Cytogenetics & molecular cytogenetics

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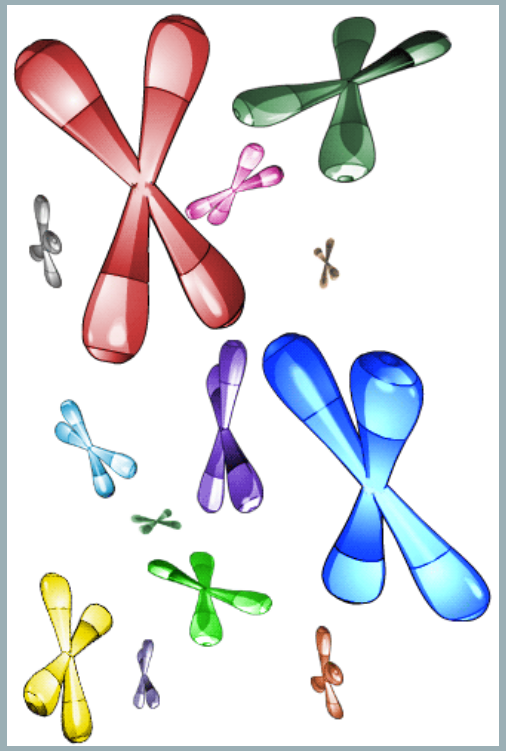

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WARNING

- This presentation is intended exclusively for educational purposes

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WHAT ARE WE GOING TO TALK ABOUT?

1. Department of Medical Genetics, genetic laboratories
2. What is cytogenetics
3. History
4. Chromosome morphology and aberrations
5. Molecular cytogenetics and its techniques
6. Case interpretation
7. Our laboratory and work

DEPARTMENT OF MEDICAL GENETICS

- Medical specialists providing highly specialized and complex genetic counselling for different types of patients and diagnoses
  - infertility, recurrent abortions
  - preimplantation and prenatal diagnostics (abnormal screening)
  - developmental delay, intellectual disability, congenital somatic abnormalities, monogenic disorders.. (solitary or familial)
  - Familial segregation of various types of disorders (chromosomal abnormalities, monogenic disorders, etc.)
  - Familial occurrence of cancers
  - Oocyte and sperm donors
  - ……

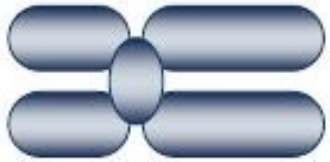
Clinical diagnoses must by confirmed on the molecular level (chromosomes, DNA)!

Services and types of analyses

Ambulance of clinical genetics

Laboratories of cytogenetics

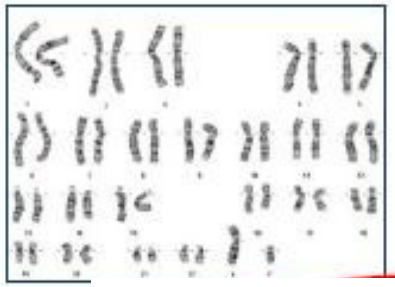
Laboratories of molecular diagnostics

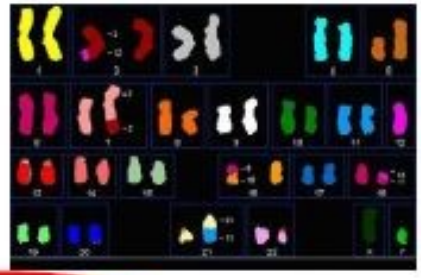


chromosomes

DNA/RNA

patient





Molecular genetics analyses

Cytogenetics analyses

genealogy

Classical and banding cytogenetics

Molecular cytogenetics

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. A BRIEF HISTORY OF (CYTO)GENETICS

- 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

HISTORY OF HUMAN CYTOGENETICS

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

  - hypotonization of cell samples (1951 - 0,075 m KCl)
  - utility of phytohaemagglutinin (PHA) - stimulation of peripheral blood lymphocytes - 1960

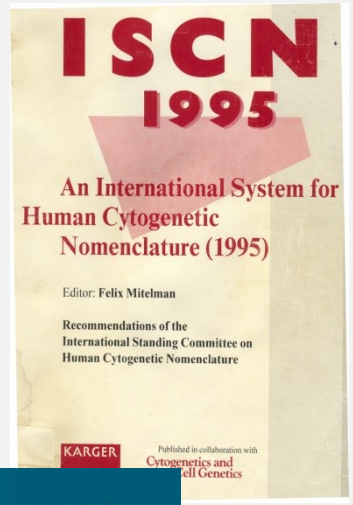
- **„Trisomy Period"** - trisomy of chromosome 21-1959
- The first deletion syndrome - "Cri du chat" - 1963
- **„Banding Area"** - chromosome banding techniques 1968 – 1970
- **„Molecular Area"**

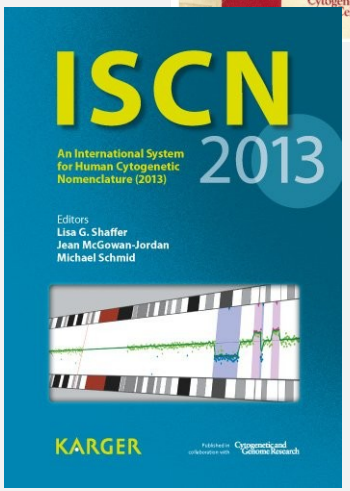
  - in situ hybridization technique – 1970
    - FISH – 1986
    - Comparative genomic hybridization (CGH) – 1992
    - spectral karyotyping (SKY), multicolor FISH (M-FISH) – 1996
    - m-banding – 2001
    - **array-CGH (molecular karyotyping)**

NOMENCLATURE OF HUMAN CHROMOSOMES

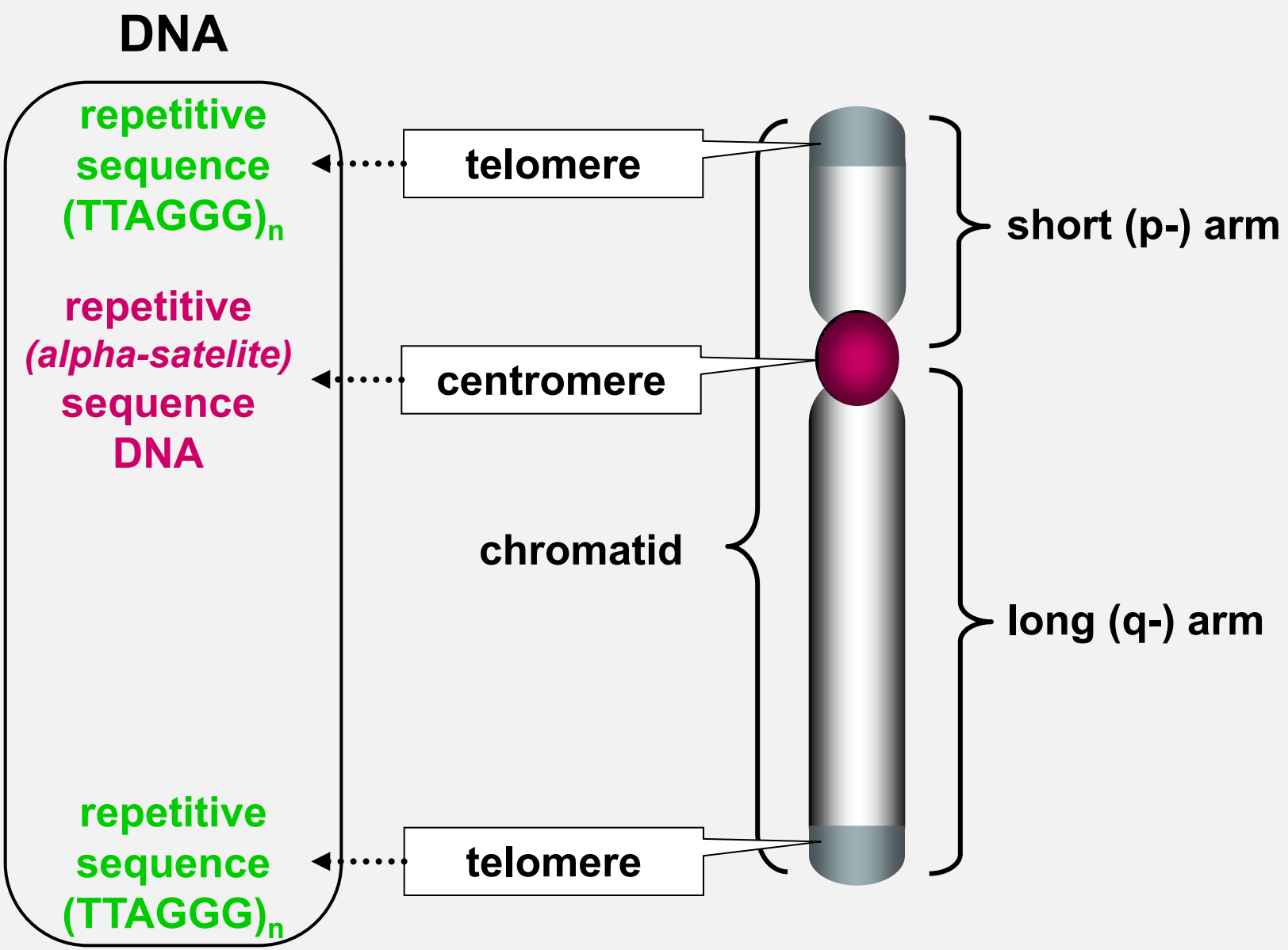
- **1960:** *Denver Conference* - sort of human chromosomes into groups according to size and shape
- **1963:** *London Conference* - chromosomes are sorted into 7 groups A – G
- **1966:** Chicago *Conference* - the description of chromosome changes
- **1971:** *Paris Conference* - the identification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

(ISCN 1978; ISCN 2016 *in present*)

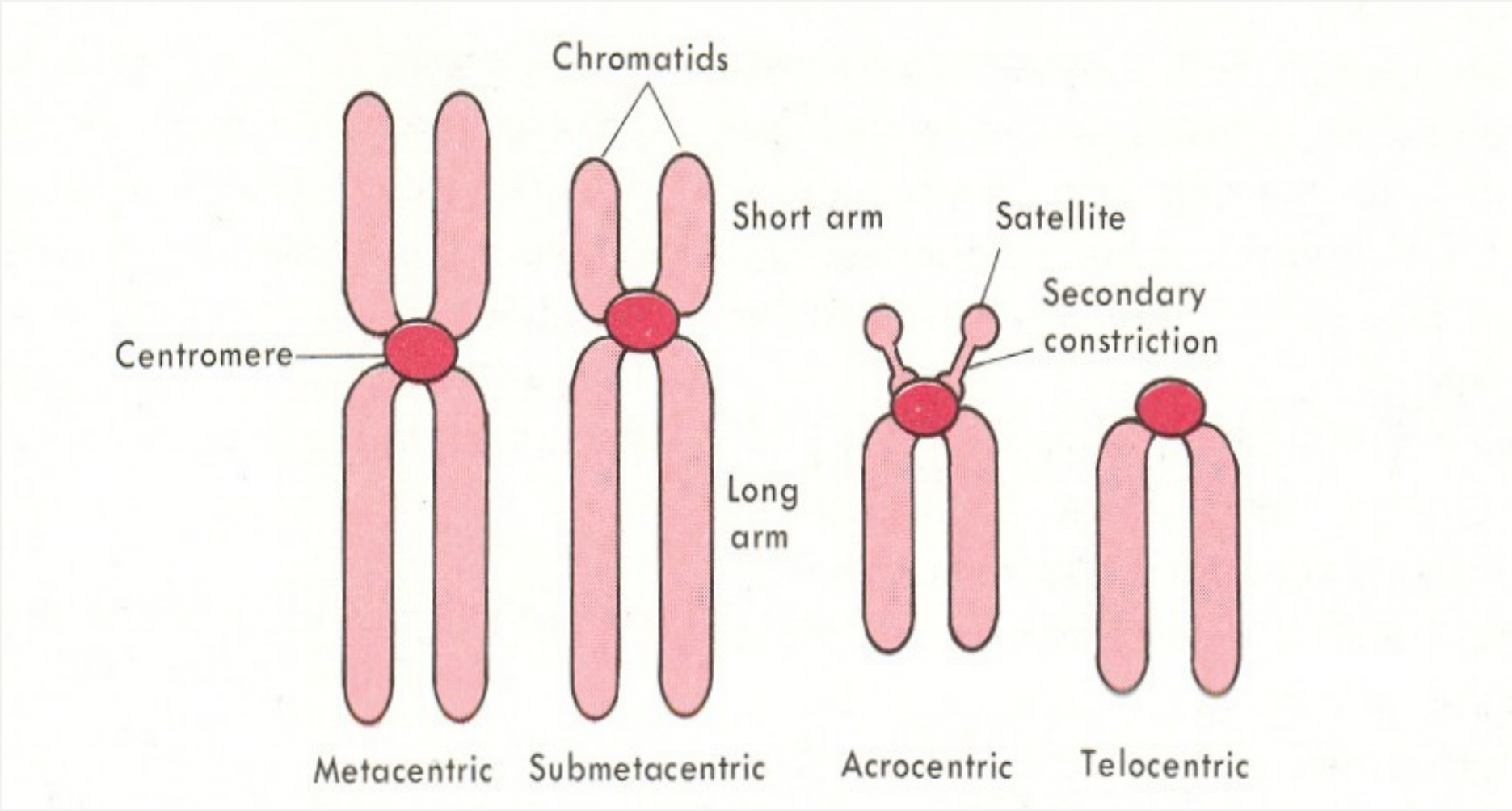




3. CHROMOSOME MORPHOLOGY



CHROMOSOME MORPHOLOGY



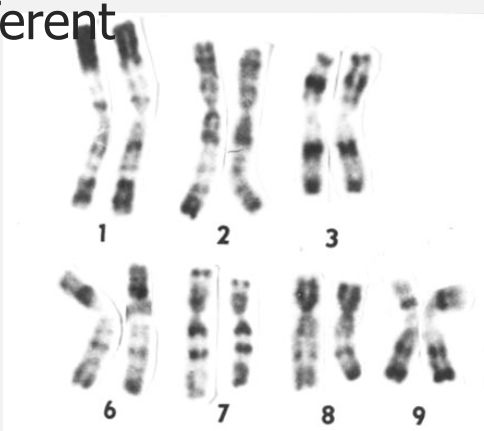
*note: telocentric chromosomes are not present in human karyotype

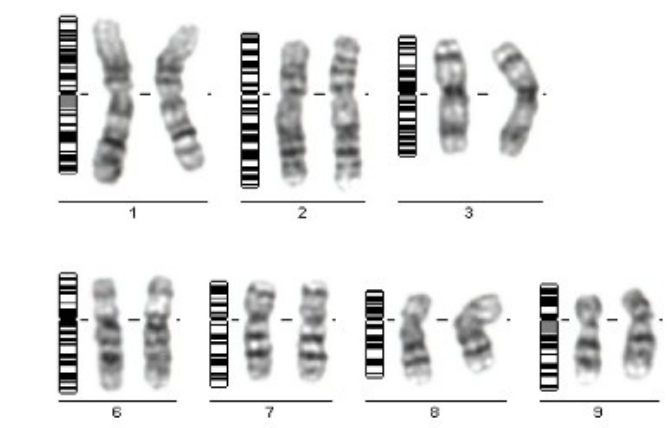
CHROMOSOME STAINING

- Classical painting
  - using Giemsa Romanowski solution
  - Detection of acquired chromosome aberations

- G – bands
  - using trypsin, salty solution and Giemsa
  - each chromosome has characteristic pattern
    (dark bands – A/T rich, light bands – G/C rich)
  - Detection of congenital chromosomes aberations (mostly > 10 Mb)

- R – bands
  - using salty solution of different pH and temperature
  - reverse to G - bands

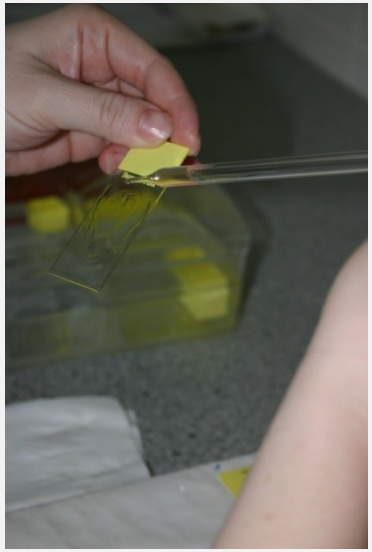


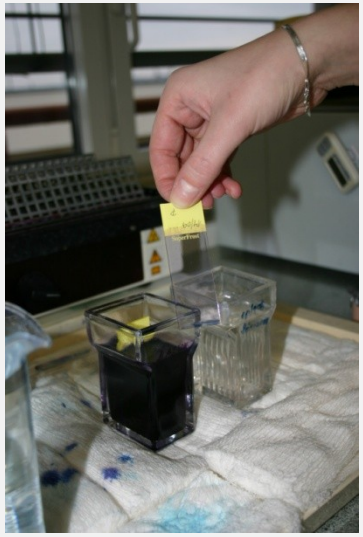


METHODOLOGY FOR CLASSICAL AND BANDING CYTOGENETICS

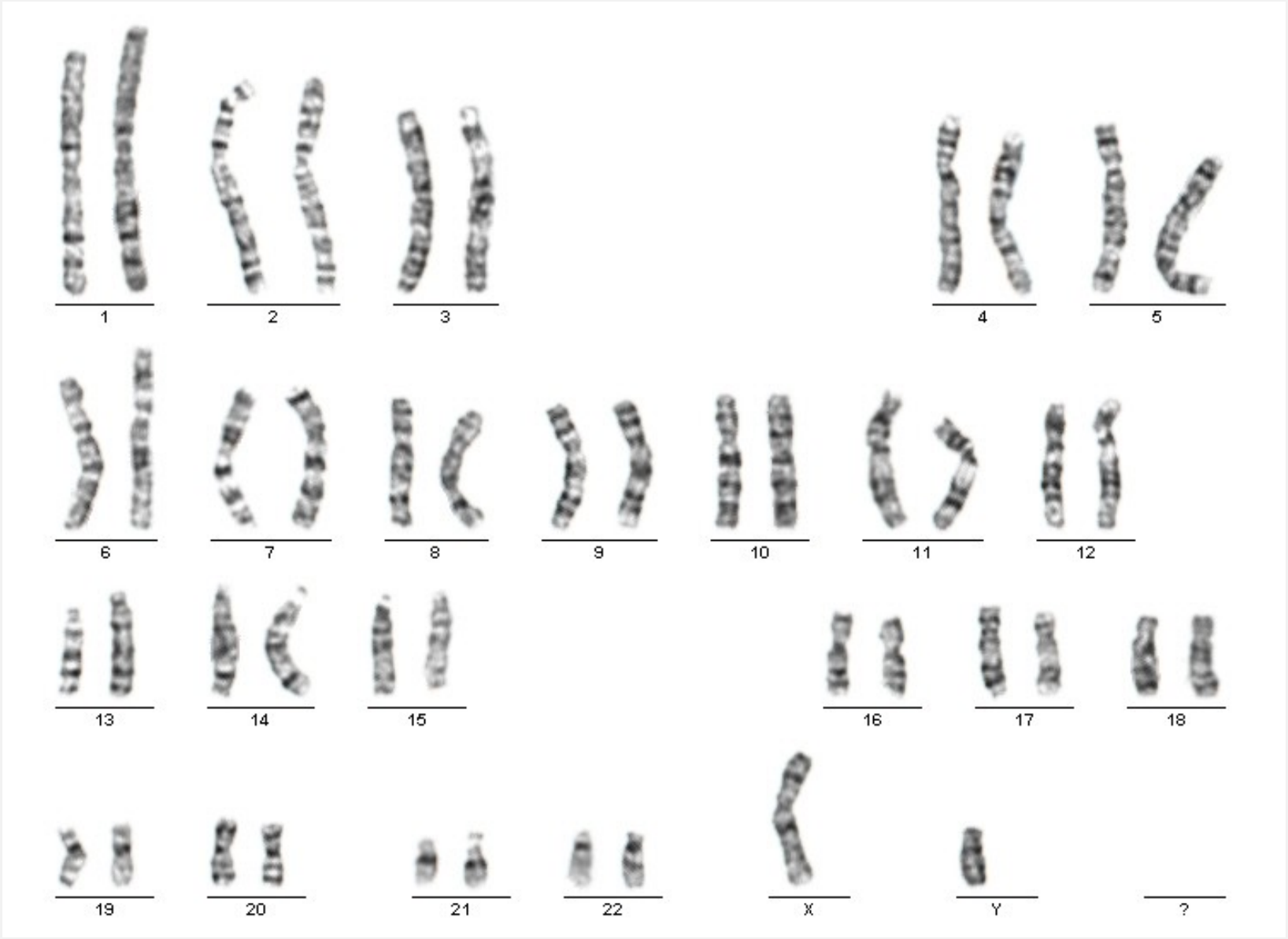
- To drop processed cell suspension with mitoses on microscopic slides
- Staining to obtain chromosomal bands
  - 1) digestion the specimen in trypsin solution
  - 2) staining in Giemsa-Romanovski dye
- Evaluation in light microscope with CCD camera

-> to get karyotype (computational analysis of image)

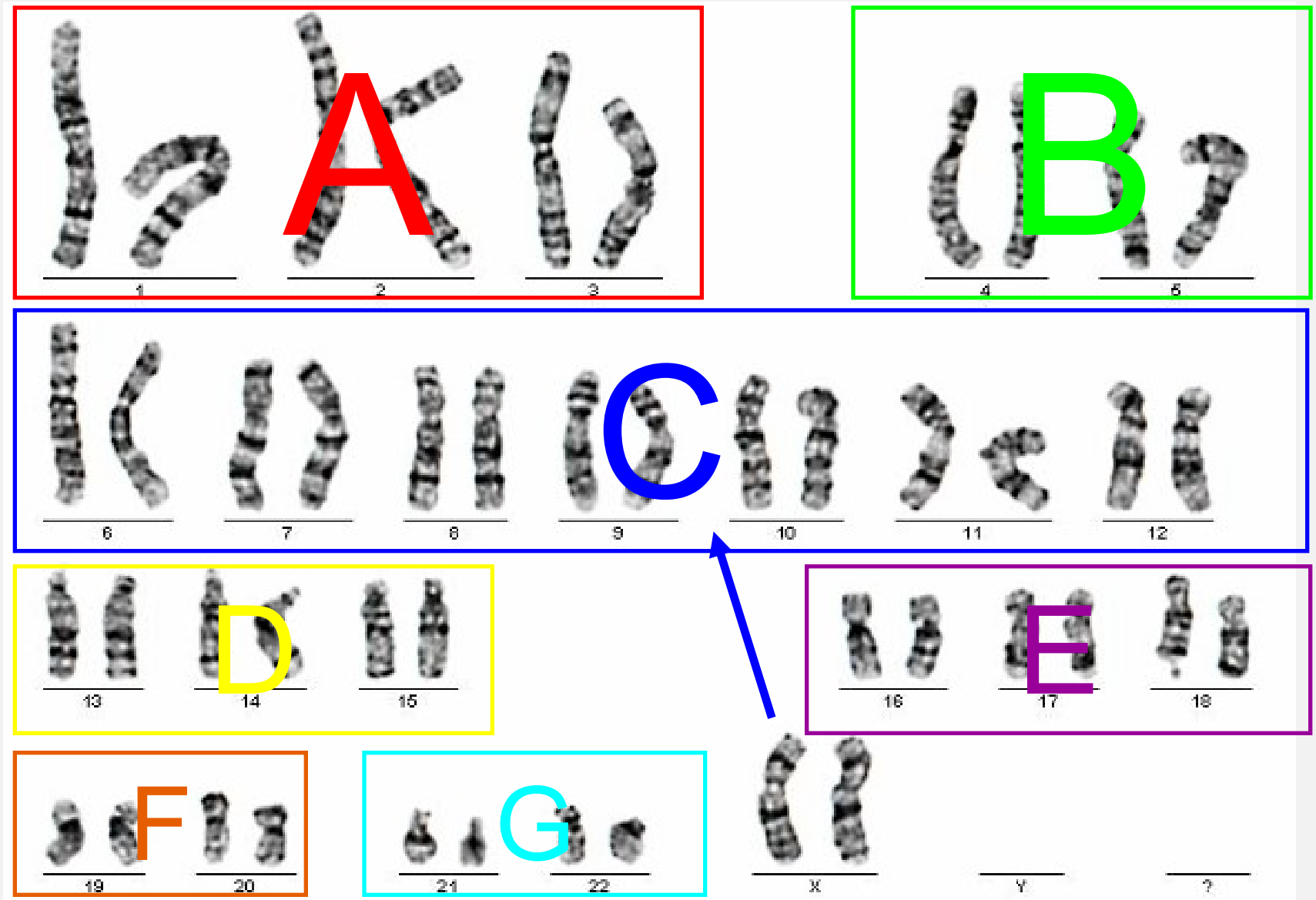




NORMAL MALE KARYOTYPE (46,XY)



HUMAN KARYOTYPE – 7 GROUPS OF CHROMOSOMES



HUMAN SOMATIC CELL CONTAINS:

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

A – large metacentric chromosomes

B – large submetacentric chromosomes

C – middle-sized submetacentric chromosomes and chromosome X

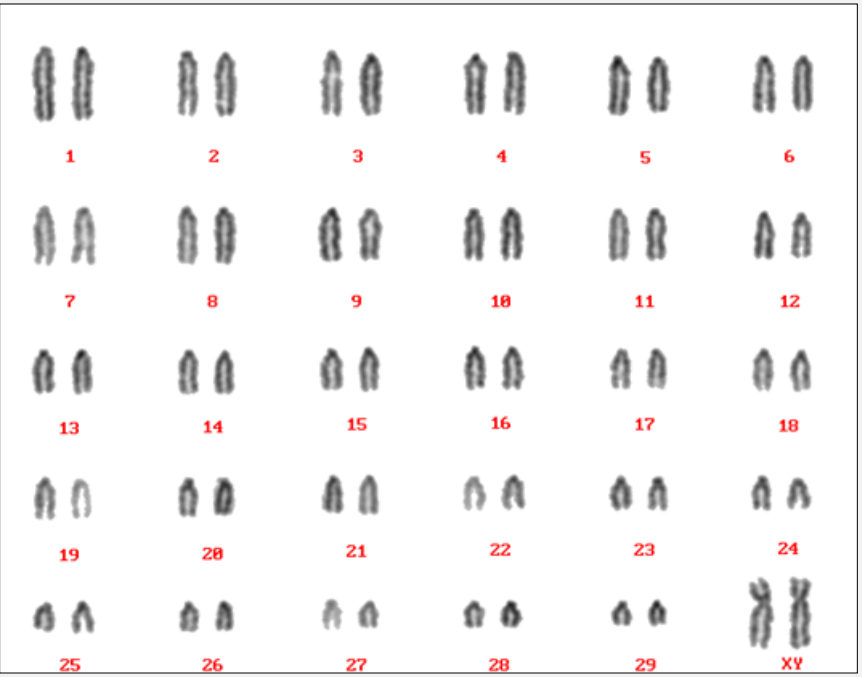
D – large acrocentric chromosomes

E – small meta- to submetacentric chromosomes

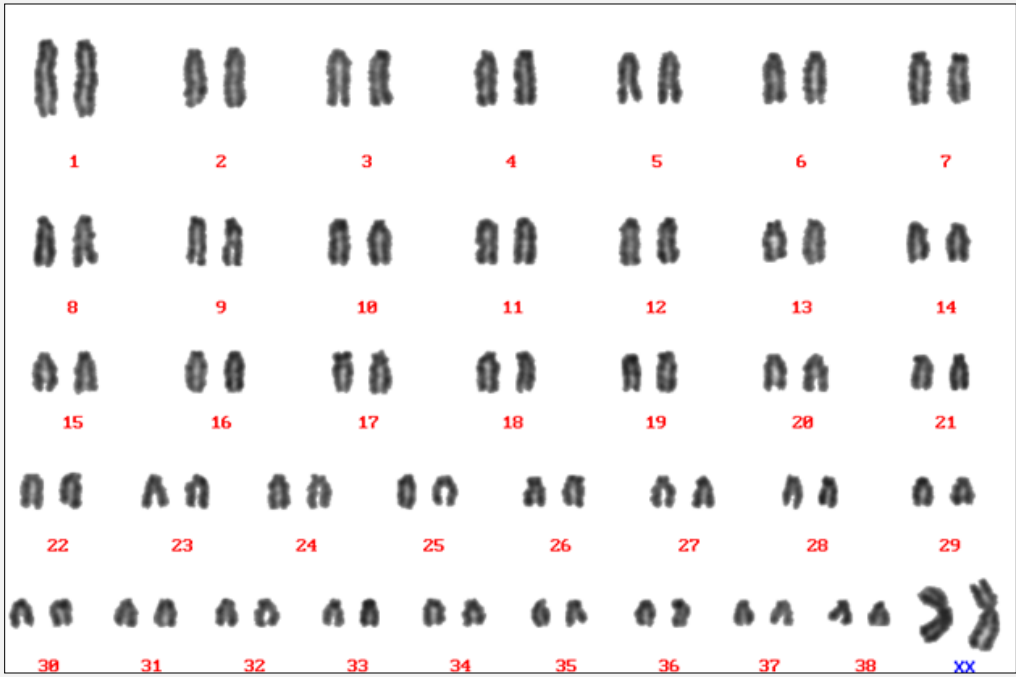
F – the smallest metacentric chromosomes („ribbons")

G – small acrocentric chromosomes and chromosome Y

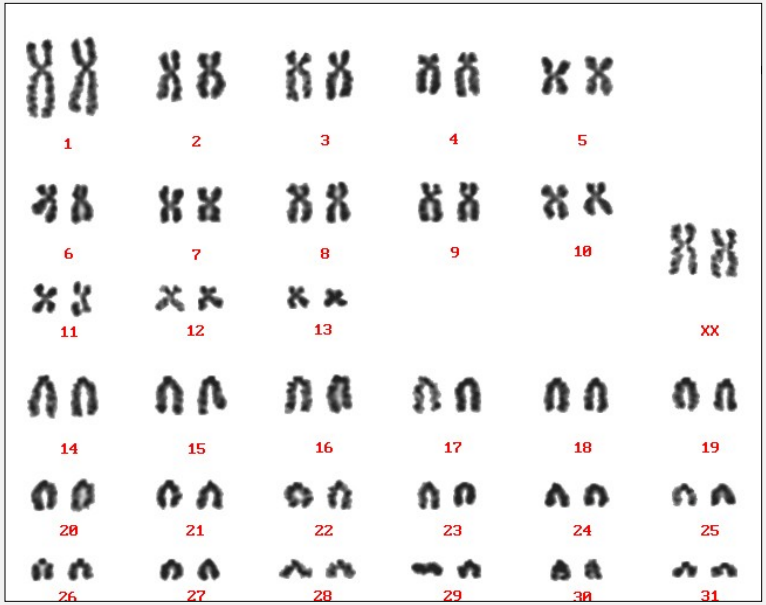
KARYOTYPES OF ANIMALS (EXAMPLES)



Cattle (2n=60)



Dog (2n=78)

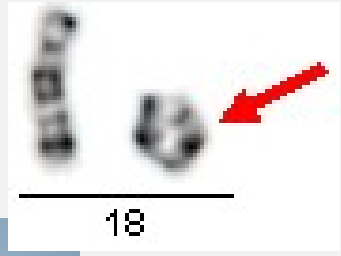


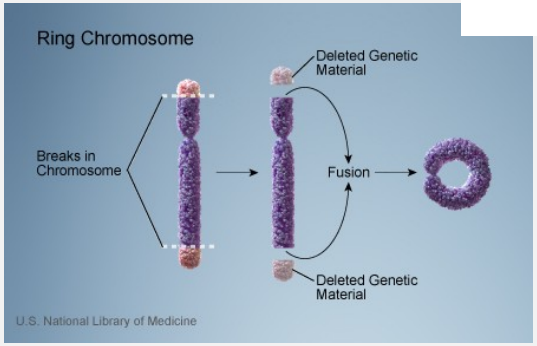
Horse (2n=64)

CHROMOSOME ABERRATIONS

AUTOSOMES

- *1. Structural chromosomal aberrations*
  - **Polymorphisms**
    - different lenght of chromosomes in homologous pair
    - no phenotype effect
  - **Inversion**
    - pericenric – including centromere
    - paracentric – does not include centromere
    - usually has no phenotype effect in its carrier
  - **Ring chromosomes**
    - breaks on both chromatides and their connection
    - loss of telomeric parts
    - intellectual and physical impairment
    - always newly created

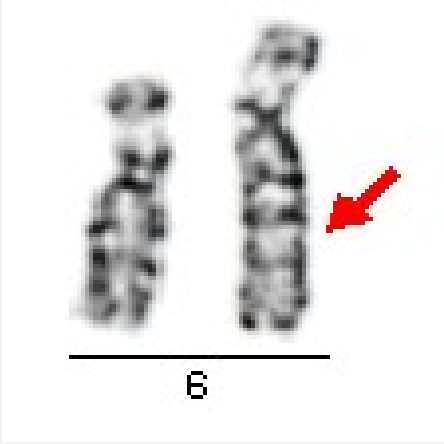




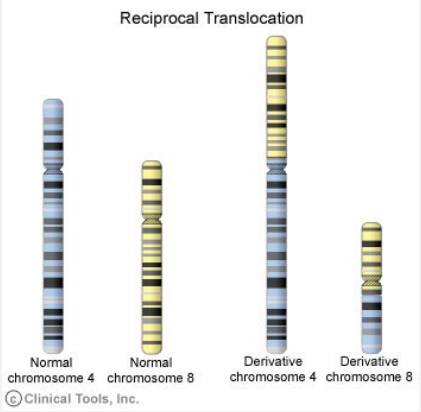
http://ghr.nlm.nih.gov/handbook/illustrations/ringchromosome

CHROMOSOME ABERRATIONS

- **Deletion** = loss of part of chromosome ->unbalanced karyotype
  - terminal – one break and loss of a terminal part
  - interstitial – two breaks and loss of a part between centromere and terminal part
  - deletion syndromes - examples:
    - Wolf-Hirschhorn syndrome (4p deletion), Cri-Du-Chat syndrome (5p deletion)
  - microdeletion syndromes - examples:
    - Prader-Willi syndrome; 15q11-q13 deletion
    - DiGeorge syndrome; 22q11.2 deletion
    - Angelman syndrome; 15q11-q13 deletion
    - Williams-Beuren syndrome; 7q11.23 deletion
- **Duplication** = gain of chromosomal part -> unbalanced karyotype
  - Typically less harmful than deletions
- **Insertion**
  - inserted part can be in the same or inverted position

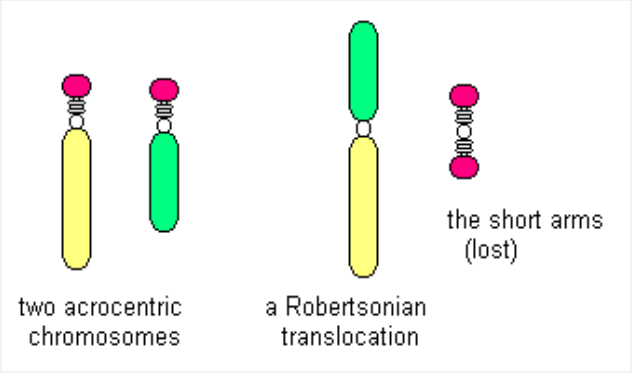


- **Translocation**
  - reciprocal
    - mutual exchange between two or more nonhomologic chromosomes
    - balanced - no phenotype effect for carriers
    - genetic risk of unbalanced genomes in carrier's offspring



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

  - 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
    - genetic risk for offspring

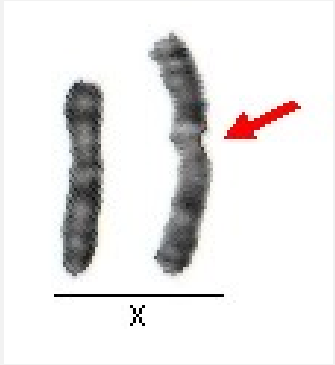


http://drugline.org/medic/term/robertsonian-translocation/

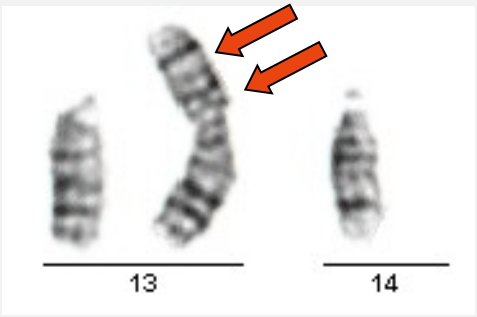
  - Simple
    - One break in the arm of one chromosome
    - Fusion of the broken part with another chromosome

- **Marker chromosomes**
  - Small supernumerical chromosomes (with centromere)
  - Often in mosaic form
  - Sometimes difficult to identify the origin

- **Isochromosomes**
  - Metacentric chromosomes – one arm is lost, the second one is duplicated



- **Dicentric chromosomes**
  - Breaks on two chromosomes
  - Fusion of parts with centromeres
  - Acentric fragment is lost



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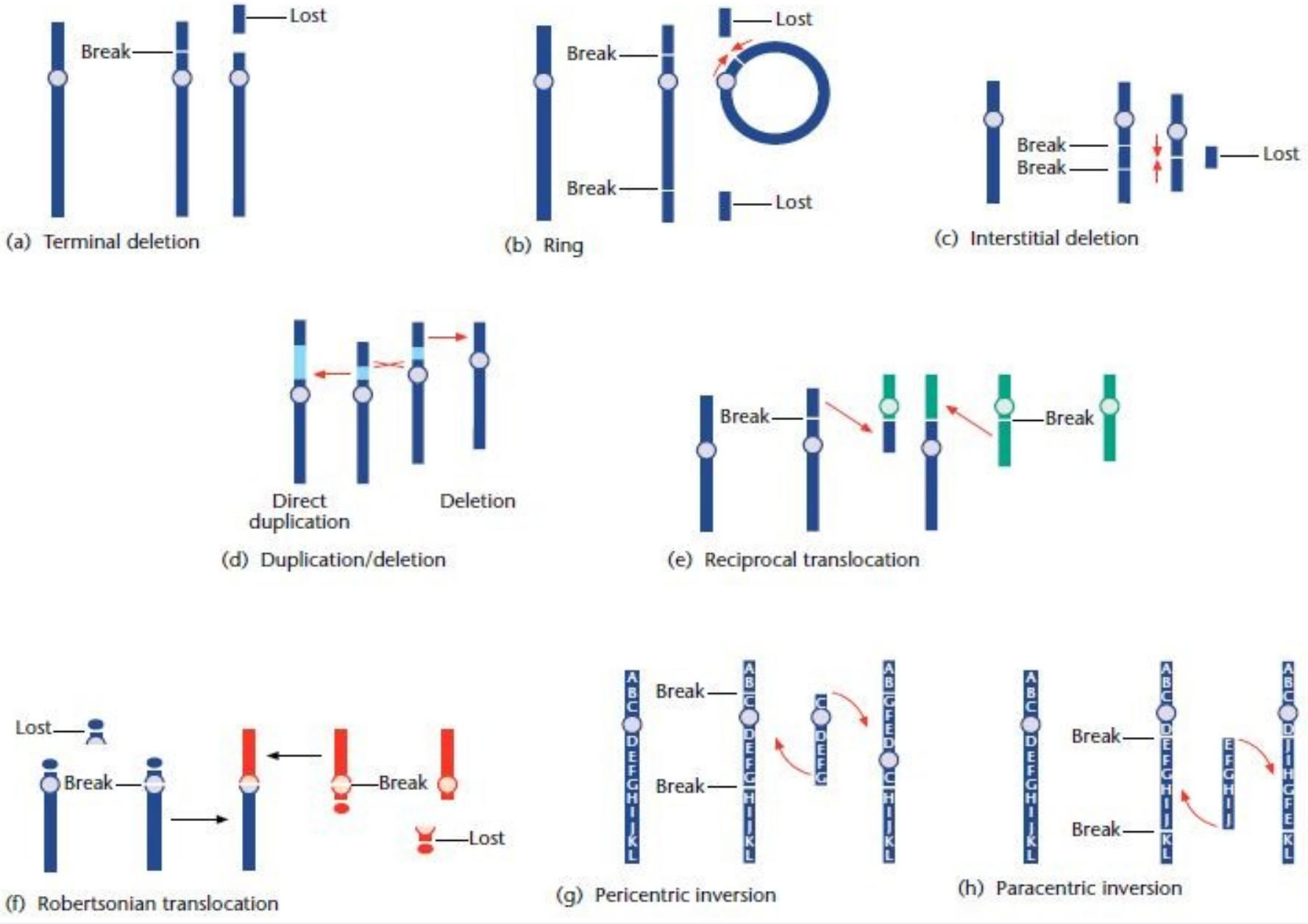
CHROMOSOMAL ABERRATIONS – OVERVIEW



M Moore, Charleen & Best, Robert. (2001). Chromosomal Genetic Disease: Structural Aberrations. 10.1038/npg.els.0001452.

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

GONOSOMES

- **Chromosome Y**
  - structural aberrations – very rare
  - numerical aberrations
    - 47, XYY – supermale syndrom
- **Chromosome X (males)**
  - Numerical aberration
    - 47, XXY – Klinefelter syndrom
- **Chromosome X (females)**
  - numerical aberrations
    - 45, X – Turner syndrom
    - 47, XXX – XXX syndrom
- **Fragile X – FRA-X**
  - the most common cause of intellectual disability (excluding trisomy 21)
  - nonspecific phenotype (intellectual impairment, facial dysmorphology, ...)

CHROMOSOMAL MOSAICISM

- Chromosomal aberrations are mostly in all human cells

- Mosaicism = 2 or more cell lines with different karyotype in human body

- Nondisjunction in early post-zygotic mitotic division (prenatal period)
- Numerical more frequent than structural

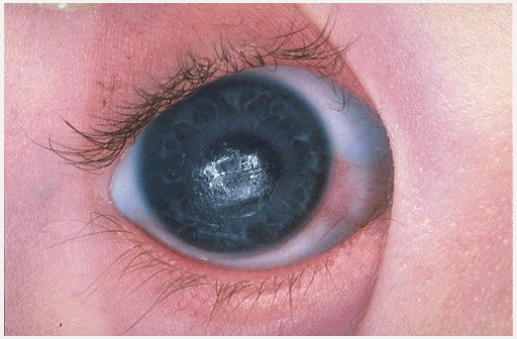
- Most often in gonosomal aneuploidies (-> spontaneous abortions, infertility)
  - Example: 45,X[6]/47,XXX[4]/46,XX[190]

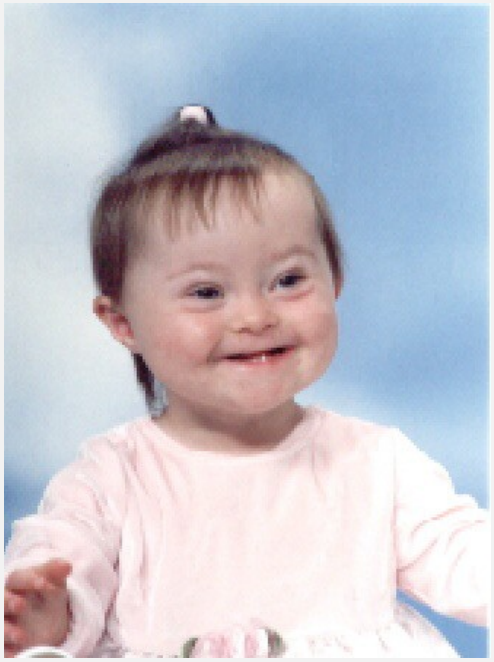
- Autosomal aneuploidies
  - Example: mosaic form of Down syndrome

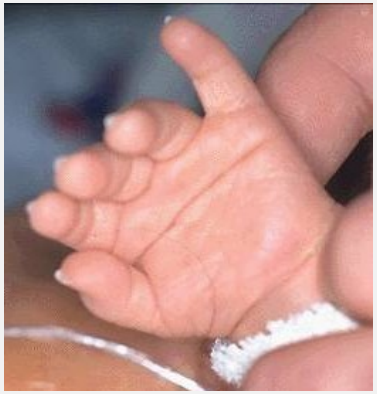
    47,XY,+21[172]/46,XY[28]

DOWN SYNDROME (47,XX OR XY,+21)

- 1866 J.L.Down
- IQ 25-50
- short stature
- round face
- short neck
- mongoloid eyes
- epicanthus
- wide nose root and flattened nose
- small mouth, large tongue, small teeth
- single transverse palmar crease
- heart diseases

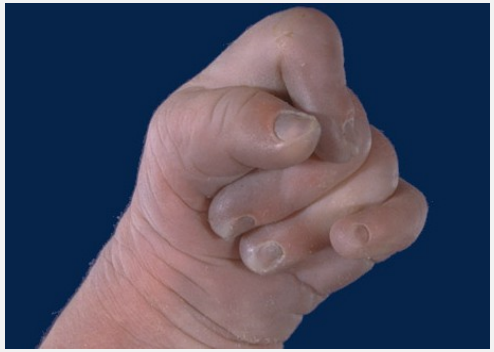


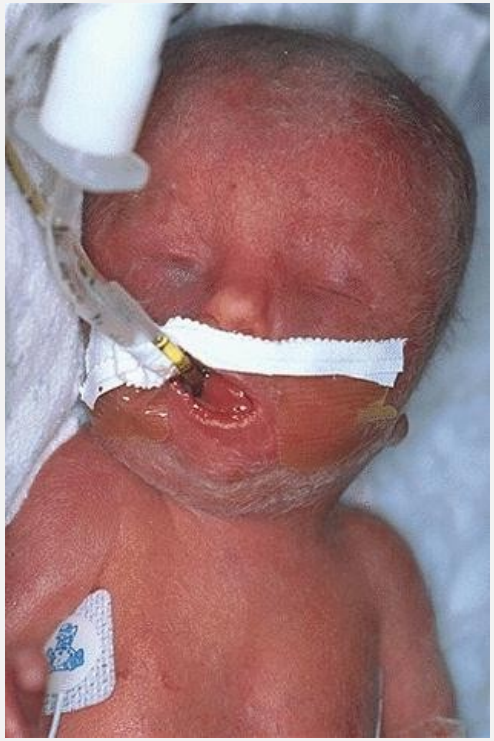


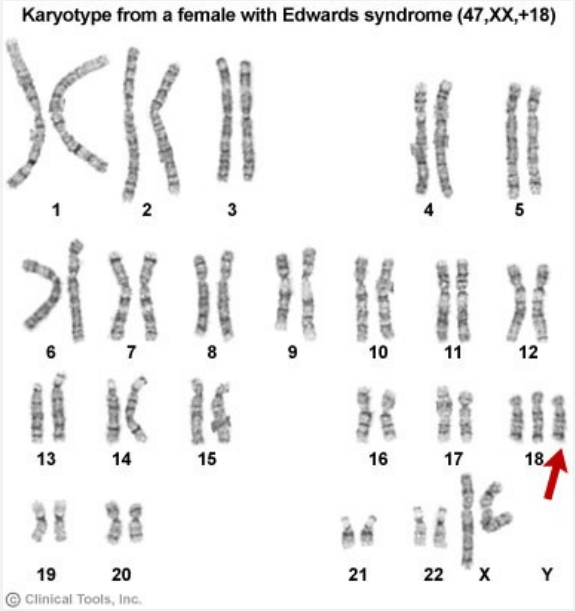


EDWARDS SYNDROME (47, XX OR XY,+18)

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

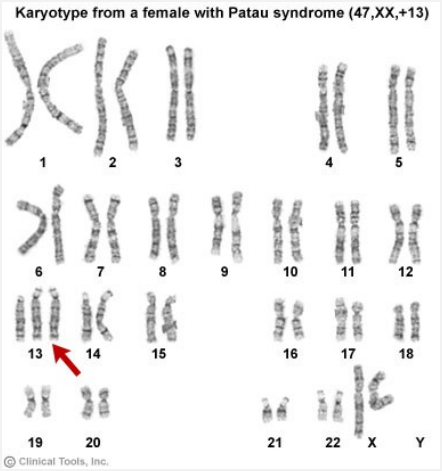


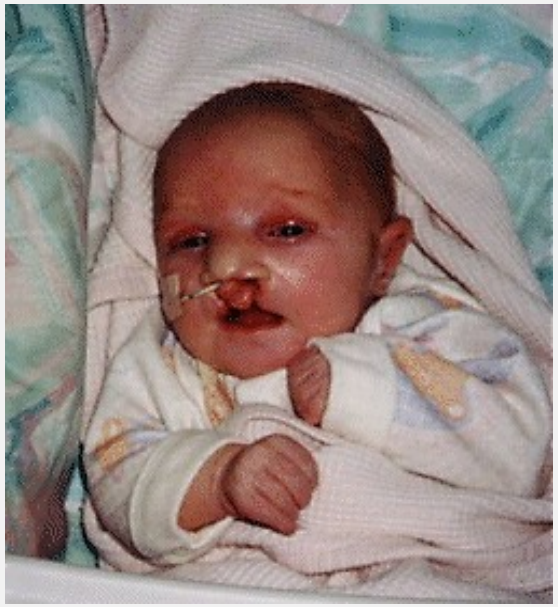


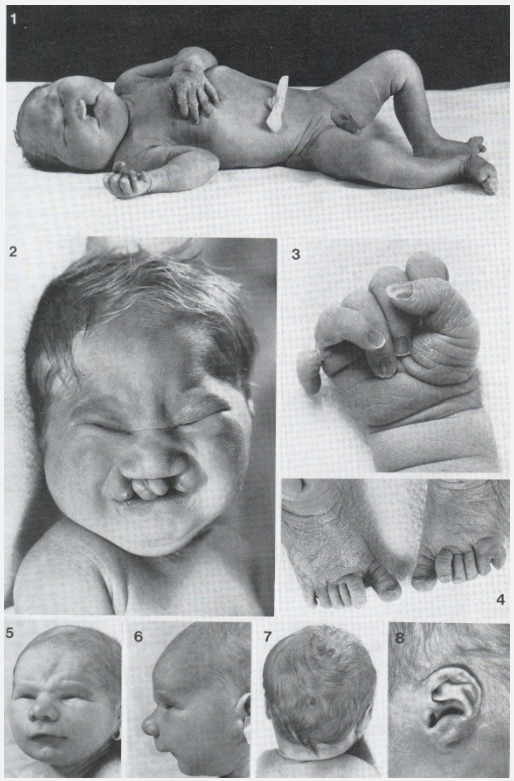


PATAU SYNDROME (47,XX OR XY,+13)

- severe somatic retardation and neurodevelopmental disorders
- microcephaly
- trigonocephaly
- cutis aplasia
- congenital brain defects
- cleft palate
- hexadactyly
- kidney defects

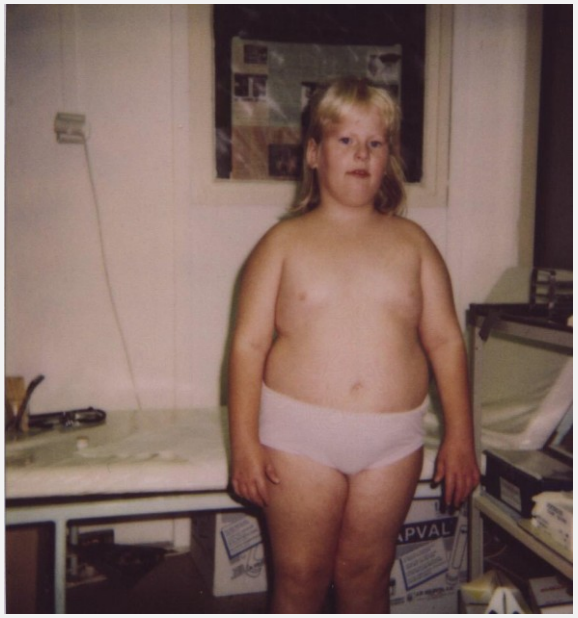


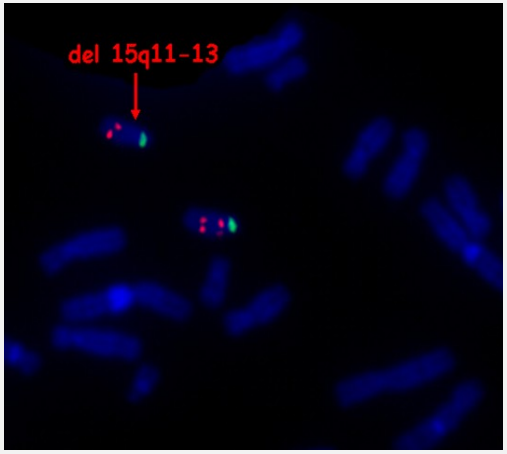




PRADER-WILLI SYNDROME (DEL 15Q11-Q13)

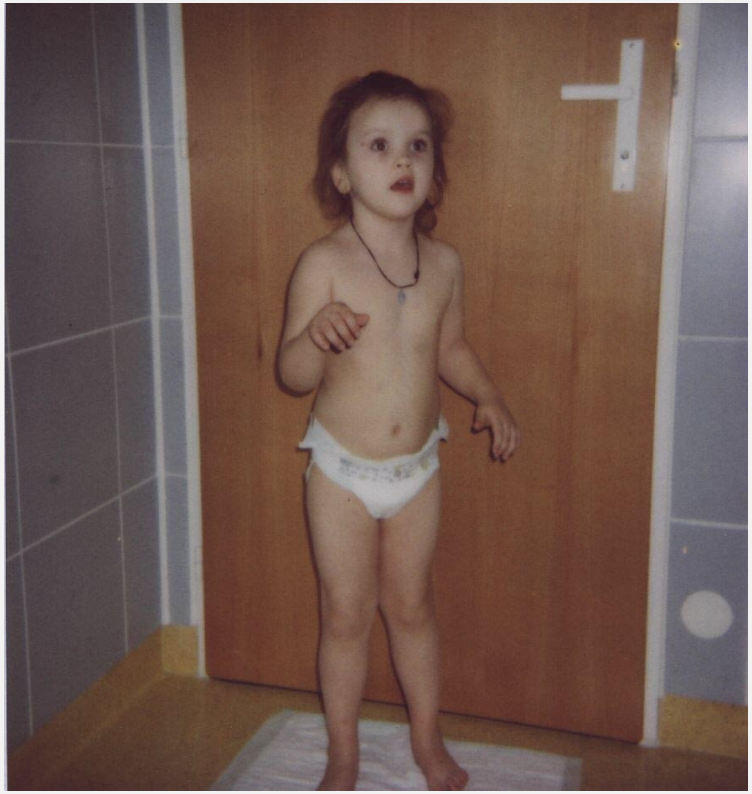
- deletion of paternal locus 15q11-q13
- low fetal activity
- hypotonia
- excessive weight gain, hyperphagia
- short stature
- hypogonadism
- intellectual disability
- hypopigmentation
- skeletal development delay (acromicria)





