Cytogenetics

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What´s the story today?

1. Historical overview of cytogenetics
2. Current techniques and methods used in cytogenetics
3. Utilization of cytogenetics in clinical praxis

What is cytogenetics

- The branch of biology that deals with heredity and the cellular components, particularly chromosomes, associated with heredity.

or more precisely we could say

- Cytogenetics is branch of the genetics, focusing on the study of chromosomes (the number, morphology, numerical and structural abnormalities, segregation in normal and pathological conditions) and the correlation of these findings with the phenotype

How and when has it begun ?

- **J. and Z. Jansen (1590)**

Father and son from Midlenndburg (Netherlands), experiments with lenses, first microscope = tube with lenses on both sides

- **G. Galiley (1564 – 1642)**

Improvements in Jansen´s construction

- **A. van Leevenhook (1632 – 1723)**

„father of microbiology“

He constructed first trully microscope, used for biology

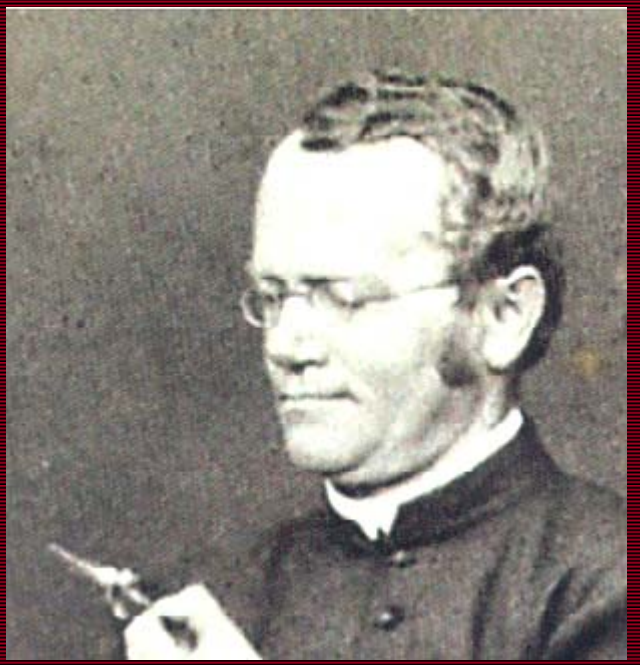
1676 – fisrt observation of bacteria under microscope „*Animalcules*“

19th century

- Two major events in the mid-1800's led directly to the development of modern genetics.

- 1859: **Charles Darwin** publishes *The Origin of Species*, which describes the theory of evolution by natural selection. This theory requires heredity to work.

- 1866: **Johann Gregor Mendel** publishes his *Experiments in Plant Hybridization*, which lays out the basic theory of genetics.

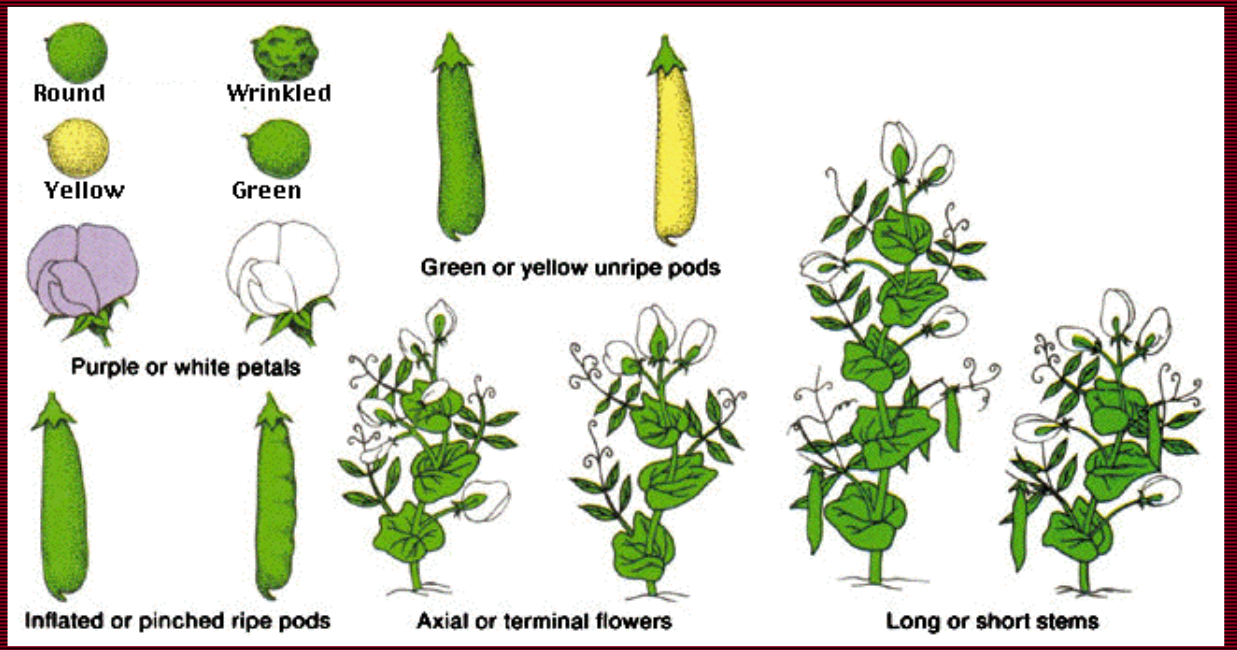


Augustinian monks of St. Thomas monastery in Brno

Gregor Mendel
defined basic principles of the heredity

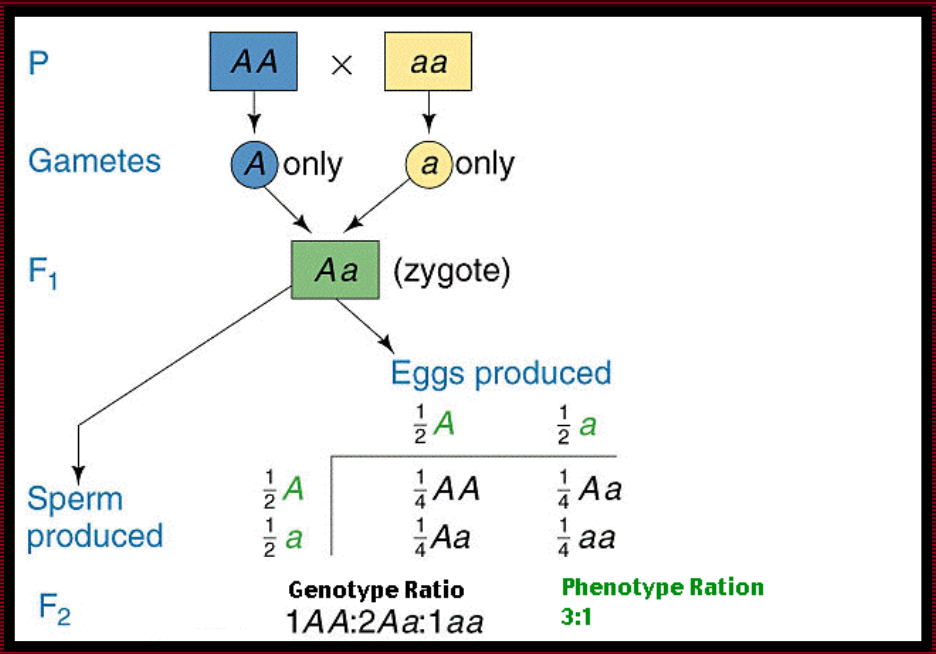
principle of segregation

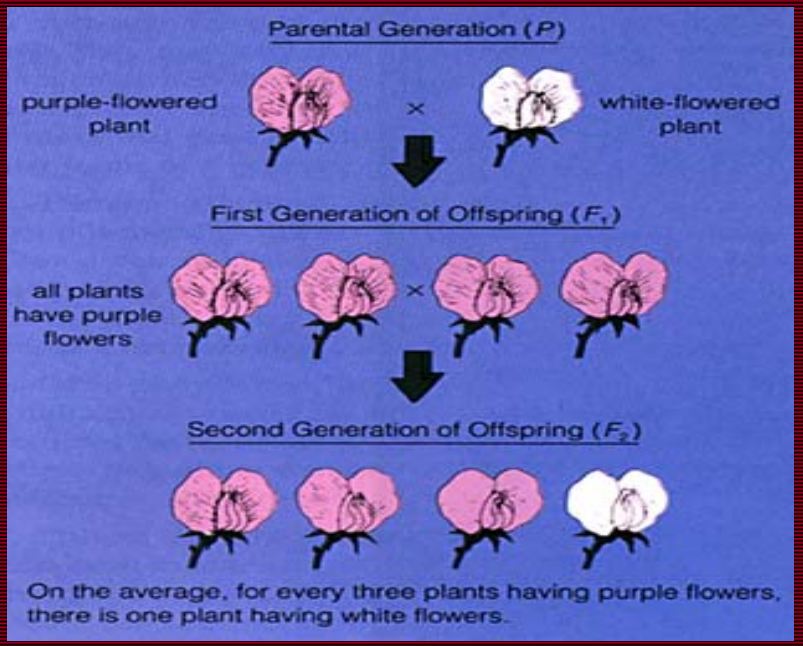
principle of combination



Law of Segregation

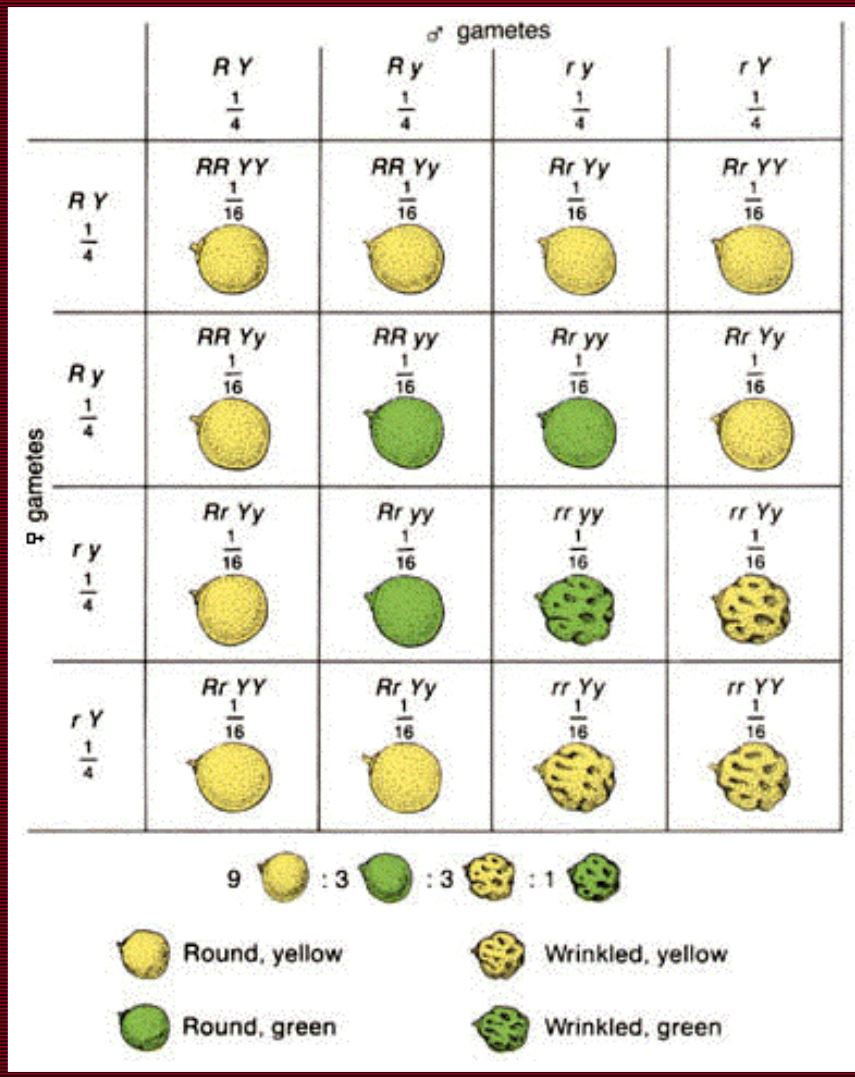
„during gamete formation allele pairs separate or segregate, into different gametes“





Law of Independent Assortment

suggested that each allele pair segregates independently of other gene pairs during gamete formation



20th century I.

