Premplantation genetic testing

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Outline

- Definitions and background
- Workflow
- Clinical cycle and embryo biopsy
- PGT-M: indications
- PGT-M: targeted versus genome-wide testing

Definitions (new)

Preimplantation genetic testing

A test performed to analyse the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for HLA-typing or for determining genetic abnormalities

- PGT for aneuploidies (chromosomal numerical) (PGT-A)
- PGT for monogenic/single gene defects (PGT-M)
- PGT for chromosomal structural rearrangements (PGT-SR)

International glossary on fertility and infertility care

Zegers-Hochschild et al., Human Reproduction, 2017

Definition (old)

Preimplantation genetic diagnosis (PGD)

- 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 *<u>high</u>* risk of transmitting a genetic condition to their children
- PGT-M, PGT-SR and high risk PGT-A
- (not necessarily infertile, but undergo IVF as part of PGT treatment)

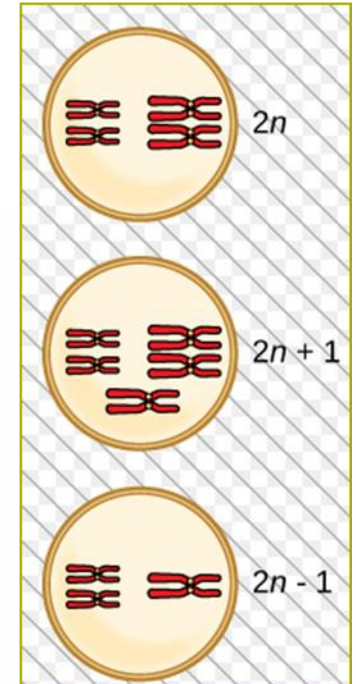
Definition (old)

Preimplantation genetic screening (PGS)

- involves selection of euploid embryos
- to improve IVF results and reduce miscarriage rates for specific IVF patients groups at *low* risk (advanced maternal age, recurrent IVF failure or repeated miscarriages)

Current definition of PGT-A also includes:

- aneuploidy testing for couples at *high* genetic risk (example: XXY or mosaic 45,X/46,XX)



Definitions

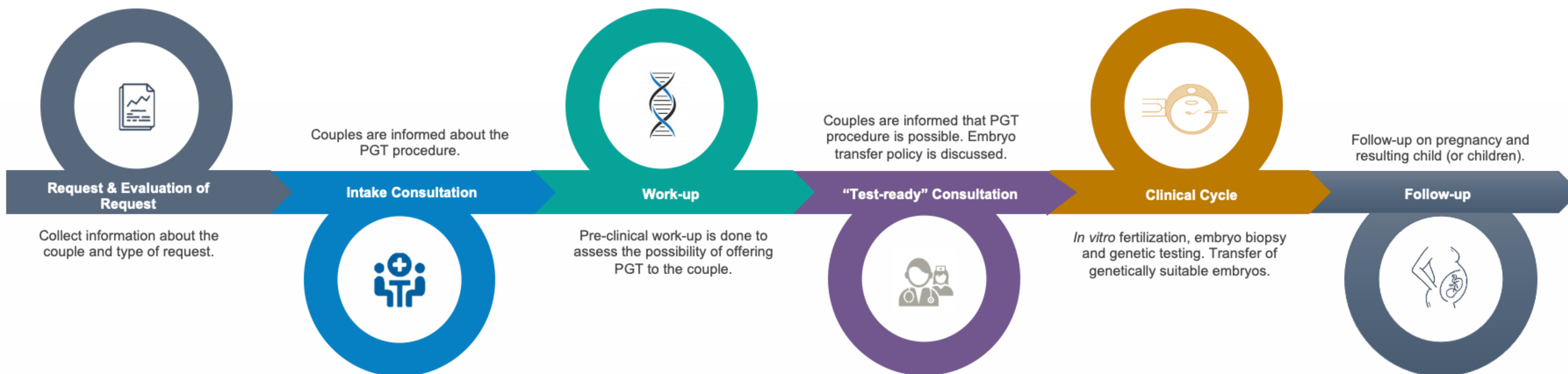
New terminology => PGT or preimplantation genetic testing

Old terminology => PGD or preimplantation genetic diagnosis and PGS or preimplantation genetic screening

PGD/PGS > PGT

PGT-A for low genetic risk IVF patient groups is a screening test

PGT: daily workflow



PGT: daily workflow

- Intake and evaluation of PGT request
  - is PGT acceptable, is PGT possible?
- Consultation at the IVF/genetics unit
  - counselling and informed consent, sample collection
- Pre-PGT workup in the genetics lab
- PGT clinical cycle
- Follow-up of cycles, pregnancies and children

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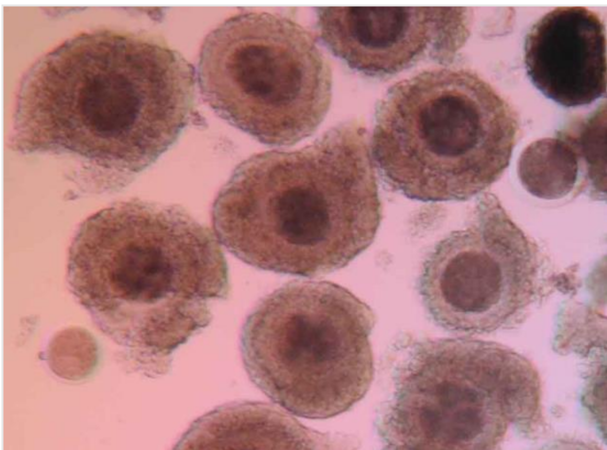
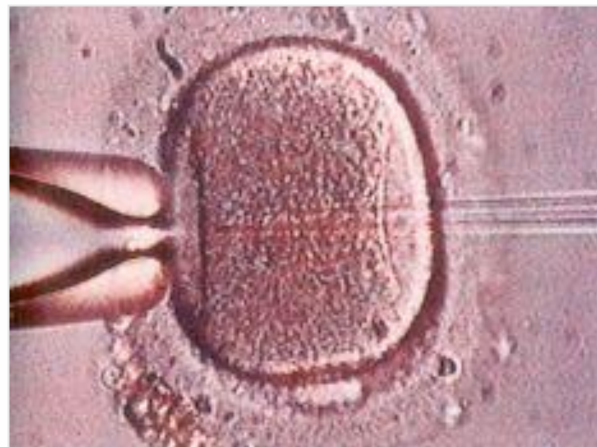
PGT cycle

oocyte collection
after hormonal
stimulation



IVF with
Intracytoplasmic sperm
injection



Day 0

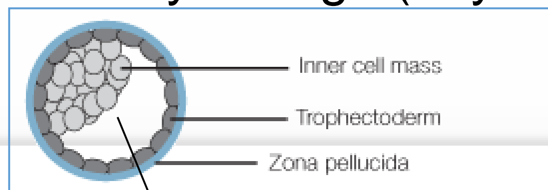
PGT cycle

in vitro embryo culture

cleavage stage (day 3)

morula stage (day 4)

blastocyst stage (day 5/6)



blastocoele

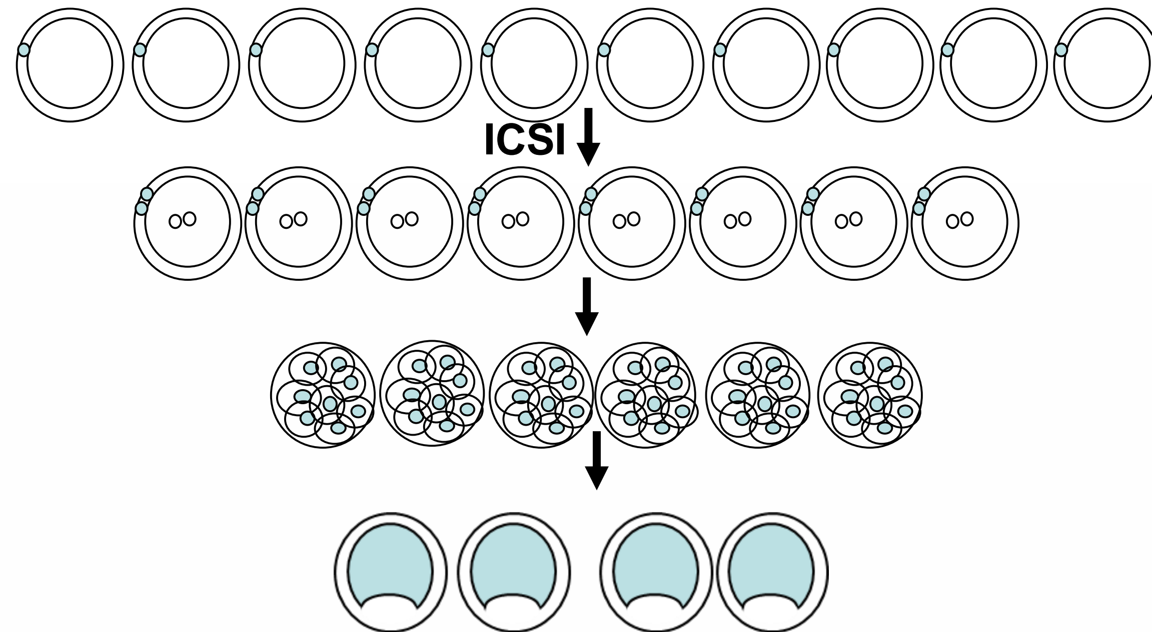
PGT cycle

10 oocytes
day 0

8 normally fertilised oocytes
day 1

6 embryos for biopsy
day 3

4 embryos for biopsy
day 5/6



PGT cycle: biopsy

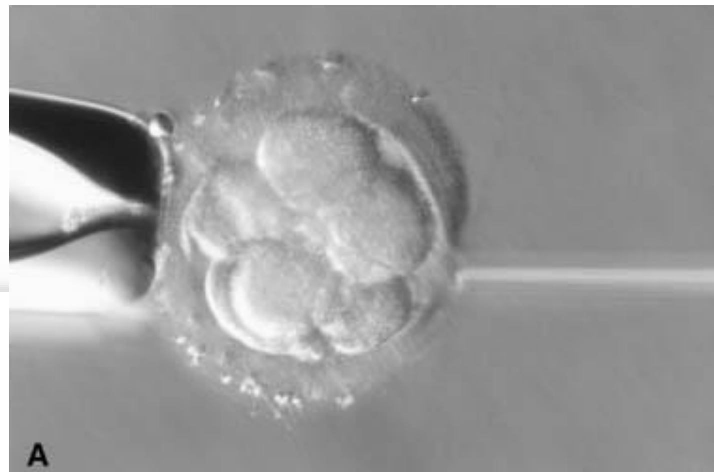
biopsy is a two step process:

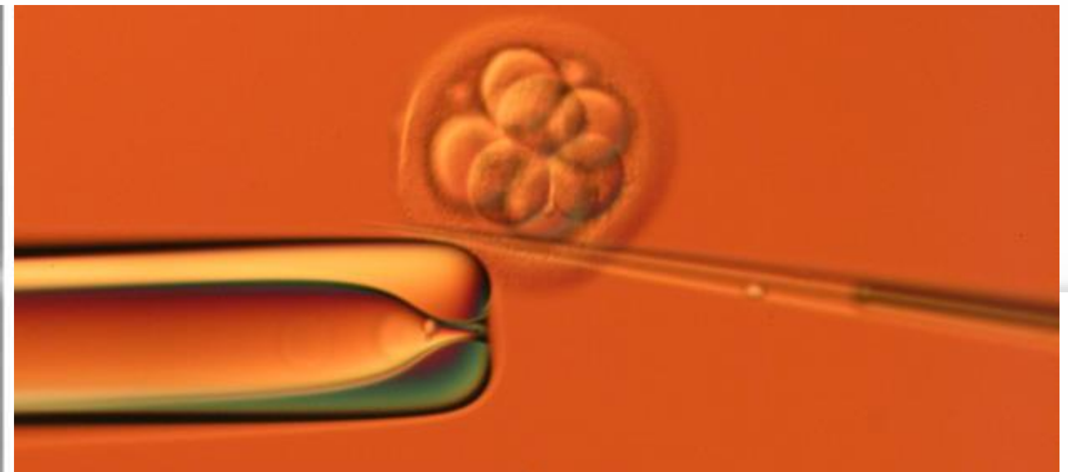
1) punction or removal of part of the ZP
   - mechanical opening (microneedle) (a)
   - chemical opening (Acidic Tyrode) (b)
   - laser pulses (most common) (c)

2) removal of nucleated cell(s)

c

b





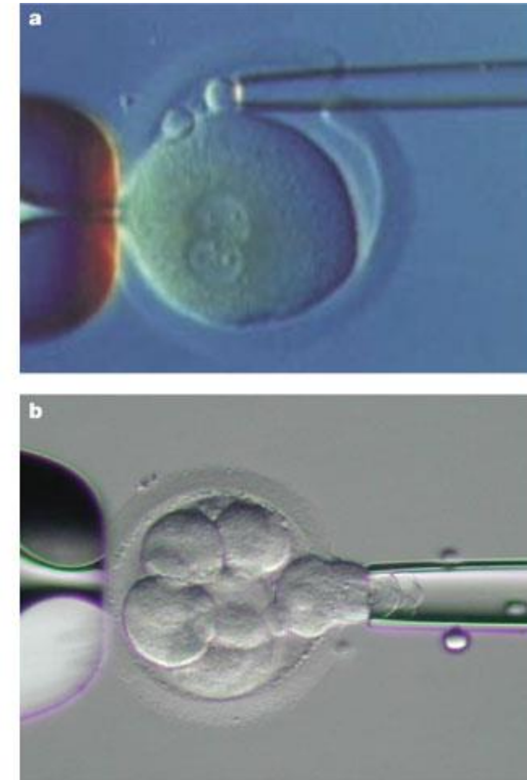
a

PGT cycle: biopsy

biopsy is a two step process:

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

2) removal of nucleated cell(s) by extrusion or aspiration

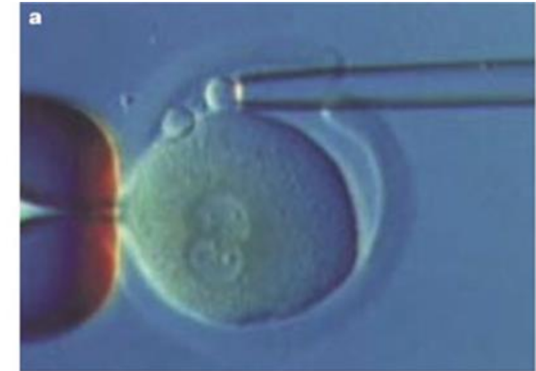


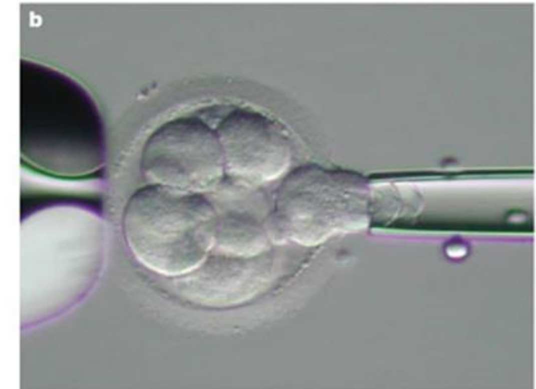
PGT cycle: biopsy

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





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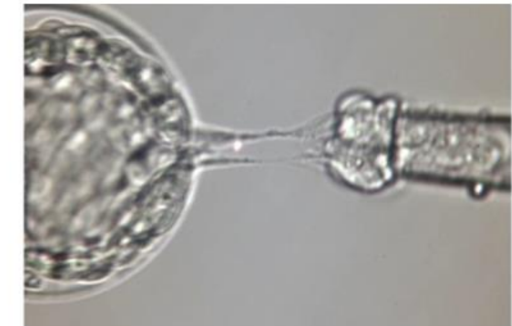
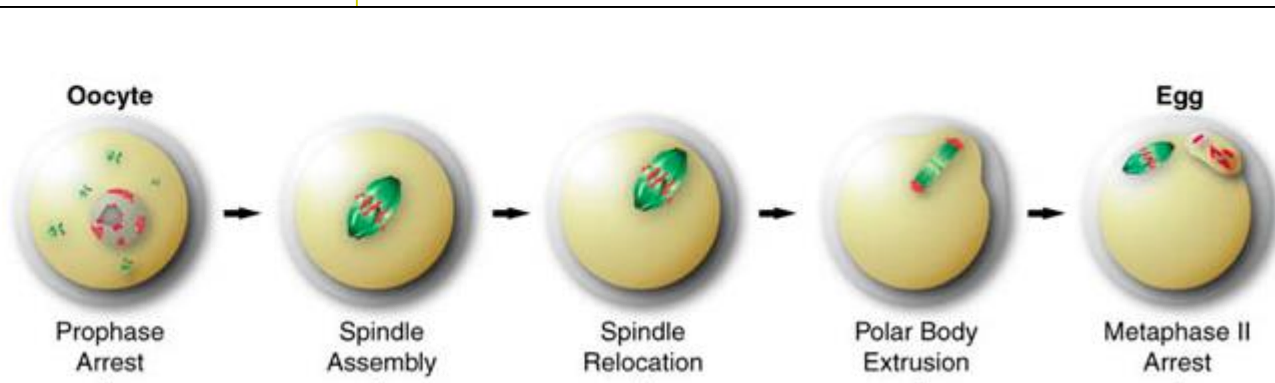
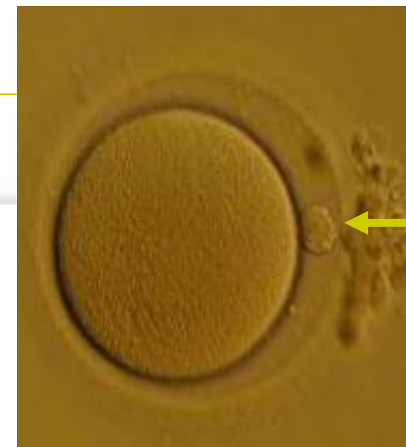


PGT cycle: polar body biopsy

polar body biopsy: removal of PB1 (at MII oocyte, before fertilization) and PB2 (extruded after fertilization): day 0/1

- both PBs are required for accurate diagnosis
- before syngamy
- sequential removal => labour intensive
- no effect on fertilisation and embryonic development
- only for maternally inherited disorders





PB1 at MII oocyte

PGT cycle: day 3 biopsy

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

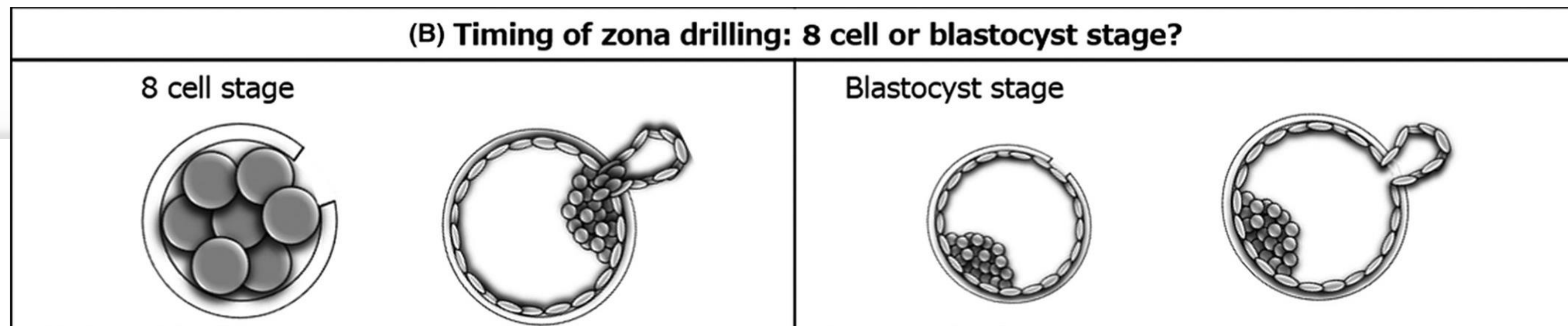
- still used in many PGT centres
- embryo incubation in Ca/Mg-free medium to disassemble cellular junctions
- for maternally and paternally inherited disorders
- gender determination possible
- fresh ET at day 5 possible
- inherent high chromosomal mosaicism rate
- impact of 1 or 2 cell removal on embryonic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

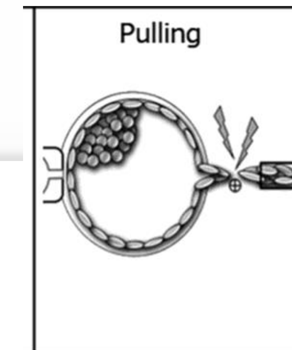
PGT cycle: day 5/6 biopsy

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

- TE cells = extraembryonic cells / safer option
- multiple cells / higher diagnostic accuracy
- no negative impact on implantation
- lower chromosomal mosaicism level
- less samples for testing
- need for good blastocyst culture systems
- need for vitrification and transfer in natural cycle

