# Manama course in Clinical Genetics - day 6
# Preconception Genetics, PGD, Reproductive Genetics

**Eftychia Dimitriadou, PhD**
**Reproductive Genetics Unit**
**eftychia.dimitriadou@uzleuven.be**

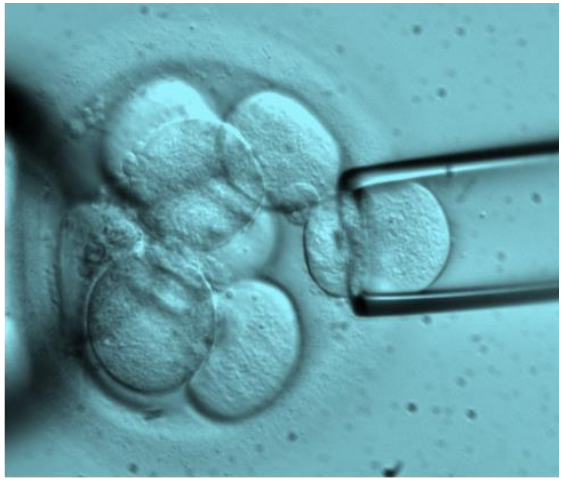
**March 7th 2023**

# Pre-implantation genetic testing

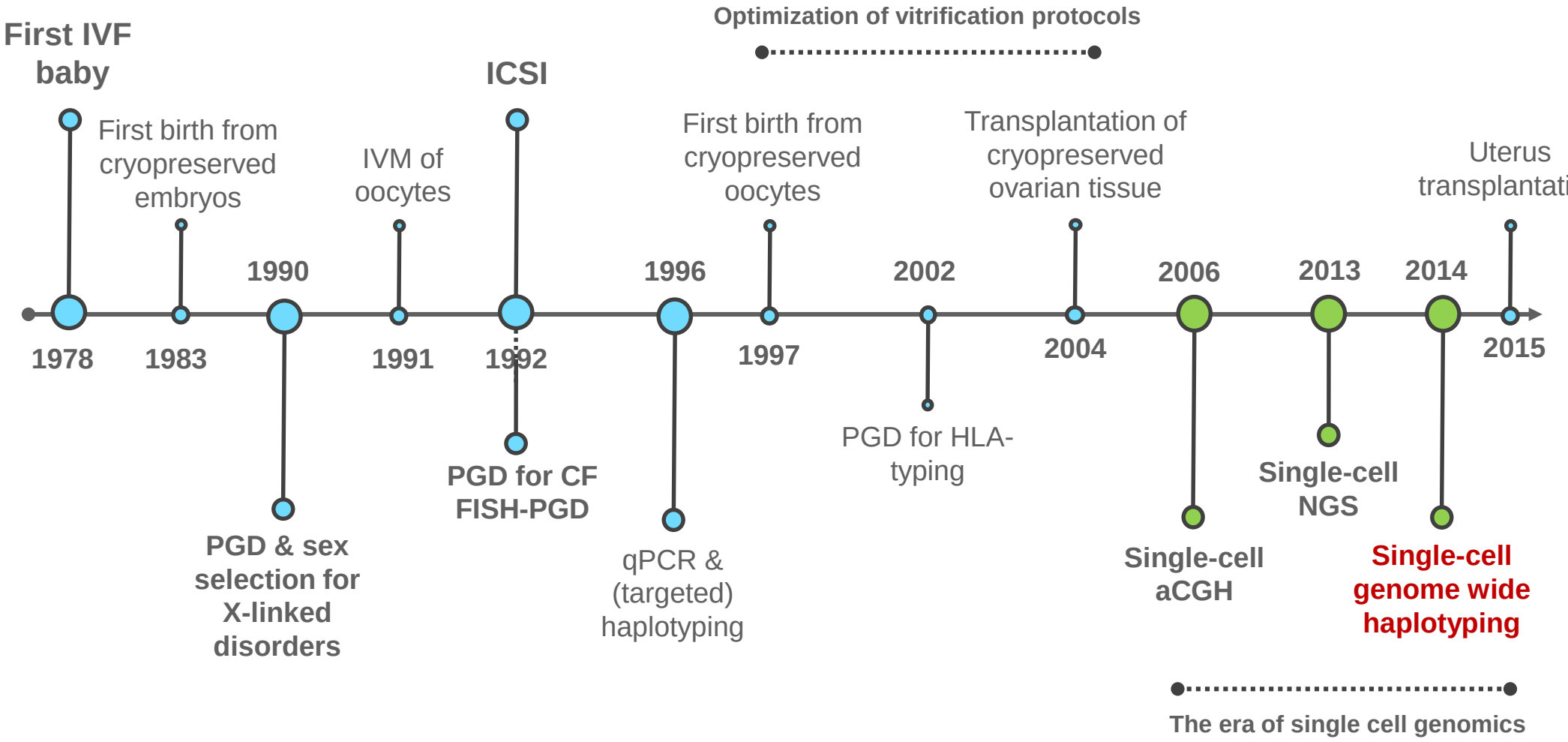
PGT

- Aim of PGT: detection of genetic abnormalities in embryos before they are transferred to the uterus

- MAR (mostly ICSI) required
  - Embryo biopsy (D3 or D5/6)
  - Genetic analysis
  - Transfer of unaffected embryos



# 40+ years of assisted reproduction



# Types of PGT based on indication

- PGT-SR — PGT for chromosomal structural rearrangements
- PGT-M — PGT for monogenic disorders
- PGT-A — PGT for aneuploidy
- Coprehensive/Universal PGT — PGT-M/SR+A

The international glossary on infertility and fertility care. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, Rienzi L, Sunde A, Schmidt L, Cooke ID et al. Hum Reprod 2017;32:1786–1801

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

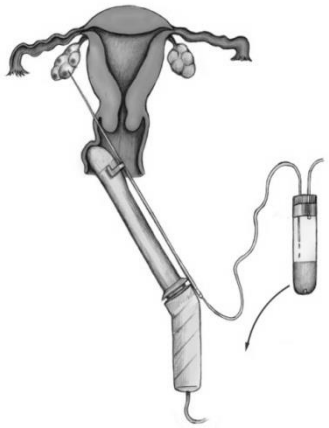
https://pgdis.org/

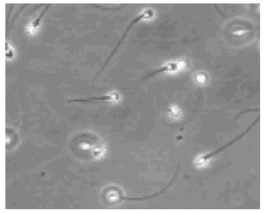
eshre
SCIENCE MOVING PEOPLE MOVING SCIENCE