- During his live, Mendel´s work was widely ignored
- 1900: rediscovery of Mendel's work by Robert Correns, Hugo de Vries, and Erich von Tschermak .
- 1904: Gregory Bateson discovers linkage between genes. Also coins the word "genetics".
- 1910: Thomas Hunt Morgan proves that genes are located on the chromosomes (using Drosophila).

Thomas Hunt Morgan

(September 25, 1866 – December 4, 1945)

Sex linked inheritance of the white eyed mutation.

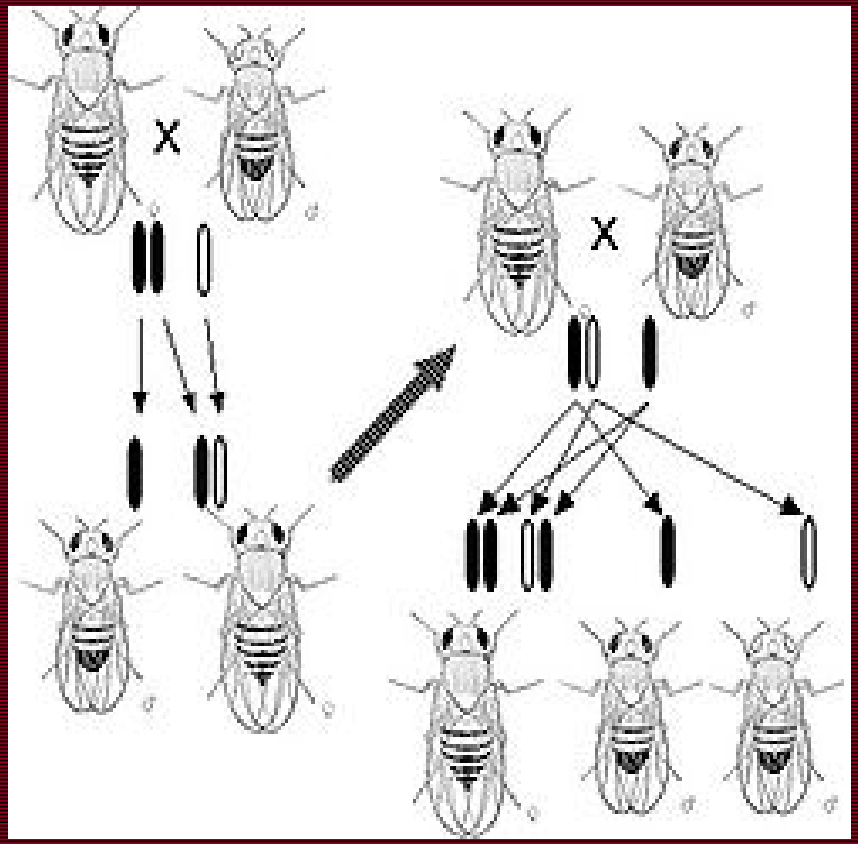


FIGURE 1.30. T.H. Morgan in his Columbia University fly room. Morgan and his coworkers, who included Calvin B. Bridges, Alfred Sturtevant, Theodosius Dobzhansky, and Hermann Muller, made seminal discoveries regarding mutations, linkage groups, and genes and established paired chromosomes as the physical basis of Mendel's laws of heredity.

1.30, courtesy of American Philosophical Society

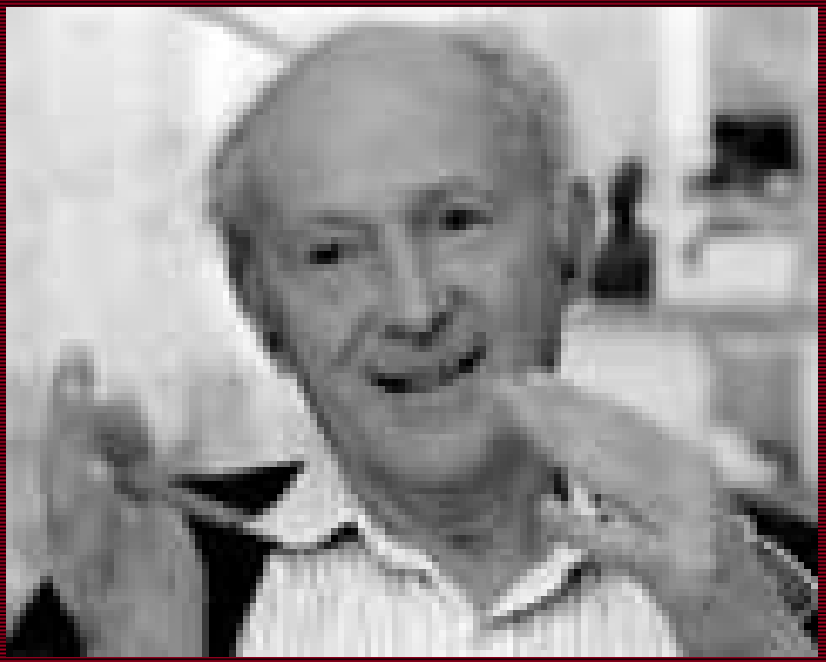
Source: Wikipedia, 2010

20th century II.

- **1926**: *Hermann J. Muller* shows that X-rays induce mutations.
- **1944**: *Oswald Avery, Colin MacLeod and Maclyn McCarty* show that DNA can transform bacteria, demonstrating that DNA is the hereditary material.
- **1953**: *James Watson and Francis Crick* determine the structure of the DNA molecule, which leads directly to knowledge of how it replicates

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

Joe Hin Tjio (1919 - 2001)



Albert Levan (1905 -1998)

Human somatic cells nuclei contain 23 pairs of chromosomes, thus overall 46 chromosome

HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ARNE MUNTZING

BAND 42: 1—2 1956

BERLINGSKA BOKTRYCKERIET, LUND

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^{-9} 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

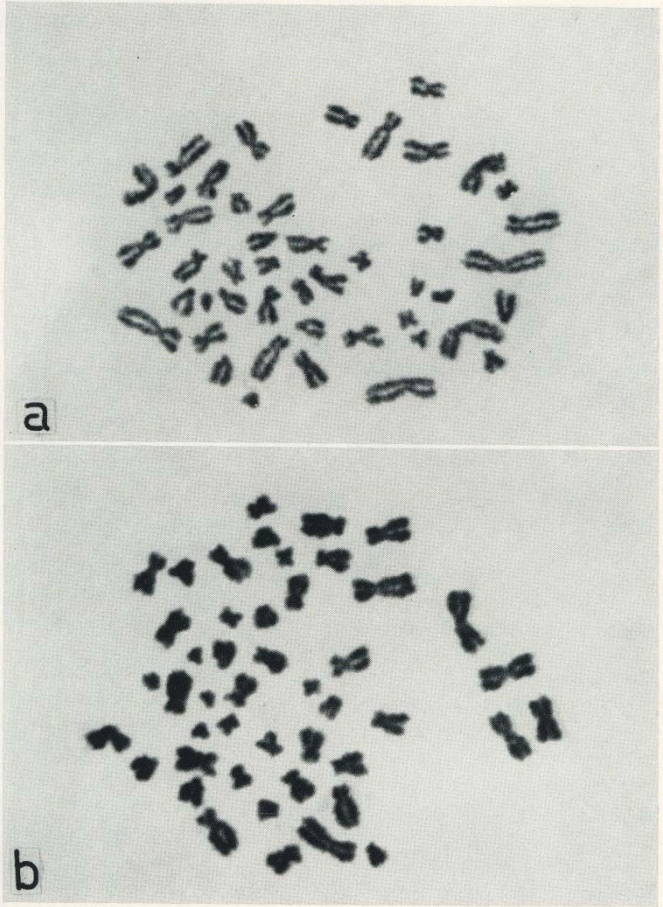


Fig. 1. Colchicine-metaphases of human embryonic lung fibroblasts grown *in vitro*. *a*: early metaphase, *b*: full metaphase. The two cells are from embryos 2 and 3 (Table 1), respectively. — ×2300.

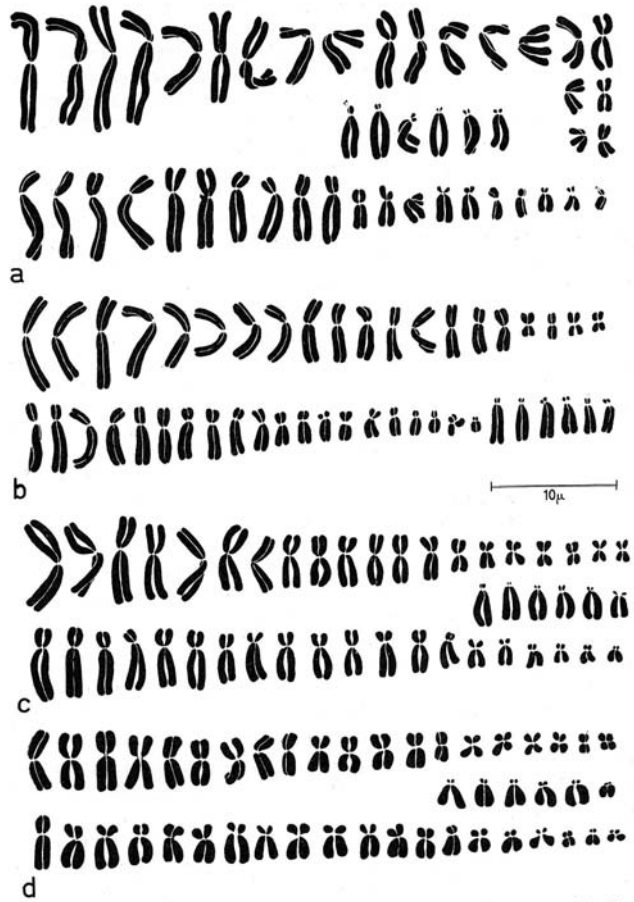


Fig. 2. Four idiogram analyses of human embryonic lung fibroblasts grown *in vitro*. The chromosomes have been grouped in three classes: M (top row), S (bottom row), and T (in between, except in *b*, where T is at the end of the S row). Within each class the chromosomes have been roughly arranged in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

