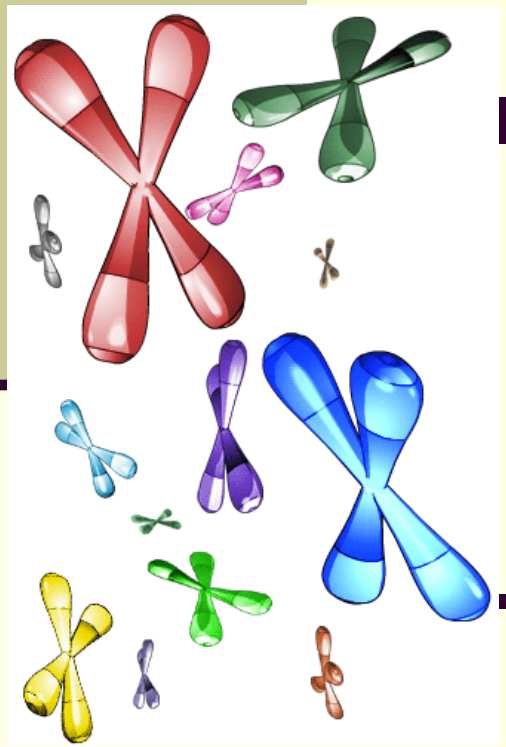
Cytogenetics & Integrated laboratory of molecular cytogenetics, Brno



What are we going to talk about?

- 1. What is cytogenetics
- 2. History
- 3. Chromosome morphology and aberrations
- 4. Molecular cytogenetics and its techniques
- 5. Our laboratory and work

1. 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.

2. Just a little history…

- 1866 Gregor Johan Mendel – Experiment in Plant Hybridization
- Father of genetics
- Defined the basic principals of heredity (principle of segregation and combination)
- During his life, his work was ignored
- Later, Mendel s work was rediscovered
- 1910 Thomas Hunt Morgan proved that genes are located on chromosomes (using Drosophila)
- 1953 James Watson and Francis Crick determined DNA structure
- 1956 Tjio, Levan – Human chromosome number is 46

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

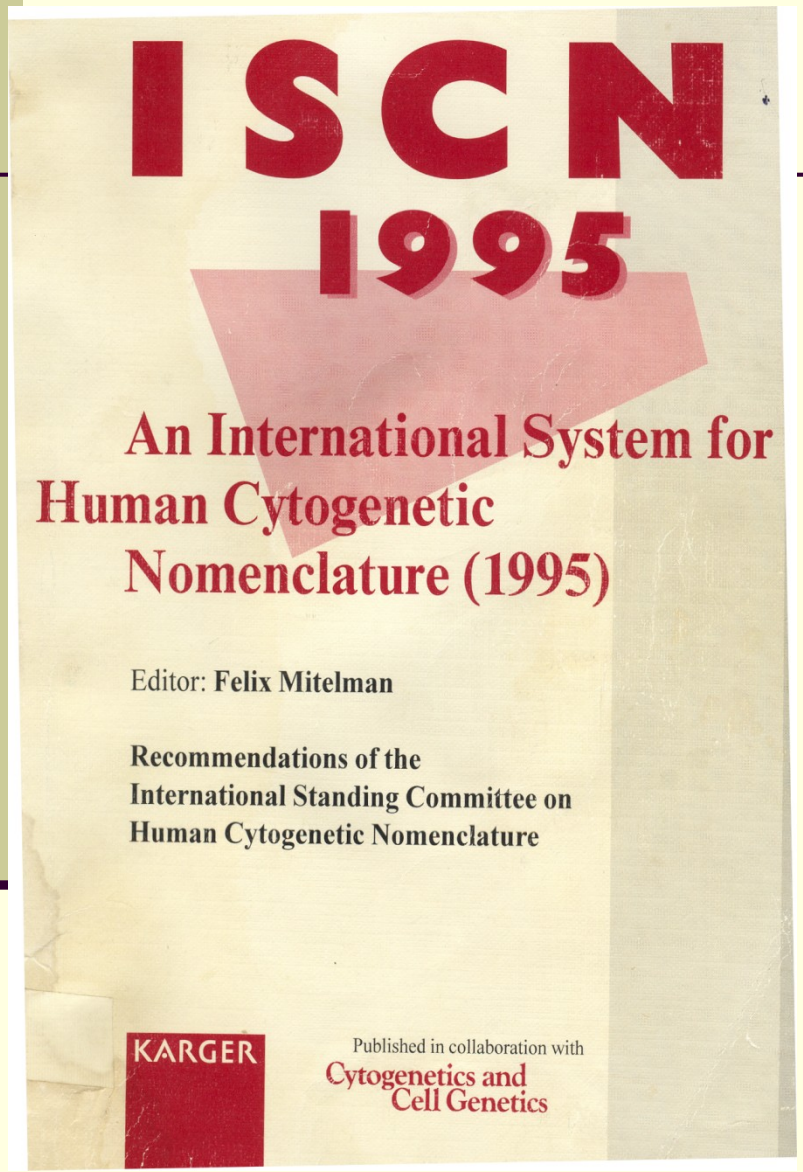
„take home message“

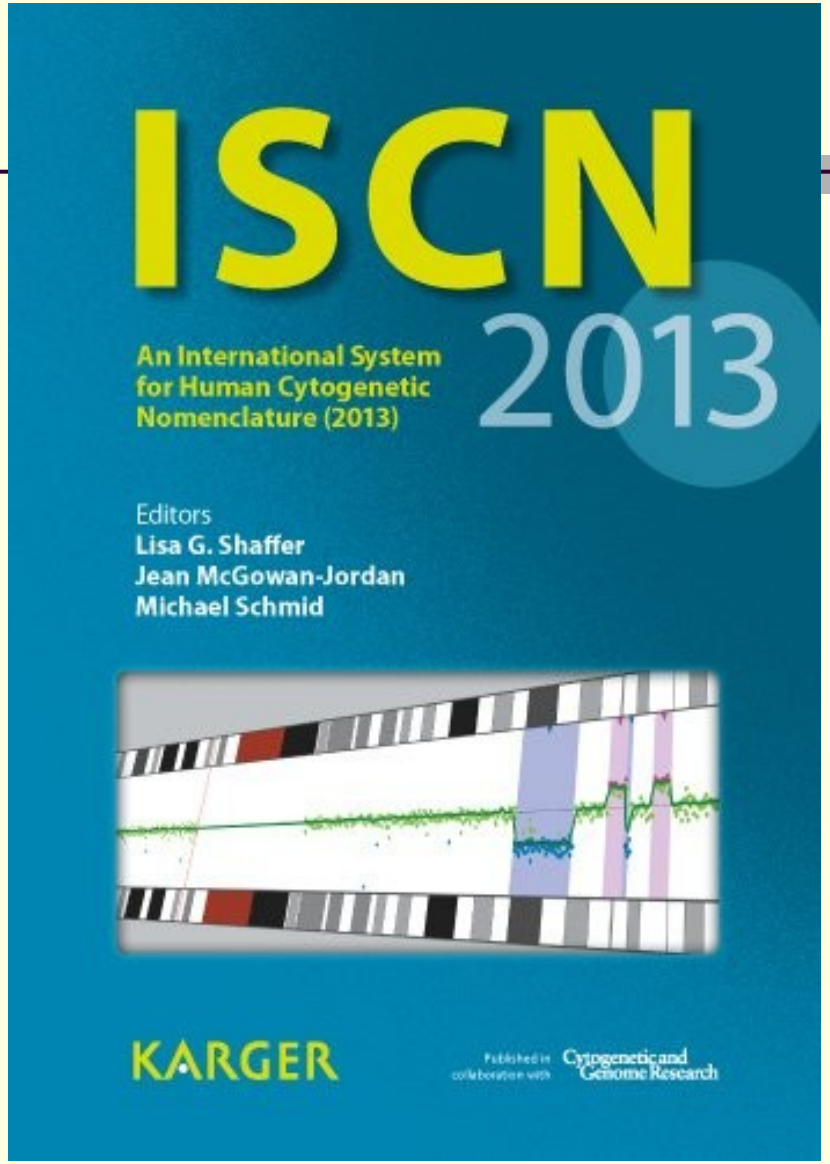
Basic conditions for development of human cytogenetics

- 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

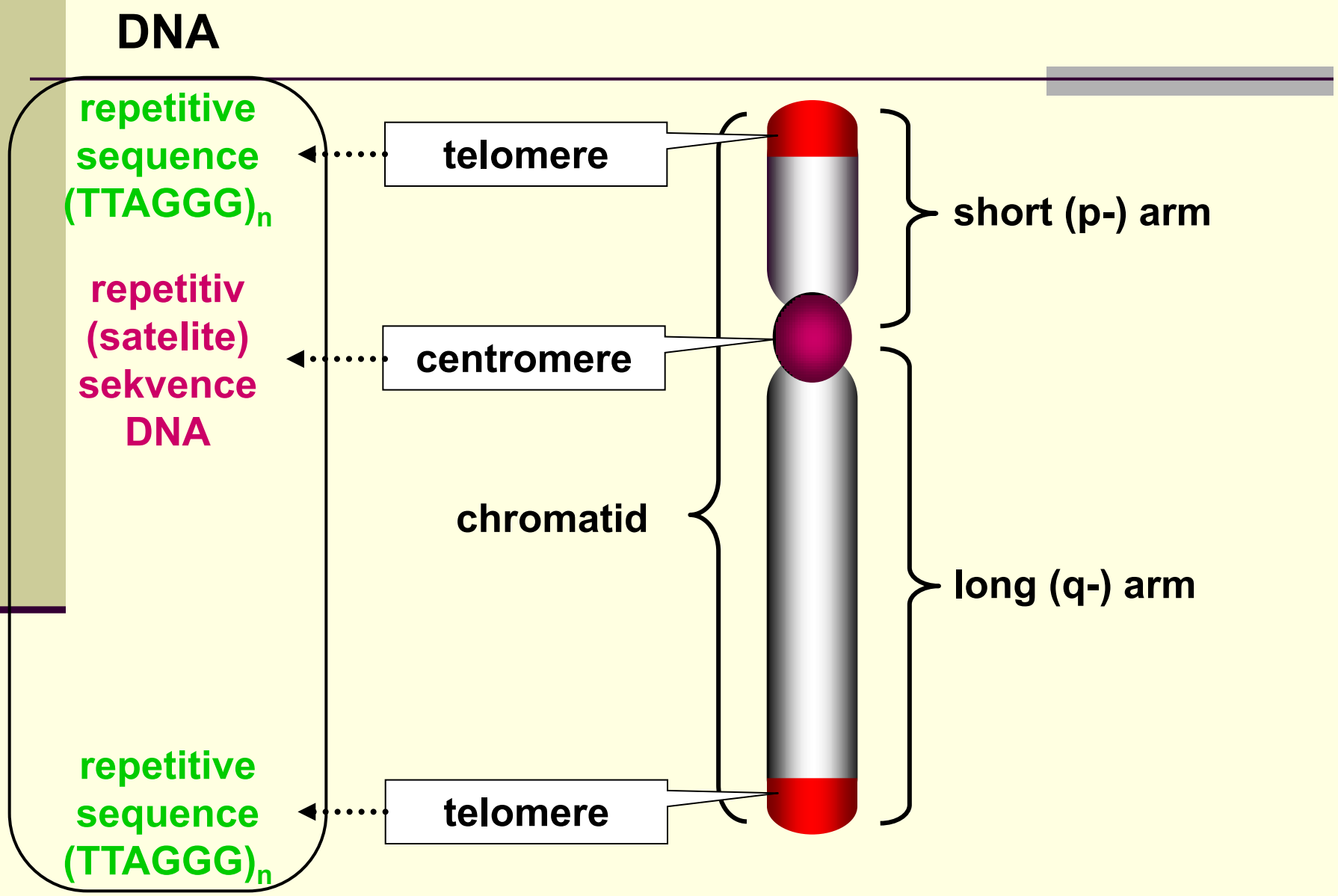
Nomenclature of human chromosomes

- **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)

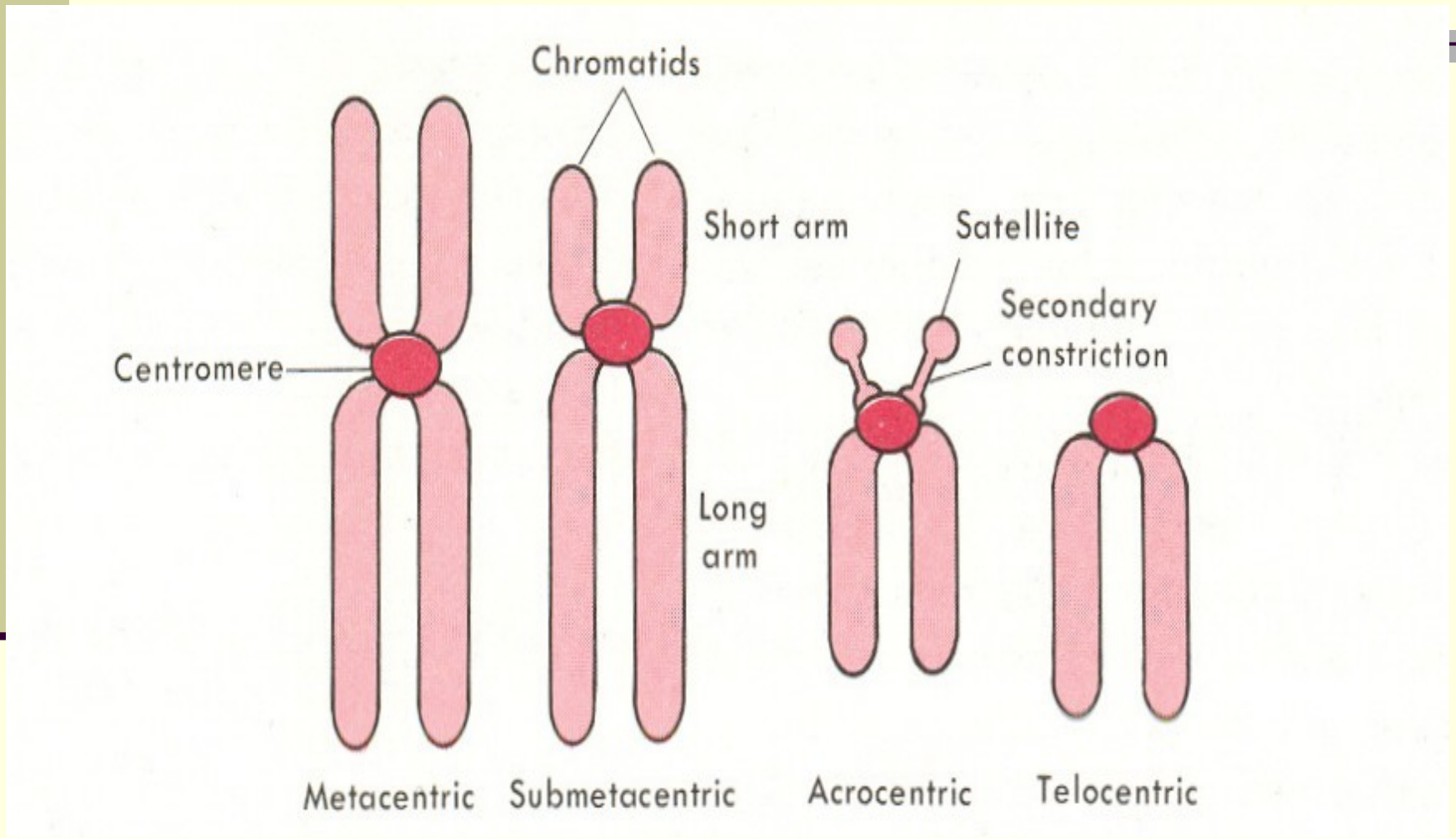

ISCN
1995
An International System for Human Cytogenetic Nomenclature (1995)
Editor: Felix Mitelman
Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature
KARGER
Published in collaboration with
Cytogenetics and Cell Genetics


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

3. Chromosome morphology



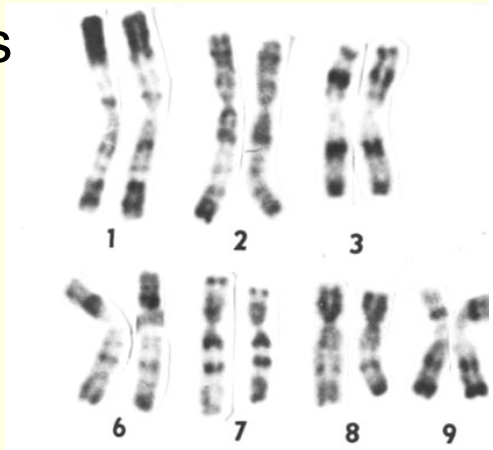
Chromosome morphology

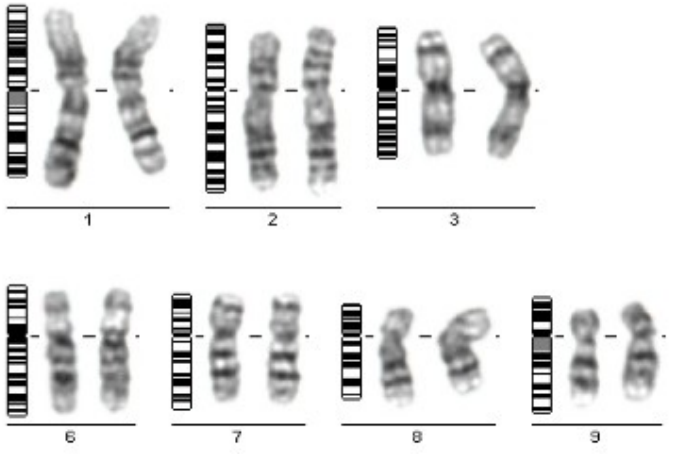


Chromosome staining

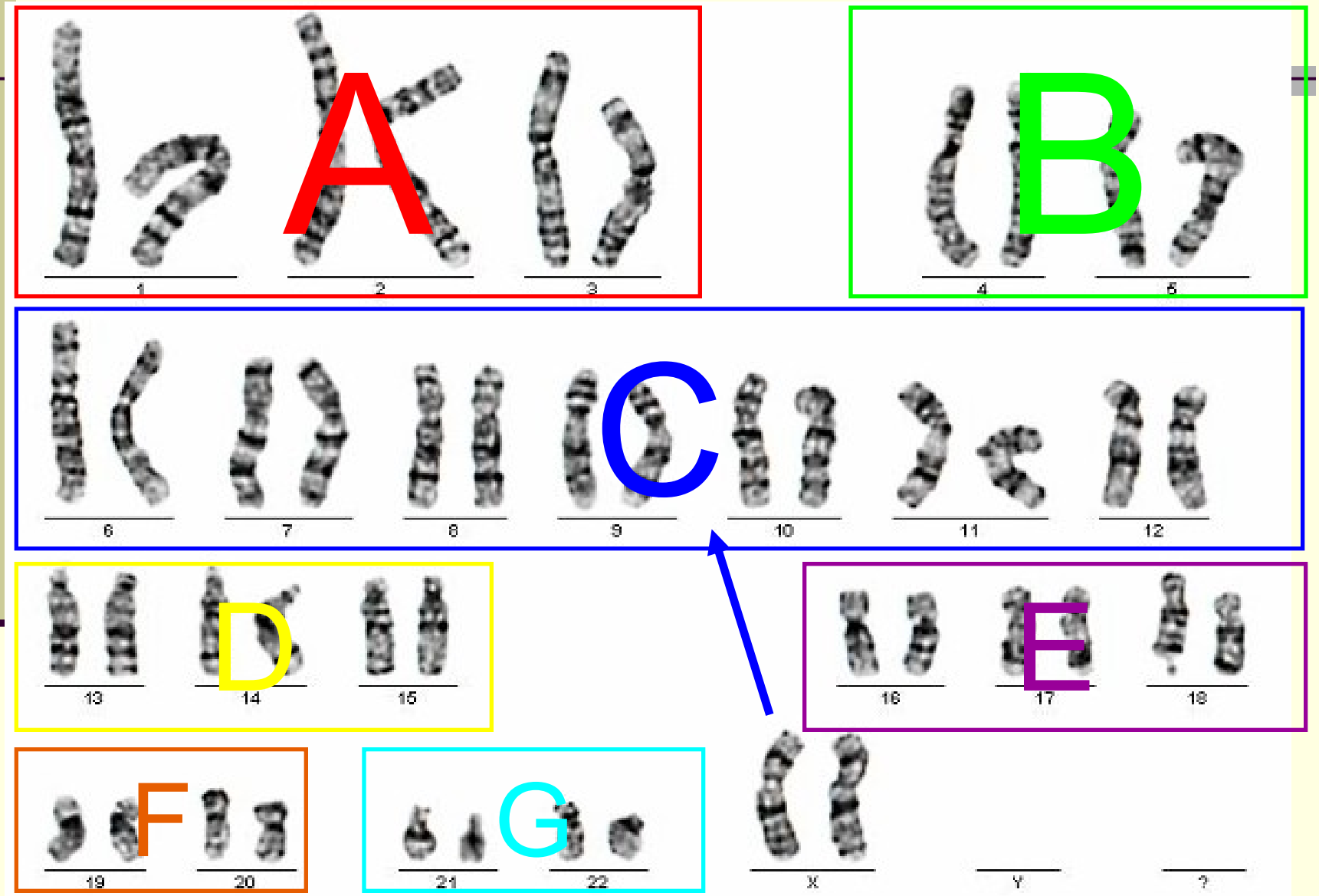
- Classical staining
  - using Giemsa Romanowski solution
  - gained chromosome aberation detection

