PGT for chromosomal abnormalities.

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BeSHG course 2023 - 2024

Universitair Ziekenhuis Brussel

Outline

- PGT-A vs PGT-SR
- Segregations of translocations
- Technologies
  - → FISH
  - → Array CGH
  - → Shallow Whole Genome Sequencing
  - → SNP array
  - → Genotyping by sequencing

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Nomenclature

- PGT= Preimplantation Genetic Testing
- PGT-A = PGT for Aneuploidy
- PGT-SR = PGT for Structural Rearrangements

- Derivative chromosome = structurally abnormal chr

PGT-A: indications

- Former PGS (screening)
  - → Couple has a normal karyotype
    - Recurrent implantation failure
    - Recurrent miscarriage
    - Advanced maternal age
    - Antecedents trisomy
    - Severe oligo-astheno-teratozoospermia (OAT)
- Numerical abnormalities (rare indication).
  - → 47,XXX; 47,XXY, 47,XYY
  - → Mosaic 45,X/46,XX
  - → Germline mosaic

PGT-SR: indications

- Balanced structural rearrangements
  - → Reciprocal and Robertsonian translocations
  - → Paracentric and pericentric inversions
  - → Insertions (rare indication)
- Unbalanced structural rearrangements
  - → Deletions, duplications
  - → Unbalanced reciprocal translocations (rare indication)

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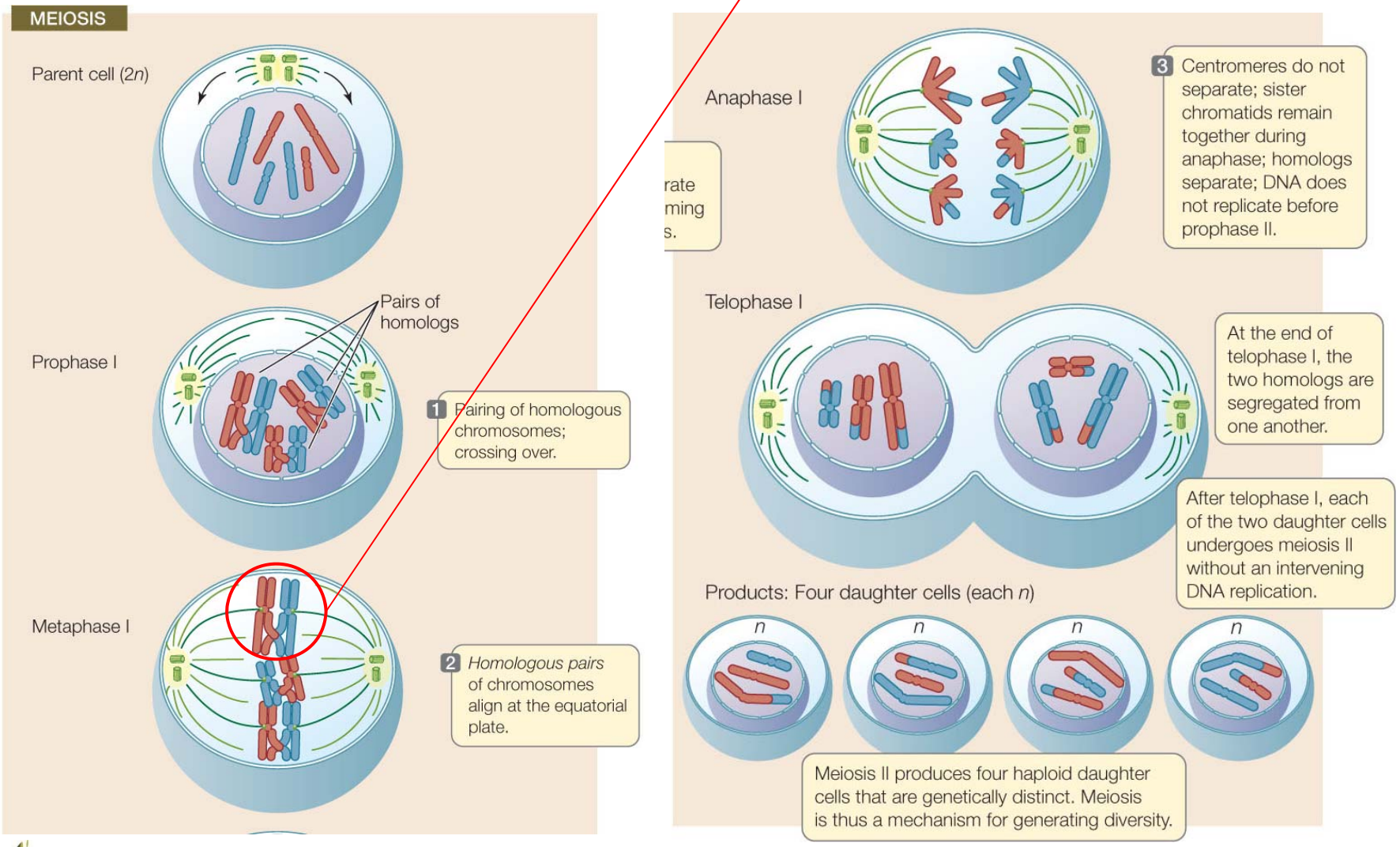
Robertsonian translocation

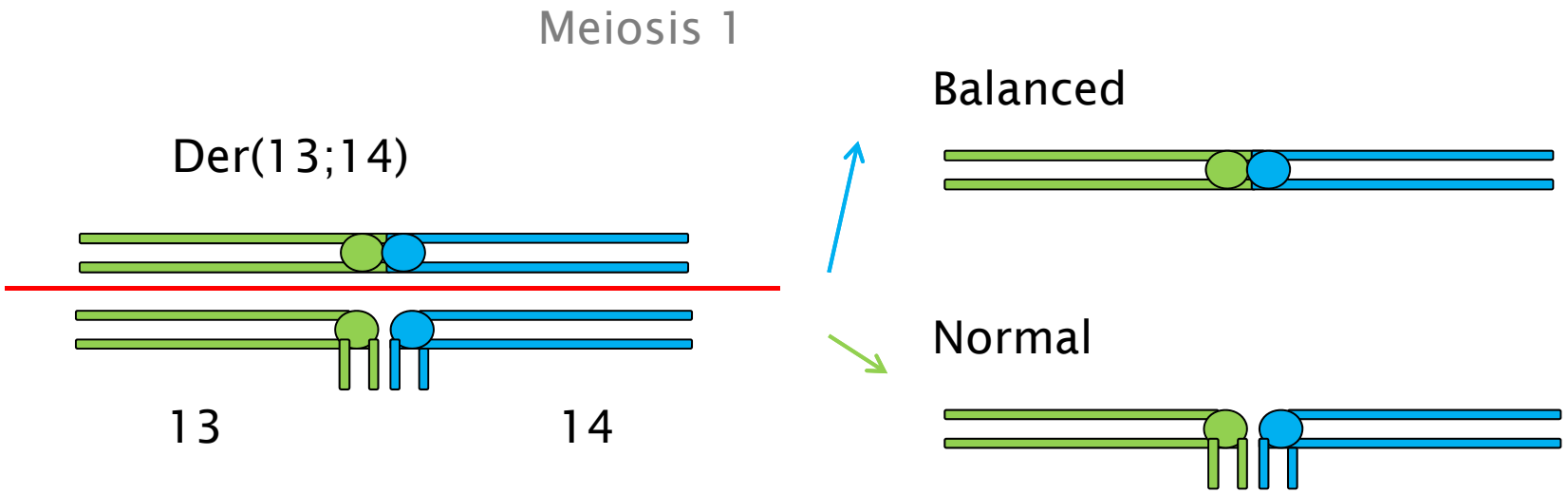
- Example 45,XX,der(13;14)(q10;q10)
- Robersonian translocation
  - → Fusion of long arms of 2 acrocentric chromosomes: 13, 14, 15, 21, 22
  - → Most often dicentric
  - → der(13;14) most frequent (75%)
  - → Viable trisomies possible with Rob involving chromosomes 13 and 21. Highest risk for trisomy 21 pregnancy in female carriers (10-15%)
  - → Higher incidence of UPD (chr14 and 15), ~0,8%
  - → 6 segregation products are expected

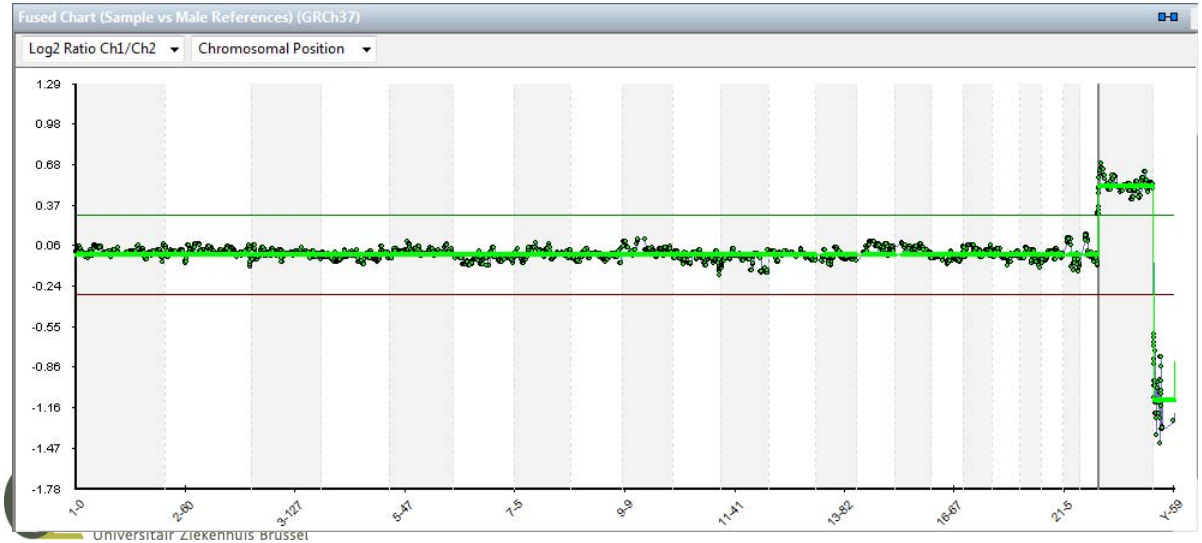
Normal meiosis

bivalent



Segregations Rob - alternate

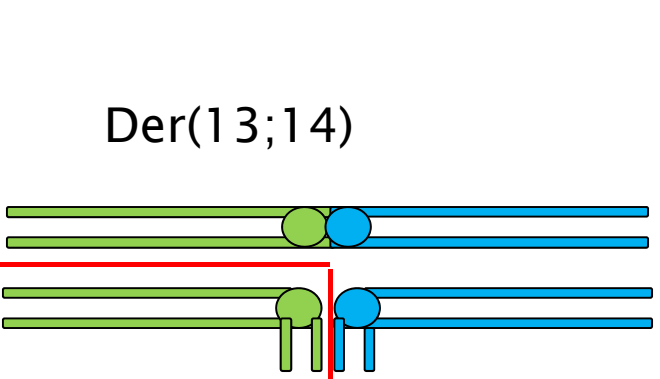




Segregations Rob - adjacent

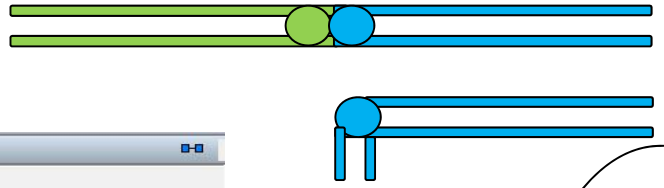
Meiosis 1

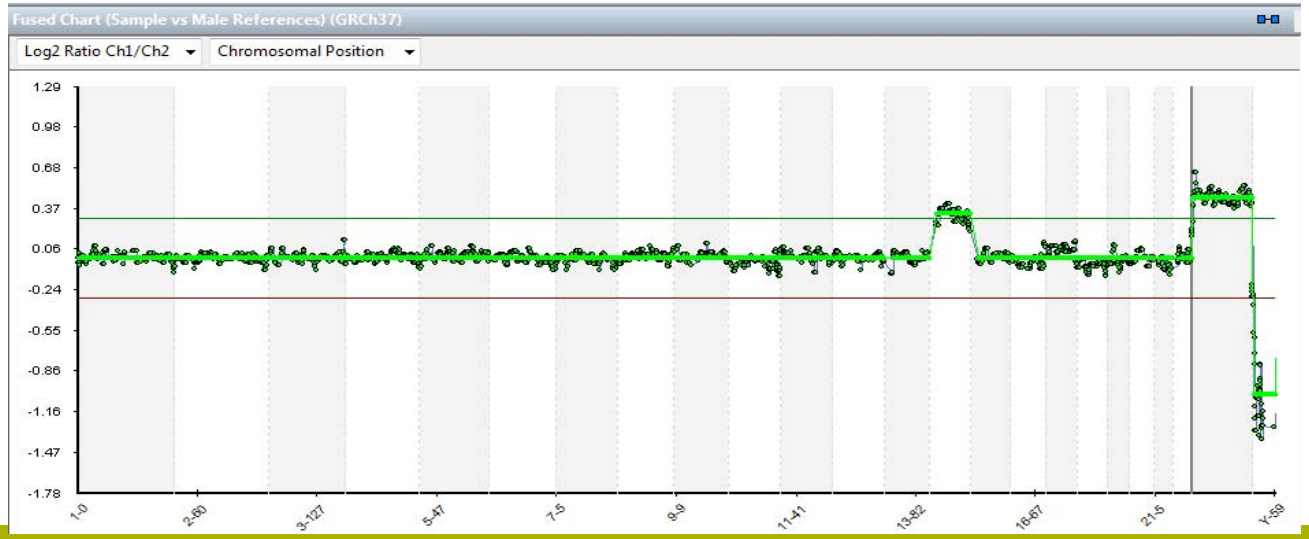
gamete nullisomy 14



Der(13;14)

gamete functional disomy 14



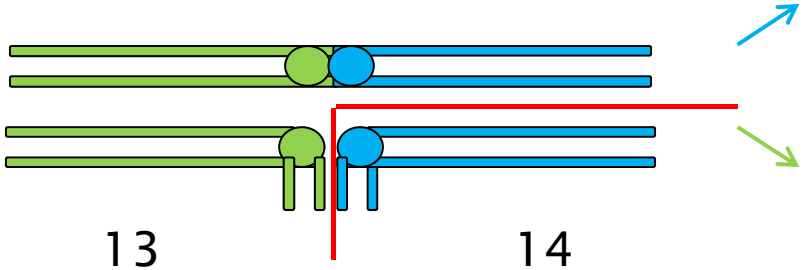


Embryo with functional trisomy 14

Segregations Rob - adjacent

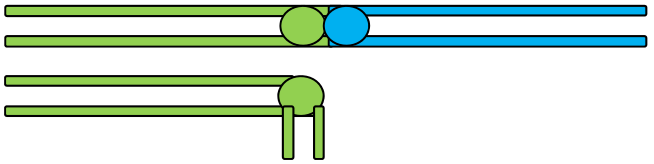
Meiosis 1

Der(13;14)

