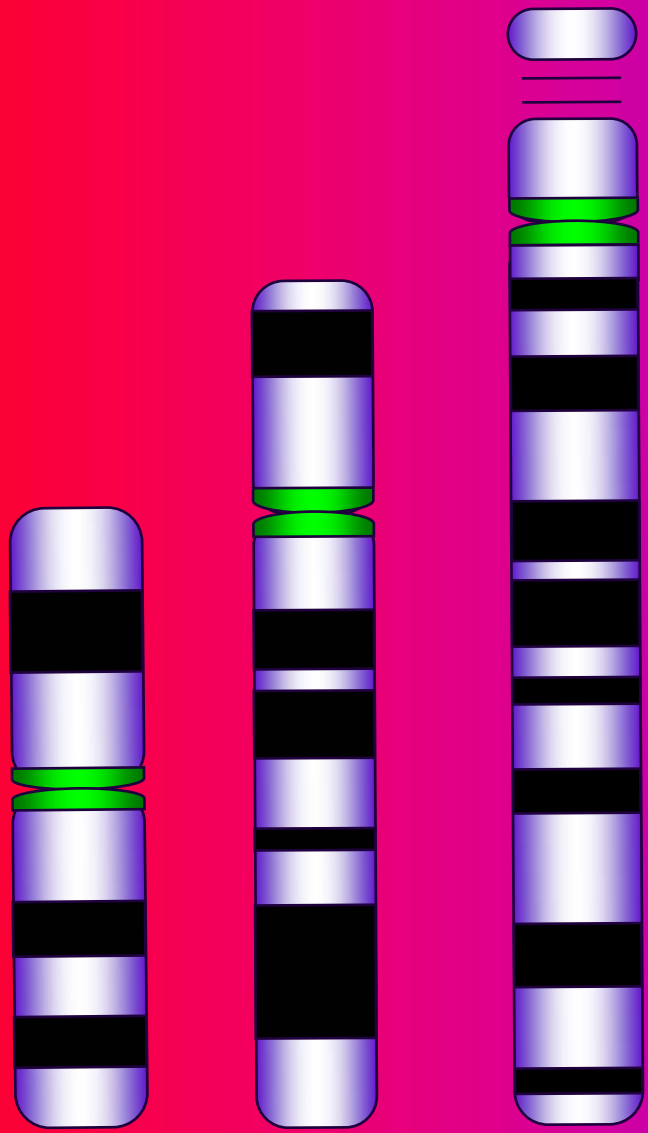
CHROMOSOME BANDING

Ivana FELLNEROVÁ, PřF UP Olomouc



Significant developments in conventional cytogenetics was the discovery of

BANDING TECHNIQUES

Chromosome banding techniques

- **Specific staining procedures developed at late 60s and early 70s**

- **Use DNA-binding chemicals to obtain reproducible <u>banding patterns</u>, unique for each chromosome.**

  [each band with characteristic **<u>location</u>**, **<u>size</u>** and **<u>staining intensity</u>**]

What is a

Chromosome band ?

- **It is part of chromosome, clearly distinguishable from other parts**

- **Crossband reflects:**
  - **The relative content of A-T versus G-C base pairs**
  - **Relative length of the repeated gene sequence**
  - **The timing of DNA replication during synthesis period**

Banding allowes:

- **Identification of each chromosome**

  The banding patterns are consistent and reproducible because chromosome coils exactly the same way every time

- **Accurate pairing**

  The banding pattern is the same for homologue chromosome but different for nonhomologue

- **Detection of chromosomal aberration**

Staining techniques

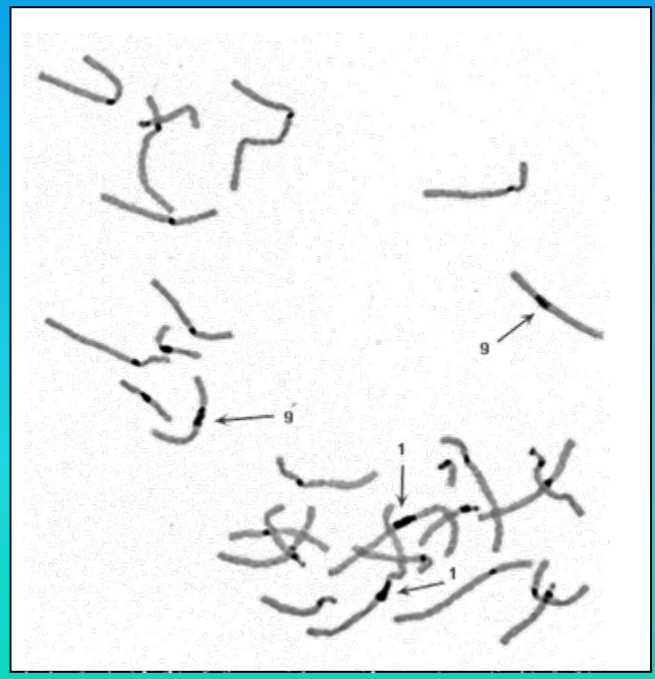
- **C-banding** Giemsa staining, acid, alkali and heat pretreatment stains constitutive heterochromatin [centromeres, telomeres]
- **G-banding** Giemsa staining + pretreatment [usually trypsin] showing more detail than C-banding
- **R-banding** reverse banding to C and G banding stain euchromatin pretreatment with hot alkali
- **Q-banding** fluorescent banding [quinacrine stain], read by fluorescence microscopy

C - banding

Dye: **Giemsa staining**

Stained: **Constitutive heterochromatin darkly**

[repetitive and satellite DNA, long arm Y human chromosome]

