Genetic Basis of Cancer

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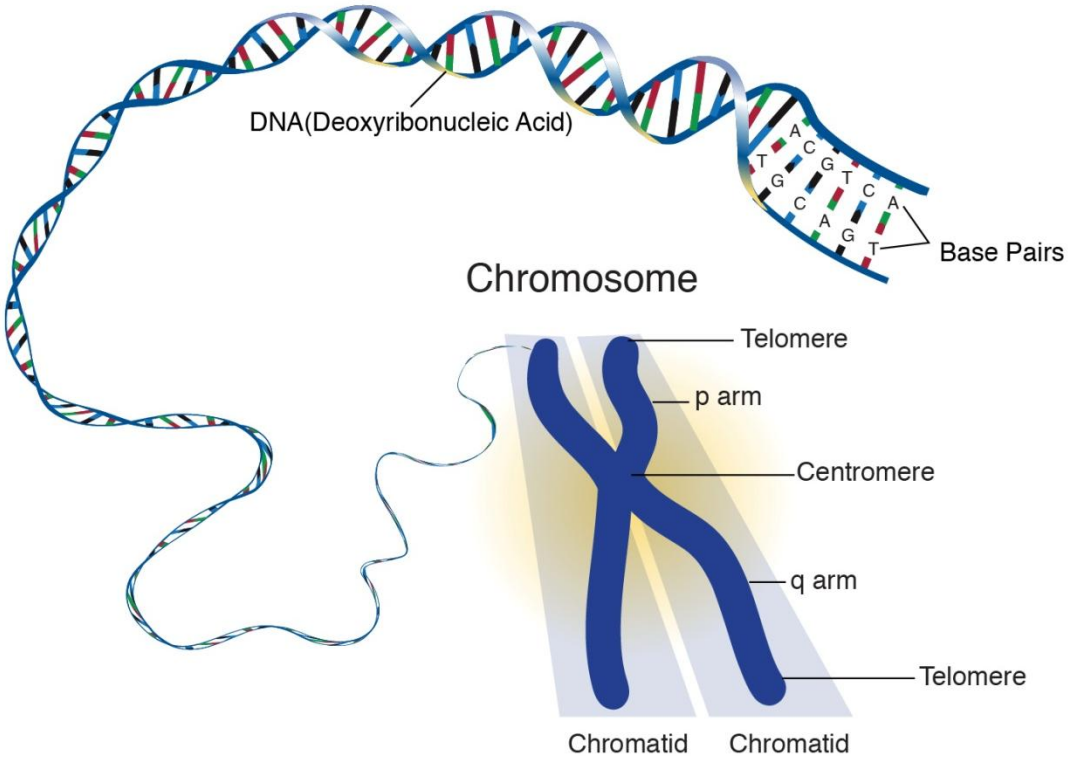
Genetics is a branch of biology concerned with the study of genes, genetic variation, and heredity in organism.

CHROMOSOMES

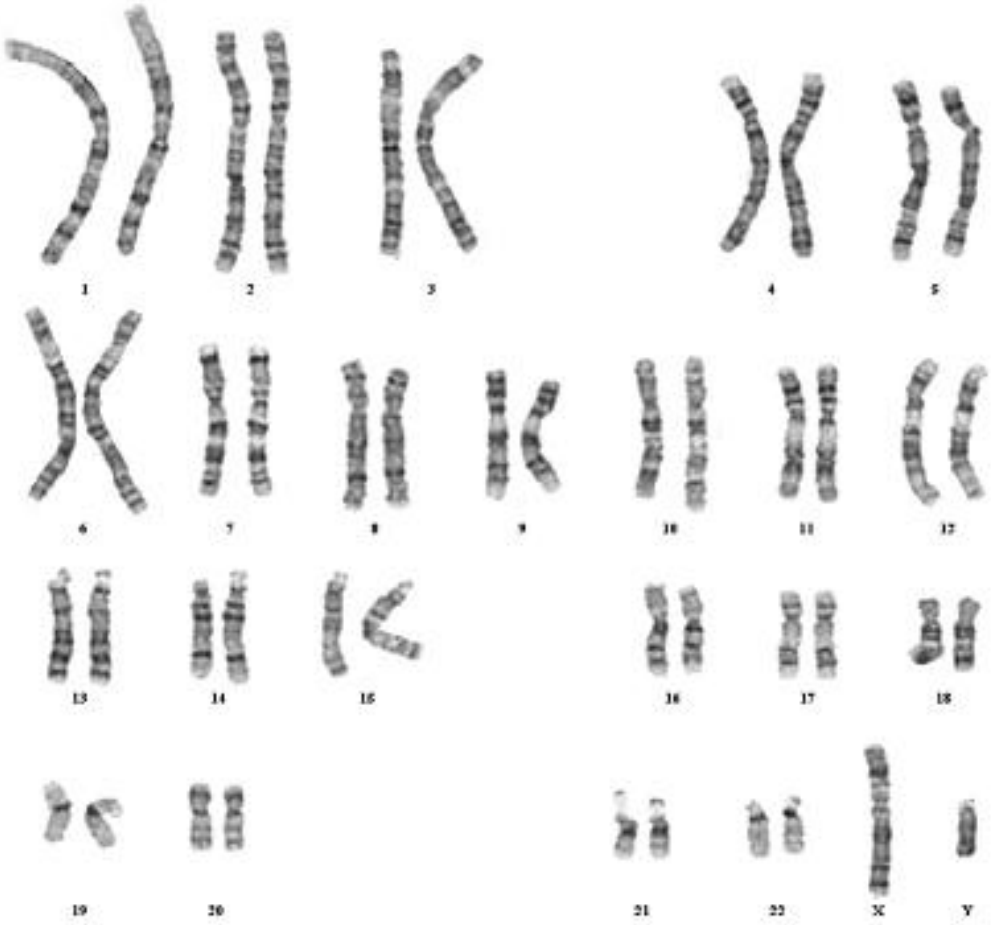


A chromosome is an organized package of DNA found in the nucleus of the cell.

Different organisms have different numbers of chromosomes.

HUMAN CHROMOSOMES

Human cells have 23 pairs of chromosomes (**22 pairs of autosomes and one pair of sex chromosomes**) for a total of 46 chromosomes per cell (Fig. 3.1).



Of the 46 chromosomes in a normal human somatic cell, 44 are autosomes and 2 are sex chromosomes.

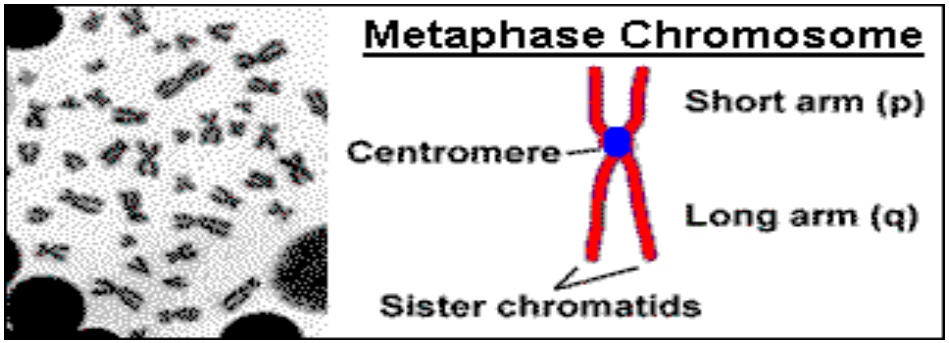
The autosomes are designated as pairs 1–22. The numbers are assigned in descending order of the length, size, and centromere position of each chromosome pair.

The sex chromosomes are noted by the letters X and Y. The female sex chromosome complement is XX and the male complement is XY.

Fig. 3.1 G-banded normal male karyotype illustrating the characteristic size, centromere position, and G-banding banding pattern for each human chromosome pair

Human Chromosome Nomenclature

Chromosomes are divided into long and short arms, separated by a centromere, or primary constriction.



A chromosome may be **metacentric**, with its centromere in the middle; **submetacentric**, with the centromere closer to one end of the chromosome; or **acrocentric**, in which the centromere is near one end of the chromosome and the short arm is essentially comprised of repetitive DNA that constitutes the satellites and nucleolar organizing regions.

Chromosomes 1 and 3 are examples of metacentric chromosomes, Chromosomes 4 and 5 are large submetacentric chromosomes, and Chromosomes 13–15 are considered medium sized acrocentric Chromosomes.

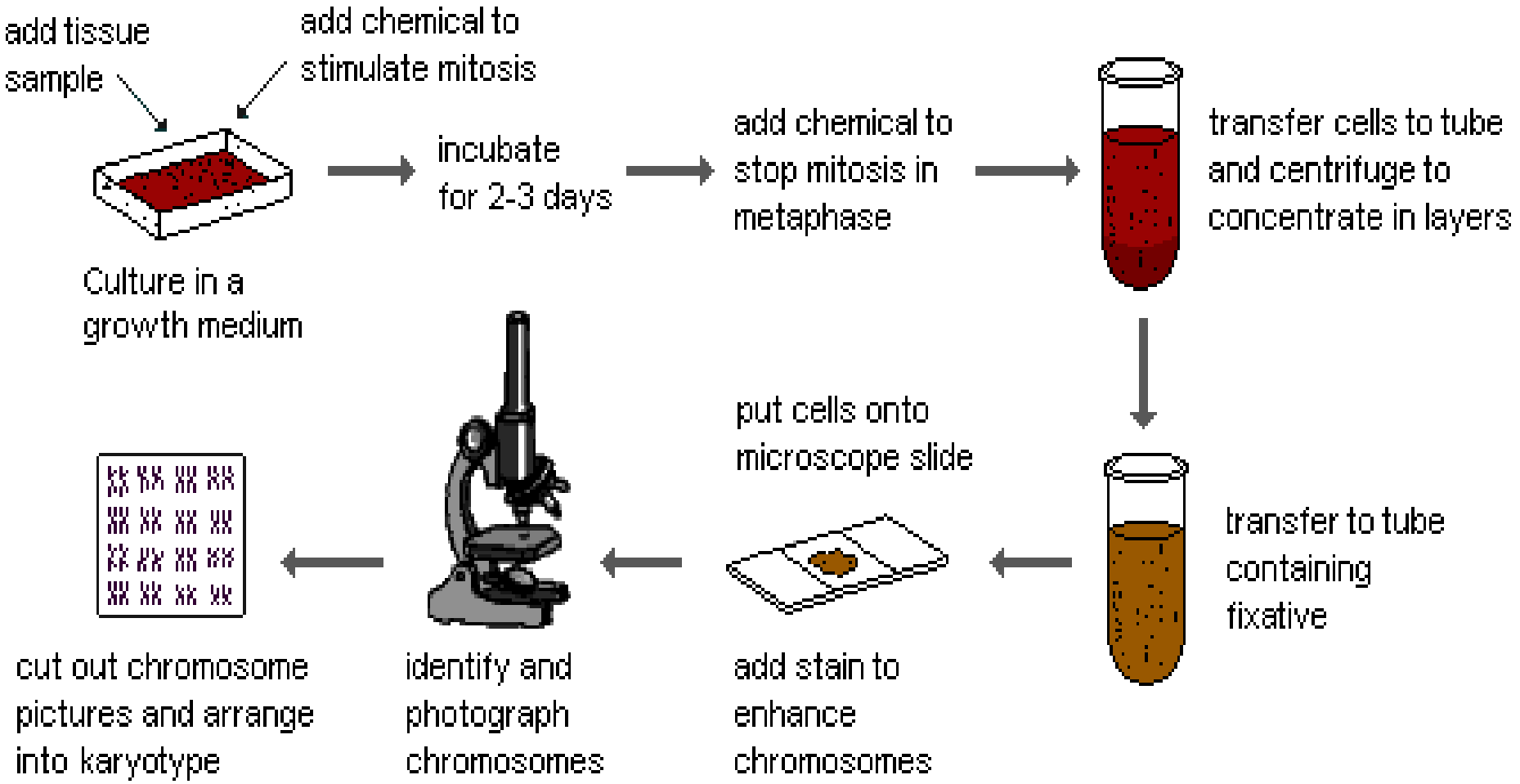
Karyotyping

Karyotyping is the process of preparing chromosomes for analysis.

The tissues used vary but the most common tissues sampled are:

Bone marrow, blood, amniotic fluid, cord blood, tumor, and tissues (including skin, umbilical cord, chorionic villi, liver, and many other organs).

Flow diagram of Karyotyping



Observations in Karyotyping

Six different characteristics of karyotypes are usually observed and compared

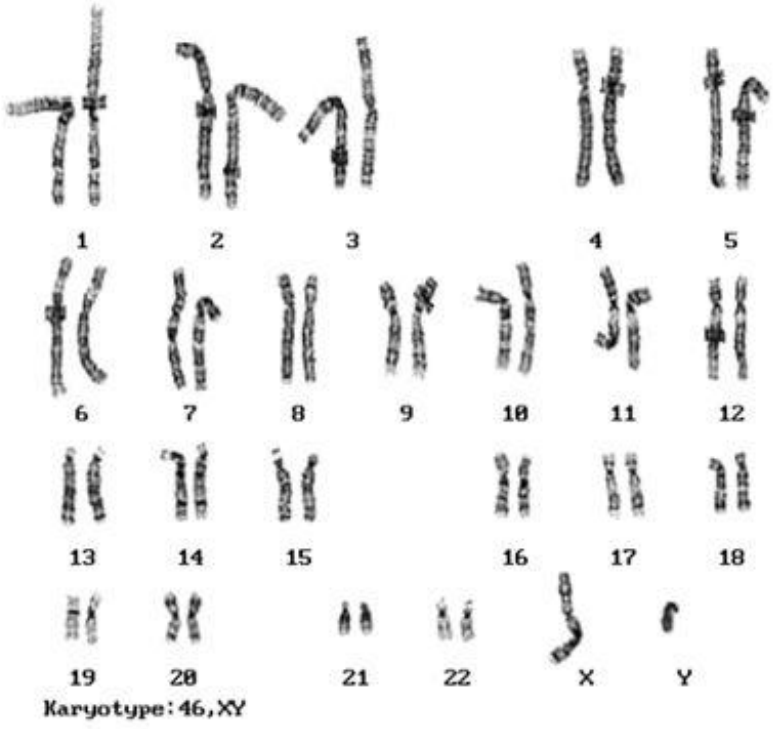
1. Differences in absolute sizes of chromosomes. Chromosomes can vary in absolute size by as much as twenty-fold between genera of the same family:
2. Differences in the position of centromeres.
3. Differences in relative size of chromosomes can only be caused by segmental interchange of unequal lengths.
4. Differences in basic number of chromosomes
5. Differences in number and position of satellites, which (when they occur) are small bodies attached to a chromosome by a thin thread.
6. Differences in degree and distribution of heterochromatic regions.

A full account of a karyotype may therefore include the number, type, shape and banding of the chromosomes, as well as other cytogenetic information.

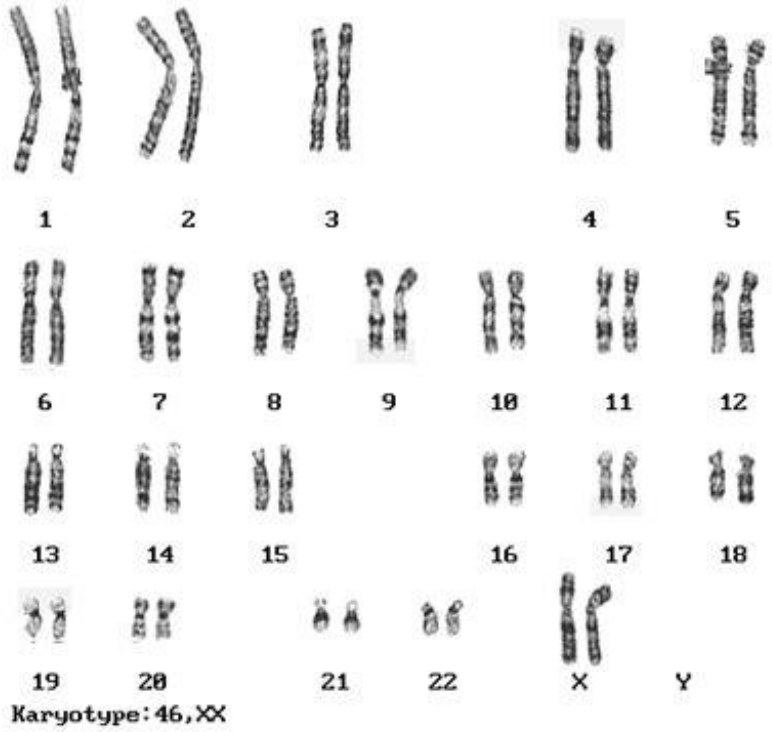
KARYOTYPE: EXAMPLE

After karyotyping- male or female?

NORMAL MALE

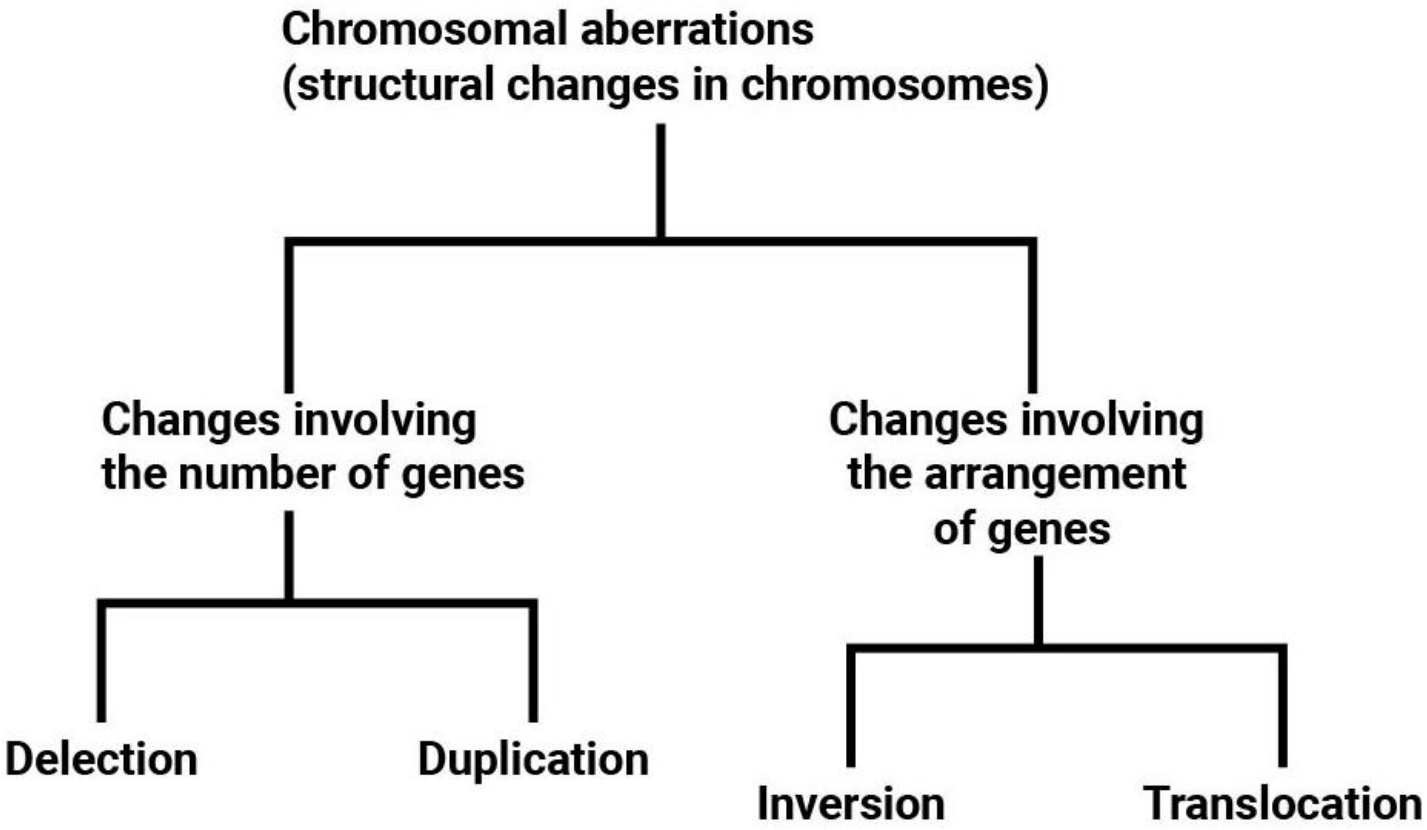


NORMAL FEMALE



Chromosomal analysis of cancer cells

Cancer arises as a result of genetic changes which consist of mutations together with numerical and structural chromosomal abberations.


