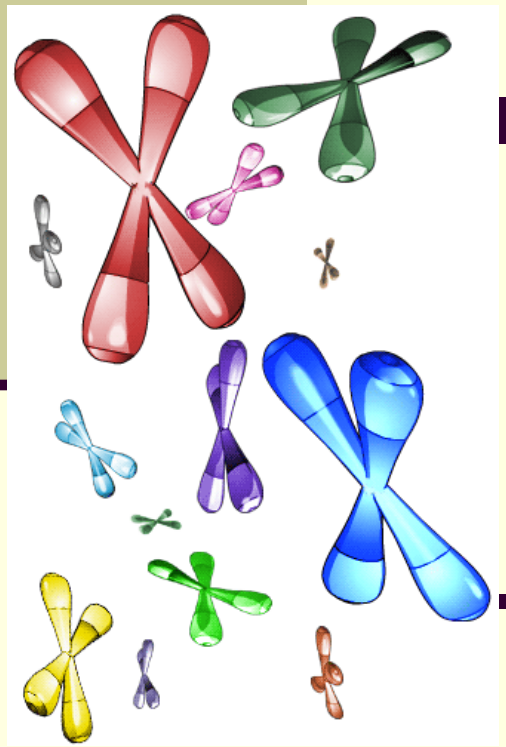
Human Cytogenetics



What is cytogenetics?

- Cytogenetics is a branch of genetics focusing on the study of chromosome changes (number, morphology, numerical and structural abnormalities, segregation in normal and pathological conditions) and their correlation with phenotype.

Development of human cytogenetics

- **„Dark Ages“** - the development and improvement of tissue culture techniques
- **„Hypotonic Period“**

  - hypotonization of cell samples (1951 - 0,075 m KCl)
  - using phytohaemagglutinin (PHA) - stimulation of peripheral blood lymphocytes - 1960
- **„Trisomy Period“** - trisomy of chromosome 21-1959
- The first deletion syndrome - "Cri du chat" - 1963
- **„Banding Area“** - chromosome banding techniques 1968 – 1970
- **„Molecular Area“**

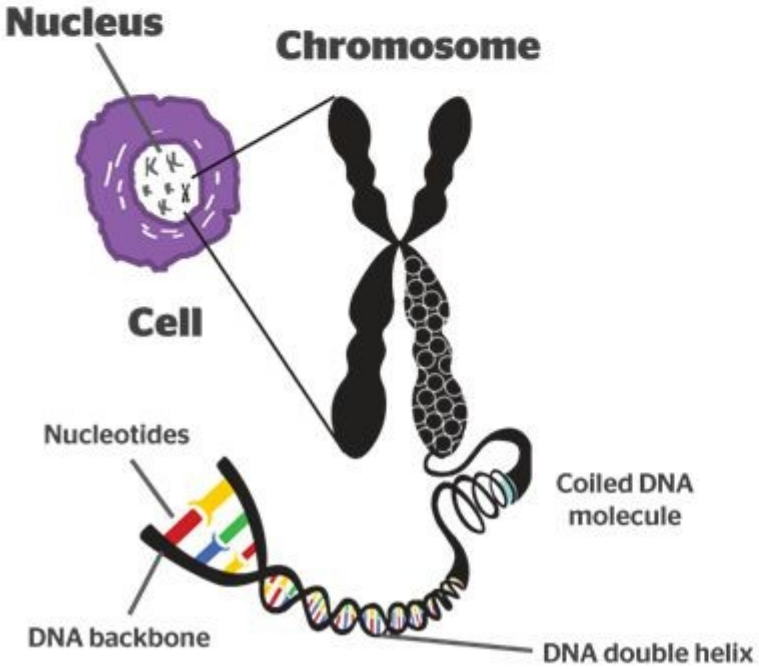
  - in situ hybridization technique – 1970
  - FISH – 1986
  - Comparative genomic hybridization (CGH) - 1992
    - Spectral karyotyping (M-FISH, SKY) - 1996
    - M - banding - 2001
    - Array - CGH - molecular karyotyping

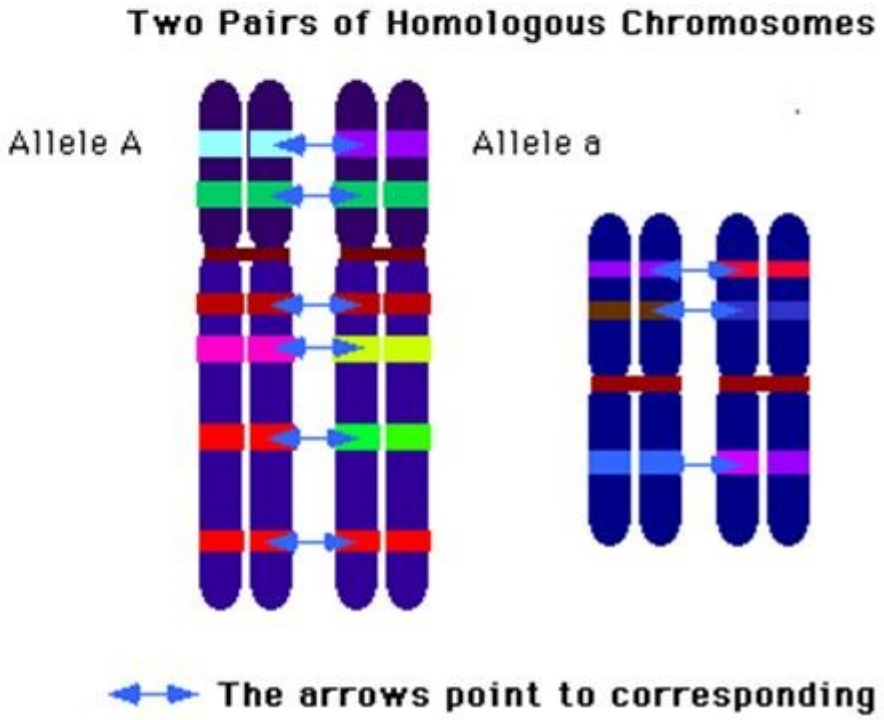
Basic conditions for development of human cytogenetics

- improved techniques of cell cultivation *in vitro*
- use of hypotonic solution (0.075 M KCl)
- establishing squash techniques
- use of colchicine – arrest of mitotic division
- 1% orcein staining

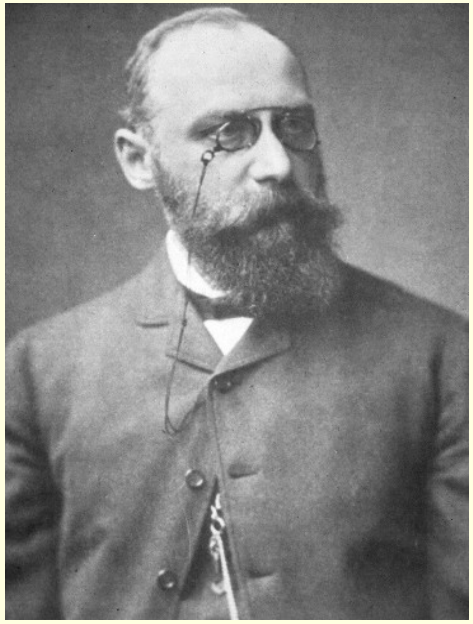
What is a chromosome?

- Structures in the nucleus that are composed of the DNA wrapped around a protein
- Humans have 46 chromosomes in their body cells (only 23 in sex cells)
  - There are 22 pairs of homologous chromosomes (chromosomes that contain the same alleles in the same location)
  - Alleles – are one type of a gene. There are dominant and recessive alleles.

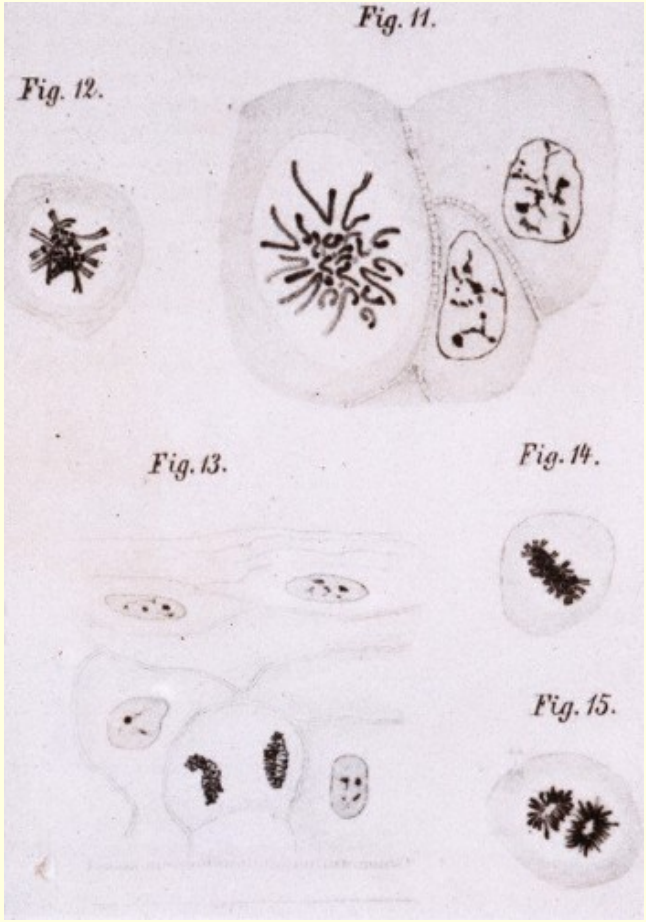




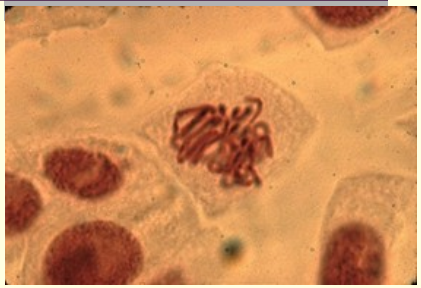
Walther Flemming (1843 – 1905) published first illustrations of chromosomes in1882



„chromo-soma“ – 1888 Waldeyer -Hartz



Chromozomes and DNA



1924 - Feulgen and Rosenback demonstrated the presence of DNA in chromosomes by histochemical staining

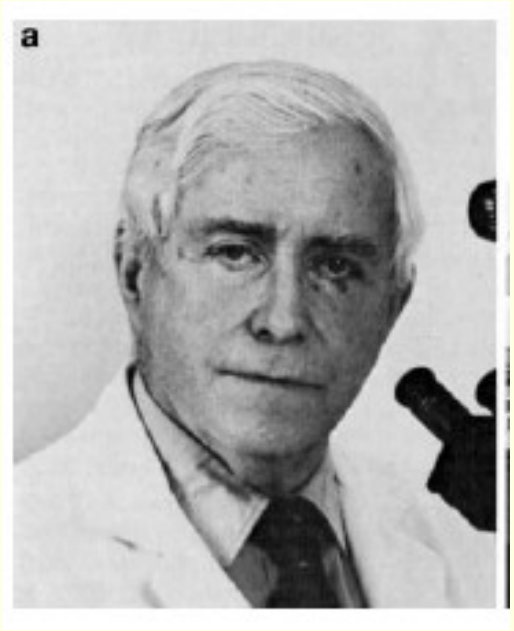
the first chromosome studies were carried out on insects (Drosophila) and plants (maize, broad beans)

1953 - Watson and Crick - deciphering the structure of DNA

but...how many chromosomes have humans?

1956 – the right number of human chromosomes

Joe Hin Tjio (1919 - 2001)



Albert Levan (1905 -1998)

Tjio, T.H., Levan, A.:
The chromosome number of man.
Hereditas 42:1, 1956

THE CHROMOSOME NUMBER OF MAN

By *JOE HIN TJIO* and *ALBERT LEVAN*

ESTACION EXPERIMENTAL DE AULA DEI, ZARAGOZA, SPAIN, AND CANCER CHROMOSOME LABORATORY, INSTITUTE OF GENETICS, LUND, SWEDEN

WHILE staying last summer at the Sloan-Kettering Institute, New York, one of us tried out some modifications of HSU's technique (1952) on various human tissue cultures carried in serial *in vitro* cultivation at that institute. The results were promising inasmuch as some fairly satisfactory chromosome analyses were obtained in cultures both of tissues of normal origin and of tumours (LEVAN, 1956).

Later on both authors, working in cooperation at Lund, have tried still further to improve the technique. We had access to tissue cultures of human embryonic lung fibroblasts, grown in bovine amniotic fluid; these were very kindly supplied to us by Dr. RUNE GRUBB of the Virus Laboratory, Institute of Bacteriology, Lund. All cultures were primary explants taken from human embryos obtained after legal abortions. The embryos were 10—25 cm in length. The chromosomes were studied a few days after the *in vitro* explantation had been made.

In our opinion the hypotonic pre-treatment introduced by HSU, although a very significant improvement especially for spreading the chromosomes, has a tendency to make the chromosome outlines somewhat blurred and vague. We consequently tried to abbreviate the hypotonic treatment to a minimum, hoping to induce the scattering of the chromosomes without unfavourable effects on the chromosome surface. Pre-treatment with hypotonic solution for only one or two minutes gave good results. In addition, we gave a colchicine dose to the culture medium 12—20 hours before fixation, making the medium 50×10^{-5} mol/l for the drug. The colchicine effected a considerable accumulation of mitoses and a varying degree of chromosome contraction. Fixation followed in 60 % acetic acid, twice exchanged in order to wash out the salts left from the culture medium and from the hypotonic solution that would otherwise have caused precipitation with the orcein. Ordinary squash preparations were made in 1 % acetic orcein. For chromosome counts the squashing was made very mild in order to keep the chromosomes in the metaphase groups. For idiogram studies a more thorough squashing was preferable. In many cases single cells were squashed

1 — *Hereditas 42*

Humans have 23 pairs of chromosomes in the nucleus of each of their body cells, for a total of 46 chromosomes.

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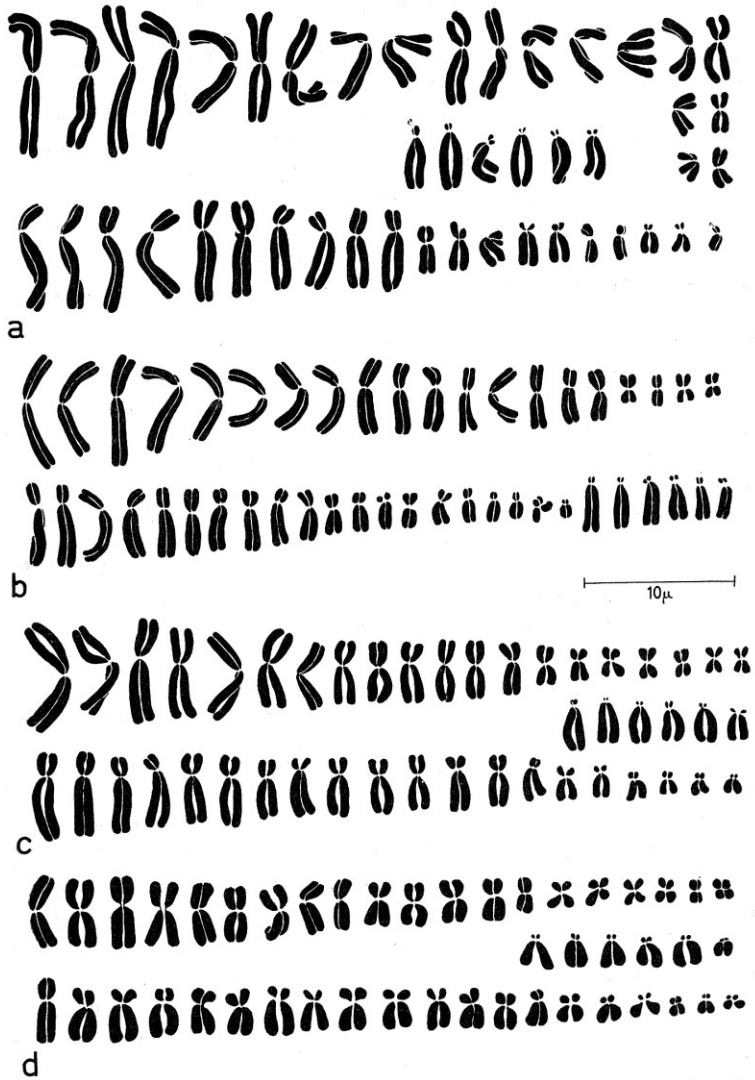
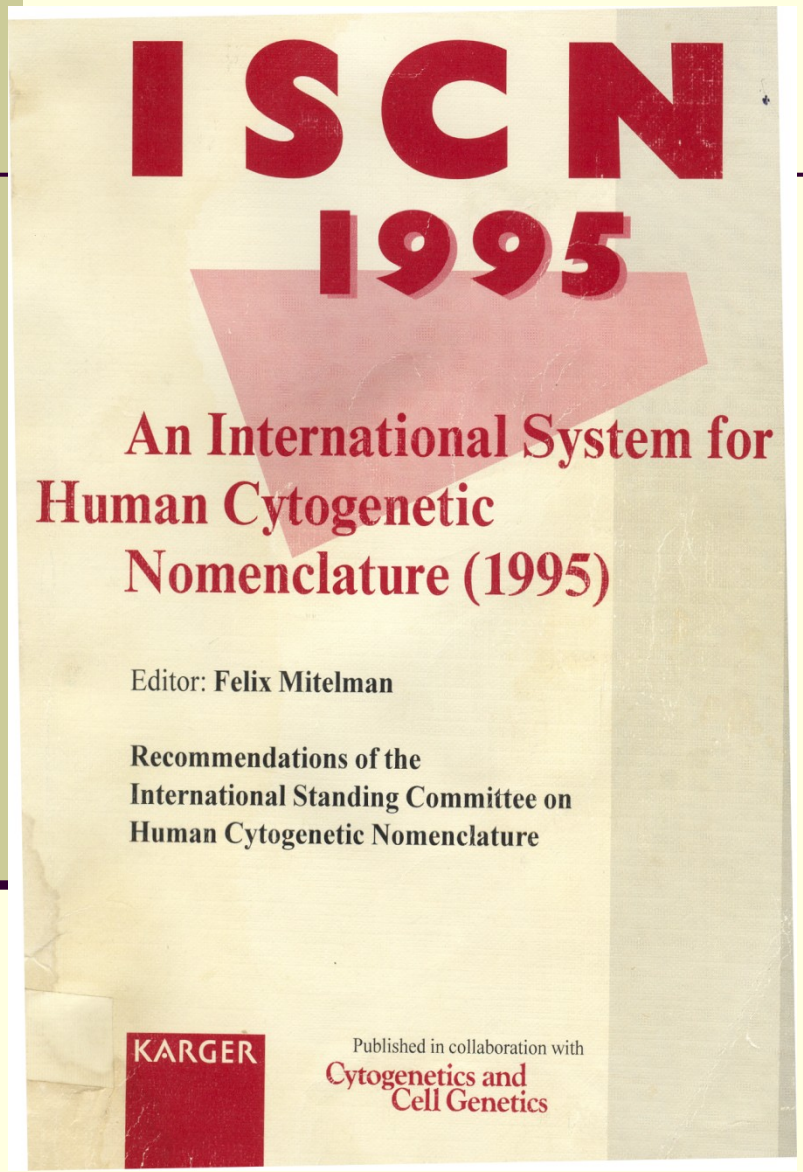
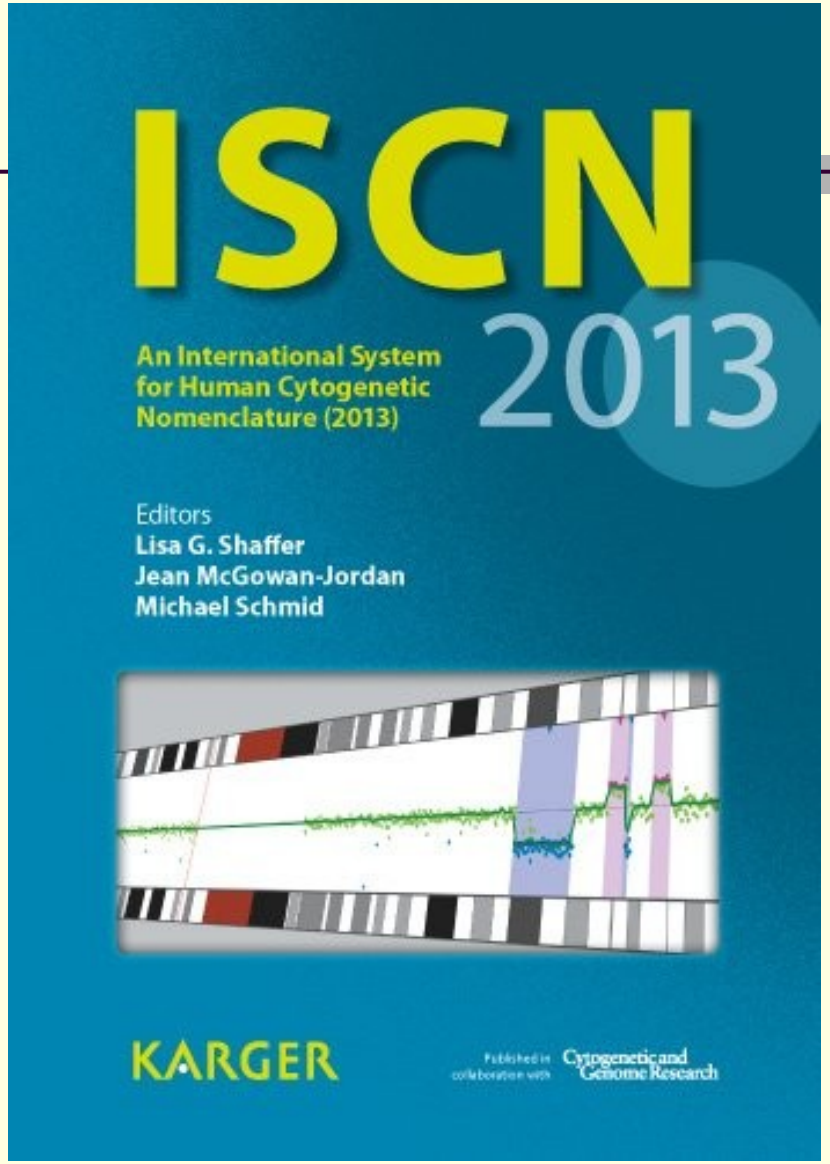


Fig. 2. Four idiogram analyses of human embryonic lung fibroblasts grown *in vitro*. The chromosomes have been grouped in three classes: M (top row), S (bottom row), and T (in between, except in *b*, where T is at the end of the S row). Within each class the chromosomes have been roughly arranged in diminishing order of size. — ×2400.