Basic conditions for development of human cytogenetics

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

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

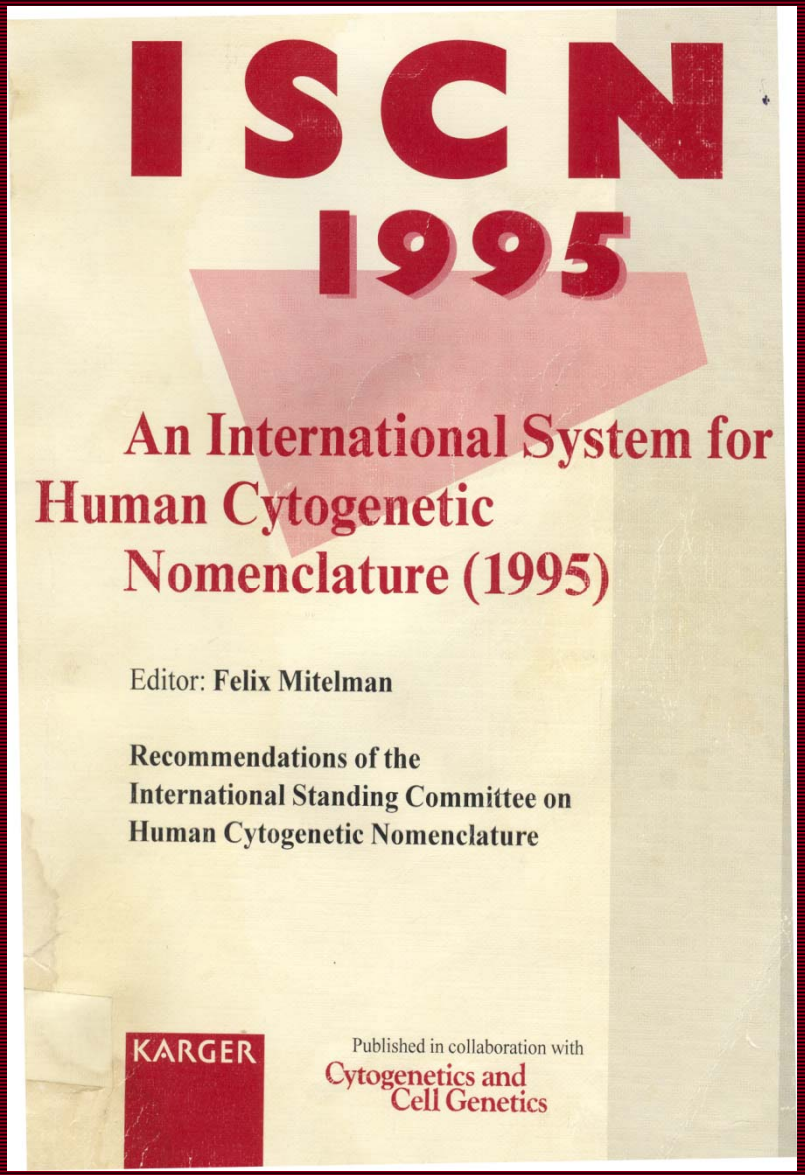
- "**Banding Area** - chromosome banding techniques 1968 – 1970

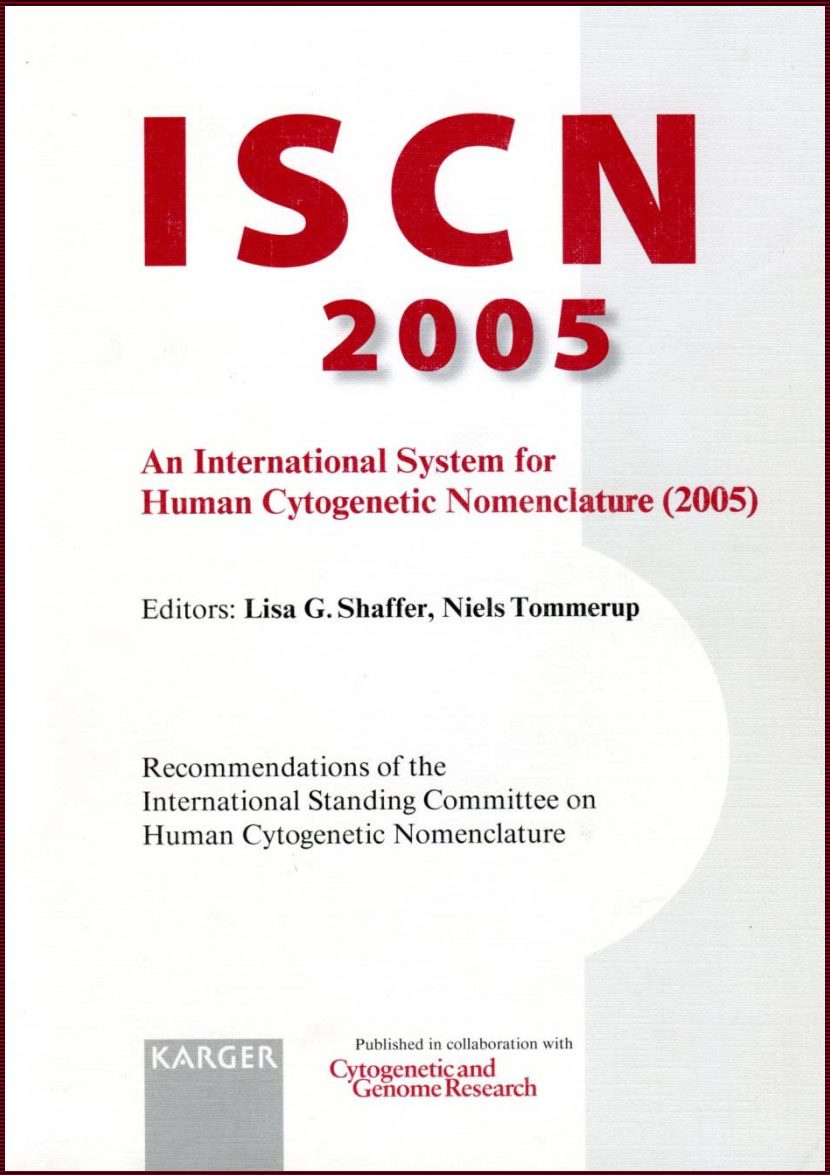
- **"Molecular Area"** –
  - *in situ* hybridization technique – 1970
  - FISH – 1986
  - Comparative genomic hybridization (CGH) - 1992
    - Spectral karyotyping (M-FISH, SKY) - 1996
    - M - banding - 2001
    - Array - CGH - molecular karyotyping

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, sign 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)





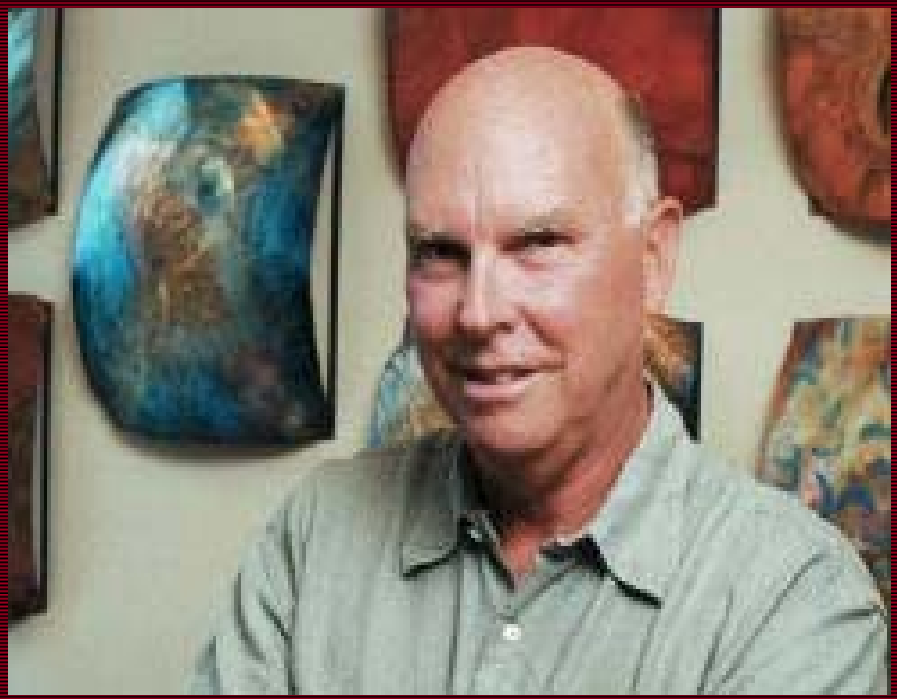
Examples of cytogenetic findings in patients enrolled under the rules of ISCN

- 46,XX or 46,XY; healty female or male
- 47,XX,+21 Down s.
- 47,XY,+18 Edwards s.
- 47,XY,+13 Patau s.
- 46,XY,del(13q)
- 45,XY,-13
- 45,X,-Y
- 46,XY,t(2;5)(q21;q31)
- 44,X,-X,der(1),del(4p),-11,der(12),-16,-22,+2mar
- 44,XX,+3,del(6q),der(8),del(10q),-13,-16
- 46,XY,-3,der(12)t(3;12)
- 47,XY,der(1),der(4),+9,-13,+20
- 46,XY,del(5q)
- 49,XY,+7,+10,+17
- 44,XY,-1,-10,der(11),-13,del(14q),-22,+2mar
- 44,XY,der(1),der(5),der(6),der(9),del(13q),der(15),-22,-22
- 62,XY,+2,+3,+5,+5,+6,+7,+7,+9,+10,+11,+14,+16,+17,+20,+20,+3mar

Human Genome Project & cytogenetics

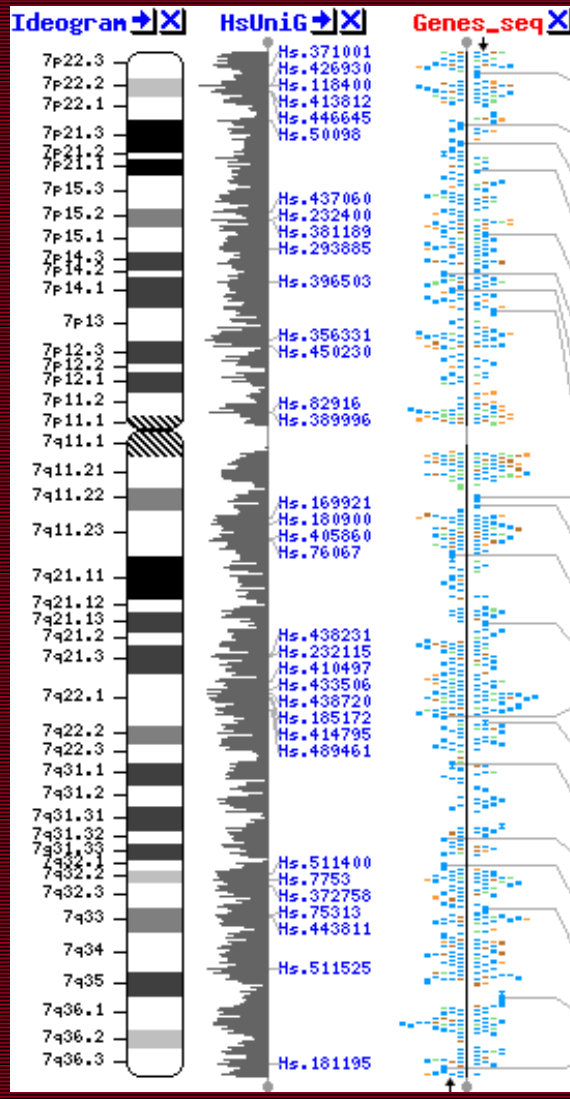
Francis Collins
Head of the Human Genome Project