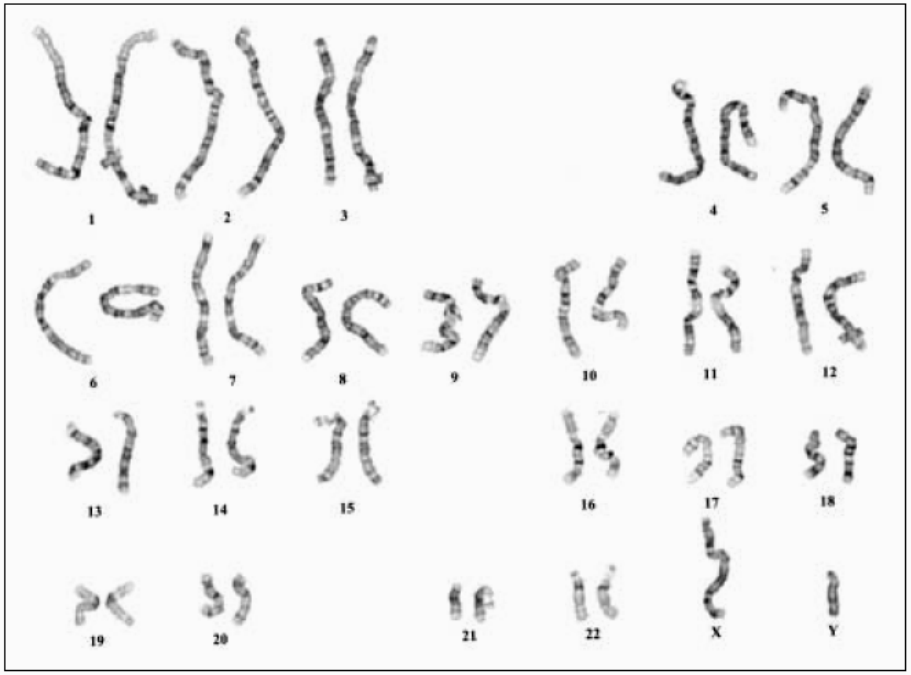


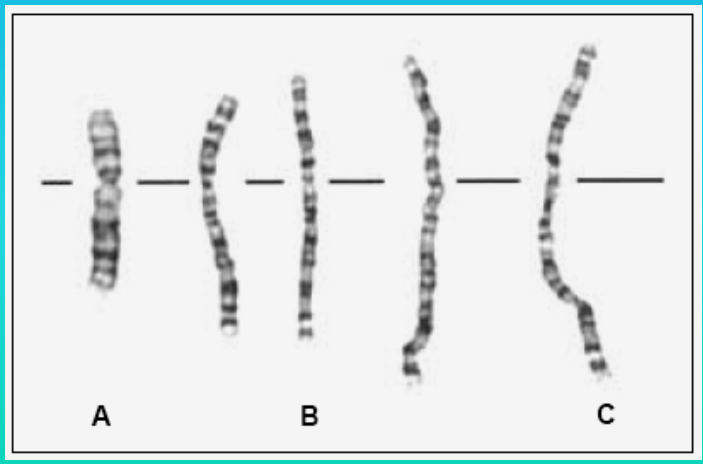
G - banding

Dye: **Giemsa staining**

Stained: **Constitutive heterochromatin dark**

G – banded human karyotype





Different stage of chromosome contraction:

A: 400-band level

B: 550-band level

C: 850- band level

The numbers are based on the total number of bands on the chromosomes of a haploid set

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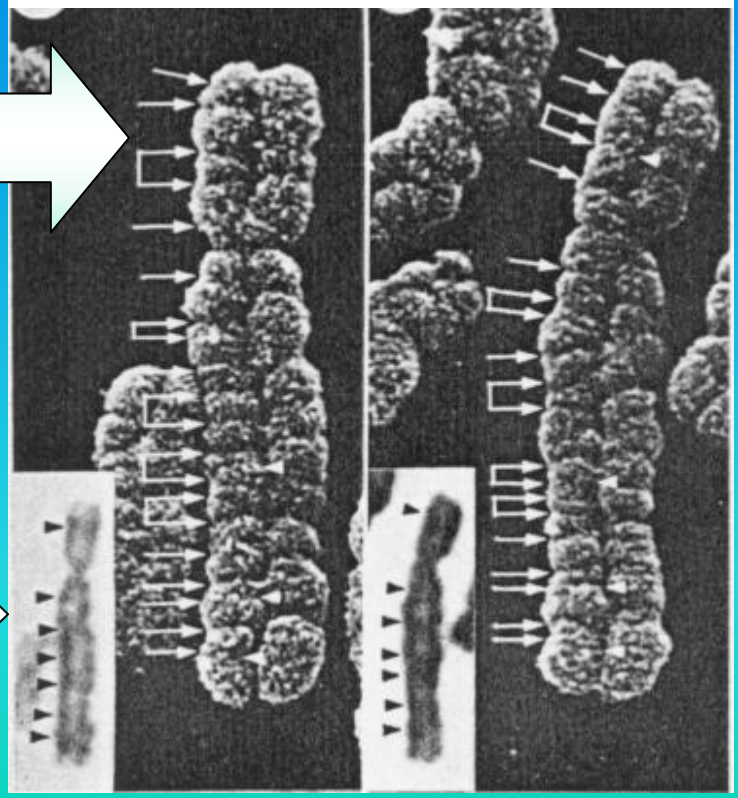
G - banding

