
# The Power of Observation

## Lessons from the Clinical Cytogenetics Laboratory

# An Introduction…….

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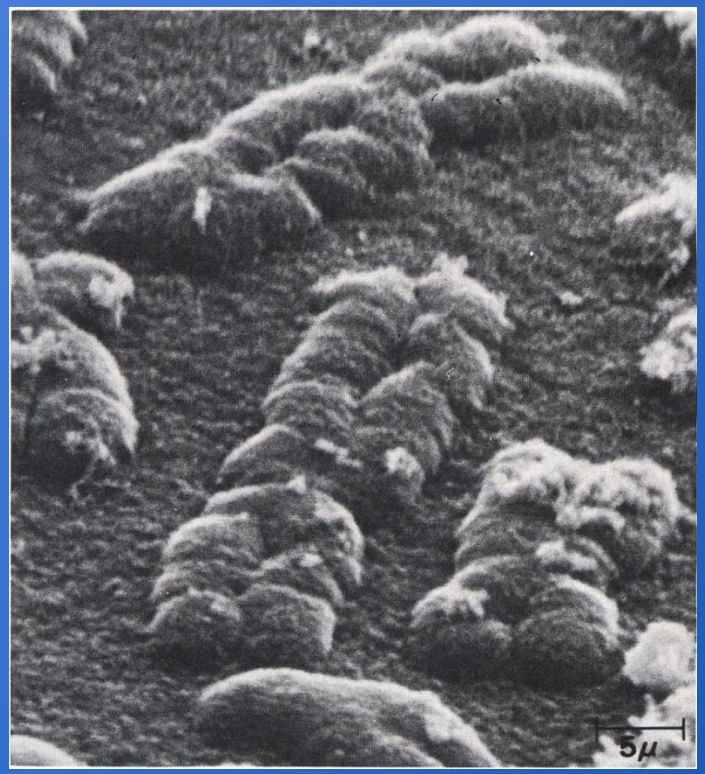
# HISTORY




Scanning electron micrograph of several human chromosomes. Source: J.B. Rattner and C.C. Lin, *Cell* 42 (1985), p. 291.

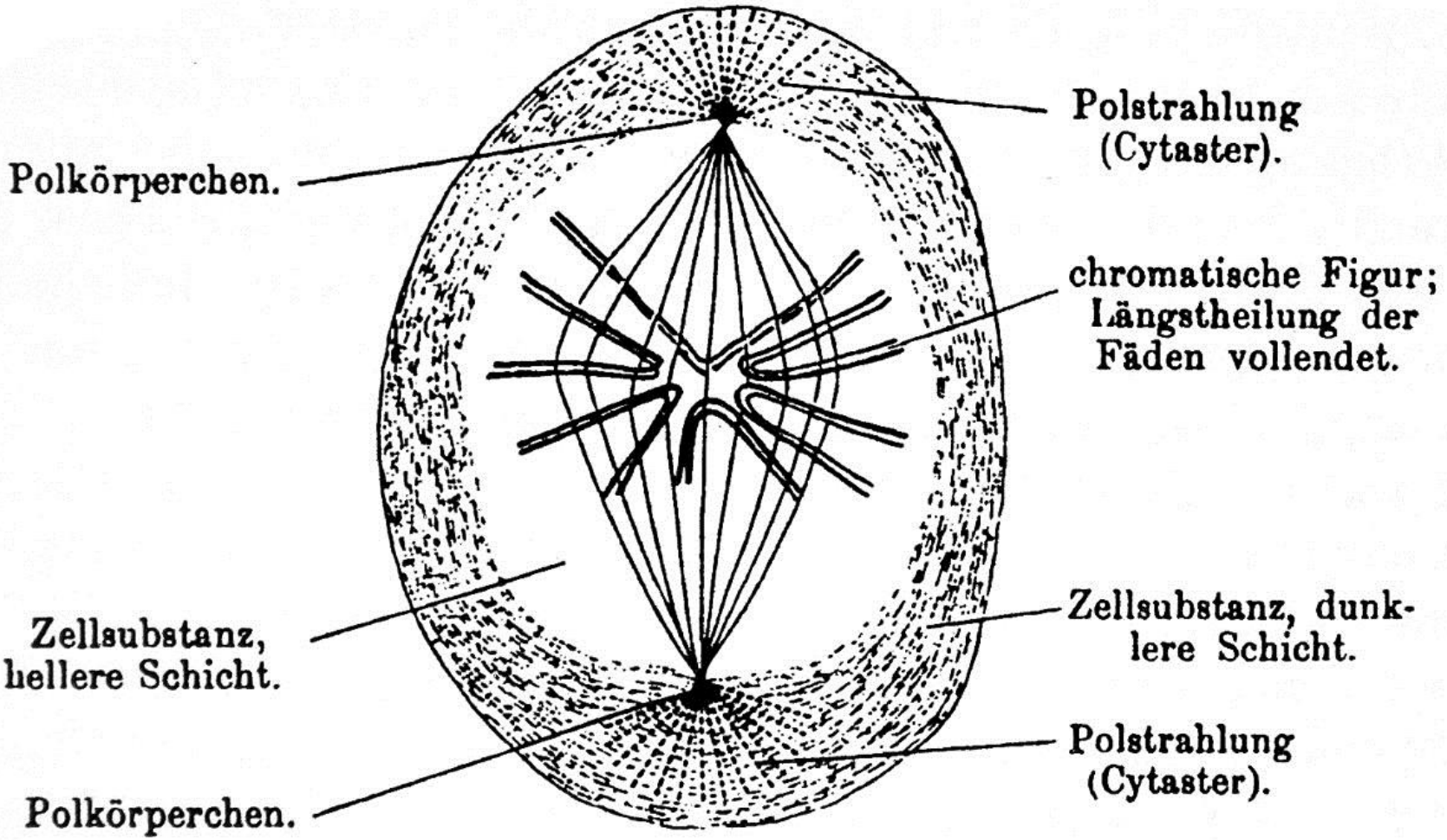
**1842: Chromosomes first observed in plant cells by Karl Wilhelm von Nägel**

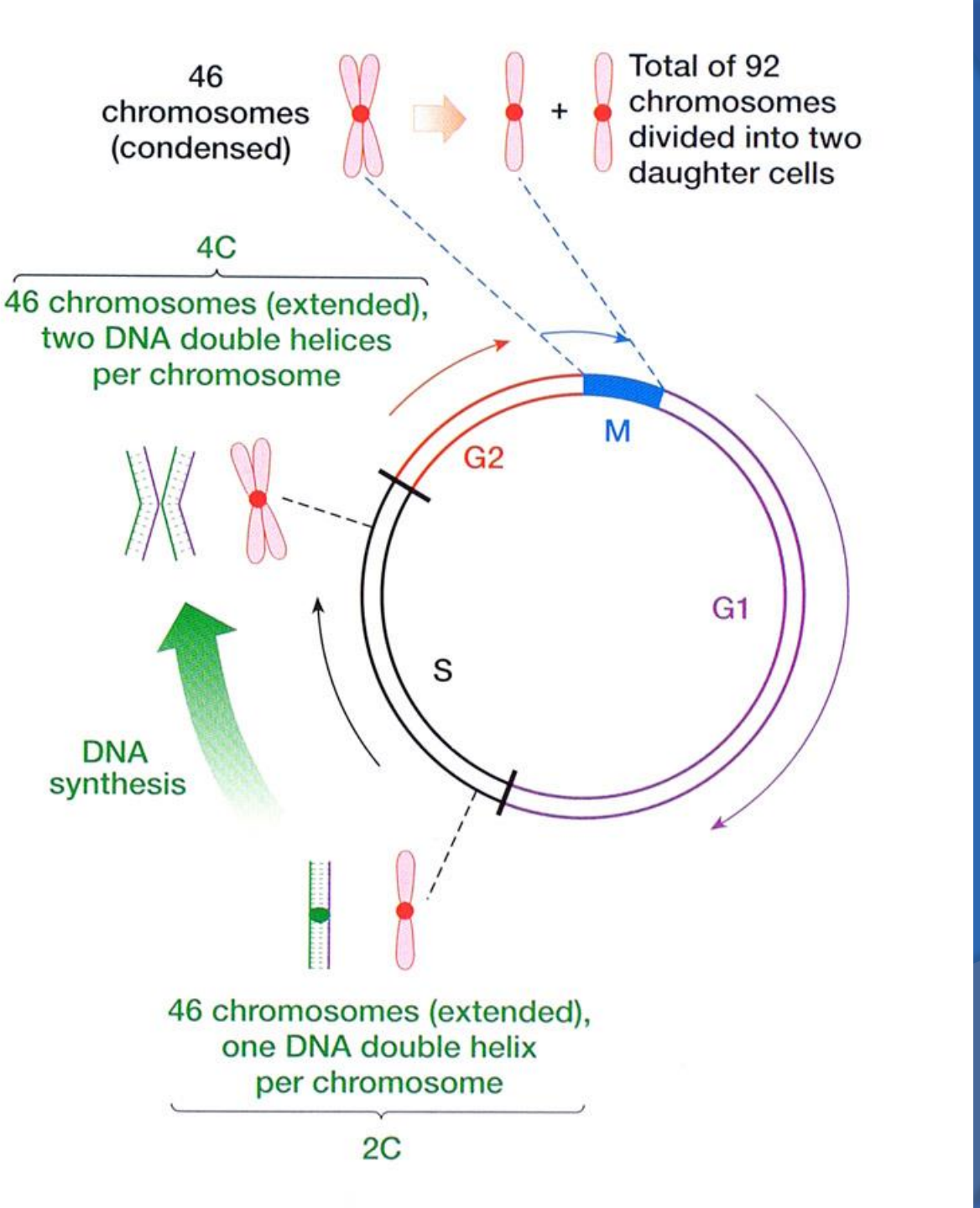
**1882: Mitosis described in animal (salmander) cells by Walter Flemming.**

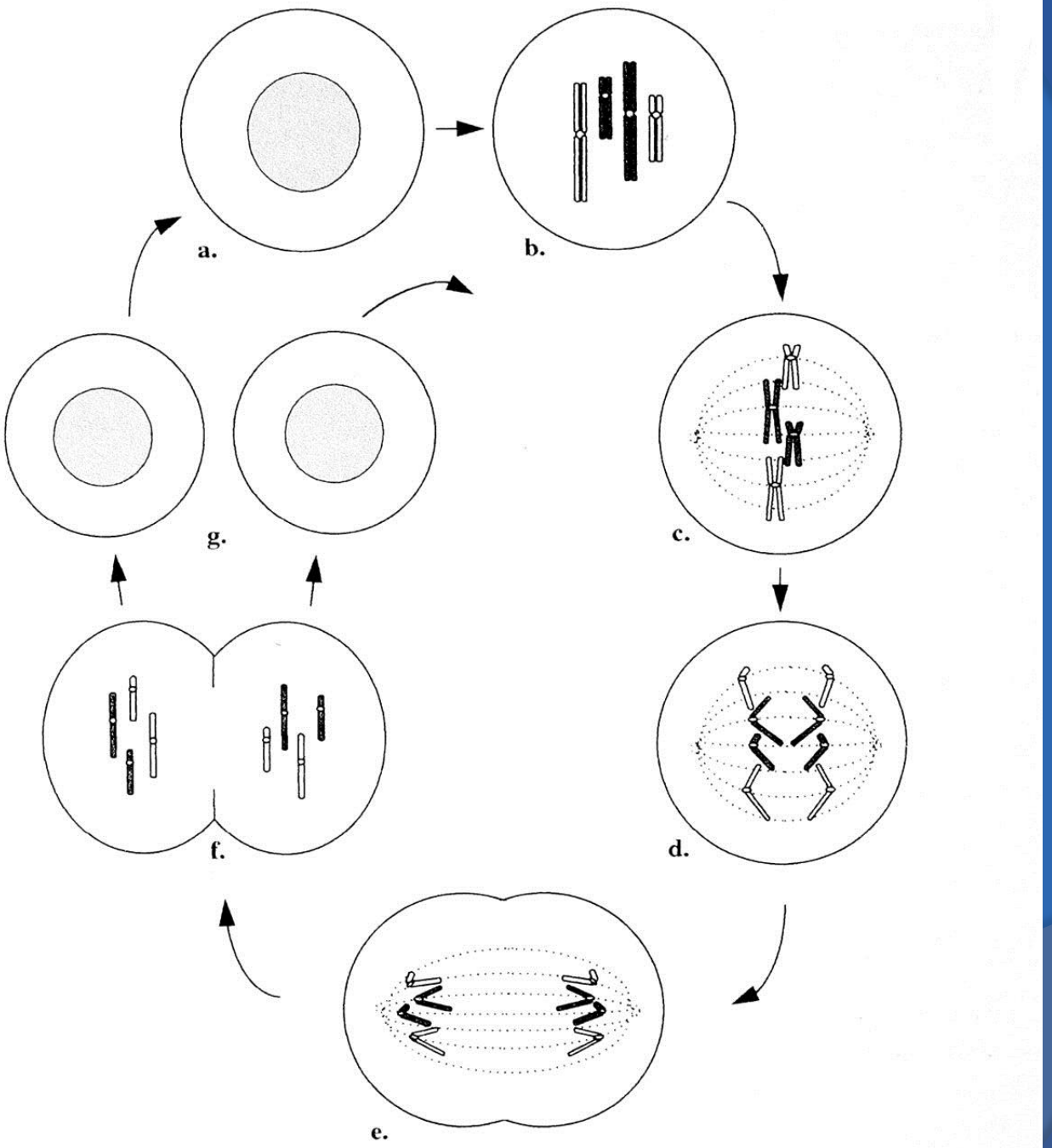
**1888: "Chromosome" coined by von Waldeyer.**

**1900s: Chromosomes carry genetic material. (Levitsky)**

**1956: Human complement of 46 chromosomes.**


Polstrahlung
(Cytaster).
Polkörperchen.
chromatische Figur;
Längstheilung der
Fäden vollendet.
Zellsubstanz,
hellere Schicht.
Zellsubstanz, dunk-
lere Schicht.
Polstrahlung
(Cytaster).
Polkörperchen.


46
chromosomes
(condensed)
+
Total of 92
chromosomes
divided into two
daughter cells
4C
46 chromosomes (extended),
two DNA double helices
per chromosome
M
G2
G1
S
DNA
synthesis
46 chromosomes (extended),
one DNA double helix
per chromosome
2C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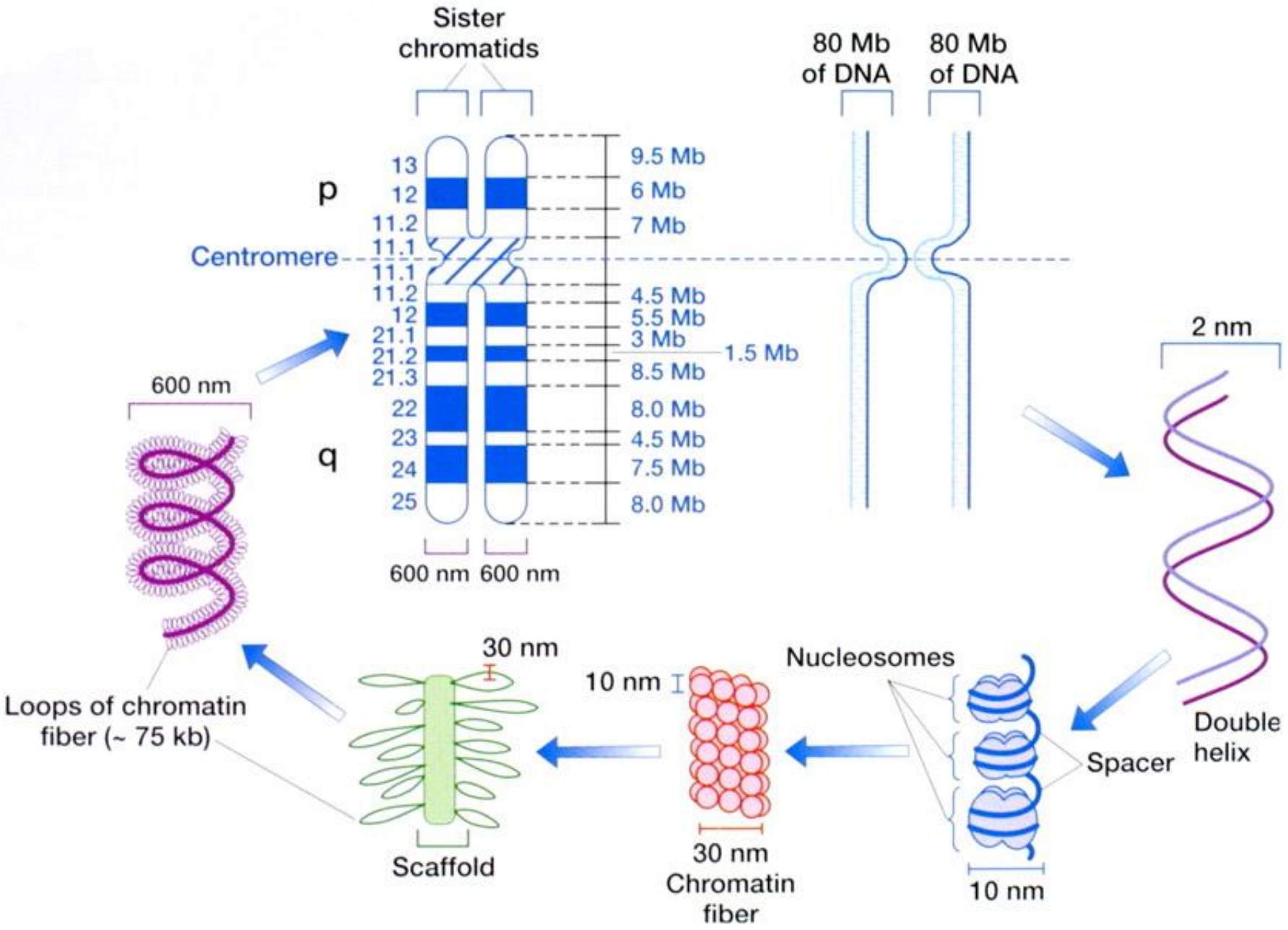
Sister chromatids
80 Mb of DNA
80 Mb of DNA
p
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13
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Centromere
9.5 Mb
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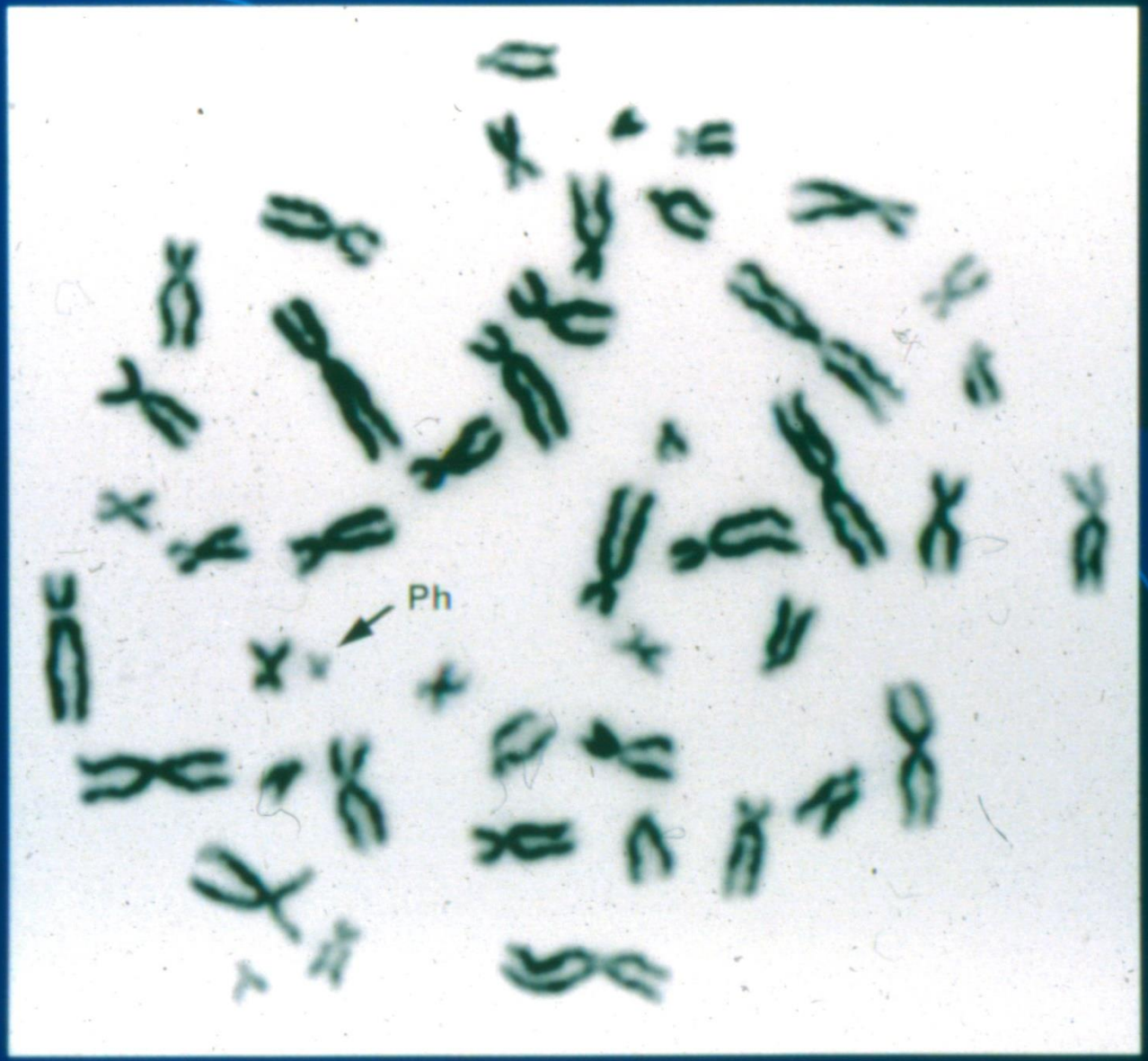
7.5 Mb
8.0 Mb
600 nm
600 nm
600 nm
2 nm
Double helix
Spacer
Nucleosomes
10 nm
10 nm
30 nm Chromatin fiber
30 nm
Scaffold
Loops of chromatin fiber (~ 75 kb)

# Size of Components of Human Genome

| | |
|---|---|
| Size of haploid genome | 3.3 X $10^9$ DNA basepairs |
| Estimated genetic constitution | 30,000 genes |
| Size of average chromosome band | 3 X $10^6$ DNA basepairs |
| Size of average gene | 5 X $10^4$ DNA basepairs |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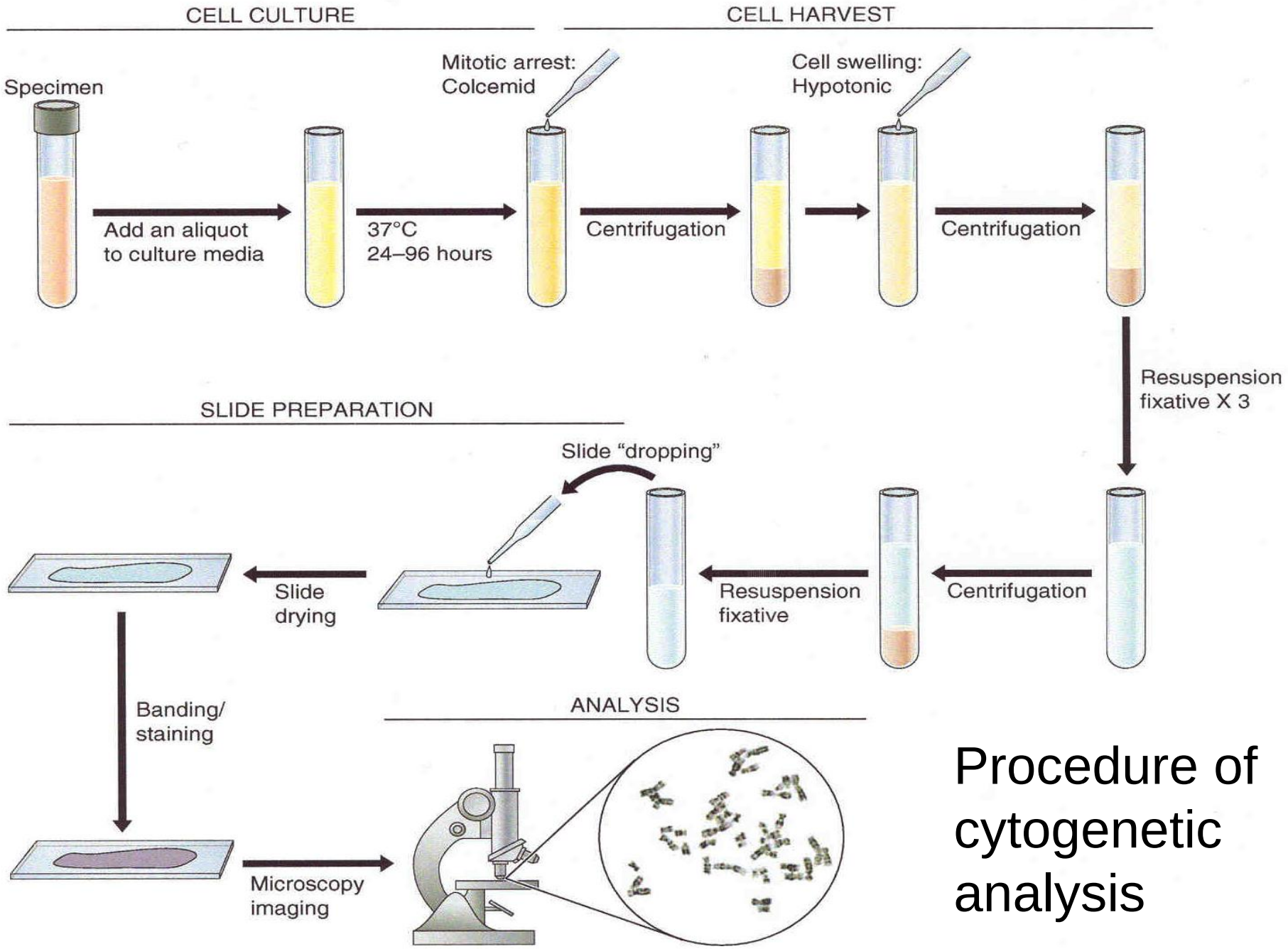
SPECIMEN TRANSPORT BAG
BIOHAZARD