- 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

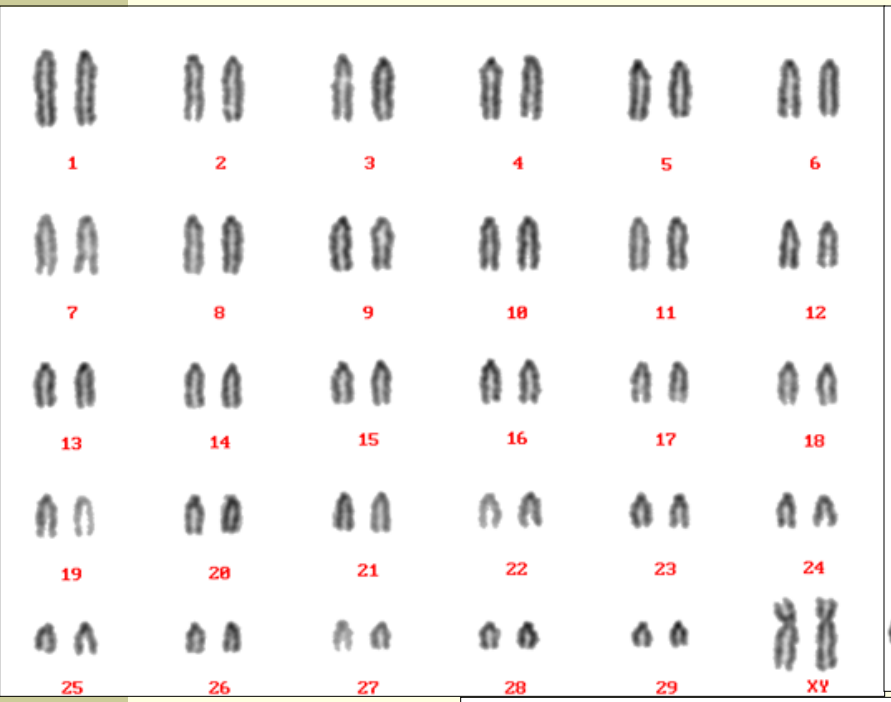




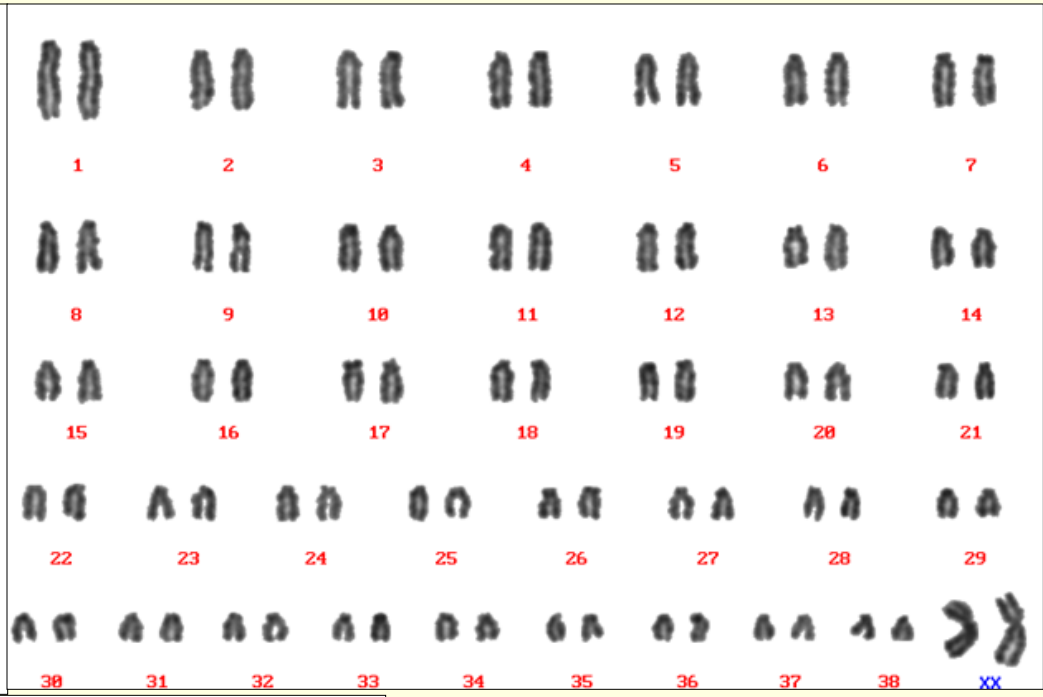
Human caryotype



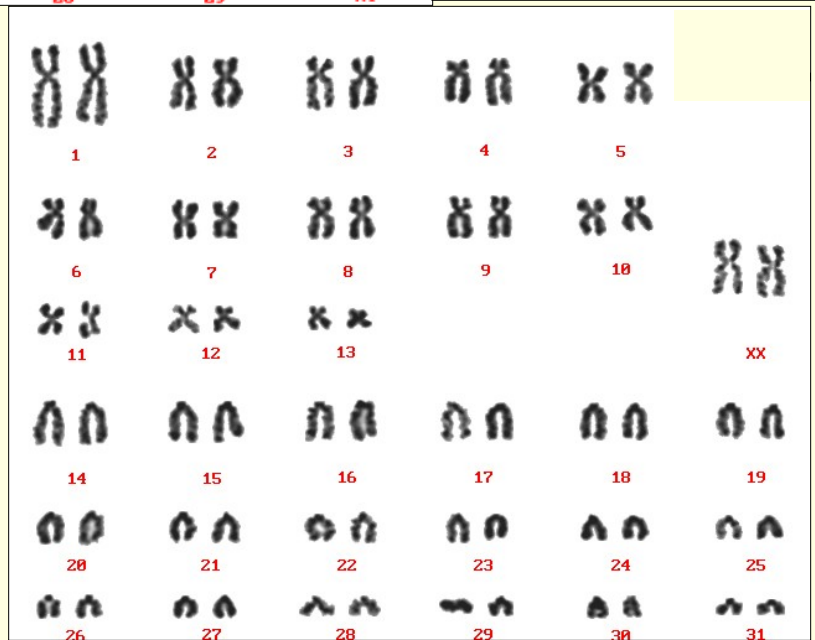
Caryotype of animals



cattle



dog



horse

„take home message“

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

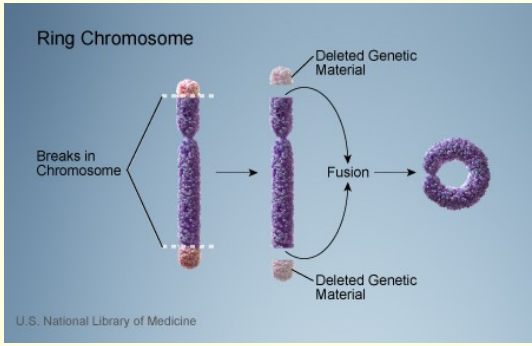
E – small meta- to submetacentric chromosomes

F – the smallest metacentric chromosomes „ribbons“

G – small acrocentric chromosomes, Y

Chromosome aberrations

- AUTOSOMIC
- 1. Structural
  - Polymorphysmus
    - different lenght of chromosomes in homologous pair
    - no phenotype effect
  - Inversion
    - pericenric – including centromere
    - paracentric – does not include centromere
    - usually has no phenotype effect
  - Ring chromosomes
    - breaks on both chromatides and their connection
    - mental and physical retardation
    - always newly created
    - sometimes redundant



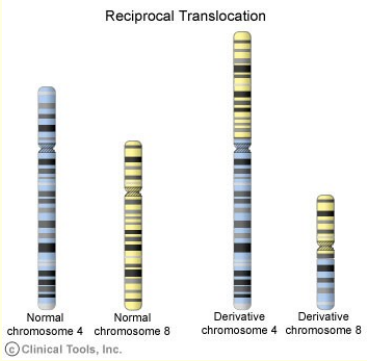
http://ghr.nlm.nih.gov/handbook/illustrations/ringchromosome

Chromosome aberrations

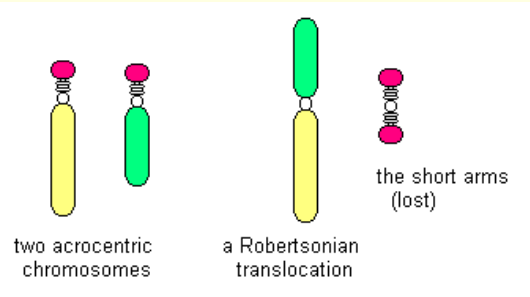
- Deletion
  - terminal – one break
  - intersticial – two breaks
  - deletion syndromes:
    - Wolf-hirschhorn syndrome; 4p deletion
    - Cri-Du-Chat syndrome; 5p deletion
  - microdeletion syndromes:
    - Prader-Willi syndrome; 15q11-12 deletion
    - DiGeorge syndrome; 22q13 deletion
    - Angelman syndrome; 15q11-13 deletion
    - Williams-Beuren syndrome; 7q11.23 deletion
- Insertion
  - inserted part can be in the same or inverted position

Chromosome aberrations

- Translocation
  - reciprocal
    - mutual exchange between two or more nonhomologic chromosomes
    - balanced - no phenotype effect
    - genetic risics of unbalanced genom gamets formation
  - robertsonian
    - between two acrocentric chromosomes
    - breaks in the area of centromeres and deletion of short arms
    - centric fusion of the remaining arms
    - balanced – normal phenotype
  - tandem
    - deletion of part of an acrocentric chromosome
    - fusion of the remaining part with another chromosome



http://www.larasig.com/node/3628



http://drugline.org/medic/term/robertsonian-translocation/

Chromosome aberrations

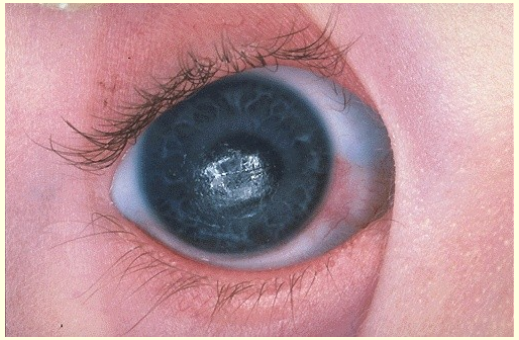
- 2. Numerical
  - Trisomy
    - 21 chromosome trisomy – Down syndrome
    - 18 chromosome trisomy – Edwards syndrome
    - 13 chromosome trisomy – Patau syndrome
  - Triploidy
    - 69 XXX, 69 XXY
    - nonviable
    - mosaic triploidy – mental retardation, syndactyly, abnormal genitals, lateral asymetry

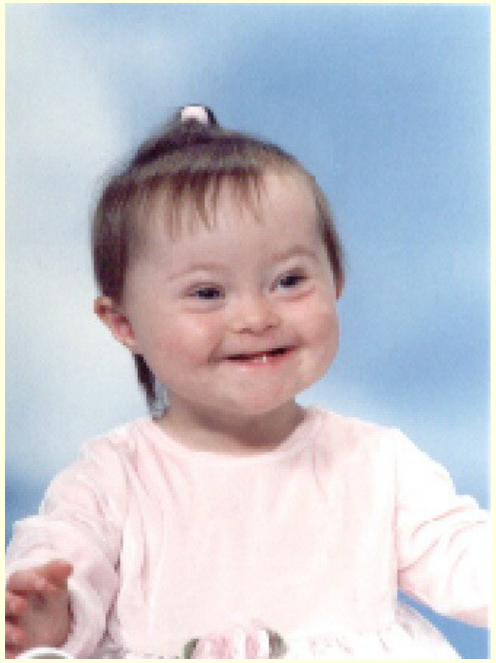
Chromosome aberrations

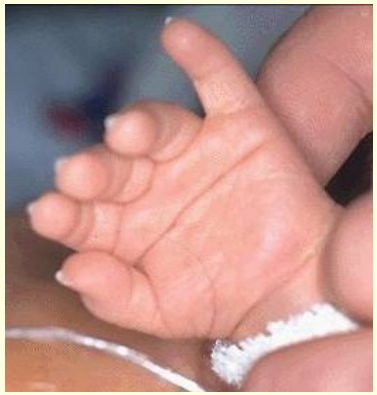
- GONOSOMIC
- Chromosome Y
  - structural aberrations – very rare
  - numerical aberrations
    - 47, XYY – supermale syndrom
- Chromosome X (male)
  - Numerical aberration
    - 47, XXY – Klinefelter syndrom
- Chromosome X (female)
  - numerical aberrations
    - 45, X – Turner syndrom
    - 47, XXX – XXX syndrom
- Fragile X – fraX
  - the most common cause of mental retardation
  - nonspecific phenotype

Down syndrome (47,XX or XY,+21)

- 1866 J.L.Down
- IQ 25-50
- small dumpy figur
- round face
- short neck
- mongoloid eyes
- epicanthic fold
- wide nose root and flattened nose
- small mouth, large tongue, small teeth
- single transverze palmar crease
- heart diseases

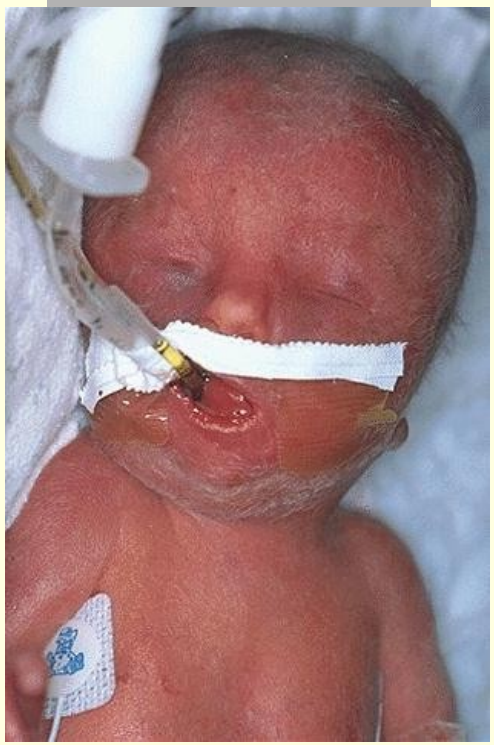


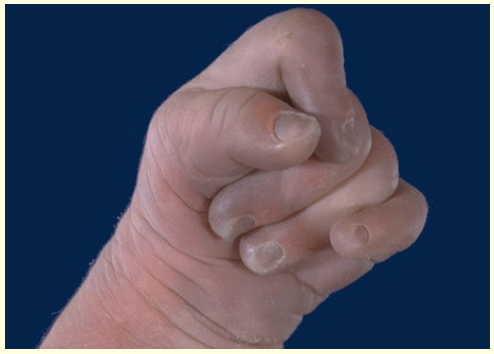


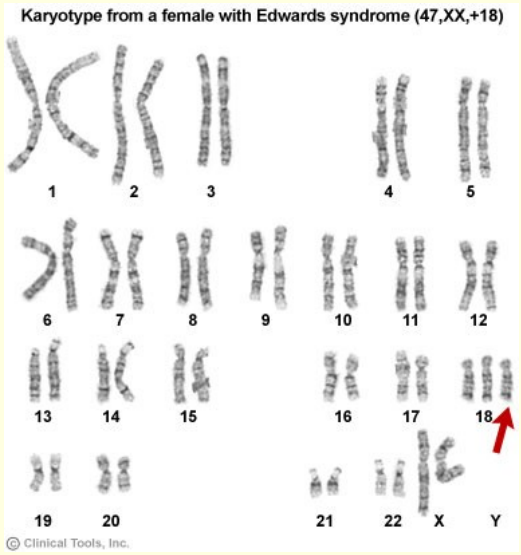


Edwards syndrome (47, XX or XY,+18)

- growth retardation
- microcephaly
- dolichocephaly – elongate head
- cleft palate
- low-set malformed ears
- finger holding
- structural heart defect at birth
- survive only few months

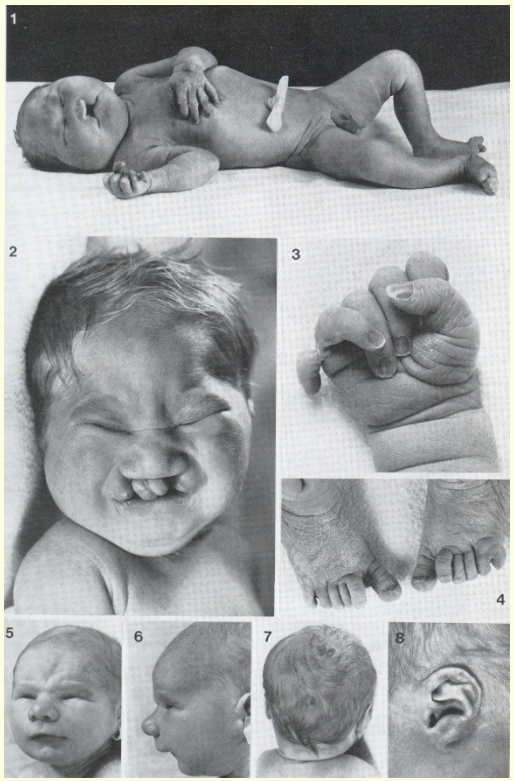


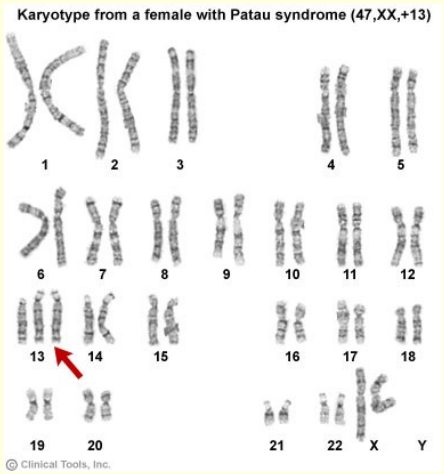


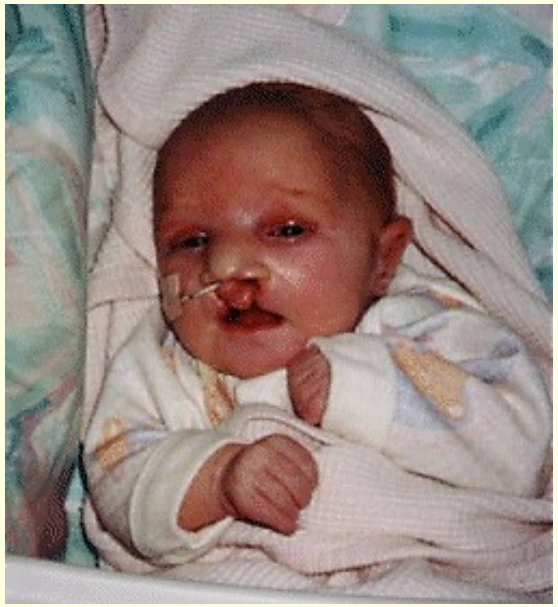


Patau syndrome (47,XX or XY,+13)

- hard growth and mental retardation
- microcephaly
- trigonocephaly
- cutis aplasia
- congenital brain defects
- cleft palate
- hexadactily
- kidney defects

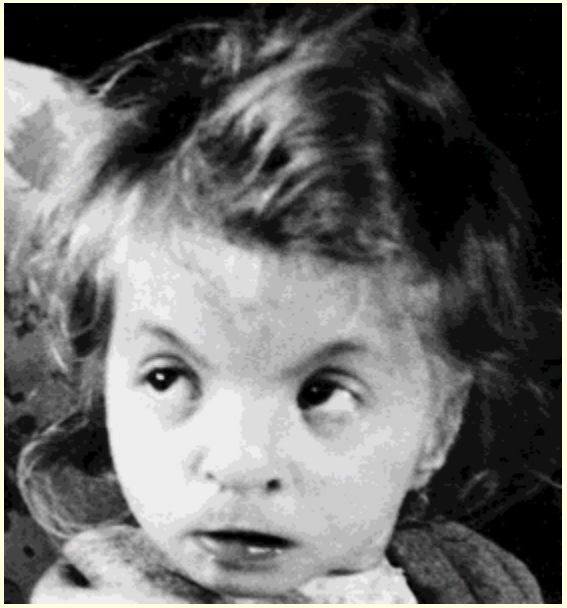






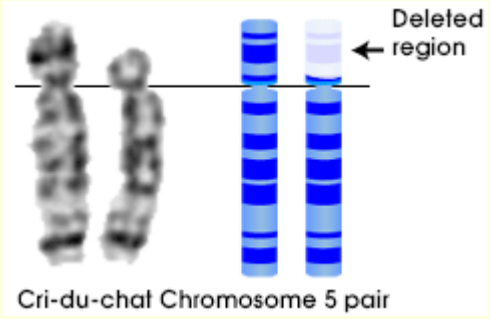
Wolf-hirschhorn syndrome (del 5p)

- microcephaly
- micrognathia (small jaw)
- ocular hypertelorism
- dysplastic ears
- growth and mental retardation
- muscle hypotonia
- seizures
- congenital heart defects



Cri-Du-Chat syndrome (del 5p)

- low birth weight, poor growth
- hypotonia
- severe cognitive speech and motor delays
- behavioral problems – hyperactivity, agression
- small head and jaw
- wide eyes
- constipation
- abnormal larynx development
  - difficulty of swallowing and sucking
  - drooling
  - cat-like cry

