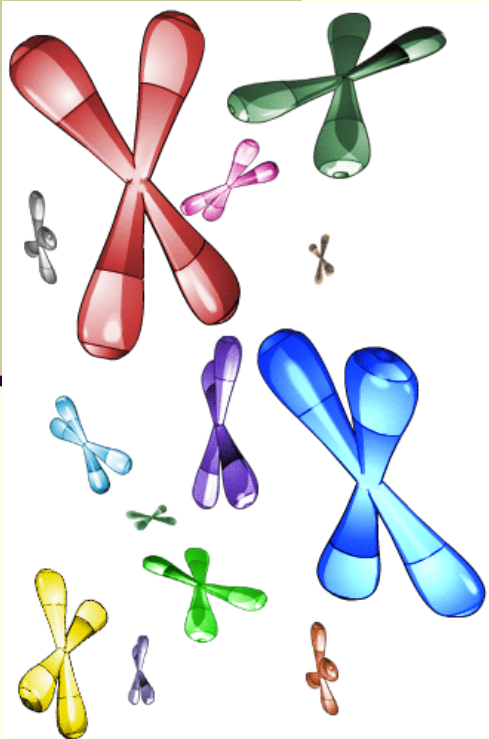


Cytogenetics & Integrated laboratory of molecular cytogenetics, Brno



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

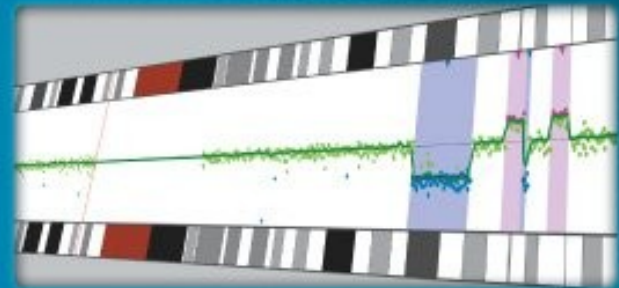
An International System
for Human Cytogenetic
Nomenclature (2013)

Editors

Lisa G. Shaffer

Jean McGowan-Jordan

Michael Schmid

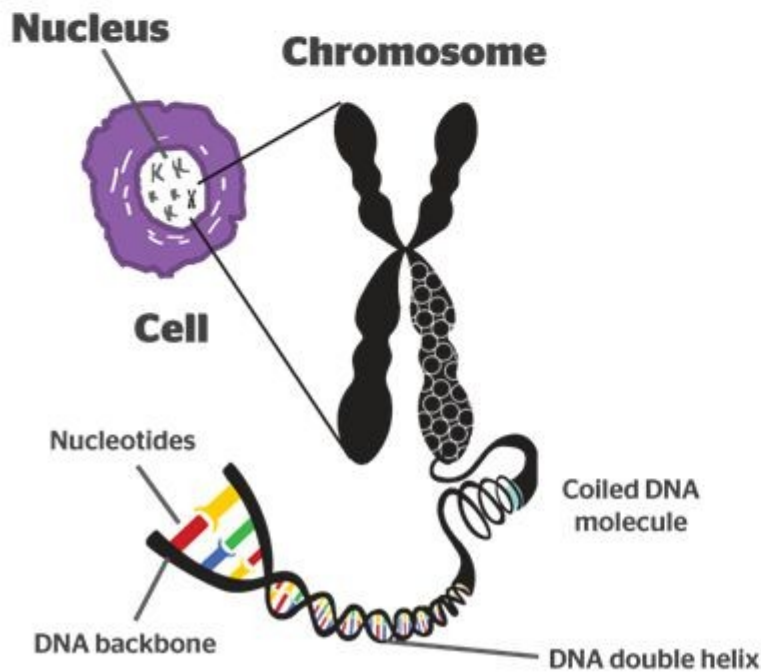


KARGER

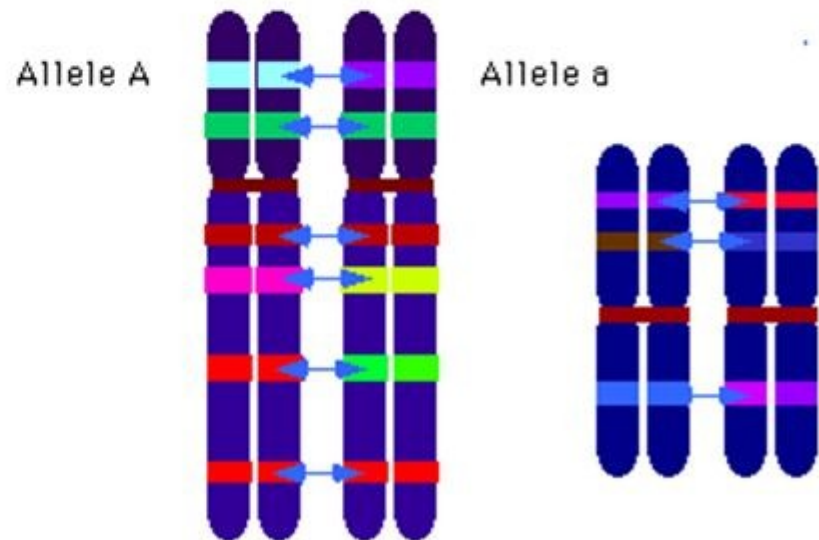
Published in
collaboration with
**Cytogenetic and
Genome Research**

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.



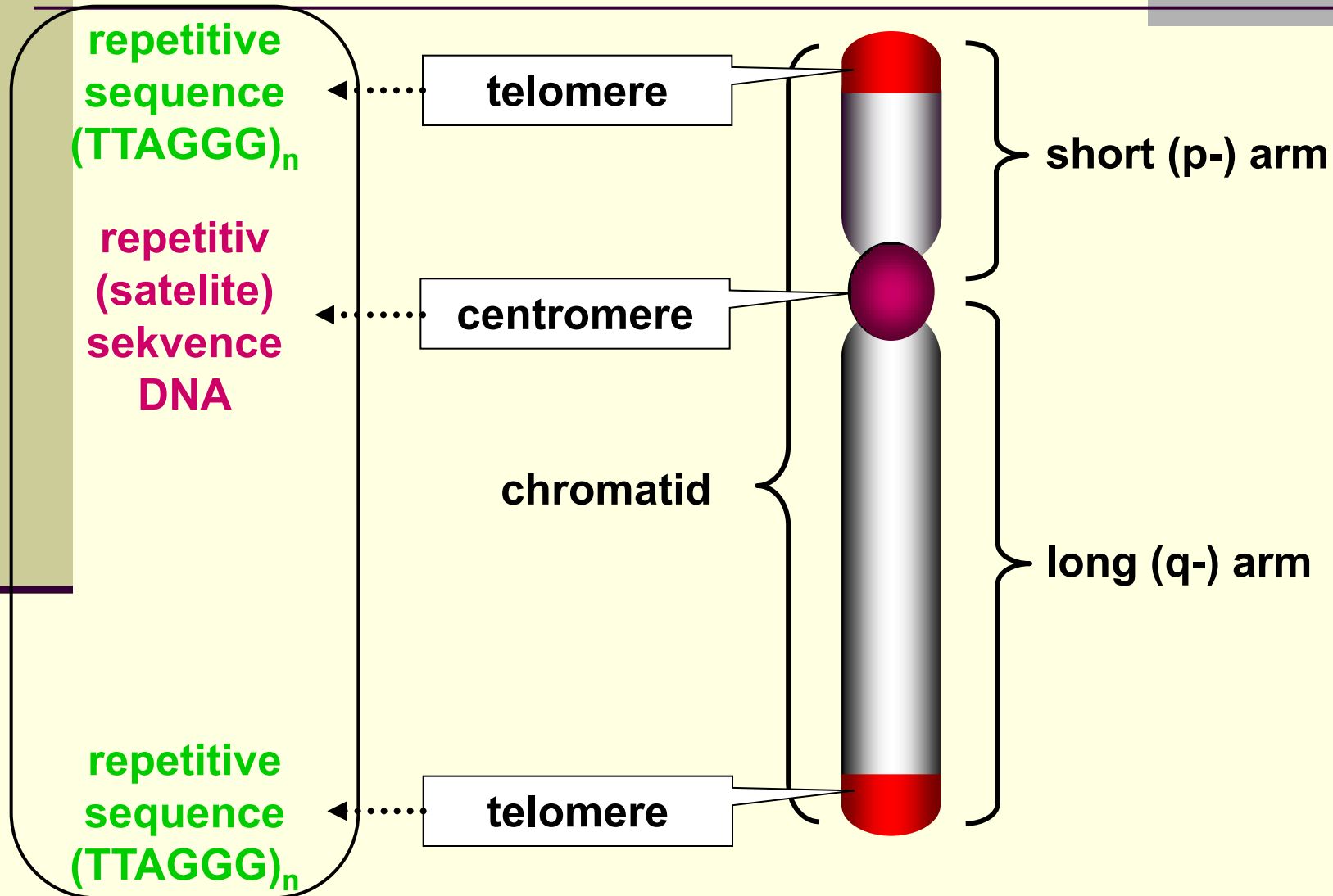
Two Pairs of Homologous Chromosomes



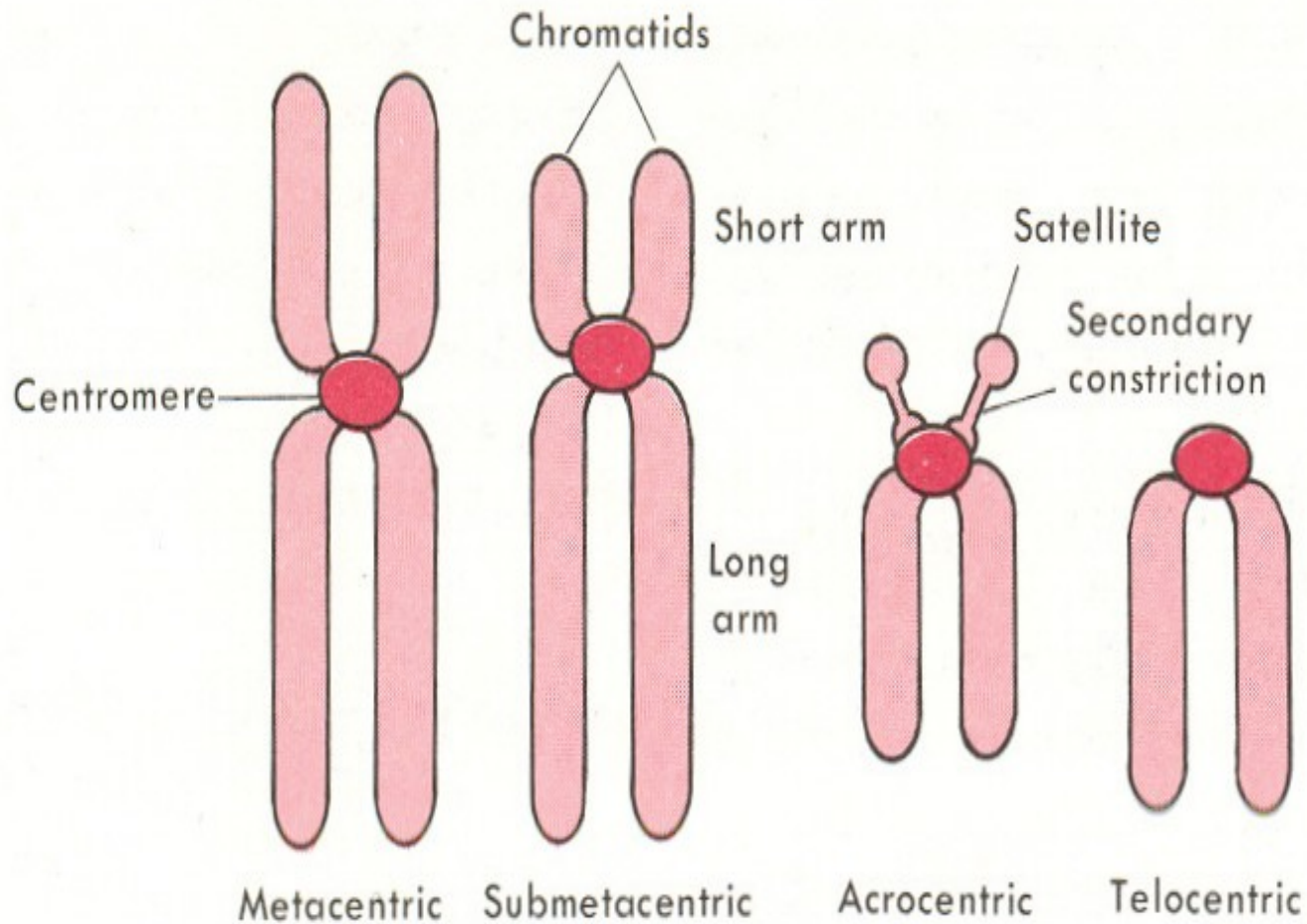
↔ The arrows point to corresponding genes

3. Chromosome morphology

DNA



Chromosome morphology



Chromosome staining

Classical staining

- using Giemsa Romanowski solution

- gained chromosome aberation detection

G – bands

- using trypsin, salty solution and Giemsa

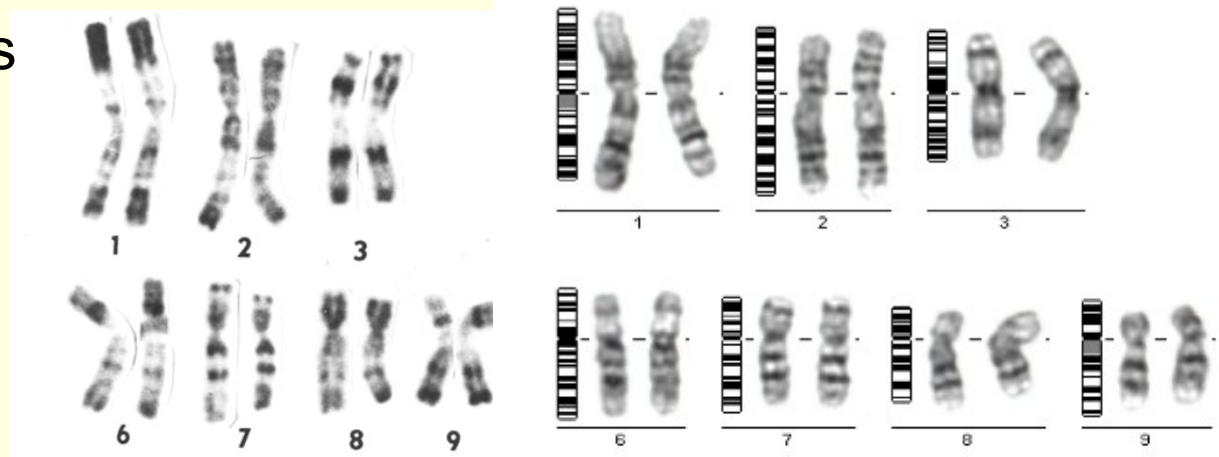
- each chromosome has characteristic stripes