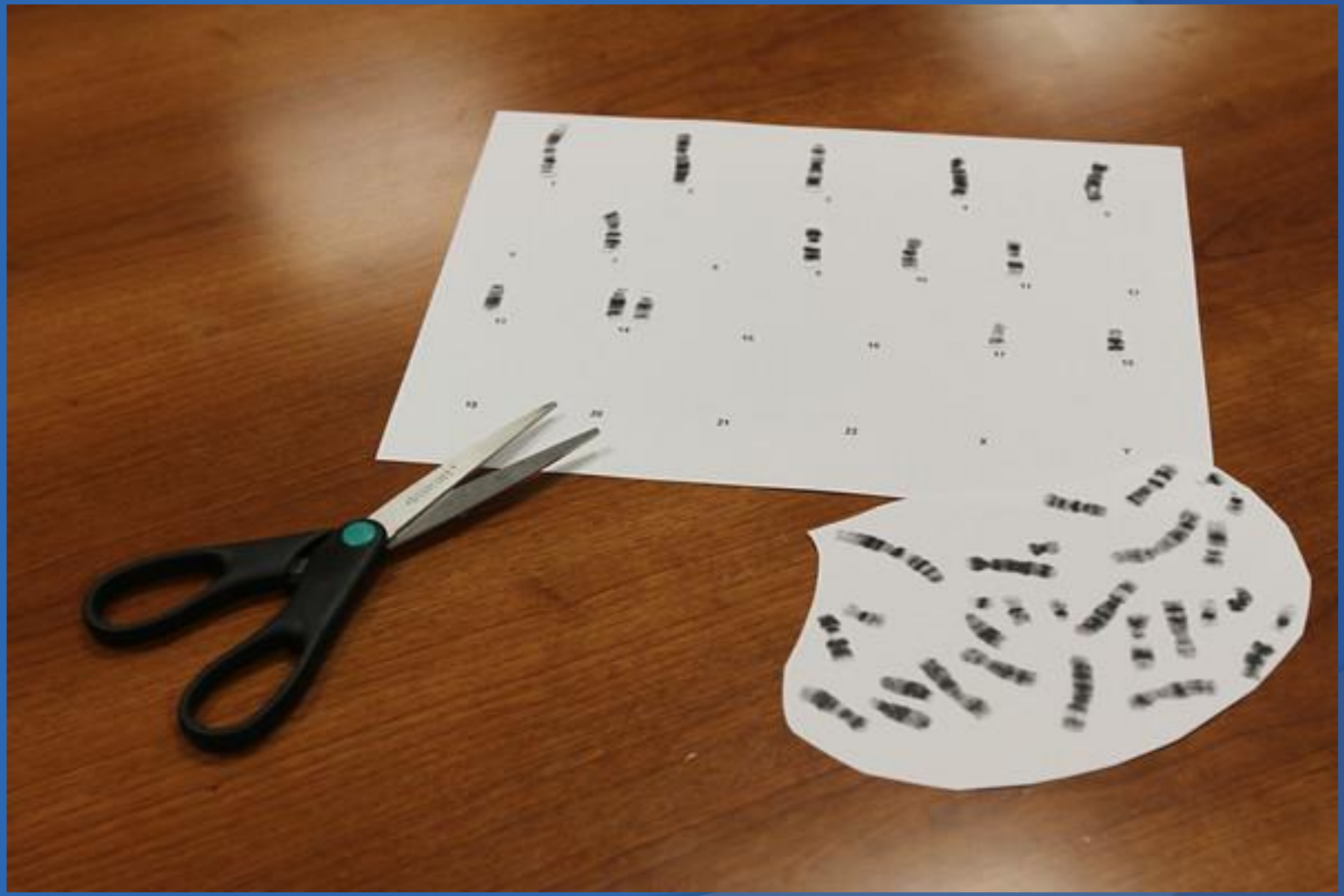

# Procedure of cytogenetic analysis

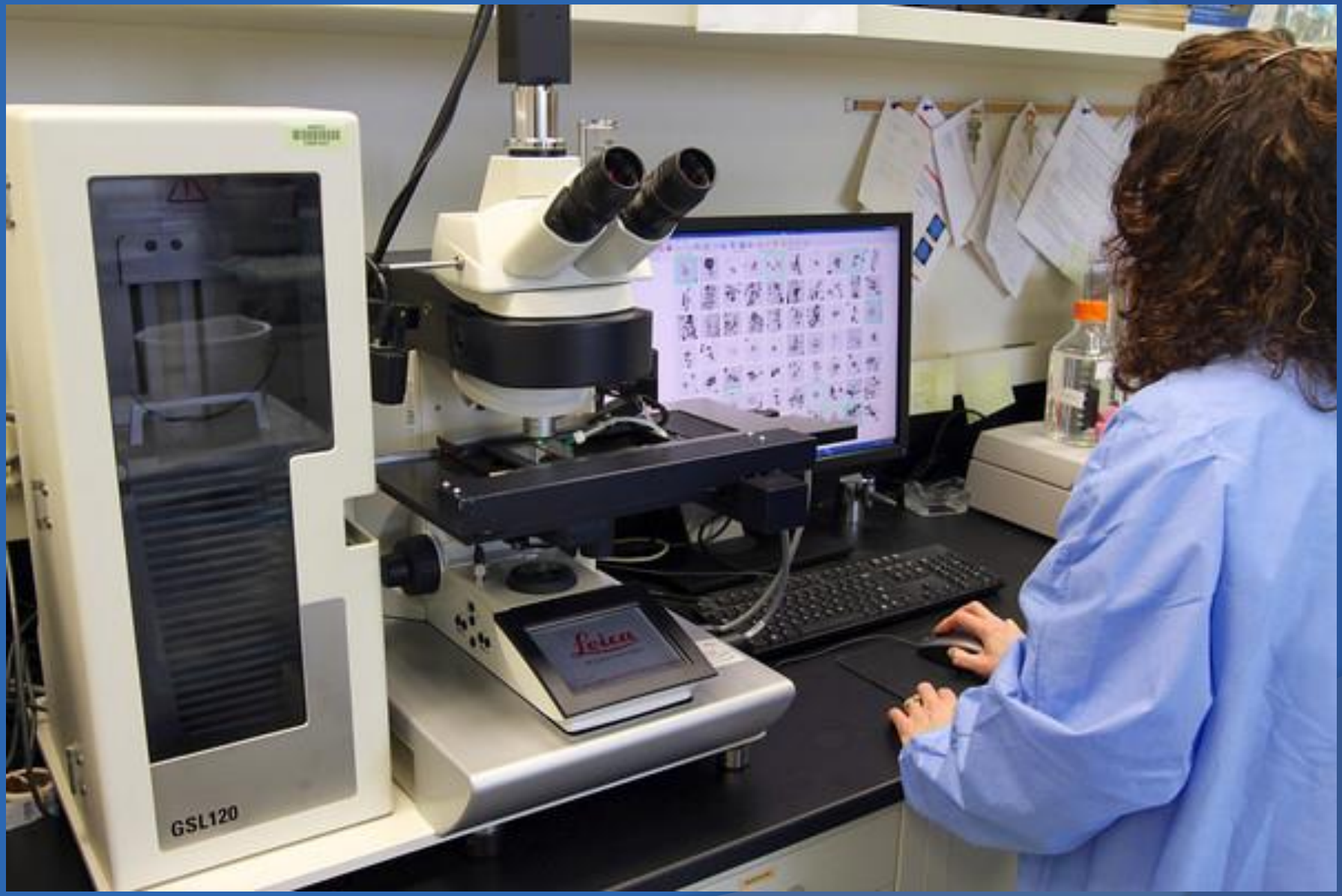

Fisherbrand
Fisherbrand
Fisherbrand
Kimwipes
Fisher

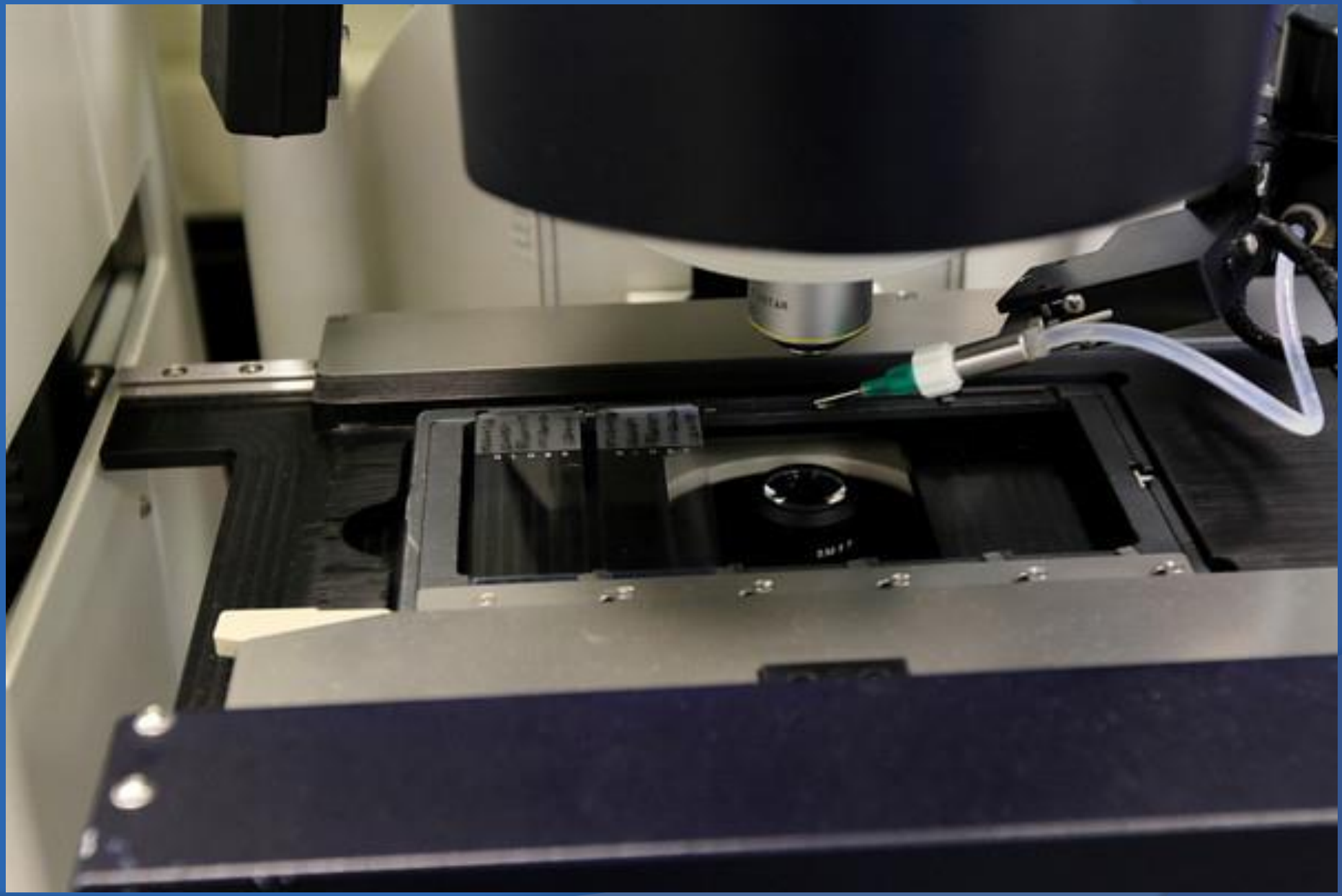

PRECISION

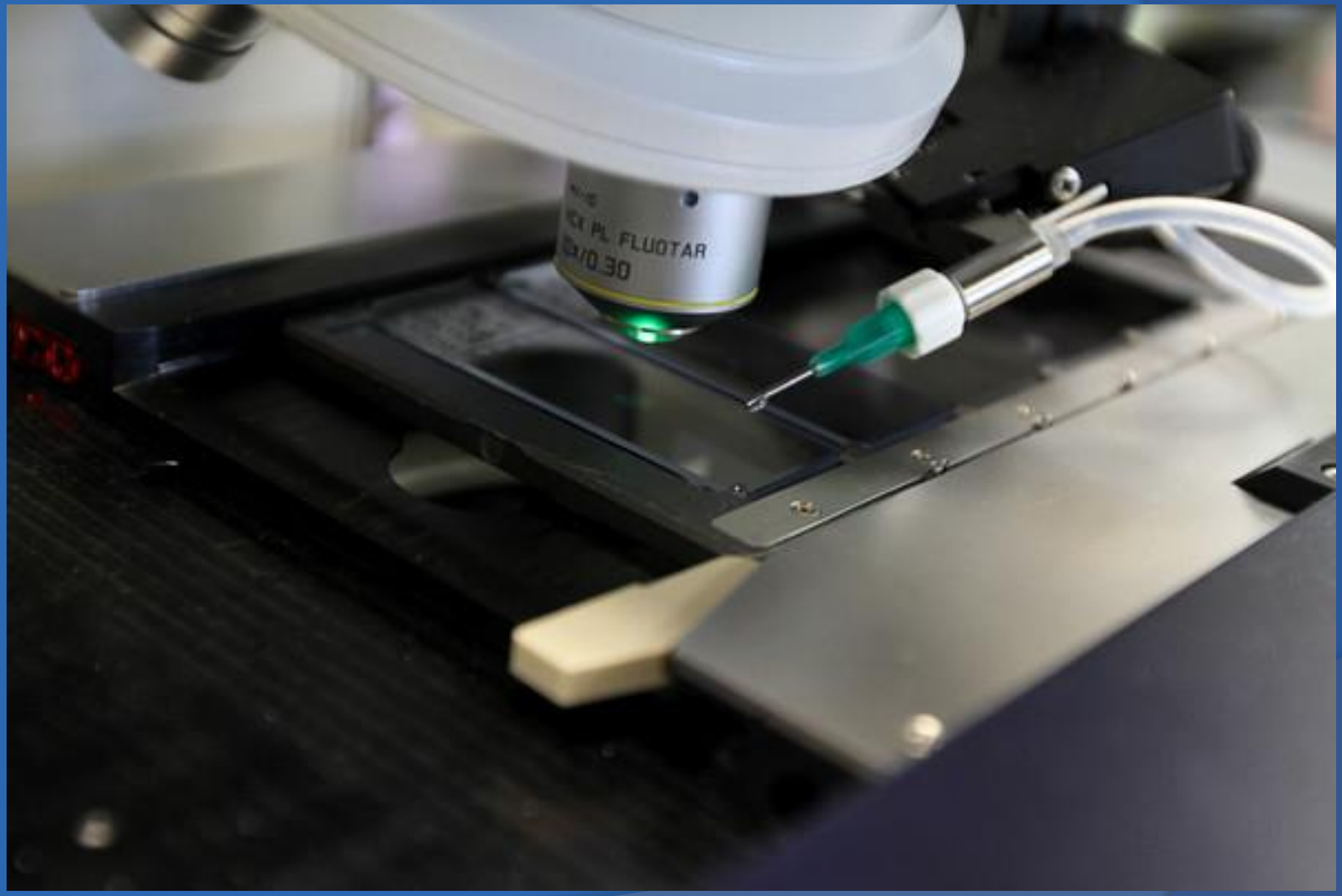







GSL120
Leica

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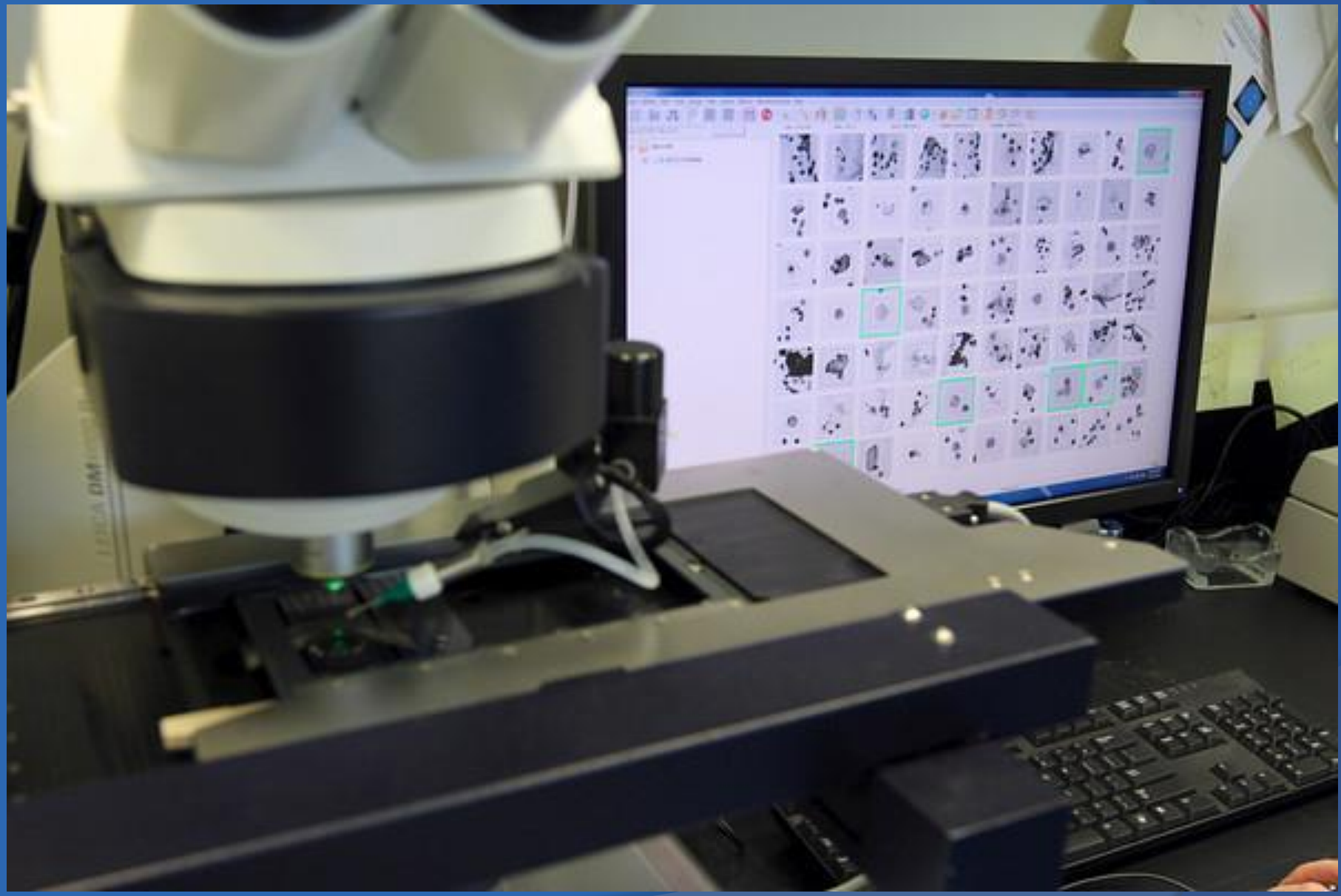
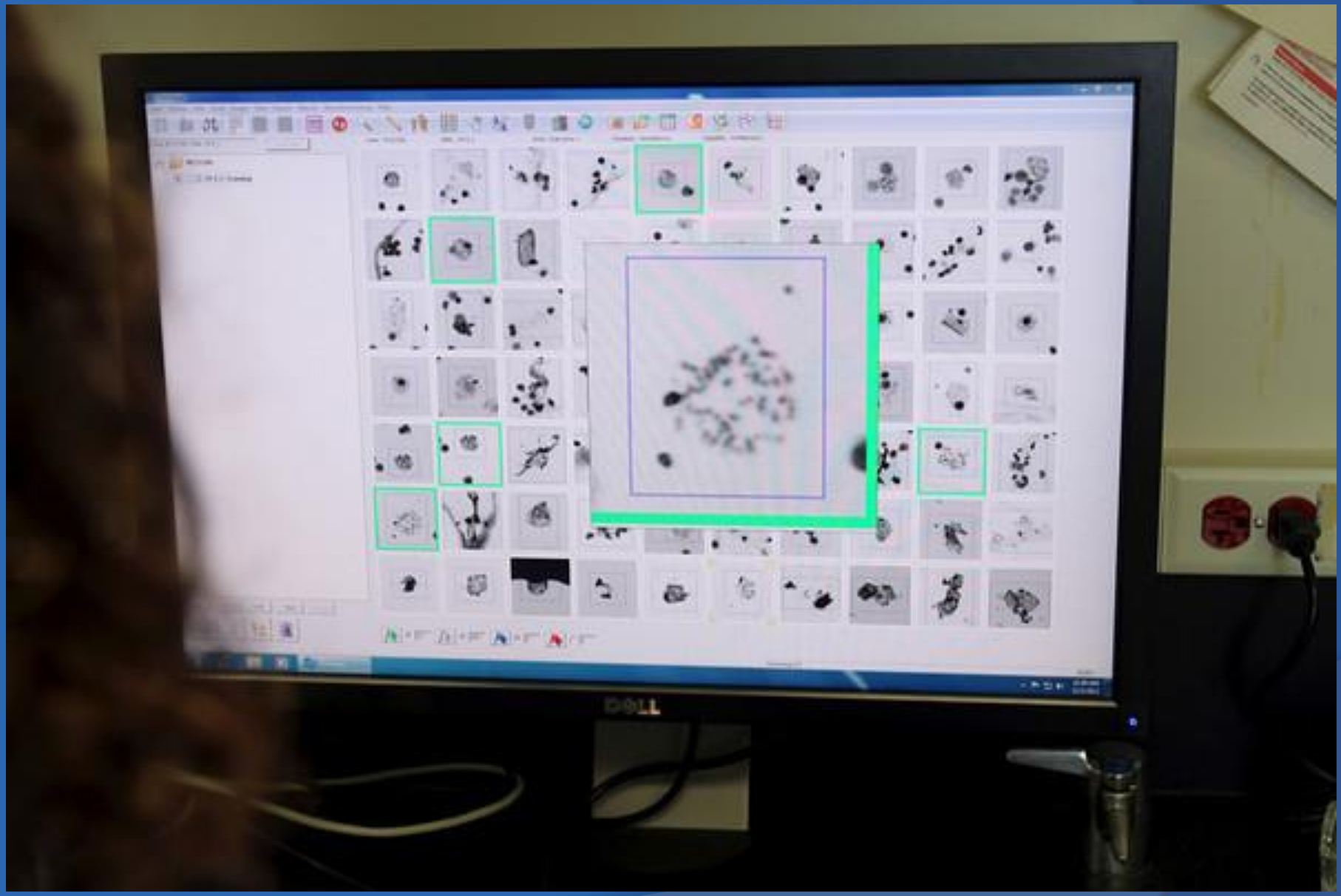
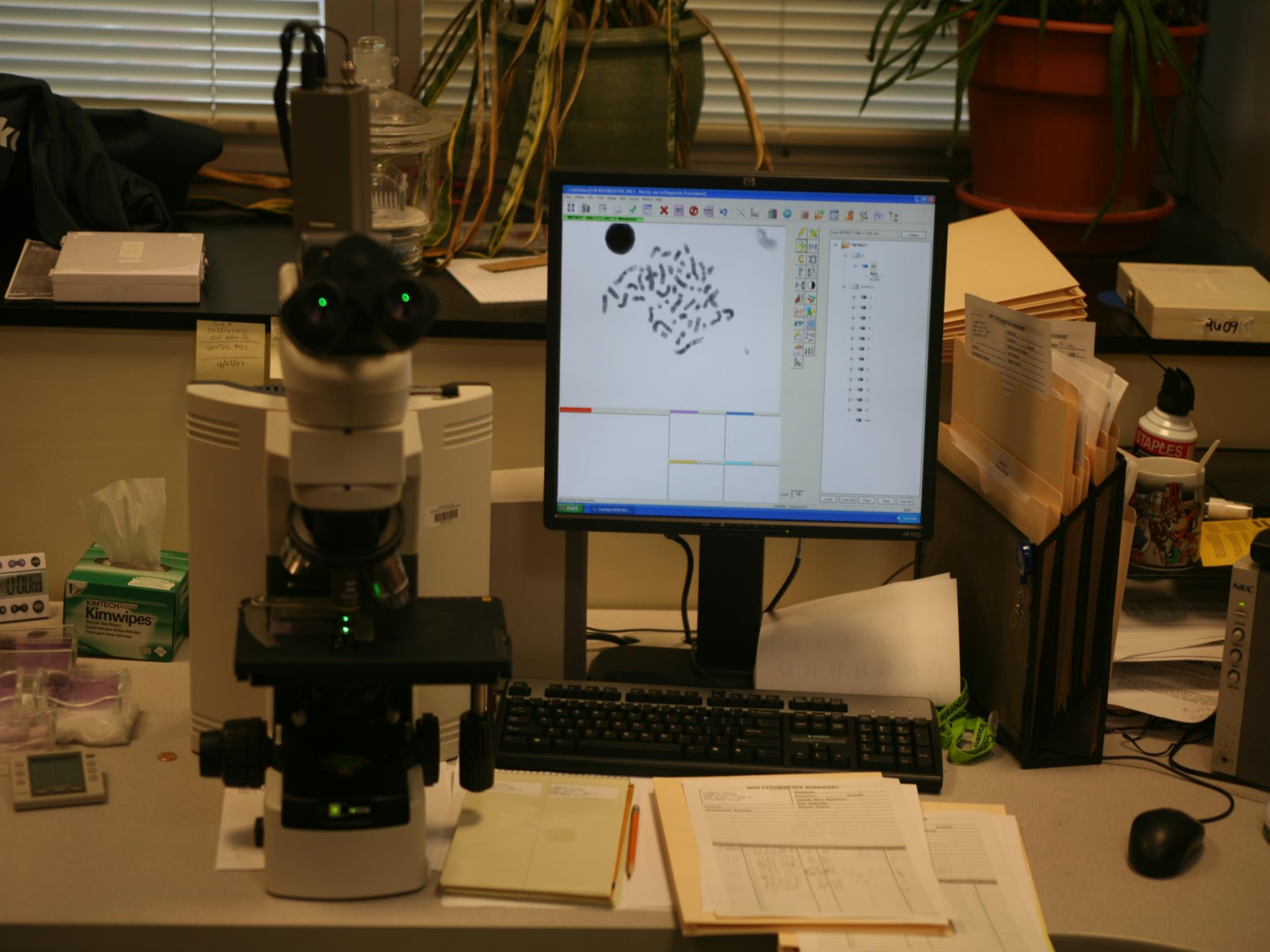
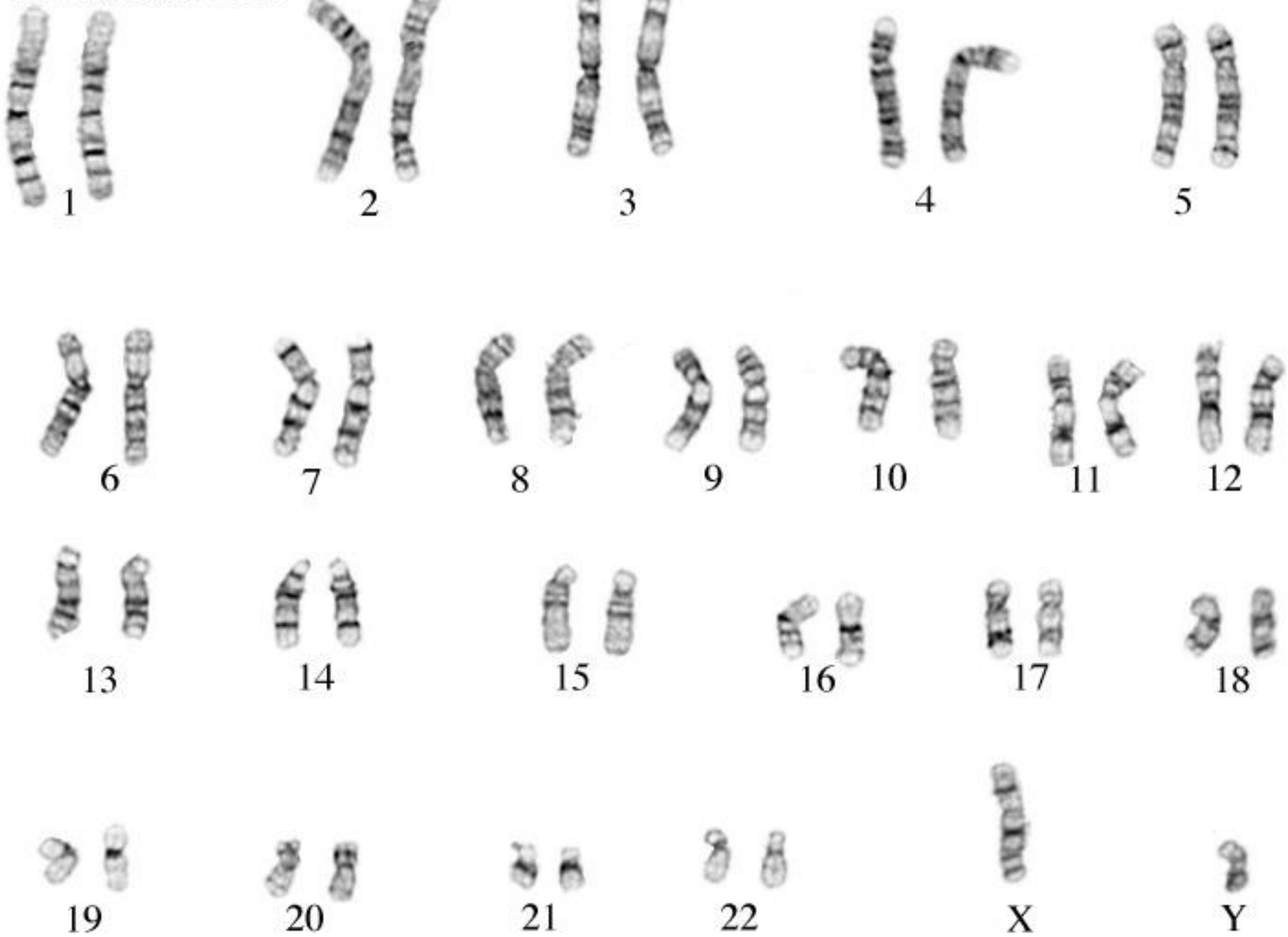
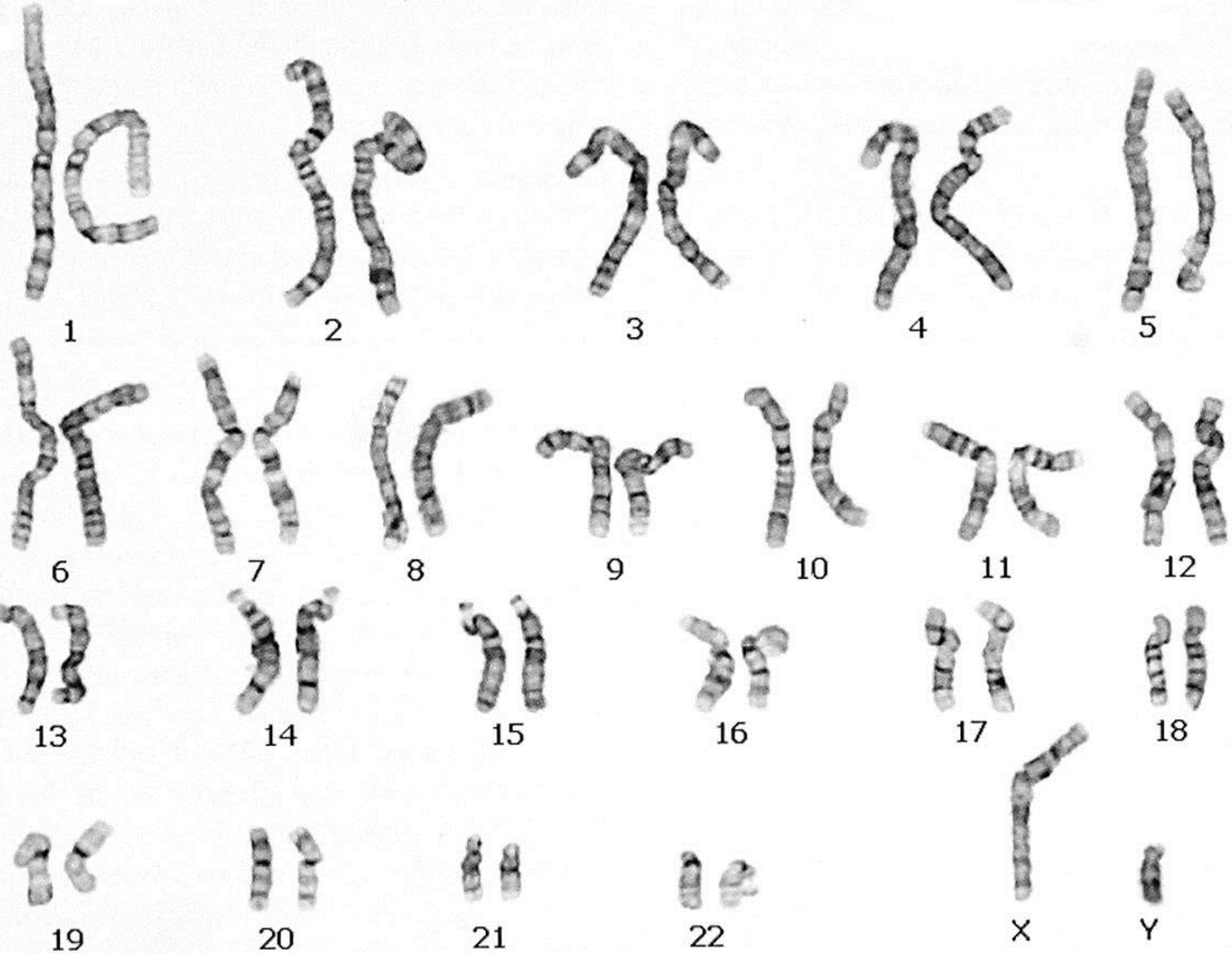





Kimwipes
STAPLES
NEC

ZWK99010 KEY

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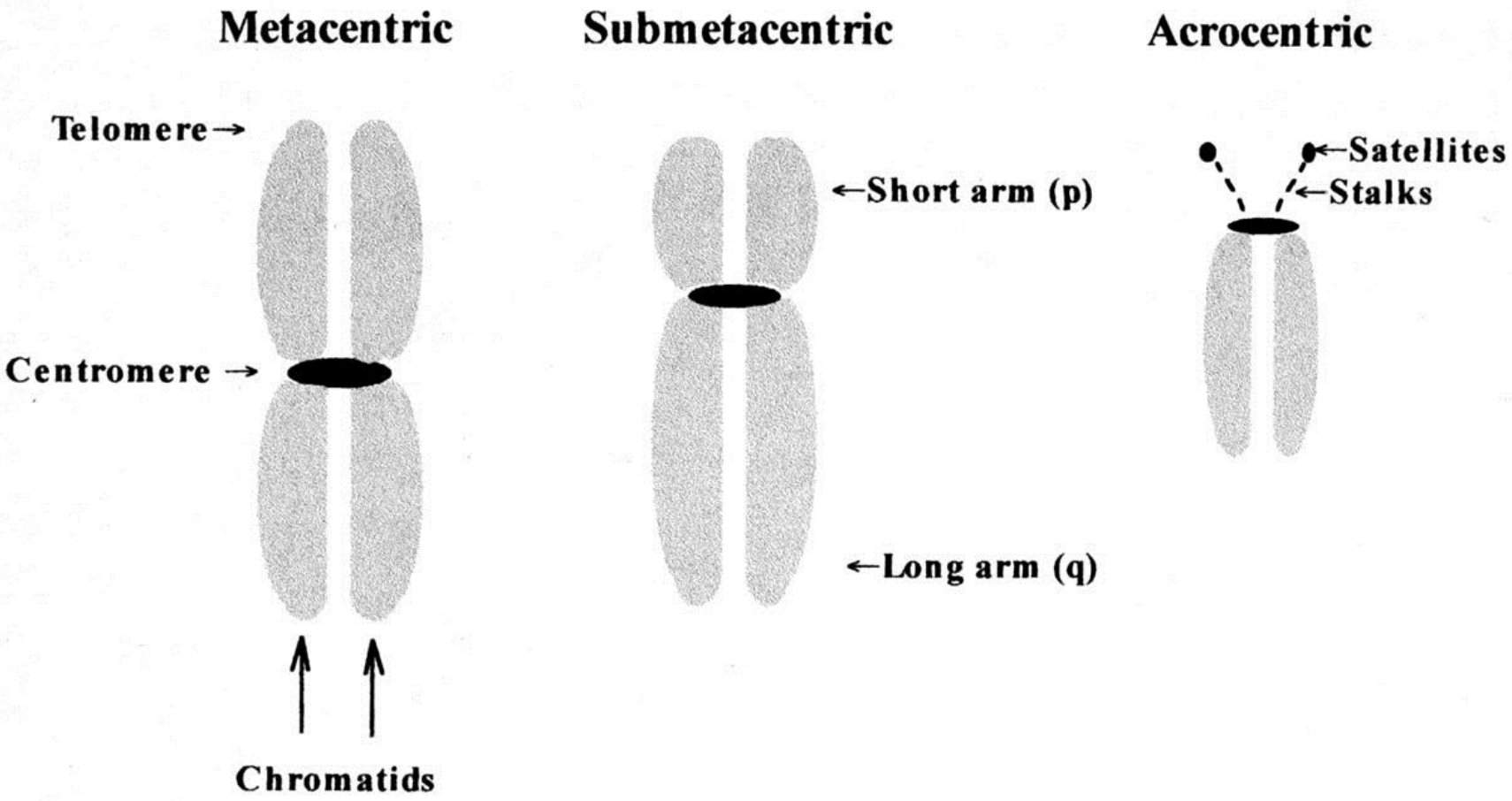
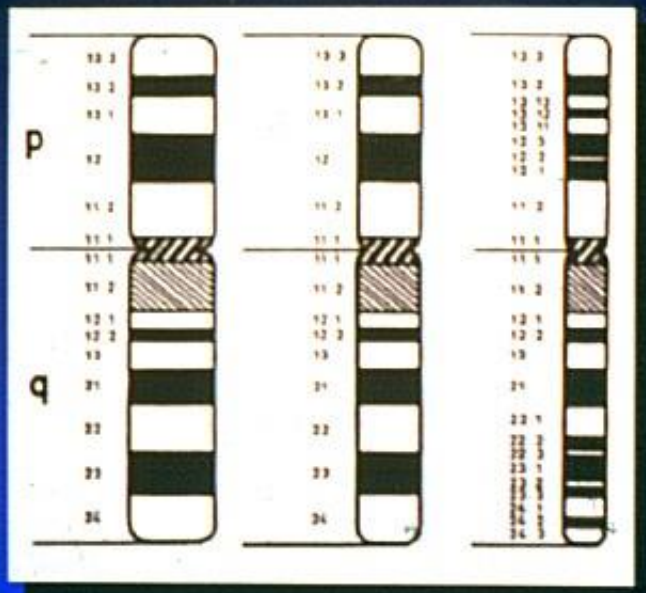


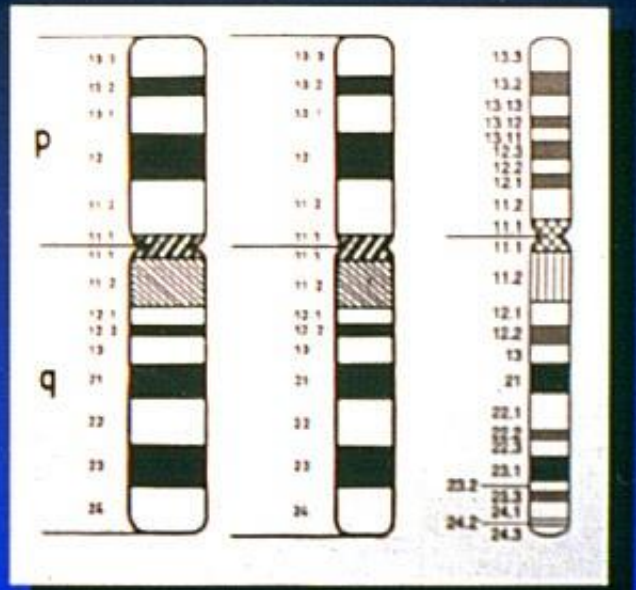

**Fig. 8.** The functional and structural components of metaphase chromosomes.

# IDEOGRAM OF CHROMOSOME 16

## ISCN 1985




## ISCN 1995



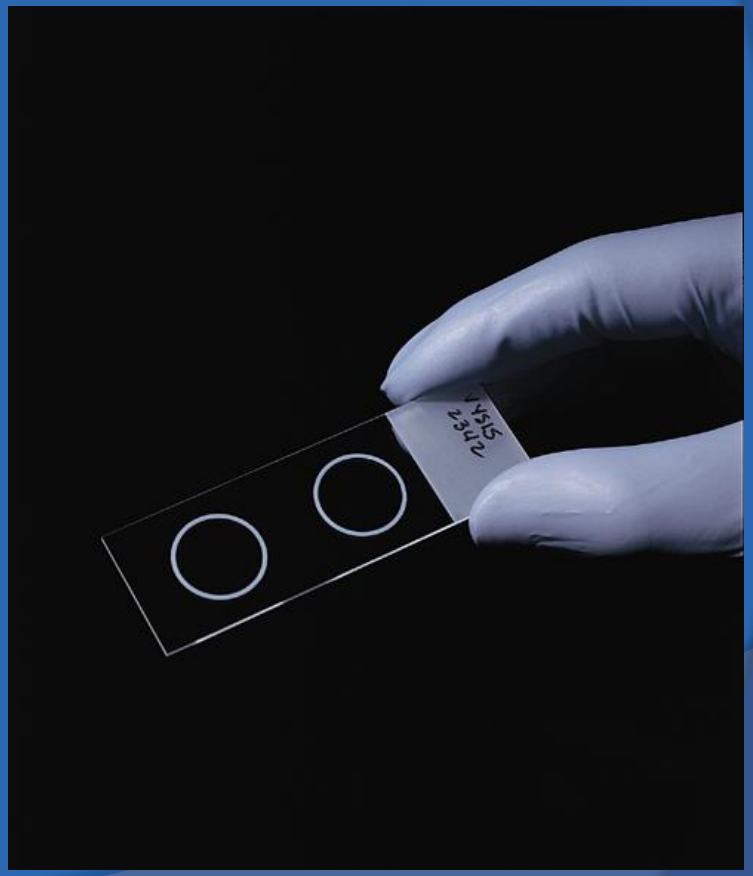

KARGER Basel

# Molecular Cytogenetics

# Fluorescence *in situ* Hybridization (FISH)

# Fluorescence *in situ* Hybridization (FISH)

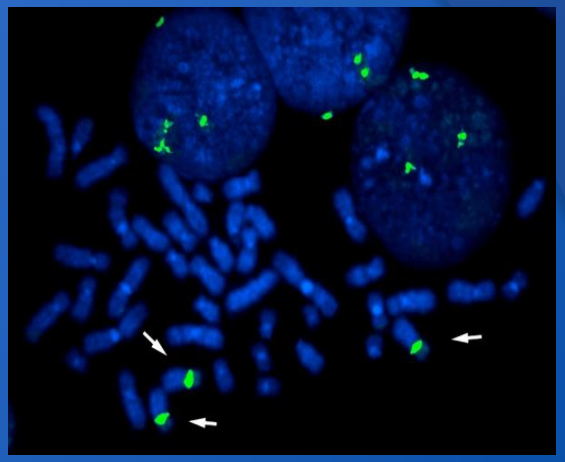
***Hybridization of a fluorescently labeled nucleic acid probe to a target sequence within a specimen on a microscope slide.***

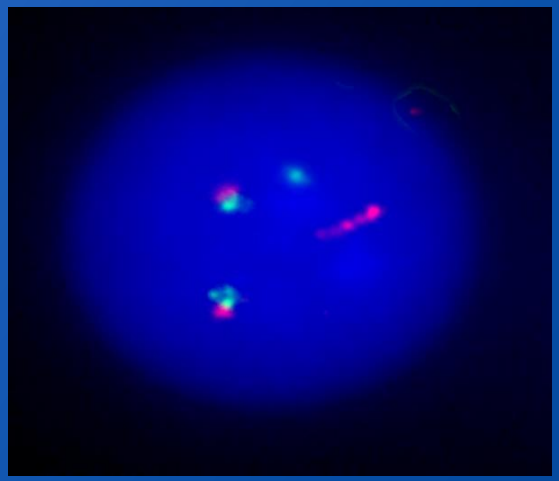


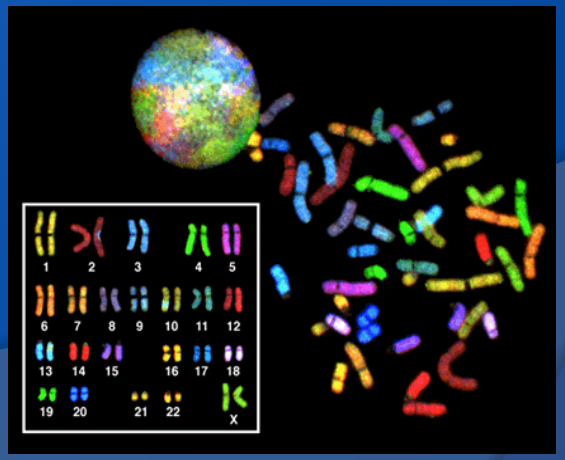

Kimwipes
HYBrite™

# Three types of FISH probes:

- Centromeric probes: trisomy/monosomy
- Locus specific probes: gain or loss, and translocations.
- Chromosome or arms/bands painting probes: structural abnormalities (SKY, M-FISH).







