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Principles of clinical cytogenetics and Genome analysis

Joris Vermeesch

October 2021,
Interuniversity course in human genetics
Thompson & Thompson chapter 5

Overview

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- Introduction
- Chromosomal rearrangments
- Technologies for CNV detection
- Clinical consequences
- Mechanisms of origin

Chromosomal abnormalities

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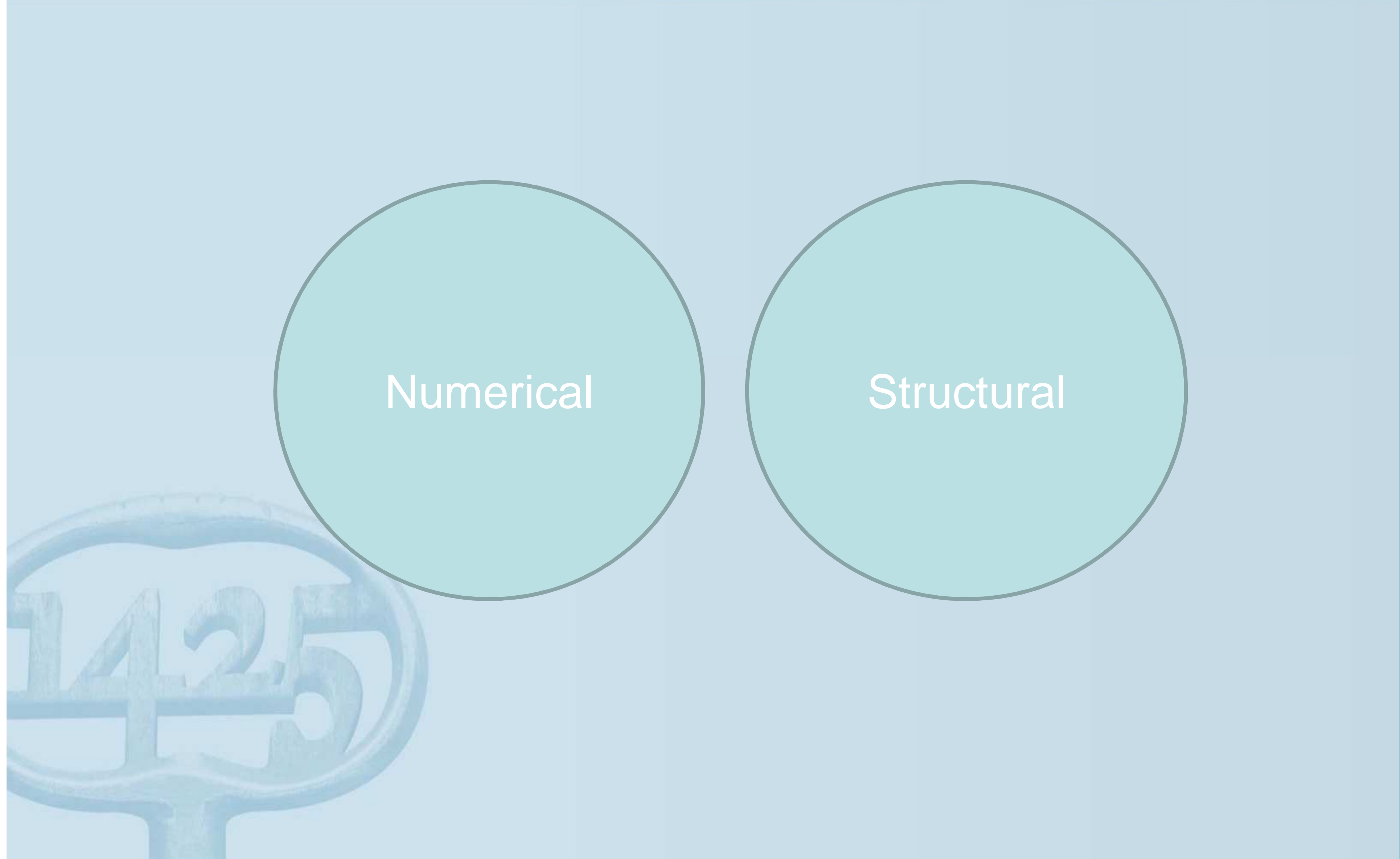

Numerical chromosomal abnormalities

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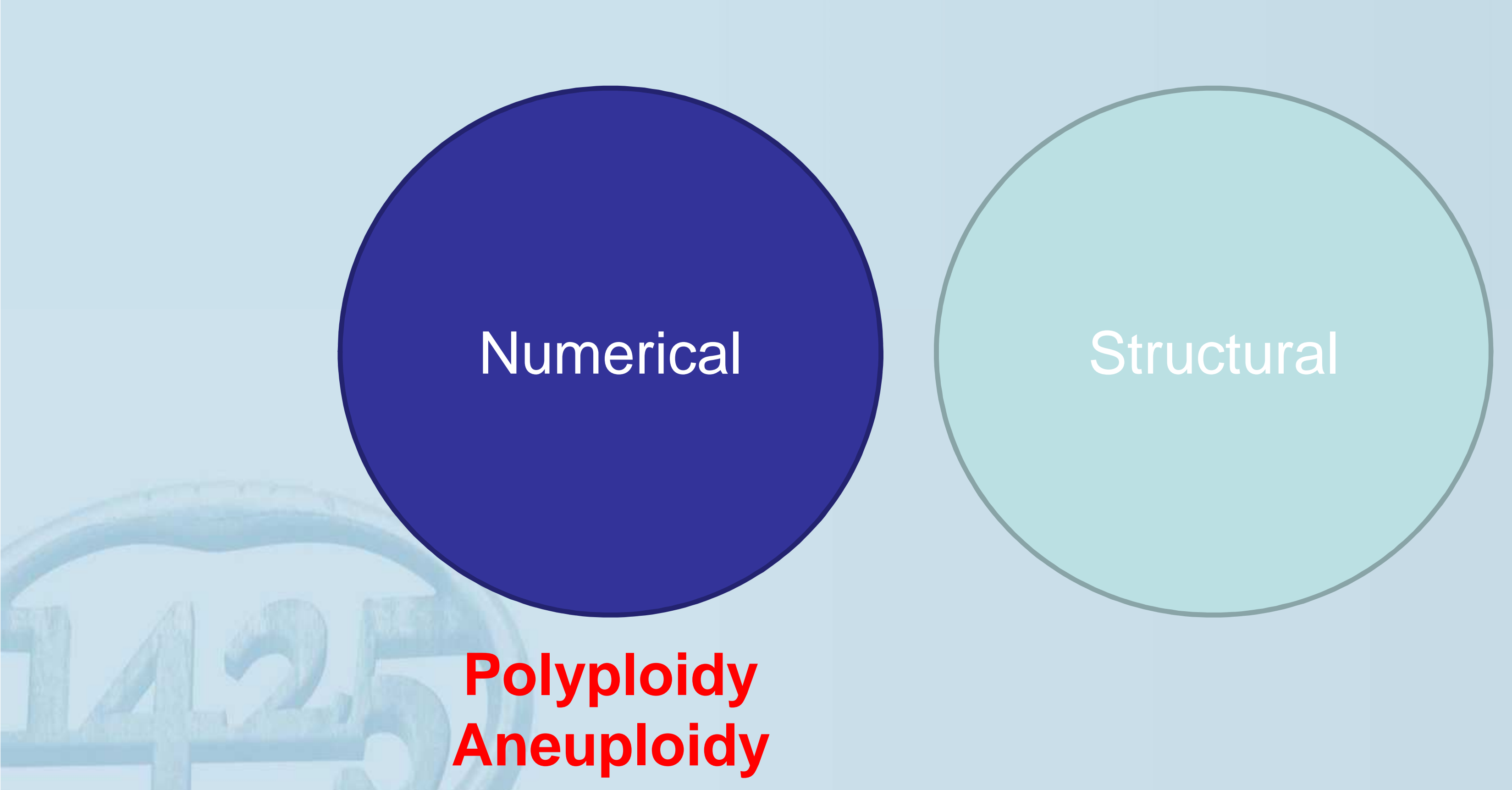
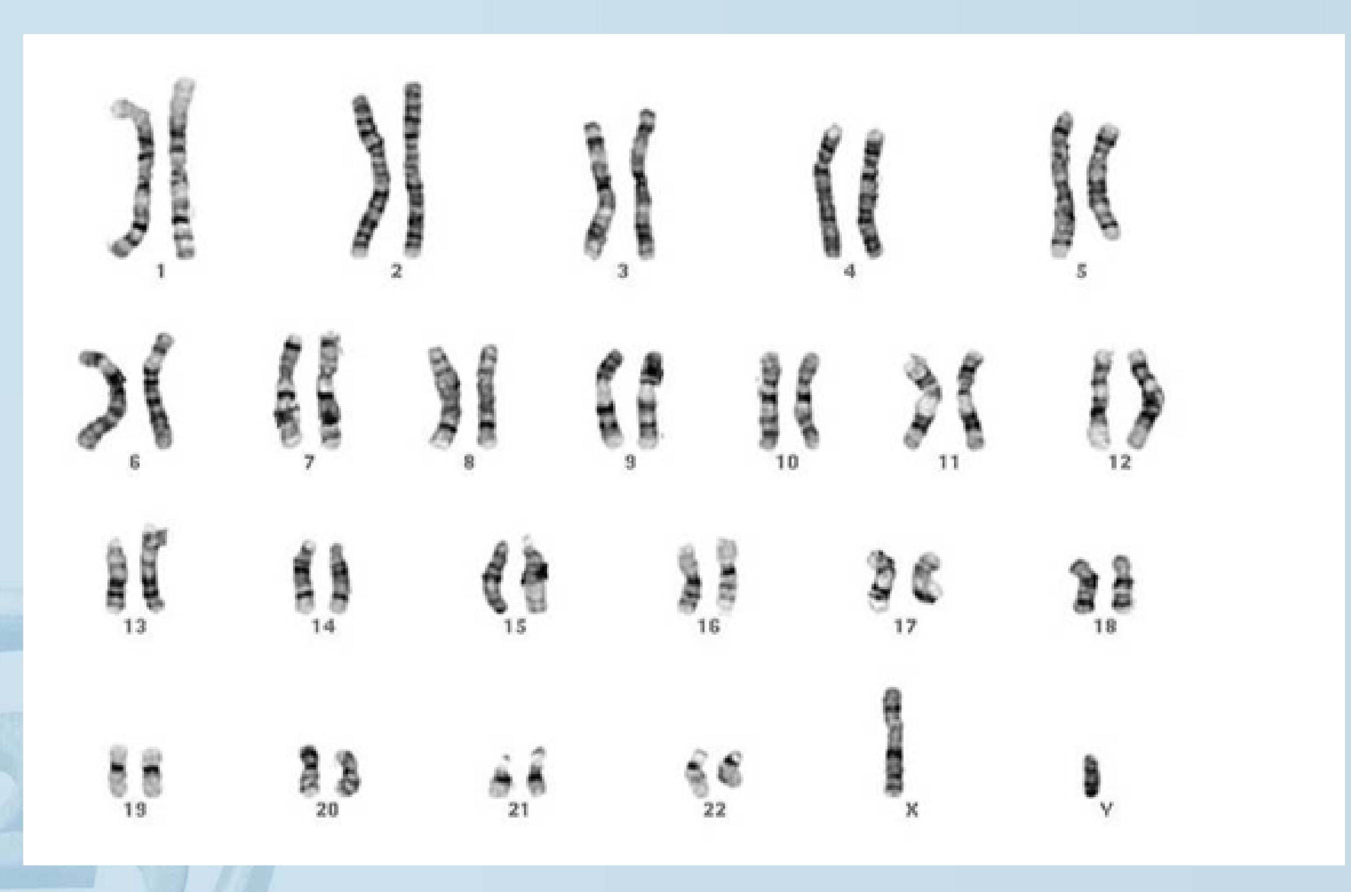


Normal Diploid



Triploidy

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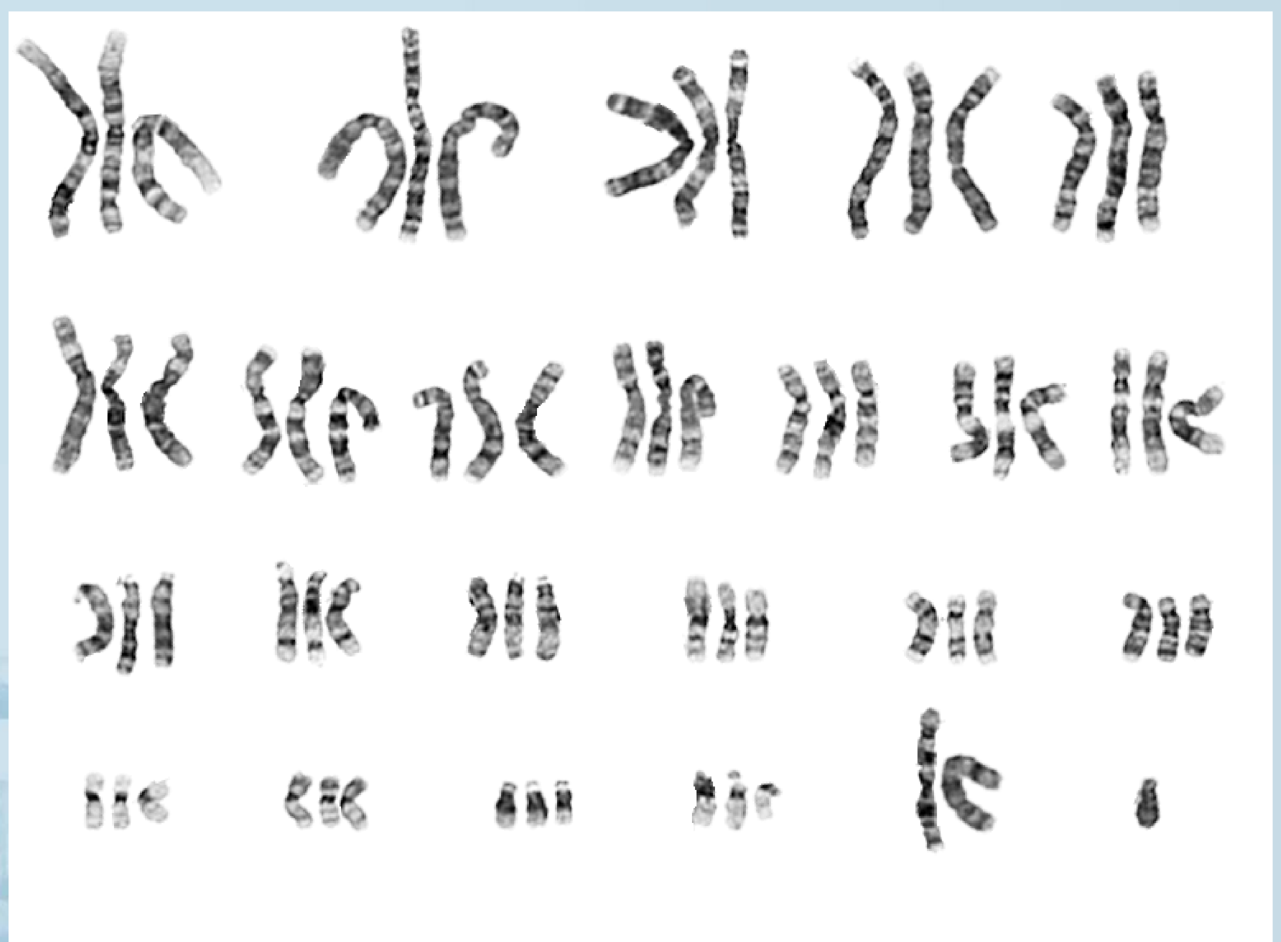

1-3% of all pregnancies
15-20% of all chromosomally abnormal miscarriages

Triploidy phenotype

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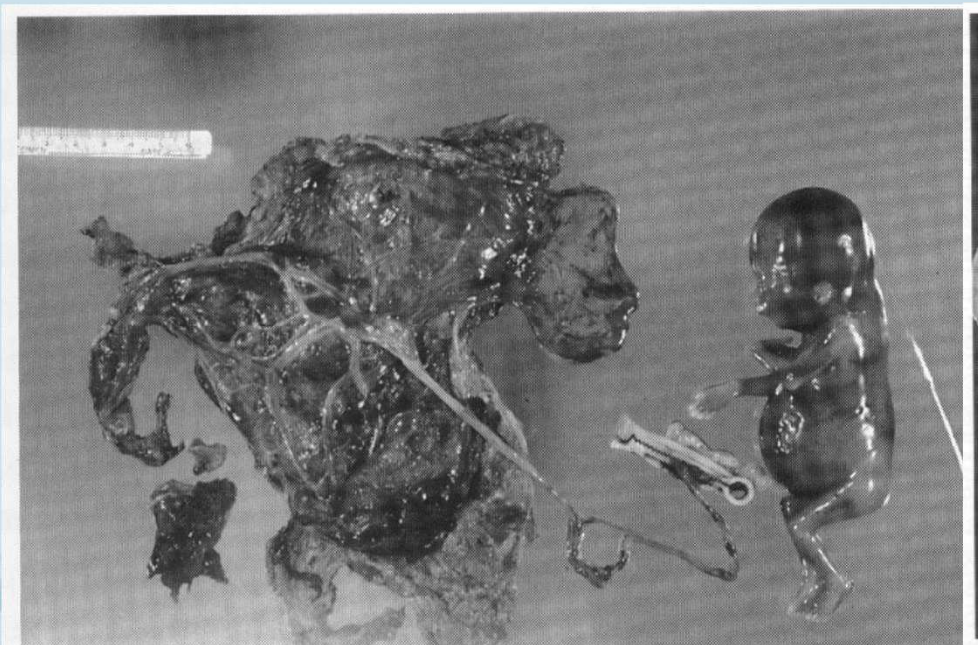
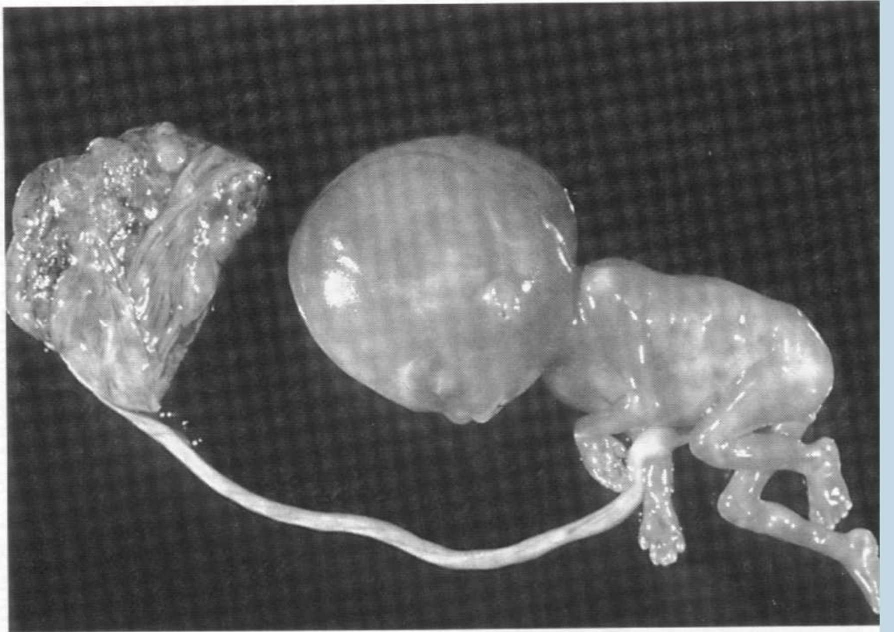
Paternal triploidy (diandry)

(often partial hydatiform moles)

Maternal triploidy (digyny)

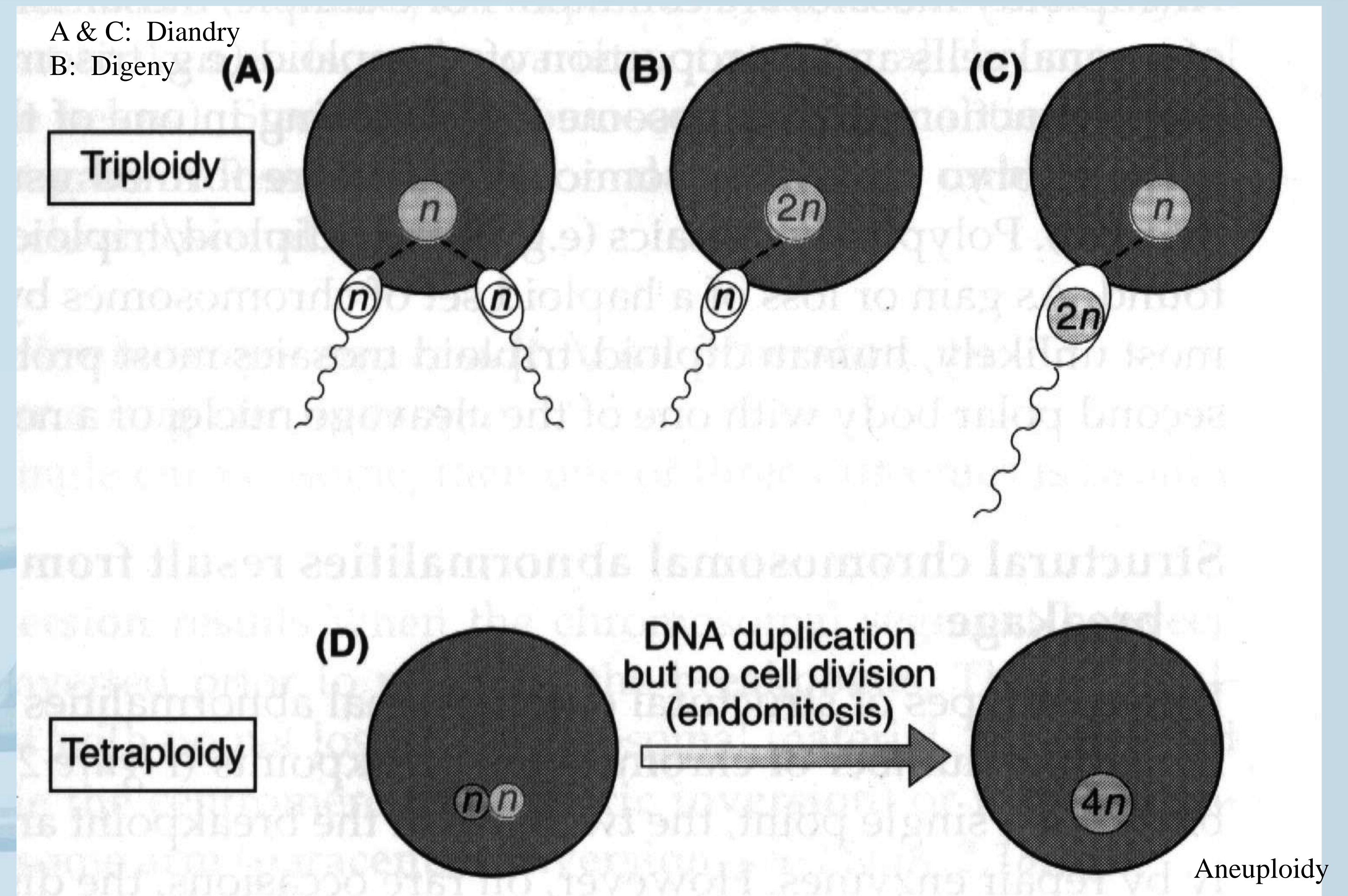
(aborted during early pregnancy)





The phenotype of a triploïde foetus is dependent on the parental origin (maternal or paternal). Dit verschil wordt veroorzaakt door imprinting.

Origins of Triploidy



Haploidy

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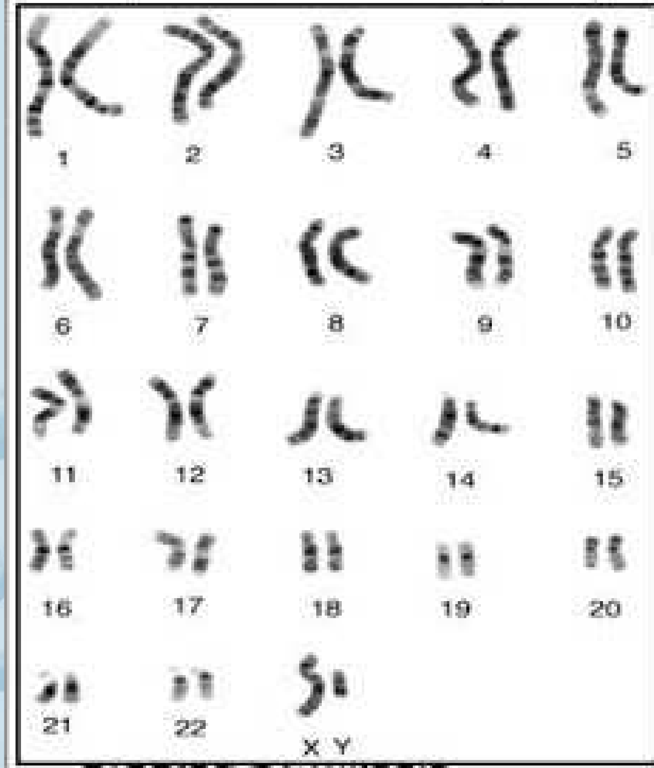
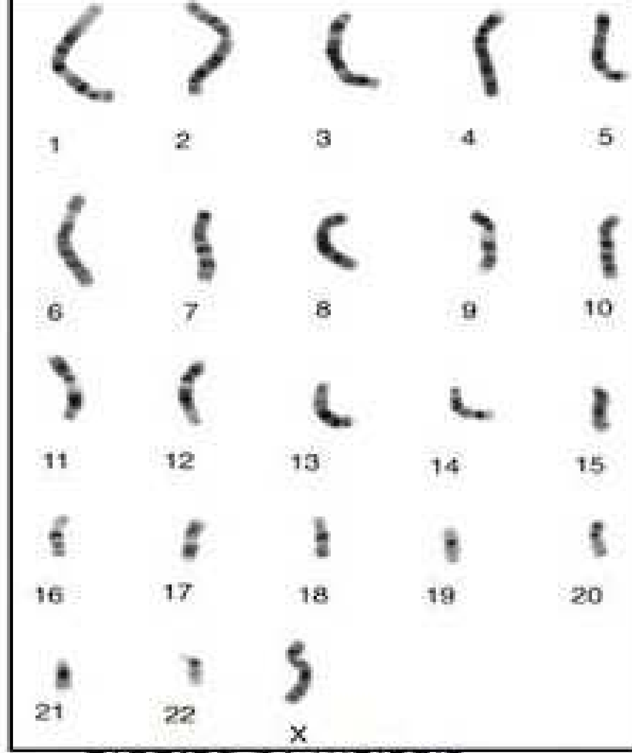


Haploid Cells (n)



Phenotypes of maternal or paternal only genomes (endoreplicated haploid)

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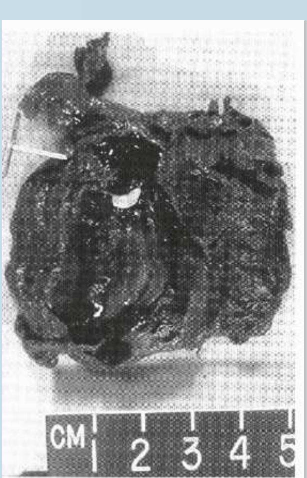
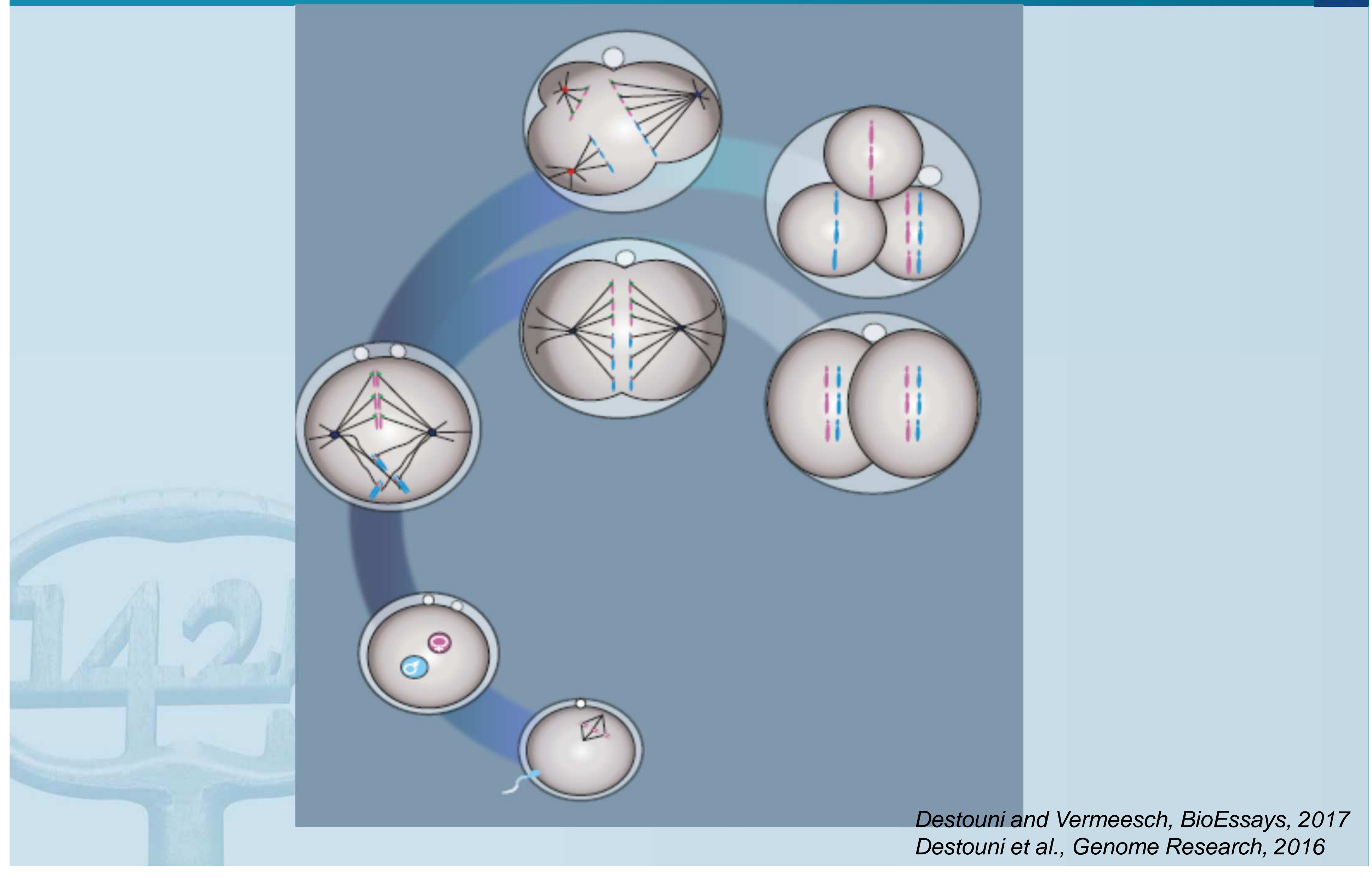
- ***Ovarium teratoma***: Germ line tumors with only maternal genome. (parthenogenetic?).

- ***Mola hydatiformis (schijnzwangerschap)***: Only the development of a trophoblast but not of a fetus. Contains only a paternal chromosome.



“Heterogoneic” genome segregation: Maternal and paternal genomes segregate?



Destouni and Vermeesch, BioEssays, 2017
Destouni et al., Genome Research, 2016

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Tetraploidy

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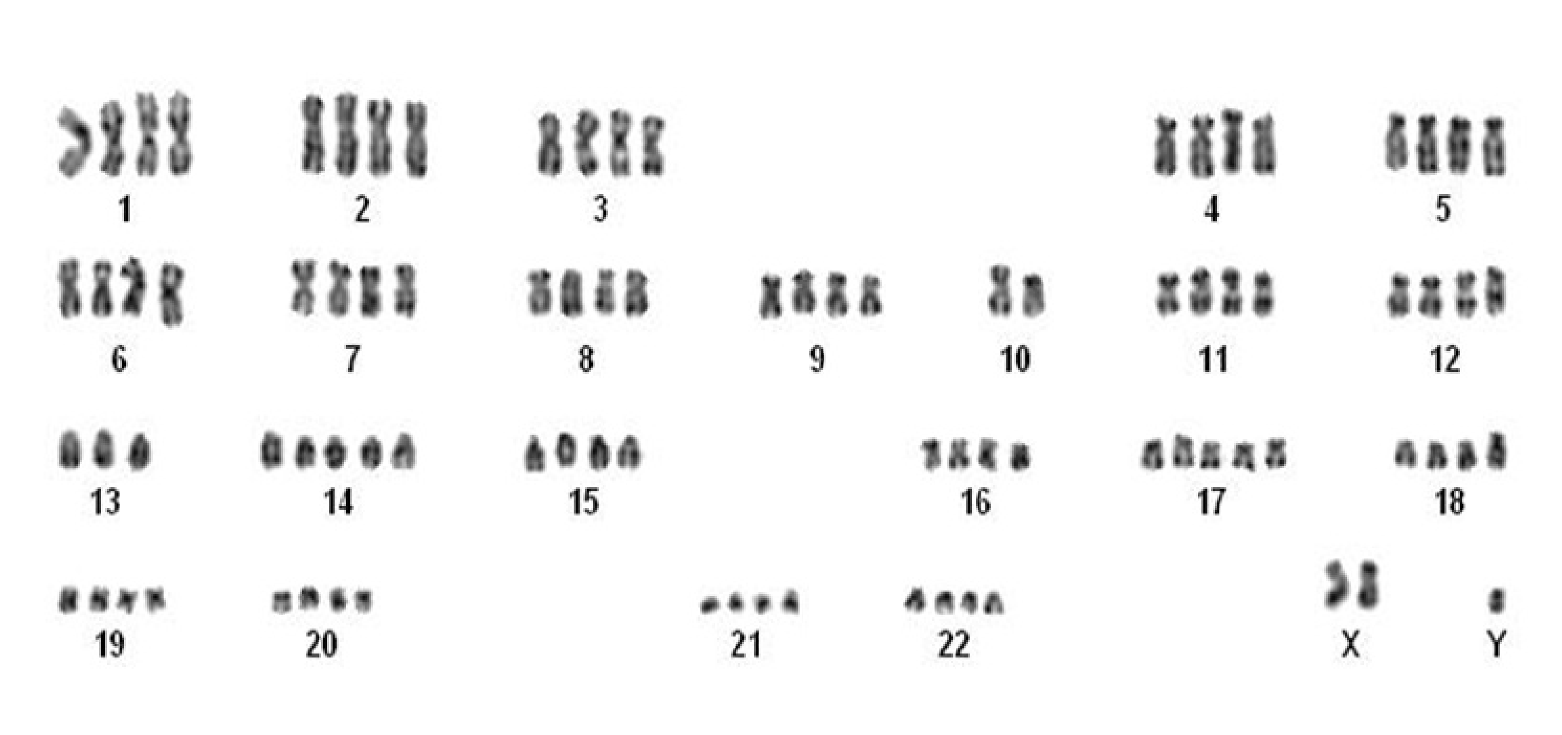
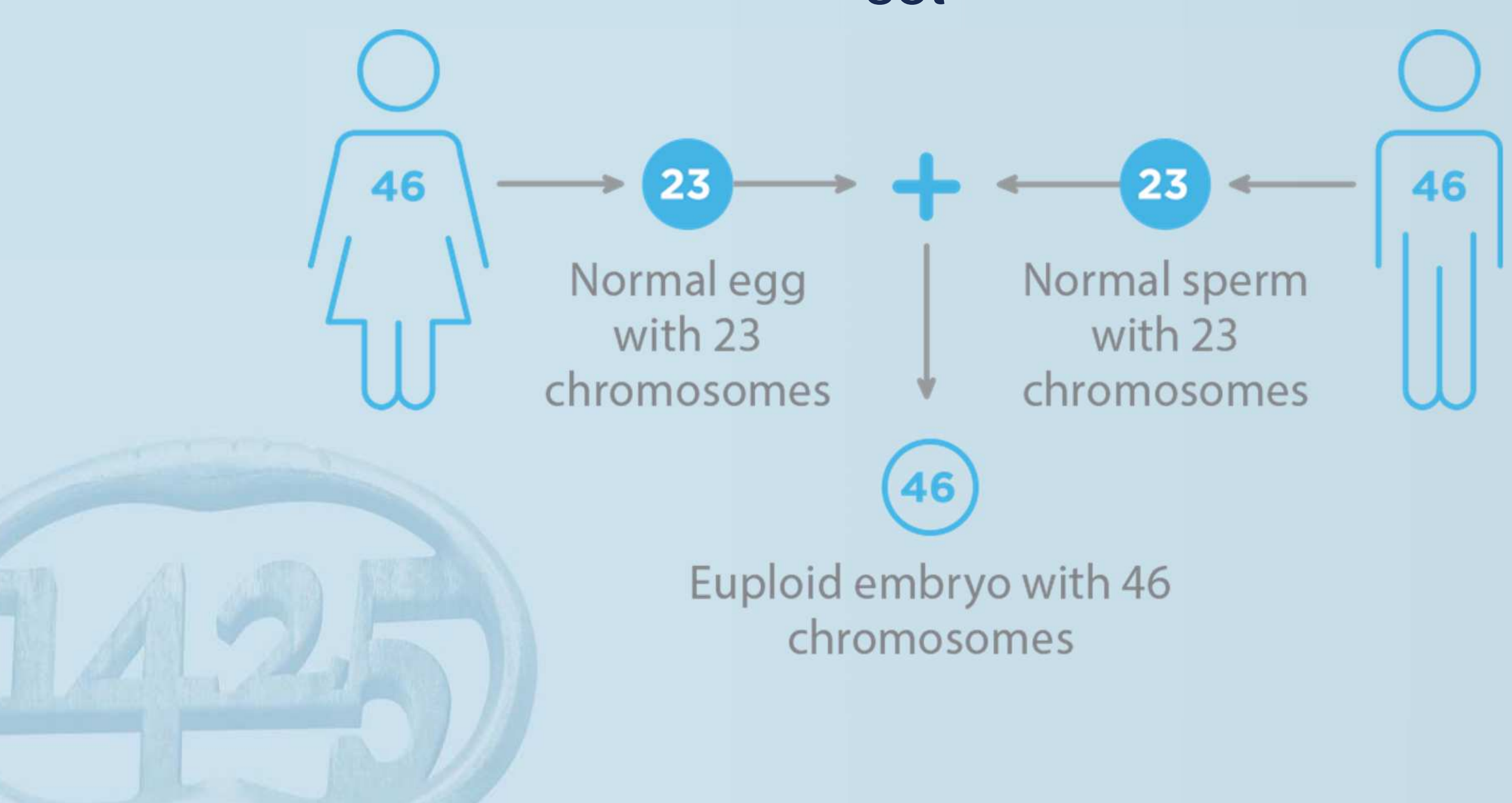


Likely the result of failure of
completion of early zygotic division

Aneuploidy

Variation in the number of particular chromosomes within a set



Aneuploidy

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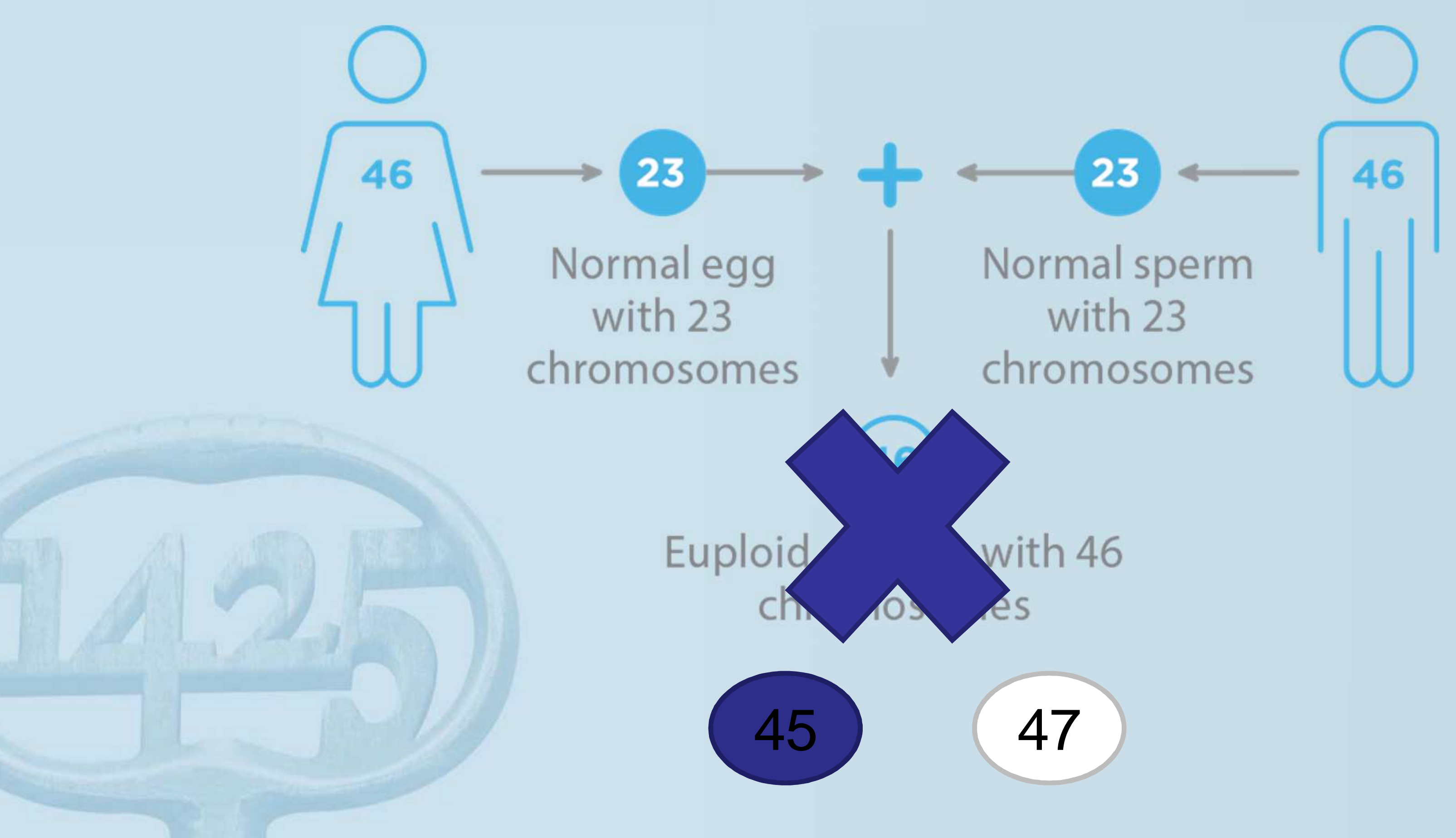
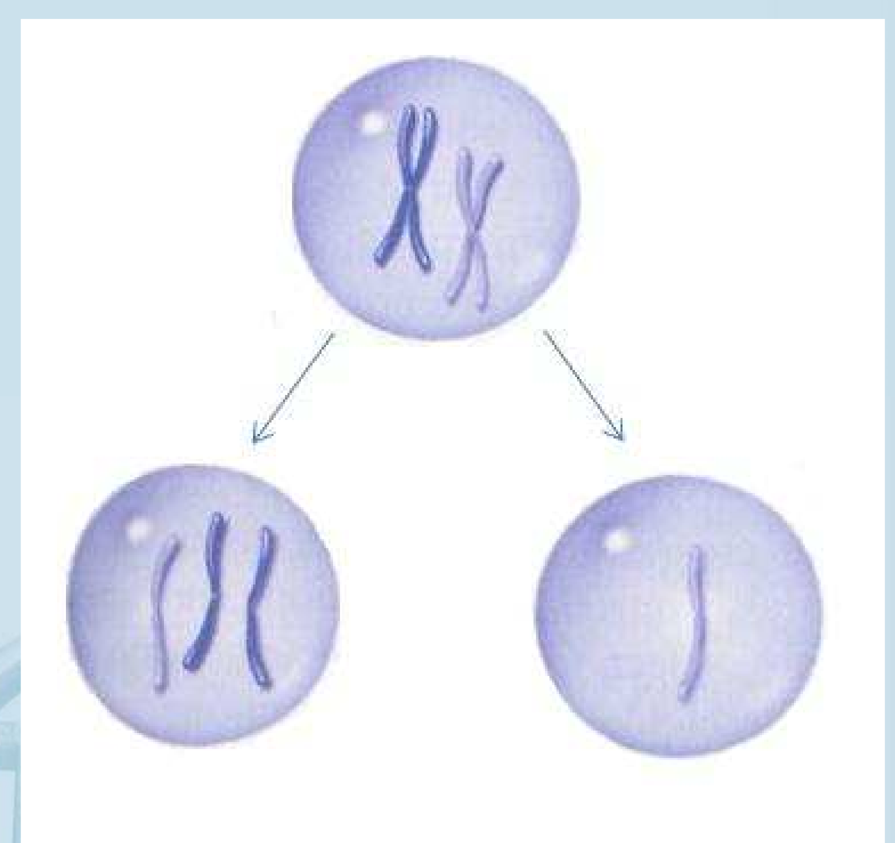
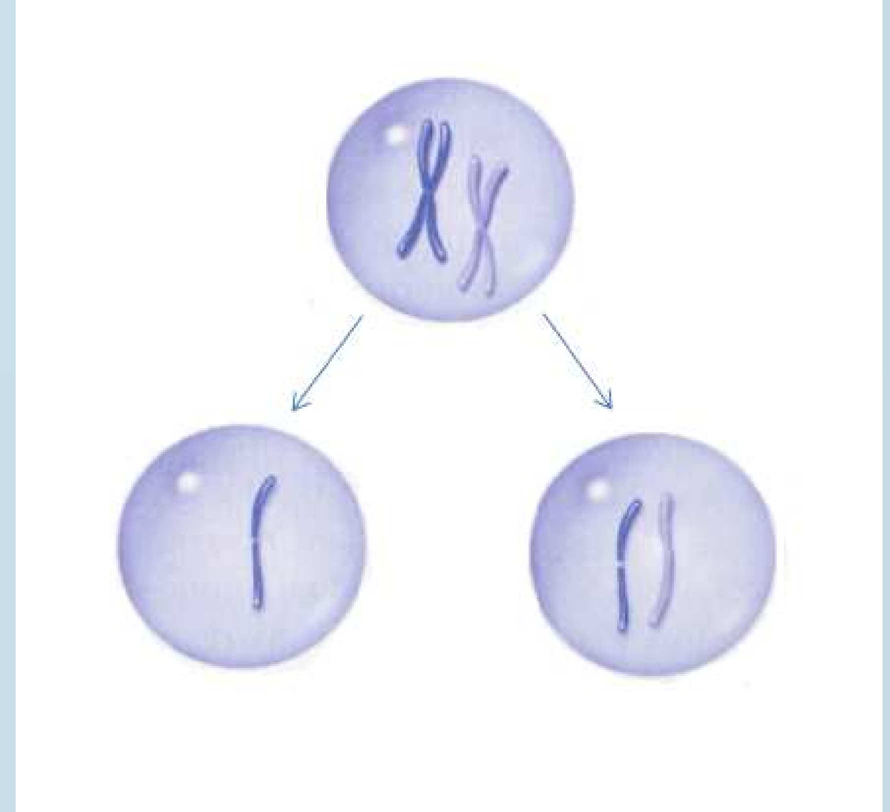
- Variation in the number of particular chromosomes within a set



Aneuploidy due to mitotic errors



Chromosome gain and loss due to **non-disjunction**



Chromosome loss due to **anaphase lagging**

Trisomy is the most frequent genetic anomaly in human and the most important cause of miscarriages

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Chromosome abnormality	Frequency of abnormality (%) Spontaneous abortions	Stillbirths	Livebirths	Probability of abnormal fetus surviving to term (%)
All abnormalities	50	5	0.5	5
Trisomy: 16	7.5	-	-	0
13, 18, 21	4.5	2.7	0.14	15
XXX, XXY, XYY	0.3	0.4	0.15	75
All others	13.8	0.9	-	0
Sex chromosome monosomy (45, X)	8.7	0.1	0.01	1
Triploidy	6.4	0.2	-	0
Tetraploidy	2.4	-	-	0
Structural abnormality	2.0	0.8	0.3	45

Trisomy totals (bracketed): Spontaneous abortions 26; Stillbirths 4; Livebirths 0,3

Hassold, 1986

Incidence of chromosome abnormalities in newborns

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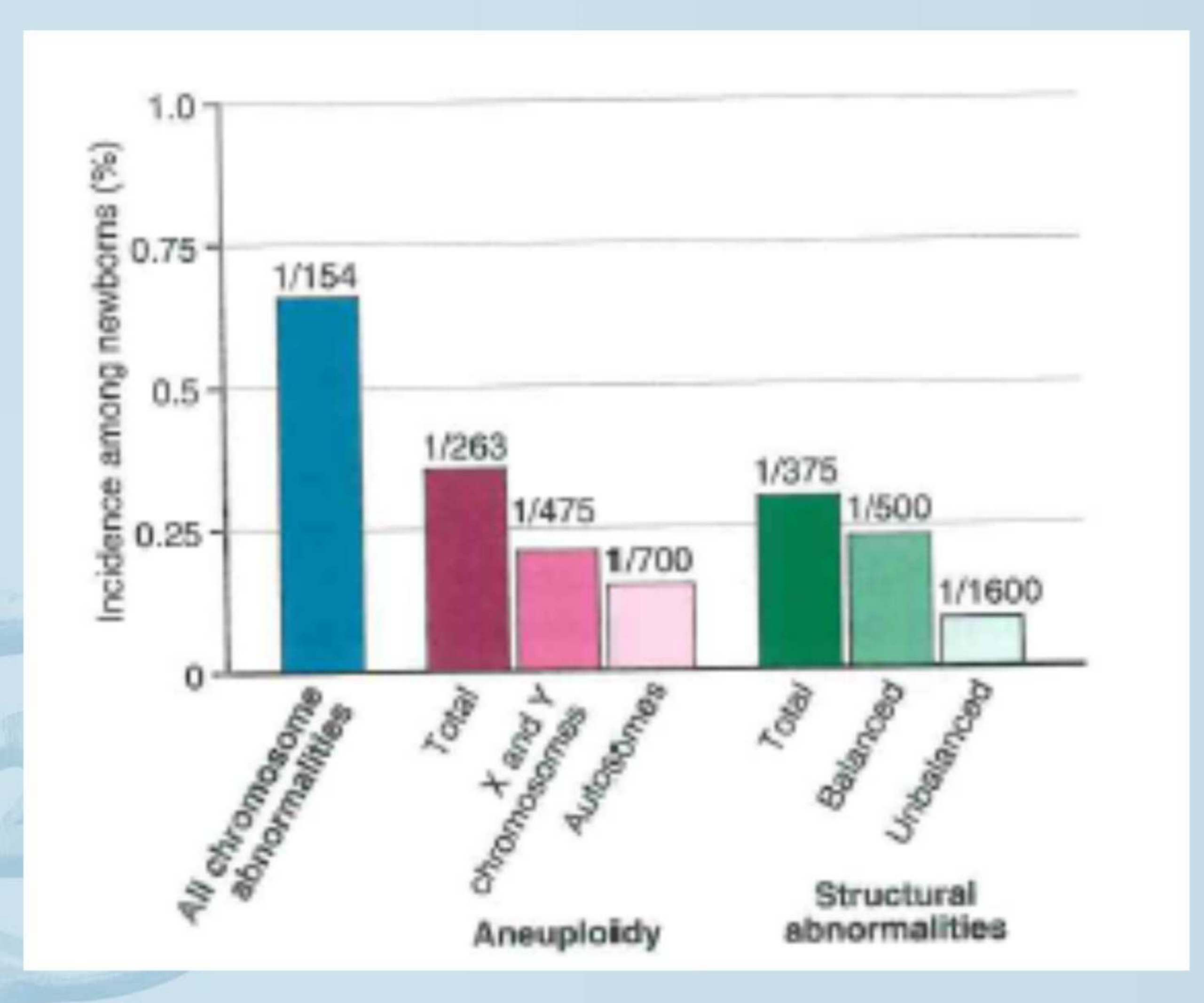
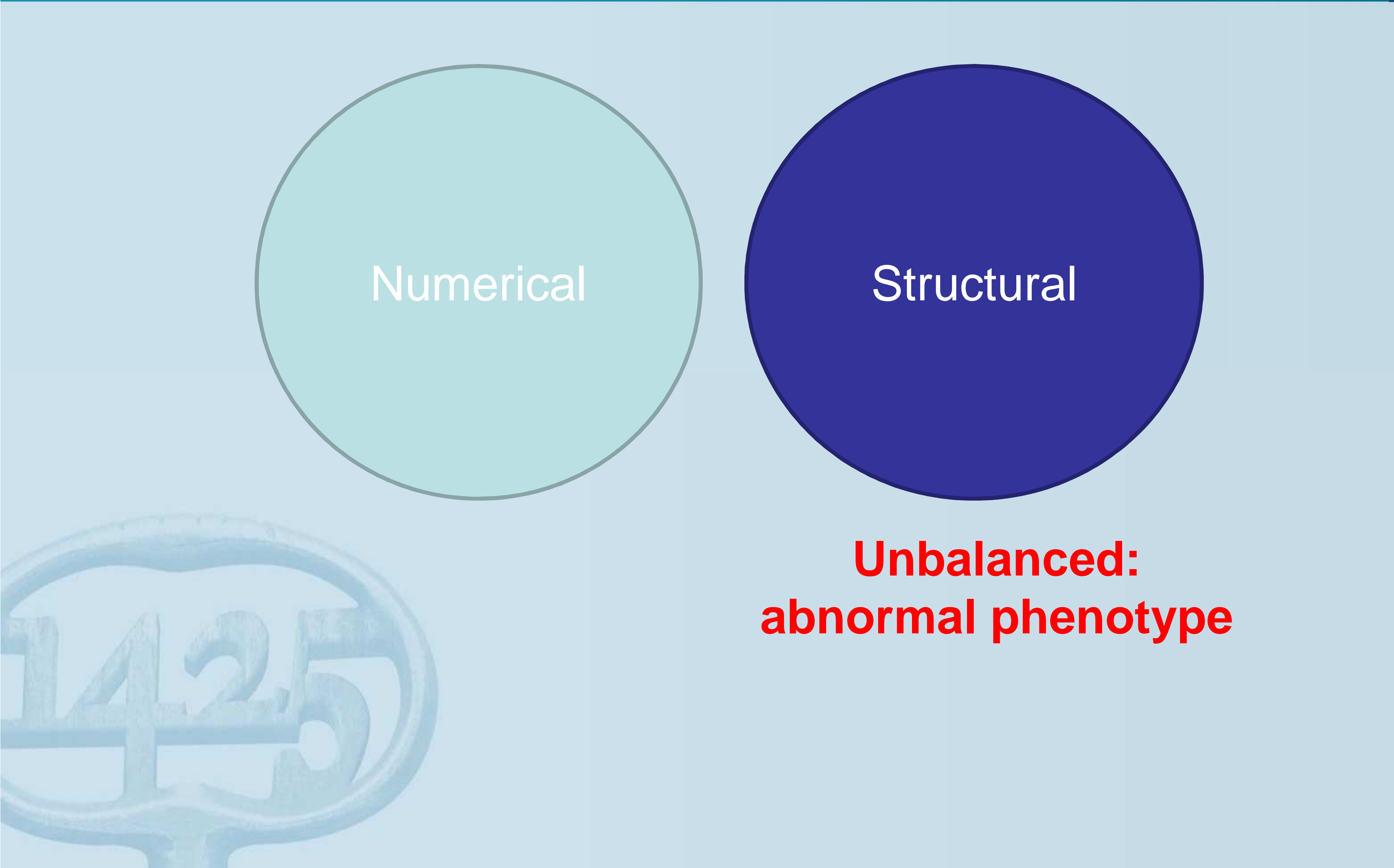