European Society of **Human Reproduction and Embryology**

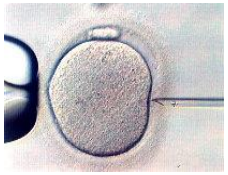
https://www.eshre.eu/

# MAR cyclus



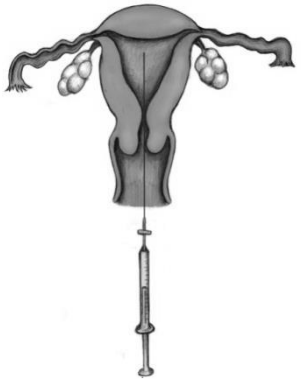


gametes



IVF/ICSI

Cryopreservation

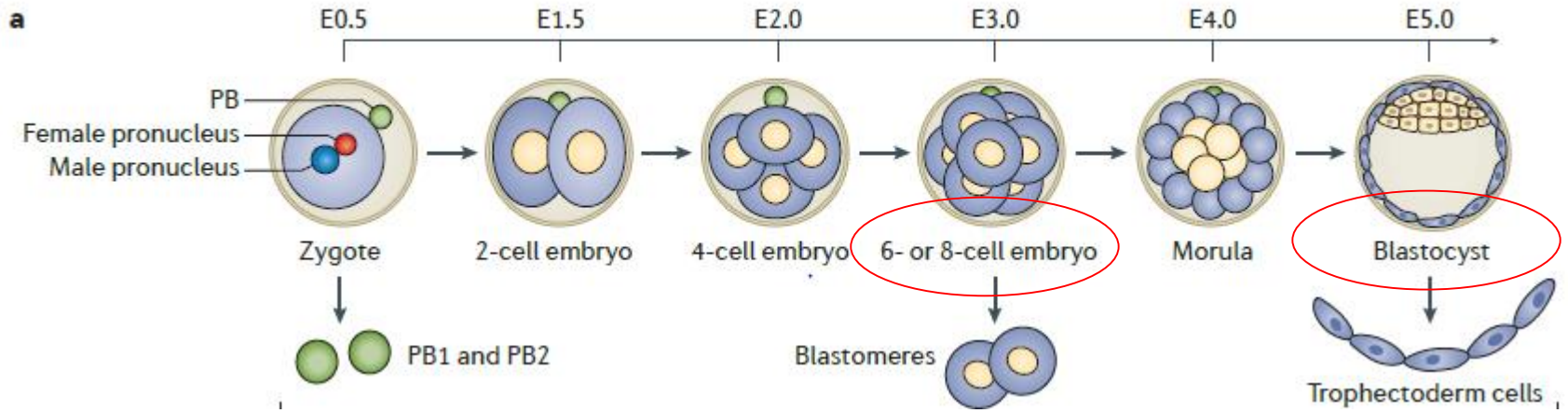


Embryo transfer

Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 5/6

Embryo development and evaluation

# Timing of embryo biopsy

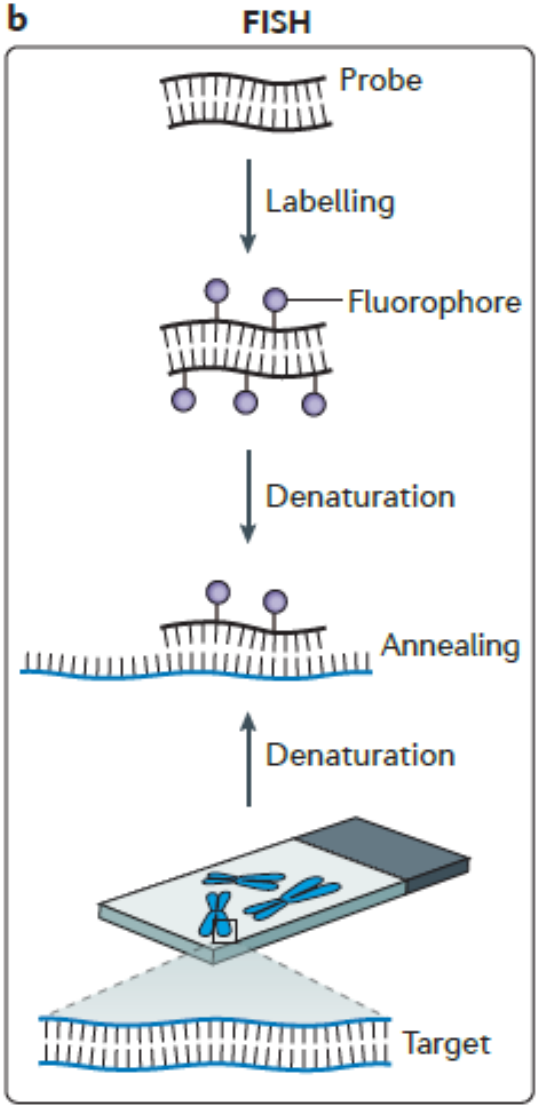



*Prenatal and pre-implantation genetic diagnosis, Vermeesch JR, et al. Nat Rev Genet. 2016 Sep*

# Techniques

- FISH
- PCR-based
- Array CGH
- Low-pass short read sequencing (GW)
- SNP array-based
- Reduced genome representation - Genotyping by sequencing

# FISH

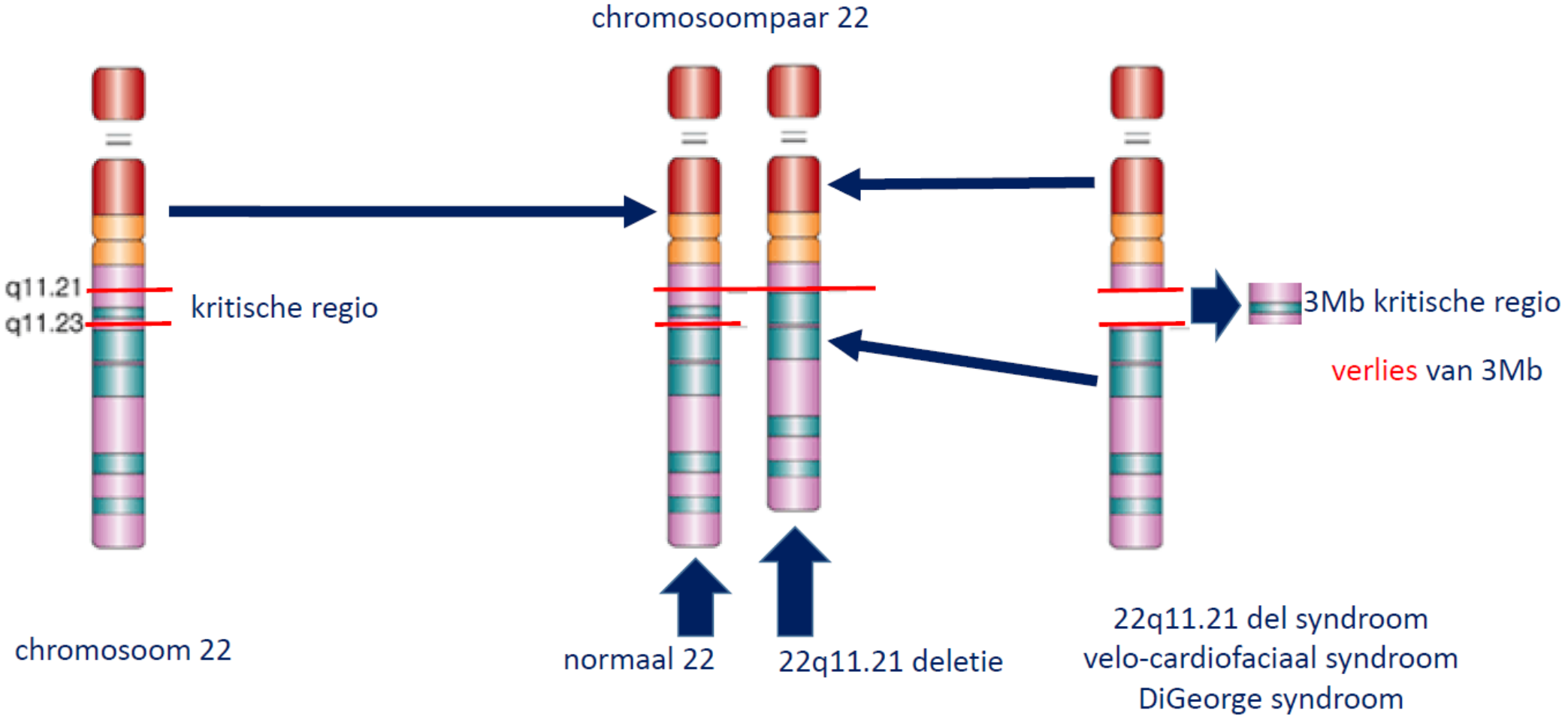



*Prenatal and pre-implantation genetic diagnosis, Vermeesch JR, et al. Nat Rev Genet. 2016 Sep*

# PGT via FISH: indications

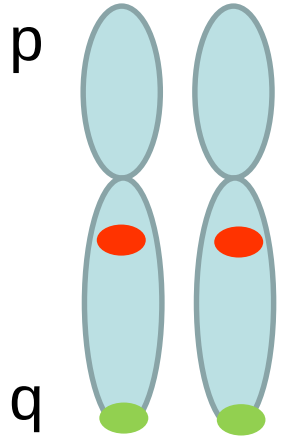
- Sex selection (X-linked or Y-linked disorders)
- Microdeletions
- Translocations