# Molecular Cytogenetic Analysis

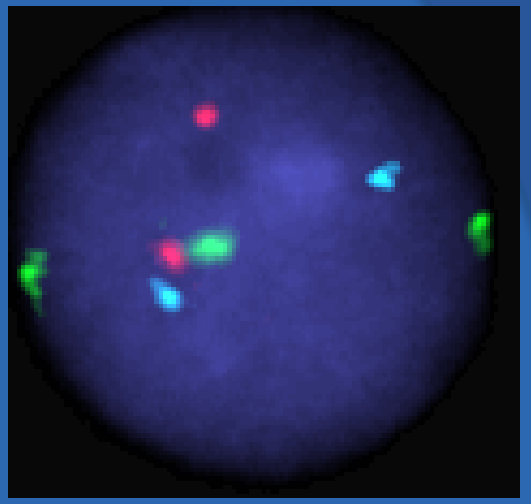
- In situ Hybridization Targets
  - Metaphase chromosomes
  - Interphase nuclei
  - Formalin-fixed, paraffin-embedded tissues

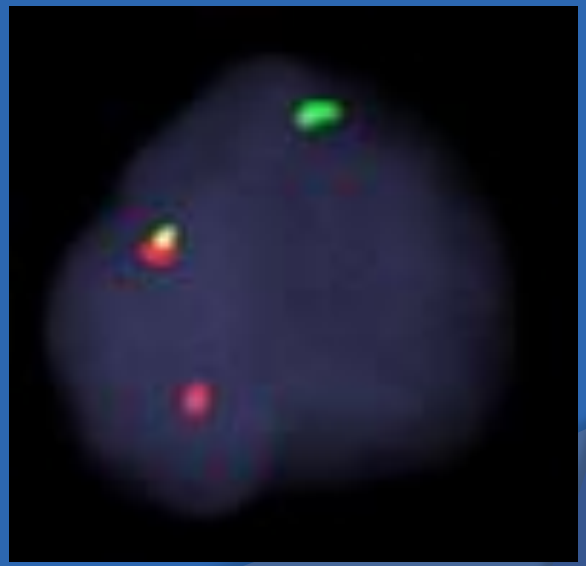
# Sample types and preparation for FISH

- Bone Marrow
- Peripheral blood
- Lymph node
- Tumor mass
- CSF
- Plural fluid

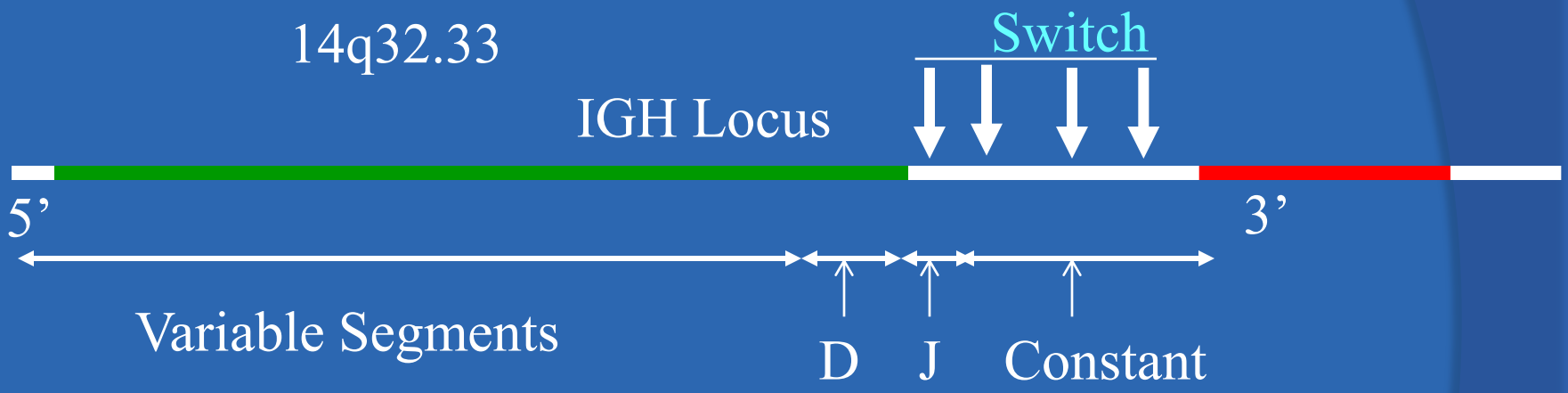
- Fresh BM/PB/LN
- Cytospin slides
- BM/PB smear
- G-banded cytogenetic slides
- H &E stained slides
- PET section

- Each probe attaches to specific  target.
- Differently colored probes simultaneously determine both number and location of different loci.
- Metaphase & interphase
- Quantitative assessment in intact tissue, cells, nuclei or chromosomes.





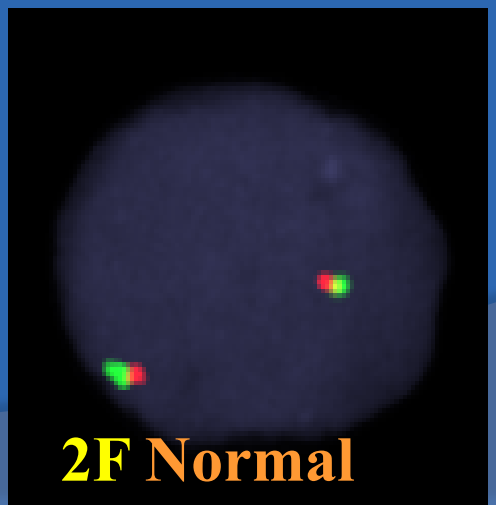
# Dual Color, Break-apart: Both Probes Flank One Breakpoint

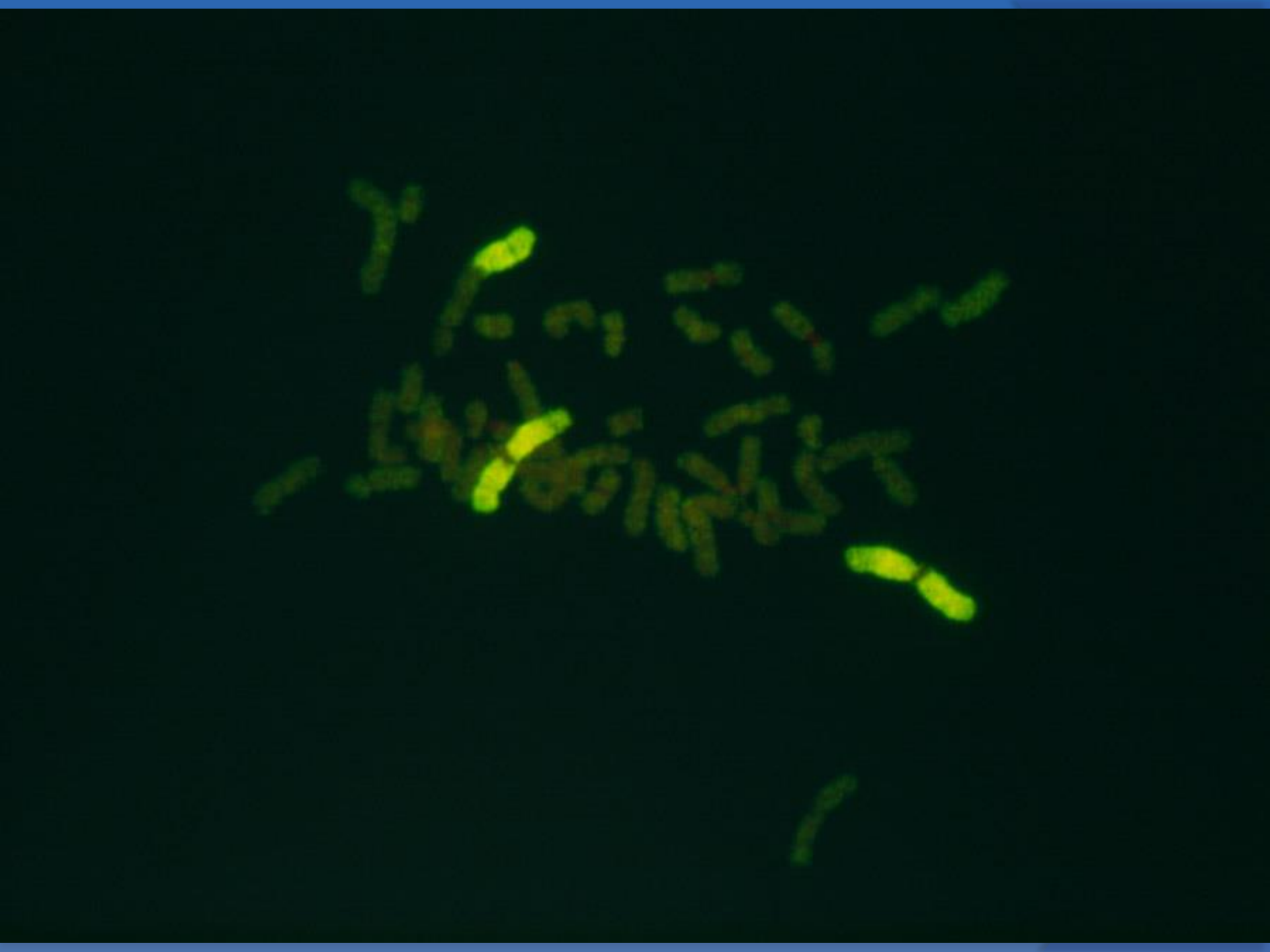



Features:

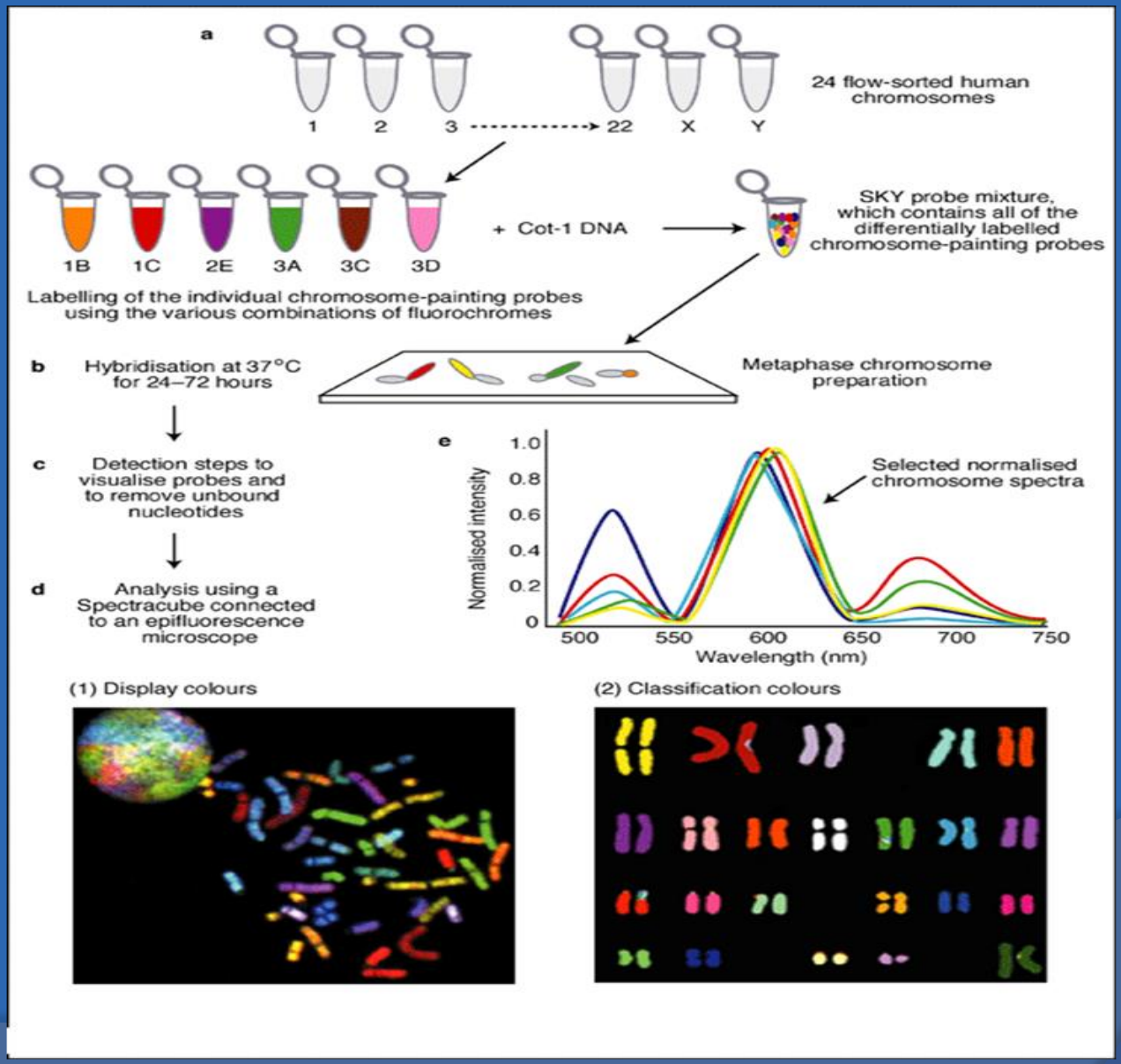
- Cutoff for probe design~1-3 %*
- Best design for intact sections
- Useful for multiple translocation partners: IGH, MLL, BCL6, MYC, ALK

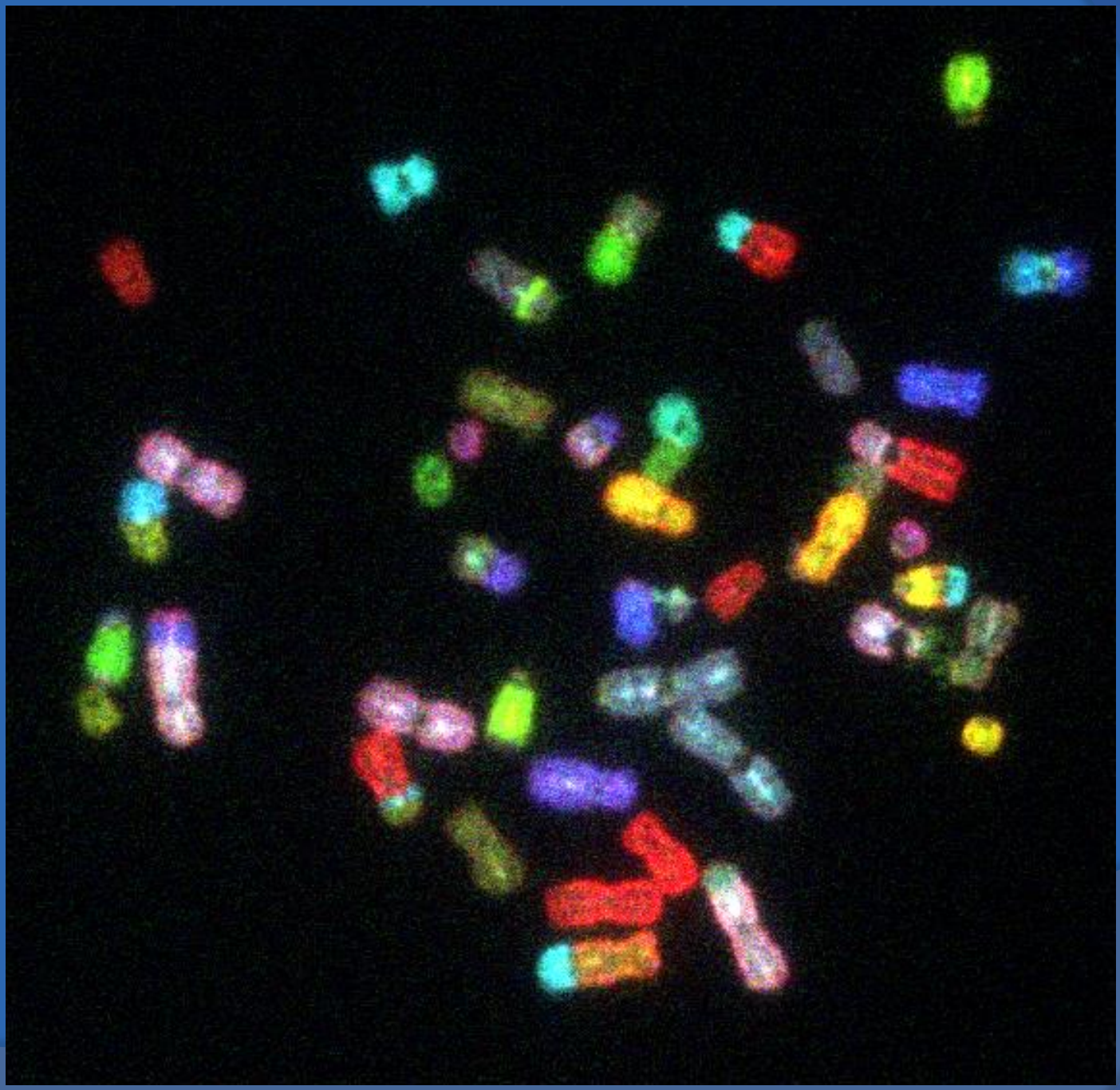
*** Cutoff values for probe types from Dewald et al, Chap 32,Clinical Laboratory Medicine, K. McClatchey Ed., 2002**

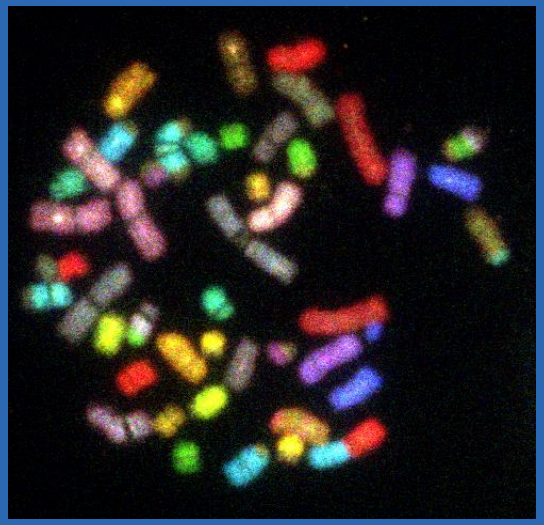


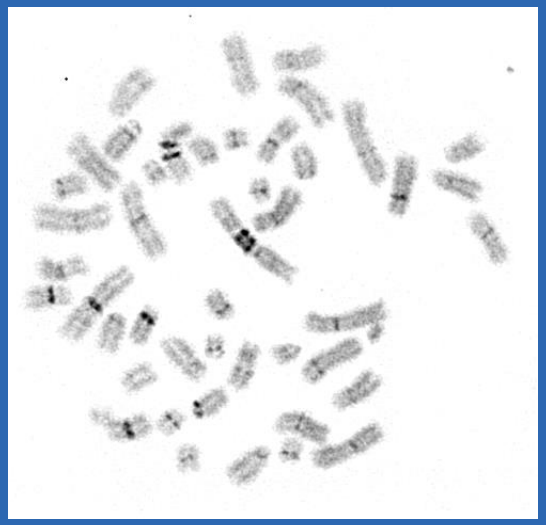



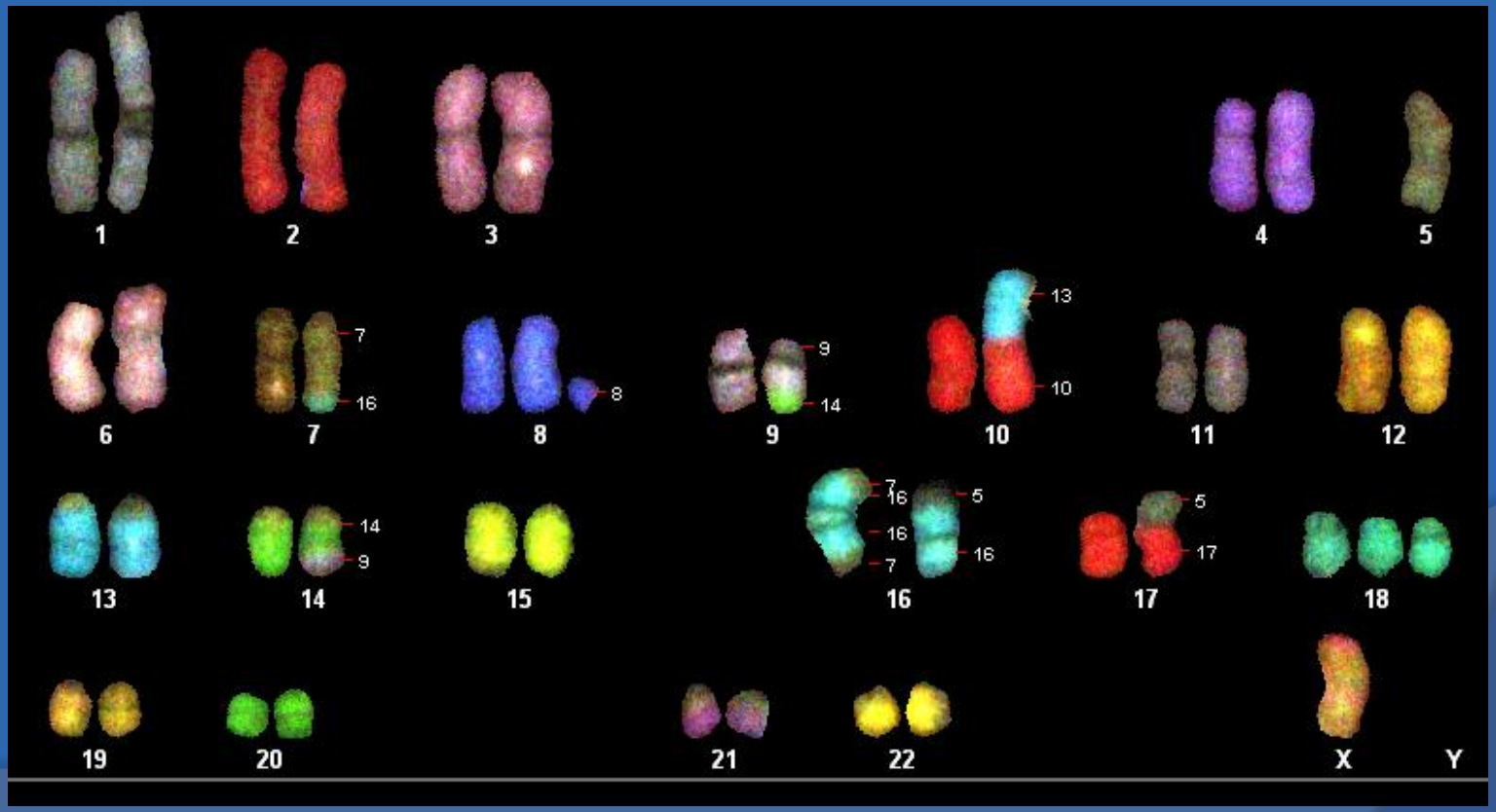
# SKY Procedure Overview







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# CYTOGENETICS VS FISH: PLUS AND MINUS

## Cytogenetics

**Plus:**

- Scan for abnormalities of all chromosomes, arms, regions and bands of a cell.
- Diagnostic: specific chromosome abnormalities.
- Identify new tumor clone markers for follow-up.
- Clonal evolution evidence.

**Minus:**

- Needs fresh samples,
- Need dividing cells and analyzable metaphase cells.
- Low sensitivity (1/20).
- Low resolution (>10 Mb): missing subtle and cryptic changes.
- Heavily rely on technicians' experience.

## FISH

**Plus:**

- Easier, simpler and faster.
- High sensitivity (of 200 cells),
  i.e., follow-up of RD.
- High resolution(>100 kb).
- Single cell analysis; Correlate with morphology and immunophenotyping.
- No metaphase cells needed.
  Fresh tissue or fixed section.
  Terminally differentiated cells.
  Low mitotic cells (CLL).

**Minus:**

- Target regions only.
- No whole chromosome pictures.
- Limited probes.

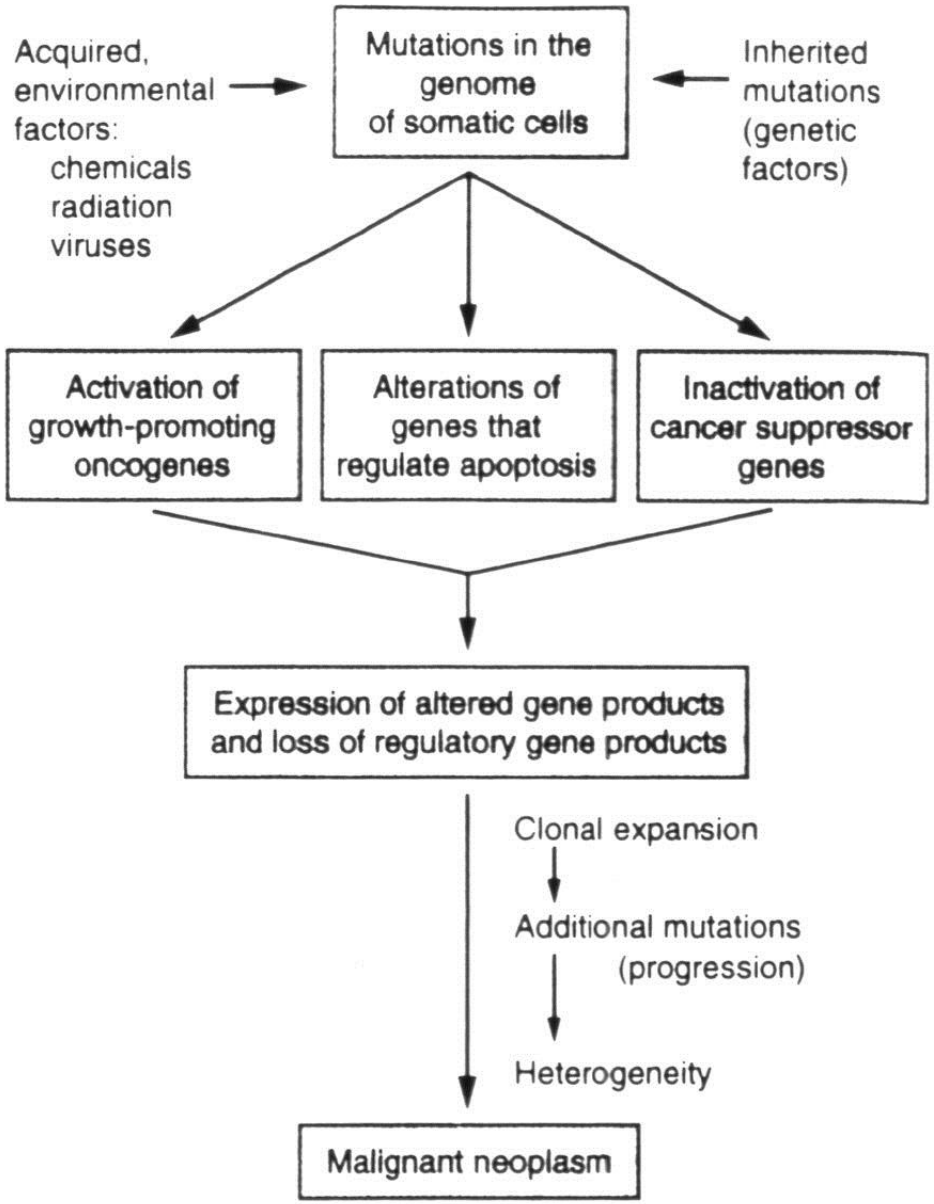
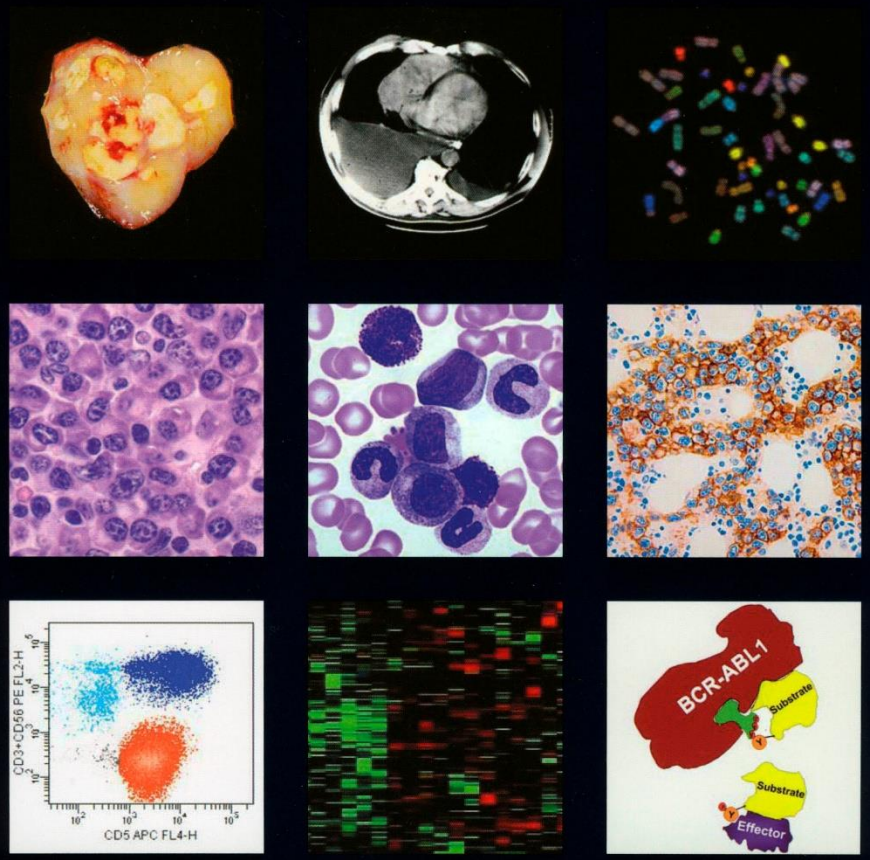



**Figure 7–22. Flow chart depicts a simplified scheme of cancer pathogenesis.**

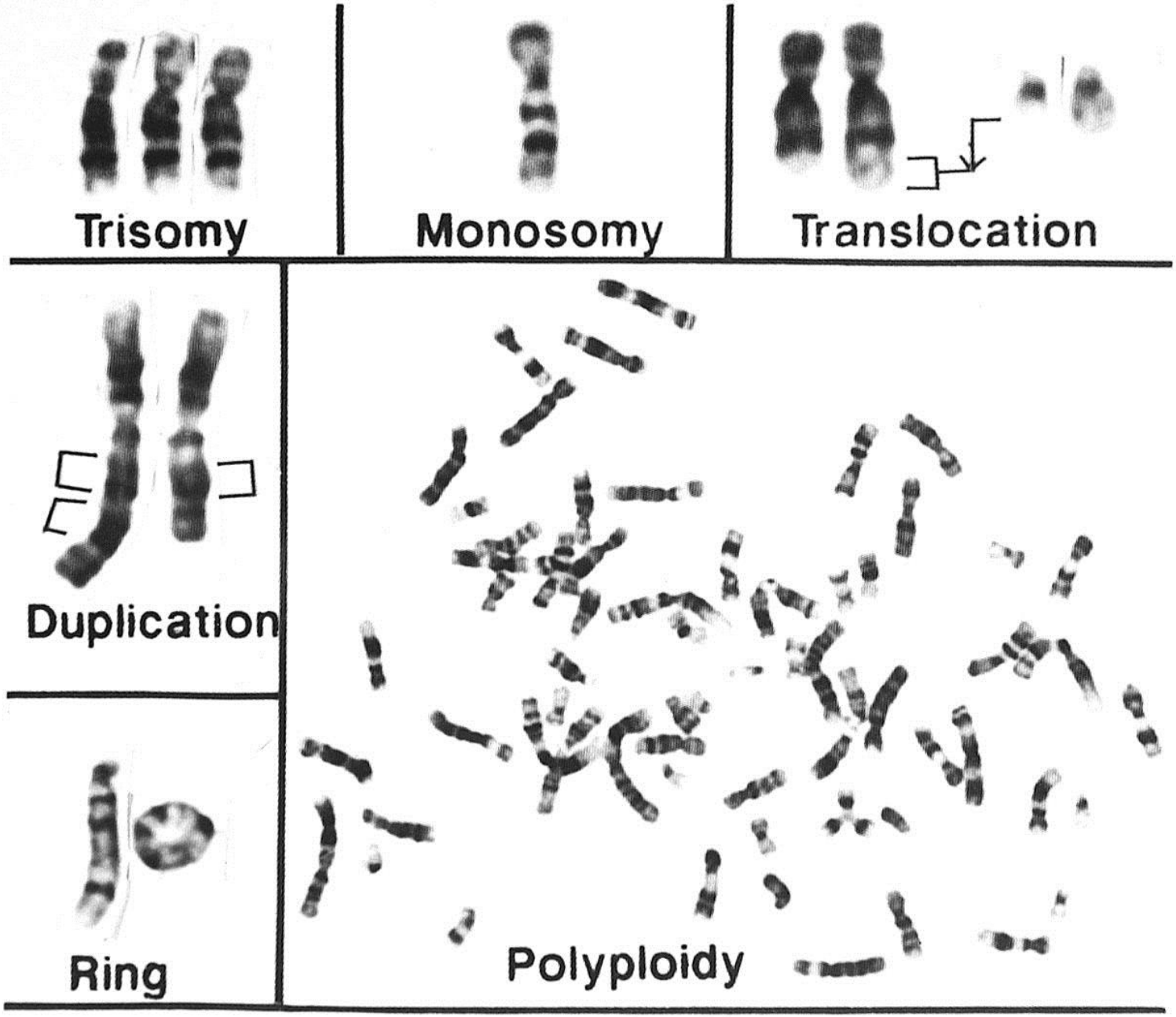
# WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

Edited by Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe,
Stefano A. Pileri, Harald Stein, Jürgen Thiele, James W. Vardiman

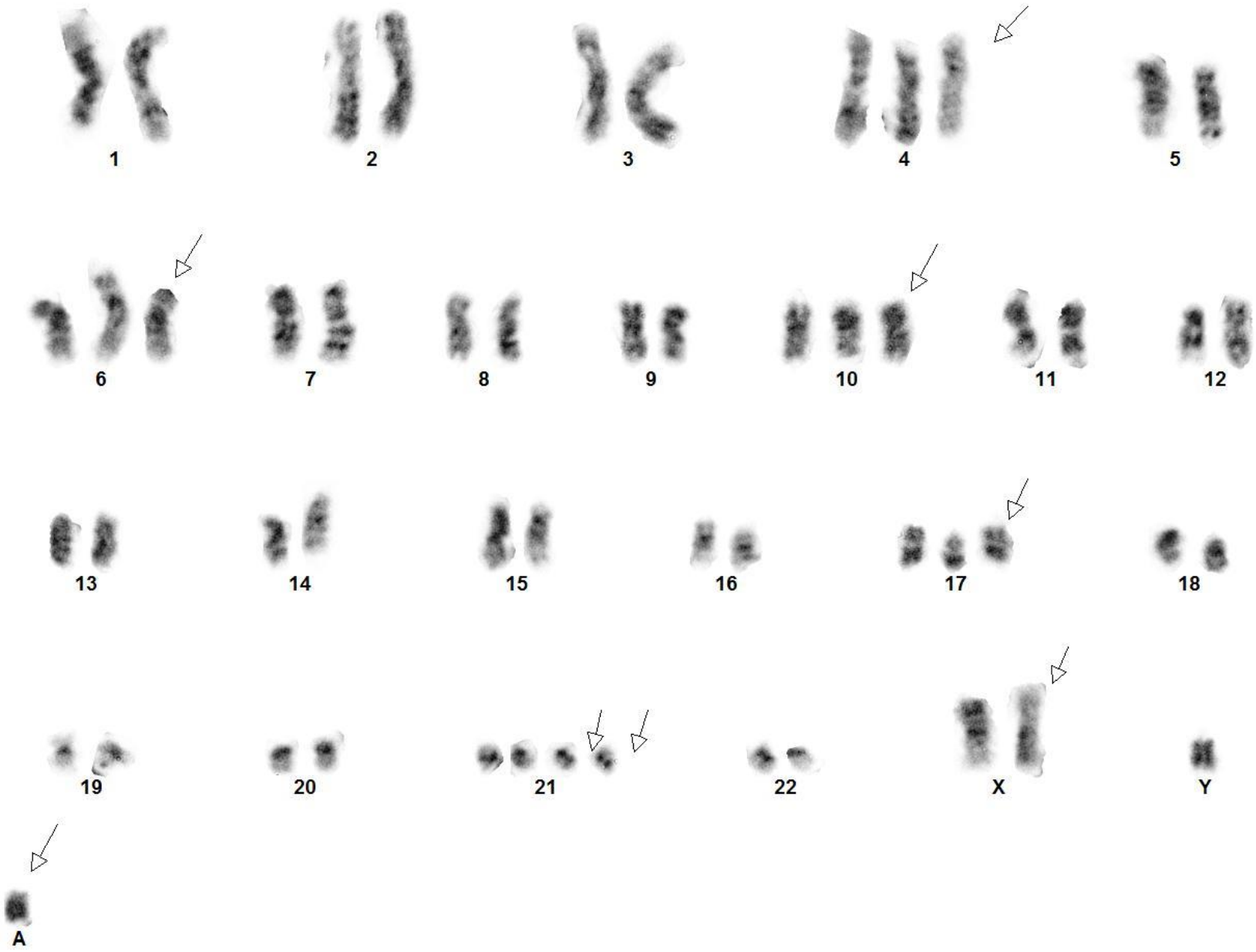



WHO

- **Mitotic events affecting chromosomes in neoplastic cells:**
  - **Abnormal segregation**
  - **Chromosome breakage**