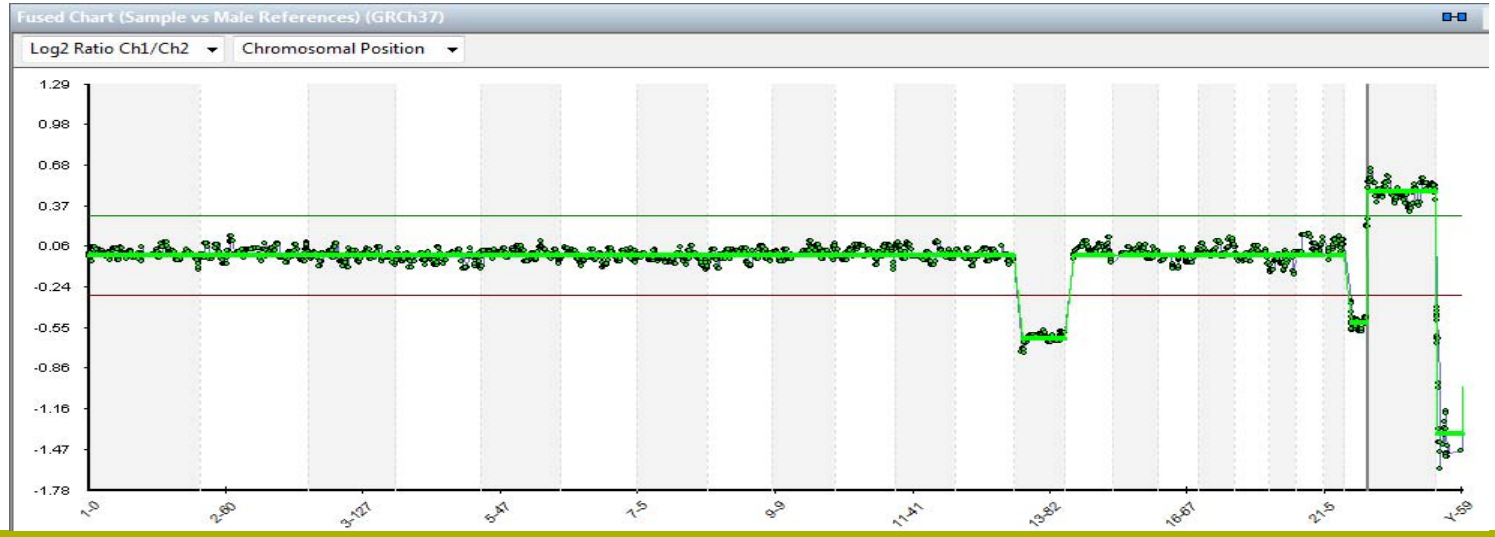


13 14

Gamete disomy 13



Gamete nullisomy 13



Embryo monosomy 13

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Reciprocal translocation

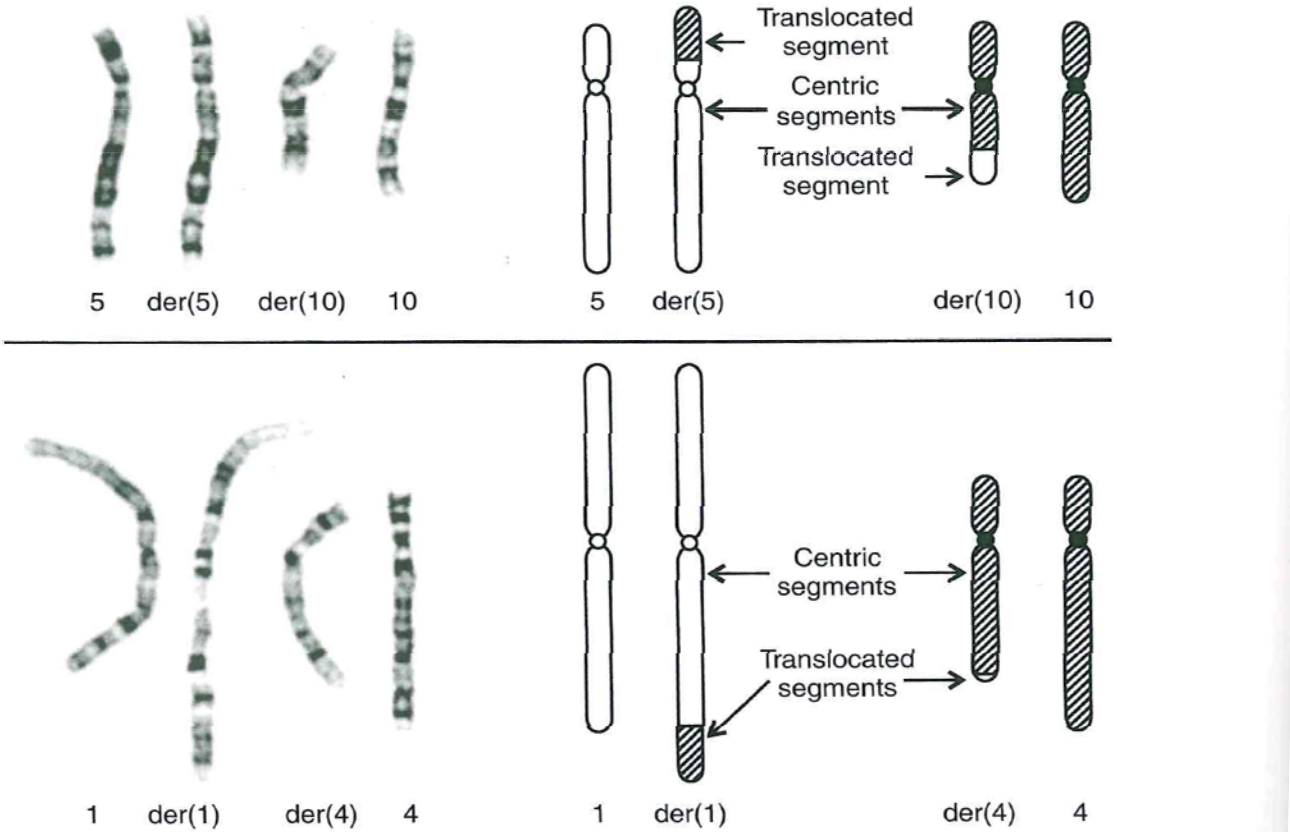

FIGURE 5–1 Reciprocal translocations demonstrating (*above*) double-segment and (*below*) single-segment exchange. The translocations are t(5;10)(p13;q23.3) and t(1;4)(q44;q31.3). (Cases of M. A. Leversha and N. A. Adams.)

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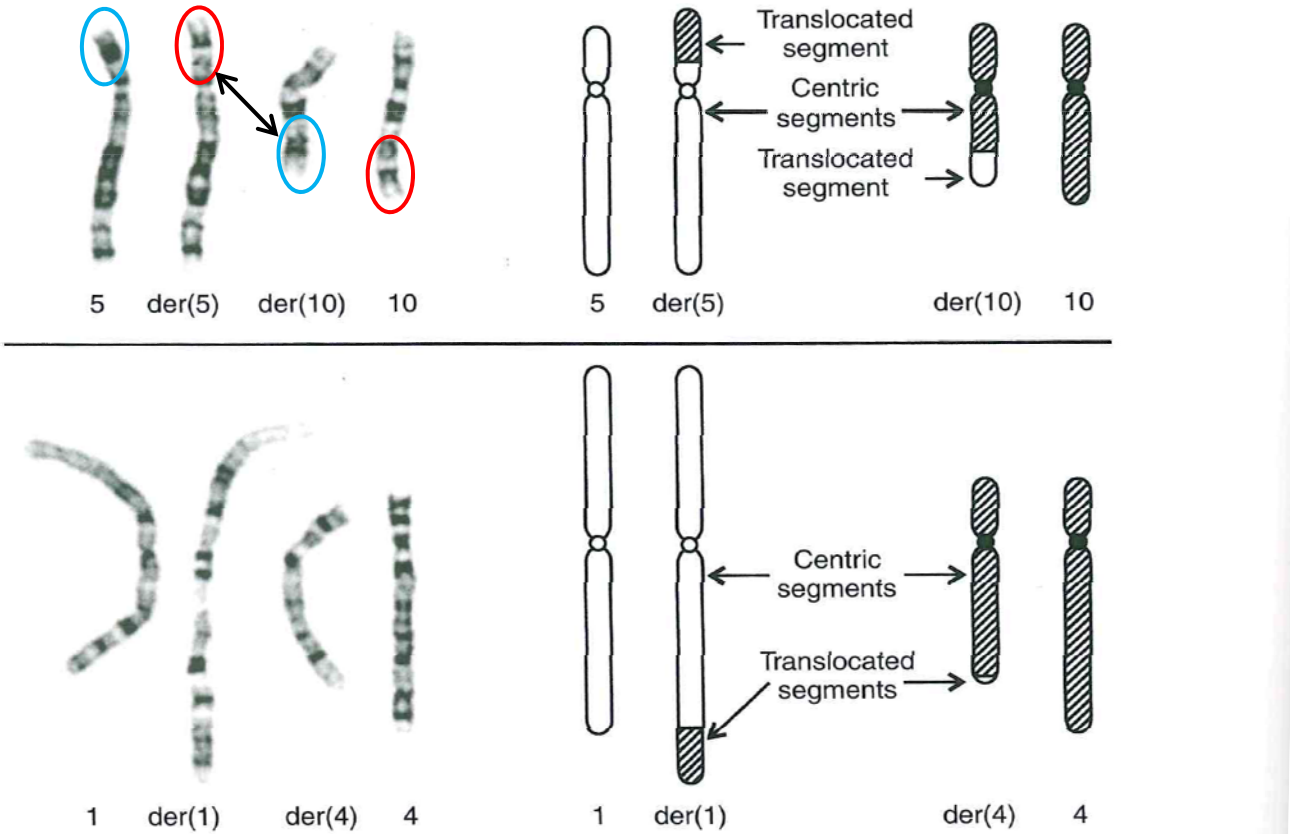

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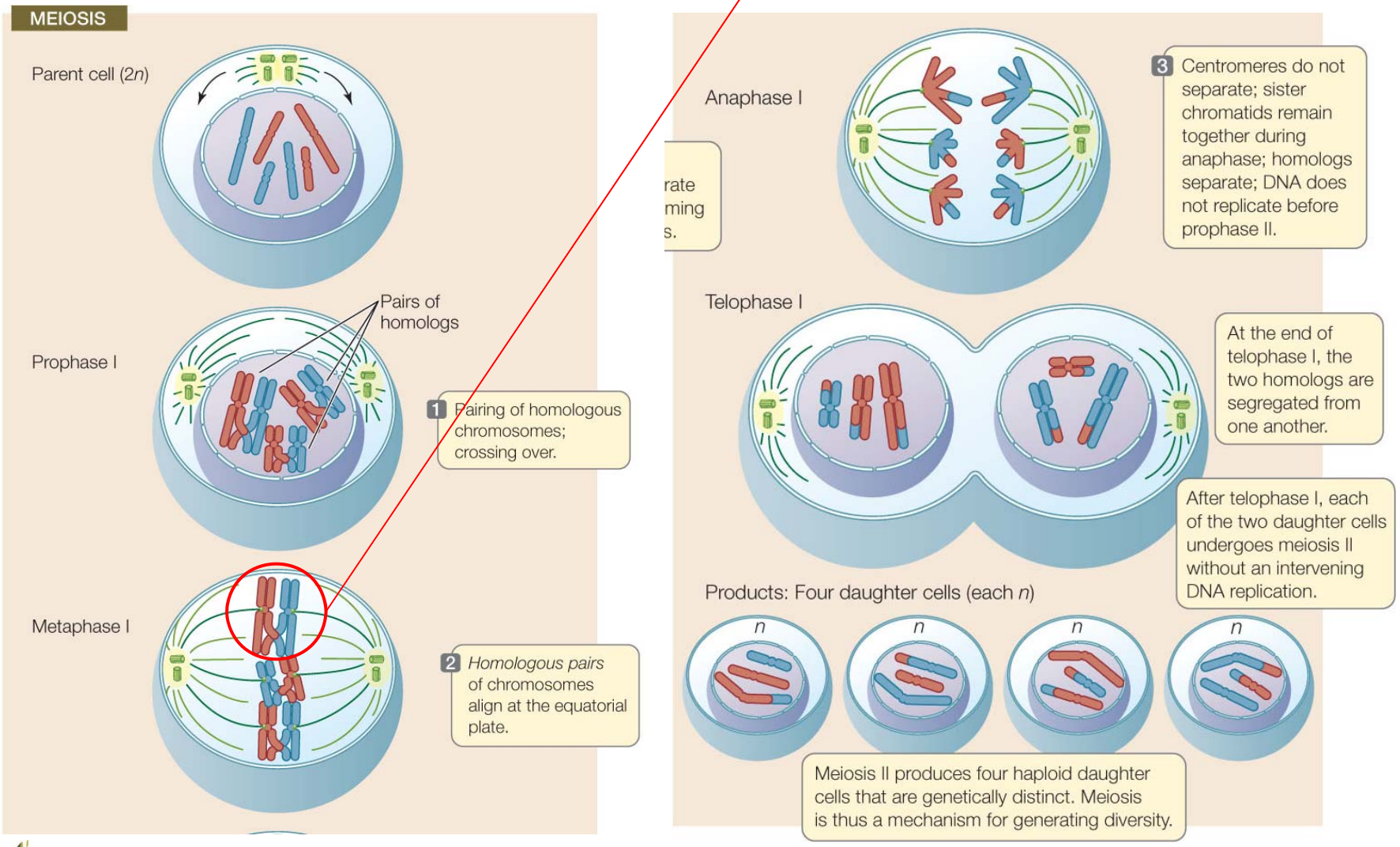

Segregations for a reciprocal transloc.