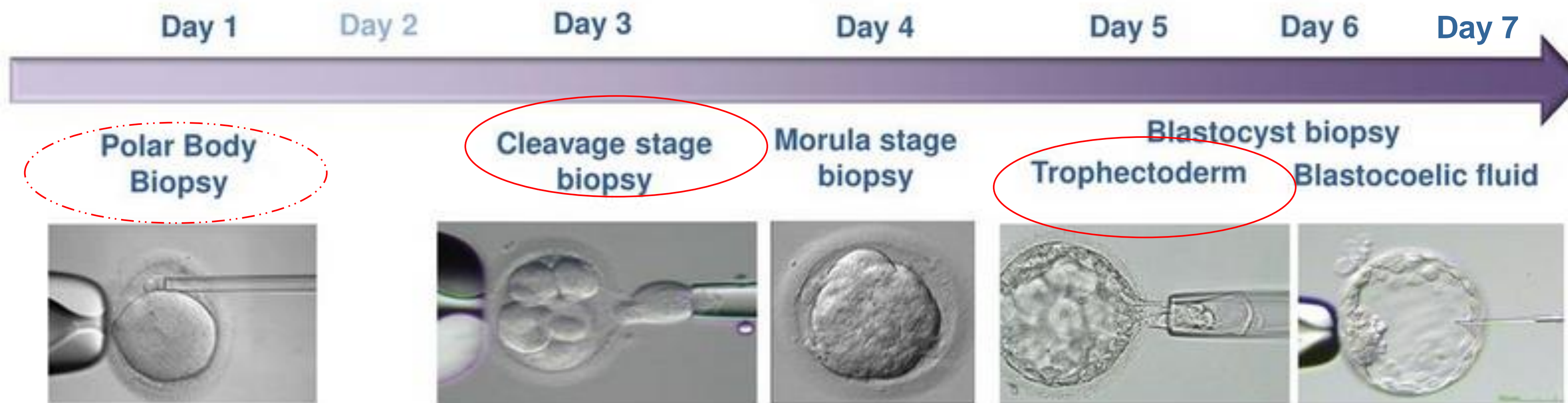
Reproductive Medicine and Biology, First published: 26 January 2020, DOI: (10.1002/rmb2.12318)



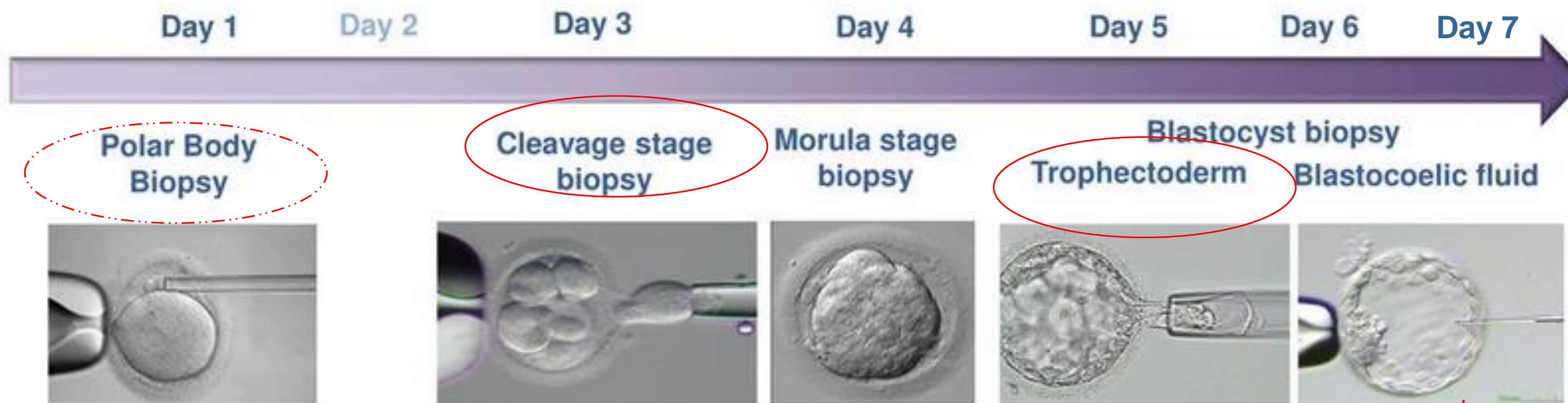


PGT cycle: biopsy

Courtesy C. Magli



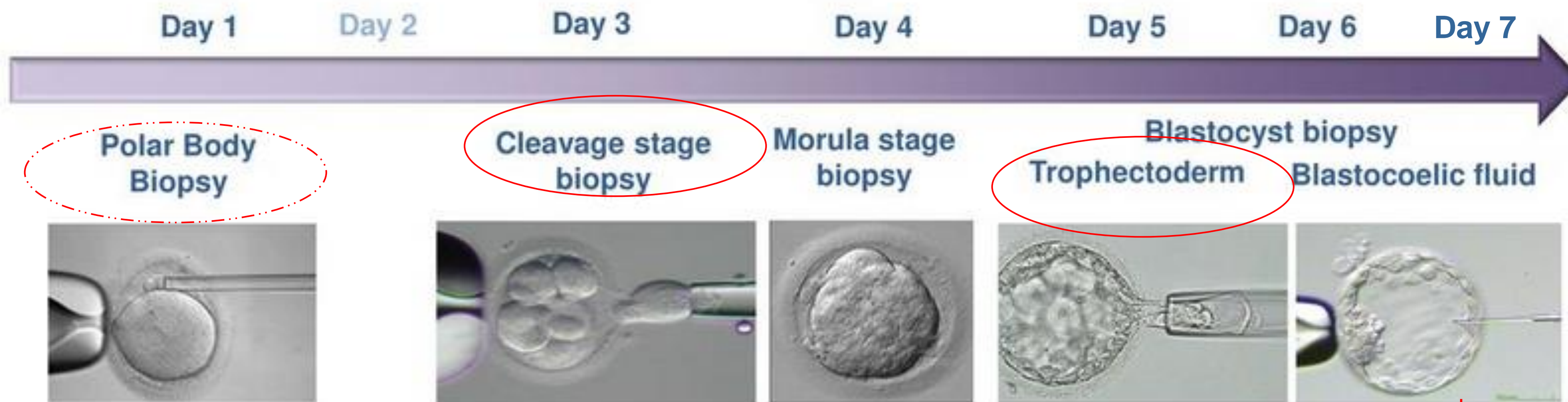
PGT cycle: biopsy



Courtesy C. Magli

minimally invasive
origin of cell-free DNA?

PGT cycle: biopsy



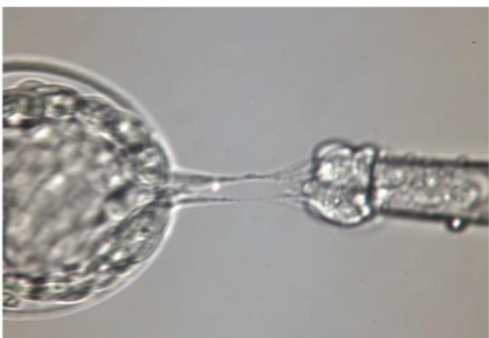
Courtesy C. Magli

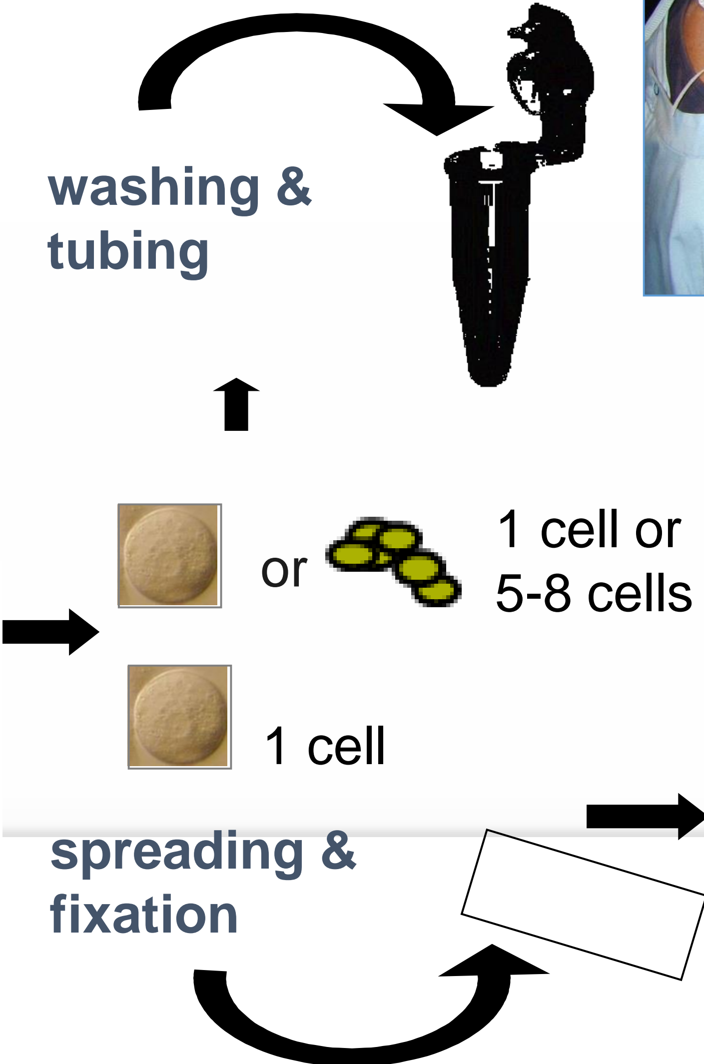
minimally invasive
origin of cell-free DNA?

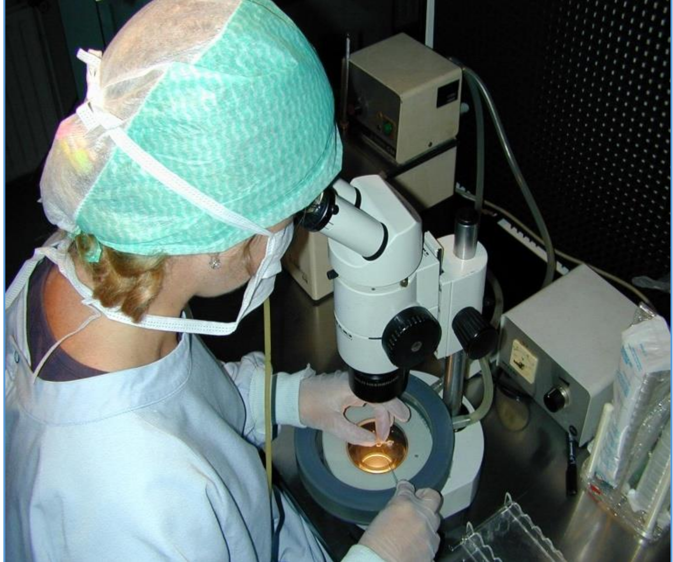
non-invasive
spent embryo culture medium