Trisomy
Monosomy
Translocation
Duplication
Ring
Polyploidy

**Karyotype 1**

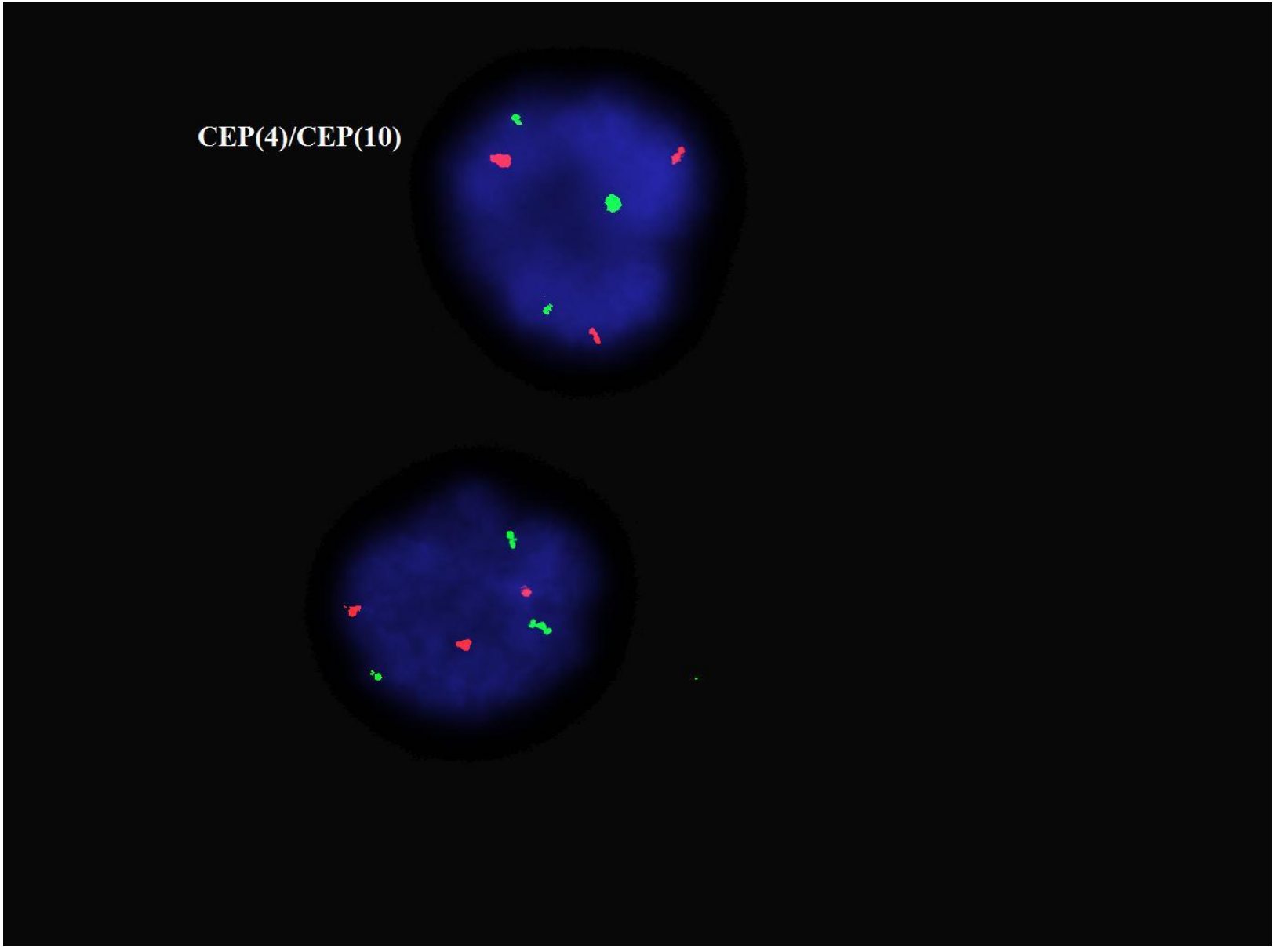



54,XY,+X,+4,+6,+10,+17,+21,+21,+mar

# FISH CEP(4)(SO)/CEP(10)(SG)

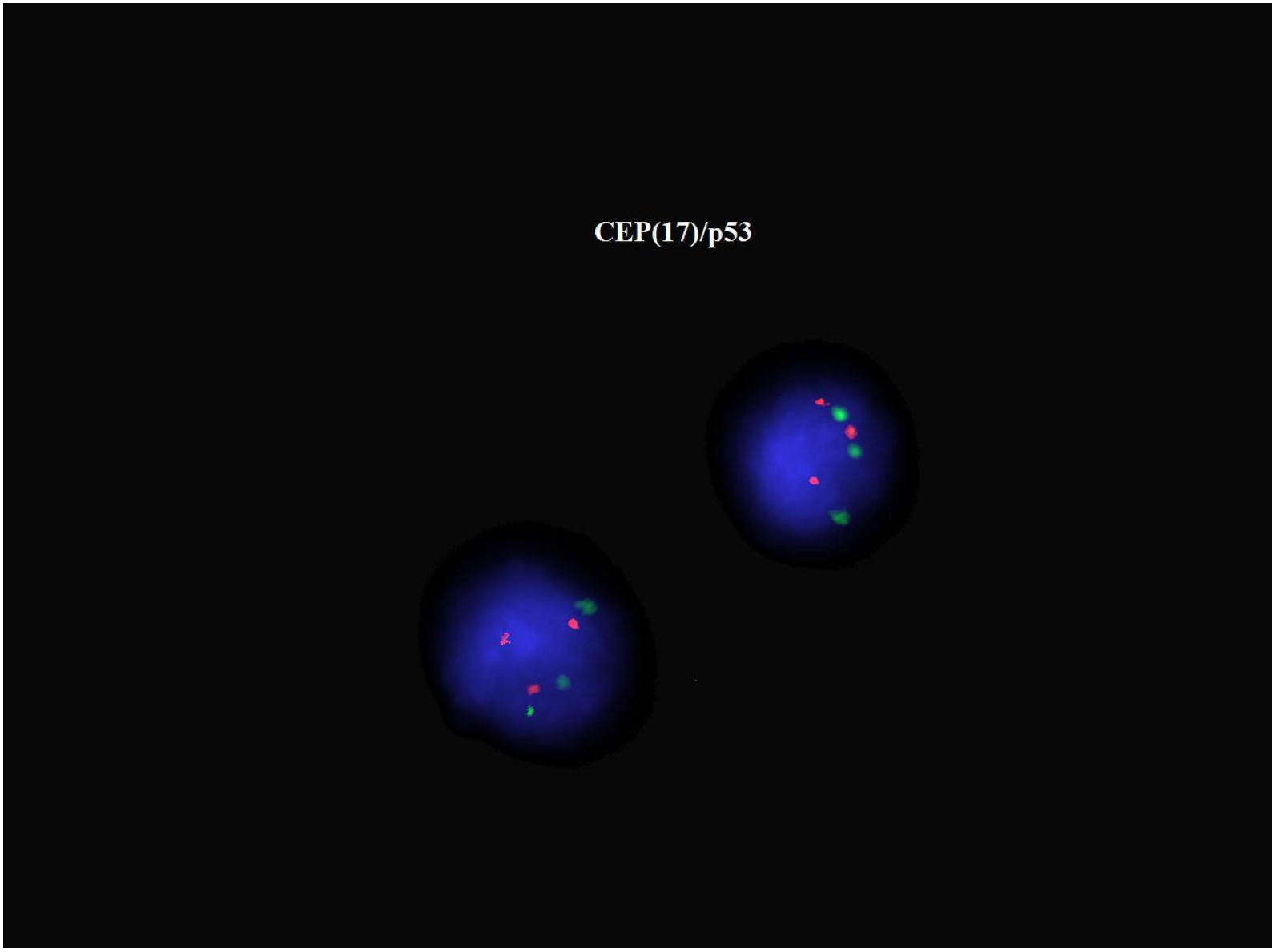
**probe**

- **CEP(4)(SO)/CEP(10)(SG)**
- **3O3G**
- **abnormal**

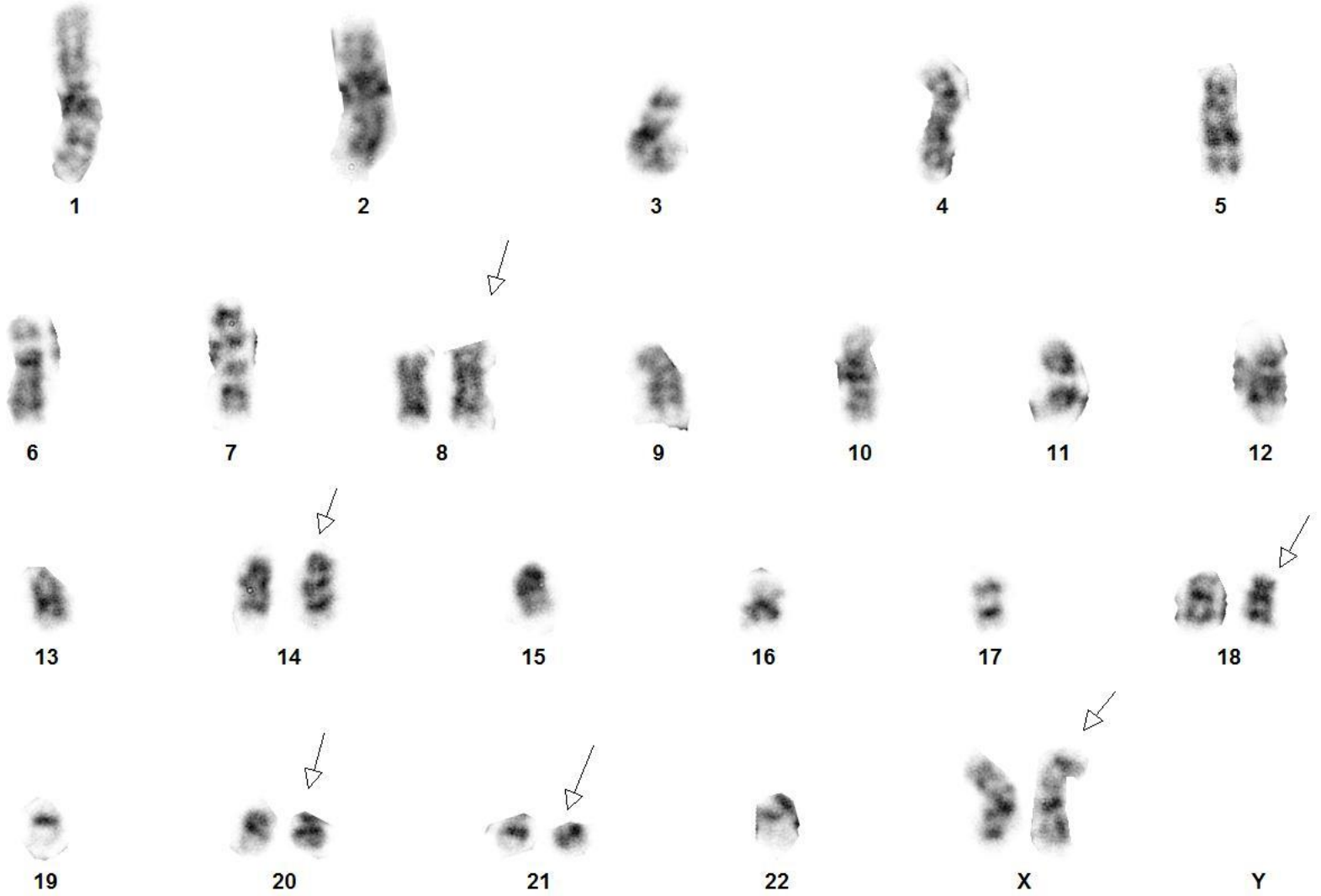


# FISH D17Z1(G)/P53(O)

**probe**
- **D17Z1(G)/P53(O)**
- **3G/3O**
- **abnormal**

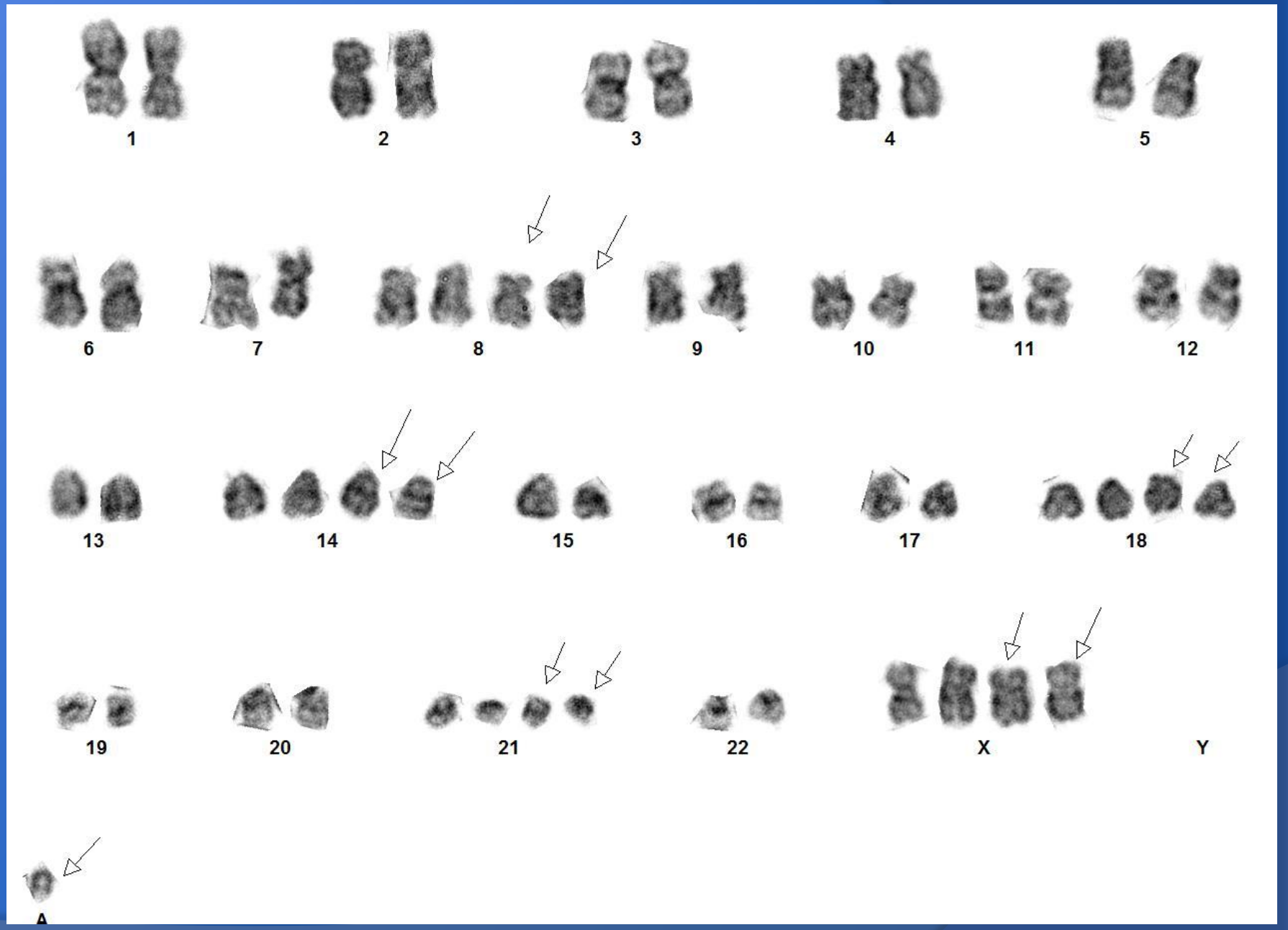


# Karyotype 1




29<n>,X,+X,+8,+14,+18,+20,+21

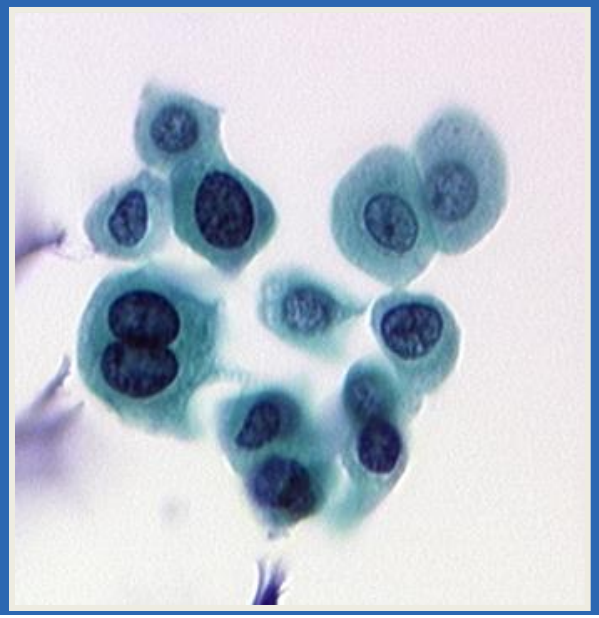
# Karyotype 4




57<2n>,XX,+X,+X,+8, +8,+14,+14,+18,+18,+21,+21,+mar

Cytology

UroVysion (FISH)



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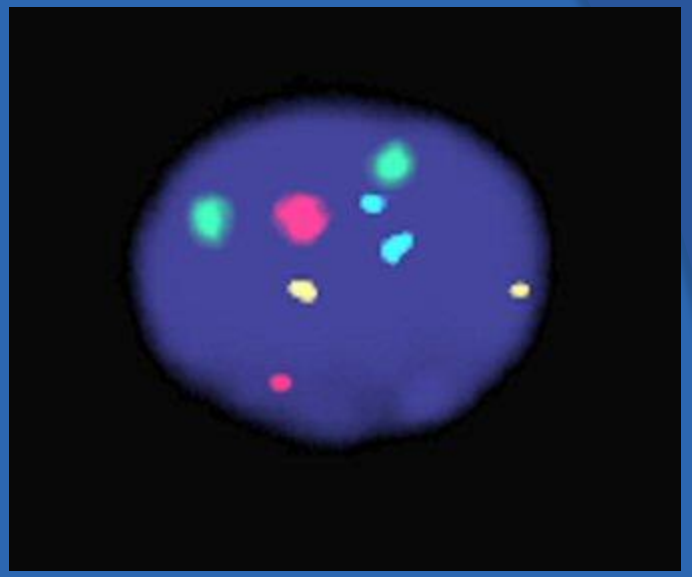
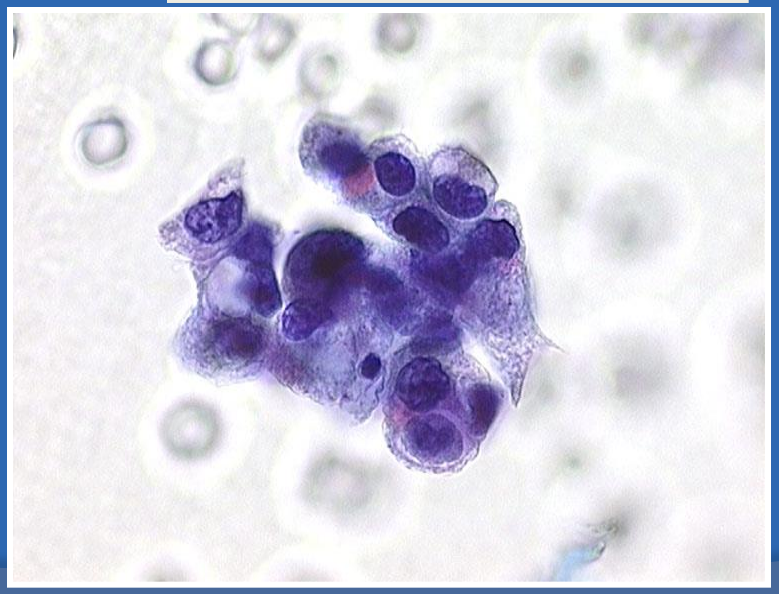
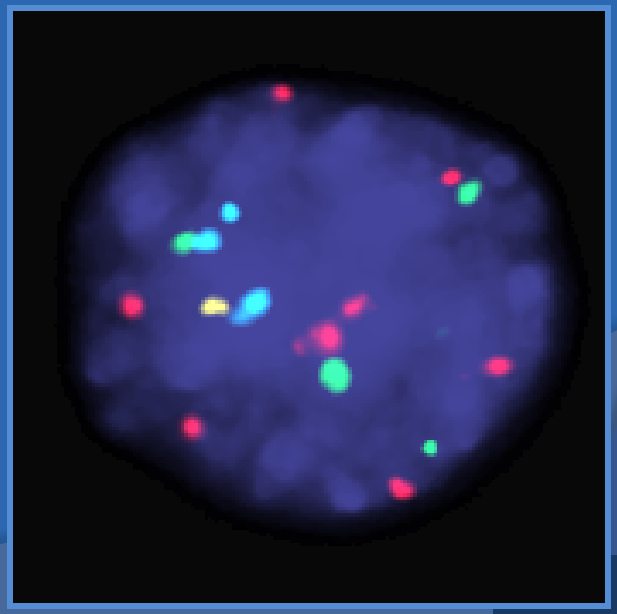
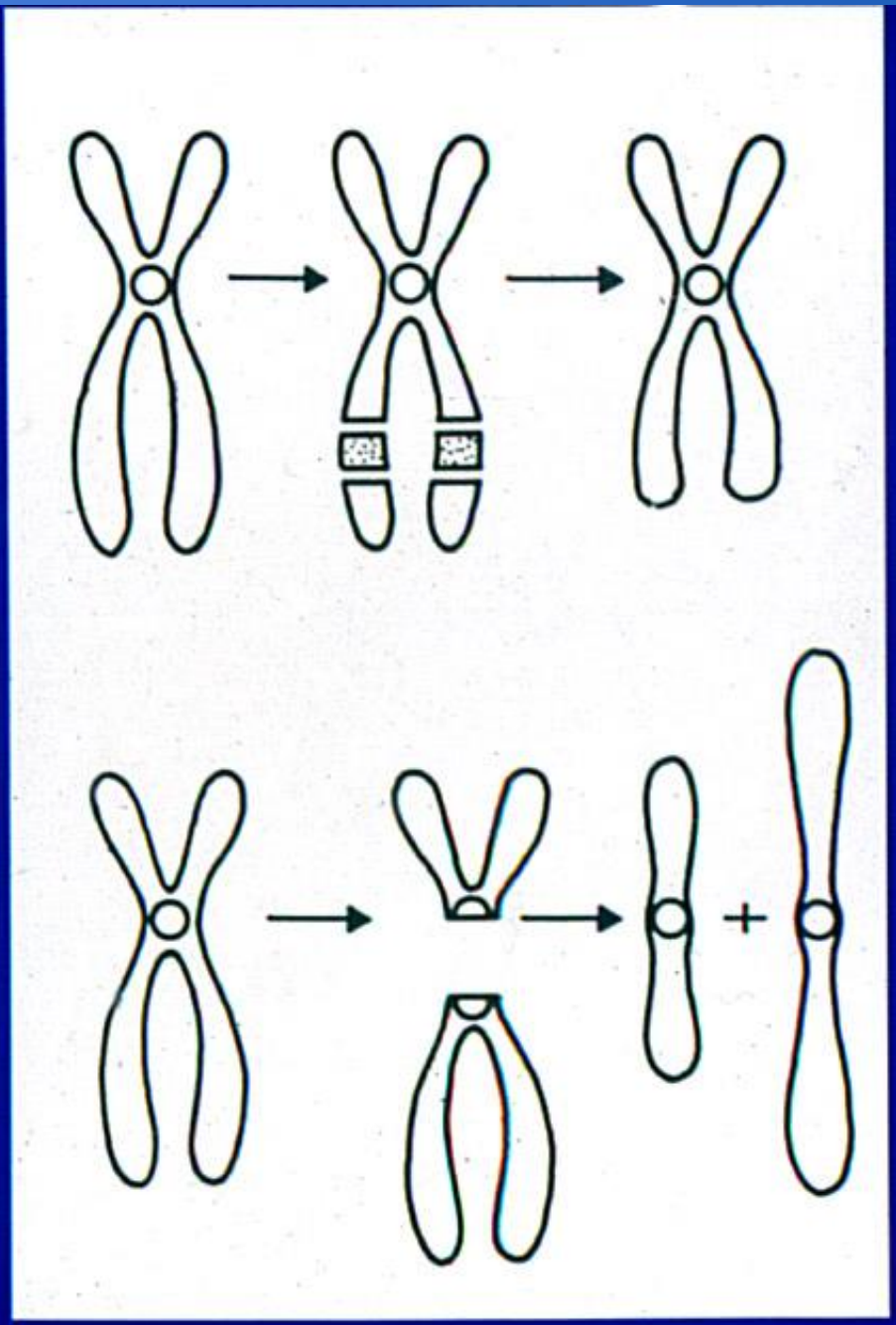
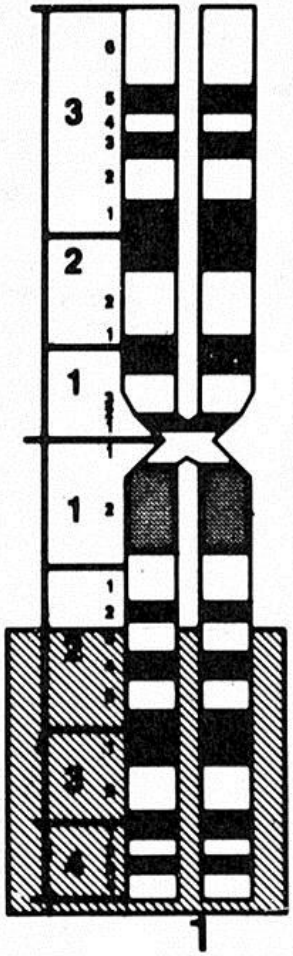


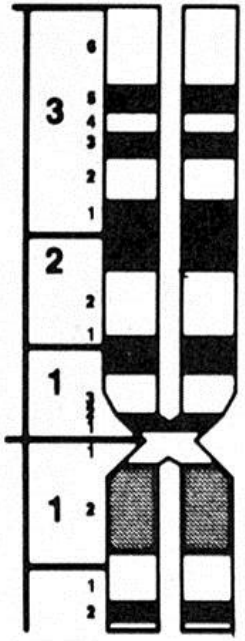


Abnormal

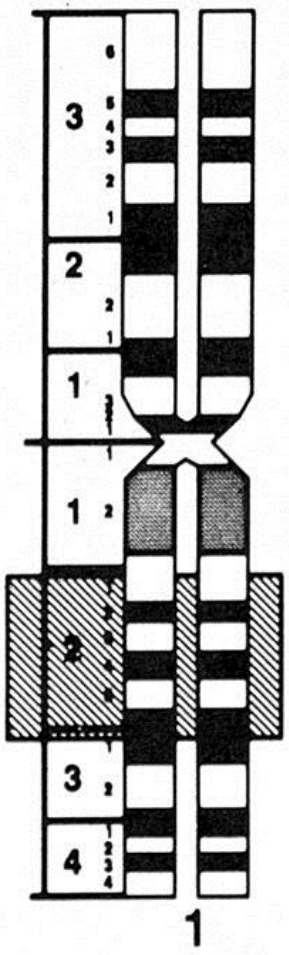


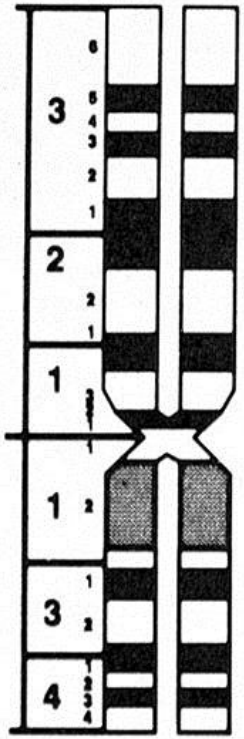




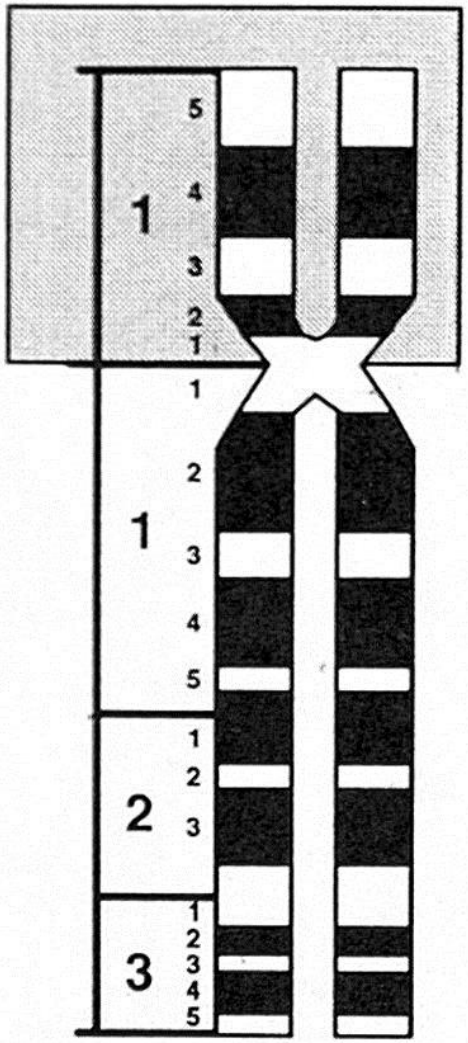




del(1)(q23)

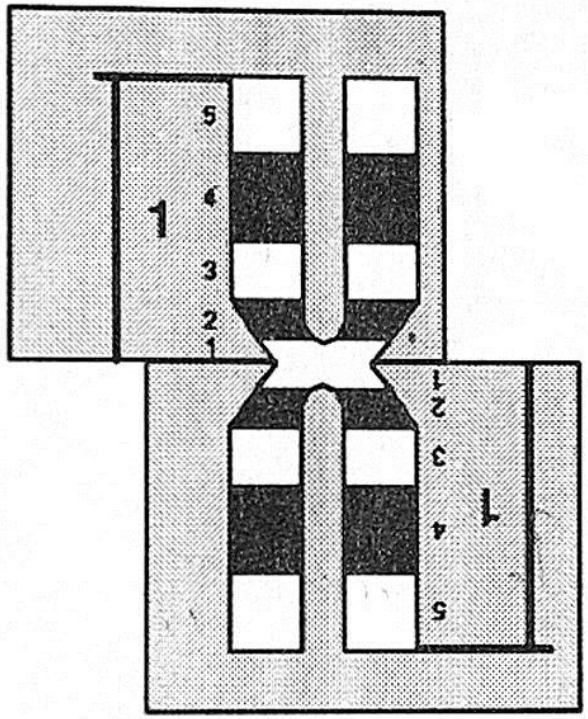


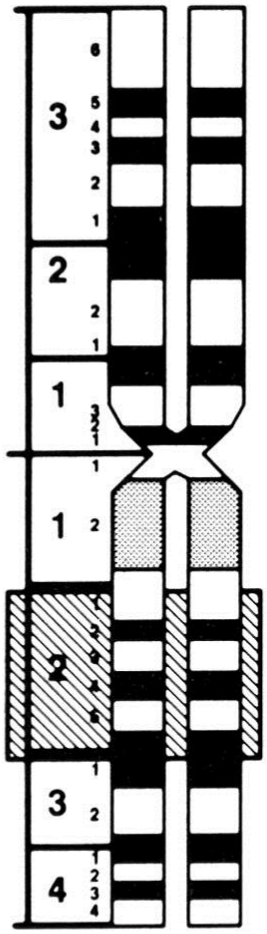

del(1)(q21q31)




5




i(5)(p10)