Chromosomal aberrations
(structural changes in chromosomes)
Changes involving
the number of genes
Changes involving
the arrangement
of genes
Delection
Duplication
Inversion
Translocation

Chromosomal Aberrations

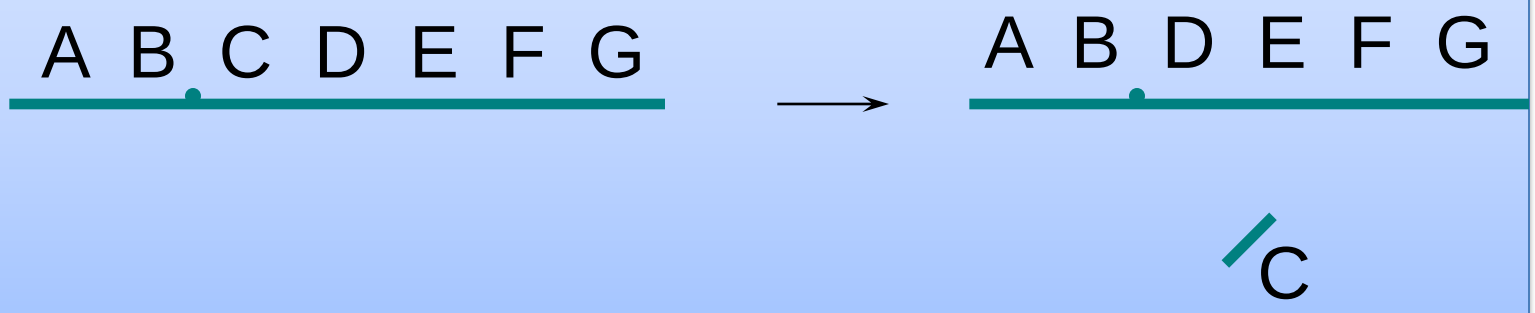
- **Changes in structure**
  - **Changes in the number of genes**
    - **deletions: genes missing**
    - **duplications: genes added**

Chromosomal Aberrations

- **Changes in structure**
  - **Changes in the location of genes**
    - **inversions: 180º rotation**
    - **translocations: exchange**
    - **transpositions: gene "hopping"**
    - **Robertsonian changes: fissions or fusions**

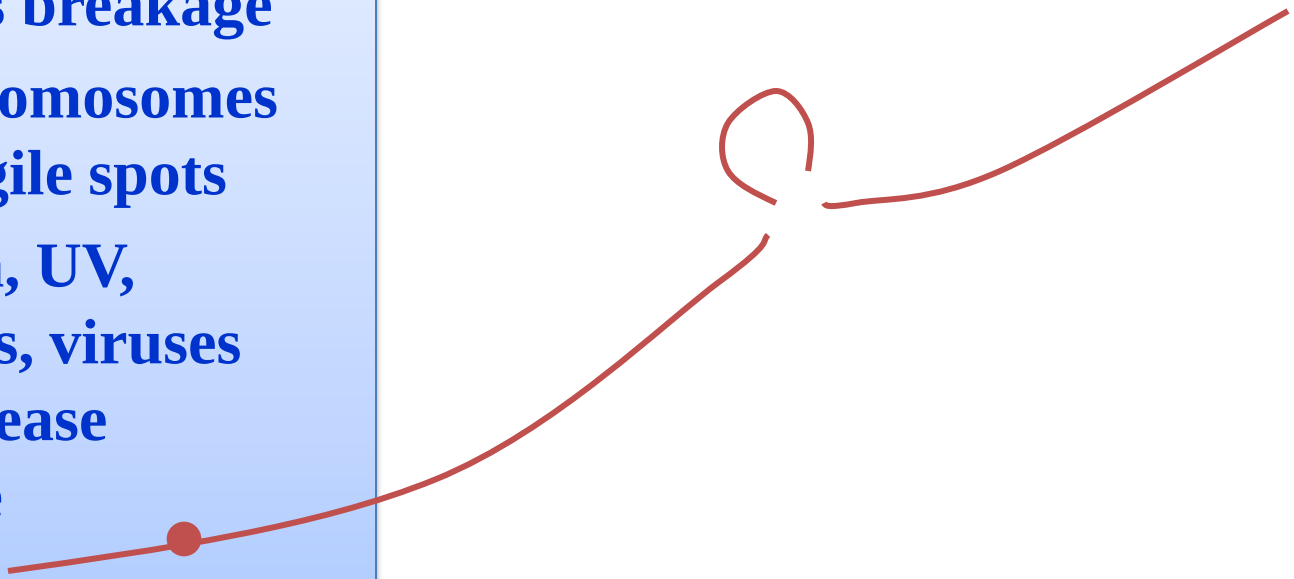
Deletions

Loss of a (generally small) segment of chromosome



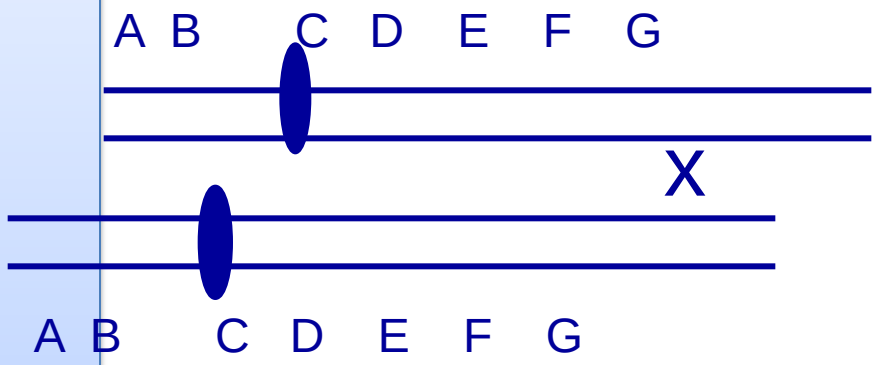
Deletions

- Arise through spontaneous breakage
  - some chromosomes have fragile spots
  - radiation, UV, chemicals, viruses may increase breakage



Deletions

May arise through unequal crossing over



Deletion

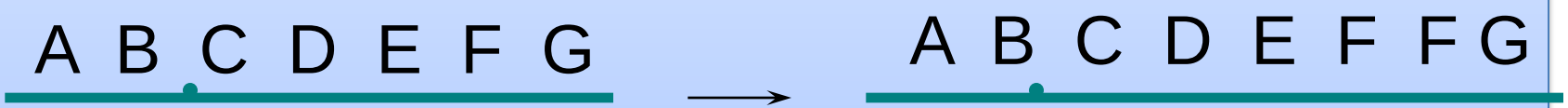
Duplication

Deletions

- **Large deletions will most probably be lethal**
- **Smaller deletions may allow survival**
  - ***E. coli*: deletions of up to 1% have been observed in living cells**
  - ***D. melanogaster* : deletions of up to 0.1% observed**

Duplications

Redundant segment of a chromosome



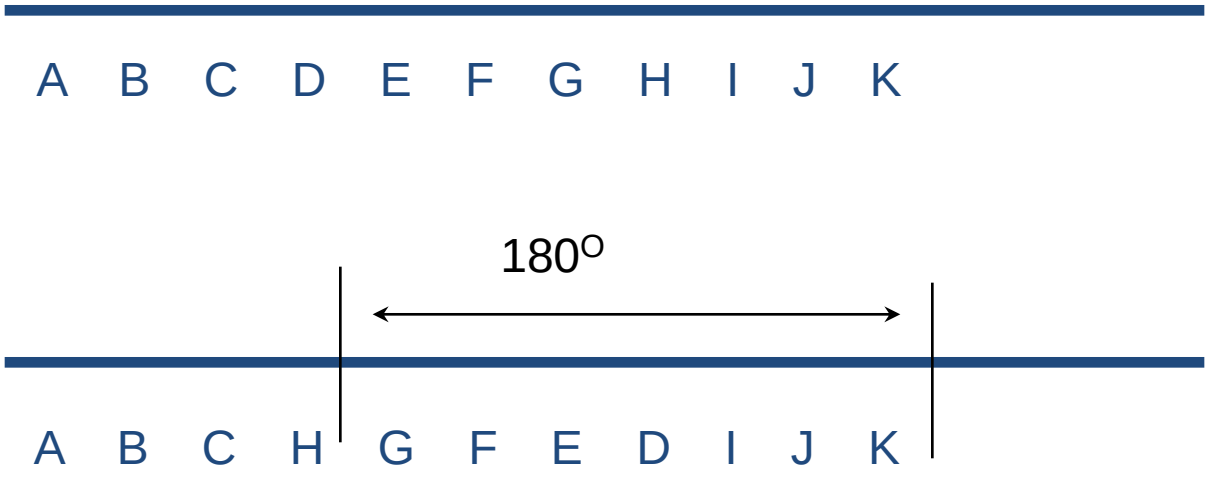
Changes in the Location of Genes

No gain or loss of information; just rearrangement of genome.

- Inversions
- Translocations
- Transpositions
- Robertsonian changes

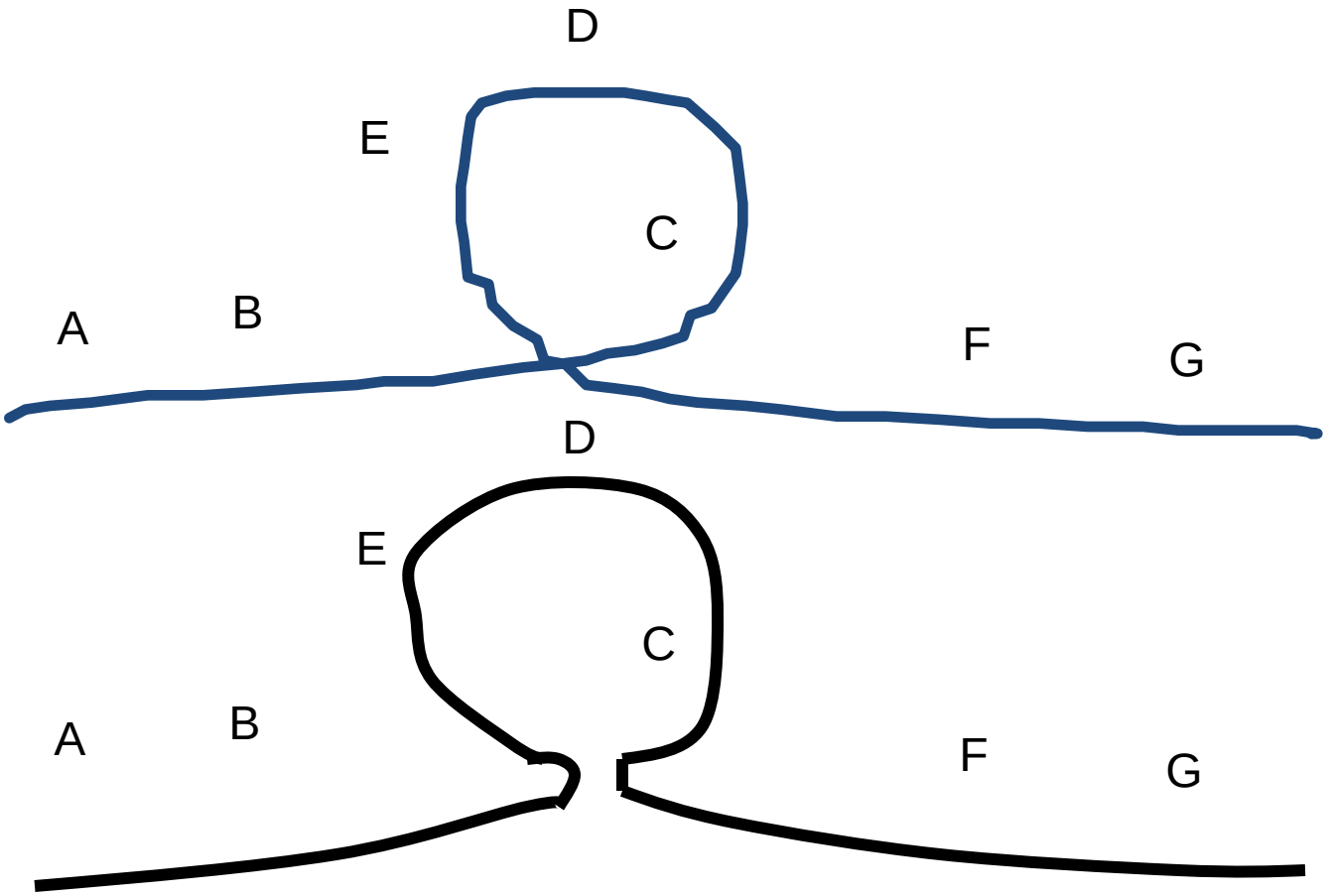
Inversions

180° reversal of chromosome segment



Inversions

Produced through breakage and reassociation of chromosome

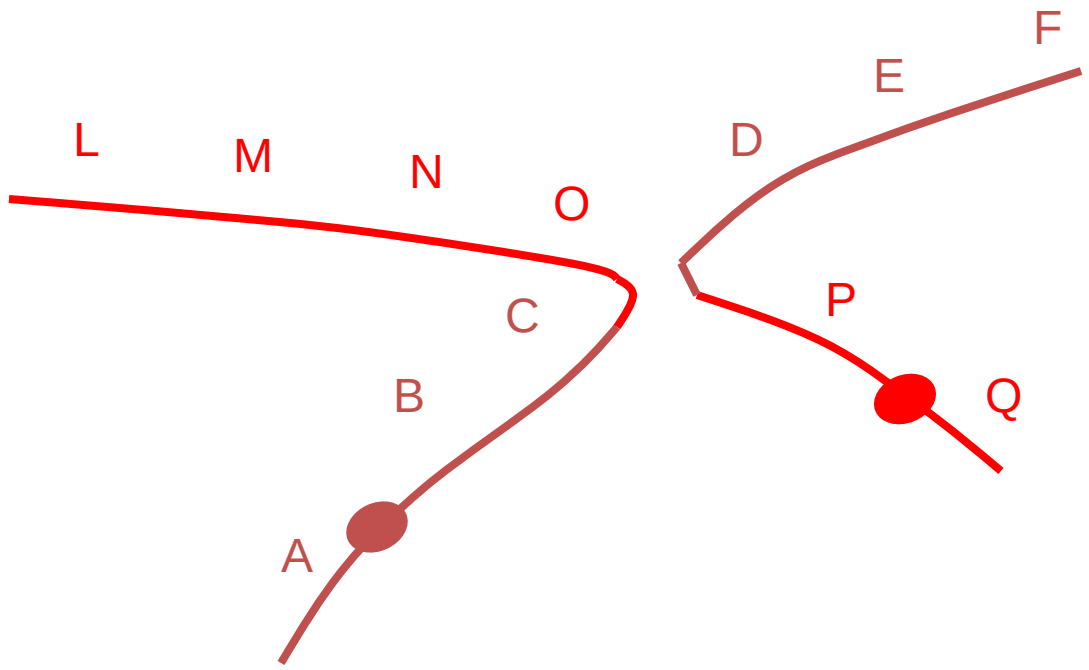


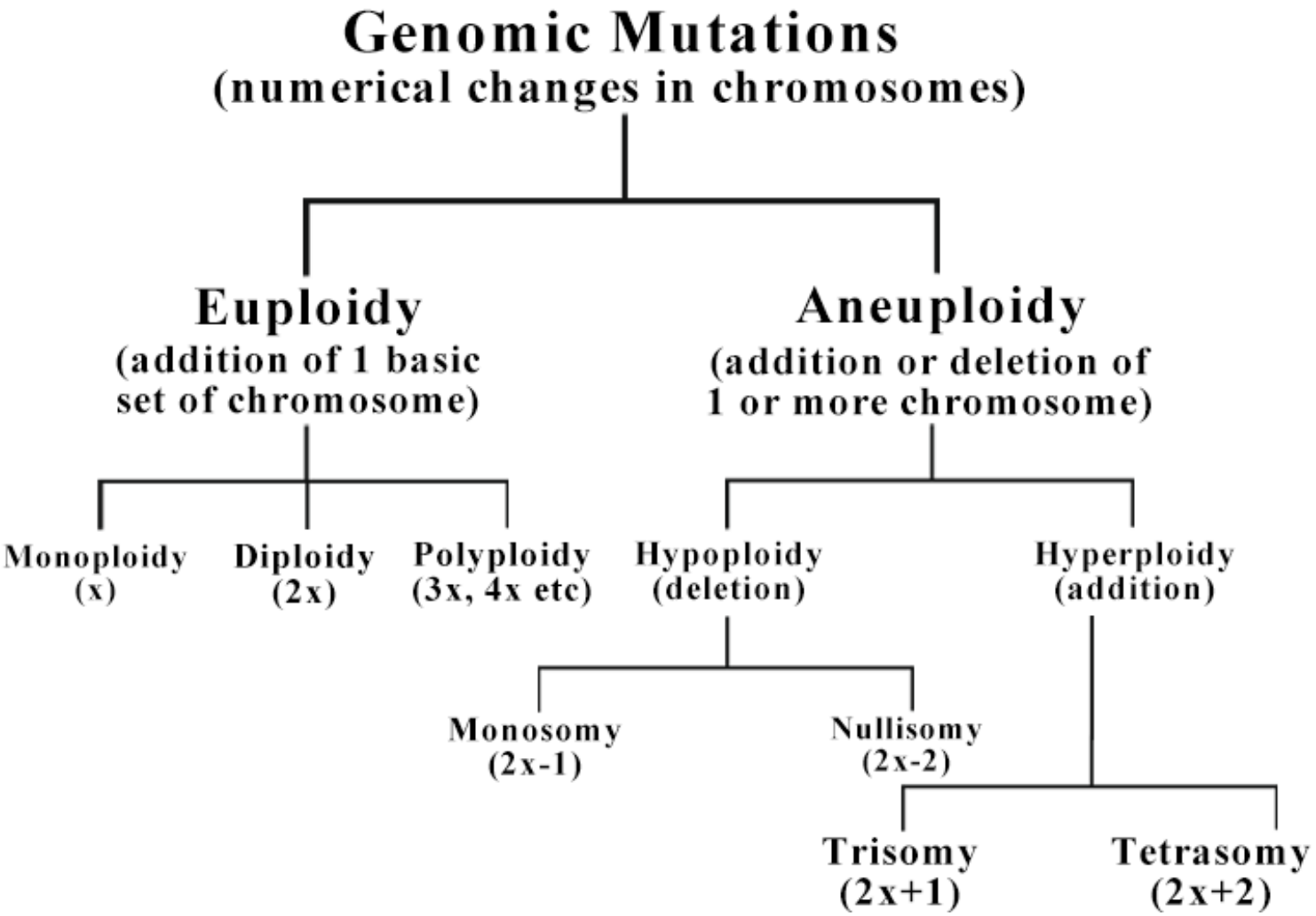
Inversions

- **May change phenotype through "position effects"**
  - **move active genes to sites generally inactive; lose gene function**
  - **move inactive genes to sites generally active; gain gene function**
- **May act to preserve blocks of genes (specific alleles) which function well together**

Translocations

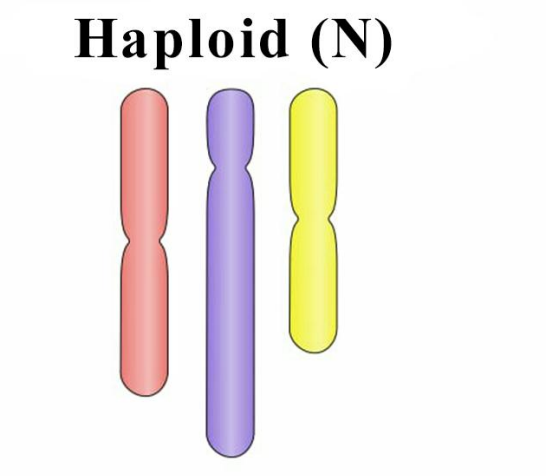
Exchange of segments between non-homologous chromosomes



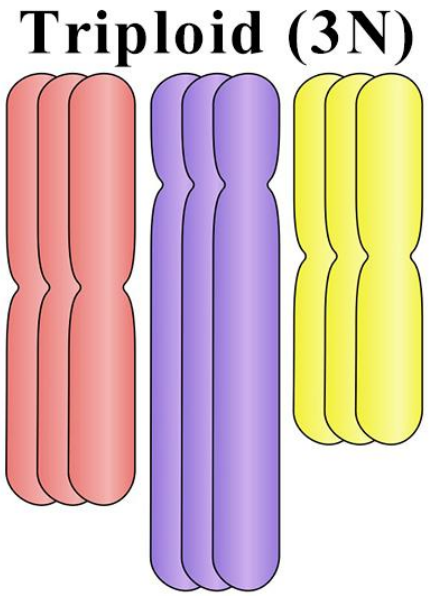

Genomic Mutations
(numerical changes in chromosomes)
Euploidy
(addition of 1 basic set of chromosome)
Aneuploidy
(addition or deletion of 1 or more chromosome)
Monoploidy (x)
Diploidy (2x)
Polyploidy (3x, 4x etc)
Hypoploidy (deletion)
Hyperploidy (addition)
Monosomy (2x-1)
Nullisomy (2x-2)
Trisomy (2x+1)
Tetrasomy (2x+2)

Euploidy

Euploidy is the addition or loss of one complete set of chromosomes



Haploidy involves the loss of one complete set of the chromosome from diploid parent thus offspring have just one set of chromosomes.



In polyploidy, more than two pairs of chromosomes are found.

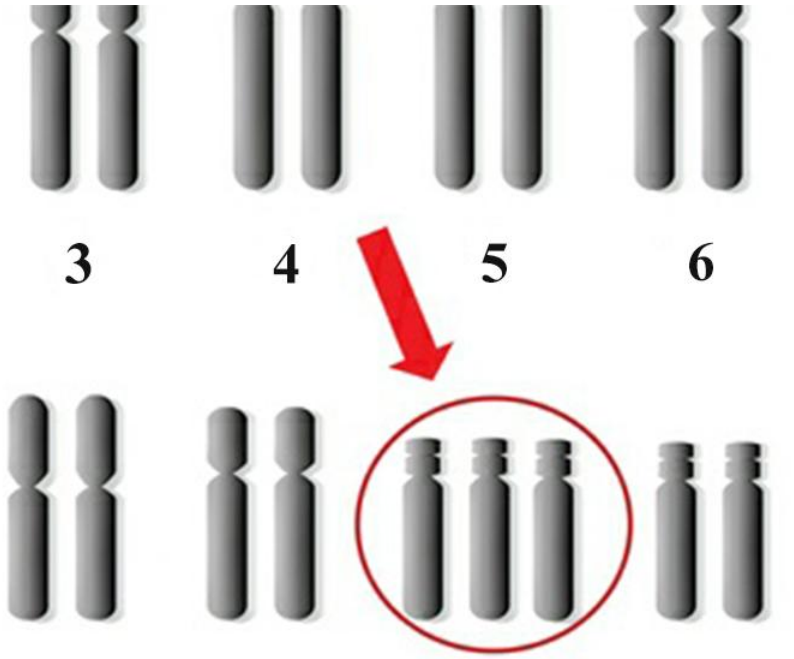
Aneuploidy

Aneuploidy is the loss of or addition of one or more chromosomes to a haloid genome. There are two types of Aneuploidy.

Hypoploidy is the loss of one or more chromosomes to the diploid genome.

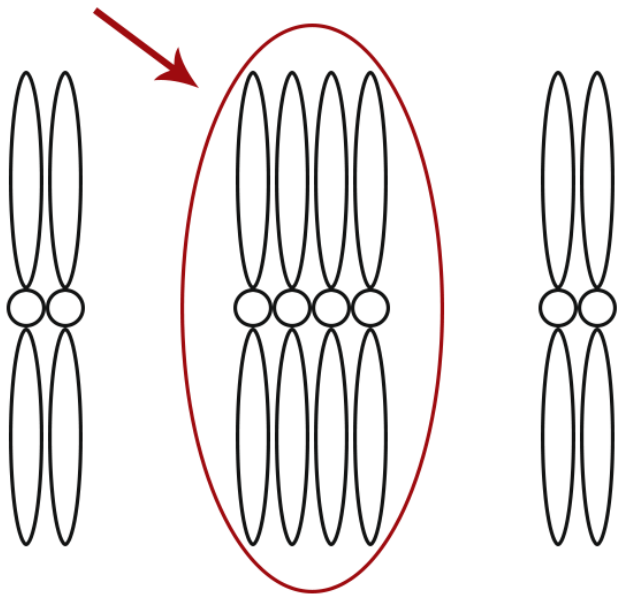
Hyperploidy is the addition of one or more chromosomes to the diploid genome.

