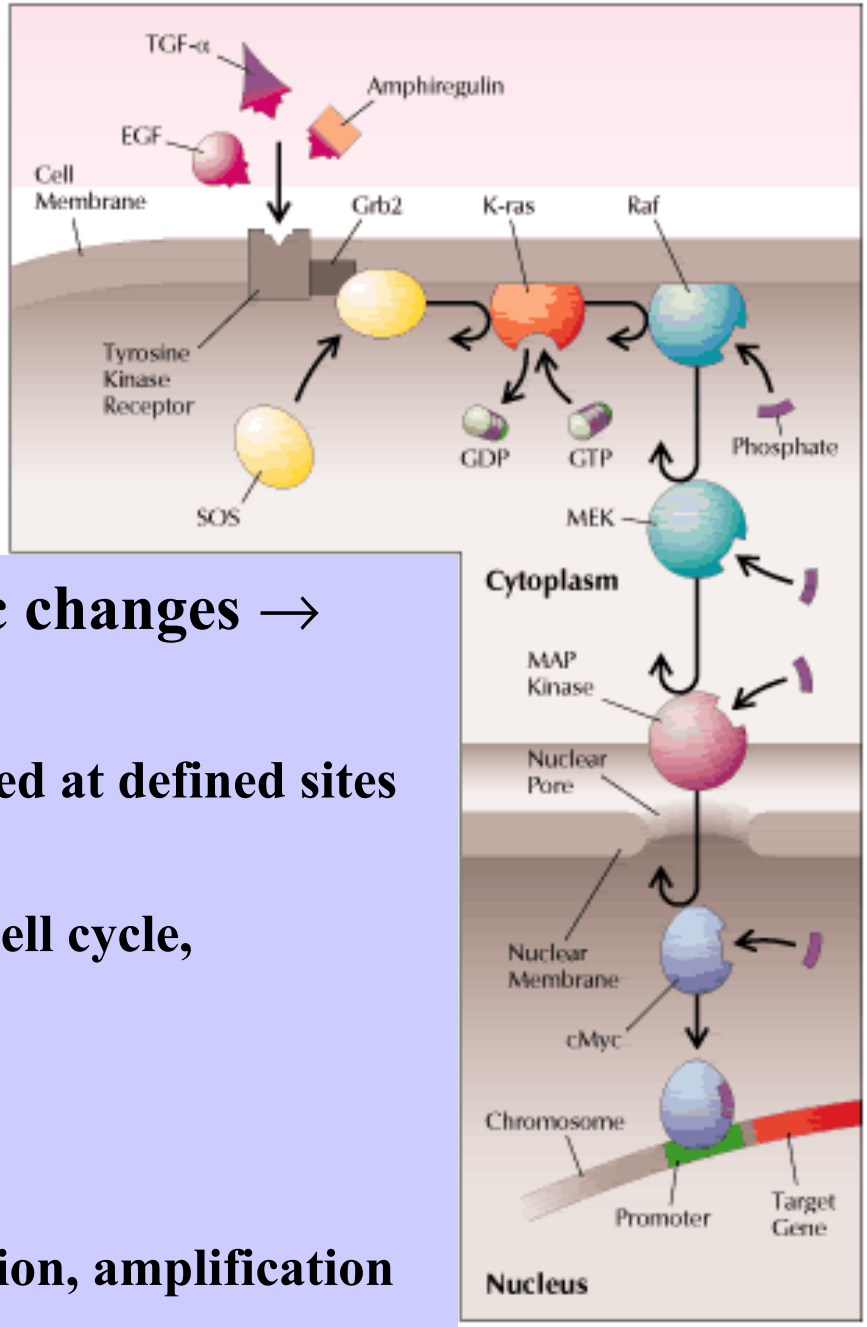
Protooncogenes



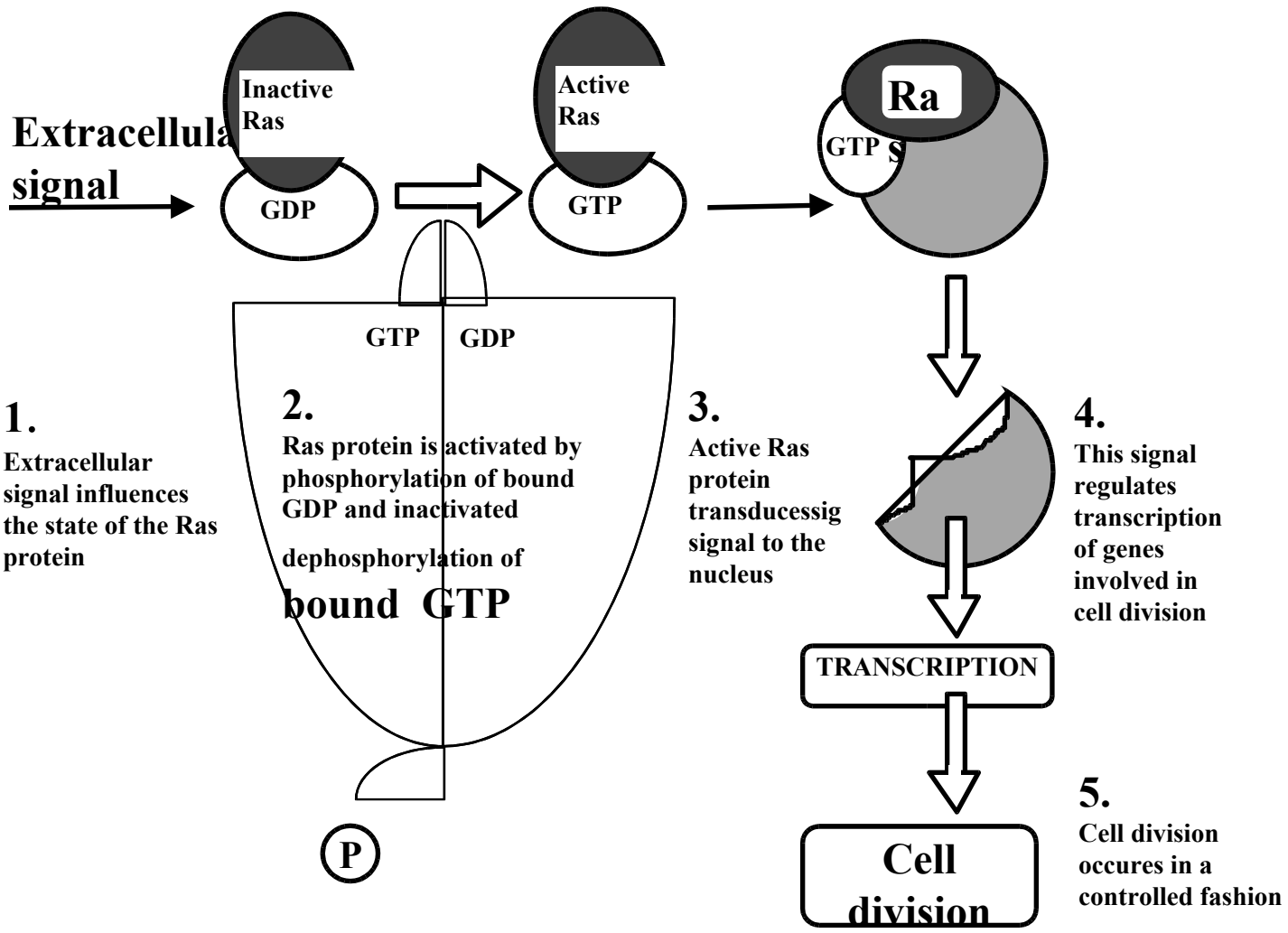
PROTOONCOGENES → **genetic changes → tumor-causing genes (c-onc)**

- **They encode proteins that are required at defined sites throughout the cell**
- **Control: cell division, proliferation, cell cycle, differentiation …**

MUTATIONS - *somatic* – dominant

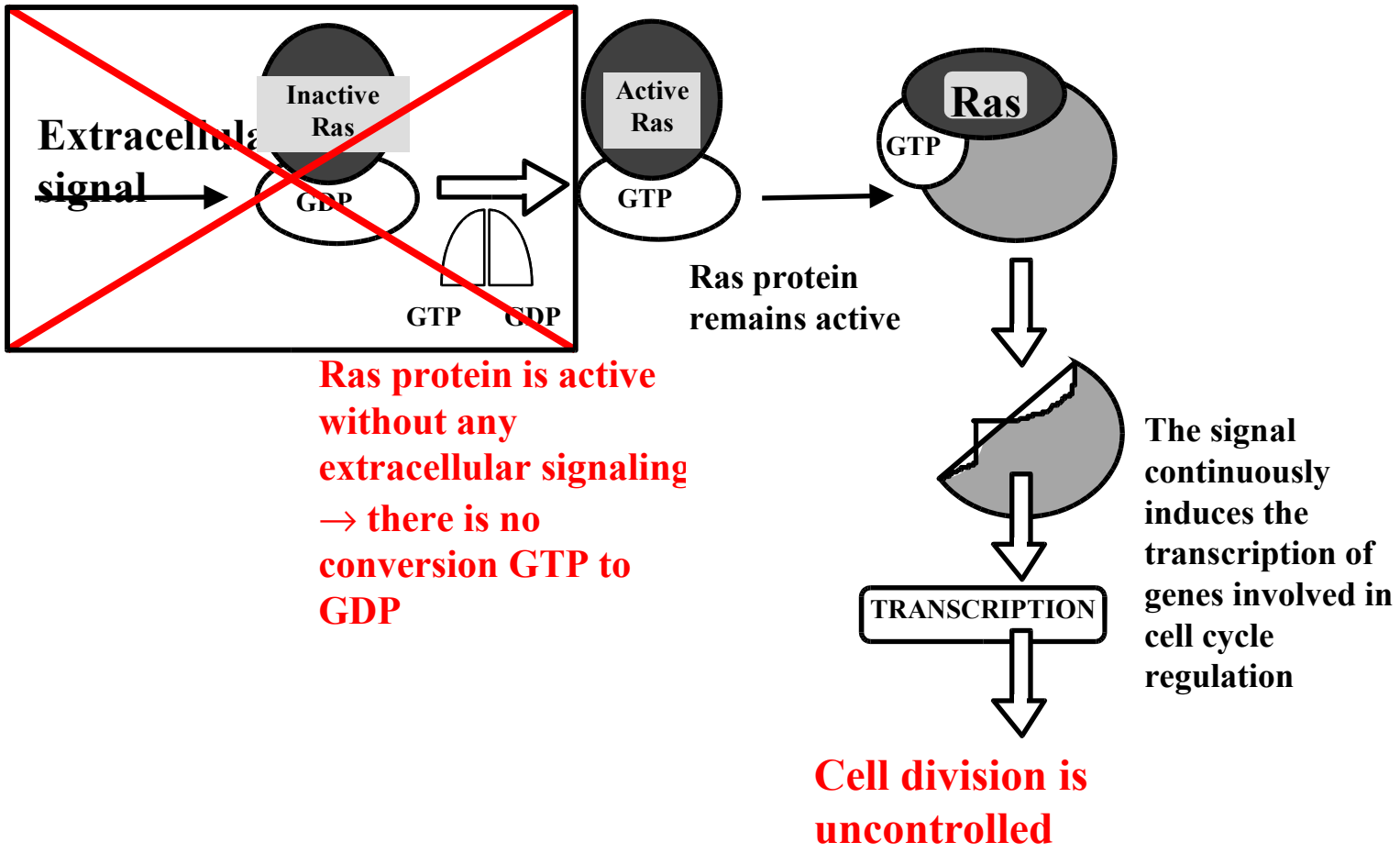
- ***Point mutations in a critical region***
- ***Chromosomal aberrations:*** **translocation, amplification**
- ***Viruses***