Tetravalent

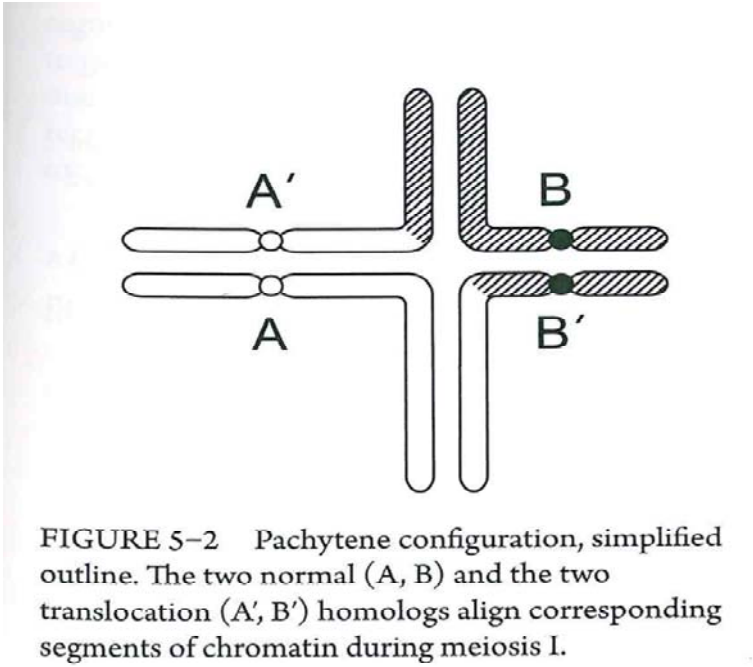

FIGURE 5–2 Pachytene configuration, simplified outline. The two normal (A, B) and the two translocation (A′, B′) homologs align corresponding segments of chromatin during meiosis I.

Table 5–1.

ONE DAUGHTER GAMETOCYTE WITH:	OTHER DAUGHTER GAMETOCYTE WITH:	SEGREGATION MODE
2:2 Segregations		
A and B	A′ and B′	Alternate segregation
A and B′	B and A′	Adjacent-1 segregation
A and A′	B and B′	Adjacent-2 segregation
3:1 Segregations		
A B A′	B′	3:1 segregation with tertiary trisomy or monosomy
A B and B′	A′	
A′ B′ and A	B	3:1 segregation with interchange trisomy or monosomy
A′ B′ and B	A	
4:0 Segregation		
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From Gardner and Amor, 'Chromosome abnormalities and genetic counseling' 5th edition, Oxford University press 2018.

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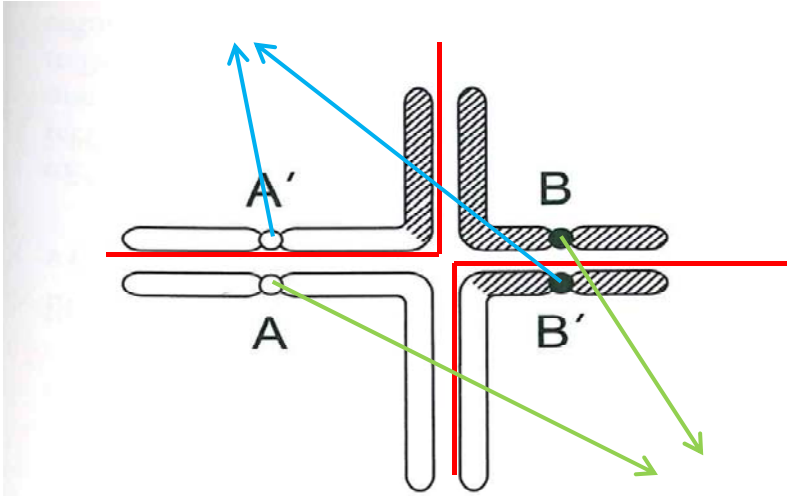

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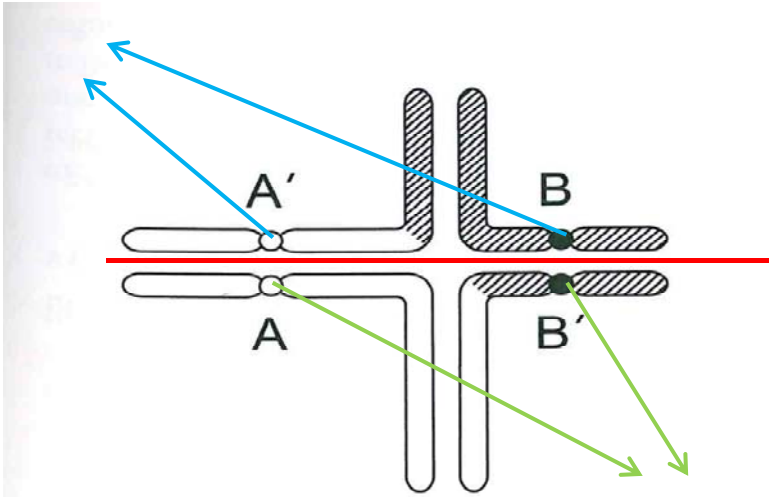

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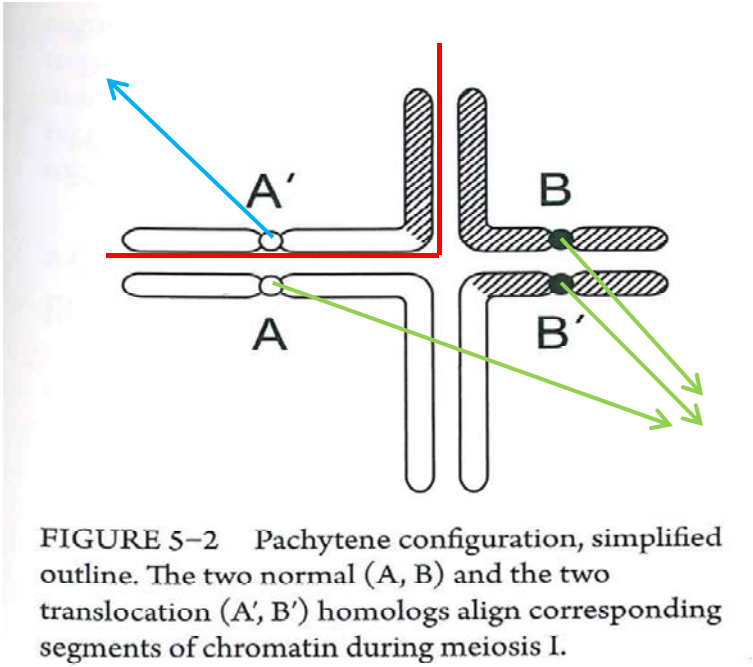

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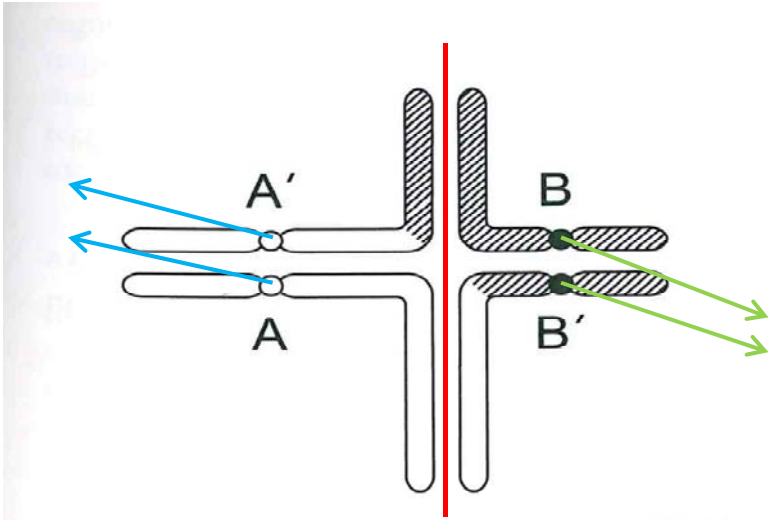

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Reciprocal translocations

- Rule of thumb:

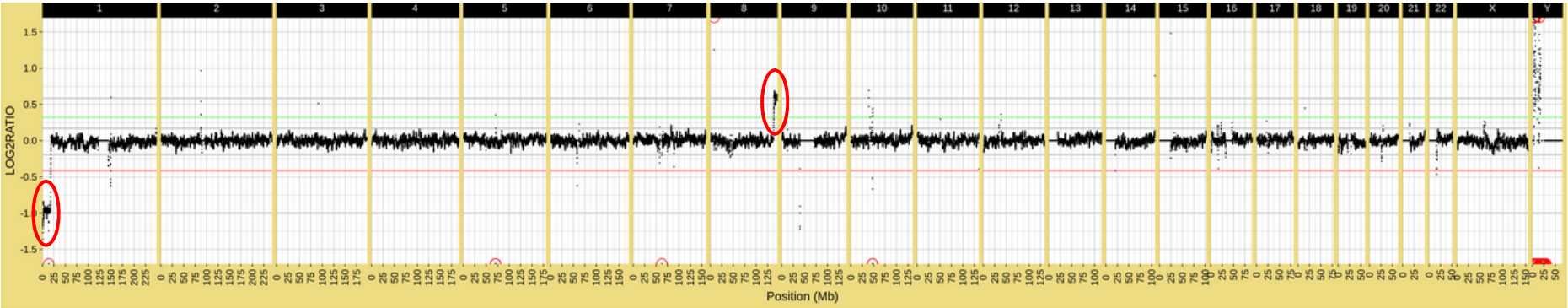
Sum of the copynumber of the centric segments

=

Sum of the copynumber of the translocated segments

->If you know the CN of 3 out of 4 segments, you know what the CN of the 4th segment should be

Rec. Transloc 46,XX,t(1;8)(p36,13;q24,23)



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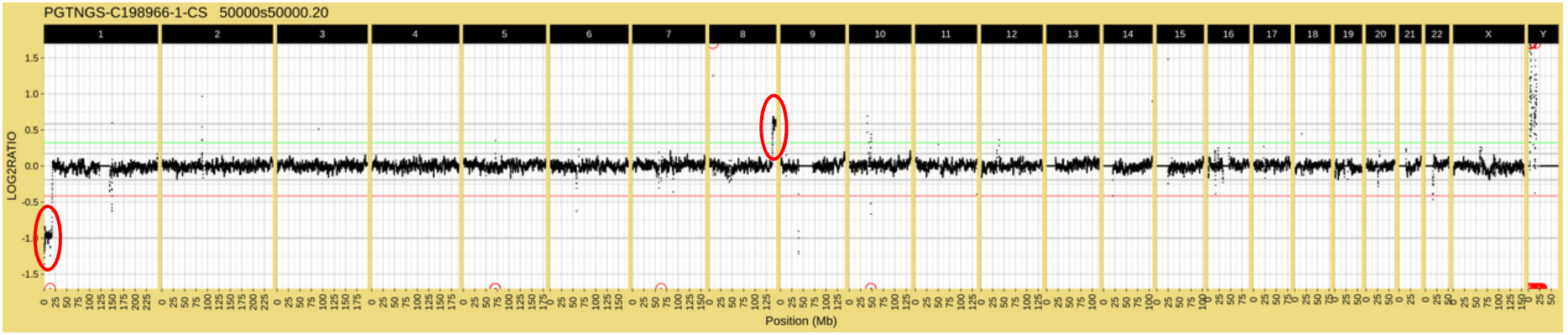
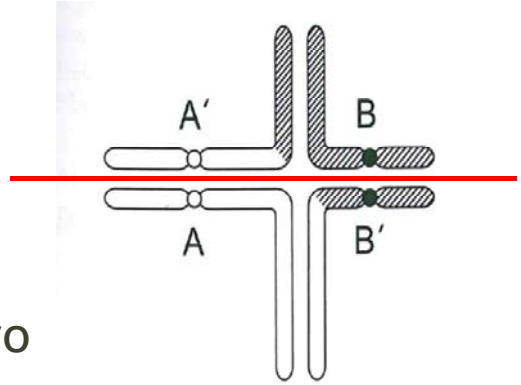


->Adjacent 1 segregation in gamete

1 derivative maternal chromosome 1 (A')

1 normal maternal chromosome 8 (B)



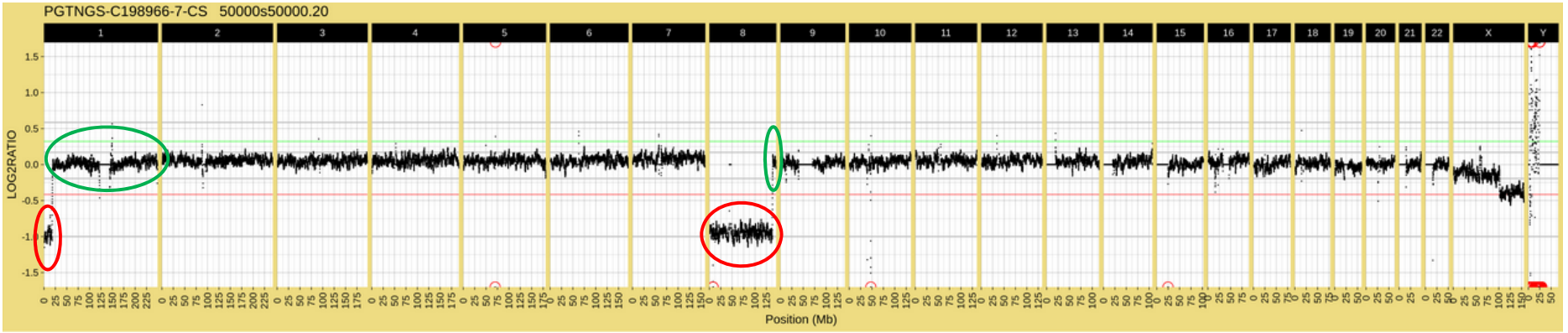
-> translocated segment on chr1 deleted in embryo

-> $Log_2R = -1$

-> translocated segment on chr 8 duplicated in embryo

-> $Log_2R = 0.58$

Rec. Transloc 46,XX,t(1;8)(p36,13;q24,23)



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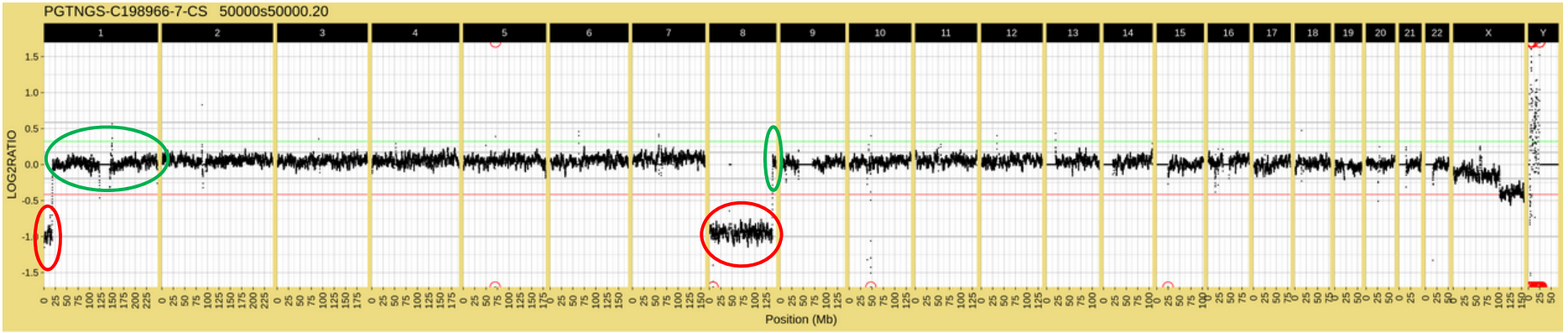
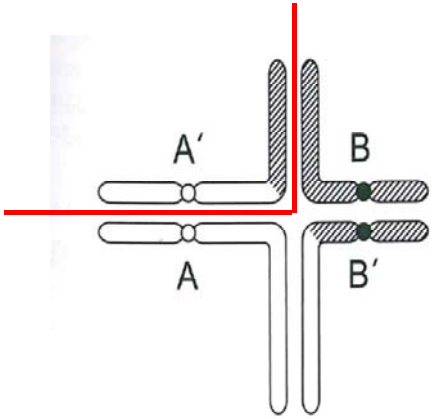


