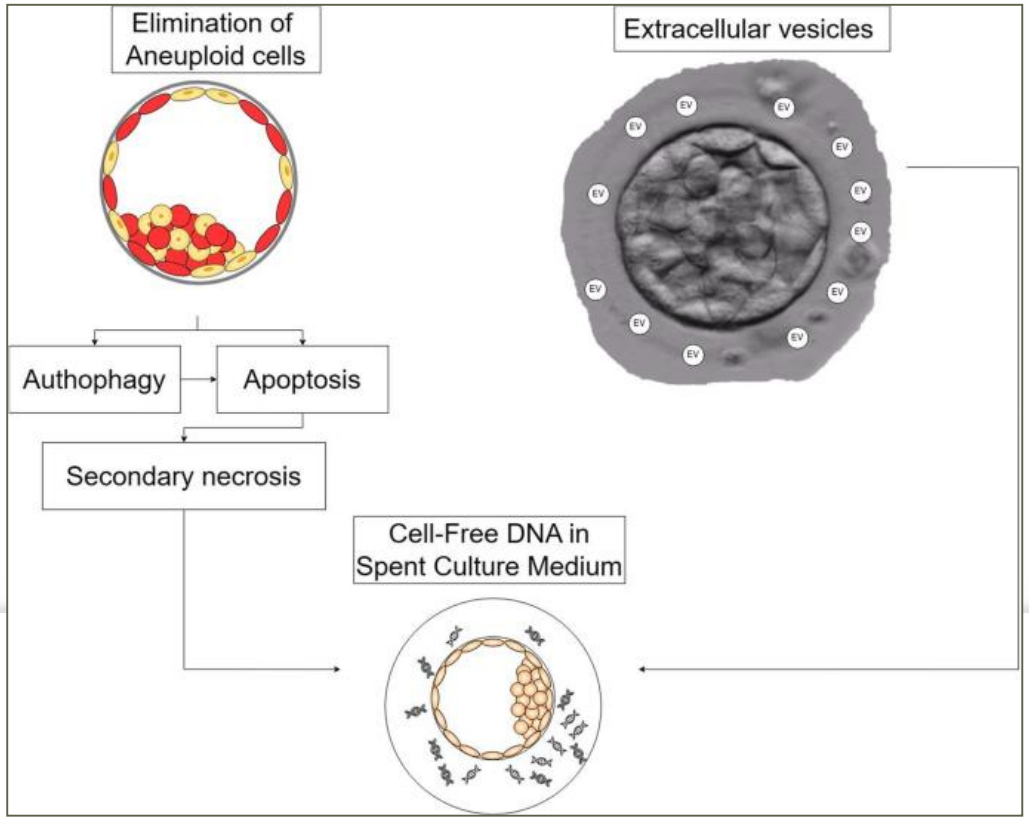


minimally invasive
low yield of cell-free DNA

non-invasive
spent embryo culture medium

PGT clinical cycle: biopsy

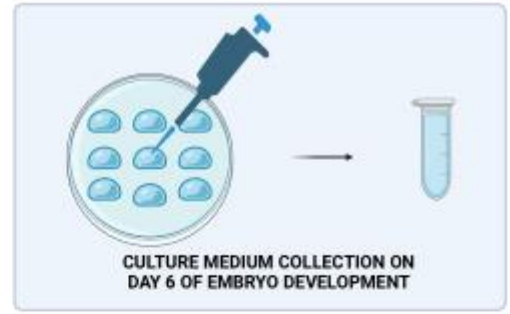
cfDNA (100-1000bp)
origin of cfDNA?

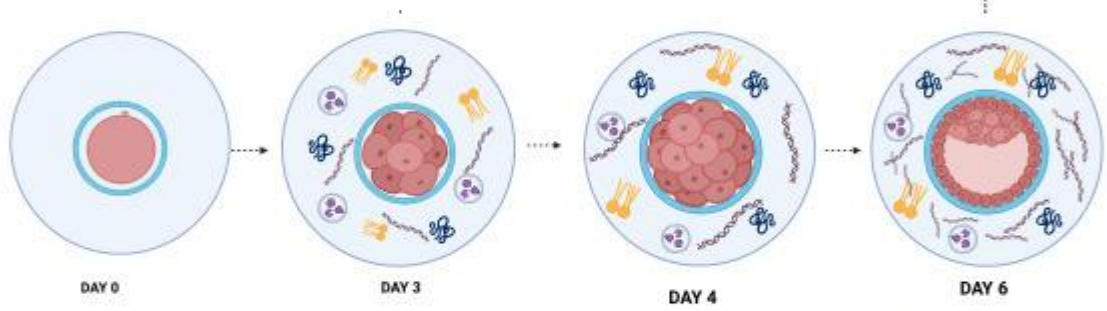


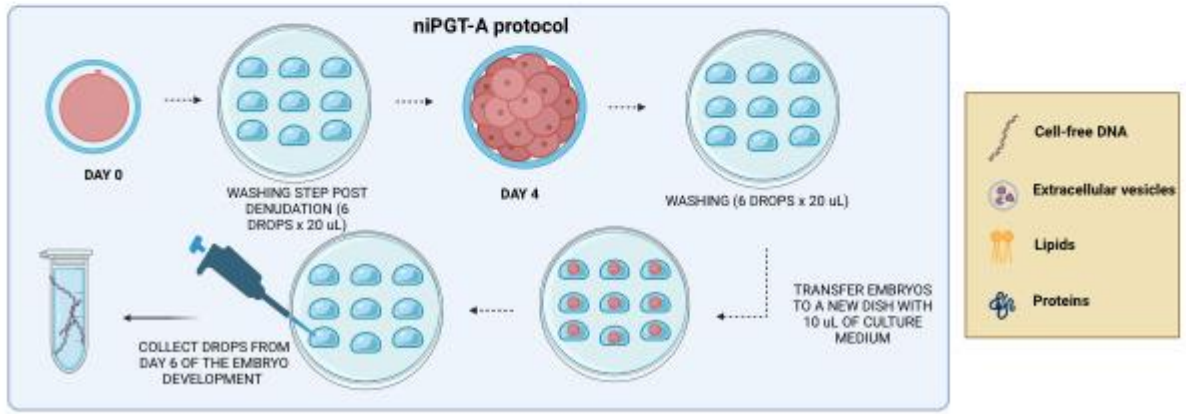
Handayani. *J Assist Reprod Genet* **40**, 1231–1242 (2023

PGT-A

non-invasive
spent embryo culture medium

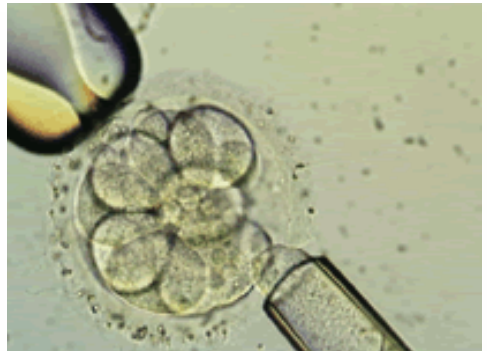


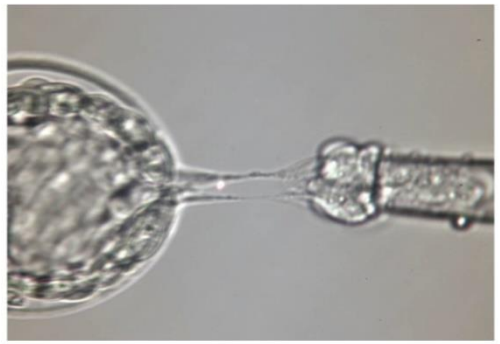




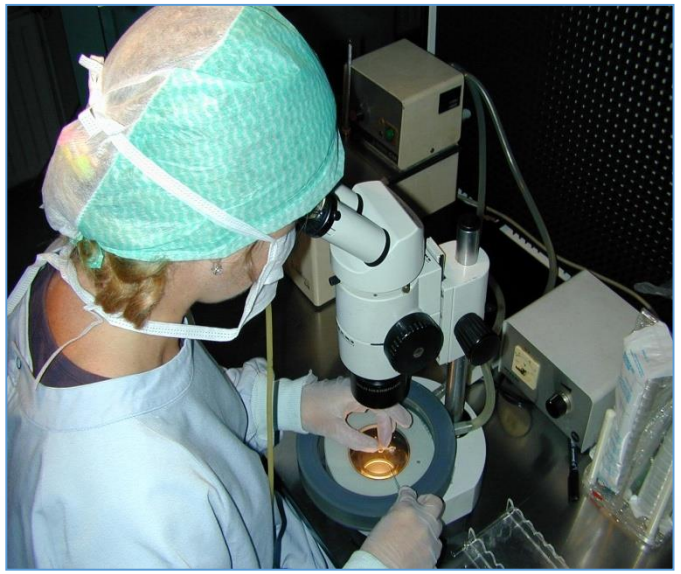
PGT cycle: biopsy sample collection

embryo biopsy
with laser (day 3 or 5/6)





washing & tubing

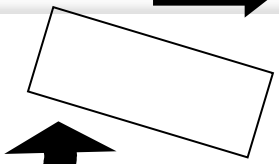


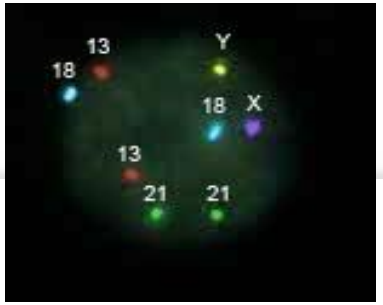
amplification

 or  1 cell or 5-8 cells

 1 cell

spreading & fixation





interphase FISH

PGT cycle: genetic testing

targeted testing
FISH: for PGT-SR and sexing
PCR amplification: for PGT-M

genome wide testing

↓

first step = whole genome amplification

(WGA)

↓

various platforms:
arrayCGH, low coverage NGS: for PGT-A, PGT-SR
SNParray, NGS with haplotyping: for PGT-M, PGT-SR

PGT for monogenic disorders: indications

- autosomal dominant, autosomal recessive and X-linked disorders

- inclusion: cases with (likely) pathogenic germline genetic variant(s) of class 4-5 in nuclear or mitochondrial DNA, proven to be disease-causing

- molecular genetic report stating the disease-causing genetic variant

(PGT-A and PGT-SR: see presentation of P. Verdyck)