- congenital chromosomes aberation detection

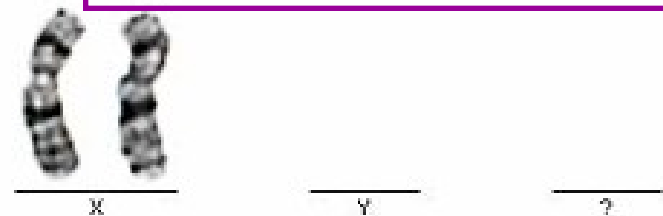
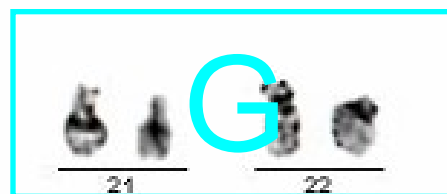
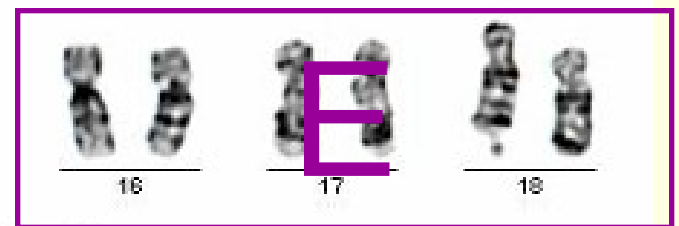
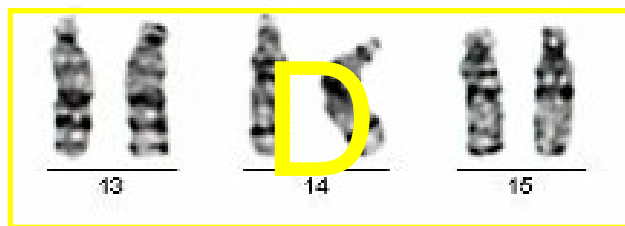
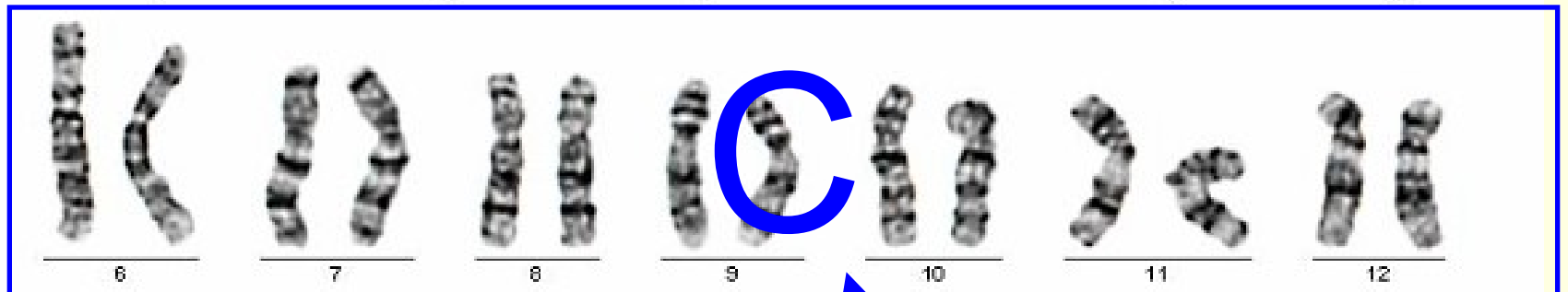
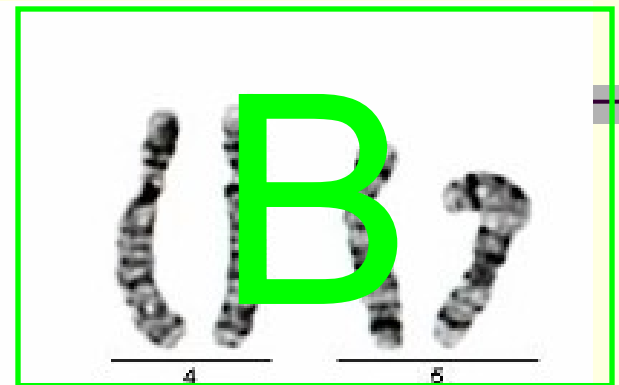
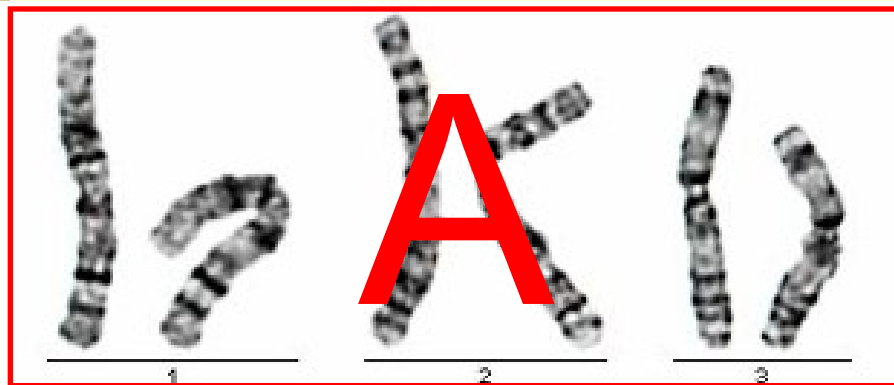
R – bands

- using salty solution of different pH and temperature

- reverse to G - bands



Human caryotype



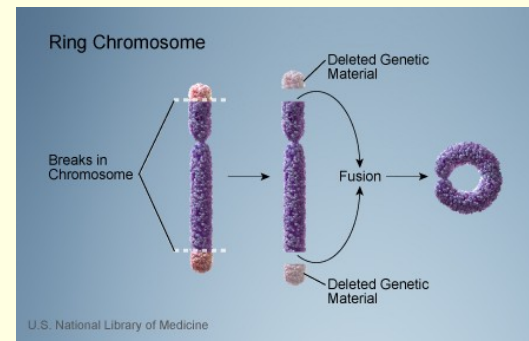
„take home message“

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
 - pericentric – 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



Chromosome aberrations

■ Deletion

- terminal – one break
- interstitial – 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

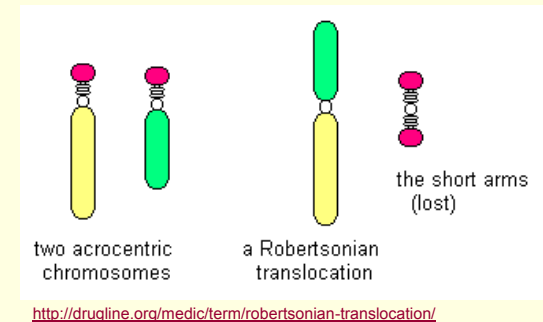
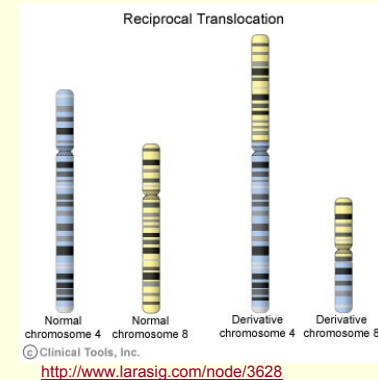
- mutual exchange between two or more nonhomologous chromosomes
- balanced - no phenotype effect
- genetic risks of unbalanced genome gametes formation

■ 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



Chromosome aberrations

■ 2. Numerical

■ Trisomy

- 21 chromosome trisomy – Down syndrome
- 18 chromosome trisomy – Edwards syndrome
- 13 chromosome trisomy – Patau syndrome

■ Triploidy

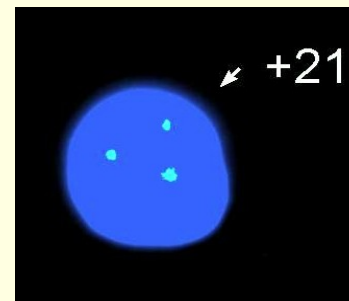
- 69,XXX; 69,XXY
- nonviable
- mosaic triploidy – mental retardation, syndactyly, abnormal genitals, lateral asymmetry

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

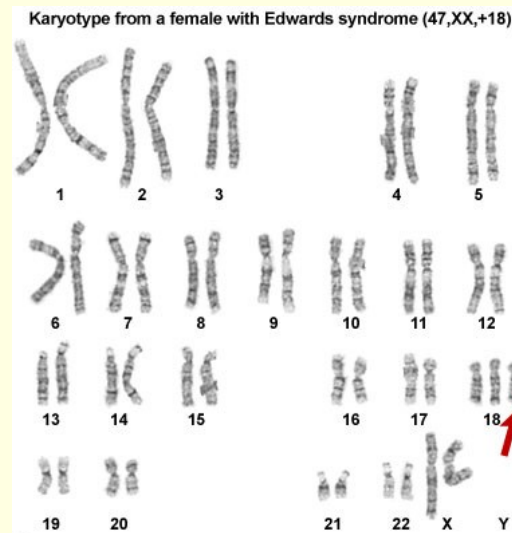
Down syndrome (47,XX or XY,+21)

- 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



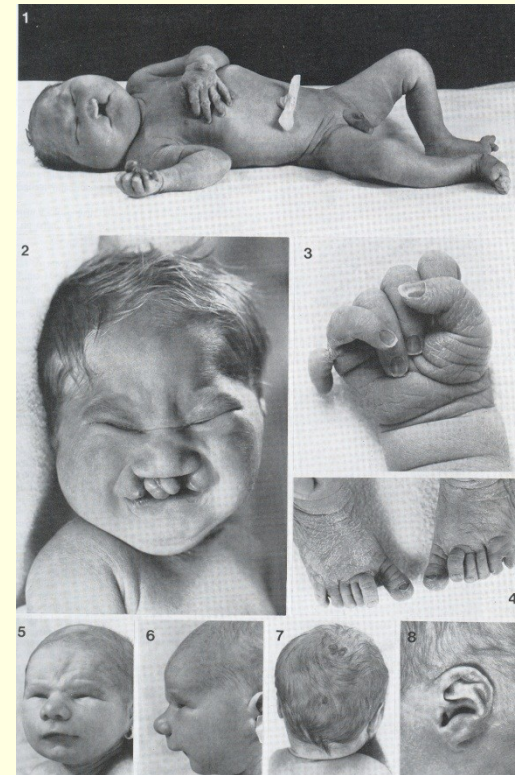
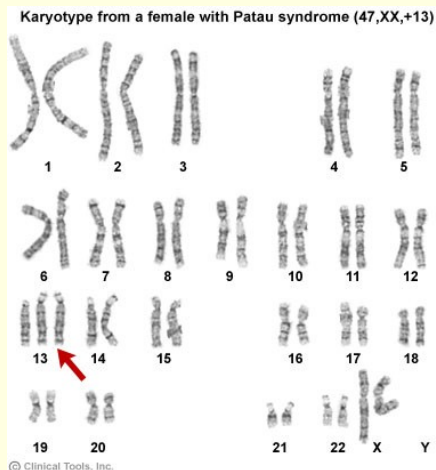
Edwards syndrome (47,XX or XY,+18)

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



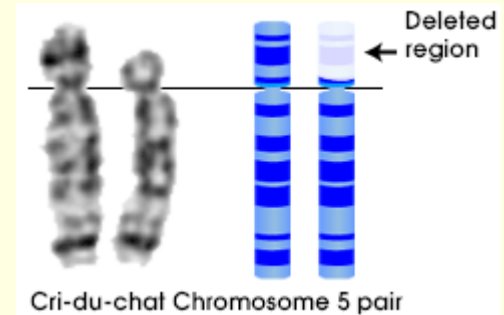
Patau syndrome (47,XX or XY,+13)

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



Cri-Du-Chat syndrome (del 5p)

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



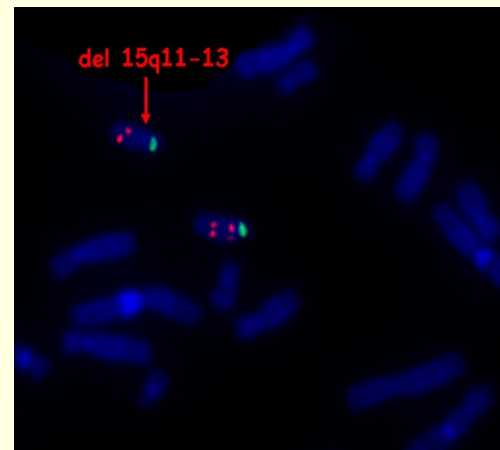
Wolf-hirschhorn syndrome (del 4p)

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



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)



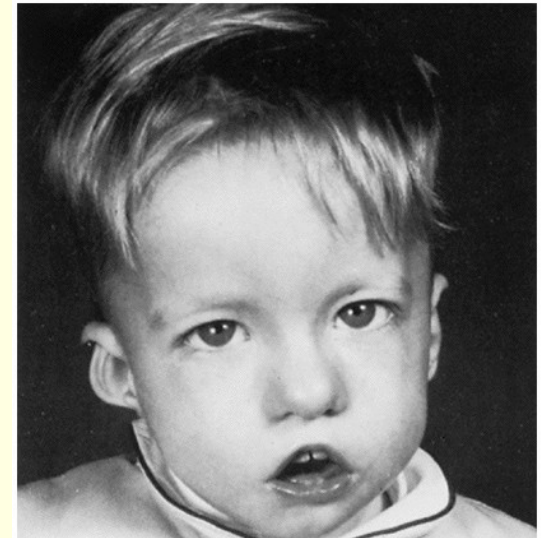
Angelman syndrome (del 15q11-q13)

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

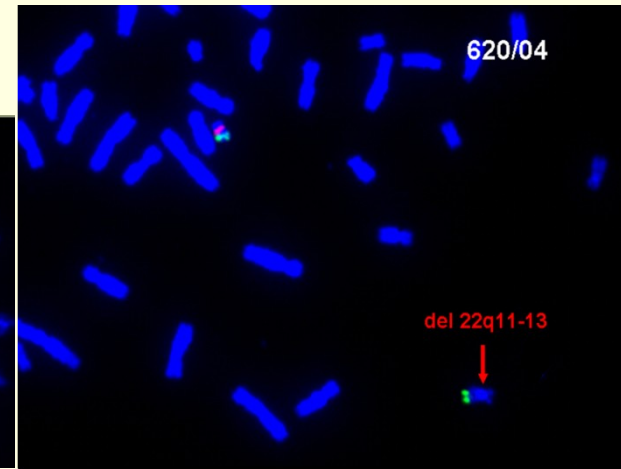
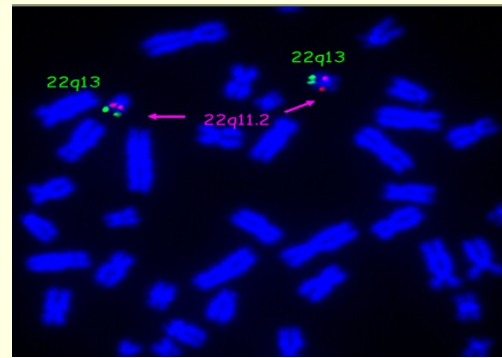


DiGeorge syndrome (del 22q11.2)

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

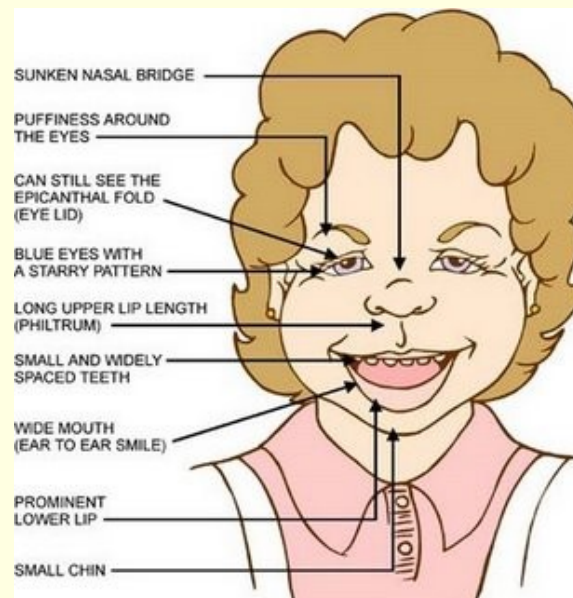
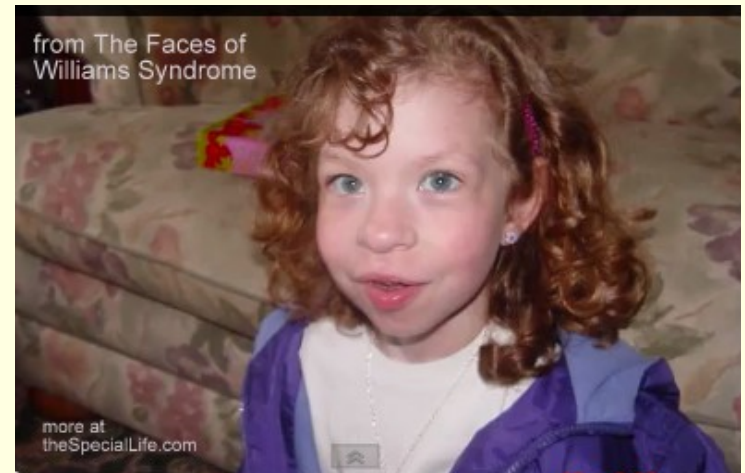


https://www.google.cz/search?q=diGeorge+syndrome&espv=210&es_sm=93&source=imgs&tbm=isch&sa=X&ei=P9CFUo21HsqR7AbP1BI&ved=0CAkQ_AUoAQ&biw=1920&bih=989&facrc=_&imgdii=&imgcr=0EhFFG2IOAvB3M%3A%3BFG4R33YXExVhsM%3Bhttp%253A%252F%252F



