Mohammed El-Khateeb

Cytogenetics

MGL-3

Feb 17th 2013

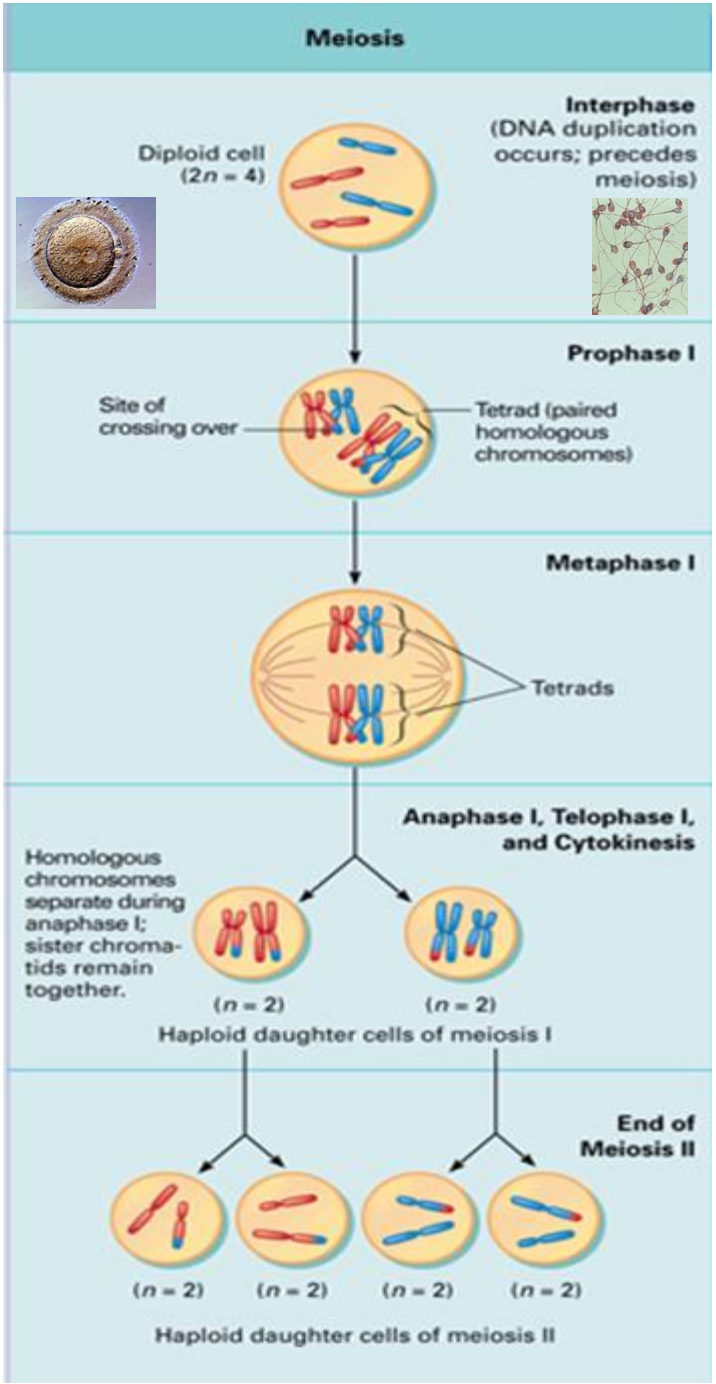
CYTOGENETICS

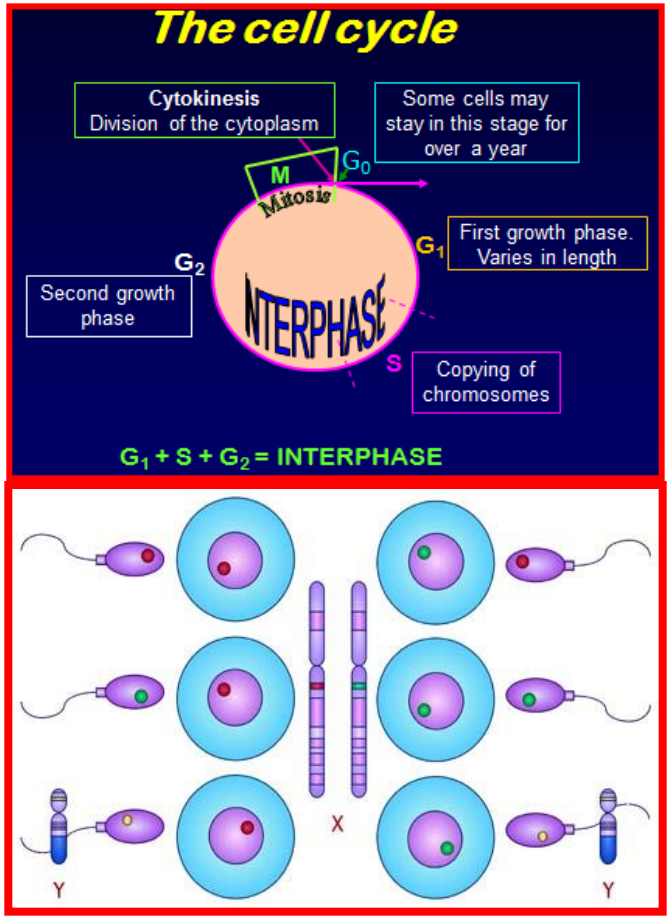
- Chromosome Structure
- Methods of Chromosome Analysis
- Molecular Cytogenetics
- Chromosome abnormalities
- Chromosome Nomenclature

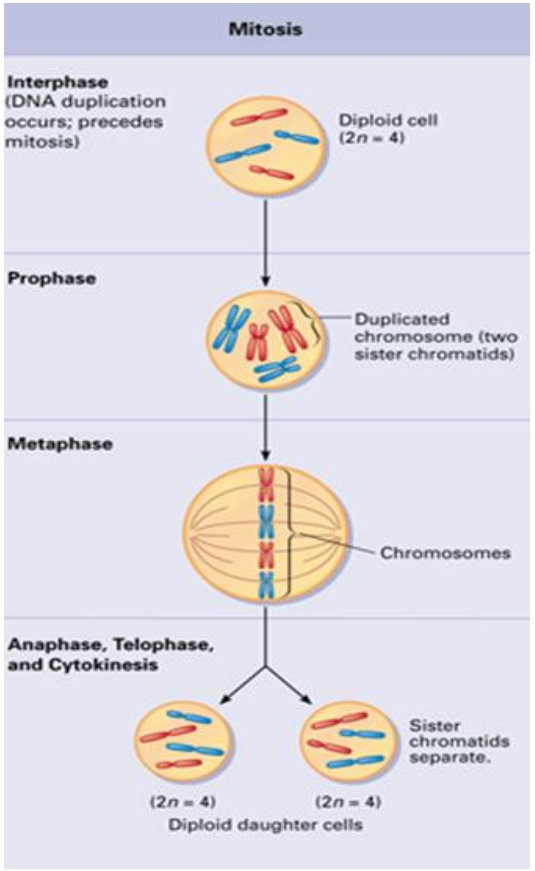
Cytogenetics

The study of chromosome number, structure, function, and behavior in relation to gene inheritance, organization and expression

Fertilization
Diploid Genome

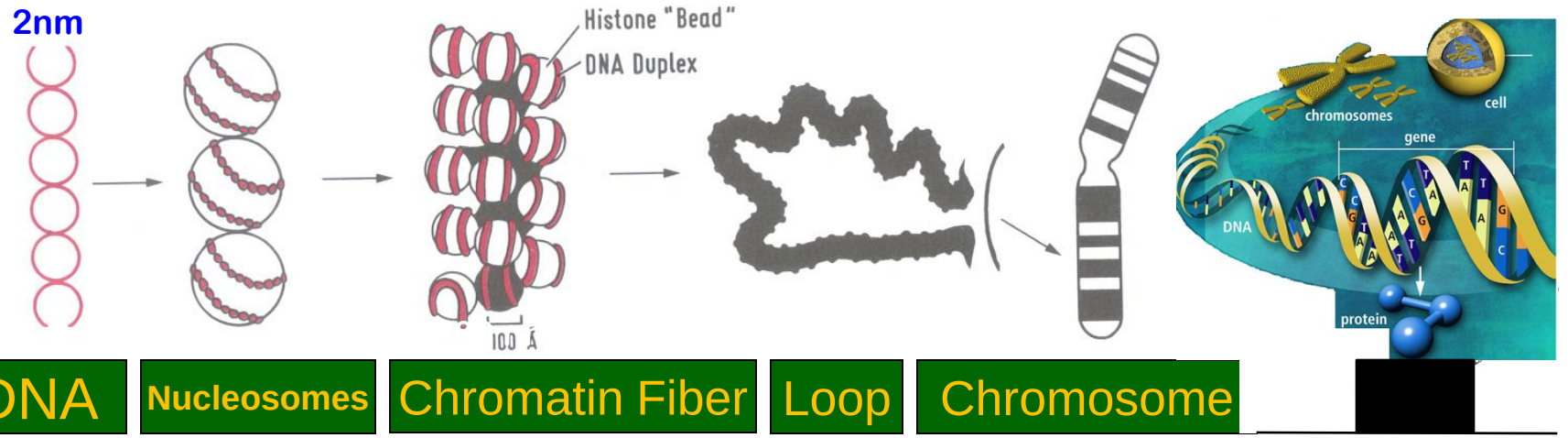


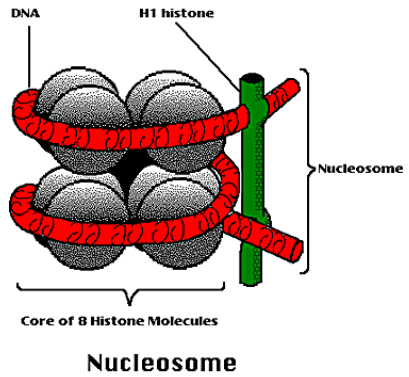




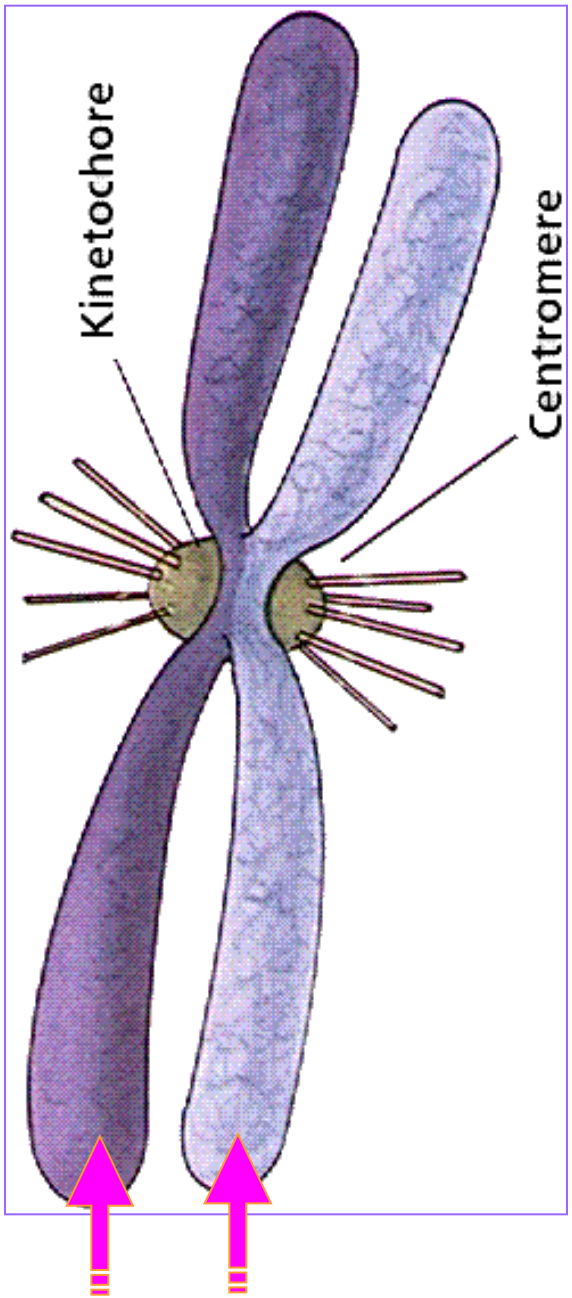
- Each parent contributes one genome copy
- Offspring cells have two near-identical copies

DNA Coiling Leading to the Visible Structure of Chromosomes





- Primary coiling of DNA double helix
- Secondary coiling of DNA double helix around the histone proteins to form nucleosomes
- Tetiary coiling of nucleosomes to form chromatin fibres
- Loops of chromatin fiber forming the chromosome

