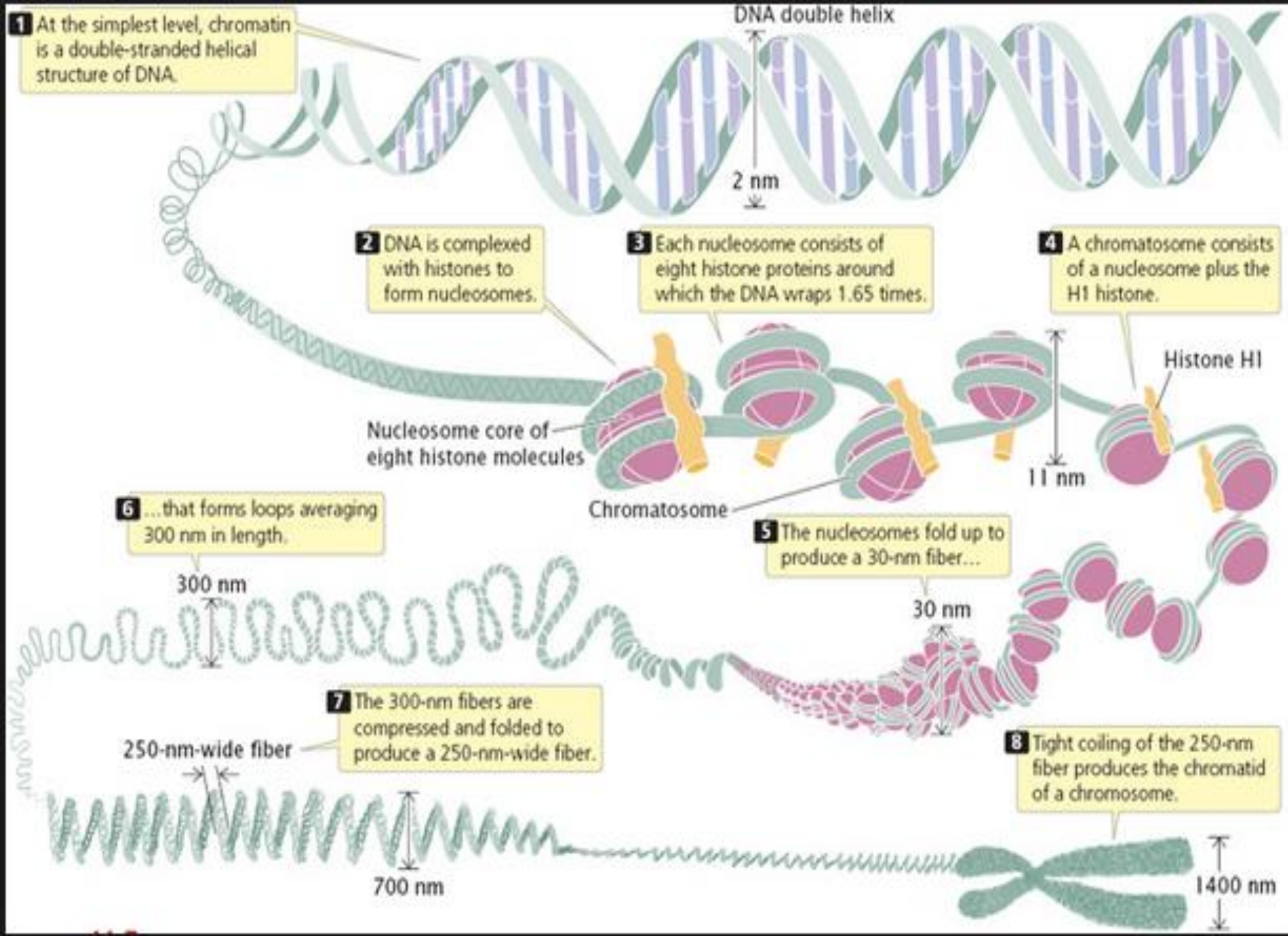
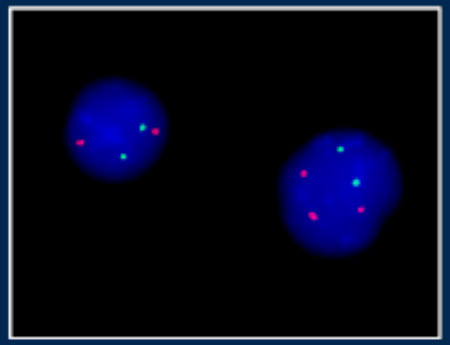
Chromosome Bands and In Situ Hybridisations

Dr.Rasime Kalkan

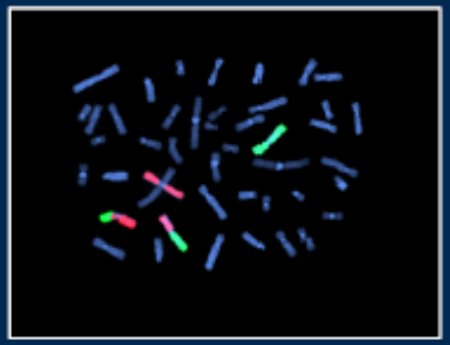

1 At the simplest level, chromatin is a double-stranded helical structure of DNA.
DNA double helix
2 nm
2 DNA is complexed with histones to form nucleosomes.
3 Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.
4 A chromatosome consists of a nucleosome plus the H1 histone.
Histone H1
Nucleosome core of eight histone molecules
11 nm
Chromatosome
6 ...that forms loops averaging 300 nm in length.
300 nm
5 The nucleosomes fold up to produce a 30-nm fiber...
30 nm
7 The 300-nm fibers are compressed and folded to produce a 250-nm-wide fiber.
250-nm-wide fiber
8 Tight coiling of the 250-nm fiber produces the chromatid of a chromosome.
700 nm
1400 nm

Methods

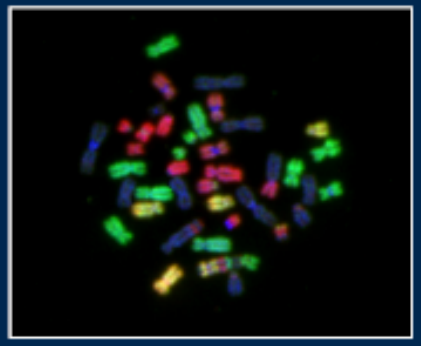
Conventional cytogenetic analysis



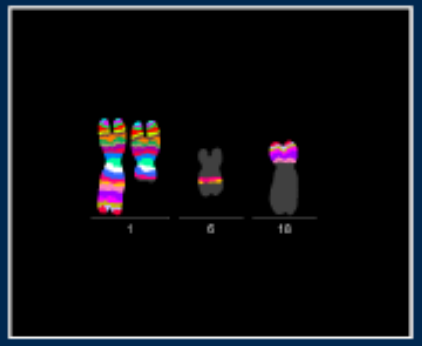
I-FISH



WCP-FISH



mFISH

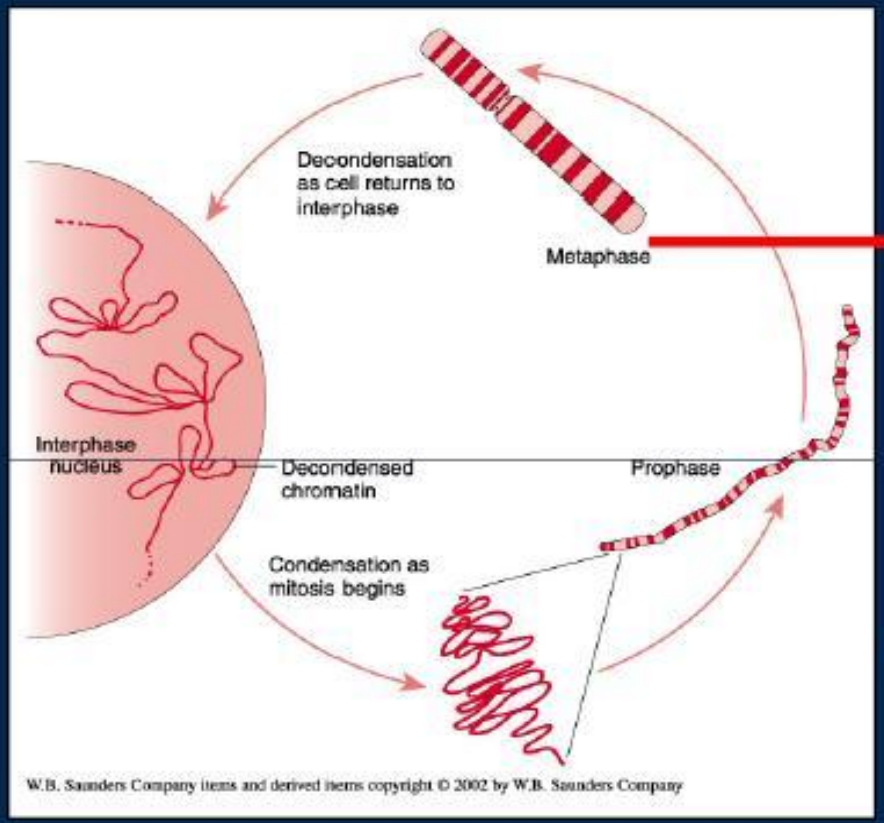


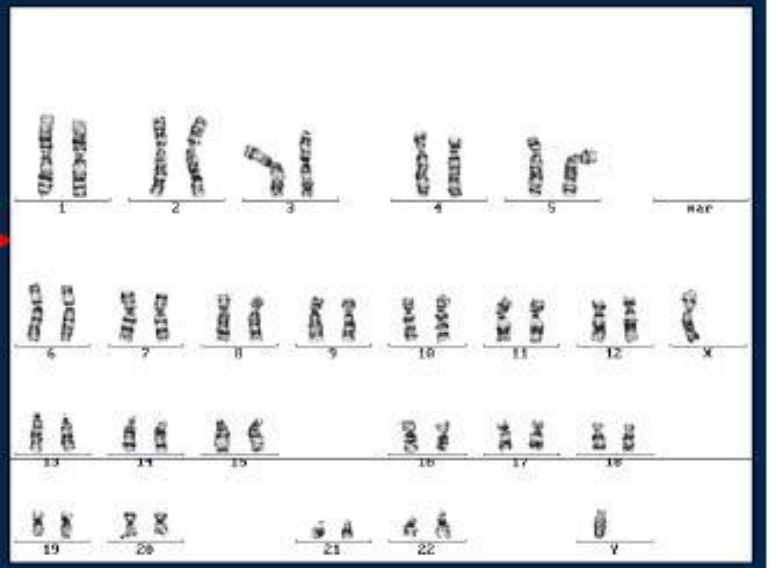
mBAND

CGH

Array CGH

Conventional cytogenetic analysis

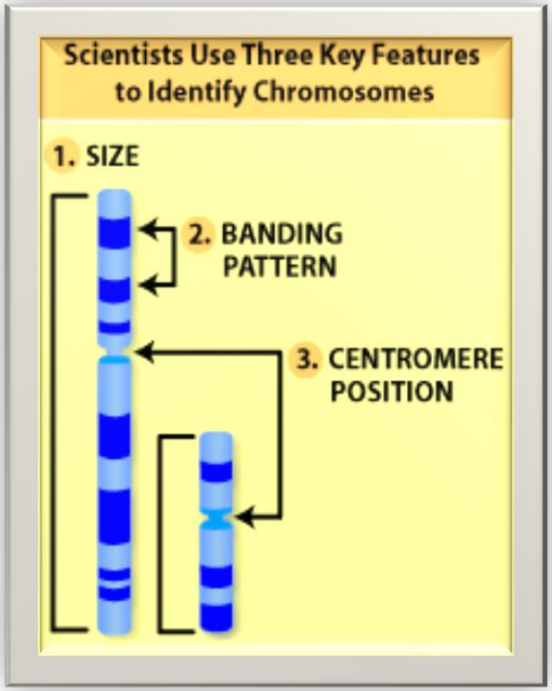


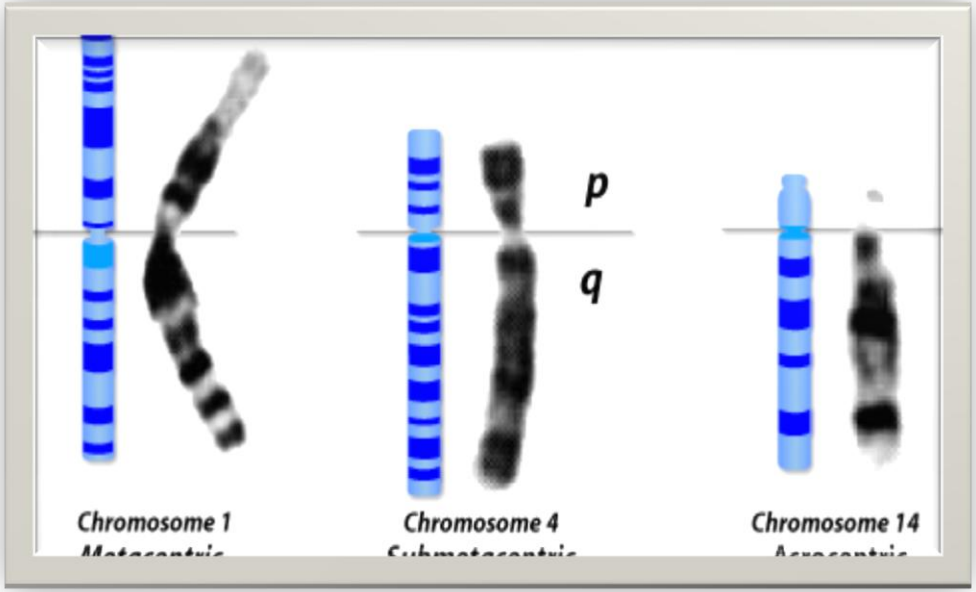


- Karyotype analysis
- Detection of chromosomal aberrations (numerical x structural)

Chromosomes can be differentiated by their characteristics such as size, position of the centromere and banding pattern.

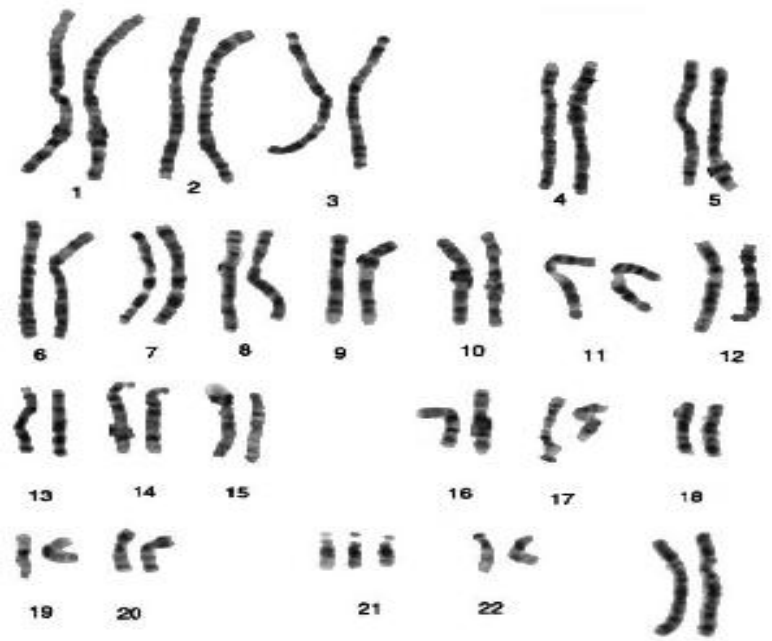
The resolution of this map is about one chromosome band that corresponds to a size of 5-10 Mega base pairs (Mbp).

