Medical Cytogenetics

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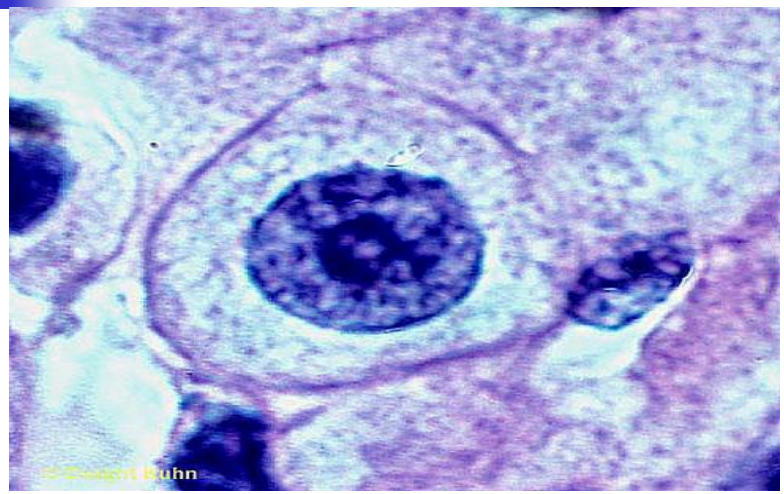
1. Chromatin and chromosome

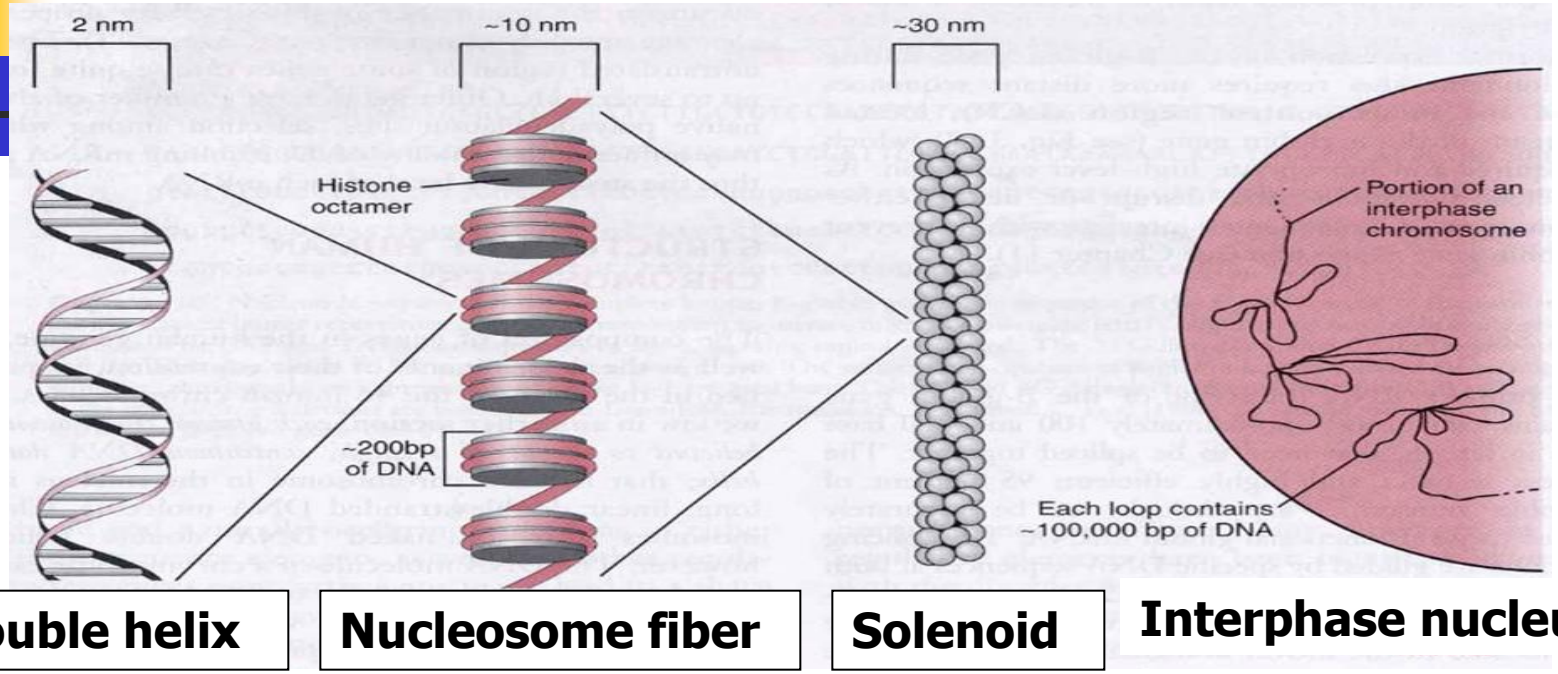


Chromatin in nucleus

Euchromatin: Slightly and evenly stained, non- or low-repetitive DNA regions

Heterochromatin: Darkly and unevenly stained, highly repetitive DNA regions

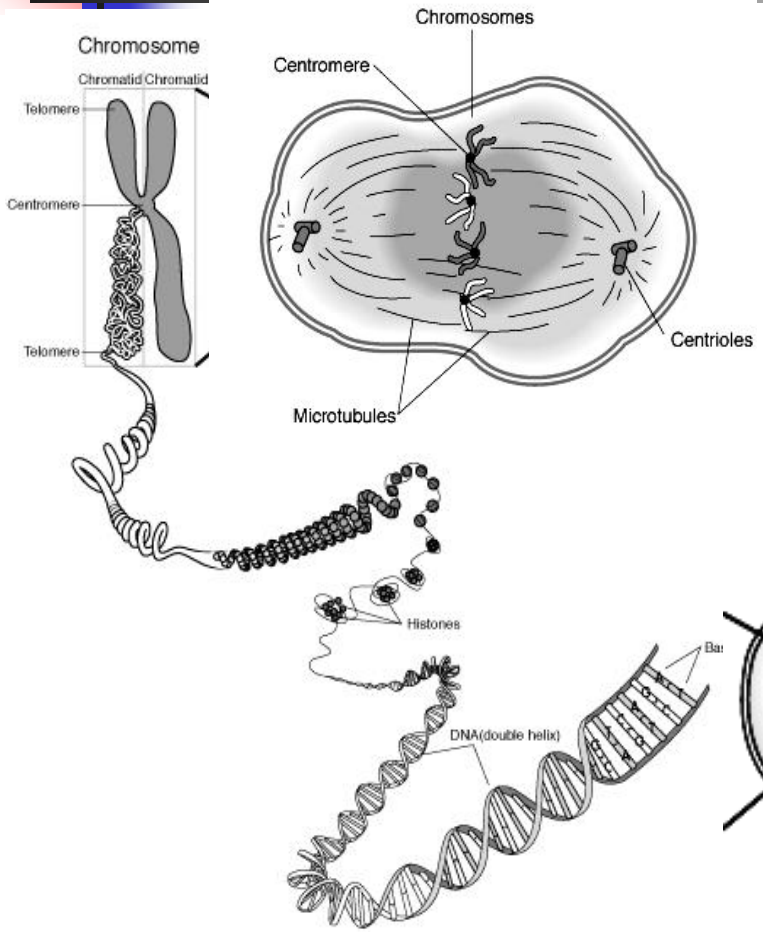
Chromatin composition

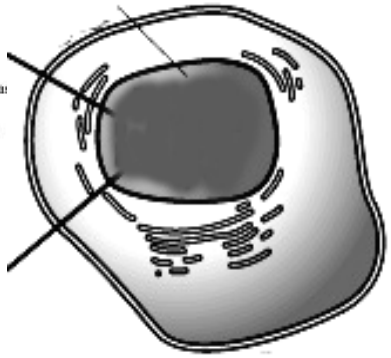


Chromatin is the basic components in the cell nucleus

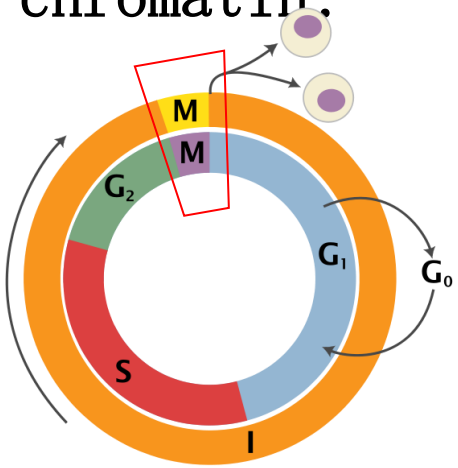
Composed of DNA, histones and non-histone proteins

From Chromatin to Chromosome



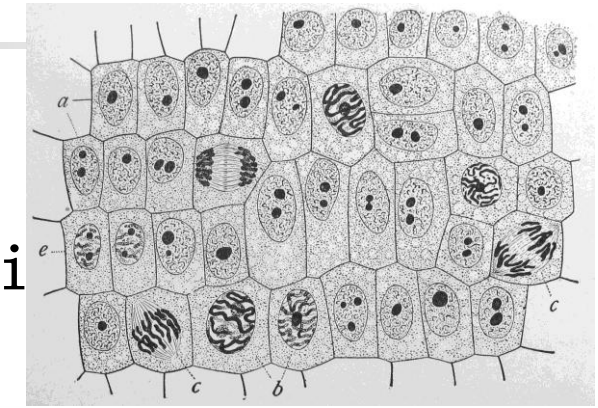


- Different mode of chromatin in metaphase
- Resulted from highly compaction of chromatin:

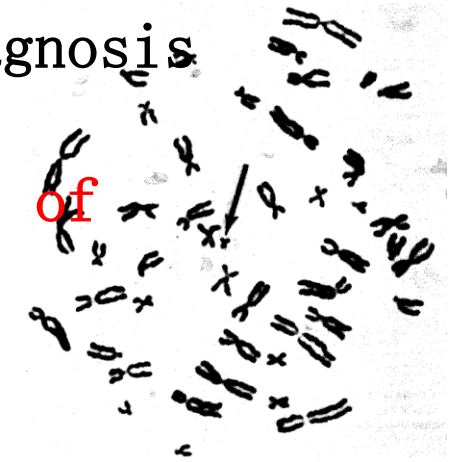


Cell Cycle

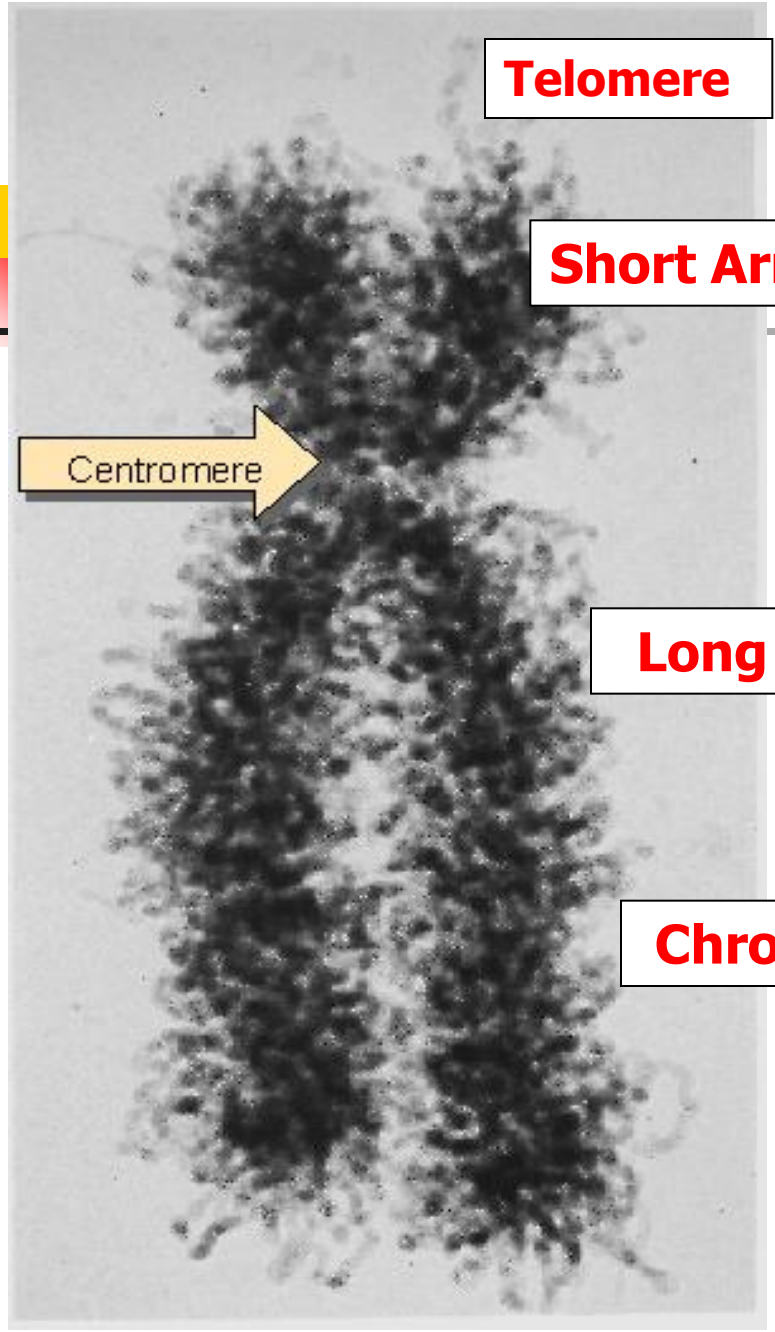
Chromosome Preparation



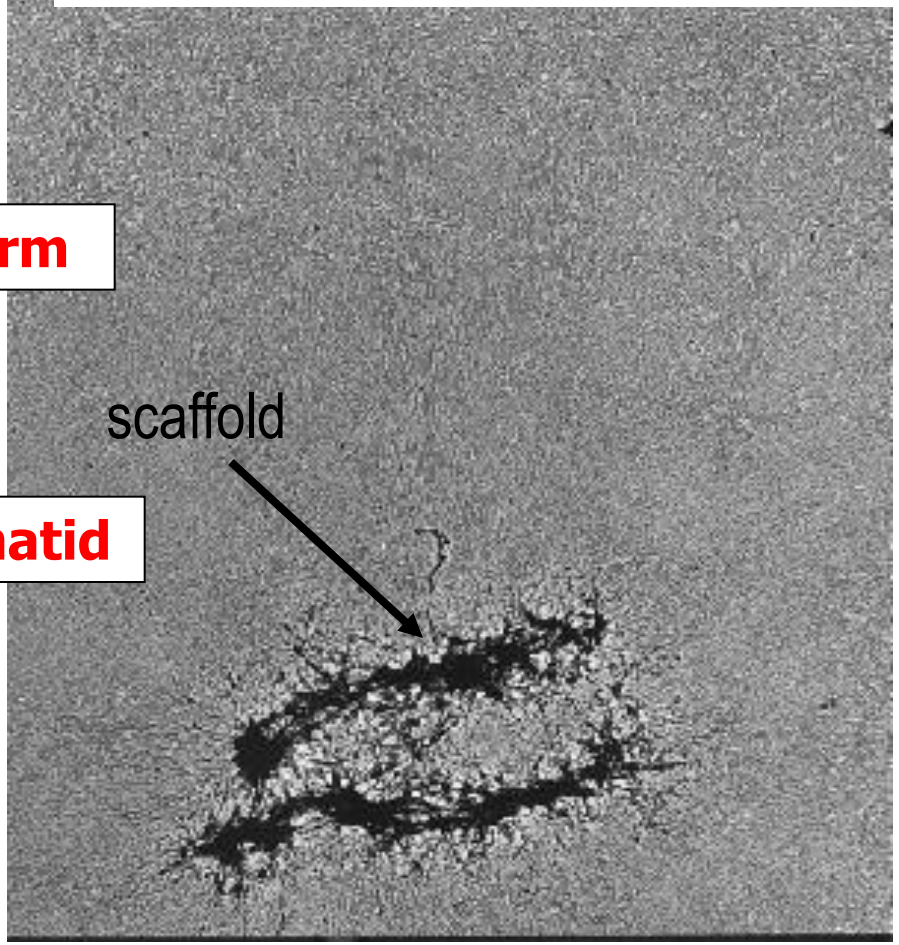
- Cell culture:
  - Peripheral blood: PHA stimulati
  - Fibroblast from varous cells
  - Bone marrow for leukemia
  - Amniotic fluid cell for fetal diagnosis
- Colchicine arresting metaphase – Harvest a great number of metaphases
- Hypotonic treatment
- Chromosome spread preparation



Identify each chromosome



- Chromatids, two after S-phase
- Centromere
- Long arm and short arm
- Telomere



2. Chromosome identification

1. Morphology of chromosome

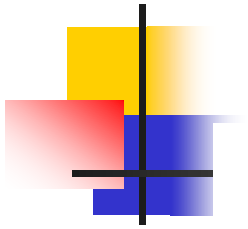
Length

RL: Relative length, Ch L / total L of a haploid set

Position of centromere

AI: Arm index, Long arm L(q) / Short arm L (p)

CI: Centromere index, q / Ch L



Metacentric Ch

Submetacentric Ch

Acrocentric Ch

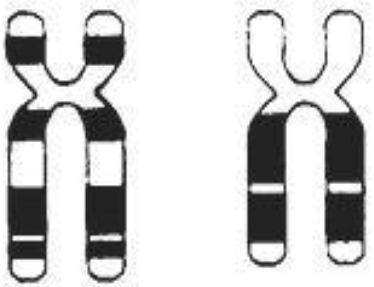
Short Arm

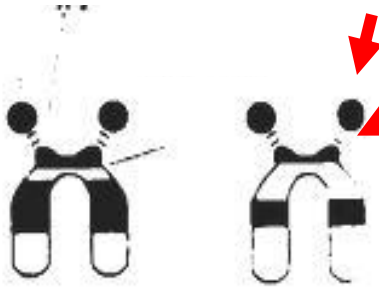
p

Centromere

Long Arm

q





Satellite

Satellite arm

3

17

18

21

22

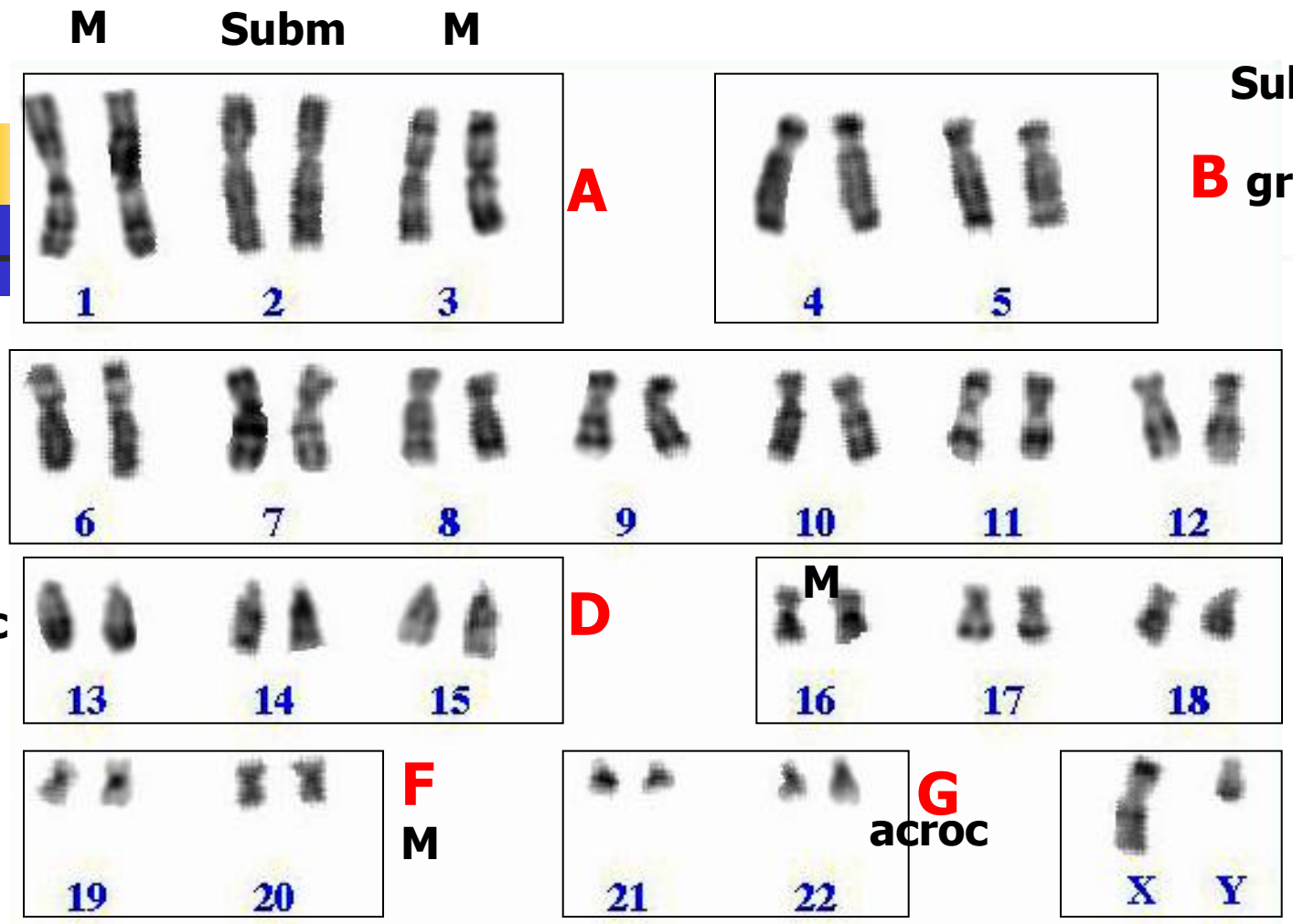
Metacentric Ch. CI: 1/2～5/8

Submetacentric Ch. CI: 5/8～7/8

Acrocentric Ch. CI: >7/8

International System for Human Cytogenetic Nomenclature ISCN, Denver

1. Rl: from large to small
   Chromosome length: from long to short
2. CI: from small to large
   Centromere position: from low to high
3. Variable heterochromatic region:
   1qh, 9qh, 16qh, Yq
   Satellite and satellite arm of acrocentric chromosomes