# microdeletion

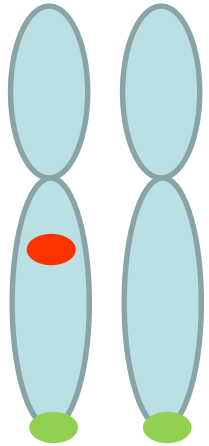


# PGT via FISH voor microdeletion syndromes

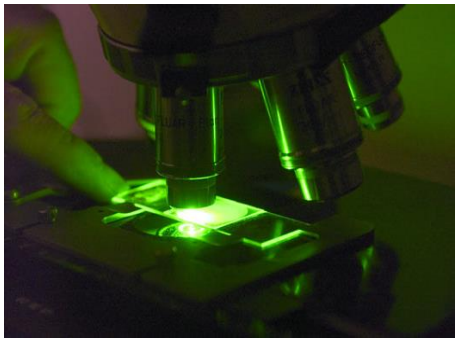
**NF1 gene**
**17q11**

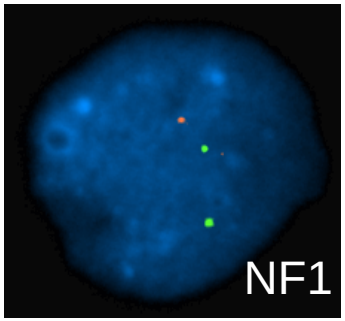


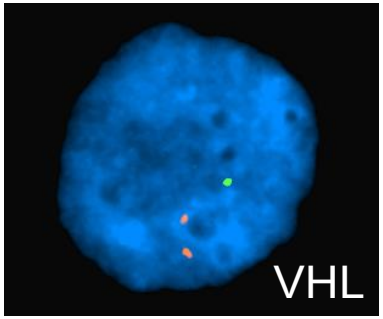

Normal
2 copies

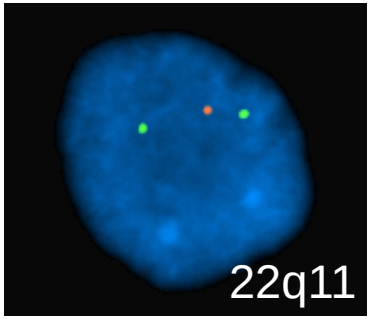


Affected
1 copy = deletion

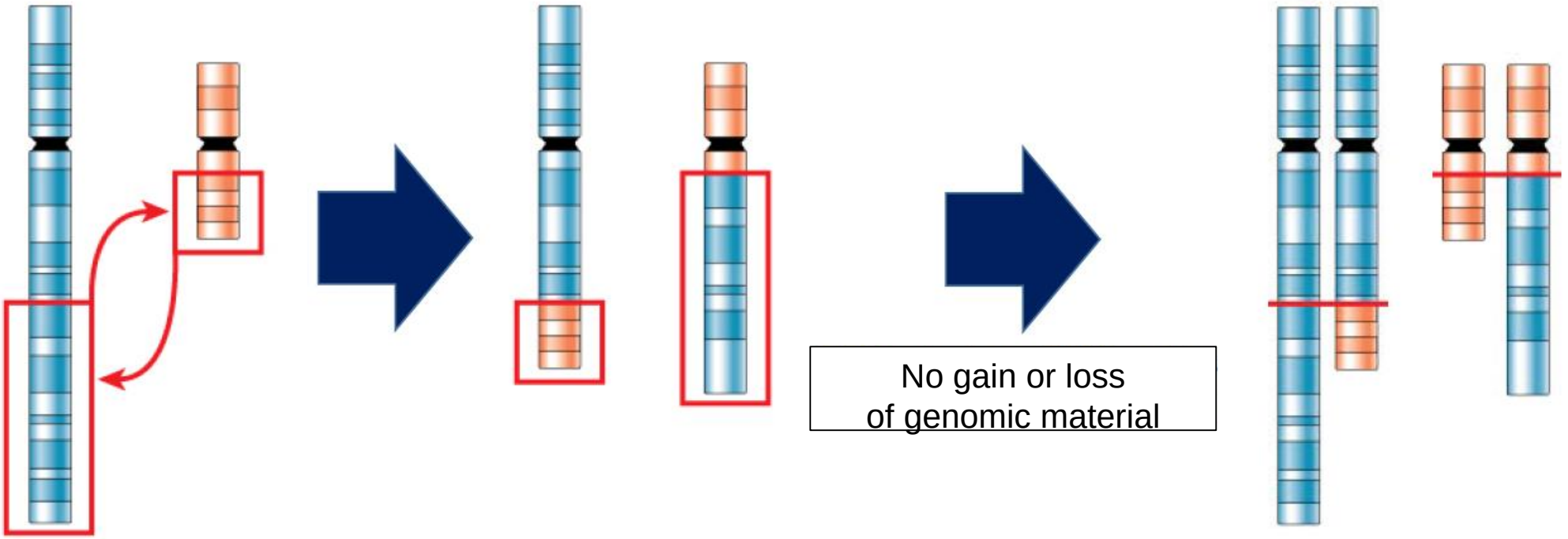




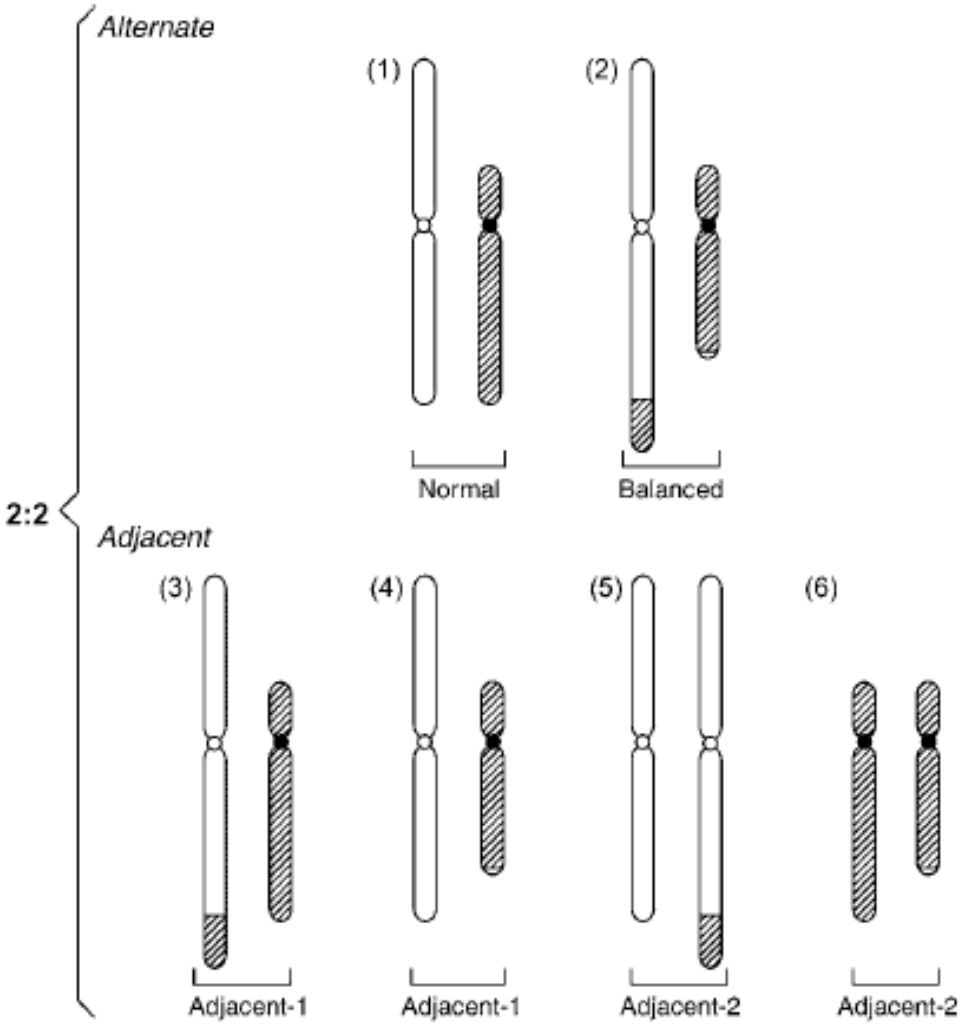



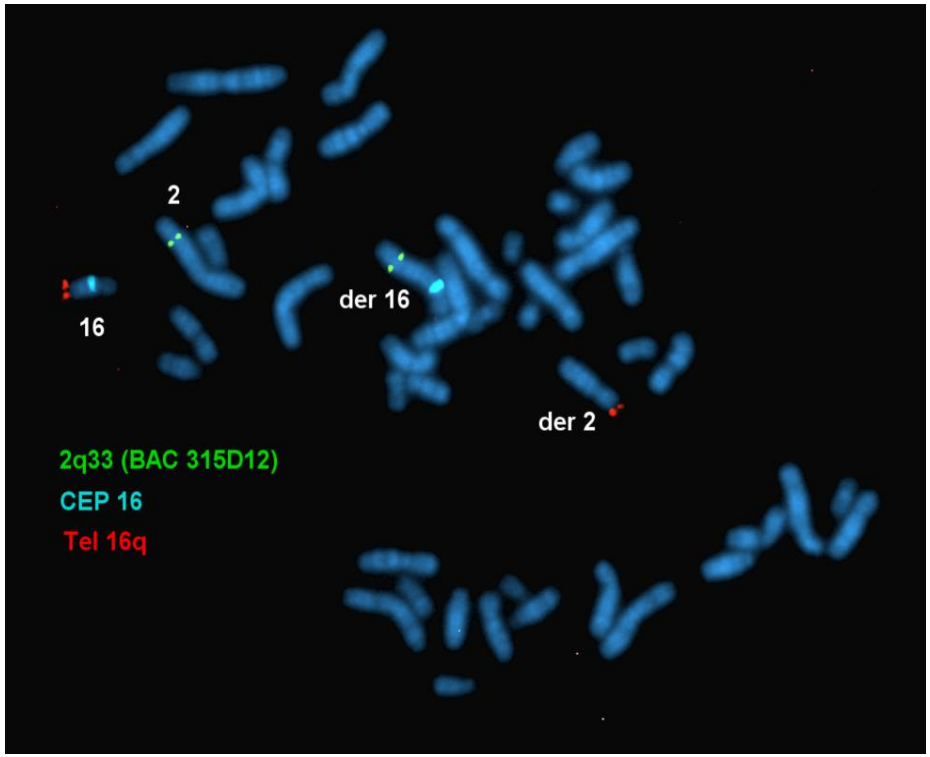
# Reciprocal translocation



# The full range of segregant gametes

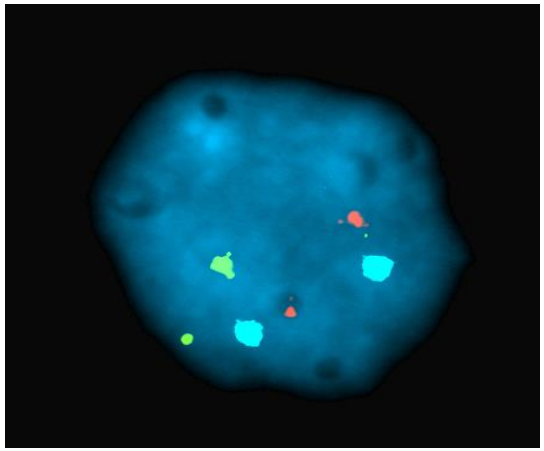


