KATHOLIEKE UNIVERSITEIT
LEUVEN

Principles of clinical cytogenetics and Genome analysis

Joris Vermeesch

October 2023,

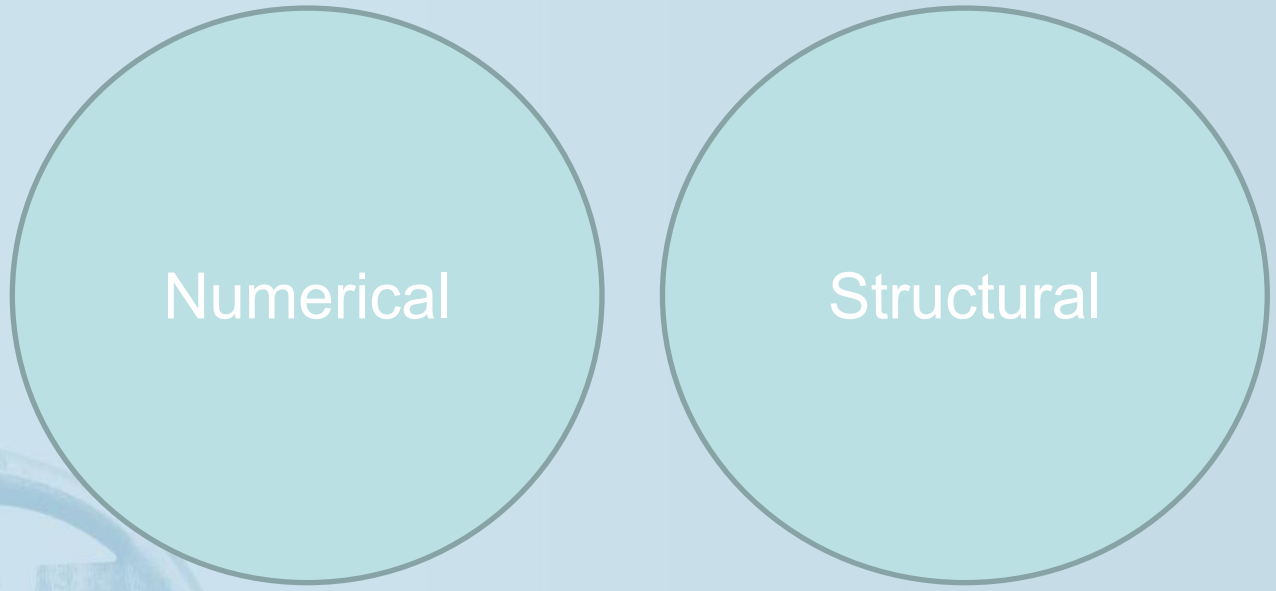
Interuniversity course in human genetics

Thompson & Thompson chapter 5

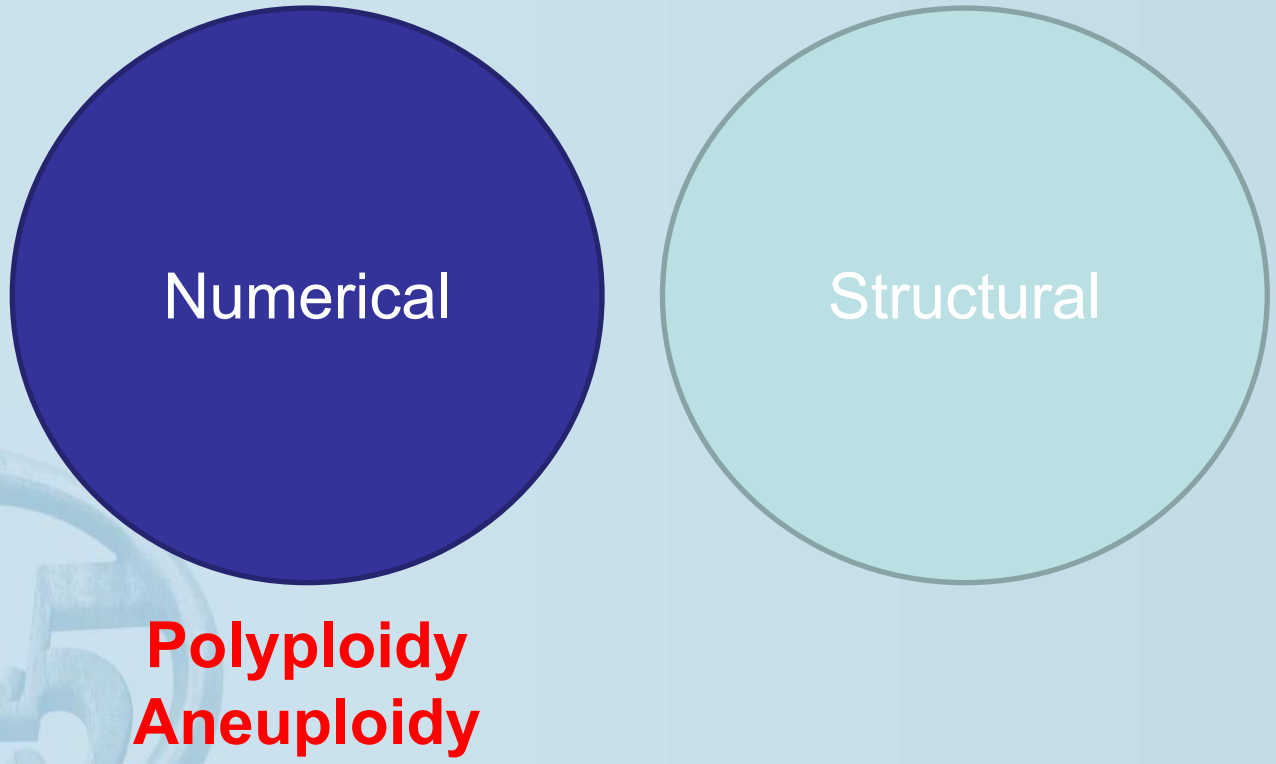
Overview

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

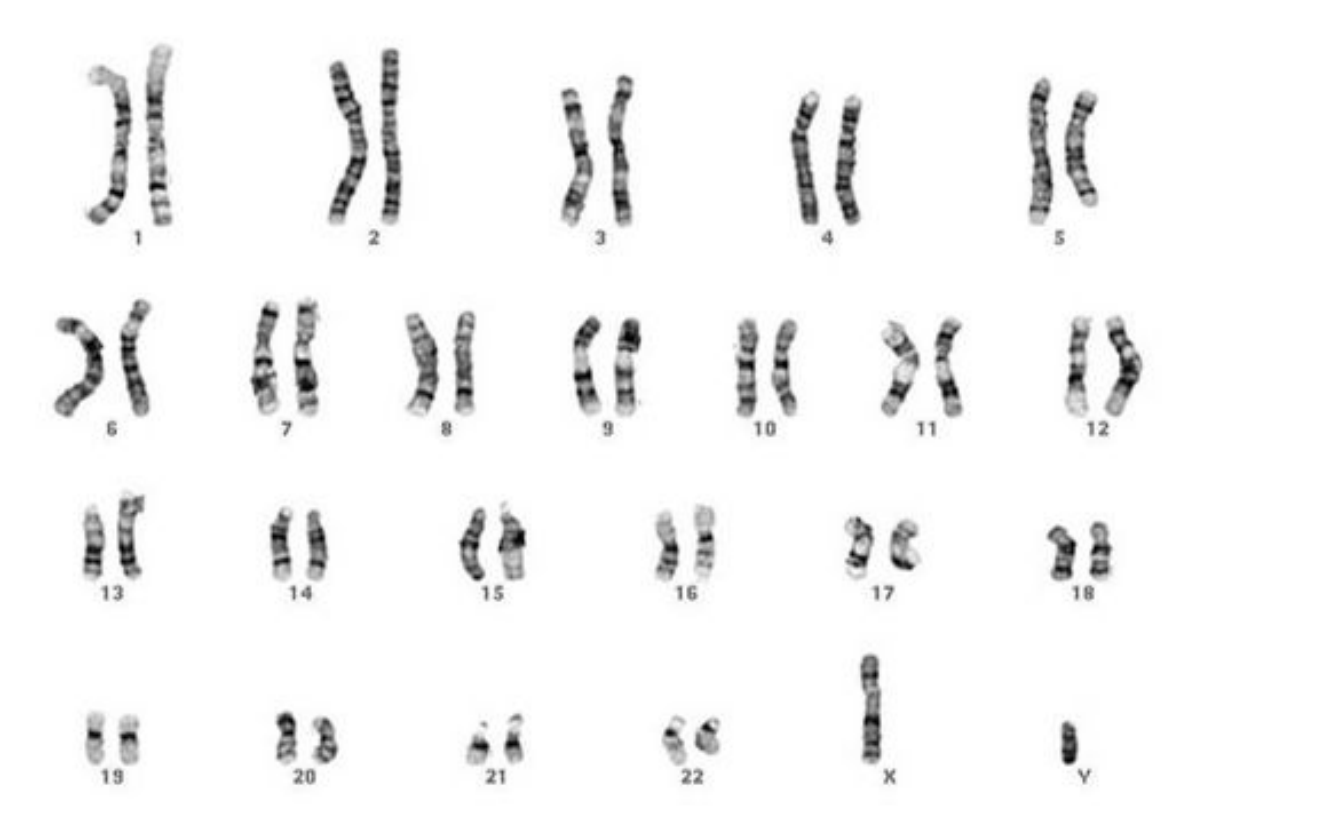
Chromosomal abnormalities



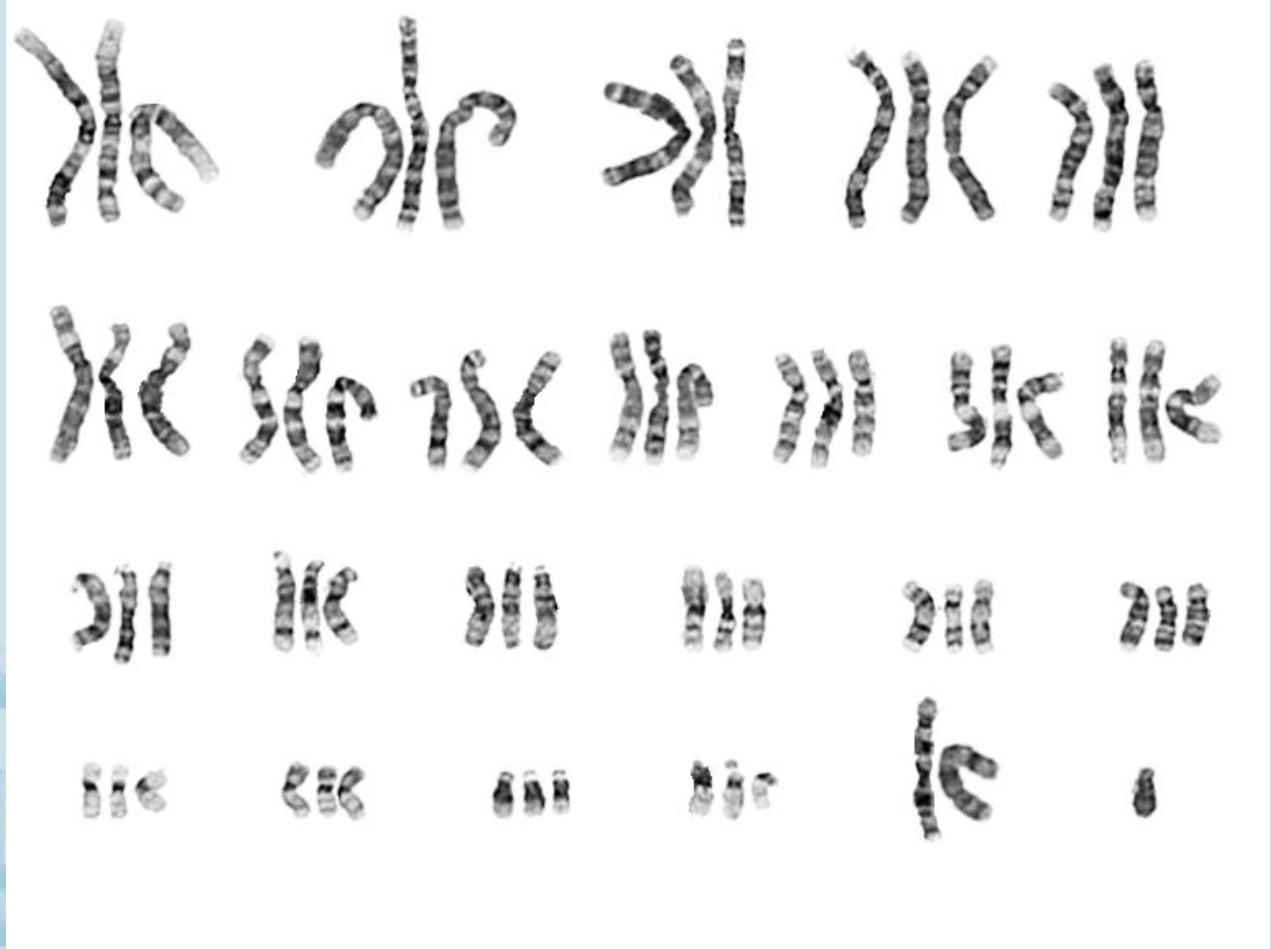
Numerical chromosomal abnormalities



Normal Diploid



Triploidy

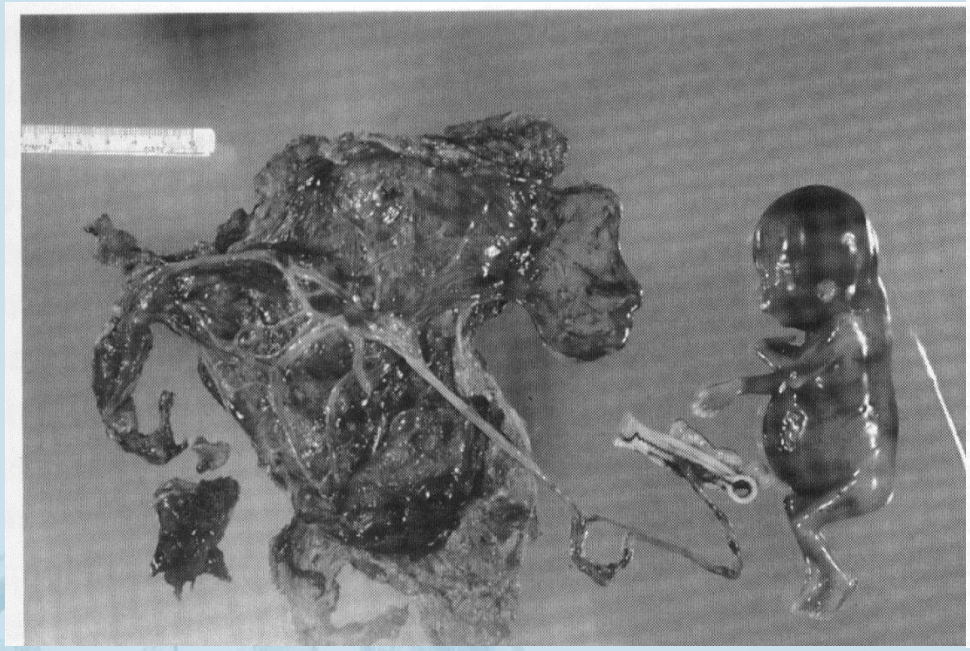


1-3% of all pregnancies
15-20% of all chromosomally abnormal miscarriages

Triploidy phenotype

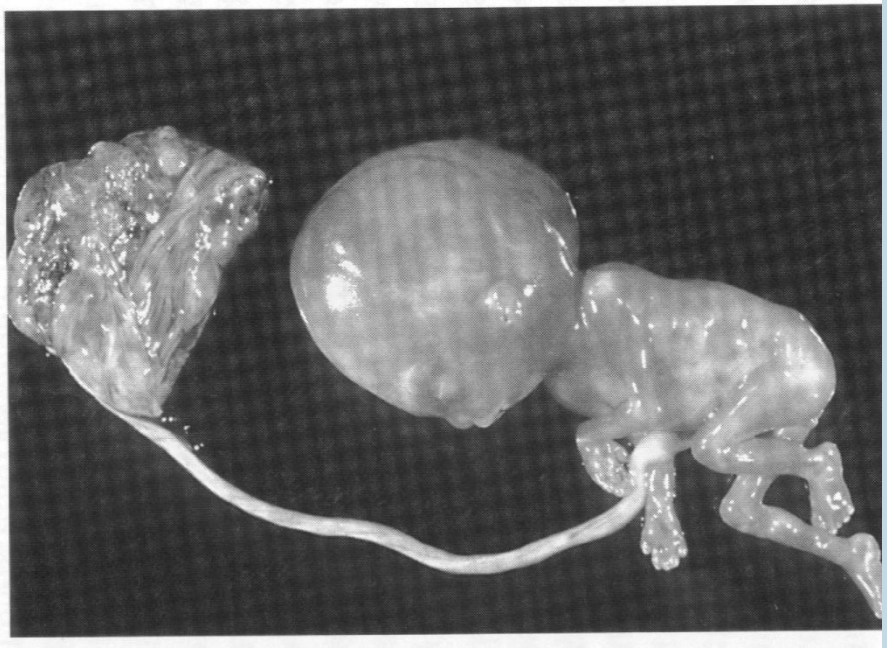
Paternal triploidy (diandry)

(often partial hydatiform moles)



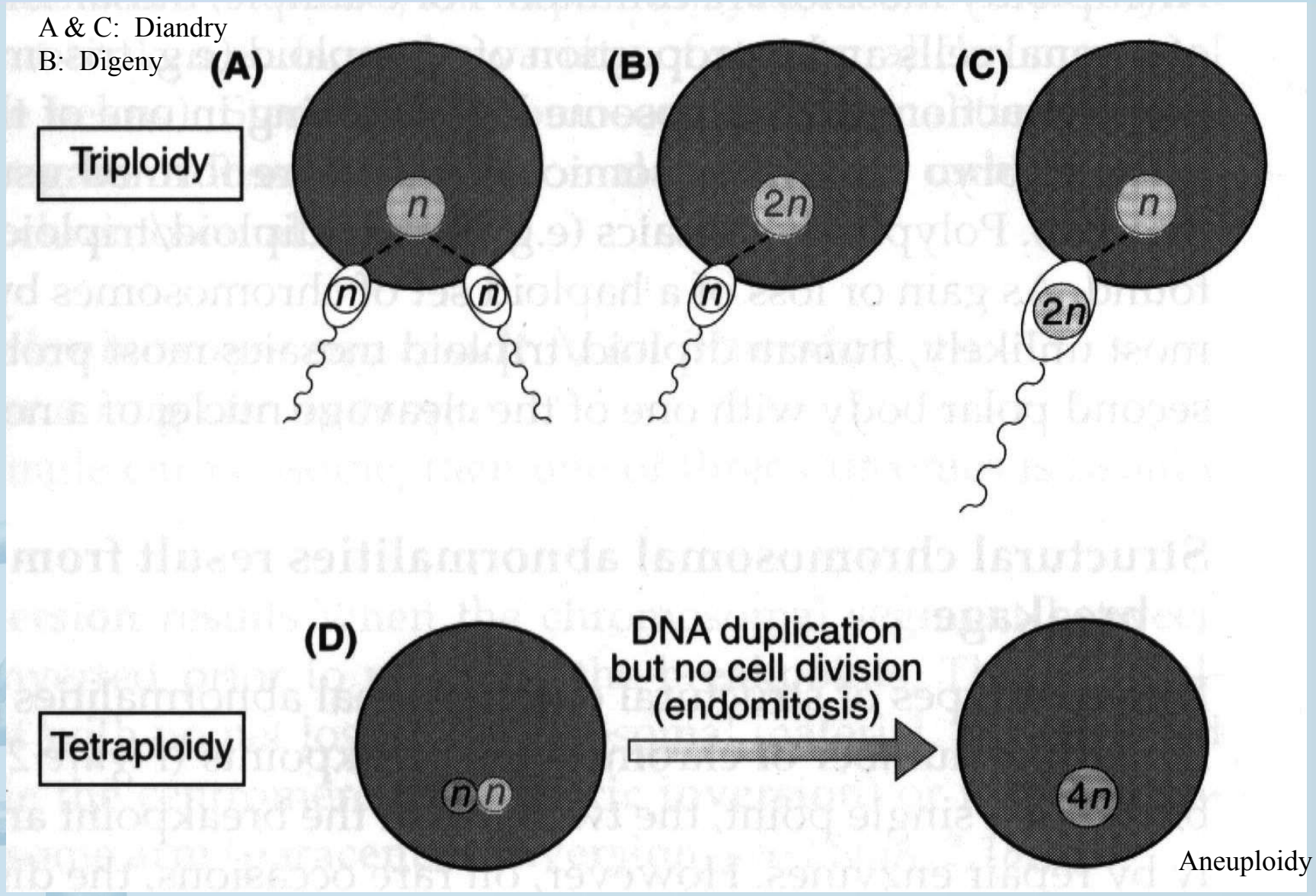
Maternal triploidy (digyny)

(aborted during early pregnancy)



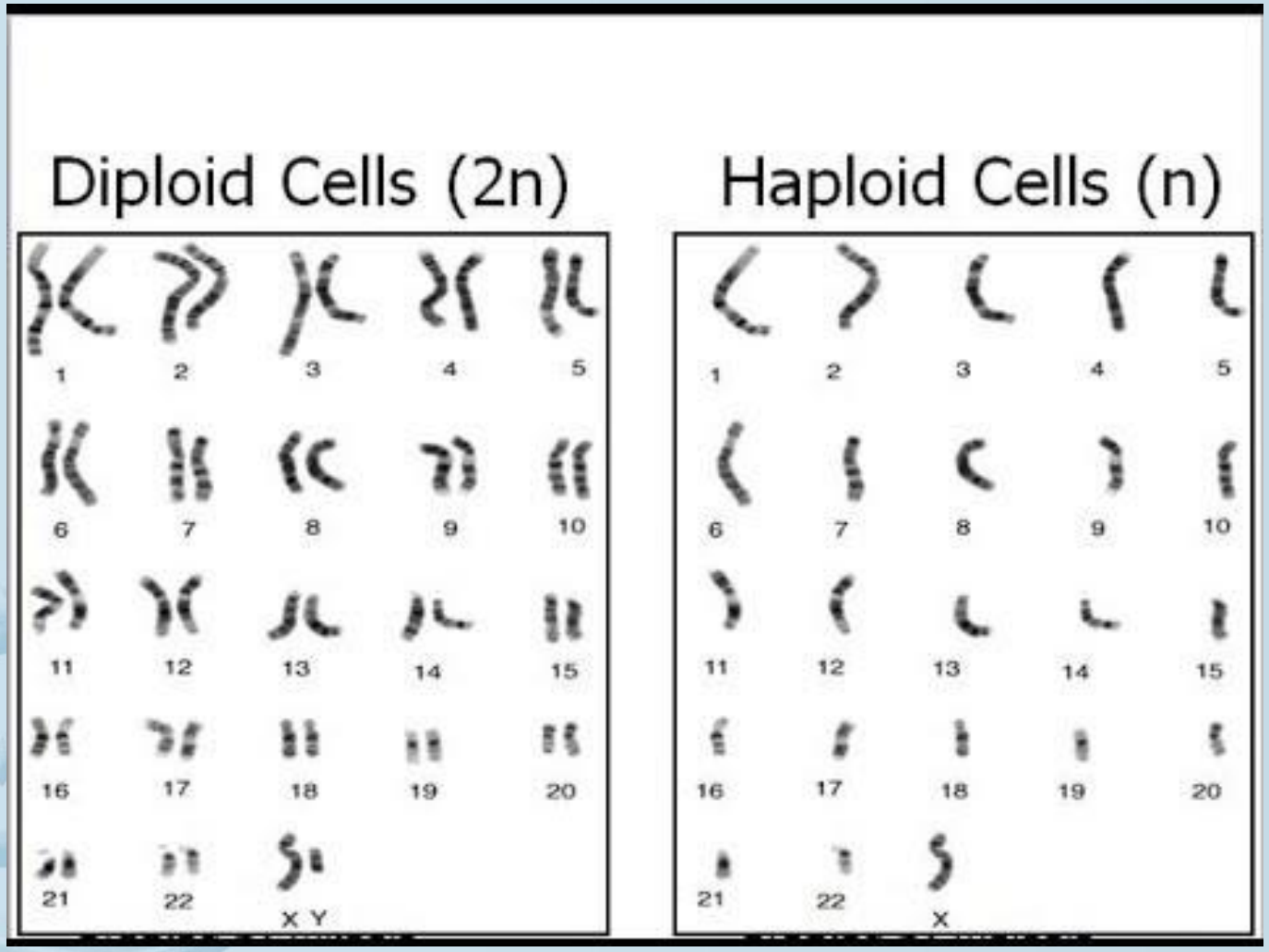
The phenotype of a triploïde foetus is dependent on the parental origin (maternal or paternal). Dit verschil wordt veroorzaakt door imprinting.

Origins of Triploidy



Aneuploidy

Haploidy

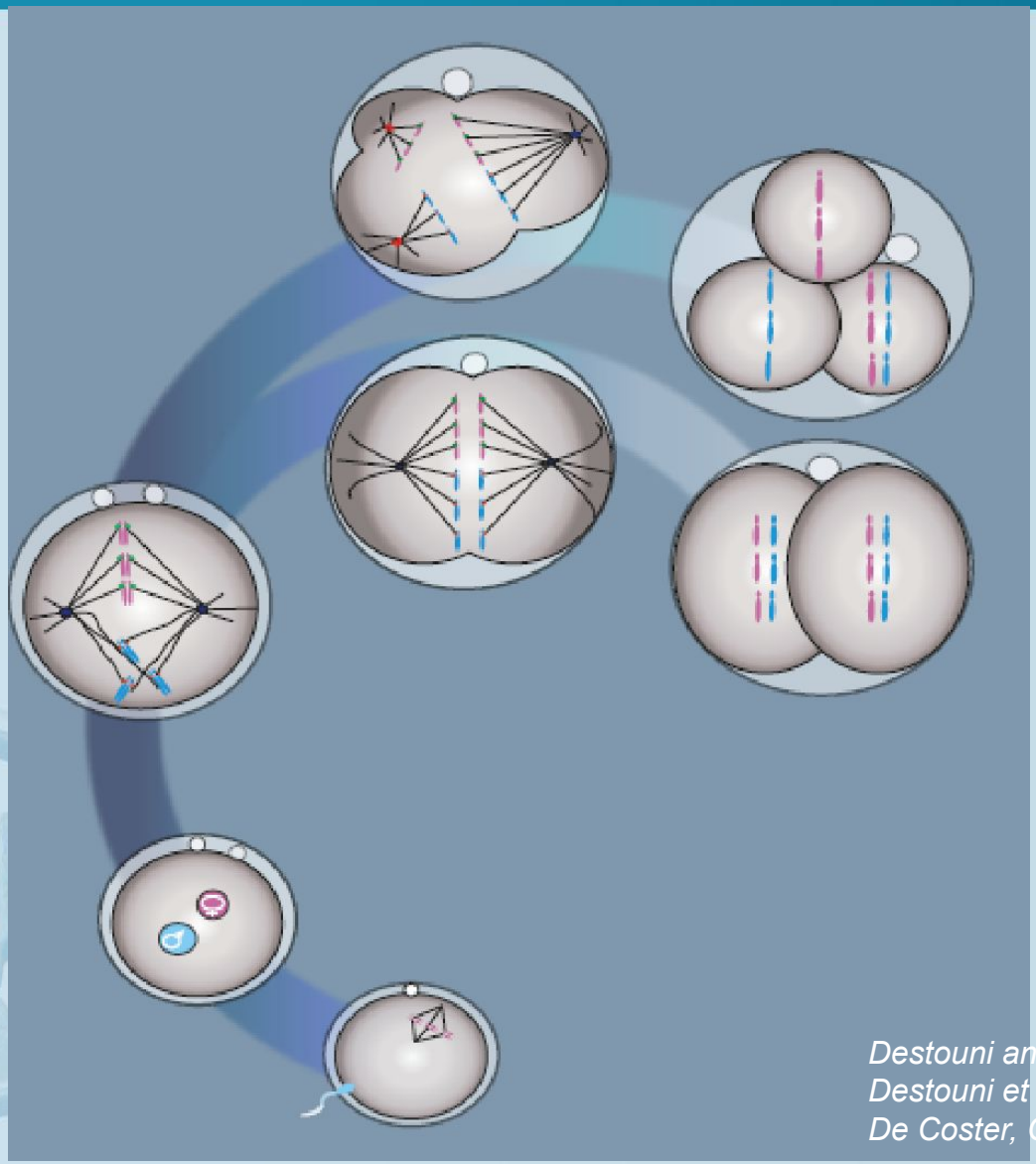

Diploid Cells (2n)
1 2 3 4 5
6 7 8 9 10
11 12 13 14 15
16 17 18 19 20
21 22 X Y
Haploid Cells (n)
1 2 3 4 5
6 7 8 9 10
11 12 13 14 15
16 17 18 19 20
21 22 X

Phenotypes of maternal or paternal only genomes (endoreplicated haploid)

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

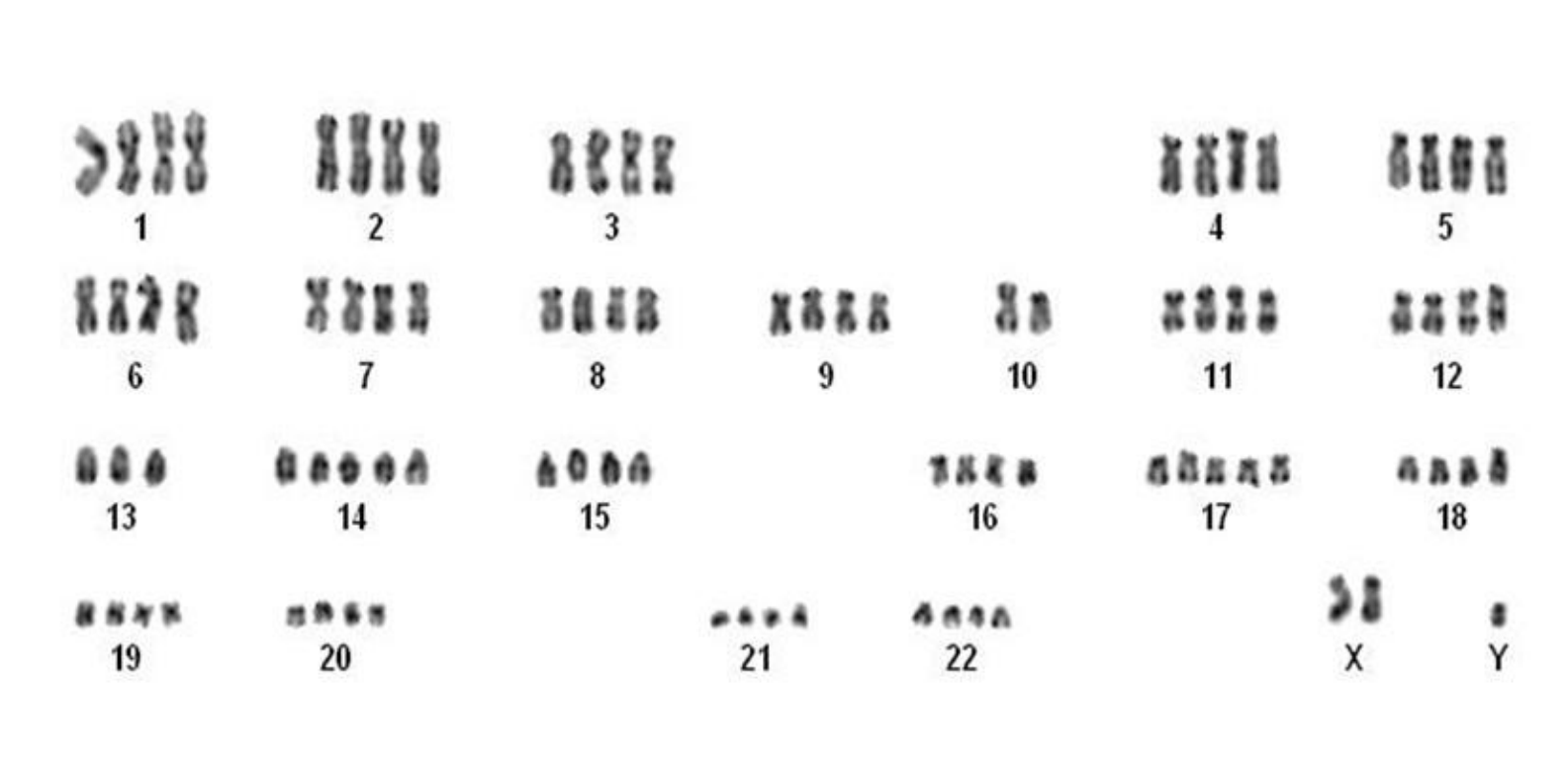
- ***Mola hydatiformis (schijnzwangerschap)***: Only the development of a trophoblast but not of a fetus. Contains only a paternal chromosome.

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



Destouni and Vermeesch, BioEssays, 2017
Destouni et al., Genome Research, 2016
De Coster, Genome Biology, 2022

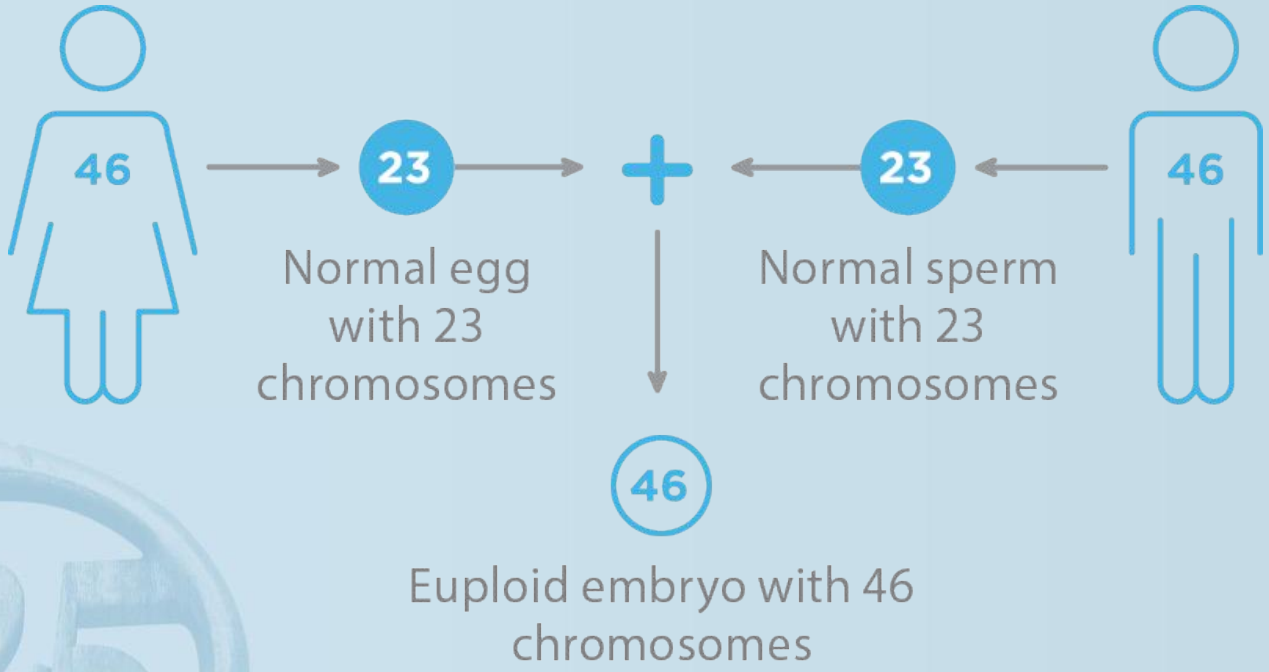
Tetraploidy



Likely the result of failure of completion of early zygotic division

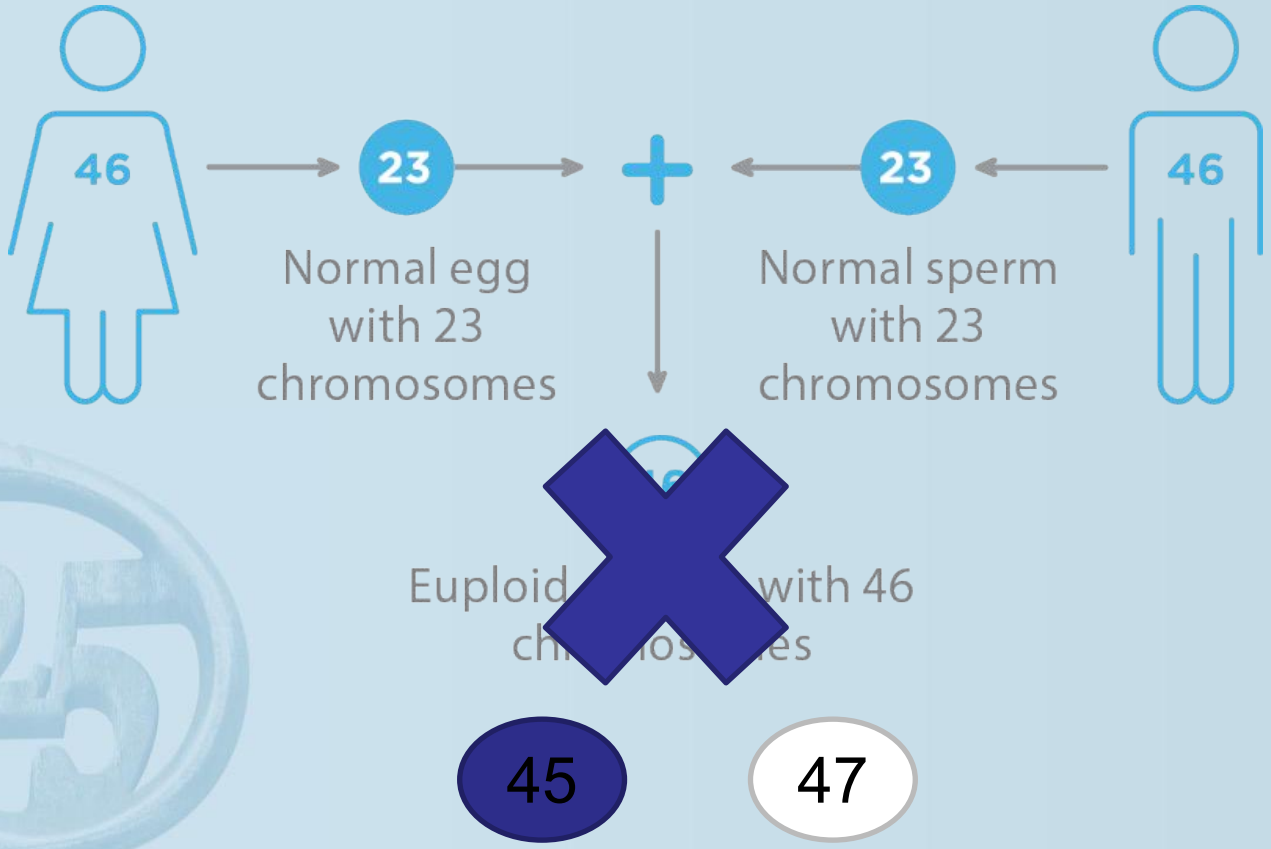
Aneuploidy

Variation in the number of particular chromosomes within a set

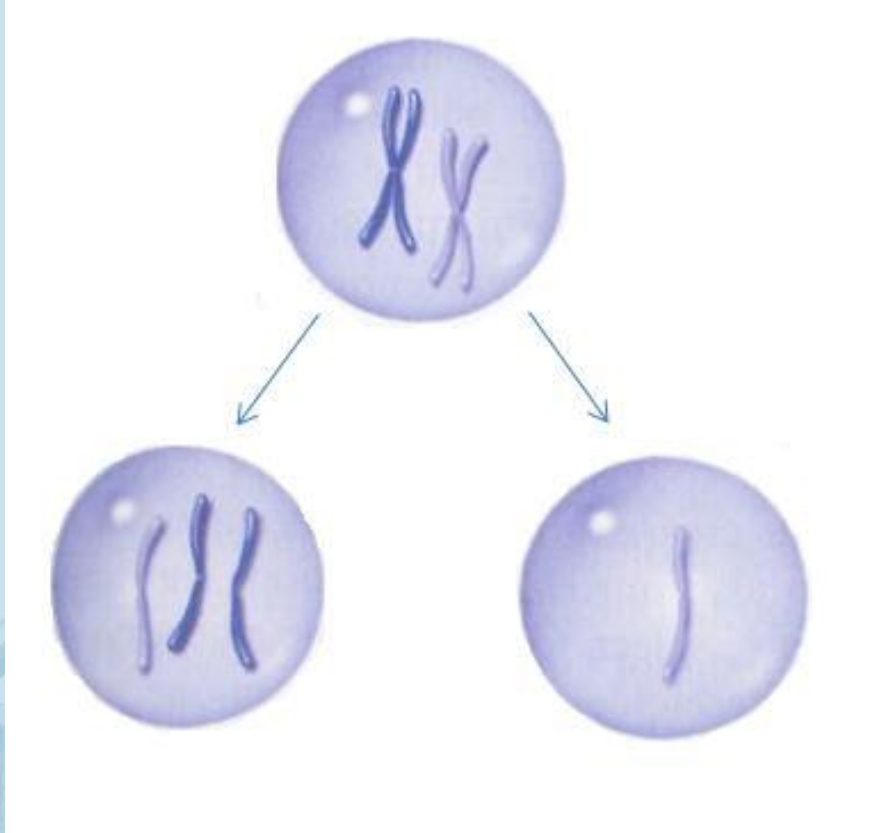


Aneuploidy

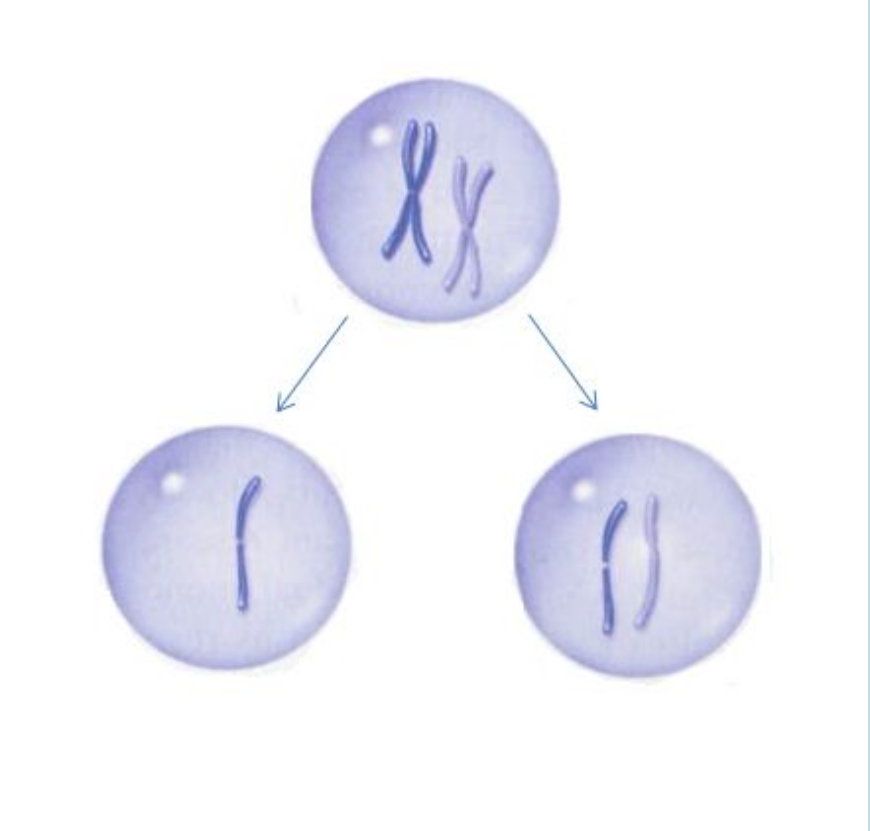
- Variation in the number of particular chromosomes within a set



Aneuploidy due to mitotic errors



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



Chromosome loss due to **anaphase lagging**

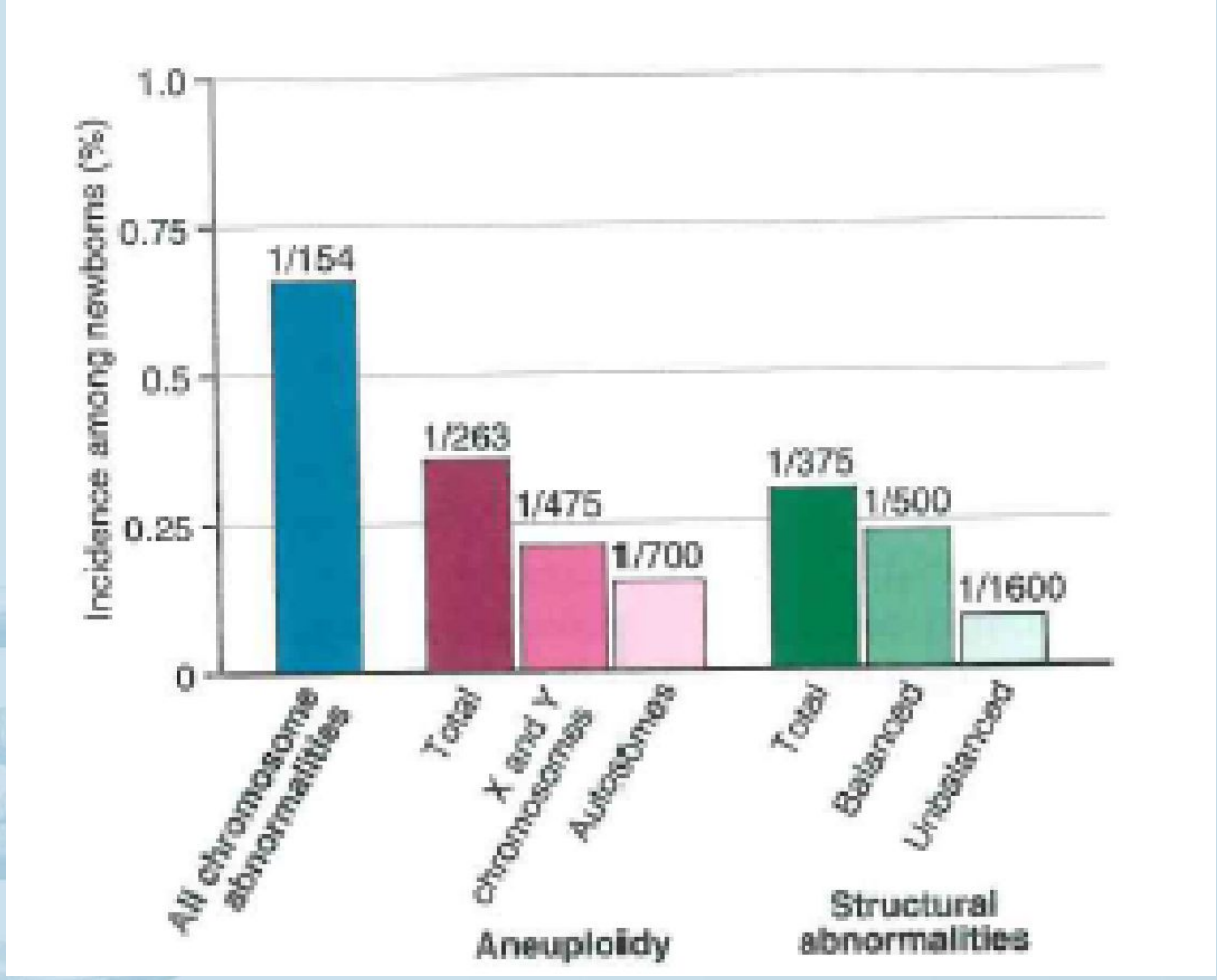
Trisomy is the most frequent genetic anomaly in human and the most important cause of miscarriages

