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MSC-ZOOLOGY

BIO301-Essentials of Genetics



HANDOUTS TOPIC NO 1 TO 250

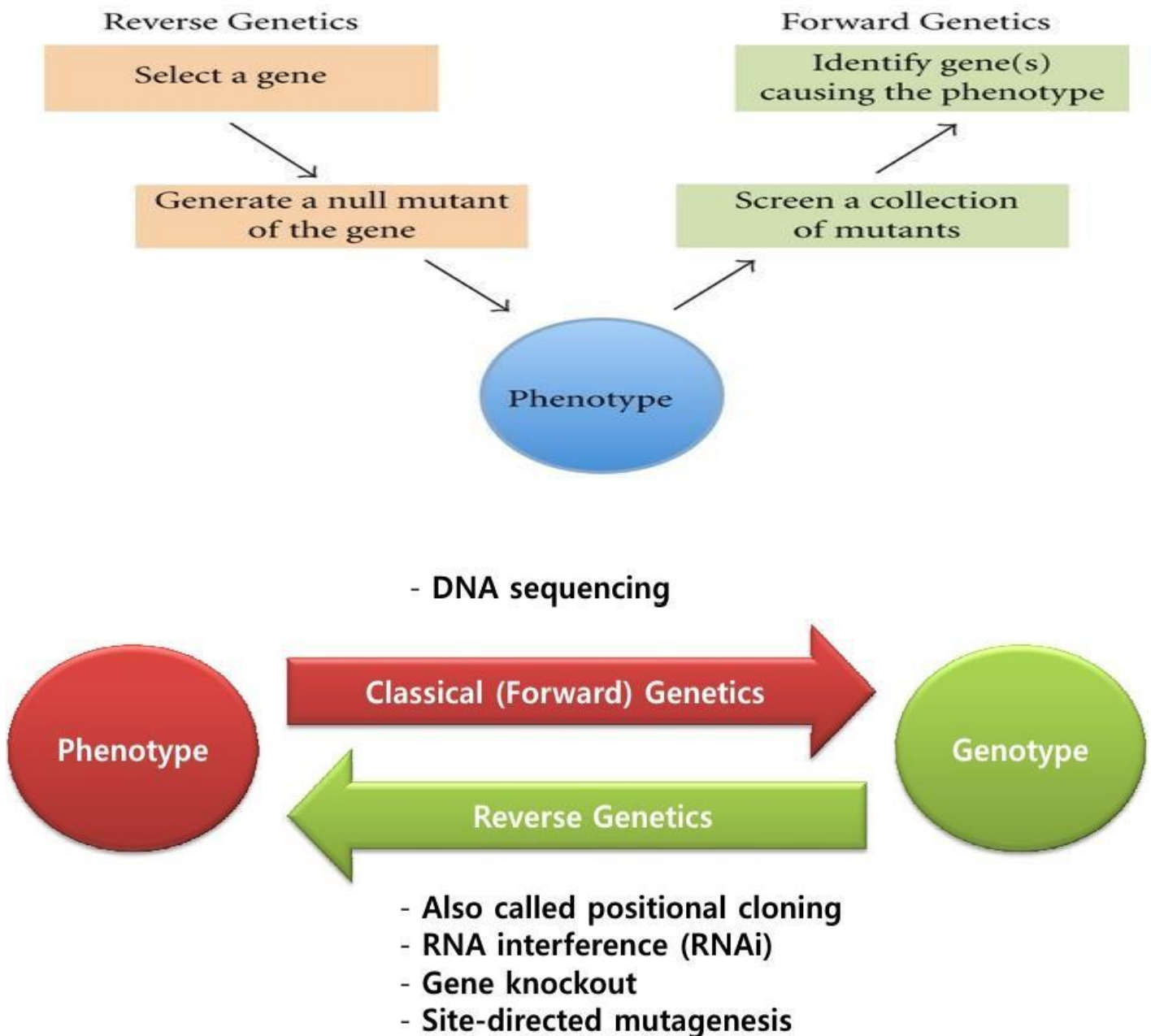
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1 Genetics Definition

Genetics is the study of genes, heredity and variation.

It is considered as a field of Biology.

The principles of heredity were explained by Gregor Mendel in 1866.



Gregor Mendel used garden pea as experimental plant for formulating the laws of heredity, following were the properties of garden pea

- Seed in a variety of shapes and colors.
- Self, cross pollinate.
- Takes up little space.
- Short generation time
- Produces many offspring.

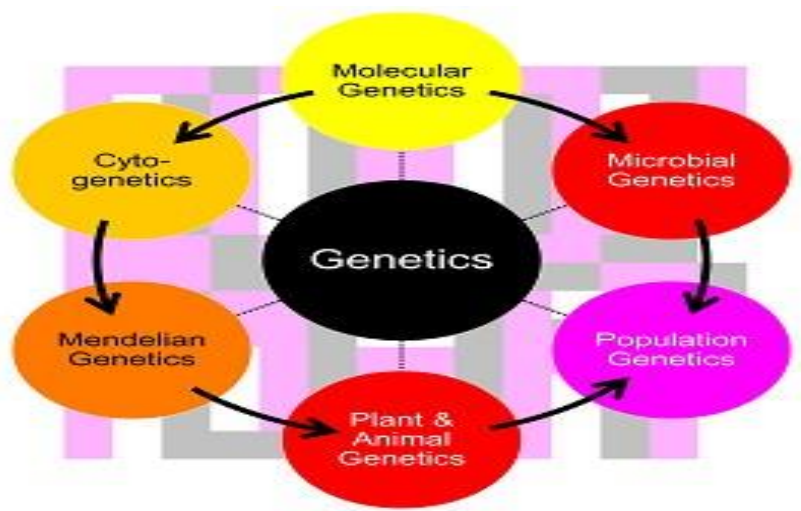
2 Types of Genetics

There are four sub disciplines of genetics which are as follows, although some of the Geneticist classify Genetics in many sub disciplines

- Transmission (Classical) Genetics
- Population Genetics
- Quantitative Genetics
- Molecular Genetics

Historically, transmission genetics developed first, followed by population genetics, quantitative genetics and finally molecular genetics.

- Transmission or classical genetics deals with movement of genes and genetic traits from parents to offspring. Mendel's Laws. It also deals with genetic recombinations.
- Population genetics is the study of traits in a group of population. In this type of genetics we study heredity in groups for traits determined by one or a few genes.
- Quantitative genetics is the study of group hereditary for traits determined by many genes simultaneously such as skin color, height, eye color etc
- Molecular genetics this branch of genetics deals with the molecular structure and function of genes.



3 Genetics Terminologies

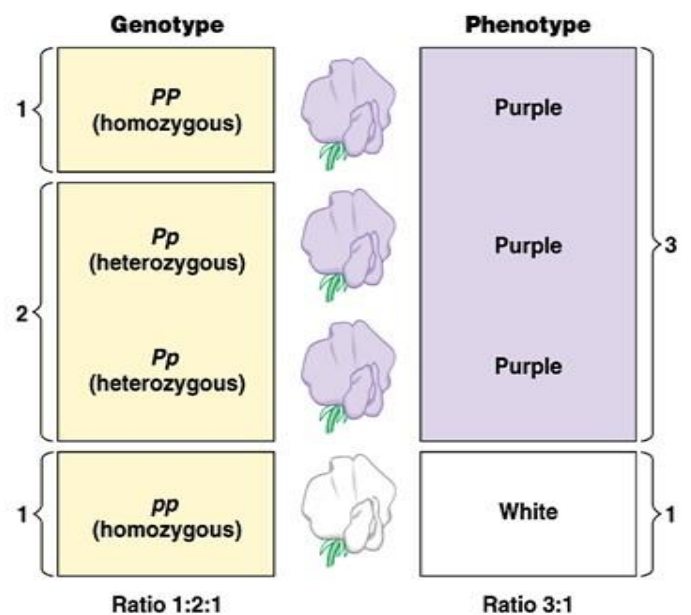
Common Genetics Terminologies

What is Character: A heritable feature (skin color, height etc).

What is Trait: variant for a character (i.e. brown, black, white etc).

What is True-breed: all offspring of same variety.

- Different generations of a cross can be
 - P generation (parents)
 - F1 generation (1st filial generation)
 - F2 generation (2nd filial generation)
- Pure Cross: A cross between a true breed plant/animal with another true breeds plant/animal is called pure cross



True breeding X True breeding

WW X ww

- Hybrid Cross:

F1 generation X F1 generation

Ww X Ww

- Genotype and Phenotype: Genetic make-up of an organism is called Genotype while physical appearance of an organism is called **Phenotype**.
- Dominant and Recessive: when one characteristic expresses itself over the other i.e. round over wrinkled was dominant in Gregor Mendel experiments while the trait that does not show through in the first generation is called as recessive trait i.e. wrinkled.

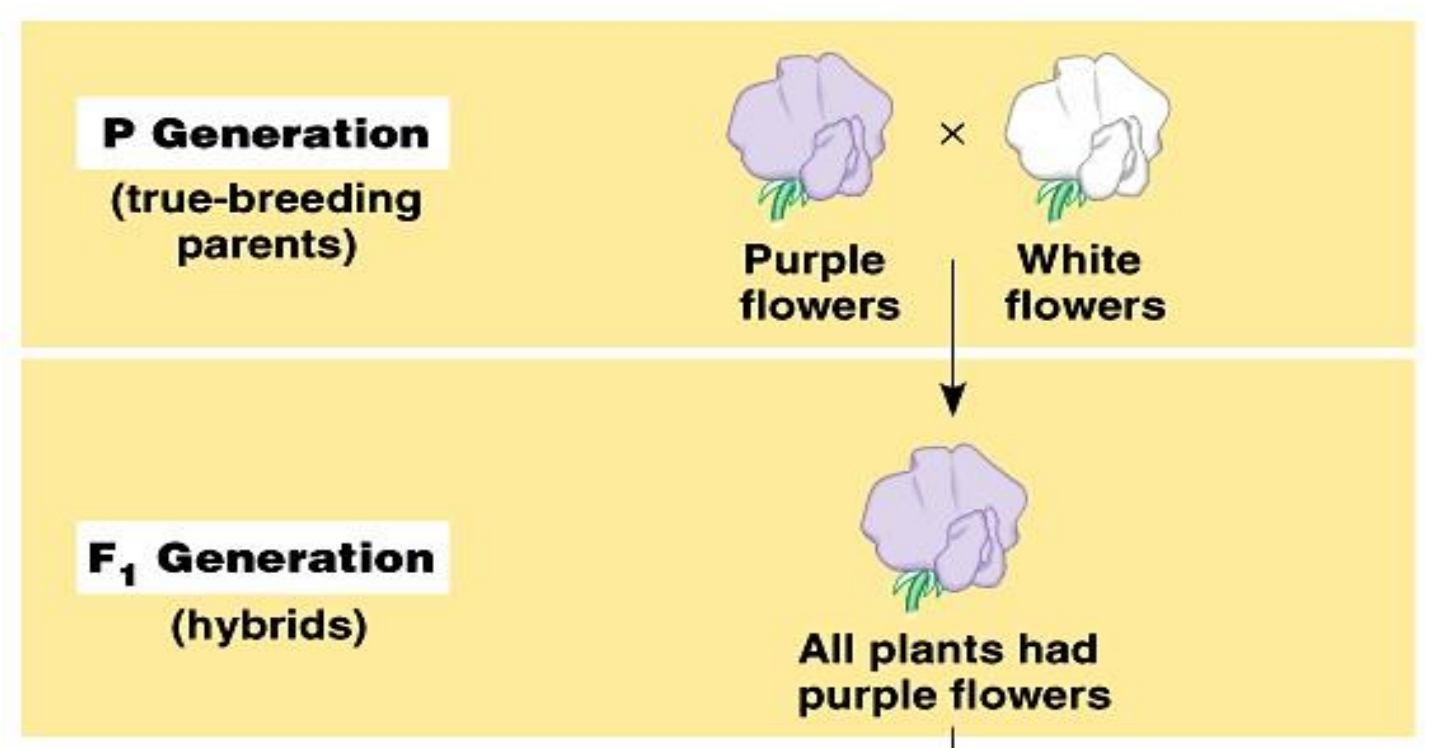
4 Mendel's law of Dominance

Mendel's law of dominance

Mendel s’ law of dominance describes that in a cross of parents, that are pure for contrasting traits, only one form of the trait will appear in the next generation. In the monohybrid cross, one version will be disappeared

Example:

Cross of two true-breeding plants. First plant is with purple color flowers while second is with white color flowers. During F1 generation, all plants will be with purple color flowers. It can be concluded that purple color dominant to white.



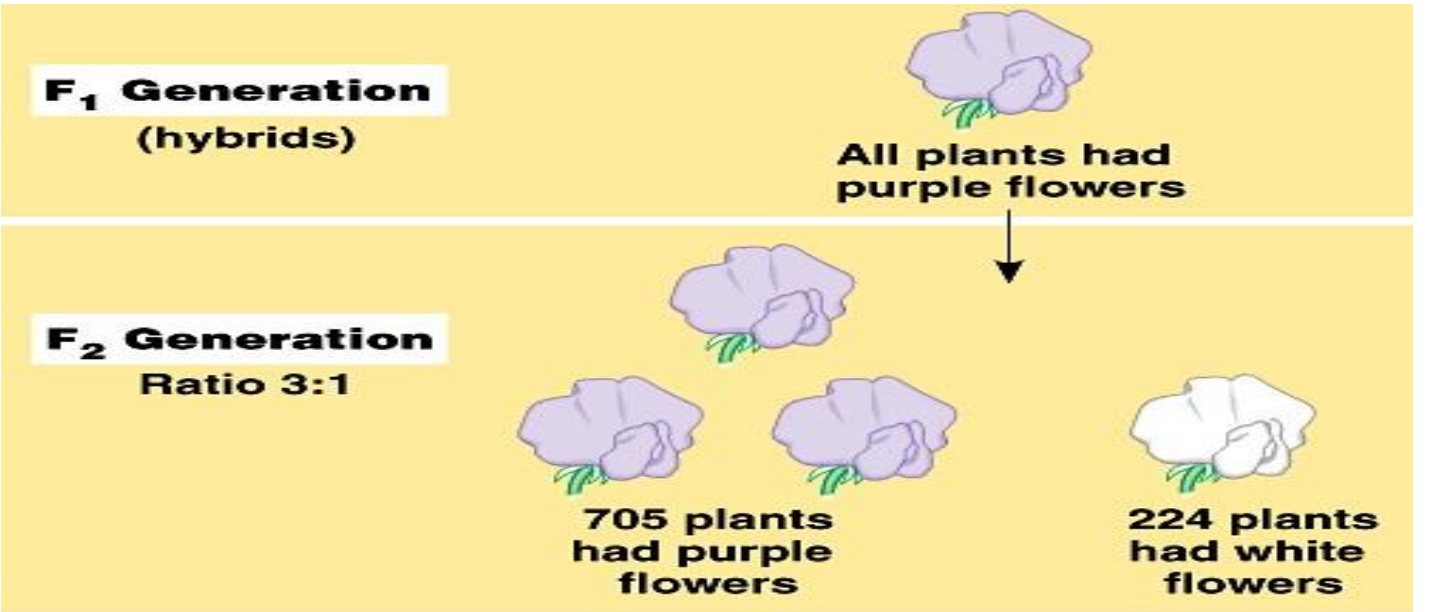
It can concluded that hybrids will show dominant phenotype

Example:

PP = purple pp = white Pp = purple

Law of dominance and current model of inheritance

Plants of F1 generation were interbred which produced F2 plants. Lost-trait was re-appeared that was the formulation of the current model of inheritance.

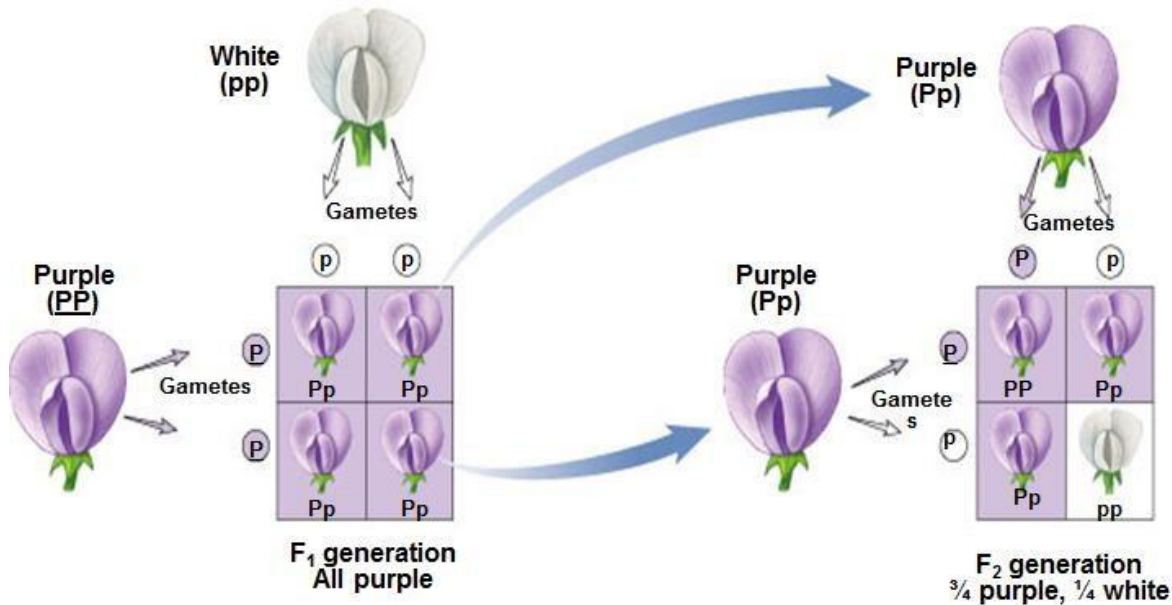


Every gene has two alleles that can code for a trait. One allele is dominant while other allele is recessive. The gene for a particular inherited character resides at a specific locus (position) on homologous chromosome. For each character, an organism inherits two alleles, one from each of the parent.

5 Monohybrid Cross and Dihybrid Cross

Monohybrid cross and Dihybrid cross

Cross-fertilization of true-breeding plants which are different in just one character is called as monohybrid cross. With monohybrid cross, Mendel determined the segregation of alleles at single gene locus.

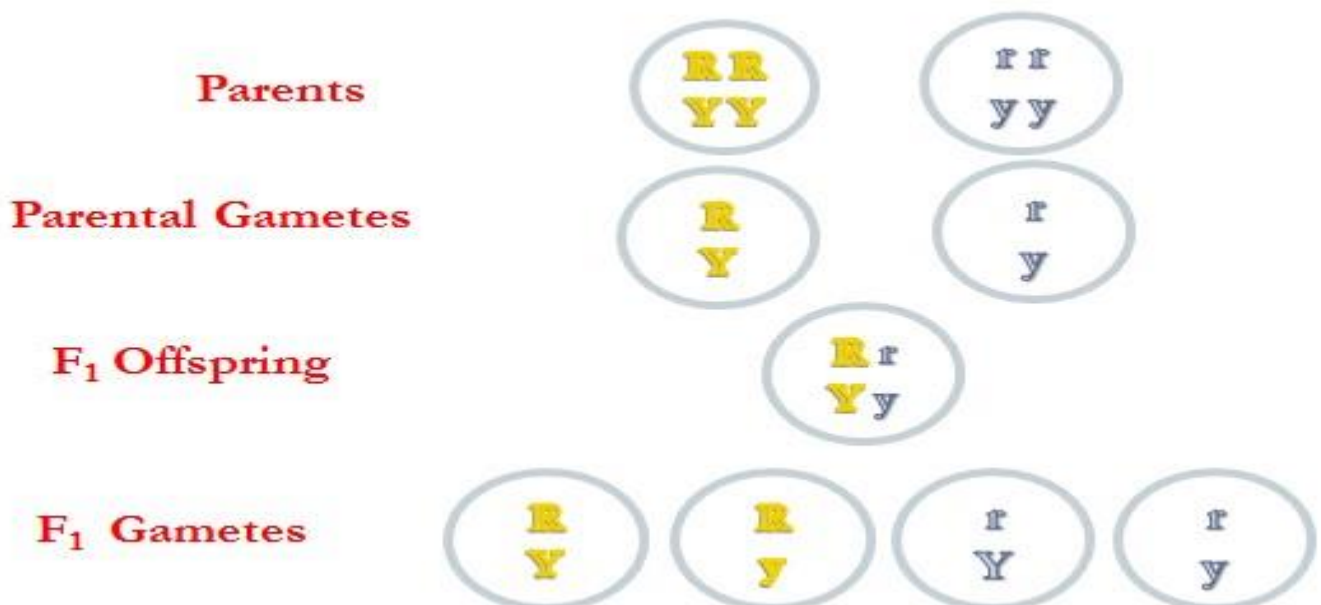


Dihybrid cross determines that alleles at two different gene loci segregate dependently or independently. Segregation is dependent or independent ?

Example:

Seed shape is controlled by one gene while seed color is controlled by a different gene. Mendel crossed two pure-breeding plants: one with round/yellow seeds, other with green/wrinkled seeds.

Dependent segregation: Alleles at the two gene loci segregate together, and are transmitted as a unit.



Independent assortment: Alleles at the two gene loci segregate independently, and are not transmitted as a unit. Plants producing gametes with different allele combinations.

Independent assortment

How are two characters transmitted from parents to offspring, if they are away from each other or on different chromosomes? They will transmit

- As a package?

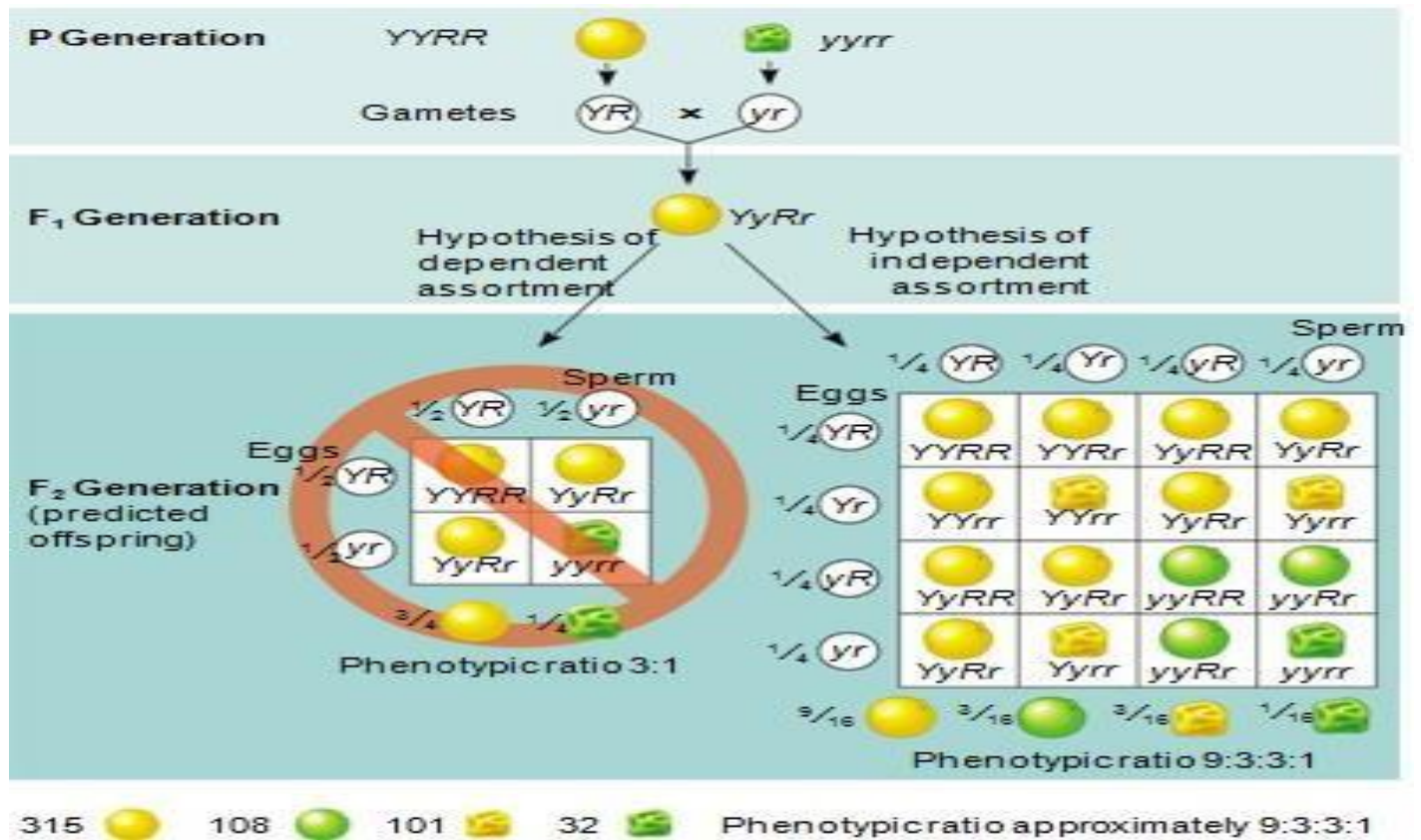
- Independently?

Answer: independently

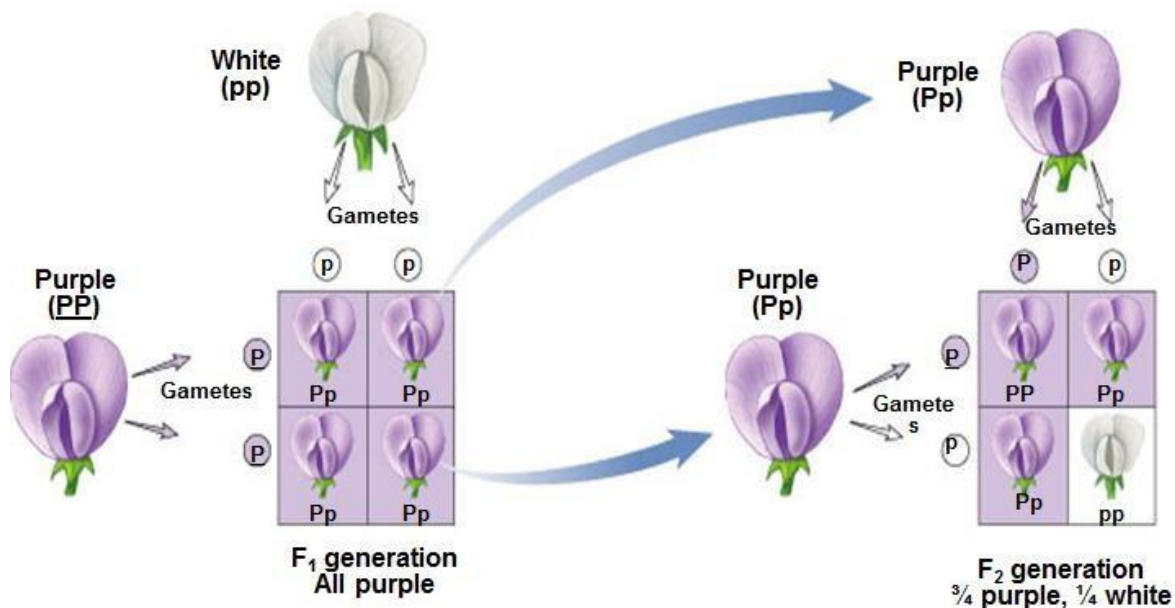
6 Mendel s' law of Segregations

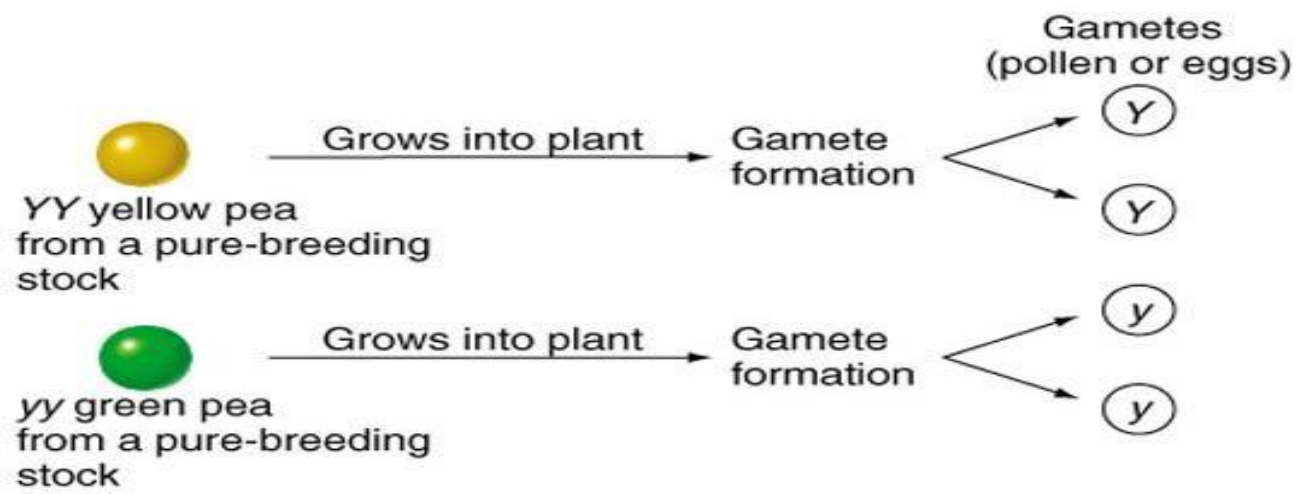
During the formation of gametes, the two alleles responsible for a trait separate from each other. Alleles are then "recombined" during the process of fertilization.

Example 1

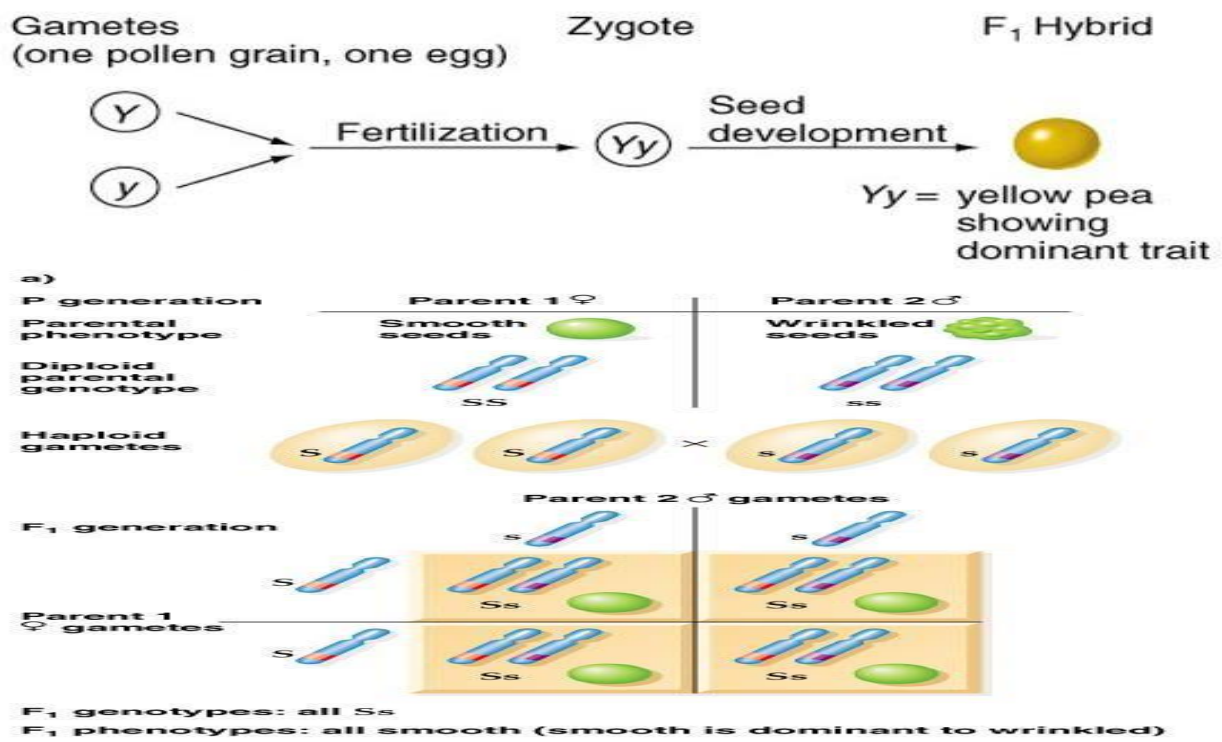


During a cross, gametes of each parent first segregate. Then, gametes combine to give new genotype. Resulting phenotype will be according to genotype.





Example 2: Gametes formation, where alleles separate.



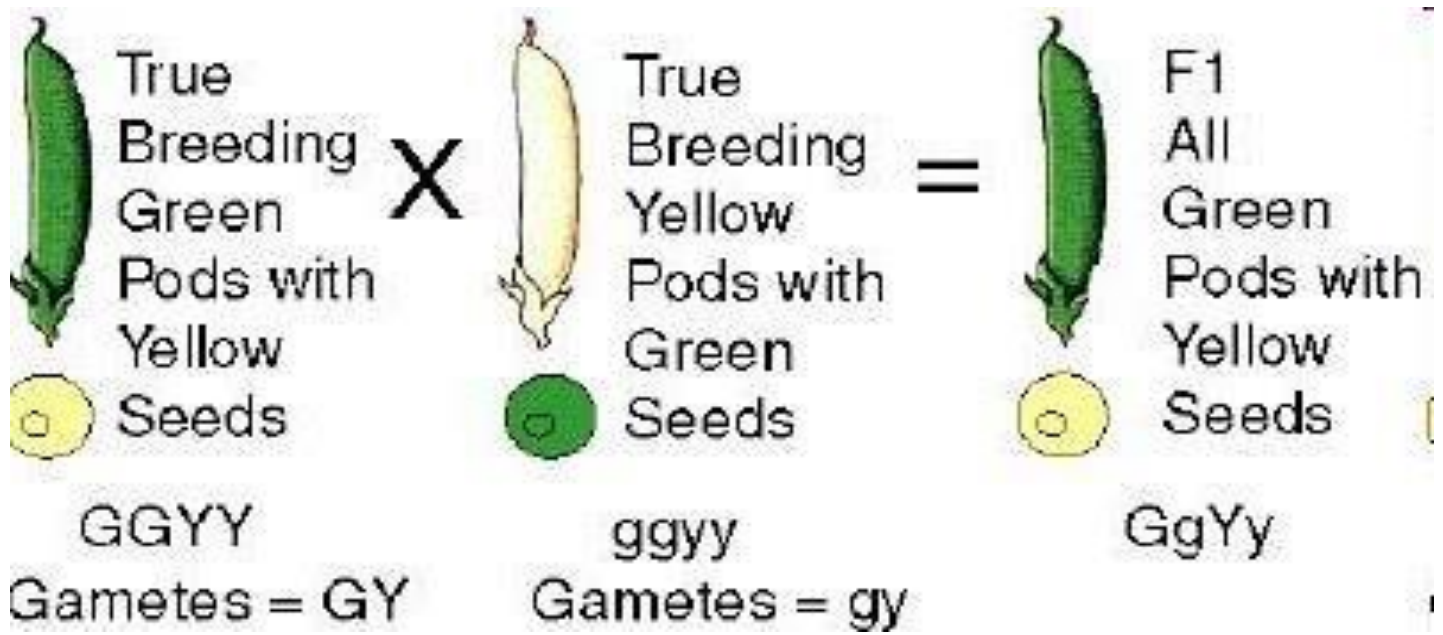
During fertilization, gametes re-unite.
 Modern Concept of Segregation states that every gene has two alleles that can code for a trait.
 Alleles separate during gametogenesis and reunite during fertilization

7 Mendel s' law of Independent Assortment

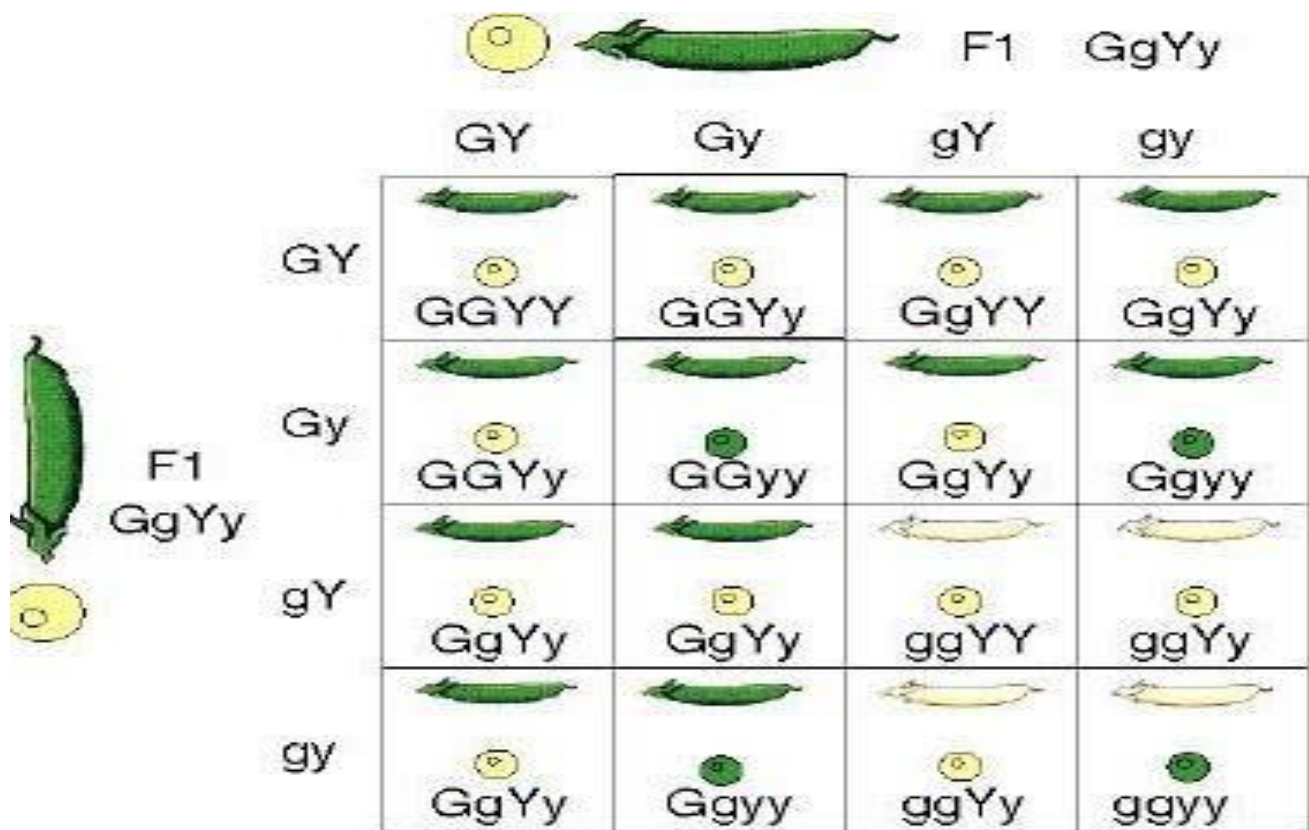
Mendel s' Law of Independent Assortment

Alleles for different traits are distributed to sex cells independently of one another. Traits are transmitted to offspring independently of one another.

Example: Dihybrid cross, true-breeding plants for two traits. For example, a plant that had green pod color and yellow seed color was cross-pollinated with a plant that had yellow pod color and green seeds. The traits for green pod color (GG) and yellow seed color (YY) are dominant. Yellow pod color (gg) and green seed color (yy) are recessive. F1 plants heterozygous GgYy

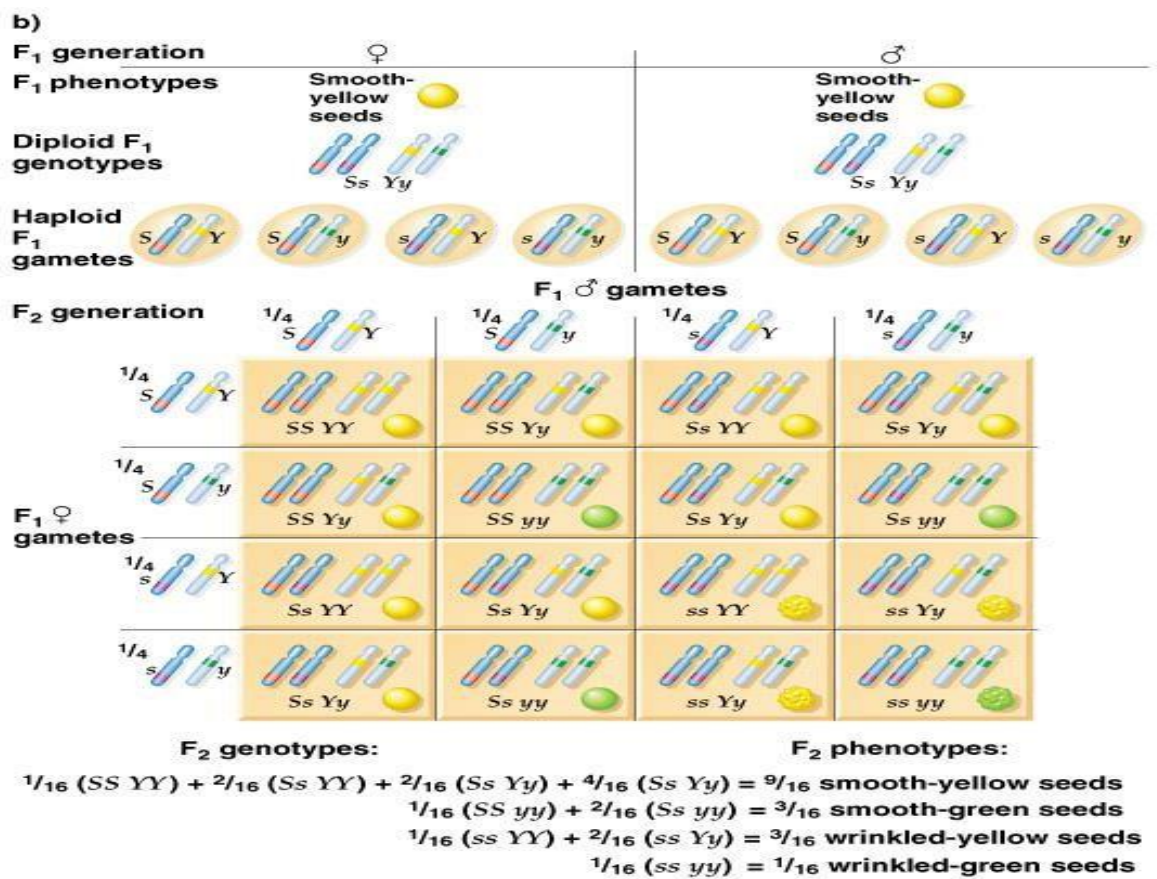


After observing the results of dihybrid cross, Mendel allowed all of the F1 plants to selfpollinate. He referred to these offspring as F2 generation. Mendel noticed a 9:3:3:1 ratio.



The results support the hypothesis of independent assortment. The alleles for seed color and seed shape sort into gametes independently of each other.

Modern Concept of Law of Independent Assortment

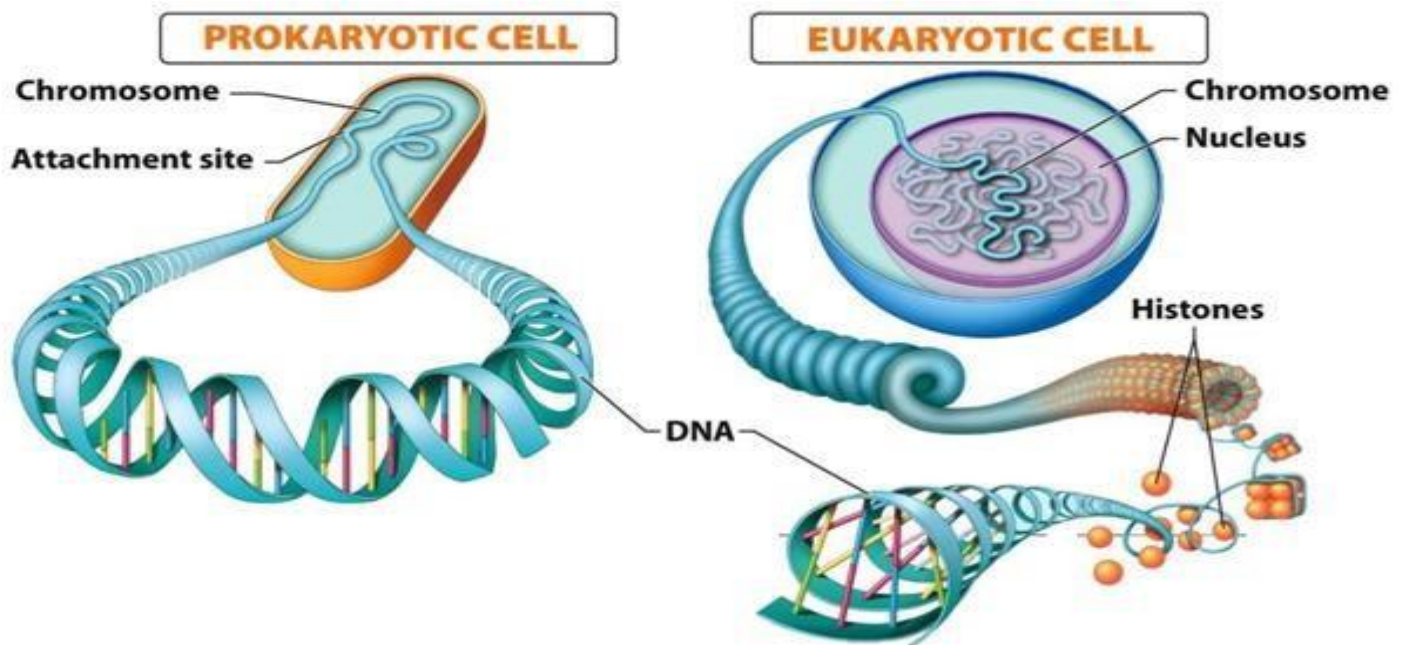


8 Modern Genetics Concepts

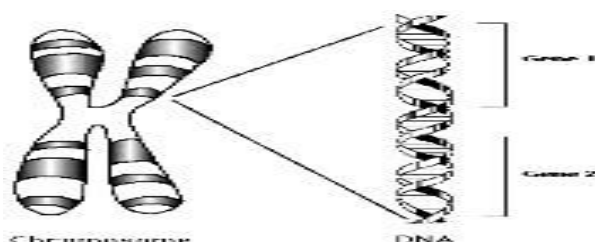
Modern Genetics Concepts

- Chromosomes and Gene (s)
- Mendelian Genetics and Non-Mendelian Genetics
- Genotypes and Phenotypes
- Mutations and Polymorphism

What is chromosome: Genetic material in cells is organized into chromosomes. Prokaryotes generally have one circular chromosome. Eukaryotes - linear chromosomes in their nuclei.






What is gene : A heredity unit on chromosomes.




Mendelian genetics: Mendelian genetics - genes that obey Mendel's laws. Most genes follow a Mendelian pattern of inheritance, However, there are many that don't.

Non-Mendelian genetics: Patterns of inheritance that deviate from a Mendelian pattern. Linkage - Non-Mendelian. Maternal effect, epigenetic inheritance, Mitochondrial inheritance etc.

Genotype and phenotype: Genetic make-up of an organism while physical appearance of a organism.


Genotype	Phenotype
EE Homozygous dominant	Detached Earlobes 
Ee Heterozygous	Detached Earlobes 
ee Homozygous recessive	Attached Earlobes 

Phenotype = Blue Eyes



Genotype = **bb**

Phenotype = Brown Eyes



Genotype = **Bb** or **BB**

What is mutation: Indicate "a change" while in other disciplines it is used to indicate "a diseasecausing change". Mutations may be harmful, beneficial, may have no effect.

9 Importance of Genetics

Importance of Genetics: Genetics is important to everyone

All patients

Family history

Medical history

Scientists

Characteristic of our genetic data

Genetic information is Personal, Permanent and Predictive

Genetics helps us in many ways

Diagnostics and treatments

Predictive, we can do planning

Screening (newborn, selected population, carriers)

Pharmacogenetic (individualized medicine)

Long term effects of genetics

Insurance

Employment

Disability

Medical care

Lifestyle, Family/children

Genetics and evolution: Genetics helps us to understand the process of evolution.

10 Heredity

What is Heredity?

- Transmission of traits from parents to offspring.
- Is transmission blending or particulate.

Is inheritance blending or particulate

- Till 19th century ...biologists believed, it was blending.
- Problematic, new genetic variations would quickly be diluted, not passed onward.

Blending hypothesis

- According to blending hypothesis, genetic material of the parents mixes during fertilization.
- Blue and yellow paints blend to make green paint.