Homologous pair of # 5 human chromosomes

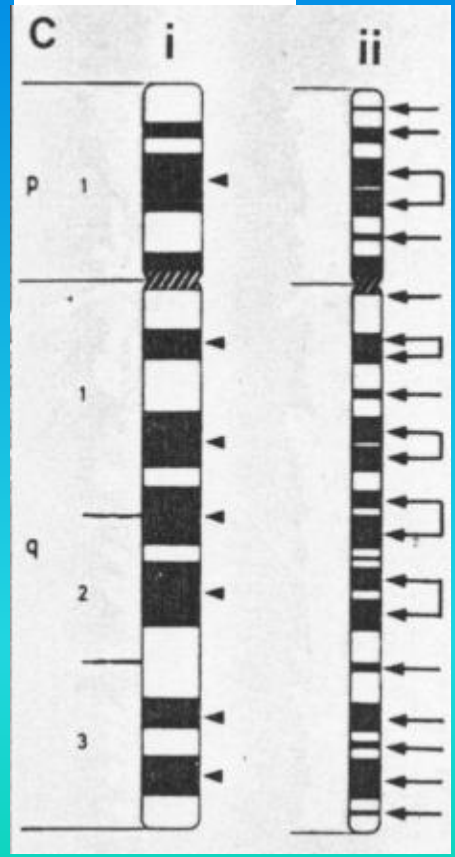
Scan electron microscope picture with grooves

Light micrograph with major G - bands

Scan electron microscopy image



IDIOGRAM



The major G-band in light micrograph are consistent with bands in the idiogram

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R - banding

DYE: fluorescent staining

STAINED: euchromatin

Q - banding

DYE: fluorescent staining

STAINED: constitutive heterochromatin

Powerful adjunct
to conventional
cytogenetic analysis
serve

MOLECULAR CYTOGENETIC TECHNIQUES

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FISH

Fluorescent In Situ Hybridization

It is a molecular cytogenetic technology utilizing fluorescently labeled DNA probes to detect or confirm gene or chromosome abnormalities, that are beyond the resolution of routine [convention] cytogenetic

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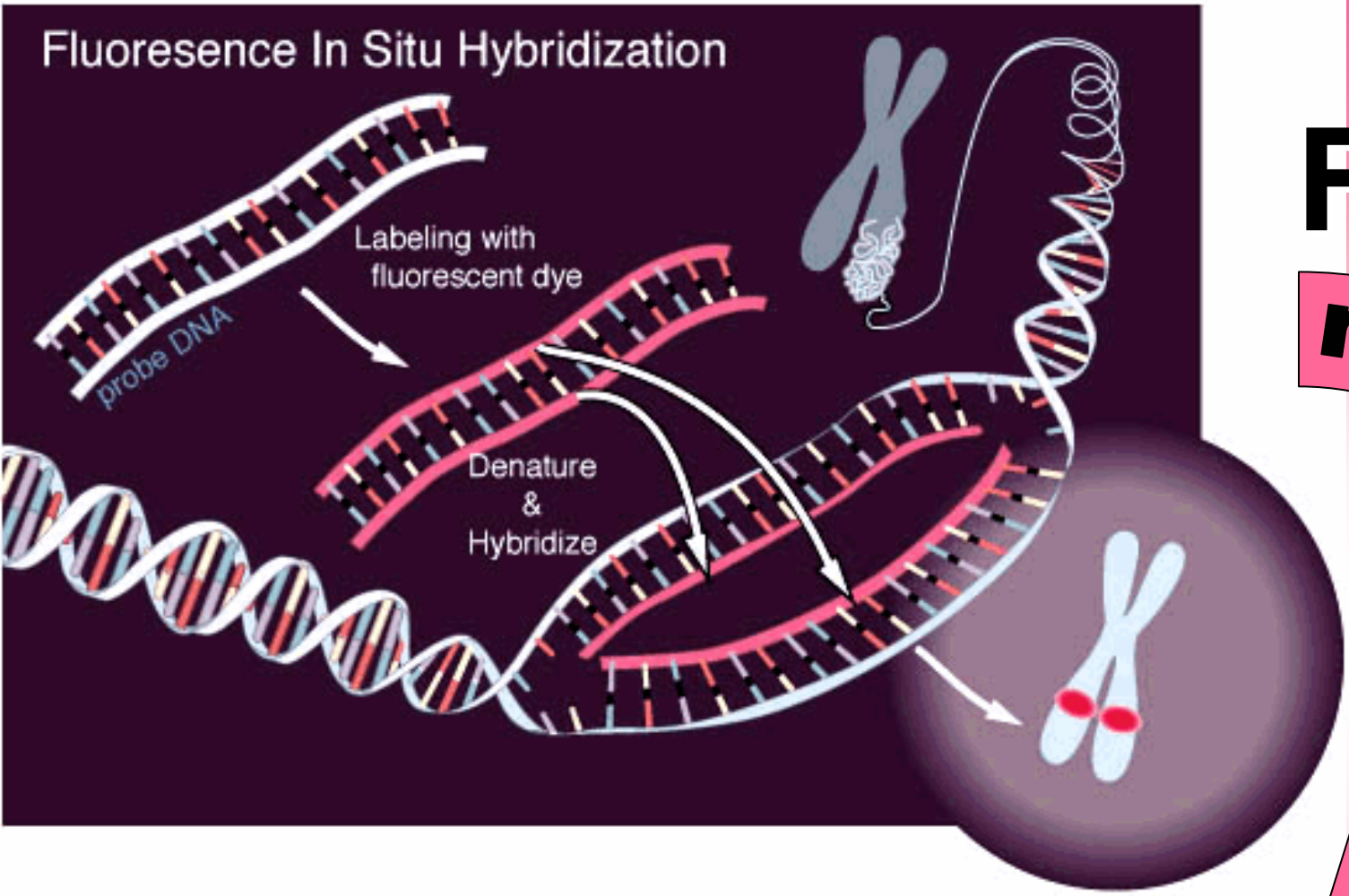

FISH

methodology

1. Making DNA probe complementary to known sequence
2. Labeling the probe with fluorescent marker
3. Denaturizing both, the probe and the sampling DNA: mix, hybridize
4. Wash of excess probe that did not bind to tested chromosome
5. Sample DNA is tested for presence or absence of the fluorescent signal