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

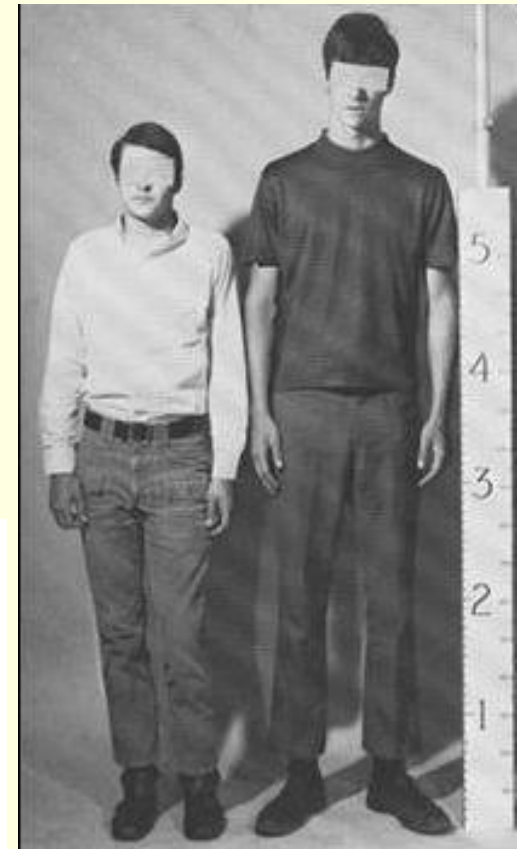
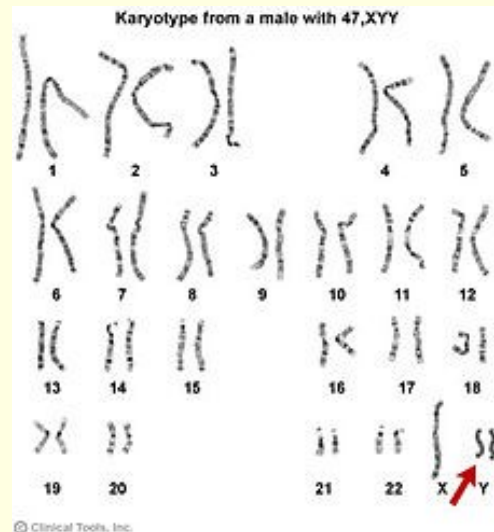


https://www.google.cz/search?q=williams+beuren+syndrome&source=inms&tbm=isch&sa=X&ei=MimGUvDpC4GctQaJhYGwCg&ved=0CAcQ_AUoAQ&biw=1920&bih=989&focr=._imgdli=._imgrc=MdTdkoWBwg-WM%3A%3BTkKZzTKDfnYIM%3Bhttp%253A%252F%252Fwww.thespeciallife.com%252Fimages%252F

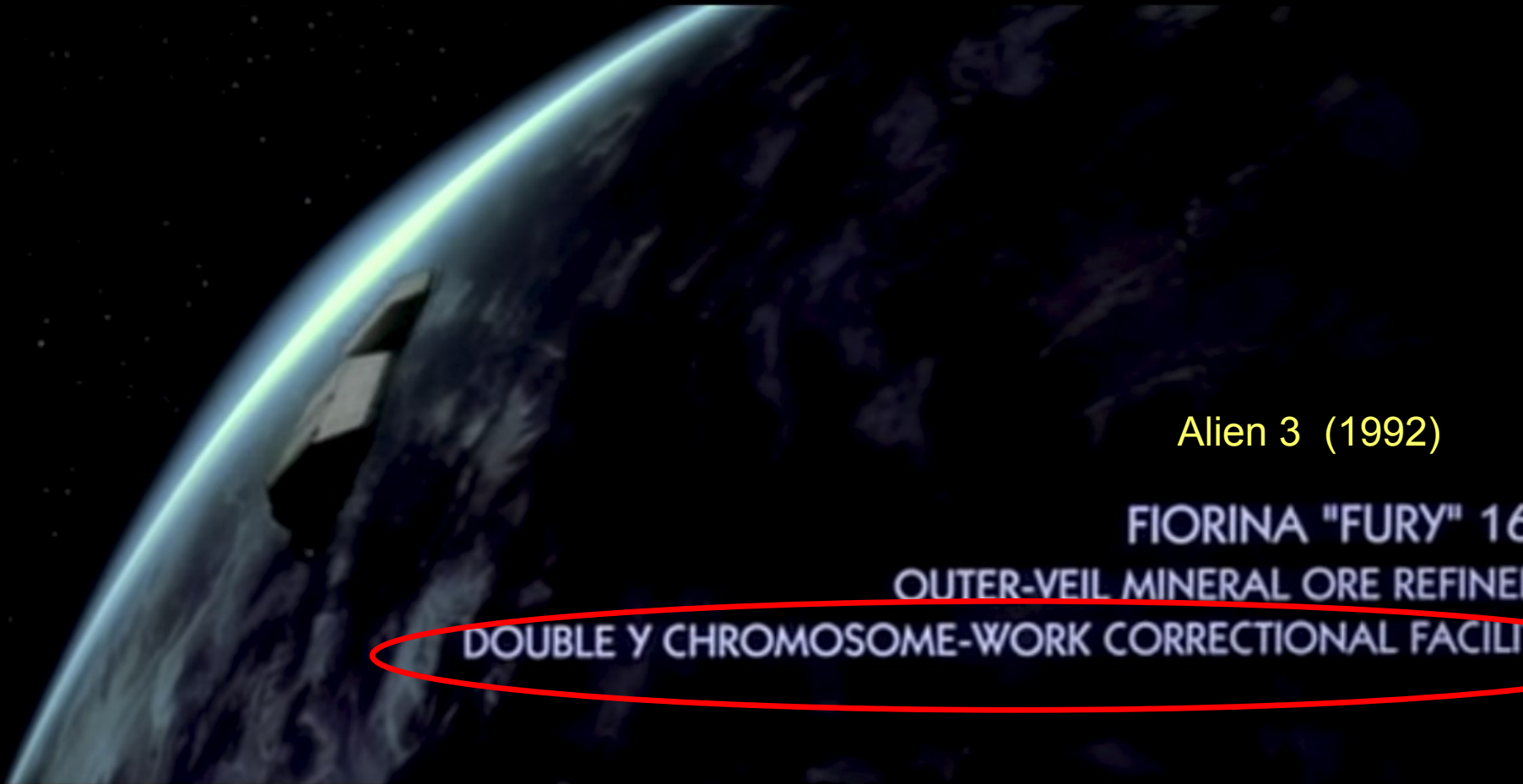
https://www.google.cz/search?q=williams+beuren+syndrome&source=inms&tbm=isch&sa=X&ei=MimGUvDpC4GctQaJhYGwCg&ved=0CAcQ_AUoAQ&biw=1920&bih=989&focr=._imgdli=._imgrc=HTJyFEuSnZo4JM%3A%3BkqK81uaGRtK-PIM%3Bhttp%253A%252F%252Fgeneticsf.laba

Supermale syndrome (47,XYY)

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



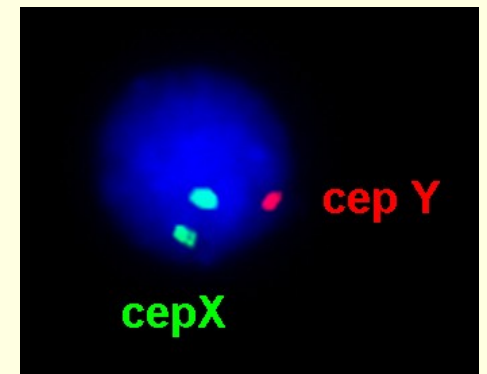
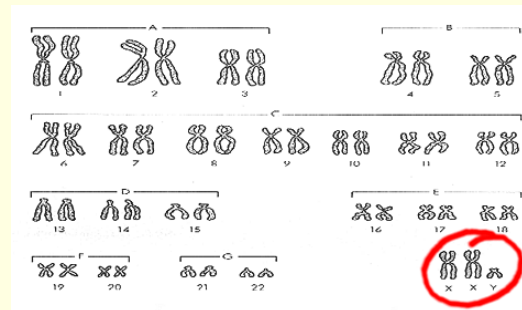
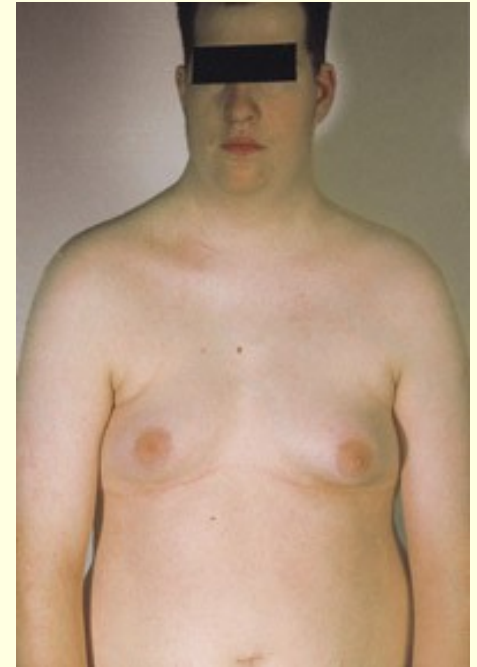
80 s – „double Y“ (Alien 3 – 1992)



Klinefelter syndrome (47,XXY)

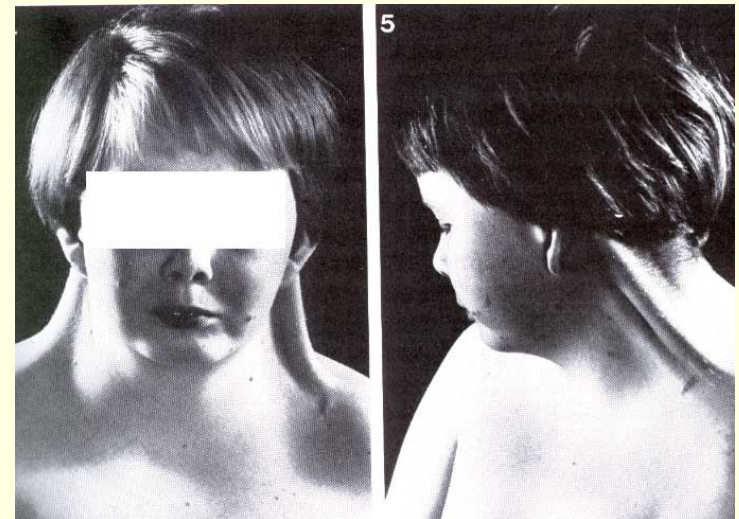
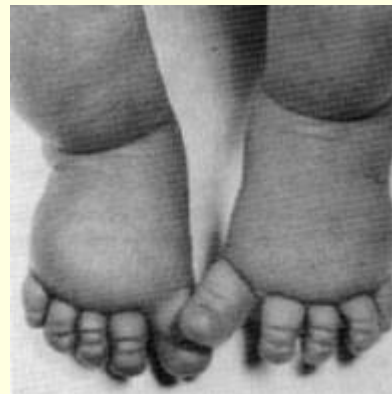
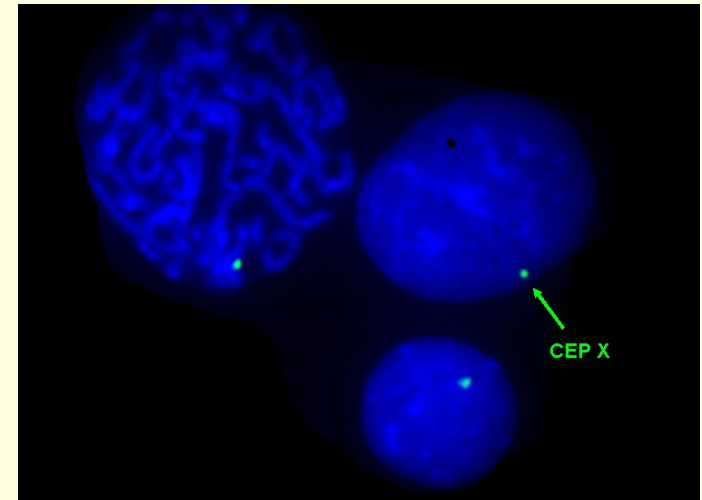
48,XXYY; 48,XXXY; 49,XXXXY

- 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
- lymphedema



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

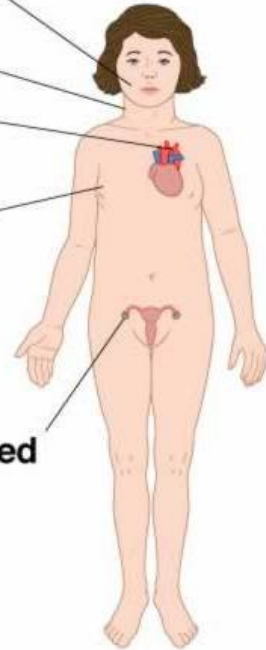
Characteristic facial features

Web of skin

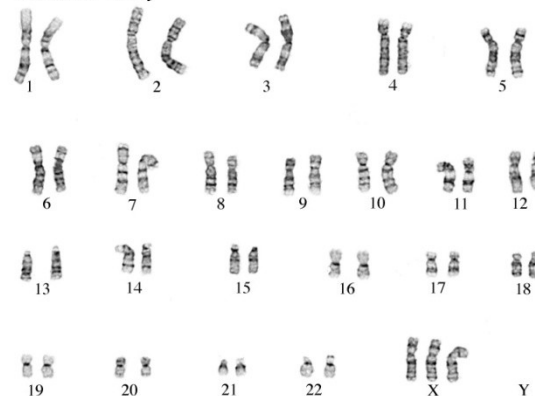
Constriction of aorta

Poor breast development

Under-developed ovaries



ZWK01047 key



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

https://www.google.cz/search?q=xxx+syndrome&source=lnms&btn=isch&sa=X&ei=xMIUvHgrG0BpOYgL&ved=0CAcQ_AUoAQ&biw=1440&bih=783#facrc=_&imgdl=1&imgcr=Hz1JqGzTyKpBM%3A%3BFZOiqHDJFB267M%3Bhttp%253A%252F%252Fworms.zoology.wisc.edu%252Fzooweb%252FPhelps%252FZWK01047k.jpg%3Bhttp%253A%252F%252Fwww.zappa.com%252Fmessageboard%252Fviewtopic.php%253F%253D5%2526%253D7057%3B768%3B576

4. Molecular cytogenetics

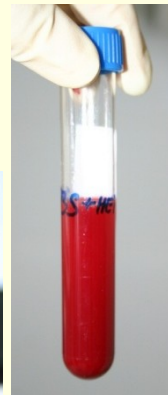
- presents the connections between classical cytogenetics and molecular biology
- utilizes the latest knowledge of molecular biology, microscopy and computer image analysis to study the structure and properties of chromosomal changes
- allows the analysis of numerical and structural chromosomal imbalances 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



