Chromosomal Analysis

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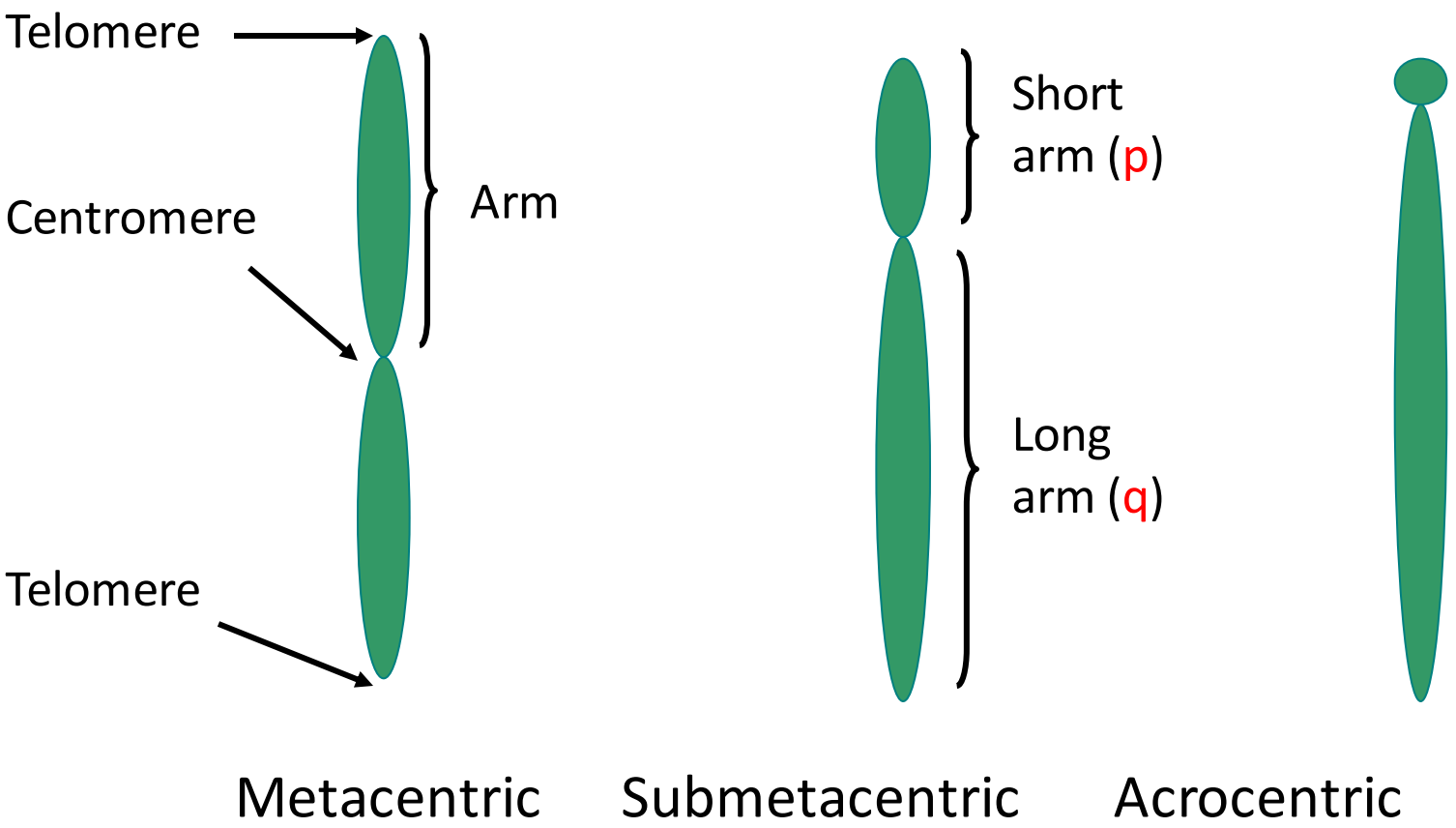
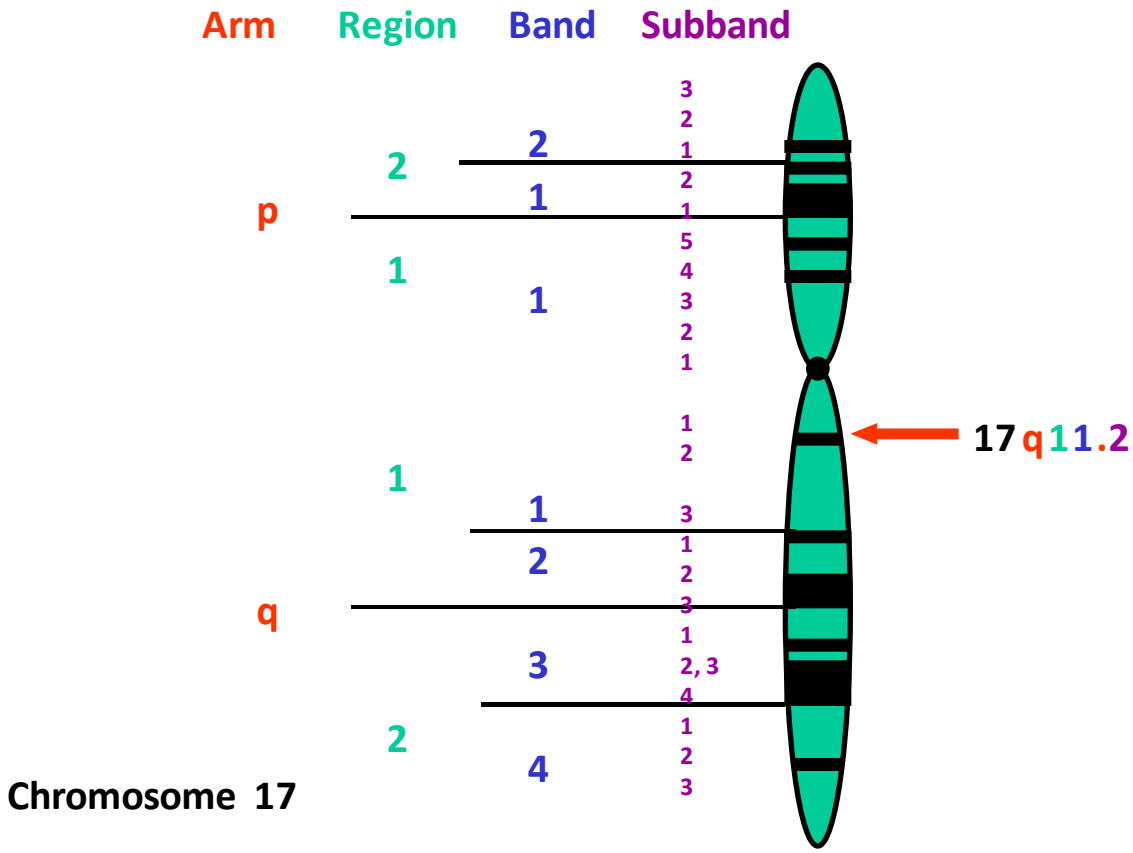
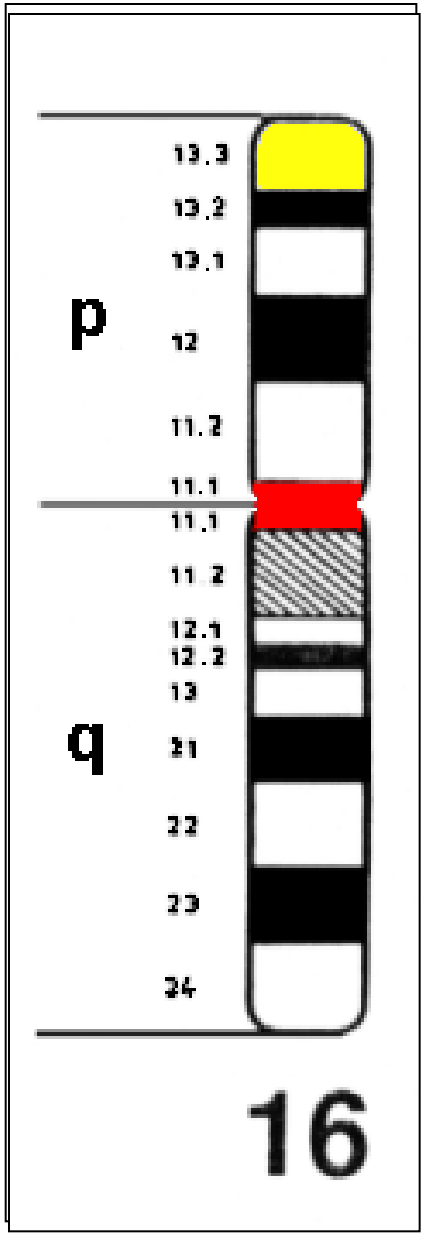
Chromosome Morphology



Defining Chromosomal Location



Nomenclature system

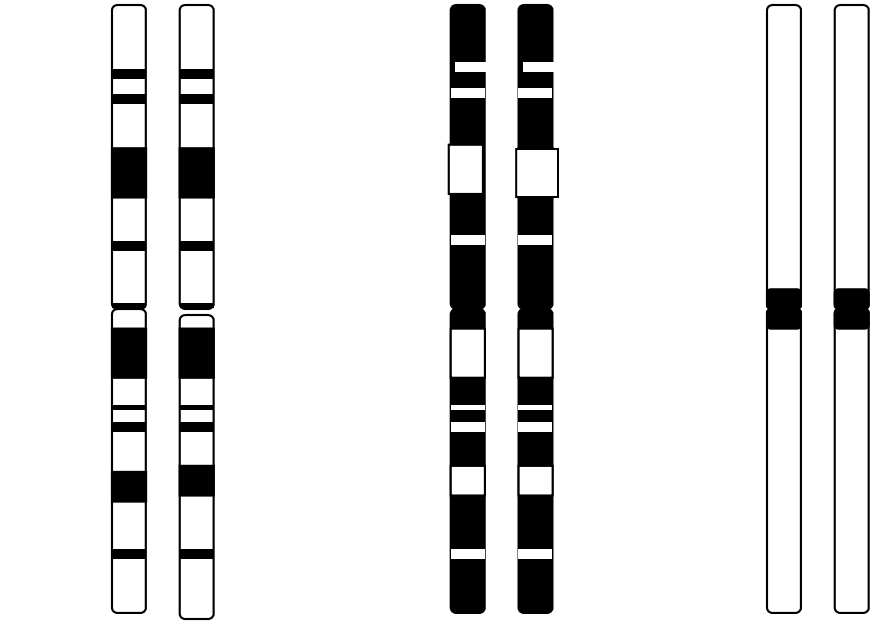


Visualizing Metaphase Chromosomes

- Patient cells are incubated and divide in tissue culture.
- Phytohemagglutinin (PHA): stimulates cell division.
- Colcemid: arrests cells in metaphase.
- 3:1 Methanol:Acetic Acid: fixes metaphase chromosomes for staining.

Visualizing Metaphase Chromosomes (Banding)

- Giemsa-, reverse- or centromere-stained metaphase chromosomes

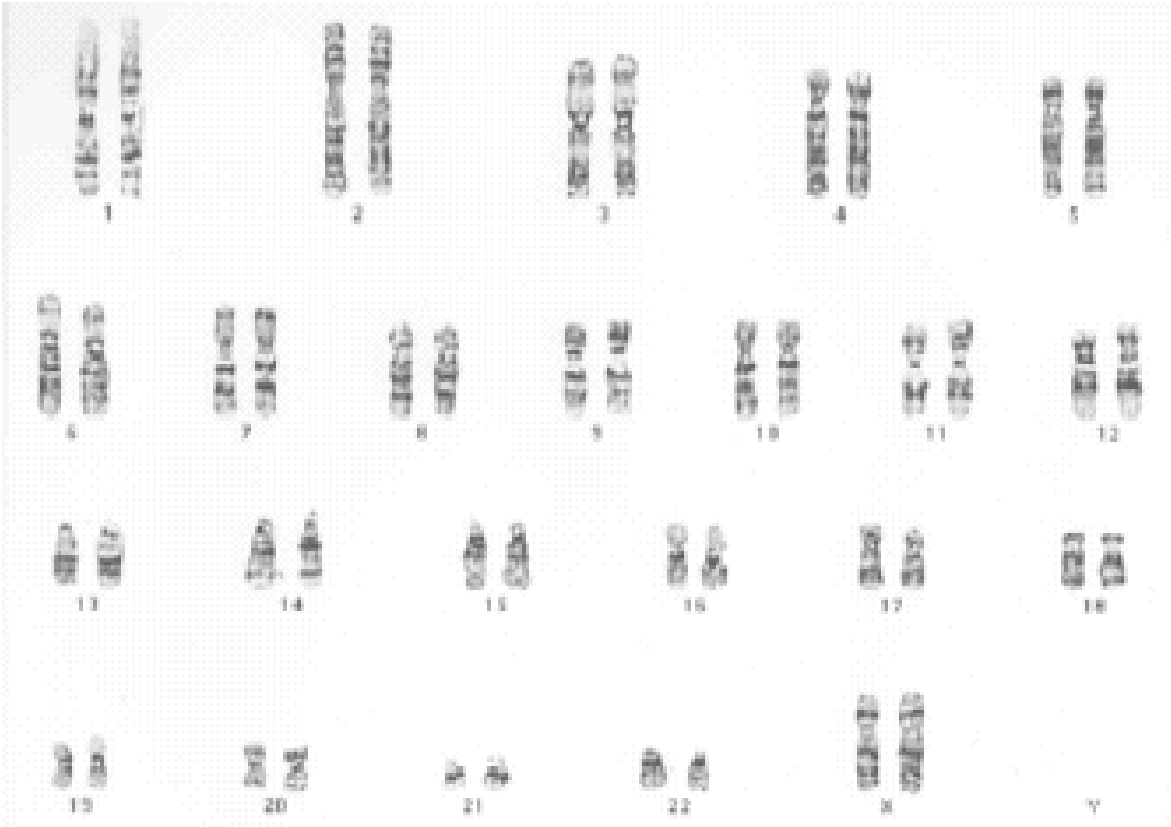


G-Bands R-Bands C-Bands

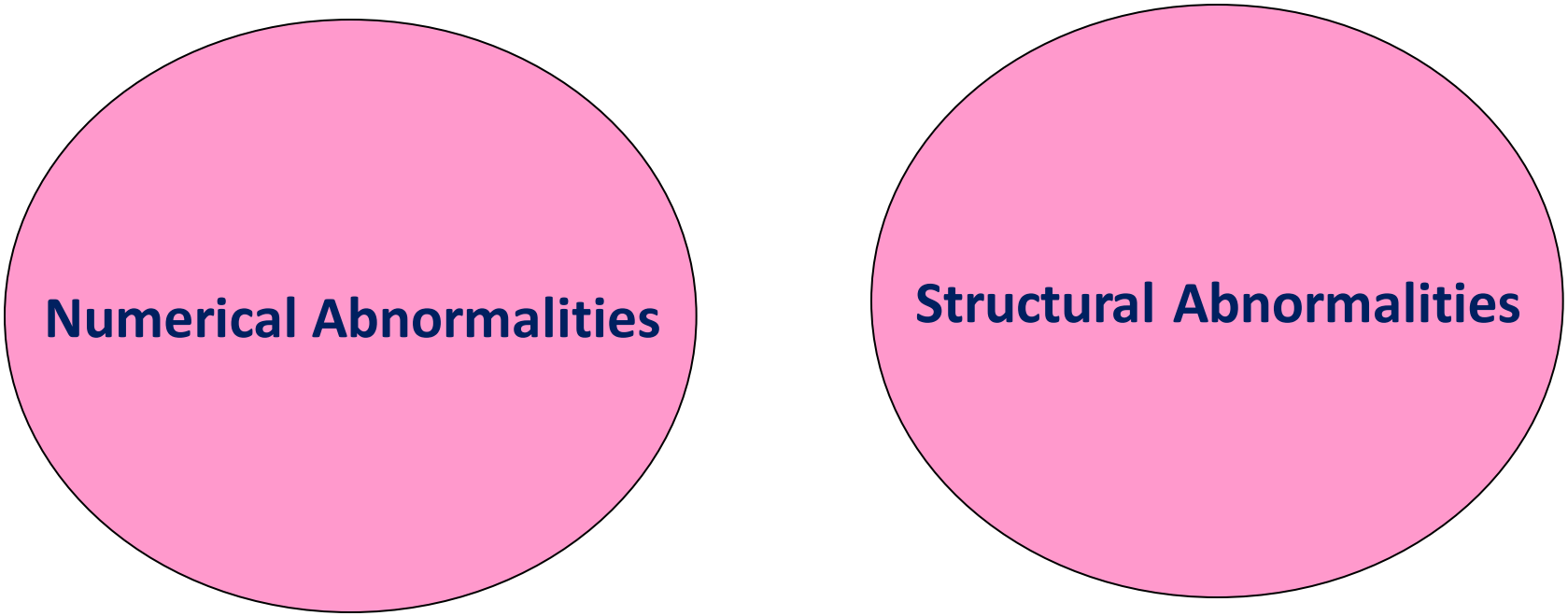
Karyotype

- International System for Human Cytogenetic Nomenclature (ISCN)
  - 46, XX – normal female
  - 46, XY – normal male
- G-banded chromosomes are identified by band pattern.