Trisomy



Trisomy is the addition of one chromosome to the diploid genome. Hence, three copies of one chromosome are present.

Tetrasomy



Tetrasomy is the addition of a pair of the chromosome to the diploid genome. Hence, four copies of one chromosome are present.

Typical Female Karyotype

46 Total Chromosomes

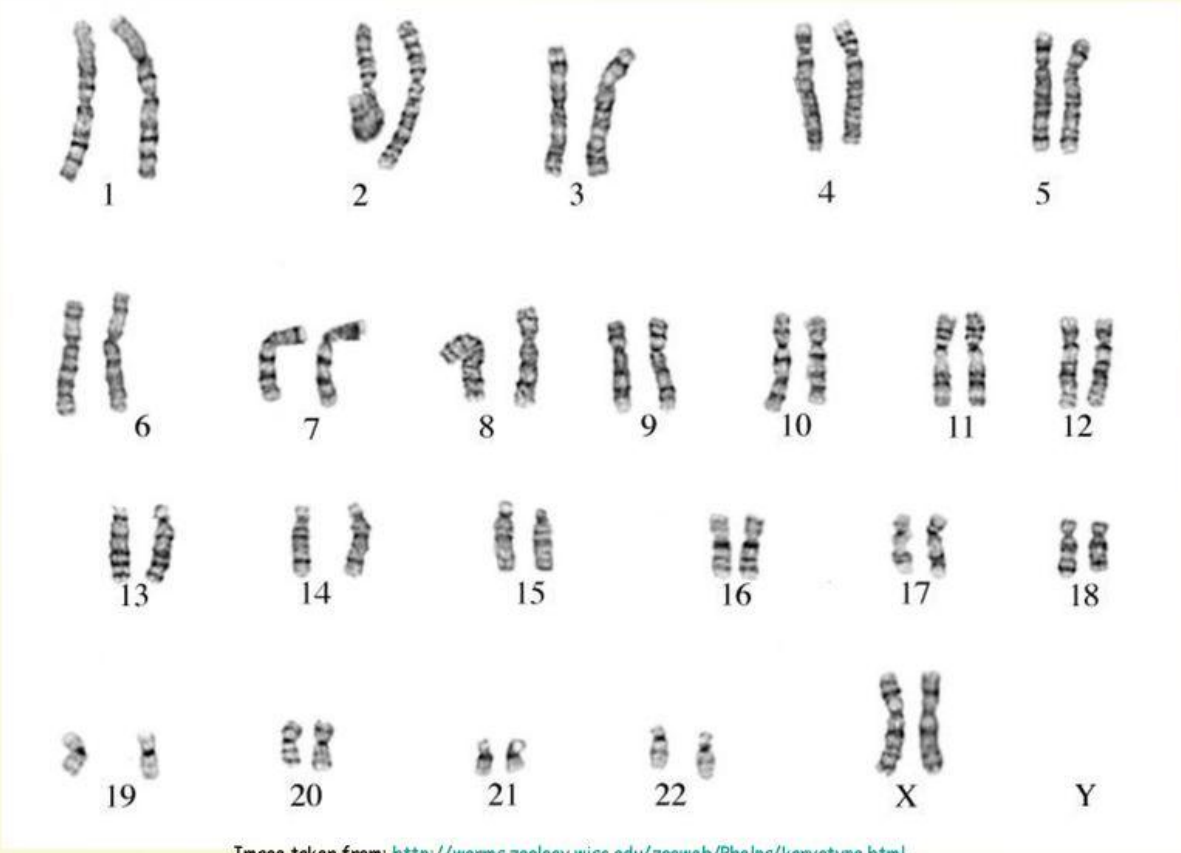


Image taken from: http://worms.zoology.wisc.edu/zooweb/Phelps/karyotype.html

Typical Male Karyotype

46 Total Chromosomes

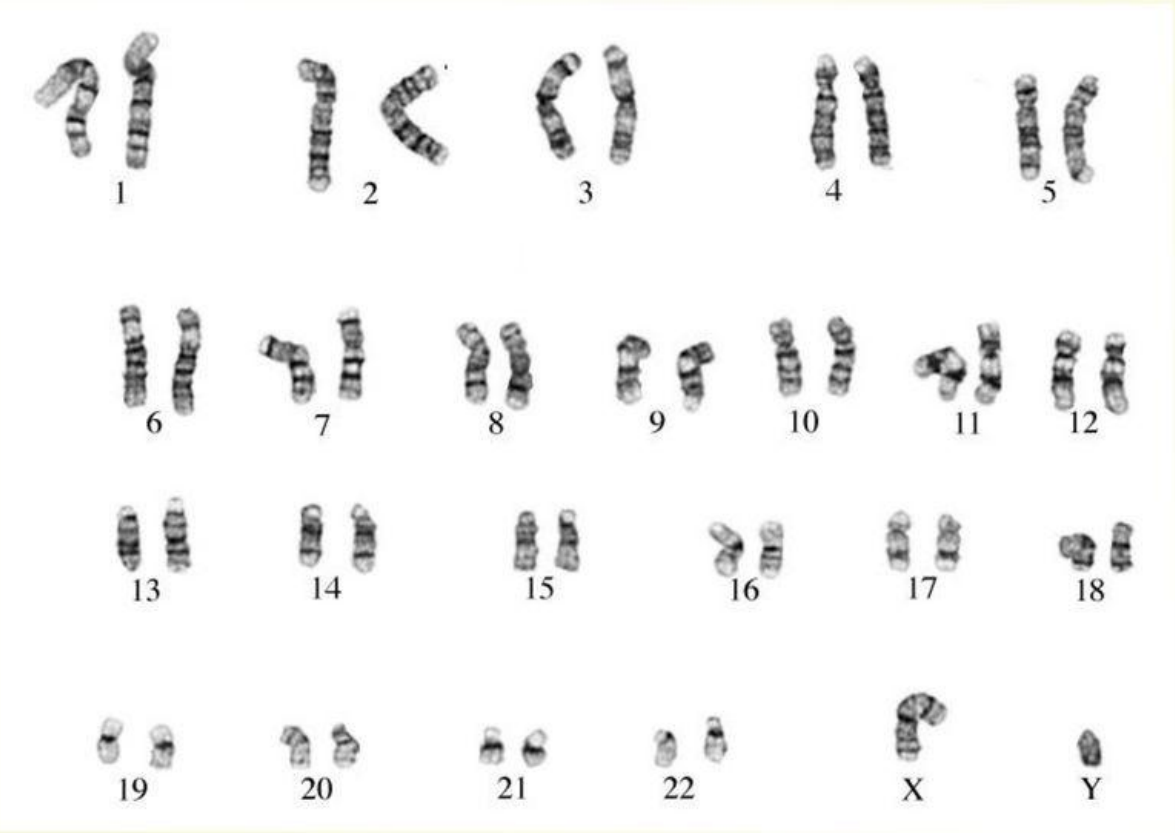
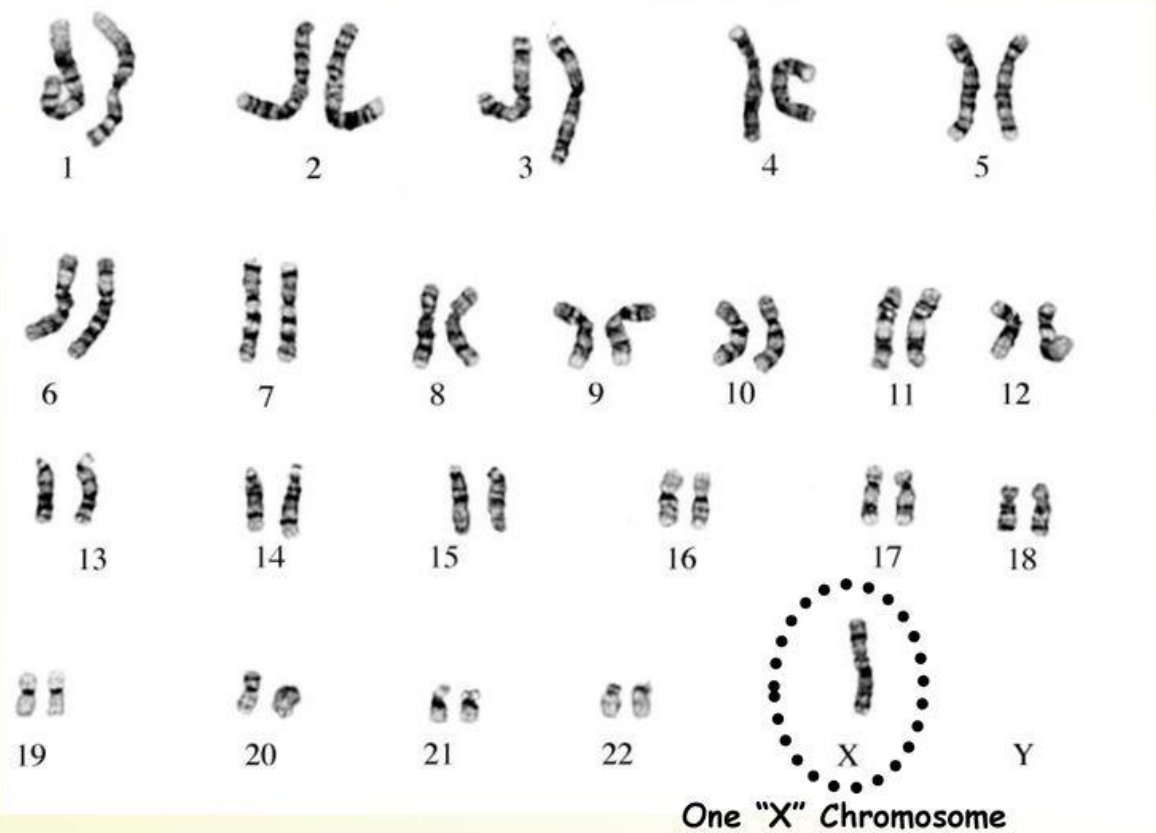