PGT and HLA matching

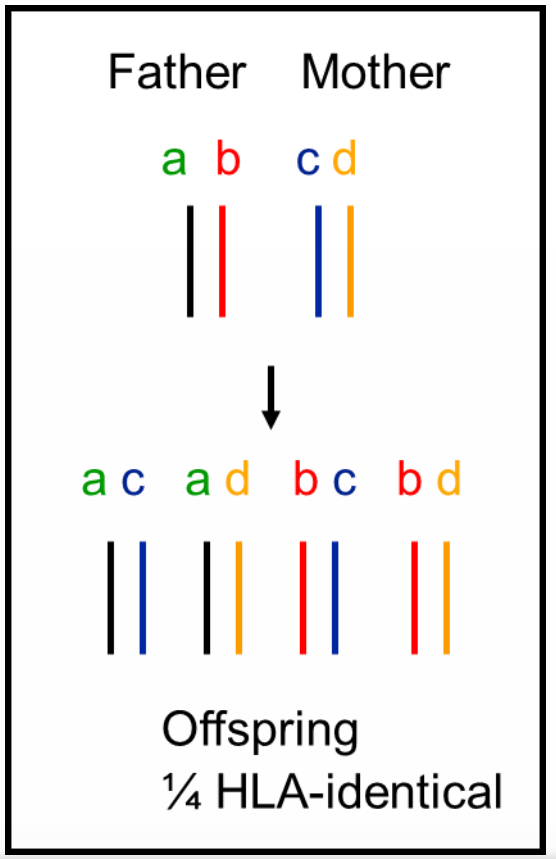
involves Human Leucocyte Antigen (HLA) typing of single/few cells biopsied from *in vitro* fertilized preimplantation embryos

aim: select an embryo which is HLA compatible with an ill sibling in need of a hematopoetic stem cell (HSC) transplantation
HSC collected (cord blood/bone marrow)
for transplantation and cure of the ill child

PGT and HLA matching

PGT-HLA only
for acquired diseases (leukemia)
25% of biopsied embryos are genetically transferable

PGT-M-HLA
for monogenic disorders (immunodeficiencies or hemoglobinopathies)
18,8% (¼ x ¾) for AR or X-linked R
12,5% (¼ x ½) for AD disorder
of biopsied embryos are genetically transferable

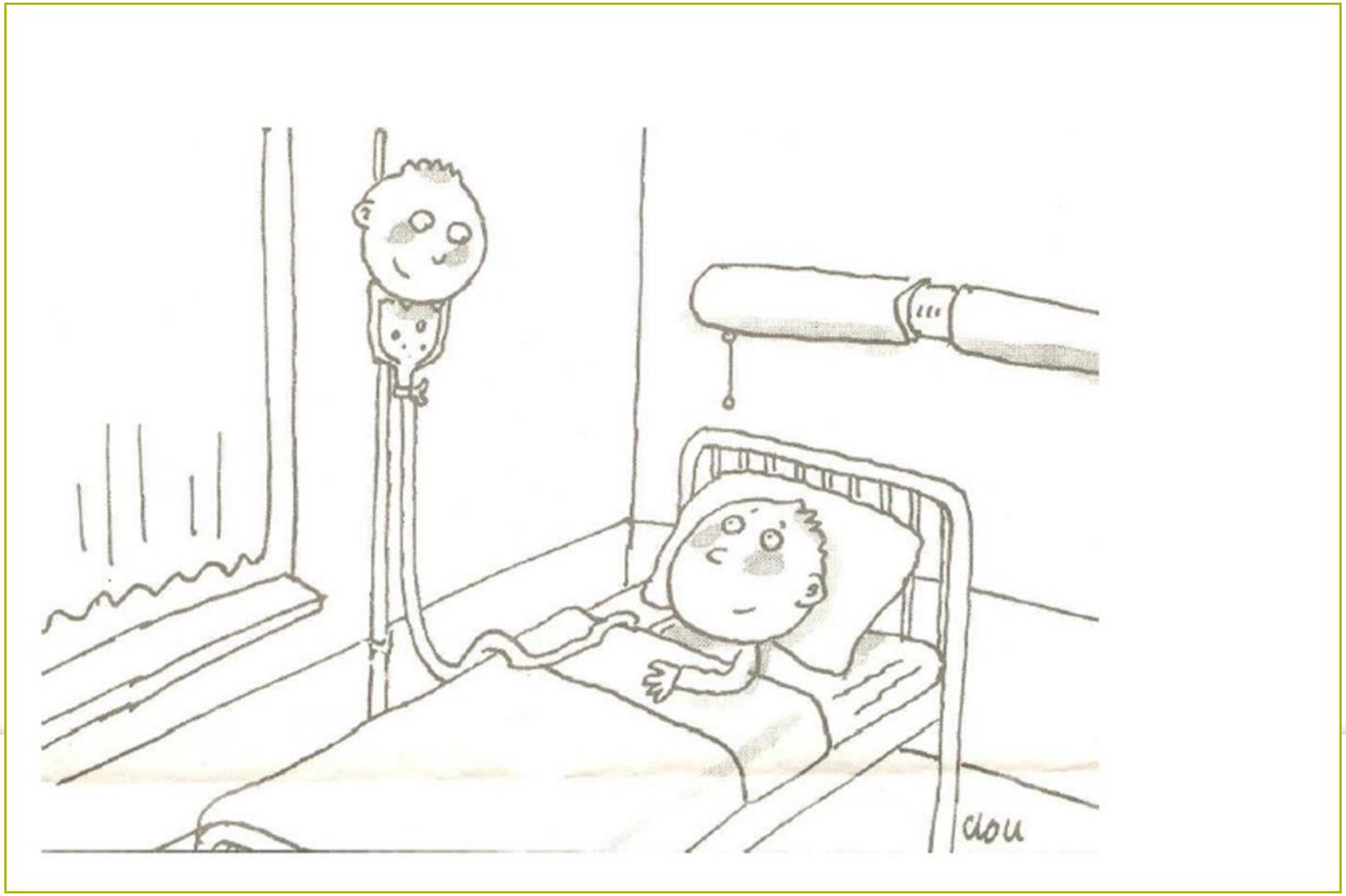


PGT and HLA matching

- Art. 67. Verboden zijn :
  1° Genetische pre-implantatiediagnostiek met het oog op eugenetische selectie, zoals gedefinieerd in artikel 5, 4°, van de wet van 11 mei 2003 betreffende het onderzoek op embryo's in vitro, dat wil zeggen gericht op de *selectie of de verbetering van niet-pathologische genetische kenmerken van de menselijke soort*;
  2° Genetische pre-implantatiediagnostiek met het oog op geslachtsselectie, zoals gedefinieerd in artikel 5, 5°, van de wet van 11 mei 2003 betreffende het onderzoek op embryo's in vitro, dat wil zeggen gericht op geslachtsselectie, met *uitzondering van de selectie ter voorkoming van geslachtsgebonden ziekten*.
  Art. 68. In afwijking van artikel 67 is pre-implantatie genetische diagnostiek uitzonderlijk toegestaan in het therapeutisch belang van een reeds geboren kind van de wensouder(s). Het geraadpleegde fertiliteitscentrum moet, in het geval bedoeld in het eerste lid van dit artikel, beoordelen of de kinderwens niet uitsluitend ten dienste staat van dat therapeutisch belang.

PGT and HLA matching

psychological support:
is offered to all PGT couples
is compulsory for
HLA requests, single parents,
cases with neurodegenerative
disorders,…



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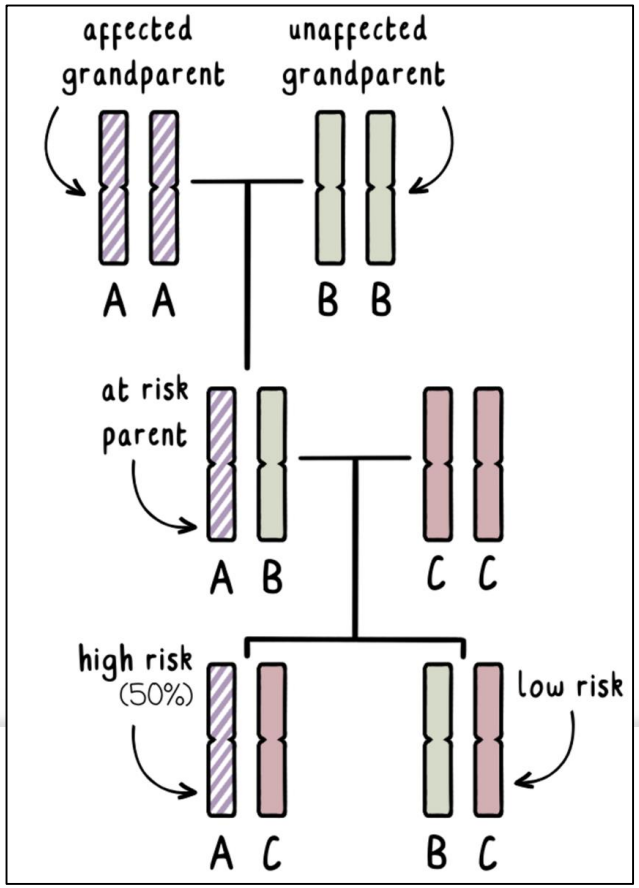
PGT-M: exclusion testing

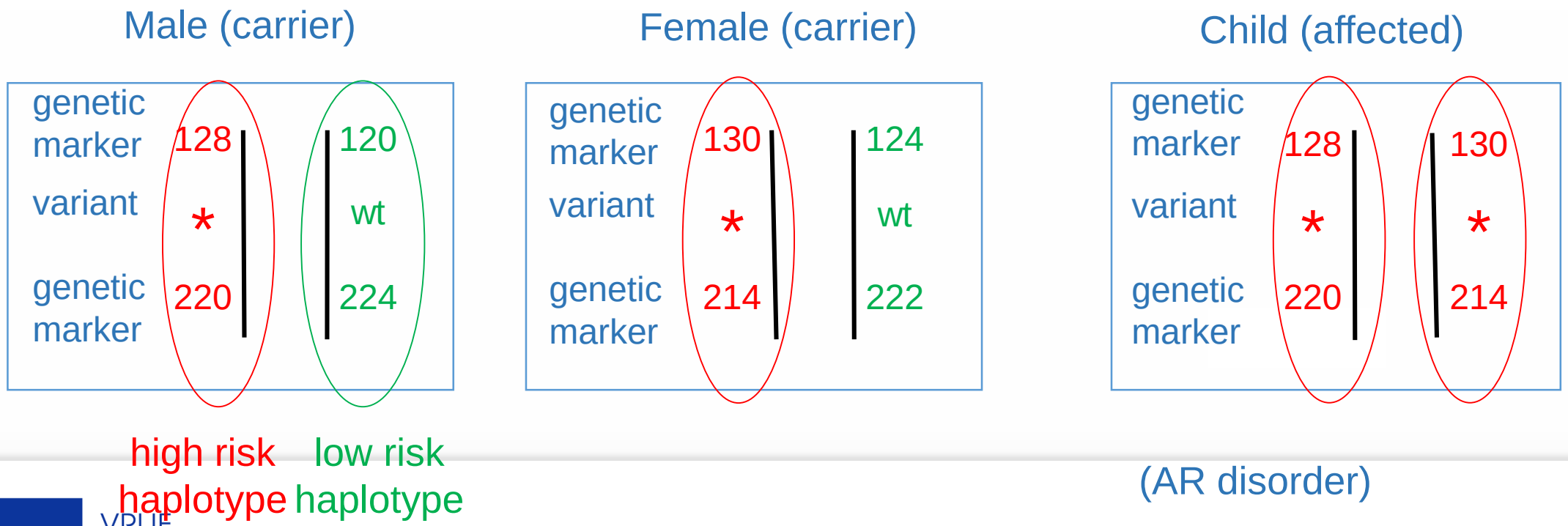
in case of late-onset neurodegenerative disorders