Craig Venter
Head of Celera Genomics

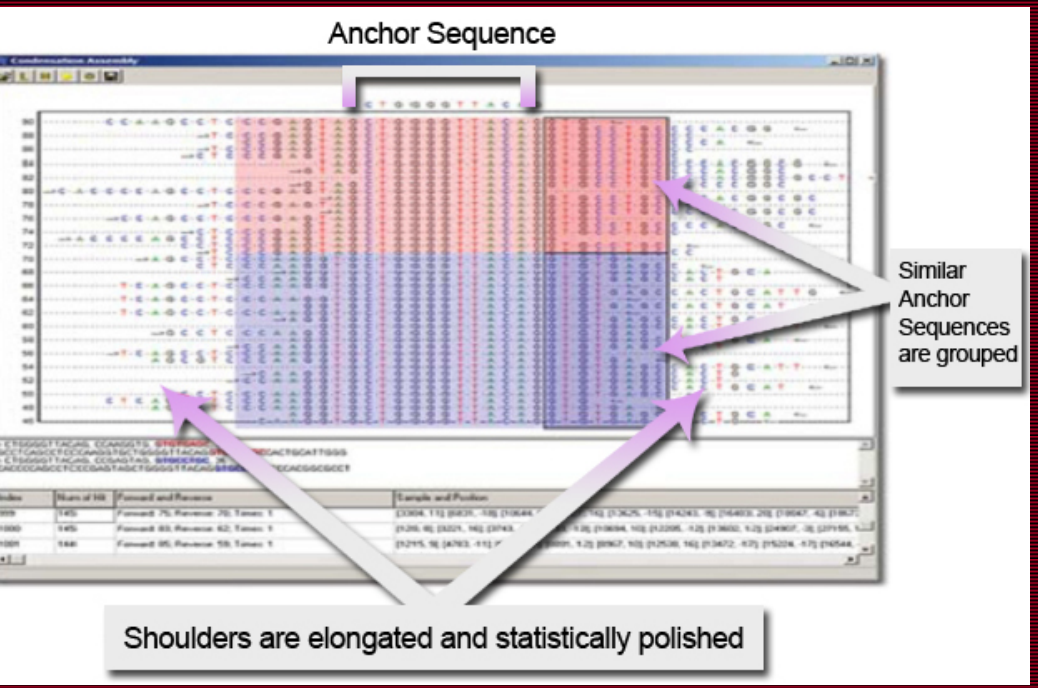


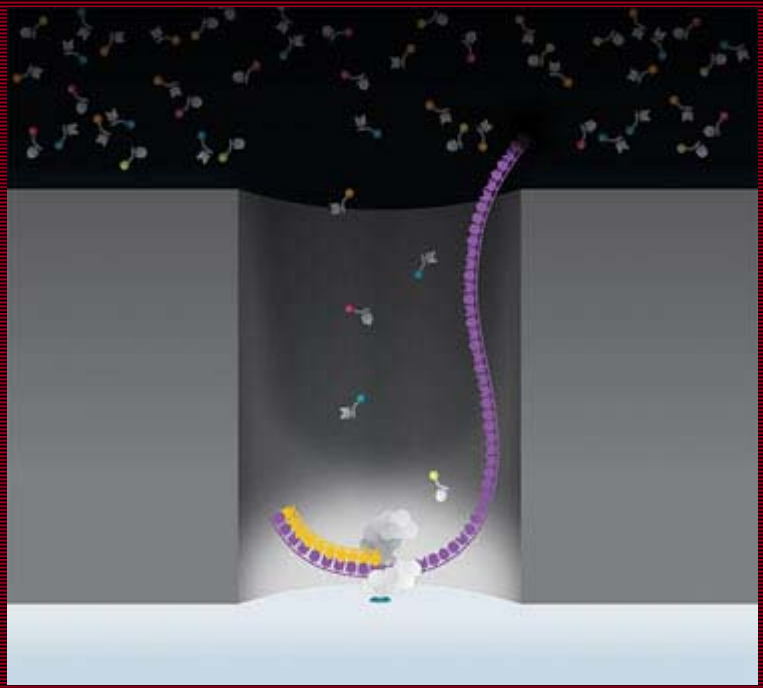
Human Genome Project & cytogenetics

- **Chromosomome 22** - first “decoded” chromosome (1999) contains 450 genes
- **Chromosomes 19 a 22** - the biggest density of genes per Mb
- **Chromosomes 13 a Y** the lowest number of genes / Mb

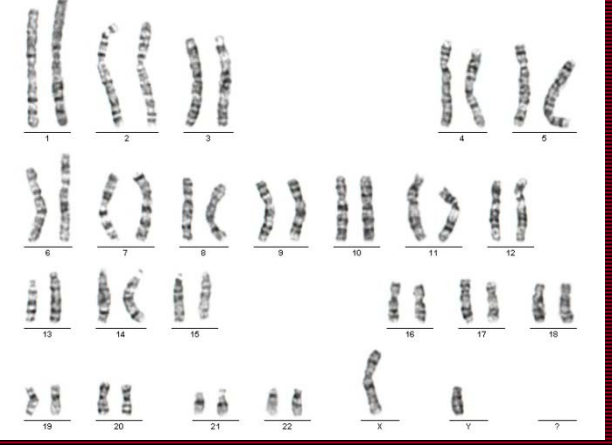


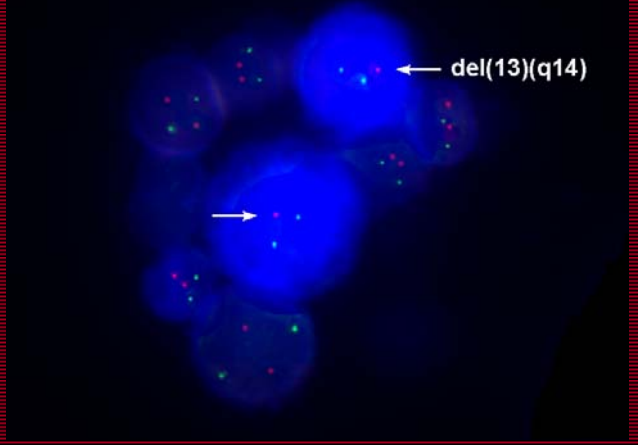
Nowadays and future

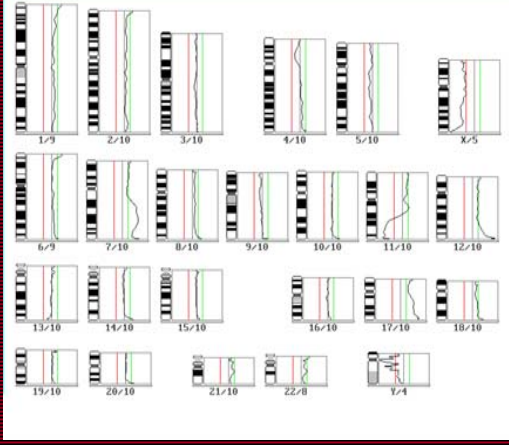




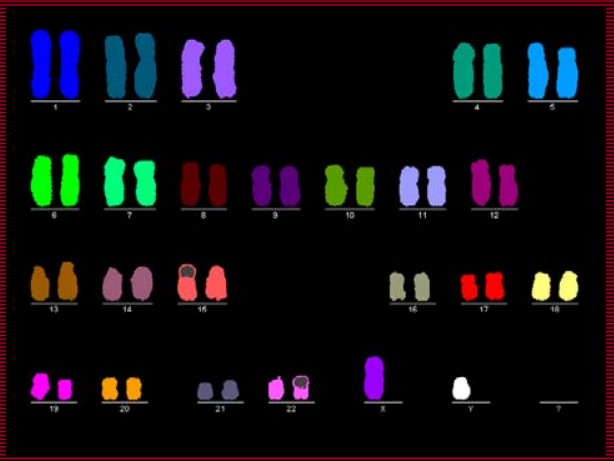
- Next (second) and third (single molecule) generation sequencing
- SOLiD (ABI), SOLEXA (Illumina), 454 pyrosequencing (Life Science)
- Key challenge = sequencing whole human genome during 24 hours for less than $1000

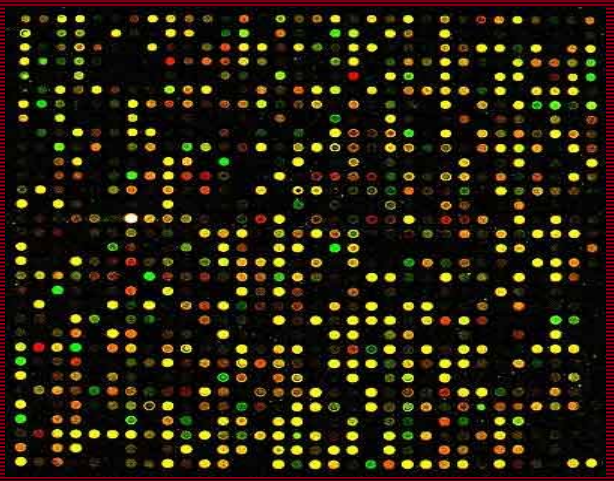






Techniques and methods used for cytogenetic analyses in OLG Brno





Chromosome morphology

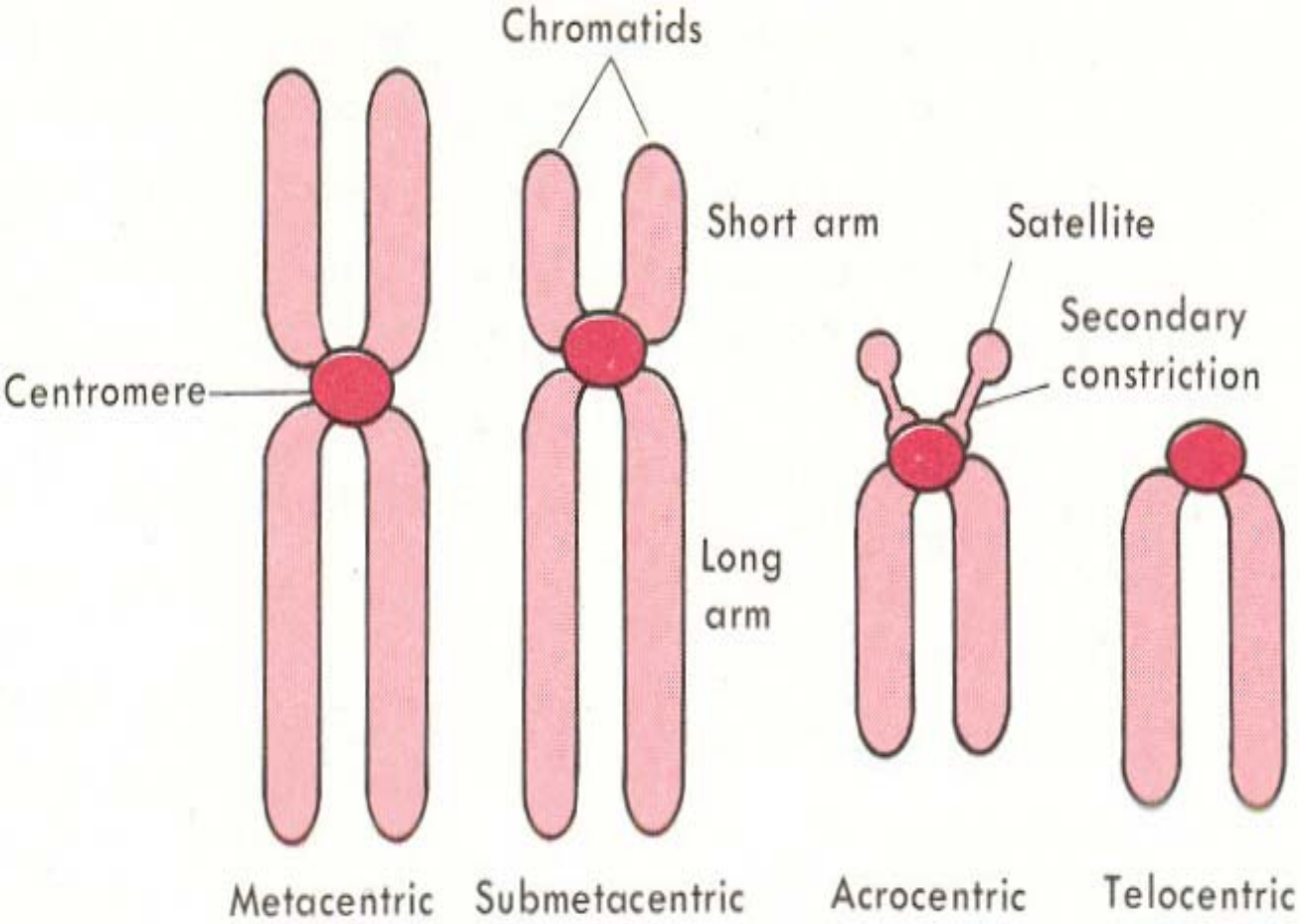
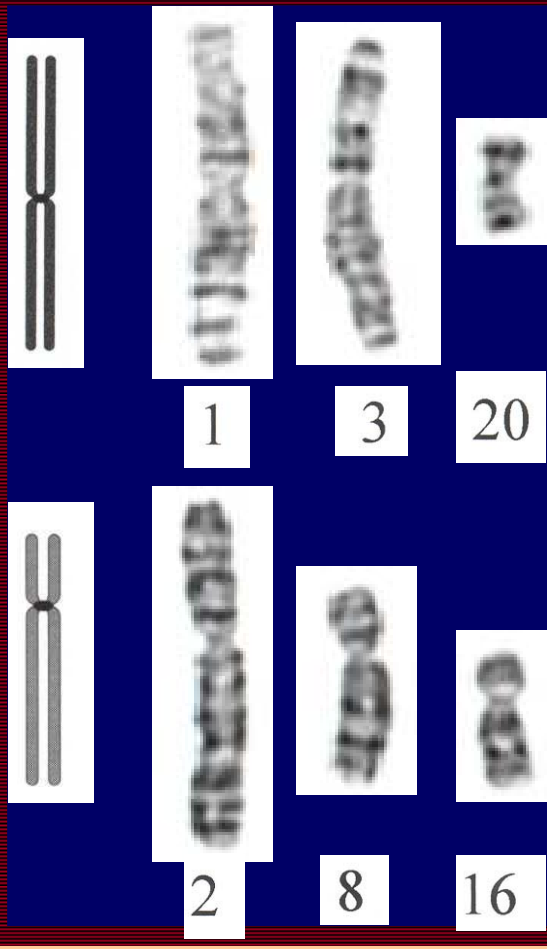