# PGT via FISH for reciprocal translocations

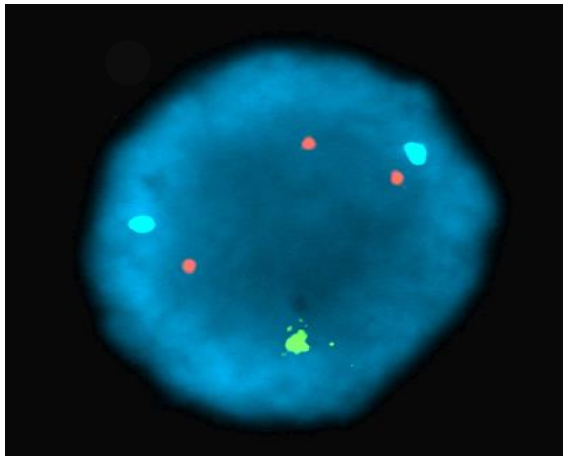



Pre-PGT: bloodsample of the prospactive parents:

- Detection of the balanced translocation (2q; 16q)
- Optimisation of FISH probe combination for PGT

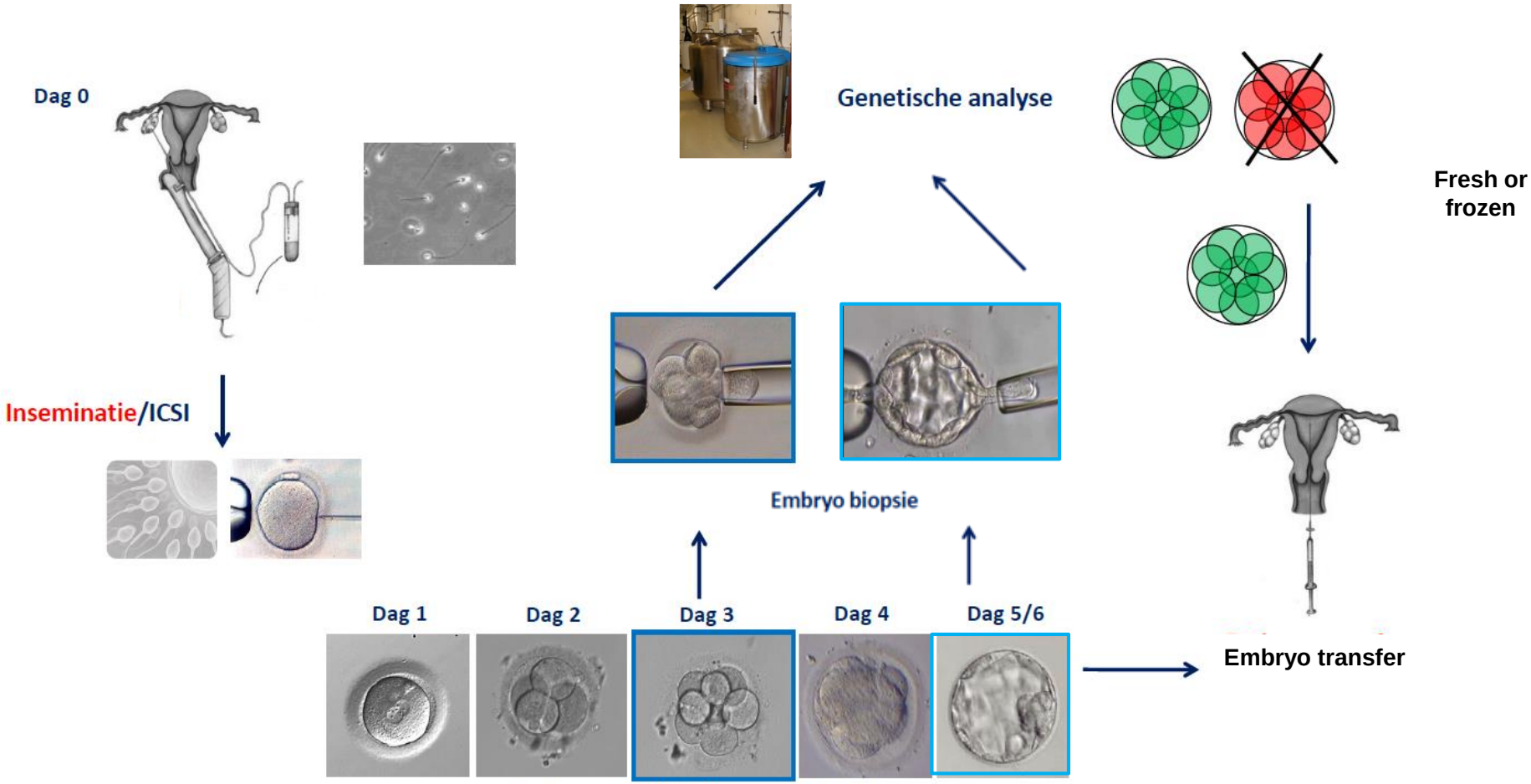


PGT: blastomere with normal diploid result for 2q and 16q



PGT: blastomere with loss of 2q and gain of 16q

# Schema: procedure PGT via FISH

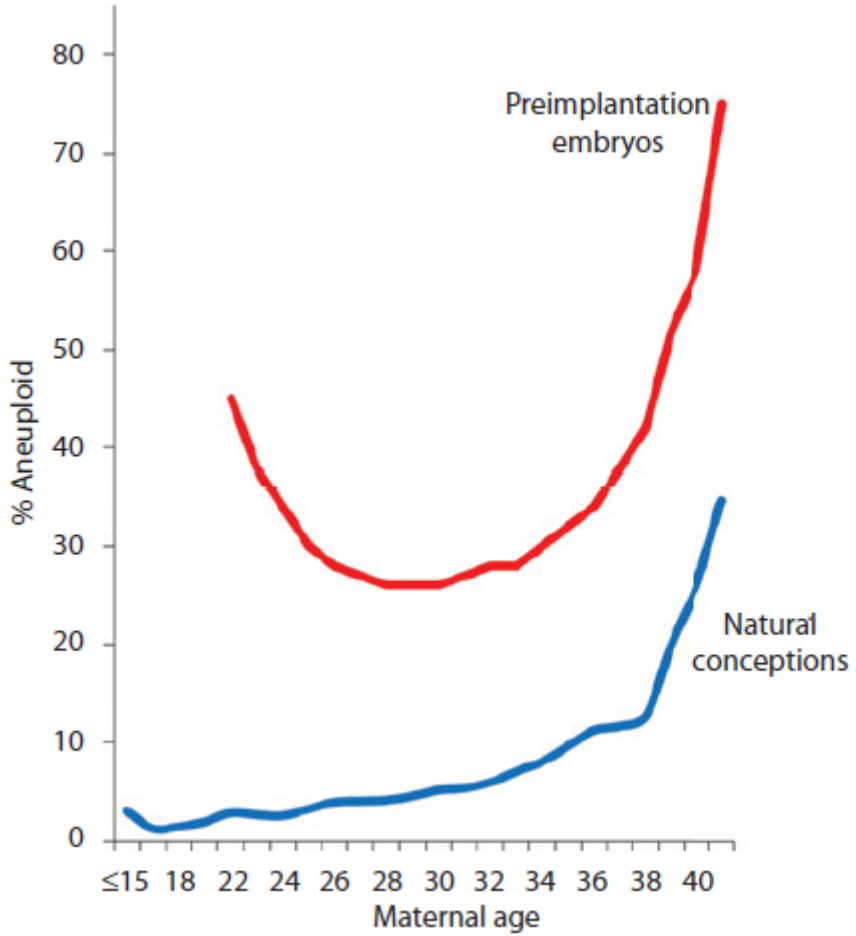


# Pros and cons of PGT-SR via FISH

+ Suitable for detecting minor chromosomal abnormalities
+ Result available within 24h/48h