Unbalanced rearrangements



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Chromosomal rearrangements resulting in copy number variation

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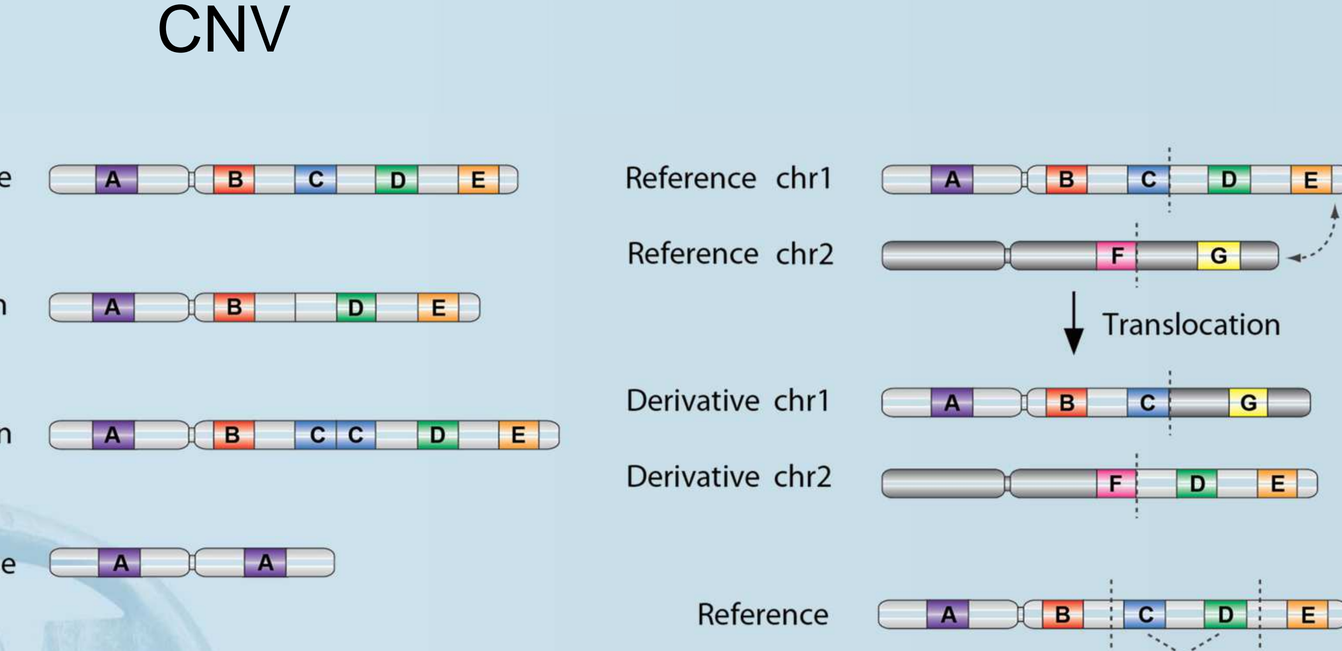
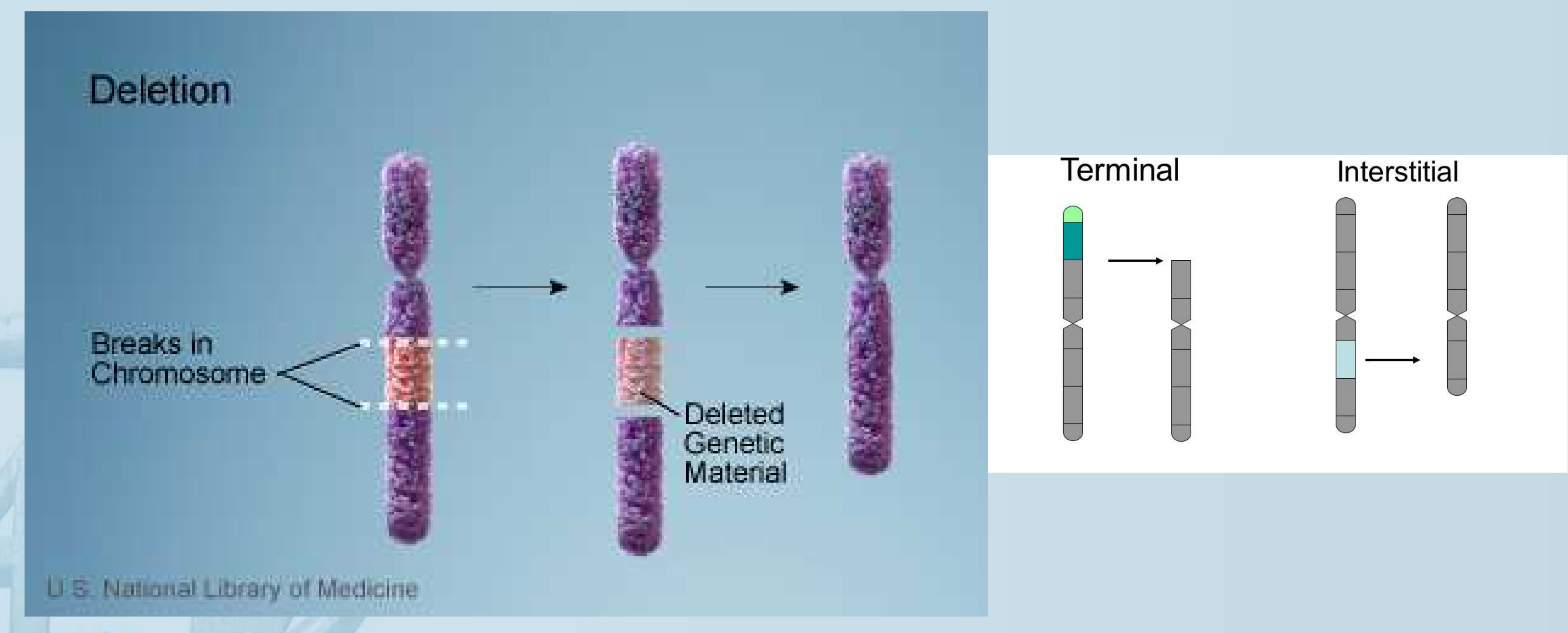


Deletions

1. Deletions: may be terminal or interstitial

The clinical effect depends on the size of the deleted segment and the number and function of the genes it coded for



Duplications

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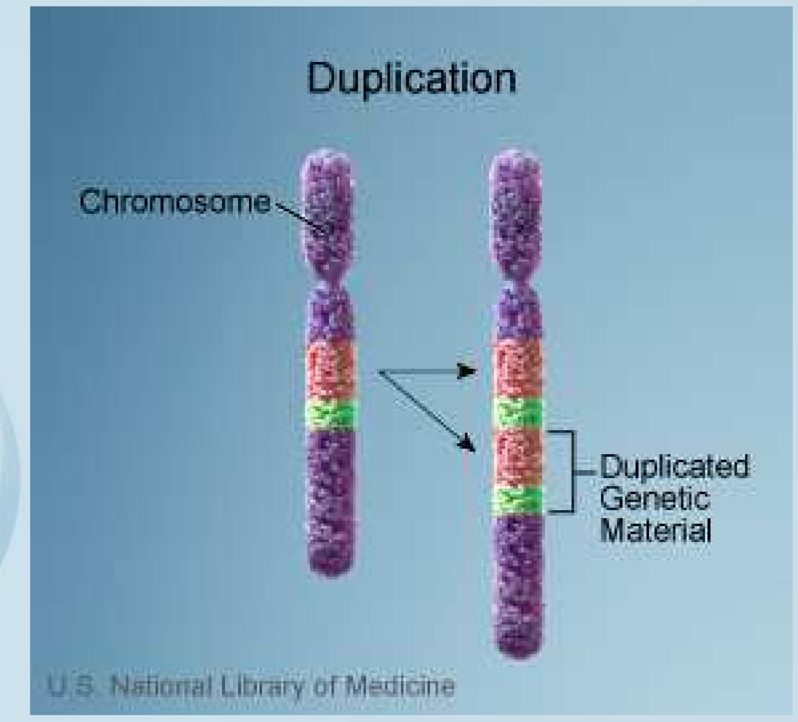
1. Deletions: may be terminal or interstitial

The clinical effect depends on the size of the deleted segment and the number and function of the genes it coded for

2. Duplications: Less harmful than deletions



Ring Chromosome

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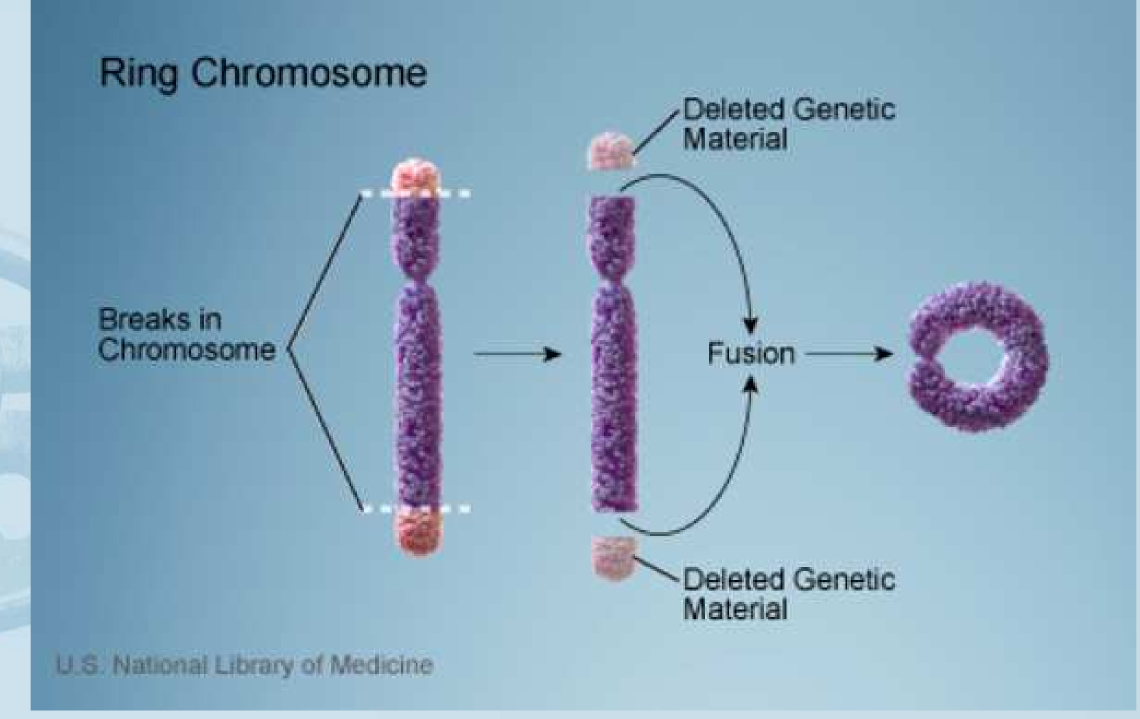
1. Deletions: may be terminal or interstitial

The clinical effect depends on the size of the deleted segment and the number and function of the genes it coded for

2. Duplications: Less harmful than deletions

3. Ring chromosomes: chromosome undergoes two breaks and the broken ends unite



Isochromosomes

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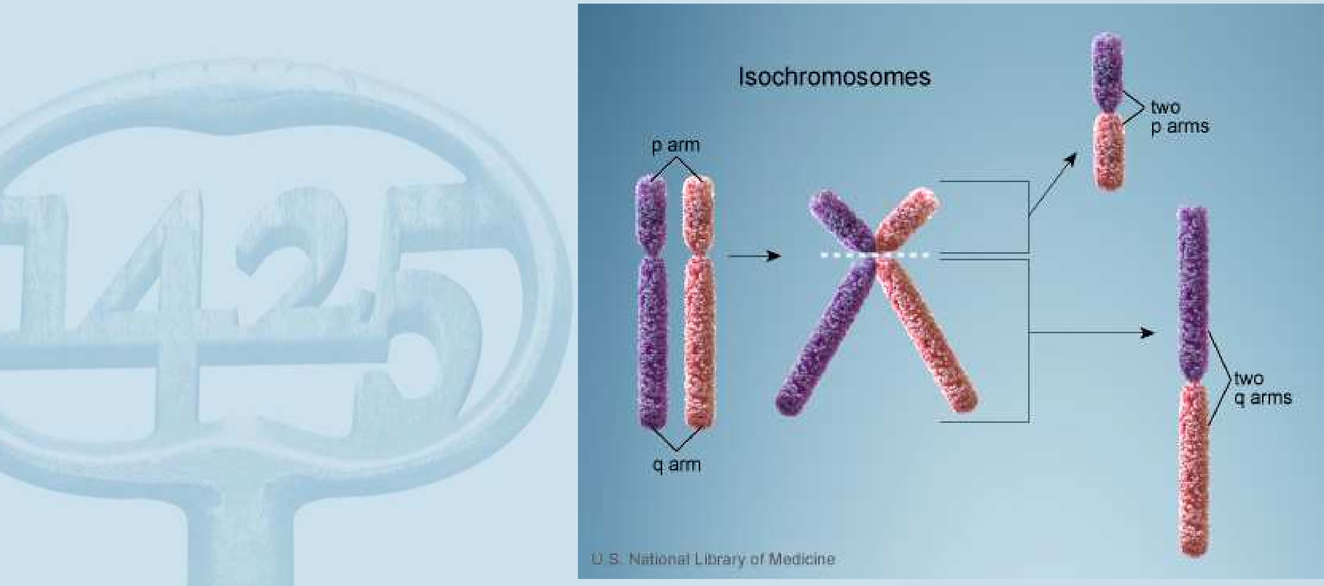
1. Deletions: may be terminal or interstitial

The clinical effect depends on the size of the deleted segment and the number and function of the genes it coded for

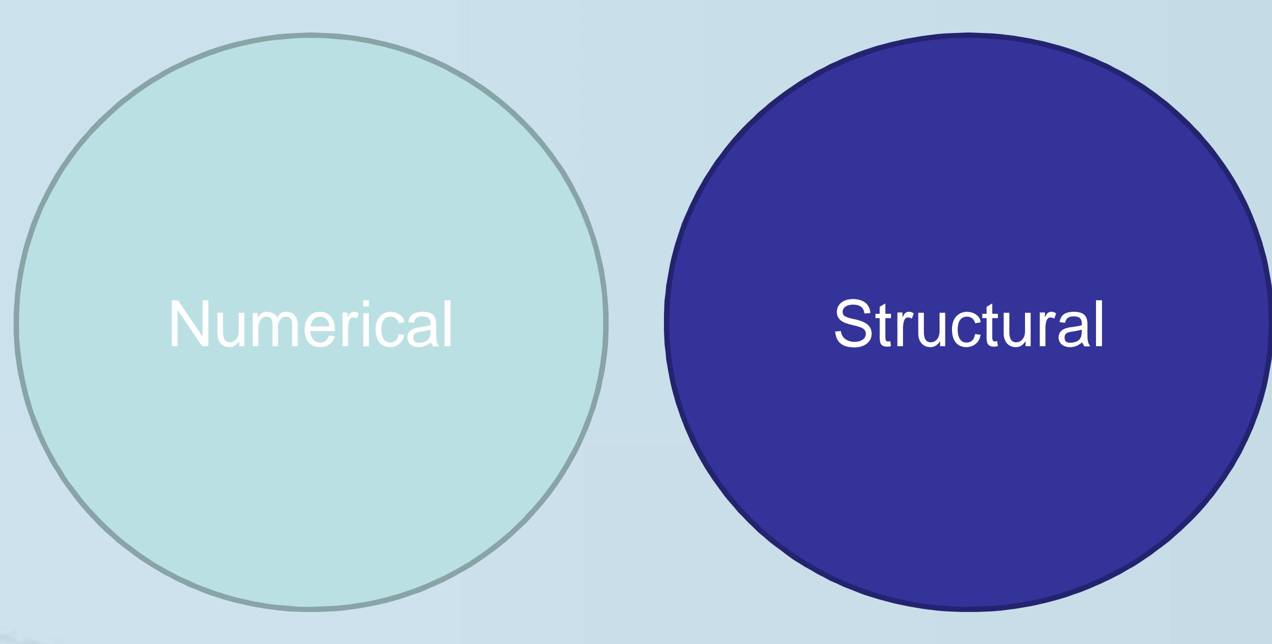
2. Duplications: Less harmful than deletions

3. Ring chromosomes: chromosome undergoes two breaks and the broken ends unite

4. Isochromosomes: chromosomes that have one arm missing and the other duplicated.



Balanced rearrangements



Balanced: normal

NO abnormal phenotype, but can pose a threat to subsequent generations because carriers are more likely to produce unbalanced gametes.

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Inversions

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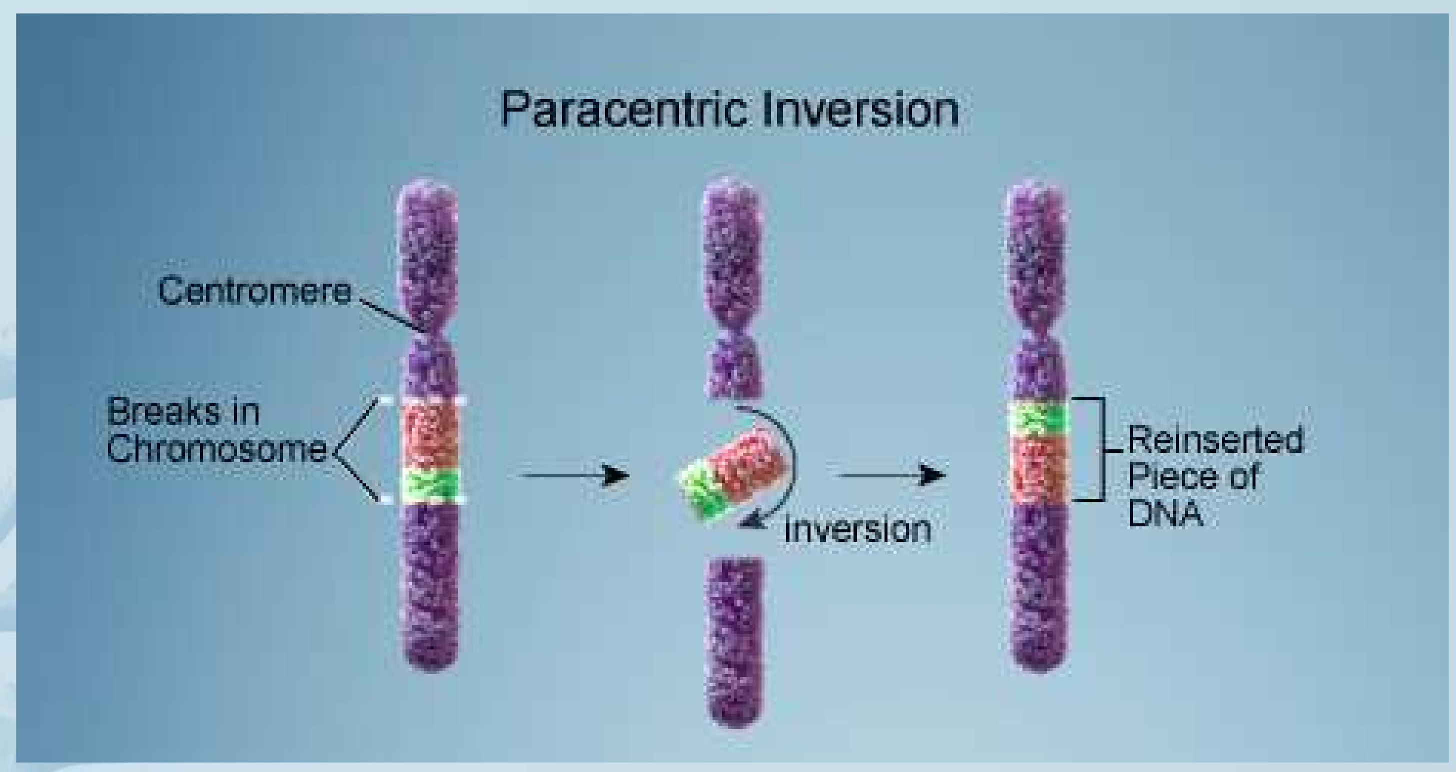
1. Inversions: a chromosome sustains two breaks and the segment inverts before rejoining the chromosome.

- **Paracentric inversion**: If both breaks occur in the same arm of a chromosome



Inversion loop at meiosis

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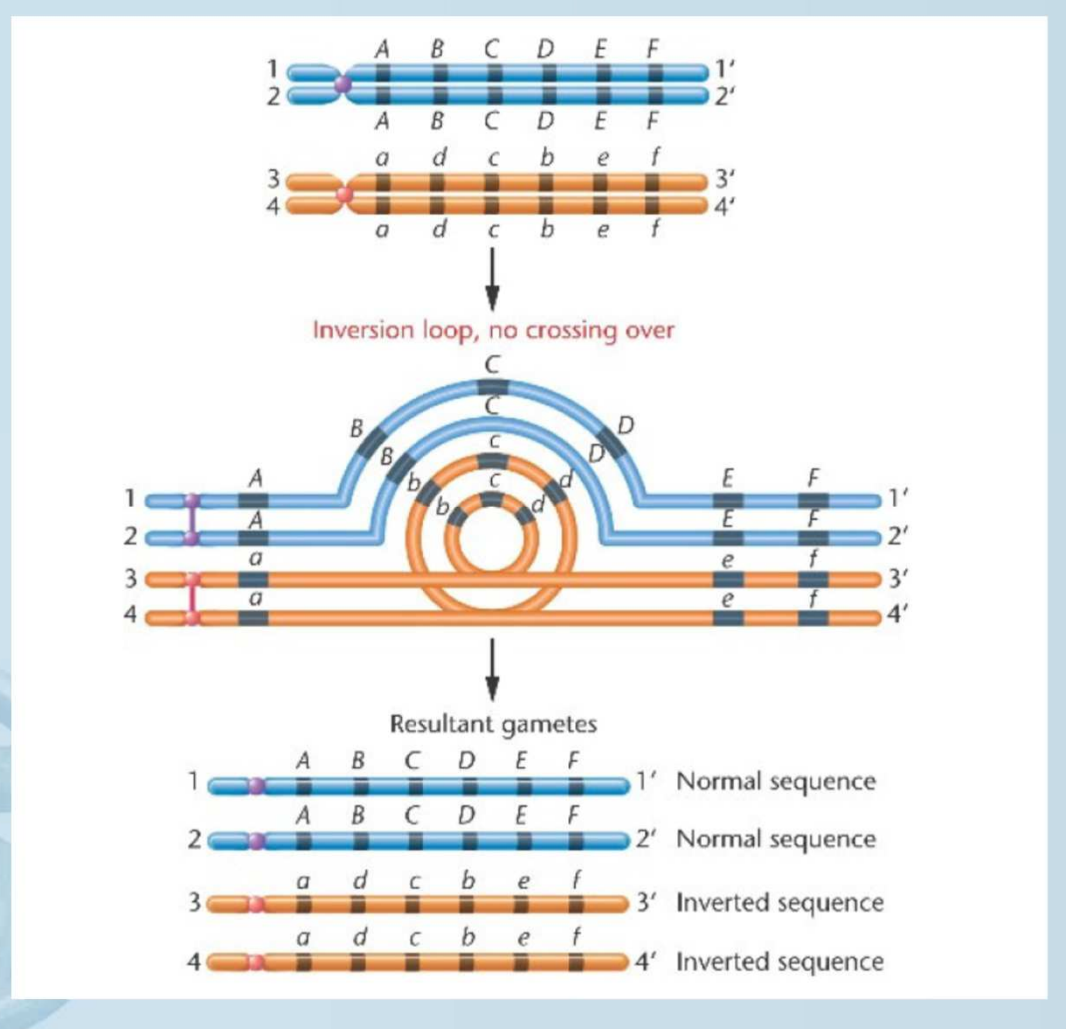

Inversions

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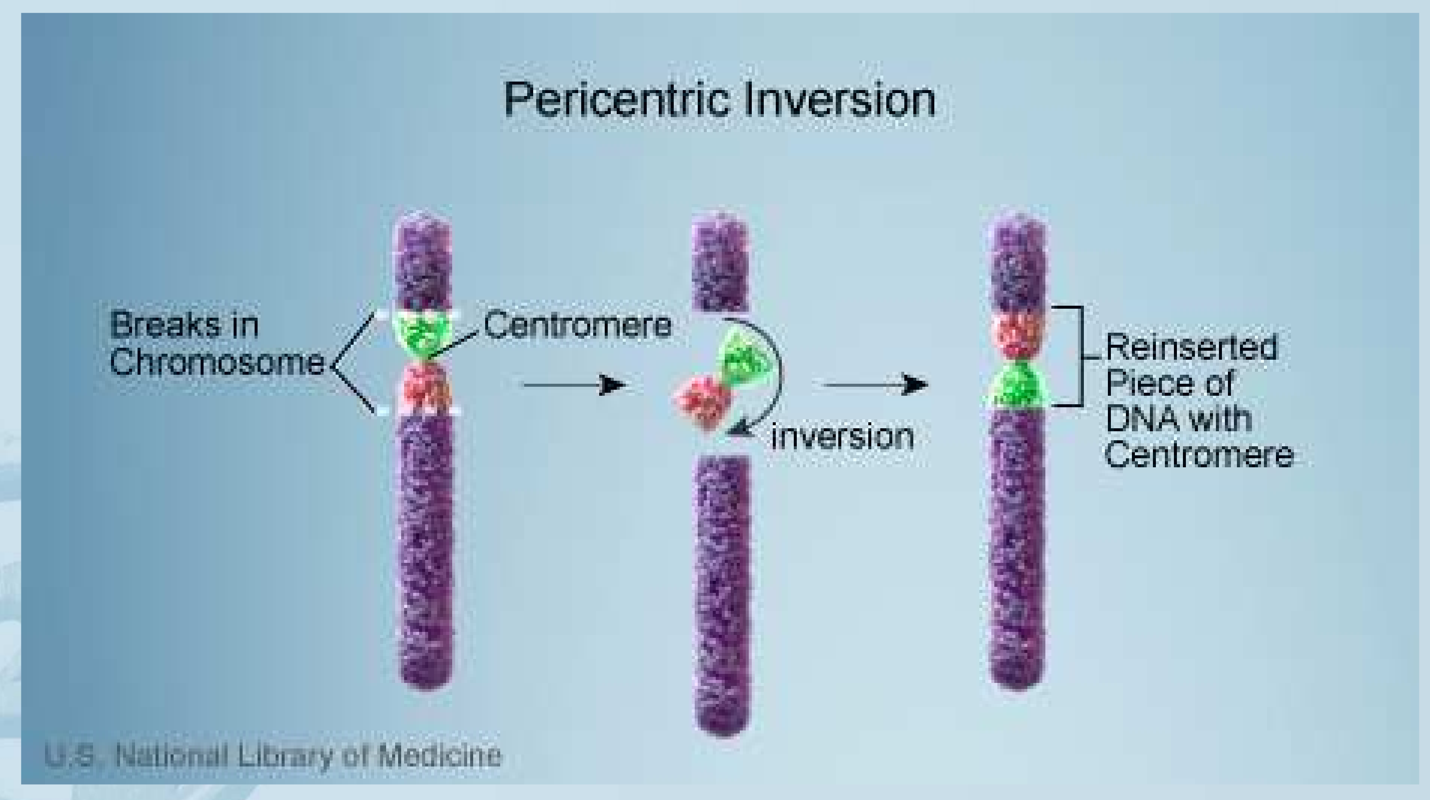
1. Inversions: a chromosome sustains two breaks and the segment inverts before rejoining the chromosome.

- **Pericentric inversion**: If the inverted segment includes the centromere



Crossing-over in pericentric inversion loop

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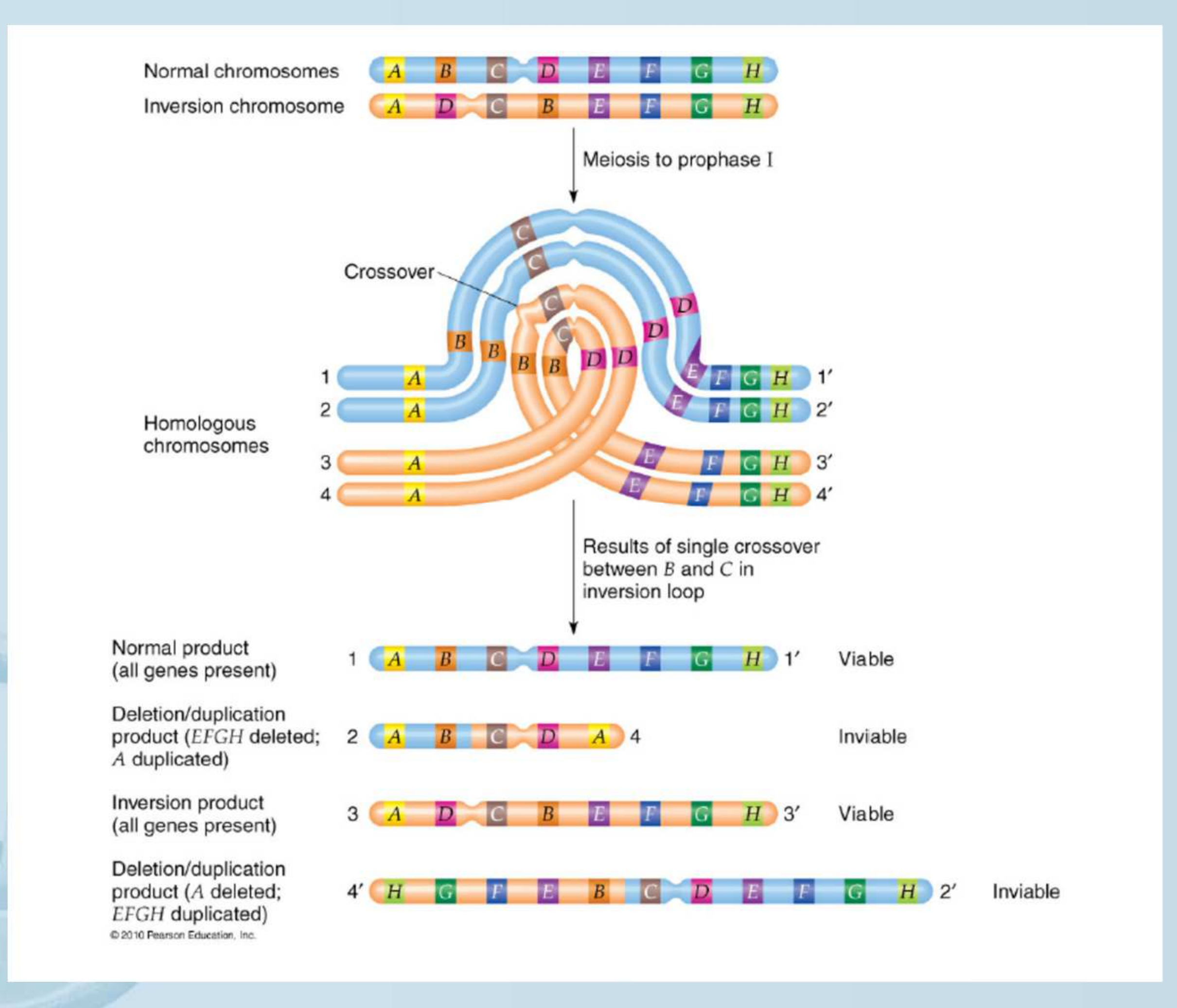

Translocations

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2. Translocations: Exchange of chromosome segments between non-homologous chromosomes.

- **Reciprocal translocation**: reciprocal exchange of the broken-off segments "the total number of chromosomes is unchanged"

- **Robertsonian translocation**: rearrangement that involves two acrocentric chromosomes that fuse near the centromere, with subsequent loss of the short arms. Although the balanced karyotype has only **45 chromosomes** (including the translocation chromosome), the phenotype is invariably unaffected *as the short arms of all five pairs of acrocentric chromosomes have multiple copies of genes for ribosomal RNA*. Therefore deletion of two short arms is not deleterious to the carrier.

Reciprocal translocations

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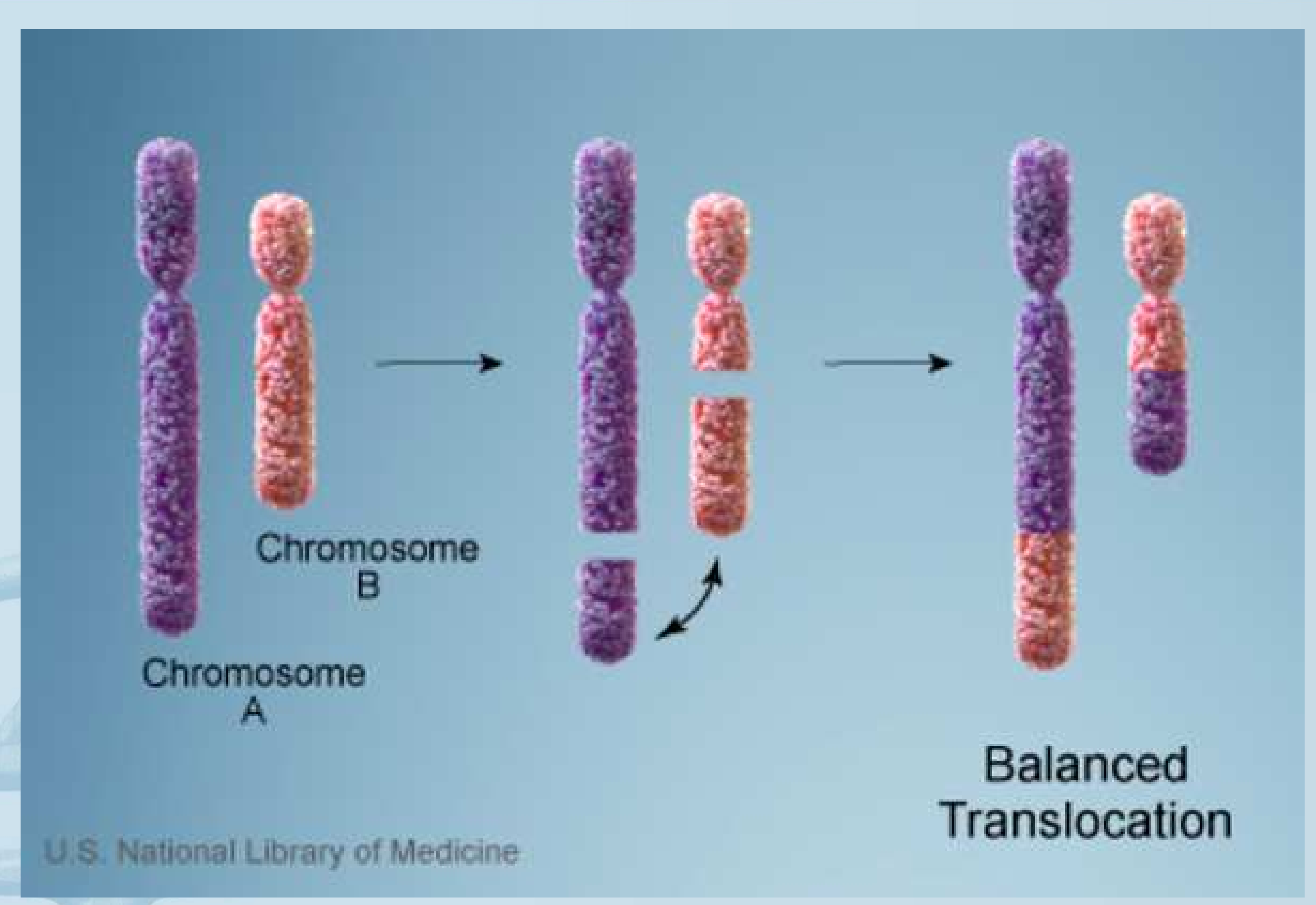

Reciprocal translocatins: quadrivalent

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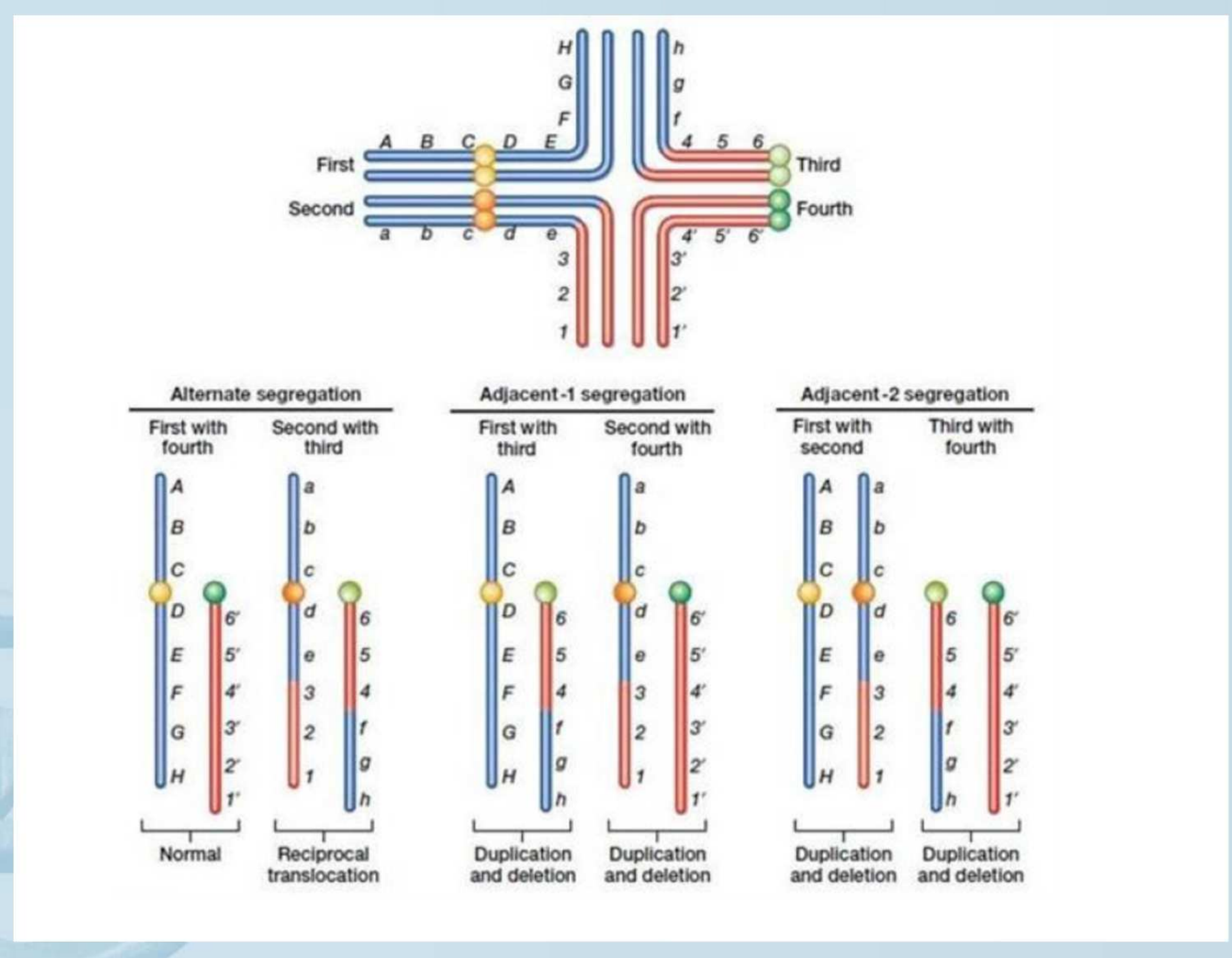

Robertsonian translocations: trivalent

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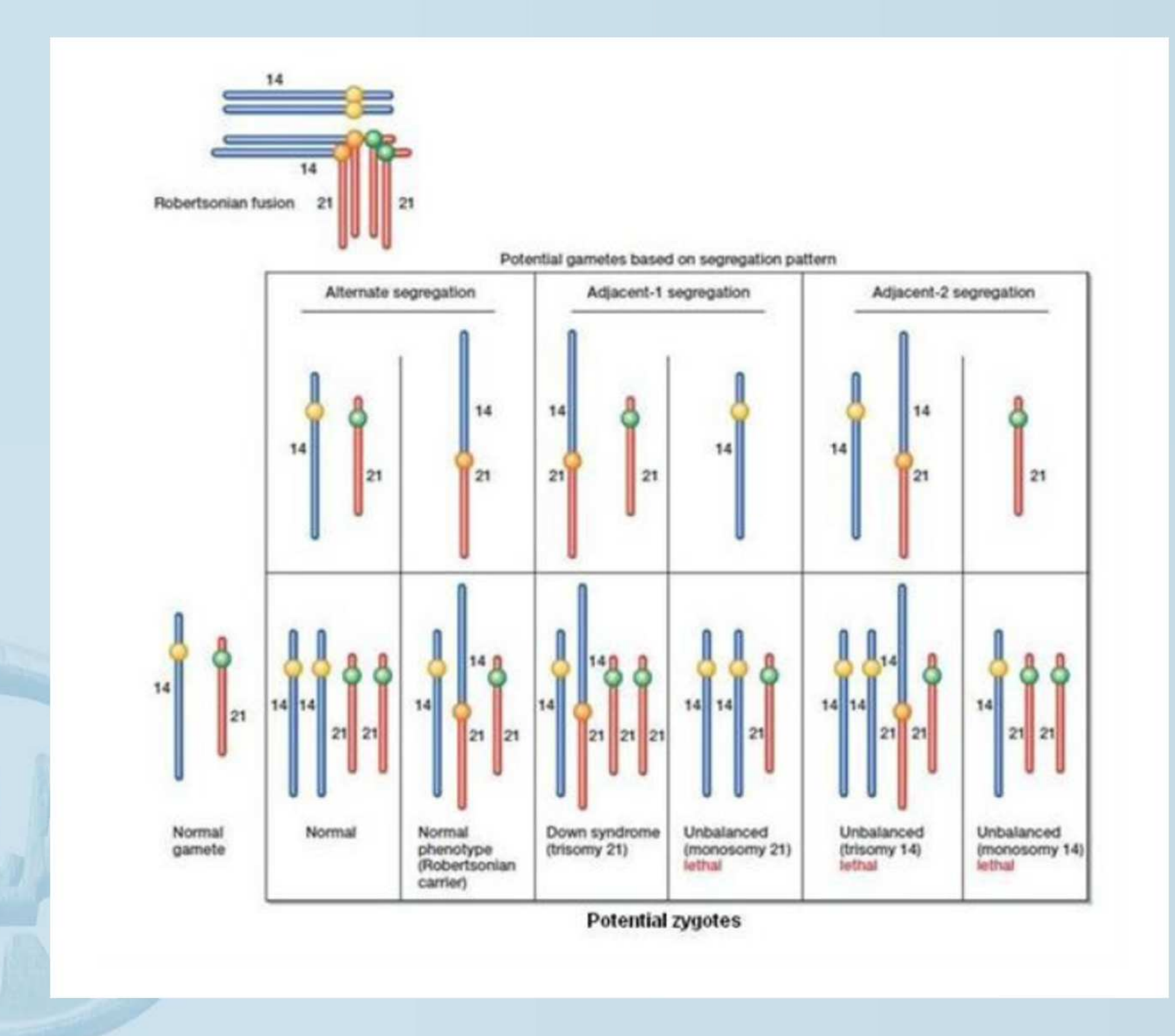

Insertion = non-reciprocal translocation

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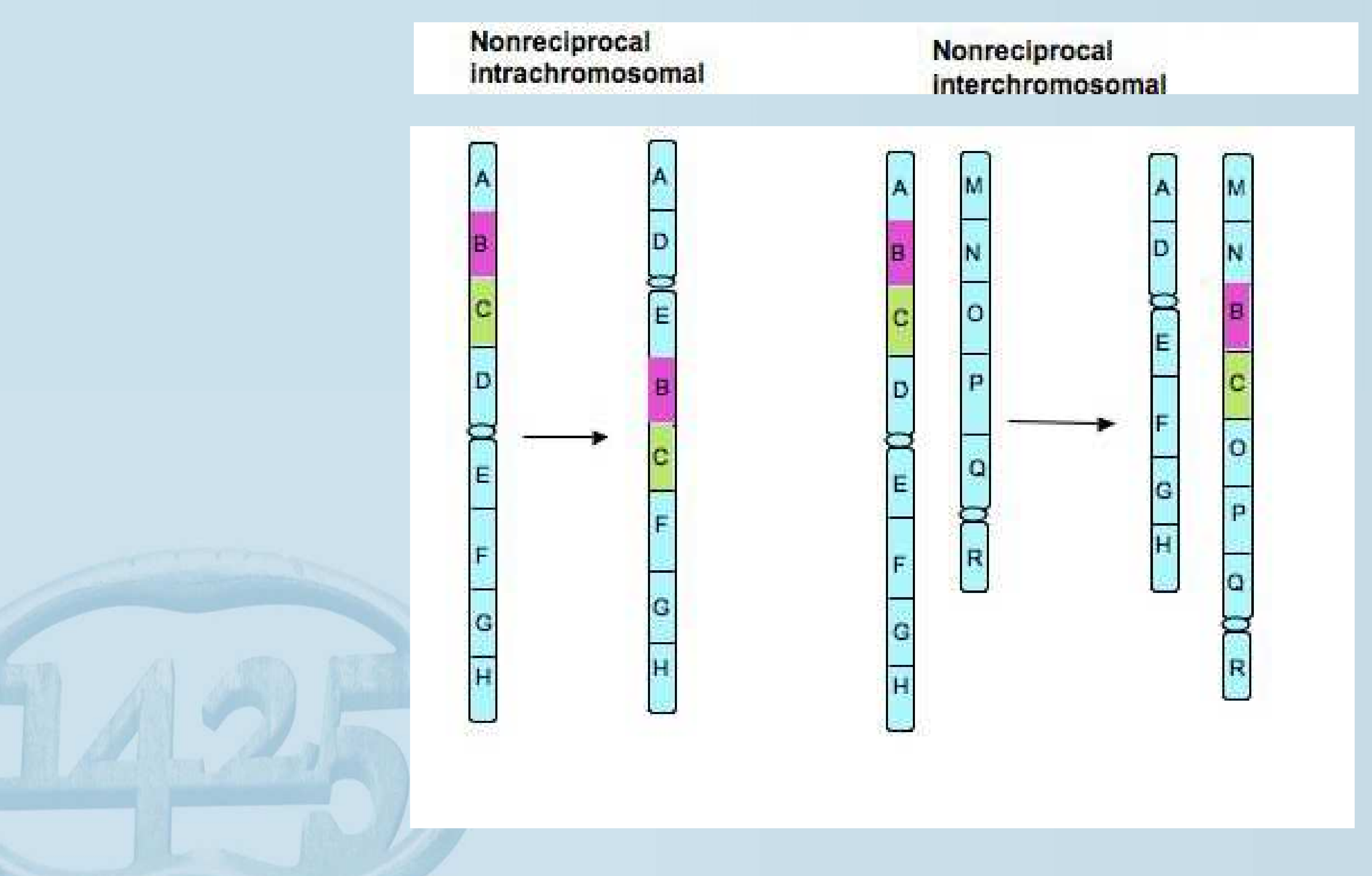

Overview

- Introduction
- Technologies for CNV detection
- Mechanisms of origin
- Clinical consequences
- Technical aspects

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Techniques to study chromosomes

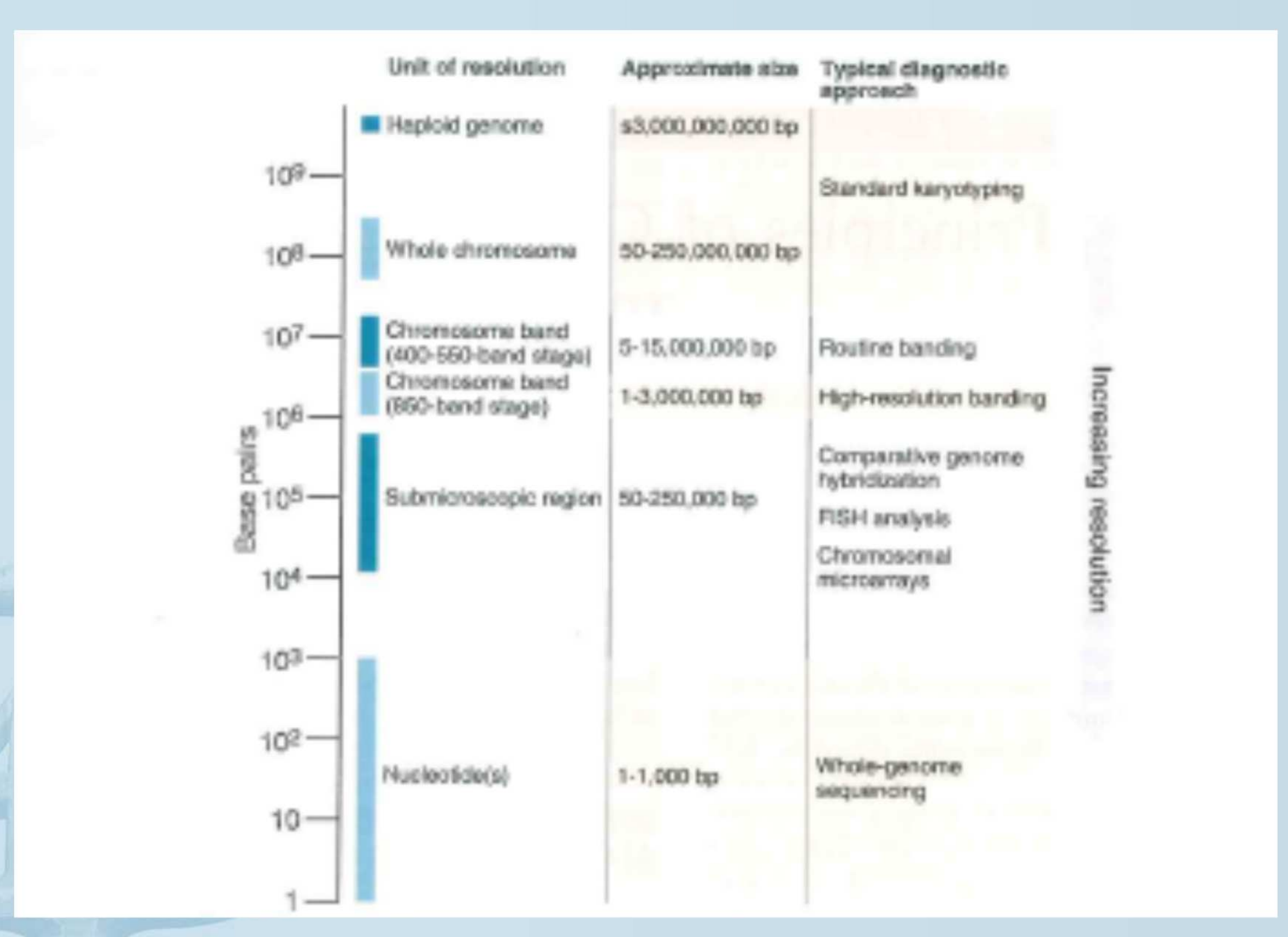
Conventional karyotyping

Fluoresence In-Situ Hybridisation

Molecular karyotyping

Massive parallel sequencing

Spectrum of resolution in chromosome and genome analysis



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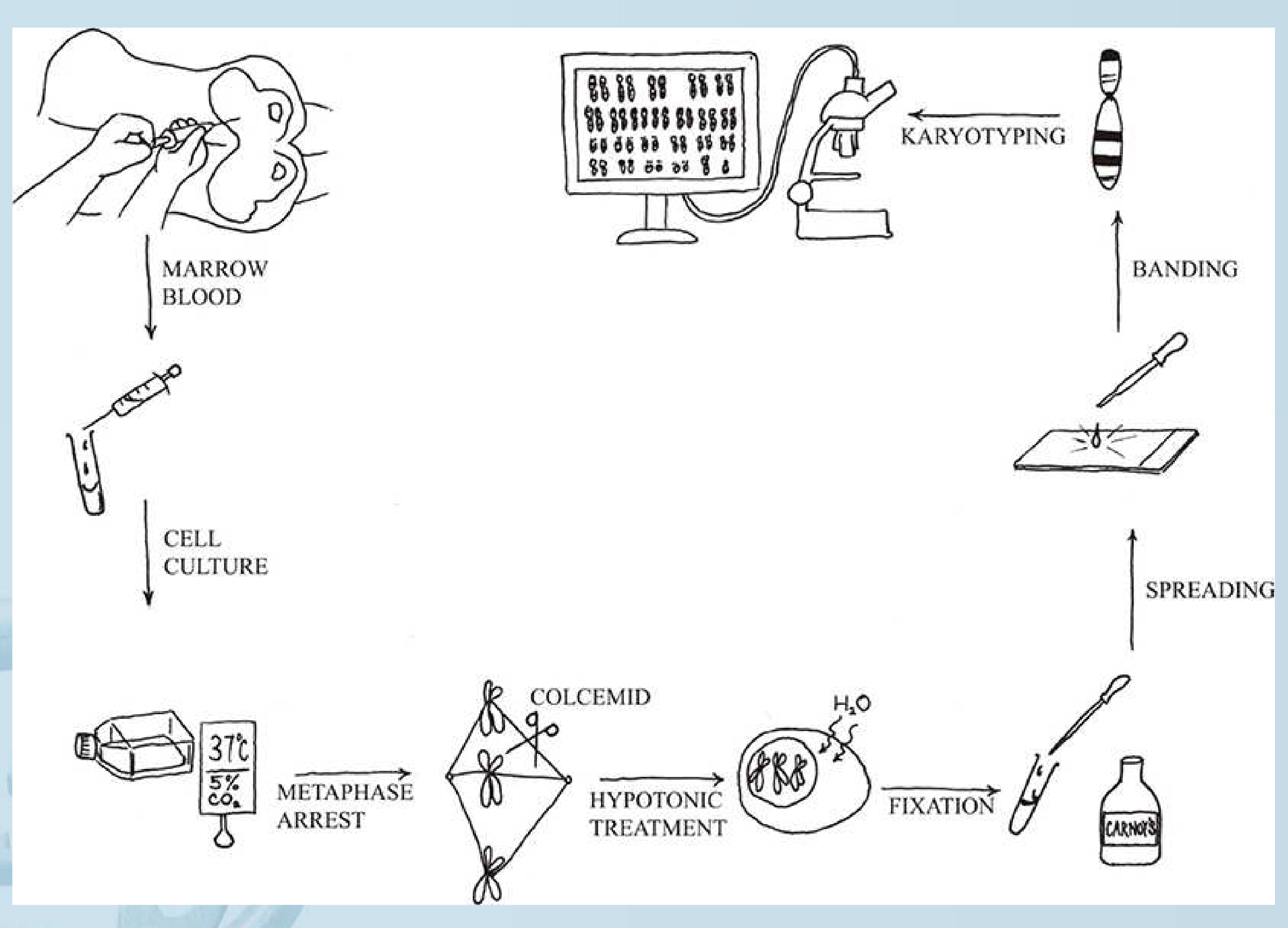
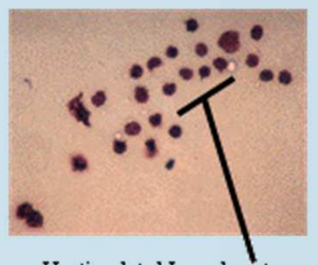
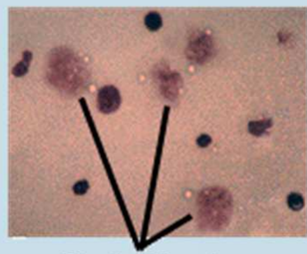


PHA stimulation

Blast Transformation of Lymphocytes



Unstimulated Lymphocytes



Stimulated Lymphocytes

- Lymphocytes are differentiated cells which do normally no undergo subsequent cell divisions.
- By culturing lymphocytes in the presence of a mitogen, they are stimulated to replicate their DNA and enter into mitosis.
- Transformation of lymphocytes into lymphoblasts can be induced by phytohemagglutinin (PHA), a mitogenic lectin extracted form red kidney beans.