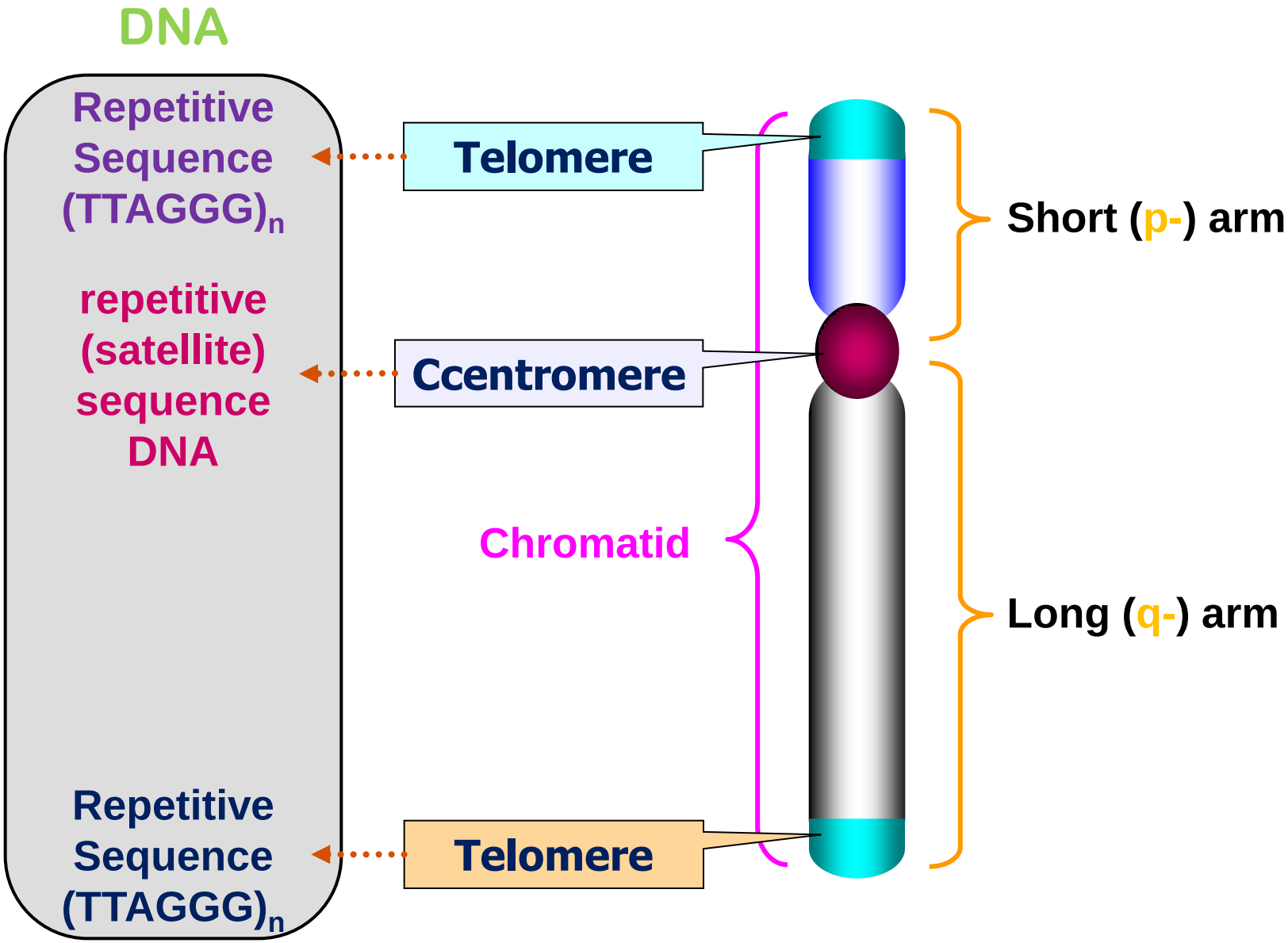


Chromosome

Chromo = colored in response to dye
Some = body

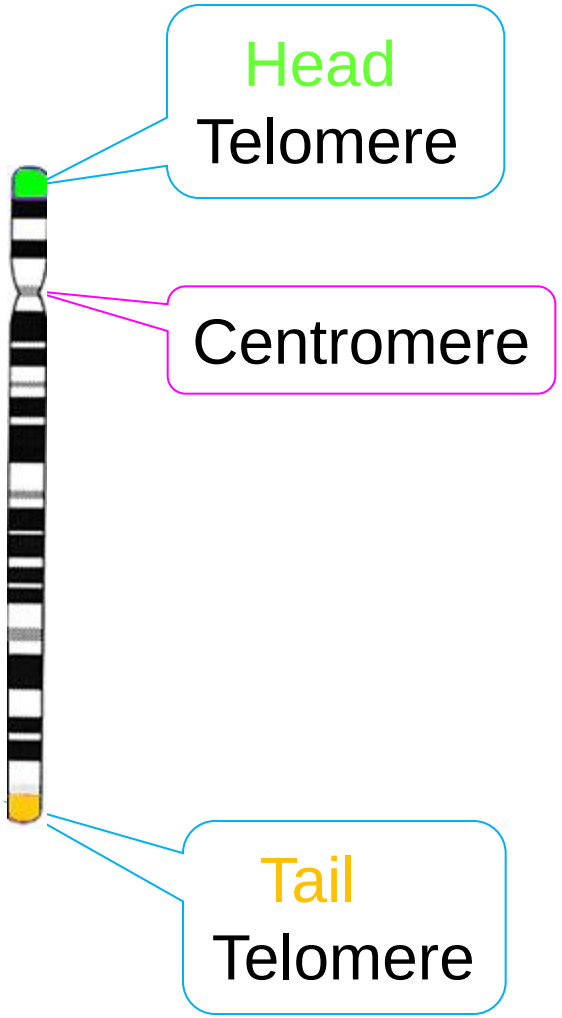
Chromosome of Eukaryotes have been the traditional subject for cytogenetic analysis because they are large enough to be examined using light microscope

Chromosome


DNA
Repetitive Sequence $(TTAGGG)_n$
repetitive (satellite) sequence DNA
Repetitive Sequence $(TTAGGG)_n$
Telomere
Ccentromere
Telomere
Chromatid
Short (p-) arm
Long (q-) arm

What are telomeres?

- Like the rest of a chromosome and its genes, telomeres are sequences of DNA - chains of chemical code.
- Like other DNA, they are made of four nucleic acid bases: A, T, G, C.
- Telomeres are made of repeating sequences of **TTAGGG** on one strand of DNA bound to **AATCCC** on the other strand. Thus, one section of telomere is a "repeat" made of six "base pairs."



DNA

DNA Sequence for **Telomeres**:

ttagggttagggttaggg…

||||||||||||||||||

aatcccaatcccaatccc…

NOTICE:

Tandem Repeats in Telomeres:

ttagggttagggttaggg…

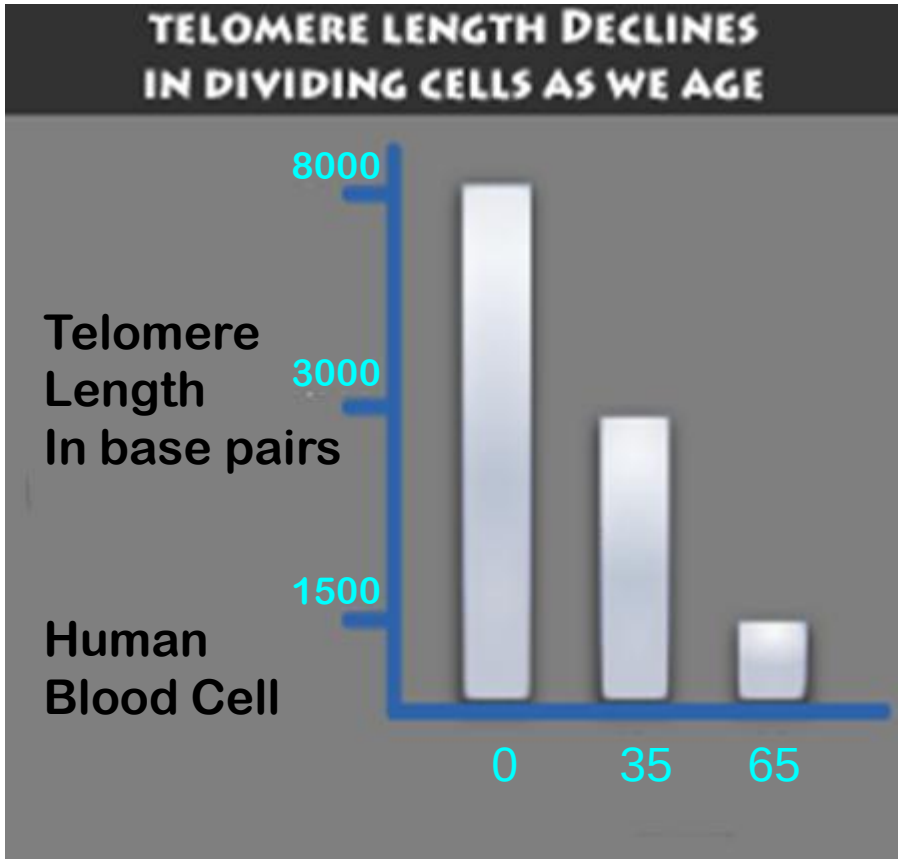
||||||||||||||||||

aatcccaatcccaatccc…

Repeated 800-1600 times in each Telomere

Telomere

- Tip of each chromosome
  - Seal chromosomes and retain chromosome integrity
  - Maintained by enzyme - telomerase
  - Reduction in telomerase and decrease in number repeats important in ageing and cell death

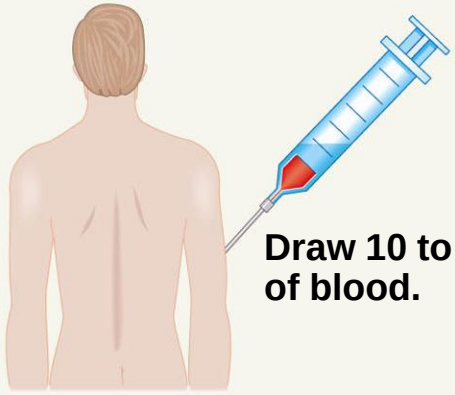


Visualizing Metaphase Chromosomes

- 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

The steps in the process of creating a karyotype for chromosome analysis.

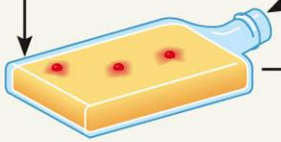
Preparation of G banded karyotype



Draw 10 to 20 ml of blood.

Add a few drops of blood.

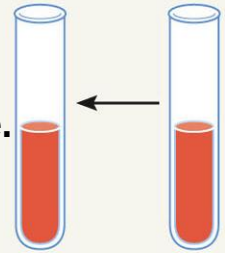
Add phytohemagglutinin to stimulate mitosis.



Incubate at 37°C for 2 to 3 days.

Add Colcemid to culture for 1 to 2 hours to stop mitosis in metaphase.

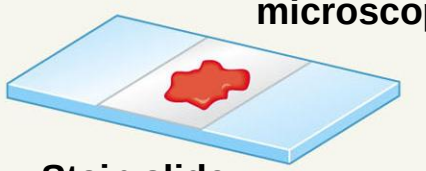
Transfer cells to tube.



Transfer to tube containing fixative.

Centrifuge to concentrate cells. Add low-salt solution to eliminate red blood cells and swell lymphocytes.

Drop cells onto microscope slide.



Stain slide with Giemsa.

Examine with microscope.

Digitized chromosome images processed to make karyotype.

Specimens

- Peripheral blood
- Fibroblasts from skin bx
- Epithelial cells from buccal smear
- Bone marrow
- Solid tumor biobsies

Chromosome Number in different animals and plants

Human	46
Chimpanzee	48
Dog	78
Horse	64
Chicken	78
Goldfish	94
Fruit fly	8
Mosquito	6
Nematode	11(m), 12(f)
Horsetail	216
Sequoia	22
Round worm	2

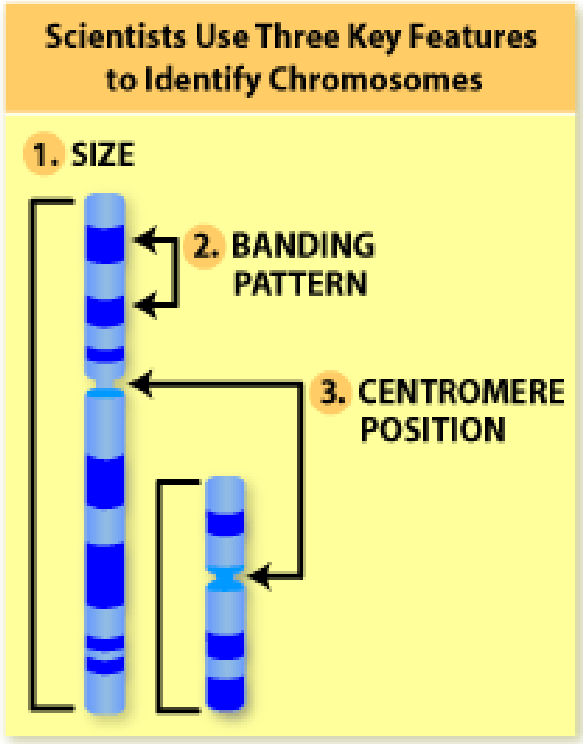
Onion	16
Mold	16
Carrot	20
Tomato	24
Tobacco	48
Rice	24
Maize	20
Haploppus gracilis	4
Crepis capillaris	6

Cytogenetics?