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
XX X, XX Y, XY Y	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

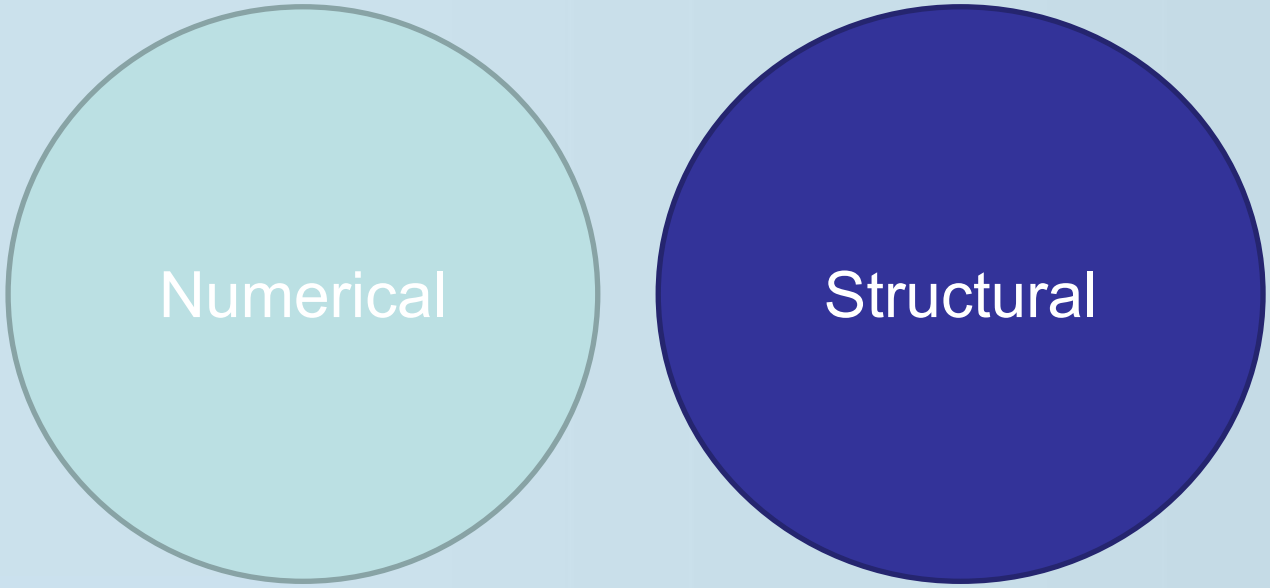
26 4 0,3

Hassold, 1986

Incidence of chromosome abnormalities in newborns



Unbalanced rearrangements

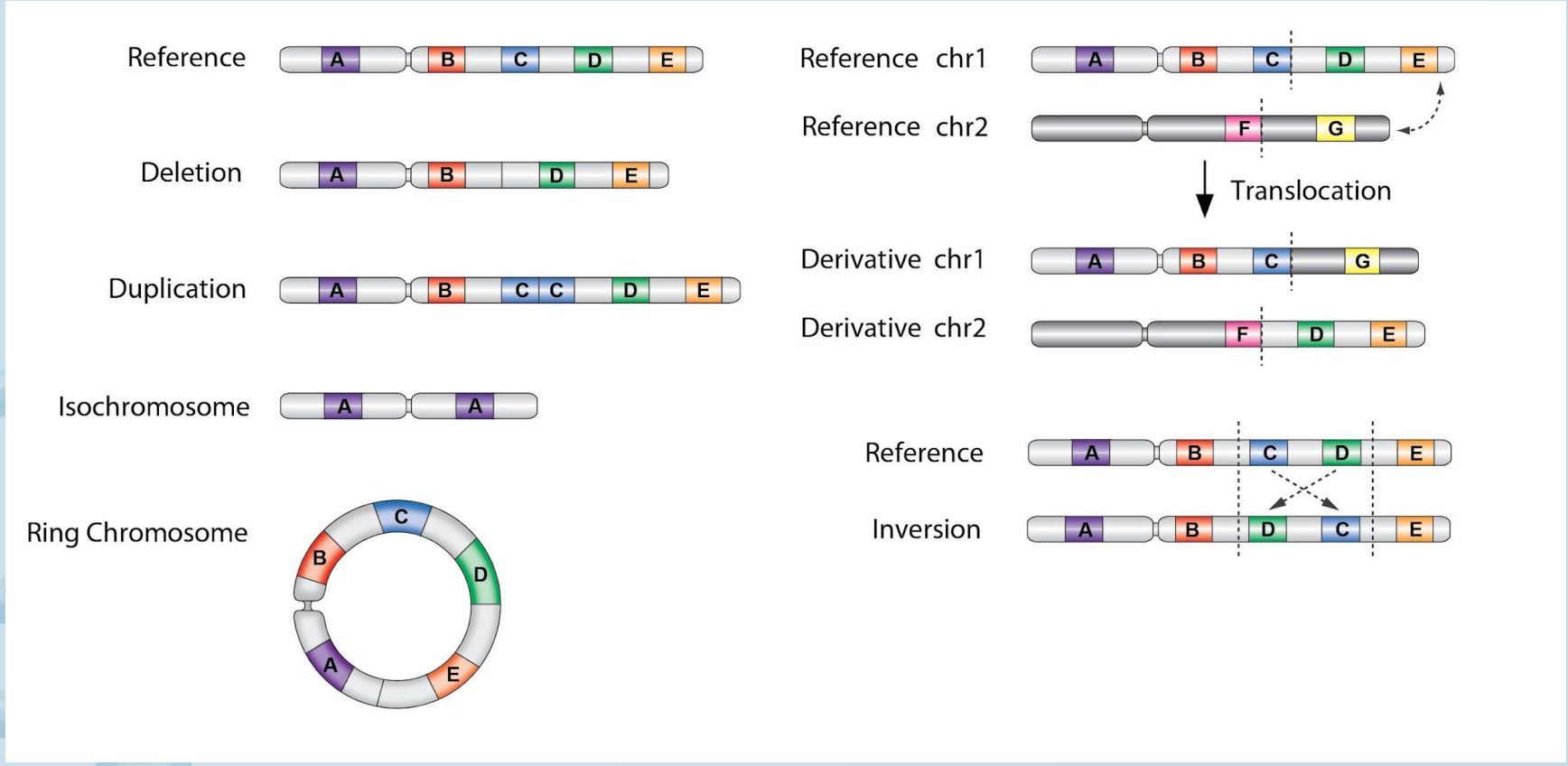


Unbalanced:
abnormal phenotype

Chromosomal rearrangements resulting in copy number variation

Unbalanced CNV

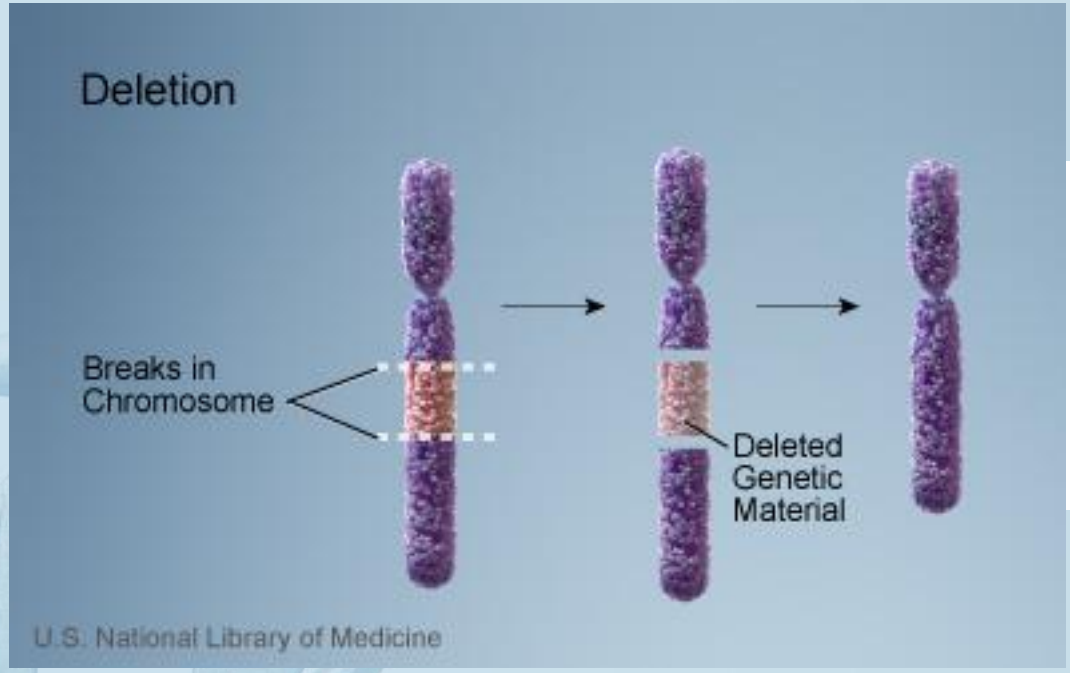
balanced

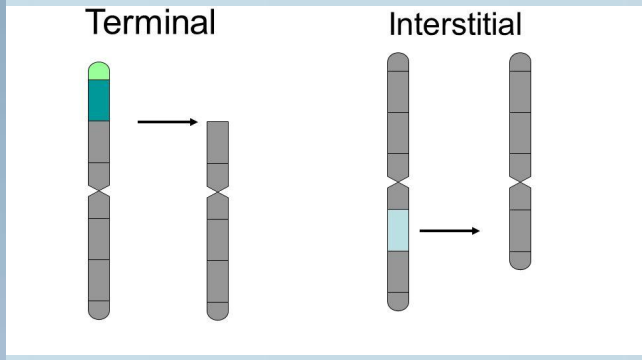


Deletions

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



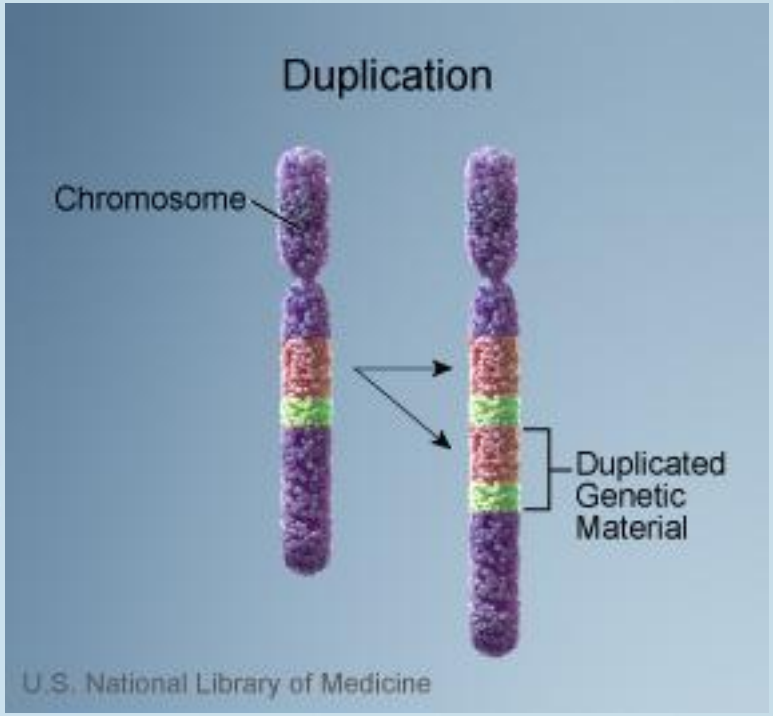


Duplications

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



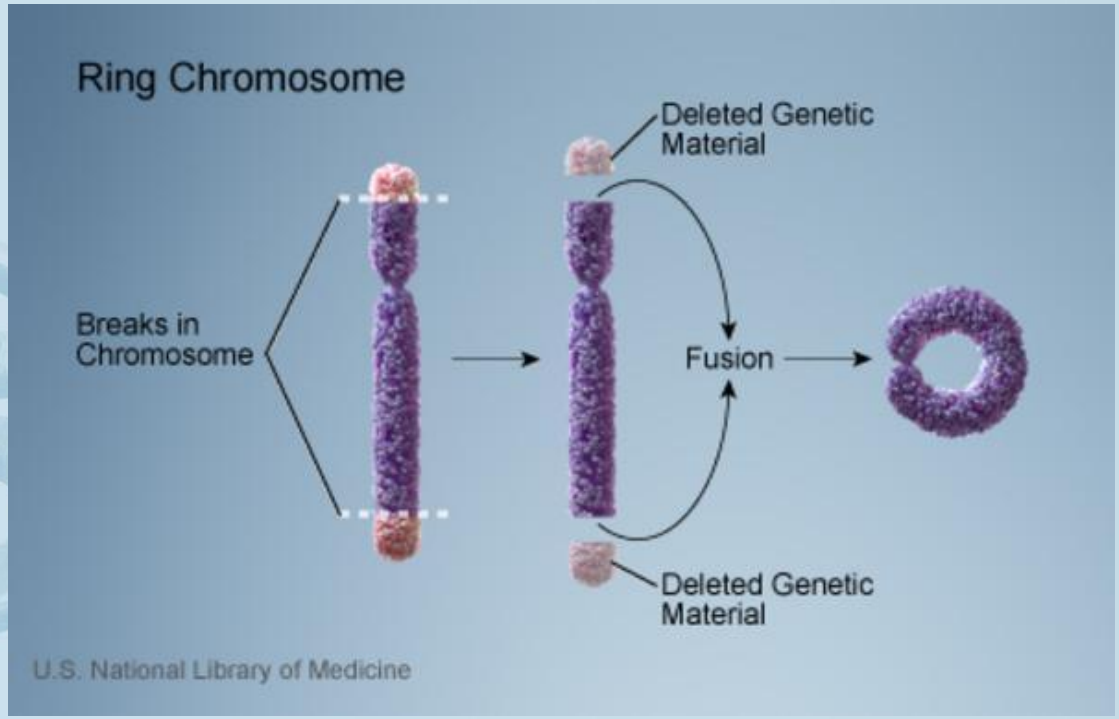
Ring Chromosome

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

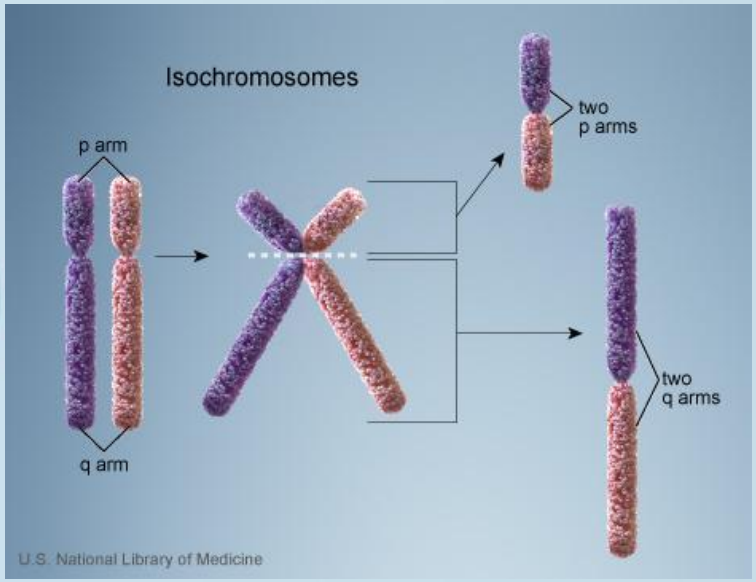
1. **Deletions:** may be terminal or interstitial

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

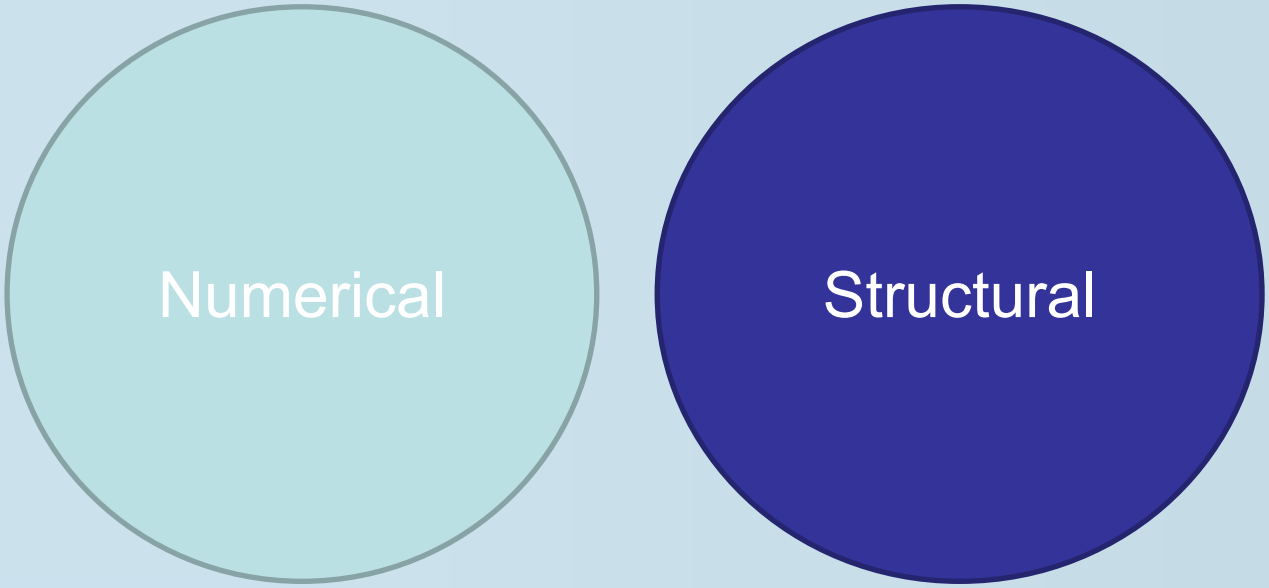
2. **Duplications:** Less harmful than deletions

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



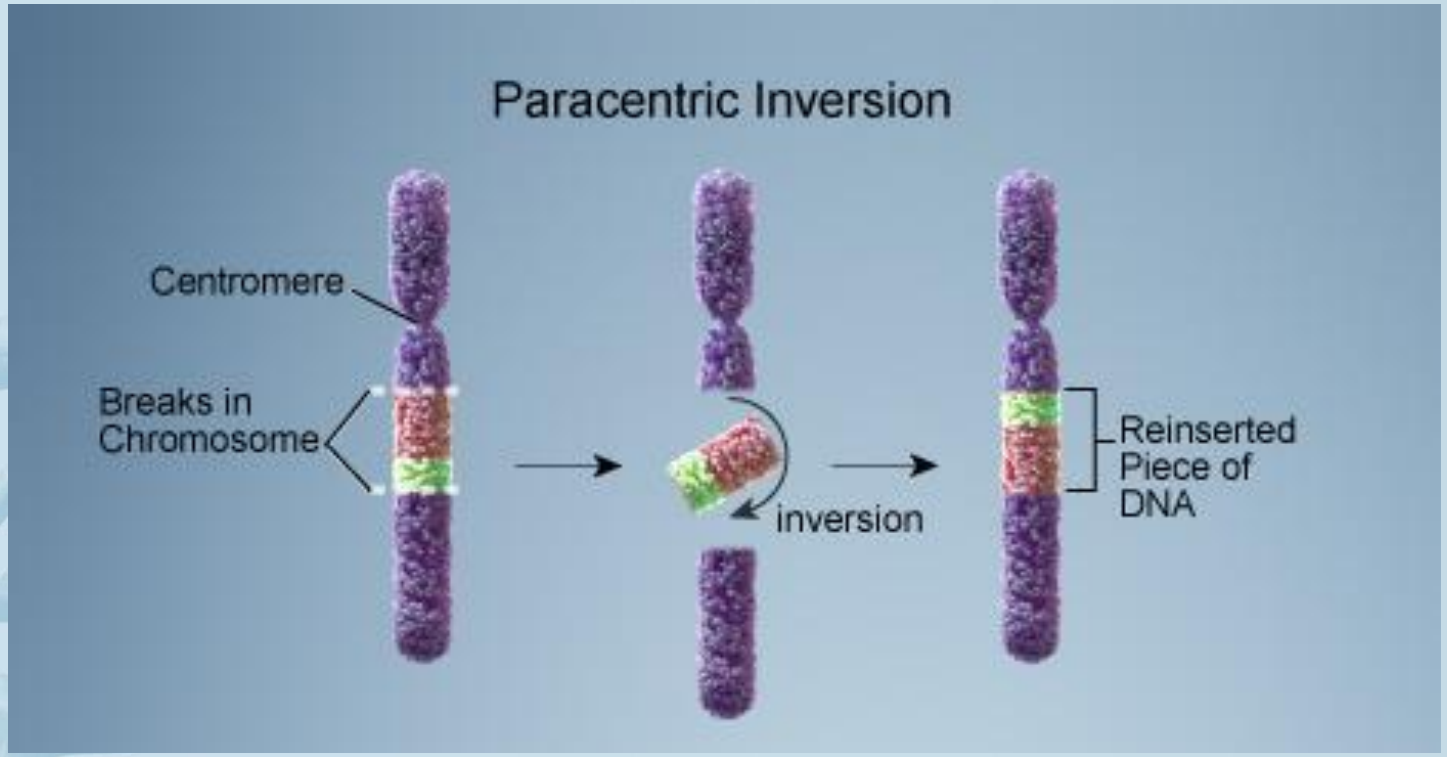
Balanced: normal

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

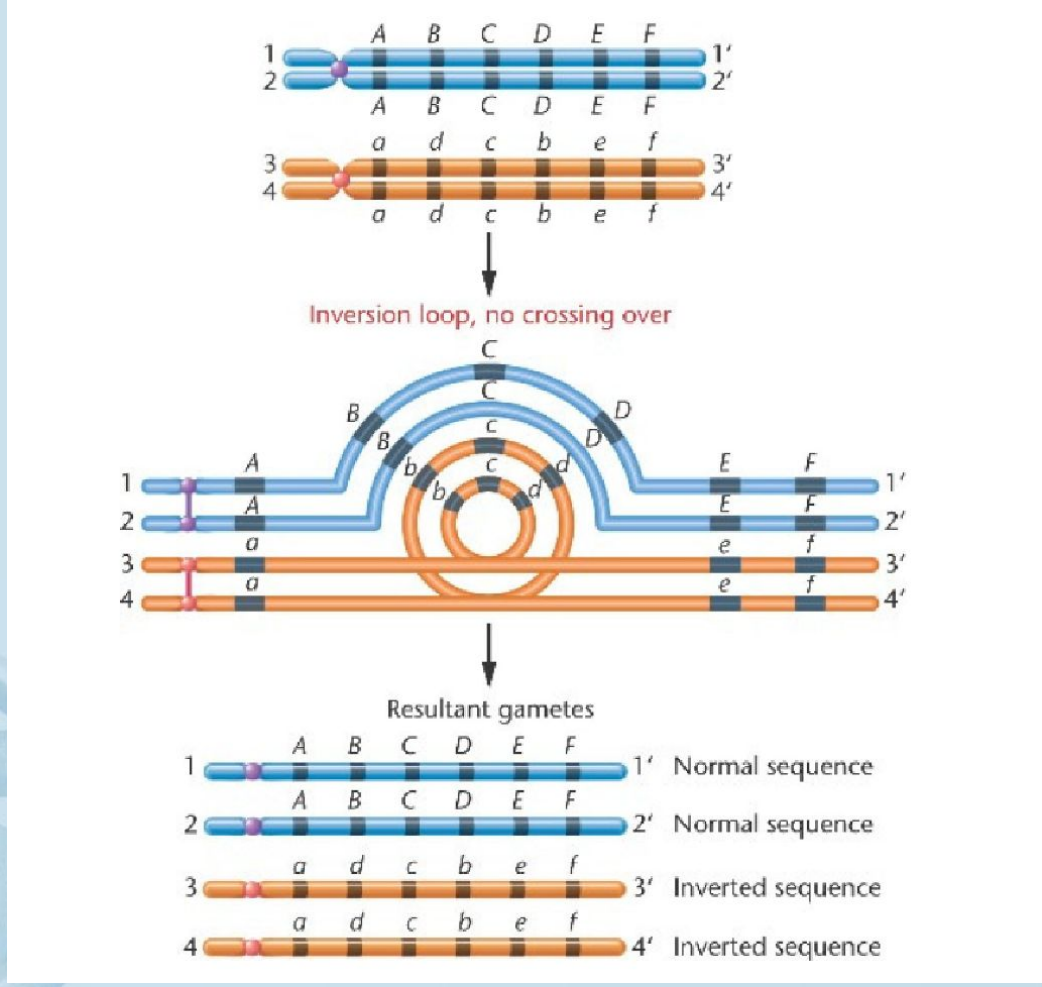
Inversions

1. Inversions: a chromosome sustains <u>two</u> breaks and the segment <u>inverts</u> before rejoining the chromosome.

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



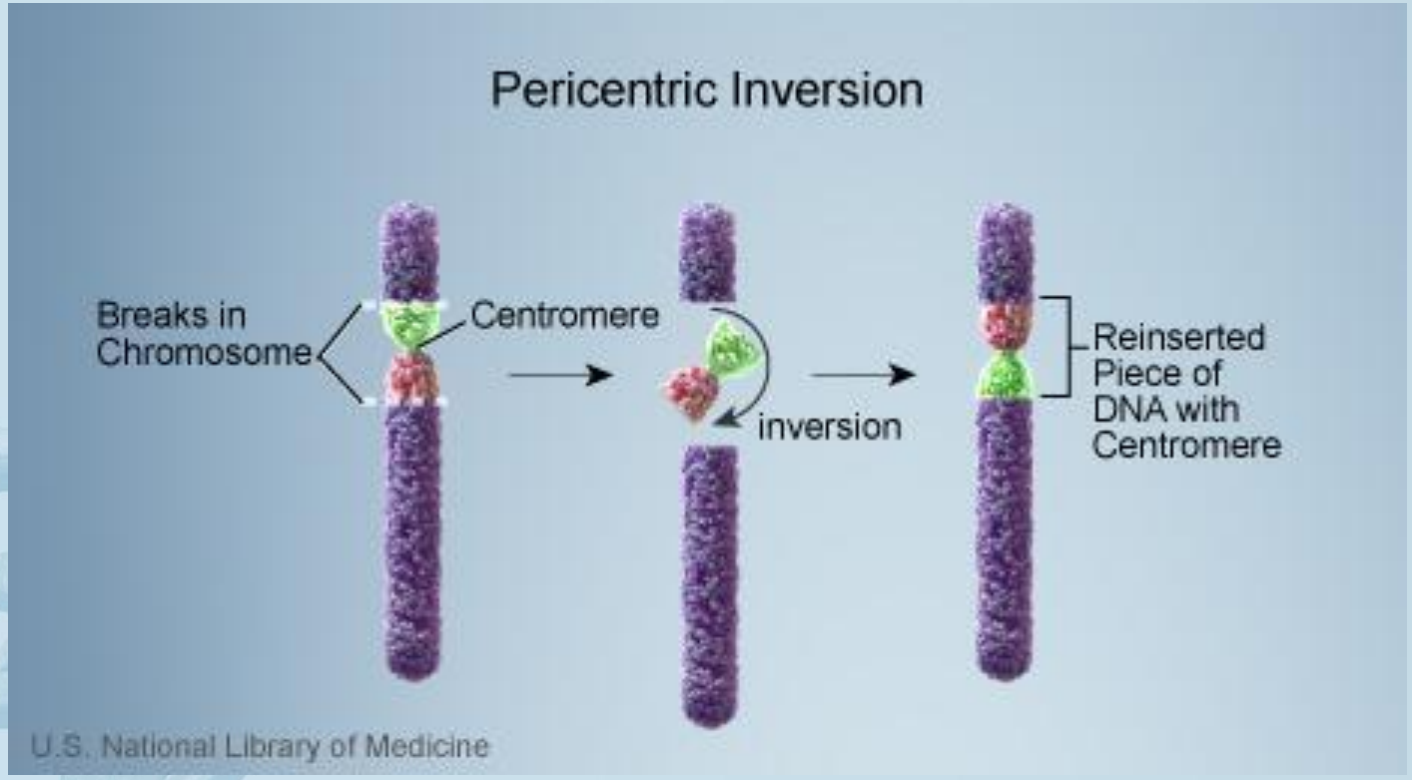
Inversion loop at meiosis



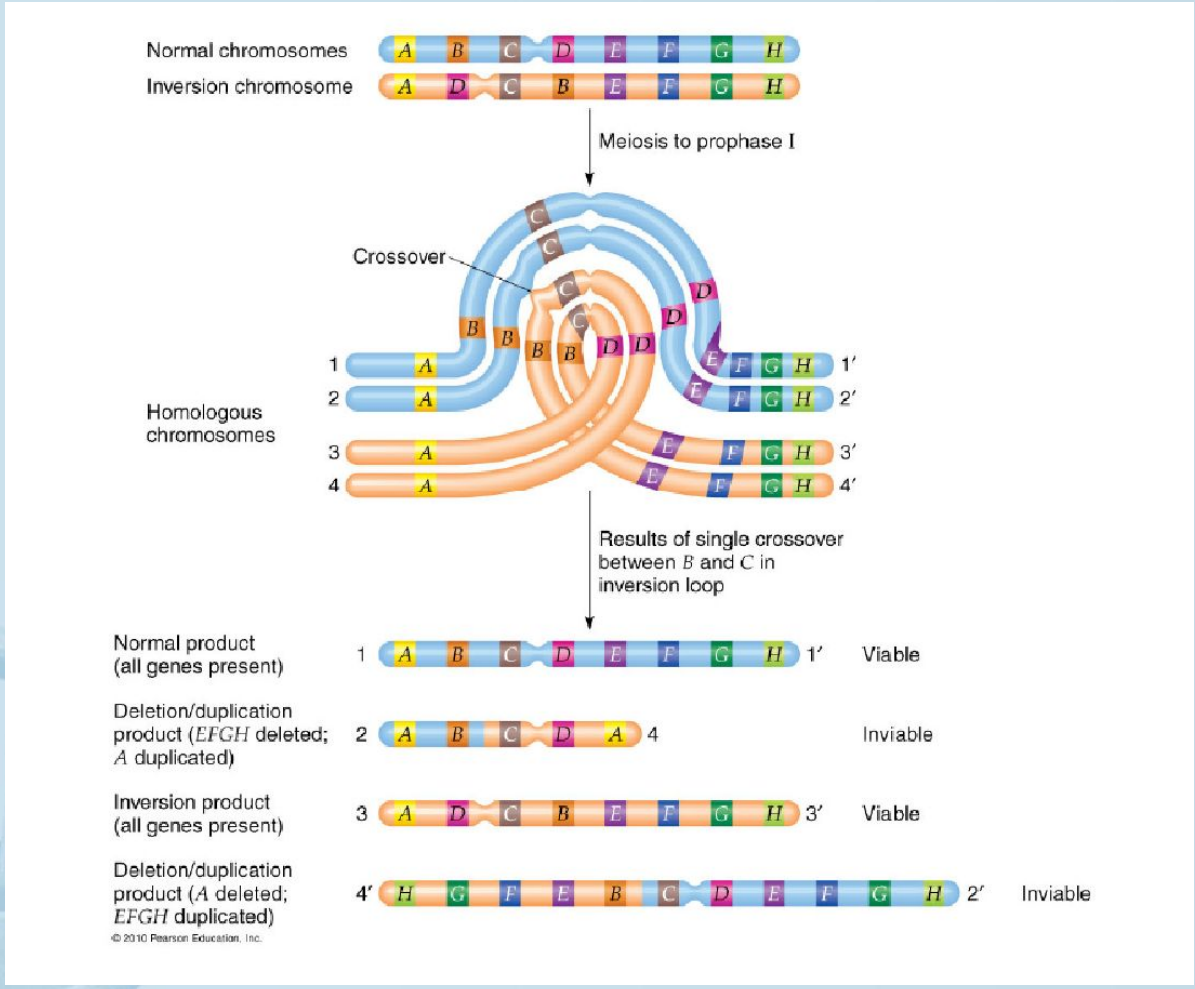
Inversions

1. Inversions: a chromosome sustains <u>two</u> breaks and the segment <u>inverts</u> before rejoining the chromosome.

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



Crossing-over in pericentric inversion loop



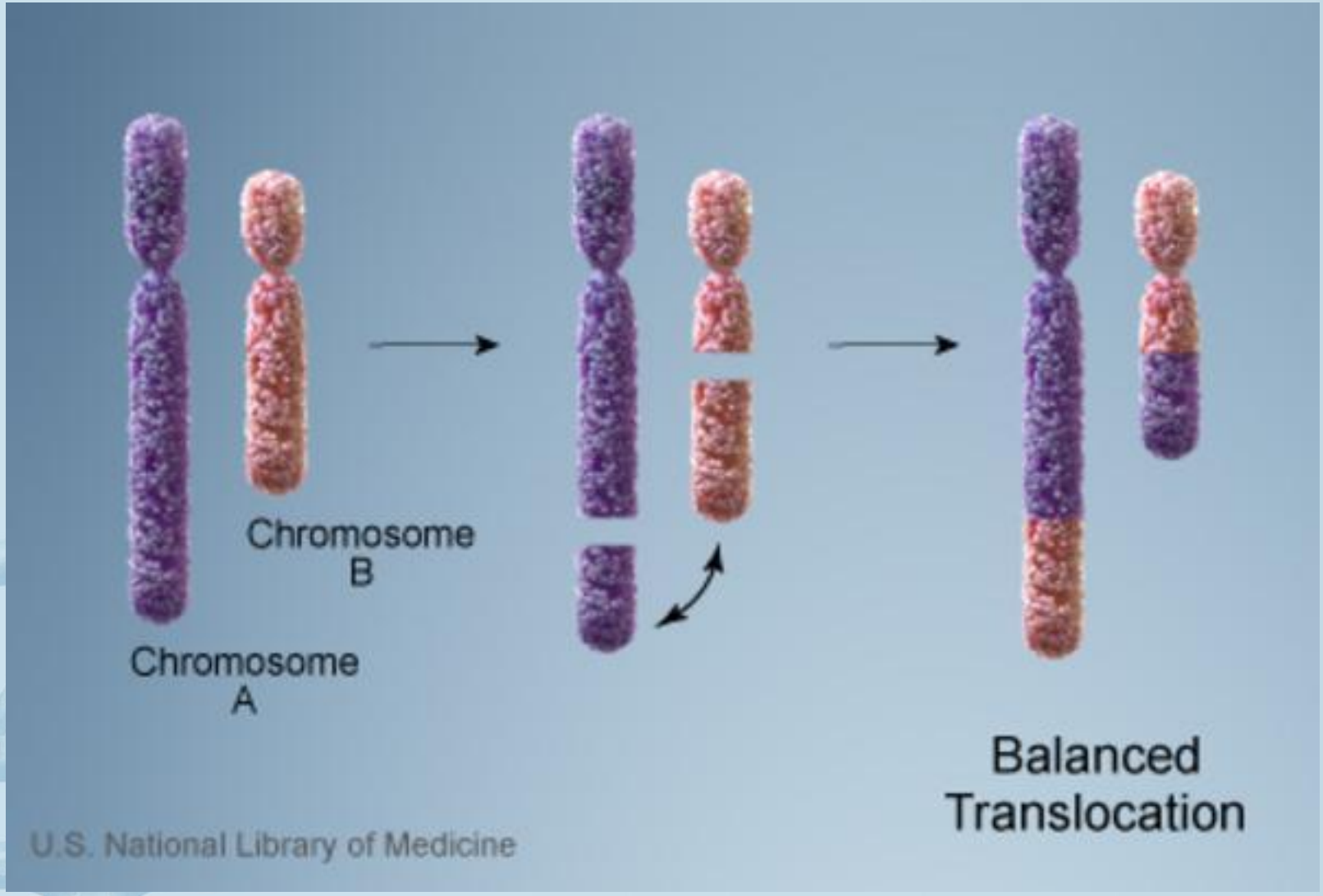
Translocations

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

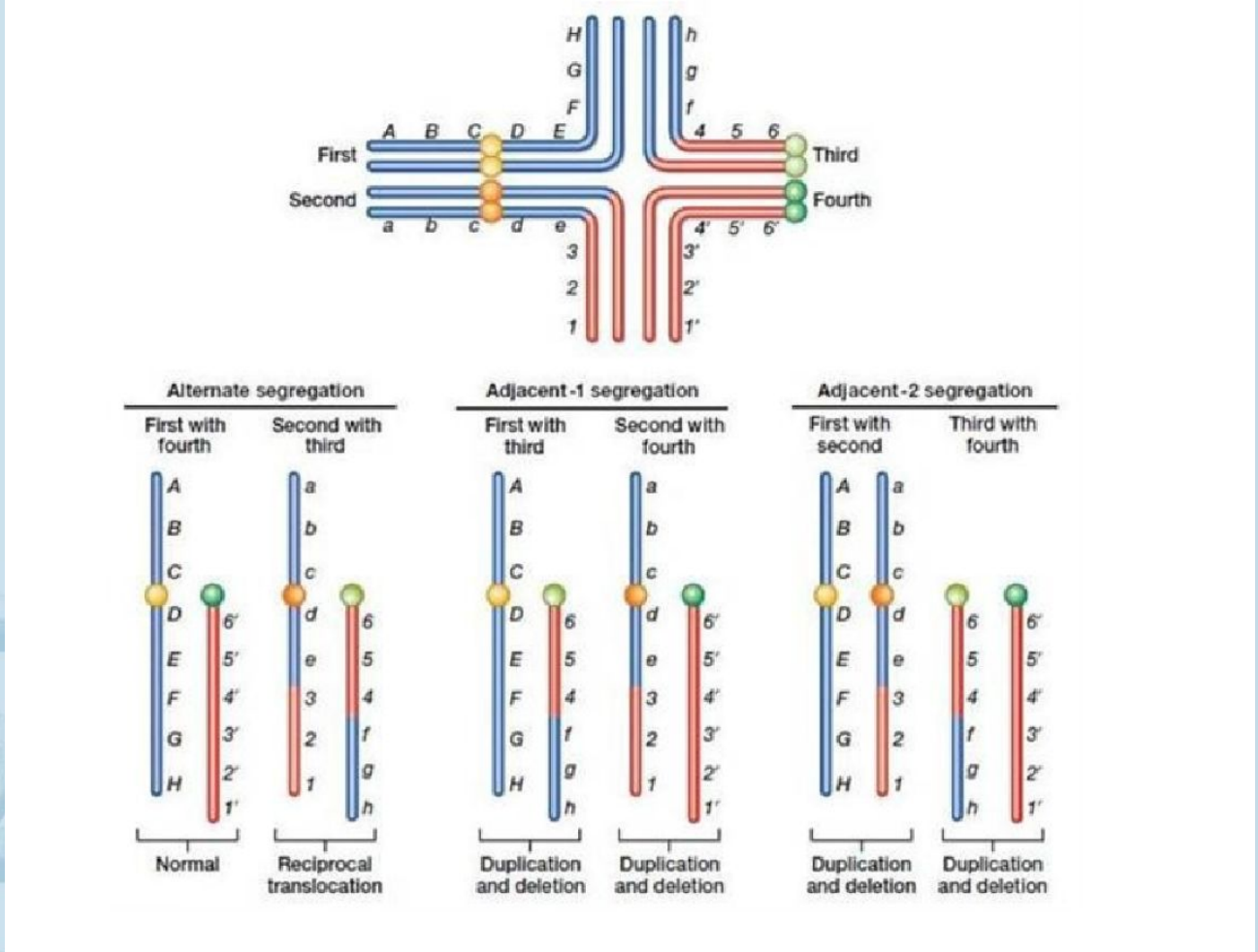
- Reciprocal translocation: reciprocal exchange of the broken-off segments "the total number of chromosomes is unchanged"

- 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.