Cell synchronisation

Methotraxate (MTX):

- Inhibits dihydrofolate reductase
- blocks cell division at the G1/S border

5-bromodeoxyuriding (BrdU):

- an analog of thymidine
- releases the block

Folic acid cycle: Folic acid is required for incorporation of thymidine during DNA synthesis

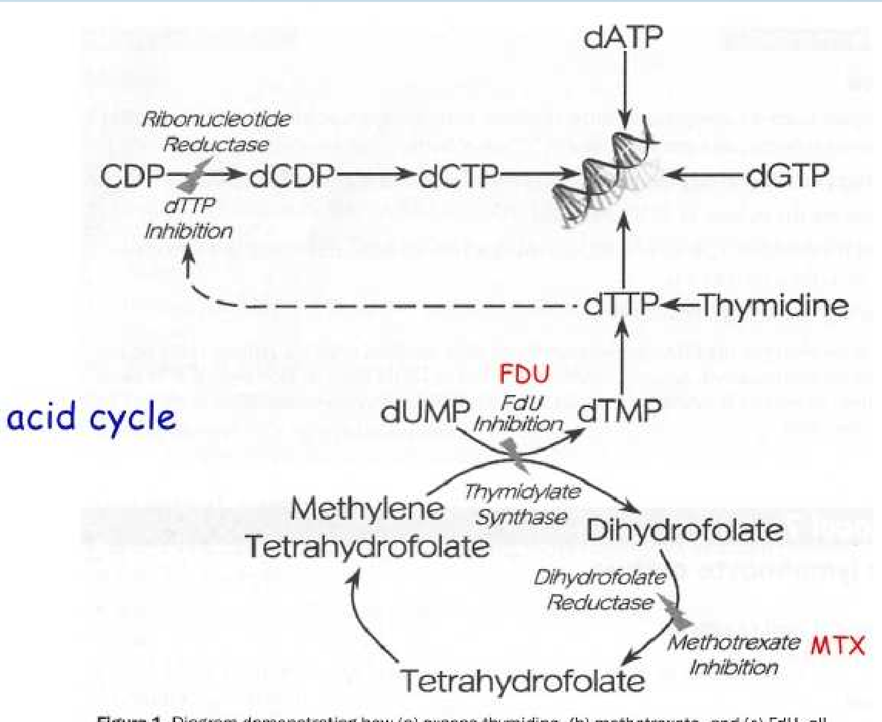
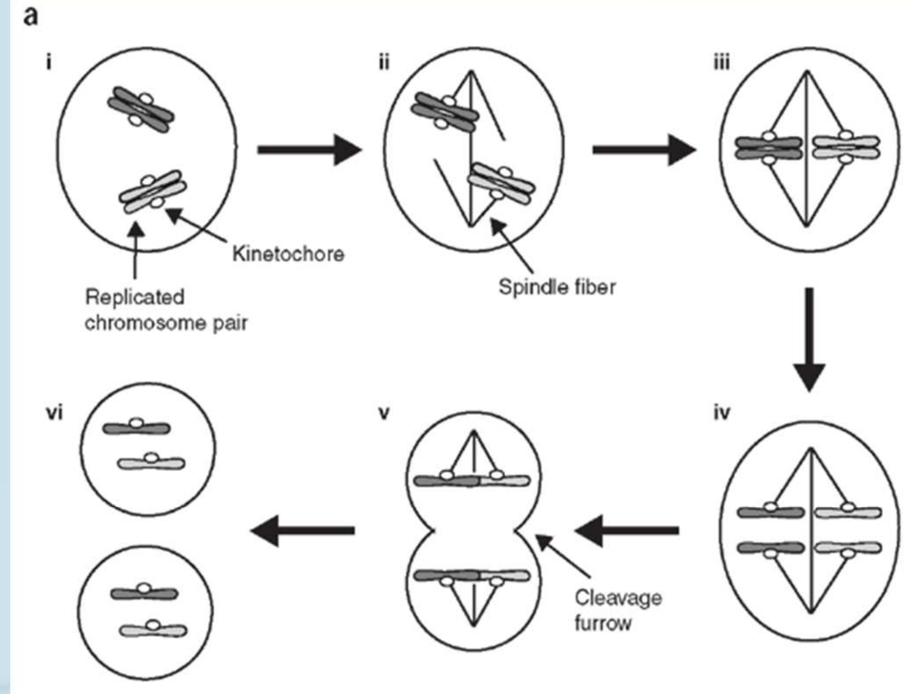


Figure 1 Diagram demonstrating how (a) excess thymidine, (b) methotrexate, and (c) FdU, all inhibit DNA synthesis. For full details see sections 5. and 5.1.

Colchicine

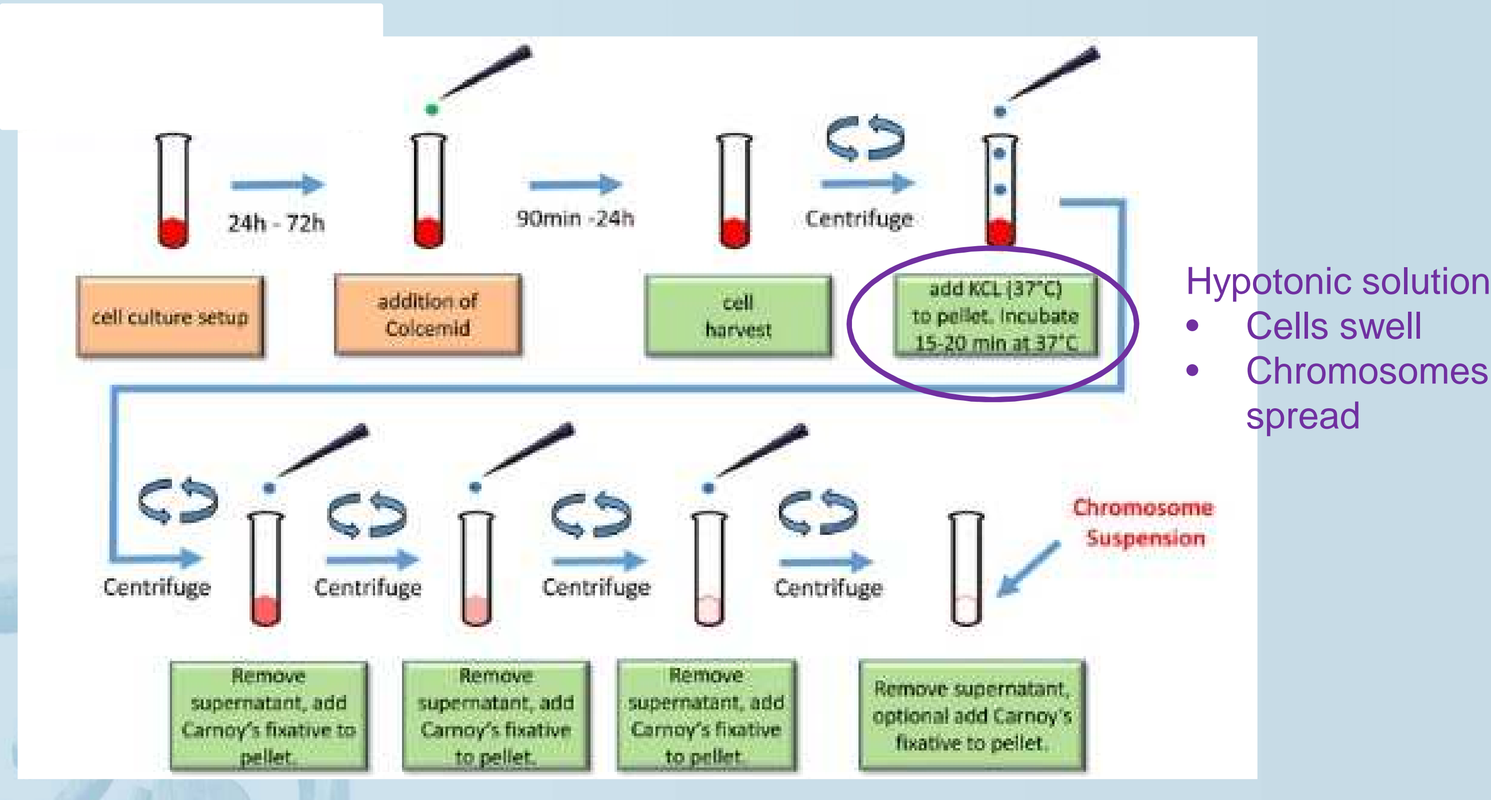


MITOSIS

Colchine:

- acts to prevent the synthesis of spindle fibers
- inhibits microtubule polymerization by binding to tubulin
- stops mitosis in metaphase

Harvesting



Hypotonic solution:

- Cells swell
- Chromosomes spread

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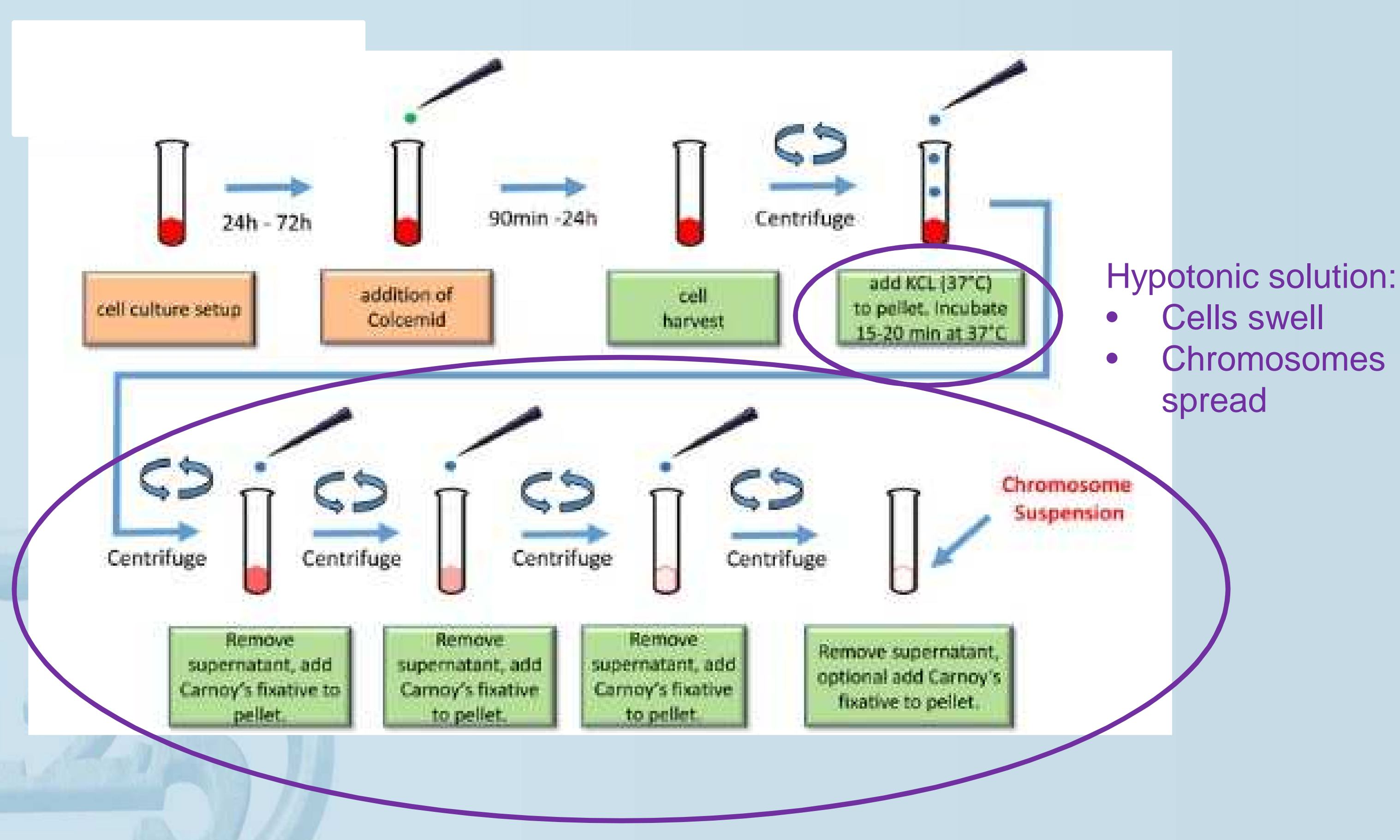
Harvesting



Hypotonic solution:
- Cells swell
- Chromosomes spread

Fixation = Methanol : Acetic Acid 3:1

Chromosome spreads

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Chromosomes are spread onto microscopic glass slides under temperature and humidity (60%) controlled conditions.

Chromosome banding

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DIFFERENT TYPES OF BANDING

G–Banding:

- Staining a metaphase chromosome with Giemsa stain is called G-Banding.
- Preferentially stains the regions that are rich in adenine and thymine and appear dark.

C-Banding:

To specifically stain the centromeric regions and other regions containing constitutive heterochromatin.

Chromosome banding

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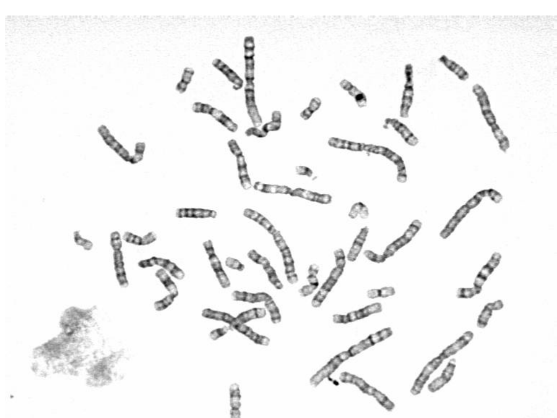
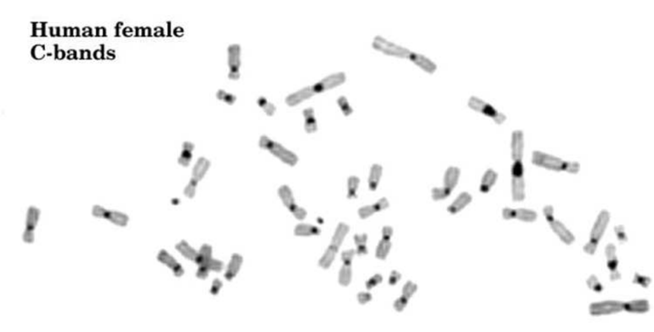
DIFFERENT TYPES OF BANDING

G–Banding:



C-Banding:



Chromosome banding

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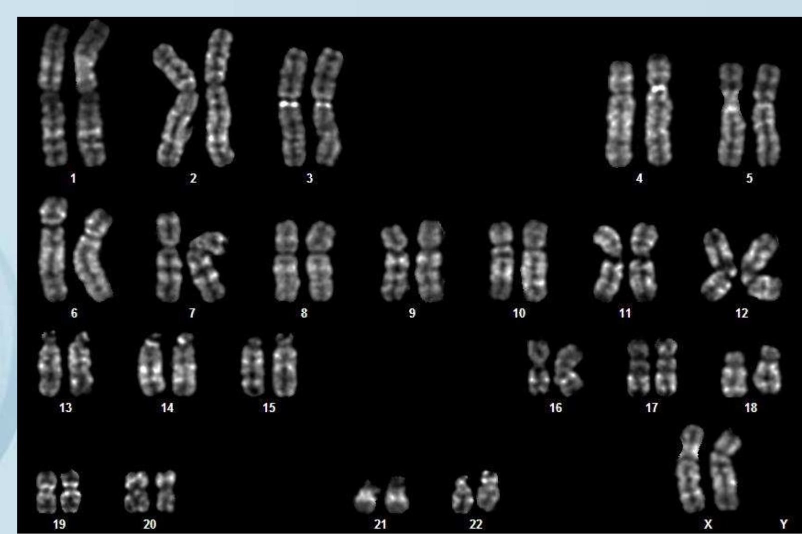
Q-Banding

- Quinacrine mustard (a fluorescent stain), an alkylating agent, was the first chemical to be used for chromosome banding.
- Quinacrine bright bands were composed primarily of DNA rich in bases adenine and thymine.



Chromosome banding

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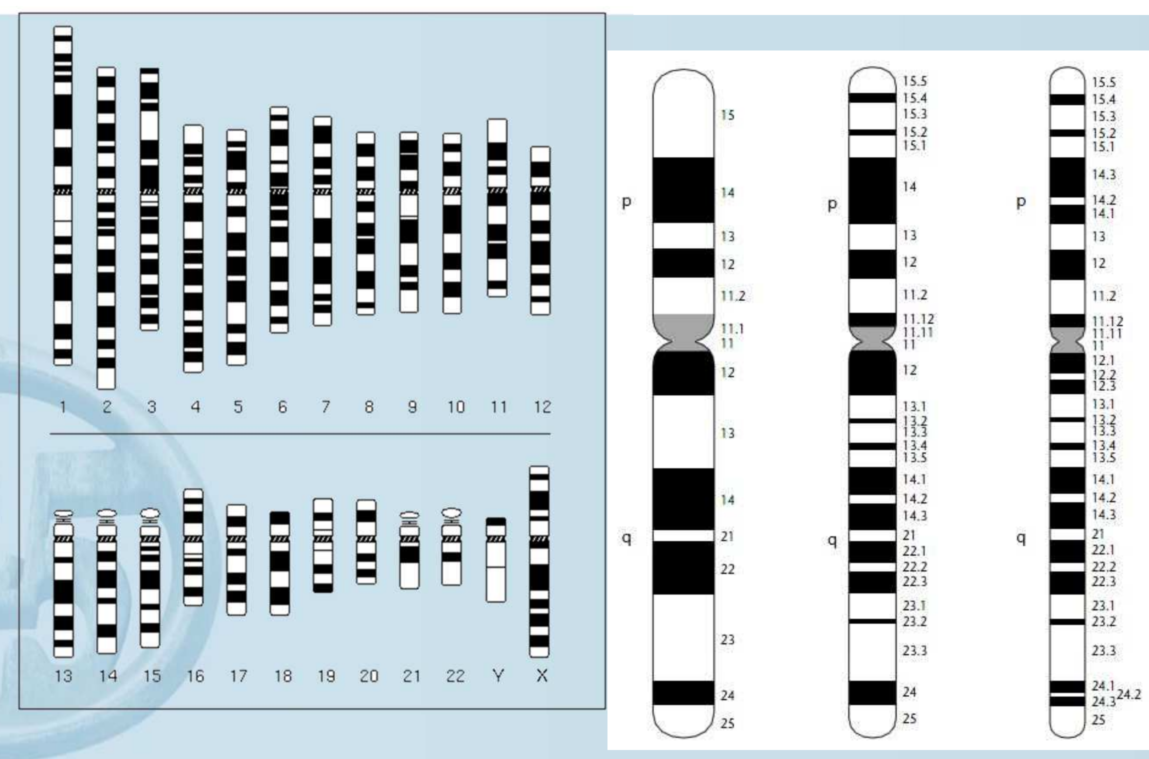
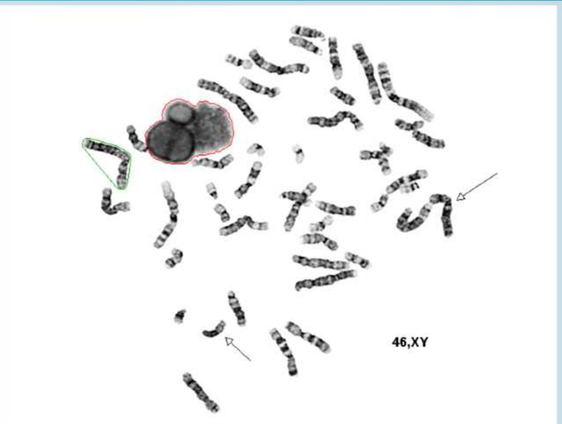
Used to identify

- Specific chromosomes and structural rearrangements.
- Various polymorphisms involving satellites and centromeres of specific chromosomes.



Microscopic imaging



Karyotyping

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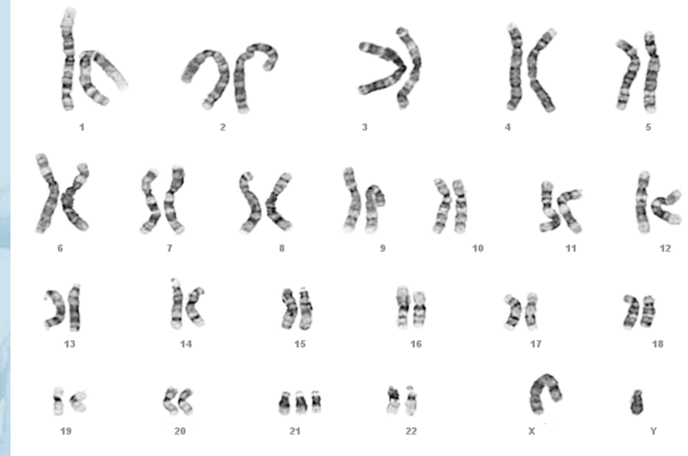
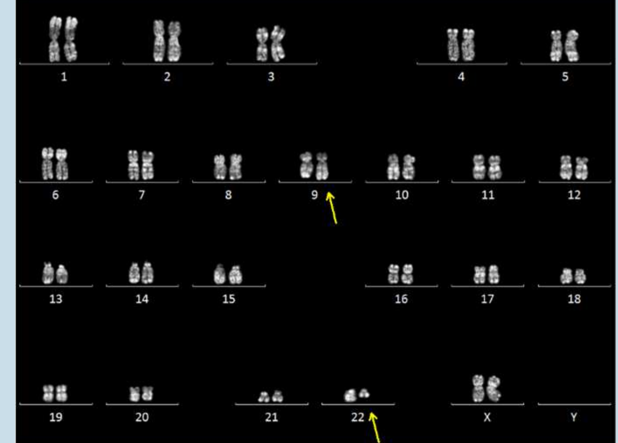


47,XY,+21



46,XX,t(9;22)(q34;q11)

ISCN: International standards cytogenetic nomenclature

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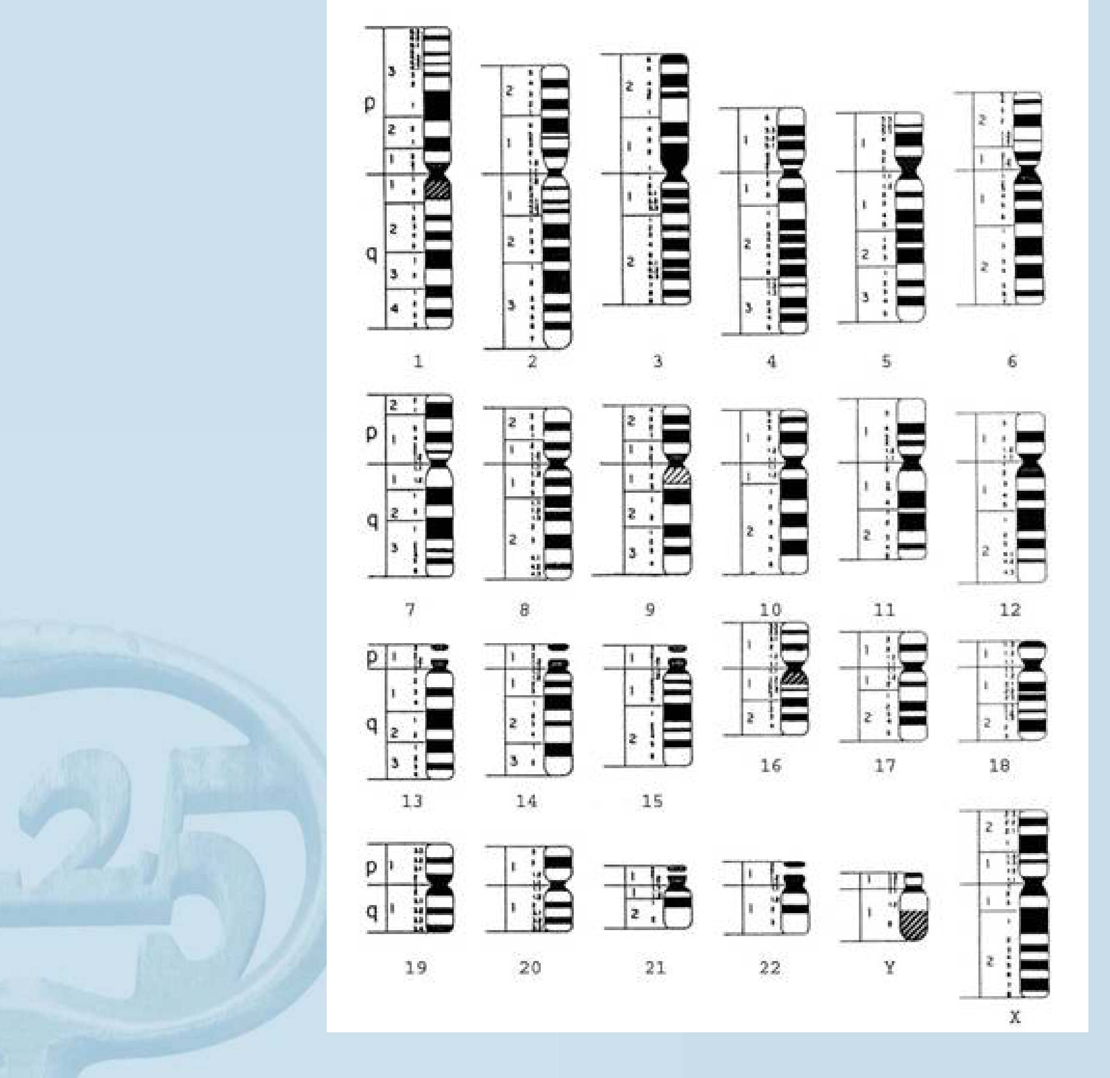

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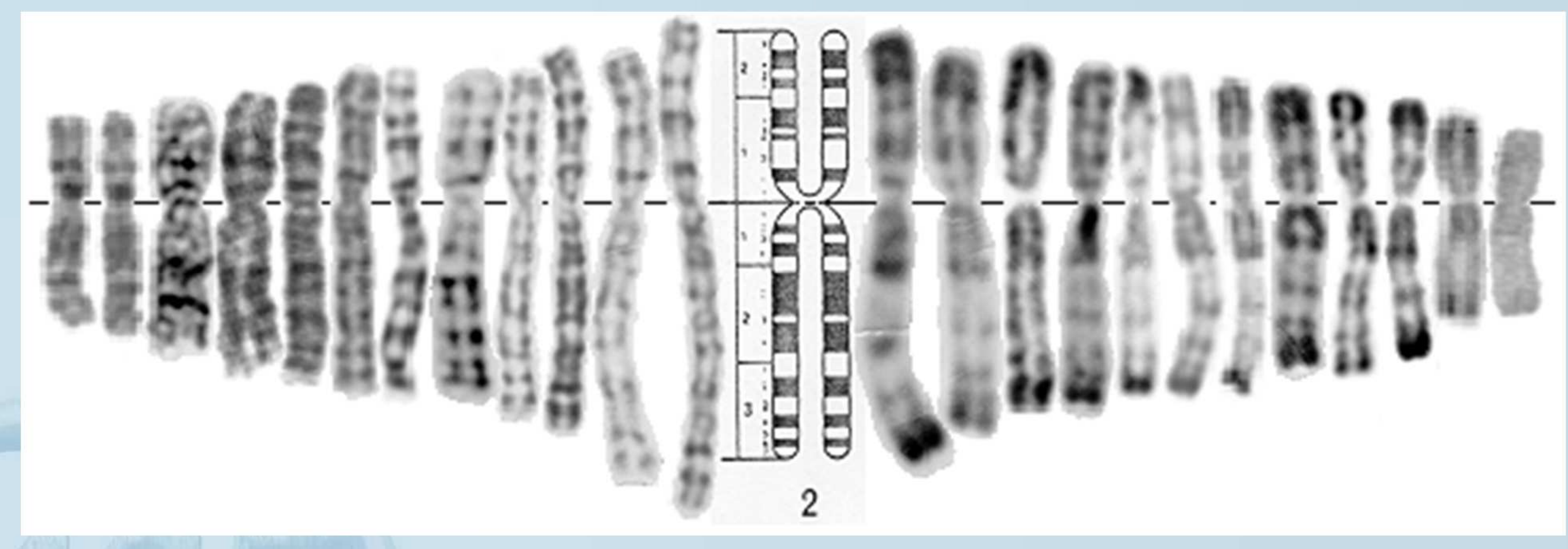

STRUCTURAL CHROMOSOMAL ANOMALIES: ISCN

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Deletion	del(1)
Duplication	dup(1)
Inversion	inv(1)
Isochromosome	i(1)
Ring chromosome	r(1)
Marker chromosome	+mar
Translocation	t(1;2)
Robertsonian translocation	t(13;14)
Insertion	

Techniques to study chromosomes

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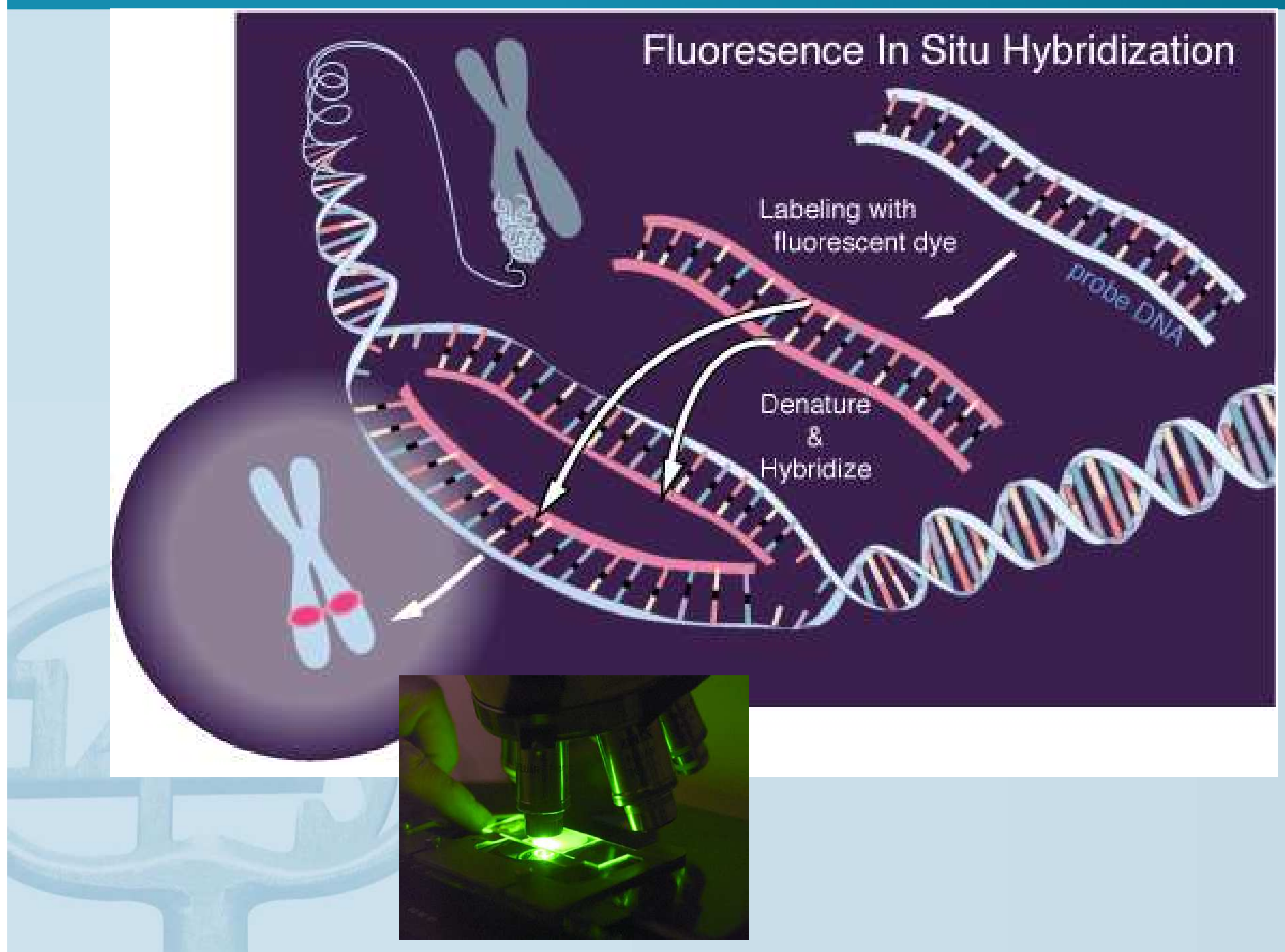
Conventional karyotyping

Fluoresence In-Situ Hybridisation

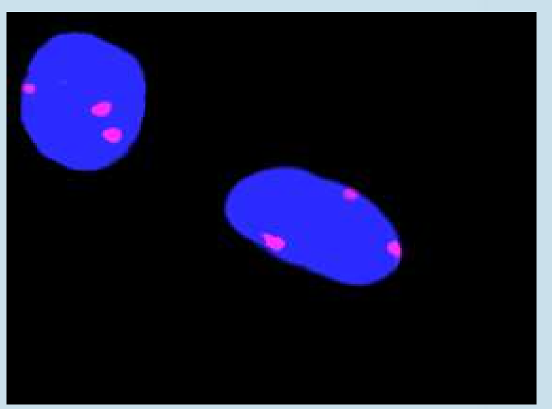
Molecular karyotyping

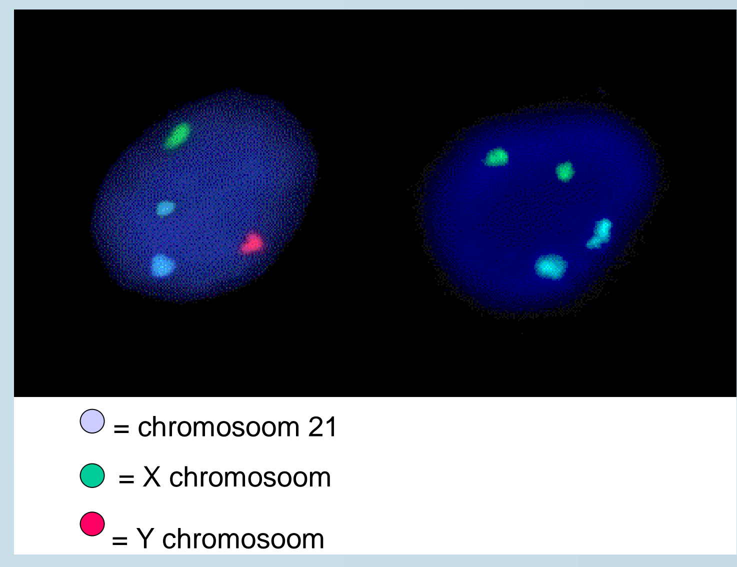
Massive parallel sequencing

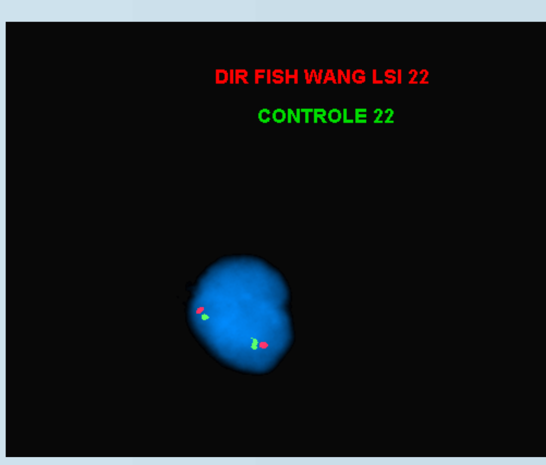
Fluorescence In Situ Hybridisation



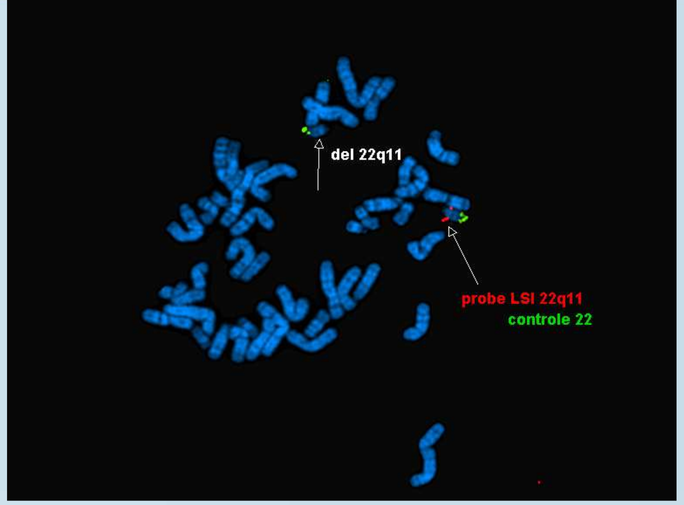
Interphase FISH

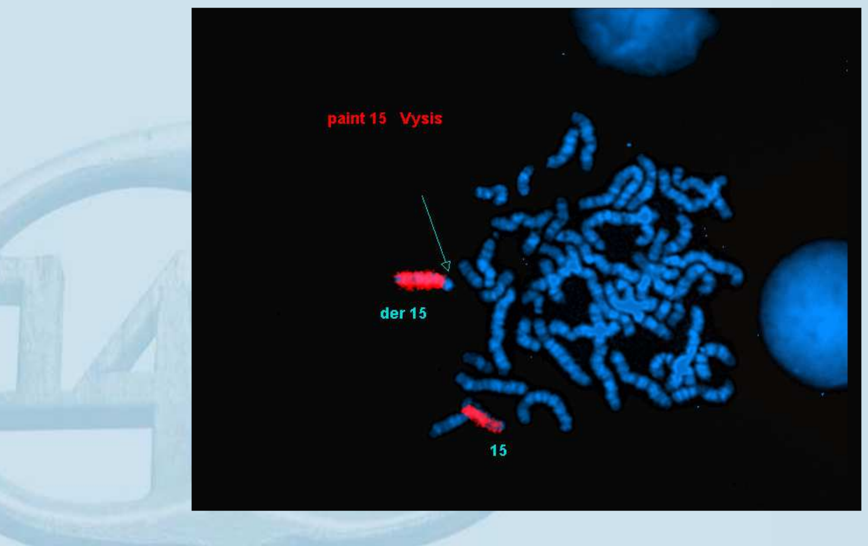


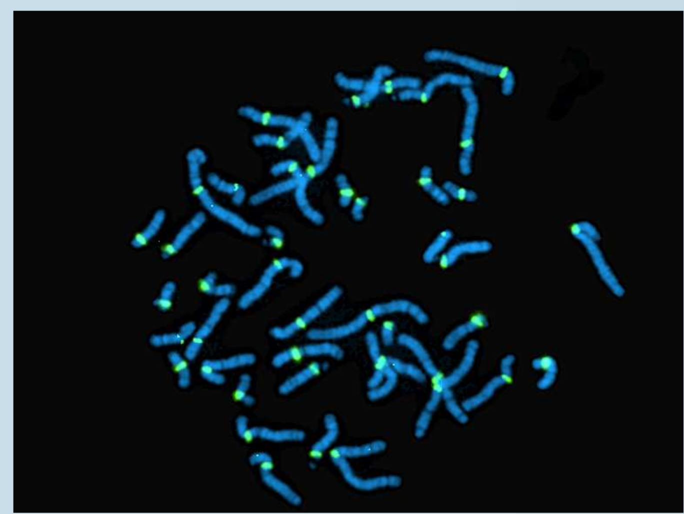




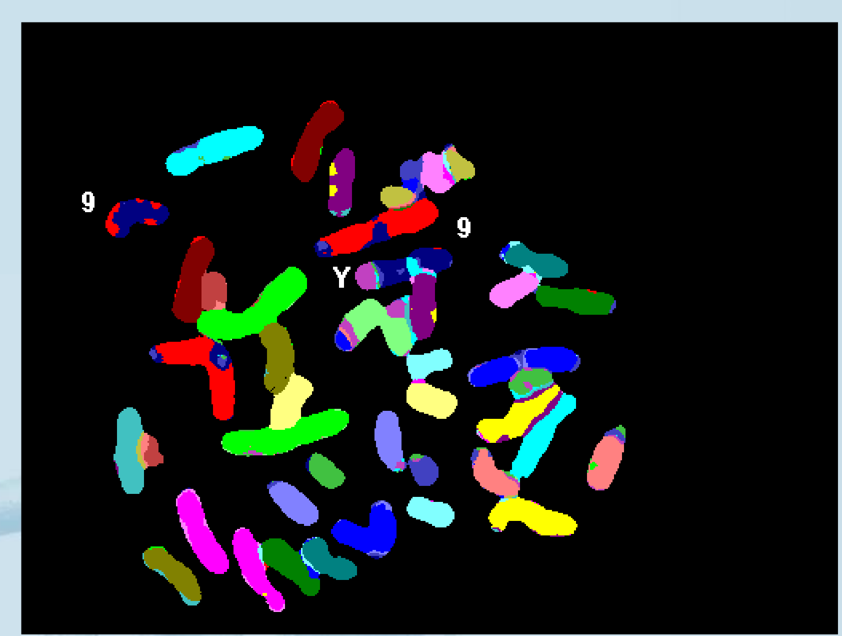
Metaphase FISH

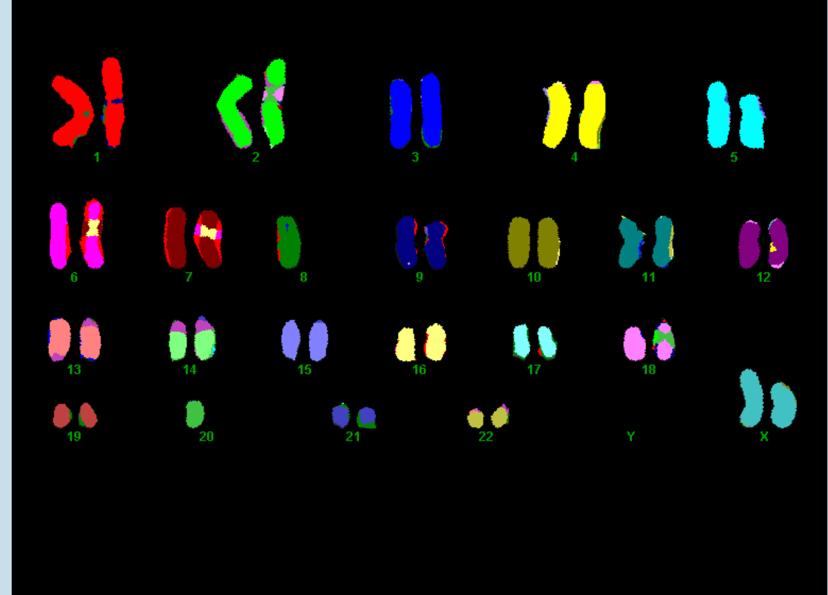




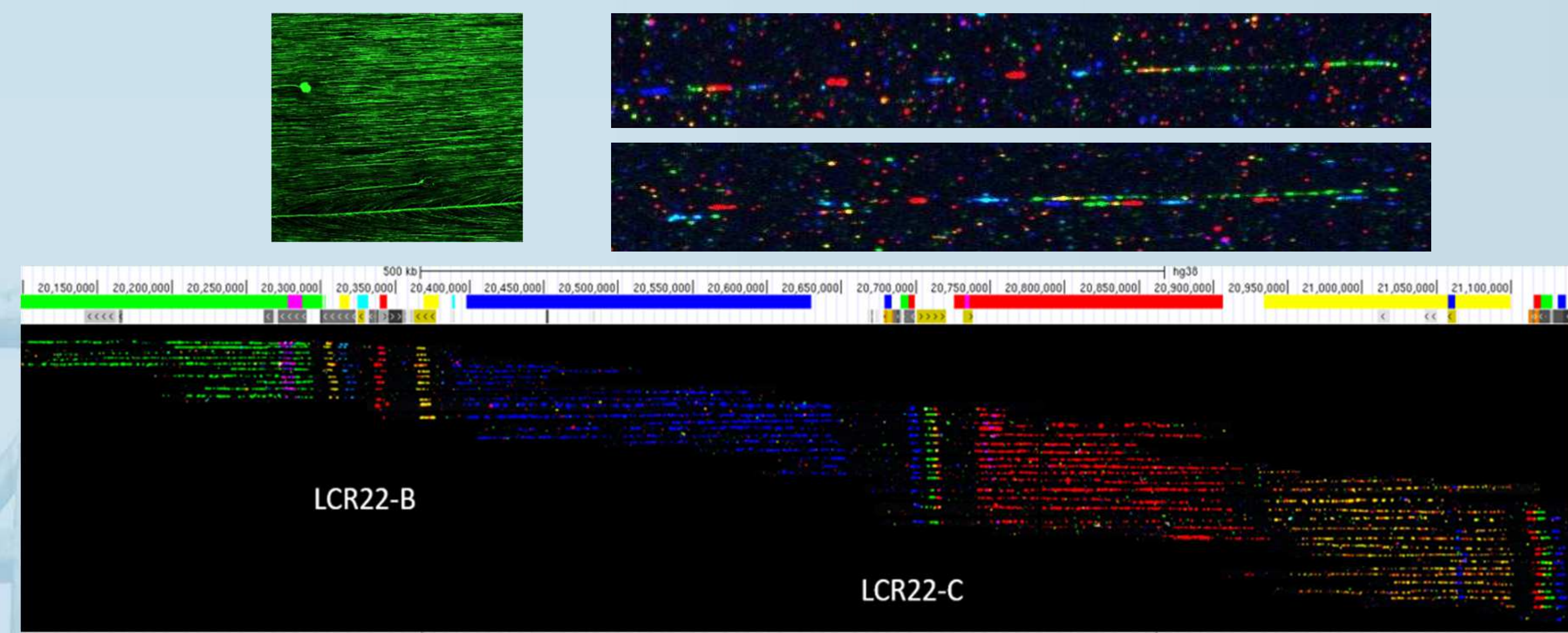


Multicolor FISH



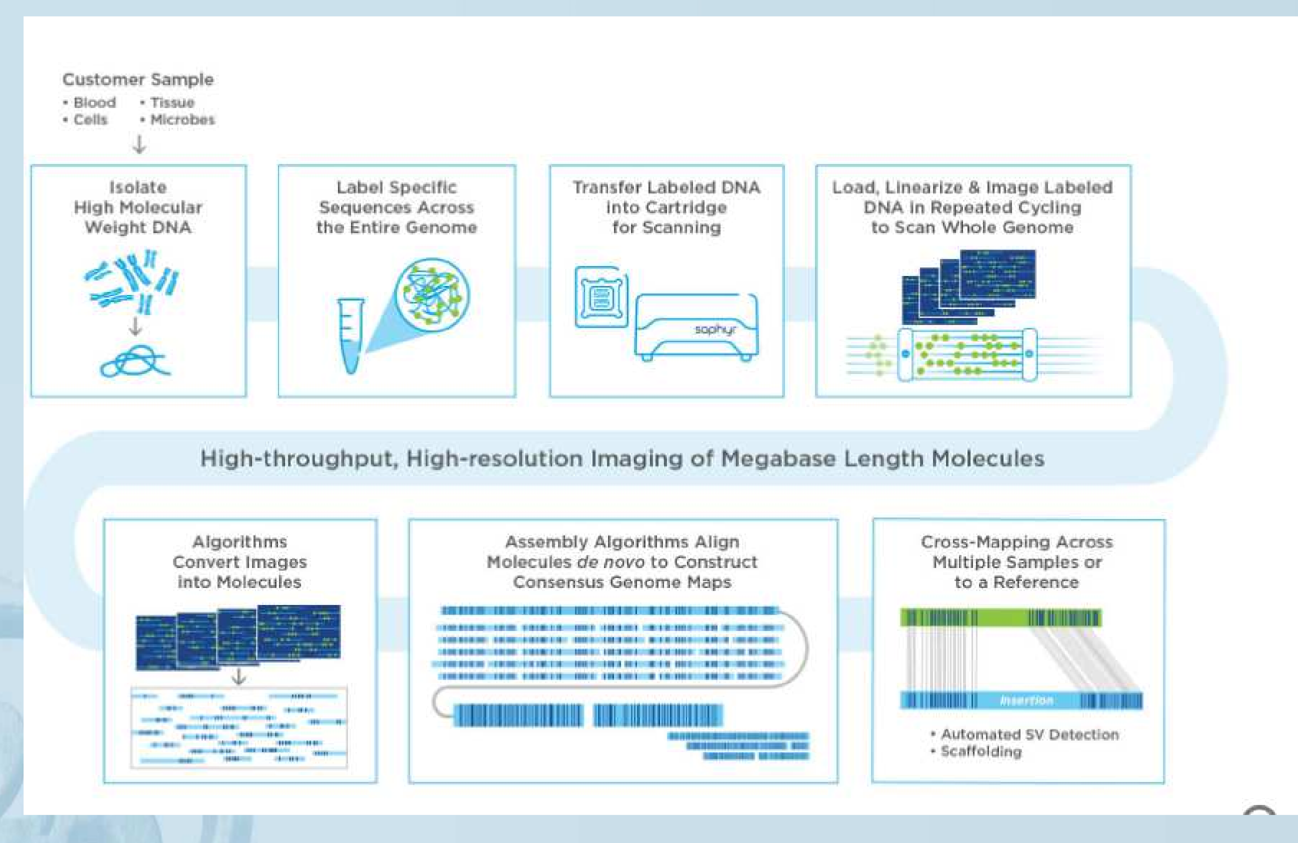


Fiber FISH



Demaerel et al., Genome Research, 2019

Optical mapping
Bionano mapping



https://www.youtube.com/watch?v=S2ng6glu04I

https://vimeo.com/116090215

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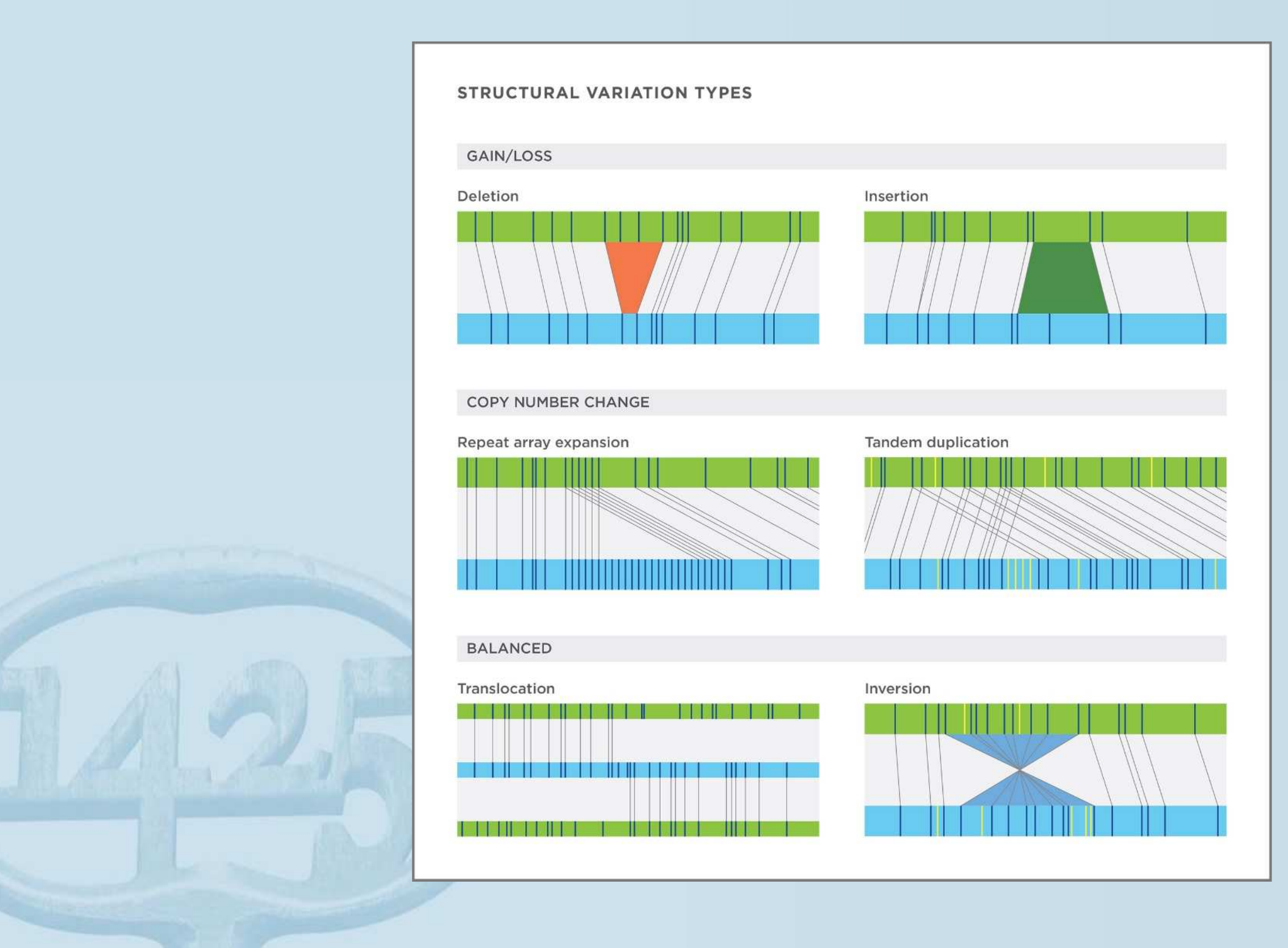
Structural variation types



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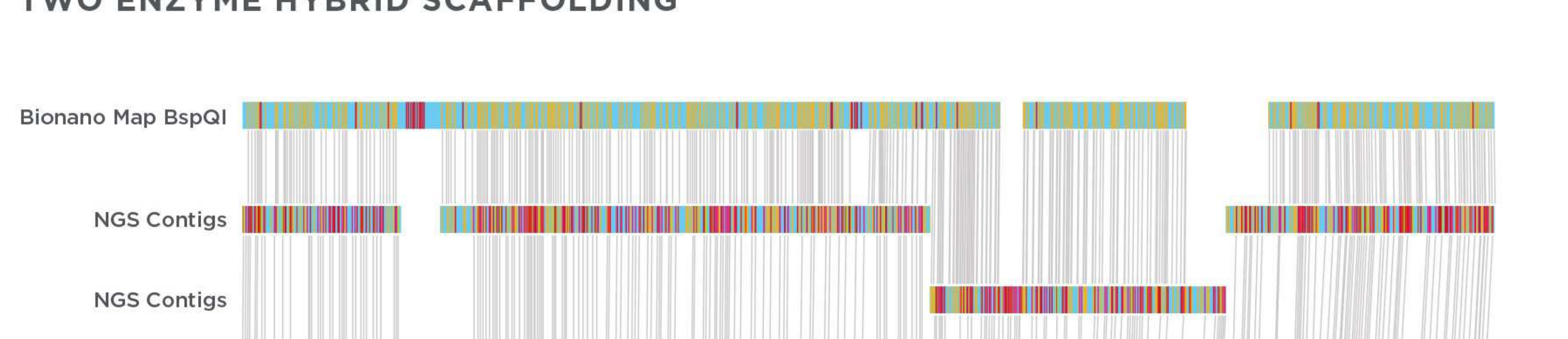
Bionano genome assembly