- **The study of the genetic constitution of cells through the visualisation and analysis of chromosomes.**
  - **G-banding**
    - **(and other traditional techniques)**
  - **Fluorescence in situ hybridization (FISH)**
  - **Molecular techniques**
    - **(QF-PCR, MLPA)**

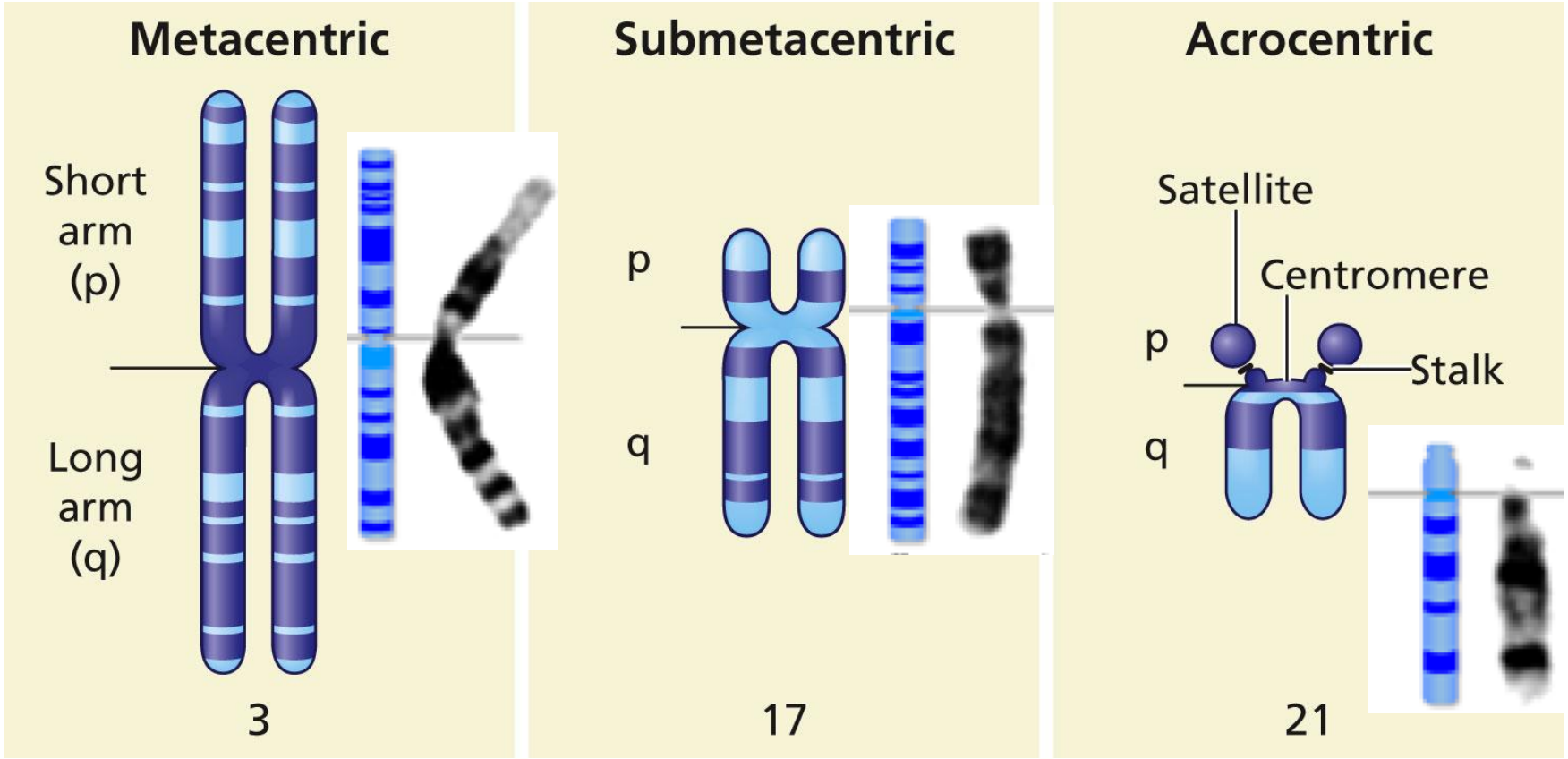
HOW DO SCIENTISTS READ CHROMOSOMES?

- **Size.** This is the easiest way to tell two different chromosomes apart.
- **Banding pattern.** The size and location of Giemsa bands on chromosomes make each chromosome pair unique.
- **Centromere position.** Centromeres are regions in chromosomes that appear as a constriction. They have a special role in the separation of chromosomes into daughter cells during mitosis cell division (mitosis and meiosis).

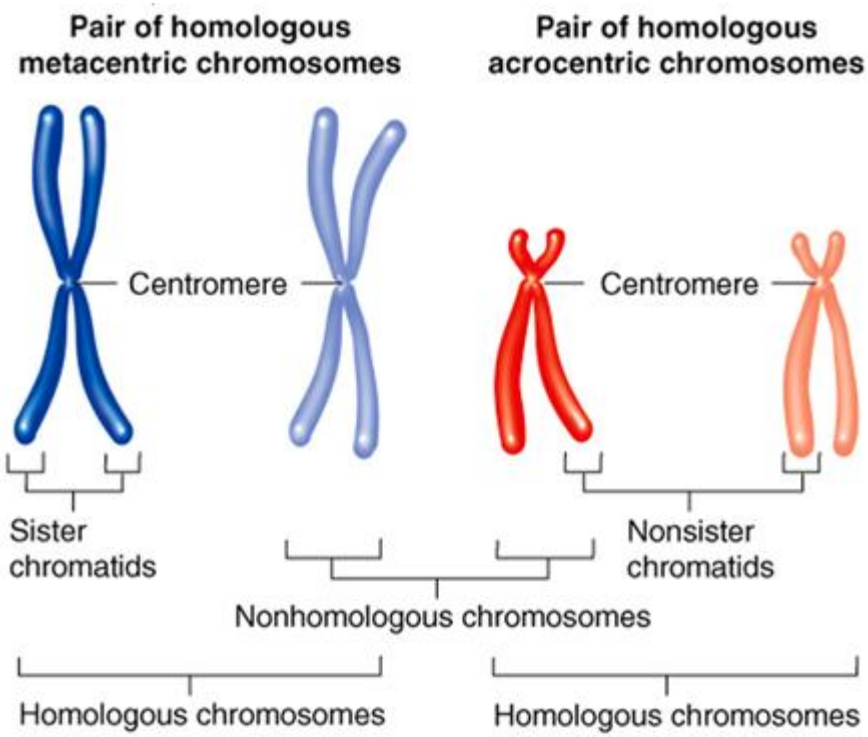


Metaphase Chromosomes

- Length
- Centromere location
- Satellite



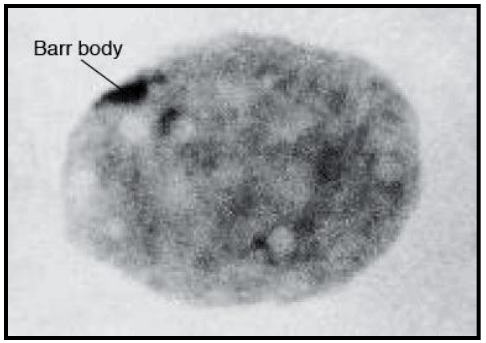
Chromosome in general (size, shape and number)

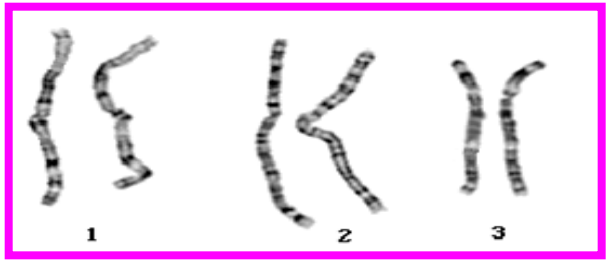


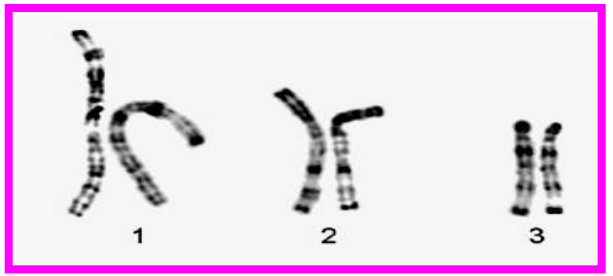
- Two sister chromatids per chromosome
- DNA replication ➡ chromatids
- Two sister chromatids joined together at centromeres
- chromosomes differ in size and appearance with staining

Basic cytogenetic examinations

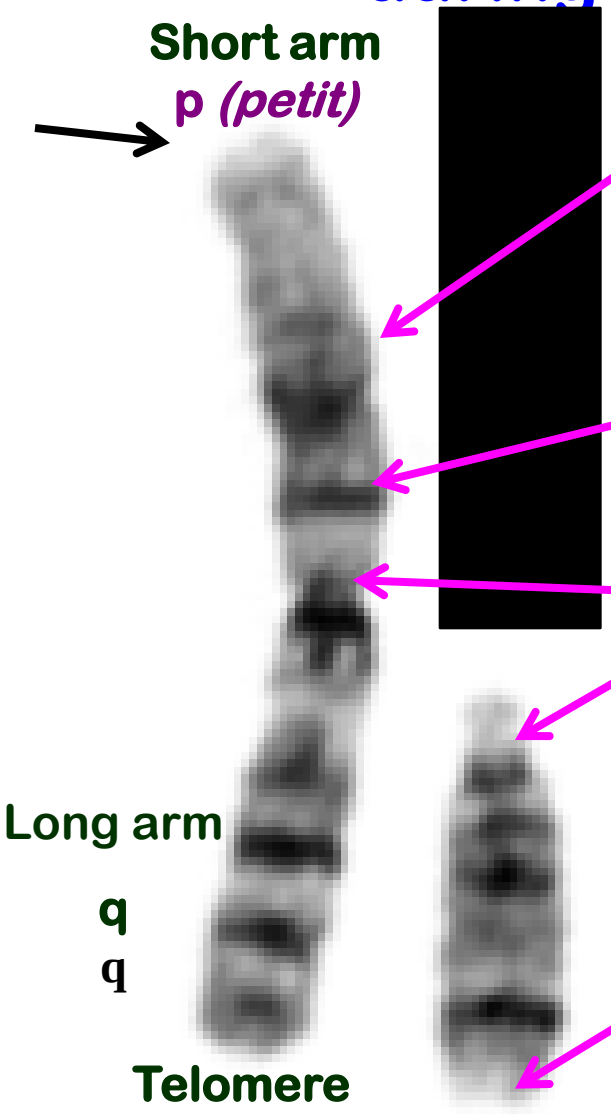
- **Interphase cells**
  - Barr body (sex chromatin)
- **Metaphase cells** – staining of chromosomes
  - Solid staining
  - G-banding
  - R-banding
  - C-banding
  - Q-banding
  - Ag-NOR







Chromosomes as seen at metaphase during cell division



Light bands

- Replicate early in S phase
- Less condensed chromatin
- Transcriptionally active
- Gene and GC rich

Dark (G) bands

- Replicate late
- Contain condensed chromatin
- AT rich

Centromere

- Joins sister chromatids
- Essential for chromosome segregation at cell division
- 100s of kb of repetitive DNA: some non-specific, some chromosome specific

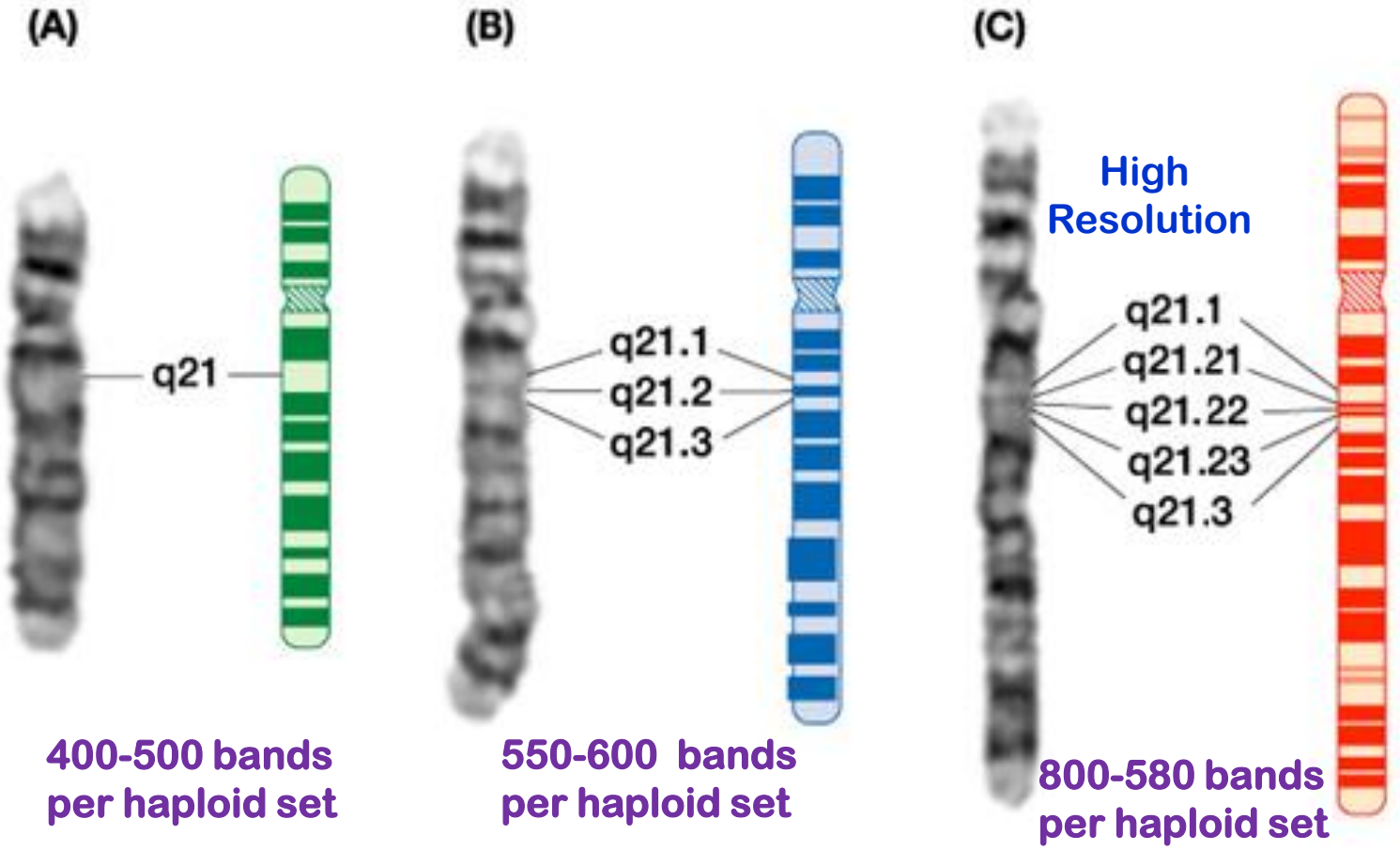
Telomere

- DNA and protein cap
- Ensures replication to tip
- Tether to nuclear membrane
- provide terminal stability to the chromosome and ensure its survival