-> Tertiairy monosomy in embryo

1 maternal derivative chromosome 1 (A')

no maternal chromosome 8 (/)



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PGT with FISH

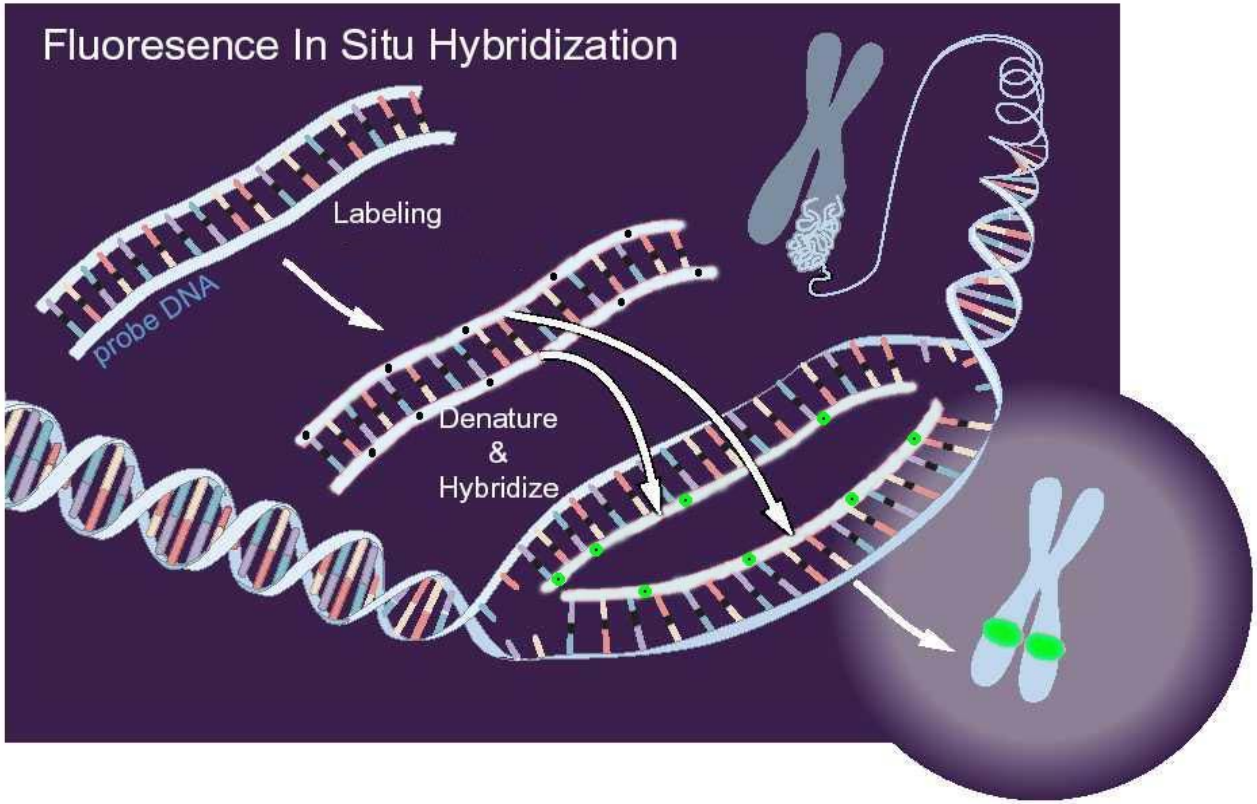
-Fluorescent *In-situ* Hybridization: hybridization of fluorescently labelled probes directly onto a fixed nucleus.

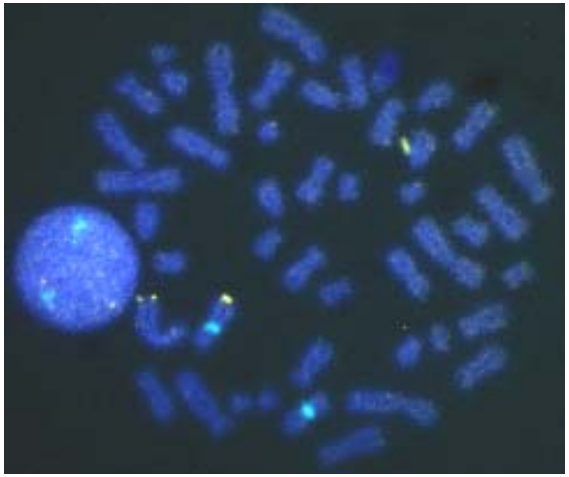
-One to three FISH hybridization rounds are possible (wash and hybridize again)

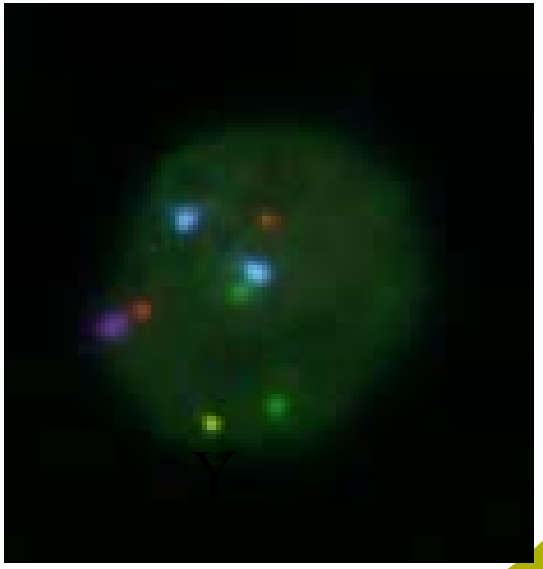
-Up to ~12 probes

-Oldest technique for chromosomal PGT

FISH:principle

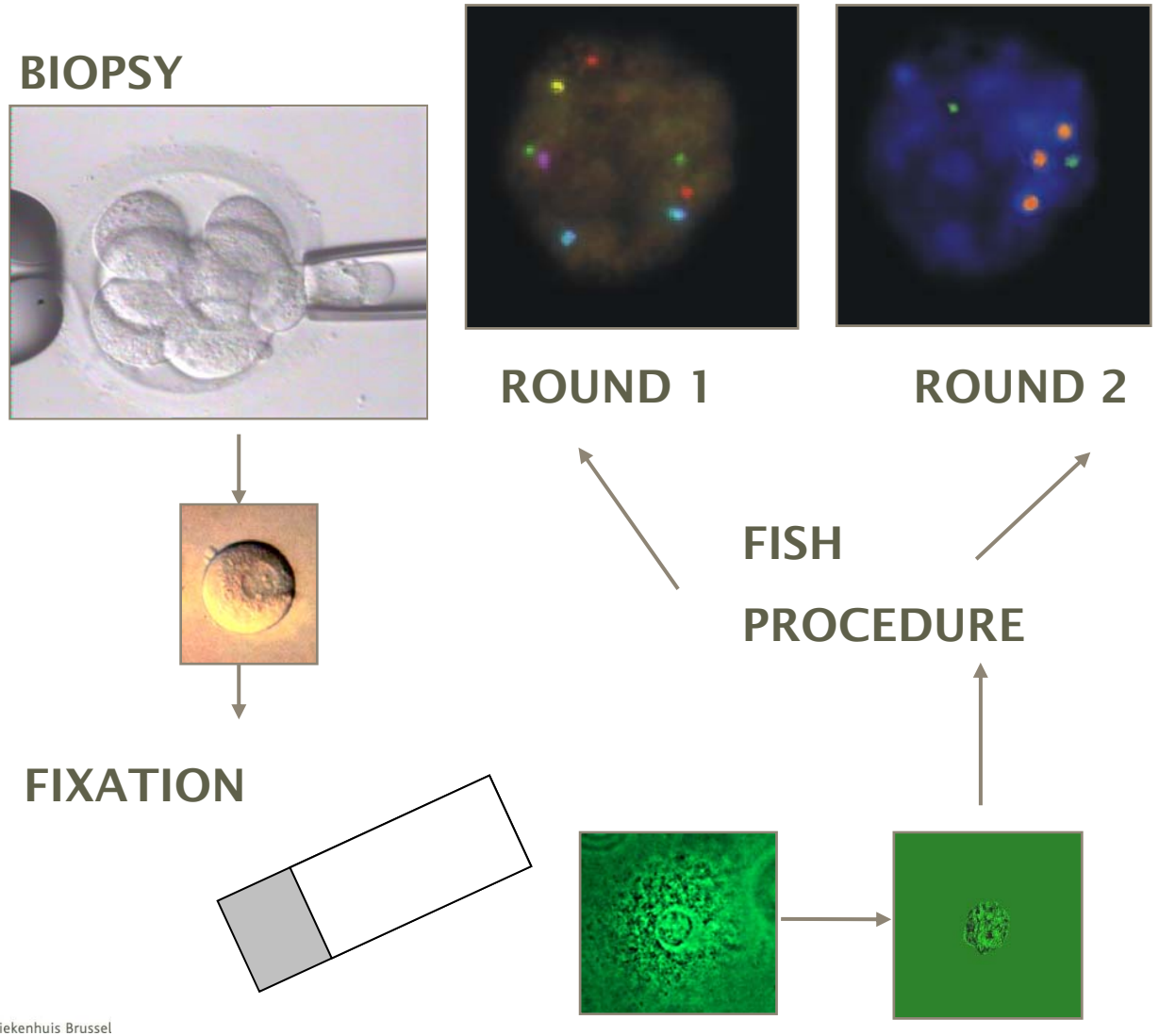






Multi - color FISH
1 → 3 consecutive FISH procedures

PGD- FISH cycle: day 3 biopsy



Example FISH - 46,XX,del(22)(q11.21q11.21)

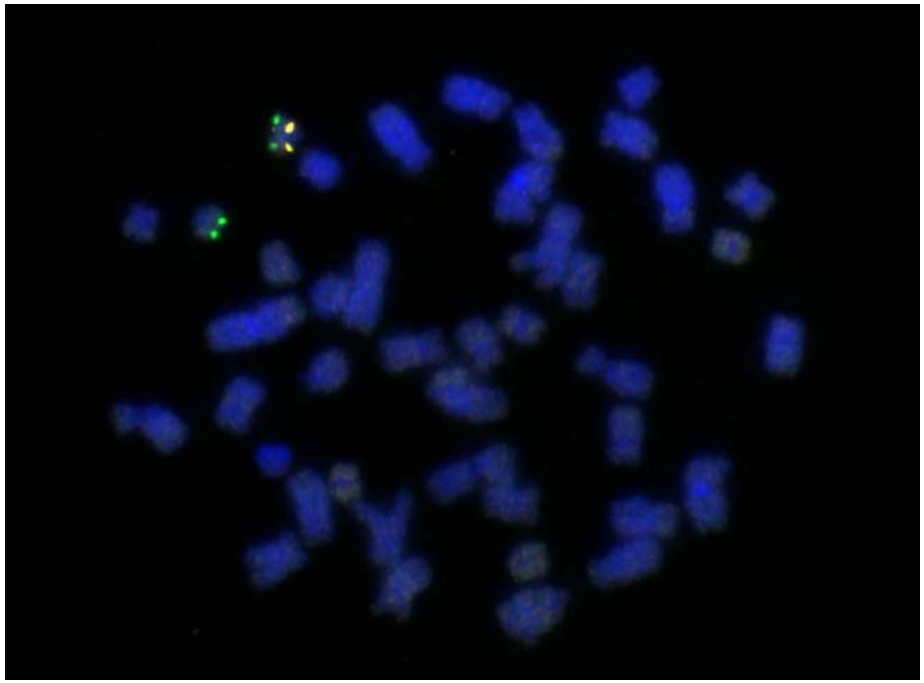
- Workup
  - → 10 Metaphase nuclei
  - → 100 Interphase nuclei

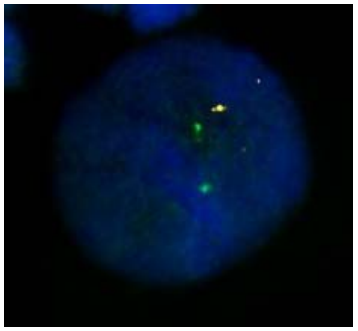
Round 1:

22q11.2 probe (Vysis, LSI TUPLE 1, Orange)
22q13.3 probe (Vysis, LSI ARSA, Green)

Round 2 (not shown):

PGT-kit
13q14 Red
18p11.1-q11.1 Alpha Satellite DNA Aqua
21q22.13-21q22.2 Green
Xp11.1-q11.1 Alpha Satellite DNA Blue
Yp11.1-q11.1 Alpha Satellite DNA Gold

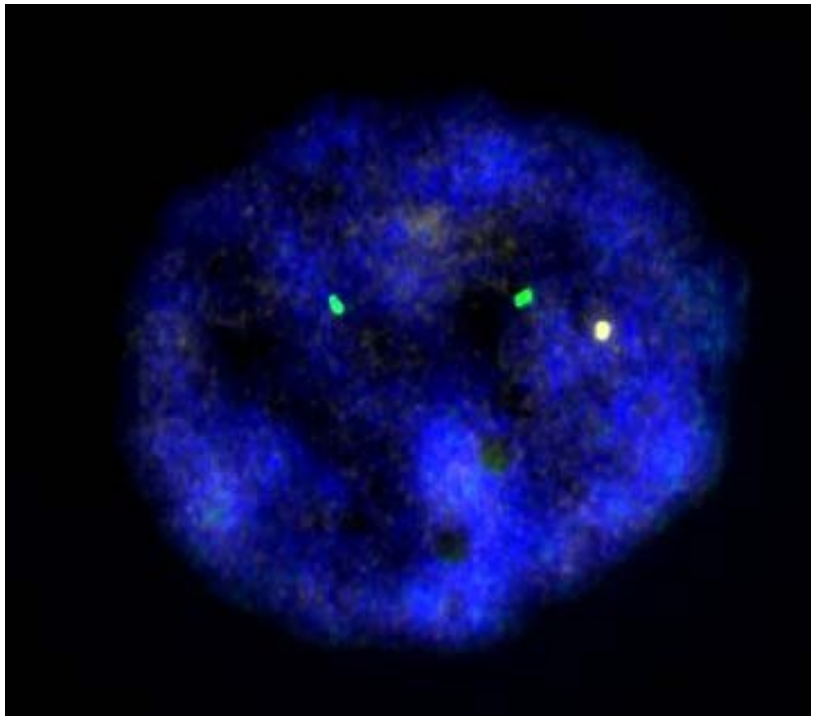




Example FISH - 46,XX,del(22)(q11.21q11.21)