Nomenclature of human chromosomes

- **1960:** *Denver Conference* - sort of human chromosomes into groups according to size and shape
- **1963:** *London Conference* - chromosomes are sorted into 7 groups A – G
- **1966:** Chicago *Conference* - the description of chromosome changes
- **1971:** *Paris Conference* - the identification and labeling of chromosomes using banding techniques
- An International System for Human Cytogenetic Nomenclature (ISCN 1978)


ISCN
1995
An International System for Human Cytogenetic Nomenclature (1995)
Editor: Felix Mitelman
Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature
KARGER
Published in collaboration with
Cytogenetics and Cell Genetics


ISCN
2013
An International System for Human Cytogenetic Nomenclature (2013)
Editors
Lisa G. Shaffer
Jean McGowan-Jordan
Michael Schmid
KARGER
Published in collaboration with
Cytogenetic and Genome Research

Human somatic cell contains

- **23 pairs or 46 chromosomes**
  - **22 autosomic pairs**
  - **1 gonozomic pair (XX or XY)**
- **7 groups ordered according to chromosome size and morphology**

A – large metacentric chromosomes

B – large submetacentric chromosomes

C – middle size submetacentric chromosomes, X

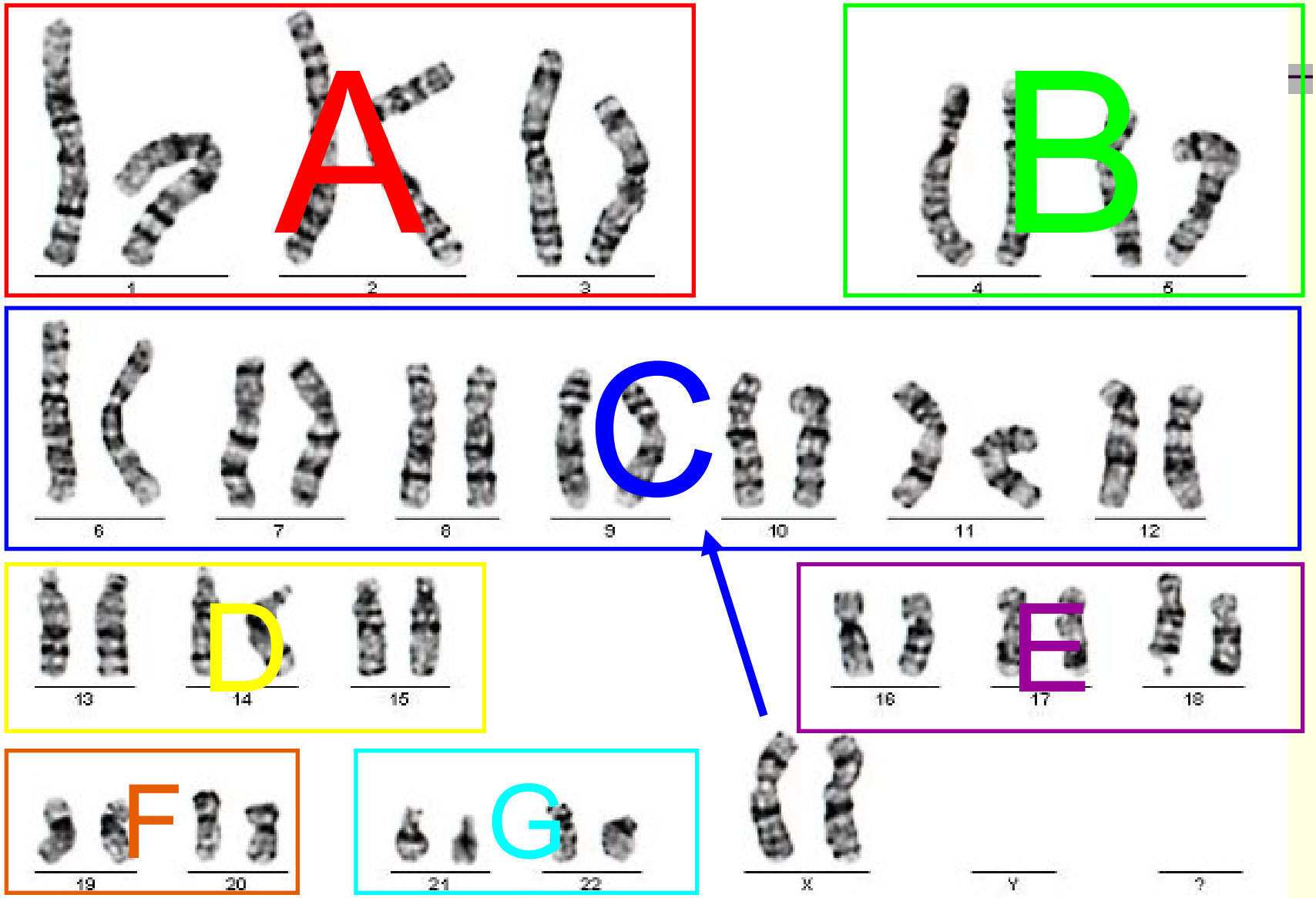
D – large acrocentric chromosomes

E – small meta- to submetacentric chromosomes

F – the smallest metacentric chromosomes „ribbons“

G – small acrocentric chromosomes, Y

Human Karyotype



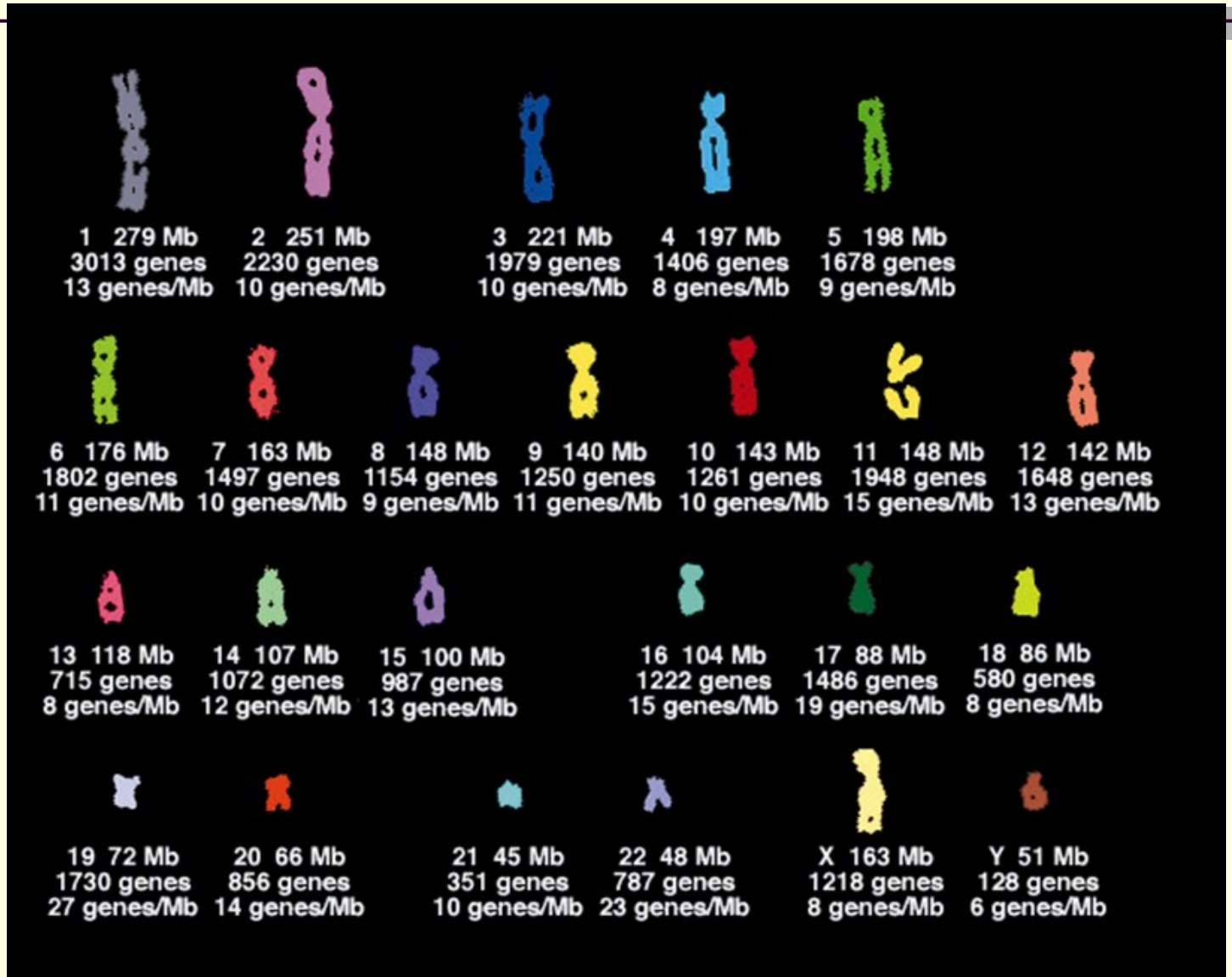
Chromosomes and few numbers

- Genomic DNA of eukaryotes - chromosomes – **linear double helix DNA <u>3 billion pb</u>**
- The human haploid genome contains:
  - **1000 mm of DNA = 2 m of DNA in a diploid cell**

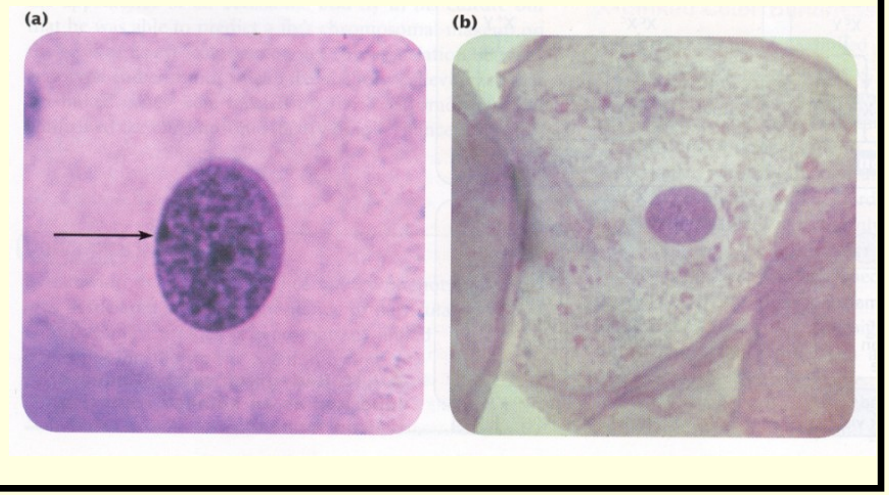
  = 23 molecules of DNA

- a typical chromosome of a eukaryotic cell contains 1 to 20 cm of DNA
- chromosome size in mitosis **1 to 10 um**
- sum of all chromosome sizes - **115 um**

Human chromosomes and genes



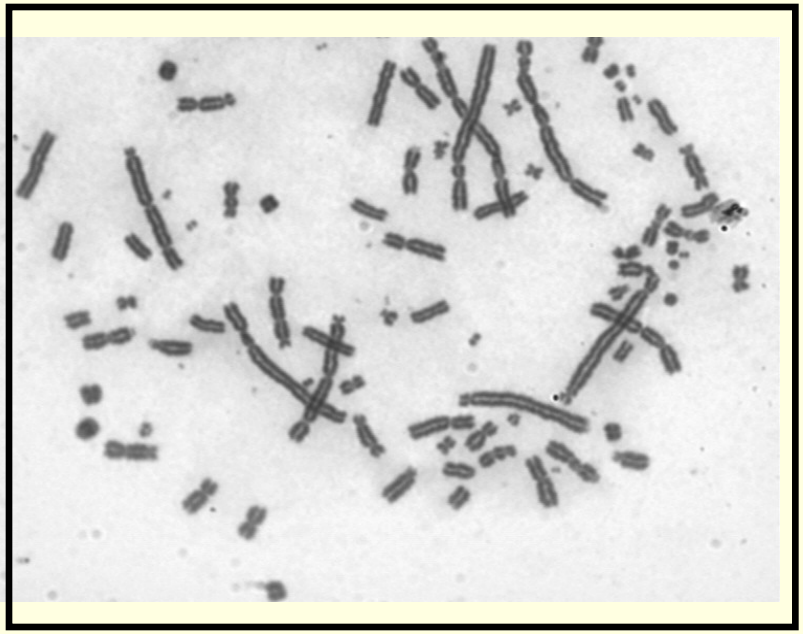
Microscopic and submicroscopic structure of chromosomes



← **Interphase - despiralization**

Mitosis, meiosis -spiralized form

↓



Interphase - chromatin

a) **Eu**chromatin (eu = true)

b) **Hetero**chromatin (hetero = different)

Characteristics of chromatin

Euchromatin

- despiralized in interphase
- spiralized in mitosis
- contains structural genes (unique sequences)
- gene expression

Heterochromatin

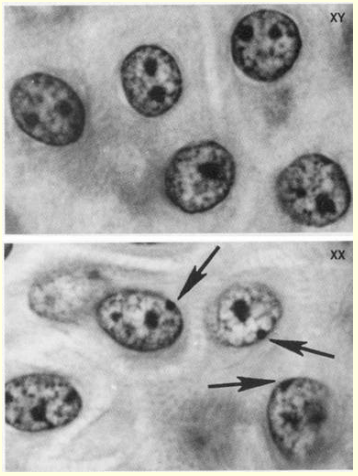
a) Constitutive repetitive sequences - satellite DNA, **does not contain active genes** (tandem repeats), somewhat spiralised in interphase – **staining** in humans especially in **centromeres**, **Yq chromosome**

b) Facultative = structurally euchromatin, behaves like heterochromatin

inactive X = sex chromatin = **Barr's body = X chromatin**

one of the two X chromosomes in female mammals is inactive

Heterochromatin - later replication in S phase (inactive X at the end of S)



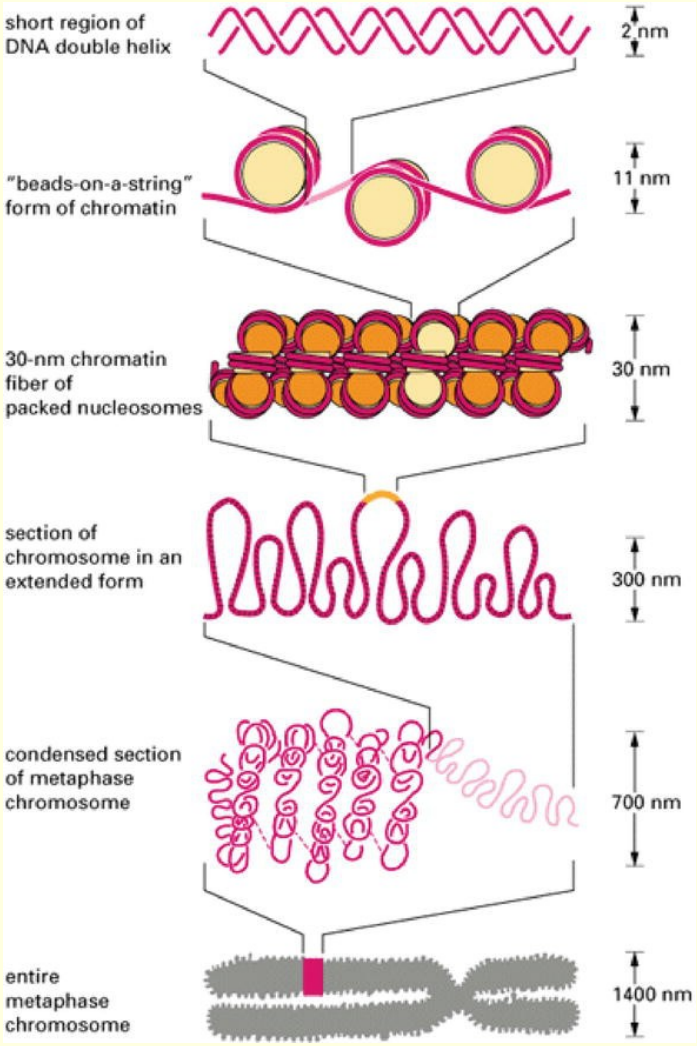
Composition of chromatin

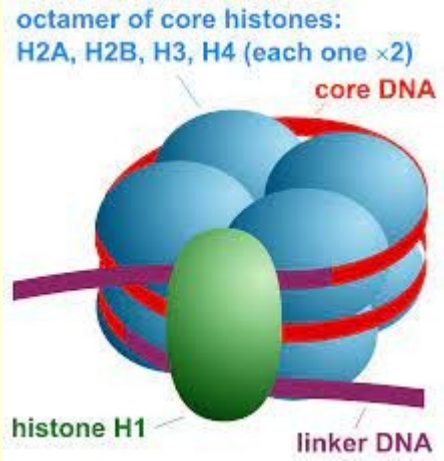
Composition of chromatin:

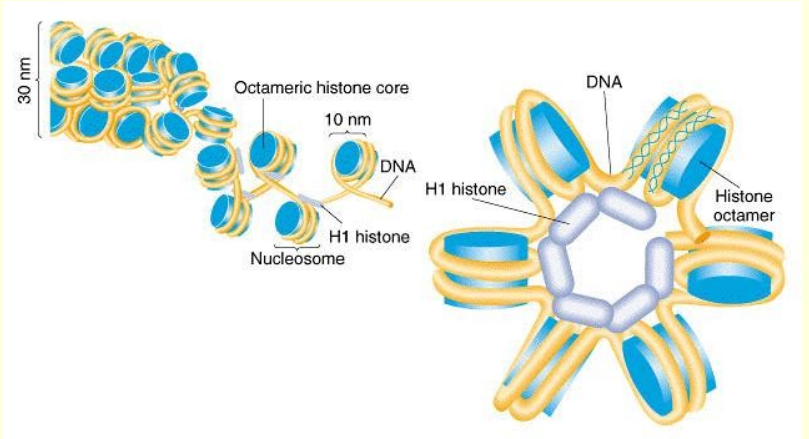
- **DNA, RNA (30 %)**
- **Proteins (70%)**

