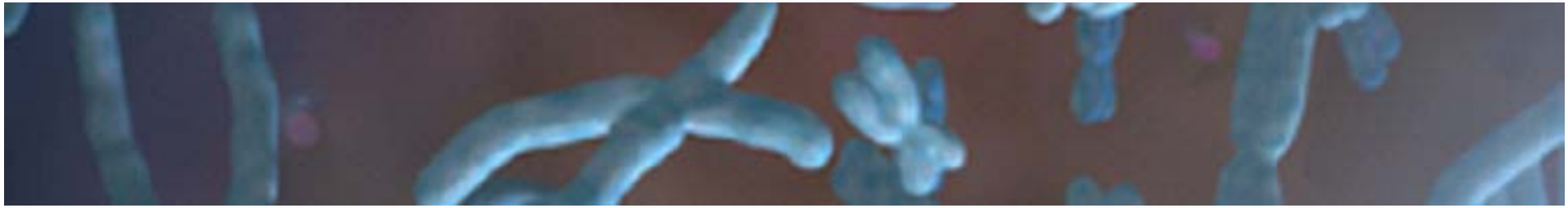
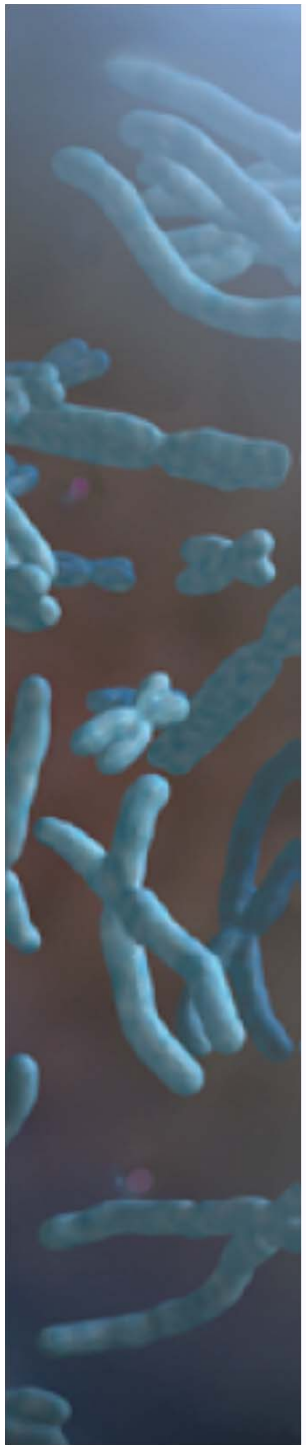
Cytogenetic analyses
in malignant hematological disorders



general concepts

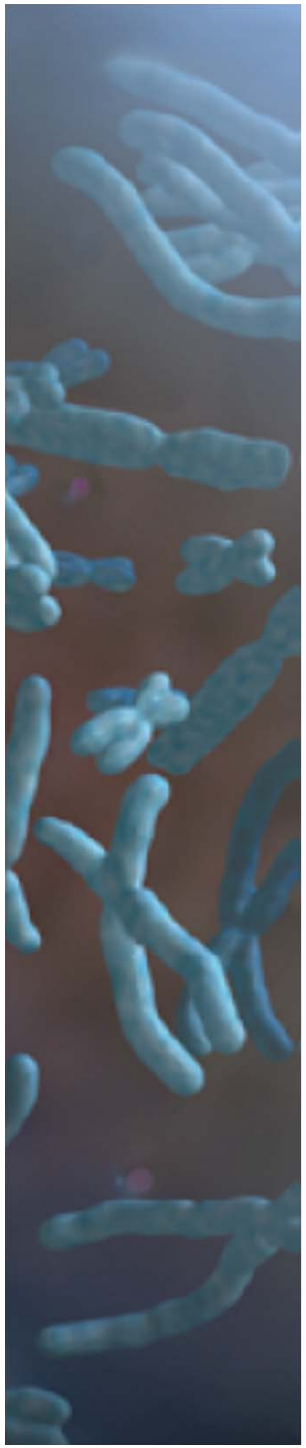
Lucienne Michaux

Lessenreeks
21/11/2017



Plan

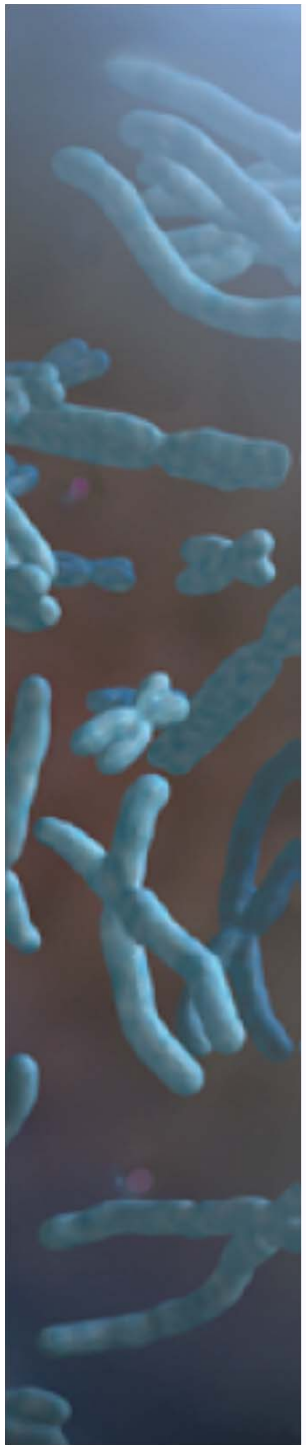
- Definition
- History
- Pathophysiology of malignant hematological disorders
- Techniques
  - Conventional cytogenetics
  - Molecular cytogenetics
- Indications of cytogenetic analyses
- Interest of cytogenetic analyses



Definition

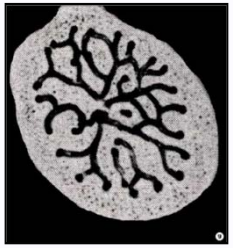
= cell genetics

- Conventional: karyotype (1950-…)
- Molecular: isotopic followed by non isotopic techniques (1985-…):
  - Immuno-enzymatic,
  - immunofluorescence (FISH)



History

- **1890**: nuclear and mitotic abnomalities in carcinoma cells (*Von Henseman*)
- **1914**: clonal chromosomal aberrations responsible for malignant transformation - theory (*Boveri*)
- **1956**: number of human chromosomes (*Tjio & Levan*)
- **1960**: small « marker » chromosome = « Philadelphia chromosome» in chronic myeloïd leukemia (*Nowell & Hungerford*)



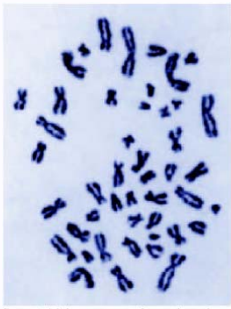
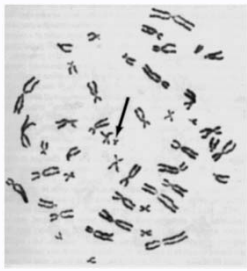
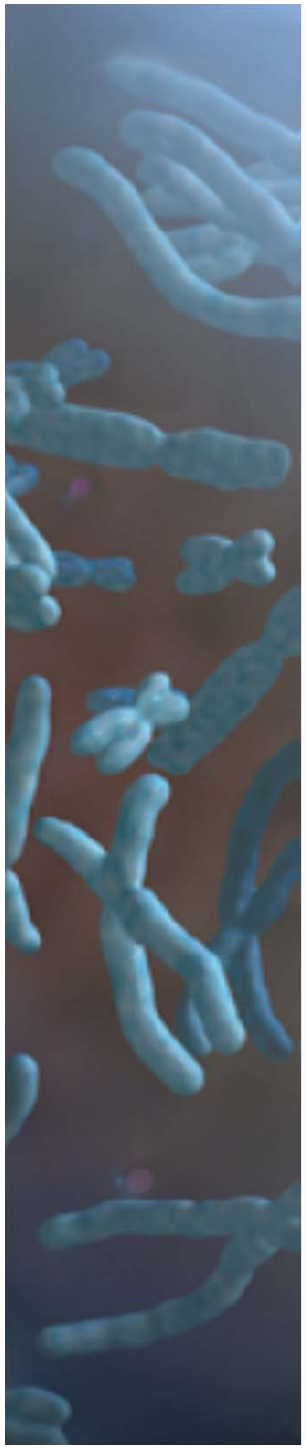


Figure 5 | **A human metaphase plate, from the original Tjio and Levan paper, showing 46 chromosomes.** Reproduced with permission from REF. 14 © (1956) Blackwell Publishing.

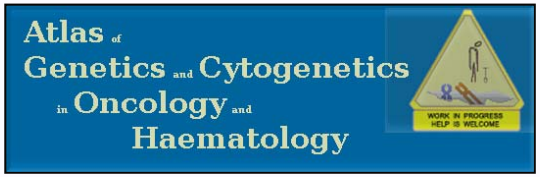


«A minute chromosome in human chronic granulocytic leukemia», ***Science, 1960***



- **1970**: chromosomal banding (*Casperson*)

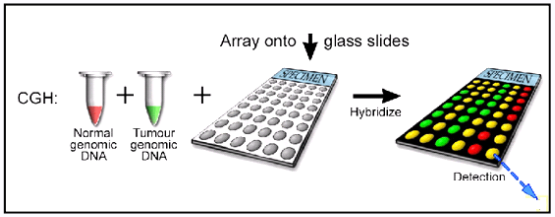
- **1970**-…: discovery of recurrent cytogenetic aberrations;
  correlations with diagnosis and prognosis

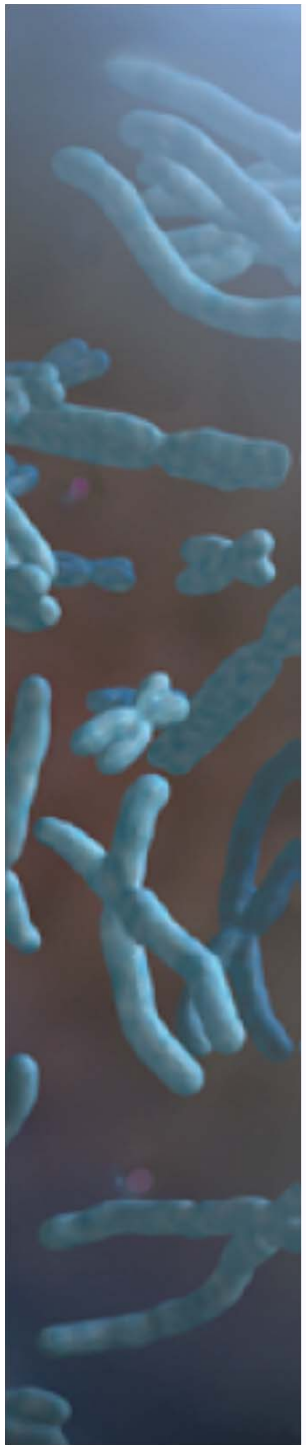


- **1975-**…: development of molecular biology, cloning of involved genes, functional studies

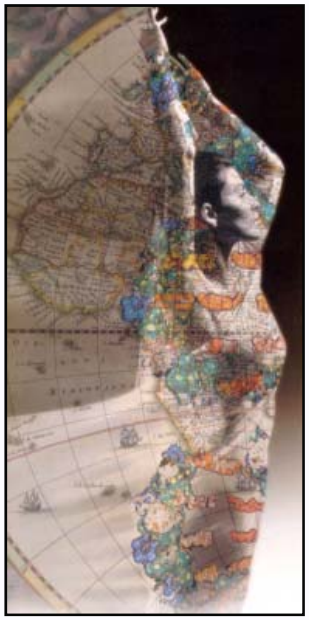
- **1990-**…: 1st therapeutic applications

- **1990-**…improvement of culture techniques and « onset » of molecular cytogenetics → progress +++
  - FISH, variants (microarrays)

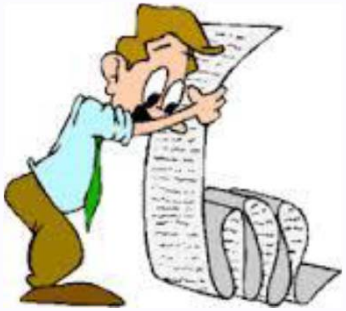


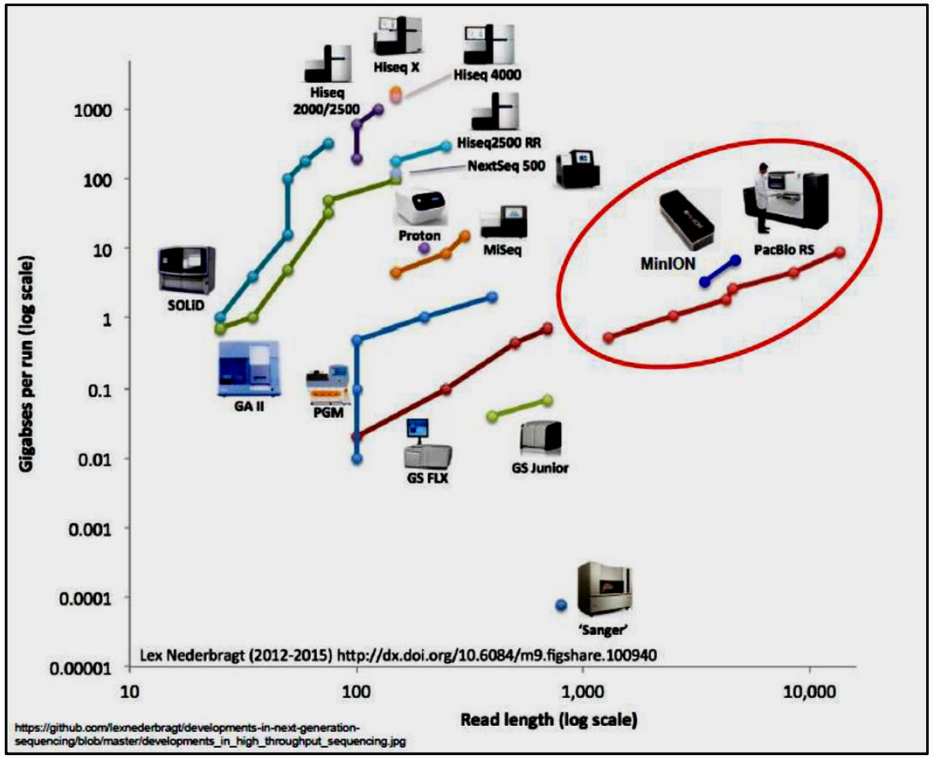


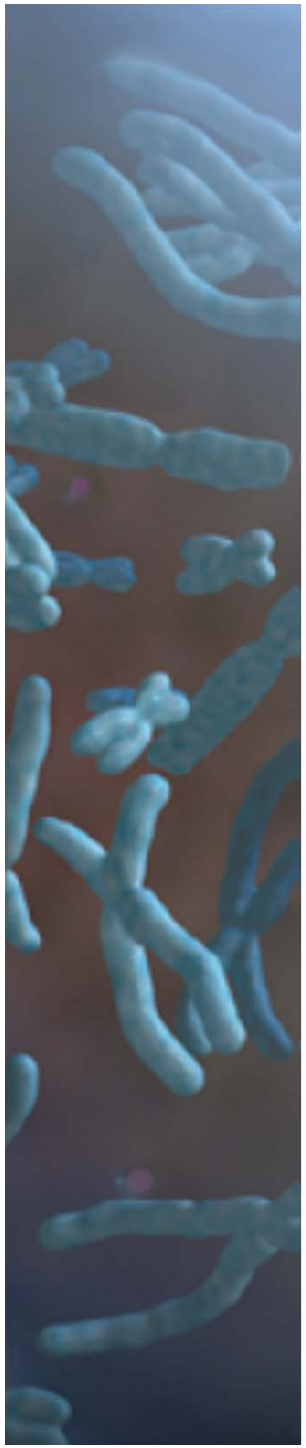
- **2001**: human genome mapped (***Hu**man **G**enome **O**rganisation*)



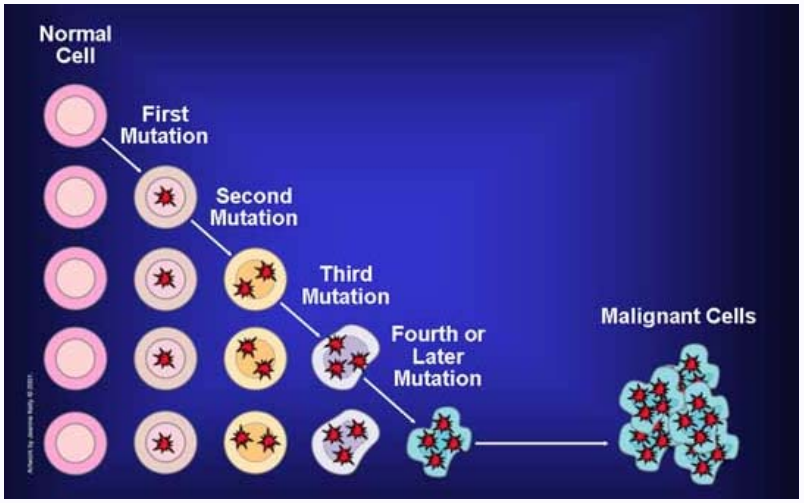
- **2009**: first whole genome sequencing of an AML (*Mardis*), ...
  - → high-throughput





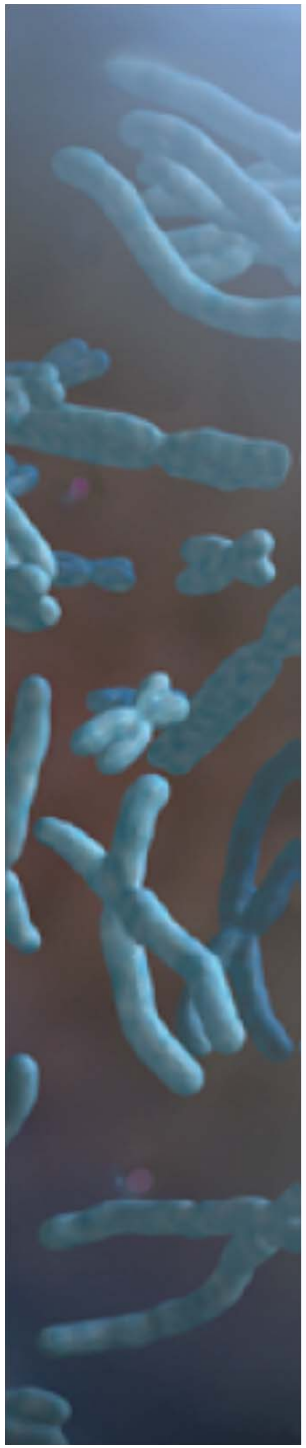


Pathophysiology



- Malignant hemopathies are acquired diseases characterized by genetic aberrations which persist (= clonality) and accumulate (= clonal evolution)
- Clonality detection is useful (☝: clonality ≠ always malignancy)
- Some aberrations are disease-specific

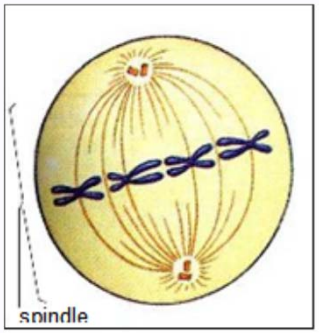
→ Clonality = diagnostic classifier & follow-up tool

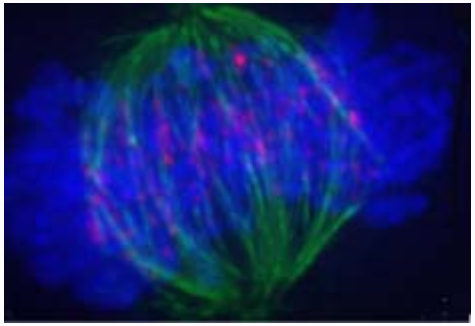


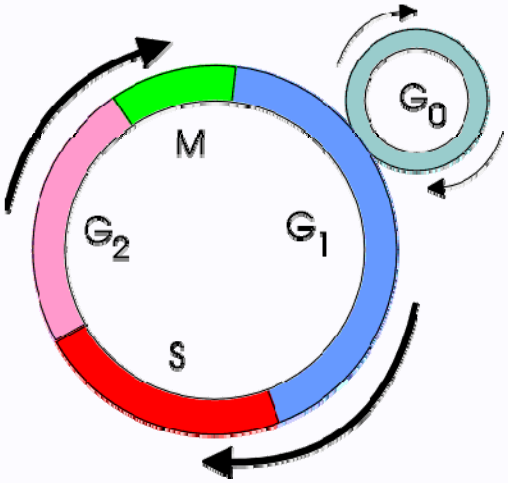
Karyotype

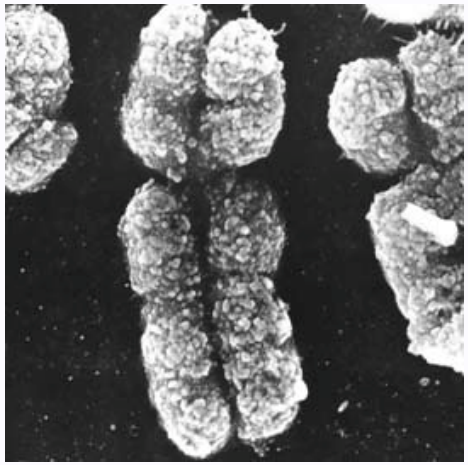
Principle: detection of chromosomal aberrations in dividing malignant cells (mitoses; in particular metaphases), using cell biology techniques