From https://en.hdbuzz.net/036

PGT-M: haplotyping

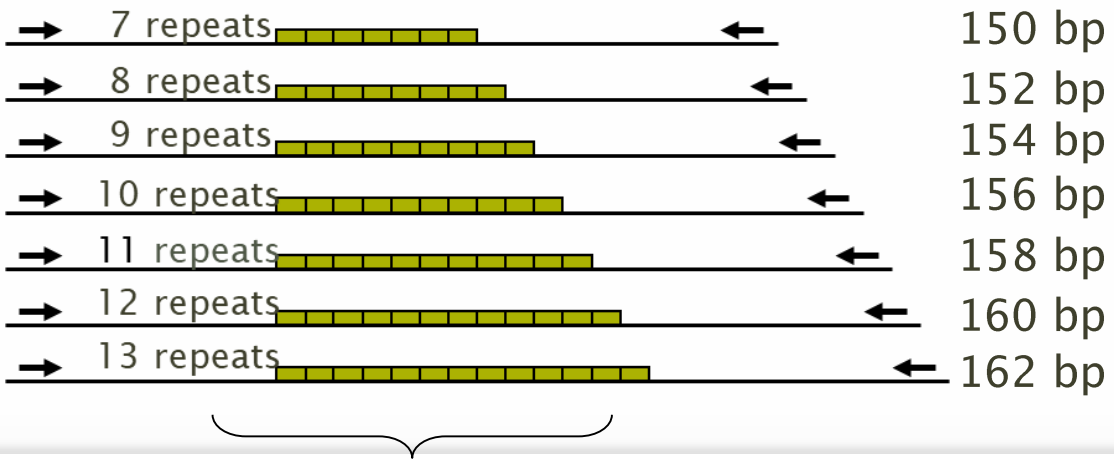
Principle: alleles of the genetic variant (* = class 4/5) and genetic markers such as STRs or SNPs located near each other on the same chromosome are inherited together



PGT-M: genetic markers for haplotyping

STR: Short Tandem Repeat
variation in number of 2, 3 or 4 bp repeats -> many alleles

gagaaaaacccctgtgctggcacacacaca cacacacacatggtgagtgtattaacaacc
gagaaaaacccctgtgctggcacacacaca cacacacacacatggtgagtgtattaacaacc



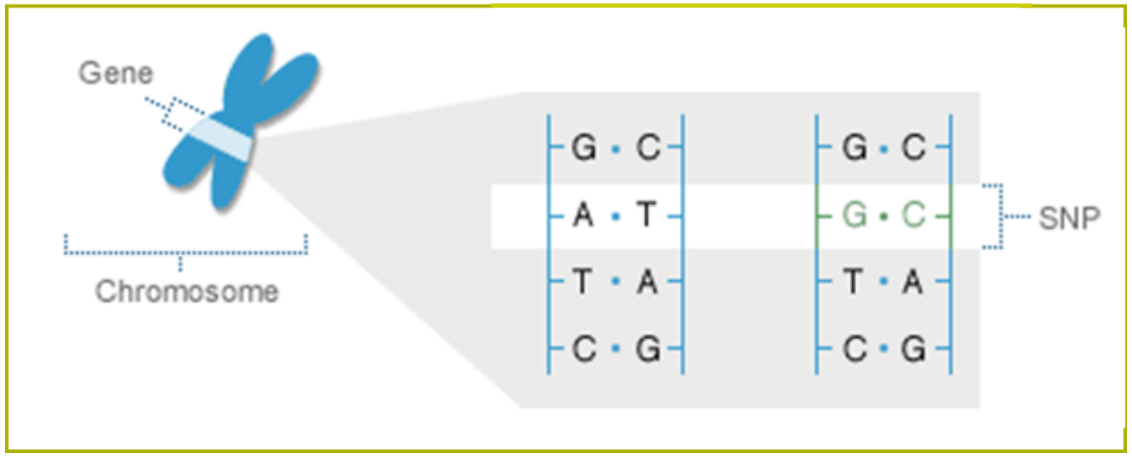
$(CA)_n$ $(CAG)_n$ $(GATA)_n$

PGT-M: genetic markers for haplotyping

SNP = Single Nucleotide Polymorphism:

AGTCATGGGCAGCCTGTT

AGTCATGGACAGCCTGTT



substitution of 1bp, =normal variants

SNP -> two alleles (lower info level)

many present in the genome (about 1 common SNP every 300bp) (human genome = 3000.10^6 bp)

PGT-M: haplotyping

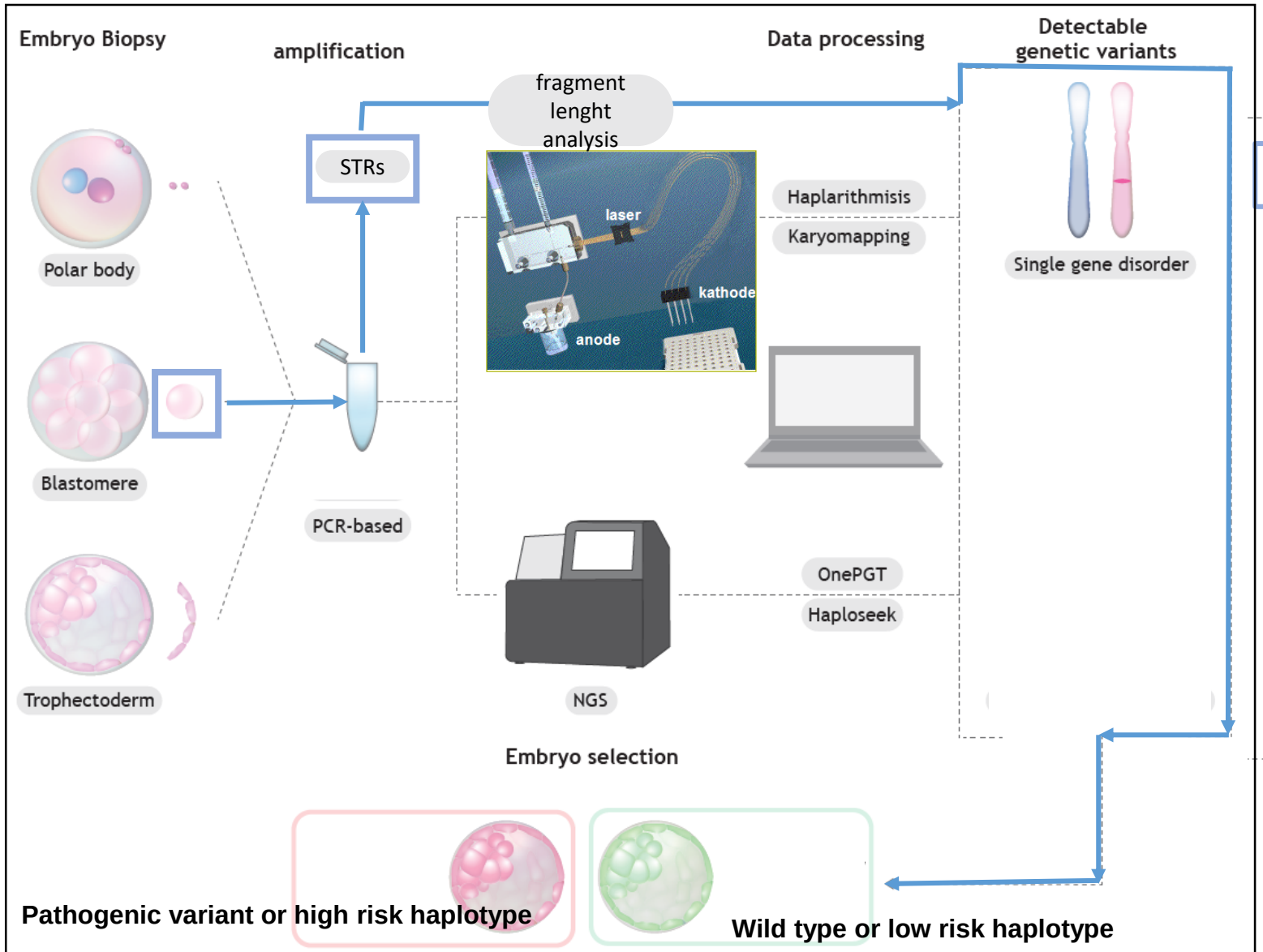
targeted haplotyping: PGT-M
genome-wide testing: haplotyping for PGT-M plus chrom copy number for PGT-A

direct haplotyping: STRs or SNPs with variant detection
indirect haplotyping: STRs or SNPs without variant detection

1 step amplification + haplotyping: single cell multiplex PCR
2 step amplification + haplotyping: WGA followed by PCR, SNParray, NGS

haplotyping at the level of single or few cells
=> pitfalls of amplification failure (AF), ADO, contamination

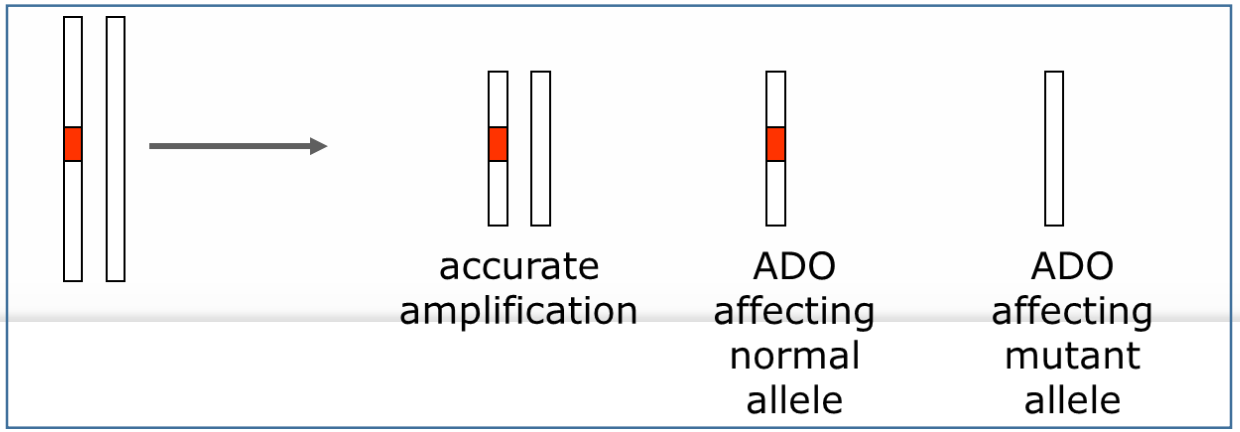
PGT-M: haplotyping: single cell multiplex PCR



PGT-M: single cell multiplex PCR

Pitfalls:

- regular PCR: 100-500 ng purified genomic DNA
- single cell PCR: lysed single cell with 6 pg or 2 DNA copies
  requires extensive optimisation of PCR conditions
- contamination: from carry-over or extraneous DNA
- allele drop out: random amplification failure of one of two alleles in a single/few heterozygous cell(s) – may affect up to 5% of single-cell PCR