Average fragment lengths: 250kb
Range: 100-500kb

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Strand-Seq

(inversions, larger SVs)

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A i. Chromosome homologues
ii. Sister chromatids with BrdU
iii. Sequenced template strands in daughter cells

3′5′ Watson Crick 5′3′
M P
1. DNA synthesis in BrdU
M M P P
2. DNA segregation & mitosis
3. isolation of single cells
4. library construction
5. Removal of BrdU+ strand
WW WC CC WC

B Chromosome
reference inversion
3′5′ 5′3′
Sister chromatids after replication

C Normal male cell (HsSs_0123)
Chr 1 2 3 4 5 6 7 8 9 10 11 12
Chr 13 14 15 16 17 18 19 20 21 22 X Y

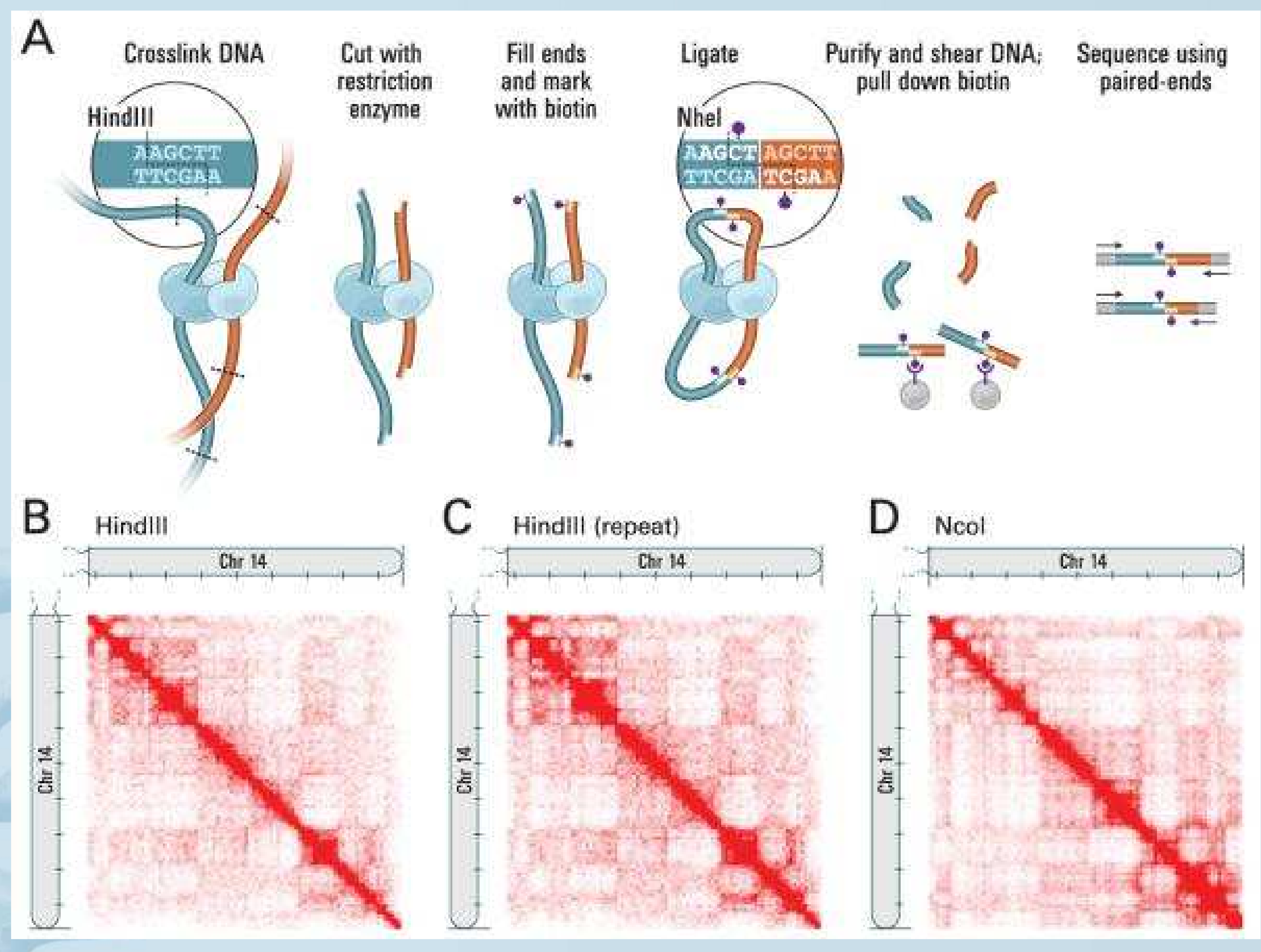
D Homozygous Reference
Heterozygous Inversion
Homozygous Inversion

E i. Chromosome 8 (Chr 8 p23.2-p21.3)
Chromosome 7 (Chr 7 q11.22-q21.11)
HsSs_0123
ii. Chr 8: 5 Mb 10 Mb 15 Mb
7,224,122 - 12,417,334
Chr 7: 70 Mb 75 Mb 80 Mb
72,312,825 - 75,040,174

© 2016 Sanders et al.; Published by Cold Spring Harbor Laboratory Press

Chromosome conformation capture

(changes in TADs structure can identify SVs)



Lieberman-Aiden et al., 2009, Science

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Techniques to study chromosomes

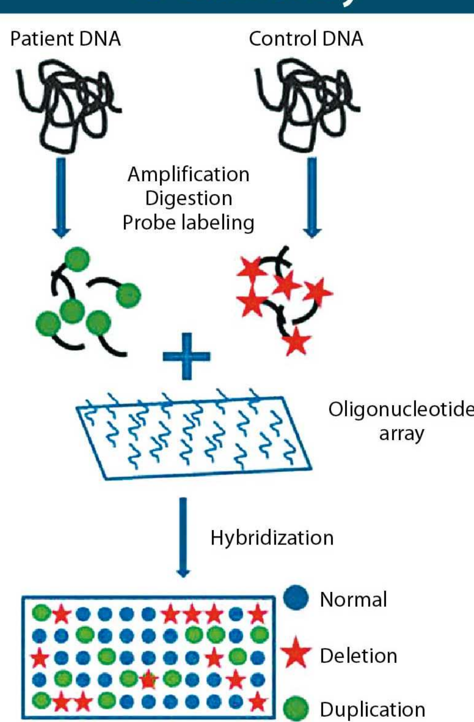
Conventional karyotyping

Fluoresence In-Situ Hybridisation

Molecular karyotyping

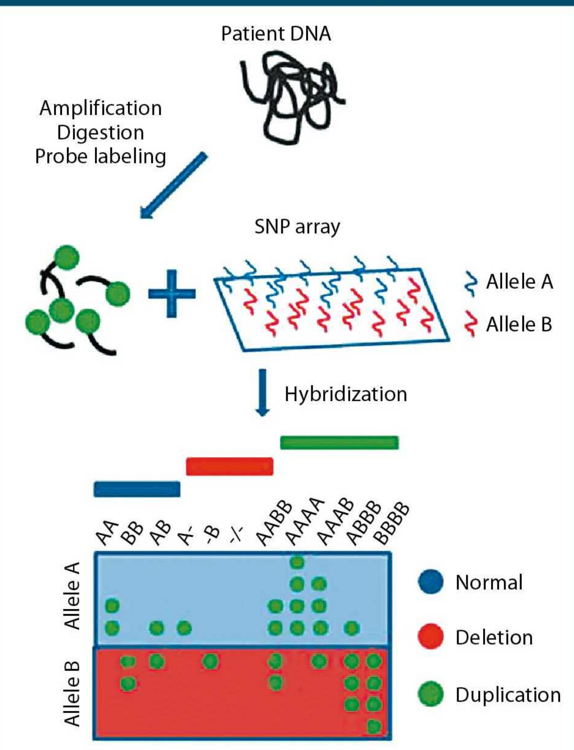
Massive parallel sequencing

Microarrays



The comparative genomic hybridization (CGH) array compares the patient's DNA to control DNA using 2 different fluorescent labels. Labeled control and patient DNA fragments are hybridized to an array containing oligonucleotide DNA sequences from genes throughout the human genome. Each position on the array correlates to a different part of the genome. The relative intensity of the 2 different labels indicates copy-number changes. When only the red label (control DNA) is present, it indicates an absence of patient DNA and therefore a deletion (red stars). When there is more patient than control DNA, the patient label is overrepresented (green circles) and indicates duplication. When there are no copy-number changes, there should be equal amounts of control-labeled and patient-labeled DNA (indicated with blue circles).

B. SNP array



A single-nucleotide polymorphism (SNP) array contains small fragments of DNA from the human genome where there are known to be multiple alleles. Each allele is represented on the array and each position on the array corresponds to a genetic locus. DNA from the patient is hybridized to the array. Patients who have the A allele at a specific locus will bind to the A allele on the array. If the patient is homozygous, the sample will bind only to A or B (AA or BB). If the patient is a heterozygote, the sample will be label hybridized to A and B (AB). Copy-number changes are determined by the relative intensity of bound DNA at each allele with a relative decrease in deletions (red bar) and an increase in duplications (green bar). Consanguinity is indicated by a loss of heterozygosity over large spans of DNA.

FIGURE 1

Overview of chromosomal microarrays.

Array Comparative Genomic Hybridization

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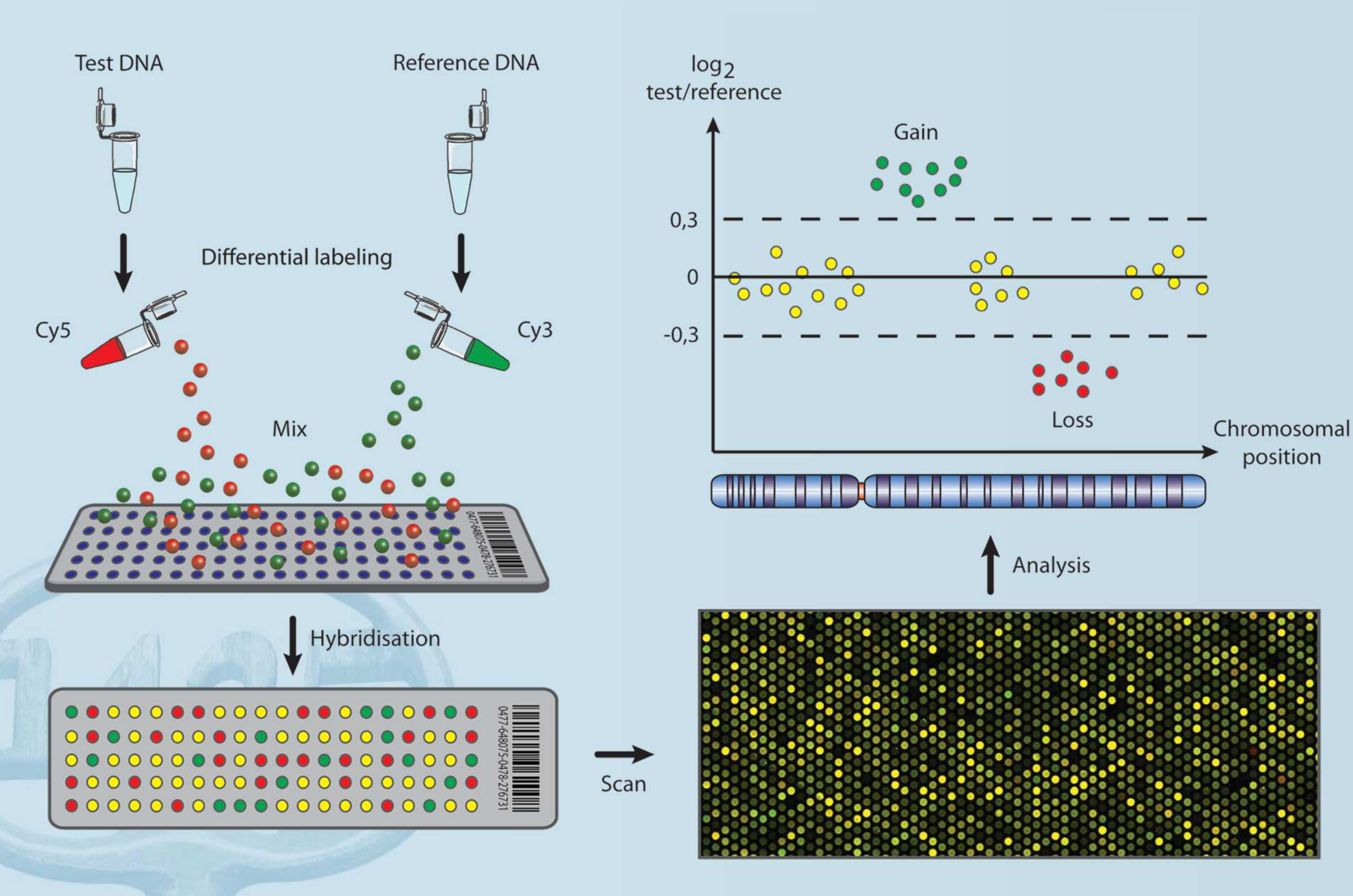
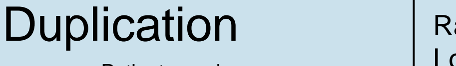


Deletions and duplications

Duplication

Patient rood
Controle groen

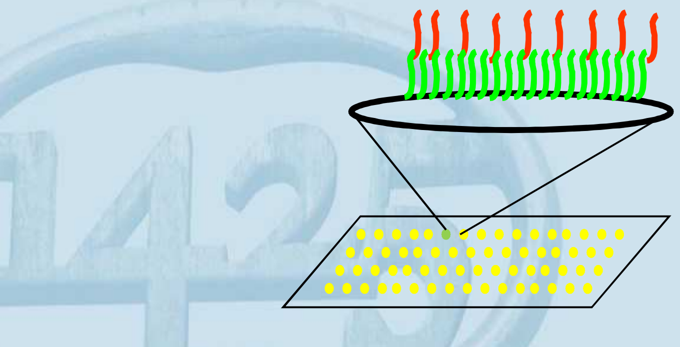
Ratio Red/green= 3/2=1.5
Log2 = 0.56

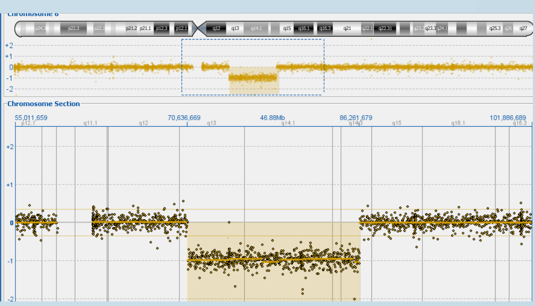


Deletion

Patient rood
Controle groen

Ratio Red/green= 1/2=0.5
Log2 = -1





Oligonucleotide-based Array CGH: genome wide view

Oligonucleotide-based Array CGH

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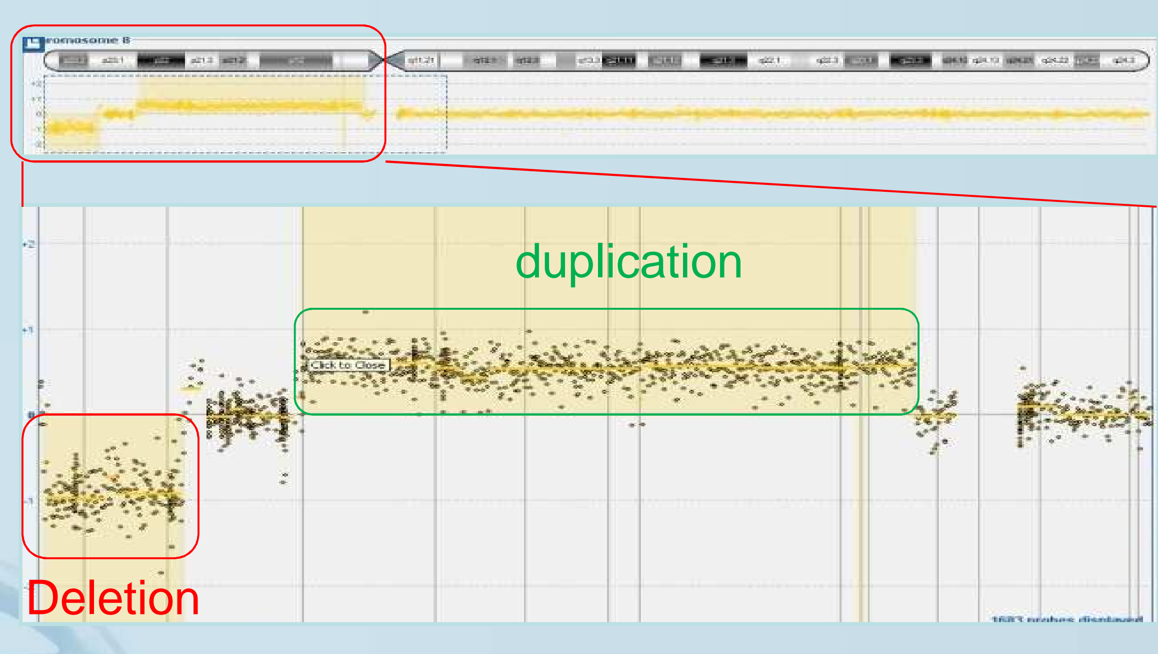

Unbalanced translocation: der(7)t(5;7)(q35.2qter;q36.1qter)

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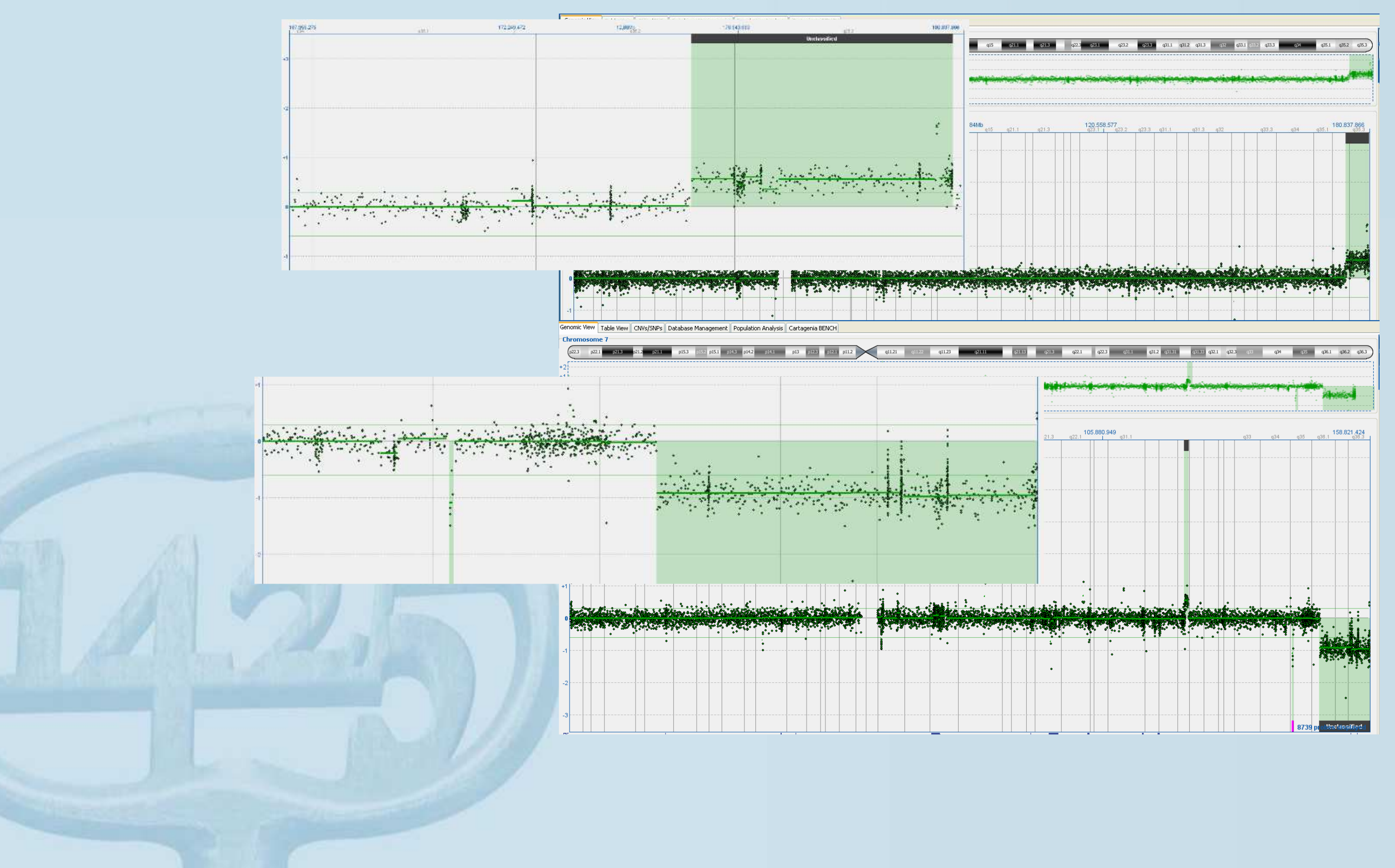
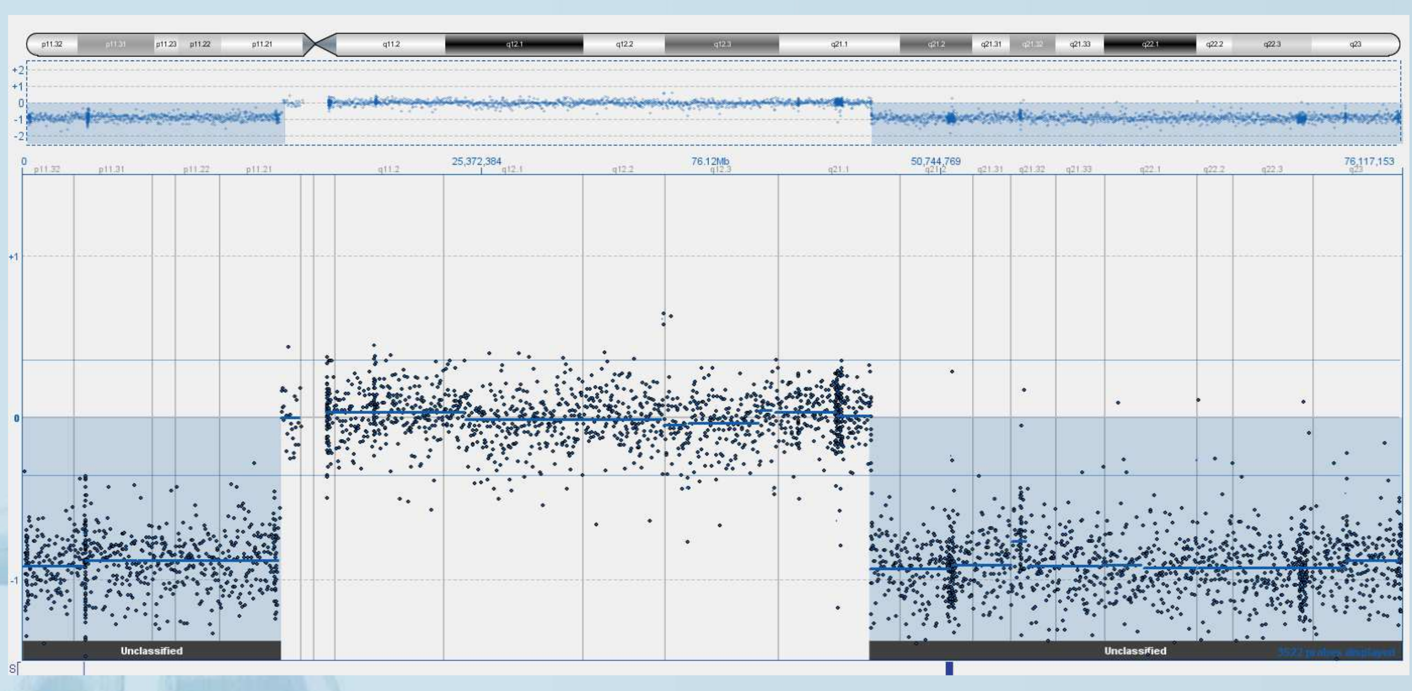
- 5 Mb gain of 5q and 8 Mb loss of 7q
- Typical pattern associated with an unbalanced translocation



?



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Array resolution depends on

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Depends on number of targets

(The more targets the higher the resolution)

and

–STANDARD DEVIATION (the variability of intensity ratio)

- DYNAMIC RANGE of individual targets

- DATA ANALYSIS

Reference

- DNA from normal individual
  - Who’s normal?
- DNA from a mixture of individuals
  - How many?
  - Which?
  - Value?
- DNA from other patients
  - When?
  - Three way hybridisations
- DNA from same individual (for acquired disorders only)

Genome wide genotyping techniques

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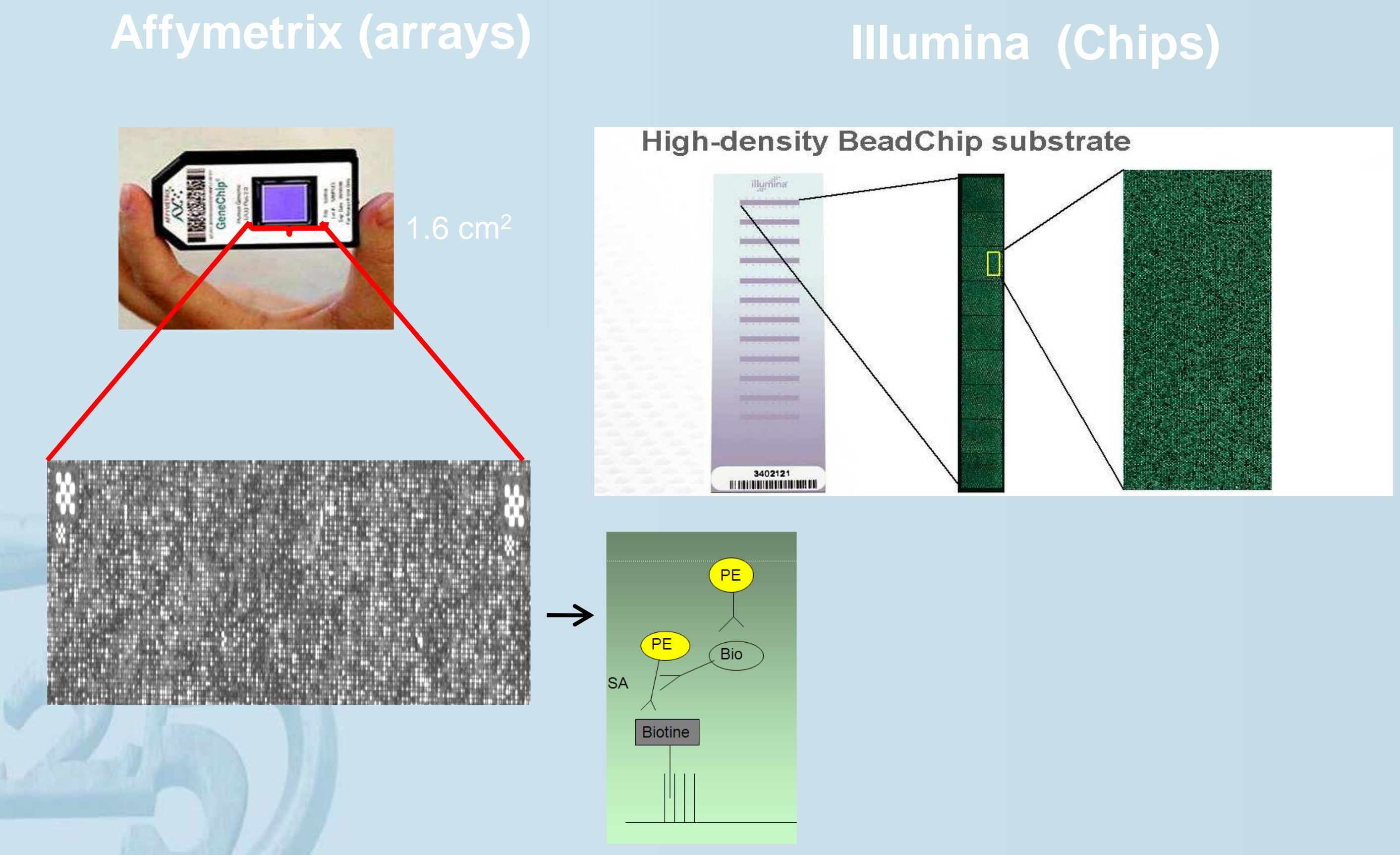
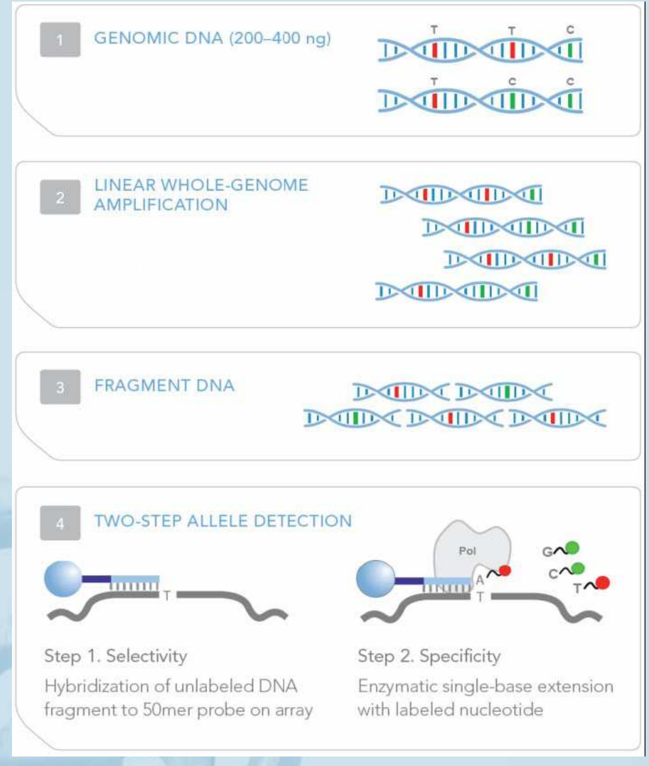
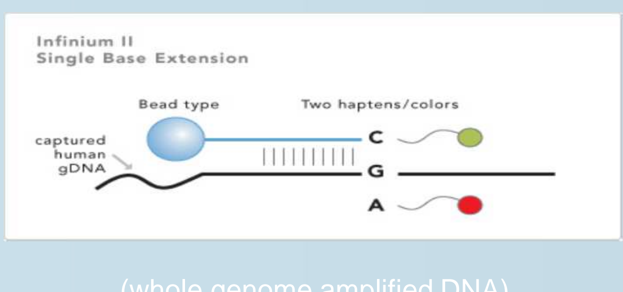
Affymetrix (arrays) **Illumina (Chips)**

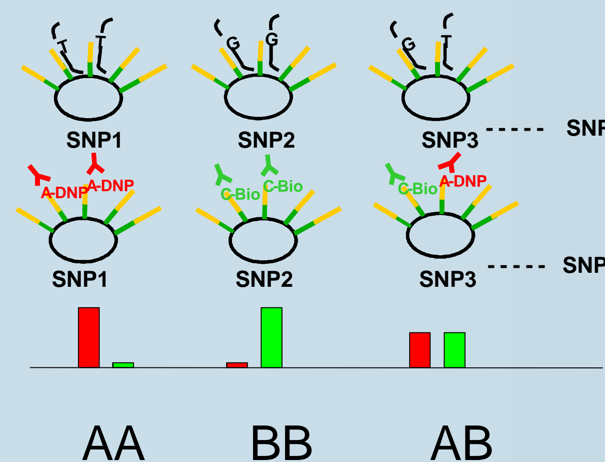


Illumina: Infinium set-up





(whole genome amplified DNA)



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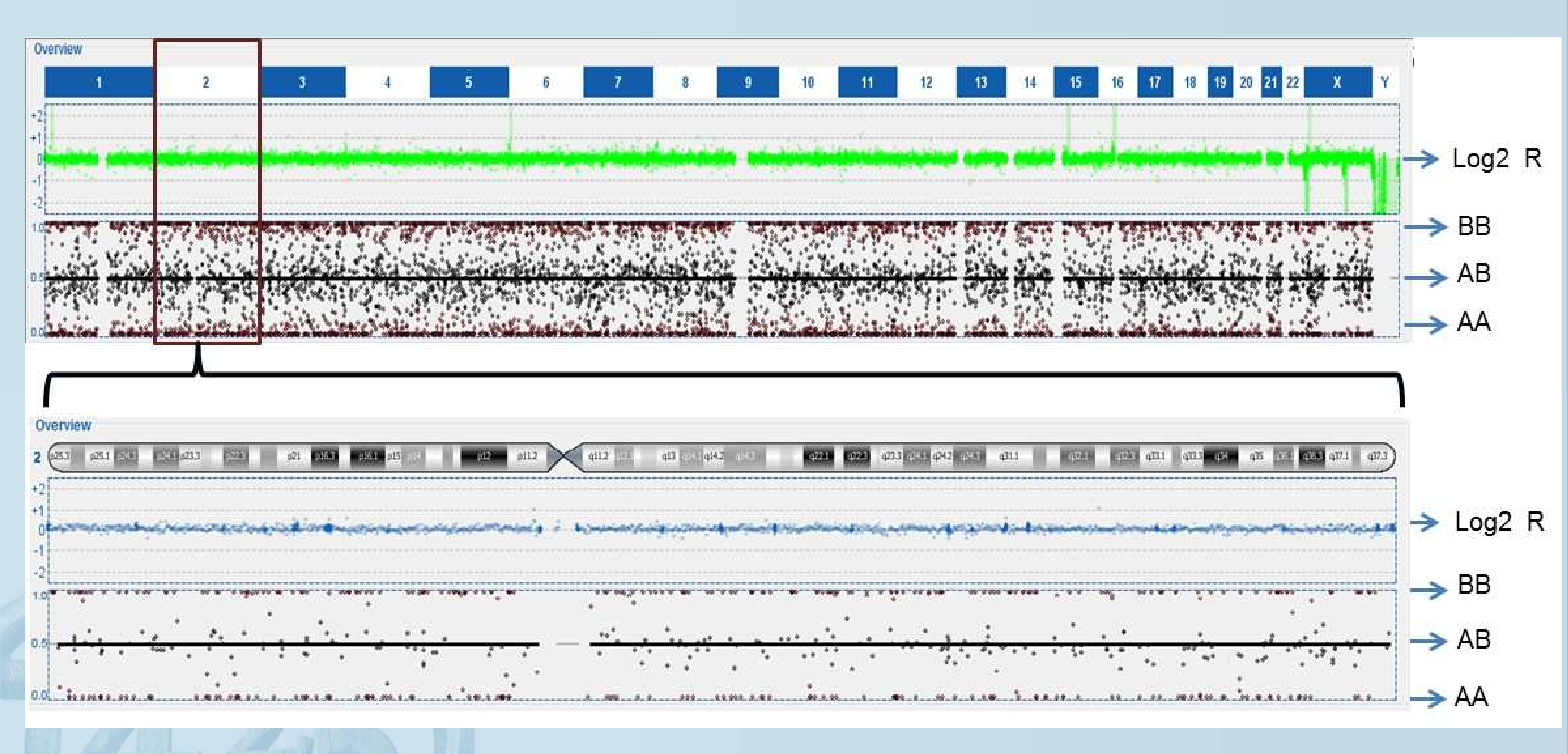
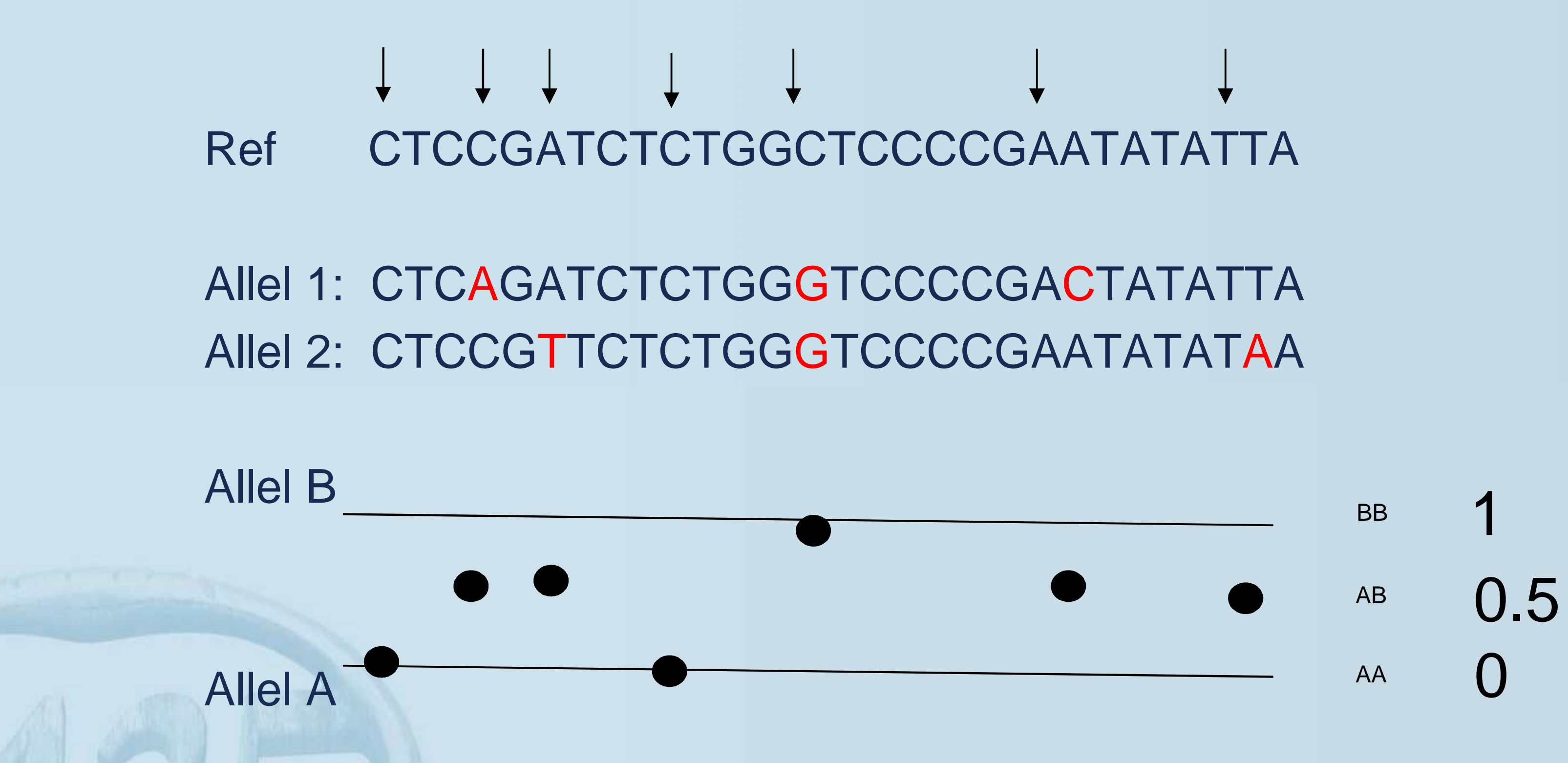
Visualisation of CNV & SNP data

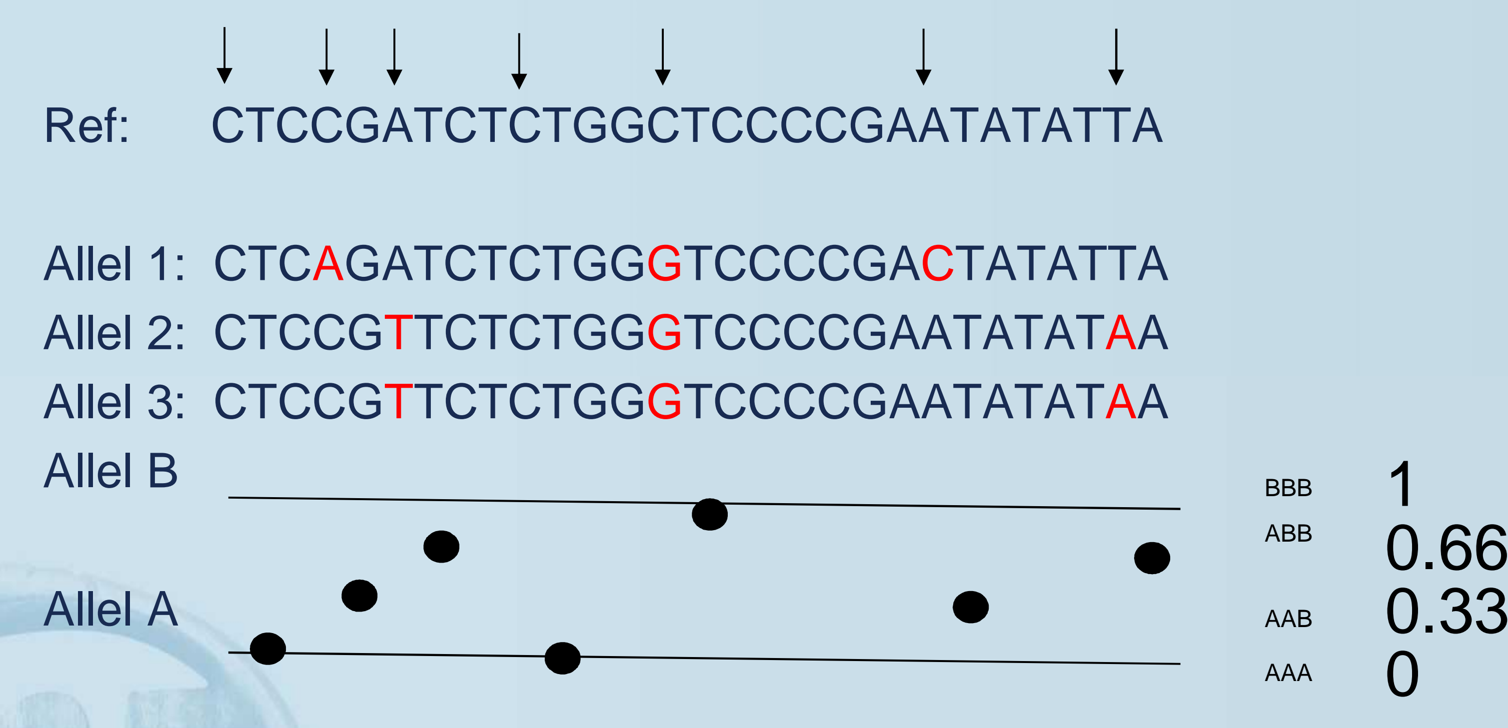


B-allele frequency plot

Principles of B-allele frequency plot
Disomy



Principles of B-allele frequency plot
Trisomy



Visualisation of CNV & SNP data

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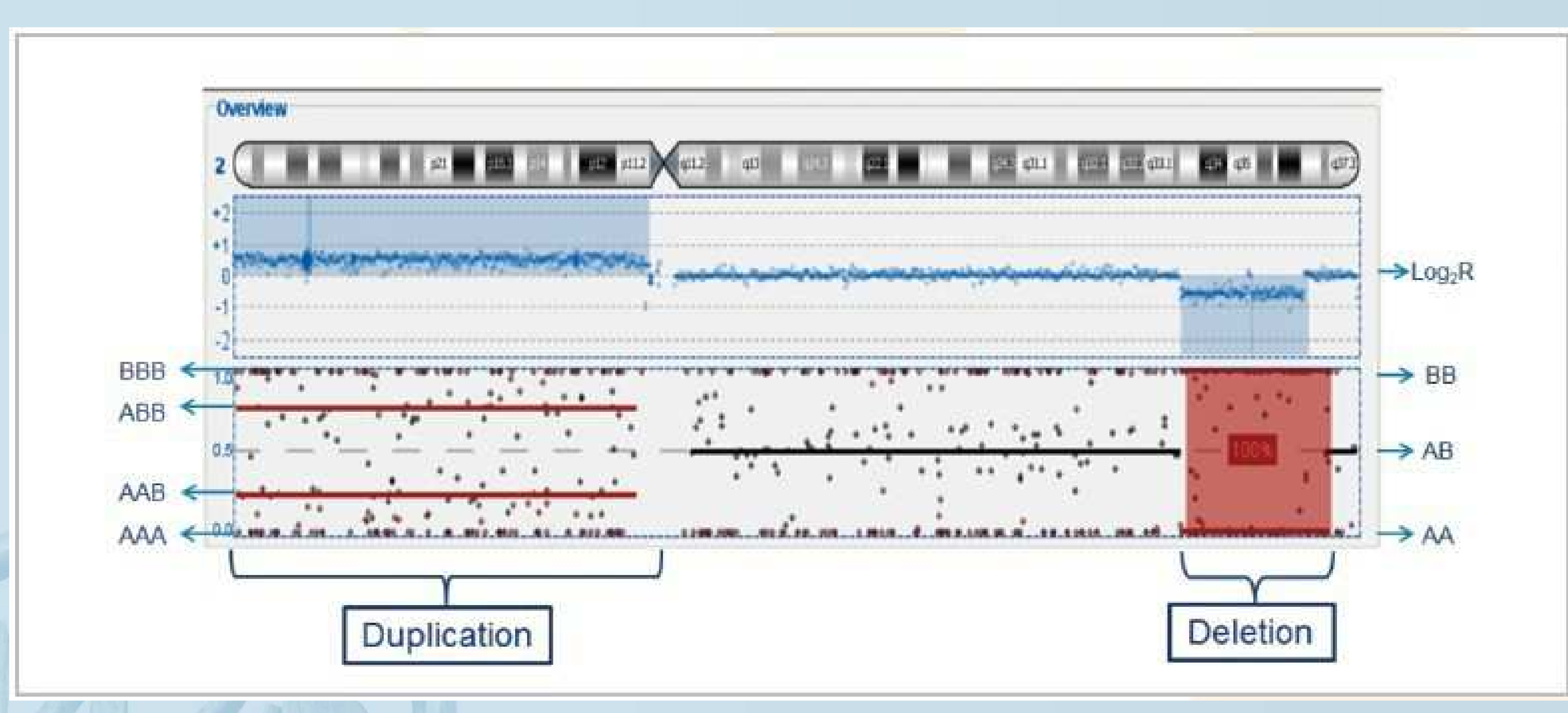
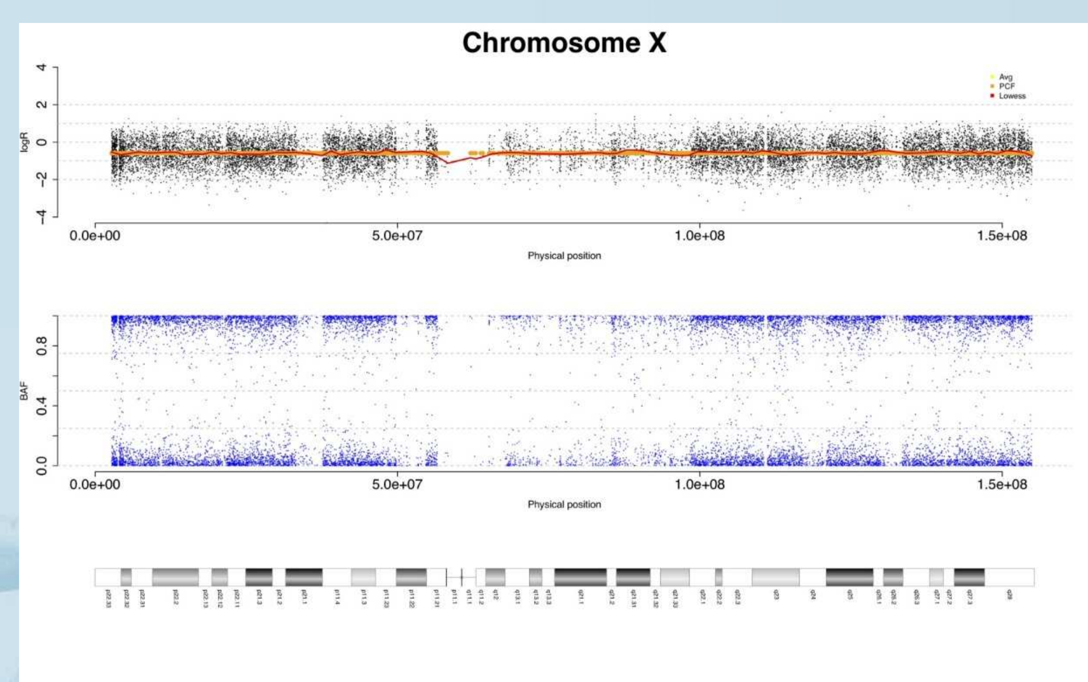


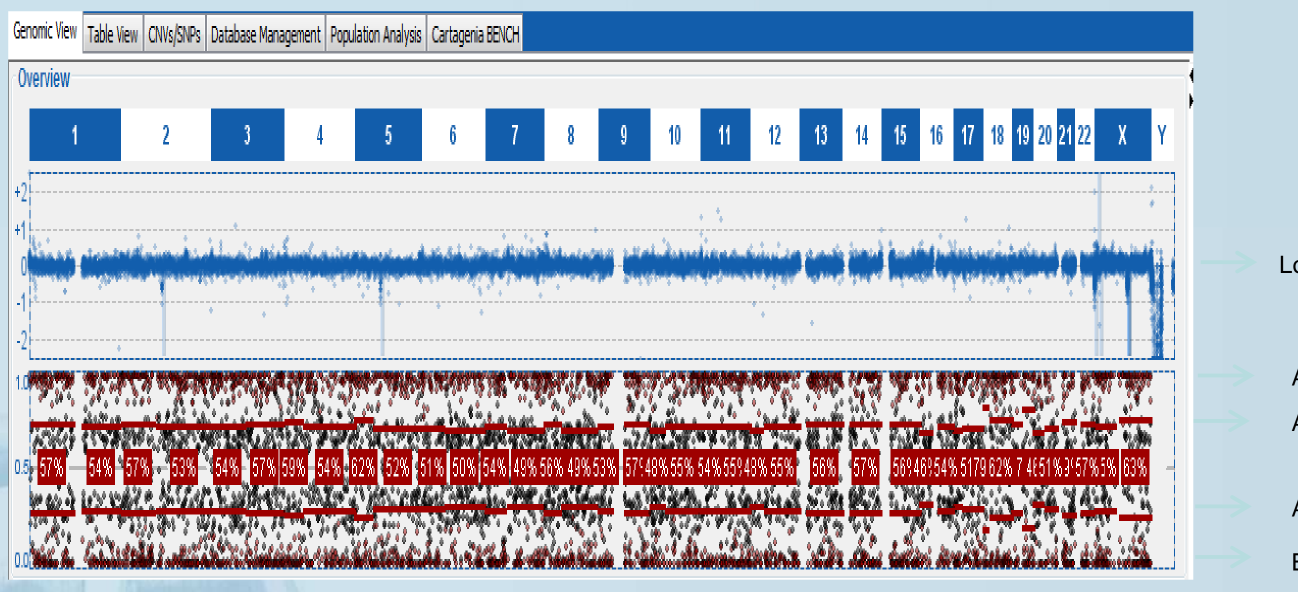
Monosomy



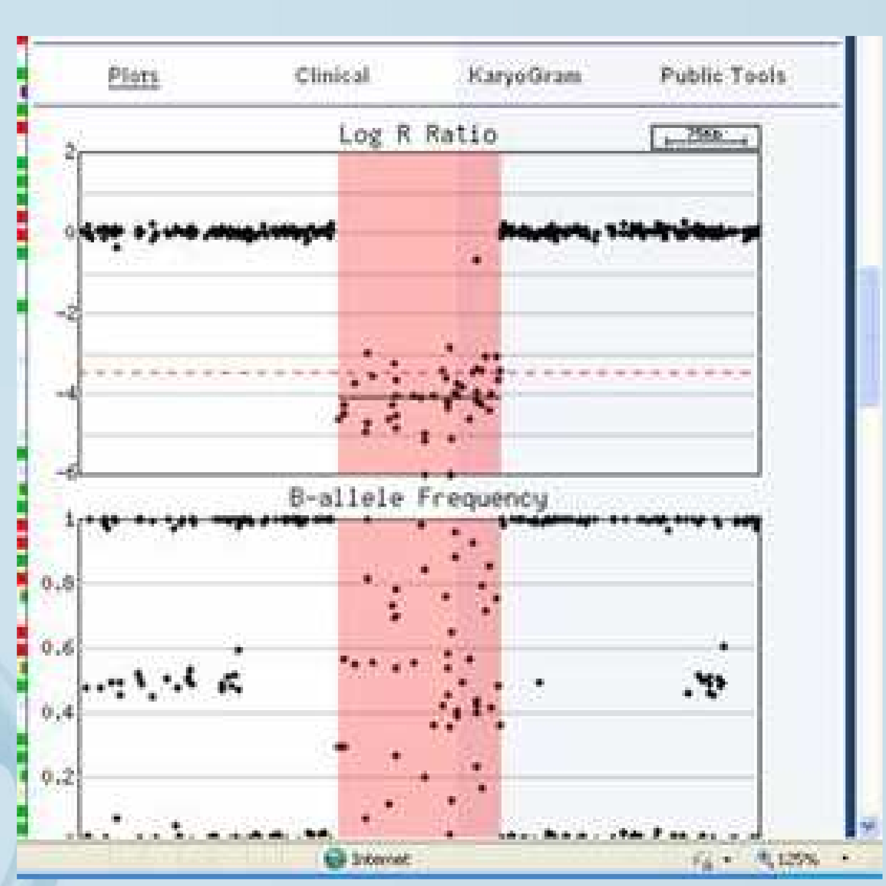
Allele A

Allele B

Triploidy



Copy number?