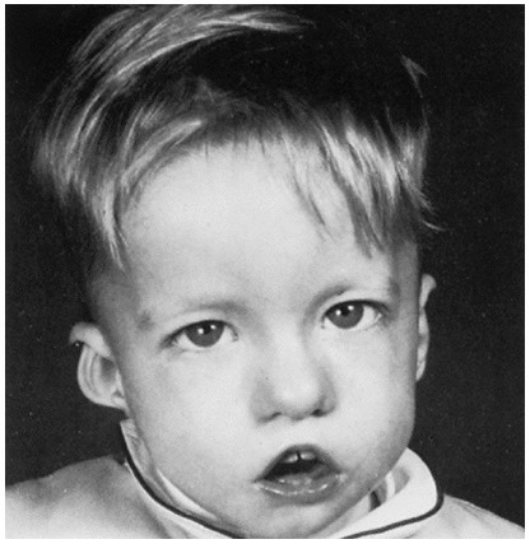
ANGELMAN SYNDROME (DEL 15Q11-Q13)

- deletion of maternal locus15q11-q13
- severe intellectual impairment
- hypotonia
- epilepsy, seizures
- hypopigmentation
- hyperactivity
- speech absence
- prominent scull shape (mandibular shape, microcephaly, flat back of head..)
- „happy puppet" syndrome
- movement/balance disorder



DIGEORGE SYNDROME (DEL 22Q11.2)

- low-set malformed ears
- small mouth and lower jaw
- narrow eyelids
- 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



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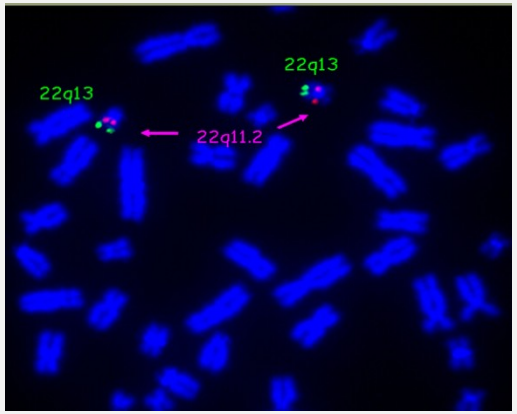
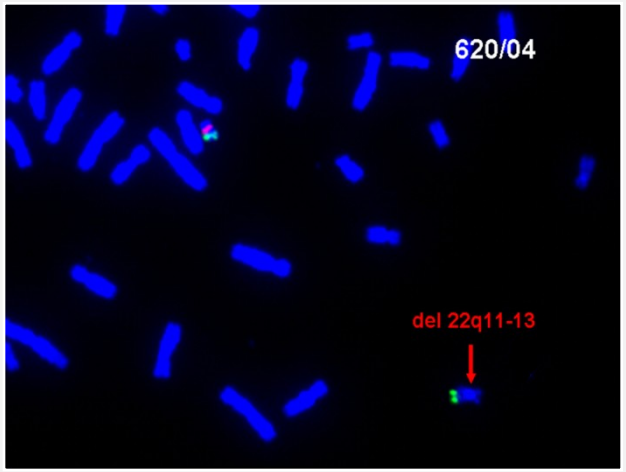
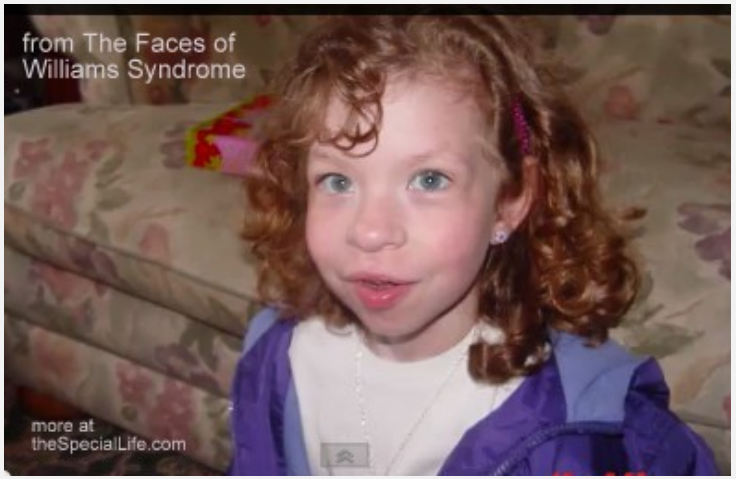
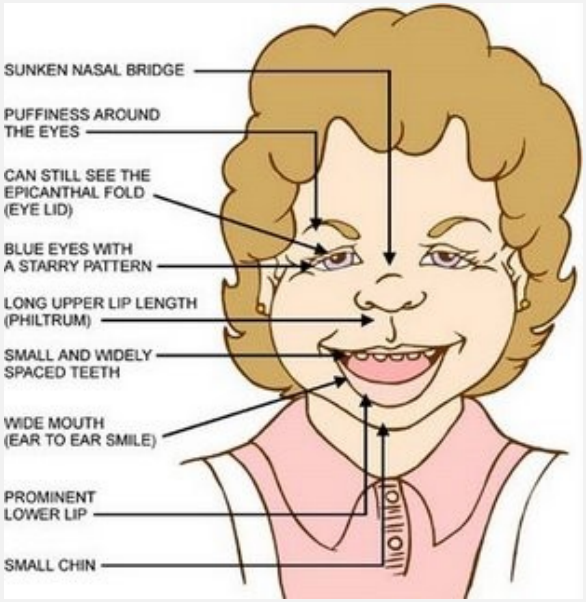




WILLIAMS-BEUREN SYNDROME (DEL 7Q11.23)

- 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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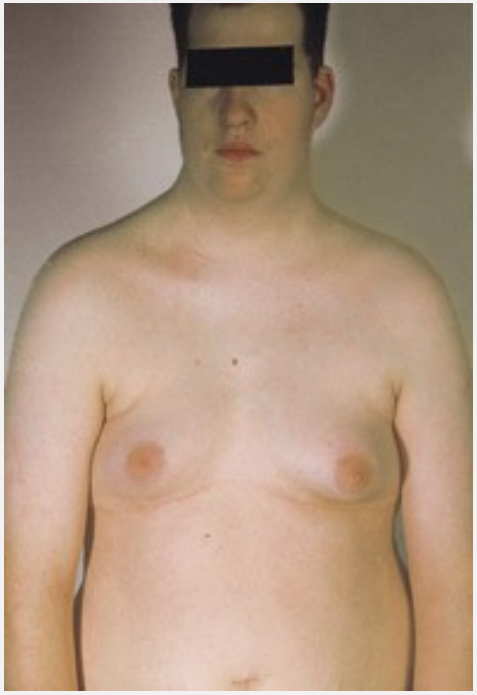
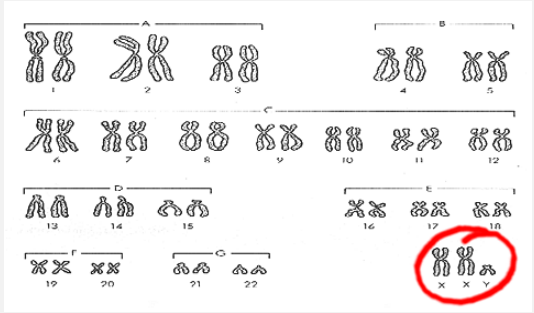
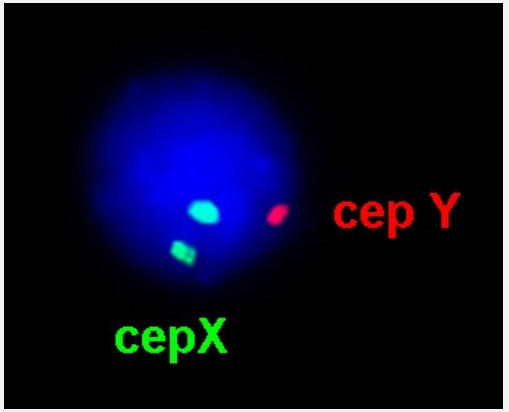
KLINEFELTER SYNDROME (47,XXY)

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

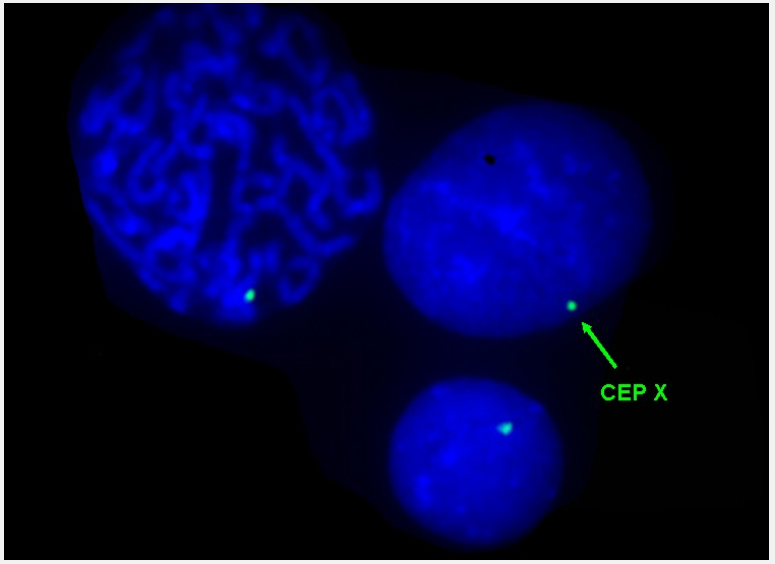


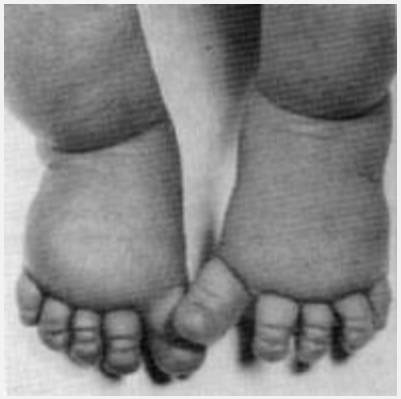


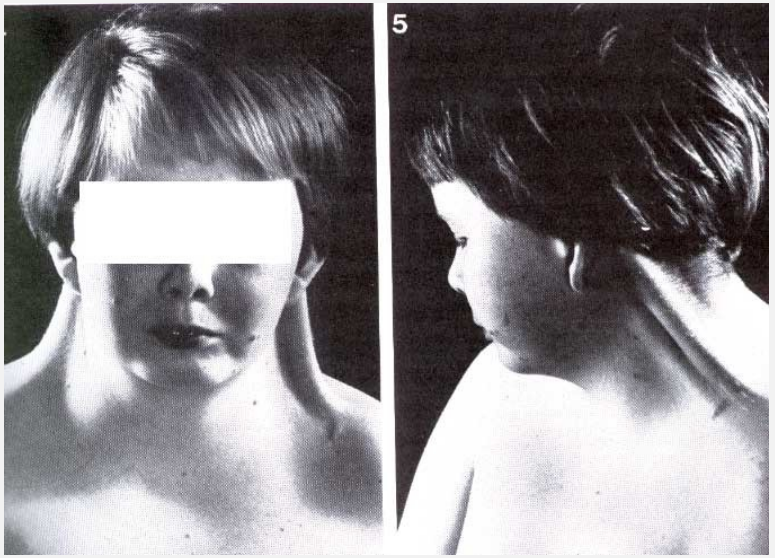


TURNER SYNDROME (45,X)

- lower birth length and weight
- low hairline
- pterigyum
- broad chest, widely spaced nipples
- small growth
- infertility, amenorrhea
- coarctation of the aorta
- webbed neck
- lymphedema







THE LINK BETWEEN KARYOTYPING (K) AND MOLECULAR CYTOGENETICS (MC)

- Using MC methods to confirm and specify pathological chromosomal aberrations detected by G-banding K
  - Aneuploidies -> FISH
  - Structural chromosomal rearrangements -> FISH, array-CGH

- MC methods can detect very subtle chromosomal rearrangements, which escaped detection using G-banding K (due to its low resolution > 5-10 Mb)

4. MOLECULAR CYTOGENETICS

- Interconnection and combination of approaches of classical cytogenetics and molecular biology
- Utility of the latest knowledge of molecular biology, microscopy and computer image analysis to study the structure and properties of chromosomal changes
- Ability to analyse both numerical and structural chromosomal imbalances unidentified classical cytogenetic techniques
- does not require the presence of mitosis for most applications
- 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

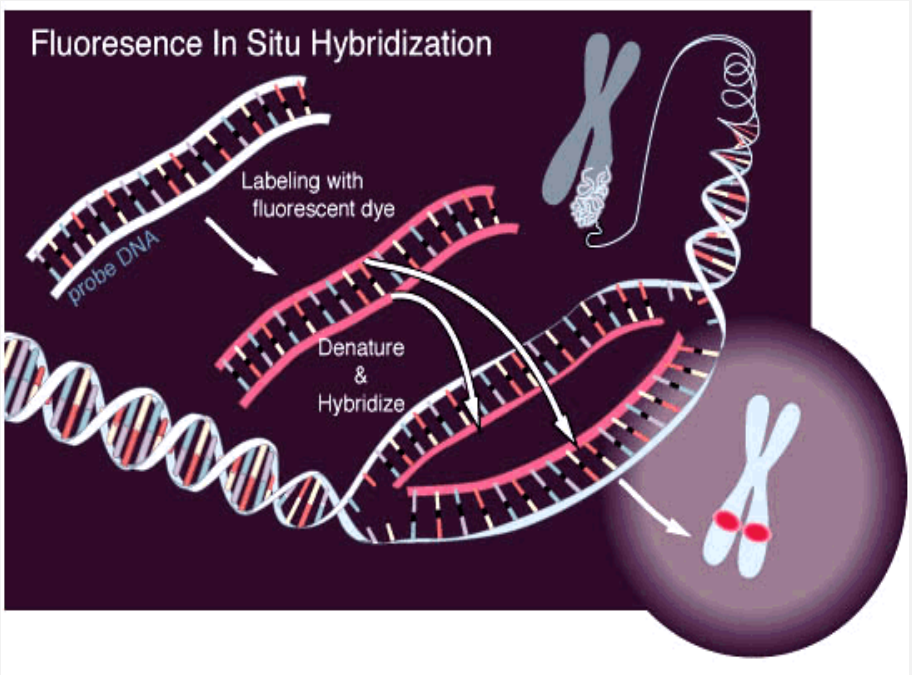
peripheral blood

solid tumor

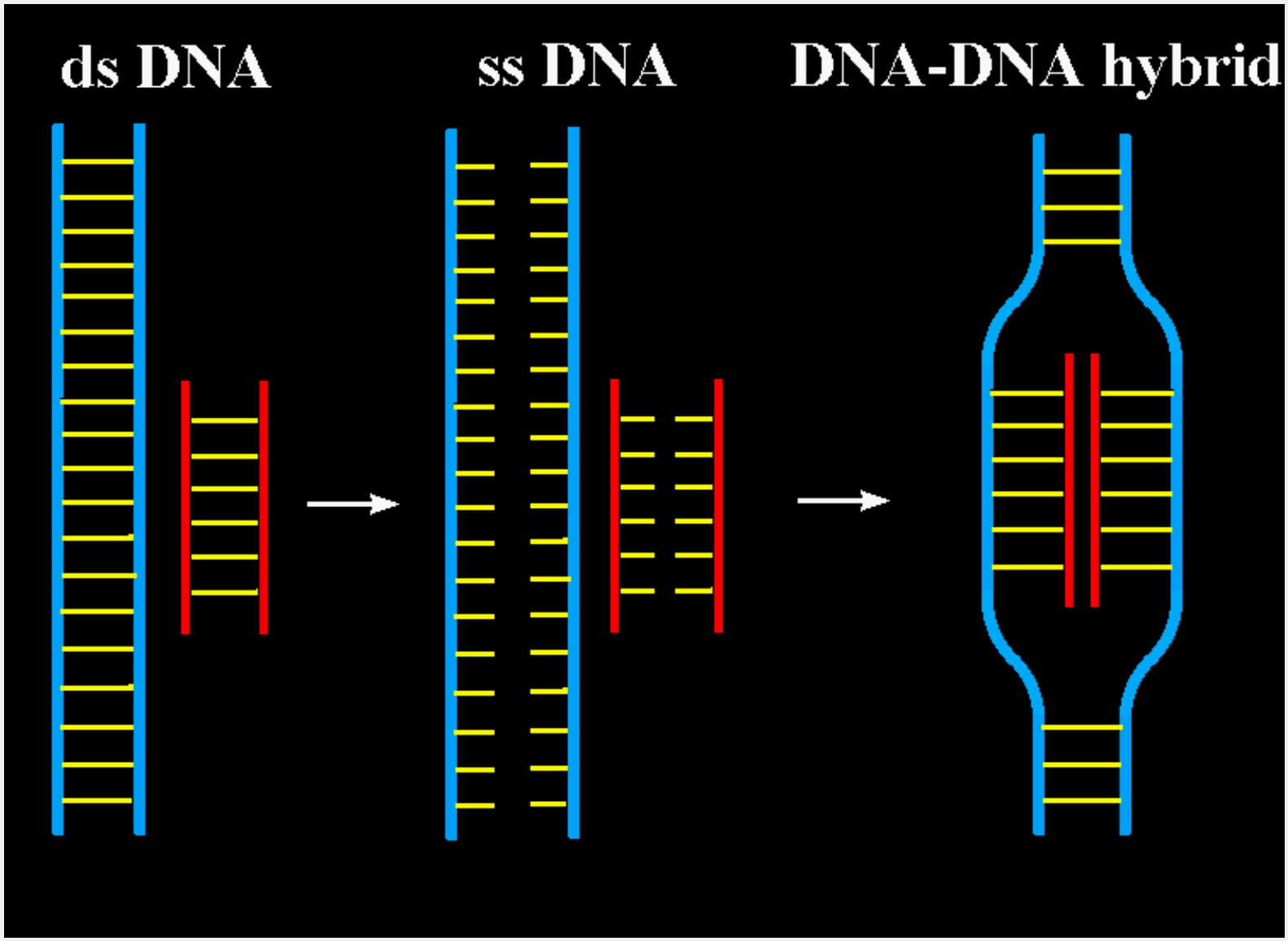
bone marrow

MOLECULAR CYTOGENETICS

The key interest of the laboratory of molecular cytogenetics is the **identification and analysis of chromosomal aberrations using molecular cytogenetic approaches**



FISH
FLUORESCENT IN SITU HYBRIDIZATION



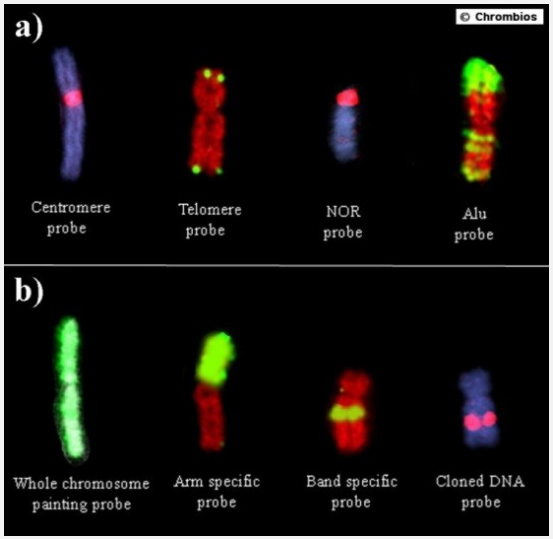
FISH

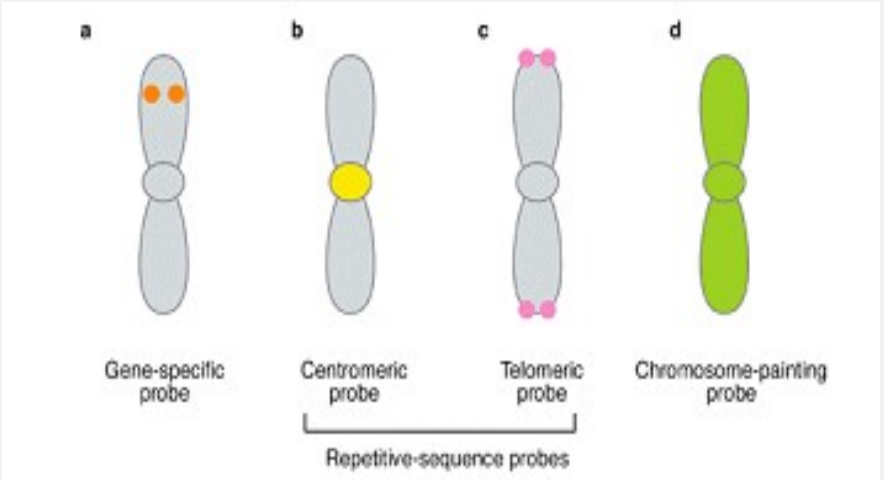
- detection of the fluorescent signals through microscope equipped with specific fluorescent filters
- material
  - cultivated peripheral blood
  - cultivated bone marrow
  - cultivated amniotic fluid cells
  - uncultivated amniocytes
  - tumor and bone marrow prints
- we determine:
  1. presence of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

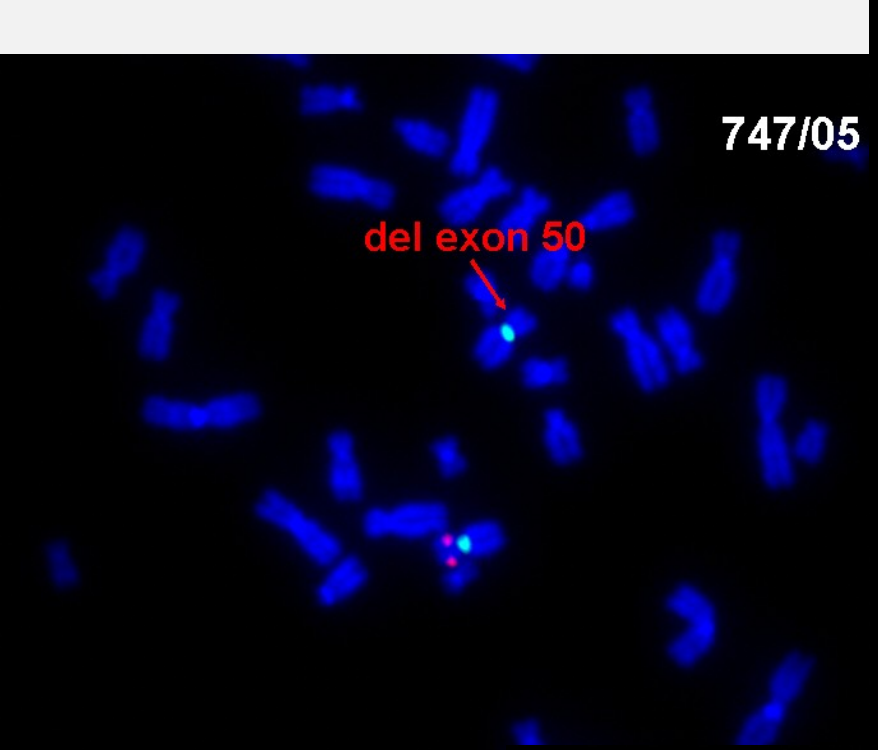
  - clinical cytogenetics

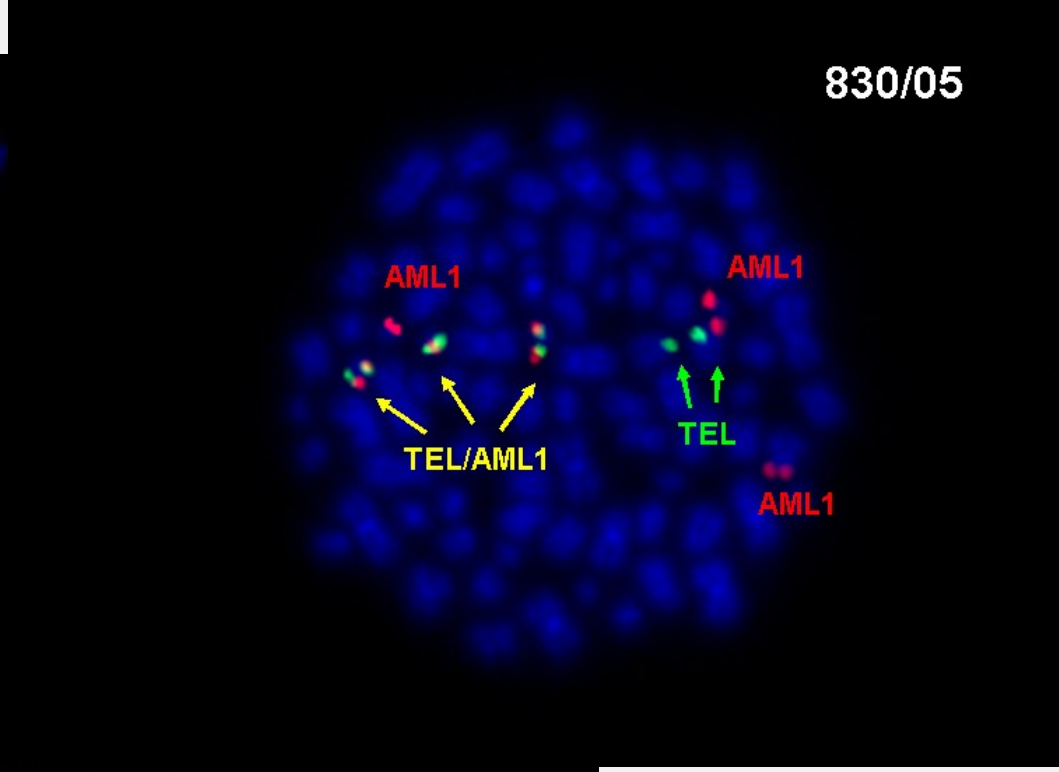
  - oncocytogenetics

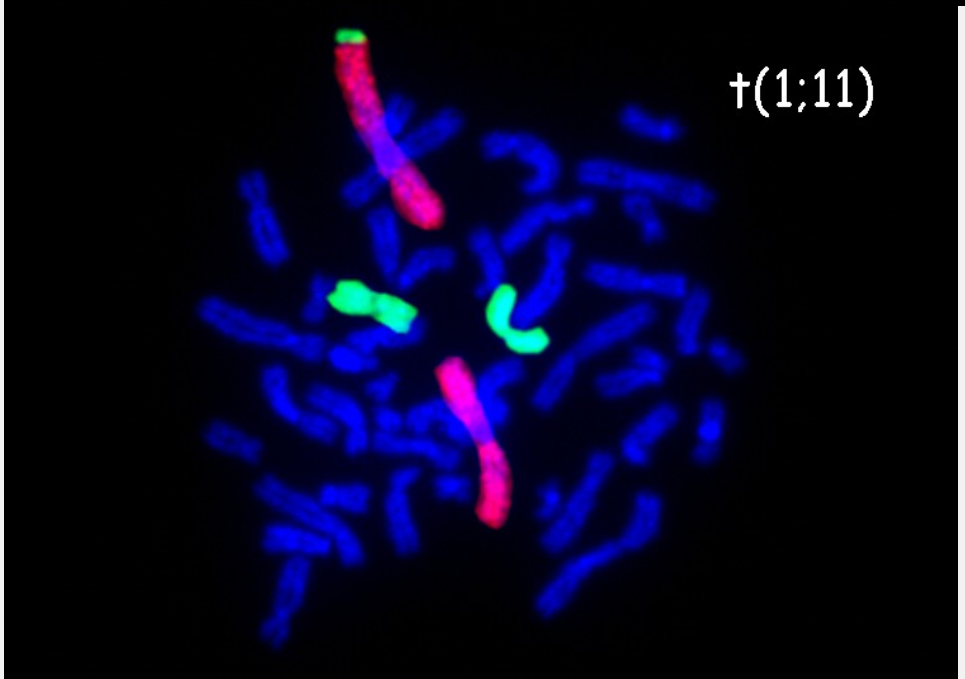
  - human genome mapping





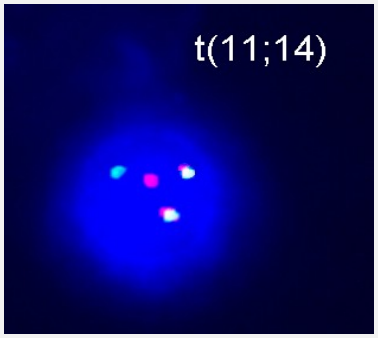

747/05
del exon 50

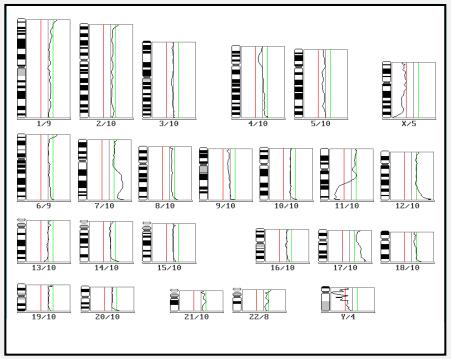

830/05
AML1
AML1
TEL/AML1
TEL
AML1

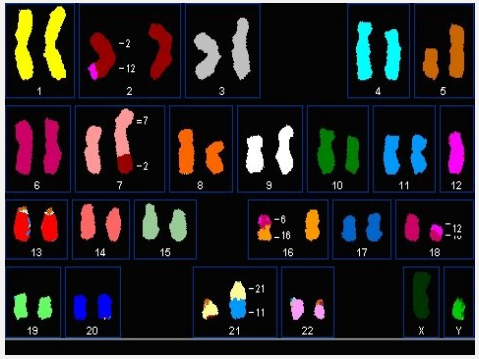

t(1;11)