Figure 15–14 Chromosomes are classified according to the position of the centromere

Chromosome classification according to position of the centromere

- Metacentric chromosomes
  centromere entirely or almost entirely in the middle, short and long arms are (almost) the same length

- Submetacentric chromosomes
  centromere of chromosome off-center, p q arms are clearly distinguished by length



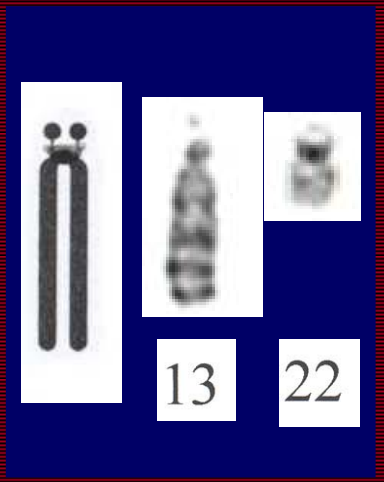
Chromosome classification according to position of the centromere

Acrocentric chromosomes

- centromere is located very close to one end from the short arms are constricted so called *satellites*

- constricted position = secondary constriction

- secondary constriction contains copies of genes coding for rRNA nucleolar organizer =NOR



ISCN classification according to size and shape of chromosomes

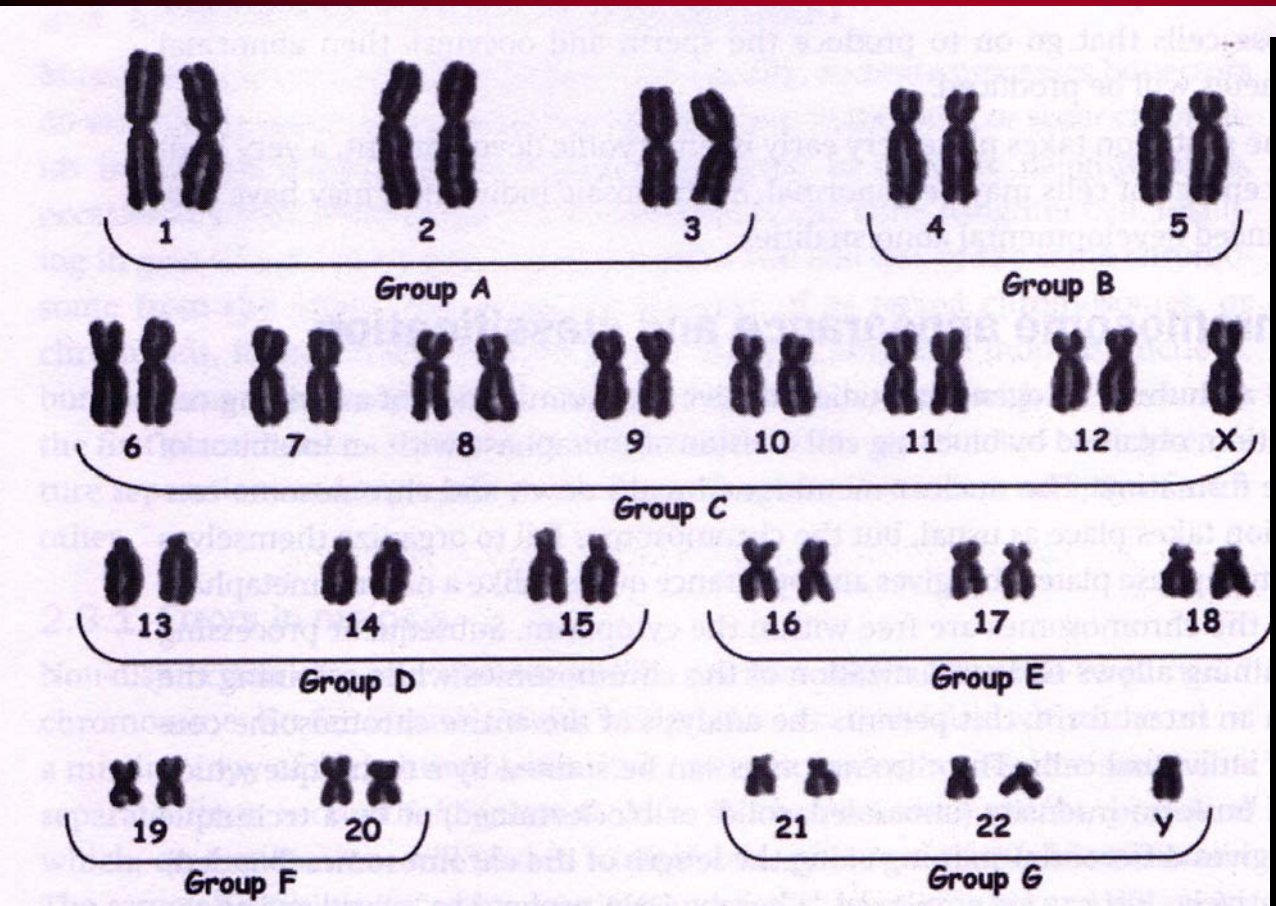
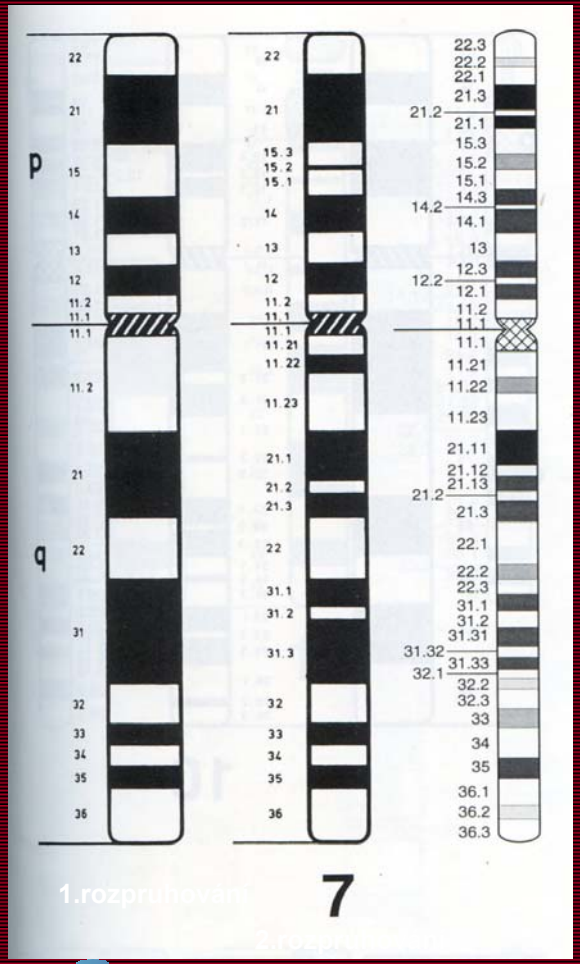


Figure 3 A solid-stained male karyotype showing classification of chromosomes into groups.

Group 1-3 (A)	Large chromosomes with approximately median centromeres; 1,2, and 3 can usually be identified morphologically.
Group 4-5 (B)	Large submetacentric chromosomes.
Group X, 6-12(c)	Medium sized submetacentric chromosomes.
Group13-15(D)	Large acrocentric chromosomes.
Group 16-18(E)	No.16 is metacentric; No.17-18 are small sub-meta-centric chromosomes.
Group 19-20(F)	Small metacentric chromosomes.
Group 21-22 + Y(G)	Short acrocentric chromosomes. (The Y chromosome belongs to this group, but has no satellites; it is of variable size and can usually be identified morphongenically.

Band numbering of chromosomes



Band numbering of chromosomes

Bands on each arm are numbered in ascending order from centromere to telomere

with progressive chromosome condensation reduces the number of bands

Number of band allows its unique identification

Source 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

Source of material for cytogenetic investigation

Peripheral blood

Solid tumor

Bone marrow

Sample preparation

According to type of investigation we could

1.Cultivate cells – obtaining mitosis

2.Sort just specific types of cells – MACS, FACS

3.DNA or RNA extraction

Sample preparation

Cultivation of amniotic fluid cells

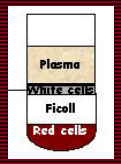
Sample preparation

Sorting of sample cells

MACS – magnetic activated cells sorting

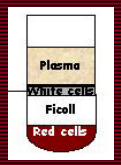
FACS – fluorescent activated cell sorting

bone marrow



gradient centrifugation

magnetic separation



gradient centrifugation

FACS Aria

erythrolysis

FACS Aria

Isolation of nucleic acids

- Phenol – chloroform extraction

- Isolation using commercial kits

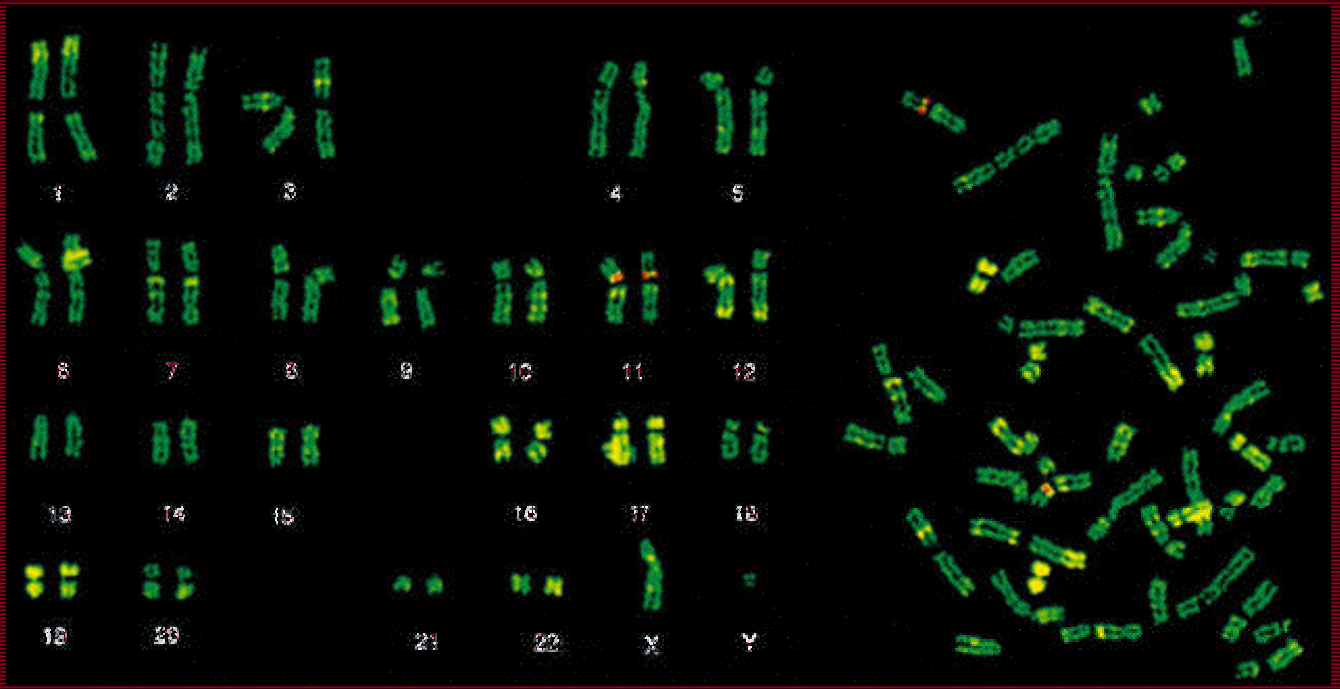
Methods for identification of chromosomal changes used in cytogenetics

A)Conventional staining (acetoorcein, Giemsa)

B)Banding technology (processing chromosomes + Giemsa or fluorochromes ....)