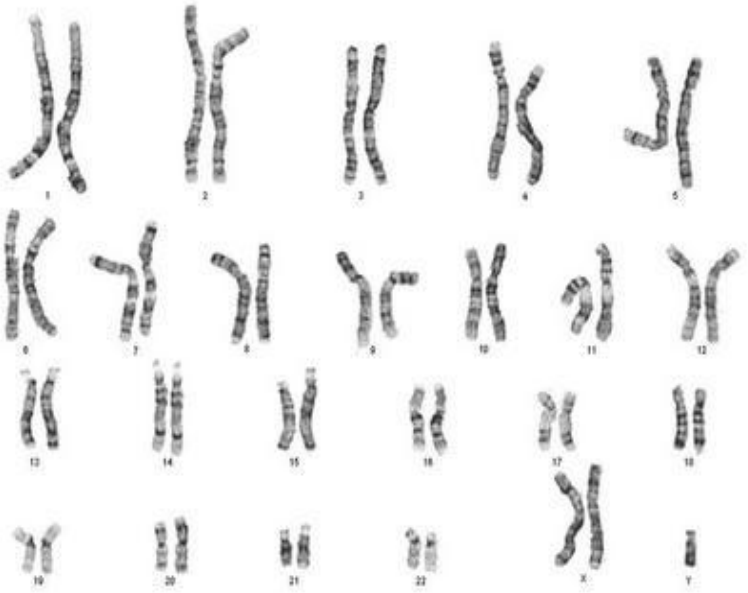




Visualizing Metaphase Chromosomes (Banding)

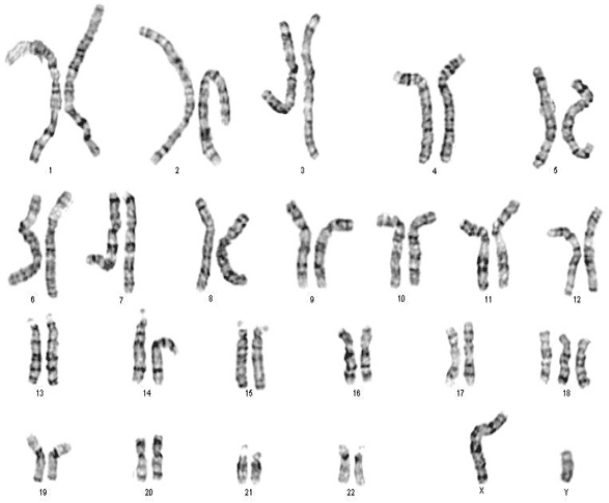
Positive G – bands	Negative G – bands
Positive Q – bands	negative Q – bands
Negative R – band	positive R bands
Early condensation	late condensation
Late replicating DNA	early replicating DNA
Tissue specific genes	house keeping genes
A+T rich region	G+C rich region

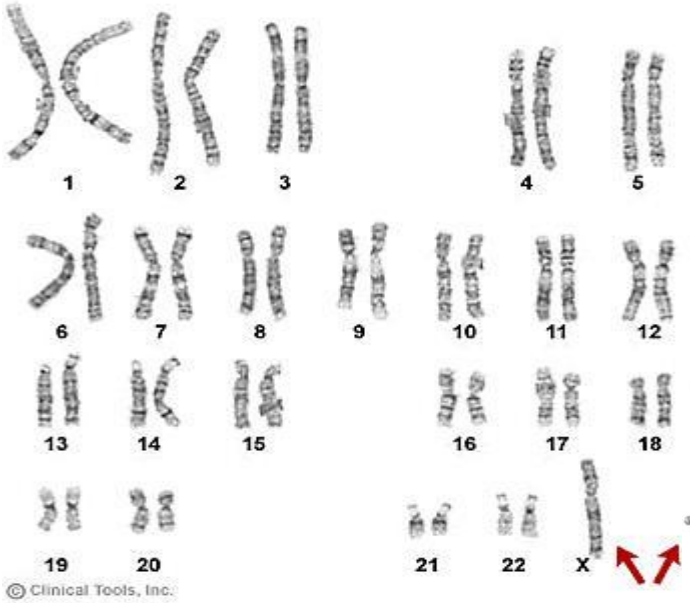




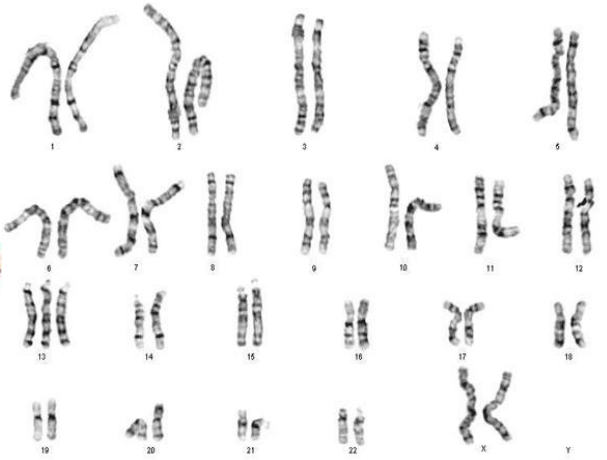
Klinefelter's syndrome

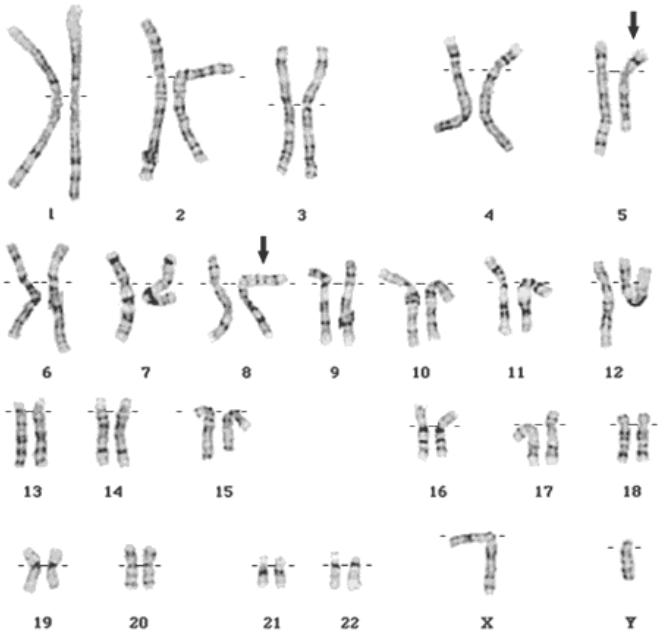
Numerical abnormalities

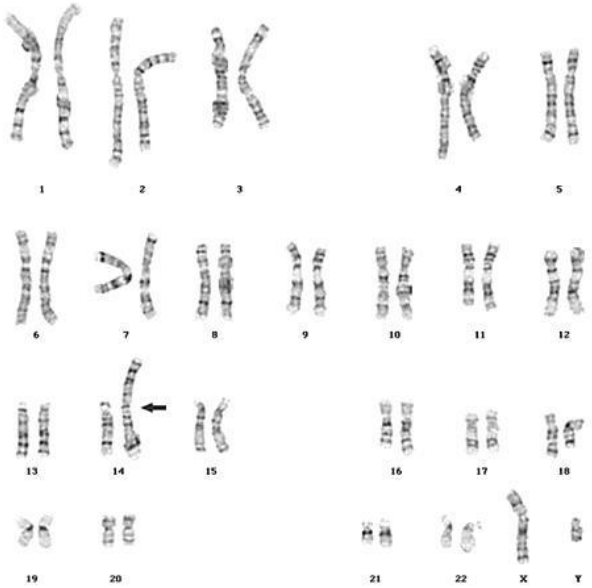




Turner syndrome (45,X)

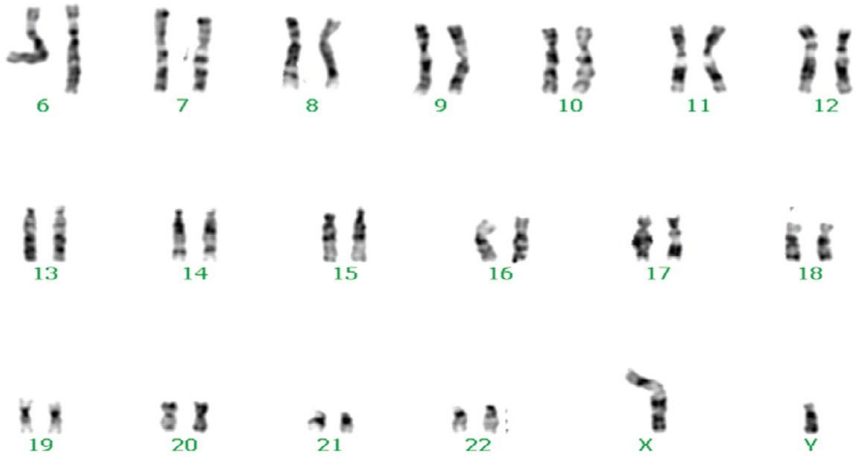




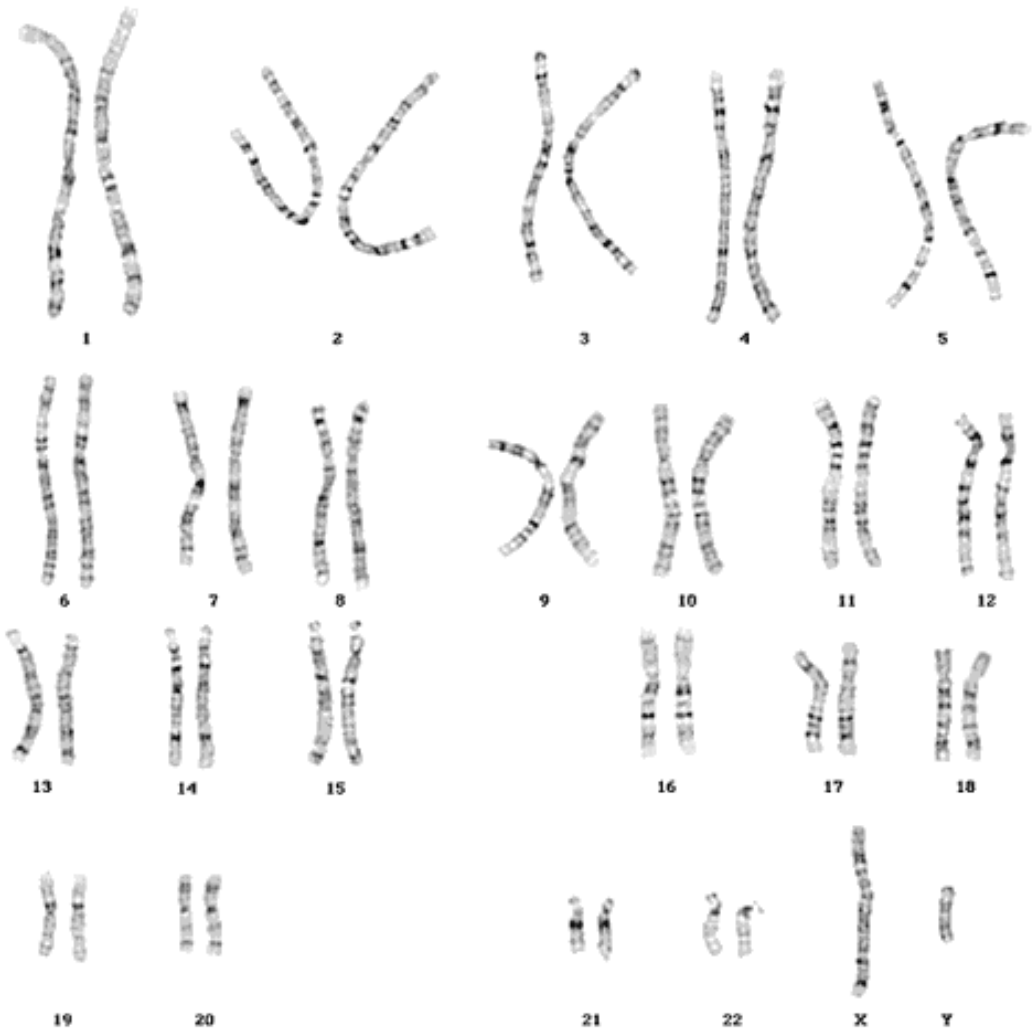


46,XY,+13,dic(13;14)(p11.2;p11.2).

Structural aberratio



High resolution karyotype



Advantages

- "Whole genome scan"
- Relative low cost

Disadvantages

- Labor intensive
- Detection above 5 Mb

Limitations of classical cytogenetics

sensitivity of chromosomal banding techniques is limited

these techniques require a high rate of dividing cells with good chromosomal morphology (resolution limit of 6 Mb)

Advantages and Disadvantages of conventional cytogenetic technique

Advantages

- Enable the entire genome to be viewed at one time.
- Suitable when a specific anomaly is suspected (e.g. Philadelphia in CML) and as a general diagnostic tool to detect additional chr. Abnormalities commonly seen in disease progression of CML.

Disadvantages

- Detect major structural abnormalities (one band = 6mb of DNA ~ 150 genes).
- Labor intensive and highly dependent upon operator experience and skills.

Indications for chromosomal analysis

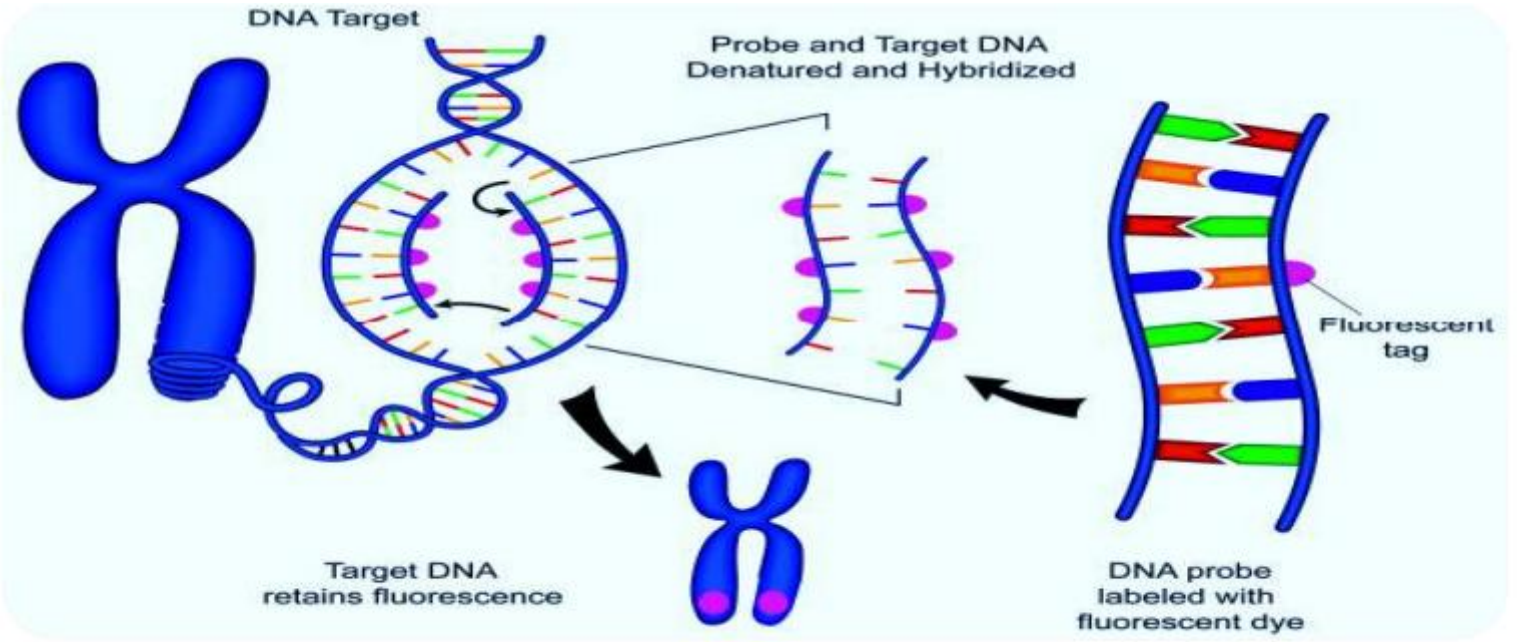
- Suspicion to concrete chromosomal abnormality (concrete syndrome)
- Multiple congenital anomalies or developmental delay
- Mental retardation
- Gonadal dysgenesis
- Infertility
- Miscarriages
- Delivery of dead fetus or death of a newborn child
- Occurrence of certain malignancies

In which conditions we have to indicate FISH analysis?

- The material doesn't contain metaphase chromosomes
  - Unsuccessful cultivation
  - It isn't possible to cultivate the tissue from patient (preimplantation analysis, rapid prenatal examinations, examinations of solid tumors or autopsy material)
- Analysis of complicated chromosomal rearrangements
- Identification of marker chromosomes
- Diagnosis of submicroscopic (cryptic) chromosomal rearrangements
  - Microdeletion syndromes
  - Amplification of oncogenes and microdeletion of tumor-suppressor genes in malignancies

Fluorescence in situ hybridization (FISH)

- In situ hybridization is the method of localizing/ detecting specific nucleotide sequences in morphologically preserved tissue sections or cell preparations by hybridizing the complementary strand of a nucleotide probe against the sequence of interest.



Fluorescence in situ hybridization (FISH)

- In general, in situ techniques are used within the area of diagnostics in order to demonstrate abnormalities in gross organization or in the localization of endogenous or exogenous DNA or RNA molecules that are causing—or are at least associated with—human disease.

Fluorescence in situ hybridization (FISH)

permits detection of selected acquired genetic changes in dividing (metaphase) and nondividing (interphase nuclei) cells

ISH is the only method that can simultaneously give information at both molecular and cellular levels, namely by visualizing DNA sequences on chromosomes and in cells and tissue sections, thereby enabling specific nucleic acid sequences to be visualized in their natural biological microenvironment.