- PGD

  → Embryo inherited del(22)(q11.21q11.21)



Strengths and limitations of FISH

- Strengths:
  - → Structural rearrangements with small unbalanced segments can be diagnosed.
  - → Haploidy and polyploidy can be detected
- Limitations:
  - → Often patient-specific workup required
  - → Often subjective interpretation (low signal to background). Frequent FISH errors (splitting or overlapping signals)
  - → Few chromosomes are tested (probemix)
  - → Uniparental disomy (UPD) is not detected.
  - → Not useful for duplications
  - → Normal and balanced segregations are not distinguishable

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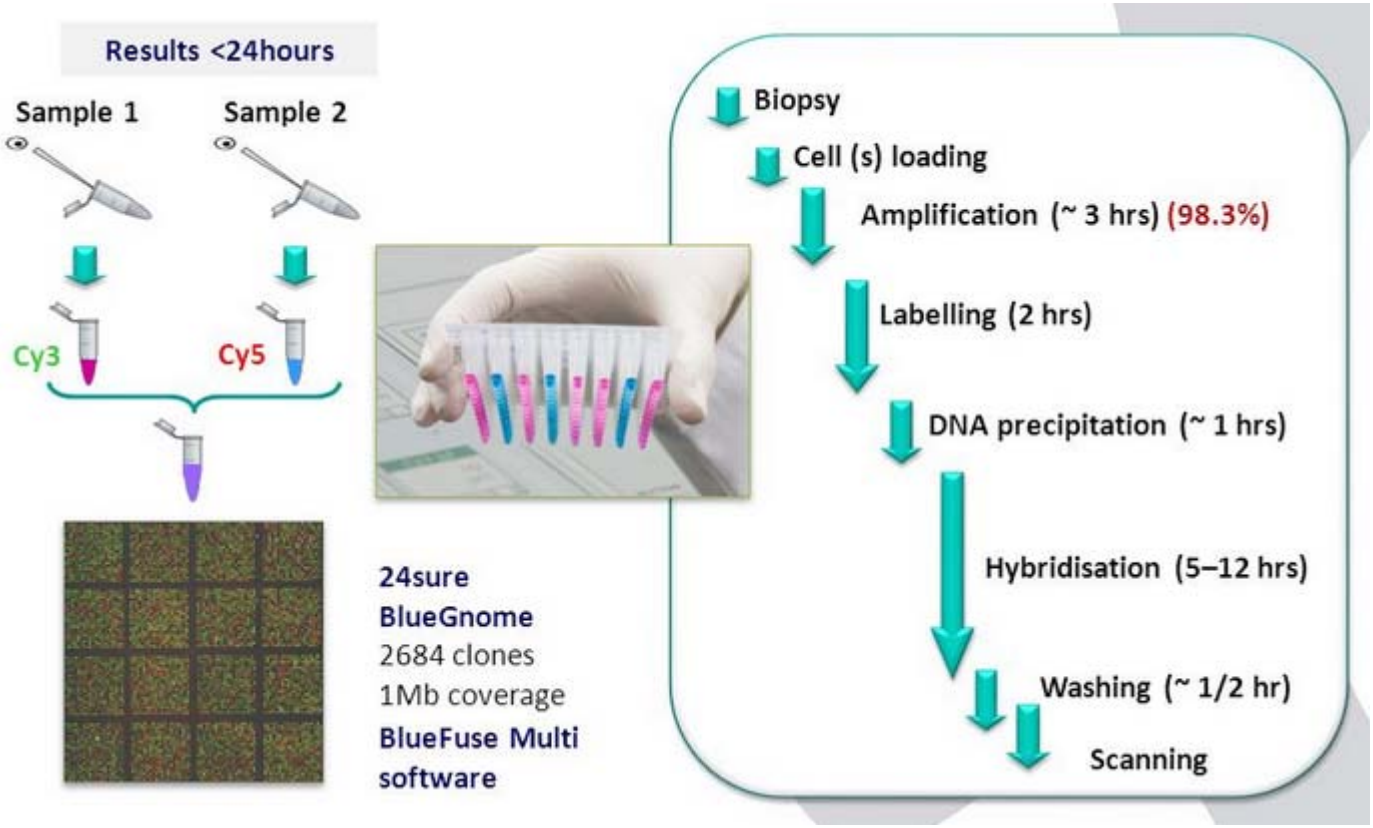
Array CGH



If is sample 2 is normal reference
In Cy5 (red)

→ Deletion sample 1; theoretical log2R = -1

→ Duplication sample 1; theoretic log2R = 0,58

→ Normal sample 1; theoretical log2R = 0

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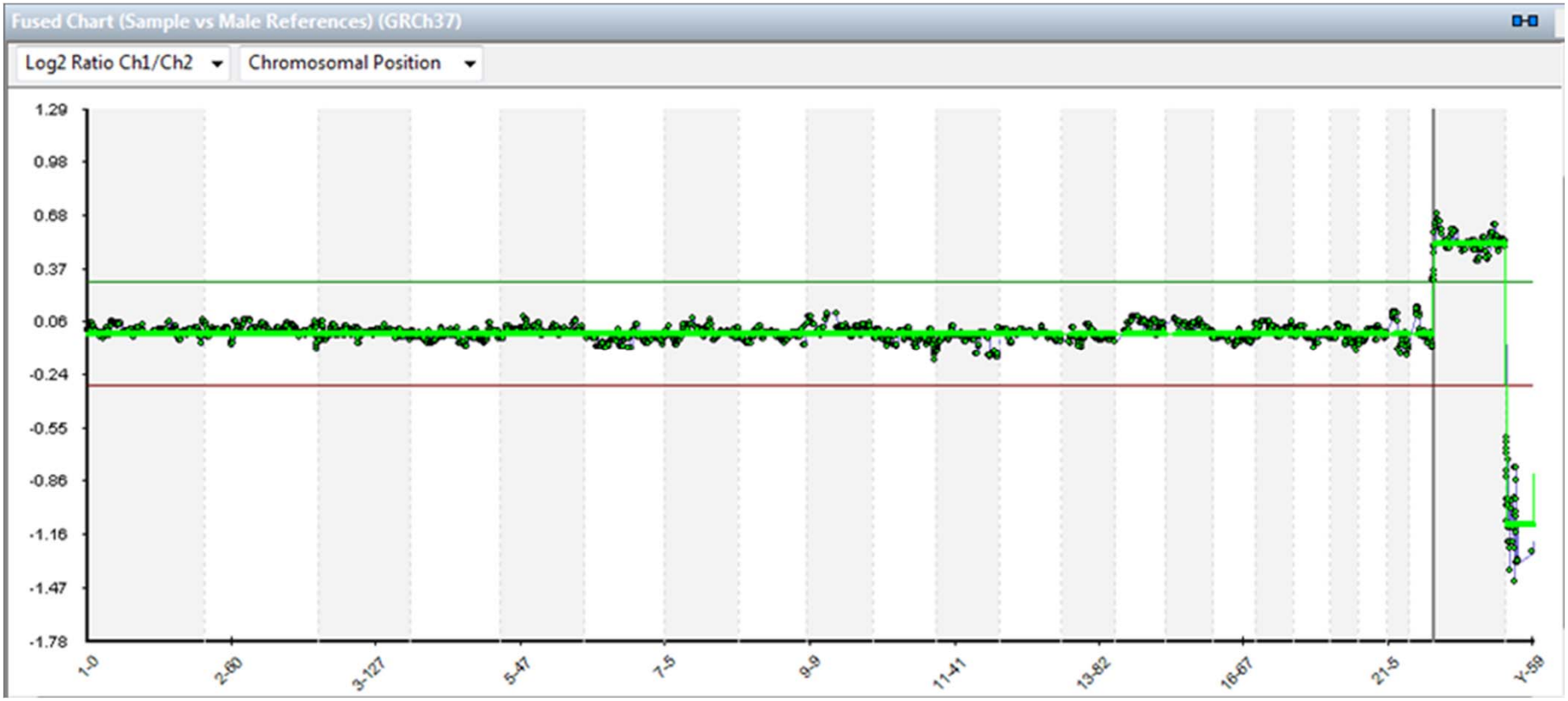
Example 46,XX



Strengths and limitations of aCGH

- Strengths:
  - → No patient-specific workup required
  - → All chromosomes are tested
  - → Straightforward interpretation
- Limitations:
  - → Uniparental disomy (UPD) is not detected.
  - → Normal and balanced segregations are not distinguishable
  - → Structural rearrangements with small exchanged segments (<10 Mb) cannot be diagnosed.
  - → Haploidy and polyploidy cannot be detected
  - → Main supplier abruptly ceased production in 2018

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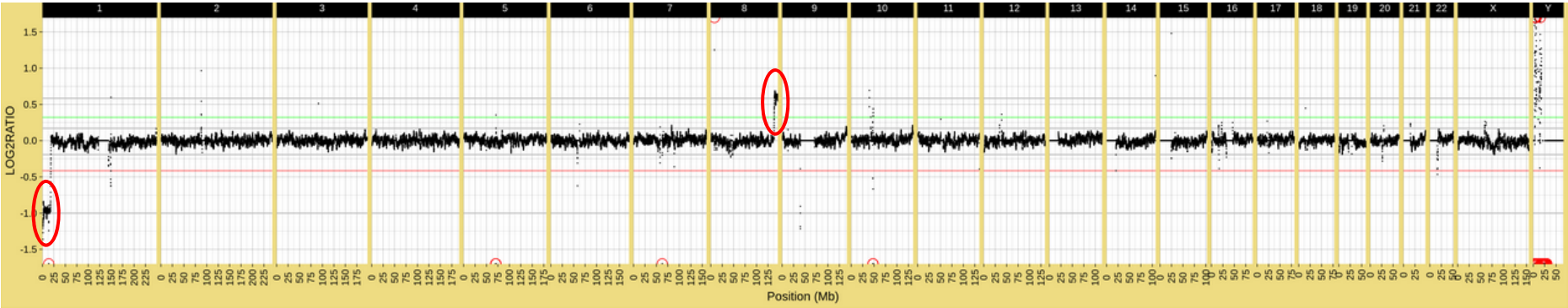
Shallow Whole Genome Sequencing

- A.k.a. low pass sequencing, low coverage NGS,..
- Massive parallel sequencing with low sequencing depth. Typically <0,3X or $<10^7$ reads.
- The number of reads is counted between specified intervals; “bins” (e.g. 1Mb) and normalized (GC content).
- The number of reads is a measure for the number of copies present

PGT - Shallow Whole Genome Seq

- Our method
  - → Trophectoderm biopsy
  - → Whole genome amplification (Sureplex Illumina)
  - → Bead cleanup
  - → Library preparation (adding adaptors for sequencing) using KAPA HyperPlus (Roche)
  - → Sequencing on NovaSeq (Illumina)
  - → Data analysis

Rec. Transloc 46,XX,t(1;8)(p36,13;q24,23)



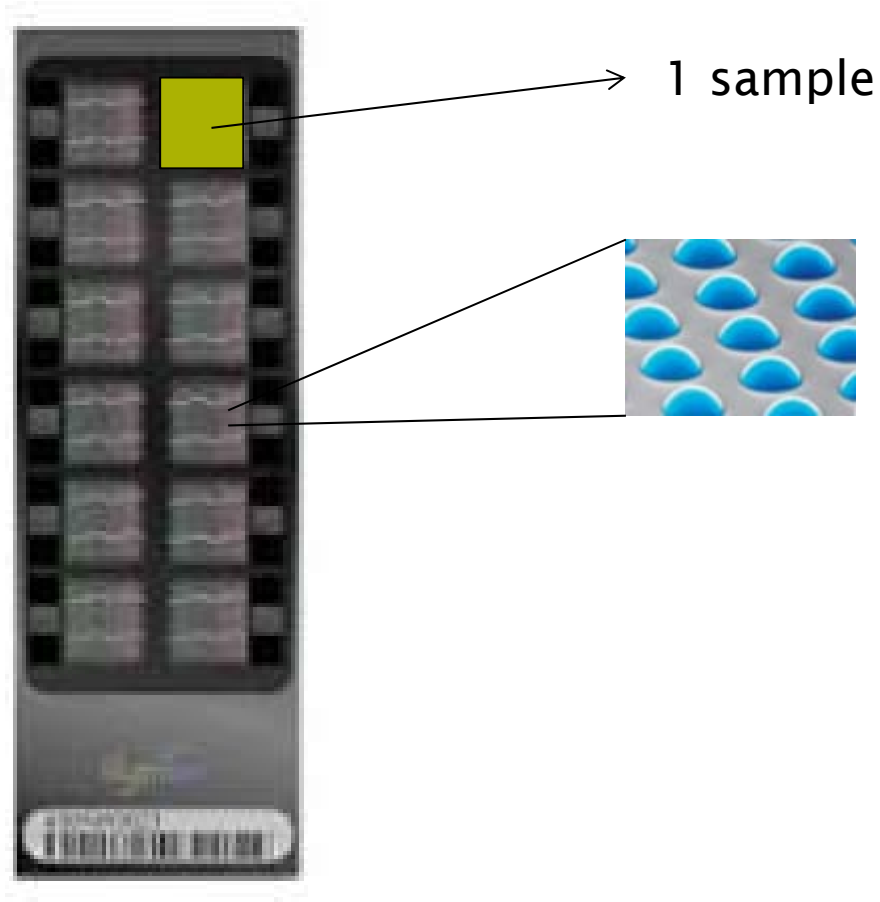
Strengths and limitations of sWGS

- Strengths:
  - → No patient-specific workup required
  - → All chromosomes are tested
  - → Straightforward interpretation
  - → Method of choice for copy-number detection (PGT-A).
- Limitations:
  - → Uniparental disomy (UPD) is not detected.
  - → Normal and balanced segregations are not distinguishable
  - → Structural rearrangements with small exchanged segments (<5 Mb) cannot be diagnosed.
  - → Haploidy and polyploidy cannot be detected