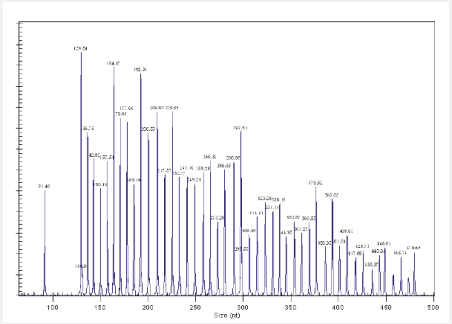
METHODS

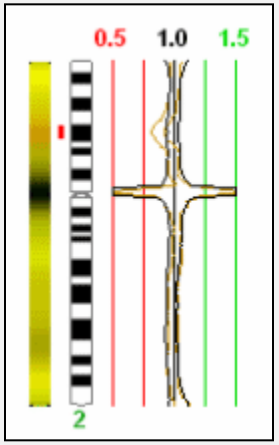
- Fluorescence *in situ* hybridization (FISH)
- Spectral karyotyping (SKY) or M-FISH
- Comparative genomic hybridization (CGH) or High resolution-CGH (*in past)*
- array-CGH
- MLPA

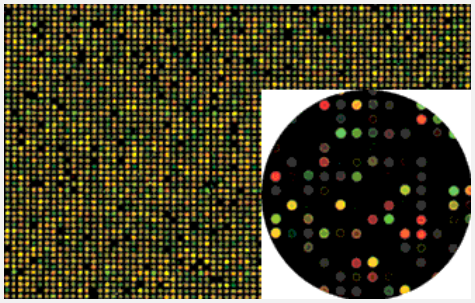












THE EQUIPMENT

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: SureScan Microarray Scanner (Agilent Tech.)

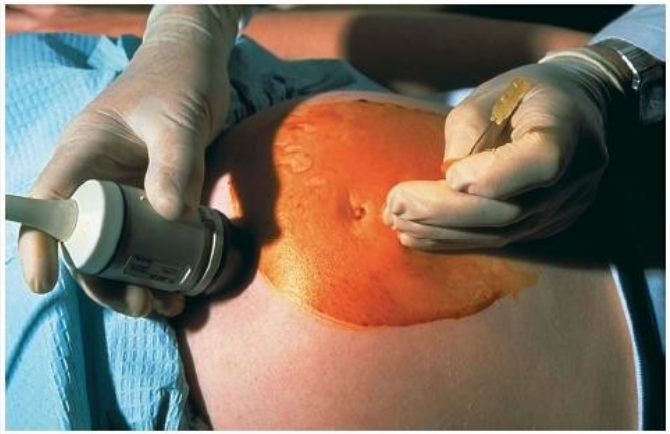
System for MLPA: capillary electrophoresis (Beckman Coulter)

MOLECULAR CYTOGENETIC INVESTIGATIONS (LABORATORIES OF CYTOGENOMICS)

APPLICATIONS OF FISH

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

PRENATAL CYTOGENETIC ANALYSES



- Uncultered and cultured amniotic cells, fetal blood cells, chorionic villi cells

- interphase FISH (I-FISH) for aneuploidies
- metaphase FISH (in specific cases)

FAST FISH Prenatal Enumeration Probe Kit (Cytocell)

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, Prader-Willi, Angelman, Williams-Beuren, ...)


2

DNA FISH Probes for Prenatal, Postnatal and Preimplementation Genetics

CHROMOSOME ANEUPLOIDY

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.

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

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

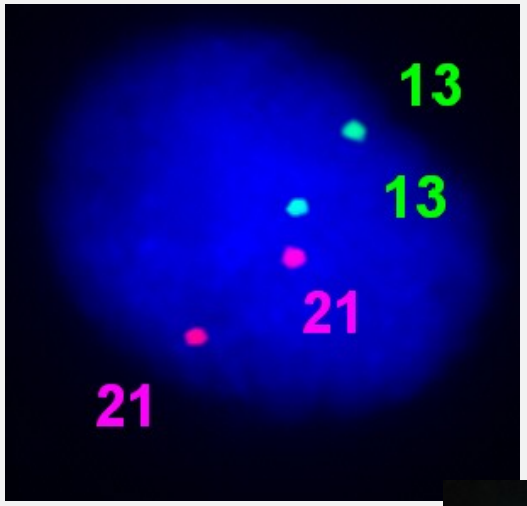
18p11.1-q11.1 CEP 18 alpha satellite SpectrumAqua
Xp11.1-q11.1 CEP X alpha satellite SpectrumGreen
Yp11.1-q11.1 CEP Y alpha satellite SpectrumOrange
13q14 LSI 13 SpectrumGreen
21q22.13-q22.2 LSI 21 SpectrumOrange

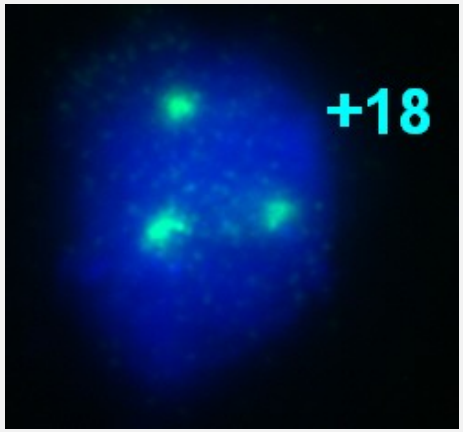
18

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

Prenatal cytogenetic analyses

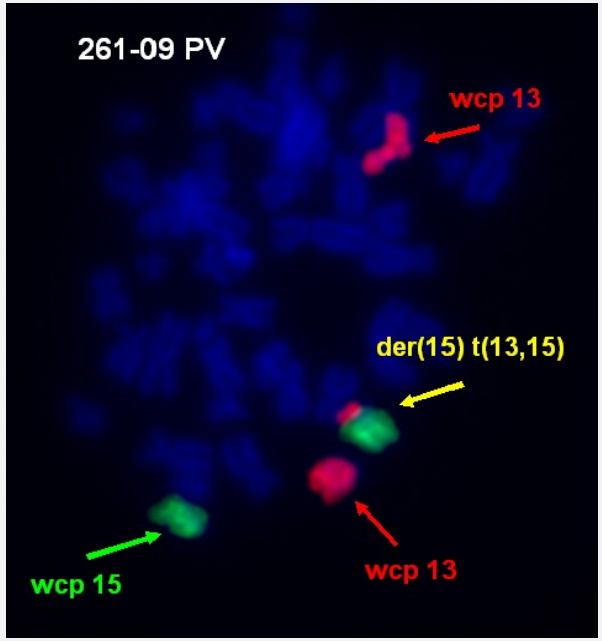
Example: FISH on uncultured and cultured cells/mitoses



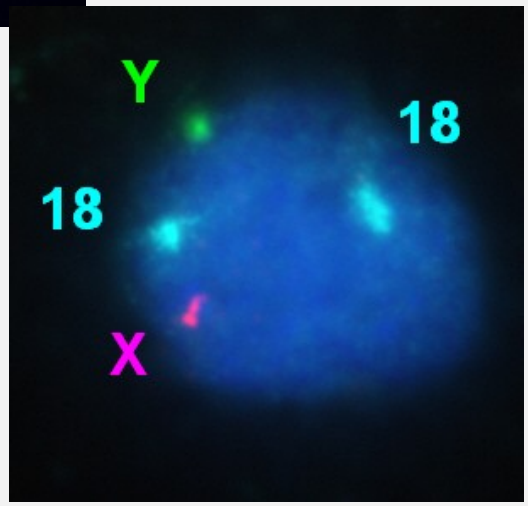


trisomy of chr. 18

t(13;15)



Normal cells



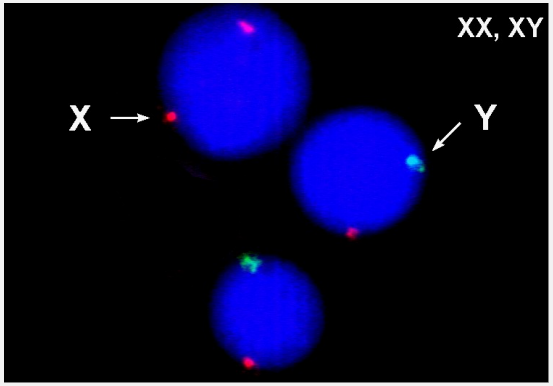
POSTNATAL CYTOGENETIC ANALYSES

ToTel Vysion Kit, Abbott-Vysis

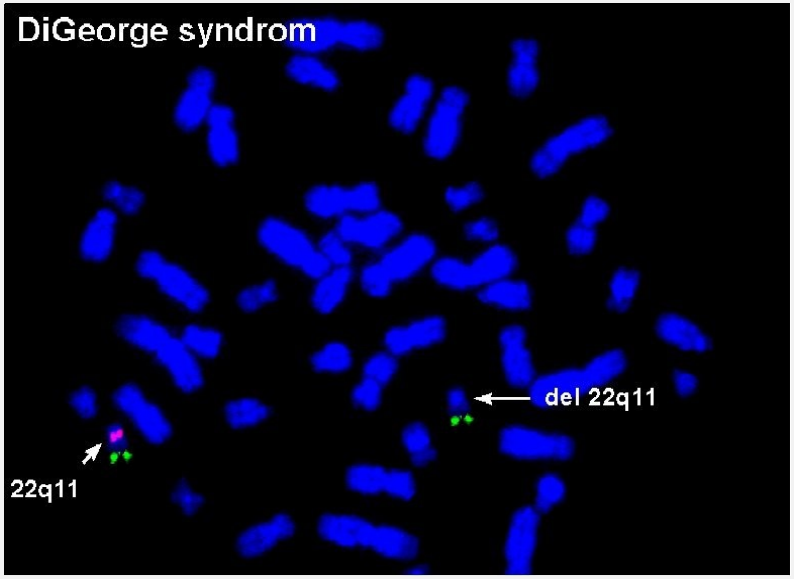
- material: peripheral lymphocytes, buccal swab
- methods: FISH, array-CGH, MLPA

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

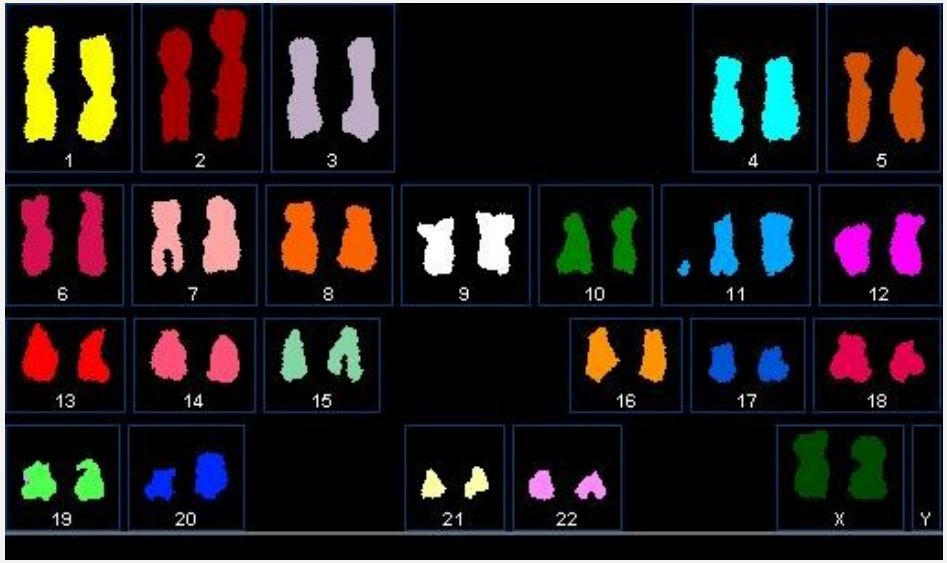
Postnatal cytogenetic analyses



FISH: 46,XX/46,XY (mosaicism)



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

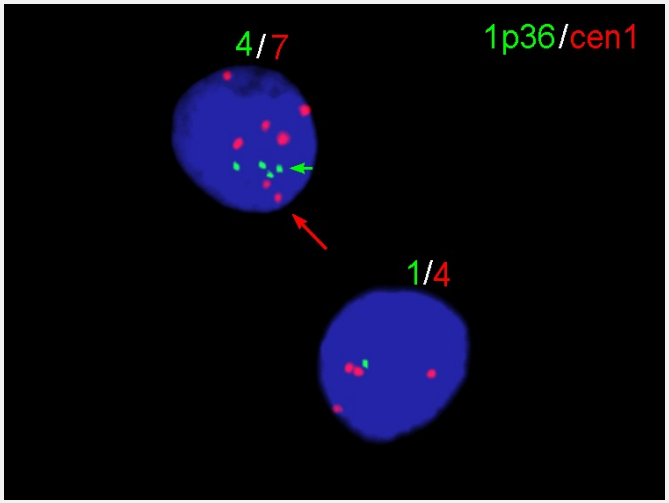


SKY: identification of marker chromosome (chr. 11)

CANCER CYTOGENETIC ANALYSES – SOLID TUMOURS

Cultivated and uncultivated solid tumors (tumour prints)

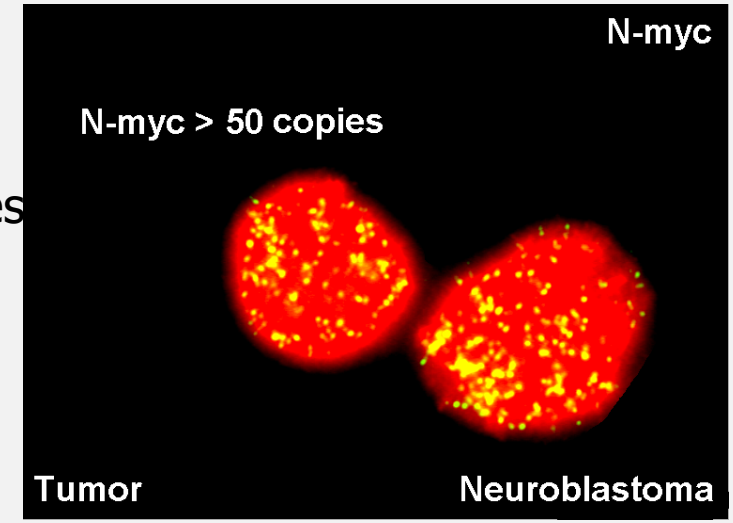
- **FISH, M-FISH/SKY, CGH**



Children solid tumours

- FISH: **targeted analysis (panel testing)**
  - **Neuroblastoma** –MYCN amplification, 1p36 deletion, gain 17q, 11q deletion;
  - **Medulloblastoma** – MYCN, MYCC amplification

- array-CGH: whole genome screening of unbalanced chromosomal aberrations (and losses of heterozygosity)



ADVANTAGES AND DISADVANAGES OF FISH

- advantages
  - does not require the presence of mitoses (for most applications)
  - quick assessment of big amount of cells

- disadvantages
  - does not provide whole genomic view
  - can detect only a specified locus or a limited number of loci (using fluorescent-labeled DNA probes)

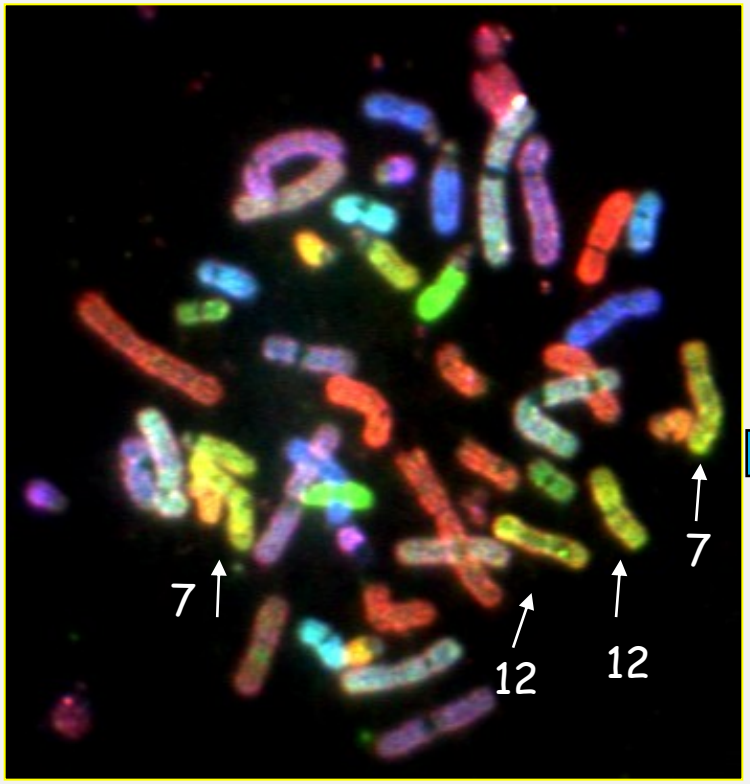
SKY
SPECTRAL KARYOTYPING

- Microscope equipped with **2 fluorescent filters (SKY, DAPI)**

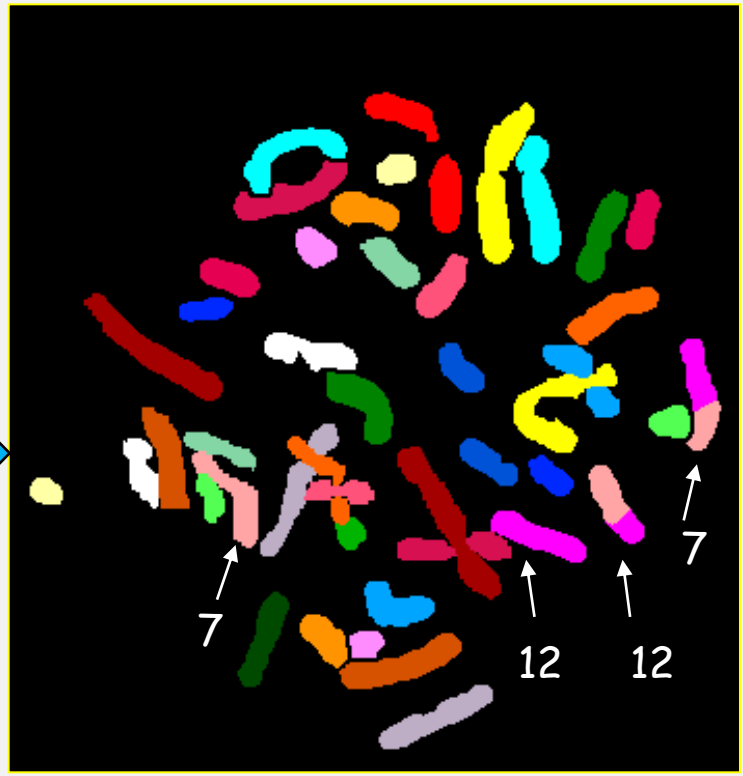
- fluorochromes (**FITC**, **Rhodamin**, **TexasRed**, **Cy5**, **Cy5.5**) **scanned by one filter**, based on a wave lenght each chromosome pair is coloured ⟶ pseudocolours

Image Acquisition with SkyVision™

Image analysis using SkyView

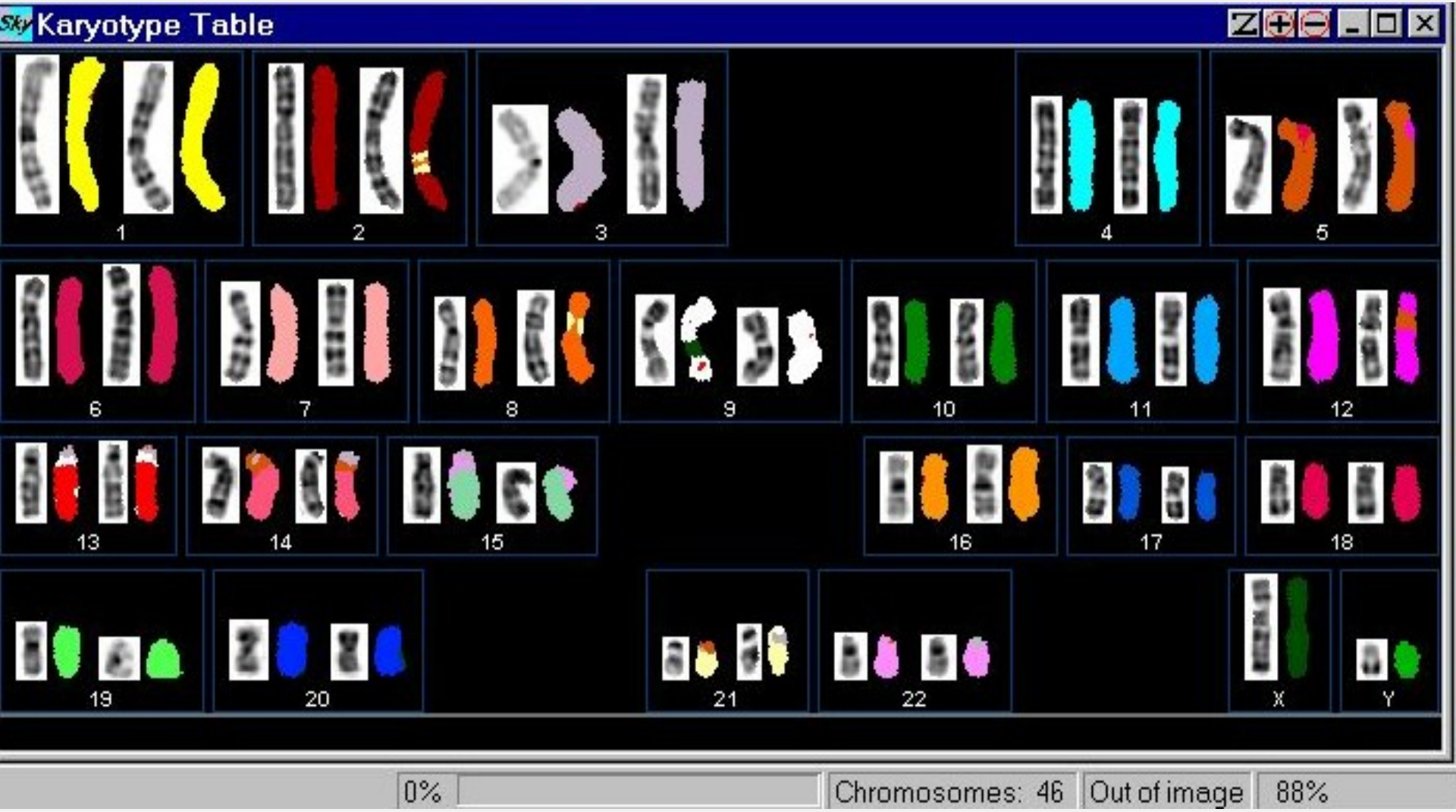


Display Image



Classified Image

The SkyView spectral karyotyping software is able 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
    - cryptic translocations and insertions
    - marker chromosomes
    - redundant material with unknown origin
    - complex rearrangements

- disadvantages
  - need of quality mitoses
  - succesful hybridisation
  - expensive method

CGH - COMPARATIVE GENOMIC HYBRIDIZATION
HR-CGH – HIGH-RESOLUTION CGH
ARRAY-CGH

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

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

- is based on **<u>comparison</u>** of two genomes

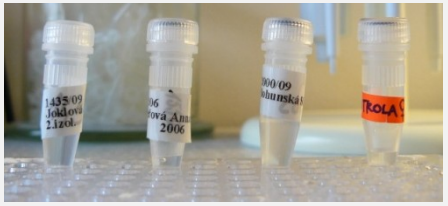
FISH

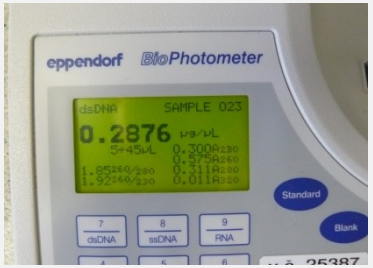
normal DNA → select DNA → **make probe (limited number of targets in one hybridization)** → label abnormal target → abnormal target identified