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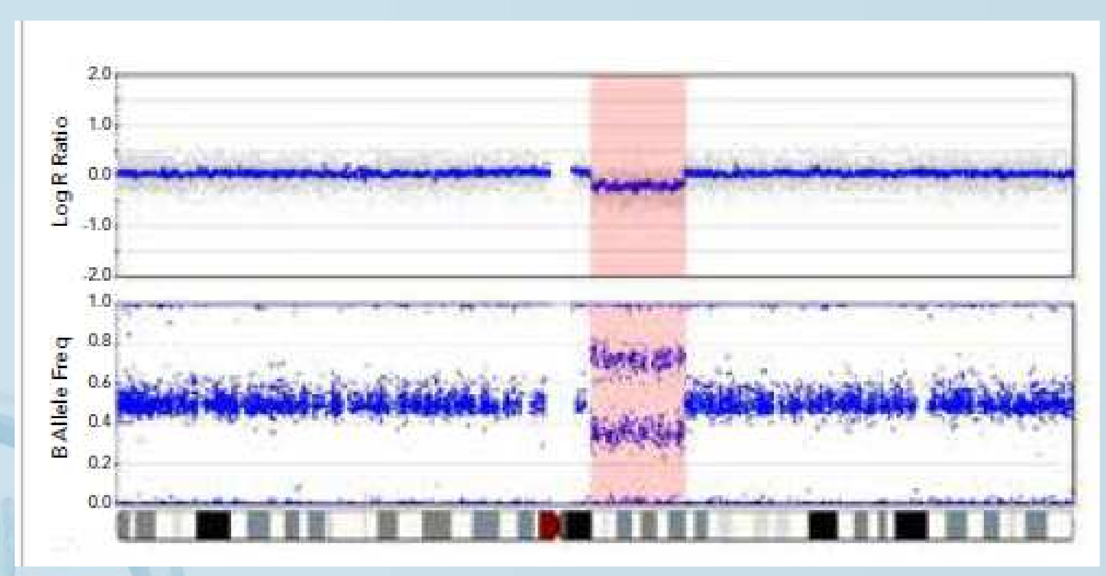
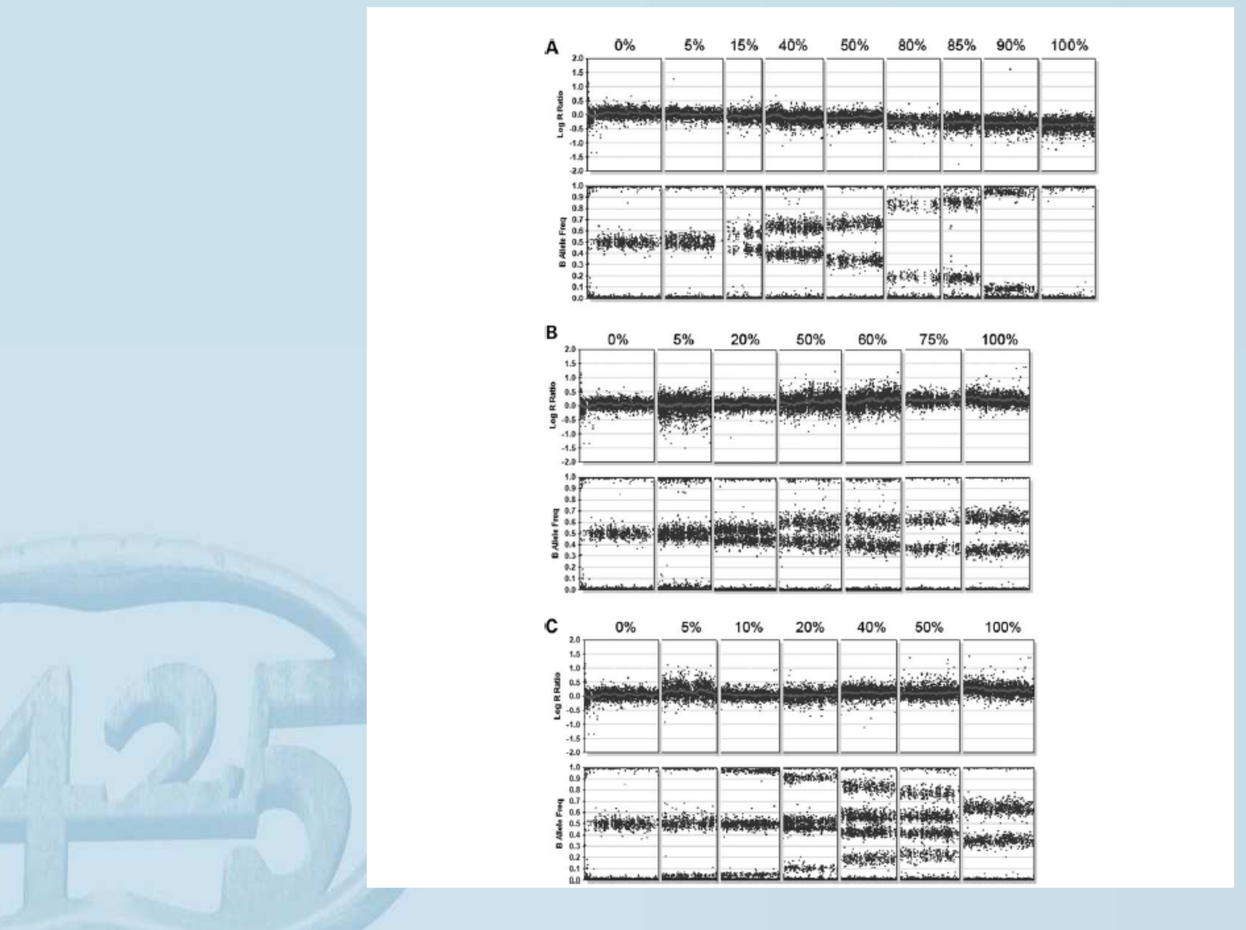
???



Log R Ratio
2.0
1.0
0.0
-1.0
-2.0
B Allele Freq
1.0
0.8
0.6
0.4
0.2
0.0

Mosaic aneuploidies



Conlin et al., Hum. Mol.Gen.,2010

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	Karyotyping	FISH	Micro-array
👍	Genome-wide Detection of balanced and unbalanced rearrangements	High resolution Fast	Genome-wide High resolution
👎	Low resolution Labour-intensive Subjective => skilled personnel	Locus specific A priori knowledge necessary	No detection of balanced rearrangements

Advantages of SNP arrays

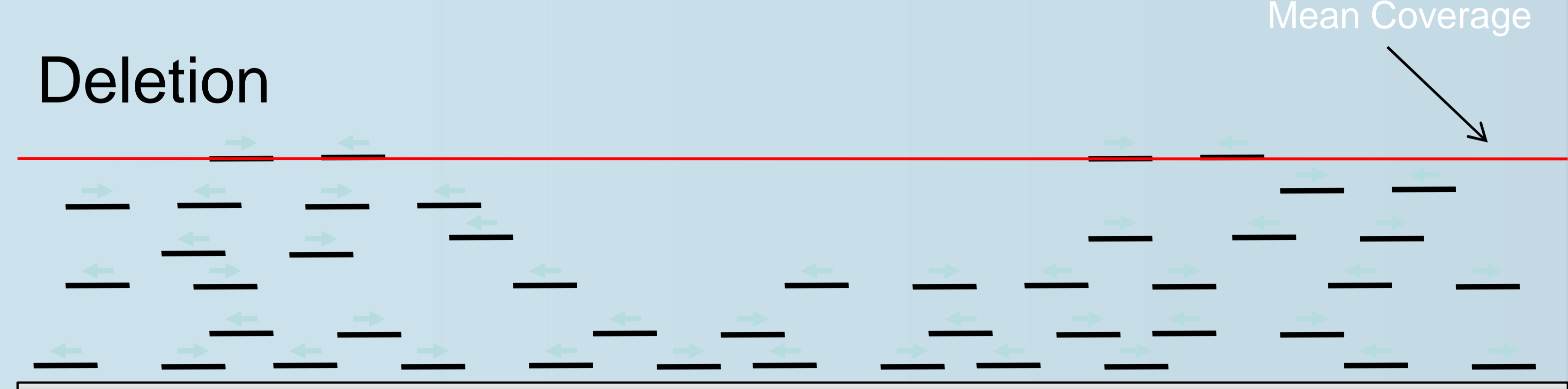
- SNP arrays have the added advantage of obtaining genotyping, which can be used to identify regions of homozygosity and can detect triploidy
- Homozygosity may indicate
  - Uniparental disomy (UPD) –although only isodisomy can be identified with SNP arrays
  - Absence of heterozygosity (AOH) in constitutional postnatal, prenatal) cases
  - Loss of heterozygosity (LOH) in cancer cases (acquired regions of homozygosity)

Massive parallel sequencing

Read-depth Analysis

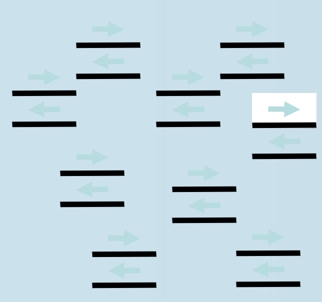
Mean Coverage

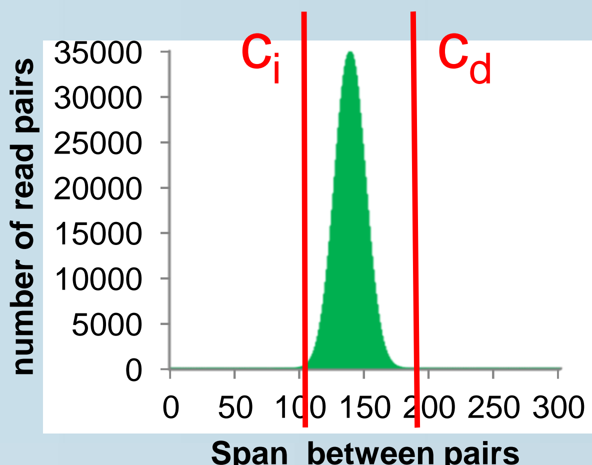
Deletion

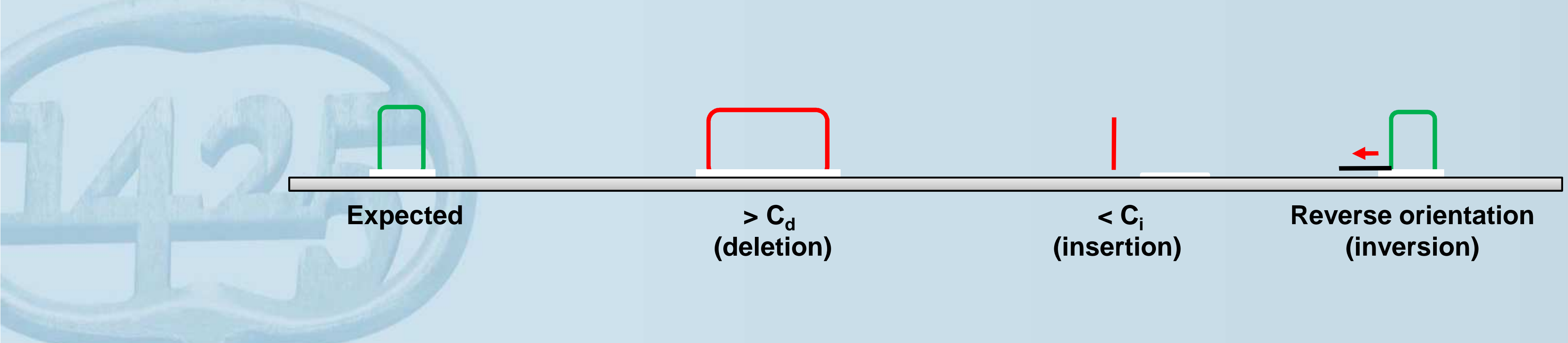


Duplication

Paired-end Mapping







Split-read Analysis

Deletion

Insertion

Read-depth reveals copy number variation

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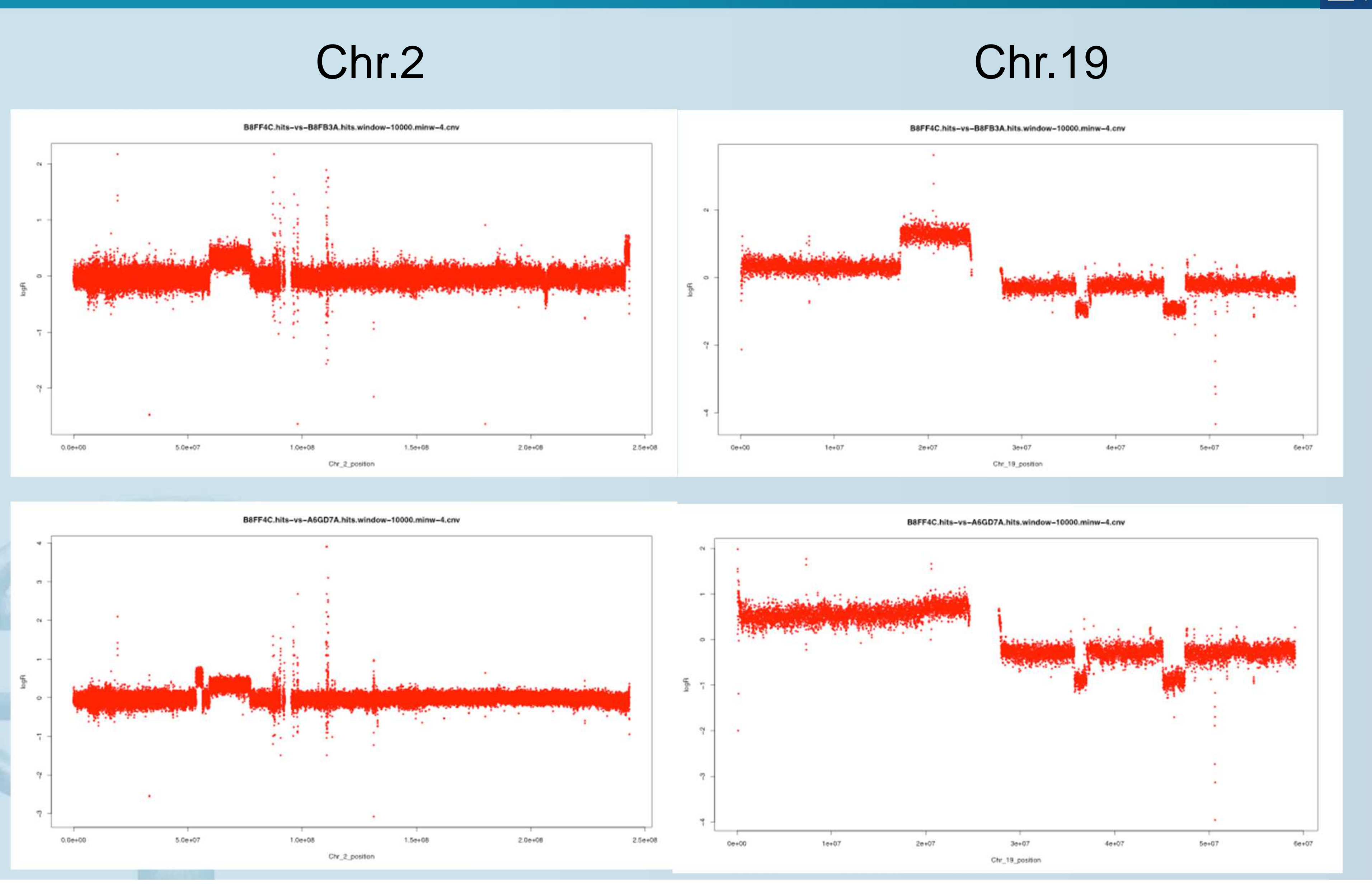

Overview

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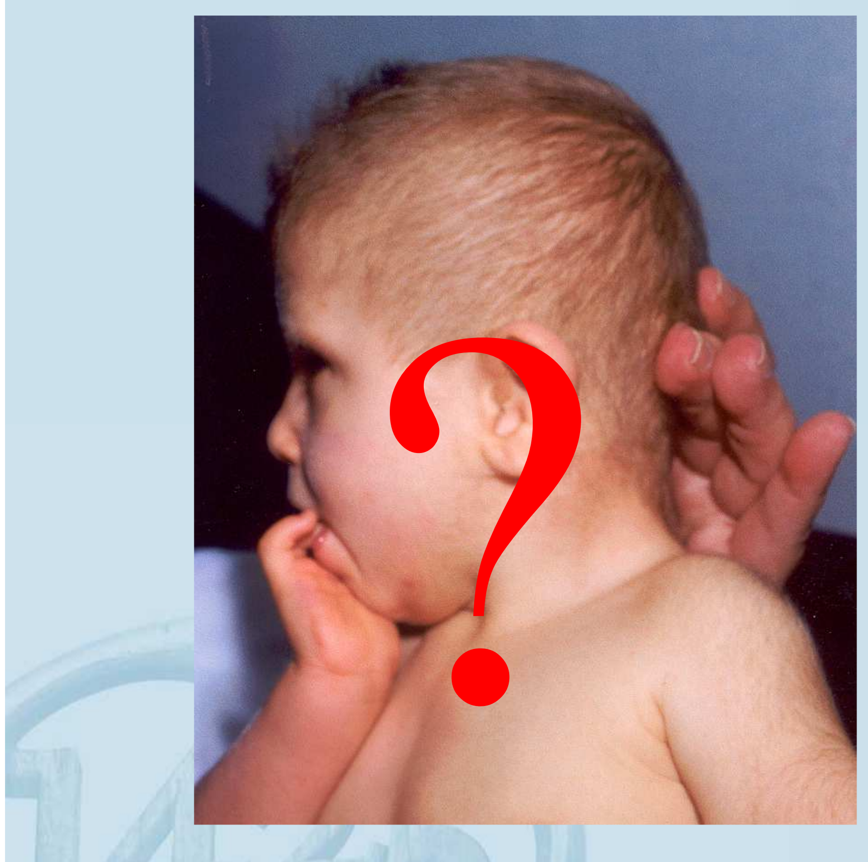
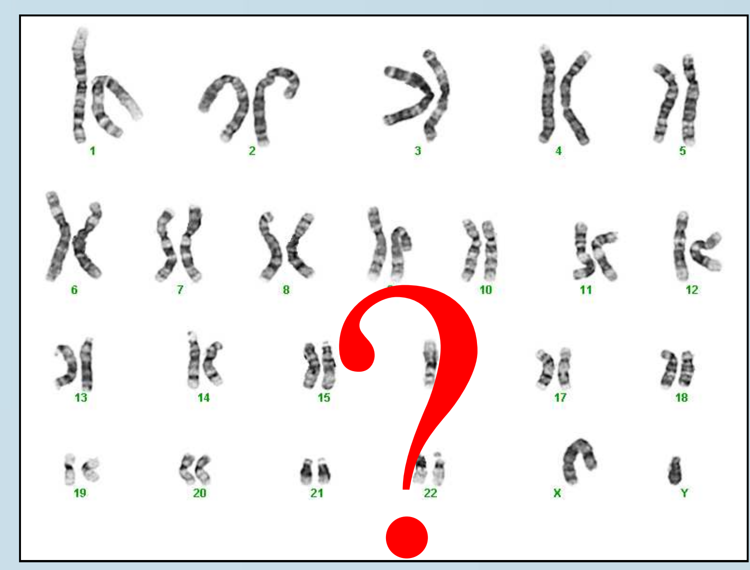
- Introduction
- Technologies for CNV detection
- Clinical interpretation & consequences
- Mechanisms of origin

15 years ago





Question: Can submicroscopic imbalances explain the cause of the MCA/MR?

15% of developmental anomalies can be explained by CNV's

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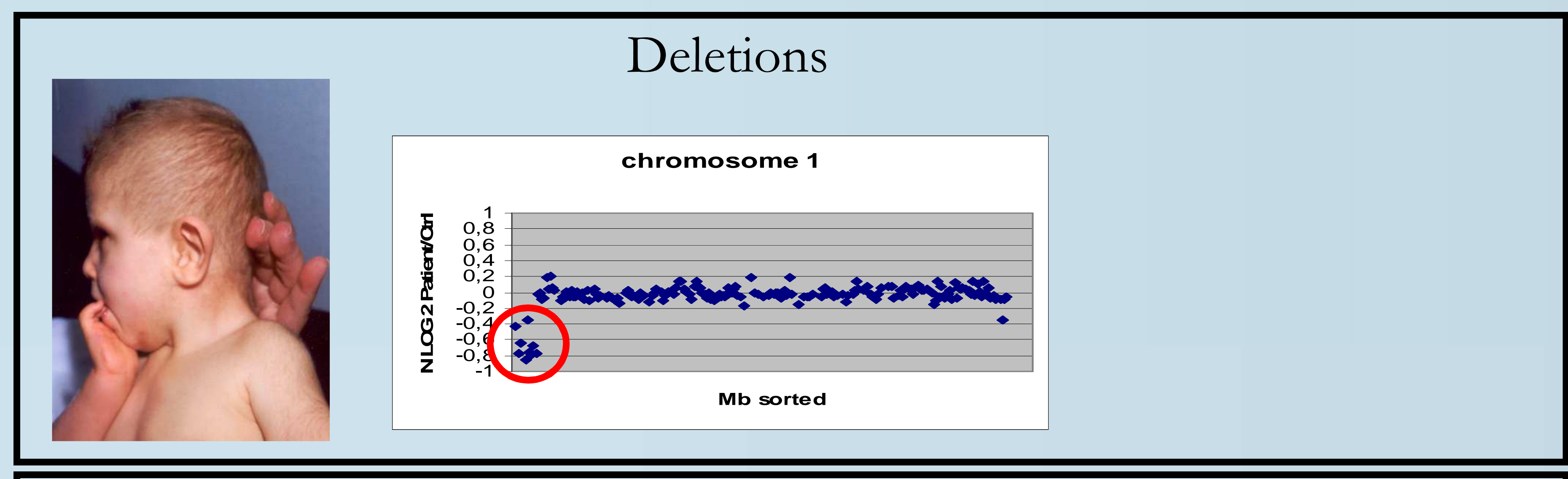
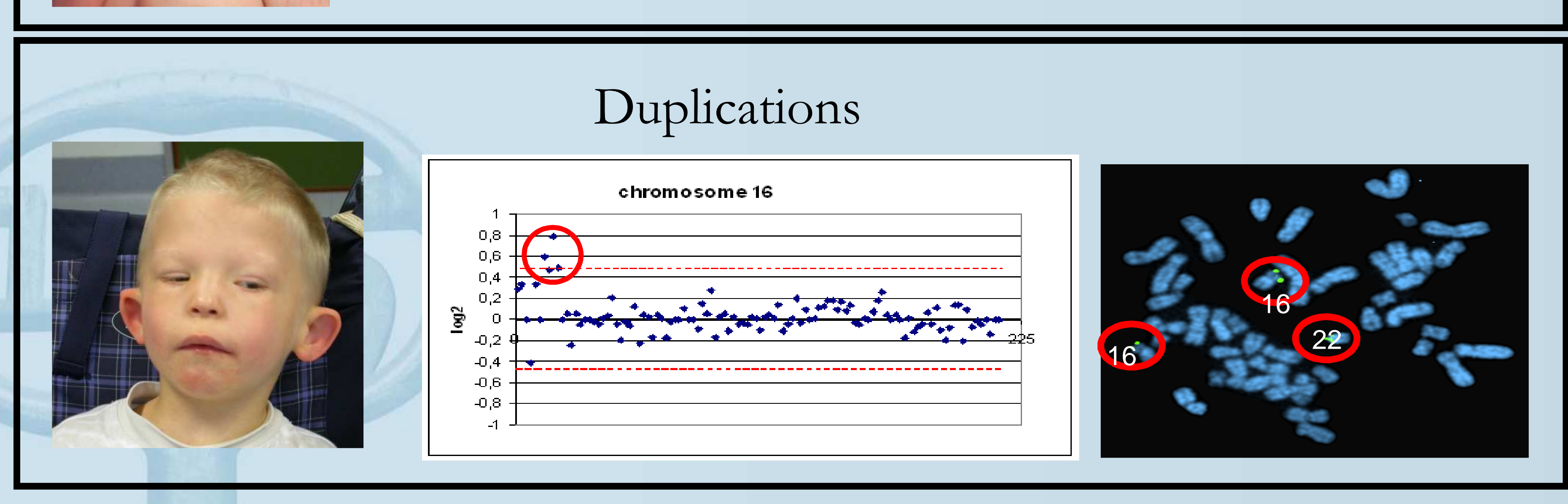
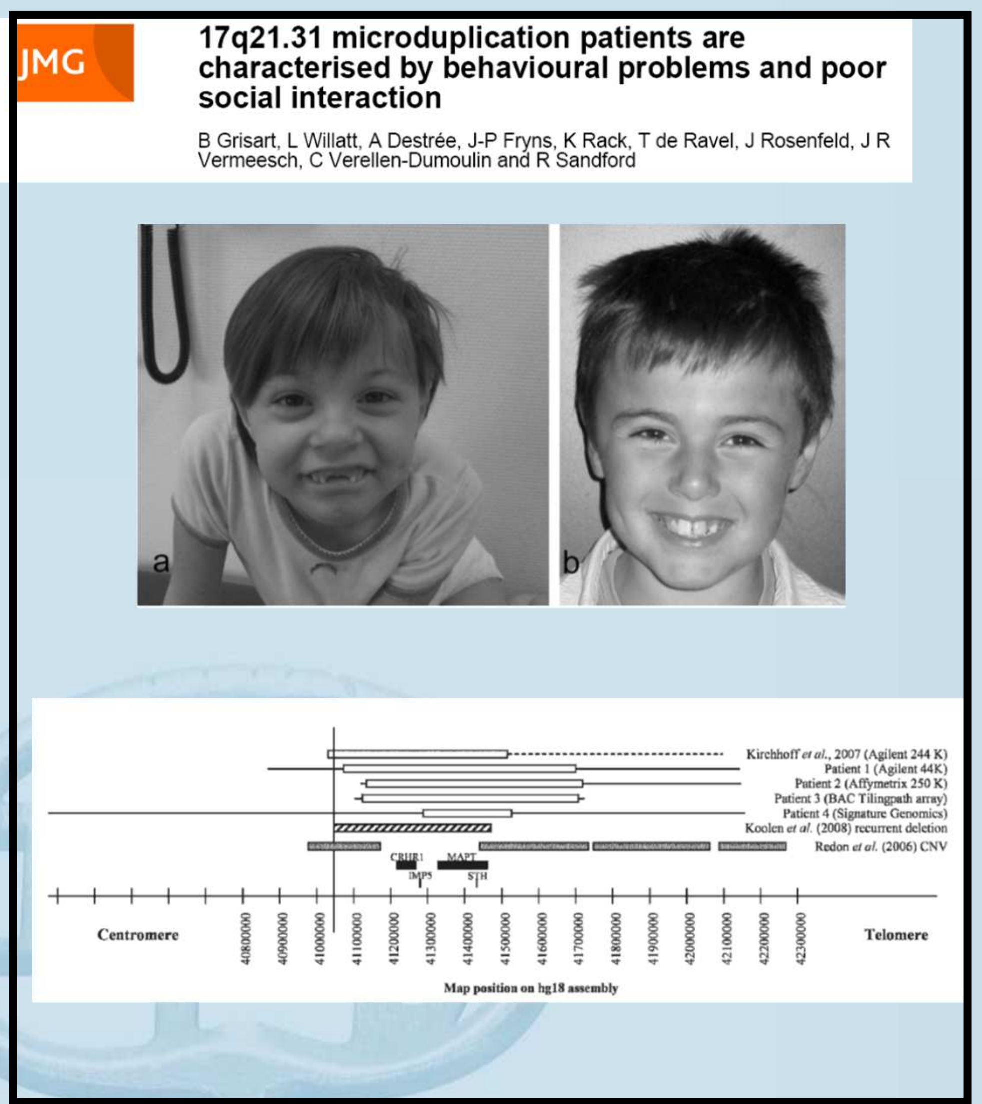
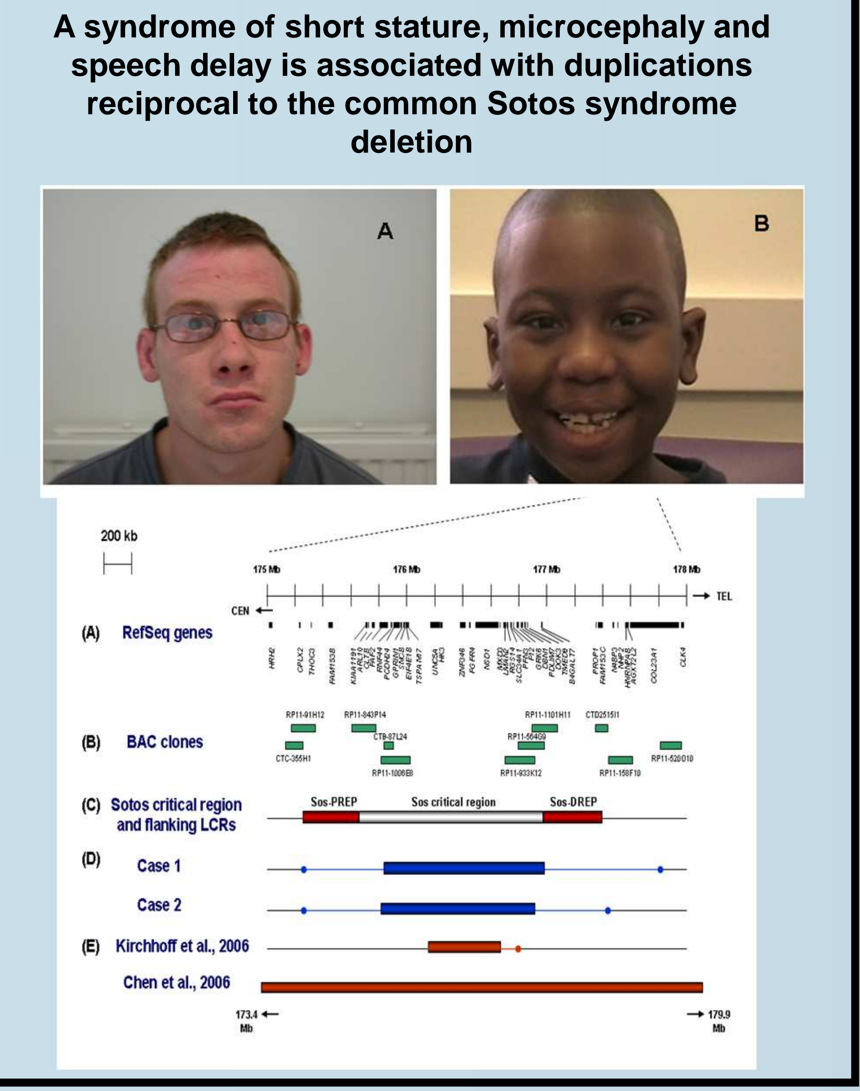




For all recurrent deletion syndromes the reciprocal duplication is now identified





Franco et al., Eur.J. Hum. Gen., 2011

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Accumulation of non-recurrent imbalances leads to the functional identification of genes

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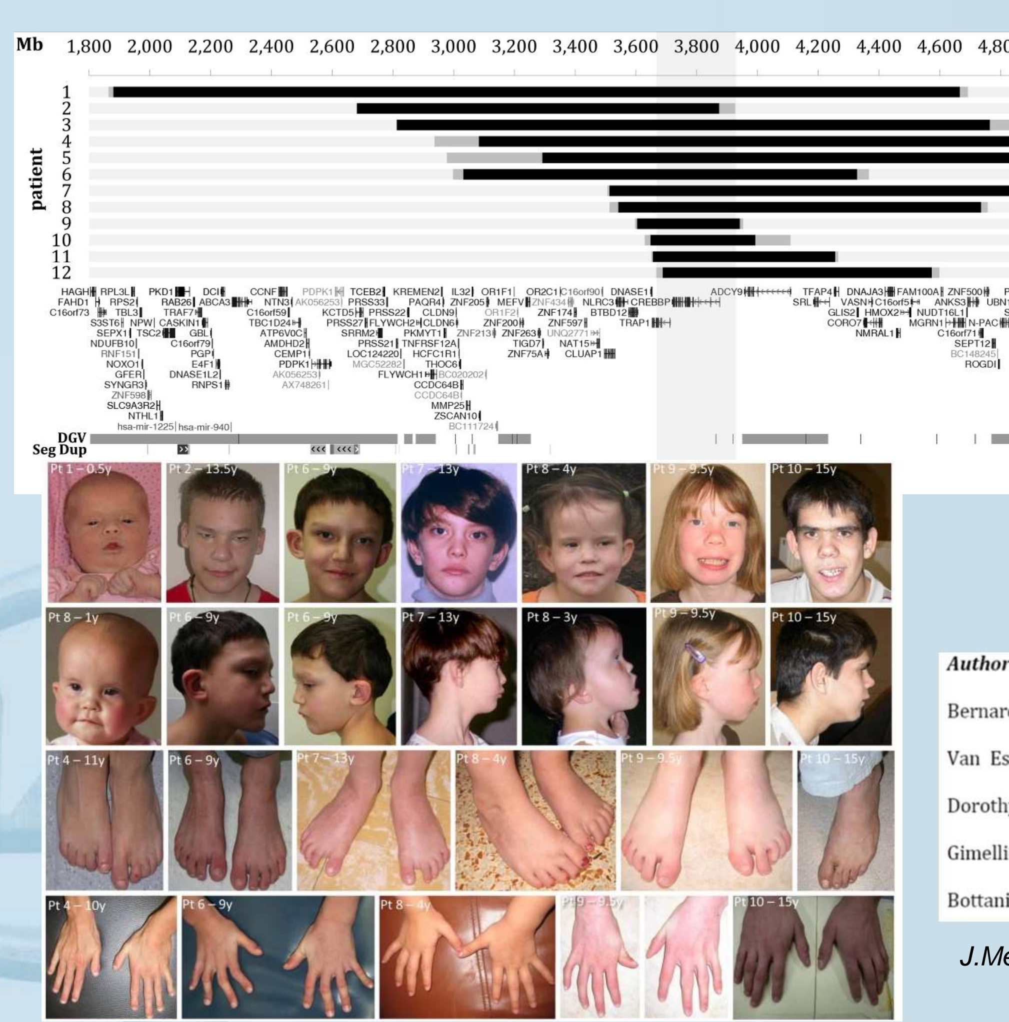

Authors:

Bernard Thienpont[1], Frédérique Béna[2], Jeroen Breckpot[1], Nicole Philip[3], Björn Menten[4], Hilde Van Esch[1], Emmanuel Scalais[5], Jessica M. Salamone[6], Chin-To Fong[7], Jennifer L. Kussmann[8], Dorothy K. Grange[9], Jerome L. Gorski[8], Farah Zahir[10], Siu Li Yong[11], Michael M. Morris[2], Stefania Gimelli[2], Jean-Pierre Fryns[1], Geert Mortier[4], Jan M. Friedman[10], Laurent Villard[12], Armand Bottani[2], , Joris R. Vermeesch[1], Sau Wai Cheung[13] & Koen Devriendt[1]

J.Med.Gen., 2011

Molecular mechanisms by which chromosomal rearrangements can influence phenotypes

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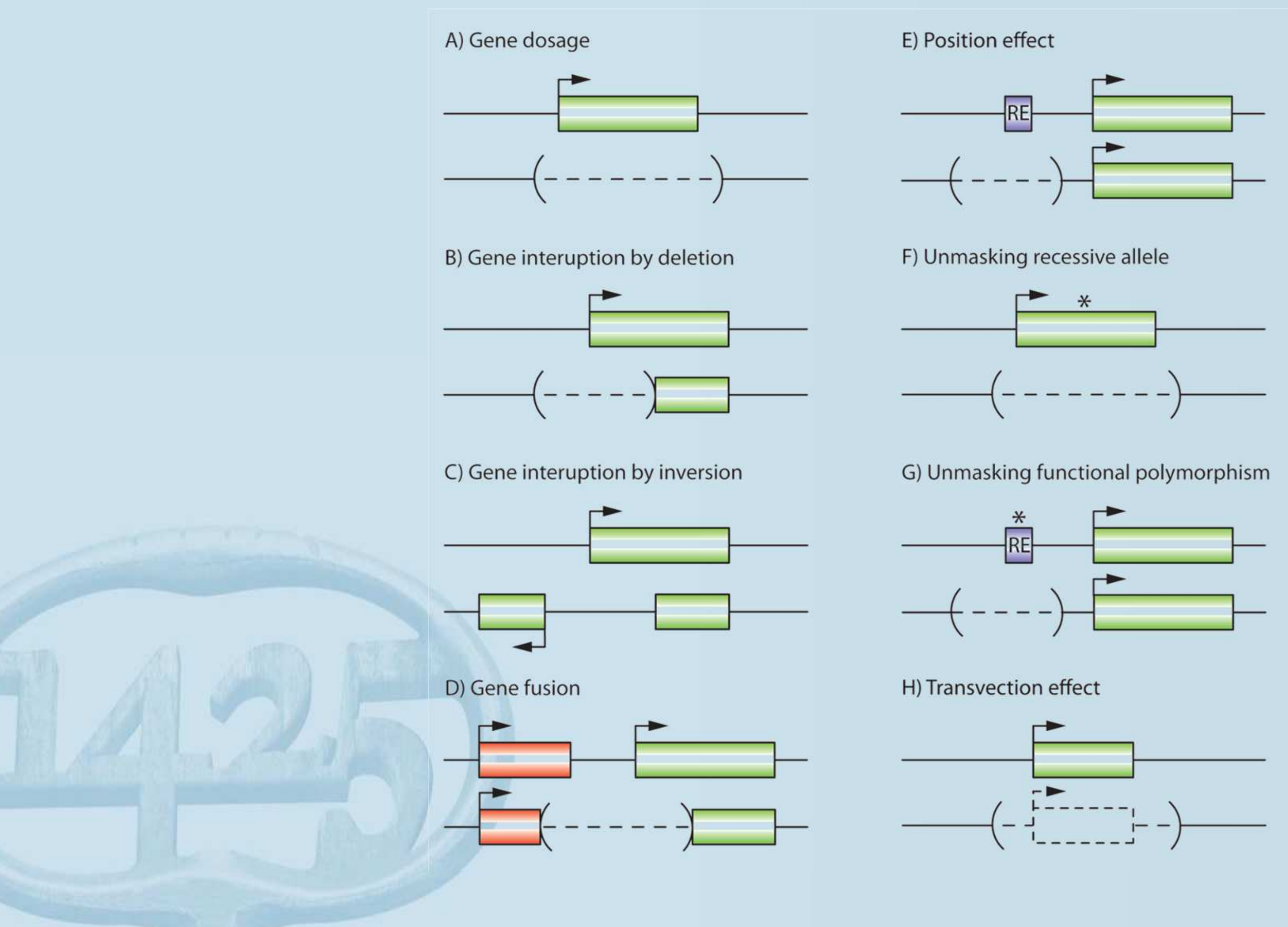
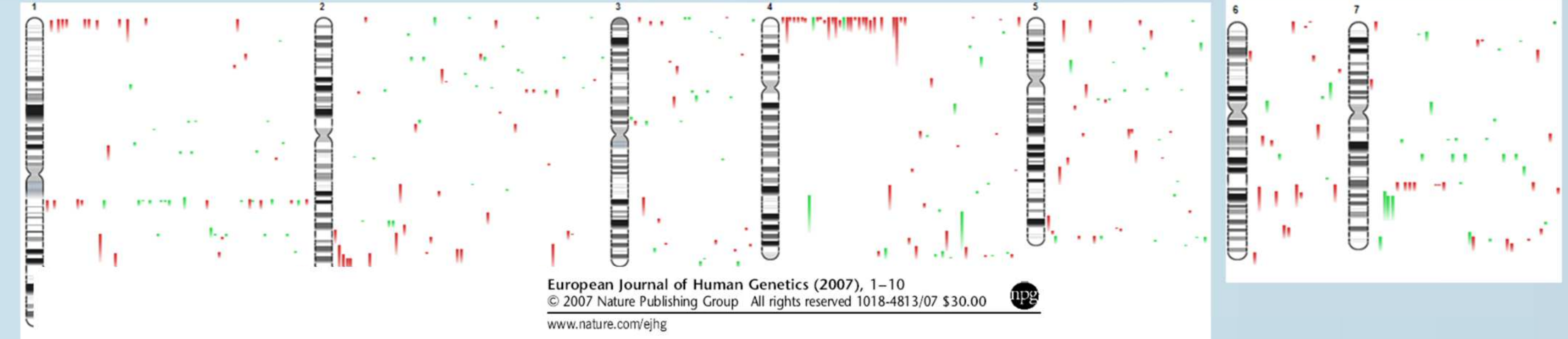


CNVs as cause of developmental disorders



European Journal of Human Genetics (2007), 1–10
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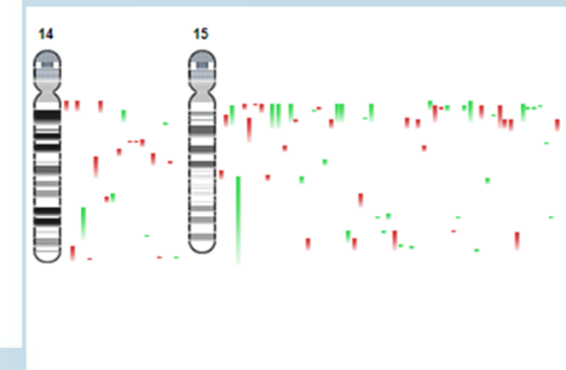
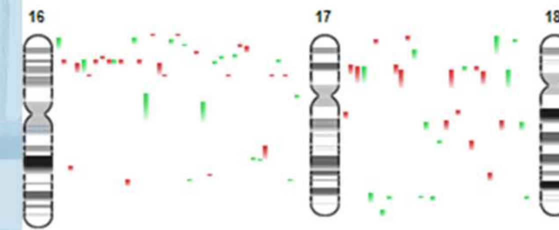
POLICY

Guidelines for molecular karyotyping in constitutional genetic diagnosis

Joris Robert Vermeesch*[,1], Heike Fiegler[2], Nicole de Leeuw[3], Karoly Szuhai[4], Jacqueline Schoumans[5], Roberto Ciccone[6], Frank Speleman[7], Anita Rauch[8], Jill Clayton-Smith[9], Conny Van Ravenswaaij[10], Damien Sanlaville[11], Philippos C Patsalis[12], Helen Firth[13], Koen Devriendt[1] and Orsetta Zuffardi[6]





Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies

David T. Miller,[1,*] Margaret P. Adam,[2,3] Swaroop Aradhya,[4] Leslie G. Biesecker,[5] Arthur R. Brothman,[6] Nigel P. Carter,[7] Deanna M. Church,[8] John A. Crolla,[9] Evan E. Eichler,[10] Charles J. Epstein,[11] W. Andrew Faucett,[2] Lars Feuk,[12] Jan M. Friedman,[13] Ada Hamosh,[14] Laird Jackson,[15] Erin B. Kaminsky,[2] Klaas Kok,[16] Ian D. Krantz,[17] Robert M. Kuhn,[18] Charles Lee,[19] James M. Ostell,[8] Carla Rosenberg,[20] Stephen W. Scherer,[21] Nancy B. Spinner,[17] Dimitri J. Stavropoulos,[22] James H. Tepperberg,[23] Erik C. Thorland,[24] Joris R. Vermeesch,[25] Darrel J. Waggoner,[26] Michael S. Watson,[27] Christa Lese Martin,[2] and David H. Ledbetter[2,*]

The American Journal of Human Genetics 86, 749–764, May 14, 2010

The Bad News : We Are All Variable

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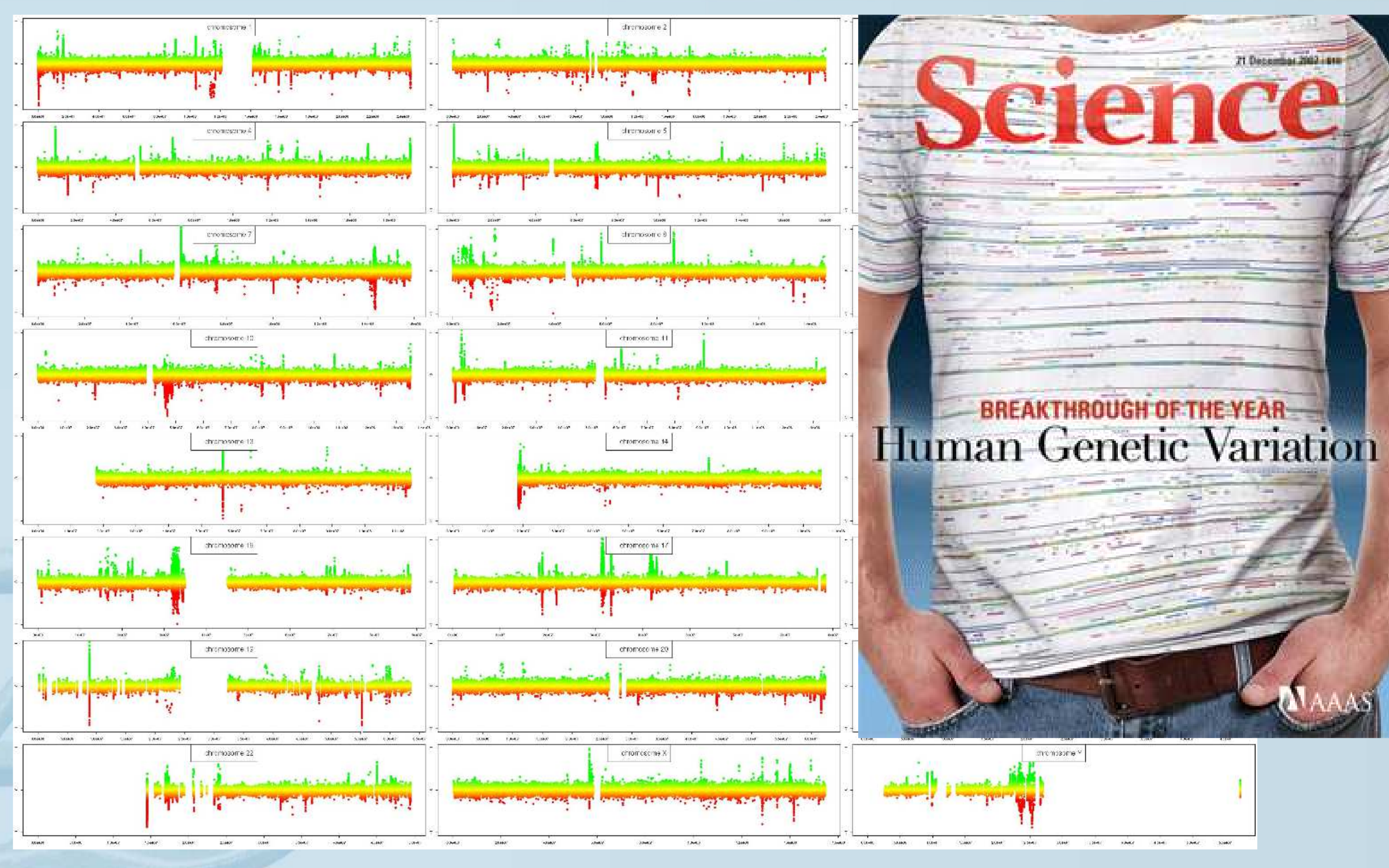

~35% of the Genome is Copy Variable in Normal Individuals

Criteria For Determining Pathogenicity

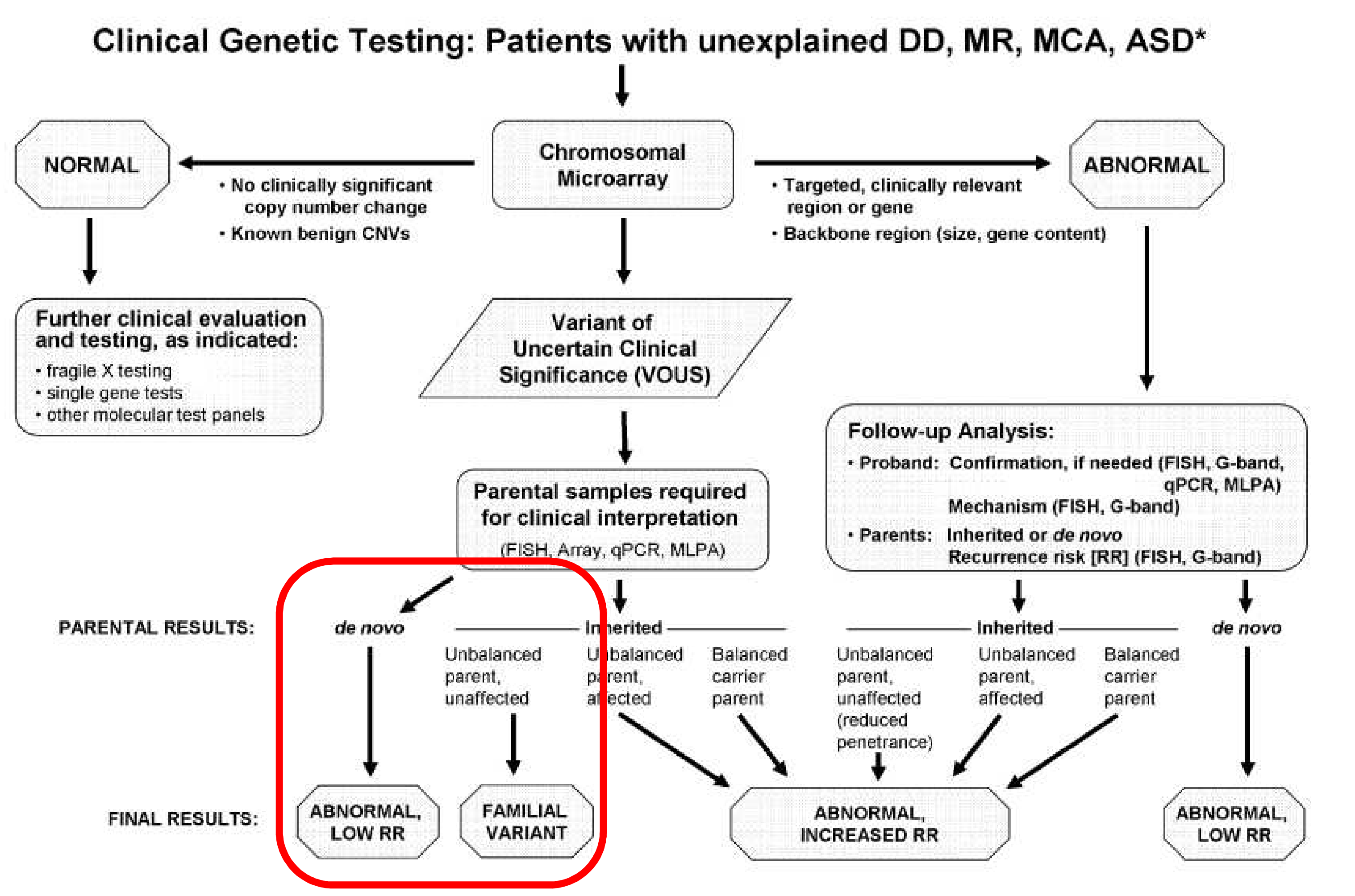

Figure 3. Algorithm for CMA Testing in Patients with Unexplained DD, MR, MCA, and A

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The Challenge : Which Variants Are Causal For The Phenotype?

KATHOLIEKE UNIVERSITEIT LEUVEN

Conventional Wisdom:

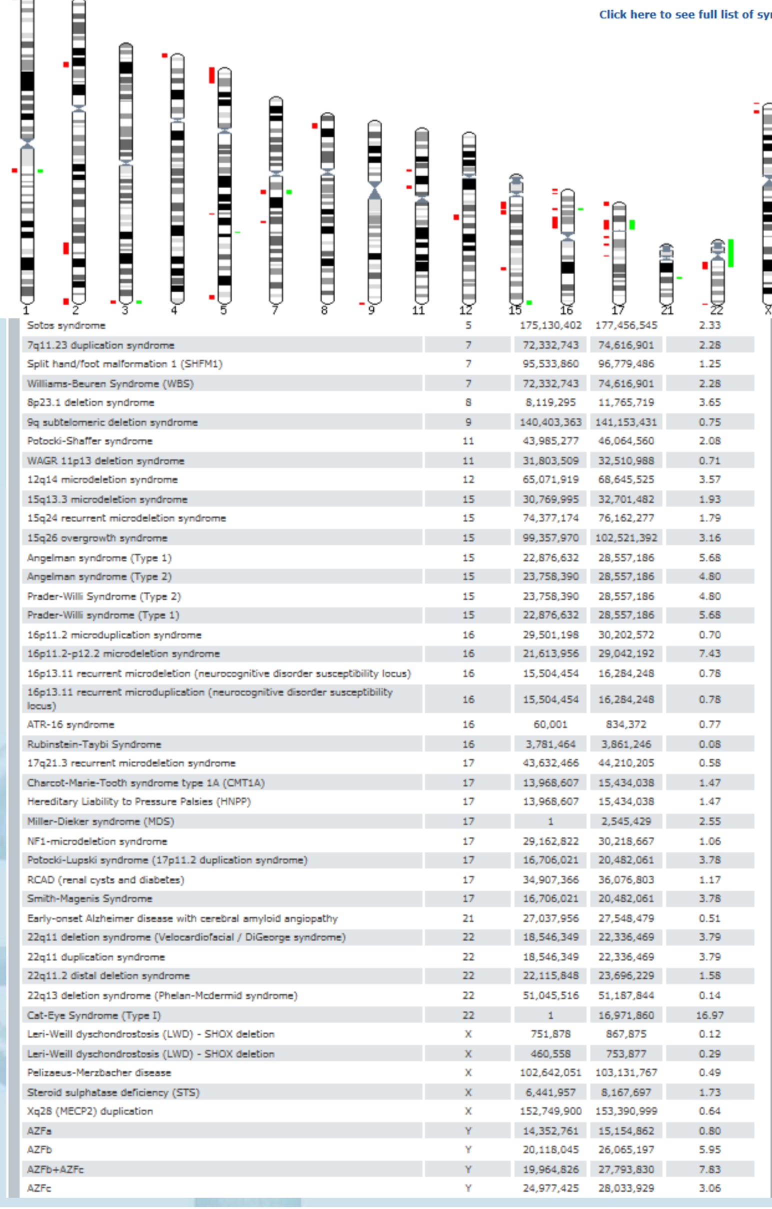
Recurrent imbalances with same phenotype are causal

The larger the size, the more likely causal

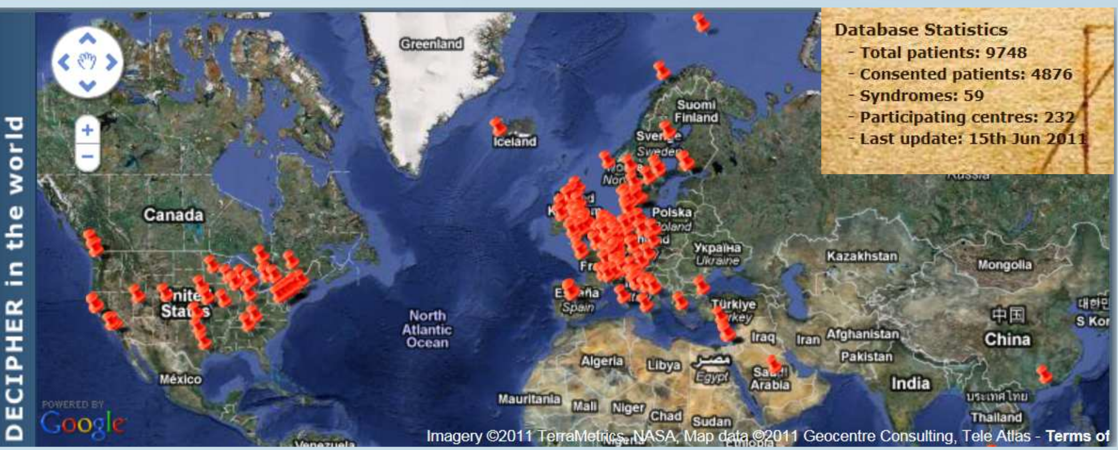
Inherited imbalances are benign whilst *de novo* imbalances are causal

Population embedded CNVs are benign

Identification of Recurrent Imbalances & Associated Phenotypes



Sotos syndrome	5	175,130,402	177,456,545	2.33
7q11.23 duplication syndrome	7	72,332,743	74,616,901	2.28
Split hand/foot malformation 1 (SHFM1)	7	95,533,860	96,779,486	1.25
Williams-Beuren Syndrome (WBS)	7	72,332,743	74,616,901	2.28
8p23.1 deletion syndrome	8	8,119,295	11,765,719	3.65
9q subtelomeric deletion syndrome	9	140,403,363	141,153,431	0.75
Potocki-Shaffer syndrome	11	43,985,277	46,064,560	2.08
WAGR 11p13 deletion syndrome	11	31,803,509	32,510,988	0.71
12q14 microdeletion syndrome	12	65,071,919	68,645,525	3.57
15q13.3 microdeletion syndrome	15	30,769,995	32,701,482	1.93
15q24 recurrent microdeletion syndrome	15	74,377,174	76,162,277	1.79
15q26 overgrowth syndrome	15	99,357,970	102,521,392	3.16
Angelman syndrome (Type 1)	15	22,876,632	28,557,186	5.68
Angelman syndrome (Type 2)	15	23,758,390	28,557,186	4.80
Prader-Willi Syndrome (Type 2)	15	23,758,390	28,557,186	4.80
Prader-Willi syndrome (Type 1)	15	22,876,632	28,557,186	5.68
16p11.2 microduplication syndrome	16	29,501,198	30,202,572	0.70
16p11.2-p12.2 microdeletion syndrome	16	21,613,956	29,042,192	7.43
16p13.11 recurrent microdeletion (neurocognitive disorder susceptibility locus)	16	15,504,454	16,284,248	0.78
16p13.11 recurrent microduplication (neurocognitive disorder susceptibility locus)	16	15,504,454	16,284,248	0.78
ATR-16 syndrome	16	60,001	834,372	0.77
Rubinstein-Taybi Syndrome	16	3,781,464	3,861,246	0.08
17q21.3 recurrent microdeletion syndrome	17	43,632,466	44,210,205	0.58
Charcot-Marie-Tooth syndrome type 1A (CMT1A)	17	13,968,607	15,434,038	1.47
Hereditary Liability to Pressure Palsies (HNPP)	17	13,968,607	15,434,038	1.47
Miller-Dieker syndrome (MDS)	17	1	2,545,429	2.55
NF1-microdeletion syndrome	17	29,162,822	30,218,667	1.06
Potocki-Lupski syndrome (17p11.2 duplication syndrome)	17	16,706,021	20,482,061	3.78
RCAD (renal cysts and diabetes)	17	34,907,366	36,076,803	1.17
Smith-Magenis Syndrome	17	16,706,021	20,482,061	3.78
Early-onset Alzheimer disease with cerebral amyloid angiopathy	21	27,037,956	27,548,479	0.51
22q11 deletion syndrome (Velocardiofacial / DiGeorge syndrome)	22	18,546,349	22,336,469	3.79
22q11 duplication syndrome	22	18,546,349	22,336,469	3.79
22q11.2 distal deletion syndrome	22	22,115,848	23,696,229	1.58
22q13 deletion syndrome (Phelan-Mcdermid syndrome)	22	51,045,516	51,187,844	0.14
Cat-Eye Syndrome (Type I)	22	1	16,971,860	16.97
Leri-Weill dyschondrostosis (LWD) - SHOX deletion	X	751,878	867,875	0.12
Leri-Weill dyschondrostosis (LWD) - SHOX deletion	X	460,558	753,877	0.29
Pelizaeus-Merzbacher disease	X	102,642,051	103,131,767	0.49
Steroid sulphatase deficiency (STS)	X	6,441,957	8,167,697	1.73
Xq28 (MECP2) duplication	X	152,749,900	153,390,999	0.64
AZFa	Y	14,352,761	15,154,862	0.80
AZFb	Y	20,118,045	26,065,197	5.95
AZFb+AZFc	Y	19,964,826	27,793,830	7.83
AZFc	Y	24,977,425	28,033,929	3.06



Home

The International Standards For Cytogenomic Arrays Consortium

The Challenge : Which Imbalances Are Causal For The Phenotype?