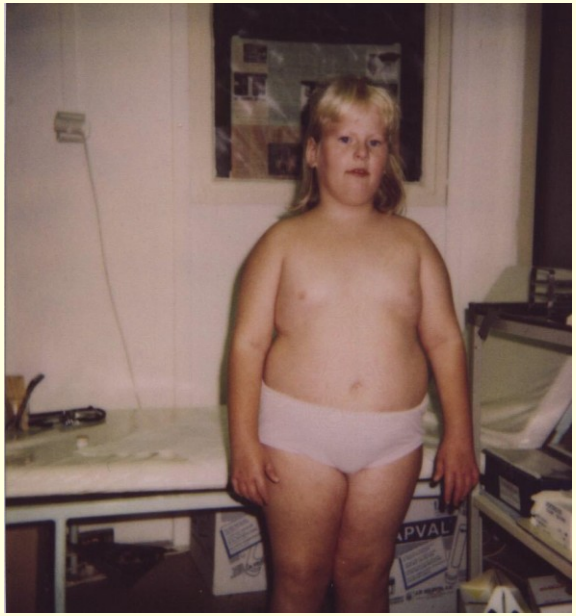


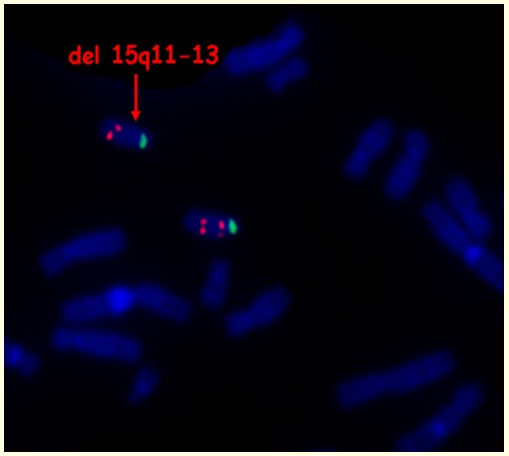
Cri-du-chat Chromosome 5 pair

http://learn.genetics.utah.edu/content/disorders/whataregd/cdc/

Prader-Willi syndrome (del 15q11-q13)

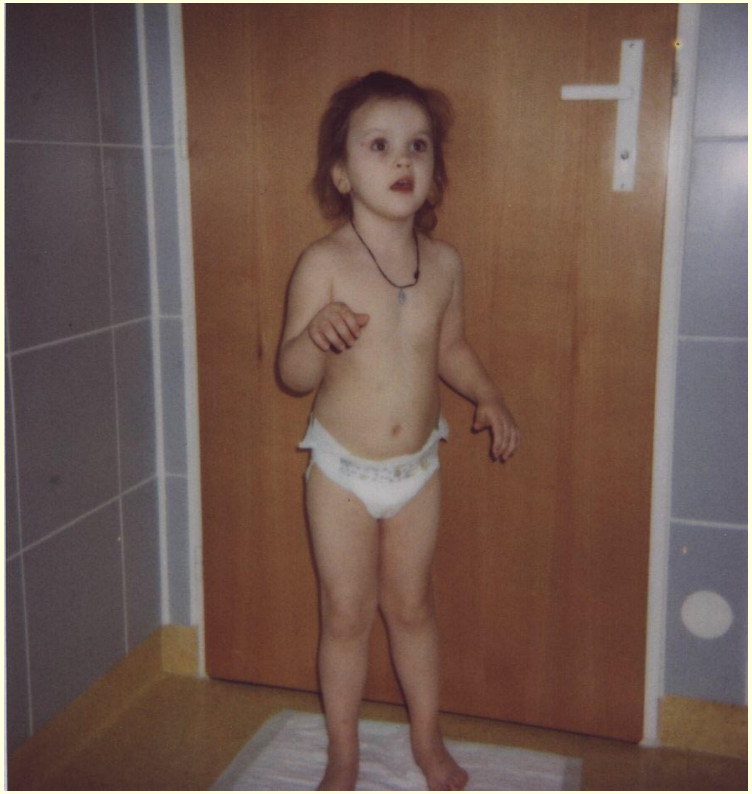
- paternal deletion
- low fetal activity
- hypotonia
- excessive weight gain, hyperphagia
- short stature
- hypogonadism
- mental retardation
- hypopigmentation
- skeletal development delay (acromicria)





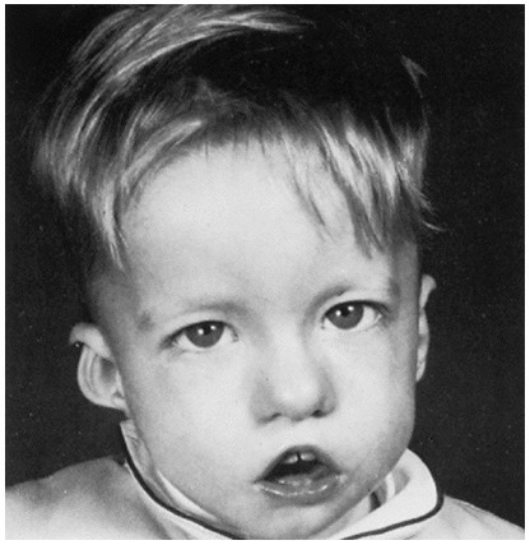
Angelman syndrome (del 15q11-q13)

- maternal deletion
- hard mental retardation
- hypotonia
- epilepsia, seizures
- hypopigmentation
- hyperactivity
- speech absence
- prominent scull shape (mandibul, microcephaly, flat back of head..)
- „happy character“
- movement or balance disorder



DiGeorge syndrome (del 22q11.2)

- low-set malformed ears
- small mouth and lower jaw
- narrow eye-lits
- submucosal or visible cleft palate
- hypocalcemia
- interupted aortic arch
- cardiac abnormality – tetralogy of Fallot
  - incomplete ventricular septum
  - right-to-left shunt of aorta
  - left ventricle hypertrophy
  - lung stenosis



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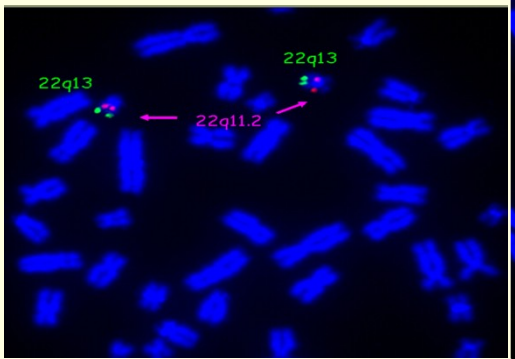
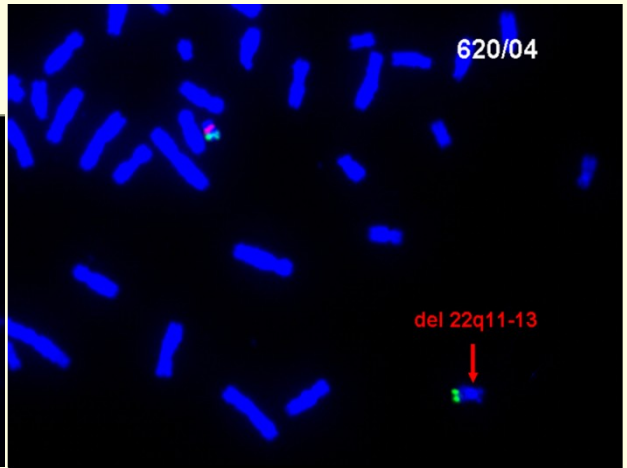
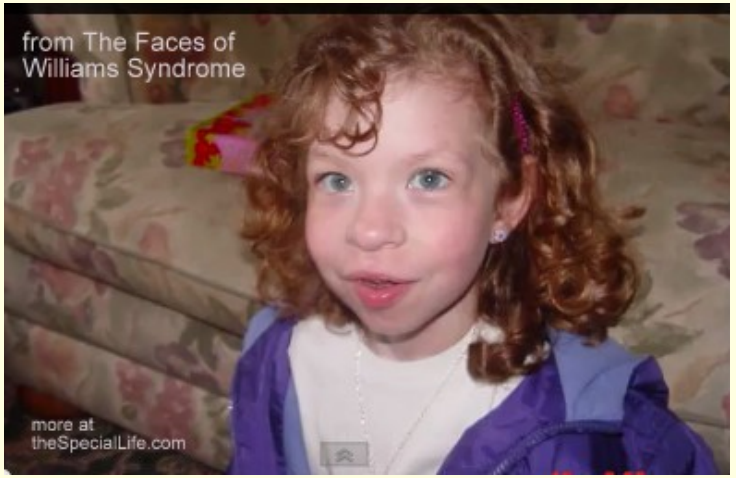




Williams Beuren syndrome (del 7q11)

- developmental delay
- mental disability
- failure to thrive
- heart defects (heart murmur, narrowing of main blood vessels)
- flattened nasal bridge
- widely spaced teeth
- hypercalcemia
- gastrointestinal problems
- urinary difficulties



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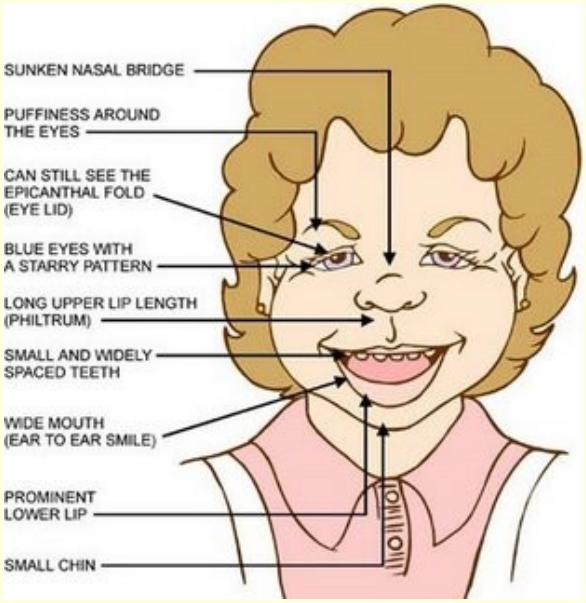

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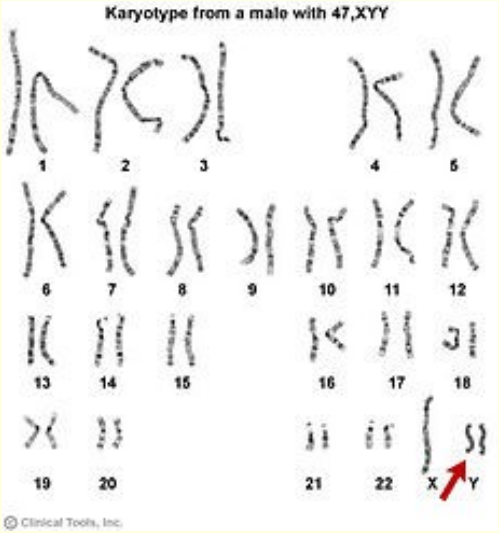
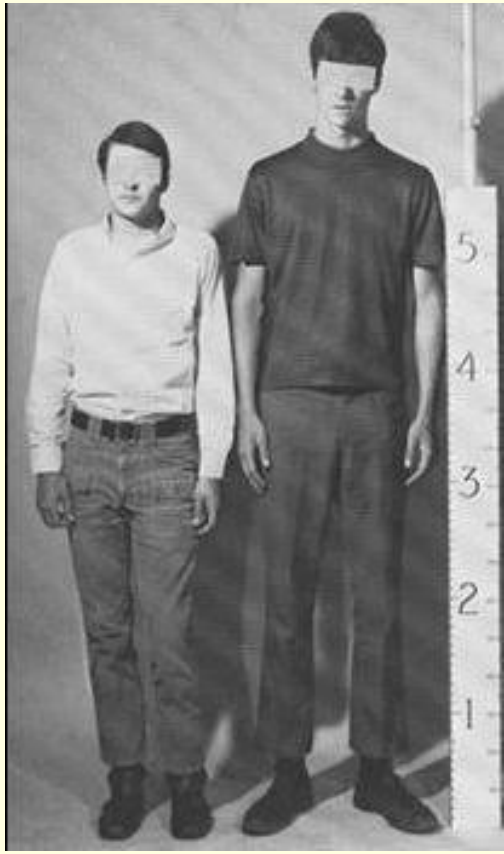
Supermale syndrome (47,XYY)

- increased growth velocity
- no unusual physical features
- normal testosteron level, fertility and sexual development
- possible learning disabilities
- delayed development of speech and language skills
- behavioral and emotional difficulties

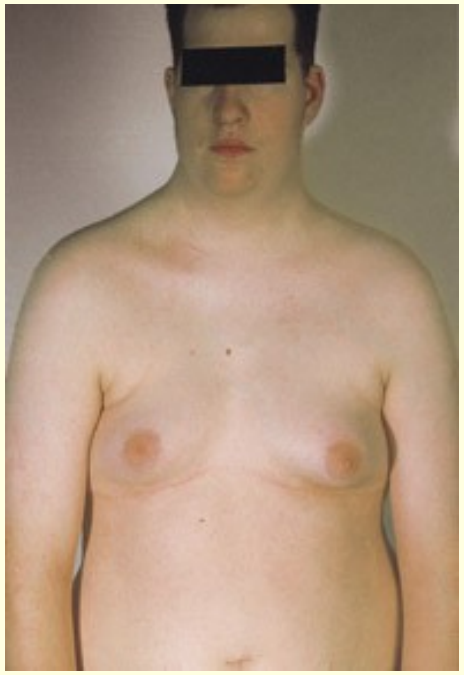


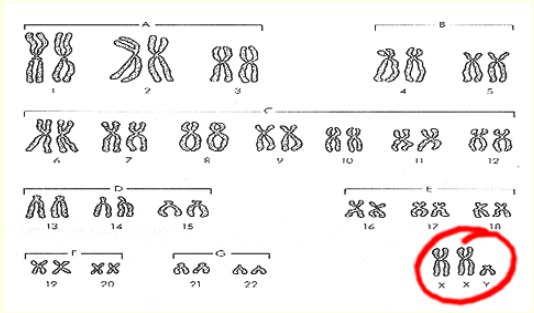


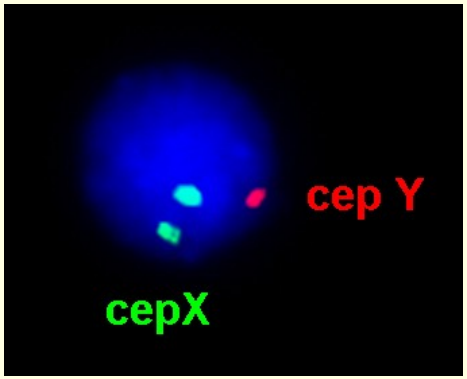
Klinefelter syndrome (47 XXY)

48,XXYY; 48,XXXY; 49,XXXXY

- tall figure
- less facial and body hair
- female distribution of body fat
- hypogonadism (decreased testicular hormon function)
- infertility
- gynecomastia (increased breast tissue)
- lower intelect degree
- variations: 48, XXYY; 48, XXXY; 49,XXXXY

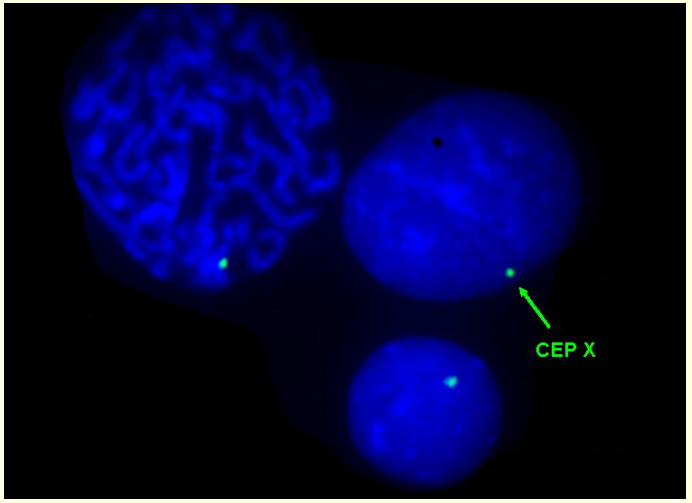


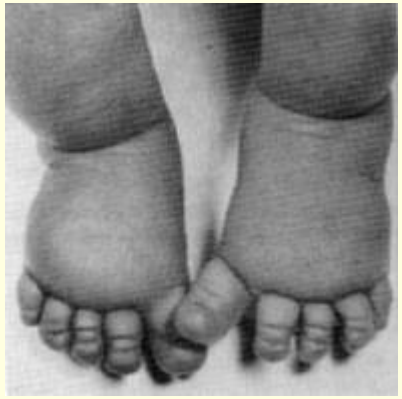


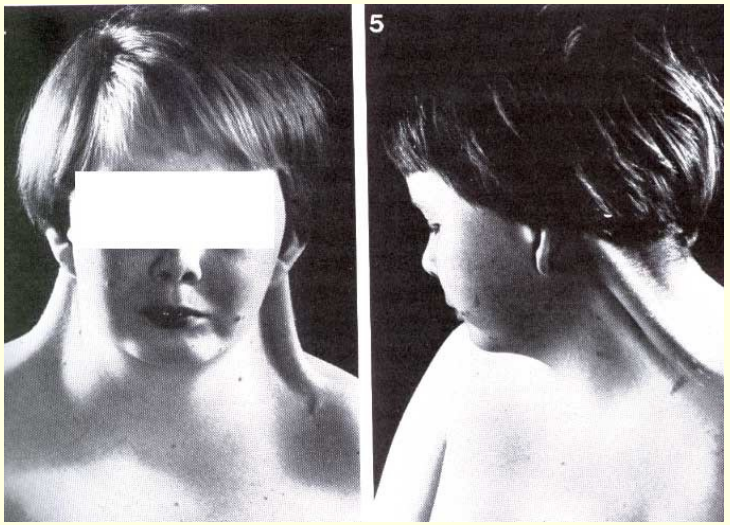


Turner syndrome (45,X)

- lower birth length and weight
- low hairline
- pterigya
- broad chest, widely spaced nipples
- small growth
- infertility, absence of menstrual period
- coarctation of the aorta
- webbed neck
- lymphederma

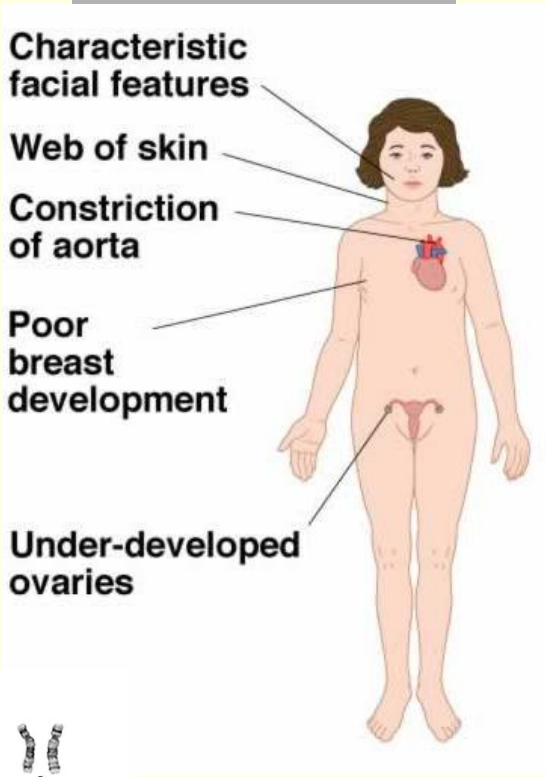




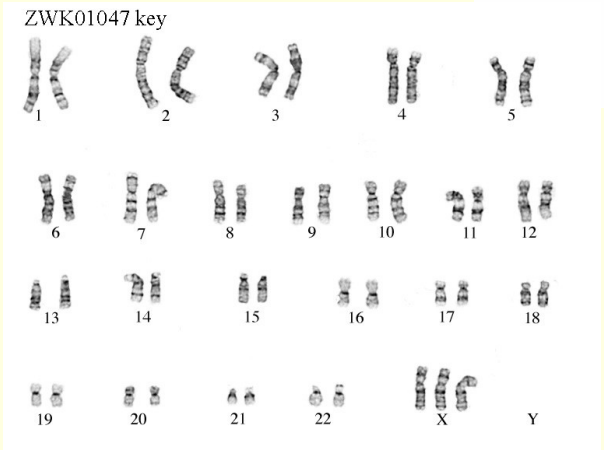


XXX syndrome (47,XXX)

- majority of triple X females are never diagnosed
- normal fertility
- inactivated Barr body
- most often only mild effects
  - tall stature
  - small head
  - speech, language and learning disabilities
  - weak muscle tone



http://pics2.this-pic.com/image/triple%20x%20syndrome



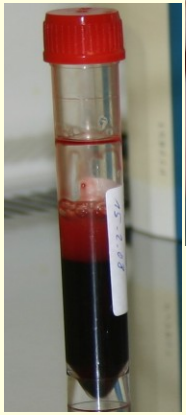
https://www.google.cz/search?q=xxx+syndrome&source=lnms&tbm=isch&sa=X&ei=xMiIUvlHgrG0BpOYgLgL&ved=0CAcQ_AUoAQ&biw=1440&bih=783#facrc=_&imgdii=_&imgrc=Hz1JqrGzTyKpBM%3A%3BfZOiqHDJFB267M%3Bhttp%253A%252F%252Fworms.zoology.wisc.edu%252Fzooweb%252FPhelps%252FZWK01047k.jpg%3Bhttp%253A%252F%252Fwww.zappa.com%252Fmessageboard%252Fviewtopic.php%253Ff%253D5%2526t%253D7057%3B768%3B576