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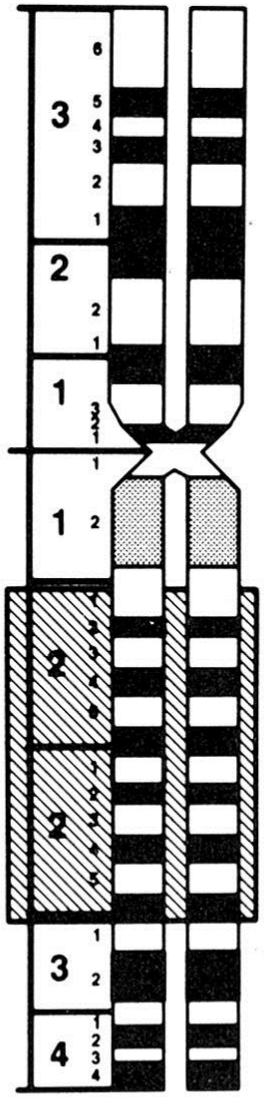
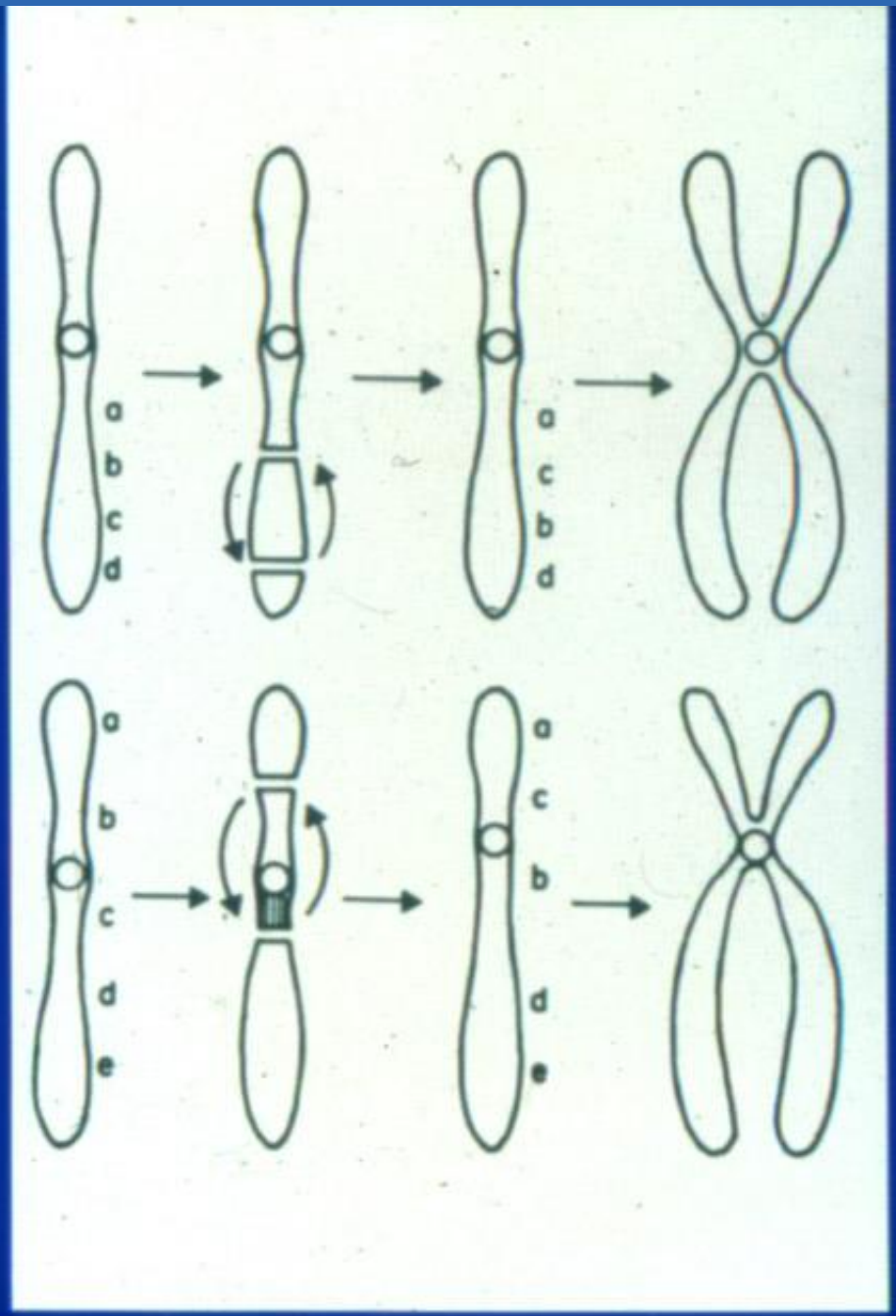


dup(1)(q21q31)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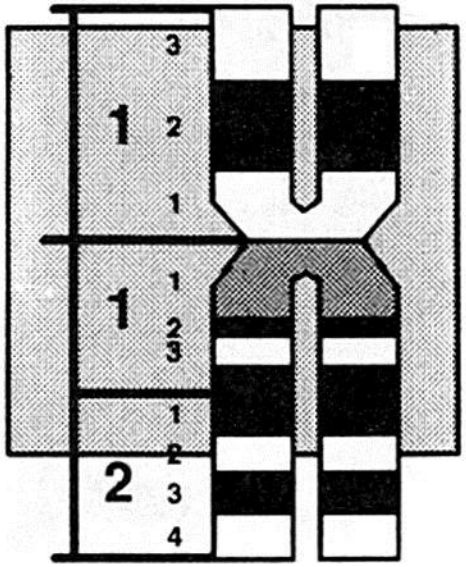
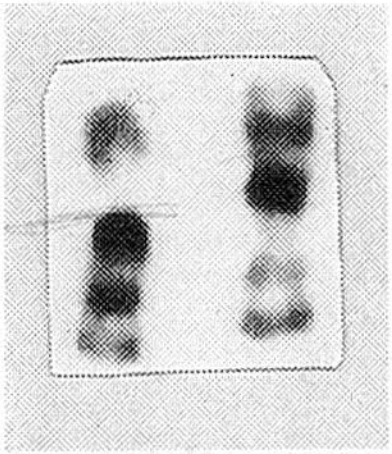
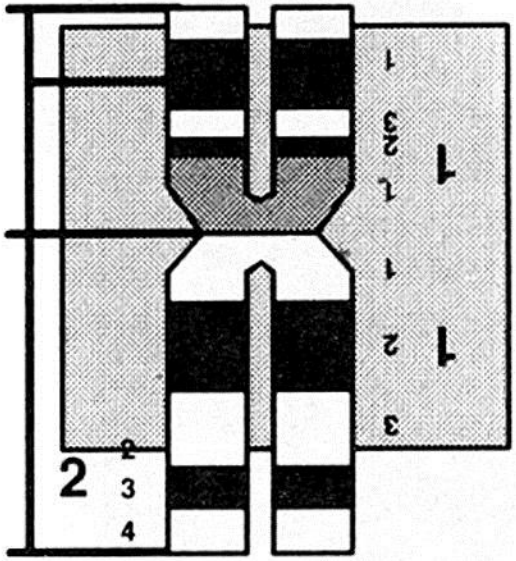
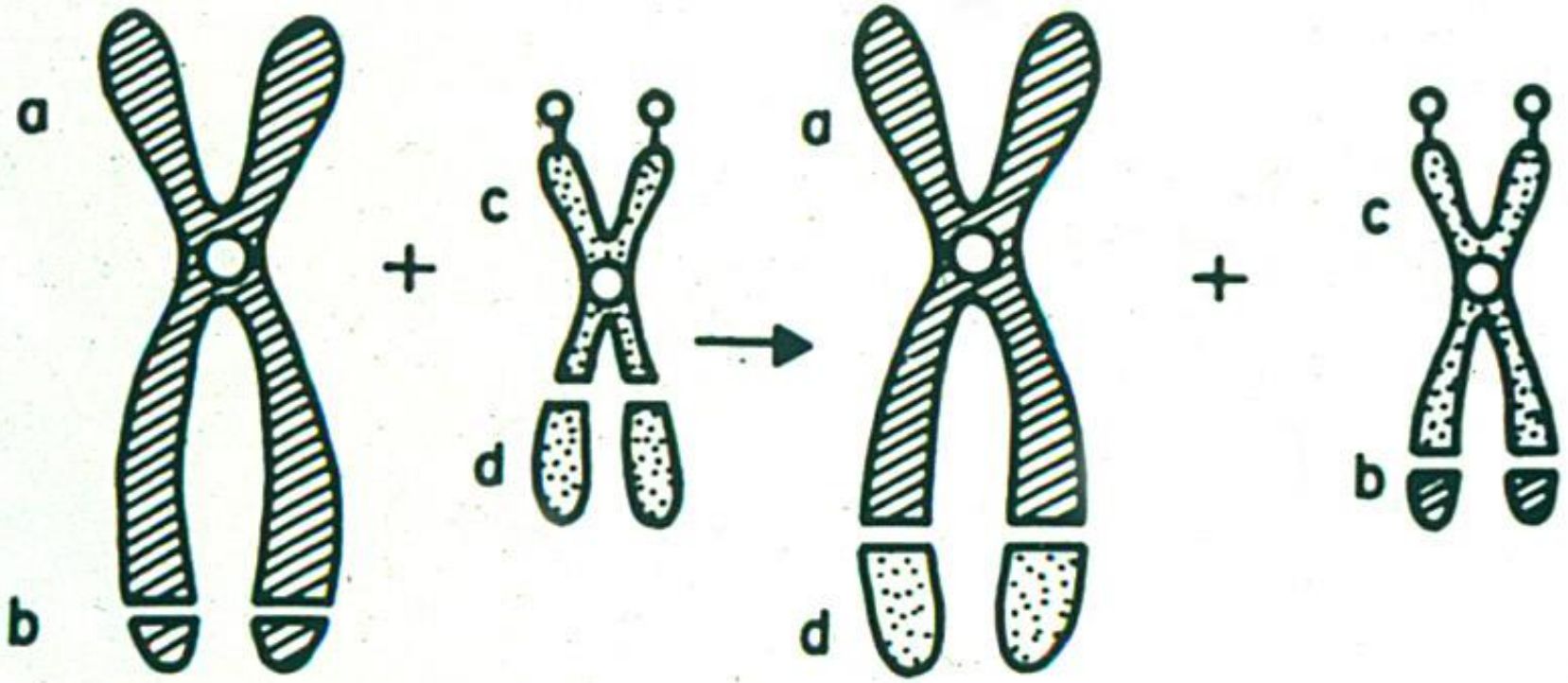



16






inv(16)(p13q22)


a
b
+
c
d
a
d
+
c
b

# GENETIC CONSEQUENCES OF CHROMOSOMAL TRANSLOCATION

# Mechanisms of leukemia transformation

- Requirements for chromosomal translocations:
  - At least 2 double-strand breaks (misrepair of double strand breaks).
  - Most reciprocal translocations appear to arise by NHEJ (non-homologous end joining).

# Endogenous double-strand breaks

- **Double-strand breaks arise spontaneously during normal DNA metabolism, including DNA replication and repair, and during programmed genome rearrangements, such as V(D)J recombination.**

# Exogenous double-strand breaks

- Double strand-breaks are produced by a wide variety of exogenous DNA damaging agents including ionizing radiation, radiomimetic drugs and topoisomerase inhibitors.

- Tobacco smoke induces mutations and double-strand breaks in a dose-dependent manner. This damage is mediated through free radicals.

# Consequences of chromosome translocations

## 1. Deregulated expression of a normal protein

**t(8;14)**

Promoter of IgH — Coding regions of c-MYC

→ **Increased expression of c-MYC**

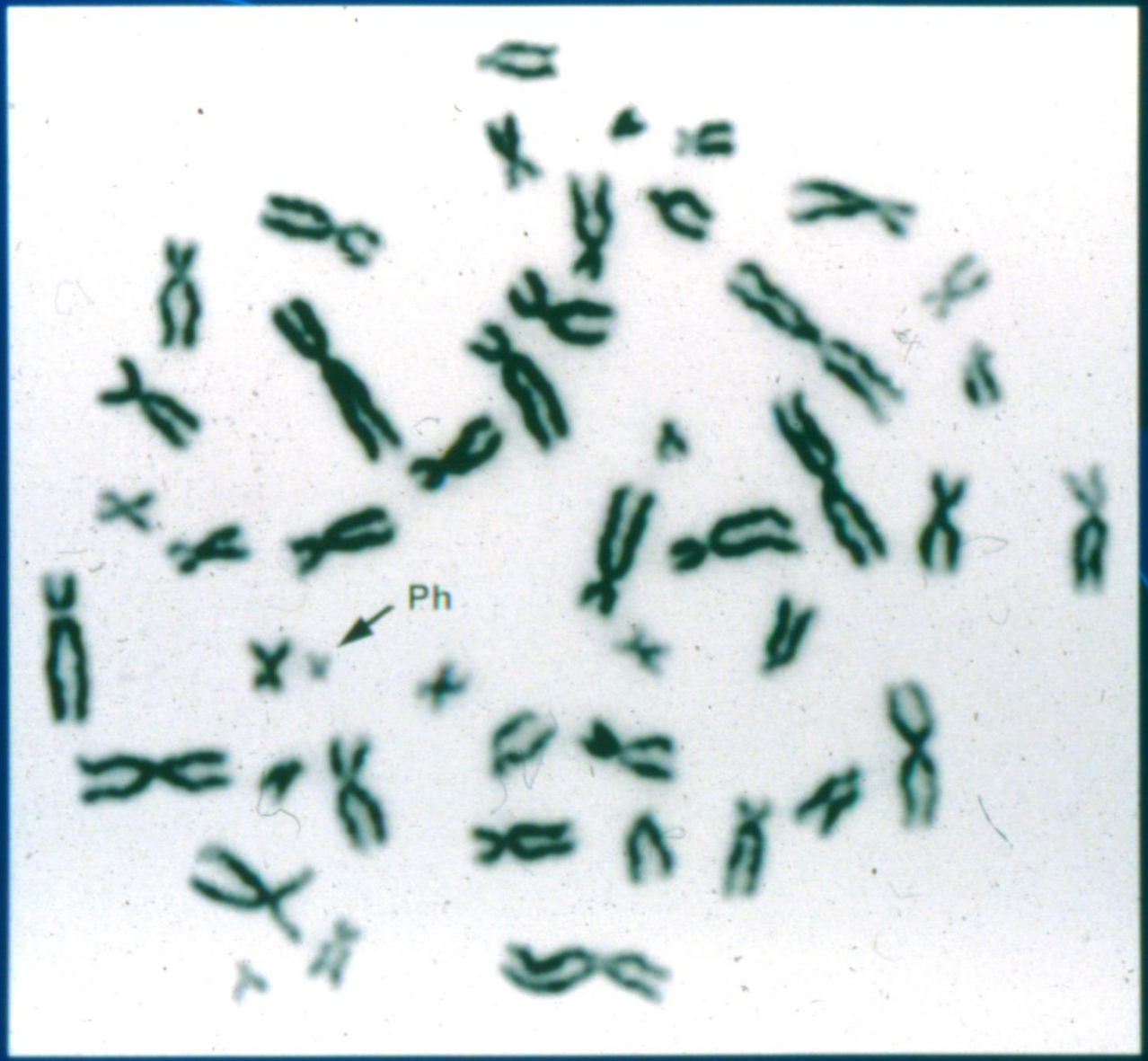
## 2. Production of a fusion protein

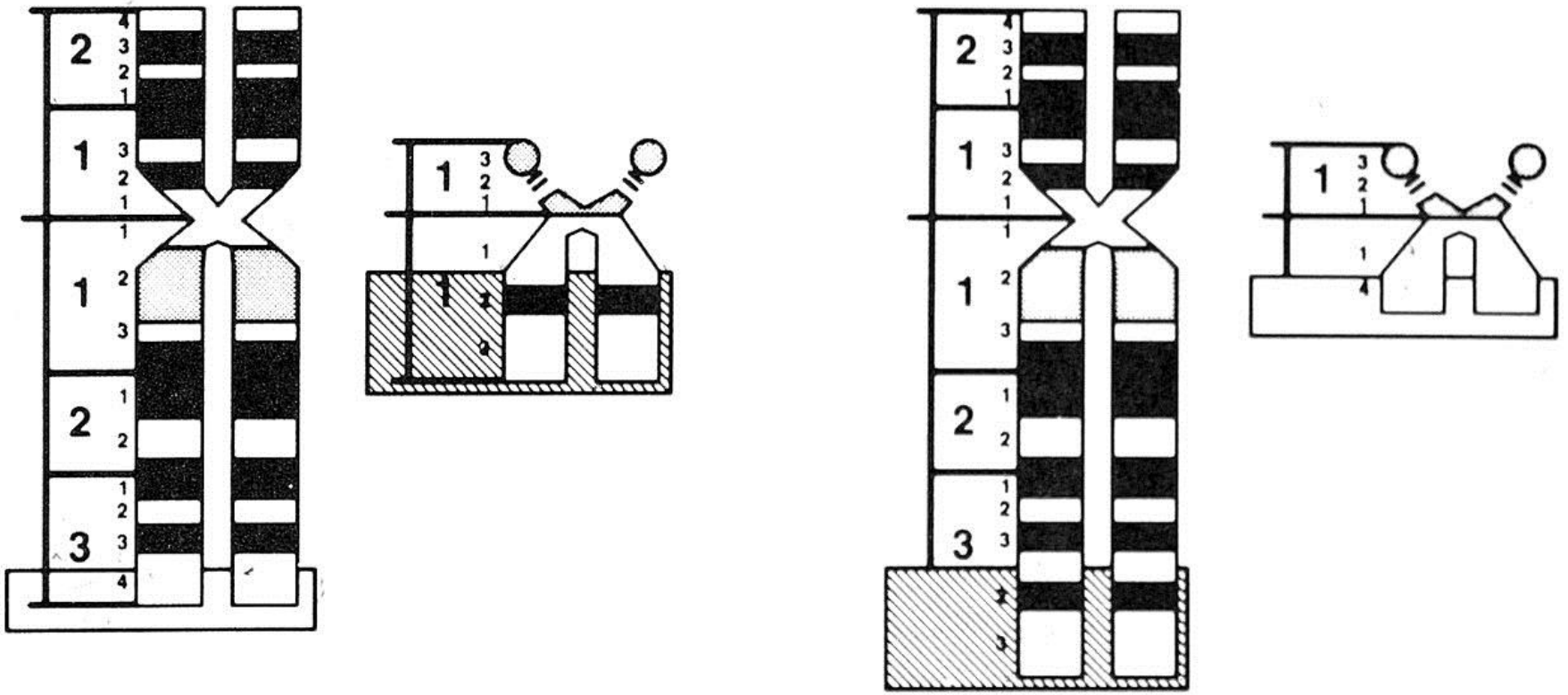
**t(8;21)**


→ **Expression of a fusion protein AML1-ETO**

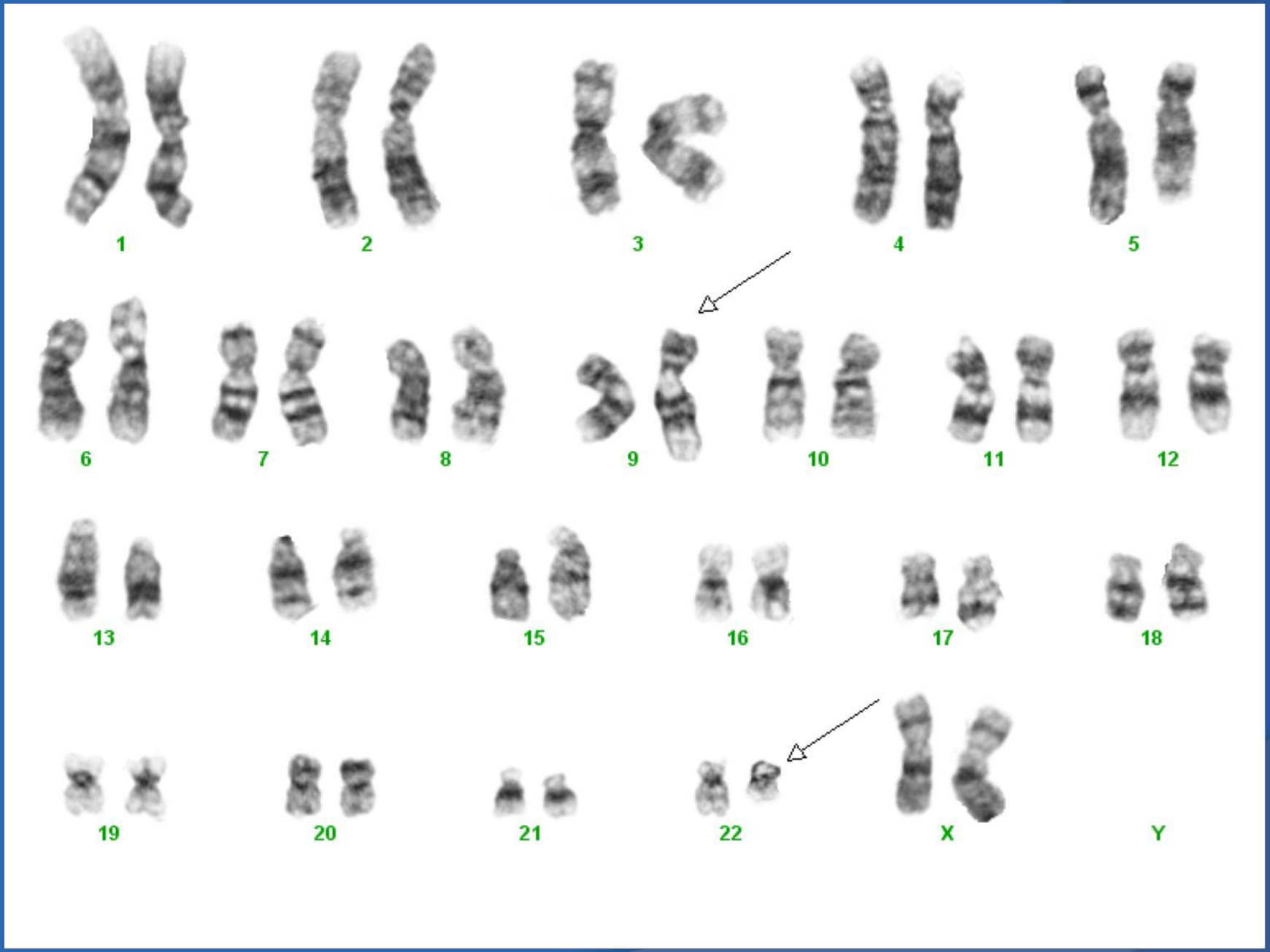
# FUNCTIONAL CLASSES:

- Proteins that regulate transcription
- Tyrosine protein kinases
- Serine protein kinases
- Cell surface receptors
- Growth factors
- Regulators of apoptosis


Ph

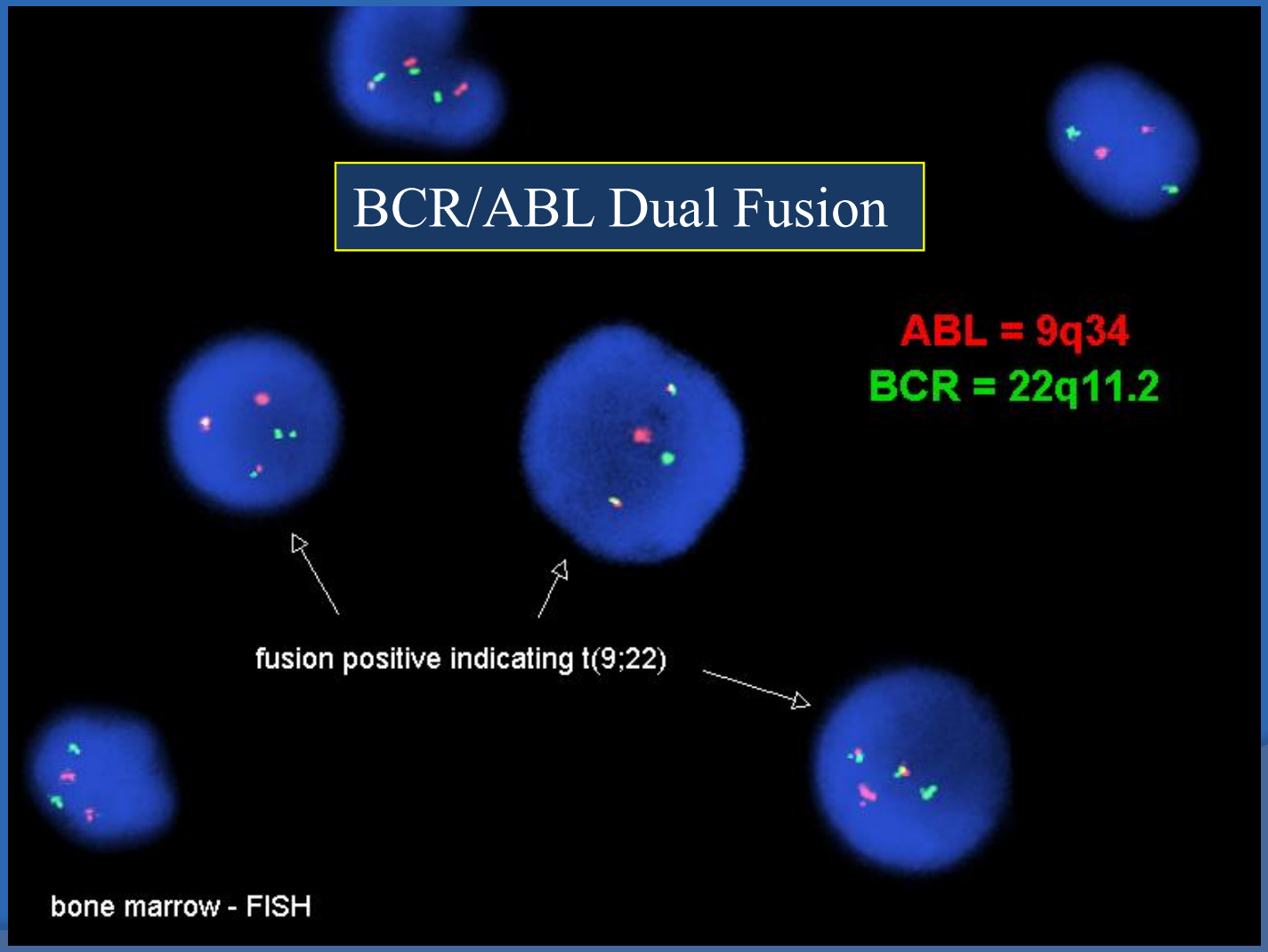


9 22 t(9;22)(q34;q11)




46,XX,t(9;22)(q34;q11.2)

# BCR/ABL Dual Fusion

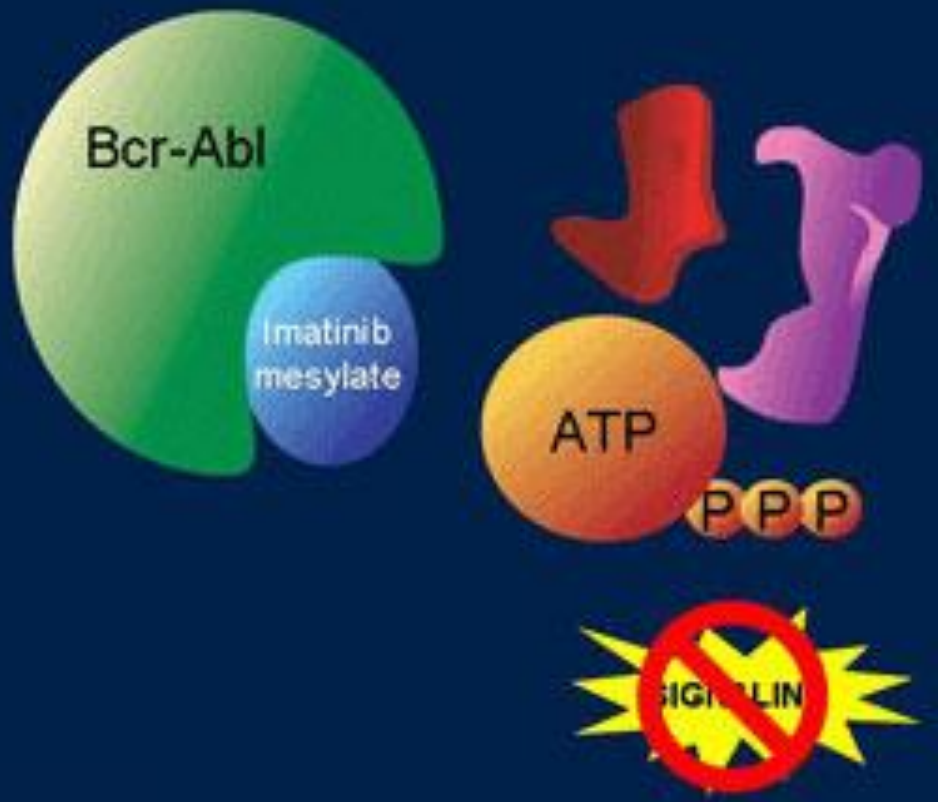


# Novel fusion protein:  BCR/ABL1

- **The BCR/ABL1 fusion protein contains the entire tyrosine kinase catalytic domain from the ABL1 gene, has constitutively increased tyrosine kinase activity, and has been implicated in the abnormal cellular proliferation seen in CML. The tyrosine kinase activity of the BCR/ABL1 fusion protein can be specifically inhibited by imatinib mesylate and several second generation ABL1 tyrosine kinase inhibitors.  ABL1 tyrosine kinase inhibitors have shown remarkable activity in all phases of CML and are the preferred therapy for most patients with newly diagnosed CML.**

# Imatinib Mesylate: Mechanism of Action

- Imatinib mesylate occupies the ATP binding pocket of the Abl kinase domain
- This prevents substrate phosphorylation and signaling
- A lack of signaling inhibits proliferation and survival




Savage DG, Antman KH. *N Engl J Med*. 2002;346:683-693.

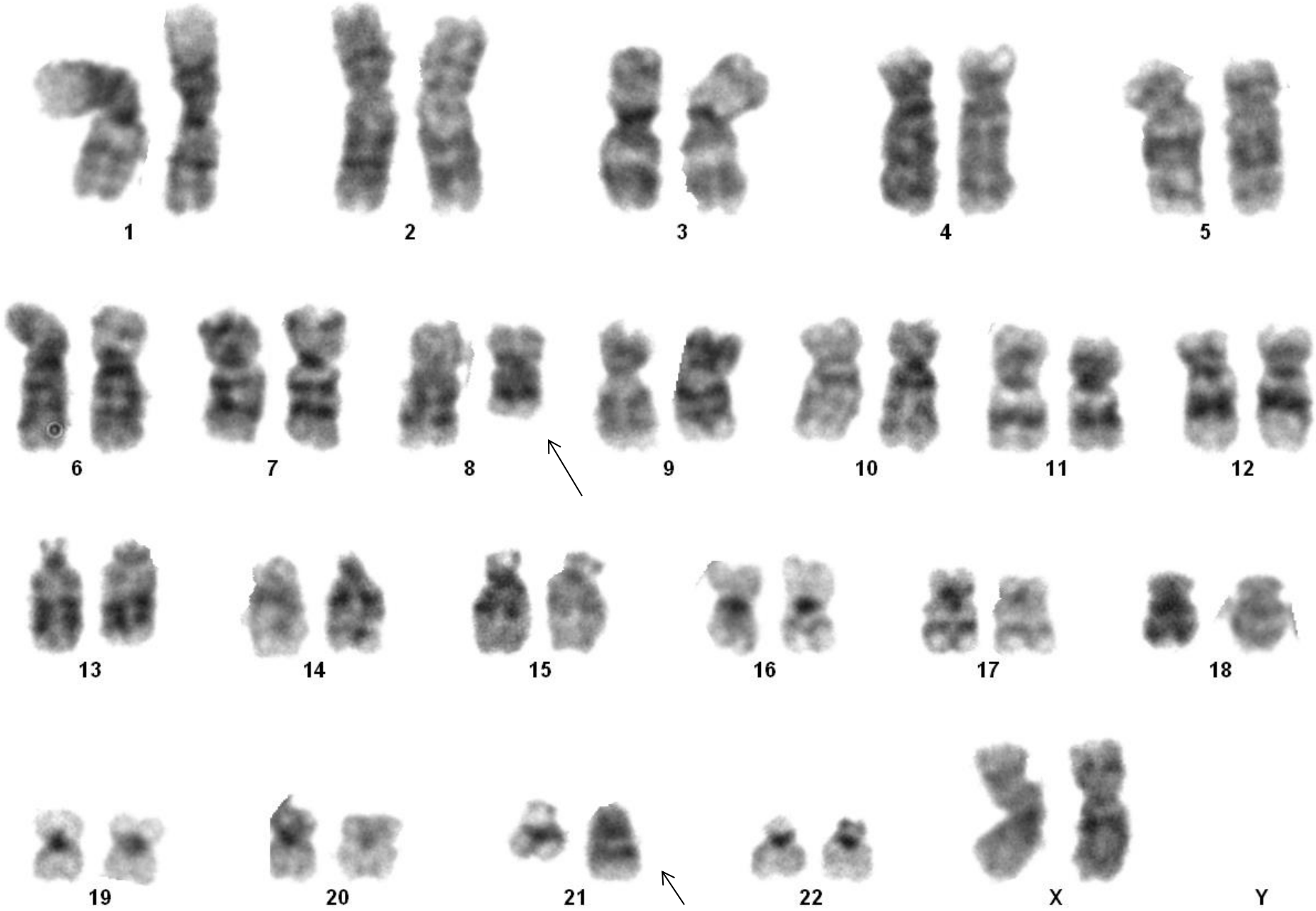
# Gleevec™


NOVARTIS
ONCOLOGY

gleevec™
imatinib mesylate

Sima Schorr began Gleevec™ therapy in May of 1999

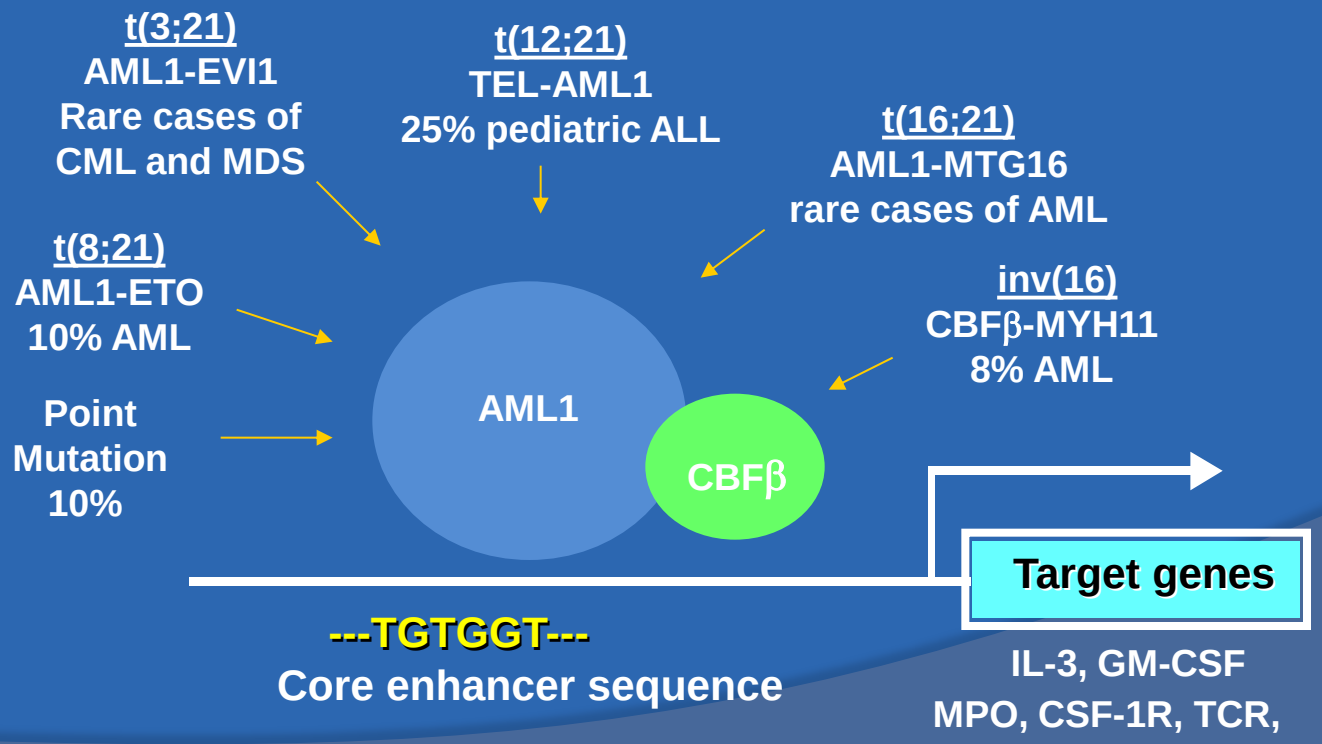

ARNOLD O. BECKMAN EXHIBIT
MAKING
MEASURING




ISCN: 46,XX,t(8;21)(q22;q22)[19]/46,XX[1]

# *AML1/(RUNX1)*

- The *AML1/(RUNX1)* gene at 21q22 codes for core binding factor (CBF) α which forms a heterodimer with CBFβ that acts as a transcriptional activating factor.
- CBF is a critical regulator in the generation and differentiation of definitive hematopoietic stem cells.

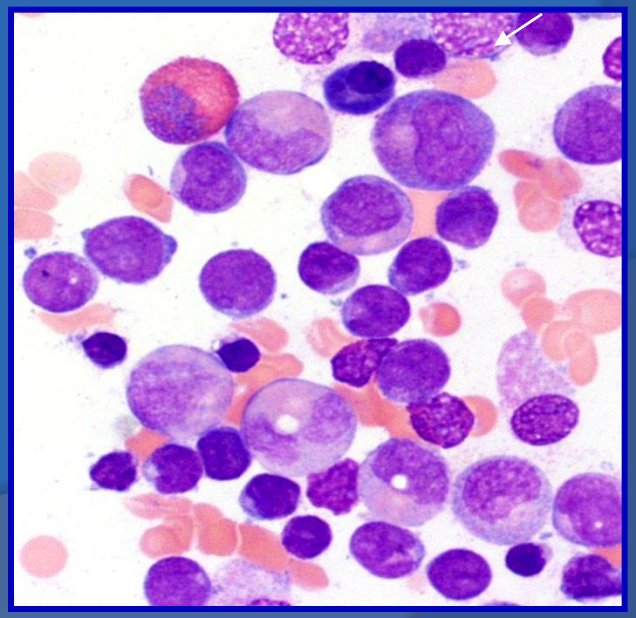


# t(8;21) in AML

- Identified by Dr. Janet D. Rowley in 1973 as the first recurring translocation in acute leukemia.
- Associated with AML-M2 (~30% of AML-M2 cases, or ~5-10% of all AML).
- Characterized by a good response to therapy (98% CR) and a prolonged disease-free survival.