Normal Female Karyotype (46, XX) (G Banding)

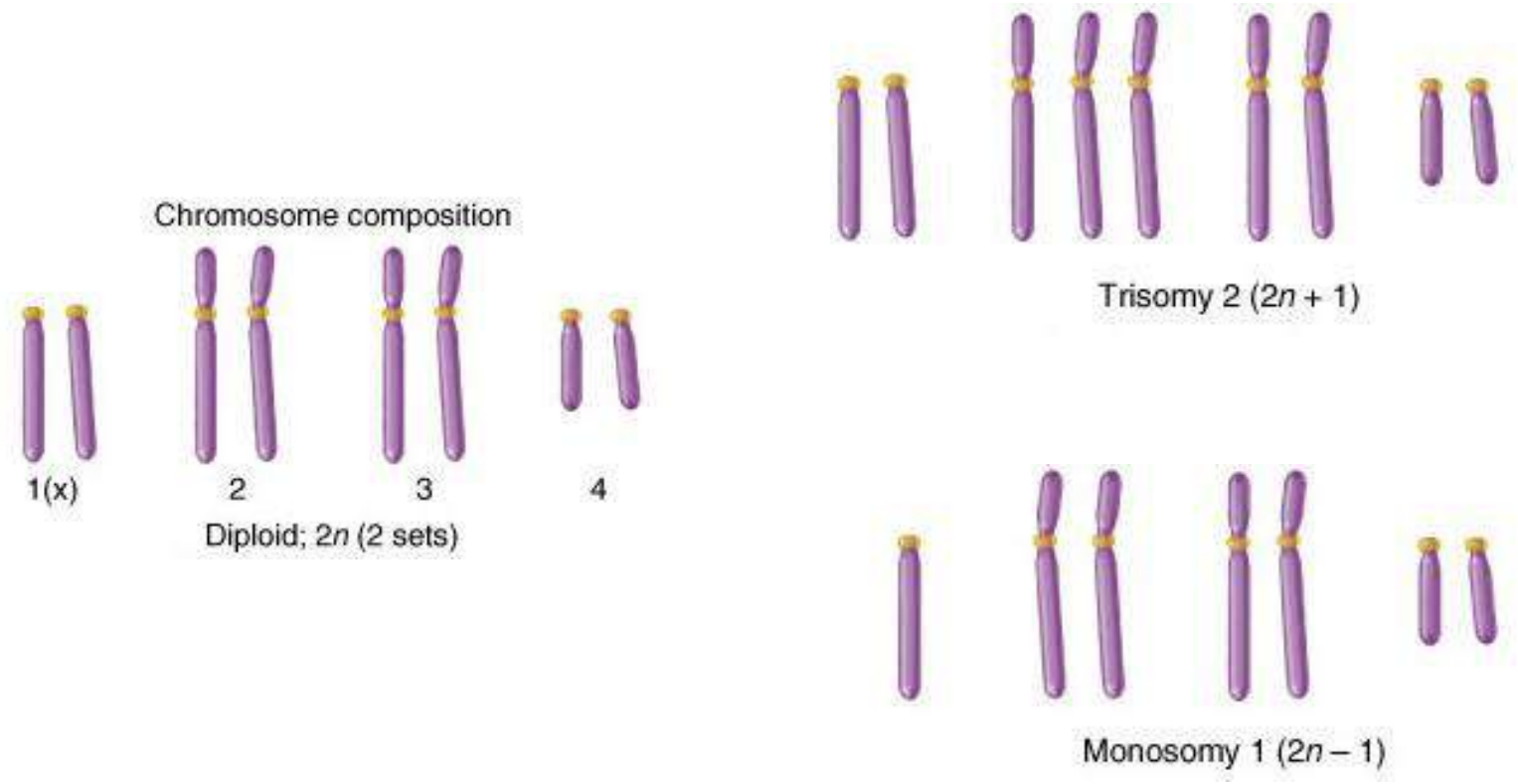


Types of Chromosomal Aberrations



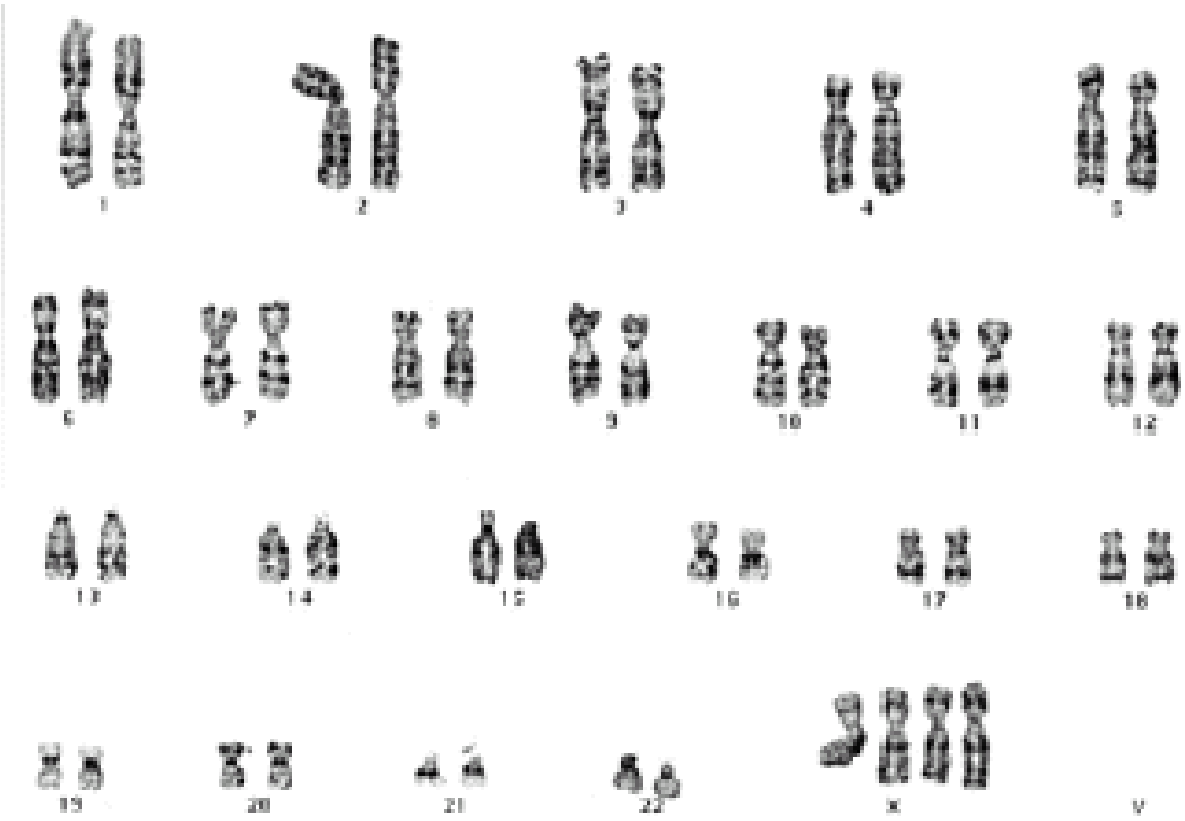
Aneuploidy

Aneuploidy occurs when one of the chromosomes is present in an abnormal number of copies.

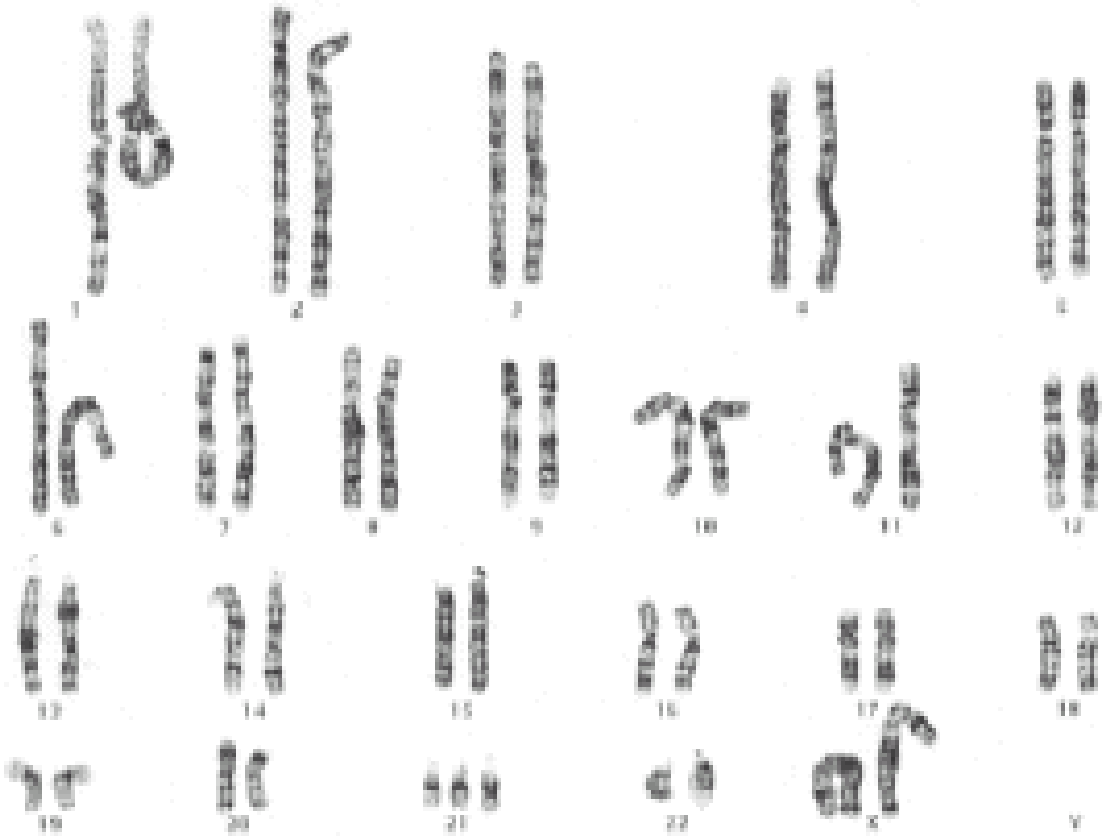


Trisomy and monosomy are two forms of aneuploidy.

Chromosome Number Abnormality
Aneuploidy (48, XXXX)



Chromosome Number Abnormality
Trisomy 21 (47, XX, +21)

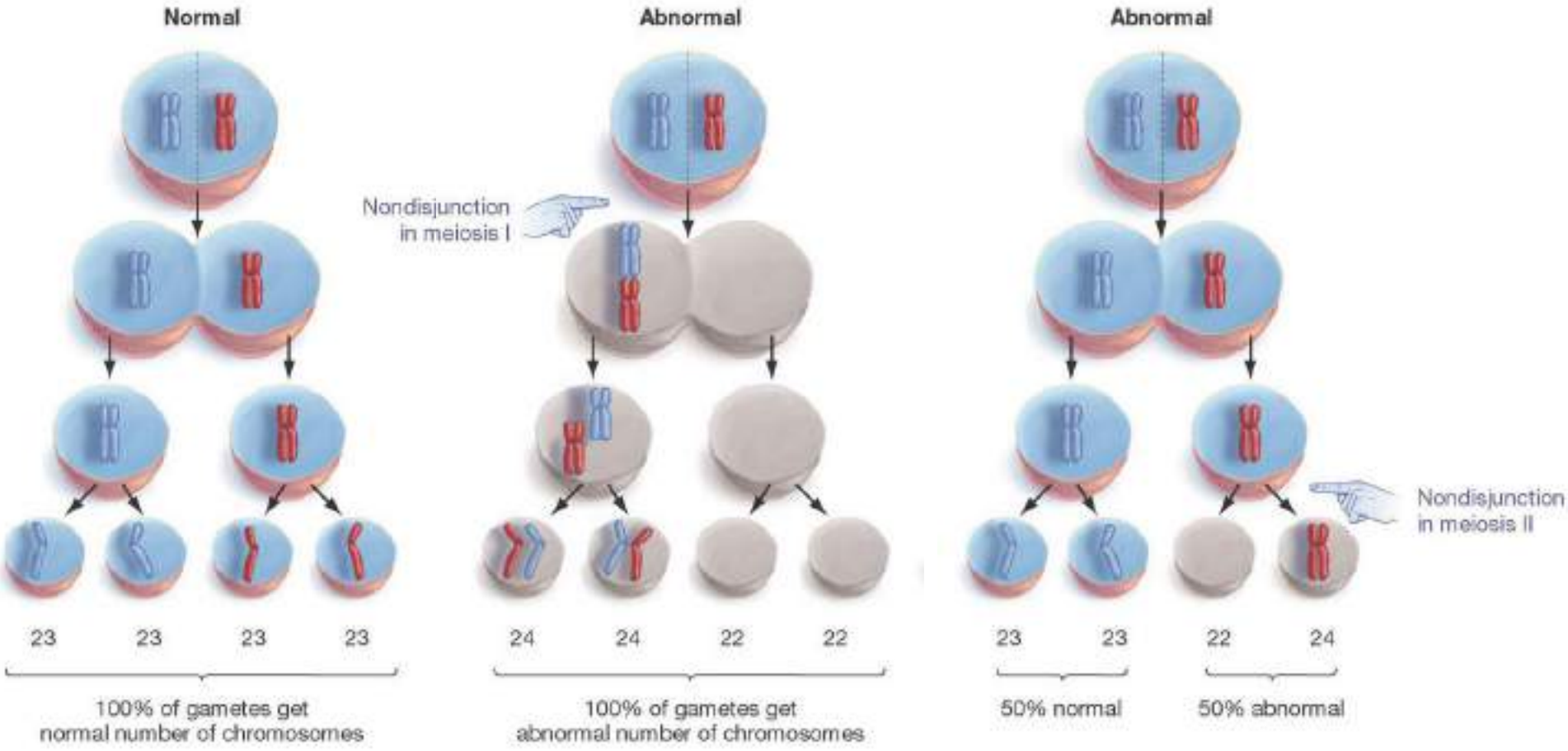


Down Syndrome is Caused by Trisomy for Chromosome 21

Aneuploidy is remarkably common, causing termination of at least 25% of human conceptions.

Aneuploidy is also a driving force in cancer progression (virtually all cancer cells are aneuploid).