- **Histones = basic proteins (20-30% arginine and lysine):** evolutionarily conserved, positively charged, important in interactions with DNA
- **H1, H2A, H2B, H3, H4** - pack chromosomes, regulate transcription, gene expression....
- Non-histone proteins = acidic proteins (e.g. HMG proteins) - regulation of gene expression

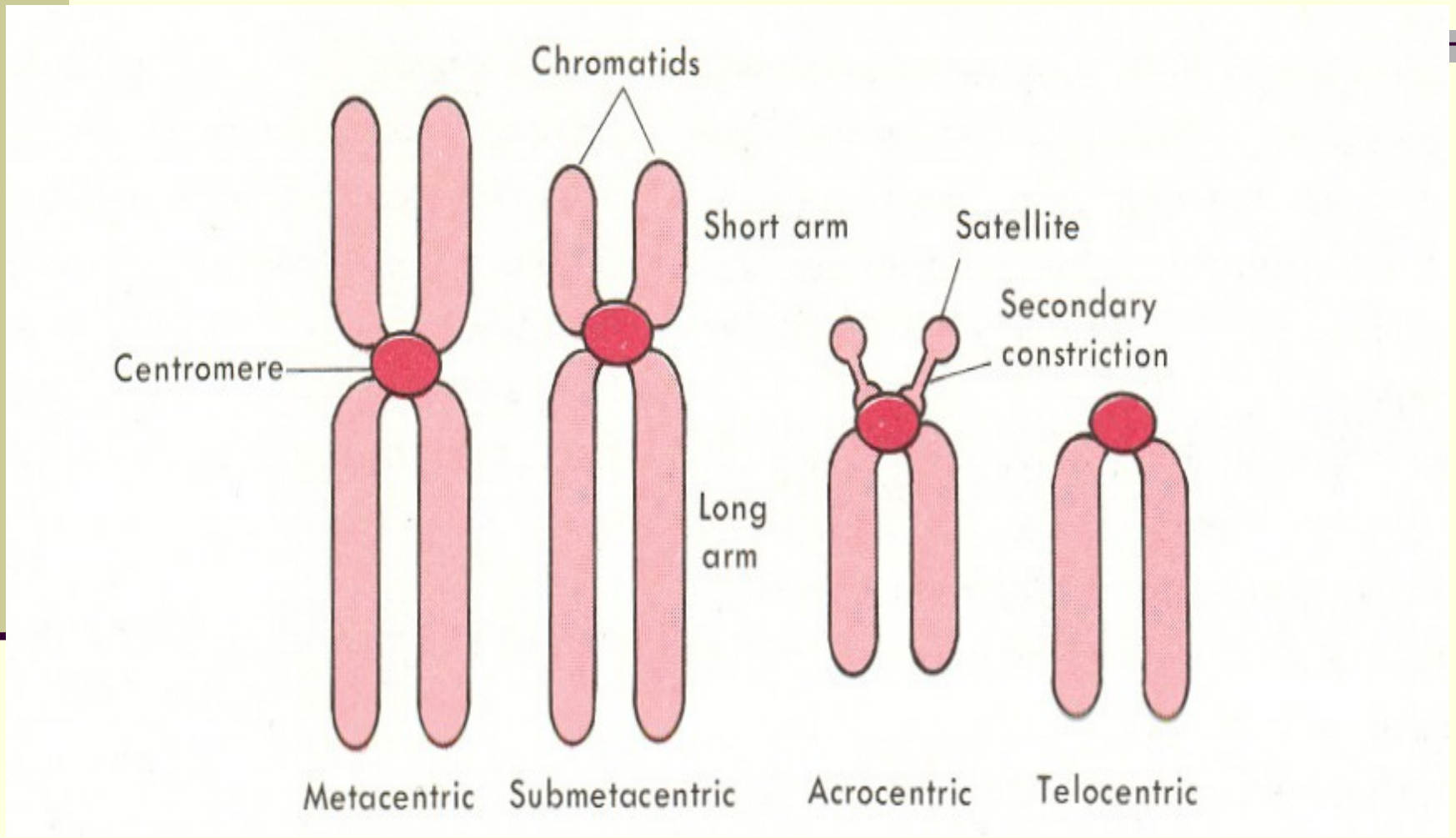
Folding of DNA – from strand to chromosome



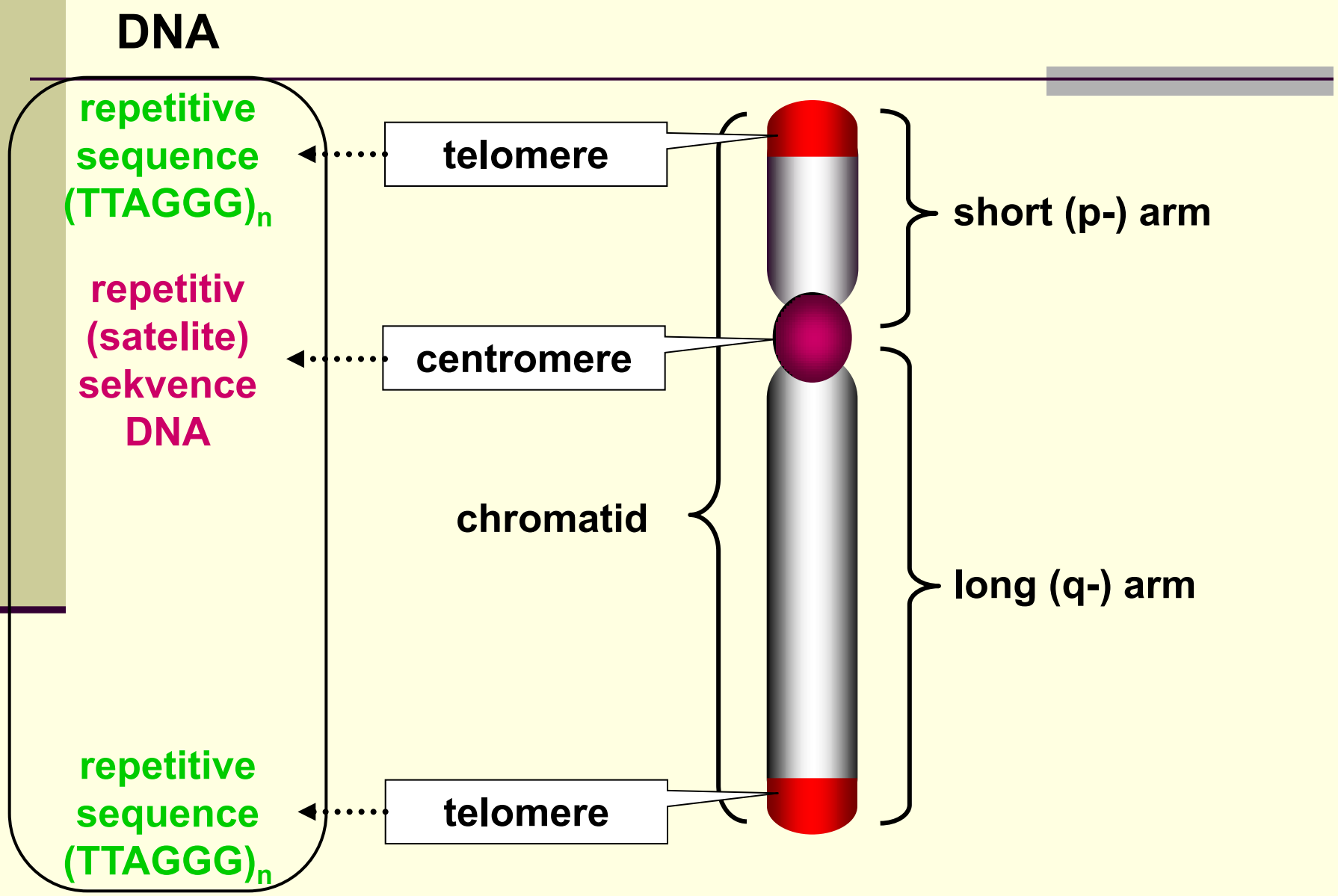




Chromosome morphology



3. Chromosome morphology



Centromere

Centromere (primary constriction) is an important part of chromosomes

- holds together sister chromatids - cohesin proteins
- essential for chromosome segregation
- visible by C-banding !

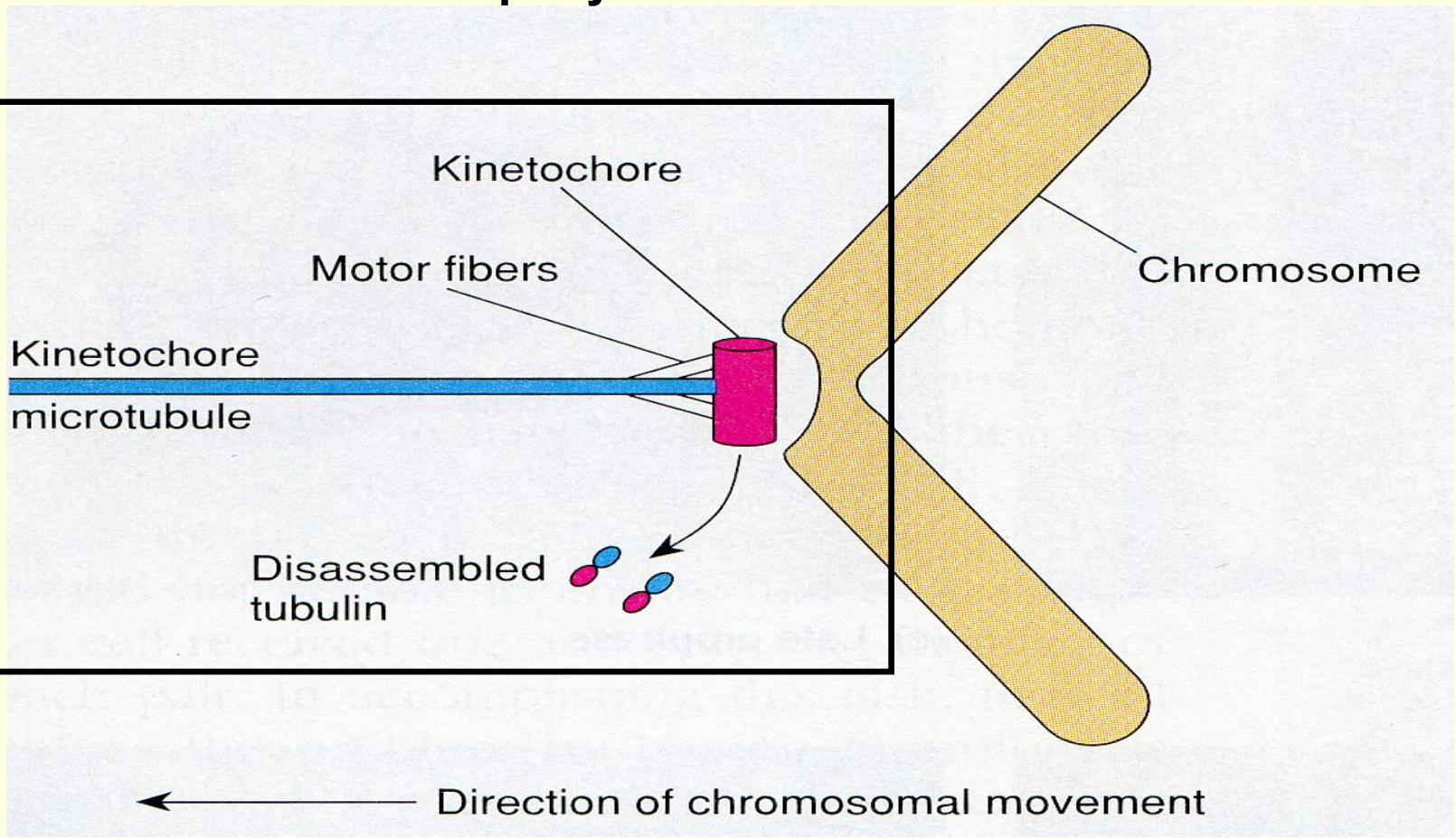
- DNA of centromeres is composed of blocks of constitutive heterochromatin = (pericentric heterochromatin) repetitive sequences create **satellite DNA**
  e.g. in humans alpha satellite DNA - unit 171 pb - blocks of 300 to 5000 kb) + binding sites for centromeric CENP proteins

Kinetochore - protein complex, microtubule binding with centormeres

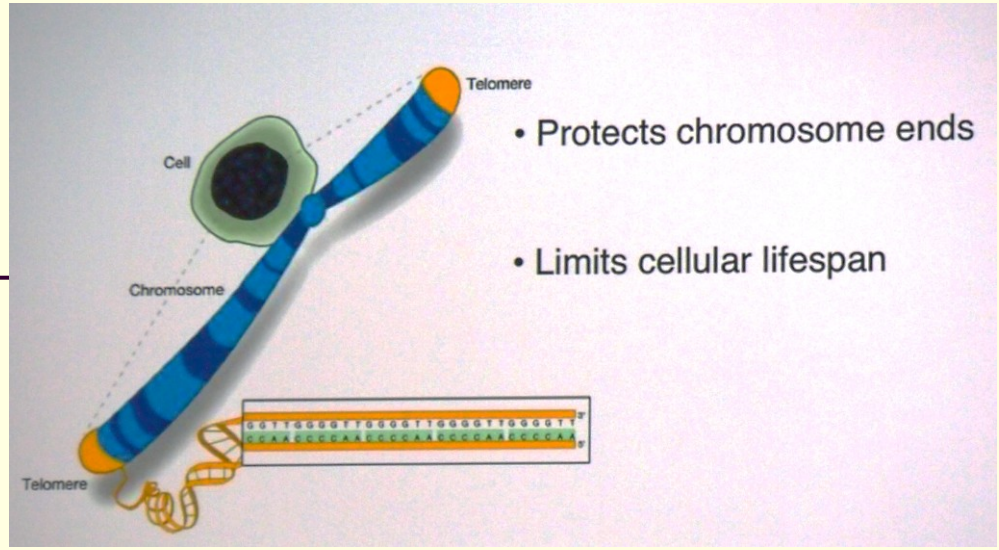
centromere disorders - nondisjunctions, acentric or dicentric chromosomes !

Centromere and kinetochore

- **zkracování či prodlužování vláken dělícího vřeténka – pohyb chromozomů**



Telomeres



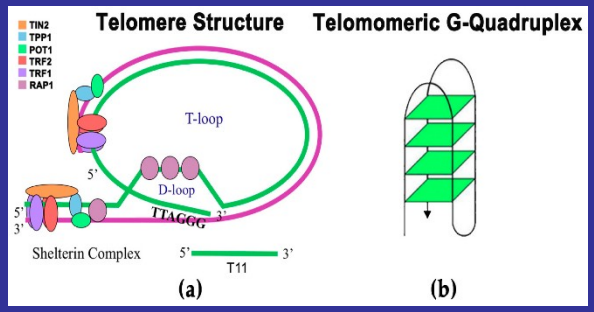
- **physical ends of eukaryotic chromosomes** - they have unique properties !
- in humans, made up of proteins and tandem repeats of **DNA (TTAGGG)n vs. (CCCTAA)n** - 500 to 3000 repeats !
- accompanied by blocks of heterochromatin

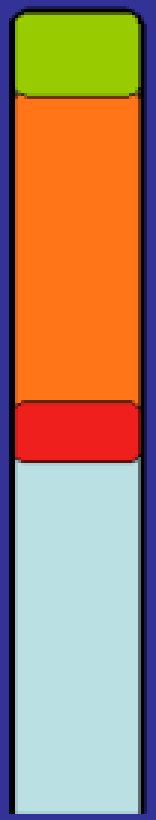
- **Absence of telomeres** → chromosome rearrangements - fusions („sticky ends“) leading to translocations, circular or unstable dicentric chromosomes

Significance of telomeres

- **protection of chromosome ends against the action of exonucleases** (ends of broken chromosomes) protection against fusion with other chromosomes – **t-loop**...
- **maintain** the **architecture** of the interphase **nucleus**
- **pairing** of homologous **chromosomes** in **meiosis**
- subtelomeric regions - most genes....
- allow r**eplication** of the ends **of linear DNA** - gap at the **5' end..** (ends are replicated by the enzyme **telomerase** ...absent in somatic cells...but active in sex cells, stem cells, tumors ...maintains telomere length)
- **cell aging** - no telomerase - shortening telomere length during division

The telomere





3–20 kb (TTAGGG)n

100-300 kb

Telomere associated repeats

Unique telomere region (site of FISH probes)

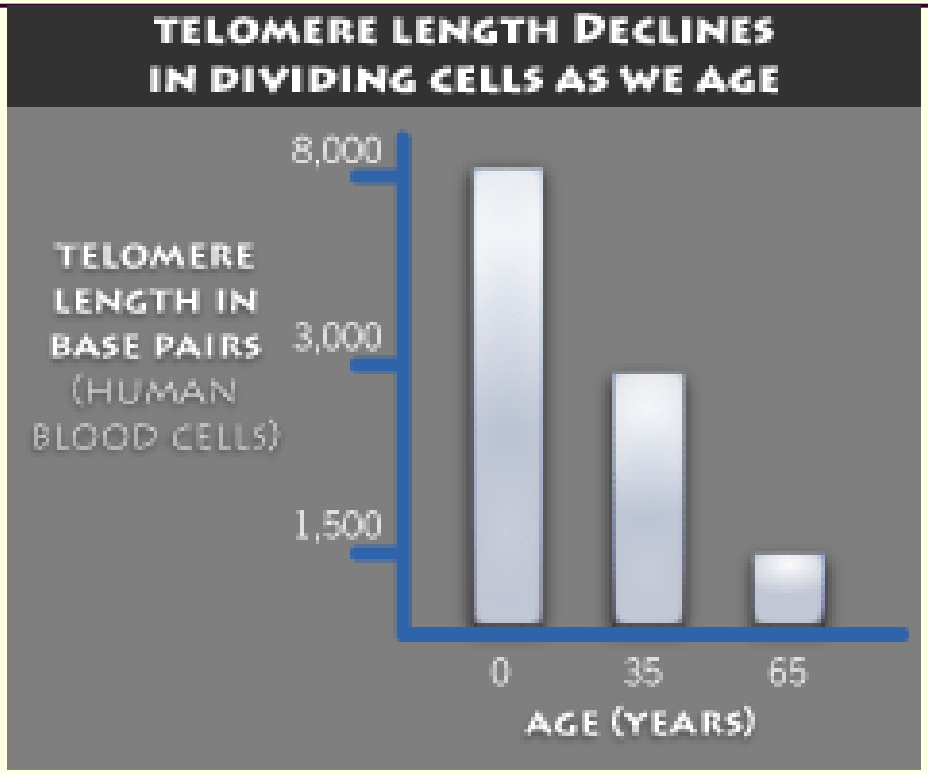
centromere

Single strand 50 - 500 pb rich overlap at 3 end rich in guanine - t-loop - end protection

Telomeres shorten during each cell division of 50 - 200 bp

- Short telomeres – blocking of cell cycle

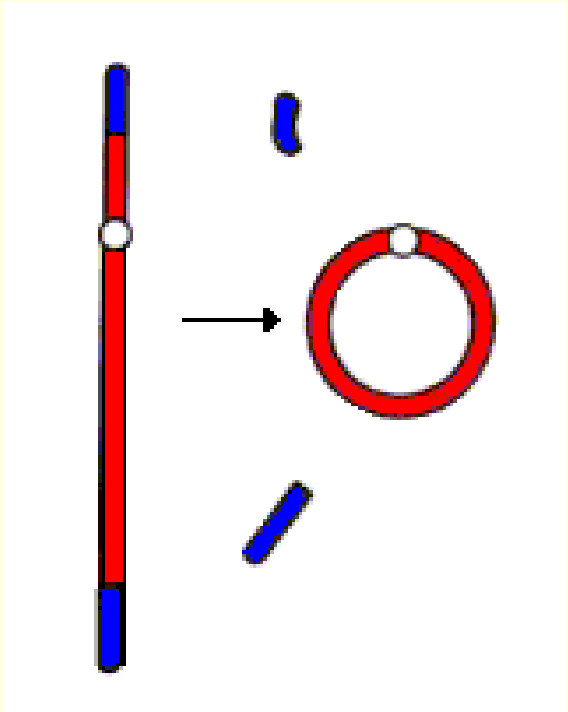
Shortenig of telomers and age

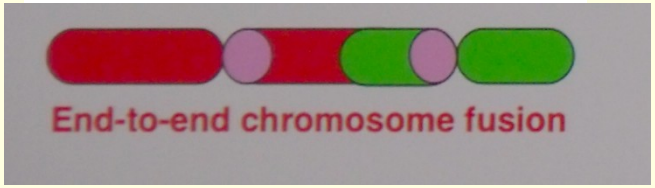


The Hayflick limit determines the maximum number of divisions a cell undergoes before it dies. Human fibroblasts in culture can divide a maximum of 50 to 70 times, after which they age and die.