KATHOLIEKE UNIVERSITEIT LEUVEN

Conventional Wisdom:

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Population embedded CNVs are benign

Rare CNVs Megabases in Size Are Observed in Normal Individuals

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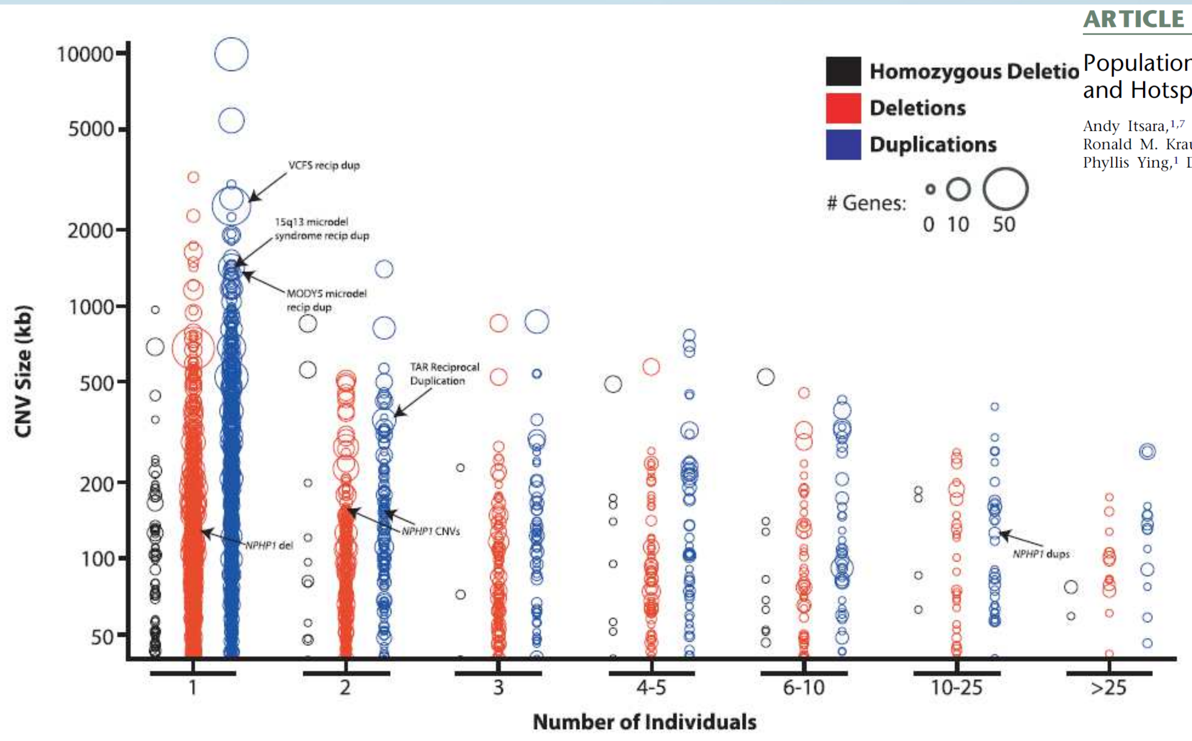

Figure 4. CNV Length, Gene Content, and Frequency Distributions
CNVs were plotted according to event type (color), length (*y* axis), frequency in the population (*x* axis, number of individuals from n = 2493), and number of RefSeq genes affected (circle size). To facilitate comparison across different platforms, events from different individuals were considered the same if their putative breakpoints were within 50 kb of one another. CNVs related to previously reported disease-causing variants are highlighted.

154 The American Journal of Human Genetics *84*, 148–161, February 13, 2009

ARTICLE

Population Analysis of Large Copy Number Variants and Hotspots of Human Genetic Disease

Andy Itsara,[1,7] Gregory M. Cooper,[1,7] Carl Baker,[1] Santhosh Girirajan,[1] Jun Li,[2] Devin Absher,[3] Ronald M. Krauss,[4] Richard M. Myers,[3] Paul M. Ridker,[5] Daniel I. Chasman,[5] Heather Mefford,[1] Phyllis Ying,[1] Deborah A. Nickerson,[1] and Evan E. Eichler[1,6,*]

Size Alone Is Not A Good Determinant
Nor Occurrence In Apparently Normal Individuals

The Challenge :
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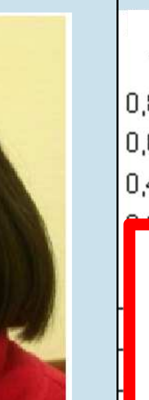
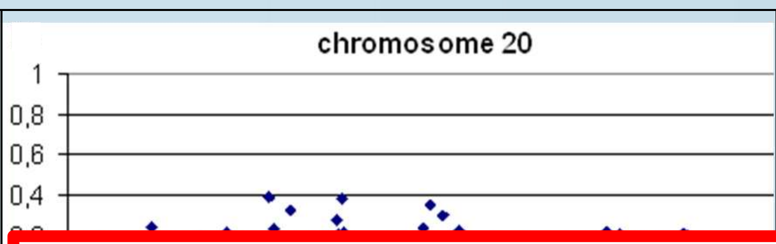
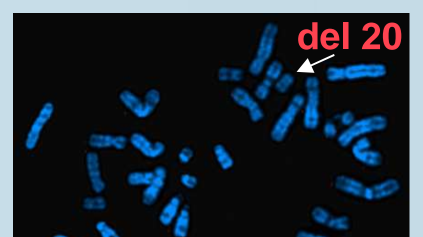
De novo is not always causal



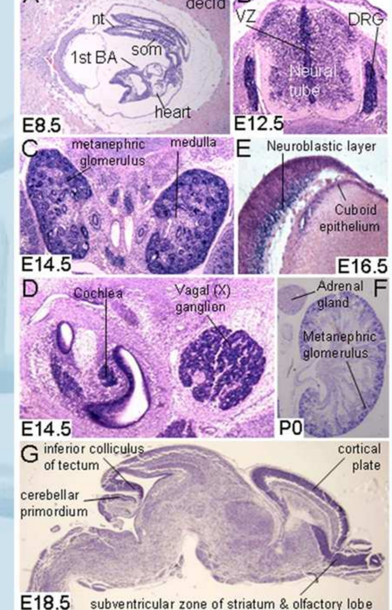




nature genetics

Exome sequencing identifies *MLL2* mutations as a cause of Kabuki syndrome

Sarah B Ng[1,7], Abigail W Bigham[2,7], Kati J Buckingham[2], Mark C Hannibal[2,3], Margaret J McMillin[2], Heidi I Gildersleeve[2], Anita E Beck[2,3], Holly K Tabor[2,3], Gregory M Cooper[1], Heather C Mefford[2], Choli Lee[1], Emily H Turner[1], Joshua D Smith[1], Mark J Rieder[1], Koh-ichiro Yoshiura[4], Naomichi Matsumoto[5], Tohru Ohta[6], Norio Niikawa[6], Deborah A Nickerson[1], Michael J Bamshad[1–3] & Jay Shendure[1]



Nicole M C Maas, Tom Van de Putte, Cindy Melotte, Annick Francis, Constance T R M Schrander-Stumpel, Damien Sanlaville, David Genevieve, Stanislas Lyonnet, Boyan Dimitrov, Koenraad Devriendt, Jean-Pierre Fryns, Joris R Vermeesch

This paper is freely available online under the BMJ Journals unlocked scheme, see http://jmg.bmj.com/info/unlocked.dtl

Vermeesch et al., EJHG, 2011

An estimated 1 out of 5 CNVs between 60 & 500 kb are benign!

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Itsara et al., Genome Research, 2010

- De novo CNV mutation rate: 2.5/100 live births
- A fourfold increase of de novo CNVs in autism spectrum patients

•=> 1/5 de novo CNVs is benign

For smaller CNVs this frequency is likely higher!

Van Ommen al. Nature Gen. 2005:

1 deletion every 8 generations and a duplication of 1/50 generations

Vermeesch et al., EJHG, 2011

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Databases of Genomic Variants : Catalogue of ‘Benign’ CNVs

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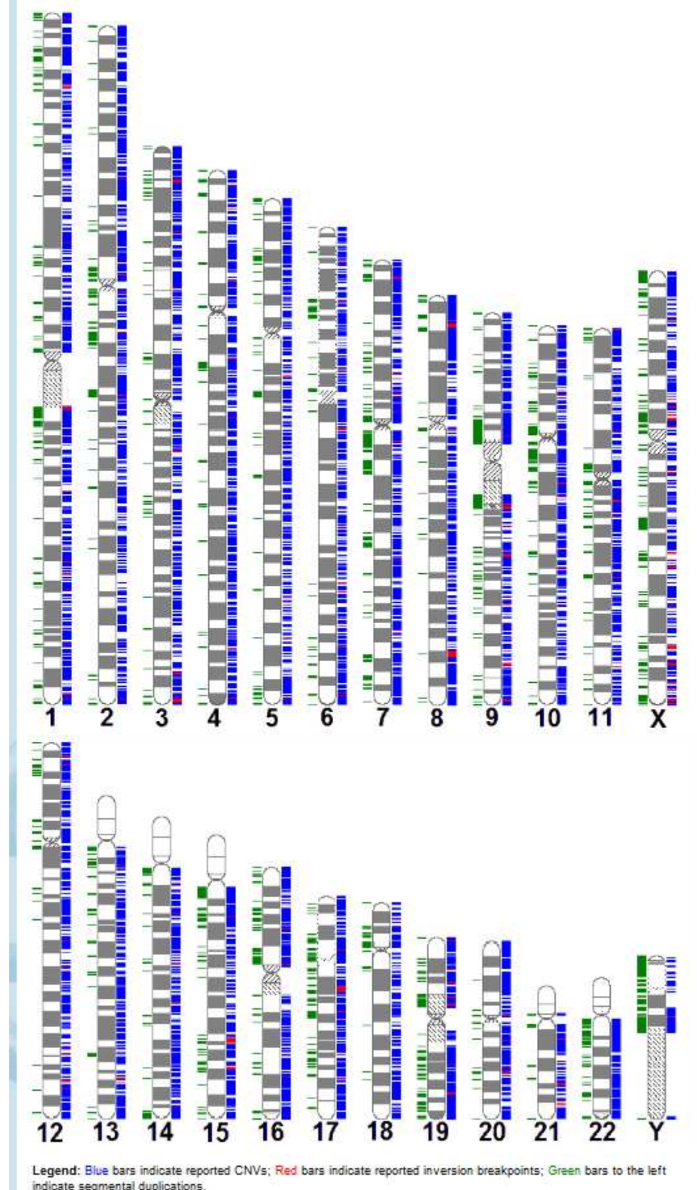

- Databases Of ‘Benign’ CNVs Have Limited Value For Clinical Assessment
- Beware of ‘HapMap bias’

Summary Statistics

Total entries: 101923 (hg18)
CNVs: 66741
Inversions: 953
InDels (100bp-1Kb): 34229
Total CNV loci: 15963
Articles cited: 42

Last updated: Nov 02, 2010
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Toronto Database of Genomic Variants

Mendelian CNVs: a paradigm shift in (cyto)genetics

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Inherited apparently benign CNVs *CAN* cause disease

"Mendelian CNVs" is the term coined here to indicate benign CNVs which can cause disease dependent on either copy number state, inheritance pattern or genetic and environmental background.

Mendelian CNVs: New wine in old bottles

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- Autosomal recessive
- Autosomal dominant
- X-linked
- Imprinted CNVs
- Variable expressivity and incomplete penetrance

Autosomal recessive CNVs

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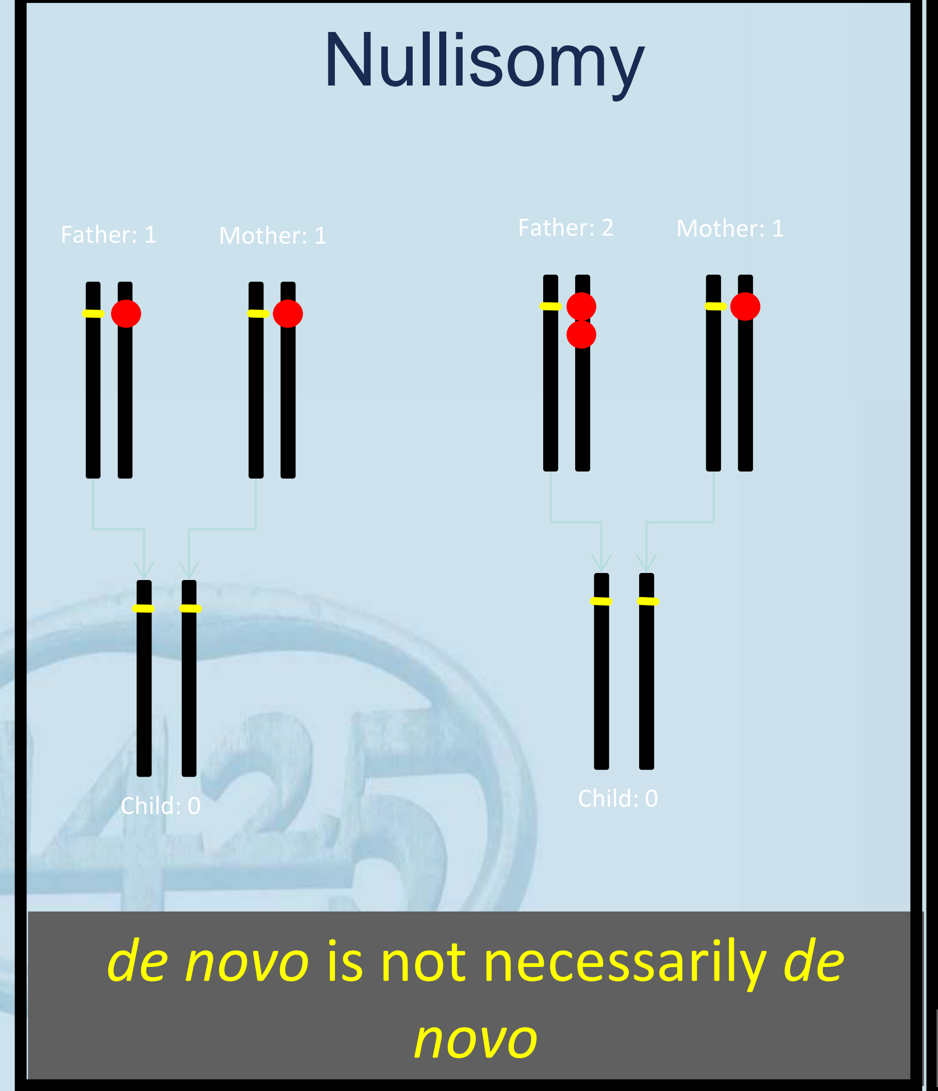


Hemizygous and mutation in second allele

Father: 2

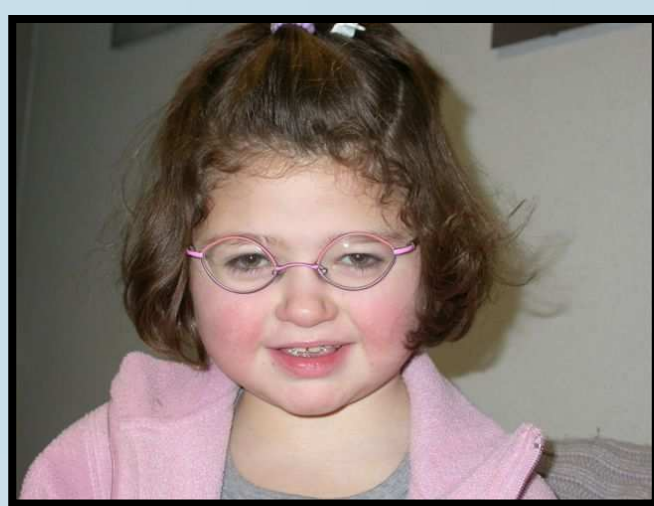
Mother: 1

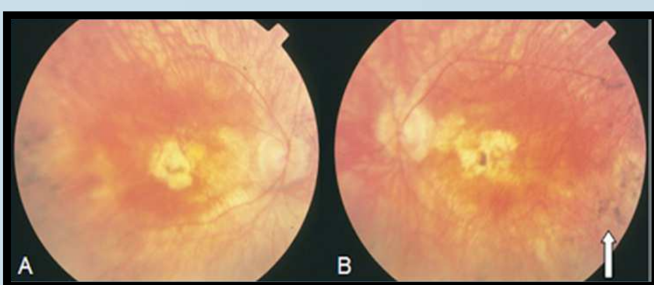
Child: 1

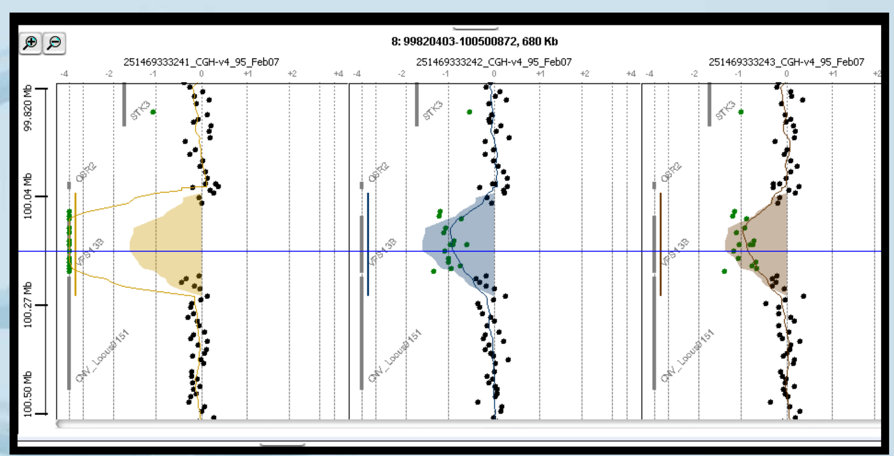
Inherited deletion IS causal

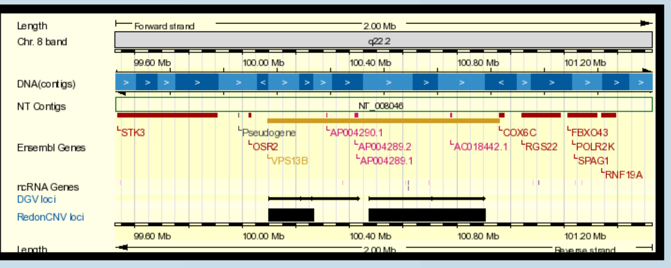
An example: Cohen syndrome

- Autosomal recessive inheritance: mutations in VPS13B (*COH1*)
- Phenotype
  - mild to severe MR
  - microcephaly
  - Truncal obesity
  - Characteristic face
  - Specific behavior
  - Retinal dystrophy , high myopia (retinal detachment, cataract)









Autosomal recessive spastic ataxia of Charlevoix-Saguenay (MIM: 270550)

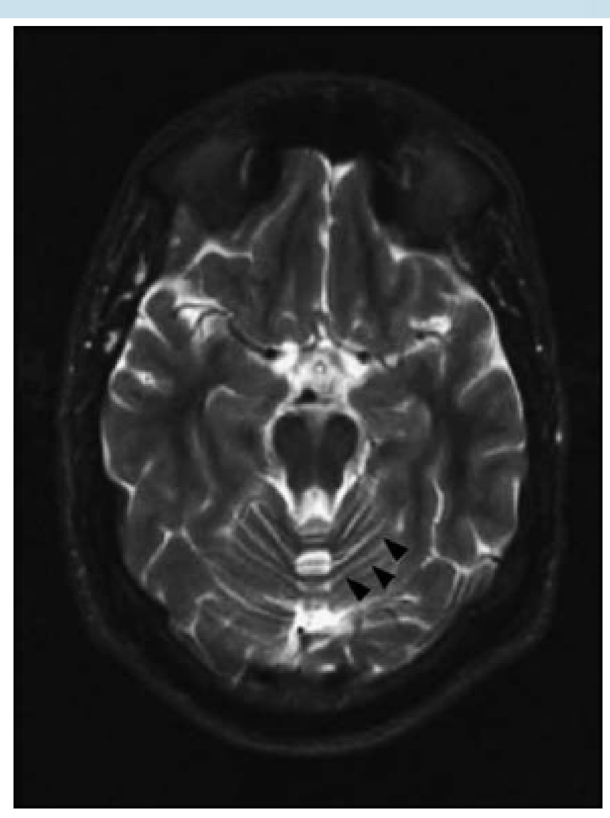


Figure 1 Brain MRI at the age of 26 years showing atrophy of the vermis superior (black arrows) and the superior cerebellar peduncles. No anomalies of the cerebral hemispheres were detected.

- Features:
  - Ataxia
  - Dysarythria
  - Spasticity
  - Distal muscle wasting
  - Nystagmus
  - Mitral valve prolapse (57%)
  - Prominent myelinated retinal nerve fibers
- Brain MRI: cerebellar atrophy of the upper part of the vermis and the superior cerebellar peduncles

Breckpot et al., EJHG 2007

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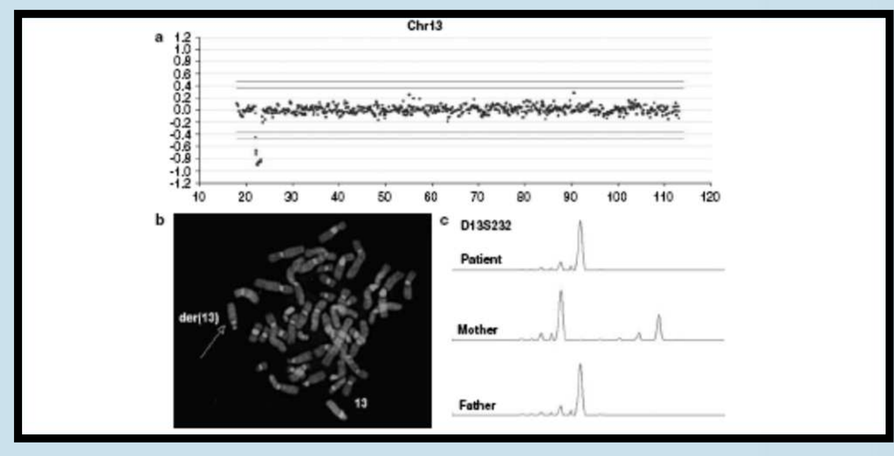
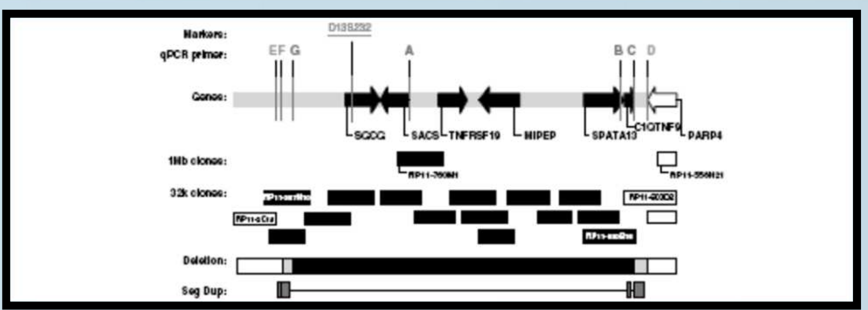
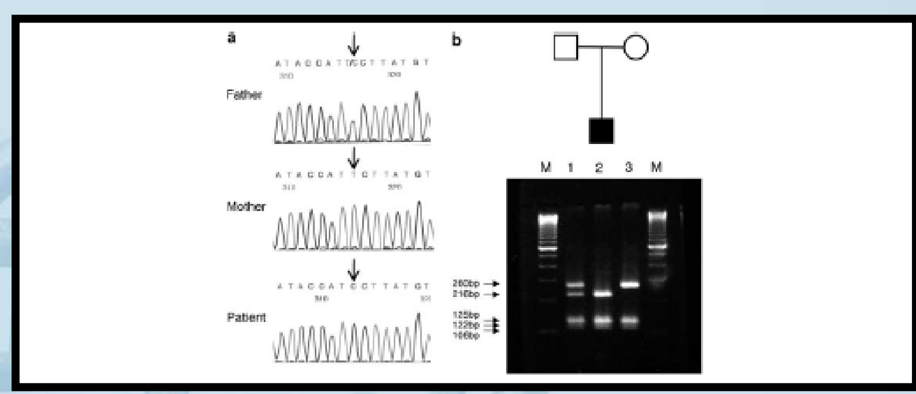
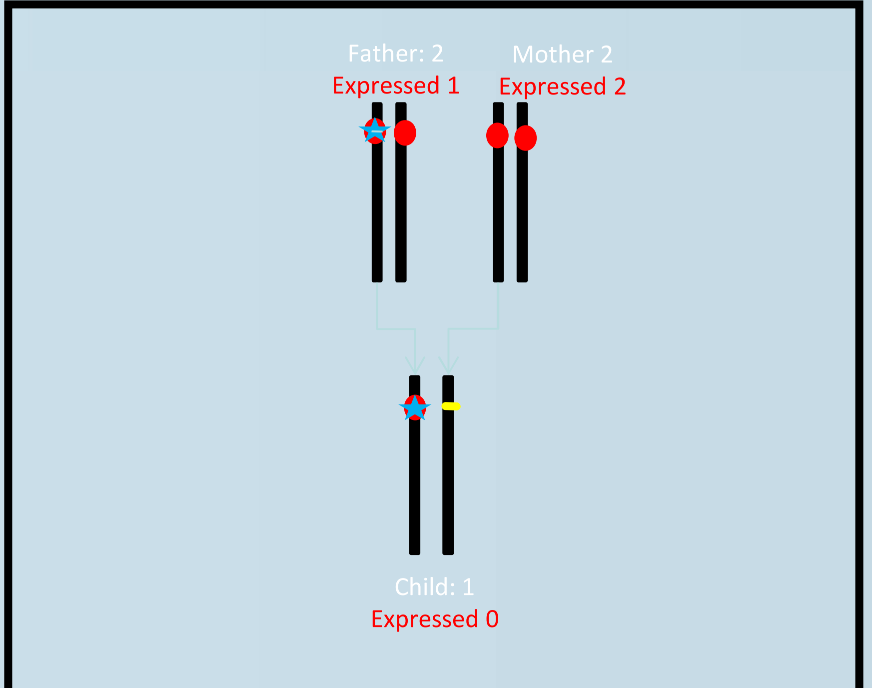
Inherited mutation, de novo deletion









Breckpot et al., EJHG 2007

Autosomal recessive CNVs

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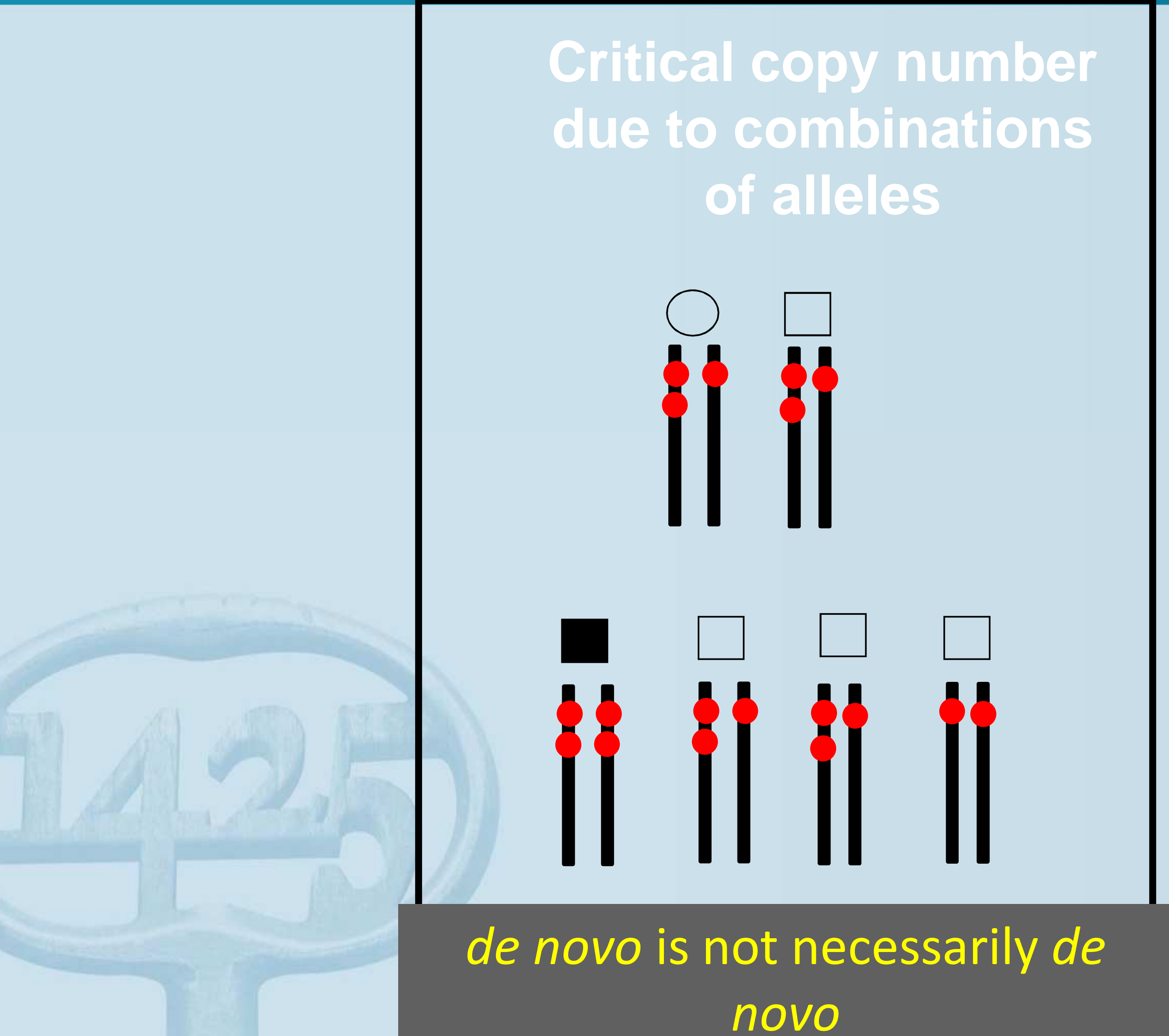

Critical copy number due to combinations of alleles

de novo is not necessarily *de novo*

Autosomal recessive CNVs: The first example?

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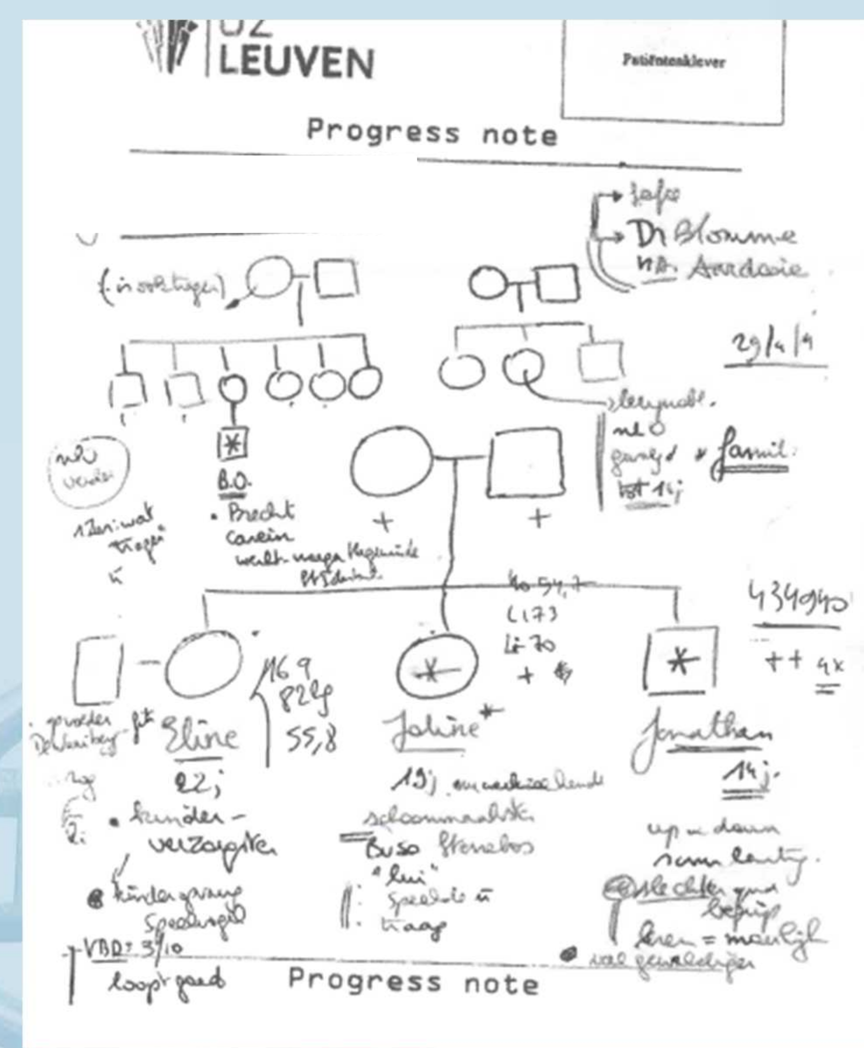

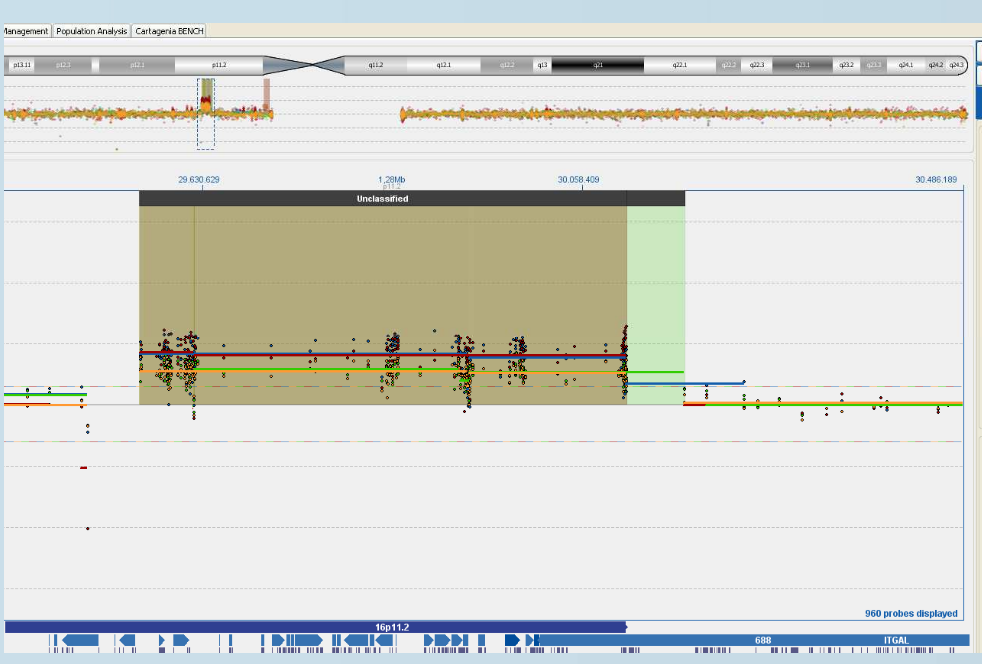

Autosomal dominant CNVs

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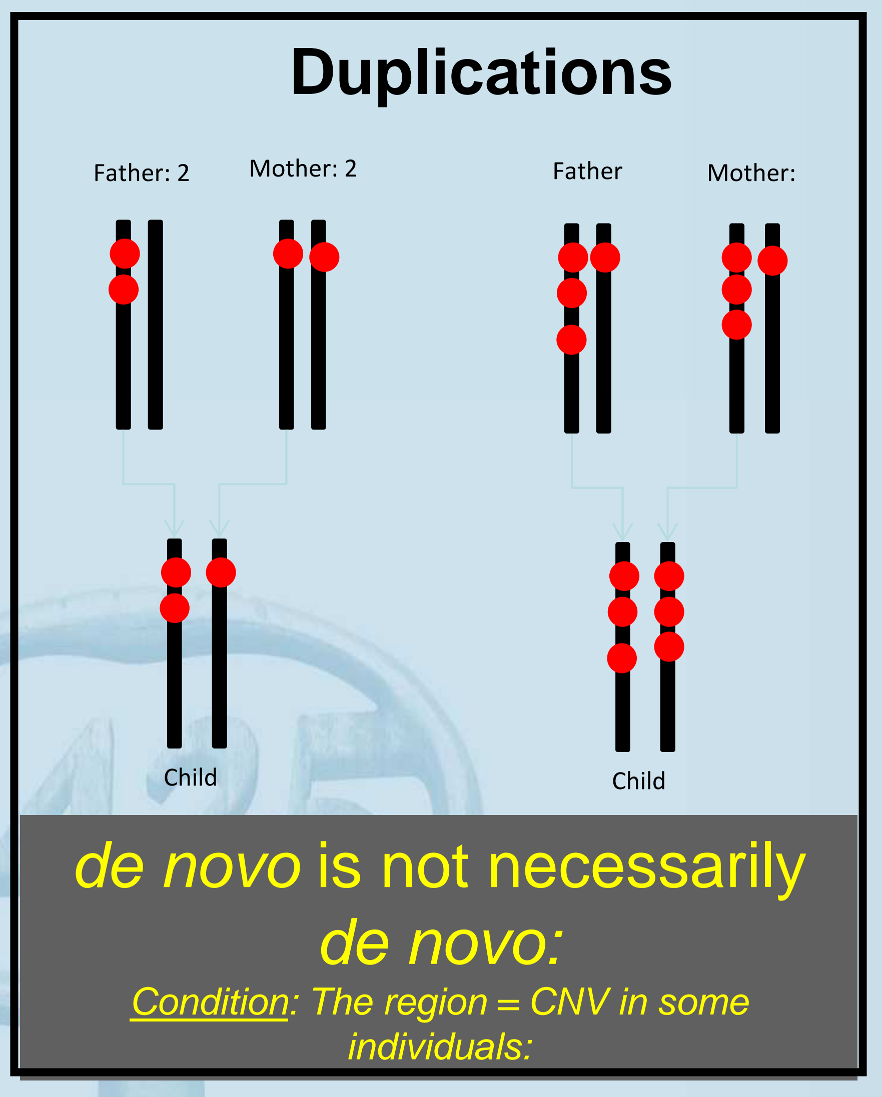

de novo is not necessarily *de novo:*

Condition: The region = CNV in some individuals:

Amplifications

"de novo = de novo"

i.e. there is no inheritance mechanism to explain a new amplification (intensity ratio difference with parents> 1.5)

Autosomal dominant CNVs

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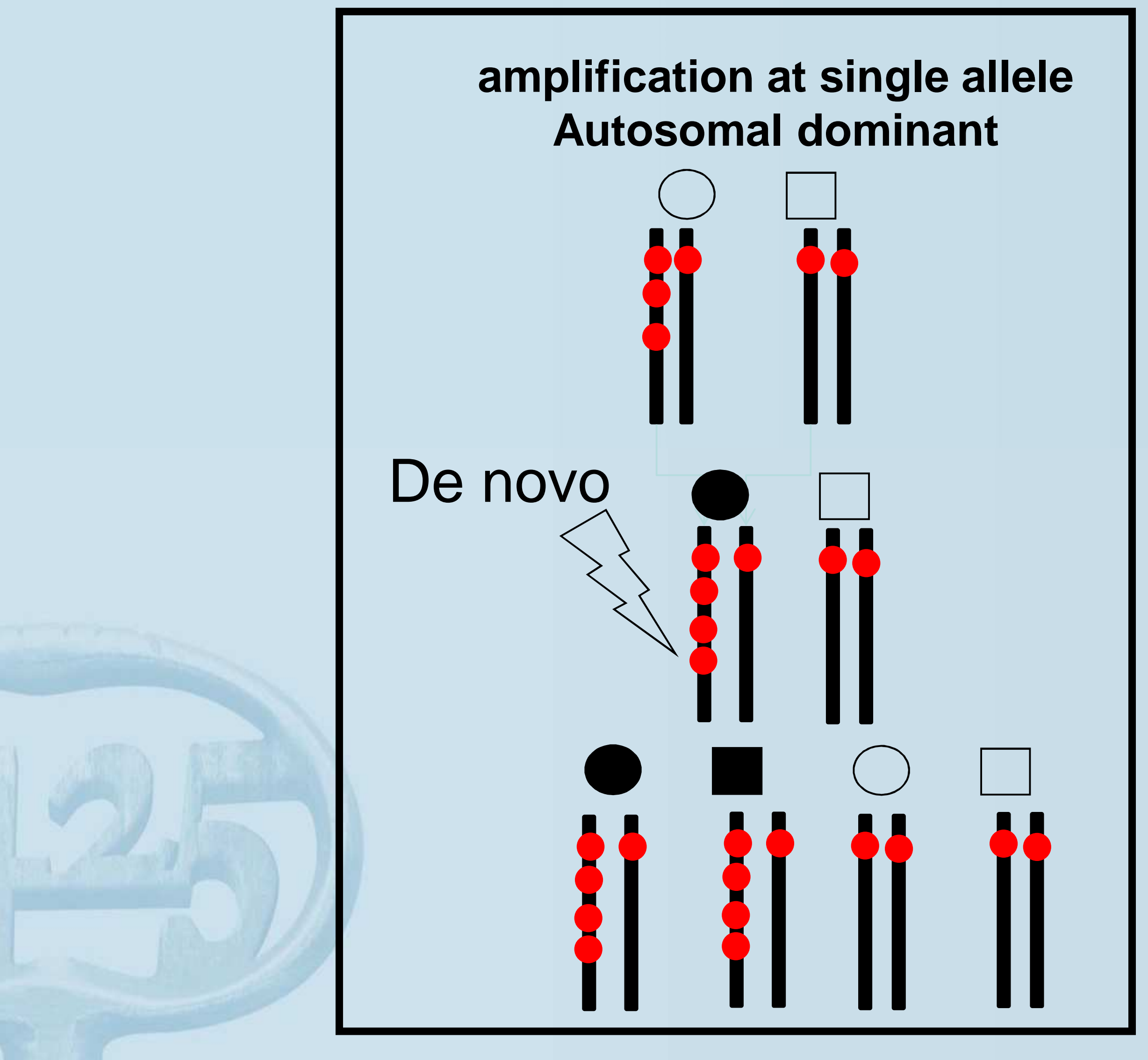
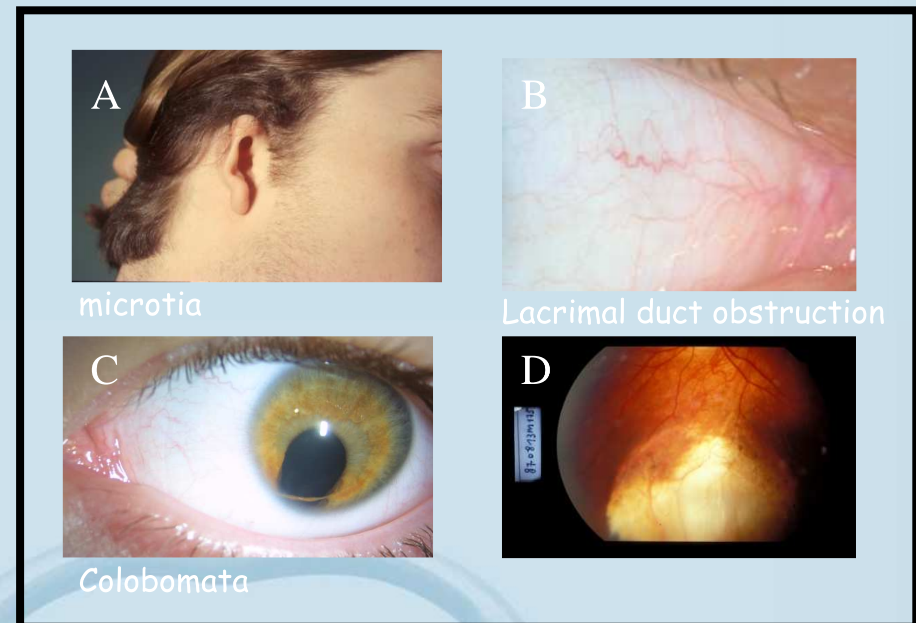
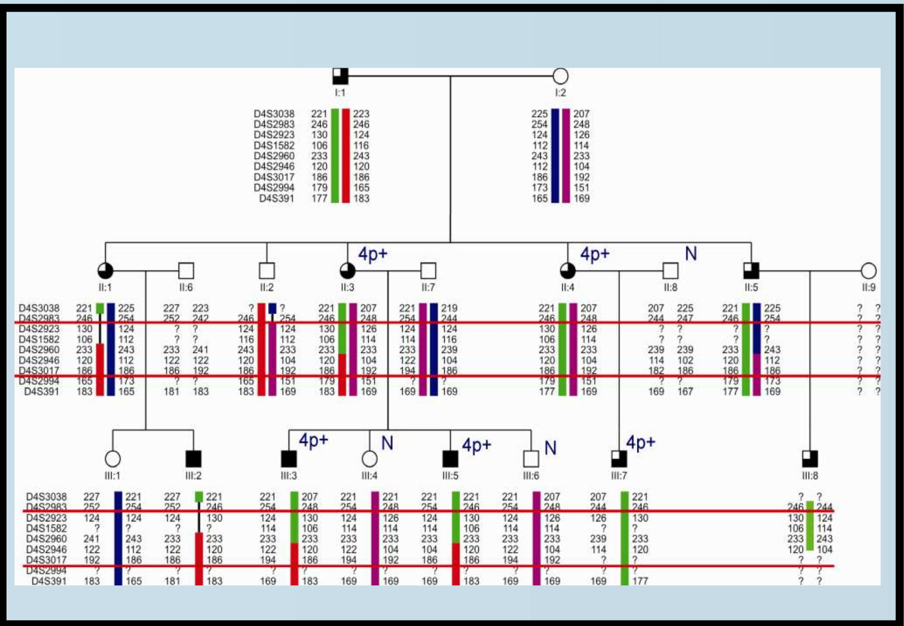


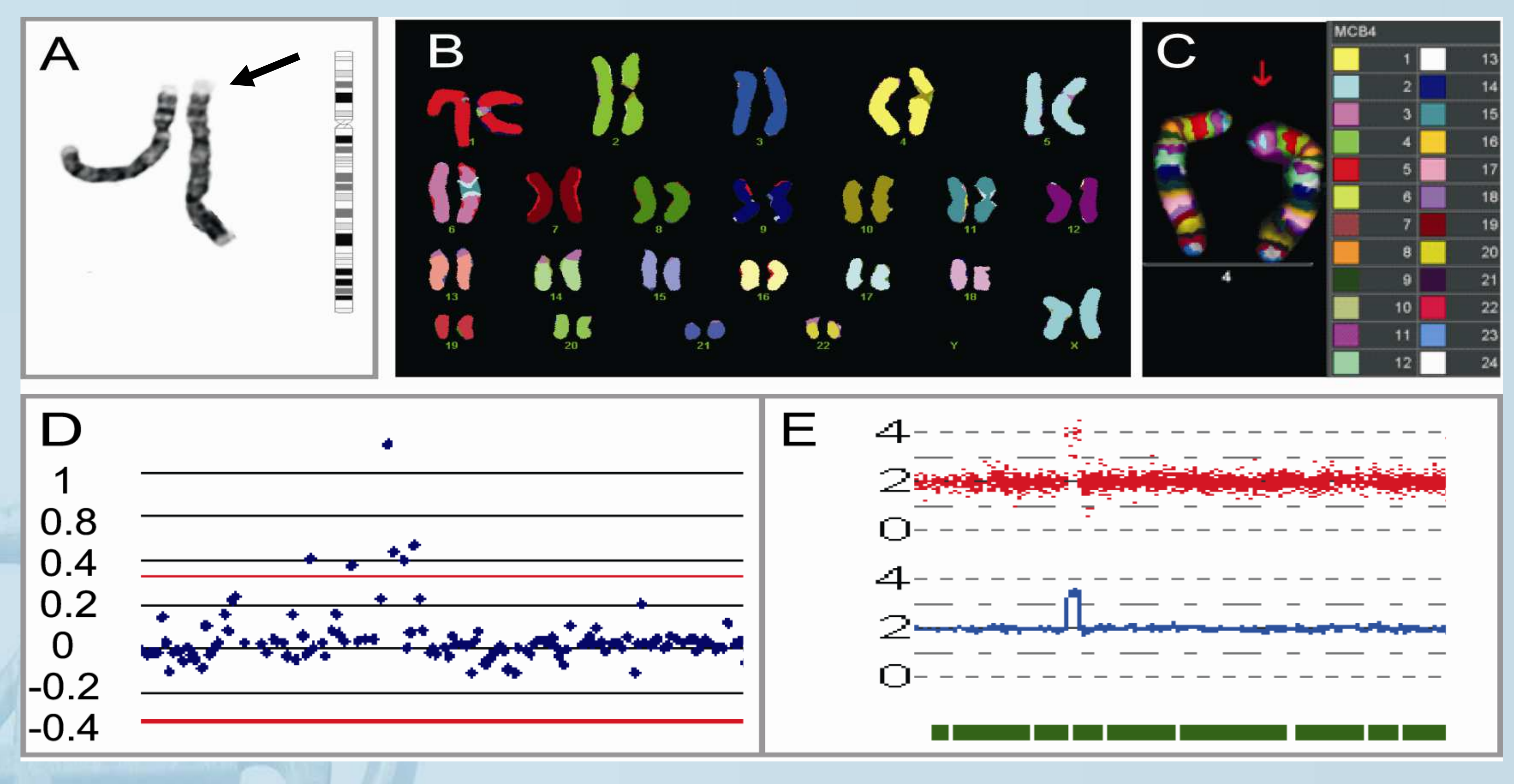
An amplification linked to autosomal dominant inherited microtia