CGH/HR-CGH/array-CGH

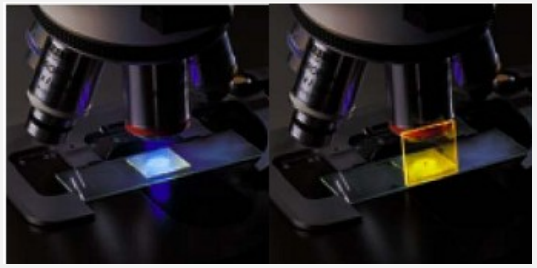
normal DNA → no DNA selection → **make probe (entire genome)** → quantify on normal target → abnormal genome quantified

Requirements





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



Equipment:

•Fluorescent microscope
(filters DAPI, Spectrum Green, Spectrum Red)

• Sensitive CCD kamera

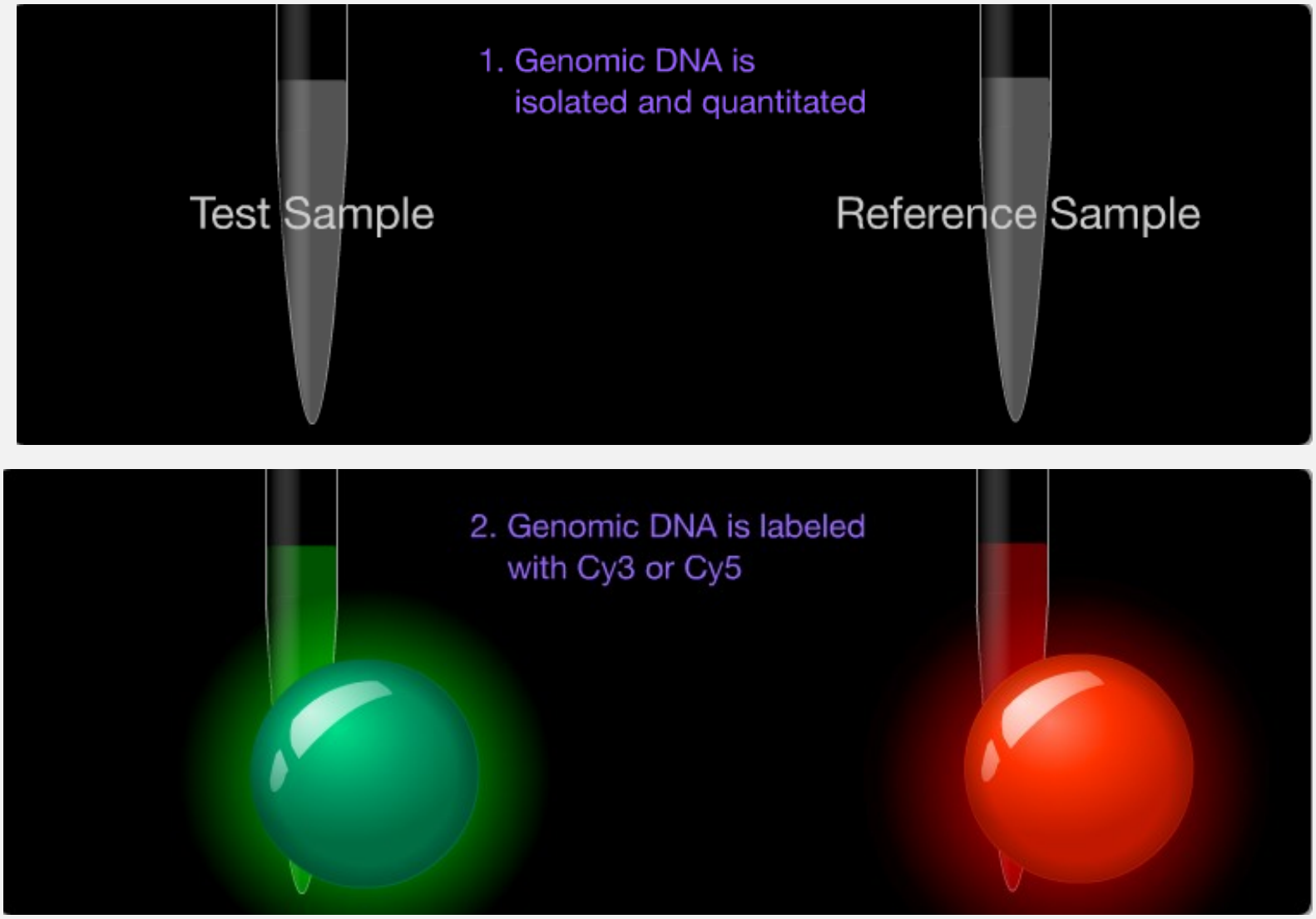
• Computer with software for CGH analysis and data interpretation, (LIM, Czech Republic)

– CGH/HR-CGH

•Hybridization oven, microarray scanner

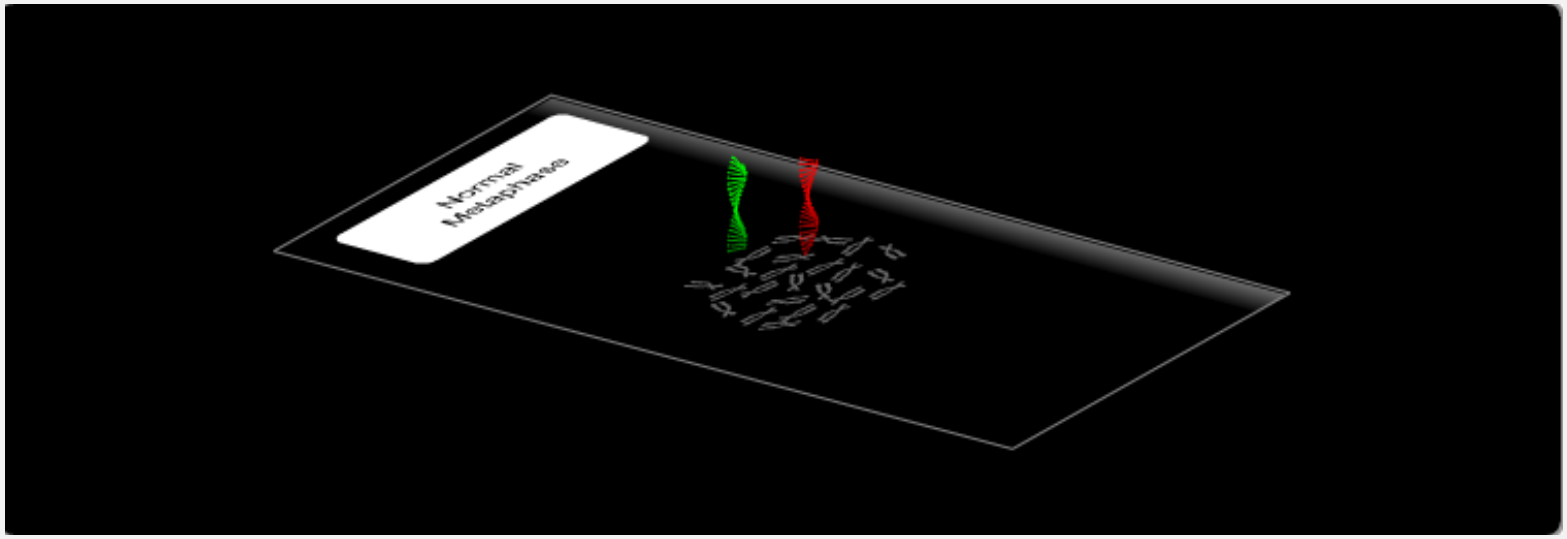
•Computer with software for array-CGH analysis and data interpretation – array-CGH

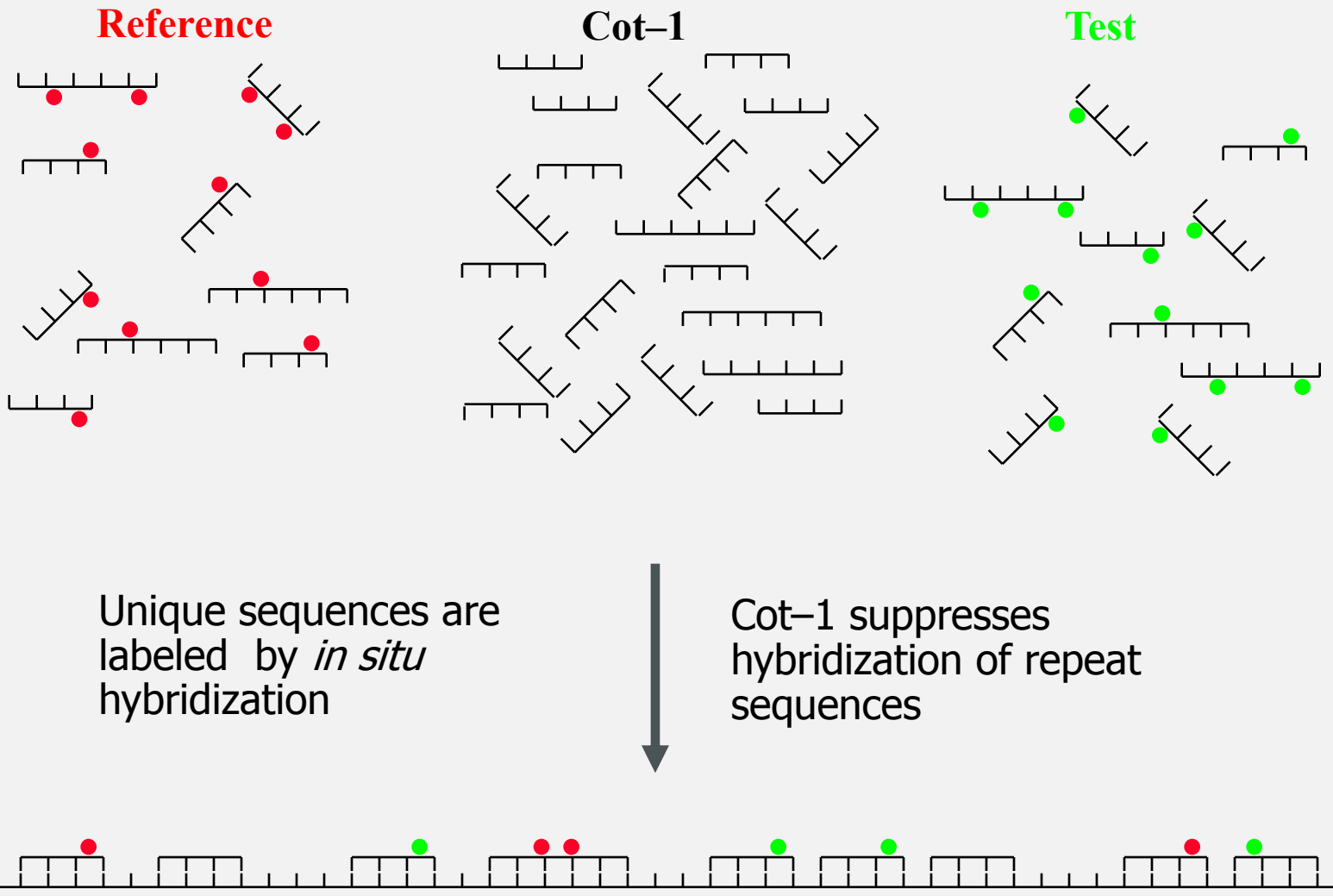
CGH principle



www.abbottmoleculars.com

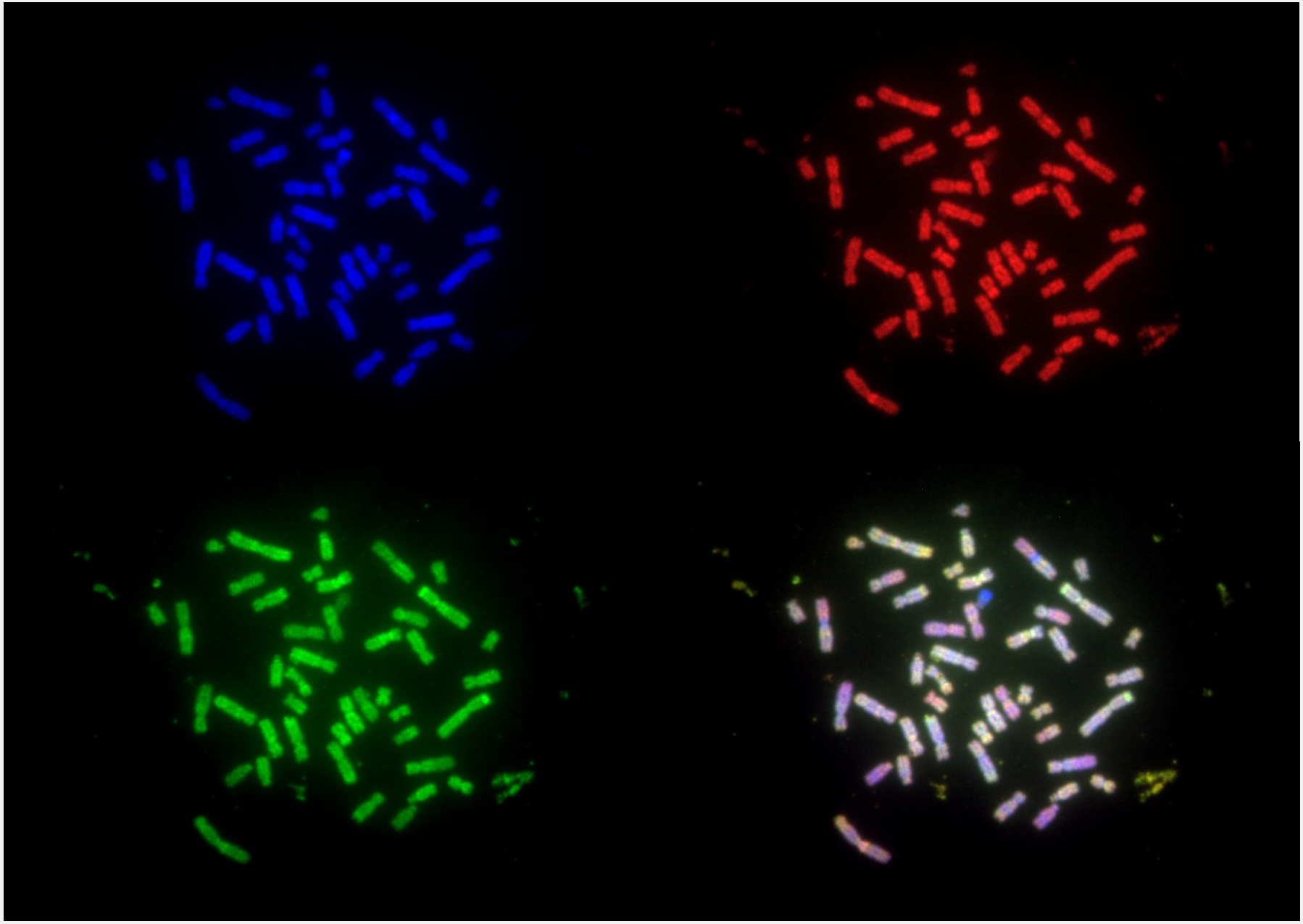
CGH principle





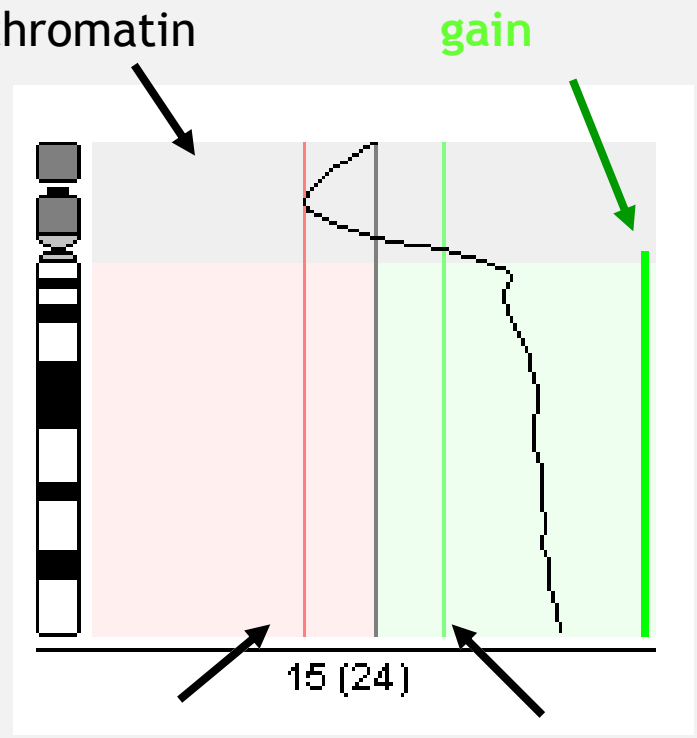
Relative brightness depends on amount of labeled DNA with appropriate complementary sequences, i.e. on the DNA copy number at this locus

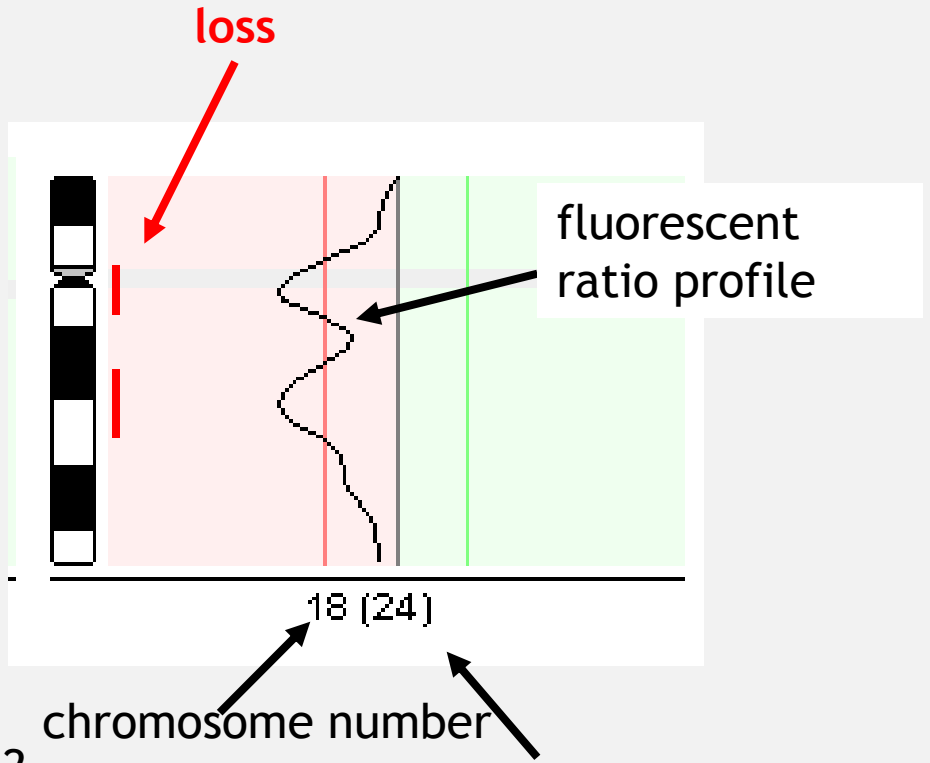
Mitoses scanning, CCD camera filters for B, G, R



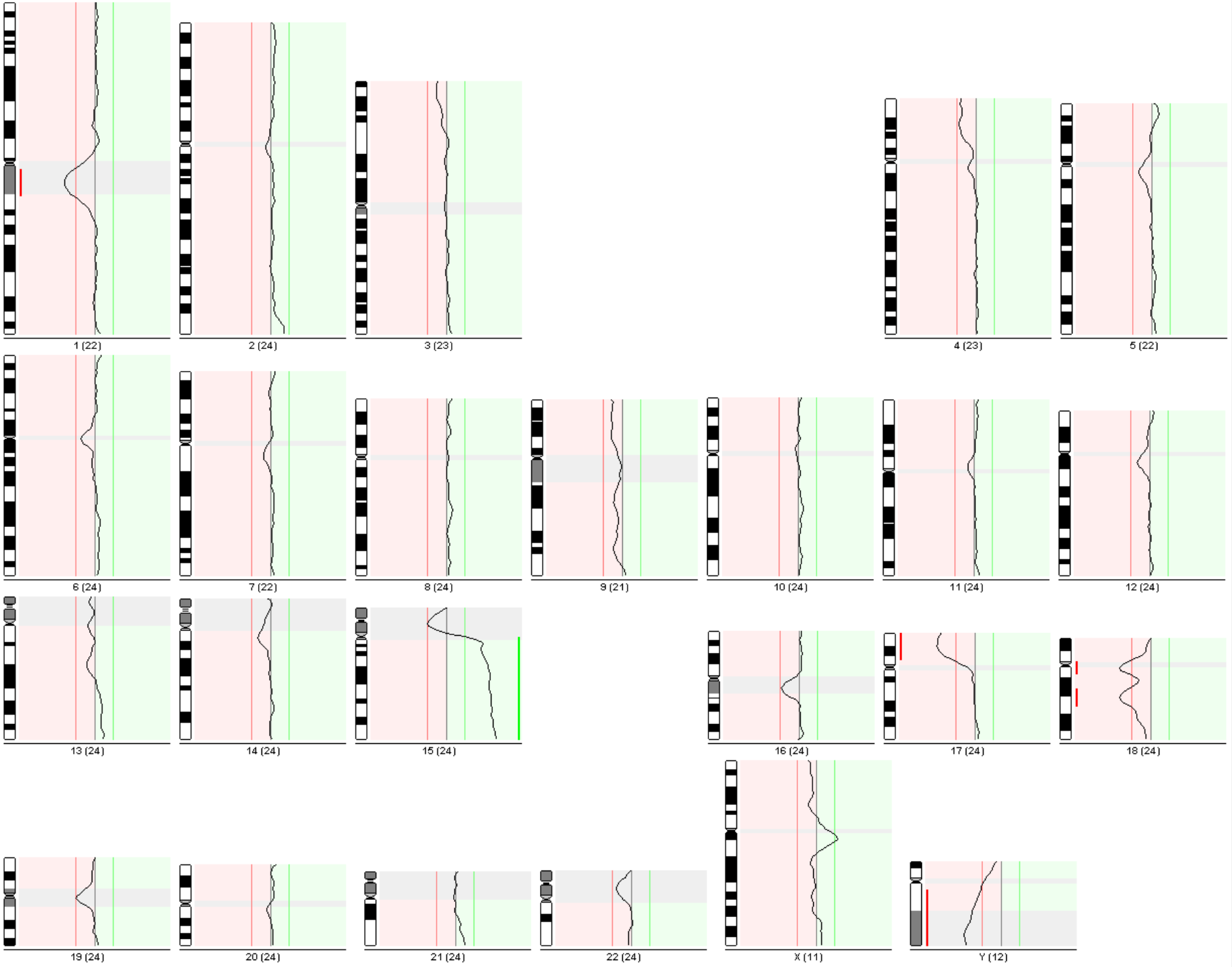
Identification of aberrations

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.





At least 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*)

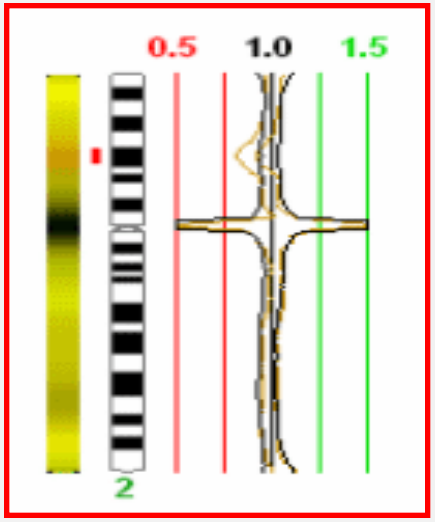
DISADVANTAGES OF CGH

- low sensitivity: **about 10 Mb for single copy changes**
  - solution: **array-CGH**
- 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, **array-CGH**

MODIFICATIONS OF CGH

High Resolution Comparative Genomic hybridization (HR-CGH)

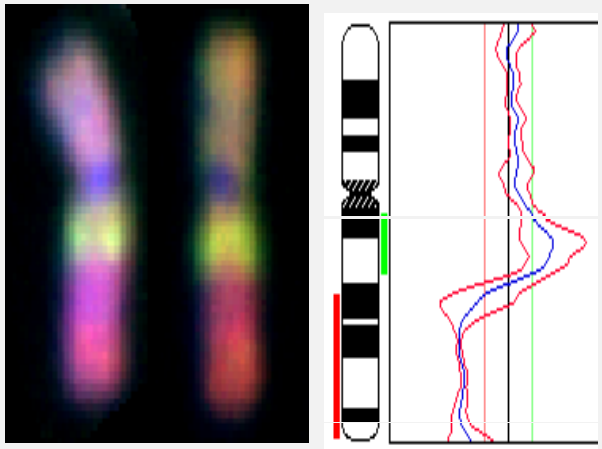
- Kirchhoff *et al.,* 1997
- the same principles and laboratory processing as CGH
- different data interpretation based on dynamic standard reference intervals – special analytical software
- genome resolution is about 4 Mb
- abnormal cell detection limit is about 30 %



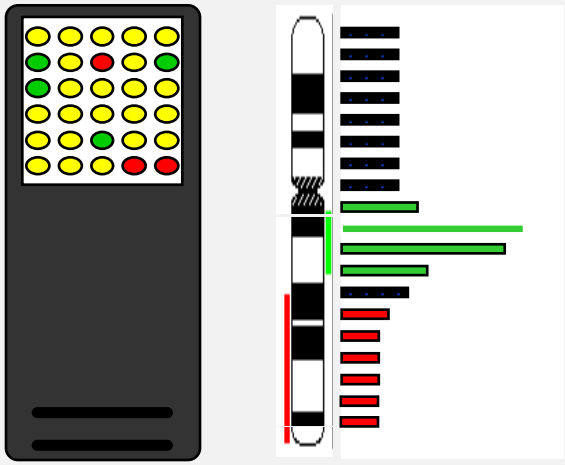
Array-based comparative genomic hybridization (array-CGH)

- Solinas-Toldo *et al.* 1997
- based on principle of CGH
- current routine approach of whole-genome screening of unbalanced chromosomal aberrations (including those of submicroscopic size < 5-10 Mb)
- nowadays fully replaces classical CGH and HR-CGH analysis
- the chromosomes on slide (CGH) are replaced by separated clones (array-CGH) (BAC – bacterial artificial chromosome, PAC – phage artificial chromosome, nowadays in most designs – oligonucleotides (miniaturized array)