Progeria – rapid ageing short telomers

Consequences of telomere errors = chromosomal aberratios



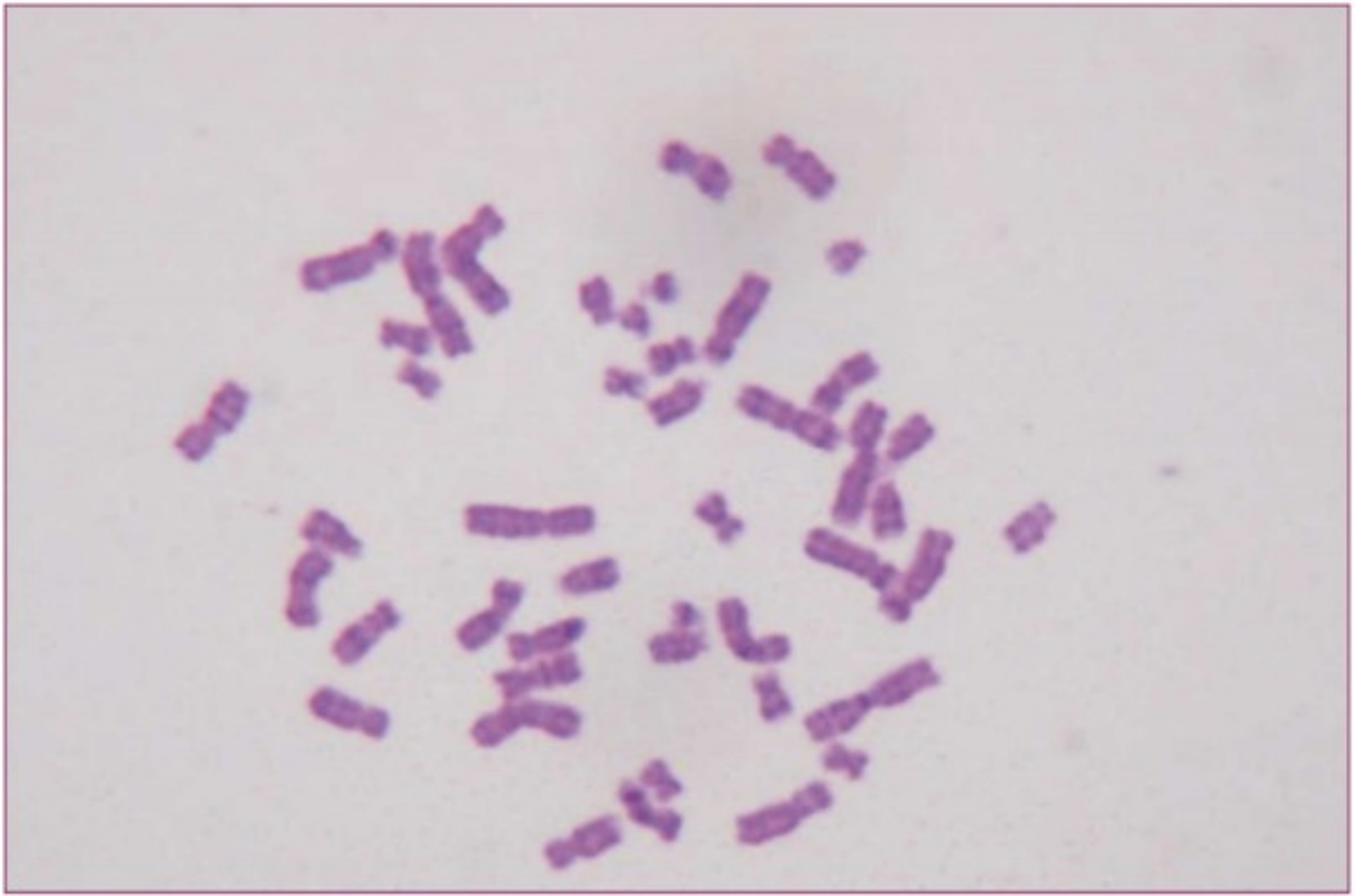


Identification of chromosomes

Chromosome staining

- **Classical staining**
  - using Giemsa Romanowski solution
  - gained chromosome aberration detection
- **G – bands**
  - using trypsin, salty solution and Giemsa
  - each chromosome has characteristic stripes
  - congenital chromosomes aberation detection
- **R – bands**
  - using salty solution of different pH and temperature
  - reverse to G - bands

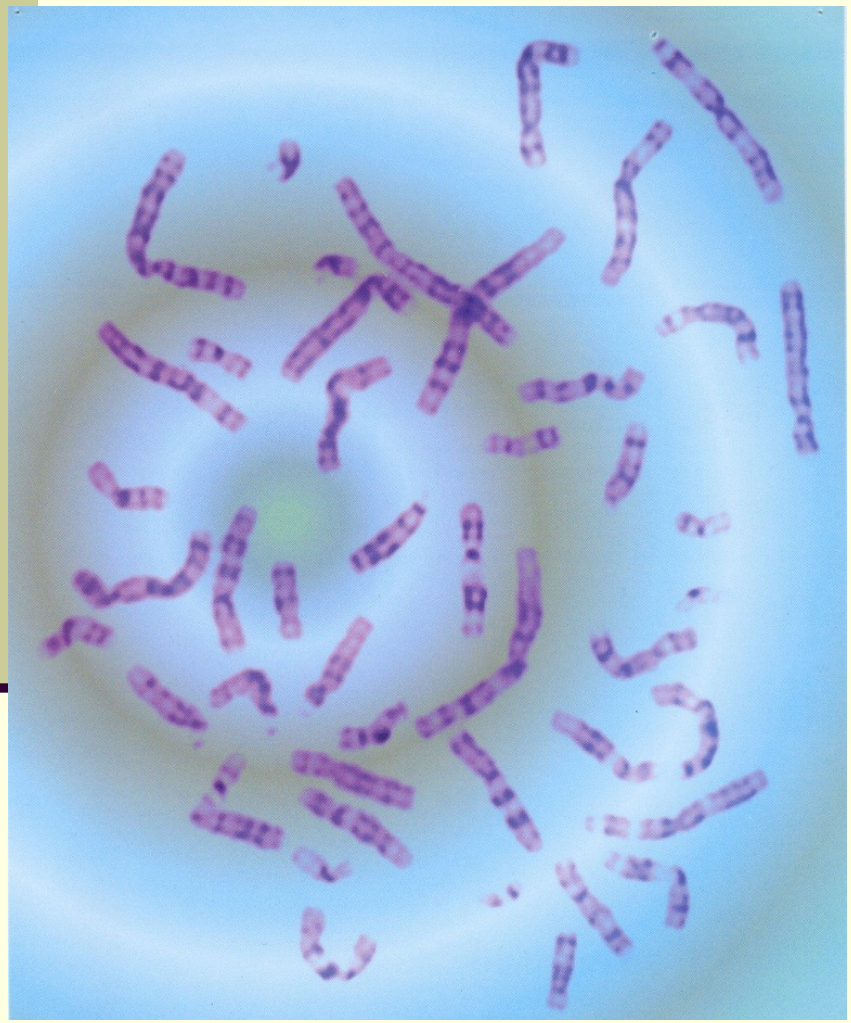
Giemsa-Romanowski staining



Characterization of G banding of human chromosomes

- **bands** on chromosomes **reflect** the structure of the **genome** and its **functional organization**
- each band contains **5 to 10 Mb** (resolution limit !!!)
- *Giemsa positive bands* - dark (G+, **rich in AT pairs**, late replicating chromosome regions
- **poor in genes**
- *Giemsa negative bands* - light (G-, **rich in GC pairs**, early replicating chromosome regions, **rich in genes**

G – banding (mitosis and descritption)



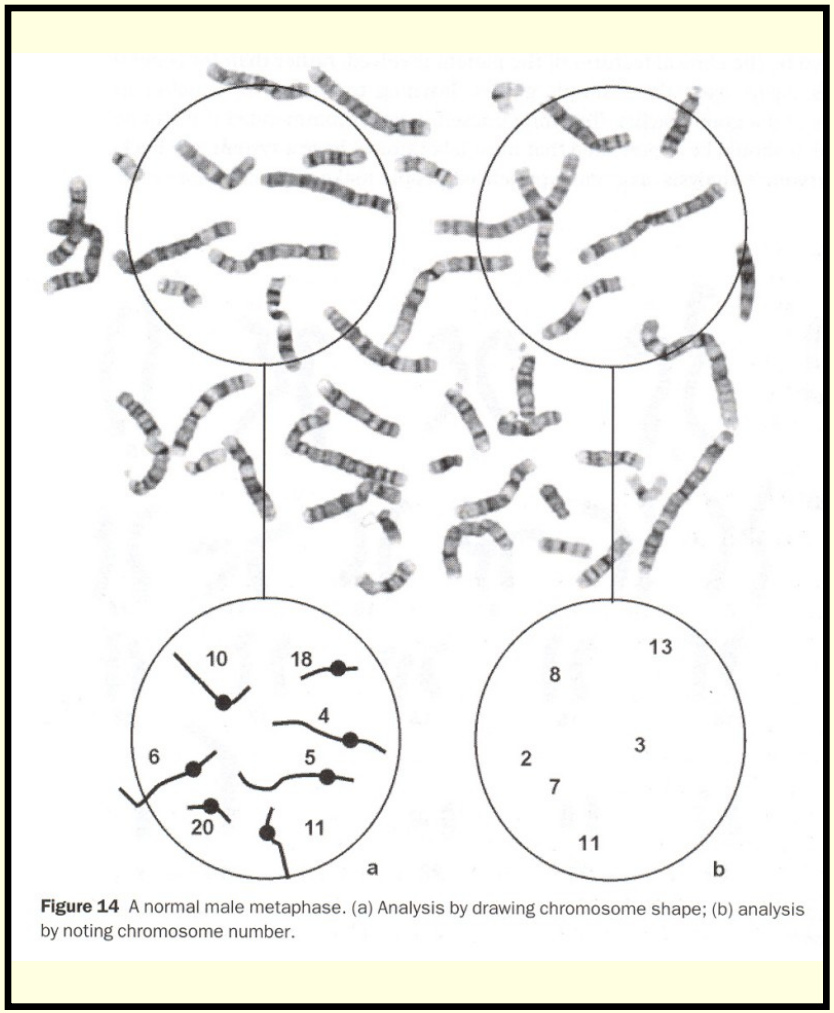
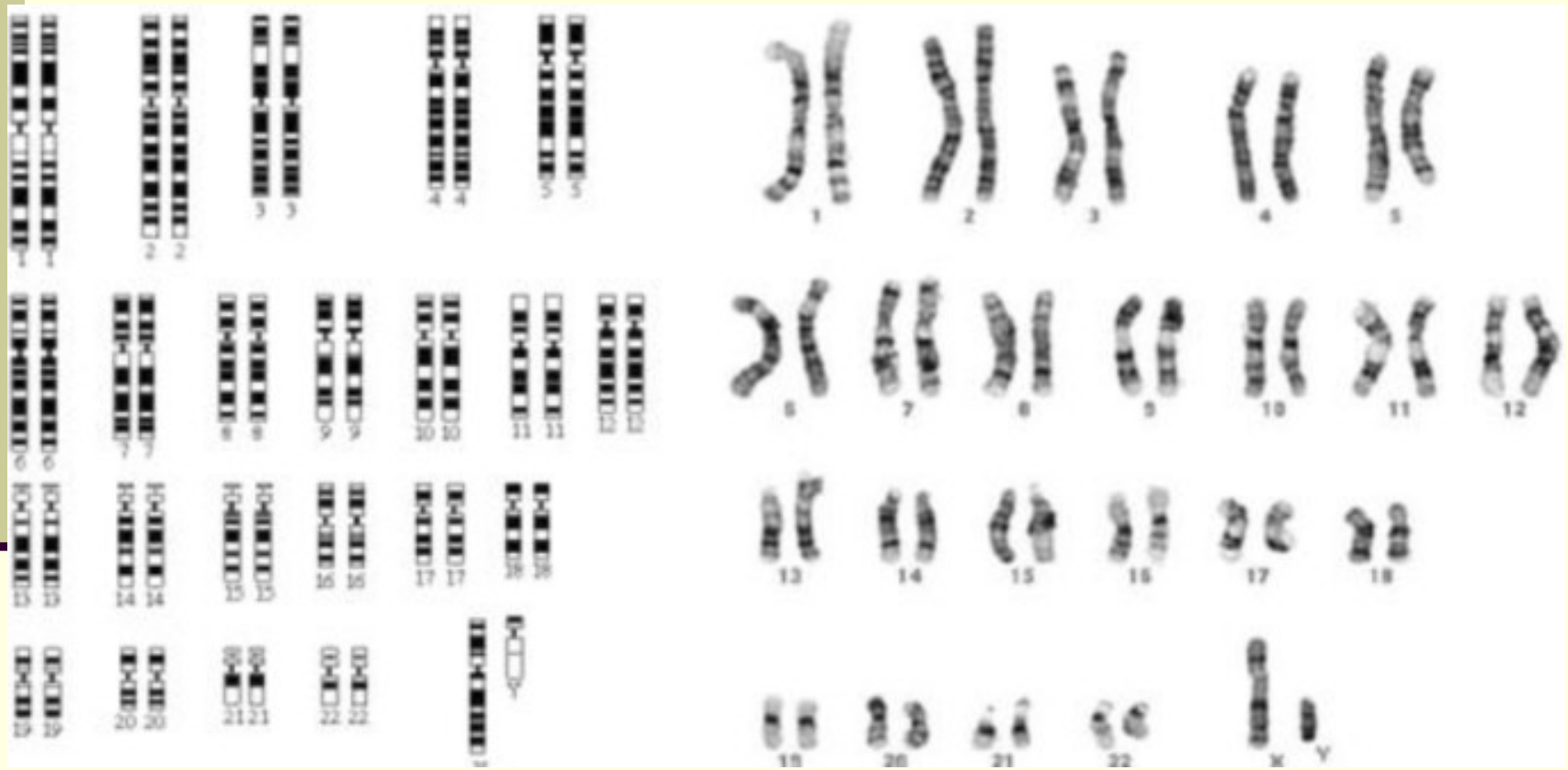


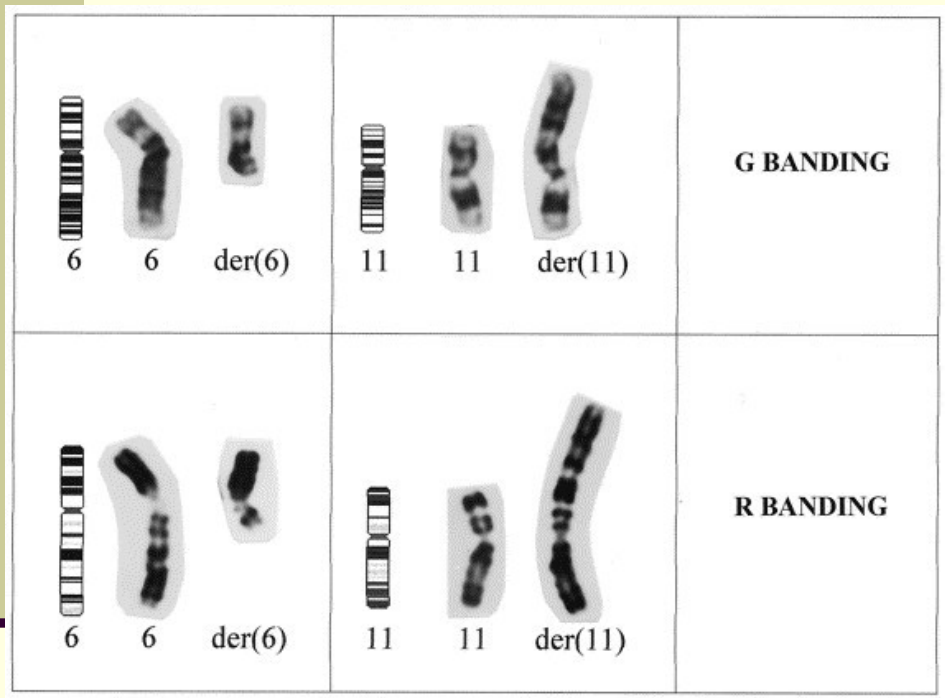
Figure 14 A normal male metaphase. (a) Analysis by drawing chromosome shape; (b) analysis by noting chromosome number.

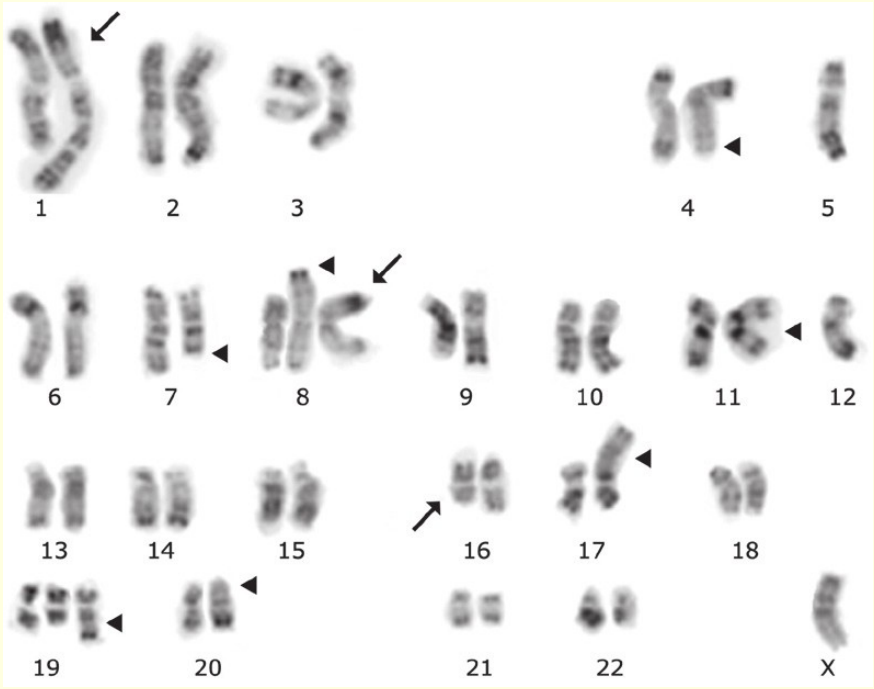
G-banding classification pattern



C - banding detects regions of centromeric heterochromatin !