peripheral blood



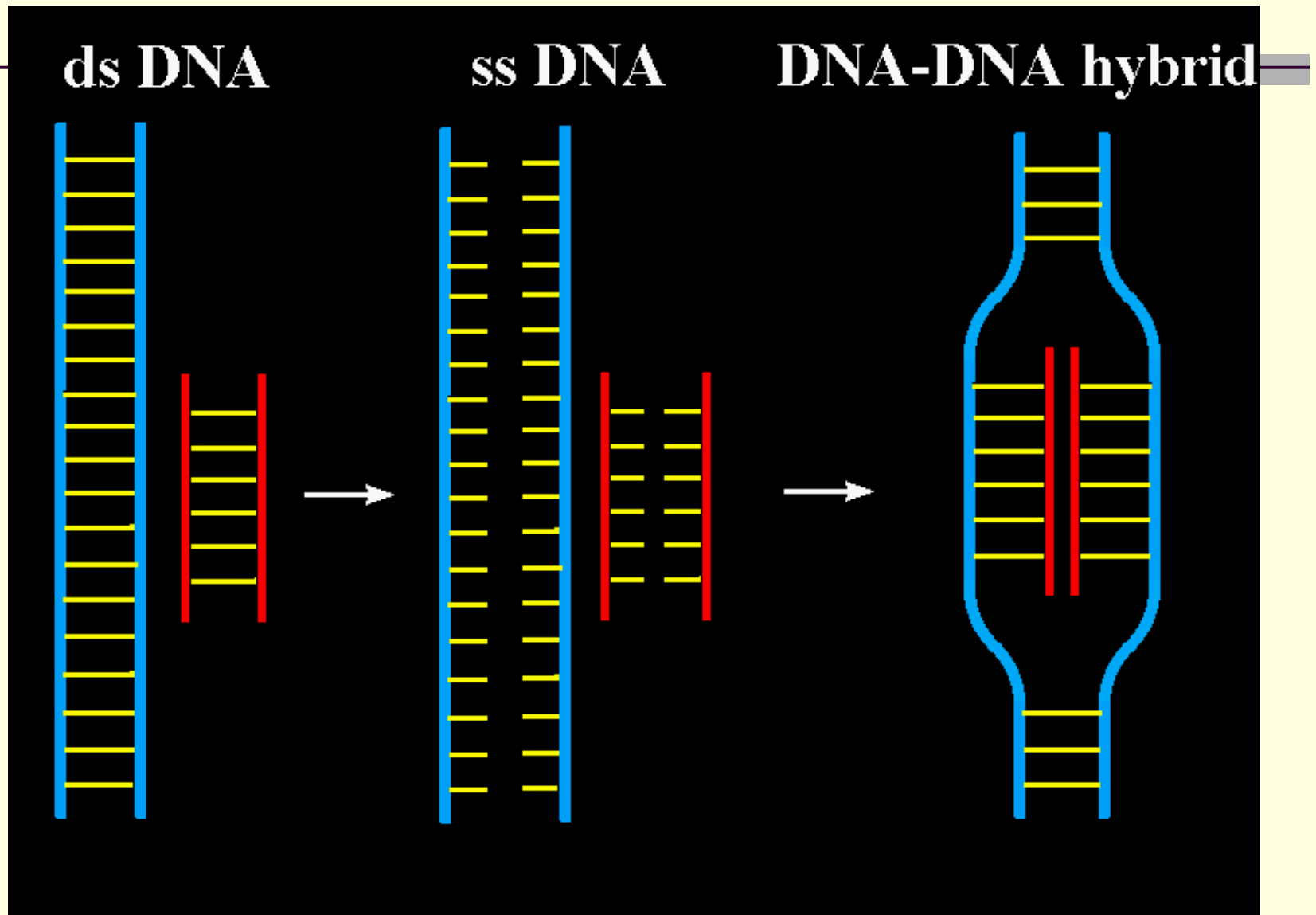
solid tumor



bone marrow

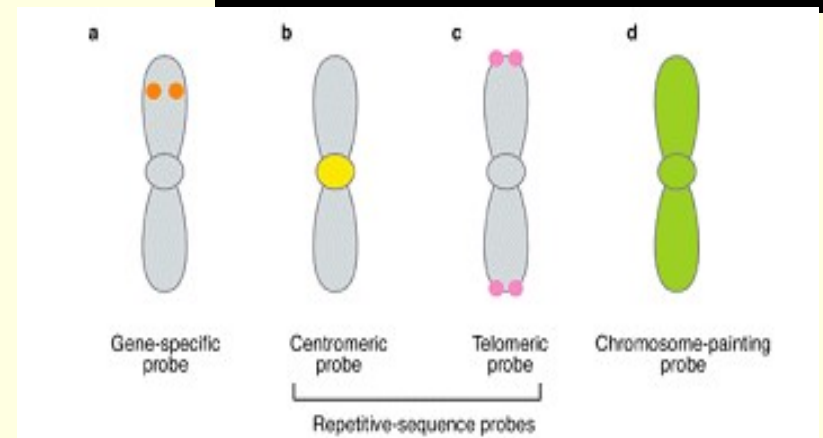
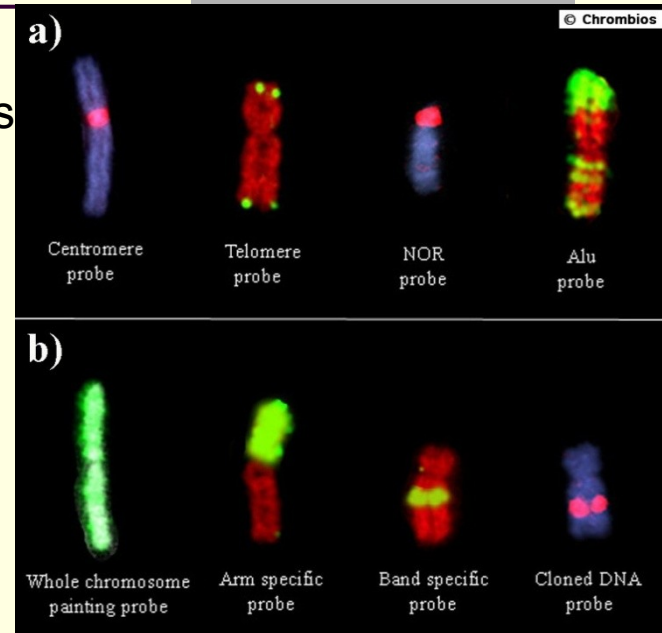
FISH

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



830/05

747/05

del exon 50

AML1

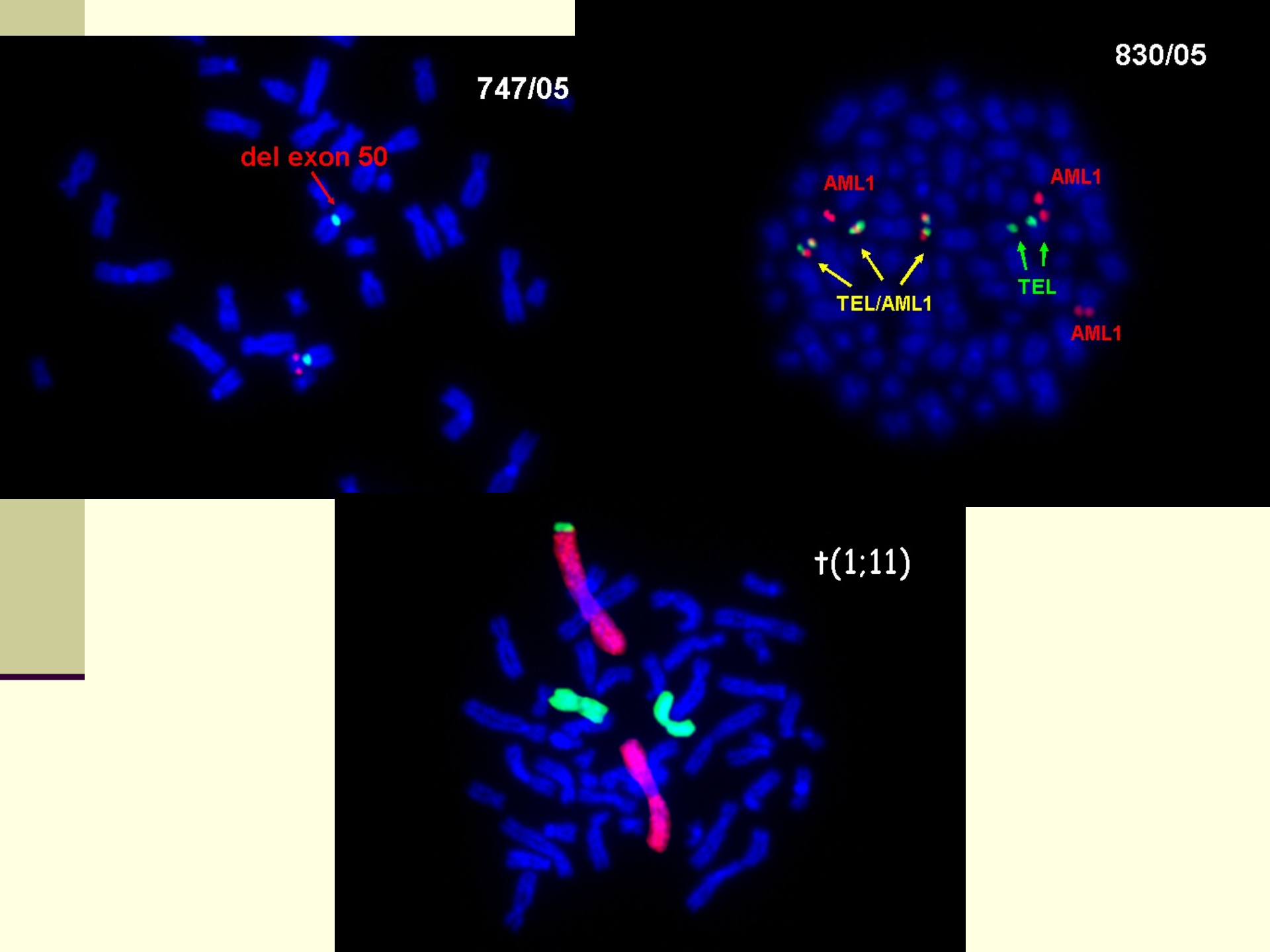
AML1

TEL/AML1

TEL

AML1

†(1;11)



Advantages and disadvantages 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

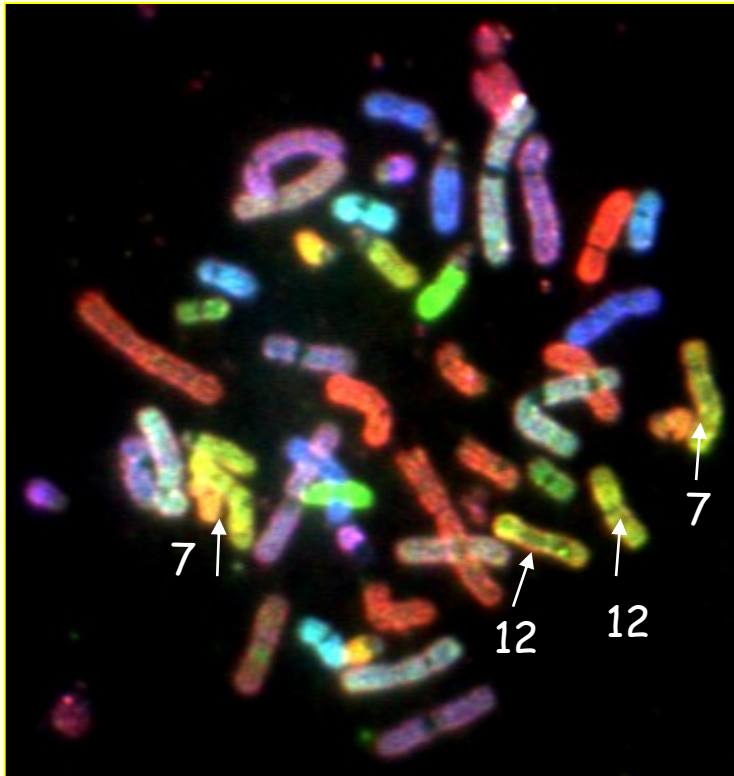
spectral caryotyping

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

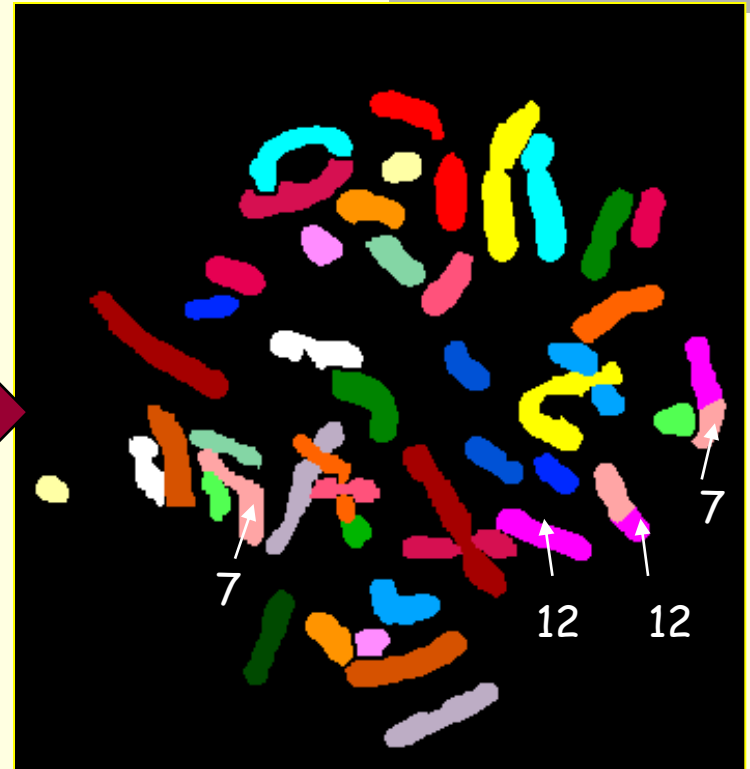


Image Acquisition with SkyVision™

Picture analyse using SkyView



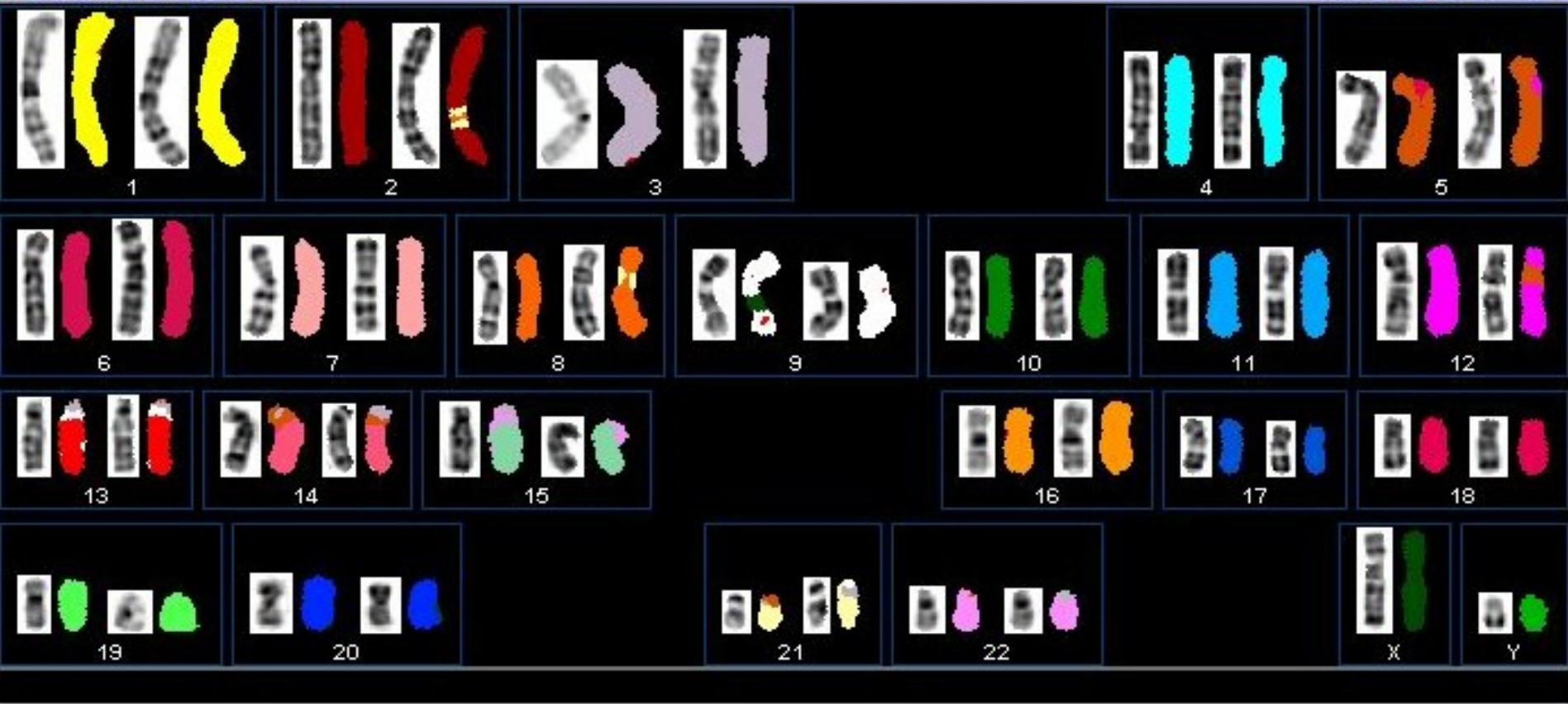
Display Image



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



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

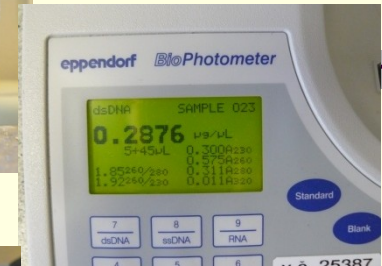
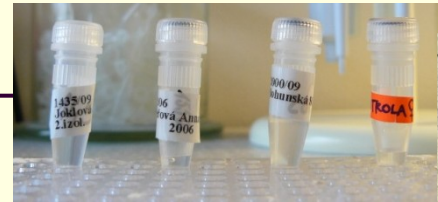
CGH requirements

Materials :

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

Equipment :

- Fluorescent microscope (filters DAPI, SpGreen, SpRed)
- Sensitive CCD camera
- Computer with software for CGH analysis and data interpretation (LUCIA CGH Advanced Statistics, Laboratory Imaging Ltd., Prague, Czech Republic)