Chromosome Non-Disjunction in Meiosis Causes Aneuploidy

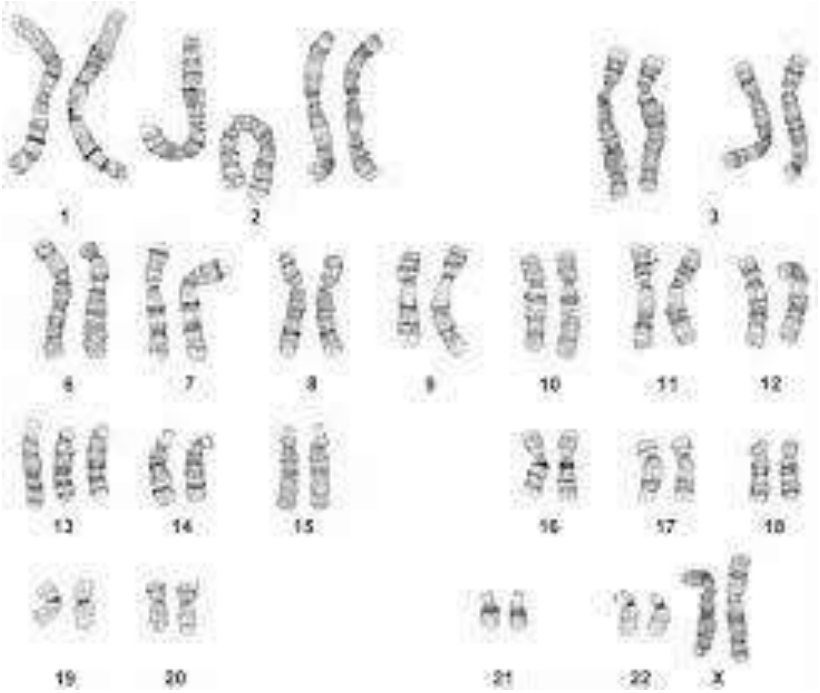


The Frequency of Chromosome Non-Disjunction And Down Syndrome Rises Sharply with Maternal Age

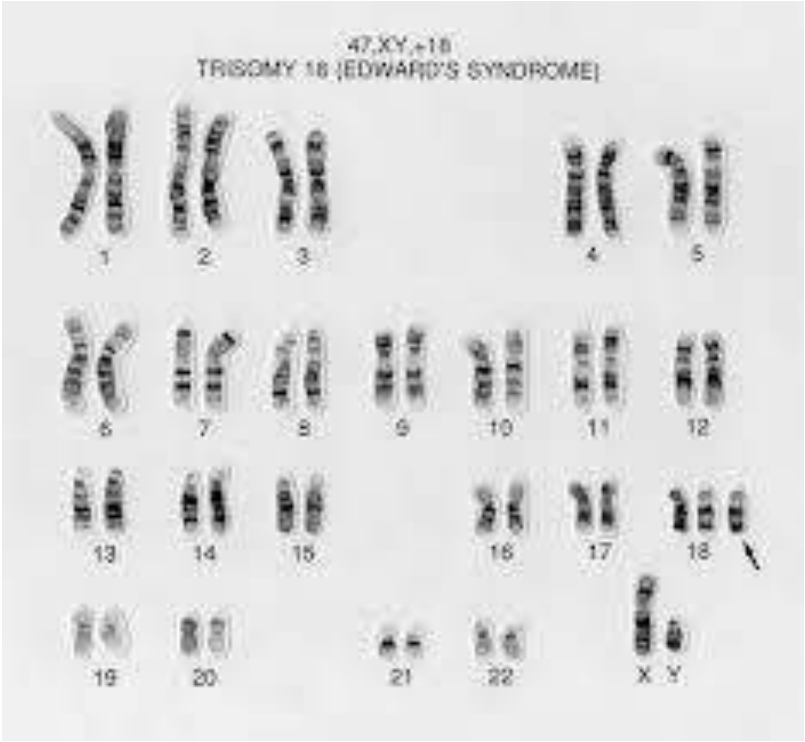
Mother's age	Chances of giving birth to a child with Down syndrome
20	1 in 1925
25	1 in 1205
30	1 in 885
35	1 in 365
40	1 in 110
45	1 in 32

Chromosome Number Abnormality

Trisomy 13 (47, XX, +13)

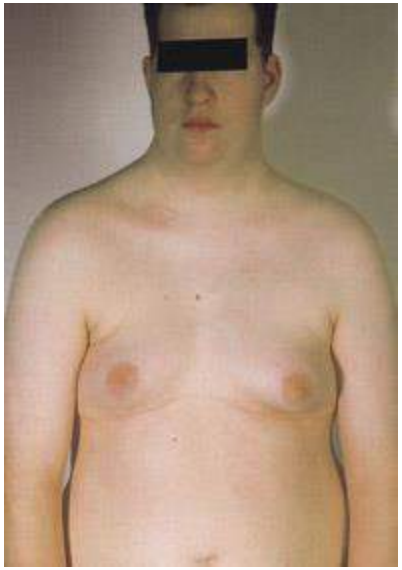


Trisomy 18 (47, XY,+18)



Sex Chromosome Aneuploid Conditions are Common

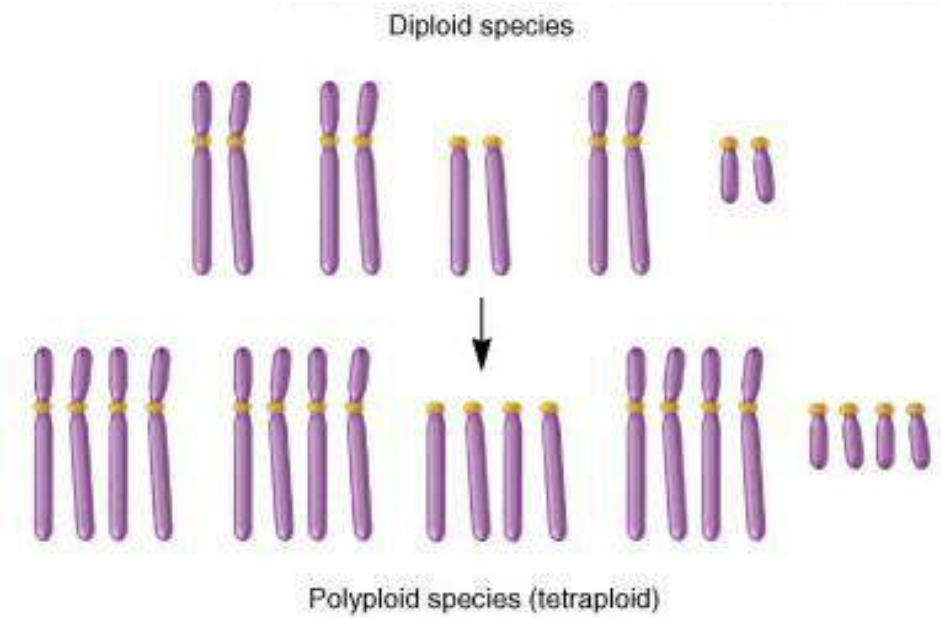
Sex Chromosomal			
XXY	1/1,000 (males)	Klinefelter	Sexual immaturity (no sperm), breast swelling
XYY	1/1,000 (males)	Jacobs	Tall
XXX	1/1,500 (females)	Triple X	Tall and thin, menstrual irregularity
X0	1/5,000 (females)	Turner	Short stature, webbed neck, sexually undeveloped



Klinefelter syndrome

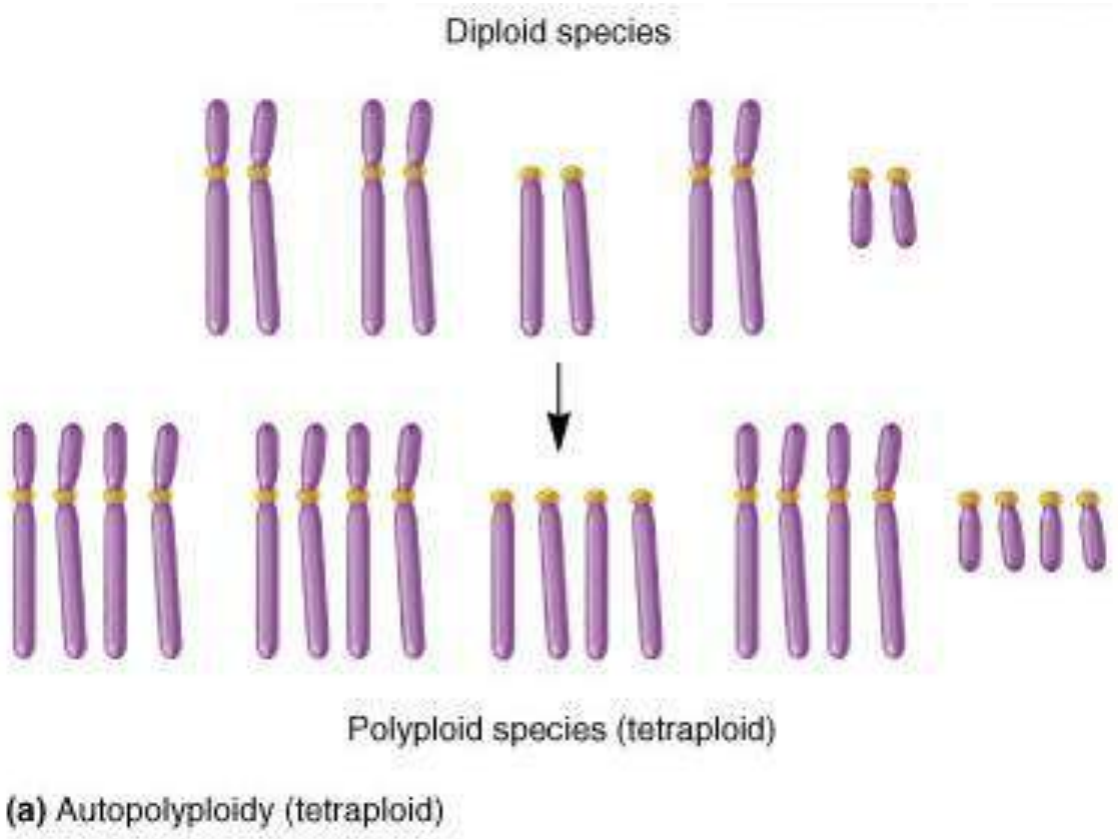
Polyploidy

Polyploidy occurs when all the chromosomes are present in three or more copies.



Polyploidy is common in plants and rare in animals.

Polyploids are Created When Chromosome Number Doubles



(a) Autopolyploidy (tetraploid)

A common way for this to occur is for the mitotic spindle to fail, leaving all chromosomes in one cell.