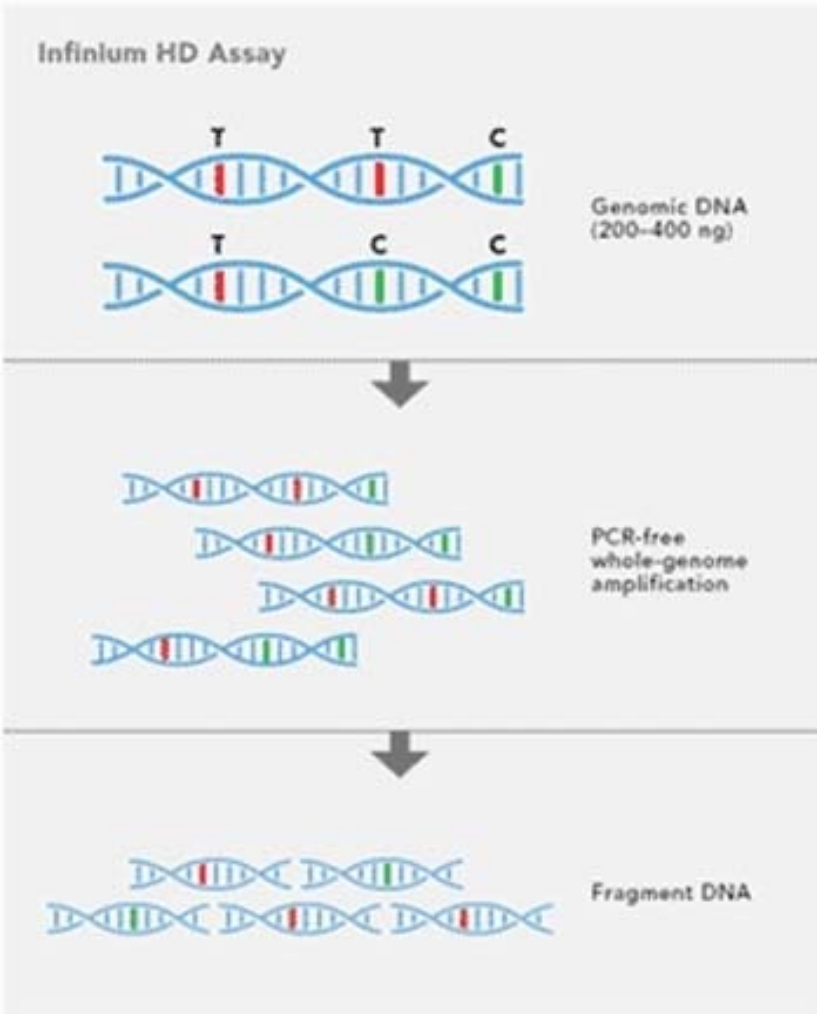
Outline

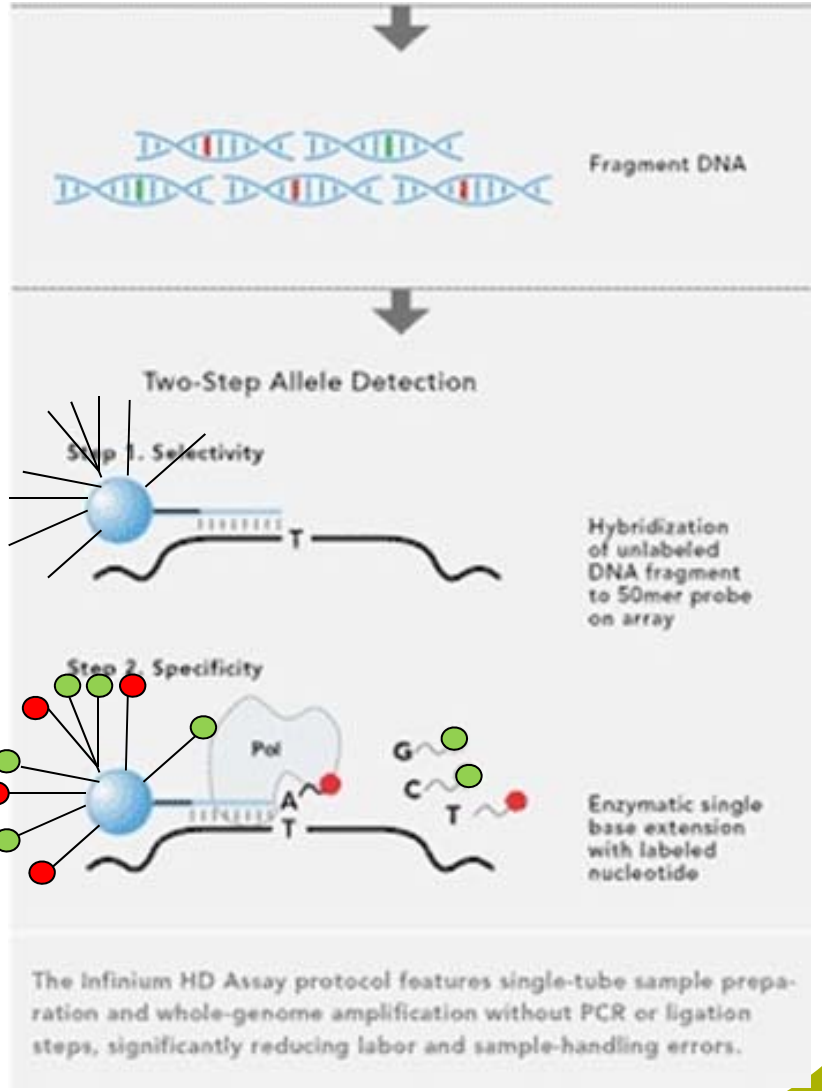
- PGT-A vs PGT-SR
- Segregations of translocations
- Technologies
  - → FISH
  - → Array CGH
  - → Shallow Whole Genome Sequencing
  - → SNP array
  - → Genotyping by sequencing

SNP array – Illumina Karyomapping



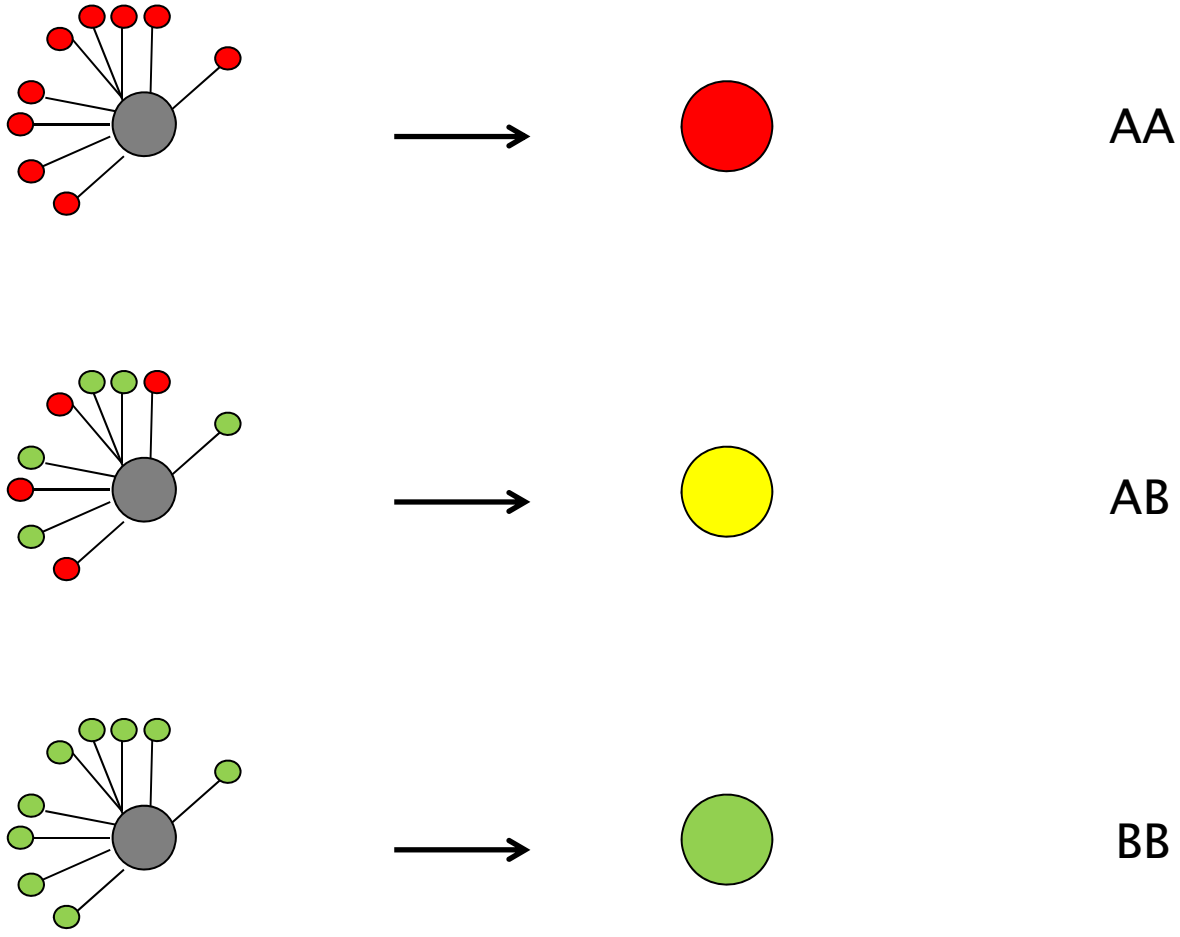
SNP array - method



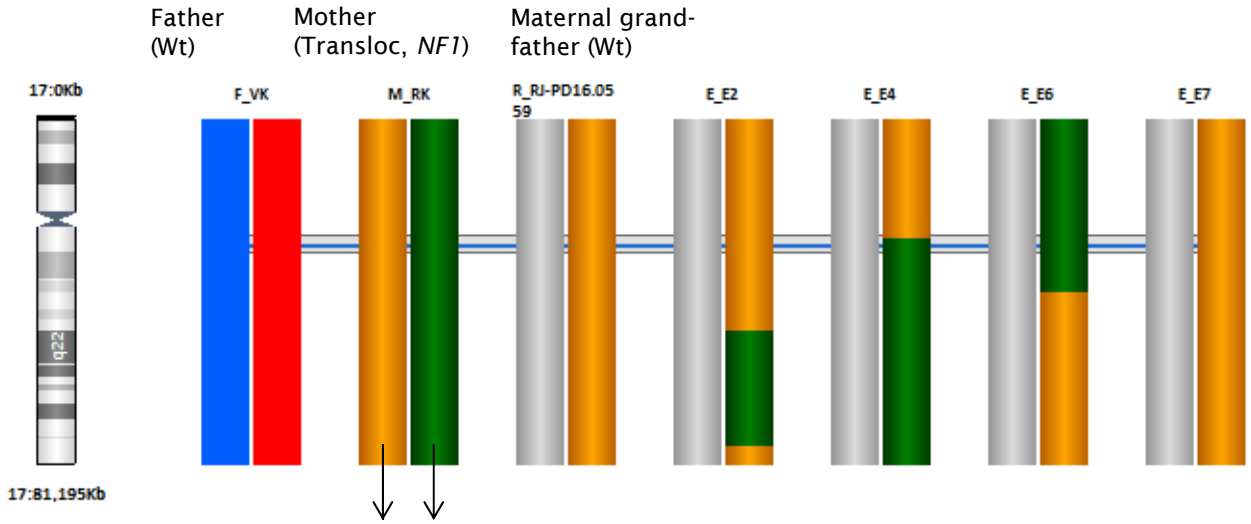


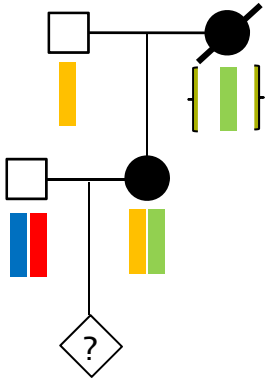
The Infinium HD Assay protocol features single-tube sample preparation and whole-genome amplification without PCR or ligation steps, significantly reducing labor and sample-handling errors.

From signal to genotype

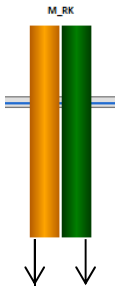


SNP array – example 46,XX,t(14;17)



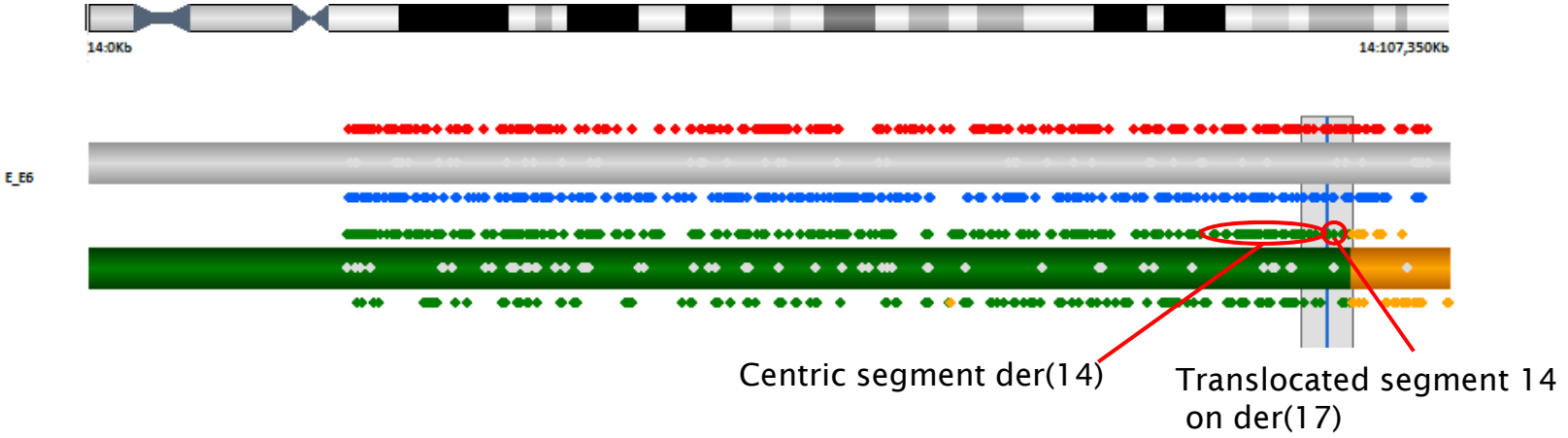


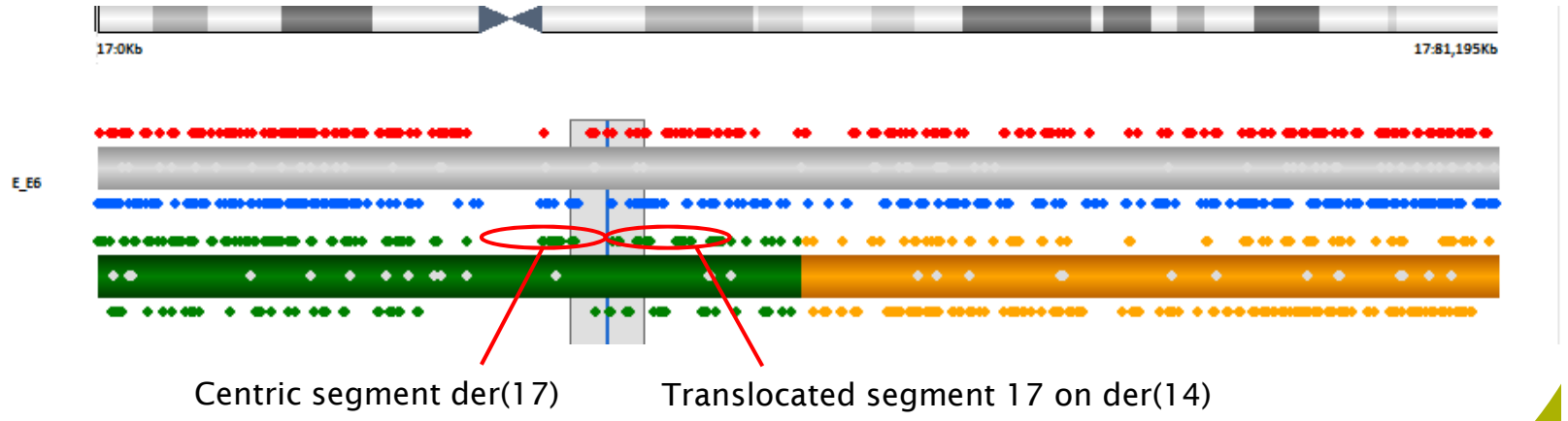
SNP array - example



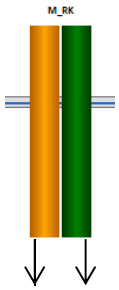
Wt

Aff: on der(14) and der(17)