**Characteristic morphology:**

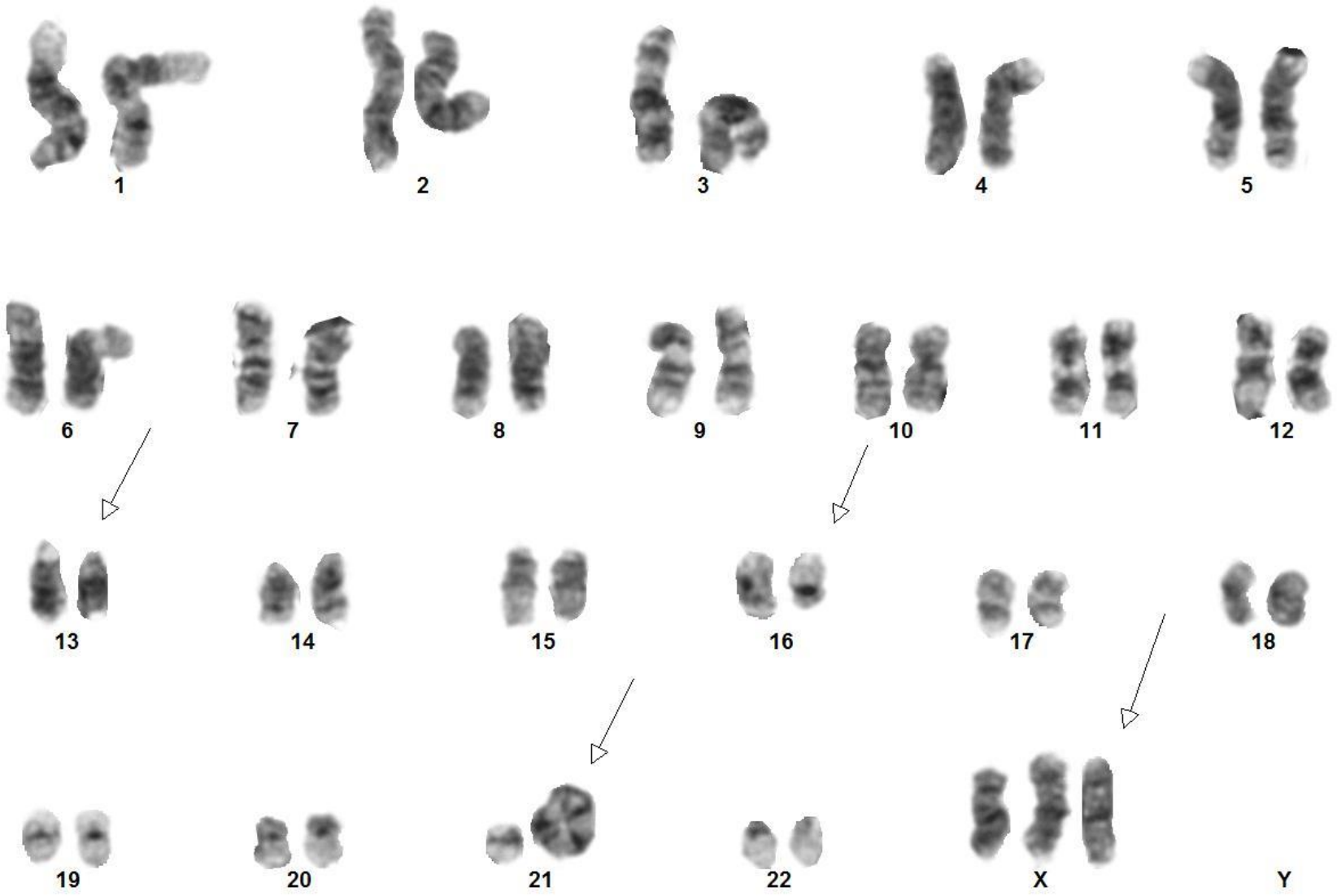
- myeloid blasts with indented nuclei.
- basophilic cytoplasm with few azurophilic granules.
- increased eosinophils in bone marrow.
- Aberrant expression of CD19, and CD56.



# Novel fusion protein: RUNX1/RUNX1T1

At the molecular level, the t(8;21) results in a RUNX1/RUNX1T1 chimeric protein. The RUNX1/AML1 gene encodes one subunit of a heterodimeric transcription factor, also known as core-binding factor, essential for hematopoiesis. Transformation by RUNX1/RUNX1T1 likely results from transcriptional repression of normal RUNX1 target genes via aberrant recruitment of nuclear transcriptional co-repressor complexes.

**Karyotype 1**

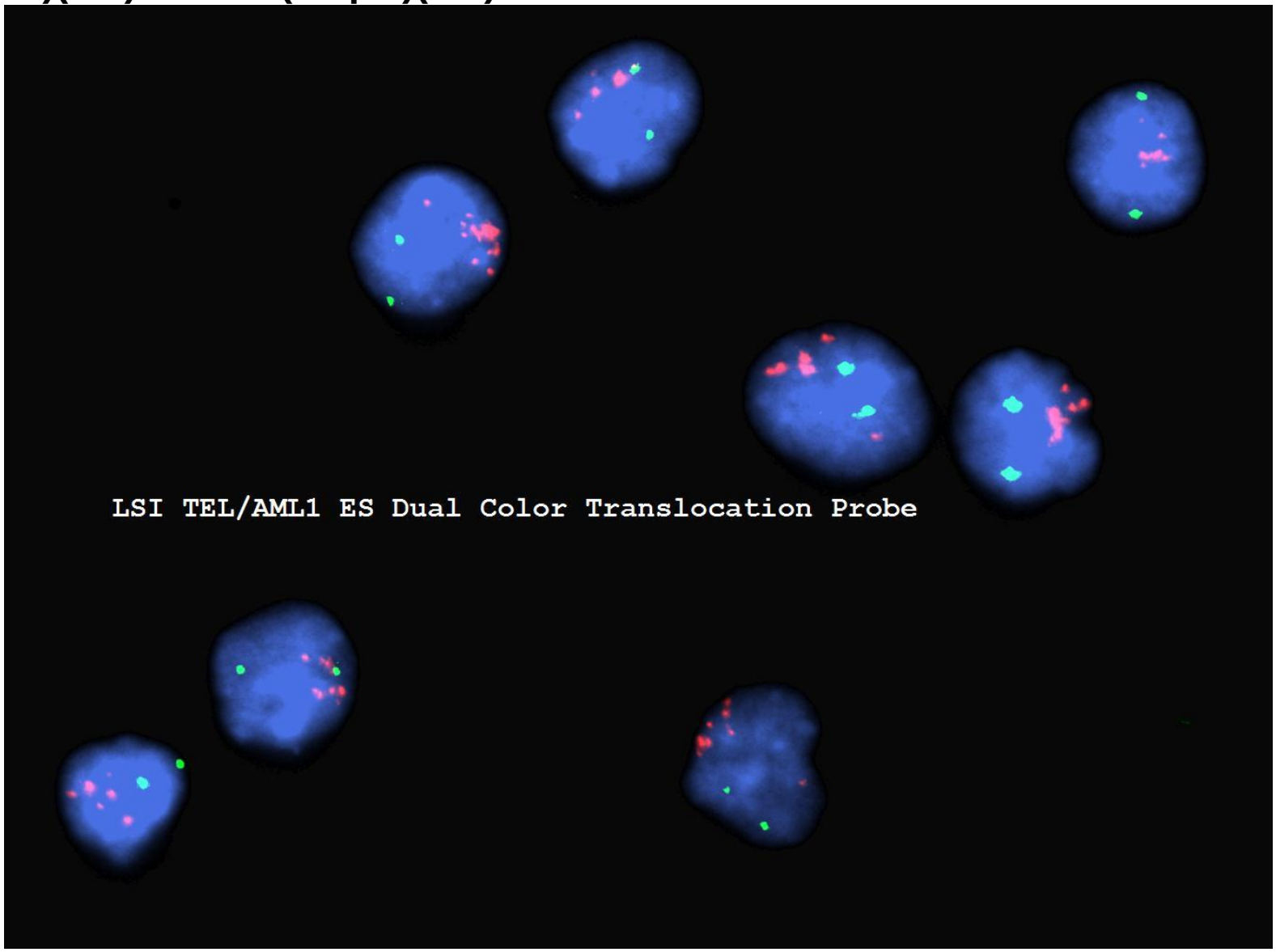


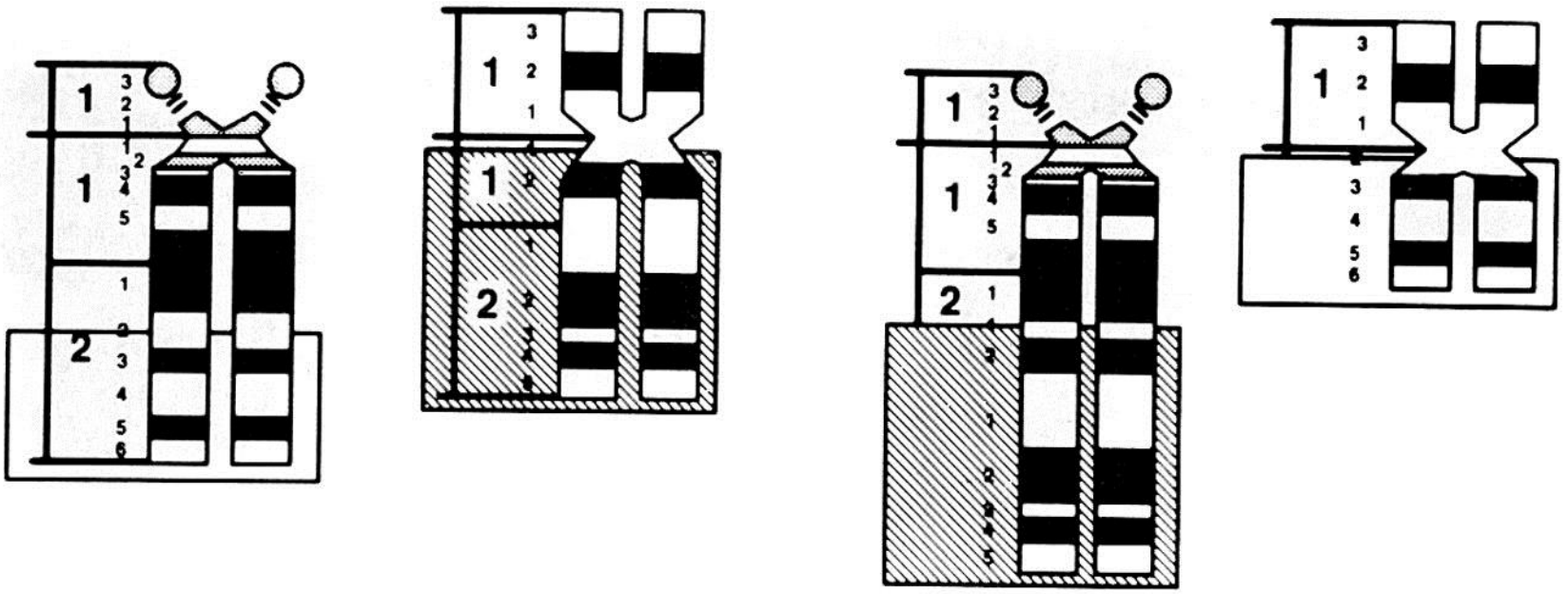

47,XX,+X,del(13)(q12q14),del(16)(q12.2q24),r(21)amp(RUNX1)

# FISH LSI ETV6(12p13)(SG)/RUNX1(21q22)(SO)

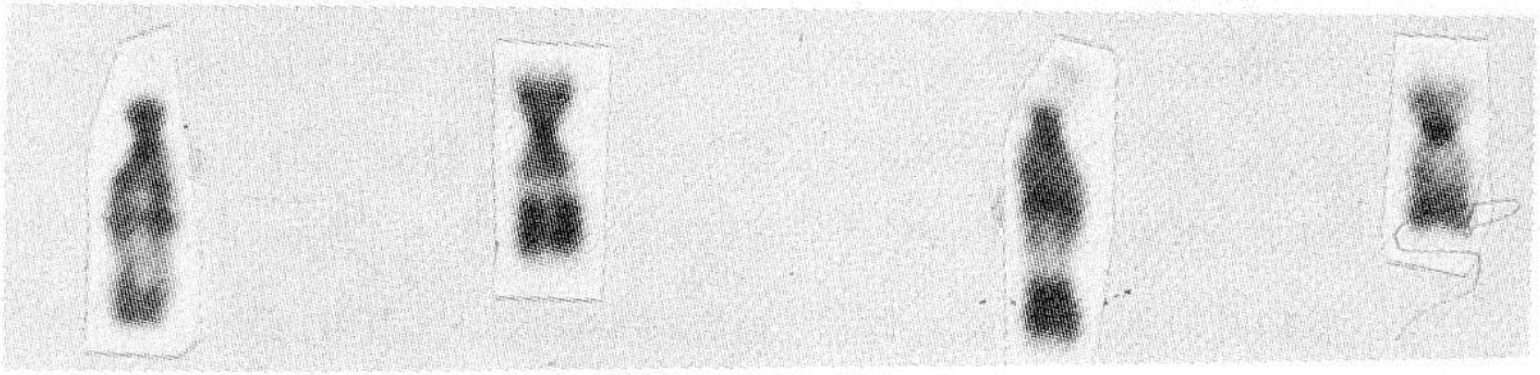
**probe**

- **LSI ETV6(12p13)(SG)/RUNX1(21q22)(SO)**
- **2G/4~12O**
- **abnormal**



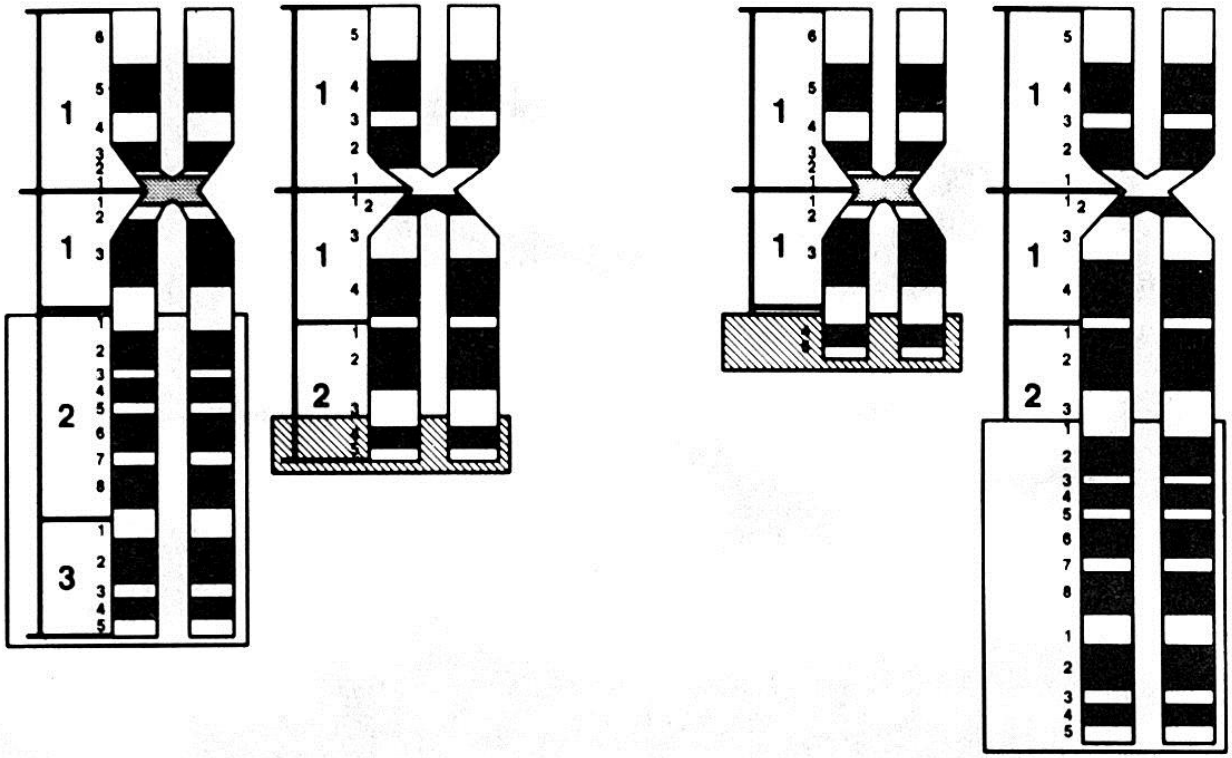


15 17 t(15;17)(q22;q11)

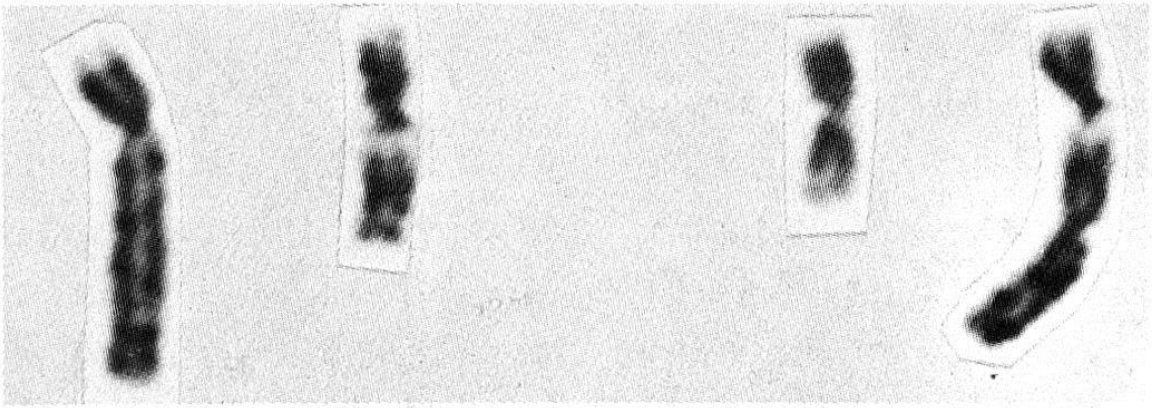


# Novel fusion protein:  PML/RARA

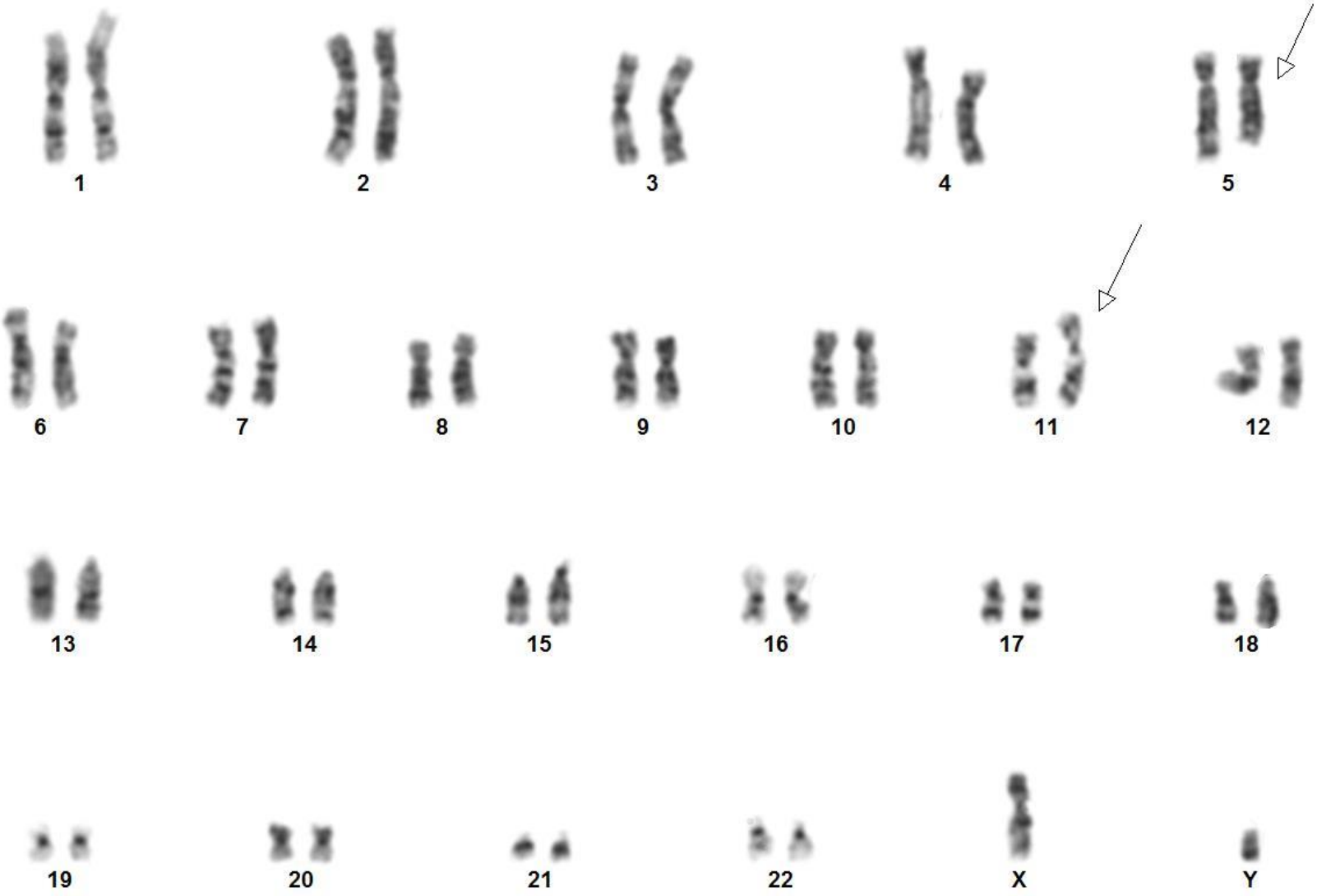
- The t(15;17) results in a fusion protein involving the promyelocytic leukemia (PML) and retinoic acid receptor-alpha proteins (PML/RARA). The oncogenic potential of the APL fusion proteins appear to result from the aberrant repression of the retinoic acid receptor-mediated gene transcription through histone deacetylase-dependent chromatin remodeling. This effect can be overcome by pharmacologic doses of retinoic acid, resulting in relief from transcriptional repression, presumably activating genes that lead to terminal differentiation of promyelocytes.



4 11 t(4;11)(q21;q23)



# Karyotype 1

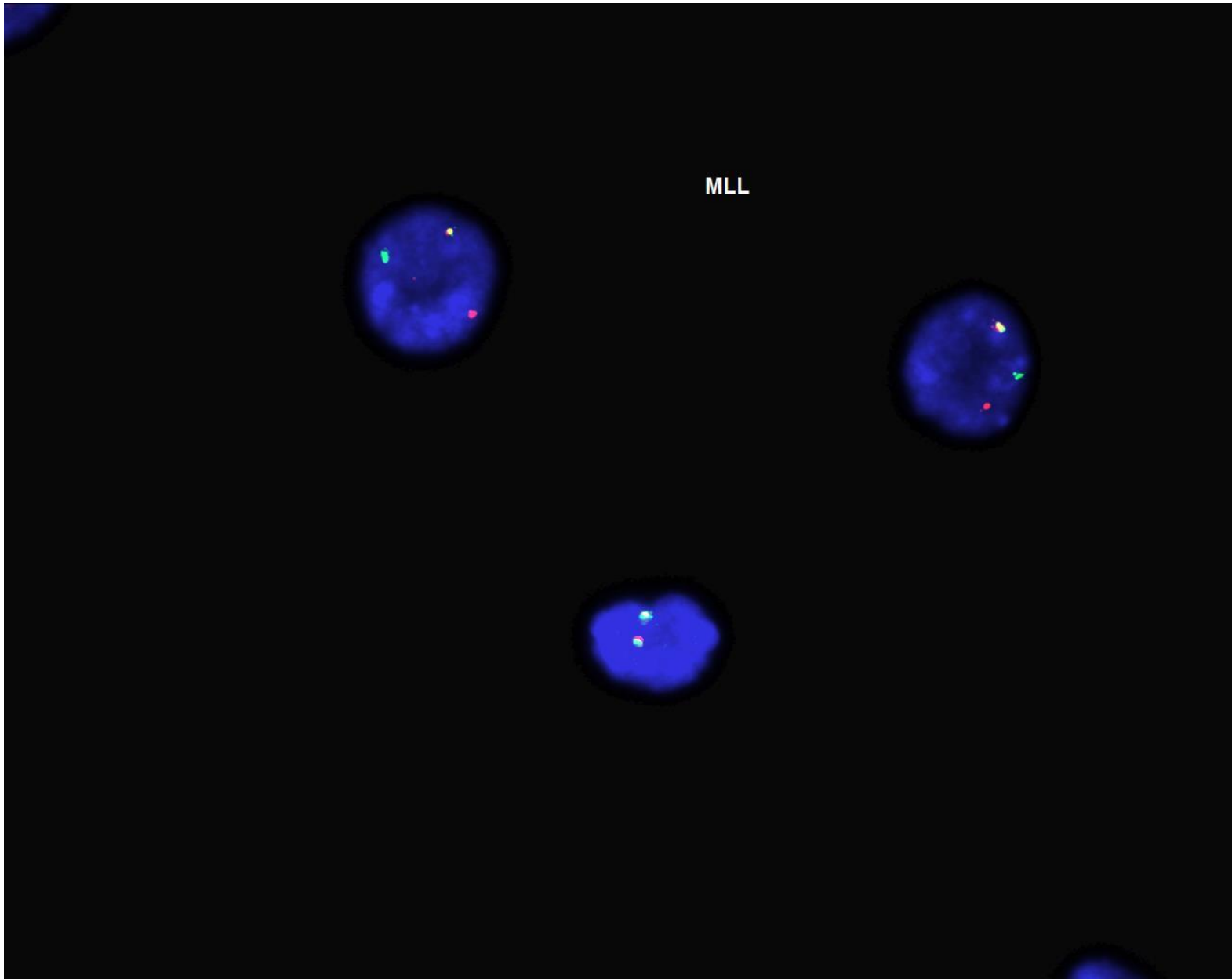


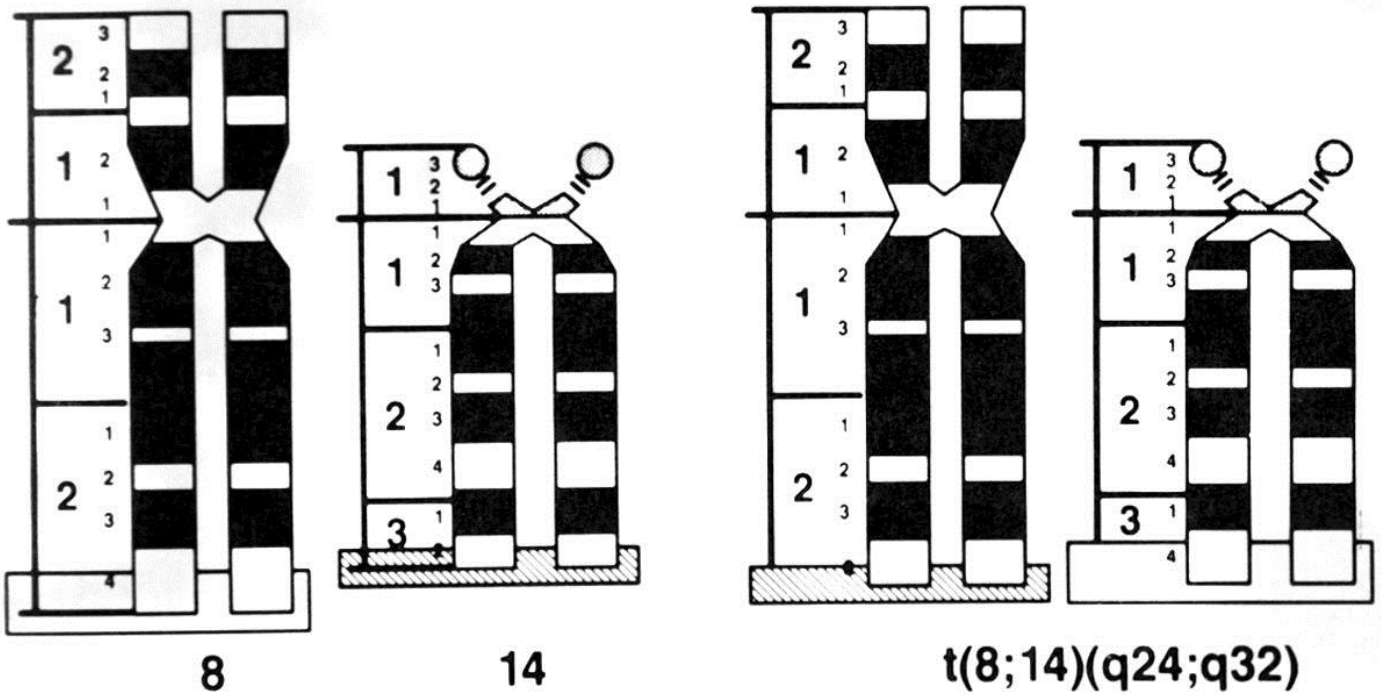

**46,XY,t(5;11)(q31;q23)**

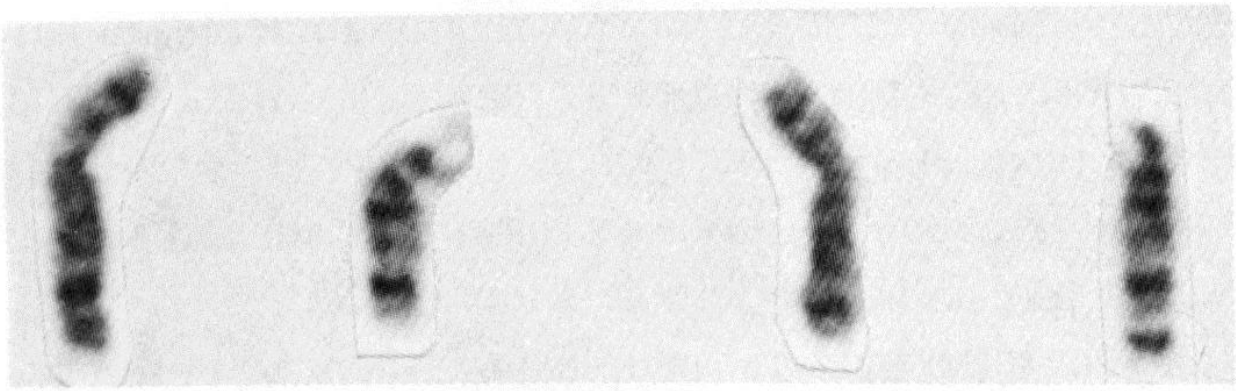
# FISH MLL(11q23) DC BA

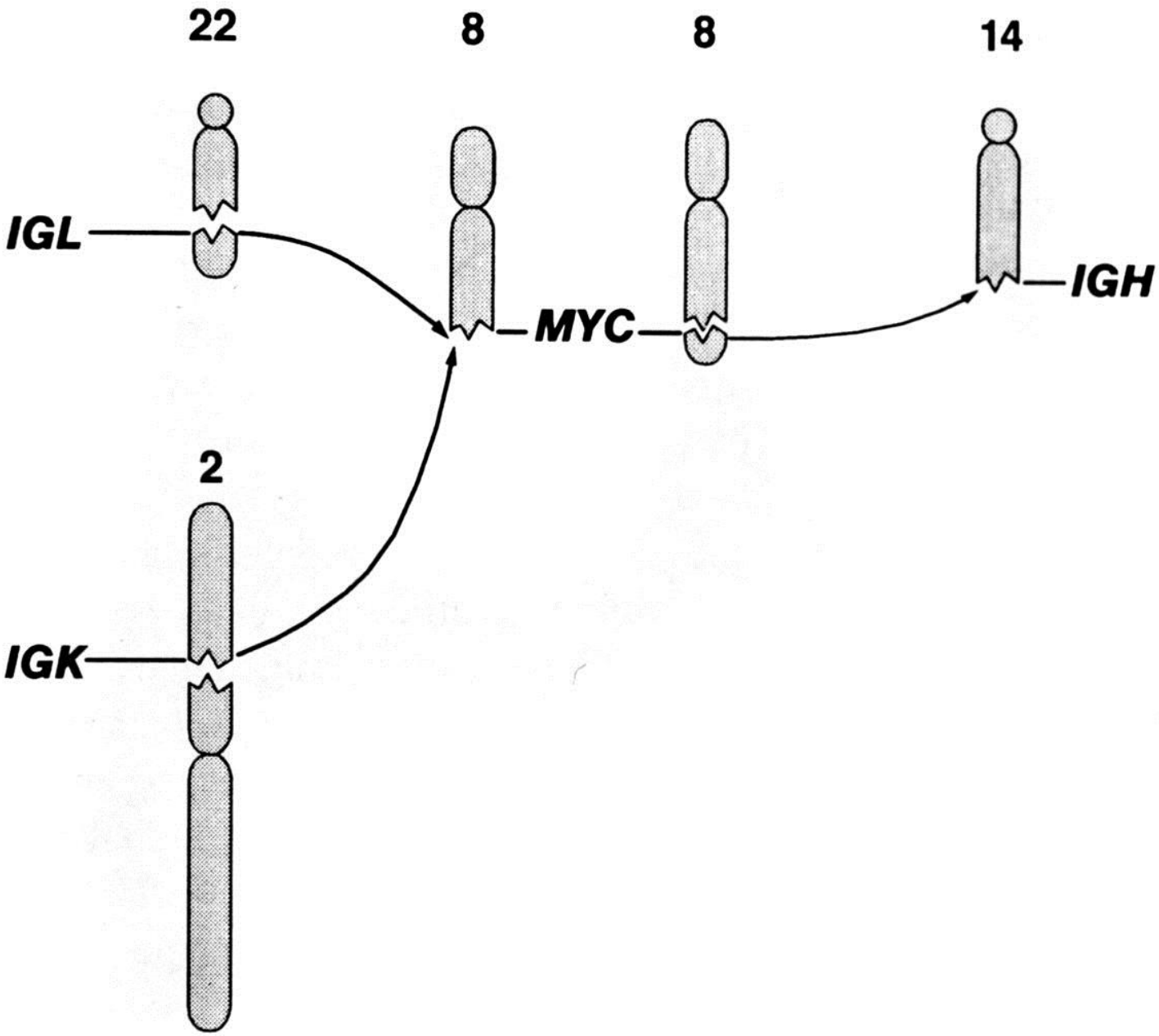
**probe**

- **MLL(11q23) DC BA**
- **1F1O1G/2F**
- **abnormal**




8
14
t(8;14)(q24;q32)