CGH

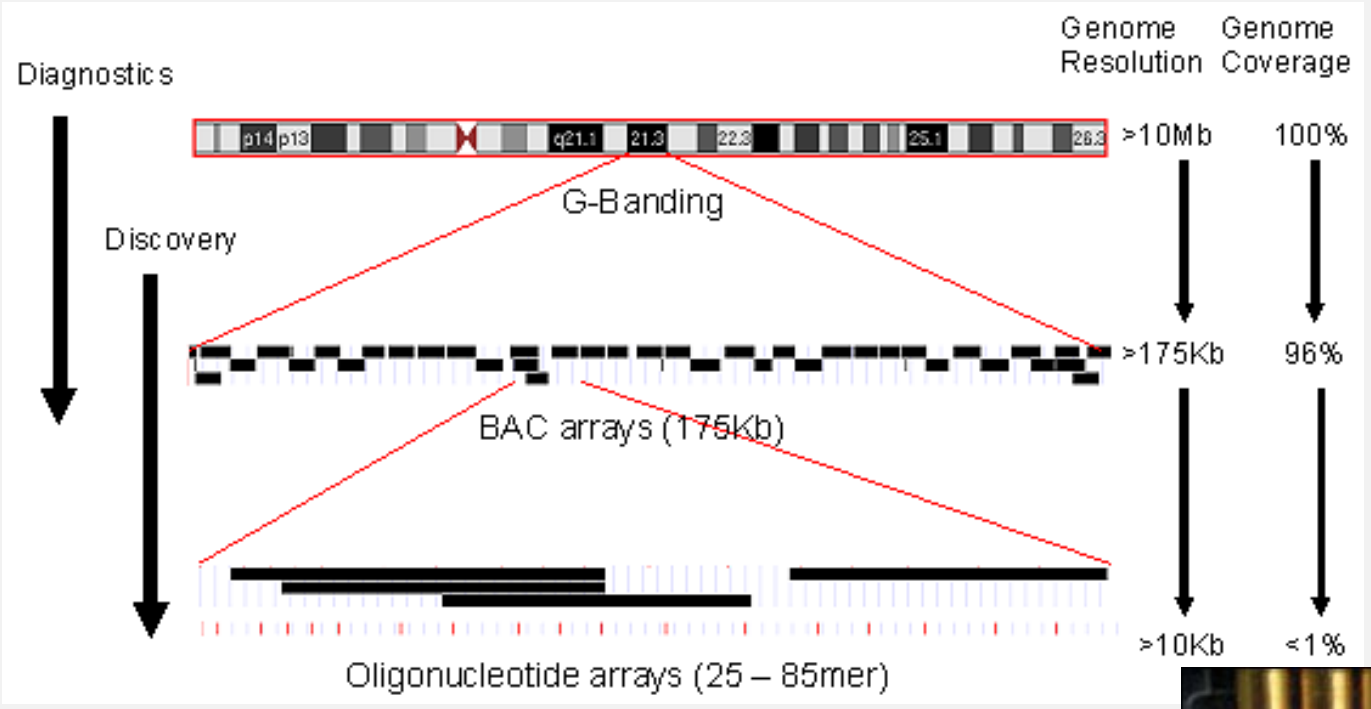


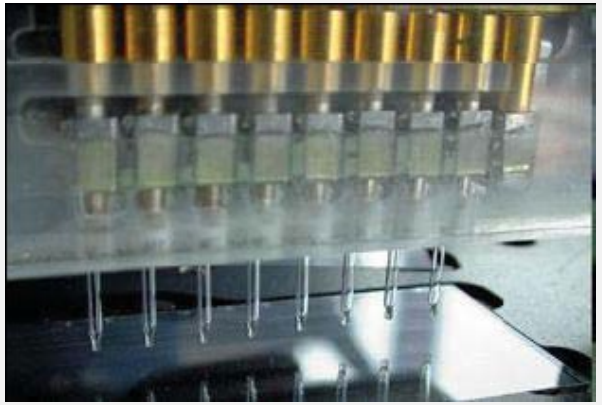
array-CGH



The origin of clones

BAC, PAC, c-DNA clones (in past), oligonucleotides





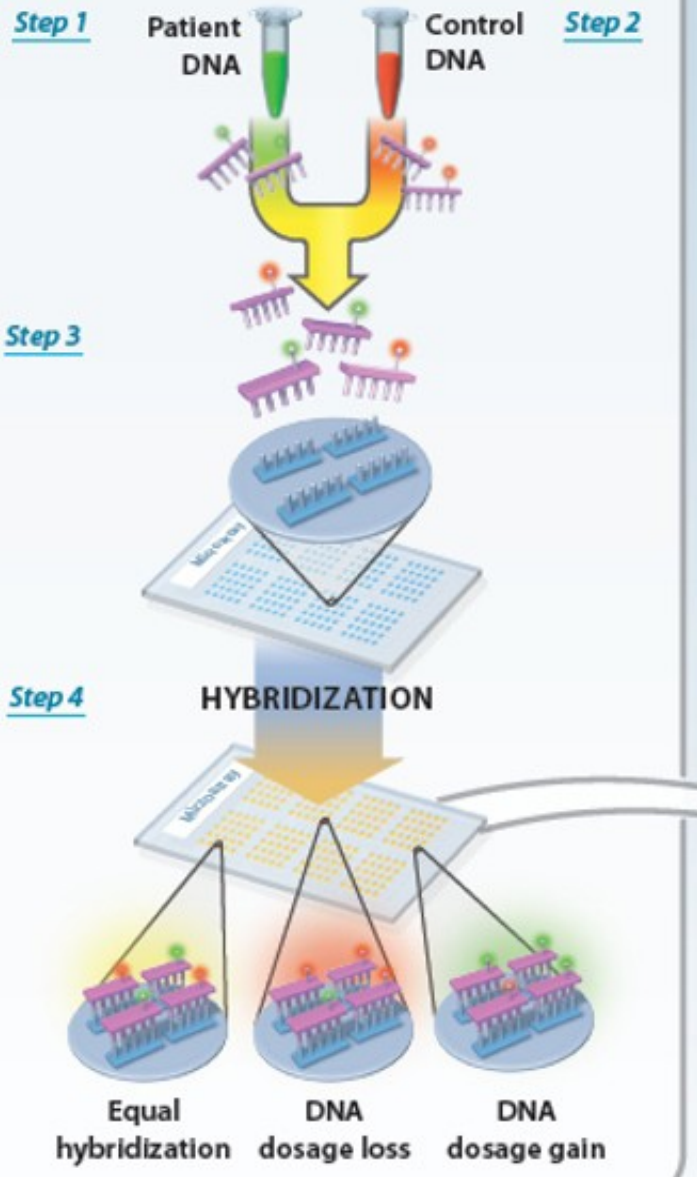
GENERAL PRINCIPLE OF ARRAY-CGH

- Effective approach of the whole-genome screening of unbalanced chromosomal rearrangements in one hybridization reaction
- Co-hybridization of differently labeled DNA samples (patient‘s DNA and reference DNA) on DNA microarray covered by a large amount of oligonucleotide fragments (representing whole genome)
- Losses and gains of genetic material in patient‘s DNA are assessed from spots with abnormal ratios of signal intensities

Who are the most suitable patients?

- Individuals with neurodevelopmental disorders (intellectual disability, autism spectrum disorders), multiple congenital abnormalities, facial dysmorphology, …
- Prenatal samples – abnormal pregnancy (prenatal screening)
- Fetal tissue (abortions)
- Tumor tissues (solid tumors, bone marrow, …)

Array CGH: The Complete Process

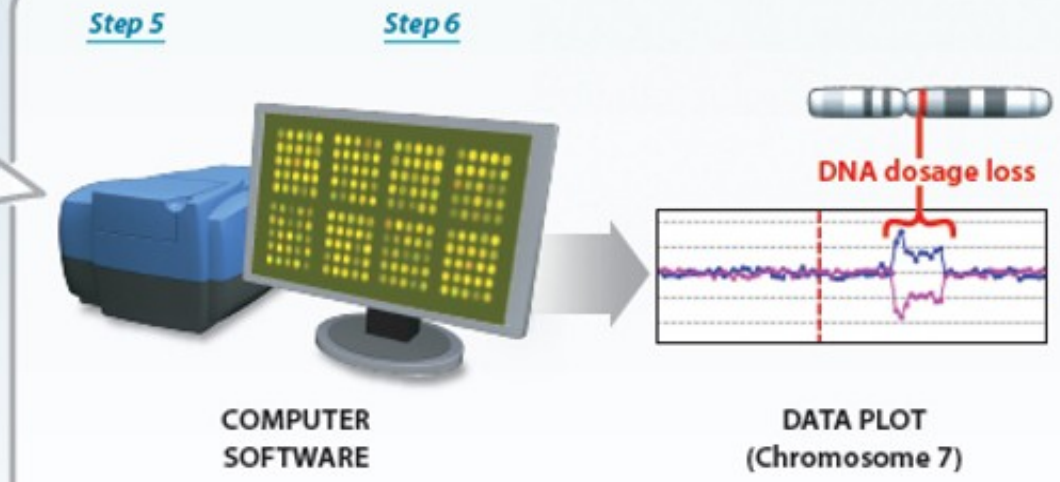


Steps 1-3 Patient and control DNA are labeled with fluorescent dyes and applied to the microarray.

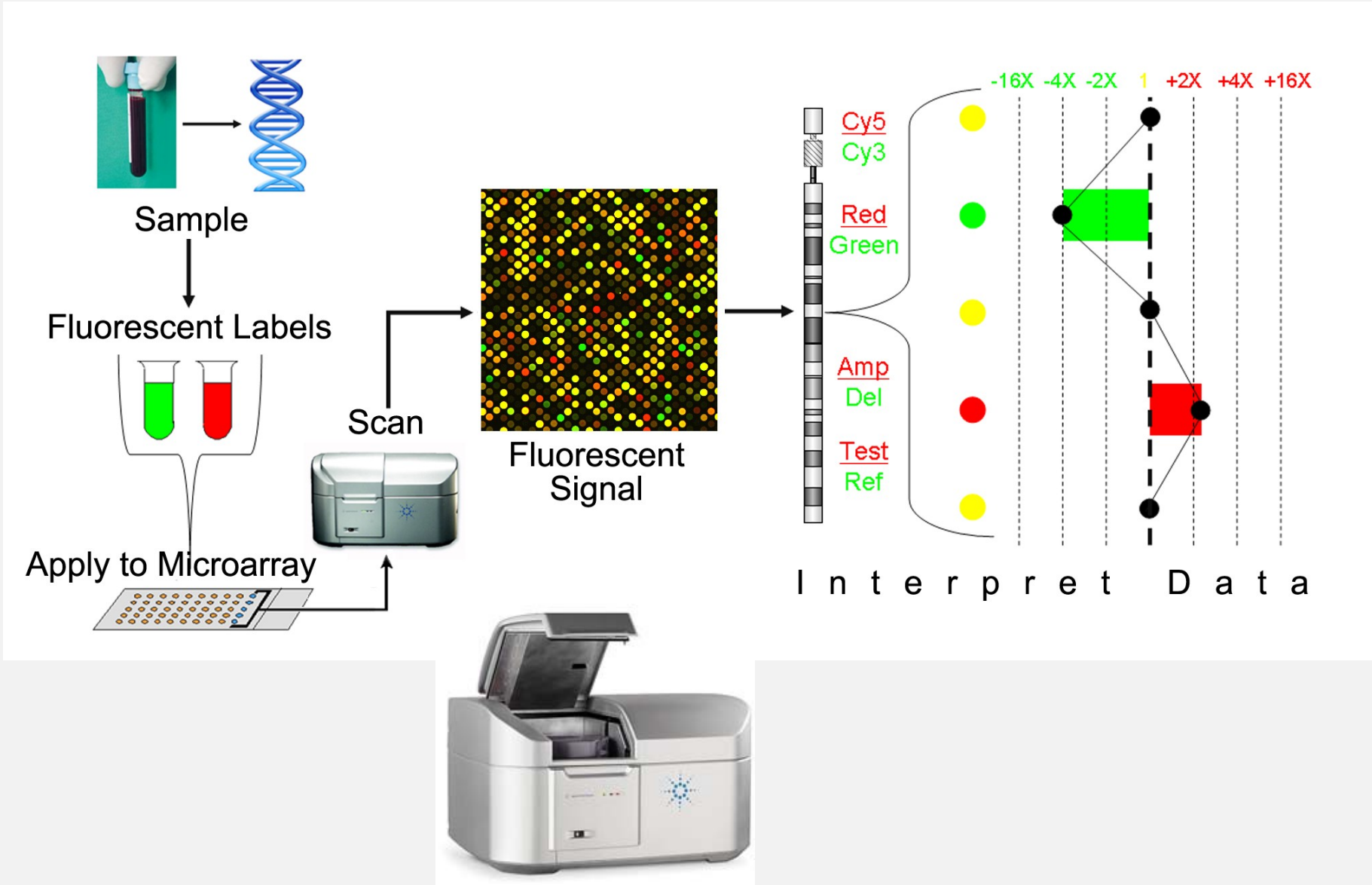
Step 4 Patient and control DNA compete to attach, or hybridize, to the microarray.

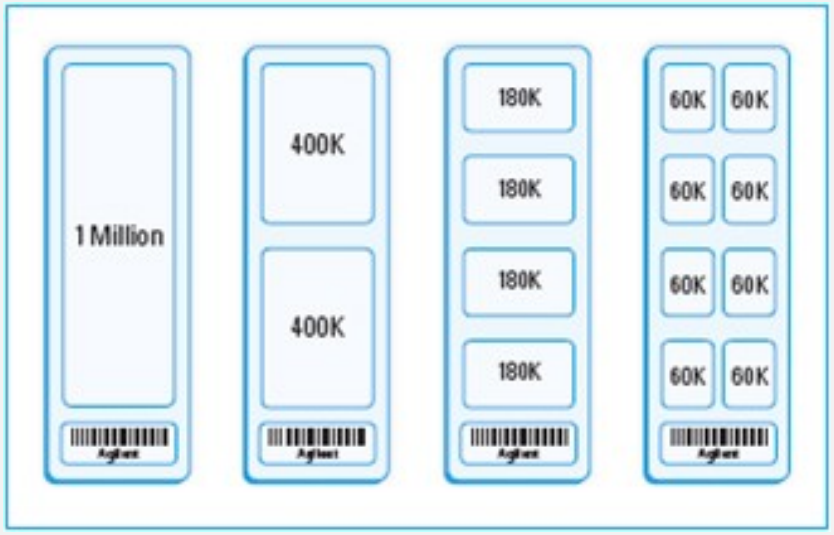
Step 5 The microarray scanner measures the fluorescent signals.

Step 6 Computer software analyzes the data and generates a plot.



Brief schema of sample processing using array-CGH



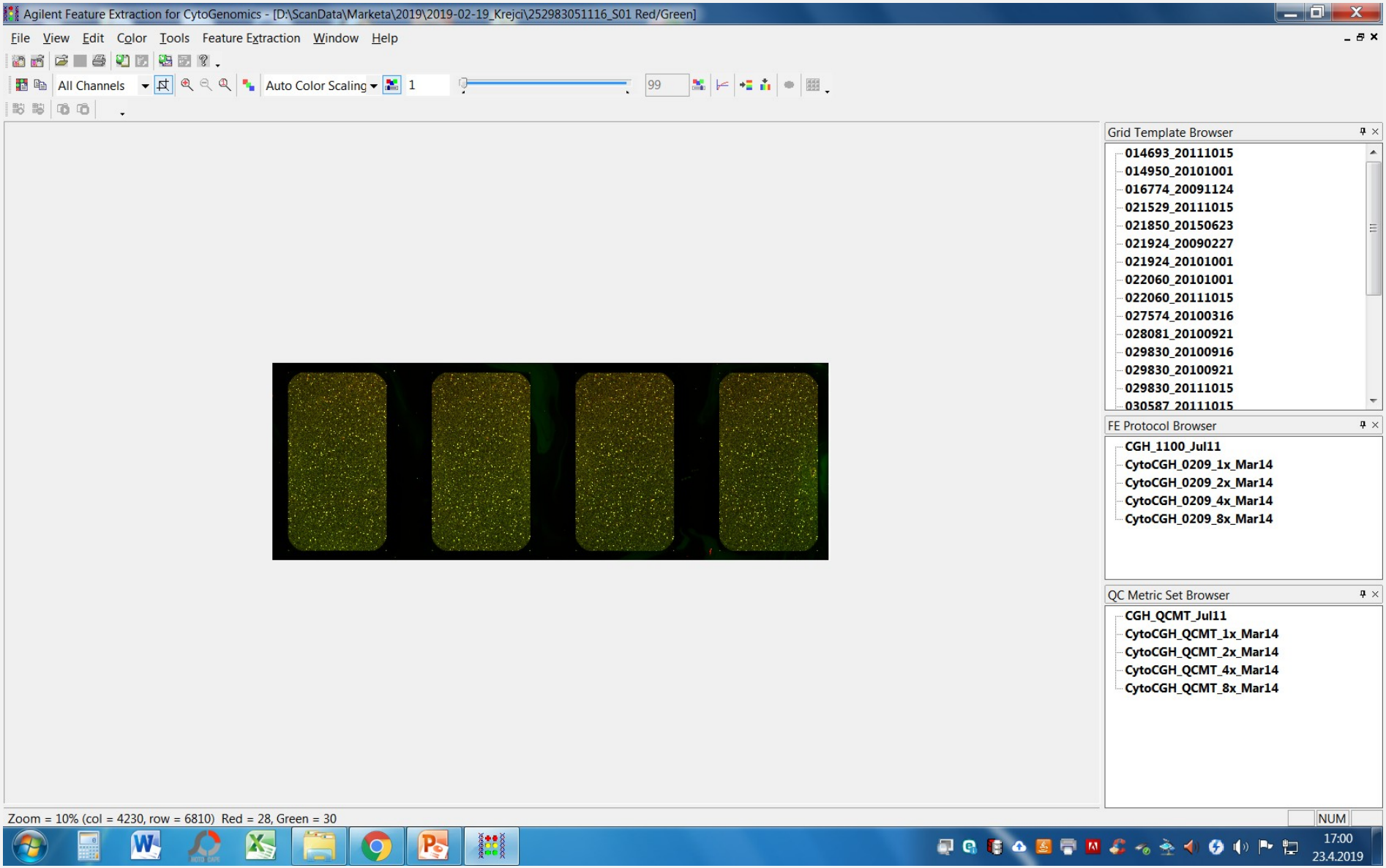


Types of DNA microarrays
(Agilent Technologies)

- ✓ 1X1M
- ✓ 2X400K
- ✓ 4X180K
- ✓ 8X60K

(parametres on www.agilent.com)

Agilent Feature Extraction for Cytogenomics – microarray scan 4X180K CGH+SNP

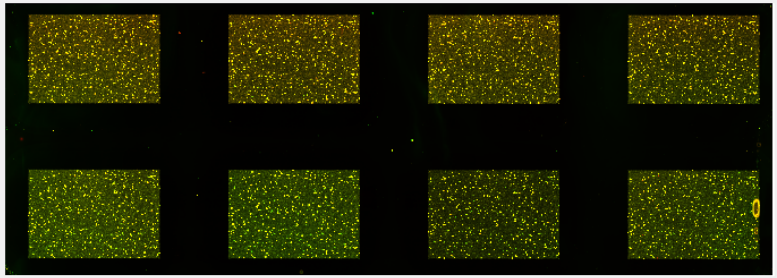


Agilent Feature Extraction for Cytogenomics – microarray scan 8X60K CGH

Agilent Feature Extraction for CytoGenomics - [D:\ScanData\Marketa\2019\2019-04-02\252192446021_S01 Red/Green]

File View Edit Color Tools Feature Extraction Window Help

All Channels | Auto Color Scaling | 1 | 99



Grid Template Browser

- 014693_20111015
- 014950_20101001
- 016774_20091124
- 021529_20111015
- 021850_20150623
- 021924_20090227
- 021924_20101001
- 022060_20101001
- 022060_20111015
- 027574_20100316
- 028081_20100921
- 029830_20100916
- 029830_20100921
- 029830_20111015
- 030587_20111015

FE Protocol Browser

- CGH_1100_Jul11
- CytoCGH_0209_1x_Mar14
- CytoCGH_0209_2x_Mar14
- CytoCGH_0209_4x_Mar14
- CytoCGH_0209_8x_Mar14

QC Metric Set Browser

- CGH_QCMT_Jul11
- CytoCGH_QCMT_1x_Mar14
- CytoCGH_QCMT_2x_Mar14
- CytoCGH_QCMT_4x_Mar14
- CytoCGH_QCMT_8x_Mar14

Zoom = 10% (col = 0, row = 0) Red = 9, Green = 59

NUM

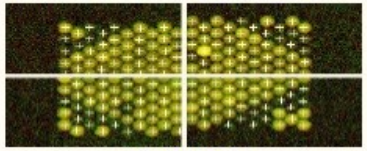
16:58
23.4.2019

Agilent Feature Extraction
–
Microarray scan and quality control (QC Report)

QC Report - Agilent Technologies : 2 Color CGH

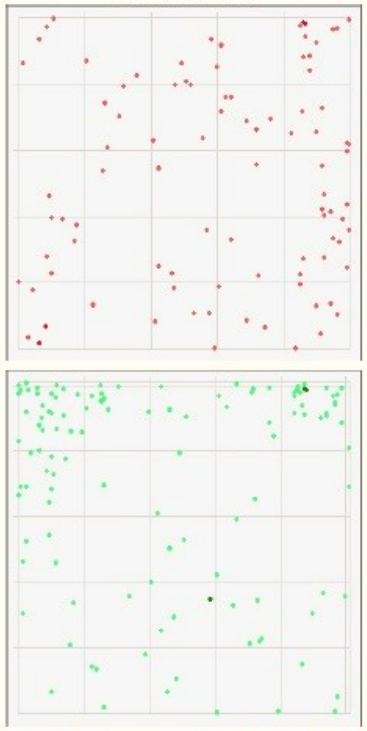
Date	Friday, March 22, 2019 - 14:51	Sample(red/green)	
User Name	admin	FE Version	2.9.2.4
Image	252192446022_S01 [1_1]	BG Method	Detrend on (NegC)
Protocol	CytoCGH_0209_4x_Mar14 (Read Only)	Multiplicative Detrend	True
Grid	021924_20101001	Dye Norm	Linear
Saturation Value	779236 (r), 778363 (g)		
DyeNorm List	NA		
No of Probes in DyeNorm List	NA		

Spot Finding of the Four Corners of the Array



Grid Normal

Outlier Numbers with Spatial Distribution
384 rows x 164 columns



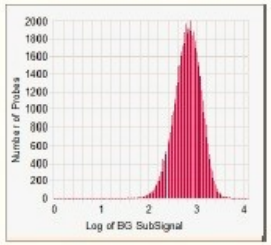
*Red FeaturePopulation
*Green FeaturePopulation
*Red Feature NonUniform
*Green Feature NonUniform

Evaluation Metrics for CytoCGH_QCMT_4x_Mar14
Excellent (10) ; Good (2) ; Evaluate (1)

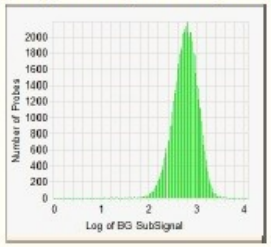
Metric Name	Value	Excellent	Good	Evaluate
IsGoodGrid	1.00	>1	NA	<1
AnyColorPrcntFeatNonU...	0.01	<1	1 to 5	>5
DerivativeLR_Spread	0.13	<0.20	0.20 to 0.30	>0.30
gRepro	0.07	0 to 0.10	0.10 to 0.20	<0 or >0.20
g_BGNoise	5.56	<10	10 to 20	>20
g_Signal2Noise	90.84	>100	50 to 100	<50
g_SignalIntensity	505.16	>400	200 to 400	<200
rRepro	0.09	0 to 0.10	0.10 to 0.20	<0 or >0.20
r_BGNoise	9.45	<10	10 to 20	>20
r_Signal2Noise	59.45	>100	50 to 100	<50
r_SignalIntensity	561.64	>400	200 to 400	<200
RestrictionControl	-1.00		0.80 to 1	<0.80 or >1
LogRatioImbalance	0.00	-0.26 to 0.26	(-0.75 to -0.2...	<-0.75 or >0.75

• Excellent • Good • Evaluate

Histogram of Signals Plot (Red)



Histogram of Signals Plot (Green)



Evaluation Metrics for CytoCGH_QCMT_4x_Mar14
Excellent (10) ; Good (2) ; Evaluate (1)

Metric Name	Value	Excellent	Good	Evaluate
IsGoodGrid	1.00	>1	NA	<1
AnyColorPrcntFeatNonU...	0.01	<1	1 to 5	>5
DerivativeLR_Spread	0.13	<0.20	0.20 to 0.30	>0.30
gRepro	0.07	0 to 0.10	0.10 to 0.20	<0 or >0.20
g_BGNoise	5.56	<10	10 to 20	>20
g_Signal2Noise	90.84	>100	50 to 100	<50
g_SignalIntensity	505.16	>400	200 to 400	<200
rRepro	0.09	0 to 0.10	0.10 to 0.20	<0 or >0.20
r_BGNoise	9.45	<10	10 to 20	>20
r_Signal2Noise	59.45	>100	50 to 100	<50
r_SignalIntensity	561.64	>400	200 to 400	<200
RestrictionControl	-1.00		0.80 to 1	<0.80 or >1
LogRatioImbalance	0.00	-0.26 to 0.26	(-0.75 to -0.2...	<-0.75 or >0.75

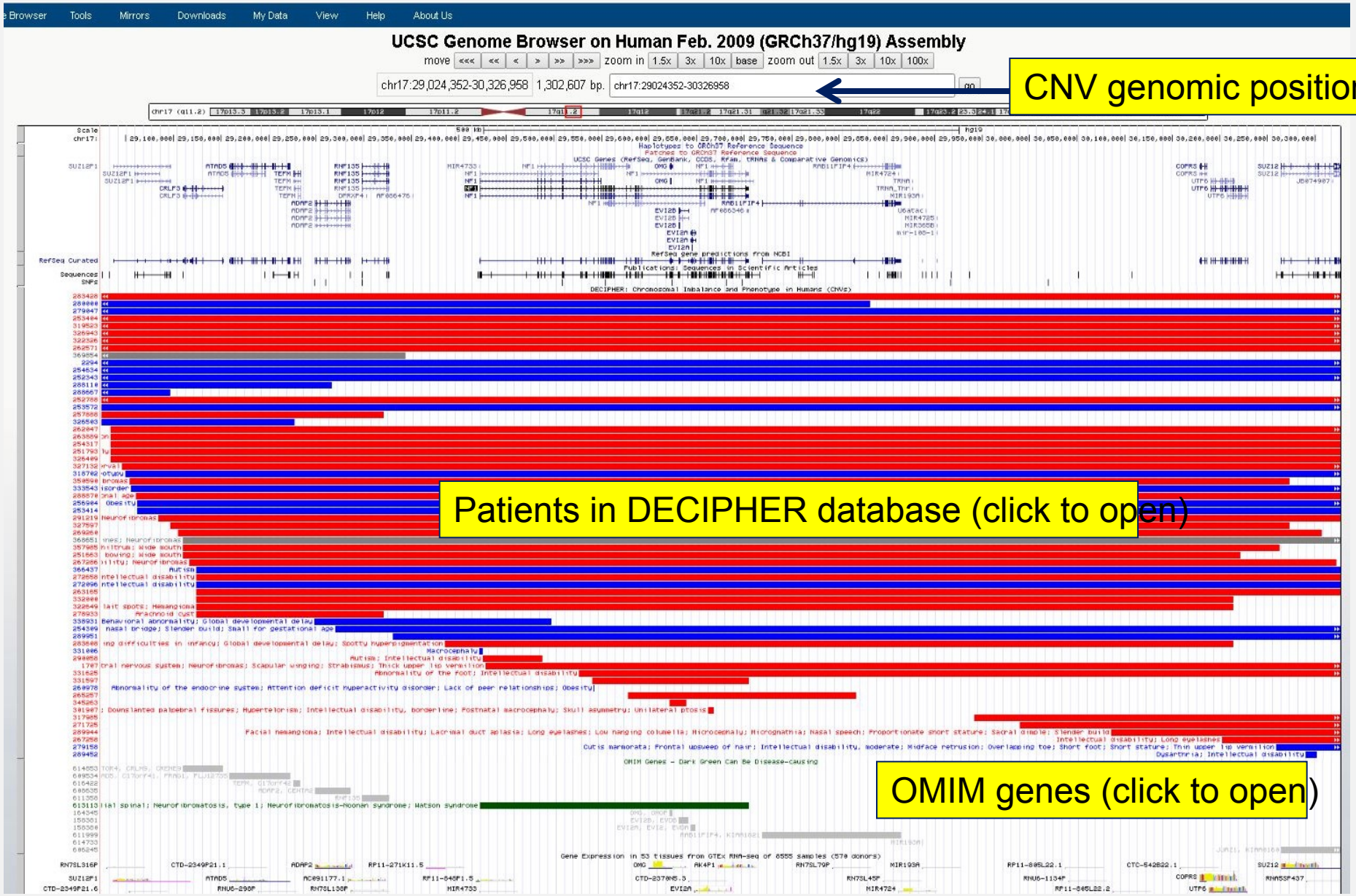
• Excellent • Good • Evaluate

AGILENT GENOMIC WORKBENCH – COPY-NUMBER VARIATION EVALUATION (GLOBAL VIEW ON CHROMOSOMES –> CHROMOSOME 17 –> DETAIL OF 17Q11.2 MICRODELETION)