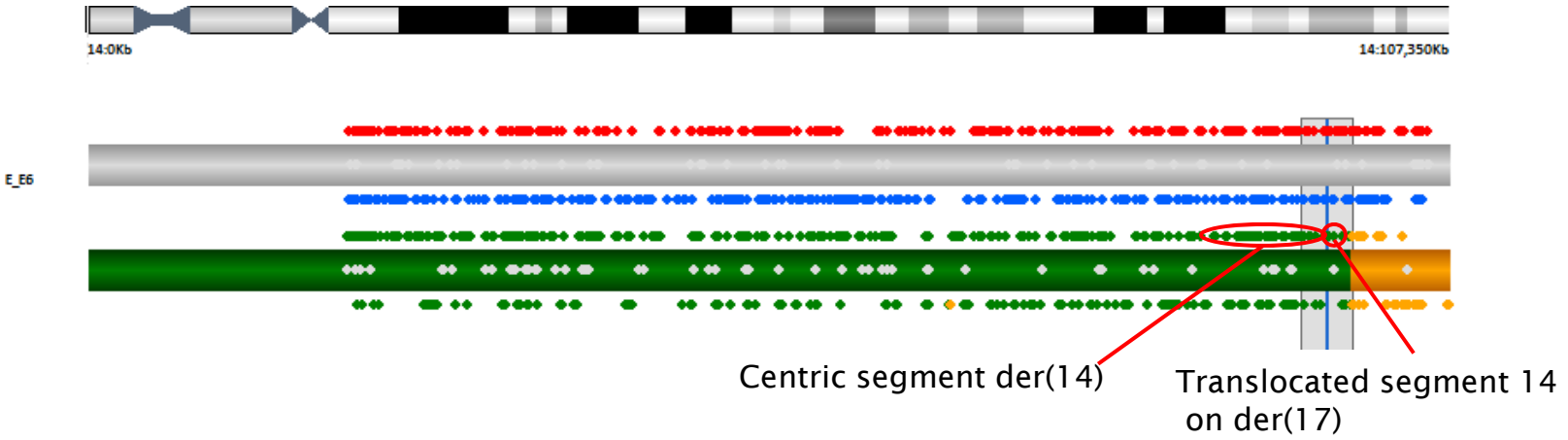


SNP array – Balanced t(14;17) carrier



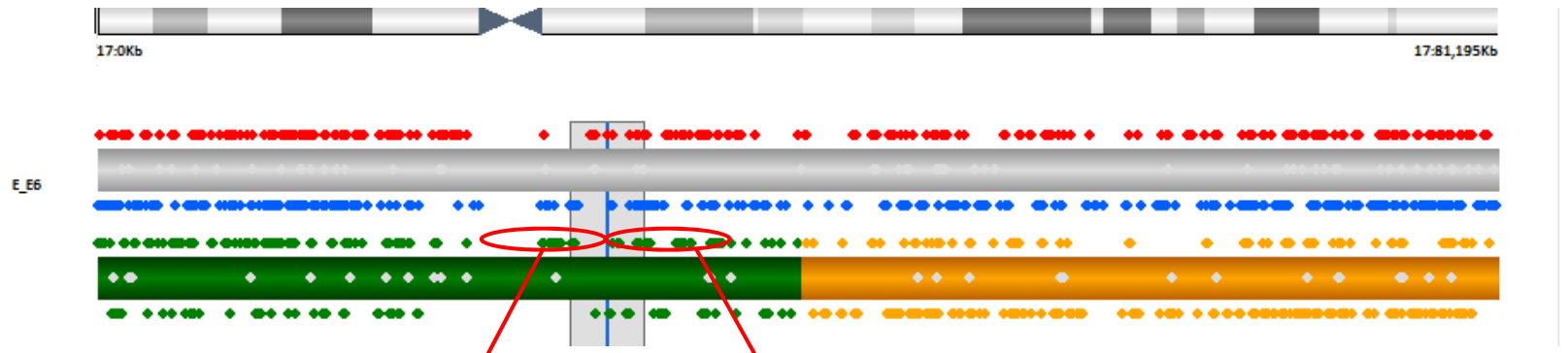
Wt

Aff: on der(14) and der(17)



Centric segment der(14)

Translocated segment 14 on der(17)



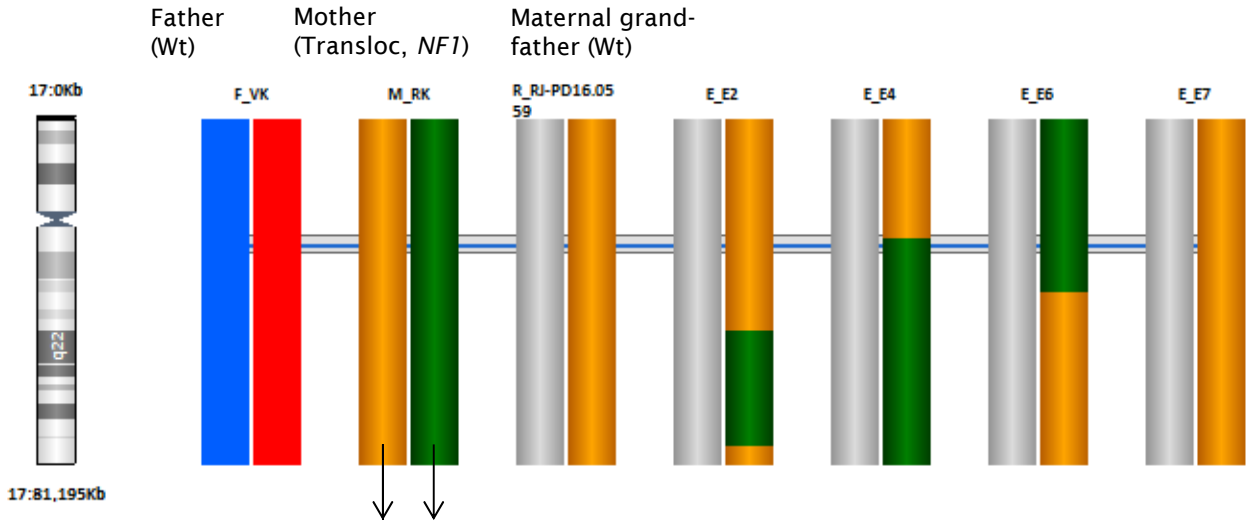
Centric segment der(17)

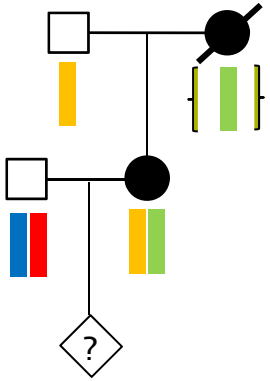
Translocated segment 17 on der(14)

Strengths and limitations of SNPa

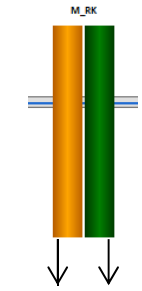
- Strengths:
  - → Uniparental disomy (UPD) can be detected.
  - → Normal and balanced segregations can be distinguished
  - → Inherited structural rearrangements with small exchanged segments (<5 Mb) can be diagnosed.
  - → Haploidy and polyploidy can be detected
  - → All chromosomes are tested
  - → Detection of PGT-SR can be combined with PGT-M or A
- Limitations:
  - → Workup is required. DNA samples from family members is required.
  - → Sensitivity for detection of *de novo* duplications and trisomies depends on the quality of the array data and the platform used

SNP array – example 46,XX,t(14;17)



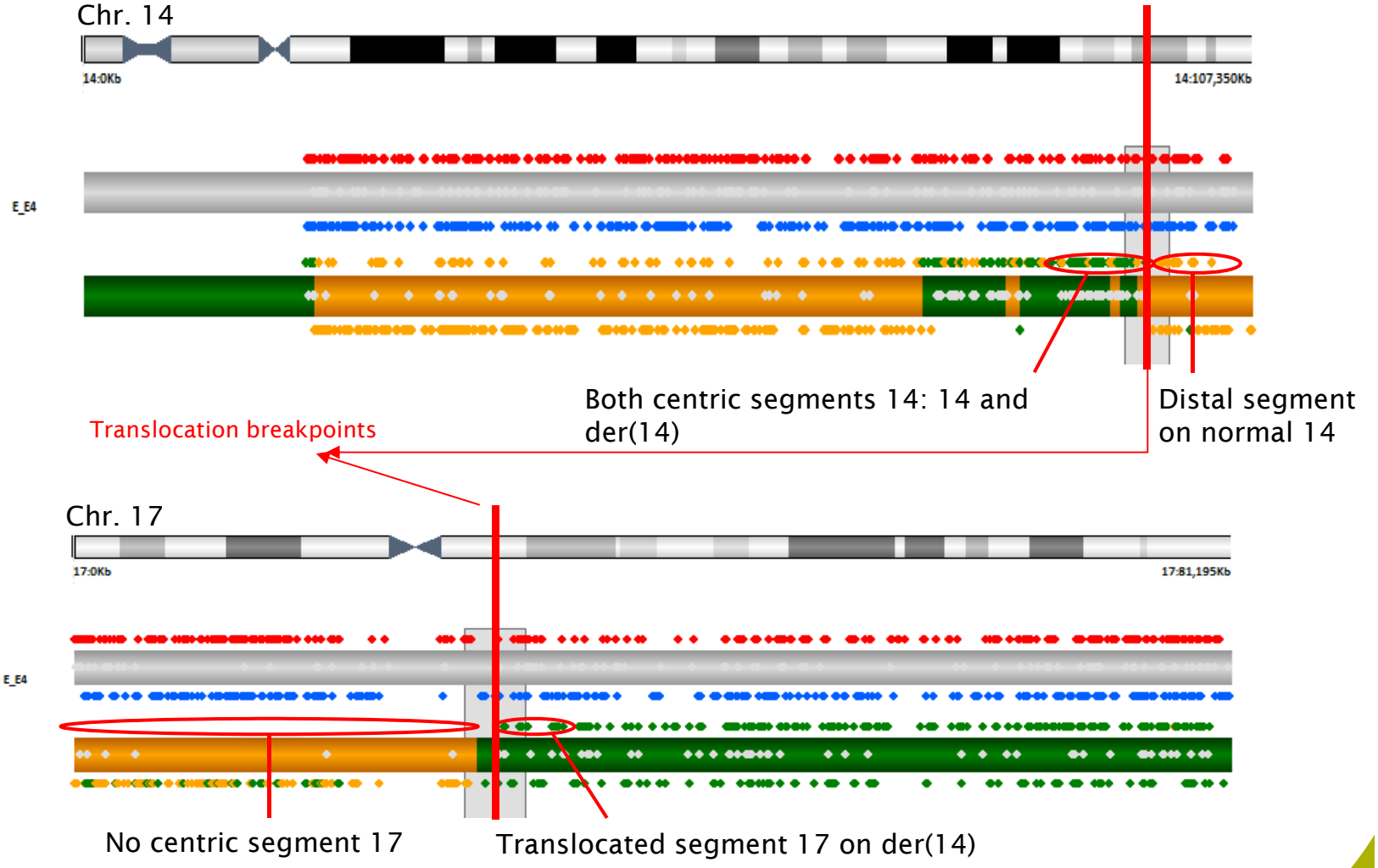


SNP array - Example

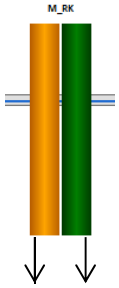


Wt

Aff: on der(14) and der(17)