Chromosomes Banding

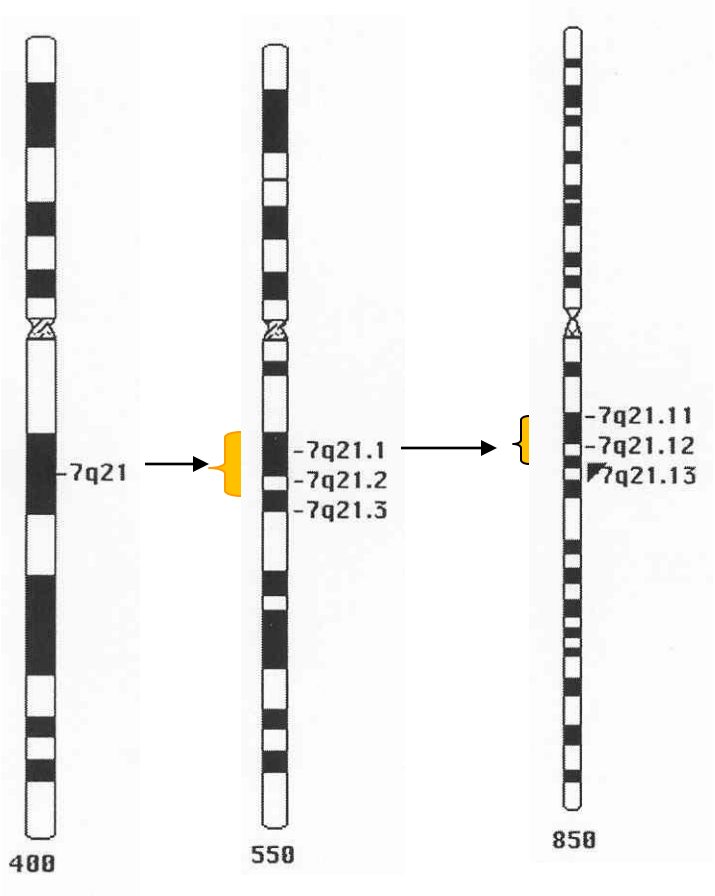
Type	Stain	Area Stained	Effect
Q-banding	Quinacrine	Chromosome arms; mostly repetitive AT-rich DNA	Under UV light, distinct fluorescent banded pattern for each chromosome.
G-banding	Giemsa	Chromosome arms; mostly repetitive AT-rich DNA	Distinct banded pattern for each chromosome; same as Q-banding pattern except single additional band near centromere of chromosomes 1 and 16
R-banding	Variety of techniques	Chromosome arms; mostly unique GC-rich DNA	Reverse banding pattern of that observed with Q- or G-banding
C-banding	Variety of techniques	Centromere region of each chromosome and distal portion of Y chromosome; highly repetitive, mostly AT-rich DNA	Largest bands usually on chromosomes 1, 9, 16, and Y; chromosomes 7, 10, and 15 have medium-sized bands; size of C-bands highly variable from person to person

High Resolution G banding



- Human chromosome 4 at varying resolutions due to exact mitotic stage, (or degrees of spreading - squashing - stretching)
- Each band corresponds to about 5000-10000 kb

Low/High Resolutions Karyotype



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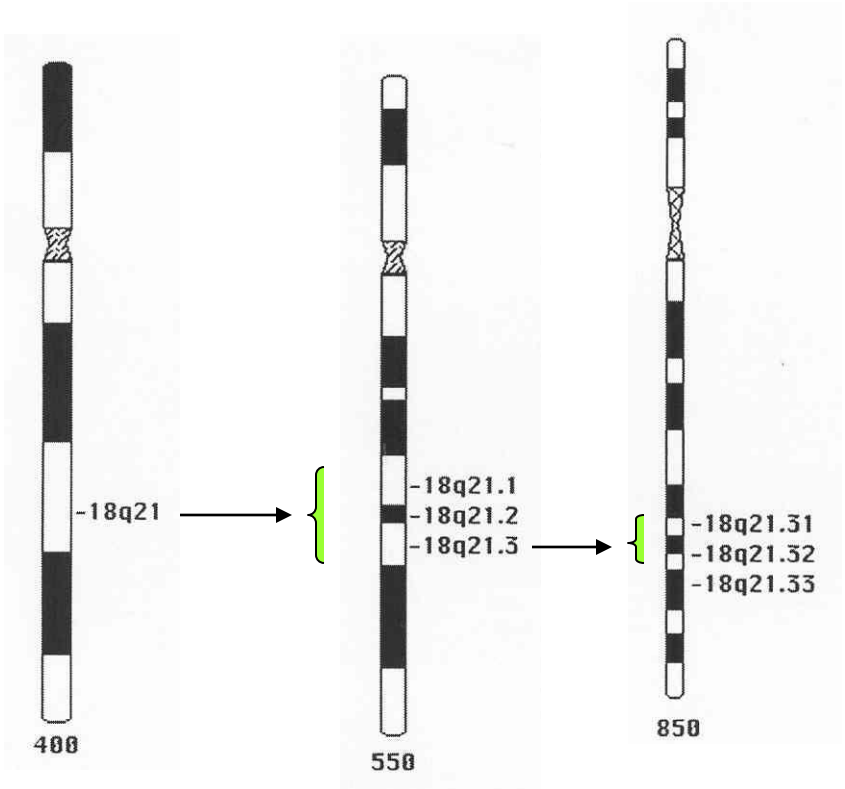
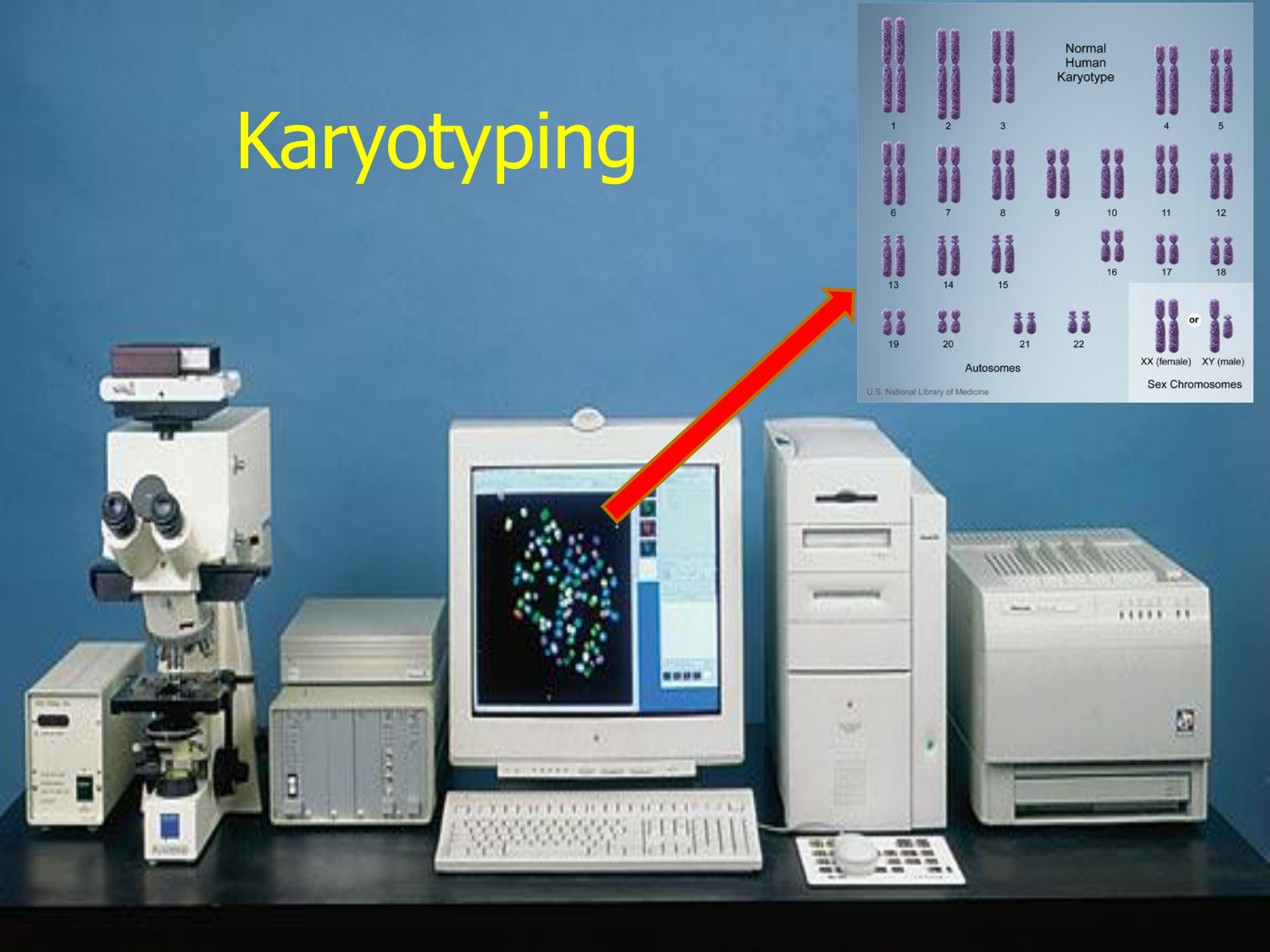


18

Karyotyping

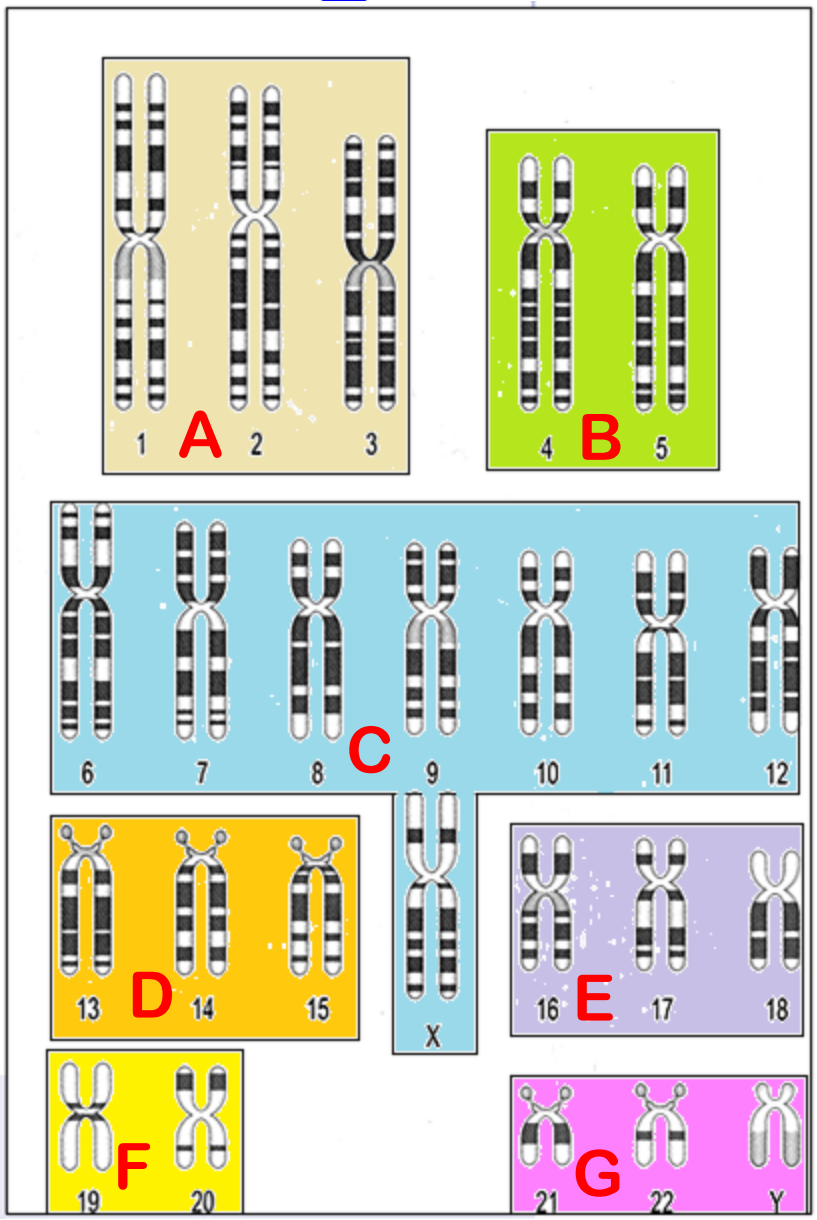


Idiograme

Autosomes are the first 22 homologous pairs of human chromosomes that do not influence the sex of an individual.