Polyploidy is a Major Force in Plant Evolution

(a) A hexploid species

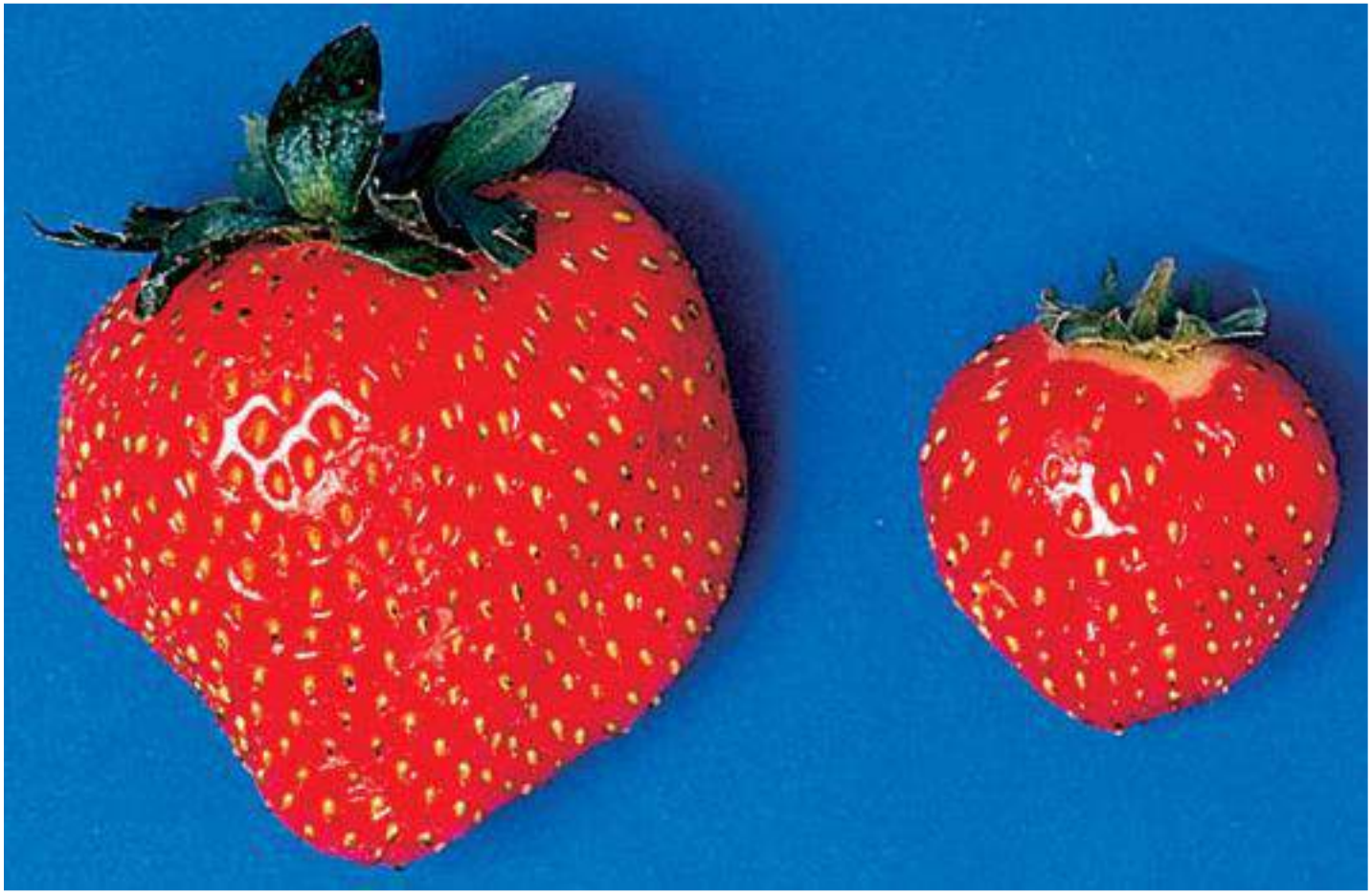
Diploid

Tetraploid

(b) A comparison of diploid and tetraploid petunias

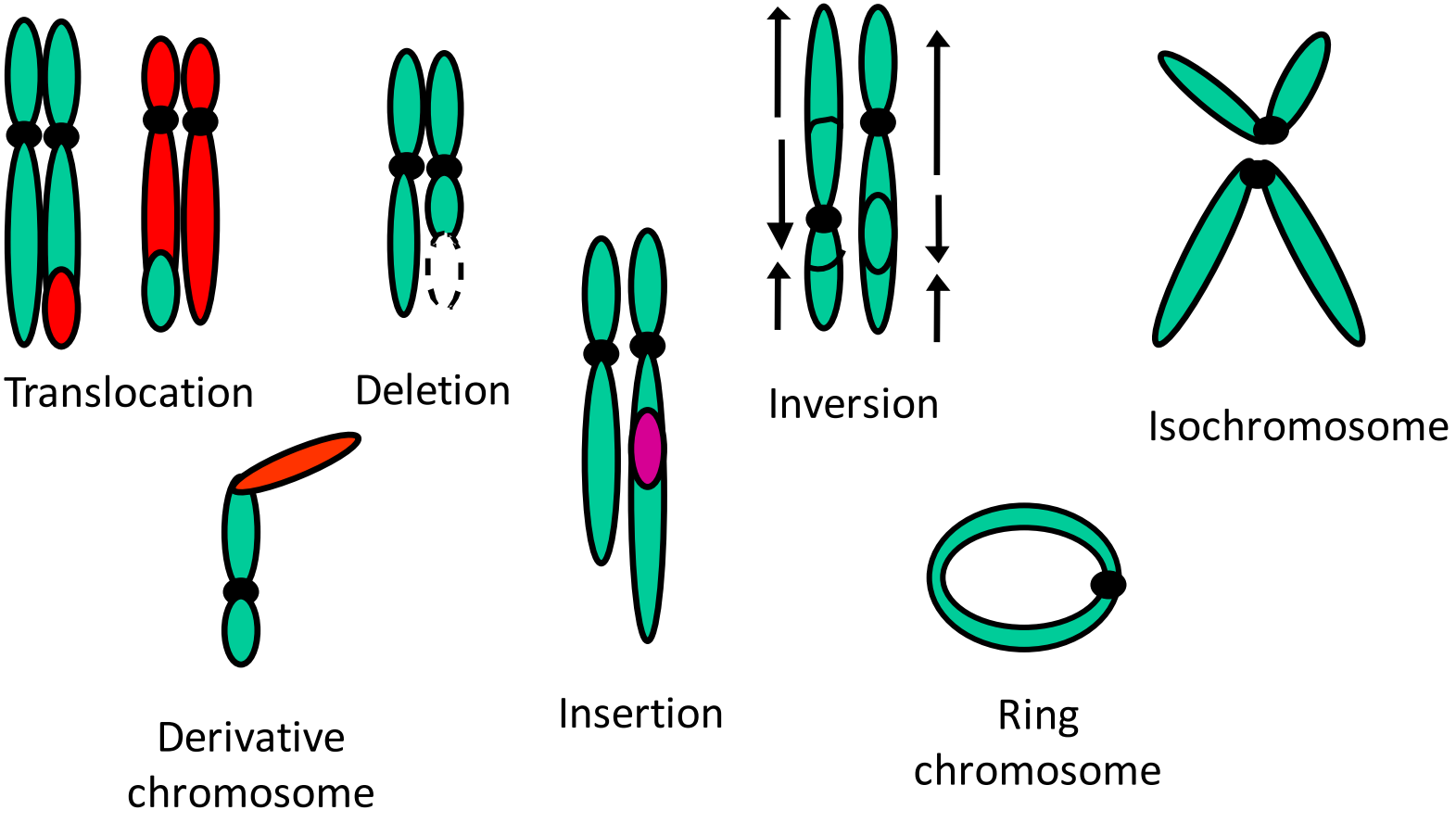
Roughly 35% of flowering plants (the most familiar plant species) arose through polyploidization.

Most Crop Species are Polyploid

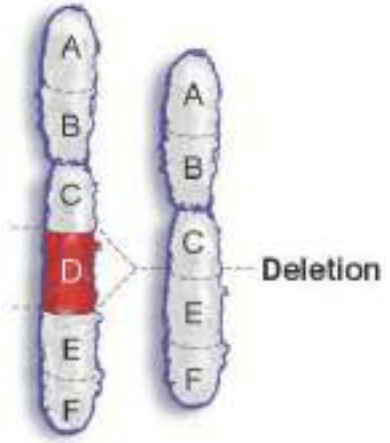


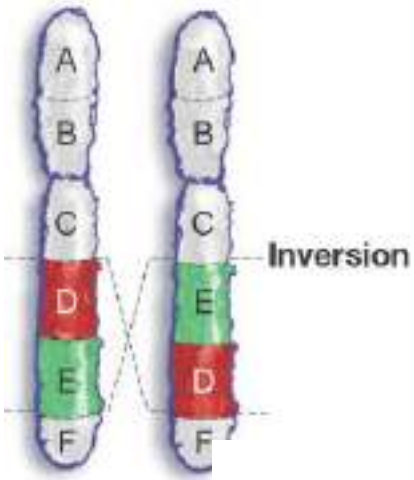
Polyploids, like the one on the left, are larger than their diploid progenitors (strawberry on right).

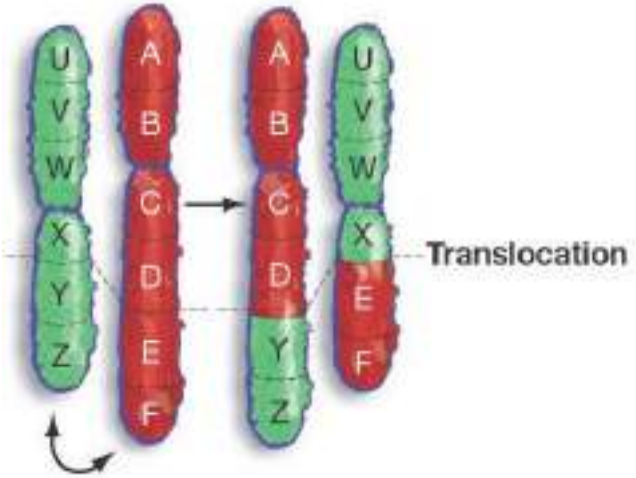
Chromosome Structure Abnormalities

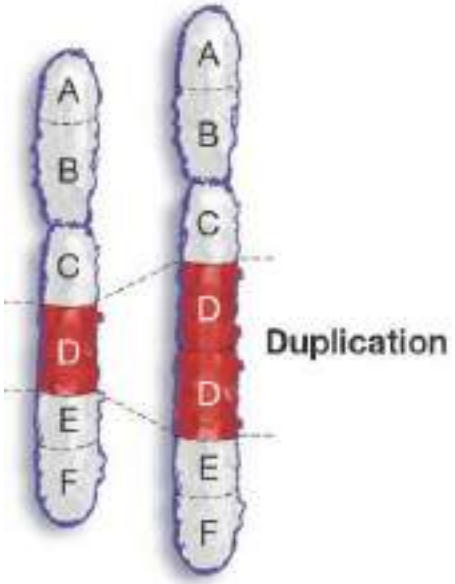


Chromosome Structural Changes

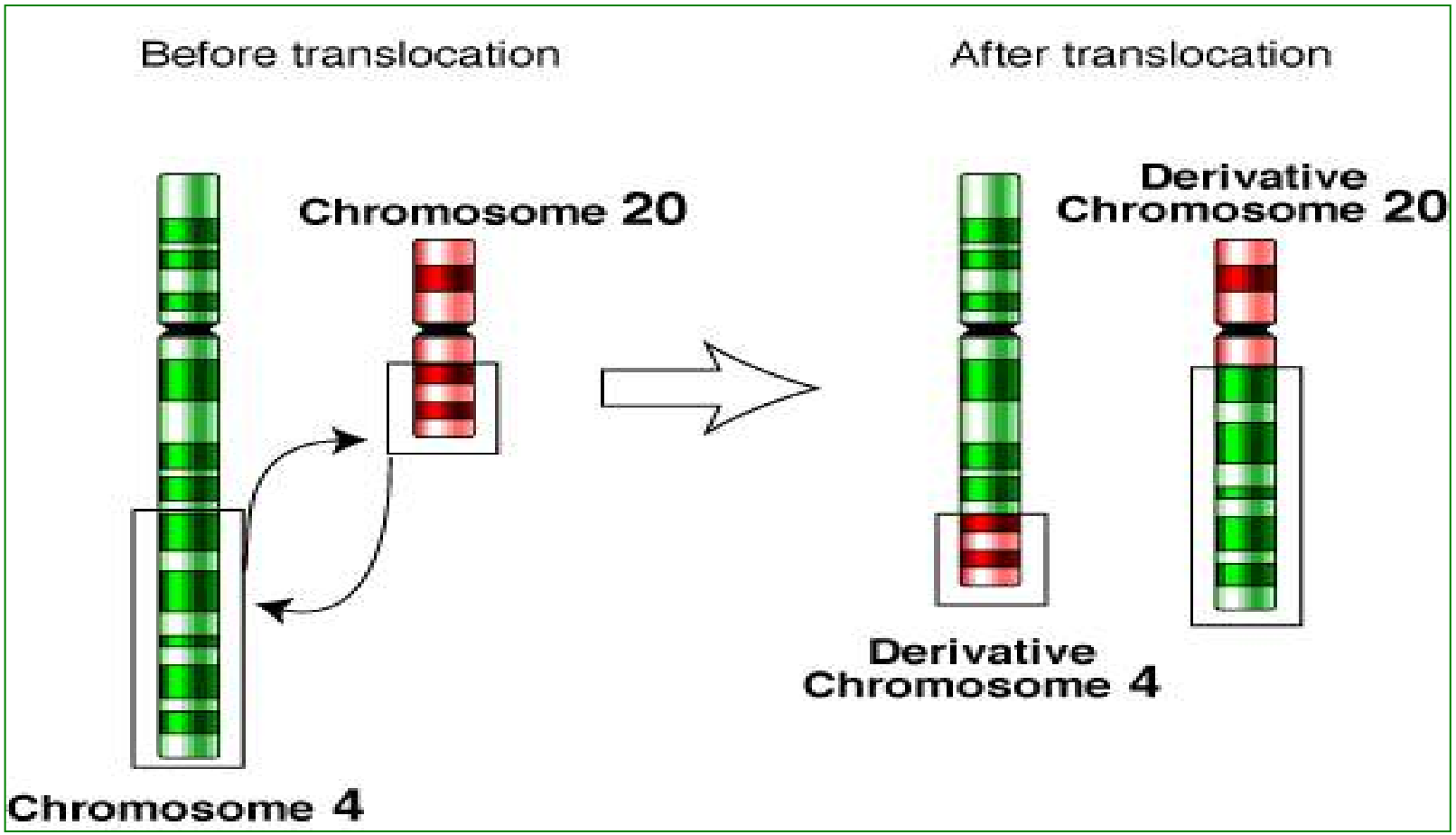








There are 4 types of chromosome structural change – all of them associated with human disorders

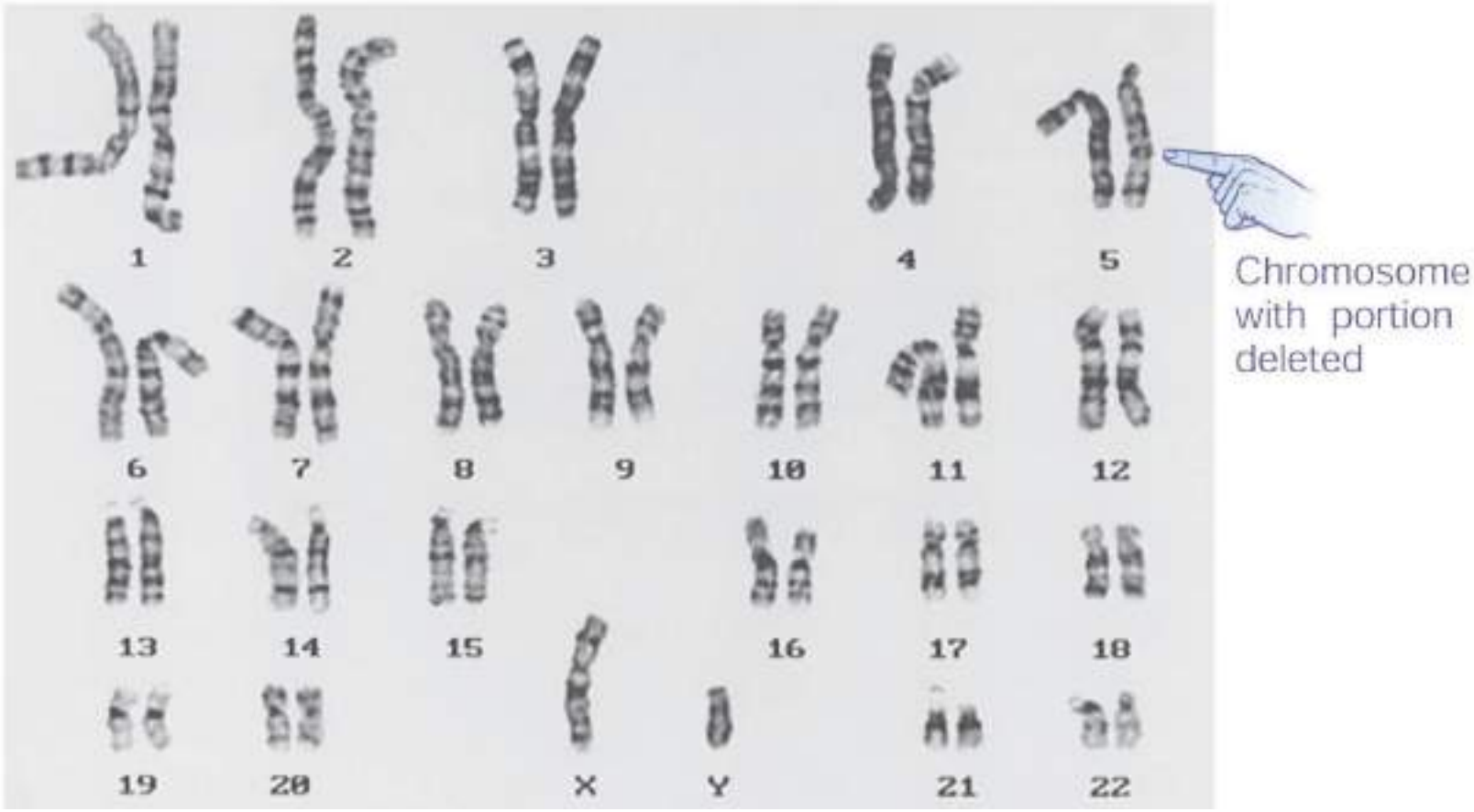

Before translocation
After translocation
Chromosome 20
Derivative
Chromosome 20
Derivative
Chromosome 4
Chromosome 4

X-linked Inheritance

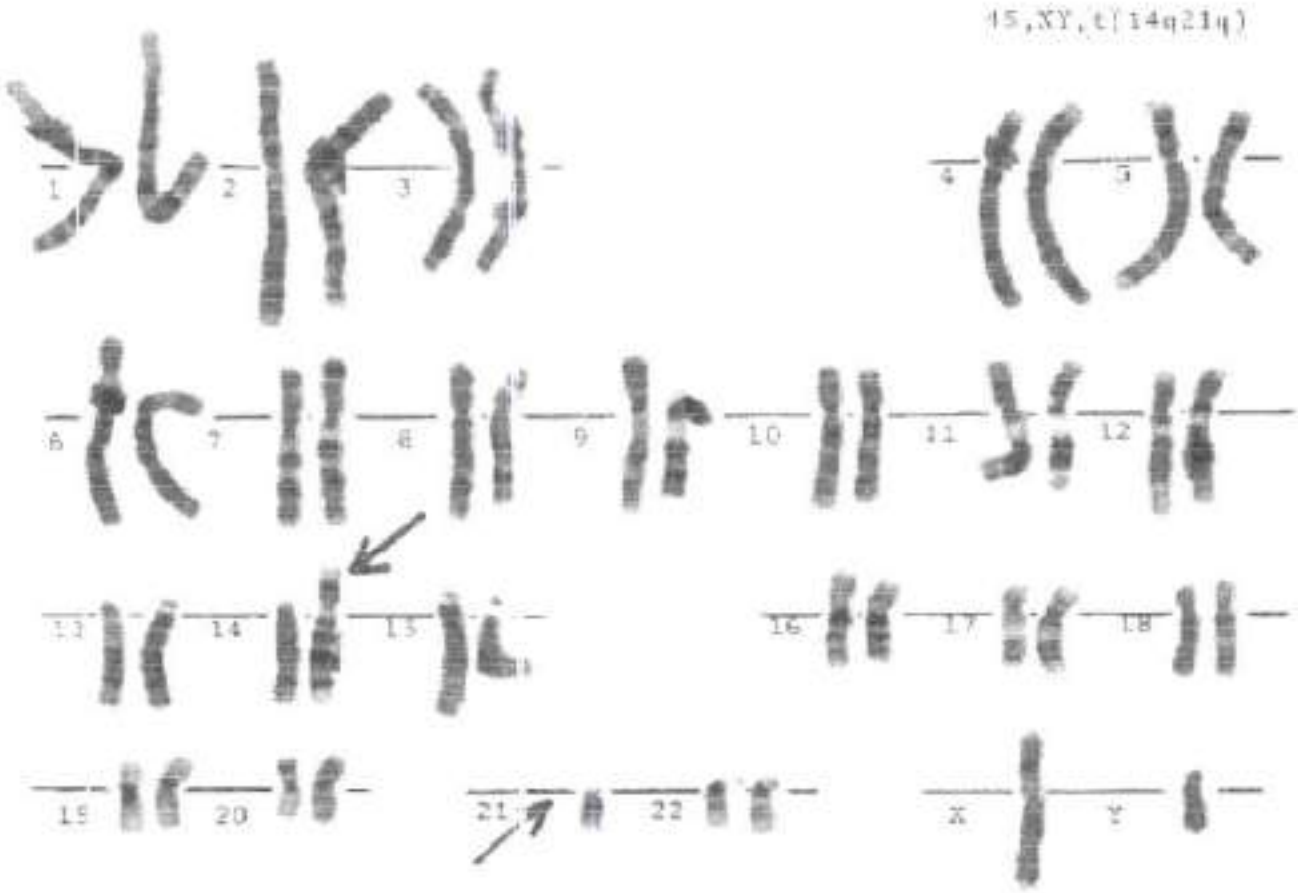
There are many X-linked recessive traits.