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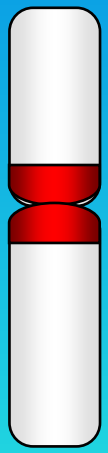
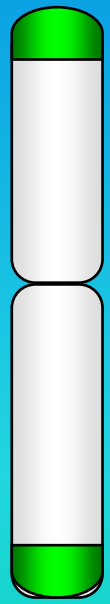
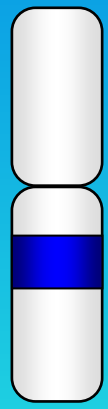
FISH PROBES



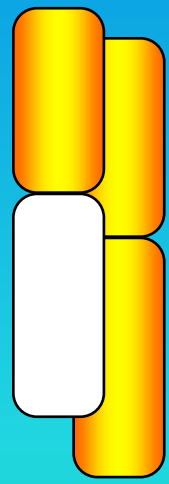
centromeric



telomeric



locus specific



chromosome arm or whole chromosome painting

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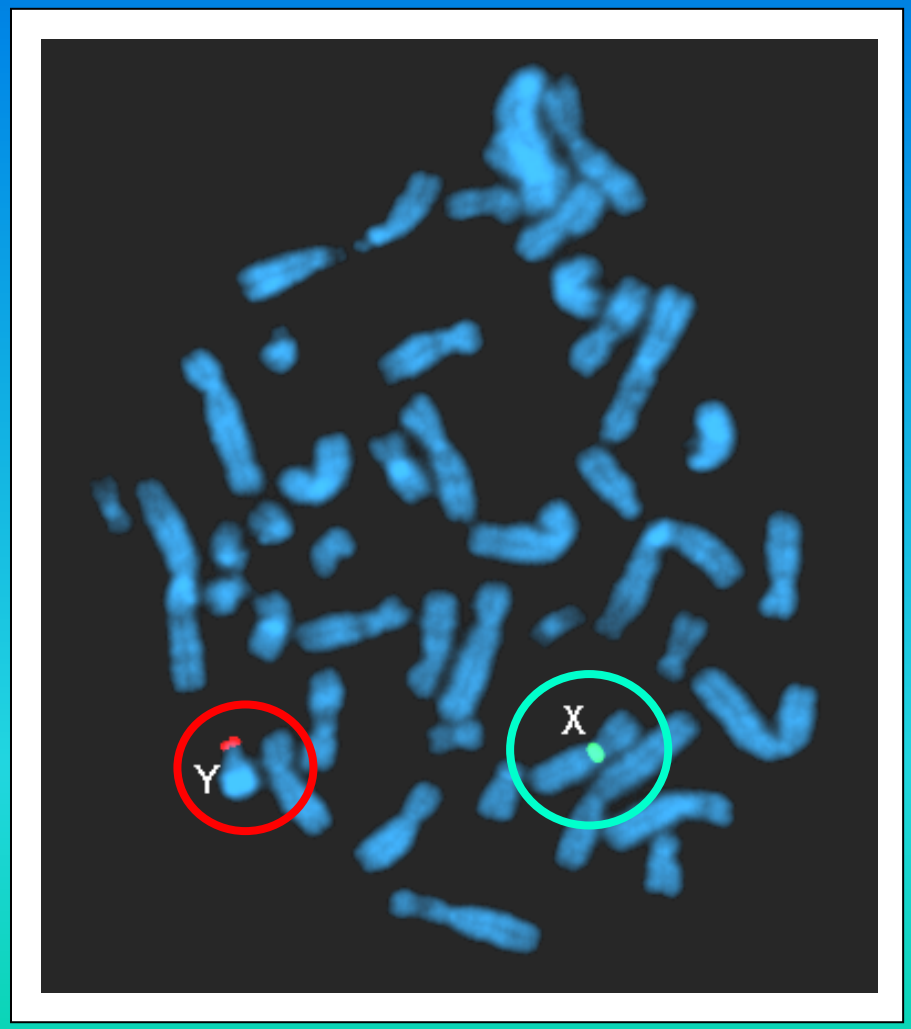
Centromer specific probe



Centromers of chromosome “X” and “Y” are labeled

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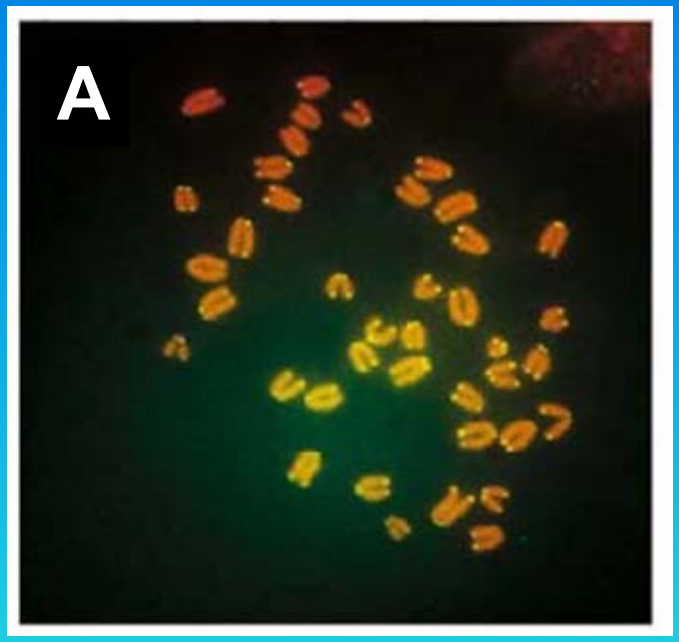
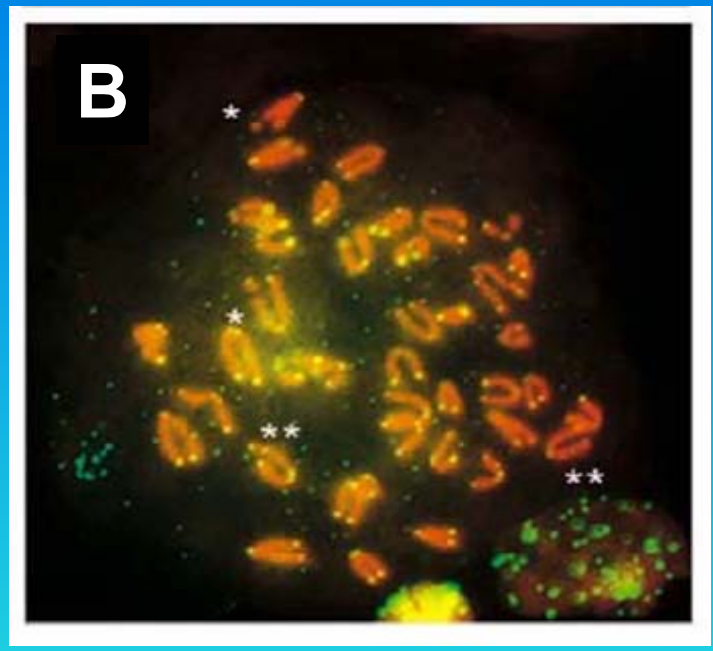
Telomere specific FISH probe