22
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8
14
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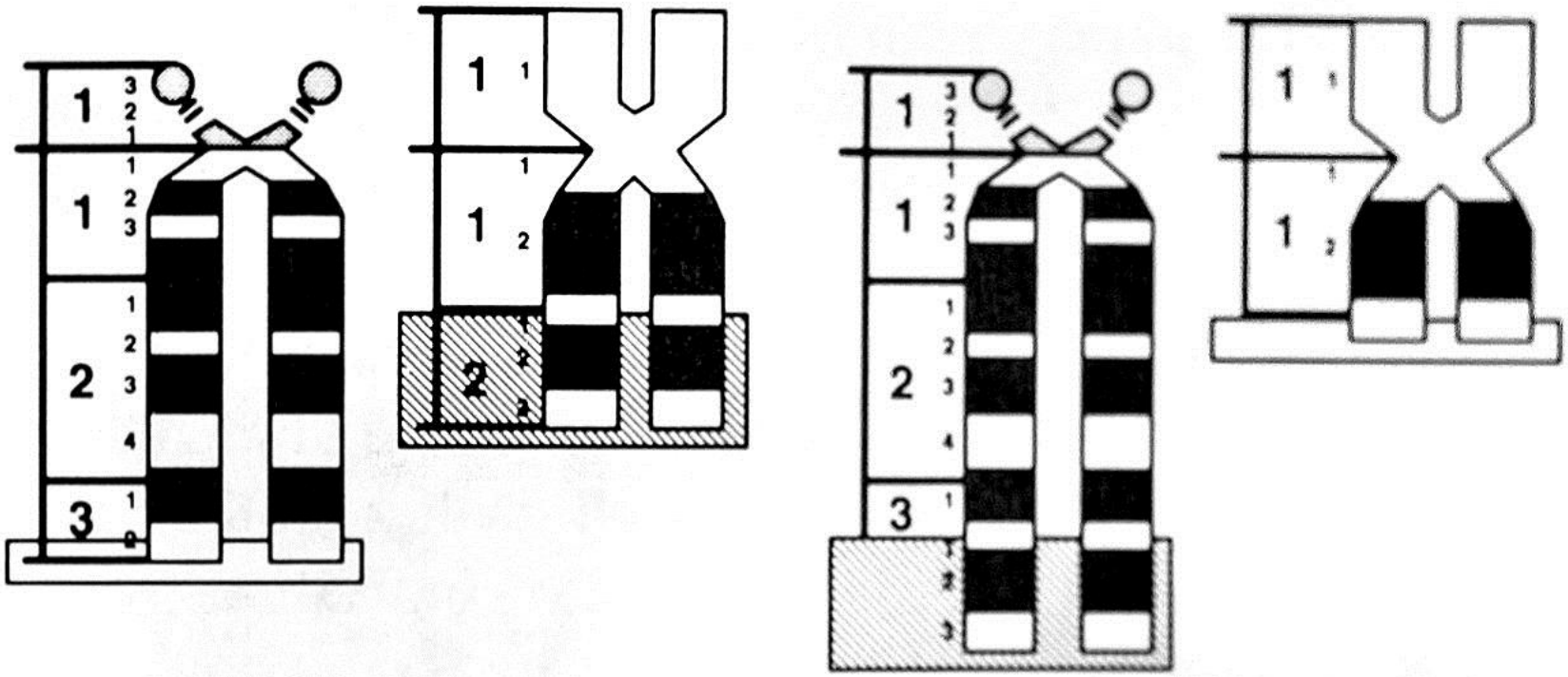
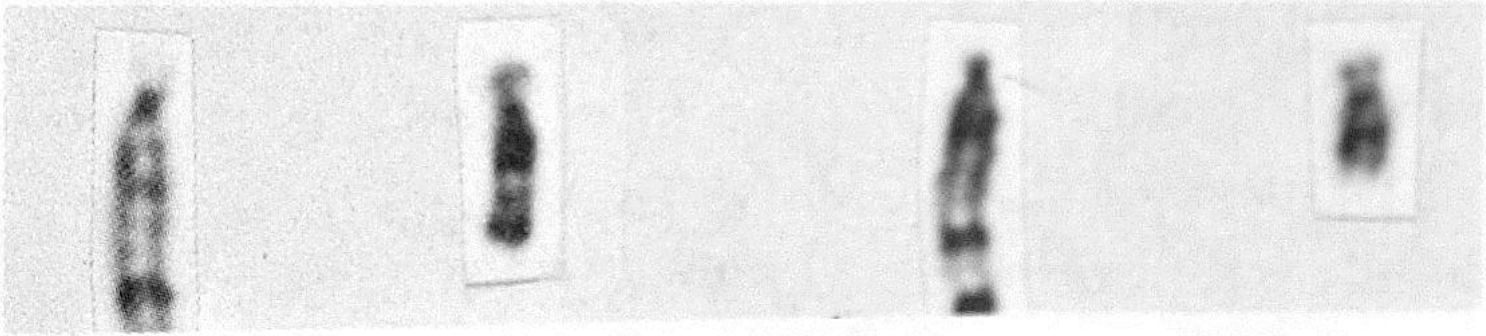
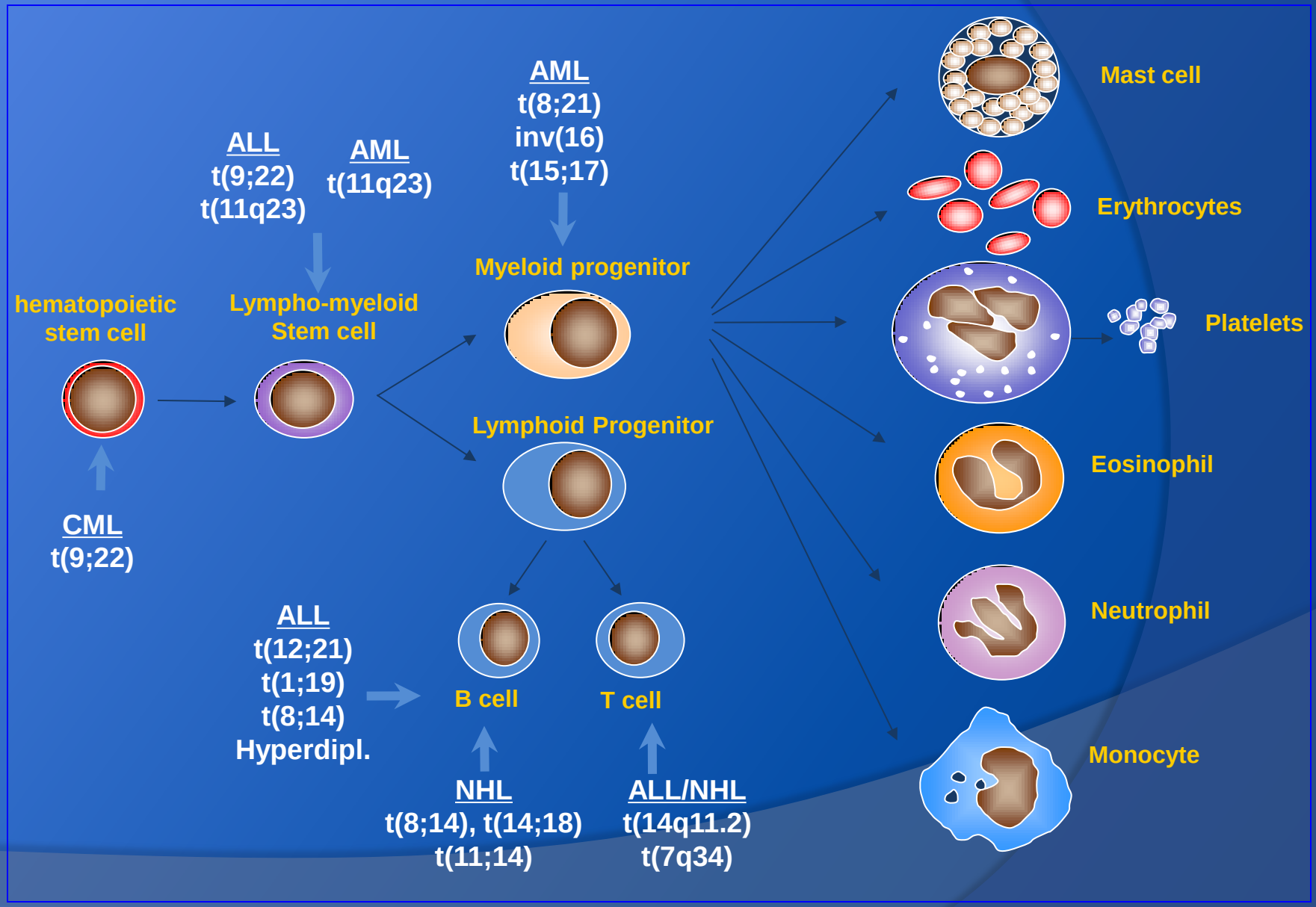
MYC
IGH
2
IGK



14 18 t(14;18)(q32;q21)



# Hematopoietic cell differentiation and chromosome abnormalities in leukemia and lymphoma

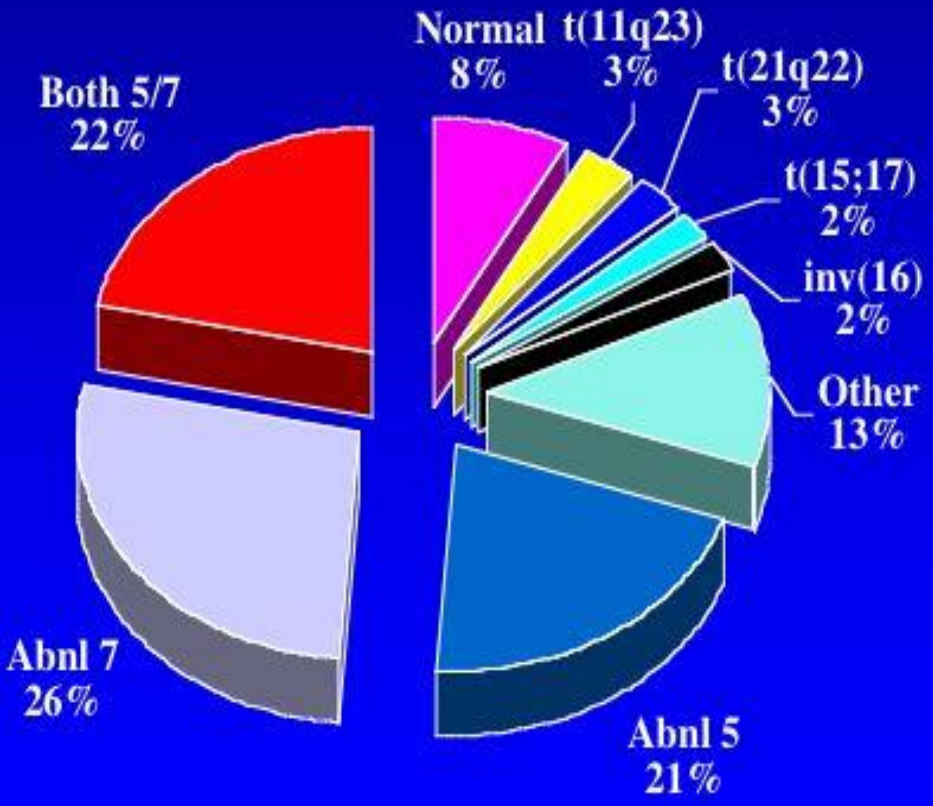


# Features of therapy-related AML

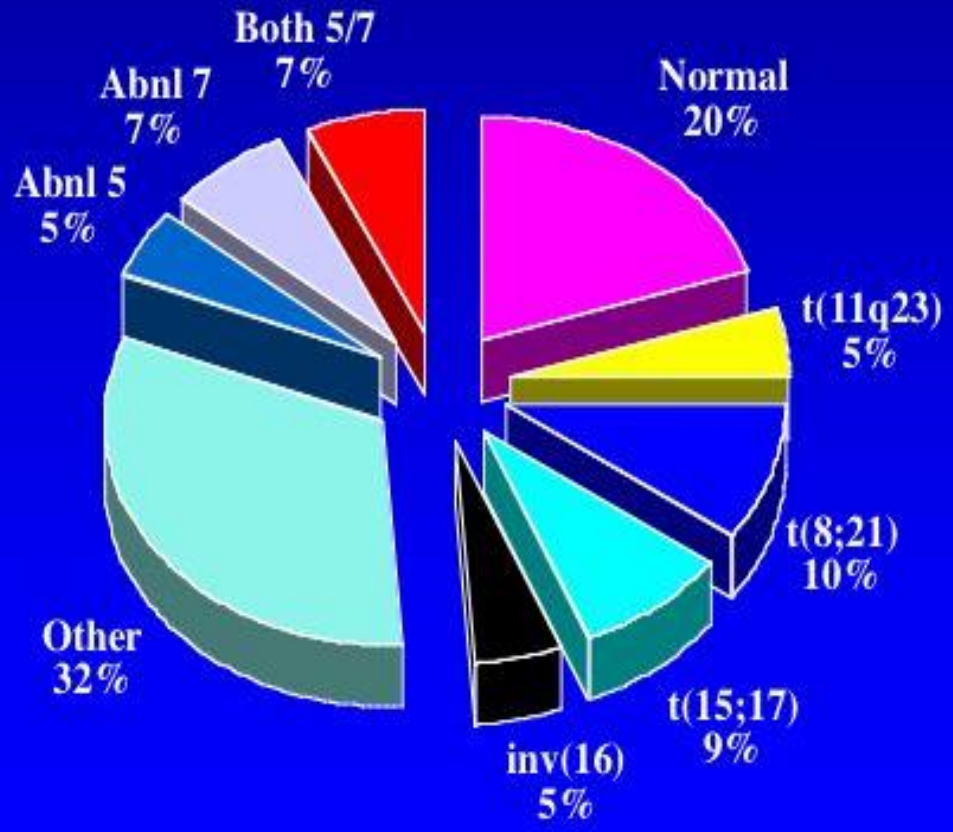
| | Alkylating agents Radiation | Topo II inhibitors (VP16, Dox) |
|---|---|---|
| Cytogenetics | -5/del(5q)/-7/del(7q) | 11q23, 21q22 |
| Latency | 5-7 yrs | 2-3 yrs |
| Presentation | Insidious (t-MDS) | acute |
| Prognosis | poor | poor |

# CYTOGENETIC ABNORMALITIES IN AML

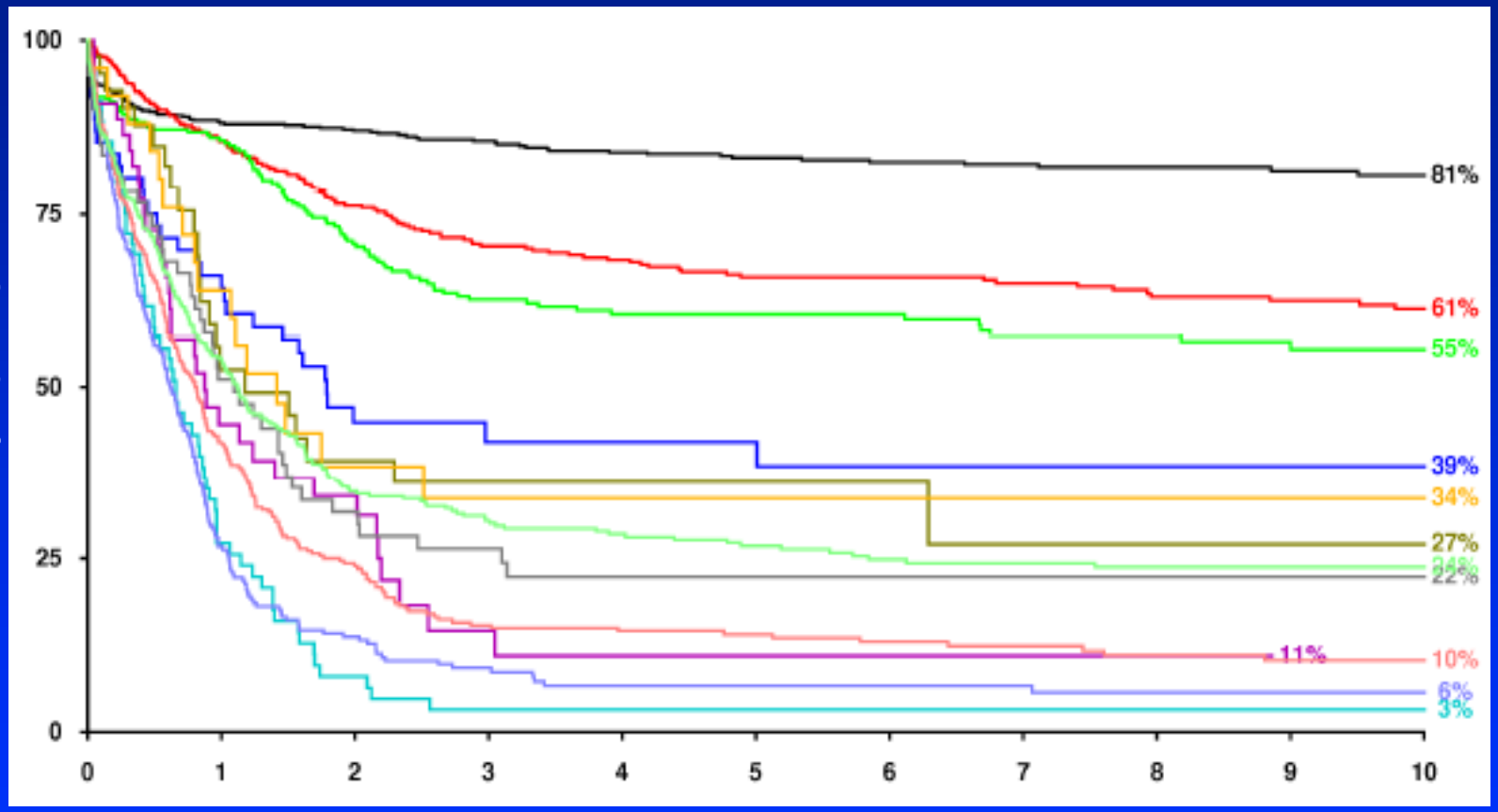
## t-MDS/t-AML




## AML de novo



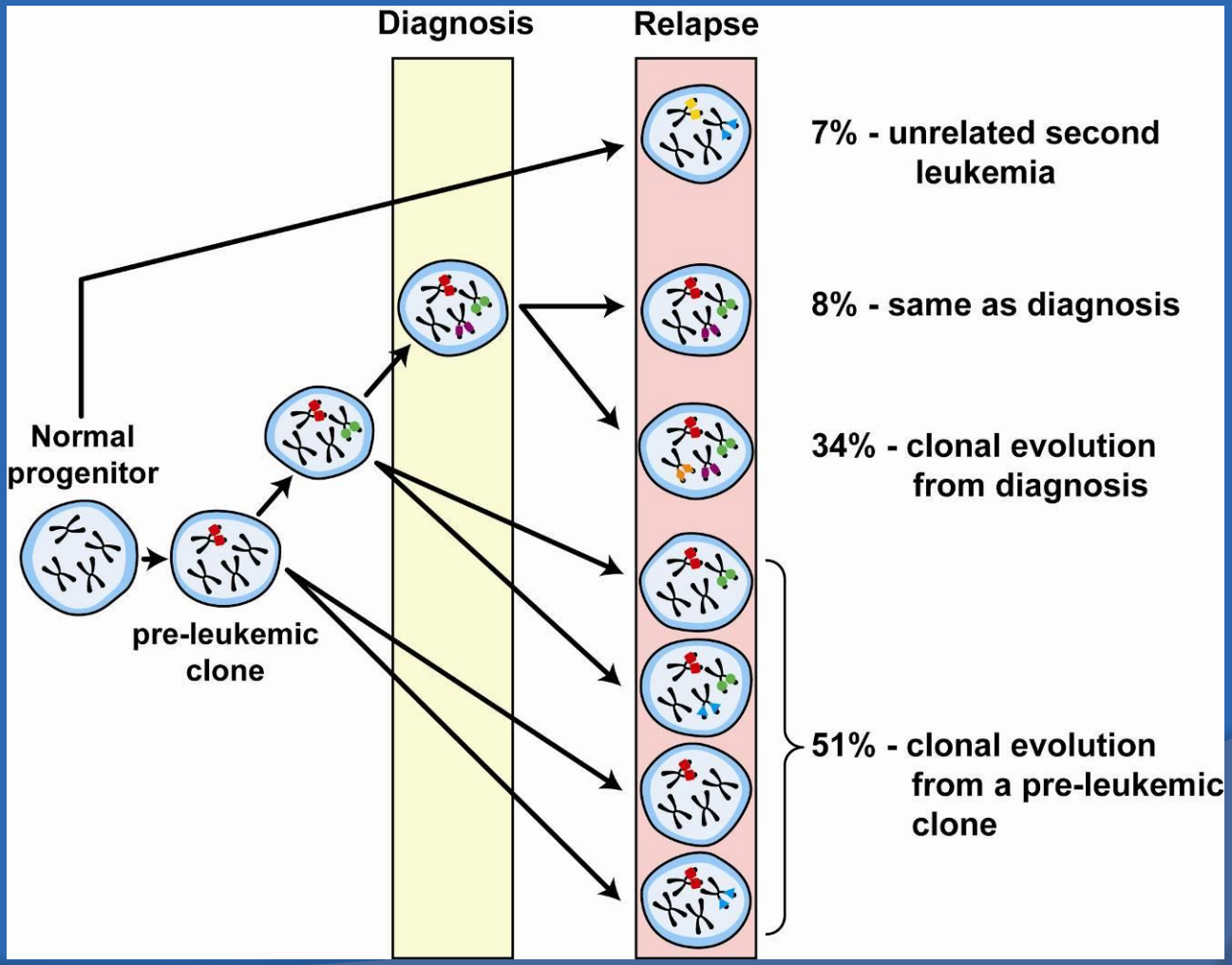
# MRC/NCRI AML Trials: Overall Survival
# ages 16-59, 2550 patients, 10 years follow-up




* Normal karyotypes: 38% OS

Grimwade et al., Blood, April 12, 2010

# Evolution of diagnosis and relapse clones

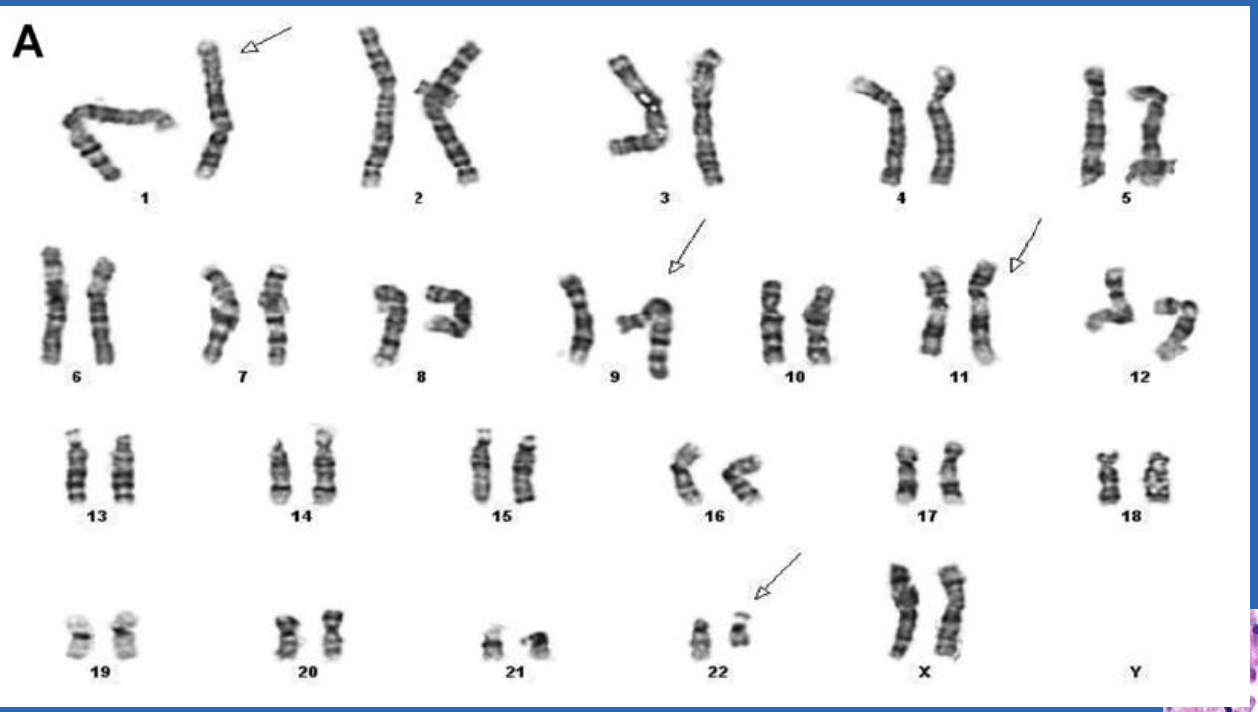


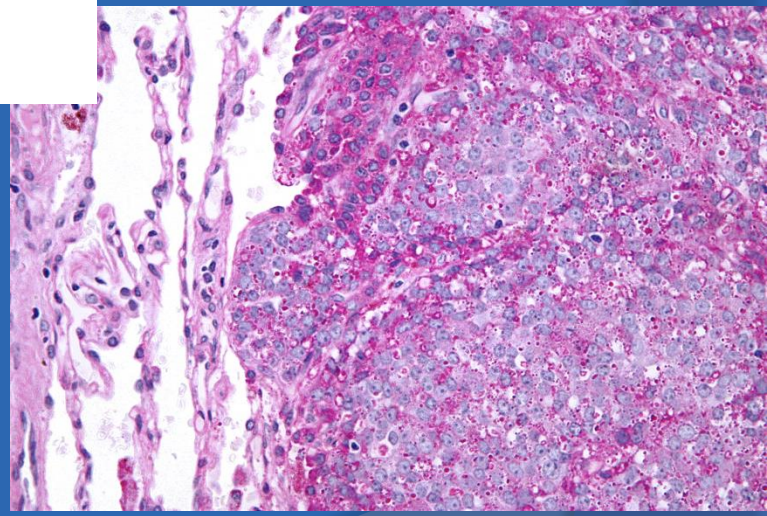
# Solid Tumor Cytogenetics: art or science?

1. Unpredictable growth of the neoplastic cells in tissue culture.
2. Overgrowth of neoplastic cells by “reactive” non-neoplastic cells.
3. Destruction of tumor cultures by bacterial of fungal infection.
4. Failure of tumor cultures to grow because of non-viable tumor.

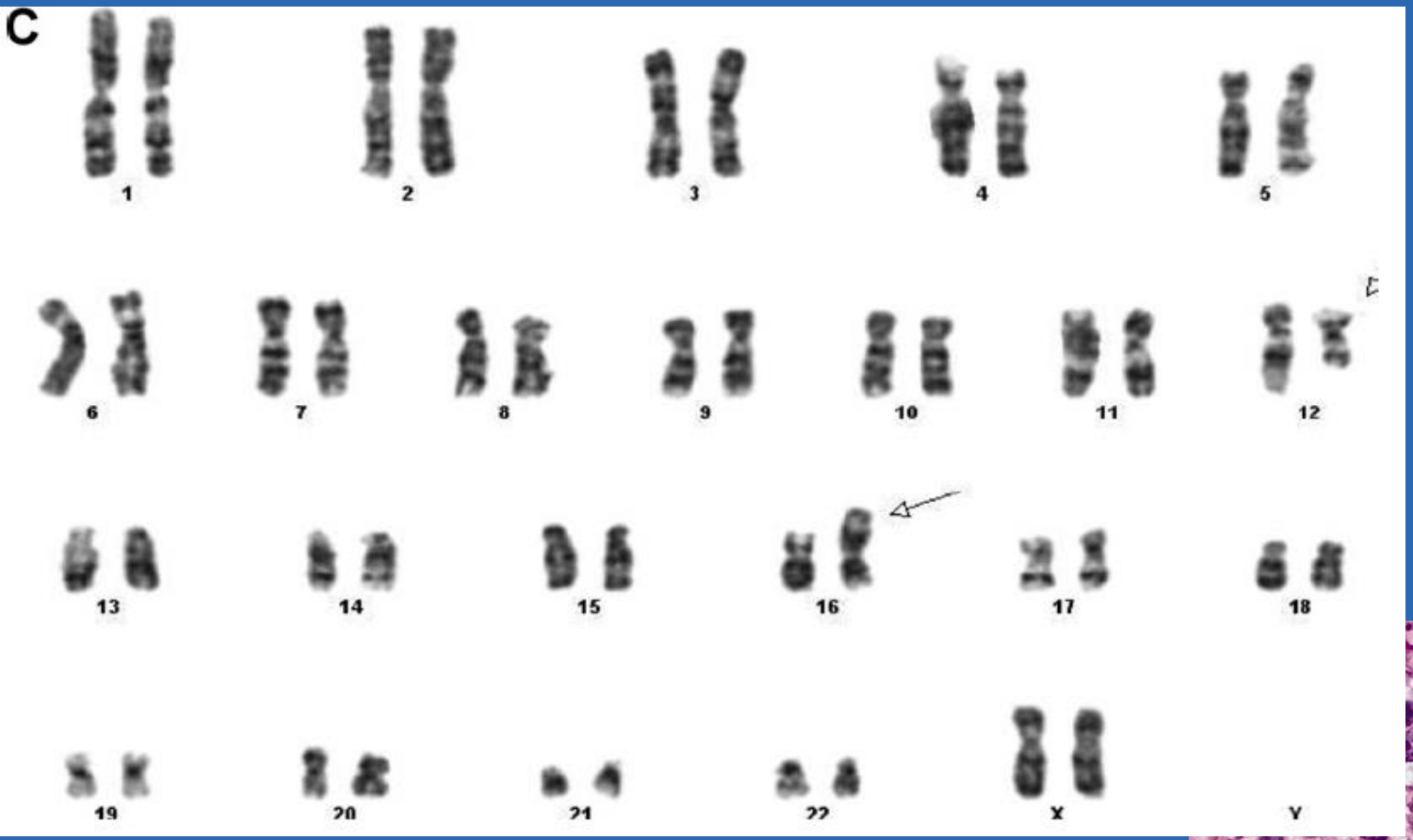
# Chromosomal changes in solid tumors

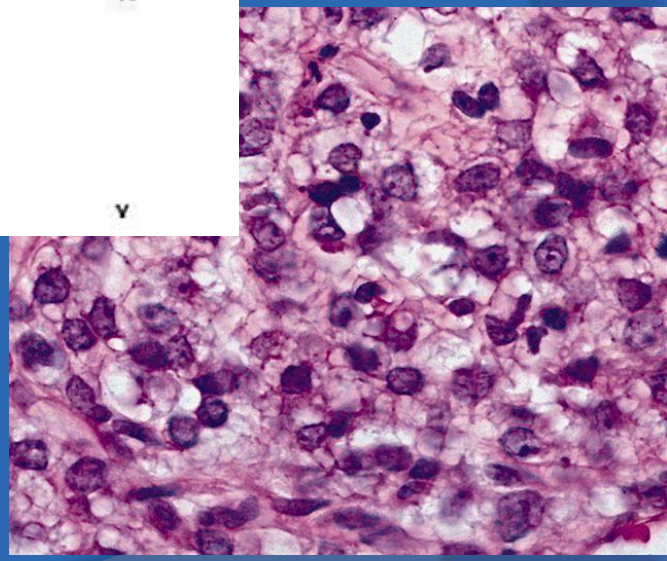
- Numerical changes (aneuploidy) – loss or gain of whole chromosomes
- Structural changes – balanced and unbalanced translocation, deletion, insertion, or inversion of specific chromosomal regions
- Amplification – high-level gain of specific chromosomal regions which manifest as double minutes (dm), homogenously staining regions (hsr), or ring (r) chromosomes.



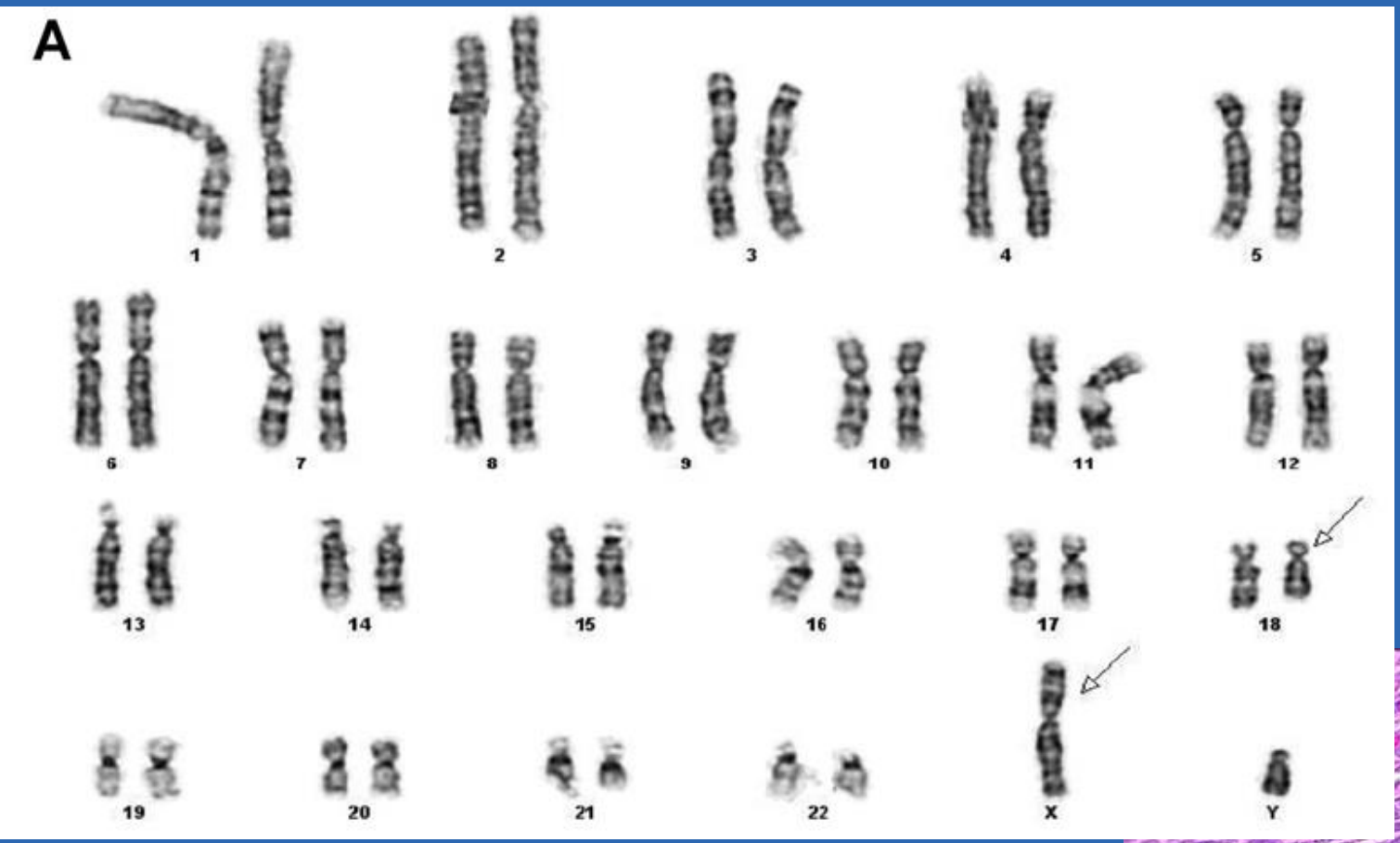



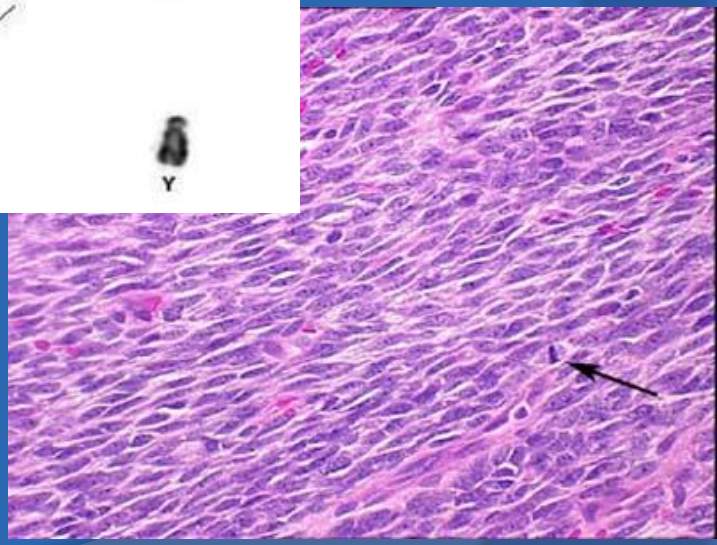
**Ewing's sarcoma with the characteristic t(11;22)(q24;q12) (*EWSR1-FLI1*) and a secondary rearrangment t(1;9)(q21;q22).**