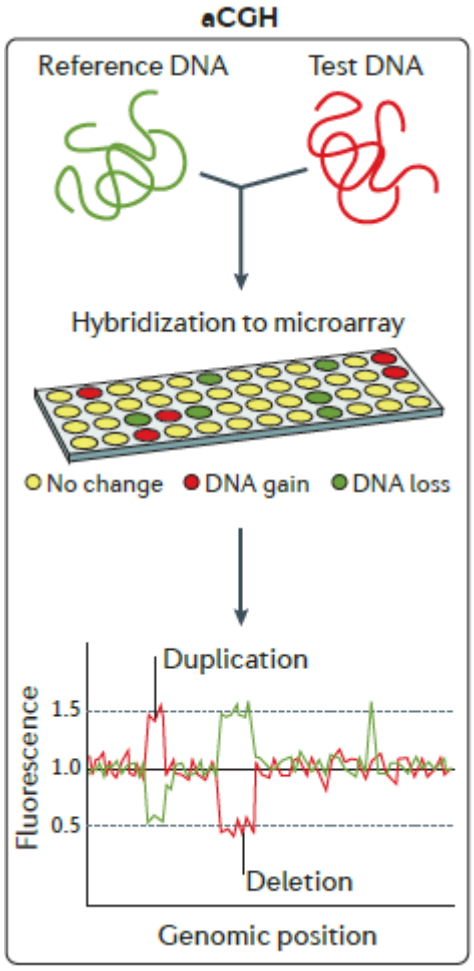
- Development of a family-specific test – pre-PGT optimisation necessary
- Restricted number of chromosomal regions can be ‘interogated’ per experiment (~ availibility of fluorochromes )
- In case of D3 biopsy, 2 cells for a relible result (~ technical artifacts)
- GW view missing: no information on other chromosomes / possible aneuploidies

# Risk on aneuploidy increases with maternal age

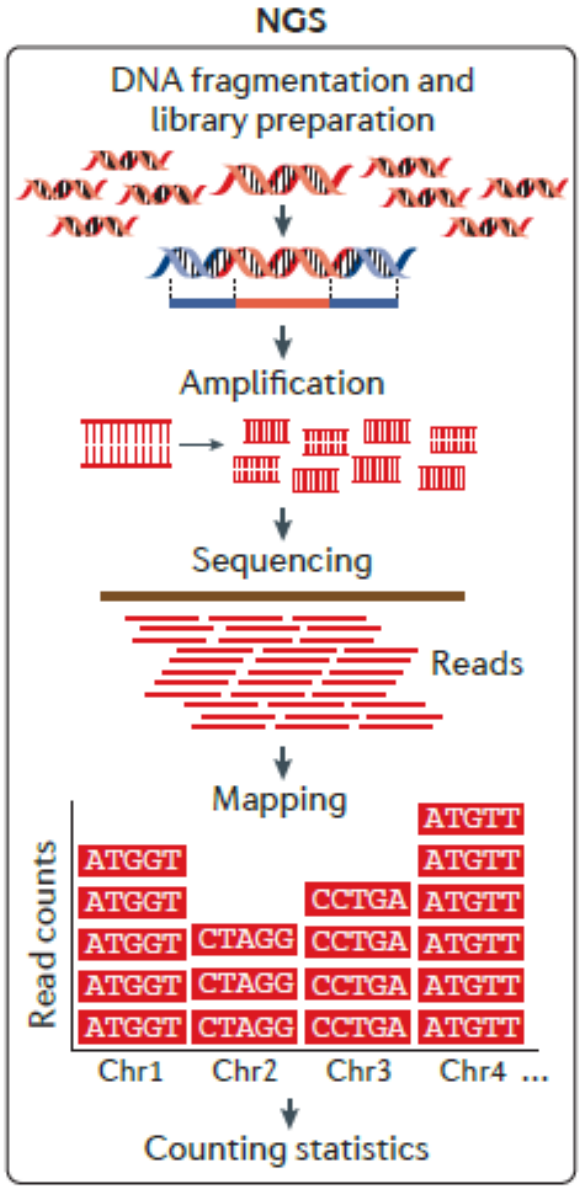



*Handbook of New Genetic Diagnostic Technologies in Reproductive Medicine, C. Simón and C. Rubio, 2018*

# Array CGH

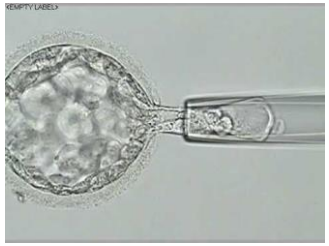

aCGH
Reference DNA
Test DNA
Hybridization to microarray
No change
DNA gain
DNA loss
Duplication
Fluorescence
1.5
1.0
0.5
Deletion
Genomic position