Image taken from: http://worms.zoology.wisc.edu/zooweb/Phelps/karyotype.html

Monosomy X - Turner Syndrome - Karyotype

45 Total Chromosomes

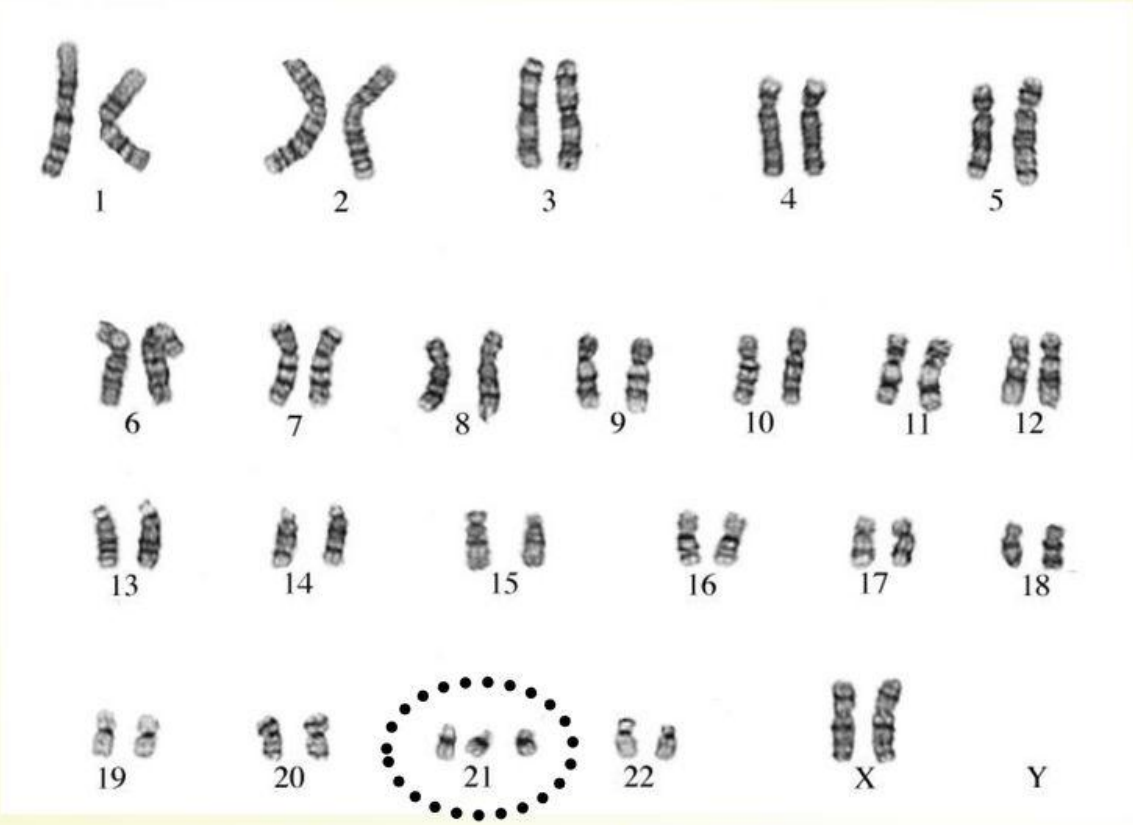


One "X" Chromosome

Image taken from: http://worms.zoology.wisc.edu/zooweb/Phelps/karyotype.html

Trisomy 21- Down's Syndrome - Karyotype

47 Total Chromosomes

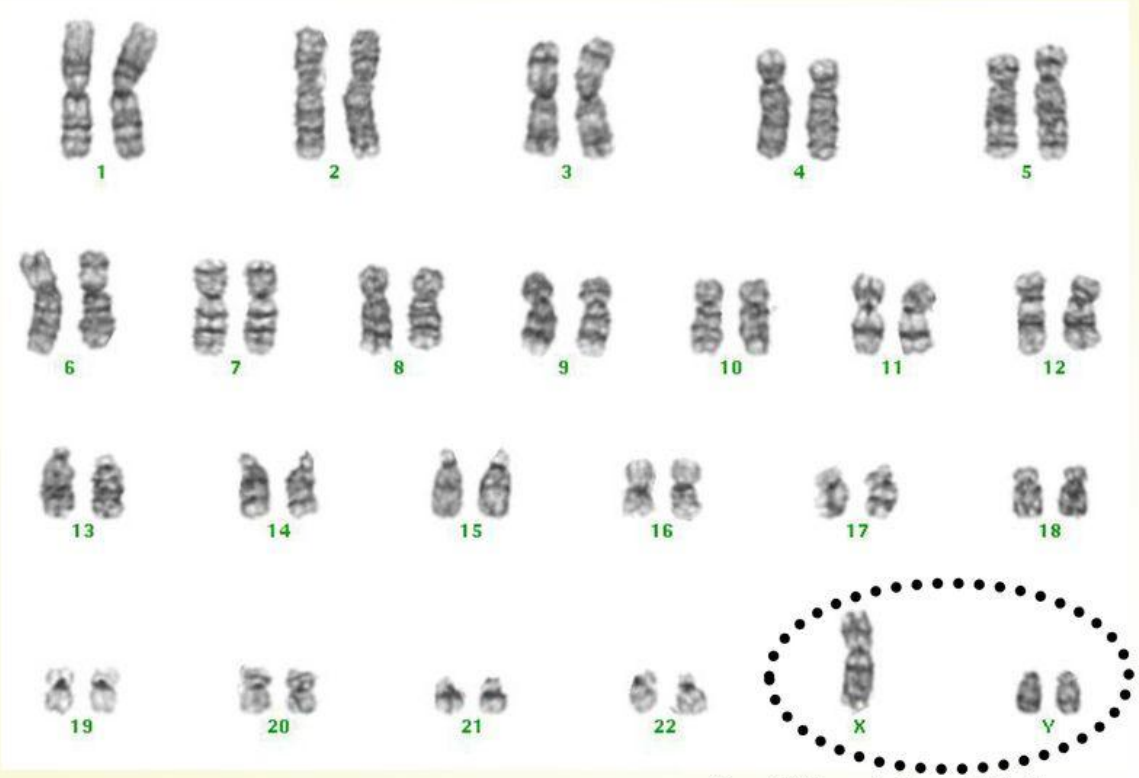


Three Chromosomes at the 21st Pair

Image taken from: http://worms.zoology.wisc.edu/zooweb/Phelps/karyotype.html

XYY Karyotype

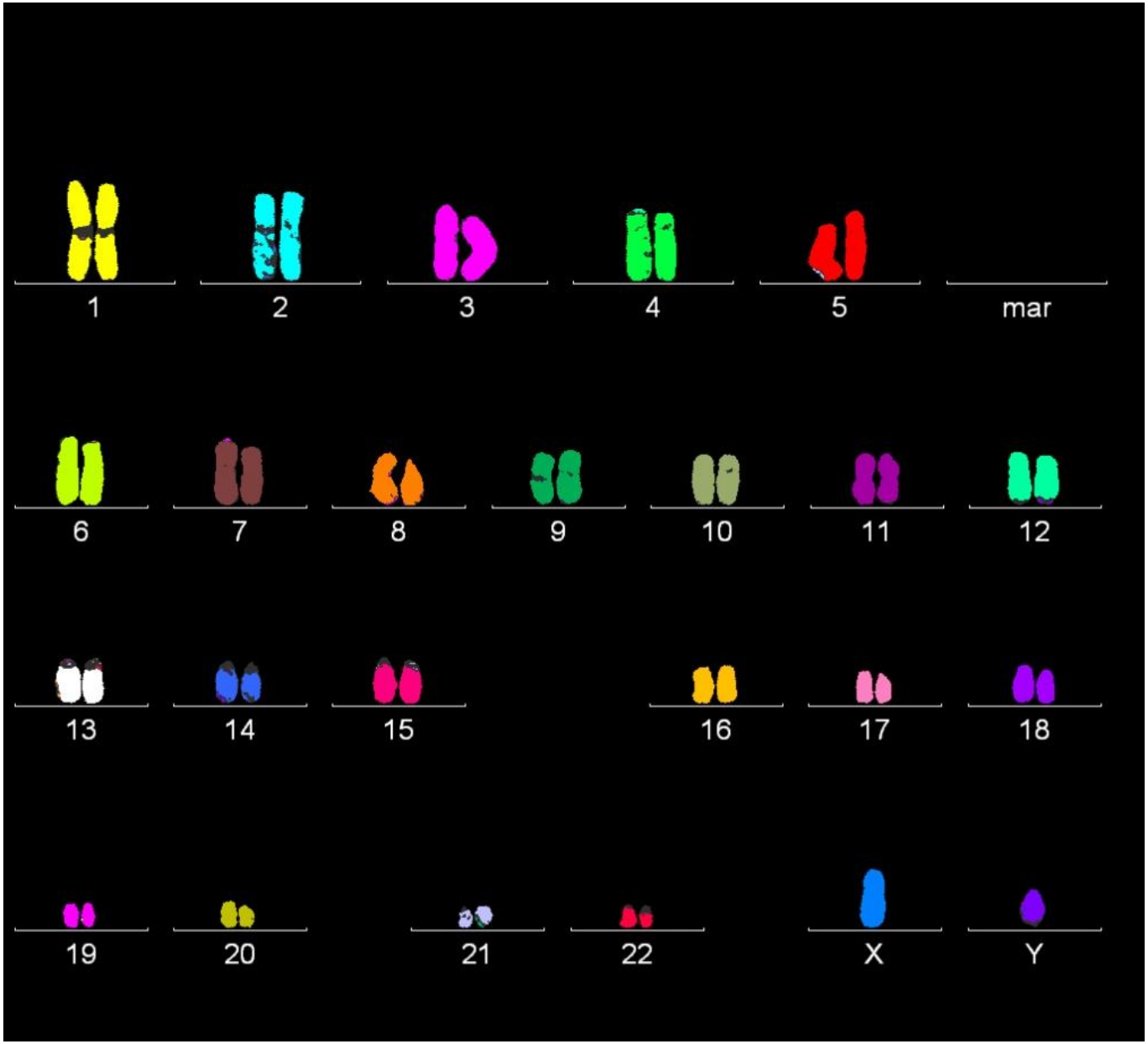
47 Total Chromosomes



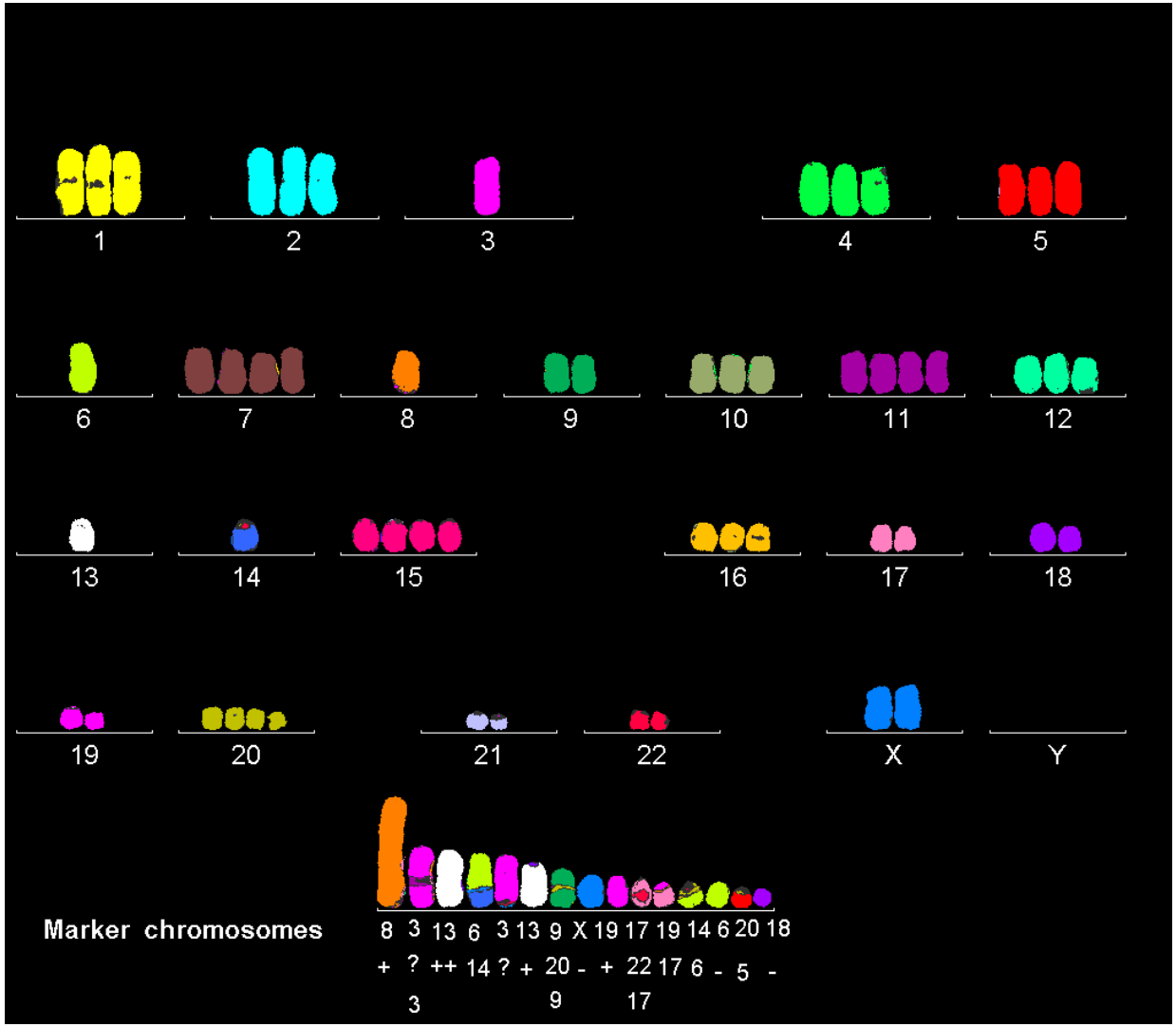
One "X" and Two "Y" Chromosomes

Image taken from: http://worms.zoology.wisc.edu/zooweb/Phelps/karyotype.html

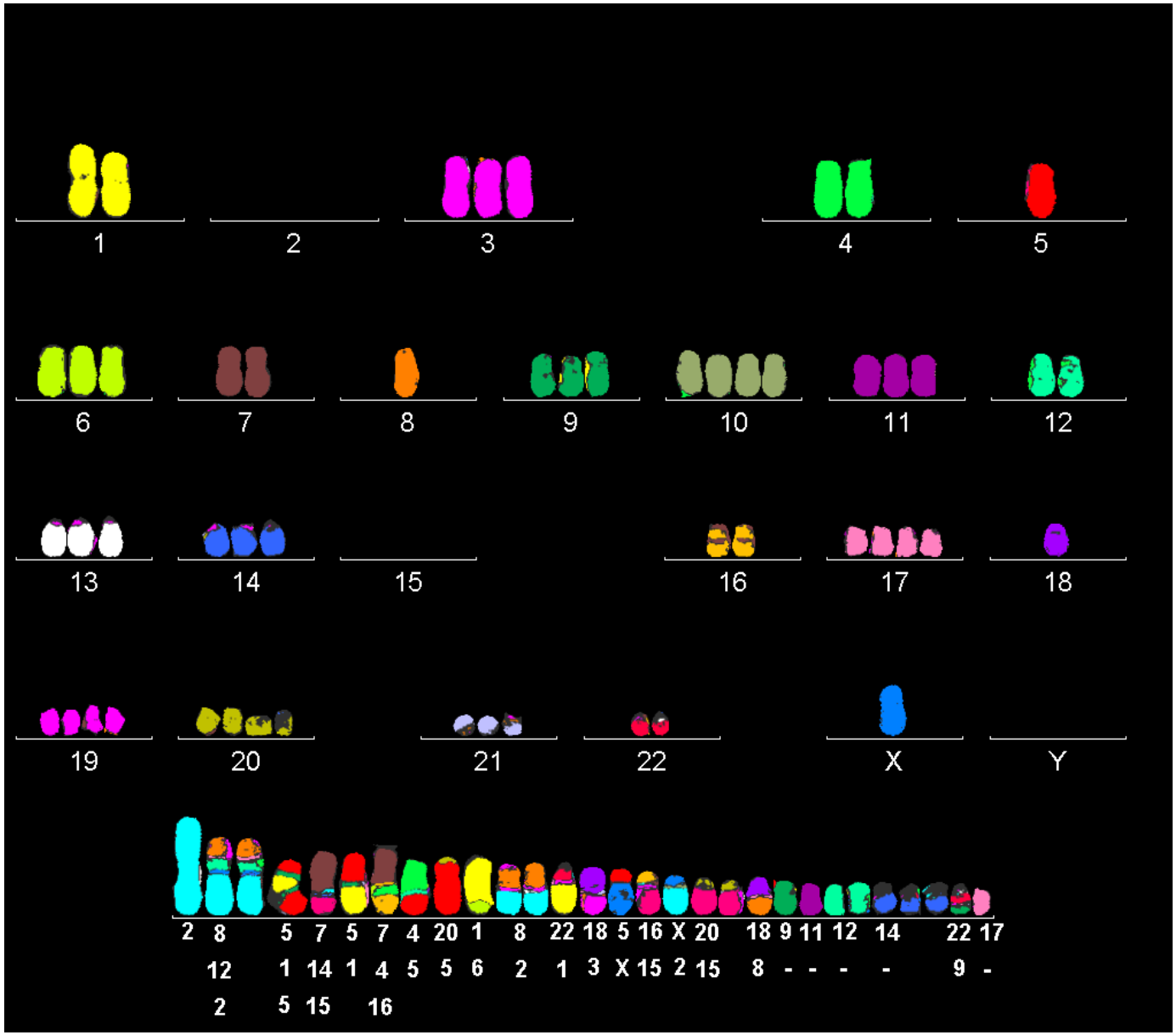
Karyotype of a diploid human cell (male)



Karyotype of a human colon cancer: Highly aneuploid, with 15 rearranged marker chromosomes



Karyotype of a breast cancer cell



MDA231-4

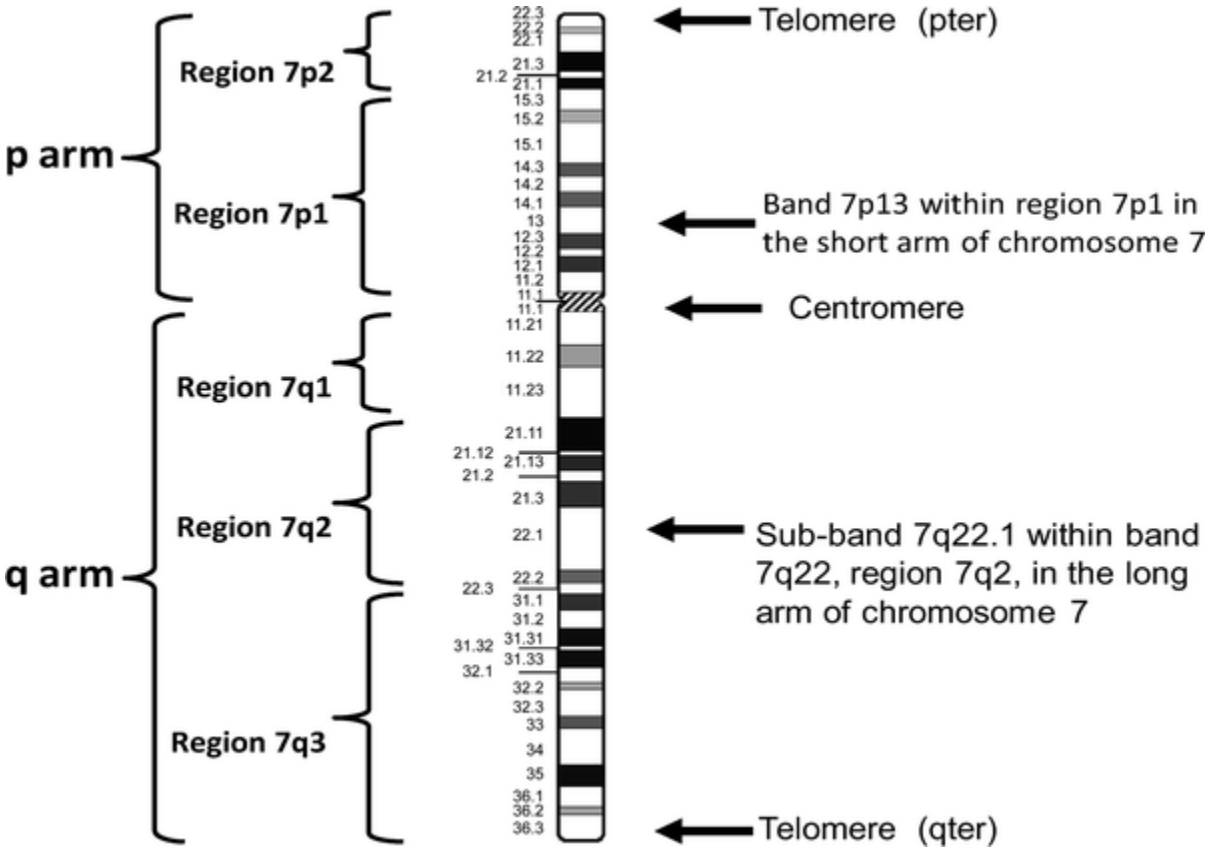
Human Chromosome Nomenclature

To effectively describe chromosomal changes in a systematic manner, a group of 17 forward-thinking investigators who had previously published human karyotypes teamed up in Denver, Colorado, in 1960 to create the foundation of the celebrated communication tool known today as ***An International System for Human Cytogenetic Nomenclature*** or **ISCN**. ISCN is an abbreviated symbolic writing method used to describe genetic changes by **copy number (dosage)** and **position (locus)**.

Situations where analysis is strongly recommended

Problems with early growth & development
Fertility problems
Neoplasia
Pregnancy in older women

Chorionic villus sampling (CVS) is a form of prenatal diagnosis to determine chromosomal or genetic disorders in the fetus. It entails getting a sample of the chorionic villus (placental tissue) and testing it. The advantage of CVS is that it can be carried out weeks after the last period, earlier than amniocentesis (which is carried out at weeks).


p arm
Region 7p2
Region 7p1
q arm
Region 7q1
Region 7q2
Region 7q3
Telomere (pter)
Band 7p13 within region 7p1 in the short arm of chromosome 7
Centromere
Sub-band 7q22.1 within band 7q22, region 7q2, in the long arm of chromosome 7
Telomere (qter)

Nomenclature for chromosomes and their abnormalities

TABLE 2–1 Nomenclature for chromosomes and their abnormalities.

Description	Meaning
–1	Loss of one chromosome 1
+7	Gain of extra chromosome 7
2q⁻ or del (2q)	Deletion of part of long arm of chromosome 2
4p*	Addition of material to short arm of chromosome 4
t(9;22)(q34;q11)	Reciprocal translocation between chromosomes 9 and 22 with break points at q34 on chromosome 9 and q11 on chromosome 22
iso(6p)	Isochromosome with both arms derived from the short arm of chromosome 6
inv(16)(p13q22)	Part of chromosome 16 between p13 and q22 is inverted

Nomenclature for chromosomes and their abnormalities

TABLE 2–2 Common chromosomal abnormalities in lymphoid and myeloid malignancies.

Malignancy	Chromosomal Aberration*	Molecular Lesion
Acute myeloid leukemia (AML)		
M1, M2 subtypes	t(8;21)(q22;q22)	*AML1-MTG8* fusion
M3 subtype	t(15;17)(q22;q11.2)	*PML-RARA* fusion
M4Eo subtype	inv(16)(p13;q22) or t(16;16)(p13;q22)	*MYH11-CBFB* fusion
M2 or M4 subtypes	t(6;9)(p23;q24)	*DEK-CAN* fusion
Therapy-related AML	~5/del(5q), ~7/del(7q)	
Chronic myeloid leukemia (CML)	t(9;22)(q34;q11) (Ph^1 chromosome)	*BCR-ABL* fusion encoding p210 protein
CML blast crisis	t(9;22)(q34;q11), 8, $+Ph^1$, 19, or i(17q)	*BCR-ABL* fusion encoding p210 protein, *TP53* mutation
Acute lymphocytic leukemia (ALL)	t(9;22)(q34;q11)	*BCR-ABL* fusion encoding p190 protein
Pre-B ALL	t(1;19)(q23;p13.3)	*E2A-PBX1* fusion
Pre-B ALL	t(17;19)(q22;p13.3)	*E2A-HLF* fusion
B-ALL, Burkitt lymphoma	t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)	Translocations between *myc* and *IgH*, *IgL*κ and *IgL*λ loci
B-Chronic lymphocytic leukemia	+12,t(14q32)	Translocations of *IgH* locus

*For an interpretation of the nomenclature of chromosomal rearrangements, see Table 2–1.

Cancer Risk Factors

Risk Factors of Cancer

A risk factor is anything that increases a person's chance of getting a disease.
Some risk factors can be changed, and others cannot.

Different cancers have different risk factors

Risk factors

Old age

- Unhealthy lifestyle (Western lifestyle), Poor diet, lack of --physical activity, or being overweight.
- Environmental factors, defined broadly to include tobacco use, diet, sunlight and infectious diseases.
- Occupational carcinogens
- Radiation
- Family history of cancer (Genetic susceptibility)
- Alcohol
- Chemicals and other substance

Risk factors: Cancer Type

Risk Factor/ Cancer Type

Cancer Type	Risk Factor
Lung Cancer	Tobacco smoke Radon Asbestos and other substances Air pollution
Breast	Radiation Genetic changes (Inherited mutation)
Colorectal	Cancer polyp Genetic alteration Diet Cigarette smoking Ulcerative colitis or chon's disease
Prostate	Diet Certain prostate changes Race Africans Americans

Risk factors: Cancer Type

Risk Factor/ Cancer Type

Cancer Type	Risk Factor
Liver	Hepatitis viruses (HCV.HBV)
Pancreas	Smoking Diabetes Being male Chronic pancreatitis
Kidney	Tobacco smoking High blood pressure Von-Hippel-Lindau syndrome (VHL)
Leukemia	Radiation Chemotherapy Certain disease (Down syndrome) Human T cell leukemia virus Myelodysplatic syndrome

Risk factors: Cancer Type

Risk Factor/ Cancer Type

Cancer Type	Risk Factor
Bladder	Occupation Certain infection Tobacco smoking Race Twice as often as Africans Americans Treatment with cyclophosphamide or arsenic
Uterine	Endometrial hyperplasia Race Africans Americans Hormonal replacement therapy Obesity
Melanoma	Dysplastic nevi Fai skin Weakened immune system Sever blistering/Sunburn UV irradiation
	Source:http://ishwaryatechnosolutions.com/cancer.aspx

Avoid Carcinogens at Work

Some Carcinogens in the Workplace

Carcinogen	Occupation	Type of Cancer
Arsenic	Mining, pesticide workers	Lung, skin, liver
Asbestos	Construction workers	Lung, mesothelioma
Benzene	Petroleum, rubber, chemical workers	Leukemia
Chromium	Metal workers, electroplaters	Lung
Leather dust	Shoe manufacturing	Nasal, bladder
Naphthylamine	Chemical, dye, rubber workers	Bladder
Radon	Underground mining	Lung
Soots, tars, oils	Coal, gas, petroleum workers	Lung, skin, liver
Vinyl chloride	Rubber workers, polyvinyl chloride manufacturing	Liver
Wood dust	Furniture manufacturing	Nasal

Cytogenetics

Cytogenetics

Cytogenetics is a branch of pathology and genetics concerned with the study of normal chromosomes and chromosome aberrations.

Classical cytogenetics allows microscopic visualization of whole chromosomes in order to assess their number and structure.

Molecular cytogenetics uses specialized techniques such as fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (aCGH) to evaluate submicroscopic chromosomal regions.

Both classical and molecular cytogenetic techniques are used to investigate constitutional and acquired chromosome abnormalities. Most recently, next-generation sequencing has been added to the cytogenetic lab arsenal, to allow exact identification of breakpoints in chromosome rearrangements.

Cytogenetics of Solid tumors

The study of chromosome abnormalities in solid tumours provides valuable information for the diagnosis and prognostic stratification of a variety of tumour subtypes.

Technical challenges are encountered in tissue culture, harvesting and finally the interpretation of complex chromosome abnormalities. Molecular cytogenetic studies complement karyotype analysis, but the ability to assess all chromosome abnormalities simultaneously underscores the value of conventional cytogenetic analysis. This chapter provides detailed methods for solid tumour cytogenetics from tissue culture to banding

Cytogenetics of Solid Tumors

The Karyotypic abnormalities in solid tumors are often quite complex. They constitute less than one-third of all cases with an abnormal karyotype reported in the literature. However there are cytogenetic aberrations especially balanced translocations which occur with remarkable specificity in distinct tumor sub types. Balanced simple disease specific changes are seen in about 20% of mesenchymal tumors and less than 5% of epithelial neoplasms. Specific translocations and gene amplifications are used as markers for diagnosis and prognosis in mesenchymal and epithelial neoplasms.

INDICATIONS:
Chromosomal analysis can be performed on all tumor specimens (benign and malignant).

List of Neoplasms (Cytogenetic changes are specific and consistent in certain tumor types)

Mesenchymal Neoplasms
Bone and Soft Tissue Neoplasms

Bone and Cartilage (eg., Osteosarcoma, Chondrosarcoma, enchondroma, Fibrous dysplasia)
Fibrous Tissue (eg., Fibrosarcoma, Fibromatosis, Fibroma)
Adipose tissue (eg., Lipoma, Liposarcoma)
Muscle and Myofibroblasts (eg.,Leiomyoma, Leiomyosarcoma, Rhabdomyosarcoma, Myofibroblastic tumors)
Vascular and Perivascular (eg.Hemangioma, Lymphangioma, Angiosarcoma, Hemangioendothelioma, Hemangiopericytoma)
Nerve Elements (eg.Neurofibroma, Schwannoma, Malignant peripheral Nerve Sheath Tumors)

Epithelial Neoplasms

Head and Neck (Squamous cell Carcinoma, Sinonasal undifferentiated carcinoma, Salivary Gland Neoplasms, Adenocarcinoma)
Lung , Pleura and Thymus (Squamous Cell Carcinoma, Adenocarcinoma, Small cell Carcinoma, Carcinoid, Mesothelioma, Thymoma, Thymic Carcinoma)

Digestive System –

Esophagus, Stomach, Small Intestine, Appendix, Colon, Rectum, Liver, Gall Bladder, Exocrine Pancreas (Squamous Cell carcinoma, Adenocarcinoma, Adenoma, Carcinoid, Heatocellular carcinoma, Hepatoblastoma, Cholangiocarcinoma)

Urinary System –

Kidney, Bladder(Renal Cell Carcinoma, Urothelial Carcinoma)
Male Genital Organs - Testis, Penis, Prostate (Germ Cell Tumors, Squamous Cell carcinoma, Prostatic adenocarcinoma)

Breast

Ductal adenocarcinoma, lobular adenocarcinoma

Female Genital Organs

Ovary, Uterus, Cervix, Fallopian Tube, Vagina, Vulva- (Epithelial ovarian tumors, Adenocarcinoma, Squamous cell Carcinoma)

Endocrine System

Pituitary Tumors
Thyroid (Papillary Carcinoma, Follicular Carcinoma, Medullary Carcinoma)
Parathyroid (Adenoma, Carcinoma)
Adrenal Gland (Adrenal Cortical adenoma,adrenal Cortical Carcinoma, Pheochromocytoma, Neuroblastoma)
Endocrine Pancreas (Islet Cell tumors)
Paraganglia (Paraganglioma)

Central Nervous System Tumors

- Astrocytic (Astrocytoma)
- Oligidendroglial (Oligodendroglioma)
- Ependymal (Ependymoma)
- Choroid Plexus Tumors
- Neuroepithelial Tumors
- Neuronal (Ganglioglioma, Gangliocytoma)
- Pineal (Pinealoblastoma, Pieocytoma)
- Embryonal (Medulloblastoma)
- Menigneal (Meningioma)
- Peripheral neuroblastic Tumors (Olfactory neuroblastoma)
- Retinoblastoma

SAMPLE REQUIREMENTS:

The excision of a tumor mass is performed surgically. A pathologist routinely examines the tissue. The size of the sample will vary depending on the size of the actual tumor.

SPECIMEN HANDLING:

Place tissue in a sterile container or a centrifuge tube with tissue culture media (3X) provided by the laboratory. Containers must be tightly capped to prevent leakage. If media is not available, then place in sterile gauze moistened with water and deliver immediately. DO NOT SUBMERGE THE SPECIMEN IN SALINE, WATER OR FORMALIN. Tumors in media may be stored in refrigerator for up to three days. Specimens that are dry, frozen, irradiated, in saline and formaldehyde or stored for more than three days may not grow. Tissues not collected sterilely may get contaminated. A tissue culture charge for handling these specimens will result.

Call the laboratory for a pick up or mail via Federal Express overnight. In case of late or weekend collection, store specimen in refrigerator. Do not freeze. Tissue not in 3X media must be delivered immediately. Call the laboratory for pick up at the earliest convenience.

PROCEDURE:

The tissue is processed for suspension and in situ cultures. Loose cells are released from the tissue by gently tapping with a scalpel. The cell suspension is set up in culture in media without the mitotic stimulant phytohemaglutinin (PHA) and incubated for 24 hours at 37°C. Ethidium bromide is used simultaneously with colcemid to improve chromosome morphology. Harvest is performed by routine methods.

The tissue is digested enzymatically to dissociate into a single cell suspension. The cells are plated onto several dishes according to cell density and viability. Cultures are maintained at 37°C until ready to harvest. Colcemid is added to initiate harvest. Harvest is performed by routine methods.

INTERPRETATION:

Twenty metaphase cells are analyzed under the microscope whenever possible. Two karyotypes are prepared from the mainline, and one from each sideline.

RESULTS:

Results are presented according to the International System for Human Cytogenetic Nomenclature (ISCN). Full explanation of the karyotype is provided and a short summary is written for each abnormal result.