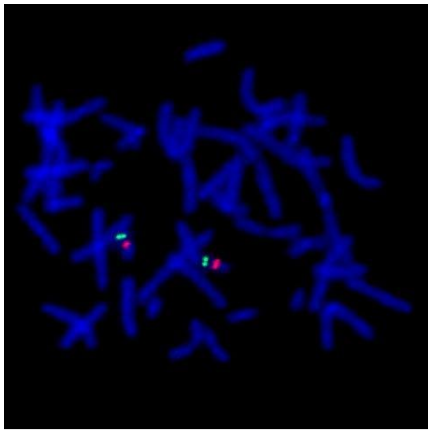
FISH studies are used to investigate the origin and progression of hematologic malignancies and to establish which hematopoietic compartments are involved in neoplastic processes

FISH Analysis

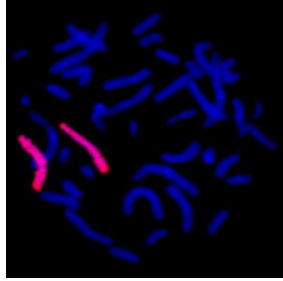
- Advantages
  - Highly specific (100 kb)
  - Microdeletions/Microduplications
- Disadvantages
  - Highly specific
  - 500-600 probes needed to match the power of karyotyping

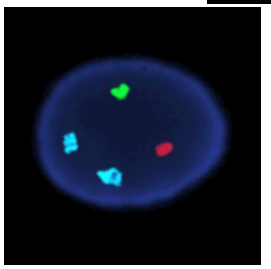
The target

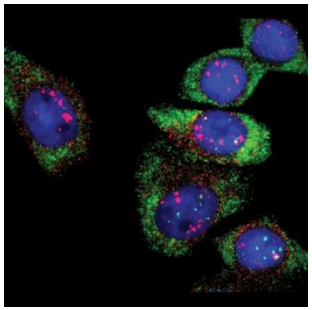
- Metaphase chromosomes
- Interphase nuclei
- Extended chromatin fibers
- Entire Cells/RNA
- Tissue sections

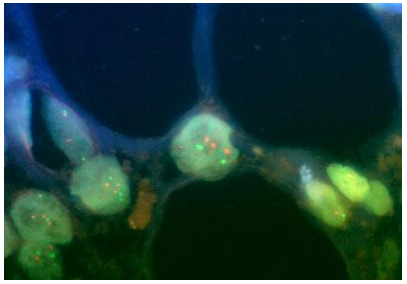


DiGeorge/Velo-Cardio-Facial/CATCH 22/Shprintzen Syndrome

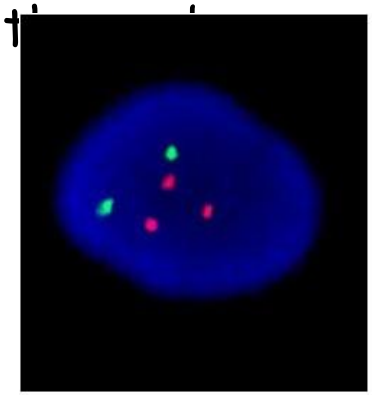


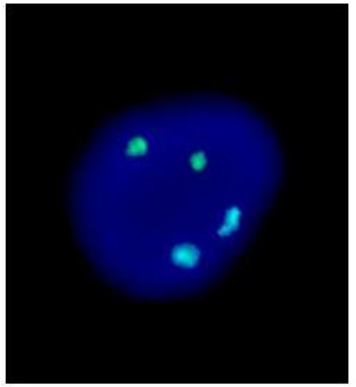






- FISH experiments can be performed with any DNA/RNA probe of sufficient size and incorporation of label to cell components that contain sequences complementary to t





Advantages of Interphase FISH

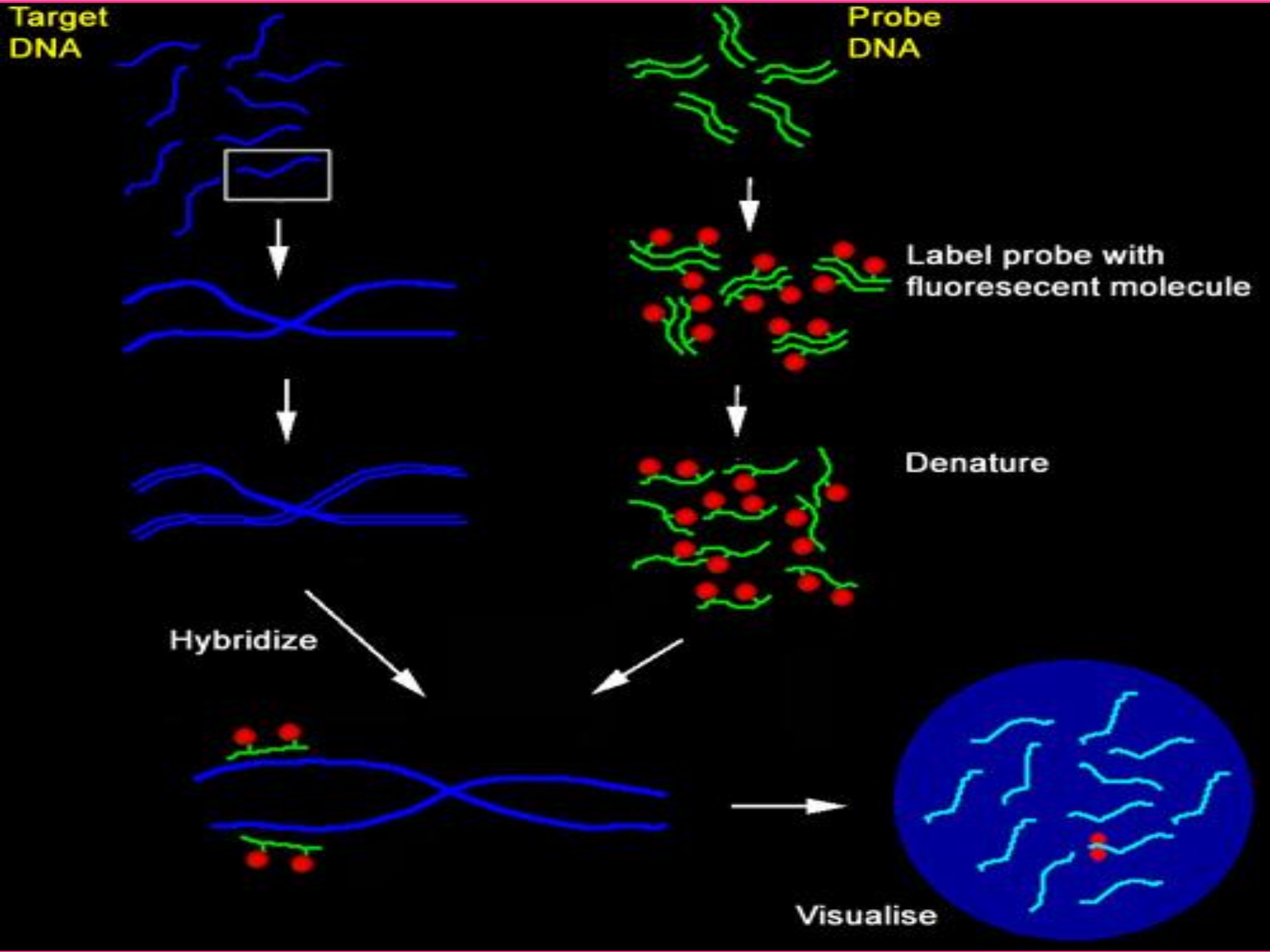
- Interphase cells for FISH do not require culturing of the cells and stimulating division to get metaphase spreads
  - interphase FISH is faster than methods using metaphase cells
  - valuable for analysis of cells that do not divide well in culture, including fixed cells.
- 200–500 cells can be analyzed microscopically using FISH
  - the sensitivity of detection is higher than that of metaphase procedures, which commonly examine 20 spreads.
- Monitor recurrent or residual disease in BMT pt.

Applications of Molecular cytogenetics

- Chromosome Identification
- Aneuploidy Detection
- Centromere Analysis
- Identification of Marker Chromosome
- Whole Chromosome Analysis (chromosome painting)
- Analysis of chromosome translocation
- Detection of unique sequence (single-copy sequence)
- Microdeletion investigation
- Analysis of gene amplification

FISH PROCEDURE

- Denature the chromosomes
- Denature the probe
- Hybridization
- Fluoresence staining
- Examine slides or store in the dark


Target DNA
Probe DNA
Label probe with fluoresecent molecule
Denature
Hybridize
Visualise

Uses of Fluorescent in situ Hybridization (FISH)

Identification and characterization of numerical and structural chromosome abnormalities.

Detection of microscopically invisible deletions.

Detection of sub-telomeric aberrations.

Prenatal diagnosis of the common aneuploidies (interphase FISH).

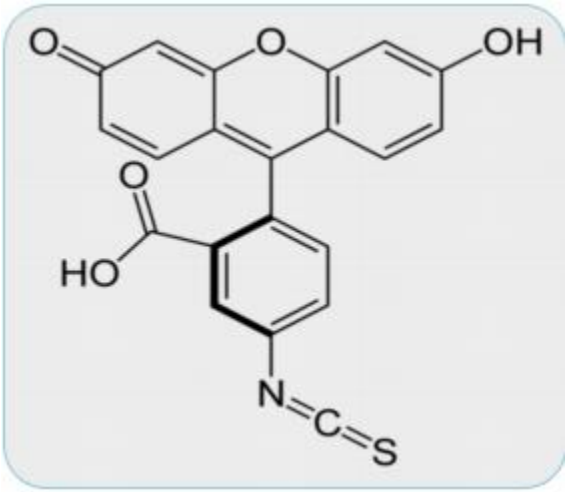
Limitations of FISH

- The inability to identify chromosomal changes other than those at the specific binding region of the probe.
- Preparation of the sample is critical in interphase FISH analysis
  - to permeabilize the cells for optimal probe target interaction
  - to maintain cell morphology.
- Cannot detect small mutations.
- Miss Uniparental disomy.
- Miss Inversions.
- Probes are not yet commercially available for all chromosomal regions
- Relativelly expensive

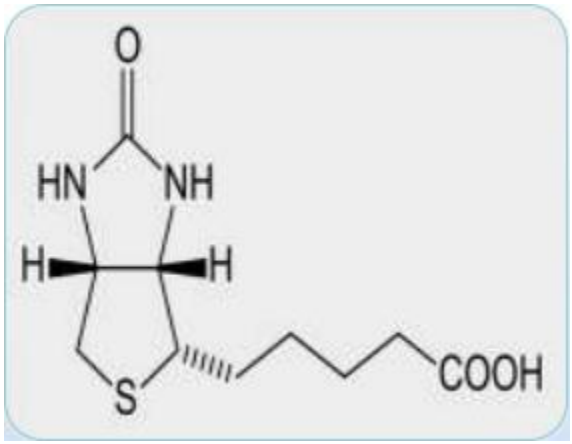

One fish
two fish
red fish
blue fish

Probes

Fluorescein



Biotin

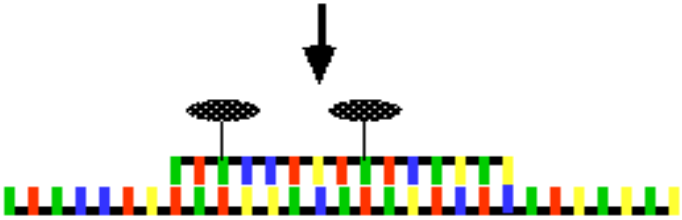


- Complementary sequences of target nucleic acids
- Designed against the sequence of interest
- Probes are tagged with fluorescent dyes like biotin, fluorescein, Digoxigenin
- Size ranges from 20-40 bp to 1000bp

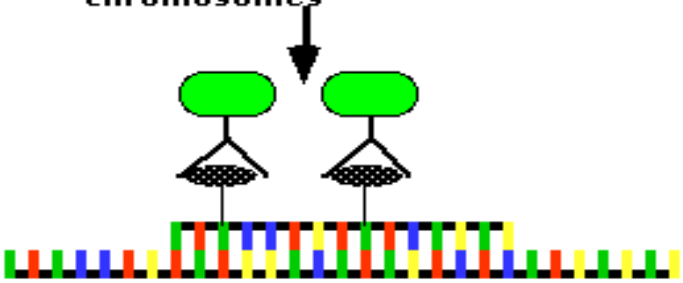
Indirect Label

Single stranded probe labeled with a non-fluorescent hapten

Single stranded DNA from denaturated chromosomes



Hybridization of probe to chromosomes

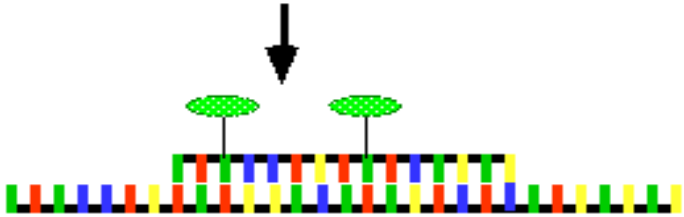


Applying fluorescent labeled antibody against hapten, visualization at the microscope

Direct Label

Single stranded probe labeled with fluorescent dUTP

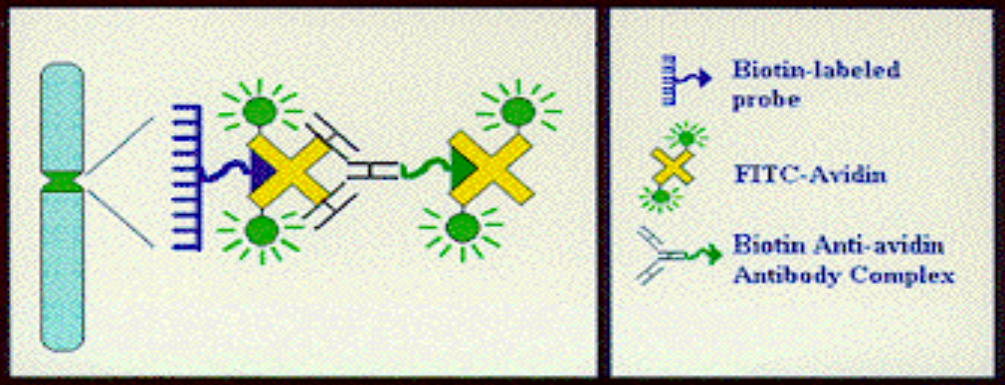
Single stranded DNA from denaturated chromosomes



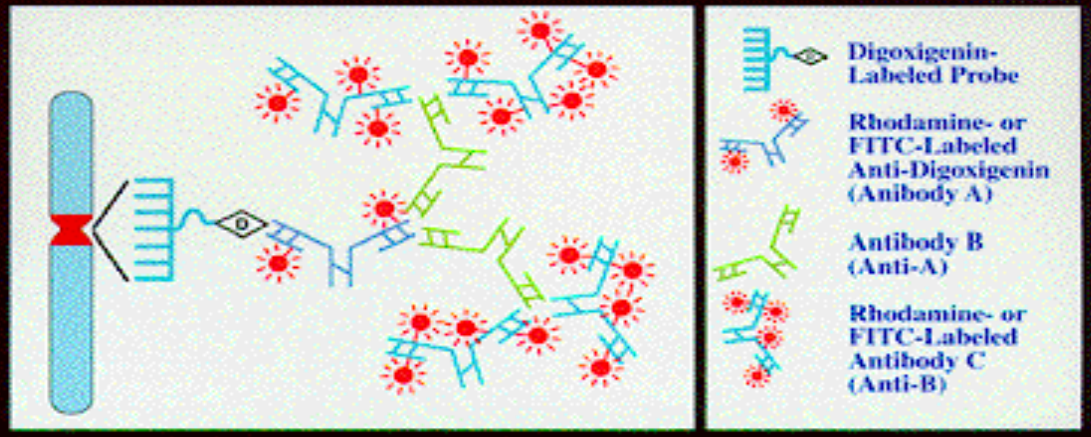
Hybridization of probe to chromosomes, direct visualization at the microscope