PGT cycle: biopsy sample collection

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







amplification

interphase
FISH

PGT cycle: genetic testing

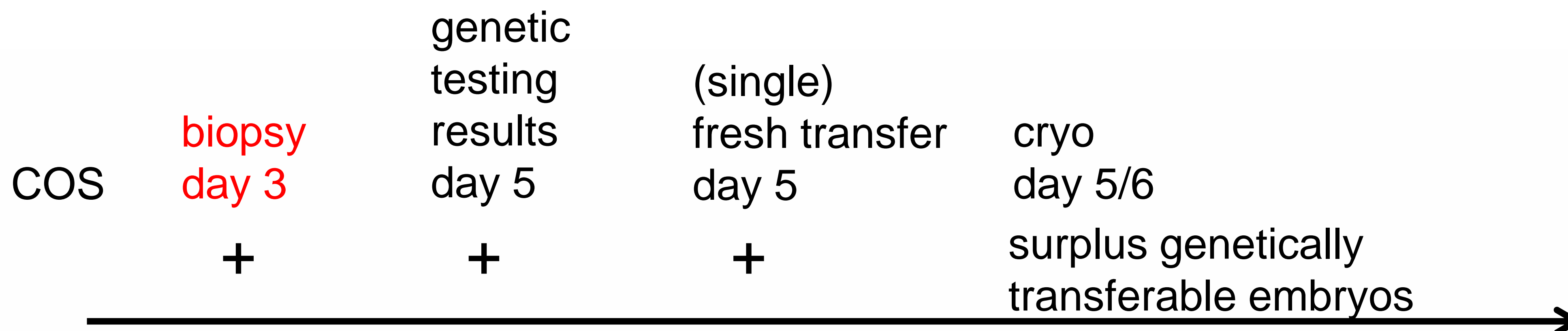
targeted testing
FISH: for PGT-SR/A and sexing
PCR amplification: for PGT-M

genome wide testing

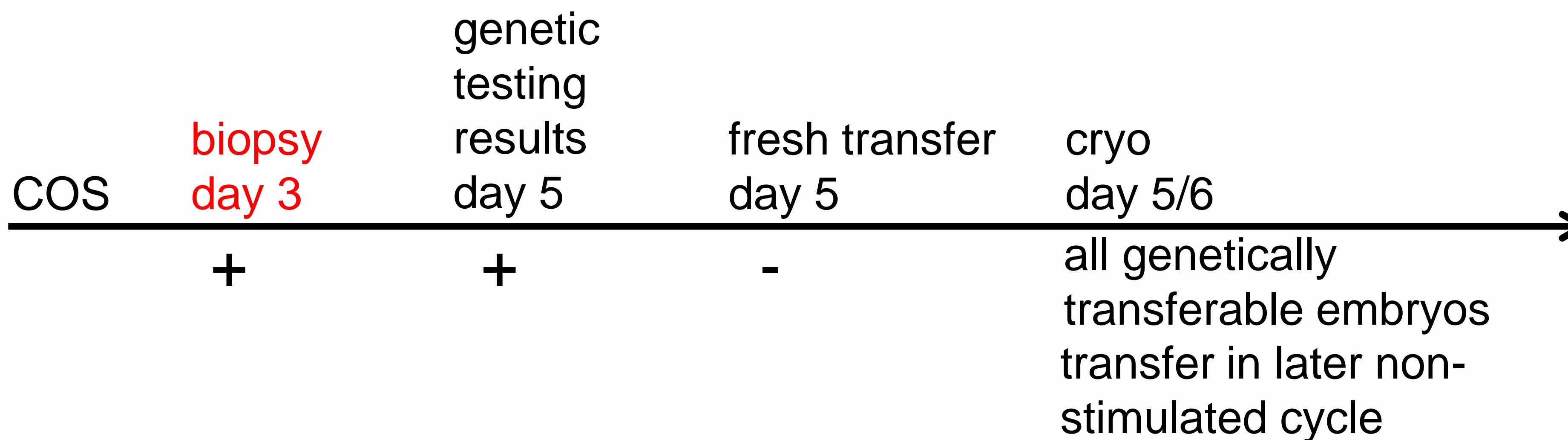
first step = whole genome amplification
(WGA)

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

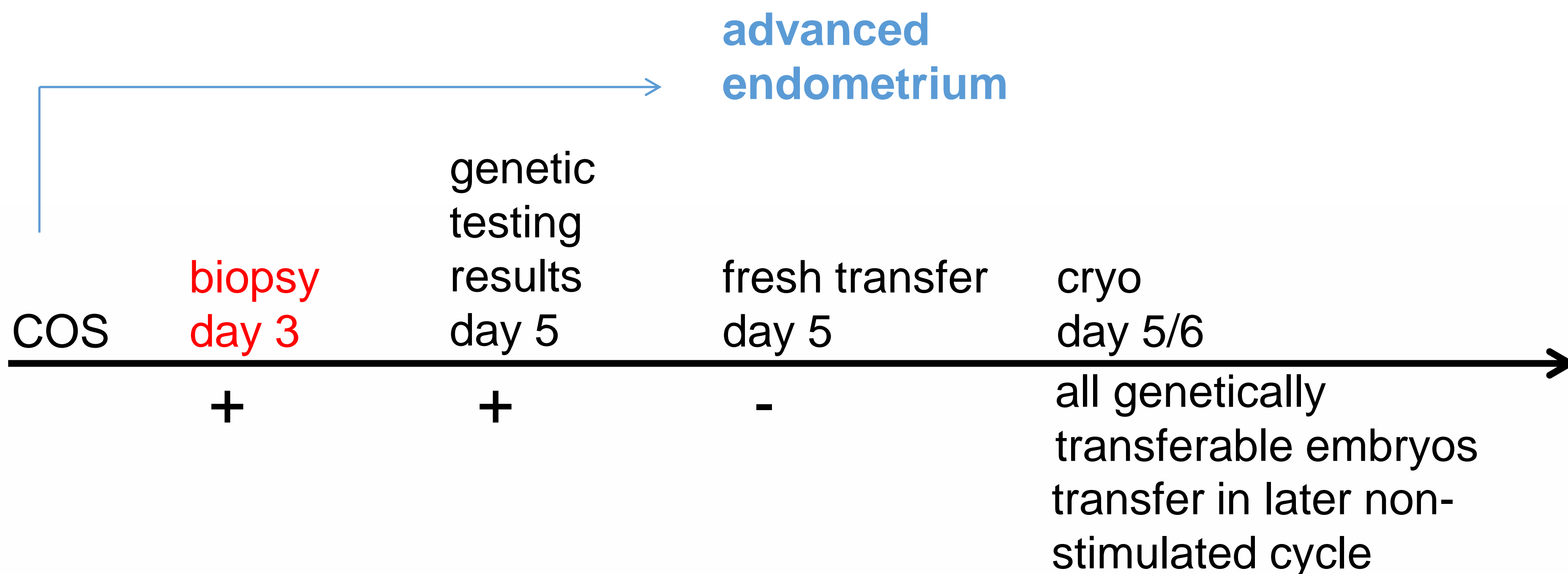
PGT cycle: 'fresh embryo transfer'



PGT cycle: ‘freeze all strategy’



PGT cycle: 'freeze all strategy'



better synchronicity between embryo and endometrium

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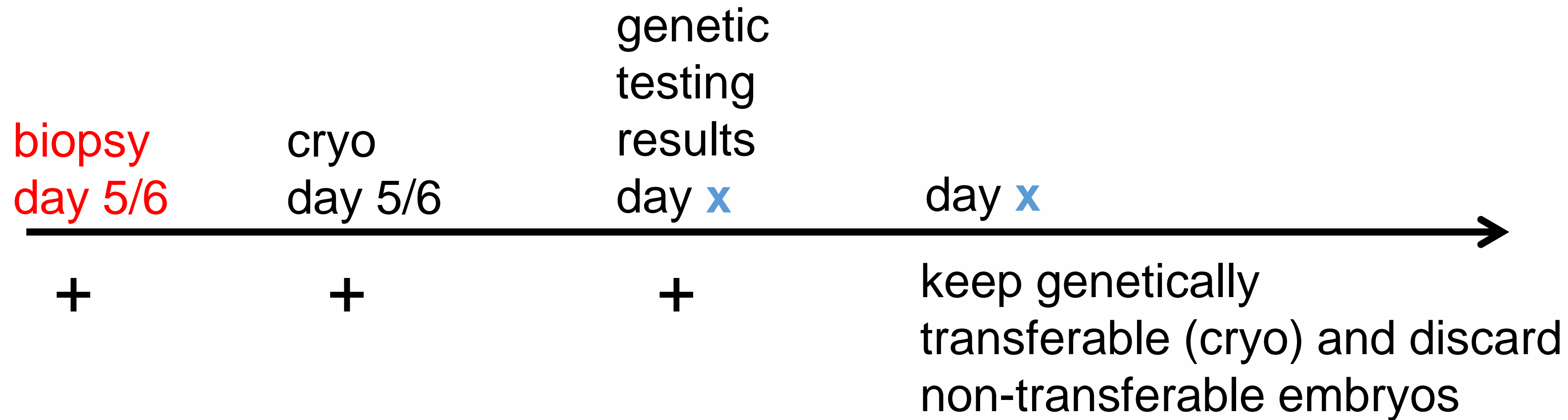
PGT cycle: ‘freeze all strategy’



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

- intake: molecular genetic report stating the disease-causing genetic variant

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

PGT and HLA matching

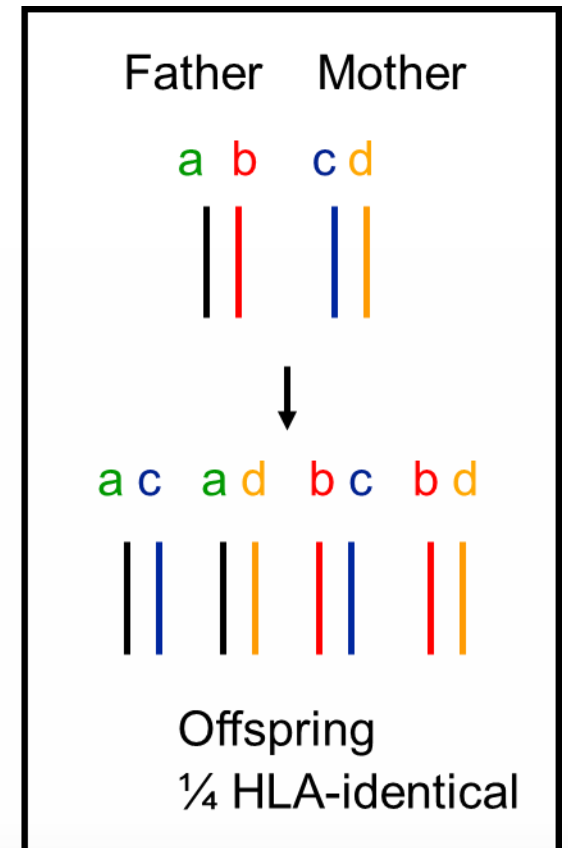
involves Human Leucocyte Antigen (HLA) typing of single/few cells biopsied from *in vitro* fertilized preimplantation embryos