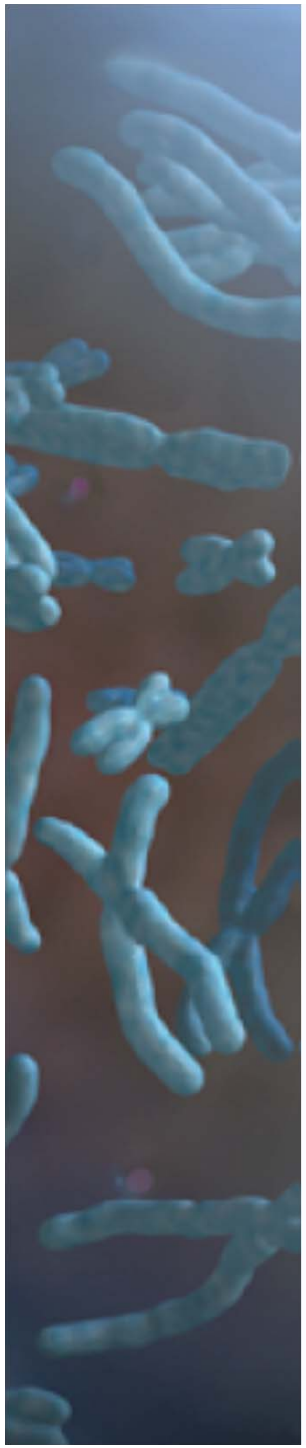
Mitosis **≤ 1 hour**

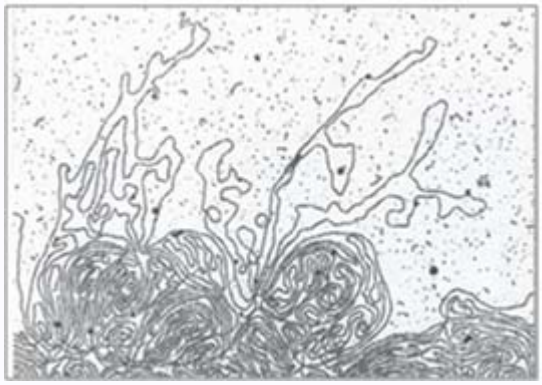


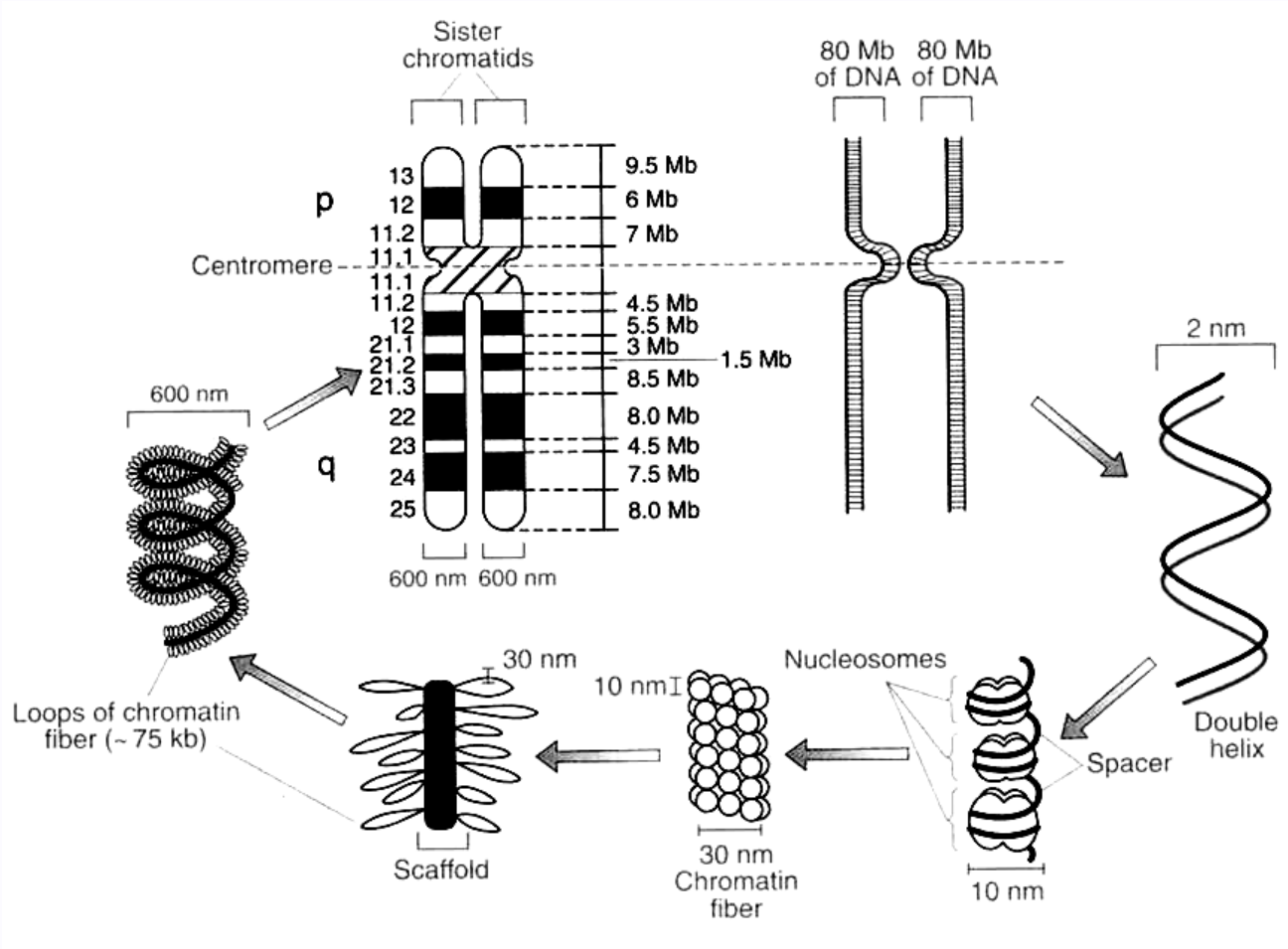











Sister chromatids
80 Mb of DNA
80 Mb of DNA
p
q
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12
11.2
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12
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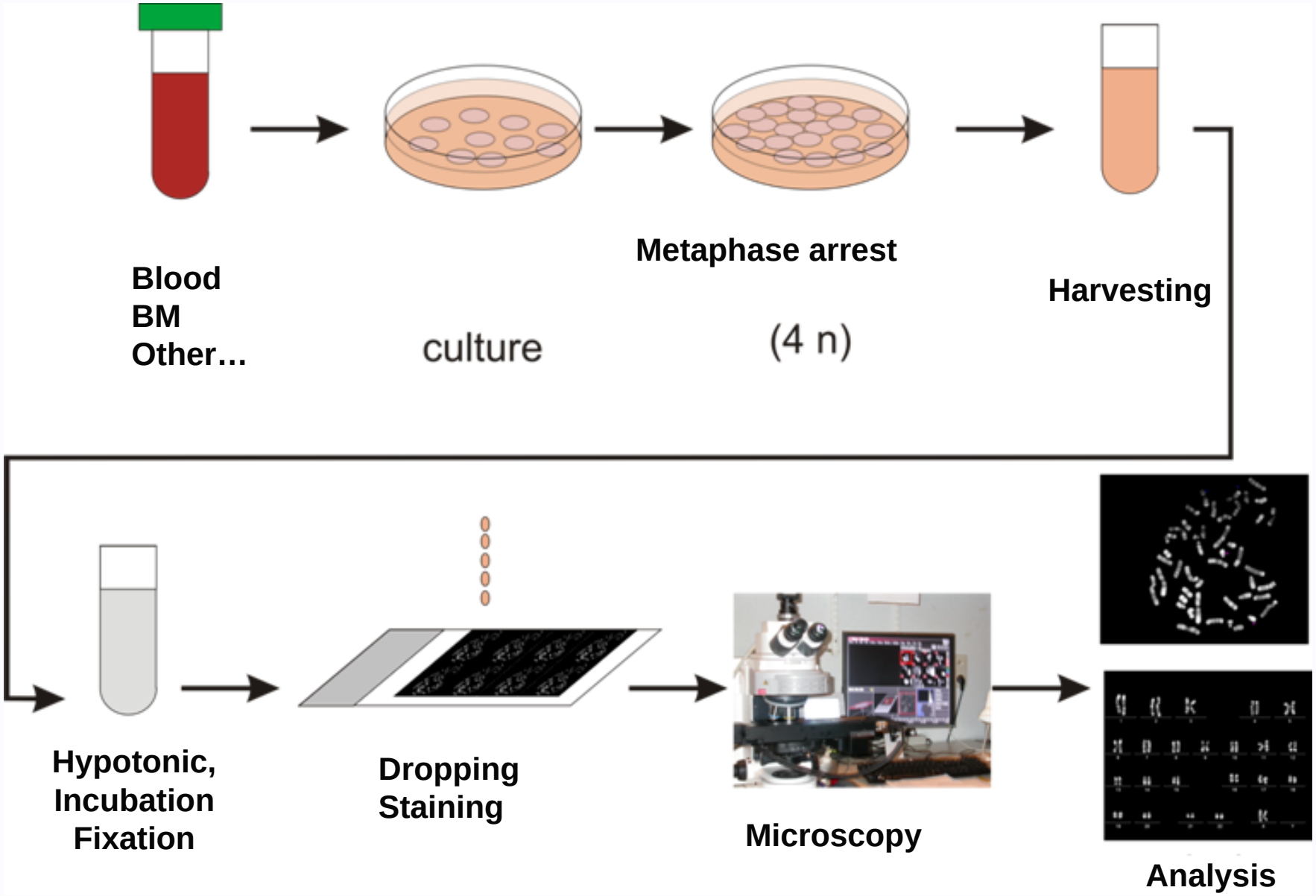
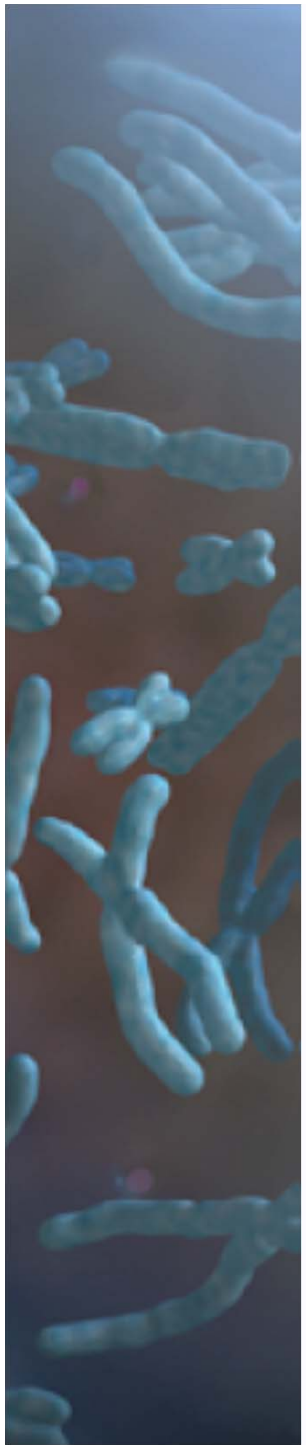
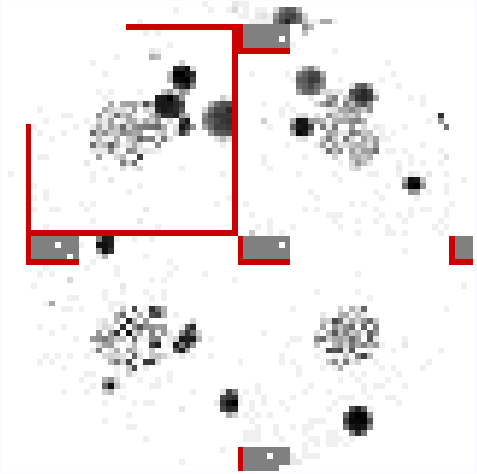
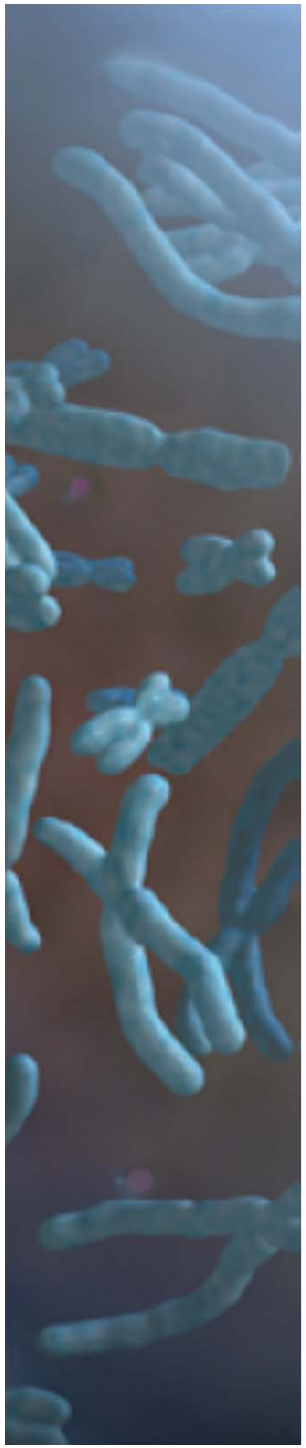
21.3
22
23
24
25
9.5 Mb
6 Mb
7 Mb
4.5 Mb
5.5 Mb
3 Mb
1.5 Mb
8.5 Mb
8.0 Mb
4.5 Mb
7.5 Mb
8.0 Mb
Centromere
600 nm
600 nm
600 nm
2 nm
Double helix
Spacer
Nucleosomes
10 nm
30 nm Chromatin fiber
30 nm
Scaffold
Loops of chromatin fiber (~75 kb)

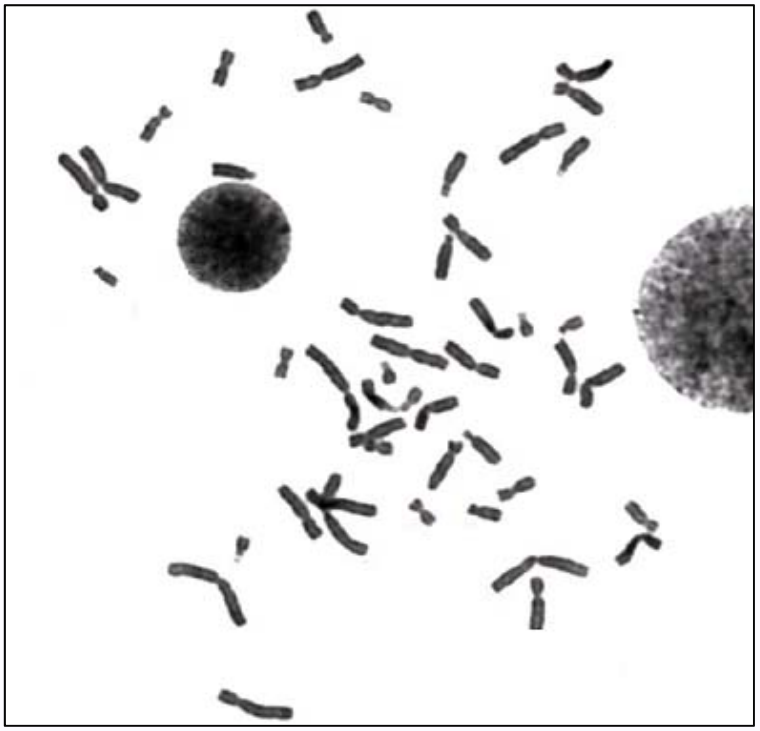
Karyotype: steps

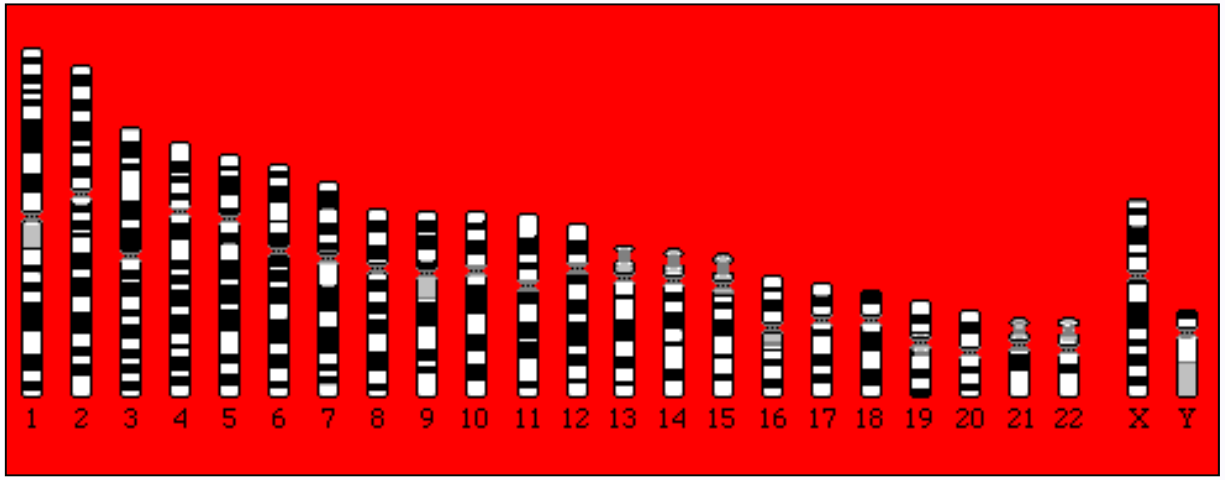








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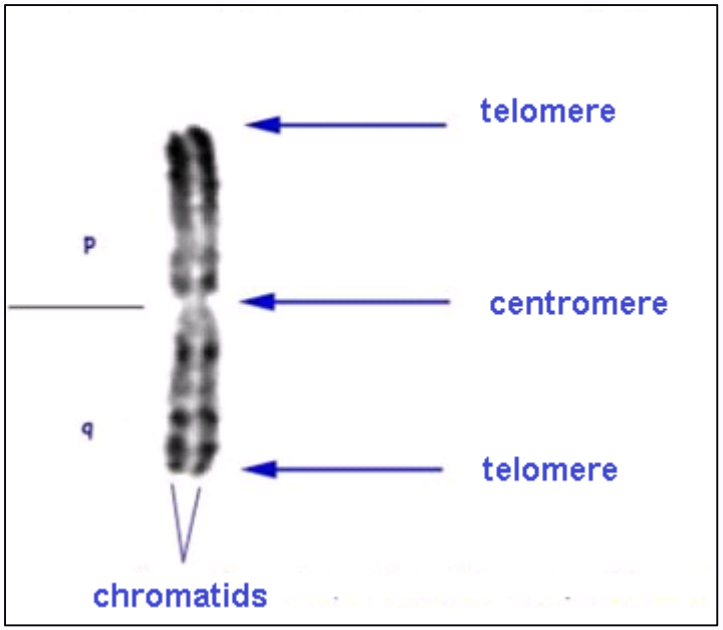
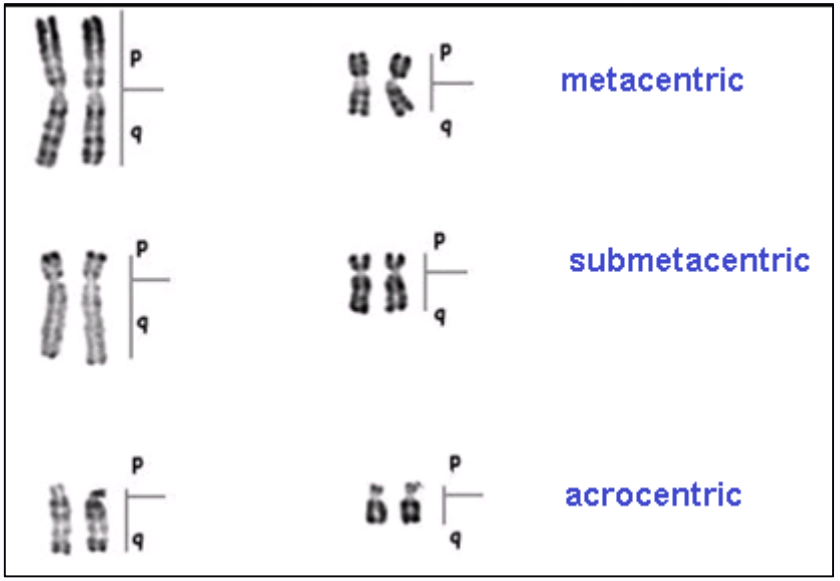
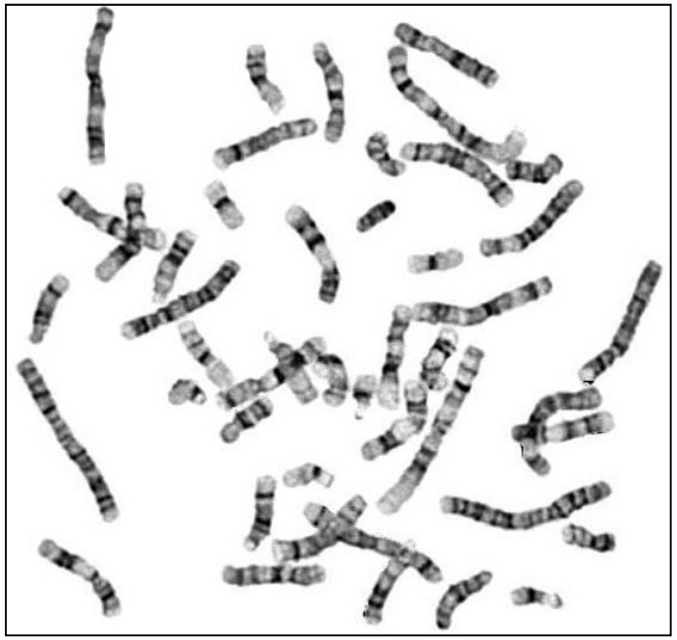
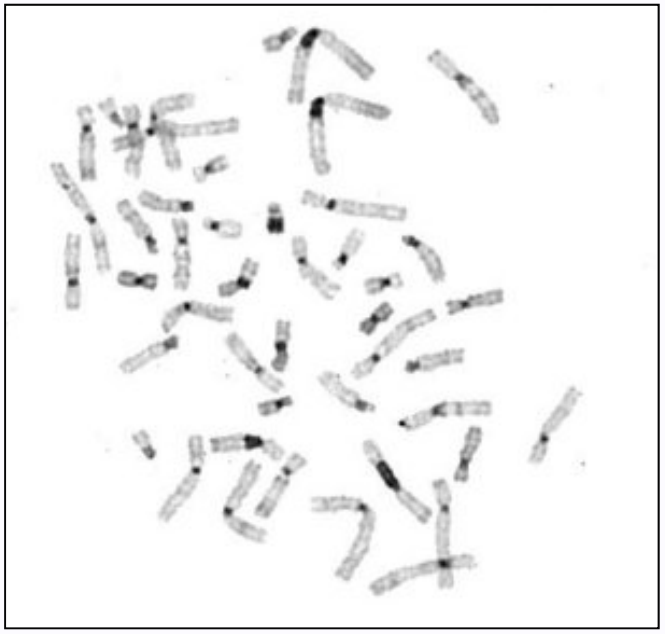

telomere
p
centromere
q
telomere
chromatids


p
q
metacentric
submetacentric
acrocentric

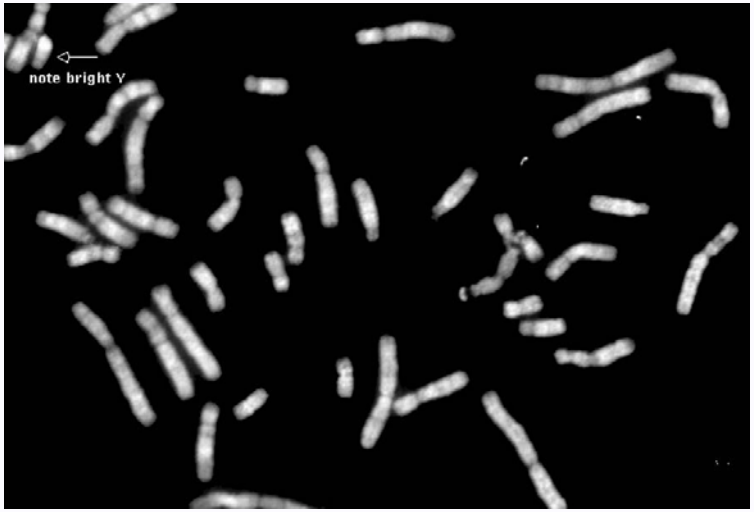


G banding (trypsin-**G**iemsa)

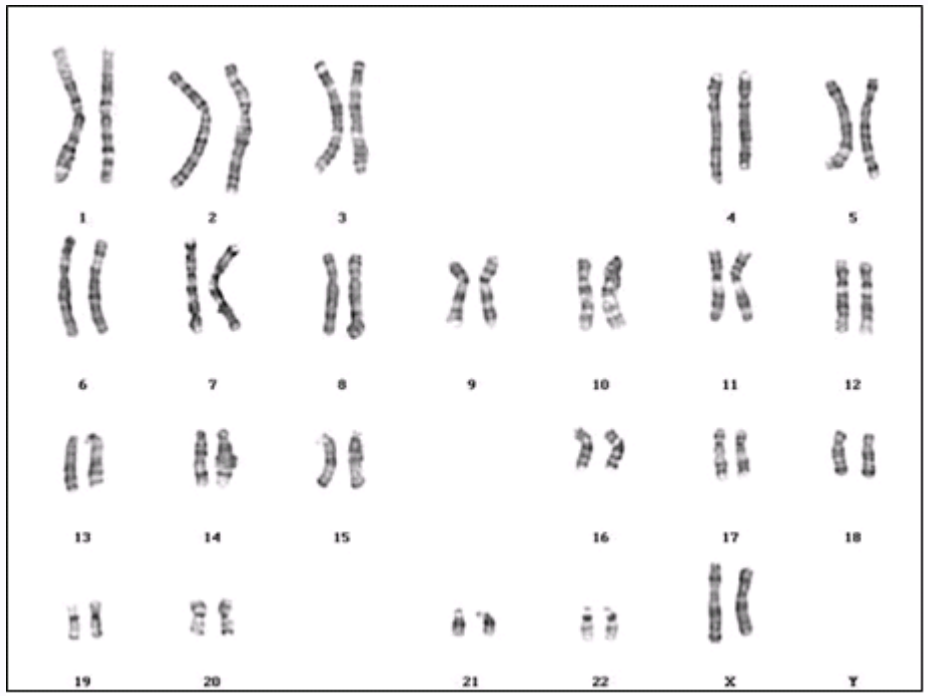


C banding (**c**entromeric,
Baryum hydroxyde)