R-banding of human chromosomes





Clinical indications for investigation of karyotypes

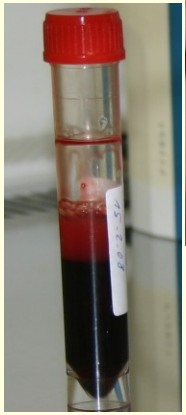
- early growth and development problems (failure to thrive, delayed growth, short stature, bilateral genitalia, mental retardation, neurodevelopmental disorders)
- stillbirth and neonatal death
- partners treated for infertility and partners with repeated spontaneous abortions
- sperm donors and egg donors
- family history (known chromosomal abnormality in first-degree relatives)
- pregnancy in women of advanced age, pregnant women with pathological findings in biochemical screening or ultrasound findings
- persons with prolonged exposure to environmental pollutants

4. Molecular cytogenetics

- presents the connections between classical cytogenetics and molecular biology
- utilizes the latest knowledge of molecular biology, microscopy and computer image analysis to study the structure and properties of chromosomal changes
- allows the analysis of numerical and structural chromosomal imbalances unidentified classical cytogenetic techniques
- does not require the presence of mitosis
- sources of material for cytogenetic investigation
  - peripheral blood
  - samples from different tissues
  - amniotic fluid cells, chorionic villi, placenta umbilical cord blood
  - bone marrow
  - samples of solid tumors

bone marrow

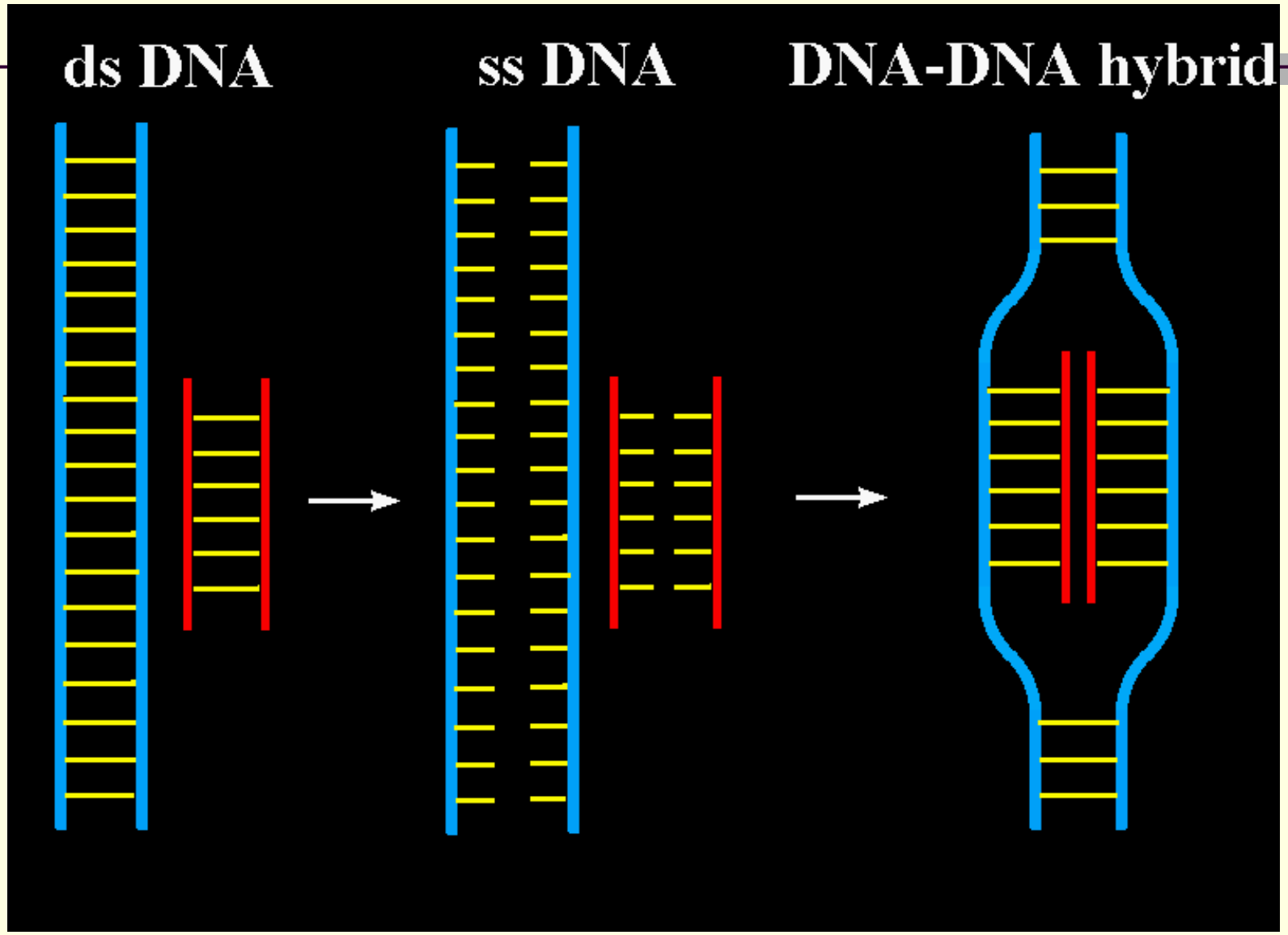
solid tumor



peripheral blood

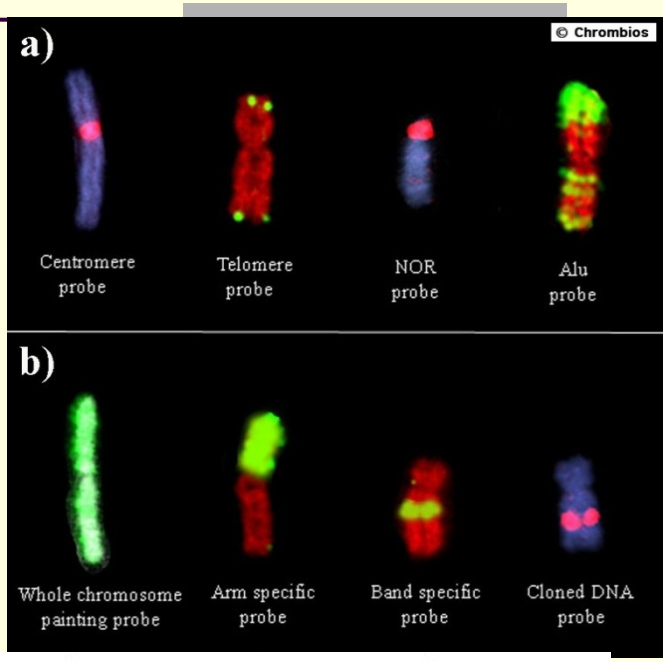
FISH

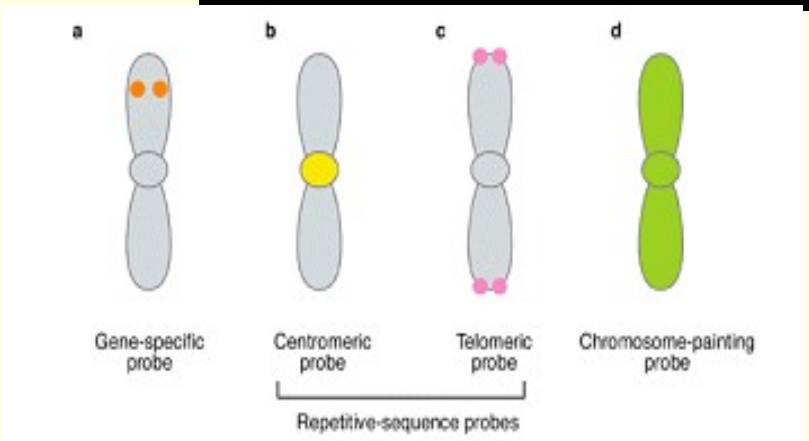
fluorescent in situ hybridization



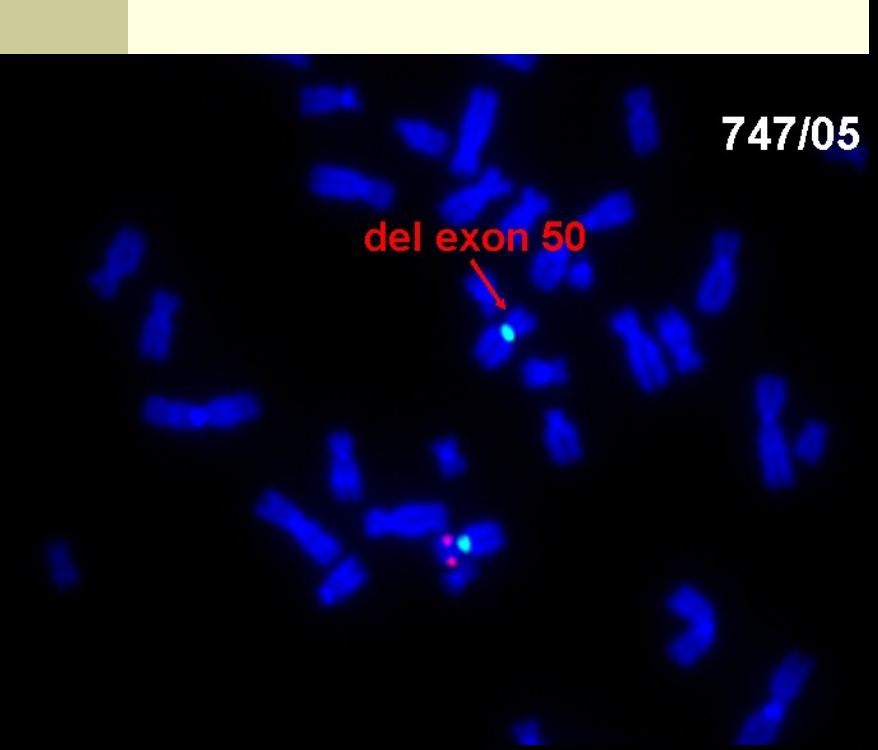
FISH

- detection of the fluorescent signals through microscope equipped with specific fluorescent filters
- material
  - cultivated peripheral blood
  - cultivated bone marrow
  - cultivated amniotic fluid cells
  - uncultivated amniocytes
  - tumor and bone marrow prints
- we determine:
  1. **presence of signals**
  2. **number of signals**
  3. **position of signals**
- the use of FISH
  - clinical cytogenetics
  - onco cytogenetics
  - human genom mapping

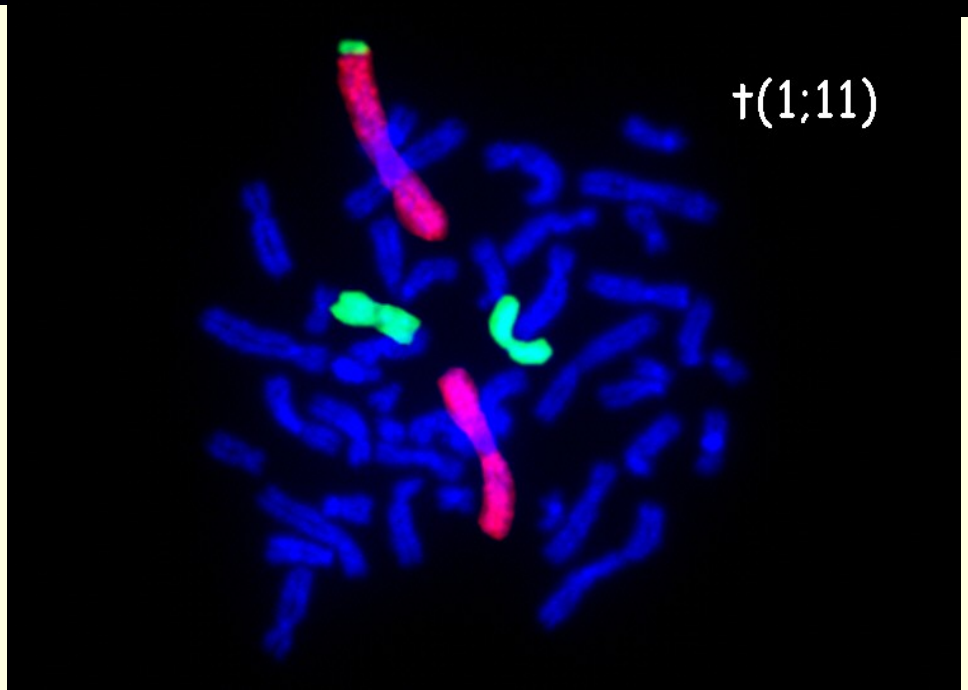




747/05



830/05



Advantages and disadvanages of FISH

- **advantages**
  - does not require the presence of mitoses (mostly)
  - quick assessment of big amount of cells

- **disadvantages**
  - does not provide whole genomic view

SKY (spectral karyotyping)

- **the images** are captured by **charge-coupled device** (CCD) imaging and analyzed by using an **interferometer** attached to a epifluorescence microscope
- Image processing software then assigns a **pseudo color** to each **spectrally different** combination, allowing the visualization of the individually **colored chromosomes**
- microscope equipped with **2 fluorescent filters (SKY, DAPI)**

  fluorochromes (**FITC** **Rhodamin** **TexasRed** **Cy5** **Cy5.5**) **scanned by one filter**, based on a wave lenght each chromosome pair is colored
- **Resolution 15-20Mbs** – complex chromosomal changes

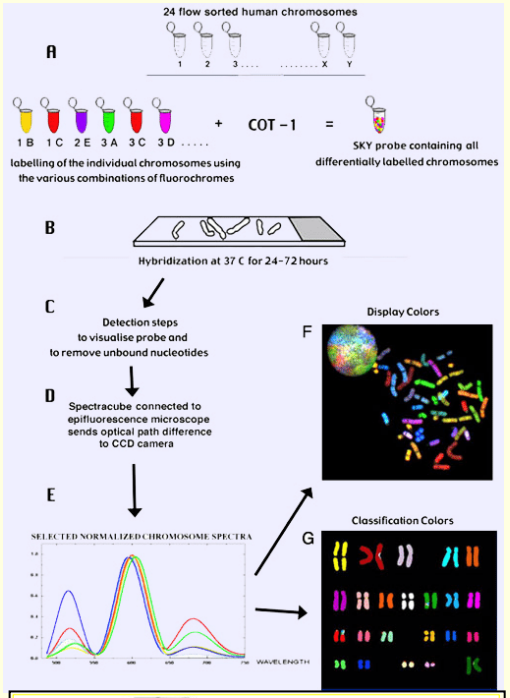
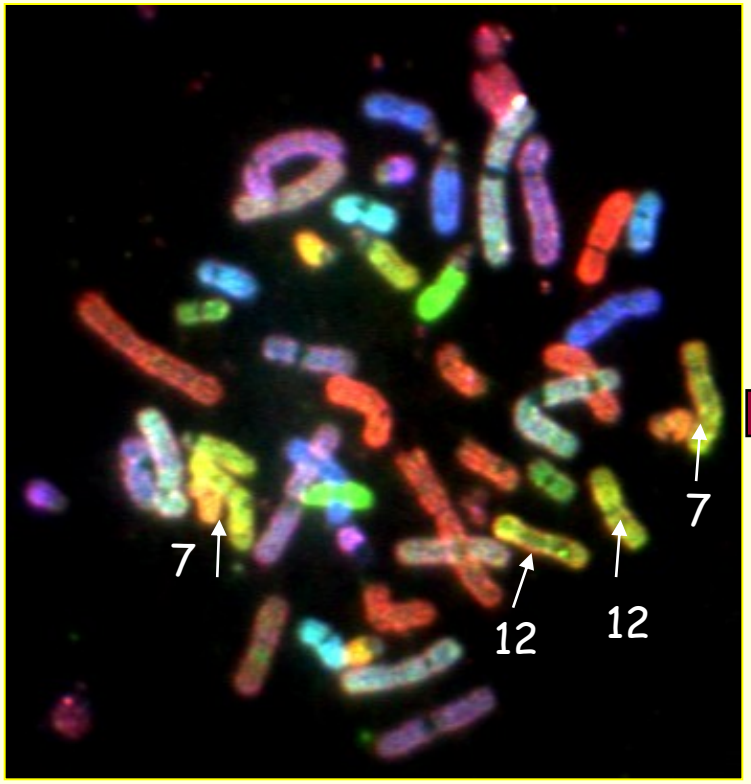
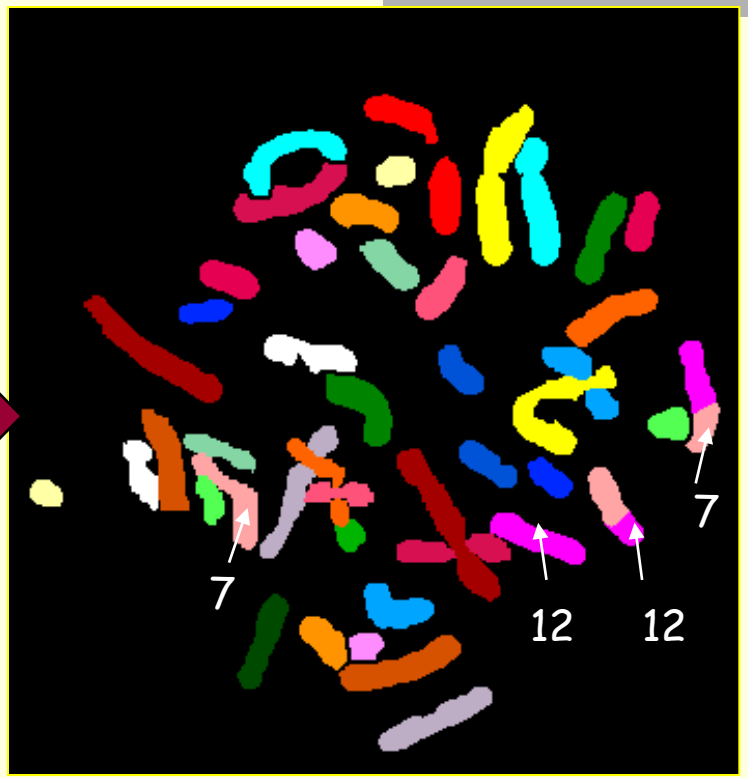


Image Acquisition with SkyVision™

Picture analyse using SkyView

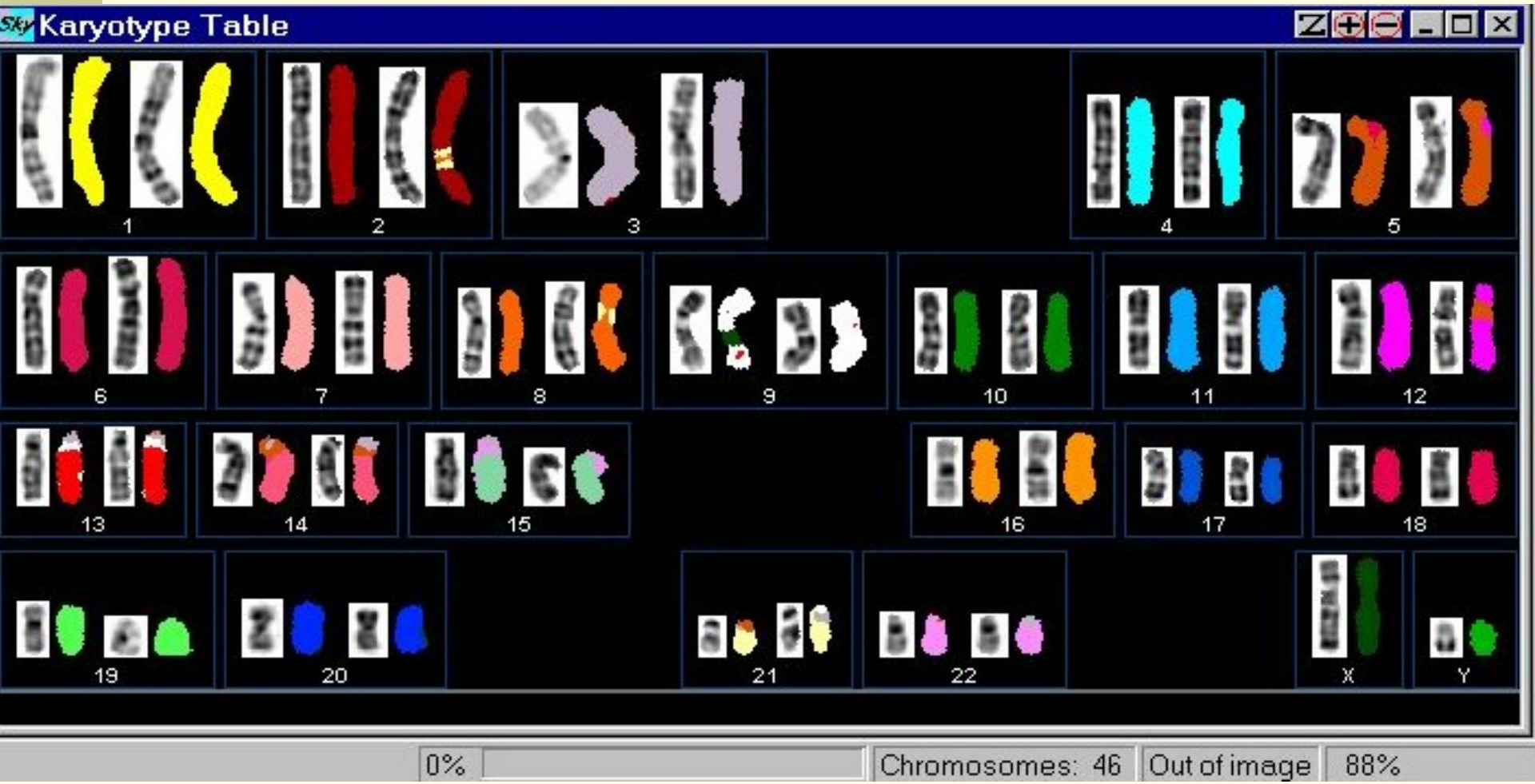


Display Image



Classified Image

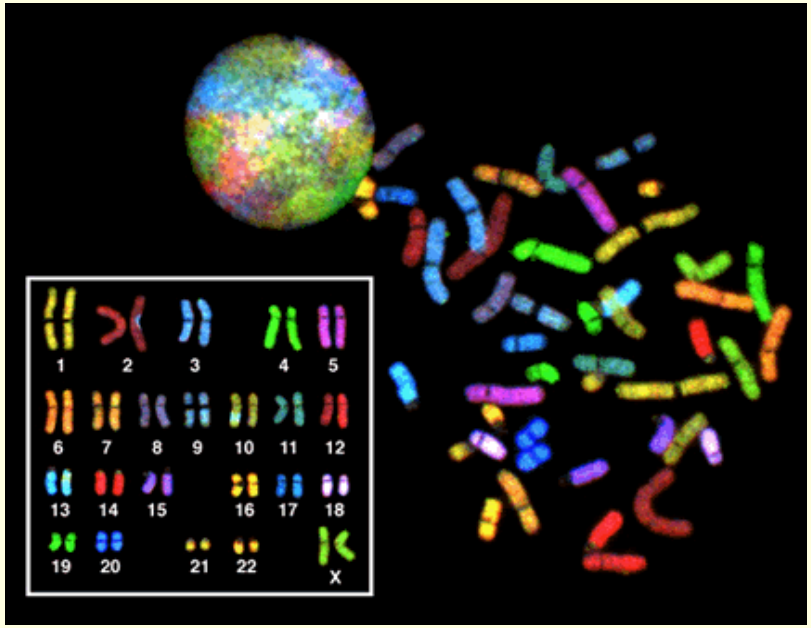
The objective of the SkyView spectral karyotyping software is to automatically classify and karyotype chromosomes in the Display image, thereby overcoming the ambiguity inherent in the display colors.