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

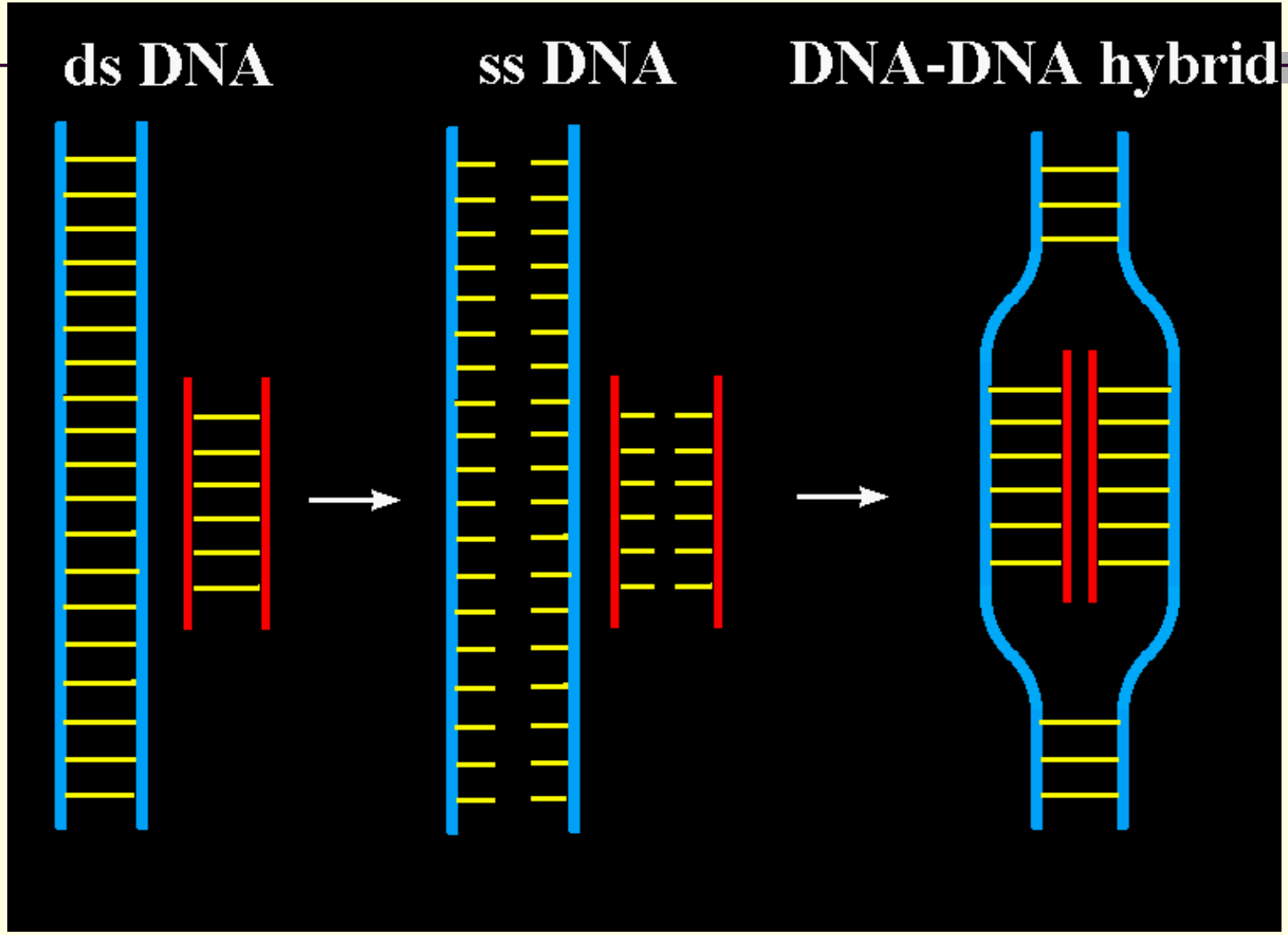
solid tumor



peripheral blood

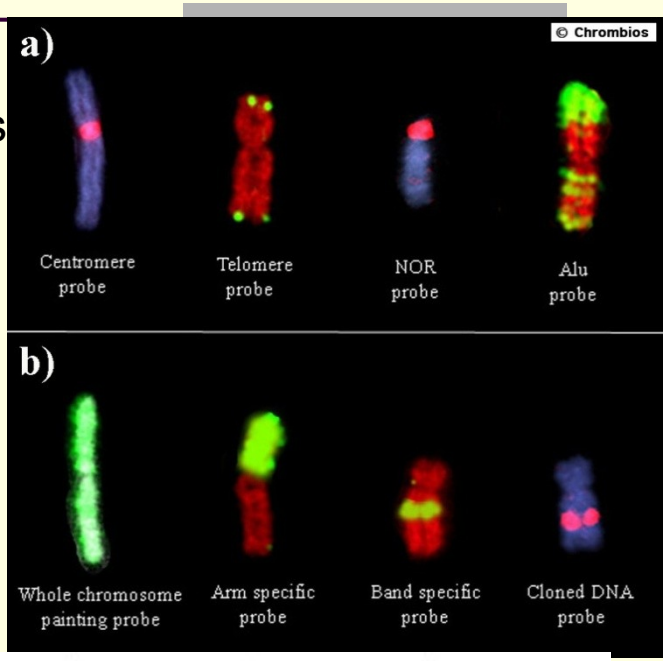
FISH

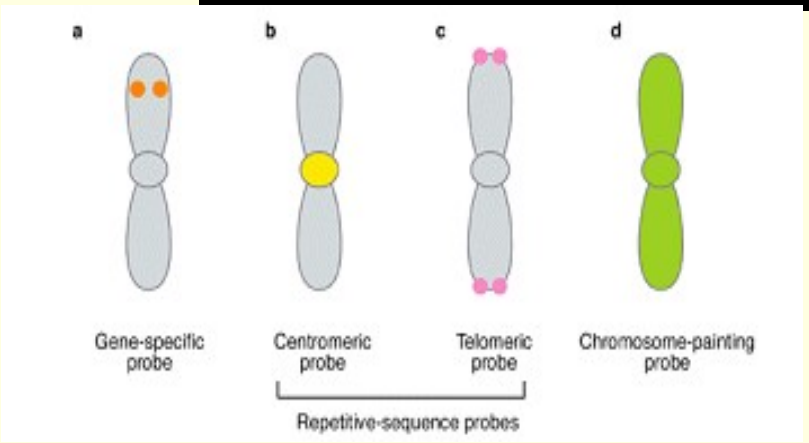
fluorescent in situ hybridization



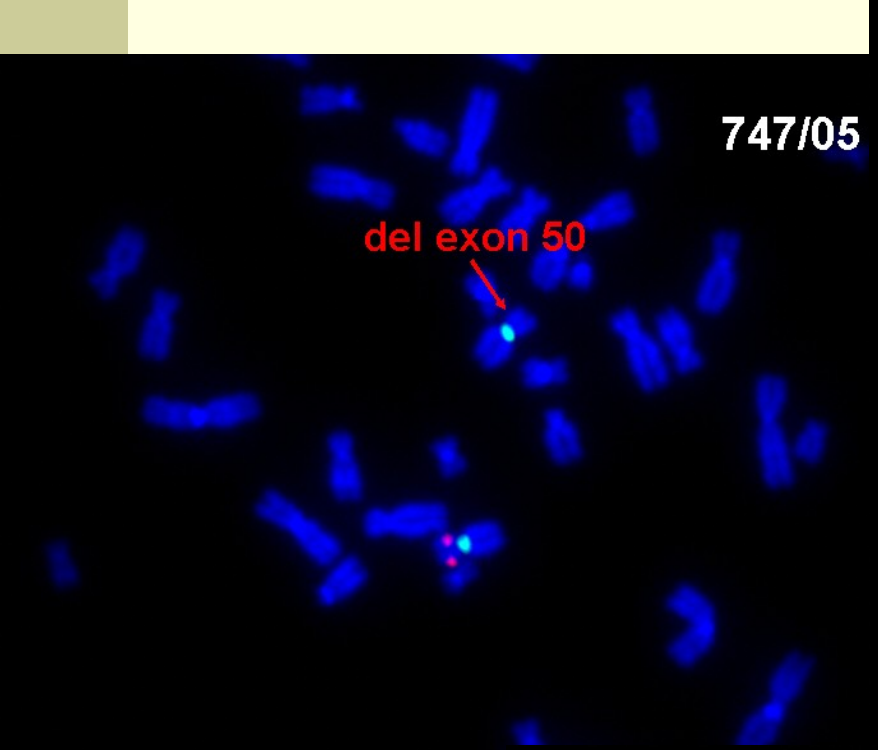
FISH

- 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

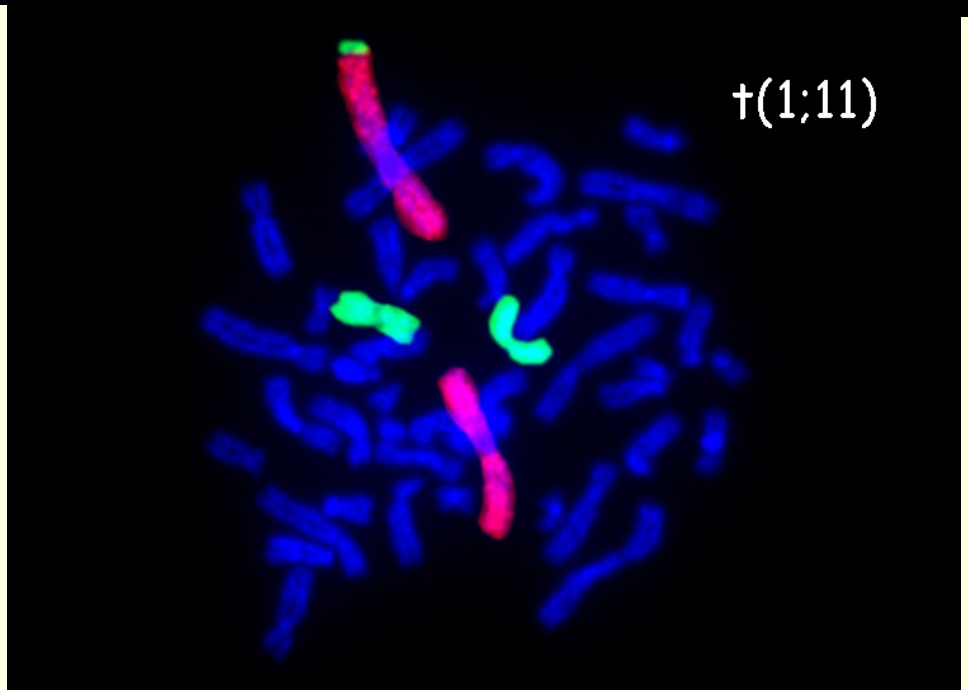




747/05



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

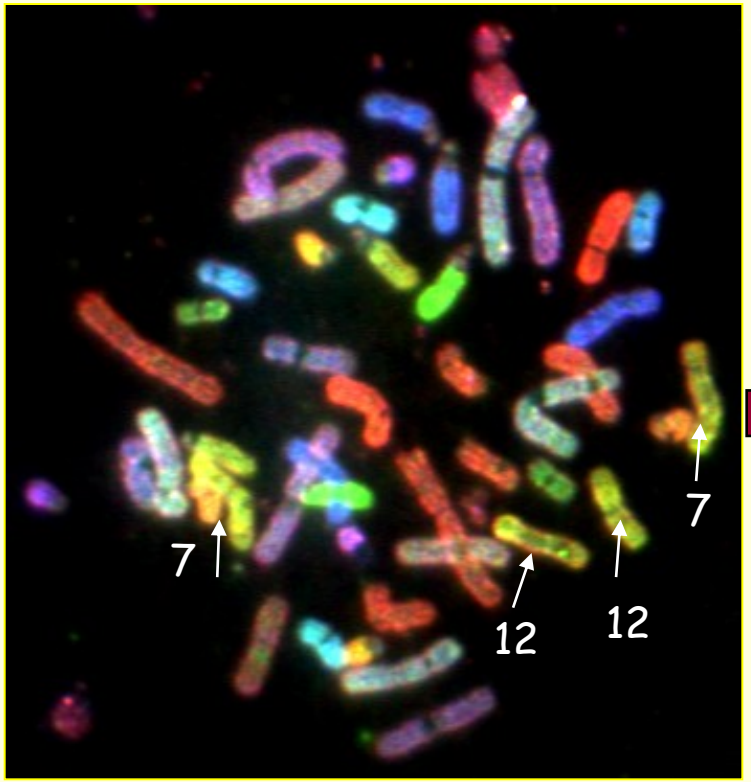
SKY

spectral caryotyping

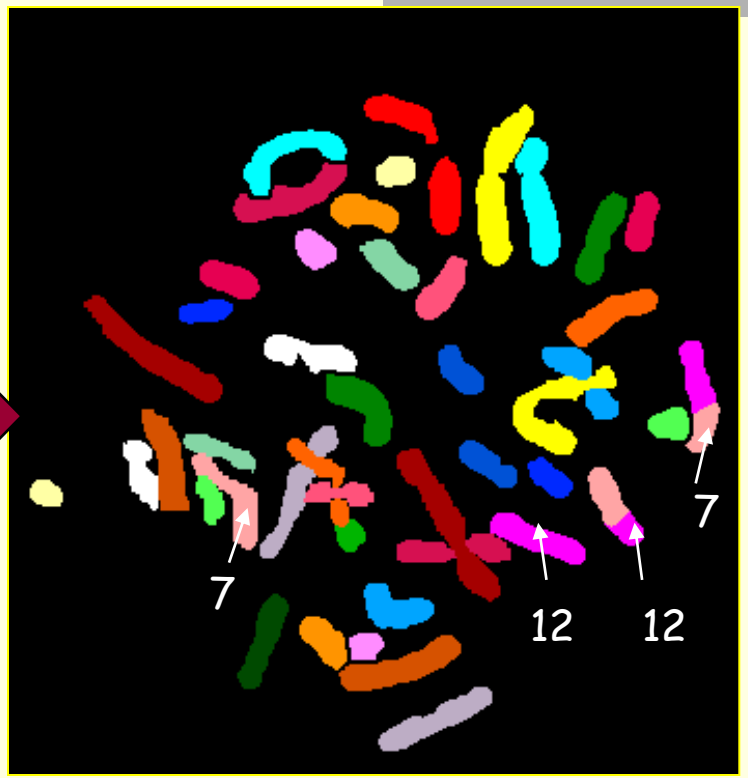
- mikroskop equipped with **2 fluorescent filters (SKY, DAPI)**
- fluorochromes (**FITC** **Rhodamin** **TexasRed** **Cy5** **Cy5.5**) **scanned by one filter**, based on a wave lenght each chromosome pair is coloured ⟶ pseudocoloures

Image Acquisition with SkyVision™

Picture analyse using SkyView

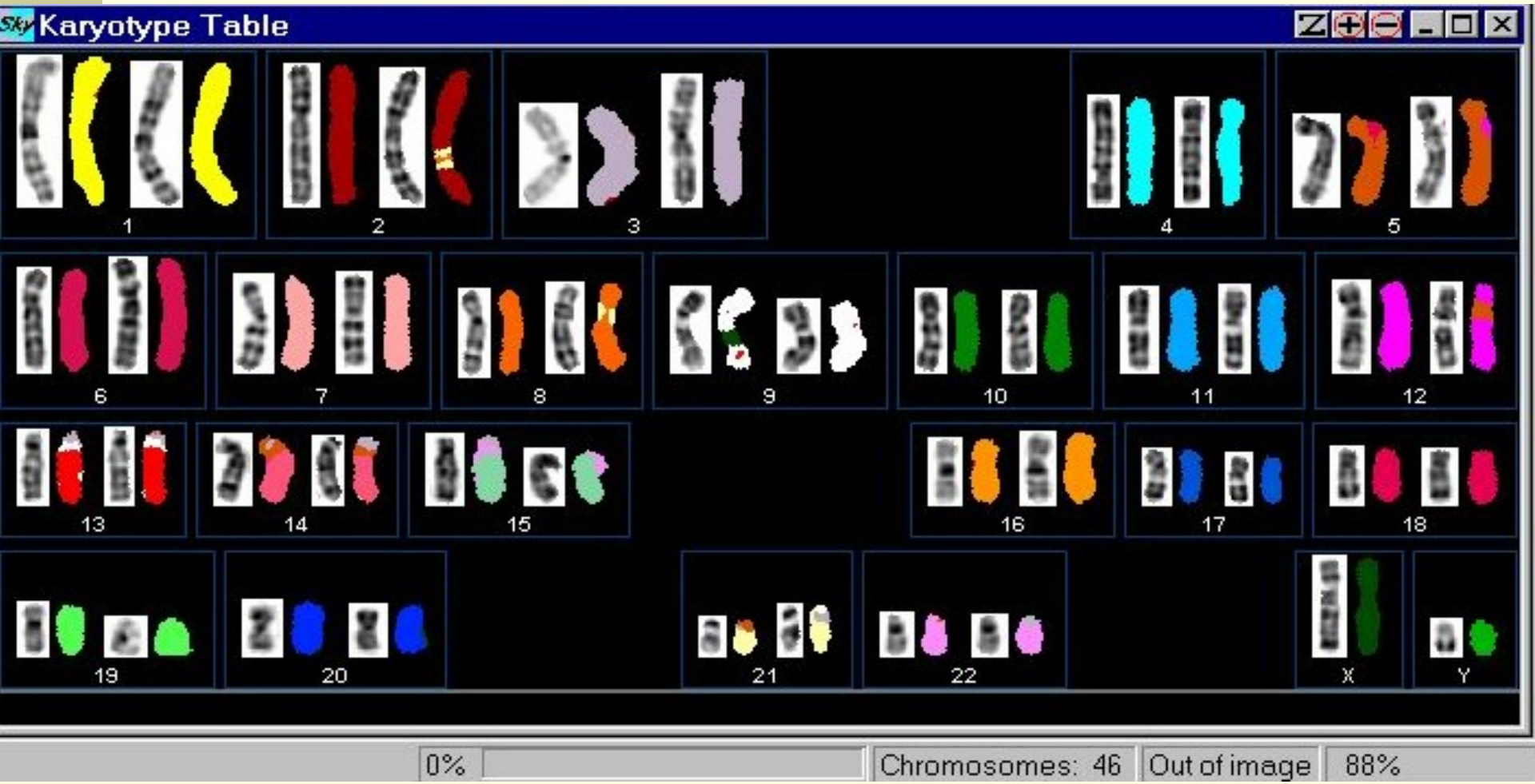


Display Image



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%

Advantages and disadvantages of SKY

- advantages
  - detects balanced rearrangements
  - detects aberations in one step
    - kryptic translocations and insertions
    - marker chromosomes
    - redundant material with unknown origin
    - komplex rearrangements
- disadvantages
  - need of quality mitoses
  - succesful hybridisation
  - expensive method

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

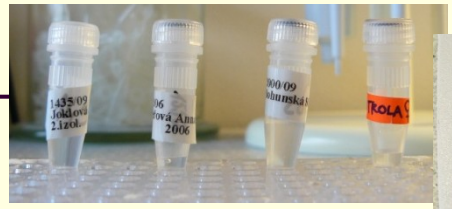
Conventional FISH

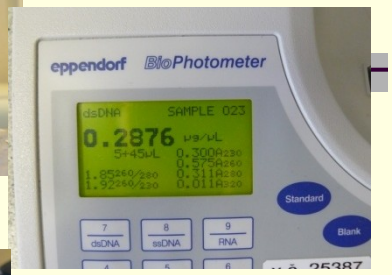
normal DNA → select DNA → make probe → label abnormal target → abnormal target identified