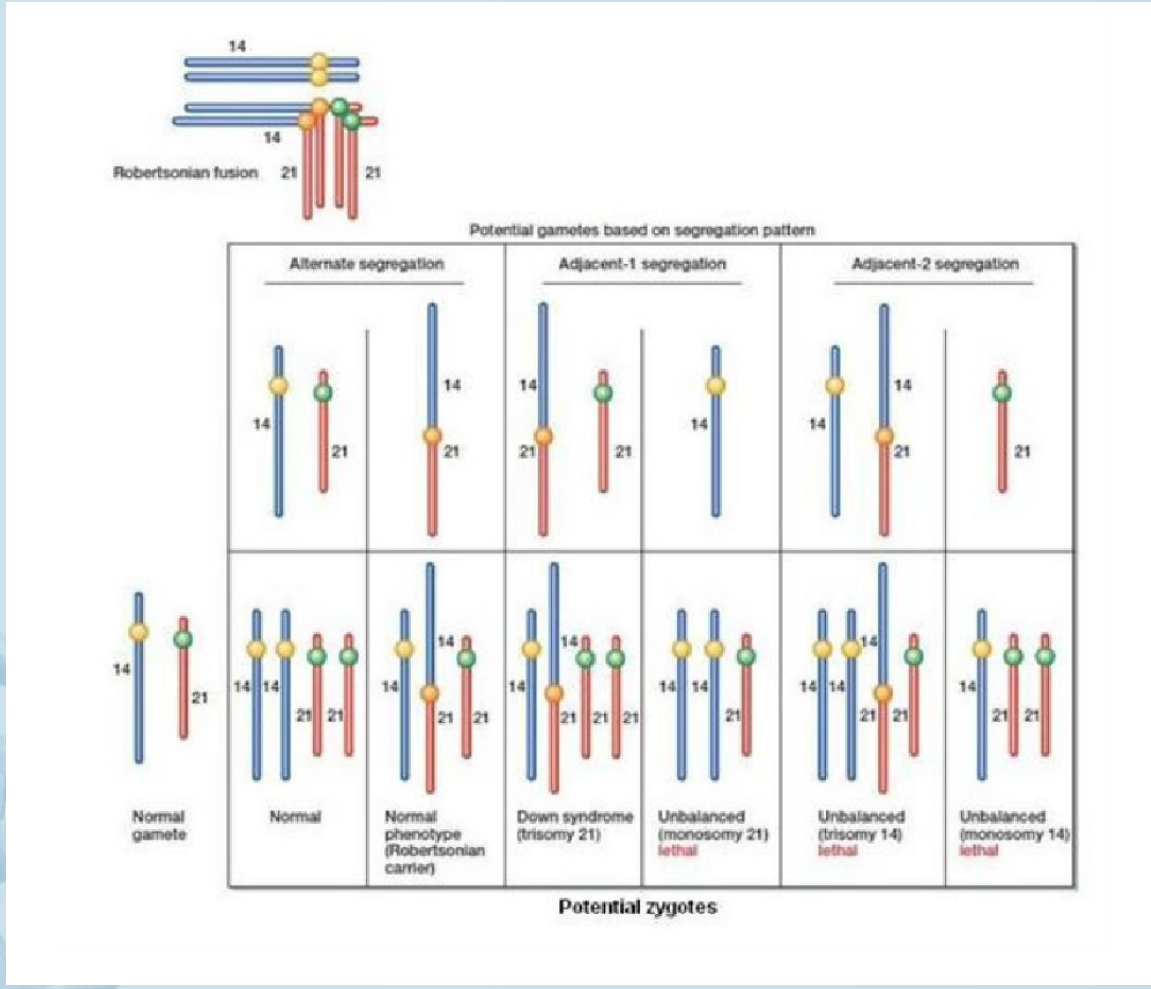
Reciprocal translocations



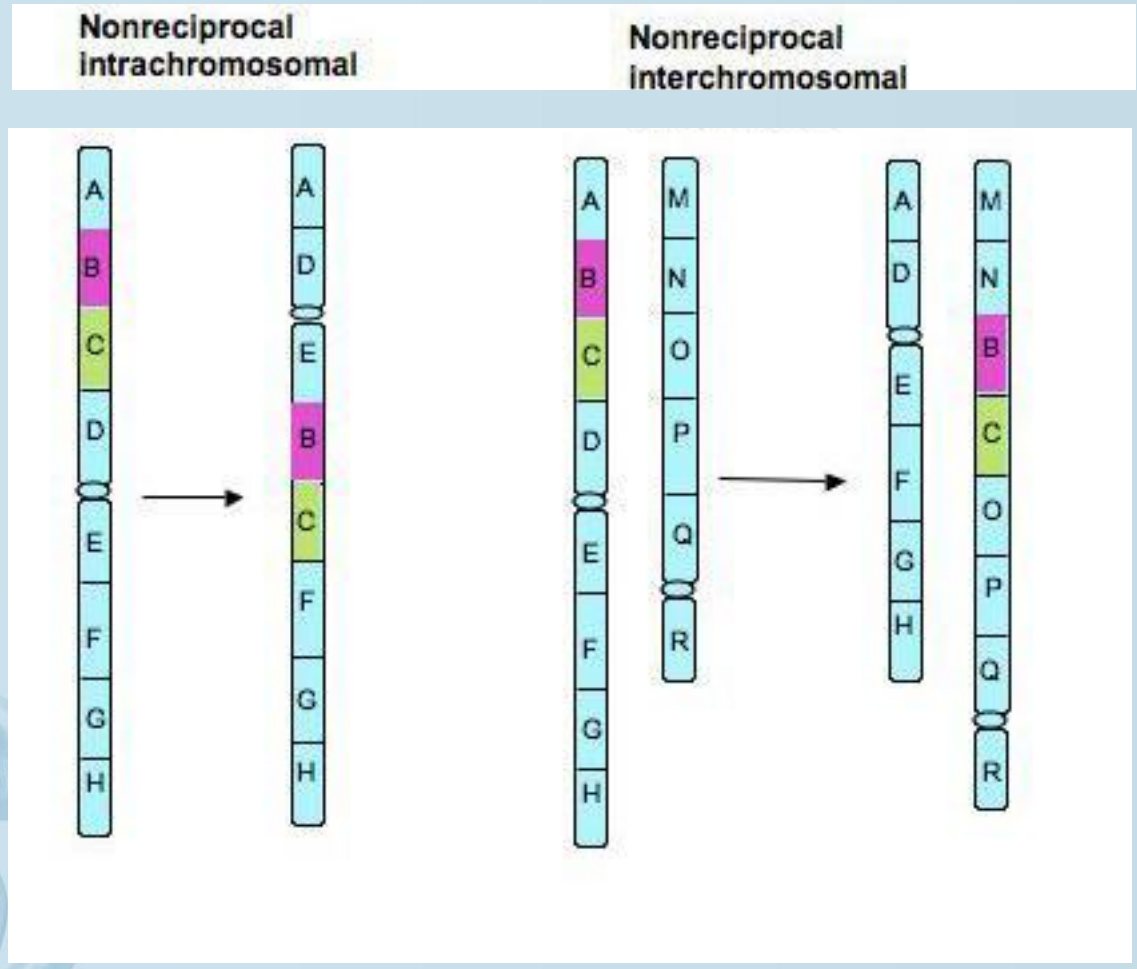
Reciprocal translocatins: quadrivalent



Robertsonian translocations: trivalent



Insertion = non-reciprocal translocation



Overview

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

Techniques to study chromosomes

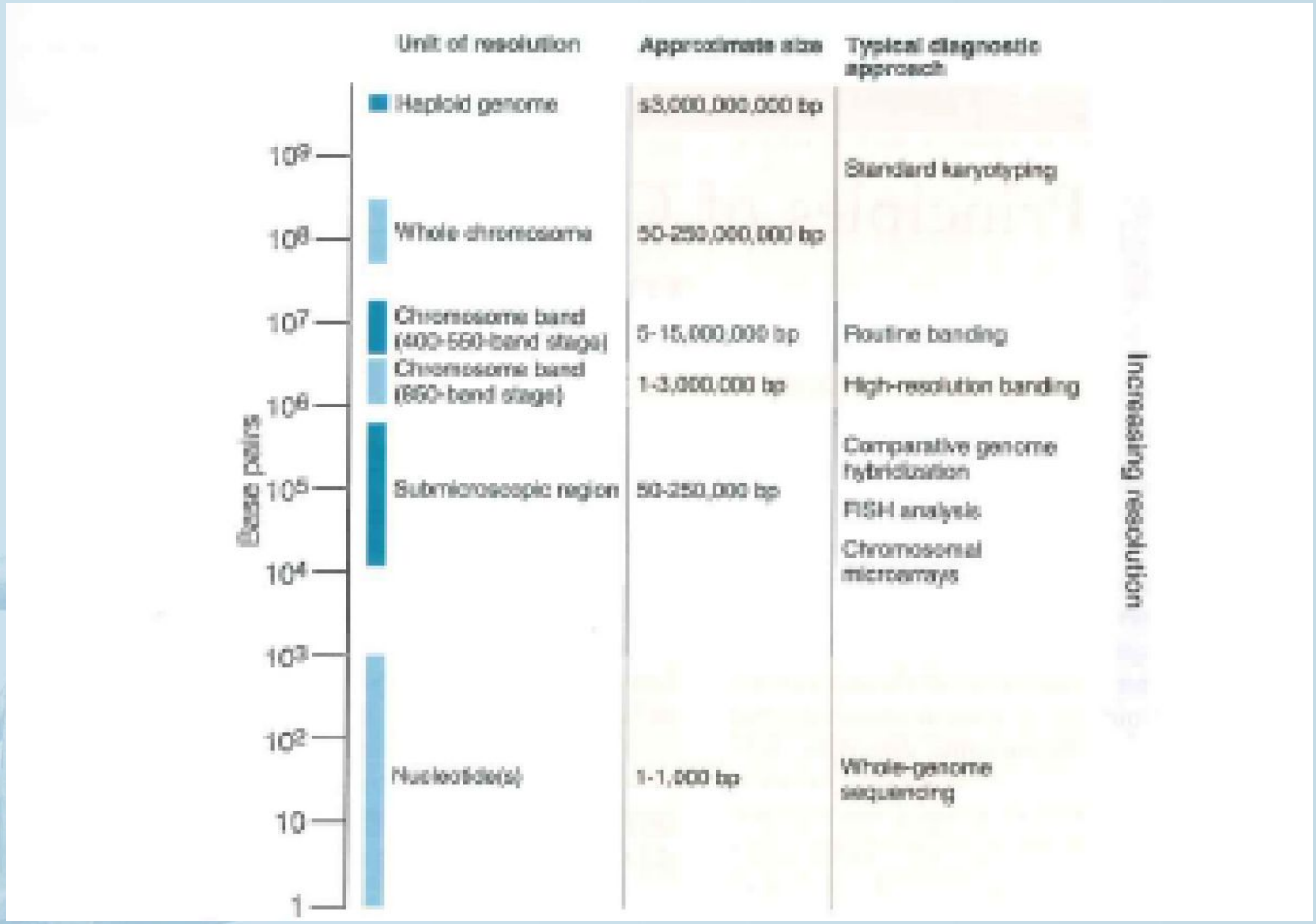
Conventional karyotyping

Fluoresence In-Situ Hybridisation

Molecular karyotyping

Massive parallel sequencing

Spectrum of resolution in chromosome and genome analysis



Techniques to study chromosomes

Conventional karyotyping

Fluoresence In-Situ Hybridisation

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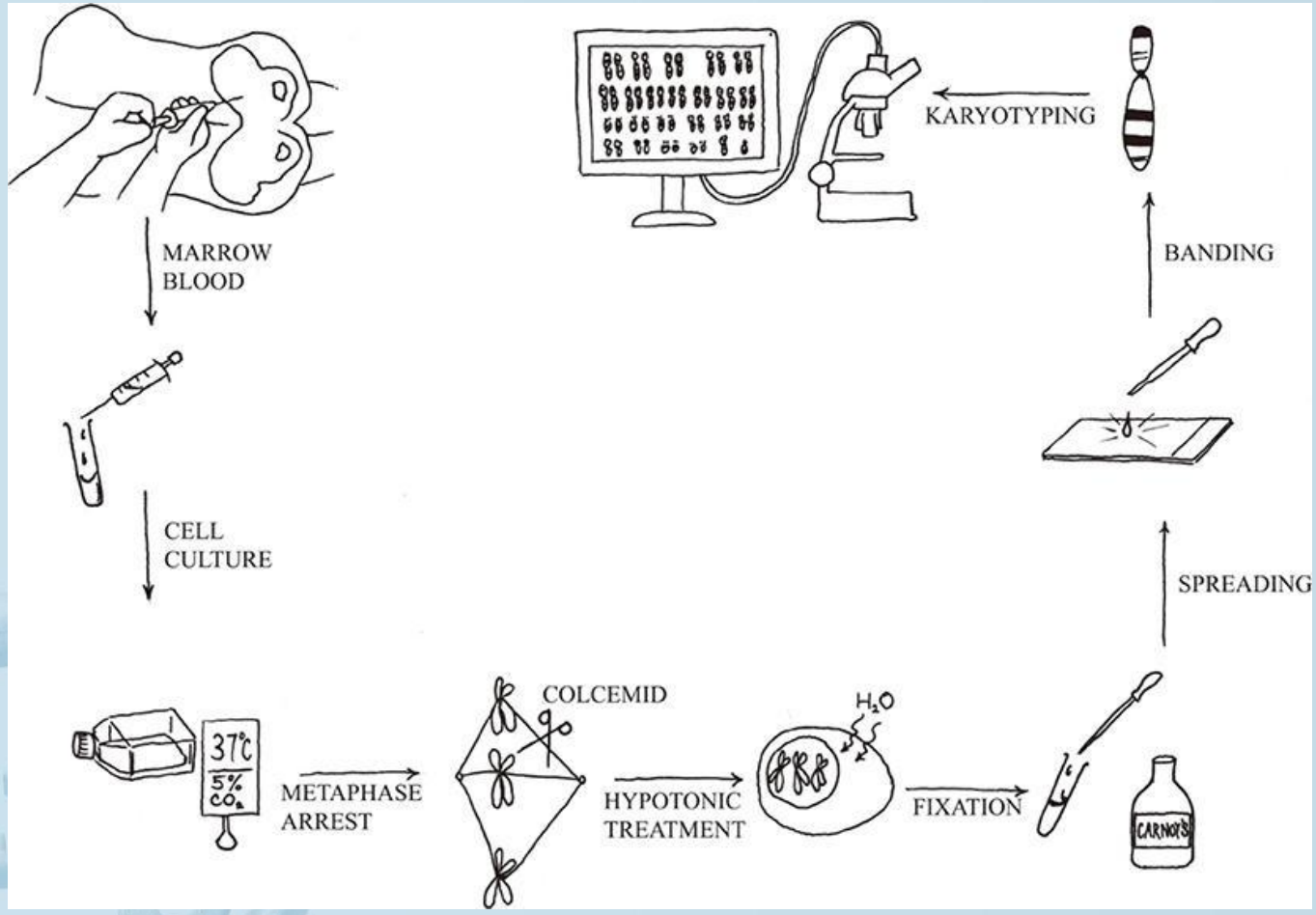
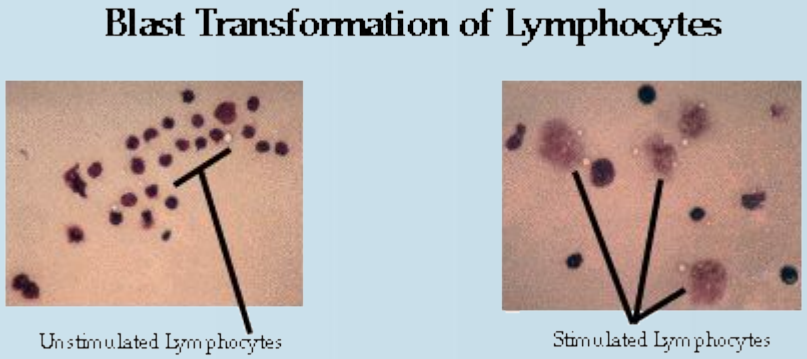
Massive parallel sequencing

Conventional karyotyping



PHA stimulation



- 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

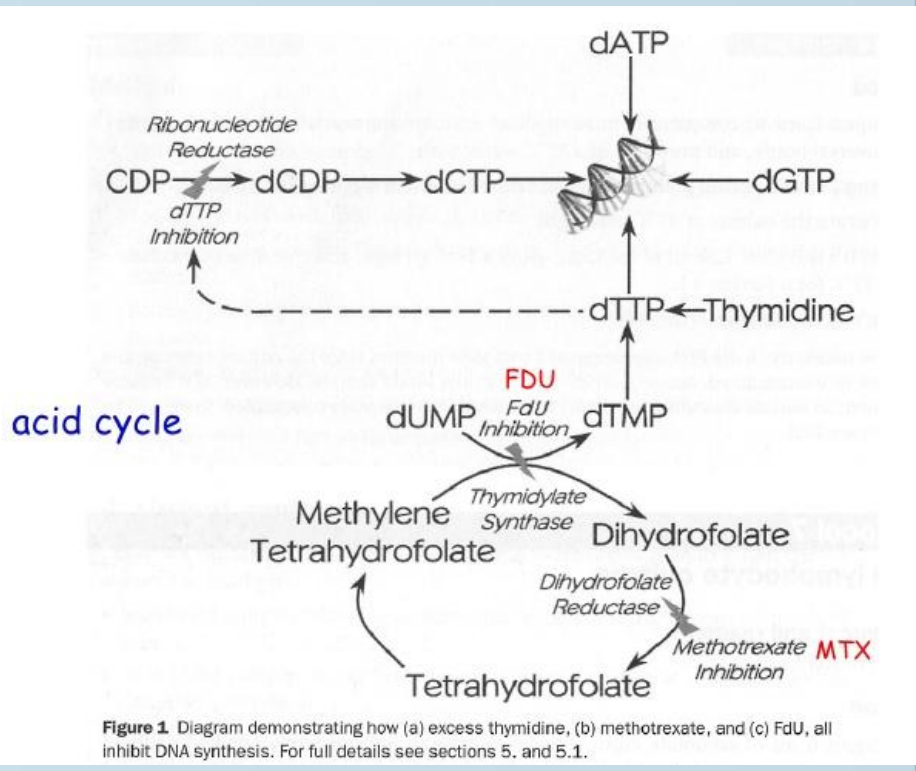
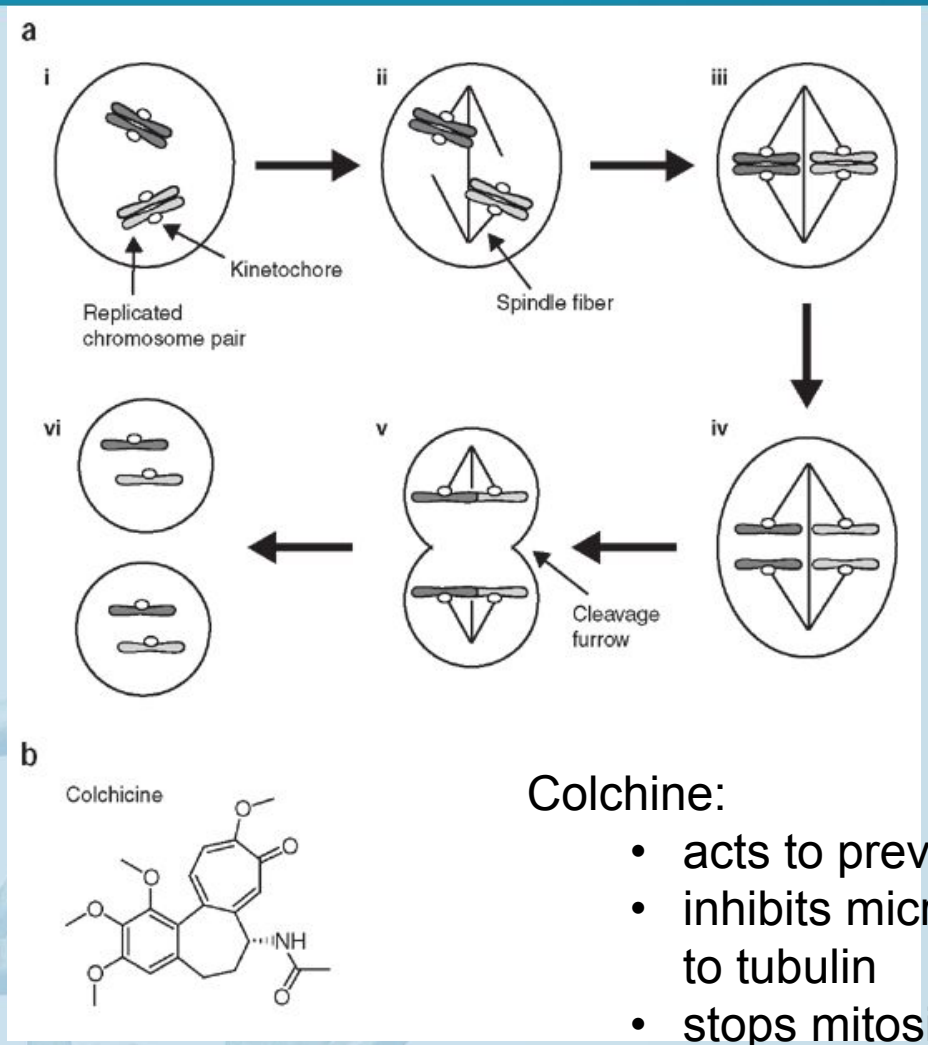


Figure 1 Diagram demonstrating how (a) excess thymidine, (b) methotrexate, and (c) FdU, all inhibit DNA synthesis. For full details see sections 5. and 5.1.

Colchicine

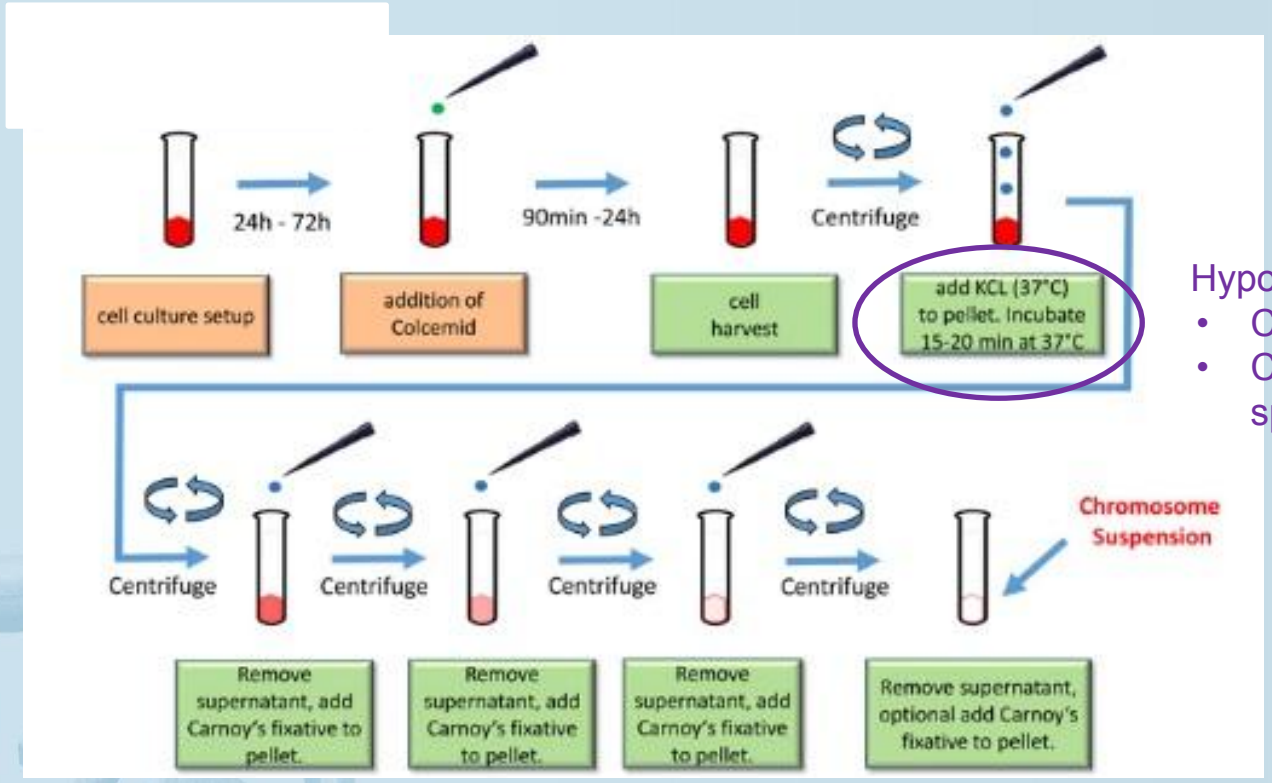


MITOSIS

Colchine:

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

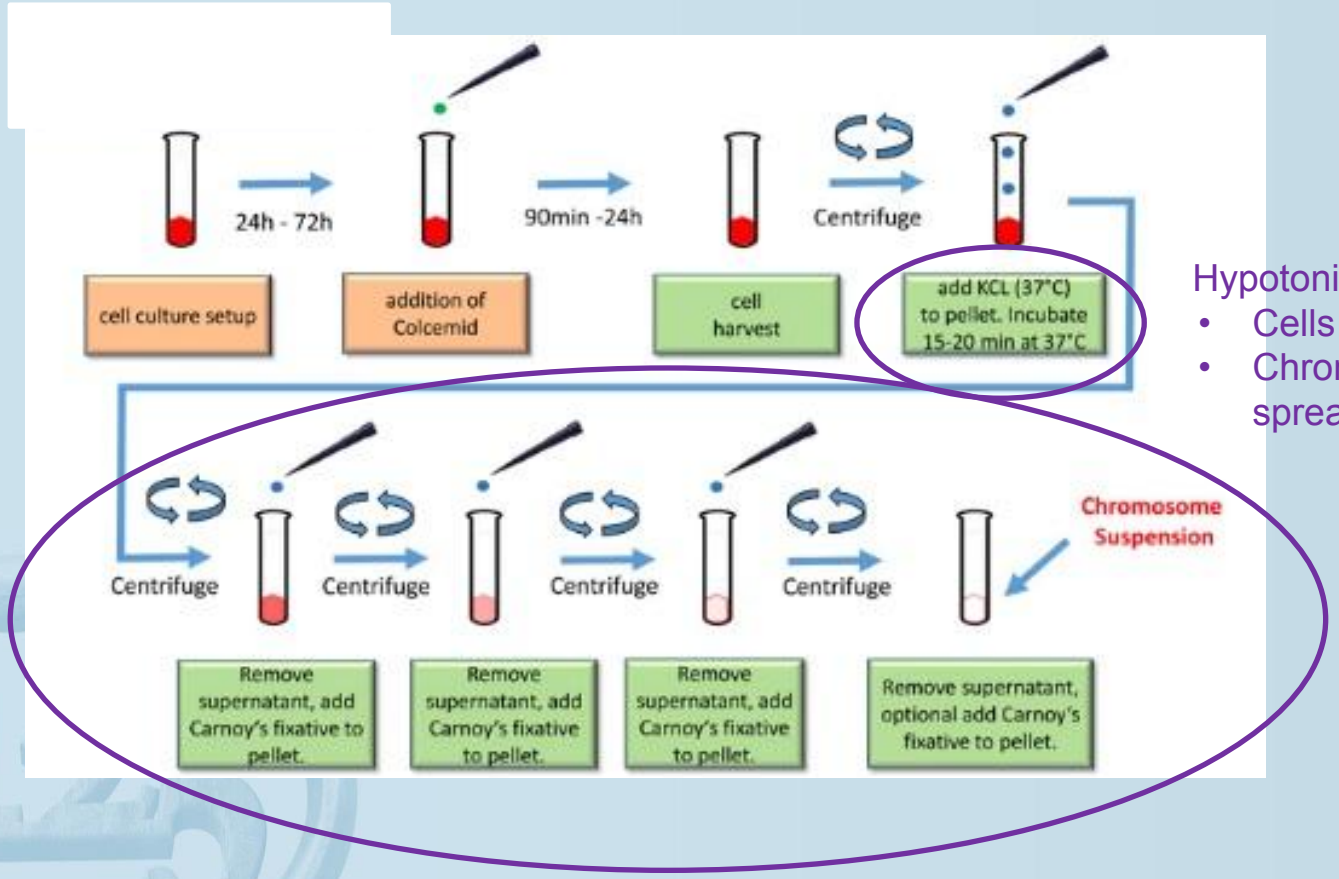
Harvesting



Hypotonic solution:

- Cells swell
- Chromosomes spread

Harvesting



Hypotonic solution:

- Cells swell
- Chromosomes spread

Fixation = Methanol : Acetic Acid 3:1

Chromosome spreads

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

Chromosome banding

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.

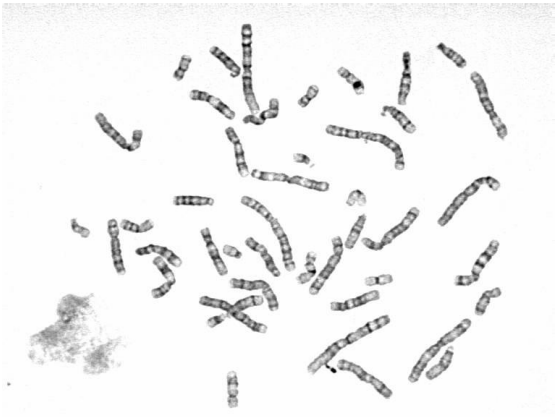
C-Banding:

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

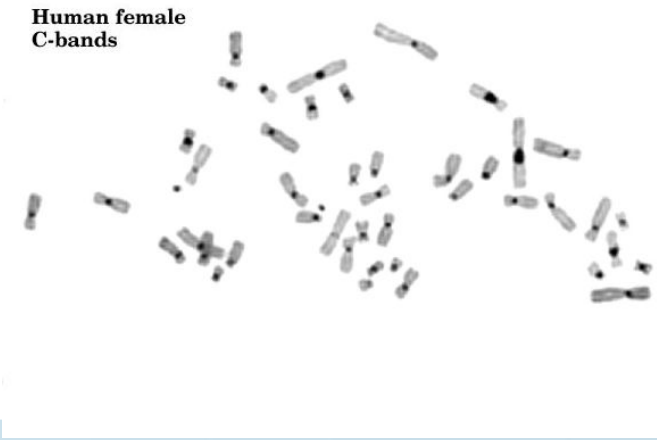
Chromosome banding

DIFFERENT TYPES OF BANDING

G–Banding:



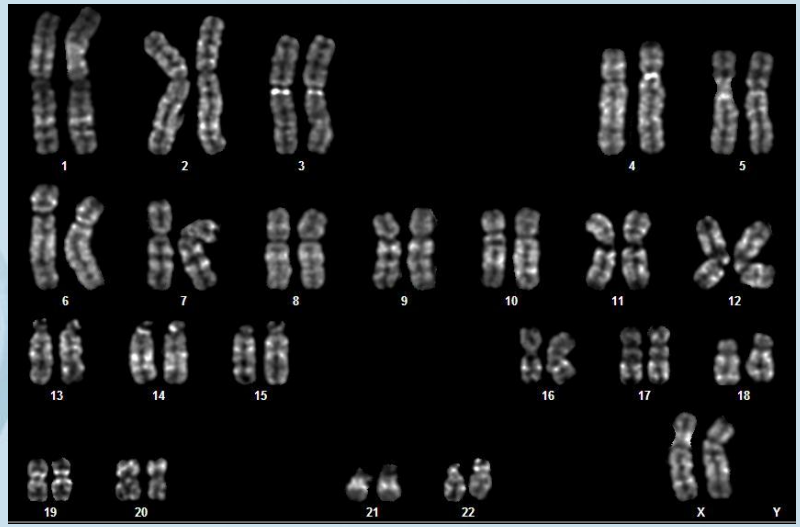
C-Banding:



Chromosome banding

Q-Banding

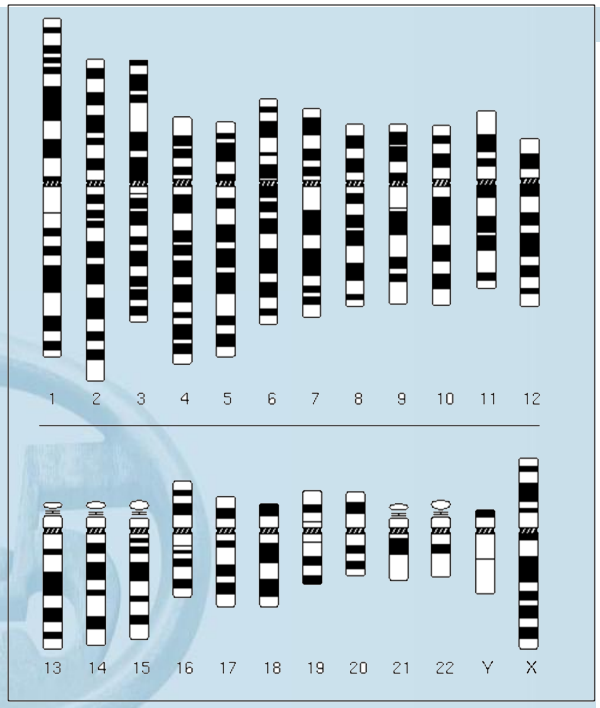
- Quinacrine mustard (a fluorescent stain), an alkylating agent, was the first chemical to be used for chromosome banding.
- Quinacrine bright bands were composed primarily of DNA rich in bases adenine and thymine.

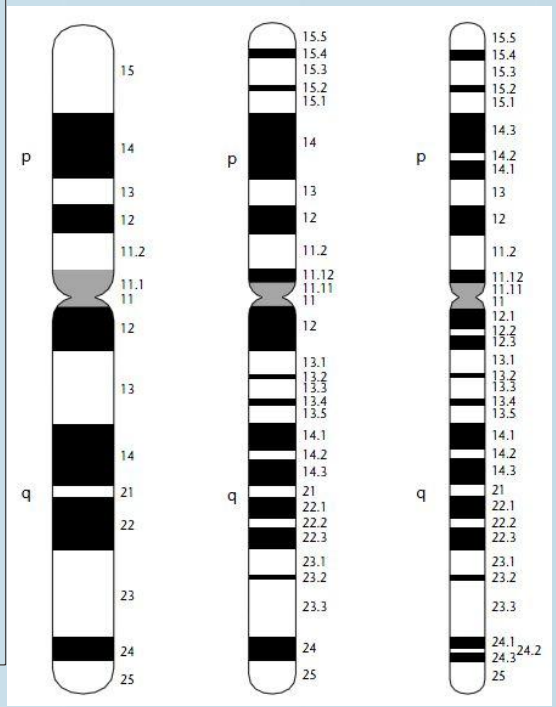


Chromosome banding

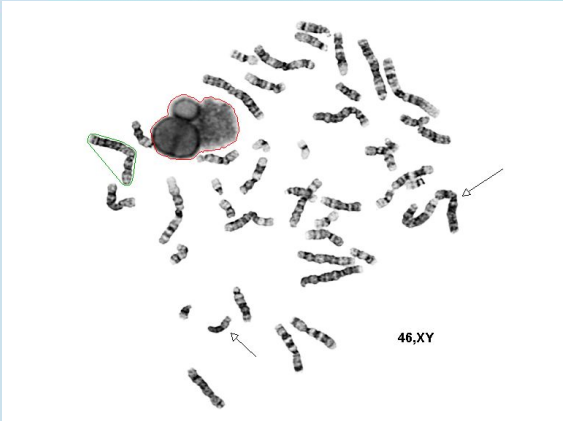
Used to identify

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

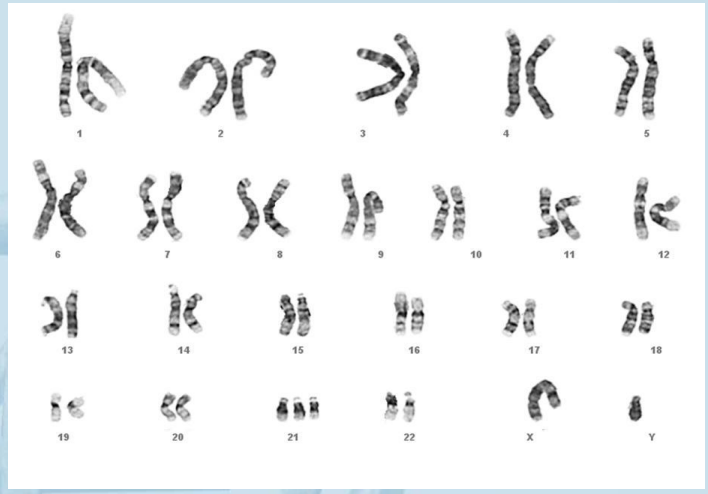




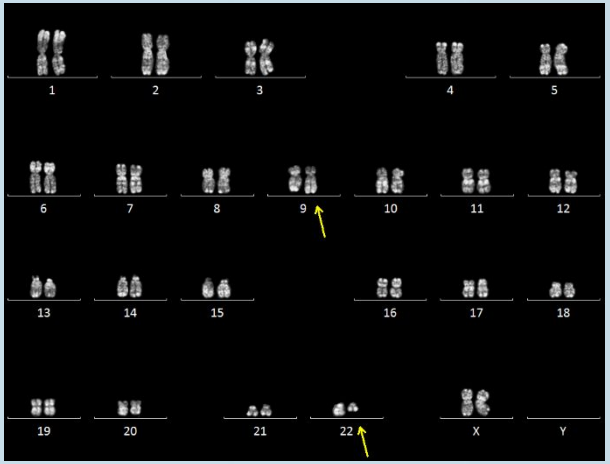
Microscopic imaging



Karyotyping

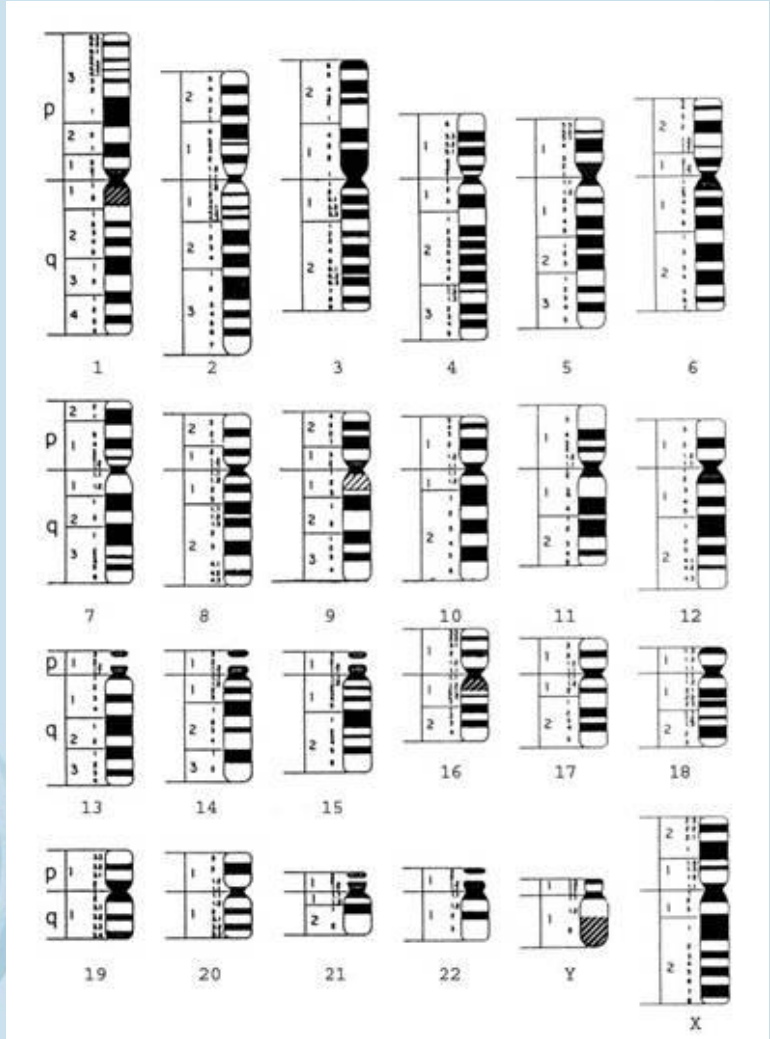


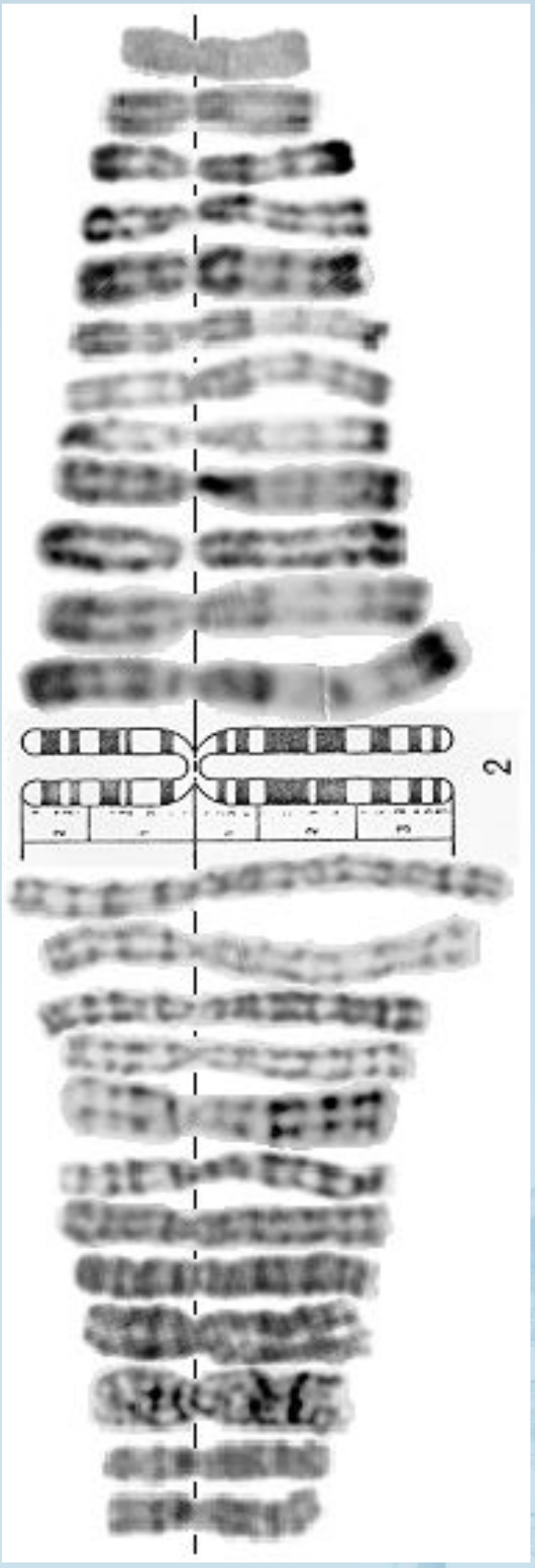
47,XY,+21



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

ISCN: International standards cytogenetic nomenclature




2

STRUCTURAL CHROMOSOMAL ANOMALIES: ISCN

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

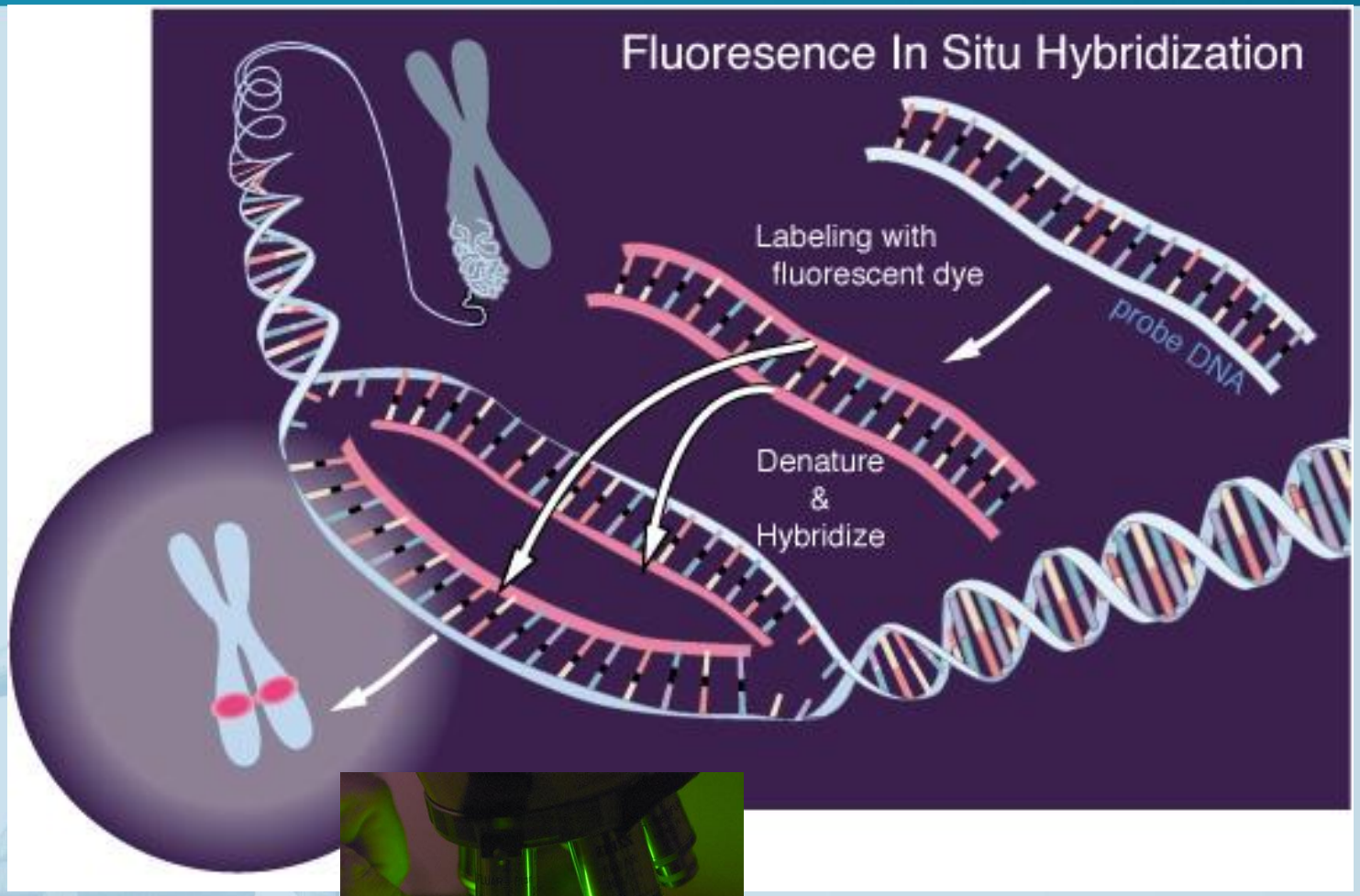
Conventional karyotyping

Fluoresence In-Situ Hybridisation

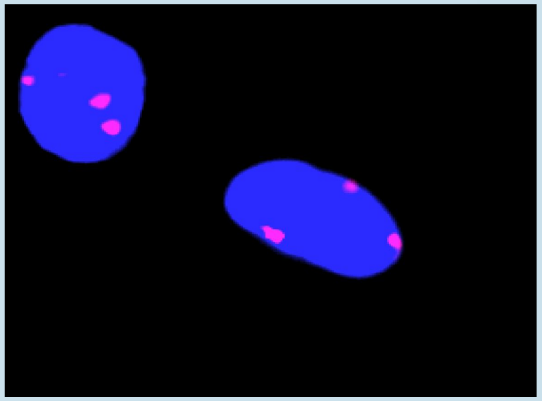
Molecular karyotyping

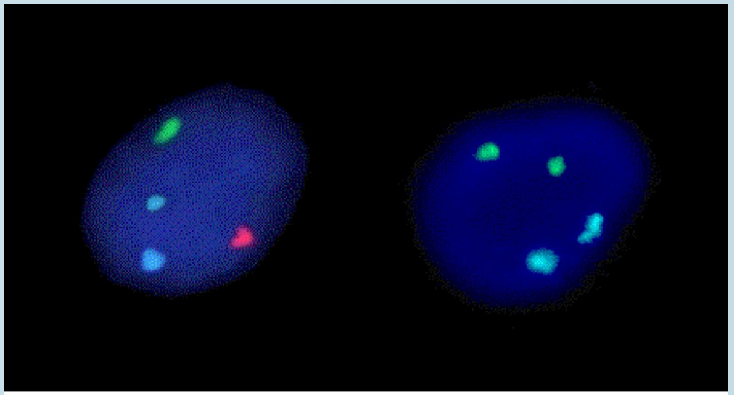
Massive parallel sequencing

Fluorescence In Situ Hybridisation



Interphase FISH

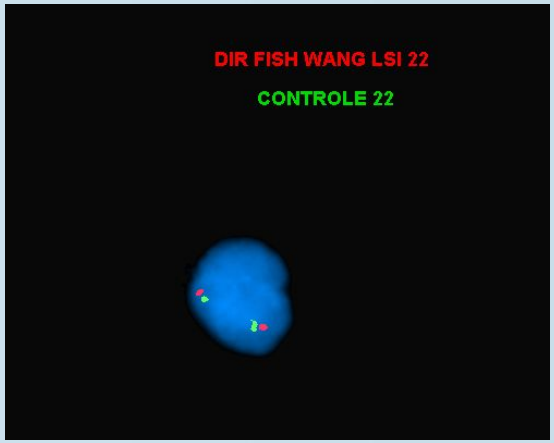




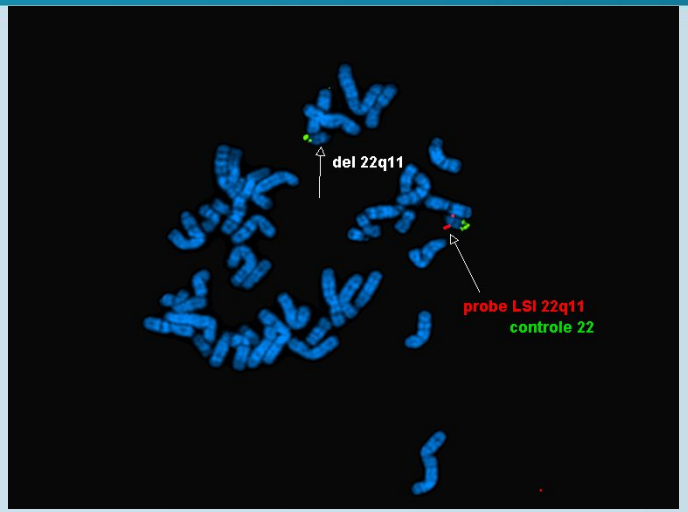
= chromosoom 21

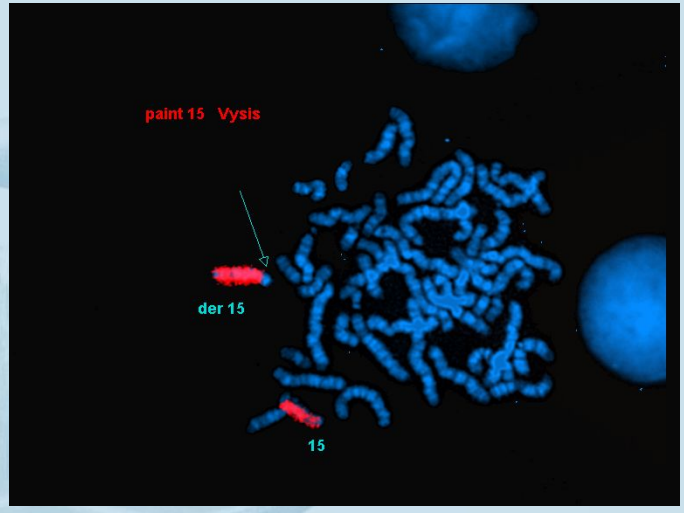
= X chromosoom

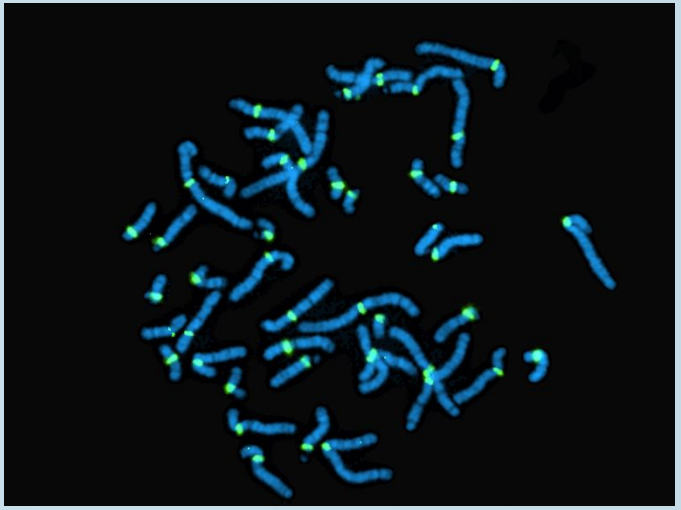
= Y chromosoom



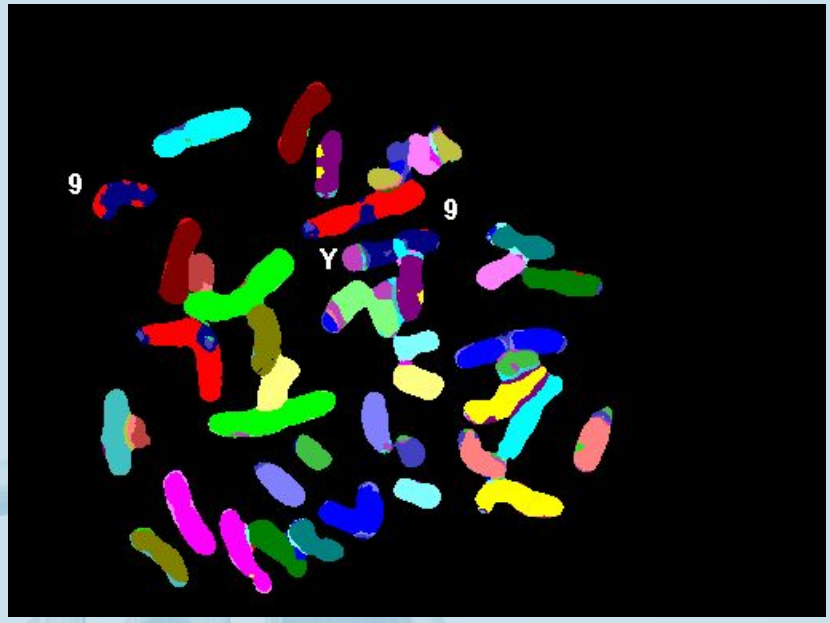
Metaphase FISH

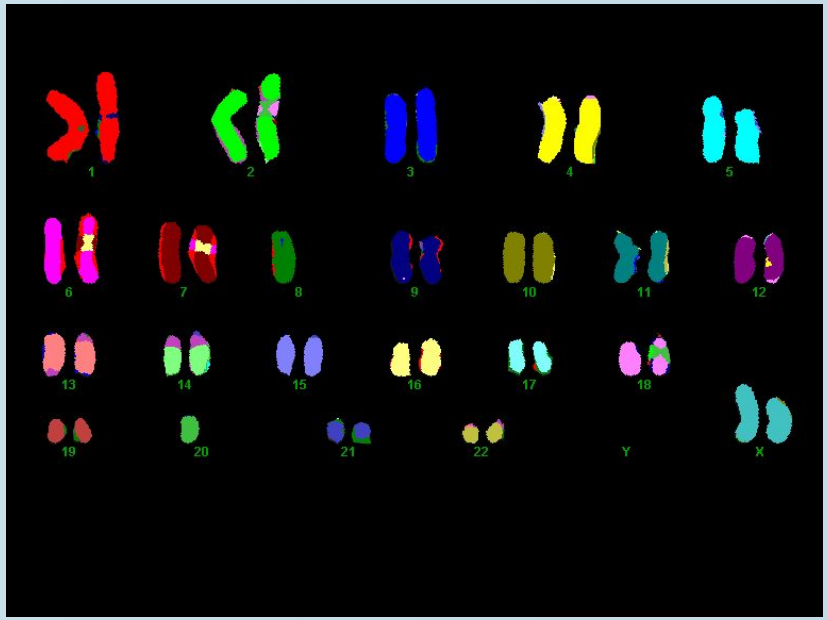






Multicolor FISH

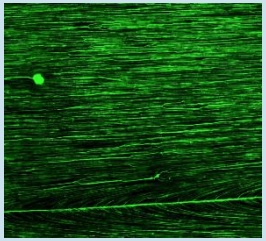


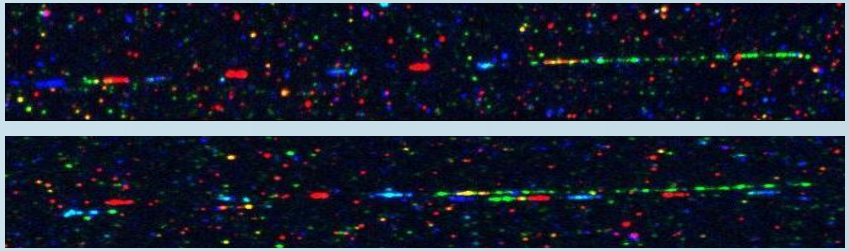


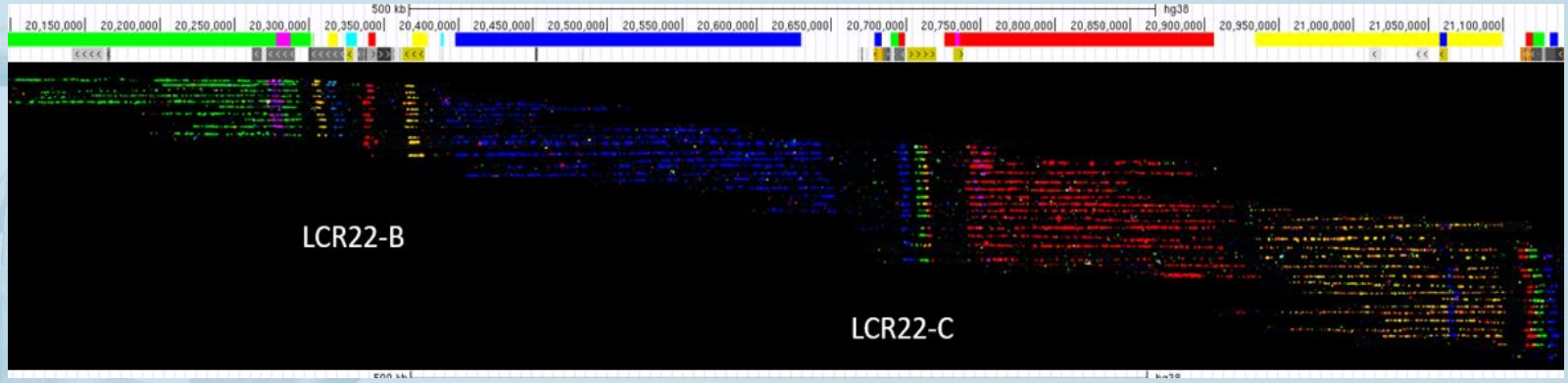
Fiber FISH

Fiber FISH mapping

- DNA is released from chromatin and stretched on slides.
- DNA probes will hybridize like arrays of dots ("beads on a string")