PGT-M: haplotyping

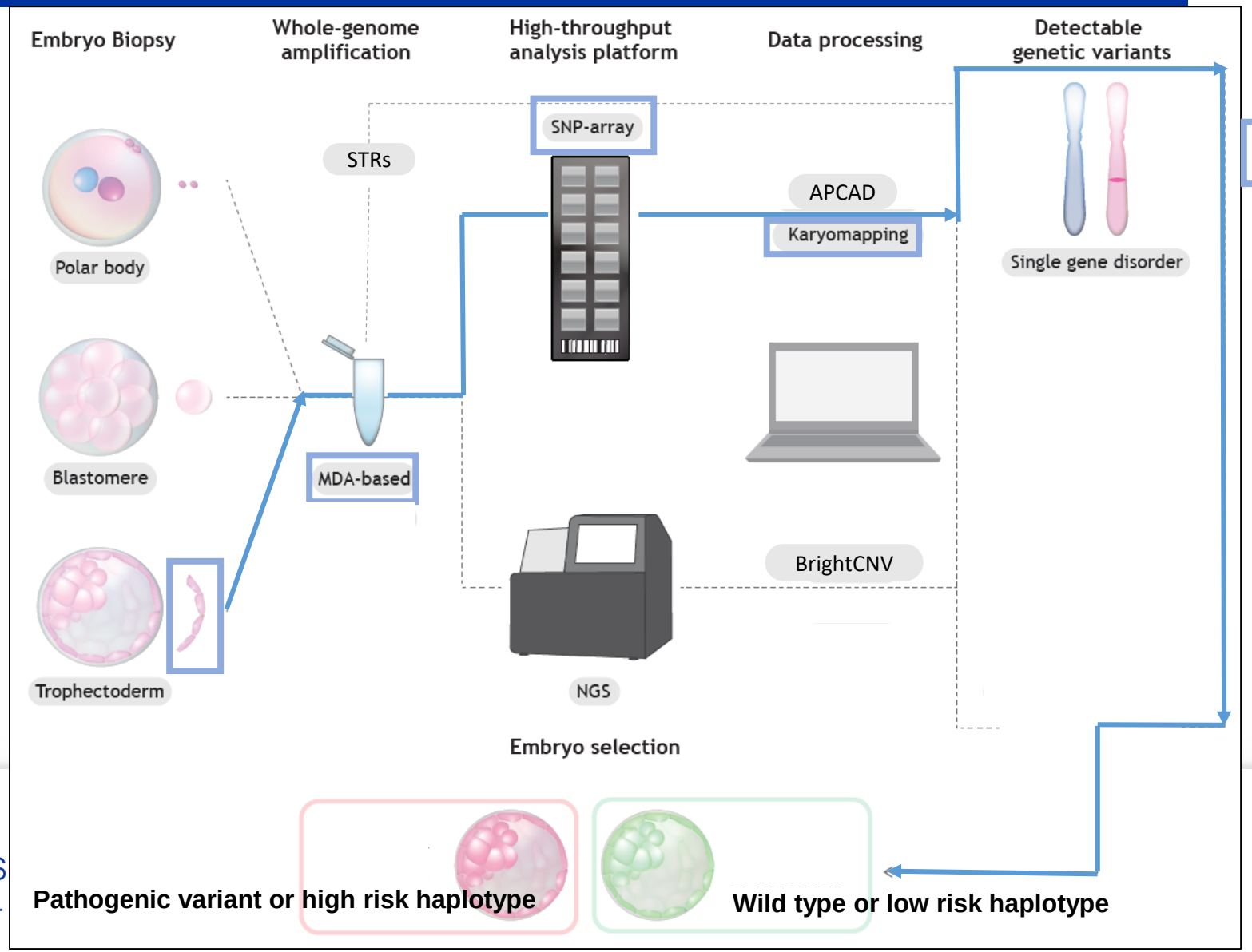
targeted haplotyping: PGT-M
genome-wide haplotyping: PGT-M plus PGT-A

direct haplotyping: STRs or SNPs with variant detection
indirect haplotyping: STRs or SNPs without variant detection

1 step amplification + haplotyping: single cell multiplex PCR
2 step amplification + haplotyping: WGA followed by PCR, SNParray, NGS

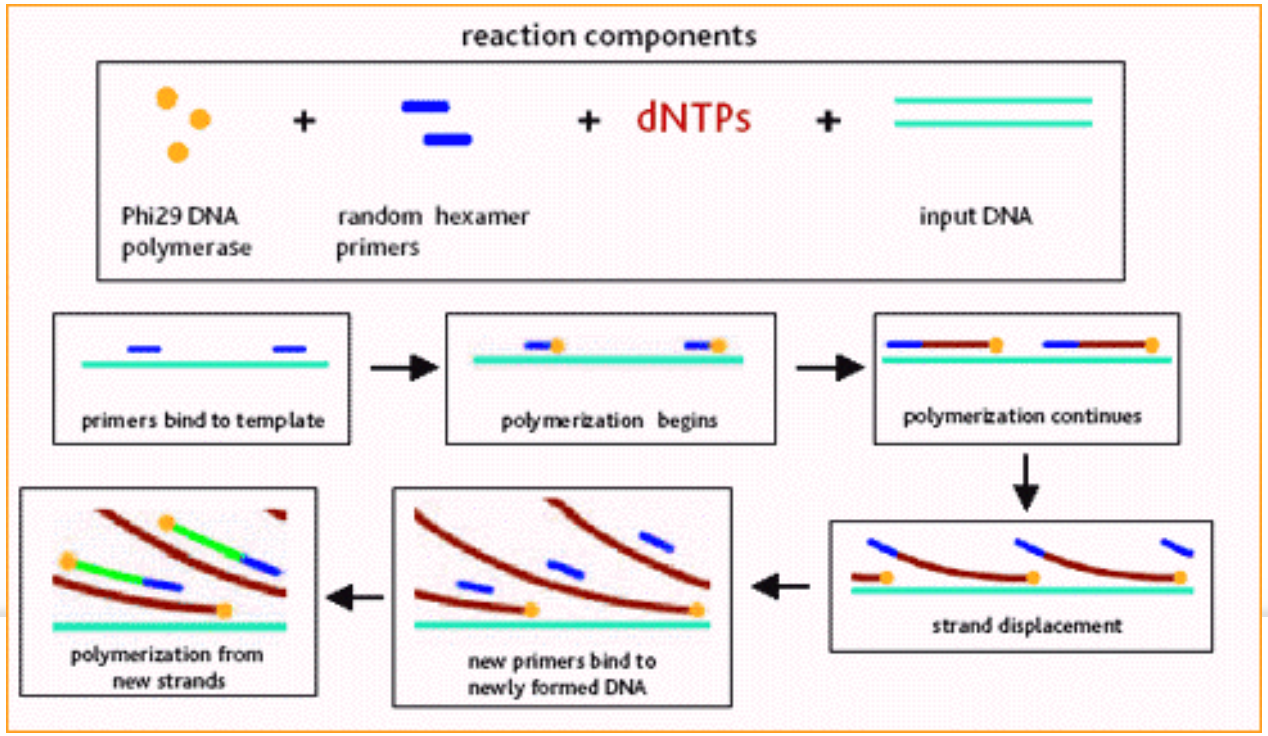
haplotyping at the level of single or few cells
=> pitfalls of amplification failure (AF), ADO, contamination

PGT-M: WGA + haplotyping



PGT-M: whole genome amplification

- Multiple Displacement Amplification, (MDA)
  isothermal amplification (30°C) => DNA fragments up to 70 kb,
  low error rates, from 6 pg (single cell) up to several µg of DNA



Dean et al., 2002

PGT-M: whole genome amplification

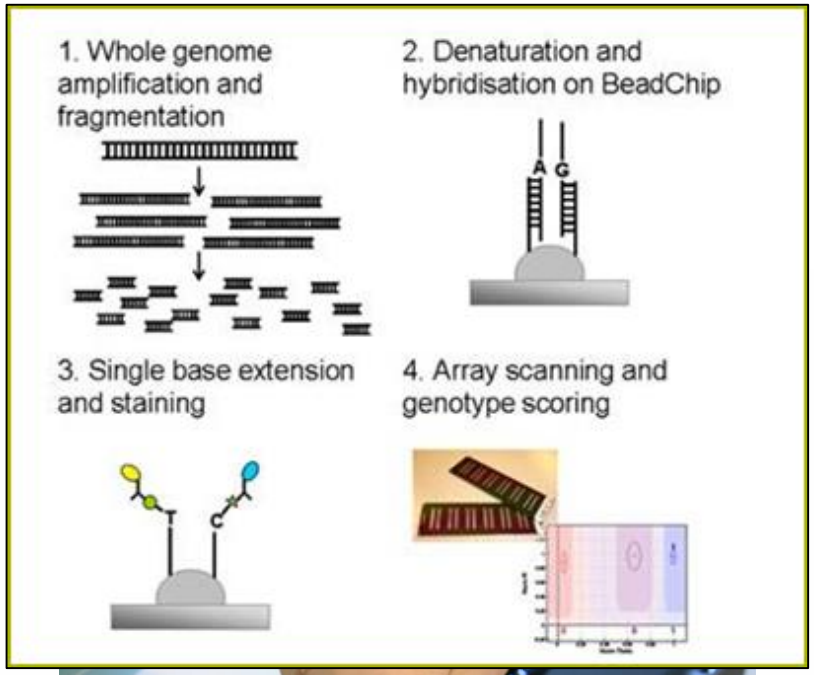
- Multiple Displacement Amplification of single/few cells
  - pitfalls of contamination, ADO
  - relative good genome coverage (better than PCR-based WGA)
    (except telomere and centromere repeats)
  - low error rates

  - amplification bias (worse than PCR-based WGA)
    (random under or overrepresentation of sequences)

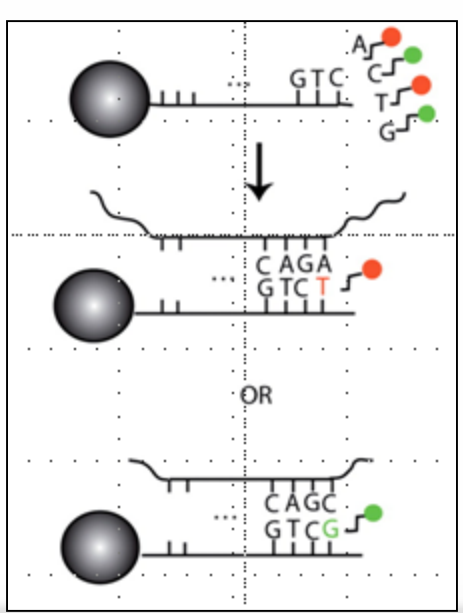
  => preferred method for haplotyping for PGT-M, suboptimal for PGT-A