46, XY Karyotyping, 7 Groups

2. Banding of chromosome

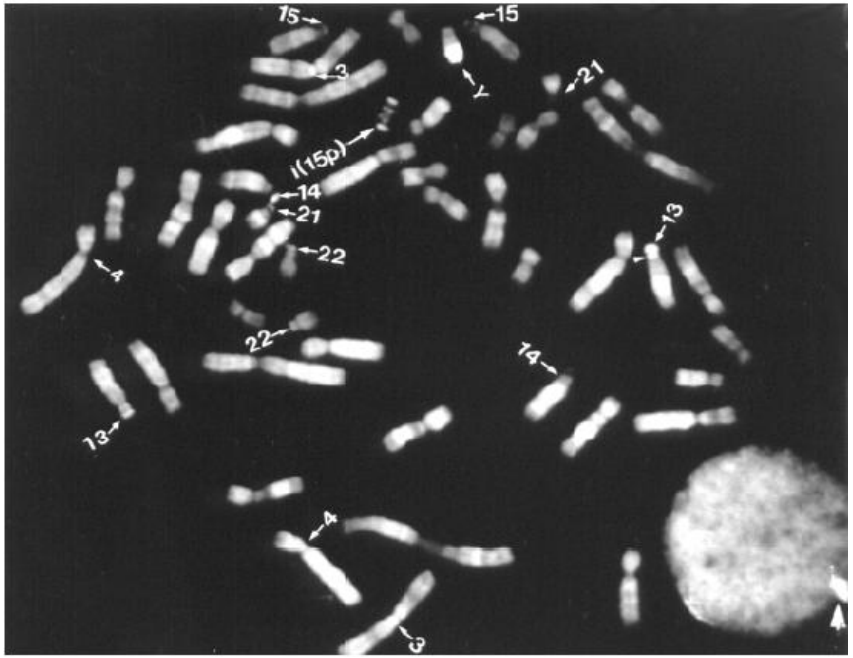
Q bands:

Caspersson (1970)

Quinacrine mustard

Fluorescence microscopy

Bright and dim bands



G Bands

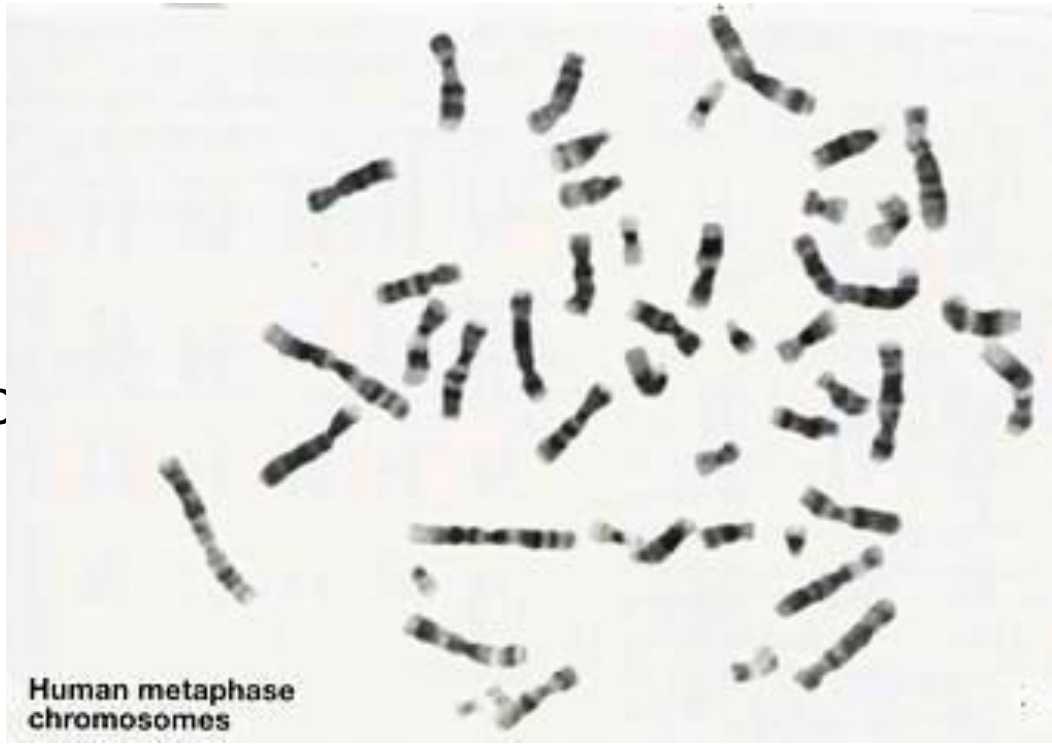
Giemsa bands:

Trypsin digestic

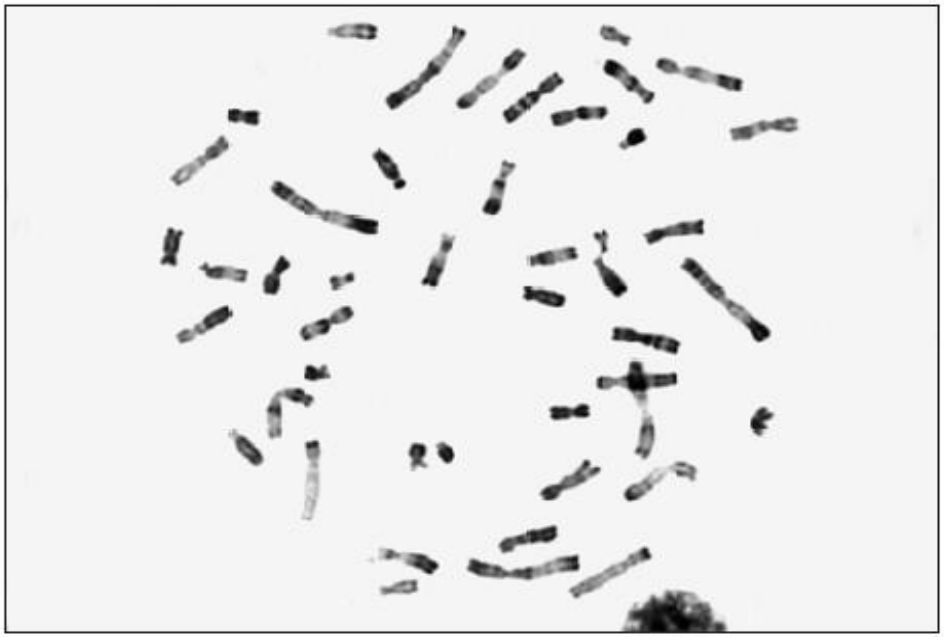
Giemsa staining

Permanent

Dark and light bands



R Banding



Reversed bands:

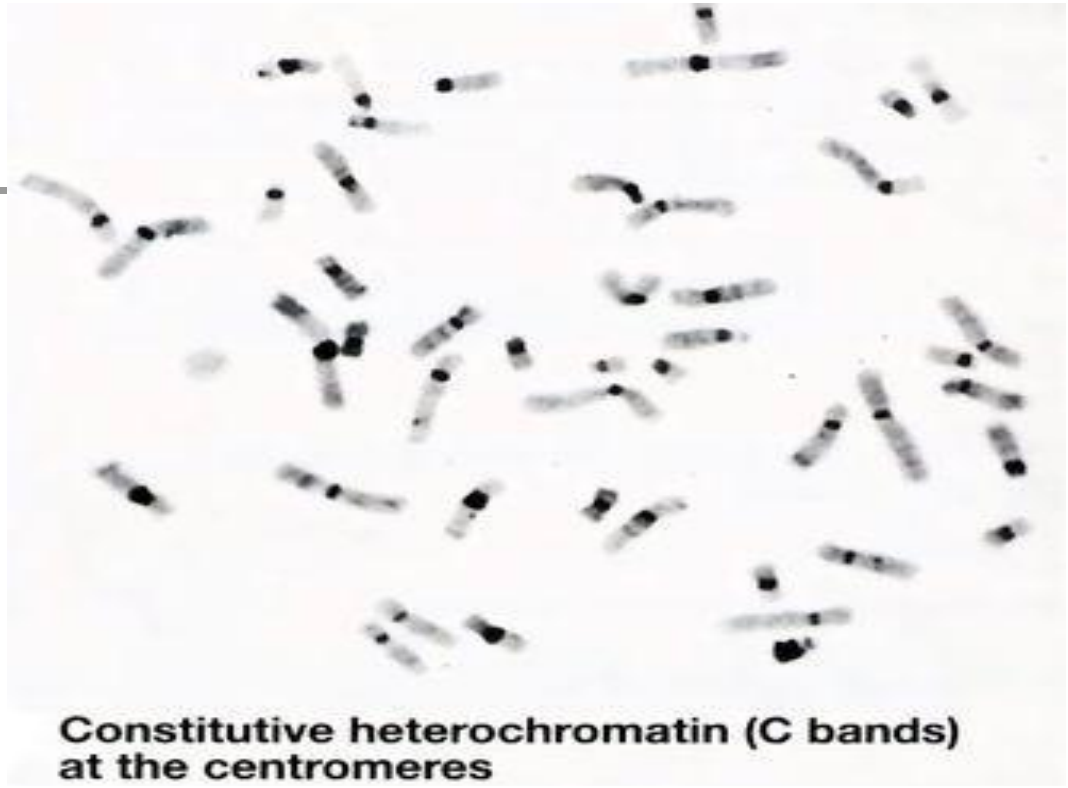
Heated, KOH

Giemsa staining

Permanent

Dark and light bands reversed to G bands

C Bands



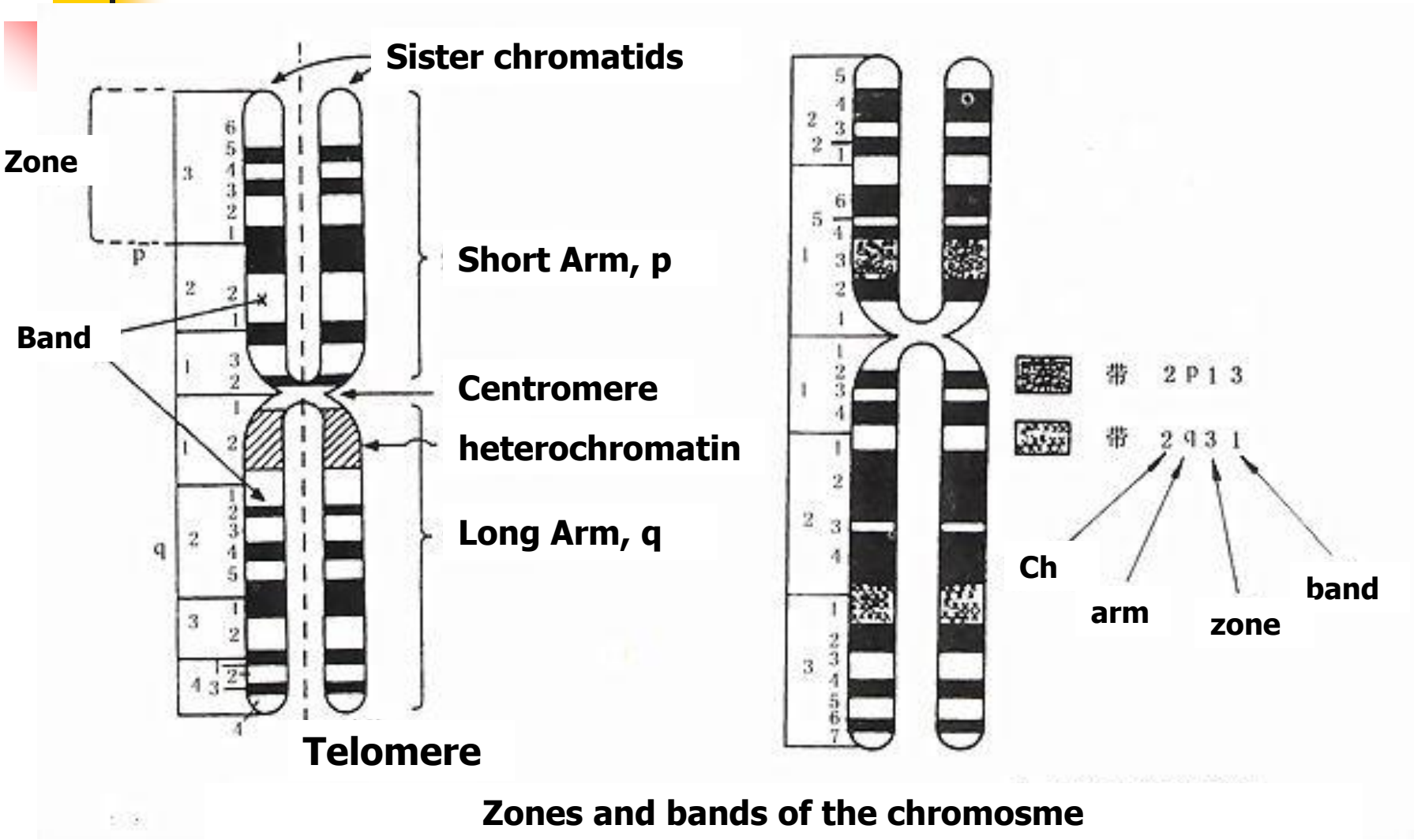
Constitutive heterochromatin (C bands) at the centromeres

Heated, KOH

Giemsa staining

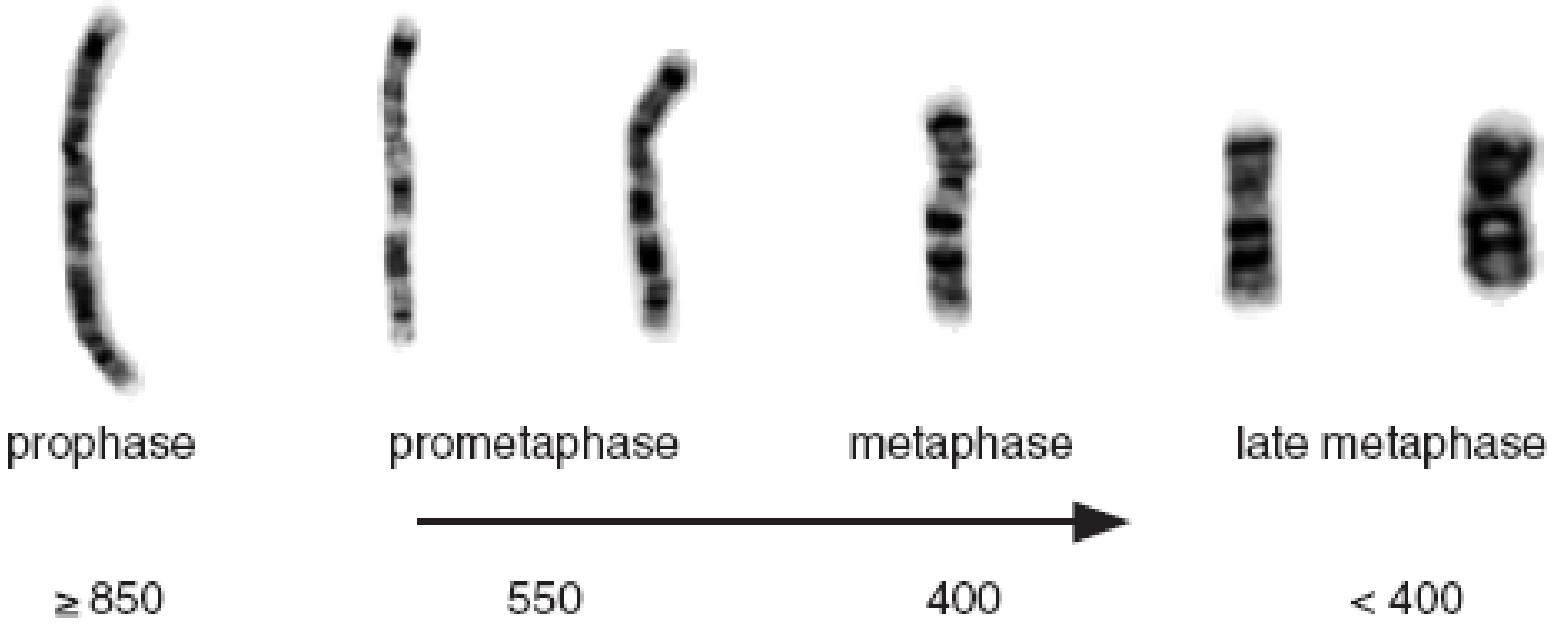
Heterochromatin in the centromeres, long arm of the Y and 1qh , 9qh and 16qh

Nomenclature of human chromosome



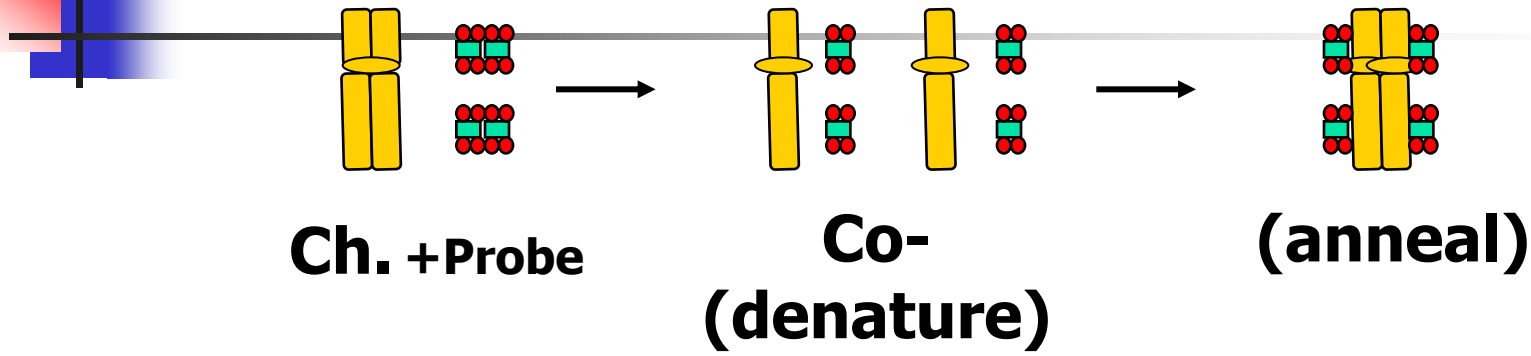
Zones and bands of the chromosme

High-resolution bands

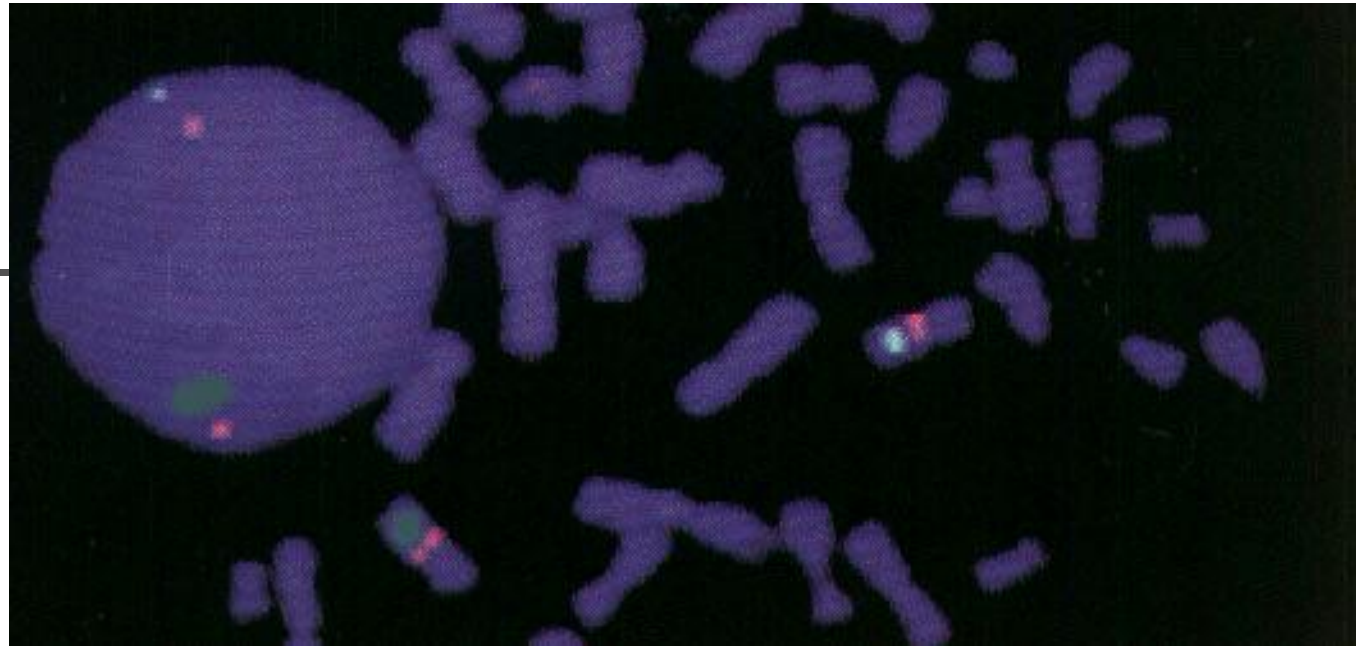


- Chromosomes in different stages of phases of cell cycle
- More detailed analysis