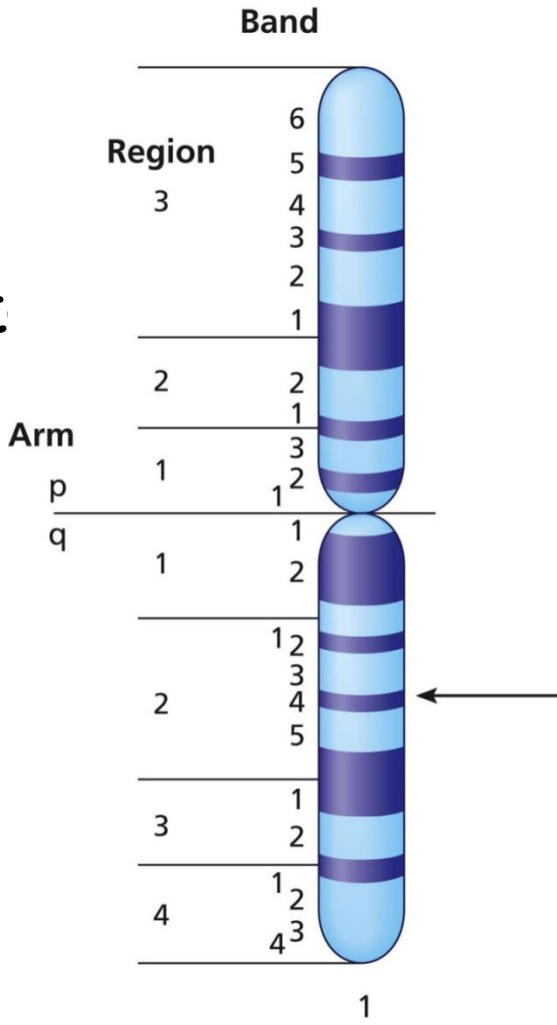
- **A** 1-3
- **B** 4-5 + X
- **C** 6-12
- **D** 13-15
- **E** 16-18
- **F** 19-20
- **G** 21-22 +Y

Sex Chromosomes are the 23rd pair of chromosomes that determine the sex of an individual.

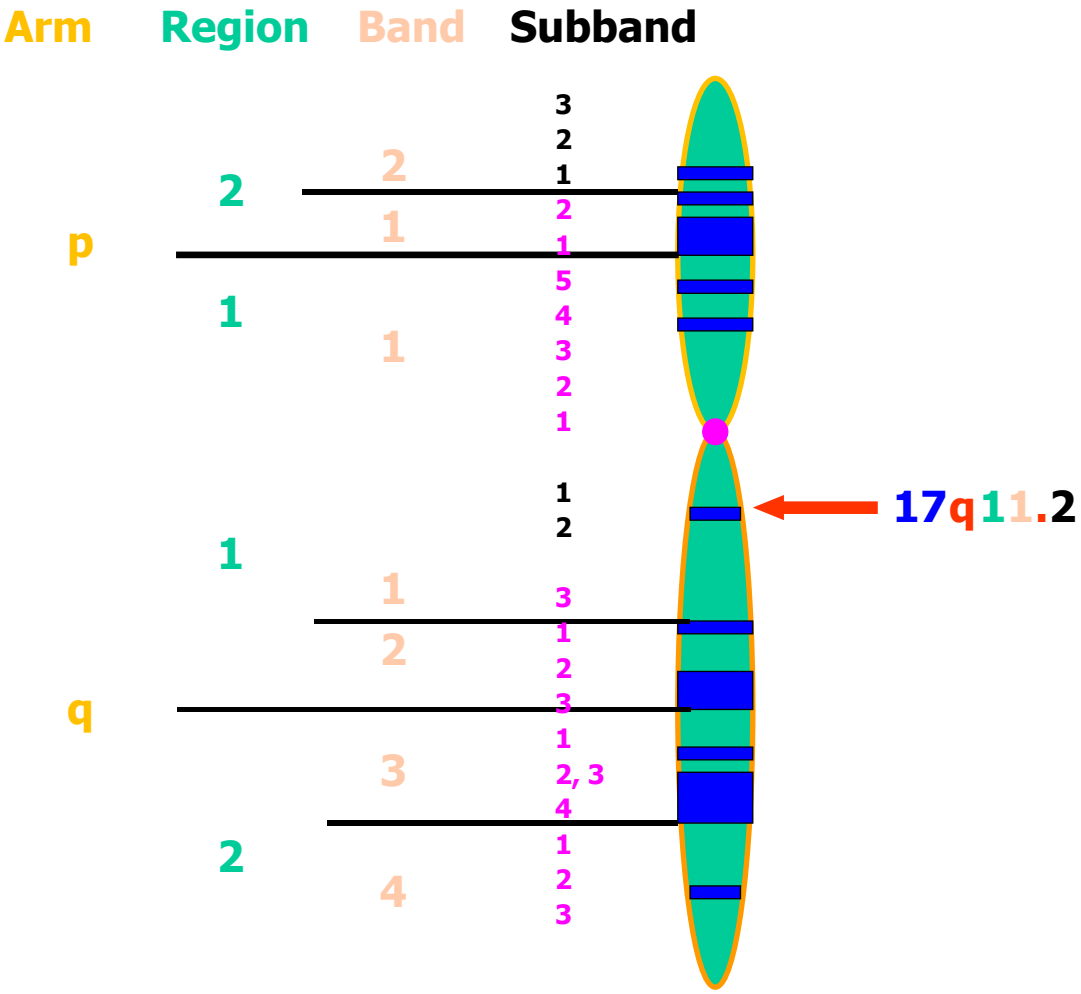


International System for Human Cytogenetic Nomenclature (ISCN)

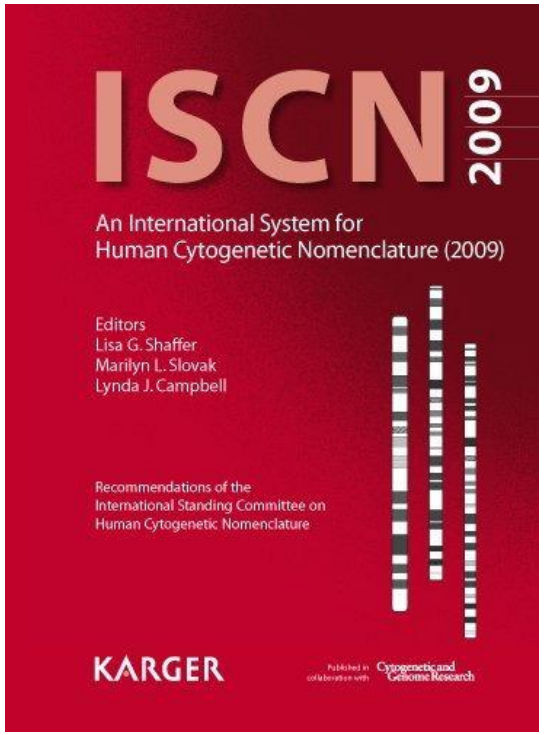
- Regions, Bands & Sub-bands
  - Each area of chromosome given number
  - Lowest number closest (proximal) t centromere
  - Highest number at tips (distal) to centromere
- 1p31.1
  - Chromosome 1
  - Short arm
  - Region 3 , band 1, sub-band 1



Defining Chromosomal Location



Chromosome 17



ISCN

- del - deletion
- dic - dicentric
- fra - fragile site
- i - isochromosome
- inv - inversion
- p - short arm
- r - ring
- der - derivative
- dup - duplication
- h - heterochromatin
- ins - insertion
- mat - maternal origin
- Pat - paternal origin
- q - long arm
- t - translocation

ISCN

46,XX,del(5p)

- Separates ,
  - Chromosome numbers
  - Sex chromosomes
  - Chromosome abnormalities

;

46,XX,t(2;4)(q21;q21)

- Separates
  - altered chromosomes
  - break points in structural rearrangements involving more than 1 chromosome

Normal male **46,XY** Normal female **46,XX**

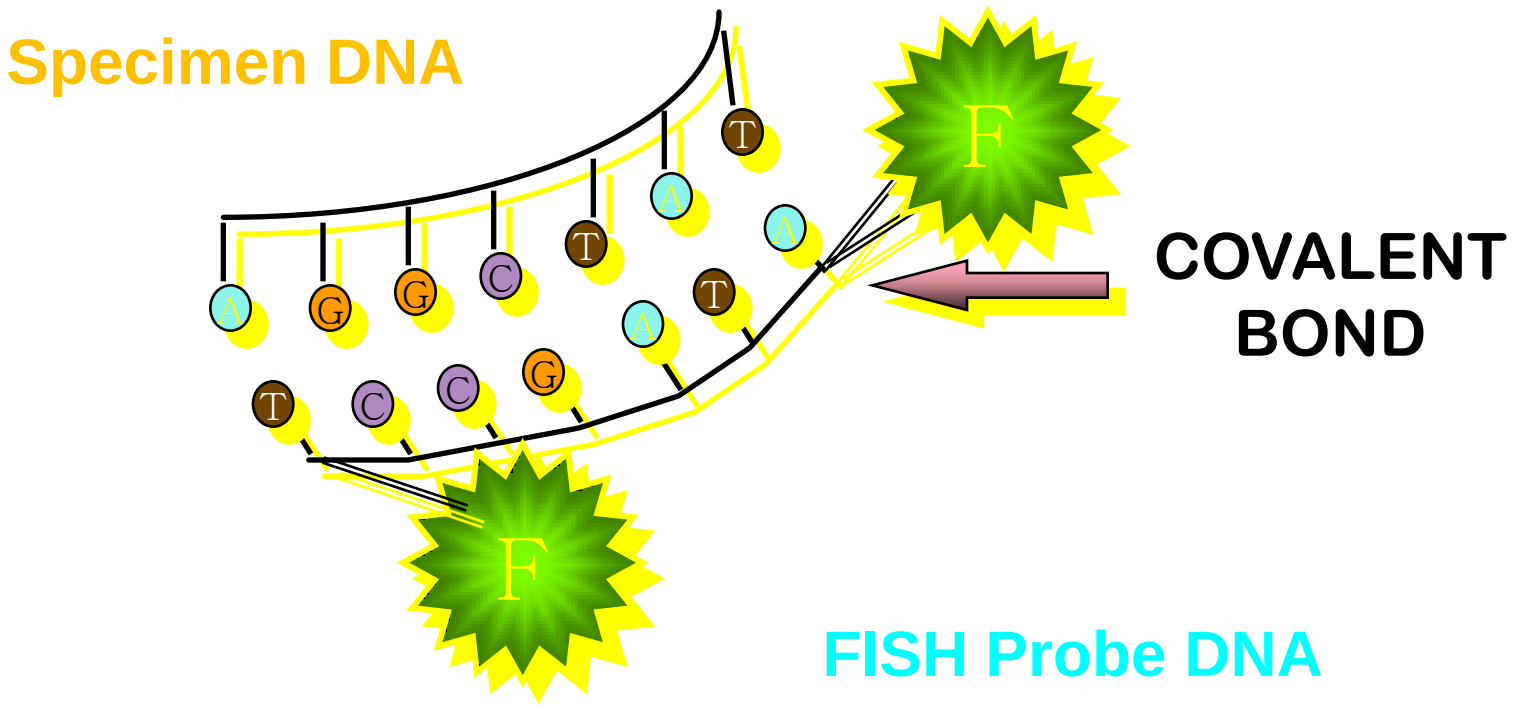
Cytogenetics?