CGH principle

1. Genomic DNA is isolated and quantitated

Test Sample

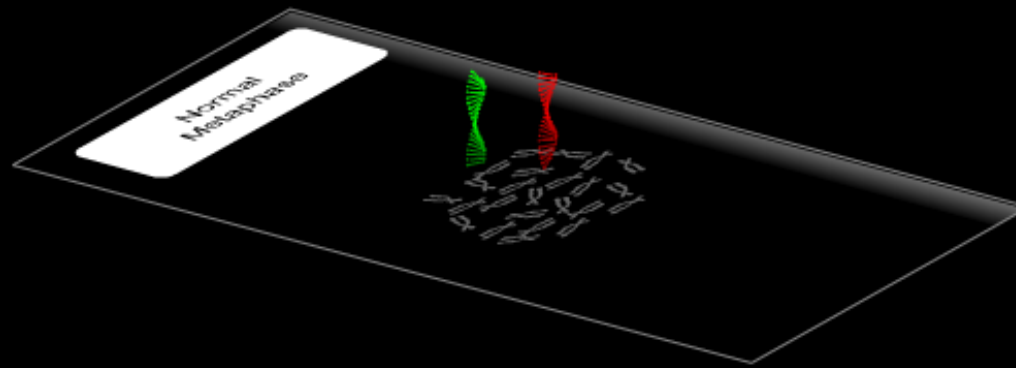
Reference Sample

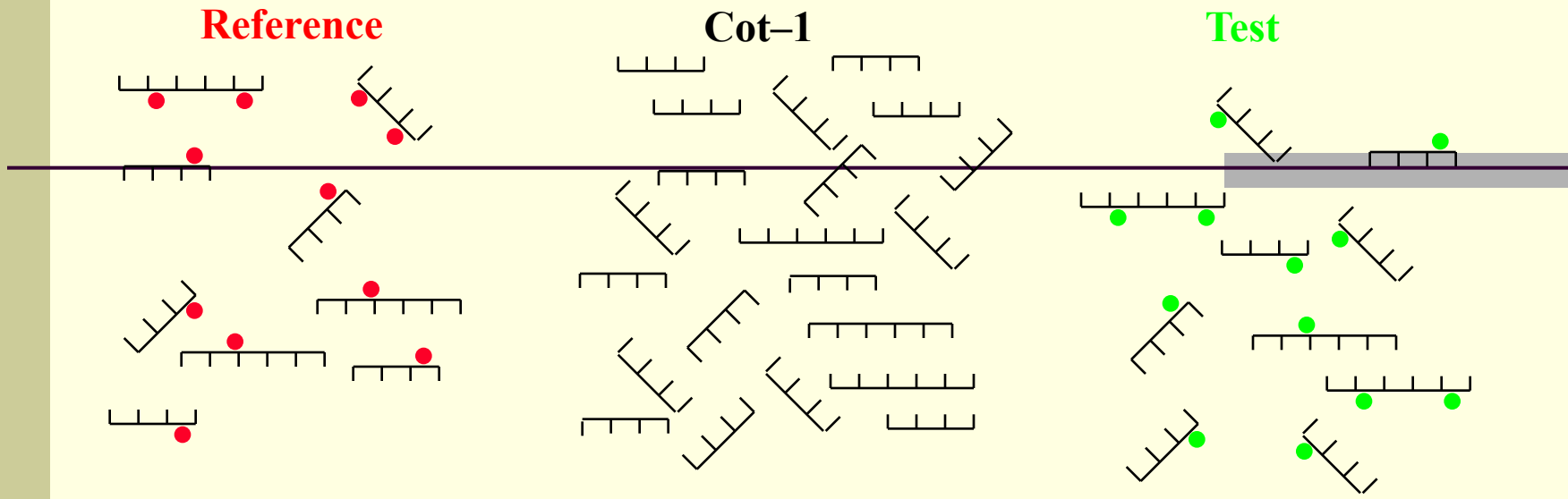
2. Genomic DNA is labeled with Cy3 or Cy5



CGH principle

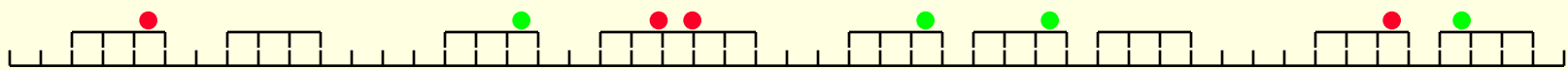
3. Labeled DNA is digested into smaller products that allow optimal hybridization





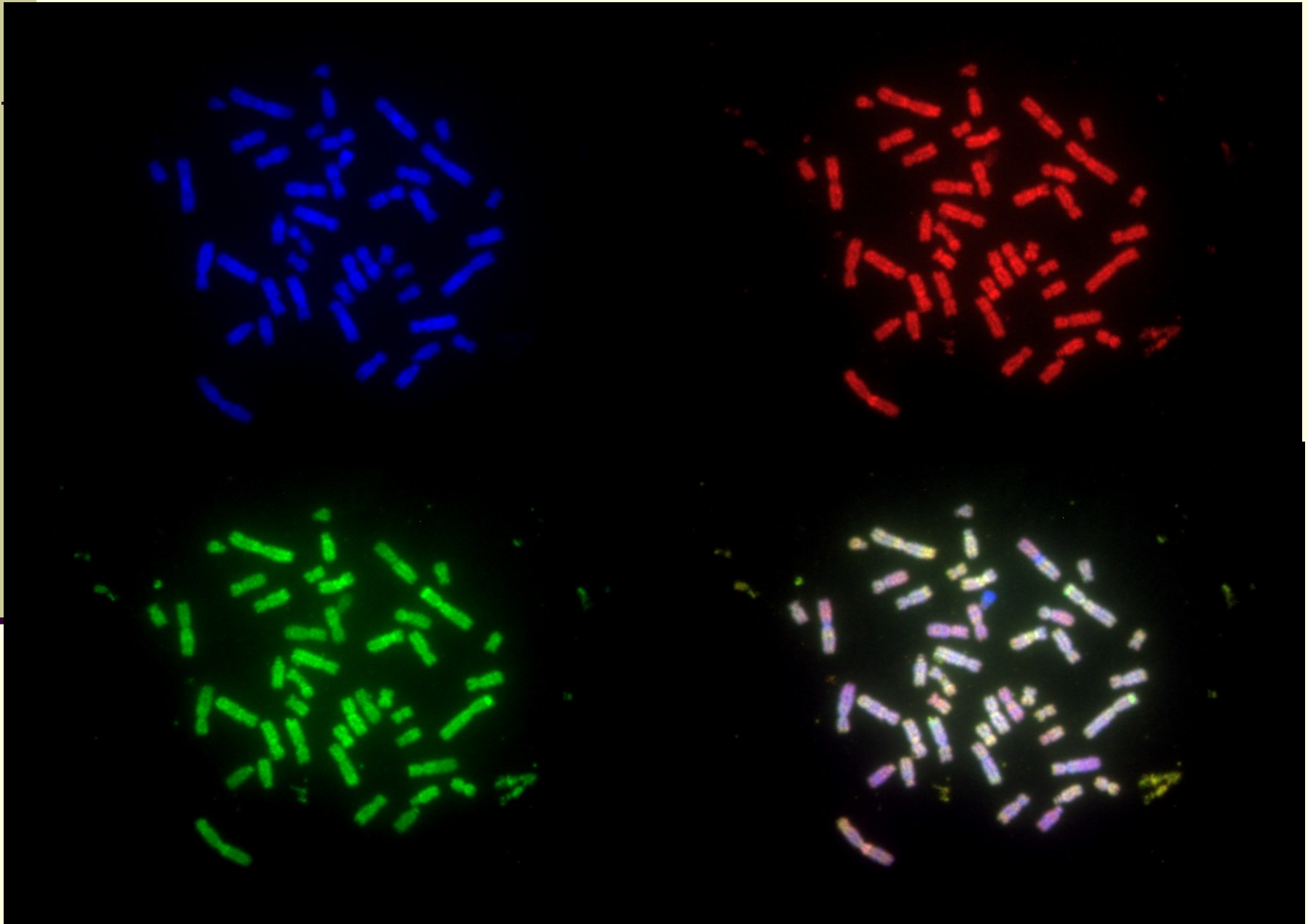
Unique sequences are labeled by *in situ* hybridization

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



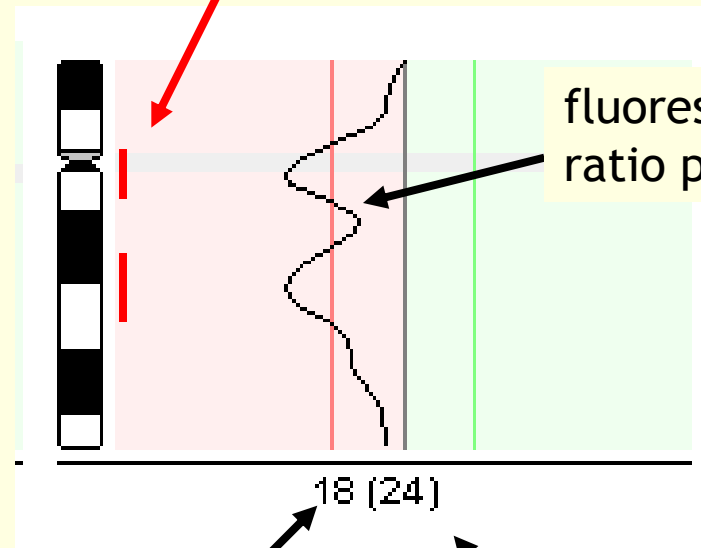
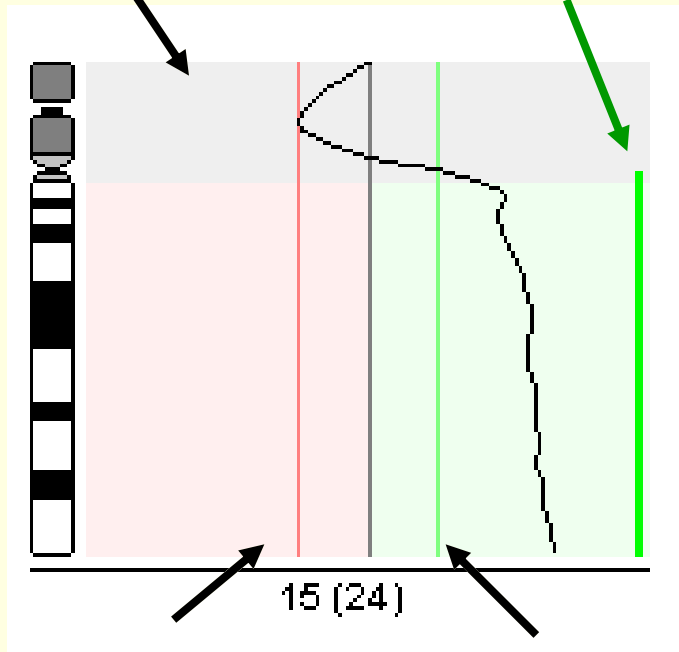
Identification of aberrations

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.

heterochromatin

gain

loss



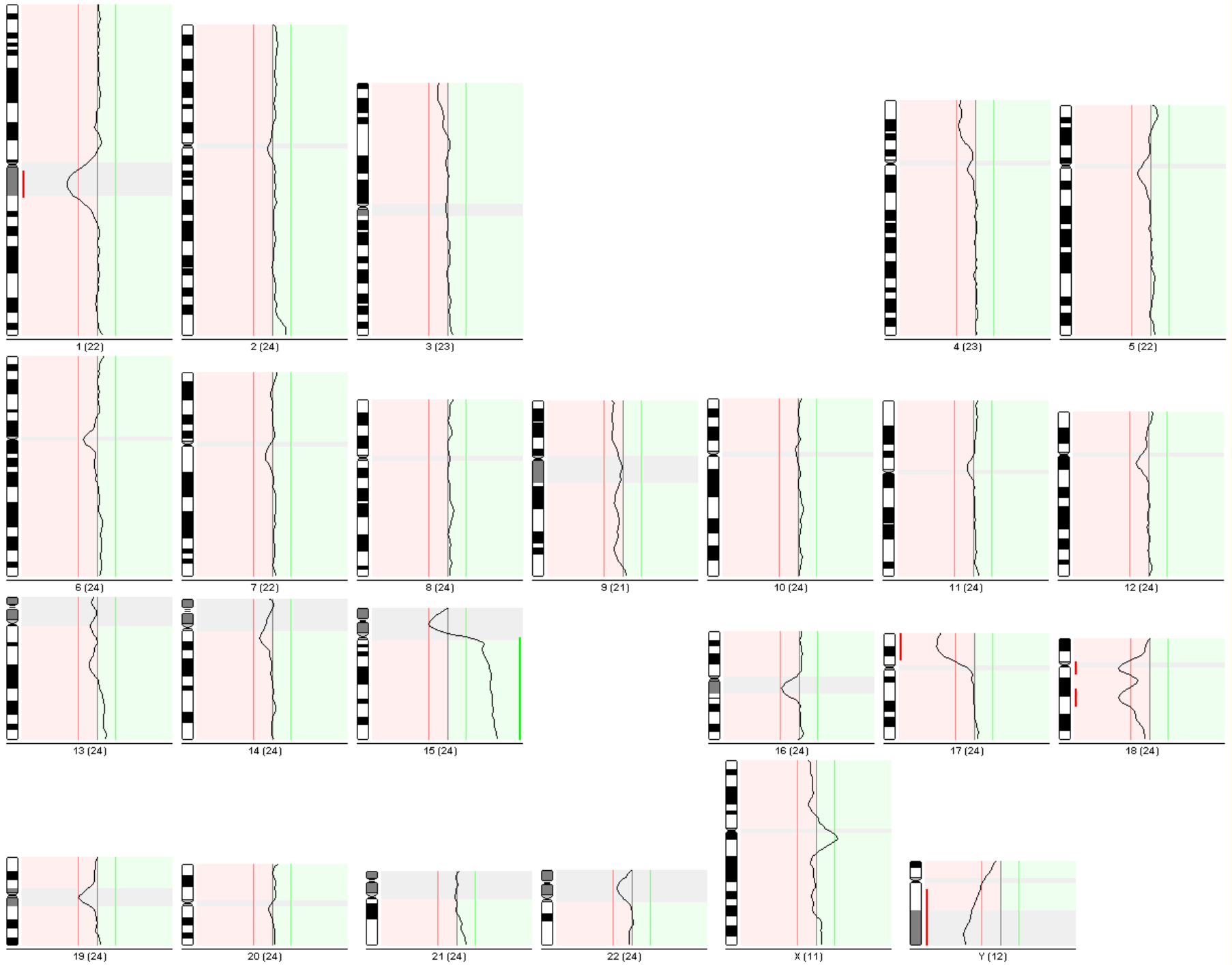
treshold 0.8

treshold 1.2

chromosome number

number of
chromosomes in
analysis

Minimally 10 metaphases should be processed.