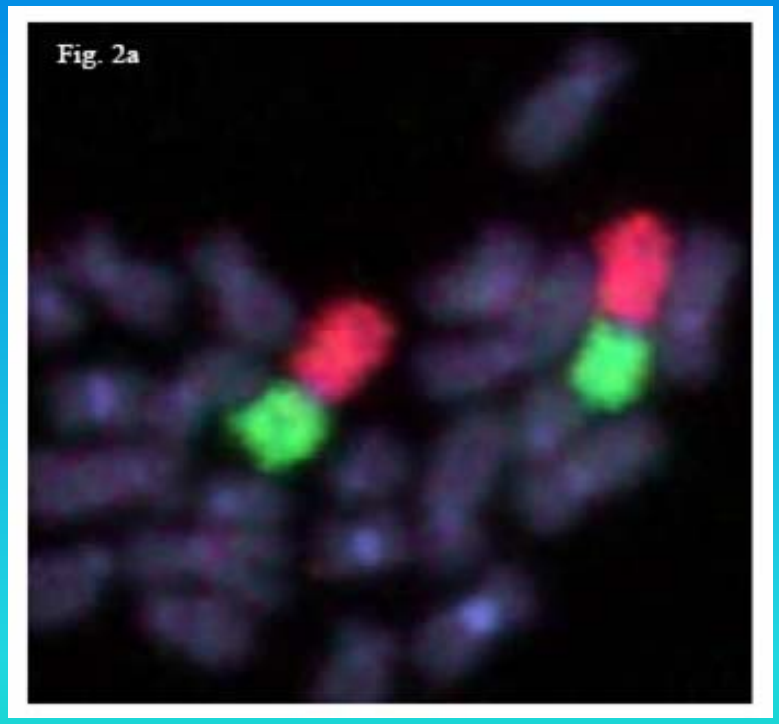
A Intact murine fibroblasts. All telomeres are associated with chromosomes

B Abundant signals obtained with probe that are not associate with chromosomes

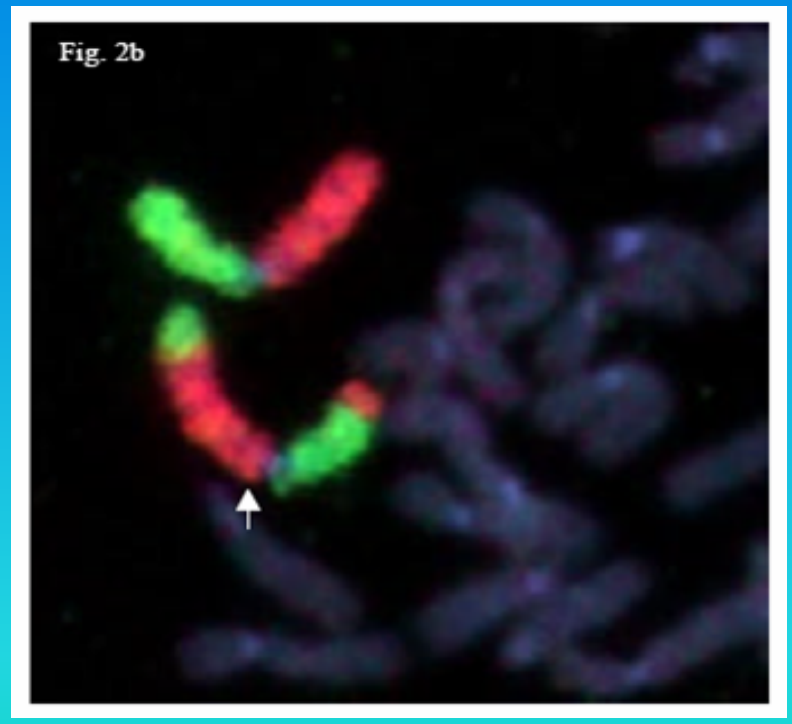
* points to chromatid breaks

Two color FISH

The p-arm is painted in green, the q-arm in read



Metaphase spread
of a normal human fibroblasts



Complete pericentric inversion
in a metaphase spread from
irradiated human fibroblasts

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ADVANTAGE of using FISH:

can be applied to

- **metaphase or non-mitotic interphase nuclei**
- **fresh or fixed archived tissues**

Clinical aplication of FISH

- characterization of structural abnormalities
- aneuploidy analysis
- cancer specific chromosome aberrations

Other FISH-based techniques

- Multi-color FISH (M-FISH)
- Spectral Karyotyping (SKY)
- Multi-color banding FISH (mBAND FISH)
- Comparative Genomic Hybridization (CGH)

Traditional FISH technique

- **uses single or couple colors**
- **paints a small number of chromosomes or their parts**

For detection more complex aberration have been developed modifications:

M-FISH AND **SKY**

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M-FISH

Multicolor [multiplex]
Fluorescent In Situ Hybridization

SKY

Spectral Karyotyping

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Visualization of

- all chromosomes
- in different colors
- at the same time

M-FISH
SKY

DYES:

to paint 24 human autosomes are used 5 basic fluorochromes and their unique combination

PROBES:

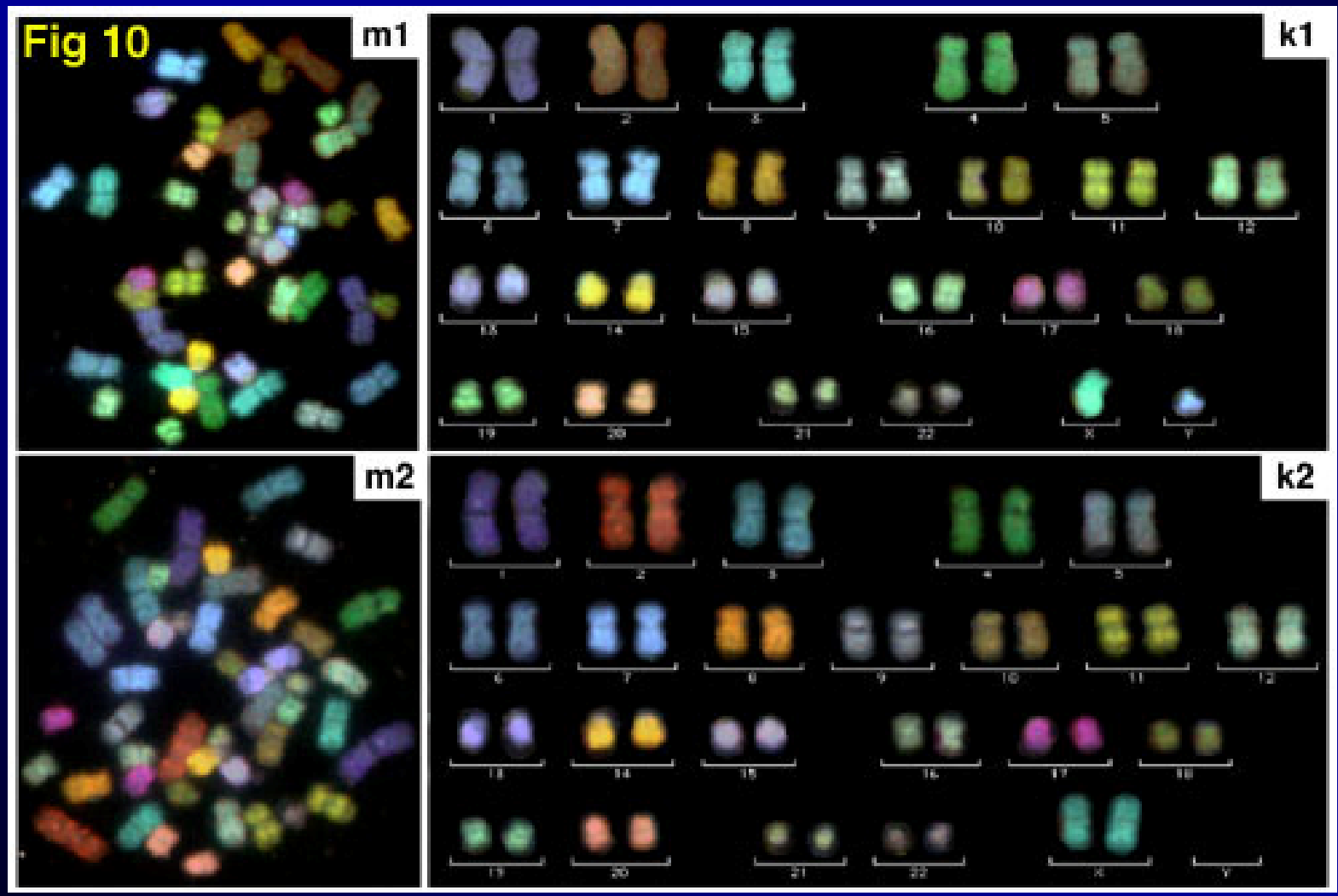
Are generating from flow-sorted chromosomes, than amplified and fluorescently labeled

READING

Microscope with designed filter set, computer analyzing
[SKY: single exposure; M-FISH: separate immages are combined by software]

M – FISH:

Normal metaphase spread [m] and karyotype [k]: male [1], female[2]



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mBAND-FISH

Multicolor BANDED Fluorescent In Situ Hybridization

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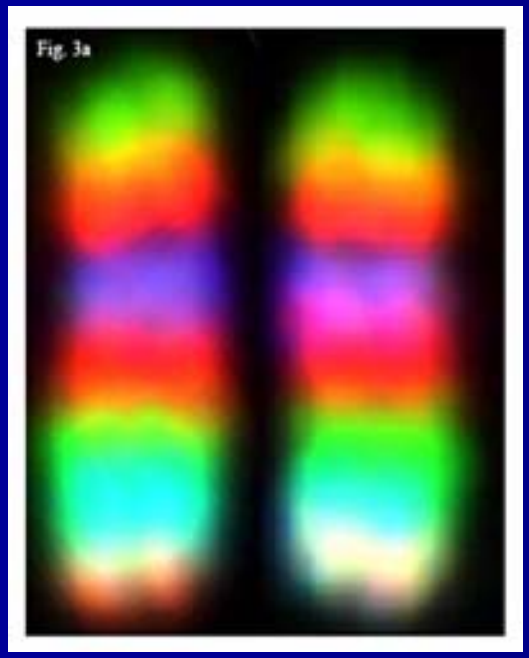
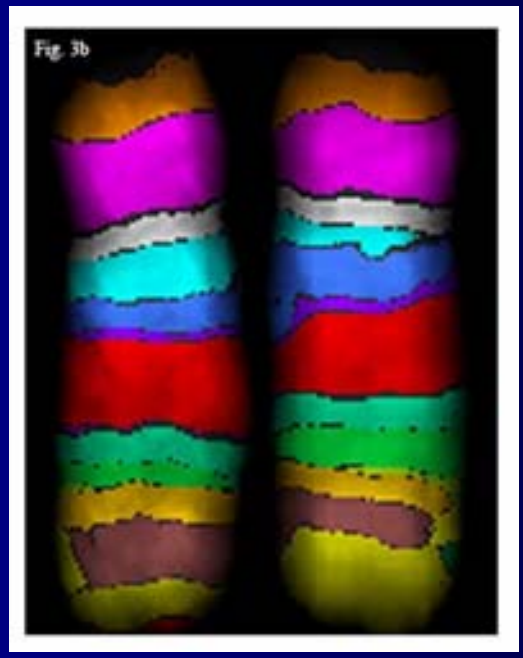
“CHROMOSOME BAR CODING”