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

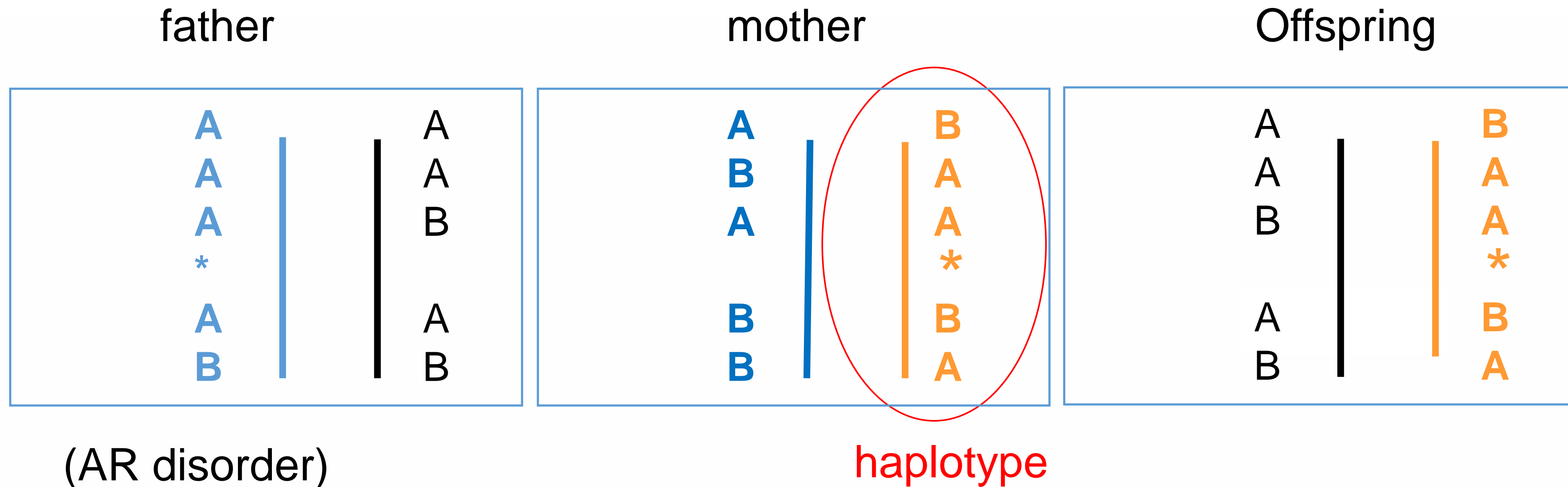


PGT and HLA matching

- 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-M: haplotyping

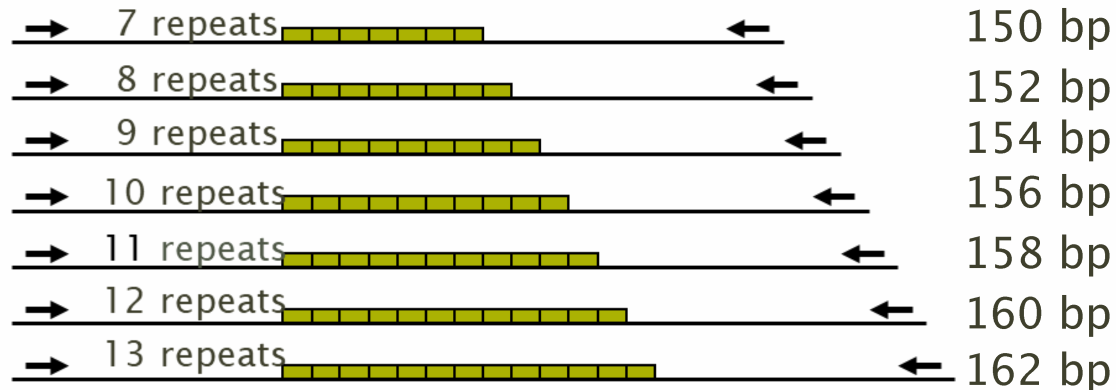
Principle: linkage-based haplotyping, alleles of genetic variant (*) and genetic markers (A,B,…) located near each other on the same chromosome are inherited together



PGT-M: genetic markers for haplotyping

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

ttactgccag ttggtccgct atctctgtca aaatggacgc tgcattccaa ctcctgggag
gagaaaaacc cctgtgctgg **cacacacaca cacacacaca** tggtgagtgt attaacaacc
agggttcgta cacctgtcag tgccgagctg gatatcagag cacactcacg cggacagaat



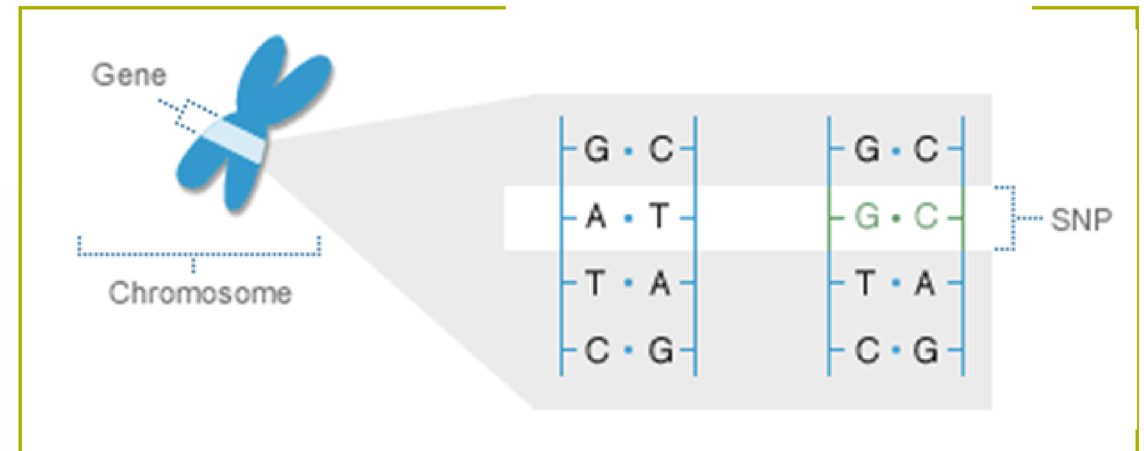
150 bp
152 bp
154 bp
156 bp
158 bp
160 bp
162 bp

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

PGT-M: genetic markers for haplotyping

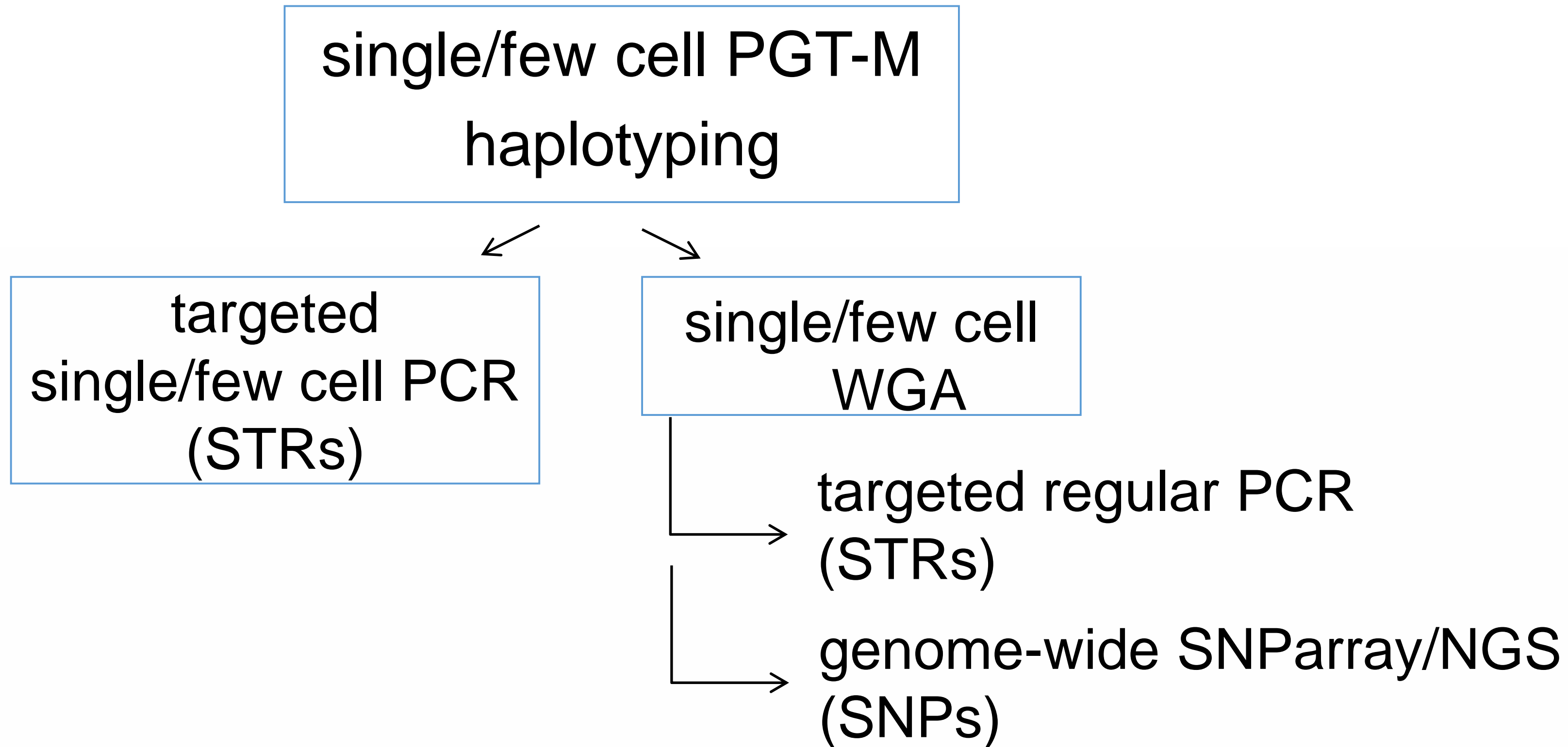
SNP = Single Nucleotide Polymorphism:

AGTCATGGGCAGCCTGTT
AGTCATGGACAGCCTGTT



substitution of 1bp, =normal variants
SNP -> two alleles
many present in the genome (about 1 common SNP every 300bp) (human genome = 3000.10^6 bp)