Fluorescence In Situ Hybridization （FISH）

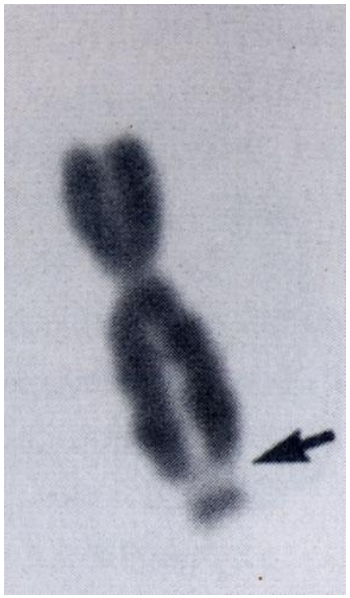


- Using DNA probe labeled with a certain marker
- Hybridizing with DNA in chromosomes and nuclei on slides
- Probes hybridized with the fragment in chromosome are detected by signals from the labeled markers



- Rapid mapping of genes and sequences in chromosome
- Detecting small fragment in interphase.
- Detecting cryptic rearrangements or small deletions – Banding could not be detected < 4Mb

Fragile Sites



46, Y, fra(X)(q27-28)

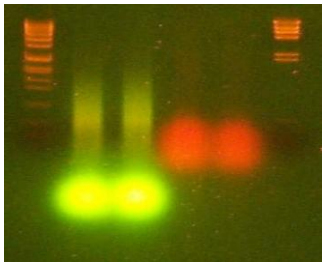
■Non-staining gaps that occasionally observer at charcteristic sites on several chromosomes

■ Depend on growth conditions

■ Heritable variants

Comparative Genomic Hybridization (CGH)

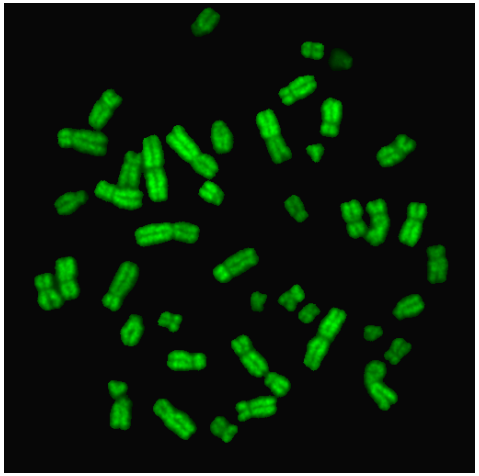
Lable test DNA with green

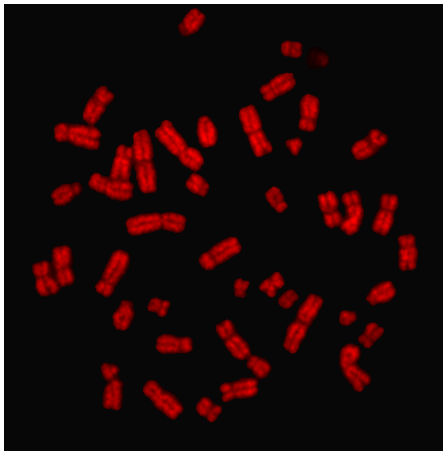


Lable normal DNA with red

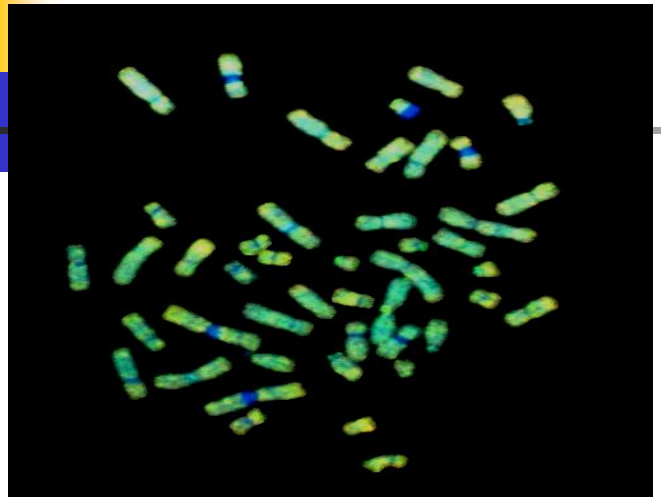
1：1 mixed

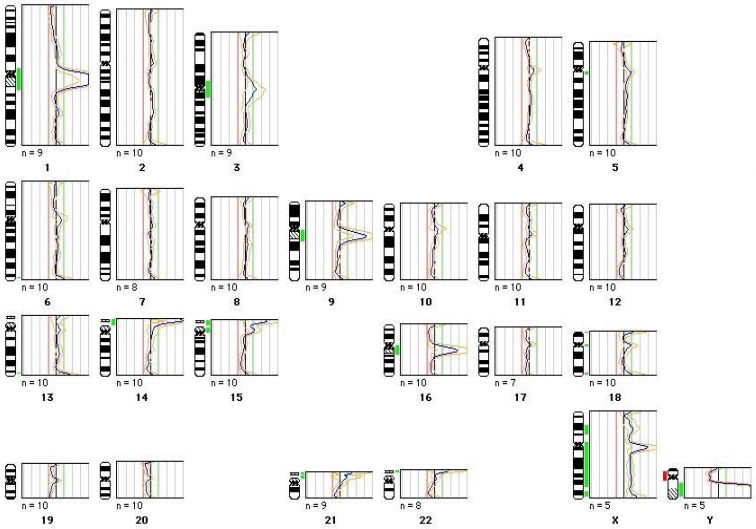
Hybrized onto male chromosomal preparation