Protooncogene *c-ras* – task 11, page 186-187



Protooncogene *c-ras* – task 11, page 186-187

Point mutation – substitution T→G (see genetic code, page 111)



Protooncogene c-ras

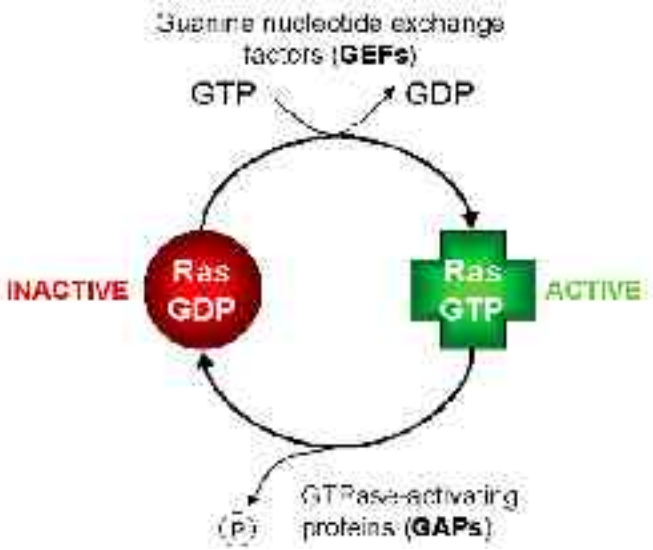
Commentary - protooncogene c-*ras*

- **G protein → GTP-binding protein with signal transmitting function**
- **Activated after binding growth factor to its receptor**
- **Activation of receptor → exchange of associated GDP to GTP**
- **GTP triggers a short time-limited Ras signaling ability**
- **Signaling pathway continues via MAP-kinases**
- **In the nucleus → activation of transcription factors (e.g. product of *c-myc*)**

Result:

***c-ras* is inactivated after short time by conversion of GTP to GDP**

Several mutations in c-*ras* can remove the time limit of the cell-stimulated signal (hydrolysis of GTP to GDP) and uncontrolled cell division leads to tumor development



Tumor suppressor genes

NORMAL GENES – CELL CYCLE REGULATION → PREVENTION OF NEOPLASIA

MUTATION → loss of gene function

MUTATION RECESIVE AT THE CELLULAR LEVEL (loss of function of both alleles)

AUTOSOMAL DOMINANT INHERITANCE

HEREDITARY PREDISPOSITION → GERMINAL MUTATION

(AT THE LEVEL OF WHOLE ORGANISM)

- ***Sporadic incidence*** **of tumor: two somatic mutations → risk = frequency neoplasia in population**
- ***Familial incidence*****: 1. germline mutation, 2. somatic mutation**
- **predisposition, early childhood, multifocal or bilateral incidence, several memmbers of family affected**
- **tumor initiation - requires two steps (Knudson – two-hit hypothesis)**
- **loss of heterozygosity in tumor cells (LOH) – e.g. linkage analysis *Rb1* gene with gene coding enzyme esterase D (marker)**

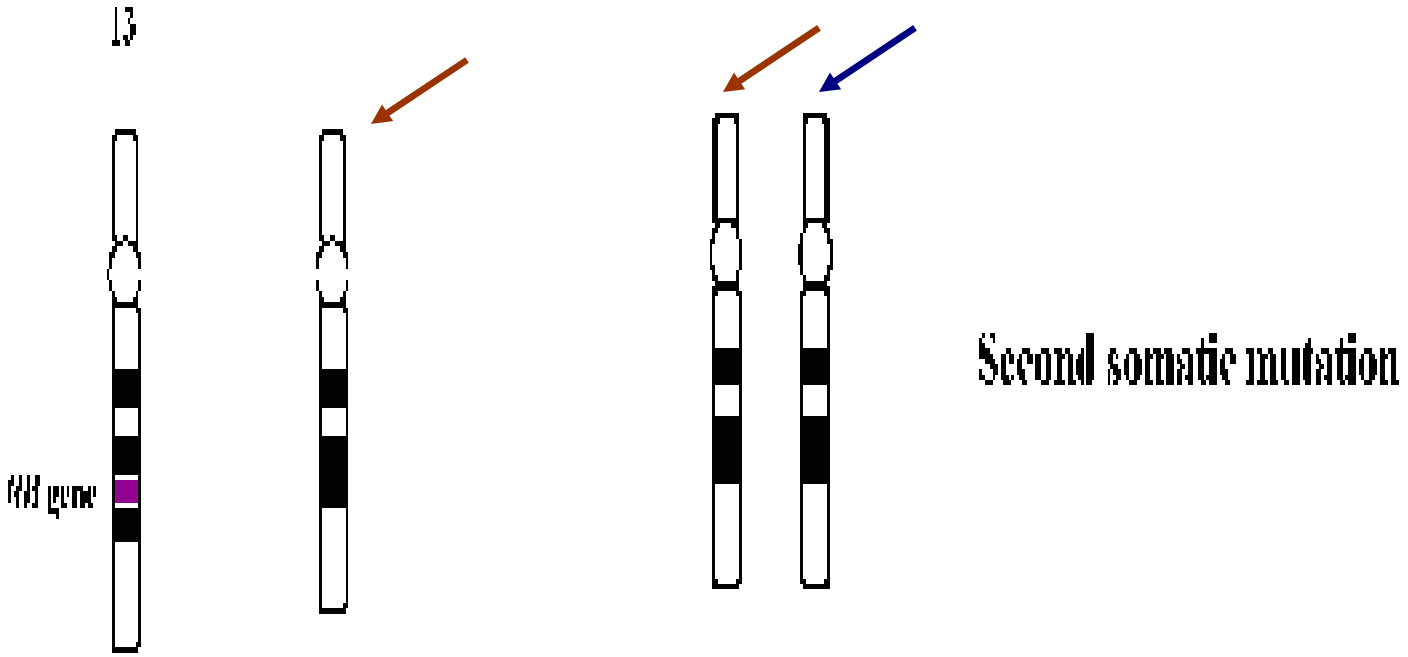
Tumor-suppressor genes – cause of loss of function

- **Deletion – could be found in different locations of a gene**
- **Mitotic nondisjunction**
- **Mitotic recombination**
- **Uniparental disomy (both chromosomes originate from the same parent)**
- **Point mutation**
- **Protein inactivation (interaction with viral antigene)**

Tumor suppressor genes – examples

Tumor localisation	Gene	Chromosome	Gene activity
eye (retinoblastoma), bone, breast, lung, urinary bladder, prostate	*Rb*	13q14	Cell cycle regulation
Kidney and other organs (WAGR syndrom)	*WT1/WT2*	11p13	Cell cycle regulation
Different types of tumors (aprox. 50% tumors – mutation of TP53)	*TP53*	17p13	Regulation of cell cycle, transcriptional factor
colon (familial adenomatosis coli), stomach, non-inherited colorectal carcinomas	*APC*	5q21	Regulation of β-catenin level, cell proliferation and adhesion
breast, ovary, prostate, larynx, digestive tract, pancreas	*BRCA1* *BRCA2*	17p21 13q12-q13	Repair of double-stranded DNA breaks

Tumor suppressor gene *Rb1 (the most frequent mutation - microdeletion)*



RECESIVE MUTATION

FIRST MUTATION – GERMLINE

SECOND SOMATIC

Two-hit hypothesis

(Knudson)

Tumor supressor gene *RB1*

Retinoblastoma → tumors originate from the retina cells

Tumor of eye - in infancy or early childhood

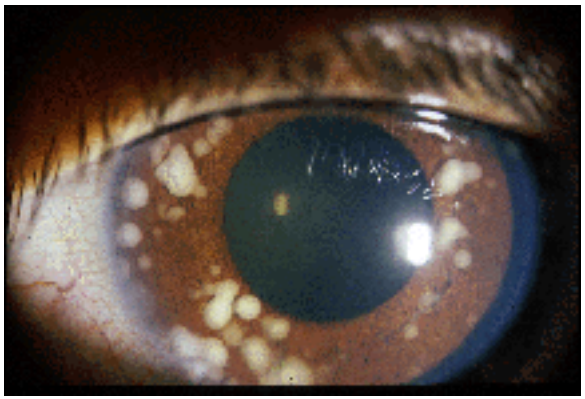
1 / 15000-18000 live birth

Non-hereditary form – 60% (one eye), two somatic mutations

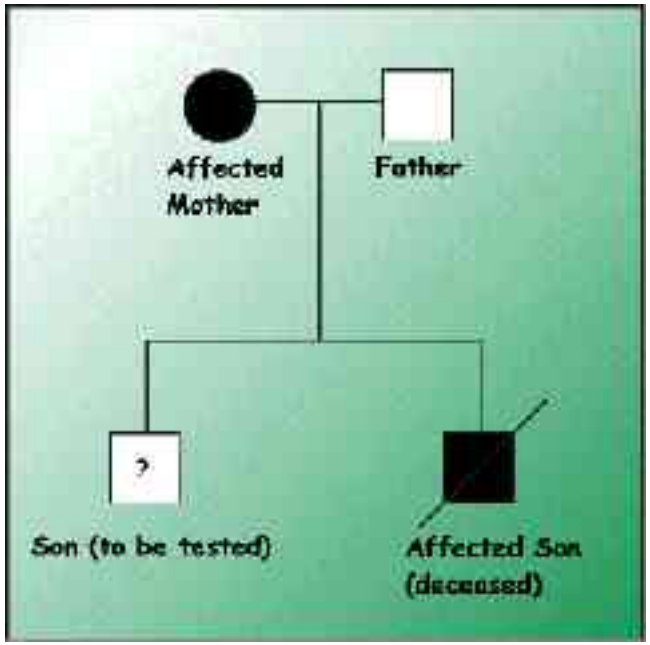
Familial incidence – 40% (bilateral, multifocal)