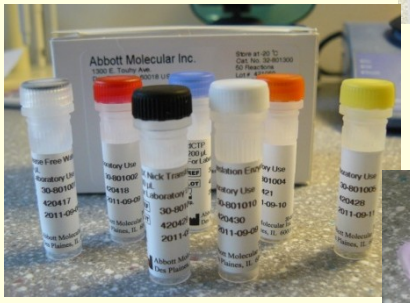
Comparative genomic hybridization

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

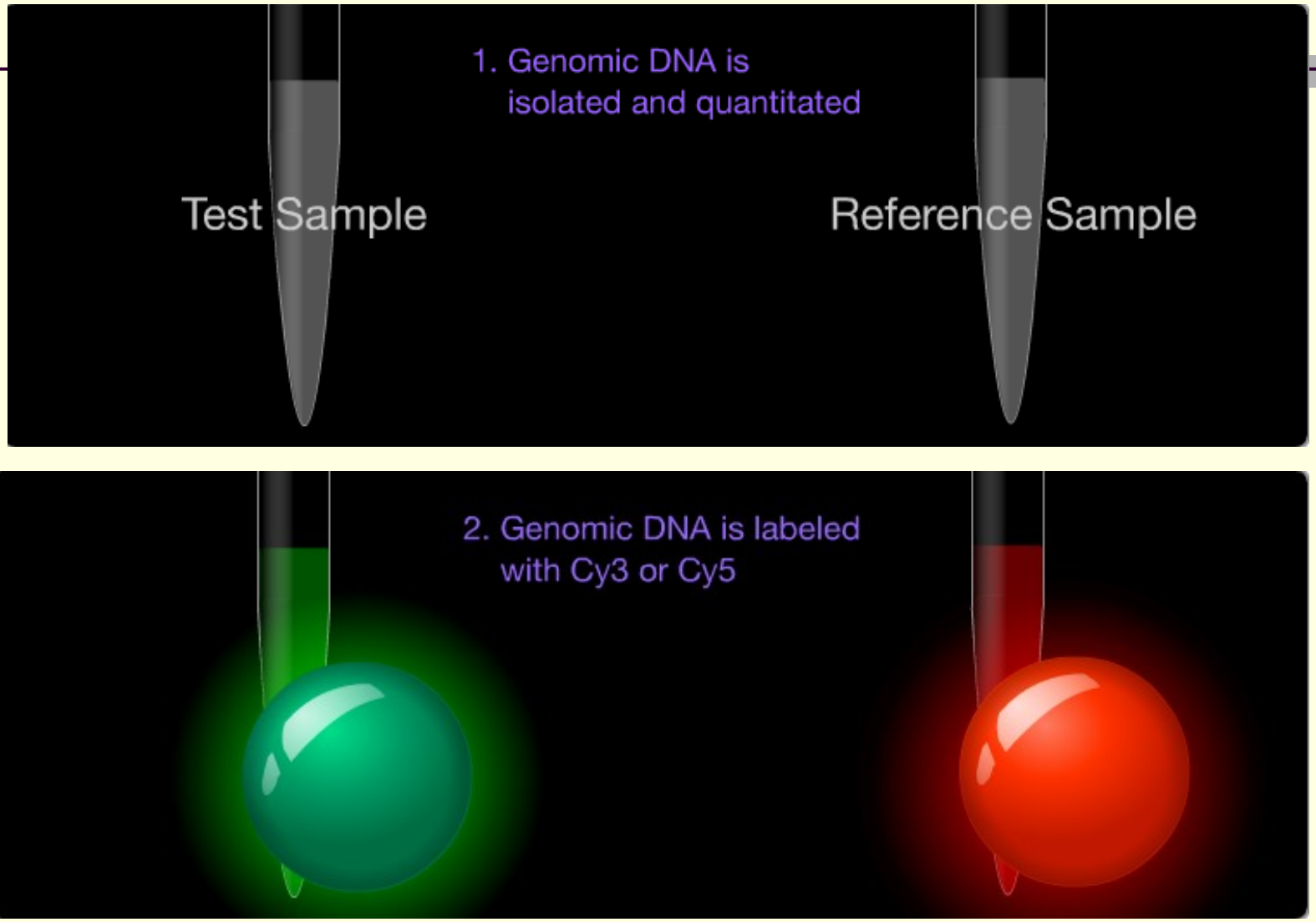
Equipment :

•Fluorescent miroscope (filters DAPI, SpGreen, SpRed)

• Sensitive CCD kamera

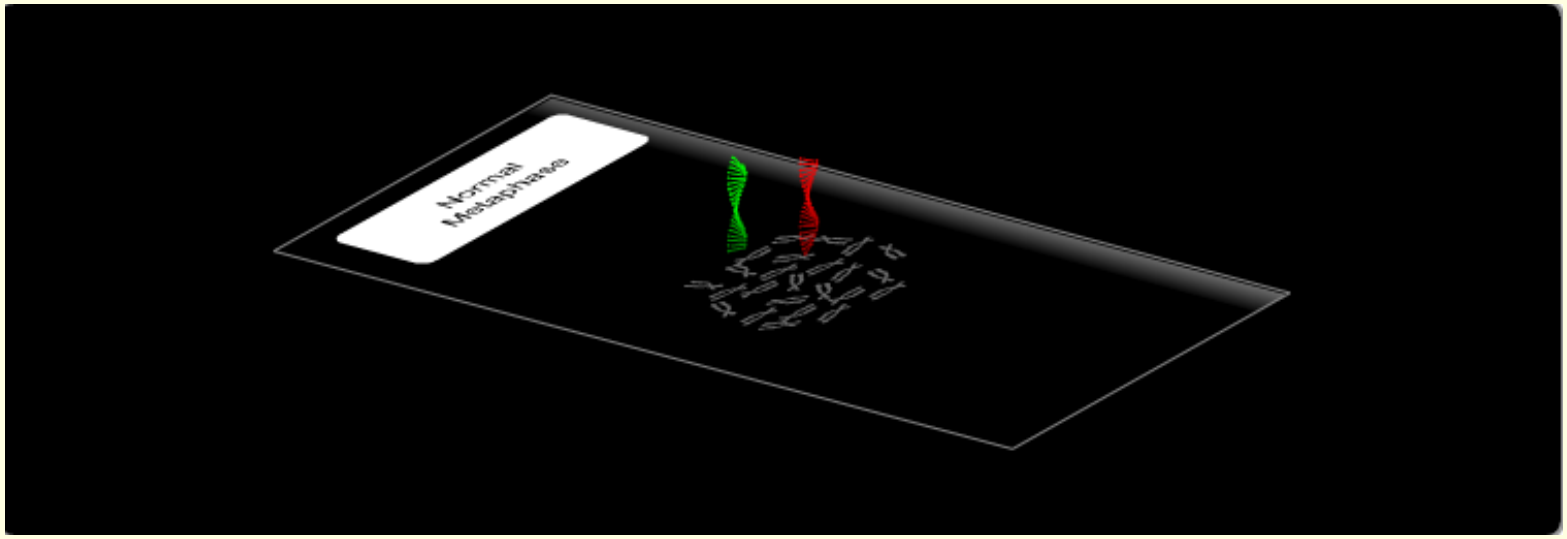
• Computer with software for CGH analysis and data interpretation (LUCIA CGH Advanced Statistics, Laboratory Imaging Ltd., Prague, Czech Republic)

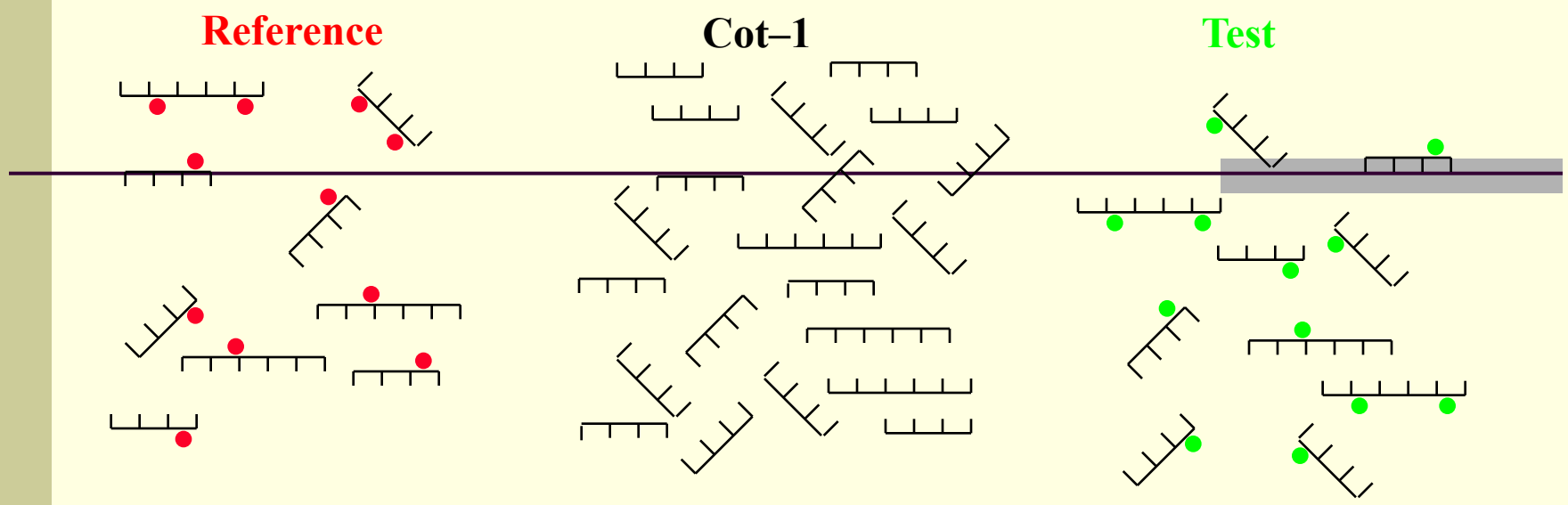
CGH principle



www.abbottmoleculars.com

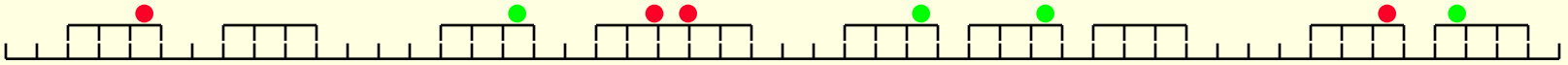
CGH principle





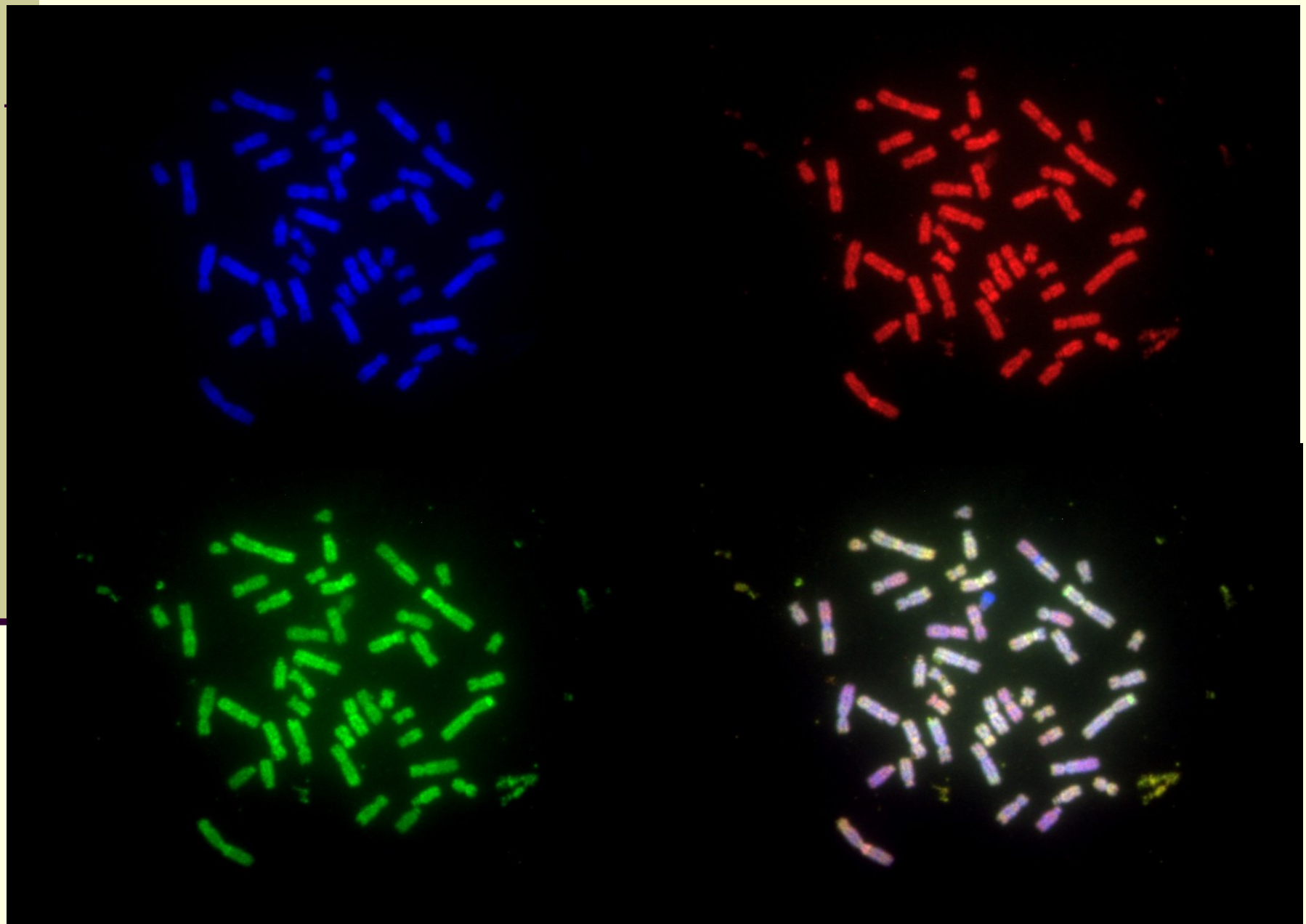
Unique sequences are labeled by *in situ* hybridization

Cot-1 suppresses hybridization of repeat sequences



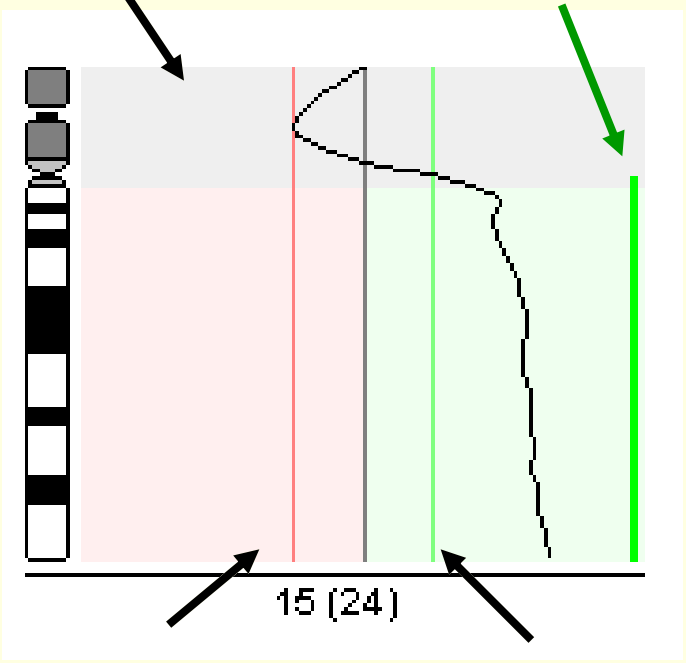
Relative brightness depends on amount of labeled DNA with appropriate complementary sequences, i.e. on the DNA copy number at this locus

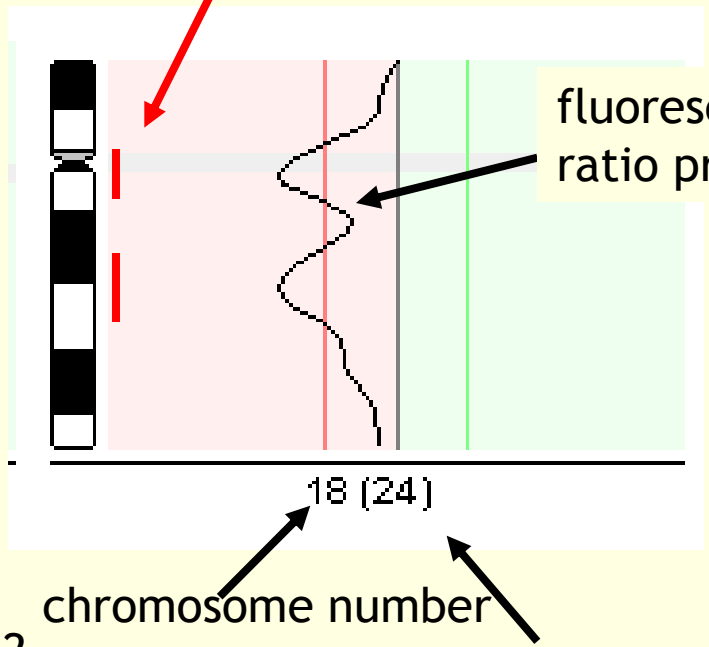
Mitoses scanning, CCD camera filters for B, G, R



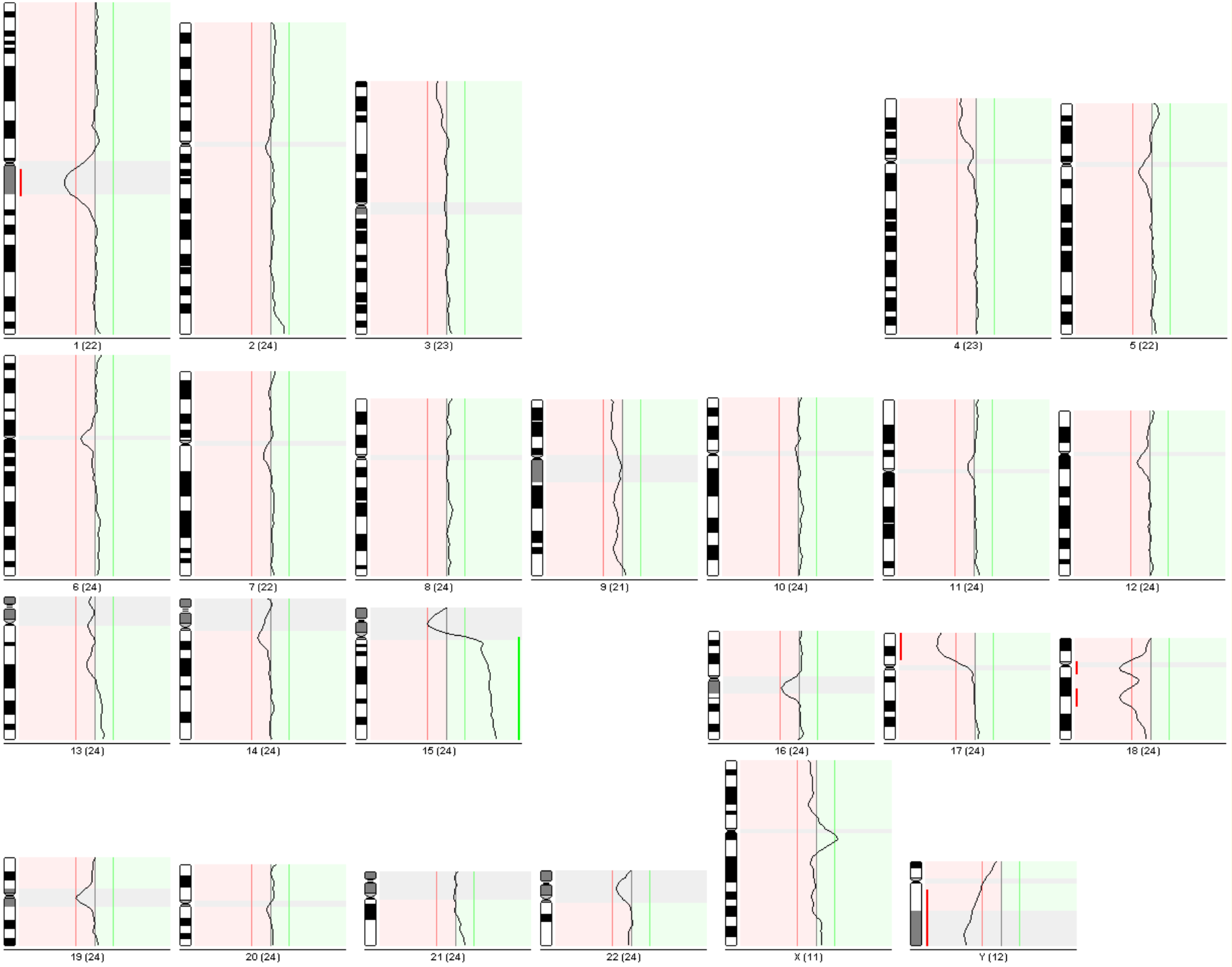
Identification of aberrations

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 not only the chromosome from which the additional unknown material is derived, but also to 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*)