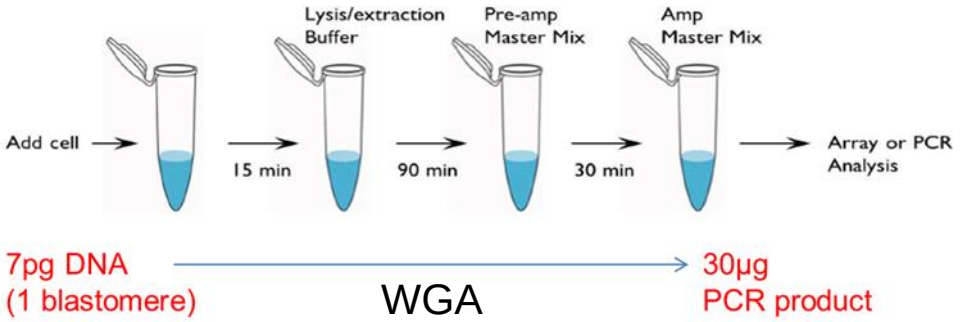
# Low pass short read sequencing

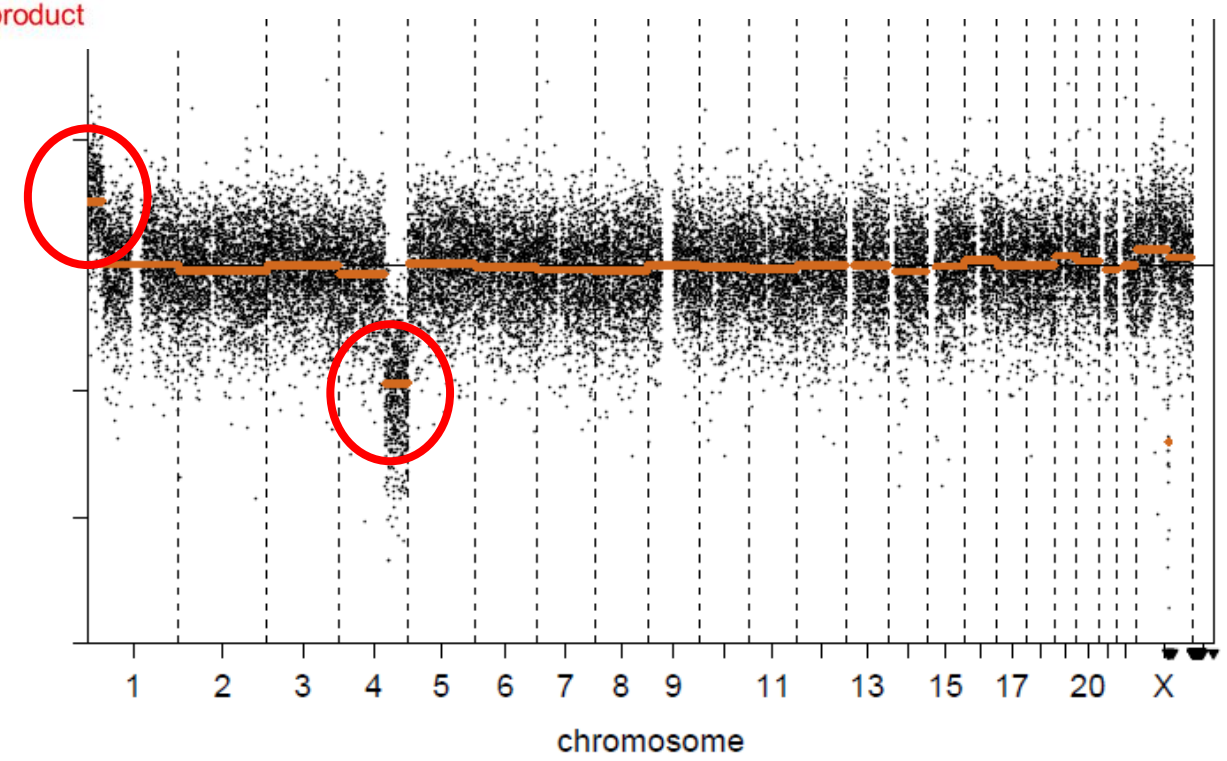


# PGT-SR via LPS

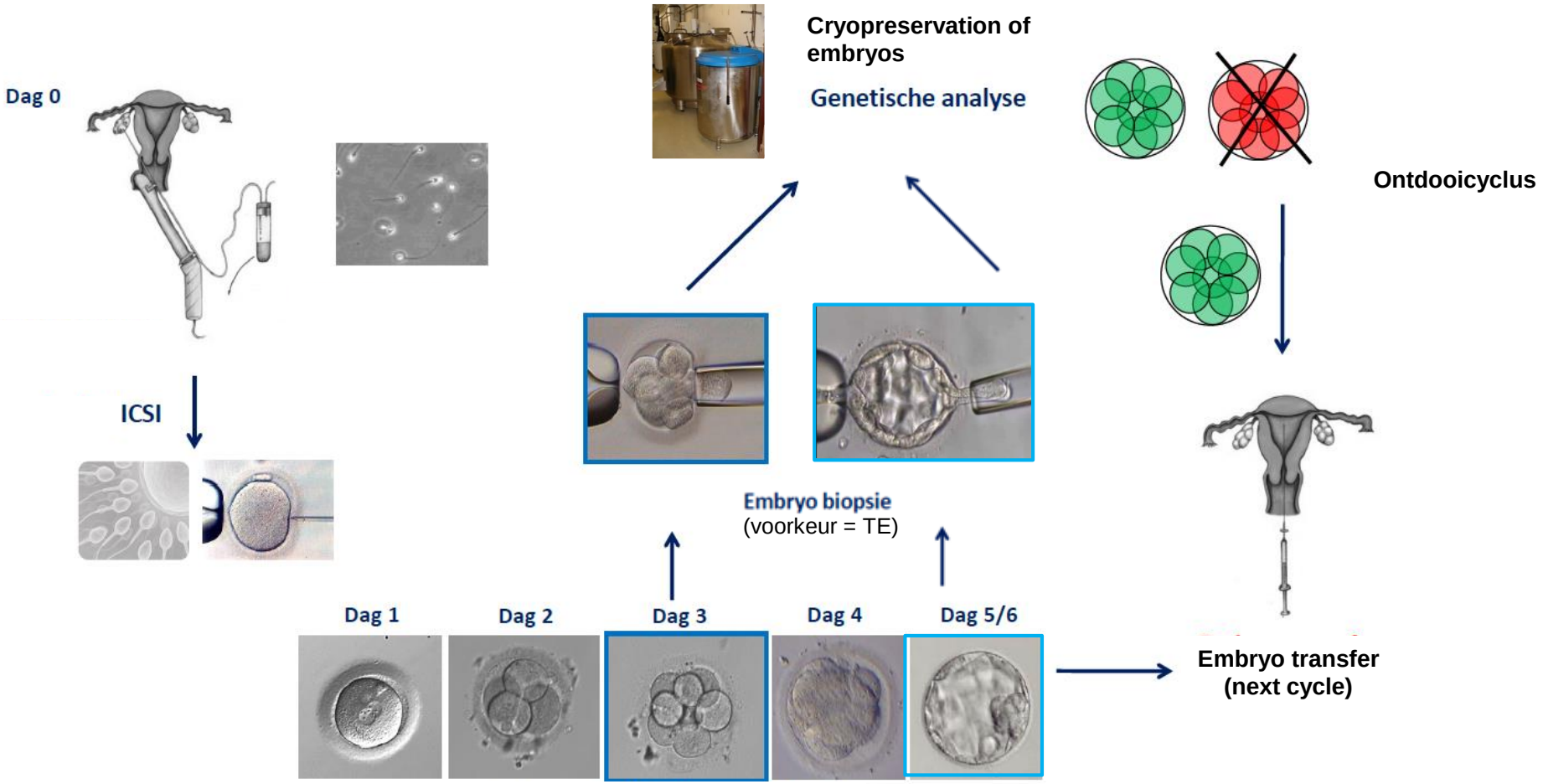
## e.g. reciprocal translocations





# Scheme: PGT-SR via LPS

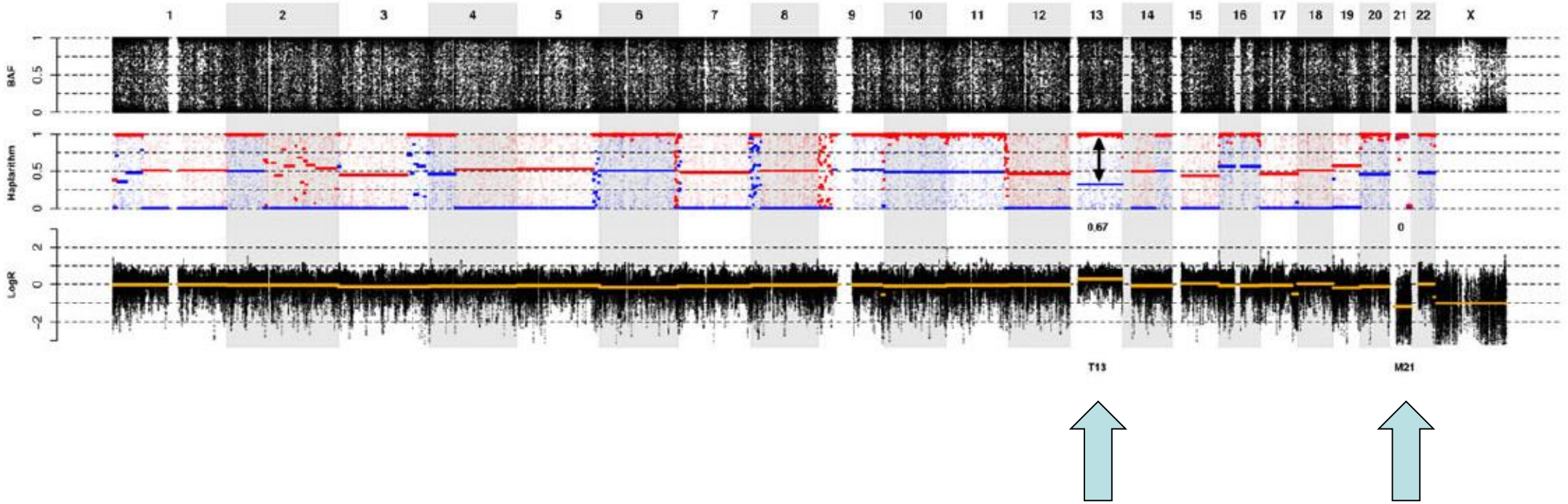


# Pros and cons PGT-SR via LPS

+ Detection of unbalanced products
+ No need for the development/optimisation of family specific tests
+ No limitation regarding the number of chromosomal regions that can be tested
+ Genome-wide CN information available (+/-)
+ High throughput

- WGA required
- Detection limit depends on biopsy type and specific platform/pipeline
- Longer TAT
- No distinction between carriers of balnced translocations and no carriers
- cryopreservation of all embryos required