FLUORESCENCE DETECTION

A BIOTIN-LABELED PROBES



B DIGOXIGENIN-LABELED PROBES



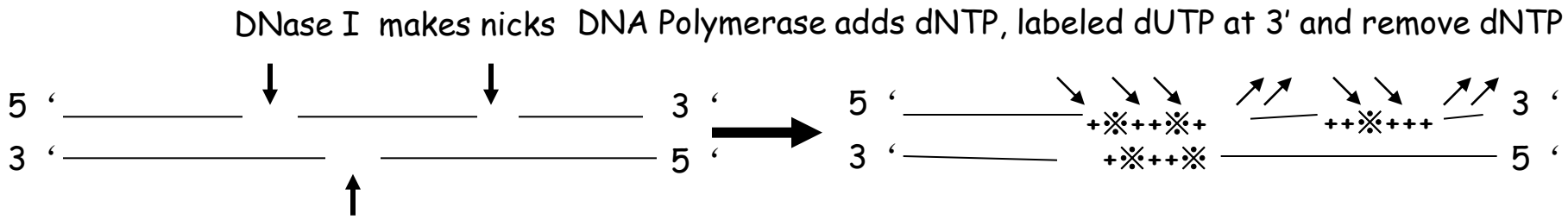
- Indirect Probe labeling, need antibodies to complete FISH procedure Haptens---Biotin-dUTP, digoxigenin-dUTP
- **Labeling techniques:**

  a) Nick translation

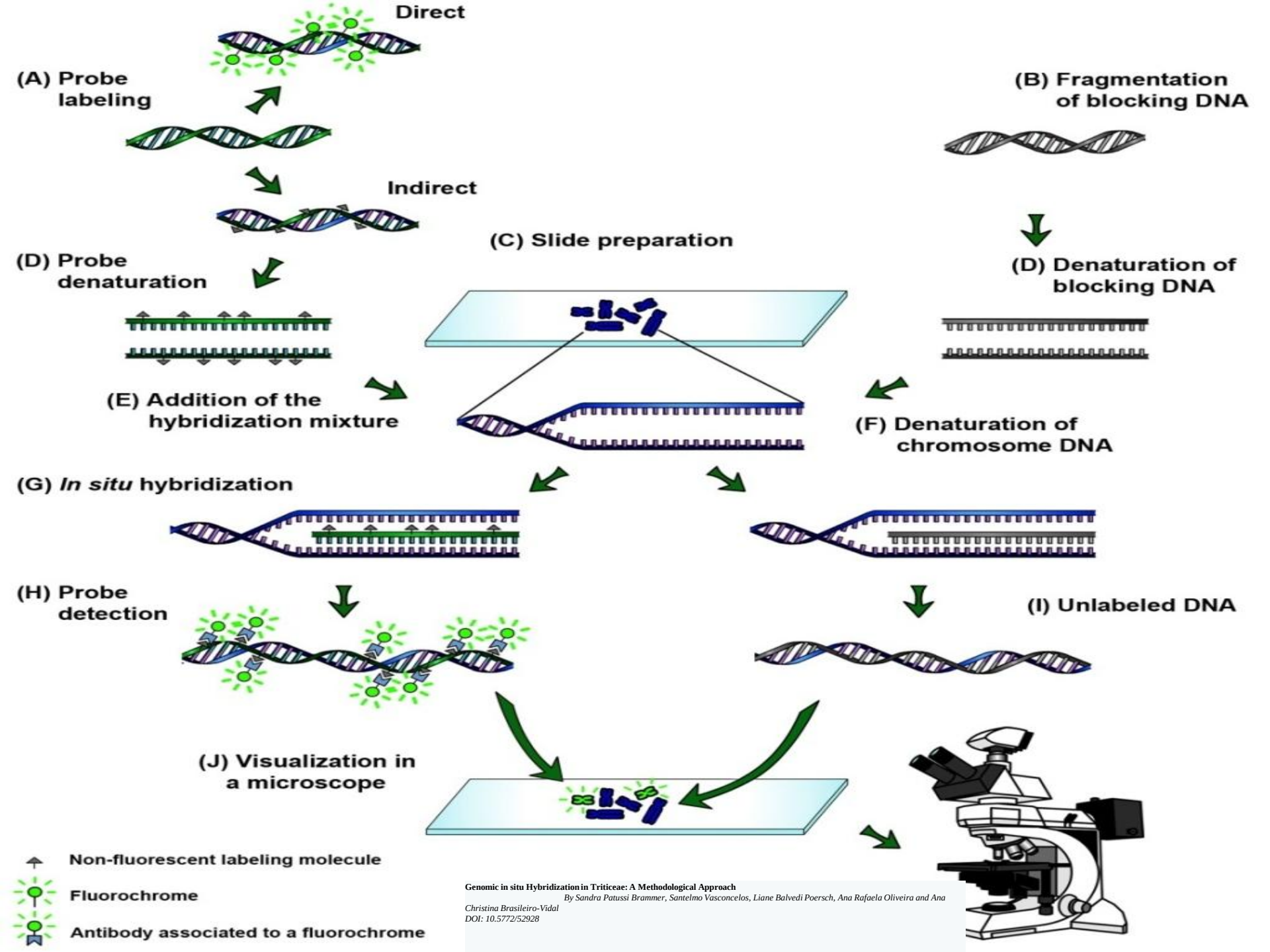
  b) Random priming

  c)PCR (Polymerase chain reaction)

- Direct Probe labeling, the probe directly labeled with fluorochromes such as SpectralGreen and SpectralOrange. **One-step hybridization.**



Principle of Nick Translation



Genomic in situ Hybridization in Triticeae: A Methodological Approach
By Sandra Patussi Brammer, Santelmo Vasconcelos, Liane Balvedi Poersch, Ana Rafaela Oliveira and Ana Christina Brasileiro-Vidal
DOI: 10.5772/52928

Denaturation → Stringency

Factors	Level	Stringency	Results (if inappropriate)
Temperature	High Low	High Low	Low efficiency High background
Concentration of salt (SSC) solution	High Low	Low High	High background Low efficiency
Concentration of formamide solution	High Low	High Low	Low efficiency High background

Types of Probes

Centromere probes

- Alpha and Satellite III probes
- Generated from repetitive sequences found in centromeres
- Centromere regions are stained brighter

Whole chromosome

- Collection of probes that bind to the whole length of chromosome
- Multiple probe labels are used
- Hybridize along the length of the chromosome

Telomere

Specific for telomeres
Specific to the 300 kb locus at the end of specific chromosome

Locus

Deletion
Translocation probes
Gene detection & localization probes Gene amplification probes

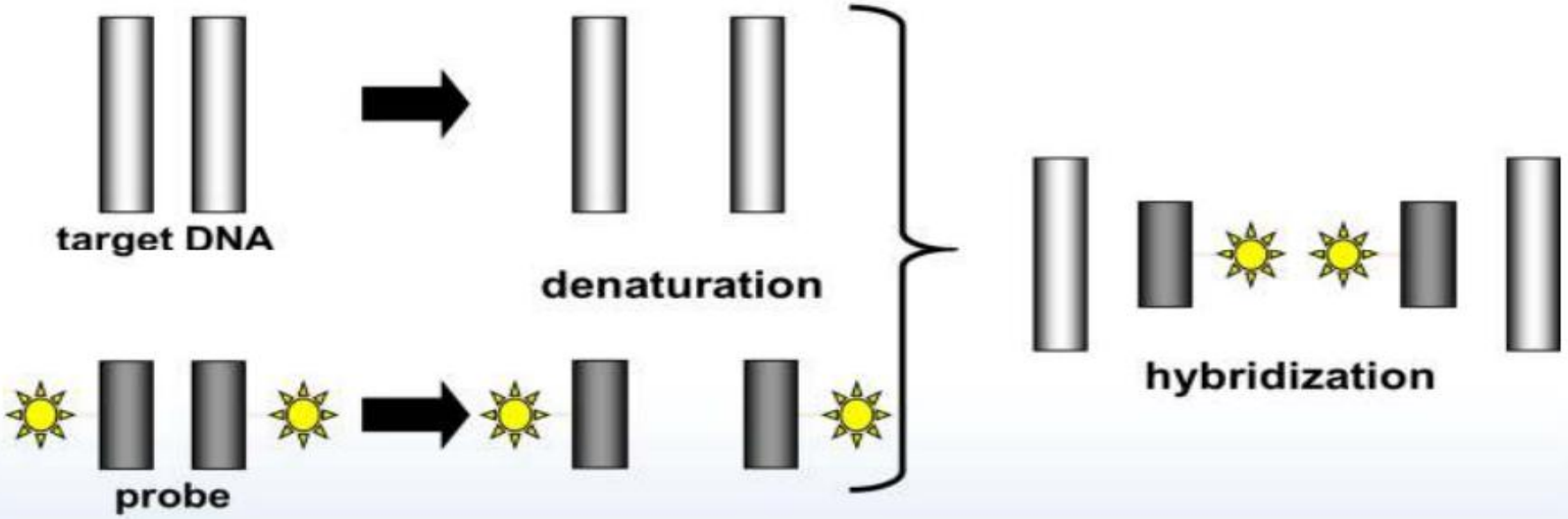
Denaturation & Hybridization

Denaturation

- Either by heat or alkaline method
- A prerequisite for the hybridization of probe and target

Hybridization

- Formation of duplex between two complementary nucleotide sequences
- Can be between
  - DNA-DNA
  - DNA-RNA
  - RNA-RNA



Detection & Visualization

Detection

- Direct labelling:
  - ➢ Label is bound to the probe
  - ➢ Less sensitive

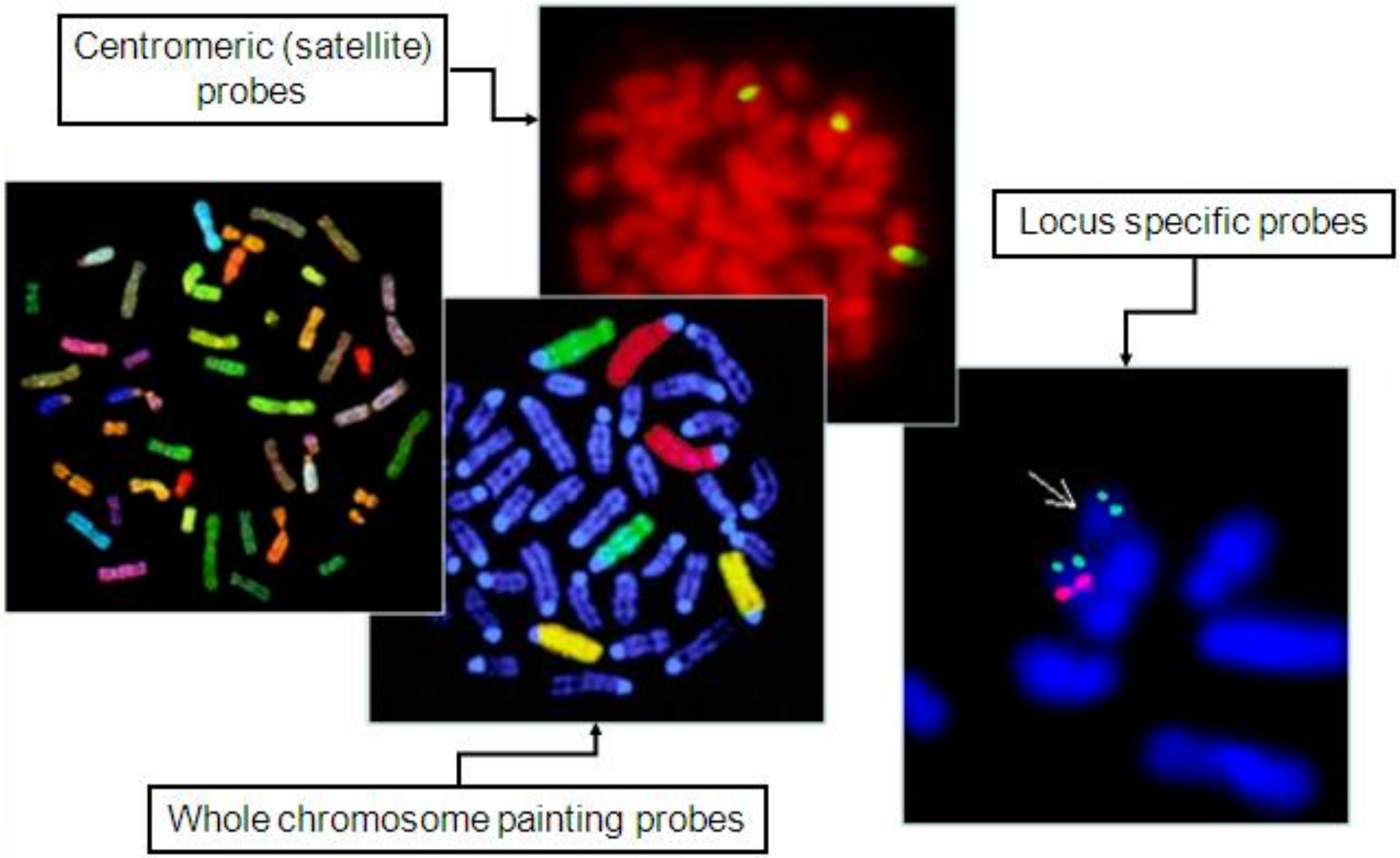
- Indirect labelling:
  - ➢ Require an additional step before detection
  - ➢ Probe detected using antibodies conjugated to labels like Alkaline phosphatase
  - ➢Results in amplification of signal

Hybridisation

- Fluorescent probe attaches to the target sequence during hybridization

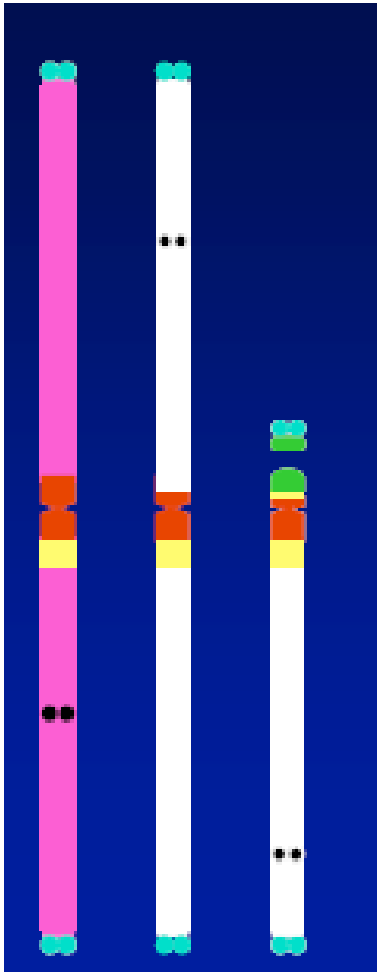
- This is visualized through a microscope with appropriate filters

Types of probes



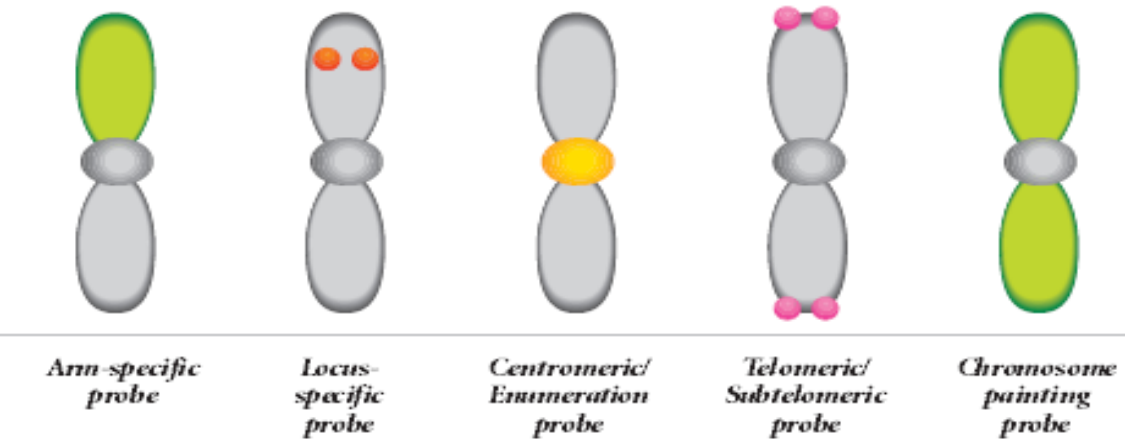
Classification of Chromosomal Sequences

- Beta satellite
- Alpha satellite
- Classical satellite
- Telomeric sequences
- Unique gene sequences