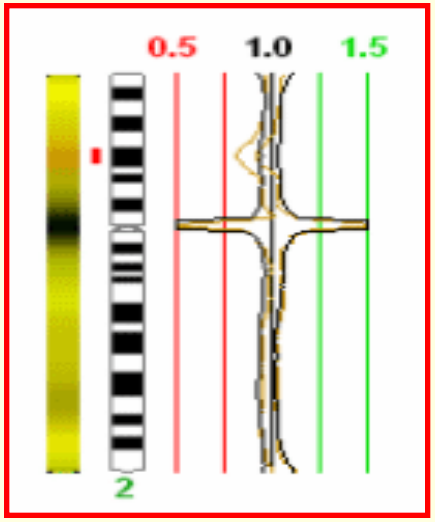
Disadvantages of CGH

- 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 %

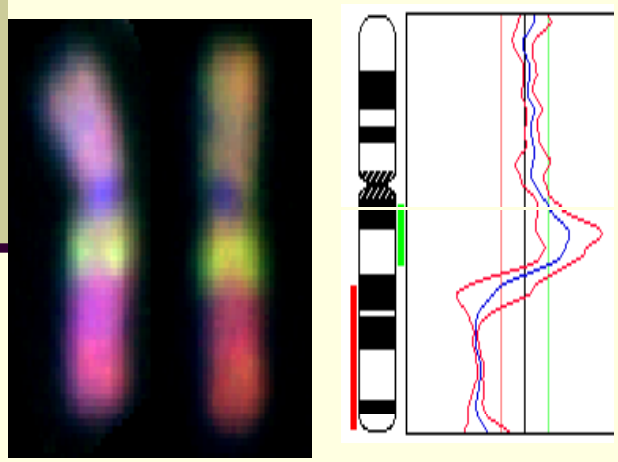


Modifications of CGH

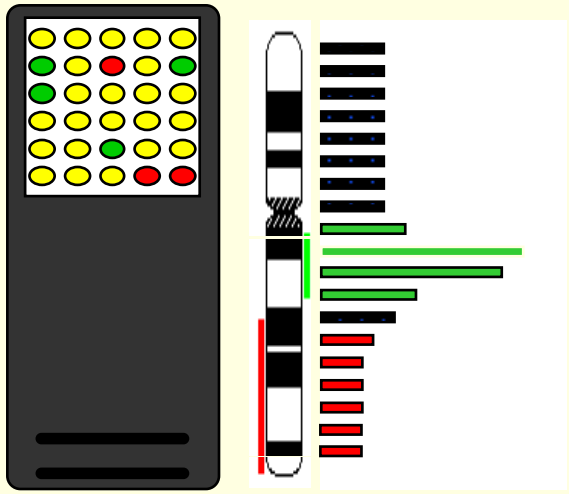
Array-CGH

- 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

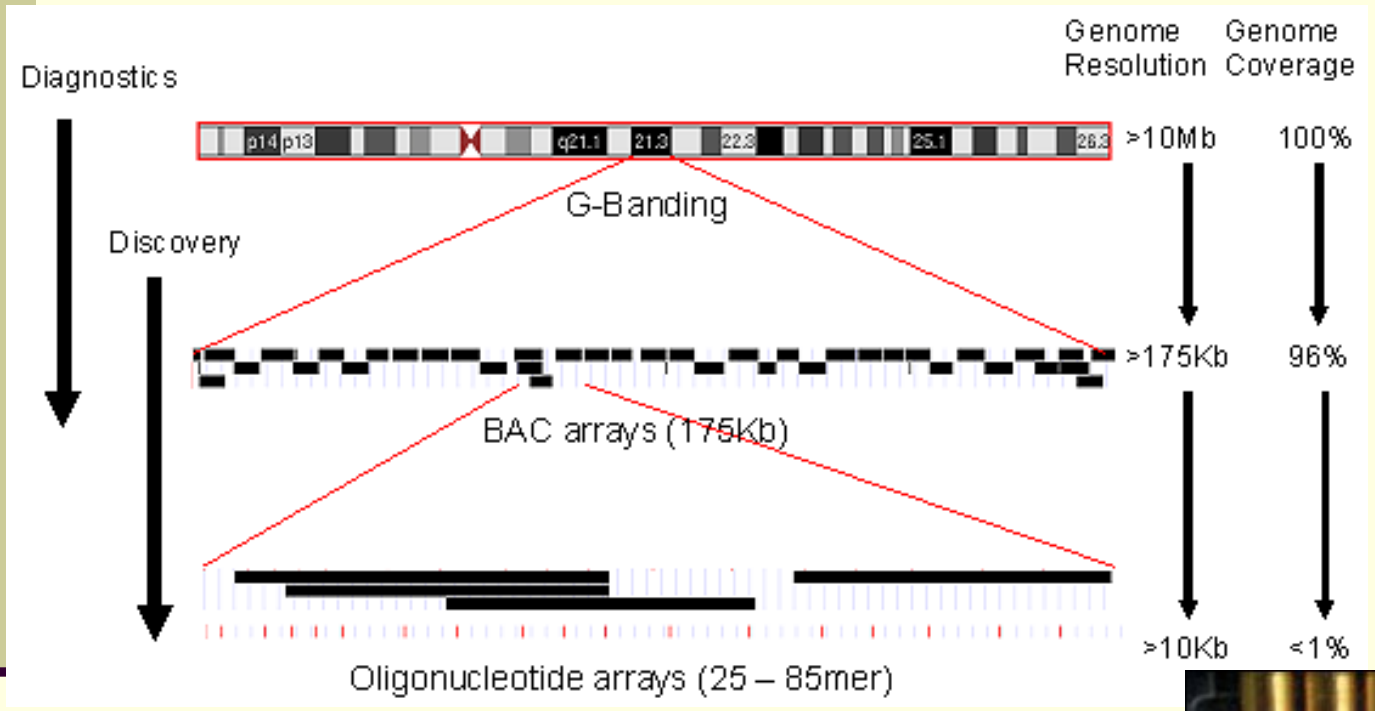


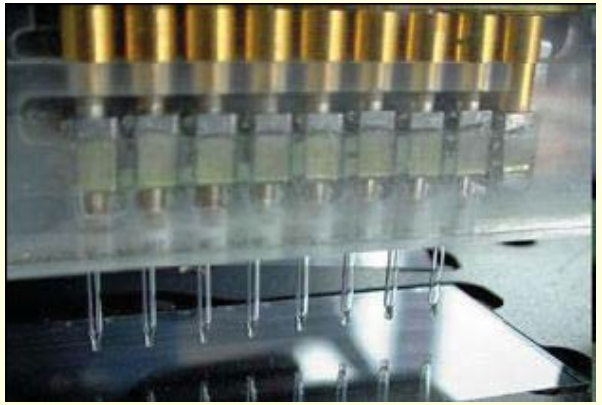
array-CGH



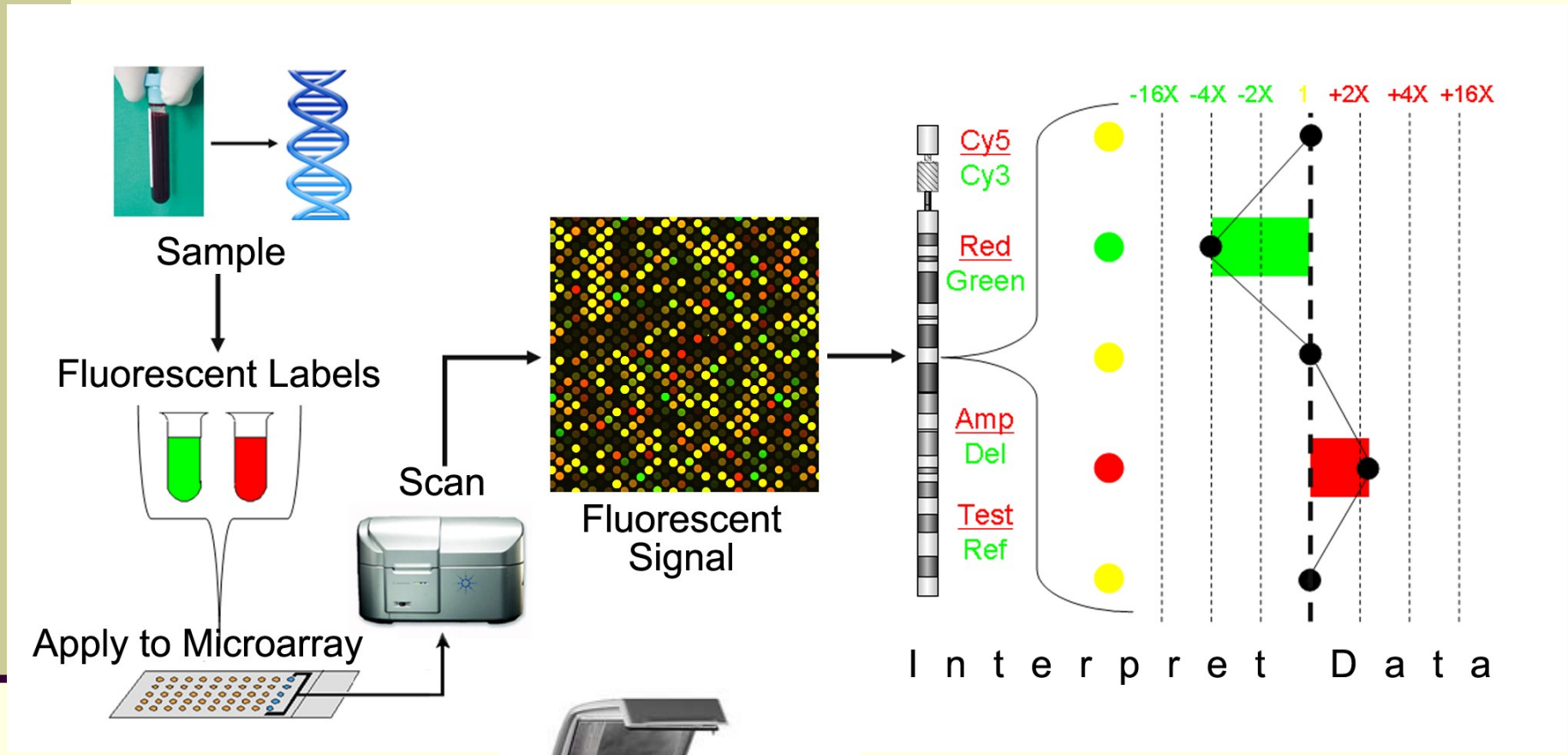
The origin of clones

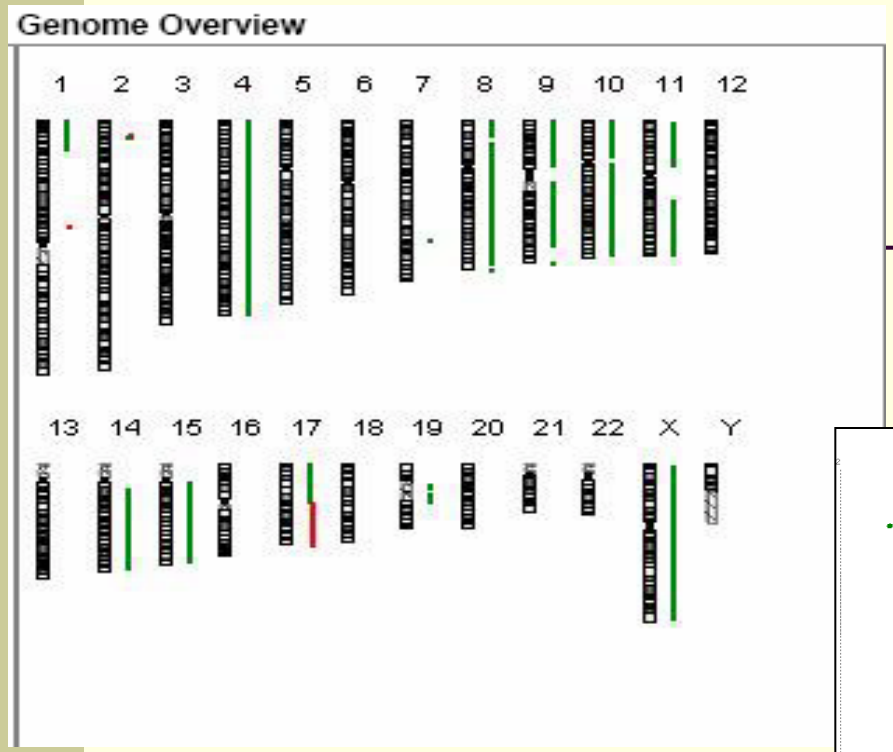
BAC, PAC, c-DNA clones, oligonucleotides

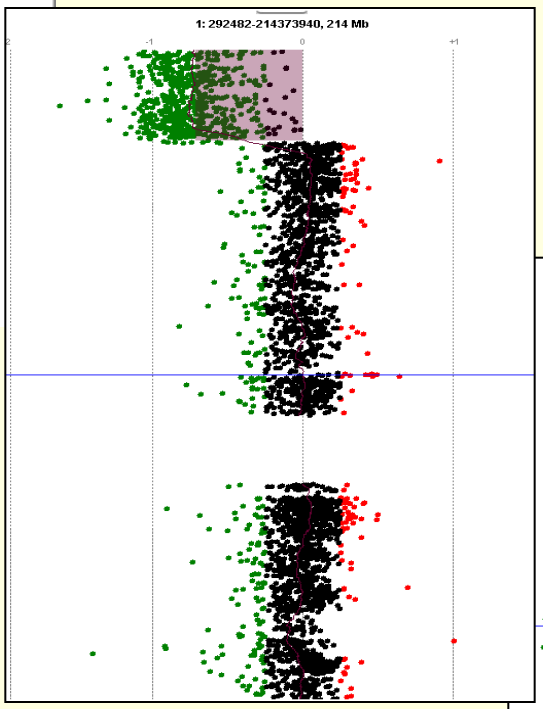


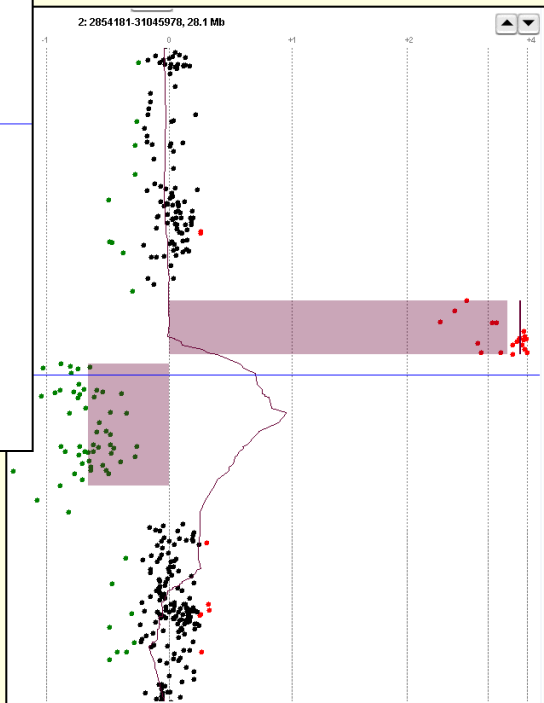


Array-CGH


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

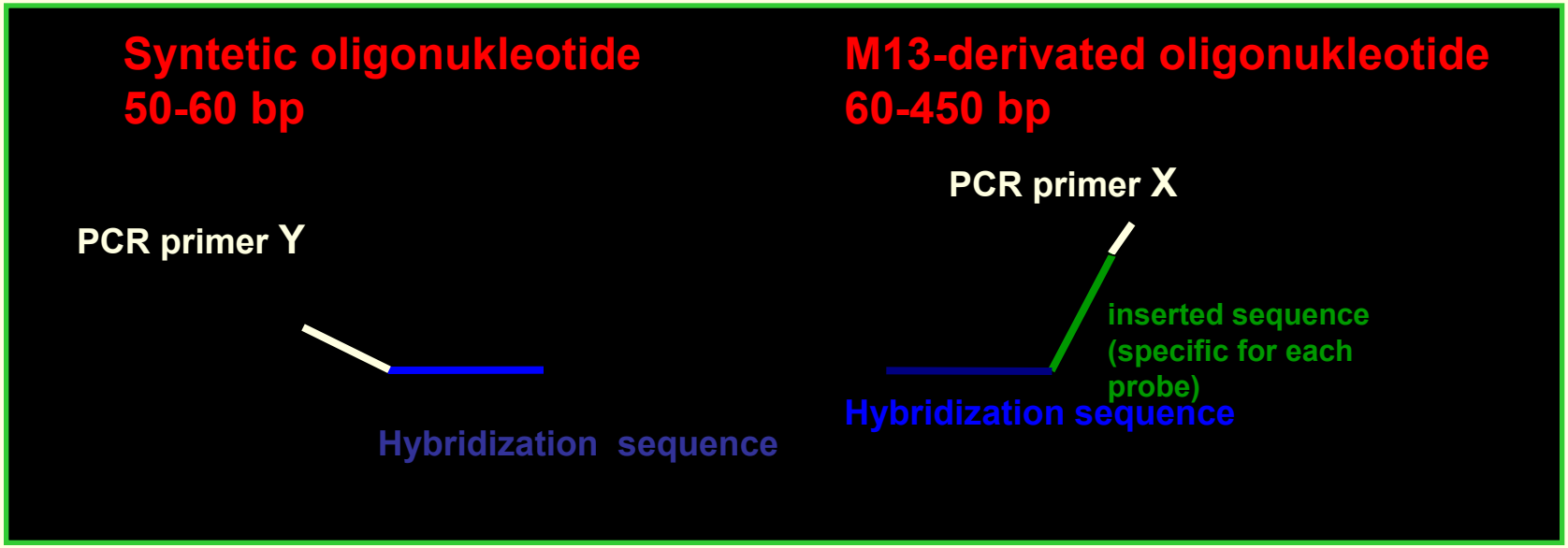
- advantages
  - detects and quantifies DNA copy number gains and losses throughout an entire genome in a single analysis
  - precise aberration locating

- disadvantages
  - does not detect balanced rearrangements (translocation, inversion)
  - does not detect ploidy changes
  - very expensive method

MLPA

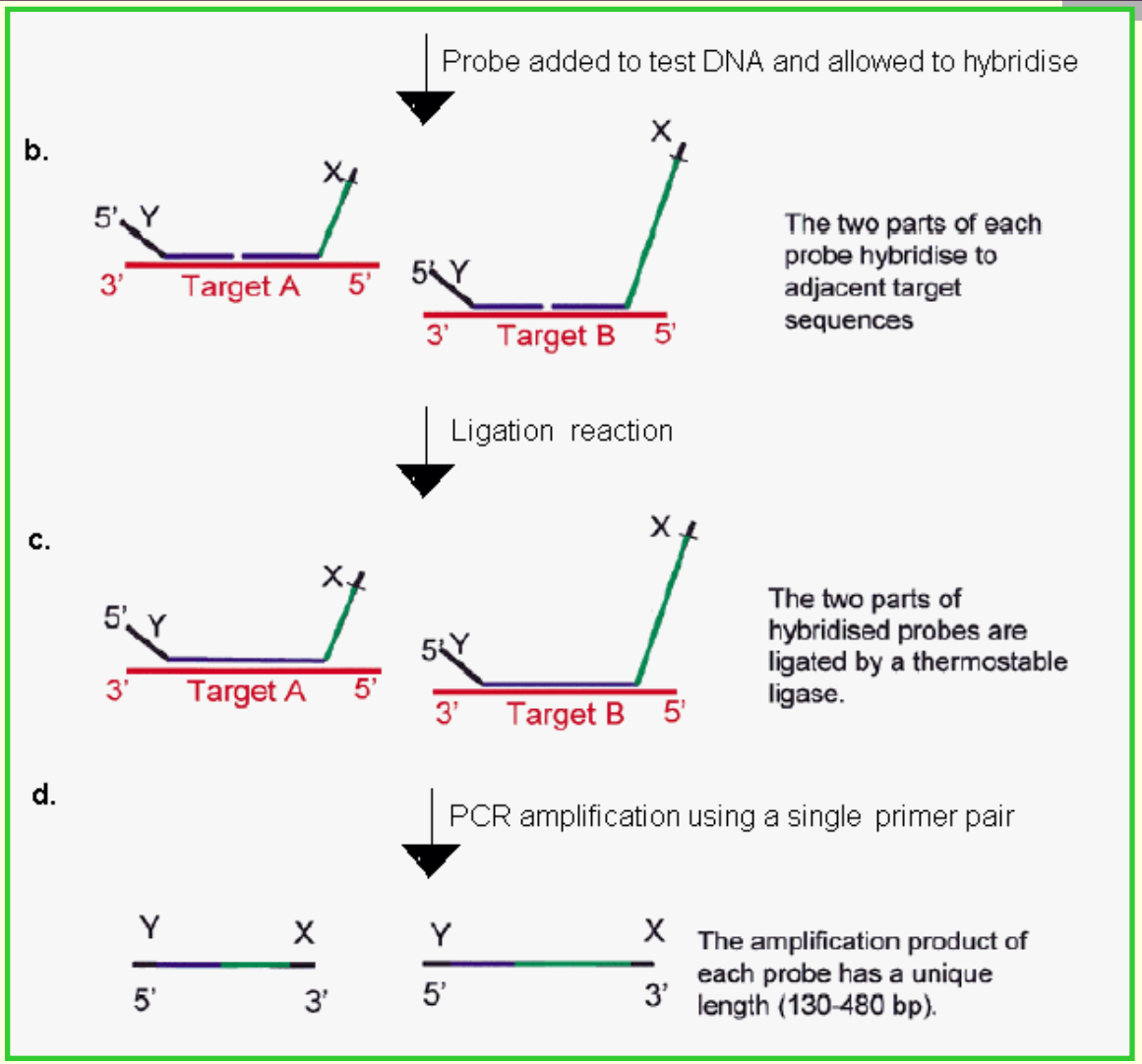
Multiplex Ligation-dependend Probe Amplification