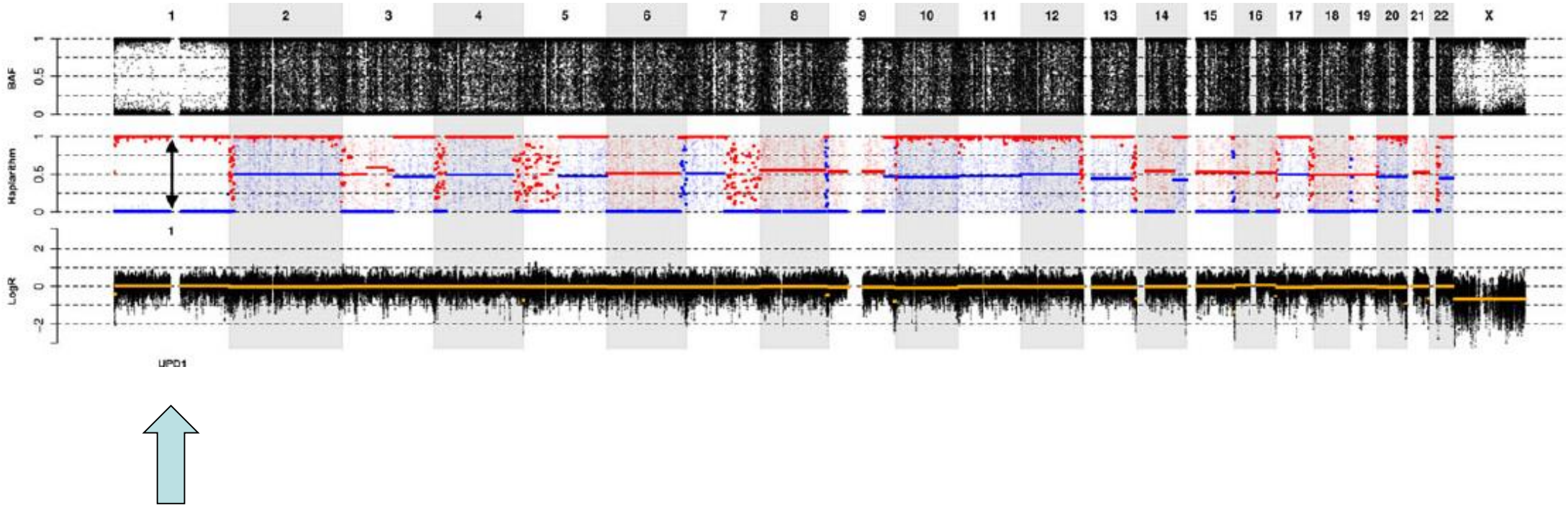
# Genome-wide findings




trisomie

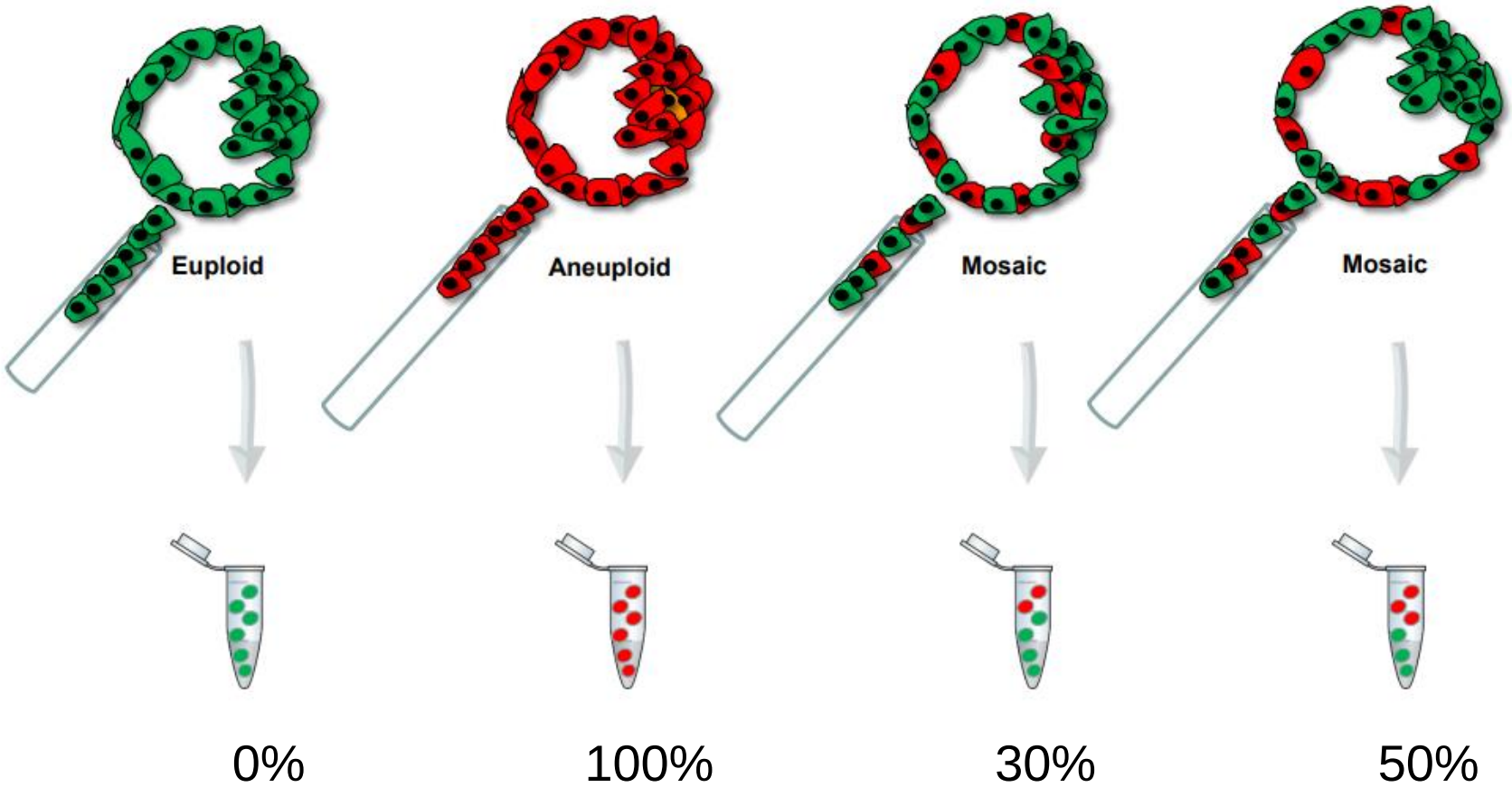
monosomie

# Genome-wide findings



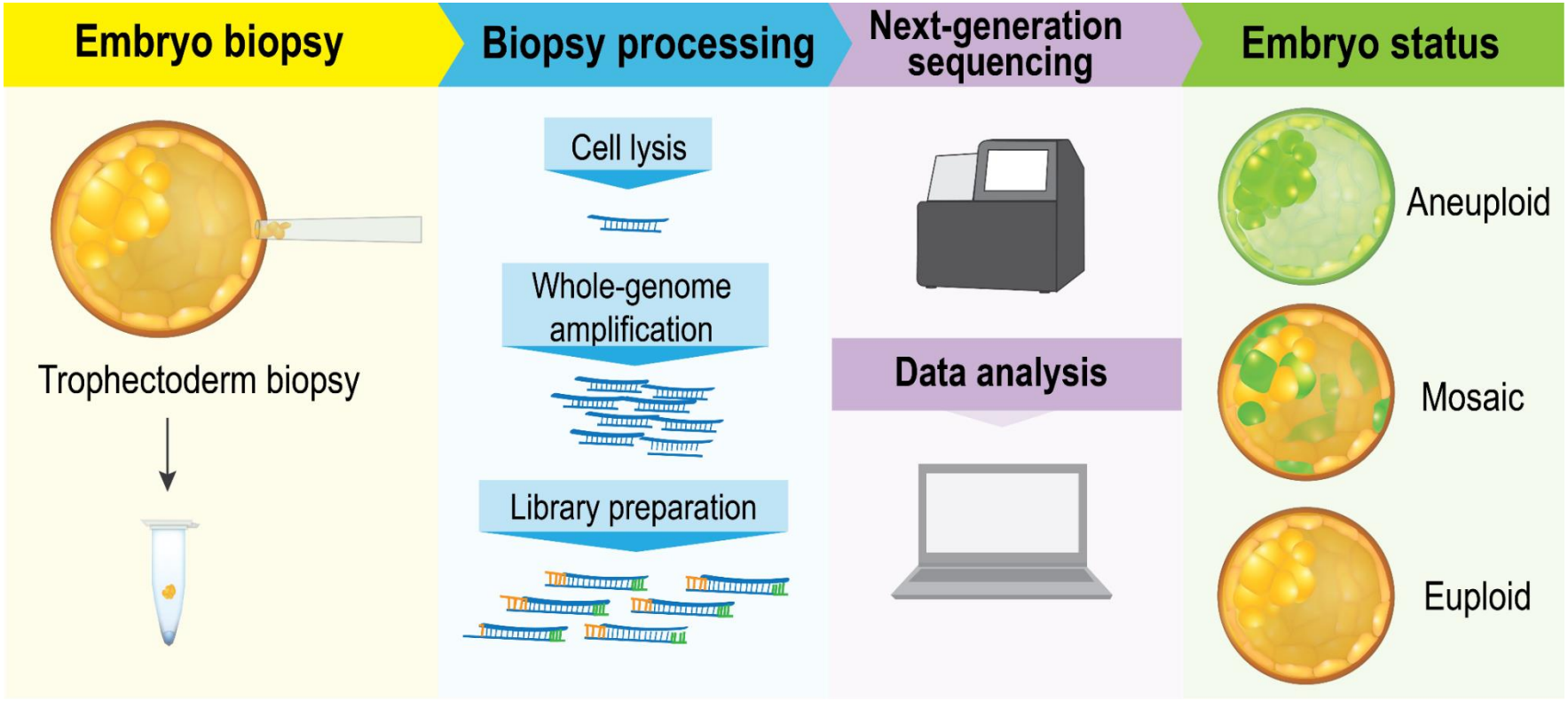

Uniparentele disomie

# Mosaicism detection




Courtesy of F. Fiorentino

# PGT-A


Embryo biopsy
Biopsy processing
Next-generation sequencing
Embryo status
Trophectoderm biopsy
Cell lysis
Whole-genome amplification
Library preparation
Data analysis
Aneuploid
Mosaic
Euploid