Probes for specific chromosomal structures

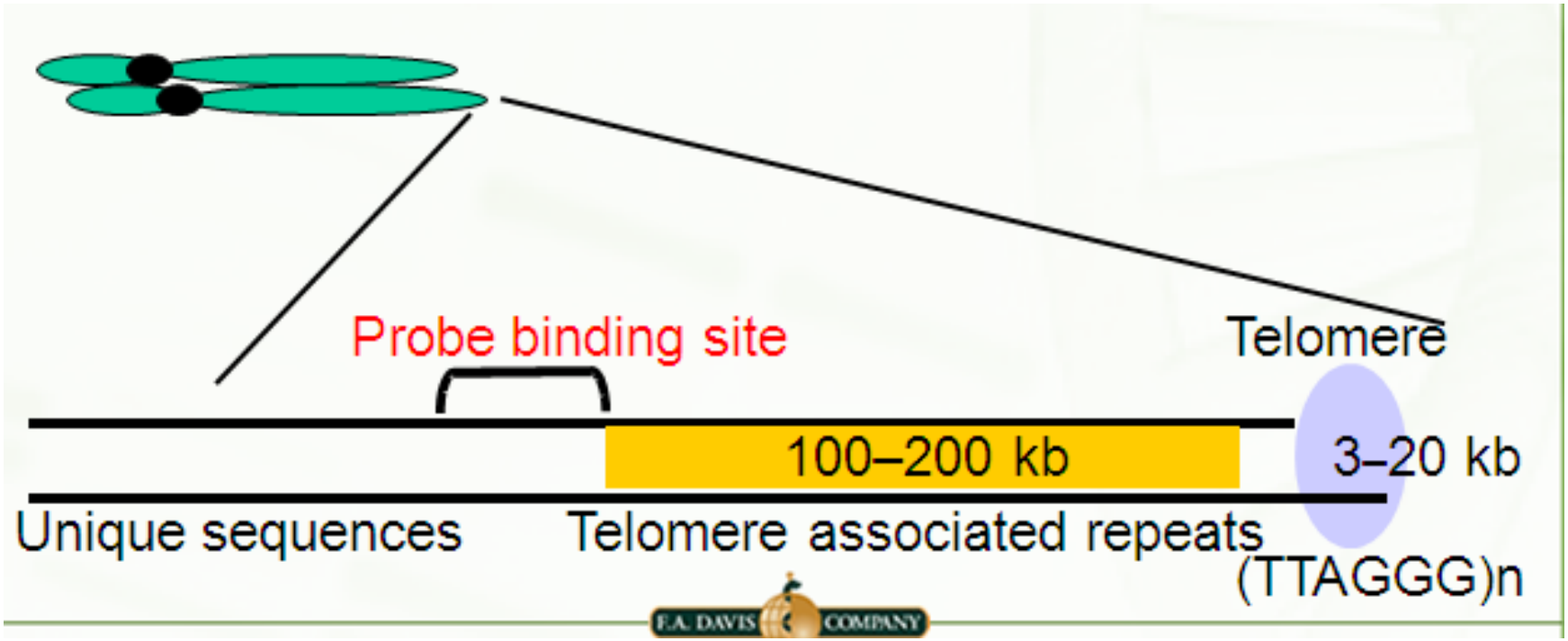
- Chromosome-specific centromere probes (CEP®)
  - Hybridize to centromere region
  - Detect aneuploidy in interphase and metaphase
- Chromosome painting probes (WCP)
  - Hybridize to whole chromosomes or regions
  - Characterize chromosomal structural changes in metaphase cells
- Unique DNA sequence probes (LSI®)
  - Hybridize to unique DNA sequences
  - Detect gene rearrangements, deletions, and amplifications



Typical probes

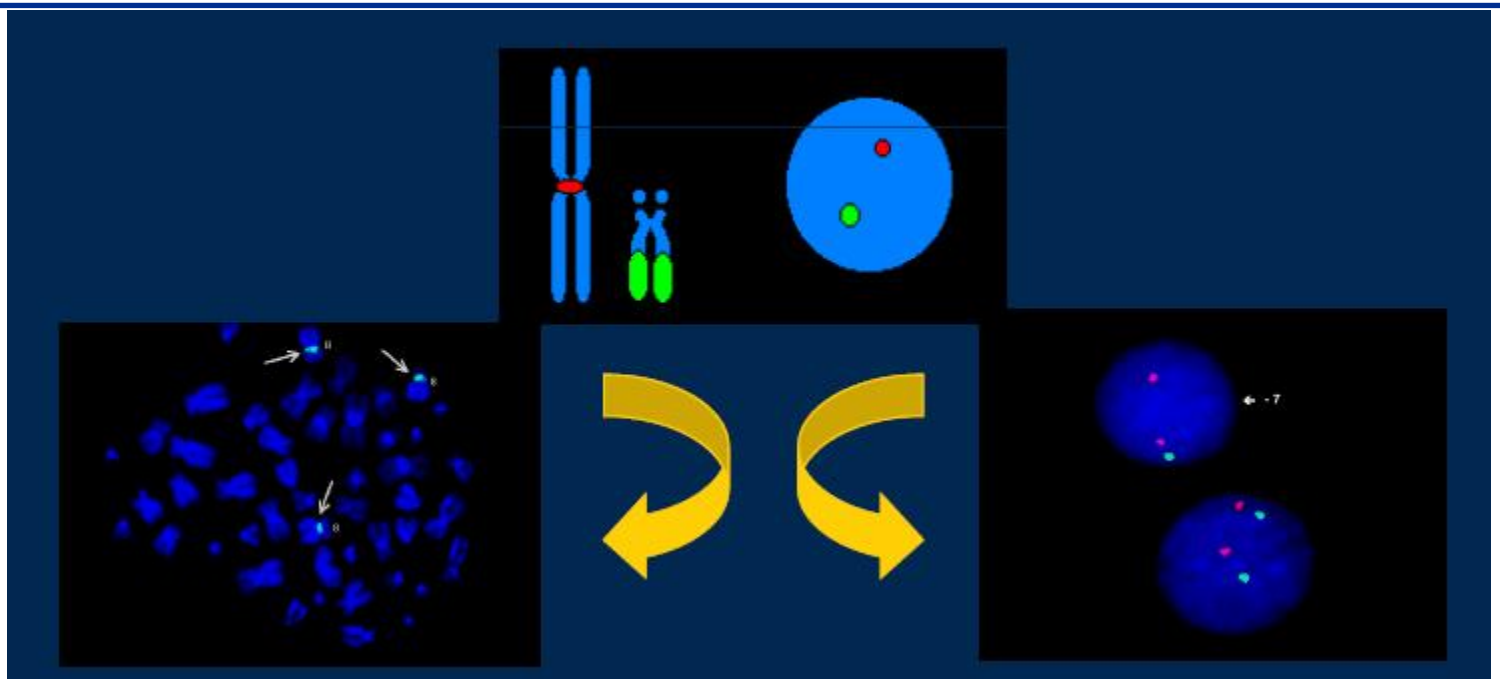
- *Locus-specific probes for marking individual gene locations.*
- *Centromeric probes for binding to repetitive satellite DNA of a chromosome.*
- *(Sub) telomeric probes for the distal sections of individual chromosome arms.*
- *Chromosome or chromosome arm-specific probes (chromosome painting probes) for dyeing a particular chromosome or chromosome arm along its entire length.*

- Telomere-specific probes (TEL)
  - Hybridize to subtelomeric regions
  - Detect subtelomeric deletions and rearrangements



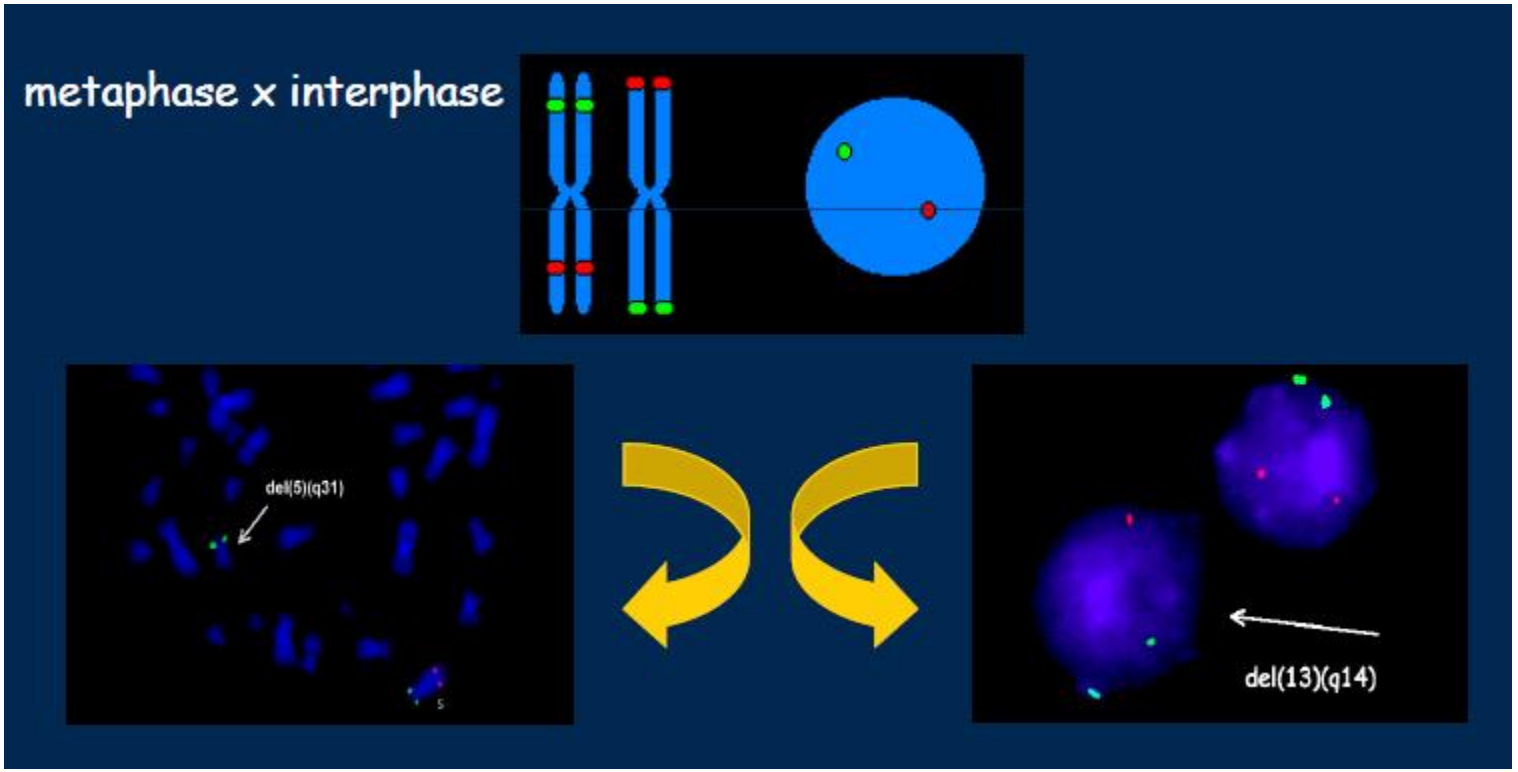
α-satellite DNA – centromeres

Determination of numerical aberrations, identification of the origin of cenromeres in marker-chromosomes, specification of cells after bone marrow transplantation (opposite sex of donor and recipient

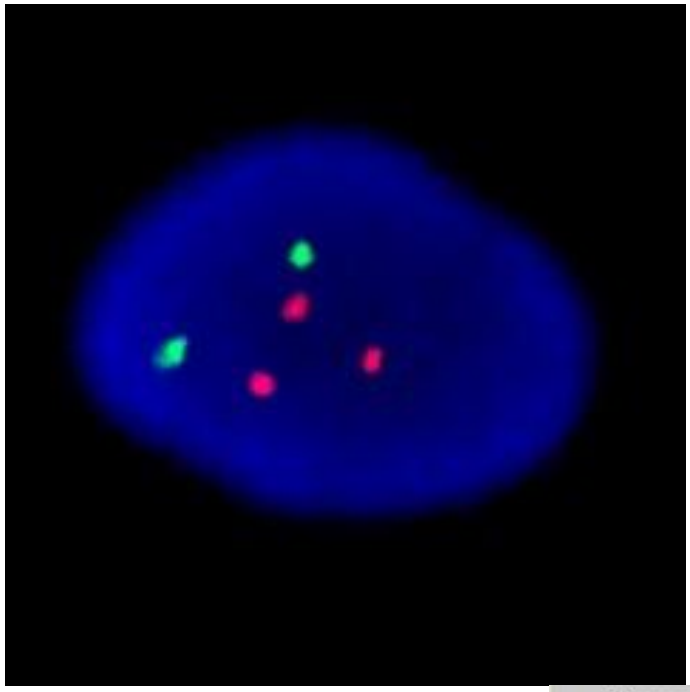


Locus specific DNA probes:

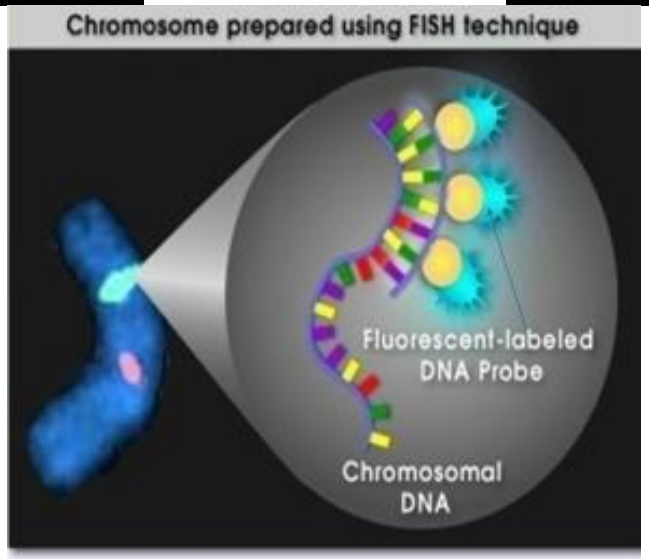
Mapping of genes on chromosomes, detection of structural rearrangements (translocations, deletions)

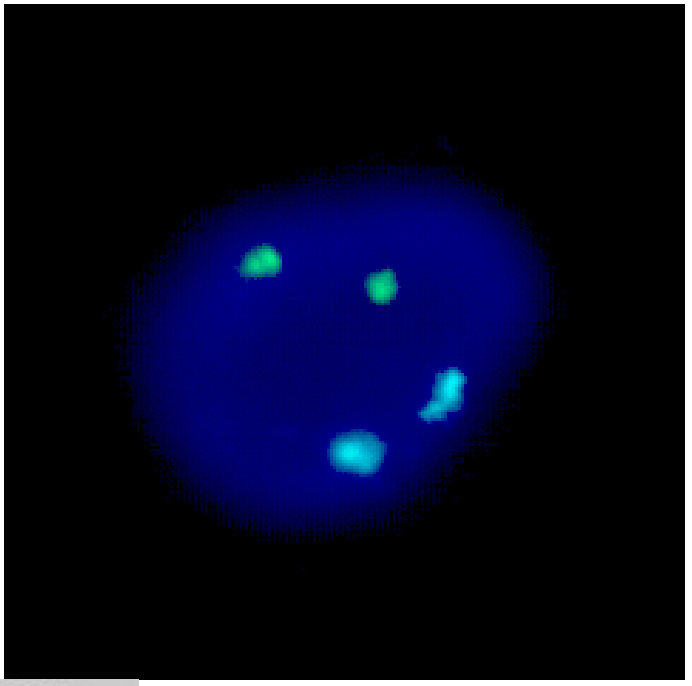


female fetus with trisomy-21



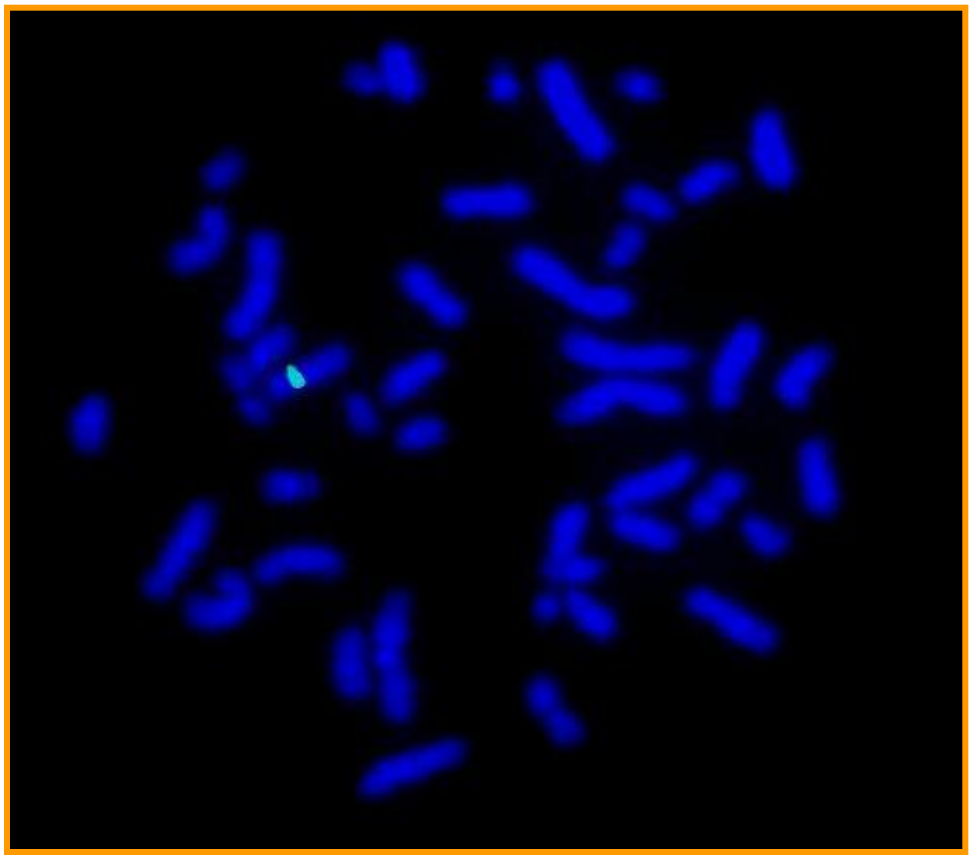
chromosomes 13 (green), and 21 (red)

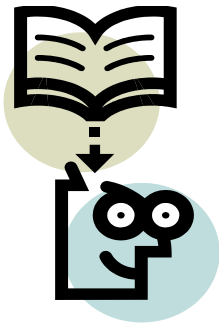




chromosomes 18 (aqua), X (green), and Y (red).