Karyotype Table
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
0%
Chromosomes: 46
Out of image
88%

M-FISH technique - (multicolor FISH)

(24 whole chromosome DNA painting probes)

- **Analysis of complex chromosomal aberrations**
- Each homologous pair of chromosomes **is uniquely labeled** with **five fluorochromes** set which are spectrally distinct in **different combinations**
- The images are captured by **band-pass filter** sets and defined **emission spectra** are measured by dedicated M-FISH software.
- Resolution **~3–10** Mb in size



Advantages and disadvantages of SKY and M-FISH

- **advantages**
  - detects balanced rearrangements
  - detects aberrations in one step
    - cryptic translocations and insertions
    - marker chromosomes
    - redundant material with unknown origin
    - komplex rearrangements

- **disadvantages**
  - need of quality mitoses
  - successful hybridization
  - expensive methods

CGH
comparative genomic hybridization

- a modification of FISH technique to measure DNA gains or losses throughout the entire genome

- enables detection of unbalanced chromosomal changes (gains or losses) throughout an entire genome in one hybridization reaction

- is based on comparison of two genomes

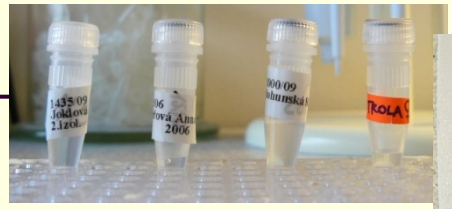
Conventional FISH

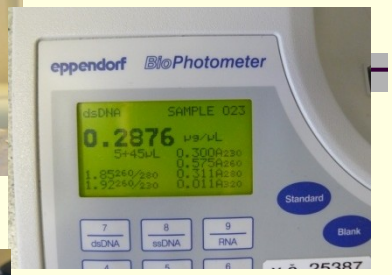
normal DNA → select DNA → make probe → label abnormal target → abnormal target identified

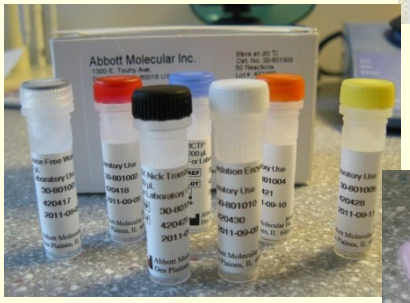
Comparative genomic hybridization

normal DNA → no DNA selection → make probe (entire genome) → quantify on normal target → abnormal genome quantified

CGH requirements







Materials :

- Good quality DNA isolated from
  - •peripheral blood
  - •bone marrow
  - •solid tumour
  - •amniocytes

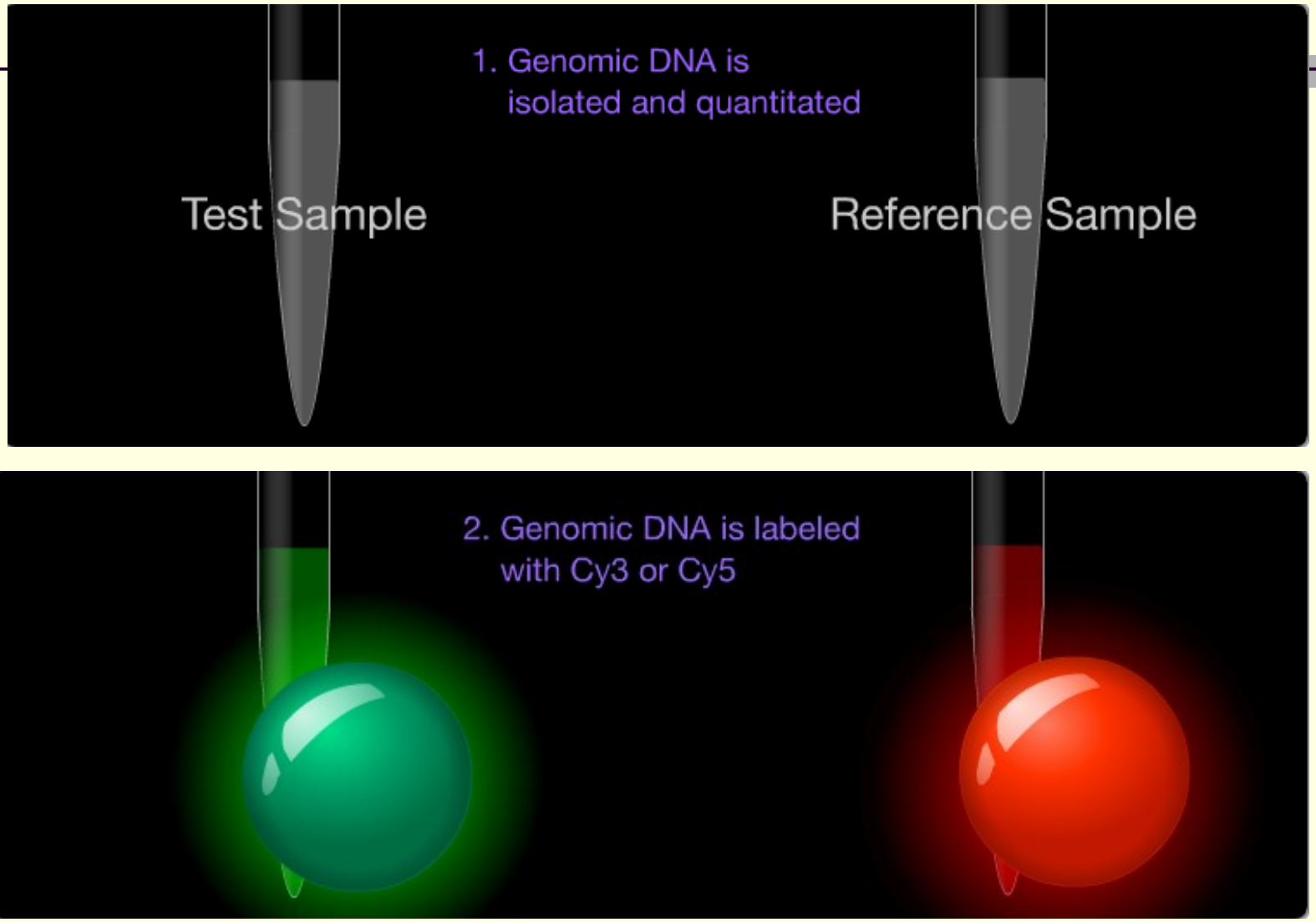
Equipment :

•Fluorescent miroscope (filters DAPI, SpGreen, SpRed)

• Sensitive CCD kamera

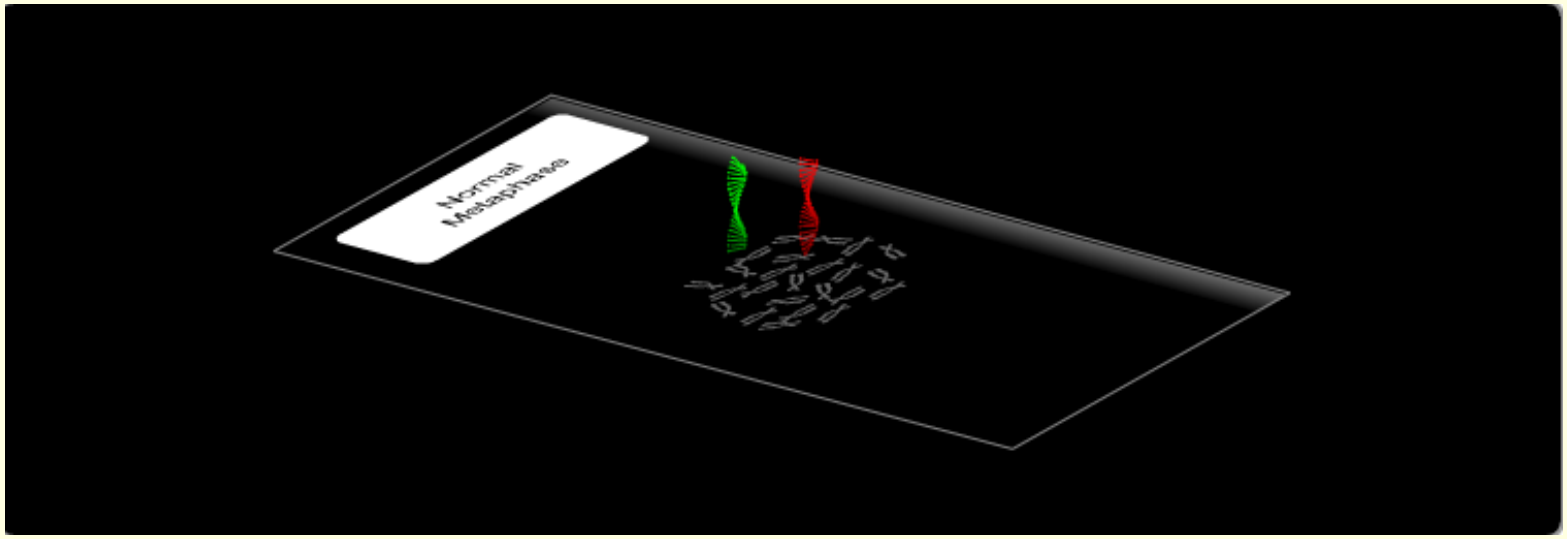
• Computer with software for CGH analysis and data interpretation (LUCIA CGH Advanced Statistics, Laboratory Imaging Ltd., Prague, Czech Republic)

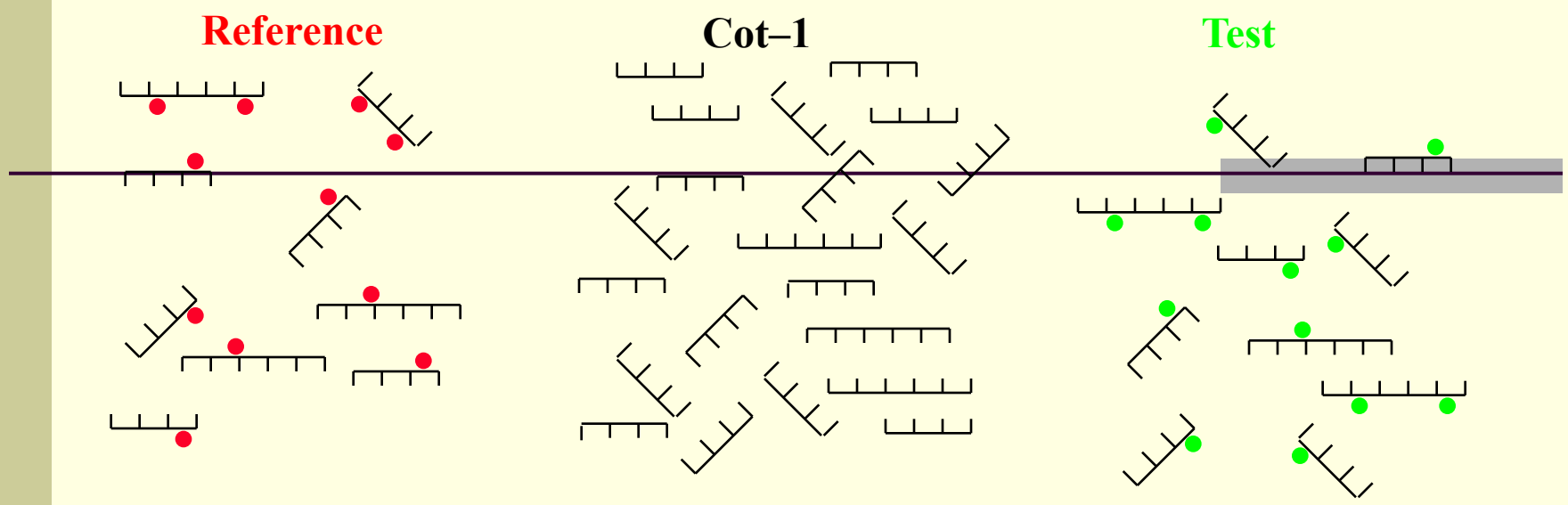
CGH principle



www.abbottmoleculars.com

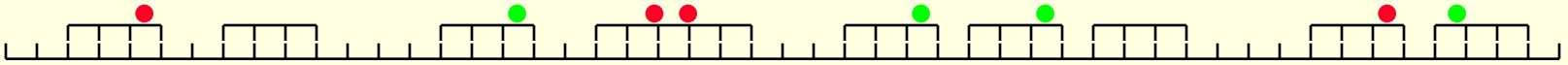
CGH principle





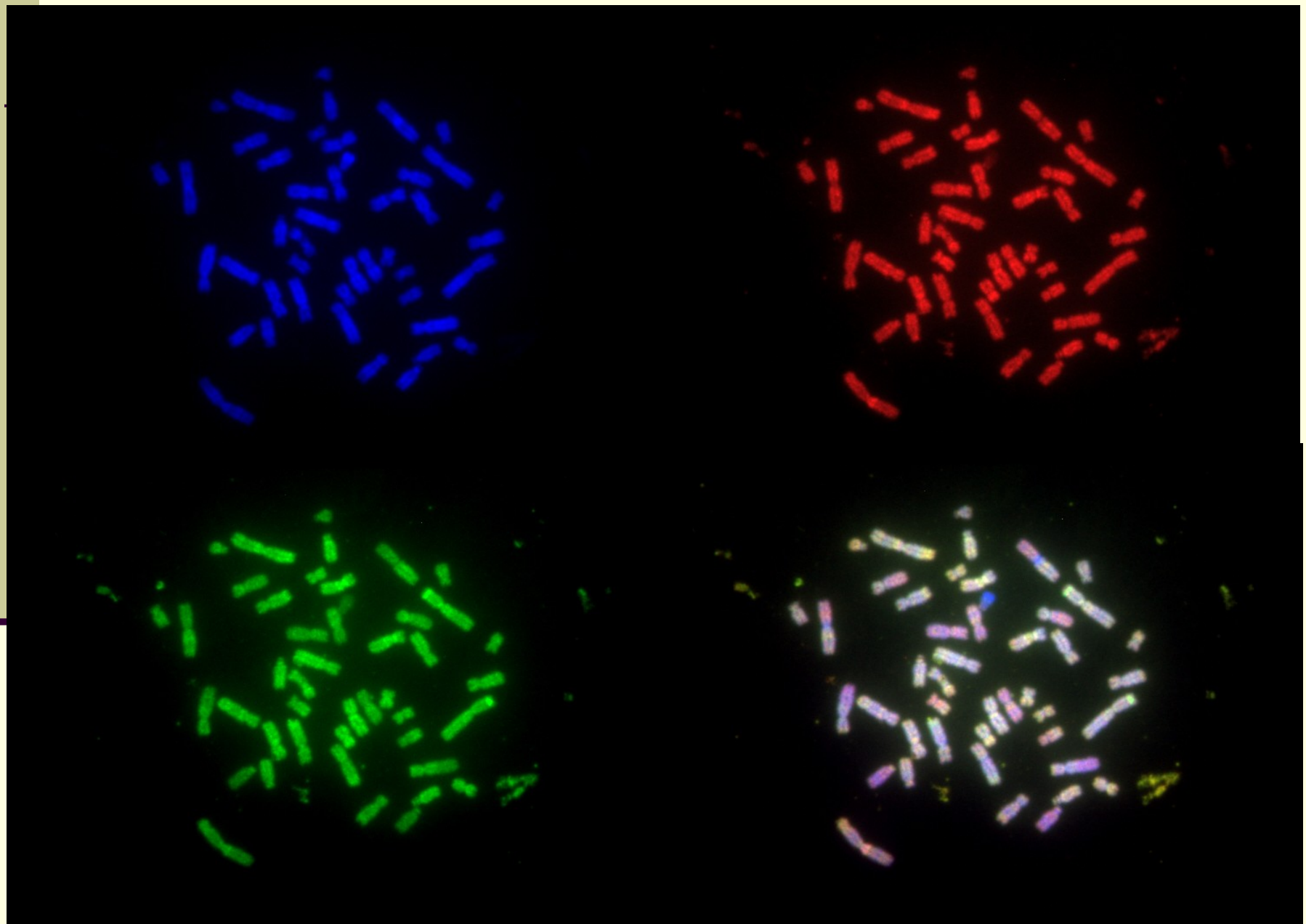
Unique sequences are labeled by *in situ* hybridization

Cot-1 suppresses hybridization of repeat sequences



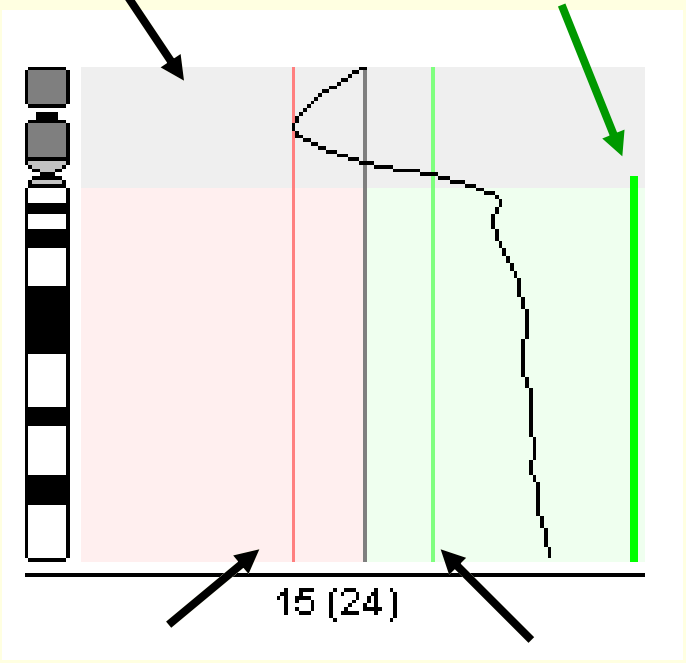
Relative brightness depends on amount of labeled DNA with appropriate complementary sequences, i.e. on the DNA copy number at this locus