C)molecular cytogenetics using DNA probes

G-banding



- Caspersson *et al*. 1968
  - using fluorescent dye (chinakrine yperit) make specific bands on chromosomes = banding

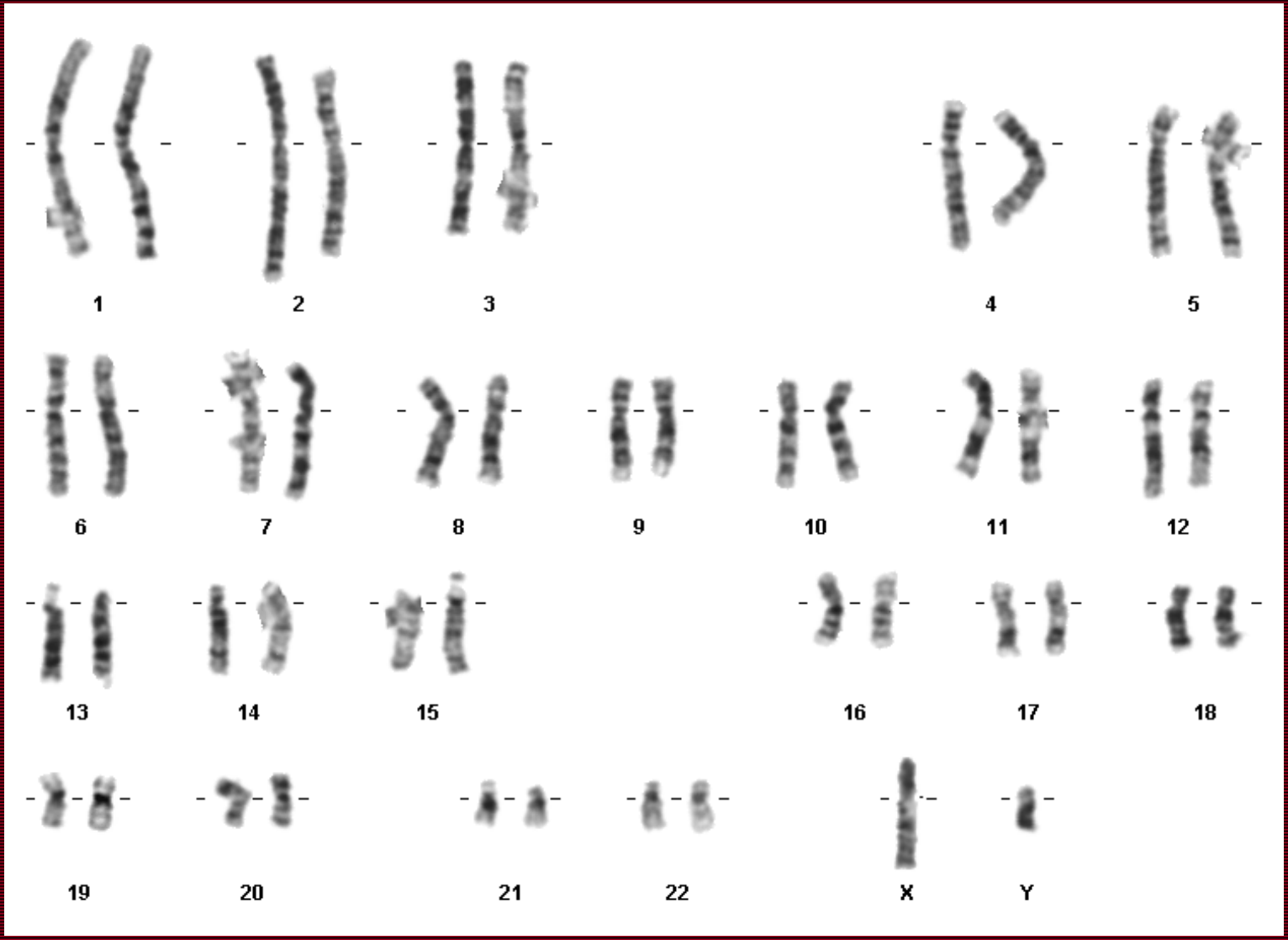
Banding techniques

- Bands = stripes on the chromosomes, which are clearly distinguishable from adjacent segments

- Methods of differentiating along the length:
  Q-bands
  G-bands
  R-bands

- Selective methods:
  C-bands
  T- bands
  NOR staining

Human karyotype: G-banding



C-bands: detection of centromeres

Staining and structure of the human genome

- bands on chromosomes reflects the structure of the genome and its functional organization
- Each band contains 5 to 10 Mbps
- Giemsa positive bands (G +, rich in AT pairs, the late replicating chromosome regions poor in genes)
- Giemsa negative bands (G-rich in GC pairs, the early replicating chromosome regions rich in genes)

Molecular cytogenetics

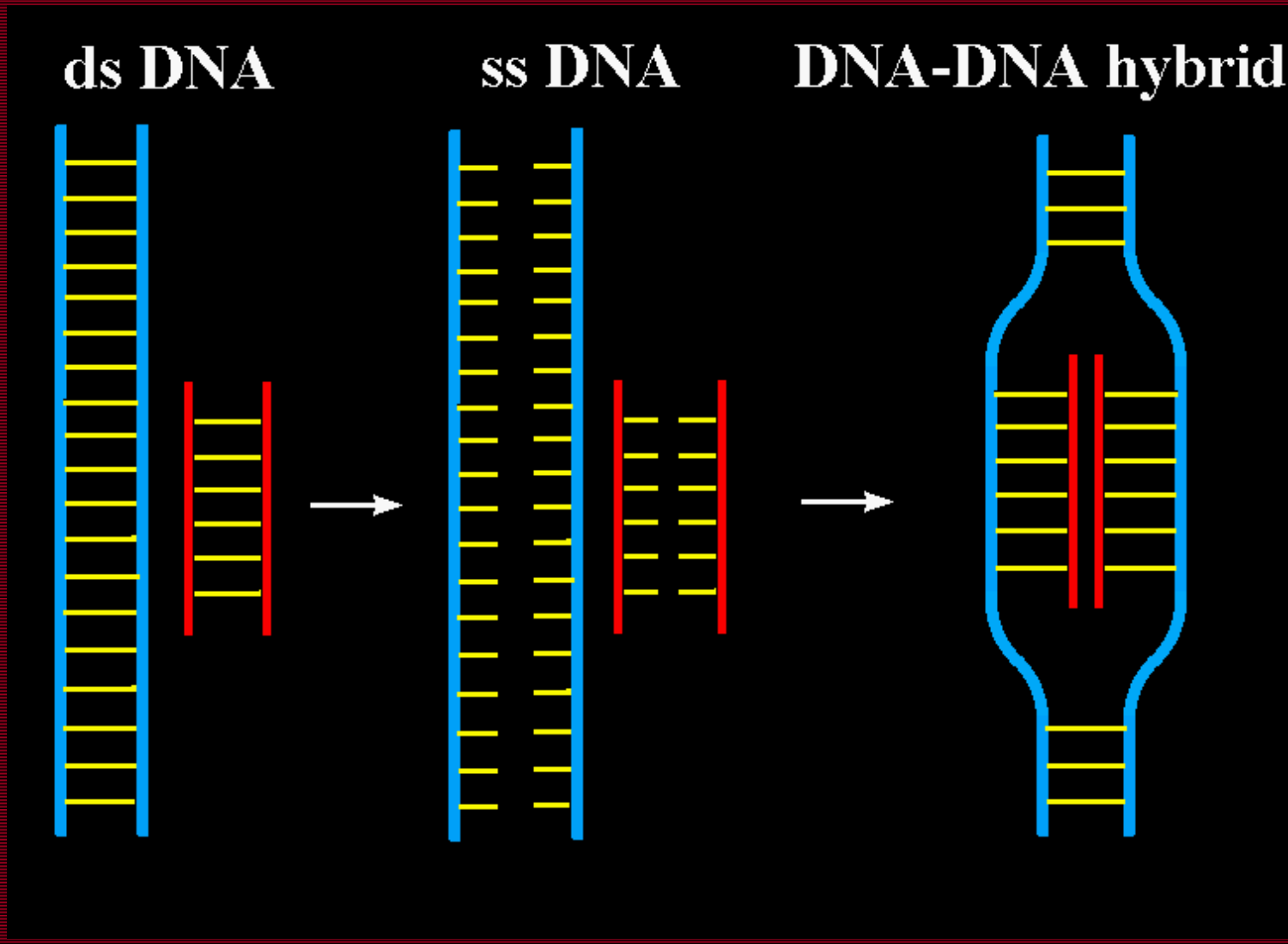
- Presents the connections between classical cytogenetics and molecular biology

- 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

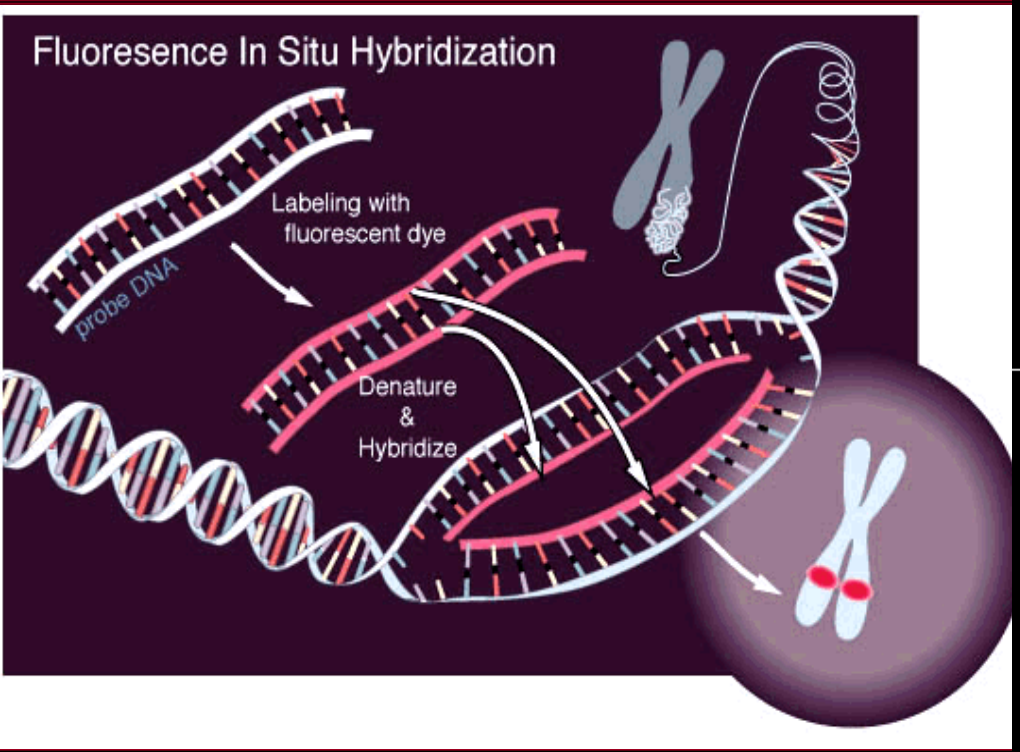
- does not require the presence of mitosis