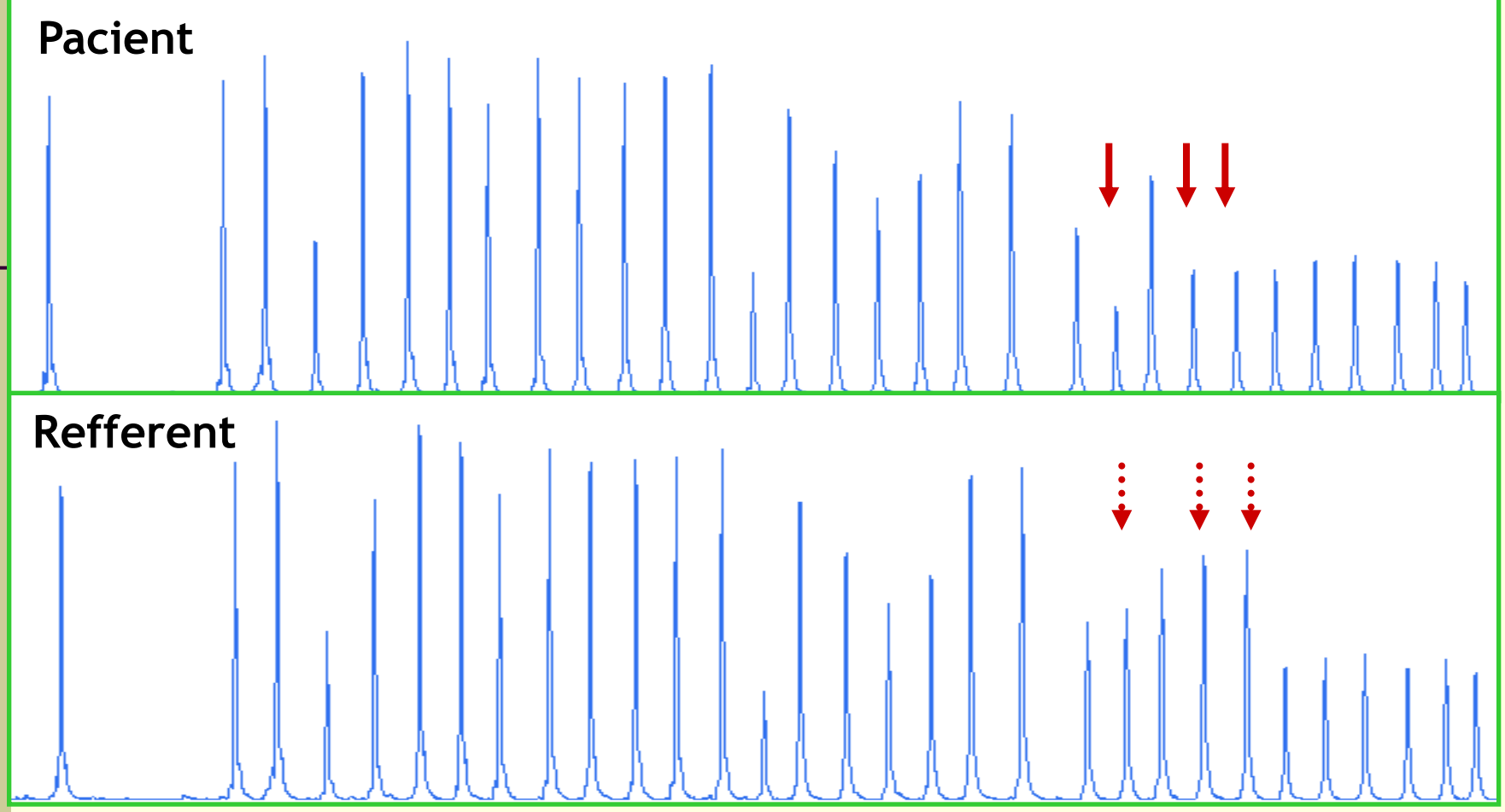
- sensitive method able to detect differences in one nucleotide
- detects changes of copy number in 45 sequences in one reaction
- simple – all the reaction takes place in one test tube
- relatively cheap method

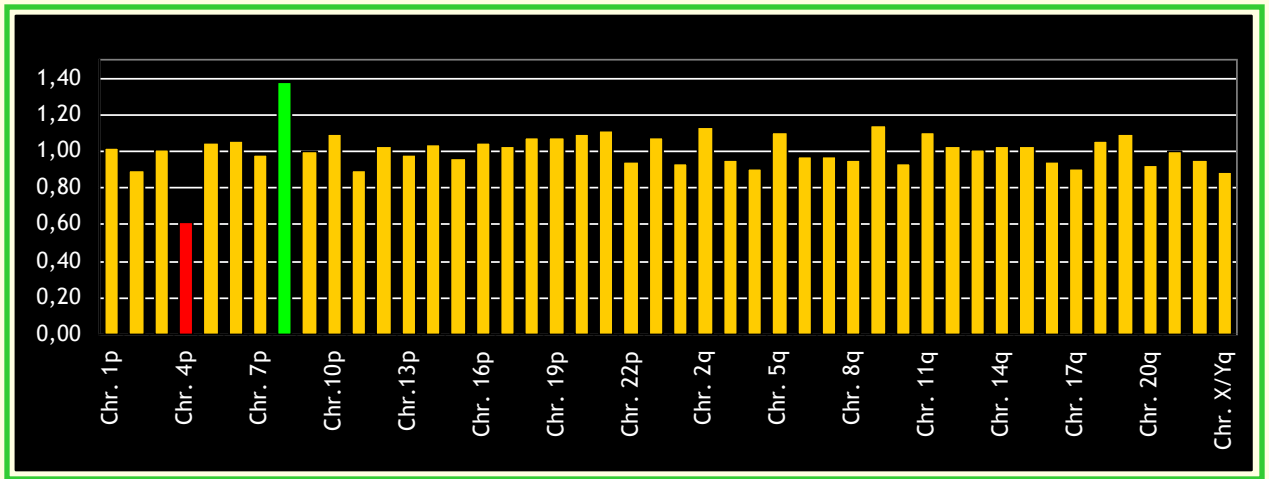


MRC Holland, www.mlpa.com

MLPA principle




Pacient
Refferent

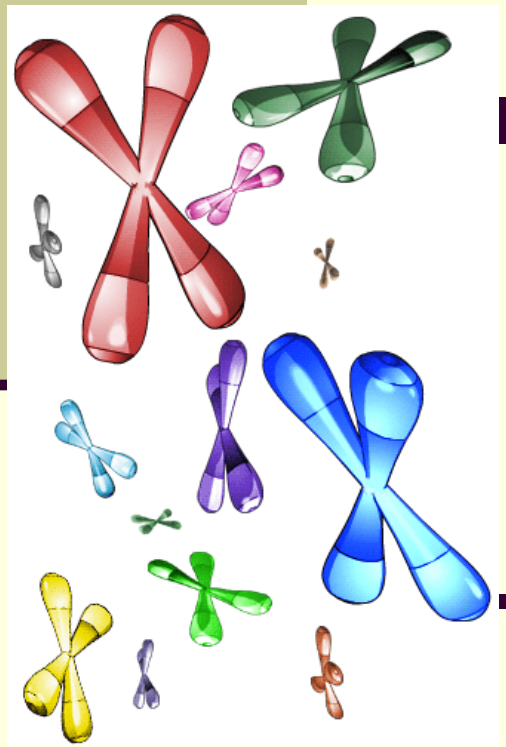

1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
Chr. 1p
Chr. 4p
Chr. 7p
Chr.10p
Chr.13p
Chr. 16p
Chr. 19p
Chr. 22p
Chr. 2q
Chr. 5q
Chr. 8q
Chr. 11q
Chr. 14q
Chr. 17q
Chr. 20q
Chr. X/Yq

Advantages and disadvantages of MLPA

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

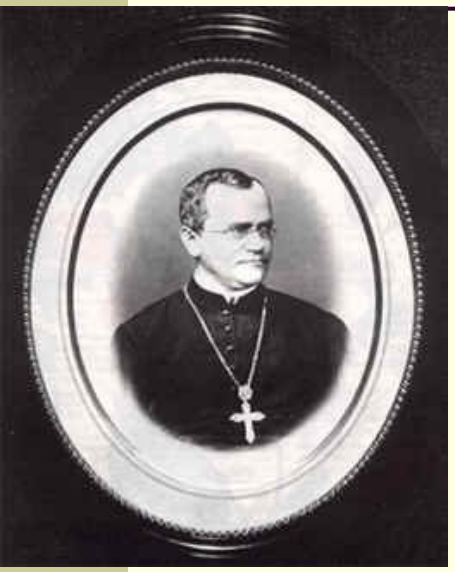
- disadvantages
  - higly sensitive to contamination
  - time difficulty
  - the aberation have to occur in 50% of cells
  - some mutations or polymorphismus can lead to false results

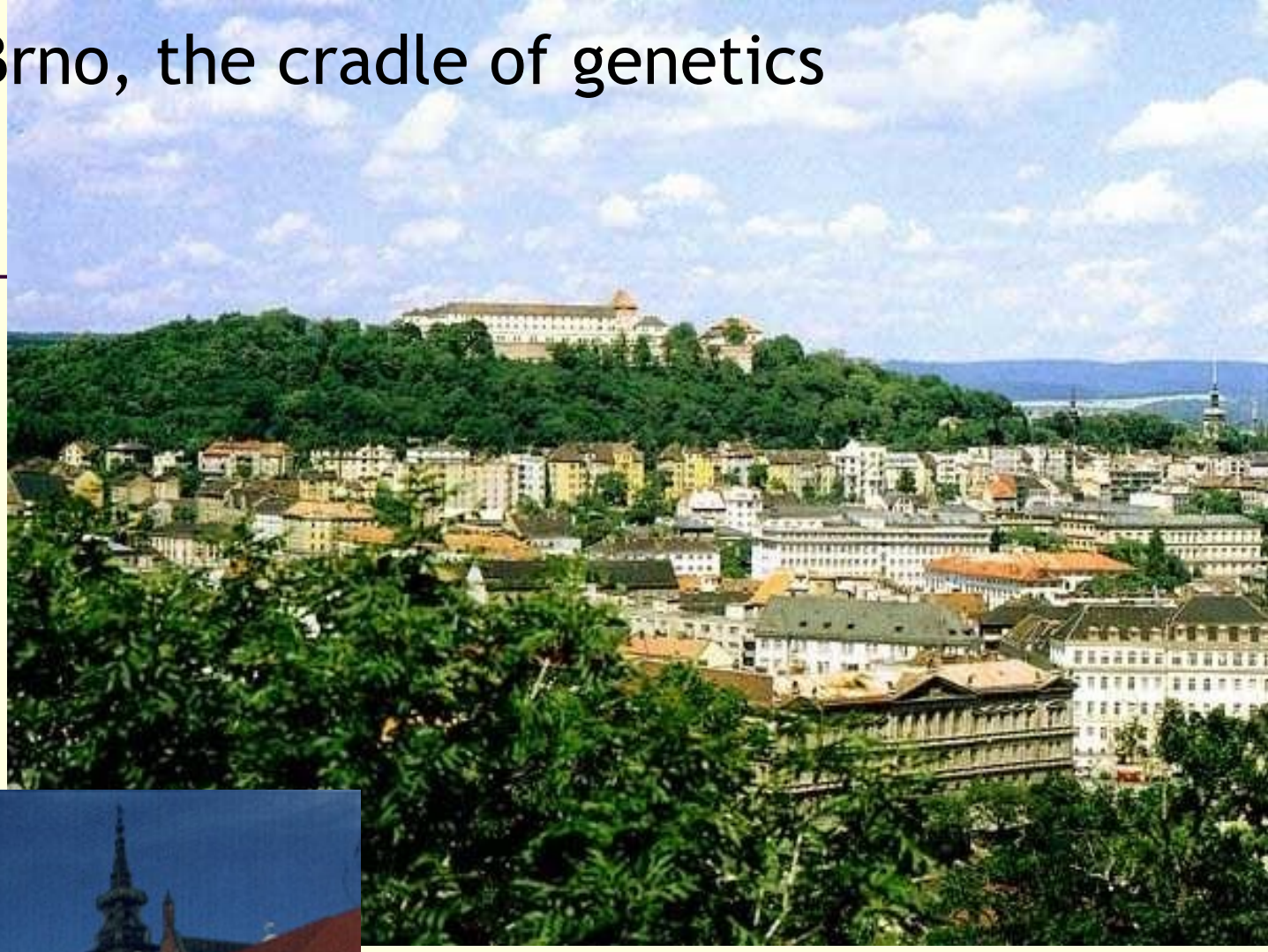
5. Integrated laboratory of molecular cytogenetics, Brno



http://www.cba.muni.cz/cytogenlab

Brno, the cradle of genetics





Augustinian monastery in Brno – place of G. J. MENDEL s work

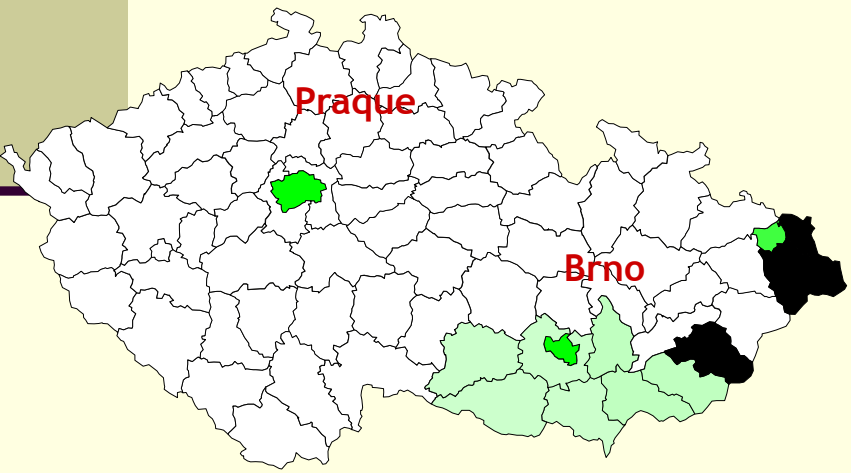
Who are we?

Integrated laboratory of molecular cytognetics is an integrated clinical and research centre, which is a result of co-operation among:

- Dept. of Genetics and Molecular Biology, Inst. of Experimental Biology, Faculty of Science, Masaryk University
- Dept. of Medical Genetics, University Hospital Brno
- University Research Centre – Czech Myeloma Group Brno

URC-CMG
UNIVERSITY RESEARCH CENTRE
CZECH MYELOMA GROUP

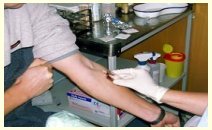
Department of Medical Genetics, University Hospital Brno: the centre for genetic investigation for South Moravia region



Genetic consulting

Cytogenetic laboratories

Laboratory of DNA diagnosis

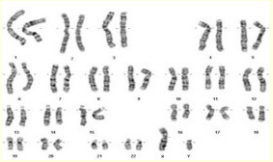


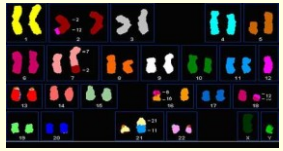
prenatal

postnatal

oncogenetic

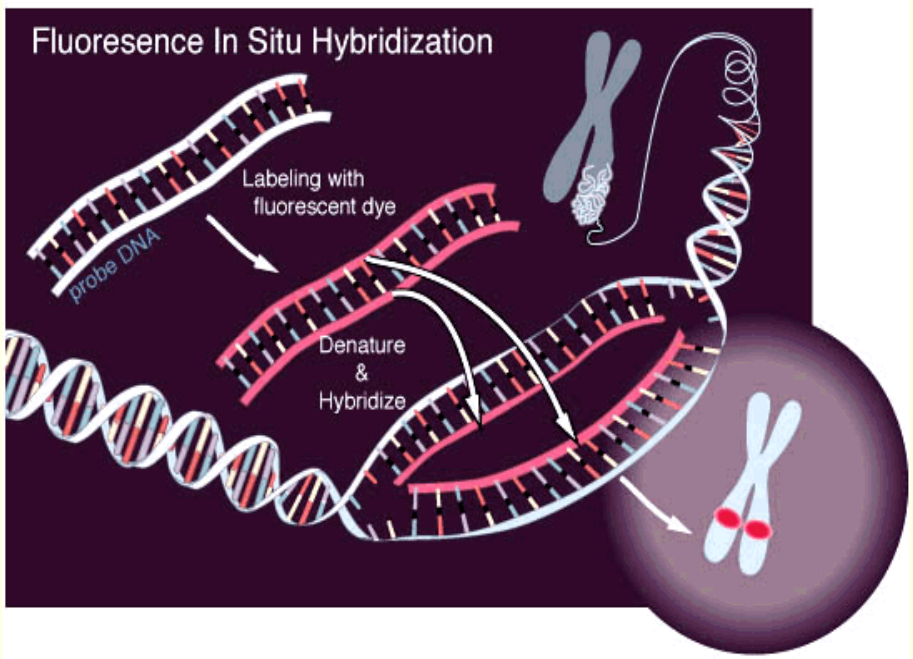
molecular cytogenetic



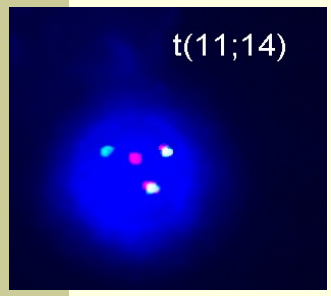


What is our interest?

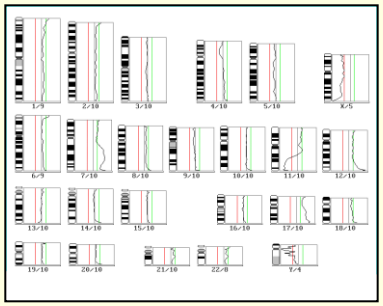
The main interest of the Integrated laboratory is the research of chromosomal aberrations using **molecular cytogenetic techniques.**

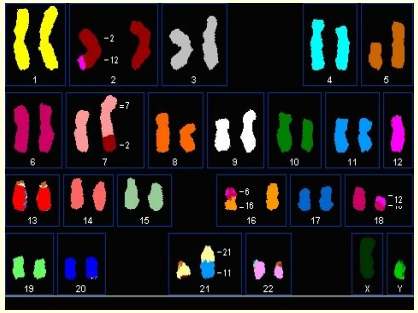


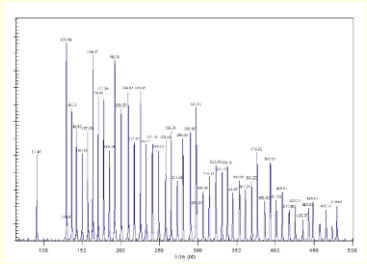
Methods

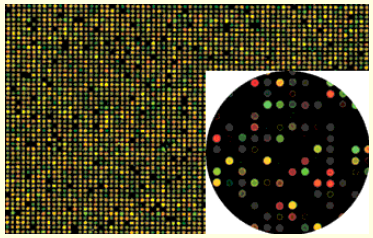


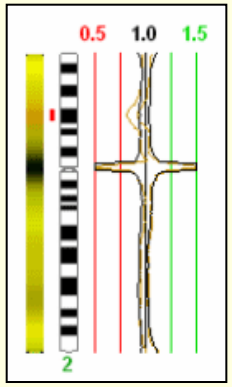
- Fluorescence *in situ* hybridization (FISH)
- Spectral karyotyping (SKY)
- Comparative genomic hybridization (CGH)
- High resolution CGH (HR-CGH)
- Array-CGH (*Agilent*)
- MLPA











The equipment

Classical Cytogenetics, FISH, CGH/HR-CGH

- Microscopes – Olympus BX61
- CCD cameras Voskuhler
- Digital Image Analysis System (LUCIA, LIM Ltd.):
  - LUCIA-KARYO
  - LUCIA-FISH
  - LUCIA-CGH/CGH Advanced Statistics

System for SKY (SKY View – Applied Spectral Imaging Ltd, Israel)

System for array-CGH: Agilent Scanner

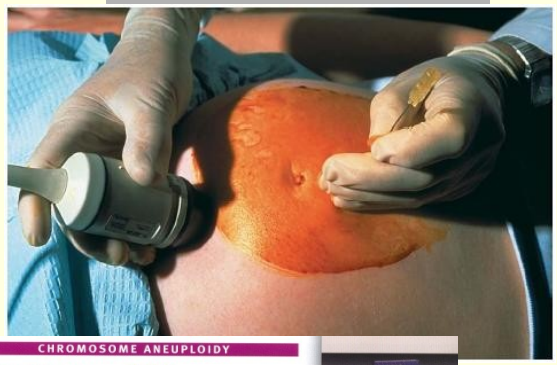
System for MLPA: capillary electrophoresis Beckman Coulter

Molecular cytogenetic investigations at Department. Of Medical Genetics

- **Prenatal cytogenetic diagnosis**
- **Postnatal cytogenetic analyses**
- **Cancer cytogenetic analyses**

Prenatal cytogenetic analyses

- **Uncultered and cultured amniotic cells, fetal blood, chorion villi**
- **I-FISH**



AneuVysion Assay Kit (Abbott Vysis)

Mix1:

- **CEP 18 Sp. Aqua**
- **CEP X Sp. Green**
- **CEP Y Sp. Orange**

Mix 2:

- **LSI 21 Sp. Orange**
- **LSI 13 Sp. Green**

Microdeletion syndromes (DiGeorge)

2

CHROMOSOME ANEUPLOIDY

DNA FISH Probes for Prenatal, Postnatal and Preimplementation Genetics

ANEUVYSION® FDA CLEARED

AneuVysion Assay Kit
33-161075 – 10 Assays
32-161075 – 30 Assays
35-161075 – 50 Assays (IVD)
(control slides not included)

The AneuVysion Assay, a Cellular Genomics assay utilizing patented fluorescence *in situ* hybridization (FISH) technology, is a prenatal test that provides a rapid (24 to 48 hour) method for detection of trisomy 13, 18, 21 (Down Syndrome) and aneuploidy of sex chromosomes X and Y. The FDA clearance for the AneuVysion Assay allows for immediate reporting of test results consistent with ACMG guidelines.

The AneuVysion Assay provides results from uncultured amniocytes within 24 to 48 hours. Rapid detection of common fetal trisomies and sex chromosome aneusomies is especially important in high risk pregnancies and medically indicated situations. These situations may include: positive maternal serum screen, abnormal fetal ultrasound, late gestation pregnancies and other medical indications requiring rapid decision making.

The AneuVysion Assay accurately detects 99.9% of all aneuploid specimens evaluated in the international, 31-site, collaborative clinical study (data on file). In a published review of experience in over 29,000 prenatal cases, the AneuVysion Assay was 99.9% accurate for the detection of aneuploidies in informative cases.