PGT-M: current diagnostic methods



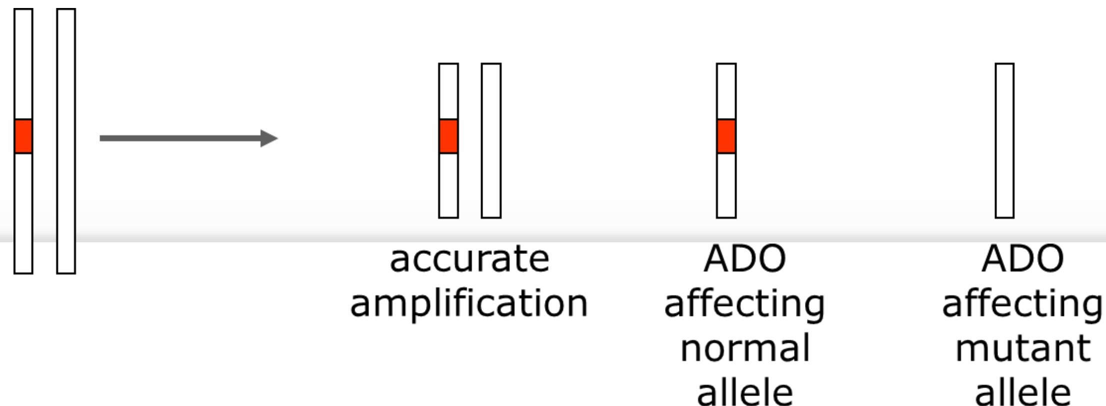
PGT-M: targeted multiplex PCR

- Regular PCR: 100-500 ng purified genomic DNA

- Single/few-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



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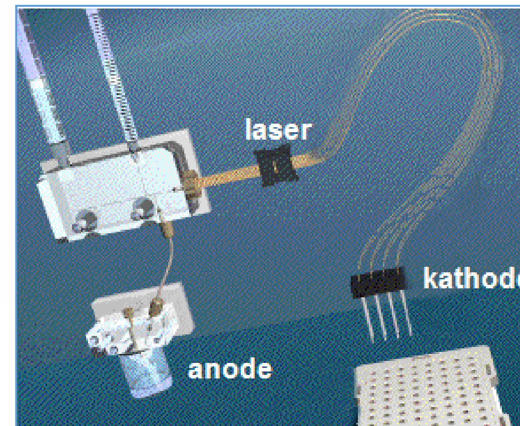
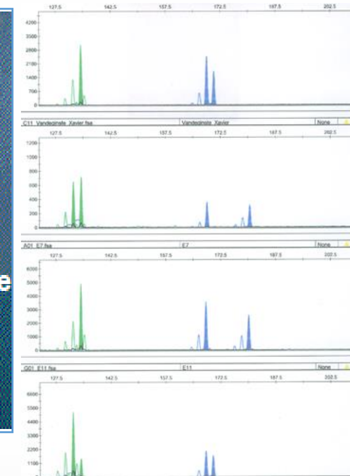
PGT-M: targeted multiplex PCR

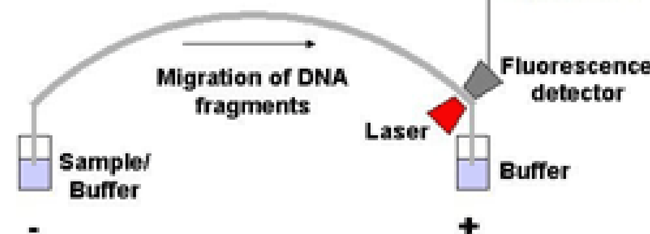
- thermal cycling reaction with fluorescently labelled primers: fragment length detection on automated sequencers (fragments up to 400 bp)
- simultaneous amplification of multiple loci per cell (flanking genetic markers +/- mutation locus)

=> more accurate: allows diagnosis AND reveals contamination & ADO

capillary
electrophoresis
5 fluorochromes

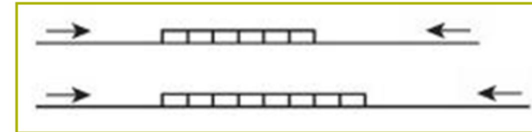






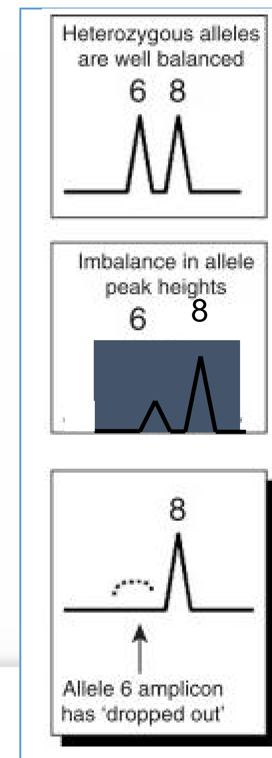
PGT-M: whole genome amplification

from 6 pg (single cell) to several µg of DNA



amplification problems =>

- bias
- allele drop-out (ADO) and preferential amplification (PA)
- amplification errors
- incomplete genome coverage



different protocols (PCR-based or MDA-based)

⇒ different downstream applications

PGT-M: MDA-based WGA

PGT-A/PGT-SR: PCR-based WGA

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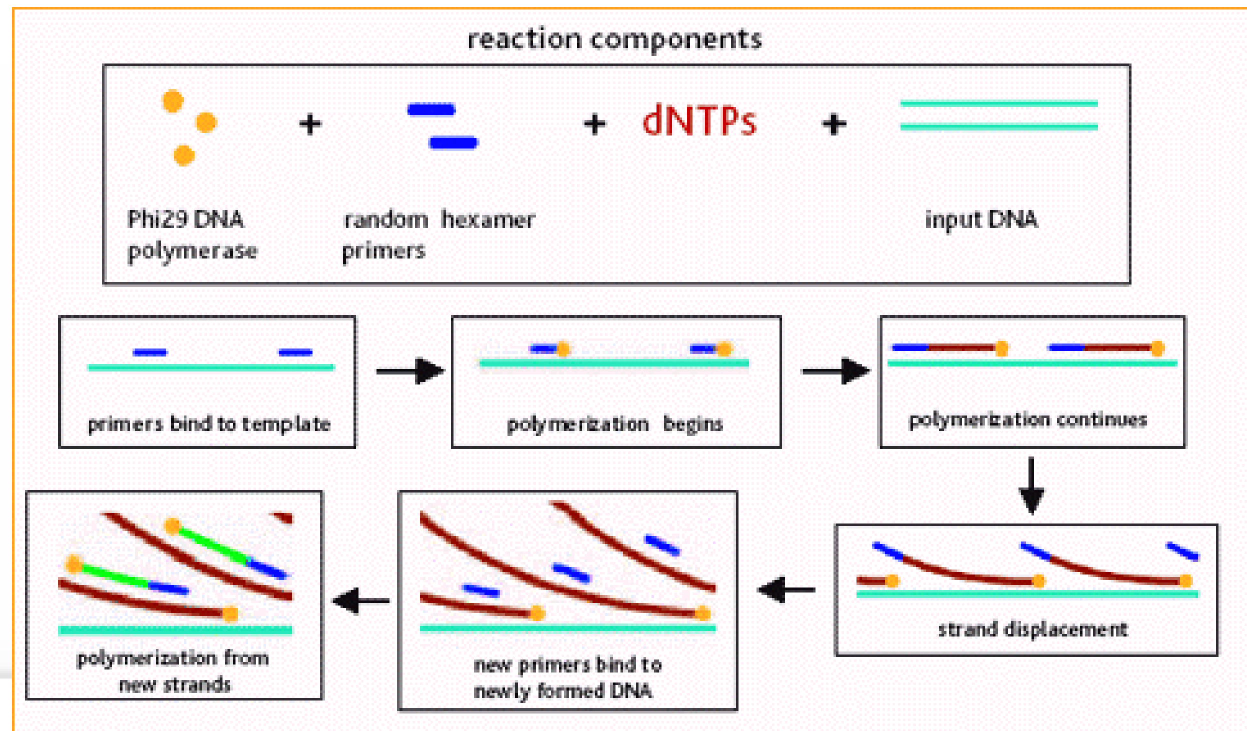
PGT-M: whole genome amplification

- Multiple Displacement Amplification, (MDA)
  isothermal amplification (30°C) => DNA fragments up to 70 kb, low error rates



Dean et al., 2002

PGT-M: whole genome amplification

- Multiple Displacement Amplification, (MDA)

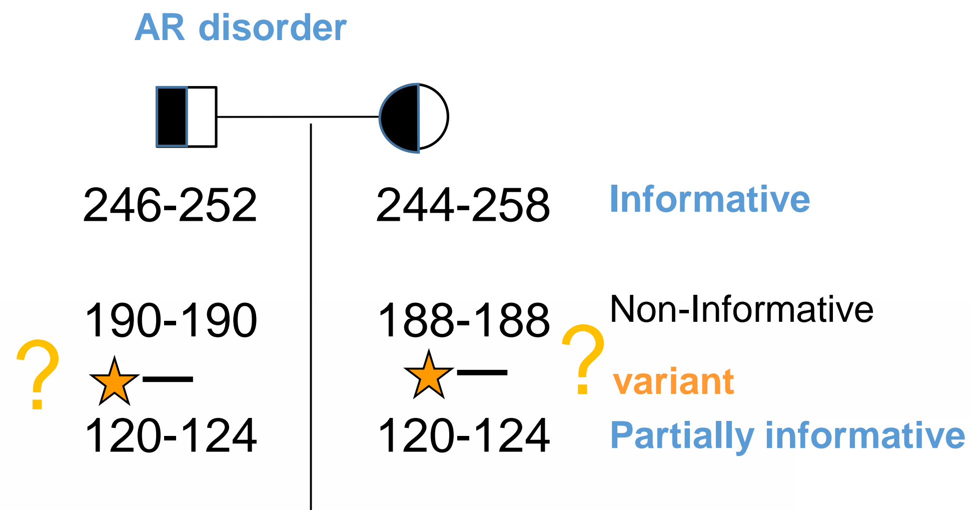
  - relative good genome coverage
    (except telomere and centromere repeats)

  -low error rates

  - amplification bias
    (random under or overrepresentation of sequences)

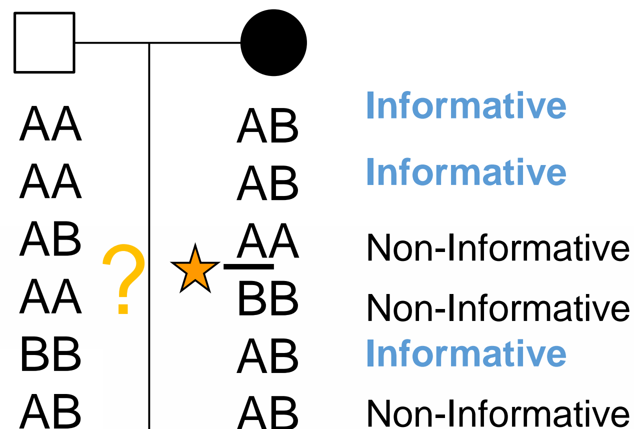
  - chimeric amplification products (branching)