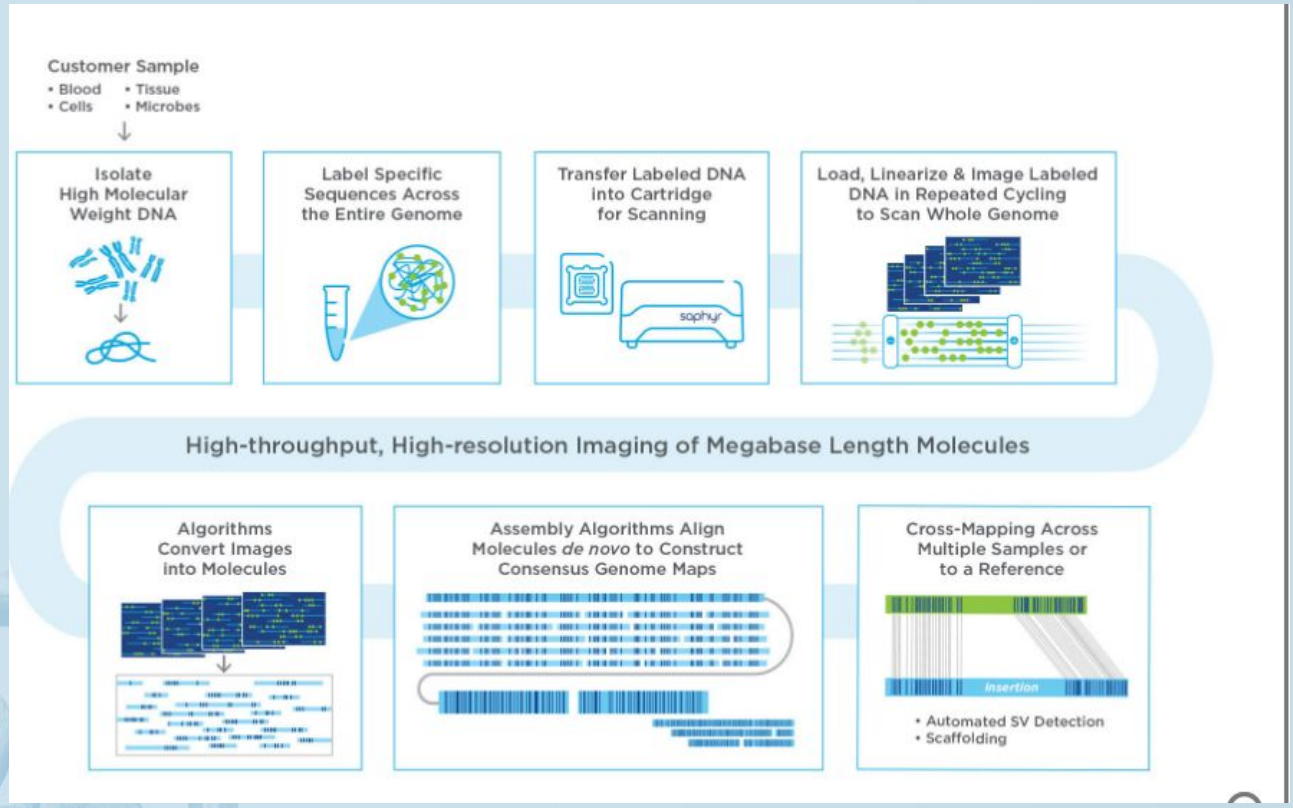






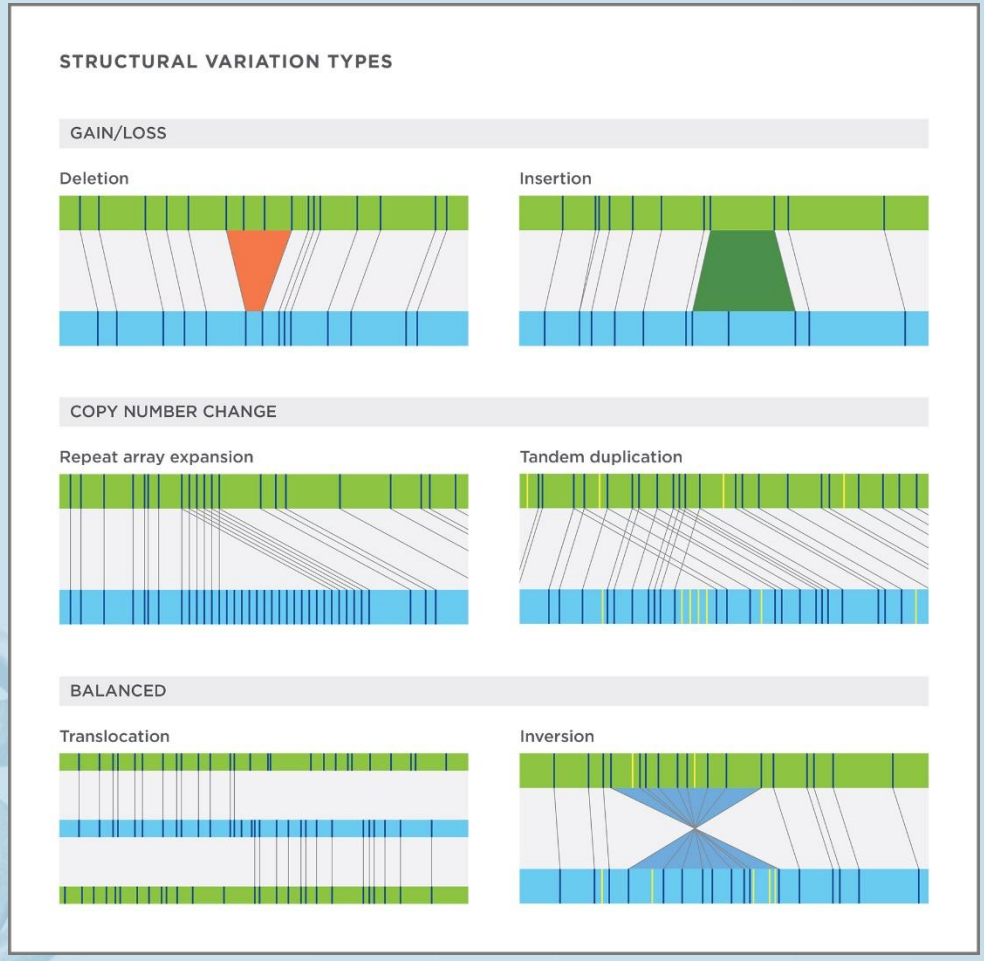
Demaerel et al., Genome Research, 2019

Optical mapping
Bionano mapping



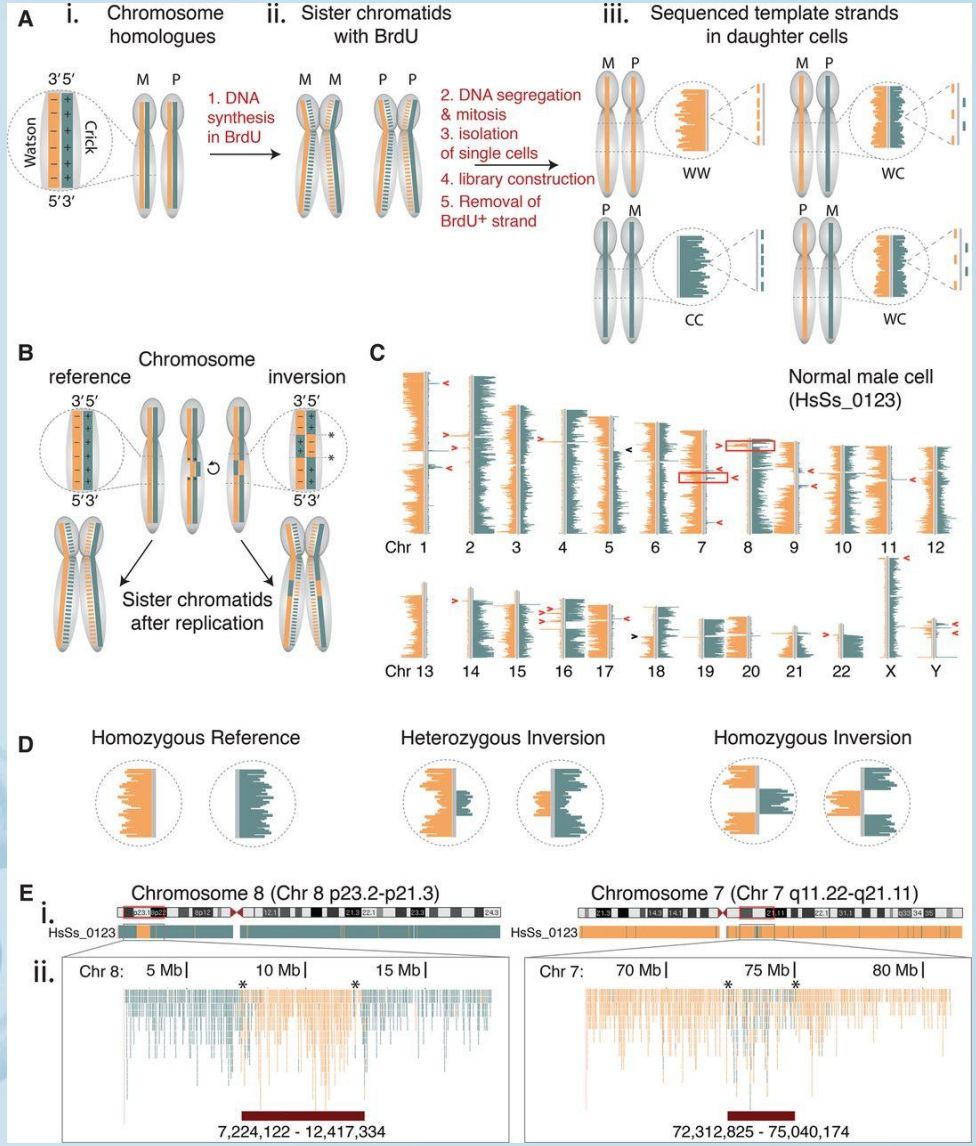
https://www.youtube.com/watch?v=S2ng6glu04I

Structural variation types



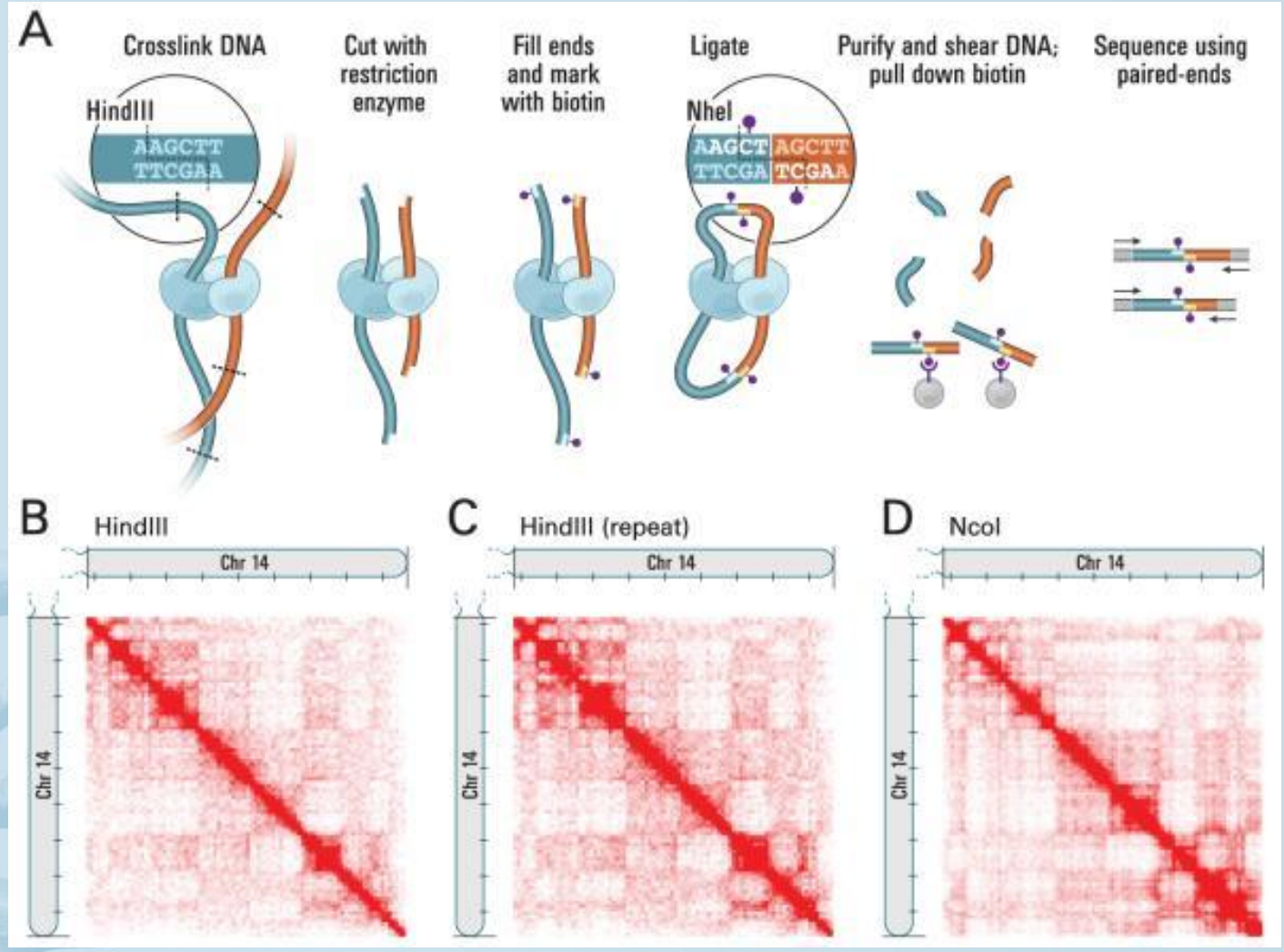
Strand-Seq

(inversions, larger SVs)



Chromosome conformation capture

(changes in TADs structure can identify SVs)



Lieberman-Aiden et al., 2009, Science

Techniques to study chromosomes

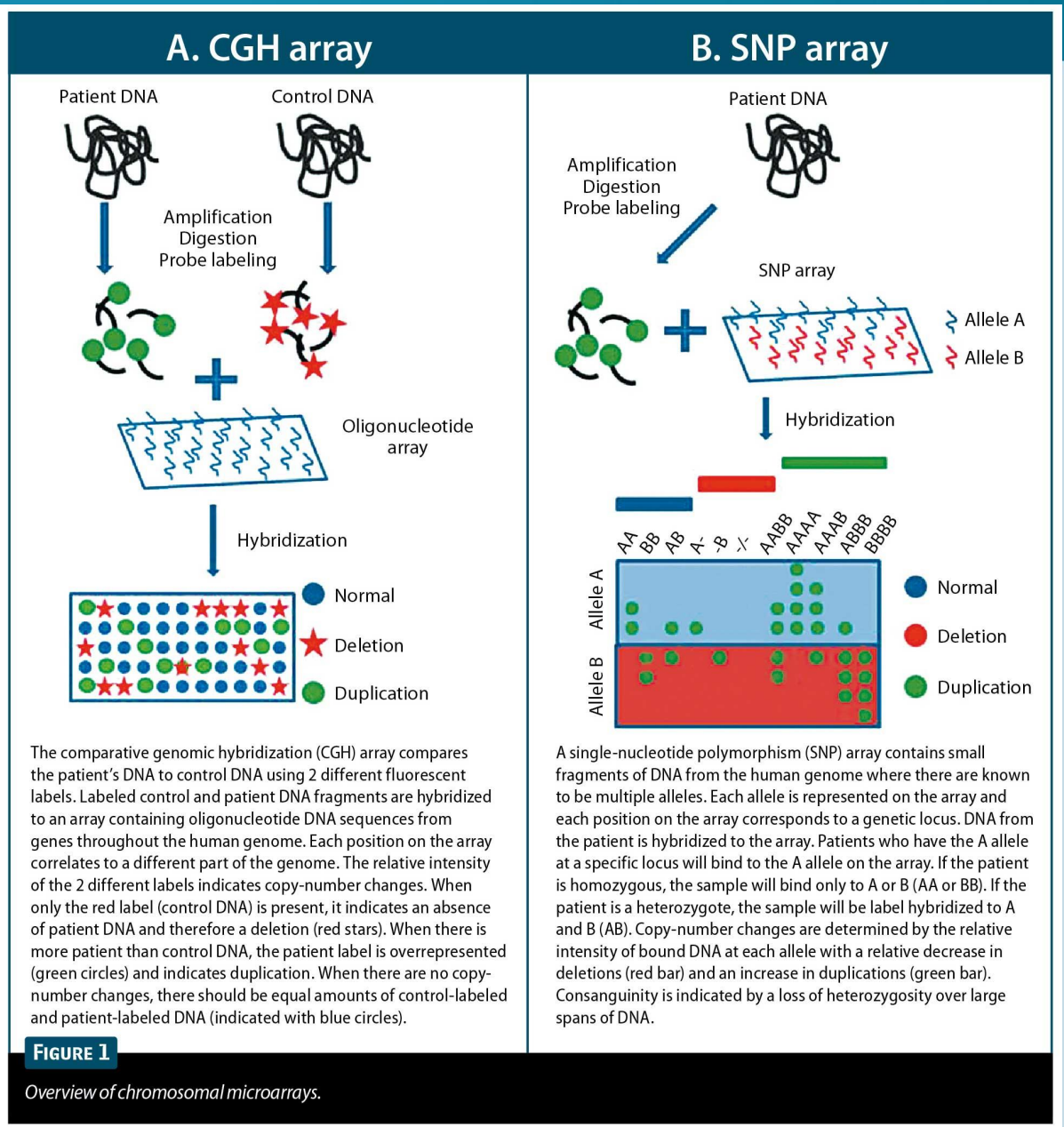
Conventional karyotyping

Fluoresence In-Situ Hybridisation

Molecular karyotyping

Massive parallel sequencing

Microarrays



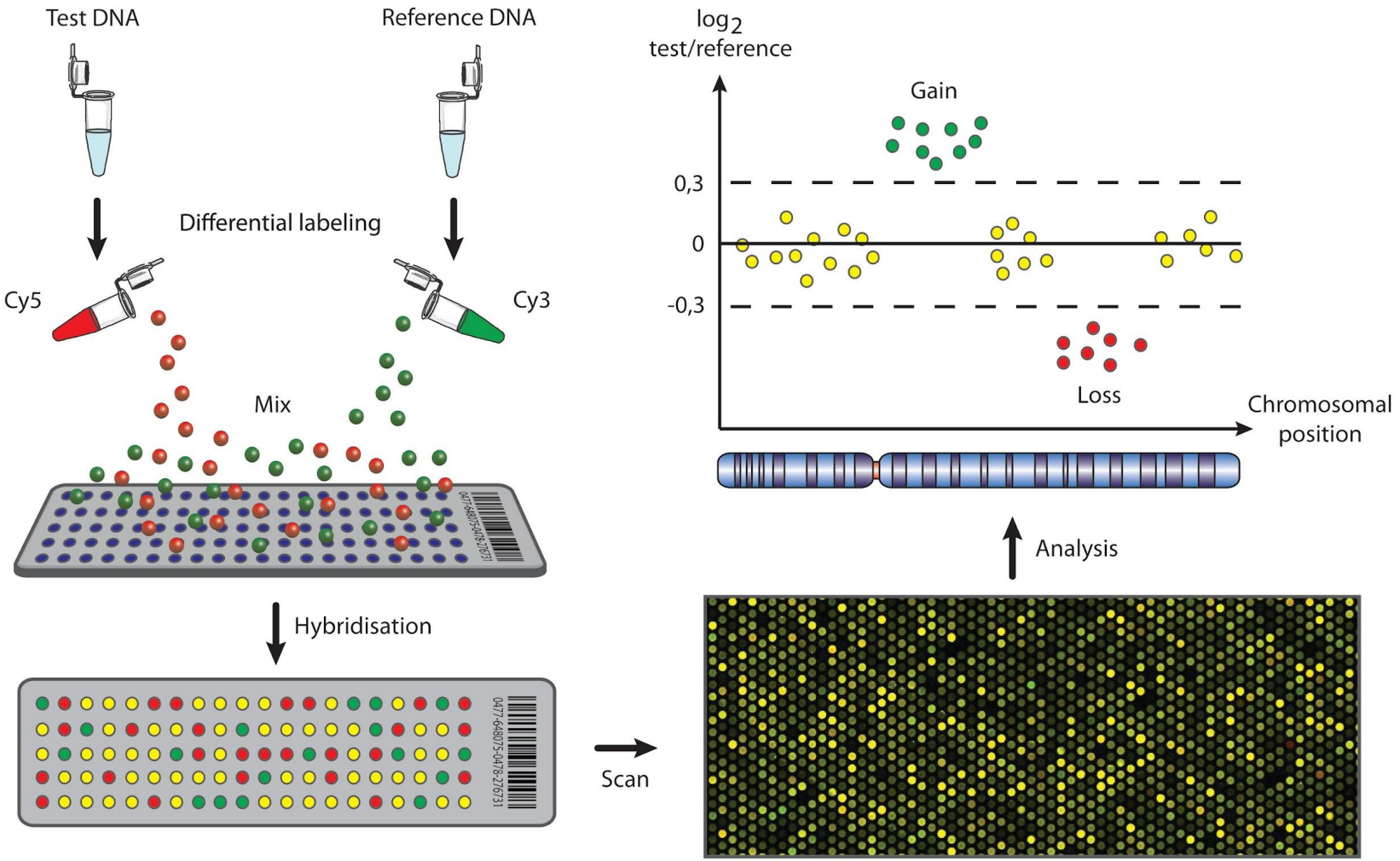
The comparative genomic hybridization (CGH) array compares the patient's DNA to control DNA using 2 different fluorescent labels. Labeled control and patient DNA fragments are hybridized to an array containing oligonucleotide DNA sequences from genes throughout the human genome. Each position on the 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).

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.

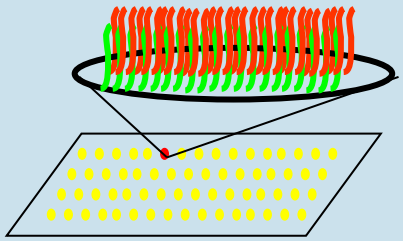
Array Comparative Genomic Hybridization



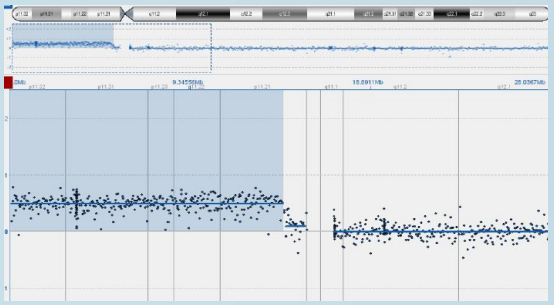
Deletions and duplications

Duplication

Patient rood
Controle groen

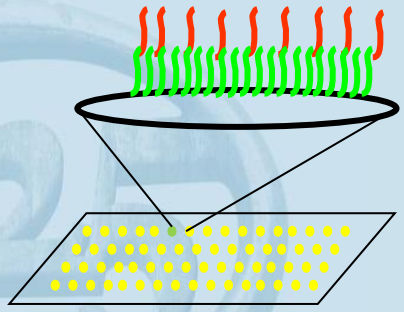


Ratio Red/green= 3/2=1.5
Log2 = 0.56

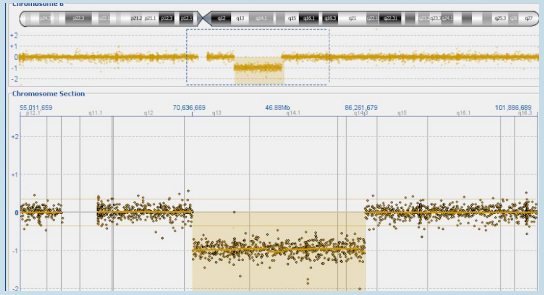


Deletion

Patient rood
Controle groen

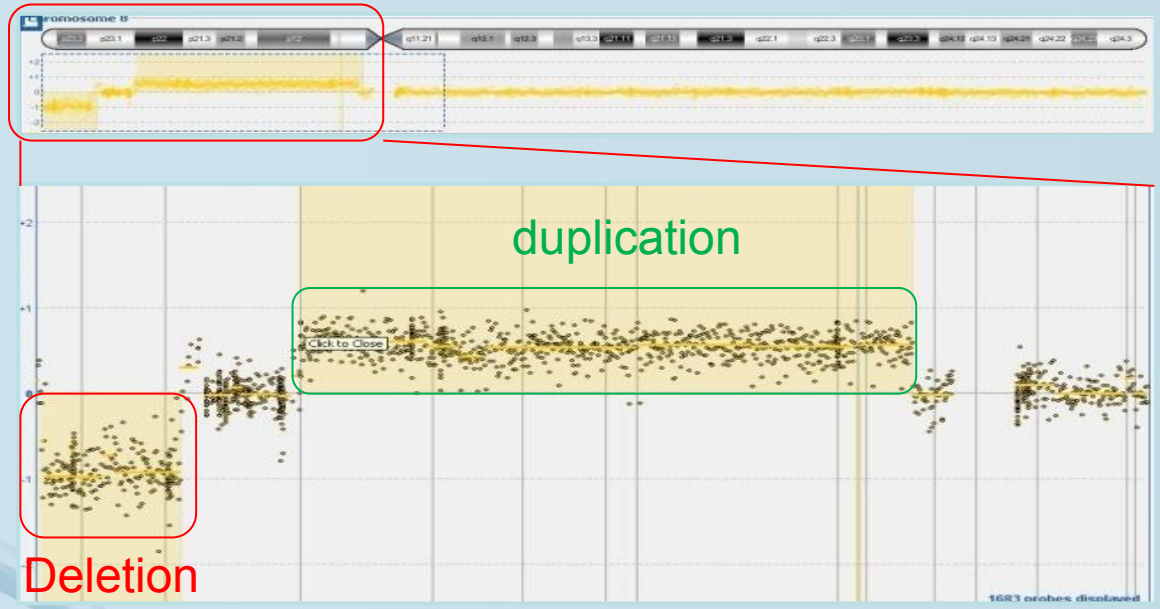


Ratio Red/green= 1/2=0.5
Log2 = -1



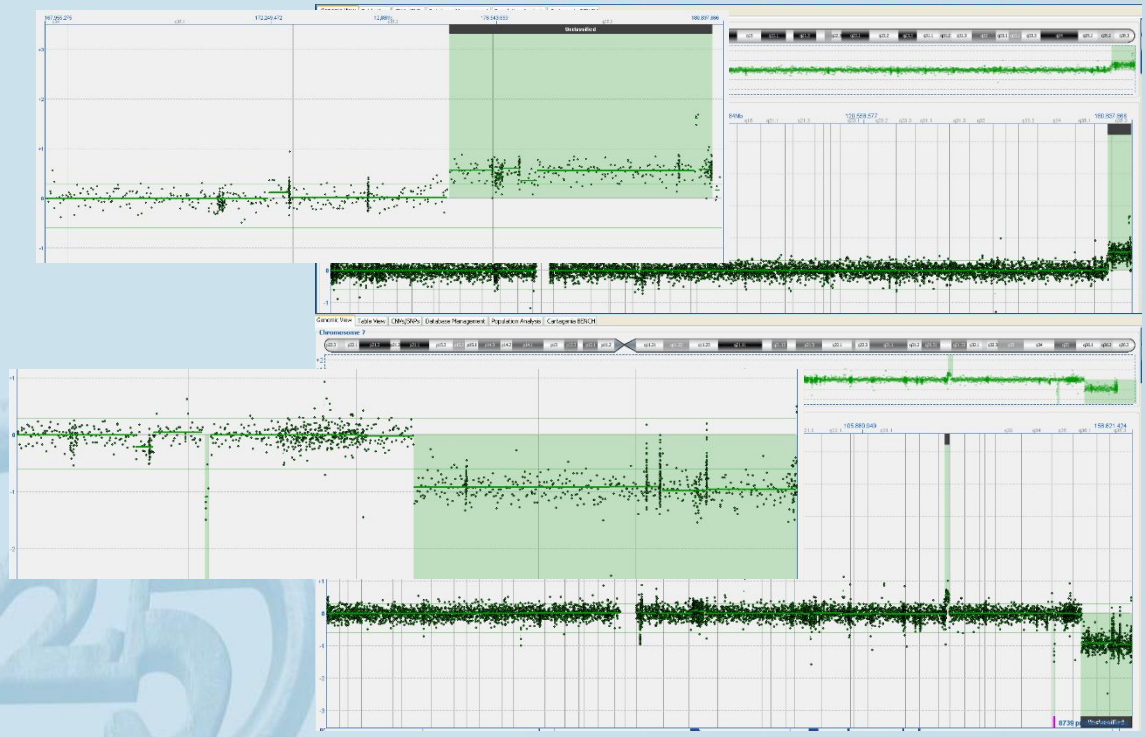
Oligonucleotide-based Array CGH: genome wide view

Oligonucleotide-based Array CGH

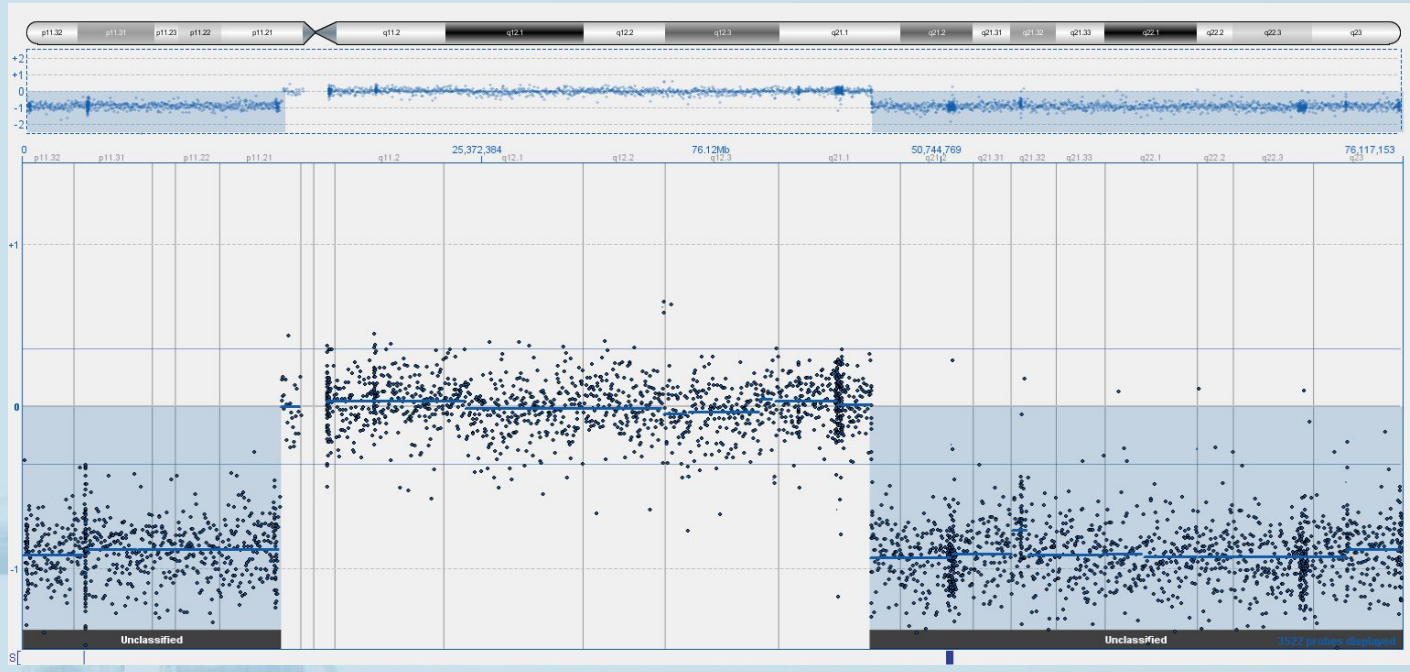


Unbalanced translocation: der(7)t(5;7)(q35.2qter;q36.1qter)

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



?


+2
+1
0
-1
-2
0
25,372,384
76.12Mb
50,744,769
76,117,153
+1
0
-1
Unclassified
Unclassified

Array resolution depends on

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

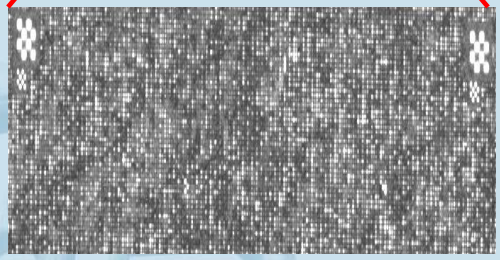
Reference

- 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)

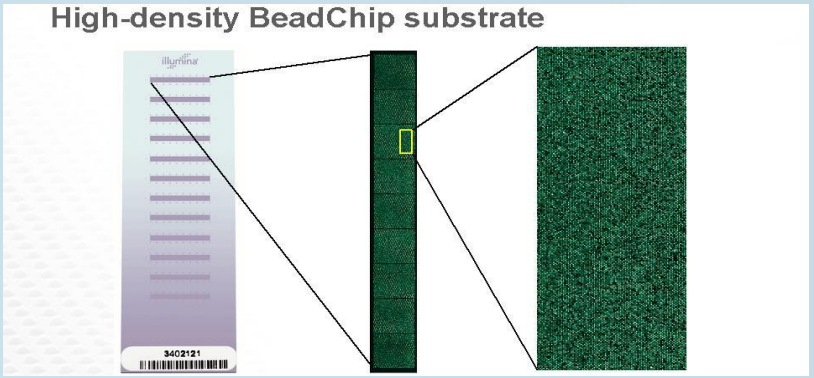
Genome wide genotyping techniques

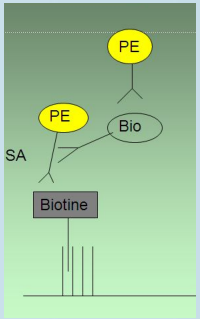
Affymetrix (arrays)

1.6 cm^2

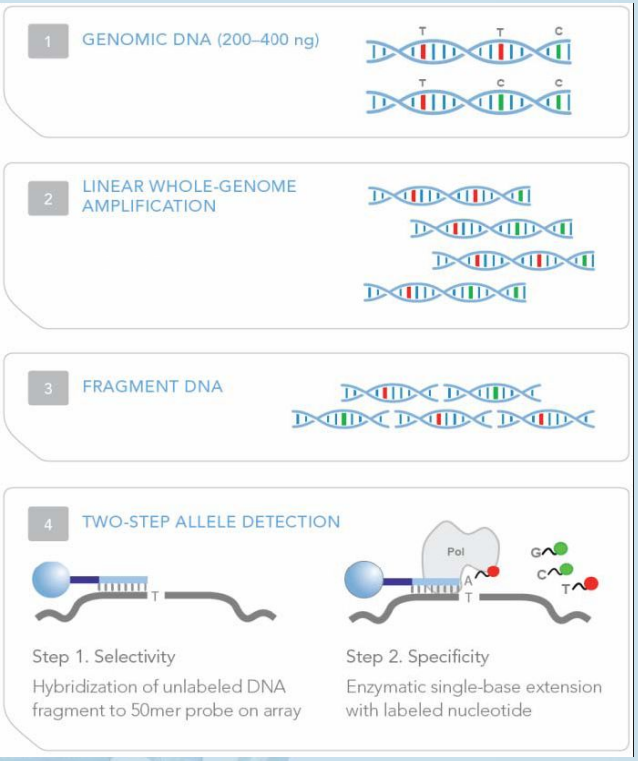


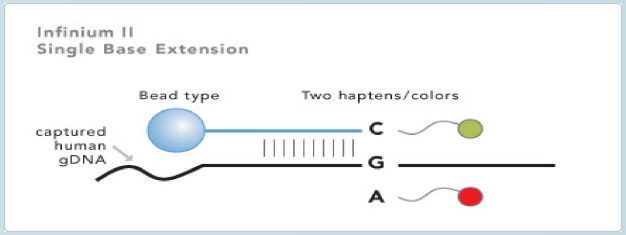
Illumina (Chips)



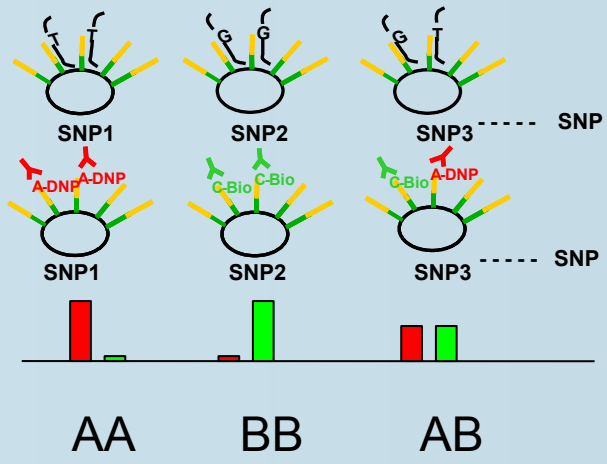


Illumina: Infinium set-up

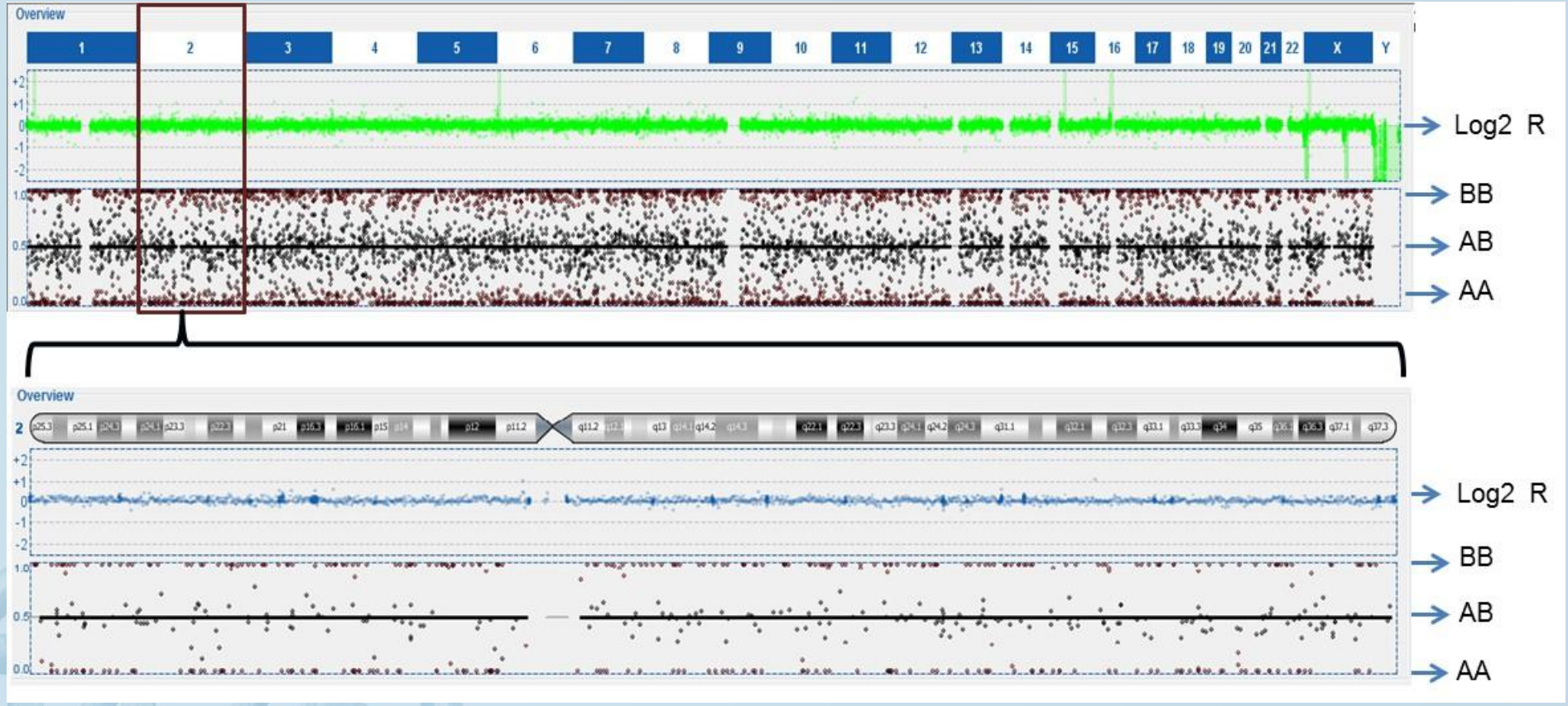




(whole genome amplified DNA)