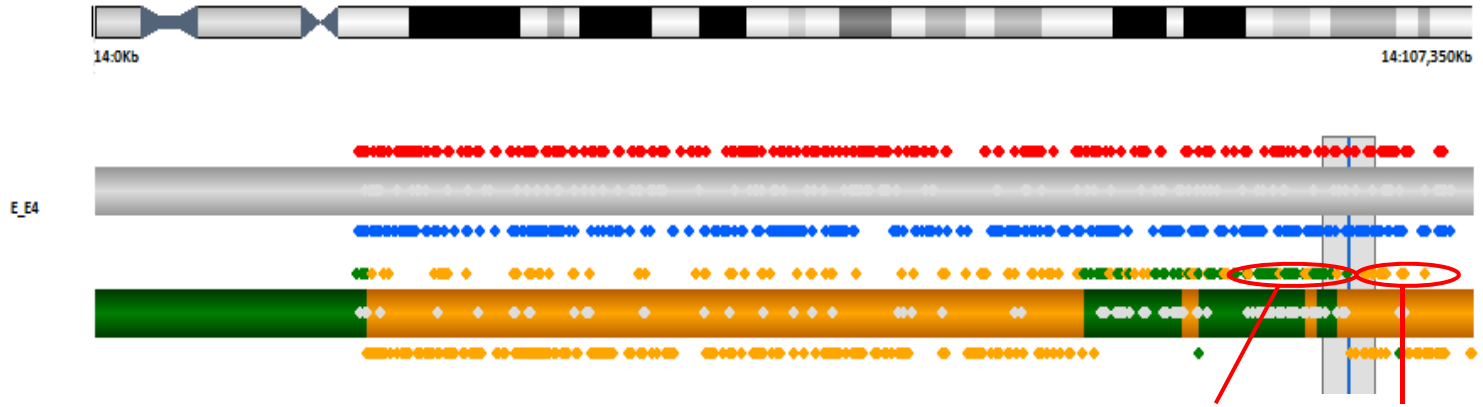


SNP array - Unbalanced



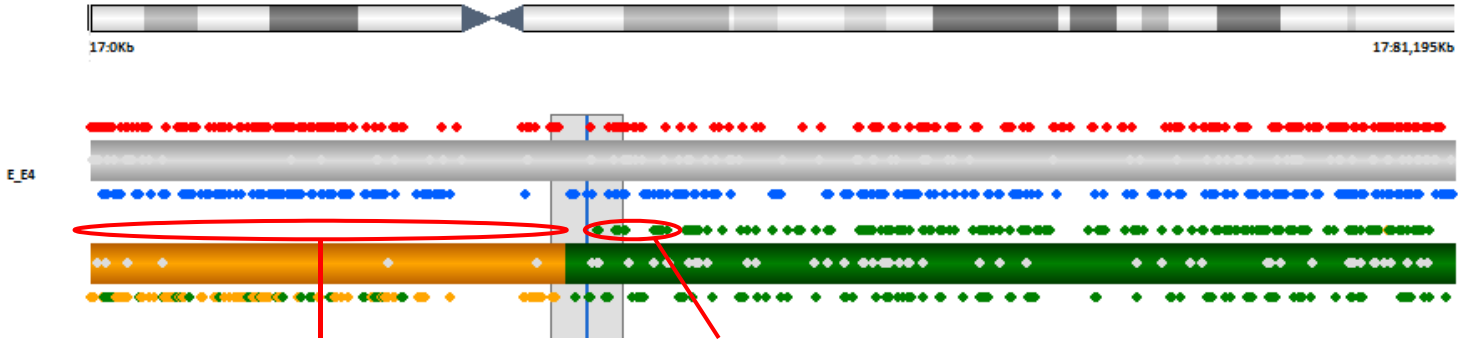
Wt

Aff: on der(14) and der(17)



Both centric segments 14: 14 and der(14)

Distal segment on normal 14



No centric segment 17

Translocated segment 17 on der(14)

Segregations for a reciprocal transloc.

Tetravalent

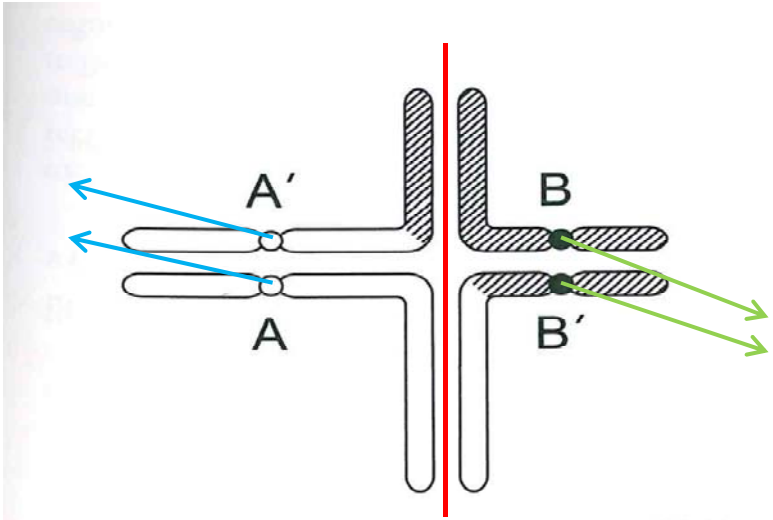


FIGURE 5–2 Pachytene configuration, simplified outline. The two normal (A, B) and the two translocation (A′, B′) homologs align corresponding segments of chromatin during meiosis I.

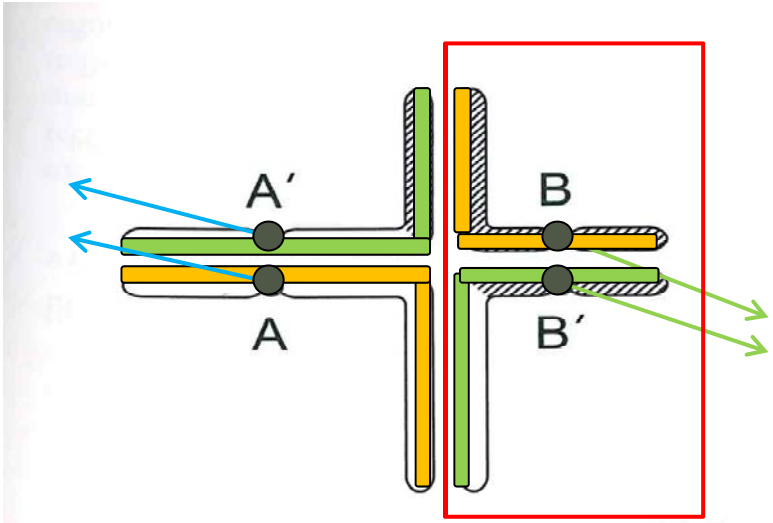
Table 5–1.

ONE DAUGHTER GAMETOCYTE WITH:	OTHER DAUGHTER GAMETOCYTE WITH:	SEGREGATION MODE
2:2 Segregations		
A and B	A′ and B′	Alternate segregation
A and B′	B and A′	Adjacent-1 segregation
A and A′	B and B′	Adjacent-2 segregation
3:1 Segregations		
A B A′	B′	3:1 segregation with tertiary trisomy or monosomy
A B and B′	A′	
A′ B′ and A	B	3:1 segregation with interchange trisomy or monosomy
A′ B′ and B	A	
4:0 Segregation		
A B A′ B′	None	4:0 segregation with double trisomy or monosomy

From Gardner and Amor, 'Chromosome abnormalities and genetic counseling' 5th edition, Oxford University press 2018.

Segregations for a reciprocal transloc.

Tetravalent



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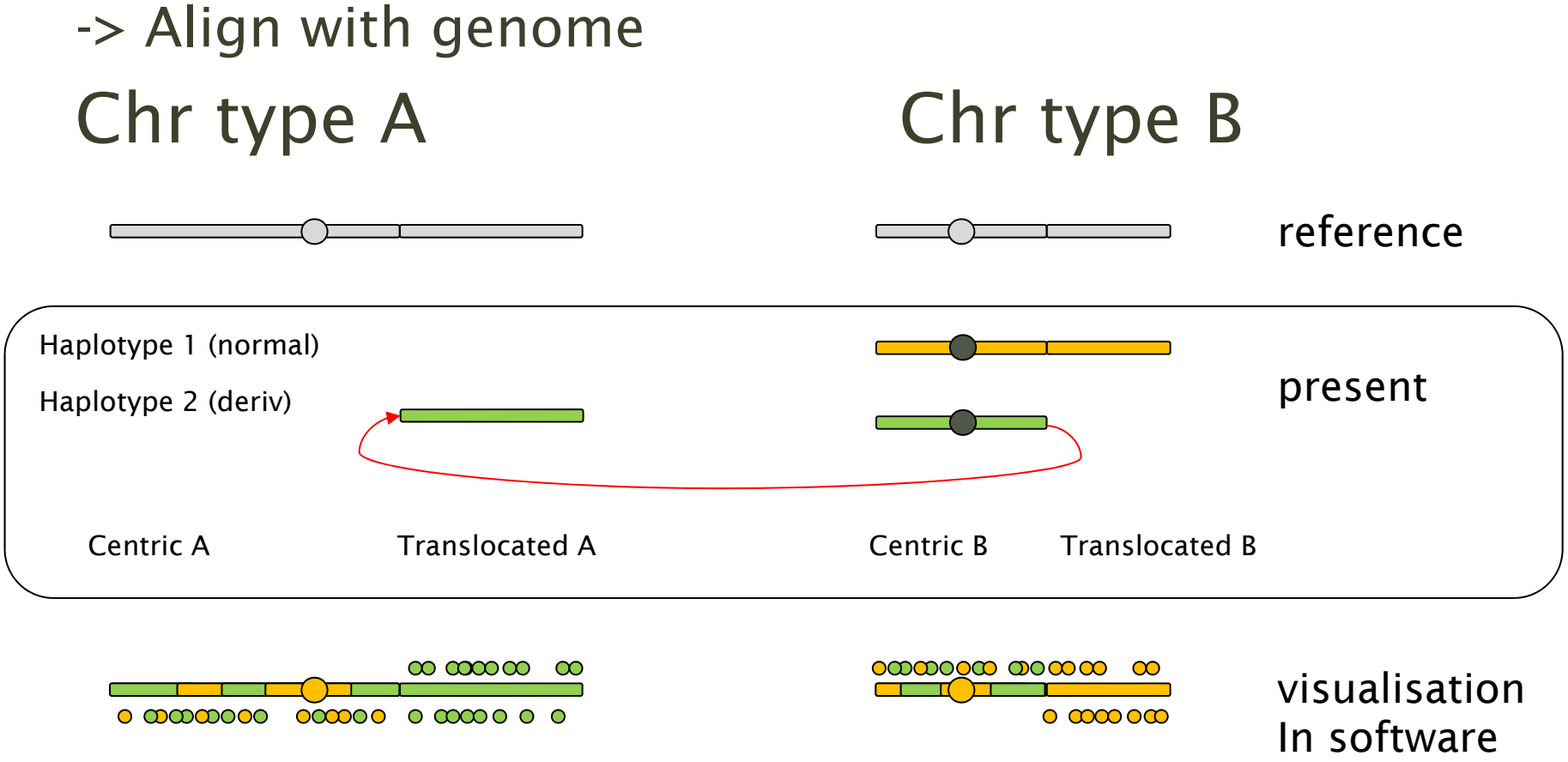
From Gardner and Amor, 'Chromosome abnormalities and genetic counseling' 5th edition, Oxford University press 2018.

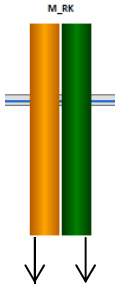
Segregations for a reciprocal transloc.

-> Align with genome



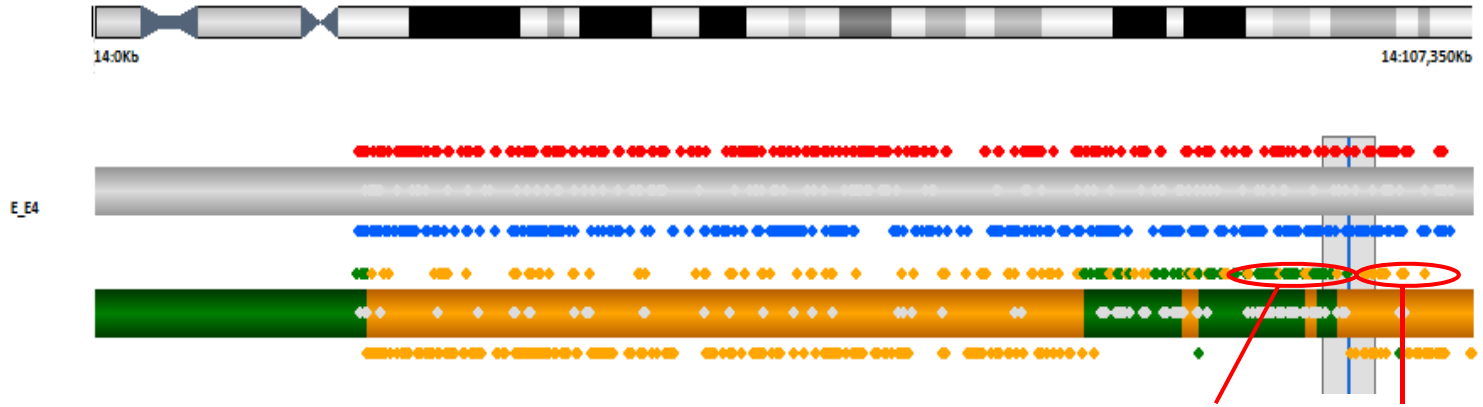
B + B' => Adjacent 2 segregation

SNP array - Unbalanced



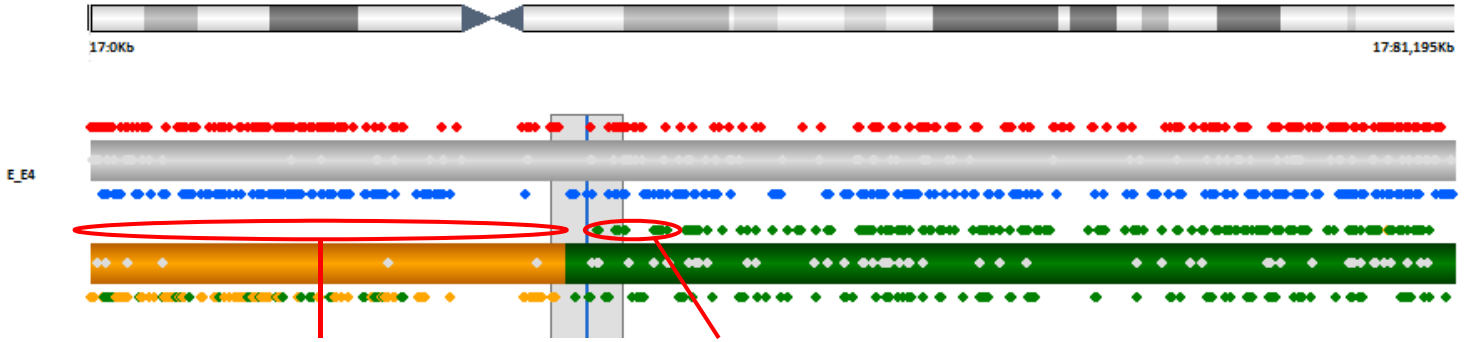
Wt

Aff: on der(14) and der(17)



Both centric segments 14: 14 and der(14)

Distal segment on normal 14



No centric segment 17

Translocated segment 17 on der(14)

Outline

- PGS vs PGD, PGT-A vs PGT-SR
- Technologies
  - → FISH
  - → Array CGH
  - → Shallow Whole Genome Sequencing
  - → SNP array
  - → Genotyping by sequencing
- Segregations of translocations

Genotyping by sequencing

- High coverage sequencing allows to determine genotypes
- Cost can be reduced by sequencing only part of the genome
  → Exome sequencing
  → Reduced representation sequencing
- Similar data compared to SNP array
- Sequencing cost has been limiting use to date