Cytogenetics of Hematologic malignancies

Cytogenetics of Hematologic malignancies

Hematologic malignancies are **cancers that affect the blood, bone marrow, and lymph nodes**. This classification includes various types of leukemia

- acute lymphocytic (ALL)
- chronic lymphocytic (CLL)
- acute myeloid (AML)
- chronic myeloid (CML))
- myeloma, and lymphoma (Hodgkin's and non-Hodgkin's (NHL)).

Recurrent chromosomal abnormalities in Hematological malignancies

CML t(9;22)

AML t(8;21), t(15;17), inv(16), t(9;11), inv(3), t(6;9), t(1;22), -5/5q-, -7/7q-

ALL t(4;11), t(1;19), t(v;11q23), t(12;21)

MDS -5/del(5q), del(20q); -7/del(7q), +8

Lymphoma

DLBCL t(3q27)
Burkitt t(8;14) and variant
Follicular t(14;18)
Mantle cell t(11;14)
Marginal zone t(11;18), t(1;14), t(14;18)

Molecular Analysis (methodology)

- Restriction Enzymes and Manipulation of Genes
- Blotting Techniques
- The Polymerase Chain Reaction
- Fluorescence in Situ Hybridization
- Comparative Genomic Hybridization
- Spectral Karyotyping/Multifluor Fluorescence in Situ Hybridization
- Single-Nucleotide Polymorphisms
- Sequencing of DNA
- Variation in Copy Number and Gene Sequence
- Microarrays and RNA Analysis

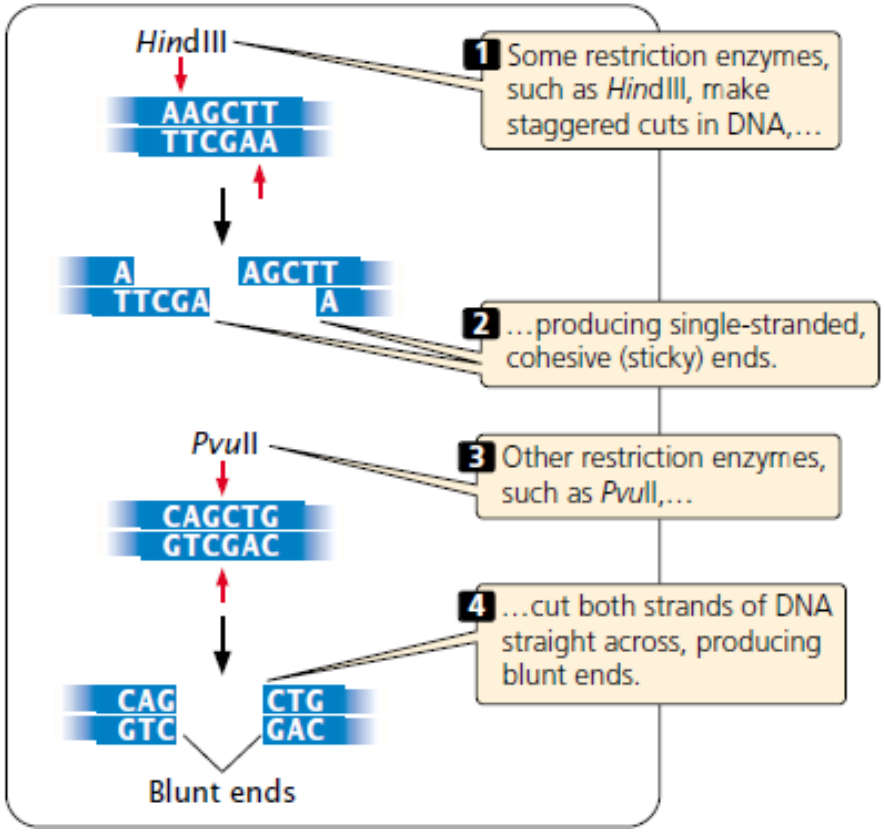
Restriction Enzymes

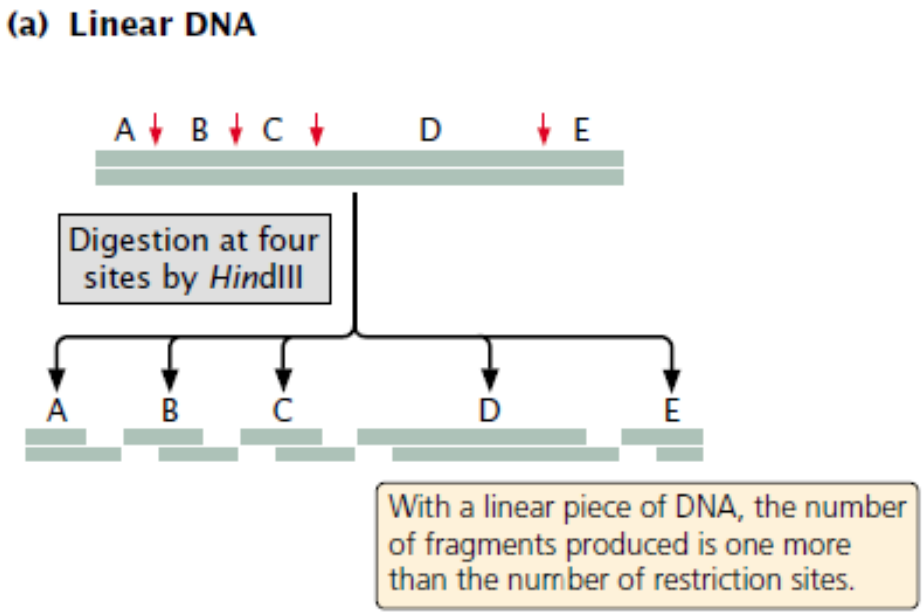
Restriction endonucleases are enzymes that make double-stranded cuts in DNA at specific base sequences.

Table 18.1 Types of restriction enzymes

Type	Activity of Enzyme	ATP Required	Cleavage Site
I	Cleavage and methylation	Yes	Random sites distant from recognition site
II	Cleavage only	No	Within recognition site
III	Cleavage and methylation	Yes	Random sites near recognition site

Type II restriction enzyme cut DNA at specific base sequences. Some restriction enzymes make staggered cuts, producing DNA fragments with cohesive ends; others cut both strands straight across, producing blunt ended fragments. There are fewer long recognition sequences in DNA than short sequences.



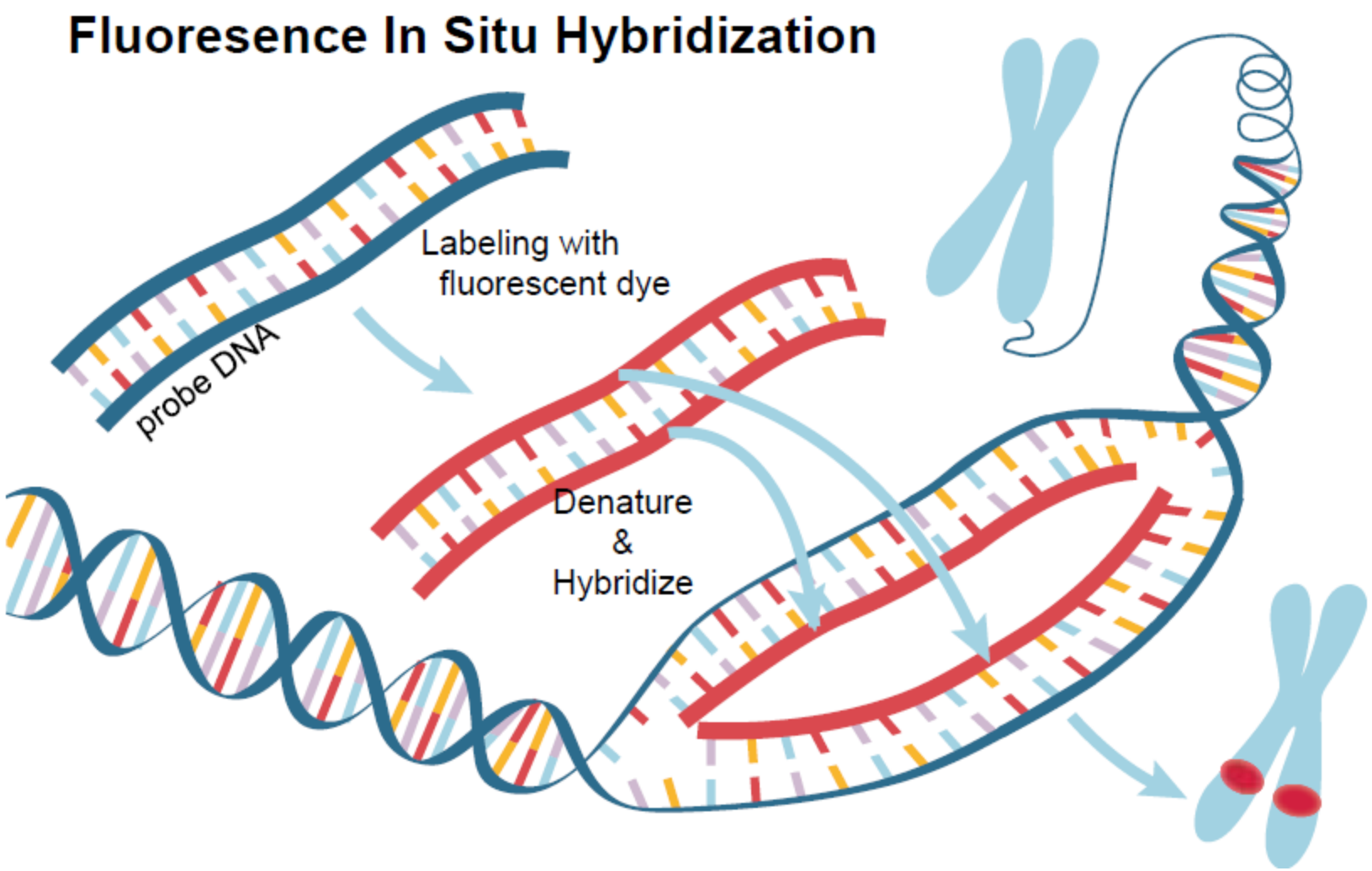


The number of restriction sites is related to the number of fragments produced when DNA is cut by a restriction enzyme.

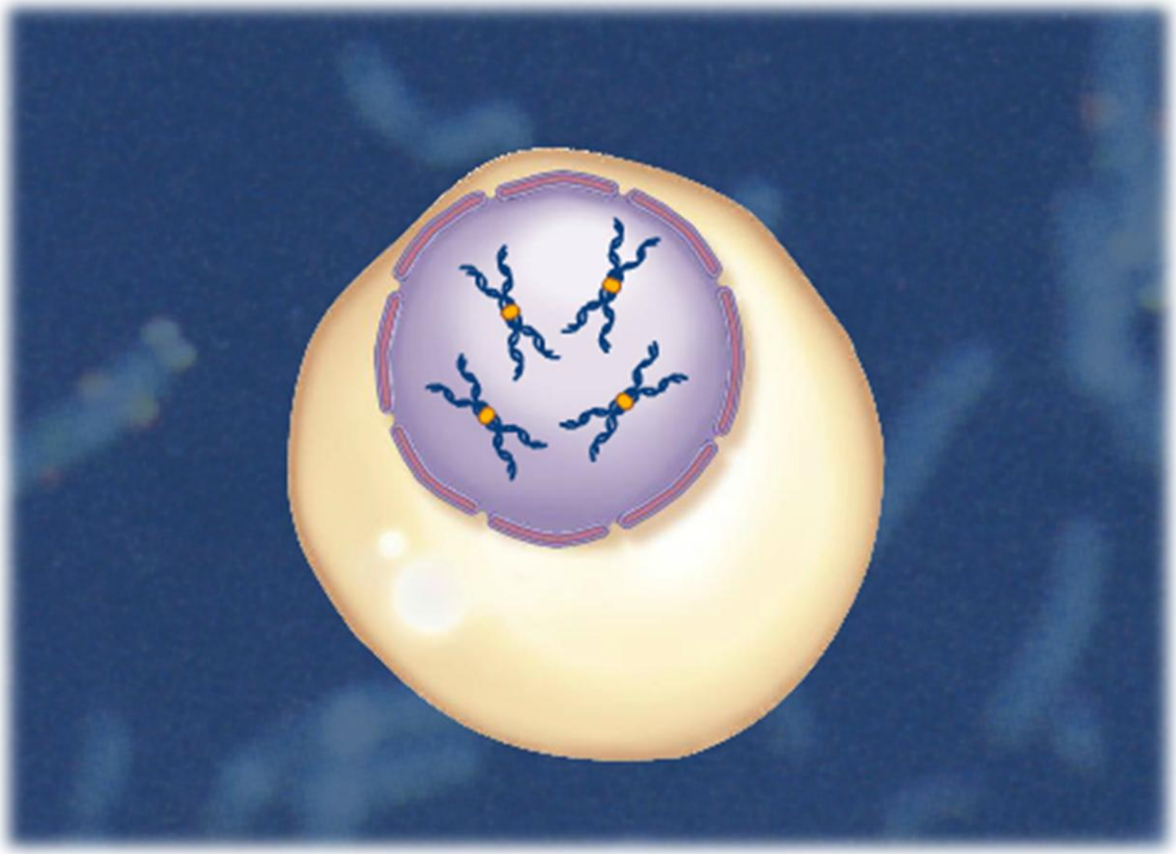
Table 18.2 Characteristics of some common type II restriction enzymes used in recombinant DNA technology

Enzyme	Microorganism from Which Enzyme Is Isolated	Recognition Sequence	Type of Fragment End Produced
*Bam*HI	*Bacillus amyloliquefaciens*	5′–G↓GATCC–3′ 3′–CCTAG↑G–3′	Cohesive
*Cof*I	*Clostridium formicoaceticum*	5′–GCG↓C–3′ 3′–C↑GCG–5′	Cohesive
*Dra*I	*Deinococcus radiophilus*	5′–TTT↓AAA–3′ 3′–AAA↑TTT–5′	Blunt
*Eco*RI	*Escherichia coli*	5′–G↓AATTC–3′ 3′–CTTAA↑G–5′	Cohesive
*Eco*RII	*Escherichia coli*	5′–↓CCAGG–3′ 3′–GGTCC↑–5′	Cohesive
*Hae*III	*Haemophilus aegyptius*	5′–GG↓CC–3′ 3′–CC↑GG–5′	Blunt
*Hind*III	*Haemophilus influenczae*	5′–A↓AGCTT–3′ 3′–TTCGA↑A–5′	Cohesive
*Hpa*II	*Haemophilus parainfluenzae*	5′–C↓CGG–3′ 3′–GGC↑C–5′	Cohesive
*Not*I	*Nocardia otitidis-caviarum*	5′–GC↓GGCCGC–3′ 3′–CGCCGG↑CG–5′	Cohesive
*Pst*I	*Providencia stuartii*	5′–CTGCA↓G–3′ 3′–G↑ACGTC–5′	Cohesive
*Pvu*II	*Proteus vulgaris*	5′–CAG↓CTG–3′ 3′–GTC↑GAC–5′	Blunt
*Sma*I	*Serratia marcescens*	5′–CCC↓GGG–3′ 3′–GGG↑CCC–5′	Blunt

FISH

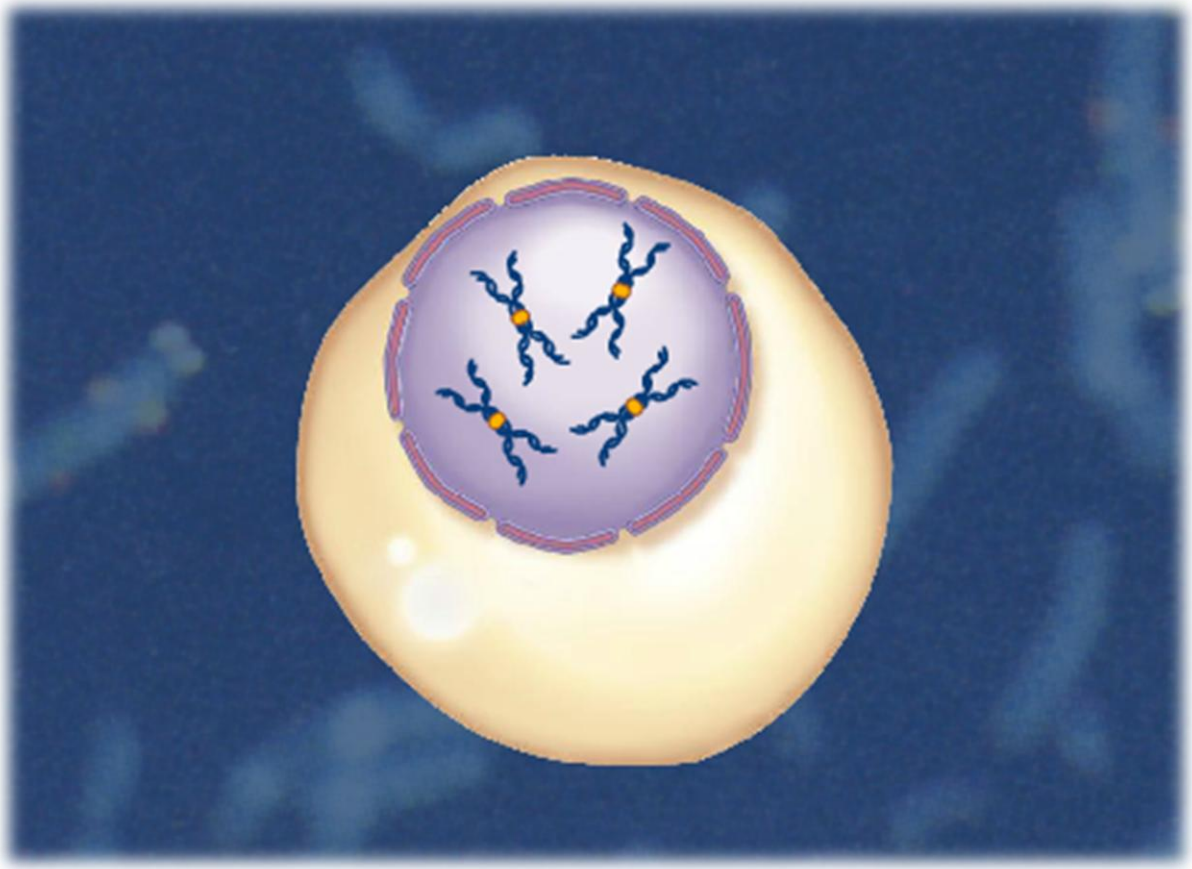

Fluoresence In Situ Hybridization
probe DNA
Labeling with
fluorescent dye
Denature
&
Hybridize

Fluorescence *In Situ* Hybridization (FISH)



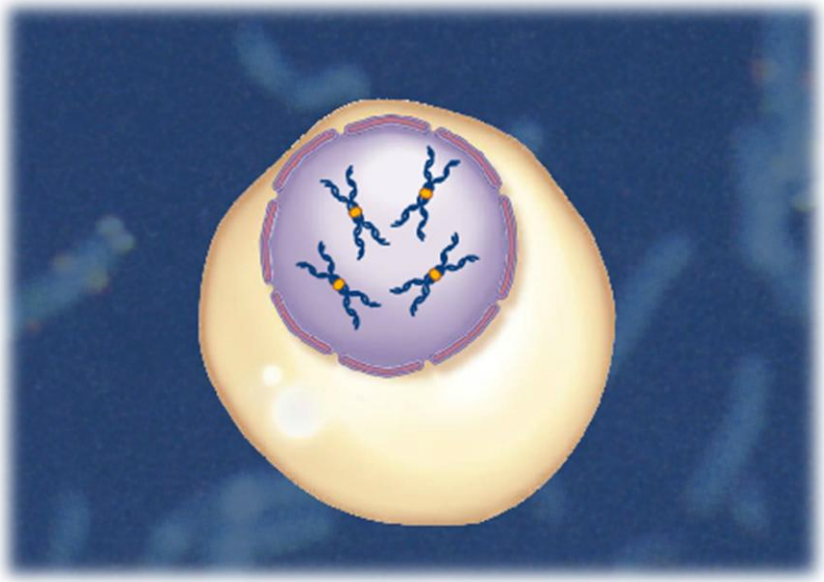
In situ hybridization is used to detect the physical location of a gene on an intact chromosome. In situ is Latin for "in place", and in this procedure the chromosomes are adhered "in place" onto a slide.

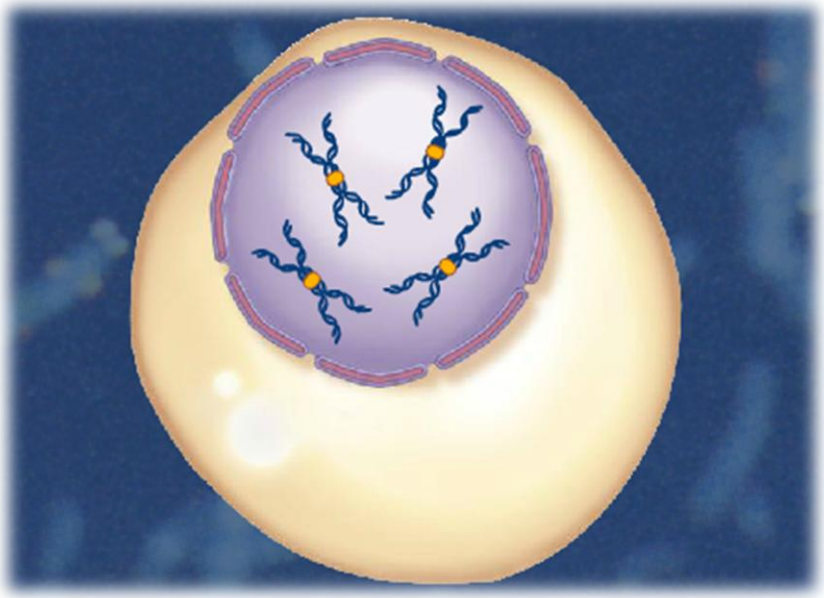
FISH



The most common method of in situ hybridization uses fluorescently labeled DNA probes. This technique is known as fluorescence in situ hybridization, or FISH.