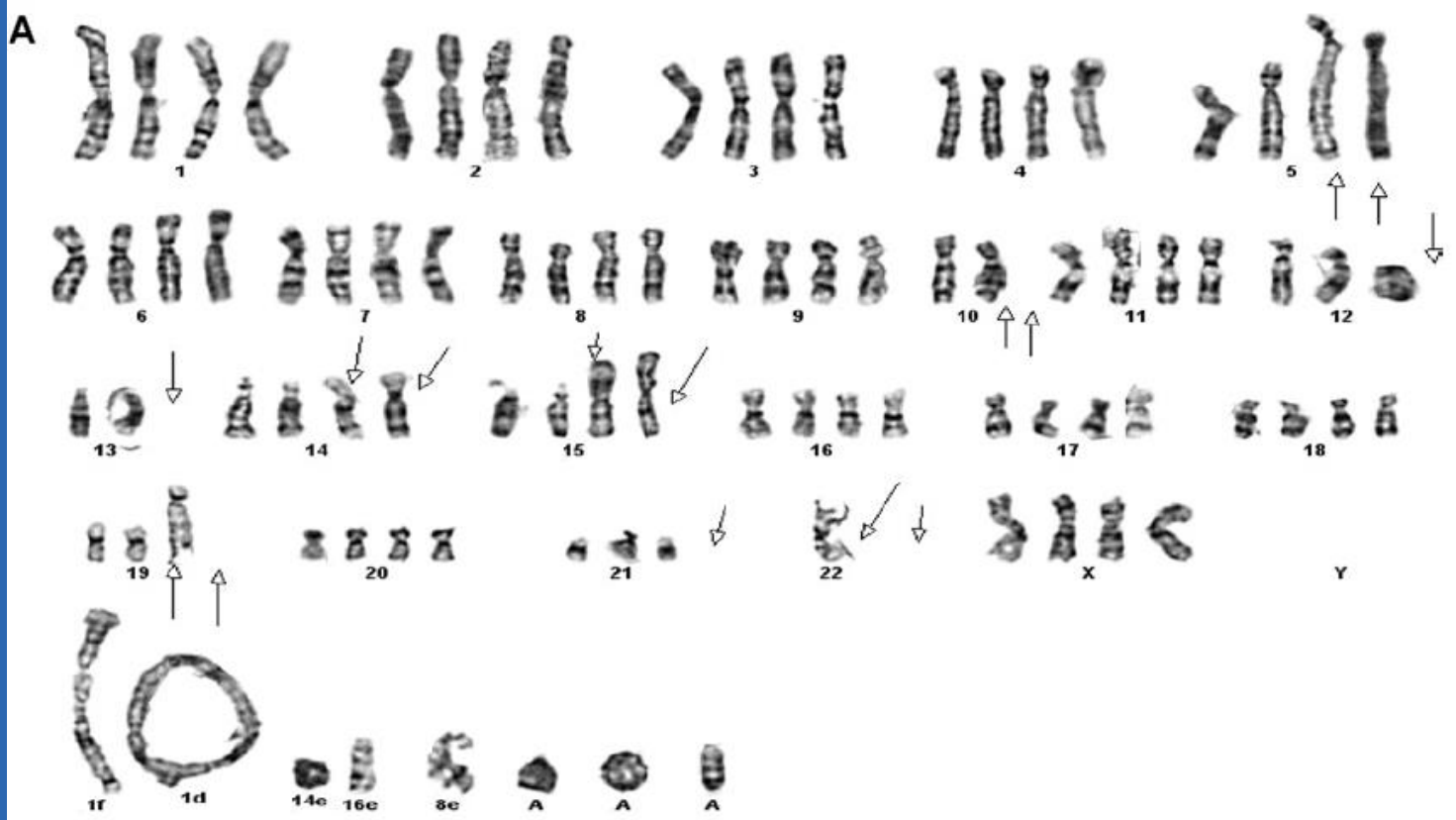



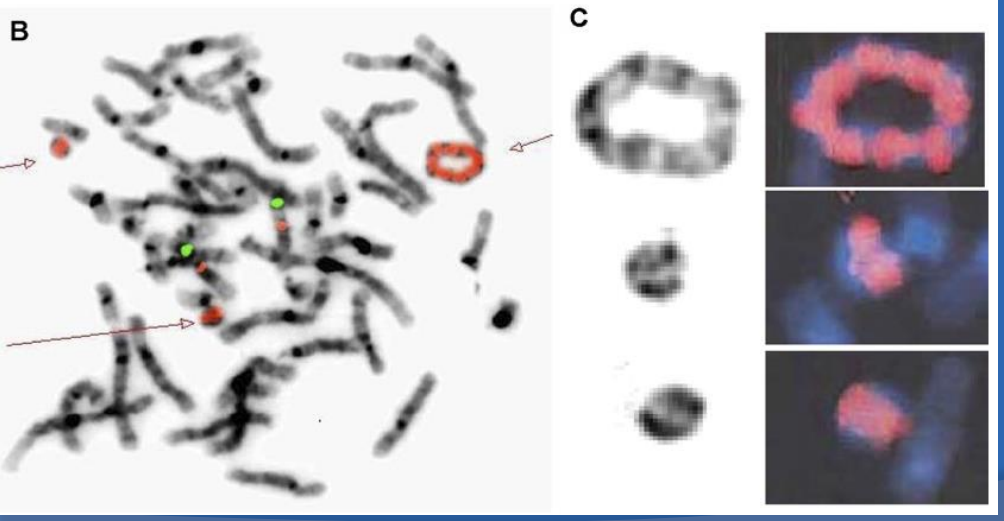
**Myxoid/round cell liposarcoma specific t(12;16)(q13;p11.2) *FUS-DDIT3***



**Synovial sarcoma with t(X;18)(p11.2;q11.2)** ***SS18-SSX1, SS18-SSX2, SS18-SSX4.***



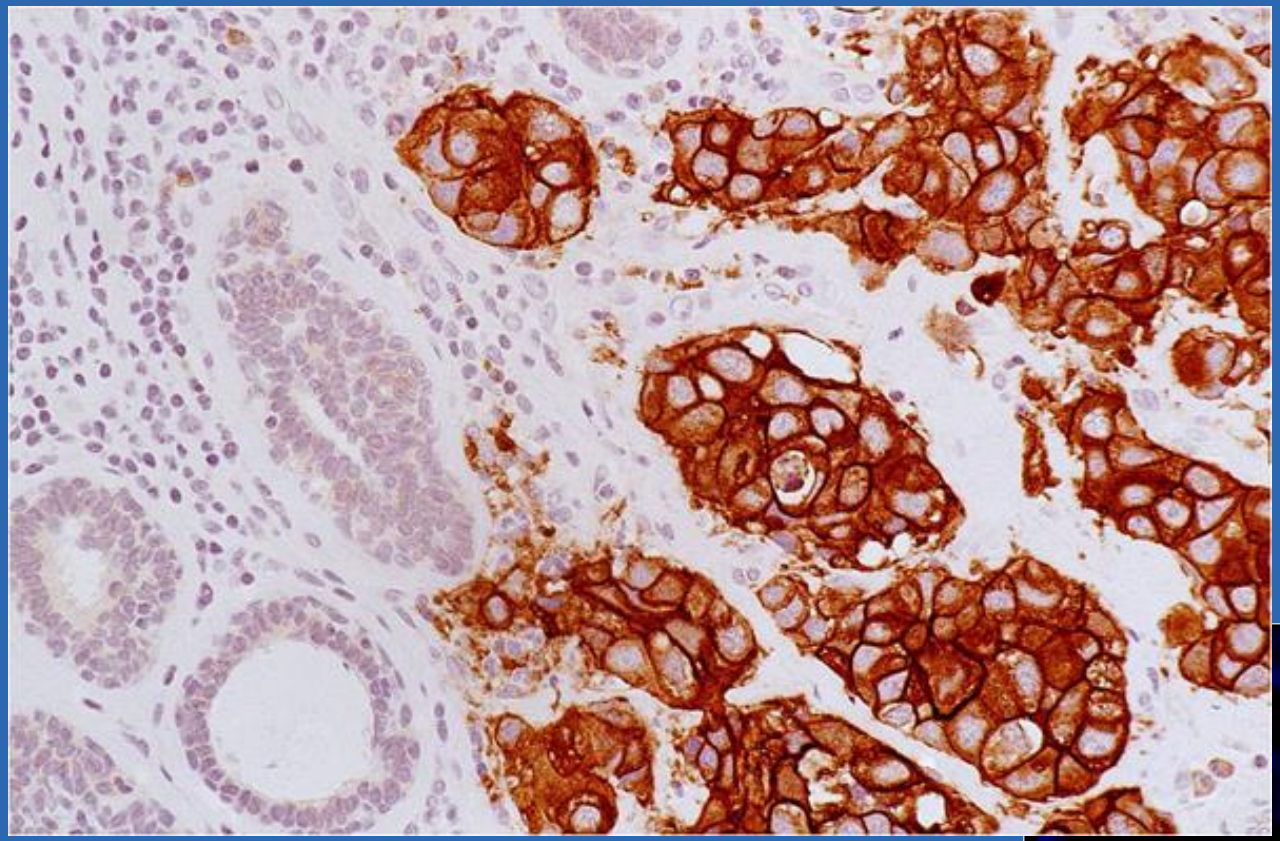



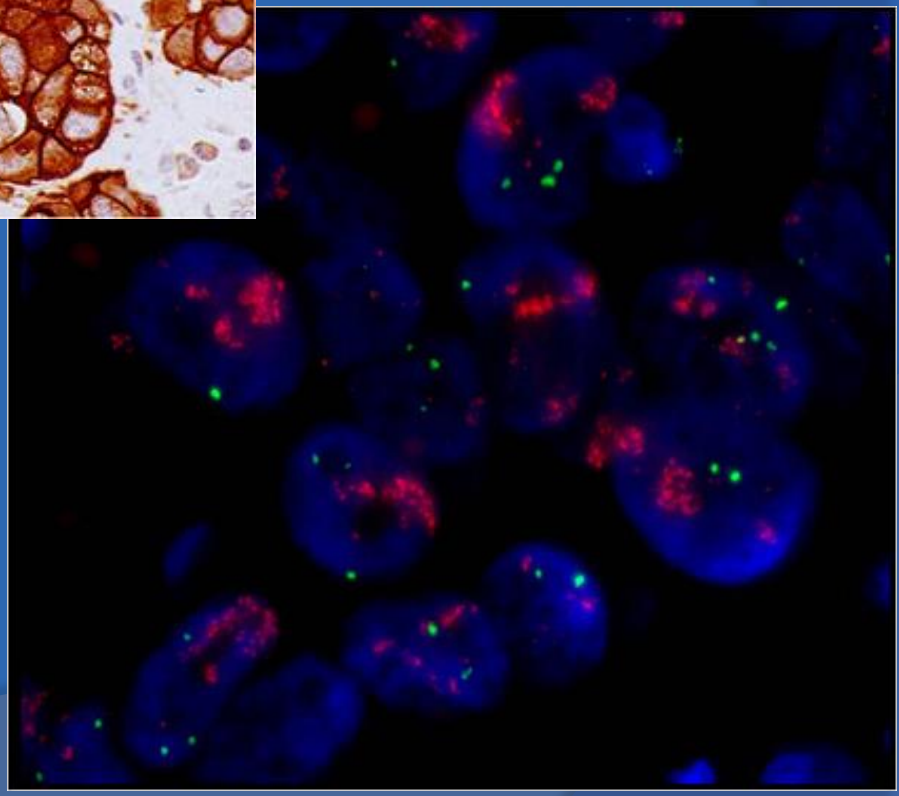

Well-differentiated liposarcoma. (A) G-banded karyotype from a high-grade tumor showing a complex near-tetraploid karyotype with multiple unbalanced structural abnormalities including the characteristic ring and giant marker chromosome. (B, C) FISH analysis with probe specific for the MDM2 gene (red) confirms the presence of MDM2 amplicons in the ring and the two marker chromosomes.

# Breast cancer

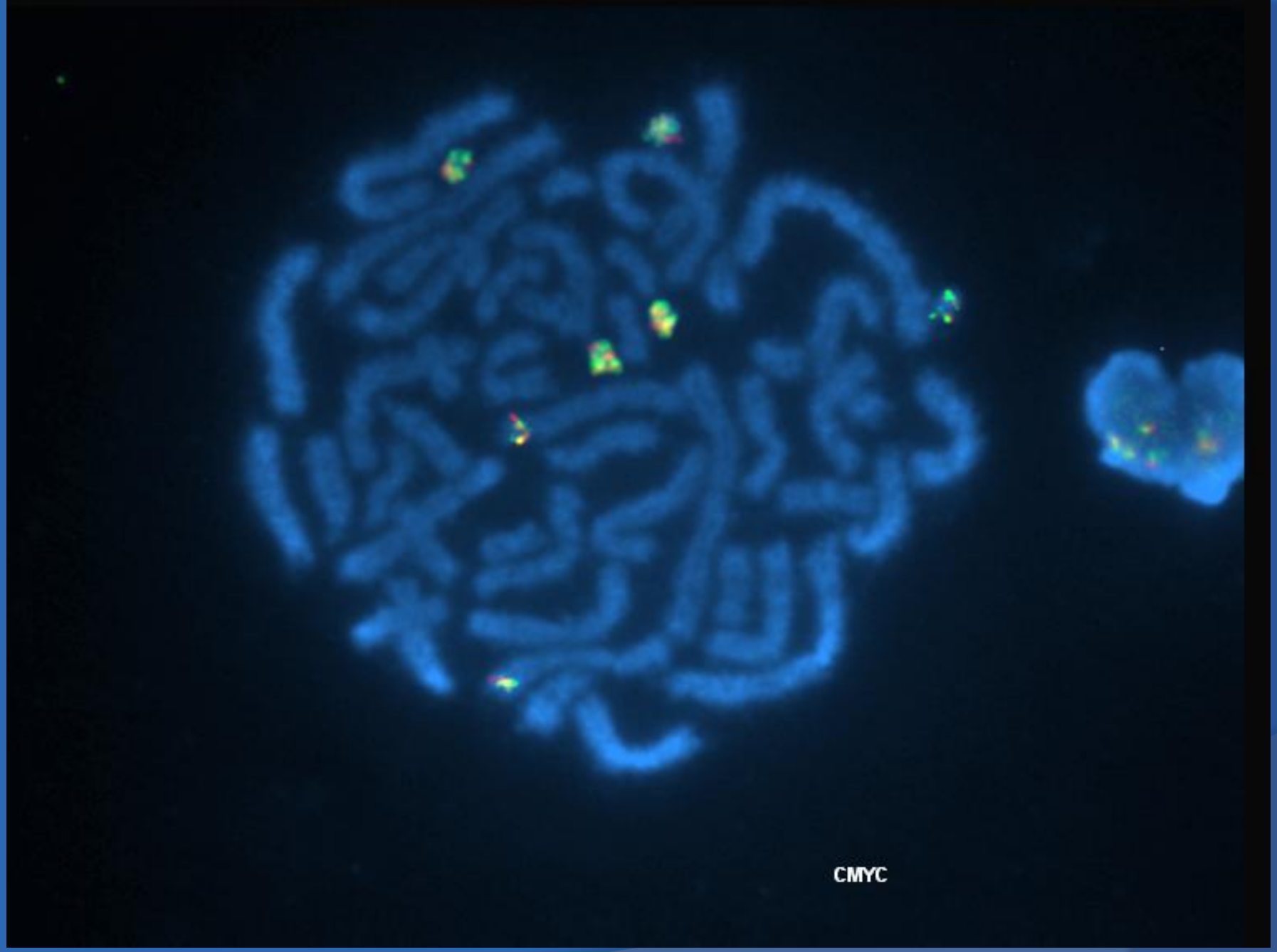
## HER2/Neu amplification


17q11.1-q11.1 CEP 17
alpha satellite
SpectrumGreen
17q11.2-q12 LSI
HER-2/neu
SpectrumOrange
17

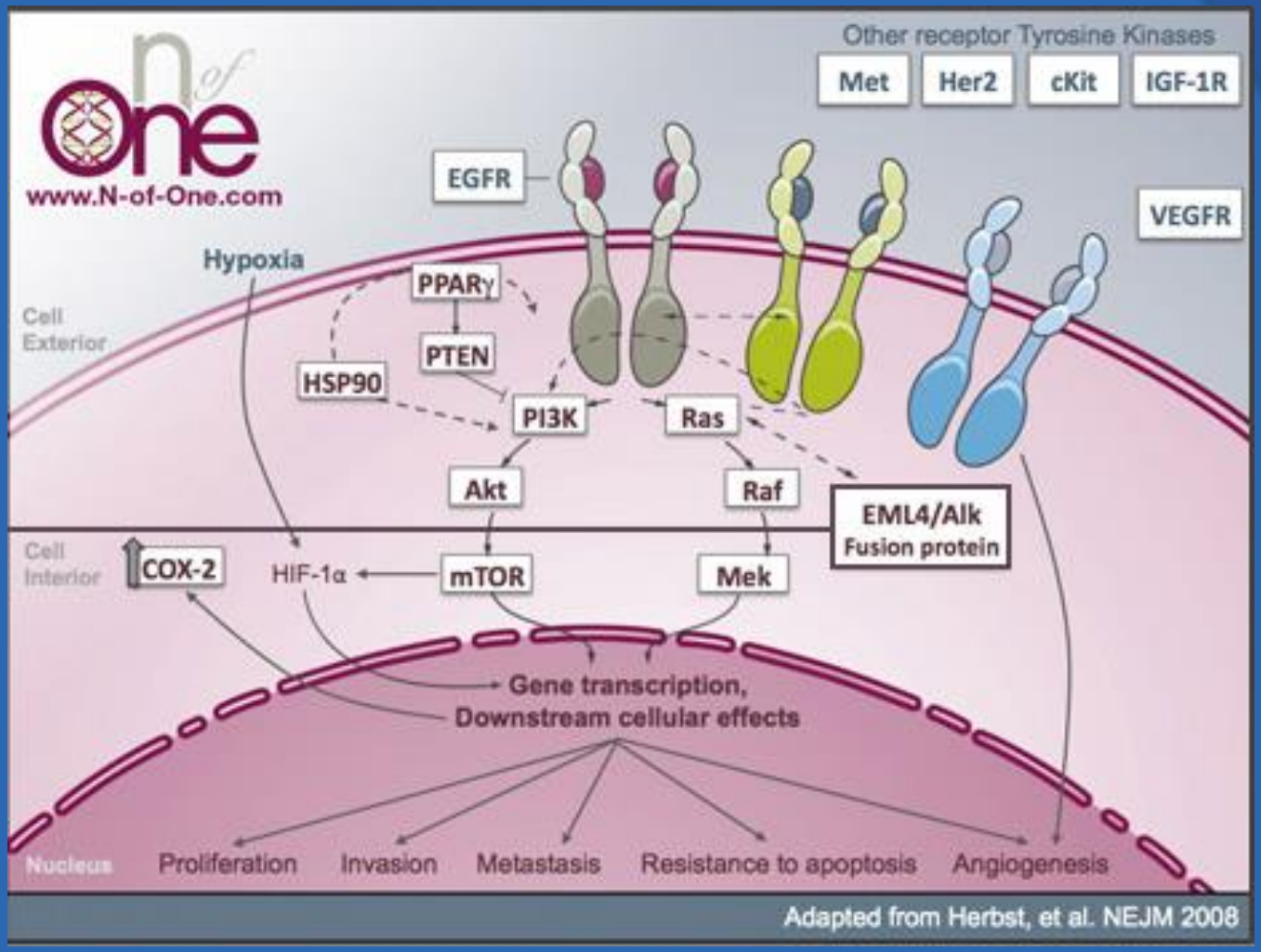




**Immunohistochemistry for Her2 (strong and diffuse, circumferential membranous staining, 3+)**




CMYC




| | | | |
|---|---|---|---|
| Lung Carcinoma | 5p15.2, 7p12, 8q24 | *?, EGFR, MYC* | Diagnosis in conjunction with cytology |
| | inv(2)(p21p23) | *EML4–ALK* | Treatment selection with ALK inhibitors |
| | 7p12 amplification | *EGFR* | Treatment selection with kinase inhibitors |
| | −1p31, −3p14, 3p21, −9p21, −17p13 | *HLJ1, FHIT, SEMA3B, CDKN2A, TP53* | |
| | −4q12−q23 | *?* | ? Metastasis |

# LUNG CANCER

**ALK (anaplastic lymphoma kinase)**

**2p23.2**

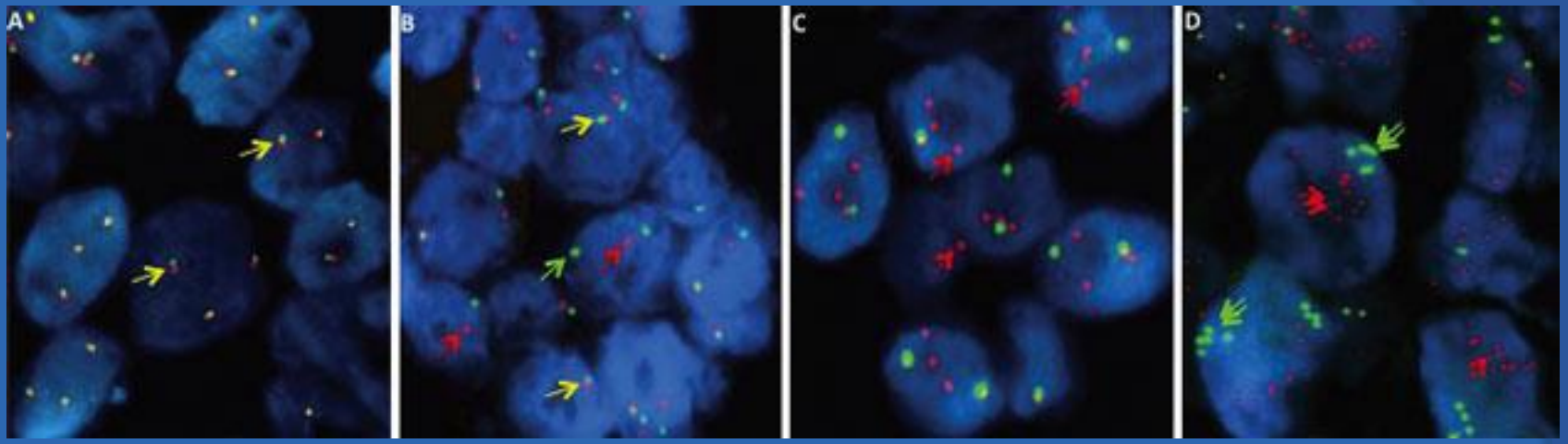
**EML4 (echinoderm microtubule associated protein-like 4) 2p21**

**Diverse small inversions on 2p**

**Non-small cell lung cancer**

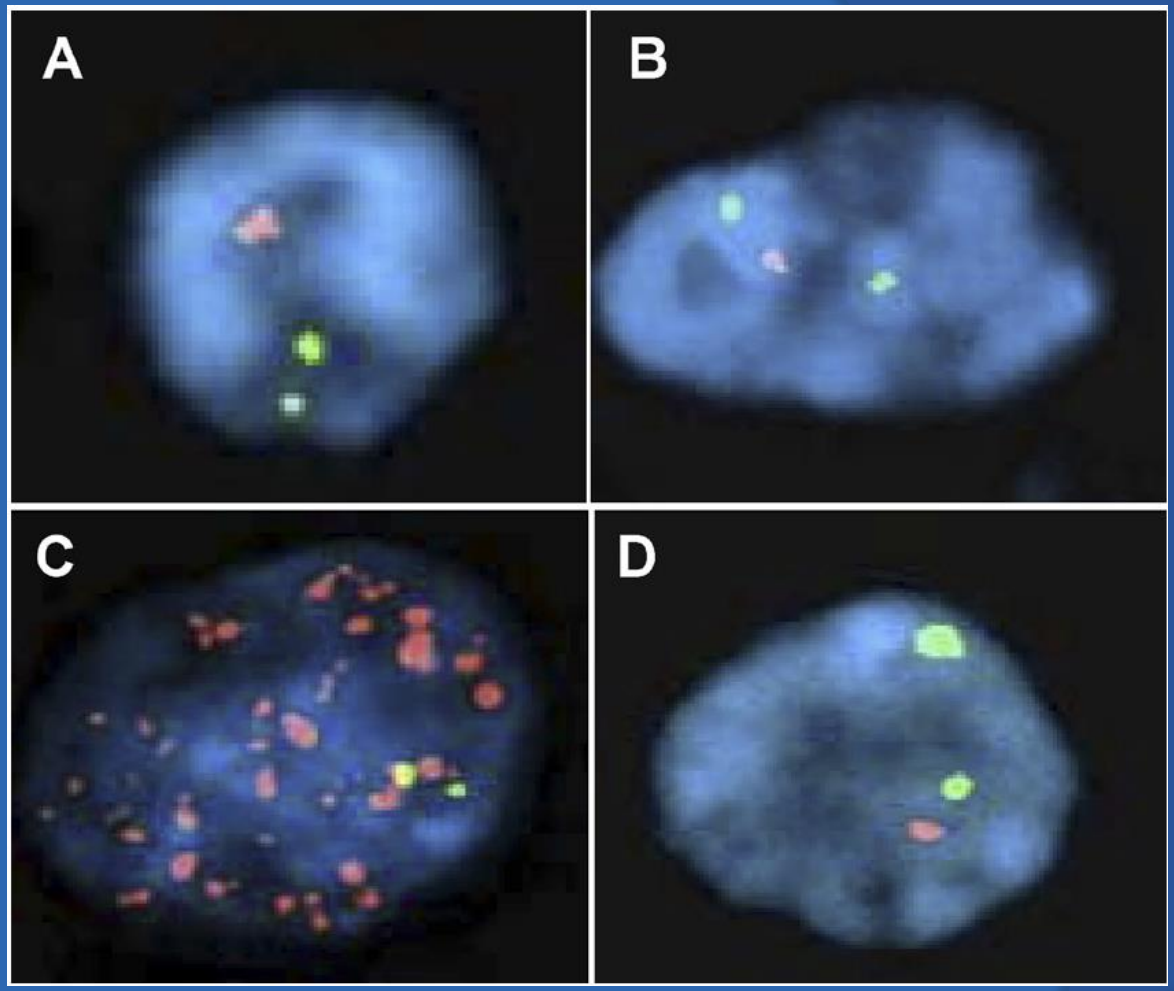
**Crizotinib**

# Break-Apart FISH Probe Kit Used to Detect ALK Positivity




When an *ALK* rearrangement occurs, the probes separate, either appearing as a classic "split" pattern (red and green arrows in panel B) or a "single red" pattern where red signals outnumber green signals, suggesting that both a rearrangement and loss of the 5' probe (non-kinase encoding) binding site has occurred (red arrows in panel C). Increases in both rearranged (double red and green arrows in panel D) and native *ALK* copy number can occur. Copy number gain (CNG) of rearranged signals has been associated with acquired resistance to crizotinib, but CNG of the native gene is not currently considered of any clinical significance.

FISH analysis in neuroepithelial tumors. (*A, B*) Two separate hybridizations identify the co-deletion of 1p36 and 19q in oligodendroglioma on the same tissue section. (*A*) Loss of 1p36 (*TP73*) (*red*) and 2 copies of the control probe at locus 1q25 (*ANGPTL*) (*green*). (*B*) Loss 19q13 (*GLTSCR*) (*red*) and 2 copies of the control probe at locus 19p13 (*ZNF443*) (*green*)




(*C*) Interphase nuclei from a patient with glioblastoma showing high level amplification of *EGFR* (7p12) (*red*) and 2 copies of centromere 7 (*green*). (*D*) The same patient also showed loss of *PTEN* (10q23) (*red*) and 2 normal copies of the control probe at centromere 10 (*green*).

- **Chromosome abnormalities in cancer**
  - **Acquired**
  - **Nonrandom**
  - **Clonal**
  - **Used for diagnosis and prognosis**

# Clinical significance of chromosome abnormalities in leukemia and lymphoma

- **Diagnosis and differential diagnosis:**

  **WHO classification based on specific cytogenetic/molecular genetic findings, such as t(8;21), t(15;17), inv(16), t(9;11) and other 11q23/MLL, inv(3)/t(3;3), t(6;9), t(1;22).**

- **Treatment protocols:**

  **APL: PML/RARa: ATRA+CT.**

  **CBF [t(8;21) and inv(16)]: HDAC consolidation.**

- **Monitoring response and engraftment of BMT**

  **cytogenetic complete remission (CR) and MRD**

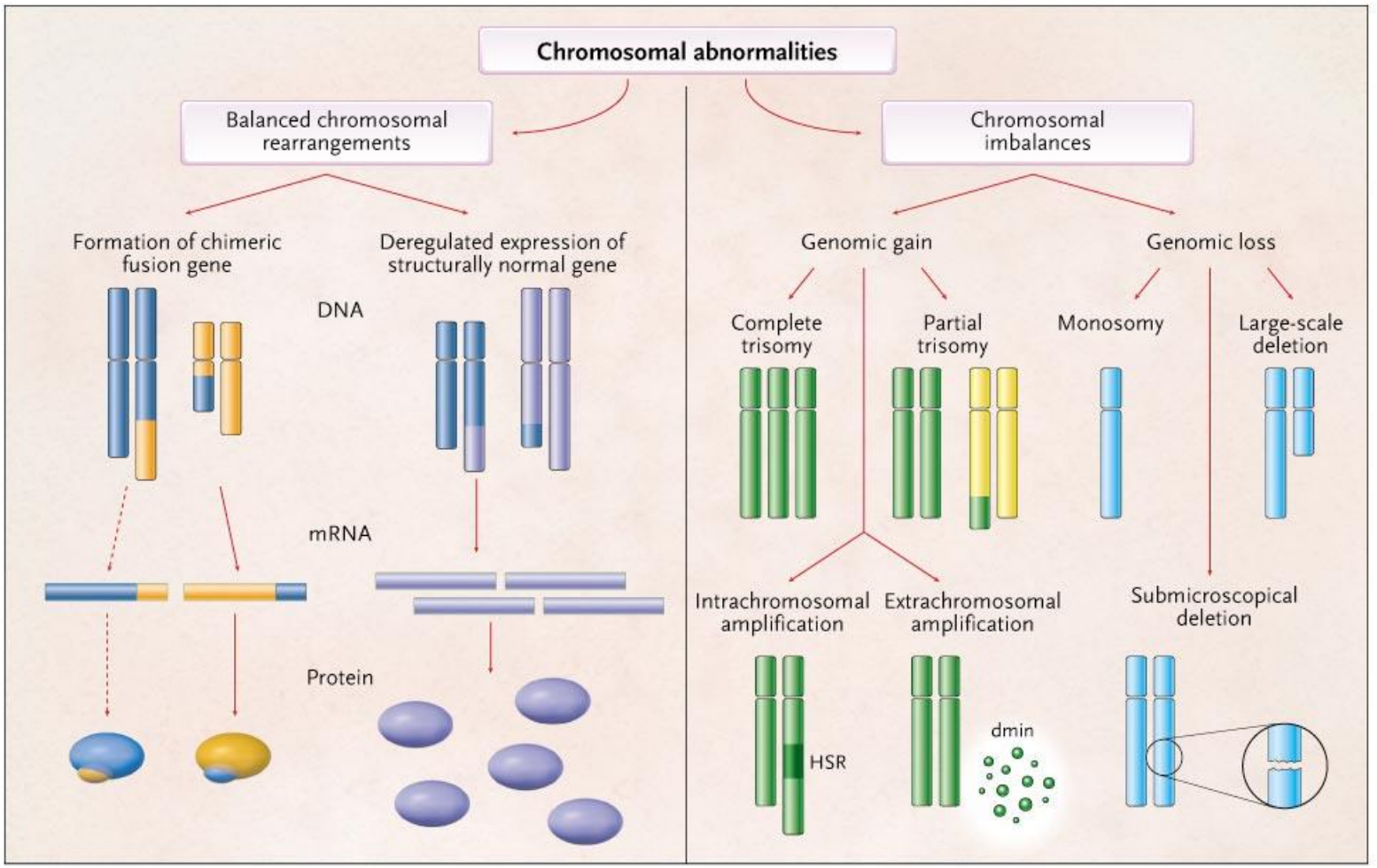
- **<u>Prognosis</u>: most critical and independent indicators.**

  **favorable (55-81% cured): t(15;17), inv(16), t(8;21);**

  **intermediate (40%): t(9;11), normal karyotype;**

  **unfavorable (<5%): complex, abnl 5 and 7, inv(3), t(6;9)**

# Chromosomal Abnormalities in Human Cancer




Fröhling S, Döhner H. N Engl J Med 2008;359:722-734

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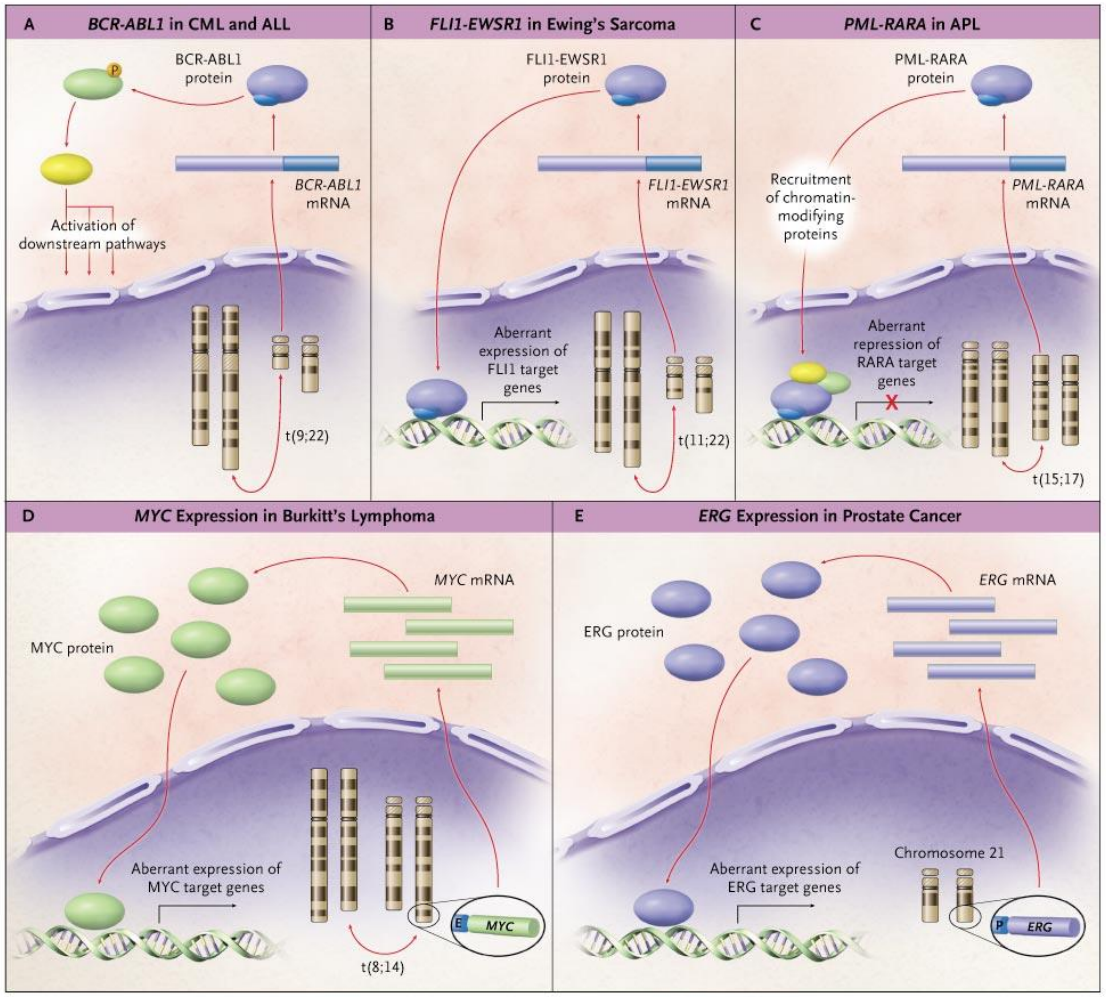
# Functional Consequences of Balanced Chromosomal Rearrangements

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# Chromosome Changes in Cancer

- Disease specific changes:
  - Leukemias
  - Lymphomas
  - Mesenchymal Tumors

# Chromosome Changes in Cancer

**Unbalanced multiple and unspecific karyotypic abnormalities:**

- **Epithelial tumors (breast, colon, lung, prostate, ovary)**
- **Mesenchymal tumors (osteosarcoma, malignant fibrous histiocytoma)**
- **Secondary hematologic malignancies**

# WEB References

- **http://www.atlasgeneticsoncology.org**
- **http://cgap.nci.nih.gov/Chromosomes/Mitelman**

# The ULTIMATE CYTOGENETICIST