PGT-M: haplotyping: SNP array

single/few cells → MDA-based WGA



karyomapping



single base extension

PGT-M: targeted vs genome-wide testing

PGT-M haplotyping

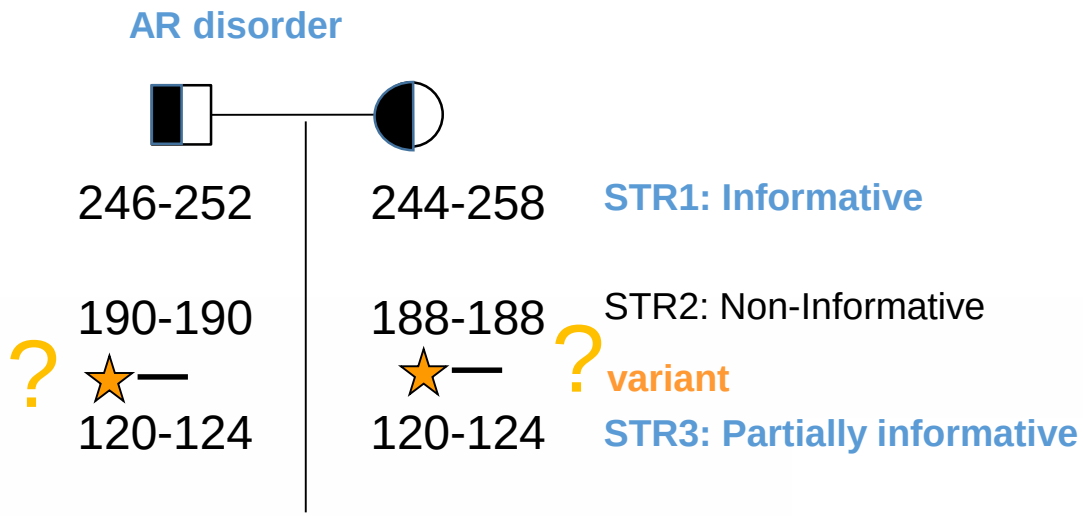
d3 biopsy + targeted multiplex PCR (STRs)

d5/6/7 biopsy + WGA + genome-wide SNP array

preclinical workup

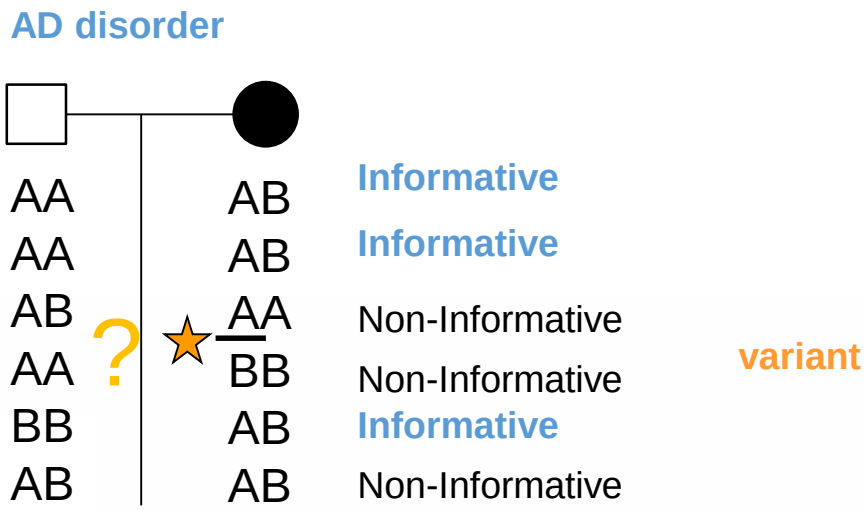
- informativity testing and haplotyping
- relevant family samples are required

PGT-M: haplotyping: preclinical workup



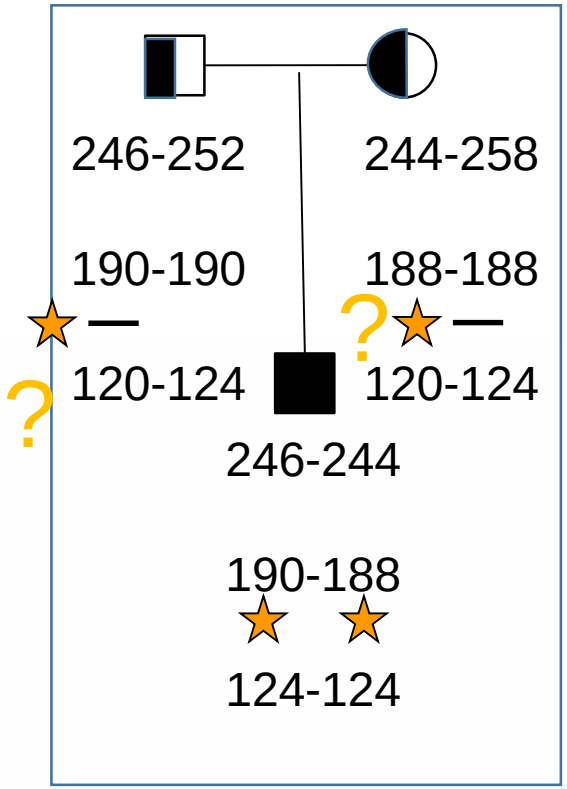
STR genotyping to select flanking STRs which are informative, allowing to distinguish alleles from each other

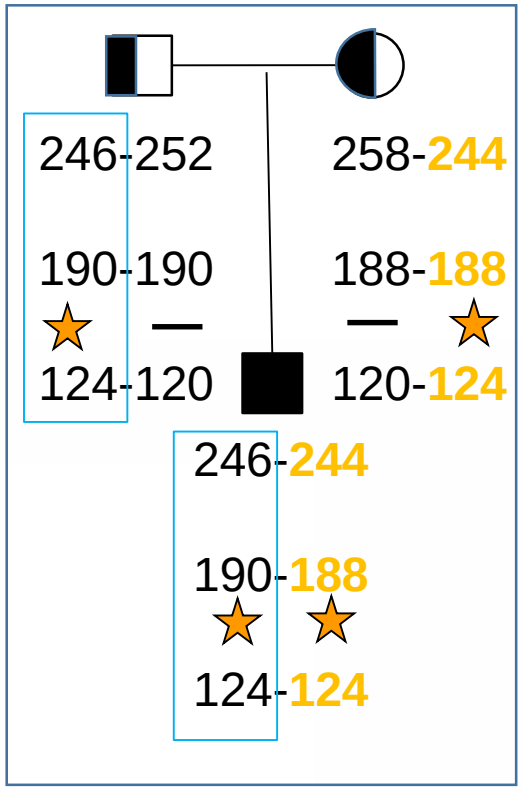
PGT-M: haplotyping: preclinical workup



SNP genotyping to select flanking SNPs which are informative, allowing to distinguish alleles from each other

PGT-M: haplotyping: preclinical workup

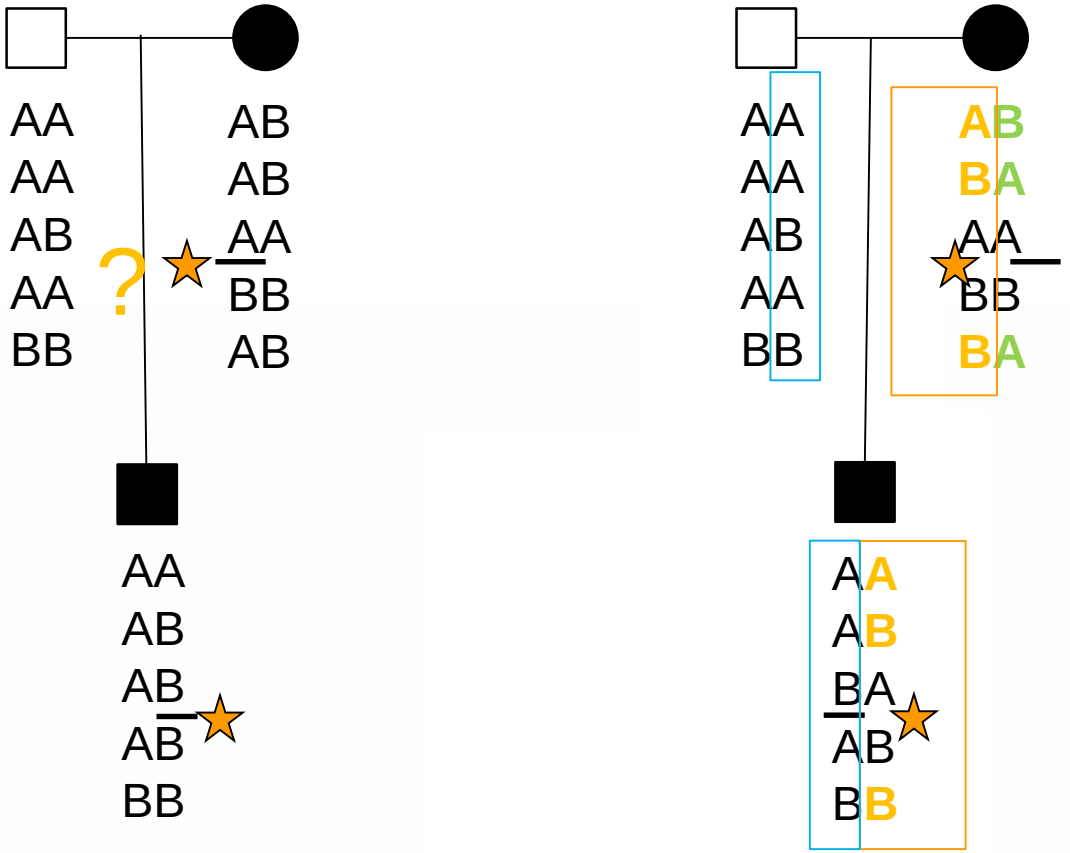




STR genotyping of family samples with known genetic status => establish parental haplotypes