Satellite (centromeric) probe on X-chromosome



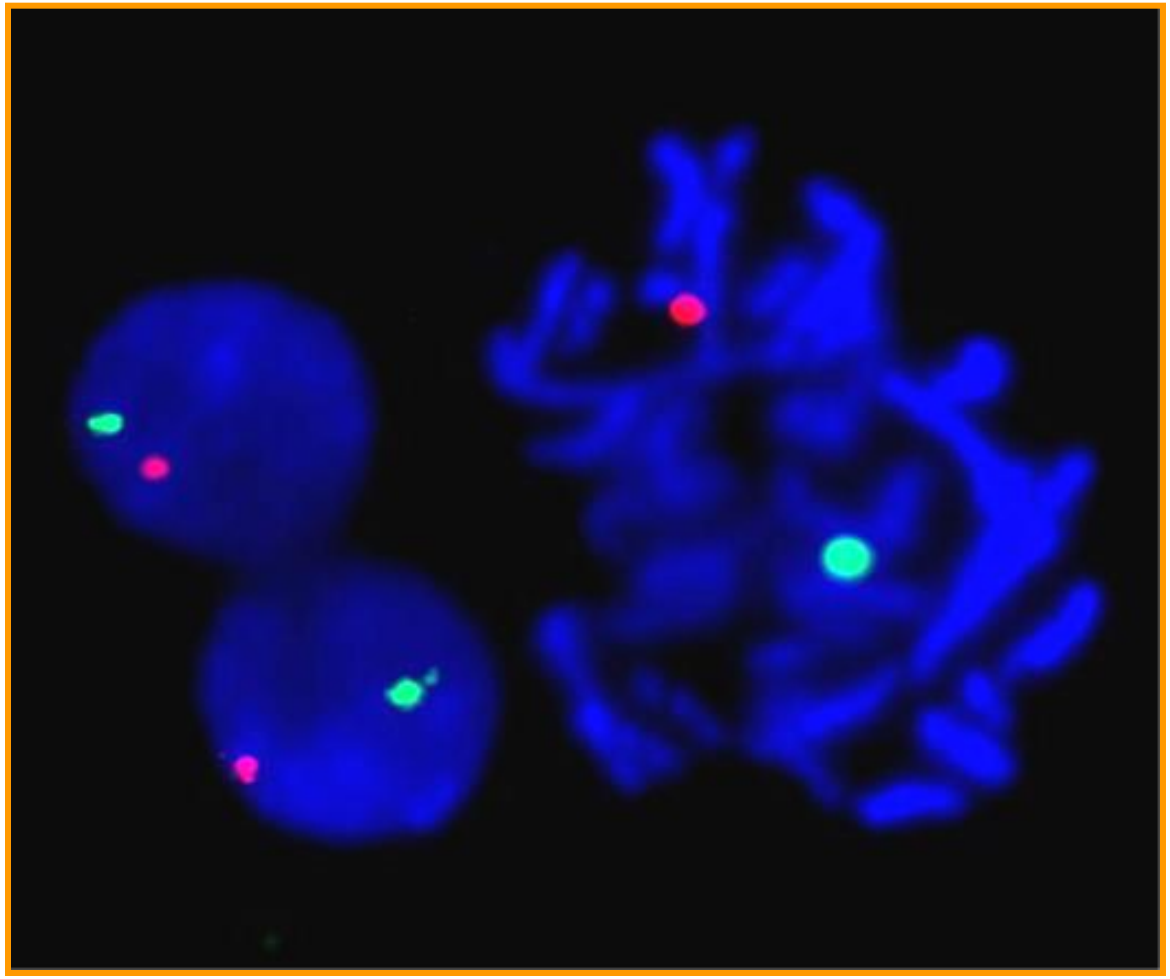


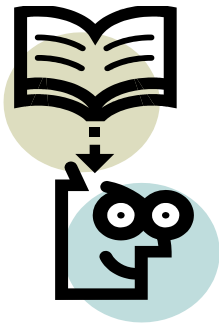
Possible karyotype? *45,X or 46,XY*

X- and Y-centromeric probes

Green = X

Red = Y

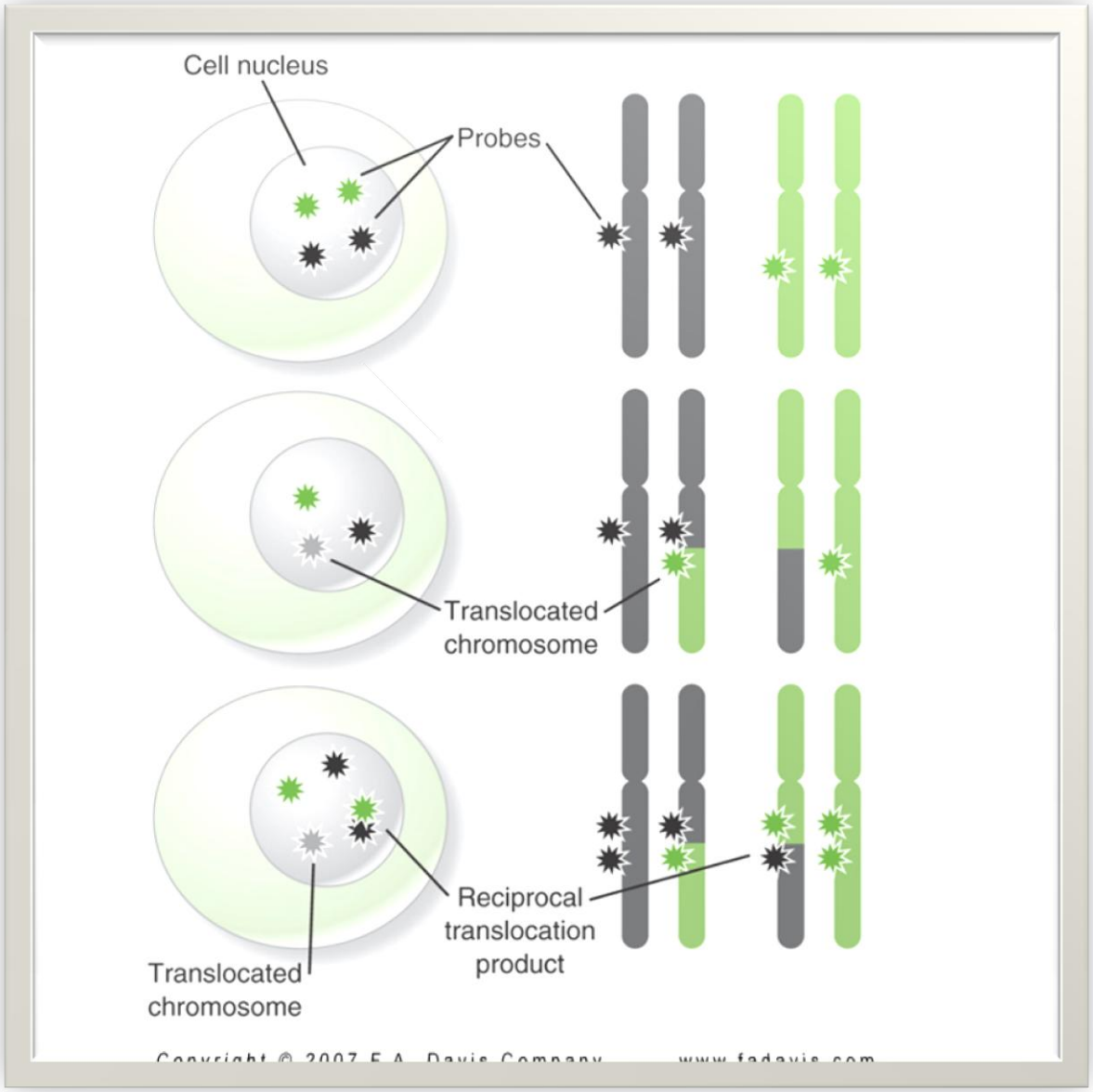




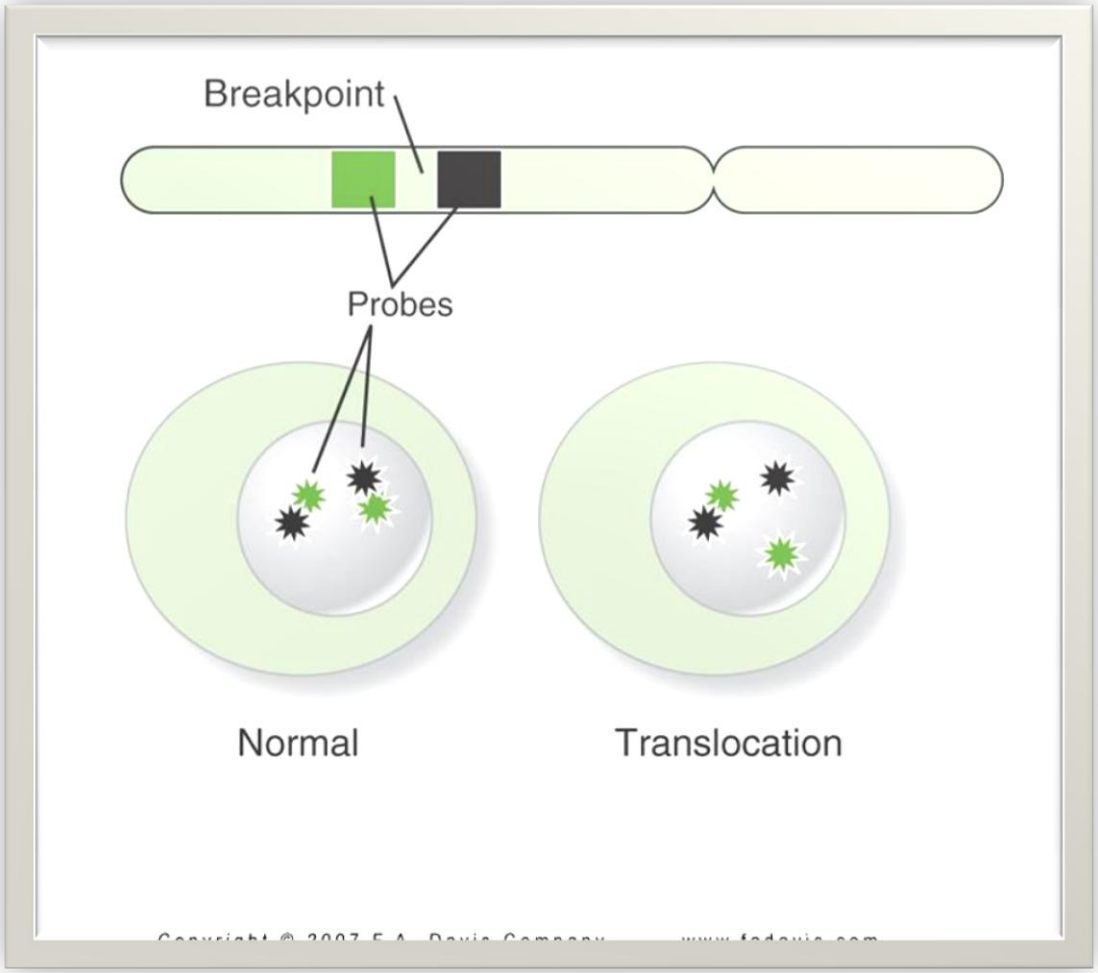
Determine probable karyotype.

46,XY

Structural Abnormality by Interphase FISH LSI® Probe (Fusion Probe)



Structural Abnormality by Interphase FISH LSI® Probe (Break Apart Probe)



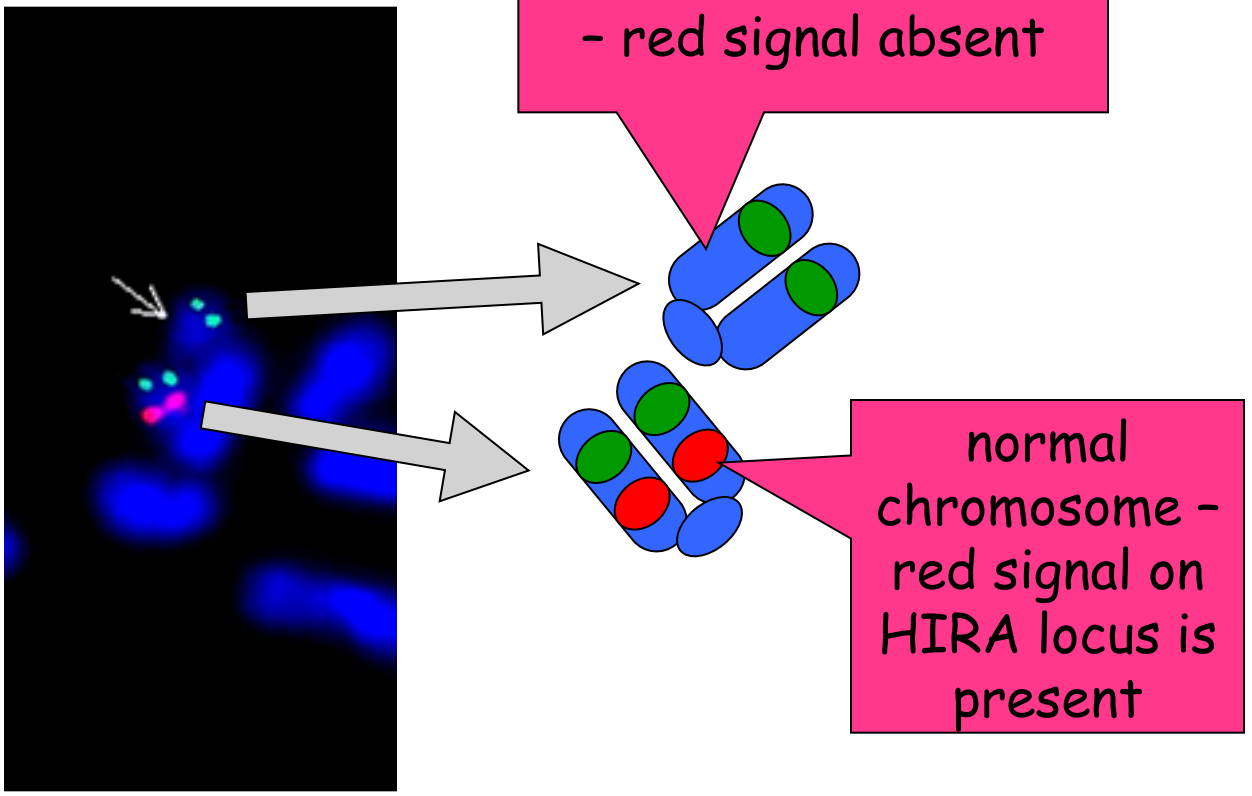
Microdeletion Studies Using FISH

Syndrome	Chromosome Location	Probe/Gene Locus
DiGeorge	22q11.2	D22S75
Velocardiofacial	22q11.2	D22S76
Miller-Dieker	17p13.3	D17S379
Smith-Magenis	17p11.2	D17S29
Prader-Willi	15q11.2	SNRPN
Angelman	15q11.12	D15S10
Williams	7q11.23	Elastin
Cri du chat	5p15.2	D5S23
Wolf-Hirschhorn	4p16.3	D4S96

Microdeletion confirmed (loss of one red signal)

Red signal – TUPLE1 (HIRA) locus

Green signal – ARSA locus (control probe)