Particulate hypothesis

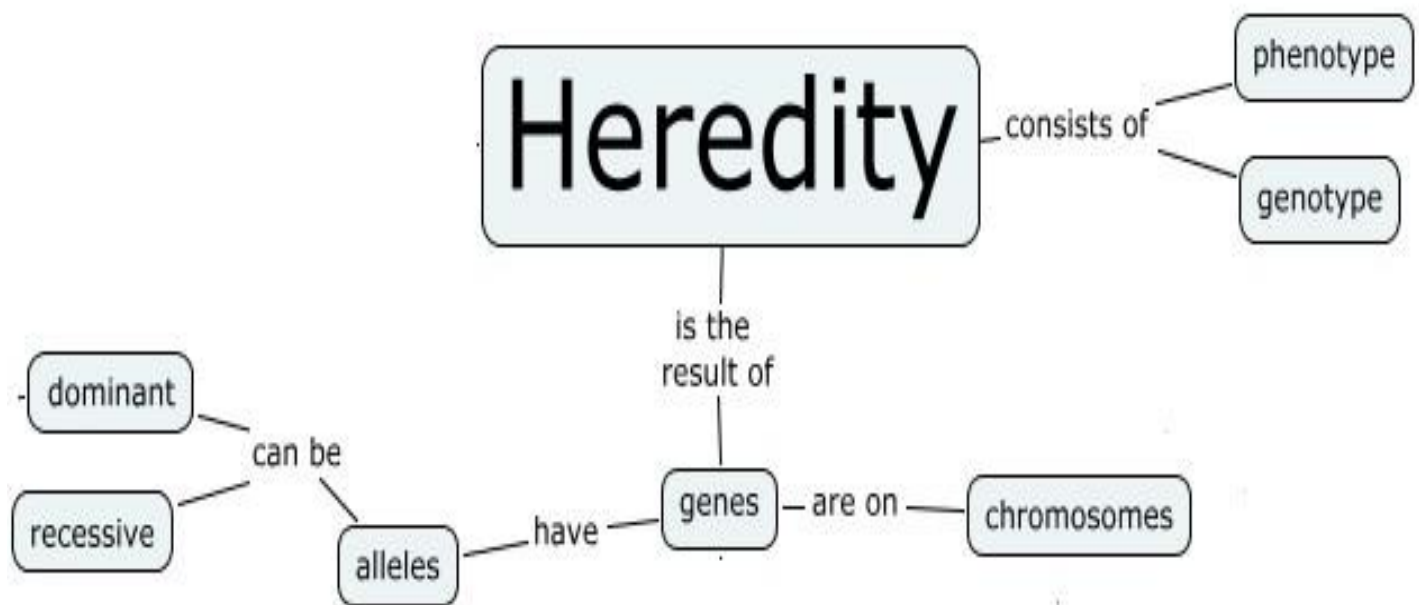
- According to particulate hypothesis, parents pass on distinct heritable units.
- Those distinct heritable units are now called as genes.

Particulate inheritance

- Mendel showed characters, or what we now call alleles, were inherited unchanged.
- Pattern of inheritance of these characters gave us the first definition of a gene.

Mendel and particulate inheritance

- Mendelian inheritance patterns involve genes that obey Mendel's laws. Most genes follow a Mendelian pattern of inheritance, However, there are many that don't.



Heredity

- Heredity is transmission of traits from parents to offspring.
- Transmission is particulate.

11 Variation

Concept of variation

- Differences can be seen in individuals of the same species, such as height, weight, color etc.
- Differences due to influence of genotype and environment.

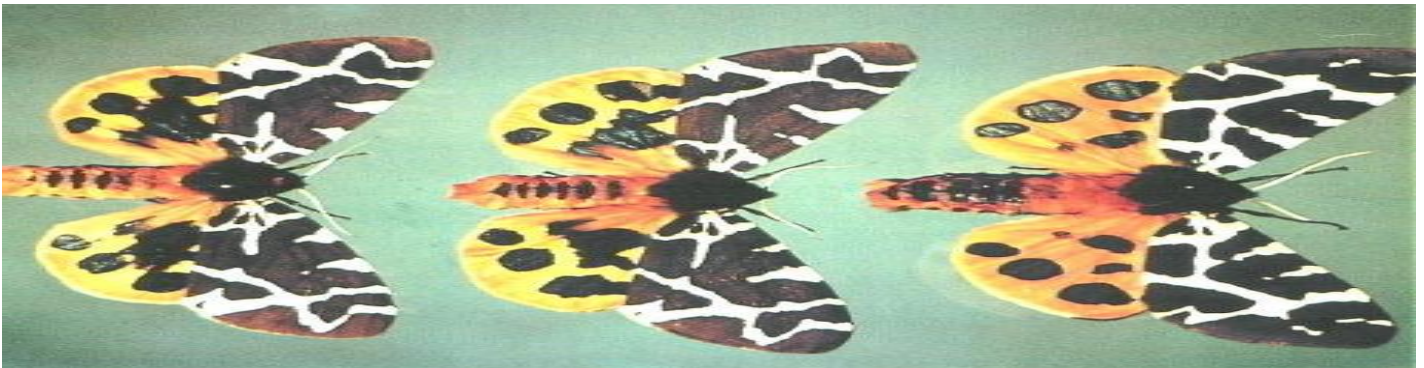
Example: snail shells

- Their color, presence/absence of bands, number of bands etc



Tiger moths

- These tiger moths are from the same family. What variations can you see ?



Example - tiger moths

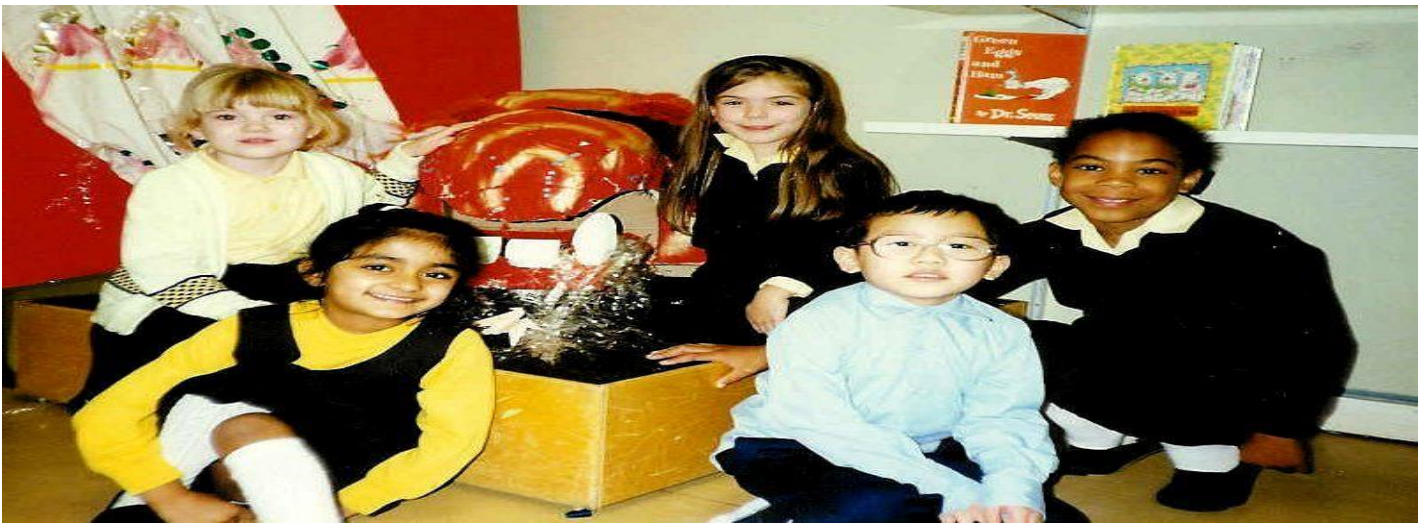
- Uppermost moth larger;
- Dark bands on abdomen differ in numbers and thickness;
- Patterns of forewings are quite different from each other;
- Number, shape distribution of black spots on hind wings.

Variation In humans

- ☐ You will already be familiar with many of the variations between individuals - humans (Homo sapiens).

Examples:

- ☐ Variations in skin color
- ☐ Variation in hair color
- ☐ Variation in hair curliness
- ☐ Variation in eye color
- ☐ Variation in sex



Conclusion

- It can be concluded that variation can be seen among individuals of the same species. It can be due to genetic makeup, environment or by combination of both.

12 Genetic and Acquired Variation

Genetic and acquired variation

What are Inherited Variations?

- Inherited variations result from the activity of genes.
- Genetically controlled and cannot be altered.
- Blood groups, finger prints and sex etc.

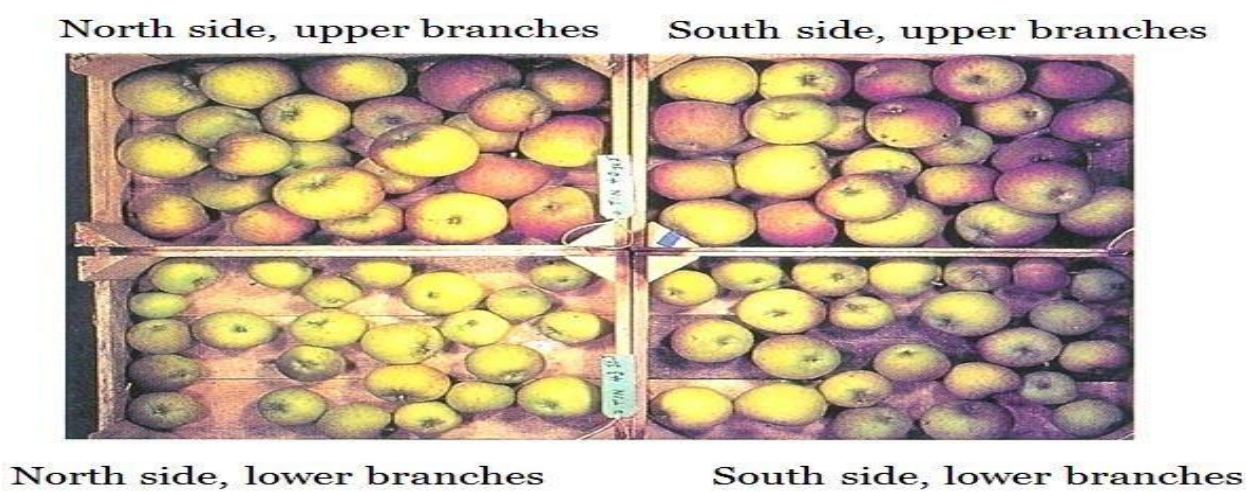
Acquired Variation

- Acquired variations result from an individual's activities or nutrition or from environmental conditions during a lifetime.

Acquired Variation

- Acquired characteristics can not be inherited. Language, obesity, athletic skills etc

Example: Apples - different position on same tree



Acquired variation

- ☐ The differences between the apples are acquired during the growing season.
- ☐ The differences will not be inherited.

Why this variation

- ☐ Environmental conditions caused the differences in size of the apples ?
- ☐ The upper branches more sunlight than lower branches.

Variation by genetic and environment

- Many variations - both genetic and environmental.
- Height depend on what genes you inherit and the amount of food you get.

Conclusion

- Variation can be seen among individuals of same species.
- It can be due to genetic makeup, environment or by combination of both.

13 Types of Variation

Types of variation: Two types of Variation

- Discontinuous Variation
- Continuous Variation

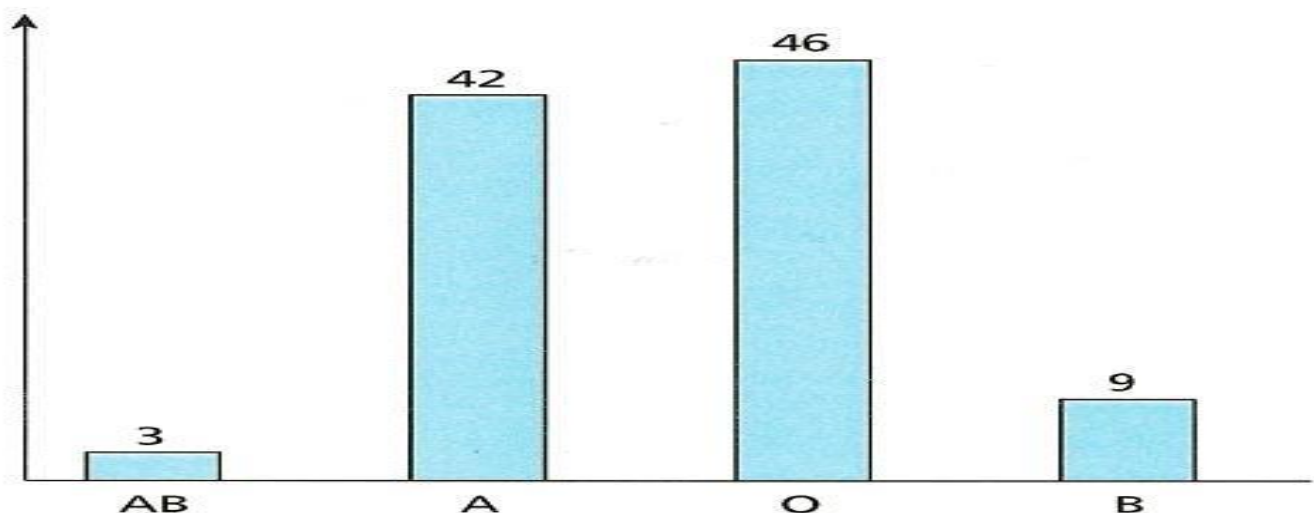
Discontinuous Variation

Discontinuous variations are entirely genetically controlled.

They cannot be altered by external conditions. Blood group, color blind, dwarfism are genetically controlled.

Graph for discontinuous

- Discontinuous variation in blood group. The figures ...not to fit a smooth curve.



Continuous variation

Continuous variation describes the situation in which there are many intermediates between the extremes. Shade of hair color between black and blond.



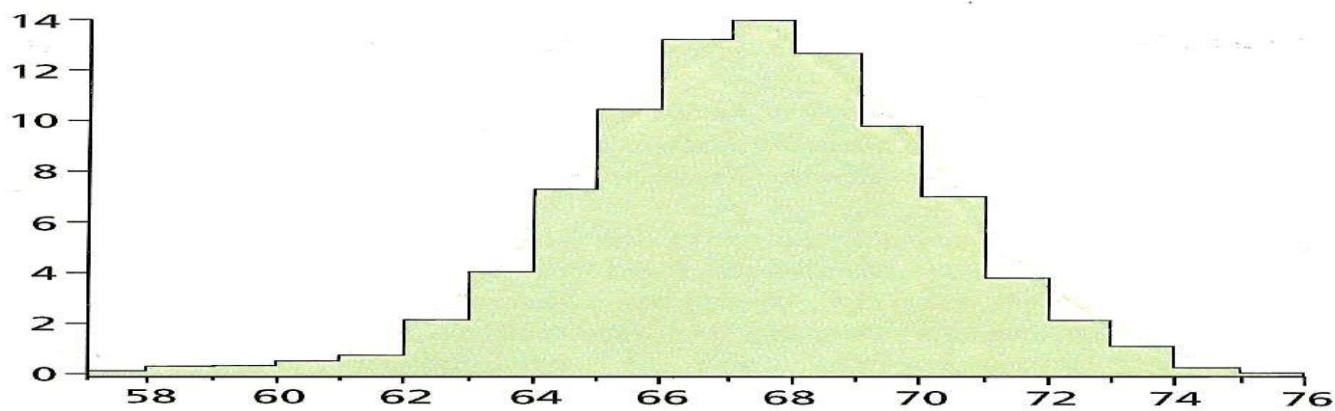
Hair color is continuous variation

Several pair of genes involved

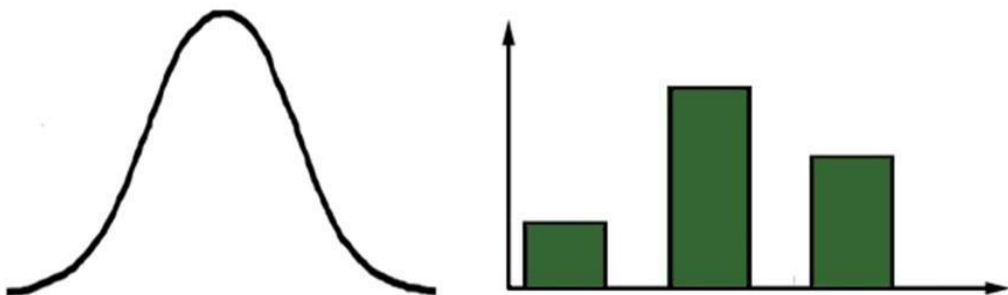
- The genome AA BB CC DD -black hair while the genome aa bb cc dd - blond hair.
- Genomes AaBbCcDd or AABbCCdd or aaBBccDd and all the other possible combinations – intermediate.

Factors - continuous Variation

- Continuous variation occurs –when controlled by the genes and the environment.
- Height depend on the genes and on the amount of food you eat during your growing.



Graph-continuous Variation - Smooth curve graph - height



- Continuous Variation**
- No distinct catagories
 - Tends to be quantitative
 - Controlled by a lot of genes
 - Strongly influenced by the environment

- Discontinuous Variation**
- Distinct catagories
 - Tends to be qualitative
 - Controlled by a few genes
 - Unaffected by the environment

14 Source of variation

Source of variation

Causes of Variation

- Genetic causes of variation
- Environmental causes of variation

Genetic causes of variation

- Meiosis
- Sexual reproduction
- Mutations

Environmental causes of variation

- Nutrients
- Drugs
- Temperature
- Physical training

GENETIC CAUSES OF VARIATION	ENVIRONMENTAL CAUSES
Meiosis – here pairs of chromosomes exchange genes & split from each other producing gametes which are not identical	Nutrients – the food we eat & minerals available to plants
Sexual reproduction – allows for recombination of genetic material from each parent in new ways in the zygote	Drugs – e.g. thalidomide was given to pregnant women in 1960s caused deformities
Mutations – these are random mistakes in the replication of DNA which make the new DNA slightly different to the original	Temperature – affects the rate of enzyme-controlled rxns, e.g. plants grown in warm conditions show faster rates of photosynthesis hence grow faster and better than those in colder environments
	Physical training – when you use muscles you increase their size & power e.g. athletes develop strong muscles as they train for their sport

Conclusion

- Variation from genetic causes are inherited from parents to offspring.
- Inherited variation - raw material for natural selection.
- Variations from environmental causes are not inherited.
- Variations that result from acquired characteristics are not acted upon by natural selection & thus do not affect evolution.

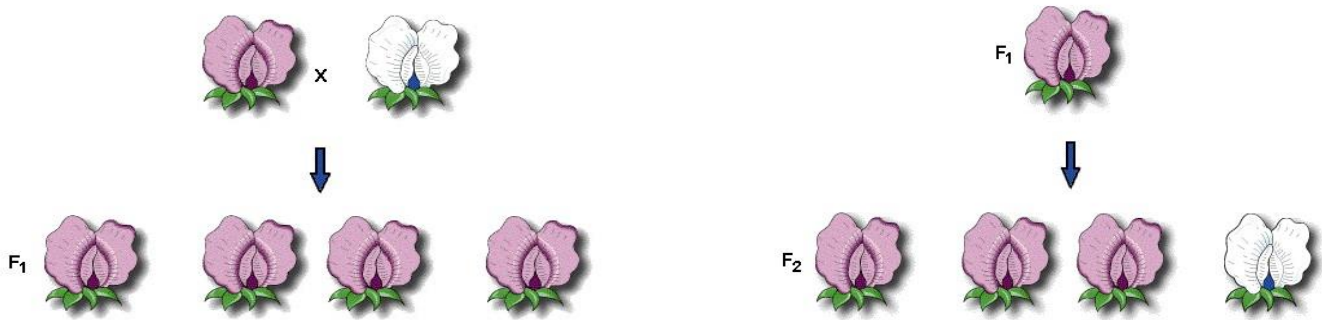
15 Heredity and Variation in Mendel s’ work

Heredity and variation in Mendel s’ work

Heredity in Mendel Work

- Gregor Mendel – inheritance how characteristics were passed ...generation.
- Pure-breeding plants e.g. tall plants produced only tall offspring & purple flowered plants produced only purple.

- There was a clear indication of heredity and variation in his work.



Both heredity and variation may be seen

- Variation is widespread in nature.
- Variation is inherited according to genetic laws and not solely by chance.

16 Mendelian and Non-Mendelian Inheritance

Mendelian and Non-Mendelian inheritance

Mendelian inheritance

- Gregor Mendel's model of inheritance describes □ Each trait is controlled by a single gene.
- Each gene has two alleles.
- A clear dominant-recessive relationship between alleles.
- Mendelian inheritance involve the genes that obey Mendel's laws: Law of dominance Law of segregation Law of independent assortment.

Mendelian inheritance

- Phenotypes will obey patterns;
- Autosomal dominant
- Autosomal recessive
- X-linked etc.

Non-Mendelian inheritance

- Pattern of inheritance that deviate from Mendelian pattern of inheritance is called as Non-Mendelian inheritance.
- Those genes that do not follow principles of heredity formulated by Gregor Mendel.

Examples of Non-Mendelian inheritance

- Mitochondrial inheritance
- Genomic (parental) imprinting
- Incomplete dominance
- Co-dominance
- Multiple alleles
- Epigenetics

17 Conclusions of Mendelian Inheritance

Conclusions of Mendelian inheritance

Mendelian inheritance

- Mendel s’ conclusions were based on three laws:
- Law of Dominance
- Law of Segregation
- Law of Independent Assortment

Conclusion- law of dominance

- Cross of true-breeding strains resemble only one of the parents.
- Smooth seeds (S) are completely dominant to wrinkled seeds (s)

Conclusion - Law of segregation

- Two members of a gene pair segregate (separate) from each other during the formation of gametes.

Conclusion-Law of independent assortment

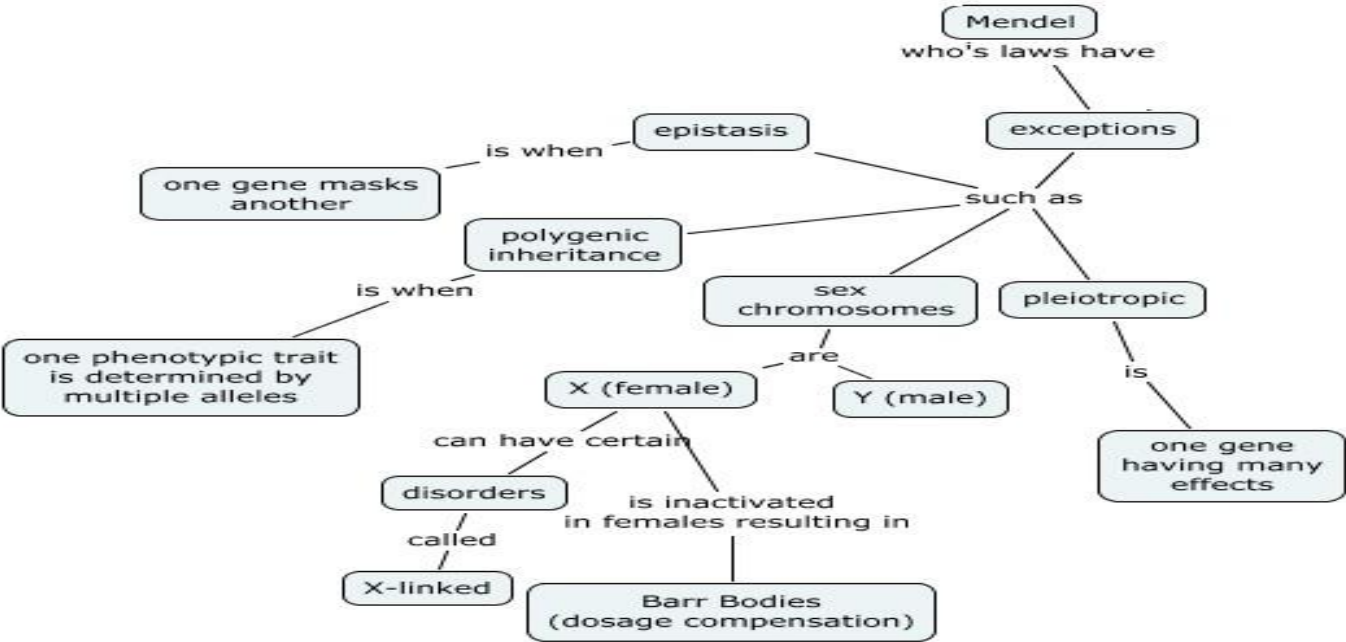
- Alleles for different traits assort independently of one another.
- Genes on different chromosomes independently behave.

18 Exceptions to Mendelian Inheritance

Exceptions to Mendelian inheritance

Exceptions to Mendelian inheritance

- Incomplete dominance
- Codominance
- Multiple alleles
- Polygenic traits
- Epistasis
- Pleiotropy
- Environmental effects on gene expression
- Linkage
- Sex linkage



19 Incomplete Dominance

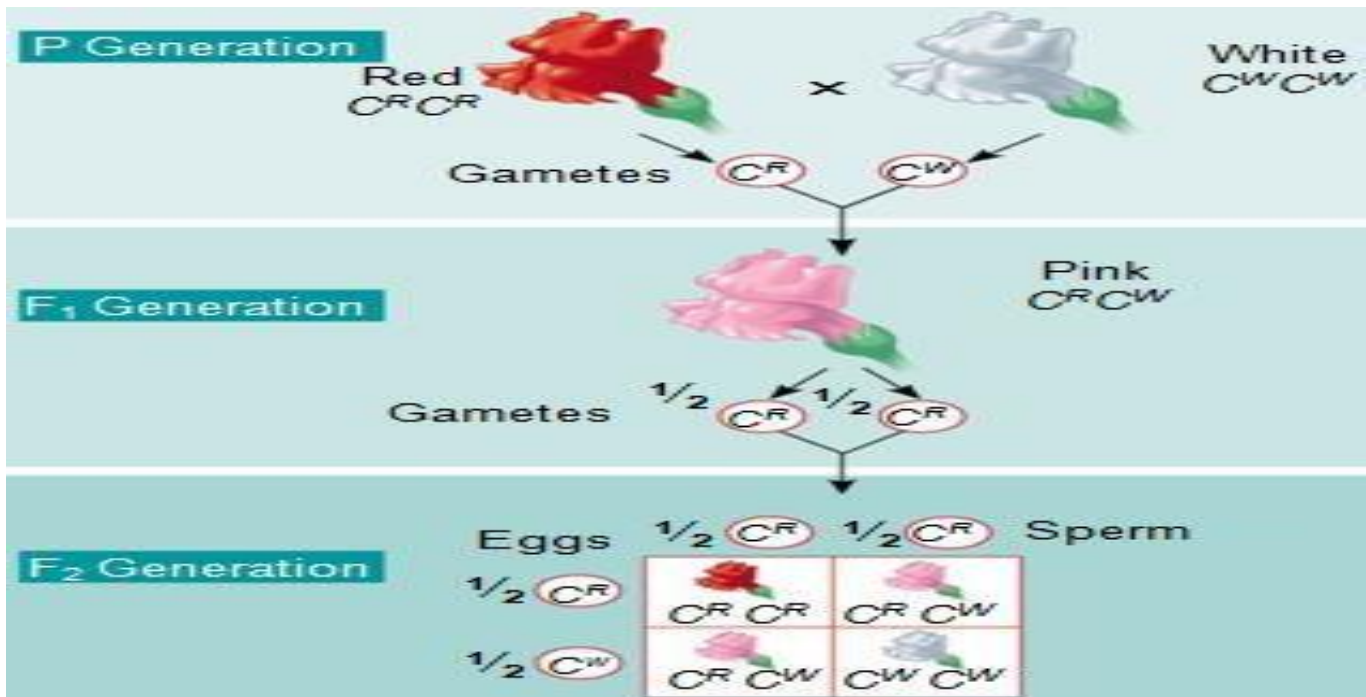
Incomplete dominance

- Some alleles for a gene are not completely dominant.
- An intermediate phenotype – between two extremes.

- None of the allele is dominant.

Incomplete dominance

- As none of the alleles is dominant. As a result of a cross, a “new” phenotype appears that is a blend of both alleles.



Example: Japanese 4 o' clock flowers

Genotypes of Japanese 4 o' clock plant

- Red flower plant genotype = **RR**
- White flower plant genotype = **WW**
- Pink flower plant genotype = **RW**

Incomplete dominance is a situation in which neither allele is dominant.





- New phenotype appears.
- New phenotype is blend of both alleles.

20 Co-dominance

Co-dominance

- A situation, when both alleles appear in the phenotype.
- Neither allele is dominant.
- Both alleles are expressed in a heterozygous individuals.
- Parallel behavior of both allele.

Example 1 : Blood group

Genotype	Phenotype (Blood Group)	Red Blood Cells
$I^A I^A$ or $I^A i$	A	
$I^B I^B$ or $I^B i$	B	
$I^A I^B$	AB	
ii	O	

Example 2: Roan cattle inheritance

- Roan color in cattle appears as a mix of red and white colors.

crcr = red hairs cwcw = white hairs

crcw = roan coat



Roan cattle inheritance

- Roan color in cattle appears as a mix of red and white.

Example 3: Appaloosa horse

- Gray horse dominant to white horse.
- Heterozygous is appaloosa –a white horse with gray spots.



- A situation, when both alleles appear in the phenotype and neither of the alleles is dominant.

21 Polygenic traits

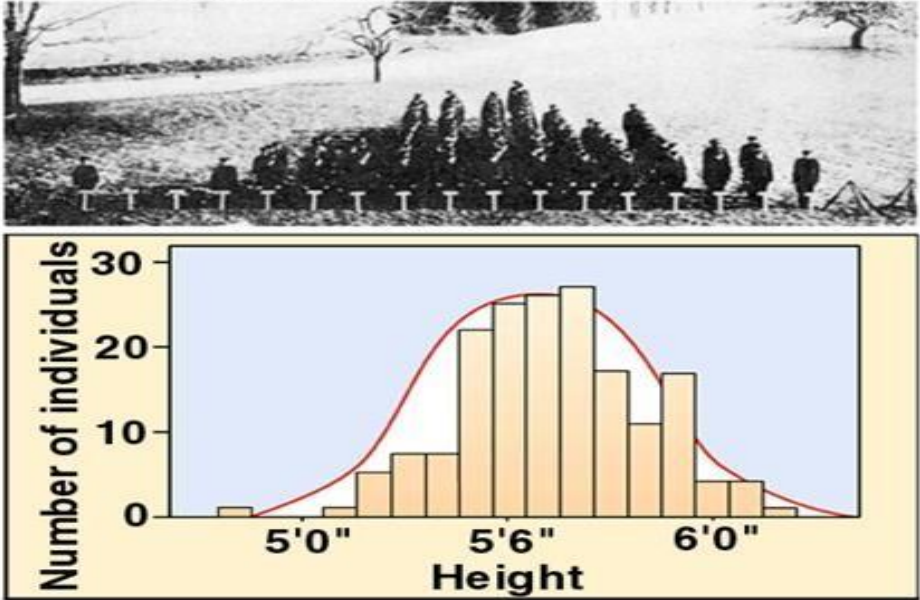
Polygenic traits

- Most traits are not controlled by a single gene locus, but by the combined interaction of many gene loci.
- These traits are called polygenic traits.

Example: Humans Height

- Graph for continuous variation

Continuous Variation



Example: Humans eyes color is controlled by many genes.

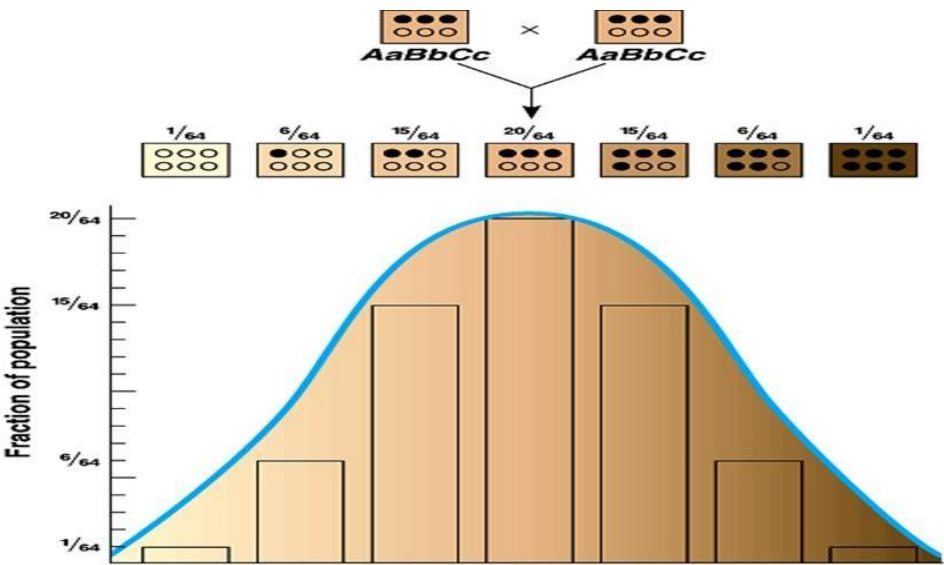


Pigmentations in humans

- Qualitative traits usually indicate polygenic inheritance; effect from two/more genes.
- Skin color pigmentation in humans is controlled by at least three separately inherited genes.

Pigmentation in humans

- Controlled by three genes.



Conclusion

- Polygenic traits are those traits which are controlled by additive effect of many genes.
- Qualitative traits are usually polygenic in nature.

22 Epistasis

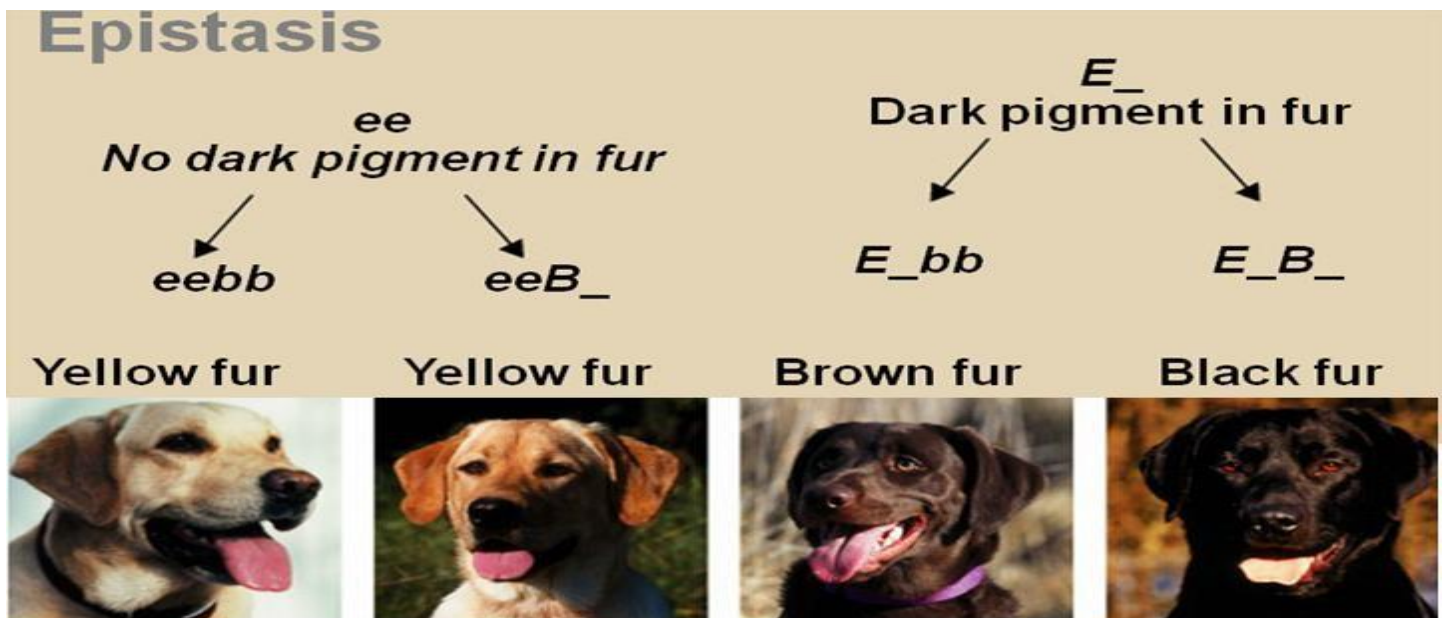
Epistasis

- Alleles at one gene locus can hide or prevent expression of alleles at second gene locus.

- Labrador retrievers one gene locus affects coat color by controlling pigment eumelanin deposited in the fur.

Example

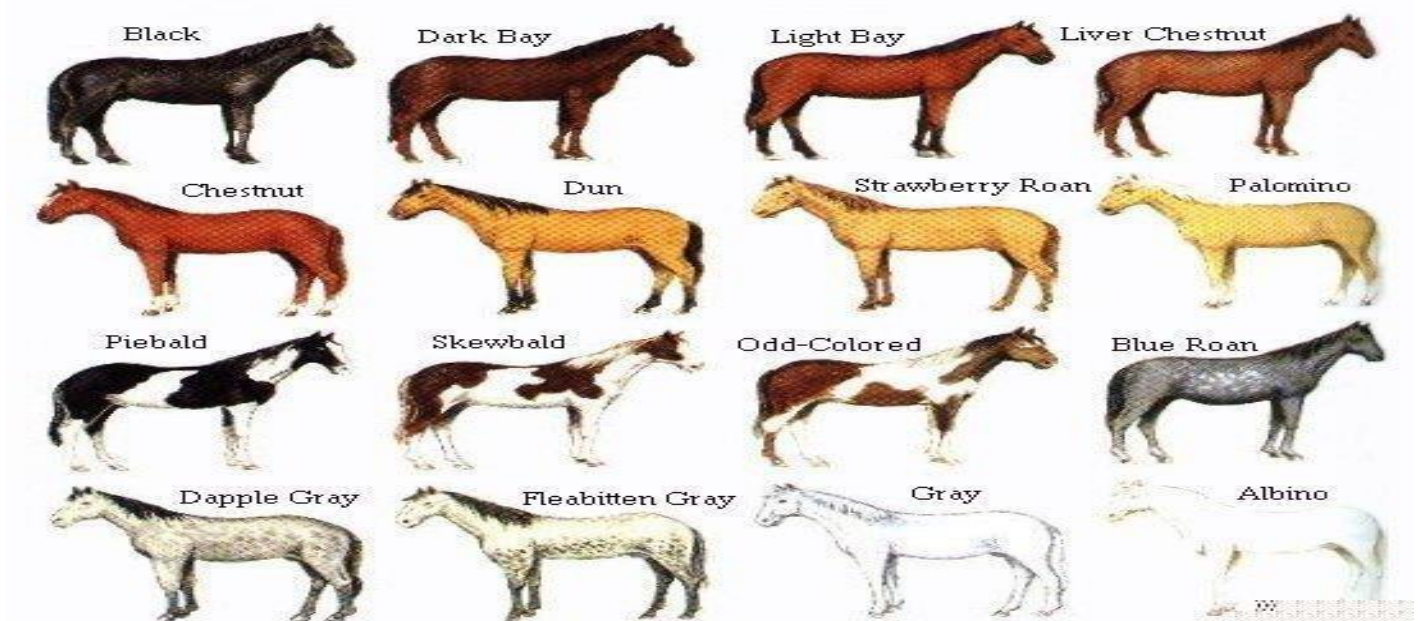
- A dominant allele B for black, recessive allele b brown coat, a second gene (e) locus controls eumelanin deposited in fur.
- Dogs homozygous recessive at this locus (ee) will have yellow fur no matter which alleles are at first locus.



Example: Epistasis in horses fur

- Horse coloration involves 2 or more gene pairings.
- EE or Ee is for black.
- ee is for red (sorrel).
- Other genes can add effects to base colors.
- Bay is AA, EE – black with agouti gene;
- Buckskin is AA, EE, CcrC – bay with cremello gene,
- Dun is AA, EE, Dd – bay with dun gene;
- Palomino is ee, CcrC – sorrel with cremello gene.

Equine's Color's and Marking's

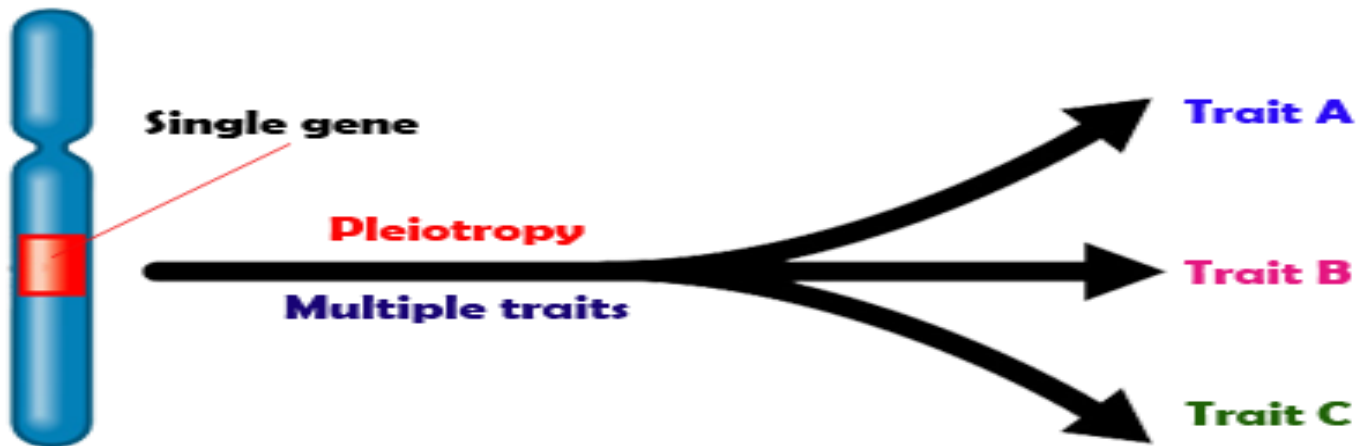


Alleles at one gene locus can hide or prevent expression of alleles at second gene locus is called as epistatsis.

23 Pleiotropy

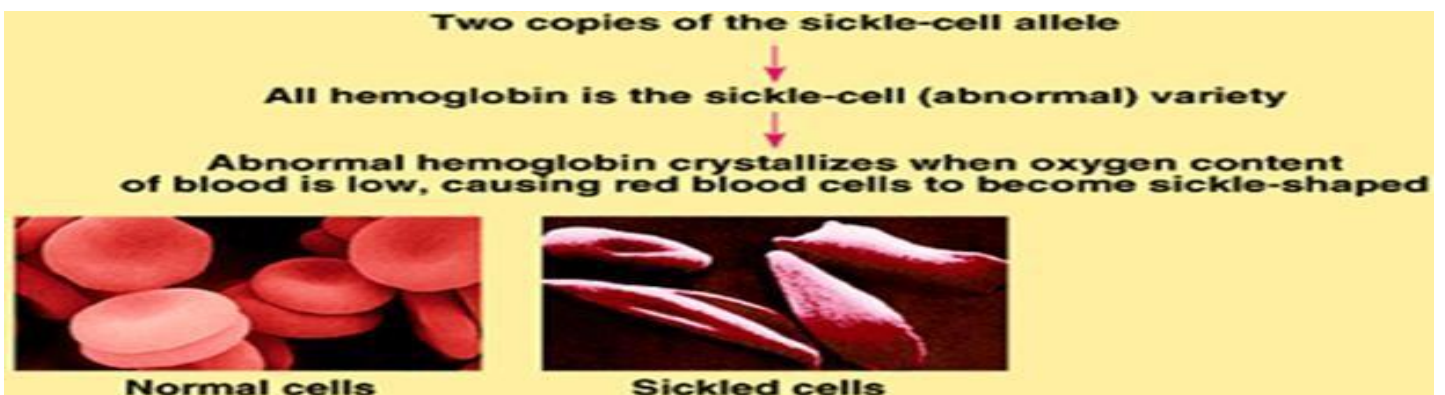
Pleiotropy

- The ability of a gene to affect an organism in many ways is called pleiotropy.
- Pleiotropy refers to an allele which has more than one effect on the phenotype.
- This phenomenon occurs when a single gene locus produces more than one traits.



Pleiotropic effects in humans

- Example:
 - Sickle cell anemia
 - Cystic fibrosis



Cystic fibrosis



Huntington disease

- One gene – more than one phenotypes

- **Phenotype 1:** progressive dementia at age of 40-50 years
- **Phenotype 2:** death in about 5 years after onset of disease.

24 Environmental Effects

Environmental effects

Environmental effects on Gene expression

- The phenotype of an organism depends not only on which genes it has (genotype), but also on the environment under which it develops.

Interaction - genotype and environment

- Although scientists agree that phenotype depends on a complex interaction between genotype and environment.
- Debate and controversy about the relative importance of both.

Environmental effects

- Allele expression may be affected by environmental conditions
- Examples:
 - Coat color in arctic foxes
 - Himalayan rabbits
 - Siamese cats

Explanation

- ch allele affected by temp >33 C tyrosinase enzyme inactivates and reduces melanin pigment.



Himalayan rabbits



Siamese cats



Environmental effect on phenotype

- pH of the soil will change the color of hydrangea flowers.



There is a strong impact of environment on the expression of certain traits.

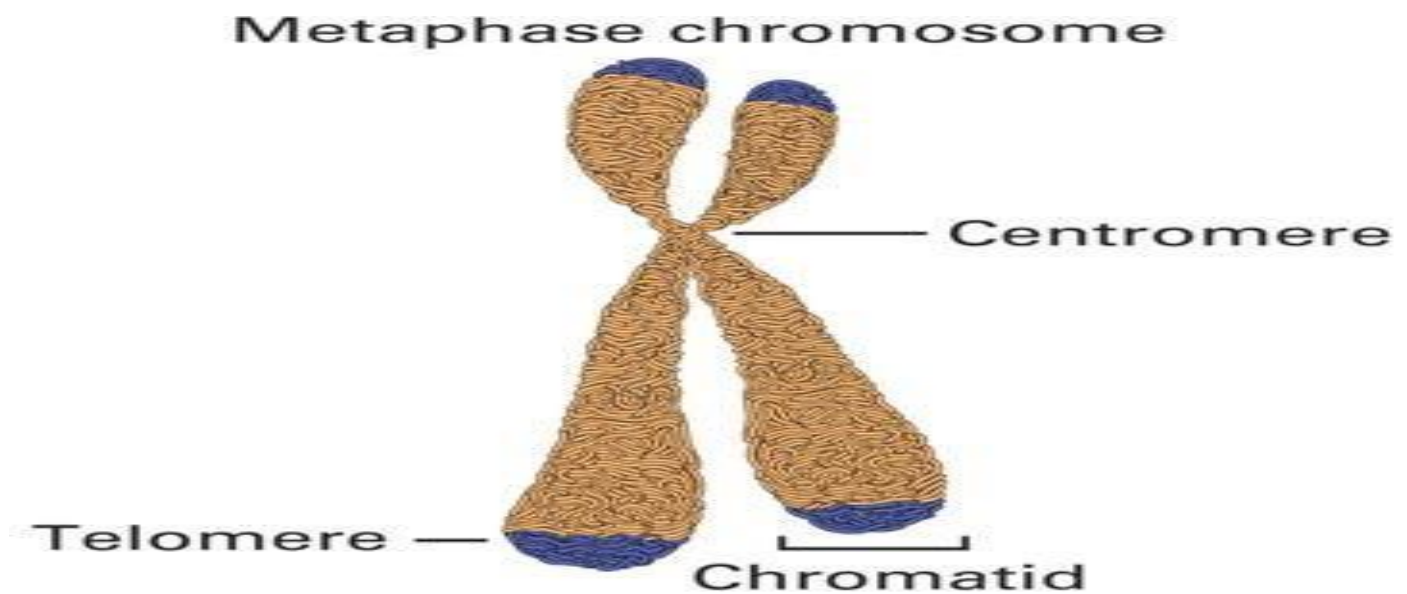
25 What are Chromosomes

What are Chromosomes:

Chromosomes are the rod-shaped, filamentous bodies present in the nucleus. They are the carriers of genes. Chromosome not visible in active nucleus due to high water content but can be seen during cell division. Chromosomes were described by Strausberger in 1875. Chromosome was first used by Waldeyer in 1888.

They were given the name chromosome (Chromo = color; Soma = body). Their numbers can be counted easily only during mitotic metaphase. Chromosomes are composed of thin chromatin threads called chromatin fibers.

Chromatin fibers folding Chromatin fibers undergo folding, coiling and supercoiling during prophase so that the chromosomes become progressively thicker and smaller and become visible. At the end of cell division, the fibers uncoil and extend as fine chromatin threads, which are not visible at light microscope.