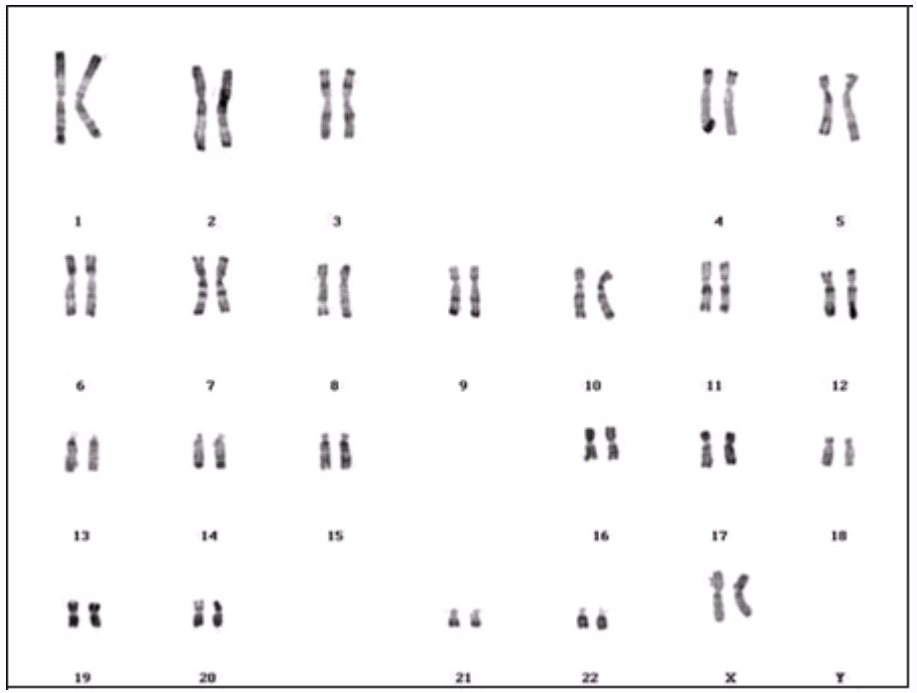
R banding (**r**everse:
heating+ acridine orange)



Q banding (**q**uinacrine)



GTG



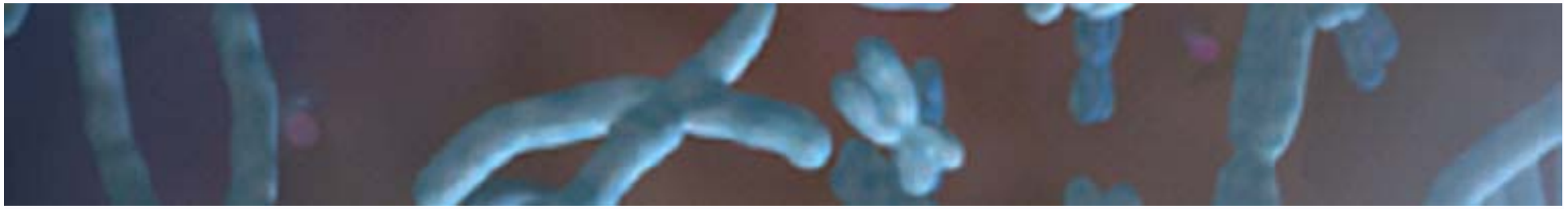
RHG

Normal human karyotype :

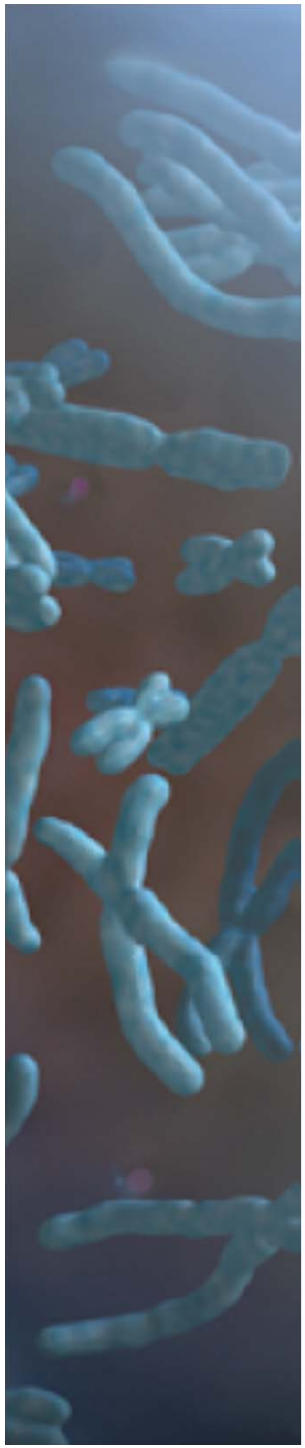
46 chromosomes

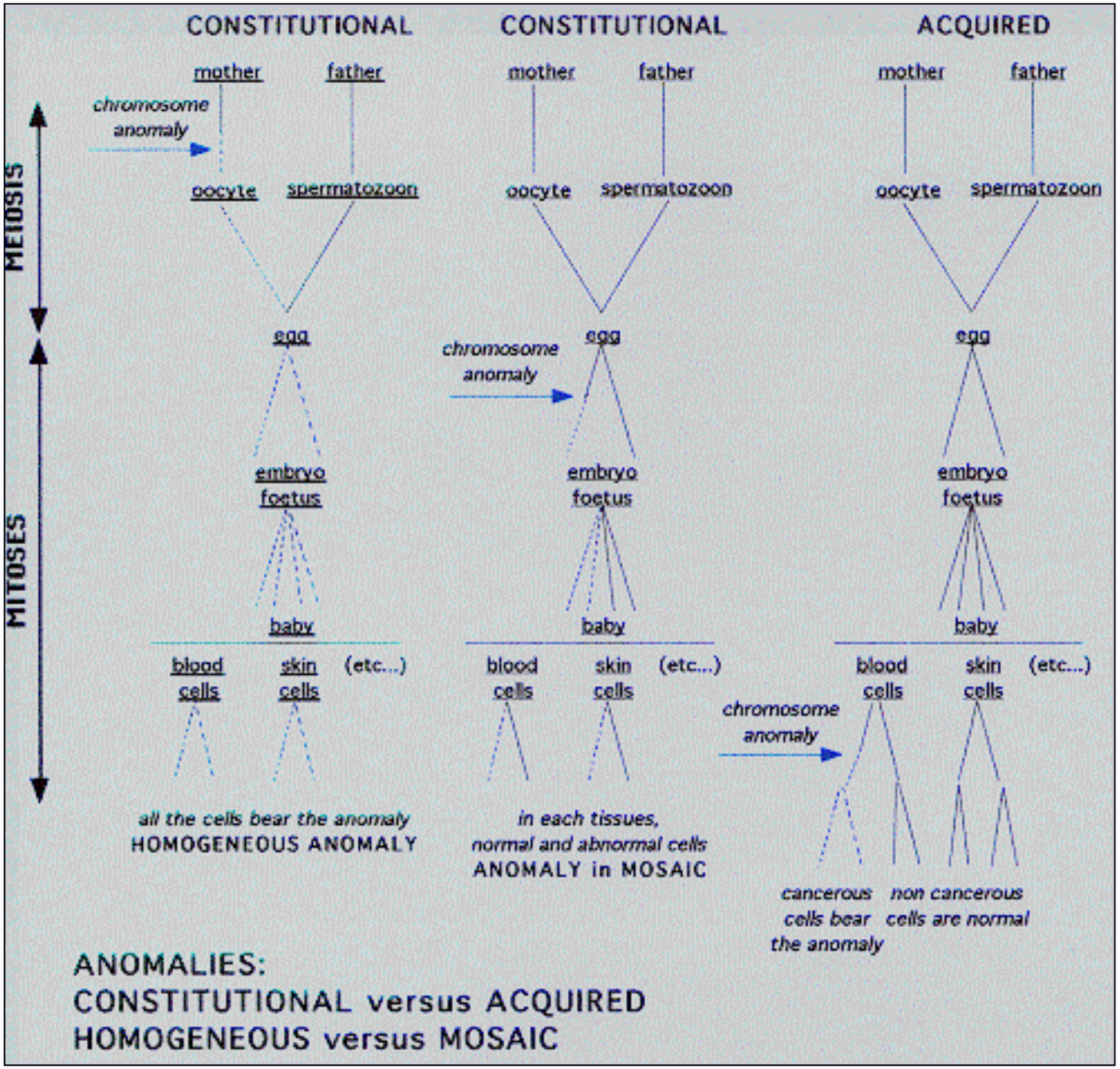
- 22 pairs of autosomes
- 2 gonosomes (XX or XY)

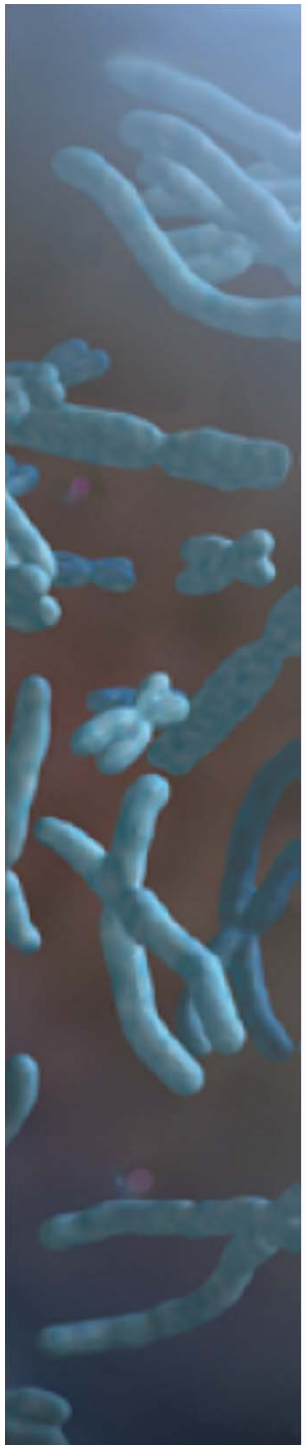
- Aim of karyotype in hemato-oncology = detection and characterization of an abnormal cell population

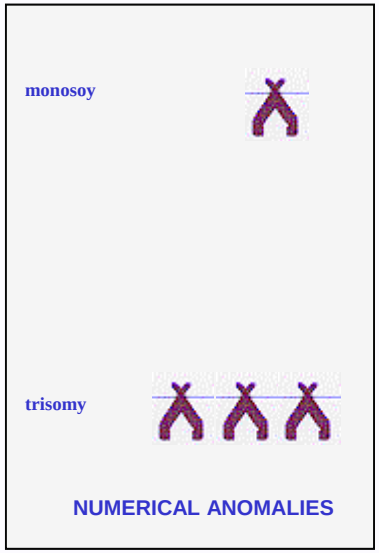


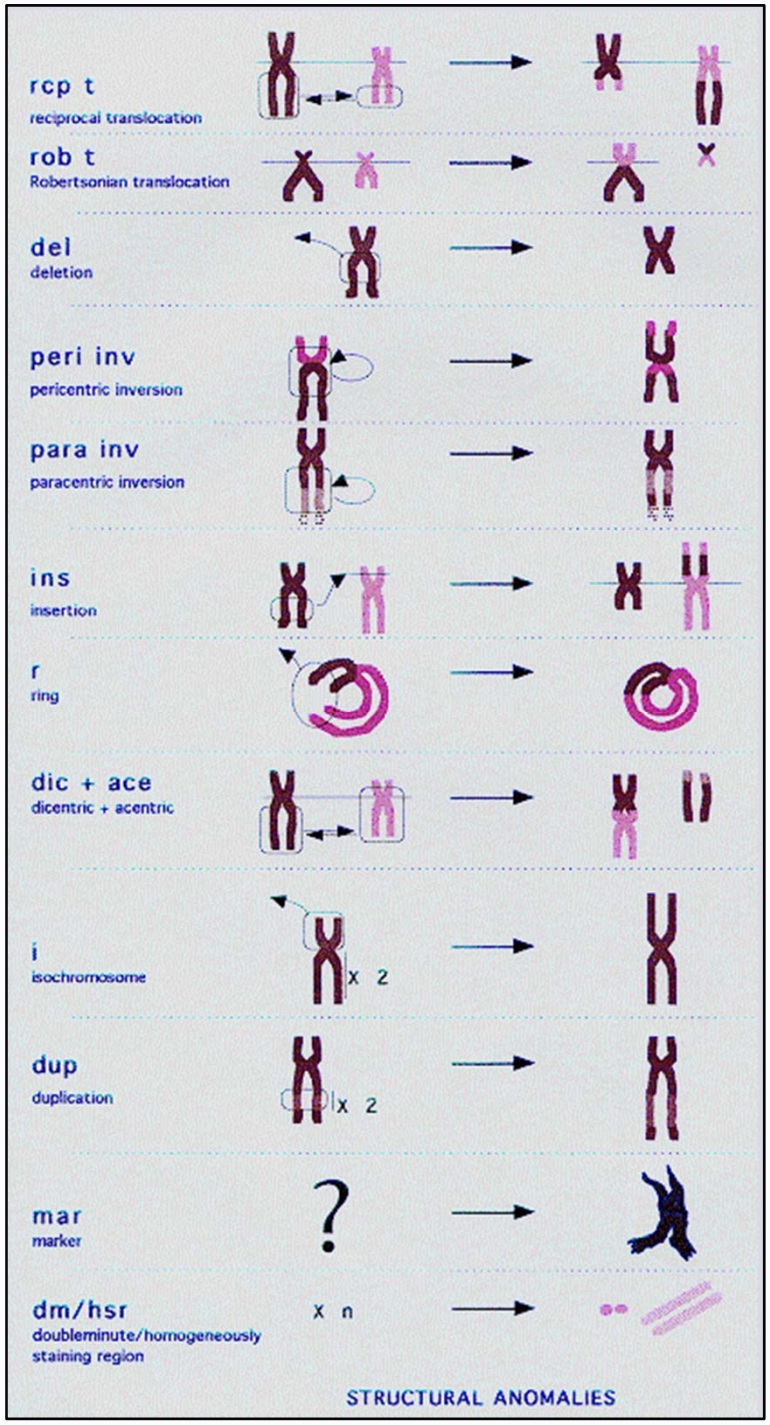
- **Clone** = cell population originating from the division of an « ancestral cell »
  - *Cytogenetic definition :**at least 2** cells with supernumerary or structurally abnormal chromosomes, **at least 3 cells** with chromosomal loss.*
  - *often (but not aklways), monoclonality = malignancy*



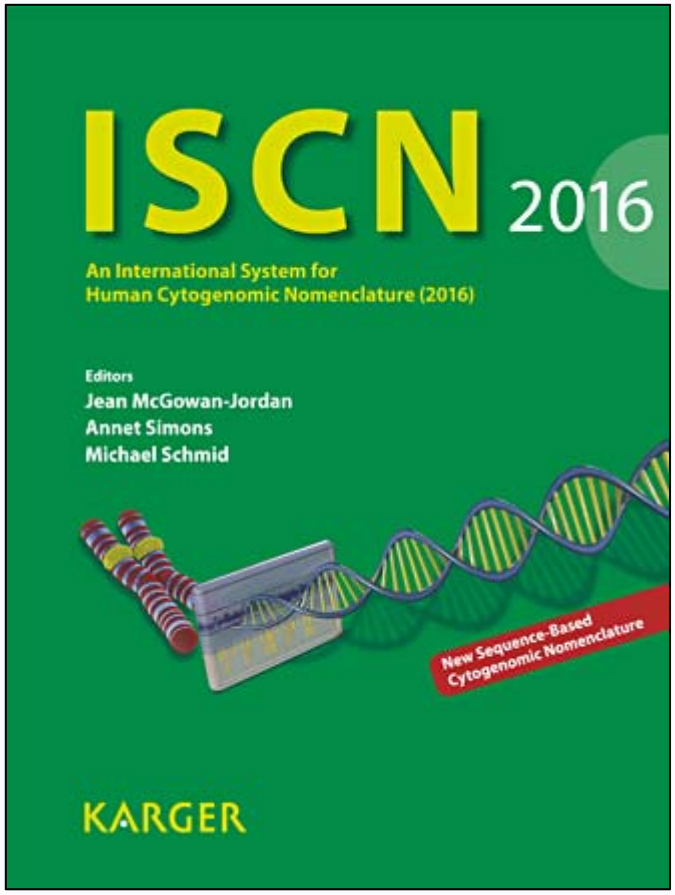

CONSTITUTIONAL
CONSTITUTIONAL
ACQUIRED
mother
father
chromosome anomaly
oocyte
spermatozoon
MEIOSIS
MITOSES
egg
embryo foetus
baby
blood cells
skin cells
(etc...)
all the cells bear the anomaly
HOMOGENEOUS ANOMALY
in each tissues, normal and abnormal cells
ANOMALY in MOSAIC
cancerous cells bear the anomaly
non cancerous cells are normal
ANOMALIES:
CONSTITUTIONAL versus ACQUIRED
HOMOGENEOUS versus MOSAIC




monosoy
trisomy
NUMERICAL ANOMALIES


rcp t
reciprocal translocation
rob t
Robertsonian translocation
del
deletion
peri inv
pericentric inversion
para inv
paracentric inversion
ins
insertion
r
ring
dic + ace
dicentric + acentric
i
isochromosome
x 2
dup
duplication
x 2
mar
marker
?
dm/hsr
doubleminute/homogeneously staining region
x n
STRUCTURAL ANOMALIES

Result (karyotype): expressed as a f**ormula**, according to rules and nomenclature (ISCN 2016)

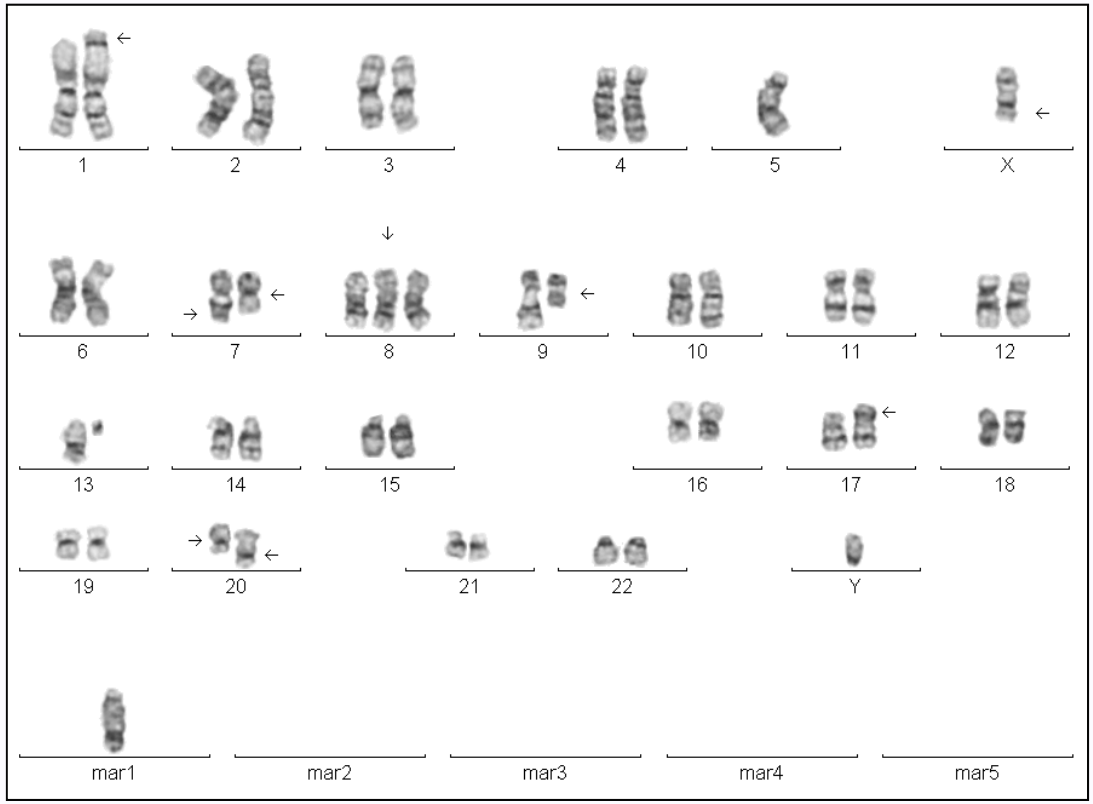


- Each clone is decribed separately (« / » between clones)
- Number of chromosomes (« modal » number) of the clone
- Gonosomes (according to ploïdy) and abnormalities
- Autosomes (ascending order: 1→22) and abnormalities
- Number of cells in the clone : [ ]

Ex:

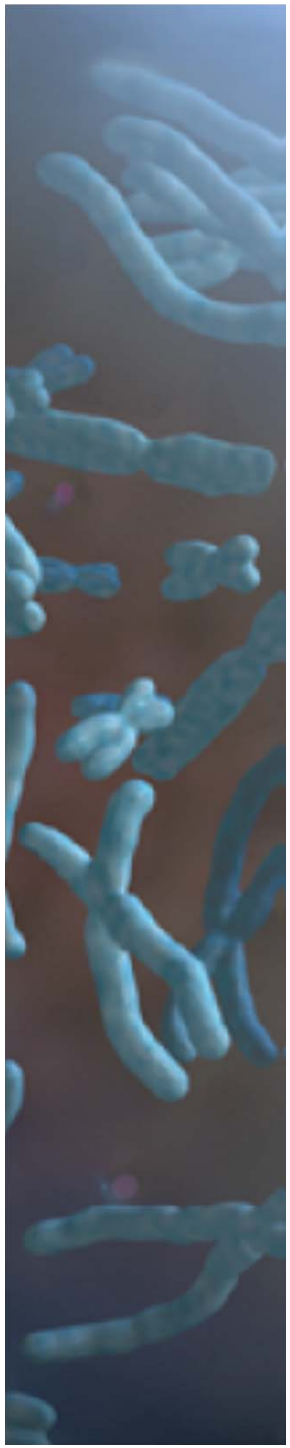
46,XY,t(9;22)(q34;q11)[4] /47,idem,+8[3]/46,XY[10]

«;» and «,», **«[»** and «(» are not the same

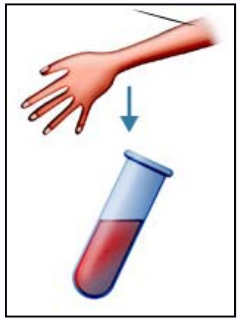


Karyotype

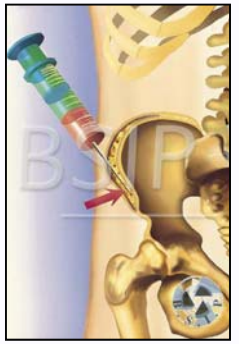
- Overview of genome
- Can miss subtle aberrations
- Requires “abnormal” cell division