- **The study of the genetic constitution of cells through the visualisation and analysis of chromosomes.**
  - **G-banding**
    (and other traditional techniques)
  - ➢ **Fluorescence in situ hybridization (FISH)**
  - **Molecular techniques**
    (QF-PCR, MLPA)

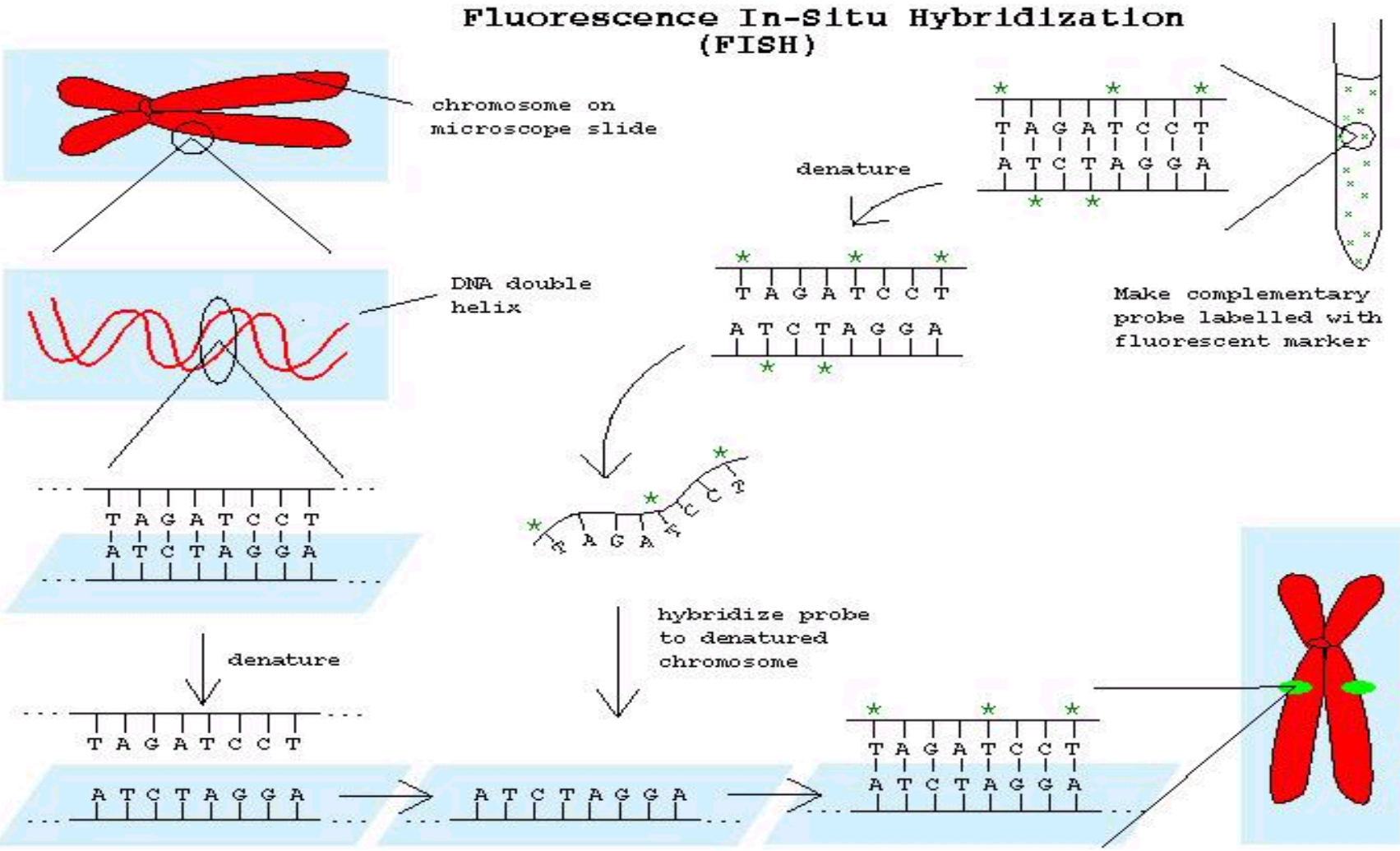
Molecular Cytogenetics

- Fluorescent Inistu Hypridization (FISH)
  - Different Fish Probes
    - Centromeric Probe
    - Chromosome specific unique sequence probe
    - Whole chromosome point probe
  - Reverse painting
  - Multicolor spectral karyotyping
- Comparative Genomic Hypridization (CGH)
- Flowcytometry

DIRECT FLUORESCENT - LABELED PROBE



FISH technique is based on the unique ability of a single stranded piece of DNA (probe) to anneal or hybridize with its complementary target sequence on the chromosome

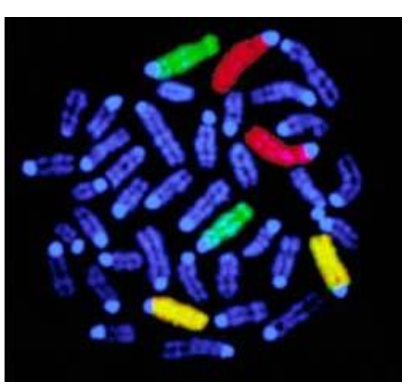


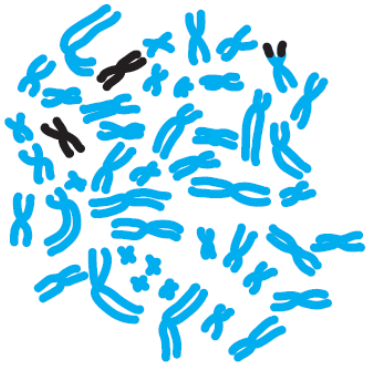
Advantages of Interphase FISH

- Interphase cells for FISH do not require culturing of the cells and stimulating division to get metaphase spreads
- 200–500 cells can be analyzed microscopically using FISH
- Monitor recurrent or residual disease in BMT pt.

Metaphase FISH

- Uses fluorescent probes that bind to metaphase chromosomal regions or to whole chromosomes.
- **Whole chromosome paints:** Probes that cover the entire chromosome, are valuable for detecting small rearrangements that are not apparent by regular chromosome banding.
- Telomeric and centromeric probes are also applied to metaphase chromosomes to detect aneuploidy and structural abnormalities





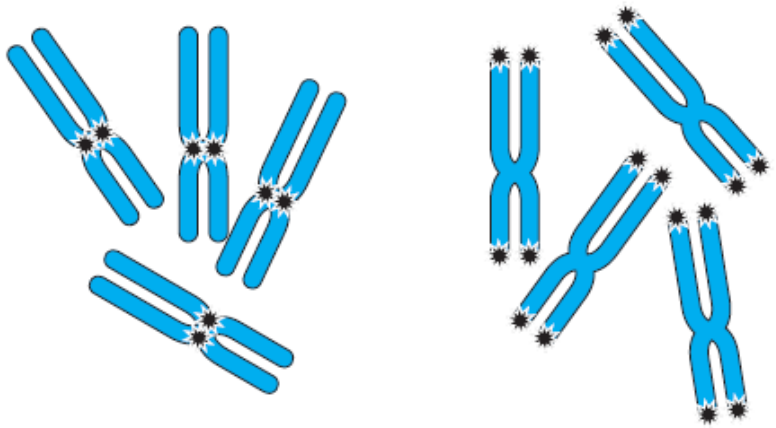
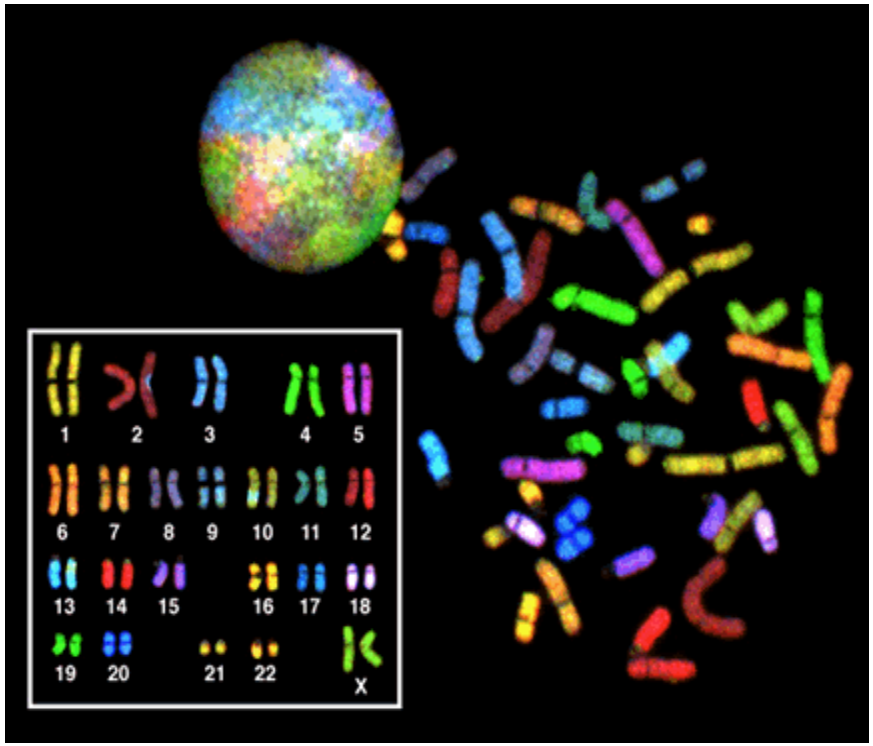


Figure 8-20 Centromeric (left) and telomeric (right) probes on metaphase chromosomes.

Spectral karyotyping (SKY) and multiple fluoeescent hybridization (M-FISH)

- By mixing combinations of five fluors and using special imaging software, can distinguish all 23 chromosomes by chromosome specific colors.
- This type of analysis can be used to detect abnormalities that affect multiple chromosomes as is sometimes found in cancer cells or immortalized cell lines.



SKY

Advantages:

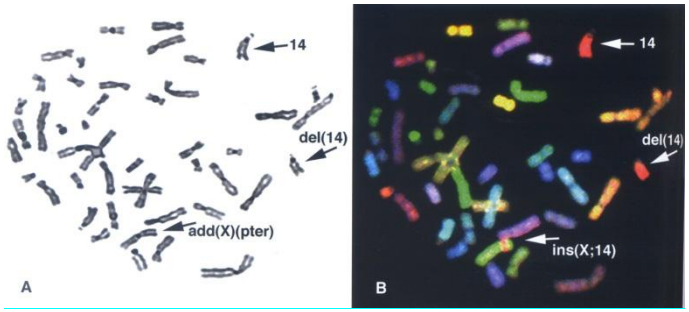
- Mapping of chromosomal breakpoints.
- Detection of subtle translocations.
- Identification of marker chromosomes, homogeneously staining regions, and double minute chromosomes.
- Characterization of complex rearrangements.

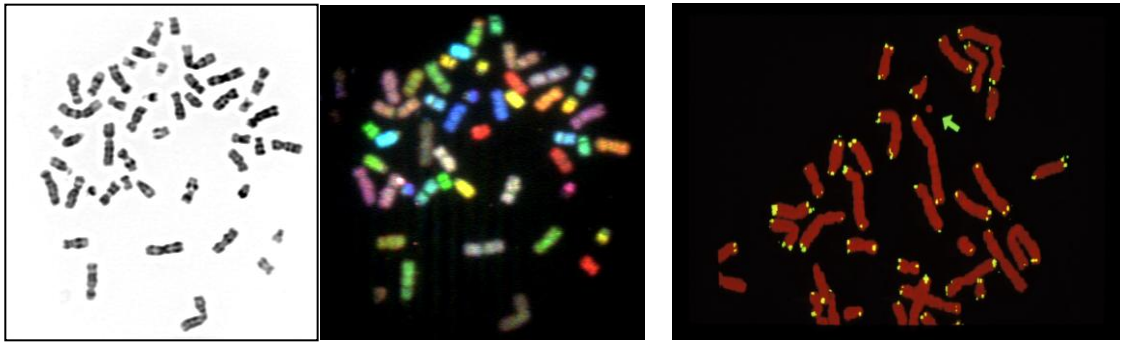
Disadvantages:

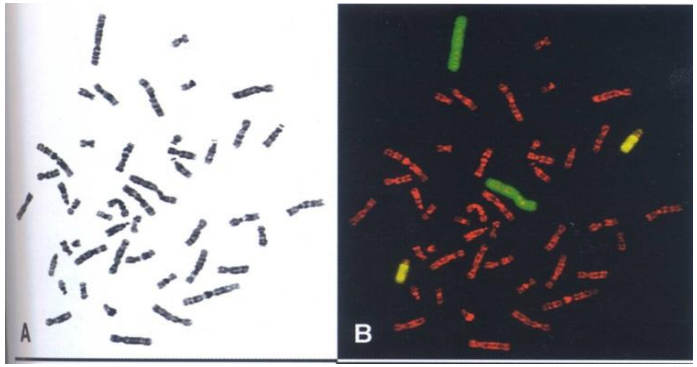
- Very expensive equipments.
- The technique is labor intensive.
- Dose not detect structural rearrangements within a single chromosome.
- Low resolution (up to 15 mb).
- Specific, not a screening method.

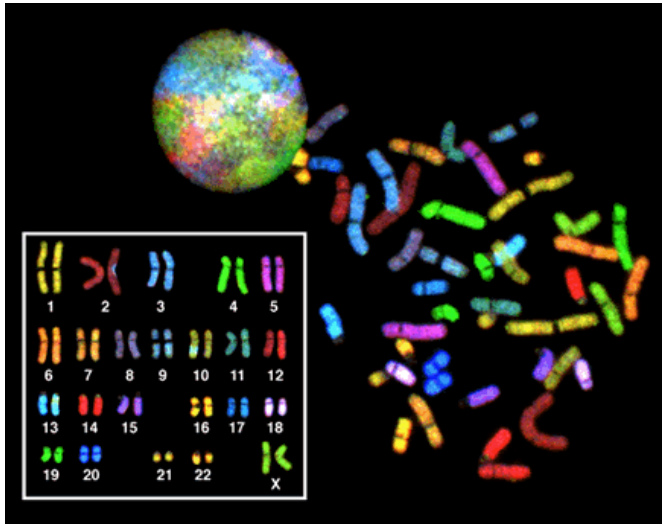
Fluorescence InSitu Hypridization

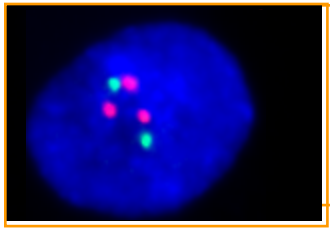
FISH



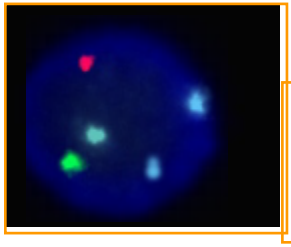




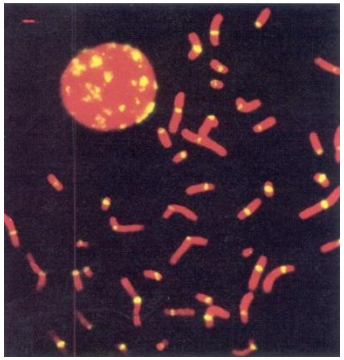




13 (green)
21 (red)



X (green),
Y (red)
18 (aqua)



Applications

- Gene Mapping
- Chromosome Identification
- Aneuploidy Detection
- Sexing for X-Linked diseases
- Marker chromosome Identification
- Total chromosome Analysis
- Translocation Analysis
- Unique Sequence DNA Detection
- Microdeletion Syndrome Analysis
- Gene Amplification Analysis

Cytogenetics?