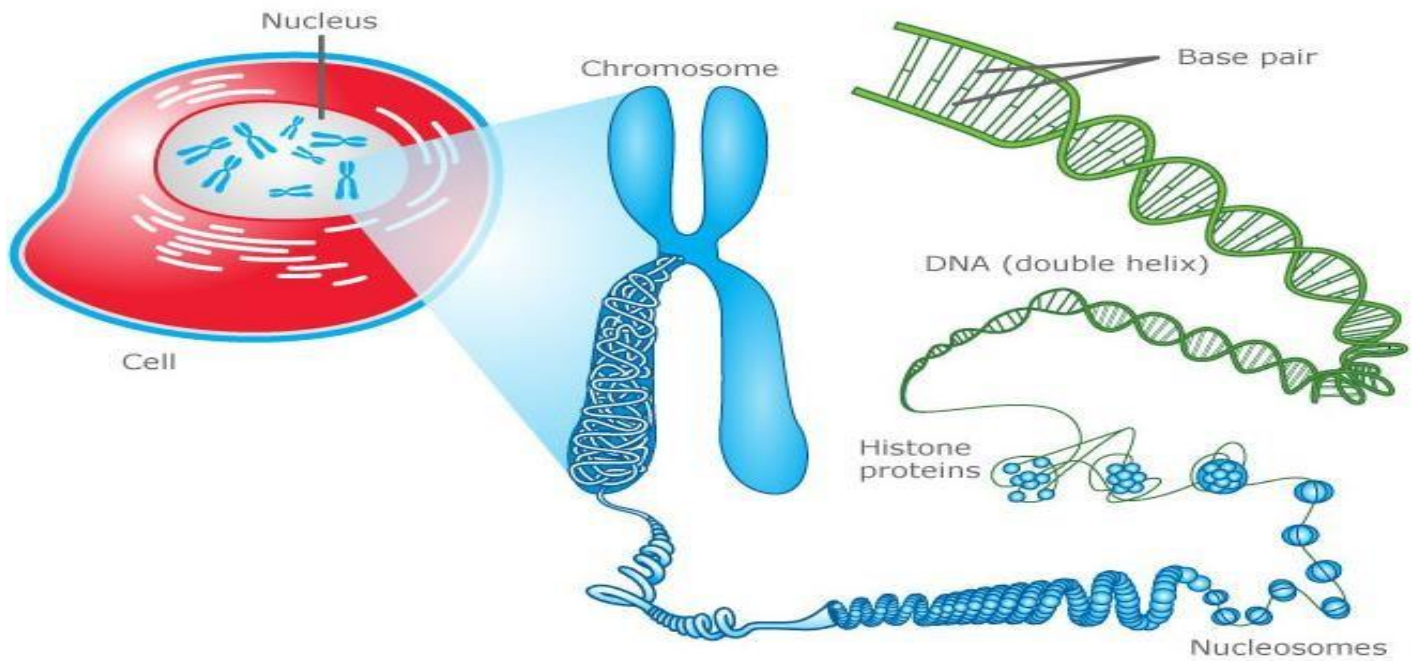
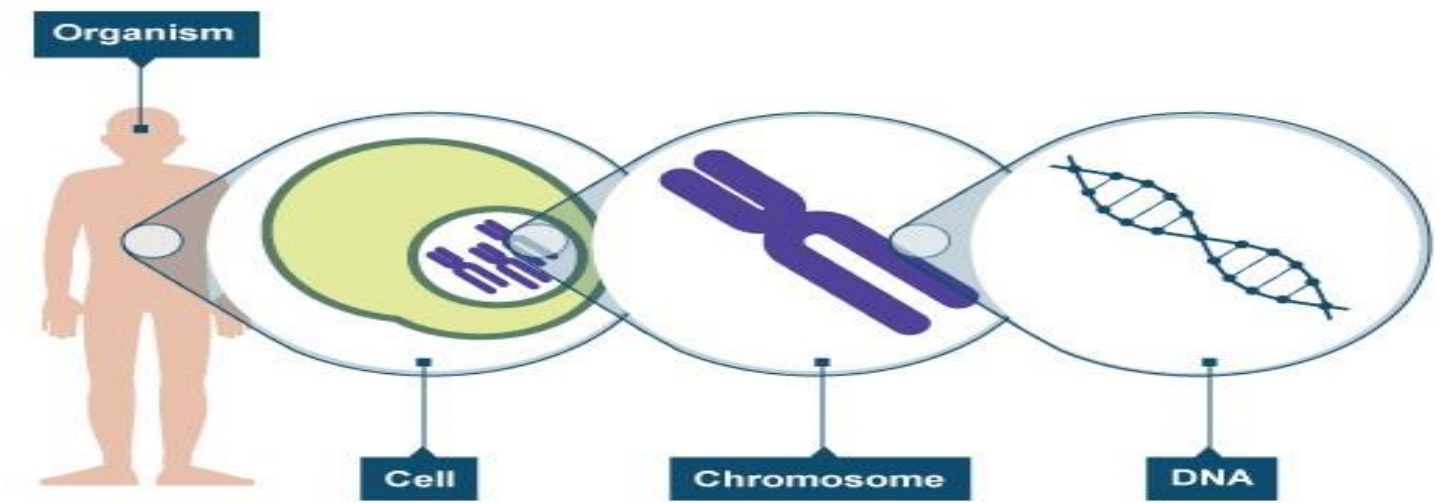
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□ Chroso

Chromosomes are rod shaped filamentous bodies present in the prokaryotic/eukaryotic cells.

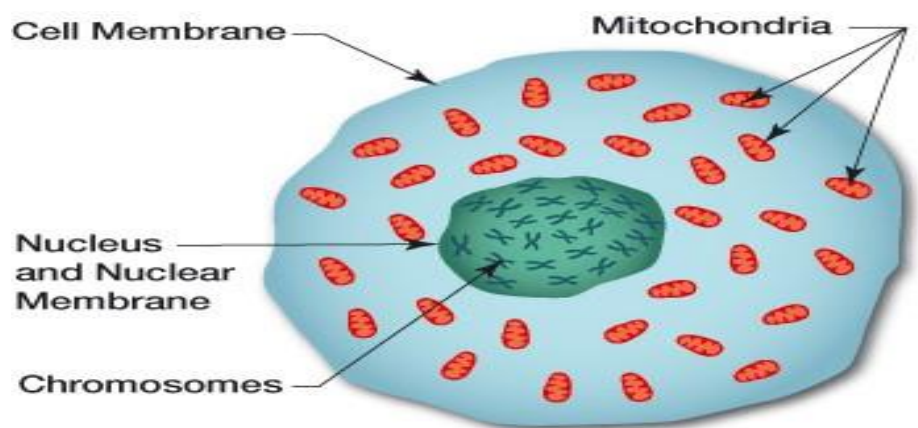
26 Cell, Nucleus and Chromosome

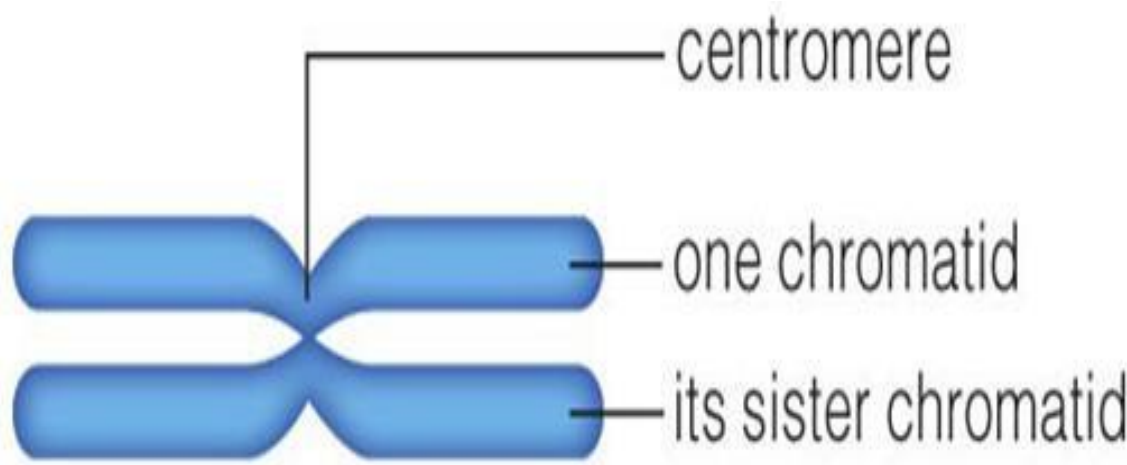
Chromosome Structure: Living organisms are made up of cells. Within cell, there is nucleus or nucleoid region. DNA or chromosomes are present in nucleus/nucleoid region.



Chromosome structure: chromosomes are made of DNA and associated proteins. Chromosome carries part or all of a cell's genetic information. Chromosomes have sister chromatids. Centromere is constricted region, where chromatids are attached.

A chromosome constitutes DNA molecule, a protein Histones and supercoiled DNA





27 Chromosome Numbers in Different Species

Variation in number of chromosomes:

Different organisms have different number of chromosomes. Most organisms have chromosomes numbers between 12 to 50. Round worm has only two chromosomes. Protozoa having 300 or more. Fungi have 3-8 chromosomes. Angiosperms have from 8 – 16 (most common number 12).

Chromosomes in humans: There are 46 chromosomes in humans. Humans have 23 pairs of chromosomes out of which 22 pair of autosomes and one pair of sex chromosomes.

Organism	No. chromosomes
<input type="checkbox"/> Human	46
<input type="checkbox"/> Chimpanzee	48
<input type="checkbox"/> Dog	78
<input type="checkbox"/> Horse	64
<input type="checkbox"/> Chicken	78
<input type="checkbox"/> Goldfish	94
<input type="checkbox"/> Fruit fly	8
<input type="checkbox"/> Mosquito	6
<input type="checkbox"/> Nematode	11(m), 12(f)
<input type="checkbox"/> Horsetail	216
<input type="checkbox"/> Sequoia	22
<input type="checkbox"/> Round worm	2

Organism	No. chromosomes
■ Onion	16
■ Mold	16
■ Carrot	20
<input type="checkbox"/> Tomato	24
<input type="checkbox"/> Tobacco	48
<input type="checkbox"/> Rice	24
<input type="checkbox"/> Maize	20

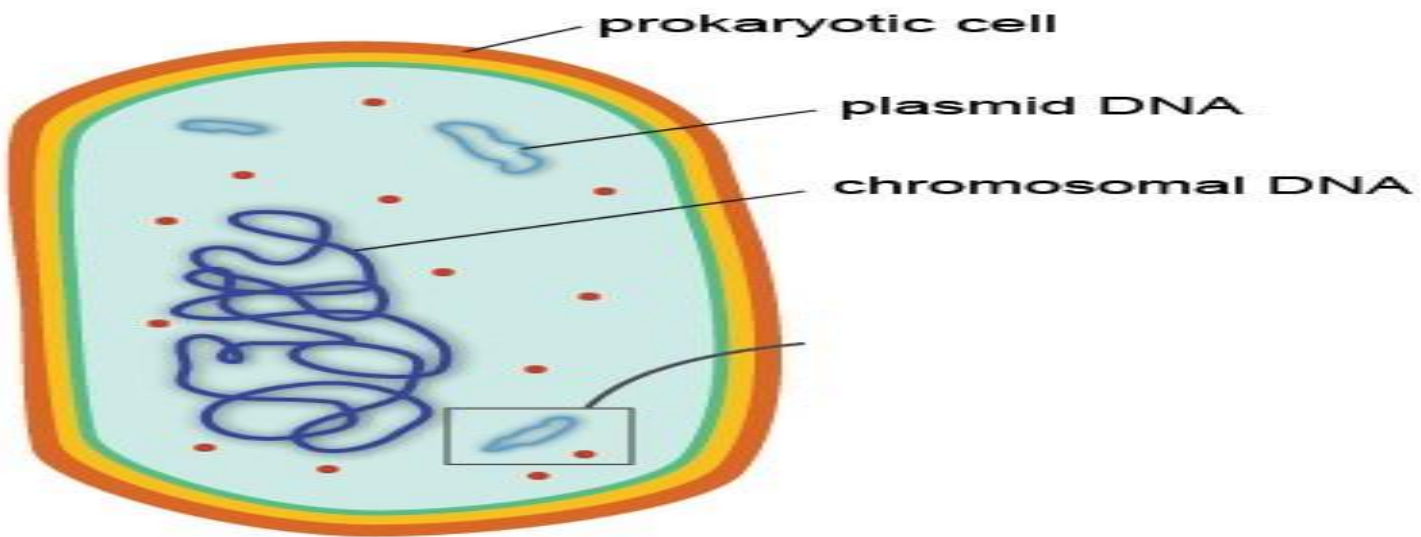
- Haploppus gracilis 4
- Crepis capillaris 6

28 Chromosomes in Prokaryotes and Eukaryotes

Chromosomes in prokaryotes and eukaryotes: Chromosomes are present in both kinds of cells. Although they are different in both type of cells.

Chromosomes in prokaryotes: Prokaryotes have single chromosome plus plasmids Circular chromosome is made up only of DNA which is found in cytoplasm

□ Chromosomes in prokaryotes



Chromosomes in Eukaryotes: There are many chromosomes in eukaryotes which are linear and made of chromatin, a nucleoprotein – histones. These chromosomes are found in a nucleus. Eukaryotic chromosomes are condensed and visible during cell division. At the beginning of mitosis can be seen - consist of two threads (sister chromatids) joined by a centromere. The sister chromatids are identical copies. During mitosis, the sister chromatids separate and are placed into two daughter nuclei.

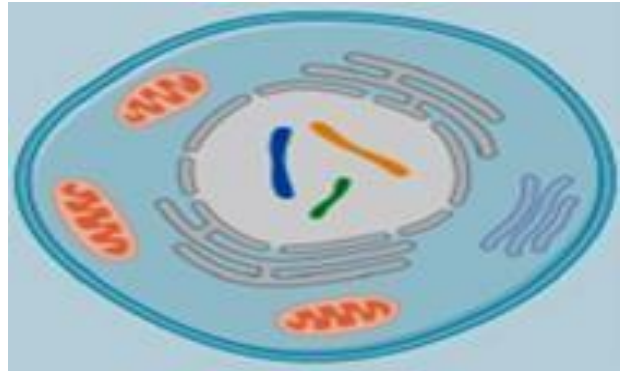
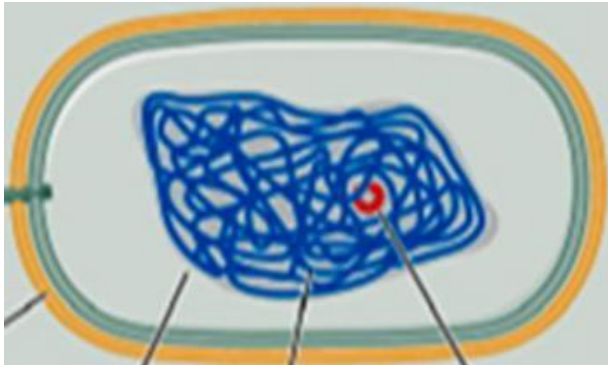
Comparison of prokaryotes and eukaryotes

PROKARYOTES	EUKARYOTES
single chromosome plus plasmids	many chromosomes
circular chromosome	linear chromosomes
made only of DNA	made of DNA and histones.
found in cytoplasm	found in a nucleus
copies its chromosome and divides immediately afterwards	copies chromosomes, then the cell grows, then goes through mitosis into two groups

29 Salient features of Chromosomes

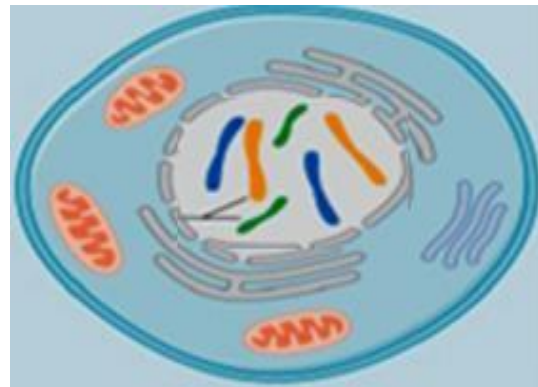
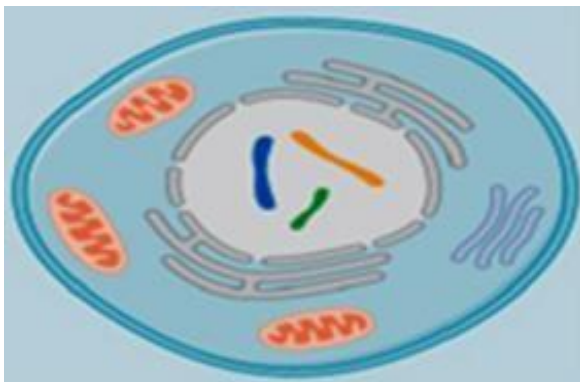
Salient features of Chromosomes: Followings are the salient features of chromosomes.

Shape of Chromosomes: Chromosomes can be circular or linear



Chromosomes numbers: Chromosome number is a characteristic of a species.

Copy number of Chromosomes: Chromosome can be haploid, diploid or polyploid.



Genes on chromosomes: Number of genes are different in each of the species.

- Humans 25,000 - 27,000
- Rice 45,000
- Drosophila 13,700
- Yeast 5,800

Gene density on Chromosomes: Prokaryotes has higher gene density than eukaryotes per Mb of DNA.

Genome characteristics: Prokaryotes have less genome size as compared to eukaryotes. Prokaryotes have less number of genes as compared to eukaryotes. Prokaryotes have higher gene density as compared to eukaryotes.

30 Size of Chromosomes

Size of chromosomes: The size of the chromosomes varies between 0.5 μ to 32 μ in length. Diameter of chromosomes varies - 0.2 μ and 3.0 μ . The longest chromosomes found in Trillium - 32 μ . The giant chromosomes found in diptera and they may be as long as 300 μ and up to 10 μ in diameter. In general, plants have longer chromosomes than animals. Species having less chromosome number usually have long chromosomes. Dicots in general, have a higher number of chromosomes than monocots. Chromosomes are longer in monocot than dicots.

Size at stages of mitosis: There is variation in the sizes of chromosomes during different stages of mitosis such as

Interphase: Chromosomes are longest & thinnest.

Prophase: there is a progressive decrease in their length accompanied with an increase in

thickness.

Anaphase: chromosomes are smallest.

Metaphase: chromosomes can be easily observed.

Chromosomes are studied during metaphase

Measurement of chromosomes: Chromosomes are generally measured during mitotic metaphase.

31 Types of Chromosomes

Types of Chromosomes: There are two types of eukaryotic chromosomes:

- Autosomes
- Sex chromosomes

Autosomes: Paired chromosomes with the same length, shape, centromere location, and genes.

Any chromosomes other than sex chromosomes are called as autosomes.

Sex Chromosomes: Members of a pair of chromosomes that differ between males and females.

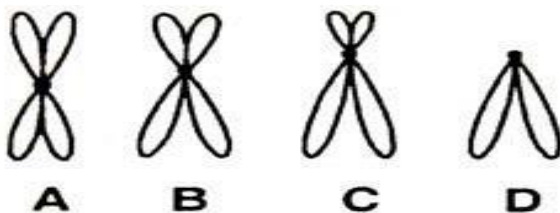
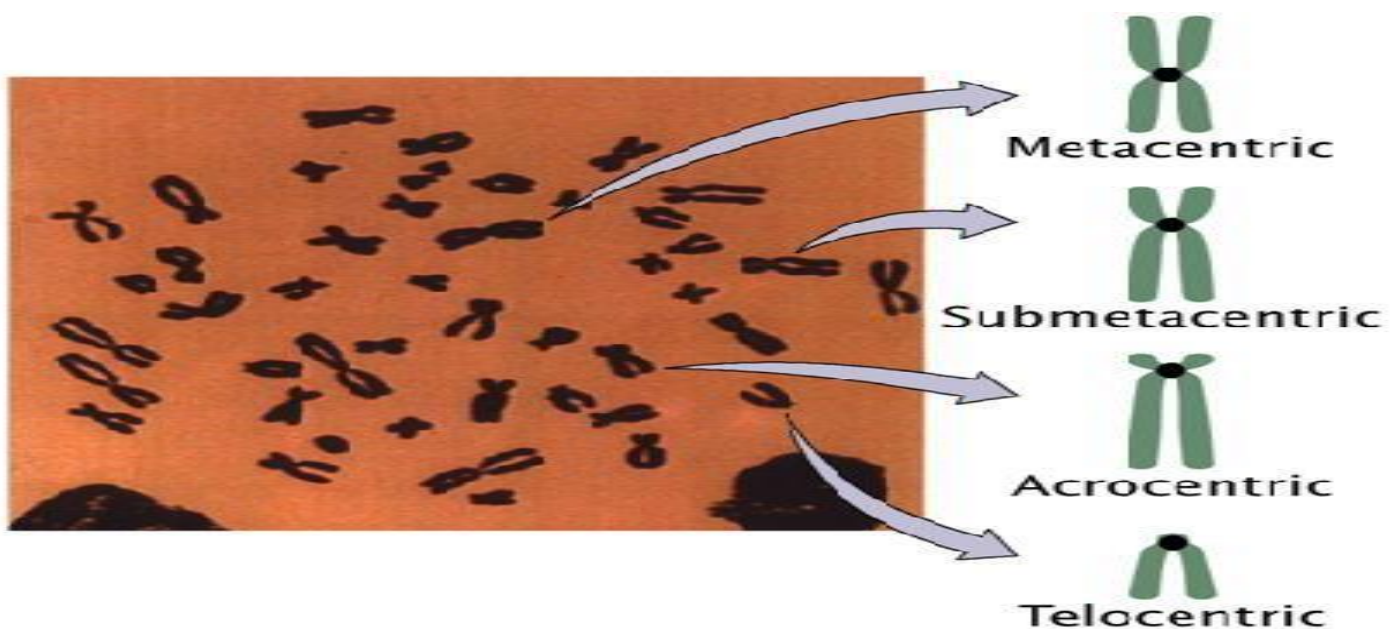
Bases on this pair of chromosomes, sex is identified.

Type of Chromosomes based on Centromere position Chromosomes may differ in the position of the centromere.

Centromere is near the middle, metacentric

Centromere is toward one end, acrocentric or sub metacentric

Centromere is very near to end, telocentric



- A - Metacentric chromosome**
B - Sub-metacentric chromosome
C - Acrocentric chromosome
D - Telocentric chromosome

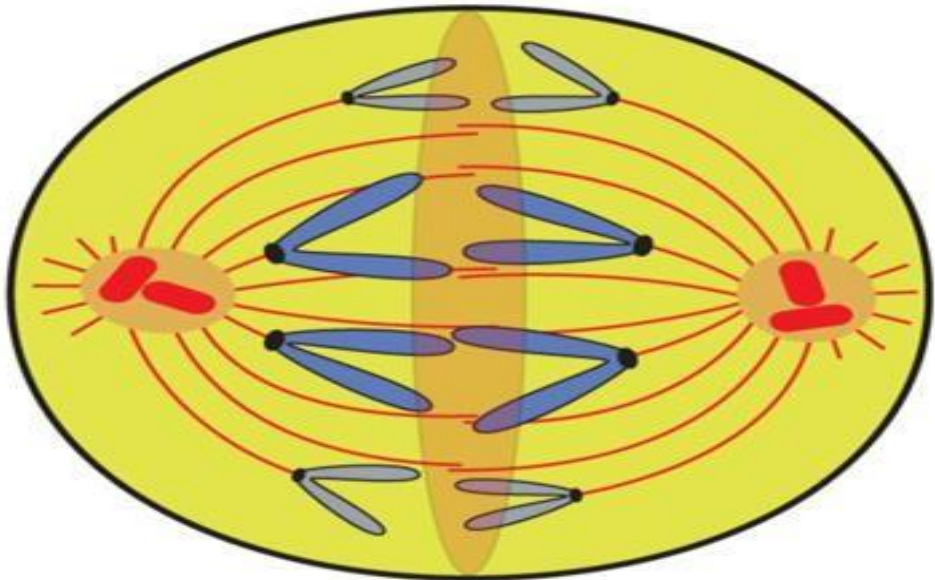
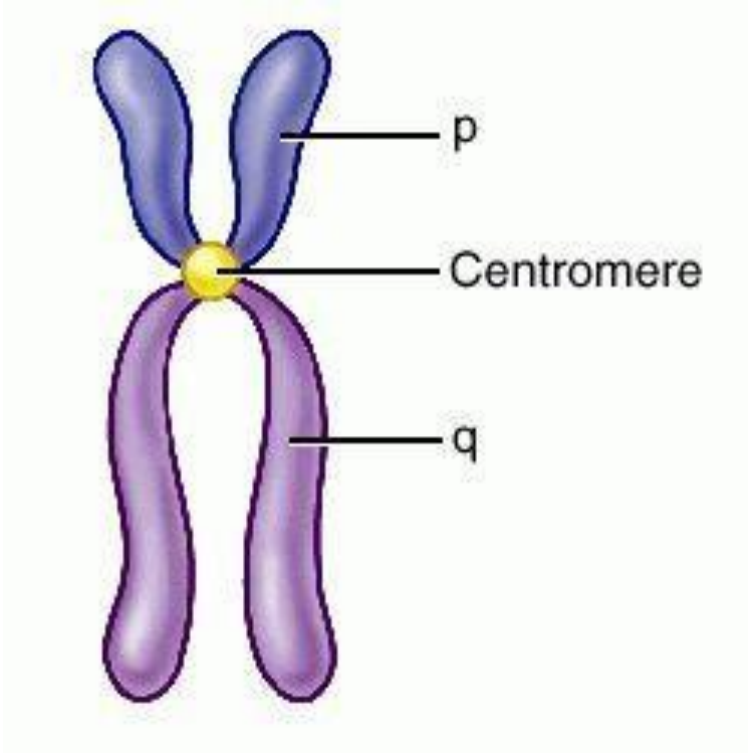
32 Centromere Functions

Centromere-function: Centromere and telomeres are two essential features of all eukaryotic chromosomes. Each provide a unique function i.e., absolutely necessary for the stability of the chromosomes. Centromeres are required for the segregation of the chromosomes during meiosis and mitosis. Telomeres provide terminal stability to the chromosome and ensure its

survival. The region where two sister chromatids of a chromosome joined or “held together” during mitotic metaphase is called centromere. When chromosomes stained they show a dark-stained region that is the centromere. The region where two sister chromatids of a chromosome joined or “held together”

During mitosis, the centromere that is shared by the sister chromatids must divide so that the chromatids can migrate to opposite poles of the cell. Centromere is an important component of chromosome structure and segregation. Centromeres are the first parts of chromosomes to be seen moving towards the opposite poles during anaphase.

Centromere moving towards the opposite poles. The remaining regions of chromosomes lag behind and appear as if they were being pulled by the centromere.



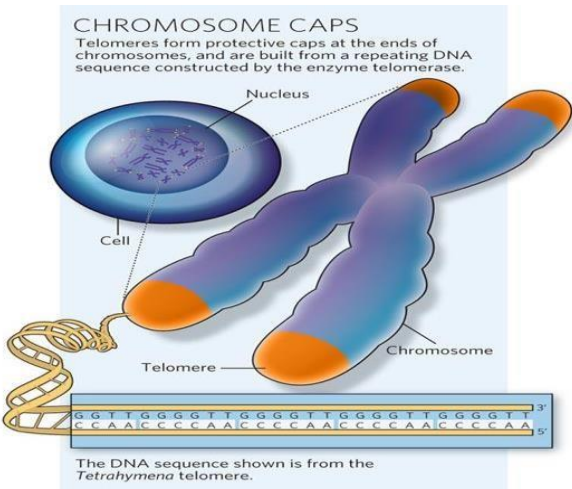
33 Telomere Functions

What are telomeres: The two ends of a chromosome are known as telomeres. These are required for the replication and stability of the chromosomes. When telomeres are damaged or removed due to chromosome breakage, ends can readily fuse or unite with broken ends of other chromosome.

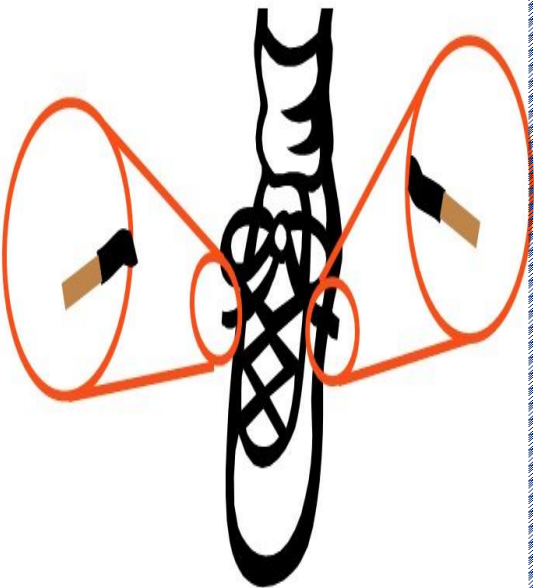
Telomeres are like the ends of shoes strings Ends of broken chromosomes are sticky, whereas the normal end is not sticky, suggesting the ends of chromosomes have unique features.

Telomeres have been isolated and characterized from several species.

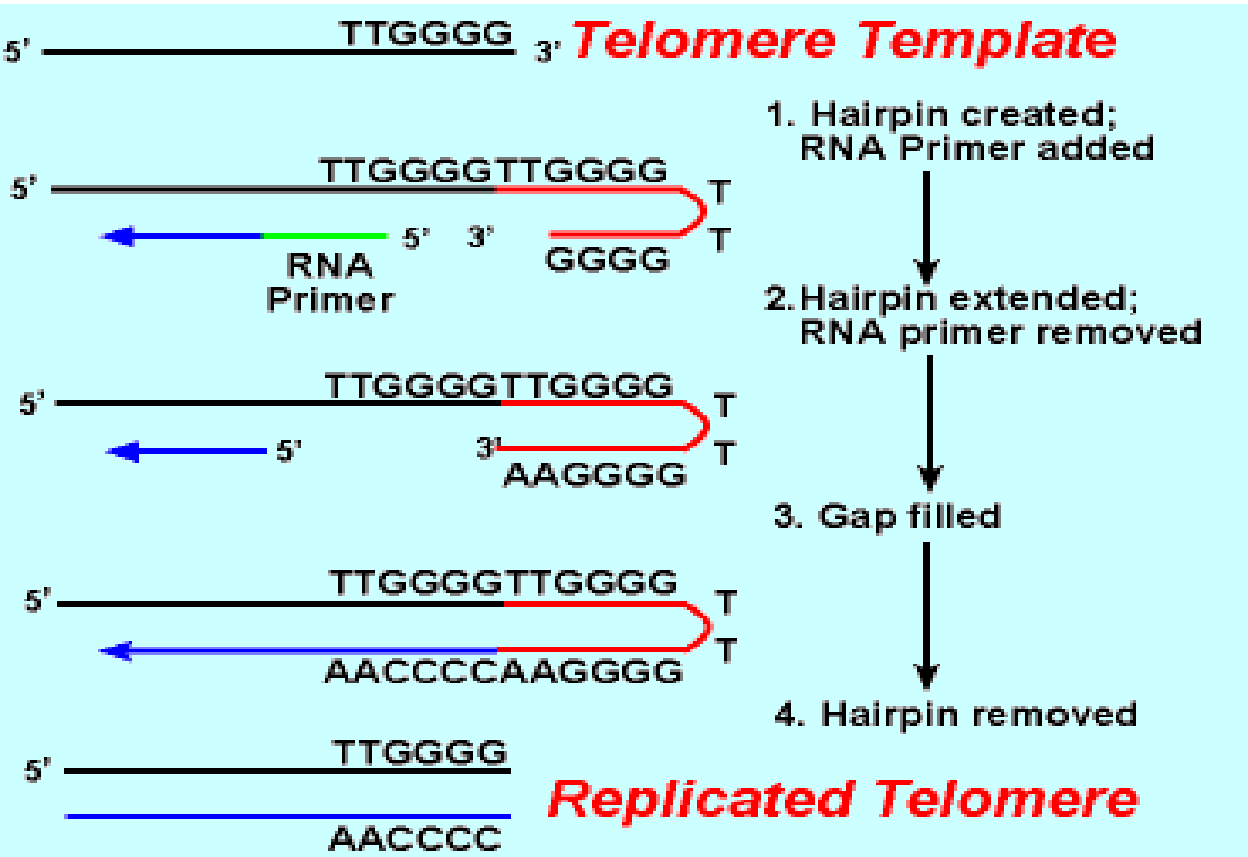
- ☐
- ☐



Species	Repeat Sequence
• <i>Arabidopsis</i>	TTTAGGG
• Human	TTAGGG
• <i>Oxytricha</i>	TTTTGGGG
• Slime Mold	TAGGG
• <i>Tetrahymena</i>	TTGGGG
• Trypanosome	TAGGG



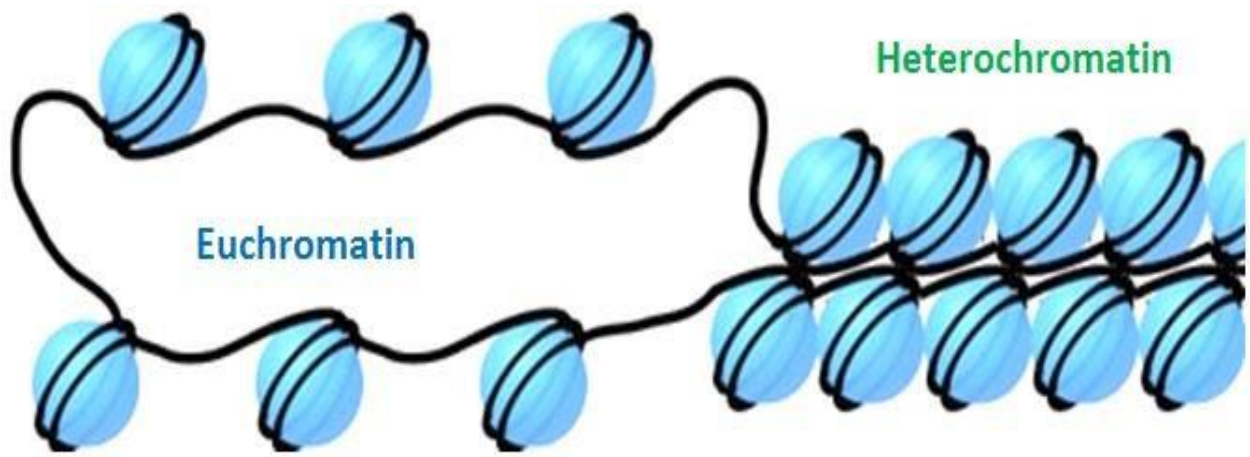
Process of Telomere replication The telomeres of this organism end in the sequence 5'TTGGGG-3'. The telomerase adds a series of 5'-TTGGGG-3' repeats to the ends of the lagging strand. Finally, the hairpin is removed at the 5'-TTGGGG-3' repeat. Thus the end of the chromosome is faithfully replicated.



RNA Primer - Short stretches of ribonucleotides (RNA substrates) found on the lagging strand during DNA replication. Helps initiate lagging strand replication .

34 Euchromatin and Heterochromatin

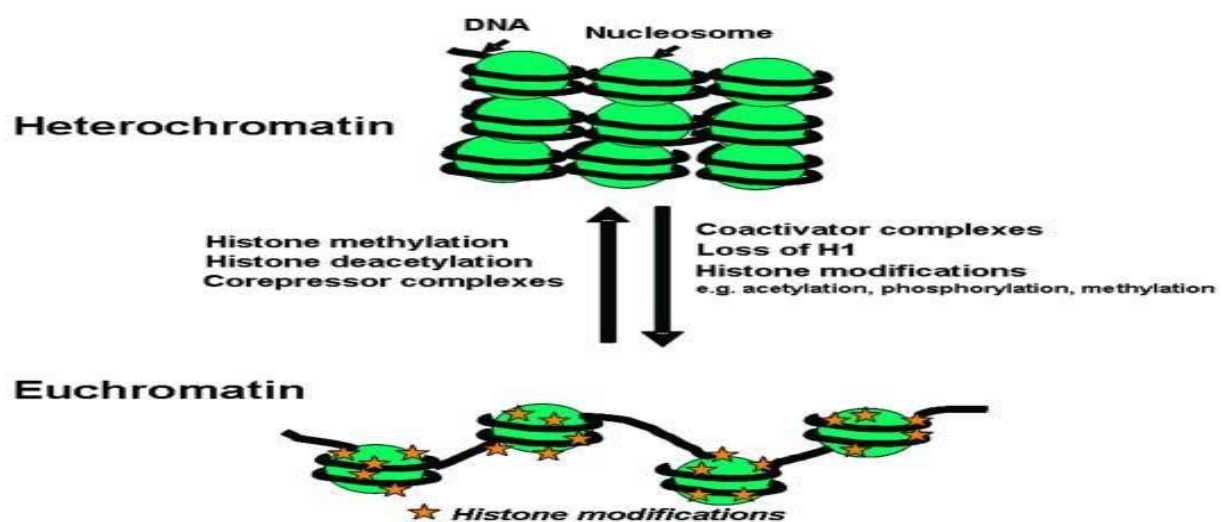
Euchromatin and Heterochromatin: Chromosomes may be identified by regions that stain in a particular manner when treated with various chemicals. Several different chemical techniques are used to identify certain chromosomal regions by staining. Darker bands are generally found near the centromere or on the ends (telomeres) of the chromosome. The position of the darkstaining is heterochromatic region or heterochromatin. Light staining regions are Euchromatin region or Euchromatin.



Heterochromatin Heterochromatin is classified into two groups:

- Constitutive
- Facultative

Constitutive heterochromatin remains permanently in the heterochromatic stage. It does not revert to the Euchromatin stage. Facultative heterochromatin consists of Euchromatin that takes on the staining and compactness characteristics of heterochromatin during some phase of development.



35 Staining and Banding Chromosomes

Staining and banding chromosomes

Staining procedures

- Staining procedures have been developed in the past two decades and these techniques help to study the karyotype in plants and animals.

Feulgen staining

- Cells are subjected to a mild hydrolysis in 1N HCl at 60 °C for 10 minutes.
- This treatment produces a free aldehyde group in deoxyribose molecules.

Q Banding

- Q bands are the fluorescent bands observed after quinacrine mustard staining with UV light.
- The distal ends not stained by this technique.

R Banding

- The R bands (reverse) are those located in the zones that do not fluoresce with the quinacrine mustard, that is they are between the Q bands and can be visualized as green.

C Banding

- The C bands correspond to constitutive heterochromatin.

The heterochromatin regions in a chromosome distinctly differ in their stainability from euchromatic region

Human chromosome banding patterns

- Human body cells contain 46 chromosomes in 23 pairs – one of each pair inherited from each parent.

Q-banding	Quinacrine	Chromosome arms; mostly repetitive AT-rich DNA	Under UV light, distinct fluorescent banded pattern for each chromosome.
G-banding	Giemsa	Chromosome arms; mostly repetitive AT-rich DNA	Distinct banded pattern for each chromosome; same as Q-banding pattern except single additional band near centromere of chromosomes 1 and 16
R-banding	Variety of techniques	Chromosome arms; mostly unique GC-rich DNA	Reverse banding pattern of that observed with Q- or G-banding
C-banding	Variety of techniques	Centromere region of each chromosome and distal portion of Y chromosome; highly repetitive, mostly AT-rich DNA	Largest bands usually on chromosomes 1, 9, 16, and Y; chromosomes 7, 10, and 15 have medium-sized bands; size of C-bands highly variable from person to person

36 Nucleosomes

Nucleosomes

- The nucleosome is composed of a core of eight histone proteins and DNA (147 bp) wrapped around them.
- The DNA between nucleosomelinker DNA.
- Linker DNA 20-60 bp.

NUCLEOSOME ASSEMBLY

- Nucleosomes are assembled immediately after DNA replication.
- Two molecules each of four types of histones are packed by DNA to form a Nucleosomes.

Histones

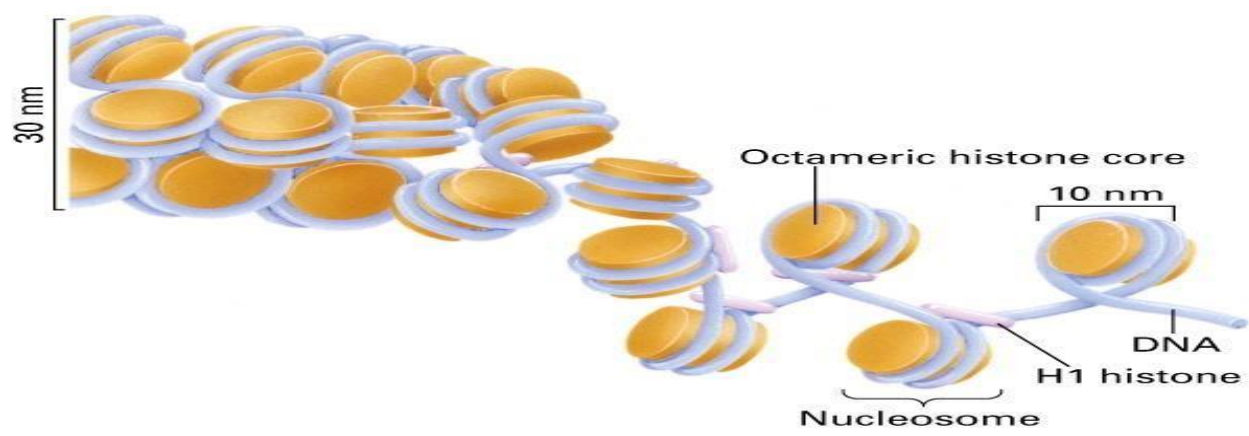
Histones are small, positively charged (basic) proteins

Nucleosomes- Five histones

- Five abundant histones are H1, H2A, H2B, H3 and H4.
- The core histones share a common structural fold, called histone-fold domain.

Nucleosomes- Five histones

- Five abundant histones are H1, H2A, H2B, H3 and H4.
- The core histones share a common structural fold, called histone-fold domain.



37 Chromatin and Chromosomes

Chromatin and chromosomes

chromatin

- The complexes between eukaryotic DNA and proteins are called chromatin.
- The major proteins of chromatin are the histones – small proteins containing a high proportion of basic amino acids arginine and lysine.

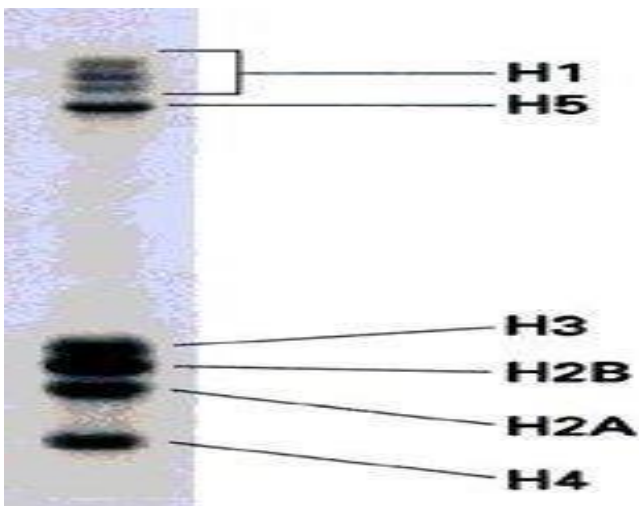
chromatin

- There are 5 major types of histones: H1, H2A, H2B, H3, and H4 – which are very similar among different sp of eukaryotes.
- The histones are extremely abundant proteins in eukaryotic cells.

Molecular weight and number of amino acids

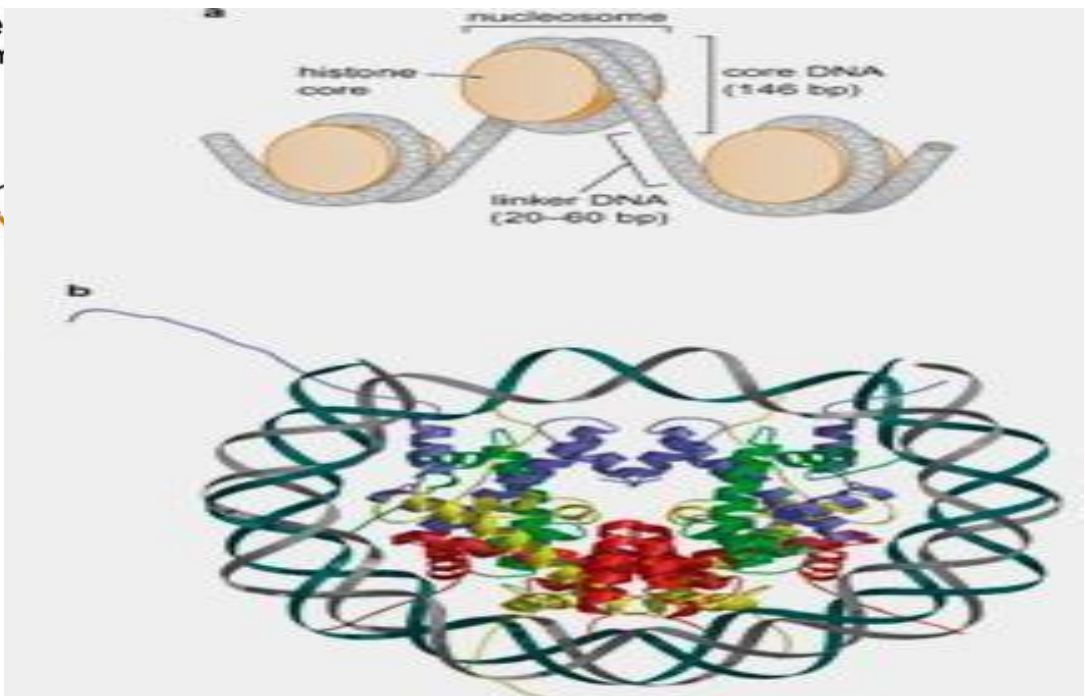
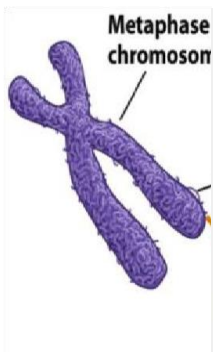
Histone	Mol. Wt	No. of Amino acid	Percentage Lys + Arg
H1	22,500	244	30.8
H2A	13,960	129	20.2
H2B	13,774	125	22.4
H3	15,273	135	22.9
H4	11,236	102	24.5

Histones - size and molecular weight



Non-histones

- Chromatin contains an approximately equal mass of a variety of nonhistone proteins.
- More than a thousand different types of proteins, which are involved in a DNA replication and gene expression.
- The DNA of prokaryotes is similarly associated with proteins, some of which presumably function as histones do, packing DNA within the bacterial cell.