Cri-du-Chat is Caused by the Loss of the Short Arm of One Copy of Chromosome 5

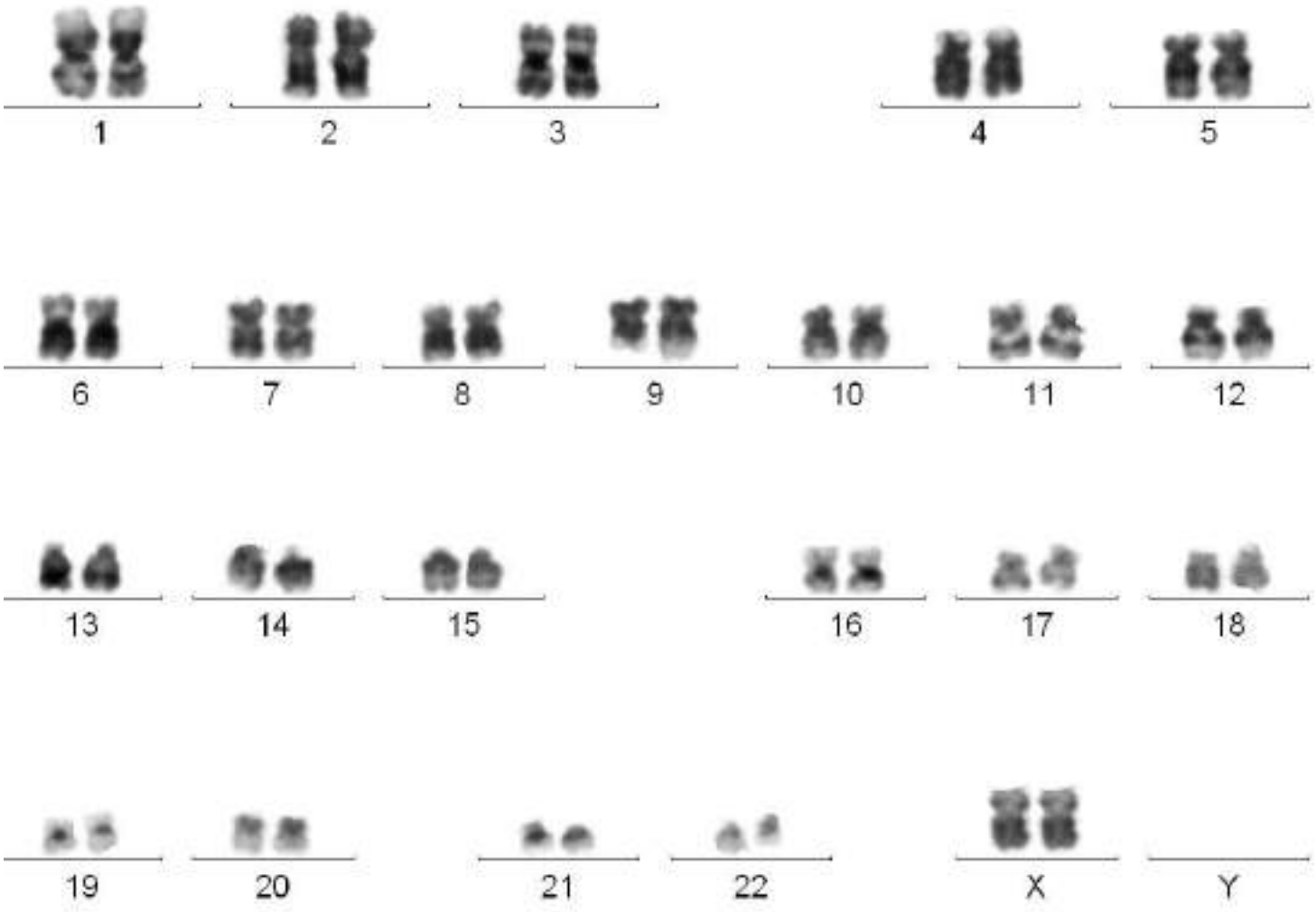
(b) Cri-du-chat karyotype

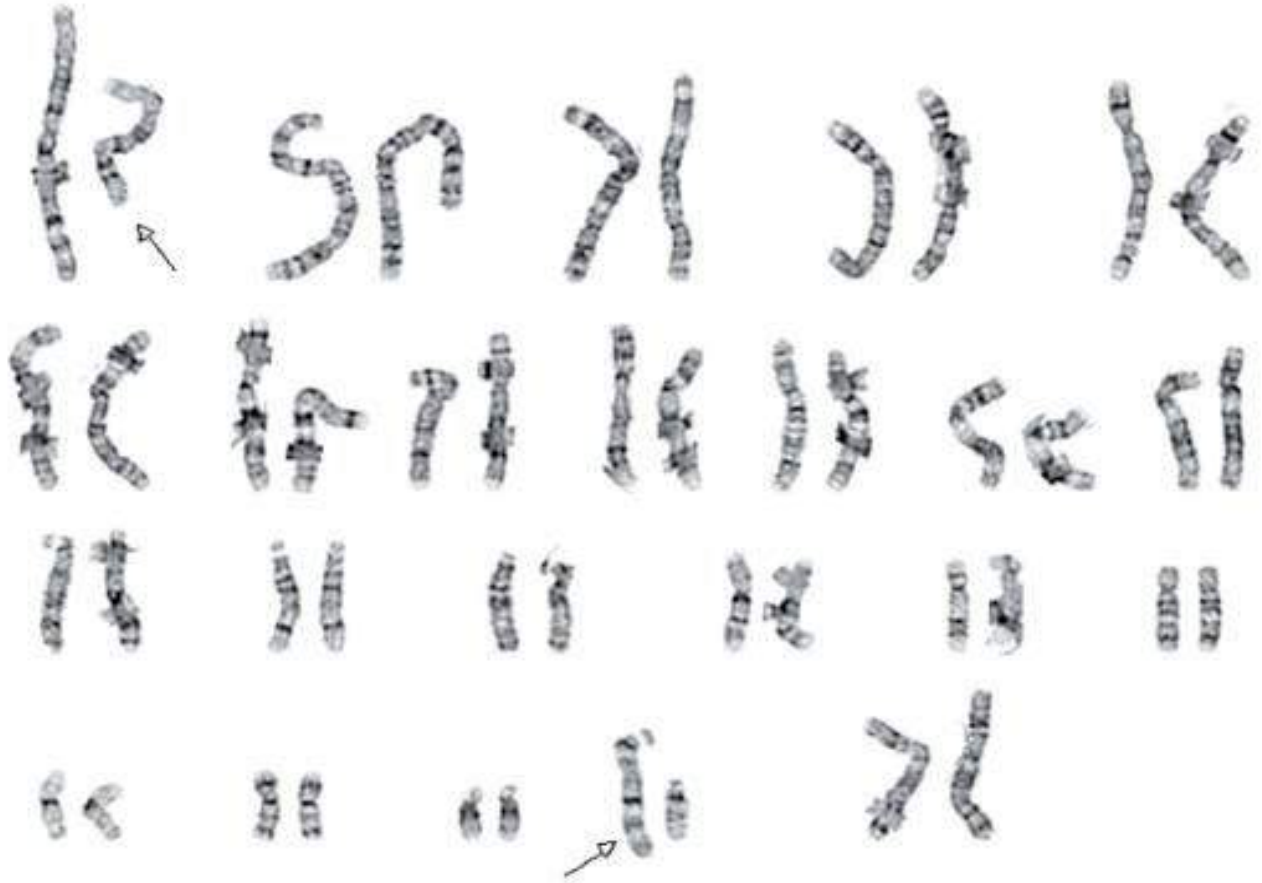


Chromosome Structure Abnormality: Balanced Translocation 45, XY, t(14q;21q)



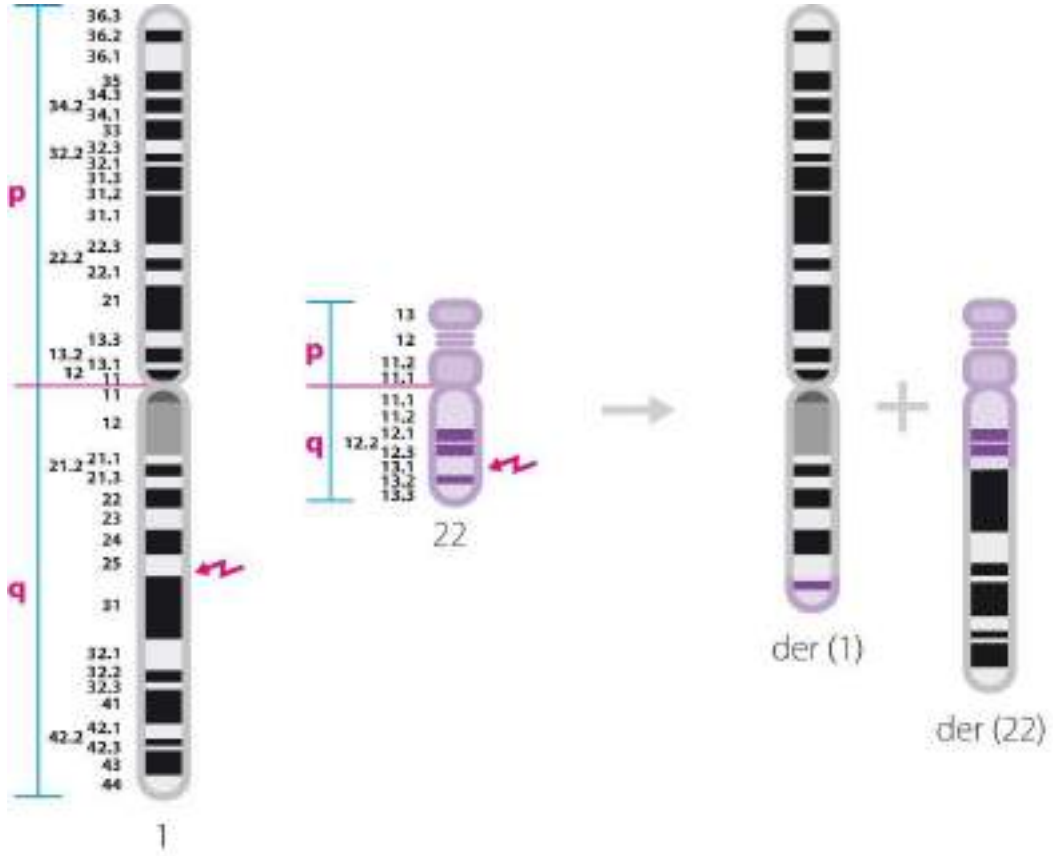
46,XX,t(9;22)(q34;q11)





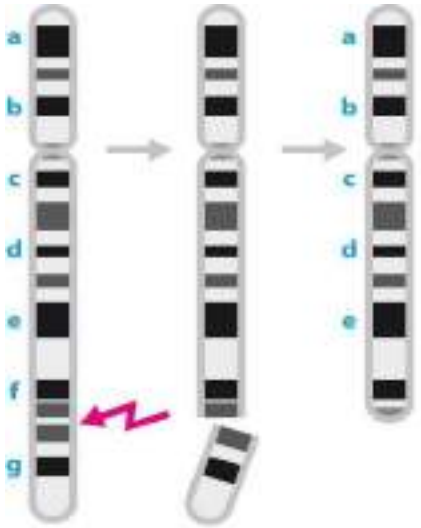
G-banded karyotypes of chromosomes

There is a balanced translocation. Chromosomes 1 and 22 have exchanged segments (arrows). The translocation is described as 46,XX,t(1:22)(q25;q13)



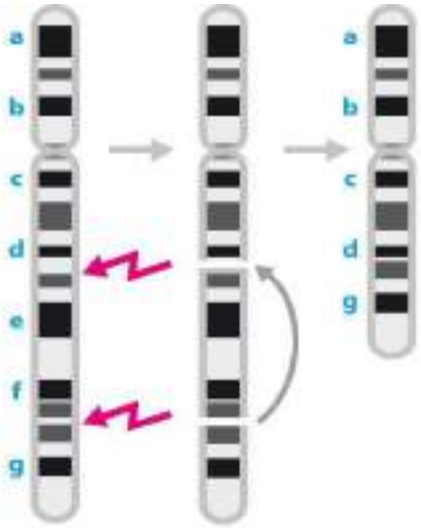
How the 1;22 translocation originated

Chromosome 1 and 22 broke at the positions indicated by the arrows, and the cell's DNA repair machinery rejoined the ends to form the two derivative chromosomes as shown. The derivative chromosomes are labelled der(1) and der(22).