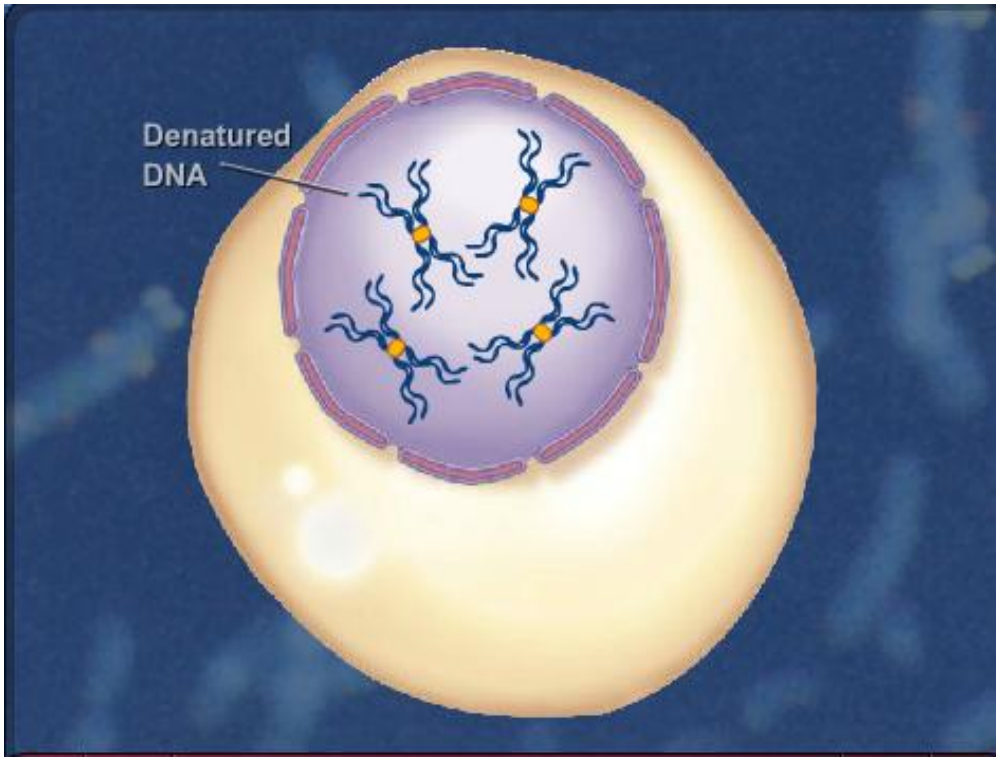
FISH





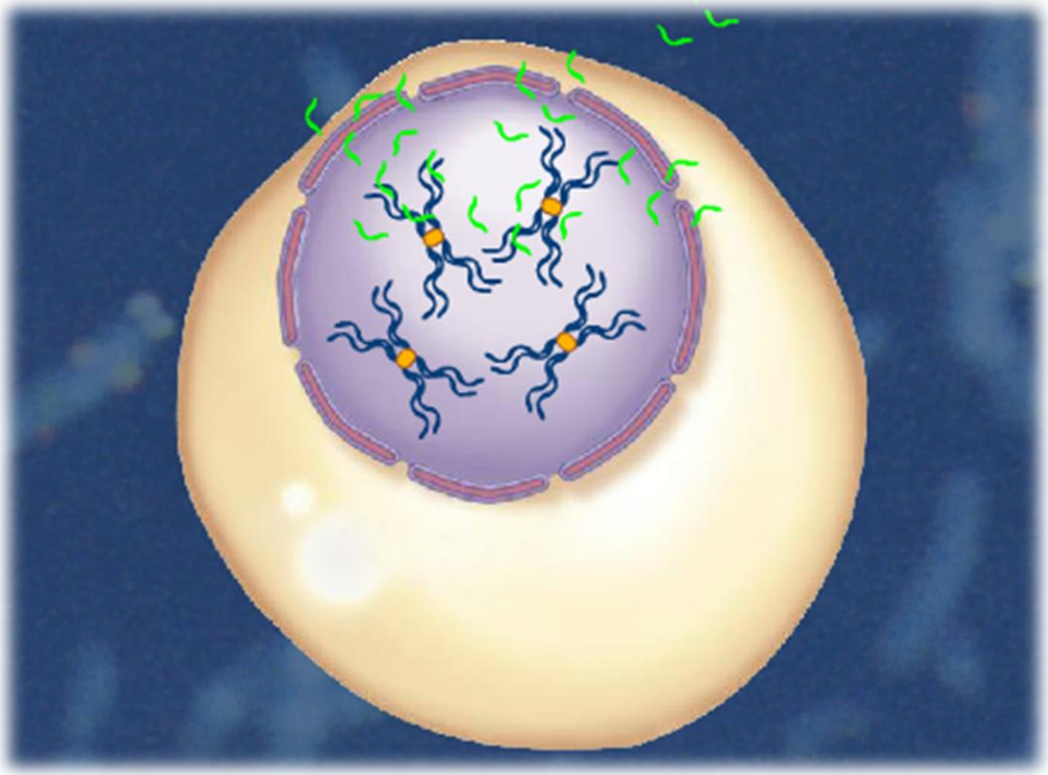
To perform FISH, first cells that have been arrested in metaphase are treated to make them swell, and then they are fixed onto the surface of a slide. This also fixes their chromosomes onto the slide.

FISH



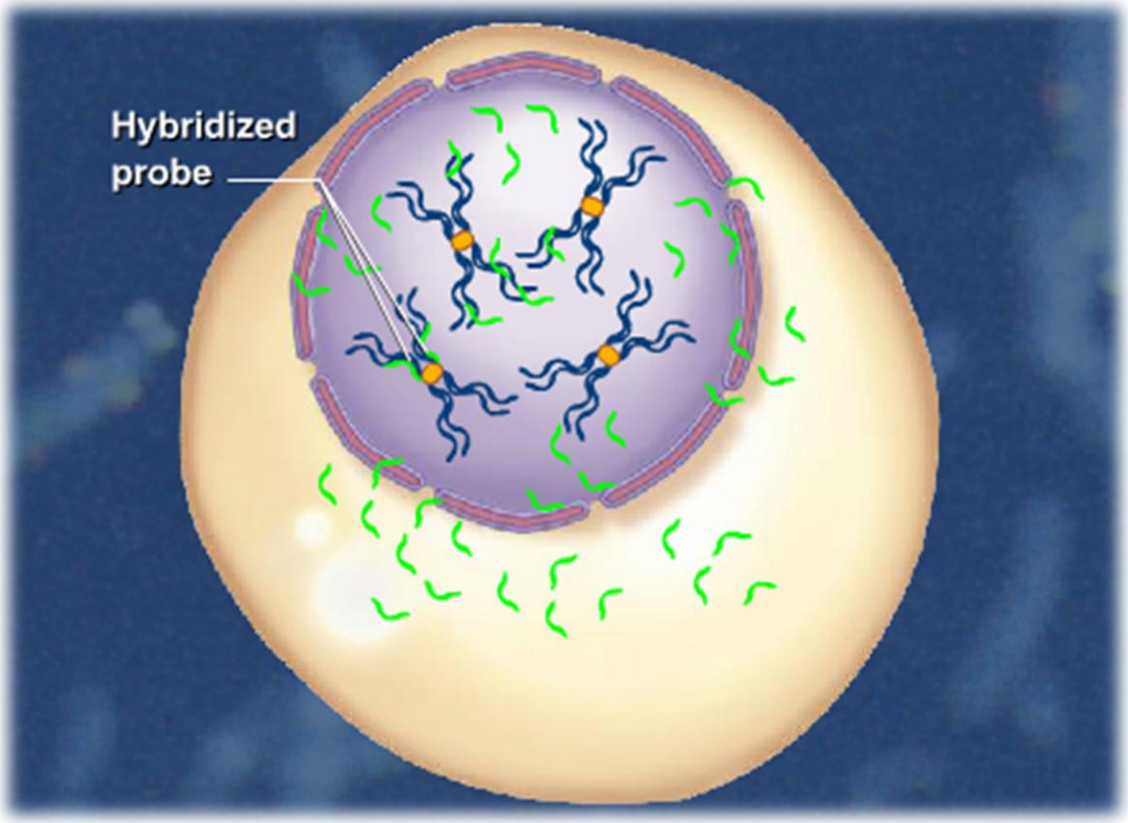
Next, the slide is treated so that the chromosomal DNA is denatured into single strands.

FISH



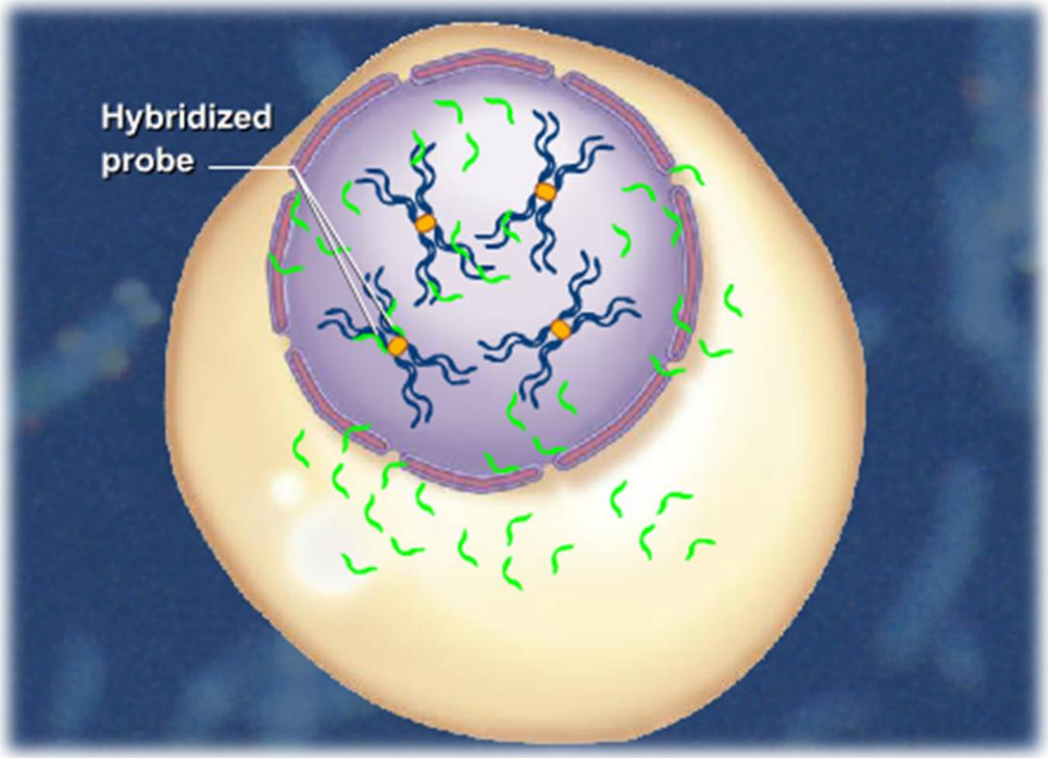
Then, special DNA probes are flooded onto the slide. The DNA probes are small pieces of single-stranded DNA with a sequence from the gene of interest.

FISH



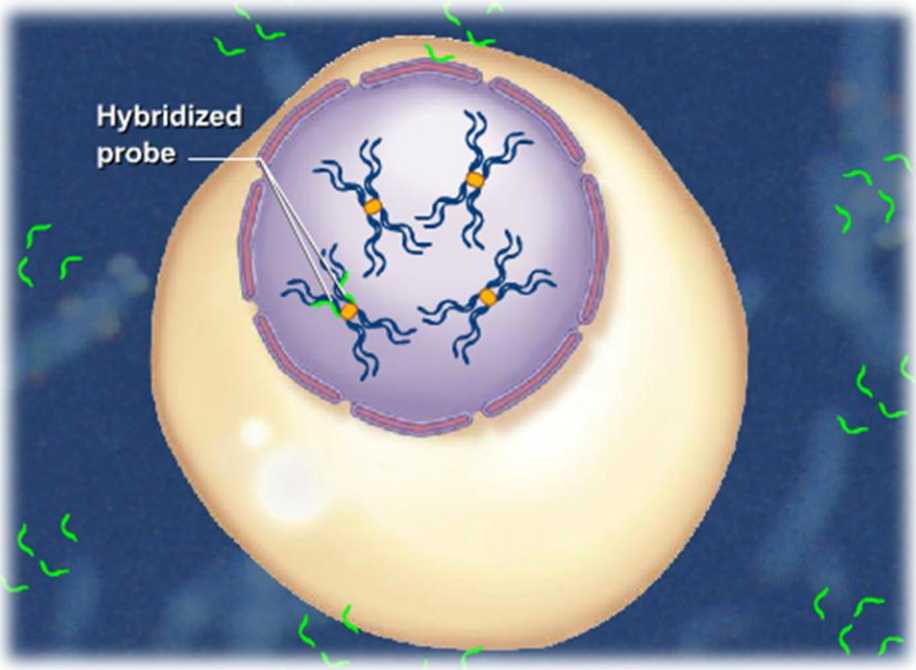
Probes are able to hybridize only with their complementary sequence, which will be at the site of the gene of interest on a particular chromosome.

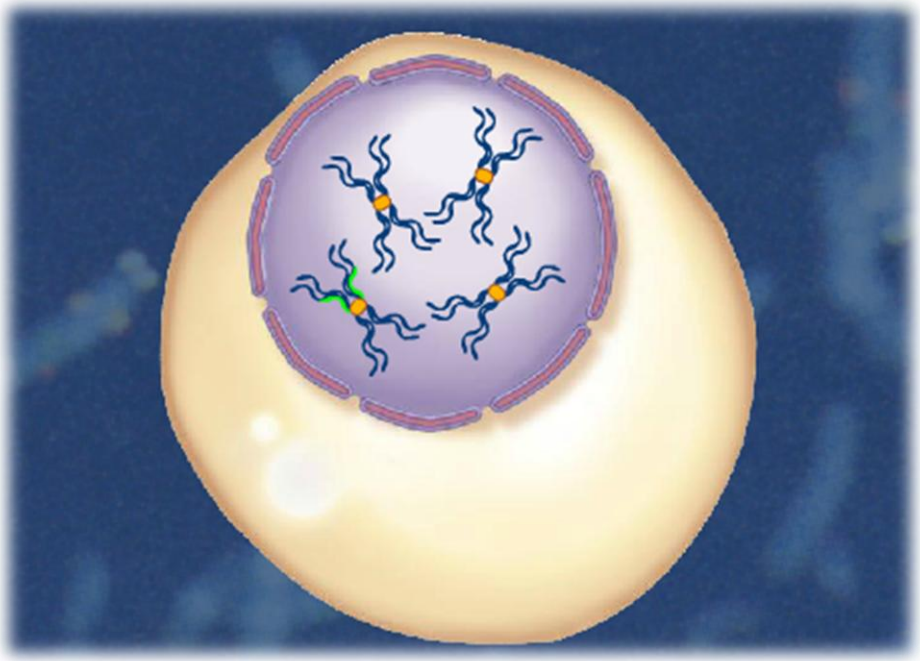
FISH



The DNA probes are allowed to hybridize with the denatured chromosomal DNA, and any excess probes are washed away.

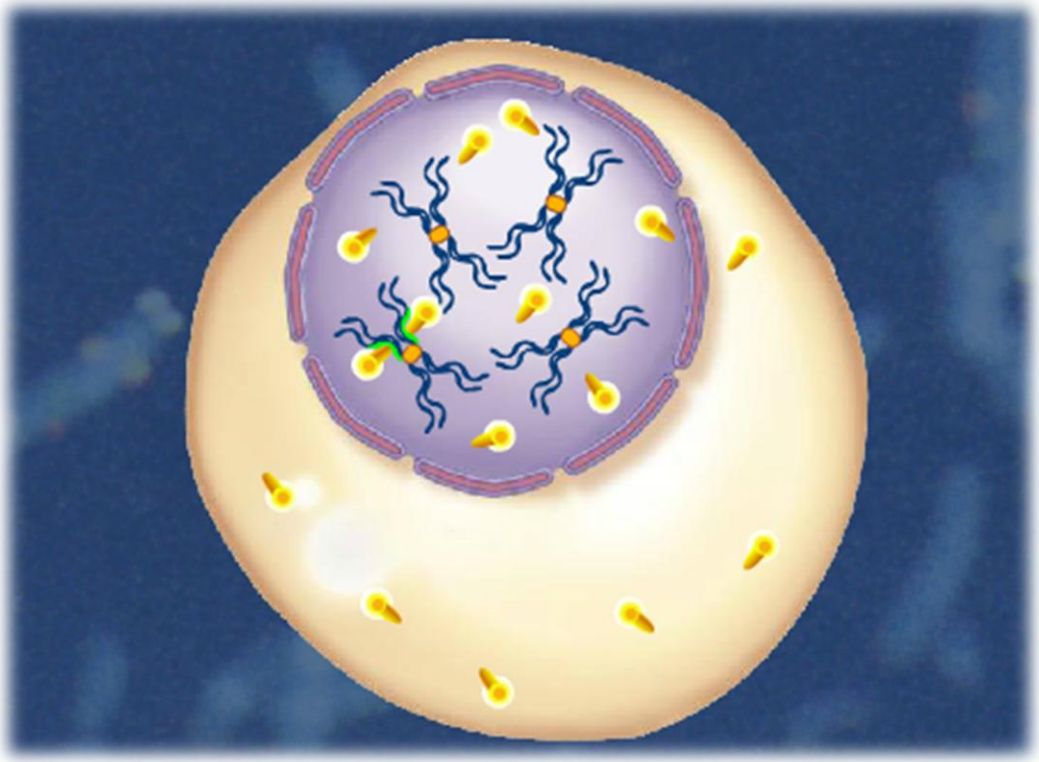
FISH





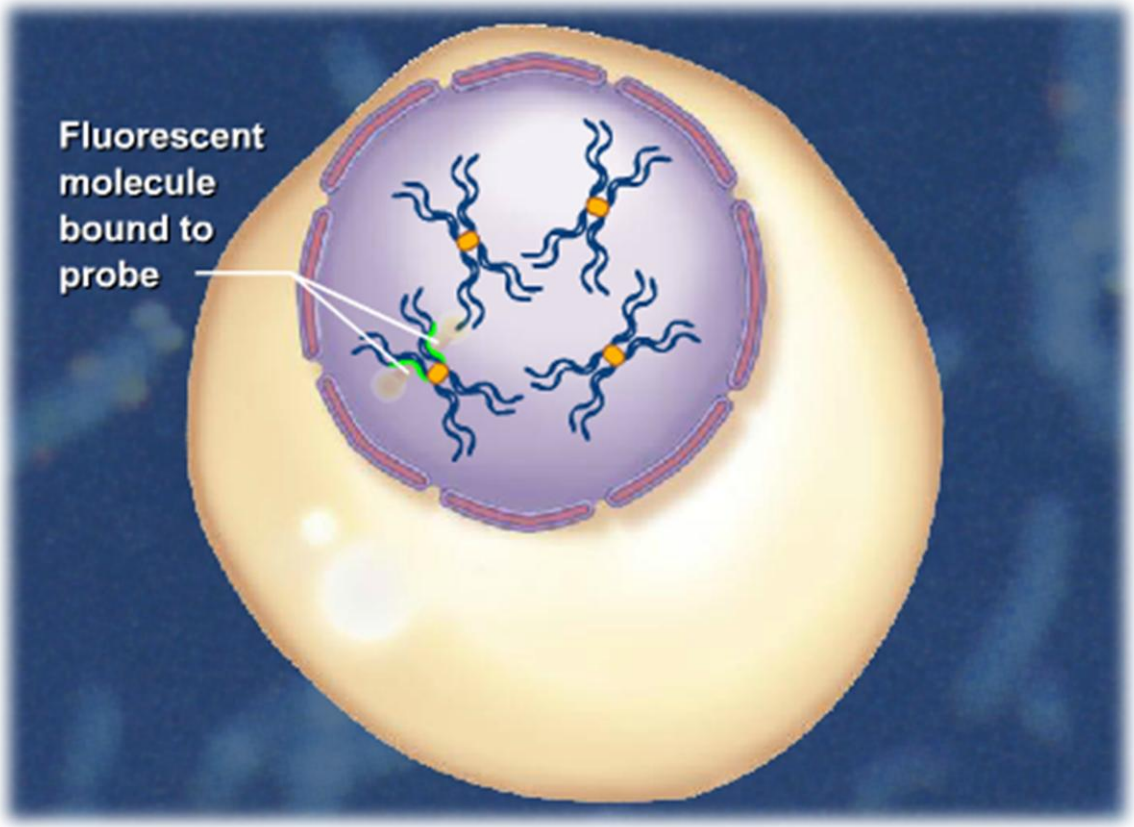
Either the DNA probes themselves are fluorescent or they are chemically modified to allow fluorescent labels to attach to them, so that when fluorescently labeled molecules are added to the slide

FISH



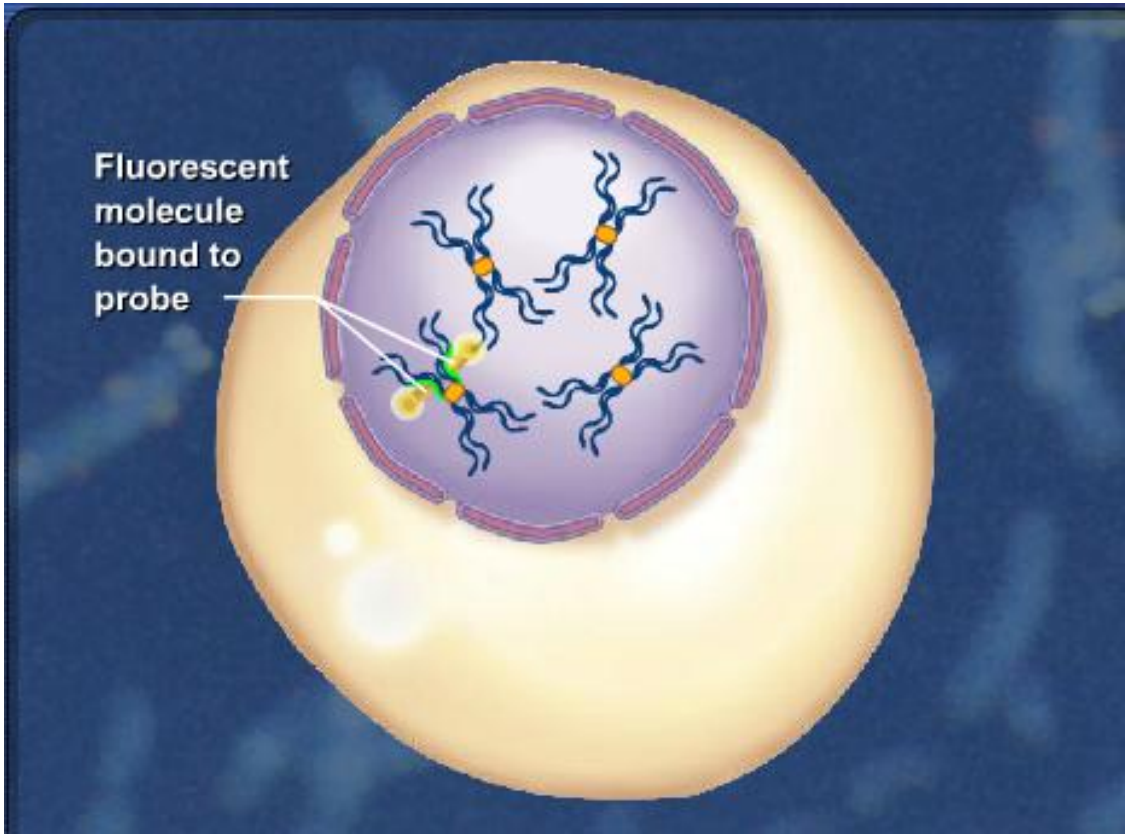
some are able to bind to the probe DNA that is bound to the site of the gene on the chromosome.