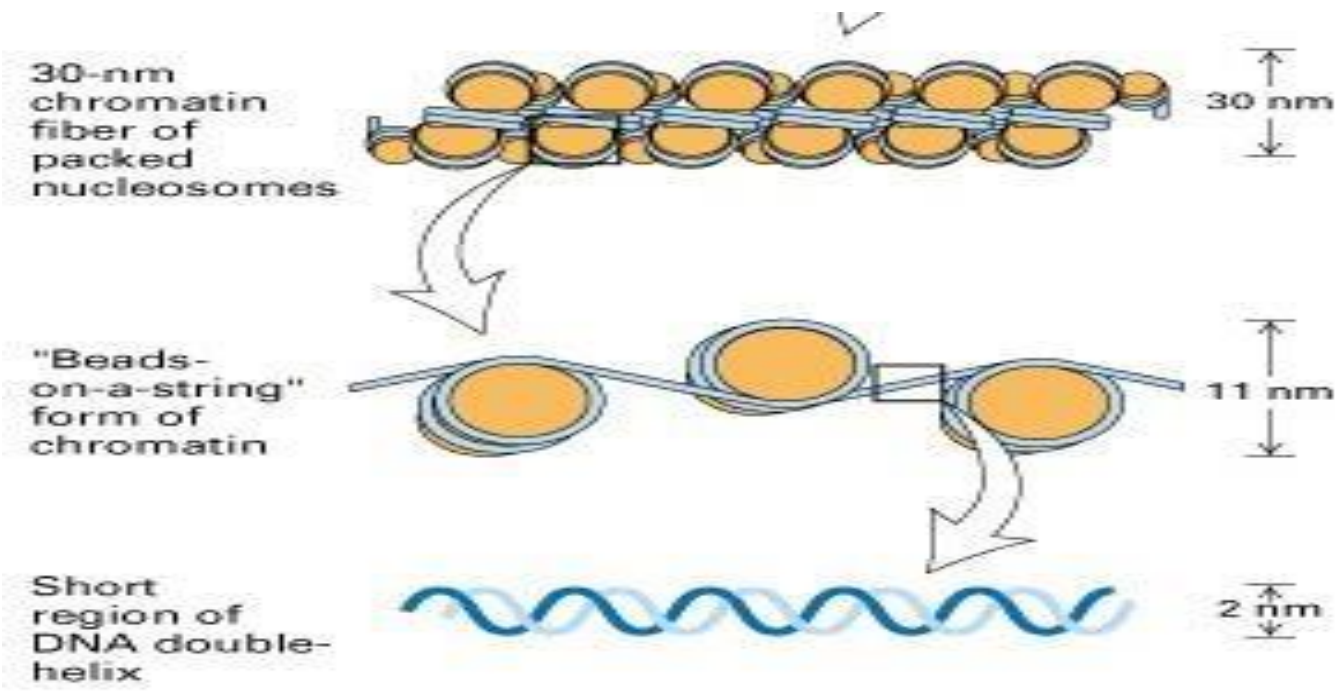
Chromosomes

- Tightly packaged DNA
- Found only during cell division
- DNA is not being used for macromolecule synthesis

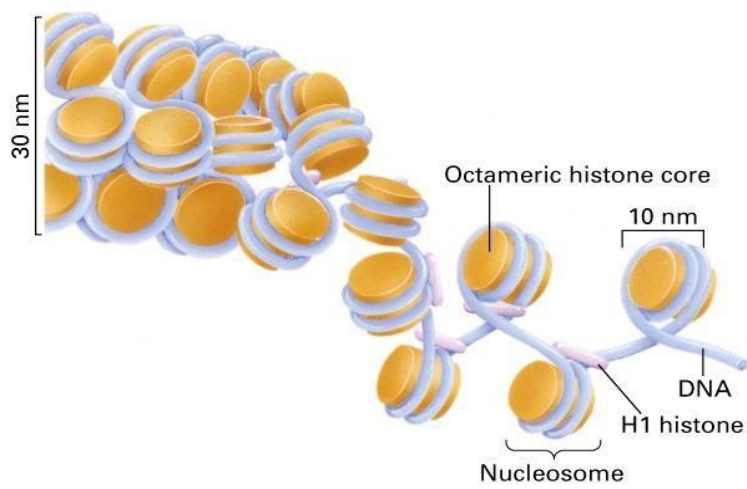
Chromatin

- Unwound DNA
- Found throughout Interphase
- DNA is being used for macromolecule synthesis

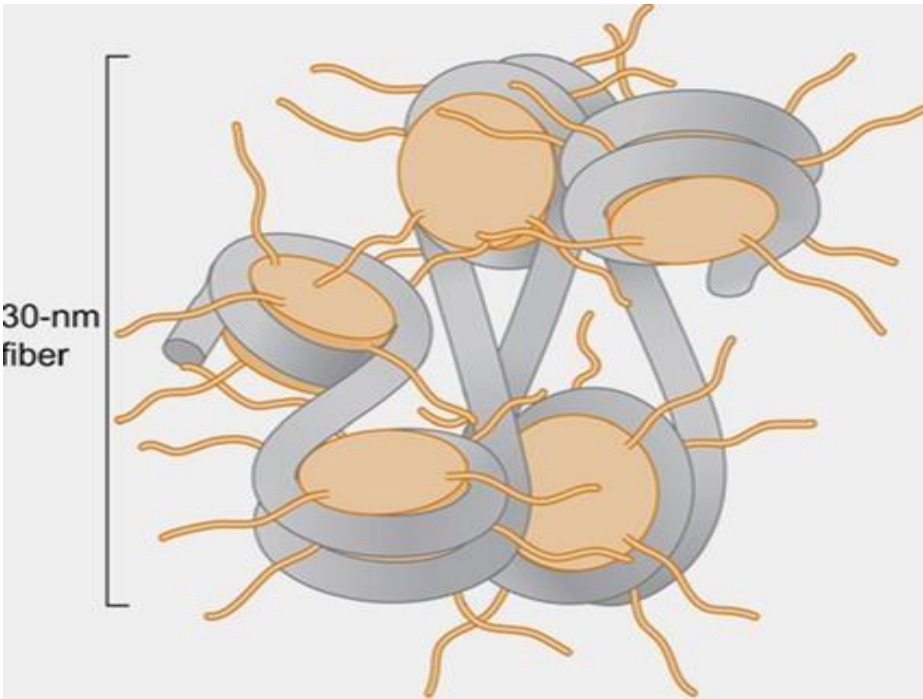
Higher order chromatin folding



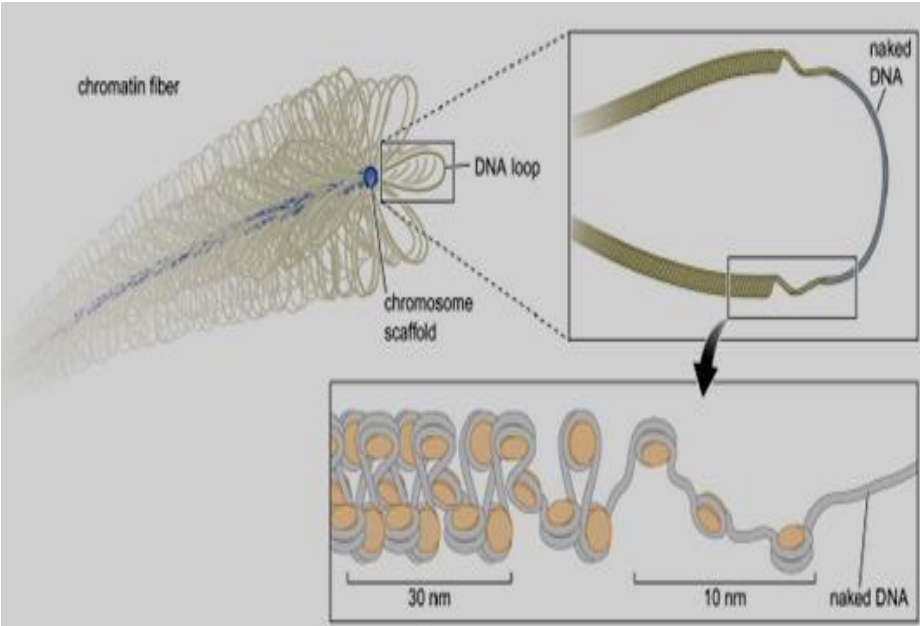
Nucleosomes and higher order



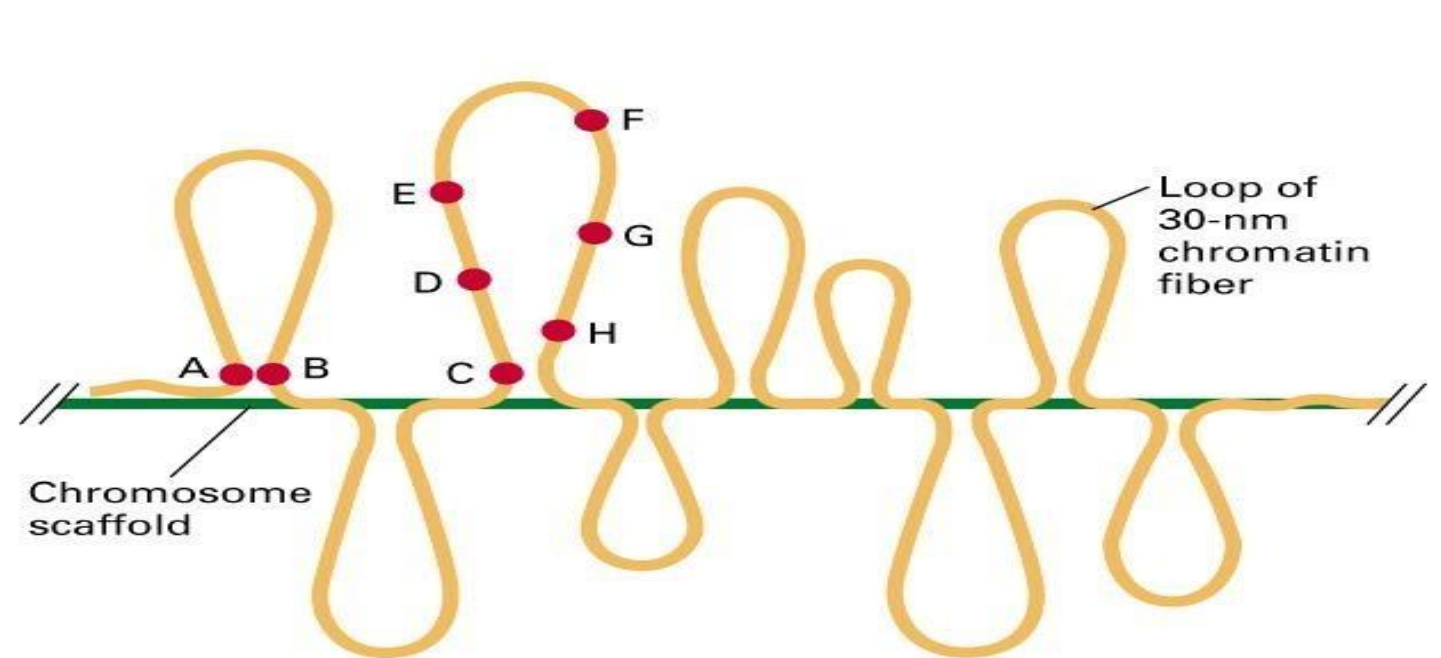
30 nm fibre



300 nm fibre



300 nm fibre



short region of
DNA double helix



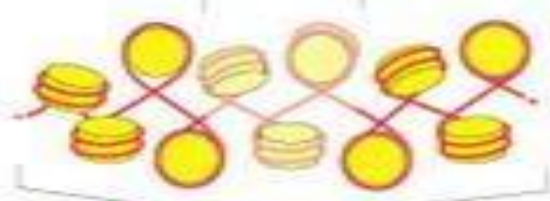
2 nm

"beads-on-a-string"
form of chromatin



11 nm

30-nm chromatin
fiber of packed
nucleosomes



30 nm

section of
chromosome in
extended form



300 nm

condensed section
of chromosome



700 nm

entire mitotic
chromosome



centromere

1400 nm

Metaphase
chromosome



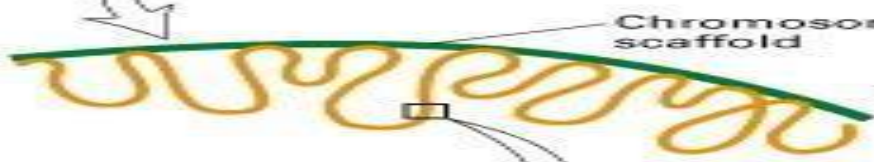
1400 nm

Condensed
scaffold-
associated
form



700 nm

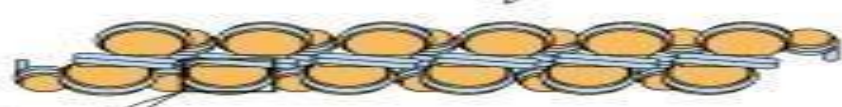
Extended
scaffold-
associated
form



Chromosome
scaffold

300 nm

30-nm chromatin
fiber of packed
nucleosomes



30 nm

"Beads-
on-a-string"
form of
chromatin



11 nm

Short
region of
DNA double-
helix



2 nm

Kinetochores

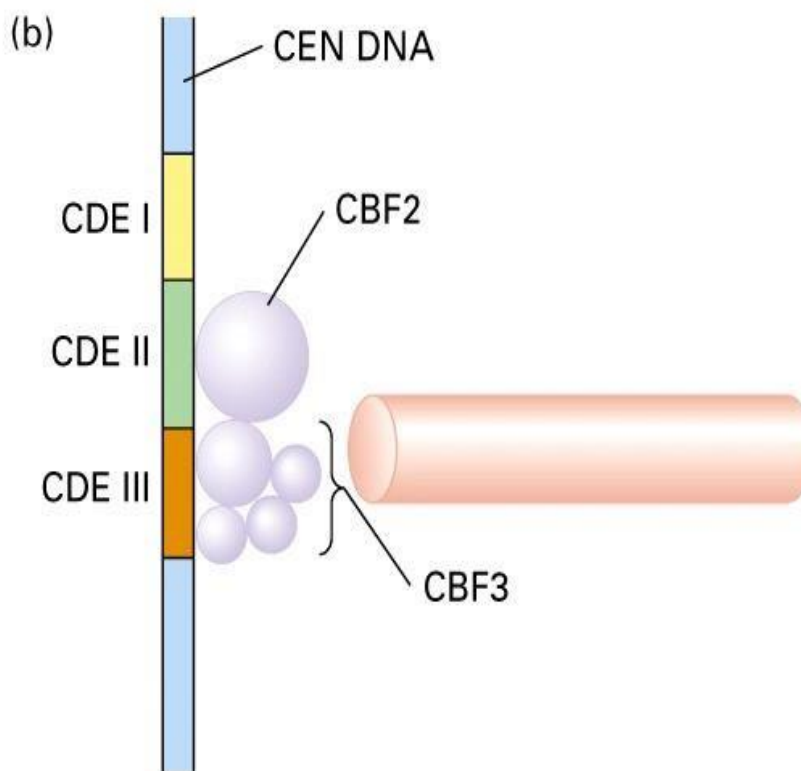
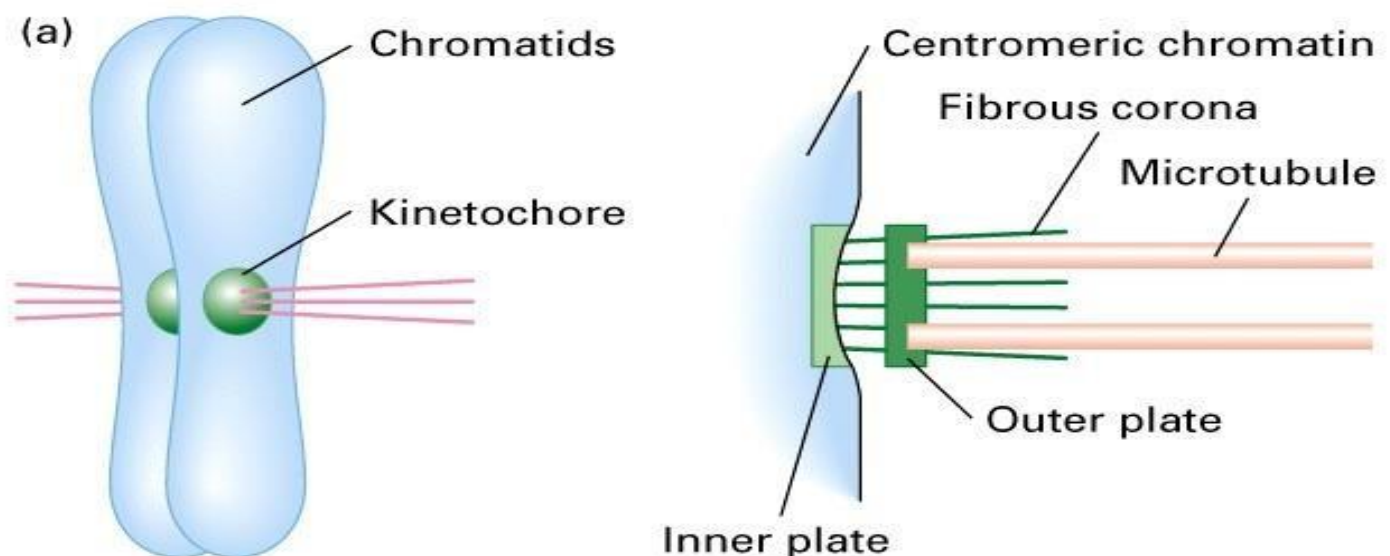
Within the centromere region, ...locations where spindle fibers attach which consist of DNA as well as protein.

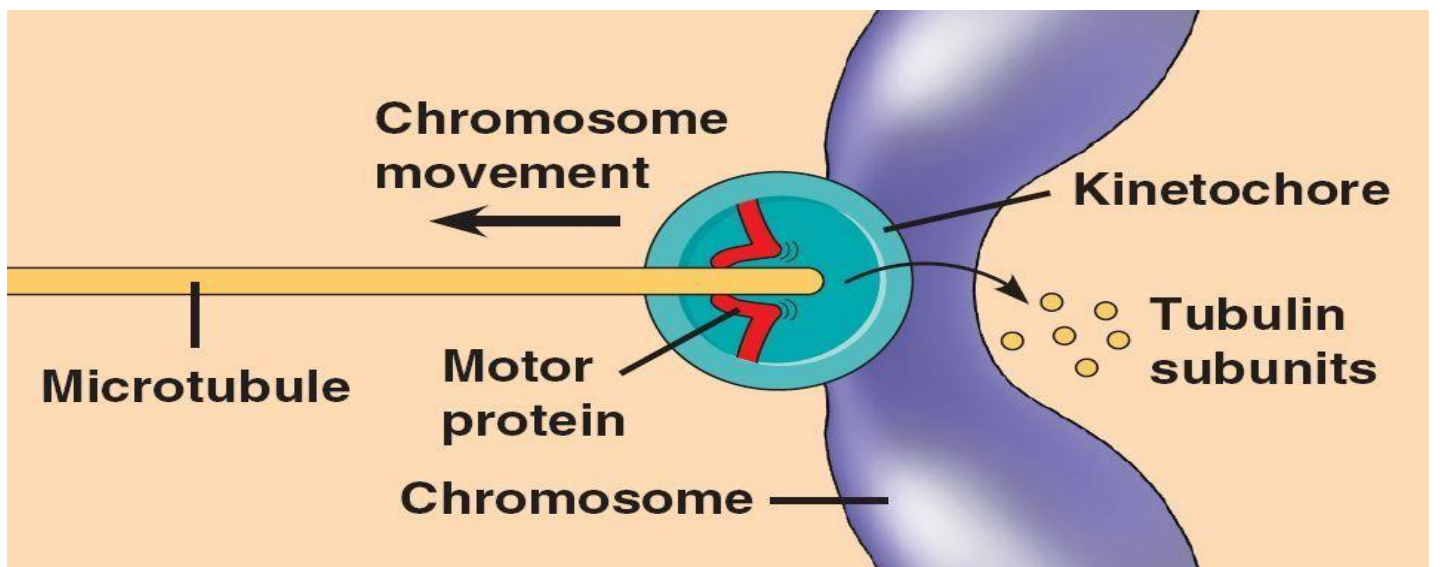
The actual location where the attachment occurs is called the kinetochore and is composed of both DNA and protein.

The DNA sequence within these regions is called CEN DNA ..120 base pairs long and consists of sub-domains, CDE-I, CDE-II and CDE-III.

Mutations in the first two sub-domains have no effect upon segregation.

But a point mutation in the CDE-III sub-domain completely eliminates the ability of the centromere to function during chromosome segregation.





40 Karyotyping

Karyotyping

IDENTIFYING CHROMOSOMES

Chromosomes identified by:

Their size

Their shape (the position of the centromere)

Banding patterns produced by specific stains (Giemsa)

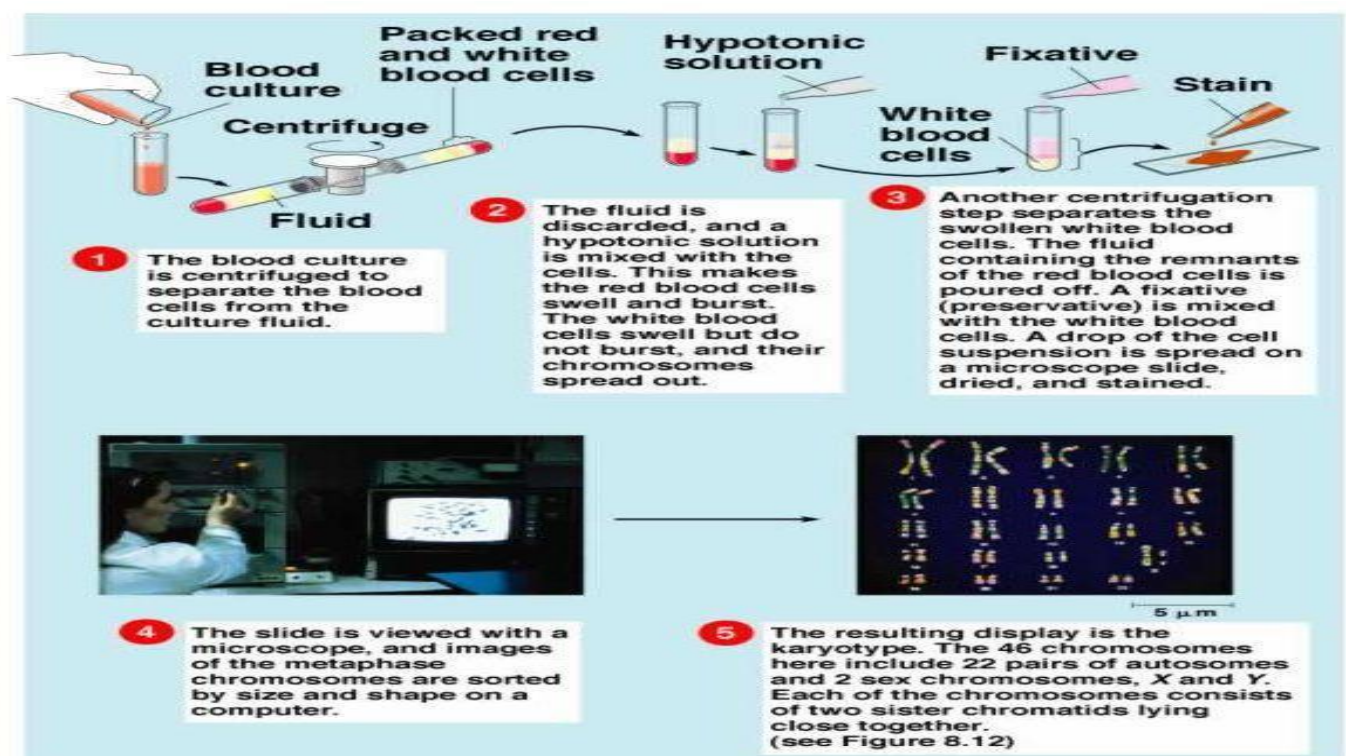
Chromosomes are analyzed by organizing them into a karyotype.

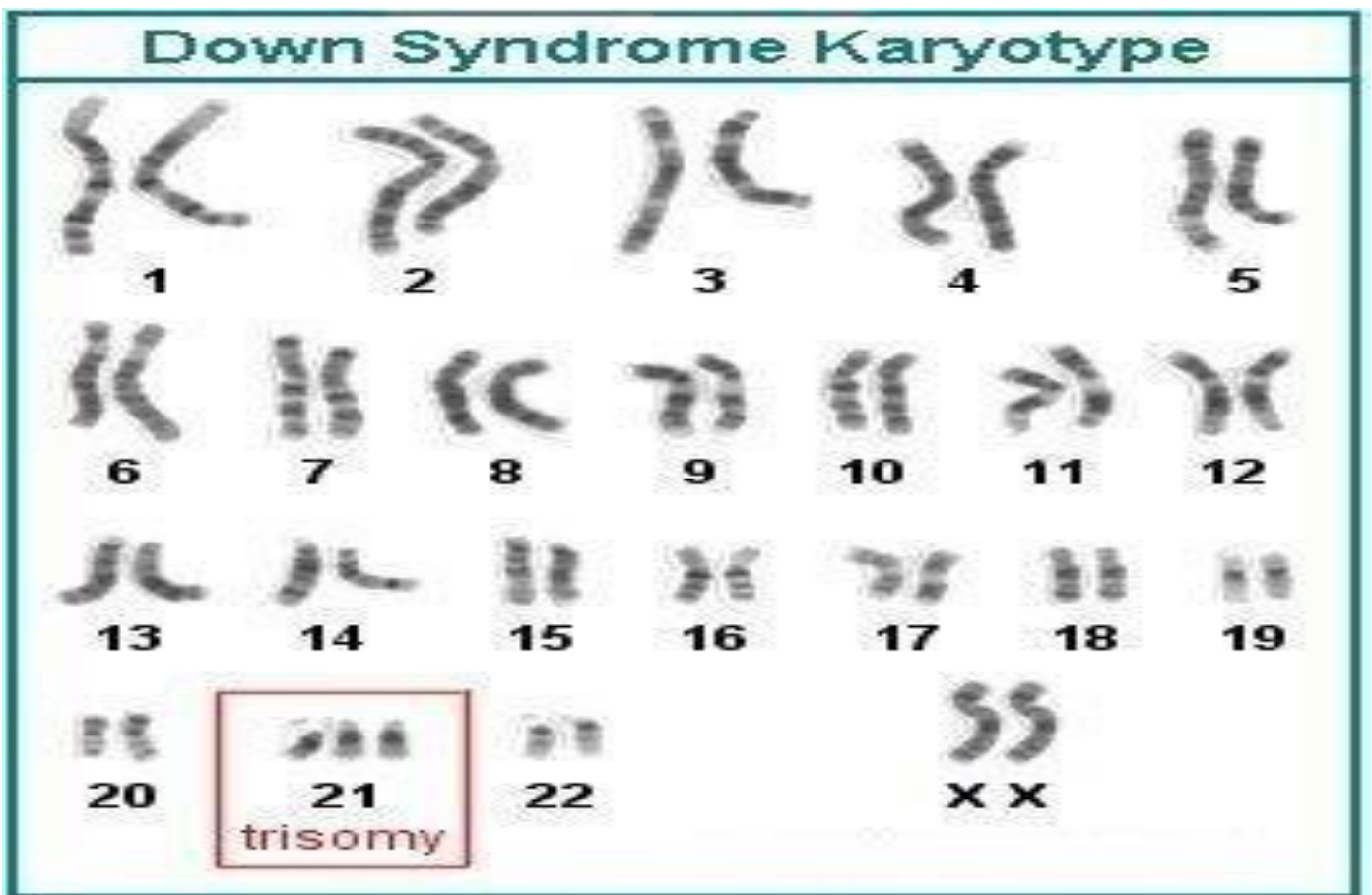
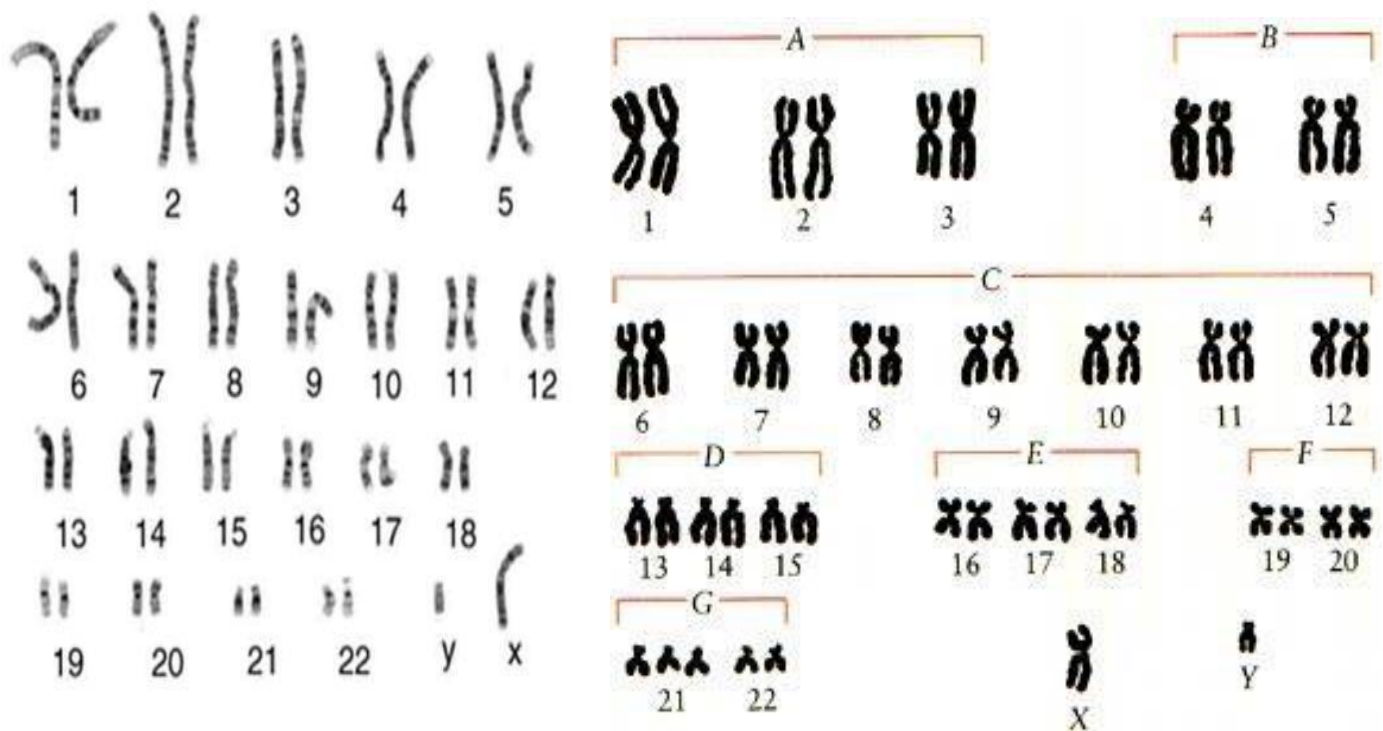
Karyotype is the general morphology of the somatic chromosome.

Generally, karyotypes represent by arranging in the descending order of size keeping their centromere in a straight line.

IDIOTYPE

Idiotype: the karyotype of a species may be represented diagrammatically, showing all the morphological features of the chromosome; such a diagram is known as Idiotype.





41 Prokaryotic Chromosomes

Prokaryotic Chromosomes

The prokaryotes usually have only one chromosome, and it bears little morphological resemblance to eukaryotic chromosomes.

The genome length is smallest in RNA viruses.

The number of gene may be as high as 150 in some larger bacteriophage genome.

In E.coli, about 3000 to 4000 genes are organized into its one circular chromosome.

The chromosome exists as a highly folded and coiled structure dispersed throughout the cell.

There are about 50 loops in the chromosome of E. coli.

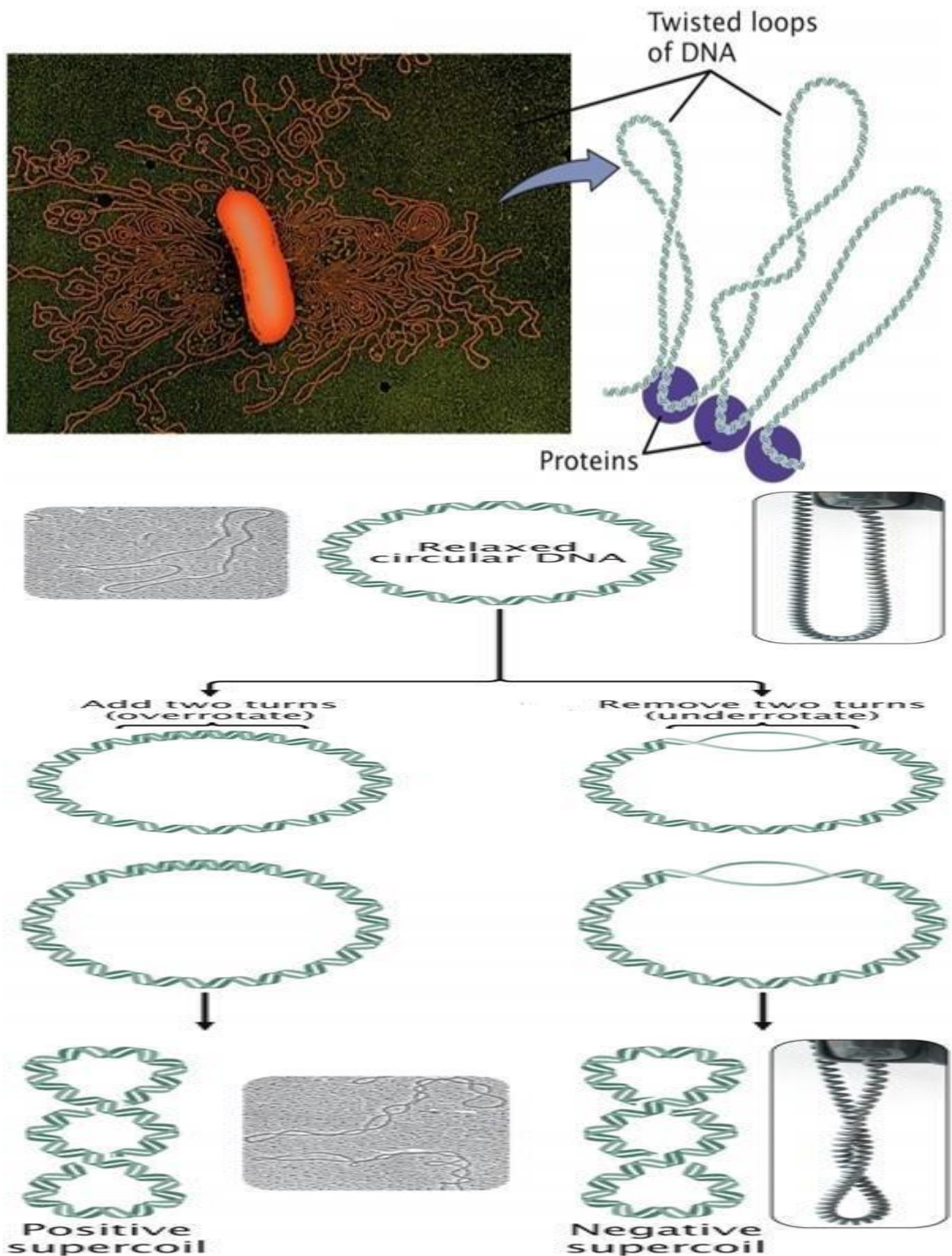
These loops are highly twisted or supercoiled structure with about four million nucleotide pairs.

During replication of DNA, the coiling must be relaxed.

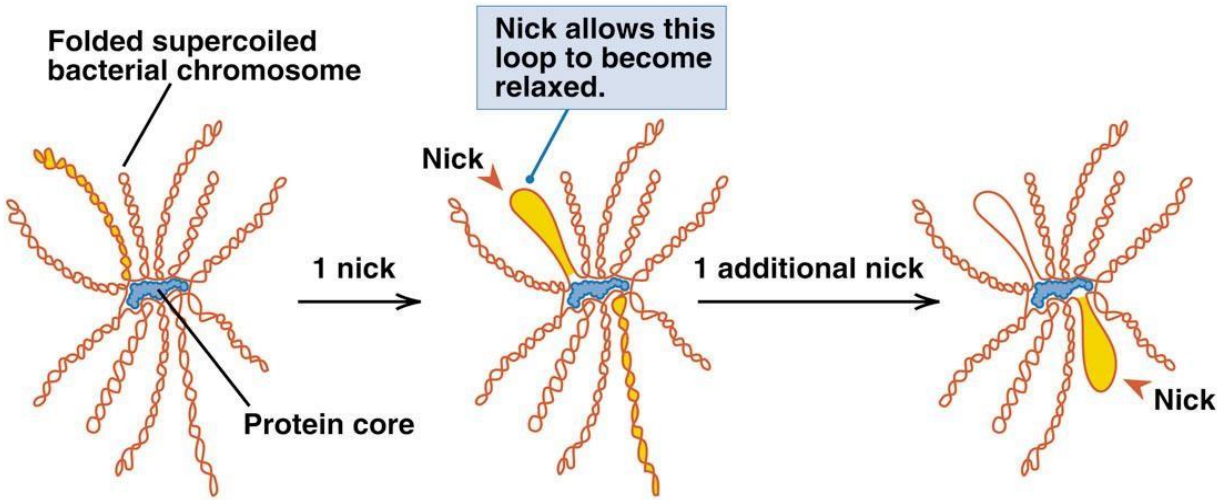
DNA gyrase is necessary for the unwinding the coils.

Bacterial Chromosome

Single, circular DNA molecule located in the nucleoid region of cell.



Mechanism of folding of bacterial CHROMOSOME



42 Sex Chromosomes and Sex determination

Sex chromosomes and sex determination

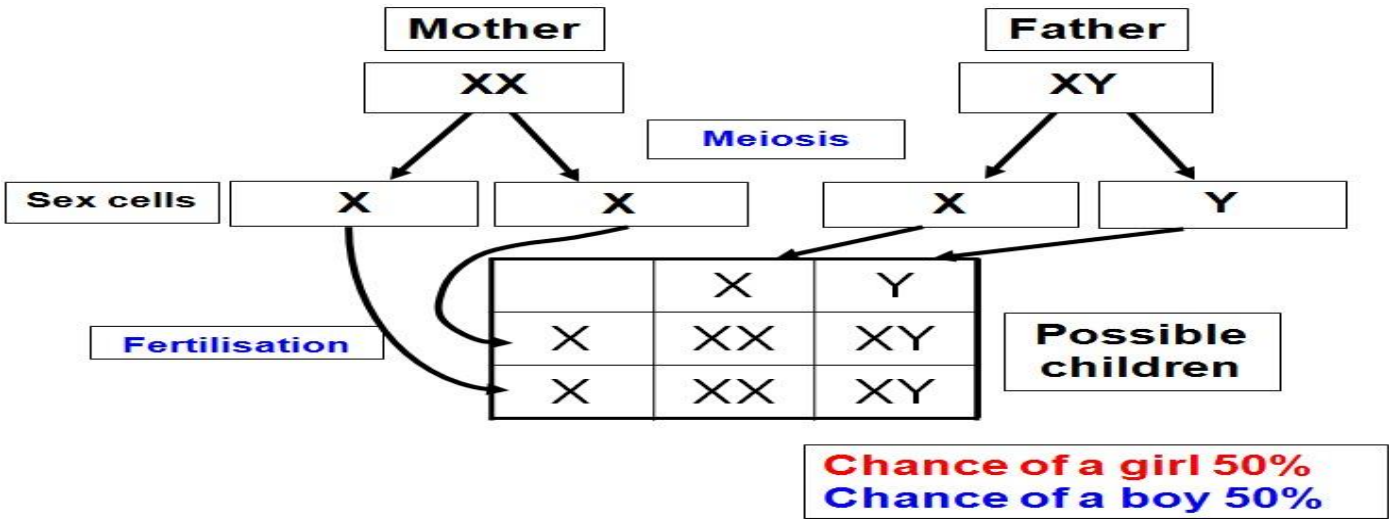
Sex Chromosomes

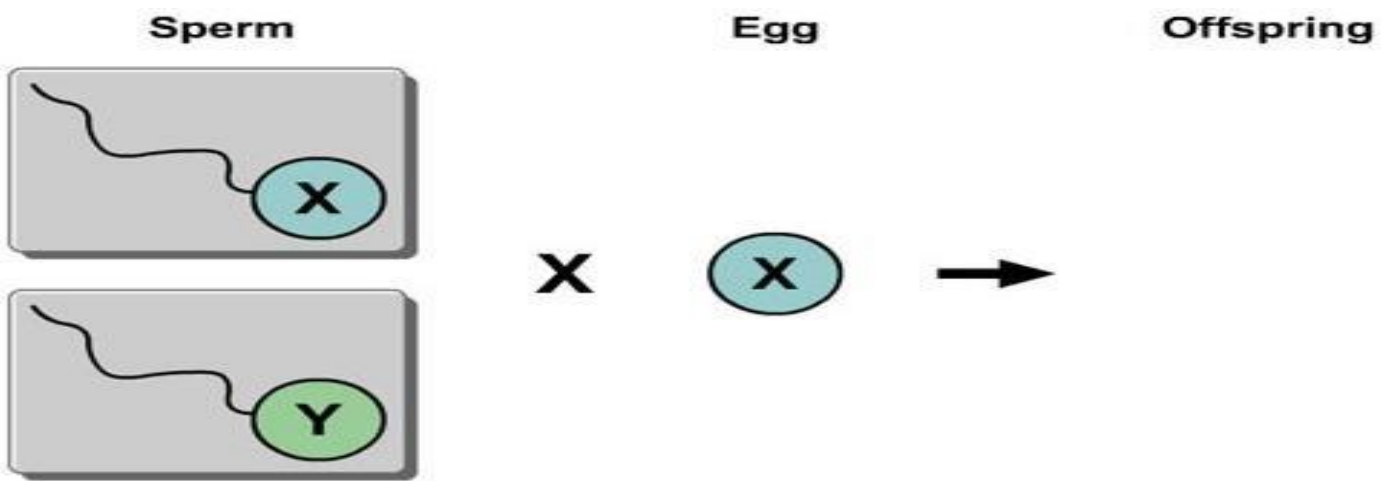
- Sex of many animals is determined by genes on chromosomes called sex chromosomes.
- Sex can be homogametic
- Sex can be heterogametic

Sex determination in different animals

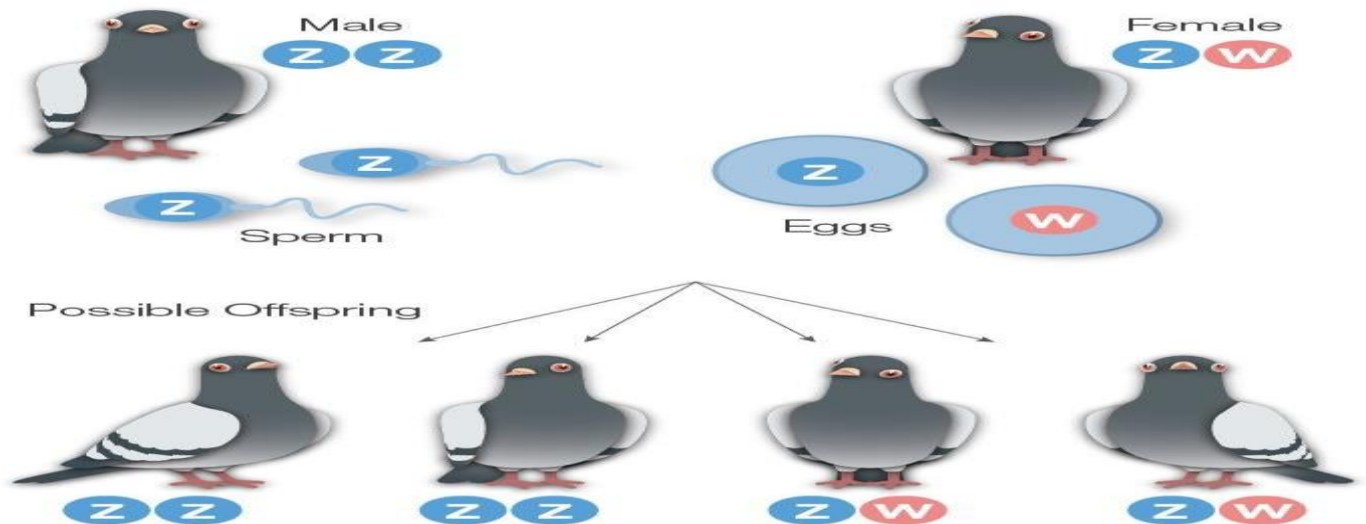
HOMOGAMETIC SEX	HETEROGAMETIC SEX	SEX DETERMINATION
Female XX	Male XY	Presence of Y-chromosome = maleness (mammals and fish) Presence of second X-chromosome = femaleness (Drosophila, the fruit fly)
Male ZZ	Female ZW	Birds, amphibians, reptiles, butterflies, moths.
Female XX	Male Xo	Grasshoppers

Inheritance of gender in humans

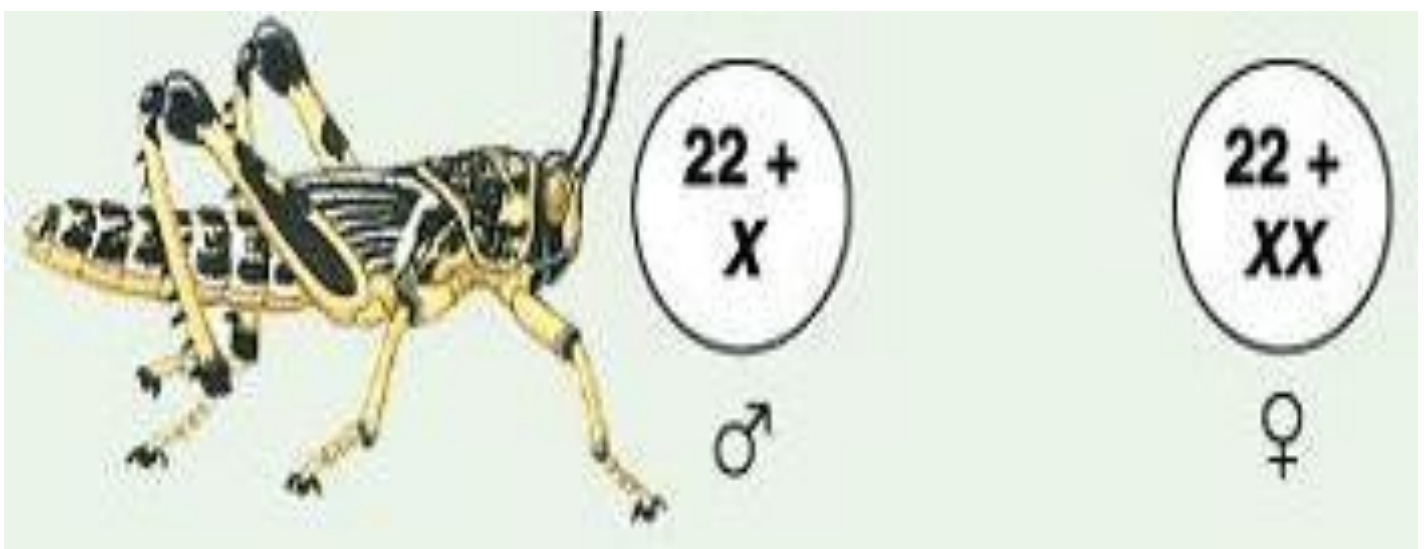




Sex system in birds



Xo system in grasshoper



43 Salient features of DNA

Salient features of DNA

Watson: X-rays diffraction pattern projected by DNA.

Erwin Chargaff demonstrated ratio of A:T are 1:1, and G:C are 1:1

Chemical structure of nucleotide identified.

Composed of 2 polymers of nucleotides.

Run antiparallel.

Molecule structure resembles a spiral staircase.

Complementary base pairing:

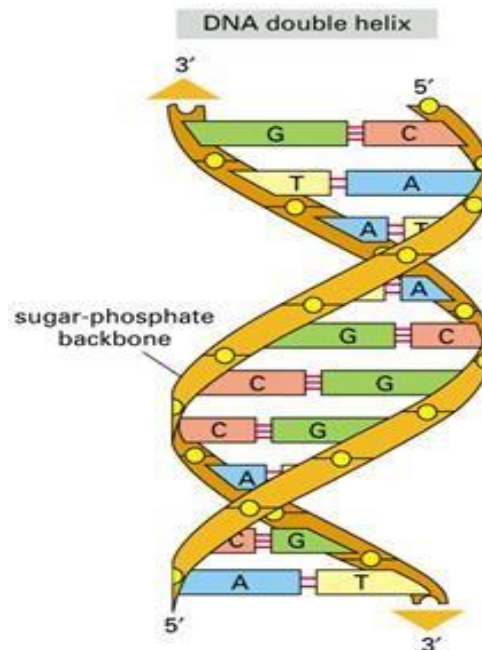
A-T, C-G

Genetic information in the linear sequence of nucleotides.

Genetic information is to synthesize proteins.

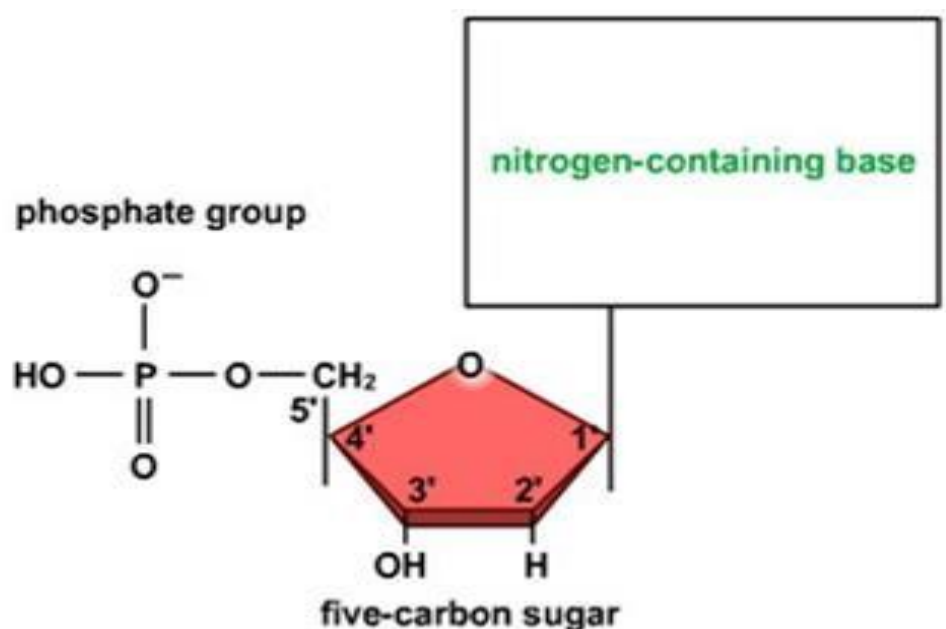
DNA forms double helix with two complimentary strands holding - hydrogen bonds.

DNA duplication occurs using one strand of parental DNA as template to form complimentary pairs with a new DNA strand.



The structure of Nucleotides includes: A phosphate sugar backbone, with one of the 5 nitrogenous bases (ATCG,U).