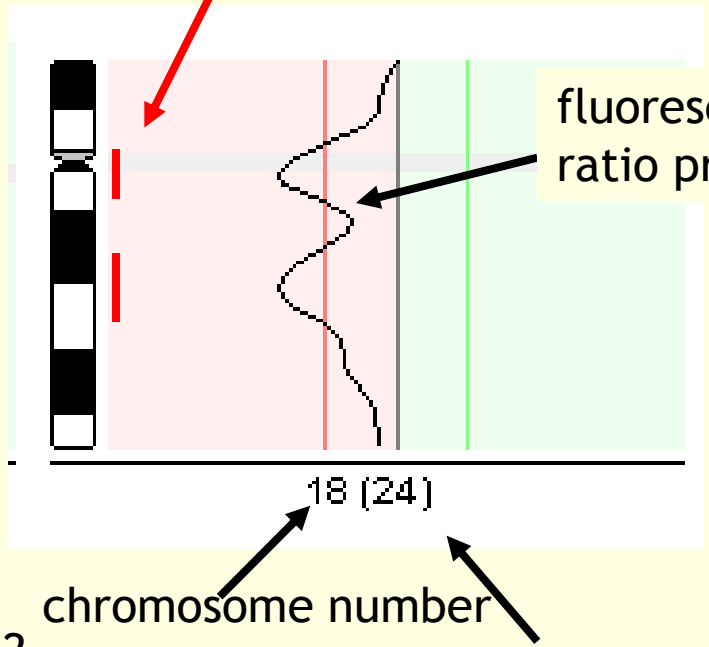
Mitoses scanning, CCD camera filters for B, G, R



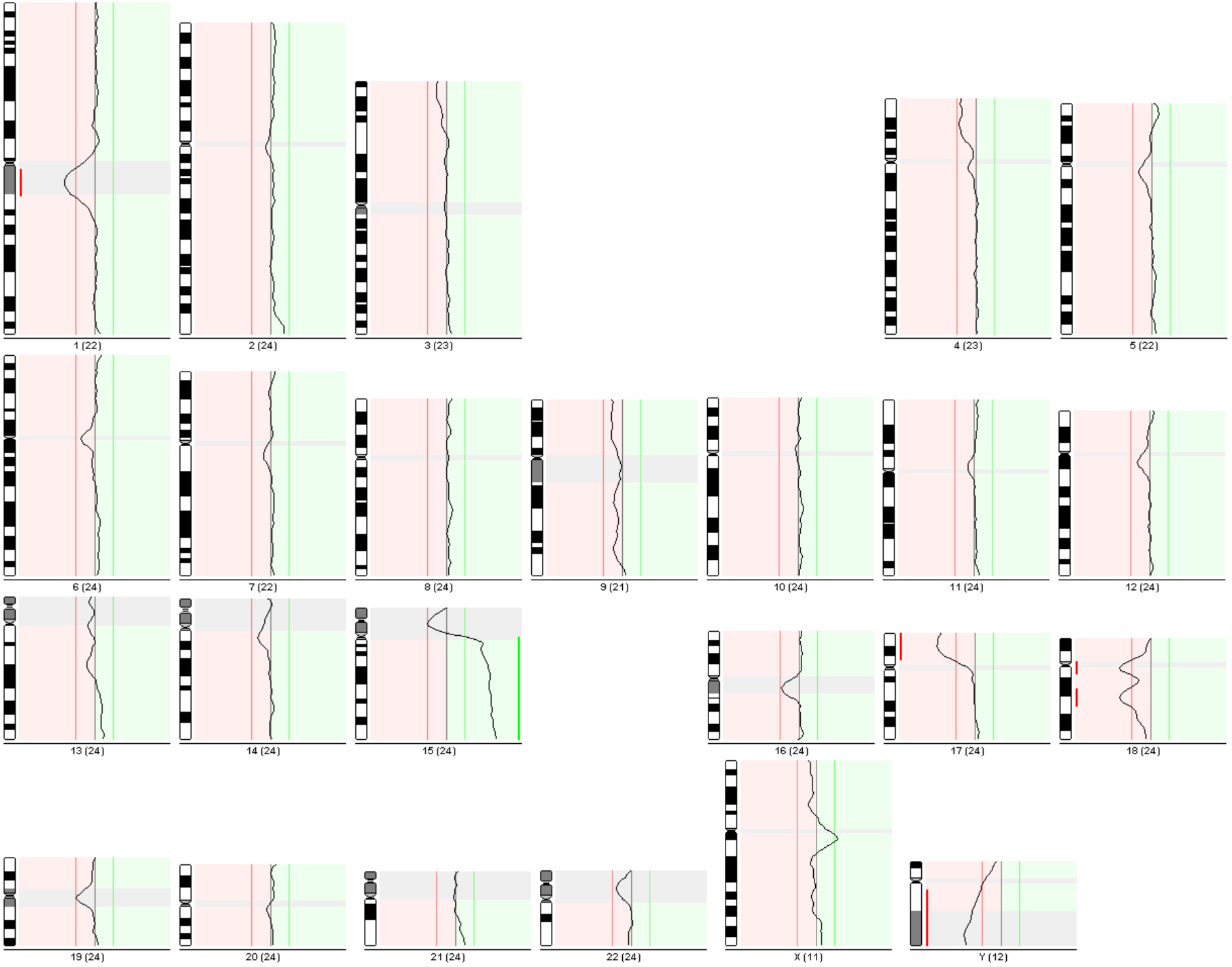
Identification of aberrations

Florescent ratio profile is compared to the fixed tresholds (15-20% from ratio 1). The ratio profile that deviates 15 % - 20 % from ratio 1.0 is typically regarded as aberrant.





Minimaly 10 metaphases should be processed.


1 (22)
2 (24)
3 (23)
4 (23)
5 (22)
6 (24)
7 (22)
8 (24)
9 (21)
10 (24)
11 (24)
12 (24)
13 (24)
14 (24)
15 (24)
16 (24)
17 (24)
18 (24)
19 (24)
20 (24)
21 (24)
22 (24)
X (11)
Y (12)

Advantages of CGH

- detects and **quantifies DNA** copy number gains and losses throughout an **entire genome** in a **single** analysis
- does **not require** cell culturing and **metaphases** from test tissue
- is able to **identify** the **chromosome** from which the additional unknown **material is derived**
- map the **region involved** to specific bands on the **source chromosome**
- in combination with whole-genome PCR, can analyze DNA from a single or very few cells

(*Nacheva et al., 1998*, *Levy and Hirschhorn, 2002*)

Disadvantages of CGH

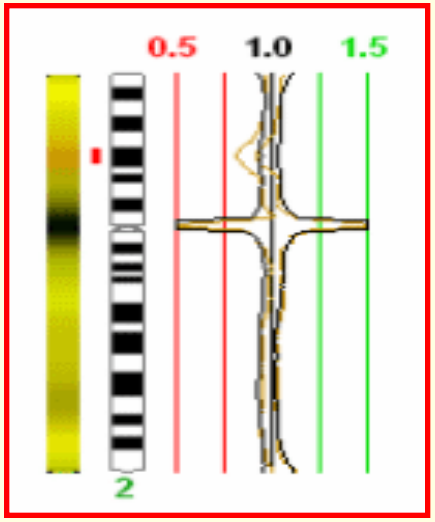
- low genomic sensitivity: about 10 Mbp for single copy changes
  - solution: microarrays
- does not detect balanced rearrangements (inversions, balanced translocations)
  - solution: mFISH
- cannot detect overall ploidy changes, e.g. tetraploid tumor
  - solution: use in conjunction with regular FISH
- requires minimally 50 % aberrant cells for reliable results
  - solution: HR-CGH, microarrrays

Modifications of CGH

High Resolution Comparative Genomic hybridization (HR-CGH)

- Kirchhoff *et al.*, 1997
- the same principles and laboratory processing as CGH
- different data interpretation based on **dynamic standard**

  reference intervals – special software
- **genome resolution is about 4 Mbp**
- abnormal cell detection limit is **about 30 %**

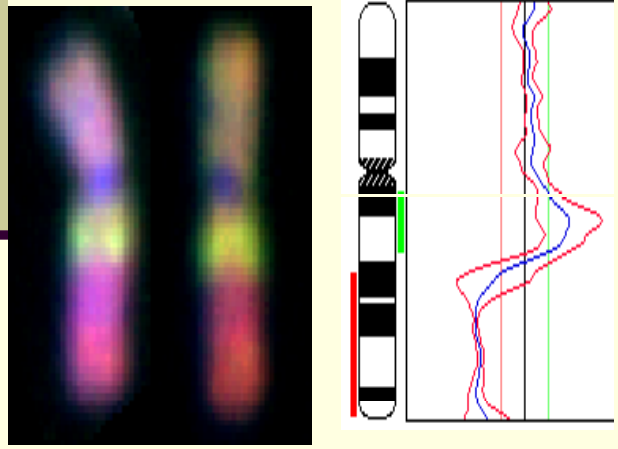


Modifications of CGH

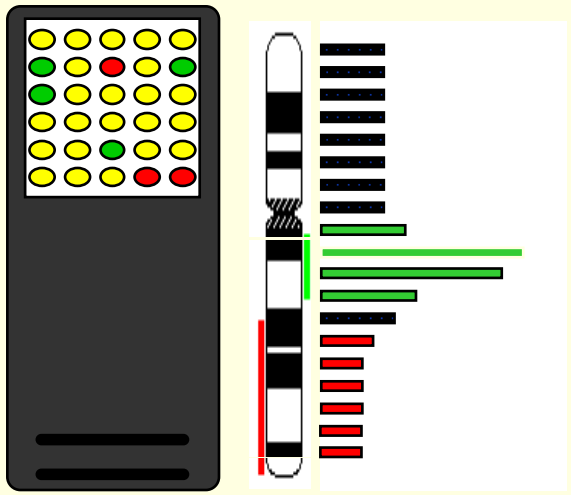
Array-CGH

- Solinas-Toldo *et al*. 1997
- based on principle of CGH
- the chromosomes (CGH) are replaced by separated clones (array-CGH)
- miniaturized array of DNA (genetic material)

CGH

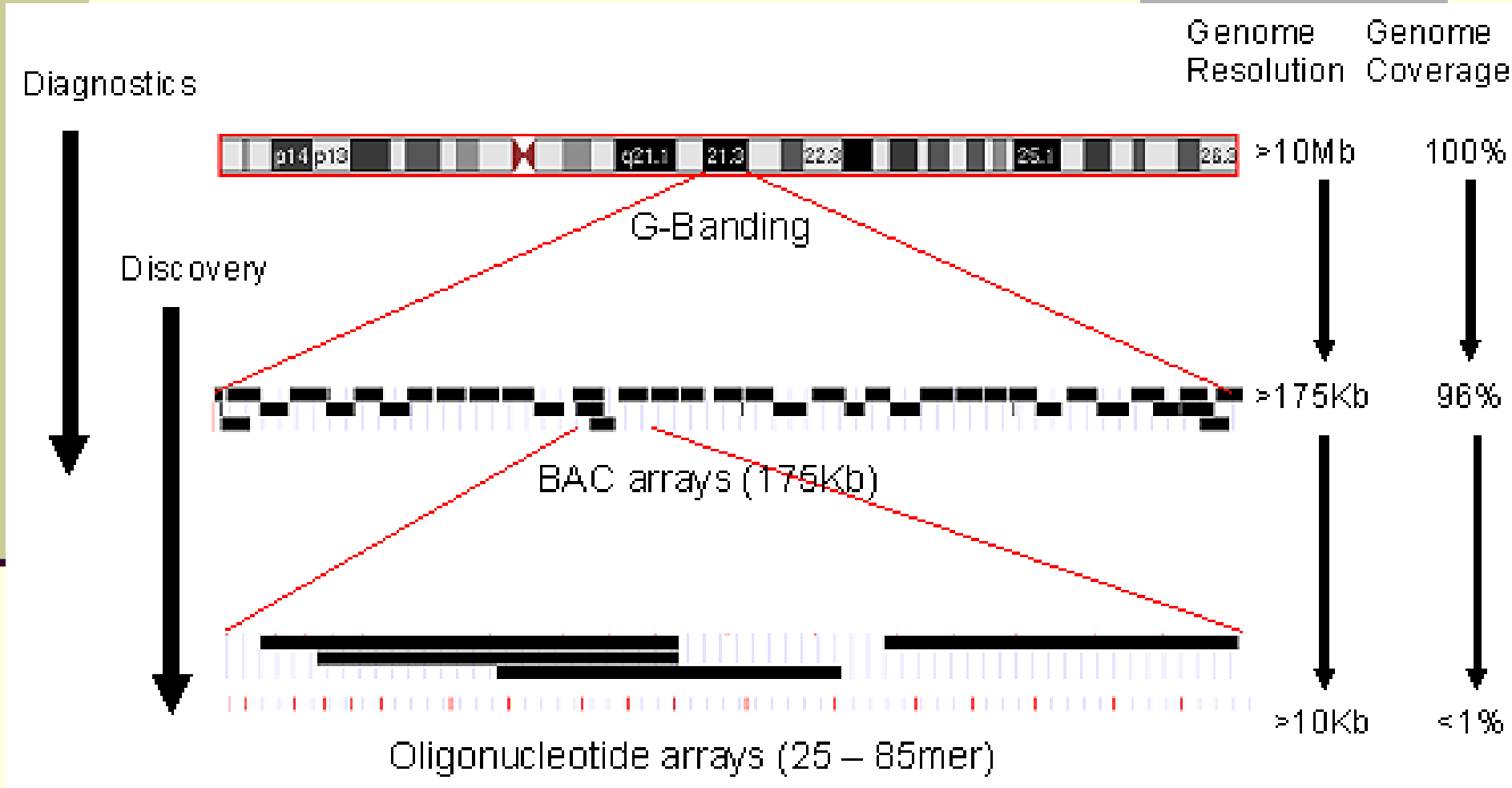


array-CGH

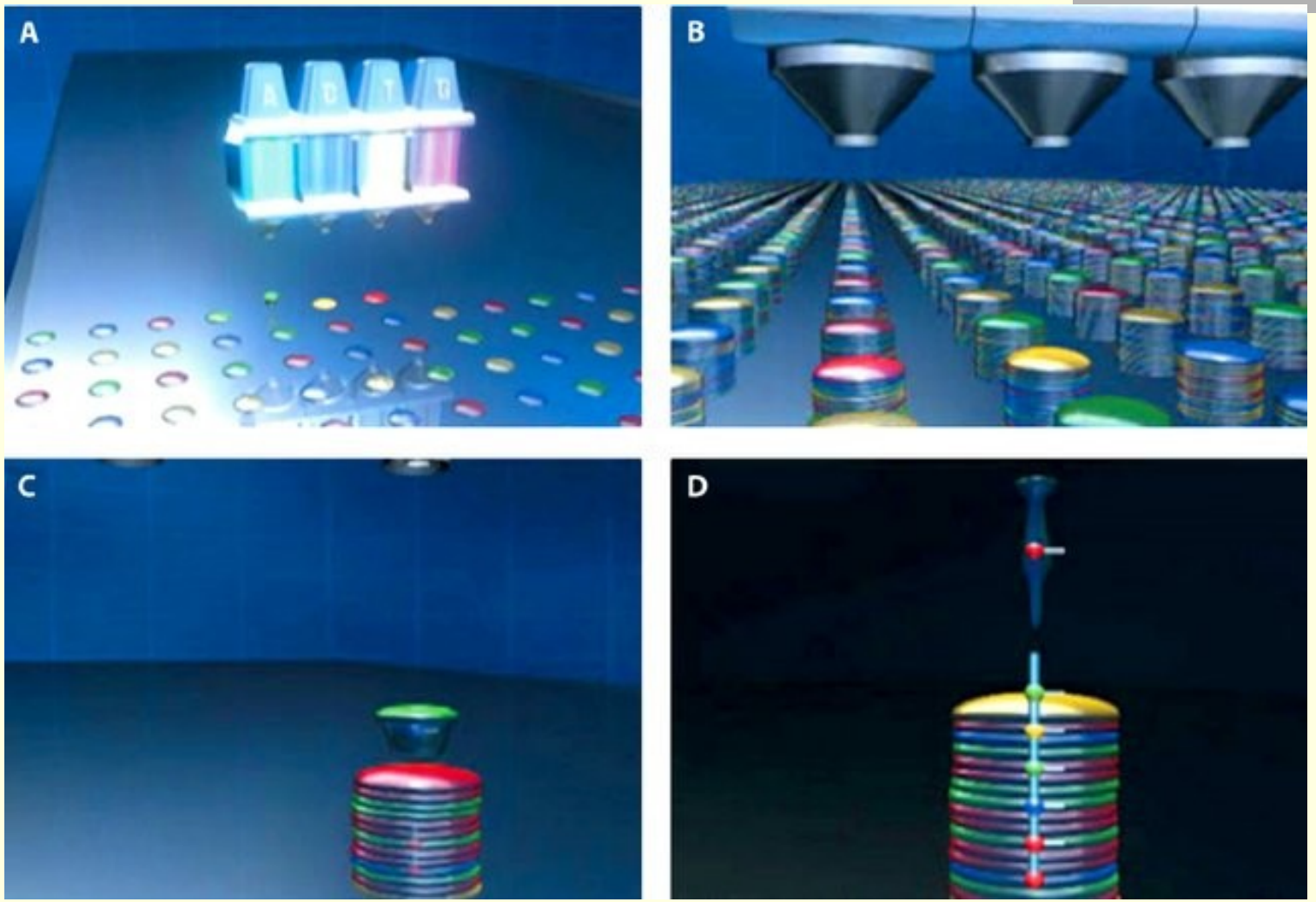


The origin of clones

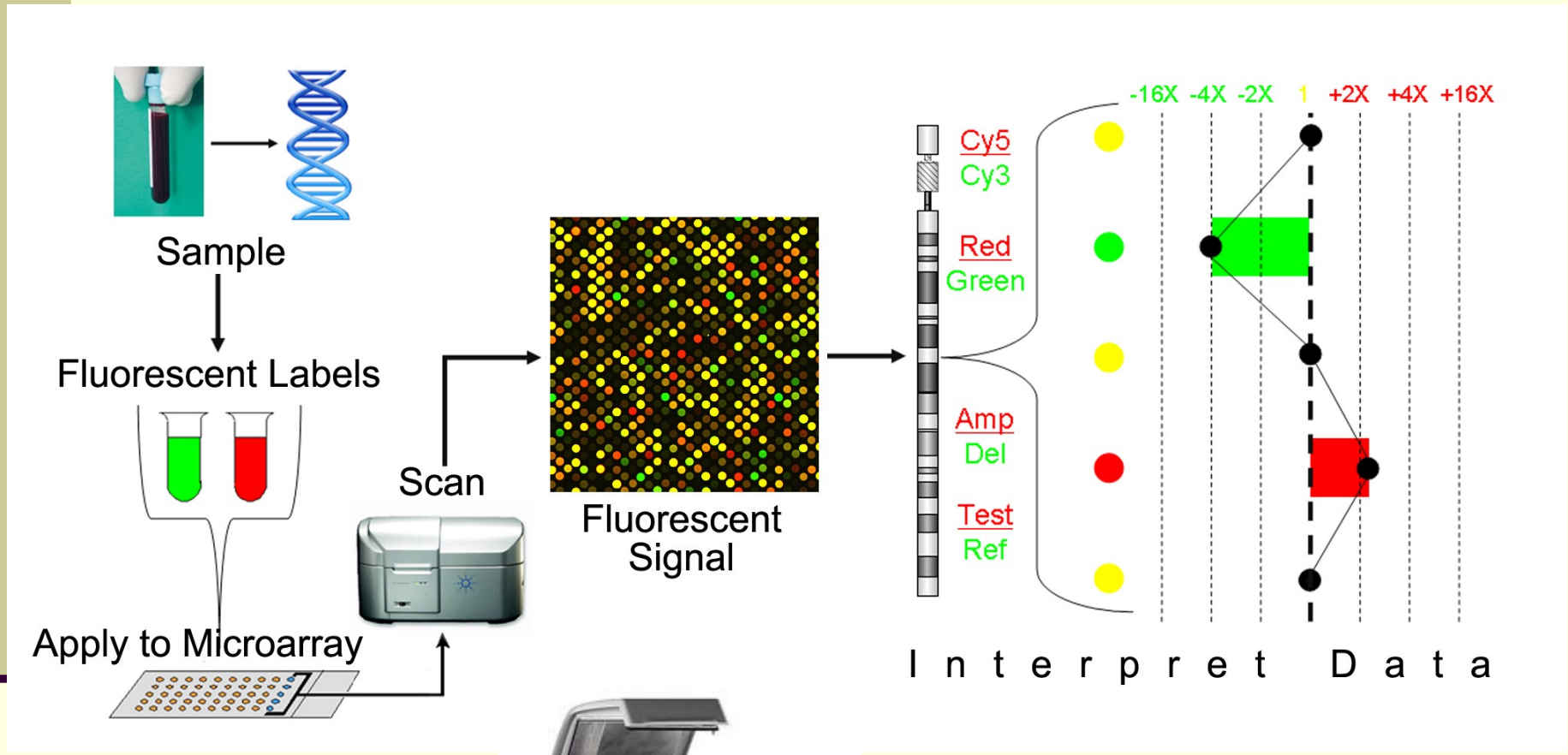
BAC, PAC, c-DNA clones, oligonucleotides