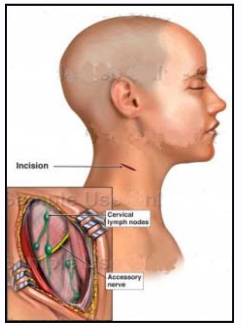
Technical constraints

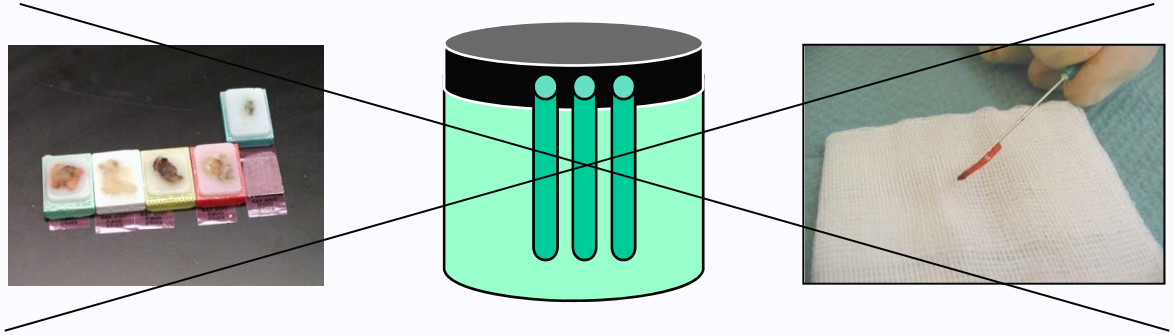


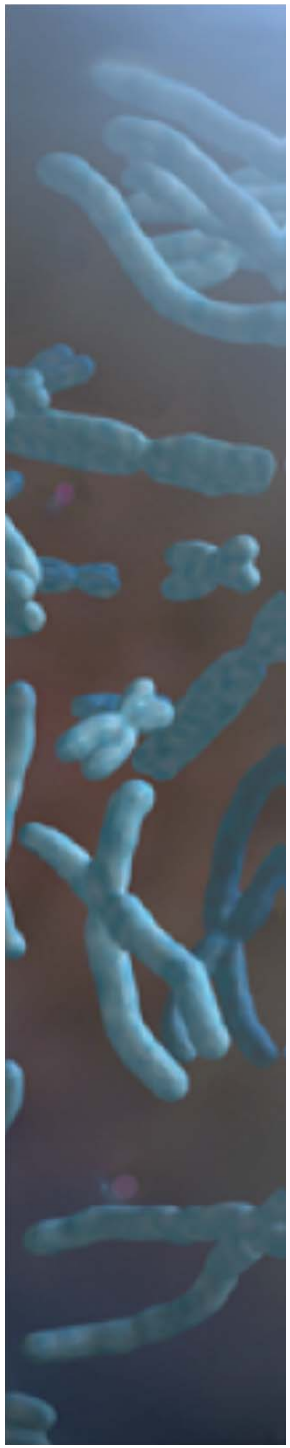
- Principle: all invaded tissues are suitable...but tissues must be viable, and the target cell capable of proliferation



- Sample: type  ! transport delay , hierarchy of sample distribution, tissue conservation
- Appropriate culture conditions (duration, mitogens/cytokines)

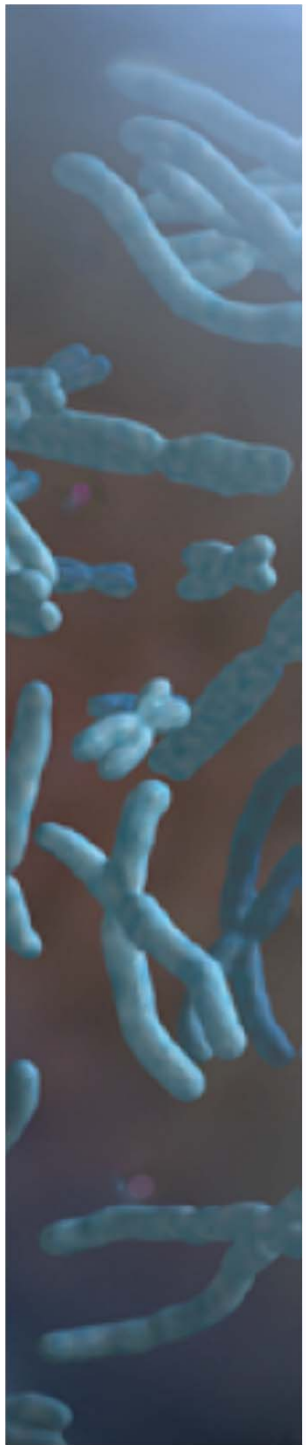




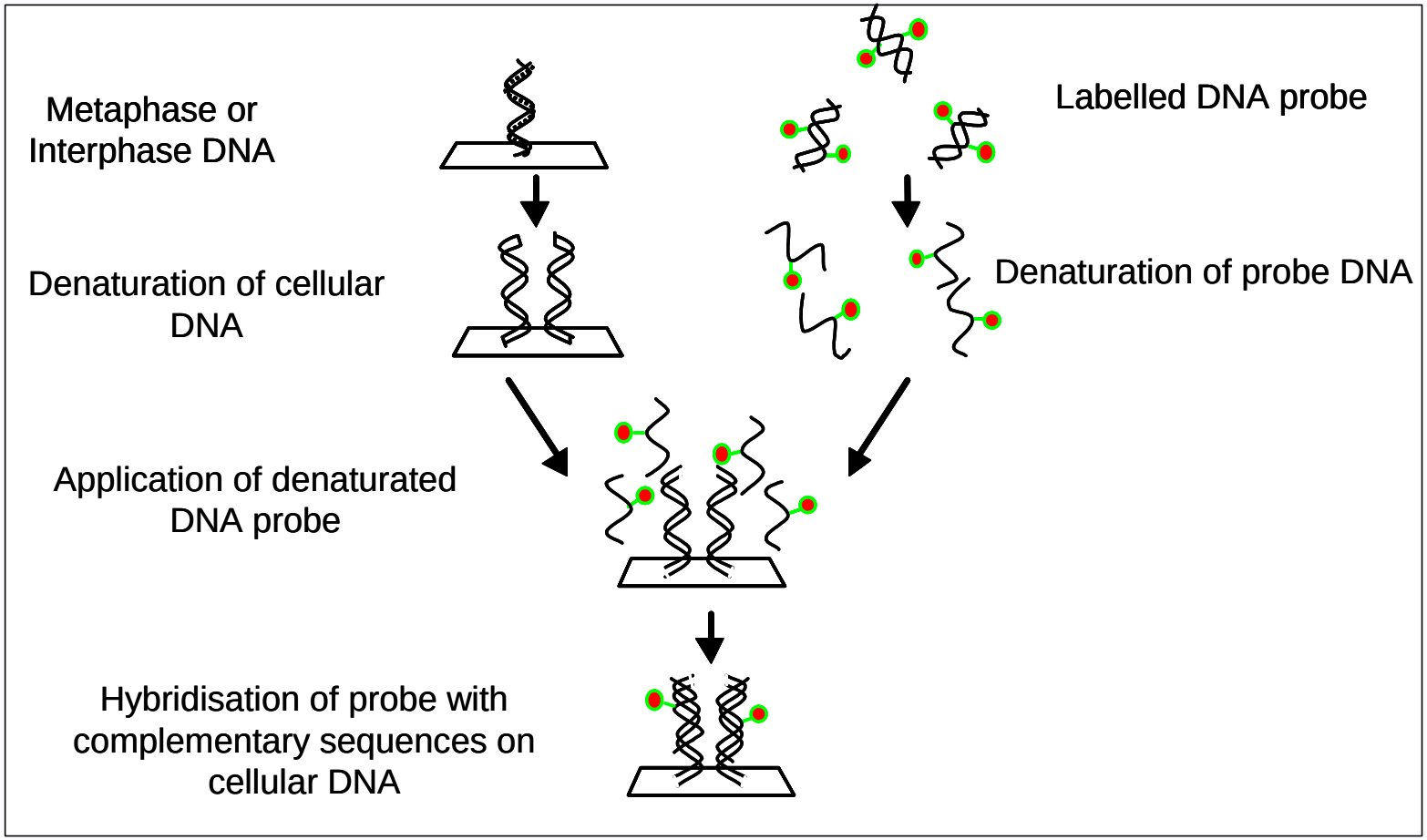


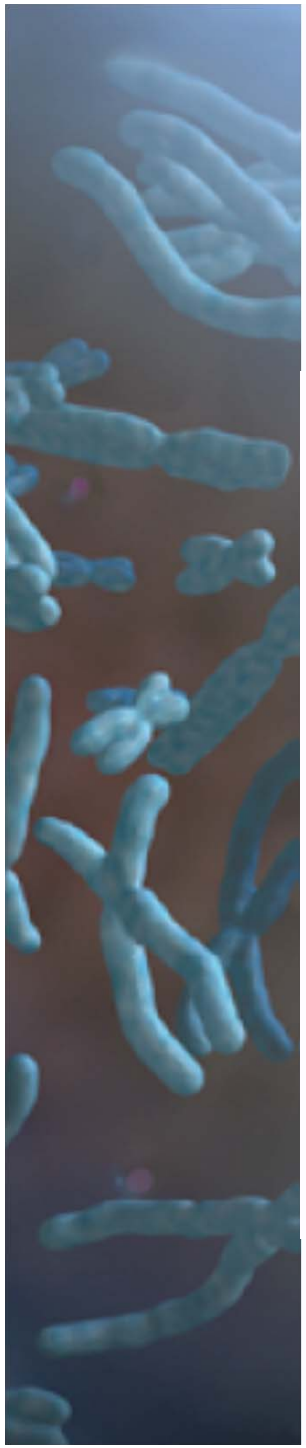
Molecular cytogenetics

- Principle: identification of chromosomal abnormalities in malignant cells...but tissues must *not* be viable
- Base: biochemical properties of DNA
- All invaded tissues are suitable
  - fresh sample: OK
  - frozen sample: OK
  - EDTA: OK
  - fixed tissue: OK (!! duration of fixation)
  - small tissue: can be OK
  - sorted cells: OK
  - FISH + immuno: OK
- Culture conditions adapted *only* in case of metaphase FISH



FISH: principle

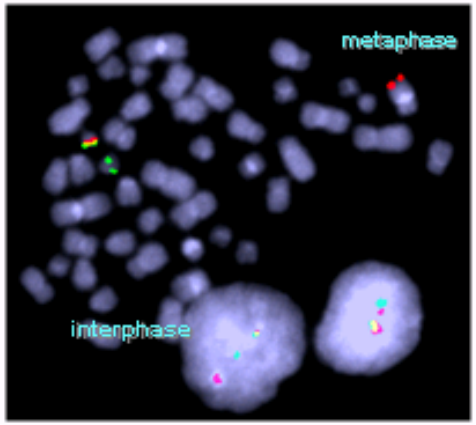


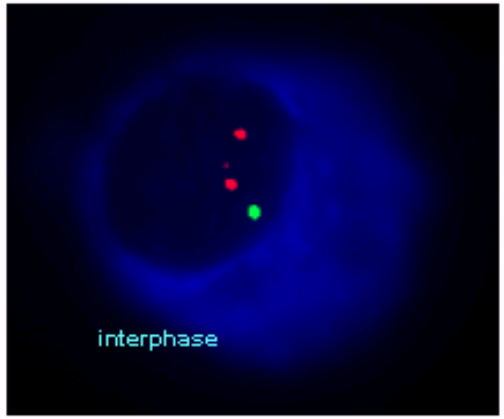


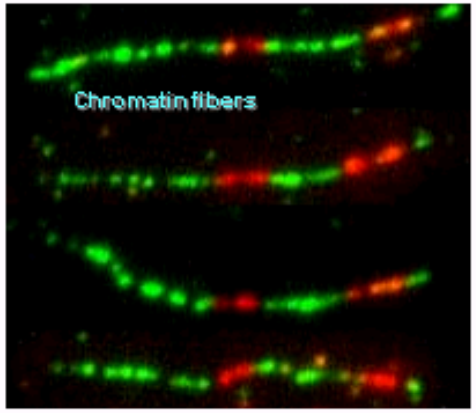
FISH: targets

Cell DNA

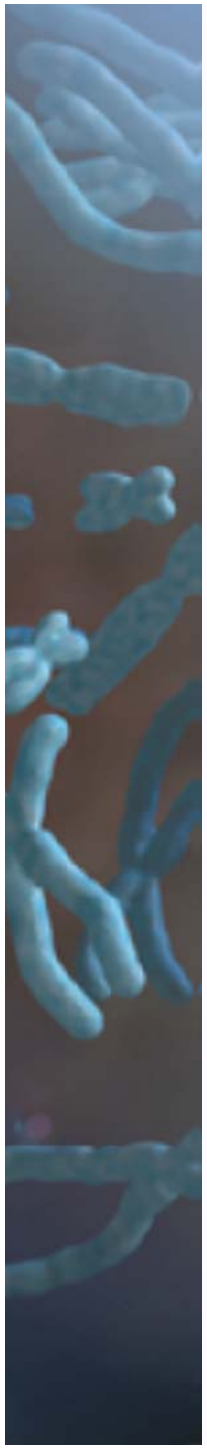
	resolution
– Conventional karyotype (smallest band)	5-10 Mb
– FISH on metaphase chromosomes	± 1 Mb
– FISH on Interphase nuclei	± 100 Kb
– FISH on chromatin fibers ("fiber FISH")	± 1 Kb



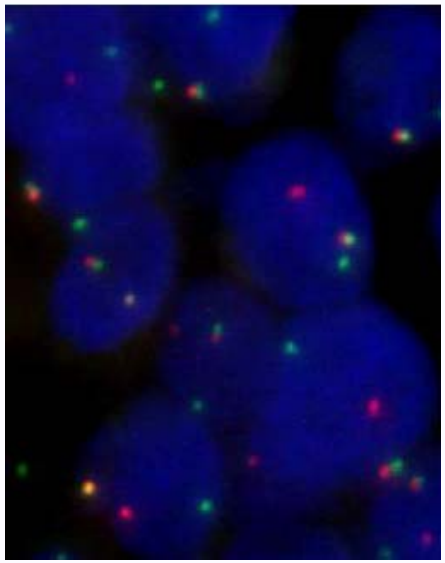


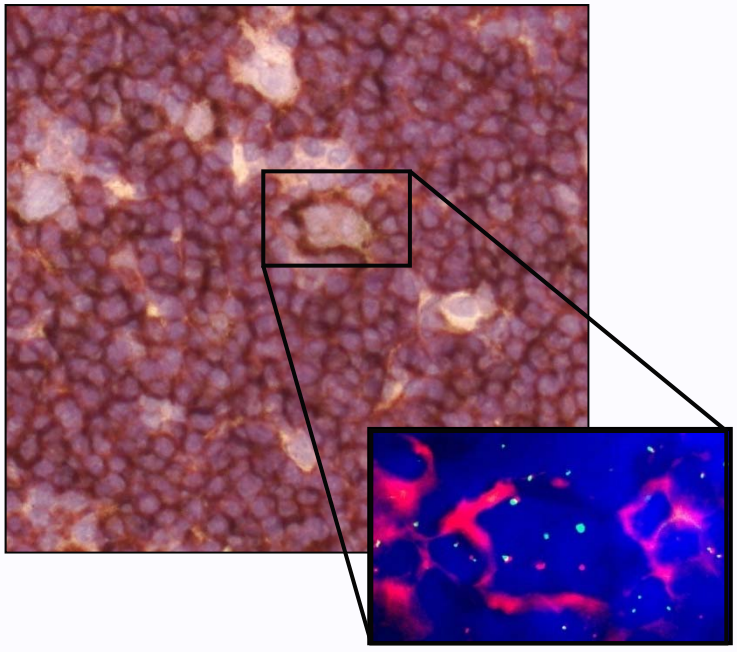


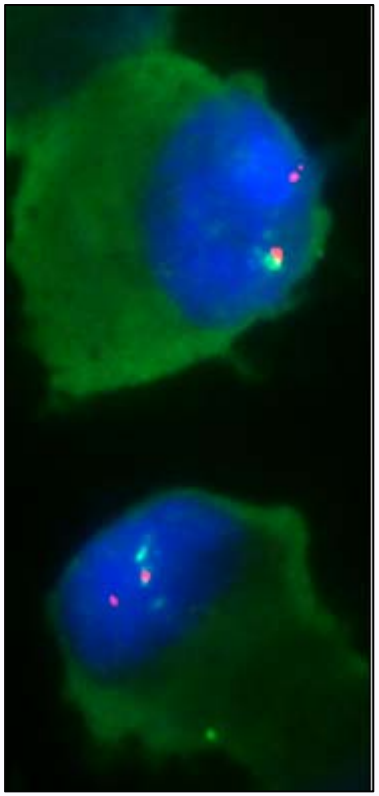
RNA

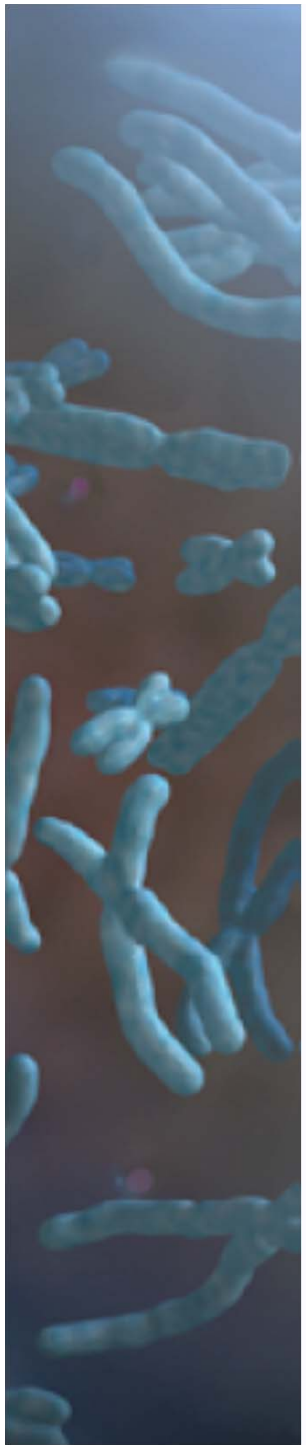


- FISH can be performed on interphase nuclei→ more sensitive than karyotype (more cells can be scored)
- Interphase FISH is possible
  - on cell suspensions/touch prints
  - on archival material
  - in combination with morphology & immunology (FICTion)

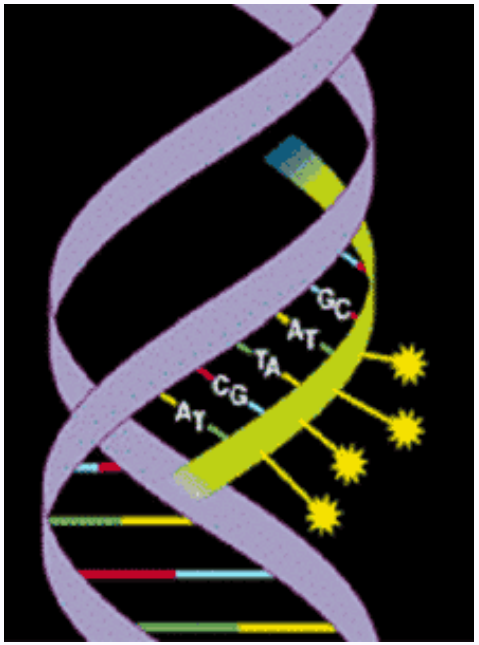




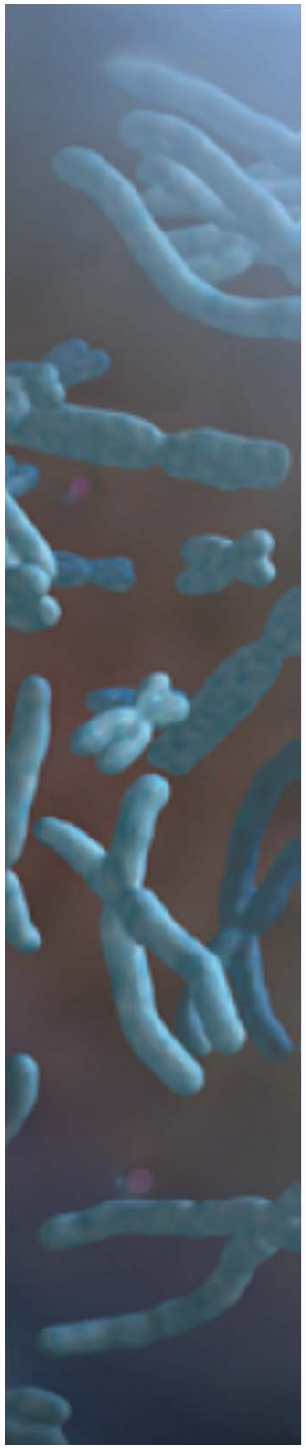


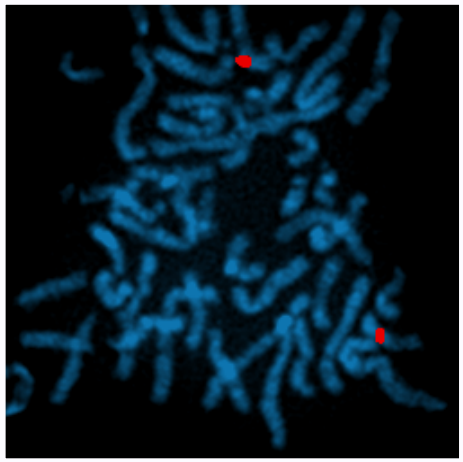


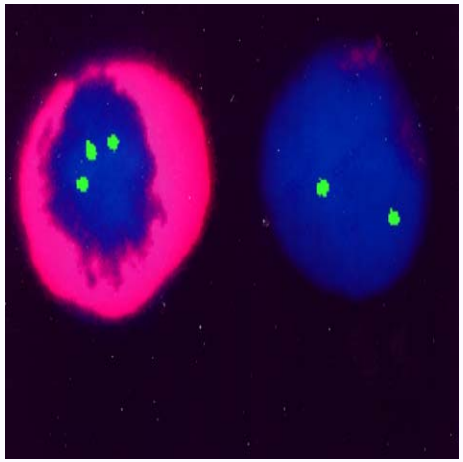
FISH: probes

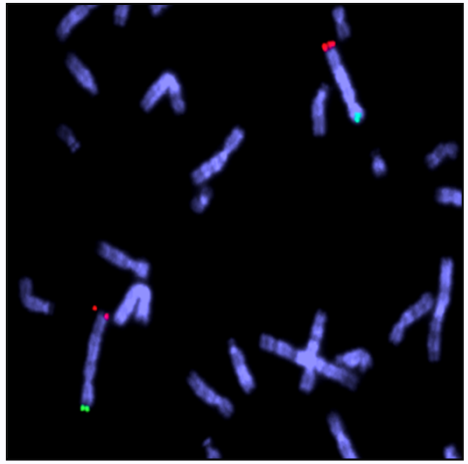


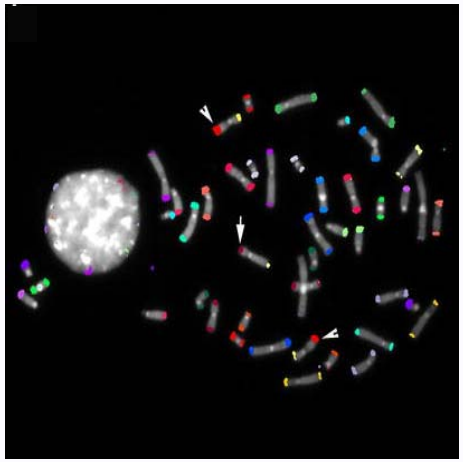
= labeled nucleic acids fragments complementary to a specific sequence of the genome