mBAND-FISH

Sub regional chromosome painting probes are used to produce a colored banding pattern

The pattern appeared to be more precise than the conventional chromosome banding





Since is not possible to present all colors in the image, the software presents the same image in computer-generated pseudo colors.

Multicolor banding FISH of human chromosome 5

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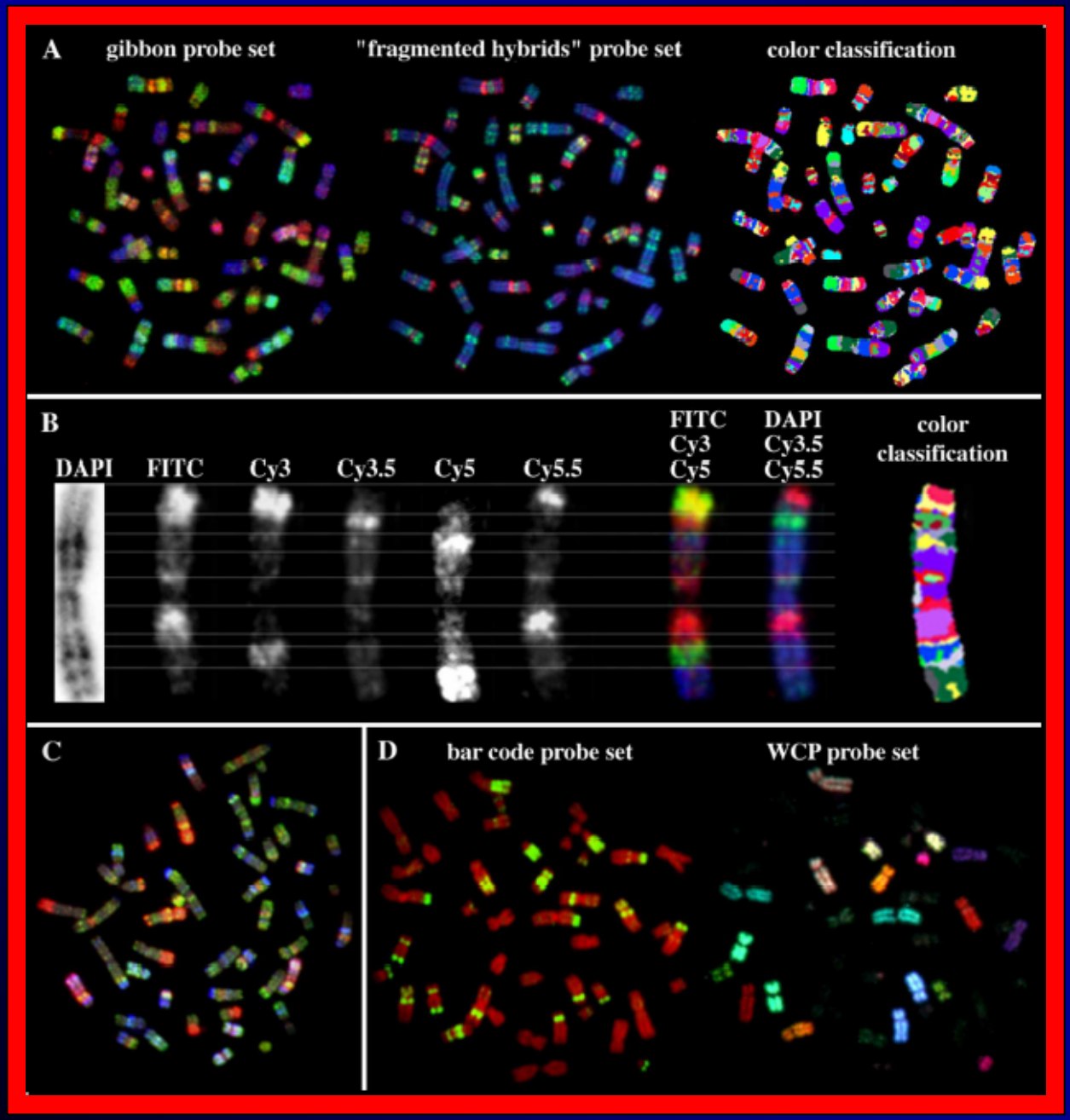