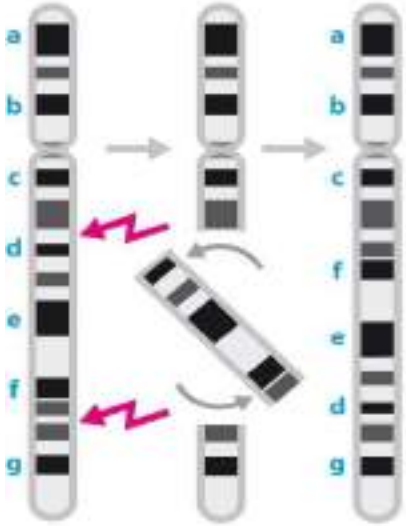


A TERMINAL DELETION

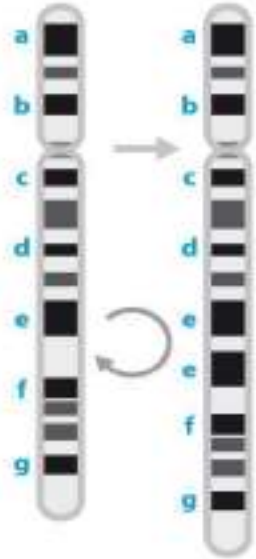
N.B. to be stable a terminal deletion must be capped by a telomere



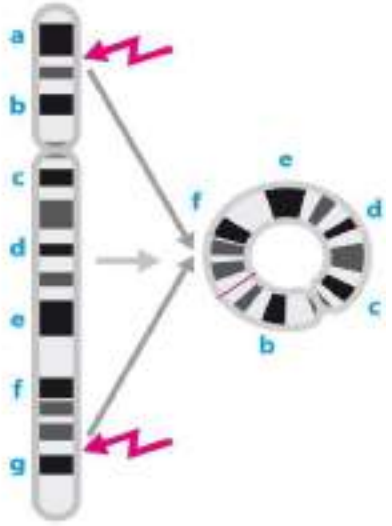
AN INTERSTITIAL DELETION



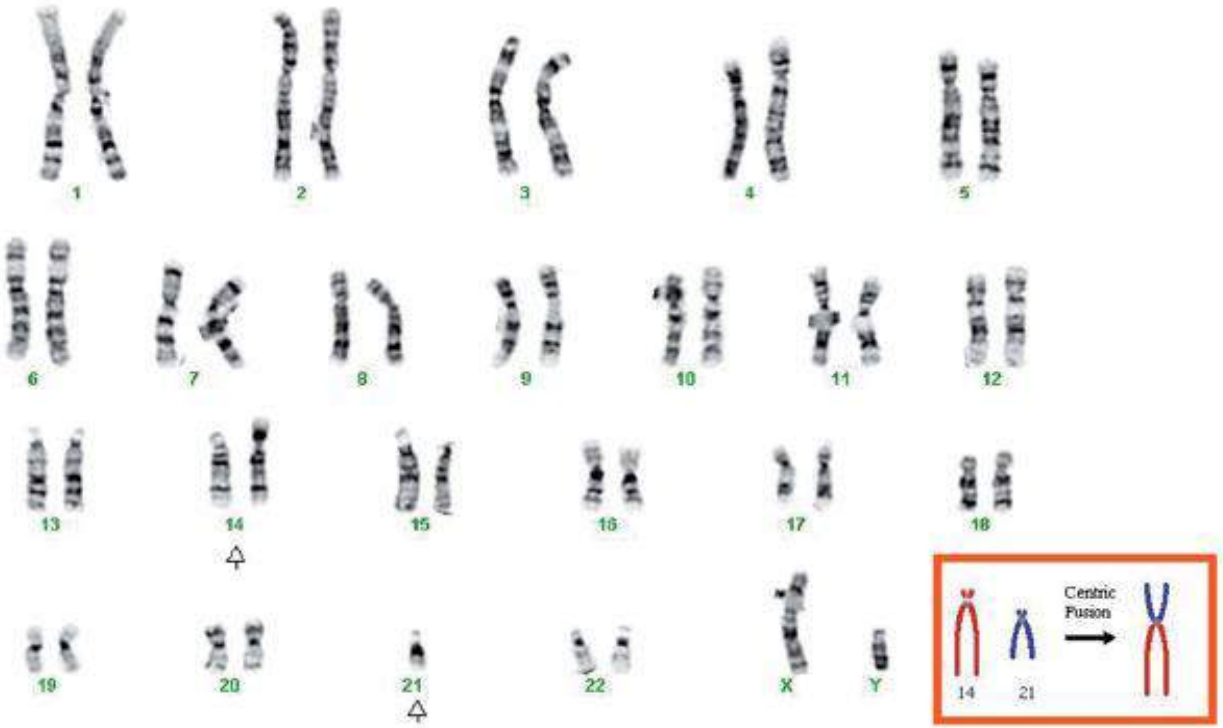
AN INVERSION



A DUPLICATION

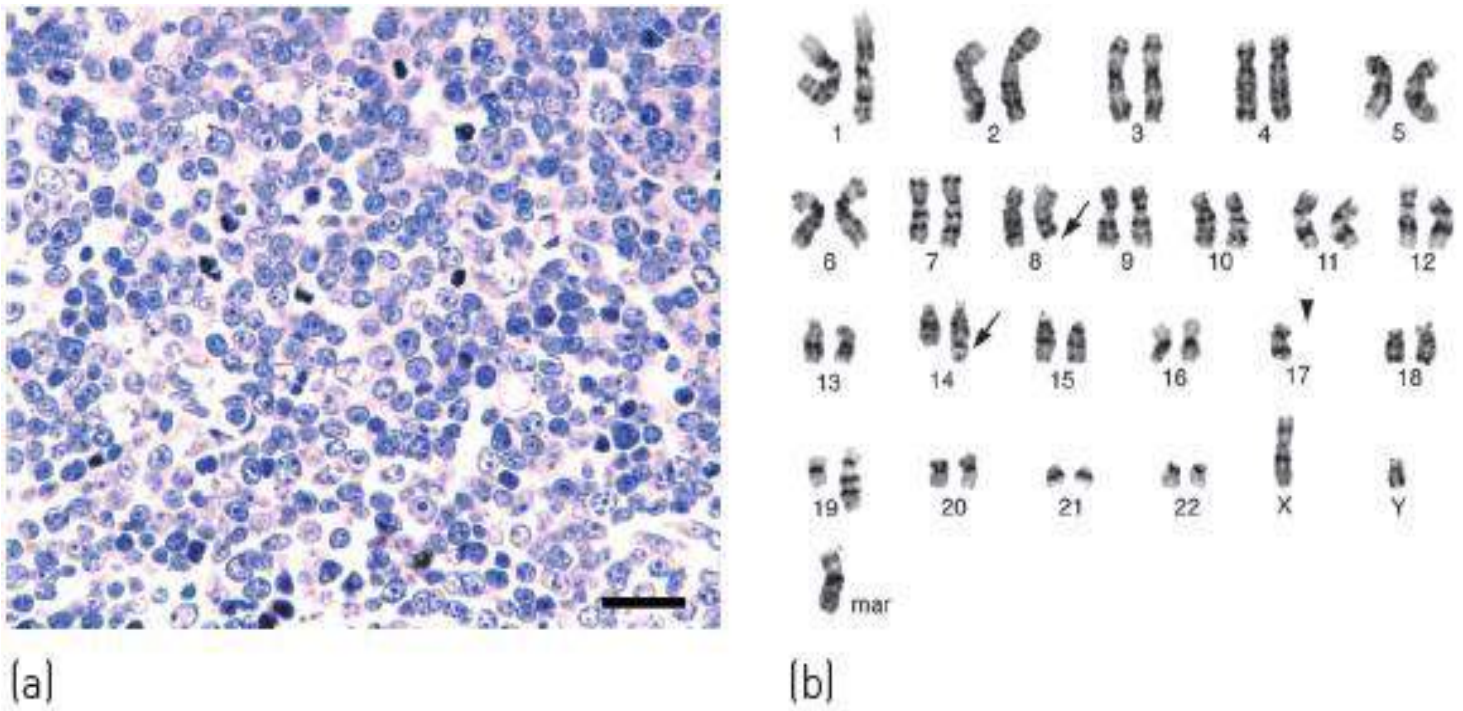


A RING CHROMOSOME



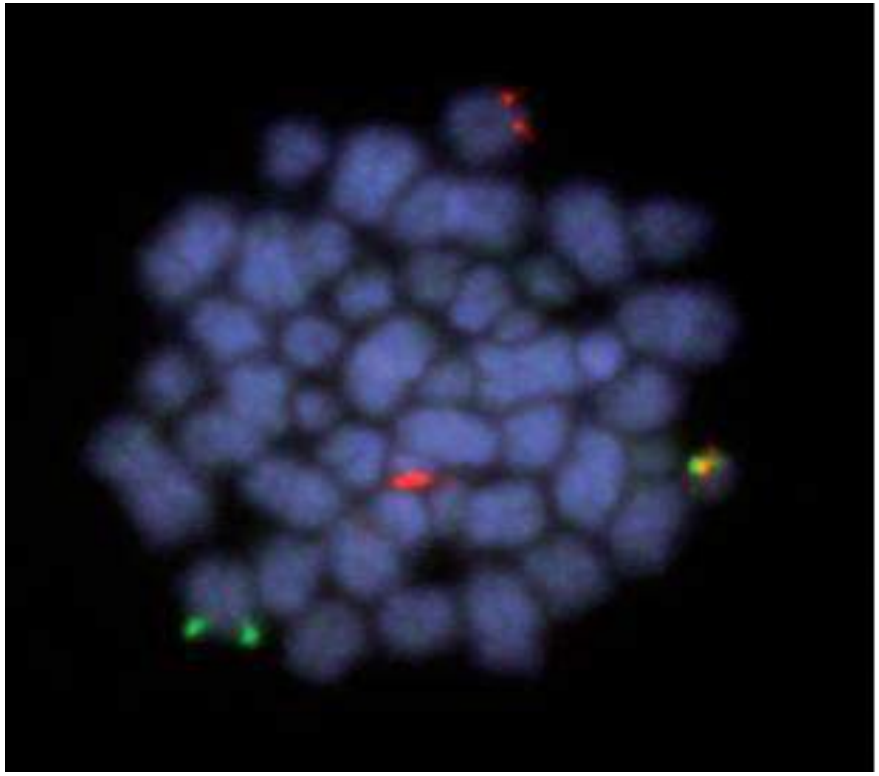
A Robertsonian translocation

The inset shows how this common type of chromosome abnormality arises. The short arms of all the acrocentric chromosomes (13, 14, 15, 21, 22) contain similar DNA. Inappropriate recombination between two non-homologous chromosomes produces the fusion chromosome, which functions as a normal single chromosome in mitosis. The small acentric fragment comprising the two distal short arms is lost.



Burkitt's lymphoma

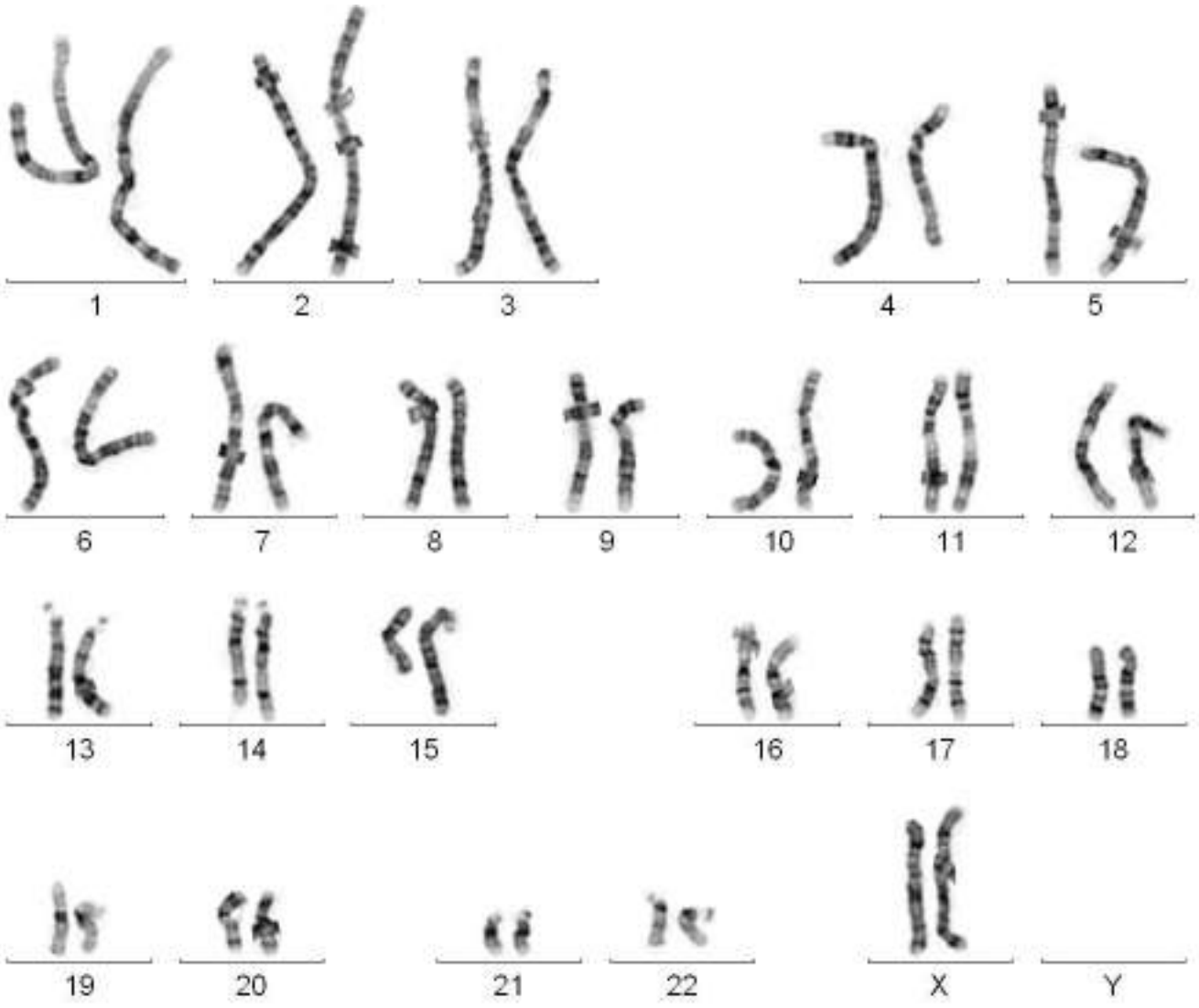
(a) Histology, and (b) a karyotype showing the characteristic 8;14 translocation. Additional chromosome abnormalities are also present, as is usually the case in neoplasia.



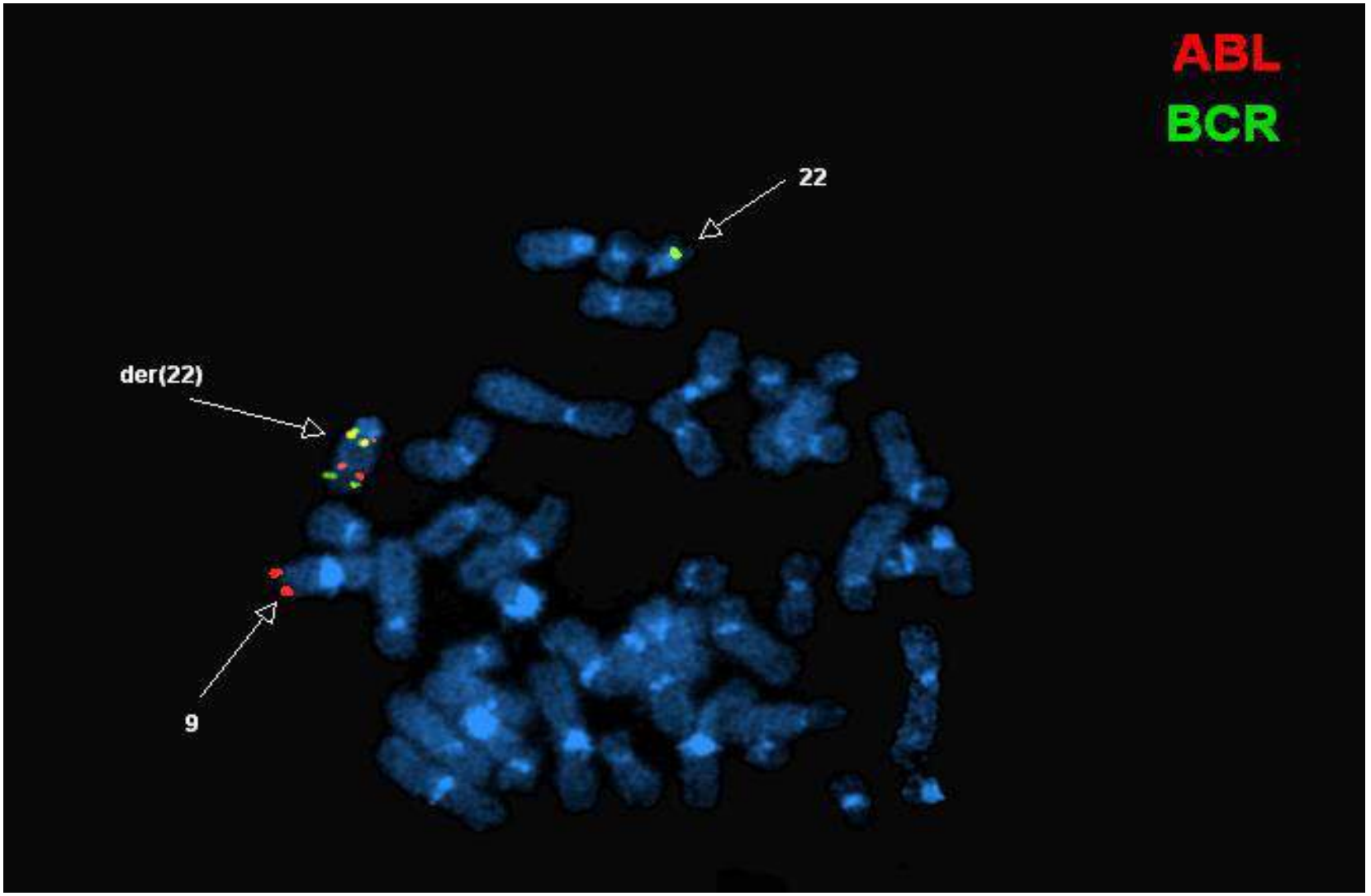
Metaphase with *TEL-AML1* fusion

The green signal is on the normal chromosome 12, one red signal is in the normal chromosome 21 and one is on the derived chromosome 12. The yellow *TEL-AML1* fusion signal is on the derived chromosome 21.