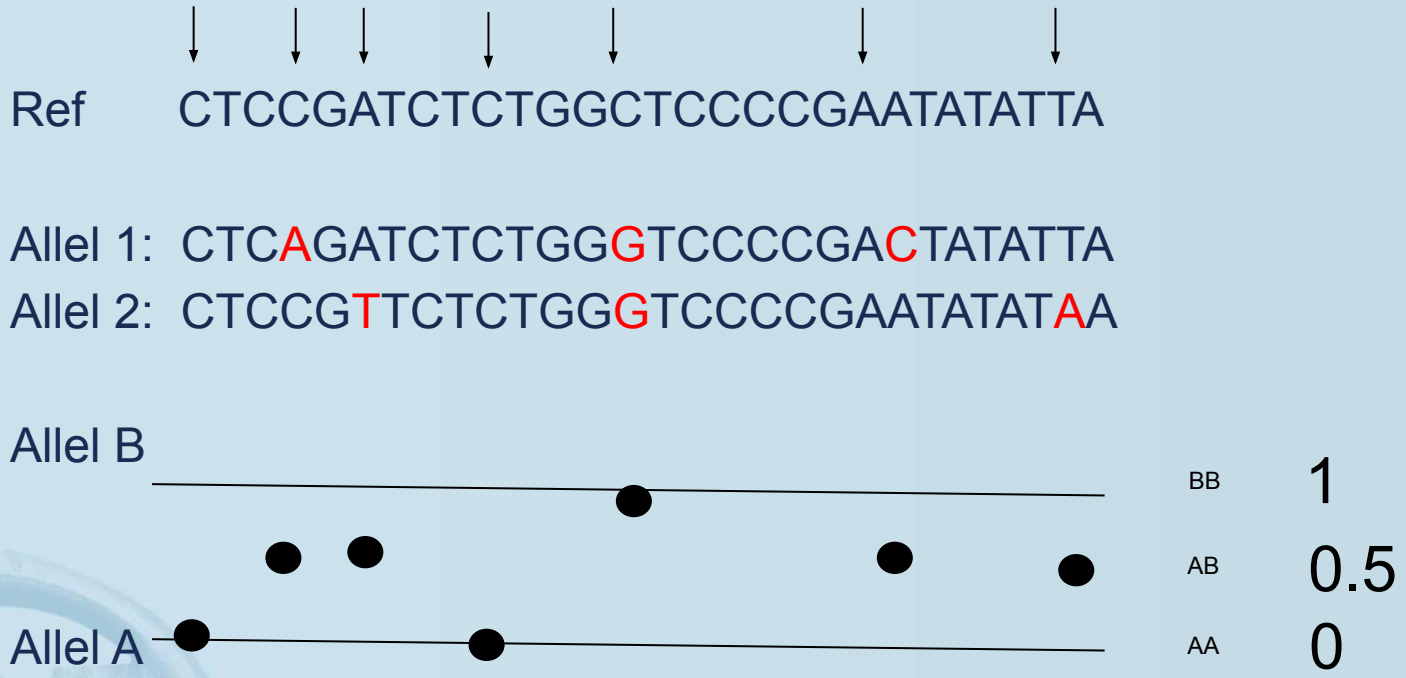


Visualisation of CNV & SNP data

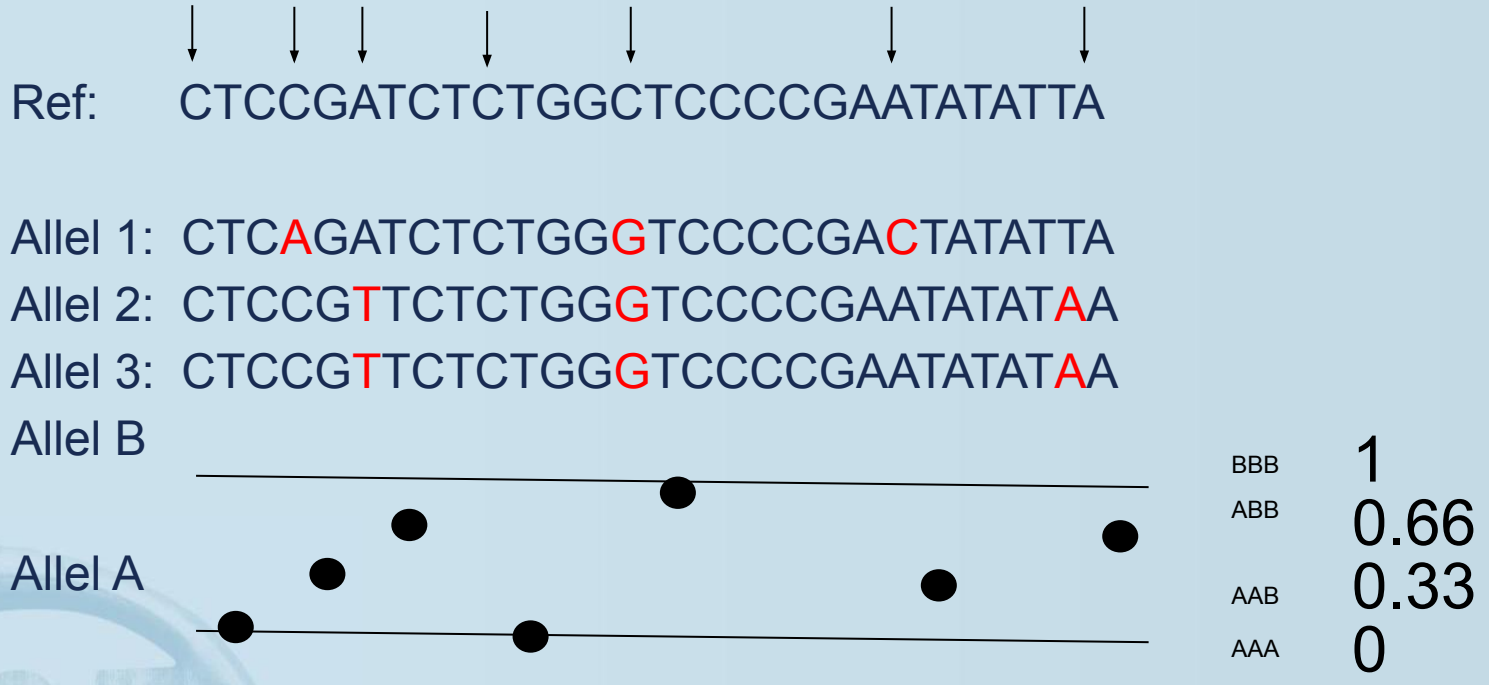


B-allele frequency plot

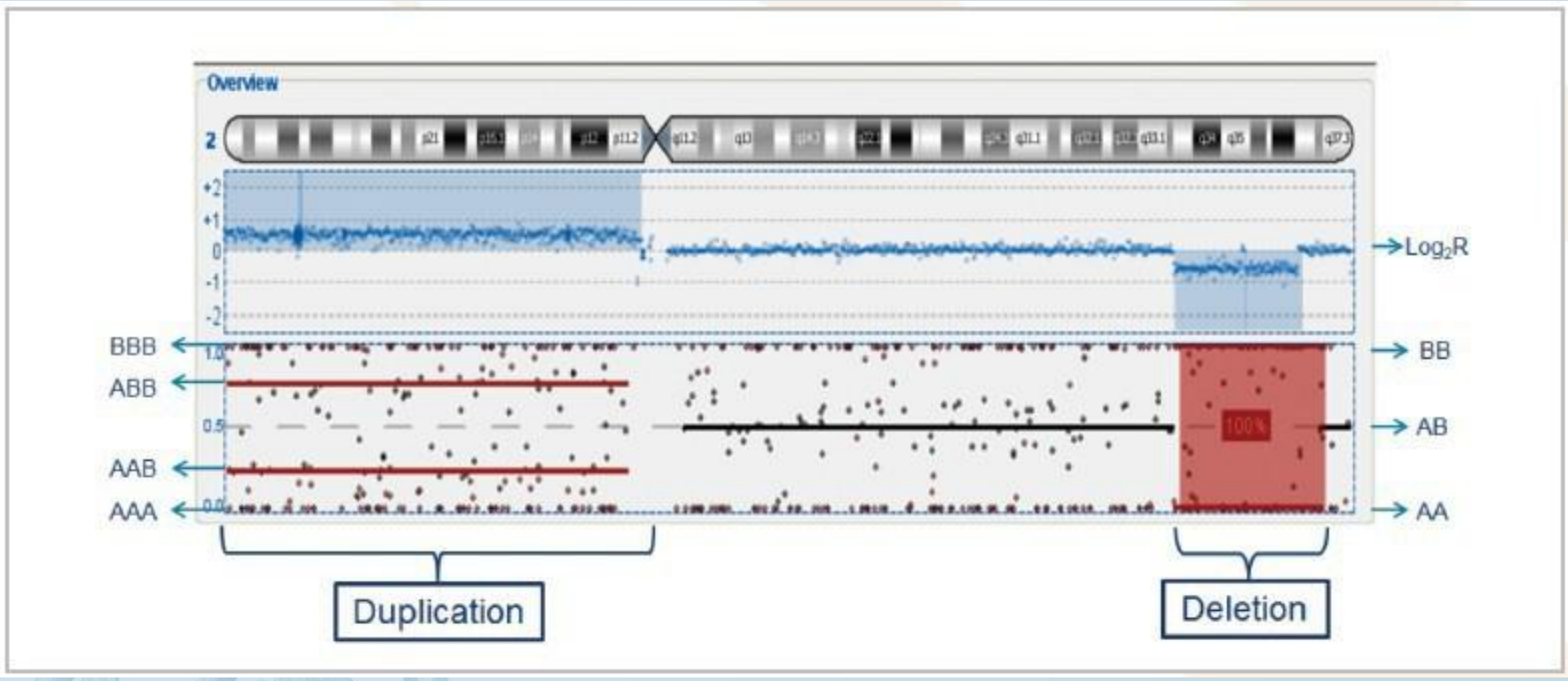
Principles of B-allele frequency plot
Disomy



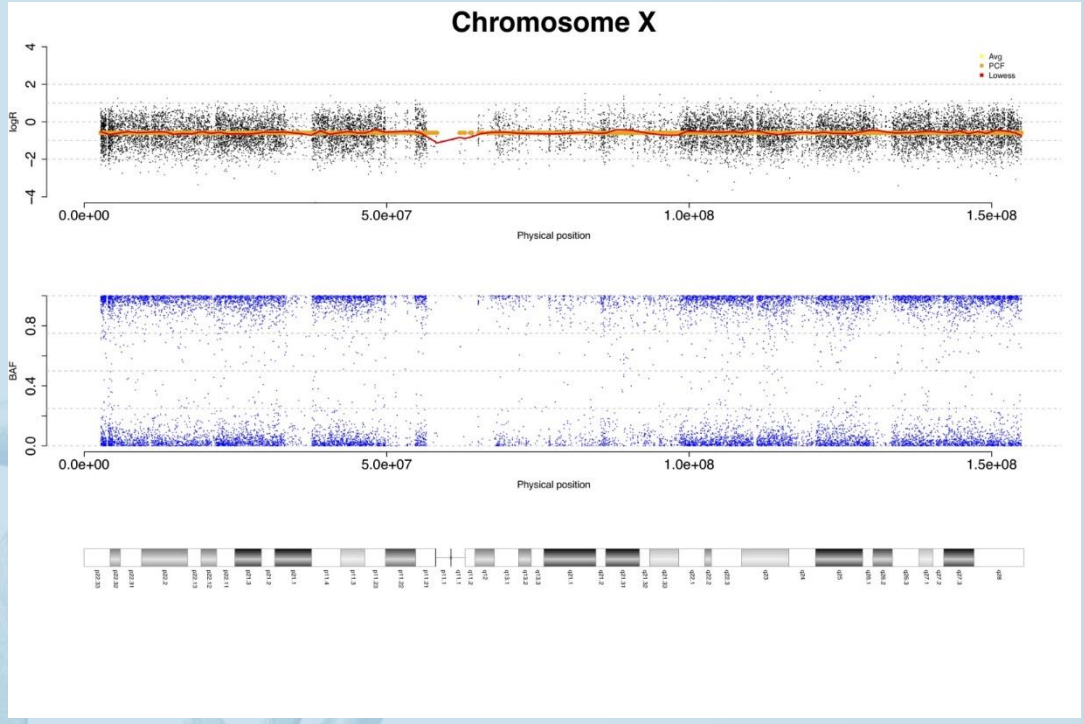
Principles of B-allele frequency plot Trisomy



Visualisation of CNV & SNP data



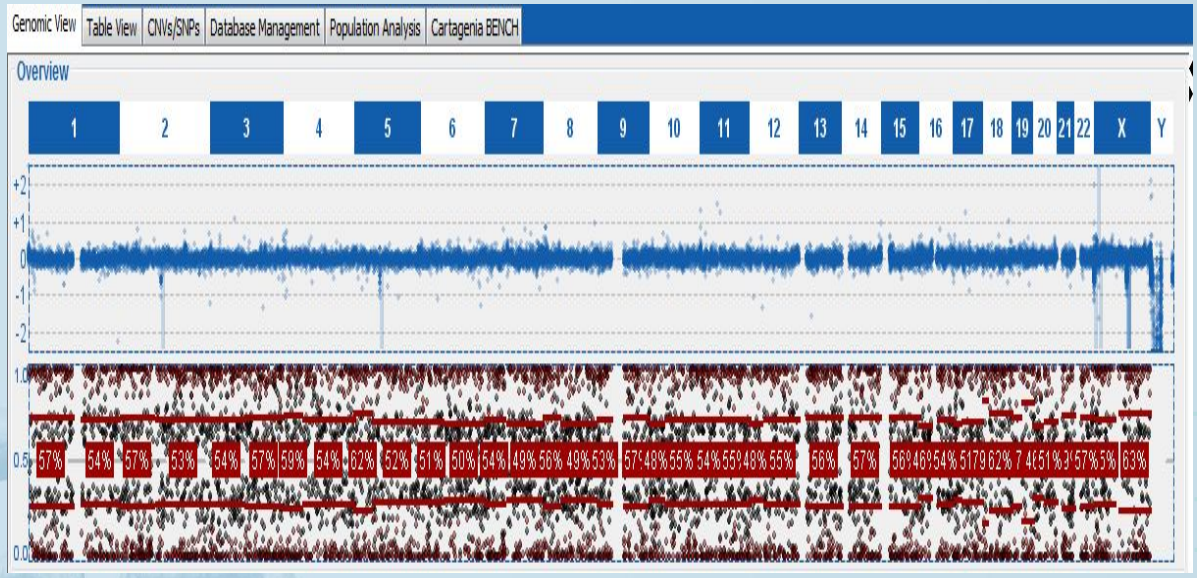
Monosomy



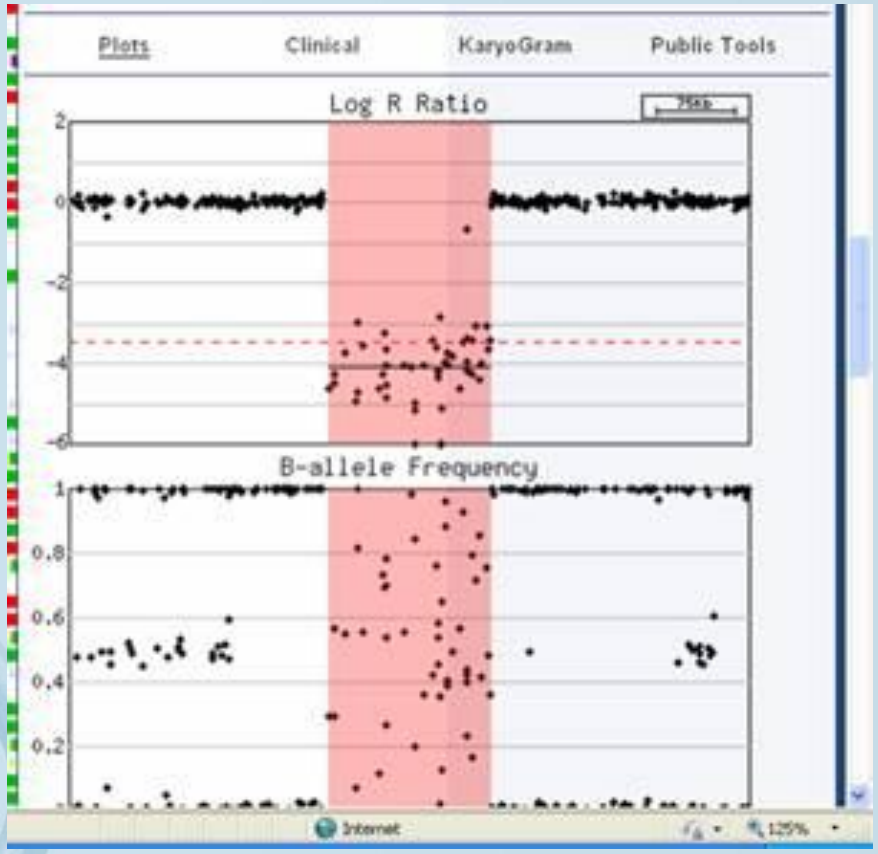
Allele A

Allele B

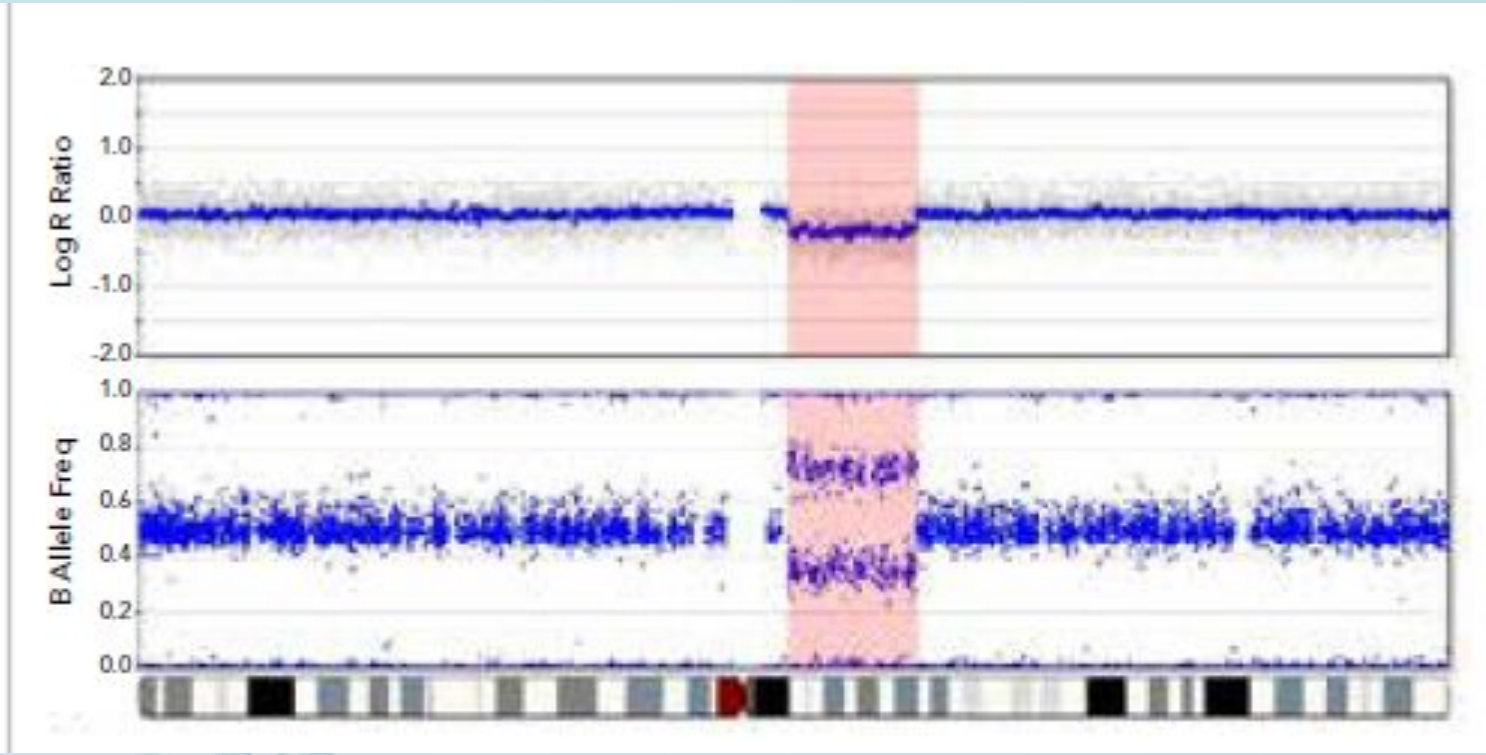
Triploidy


Genomic View
Table View
CNVs/SNPs
Database Management
Population Analysis
Cartagenia BENCH
Overview
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
+2
+1
0
-1
-2
1.0
0.5
0.0
57% 54% 57% 53% 54% 57% 59% 54% 62% 52% 51% 50% 54% 49% 56% 49% 53% 57% 48% 55% 54% 55% 48% 55% 56% 57% 56% 54% 62% 51% 57% 63%
Log2 R
AAA
AAB
ABB
BBB

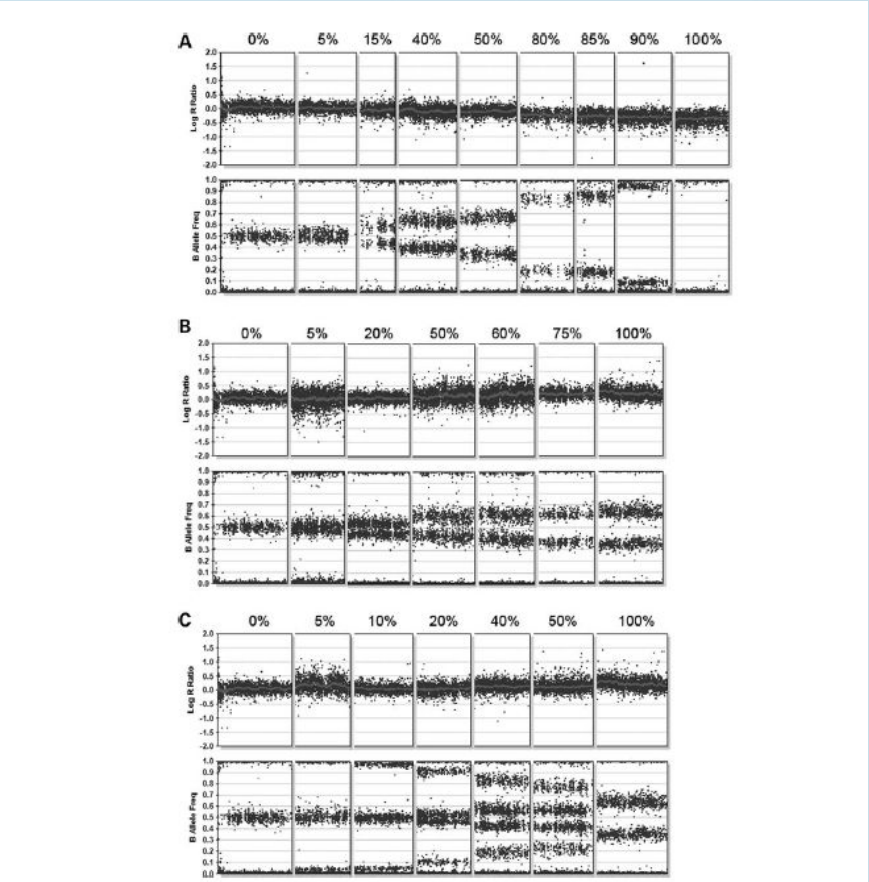
Copy number?


Plots
Clinical
KaryoGram
Public Tools
Log R Ratio
2
0
-2
-4
-6
B-allele Frequency
1
0.8
0.6
0.4
0.2
Internet
125%

???


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

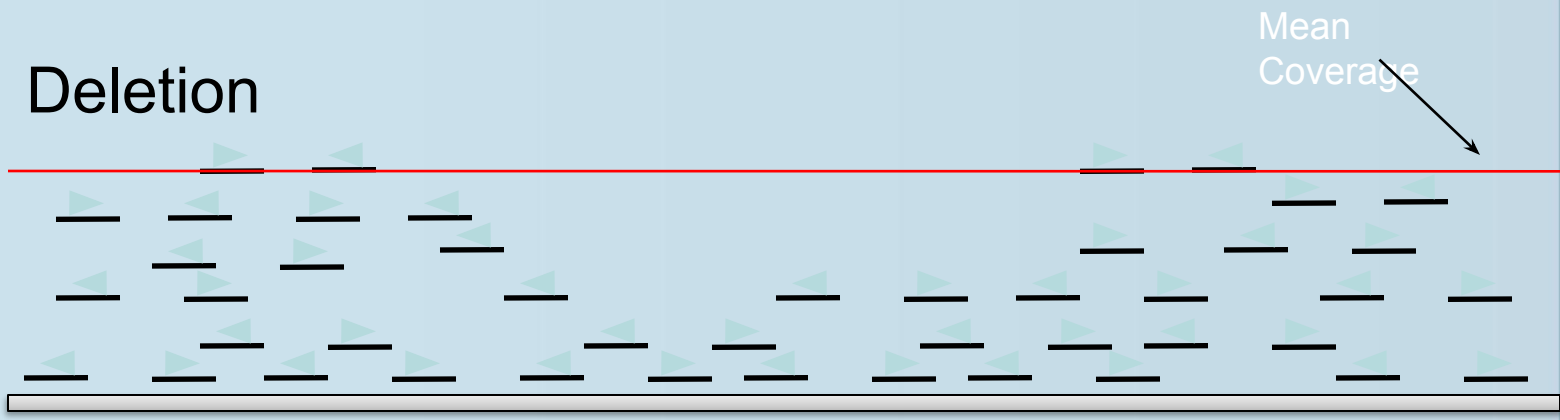
	Karyotyping	FISH	Micro-array
	Genome-wide Detection of balanced and unbalanced rearrangements	High resolution Fast	Genome-wide High resolution
	Low resolution Labour-intensive Subjective => skilled personnel	Locus specific A priori knowledge necessary	No detection of balanced rearrangements

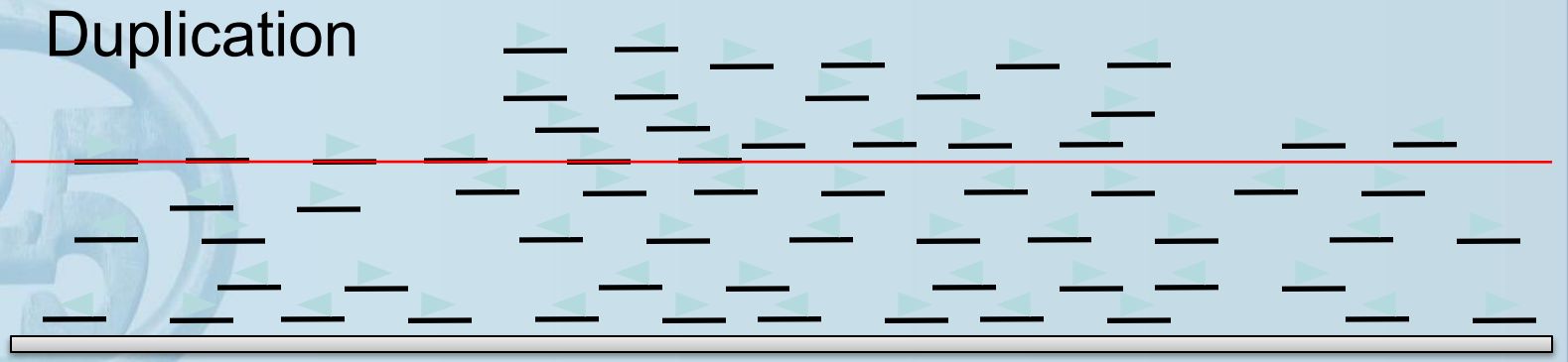
Advantages of SNP arrays

- 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)

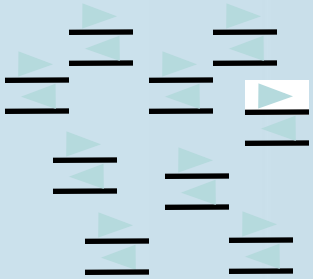
Massive parallel sequencing

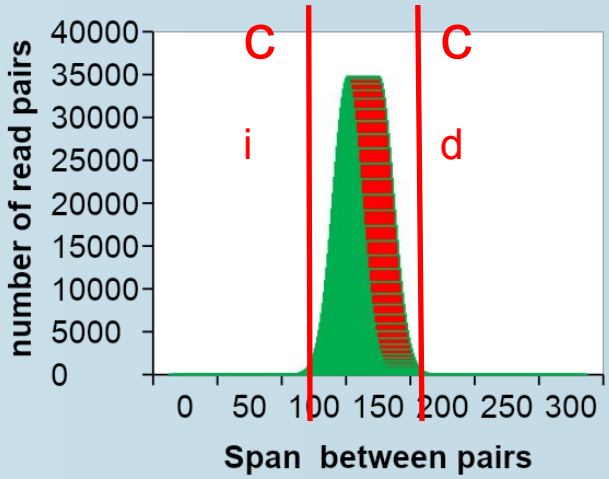
Read-depth Analysis

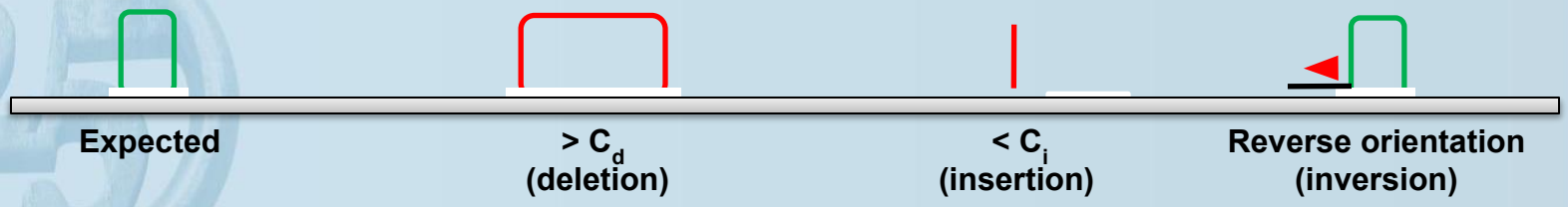




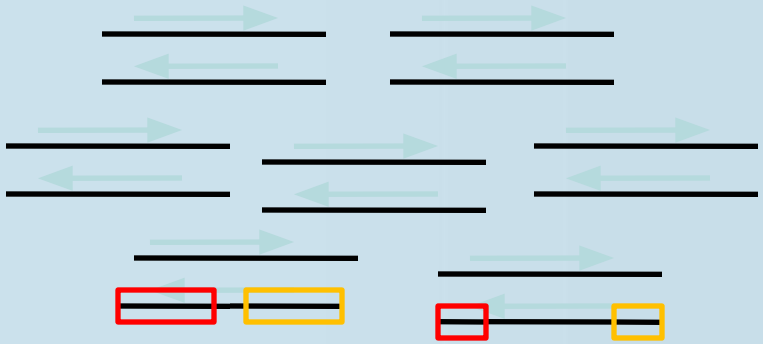
Paired-end Mapping

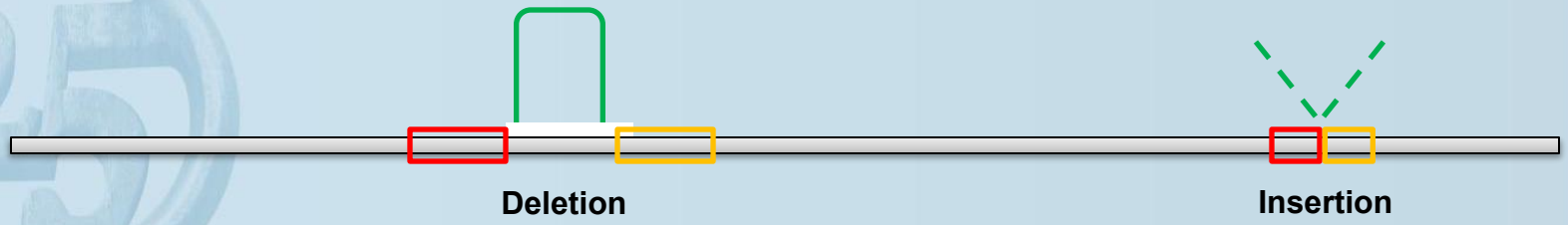






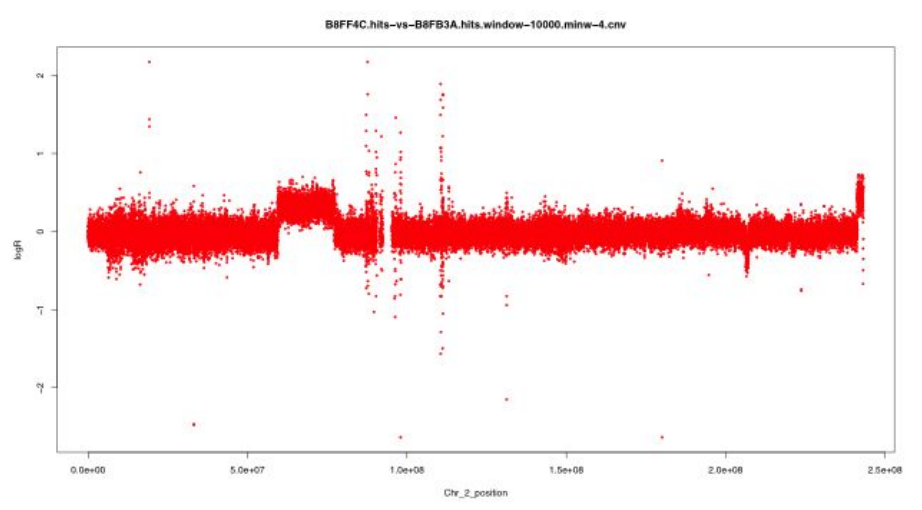
Split-read Analysis

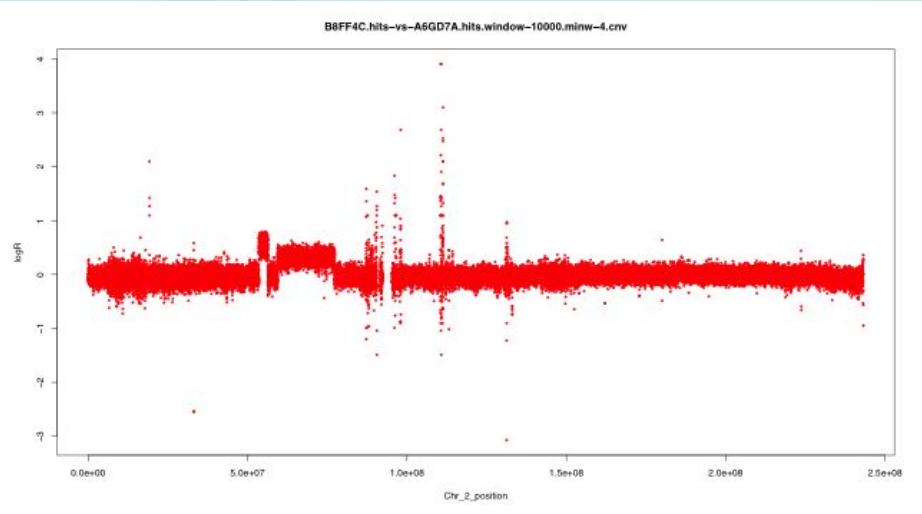




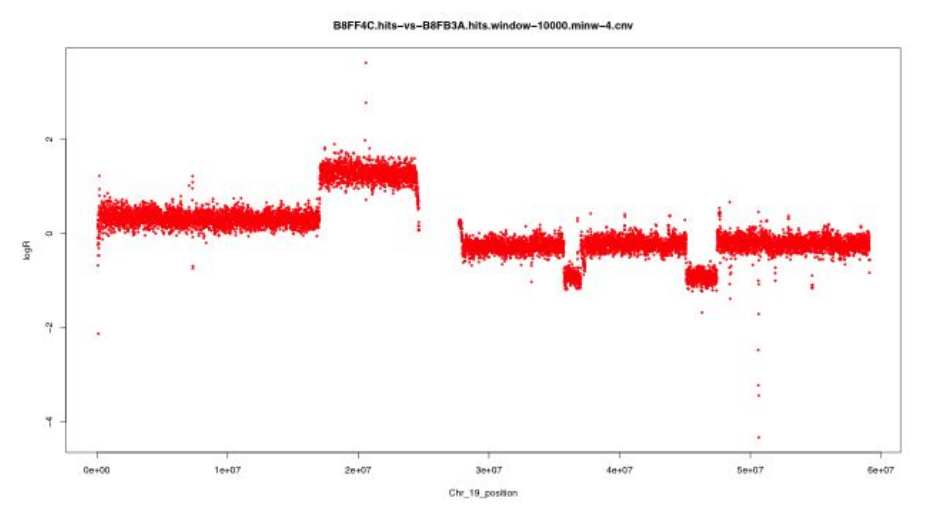
Read-depth reveals copy number variation

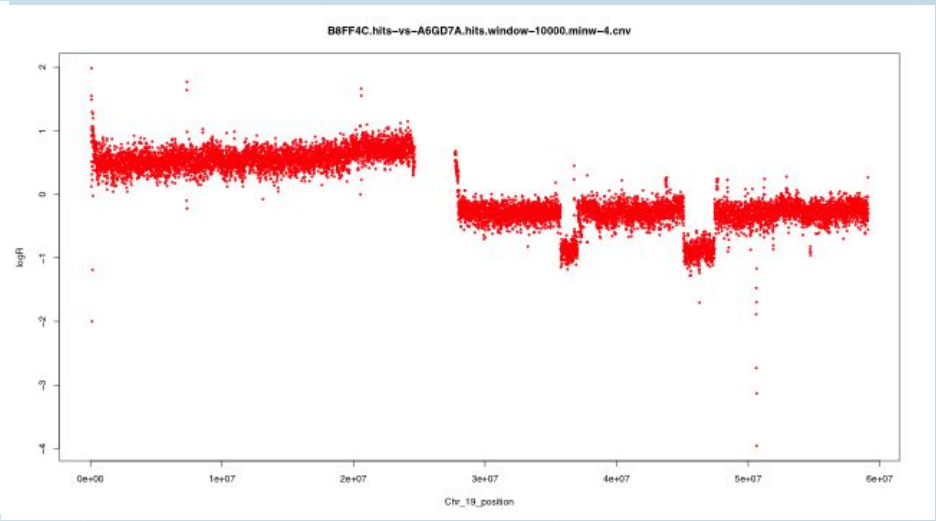
Chr.2





Chr.19

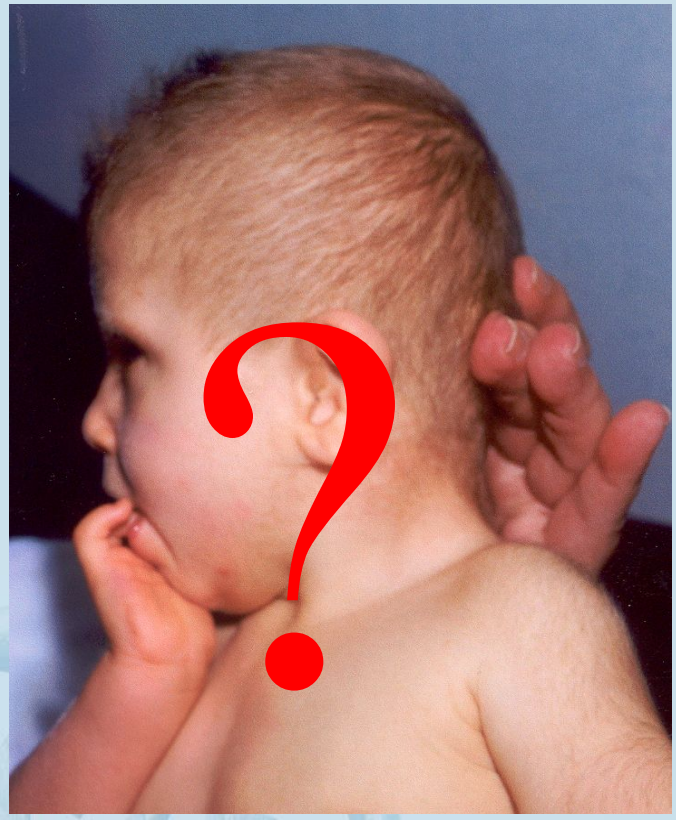


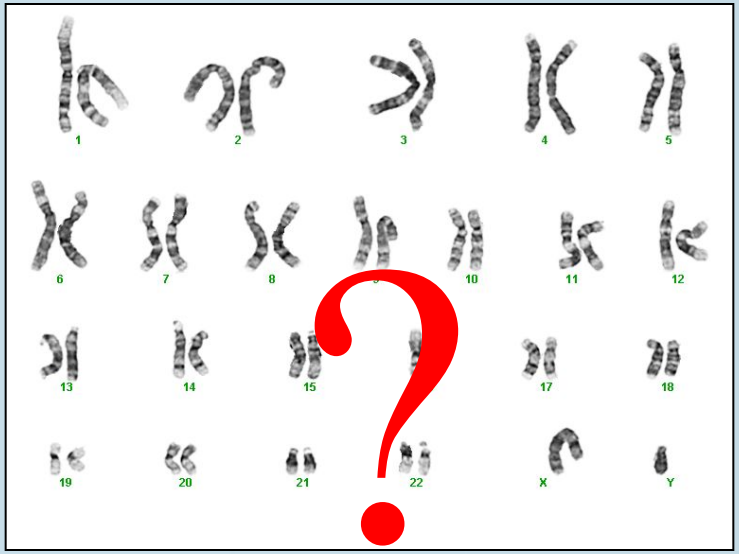


Overview

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

15 years ago

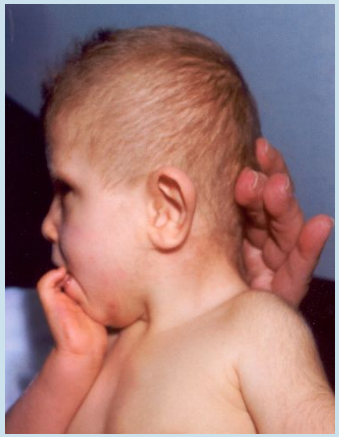


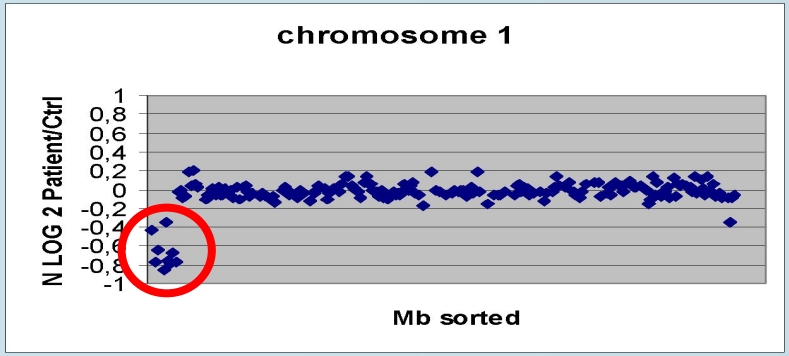


Question: Can submicroscopic imbalances explain the cause of the MCA/MR?

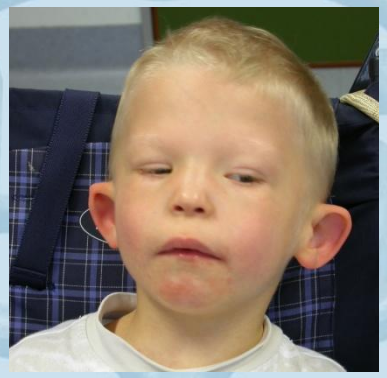
15% of developmental anomalies can be explained by CNV's

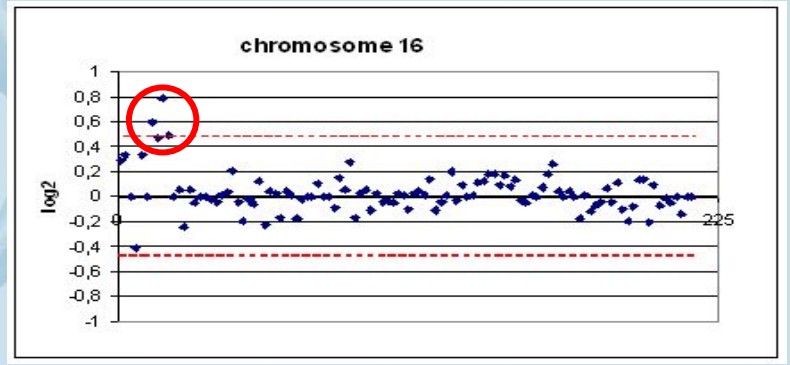
Deletions

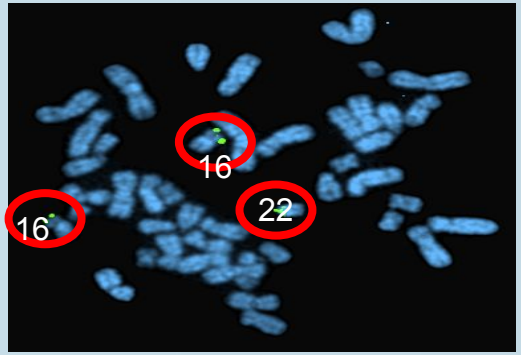




Duplications





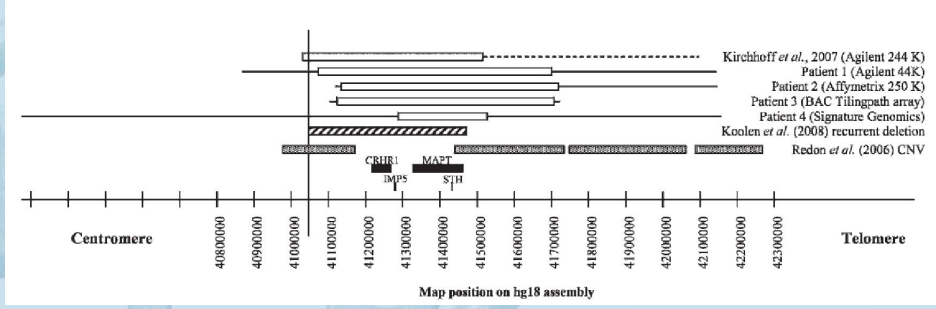


For all recurrent deletion syndromes the reciprocal duplication is now identified

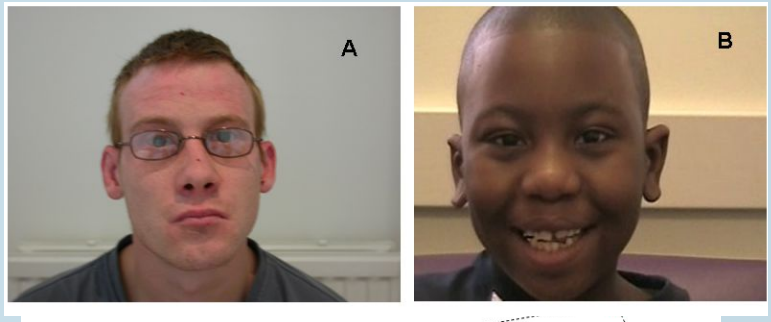
JMG

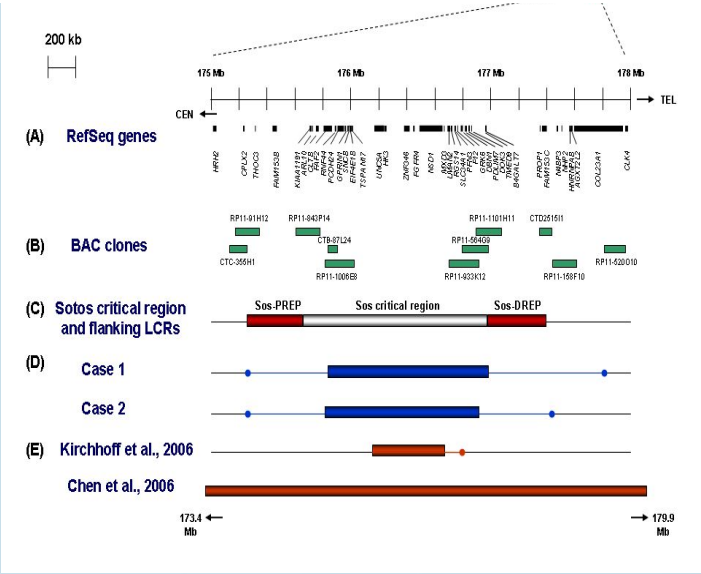
17q21.31 microduplication patients are characterised by behavioural problems and poor social interaction

B Grisart, L Willatt, A Destrée, J-P Fryns, K Rack, T de Ravel, J Rosenfeld, J R Vermeesch, C Verellen-Dumoulin and R Sandford



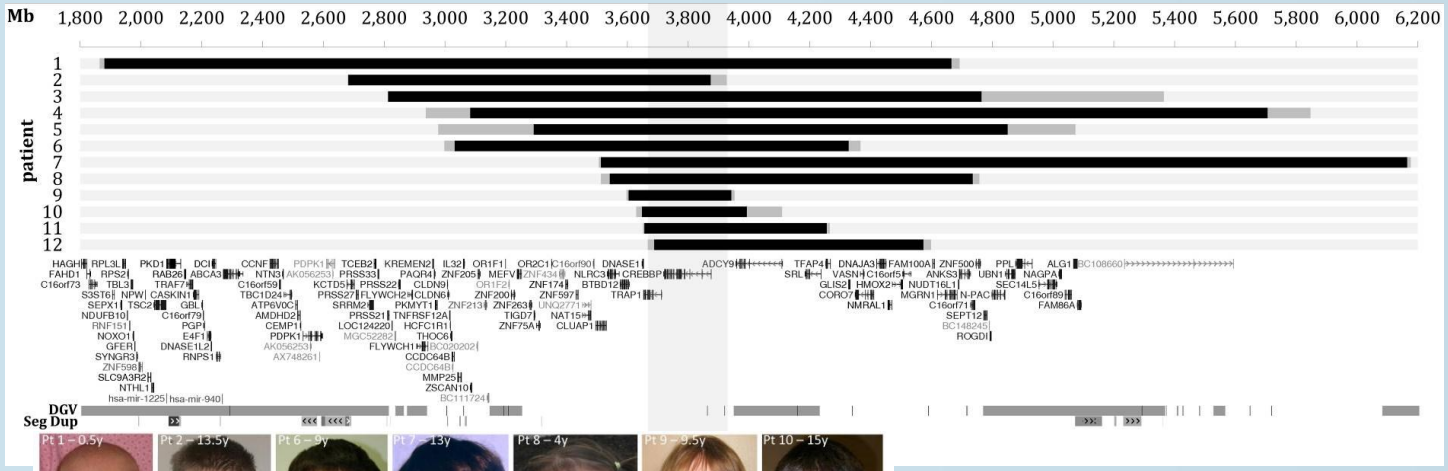
A syndrome of short stature, microcephaly and speech delay is associated with duplications reciprocal to the common Sotos syndrome deletion