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

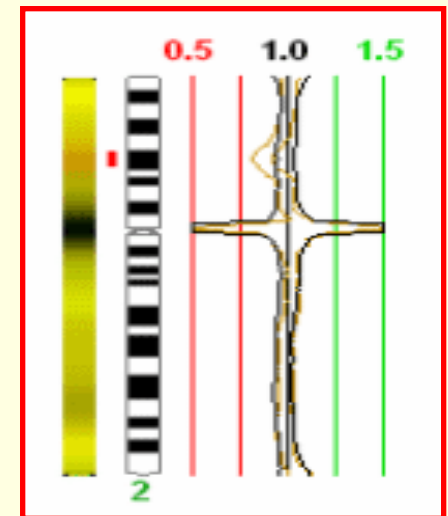
Disadvantages of CGH

- 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, microarrays

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 %

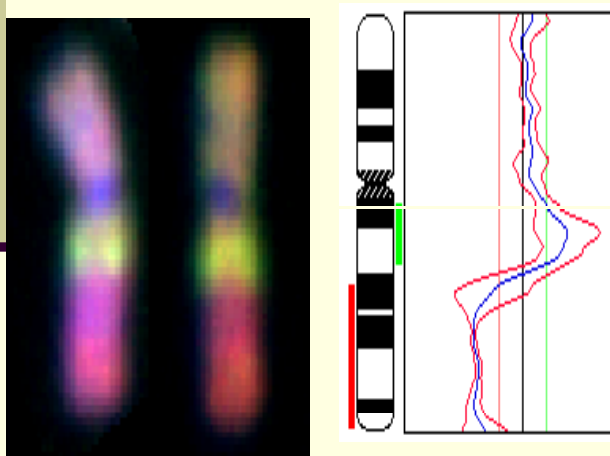


Modifications of CGH

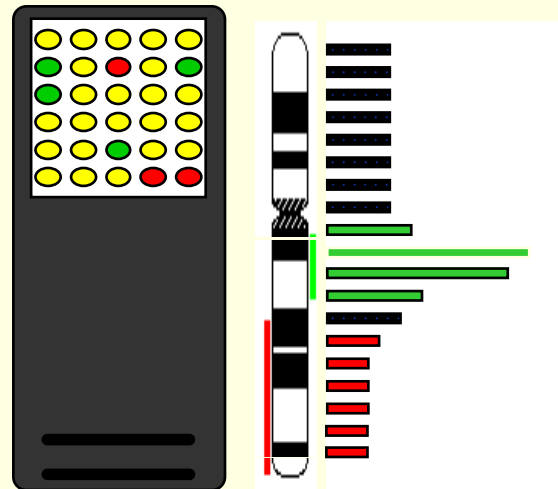
Array-CGH

- 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

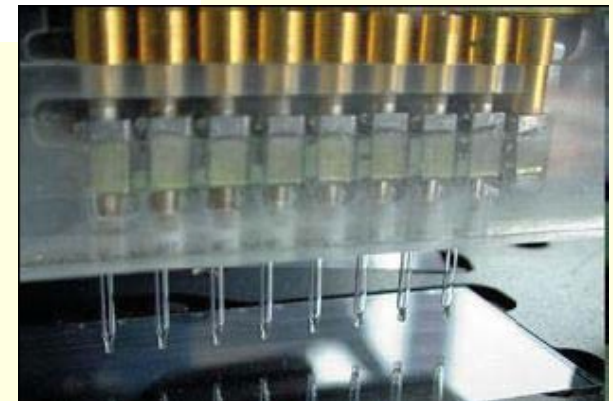
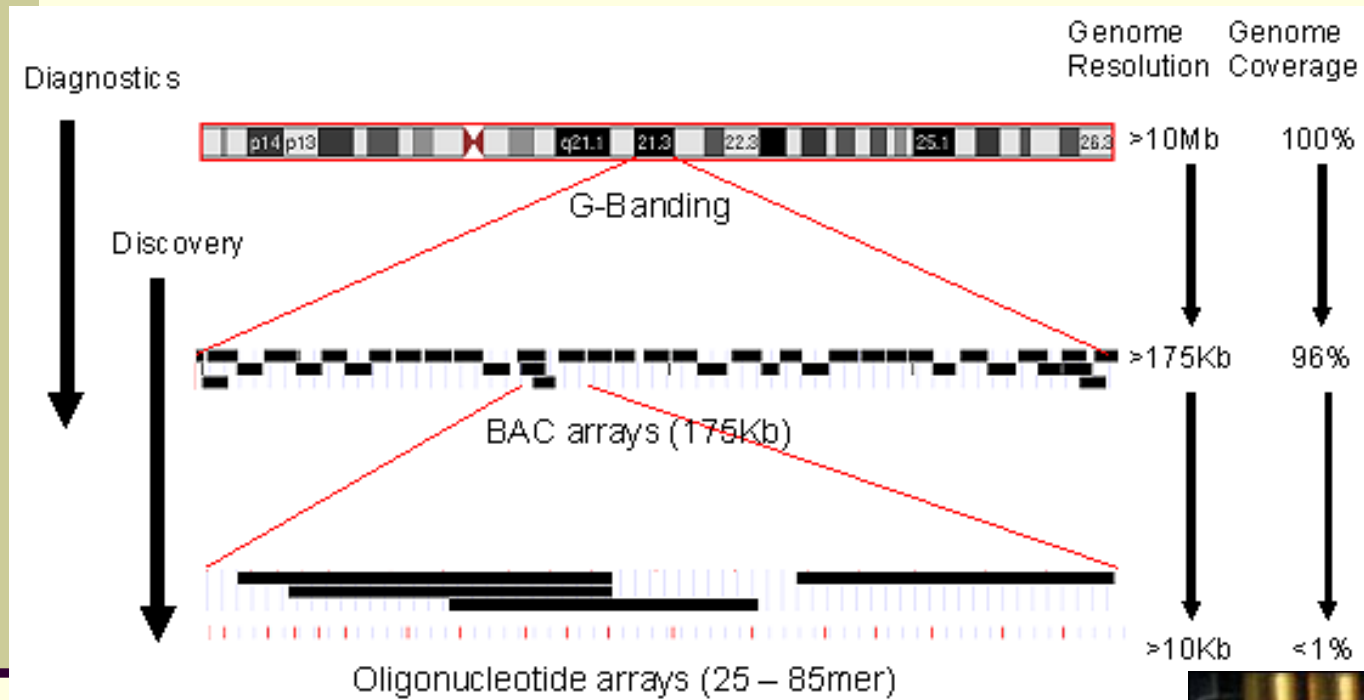


array-CGH

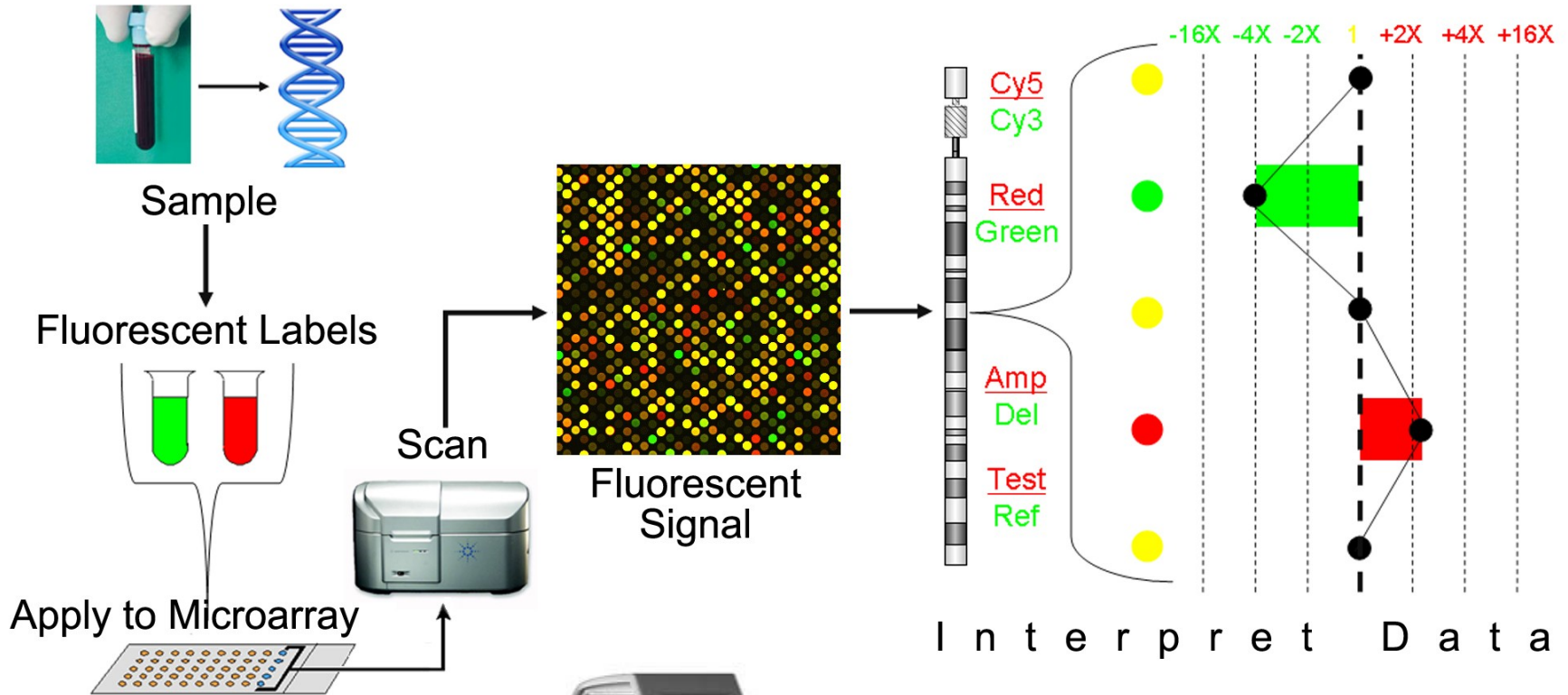


The origin of clones

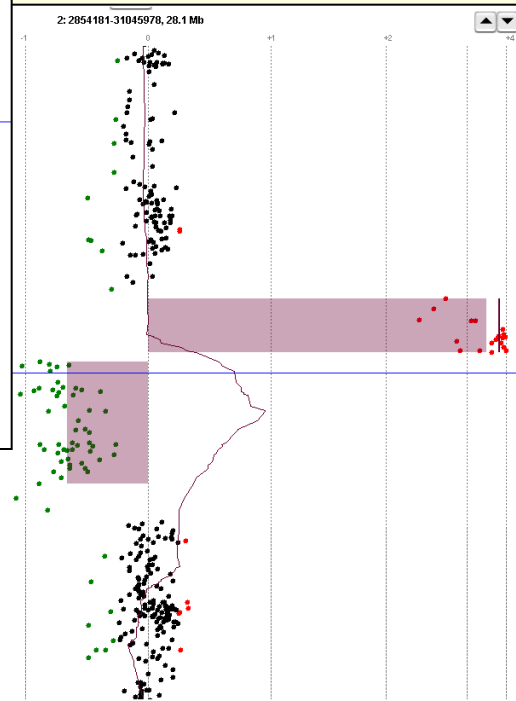
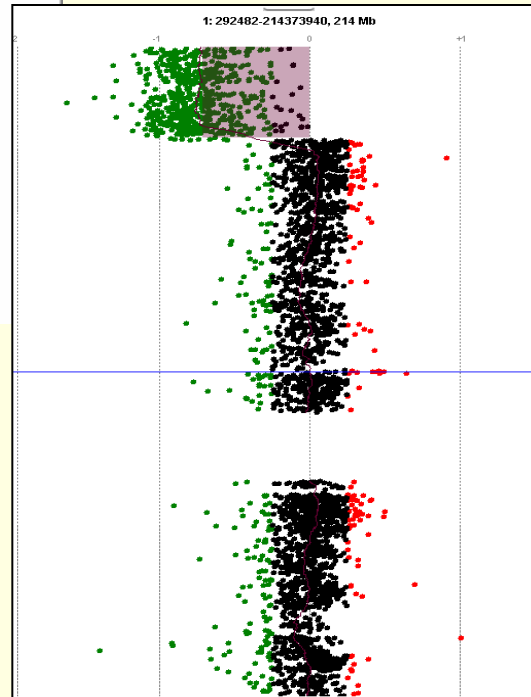
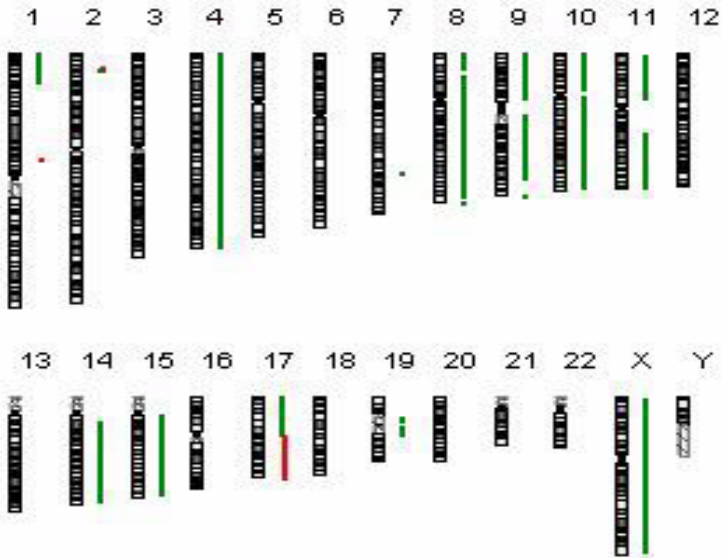
BAC, PAC, c-DNA clones, oligonucleotides



Array-CGH



Genome Overview



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-dependent 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