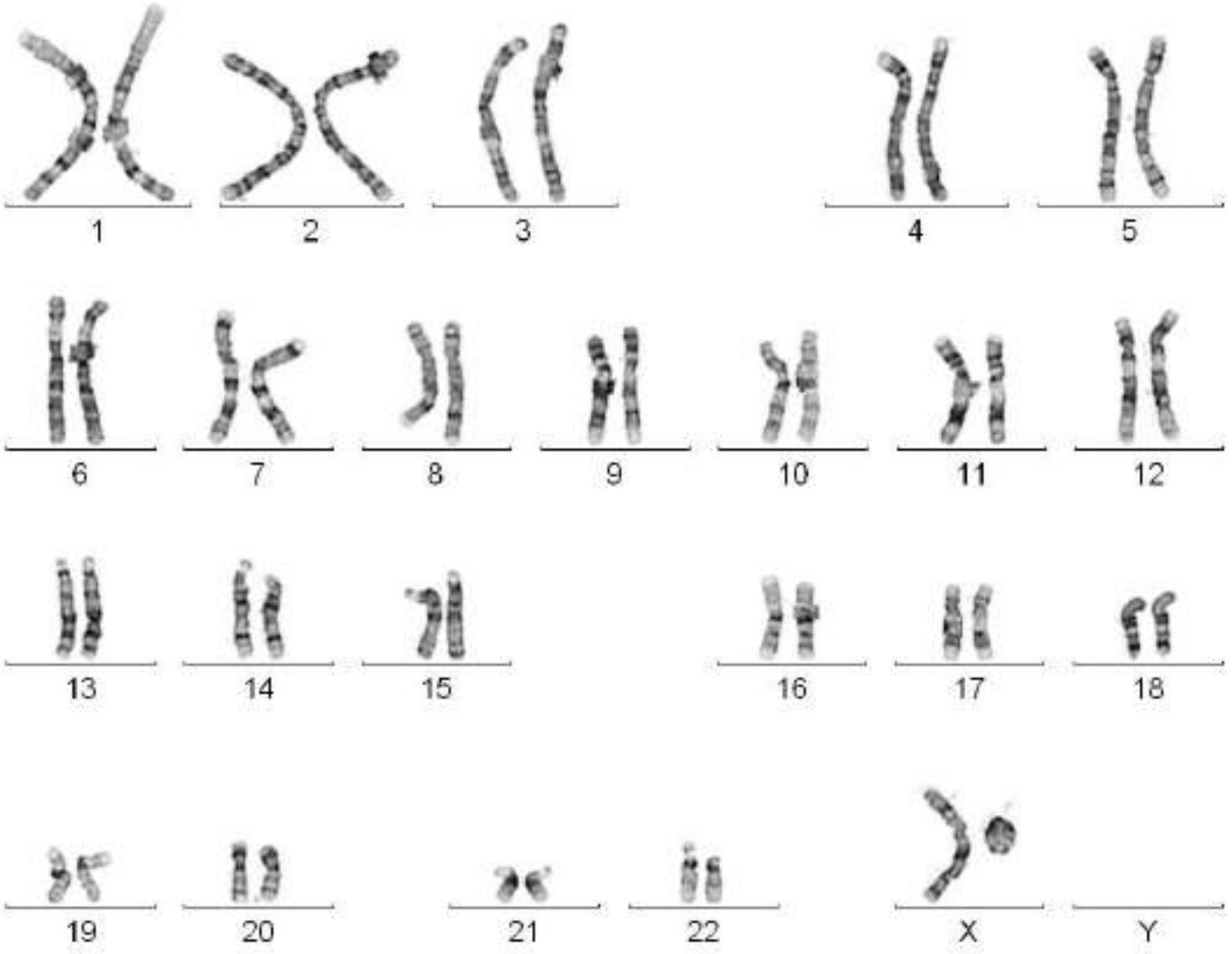
46,XX,t(4;15)(q2?1.3;q13)



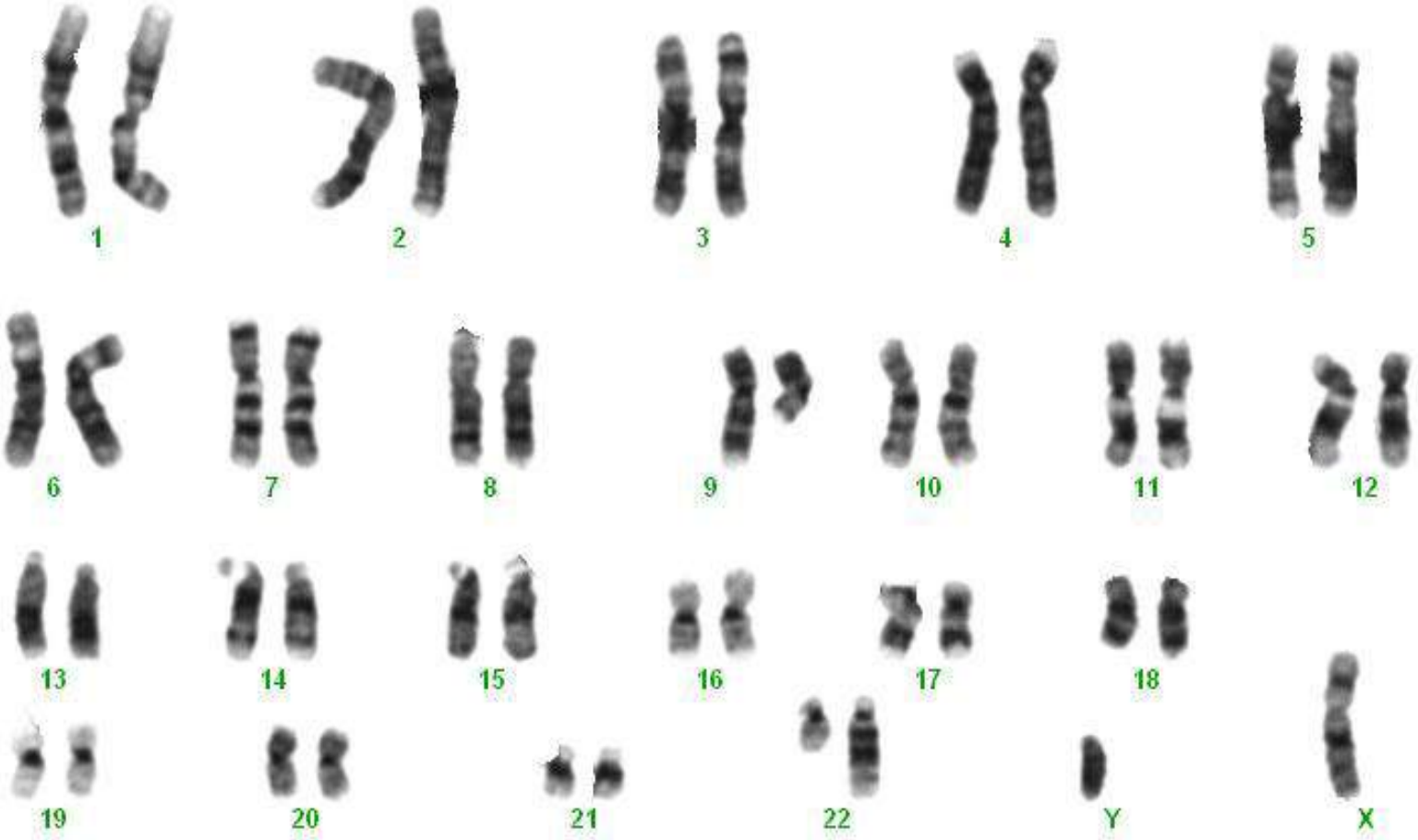
ish der(9)(ABL-),der(22)(BCRsp+conABLsp+,ABLsp+,BCRsp+)



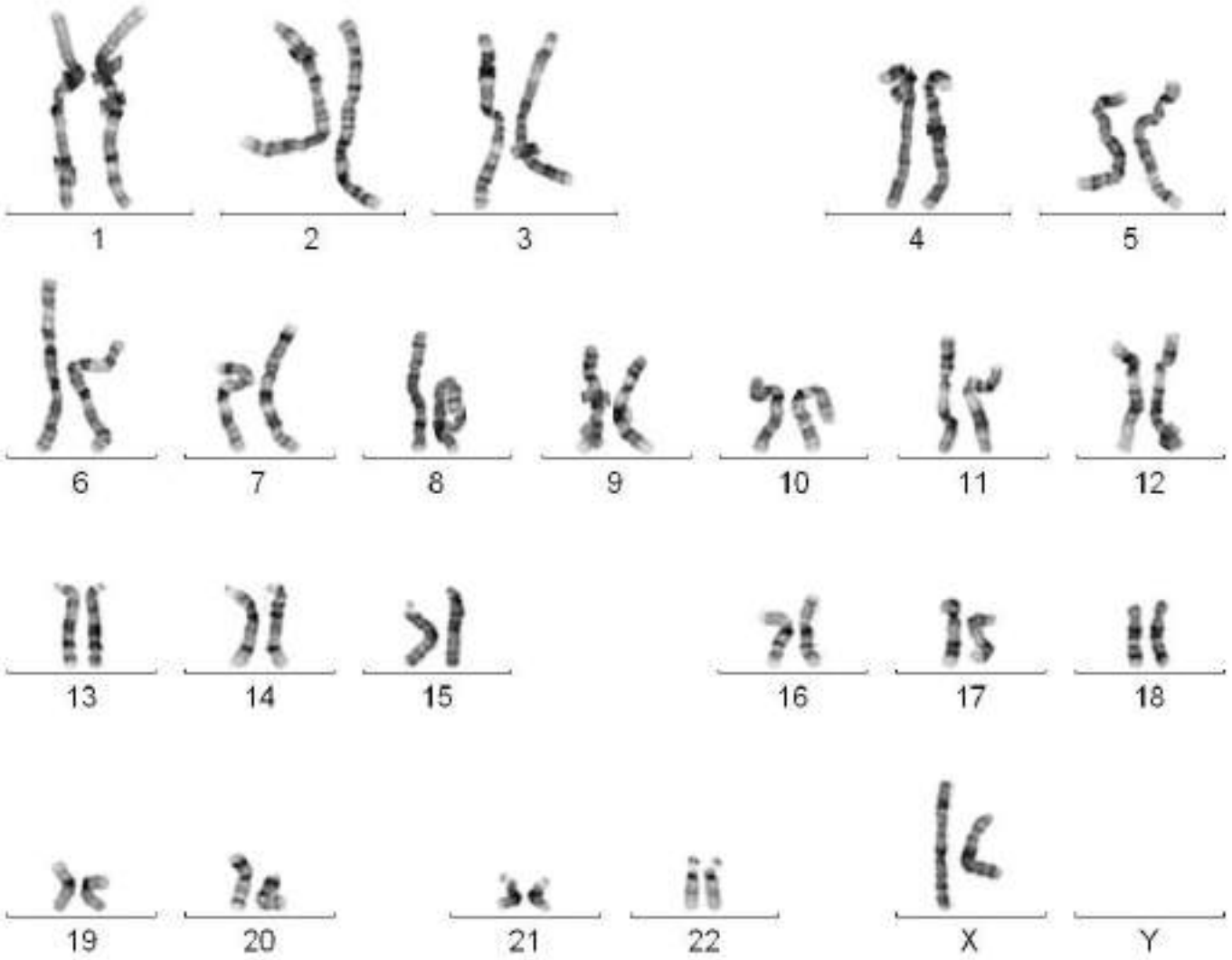
46,X,r(X)



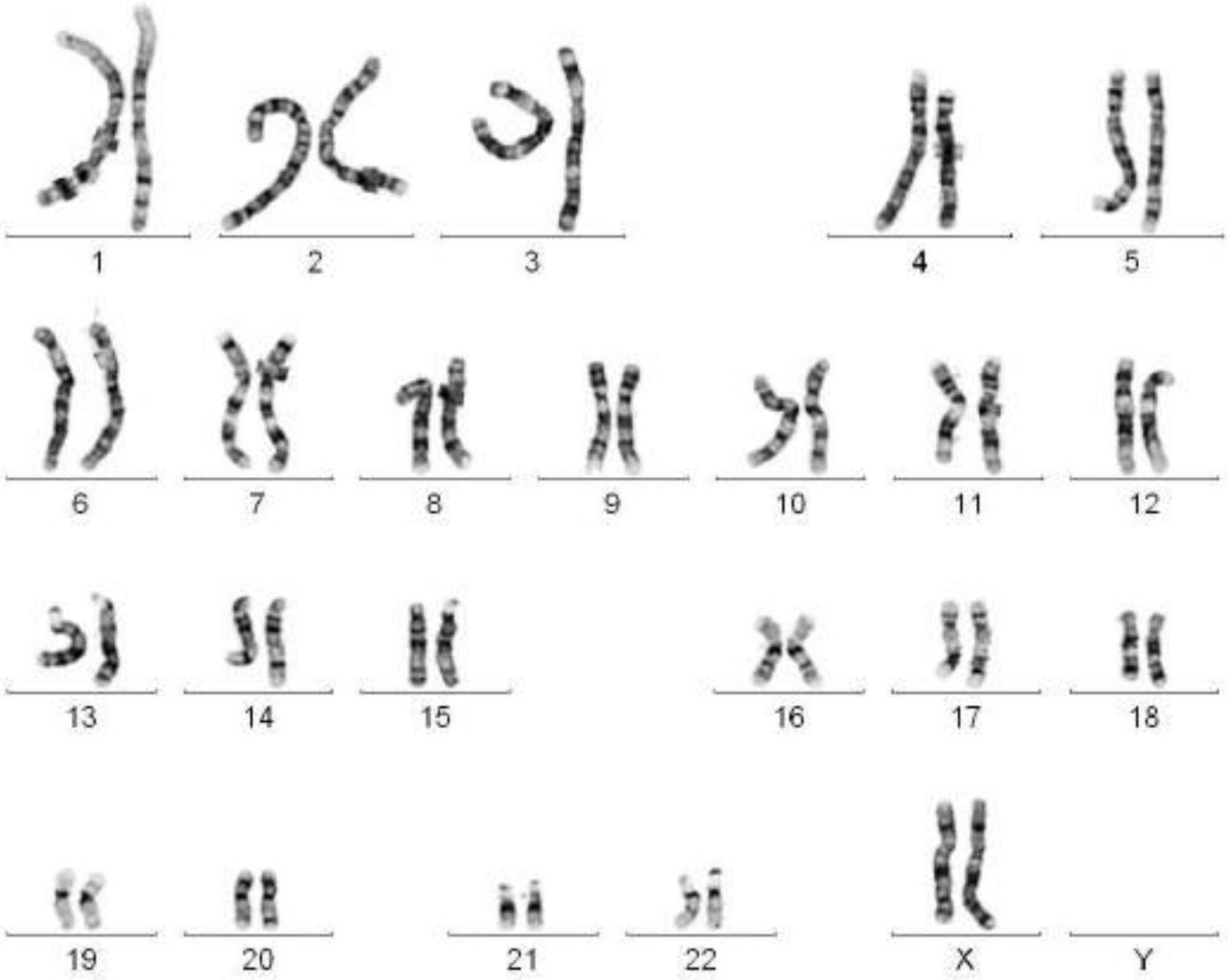
ins(22;9)(q11;q13q34)