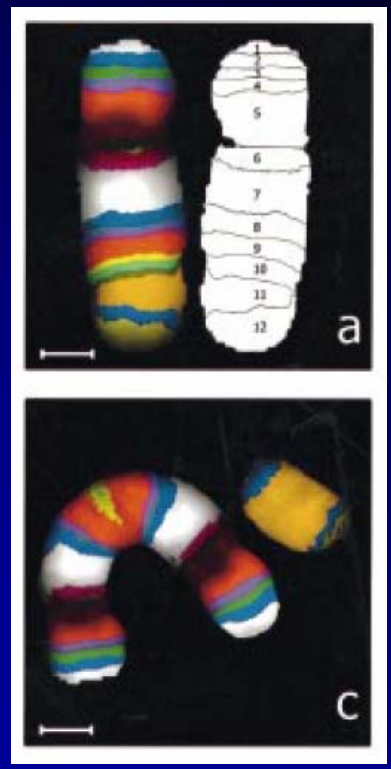

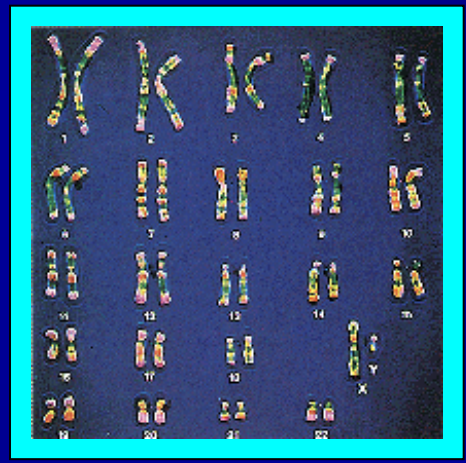

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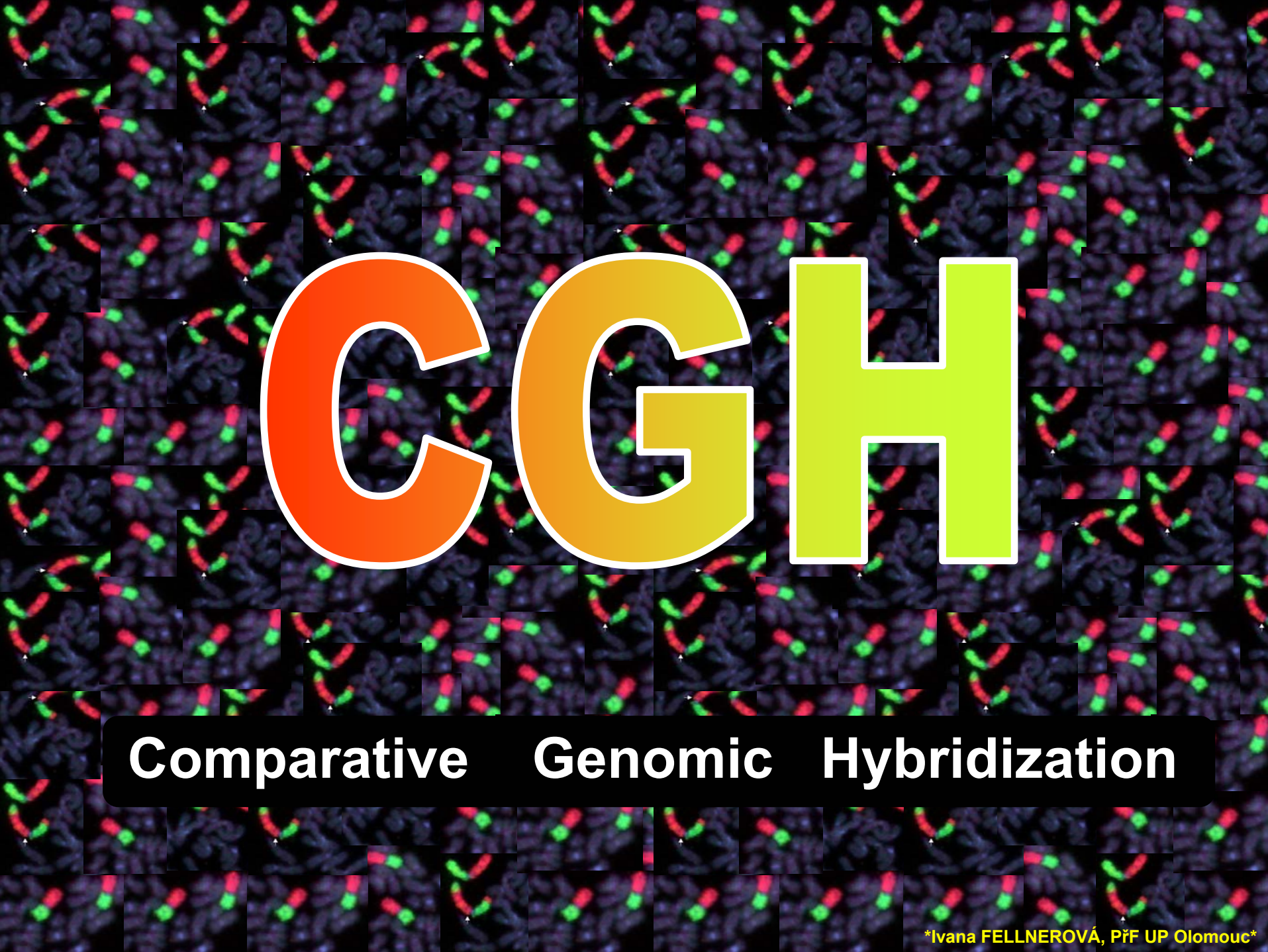

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Comparative genomic hybridization

CGH is fluorescent molecular cytogenetic technique based on quantitative two-color fluorescence in situ hybridization.

- Identifies chromosomal abnormalities where there is an:
  A) Net loss (deletion)
  B) Gain (duplication, insertion, amplification)

- Can be applied to fresh or frozen tissues, cell lines, and archival samples.

CGH methodology

control/normal DNA

Equal amounts of normal and tumor DNA are mixed together

test/patient DNA

Normal DNA

Tumor DNA

Both labeled DNAs add to normal metaphase chromosomes

FISH

Image capture

analysis the red and green ratio

LOSS

of material in the test DNA: region appears

RED

GAIN

of material in the test DNA: region appears

GREEN

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Molecular cytogenetic techniques

Powerful adjuncts to conventional cytogenetic analysis of:

- numerical aberation [aneuploidy]
- structural rearrangements
- submicroscopic rearrangements
  - microdeletions/duplications
  - subtelomere rearrangements

Sources / Links

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