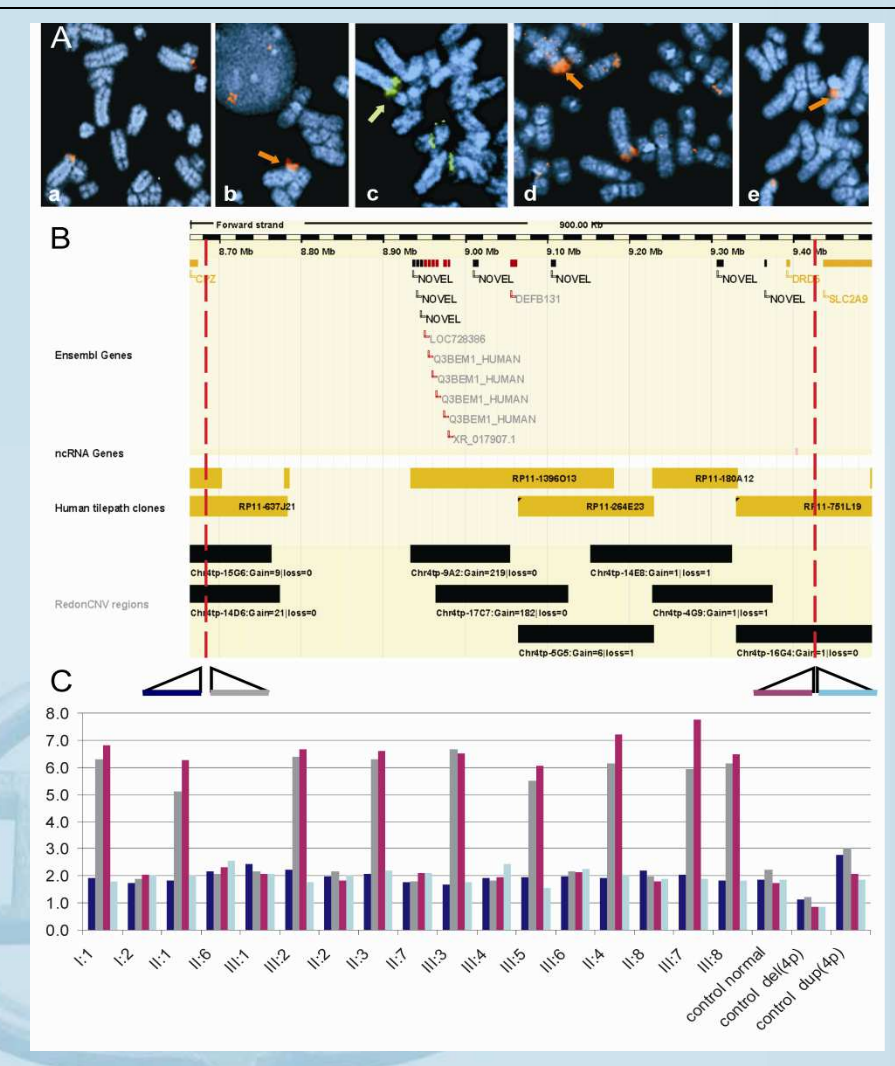
Balikova et al., AJHG, 2008

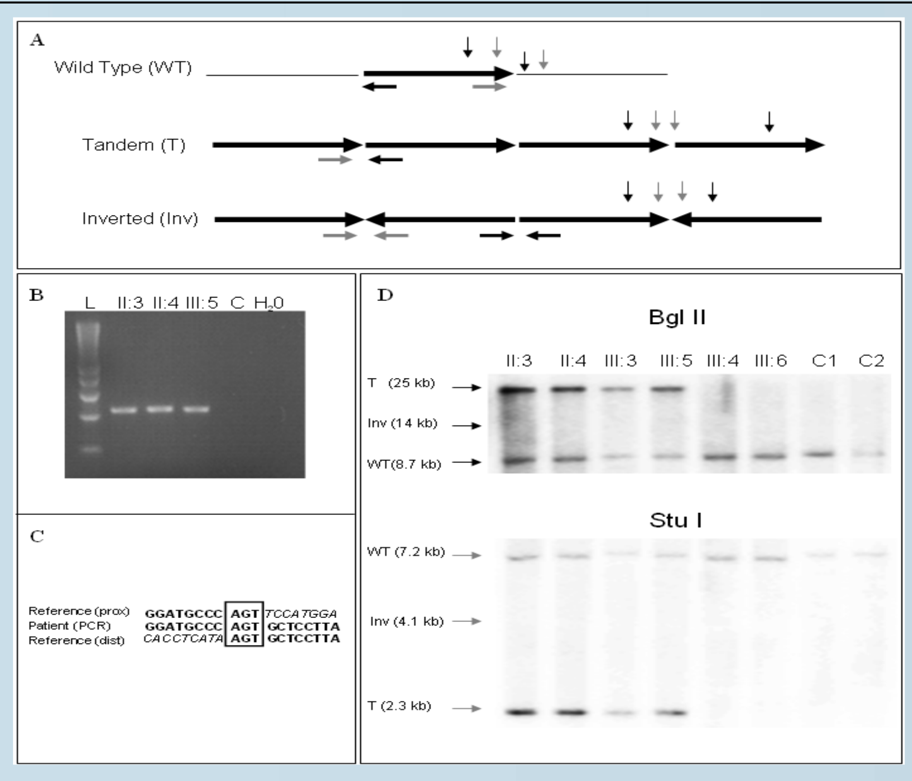
An amplification linked to autosomal dominant inherited microtia



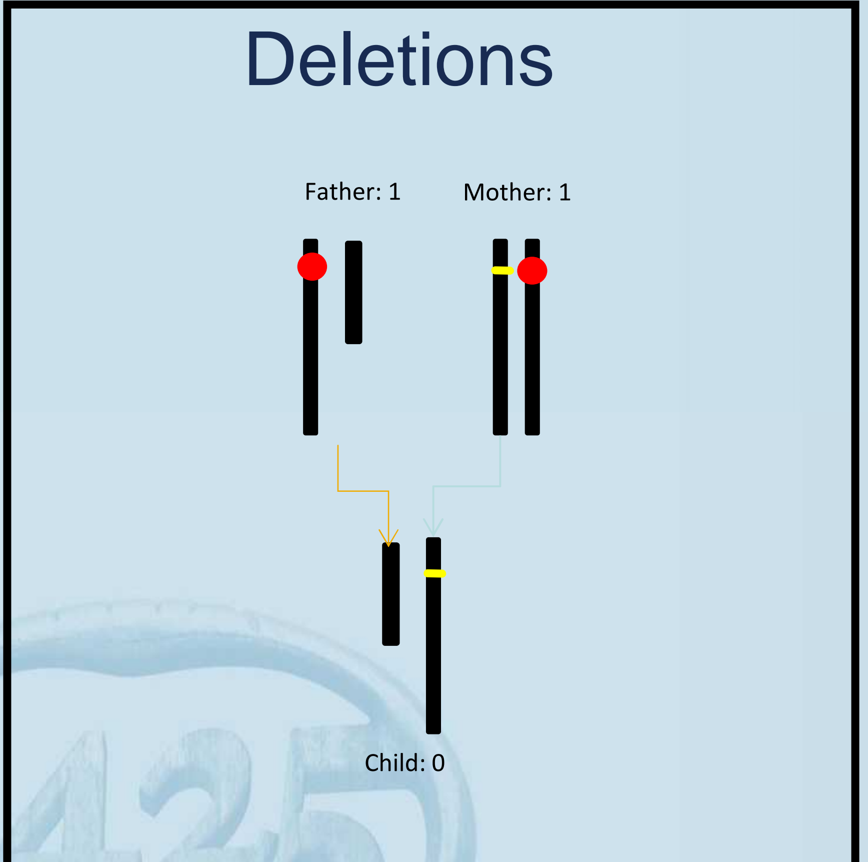
The alteration is located within the 4p olfactory receptor gene cluster

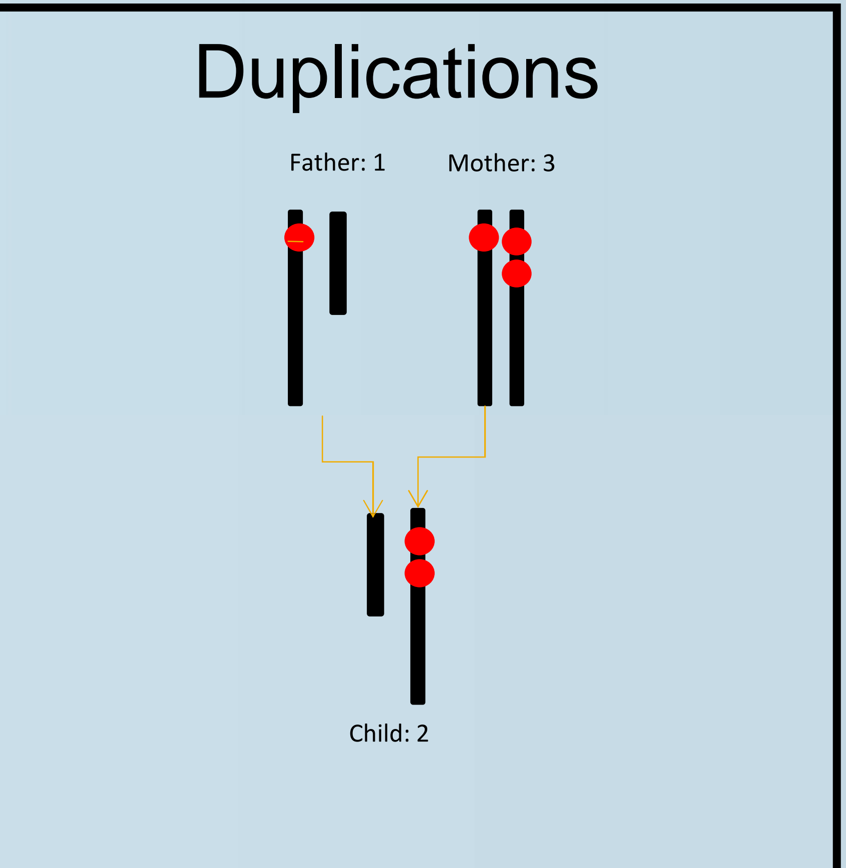
Five exact tandem copies of ~750 kb segment





X-linked CNVs





Apparently *de novo* in child is not necessarily *de novo* but inherited from mother

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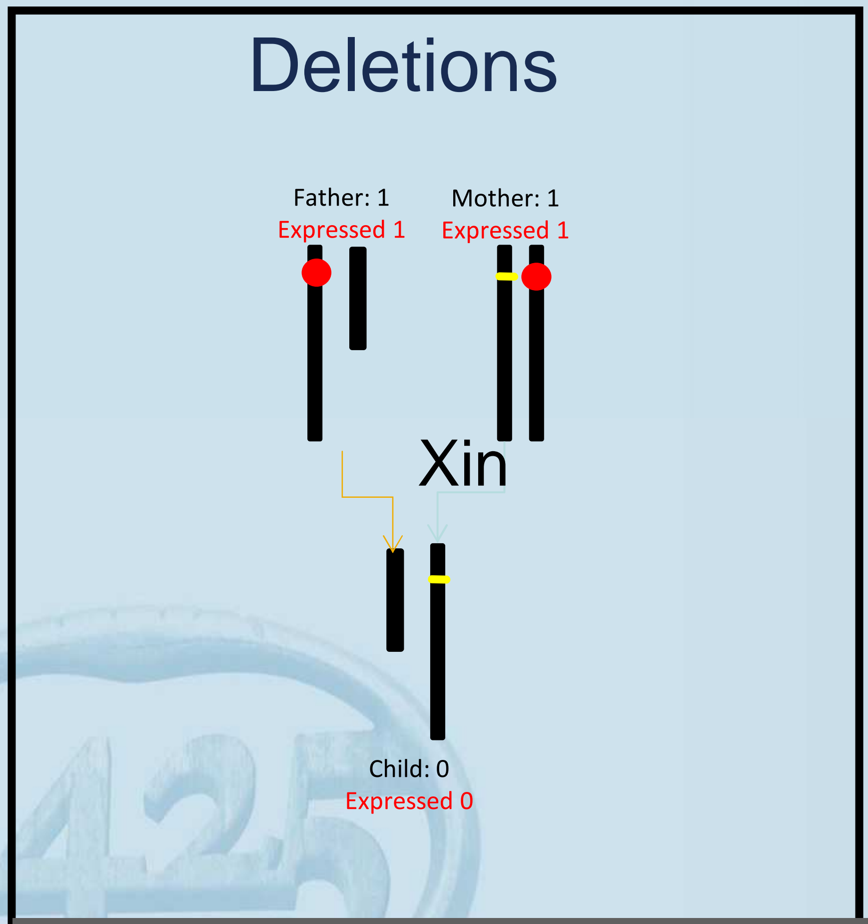
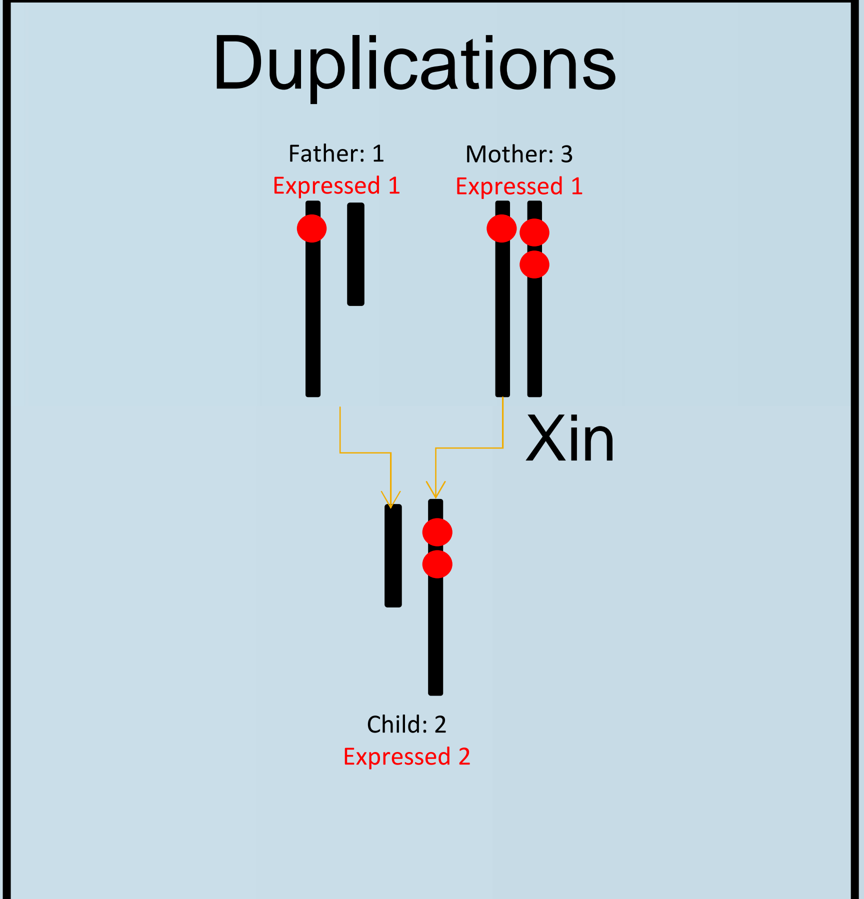
X-linked CNVs





Apparently *de novo* in child is not necessarily *de novo* but inherited from mother

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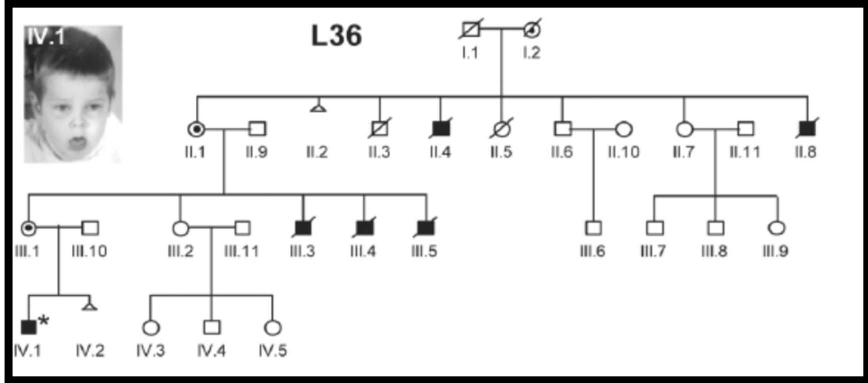
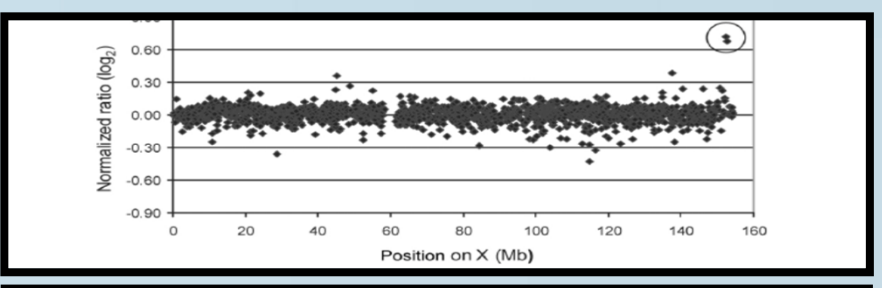
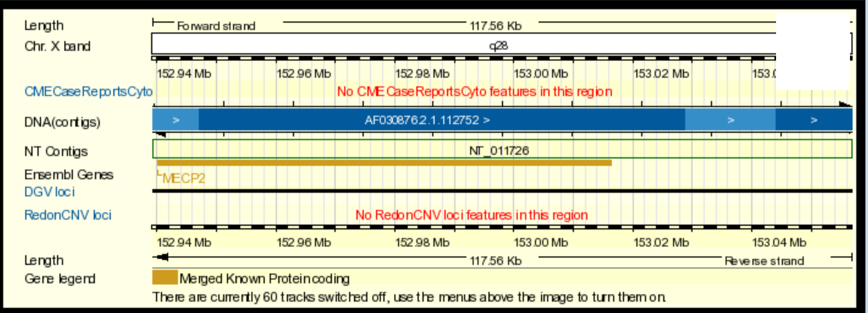
MECP2 duplication

- Deletions cause Rett syndrome
  - Progressive neurodegenerative disorder
  - Affecting mainly females
- Duplications
  - Severe-to-profound MR
  - Axial and facial hypotonia
  - Progressive spasticity
  - Seizures
  - Recurrent infections leading to early death.
  - Mild dysmorphic features
- Affect only males







Van Esch et al. Am.J.Hum.Genet. 77:442-453, 2005

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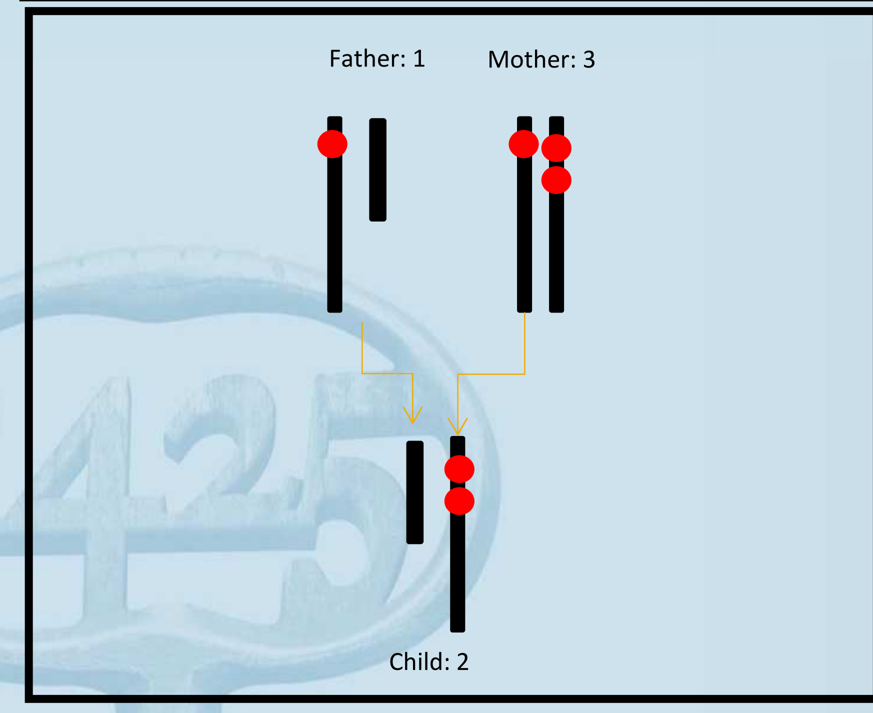
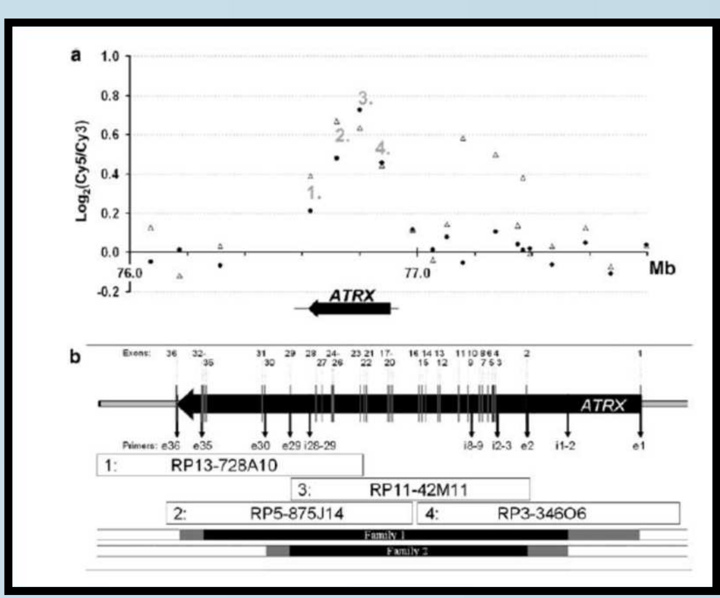
ATR-X syndrome

- Severe-to-profound MR
  - Characteristic facial appearance
  - Genital anomalies
  - Alpha thalassaemia





Thienpont et al. Eur.J. Hum. Gen. (2007) 15, 1094-1097

ATR-X syndrome

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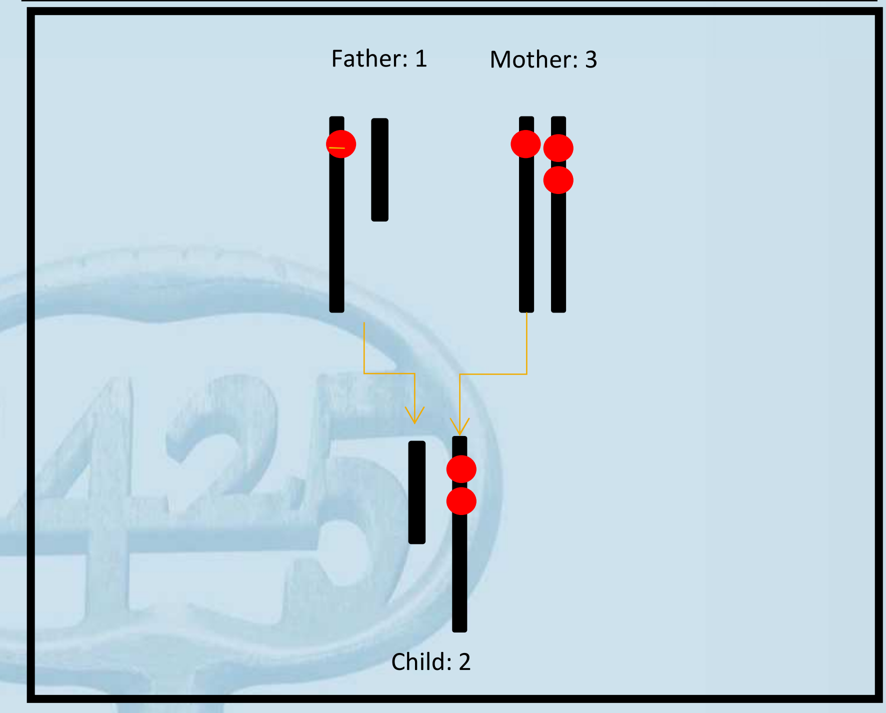

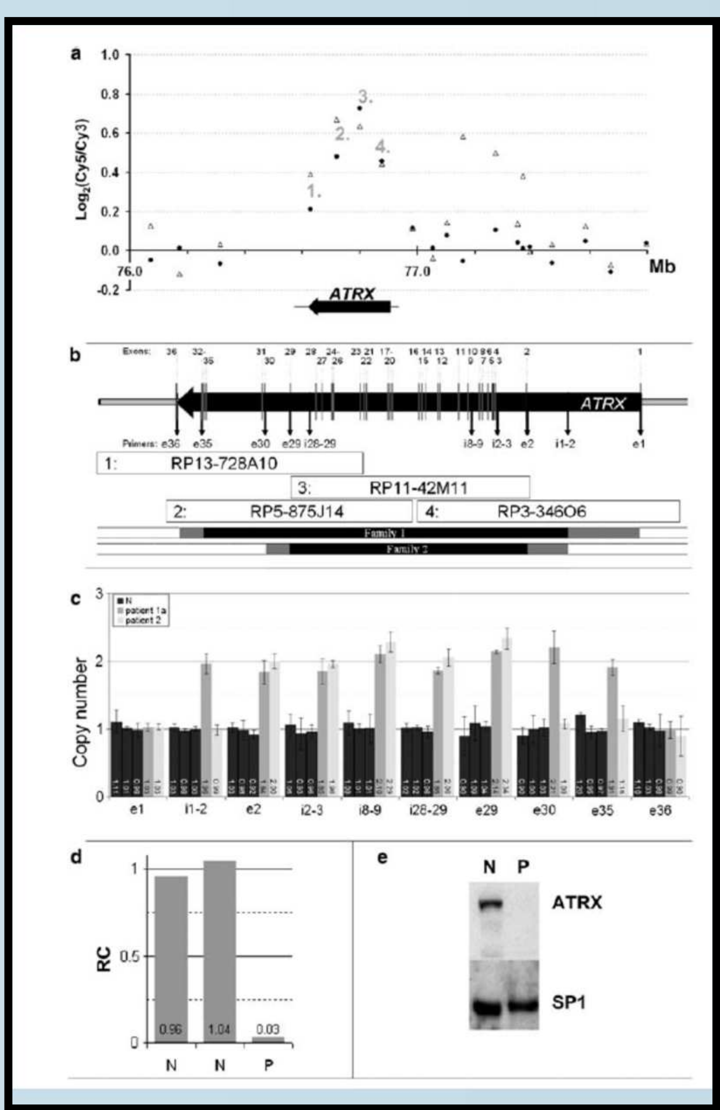

Thienpont et al. Eur.J. Hum. Gen. (2007) 15, 1094-1097

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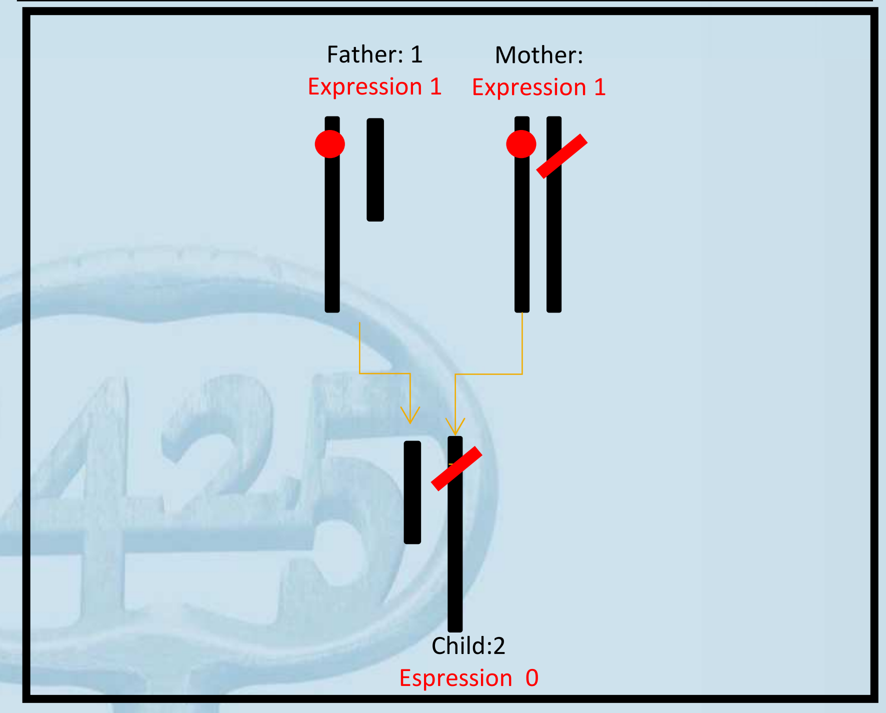

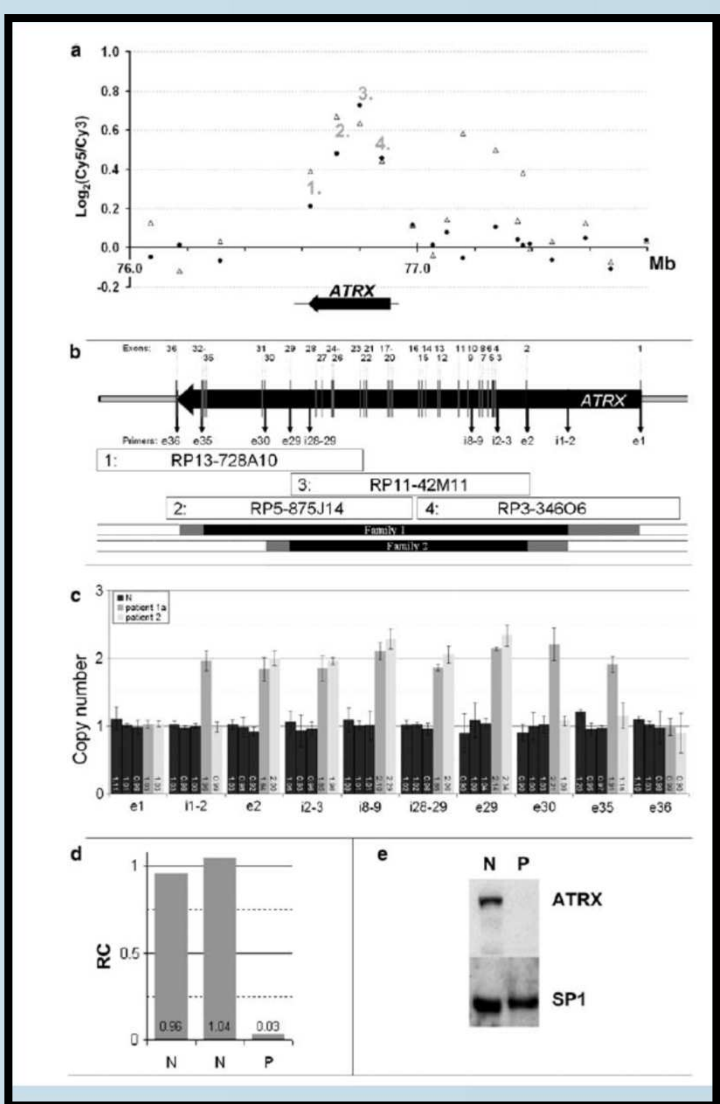

Thienpont et al. Eur.J. Hum. Gen. (2007) 15, 1094-1097

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Imprinted CNVs

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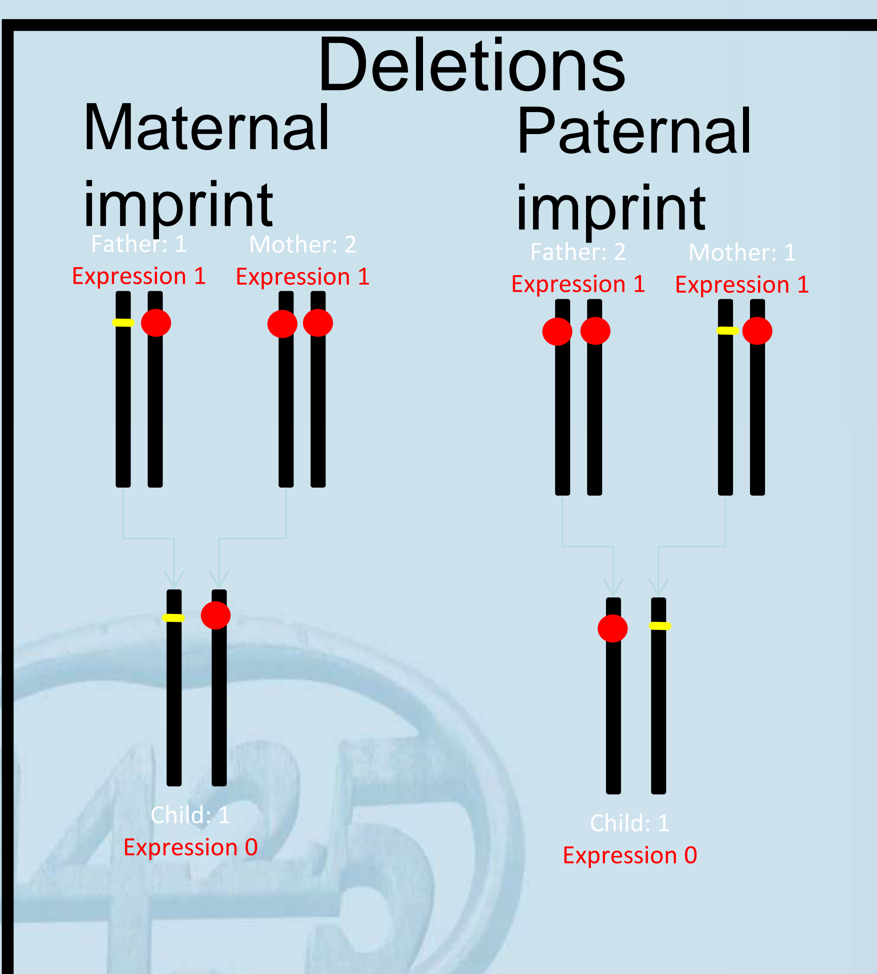
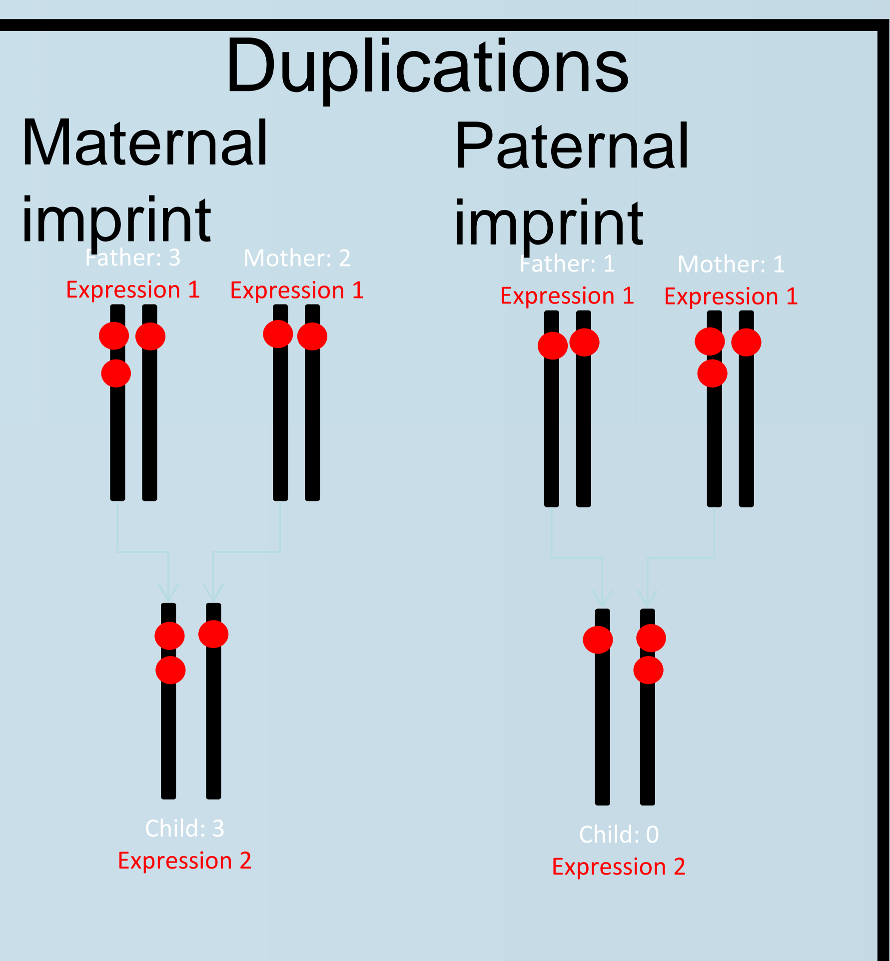
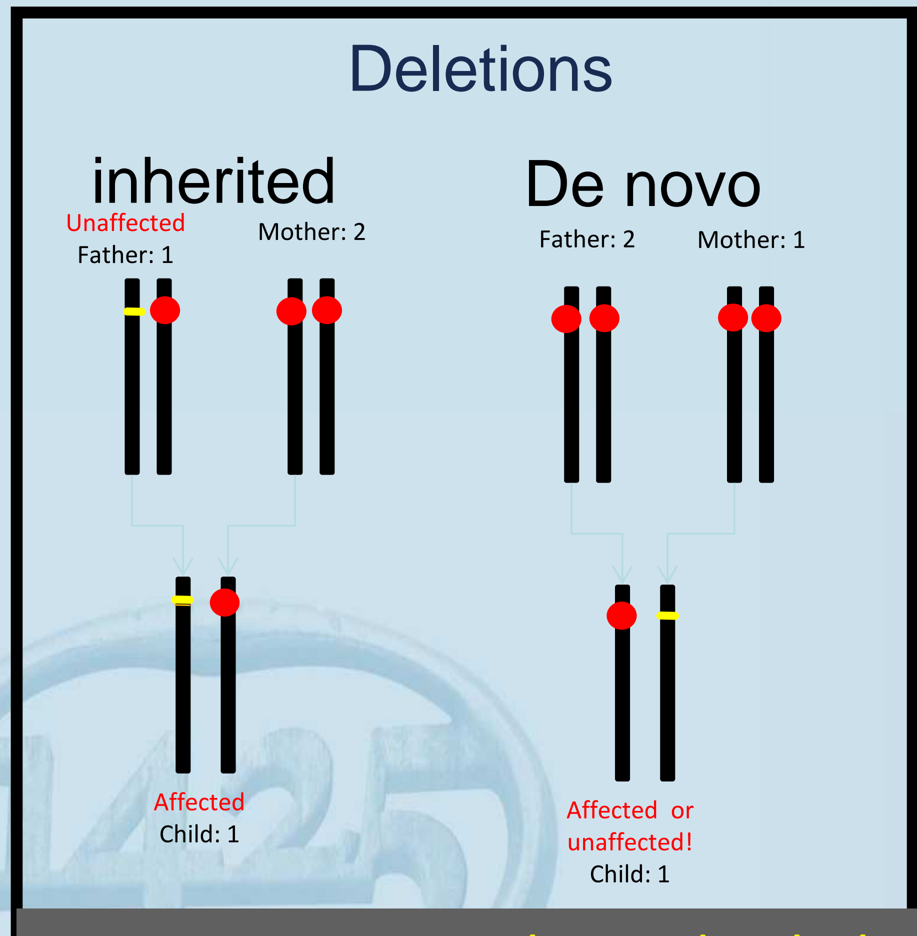
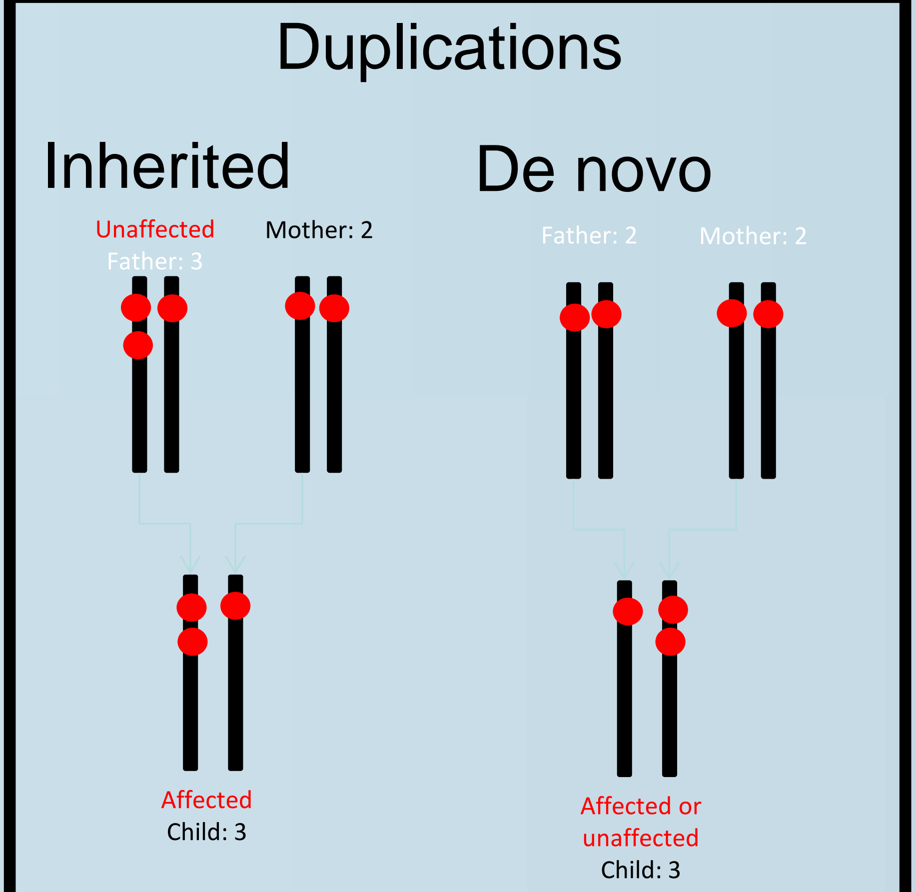




No copy number change is not necessarily *not causal*

Variable expressivity and incomplete penetrance





Inherited imbalances can be causal

A copy number change does not *necessarly* causes a phenotype

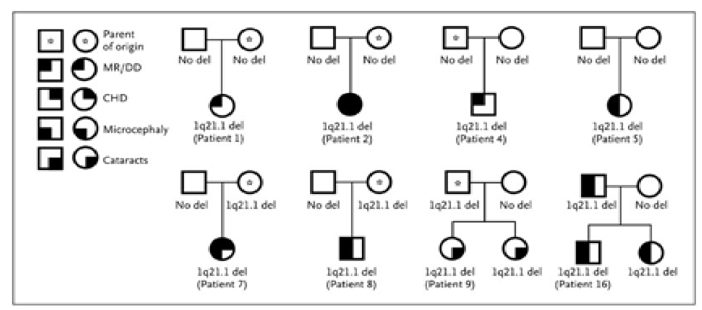
CNVs as risk factor for MR/CA (variable penetrance and expressivity)

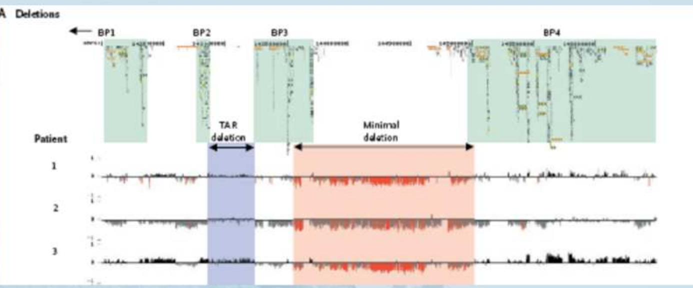
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Recurrent Rearrangements of Chromosome 1q21.1 and Variable Pediatric Phenotypes

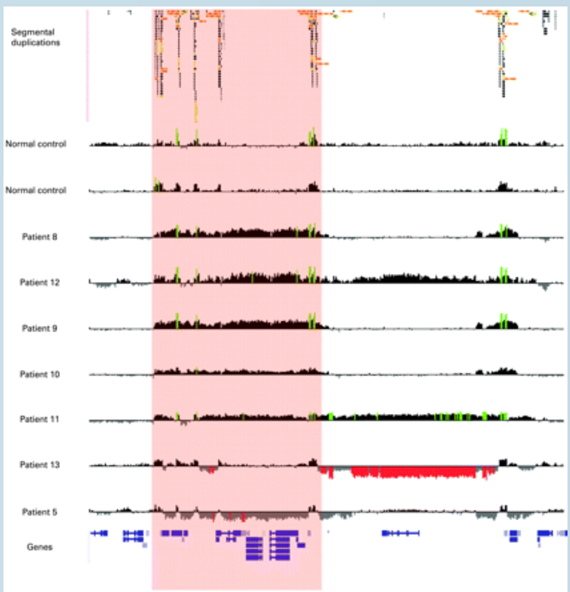
H. Mefford, A. Sharp, C. Baker, A. Itsara, Z. Jiang, K. Buysse, S. Huang,





Recurrent reciprocal deletions and duplications of 16p13.11: The deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant

Femke D Hannes, Andrew J Sharp, Heather C Mefford, Thomy de Ravel, Claudia A Ruivenkamp, Martijn H Breuning, Jean-Pierre Fryns, Koen Devriendt, Griet Van Buggenhout, Annick Vogels, Helen H Stewart, Raoul C Hennekam, Gregory M Cooper, Regina Regan, Samantha JL Knight, Evan E Eichler and Joris R Vermeesch



Deletion	Duplication
25/5218 patients	9/5218 patients
0/4737 controls	1/4737 controls
P = 1.1×10^{-7}	P = 0.02

Deletion	Duplication
5/1026 patients	5/1026 patients
0/2014 controls	5/1682 controls
P =0.0048	No Difference

CONCLUSION:

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The boundary between benign and pathogenic variation becomes blurred.

Even known disease causing imbalances can be tolerated and appears to be part of the normal phenotypic human spectrum!!!

De Ravel et al., Cyt. Genome research, 2007

Overview

- Introduction
- Technologies for CNV detection
- Clinical consequences
- Mechanisms of origin

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Mechanisms causing intrachromosomal CNVs

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Recurrent CNVs (genomic disorders)

- Non-allelic Homologous recombination (NAHR)
  - Unequal crossing over
  - Break-induced replication
  - Single-strand annealing

Non-recurrent CNVs

- Non Homologous End Joining (NHEJ)
- Microhomology mediated break induced replication (MMBIR)
- Fork stalling and template switching (FoSTeS)
- Replication slippage

Genomic disorders (recurrent CNVs)

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- Conditions that result from rearrangements of the genome rather than base pair changes of DNA
- Due to inherent genomic instability that results in susceptibility to structural variation mutagenesis.
- Structural variants:
  - Include copy number variants (CNVs), copy number neutral, inversions, insertions and translocations
  - Are not resolved by chromosome karyotype studies (< 5Mb) but at least 50bp in size, discriminating them from smaller variants, such as singe-nucleotide variants (SNVs) and short insertions and deletions (indels)
  - are an underlying factor in human evolution and in many diseases (ID/DD/cancer).

Recurrent versus non-recurrent CNVs

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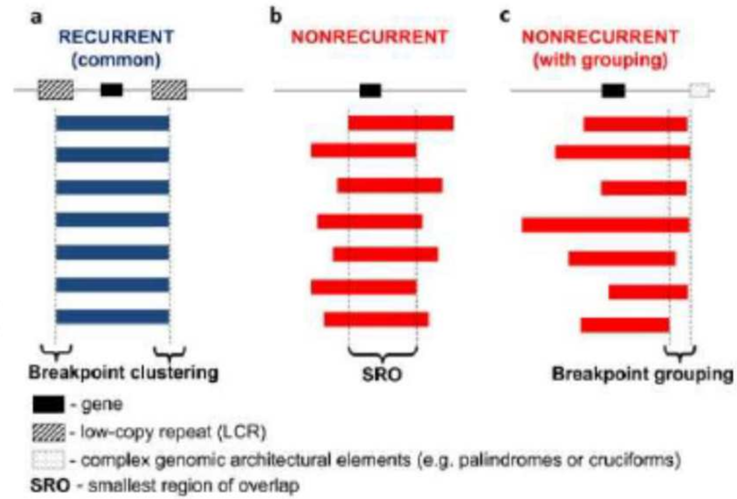
- A change in copy number requires a change in chromosome structure, joining two formerly separated DNA sequences.
- These breakpoint junctions yield insights into the mechanisms that cause the chromosomal structural change.
- Recurrent rearrangements:
  - Same size
  - Same genomic content
- Nonrecurrent rearrangements:
  - unique size
  - unique genomic content



Genomic disorders (recurrent CNVs)

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Key mechanism:

Change in copy number by homologous recombination

I) non-allelic/ectopic homologous recombination (NAHR) between low copy repeats (LCRs)/segmental duplications

II) Single-strand annealing

- Meiotic (most often)

Low copy repeats (LCRs or segmental duplicons)

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- Definition: segments of >1000 bp that are present in multiple copies in the genome
- Intrachomosomal and interchromosomal

Genome wide LCRs

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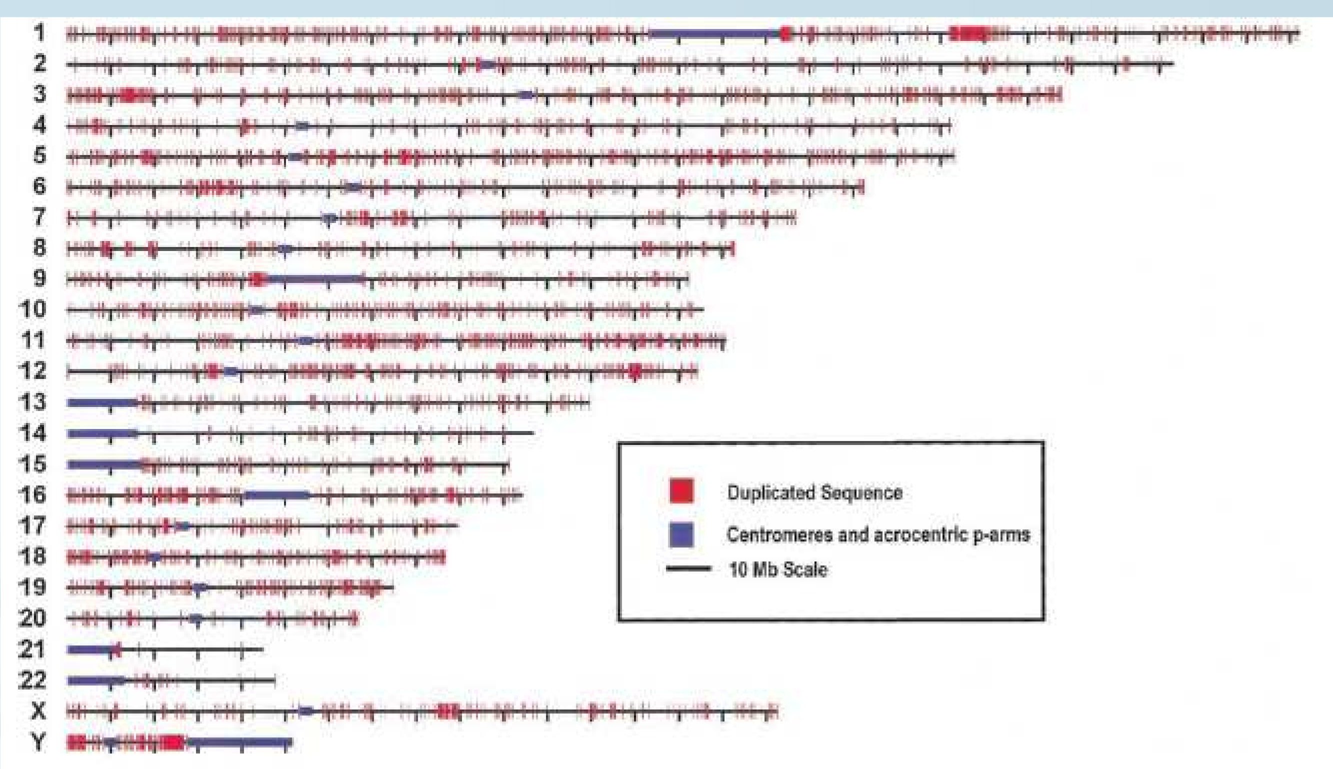

Figure 3 Genome-wide view of segmental duplications. The positions of alignments are depicted in red for each of the 24 chromosomes. Panels separate alignments on the basis of similarity: (*A*) 90%–98% identity and (*B*) 98%–100% identity. Purple bars depict centromeric gaps as well as the p-arms of acrocentric chromosomes (13, 14, 15, 21, and 22).Because of scale constraints, only alignments >5 kb are visible. Views were generated with the program PARASIGHT (J.A. Bailey, unpubl.), a graphical pairwise alignment viewer.

Baily et al. , Genome research, 2001

Segmental duplications in the human genome

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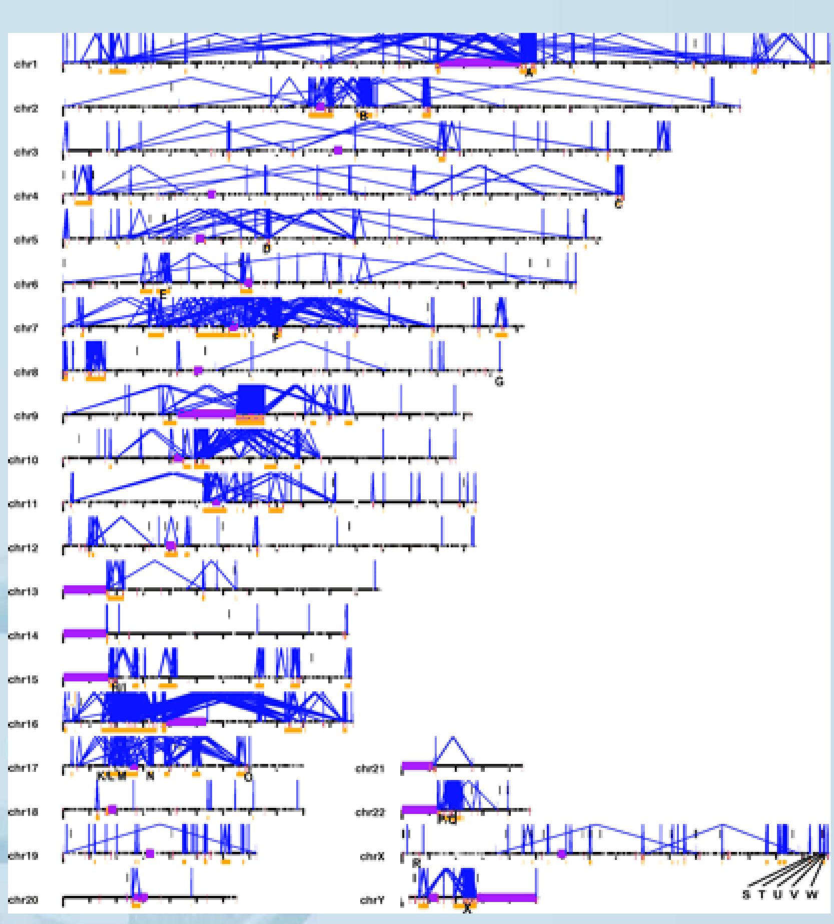

Recent Segmental Duplications in the Human Genome

Jeffrey A. Bailey,[1] Zhiping Gu,[2] Royden A. Clark,[1] Knut Reinert,[2] Rhea V. Samonte,[1] Stuart Schwartz,[1] Mark D. Adams,[2] Eugene W. Myers,[2] Peter W. Li,[2] Evan E. Eichler[1*]

Chromosome 16 segmental duplications

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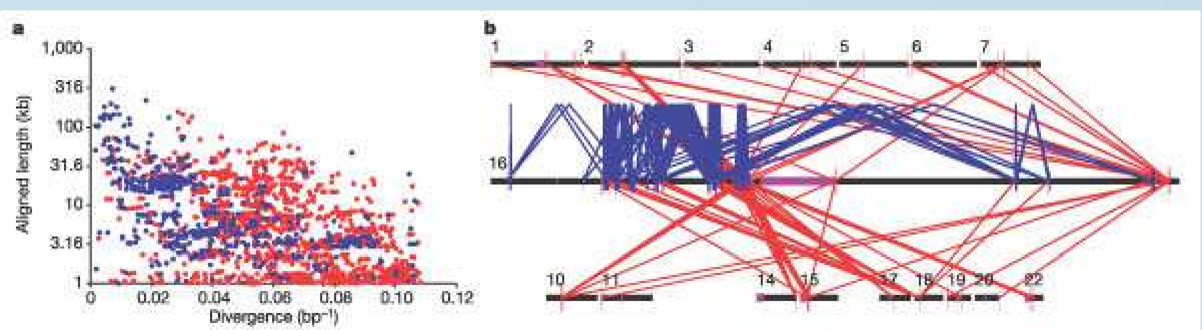

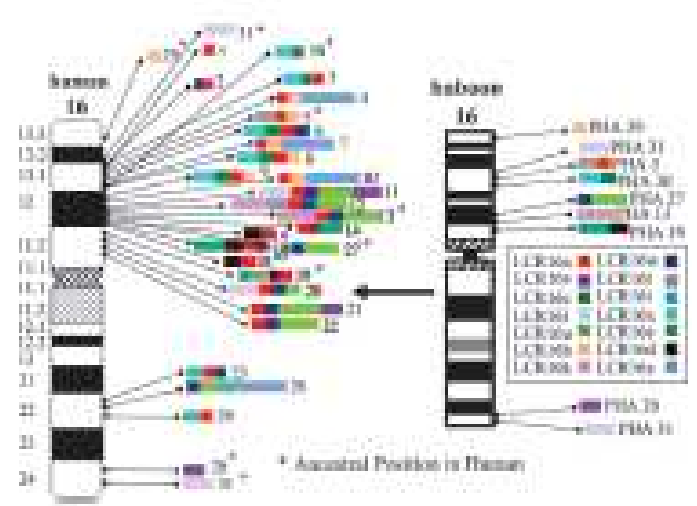

Fig. 1. LCR16 organization in human and baboon. The location, copy number, and structure of LCR16 duplications are depicted within the context of an ideogram for human (Left) and Papio hamadryas (PHA) (Right) based on the human genome reference sequence (hg16), BAC-end sequencing, and complete clone insert sequence of baboon clones. With the exception of the ancestral loci, duplication blocks are enumerated based on their position (p–q) on human chromosome 16 (Table 5).