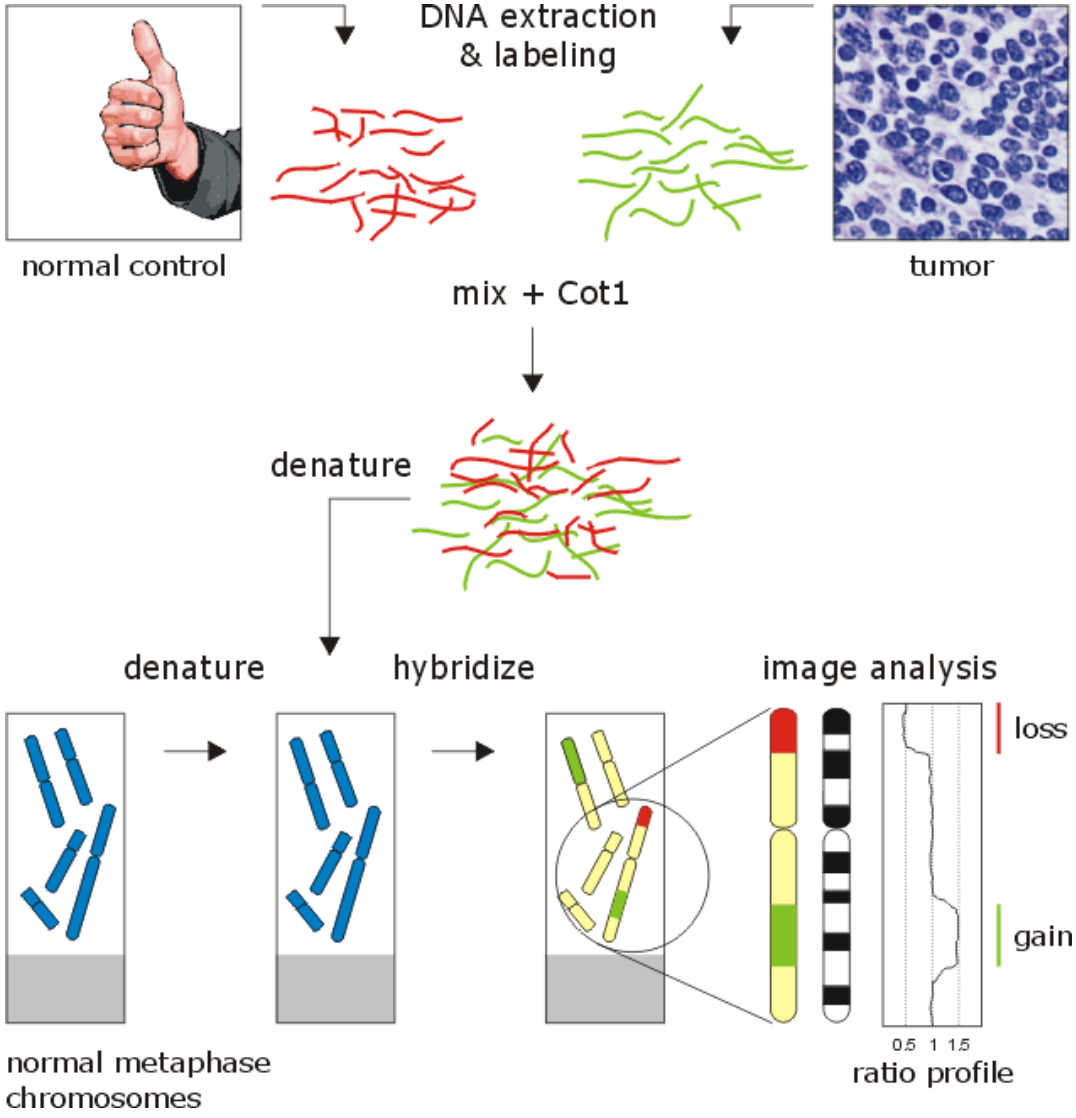
- The study of the genetic constitution of cells through the visualisation and analysis of chromosomes.
  - G-banding
    (and other traditional techniques)
  - Fluorescence in situ hybridization (FISH)
  - **Molecular techniques**
    (CGH, QF-PCR, MLPA, Microarray)

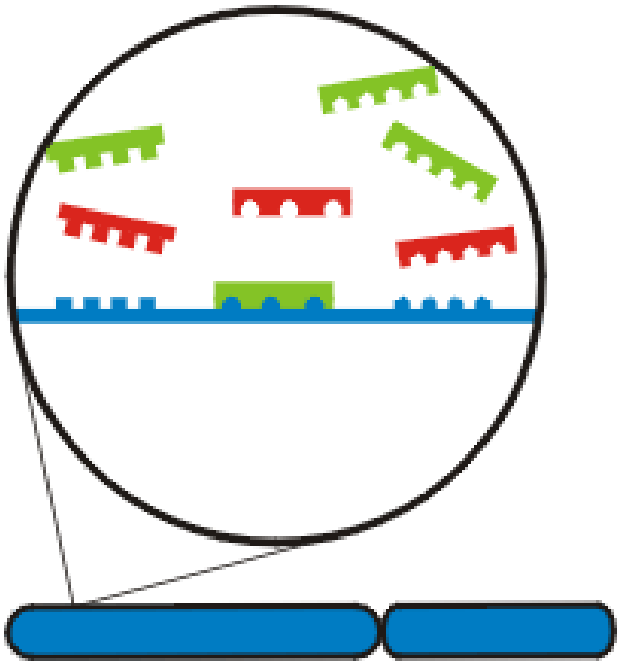
Comparative Ggenomic Hypridization (CGH)

Methods:

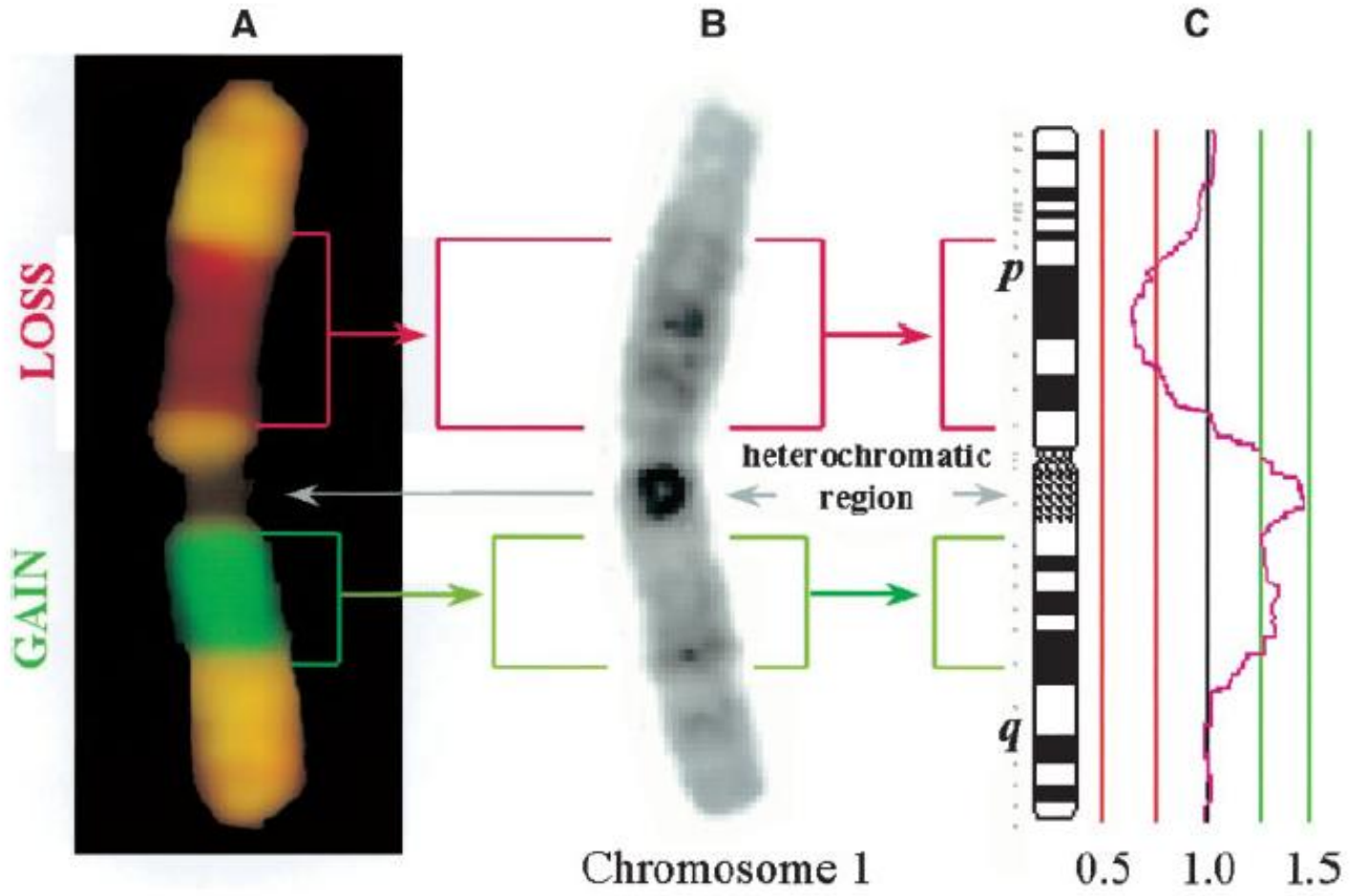
- Isolate Genomic DNA from samples
- DNA digestion
- Label patient and control samples
- Hybridize to microarray
- Post hybridization washing
- Assay scanning and data analysis

Comparative Ggenomic Hypridization (CGH)





Comparative Genomic Hybridisation (CGH)



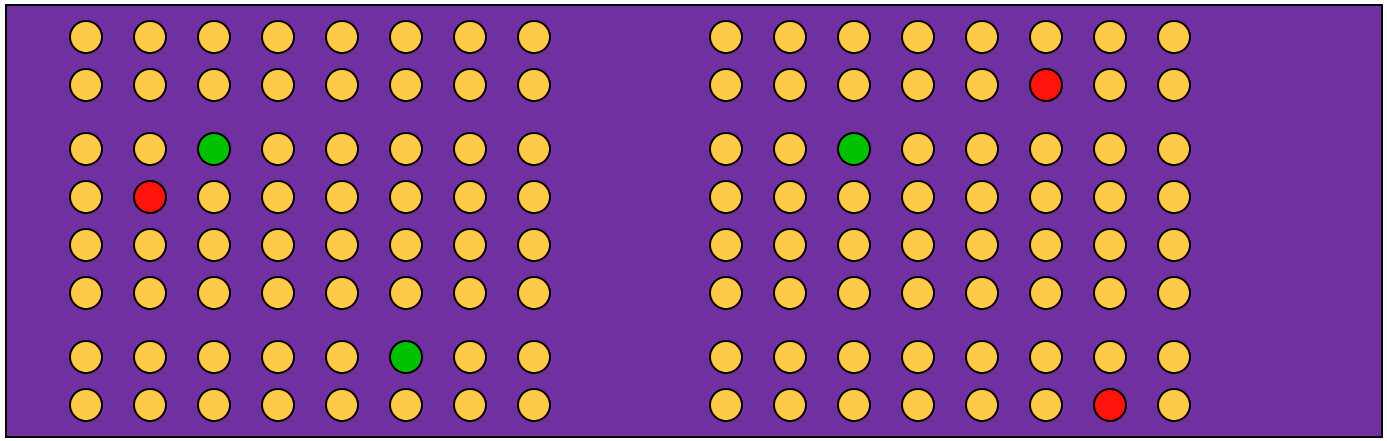
Amplified gene = Green

Reduction of gene = Red

Reading a CGH-Microarray

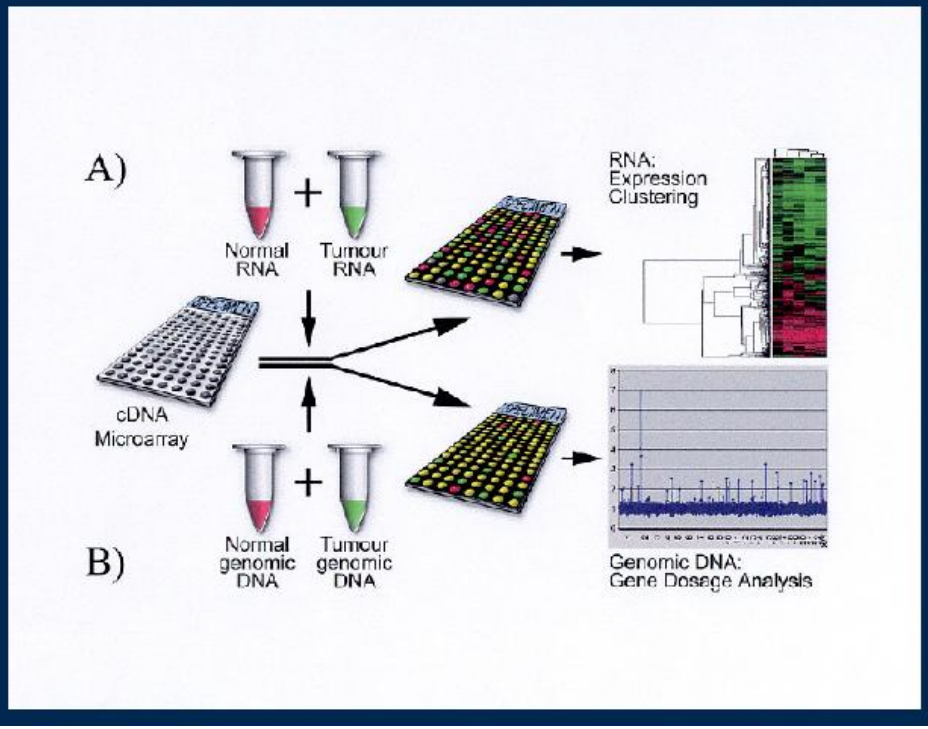
The resulting “colour” of a spot will depend on the ratio of “Red” and “Green” labeled DNA which has Hybridized to the Spot