Germline mutation, transmited as an autosomal dominant trait

Mutation - both genes



a shimmer out of the affected eye



TUMOR SUPPRESSOR GEN *Rb1*

Rb1 gene – ubiquitously expressed

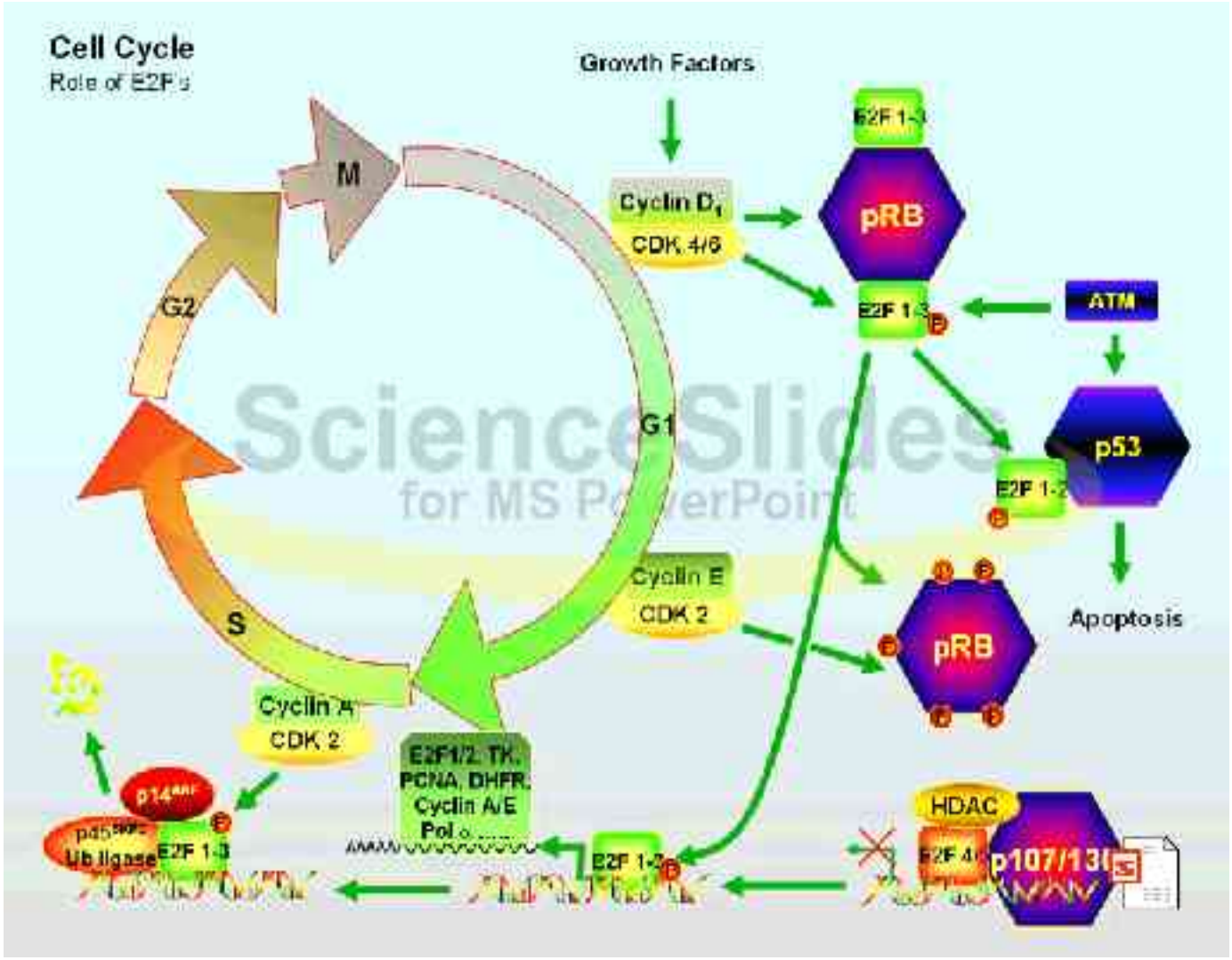
Cell cycle regulation

- **gene product – nuclear phosphoprotein (pRb - 100kD)**
- **phosphorylation / dephosphorylation (serine and threonine residues)**
- **dephosphorylation of protein Rb – active form → inactivation of transcriptional factor E2F**

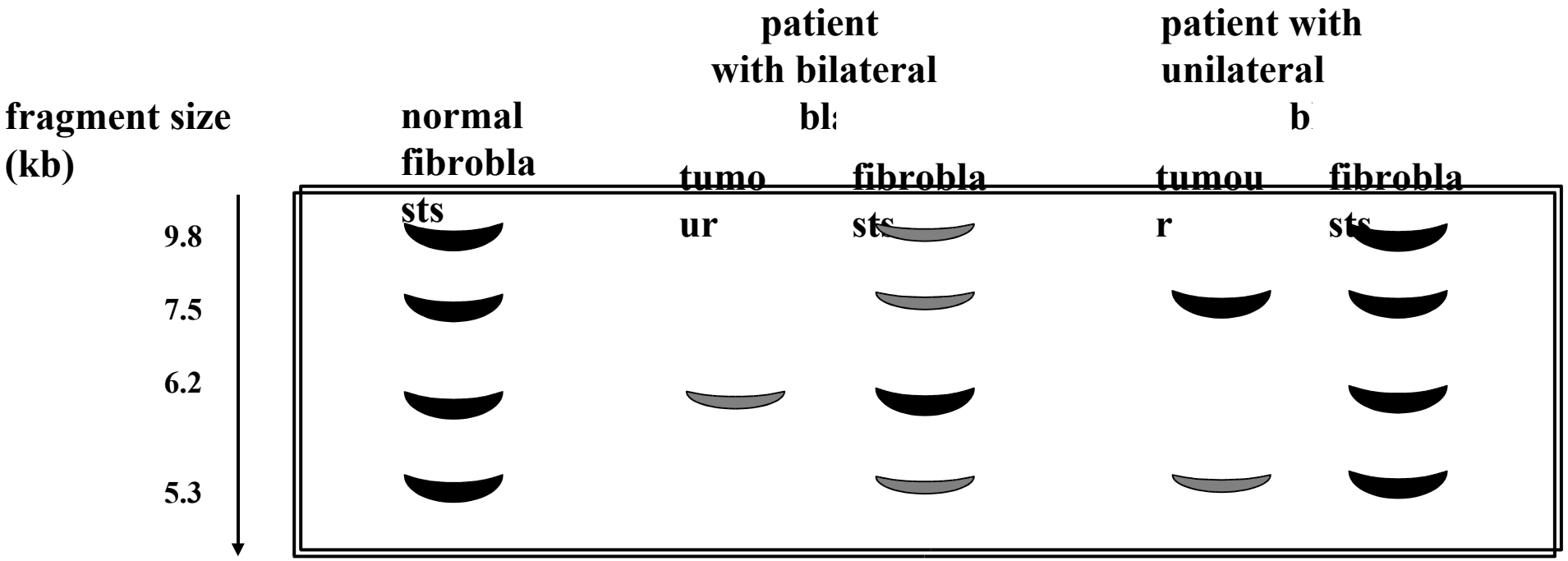
∀ → cell cycle block in G1

- **phosphorylated pRb (inactive) → E2F released from complex pRb/E2F**
- **transition fom G1 to S phase of interphase**

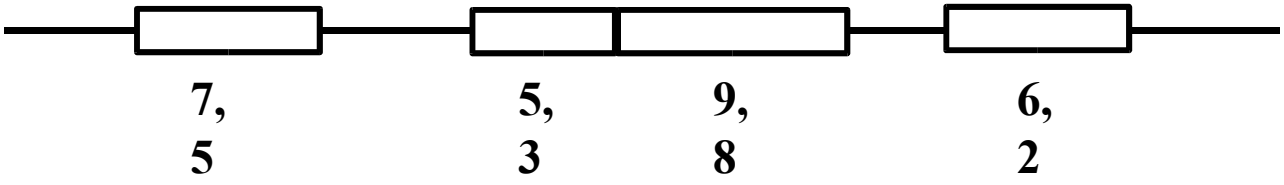
Tumor suppressor genes and cell cycle

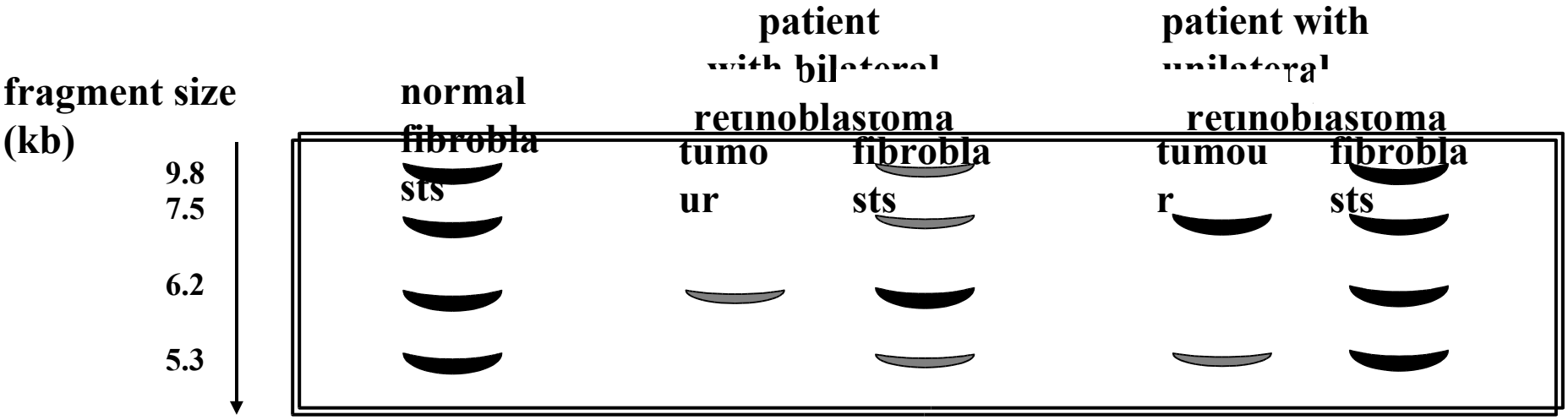


Tumor suppressor gene Rb1 – page 182-183

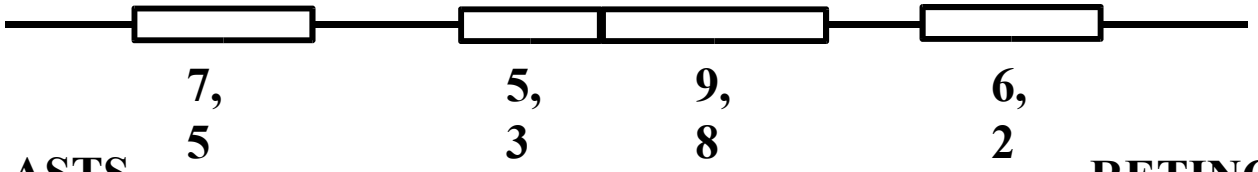


restriction map: sizes and order of HindIII restriction fragments in the retinoblastoma gene