The AneuVysion Assay Kit Includes:
5 DNA probes in a packaged set of two probe mixtures (#1 and #2) that are pre-denatured, pre-mixed in hybridization buffer and ready to apply to the denatured amniocyte specimen slide. In addition, reagents for hybridization washes and a package insert are provided.

Probe Mixture #1 (3 probes)
CEP 18: SpectrumAqua D18Z1, alpha satellite DNA (18p11.1-q11.1).
CEP X: SpectrumGreen DXZ1, alpha satellite DNA (Xp11.1-q11.1).
CEP Y: SpectrumOrange DYZ3, alpha satellite DNA (Yp11.1-q11.1).
Probe mixture #1 is complete with probes, blocking DNA, fluorophore-labeled total human genomic DNA and hybridization buffer. The fluorophore-labeled total human genomic DNA acts as a counterstain when viewing the probes using single or triple bandpass filters. See FISH Microscope Filter section.

Probe Mixture #2 (2 probes)
LSI 21: SpectrumOrange, loci D21S259, D21S341, D21S342 (21q22.13-q22.2).
LSI 13: SpectrumGreen, spans the Retinoblastoma gene (RB1);(13q14). Probe mixture #2 is complete with probes, blocking DNA and hybridization buffer.

For a complete listing of references, please visit www.vysis.com.

ProbeChek Male Amniocyte Control
30-805010 – 5 Slides
This control slide is prepared from a normal cultured male amniocyte cell line that is harvested, fixed in suspension medium and applied to glass microscope slides using a method optimal for interphase FISH.

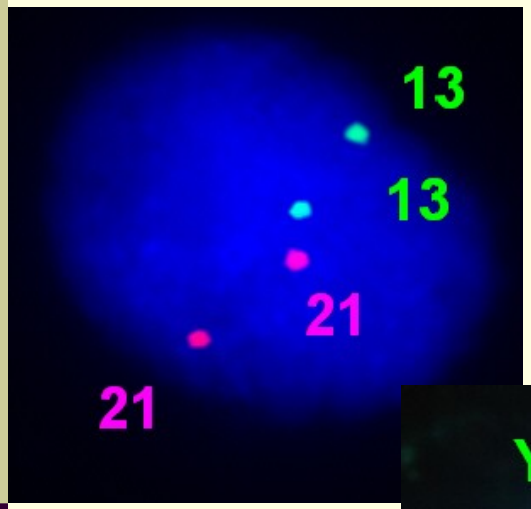
ProbeChek Positive Control
30-805017 – 5 Slides
This control slide is prepared from a cultured cell line that is harvested, fixed in suspension medium and applied to glass microscope slides using a method optimal for FISH. The slides (after hybridization with the AneuVysion Assay) will provide a result that shows cells that are aneuploid for chromosomes 13, 18, 21, X and Y. This control provides an excellent training and validation tool for the AneuVysion Assay.

18p11.1-q11.1 CEP 18 alpha satellite SpectrumAqua
Xp11.1-q11.1 CEP X alpha satellite SpectrumGreen
Yp11.1-q11.1 CEP Y alpha satellite SpectrumOrange
13q14 LSI 13 SpectrumGreen
21q22.13-q22.2 LSI 21 SpectrumOrange

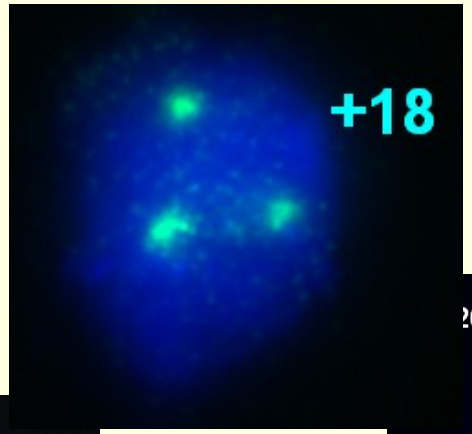
18

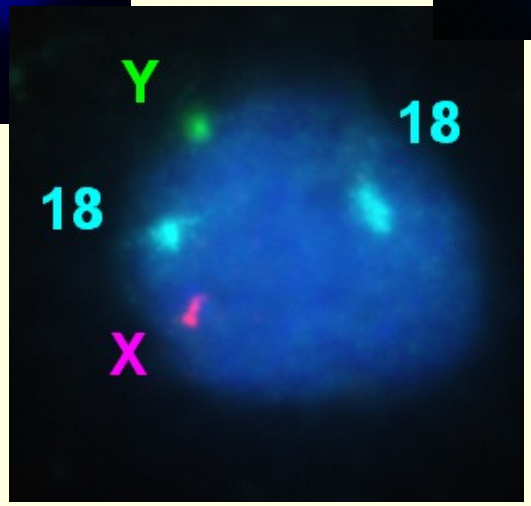
VYSIS PRODUCT CATALOG Phone 800-553-7042, extension 1

Prenatal cytogenetic analyses



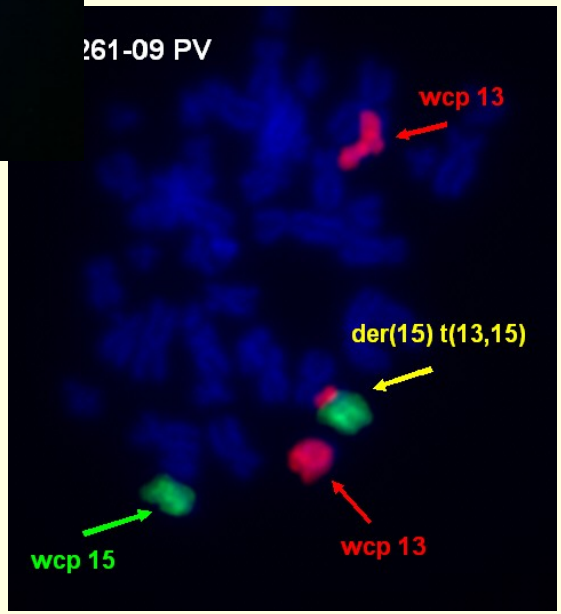
Normal cells





trizomy
of chr. 18

t(13;15)



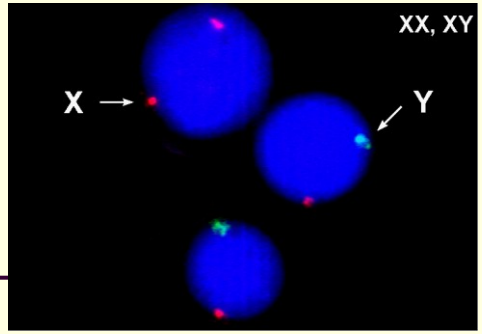
Postnatal cytogenetic analyses

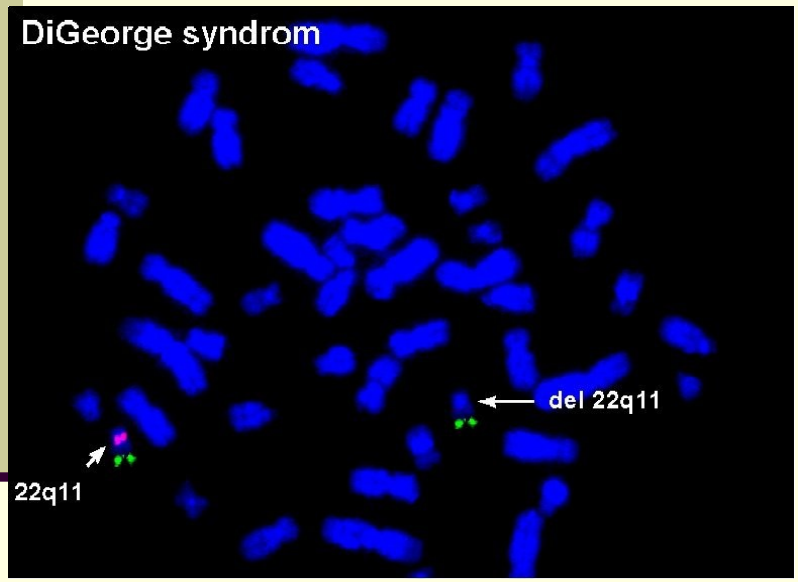
- **Peripheral lymhocytes, buccal swab**
- **FISH, CGH, HR-CGH, array-CGH, MLPA, SKY**

ToTel Vysion Kit, Abbott-Vysis

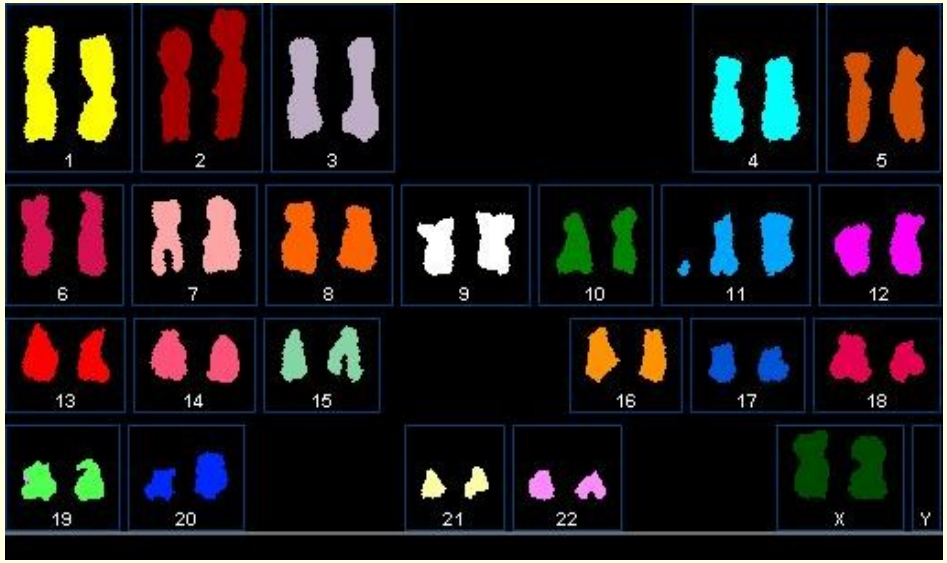
- Microdeletion syndromes – FISH probes, MLPA kits P245, P297
  - DiGeorge syndrome
  - Prader-Willi/Angelman syndrome
  - Williams-Beuren syndrome
  - 1p36 microdeletion syndrome
- Subtelomeric screening – MLPA kits P036, P070 (MRC-Holland), ToTel Vysion kit (Vysis)
- Origin of marker chromosomes – CGH, SKY, WCP FISH probes
- Identification and specification of numerical and structural aberrations – CGH, SKY
- Detection of gonosomal mozaics – FISH (X/Y probes) in infertile couples or gonosomal syndromes

Postnatal cytogenetic analyses





FISH: deletion of (22)(q11.2)
(DiGeorge syndrome)



SKY: marker chromosome identification
(chr. 11)

How do we proceed?

Karyotype G-banding

normal

MLPA

normal

aberation

specific syndrome

confirm

confirm

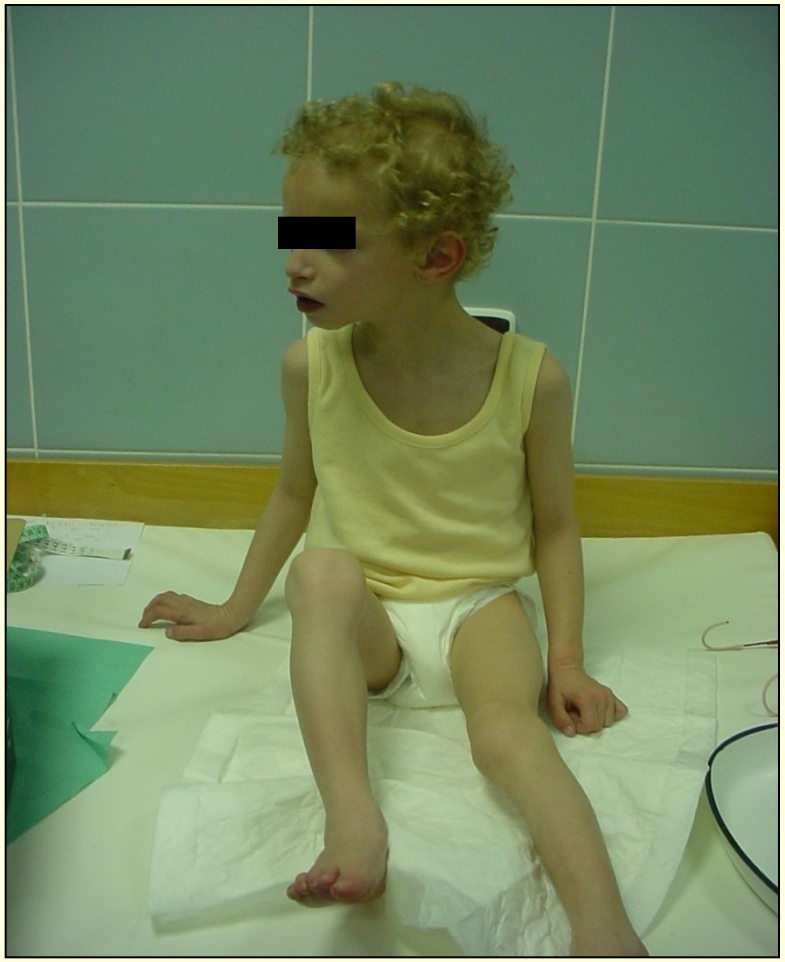
array-CGH

confirm

FISH

FISH

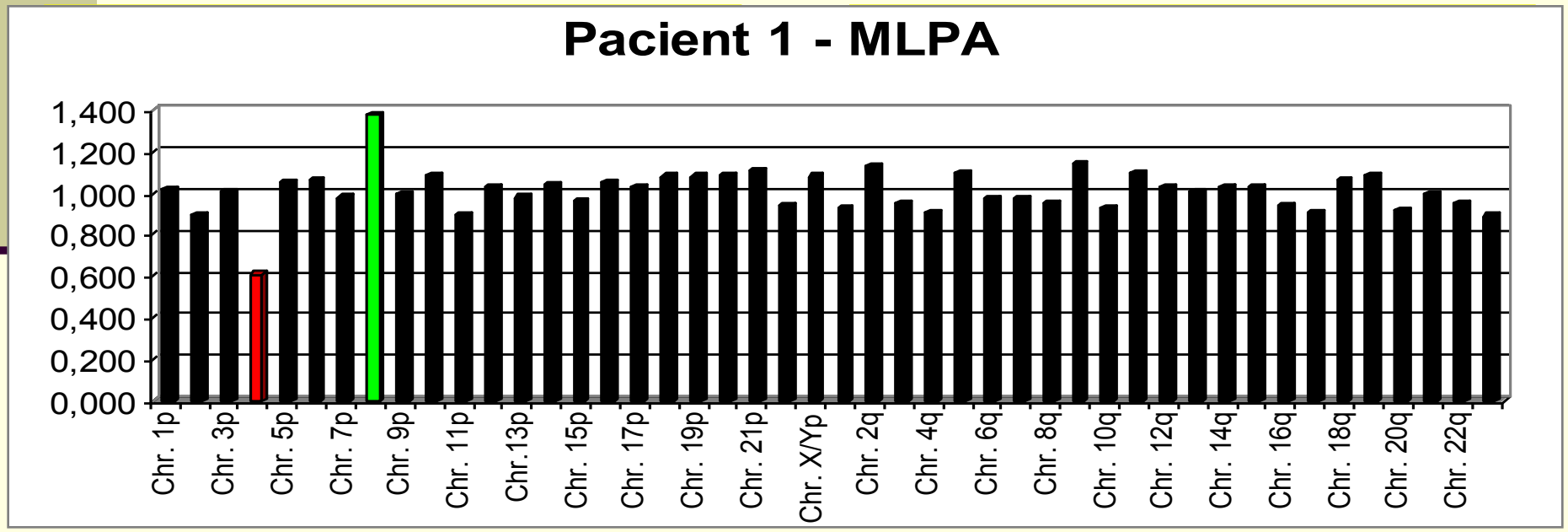
Case interpretation
Patient : del(4p)dup(8p)



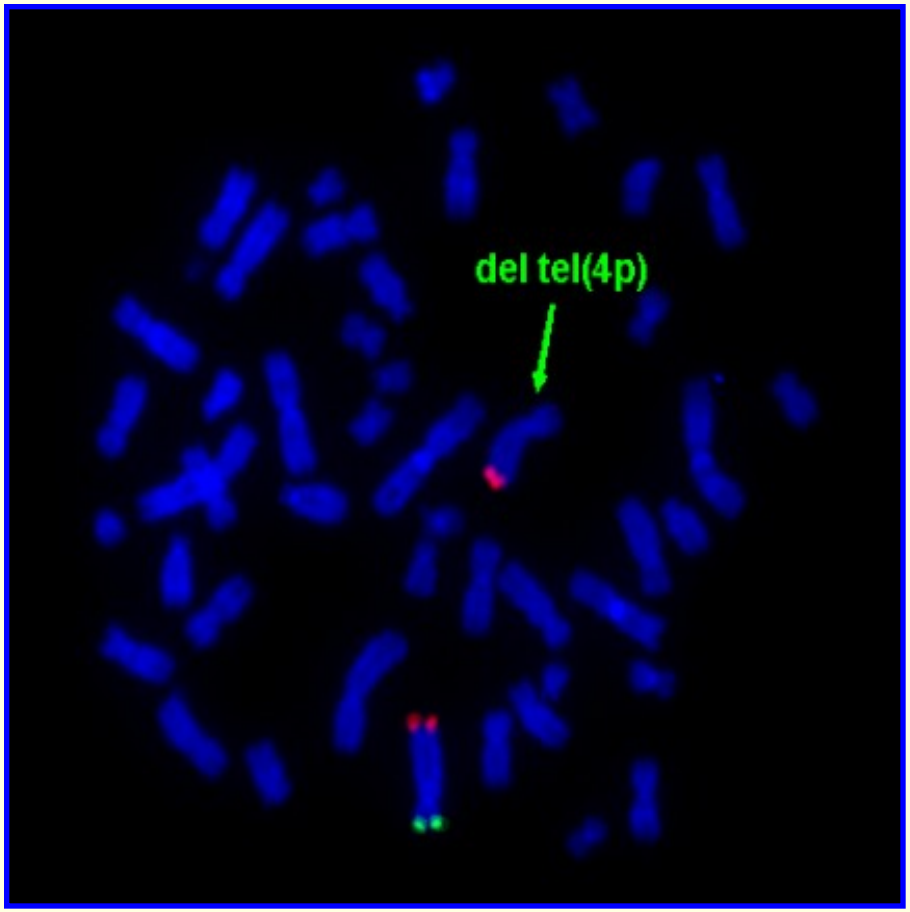
- Born in 2001
- Clinical symptoms:
  - hard PMR
  - facial dysmorphy
  - stigmata
  - hypertelorism
  - hemangiom on right eye lid

Examination

- karyotype: 46,XY,der(4)
- HR – CGH: negative
- MLPA: P036B **del(4p)dup(8p)**
  P070 **del(4p)dup(8p)**

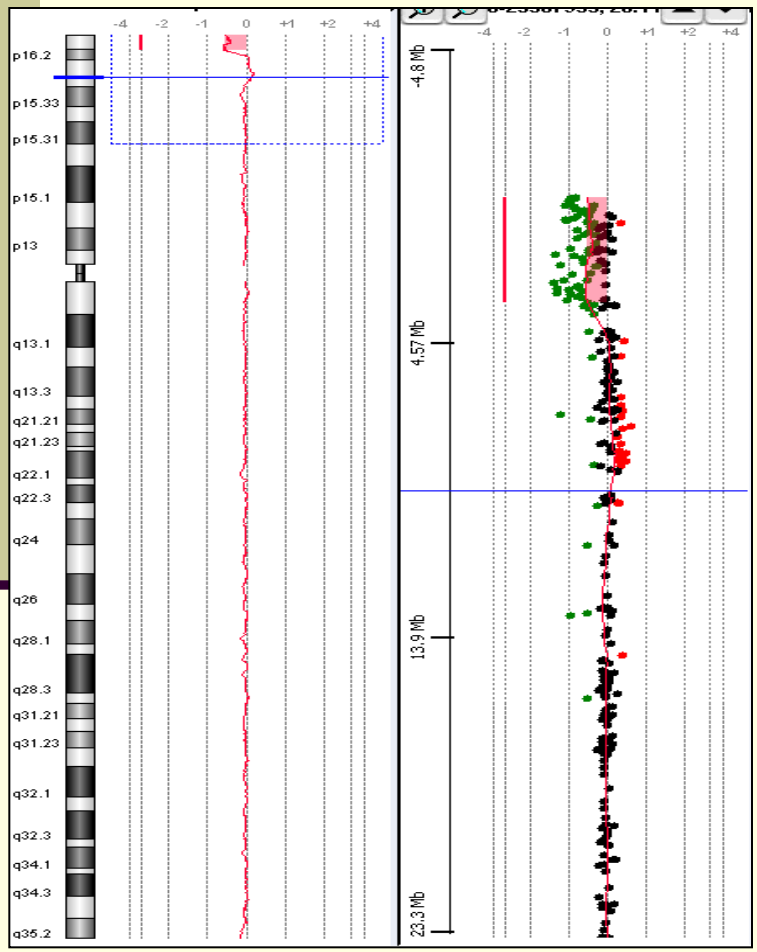


Deletion confirmation by the FISH method



Array CGH – confirmation of the aberation

dup(8)(p23.2pter) ~ 6.5 Mb



del(4)(p16.2pter) ~3.7 Mb