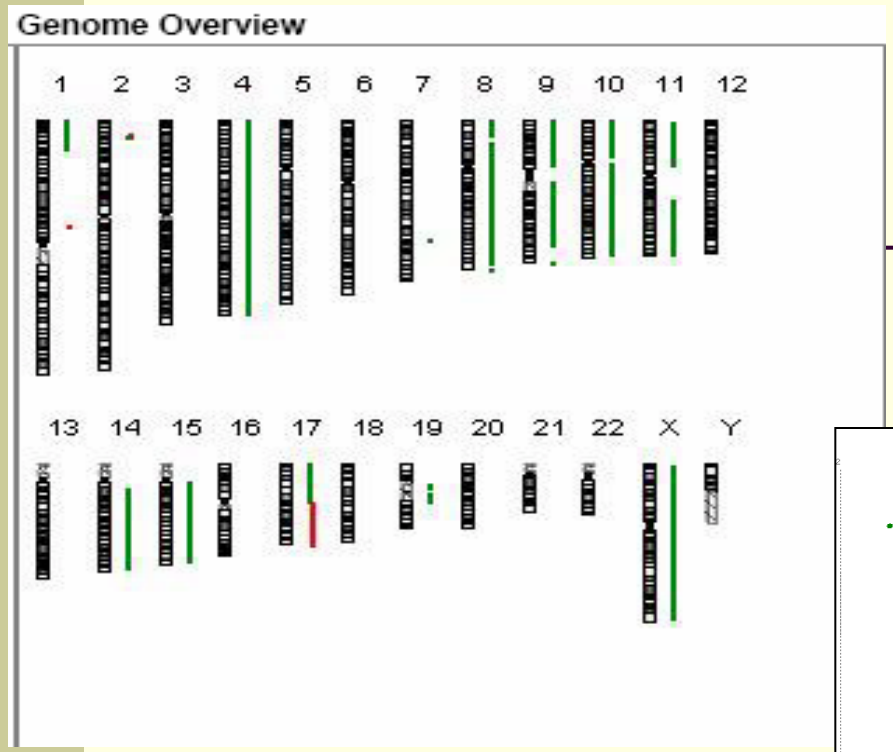


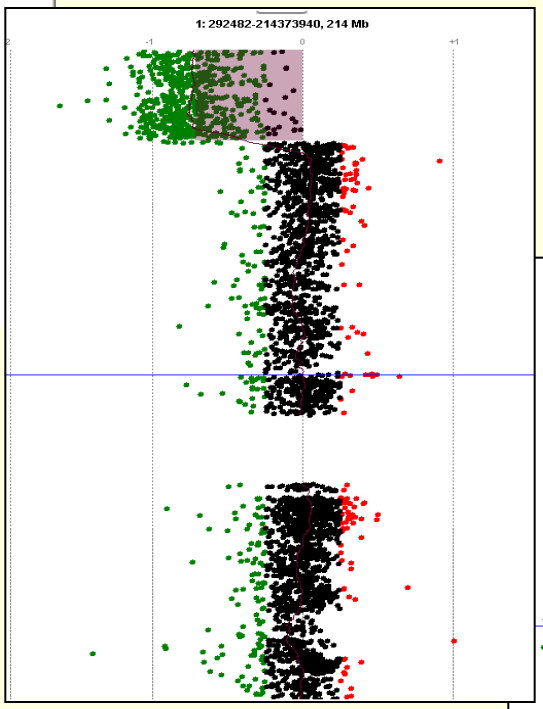
Agilent Sureprint Technology

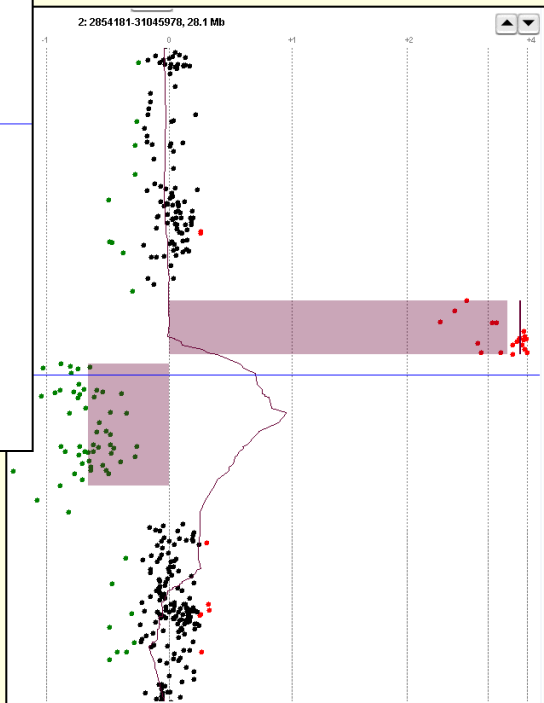


Array-CGH


Sample
Fluorescent Labels
Apply to Microarray
Scan
Fluorescent Signal
Cy5
Cy3
Red
Green
Amp
Del
Test
Ref
-16X -4X -2X 1 +2X +4X +16X
I n t e r p r e t D a t a


Genome 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


1: 292482-214373940, 214 Mb

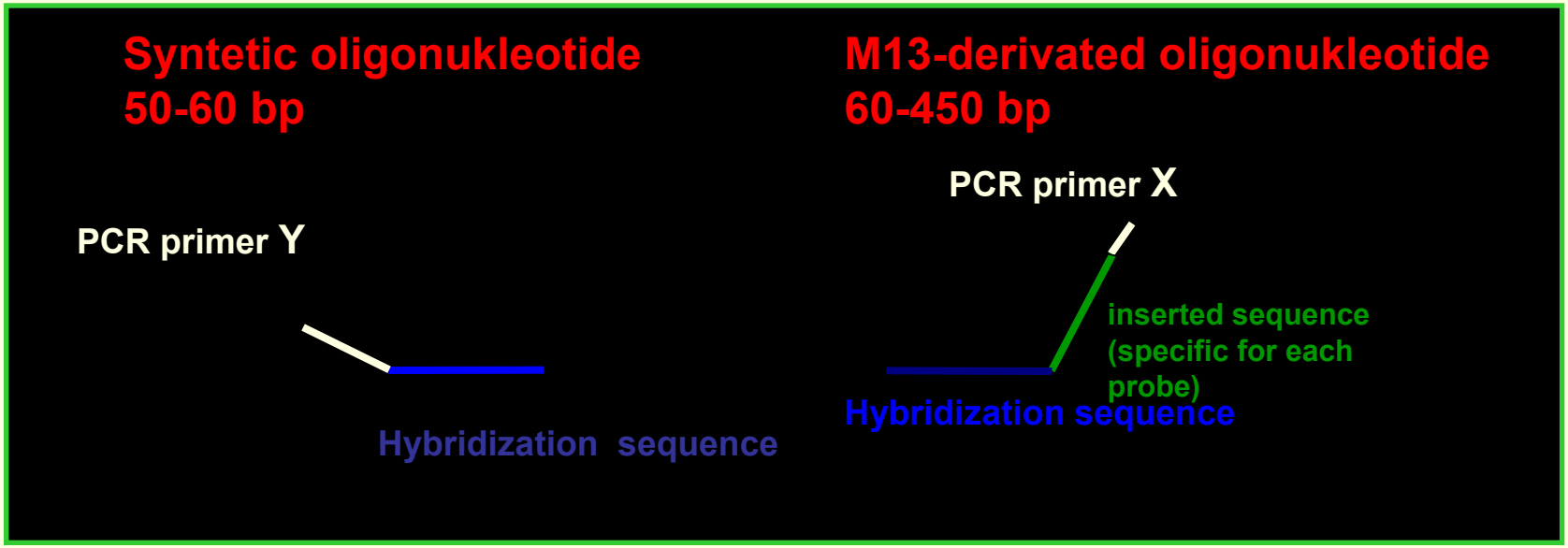

2: 2854181-31045978, 28.1 Mb

Advantages and disadvantages of array-CGH

- **advantages**
  - detects and quantifies **DNA** copy number **gains** and **losses** throughout an **entire genome** in a **single** analysis
  - **precise** aberration locating
- **disadvantages**
  - does not detect **balanced rearrangements** (translocation, inversion)
  - does **not** detect **ploidy changes**

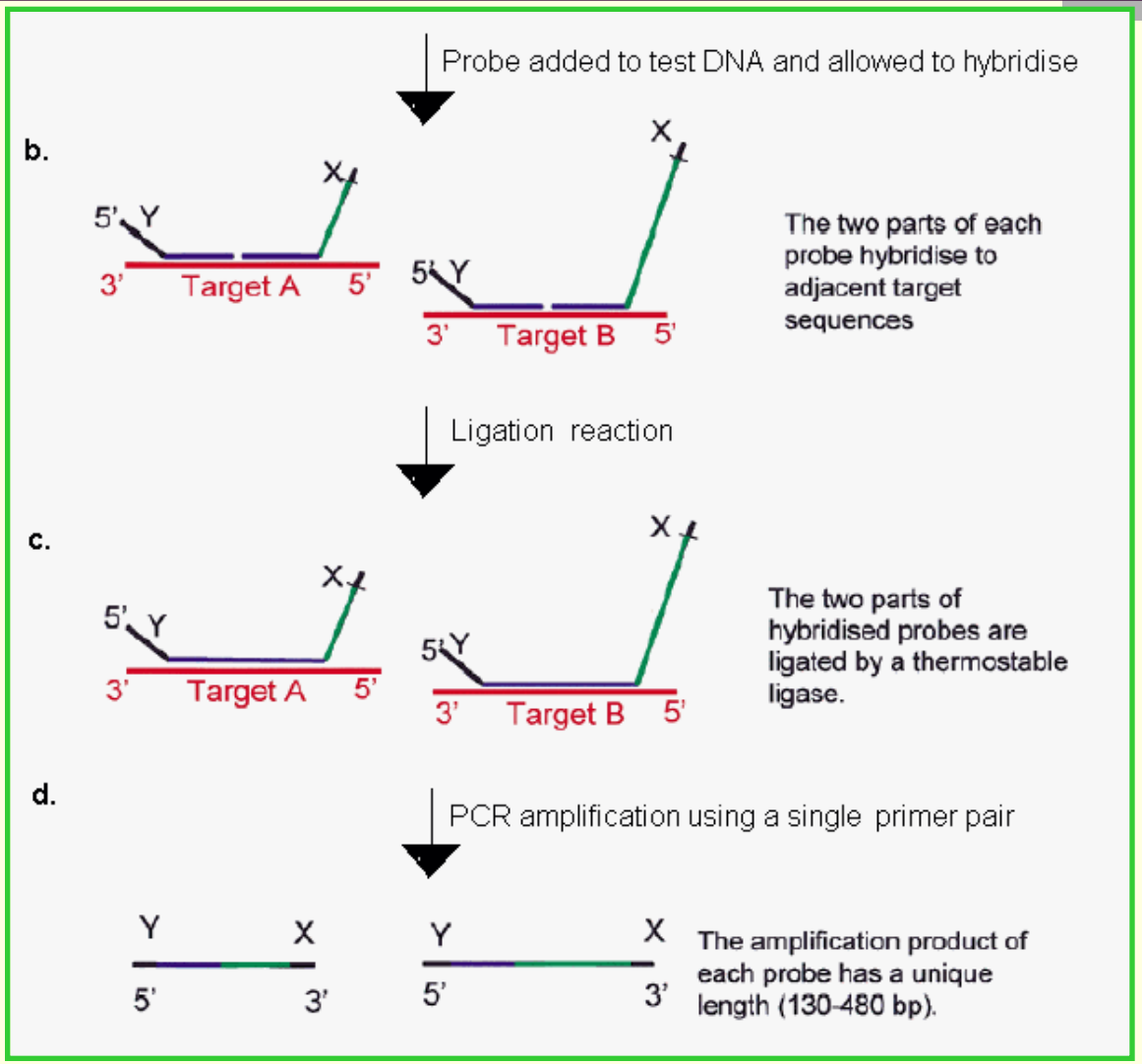
MLPA
Multiplex Ligation-dependend Probe Amplification

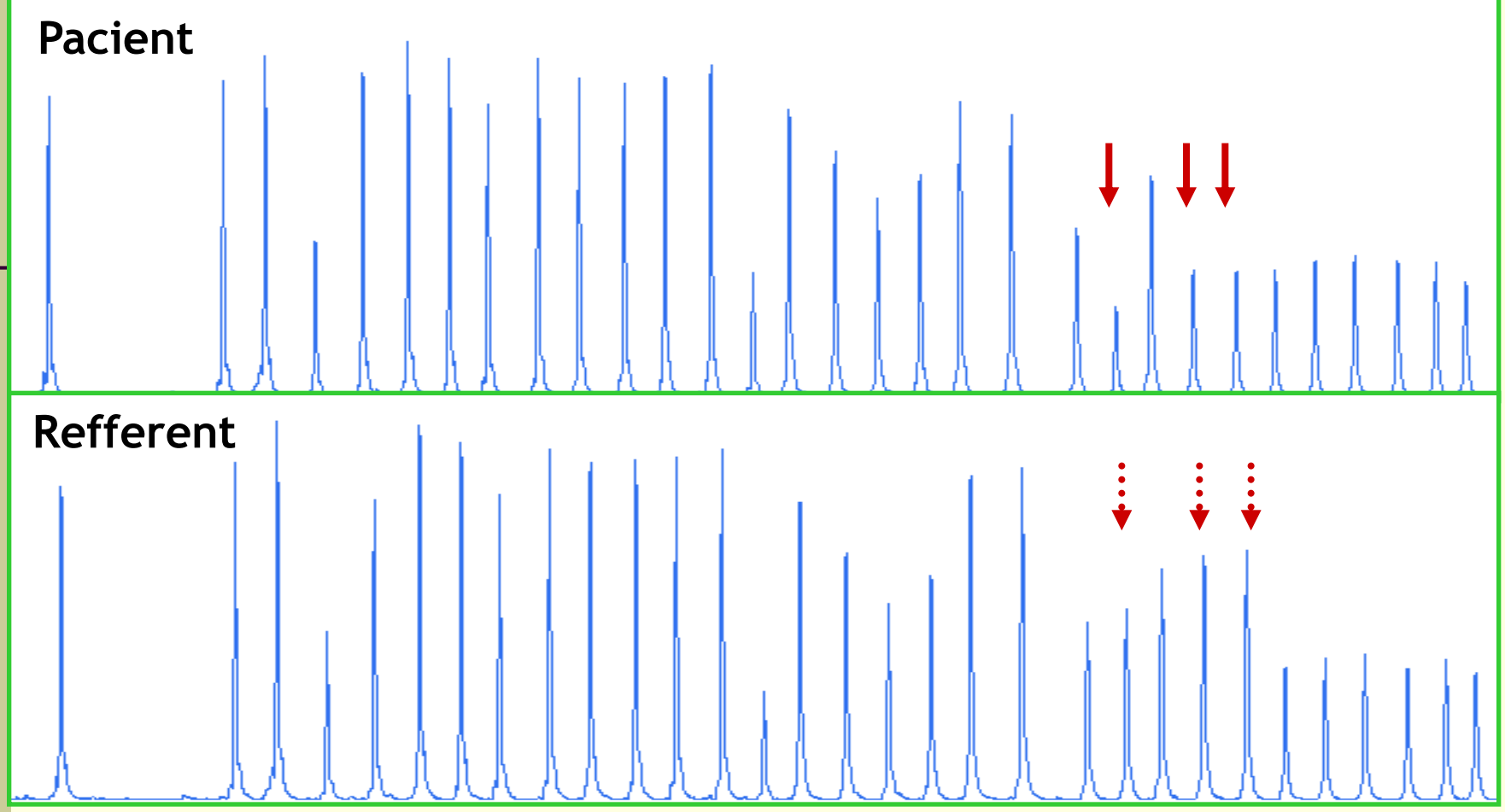
- sensitive method able to detect differences in one nucleotide
- detects changes of copy number in 45 sequences in one reaction
- simple – all the reaction takes place in one test tube
- relatively cheap method

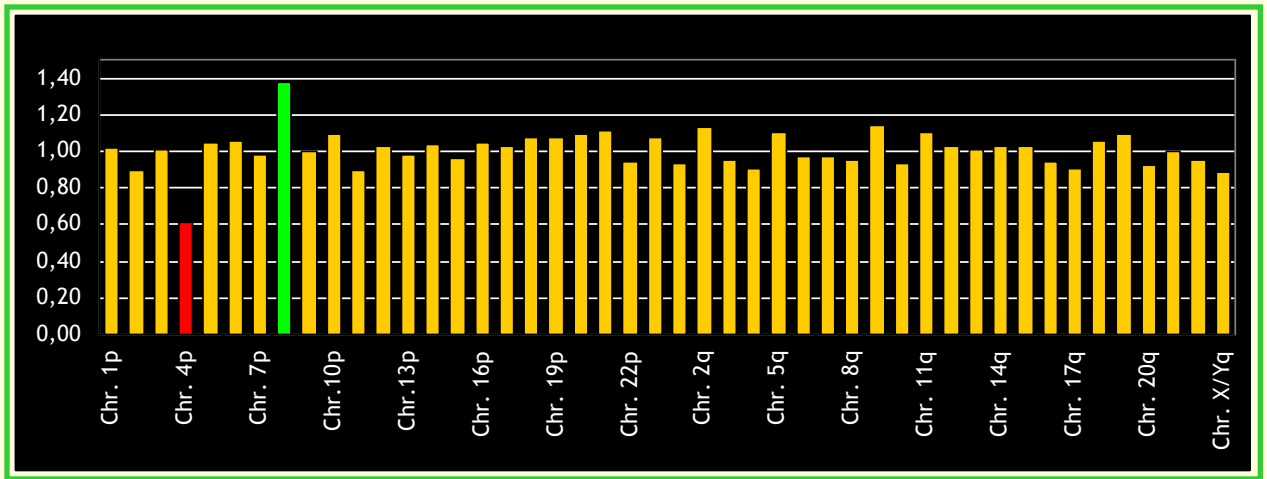


MRC Holland, www.mlpa.com

MLPA principle




Pacient
Refferent


1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
Chr. 1p
Chr. 4p
Chr. 7p
Chr.10p
Chr.13p
Chr. 16p
Chr. 19p
Chr. 22p
Chr. 2q
Chr. 5q
Chr. 8q
Chr. 11q
Chr. 14q
Chr. 17q
Chr. 20q
Chr. X/Yq

Advantages and disadvantages of MLPA

- advantages
  - sensitive
  - specific
  - multiplex
  - simple
  - cheap

- disadvantages
  - higly sensitive to contamination
  - time difficulty
  - the aberation have to occur in 50% of cells
  - some mutations or polymorphismus can lead to false results