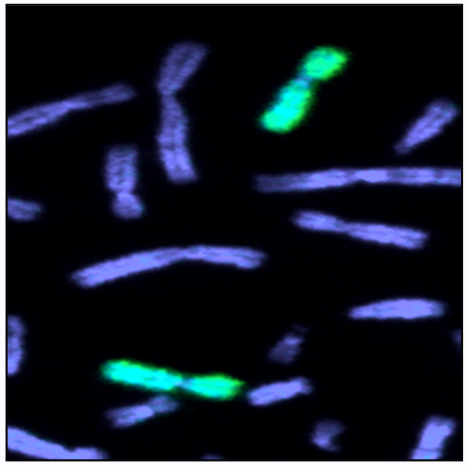


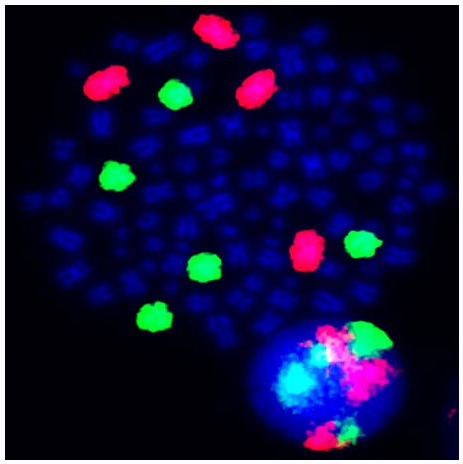






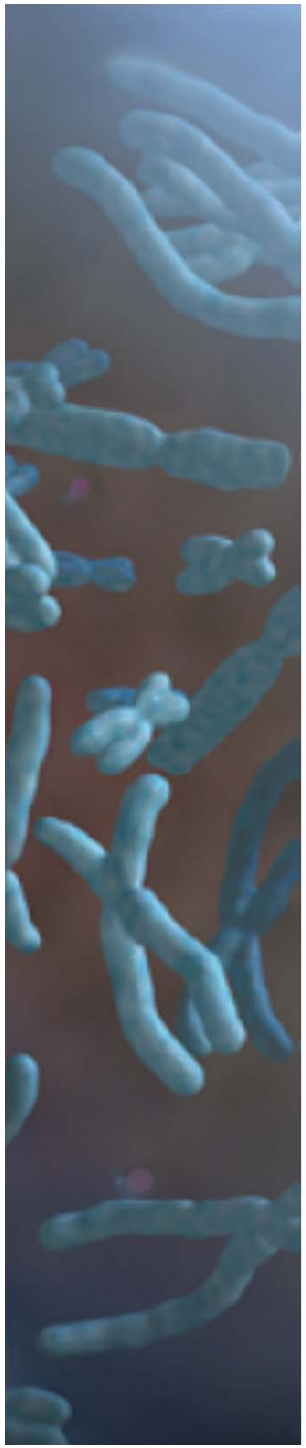






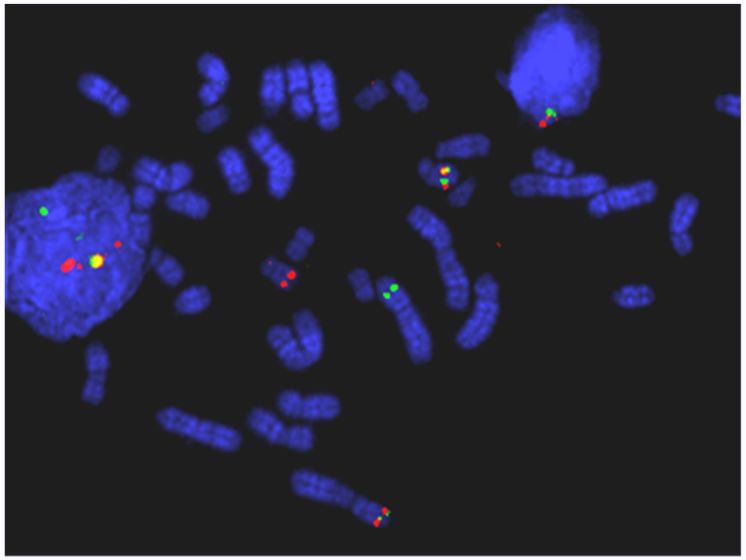
Different probes:

- centromeric
- telomeric
- painting (wcp)



- “Locus specific” probes” (*commercial or homemade*)

Vectors	**Insert size**
Plasmids	0,5-5 Kb
Phages	9-25 Kb
Cosmids	35-50 Kb
Phosmids	> 40 Kb
P1	70-85 Kb
PAC (P1-Artificial Chromosomes)	100-120 Kb
BAC (Bacterial Artificial Chromosomes)	120-150 Kb
YAC (Yeast Artificial Chromosomes)	200 Kb → > 2Mb



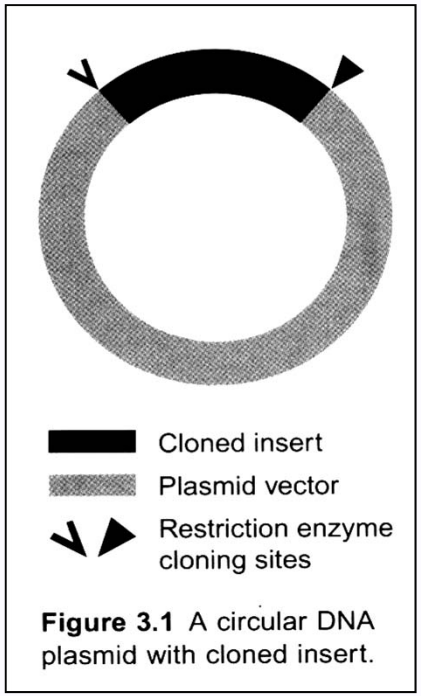
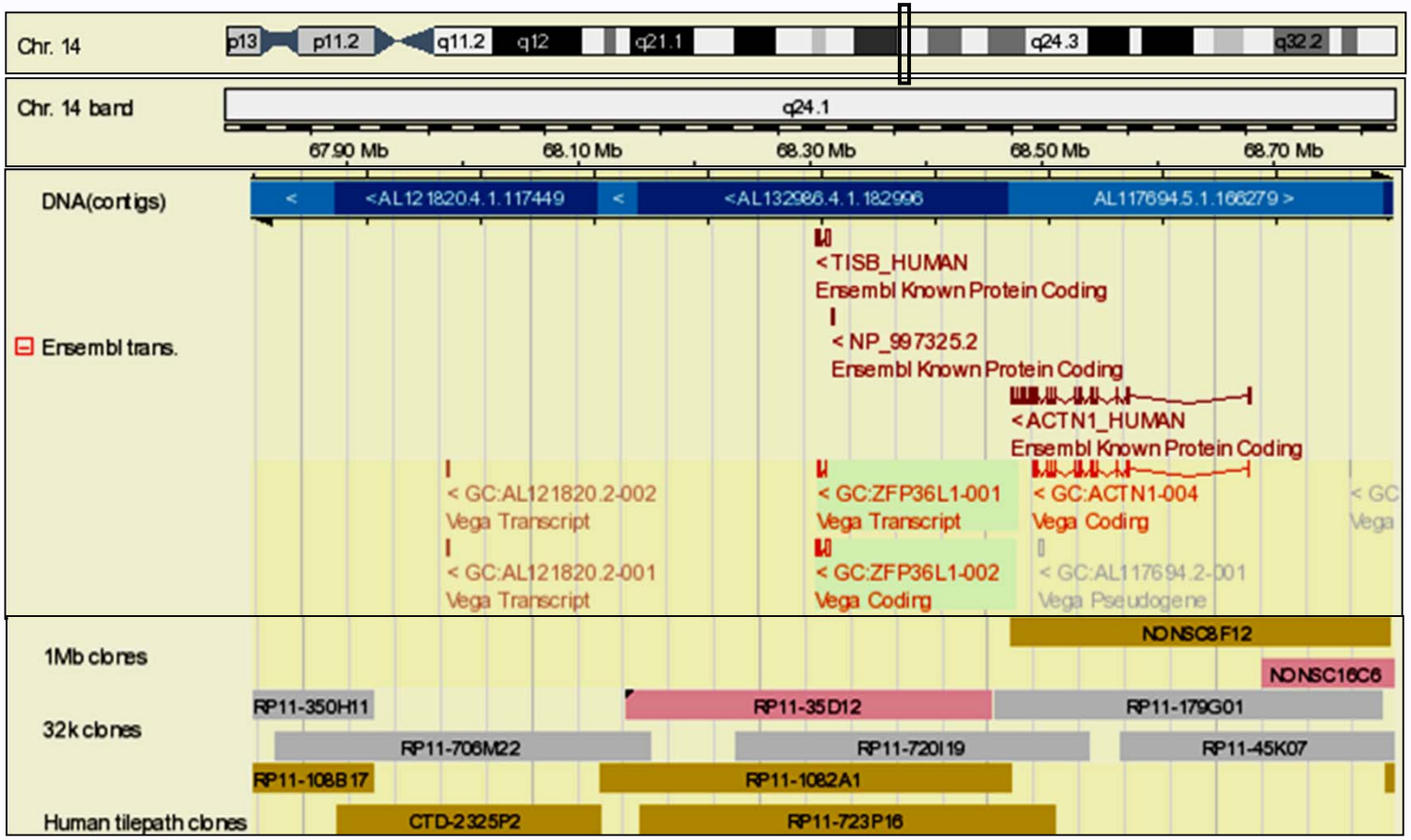
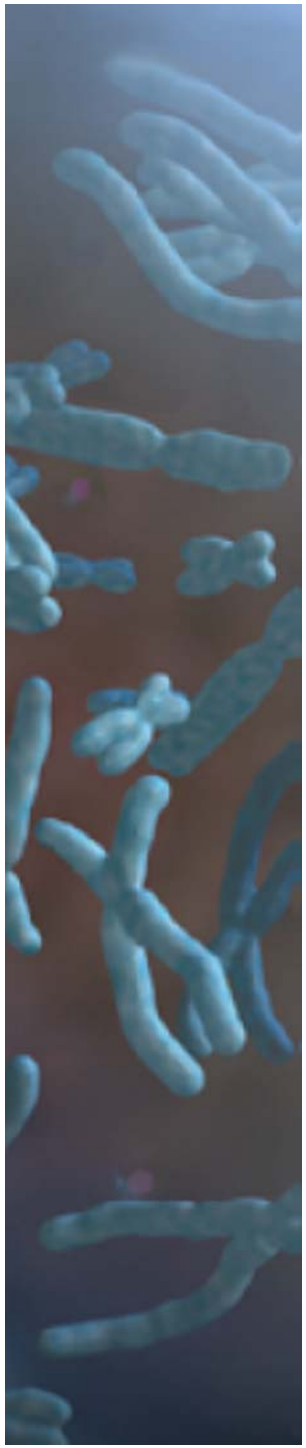


Figure 3.1 A circular DNA plasmid with cloned insert.



www.ensembl.org



National Center for Biotechnology Information

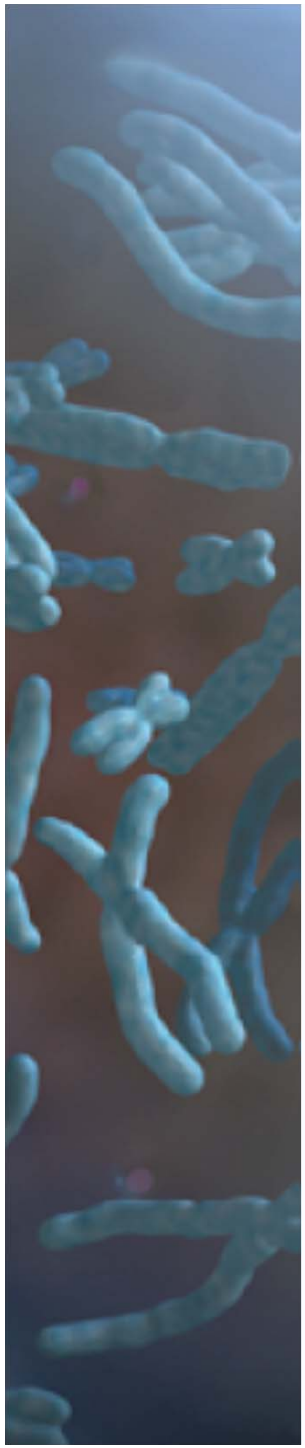
http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606

University of California Santa Cruz

http://genome.cse.ucsc.edu/cgi-bin/hgGateway

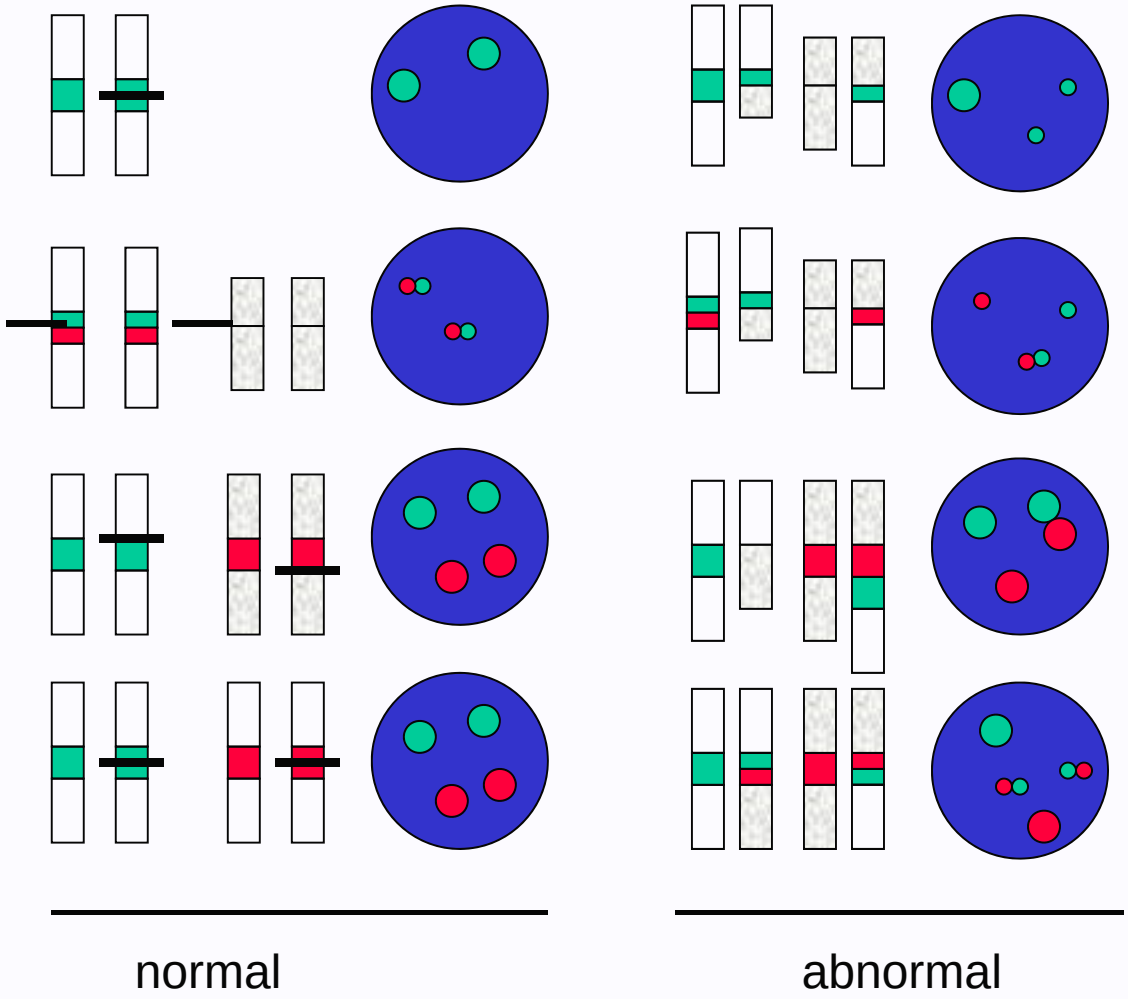
Ensembl Genome Browser

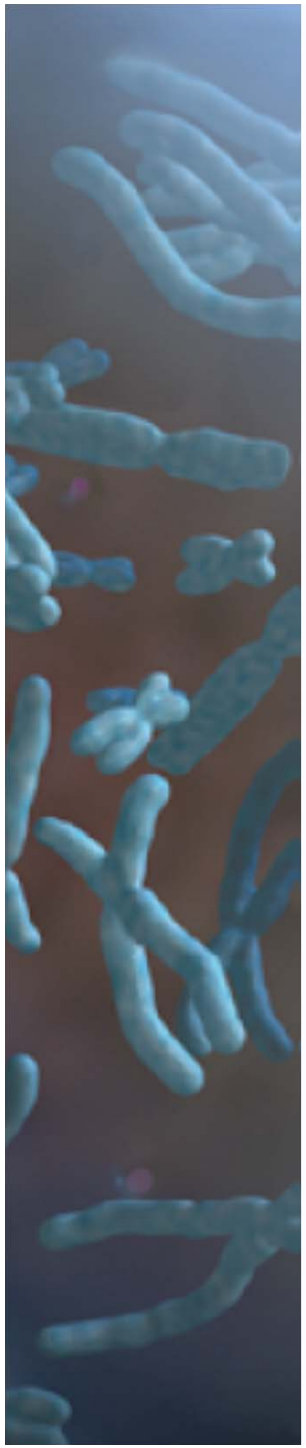
http://www.ensembl.org/Homo_sapiens/index.html

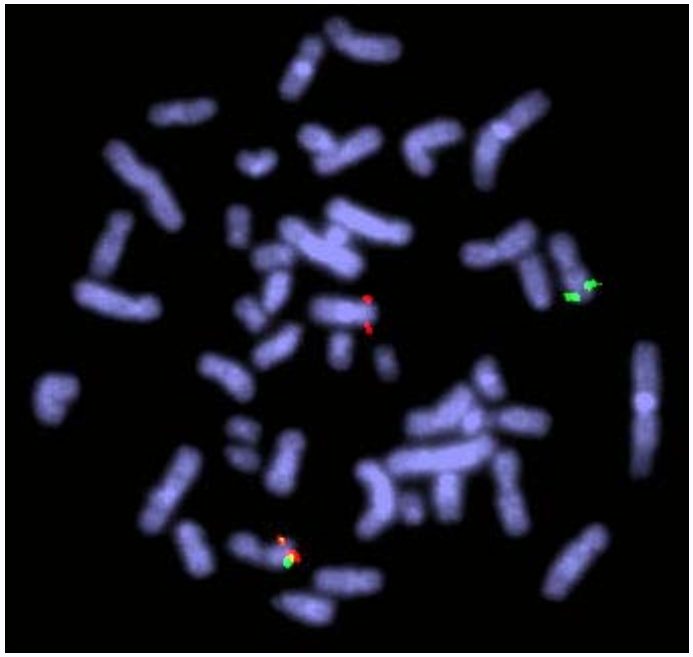


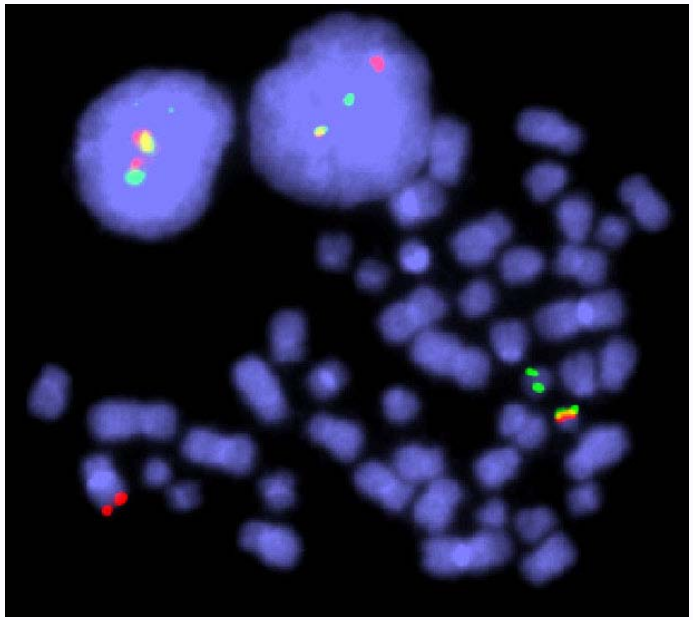
Locus-specific probes: strategies

- breakapart
- colocalization
- combination









Examples:

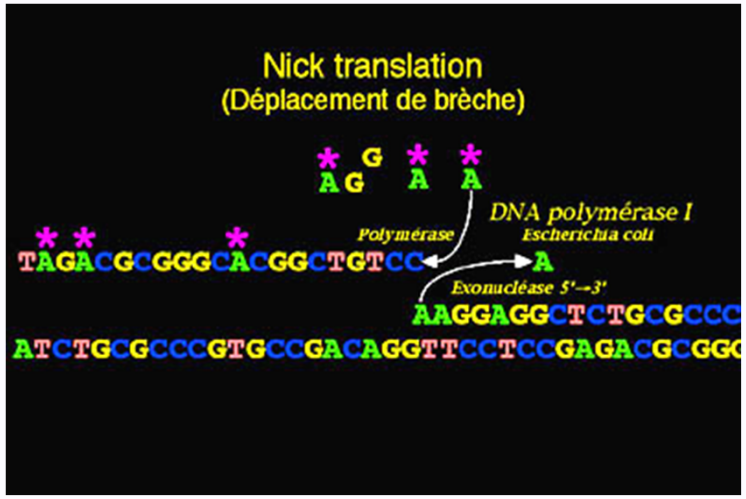
- separation/breakapart

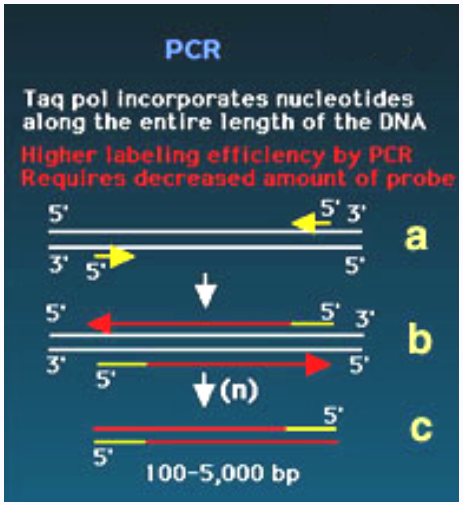
  split of *KMT2A*/11q23 in an AML with a t(9;11)

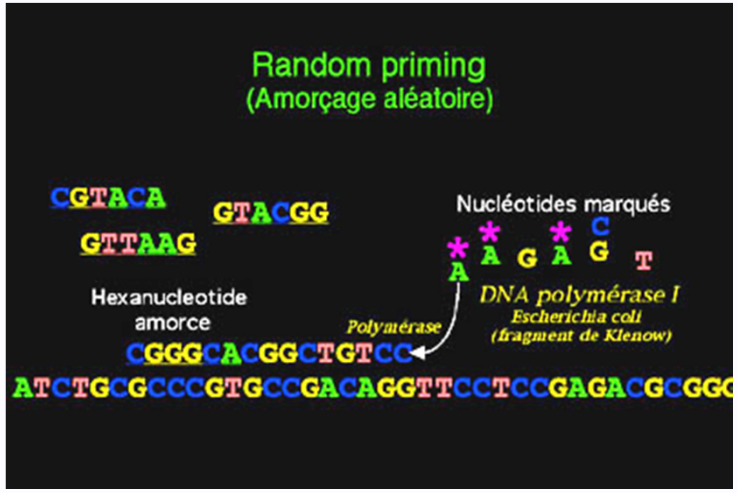
- Colocalisation/fusion

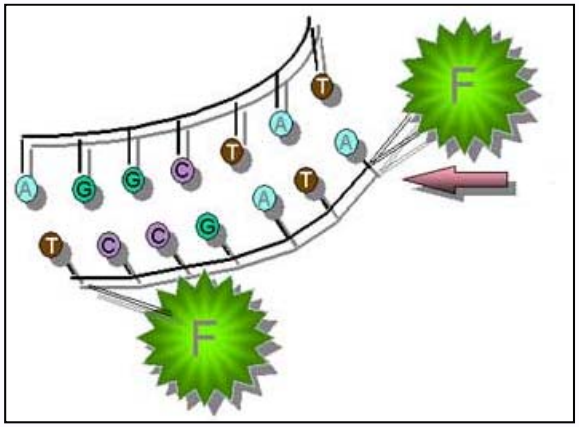
  BCR/*ABL* fusion on a der(22)t(9;22) in a CML