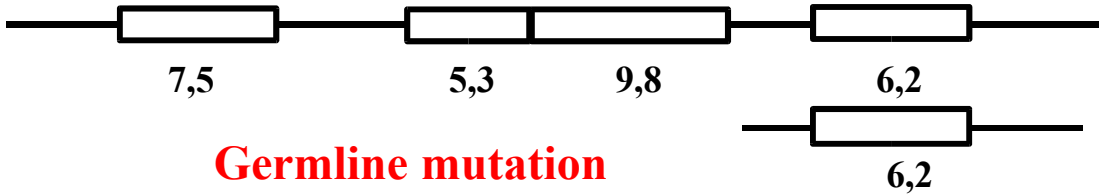




restriction map: sizes and order of HindIII restriction fragments in the retinobla



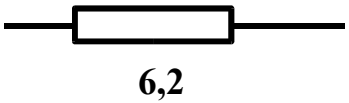
FIBROBLASTS



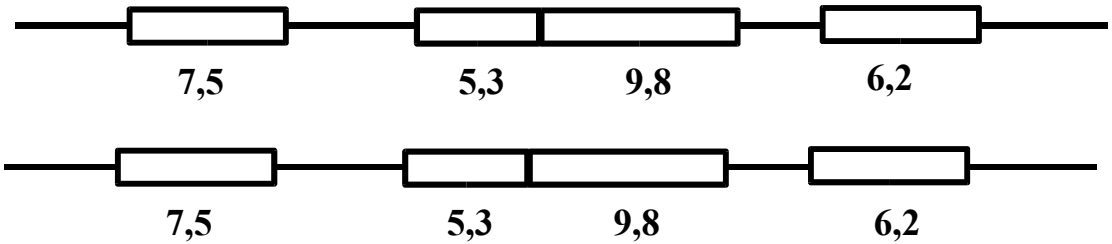
Germline mutation

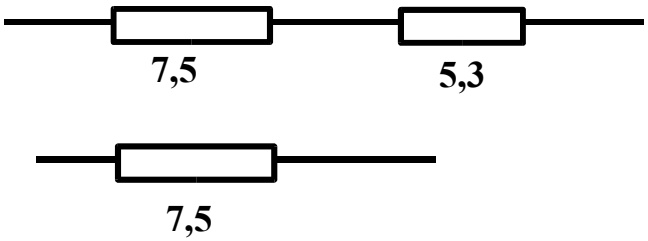
RETINOBLASTOMA

Somatic mutation



Two different somatic mutations



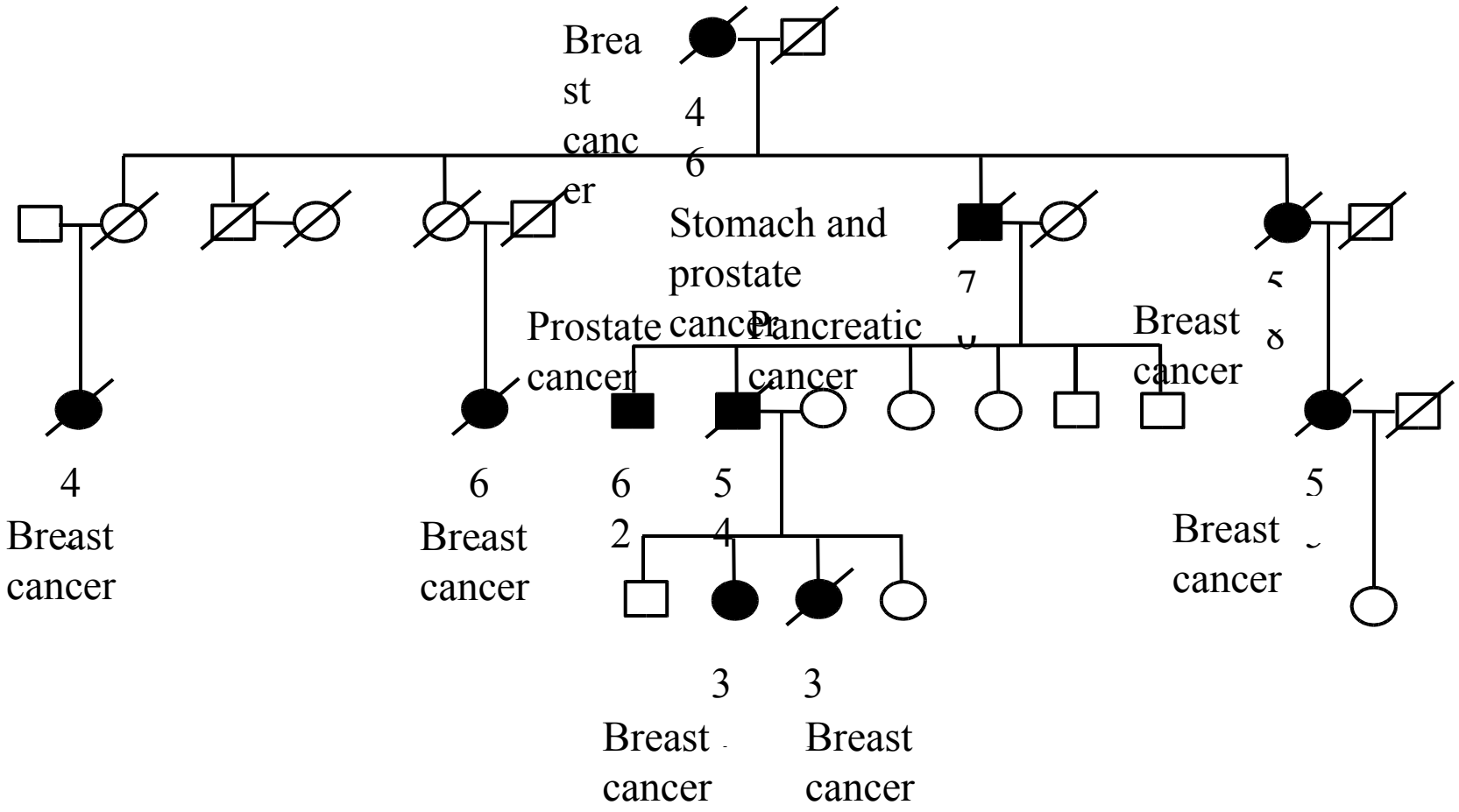


Tumor suppressor genes *BRCA1, BRCA2 – page 178*

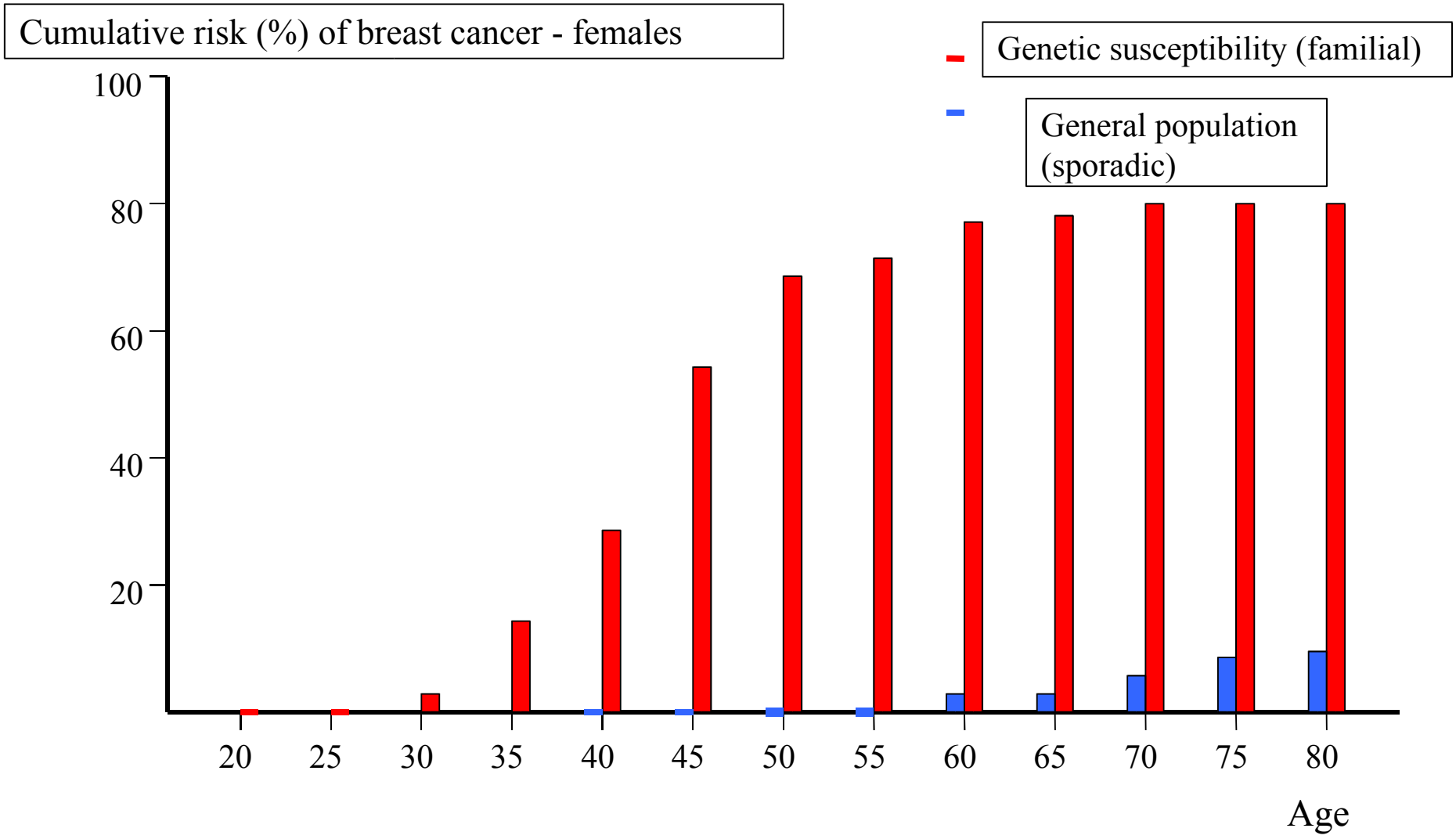
- **Products *BRCA* genes form complex with products of several genes**
- **Cell cycle regulation**
- **Repair – doublestrand breaks of DNA**
- **Inherited mutation of *BRCA1* – familial incidence breast cancer and/or cancer of ovary**
- **Germline mutation of *BRCA2* gene – association with cancer of breast (both female and male), prostate and pancreas**
- **proteine BRCA1 and BRCA2 → cooperation with protein *RAD51***