- adenine (A)
- guanine (G)
- thymine (T)
- cytosine (C)



44 Nucleotides

NUCLEOTIDE

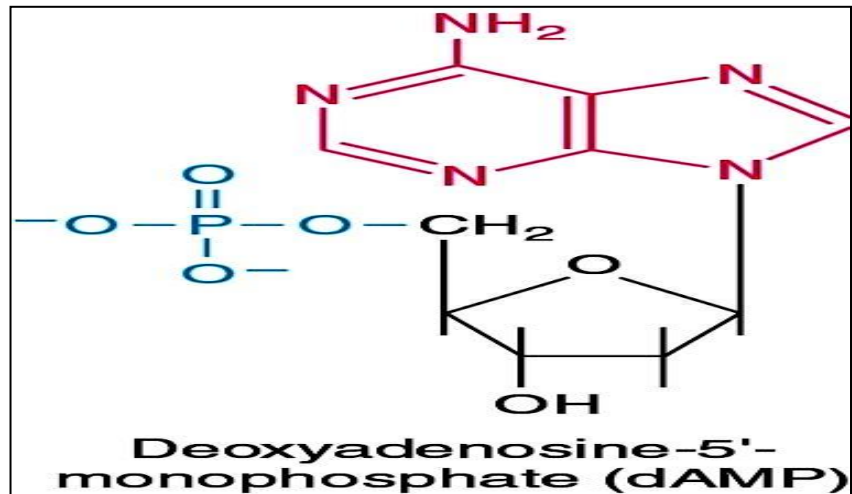
Made of three components 5 carbon sugar (pentose) nitrogenous base phosphate group

Oligonucleotide – short polymer (<10)

Polynucleotide – long polymer (>10)

Nucleoside = monomer of sugar + base

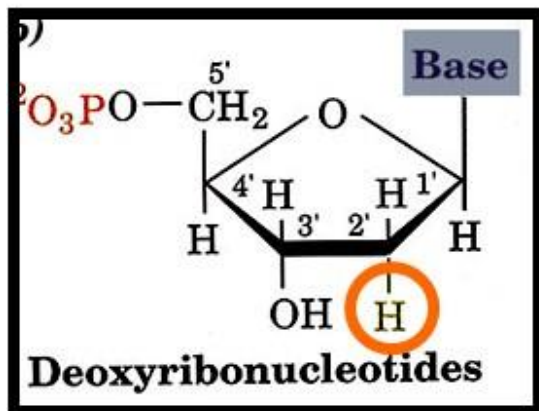
NUCLEOTIDE MONOMER



Sugar molecules

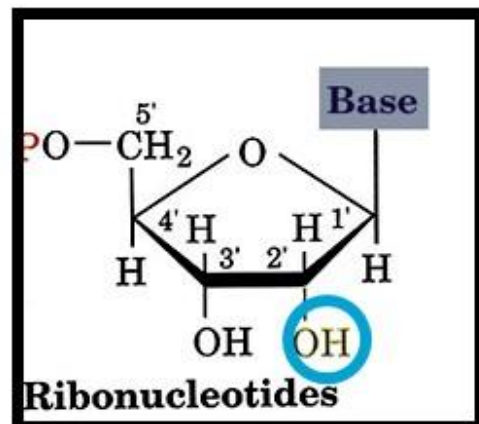
Two types of sugar molecules

DNA



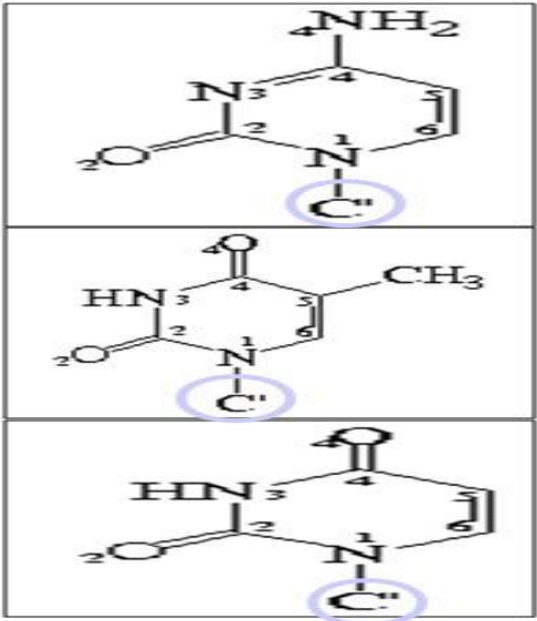
2'-deoxy-D-ribose

RNA



2'-D-ribose

Nitrogenous Bases pyrimidines

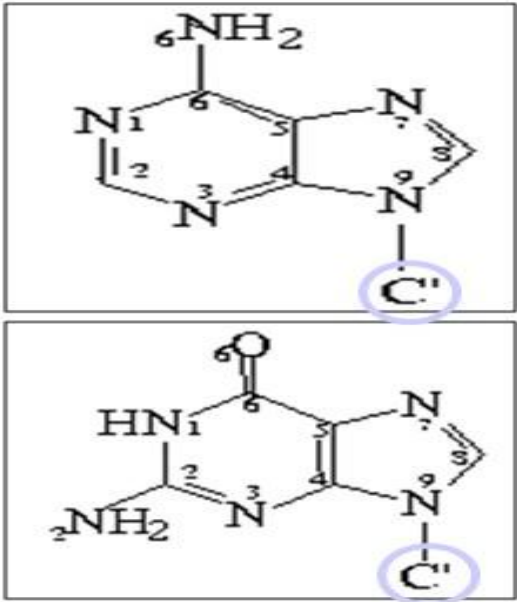


cytosine

thymine

uracil

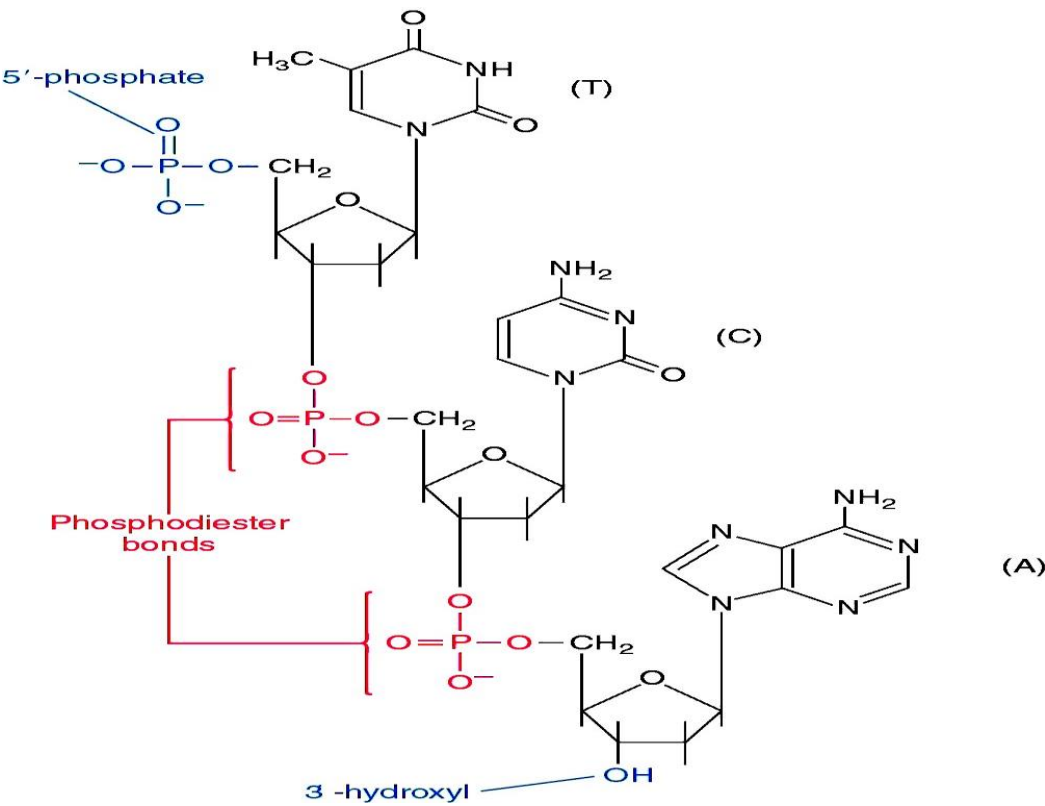
Nitrogenous bases purines



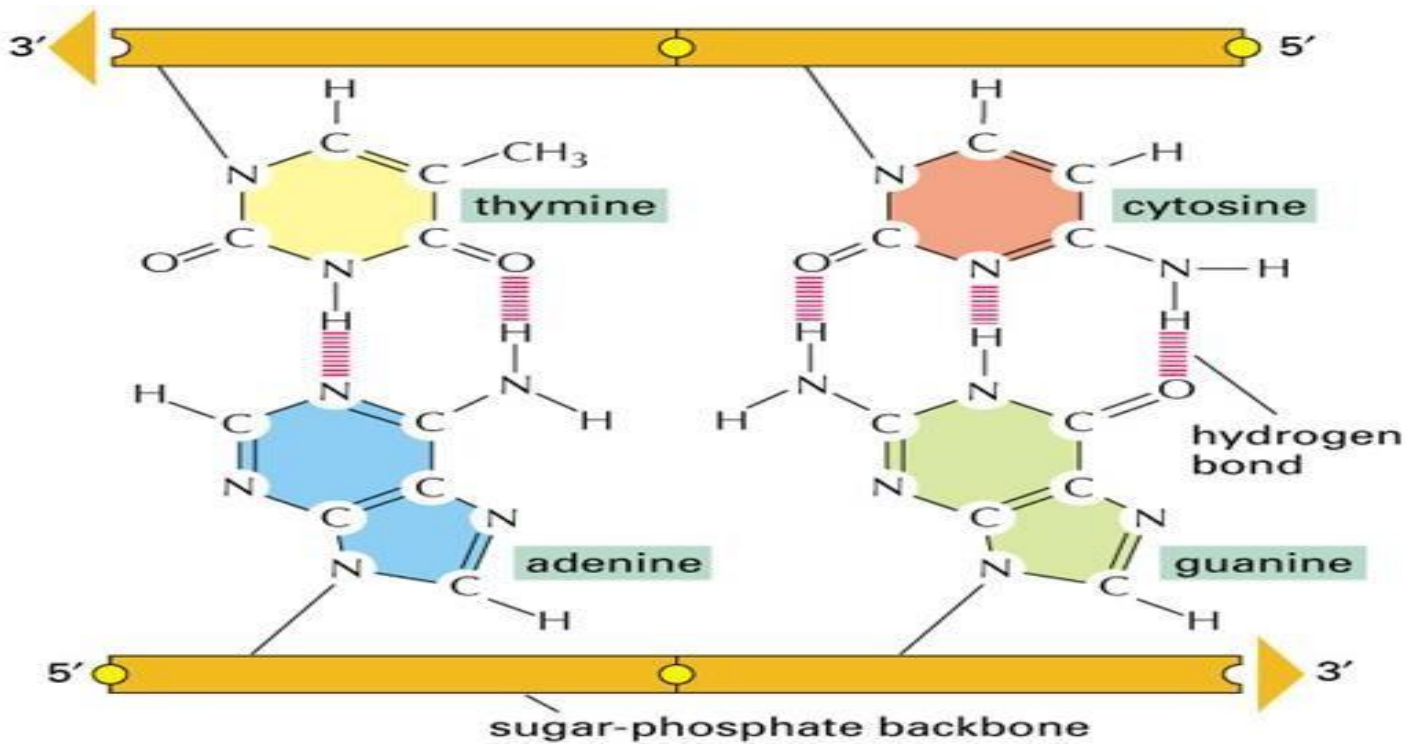
adenine

guanine

Polynucleotide linkage



Pairing of A – T , c – G



45 Conformations of DNA

Conformations of DNA

B-DNA

Right-handed helix.

Intermediate.

Planes of the base pairs nearly perpendicular to the helix axis

Tiny central axis.

Wide + deep major groove.

Narrow + deep minor groove.

A-DNA

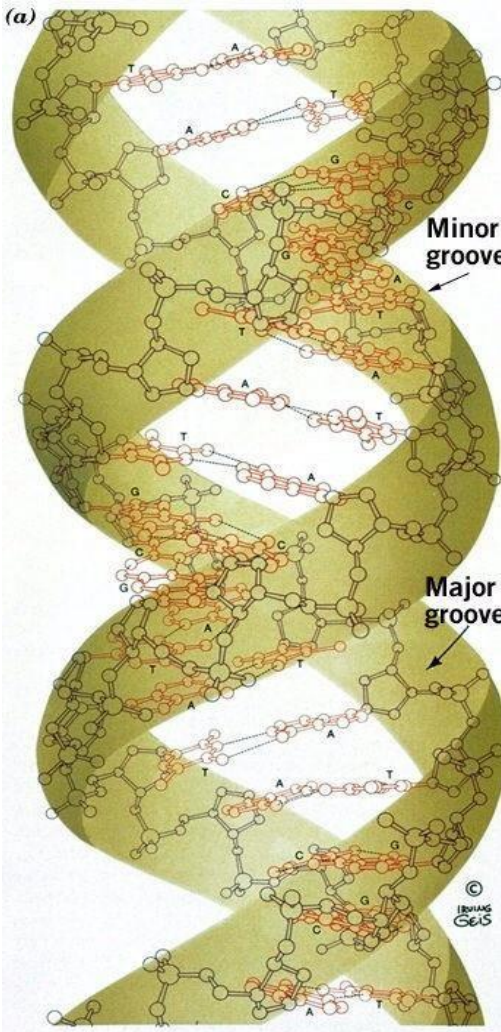
Right-handed helix.

Widest .

Planes of the base pairs inclined to the helix axis.

Narrow + deep major groove.

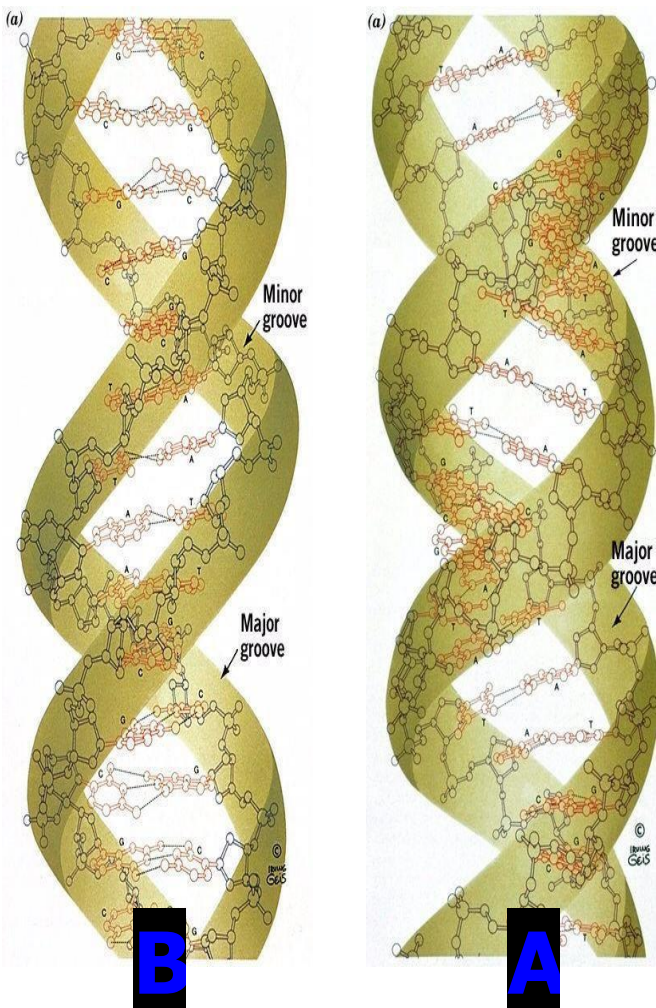
Wide + shallow minor groove.



Z-DNA

- Left-handed helix.
- Narrowest .
- Planes of the base pairs nearly perpendicular to the helix axis.
- No internal spaces.
- No major groove.
- Narrow + deep minor groove.

	A-DNA	B-DNA	Z-DNA
Helix	Right-handed	Right-handed	Left-handed
Width	Widest	Intermediate	Narrowest
Planes of bases	planes of the base pairs inclined to the helix axis	planes of the base pairs nearly perpendicular to the helix axis	planes of the base pairs nearly perpendicular to the helix axis
Central axis	6A hole along helix axis	tiny central axis	no internal spaces
Major groove	Narrow and deep	Wide and deep	No major groove
Minor groove	Wide and shallow	Narrow and deep	Narrow and deep



46 Chargaff Rule of Base Ratio

Chargaff rule of base ratios

Chargaff

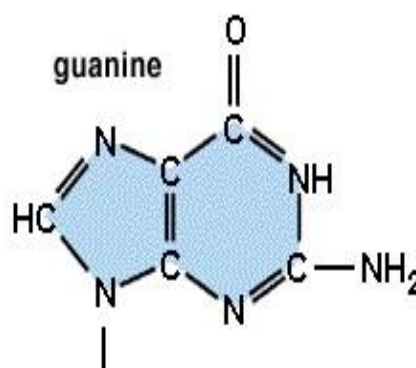
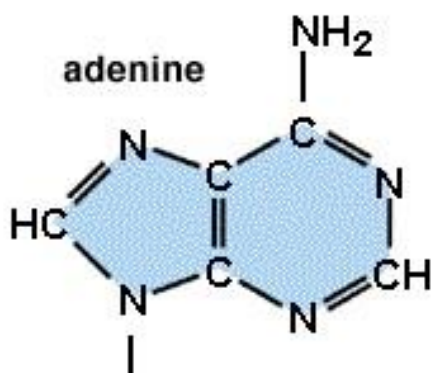
1944 - Study of DNA and its four chemical bases - adenine, cytosine, guanine and thymine.

Discover amounts of adenine and thymine were equal, as were the amounts of cytosine and guanine.

Purines

Consist of a six-membered and a five-membered nitrogen-containing ring, fused together.

Adenine = 6-amino purine Guanine = 2-amino-6-oxo purine .



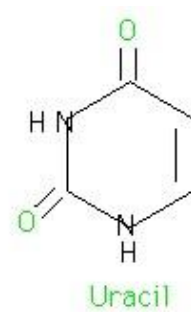
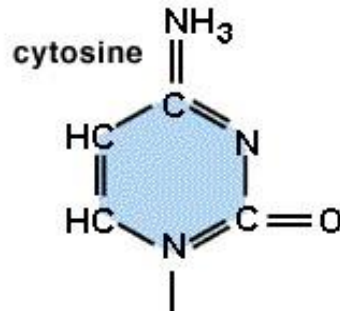
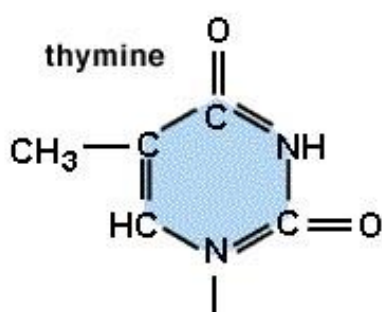
Pyrimidines

Have only a six-membered nitrogen-containing ring.

Uracil = 2,4-dioxy pyrimidine

Thymine = 2,4-dioxy-5-methyl pyrimidine

Cytosine = 2-oxy-4-amino pyrimidine



Chargaff's first parity rule

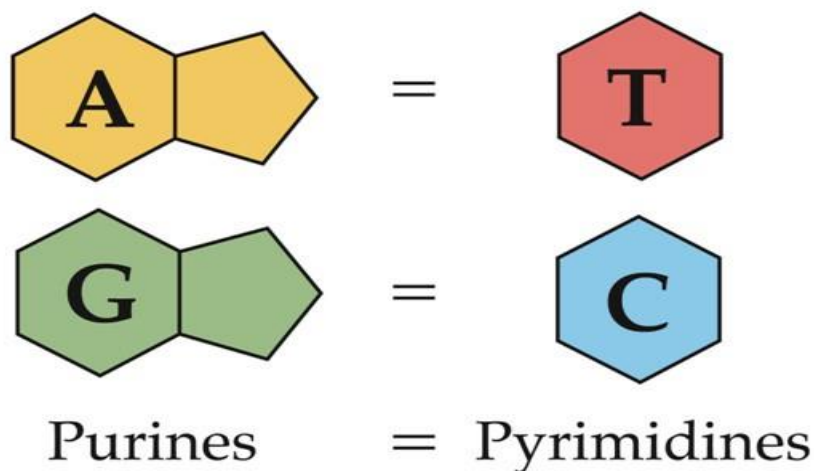
Stated that purines pair with pyrimidines, specifically A with T and C with G

Chargaff's second parity rule

% of A approximately equals % of T

% of G approximately equals % of C

Chargaff's second parity rule



G-C rule

Stated that (G+C) % is constant within a species, but often differs between species.

Cluster rule

Stated that pyrimidines often occur in clusters, and hence on the complementary strand purines do likewise.

Giant or Polytene chromosome

Giant chromosome

Found in salivary glands of some Diptera.

These chromosomes are very long and thick.

Hence they are known as Giant chromosomes.

Giant chromosome

They are first discovered by Balbiani in 1881 in dipteran salivary glands and thus also known as salivary gland chromosomes.

Giant chromosome

Giant chromosomes have also been discovered in suspensors of young embryos of many plants, but these do not show the bands so typical of salivary gland chromosomes.

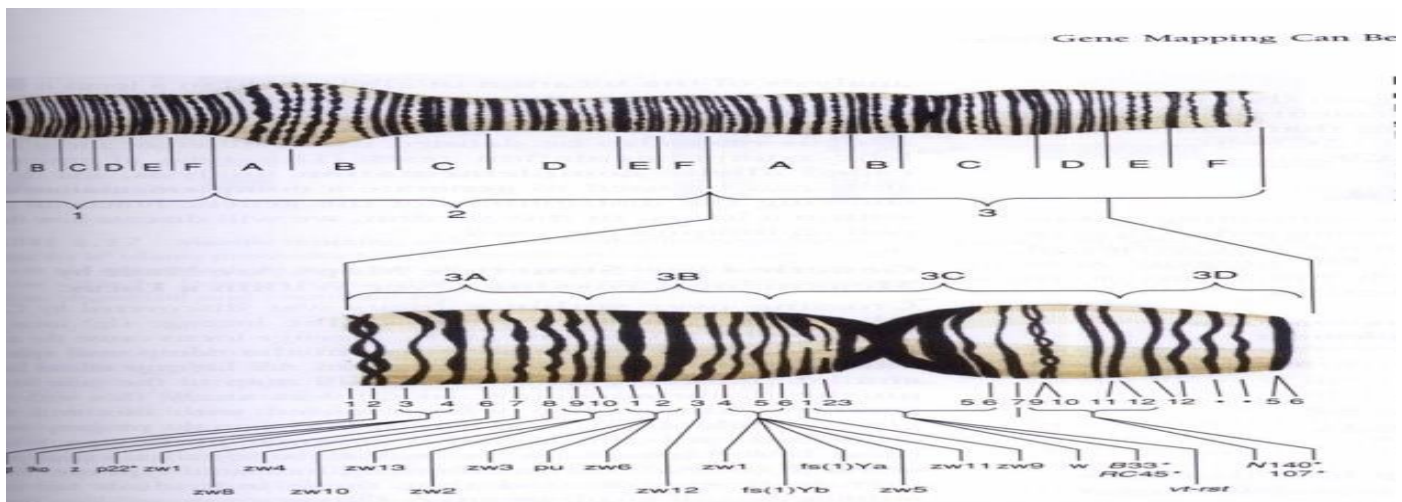
Giant chromosome

The total length of *D. melanogaster* giant chromosomes is about $2,000\mu$.

Giant chromosome

Giant chromosomes are made dark staining regions called “bands”.

Separated by relatively light or non-staining “interband” regions. In *Drosophila* about 5000 bands can be recognized.



Giant chromosome

Some of these bands are as thick as 0.5μ , while some may be only 0.05μ thick.

About 25,000 base-pairs are now estimated for each band.

Giant chromosome

These chromosomes are also known as “Polytene chromosome”, and the condition is referred to as “Polytene”

Giant chromosome

The numerous strands of these chromosomes are produced due to repeated replication of the paired chromosomes without any nuclear or cell division.

Giant chromosome

It is estimated that giant chromosomes of Drosophila have about 1,024 strands.

In the case of Chironomous may have about 4,096 strands.

48

Lampbrush chromosome

Lampbrush Chromosome

Lampbrush chromosome

It was given this name because it is similar in appearance to the brushes used to clean lamp chimneys.

First observed by Flemming in 1882.

Lampbrush chromosome

These are found in oocytic nuclei of vertebrates (sharks, amphibians, reptiles and birds) as well as in some invertebrates.

Also found in some plants.

Lampbrush chromosome

One loop represent one chromatid, i.e., one DNA molecule. The size of the loop may be ranging the average of 9.5 μm to about 200 μm

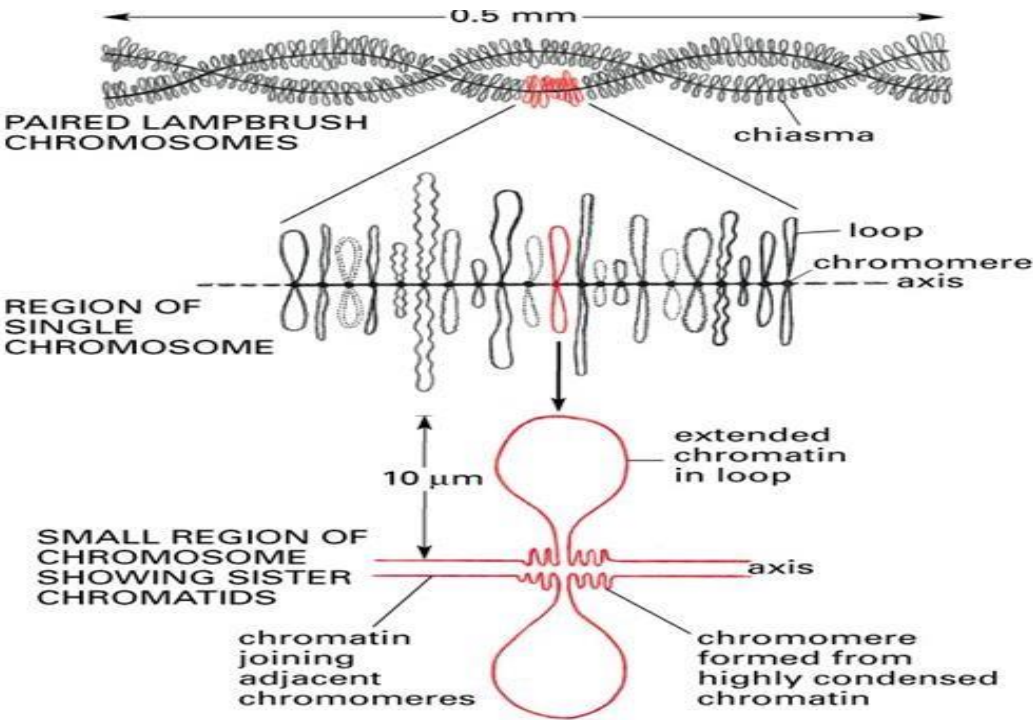
Lampbrush chromosome

Each lampbrush chromosome contains a central axial region, where the two chromatids are highly condensed.

Each chromosome has several chromomeres distributed over its length.

Lampbrush chromosome

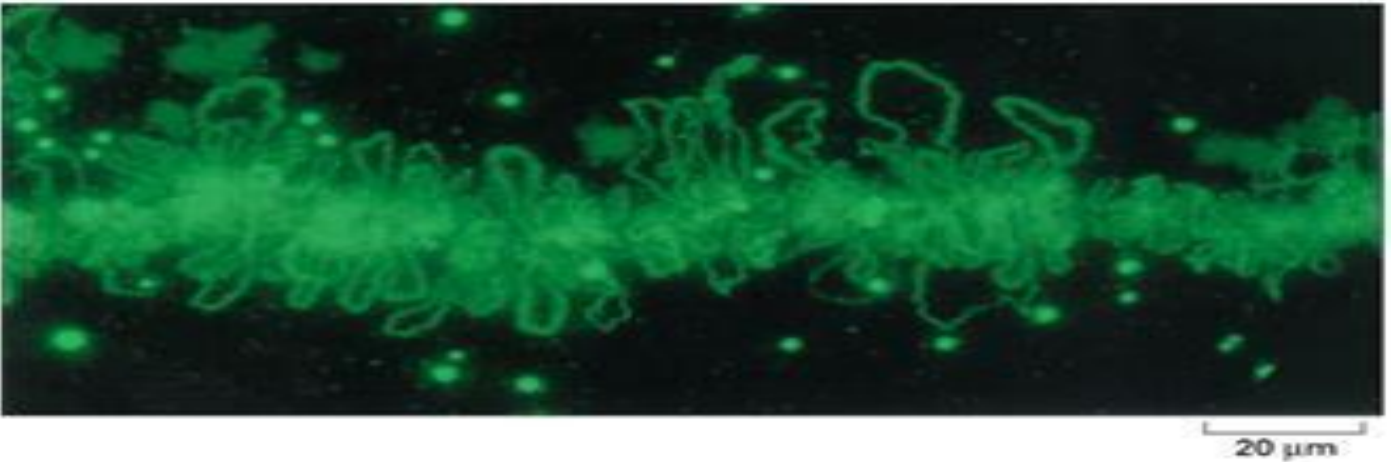
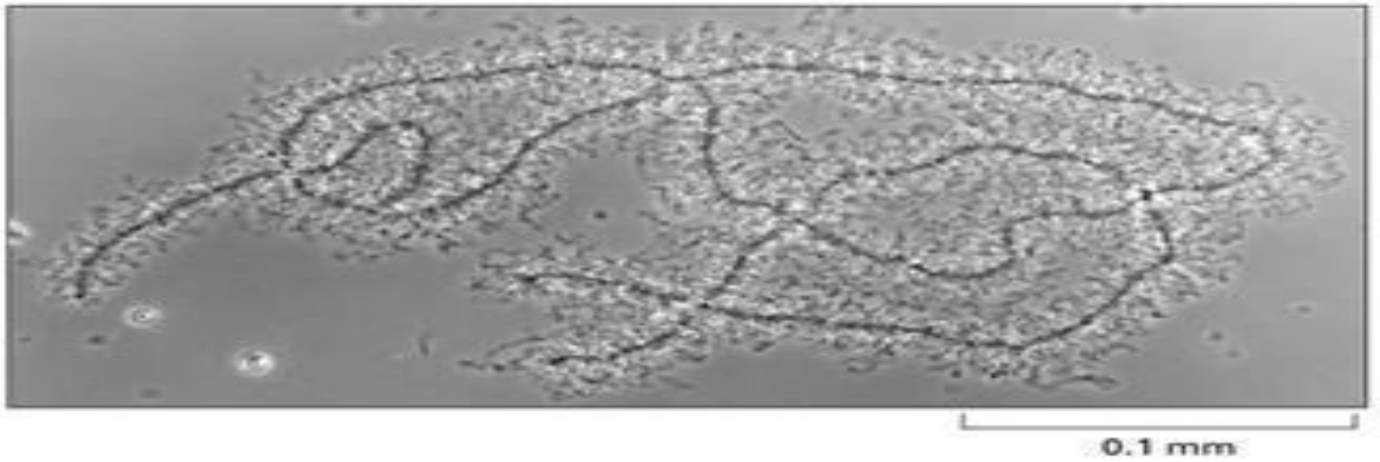
One loop represent one chromatid, i.e., one DNA molecule. The size of the loop may be ranging the average of 9.5 μm to about 200 μm



Lampbrush chromosome

One end of each loop is thinner (thin end) than the other end (thick end).

Phase-contrast and fluorescent micrographs

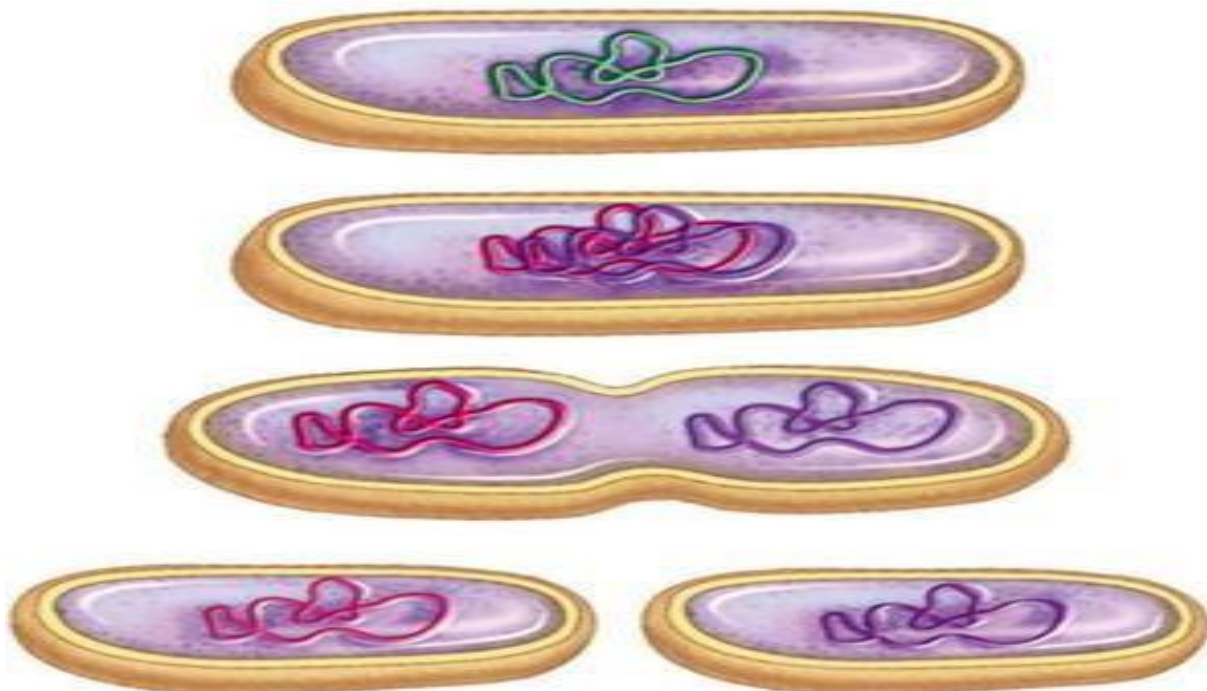


49 Cell Division

Cell division and reproduction: Cell division is a fundamental process which is required for growth in unicellular and multicellular organisms. It is a process that divides the cell into two daughter cells. It is necessary for reproduction in unicellular and multicellular organism. Cell division also helps to replace worn out cells in multicellular organisms.

Reproduction in prokaryotes - binary fission

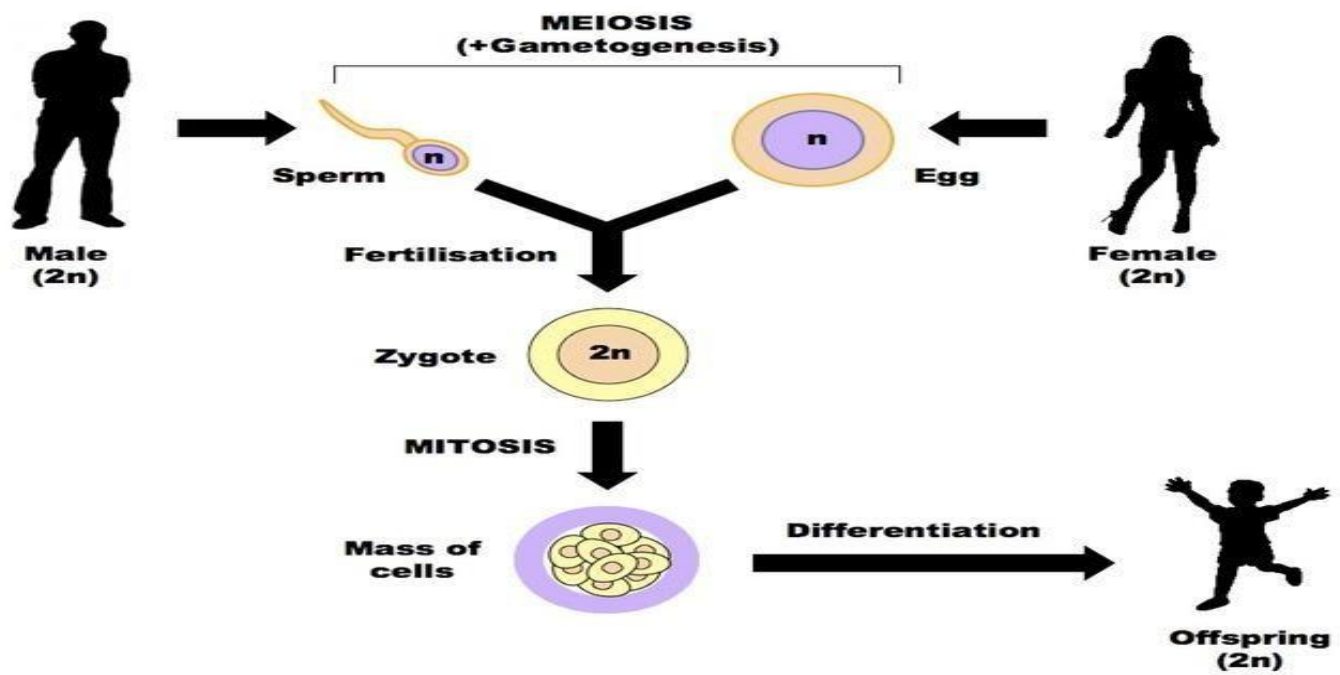
Prokaryotes simply divide their cells into two daughter cells by binary fission.



Reproduction in eukaryotes

Eukaryotes must divide their nucleus and other organelles before cell division.

Before the nucleus divides, the genetic material replicates.



Conclusion

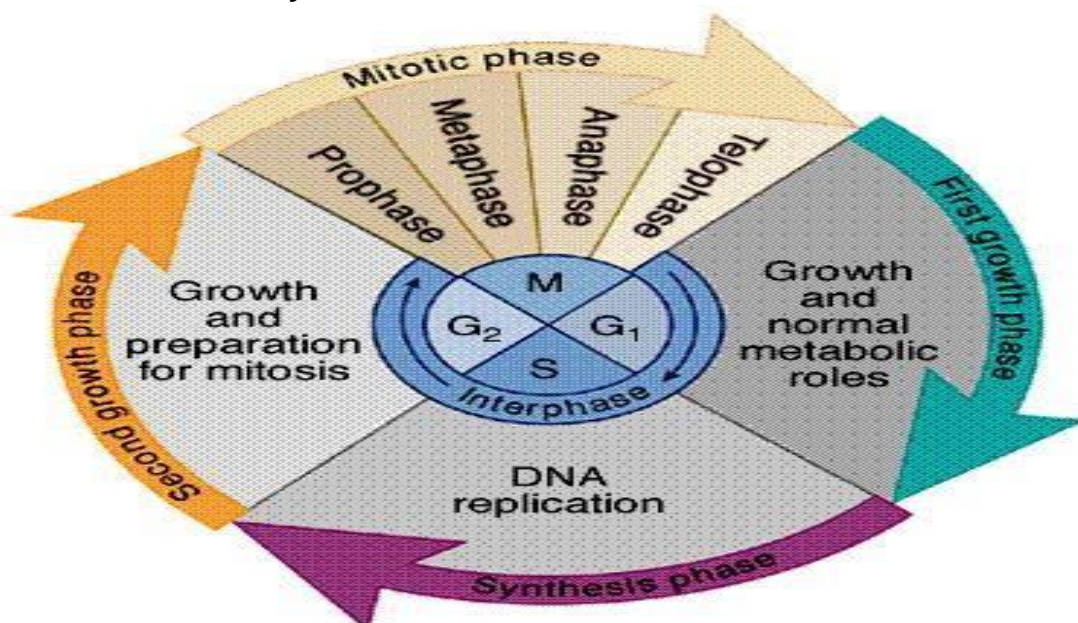
Cell division and reproduction are two fundamental processes in prokaryotes and eukaryotes

50 Cell Cycle

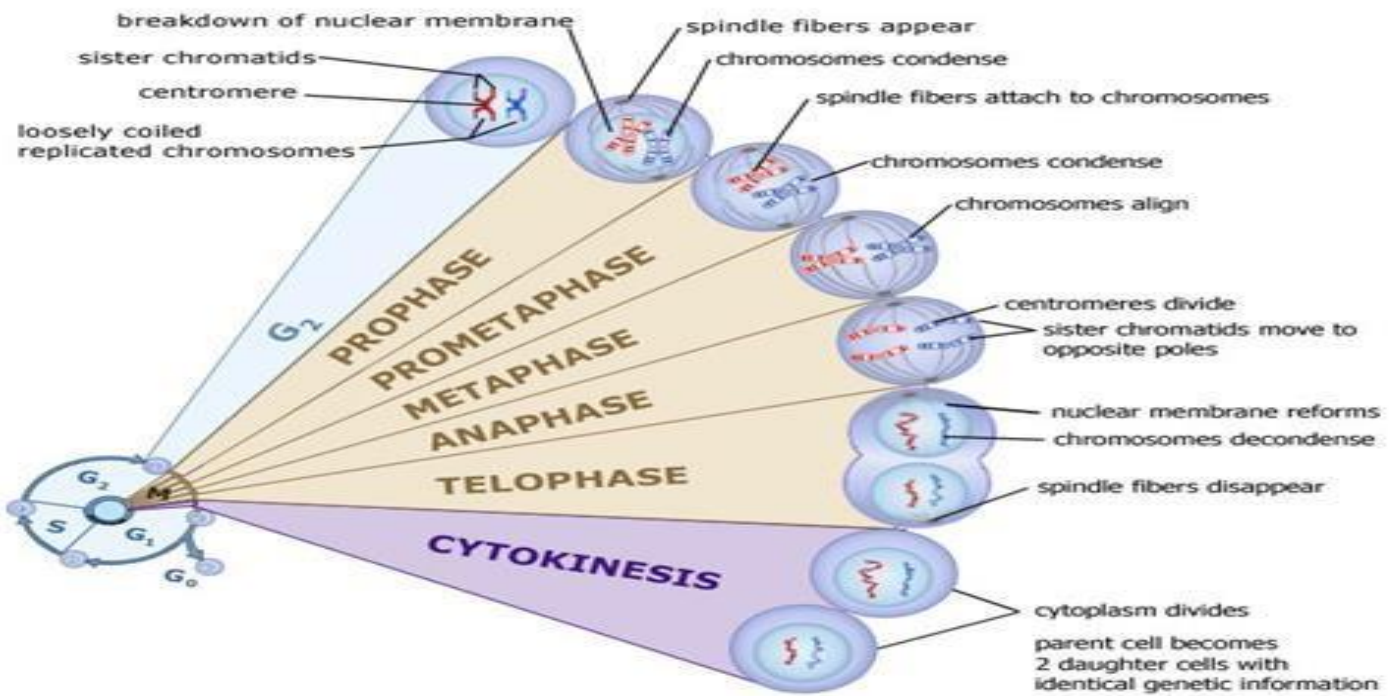
Cell cycle: The cell cycle consists of;

- Interphase ☐ Mitotic phase

Phases of cell cycle



Mitotic phase



G0 phase

G₀ phase also called as post-mitotic phase.

Eukaryotes cells generally enter into G₀ state from G₁.

Interphase

Before a cell enters into cell division, it needs to take nutrients.

Interphase is a series of changes that takes place in a newly formed cell.

Interphase proceeds in three stages;

- G₁ stage
- S stage
- G₂ stage

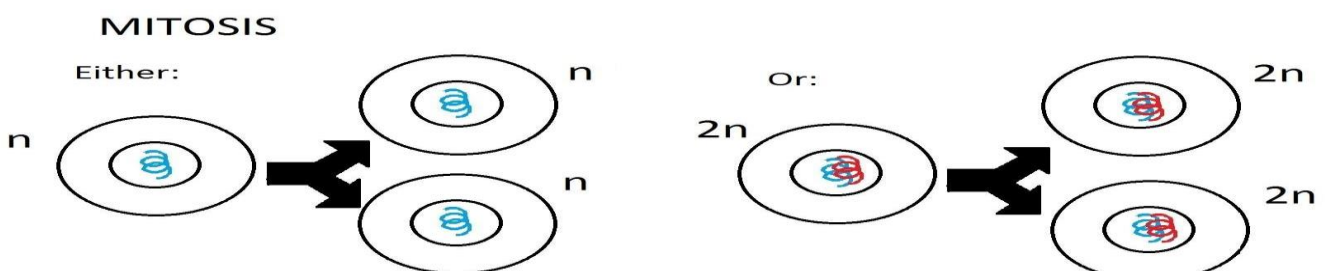
Functions of cell cycle include reproduction, growth and development after tissue renewal

51 Mitosis

Mitosis: A single cell divides into two identical daughter cells. Some haploid & diploid cells divide by mitosis. It produces two new cells from a single cell. Both new cells are genetically identical to the original cell. Each cell has to have its own cytoplasm and DNA.



Chromosomes replicate before mitosis, DNA replicate to produce identical copies. Two sister chromatids have to break apart.



A single cell divides into two identical daughter cells.

52 Unreplicated and Replicated Chromosomes

Un-replicated and replicated chromosome: before the cell division takes place; Chromosomes (DNA) are replicated (duplicated) .Duplicated chromosomes which are attached at their centromere As long as they remain attached, known as sister chromatids.

Un-replicated

Duplicated chromosomes are due to DNA replication Entire DNA of each of the chromosome replicates. Replication is semiconservative.

DNA replication: Double helical structure is opened by enzyme gyrases and topoisomerase. These enzymes remove helix and produce a nick to uncoiled DNA. One strand of DNA is called as leading strand while other strand is called as lagging strand. Continuous replication in leading strand. Discontinuous replication in lagging strands, i.e. in forms of small fragments which are called as Okazaki fragments.

Conclusion, before cell division, DNA replicates.

53 Interphase

Interphase: Interphase consists of

- G1 phase
- S phase □ G2 phase

Interphase summary Interphase ~ 90% of the time.

- G1: new cell absorbs nutrients and grows larger.
- S phase: Synthesis of new DNA for daughter cells. □ G2: Cell continues to grow, gets too large, needs to divide.

54 Prophase of Mitosis

Prophase of mitosis: Prophase is characterized by four events: Chromosomes condensed - more visible. Nuclear membrane disappears. Centrioles are separated and take positions on the opposite poles of the cell. Spindle fibers form and radiate toward the center of the cell.

- Chromosomes condense at the start of mitosis.
- DNA wraps around histones that condense it.

Prophase- chromosomes are condensed- Different levels of condensation/folding Chromosomes condense at the start of mitosis. DNA wraps around histones that condense it.

Prophase Summary Prophase is the first stage of mitotic cell division.

55 Metaphase of Mitosis

Metaphase During metaphase, two events occurs; Chromosomes line up across the middle of the cell. Spindle fibers connect the centromere of each sister chromatid to the poles of the cell, where centrioles are present. Metaphase – chromosomes are aligned in the middle

Metaphase-Summary During metaphase, two events occurs; Chromosomes lined up in the middle line. Spindle fibers connect to centromere.

56 Anaphase of Mitosis

Anaphase of mitosis During anaphase, three events occurs; Centromere split – as a result sister chromatids are separated. Sister chromatids become individual chromosomes. Separated chromatids move to opposite poles of the cell.

- Anaphase Securin is a protein which inhibits a protease known as separase. Destruction of securin results in split of centromeres and the new daughter chromosomes are pulled toward the poles.

Cell in an oval shape Chromosomes are drawn to each side of the cell.

Non-kinetochore spindle fibers push against each other,that stretch the cell into an oval shape

Summary Centromere split and chromosomes are separated. Chromosomes arrived at poles. Cell stretches into an oval shape.

57 Telophase of Mitosis

Telophase

consists of four events: Chromosomes start to uncoil. Nuclear envelope forms around the chromosomes at each pole of the cell.

Telophase Spindle fibers break down and dissolve, Daughter nucleus is formed in each of the daughter cell. Telophase Nuclear membranes are formed around each set of chromatids. Nucleoli also reappear. Chromosomes unwind back into chromatin material.

Telophase – effects of prophase are reversed During telophase, the effects of prophase and prometaphase are reversed.

Telophase Summary Spindle fibers break down and nuclear members develops around each daughter nucleus.

58 Cytokinesis

Cytokinesis

Cytokinesis is the division of the cytoplasm into two individual cells. Process differs in plants and animal cells

Cytokinesis in animal cells Cell membrane forms a cleavage furrow that eventually pinches the cell into two equal parts. Each part containing its own nucleus and cytoplasmic organelles.

Cytokinesis in Plants cells a cell plate is formed in midway that divides the nuclei. Cell plate gradually develops into a separating membrane.

Plants cell- cytokinesis

Cytokinesis - comparison

Summary Cytokinesis differs in animal and plant cells. In animal cells, the membrane pinches and divides the cell. In plant cells, a cell plate forms that divides the cell.

59 Mitotic Spindles

Mitotic apparatus/ spindles include

- Centrosomes
- Spindle microtubules □ Asters

Mitotic apparatus/ spindles the apparatus of microtubules controls chromosome movement during mitosis. Centrosome replicates Centrosome replicates, forming two centrosomes that migrate to opposite poles. Assembly of spindle microtubules begins in the centrosome. Mitotic spindles – asters Asters are the radial array of short microtubules extends from each centrosome Spindles microtubules attach to kinetochores Spindle microtubules attach to the kinetochores of chromosomes and move the chromosomes to the metaphase plate.

Mitotic spindles in anaphase, sister chromatids separate and move along the kinetochore microtubules toward opposite ends of the cell.

60 Somatic cells and Gametes

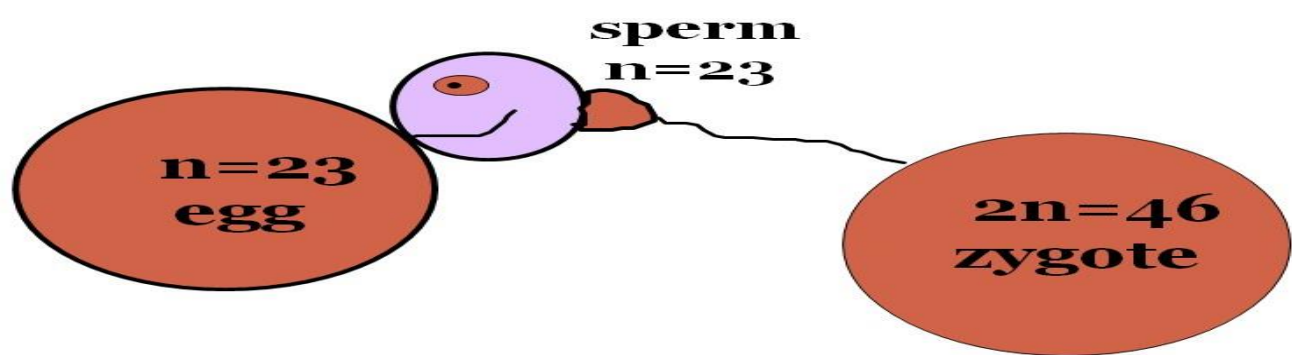
Diploid cells and haploid cells

Diploid cells are body cells. They are called as (2n) cells. Examples: skin cells, brain cells, bone cells, blood cells. In humans, ~ 220 types of somatic cells.

Haploid cells Sex cells produce gametes. Gametes are haploid (n) cells. Sperms and ova are gametes which are produced in males and females respectively.