Probes: labeling

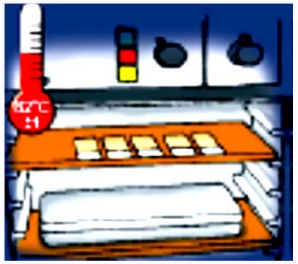


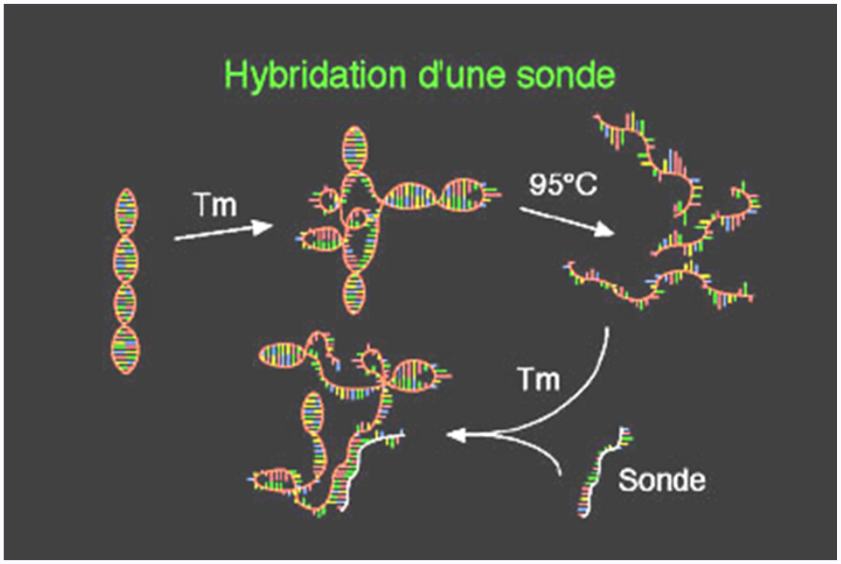


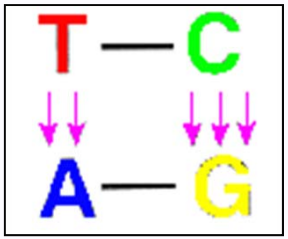


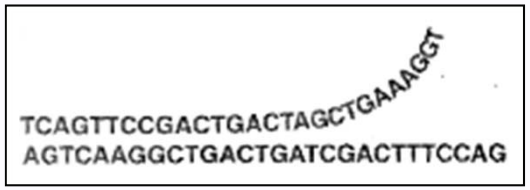


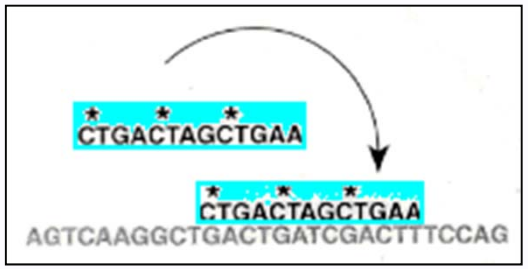
Probes: hybridization

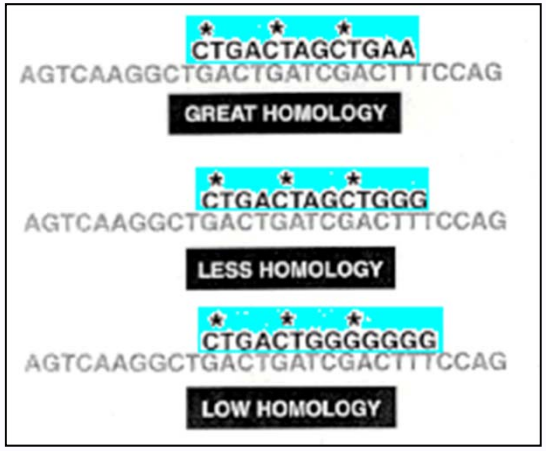




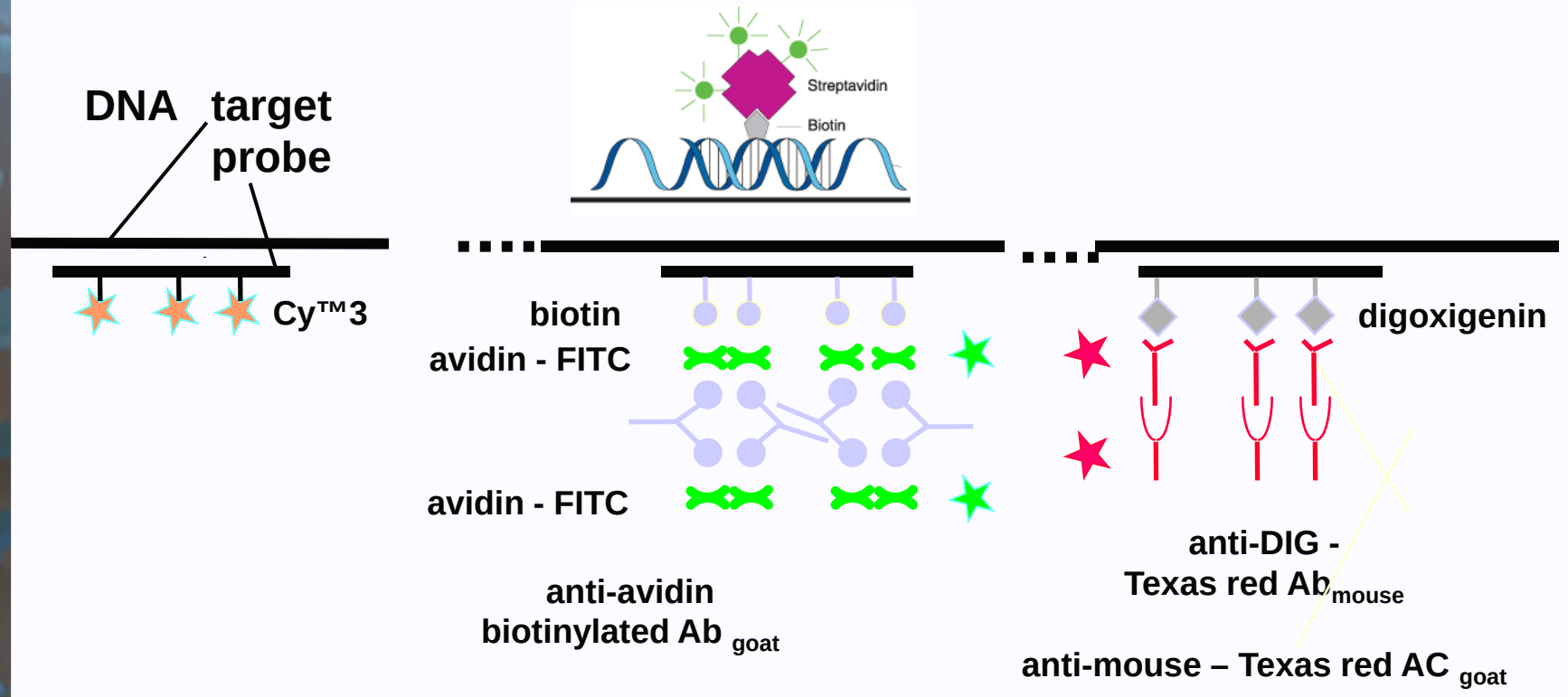


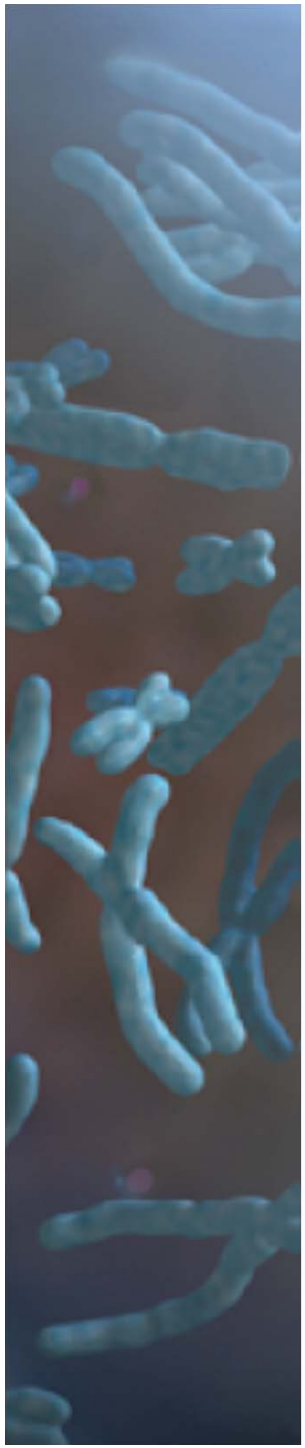


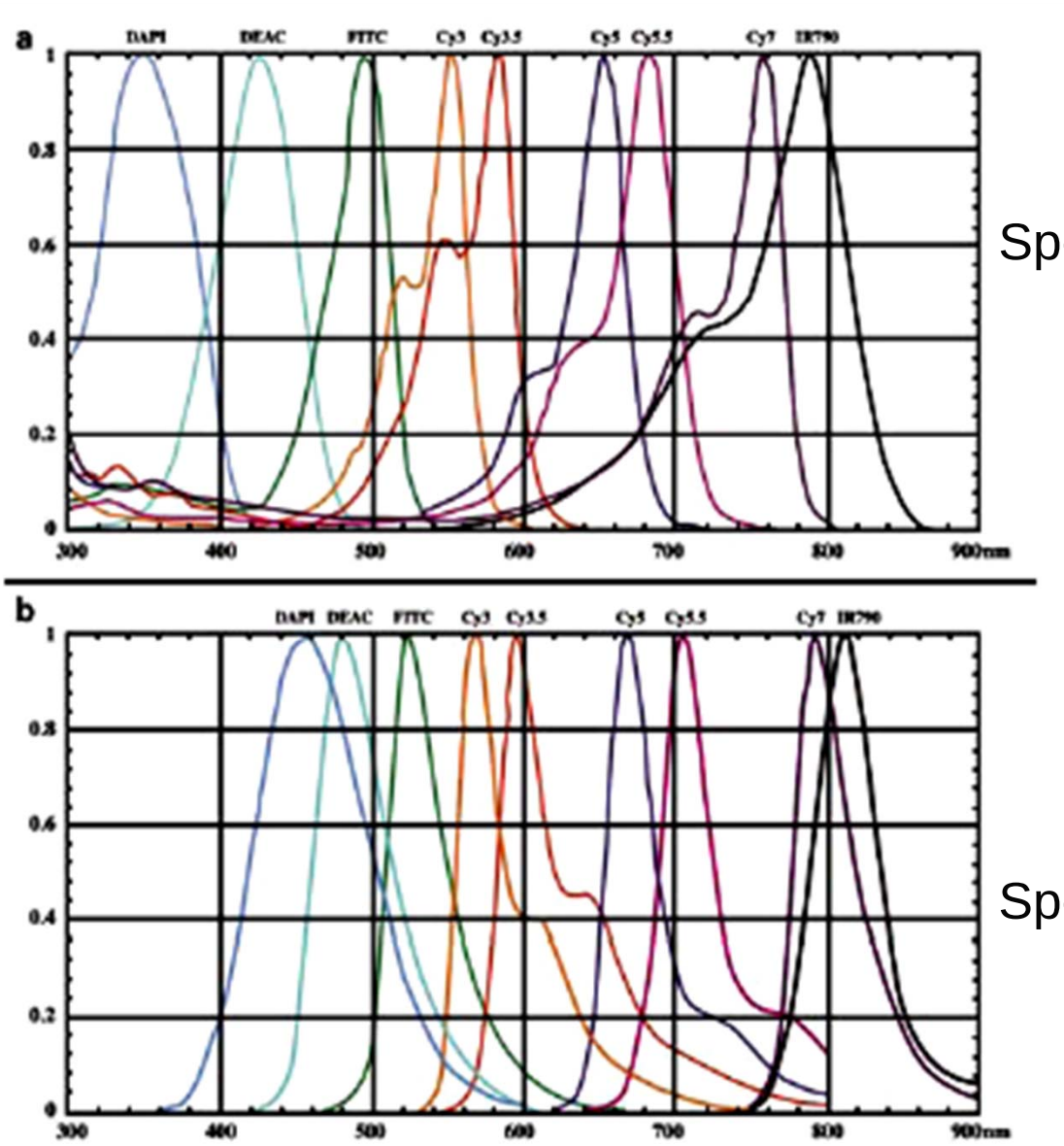




Probes: revelation

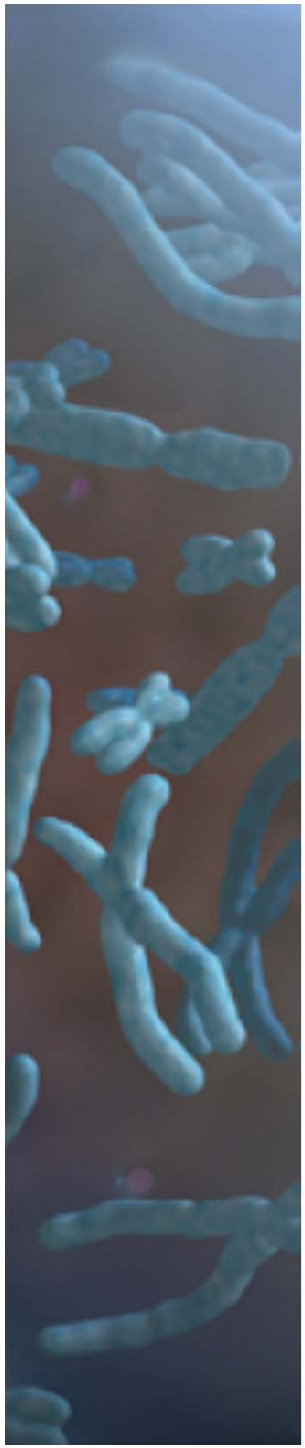


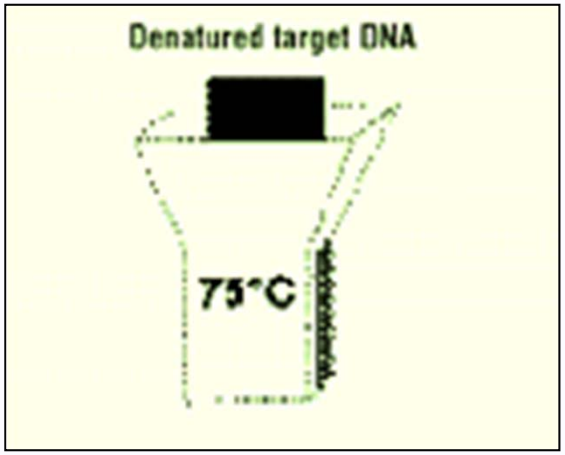


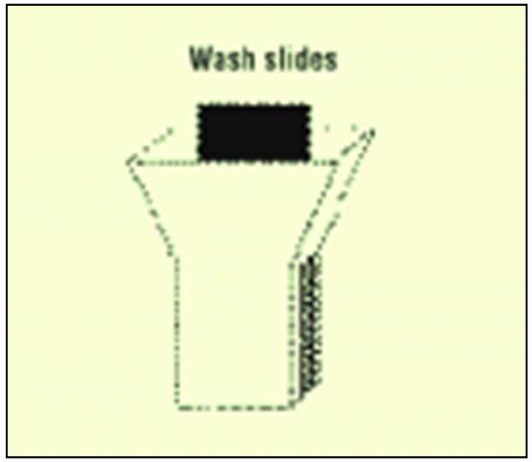


Spectrum of excitation

Spectrum of emission

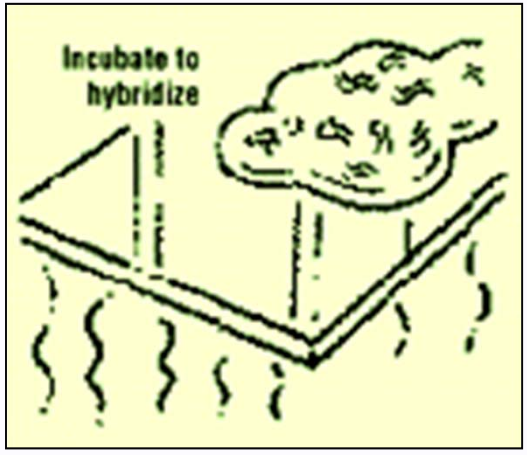



Denatured target DNA
75°C

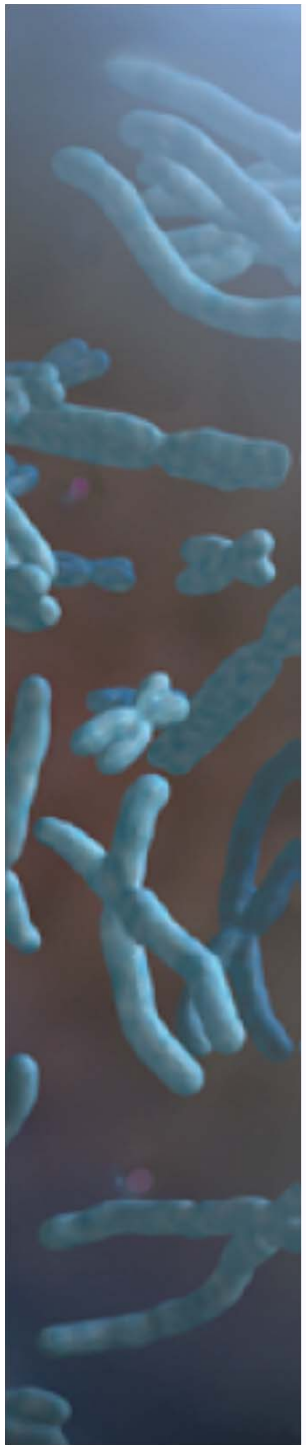

Wash slides


Apply probe to slide

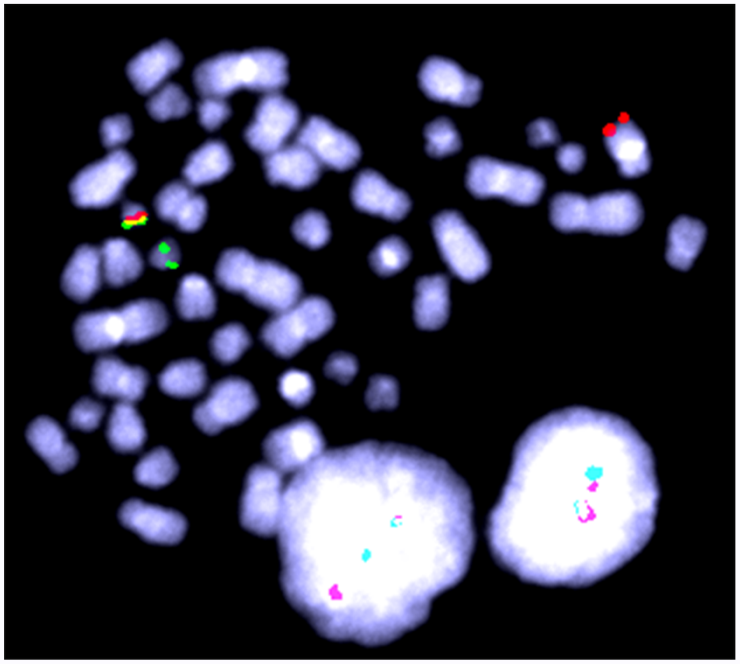

Apply counterstain
to slide


Incubate to
hybridize

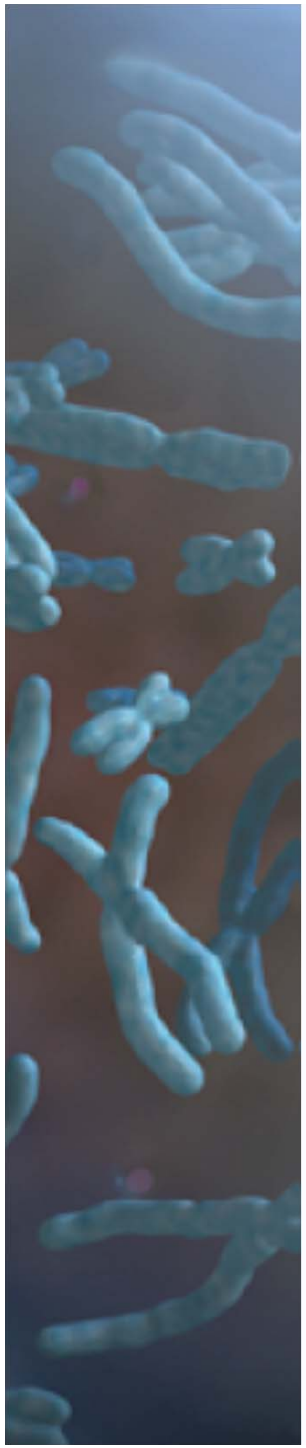

View
results

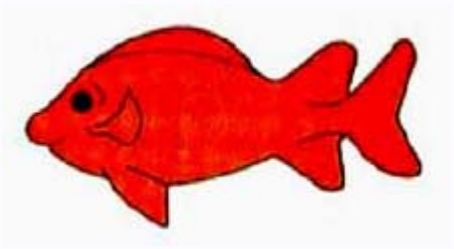


FISH

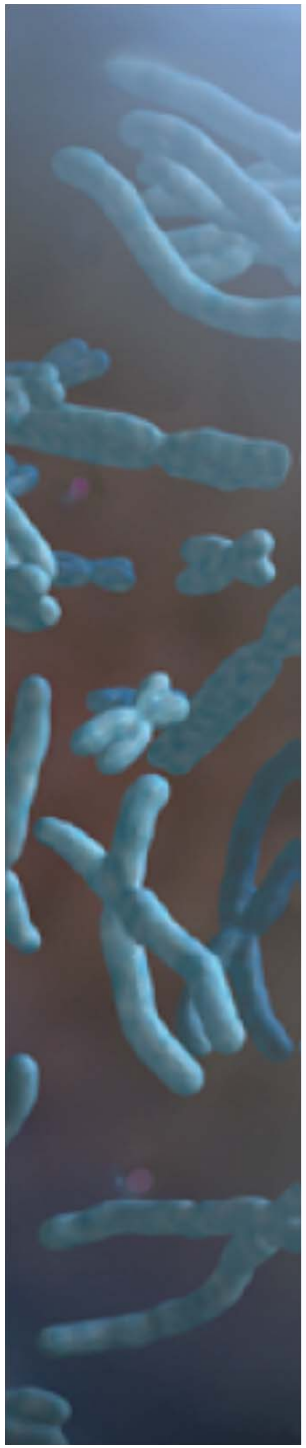


- Targeted analysis of region(s) of interest
- Does not necessarily require “abnormal” cell division





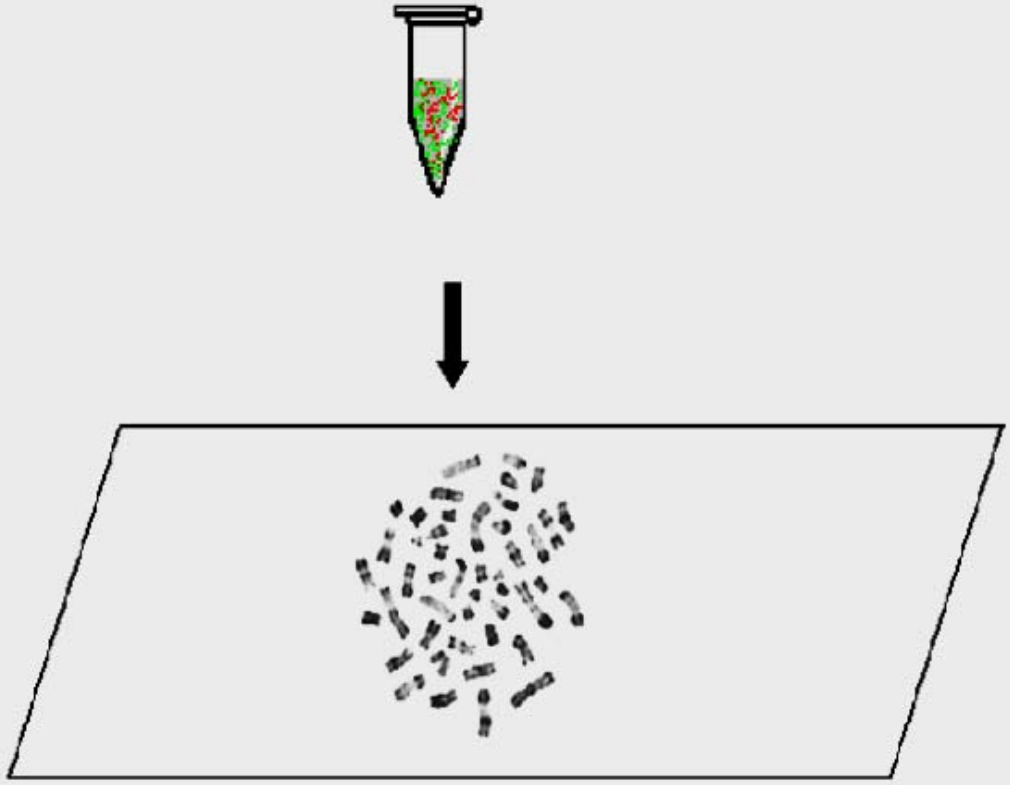
- Variants of the FISH technique
  - spectral karyotype / M-FISH
  - M-Band
  - RX-FISH
  - Comparative genomic hybridization (CGH)
  - **Array-CGH**, SNP-array
  - CESH...