Mutation of *BRCA1 / BRCA2* gene – truncated protein (loss of function)

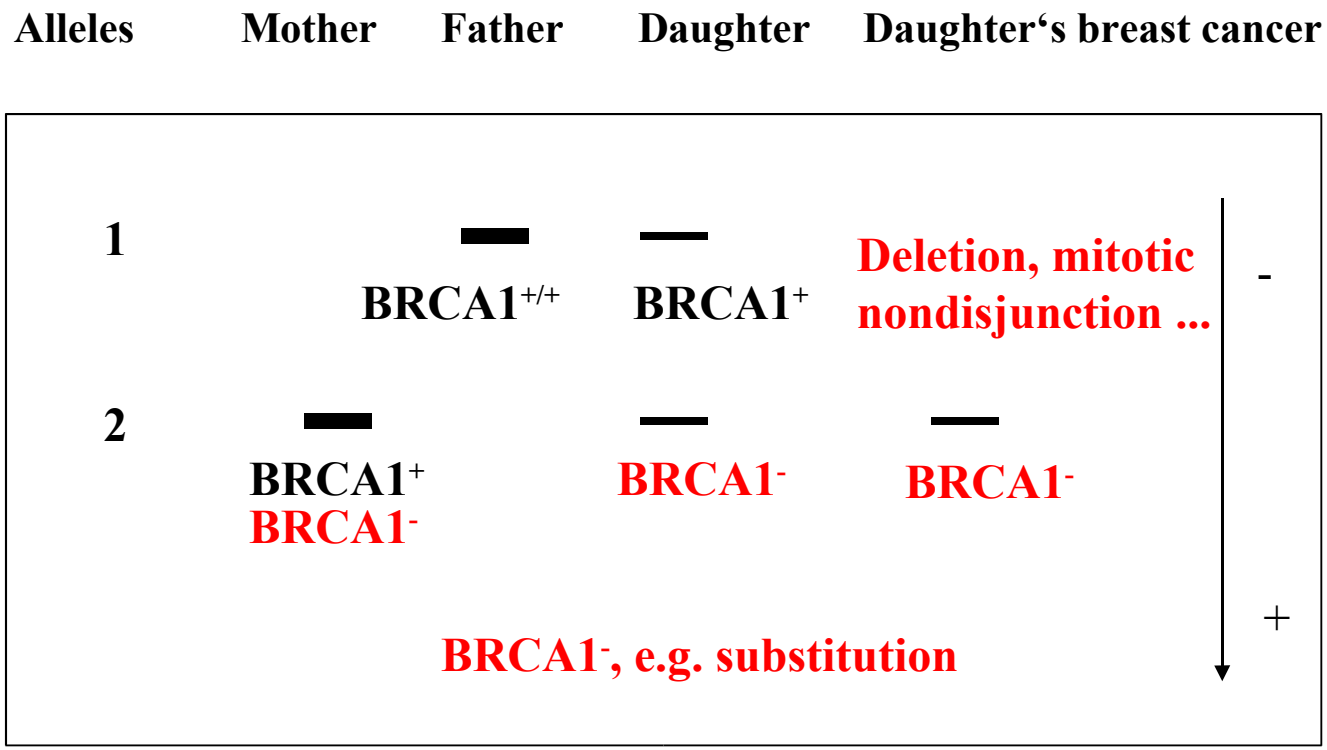
Familial incidence of tumors associated with *BRCA2*



Sporadic and familial incidence of breast cancer - females

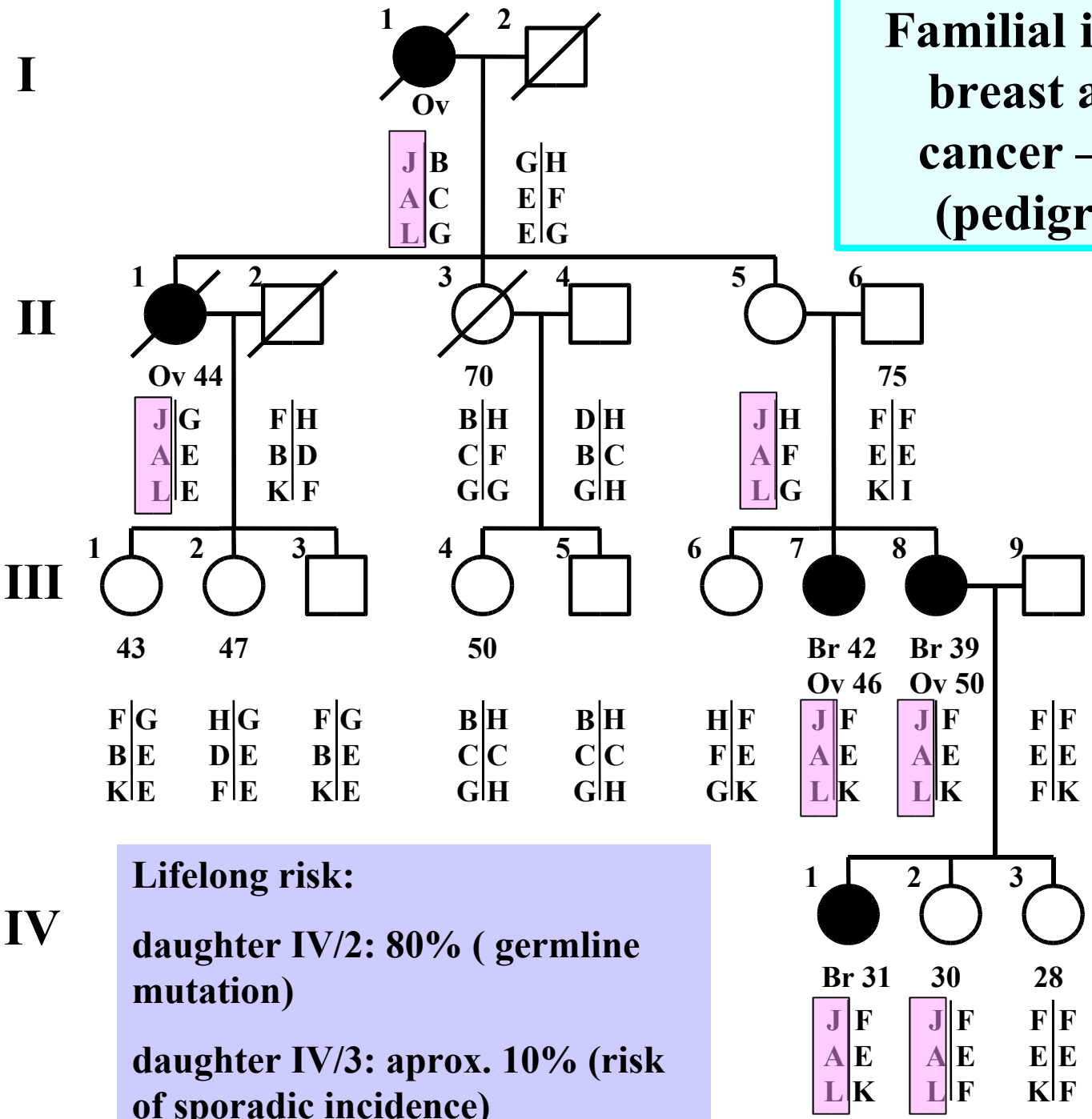


Linkage analysis: marker gene (allele 1 and 2) - gene *BRCA1 - page 179*



Examined cells of mother, father and daughter: fibroblasts or leukocytes
Loss of heterozygosity (LOH) in breast cancer cells

Familial incidence of breast and ovary cancer – page 181 (pedigree study)



Lifelong risk:

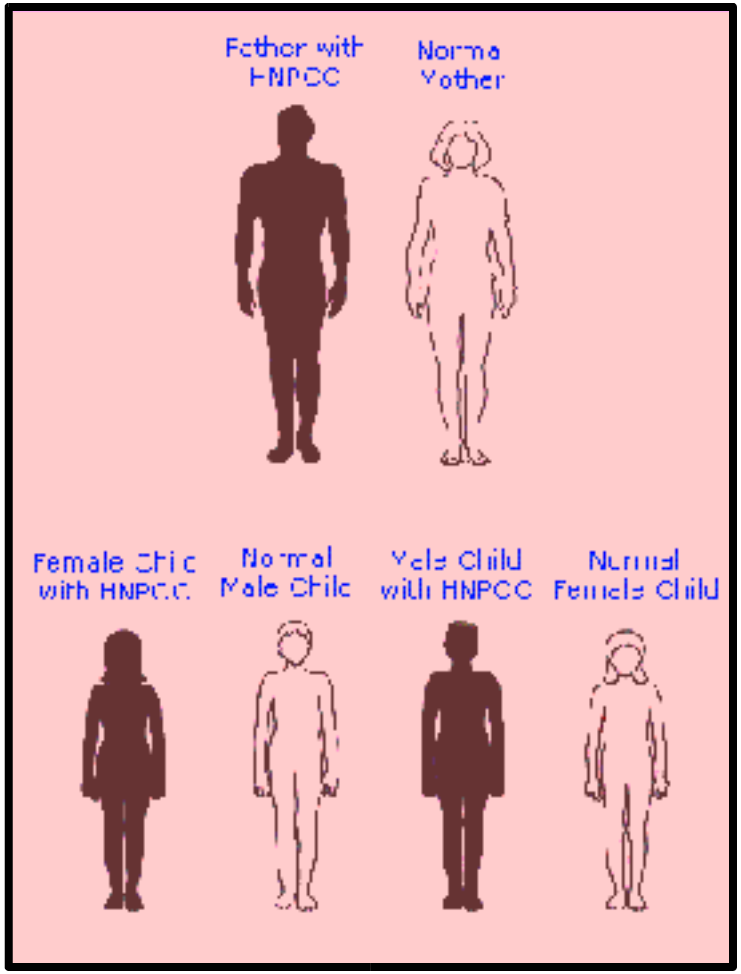
daughter IV/2: 80% (germline mutation)

daughter IV/3: aprox. 10% (risk of sporadic incidence)