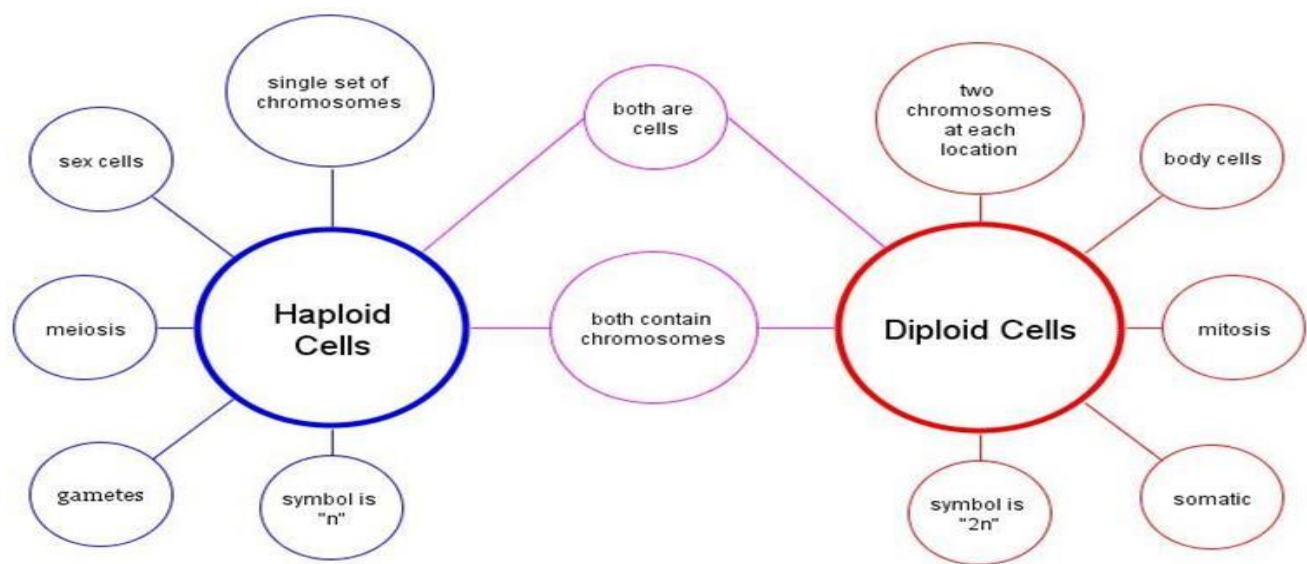
Male Gametes and female gametes the male gametes are the sperms and are produced in the male gonad - testes. The female gametes are the ova (ovum) and are produced in the female gonad - ovaries.

Fertilization is a process of fusion of spermatozoa and ova to produce a cell called zygote.



Diploid - somatic cells

Haploid - sex cells



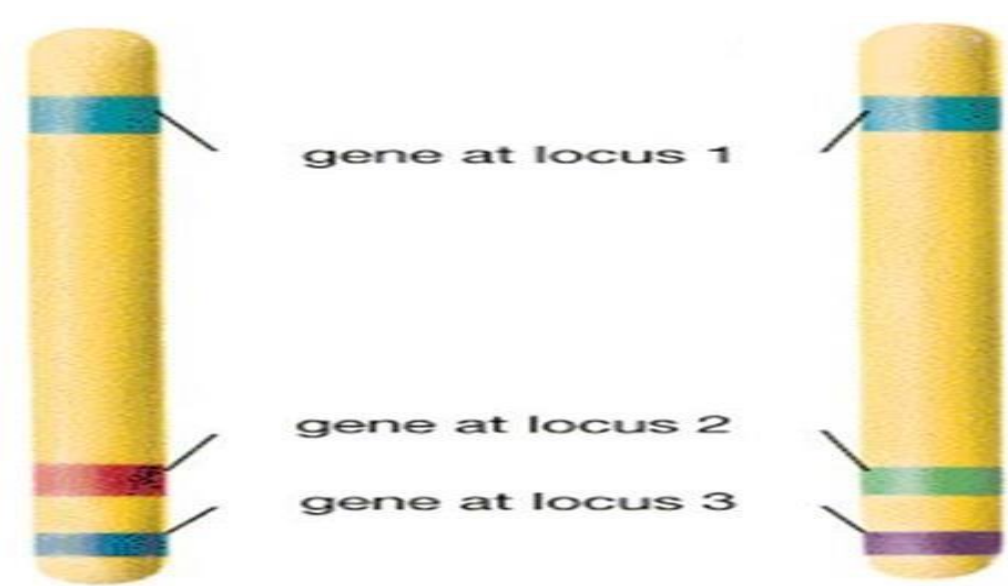
Meiosis is a process that reduces diploid number into haploid.

61 Homologous Chromosomes

Homologous chromosomes:

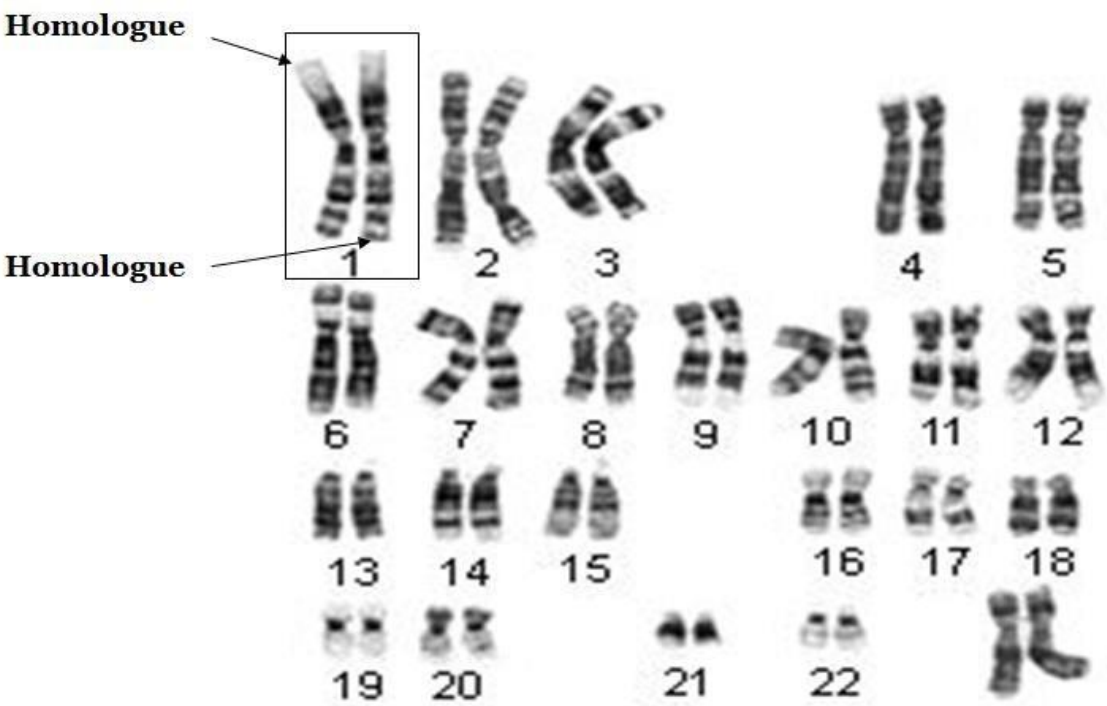
Pair of chromosomes that are similar in shape and size are called as homologous chromosomes. Homologous pairs carry genes (alleles) controlling the same inherited traits. Each gene alleles are in the same position on homologues chromosome.

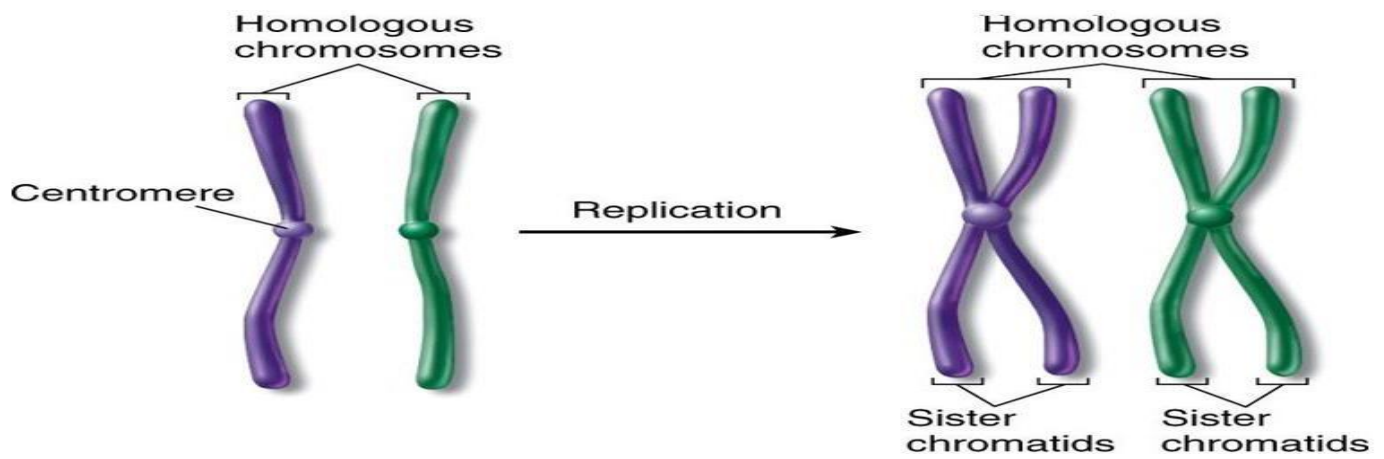
Homologous – shape and size



Homologous- one from male, one from female: For each set, one of the homologues comes from the male, while other homologue comes from the female. If an organism is diploid (2n) then it has two matching

Homologous chromosomes in humans are 46 chromosomes; 22 pair of homologous chromosomes, while one pair is of sex chromosomes. Sex chromosomes in male are XY while female is XX.





62 Meiosis

Meiosis: Meiosis - diploid cells are reduced to haploid cells. This is a process by which gametes (sex cells) are produced.

Chromosome numbers would be doubled without meiosis. If meiosis did not occur, the chromosome number in each new generation would be doubled. The offspring would die.

Meiosis is a two cell divisions process. There is only one duplication of chromosomes during meiosis I.

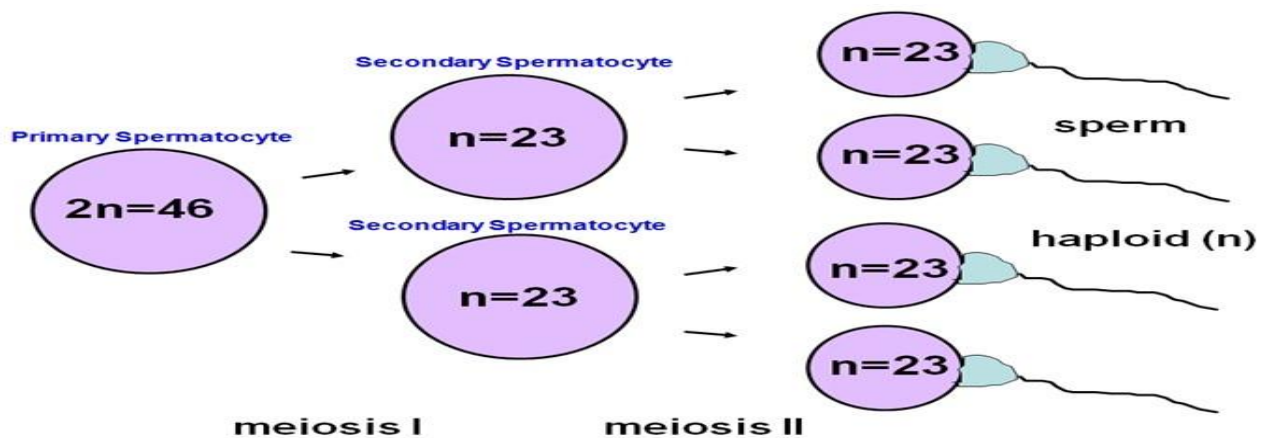
Meiosis I

Meiosis II

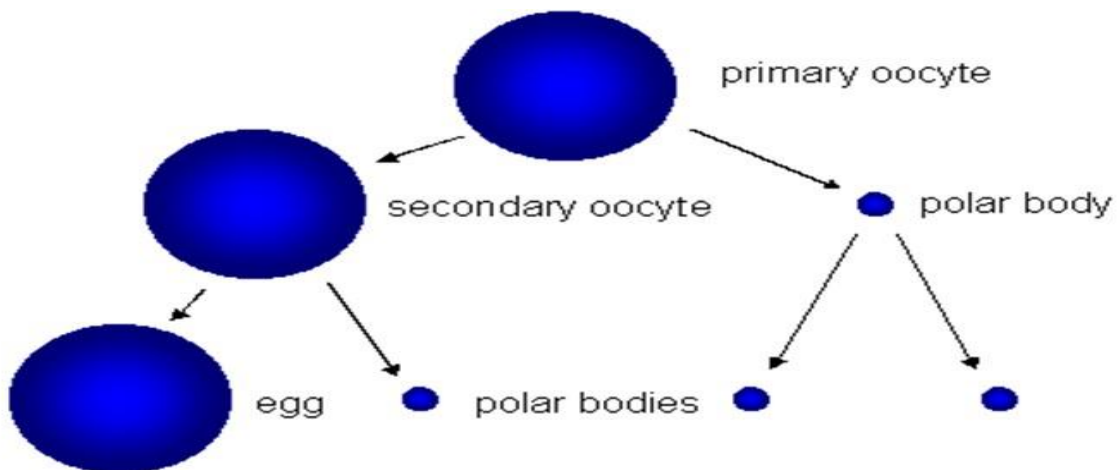
Spermatogenesis and oogenesis

Meiosis in males is called spermatogenesis and produces sperms.

Meiosis in females is called oogenesis and produces ova.



Spermatogenesis: Four sperm cells are produced from primary spermatocyte



Oogenesis: The polar bodies die. Only one ovum (egg) is produced from each primary oocyte.

Stages of meiosis: There are two stages of meiosis

Meiosis I

Meiosis II

During meiosis I, homologous chromosomes separate.

During meiosis II, sister chromatids separate.

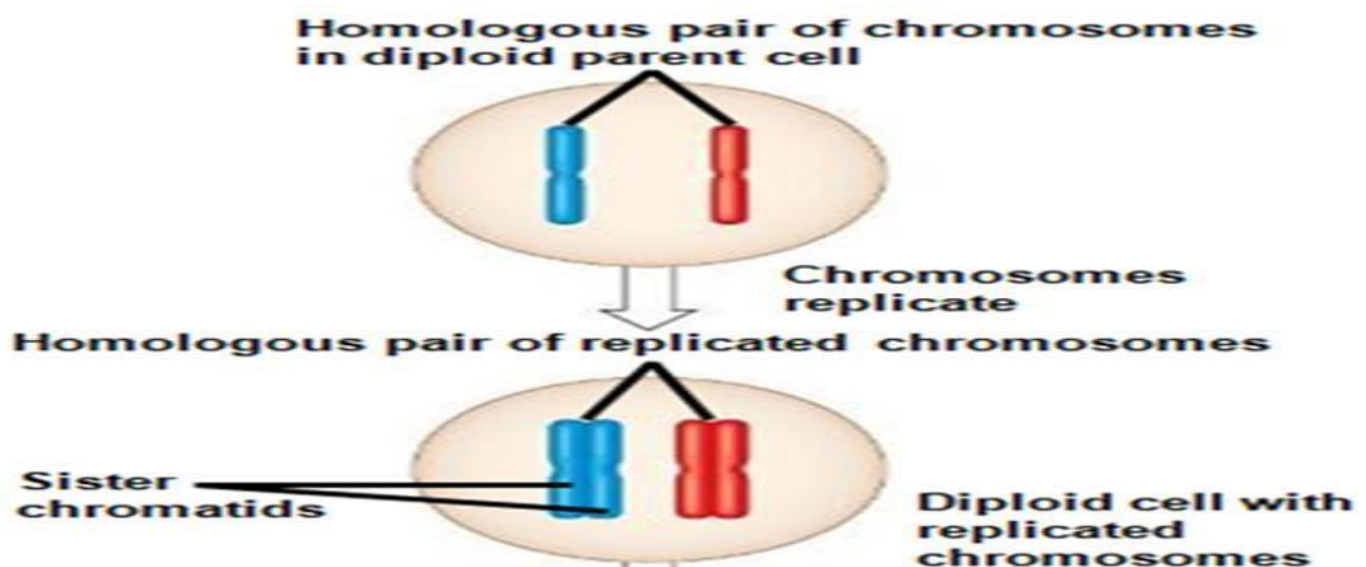
Meiosis I – Reductional division

Meiosis I results in two haploid daughter cells. It is called the reductional division. Homologous chromosomes separate.

Meiosis II: Second cell division where sister chromatids separate. As a result of meiosis II, two haploid cells become four haploid cells. Meiosis II behaves like mitosis, where no reduction in chromosomes number.

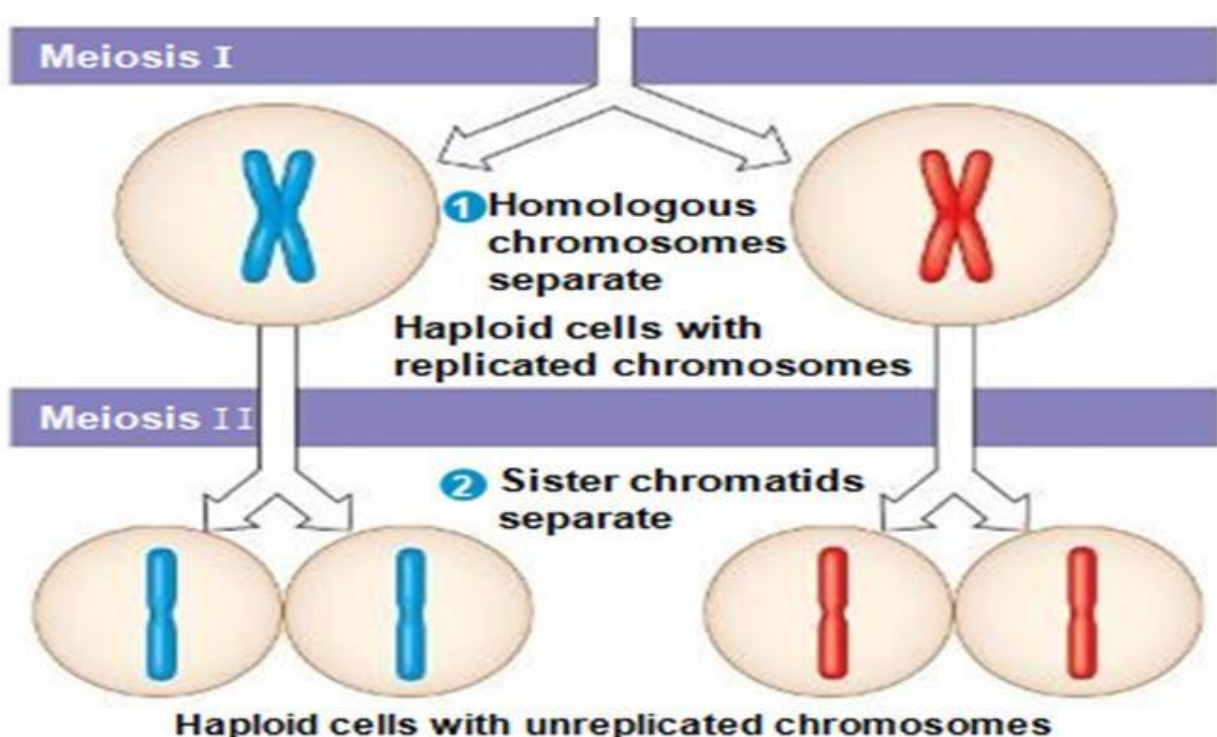
Meiosis II – Equational division: Meiosis II results in four haploid daughter cells with unreplicated chromosomes; it is called equational division.

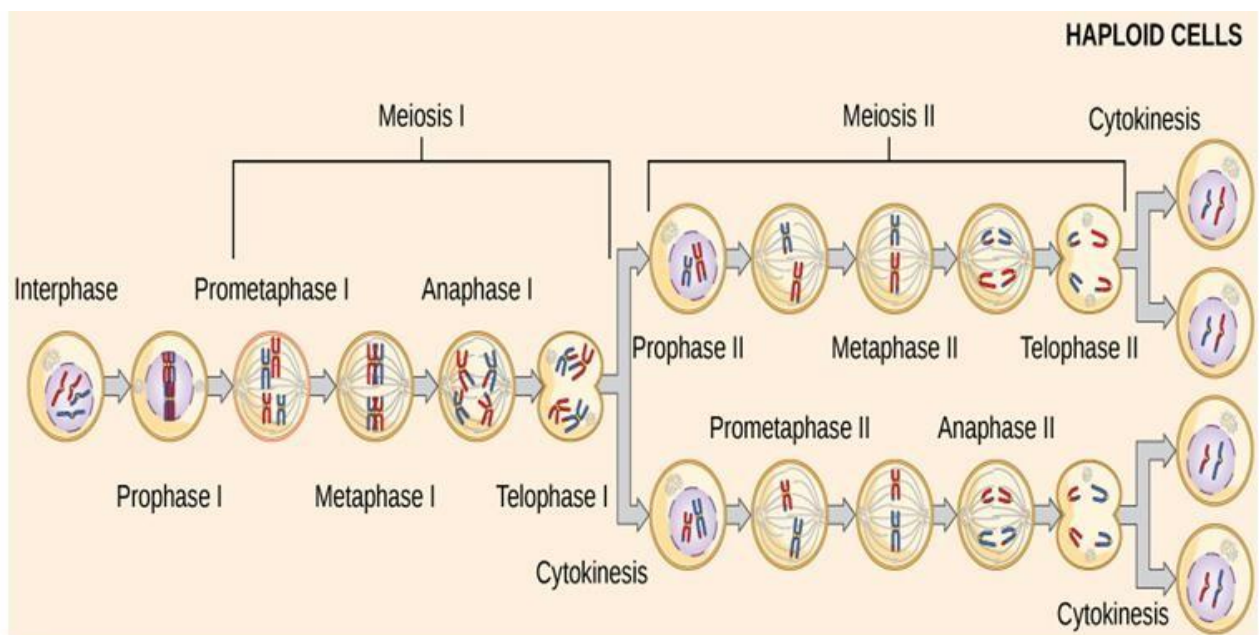
Interphase chromosomes replicates



Meiosis I, Meiosis II

Meiosis - four haploid cells





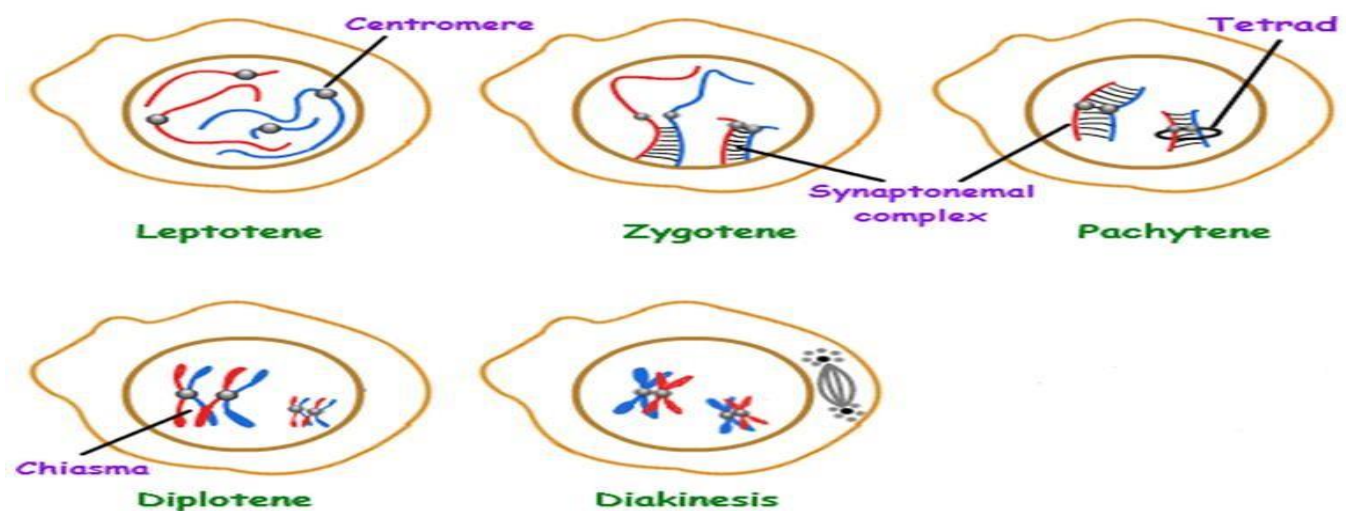
64 Prophase I of Meiosis

Prophase I of meiosis

Prophase I occupies more than 90% of the time required for meiosis. Five sub stages of prophase I

Sub stages of prophase I

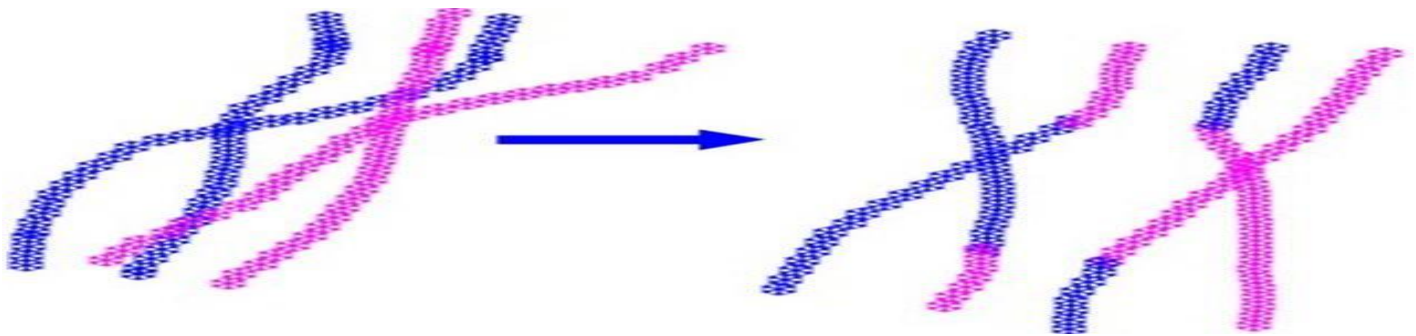
- Leptotene
- Zygotene
- Pachytene
- Diplotene
- Diakinesis



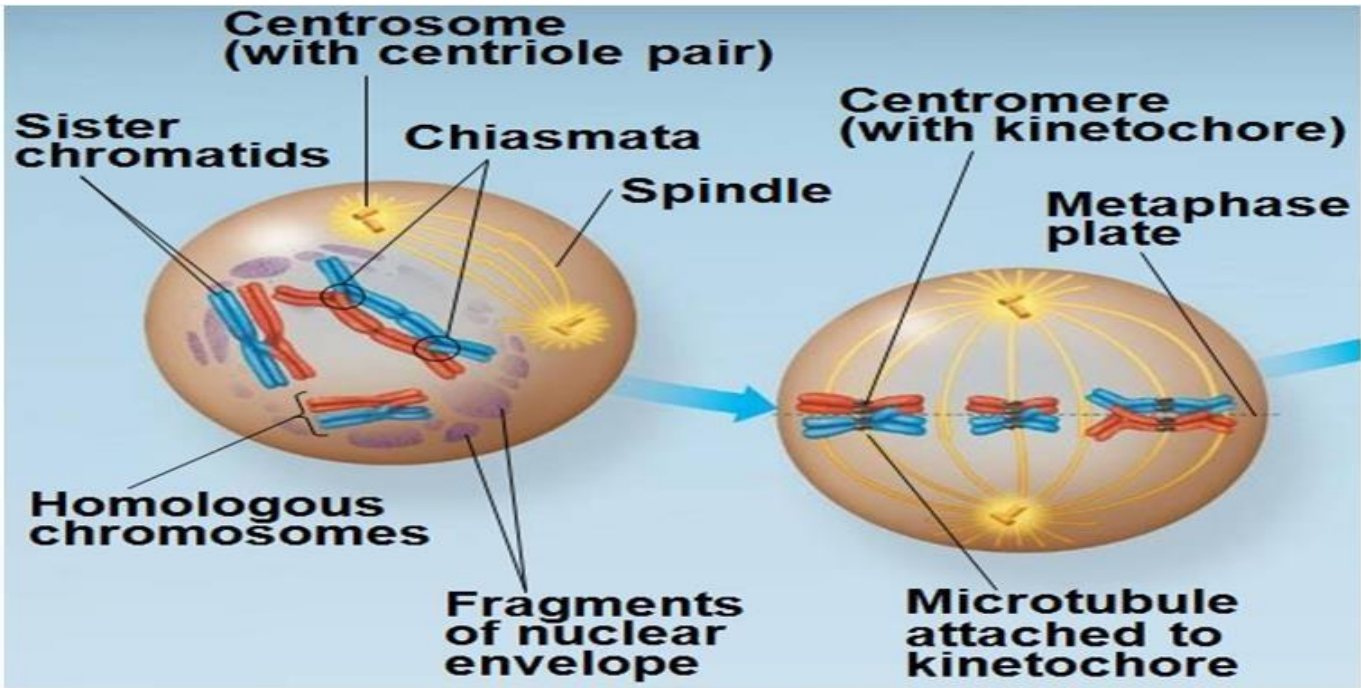
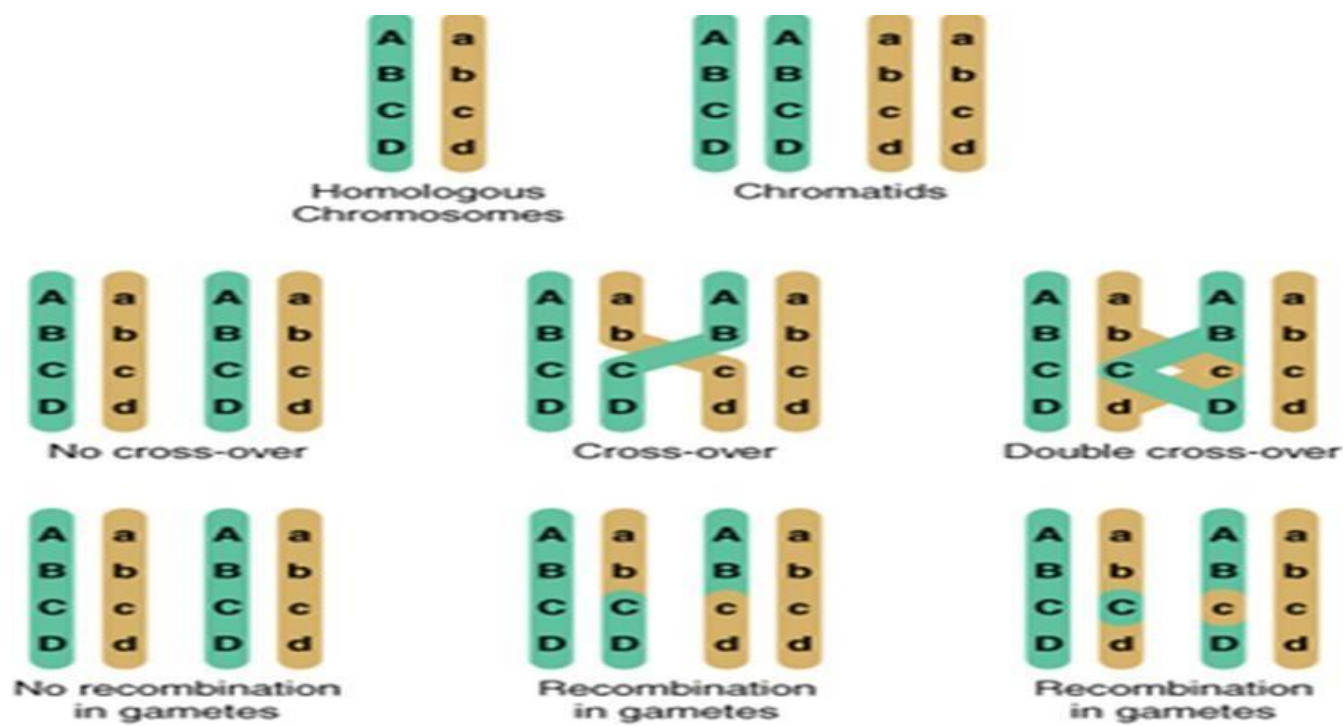
Events of prophase I: The duplicated homologous chromosomes pair. The nucleolus disappears. Centrioles migrate to opposite poles of the cell. Meiotic spindle forms between the two pairs of centrioles. Nuclear membrane disappears.

Chiasmata formation during prophase I During pairing up of chromosomes, non-sister chromatids exchange DNA segments. Each pair of chromosomes forms a tetrad, a group of four chromatids.

Pairing and exchange of chromosome fragments



Prophase I – Chiasmata



Summary - Prophase I

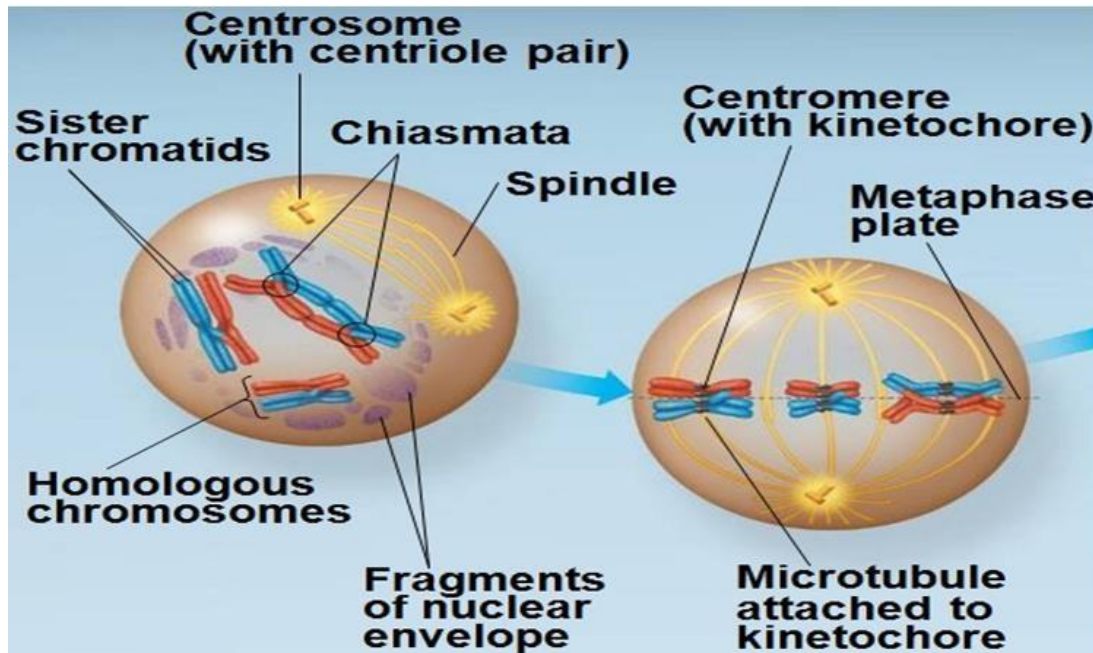
- Homologous chromosomes pairs.
- Nucleolus disappears.
- Meiotic spindle forms.
- Nuclear membrane disappears.

65 Metaphase I of Meiosis

Metaphase I of meiosis:

In metaphase I, tetrads line up at the metaphase plate, with one chromosome facing each pole. Microtubules from one pole are attached to kinetochore of one chromosome of each tetrad. Microtubules from the other pole are attached to the kinetochore of the other chromosome.

Metaphase I



66 Anaphase I of Meiosis

Anaphase I of meiosis Pairs of homologous chromosomes separate. One chromosome moves toward each pole, guided by the spindle apparatus.

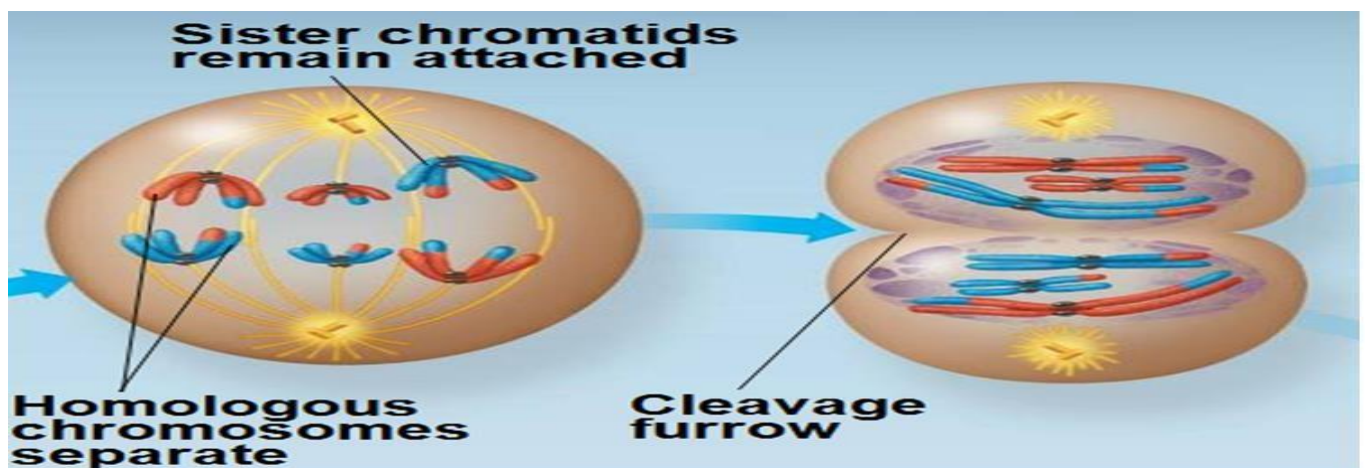
Anaphase I – sister chromatids attached at centromere Sister Chromatids remain attached at the centromere of each of the homologue chromosome and move as one unit toward the pole.

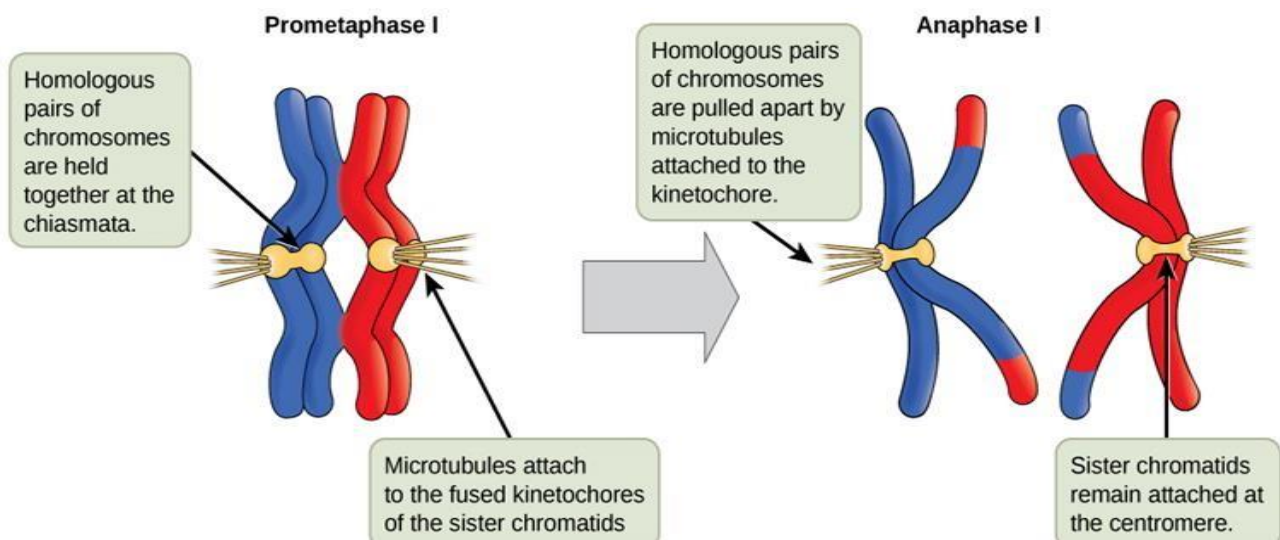
Difference between anaphase of mitosis and meiosis I

Sister chromatids remained joined in meiosis I, whereas in mitosis they separate.

In mitosis, centromere splits into two parts while in meiosis it do not splits.

Anaphase I





Summary - Anaphase I

- Pairs of homologous chromosomes separate.
- One chromosome moves toward one pole while other chromosome moves to other pole of the cell.

67 Telophase I of Meiosis

Telophase I of meiosis:

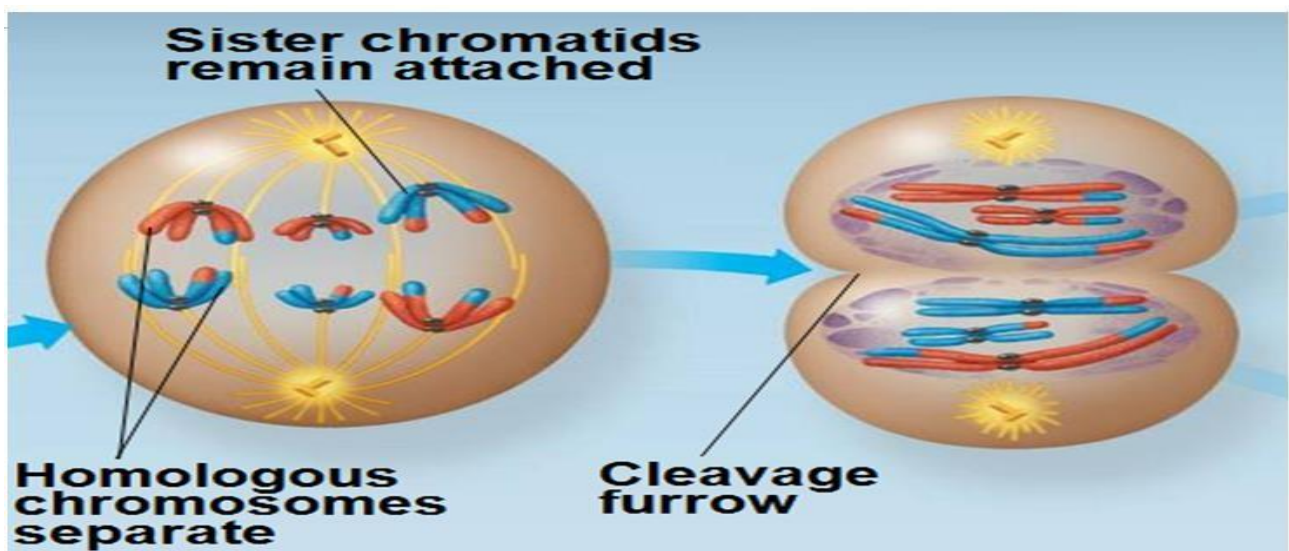
Haploid set of chromosomes arrived at each of the pole. Each chromosome still consists of two sister chromatids. Cytokinesis usually occurs simultaneously, forming two haploid daughter cells.

Cytokinesis – Animal cell and plants cell

- In animal cells, a cleavage furrow is formed.
- In plant cells, a cell plate formed.

No replication between Meiosis I and meiosis II: There is no chromosome replication between the end of meiosis I and beginning of meiosis II. Chromosomes are already replicated.

Telophase I



Haploid set of chromosomes arrived at each of the pole.

Meiosis I results, a diploid cell produced two haploid cells.

68 Prophase II of Meiosis

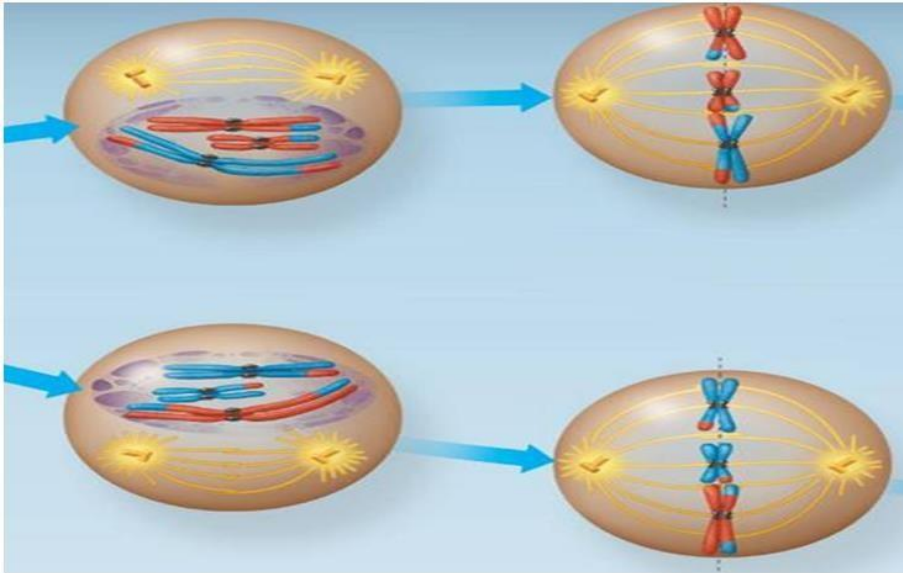
Prophase II of meiosis

Meiosis II similar to mitosis

- Meiosis II also occurs in four phases:
- Prophase II
- Metaphase II
- Anaphase II
- Telophase II and cytokinesis

Prophase II: No replication of Chromosomes as they are already replicated. Centrioles are separated and take positions on the opposite poles of the cell. Nuclear membrane disappears. Spindle fibers form and radiate toward the center of the cell.

Prophase II



Summary - Prophase II

- Nuclear membrane disappears.
- Spindle fibers form and radiate toward the center of the cell.
- Centrioles take the position on poles.

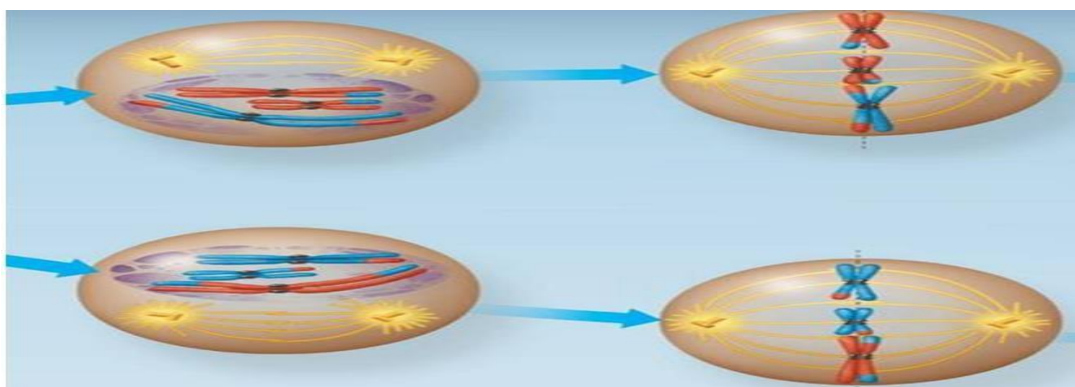
69 Metaphase II of Meiosis

Metaphase II of meiosis In metaphase II, sister chromatids are arranged at the metaphase plate.

The kinetochores of sister chromatids attach to microtubules extending from opposite poles.

Chromosomes lined up across the middle of the cell. Due to crossing over during meiosis I, the two sister chromatids of each chromosome are no longer genetically identical.

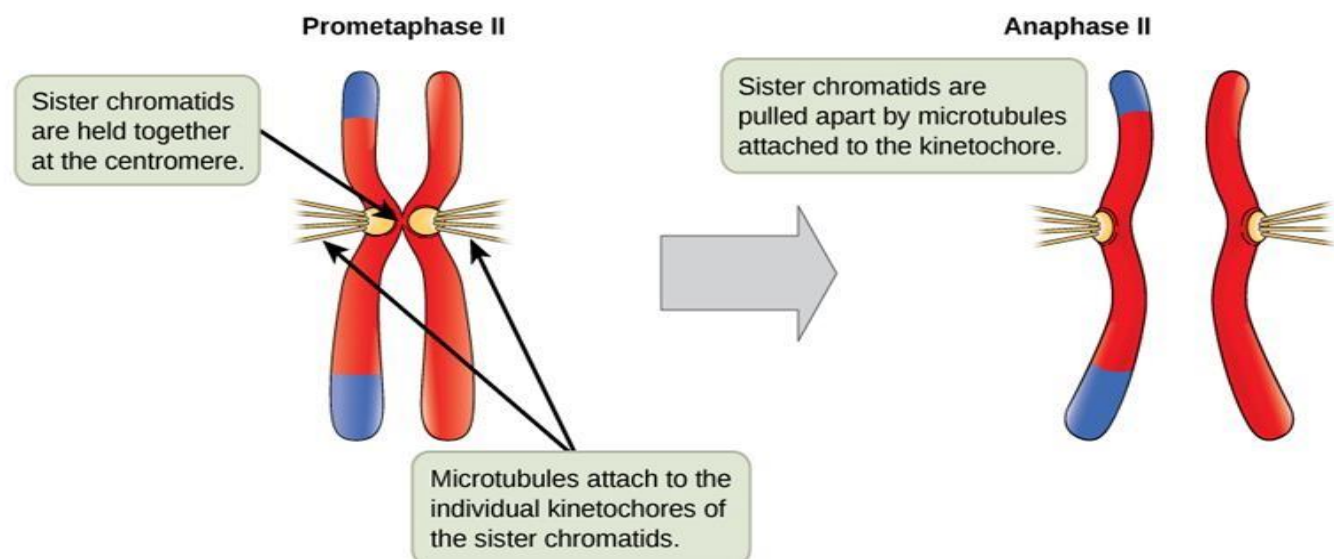
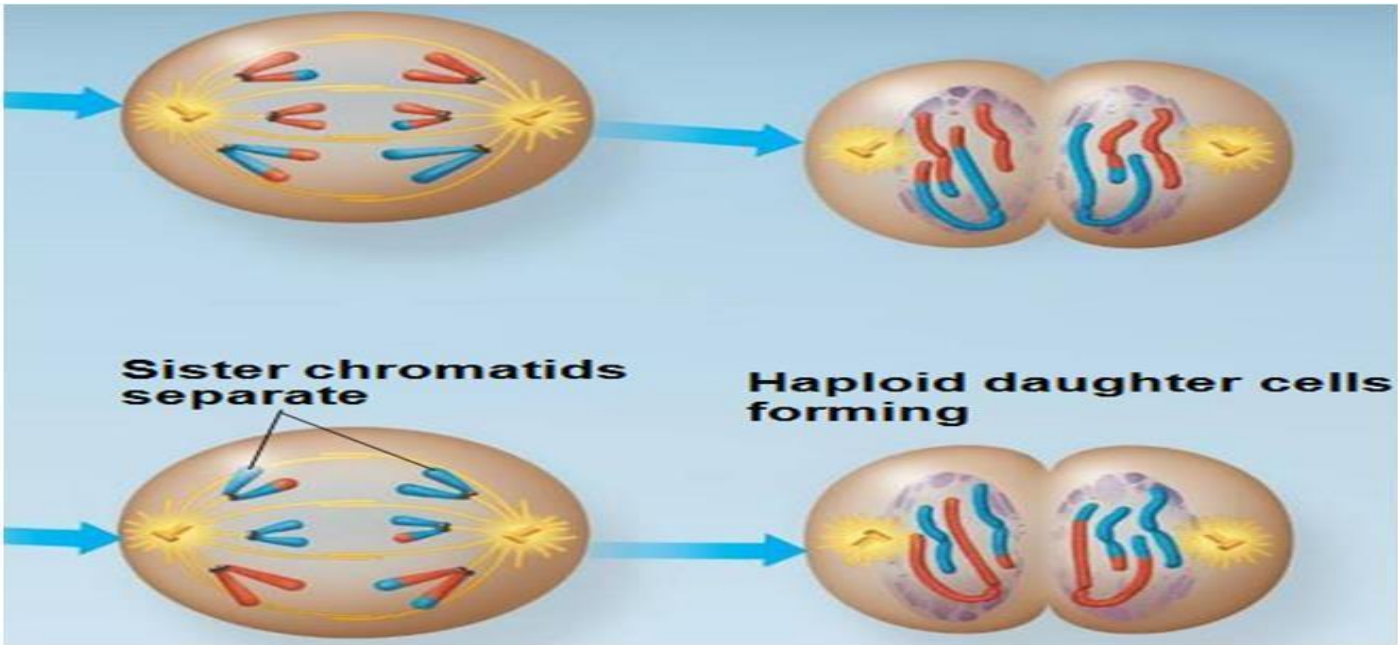
Metaphase II



Kinetochores of sister chromatids attach to microtubules extending from opposite poles. Chromosomes lined up across the middle of the cell.