Recurrent duplication-driven transposition of DNA during hominoid evolution

Matthew E. Johnson*†, NISC Comparative Sequencing Program‡§, Ze Cheng*, V. Anne Morrison¶, Steven Scherer‖, Mario Ventura**, Richard A. Gibbs‖, Eric D. Green‡††, and Evan E. Eichler*‡‡

Recurrent rearrangements

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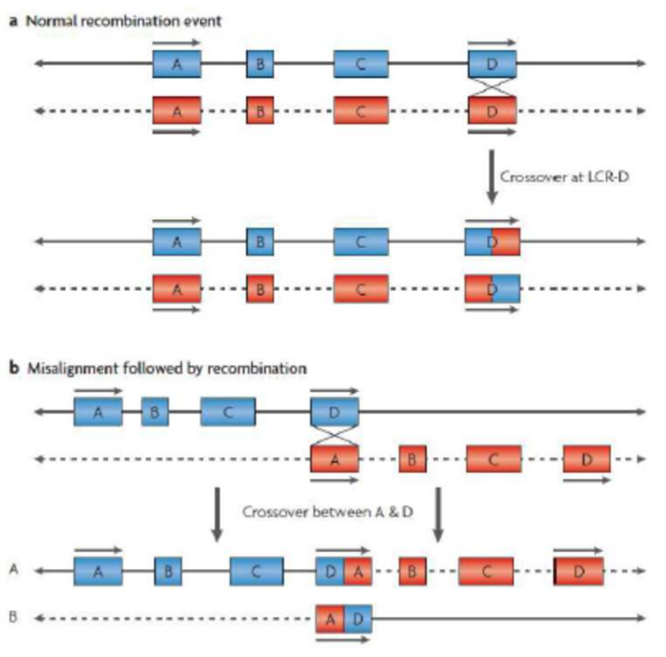

Model for interchromosomal recombination between two homologues leading to formation of a deletion and a reciprocal duplication

Chromosomes are shown as lines; solid and dotted lines are used to distinguish between the two homologues. a | Segmental duplications or low-copy repeats (LCRs) are shown as blue or red boxes with arrows to indicate the orientation of the shared modules within them. They are depicted during a normal recombination event between two properly aligned segmental duplications, A and D. b | Misalignment of segmental duplications that share sequence homology in the same orientation results in interchromosomal recombination between the two homologues of a chromosome. This results in a reciprocal duplication on homologue A and a deletion on homologue B.

Potential rearrangemetns driven by NAHR

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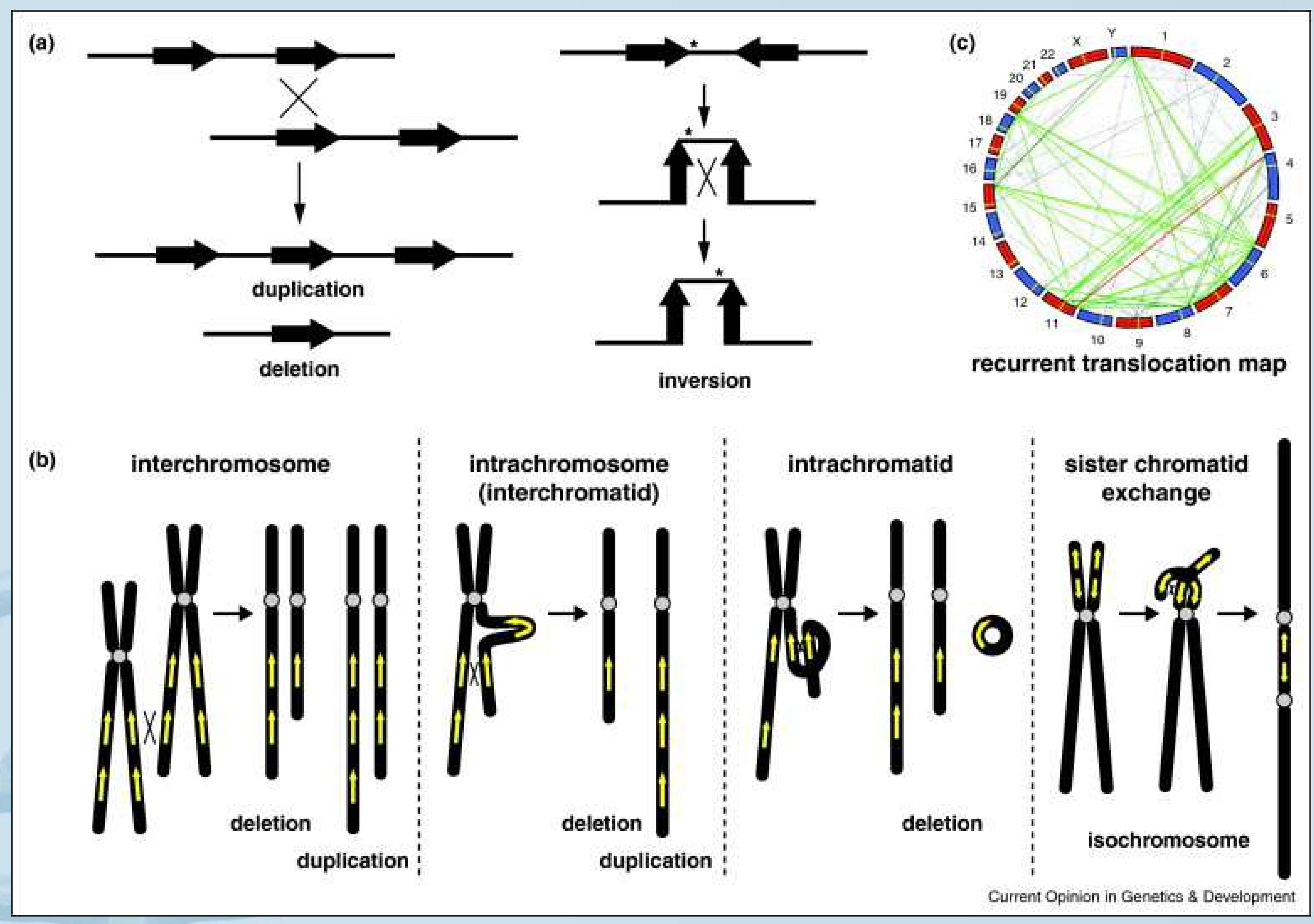

Recurrent rearrangements

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Key mechanism:

Change in copy number by homologous recombination

I) non-allelic/ectopic homologous recombination (NAHR) between low copy repeats (LCRs)/segmental duplications

II) Single-strand annealing

Recurrent rearrangements

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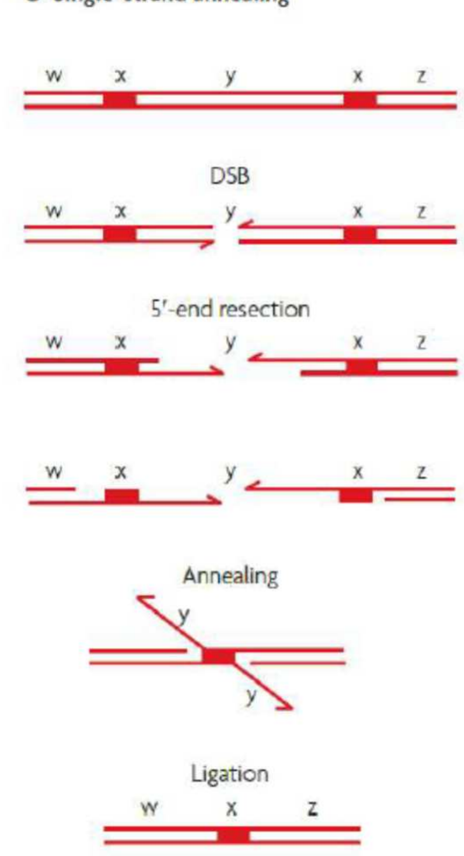
Key mechanism:

Change in copy number by homologous recombination

II) Single-strand annealing



When 5′-end resection on either side of a double-stranded break (DSB) does not lead to invasion of homologous sequence, resection continues. If this resection reveals complementary single-stranded sequence (x) shown by the filled regions, these can anneal. Removal of flaps, gap filling and ligation complete repair of the DSB with deletion of the sequence between the repeats (y) and of one of the repeats.

NAHR by break induced replication

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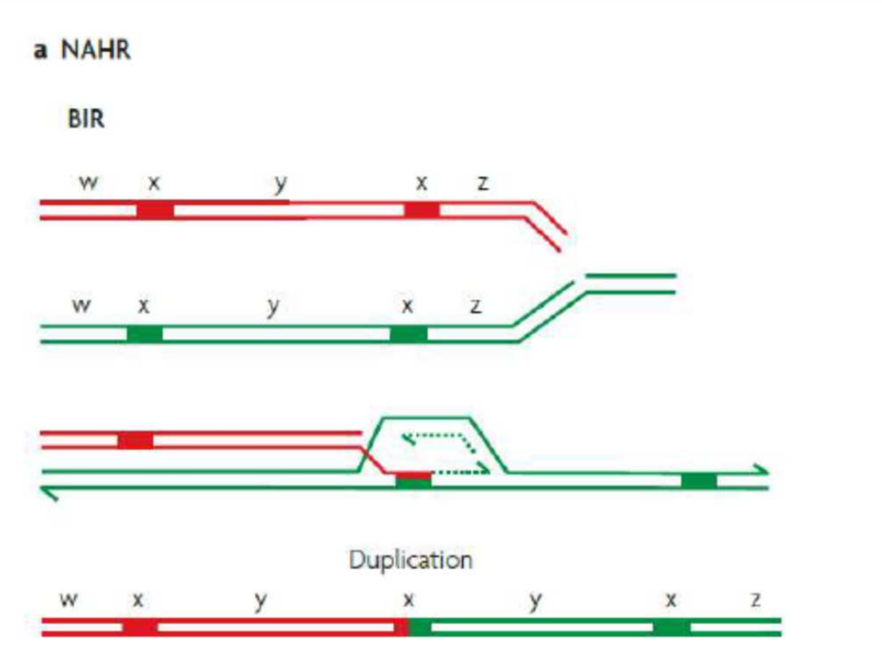

NAHR can also occur by break induced replication (BIR) when the broken molecule uses ectopic homology to restart the replication fork. BIR will form duplications and deletions in separate events.

Genomic disorders

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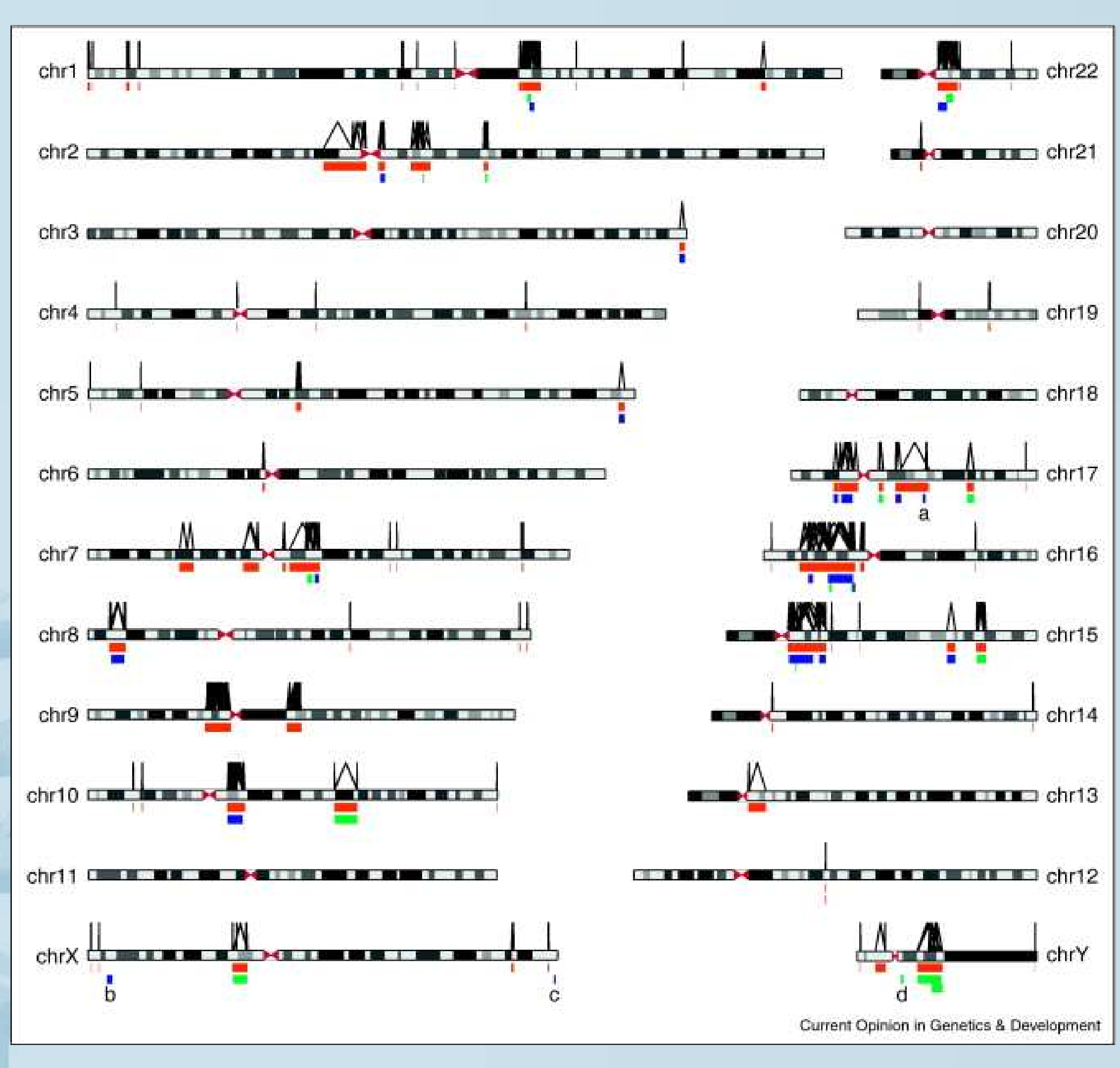

Genomic disorders

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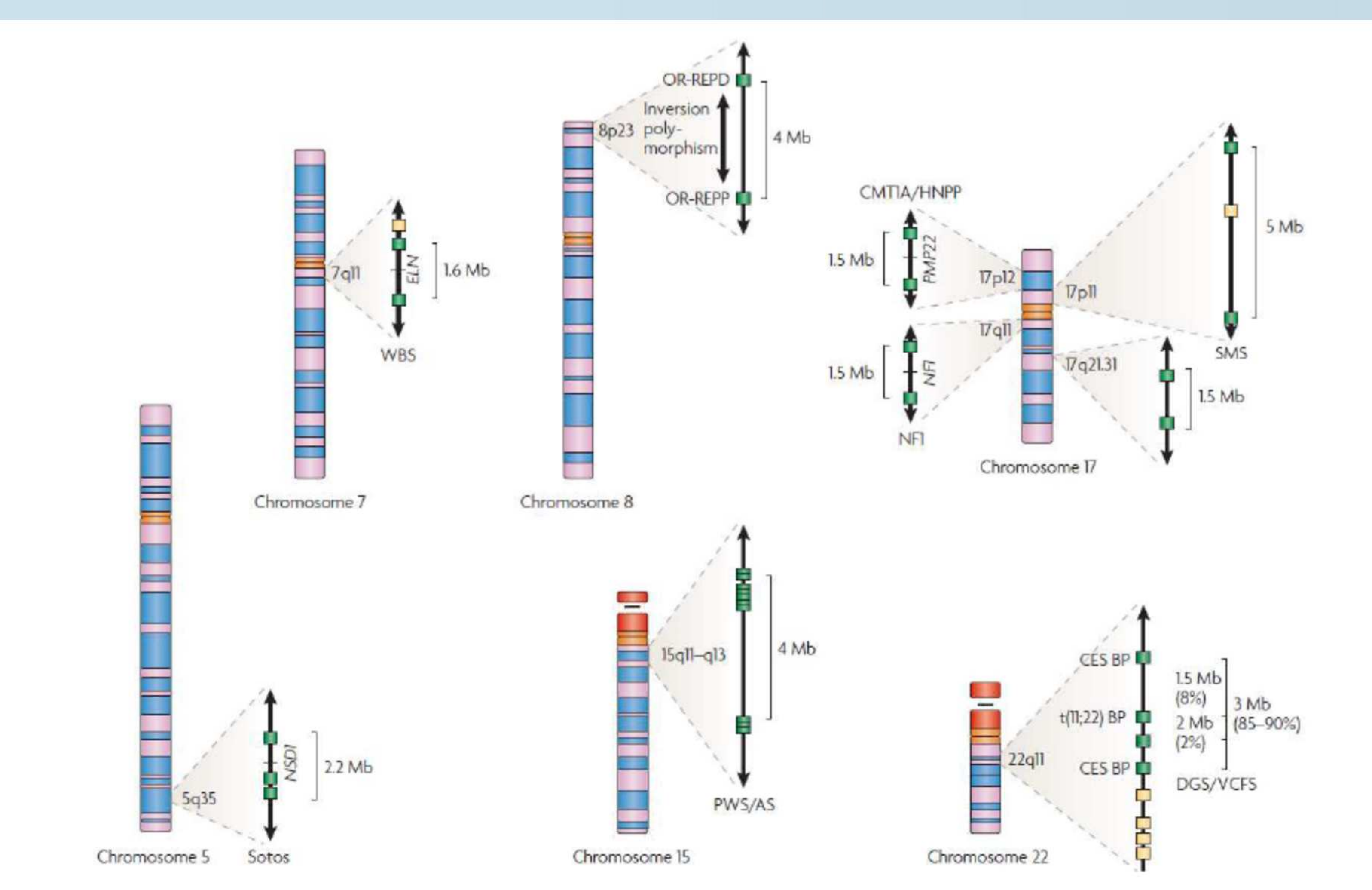
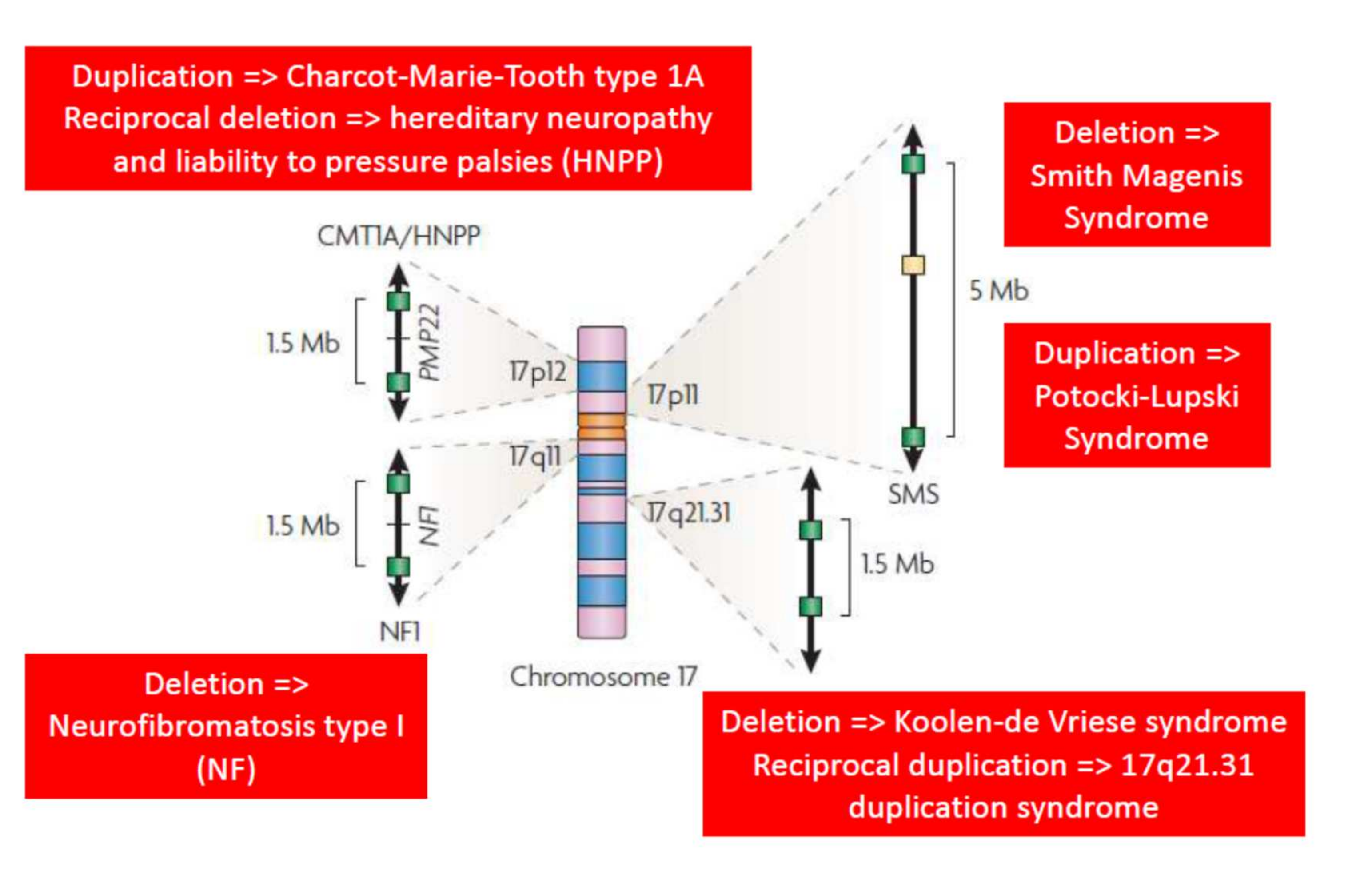


Chromosomal rearrangements mediated by segmental duplications

Genomic disorders on chromosome 17



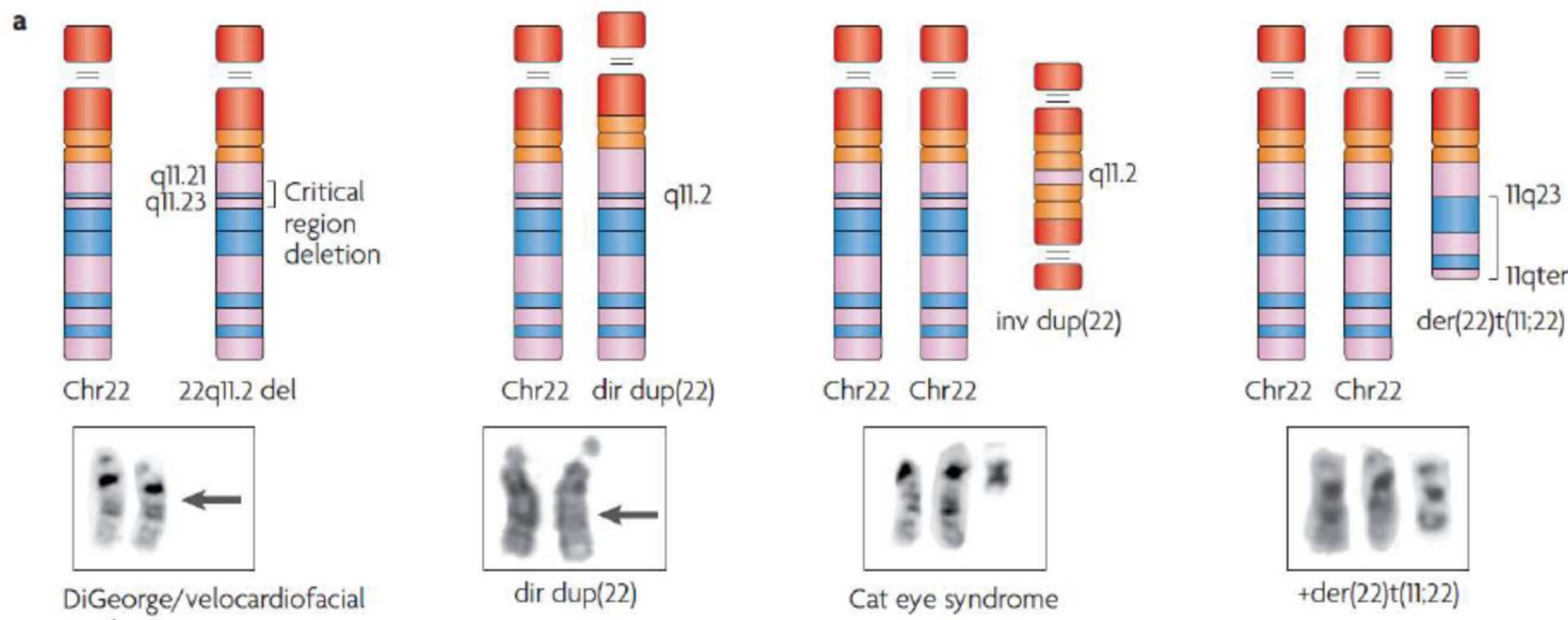
Genomic disorders on chromosome 22q11

chr22 (q11.21-q11.23) 22p13 22p12 22p11.2 22q11.21 q11.23 22q12.1 22q12.2 22q12.3 22q13.1 22q13.2 22q13.31

Scale 2 Mb hg38
chr22: 18,500,000 19,000,000 19,500,000 20,000,000 20,500,000 21,000,000 21,500,000 22,000,000 22,500,000 23,000,000 23,500,000 24,000,000
Duplications of >1000 Bases of Non-RepeatMasked Sequence
Segmental Dups

LCR-A B C D E F G H

Genomic disorders on chromosome 22



Different 22q11.2 rearrangements mediated by NAHR

- the deletion of chromosome 22q11.21–11.23 (indicated by an arrow) is associated with DiGeorge and velocardiofacial syndrome.
- the interstitial reciprocal duplication is a susceptibility locus.
- the inv dup(22) is associated with cat eye syndrome => tetrasomy for 22q11.2 = bisatellited marker.
- the +der(22)t(11;22) — a derivative chromosome 22 that is generated by the translocation between chromosomes 11 and 22 — is associated with Emanuel syndrome.

Rearrangements between different LCR22s lead to different deletion sizes

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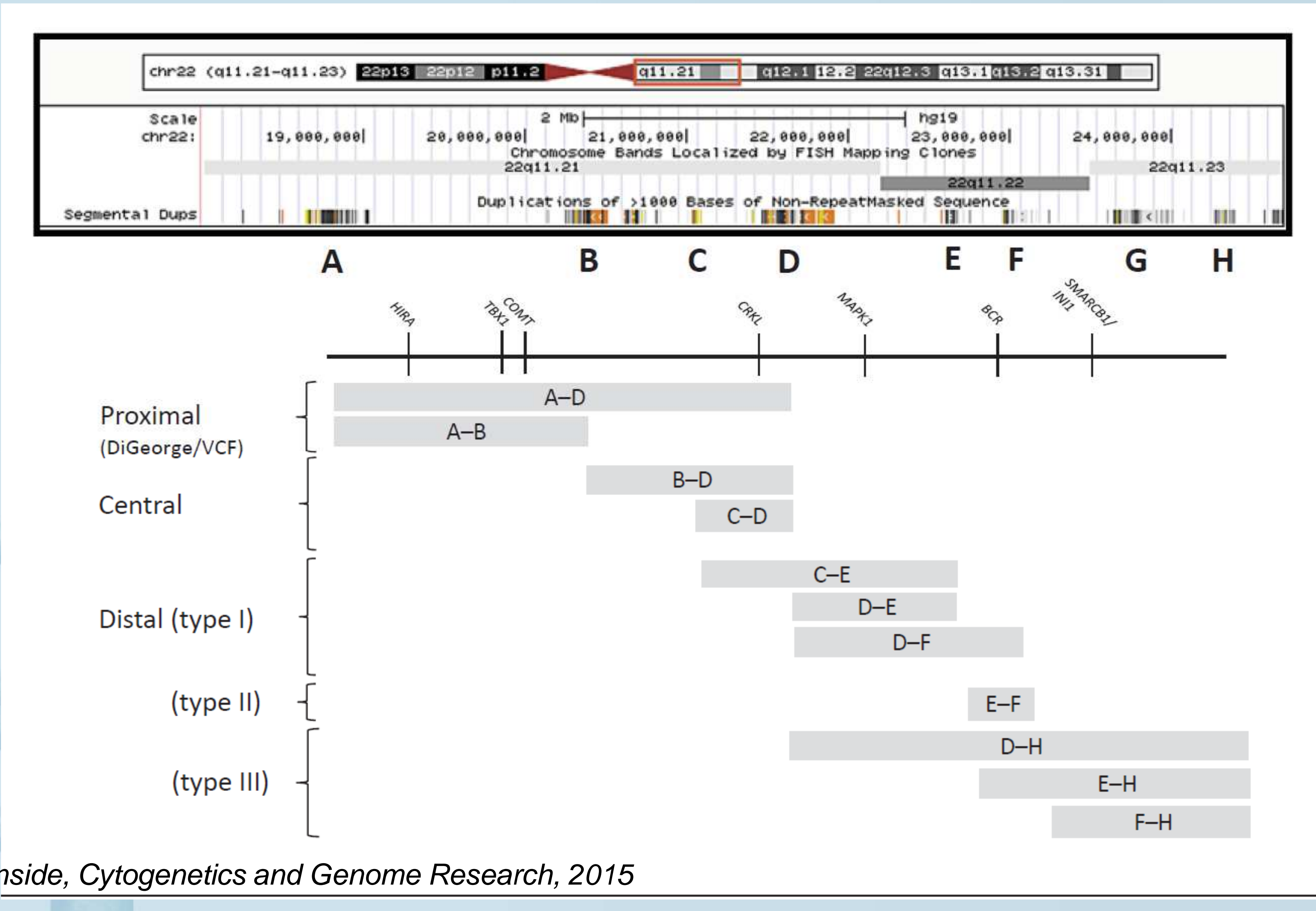
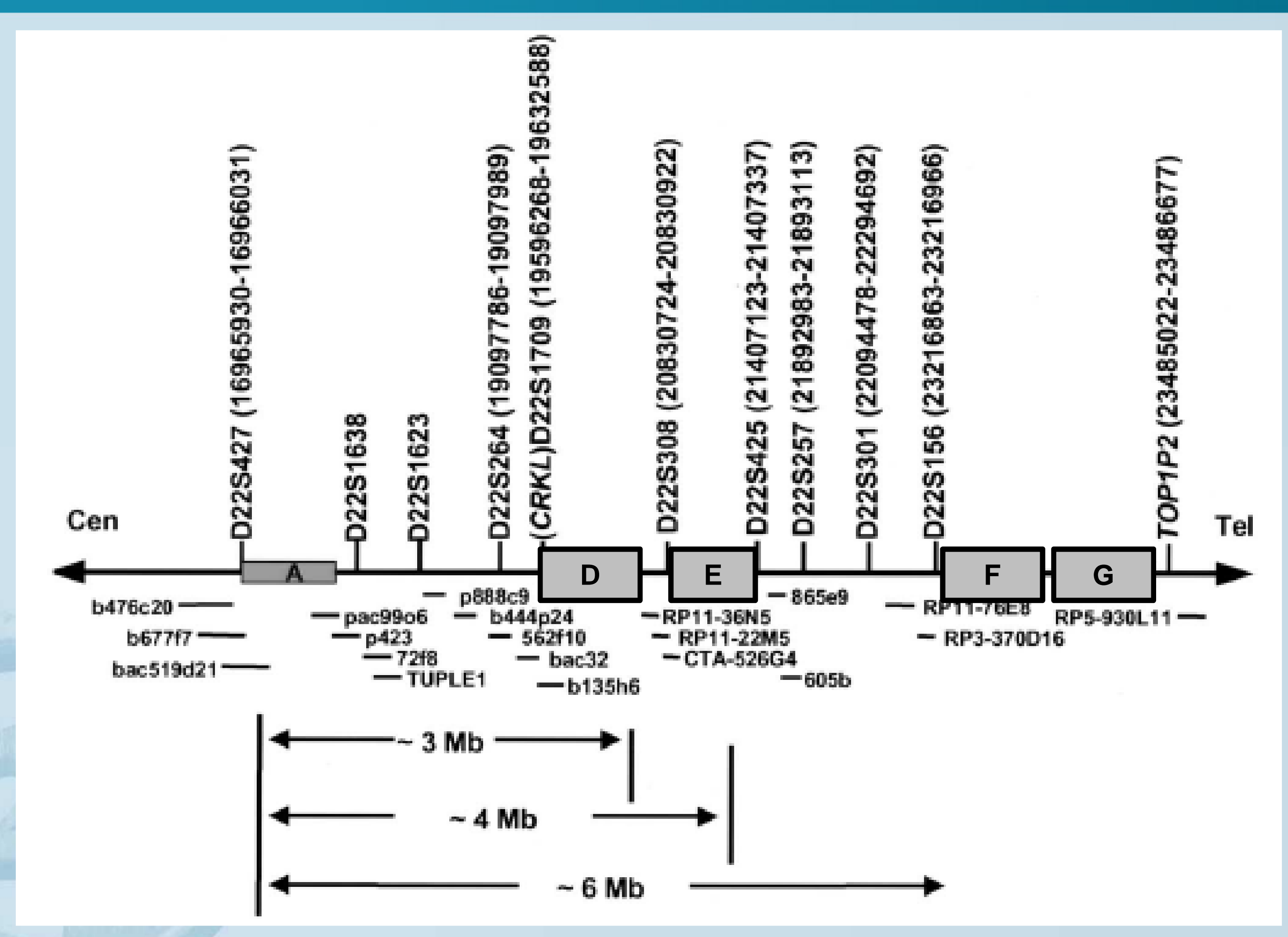


Burnside, Cytogenetics and Genome Research, 2015

The reciprocal 22q11 duplication syndrome



Ensenauer et al., Am. J. Hum. Gen., 2003

Recurrent human translocations mediated by NAHR (Ou et al., Genome research 2011)

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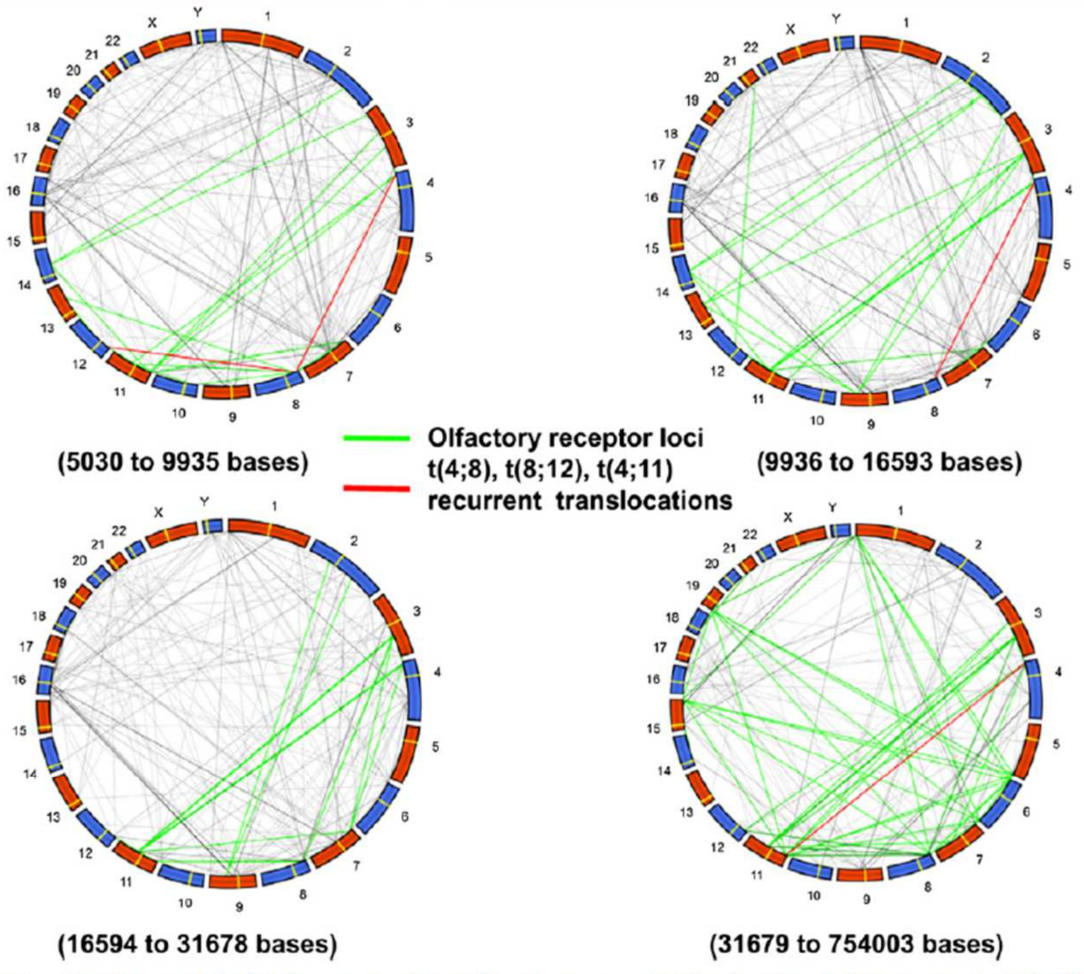

Figure 4. Recurrent translocation map. A global genomic view of interchromosomal LCR pairs with >5 kb in size and >94% DNA sequence identity represented by dotted lines and distribution divided into four groups based on the size of LCR. To create this plot we circularized the genome using polar coordinates. We then connected points between a pair of chromosomes linked by LCRs satisfying our size sequence identify criteria (see Supplemental Table 3). The midpoints of the LCRs were used to identify each segment with a single location on each chromosome. The red dotted lines indicate the translocations identified in our patient database, while the green dotted lines represent the olfactory receptor LCRs. (*A*) The size of LCR ranges from 5030 to 9935 bases in the first 25%. (*B*) The size of LCRs range from 9936 to 16,593 bases for the second 25% of LCRs. (*C*) The size of LCRs range from 16,594 to 31,678 bases for the third 25% of LCRs. (*D*) The size of LCRs range from 31,679 to 754,003 bases for the final 25% of LCRs.

Non-recurrent chromosomal rearrangements

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Mechanism of structural abnormalities

Misrepair of a double strand break/segregation error
Recombination error
DNA replication error

Non-recurrent rearrangements

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- Breakpoint junctions can be characterized by simple blunt ends or microhomologies (short stretches of shared nucleotide identity; 2-15bp)
- Chromosomal structural changes can be complex
- Junctions do not coincide with LCRs but tend to occur in the vicinity of regions that are rich in LCRs => complex regional genomic architecture

Key mechanism:

≠ homologous recombination

= <u>nonhomologous</u> repair mechanism:

- non-replicative
- replicative (linked to DNA replication)

Non-recurrent rearrangements

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Key mechanism:

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Non-recurrent structural variants

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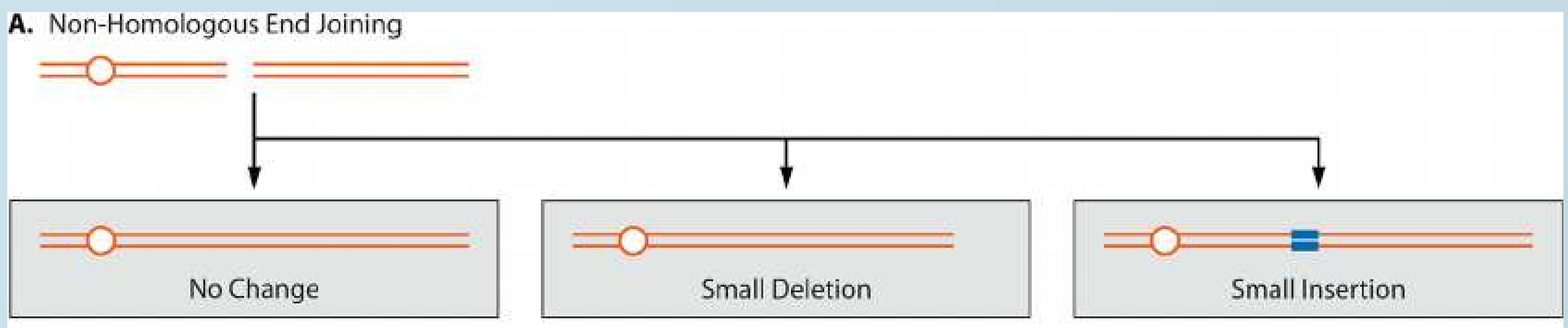
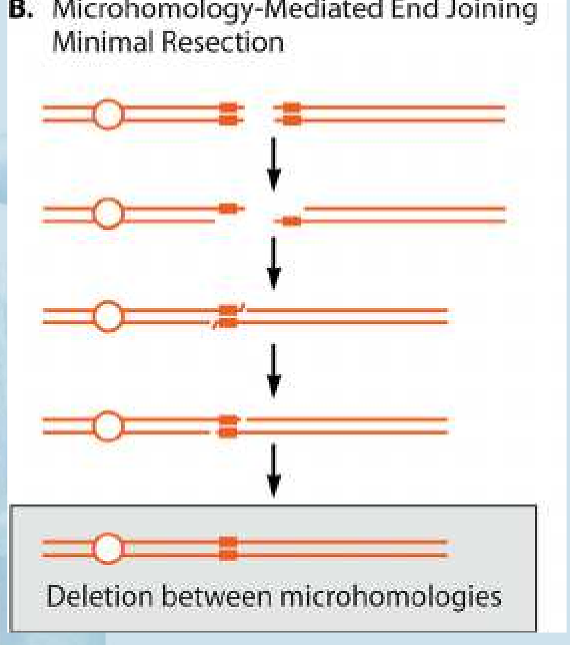
nonhomologous <u>non-replicative</u> repair mechanisms





NHEJ and MMEJ = two pathways of DSB repair that do not require homology or need very short homologies for

Non-recurrent structural variants

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nonhomologous non-replicative repair mechanisms

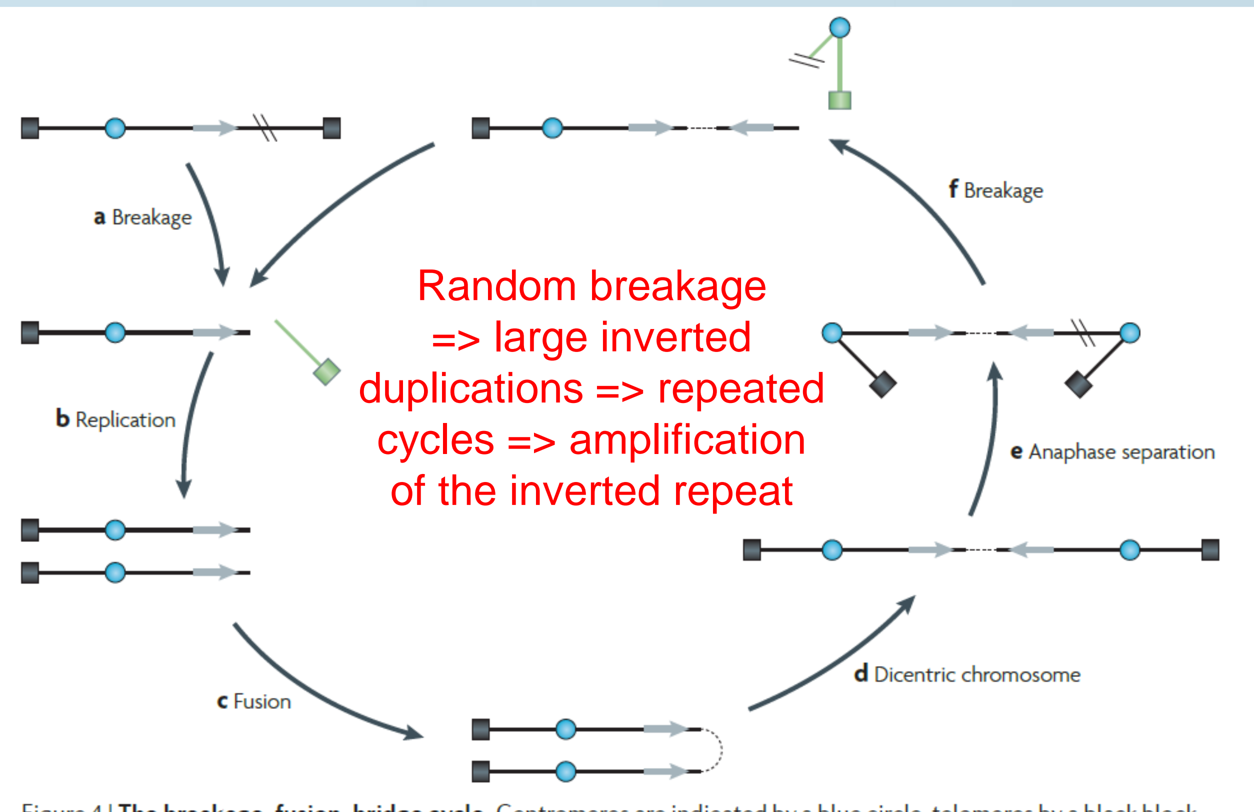

Figure 4 | **The breakage–fusion–bridge cycle.** Centromeres are indicated by a blue circle, telomeres by a black block and genomic sequence as grey arrows showing orientation. Breakage points are shown as double black lines, and fragments that are lost are in green. **a** | A doubled-stranded break (DSB) occurs in an unreplicated chromosome, causing it to lose a telomere. **b** | After replication, both sister chromatids lack telomeres. **c** | These two ends are thought to fuse. **d** | The fusion in part **c** forms a dicentric chromosome. **e** | At anaphase, the two centromeres of the dicentric chromosome are pulled apart, initially forming a bridge between the telophase nuclei. **f** | Eventually, the bridge is broken in a random position. This inevitably leads to the formation of a large inverted duplication. The chromosome once again has an unprotected end, and after replication will form two sister chromosomes that can fuse to form a new a dicentric chromosome, and so the process is repeated until the end acquires a telomere from another source.

Non-recurrent rearrangements

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- Breakpoint junctions can be characterized by simple blunt ends or microhomologies (short stretches of shared nucleotide identity; 2-15bp)
- Chromosomal structural changes can be complex
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≠ homologous recombination

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- replicative (linked to DNA replication)

Non-recurrent structural variants

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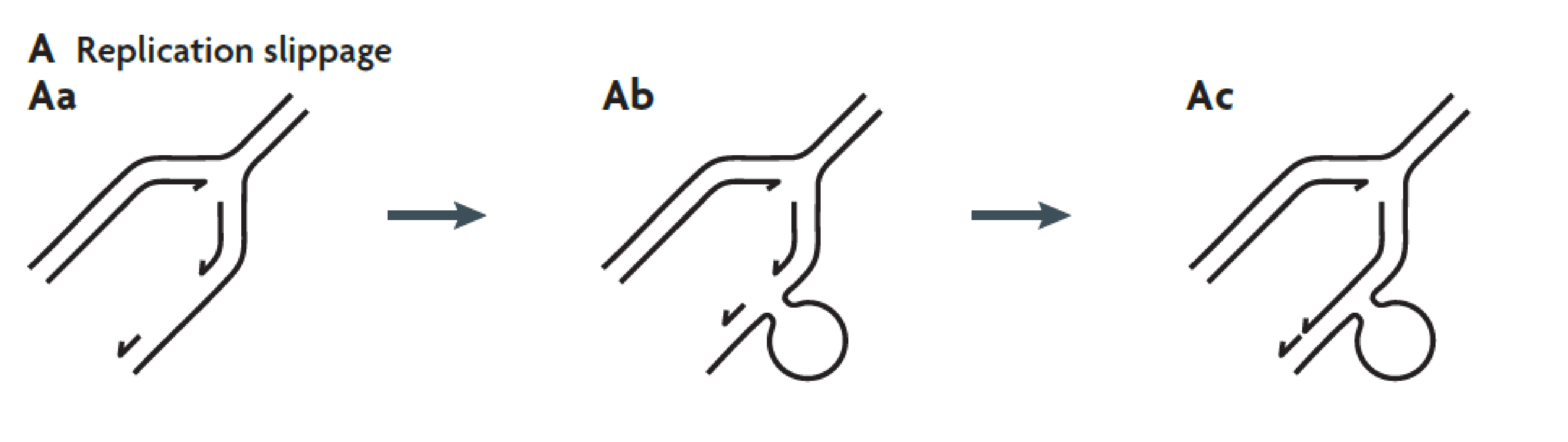
nonhomologous replicative repair mechanisms



A | Replication slippage.
During replication, a length of lagging-strand template becomes exposed as a single strand (**Aa**).
The 3′ primer end can move to another sequence showing a short length of homology on the exposed template (**Ab**); this move might occur owing to the formation of secondary structures in the lagging-strand template.
Lagging strand synthesis can continue after having failed to copy part of the template (**Ac**). As shown, this will produce a deletion. Several variations on this mechanism can also produce a duplication of a length of DNA. Events occurring by this mechanism are confined to the length of genome found in a **single** replication fork.

Non-recurrent structural variants

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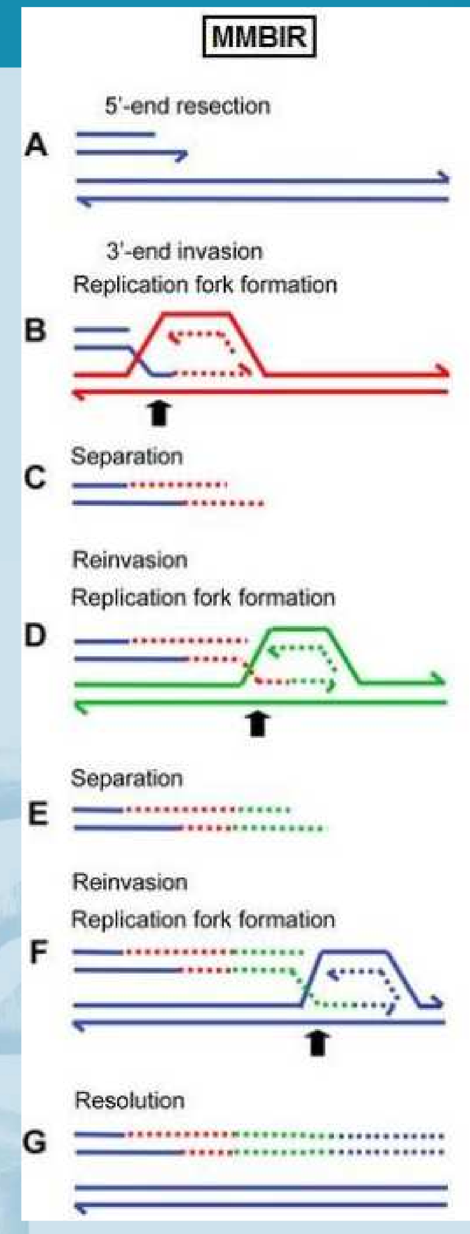
nonhomologous replicative repair mechanisms

B FoSTeS
Ba
Bb
Bc
Bd

B | Fork stalling and template switching (FoSTeS).
An exposed single-stranded lagging strand template (**Ba**) might acquire a secondary structure (**Bb**), which can block the progress of the replication fork. The 3′ end then becomes free from its template (**Bc**), and might then alight on another exposed single-stranded template sequence on **another** replication fork that shares microhomology (**Bd**), thus causing duplication, deletion, inversion or translocation, depending on the relative position of the other replication fork. Fork stalling can be caused by other situations, such as lesions in the template strand or shortage of deoxynucleotide triphosphates.

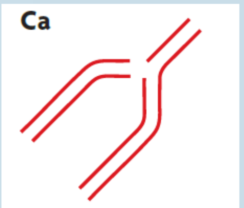
Non-recurrent structural variants



nonhomologous replicative repair mechanisms

Microhomology-mediated break-induced replication

Starts with replication fork collapse in which one arm breaks off a replication fork because the fork encounters a nick on a template strand, or can be caused by endonuclease.



The molecule that is produced carries short sequences from other genomic locations

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Chromoanagenesis

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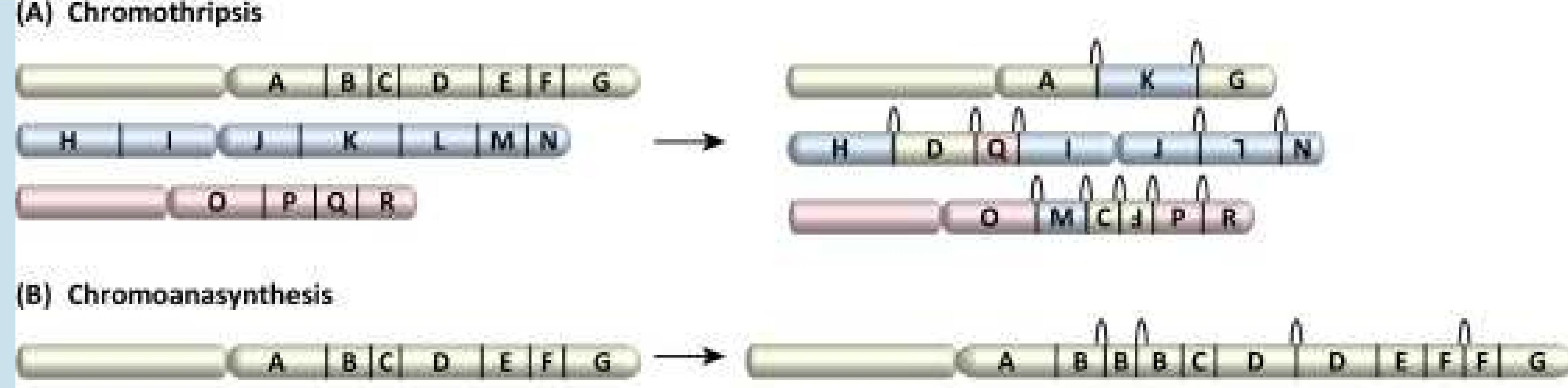

The most severe forms of genomic reorganization are described as 'chromoanagenesis,' or chromosome rebirth, so named because the chromosomes are rearranged beyond recognition. Chromosome shattering, or 'chromothripsis', and chromosome reconstitution, or chromoanasynthesis', are two types of chromoanagenesis.

(A) Chromothripsis shatters three **nonhomologous chromosomes**. The **only copy-number variations (CNVs) are deletions** of B and E, but translocating segments and inversions have shuffled the contents of the three chromosomes. The 12 breakpoint junctions have blunt ends or short microhomology.

(B) Chromoanasynthesis leads to **triplication** (B) and **duplications** (D and F) **across one chromosome**. These breakpoint junctions contain microhomology and insertions that suggest a DNA replication-based mechanism of repair.

chromoanasynthesis

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A Distinct Class of Chromoanagenesis Events Characterized by Focal Copy Number Gains

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