Mutator genes – mismatch repair (MMR)

Association with HNPCC (Hereditary Nonpolyposis Colon Cancer)

- hMSH2, hMLH1, hPMS1, hPMS2, hMSH6, hMSH3
- genome stability
- mismatch repair (replication errors)
- microsatellite loci (e.g. CA_n) instability
- increased mutation frequency 100 –1000x
- recessive character of mutation in MMR genes
- autosomal dominant heritability HNPCC
- Lynch syndrome I – colon and rectal cancer
- Lynch syndrome II - cancer of colon and rectum, 30% - tumor endometrium, stomach, pancreas, urinary tract



Mismatch repair - hMSH2

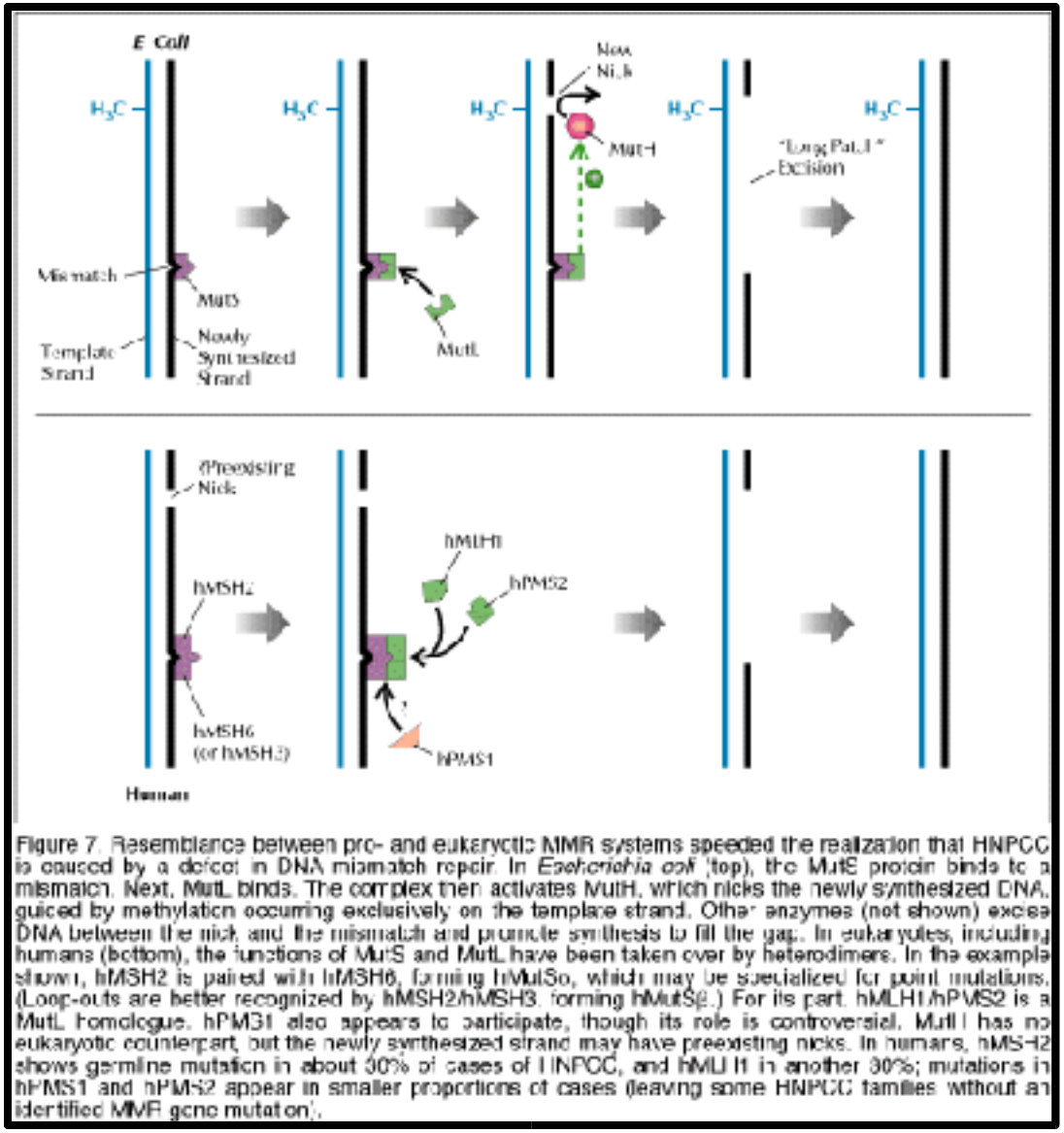


Figure 7. Resemblance between pro- and eukaryotic MMR systems speeded the realization that HNPCC is caused by a defect in DNA mismatch repair. In *Escherichia coli* (top), the MutS protein binds to a mismatch. Next, MutL binds. The complex then activates MutH, which nicks the newly synthesized DNA, guided by methylation occurring exclusively on the template strand. Other enzymes (not shown) excise DNA between the nick and the mismatch and promote synthesis to fill the gap. In eukaryotes, including humans (bottom), the functions of MutS and MutL have been taken over by heterodimers. In the example shown, hMSH2 is paired with hMSH6, forming hMutSα, which may be specialized for point mutations. (Loop-outs are better recognized by hMSH2/hMSH3, forming hMutSβ.) For its part, hMLH1/hPMS2 is a MutL homologue. hPMS1 also appears to participate, though its role is controversial. MutH has no eukaryotic counterpart, but the newly synthesized strand may have preexisting nicks. In humans, hMSH2 shows germline mutation in about 30% of cases of HNPCC, and hMLH1 in another 30%; mutations in hPMS1 and hPMS2 appear in smaller proportions of cases (leaving some HNPCC families without an identified MMR gene mutation).

Nonrandom chromosomal aberrations in tumor cells

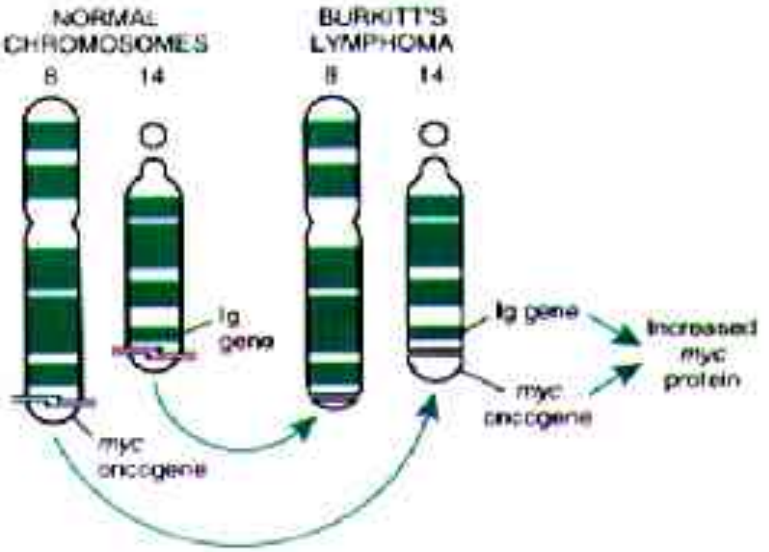
Nonrandom (primary)

- **Philadelphia chromosome (Ph1)**
- **Translocation – Burkitt lymphoma**
- **Amplification, double-minutes**

Random (secondary)

- **random chromosomal aberrations (deletions, translocations, dicentric chromosome, ring chromosome, isochromosome ….)**
- **heteroploidy, for example pseudodiploidy**

Nonrandom chromosomal aberration
Burkitt´s lymphoma



Translocation of protoonkogene c-myc t (8;14), or rarely t(8,22), t(2,8)