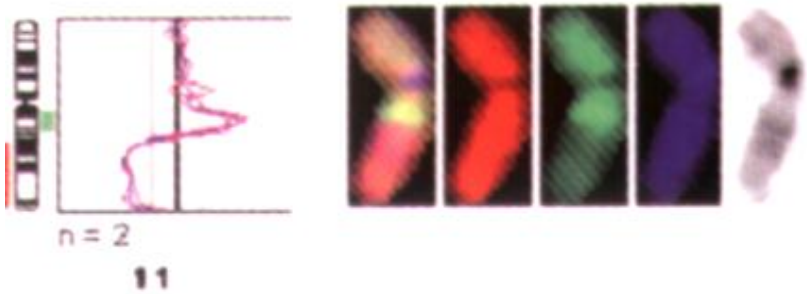


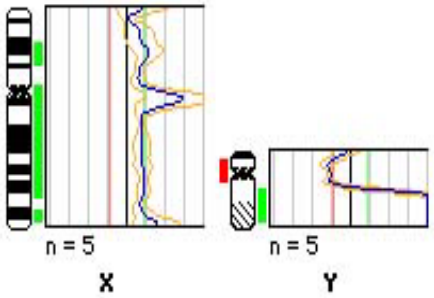


CGH





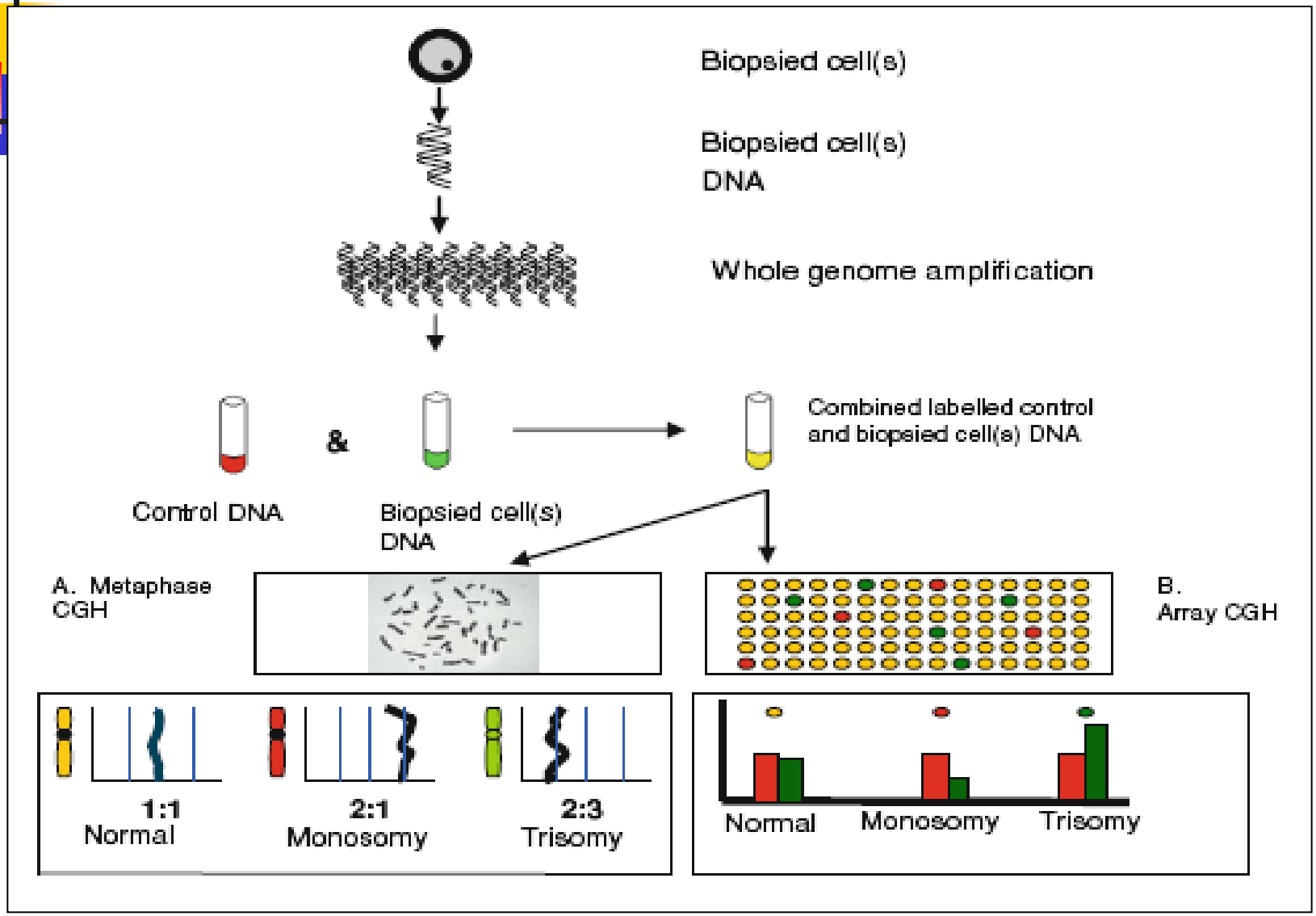




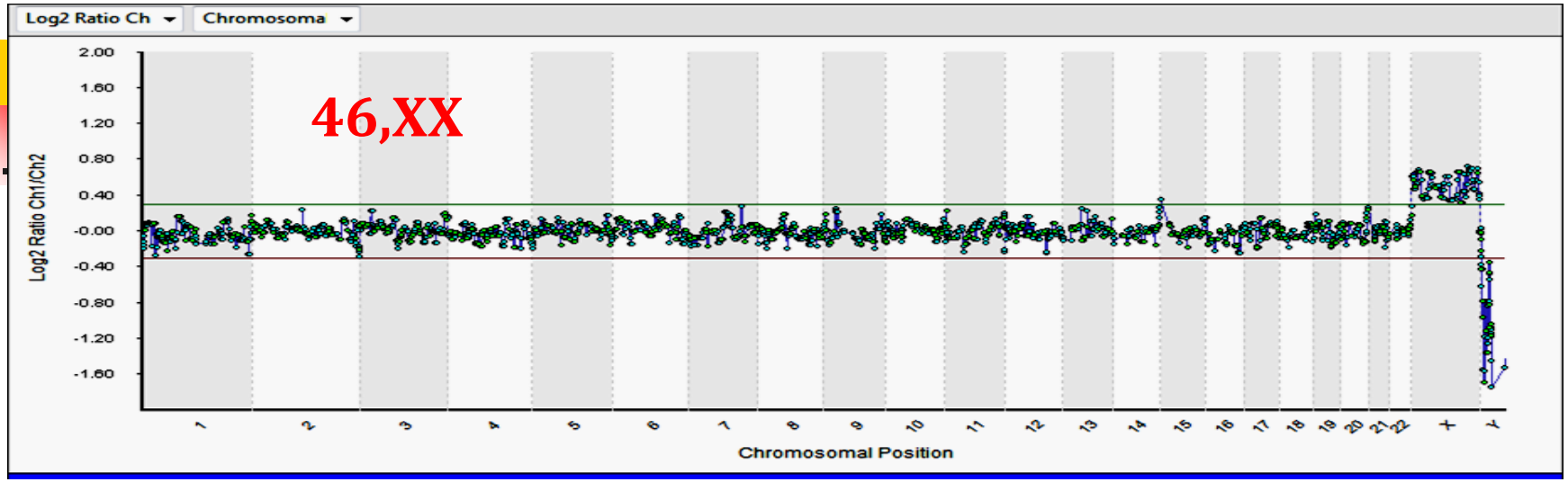
Compare the intensity of two fluorochromes along the chromosome set.

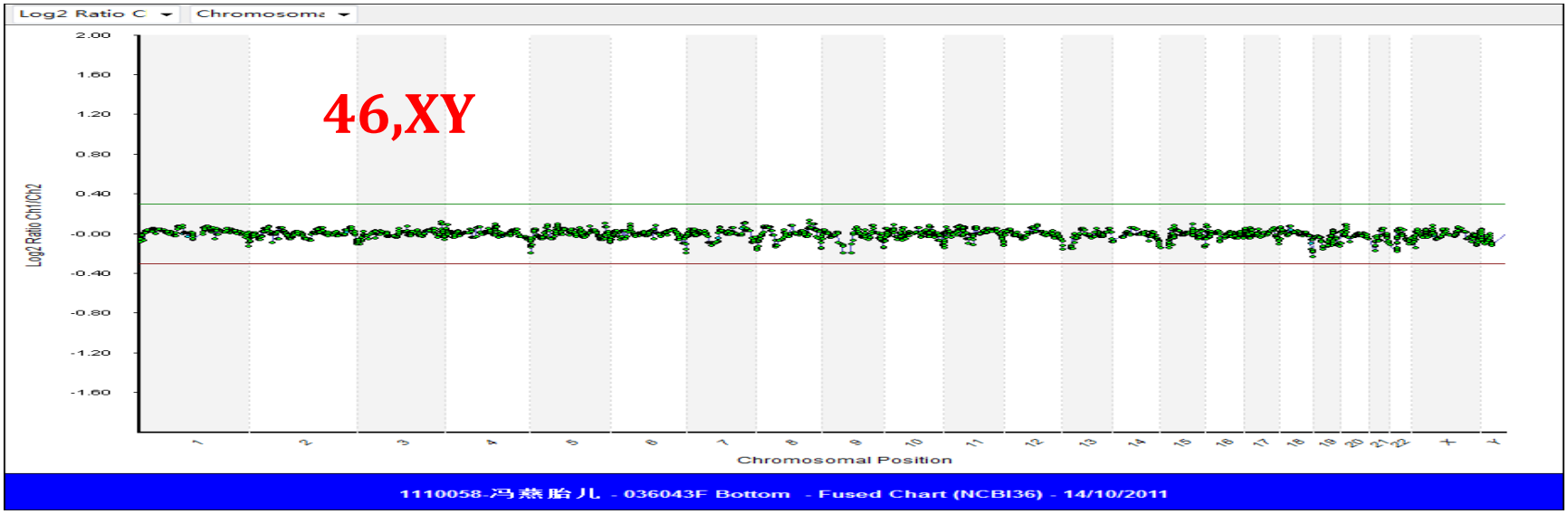
Detection of duplication or deletion of chromosomal segment.

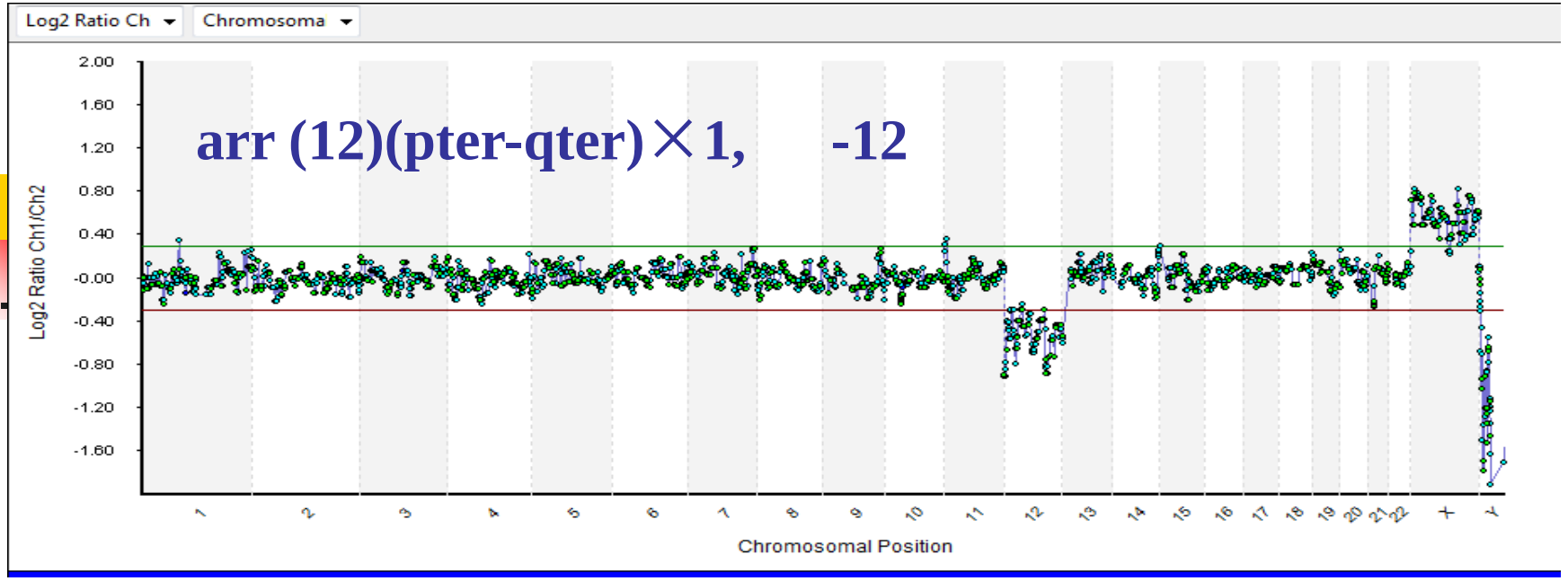
Array CGH，aCGH

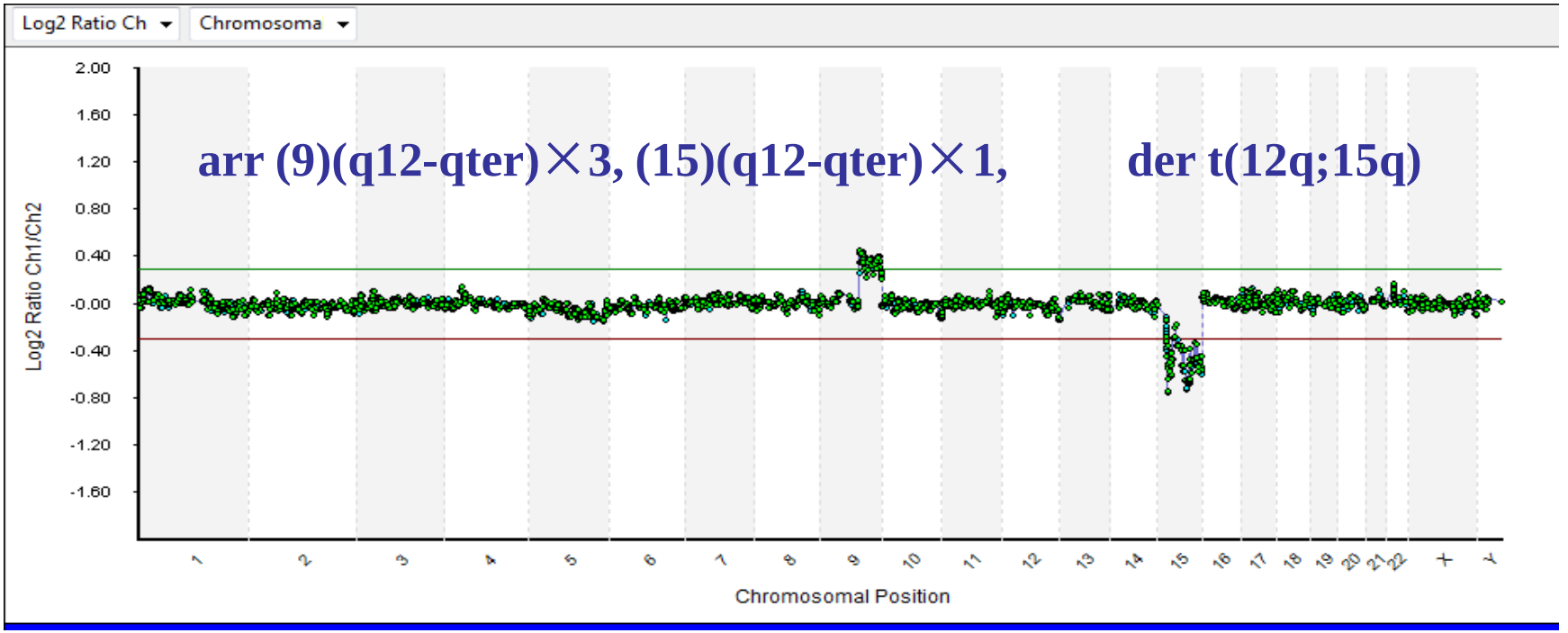


Euploid


Log2 Ratio Ch
Chromosoma
46,XX
Log2 Ratio Ch1/Ch2
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Chromosomal Position