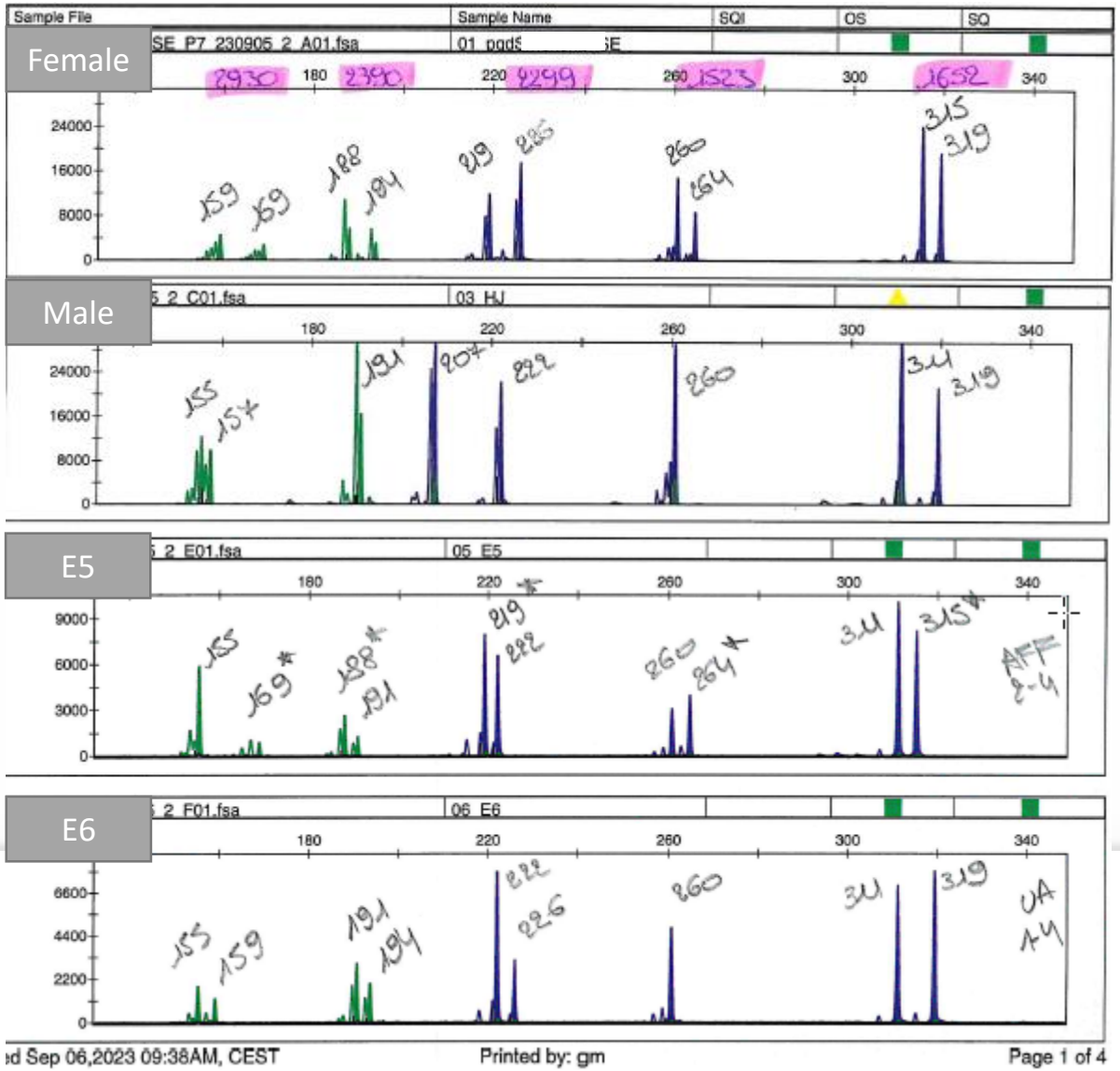
PGT-M: haplotyping: preclinical workup

AD disorder



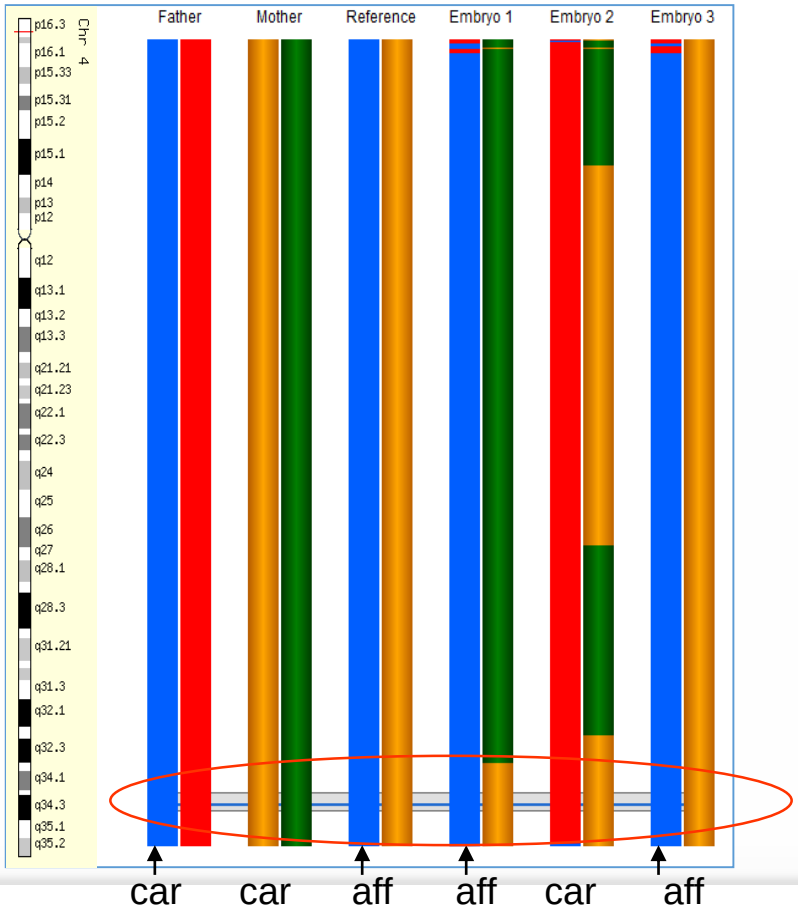
SNP genotyping of family samples with known genetic status => establish parental haplotypes

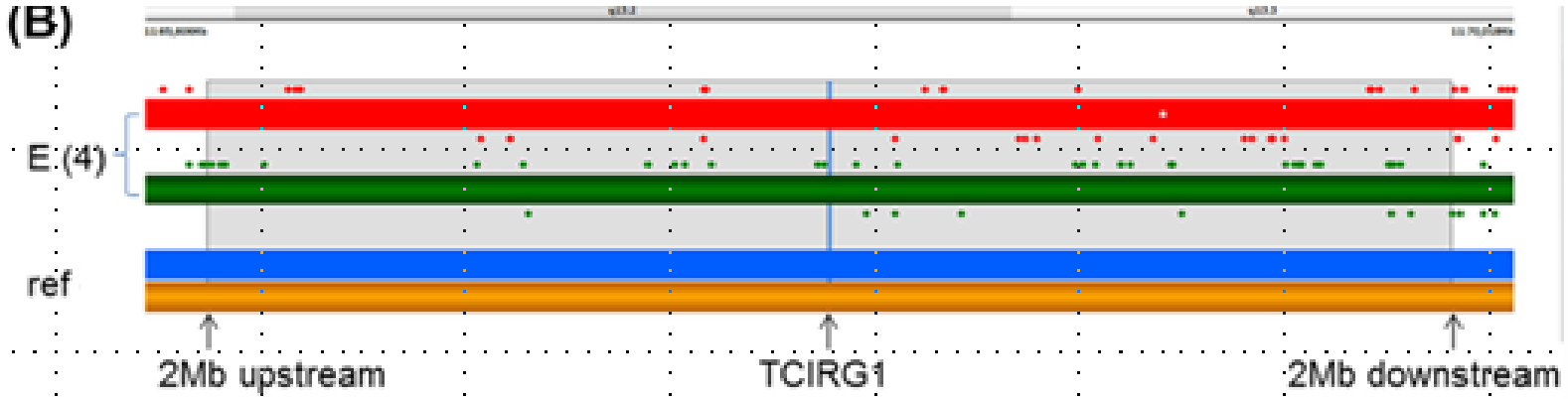
PGT-M: haplotyping: diagnosis



Female	Male	Locus	Relative location	E5.1	E6.1
DUX4					
159 - 169*	157 - 155	D4S2930 HEX	908 Kb 5'	169* - 155	159 - 155
194 - 188*	191 - 191	D4S2390 HEX	1.25 Mb 5'	188* - 191	194 - 191
226 - 219*	207 - 222	D4S2299 FAM	1.48 Mb 5'	219* - 222	226 - 222
260 - 264*	260 - 260	D4S1523OTN1 FAM	565 Kb 5'	264* - 260	260 - 260
319 - 315*	319 - 311	D4S1652 FAM	921 Kb 5'	315* - 311	319 - 311
Negative Control Intern: OK			Blanks	ok	ok
Negative Control Extern:			Remarks		
			DIAGNOSIS CELL	Affected	Unaffected
			DIAGNOSIS EMBRYO	Affected	Unaffected

PGT-M: haplotyping: diagnosis





haplotyping of SNPs in embryos
vs reference (aff son)
AR disorder

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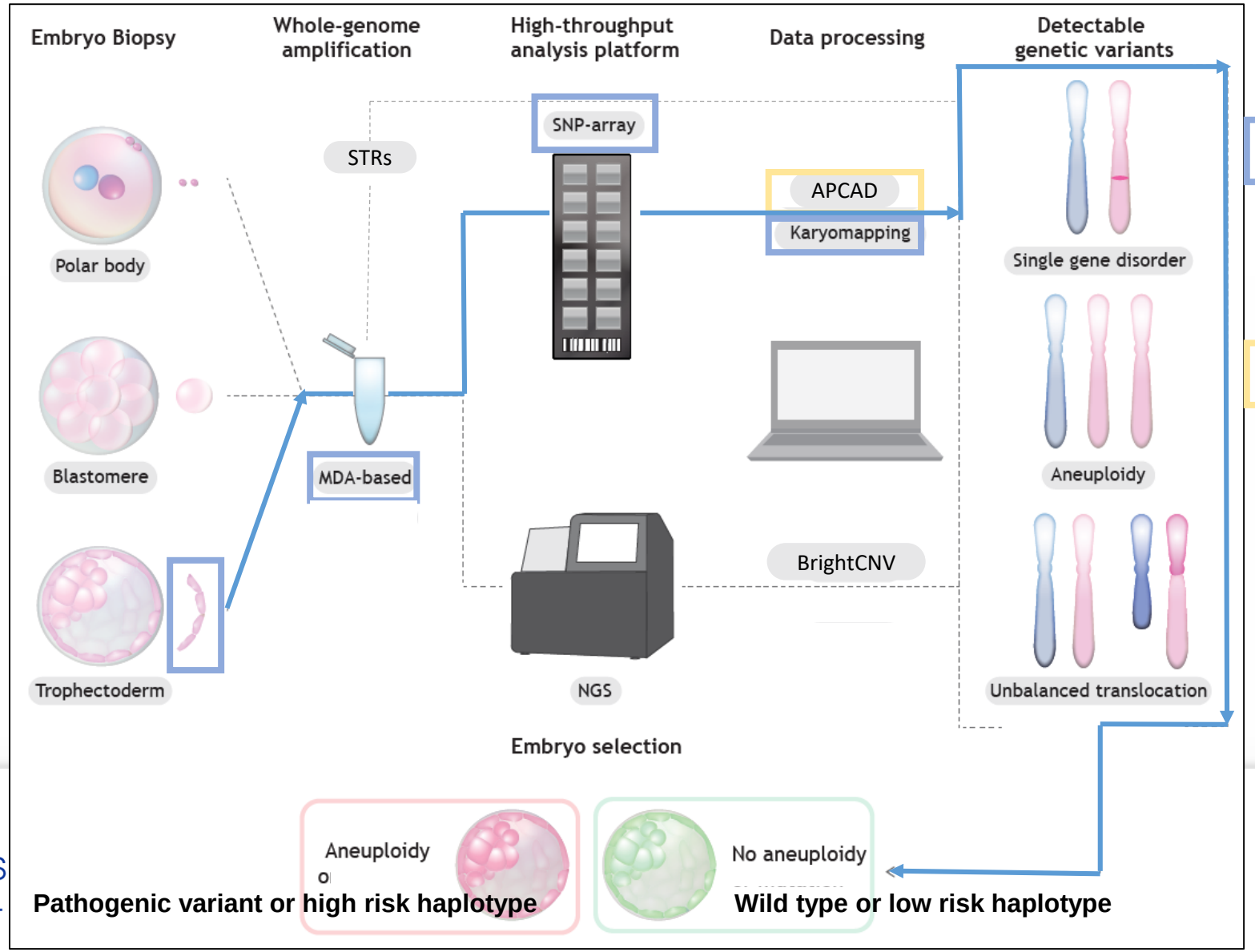
BRUSSEL