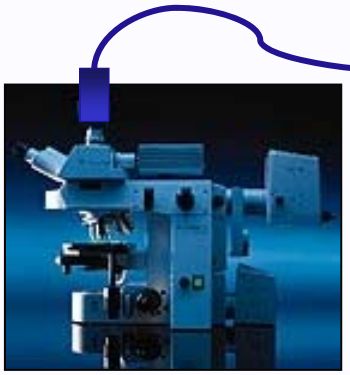
Spectral Karyotype / M-FISH

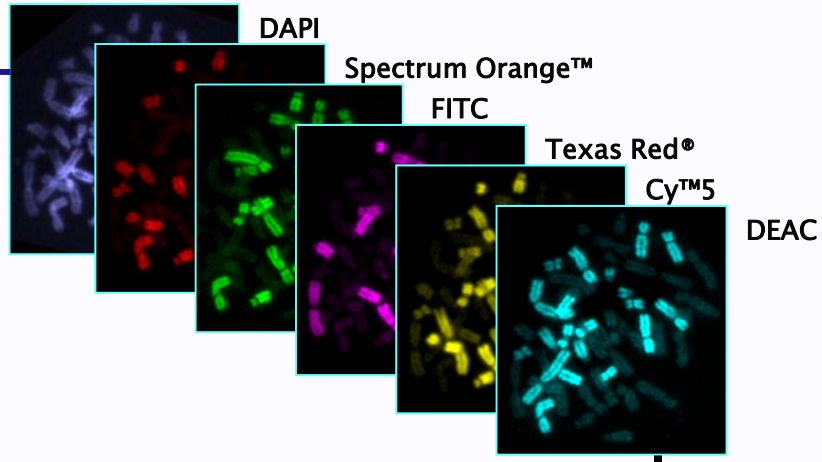
	chromosomes																						
Fluor	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
FITC			300		500			600	300				600	250	500	300			250			100	
Cy3	300								200	300	300			200	150	200	150		100	200	300		300
TR		400					300			250		350	450		150				200	200	300	150	
Cy5				500		400		400			400	500				200	250			300		200	
bio/Cy 7			250			300	350			200				250			200	400	200				300

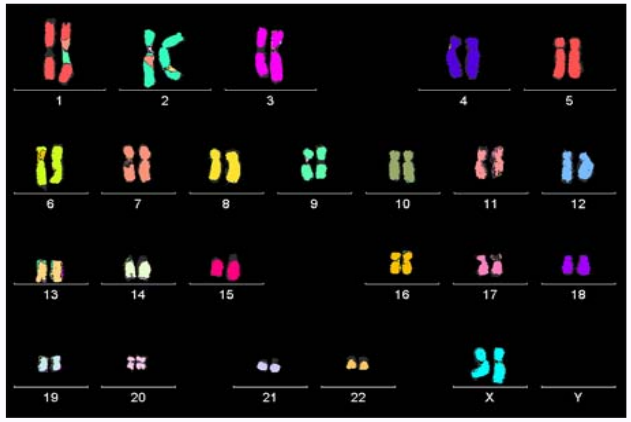
mix 24 sondes

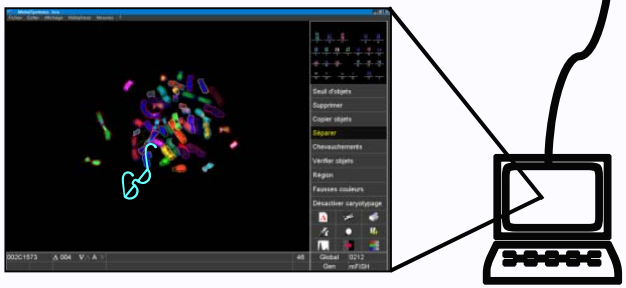


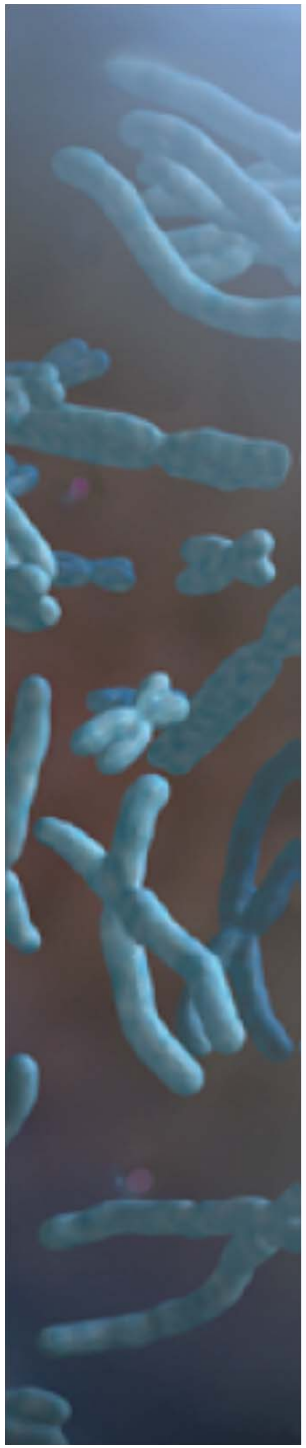
Spectral karyotype / M-FISH

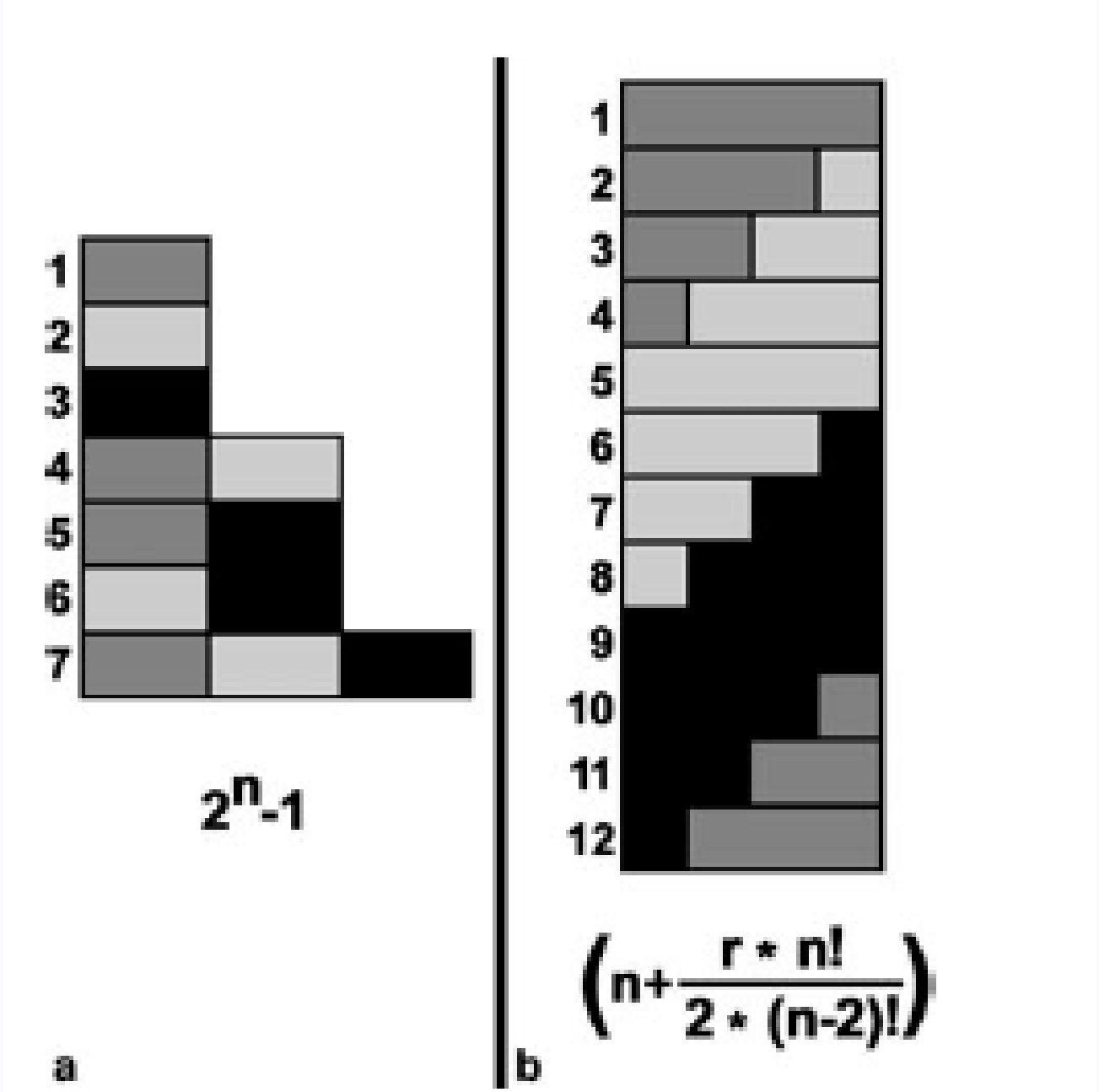


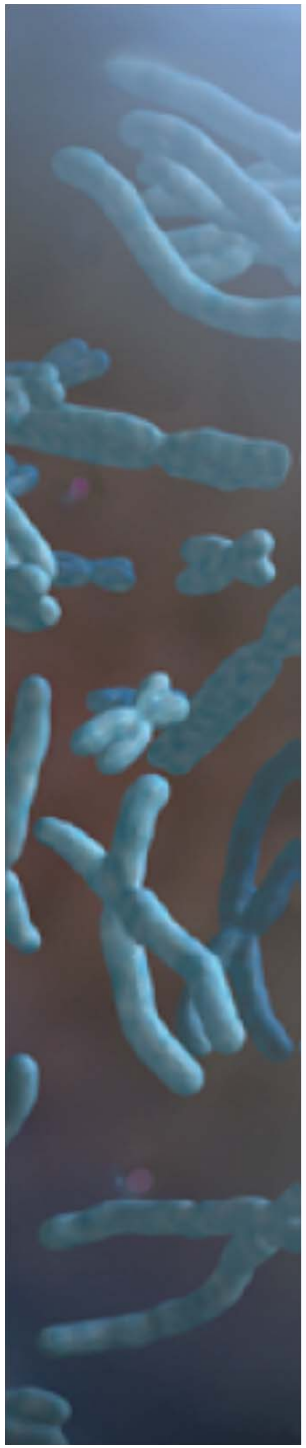




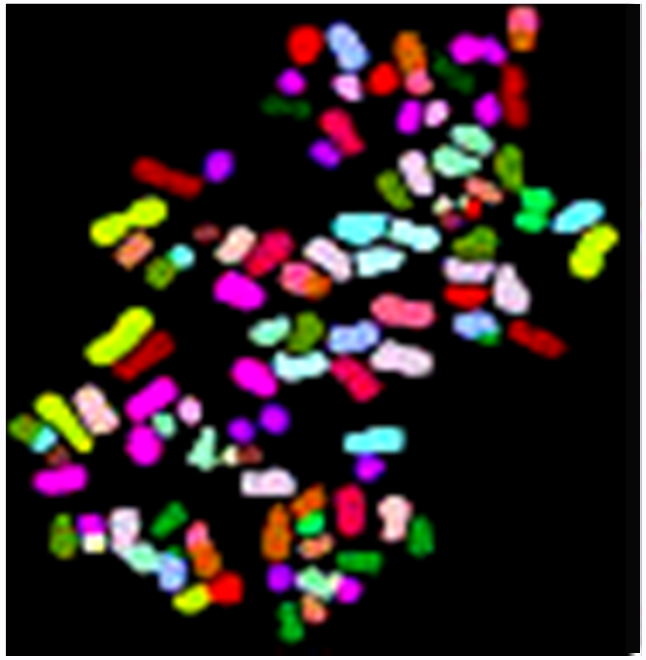





1
2
3
4
5
6
7
2^n-1
a
b
1
2
3
4
5
6
7
8
9
10
11
12
$\left(n+\frac{r * n!}{2 * (n-2)!}\right)$



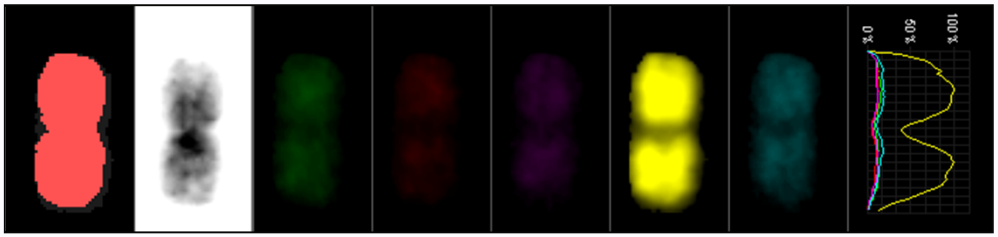
Spectral karyotype / M-FISH



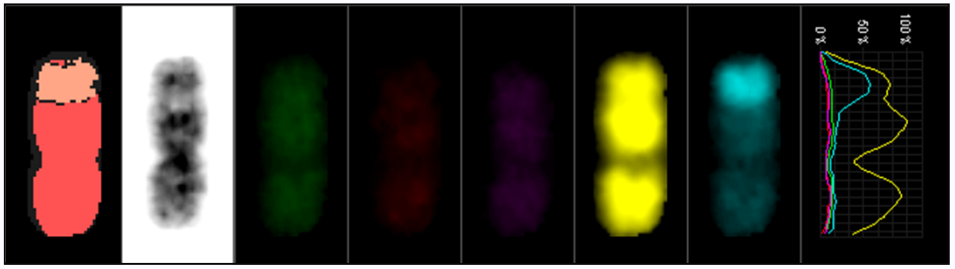
- Overview of genome
- Can miss subtle aberrations
- Requires “abnormal” cell division

Example M-FISH

→ "Profiles"



chromosome 1



der(1)t(1;7)

Acute promyelocytic leukemia → t-MDS

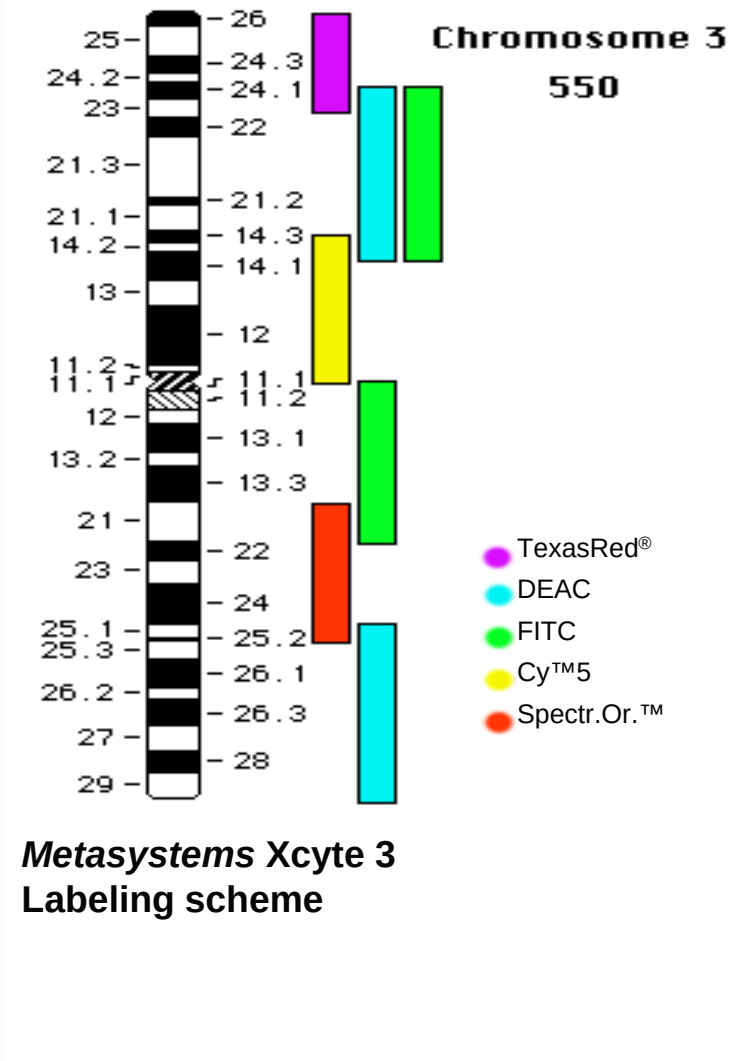
chromosome

fluorochromes

#	FITC	SpeOra	TexRed	Cy5	DEAC
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
X					
Y					

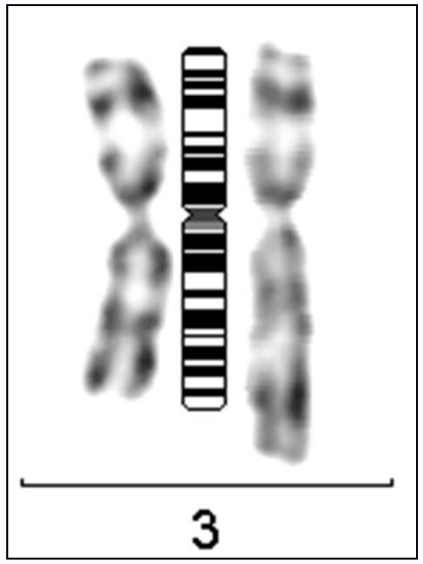
mFISH Labelling Scheme
MetaSystems

M-Band: example (and principle)

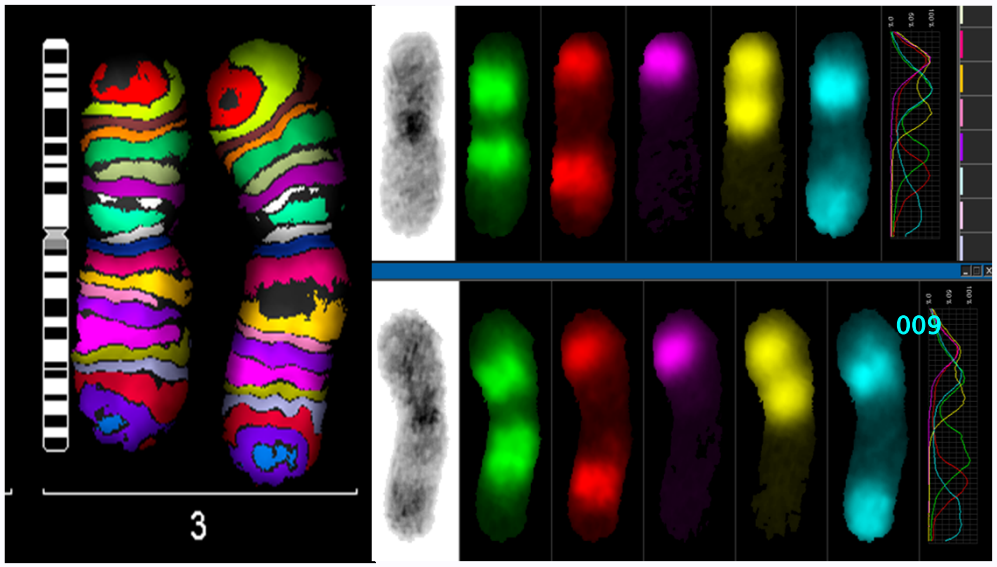


***Metasystems* Xcyte 3**
Labeling scheme

AML M4

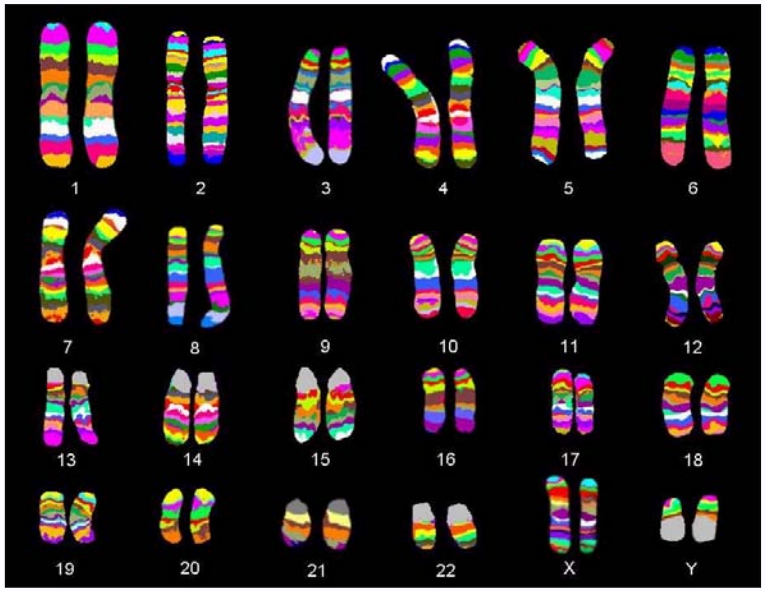


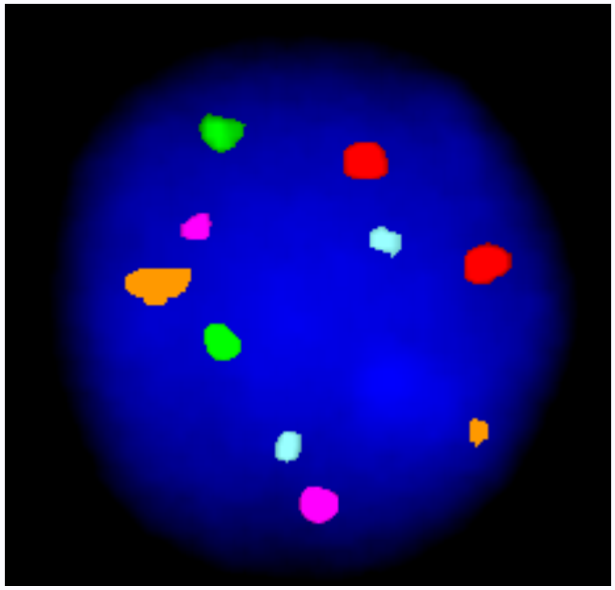
G-banding

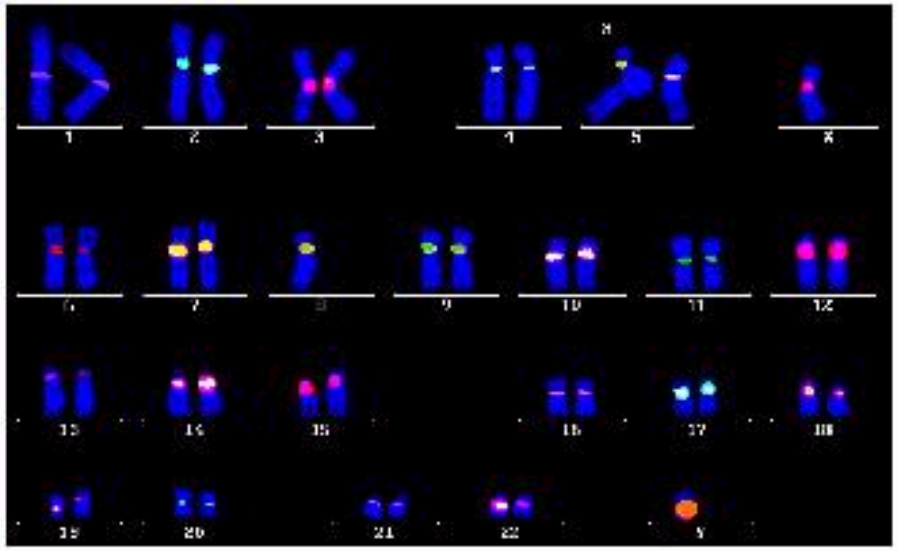


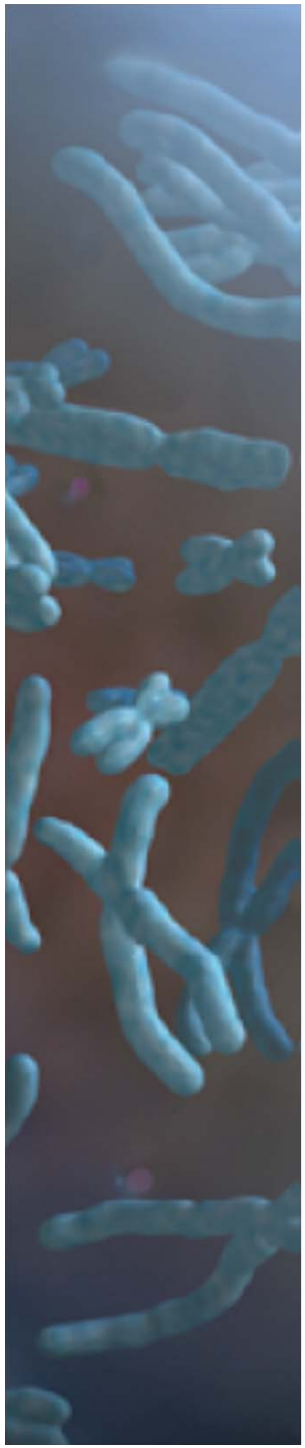
m-BAND chromosome 3

FISH: expanding!