Syntetic oligonukleotide
50-60 bp

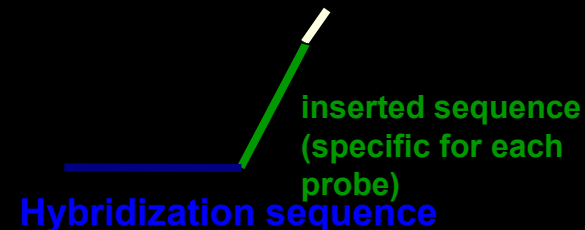
PCR primer Y



Hybridization sequence

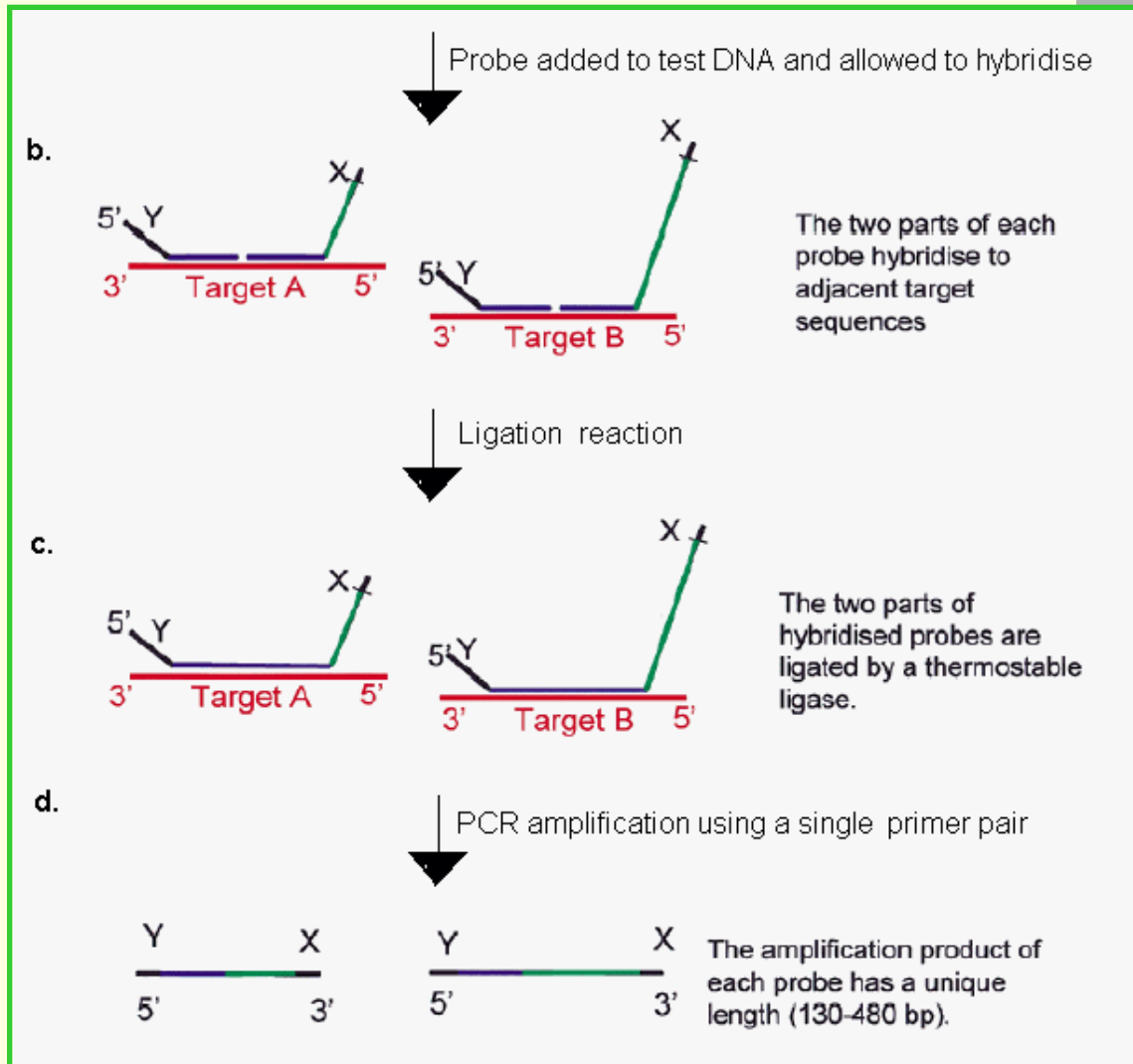
M13-derived oligonukleotide
60-450 bp

PCR primer X

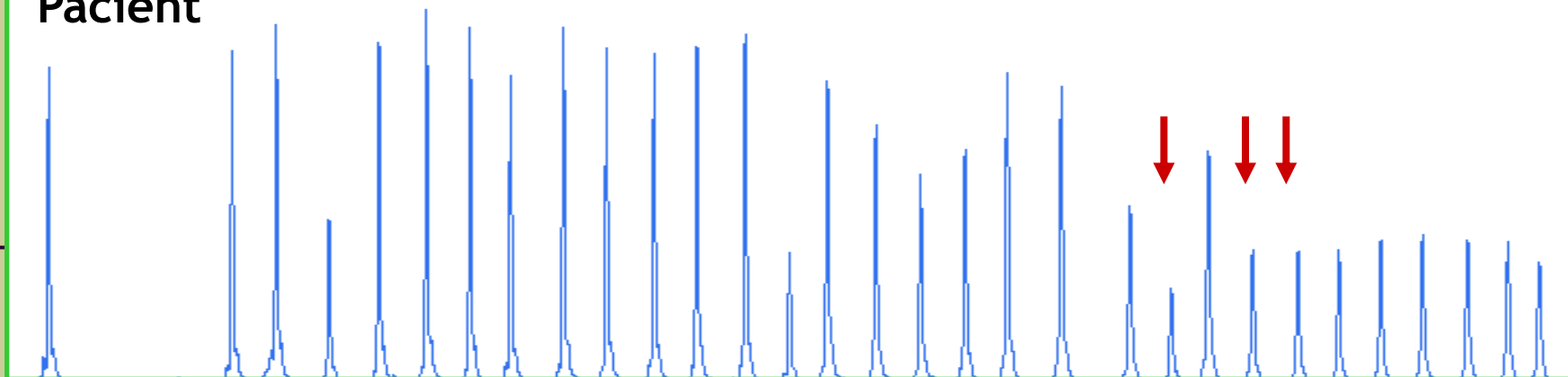


Hybridization sequence

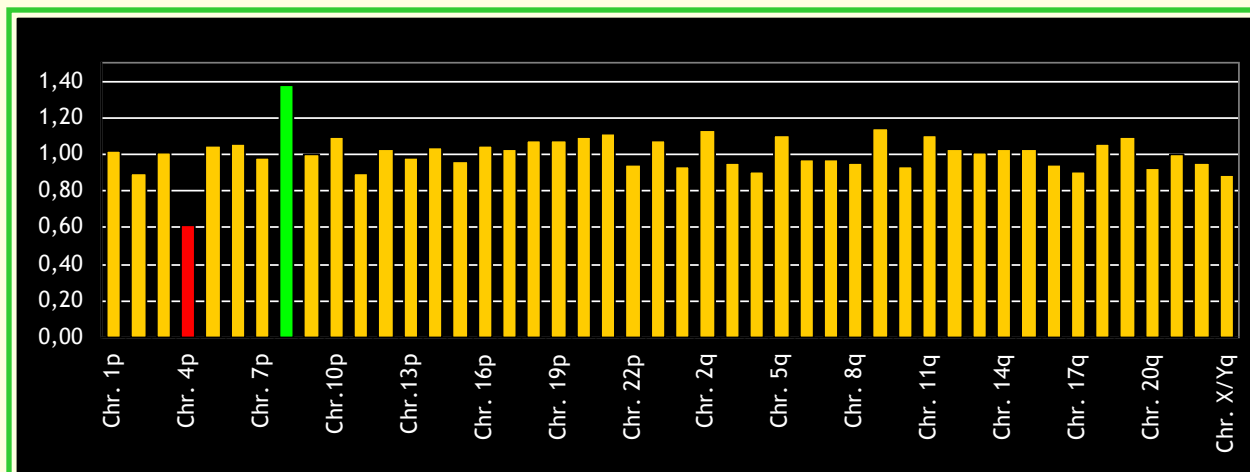
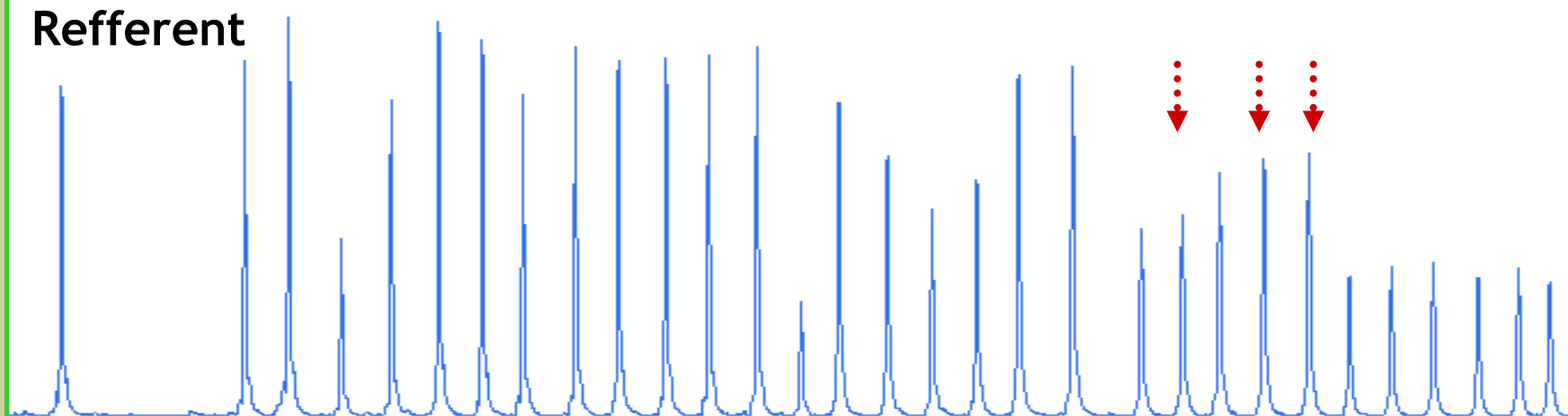
MLPA principle



Patient



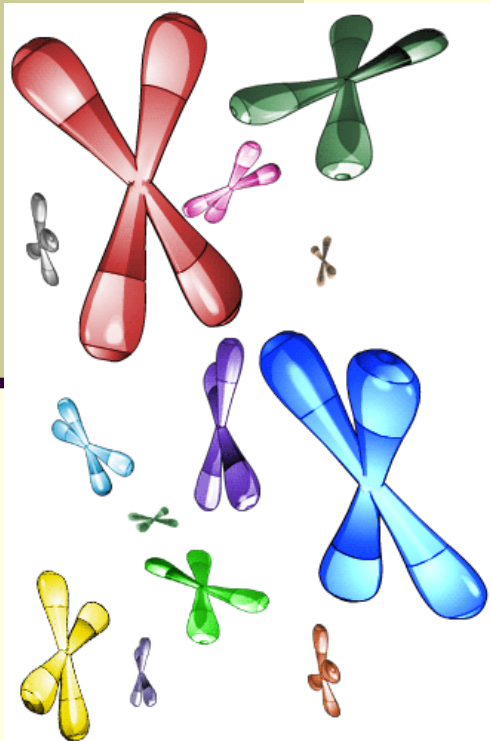
Refferent



Advantages and disadvantages of MLPA

- advantages
 - sensitive
 - specific
 - multiplex
 - simple
 - cheap
- disadvantages
 - highly sensitive to contamination
 - time difficulty
 - the aberration have to occur in 50% of cells
 - some mutations or polymorphisms 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**

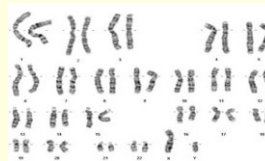
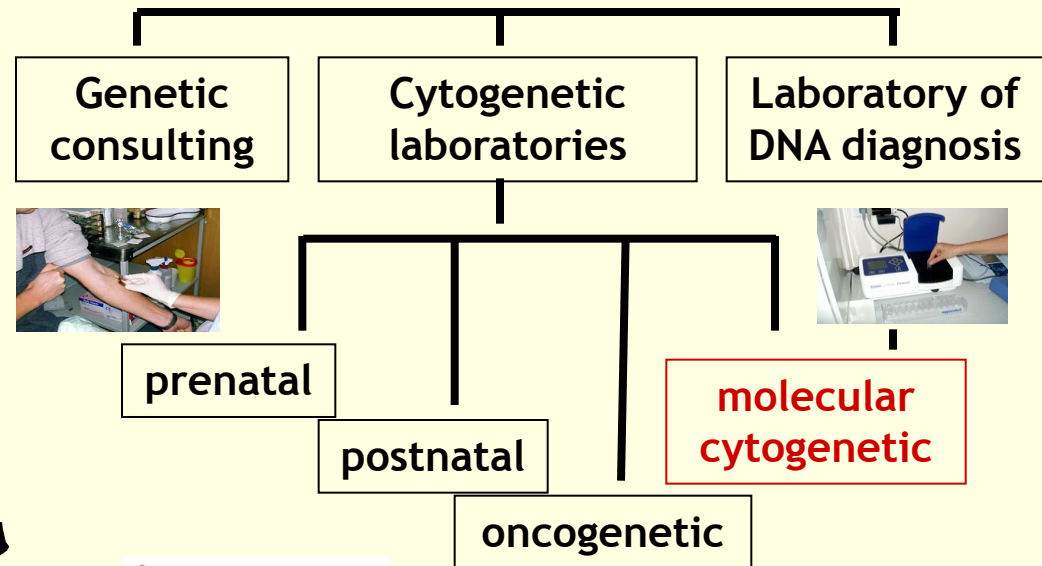
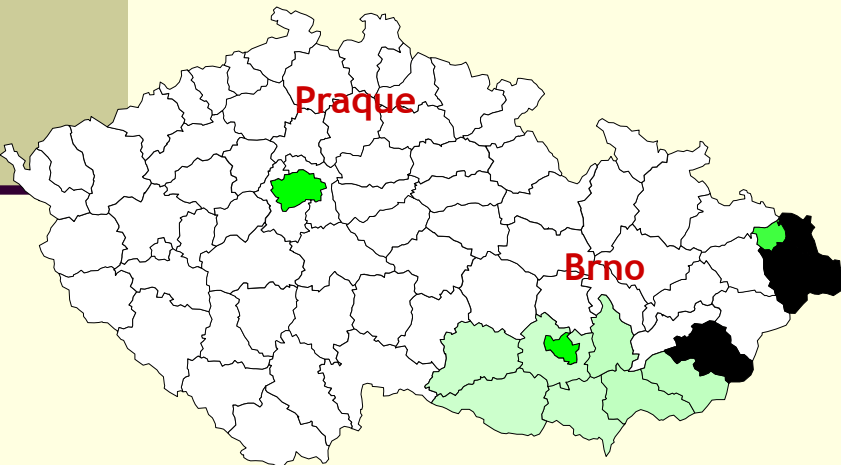
Who are we?

Integrated laboratory of molecular cytogenetics 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

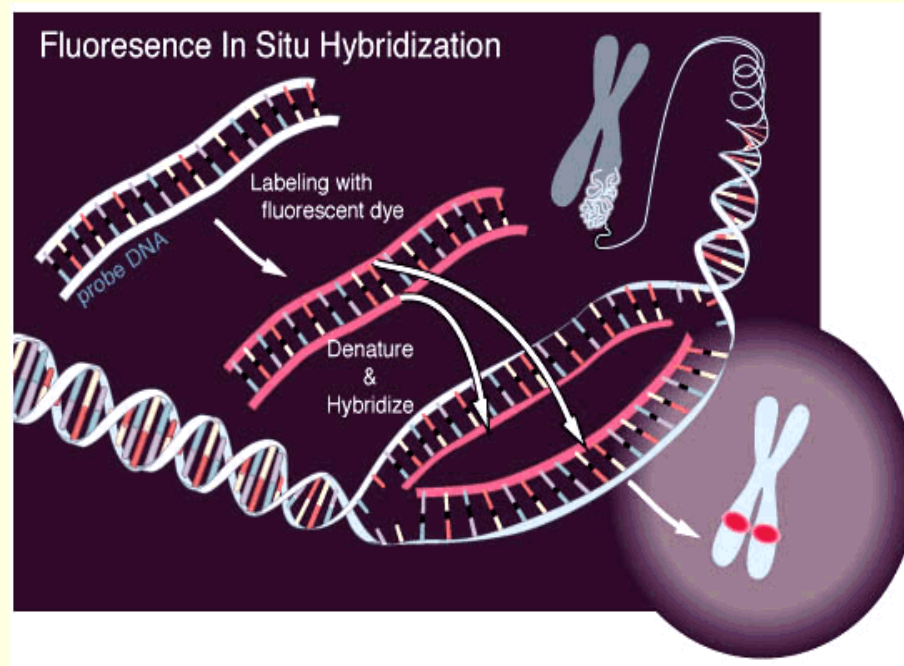


Department of Medical Genetics, University Hospital Brno: the centre for genetic investigation for South Moravia region

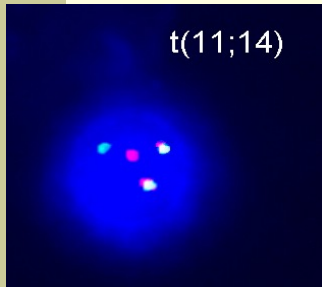


What is our interest?

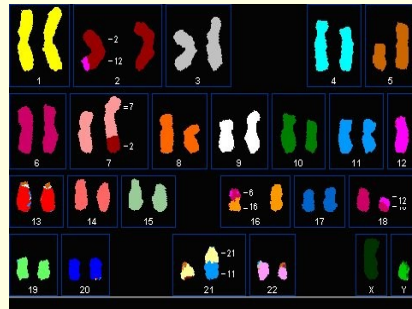
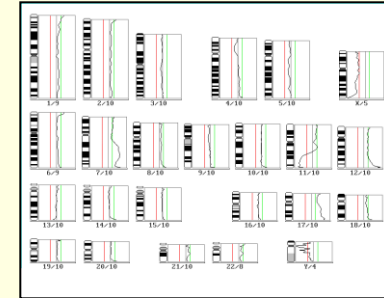
The main interest of the Integrated laboratory is the research of chromosomal aberrations using **molecular cytogenetic techniques**.



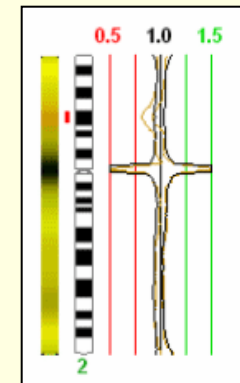
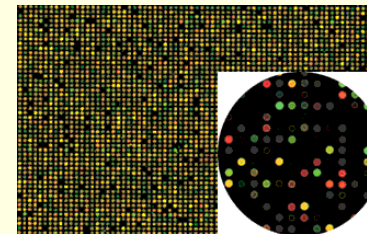
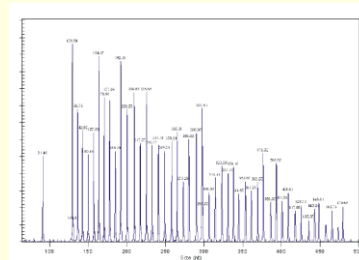
Methods



- 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

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

AneuVysion Assay Kit (Abbott Vysis)

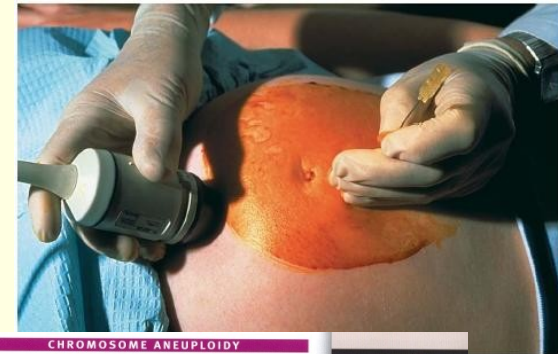
Mix1:

- 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)



DNA FISH Probes for Prenatal, Postnatal and Preimplantation Genetics

2

CHROMOSOME ANEUPLOIDY

ANEUVYSION[™]
AneuVysion Assay Kit
 33-161075 – 10 Assays
 32-161075 – 30 Assays
 35-161075 – 50 Assays (DVD)
 (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 aneuploidies is especially important in high risk pregnancies and medically indicated situations. These situations may include: positive maternal serum screens, 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 X: SpectrumGreen DYZ1, alpha satellite DNA (pYp11.1-q11.1)
 CEP Y: SpectrumOrange DYZ3, alpha satellite DNA (pYp11.1-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.

ProbeChk 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.

ProbeChk 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.

The AneuVysion Assay Kit, FDA cleared *in vitro* diagnostic use.

Direct (uncultured) amniocyte hybrid with the AneuVysion 18/XY probes. The aqua signals indicate three copies of chromosome 18, one green signal indicates copy of chromosome X and one orange signal indicates one copy of chromosome Y.