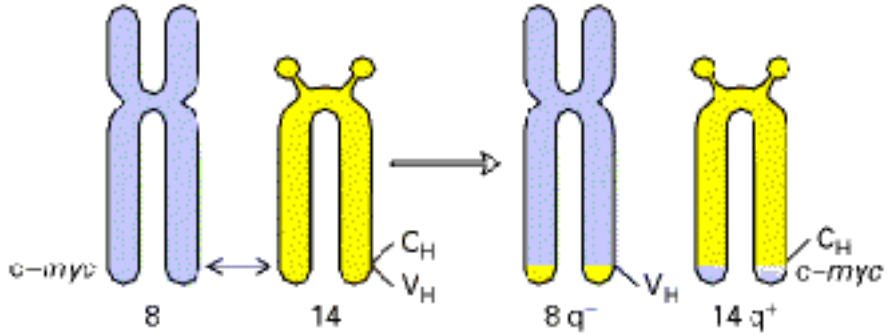
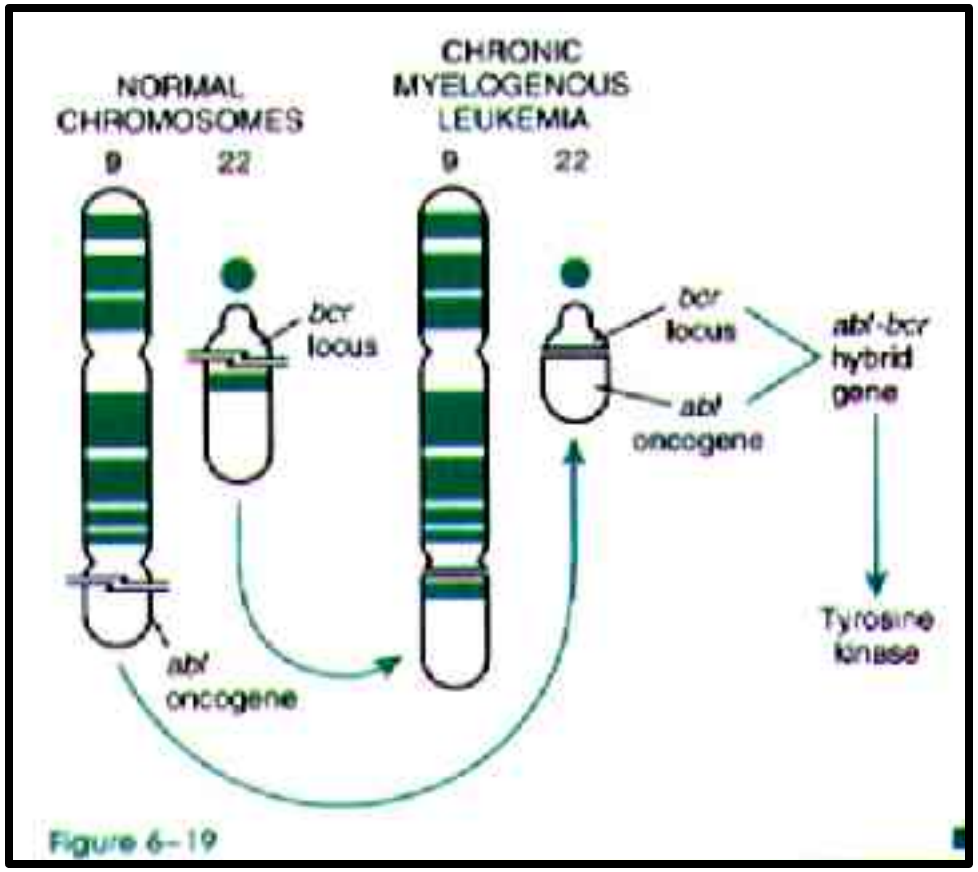
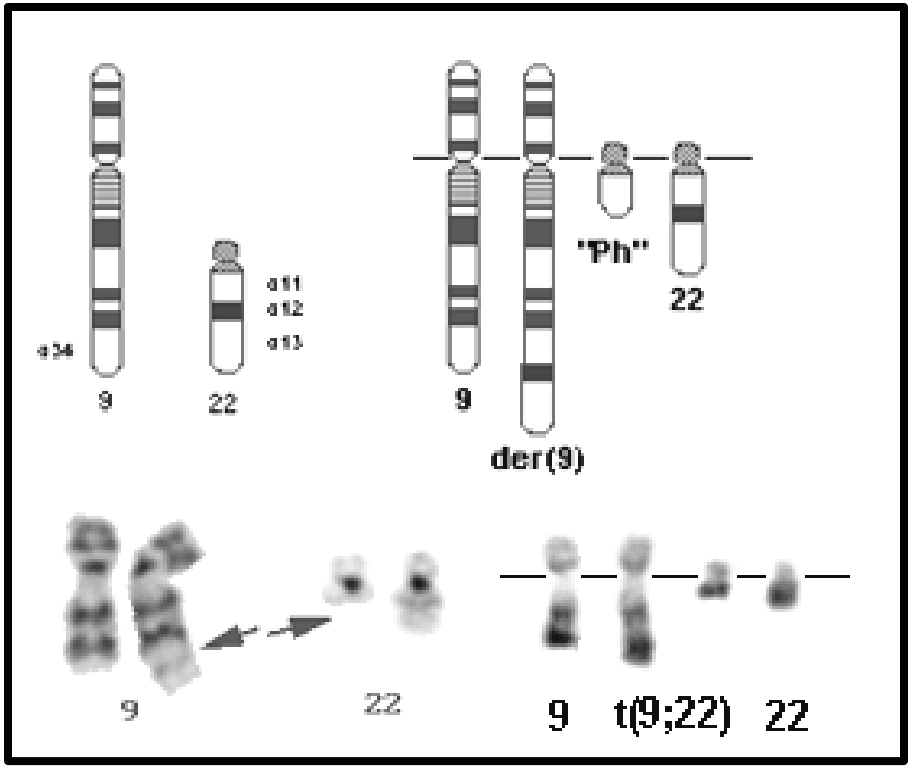


Figure 24-22. Chromosomal translocation in Burkitt's lymphoma. This leads to overexpression of the Myc transcription factor.

Nonrandom chromosomal aberration
Chronic myeloid leukemia (CML)
Philadelphia chromosome (Ph)





Chronic myeloid leukemia (CML)
Philadelphia chromosome (Ph)

Philadelphia (Ph) chromosome - reciprocal translocation - chromosome 9 and 22 - { t(9;22)(q34;q11) }

Cytogenetic prognostic marker - 90% CML

Cytogenetic marker - 5 -20% acute lymphocytic leukemia (ALL)

Translocation → fusion genes BCR-ABL, break in BCR ("breakpoint cluster region") gene (chromosome 22 and ABL protooncogene chromosom 9)

Chimeric proteine 210 kD - oncoprotein

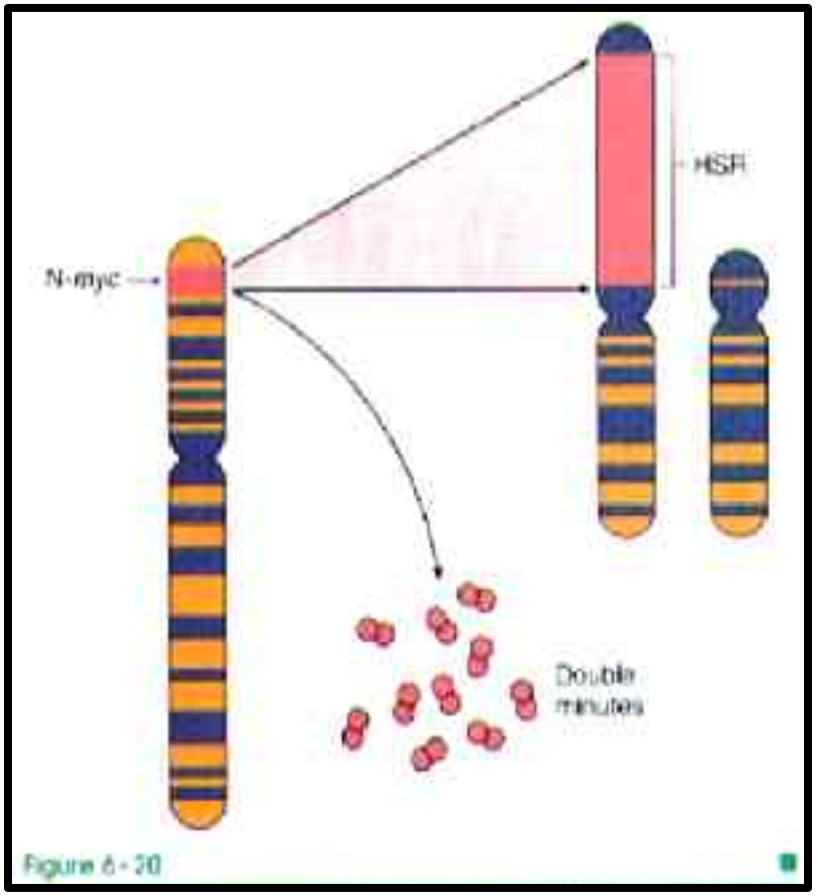
Nonrandom chromosomal aberration
Amplification - protooncogene *N-myc*

Amplification of DNA sequence

A) homogenously stained regions (HSR)

B) double minutes

- **diagnostic and prognostic marker**
- **family of myc protooncogenes (virus of chicken myeloblastosis) cell cycle regulation**
- **N-myc – neuroblastoma**
- **L-myc – small cell lung carcinoma**



FISH methods

FISH – diagnostic tool of clasical cytogenetics combined with molecular genetic approach

Based on hybridization of single stranded DNA probe with single stranded sequence of studied DNA in situ (based on the complementarity rule)

Examination of chromosomes in mitotis or interphase

DNA probe is labeled with fluorochrome (eg. Texas Red, FITC, etc.)

Types of DNA probes – centromerical, locus-specific, painting of chromosomes

***Centromeric probes*: α-satellite sequences of repetitive DNA present in centromeric regions → detection of abnormal chromosome number**

***Locus-specific probes*: hybridization with specific loci on chromosomes → detection of structural aberrations (microdeletion, translocations…)**

***Painting of whole chromosomes*: mix of chromosome-specific DNA fragments enables to distinguish different chromosomes, but cannot be used for interphase chromosomes**

Locus-specific probe – breast cancer cell in interphase

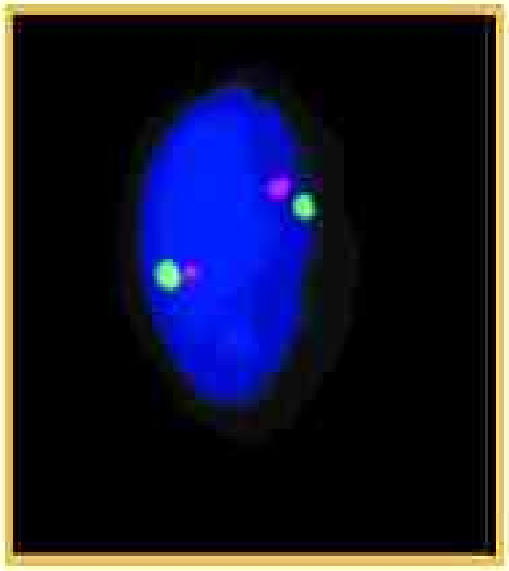
Protooncogene Her-2/neu

- epidermal growth factor receptor (tyrosine kinase activity)

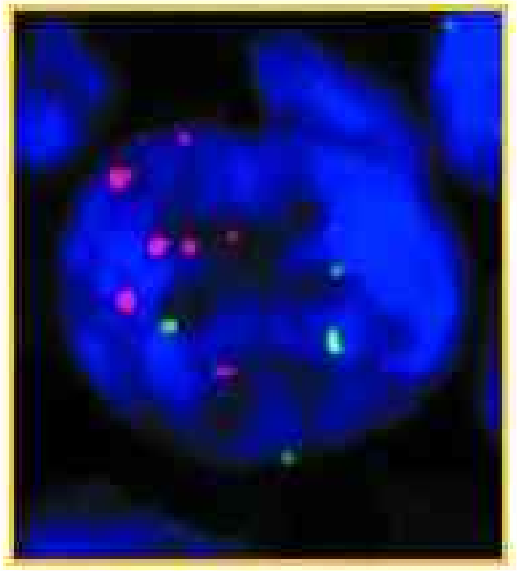
Prognostic and diagnostic marker – breast cancer, urinary bladder ….