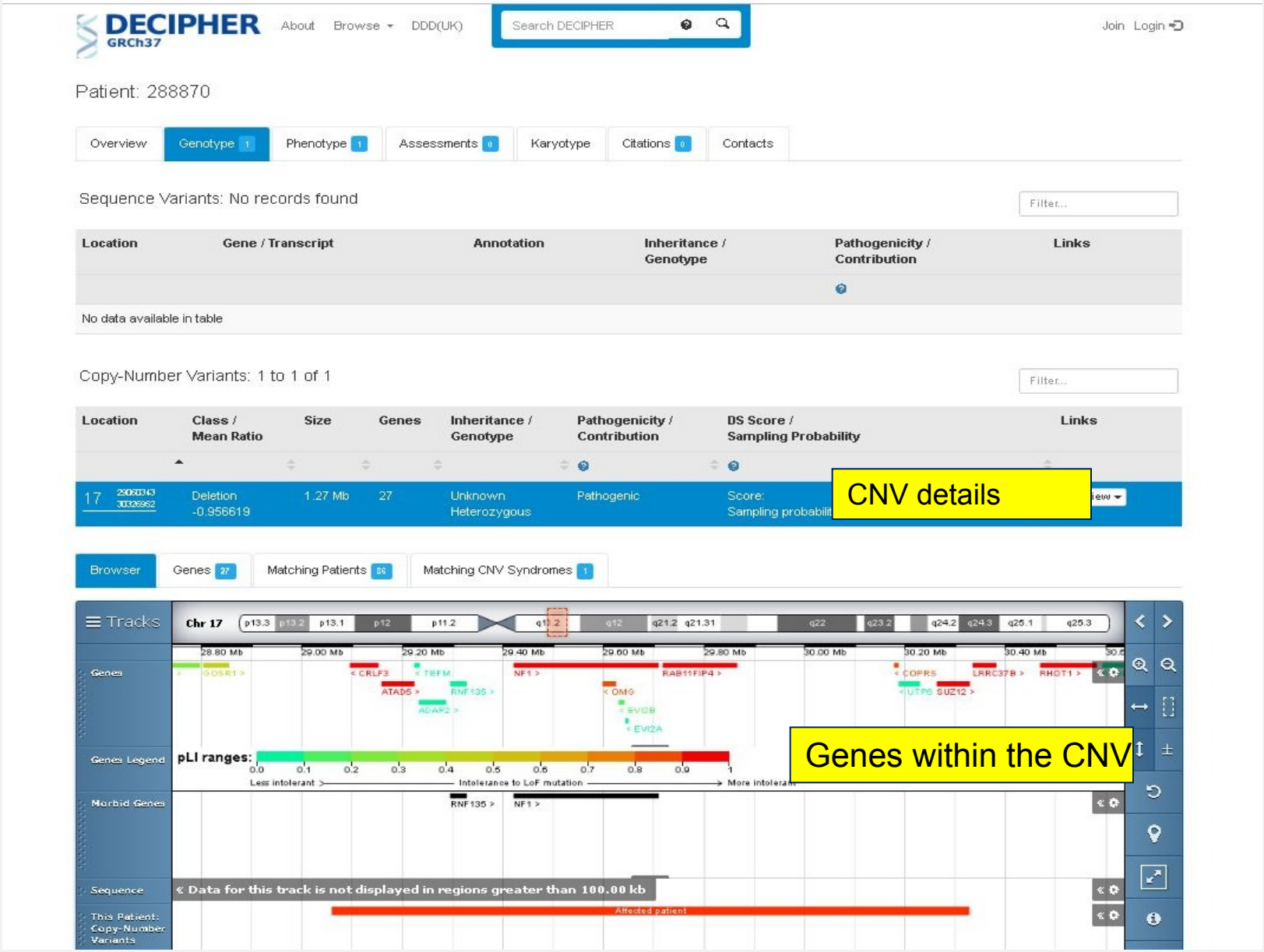
THE INTERPRETAION OF COPY-NUMBER VARIATIONS MUST BE PERFORMED IN THE CONTEXT OF:

1. Patient‘s phenotype
2. Analysis of parental genomes -> to assess CNVs origin (*de novo* or inherited CNV from parent with normal/abnormal phenotype)
3. Information in databases of genetic variants (UCSC, DECIPHER, DGV...) and about genes in CNVs (database OMIM)
4. Information in relevant scientific and clinical literature (Pubmed…)

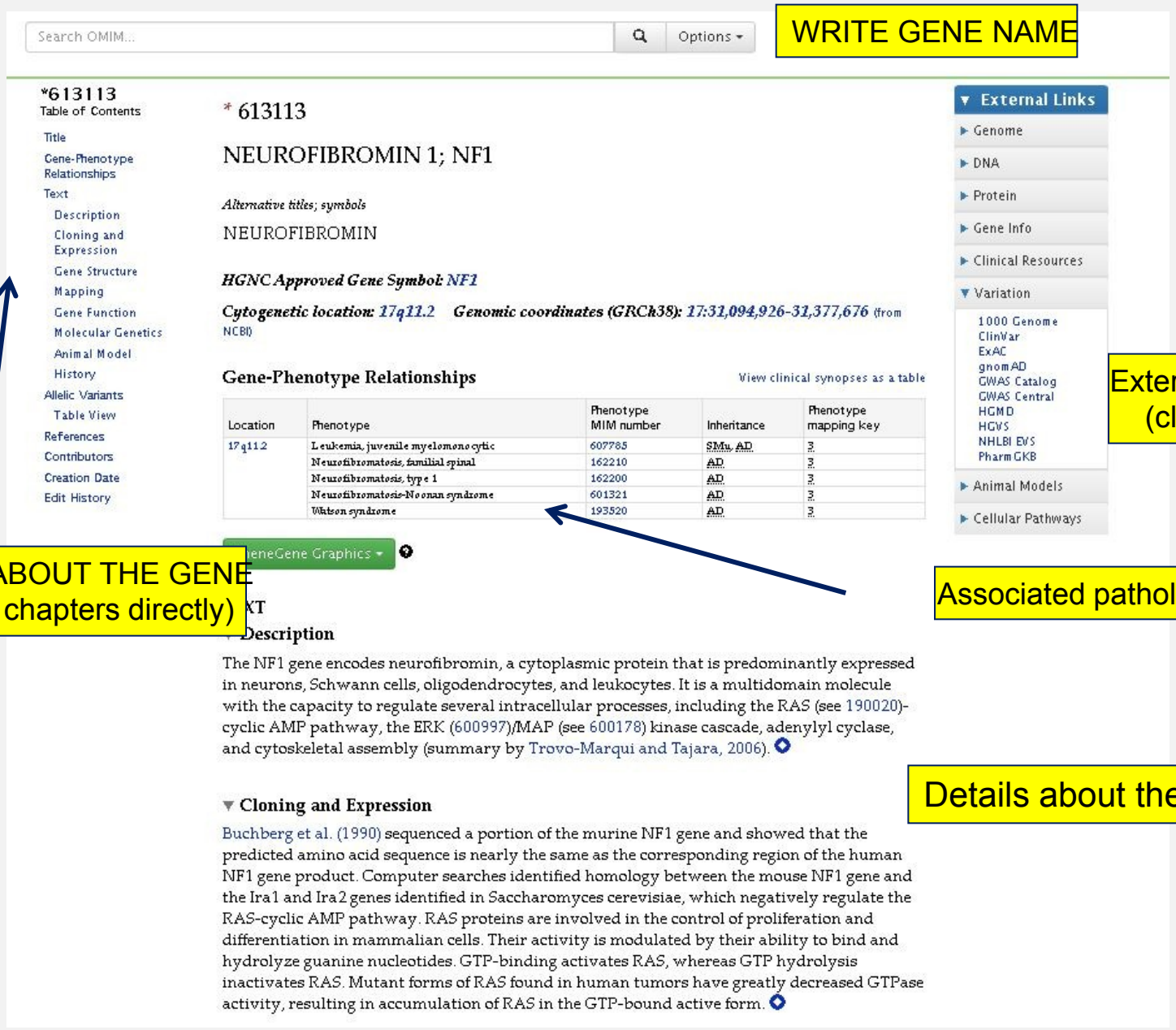
UCSC GENOME BROWSER



DECIPHER [HTTPS://DECIPHER.SANGER.AC.UK/](https://decipher.sanger.ac.uk/)



OMIM (WWW.OMIM.ORG)



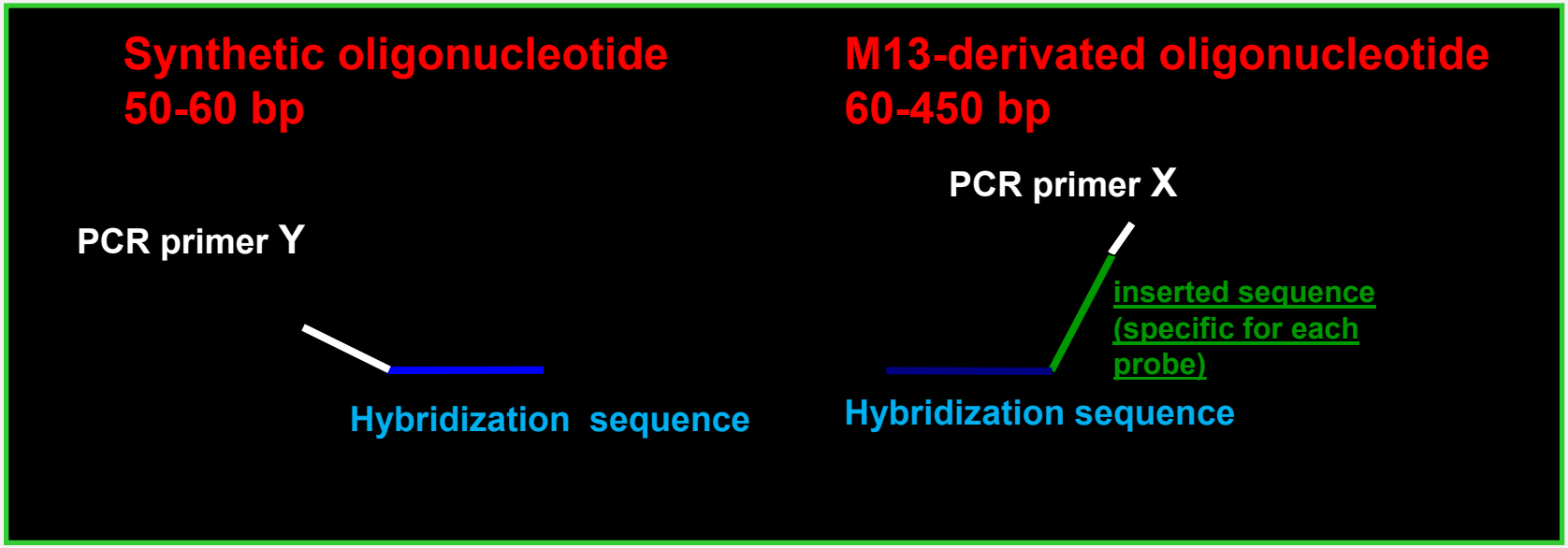
ADVANTAGES AND DISADVANTAGES OF ARRAY-CGH

- advantages
  - detects and quantifies DNA copy number gains and losses throughout an entire genome in a single analysis
  - **resolution** depends on microarray platform of the choice (**from ~50 kb**)
  - ability to detect mosaicism < 30%
  - precise aberration location (based on aberrant clone positions)

- disadvantages
  - unable to detect balanced rearrangements (translocation, inversion)
  - unable to detect ploidy changes
  - very expensive method
  - highly qualified and experienced laboratory stuff

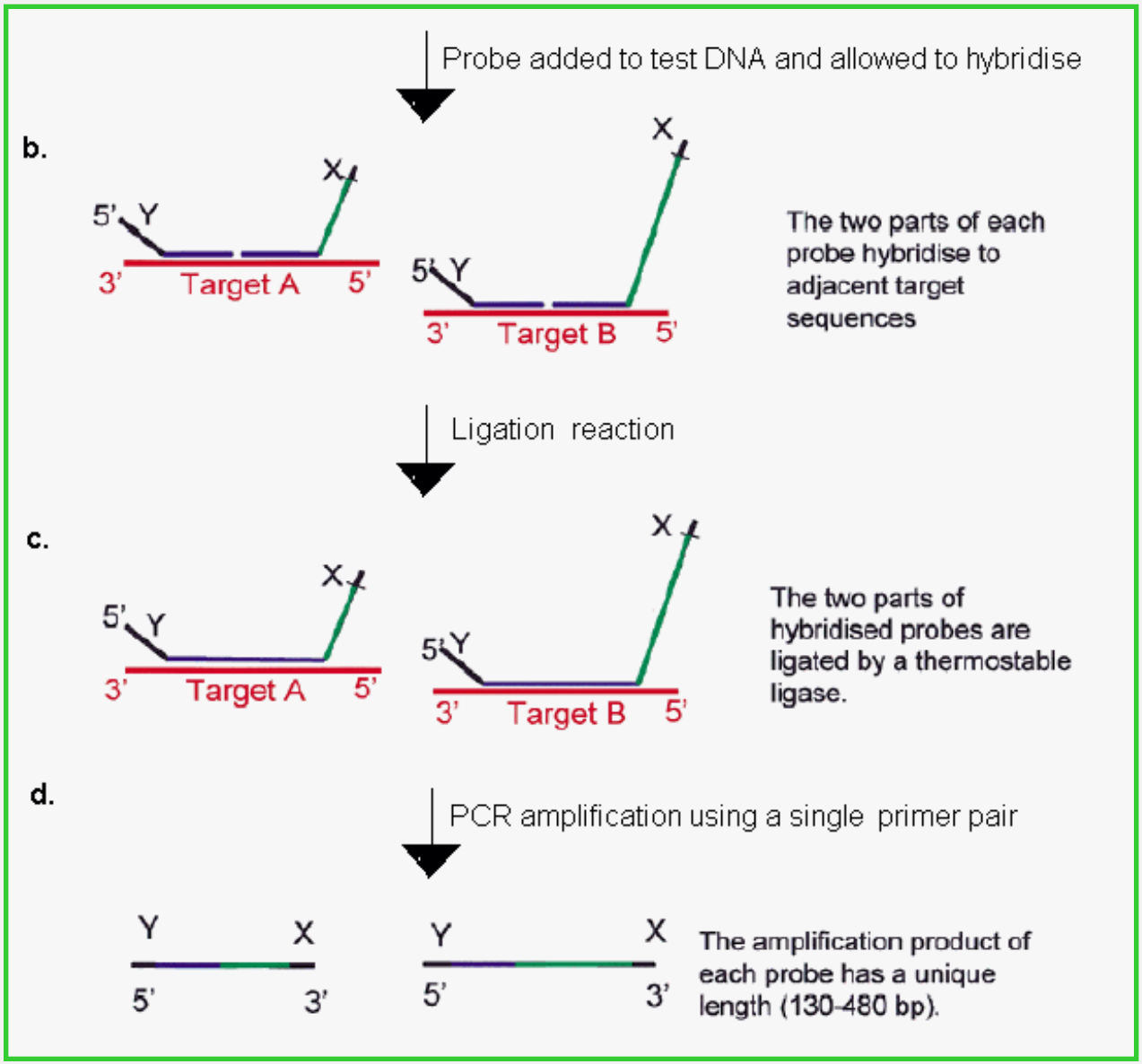
MLPA
MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION

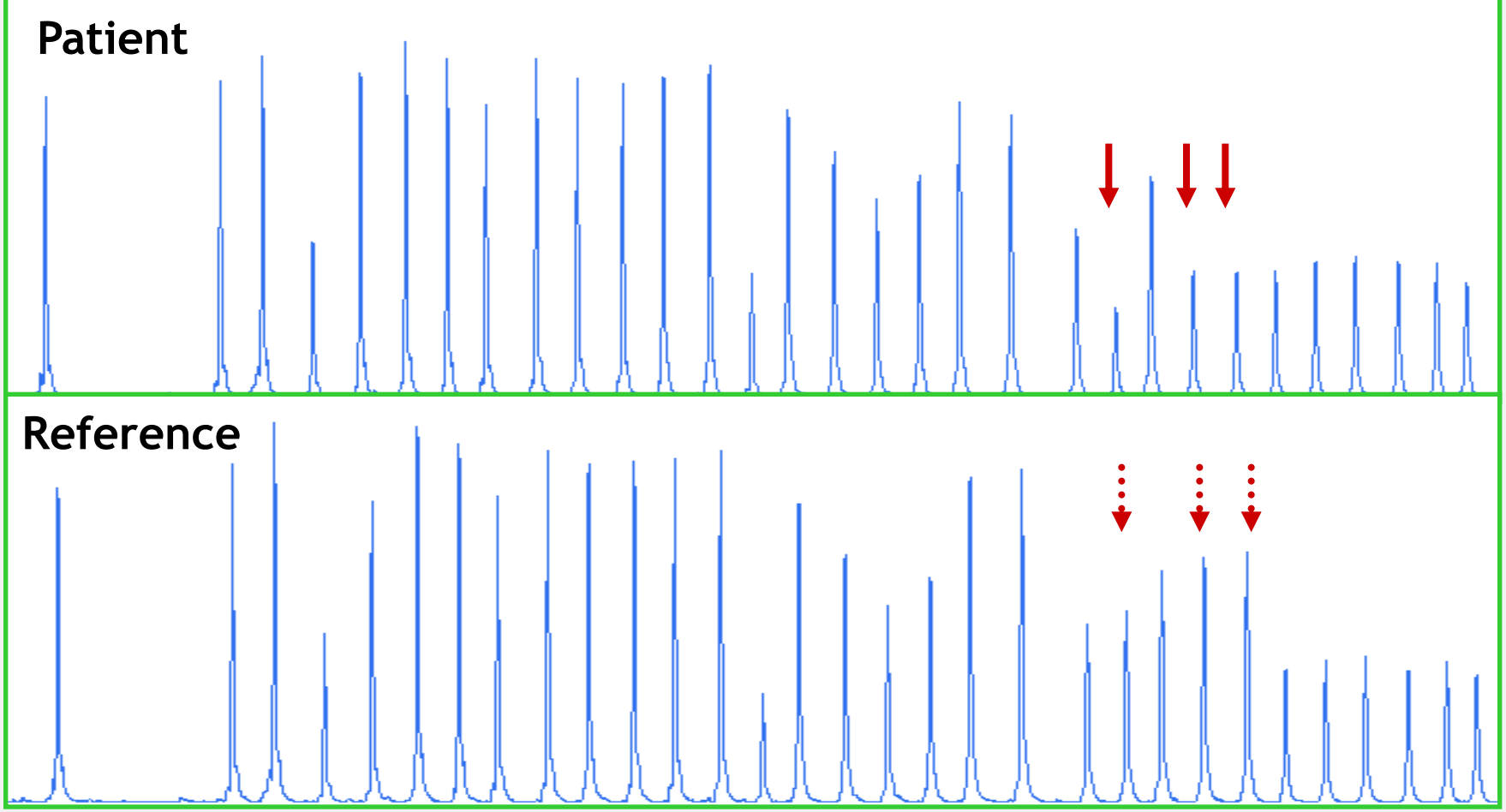
- sensitive method able to detect differences up to nucleotide level
- detects changes of copy number in 45 sequences in one reaction
- simple – all the reaction takes place in one test tube
- relatively cheap method

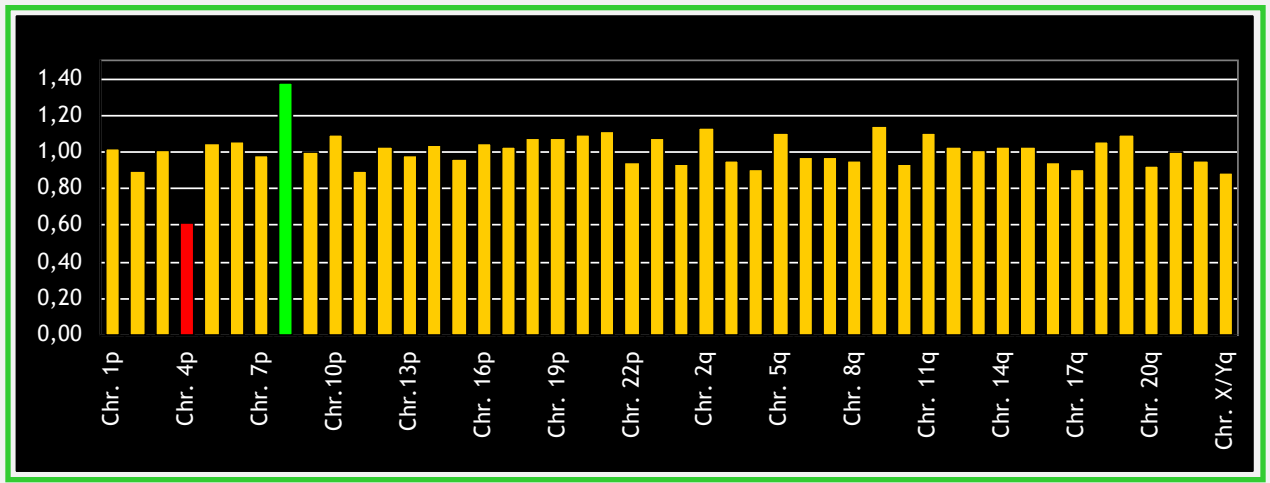


MRC Holland, www.mlpa.com

MLPA PRINCIPLE (IN BRIEF)




Patient
Reference


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

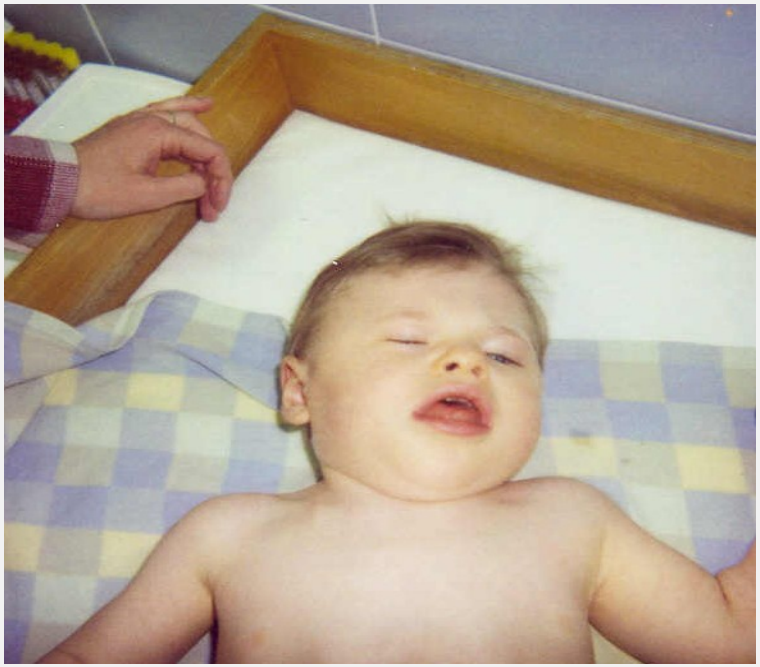
- advantages
  - sensitive
  - specific
  - multiplex
  - simple
  - cheap

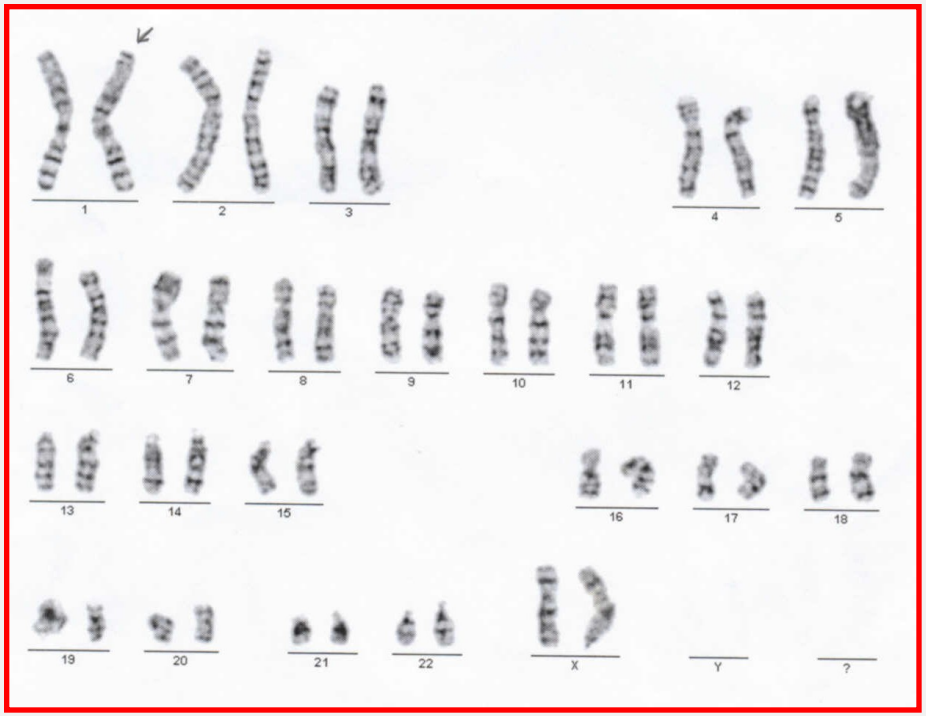
- disadvantages
  - highly sensitive to contamination
  - time difficulty
  - the aberrations have to occur in 50% of cells (mosaicism)
  - mutations or polymorphisms can lead to false results