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

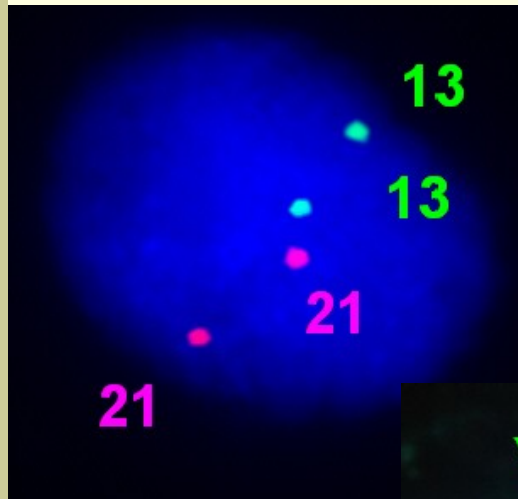
13q14 LSI 13
SpectrumGreen

21q22.13-q22.2 LSI 21
SpectrumOrange

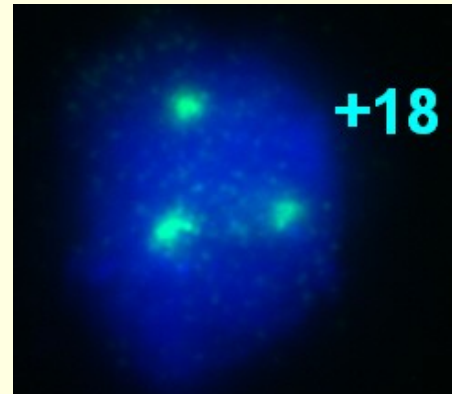
18

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

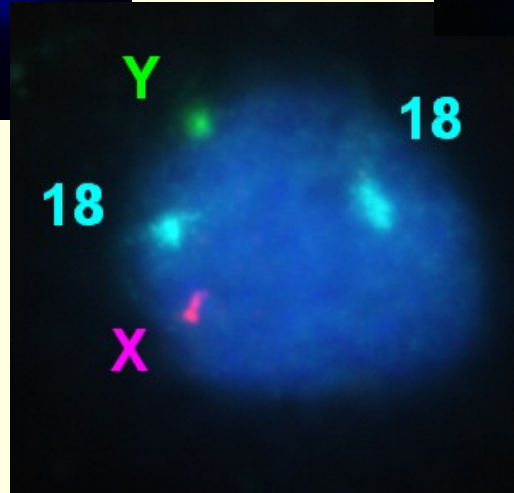
Prenatal cytogenetic analyses



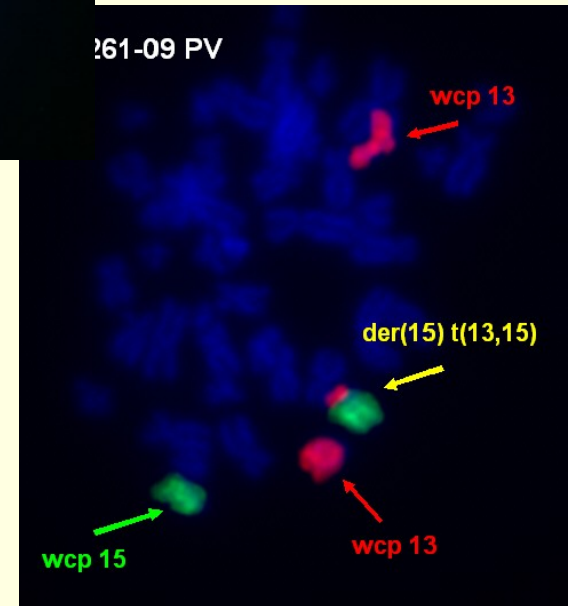
Normal cells



trisomy
of chr. 18



t(13;15)



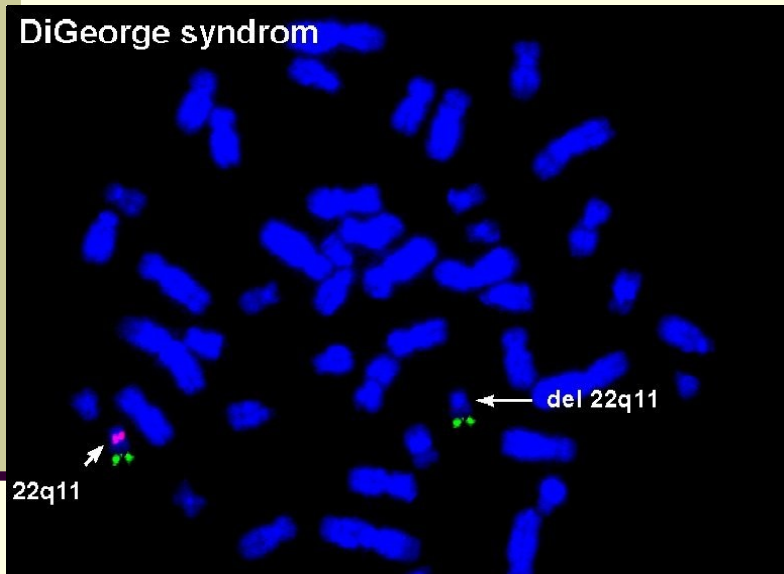
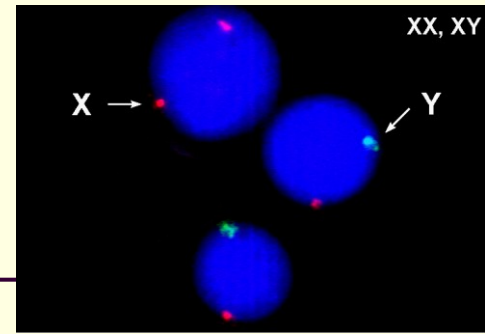
Postnatal cytogenetic analyses



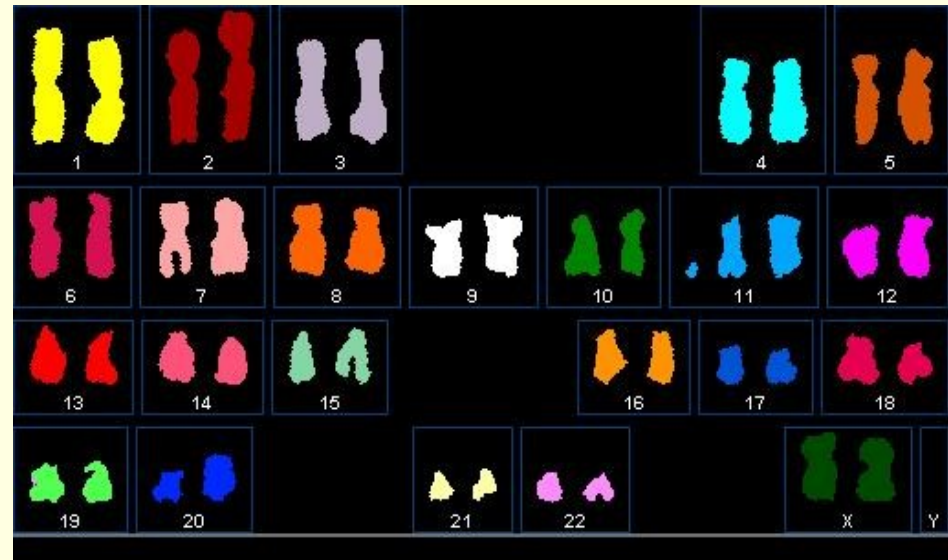
ToTel Vysion Kit, Abbott-Vysis

- **Peripheral lymphocytes, buccal swab**
- **FISH, CGH, HR-CGH, array-CGH, MLPA, SKY**
- **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 mosaics** – FISH (X/Y probes) in infertile couples or gonosomal syndromes

Postnatal cytogenetic analyses

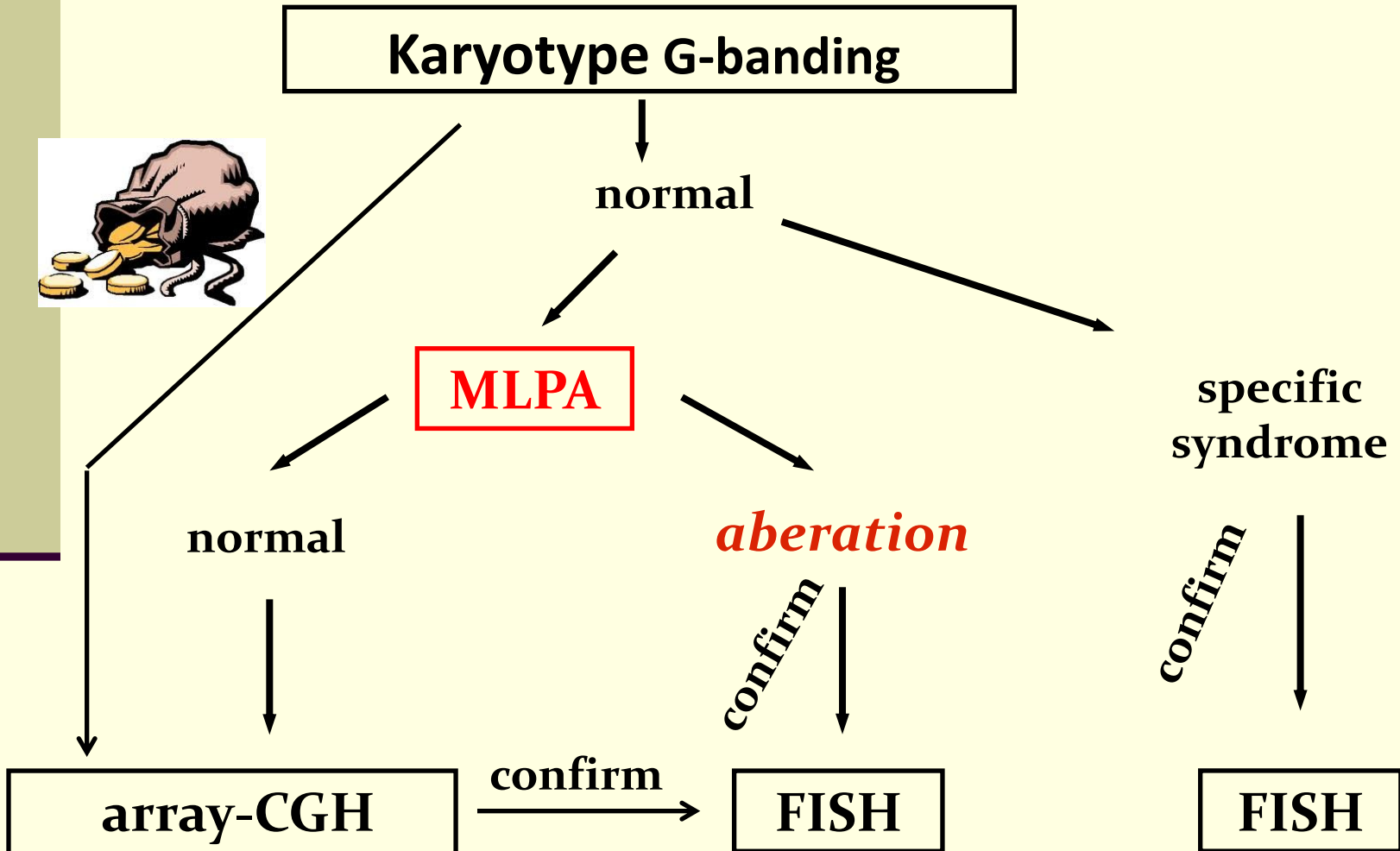


FISH: deletion of (22)(q11.2)
(DiGeorge syndrome)



SKY: marker chromosome identification
(chr. 11)

How do we proceed?



Case interpretation

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

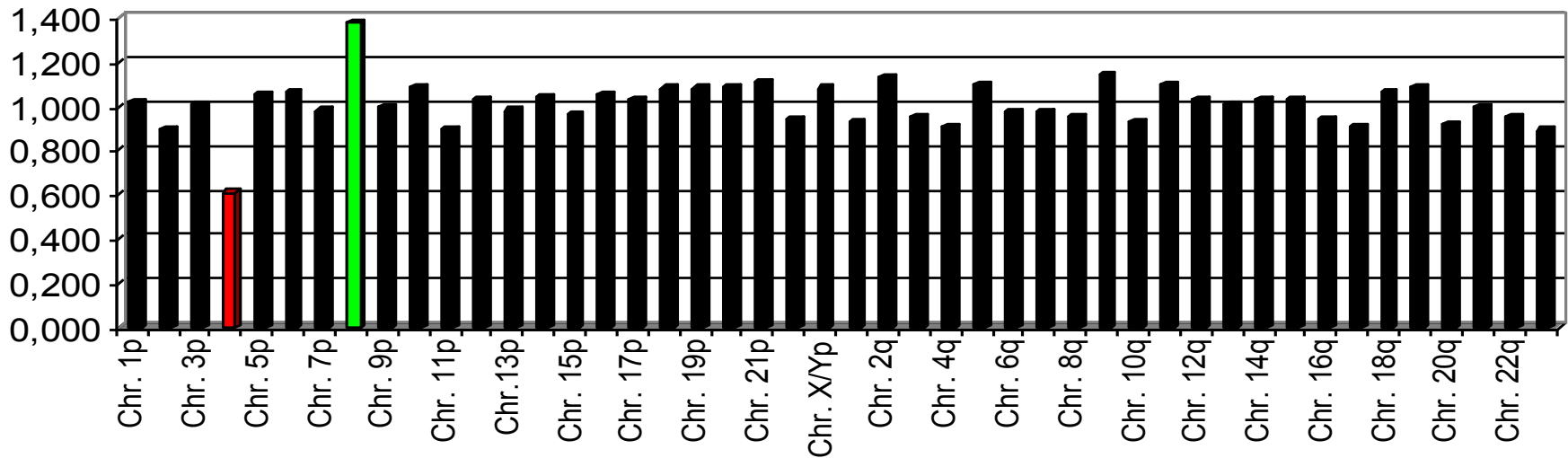


- Born in 2001
- Clinical symptoms:
 - hard PMR
 - facial dysmorphism
 - stigmata
 - hypertelorism
 - hemangioma on right eye lid

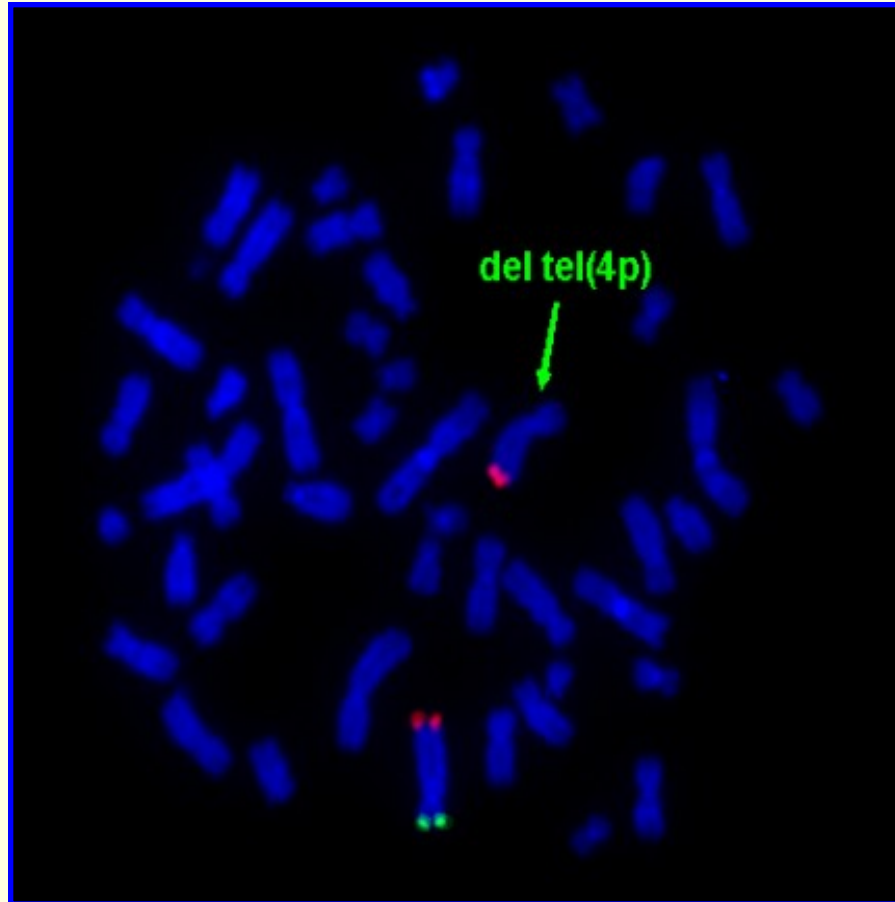
Examination

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

Patient 1 - MLPA

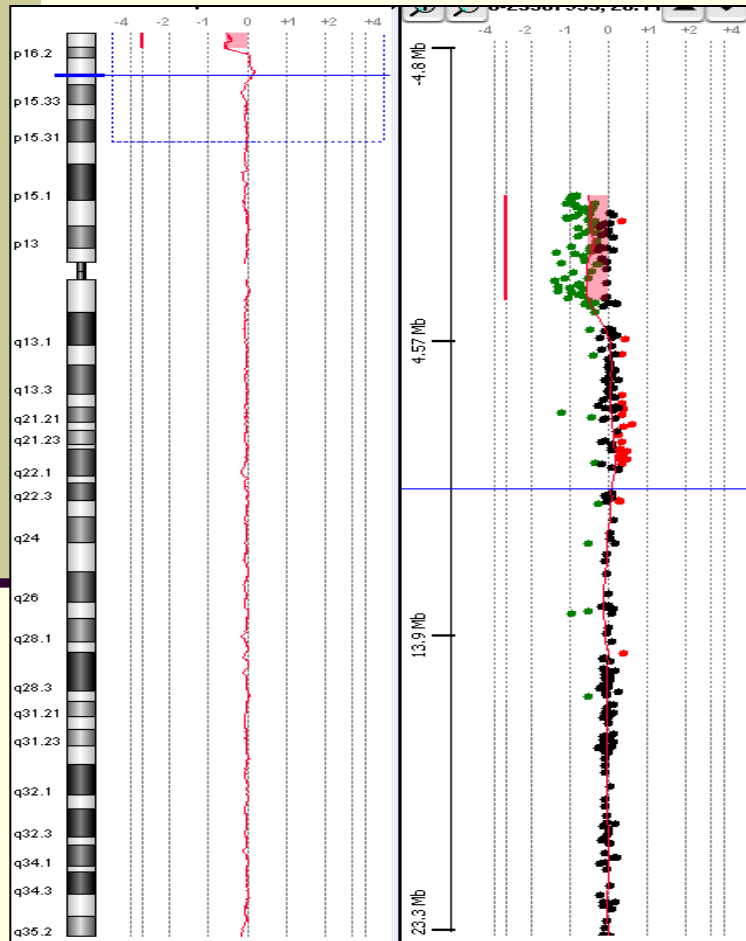


Deletion confirmation by the FISH method



Array CGH – confirmation of the aberation

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



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