70 Anaphase II of Meiosis

Anaphase II of meiosis Centromere split – as a result sister chromatids are separated. Sister chromatids become individual chromosomes. Separated chromatids move to opposite poles of the cell.

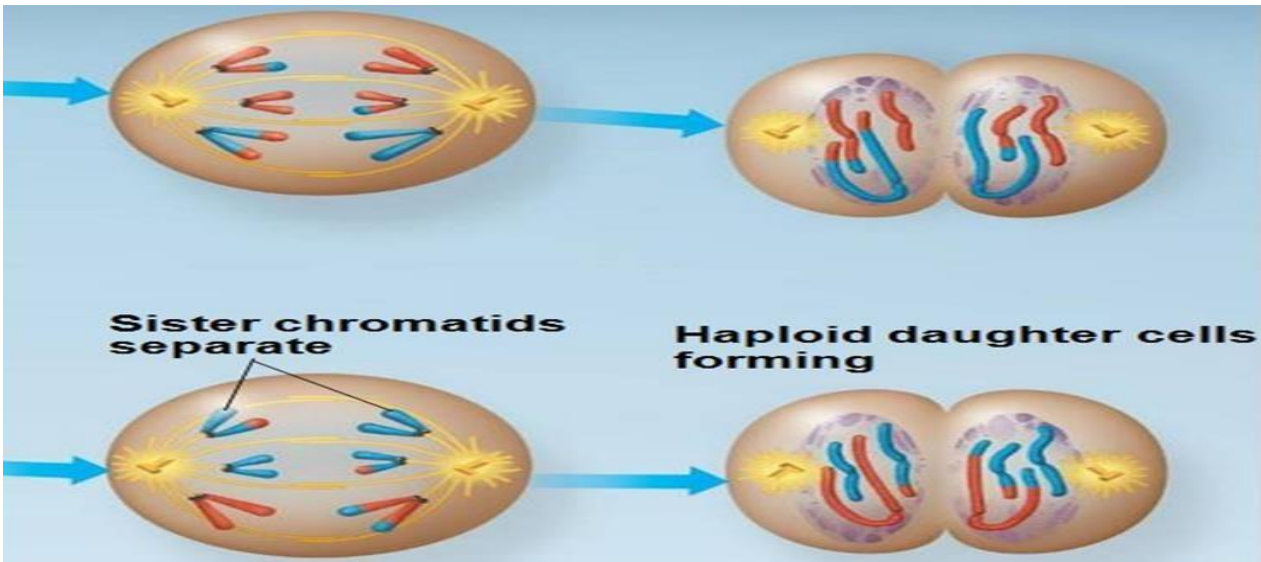


Summary - Anaphase II

- Sister chromatids become individual chromosomes.
- Separated chromatids move to opposite poles of the cell.

71 Telophase II of Meiosis

Telophase II of meiosis In Telophase II, the chromosomes arrive at opposite poles. Nuclear membrane forms and the chromosomes begin de-condensing. Cytokinesis occurs. Now, there are four daughter cells, each with a haploid set of chromosomes. Each daughter cell is genetically distinct from the others and from the parent cell.



Telophase II

Summary - Telophase II

- Four daughter cells are produced each with a haploid set of chromosomes.
- Each daughter cell is genetically distinct from the others and from the parent cell.

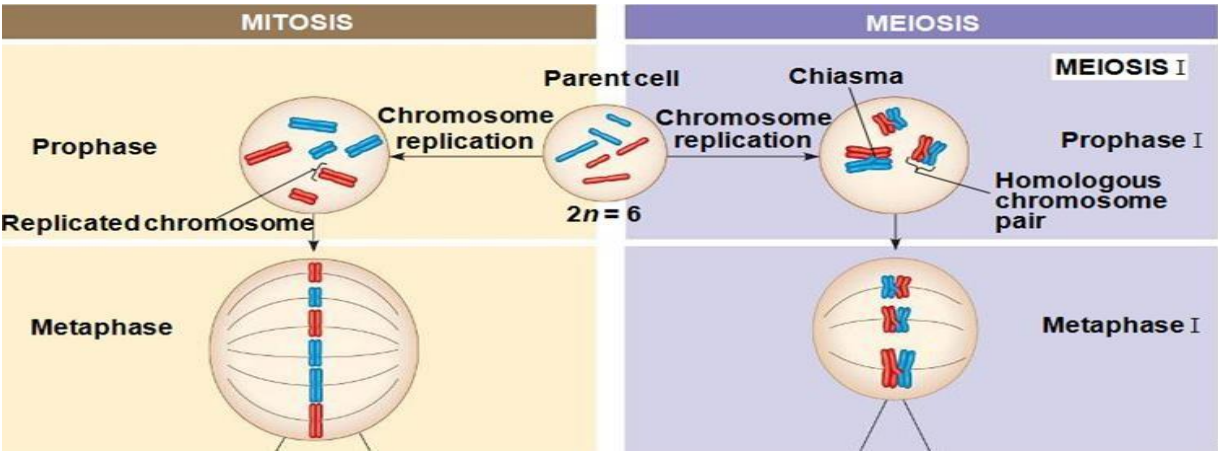
72 Mitosis and Meiosis

Mitosis and meiosis

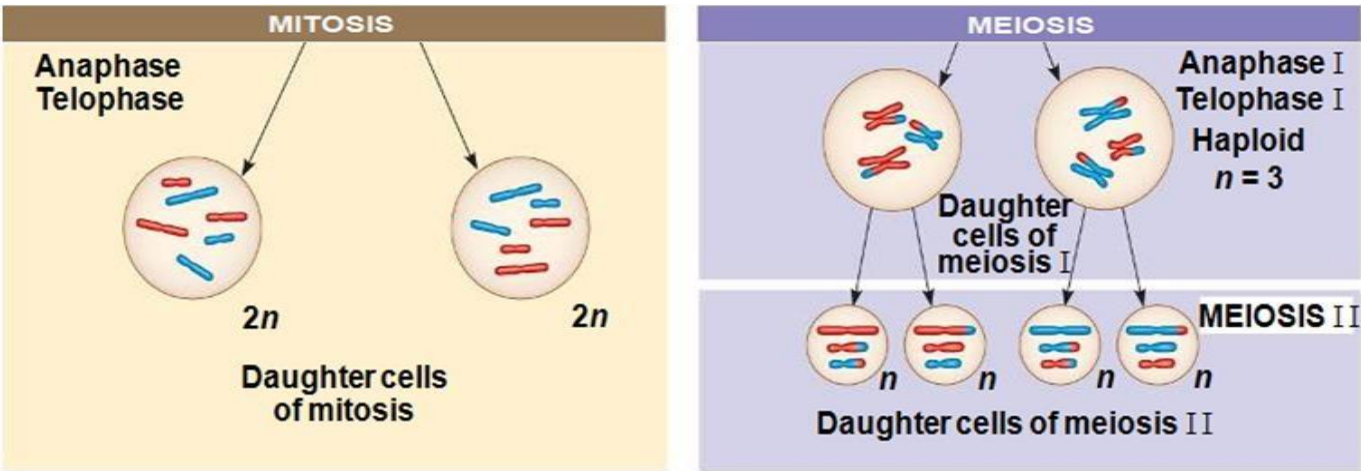
Mitosis: Mitosis conserves the number of chromosome sets, producing cells that are genetically identical to the parent cell.

Meiosis: Meiosis reduces the number of chromosomes sets from two (diploid) to one (haploid), producing cells that differ genetically from each other and from the parent cell.

Comparison



The mechanism for separating sister chromatids is virtually identical in meiosis II and mitosis.



Comparison

Meiosis reduces the number of chromosomes sets from diploid to one haploid and produced four daughter cells which not identical to each other and parent cell.

Mitosis – produces two daughter cells which are identical to parent cell.

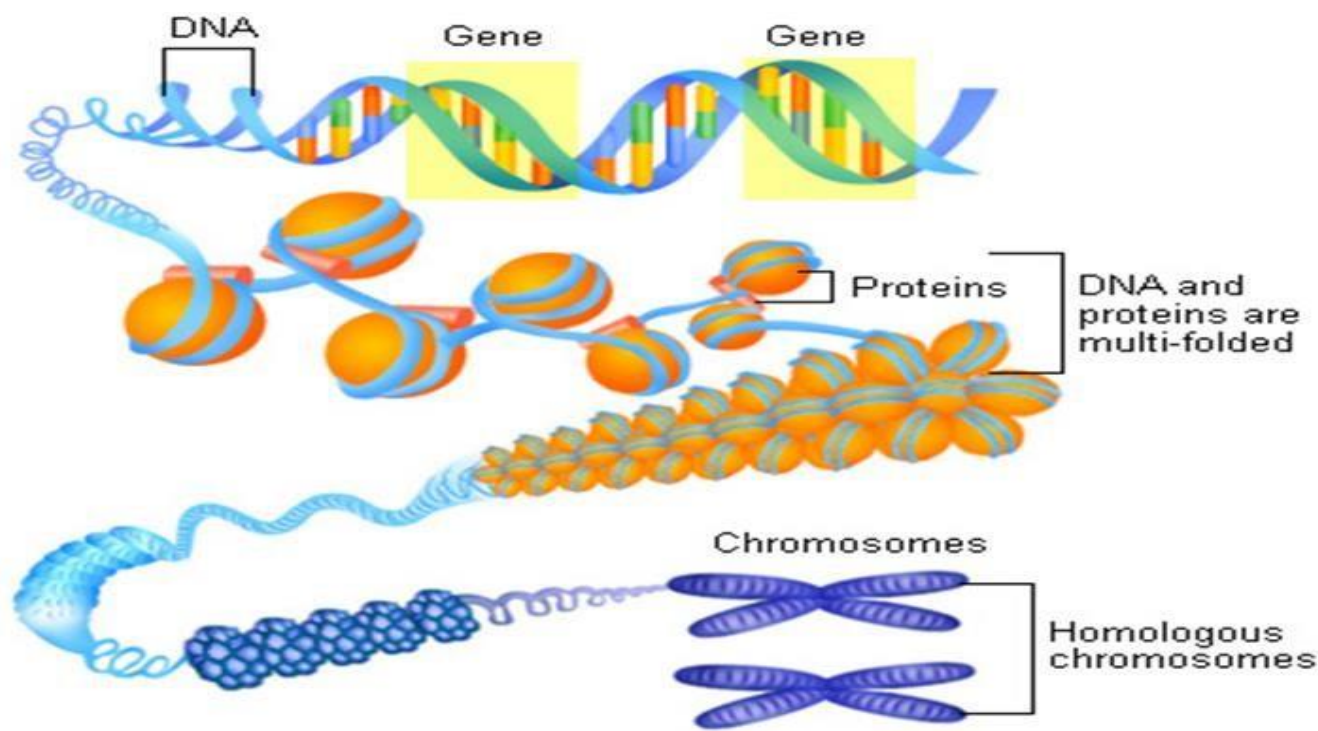
73 Summary - Mitosis and Meiosis

Summary - Mitosis and meiosis

SUMMARY		
Property	Mitosis	Meiosis
DNA replication	Occurs during interphase before mitosis begins	Occurs during interphase before meiosis I begins
Number of divisions	One, including prophase, metaphase, anaphase, and telophase	Two, each including prophase, metaphase, anaphase, and telophase
Synapsis of homologous chromosomes	Does not occur	Occurs during prophase I along with crossing over between nonsister chromatids; resulting chiasmata hold pairs together due to sister chromatid cohesion
Number of daughter cells and genetic composition	Two, each diploid (2n) and genetically identical to the parent cell	Four, each haploid (n), containing half as many chromosomes as the parent cell; genetically different from the parent cell and from each other
Role in the animal body	Enables multicellular adult to arise from zygote; produces cells for growth, repair, and, in some species, asexual reproduction	Produces gametes; reduces number of chromosomes by half and introduces genetic variability among the gametes

74 Chromosomal Theory of Inheritance

Chromosomal theory of inheritance: The chromosomal theory of inheritance states that genes have specific loci on chromosomes.



Genes on chromosomes

Behavior of chromosomes – According to Mendel s’ Laws

Theory states that behavior of chromosomes is according to Mendel’s laws of segregation and independent assortment.

Mendel s’ factors and chromosomes behavior

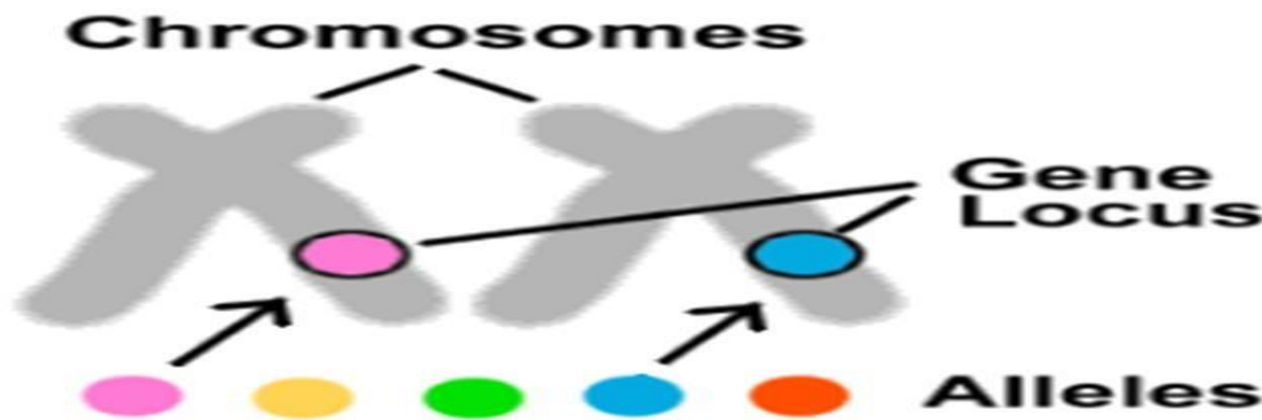
Around 1900, cytologists and geneticists began to see parallels between the behavior of chromosomes and the behavior of Mendel’s factors.

Homologous chromosomes separate and alleles segregate during meiosis.

Fertilization restores the paired condition for both chromosomes and genes.

Evidence that chromosomes are location of genes

- T. H. Morgan was the first to associate a specific gene/trait with a specific chromosome.
- Convincing evidence that chromosomes are the location of Mendel’s heritable factors.



Chromosomal theory of inheritance states that Genes are present on chromosomes.

75 Experiments of T H. Morgan

Experiment of T. H. Morgan

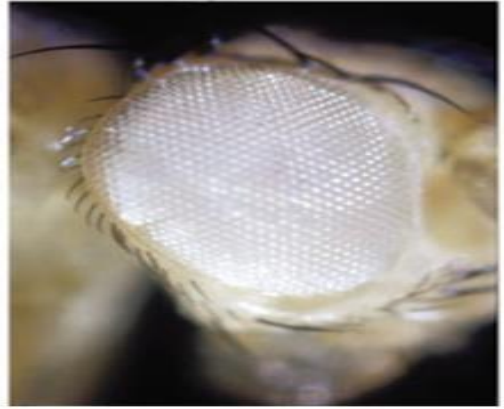
Eye color trait is linked with sex of drosophila

- Morgan with an experiment proved that eye color trait is linked with sex of the drosophila.

Experiment

- Male flies were with white eyes (mutant).
- Female flies were with red eyes (wild type).

Male flies with white eyes Female flies with red eyes



Progeny f1 was with red eyes

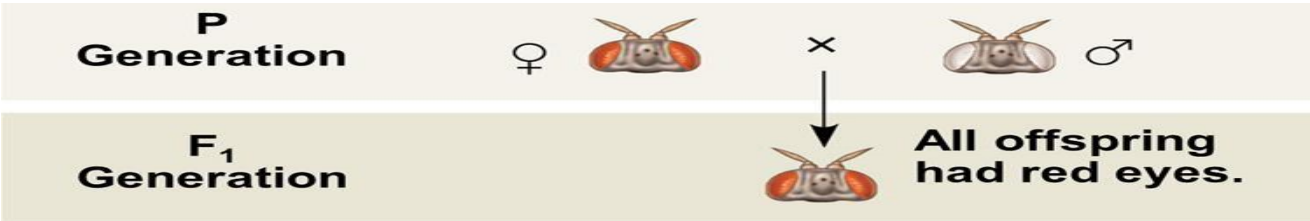
- All the flies produced during F1 generation were with red eyes.

Progeny f2 showed 3:1 ratio

- F2 generation showed 3:1 (three red eye: one white eye).
- White-eyed trait appeared only in males.
- All females and half males with red eye.

White eyed trait appeared only in males

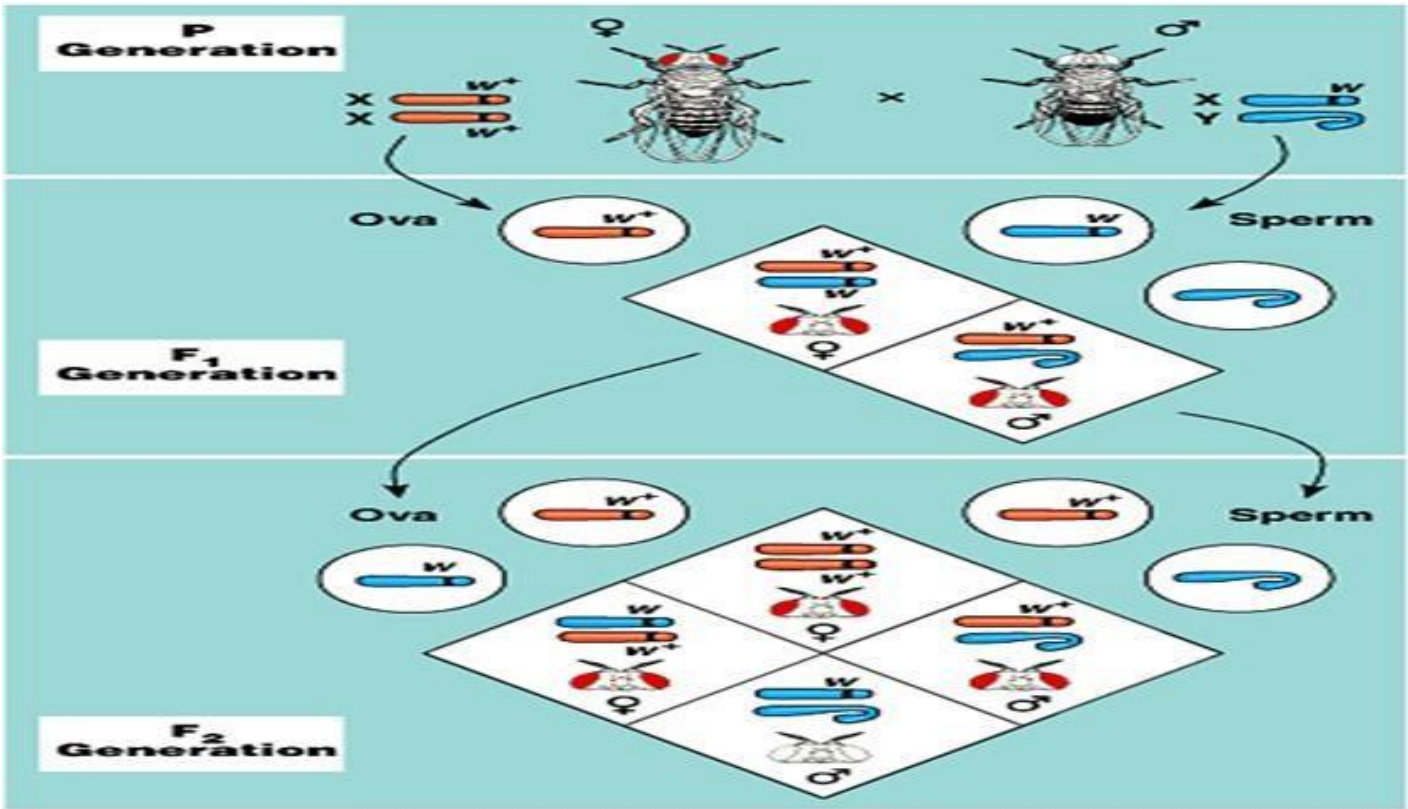
- White-eyed trait appeared only in male fruit flies (*Drosophila melanogaster*).



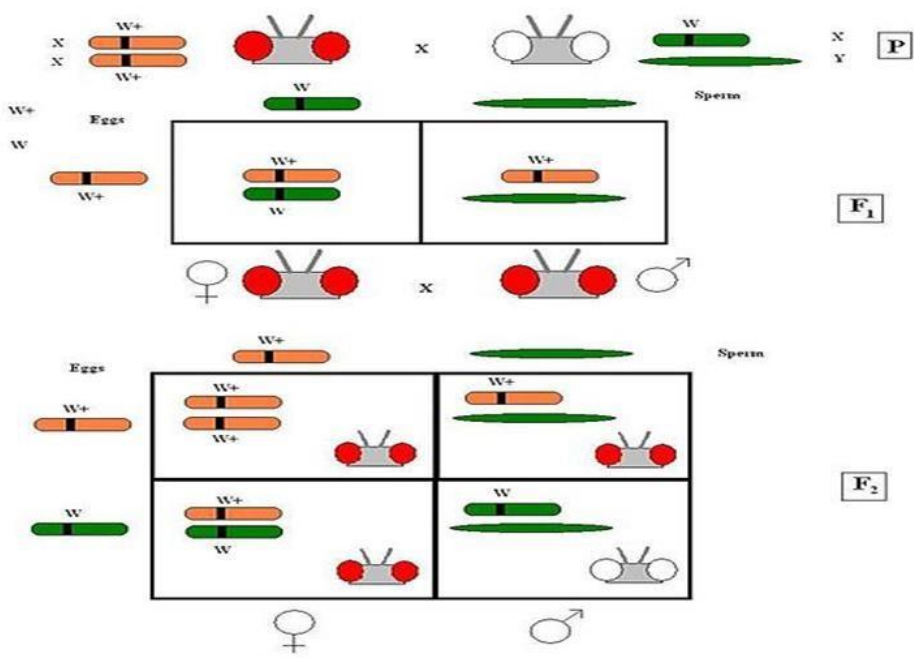
RESULTS



Morgan s' Experiment



Morgan’s Discovery: Transmission of X chromosome correlates with inheritance of eye color trait
First solid evidence indicating that a gene is associated with a specific chromosome.



76 Effect of Linkage on Inheritance

Effect of linkage on inheritance

Linked Genes inherit together

- Genes located on the same chromosome, if are linked, will inherit together.

Effect of linkage on inheritance

- Morgan did another experiment with fruit flies to see how linkage affects the inheritance of two different characters.
- body color – wild type gray, mutant black
- wing size – wild type Normal, mutant vestigial

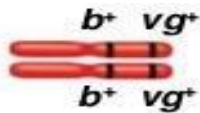


Wild type (gray body, normal wings)

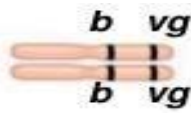


Double mutant (black body, vestigial wings)

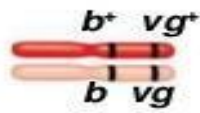
CROSS



Wild type (gray body, normal wings)



Double mutant (black body, vestigial wings)



Wild-type F₁ dihybrid (gray body, normal wings)

P Generation (homozygous)

Wild type (gray body, normal wings)

$b^+ b^+ vg^+ vg^+$



x



Double mutant (black body, vestigial wings)

$b b vg vg$

F₁ dihybrid (wild type)

$b^+ b vg^+ vg$



TESTCROSS

x

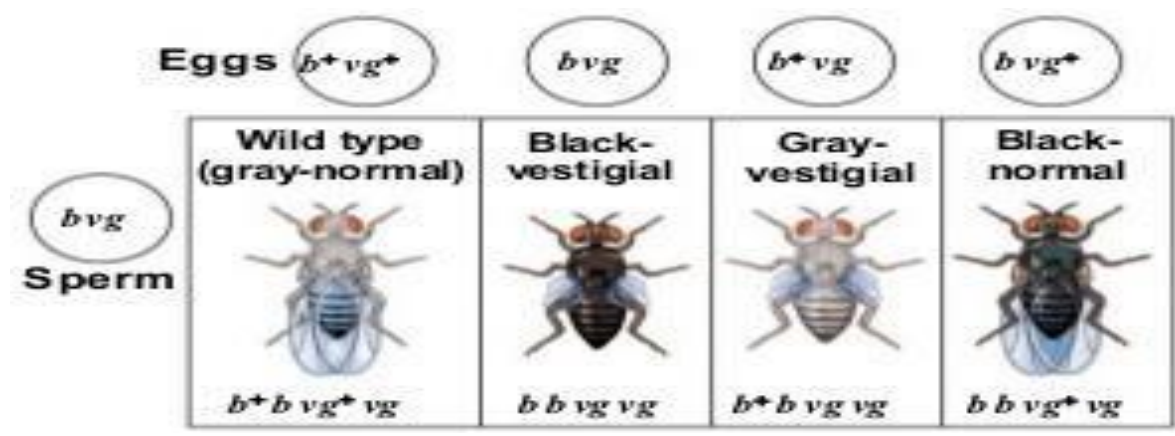


Double mutant

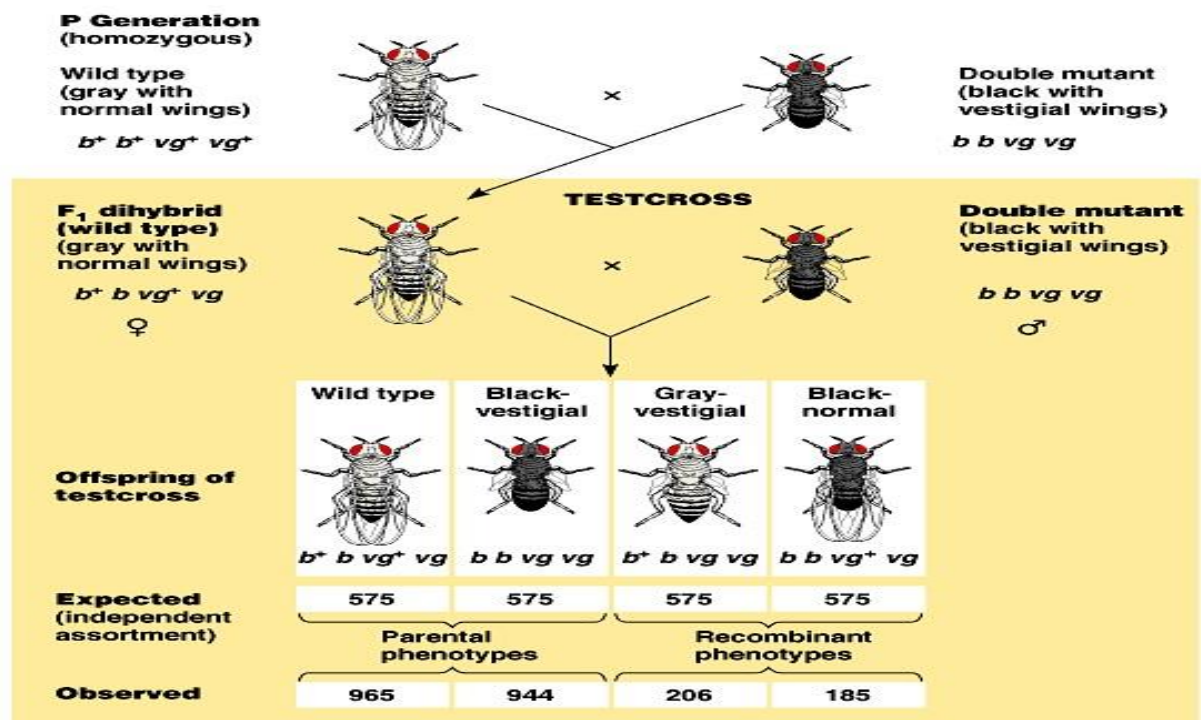
$b b vg vg$

Testcross offspring

Eggs	$b^+ vg^+$	$b vg$	$b^+ vg$	$b vg^+$
Sperm	$b vg$			
	Wild type (gray-normal)	Black-vestigial	Gray-vestigial	Black-normal
	$b^+ b vg^+ vg$	$b b vg vg$	$b^+ b vg vg$	$b b vg^+ vg$



If gene are located on different chromosomes	1 (575)	1 (575)	1 (575)	1 (575)
If genes are located on same chromosomes	1 (1150)	1 (1150)	0	0
Morgan Results	965	944	206	185



Results -linkage and cross over

- According to independent assortment, four phenotypes in 1:1:1:1 ratio.
- Observed large number of wild-type and double-mutant flies.

Morgan reasoned

- Body color and wing shape are inherited together because their genes are on the same chromosome.
- New phenotypic variations --- crossing over and linkage that break the genes physical connection.

77 Linkage Mapping

Linkage map

- Linkage map is an ordered list of the genetic loci along a particular chromosome.
- A map of the genes on a chromosome based on linkage analysis.

Linkage map does not show physical distance

- A linkage map does not show the physical distances between genes but rather their relative positions, as determined by how often two gene loci are inherited together.

Position of genes in terms of recombination frequency

- A linkage map is a genetic map of a species that shows the position of its known genes or genetic markers relative to each other in terms of recombination frequency.

Linkage mapping used for identifying location of diseased genes

- Linkage mapping is critical for identifying the location of genes that cause genetic diseases.

Genetic map is based on recombination frequencies

- A genetic map is a map based on the frequencies of recombination between markers during crossover of homologous chromosomes.

Greater frequency more distance

- The greater the frequency of recombination (segregation) between two genetic markers, the further apart they are assumed to be.

Markers used

- Microsatellites
- Restriction fragment length polymorphisms (RFLPs).

78 Linkage map of Drosophila Chromosome

Linkage map of drosophila chromosome

Linkage map

- A linkage map is a genetic map of a species that shows the position of its known genes or genetic markers relative to each other in terms of recombination frequency.

Frequency of recombinant offspring reflect the distance

- ☐ Frequency of recombinant offspring reflected distances between genes on chromosomes.

Greater the distance, more chances of cross over

- The greater the distance between two genes, the more points between them where crossing over can occur.

Sturtevant used recombination frequencies to map position

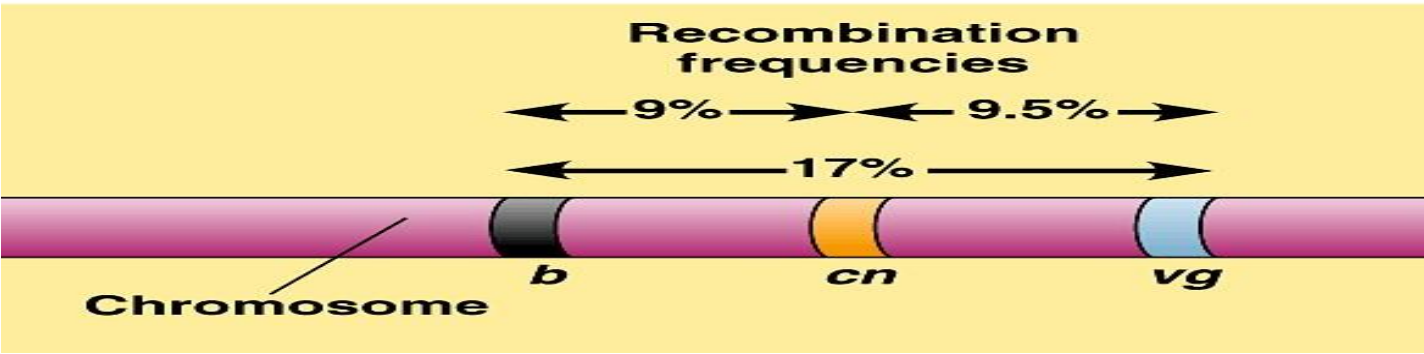
- He used recombination frequencies from fruit fly crosses to map the position of genes along chromosomes.

Test cross designed to map three genes

- The test cross design to map the relative position of three fruit fly genes, body color (b), wing size (vg), and eye color (cn).

Linkage map

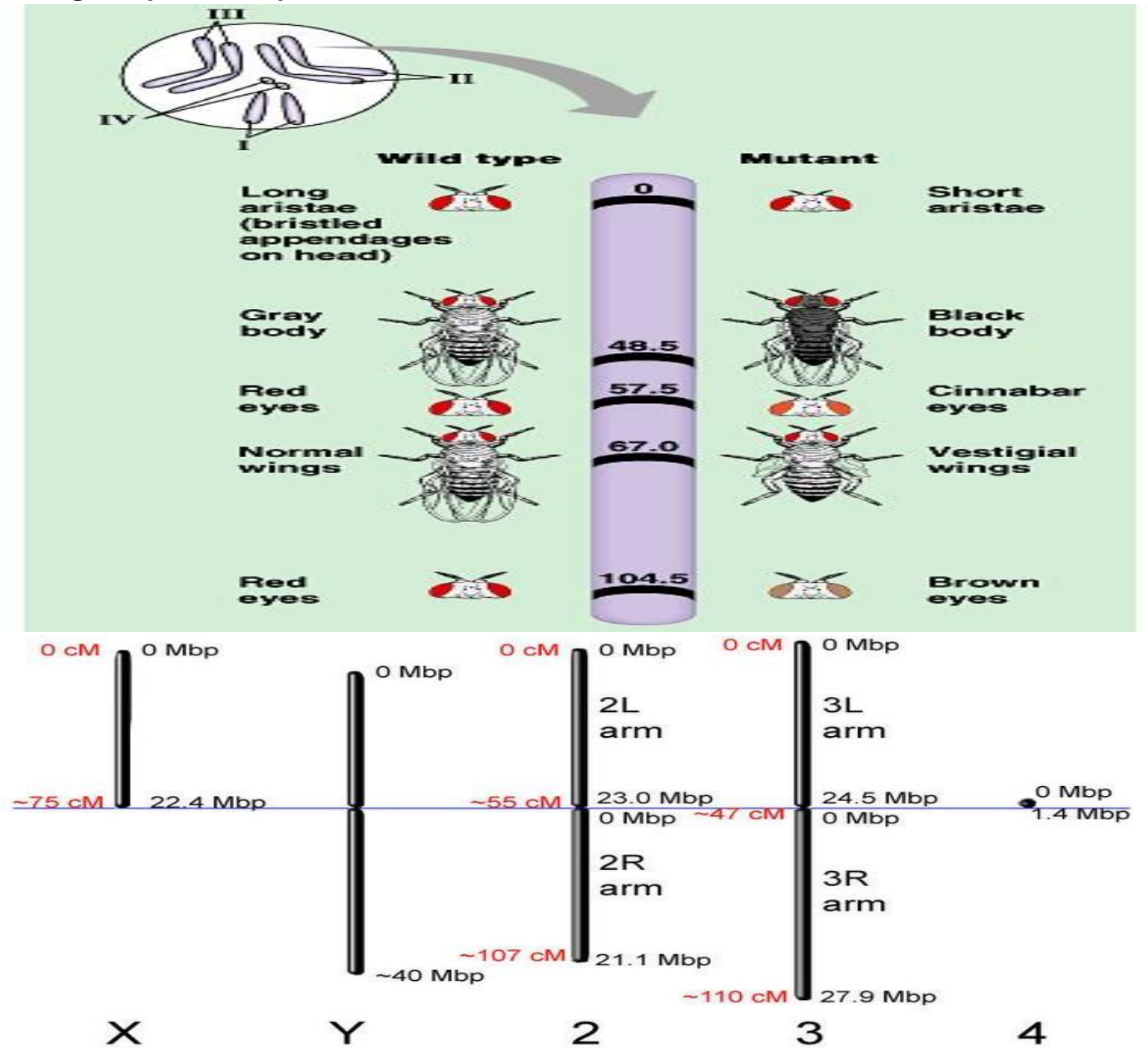
- Recombination frequency between cn and b is 9%
- Between cn and vg is 9.5%
- Recombination frequency between b and vg is 17%



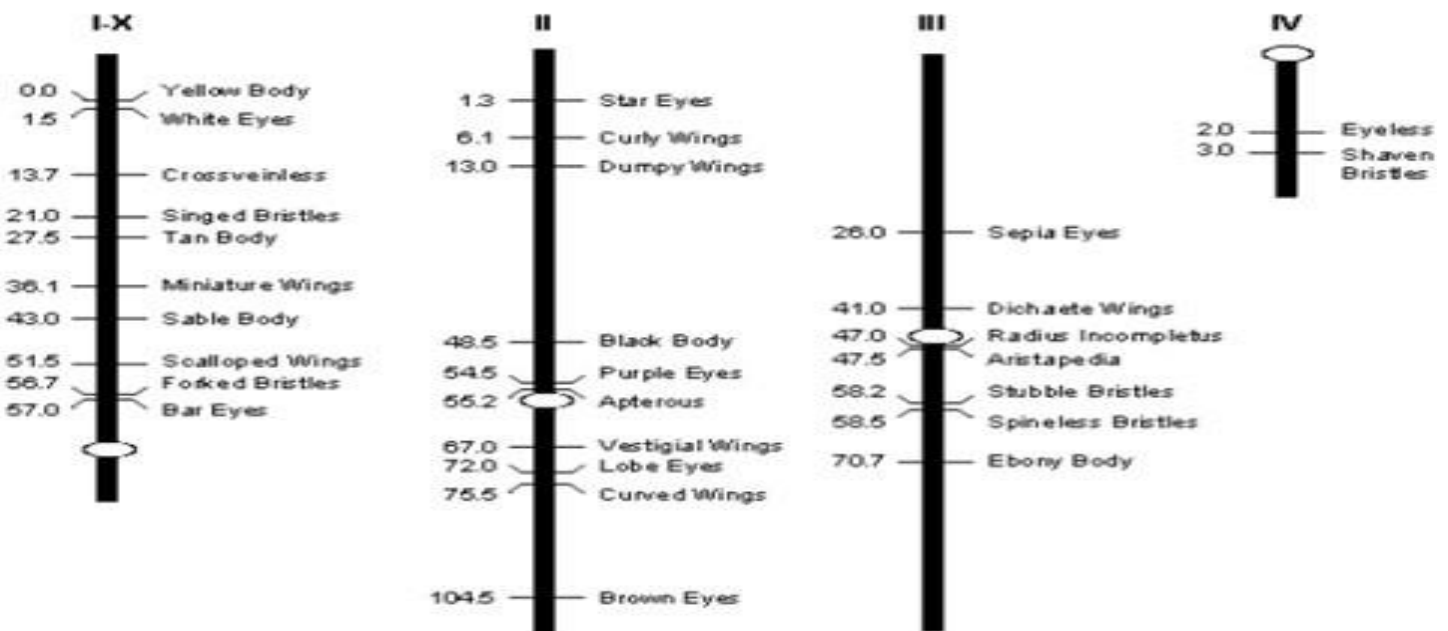
Eye color gene between the two others

- The only possible arrangement of these three genes places the eye color gene between the other two.

Linkage map of drosophila chromosome 2



Drosophila Chromosome Map



Map Units – CentiMorgan

- Sturtevant expressed distance between genes - as map units.
- One map unit (1 cM) is equivalent to a 1% recombination frequency.

79 Chromosomal Basis of Sex Determination

Sex determination and chromosomes role

Genetic basis of sex – discovery of sex chromosomes

- Understanding of genetic basis of sex was with the discovery of sex chromosomes in the early 1900s.

Insects chromosomes

- Analyses of male and female insect chromosomes.
- Most chromosomes were present in equal numbers in both males and females.
- One /two additional chromosome.

Chromosomal difference is responsible for Sex determination

- Analyses of other species revealed that chromosomal differences are primarily responsible for sex determination.

Sex determination

- Chromosomal basis for determining sex
- The X-Y System
- The X-O System
- The Z-W System
- The haploid-diploid System

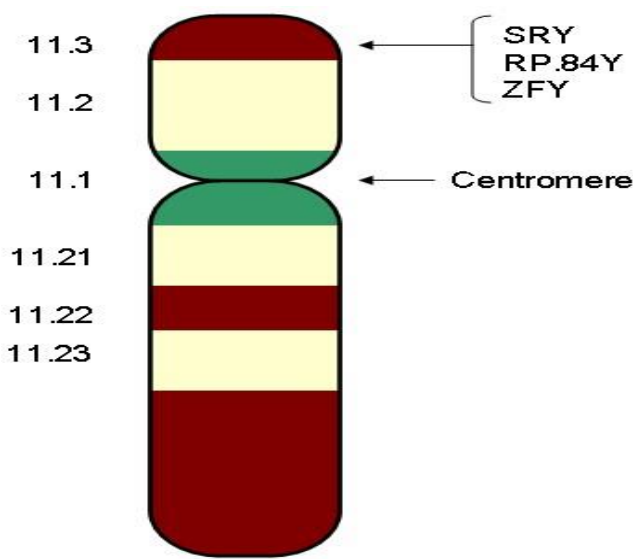
Sex determination in humans

- Female homogametic XX
- Male heterogametic XY
- Sex of offspring depends on the sperm has an X or Y

SRY gene - Y chromosome

- Researchers have found the SRY gene (sex determining region of the Y chromosome
- In individuals with the SRY gene, the generic embryonic gonads are modified into testes.

Sex determining region on Y chromosome



Other genes on Y chromosome

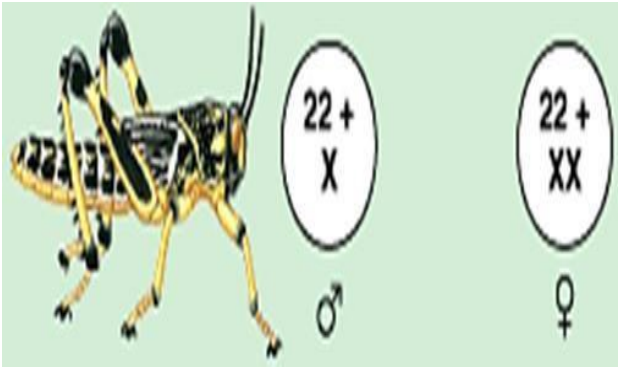
- In addition, other genes on the Y chromosome are necessary for the production of functional sperms.
- In individuals lacking the SRY gene, the generic embryonic gonads develop into ovaries.

80 Sex Determination in Other Organisms

Sex determination systems

X-O System

- In insects, only one type of chromosome.
- Females XX, Males XO
- Sex of offspring, sperm X or O



Z-W

System

- In birds, some fishes and some insects.
- Females ZW, Males ZZ.



Haploid-diploid

- No sex chromosomes in most species of ants and bees.
- Females fertilized ova diploid
- Males unfertilized ova haploid



Sex Determination - Conclusion

- Different organisms have different systems of sex determination.

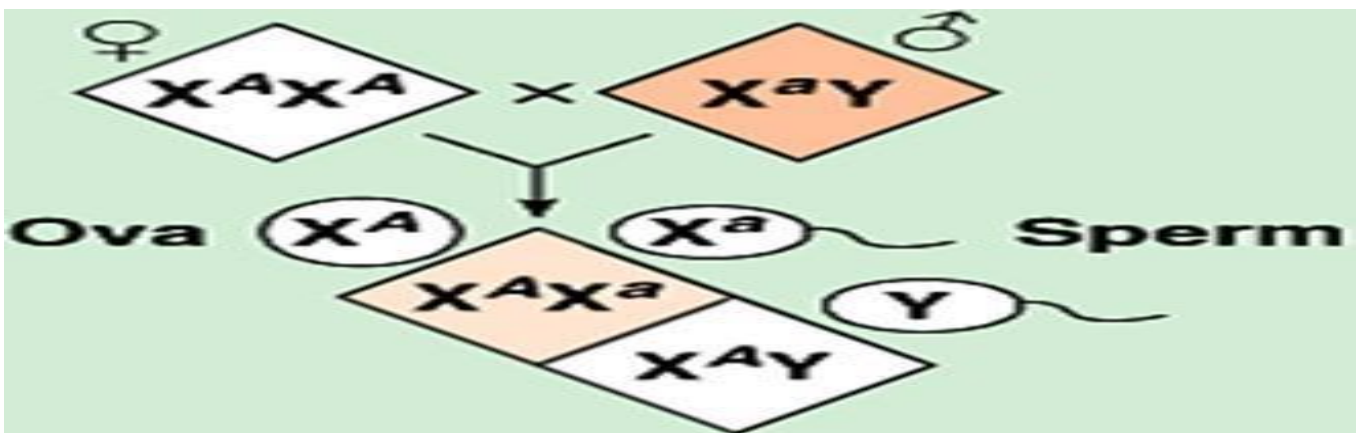
81 Inheritance of Sex linked genes

Sex linked genes

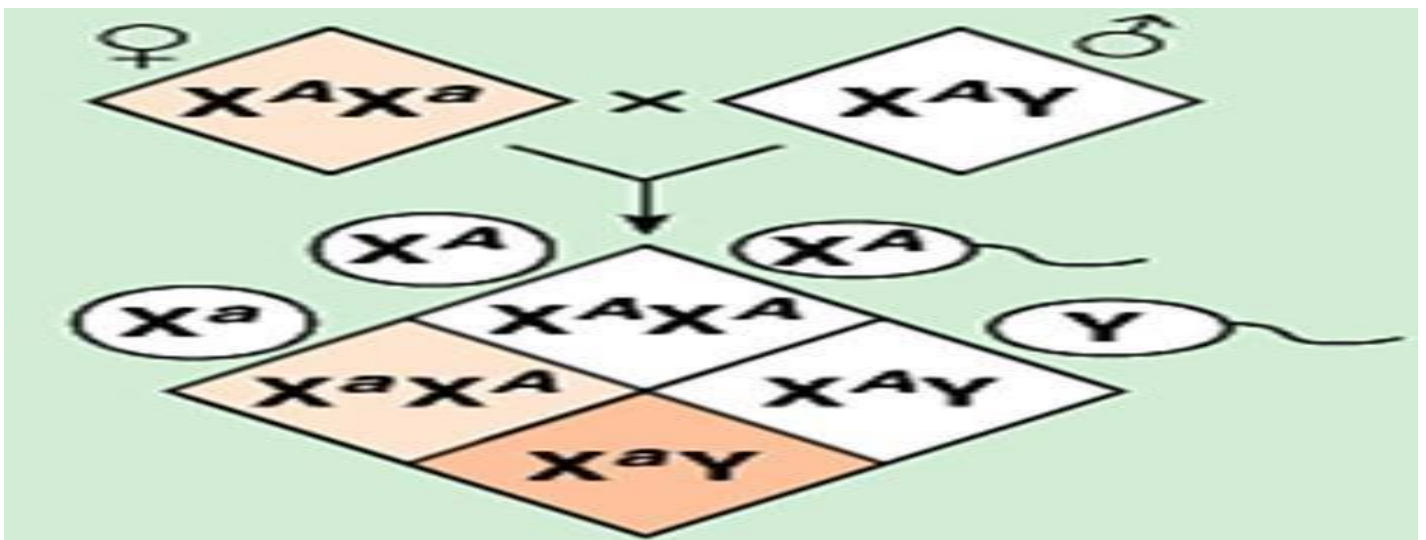
Inheritance of sex linked genes

- A gene located on either sex chromosome a sex-linked.
- The sex chromosomes have genes for many characters unrelated to sex.