5. CASE INTERPRETATION

CASE INTERPRETATION 1

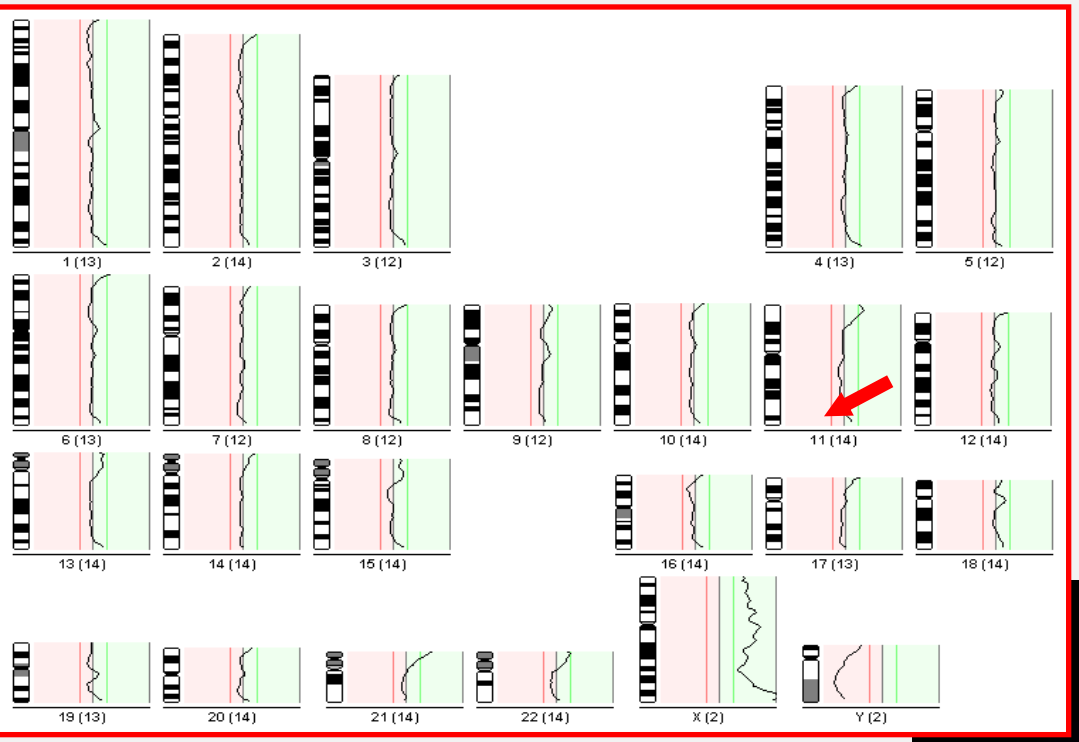
- girl, born in 2002
- dg: stigmatization – mongoloid eye position, hyperplastic gingival mucose membrane, atypical chest and tummy
- mother 46,XX, inv(9), father 46,XY,add(1)[87]/46,XY[13]



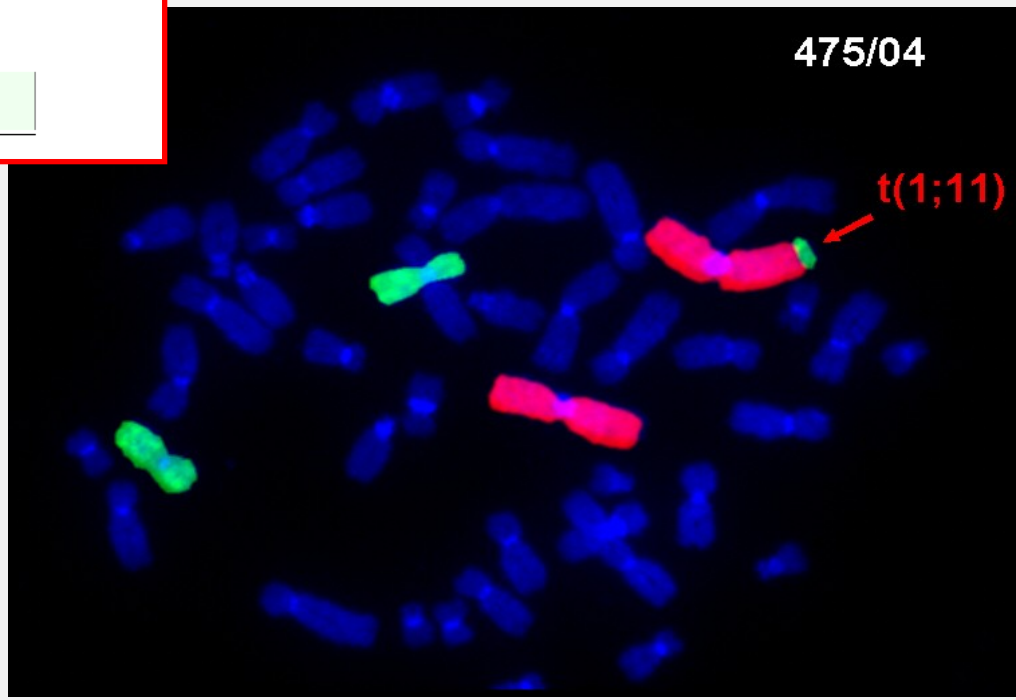


46,XX,add(1)

CGH: rev ish enh (11p15-pter) – unbalanced translocation

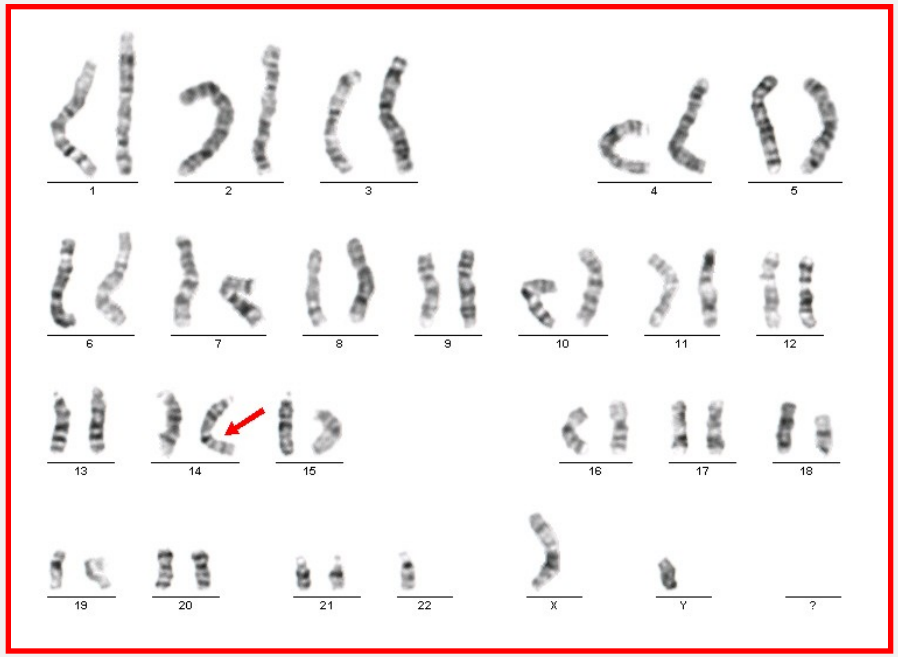


FISH: der(1)t(1;11)

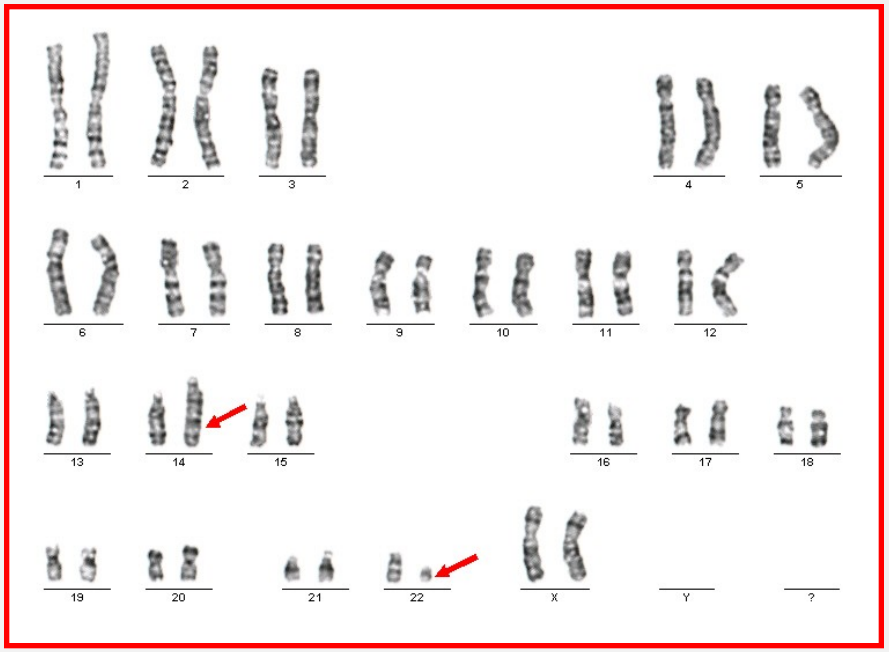


CASE INTERPRETATION 2

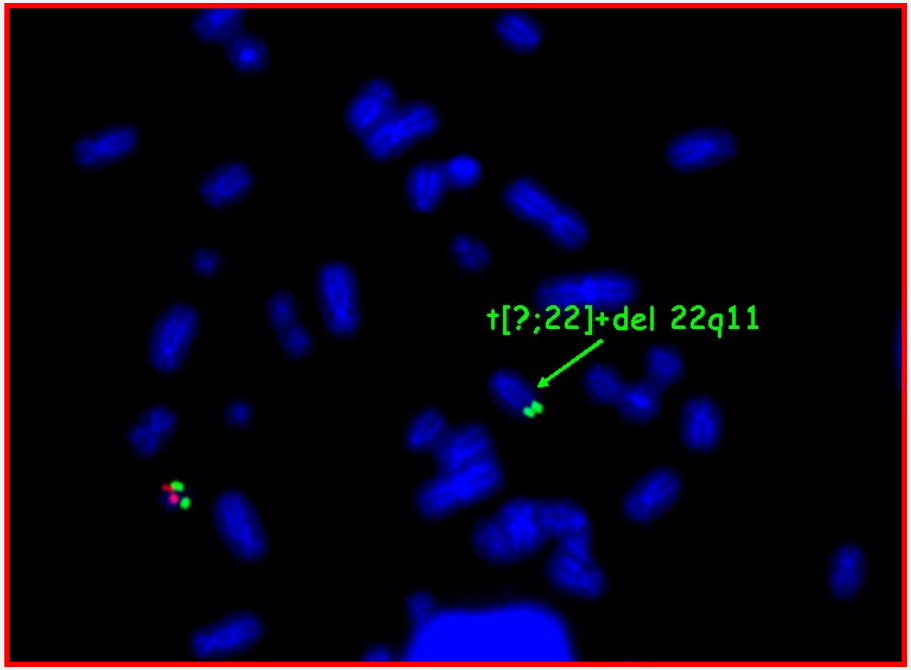
- boy, born in 2004
- heart defect, facial dysmorphology



45,XY,-22,der(14)

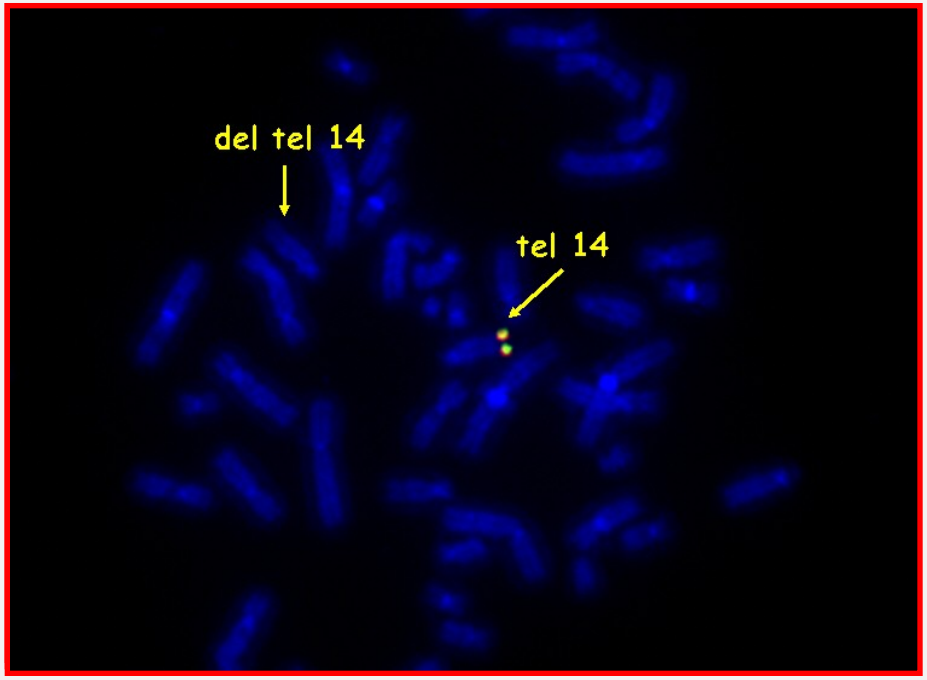


46,XX,der(14)t(14;22)(q32.3;q11.2)



45,XY,der(14)t(14;22)(q32.3;q11.2)

DiGeorge sy

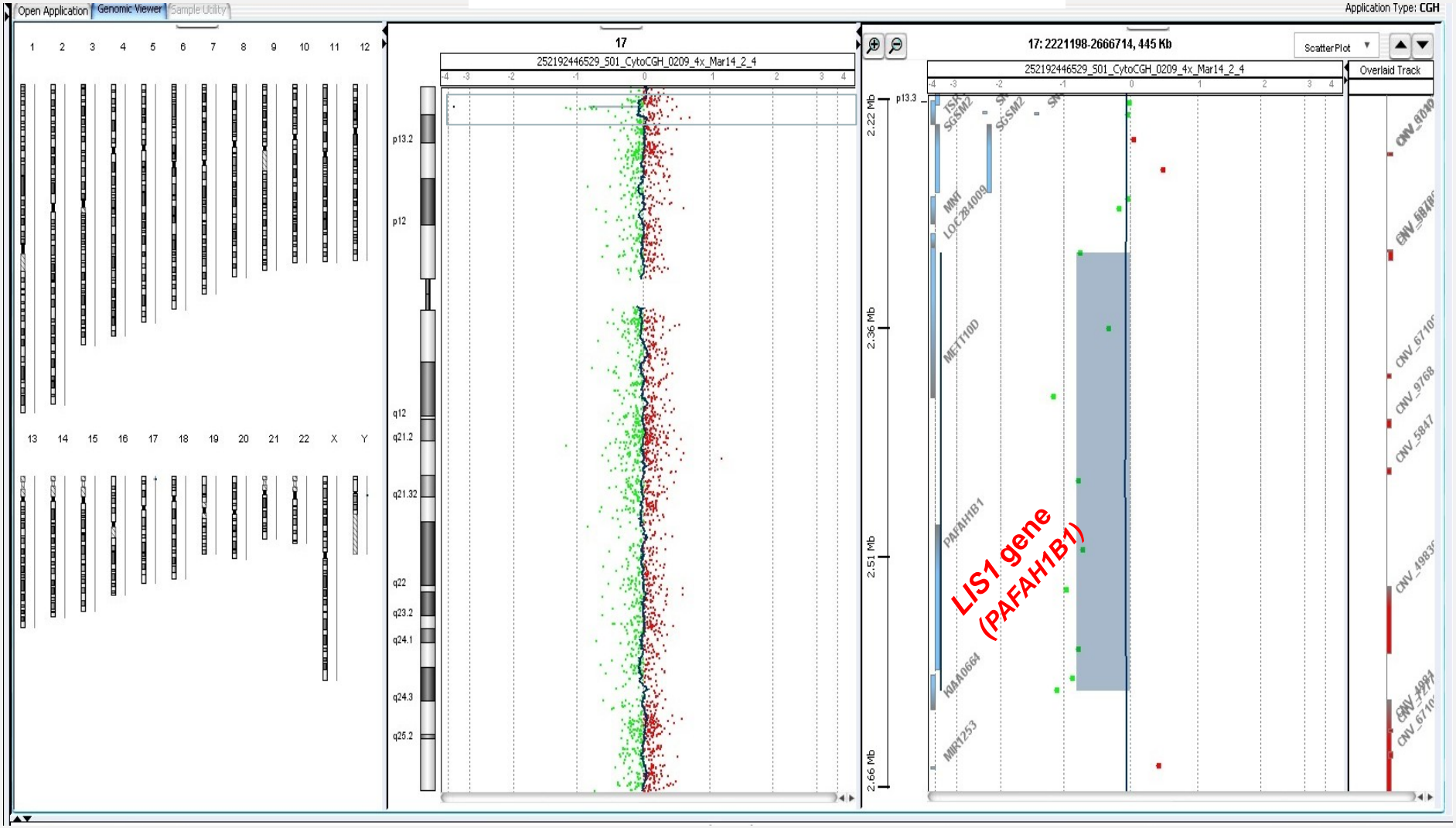


46,XX,der(14)t(14;22)(q32.3;q11.2)

CASE INTERPRETATION 3

- boy, born in 2018
- severe lissencephaly, global developmental delay

17p13.3 microdeletion (~280 kb)



GENOTYPE-PHENOTYPE CORRELATION (OMIM DATABASE)

*601545

Table of Contents

* 601545

PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE, ISOFORM 1B, ALPHA SUBUNIT; PAFAH1B1

Alternative titles; symbols

LIS1 GENE; LIS1

HGNC Approved Gene Symbol: PAFAH1B1

Cytogenetic location: 17p13.3 *Genomic coordinates (GRCh38): 17:2,593,209-2,685,616* (from NCBI)

Gene-Phenotype Relationships

View clinical synopses as a table

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key
17p13.3	Lissencephaly 1	607432	AD	3
	Subcortical laminar heterotopia	607432	AD	3

PheneGene Graphics

TEXT

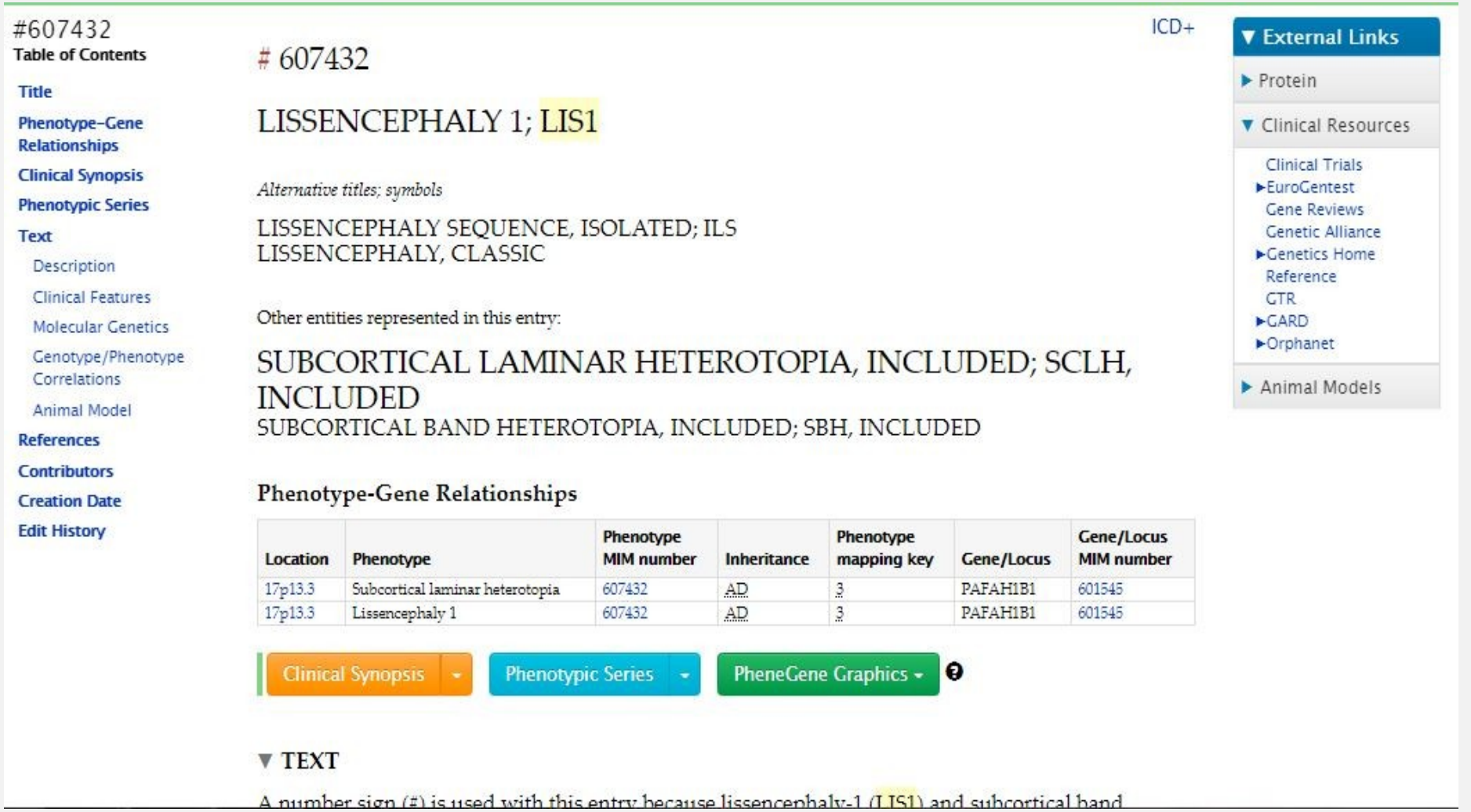
▼ Description

Platelet-activating factor acetylhydrolase (PAFAH) catalyzes the removal of the acetyl group at the

▼ External Links

- ▶ Genome
- ▶ DNA
- ▶ Protein
- ▶ Gene Info
- ▶ Clinical Resources
- ▼ Variation
  - 1000 Genome
  - ClinVar
  - ExAC
  - gnomAD
  - GWAS Catalog
  - GWAS Central
  - HGMD
  - HGVS
  - NHLBI EVS
  - PharmGKB
- ▶ Animal Models
- ▶ Cellular Pathways

GENOTYPE-PHENOTYPE CORRELATION (OMIM DATABASE)



#607432
Table of Contents

- Title
- Phenotype–Gene Relationships
- Clinical Synopsis
- Phenotypic Series
- Text
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  - Clinical Features
  - Molecular Genetics
  - Genotype/Phenotype Correlations
  - Animal Model
- References
- Contributors
- Creation Date
- Edit History

ICD+

607432

LISSENCEPHALY 1; LIS1

Alternative titles; symbols

LISSENCEPHALY SEQUENCE, ISOLATED; ILS
LISSENCEPHALY, CLASSIC

Other entities represented in this entry:

SUBCORTICAL LAMINAR HETEROTOPIA, INCLUDED; SCLH, INCLUDED

SUBCORTICAL BAND HETEROTOPIA, INCLUDED; SBH, INCLUDED

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
17p13.3	Subcortical laminar heterotopia	607432	AD	3	PAFAH1B1	601545
17p13.3	Lissencephaly 1	607432	AD	3	PAFAH1B1	601545

Clinical Synopsis | Phenotypic Series | PheneGene Graphics

TEXT

A number sign (#) is used with this entry because lissencephaly-1 (LIS1) and subcortical band

▼ External Links

- ▶ Protein
- ▼ Clinical Resources
  - Clinical Trials
  - ▶EuroGentest
  - Gene Reviews
  - Genetic Alliance
  - ▶Genetics Home
  - Reference
  - GTR
  - ▶GARD
  - ▶Orphanet
- ▶ Animal Models

GENOTYPE-PHENOTYPE CORRELATION (PUBMED)

ORIGINAL CONTRIBUTION

LIS1-Related Isolated Lissencephaly

Spectrum of Mutations and Relationships With Malformation Severity

Yoann Saillour, PhD; Nathalie Carion, MS; Chloé Quelin, MD; Pierre-Louis Leger, MD; Nathalie Boddaert, MD, PhD; Caroline Elie, MD; Annick Toutain, MD, PhD; Sandra Mercier, MD; Marie Anne Barthez, MD; Mathieu Milh, MD, PhD; Sylvie Joriot, MD; Vincent des Portes, MD, PhD; Nicole Philip, MD, PhD; Dominique Broglin, MD; Agathe Roubertie, MD, PhD; Gaelle Pitelet, MD; Marie Laure Moutard, MD; Jean Marc Pinard, MD; Claude Cances, MD; Anna Kaminska, MD; Jamel Chelly, MD, PhD; Chérif Beldjord, MD, PhD; Nadia Bahi-Buisson, MD, PhD

Objective: With the largest data set of patients with *LIS1*-related lissencephaly, the major cause of posteriorly predominant lissencephaly related to either *LIS1* mutation or intragenic deletion, described so far, we aimed to refine the spectrum of neurological and radiological features and to assess relationships with the genotype.

Design: Retrospective study.

Subjects: A total of 63 patients with posteriorly predominant lissencephaly.

Interventions: Of the 63 patients, 40 wer
either *LIS1* point mutations (77.5%) or sm
letions (20%), and 1 carried a somatic n
tion. On the basis of the severity of neur
ment, epilepsy, and radiological findings, c
the location and type of mutation were ex

Results: Most patients with *LIS1* mut
strated posterior agyria (grade 3a, 55.3%)
pus callosum (50%) and prominent perivascular spaces (67.4%). By contrast, patients without *LIS1* mutations tended to have less severe lissencephaly (grade 4a, 41.6%) and no additional brain abnormalities. The degree of neuromotor impairment was in accordance with the severity of lissencephaly, with a high incidence of tetraplegia (61.1%). Conversely, the severity of epilepsy was not determined with the same reliability because 82.9% had early onset of seizures and 48.7% had seizures more often than daily. In addition, neither the mutation type nor the location of the mutation were found to predict the sever-

Pediatr Neurol. 2007 Apr;36(4):258-60.

Partial deletion of LIS1: a pitfall in molecular diagnosis of Miller-Dieker syndrome.

Izumi K[1], Kuratsuji G, Ikeda K, Takahashi T, Kosaki K.

Author information

Abstract

Miller-Dieker syndrome represents a microdeletion syndrome spanning the LIS1 locus at 17p13.3, the deletion of which leads to lissencephaly. A fluorescence in situ hybridization study using an LIS1 probe is considered the standard laboratory diagnostic method for Miller-Dieker syndrome. This report documents a Miller-Dieker syndrome patient who tested normal when a commercially available LIS1 fluorescence in situ hybridization study probe was used but was later demonstrated to have a partial deletion of the LIS1 locus. The present case exemplifies a major shortcoming of commercially available fluorescence in situ hybridization studies for the diagnosis of microdeletion syndromes such as Miller-Dieker syndrome: that is, relatively small deletion can potentially remain undetected.

PMID: 17437911 DOI: 10.1016/j.pediatrneurol.2006.11.015

[Indexed for MEDLINE]

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