PGT-M+PGT-A/SR



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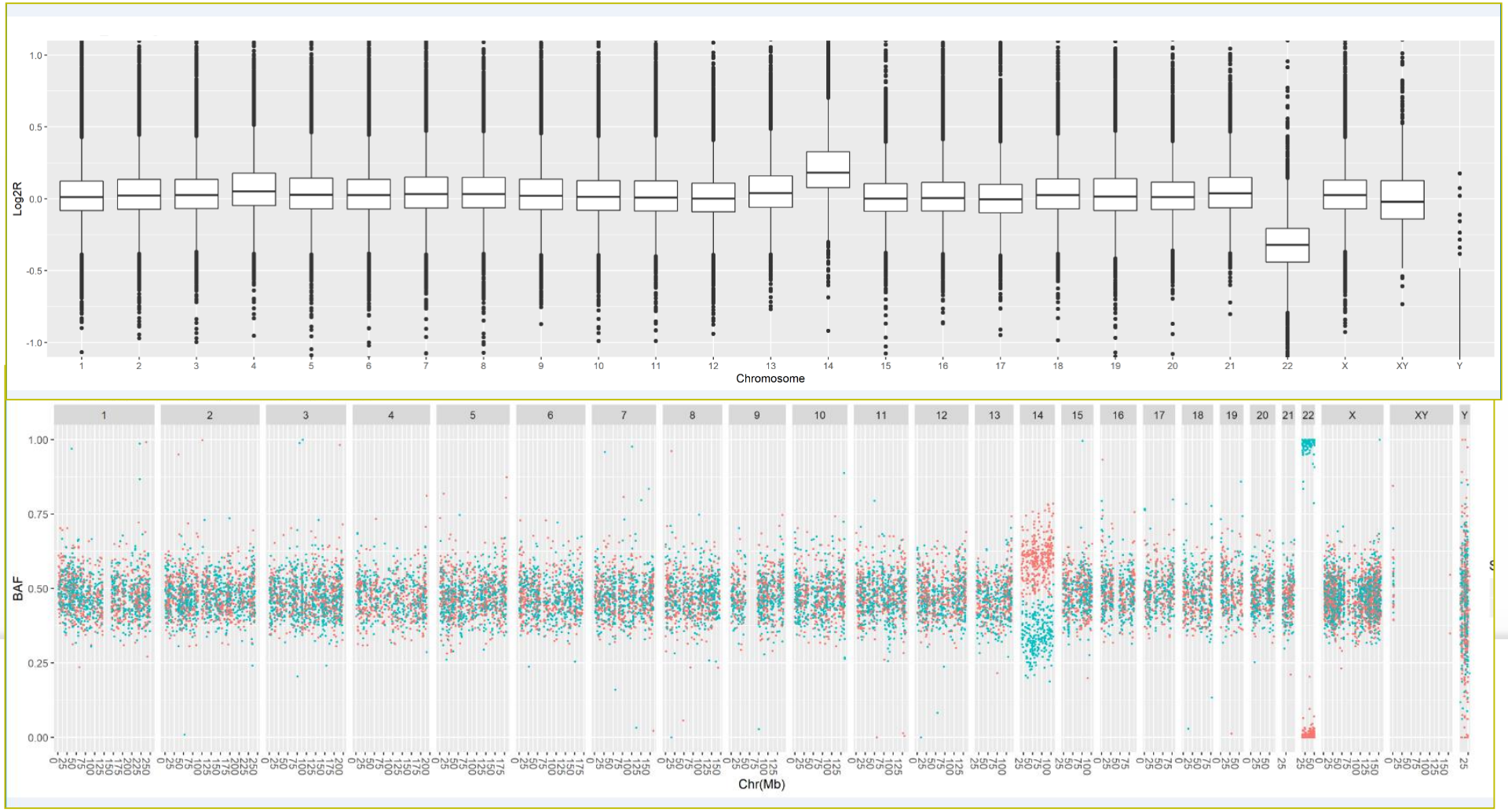
PGT-M+PGT-A/SR

APCAD algorithm (Verdyck *et al*, 2022), trisomy 14, monosomy 22



PGT-M: diagnosis

- indirect diagnosis: use of genetic markers that flank the locus/gene of interest (within 1-2 Mb) (leaning on genetic reports)
- variant detection can be added as confirmation
- convenient when direct variant detection is not possible (region too large for amplification, pseudogene interference, …)

- limiting factor: informative couples with family history and requirement for samples form affected family members

PGT-M: diagnosis

no relevant family DNA samples / *de novo* genetic variant in prospective parent =>

1) mandatory to include genetic variant detection in the test strategy

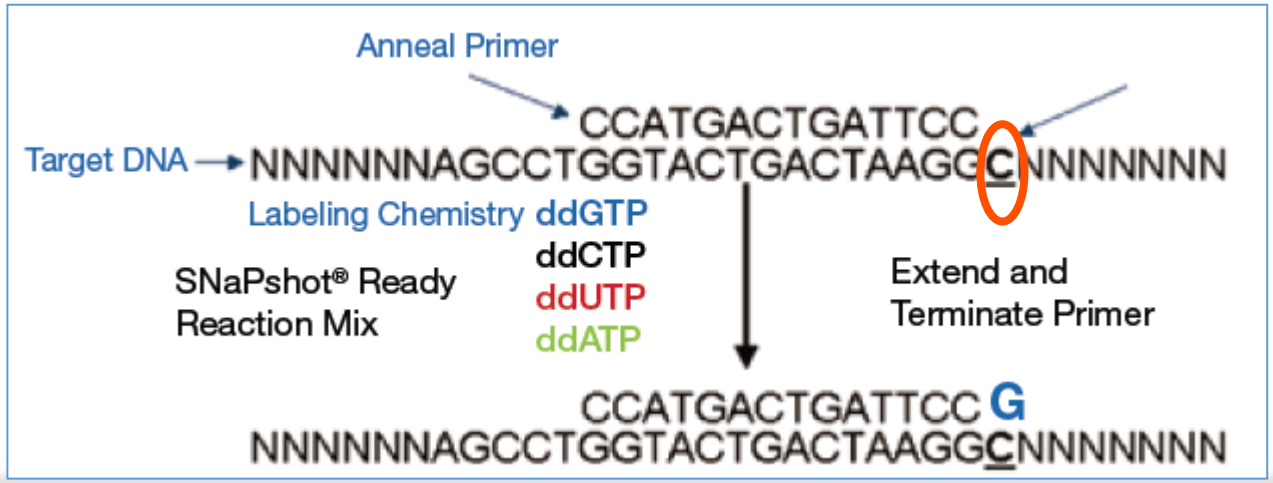
2) determination of low/high risk haplotypes: using gametes or embryos

PGT-M: diagnosis

Genetic variant detection: allele discrimination

- small dups and dels => amplification fragment length differences
- single nucleotide variants => mini-sequencing or other post-amplification methods
- more complex/larger gene rearrangements: 1) high resolution characterisation of breakpoints via long-read seq,

  2) set up of bridge-PCR



PGT-M: diagnosis

no relevant family DNA samples / *de novo* genetic variant in prospective parent =>

1) mandatory to include genetic variant detection in the test strategy

2) determination of low/high risk haplotypes: using gametes or embryos

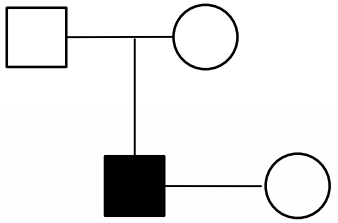
PGT-M: diagnosis

determination of low/high risk haplotypes in parent(s): embryo-based

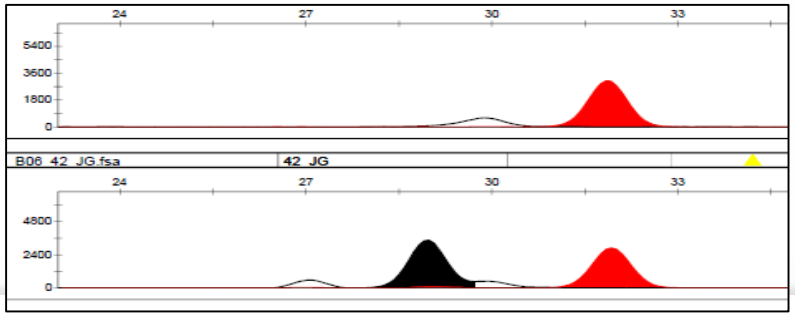
- run variant detection on all embryos => affected embryo
- select an affected embryo as phasing reference for haplotyping of embryos => concordant results between variant detection and haplotypes – ideally min 4 embryos (1 aff, 1 unaff) are analyzed
- If < 4 embryos are available for testing, add low quality embryos non-suitable for biopsy
- >1 cycle may be necessary to complete haplotyping
- be aware of mosaicism: use affected samples as phasing reference

PGT-M: diagnosis

case: couple with male, carrier of *de novo* variant c.6713A>G in *PKD1*
=> apply strategy with variant detection included



preclinical workup: validation variant detection (mini-sequencing)



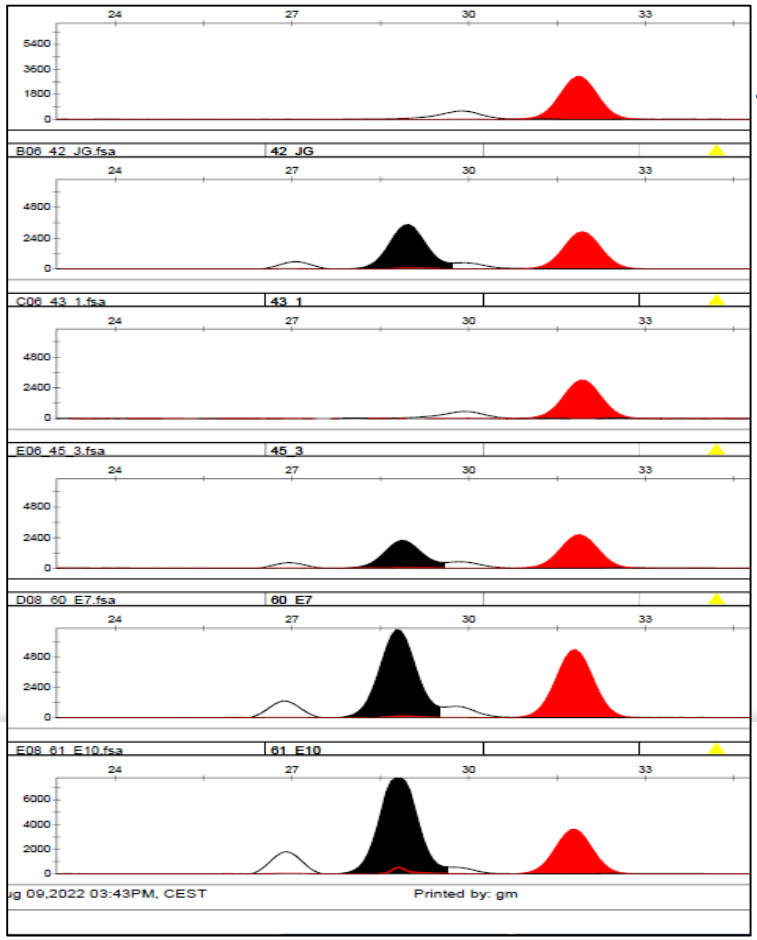
female

male (aff)