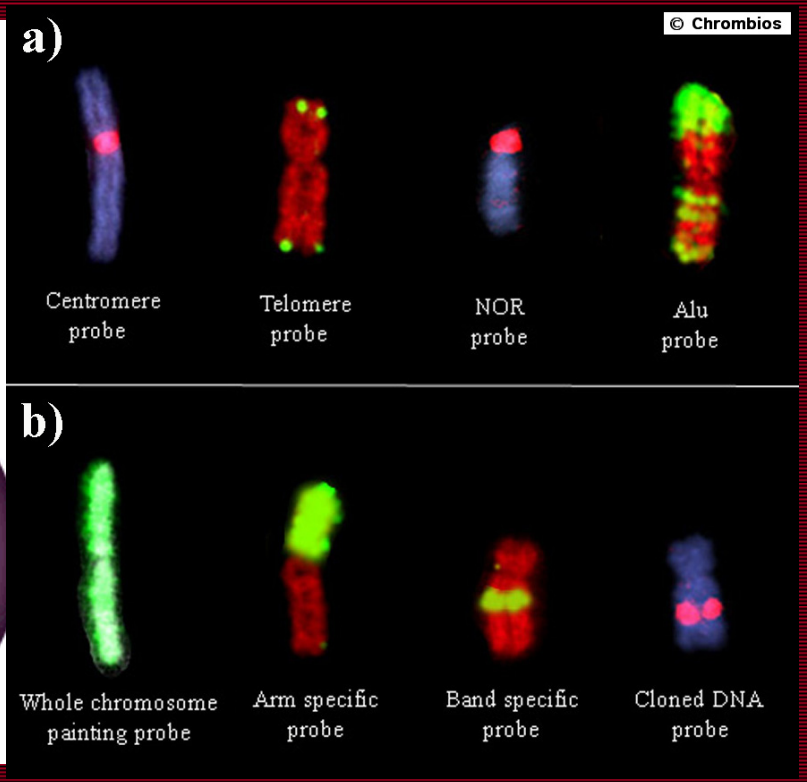
Principle of *in situ* hybridization



FISH

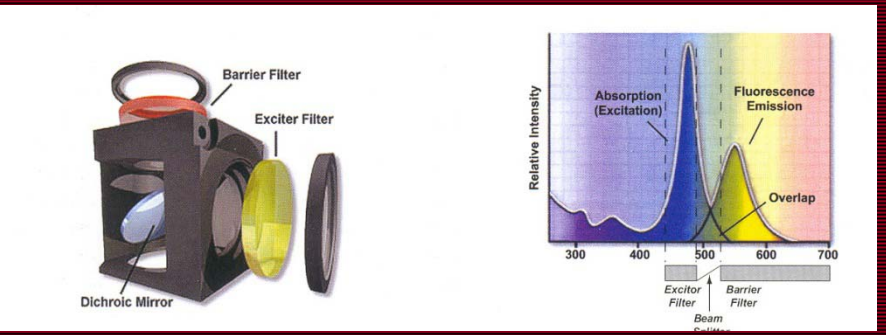
(fluorescent hybridizace *in situ*)





Basic equipment for FISH analysis

- Fluorescent microscope with appropriate objectives and fluorescent filters
- High sensitive CCD camera
- Suitable PC + software for picture analysis

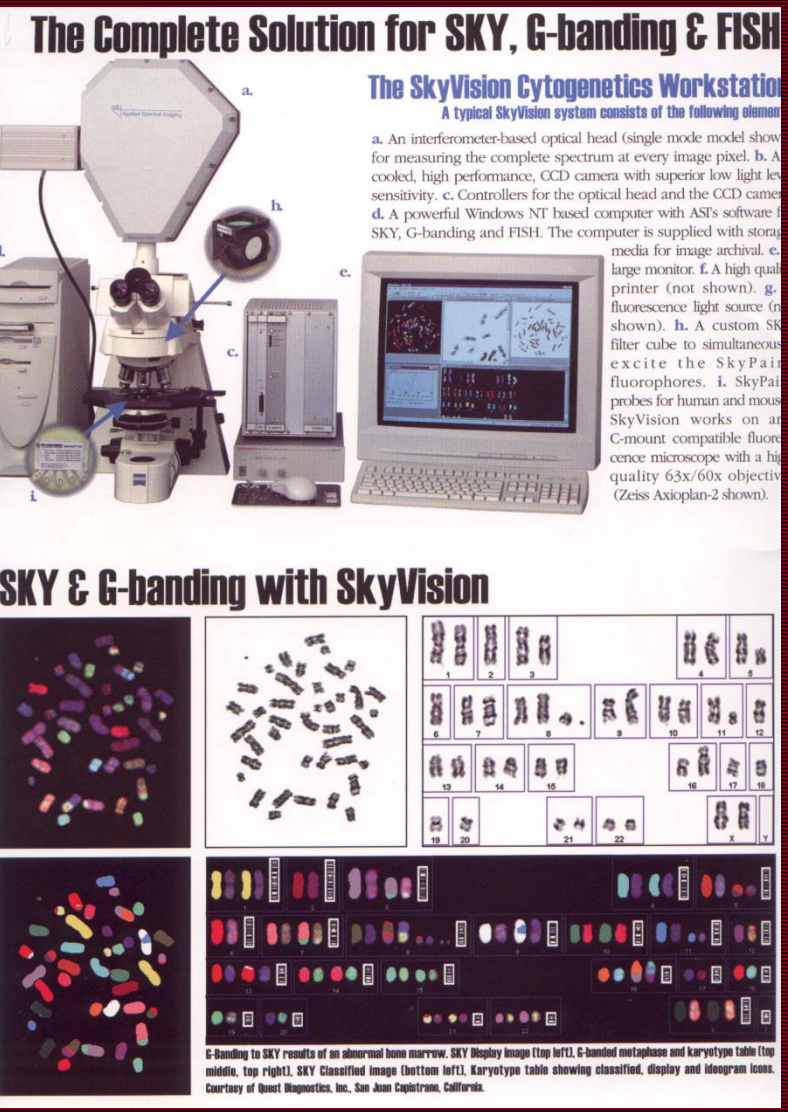


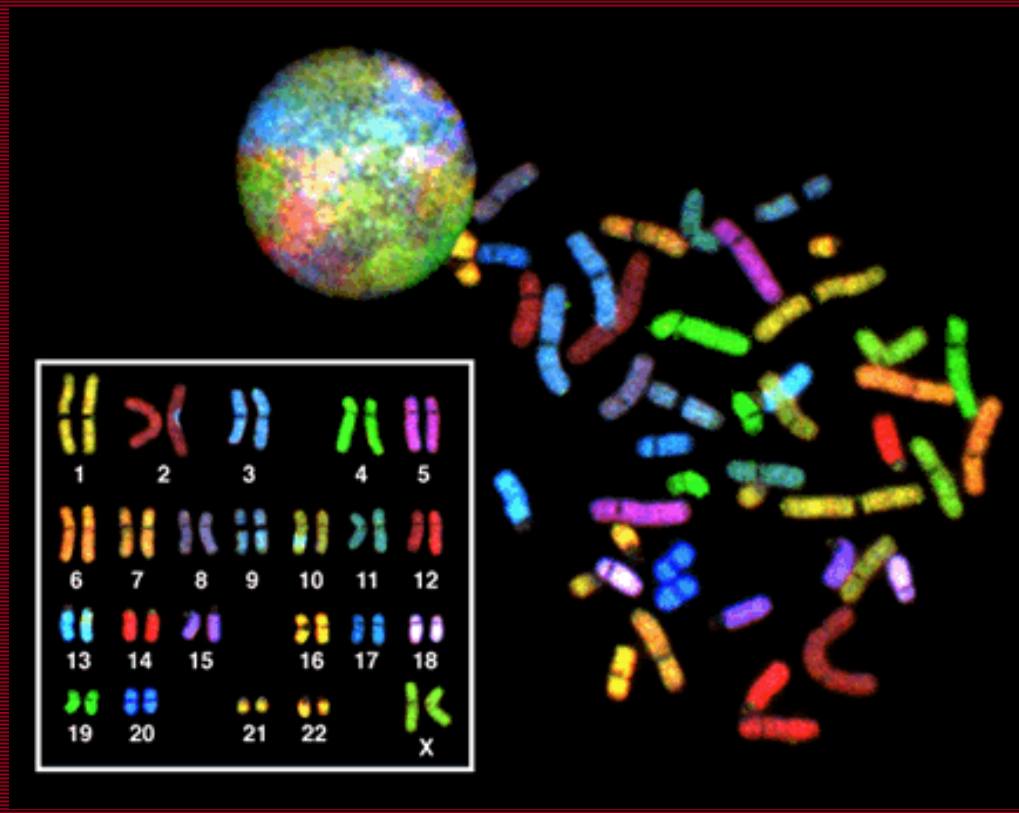
Analyses using FISH

- Based on detection of the fluorescent signals through microscope equipped with specific fluorescent filters

- We determine:
  1. Presence of the signals
  2. Number of signals
  3. Position of signals

Spectral karyotyping (SKY)





- Each chromosome is labeled with a unique combination of the five fluorescent dyes.
- This results in a unique fluorescence SPECTRUM of each chromosome.

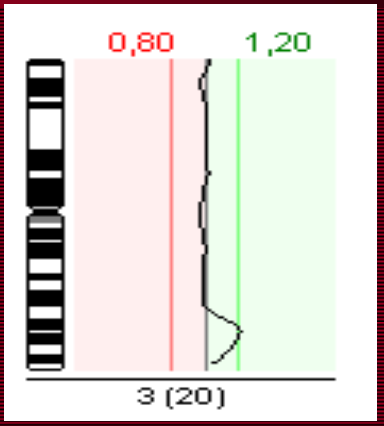
Comparative Genomic Hybridization (CGH)

- Screening and mapping unbalanced genetic alterations (deletions, duplications) across whole genome in single experiment