FISH



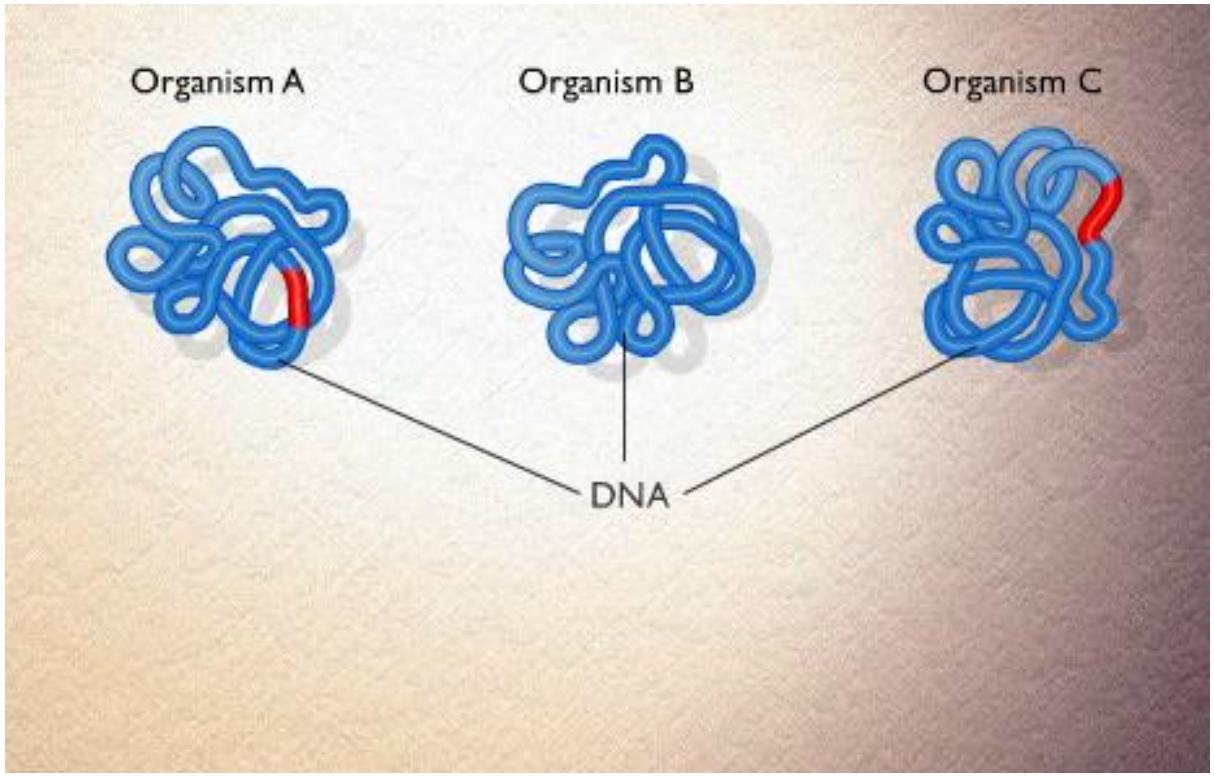
Excess fluorescently labeled molecules are then washed away.

FISH



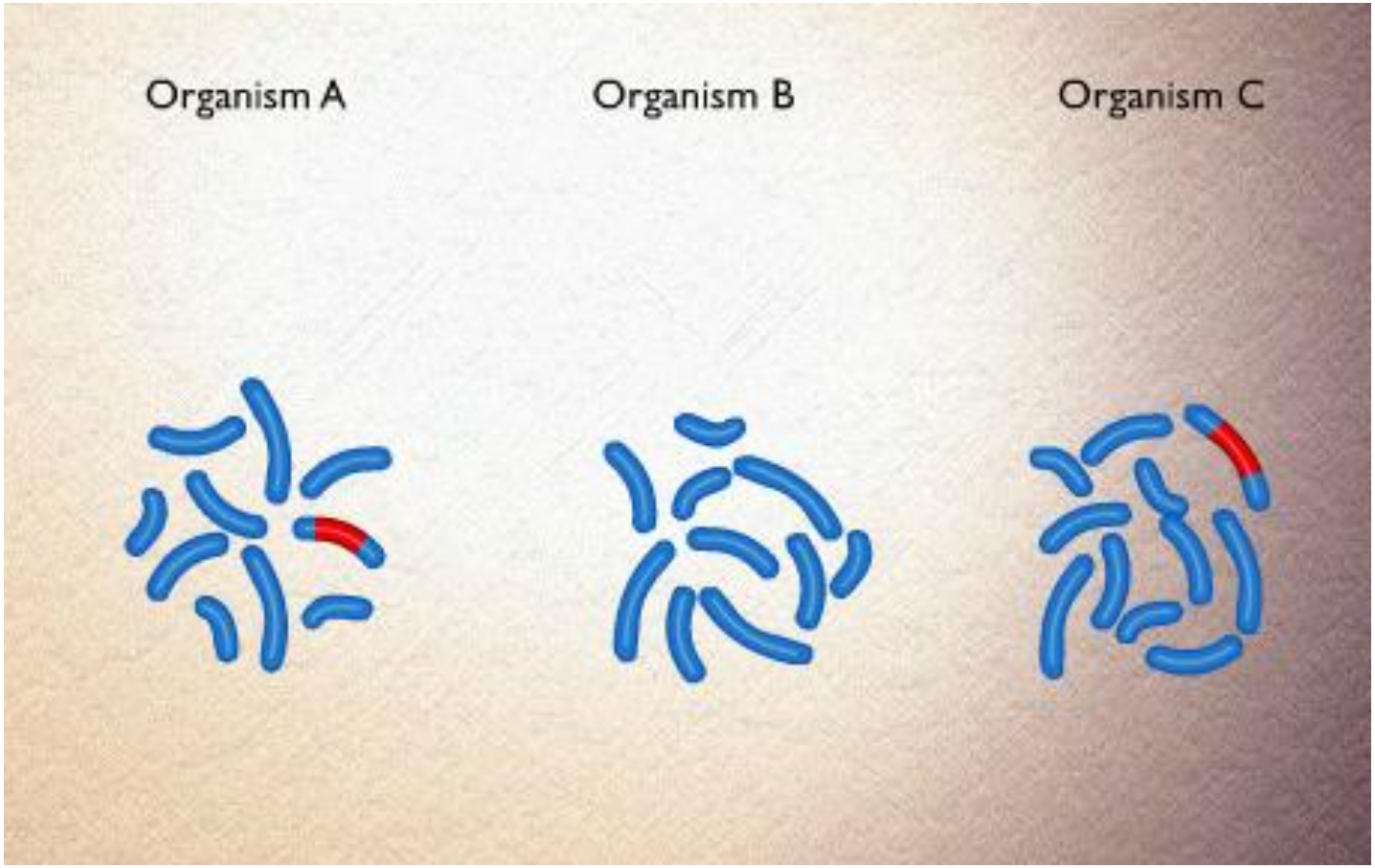
The slide is then viewed using a fluorescence microscope. The fluorescently labeled molecules reveal the physical location of the gene of interest.

Southern Blot



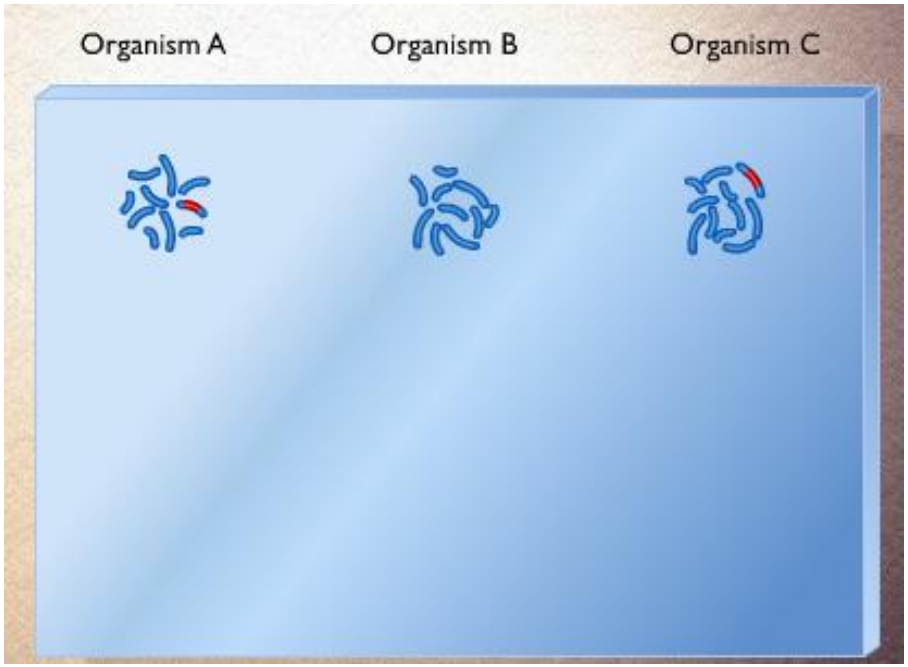
The Southern blot is used to verify the presence or absence of a specific nucleotide sequence in the DNA from different sources and to identify the size of the restriction fragment that contain the sequence.

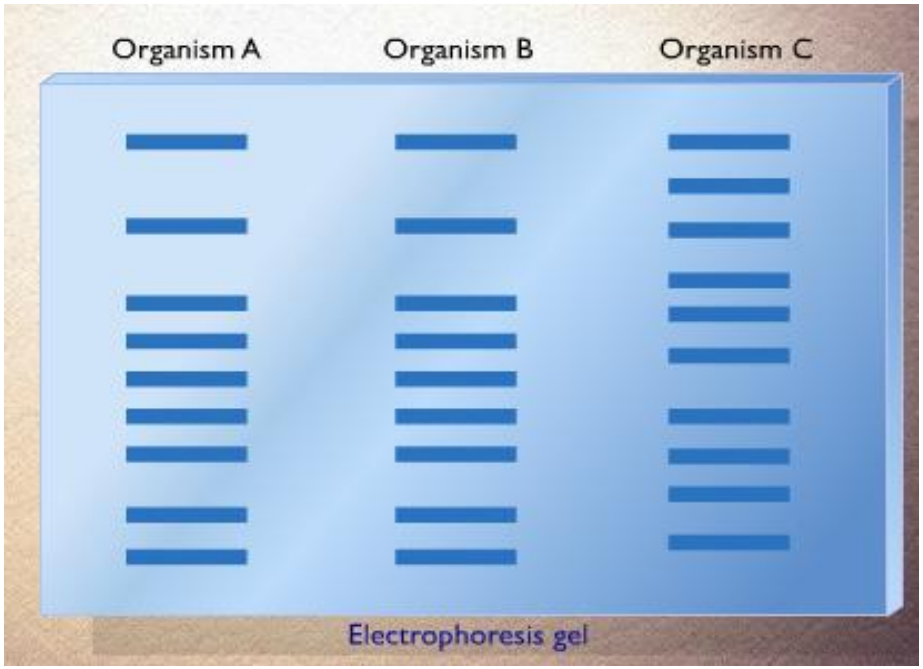
Southern Blot



In this procedure, the DNA is isolated from each source and the digested with a specific restriction enzyme.

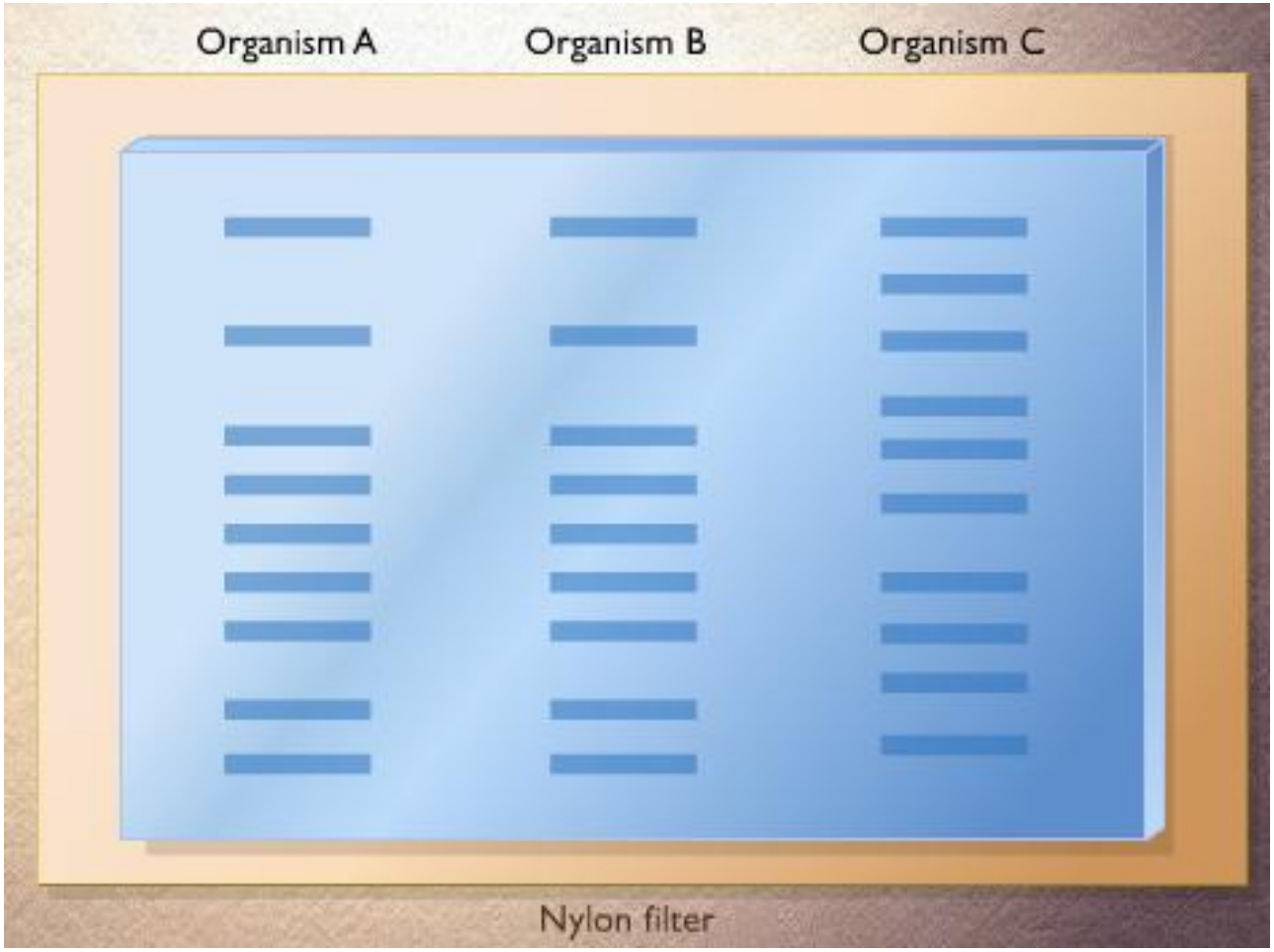
Southern Blot





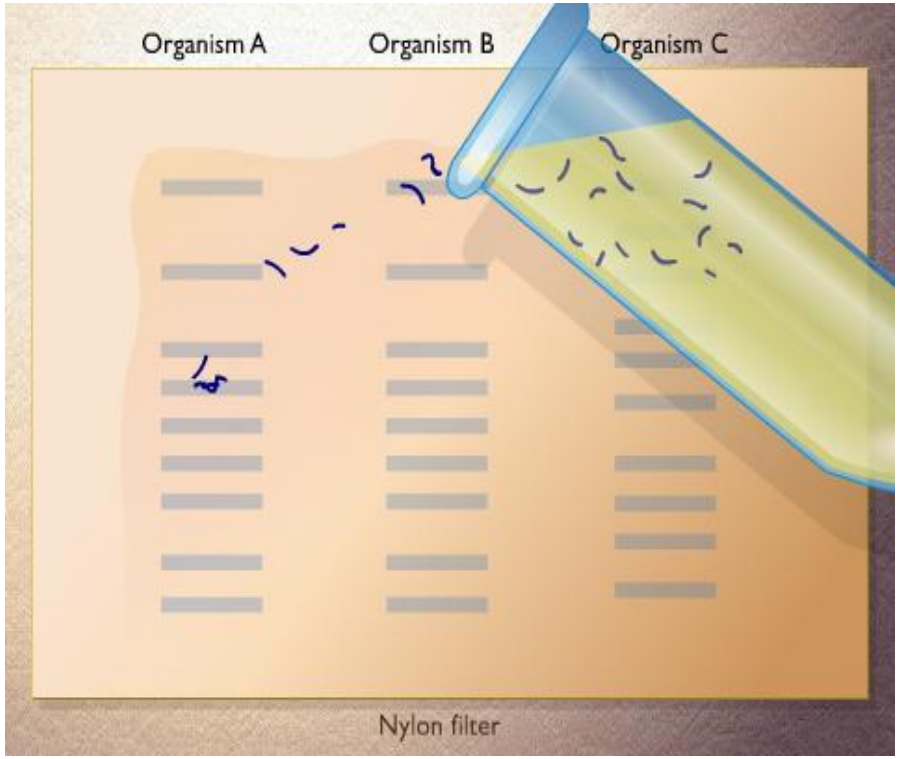
The DNA restriction fragments are then loaded onto an agarose gel and the fragments separated by electrophoresis according to size, with the smaller fragments migrating faster than larger fragments.

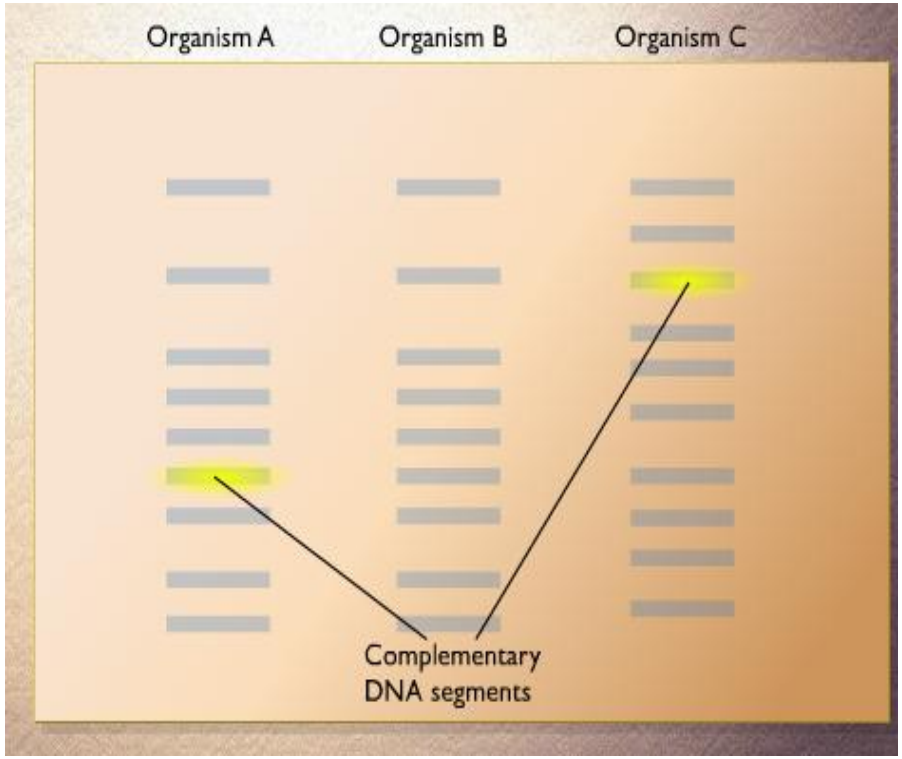
Southern Blot



The DNA is then transferred from the fragile gel to a nylon filter

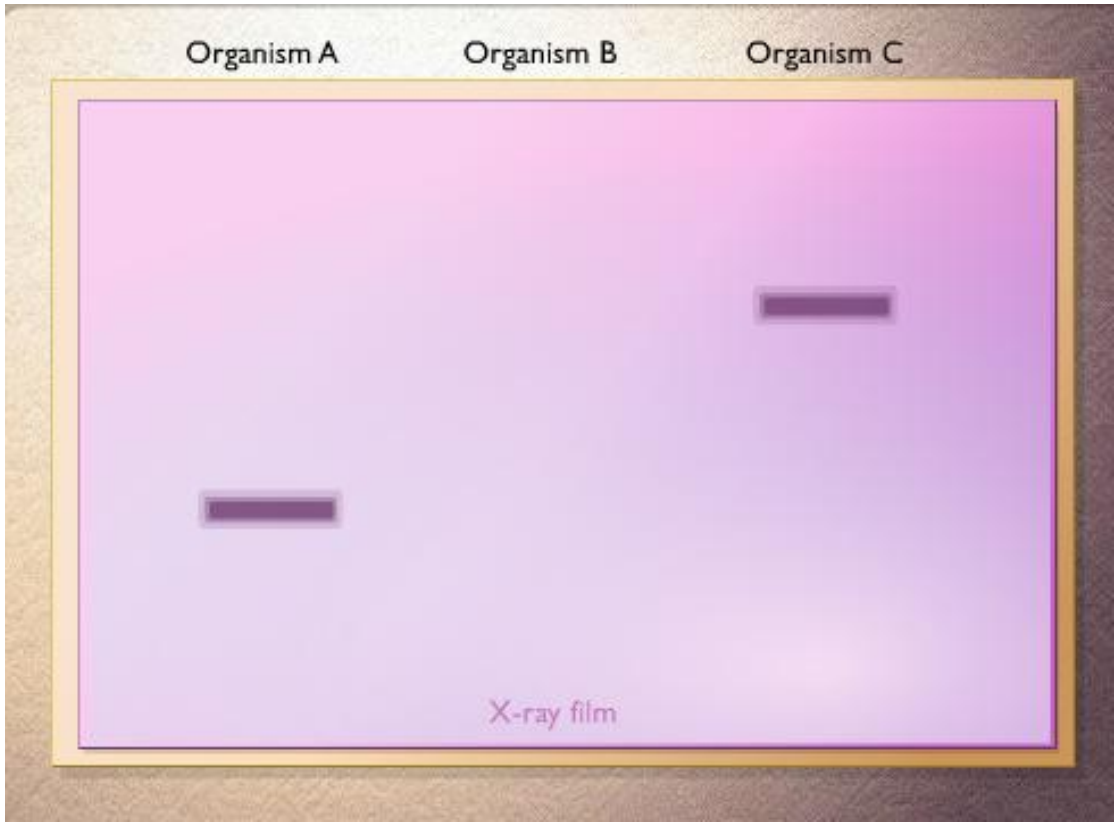
Southern Blot





The radioactively labeled nucleic acid probe is added. The probe binds to complementary DNA segments. The DNA segment being probed is not present in Organism B.