Log2 Ratio C
Chromosoma
46,XY
Log2 Ratio Ch1/Ch2
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Chromosomal Position
1110058 冯燕胎儿 - 036043F Bottom - Fused Chart (NCBI36) - 14/10/2011


Log2 Ratio Ch
Chromosoma
arr (12)(pter-qter)×1, -12
Log2 Ratio Ch1/Ch2
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Chromosomal Position


Log2 Ratio Ch
Chromosoma
arr (9)(q12-qter)×3, (15)(q12-qter)×1, der t(12q;15q)
Log2 Ratio Ch1/Ch2
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Chromosomal Position

3. Chromosome abnormalities

(1). Numerical chromosomal abnormalities

Heteroploidy: A chromosome complement with chromosome number other than 46

Euploidy: A chromosome complement with an exact multiple of the haploid chromosome number

Aneuploidy: A chromosome complement with chromosome number other than an multiple of the haploid chromosome number

A. Euploidy: Monoploidy and Polyploidy

- 1N: Monoploidy 23X or 23Y:
  in parthenogenesis
- 3N: Triploidy 69, XXX or 69, XXY
  in partial mole, aborted fetus and liveborn who does not survive long
- 4N: Tetraploid 92, XXXX or 92, XXYY
  in aborted fetus

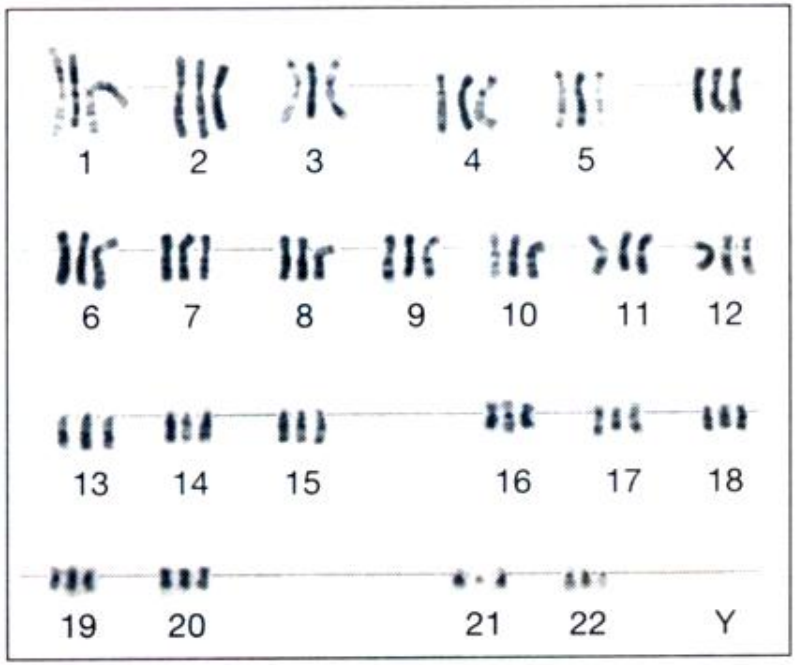
Triploidy

Triploidy

- Most frequent chromosomal aberration (15%) in fetuses following spontaneous abortion
- Severe growth retardation, early lethality
- Occasional liveborn infant with severe malformation
- Dispermia a frequent cause

1.

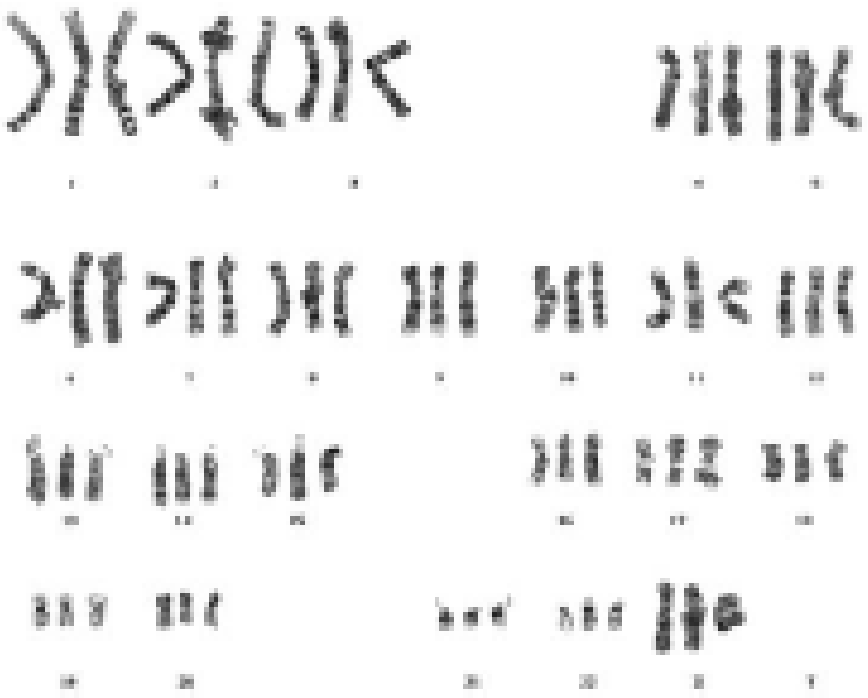
2.

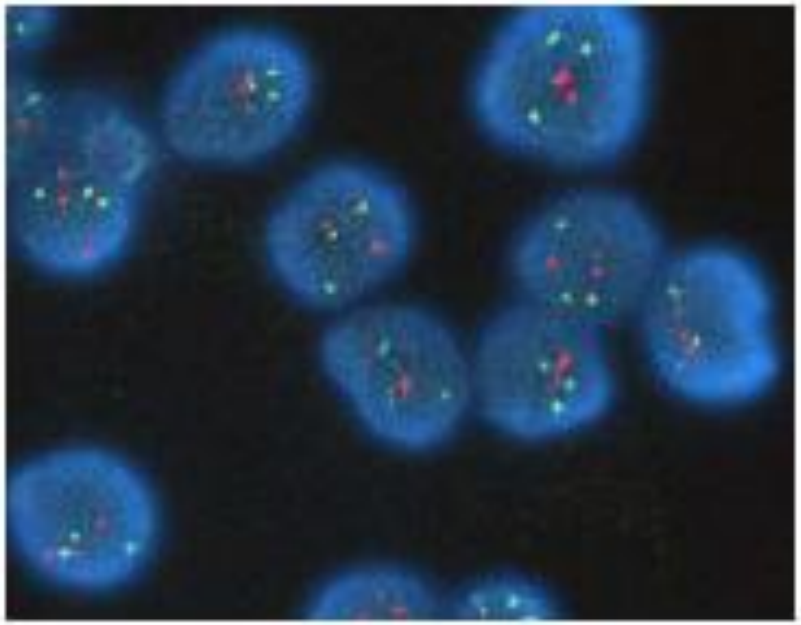


3.

Triploidy

Triploidy

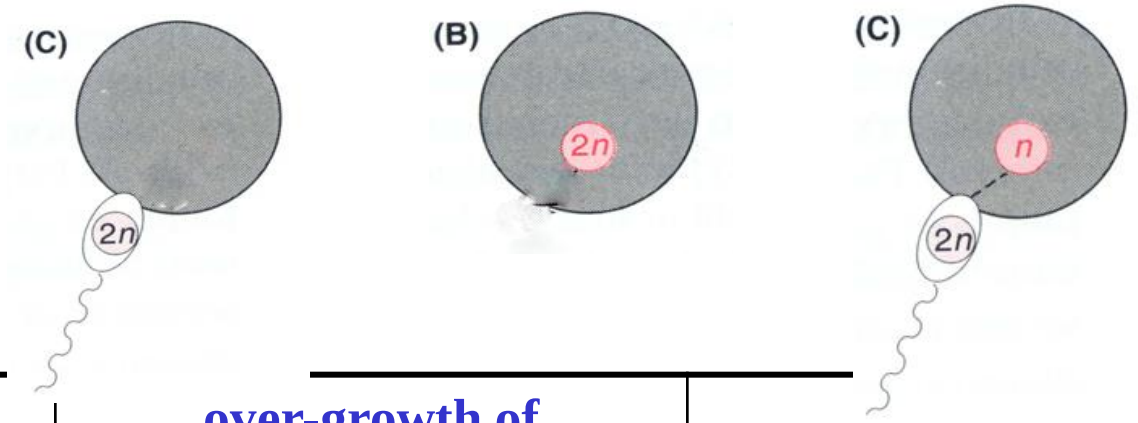




13-green, 21-red

Imprinting: The expression of the phenotype depends on whether the gene or genome inherited from the father or mother.