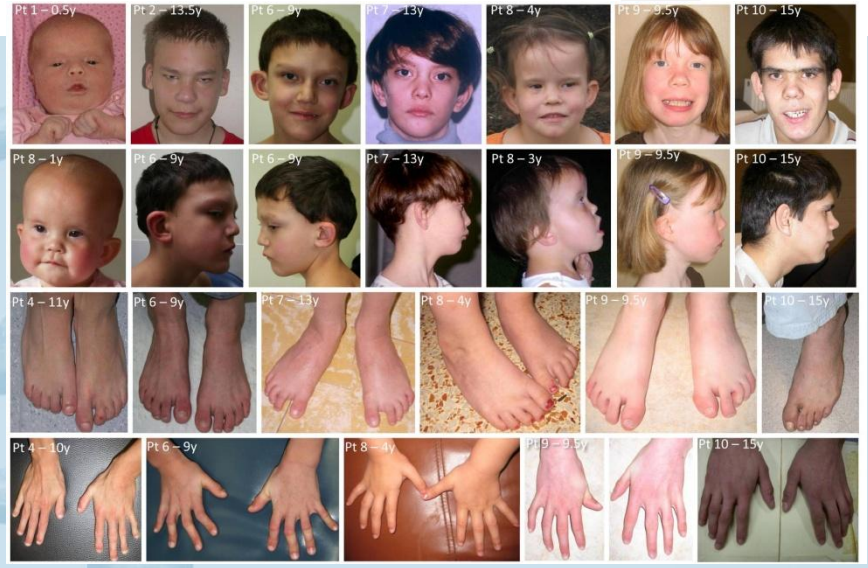




Franco et al., Eur.J. Hum. Gen., 2011

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



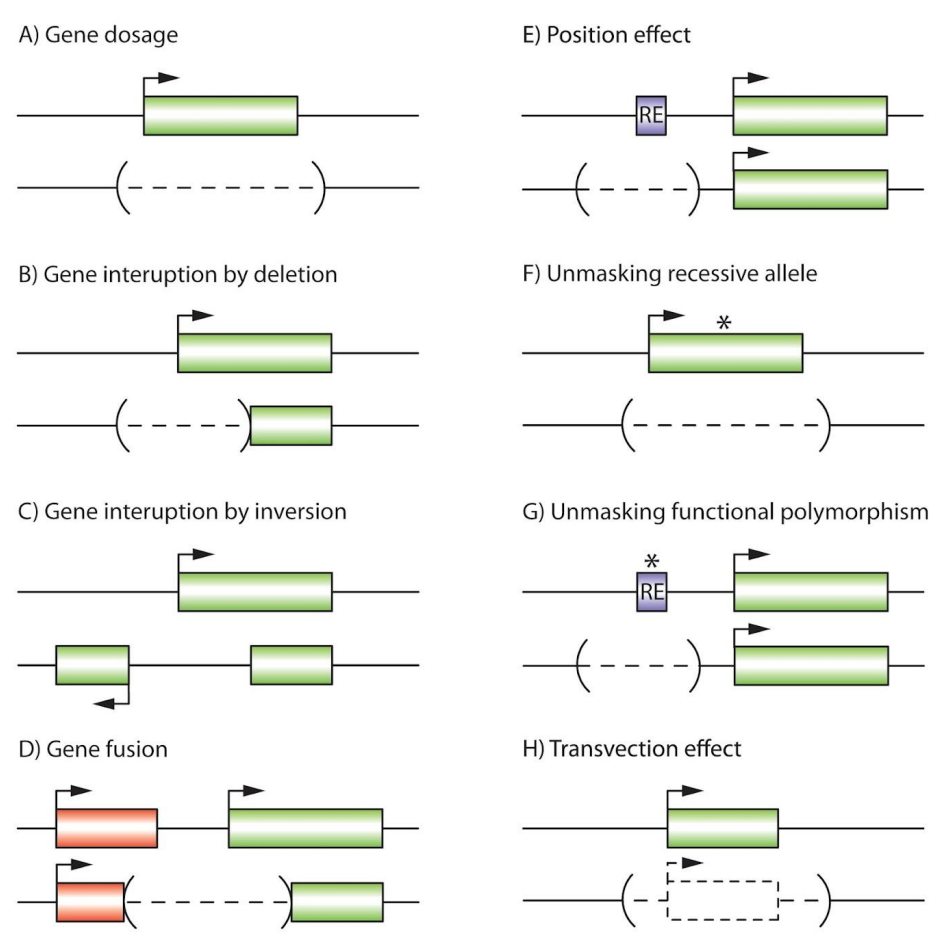


Authors:

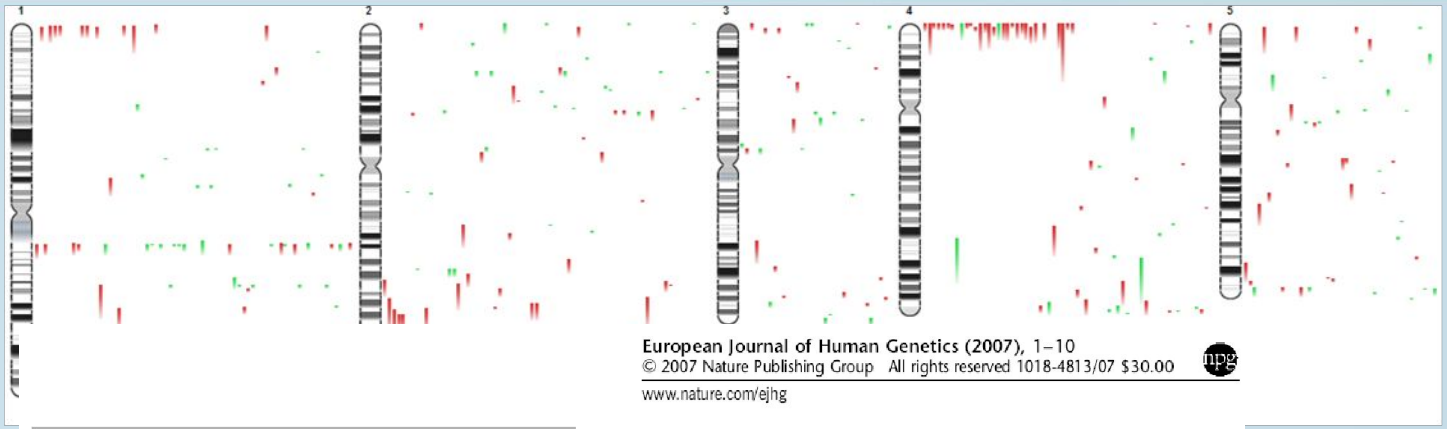
Bernard Thienpont[1], Frédérique Béna[2], Jeroen Breckpot[1], Nicole Philip[3], Björn Menten[4], Hilde Van Esch[1], Emmanuel Scalais[5], Jessica M. Salamone[6], Chin-To Fong[7], Jennifer L. Kussmann[8], Dorothy K. Grange[9], Jerome L. Gorski[8], Farah Zahir[10], Siu Li Yong[11], Michael M. Morris[2], 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

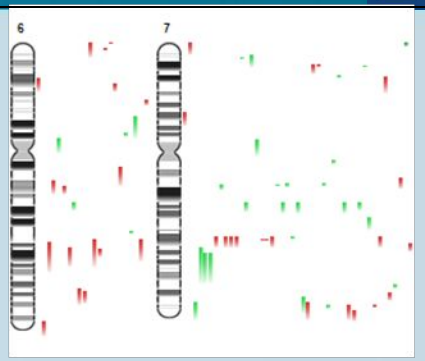


CNVs as cause of developmental disorders



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

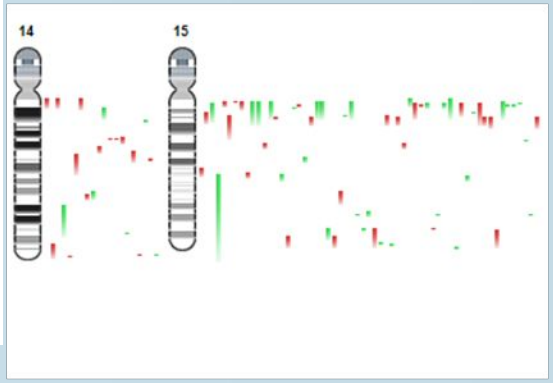
www.nature.com/ejhg

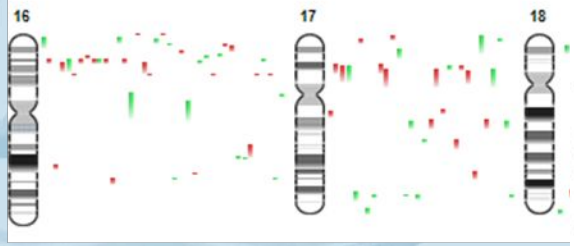


POLICY

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

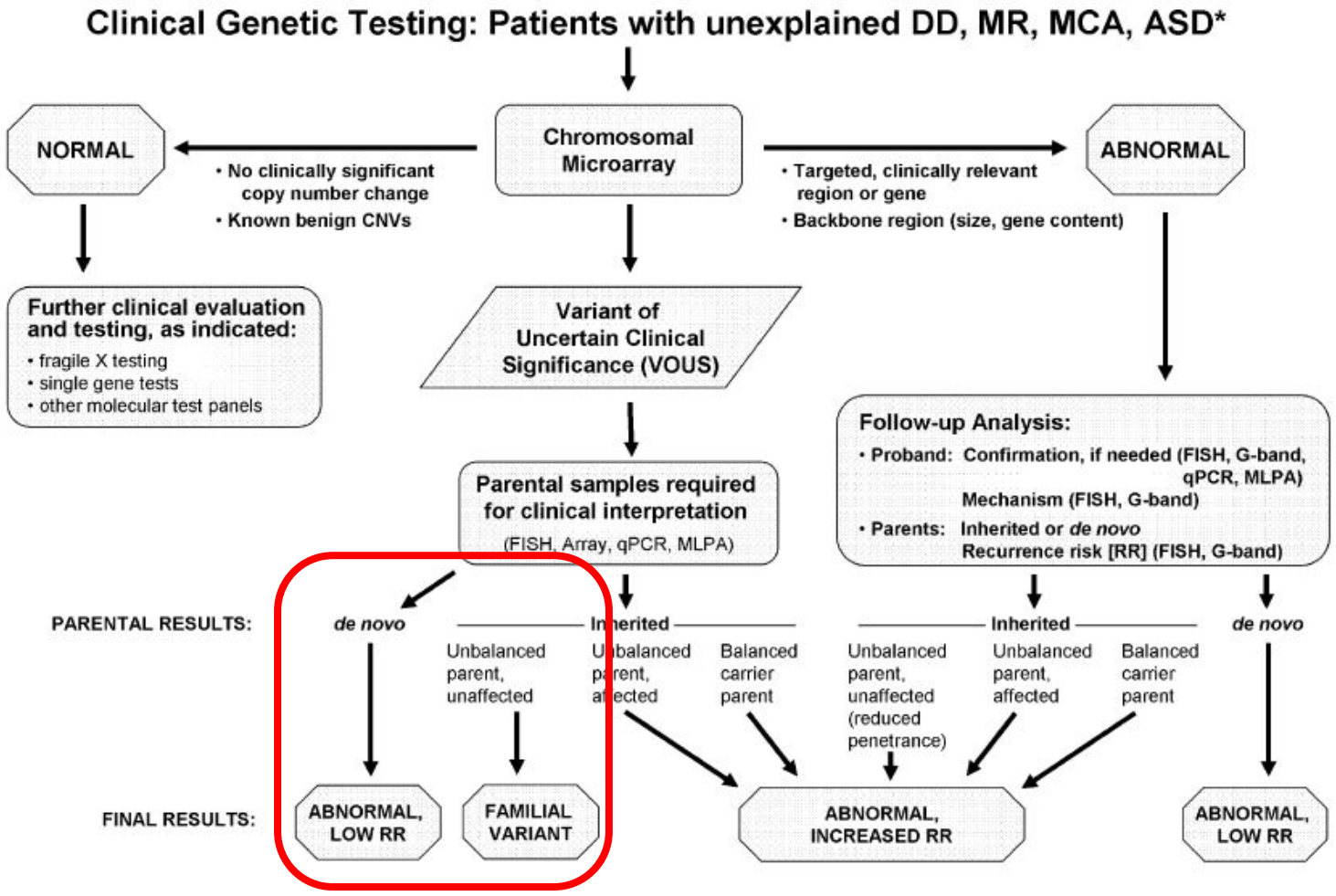
David T. Miller,[1,*] Margaret P. Adam,[2,3] Swaroop Aradhya,[4] Leslie G. Biesecker,[5] Arthur R. Brothman,[6] Nigel P. Carter,[7] Deanna M. Church,[8] John A. Crolla,[9] Evan E. Eichler,[10] Charles J. Epstein,[11] W. Andrew Faucett,[2] Lars Feuk,[12] Jan M. Friedman,[13] Ada Hamosh,[14] Laird Jackson,[15] 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

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

Criteria For Determining Pathogenicity



* Excludes patients with recognizable syndrome (e.g., Down syndrome), family history of a chromosomal rearrangement or multiple miscarriages

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

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

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Nigel P. Carter,[7] Deanna M. Church,[8] John A. Crolla,[9] Evan E. Eichler,[10] Charles J. Epstein,[11]
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Erin B. Kaminsky,[2] Klaas Kok,[16] Ian D. Krantz,[17] Robert M. Kuhn,[18] Charles Lee,[19] James M.

The Challenge : Which Variants Are Causal For The Phenotype?

Conventional Wisdom:

Recurrent imbalances with same phenotype are causal

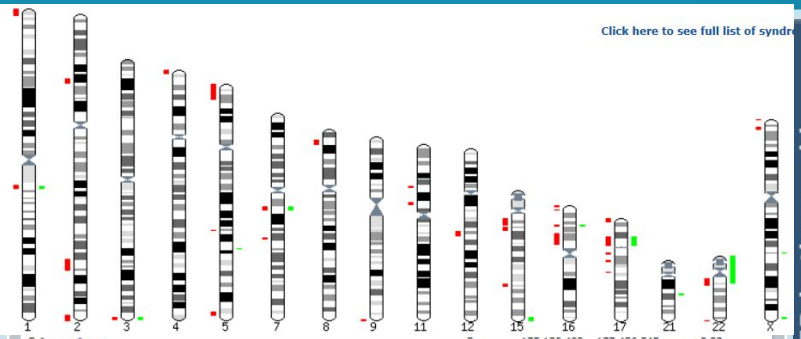
The larger the size, the more likely causal

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

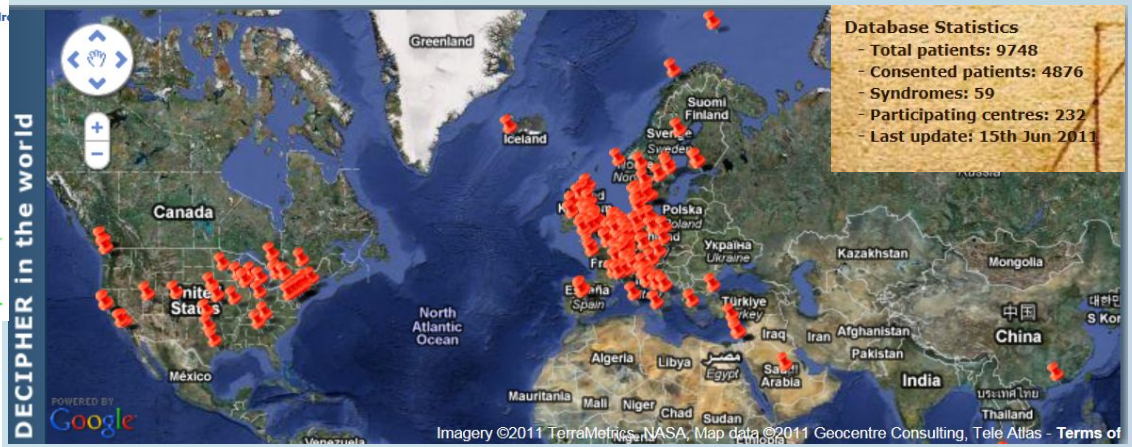
Population embedded CNVs are benign

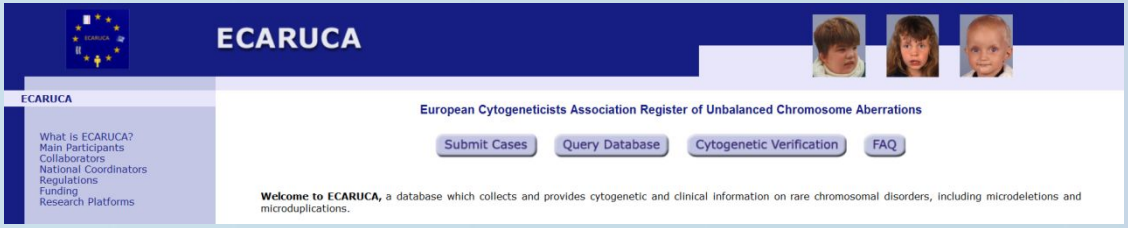
Identification of Recurrent Imbalances & Associated Phenotypes

KATHOLIEKE UNIVERSITEIT LEUVEN



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





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Population embedded CNVs are benign

Rare CNVs Megabases in Size Are Observed in Normal Individuals

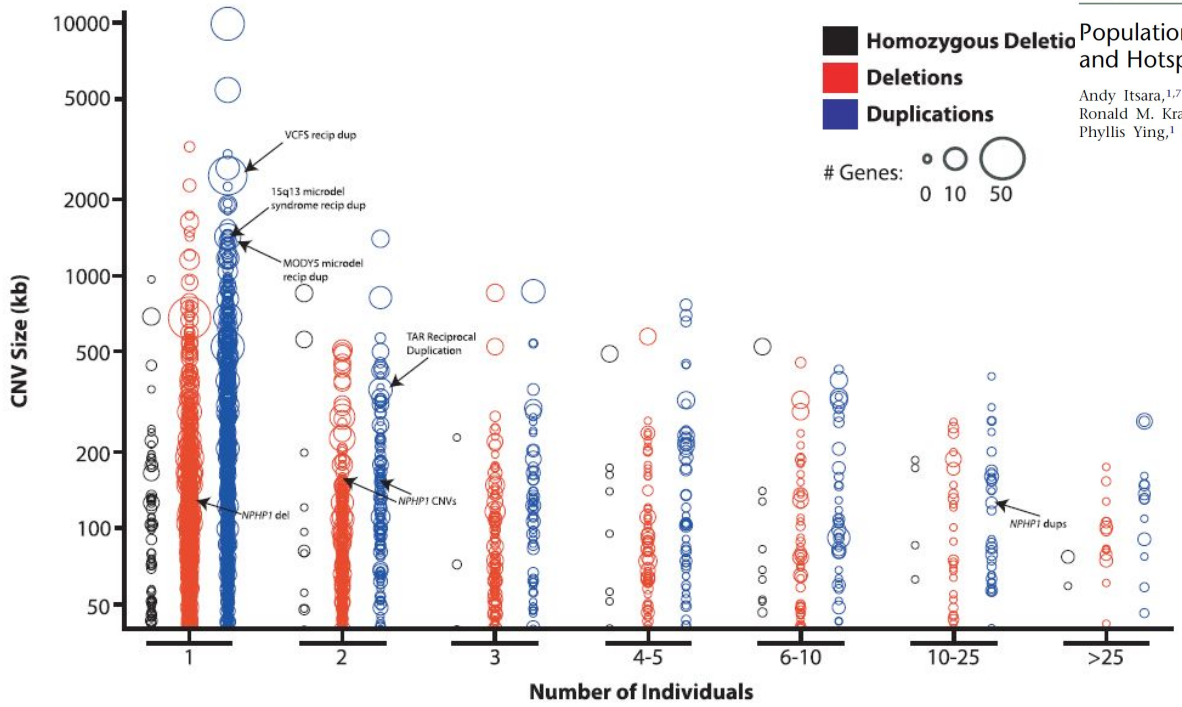

Figure 4. CNV Length, Gene Content, and Frequency Distributions

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

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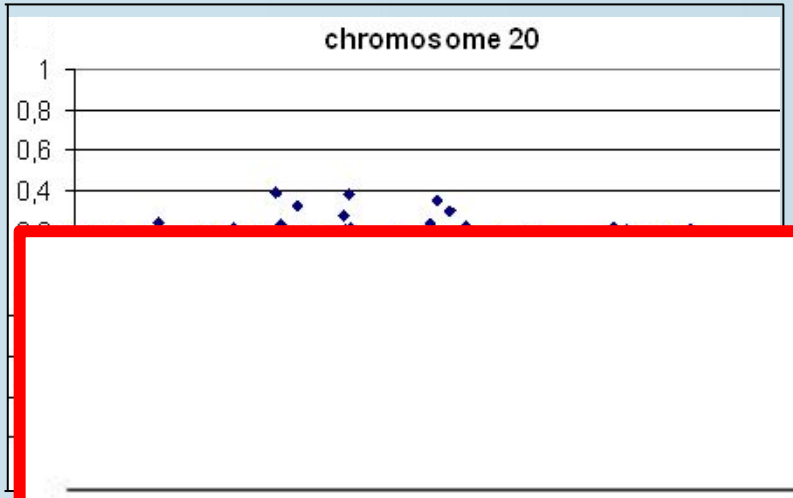
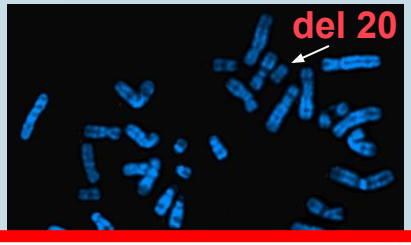
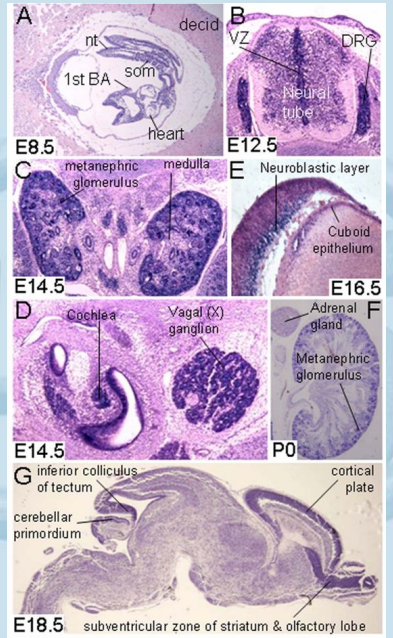
De novo is not always causal







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

Vermeesch et al., EJHG, 2011

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

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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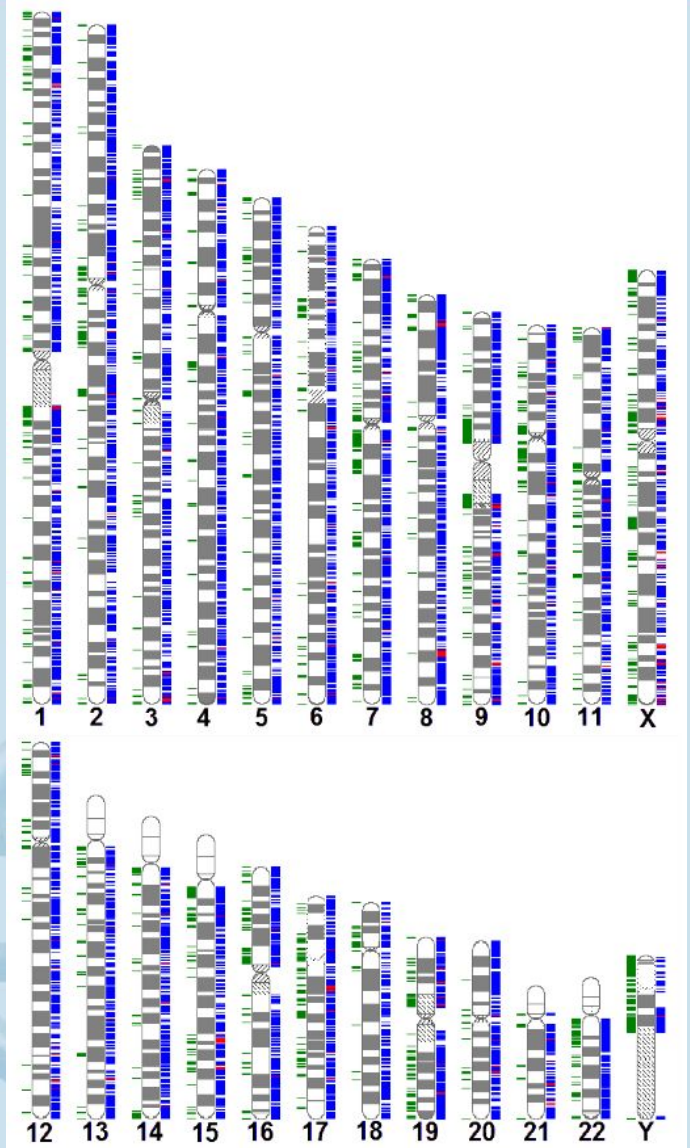
Population embedded CNVs are benign

Databases of Genomic Variants : Catalogue of 'Benign' CNVs



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

Database of Genomic Variants

Genome-wide view of CNVs

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
Join our mailing list

Toronto Database of Genomic Variants

Mendelian CNVs: a paradigm shift in (cyto)genetics

Inherited apparently benign CNVs *CAN* cause disease

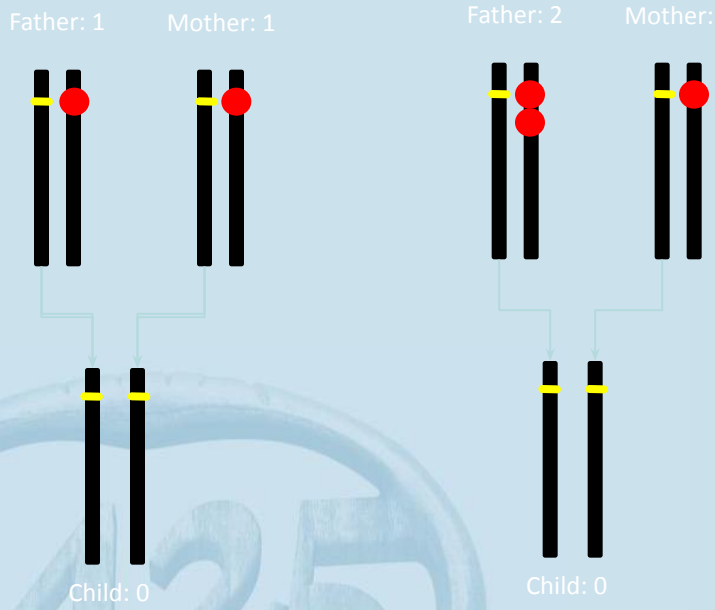
"Mendelian CNVs" is the term coined here to indicate benign CNVs which can cause disease dependent on either copy number state, inheritance pattern or genetic and environmental background.

Mendelian CNVs: New wine in old bottles

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

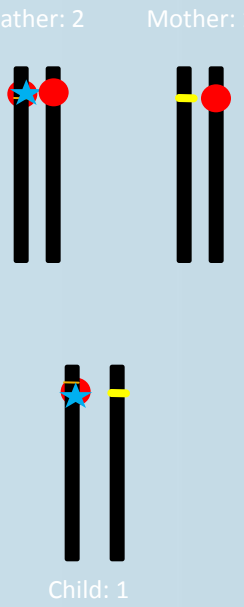
Autosomal recessive CNVs

Nullisomy



de novo is not necessarily *de novo*

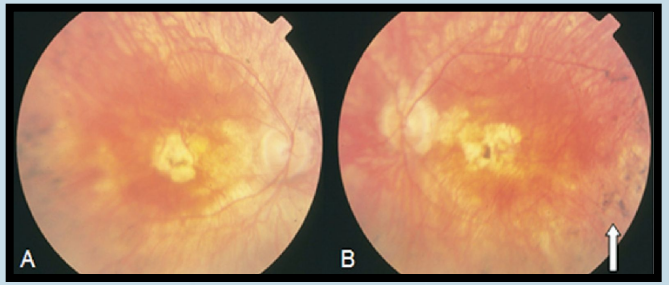
Hemizygous and mutation in second allele

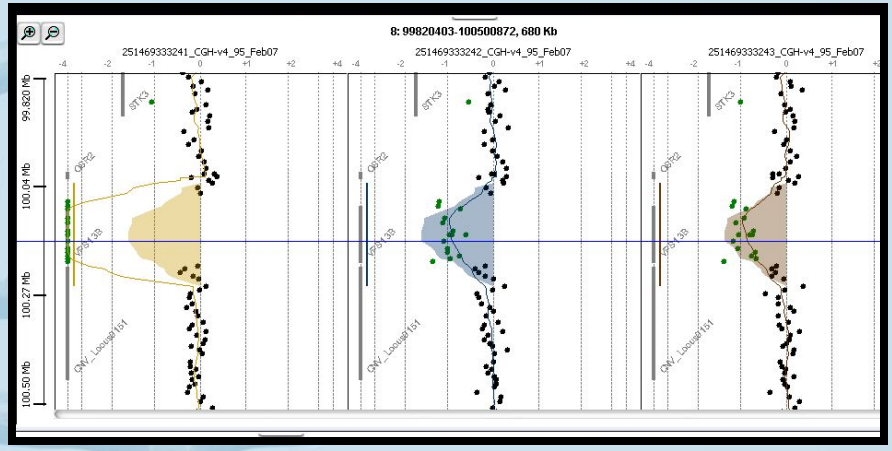


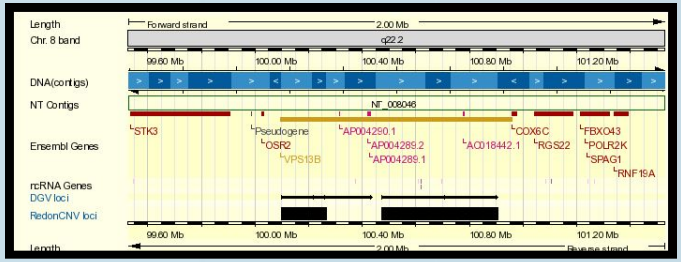
Inherited deletion IS causal

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)

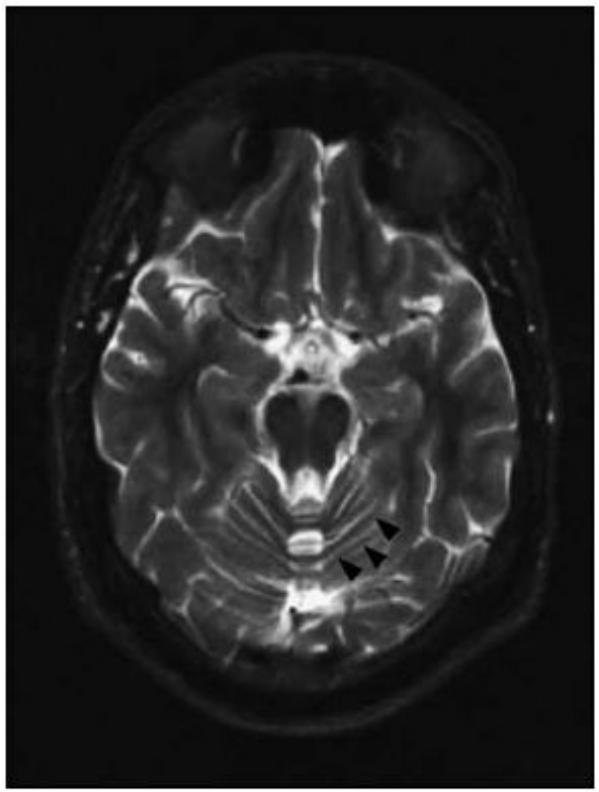
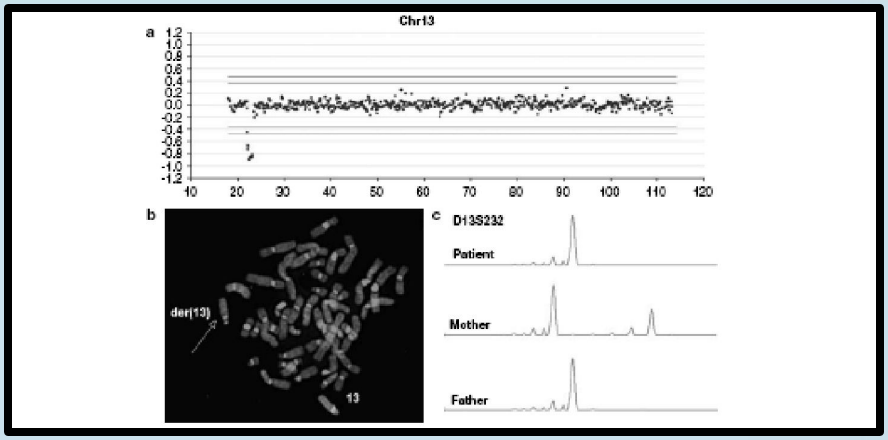


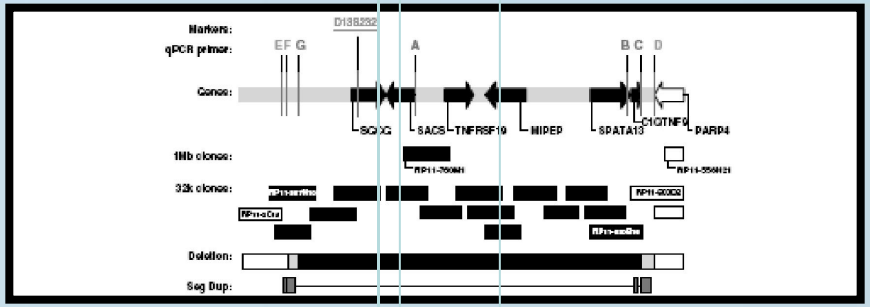
Figure 1 Brain MRI at the age of 26 years showing atrophy of the vermis superior (black arrows) and the superior cerebellar peduncles. No anomalies of the cerebral hemispheres were detected.

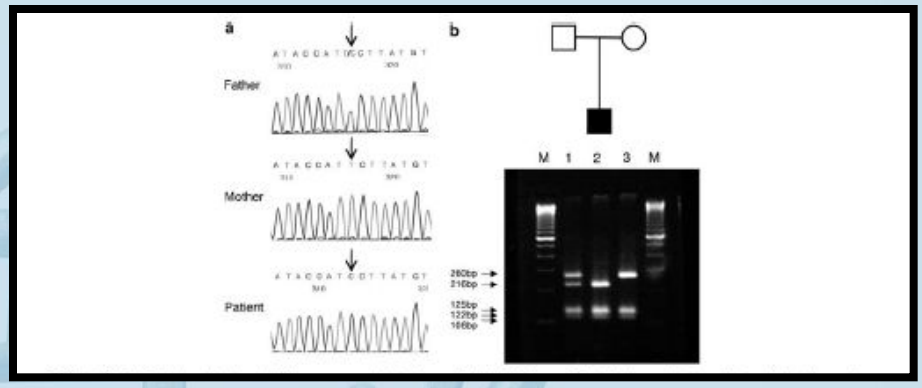
- Features:
  - Ataxia
  - Dysarythria
  - Spasticity
  - Distal muscle wasting
  - Nystagmus
  - Mitral valve prolapse (57%)
  - Prominent myelinated retinal nerve fibers
- Brain MRI: cerebellar atrophy of the upper part of the vermis and the superior cerebellar peduncles

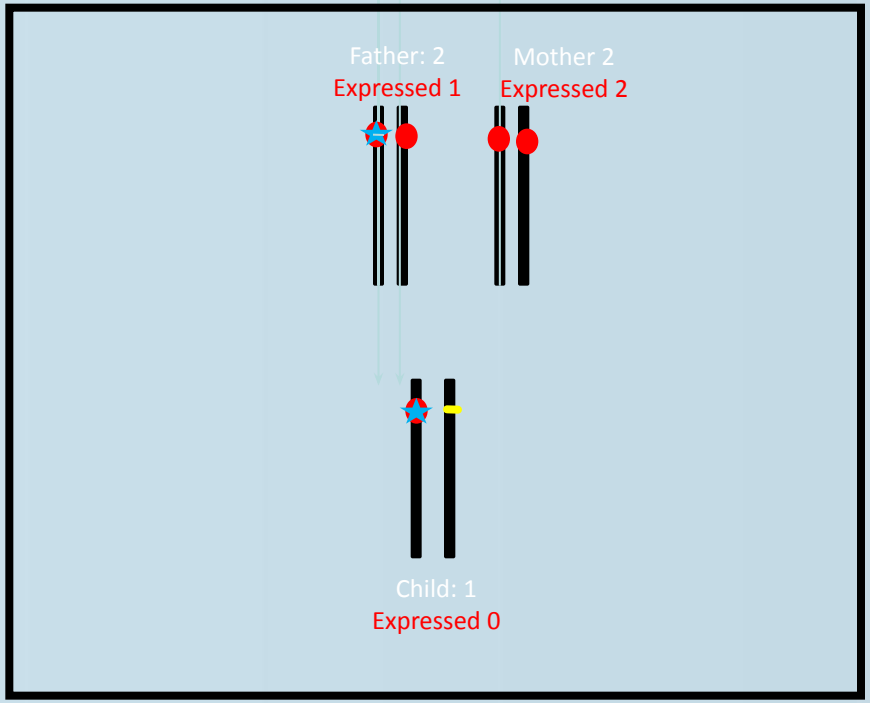
Breckpot et al., EJHG 2007

Inherited mutation, de novo deletion





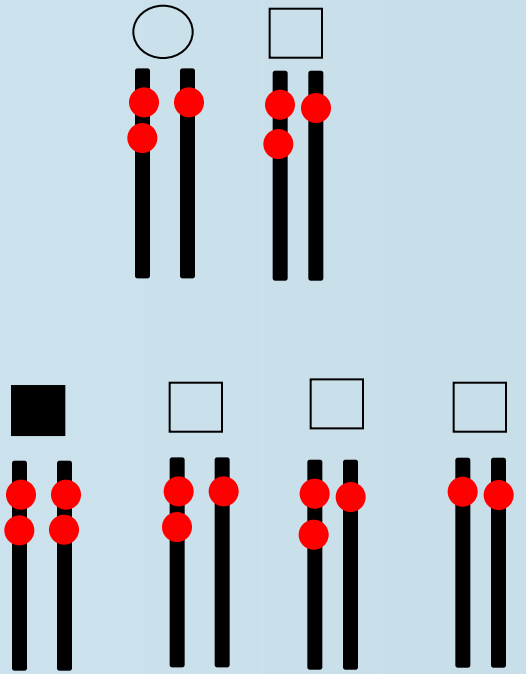




Breckpot et al., EJHG 2007

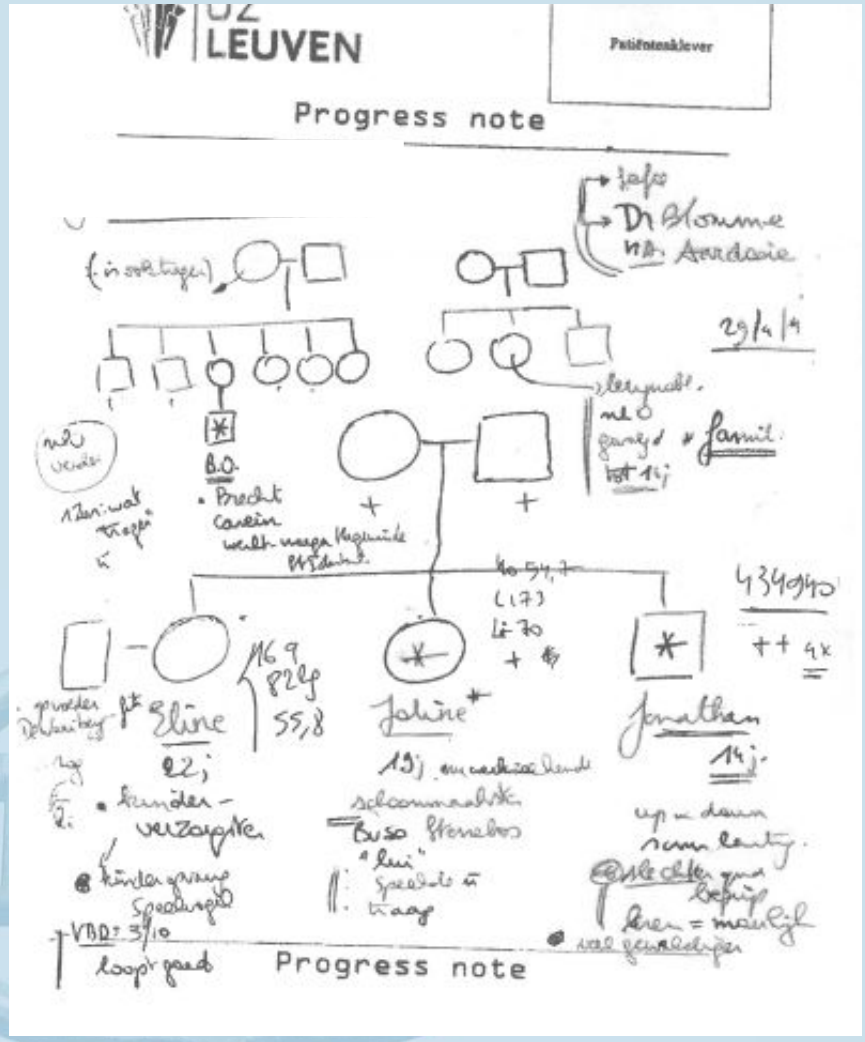
Autosomal recessive CNVs

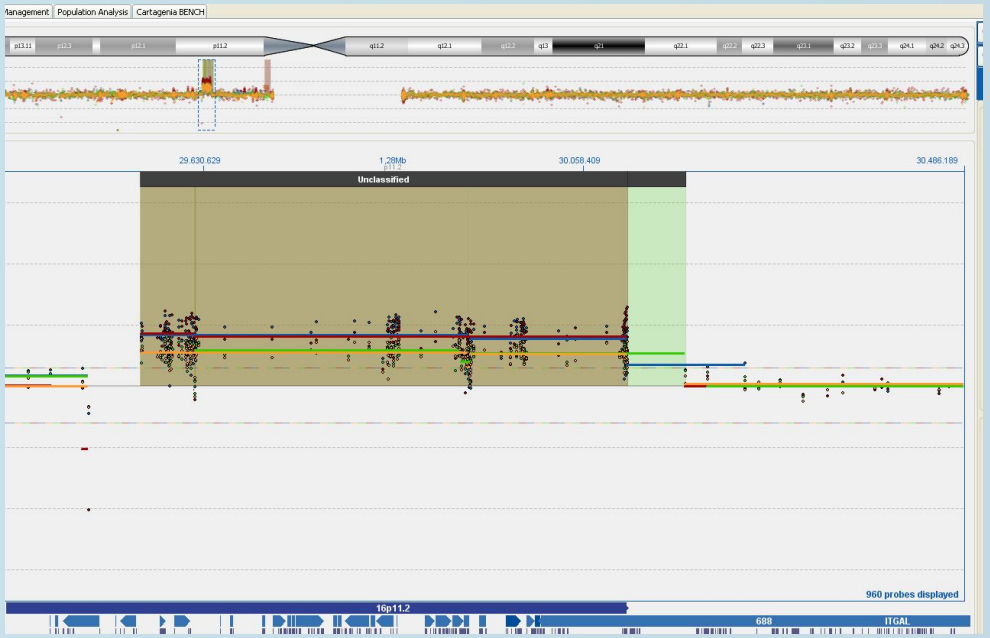
Critical copy number due to combinations of alleles



de novo is not necessarily *de novo*

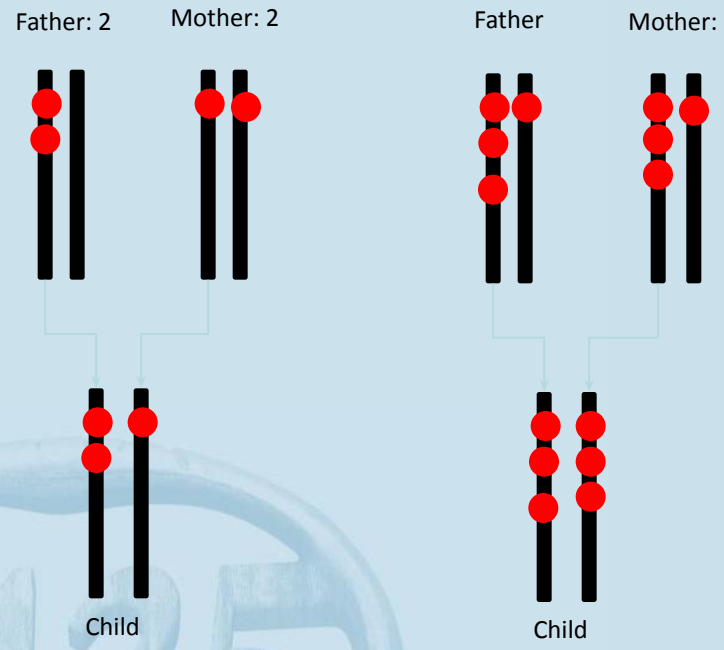
Autosomal recessive CNVs: The first example?