Southern Blot



To detect the position of the radioactive probe, the nylon membrane is covered with an X-ray film. After development, the positions of the probe become visible.

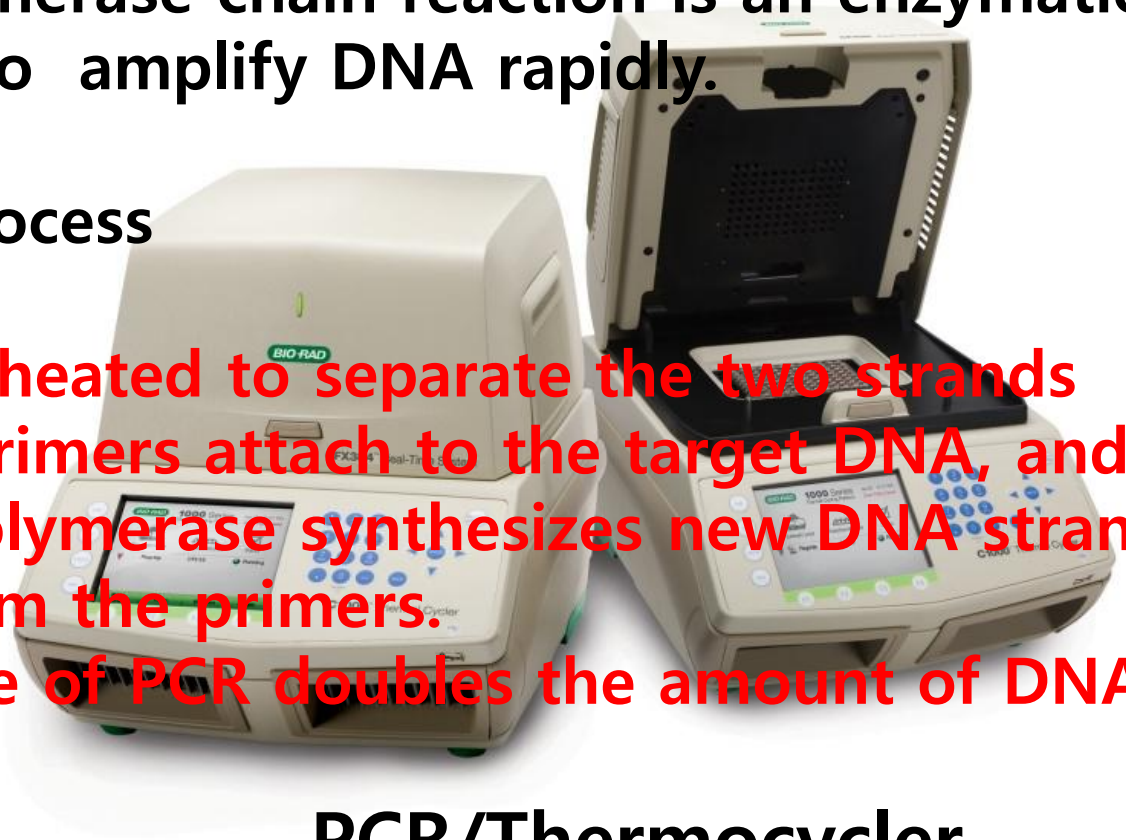
Polymerase Chain Reaction (PCR)

The polymerase chain reaction is an enzymatic, *in vitro* method to amplify DNA rapidly.

In this Process

- ✓DNA is heated to separate the two strands
- ✓Short primers attach to the target DNA, and
- ✓DNA polymerase synthesizes new DNA strands from the primers.

Each cycle of PCR doubles the amount of DNA.



PCR/Thermocycler

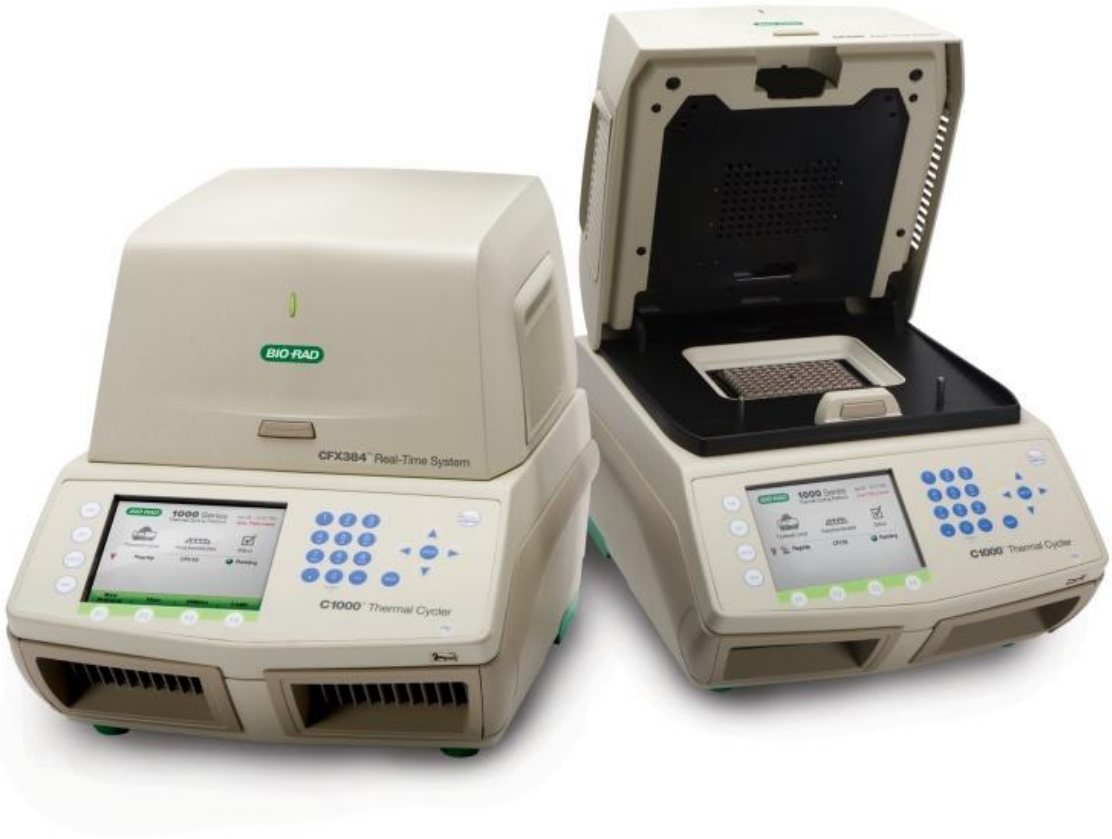
Polymerase Chain Reaction (PCR)

DNA polymerase enzyme, ***Taq*** **polymerase (resistant to denaturation at high temperatures)**

Specific oligonucleotide Primers (can then be synthesized or obtained commercially) are used to increase the amount of target DNA for analysis.

Usually DNA of about 200-1000 bp is amplified

Polymerase Chain Reaction (PCR)



The reaction sequence is accomplished simply by changing the temperature of the reaction mixture.

Polymerase Chain Reaction (PCR)

PCR steps

Step 1

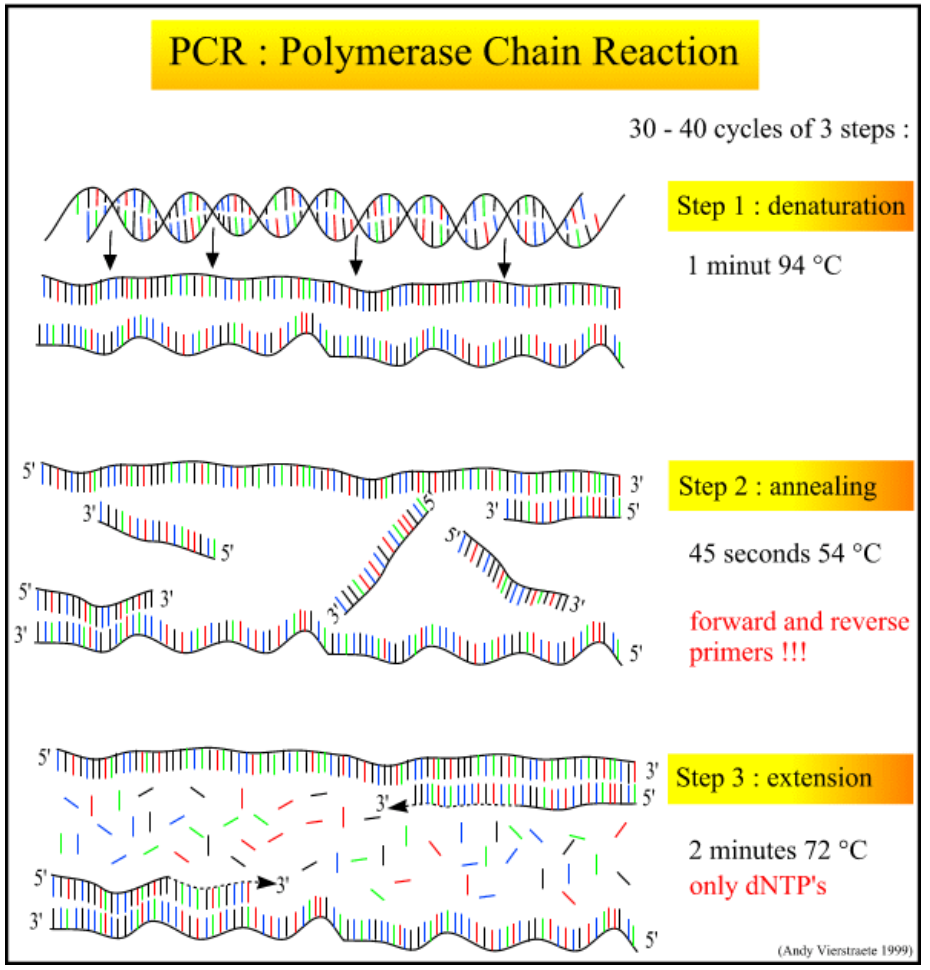
- **Incubation at 94°C denatures (seperates) the DNA duplex and creates single-stranded DNA.**

Step 2

- **Incubation at 53°C allows hybridization of new primers**

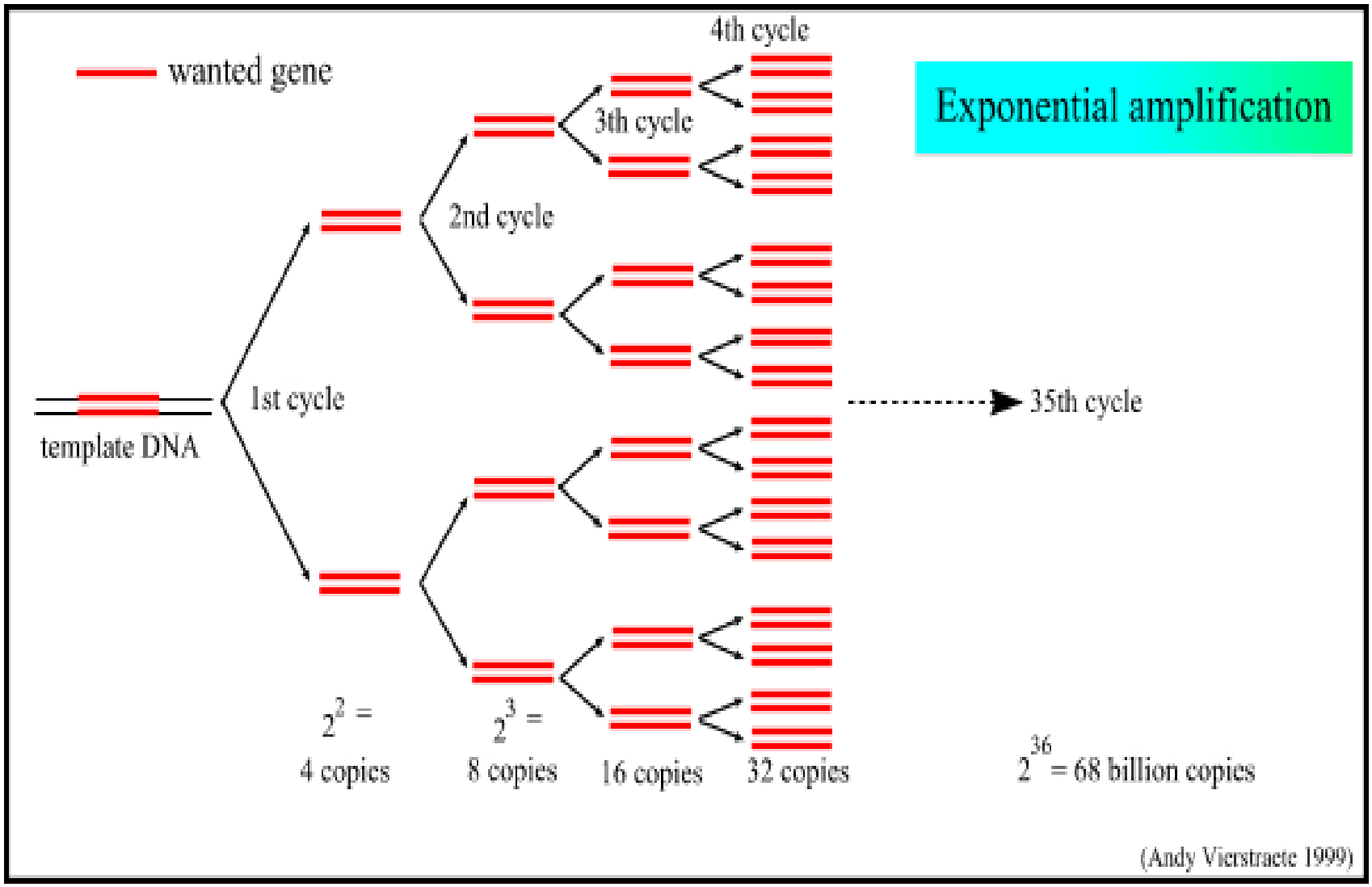
Step 3

- **Incubation at 72°C allows taq polymerase to synthesize new DNA from the primers**



PCR

Polymerase Chain Reaction (PCR)

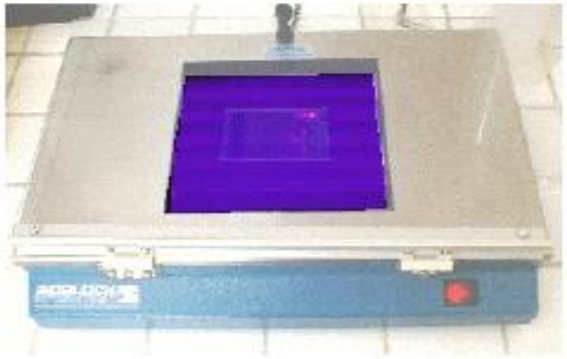


Polymerase Chain Reaction (PCR)

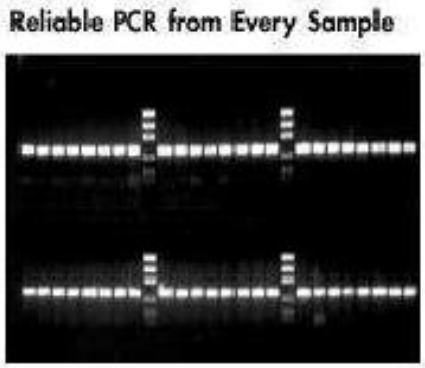
PCR

Agarose gel electrophoresis

3-4 hours



UV visualisation



The final product

Identification of Mutations in Tumors

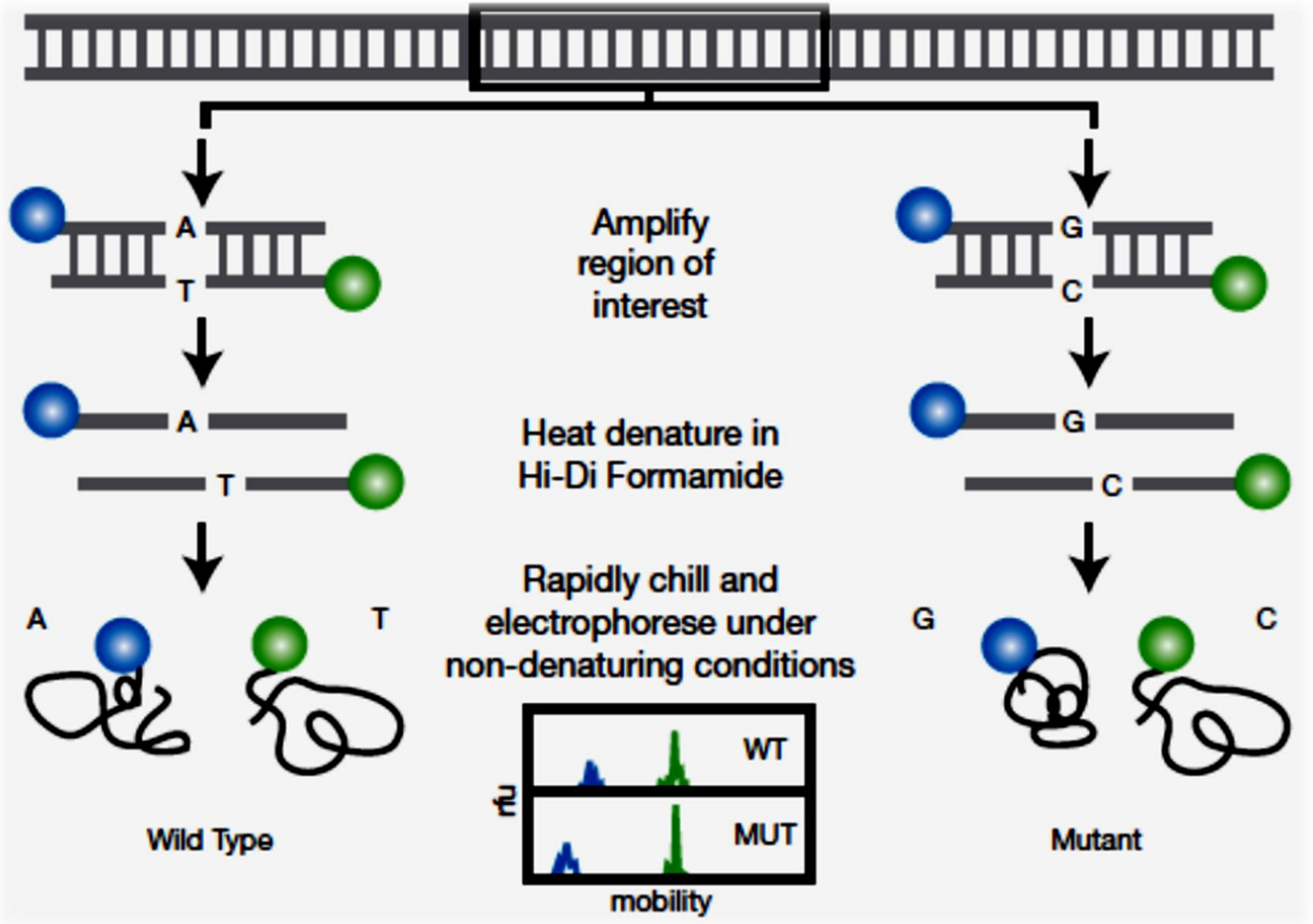
Single Strand Conformation Polymorphism (SSCP)

SSCP detects sequence variations (single-point mutations and other small-scale changes) through electrophoretic mobility differences.

These variations can potentially cause conformational changes in the DNA molecules.

Under nondenaturing conditions and often reduced temperature, single-stranded DNA molecules can assume unique conformations that vary depending on their nucleotide sequences.

SSCP


A
T
A
T
A
T
Wild Type
Amplify region of interest
Heat denature in Hi-Di Formamide
Rapidly chill and electrophorese under non-denaturing conditions
WT
MUT
nu
mobility
G
C
G
C
G
C
Mutant

Nuclease protection assays

Nuclease protection assays use a single-stranded radioactive DNA or RNA probe.

The nucleotide sequence of the probe contains at least some nucleotides that are complementary to the mRNA being analyzed. The probe is annealed to the target mRNA by base-pairing, and the regions of the probe that are complementary to the target mRNA now become double-stranded, while the noncomplementary regions of the probe remain single-stranded. The annealed mixture is then subjected to digestion with an enzyme specific for single-stranded DNA (usually S1 nuclease), when using a DNA probe, or RNA (usually a mixture of RNase A and RNase T1), when using an RNA probe. The double-stranded annealed areas resist digestion, while all the single-stranded noncomplementary parts of the probe are digested away. In essence, areas in the probe that anneal to the mRNA are "protected" from digestion by the nucleases. The surviving, undigested parts of the probe can then be analyzed by electrophoresis through an agarose or polyacrylamide gel. The amount of radiolabeled probe resistant to digestion is proportional to the amount of target mRNA in the sample.

Nuclease protection assays