PGT-M: genetic markers: preclinical workup



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

PGT-M: genetic markers: preclinical workup



variant

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

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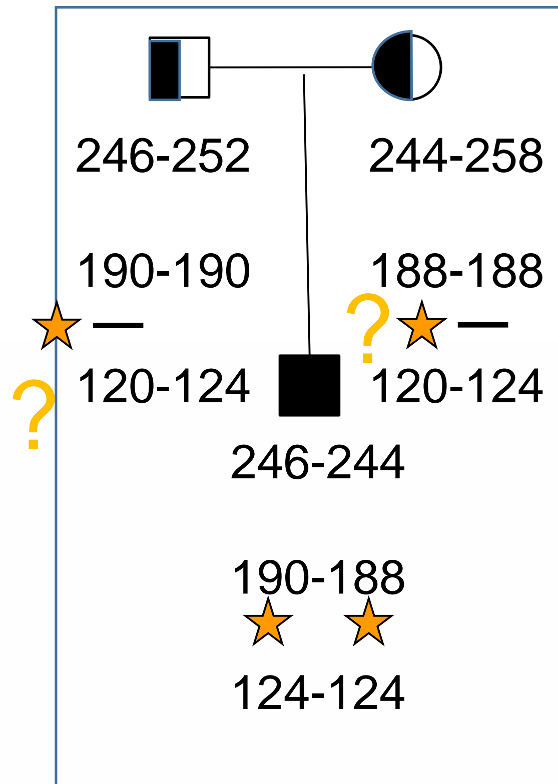
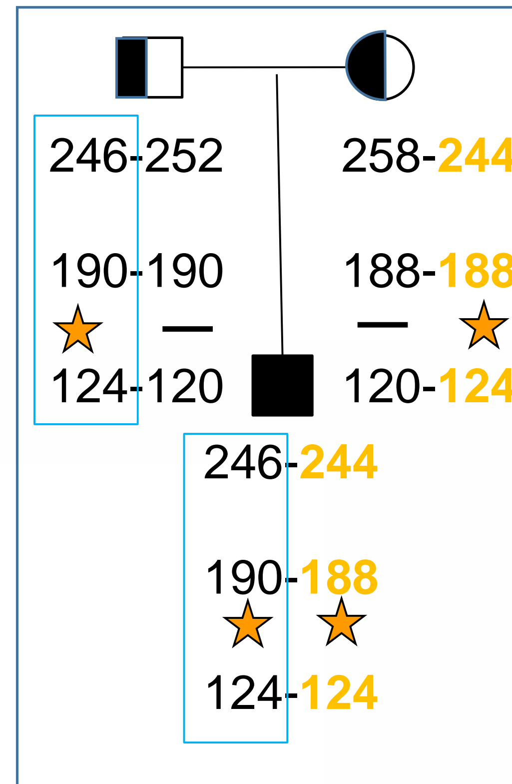
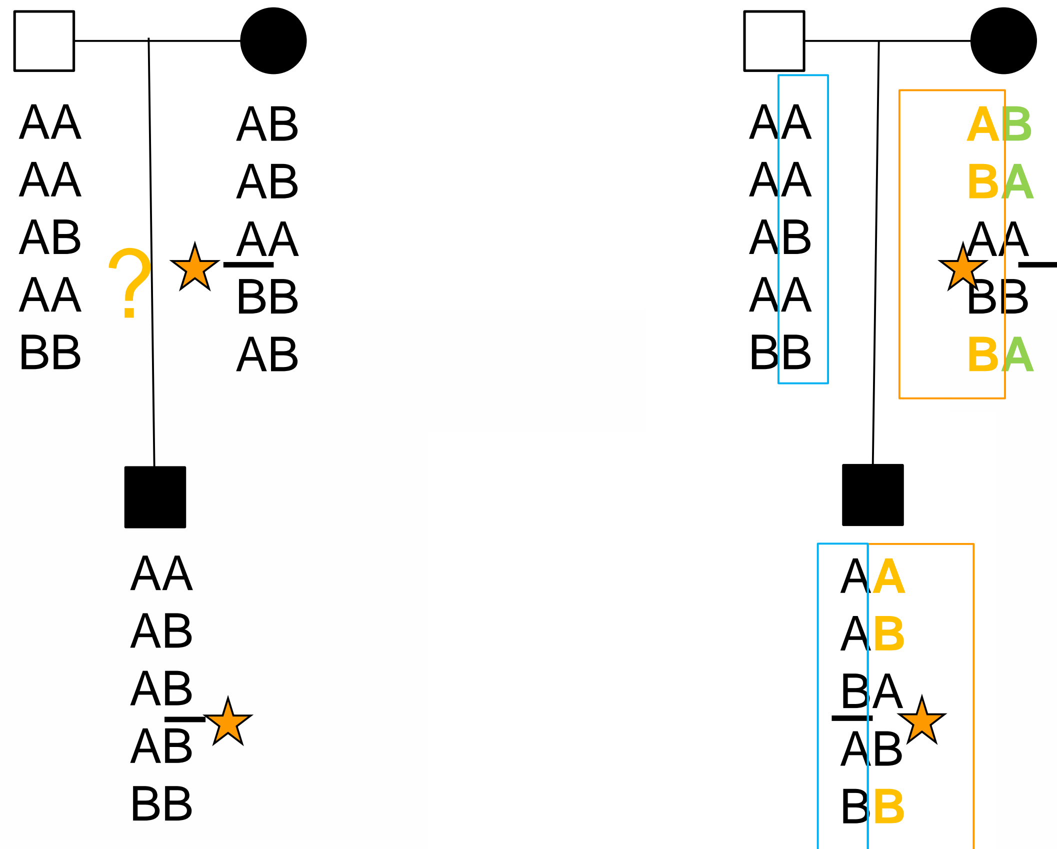
PGT-M: genetic markers: preclinical workup





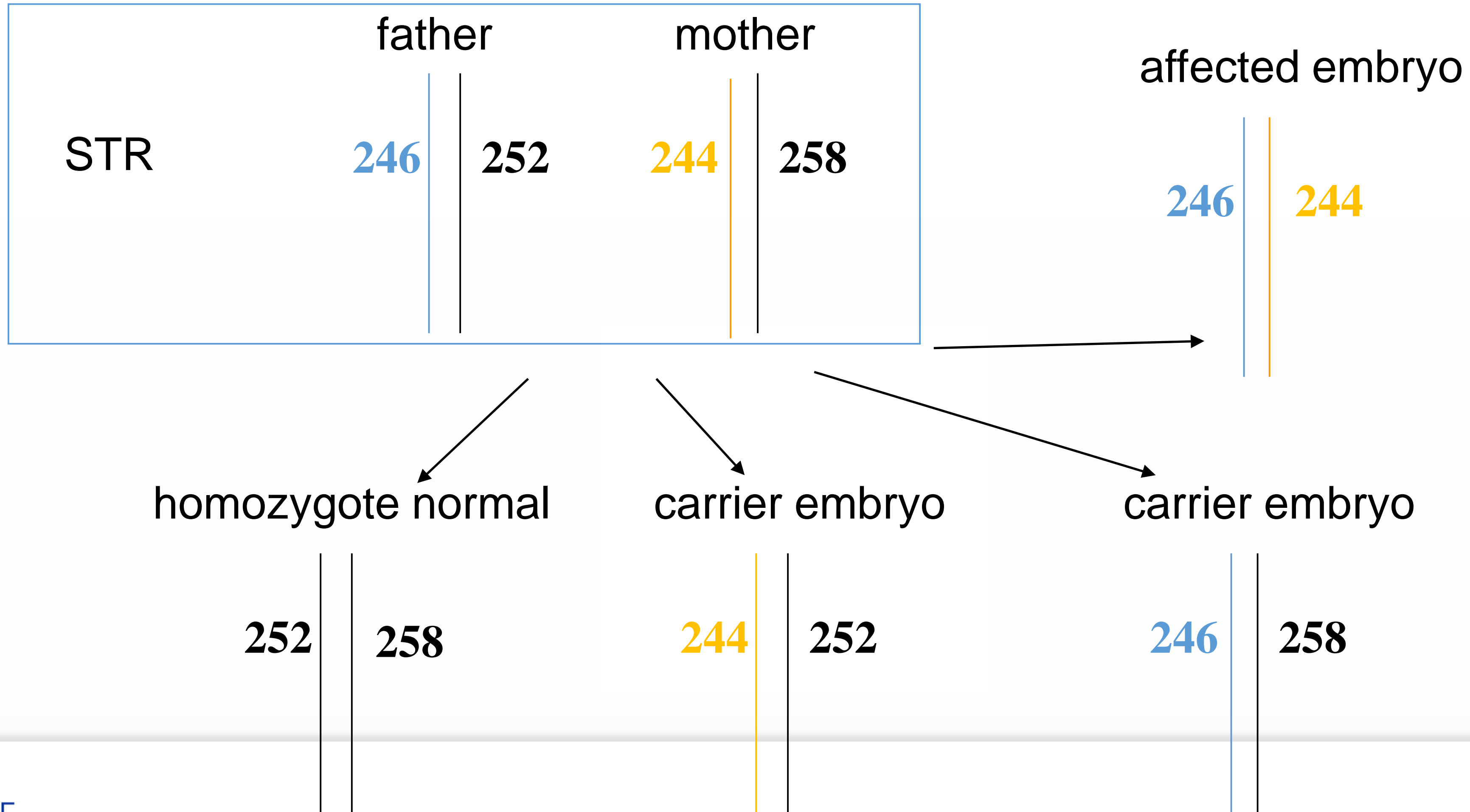
STR genotyping of family samples with known genetic status => establish parental haplotypes

PGT-M: genetic markers: preclinical workup



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

PGT-M: diagnosis



PGT-M: diagnosis

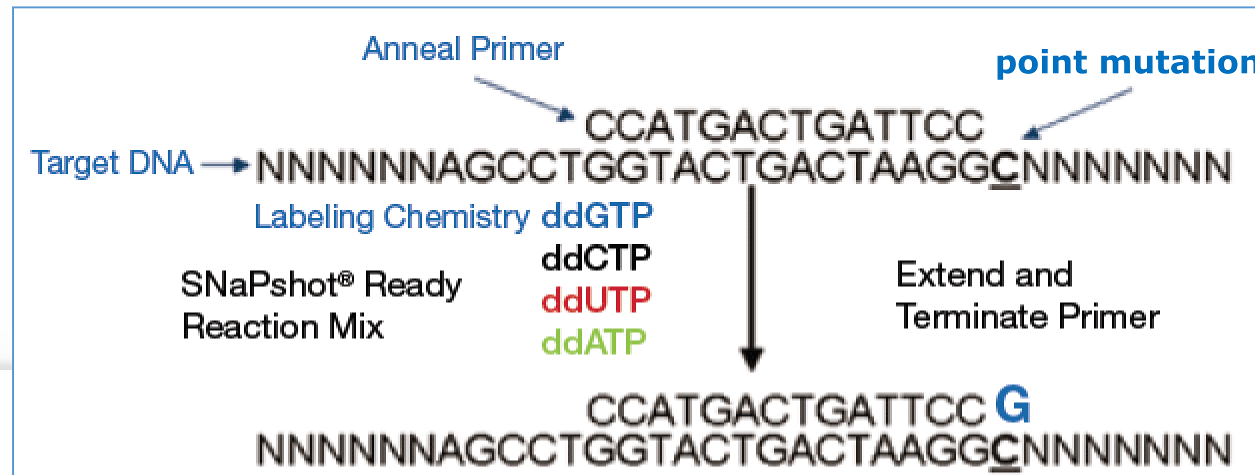
- indirect diagnosis: use of genetic markers that flank the locus/gene of interest (within 1-2 Mb)
  more markers at either side make the test more robust
  - when mutation analysis is not possible
    (region too large for amplification, pseudogene interference, …)
  - limiting factor: informative couples with family history and requirement for samples form affected family members

no family samples/*de novo* mutation?