## PGT type

- PGT-SR
- PGT-M
- PGT-A
- Coprehensive PGT

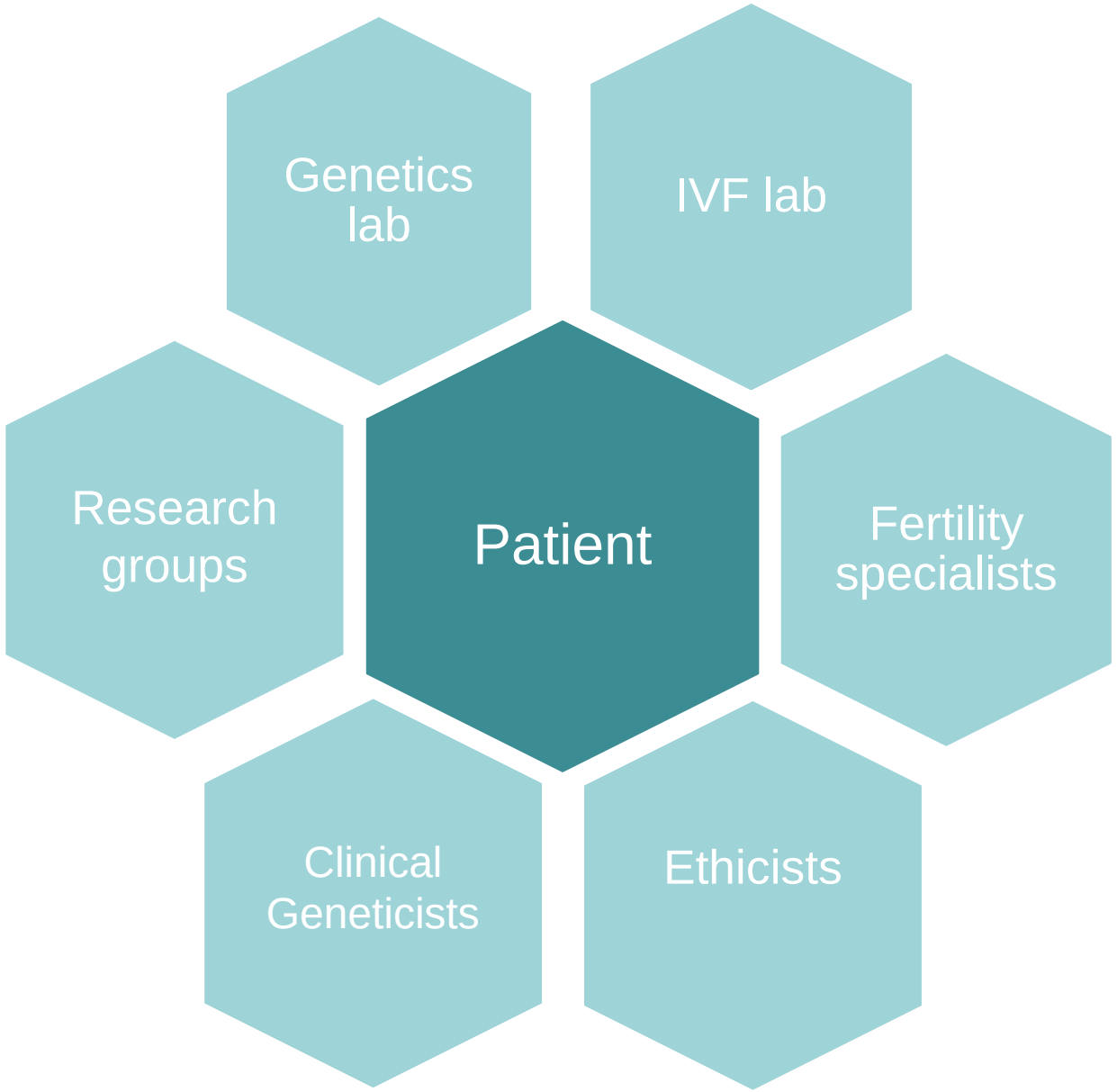
## Biopsy type

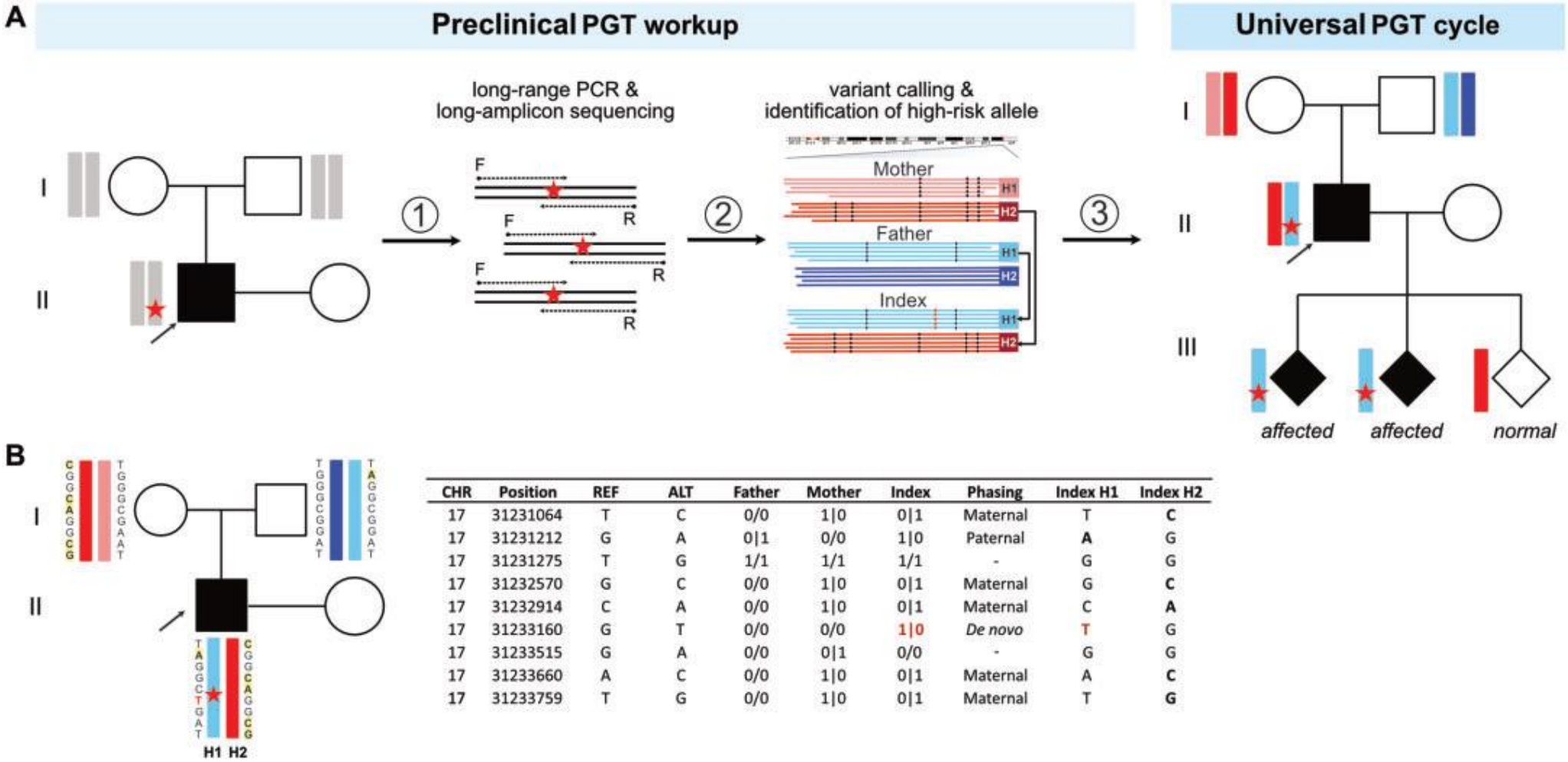
- Dag 3 (blastomere)
- Dag 5/6 (trophectoderm)

## Techniques

- FISH
- PCR-based
- Low-pass short read sequencing
- SNP array-based
- Genotyping by sequencing

# Team work


Genetics lab
IVF lab
Research groups
Patient
Fertility specialists
Clinical Geneticists
Ethicists




| 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** |

Preclinical workup using long-read amplicon sequencing provides families with de novo pathogenic variants access to universal preimplantation genetic testing. Tsuiko et al Hum Reprod 2023 Mar 1;38(3):511-519. doi: 10.1093/humrep/deac273