Autosomal dominant CNVs

Duplications



de novo is not necessarily *de novo:*

Condition: The region = CNV in some individuals:

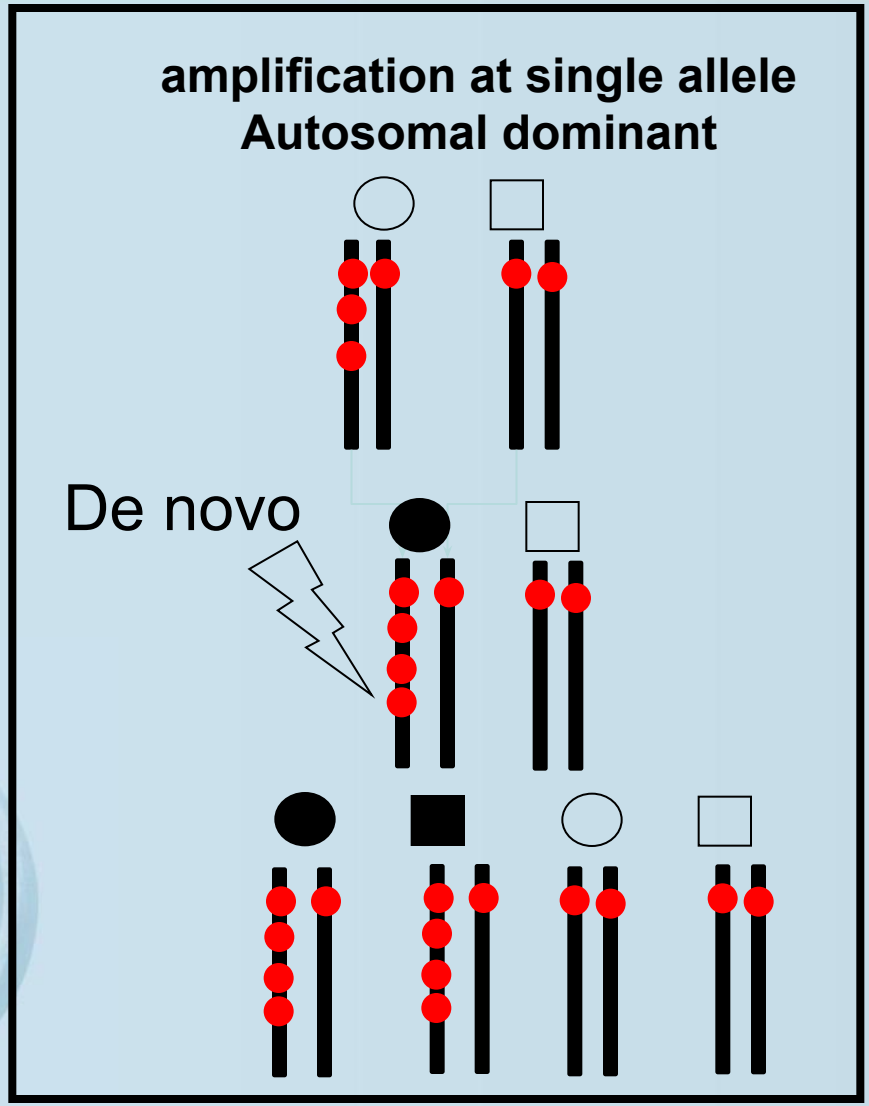
Amplifications

"de novo = de novo"

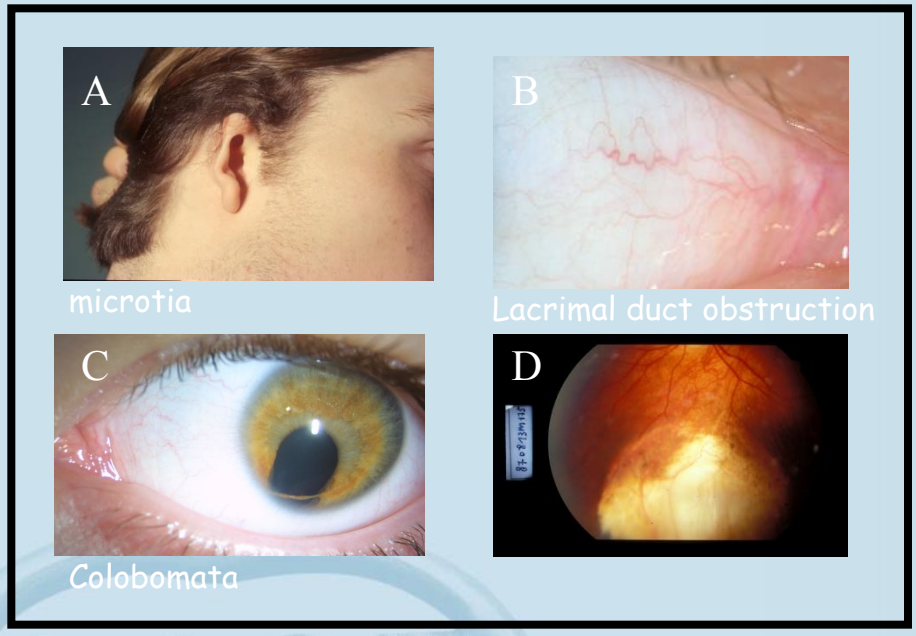
i.e. there is no inheritance mechanism to explain a new amplification

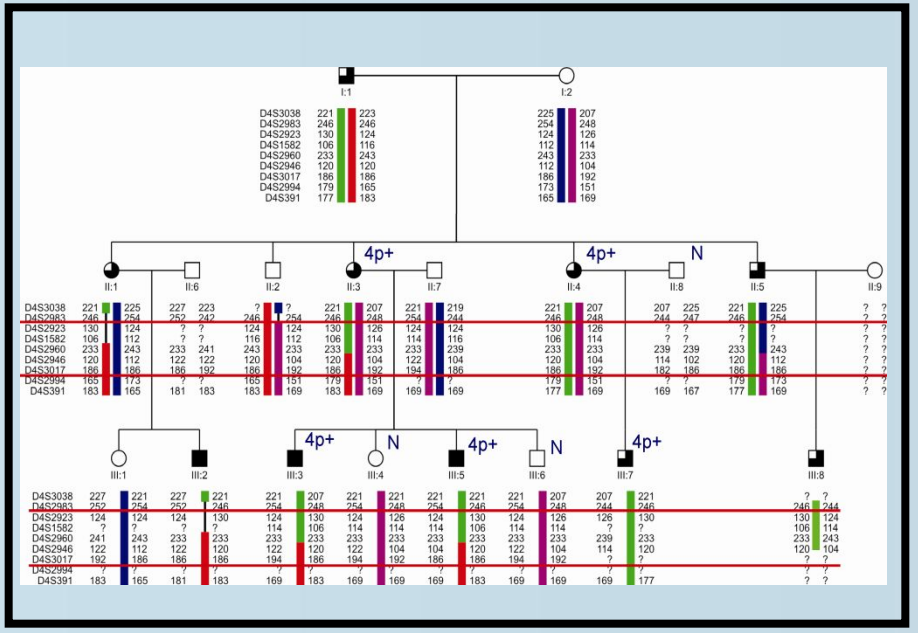
(intensity ratio difference with parents> 1.5)

Autosomal dominant CNVs



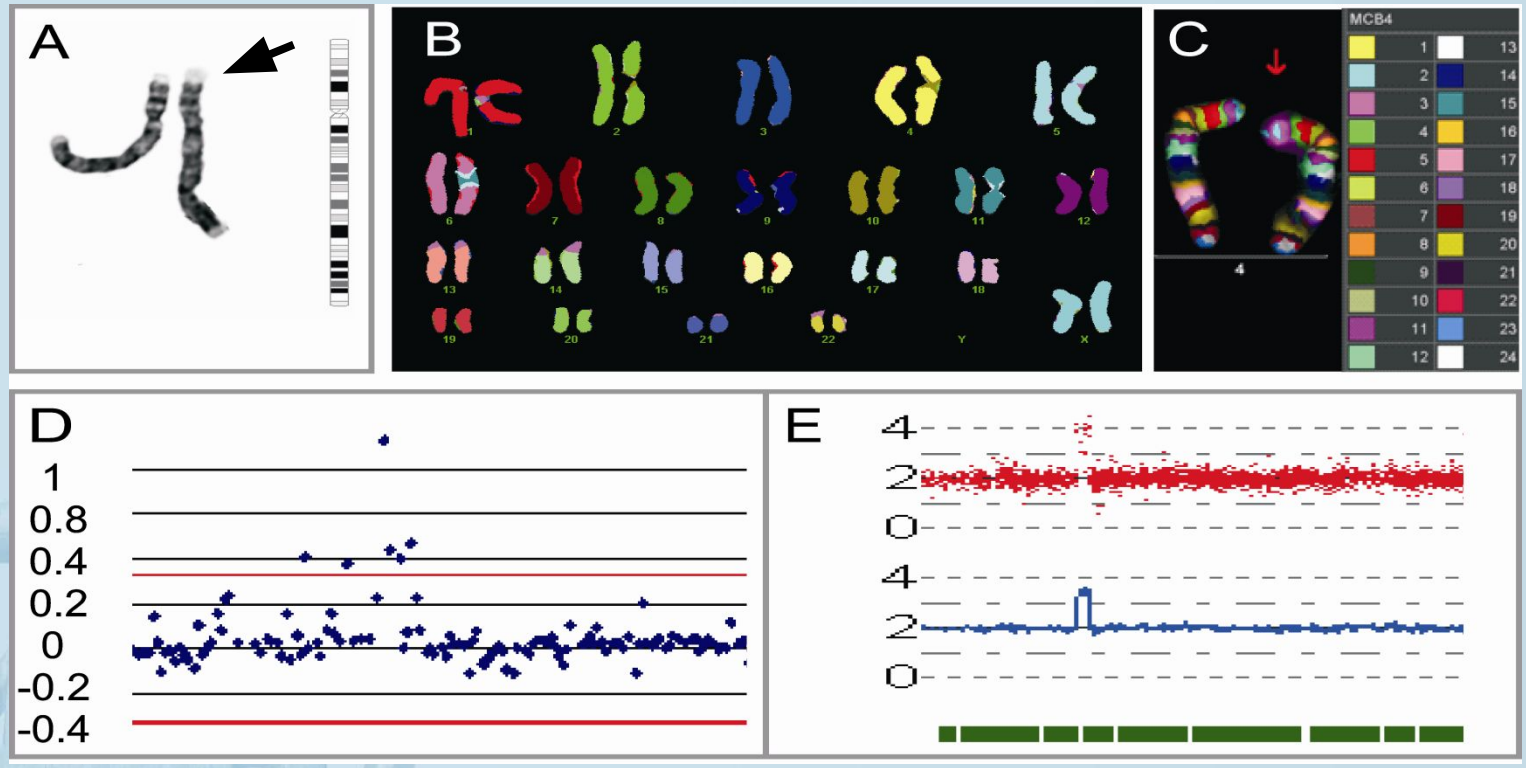
An amplification linked to autosomal dominant inherited microtia





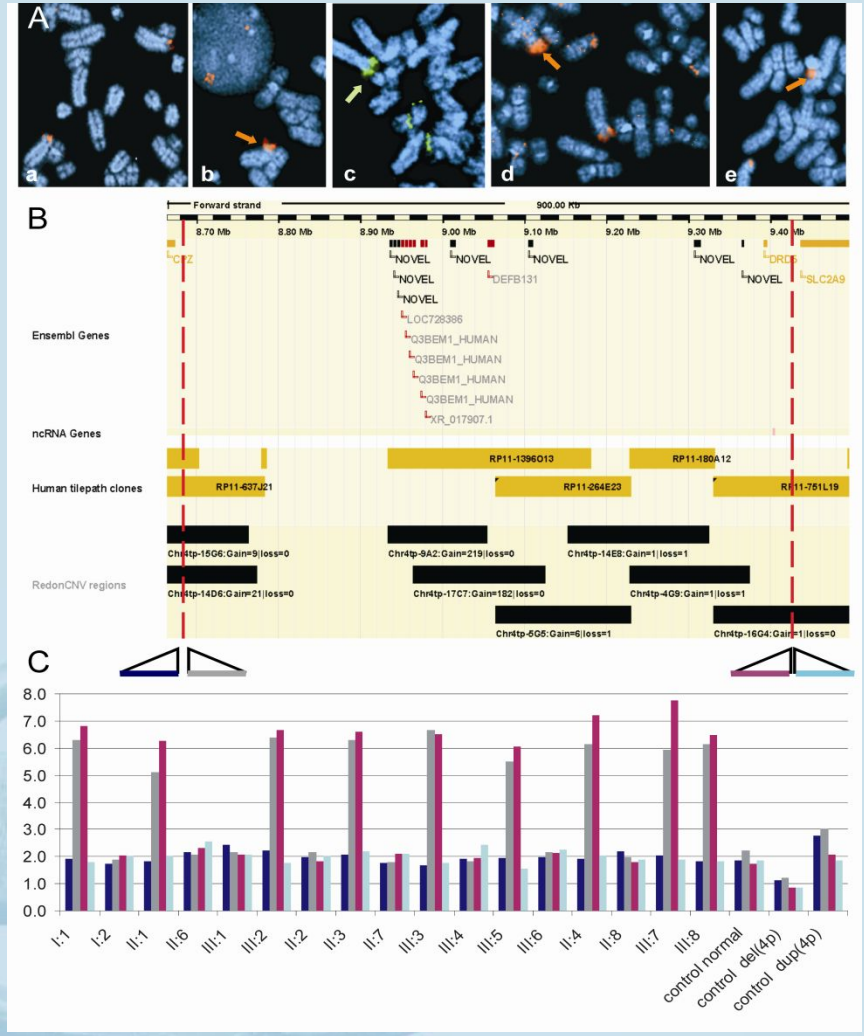
Balikova et al., AJHG, 2008

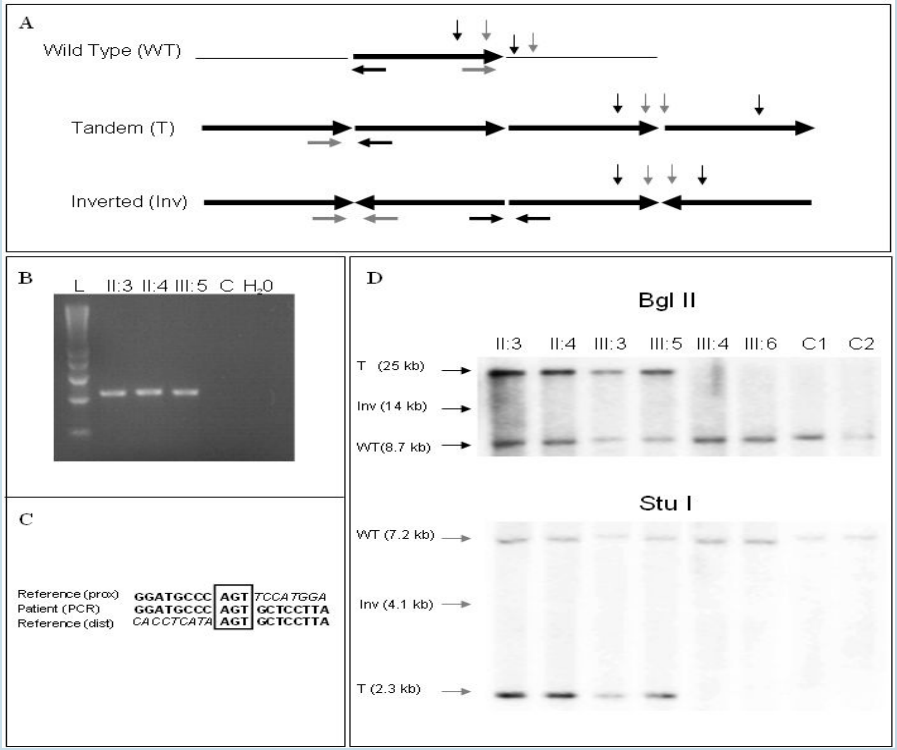
An amplification linked to autosomal dominant inherited microtia



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

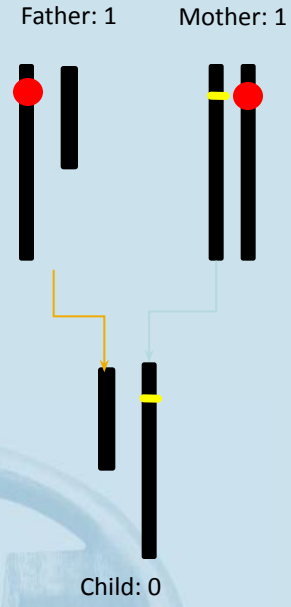
Five exact tandem copies of ~750 kb segment



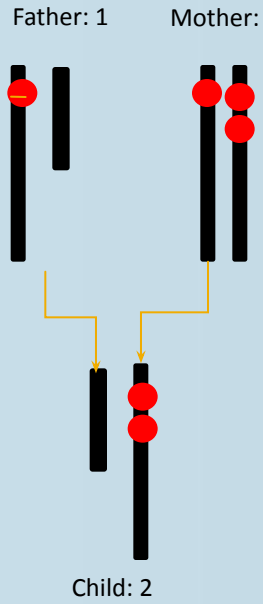


X-linked CNVs

Deletions



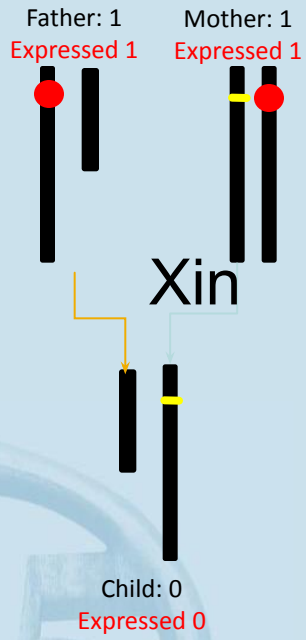
Duplications



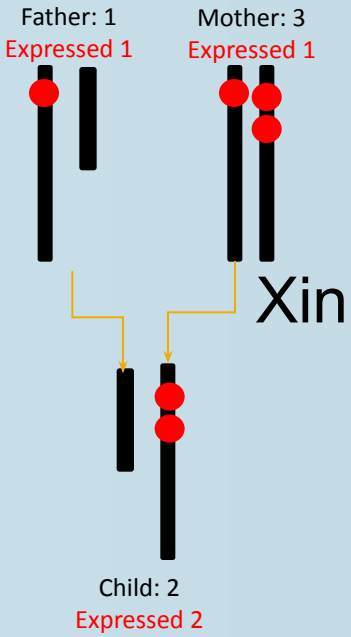
Apparently *de novo* in child is not necessarily *de novo* but inherited from mother

X-linked CNVs

Deletions



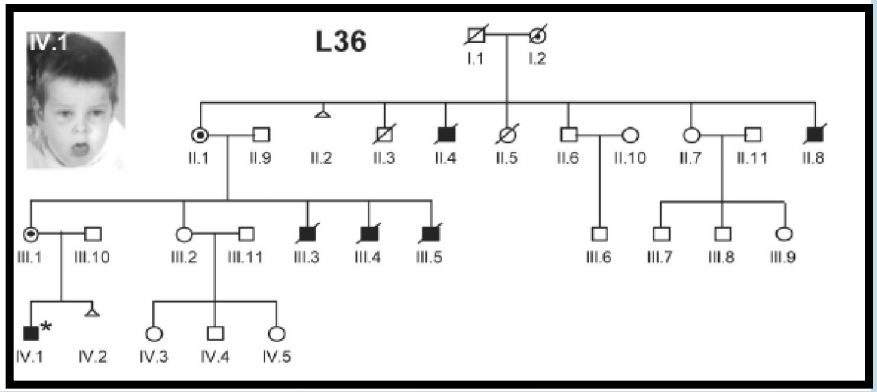
Duplications

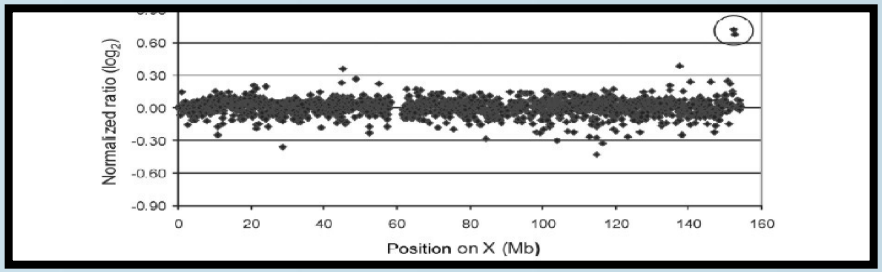


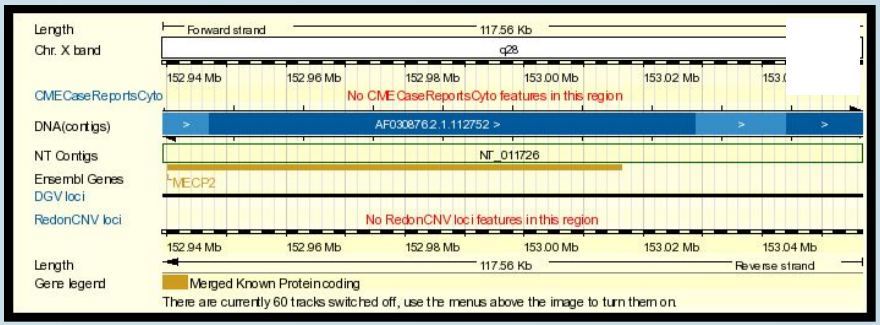
Apparently *de novo* in child is not necessarily *de novo* but inherited from mother

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



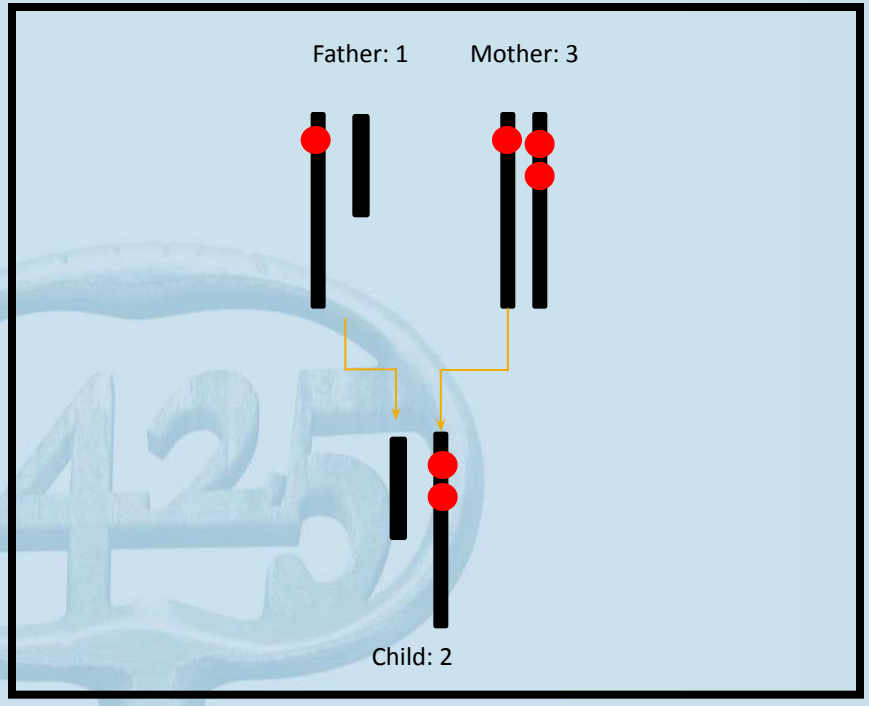


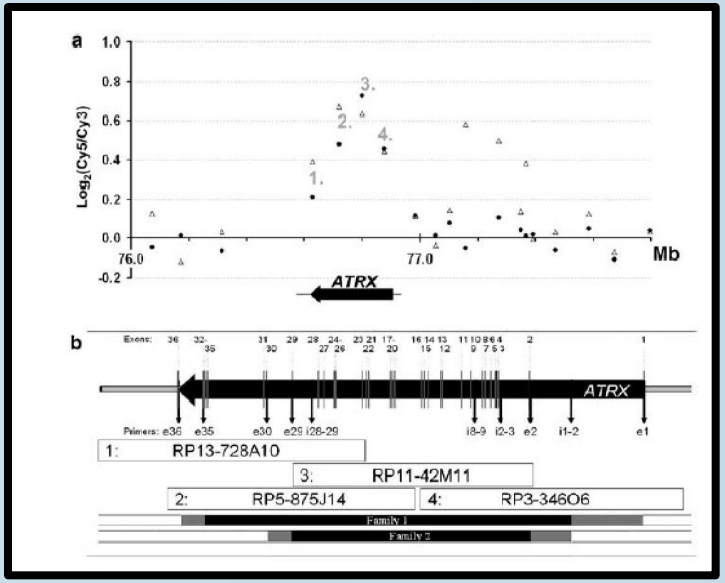


Van Esch et al. Am.J.Hum.Genet. 77:442-453, 2005

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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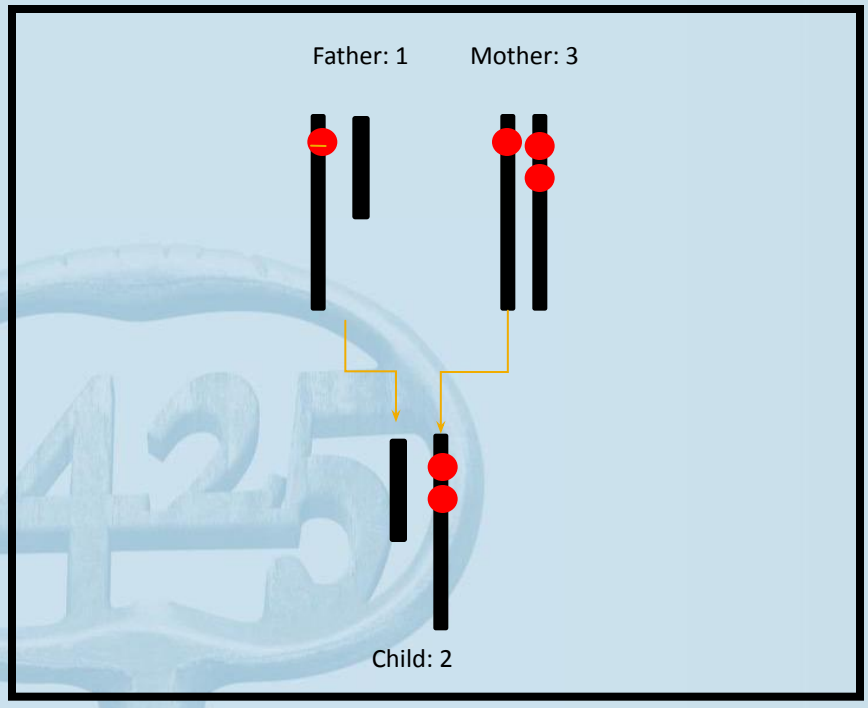

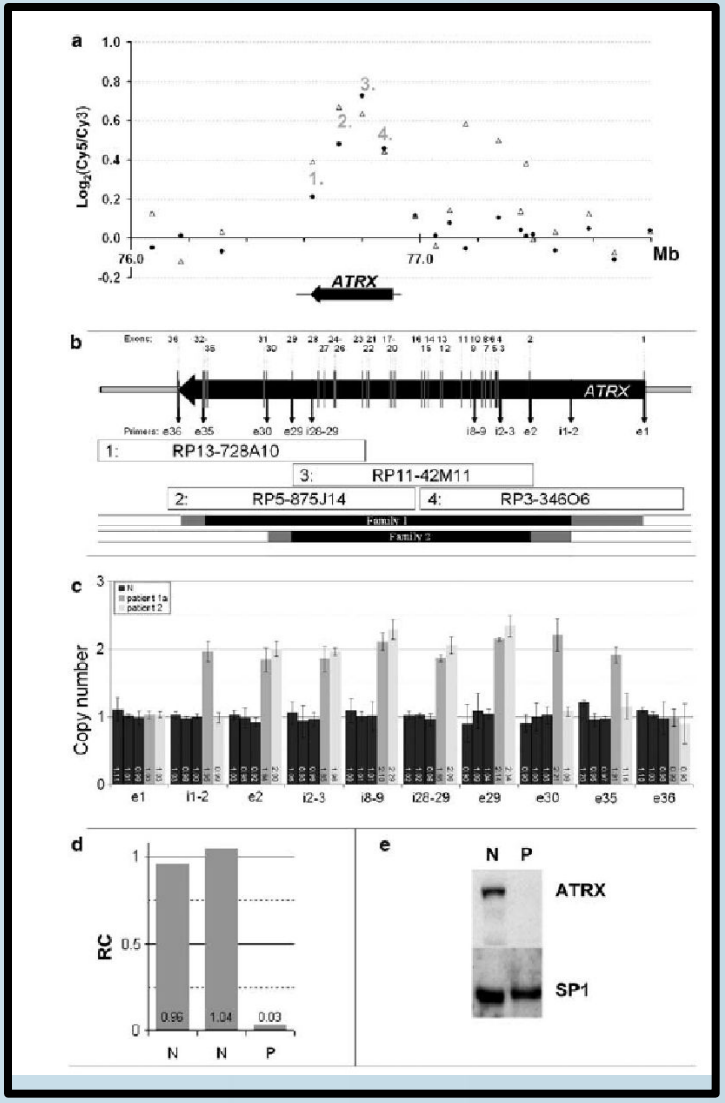

Thienpont et al. Eur.J. Hum. Gen. (2007) 15, 1094-1097

ATR-X syndrome

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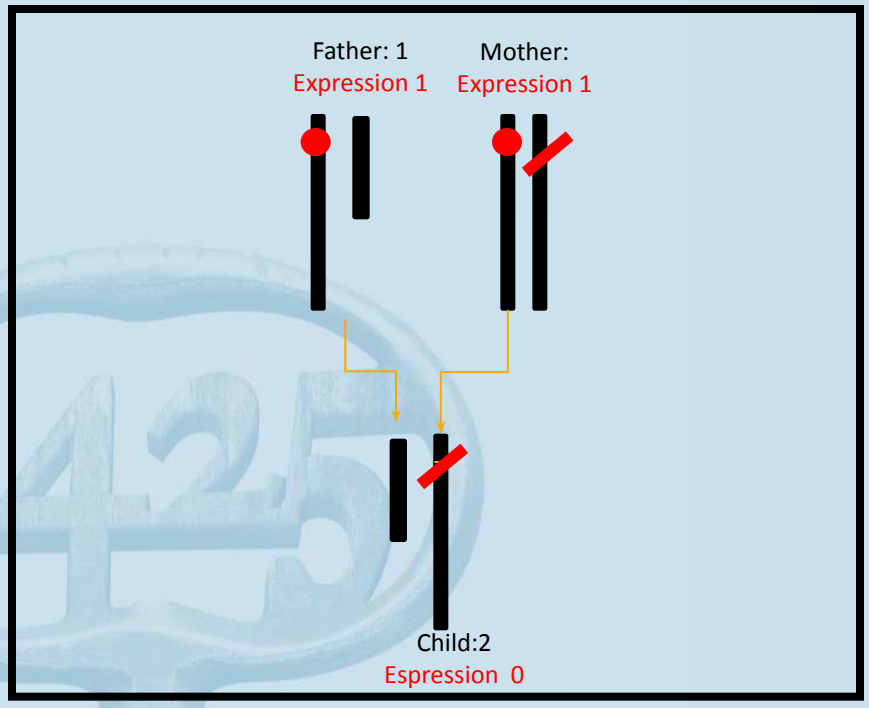
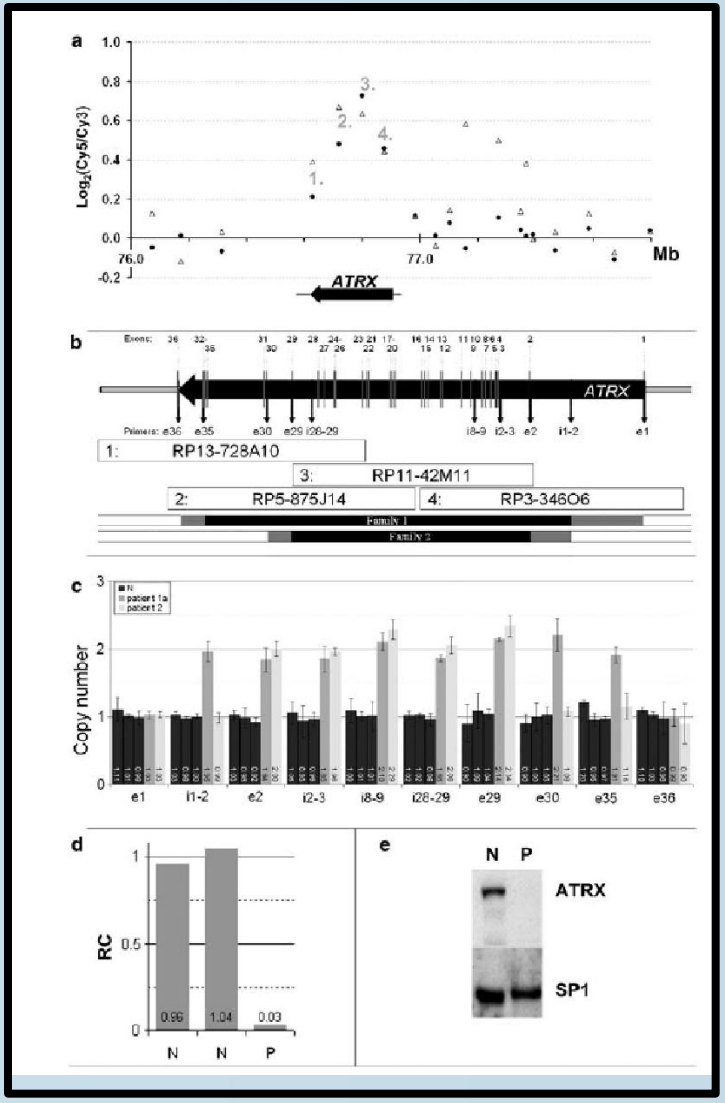


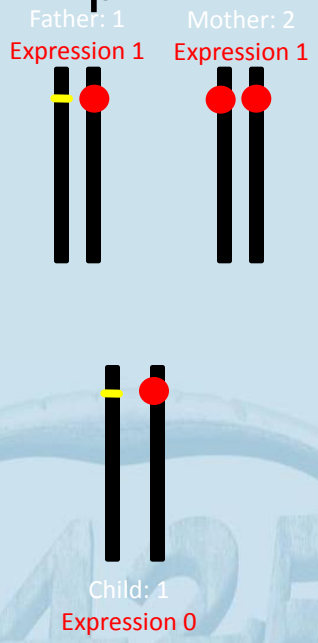


Thienpont et al. Eur.J. Hum. Gen. (2007) 15, 1094-1097

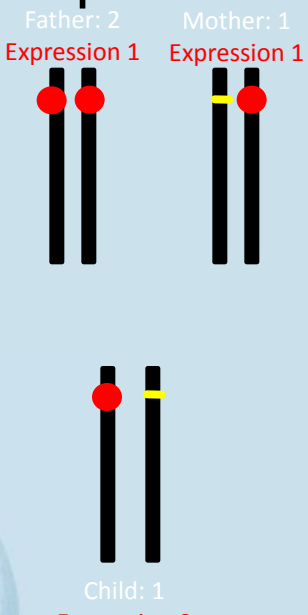
Imprinted CNVs

Deletions

Maternal imprint

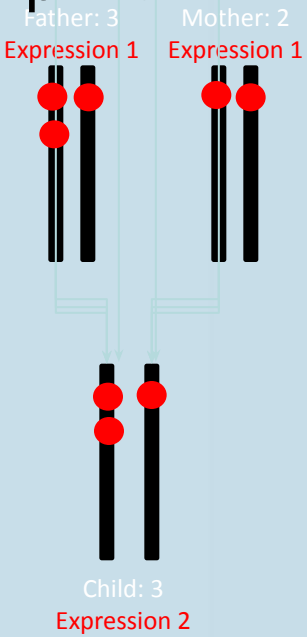


Paternal imprint

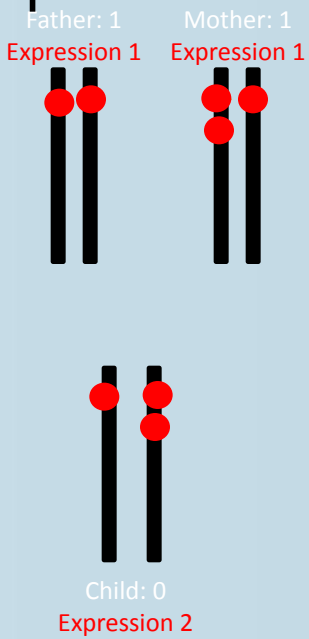


Duplications

Maternal imprint



Paternal imprint

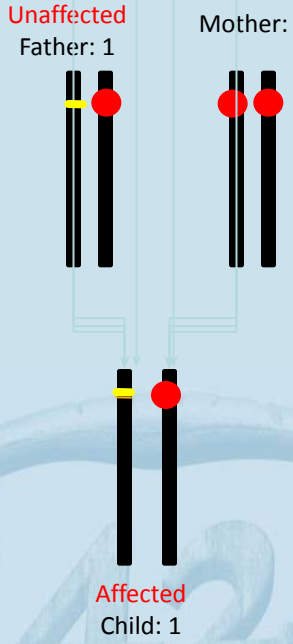


No copy number change is not necessarily *not causal*

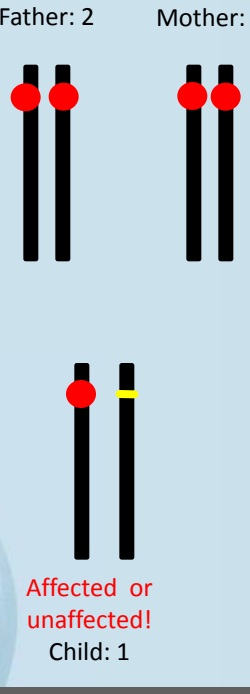
Variable expressivity and incomplete penetrance

Deletions

inherited

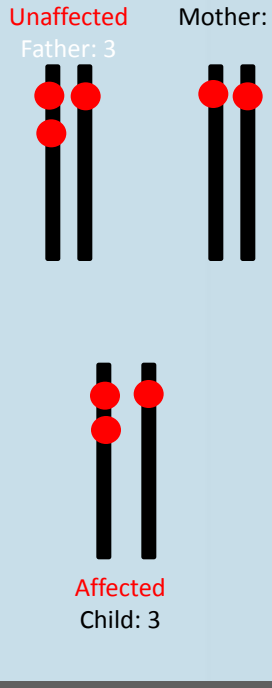


De novo

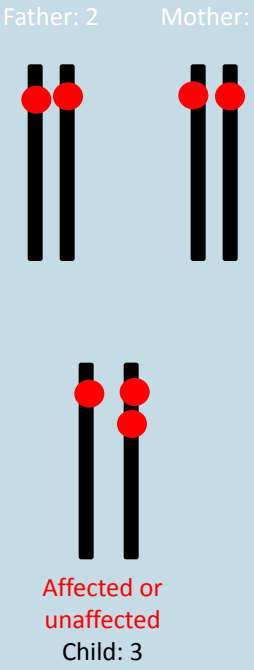


Duplications

Inherited



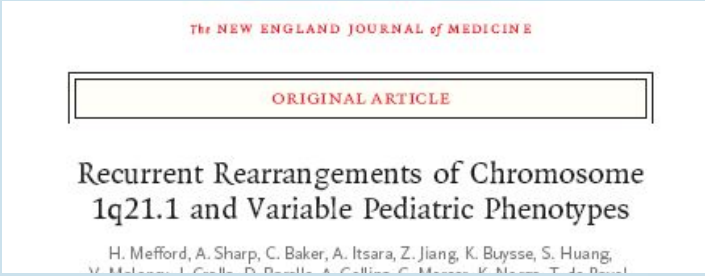
De novo



Inherited imbalances can be causal

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

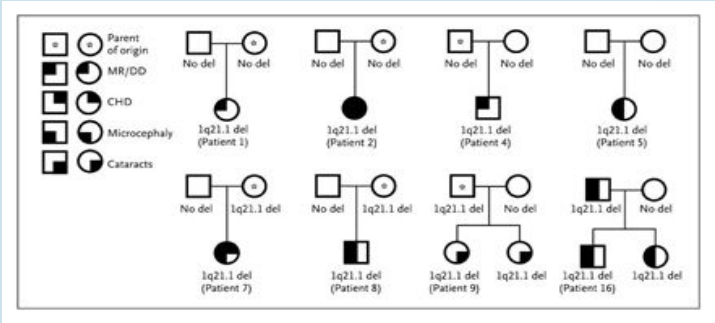
CNVs as risk factor for MR/CA (variable penetrance and expressivity)

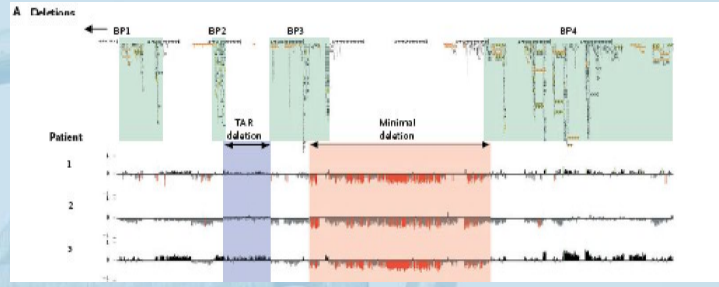

The NEW ENGLAND JOURNAL of MEDICINE

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,

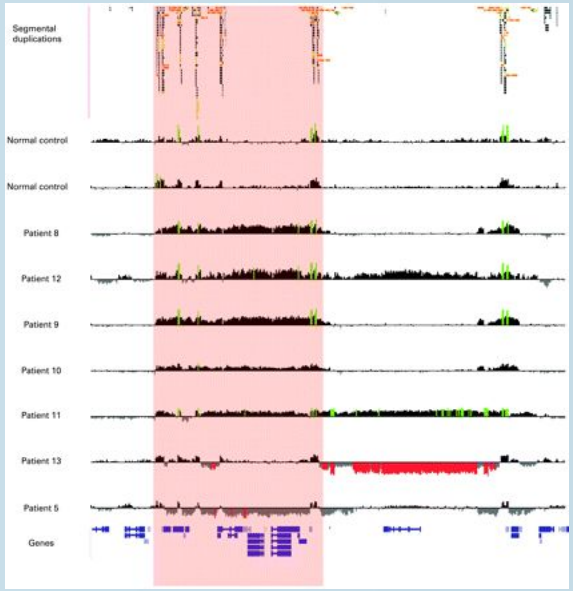




JMG ONLINE

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
$P = 1.1 \times 10^{-7}$	P = 0.02

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

CONCLUSION:

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

Mechanisms causing intrachromosomal CNVs

Recurrent CNVs (genomic disorders)

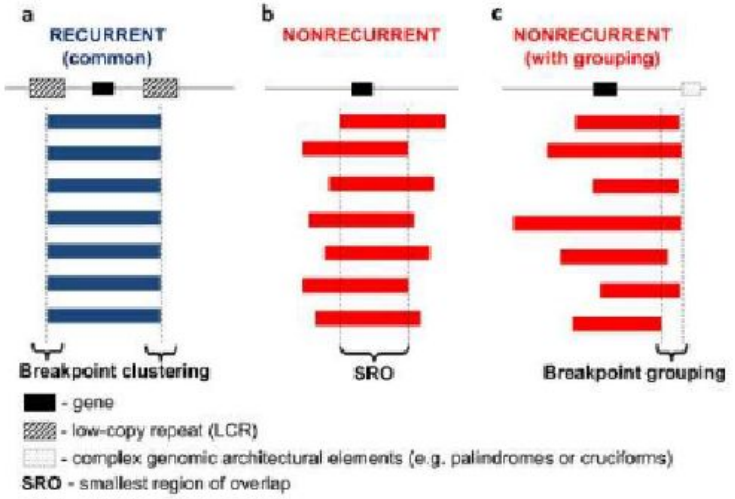
- Non-allelic Homologous recombination (NAHR)
  - Unequal crossing over
  - Break-induced replication
  - Single-strand annealing

Non-recurrent CNVs

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

Recurrent versus non-recurrent CNVs

- 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



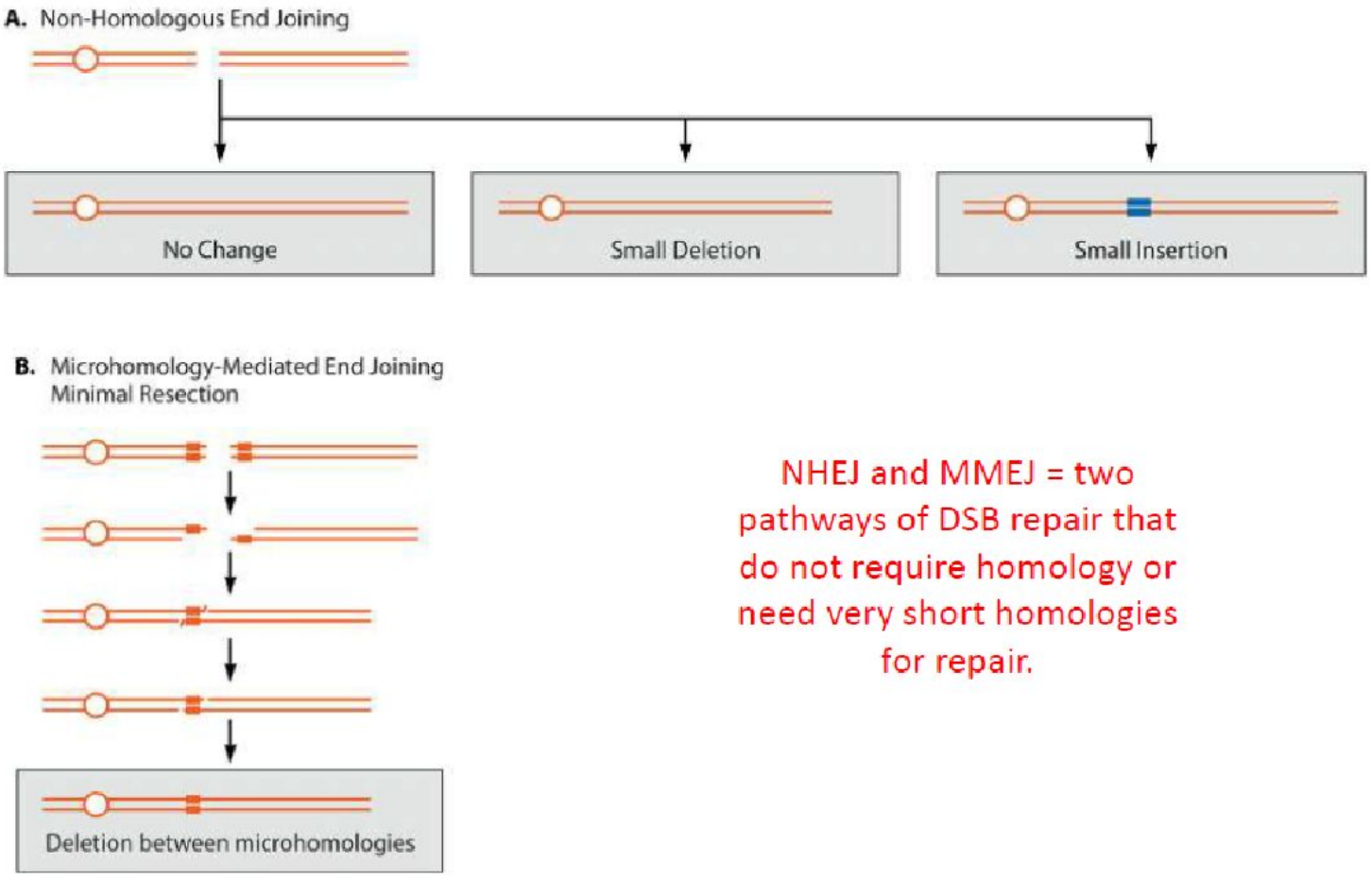
Non-recurrent chromosomal rearrangements

Mechanism of structural abnormalities

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

Non Replicative mechanisms of non-recurrent CNVs

nonhomologous non-replicative repair mechanisms



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

Genomic disorders (recurrent CNVs)

- Change in copy number driven by homologous recombination
  - Non-allelic/ectopic homologous recombination (NAHR) between low copy repeats
  - Single-strand annealing
- Meiotic (most often)

Low copy repeats (LCRs or segmental duplicons)