PGT-M: diagnosis

couple with male carrier of *de novo* c.6713A>G in *PKD1 (telomeric chr 16)*

cycle 1: 4 embryos for analysis
WGA followed by 1) variant detection 2) SNParray
select an affected embryo as phasing reference for SNParray



female

male (aff)

E1 (unaff)

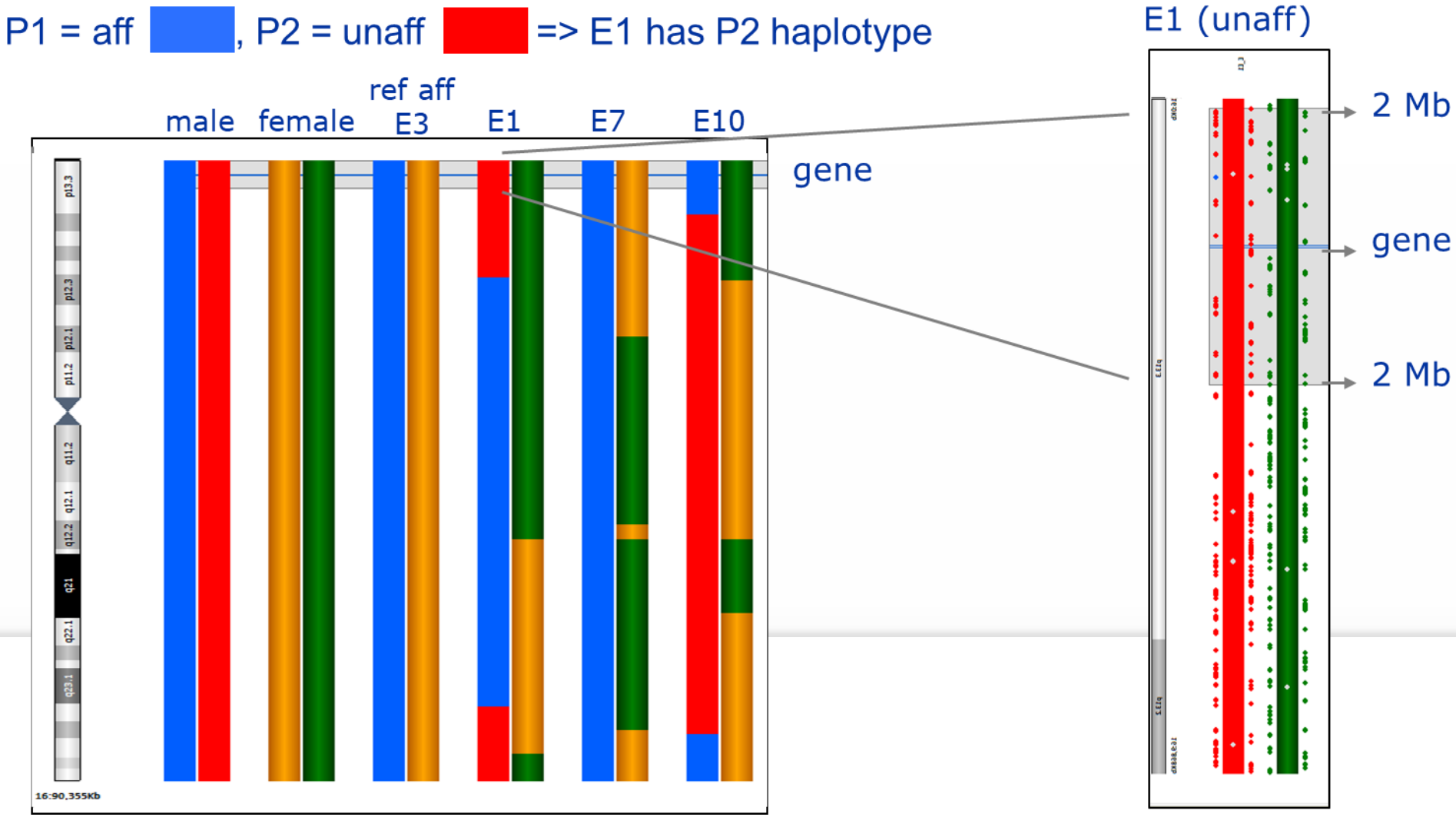
E3 (aff)

E7 (aff)

E10 (aff)

PGT-M: diagnosis

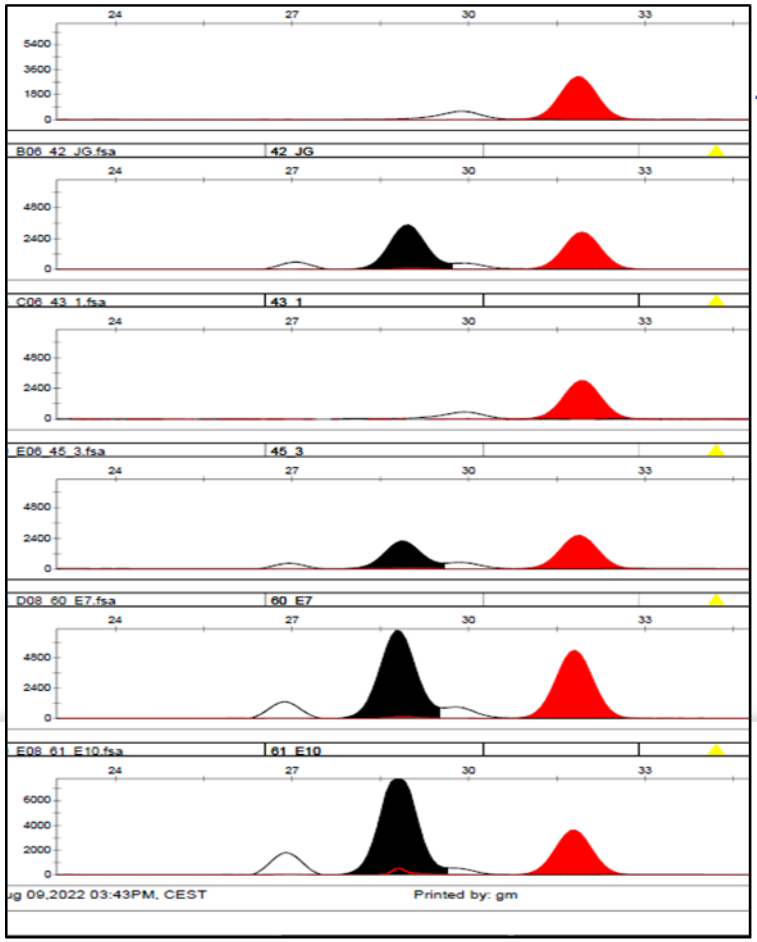
couple with male carrier of *de novo* c.6713A>G in *PKD1 (telomeric chr 16)*



PGT-M: diagnosis

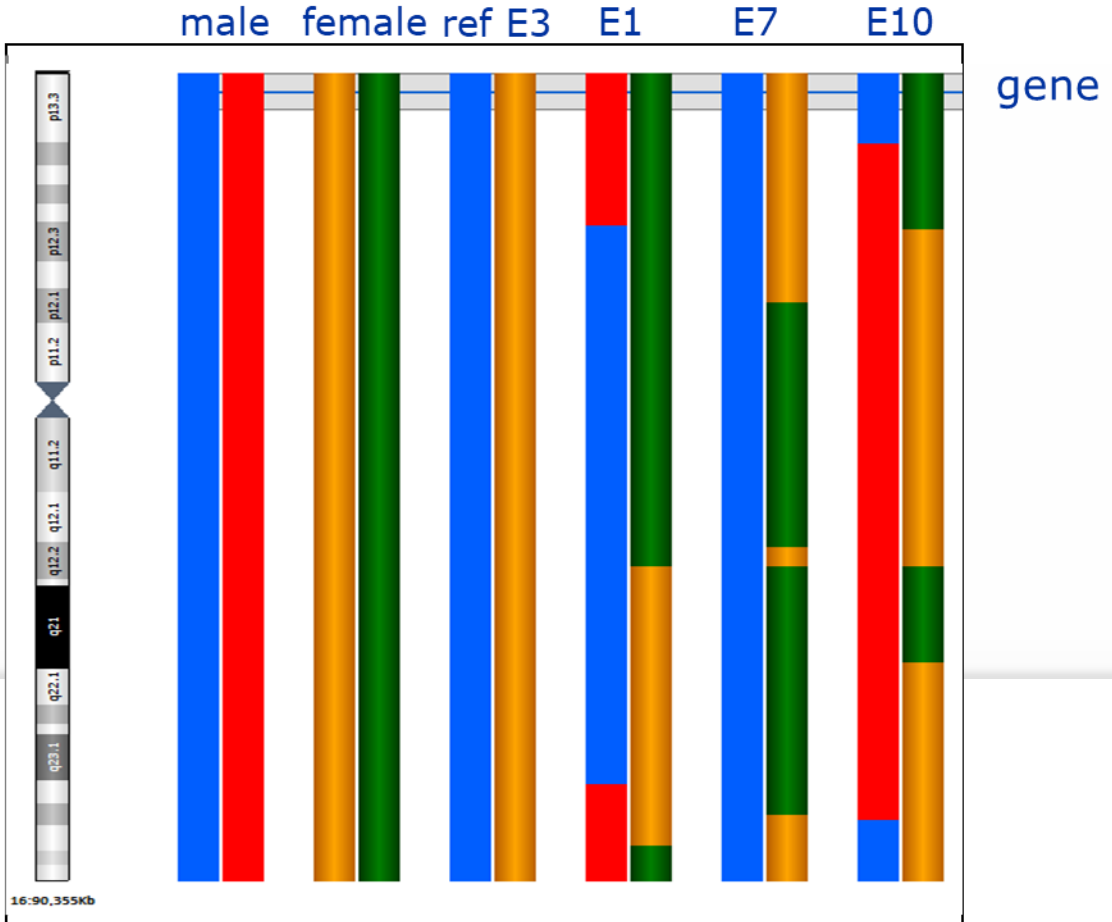
couple with male carrier of *de novo* c.6713A>C in *PKD1 (telomeric chr 16)*

variant detection results



aff E3 as ref for SNPa

P1 = aff , P2 = unaff => E1 has P2 haplotype



Questions?