46,X,del(X)(p21.1)

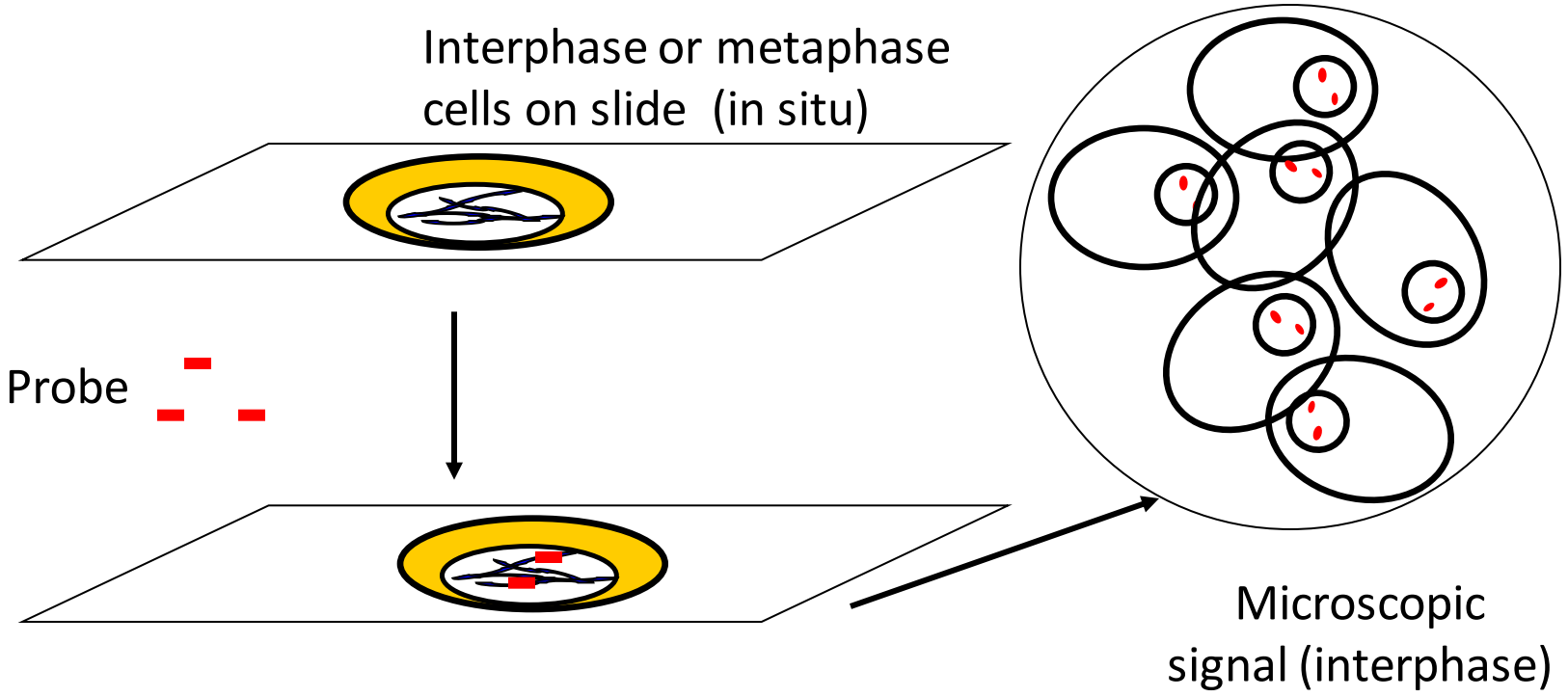


46,XX,del(4)(p15.2p16.?2)



Fluorescent *in situ* Hybridization (FISH)

- Hybridization of complementary gene- or region-specific fluorescent probes to chromosomes.



Fluorescent *in situ* Hybridization (FISH)

- Metaphase FISH
  - Chromosome painting
  - Spectral karyotyping
- Interphase FISH

Uses of Fluorescent *in situ* Hybridization (FISH)

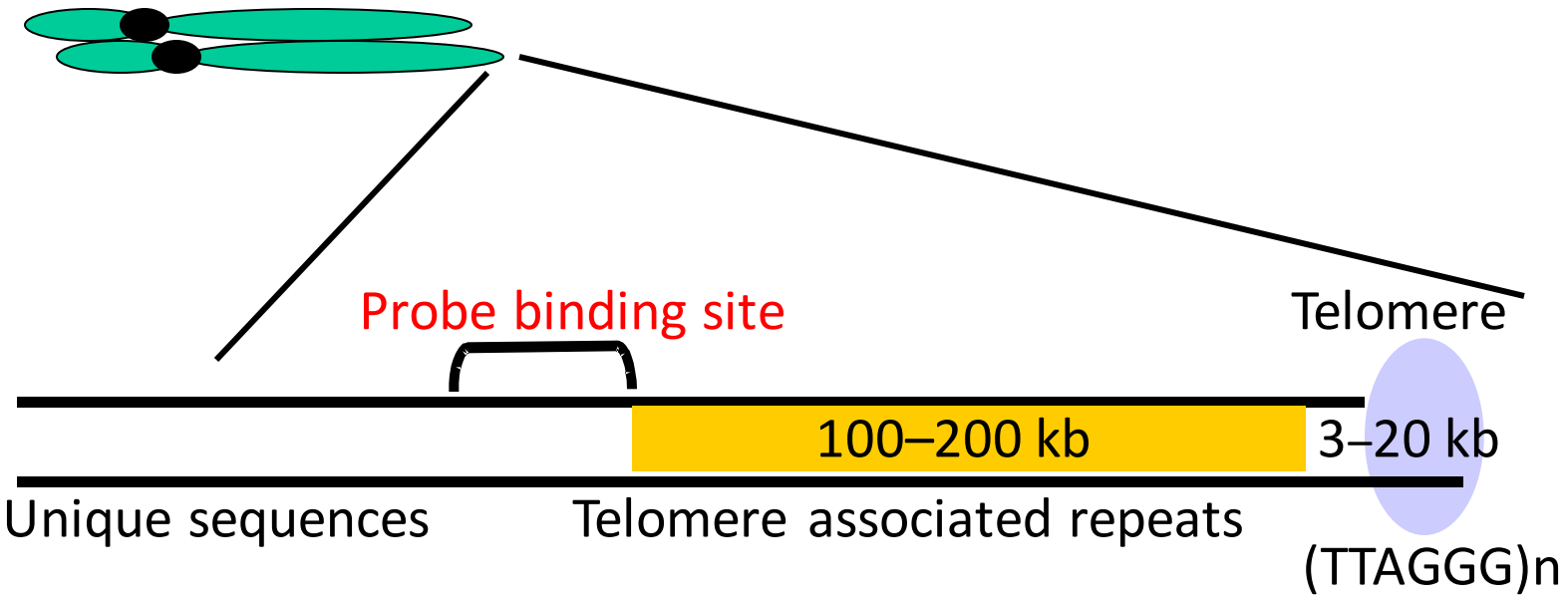
- Identification and characterization of numerical and structural chromosome abnormalities.
- Detection of microscopically invisible deletions.
- Detection of sub-telomeric aberrations.
- Prenatal diagnosis of the common aneuploidies (interphase FISH).

FISH Probes

- Chromosome-specific centromere probes (CEP®)
  - Hybridize to centromere region
  - Detect aneuploidy in interphase and metaphase
- Chromosome painting probes (WCP)
  - Hybridize to whole chromosomes or regions
  - Characterize chromosomal structural changes in metaphase cells
- Unique DNA sequence probes (LSI®)
  - Hybridize to unique DNA sequences
  - Detect gene rearrangements, deletions, and amplifications

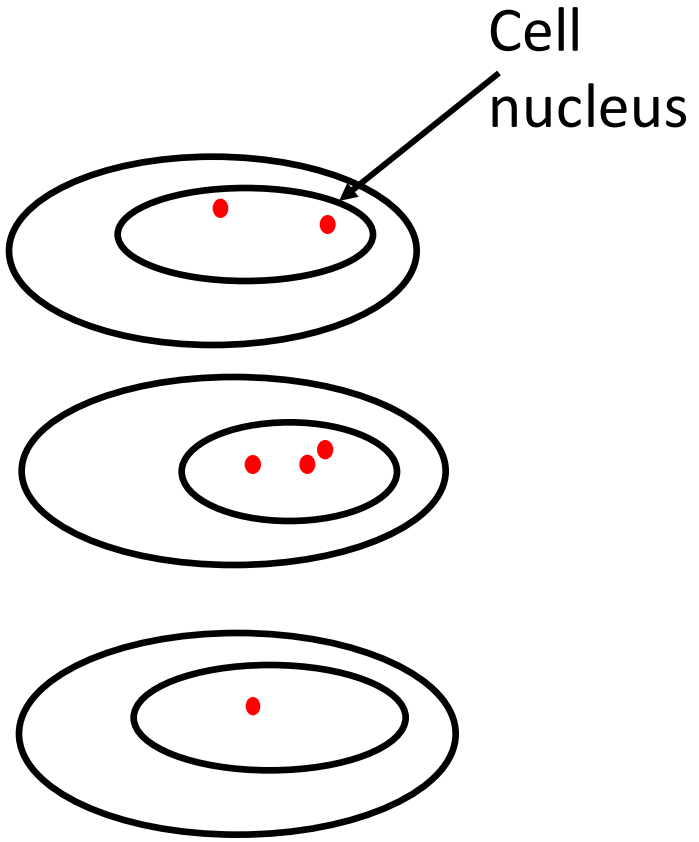
FISH Probes

- Telomere-specific probes (TEL)
  - Hybridize to subtelomeric regions
  - Detect subtelomeric deletions and rearrangements



Genetic Abnormalities by Interphase FISH LSI® Probe

- Greater or less than two signals per nucleus is considered abnormal.



Summary

- Mutations are heritable changes in DNA.
- Mutations include changes in chromosome number, structure, and gene mutations.
- Chromosomes are analyzed by Giemsa staining and karyotyping.
- Karyotyping detects changes in chromosome number and large structural changes.
- Structural changes include translocation, duplication, and deletion of chromosomal regions.
- More subtle chromosomal changes can be detected by metaphase or interphase FISH.

Genetic Diseases

- Duchenne/ Becker muscular dystrophy
- Thalassaemia
- Spinal muscular atrophy
- Fragile X mental retardation
- Hemophilia
- Cystic fibrosis
- Y-chromosome microdeletions

Muscular Dystrophy

- DMD/ BMD
- Mutations in dystrophin gene
- It is one of largest gene: 2.6Mb, 79 exons
- Large intragenic deletions in 70% patients
- Deletion hotspots: Proximal and distal
- Southern hybridization, multiplex PCR
- Absence of deletion does not rule out disease
- Detection of point mutation requires DNA sequencing

Hemophilia A

- Bleeding disorder due to defect in factor VIII gene
- Large gene and heterogeneous point mutations
- Direct mutations in limited number of patients
- Genetic diagnosis based on LINKAGE

Spinal Muscular Atrophy (SMA)

- **Autosomal recessive, neuromuscular disorder**
- **Second most lethal disorder after cystic fibrosis**
- **Incidence of ~ 1/10,000 live births**
- **Carrier frequency of ~ 1/40**

- **Degeneration of anterior horn cells of spinal cord**
- **Leads to symmetrical muscle weakness and atrophy**
- **Hypotonia, Respiratory Insufficiency and Fasciculation of tongue and palms**

- **Survival motor neuron gene *(SMN)* has been identified to cause SMA.**
- **Gene is about 27 kb in size and encodes for a 38 kDa protein consisting of 294 amino acids.**
- ***SMN* gene is present in two homologous copies: telomeric (*SMN1)* and centromeric *(SMN2*).**
- ***SMN1* gene is deleted in SMA patients but *SMN2* gene is present.**

Fragile X Mental Retardation

☆ **Fragile X syndrome, the most common cause of inherited mental retardation.**

☆ **Incidence : 1 in 4000 males and 1 in 8000 females**

☆ **Expansion of unstable CGG repeats at 5`-UTR of FMR-1 gene may lead to the disease**

Normal	**6-50**
Premutation	**50-200**
Full mutation	**>200**

☆ **Absence of FMR-1 gene product (FMRP) is the typical cause of disease**

☆ **FMR-1, a highly conserved gene, consists of 17 exons and spans ~38 kb**

Thalassemia

- Thalassemia is an inherited blood disorder in which the body makes an abnormal form of hemoglobin.
- The disorder results in excessive destruction of red blood cells, which leads to anemia. Anemia is a condition in which your body doesn't have enough normal, healthy red blood cells.
- Thalassemia is inherited, meaning that at least one of your parents must be a carrier of the disorder. It's caused by either a genetic mutation or a deletion of certain key gene fragments.
- Thalassemia minor is a less serious form of the disorder. There are two main forms of thalassemia that are more serious. In alpha thalassemia, at least one of the alpha globin genes has a mutation or abnormality. In beta thalassemia, the beta globin genes are affected.
- Each of these forms of thalassemia has different subtypes. The exact form you have will affect the severity of your symptoms and your outlook.

Cystic Fibrosis (CF)

- Cystic fibrosis (CF) is a genetic disorder, which means you get it from your parents at birth. It affects the way your body makes mucus, a substance that helps your organs and systems work. Mucus should be thin and slippery, but when you have CF, it becomes thick and glue-like. This blocks tubes and ducts throughout your body.
- Over time, this thick mucus builds up inside your airways. This makes it hard to breathe. The mucus traps germs and leads to infections. It can also cause severe lung damage like cysts (fluid-filled sacs) and fibrosis (scar tissue). That's how CF got its name.
- Cystic fibrosis is caused by a change, or mutation, in a gene called CFTR (cystic fibrosis transmembrane conductance regulator). This gene controls the flow of salt and fluids in and out of your cells. If the CFTR gene doesn't work the way it should, a sticky mucus builds up in your body.

Y Chromosome Microdeletion (YCM)

- **Y chromosome microdeletion** (YCM) is a family of genetic disorders caused by missing gene(s) in the **Y chromosome**. Many men with YCM exhibit no symptoms and lead normal lives. However, YCM is also known to be present in a significant number of men with reduced fertility.

- **Y chromosome** infertility is **caused** by **deletions** of genes in the AZF regions. These **deletions** remove several genes, or in rare cases, a single gene. Loss of this genetic material likely prevents the production of one or more proteins needed for normal sperm cell development.

- Three independent loci within the AZF region, named AZFa, AZFb, and AZFc, with each subregion associated with somewhat different effects on spermatogenesis.

Non-invasive Prenatal Diagnosis

- Long-term goal of human genetics is to develop prenatal diagnostic tests which are non-invasive with no risk to mother or fetus
- Fetal cells in maternal blood: Particularly useful if fetus is male. Requires enrichment of cells
- Fetal DNA has been detected in maternal circulation. Research is going on to successfully develop diagnostics based on presence of small amount of fetal DNA in maternal plasma or serum.

Pre-implantation Genetic Diagnosis

- Detection from single cell poses problems even with techniques like PCR
- High or total signal failure
- Selective signal failure (Allele dropout) may result in misdiagnosis
- Contaminations can pose serious problems
- PCR modification particularly using fluorescent detection can overcome some problems.

Thank You