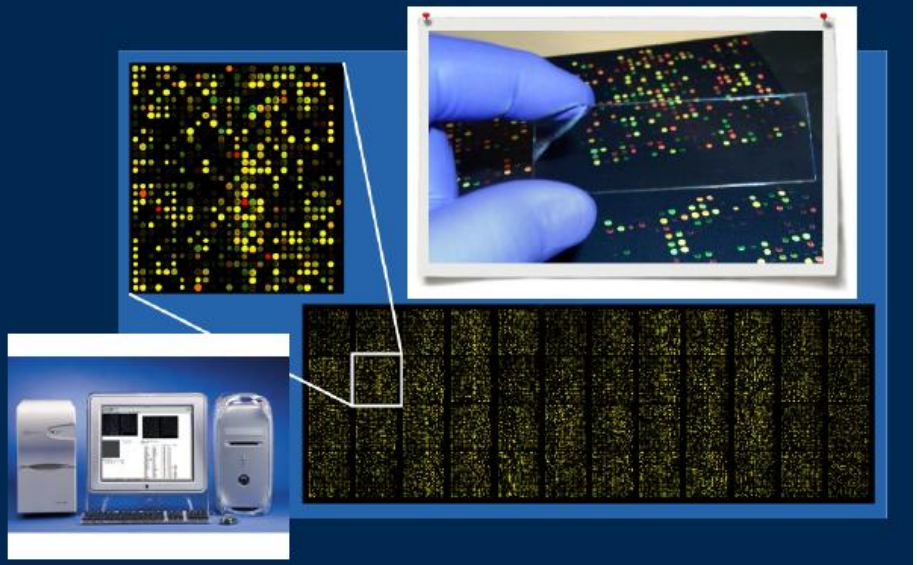
- Equal
- Excess Patient DNA (Duplication)
- Loss of patient DNA (Deletion)



Array-based comparative genomic hybridization (aCGH)

- new tool to search for recurrent gains or loss of chromosomal regions throughout the genome according to detection with very high resolution of copy number changes at DNA level
- only recently is aCGH successfully utilised in diagnostics of leukemias and the results revealed a large spectrum of genomic imbalancies, including novel recurrent deletions and amplifications





BAC arrays ~1MB
Oligo arrays ~100 kb (maximal resolution ~ 35 kb)

Indications - Postnatal

- **Multiple congenital anomalies**
- **Developmental delay/ mental retardation of unknown origin**
- **Autism**
- **Any individual suspected of a chromosomal imbalance, even with normal karyotype**
- **High resolution mapping to identify specific genes**

Current Uses of Array CGH

- Define congenital genetic defects
- Define acquired genetic changes (in cancer)
- Molecular fingerprints of specific tumors and subtypes
- Identification of novel chromosomal regions for drug targets and new treatments

CGH

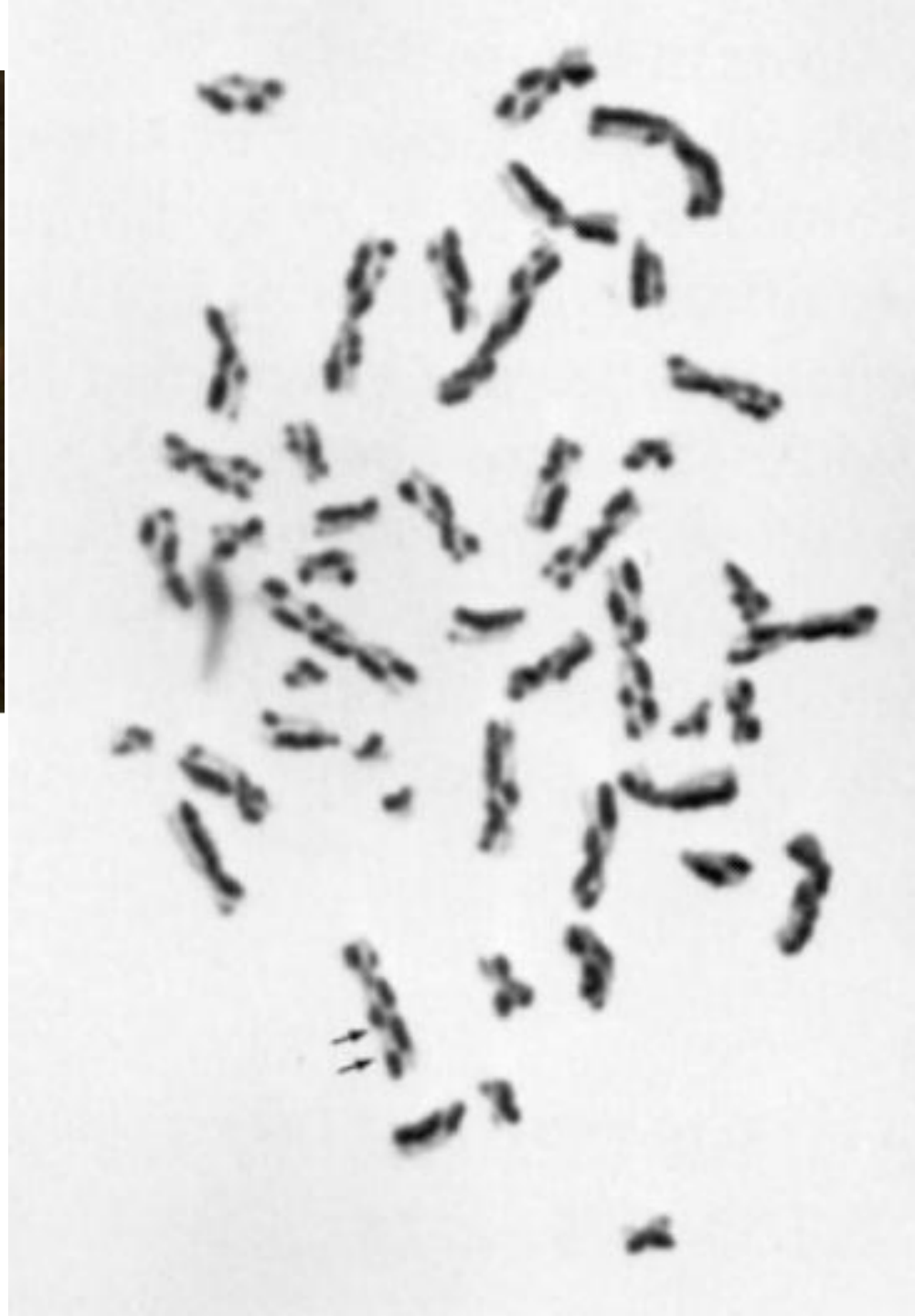
Advantages

- whole genome in 1 experiment
- no need to culture tumor cells
- sensitive detection of gene amplification
- retrospective analysis

Disadvantages

- limited resolution (~10 Mb del/dup)
- laborious
- only gains and losses / no balanced rearrangements
- no information on the nature of the aberrations

Sister chromatid exchanges



CHROMOSOMAL ABNORMALITIES

Types of chromosome abnormalities

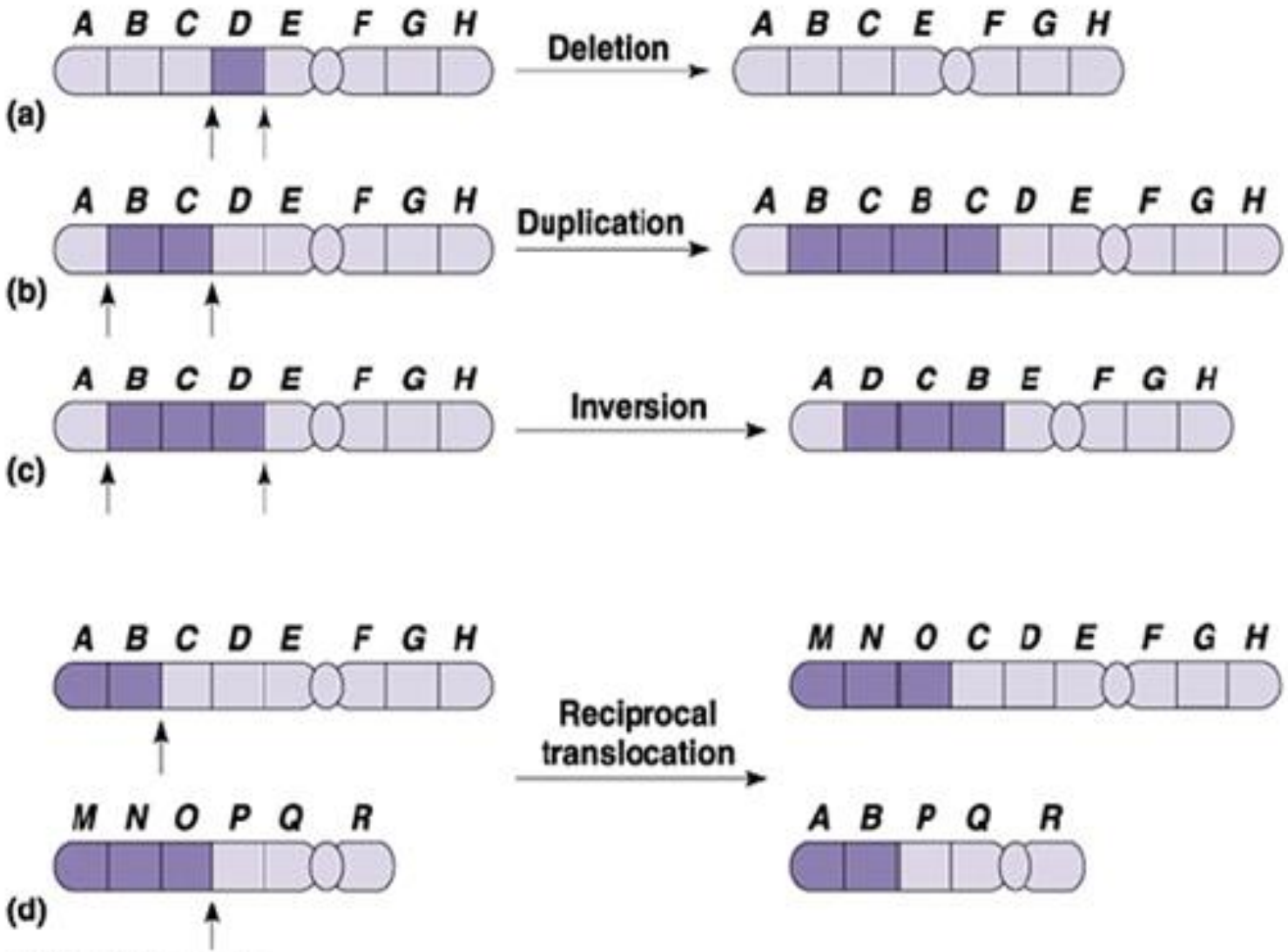
- **Numerical**
  - Aneuploidy (monosomy, trisomy, tetrasomy)
  - Polyploidy (triploidy, tetraploidy)
- **Structural**
  - Translocations
  - Inversions
  - Insertions
  - Deletions
  - Rings
  - Duplication
  - Isochromosomes

Classification of chromosomal anomalies

- **Numerical** ***(usually due to de novo error in meiosis)***
  - ***Aneuploidy*** - monosomy
    - trisomy
  - ***Polyploidy*** - triploidy

- **Structural** ***(may be due to de novo error in meiosis or inherited)***
  - ***Translocations*** - reciprocal
    - Robertsonian (centric fusion)
  - ***Deletions***
  - ***Duplications***
  - ***Inversions***

- **Different cell lines** ***(occurs post-zygotically)***
  - ***Mosaicism***



Chromosome abnormalities and maternal age