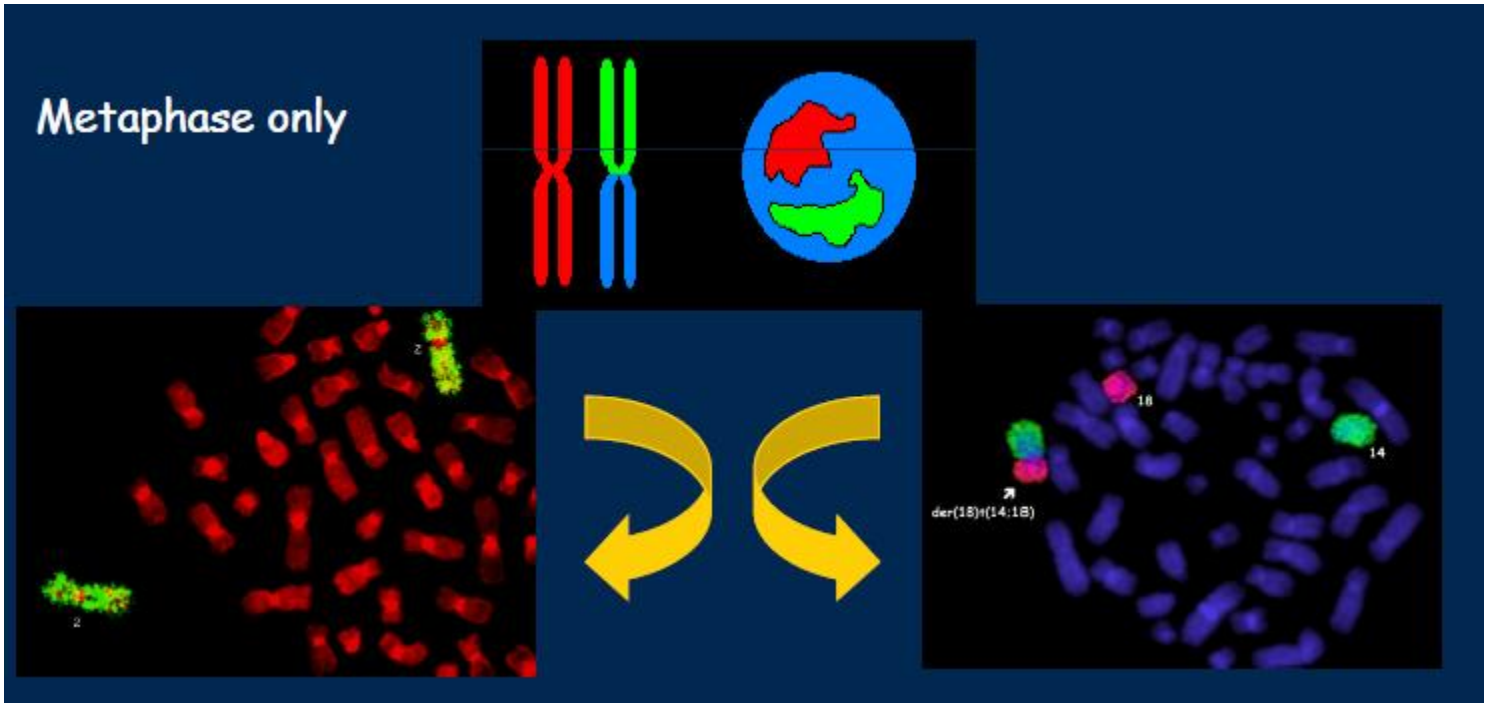


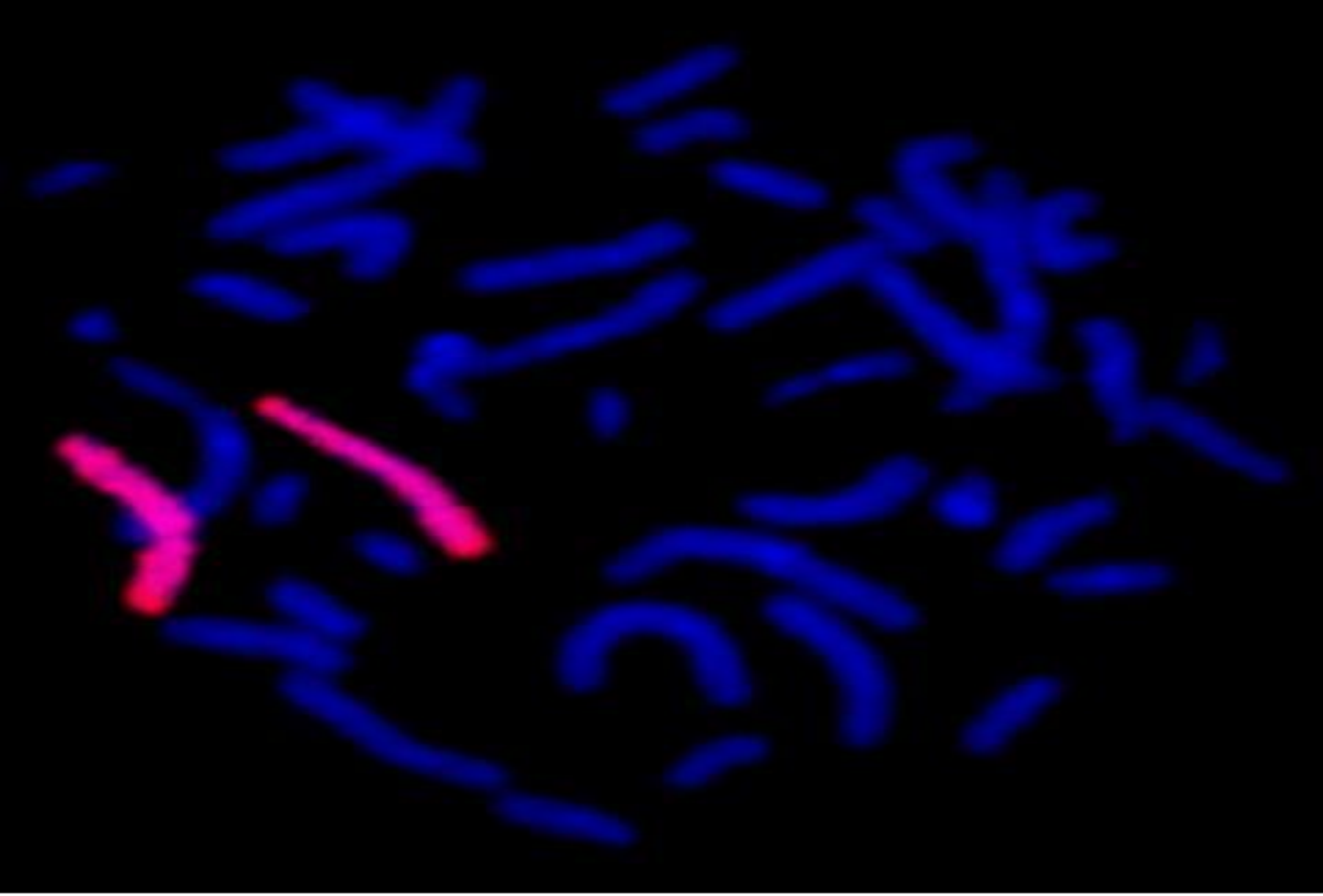
Microdeletion 22q11.2 is associated with

DiGeorge syndrome.

Chromosome painting probes:

They contain sequences from whole chromosomes or chromosomal parts (partial probes) Determination of structural rearrangements (translocations and deletions of large extent), identification of origin of marker chromosomes



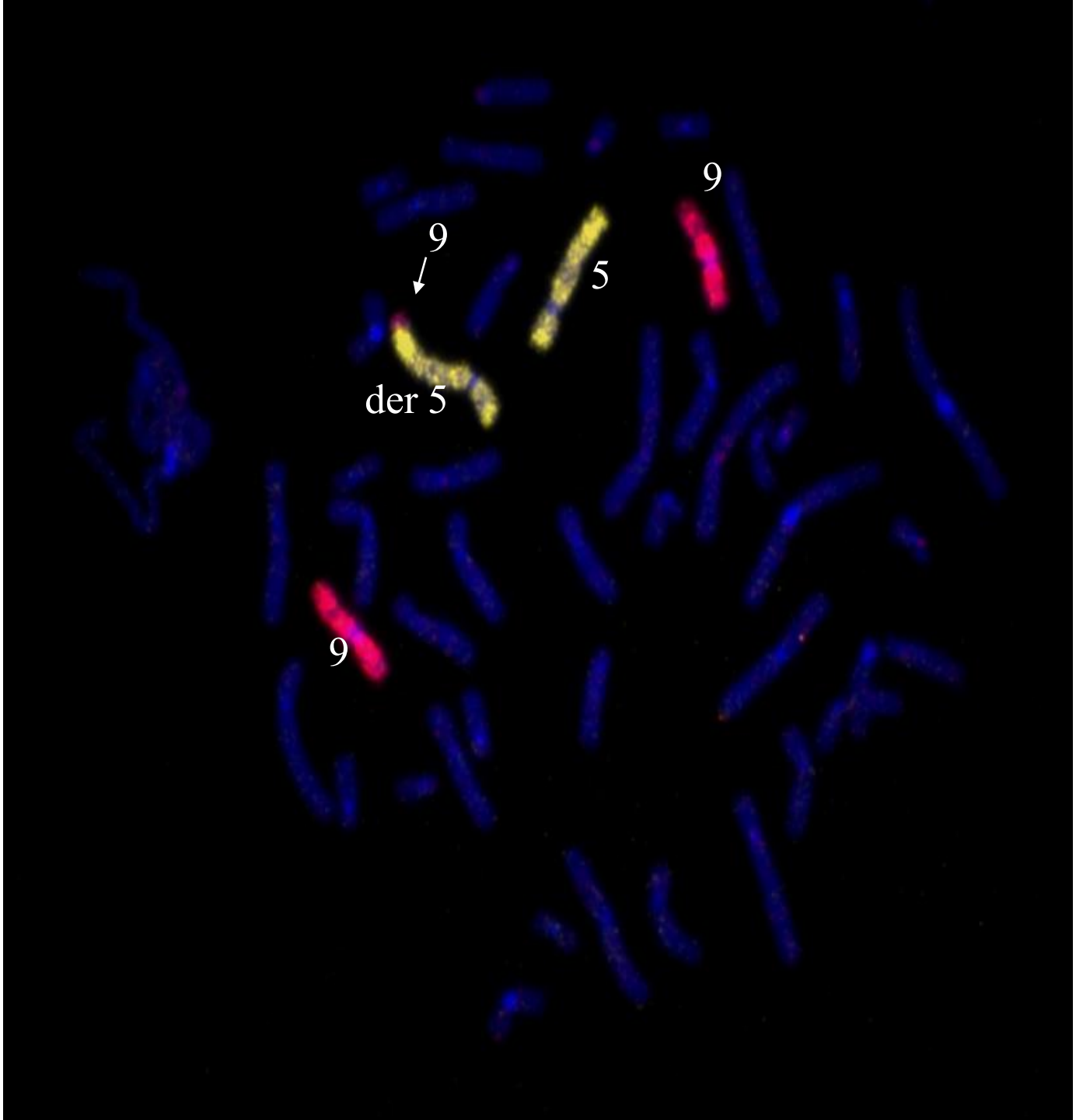


Whole Chromosome Painting 4

der 5

der 5

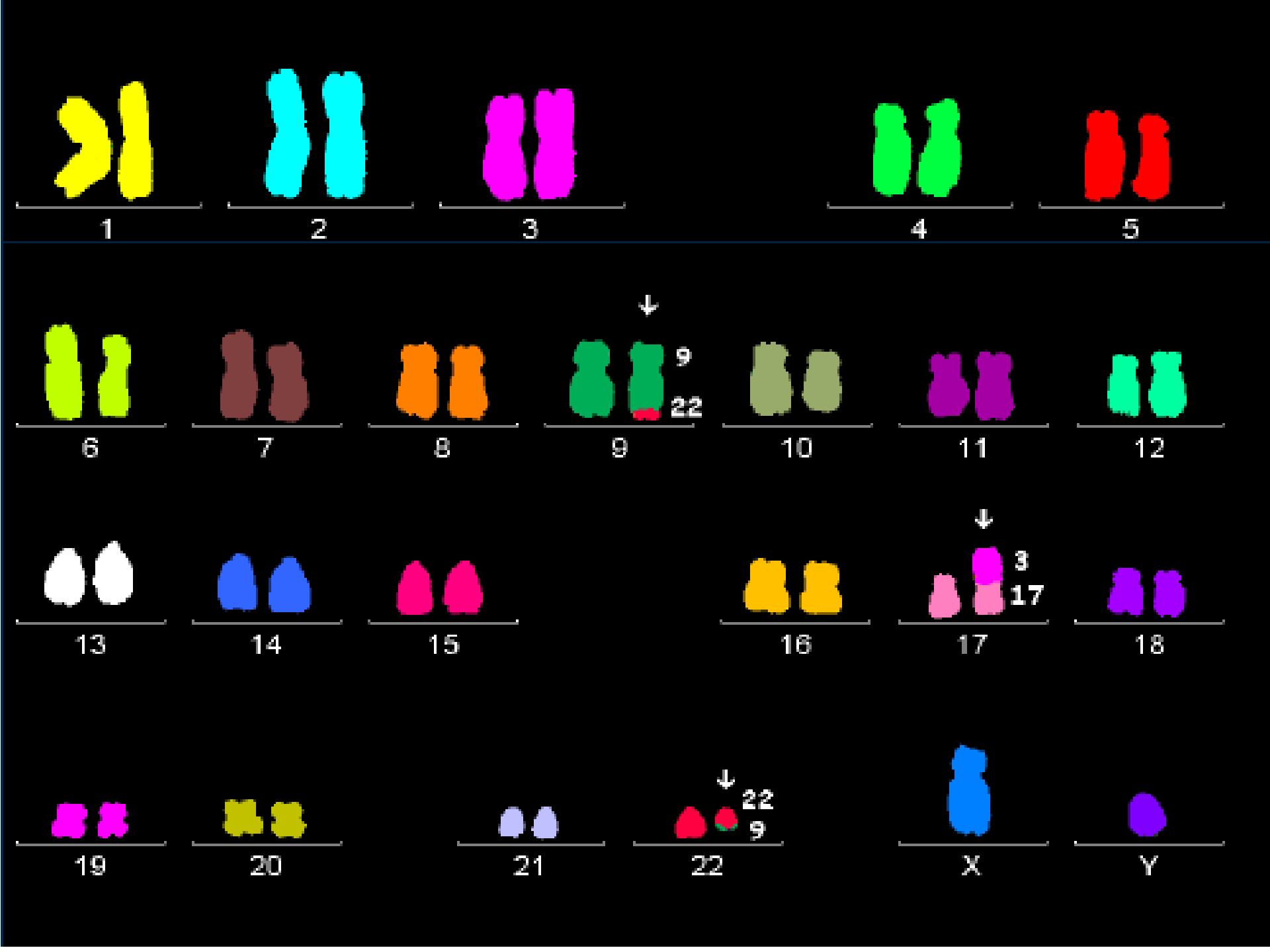
SpectrumGold
WCP 5 +
SpectrumRed
WCP 9



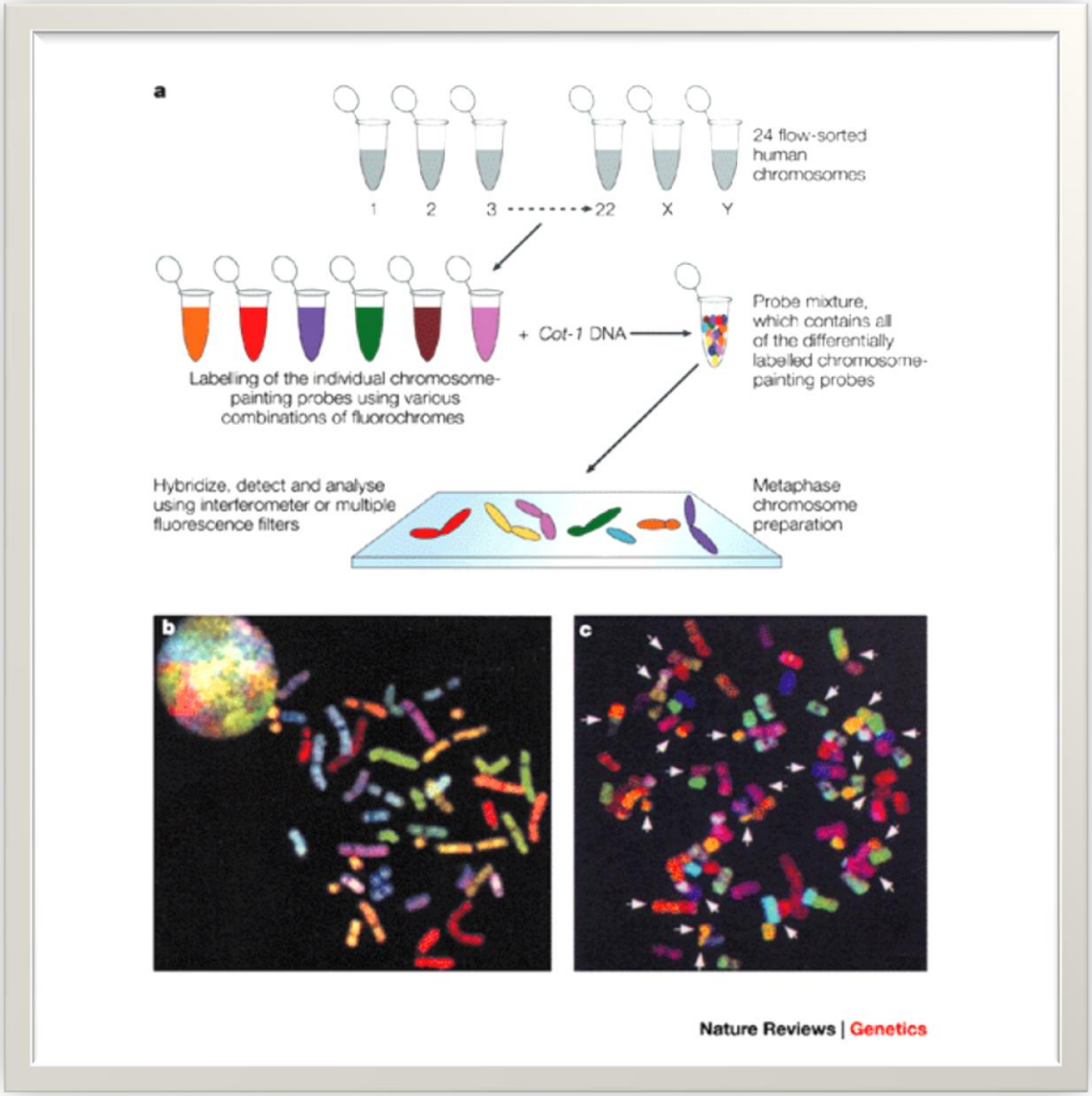
Multicolor FISH - mFISH

allows in one hybridization experiment distinguish according to different color every pair of autosomes and sex chromosomes and then it is possible to make analyses of the whole genome and every structural and numerical rearrangement

analyses of complex chromosomal rearrangements in bone marrow cells of patients with hematological malignancies will bring us detailed informations about involvement of specific chromosomes or their regions into rearrangements