PGT-M: diagnosis

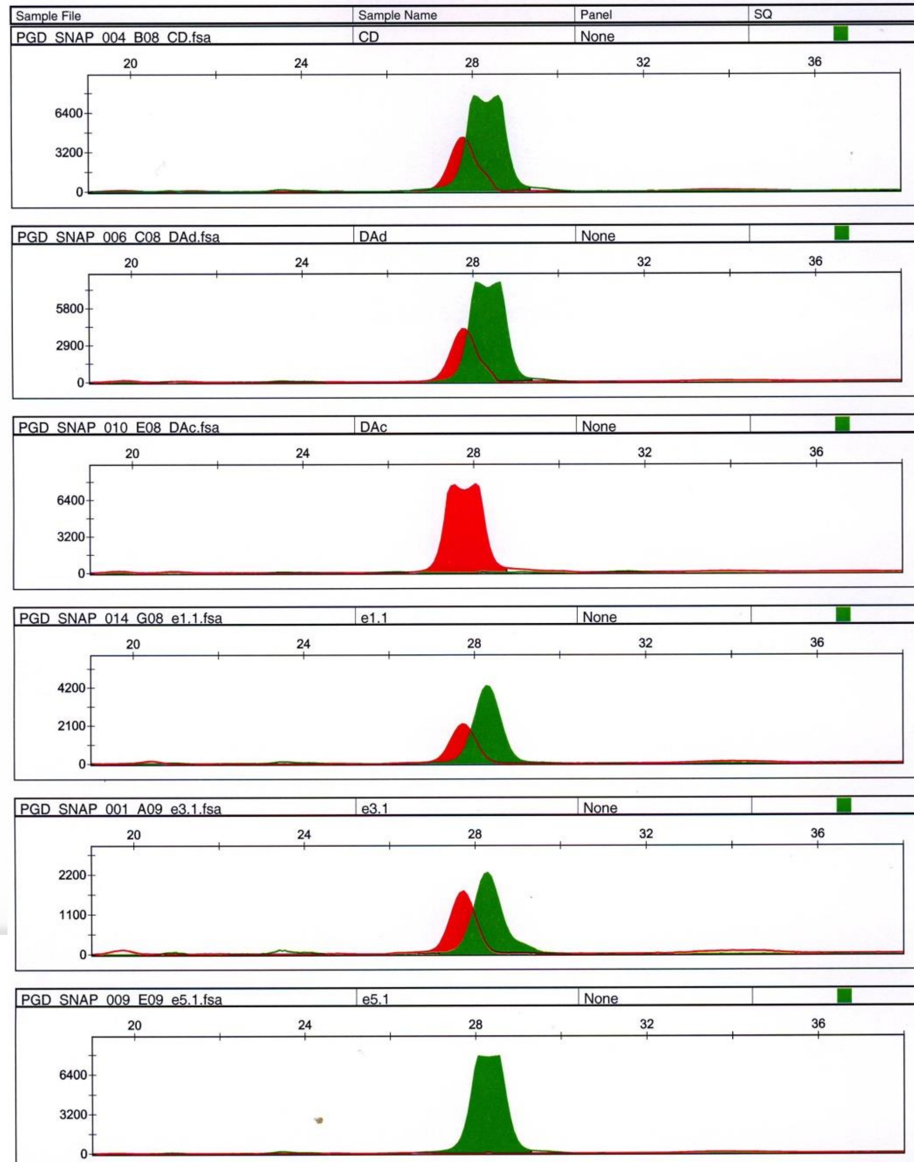
direct diagnosis: add mutation detection to STRs/SNPs and establish haplotypes during PGD cycle(s), based on affected embryos (only for point mutations, small dups and dels)

- larger dups and dels => characterization of start/endpoints > breakpoint PCR
- small dups and dels => differences in fragment length
- pointmutations => post-PCR reactions for further allele discrimination
  - → restriction enzyme analysis, ARMS, minisequencing….



PGT-M: diagnosis

c.20A>T
of sickle cell
mutation
in *HBB*



genomicDNA of carrier mother

genomic DNA of carrier father

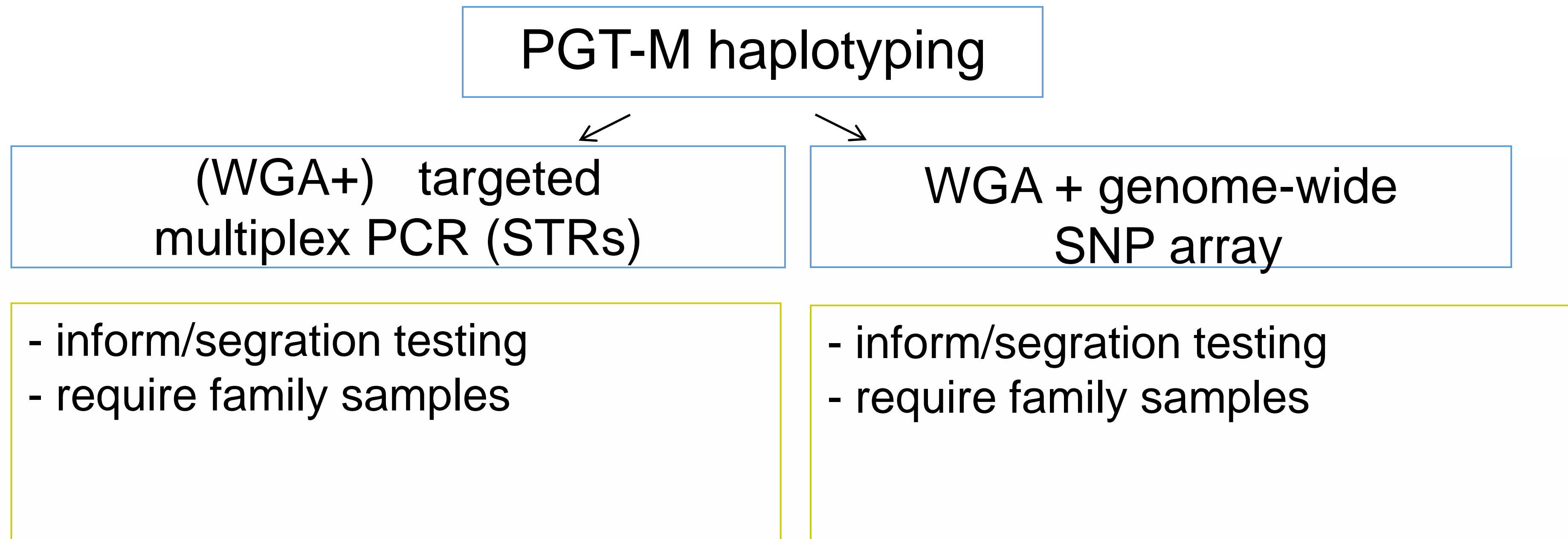
genomic DNA of affected child

cell from a carrier embryo (E1)

cell from a carrier embryo (E3)

cell from homozygous normal embryo (E5)

PGT-M: targeted vs genome-wide testing



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PGT-M: targeted vs genome-wide testing

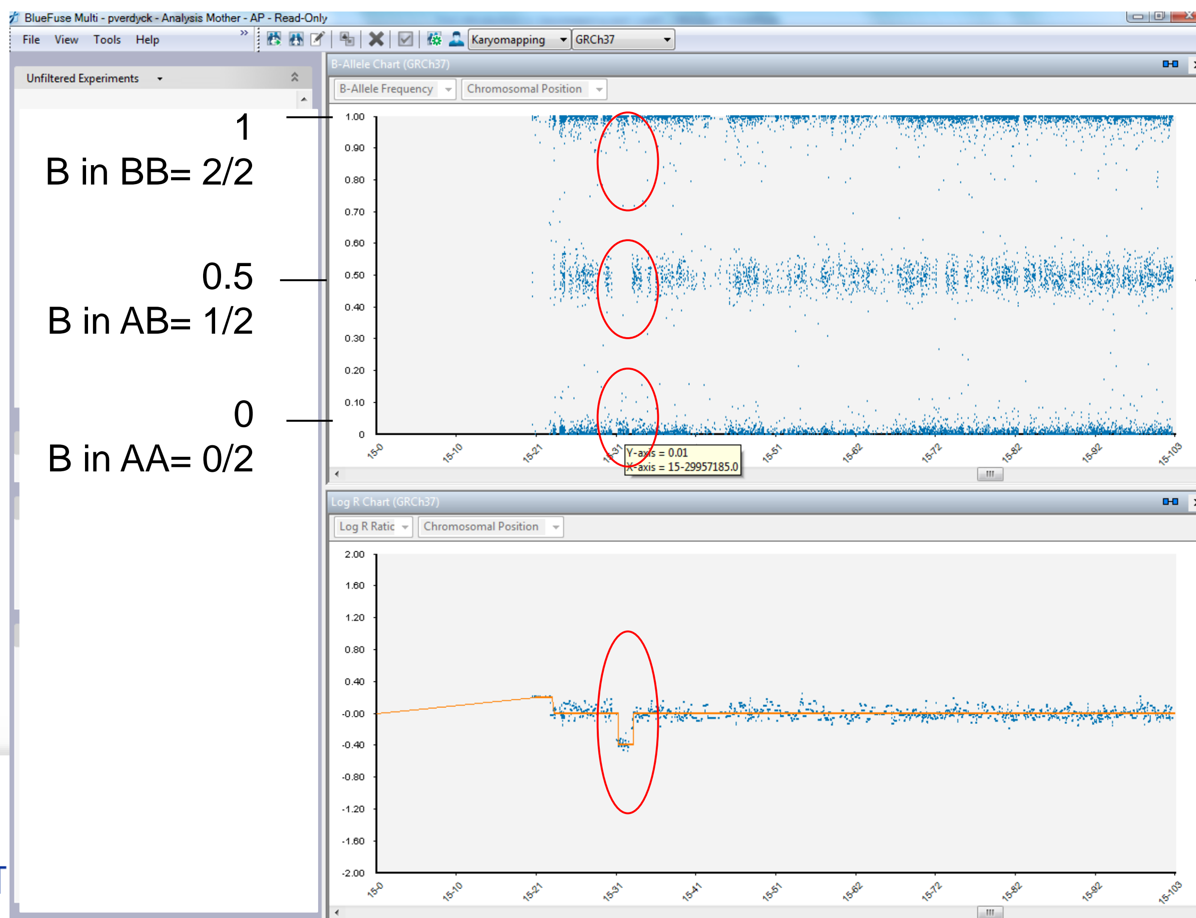
PGT-M haplotyping

(WGA+) targeted multiplex PCR (STRs)	WGA + genome-wide SNP array/NGS
- customized test	- standardized workflow
- cheap consumables	- expensive equipment/consumables (pooling of samples)
- development/validation per target WGA + regular PCR: acceptable single cell PCR: time consuming, high personnel cost	- implementation validation (1x)
	- double indications - info on chromosomal copy number (PGT-A)

PGT-M+PGT-A:SNParray/NGS

haplotypes + chromosomal copy number

BAF = colour



BB

AB

AA

Log_2 R = intensity

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PGT-M+PGT-A:SNParray/NGS

combine haplotyping and chromosomal copy number

data interpretation: well-developed algorithms

Illumina karyomapping software: restrictions in detecting copy number aberrations (PGT-A is not a supported application)

extending SNP array/NGS with additional algorithms or in-house algorithms (haplarithmisis from Zamani *et al*, 2015; APCAP from Verdyck et al, 2022)