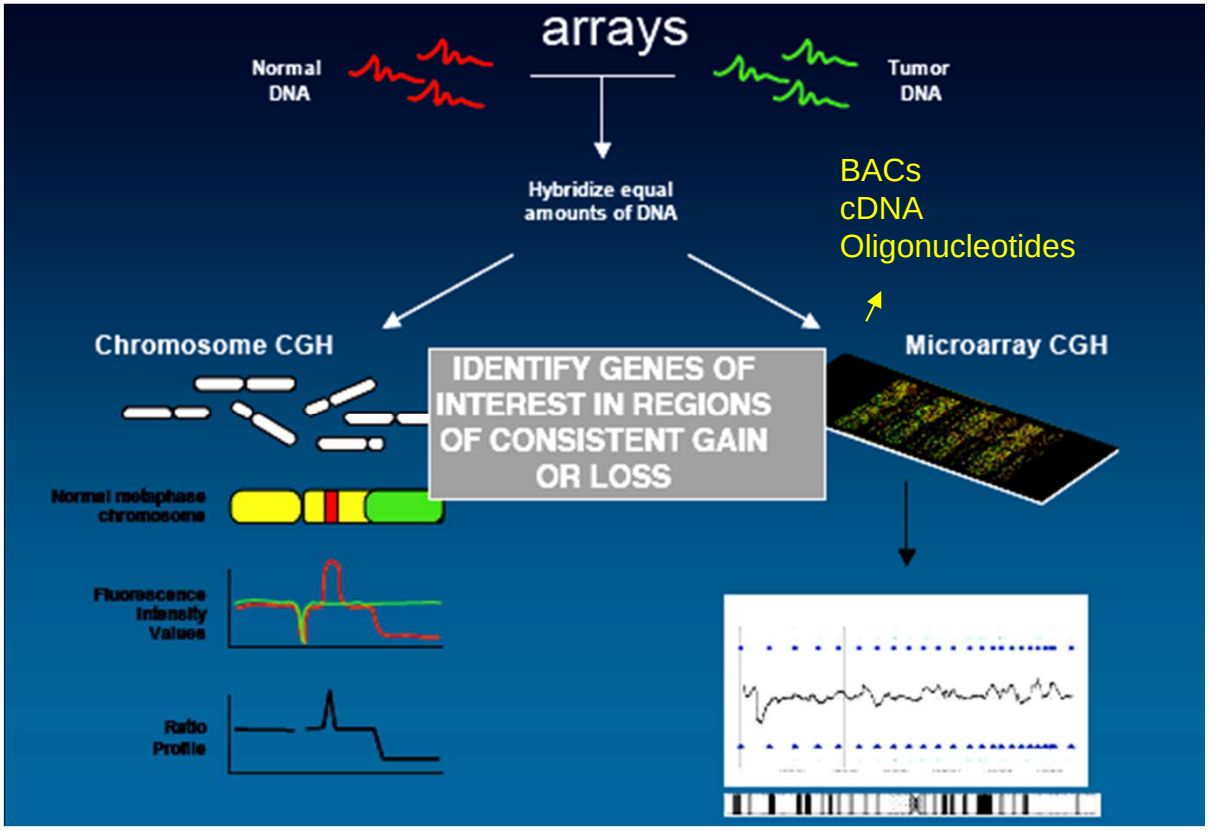




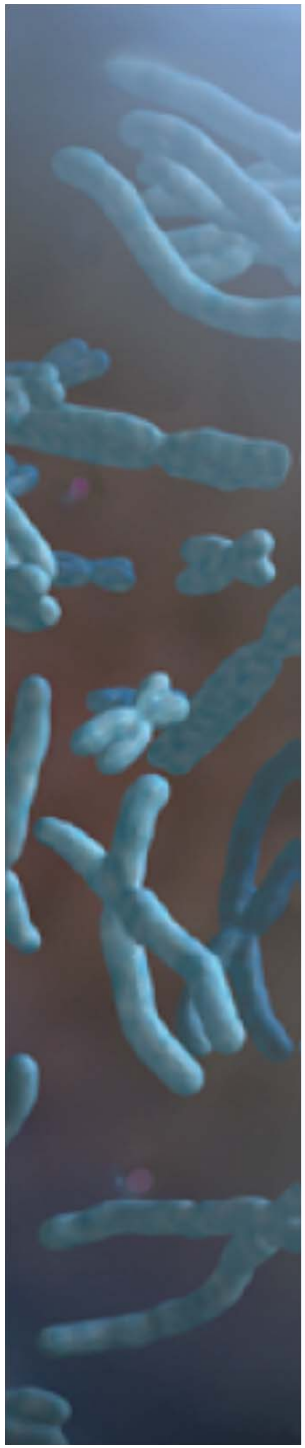




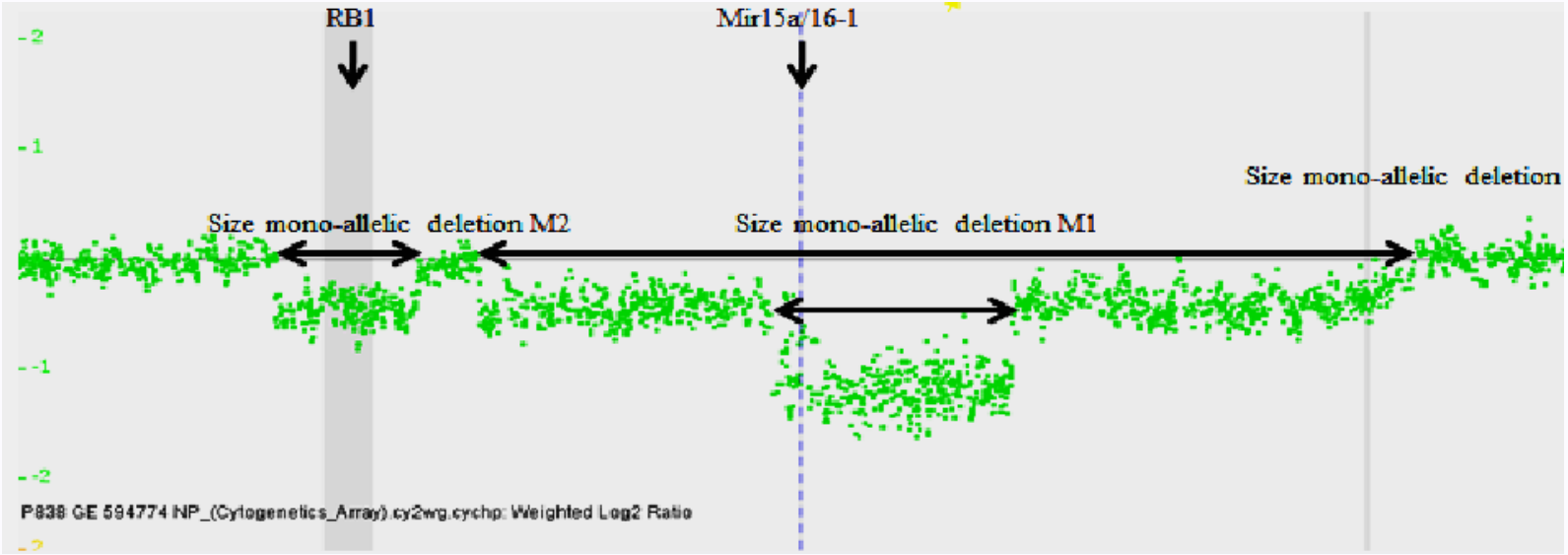
Comparative genomic hybridization (CGH)

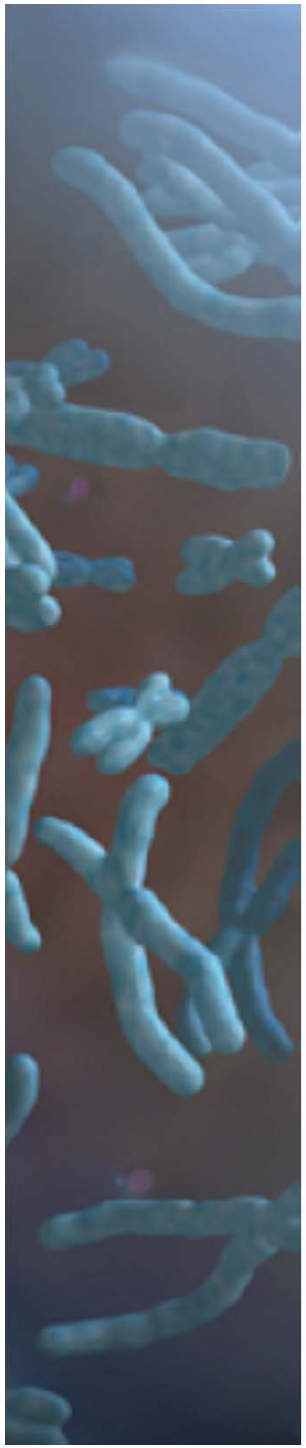


- Detects DNA gains/losses
- Does not detect balances abnormalities
- Does not require cell division



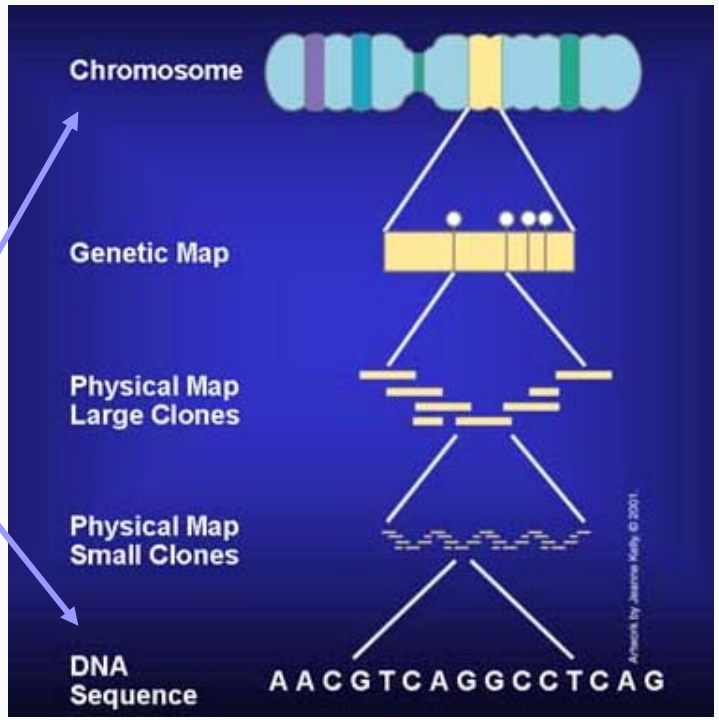
Comparative genomic hybridation (aCGH)
Example: CLL with del(13q)

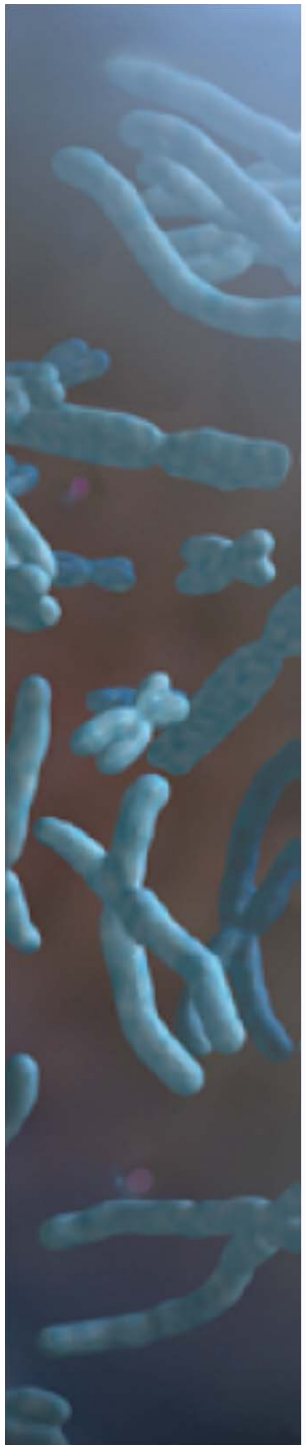




Cytogenetic analyses which tool?

- Selection based on
  - type of sample available (fresh/frozen or not, amount, access)
  - type of question (diagnostic set-up vs follow-up of MRD)
  - type of abnormality to screen for (point mutation / specific gene aberration vs genome wide screening)
  - Context: routine vs research

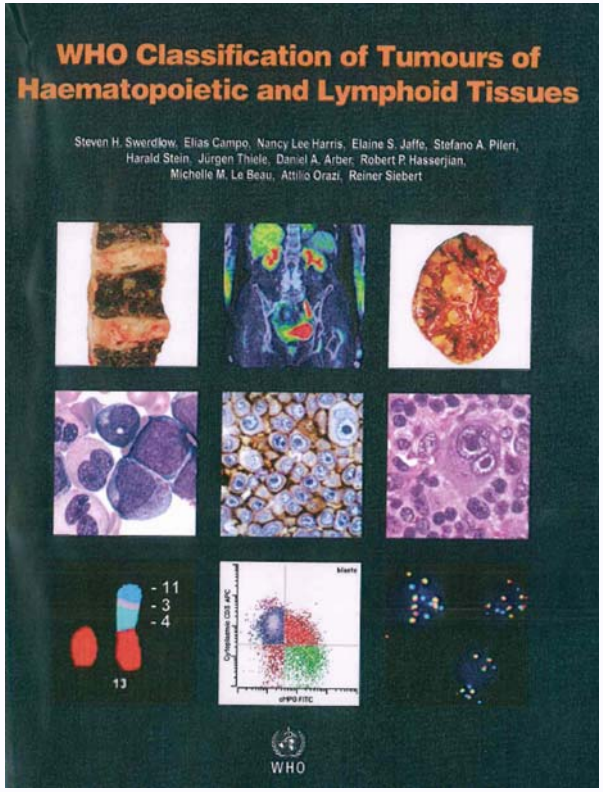


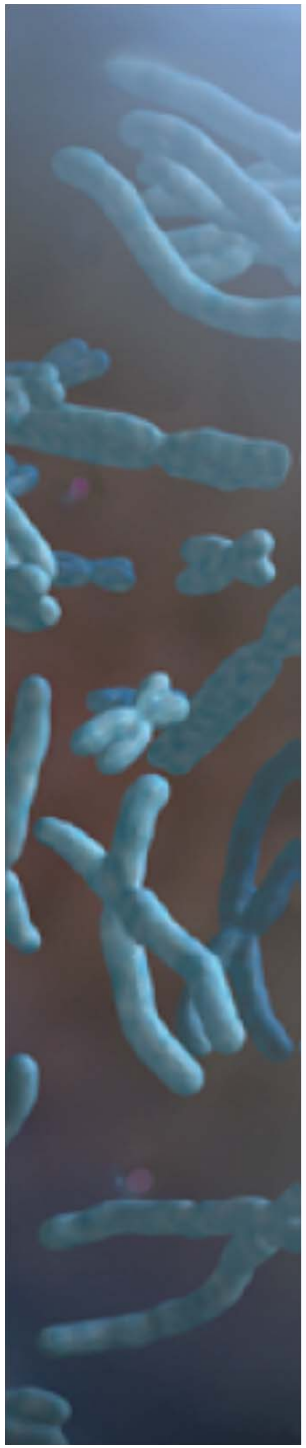


Cytogenetics: diagnostic value

The World Health Organization (WHO) classification of malignant hemopathies includes cytogenetics

- Some aberrations are subtype specific
- Some aberrations can indicate for the presence of a malignant disorder

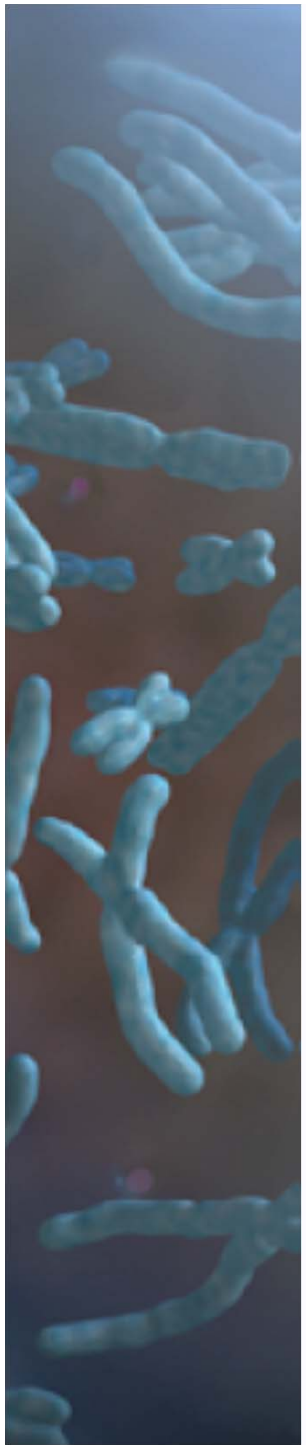




Cytogenetics: prognostic value

Type (and sometimes number) of aberrations = major prognostic factor → « stratified » treatments

Clonal evolution: often predicts poor outcome



Indications of cytogenetic analyses

	Diagnostic	FU	Prognostic
AML	+	+/-	+
ALL	+	+/-	+
MDS	+/-	+/-	+
CML	+++	+++	+
MPN (other)	+	+/-	-
NHL	+	-	-
CLL	+/-	+	+
MM	+/-	+/-	+

Other…

- pathophysiology
- & therapy!

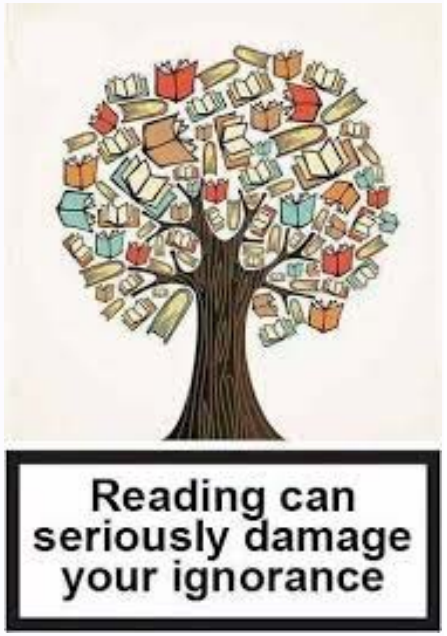
Atlas of cytogenetics (contains informations on clinico-biological entities and on specific chromosome aberrations) :
http://atlasgeneticsoncology.org/

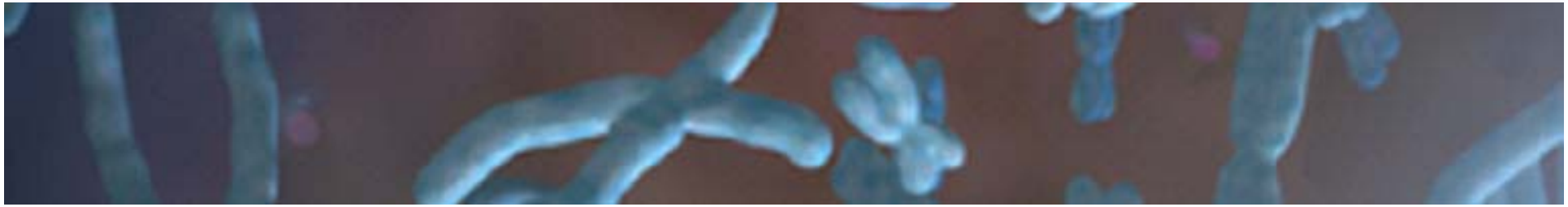
WHO 2017

Catalog of genetic anomalies in cancer (useful in case of very rare aberrations)
http://cgap.nci.nih.gov/Chromosomes/Mitelman

Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel.
Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Löwenberg B, Bloomfield CD.
Blood. 2017 Jan 26;129(4):424-447.

Current challenges and opportunities in treating adult patients with Philadelphia-negative acute lymphoblastic leukaemia.
Wolach O, Amitai I, DeAngelo DJ.
Br J Haematol. 2017 Oct 26. doi: 10.1111/bjh.14916. [Epub ahead of print]






QUESTIONS
FOUND IN GOOGLE AUTOCOMPLETE
WHY AREN'T ECONOMISTS RICH
WHY DO AMERICANS CALL IT SOCCER
WHY ARE MY EARS RINGING
WHY ARE THERE SO MANY AVENGERS
WHY ARE THE AVENGERS FIGHTING THE X MEN
WHY IS WOLVERINE NOT IN THE AVENGERS
WHY ARE THERE ANTS
WHY IS EARTH TILTED
WHY IS SPACE BLACK
WHY IS OUTER SPACE SO COLD
WHY IS NASA SHUTTING DOWN
WHY ARE THERE GHOSTS
WHY DO TWINS HAVE DIFFERENT FINGERPR
WHY ARE AMERICANS AFRAID OF DRAG
WHY DO YOUR BOOBS HURT?
WHY DO I SAY UH
SEA SALT BETTER
THERE MIRRORS ABOVE BEDS
THERE TREES IN THE MIDDLE OF FIELDS
THERE NOT A POKEMON MMO
THERE LAUGHING IN TV SHOWS
THERE DOORS ON THE FREEWAY
THERE SO MANY SHOWERS RUNNING
THERE ANY COUNTRIES IN ANTARCTICA
THERE SCARY SOUNDS IN MINECRAFT
THERE KICKING IN MY STOMACH
THERE TWO SURGEONS AFTER HTTP
RE THERE CELEBRITIES
DO SNAKES EXIST
WHY ARE THERE SPIDERS IN MY ROOM