- Huge amount of data, detection changes smaller than 5kb

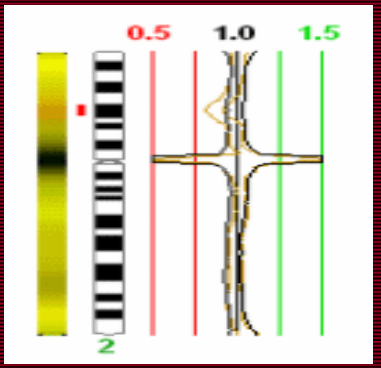
Original CGH

- Kallioniemi *et al*. 1992
- detection range: 5 - 10 Mb



High resolution CGH

- Kirchhoff *et al*. 1997
- detection range: from 3 Mb



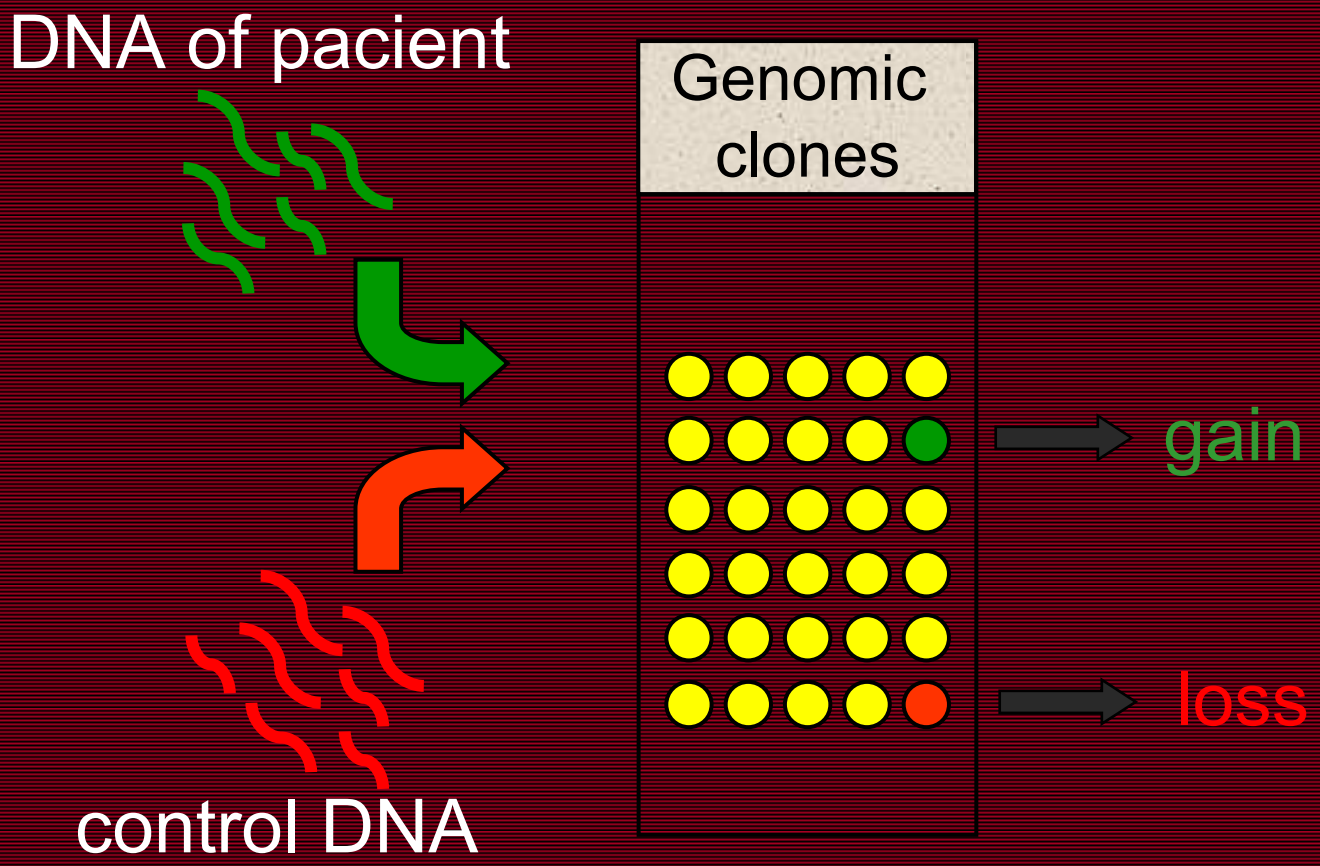
Array CGH

- Solinas-Toldo et al., 1997
  New approach of scanning – not using chromosomes, but BAC clones = detection range from ~ 0,5 Mb

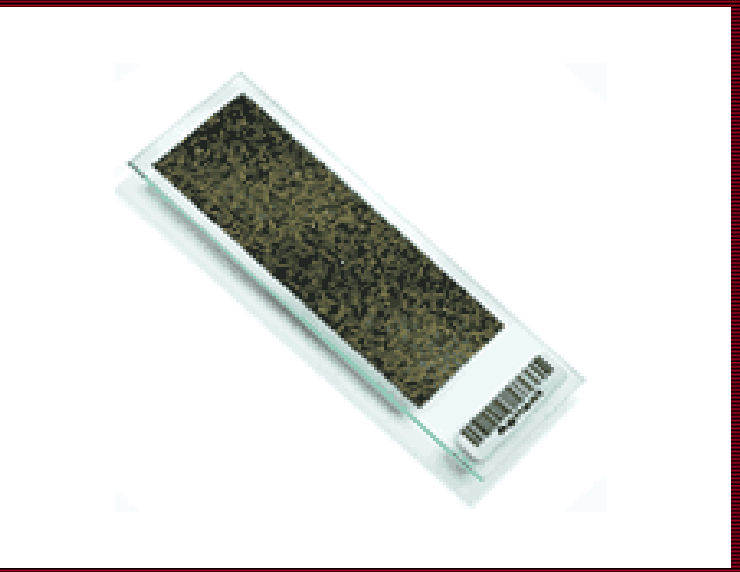
- Nowadays – BAC clones replaced by short (60 mers) oligonucleotide probes utilized on array (spots)

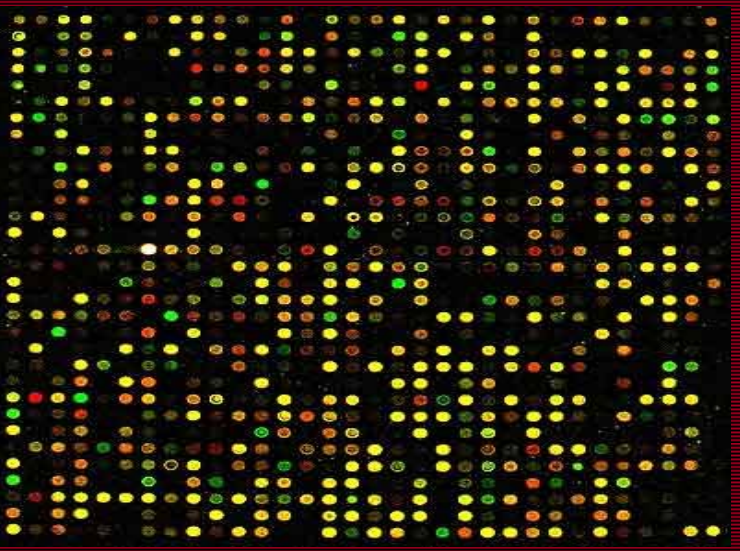
- According to density of probes, detection range starts at **5kb** (1M Agilent array)

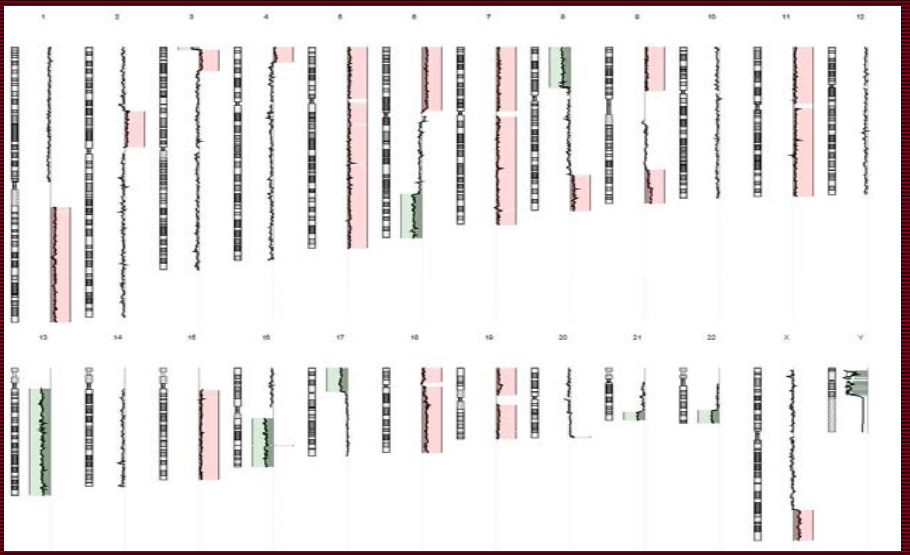
CGH-Arrays



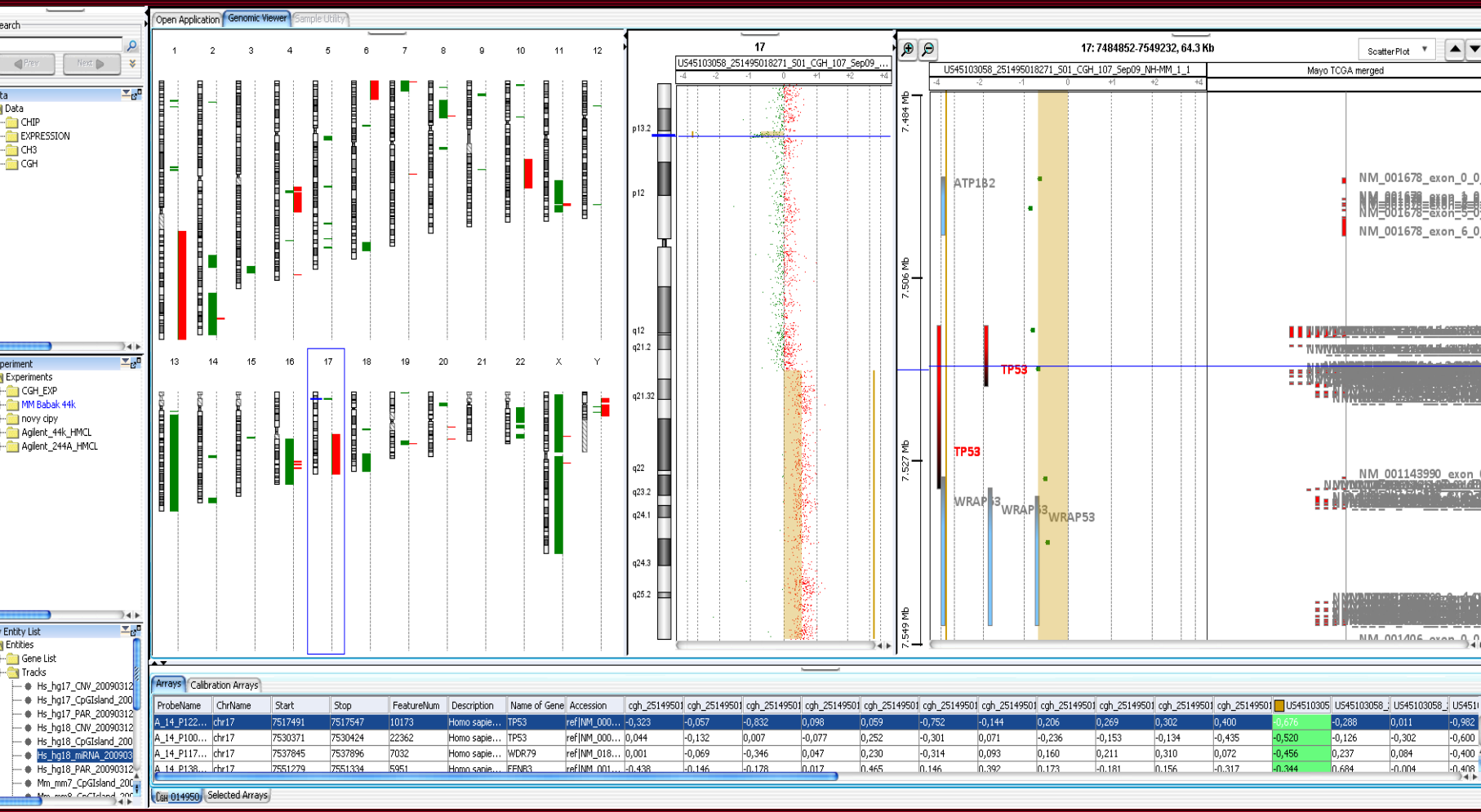
Pinkel et al., Nat Genet (1998), 20(2):207-







Agilent Workbench Standard 5.1



Babak Research Institute

All previously showed techniques allow:

- specify diagnosis
- determining and monitoring of treatment strategies
- estimates of the likely development of disease

In very fast way (24 hours average, 48 hours for array-CGH)