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

Genome wide LCRs

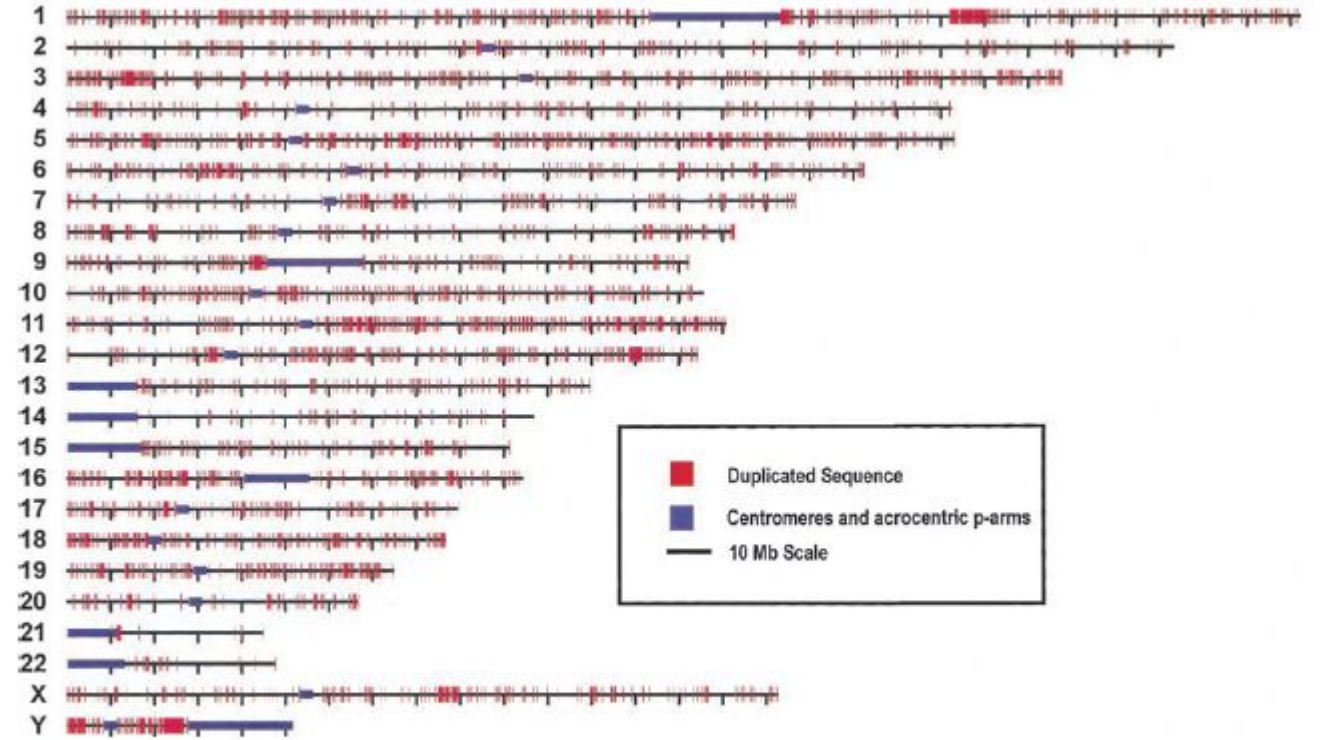
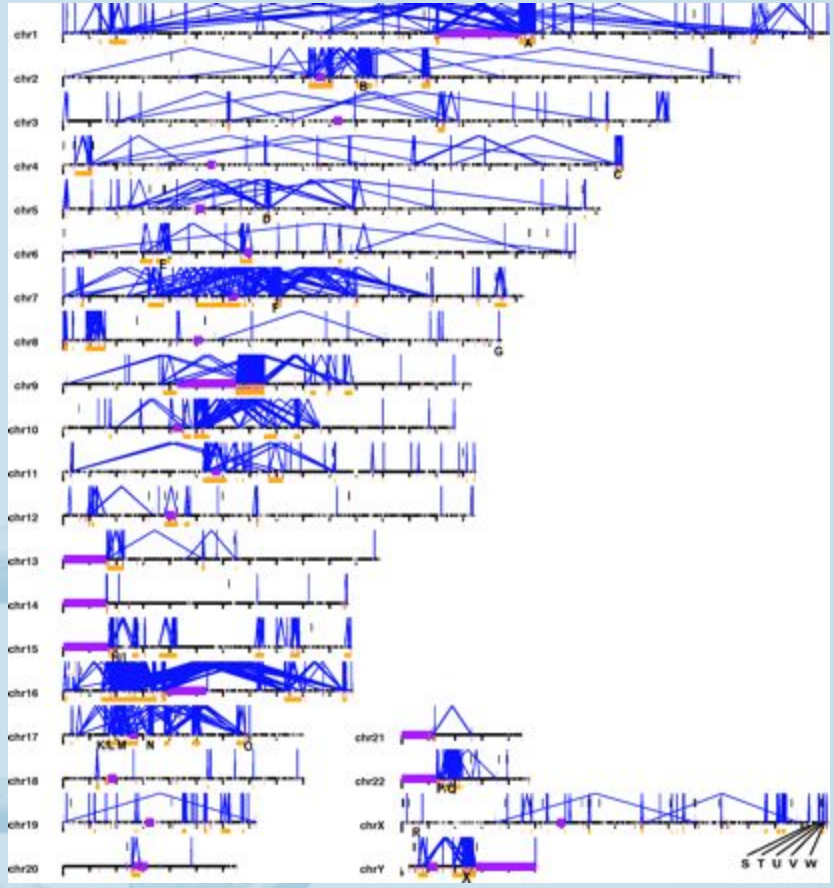


Figure 3 Genome-wide view of segmental duplications. The positions of alignments are depicted in red for each of the 24 chromosomes. Panels separate alignments on the basis of similarity: (*A*) 90%–98% identity and (*B*) 98%–100% identity. Purple bars depict centromeric gaps as well as the p-arms of 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

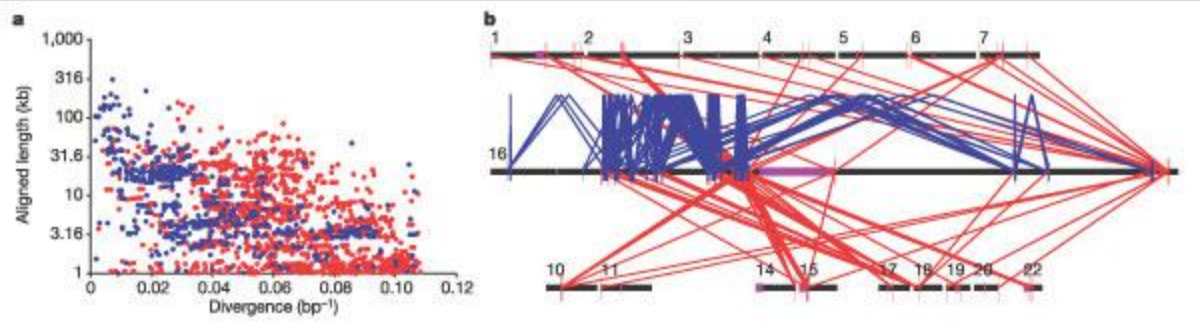
Segmental duplications in the human genome



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



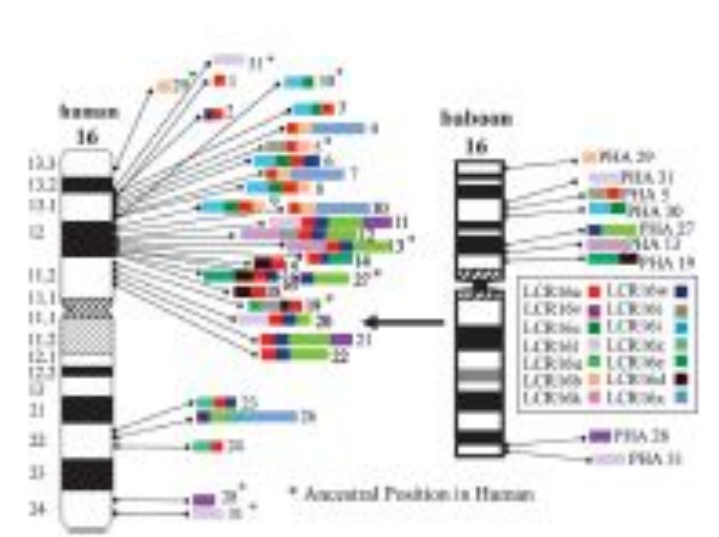
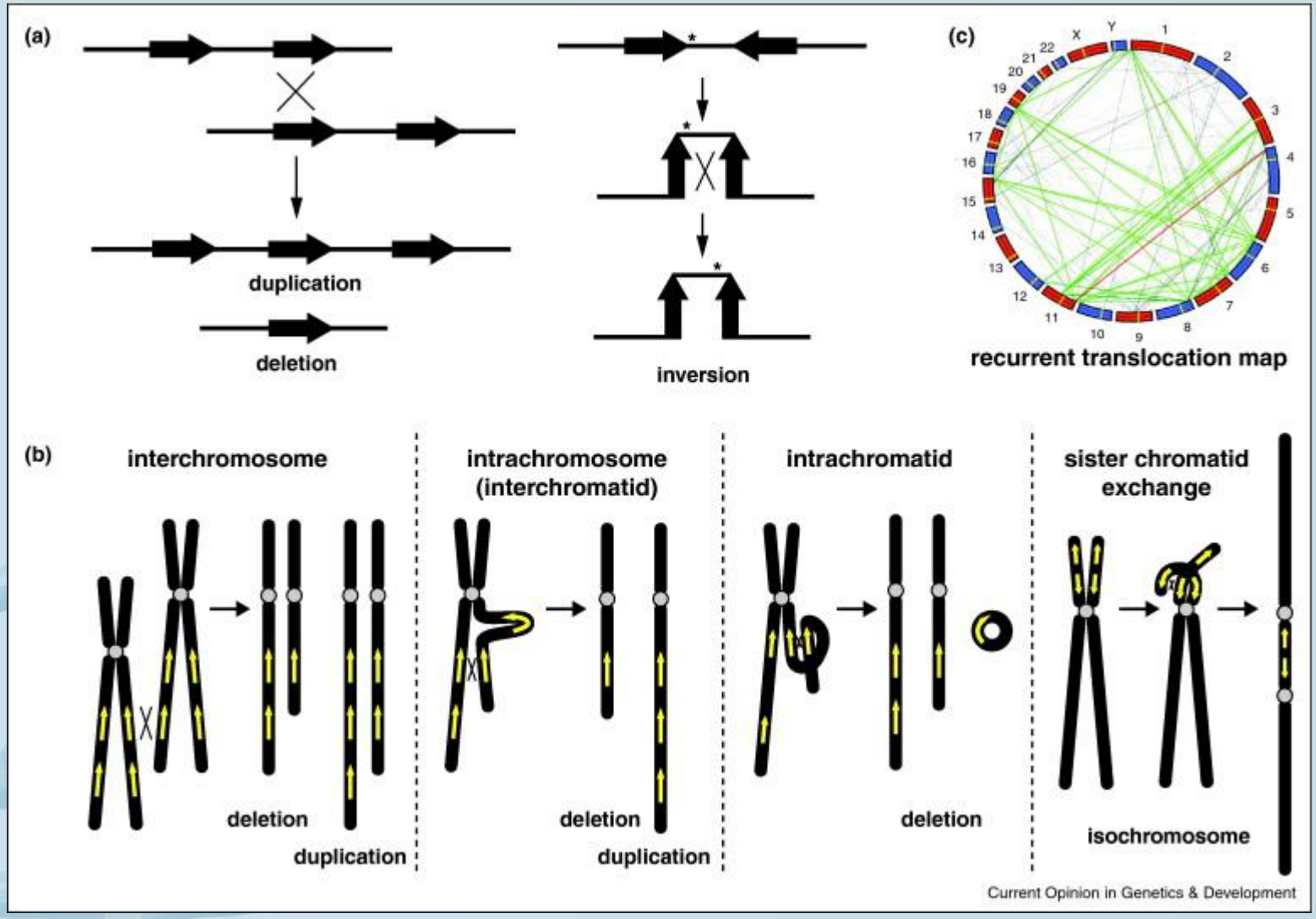


Fig. 1. LCR16 organization in human and baboon. The location, copy number, and structure of LCR16 duplications are depicted within the context of an ideogram for human (Left) and Papio hamadryas (PHA) (Right) based on the human genome reference sequence (hg16), BAC-end sequencing, and complete clone 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).

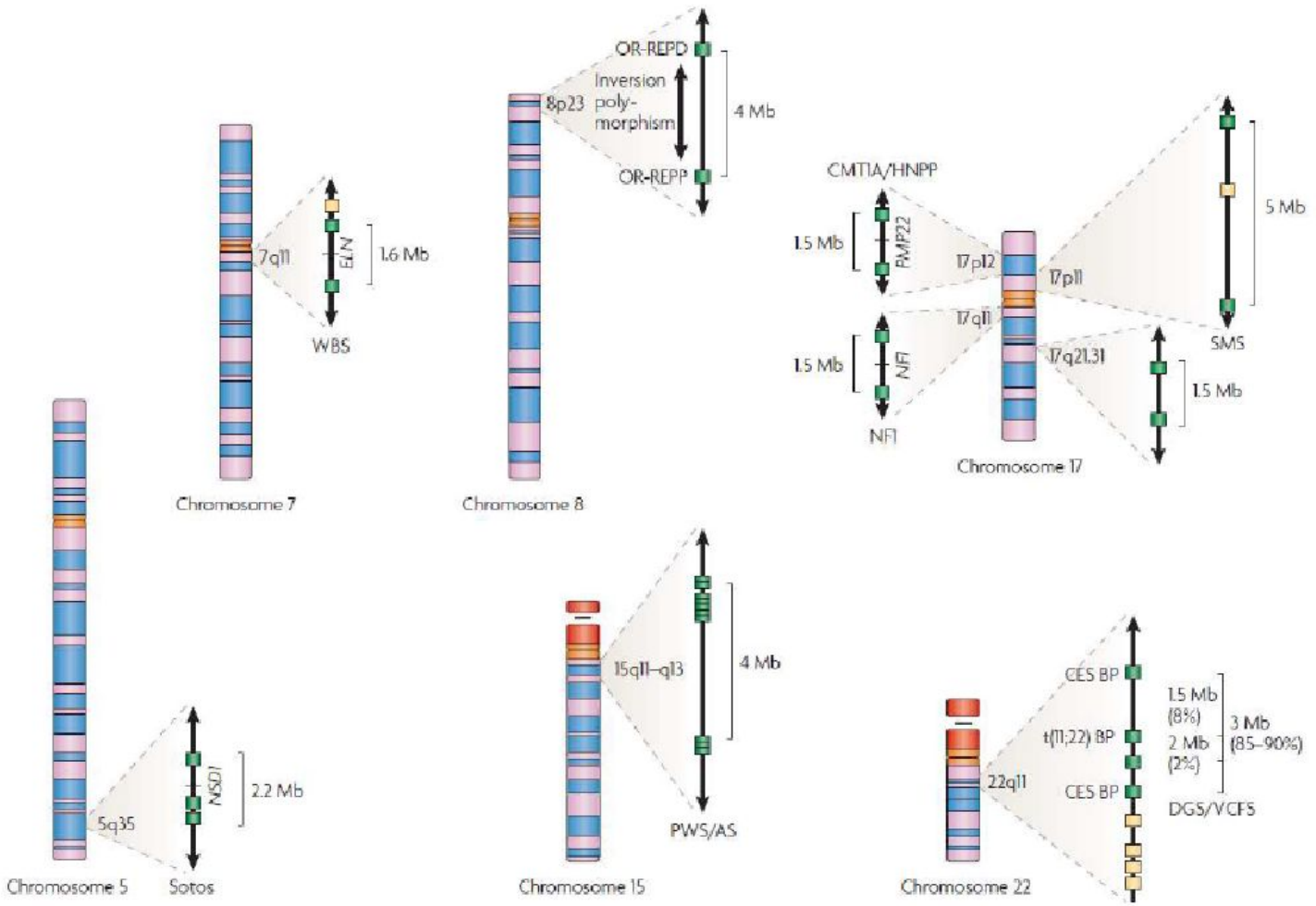
Recurrent duplication-driven transposition of DNA during hominoid evolution

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*‡‡§§

Potential rearrangemetns driven by NAHR



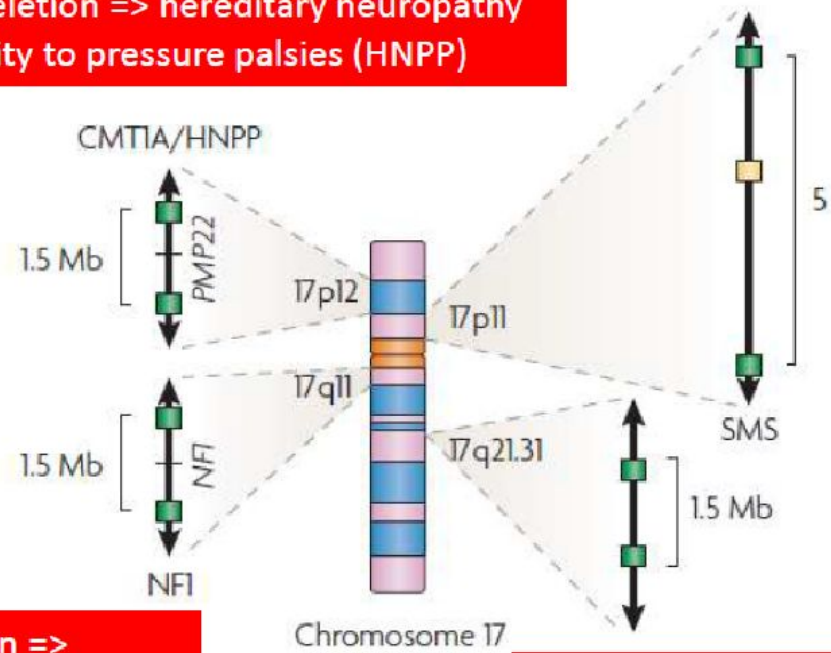
Genomic disorders



Chromosomal rearrangements mediated by segmental duplications

Genomic disorders on chromosome 17

Duplication => Charcot-Marie-Tooth type 1A
Reciprocal deletion => hereditary neuropathy and liability to pressure palsies (HNPP)



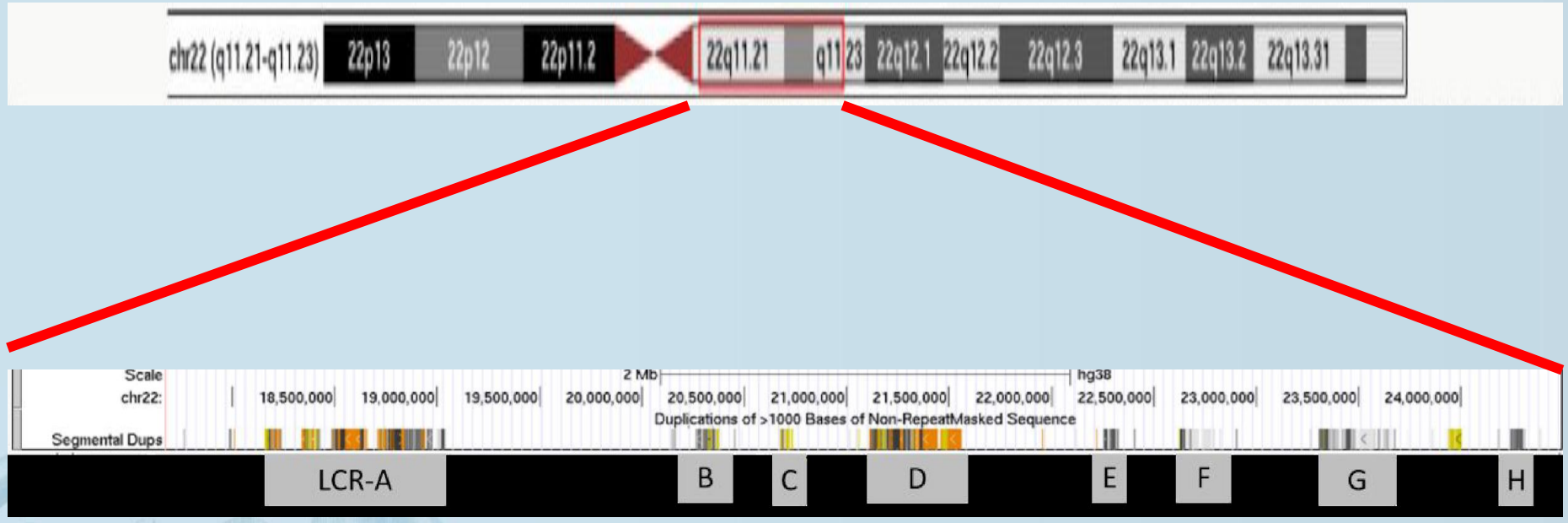
Deletion => Smith Magenis Syndrome

Duplication => Potocki-Lupski Syndrome

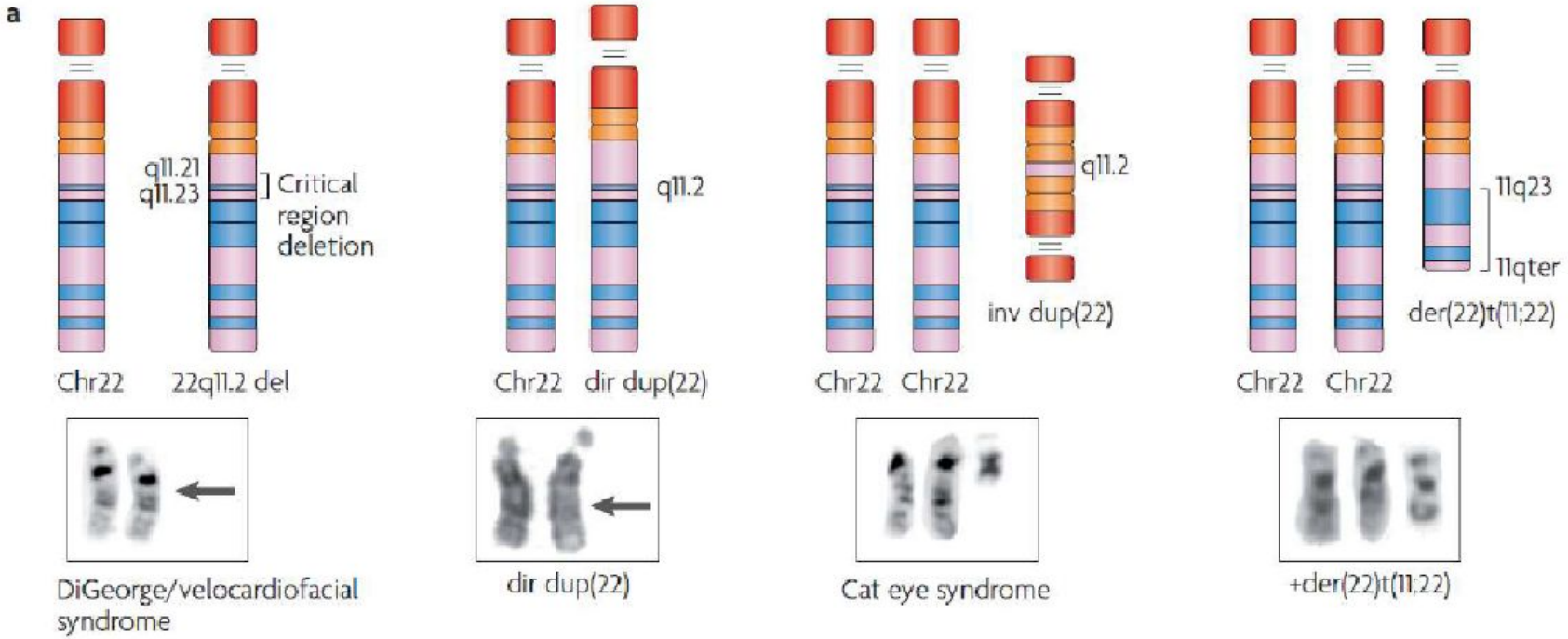
Deletion => Neurofibromatosis type I (NF)

Deletion => Koolen-de Vriese syndrome
Reciprocal duplication => 17q21.31 duplication syndrome

Genomic disorders on chromosome 22q11



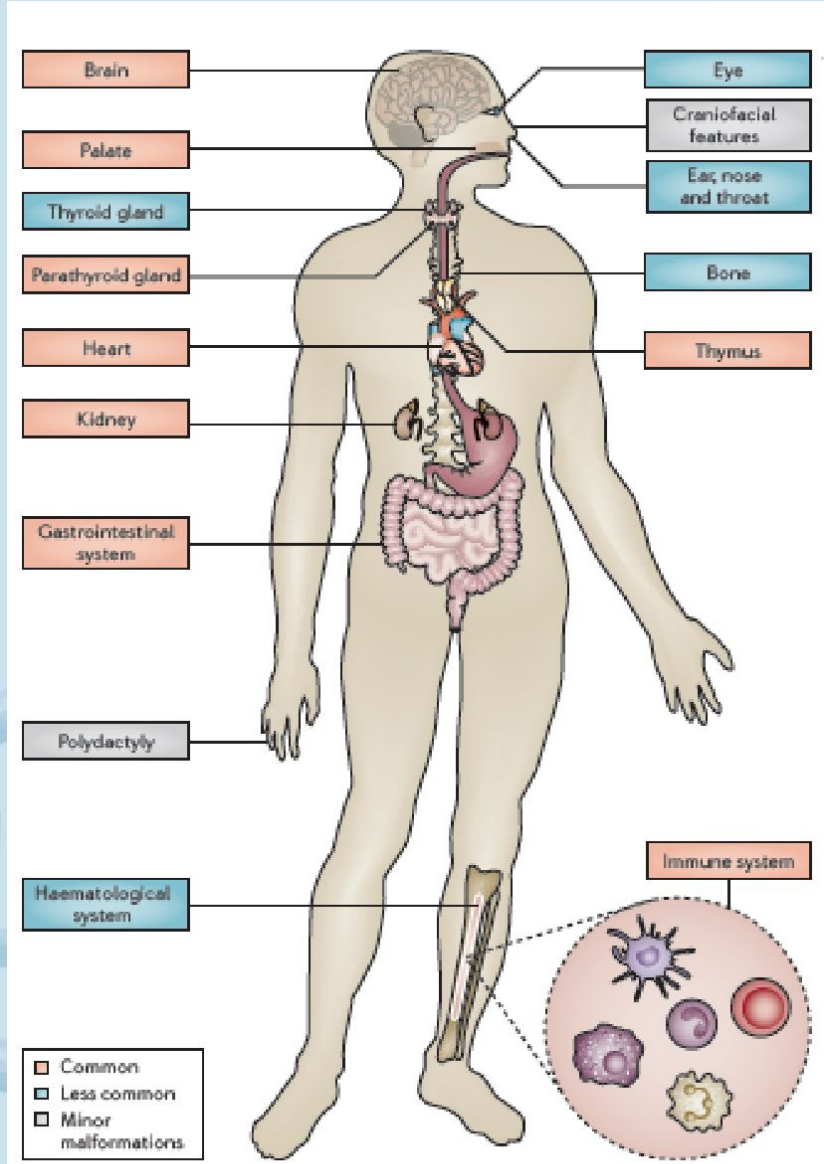
Genomic disorders on chromosome 22

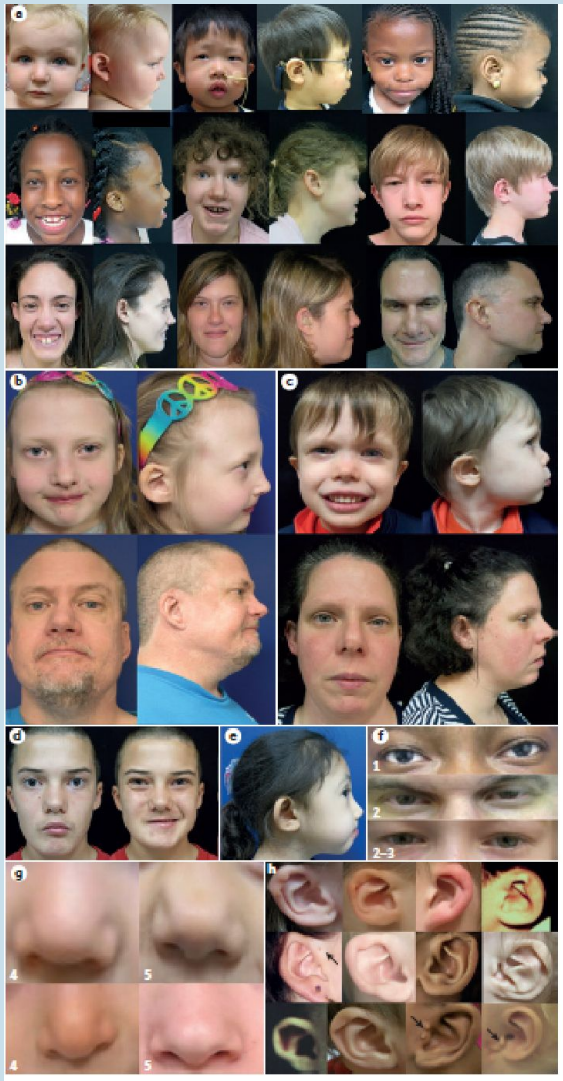


Different 22q11.2 rearrangements mediated by NAHR

- 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.

22q11DS (VCFS/Digeorge)

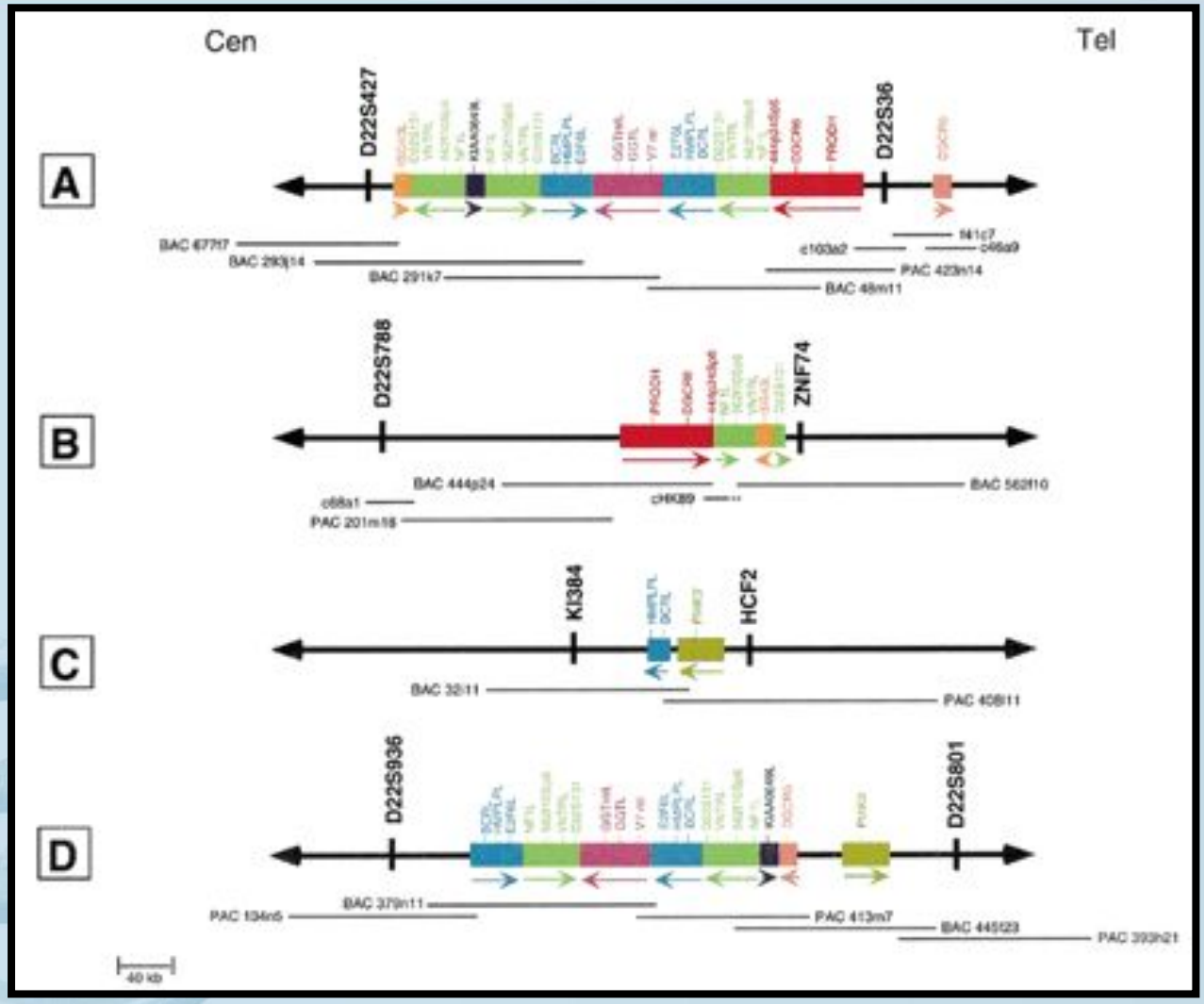




22q11.2 deletion syndrome

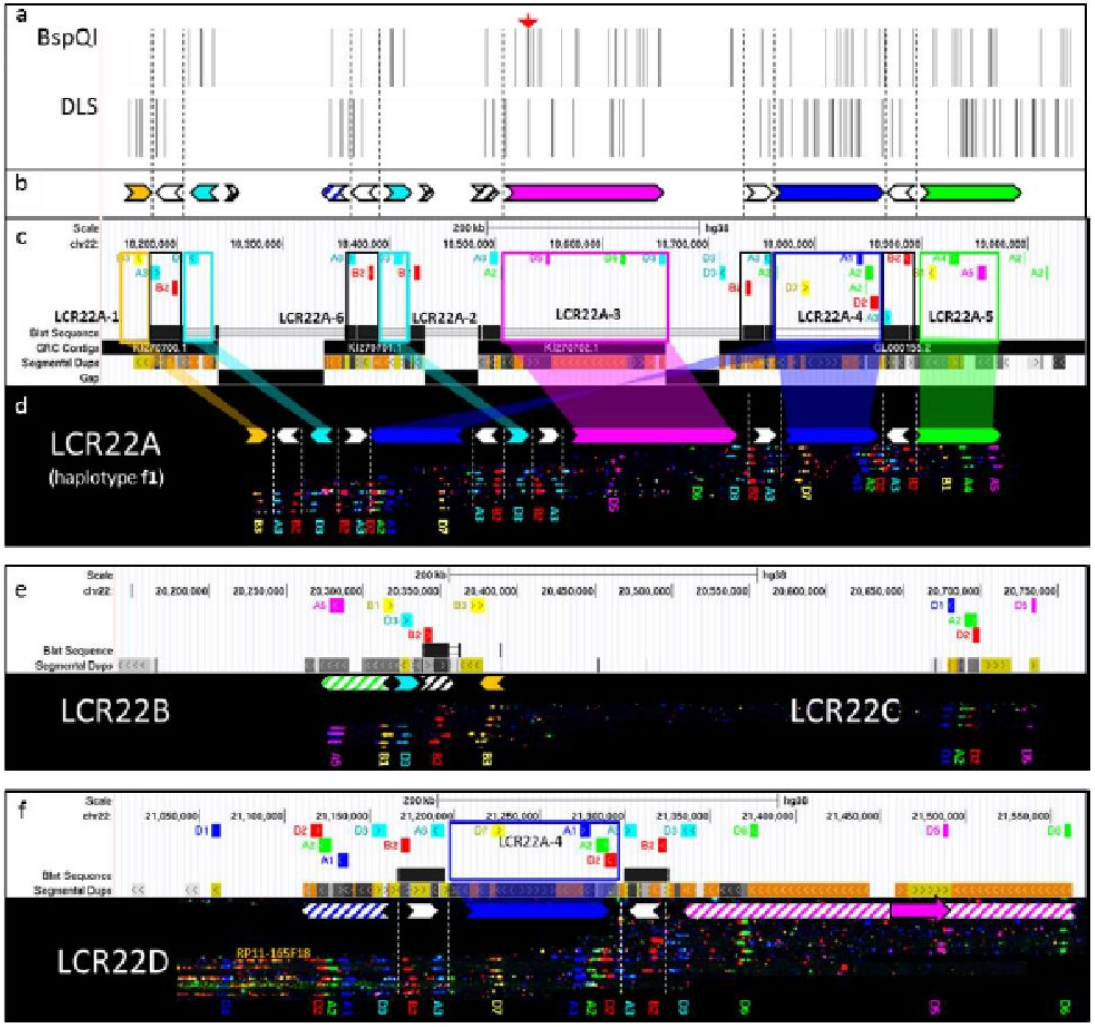
Donna M. McDonald-McGinn[1], Kathleen E. Sullivan[2], Bruno Marino[3], Nicole Philip[4], Ann Swillen[5], Jacob A. S. Vorstman[6], Elaine H. Zackai[7], Beverly S. Emanuel[7], Joris R. Vermeesch[8], Bernice E. Morrow[9], Peter J. Scambler[10] and Anne S. Bassett[11]

LCRs are patchwork of subunits



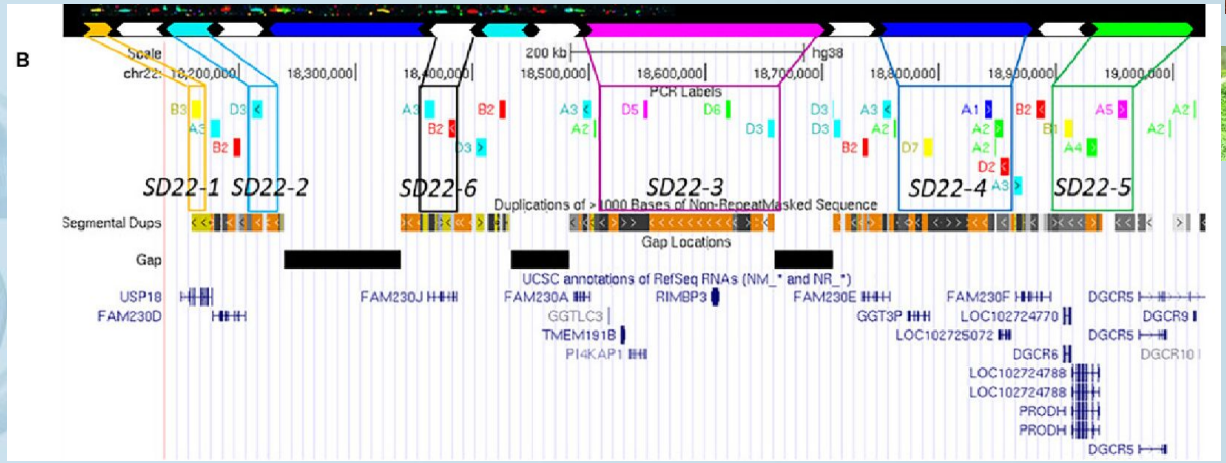
Shaikh et al., 2000
Guo et al., BMC Genomics 2011
Vervoort and Vemeesch, 2022

LCRs subunits are arranged in larger duplicons



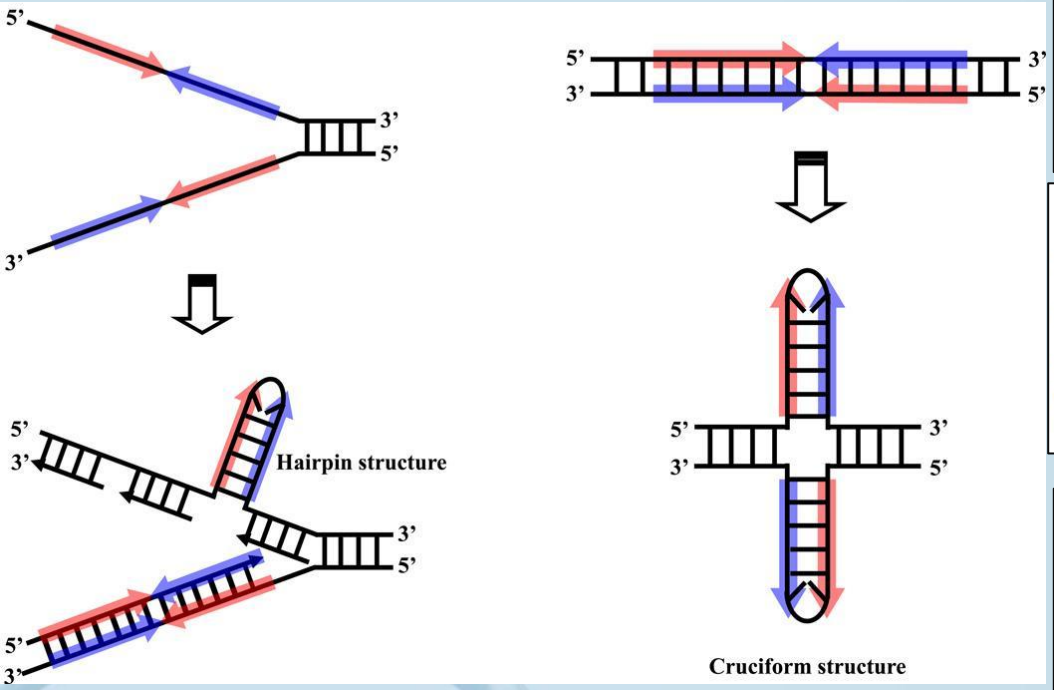
Demaerel et al., Genome Research, 2019

LCR A variability is human specific



Vervoort et al., Frontiers in Genetics, 2021

Not only NAHR but palindromes driven rearrangements can cause 22q11 rearrangements



Am J Hum Genet. 2003 Mar;72(3):733-8. doi: 10.1086/368062. Epub 2003 Jan 29.

The constitutional t(17;22): another translocatio mediated by palindromic AT-rich repeats

Hiroki Kurahashi [1], Tamim Shaikh, Masayuki Takata, Tatsushi Toda, Beverly S Emanuel

Hum Mol Genet. 2021 Feb 25;29(24):3872-3881. doi: 10.1093/hmg/ddaa251.

Double strand breaks (DSBs) as indicators of genomi instability in PATRR-mediated translocations

Sarah Correll-Tash [1], Brenna Lilley [1], Harold Salmons Iv [1], Elisabeth Mlynarski [1], Colleen P Franconi [1], Meghan McNamara [1], Carson Woodbury [1], Charles A Easley [2], Beverly S Emanuel [1 3]

Genome Res. 2007 Apr;17(4):451-60. doi: 10.1101/gr.5651507. Epub 2007 Feb 6.

AT-rich repeats associated with chromosome 22q11 rearrangement disorders shape human genome architecture on Yq12

Melanie Babcock [1], Svetlana Yatsenko, Pawel Stankiewicz, James R Lupski, Bernice E Morrow

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

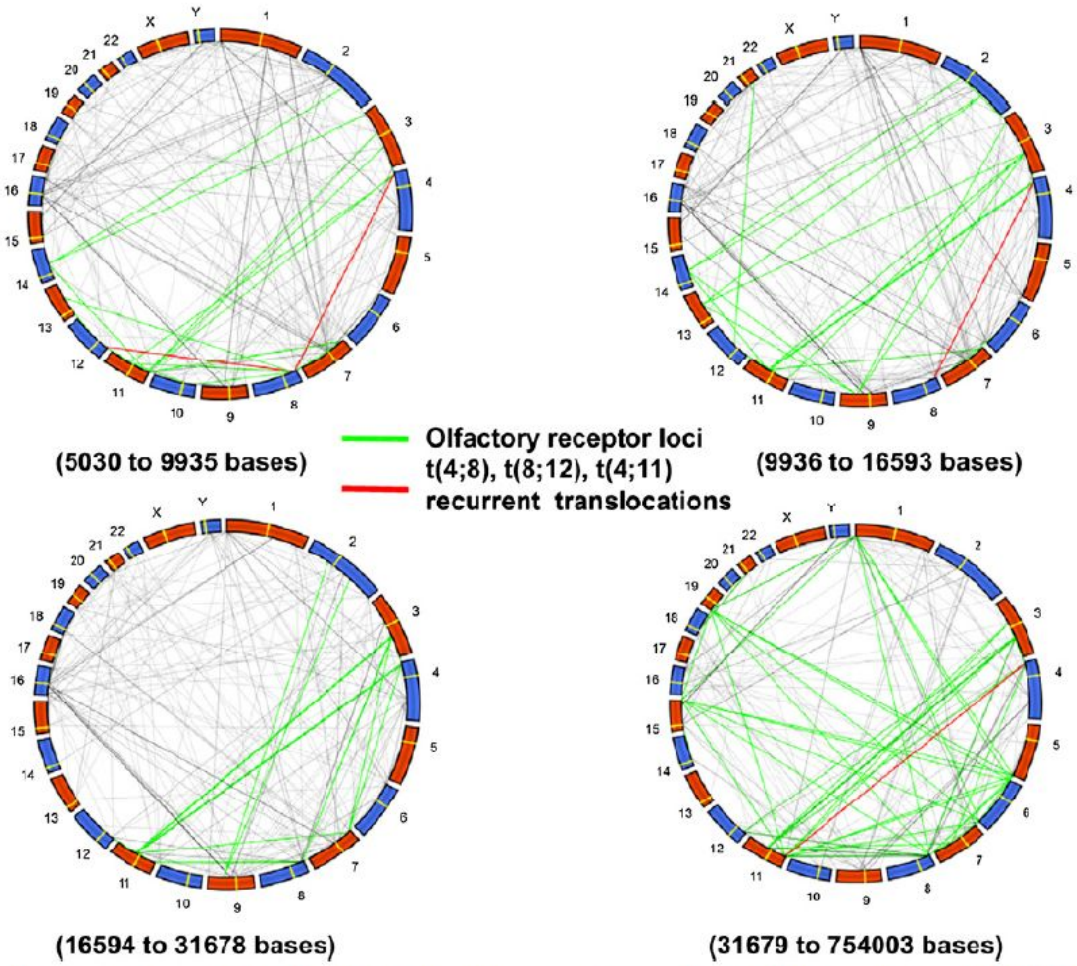
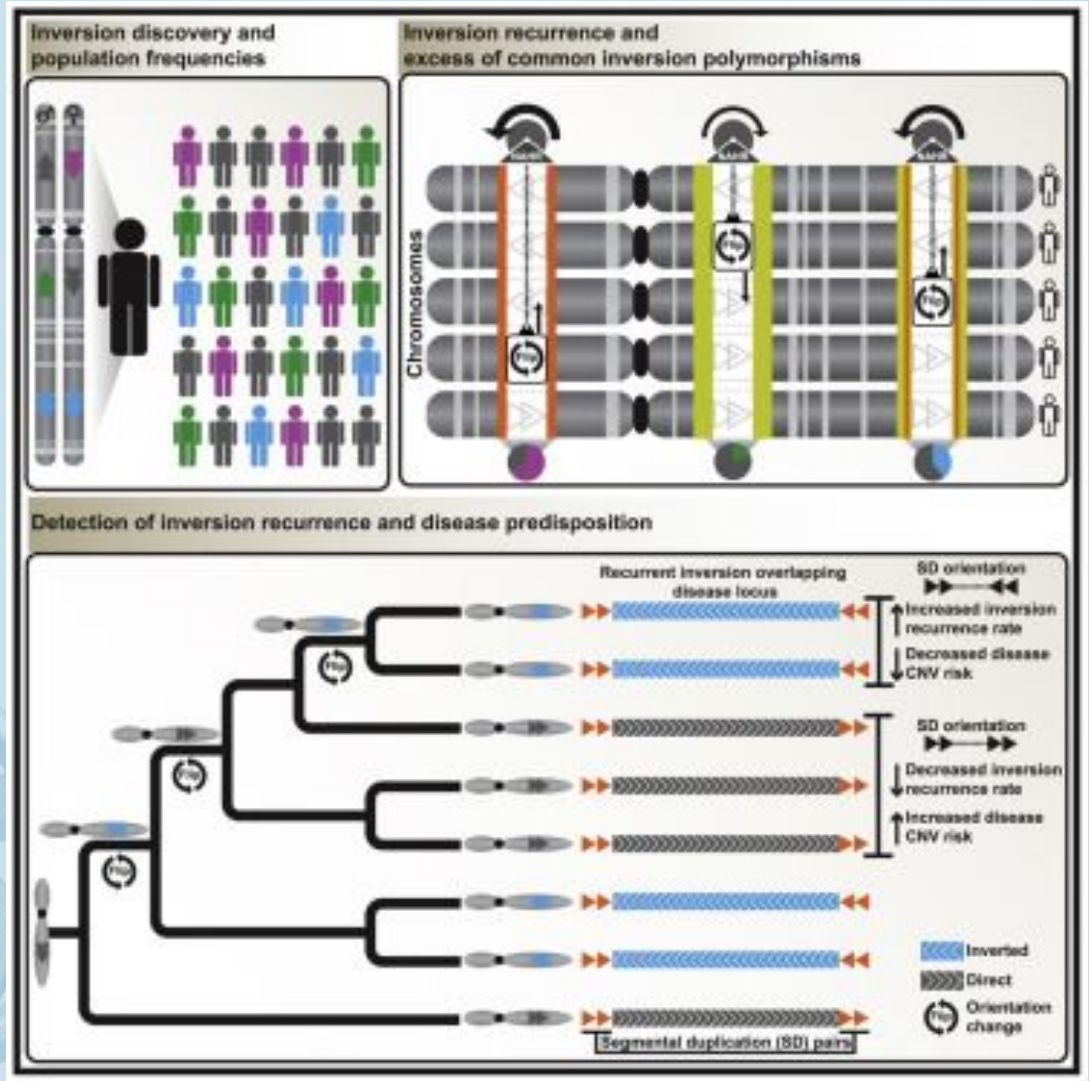


Figure 4. Recurrent translocation map. A global genomic view of interchromosomal LCR pairs with >5 kb in size and >94% DNA sequence identity represented by dotted lines and distribution divided into four groups based on the size of LCR. To create this plot we circularized the genome using polar coordinates. 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.

Inversion polymorphisms between LCRs are common in population



Porubsky et al., Cell, 2022

Take home messages

- Non-recurrent rearrangements occur at random
- Recurrent rearrangements occur via NAHR between LCRS