Determine which picture represents situation in tumor cell:

Centromere– green signal -2x
Her-2/neu – red signal – 2x
Normal cell

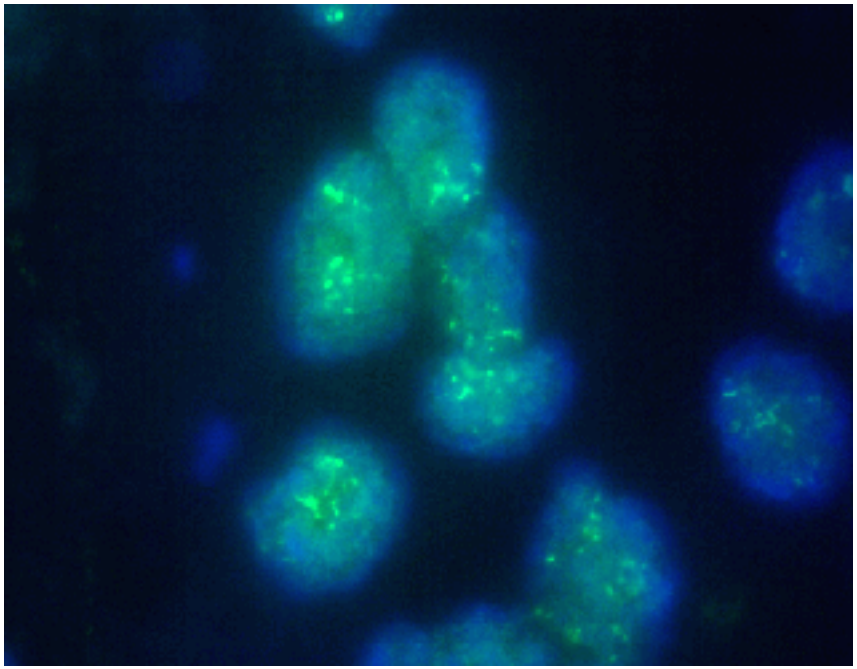


Centromere– green signal -2x (two homologous chromosomes)
Her-2/neu – red signal – amplification of gene (6x); second chromosome 1x (no mutation)
Tumor cell



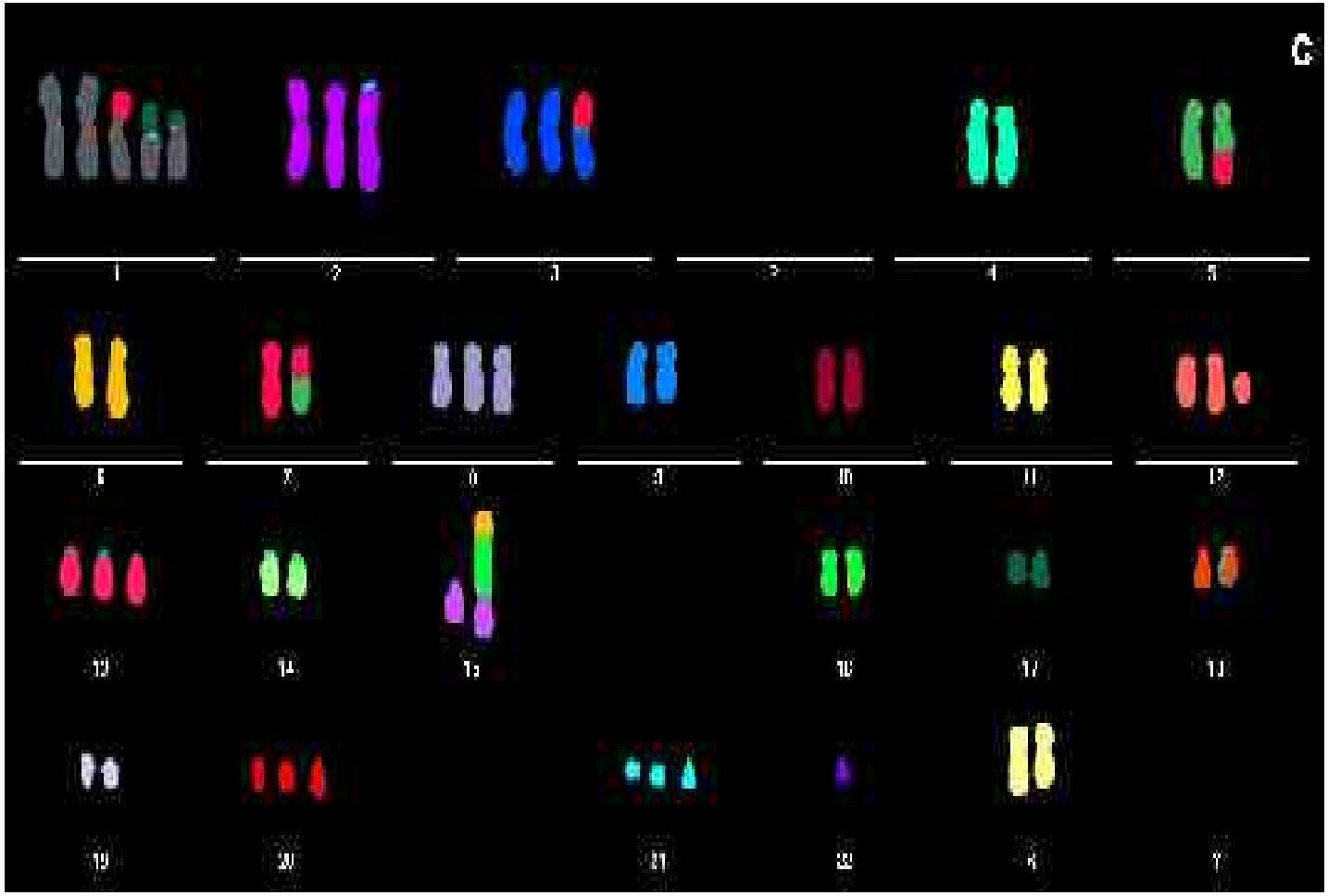
Small-cell carcinoma of lung

Determine which type of chromosomal aberration represents the picture:



- **FISH method - „smear“, single signals are undetectable**
- **Multiple copies (amplification) of protooncogene *L-myc* - non-random chromosomal aberration**
- **Bad prognosis**

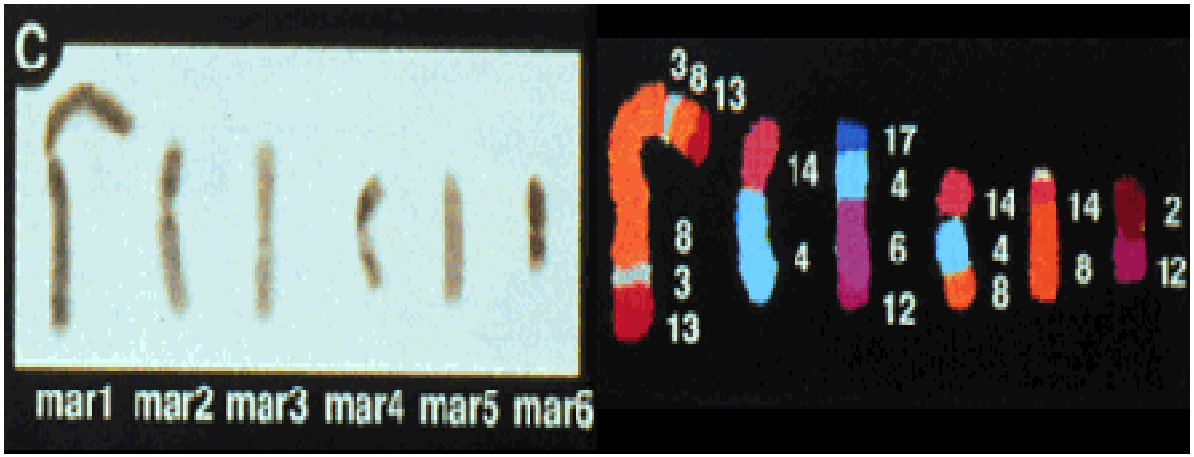
Multicolor painting probe – karyotype of lung tumor
hyperdiploidic number of chromosomes + random structural aberrations



Chromosomal aberrations in breast cancer cells

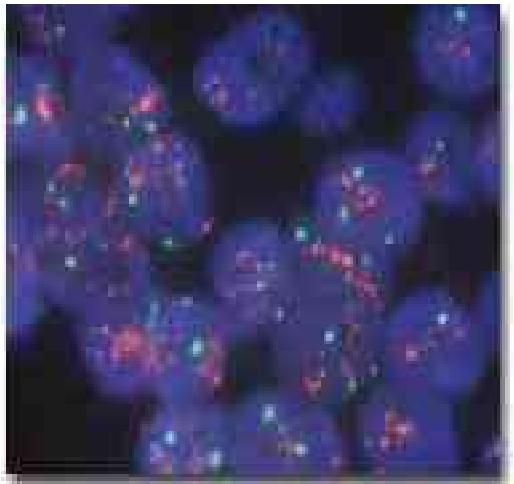
Determine which picture represents random and which one non-random chromosomal aberrations.

Multicolor FISH



Chromosomes in breast cancer cells - multiple random aberrations

Detection of Her-2/neu using FISH



Breast cancer cells – amplification of protooncogene Her-2/neu

Amplification in majority of cancer cells (many signals → "smear")

Non-random (primary) chromosomal aberration

Karyotype → diagnostic marker, associated with prognosis and choice of therapy

Comparative genome hybridization (CGH)

Comparative genome hybridization (CGH) – molecular cytogenetic method
Identification of multiple chromosomal non-balanced aberrations in tumor cells, balanced aberrations are not detected using CGH

Pathological event must be found at least in 50% cells

Genomic DNA from normal and tumor tissue → simultaneous *in situ* hybridization with methaphase chromosomes of normal (healthy) cell

Detection : (i) green fluorescent labeled probe – DNA of tumor
(ii) red fluorescent labeled probe - DNA of normal tissue (control sample of DNA)

Ratio of green to red fluorescent signal intensity gives the ratio of difference between DNA isolated from normal and tumor cells

CGH analysis requires an equipment for quantitative analysis of fluorescent signal

Comparative genomic hybridization (CGH)