parthenogenesis



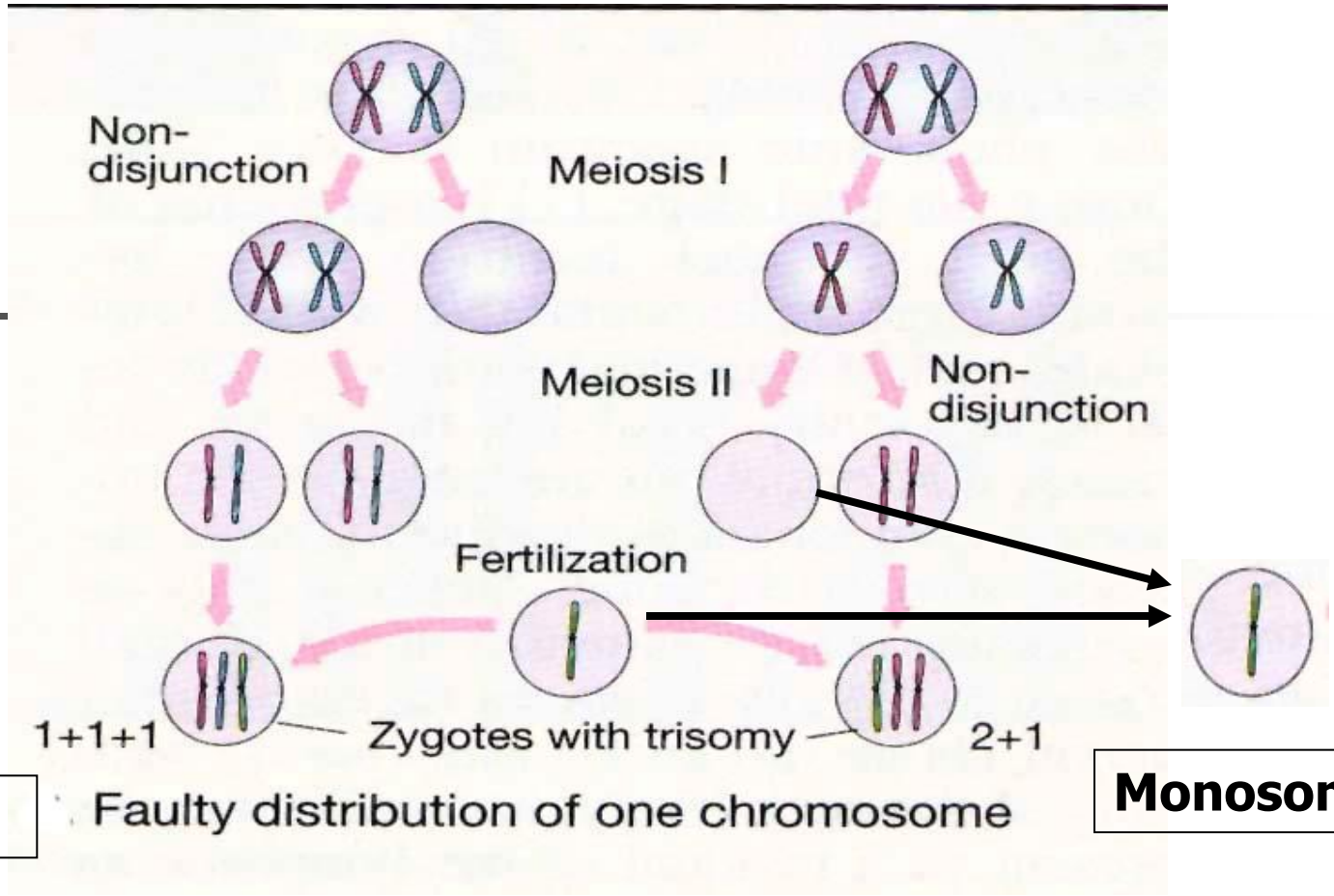
Pat. diploidy	over-growth of trophoblast	Complete mole
Mat. diploidy	over-growth of inner mass cell	Teratomas
Triploidy	Two sperm fertilized with a normal oocyte	Partial mole

(2). Aneuploidy

Loss or gain of chromosomes (not multiple of a haploidy)

Monosomy 2n-1: one instead of a pair of homologous chromosomes

Trisomy 2n+1: three instead of a pair of homologous chromosomes



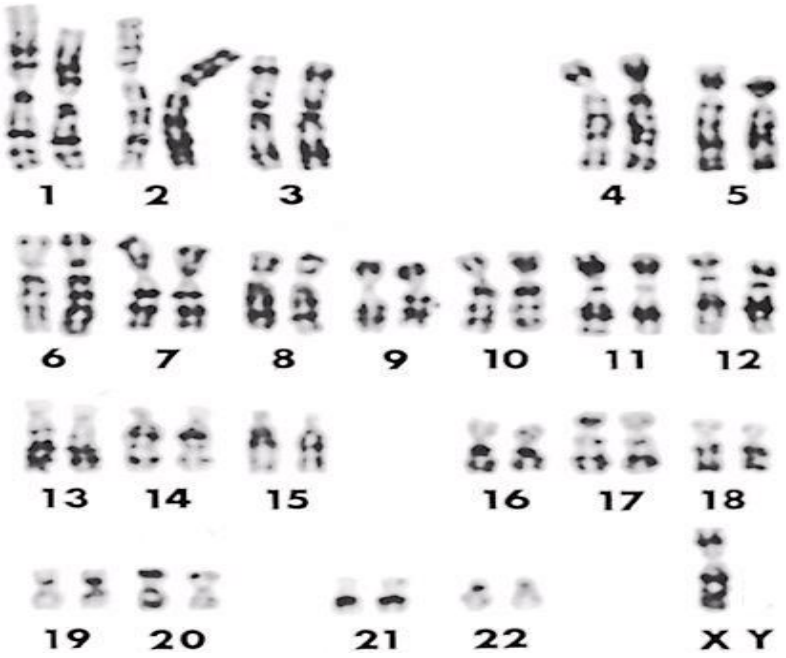
Trisomy

Monosomy

Resulted from nondisjunction of the homologous chromosomes (meiosis I) or sister chromatids (mitosis or meiosis II).

a. Monosomy

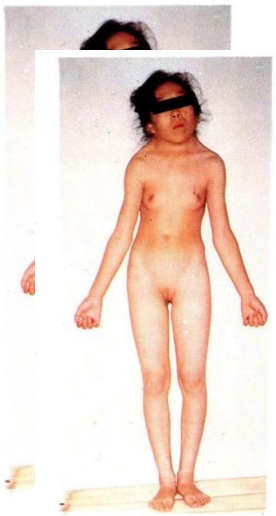
- Almost all monosomy for an entire chromosome is lethal
- Turner's syndrome: 45,X, the only monosomy can be born and survive

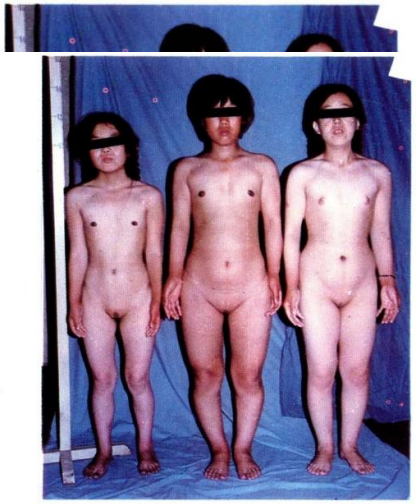


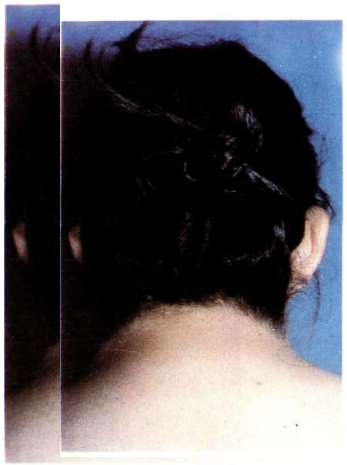
45,X

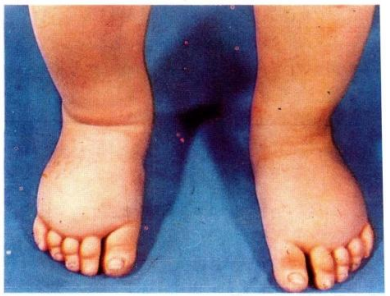
Typical Turner's syndrome

- Short stature
- Gonadal dysgenesis: steak gonads
- Unusual faces, webbed neck, low posterior hairline, broad chest with widely spaced nipples

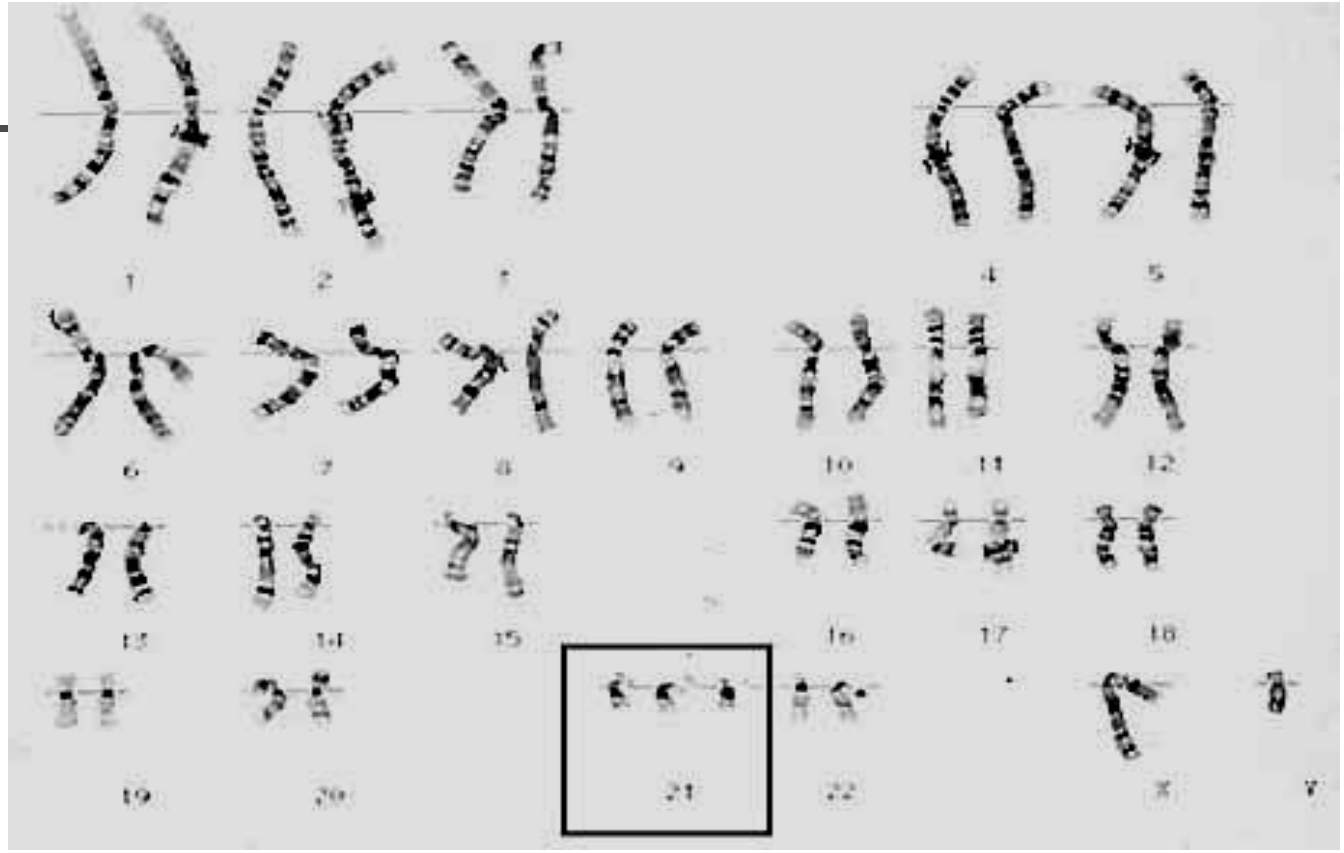








b. Trisomy



Trisomy 21, Down's syndrome

47, XY, +21