1
2
3
4
5
6
7
8
9
9
22
10
11
12
13
14
15
16
17
3
17
18
19
20
21
22
22
9
X
Y

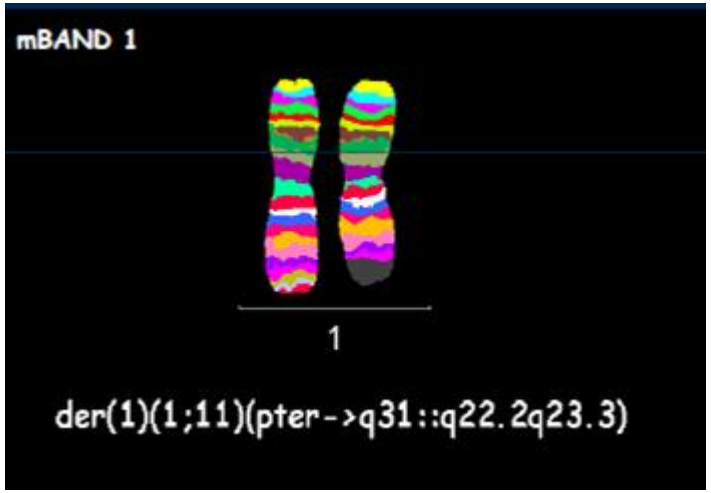
Spectral karyotyping and multifluor FISH paint each human chromosome in one of 24 colors (SKY)

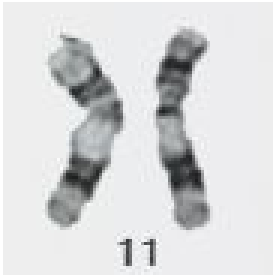


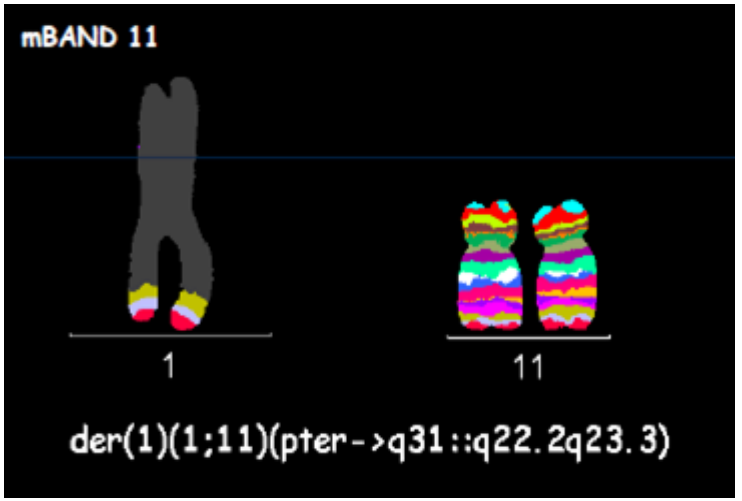
 Trask, B. Human cytogenetics: 46 chromosomes, 46 years and counting. *Nature Reviews Genetics* **3,** 776 (2002).

Multicolor banding with high resolution - mBAND

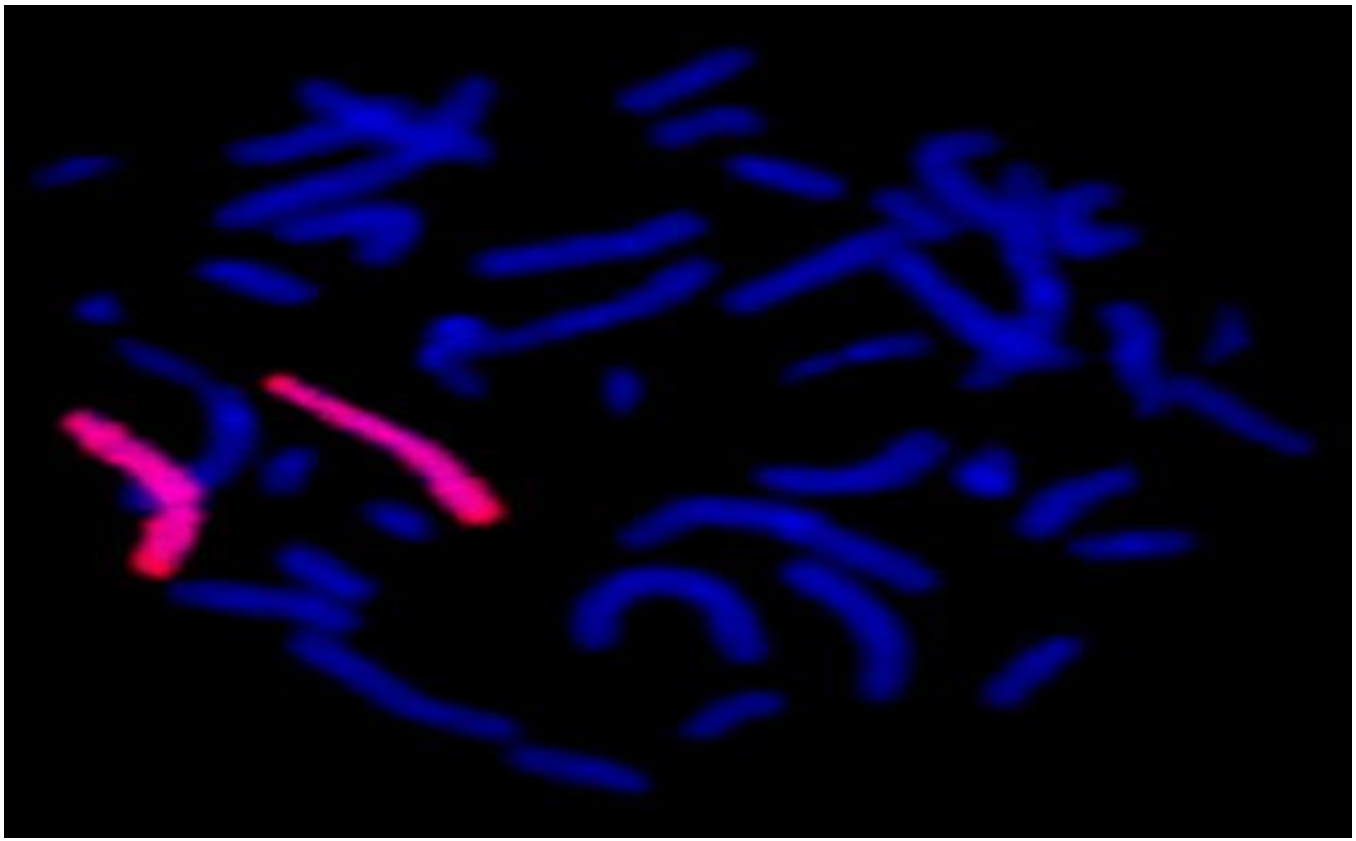
enables determination of exact breakpoints of chromosomal aberrations with much higher resolution than classical banding

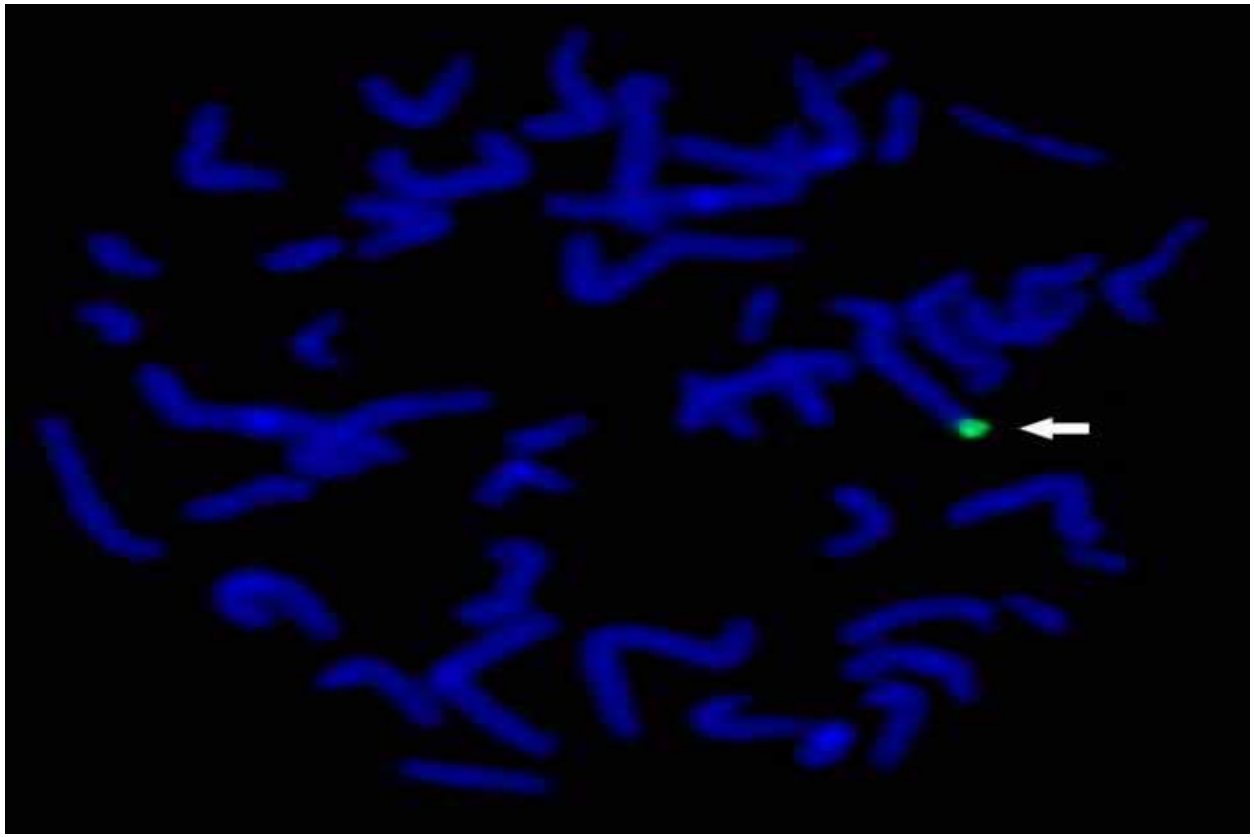






Did chr 4 have a small terminal deletion at 4q?





The impact of conventional and molecular cytogenetic analysis in oncohematology

Is part of the work up at diagnosis

Provides comprehensive information on the karyotype

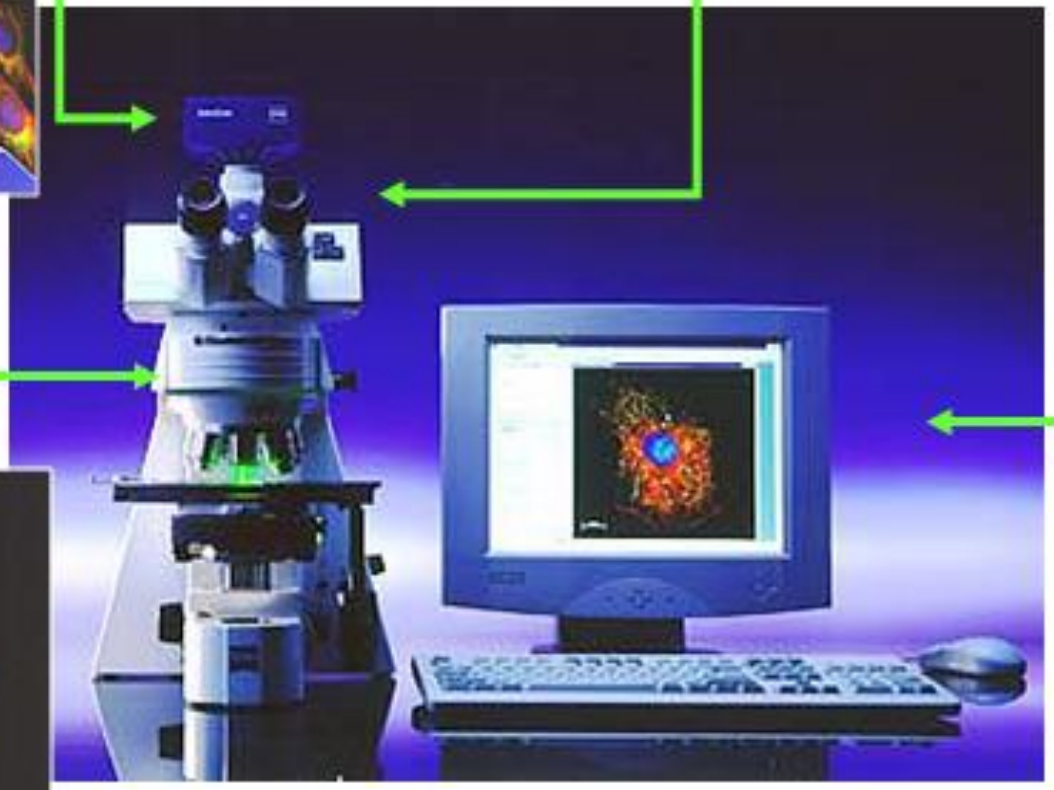
* help to specify diagnosis

* help to determine the prognosis

* help monitor effectiveness of treatment

CCD Camera

Fluorescent Microscope



Filters

FISH Analysis Software

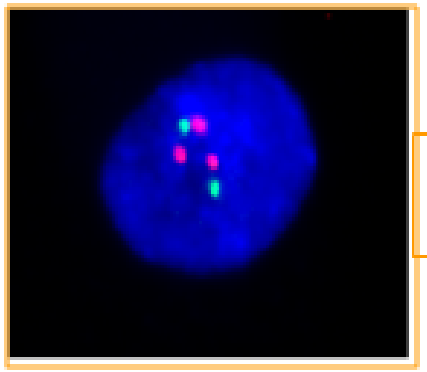
Absorption Spectra of Fluorochromes Commonly Conjugated to Secondary Antibodies

Fluorochrome	Absorption	Emission
Cascade Blue	400	420
Fluorescein	494	518
Rhodamine	570	590
Texas Red	595	615
Cy5	650	670

Diagnostic Potential For Karyotype, FISH, and Chromosomal Micro- array Analysis (CMA) For Selected Disorders

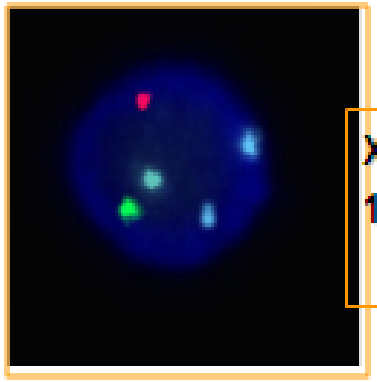
Condition	Locus studied	Karyotype	Disease specific FISH	Telomere FISH	CMA
Aneuploidy	various	~100%	Not detected	Detected by karyotype	~100%
Large deletions, large dupllications, translocation of large segments	various	~100%	Not detected	Detected by karyotype	Karyotype better for present
Cryptic Rearrangements of telomeres	various	Not detected	Not detected	~100%	~100% for unbalanced
1p36 deletion	1p36.3	Few	~99%	>95%	~99%
Wolf-Hirschhorn	4p16.3	Most	~99%	>95%	~99%
Cri-du-chat	5p15.2	Most	~99%	>95%	~99%
Williams-Beuren	7q11.2	Almost none	~99%	Not detected	~99%
Prader-Willi	15q11-q13	Unreliable	~70%	Not detected	~70%
Angelman	15q11-q13	Unreliable	~70%	Not detected	~70%
Miller-Dieker lissencephaly	17p13.3	Few	>90%	Some detected	>90%
Smith-Magenis	17p11.2	Some	>95%	Not detected	>95%
Velocardiofacial/DiGeorage 1	22q11.2	Rarely	>95%	Not detected	>95%

FISH vs. Karyotyping



13 (green)
21 (red)

99.9% correlation



X (green), Y (red)
18 (aqua)

Results: < 24 hours

Results: 7 - 10 days