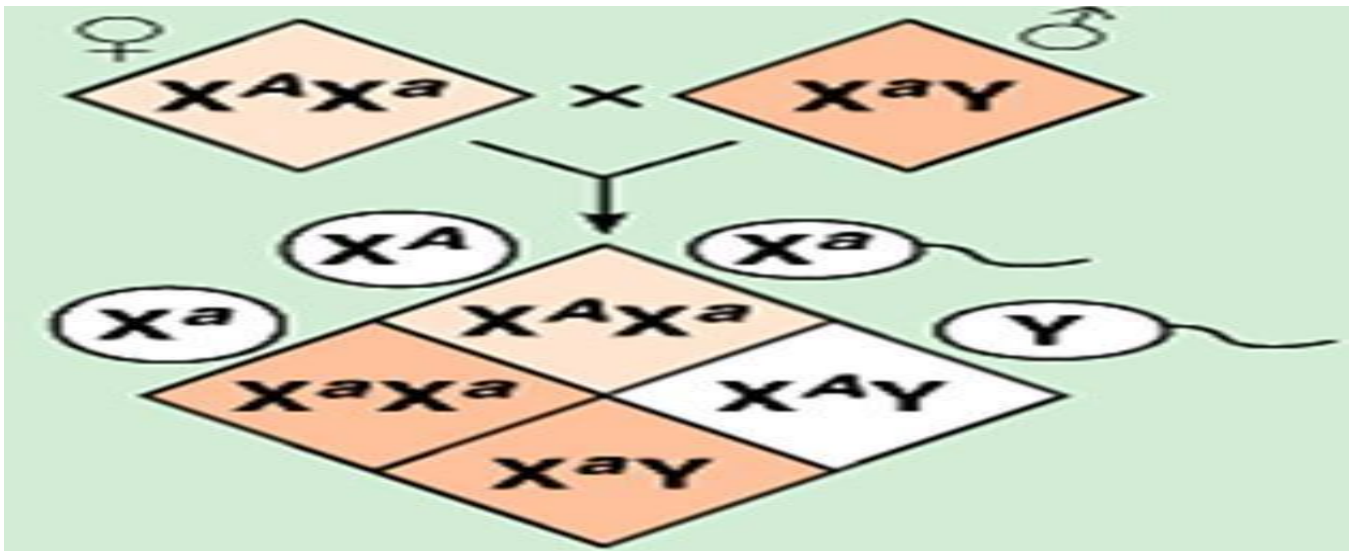
Father with disorder will transmit the mutant allele to all daughters but to no sons



Carrier female mates normal male, 50% chance that each daughter will be a carrier; 50% chance that each son will have the disorder.



If a carrier female mates a male with disorder, 50% chance that each child born with disorder, regardless of sex.

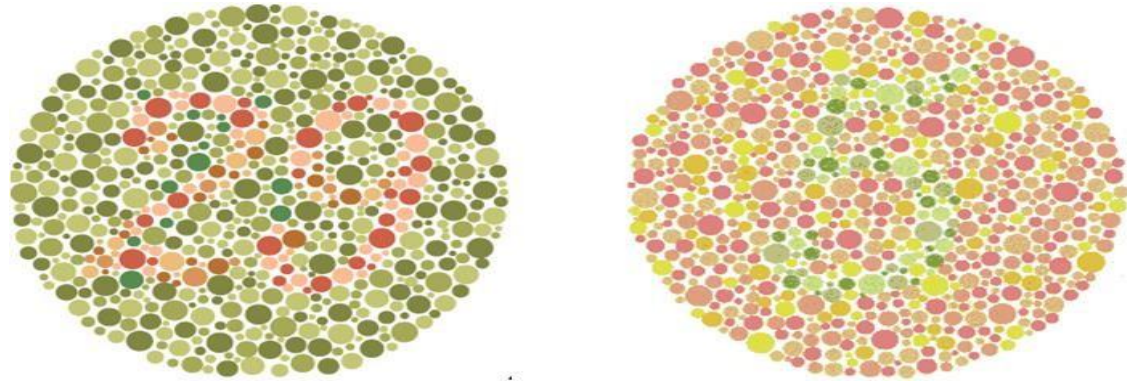


Human Sex linked Disorders

- Color blindness
- Duchenne muscular dystrophy
- Hemophilia

Color Blindness

- Normal Female XX, Normal male XY
- Color Blind $X^c X^c$, Carrier $X^c X$, Color Blind Male $X^c Y$



Normal Father, Color Blind Mother

- All daughters are carriers, sons are color blind.

	X	Y
X ^c	X X ^c	X X ^c
X ^c	X X ^c	X ^c Y

Normal Mother, Color Blind Father

- All daughters are carriers, sons are normal

	X ^c	Y
X	X X ^c	X Y
X	X X ^c	X Y

Sex linked genes/traits

- A gene located on either of sex chromosome is called sex-linked and traits are called as sex-linked traits.

82 Genetic Mapping in Humans

Genetic mapping in humans

Genetic mapping

- Genetic mapping - also called linkage mapping provides clues about which chromosome contains the gene and precisely where it lies on that chromosome.

Single gene disorders identified

- Genetic maps have been used successfully to find the single gene responsible inherited disorders, like cystic fibrosis and muscular dystrophy.

How genetic mapping performed

- To produce a genetic map, researchers collect blood or tissue samples from family members where a certain disease or trait is prevalent.

How genetic mapping performed

- Scientists isolate DNA from samples and examine it for the unique patterns of bases.
- Molecular techniques are referred to as genotyping polymorphisms or markers.

Dna markers help in location of genes

- Before researchers identify the gene responsible for the disease or trait, DNA markers can tell them roughly where the gene is on chromosome.

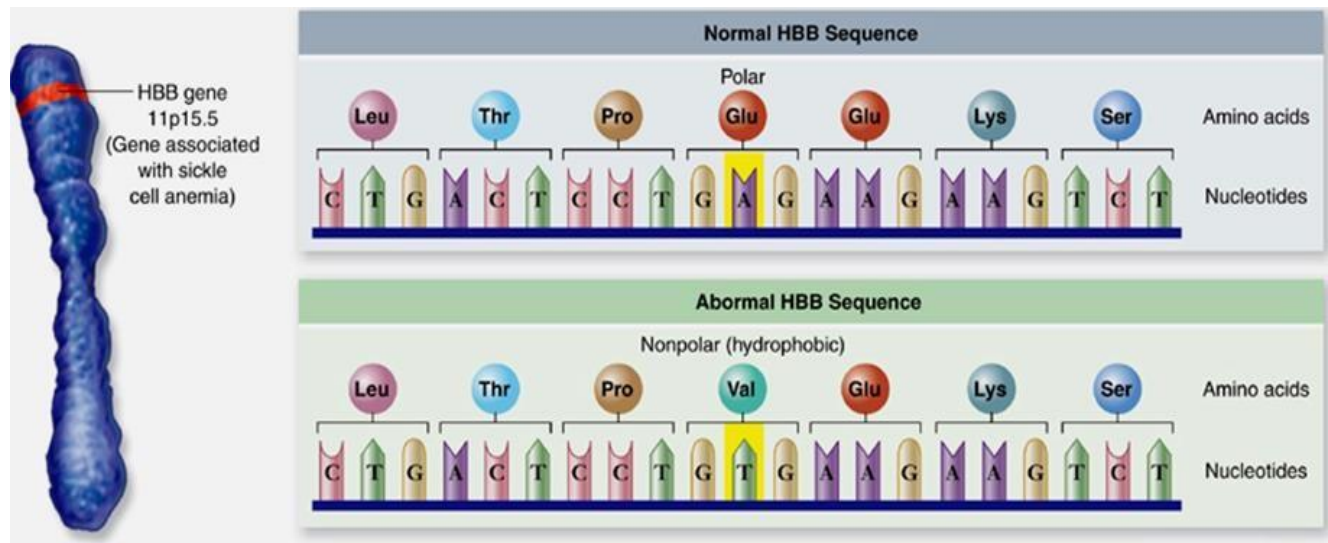
More markers increases chance of linkage

- The more DNA markers there are on a genetic map, the more likely it is that one will be closely linked to a disease gene.

Types of Genetic markers

- Several different types of genetic markers, the type most used on genetic maps is known as a microsatellite and SNPs.

Genetic mapping in humans



Genetic mapping provide clues about location of genes

- Genetic mapping provides clues about the location of genes on chromosomes.

83 Concept of Multiple Alleles

Multiple alleles

- Three or more alternative forms of a gene (alleles) that can occupy the same locus/position on a chromosome.

Genes have specific loci on chromosomes

- According to chromosomal theory of inheritance, genes have specific loci/position on chromosomes.

Diploid individuals have two alleles

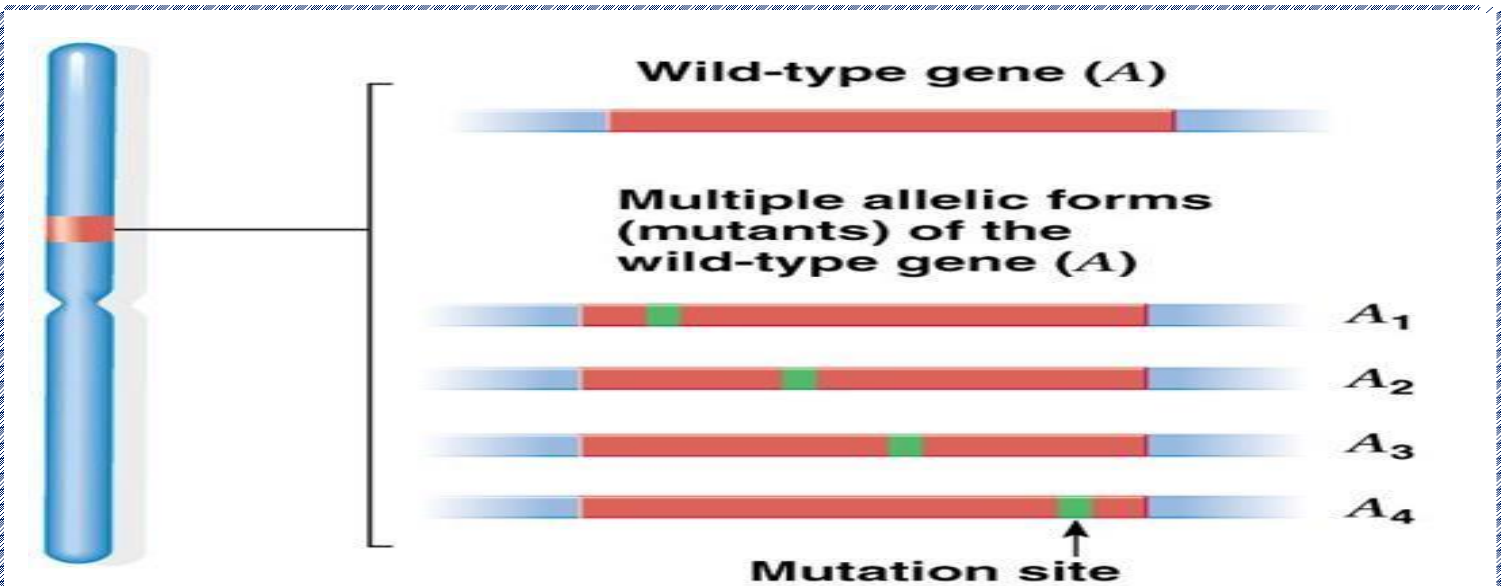
- Diploid individuals have two alleles, one on each of the homologous chromosome.

All genes do not have two forms

- All genes do not have two forms; many have multiple alleles or multiple forms.
- ABO blood groups

Mono-allelic, di-allelic and poly-allelic

- 30% genes in humans are di-allelic - they exist in two forms.
- 70% are mono-allelic; they exist only in one form.
- Few are poly-allelic.



Multiple alleles

Poly-allelic genes are tissue specific

- Poly-allelic genes are usually associated with tissue types.
- These genes are so varied that they provide us with our genetic finger print.

Number of alleles - number of genotypes ☐ Di-allelic

genes can generate 3 genotypes.

- Genes with 3 alleles can generate 6 genotypes.
- Genes with 4 alleles can generate 10 genotypes.

Multiple alleles - conclusion

- Three or more alternative forms of a gene (alleles) that can occupy the same locus/position on a chromosome.

84 Multiple Alleles - our blood group

Multiple alleles - blood groups

Abo blood system

- Controlled by a tri-allelic gene.
- Allele A, Allele B, Allele i
- Generate 6 genotypes.

Two of the alleles are co-dominant to each other .

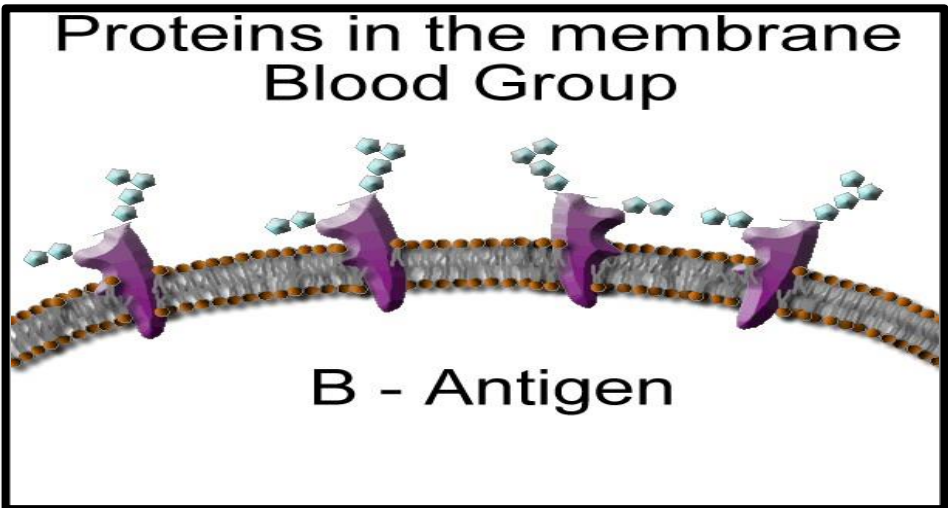
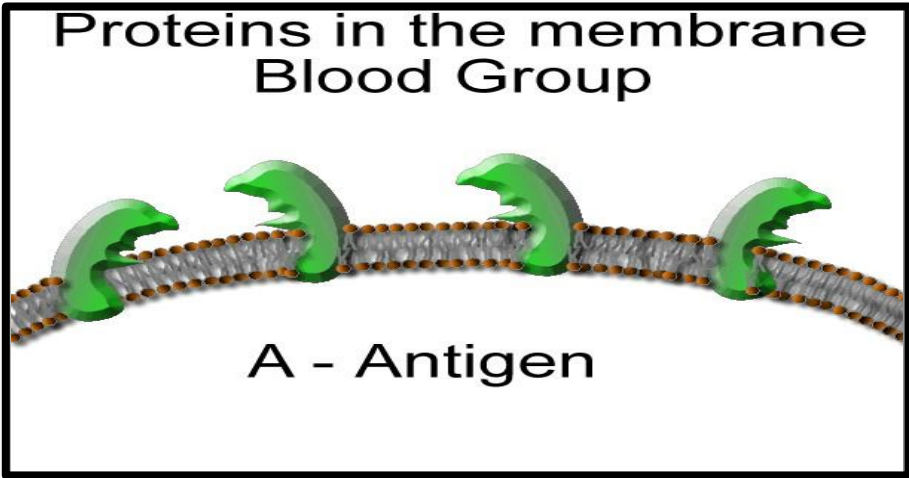
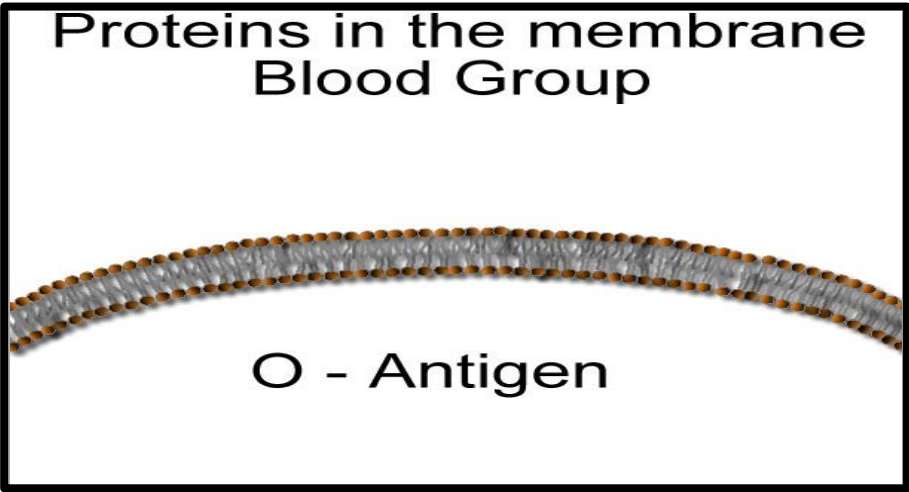
- ☐ Two of the alleles are codominant to one another.
 - Both are dominant over the third allele.
 - Allele A and Allele B when exist together will be co- dominant.

Alleles control the production of antigens

- The alleles control the production of antigens on the surface of the red blood cells.

Alleles and antigens

- Allele I^A produces antigen A
- Allele I^B produces antigen B
- Allele i produces no antigen



Alleles and antigens

$I^A I^A$ or $I^A i$	A	
$I^B I^B$ or $I^B i$	B	
$I^A I^B$	AB	
ii	O	

Blood group

6 Genotypes	4 Phenotypes (blood group types)
$I^A I^A$	A
$I^A I^B$	AB
$I^A i$	A
$I^B I^B$	B
$I^B i$	B
ii	O

Blood groups exists in homo and hetero forms .

- Blood type A - homozygous and heterozygous.
- Blood type B - homozygous and heterozygous.
- Blood types AB and O have only one genotype each.

Blood groups system - conclusion

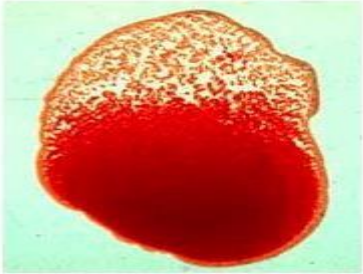
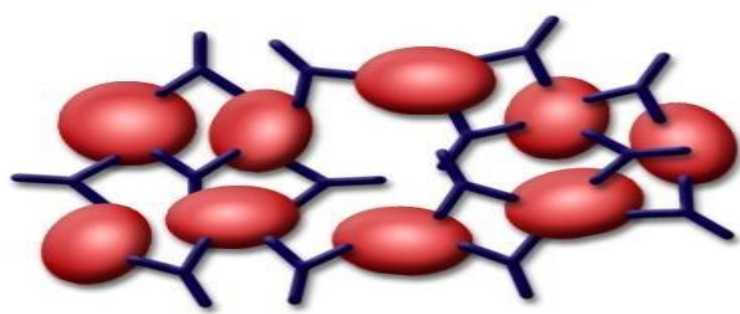
- Controlled by a tri-allelic gene.
- Four major blood groups – can be positive – negative based on Rh factor.

85 Blood groups and Transfusions

Blood groups and Transfusions

Immune system– self and non-self

- Blood types vary and your immune system recognizes your own blood type as being self.
- Other blood group types are recognized as non-self.
- Type A produce antibodies to agglutinate cells which carry Type B antigens. They recognize them as non-self
- The opposite is true for Type B.



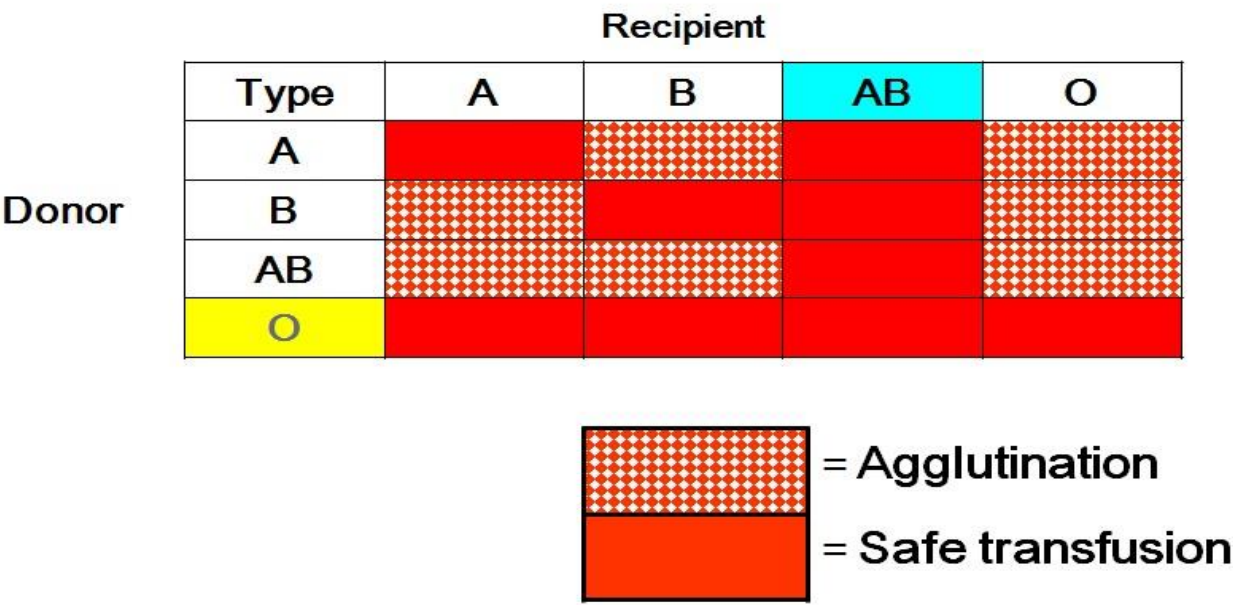
Blood group type o, do not carry any antigens

- Neither of these people will agglutinate blood cells which are Type O.

- Type O cells do not carry any antigens for the ABO system.

Universal – donor and recipient

- Type O blood may be transfused into all the other types = the universal donor.
- Type AB blood can receive blood from all the other blood types = the universal recipient.



Donor-recipient compatibility

- Person with Type A (Heterozygous) married a person Type B (Heterozygous) blood. genotypes and phenotypes ?

$I^A i \times I^B i$

Genotypic Ratio:

$I^A I^B \quad I^A i \quad I^B i \quad ii$

Phenotypic Ratio:

$AB : A : B : O$

	I^A	i
I^B	$I^A I^B$	$I^B i$
i	$I^A i$	ii

If a cross between a person with type O with heterozygous type B, what would be genotypic and phenotypic ratio

$ii \times I^B i$

GENOTYPIC RATIO

$I^B i : ii$

Phenotypic Ratio

$B : O$

	i	i
I^B	$I^B i$	$I^B i$
i	ii	ii

Blood groups- conclusion

- Immune system recognized the blood as self and non-self.

Multiple alleles- rabbits





Multiple alleles In rabbits

- Traits controlled by more than two alleles – multiple alleles.
- Fur color of rabbits – example of multiple alleles.

Fur color in rabbits

Fur color in rabbits C = Agouti (dark gray) c^{ch} = Chinchilla (light gray) c^h = Himalayan (white with black points) c = White



Allele			
C	c ^{ch}	c ^h	c
Genotype			
C ⁺ C ⁺	c ^{ch} c ^{ch}	c ^h c ^h	cc
Phenotype			
WILD TYPE: Brown fur	CHINCHILLA: Black-tipped white fur	HIMALAYAN: White fur with black paws, nose, ears, tail	ALBINO: White fur
			

Genotypes of Fur color

- ☐ White cc
- ☐ Himalayan c^hc^h , c^hc
- ☐ Chinchilla c^{ch}c^{ch}, c^{ch}c , c^{ch}c
- ☐ Gray CC , Cc^{ch} , Cc^h , Cc

Dominance of alleles

Allele C dominant to allele c^{ch} Allele c^{ch}
dominant to allele c^h
Allele c^h dominant to allele c

Multiple alleles conclusion

Traits controlled by more than two alleles.

87 Correlation of Alleles and Genotypes

Alleles and genotypes
Number of alleles and number of genotypes

- Genotypes = $n(n + 1)/2$
- Homozygotes = n
- Heterozygotes = $n(n - 1)/2$

# alleles	# genotypes	Homozygotes	Heterozygotes
1	1	1	0
2	3	2	1
3	6	3	3
4	10	4	6
5	15	5	10

Number of alleles and number of genotypes

Genotypes = $n(n + 1)/2$
Homozygotes = n
Heterozygotes = $n(n - 1)/2$

Calculate genotypes for fur color of rabbits, if Four (4) alleles for fur color.

Genotypes = $n(n + 1)/2$
Homozygotes = n
Heterozygotes = $n(n - 1)/2$

Number of alleles and number of genotypes

- Based on number of alleles, number of genotypes can be calculated.

88 Linkage and Cross over

Linkage and crossing over

Linkage

- Genes that are found on the same chromosome are said to be linked genes.
- Linked genes are inherited together.

Linkage -genetically failure of segregation

- Genetically linkage is defined as the failure of two genes to assort independently.
- Linkage occurs when two genes are close to each other on the same chromosome.

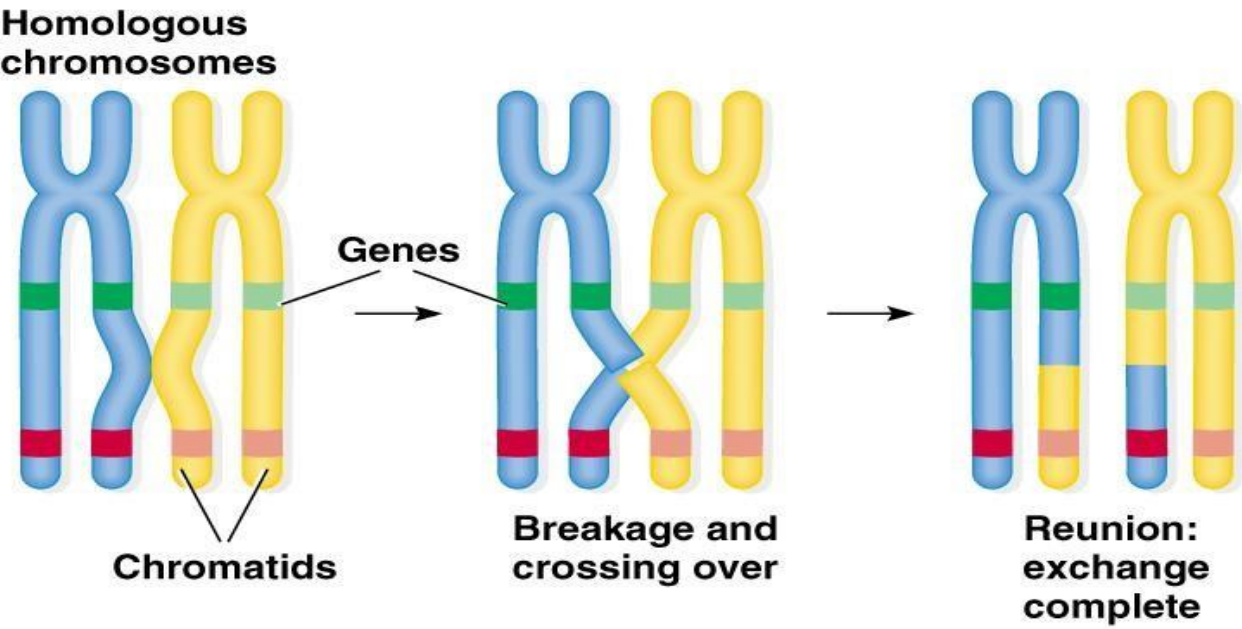
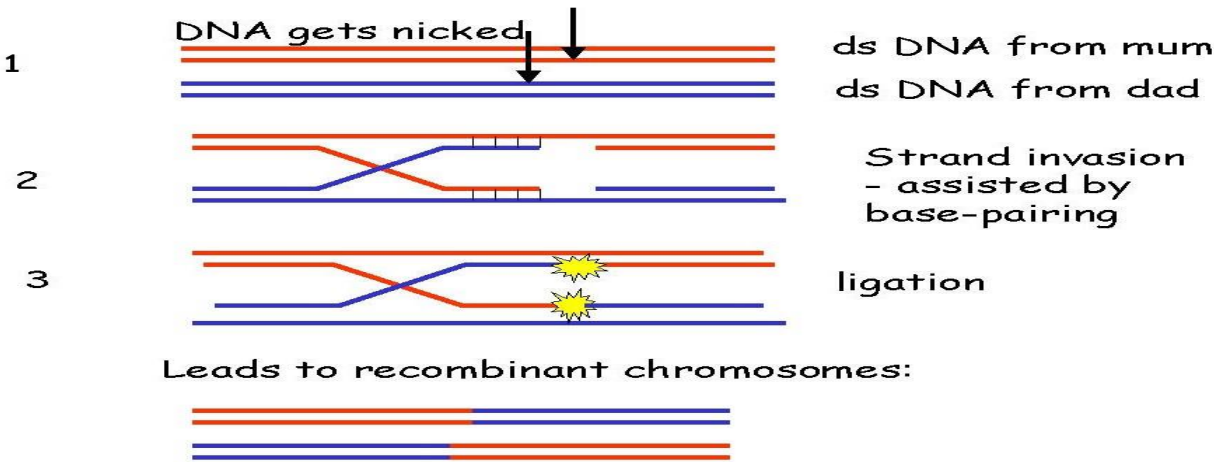
Linkage is based on frequency of crossing over

- Genes far apart on the same chromosome assort independently.
- Linkage is based on the frequency of crossing over between the two genes.

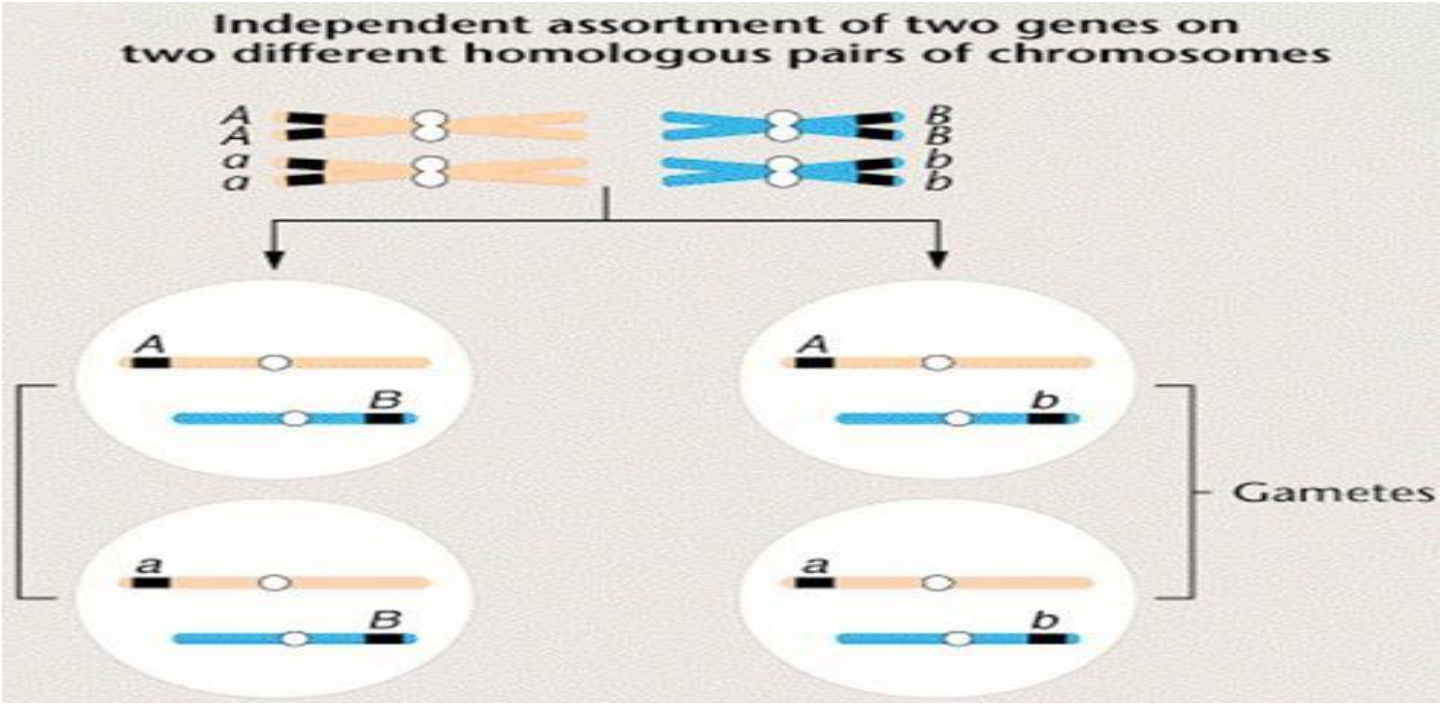
Chiasmata formation

- Chiasmata visible on chromosomes are regions of crossing over.
- Occurs between non-sister chromatids.

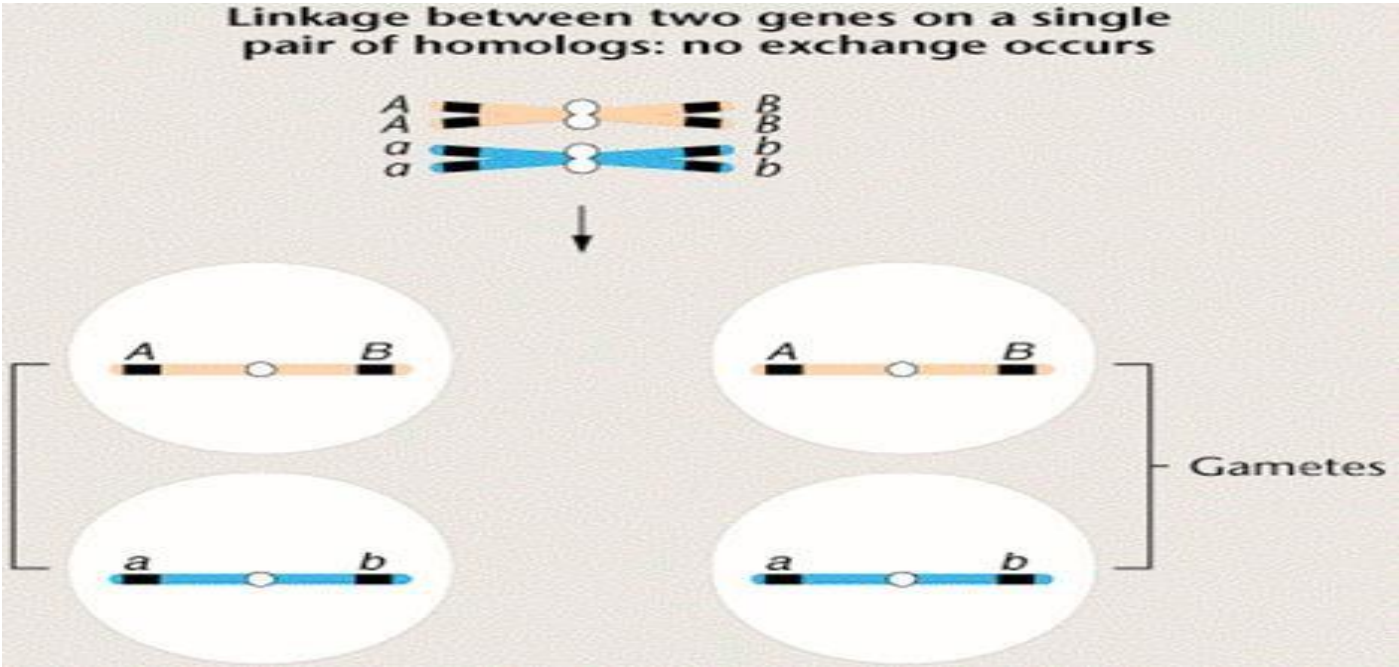
Crossing over in prophase I



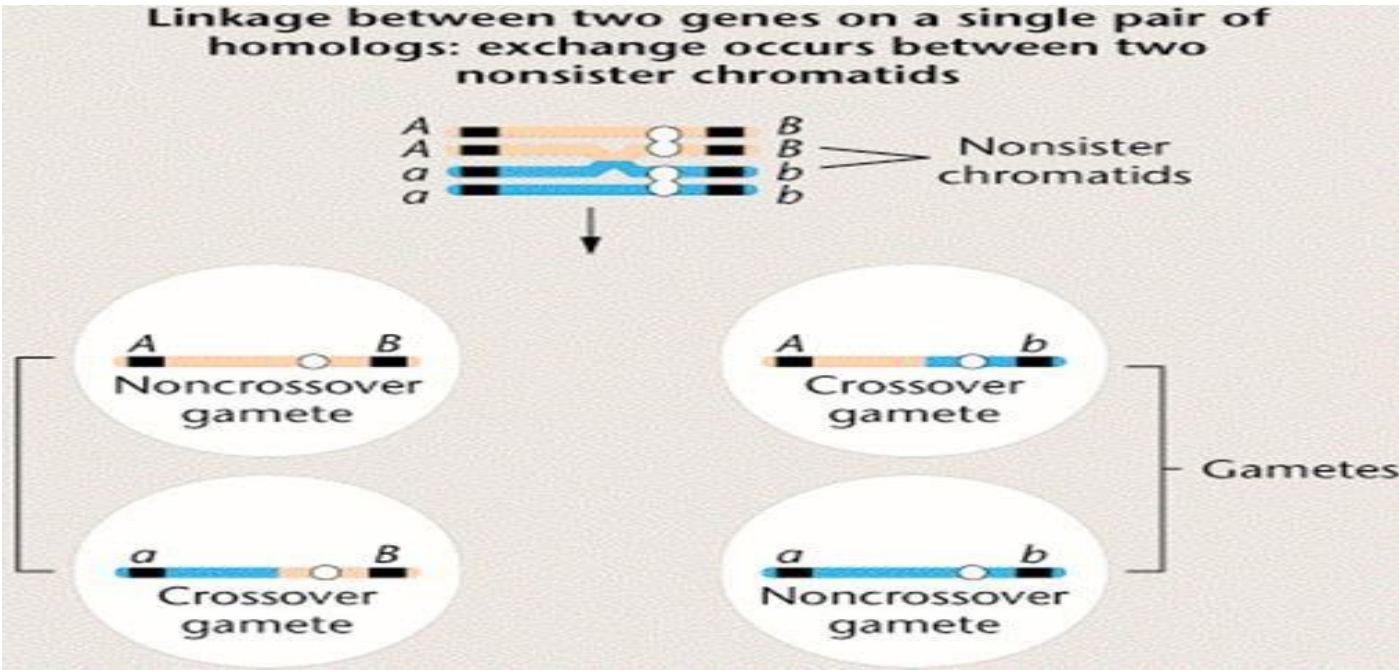
Crossing over in prophase I



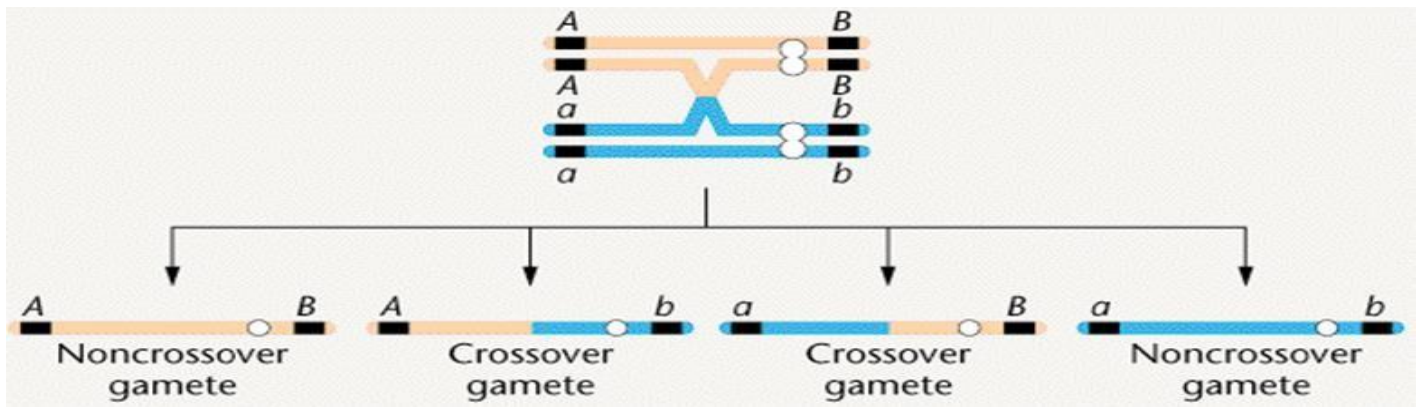
Crossing over in prophase I



Genes are linked



Exchange between two non-sister chromatids



Parental and non-parental gametes

Linkage and crossing over

- Linkage and crossing over a phenomenon that may be seen during meiosis.

89 Chromosome Mapping

Linked genes

Chromosome mapping

- Genes that are found on the same chromosome are said to be linked genes.
- Linked genes are inherited together.

Chromosome mapping

- Linkage is defined genetically as the failure of two genes to assort independently.
- Linkage occurs when two genes are close to each other on the same chromosome

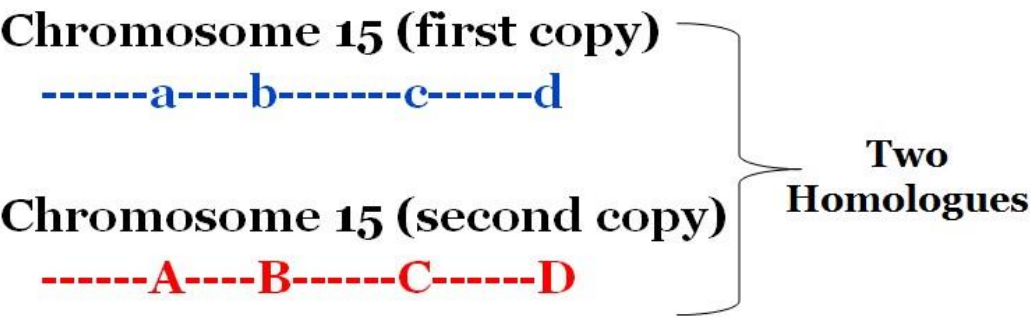
Closer the genes, fewer the cross over

- The closer genes are together on a chromosome, the fewer crossovers will occur.
- Genes farther apart, affected by more crossovers (higher crossover frequency).

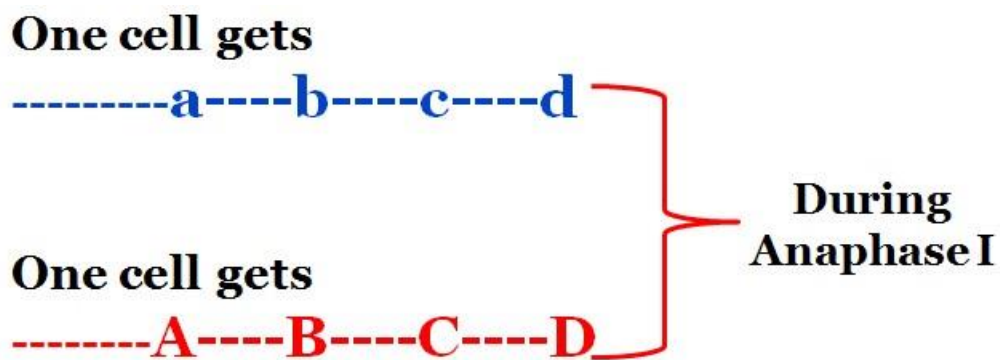
Map units

Crossover value of

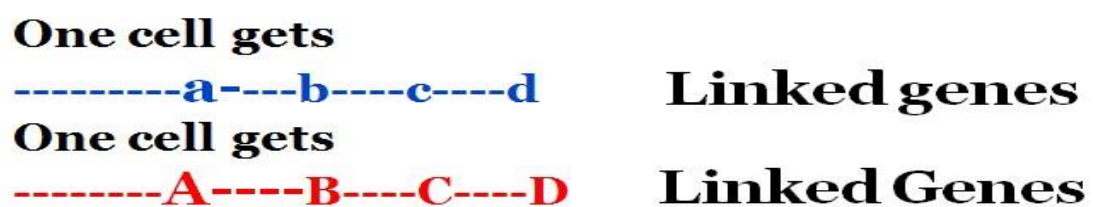
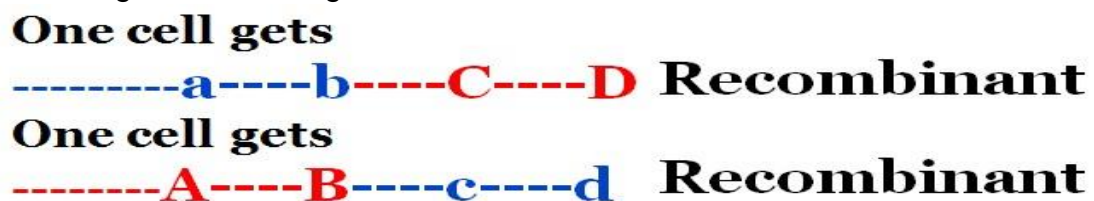
- 1% = 1 map unit apart - genes are close together.
- 12% = 12 map units apart - genes are further apart.
- Cross over frequency = Map distance/units.



Linkage and linked genes



When separated during meiosis linkage



Linked genes inherit together. This phenomenon used for identification of many diseased genes in humans.

90 Genetic and Physical Map

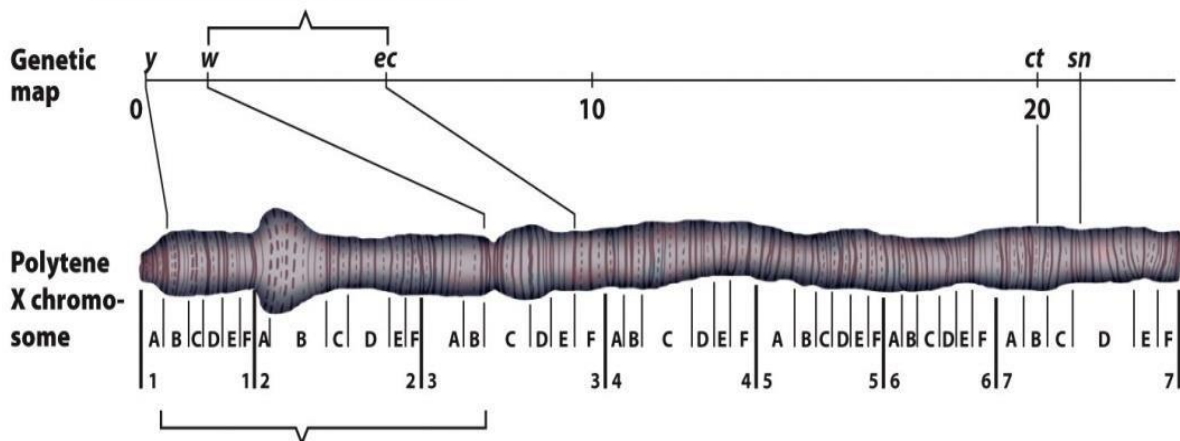
Physical mapping

- A physical map is primarily based on the locations of landmarks along a DNA molecule.
- Units of distance are expressed in base pairs or Mbs.

Different Mapping techniques

- Cytogenetic map
- In situ hybridization
- Genetic mapping
- Restriction enzymes site mapping
- Radiation hybrid mapping

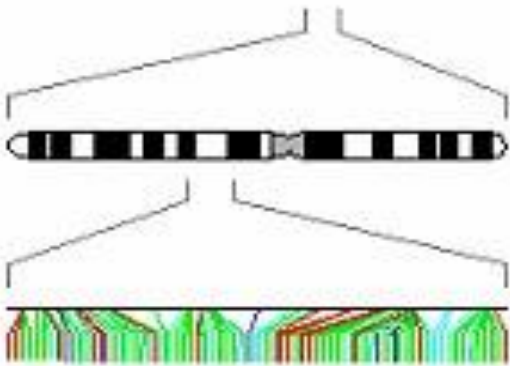
The genes *w* and *ec* are far apart on the genetic map, but close together on the physical map of the chromosome.



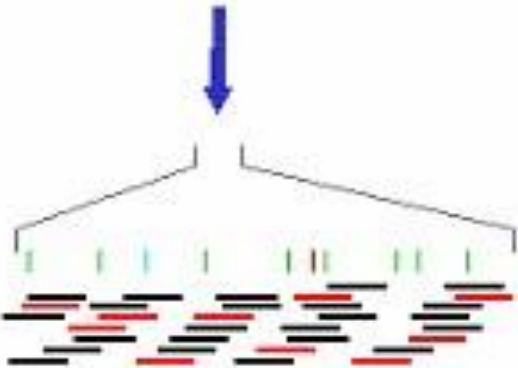
The genes *y* and *w* are far apart on the physical map of the chromosome, but close together on the genetic map.

Physical mapping - Top down

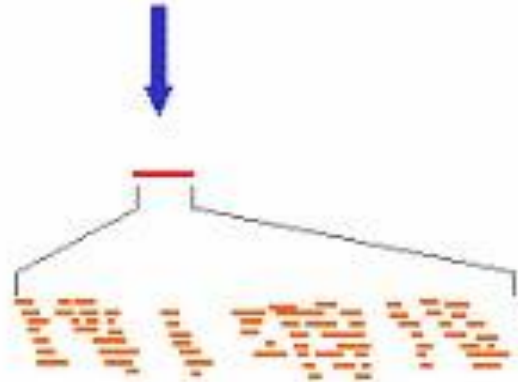
- 1. Construction of maps of ordered landmarks (genetic markers, genes): provides long-range map and organisation into individual chromosomes.



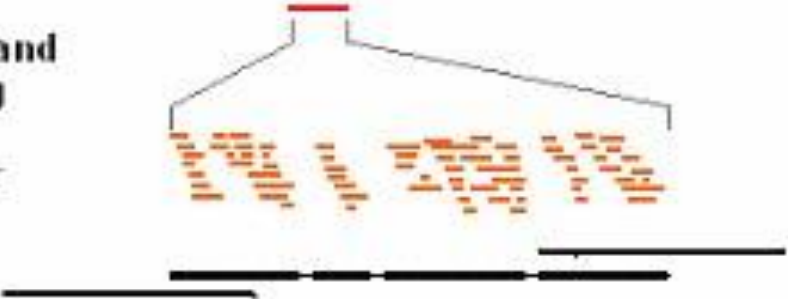
- 2. Physical maps of overlapping clones anchored to the landmark maps.



- 3. Selection of tile path (clones in red)

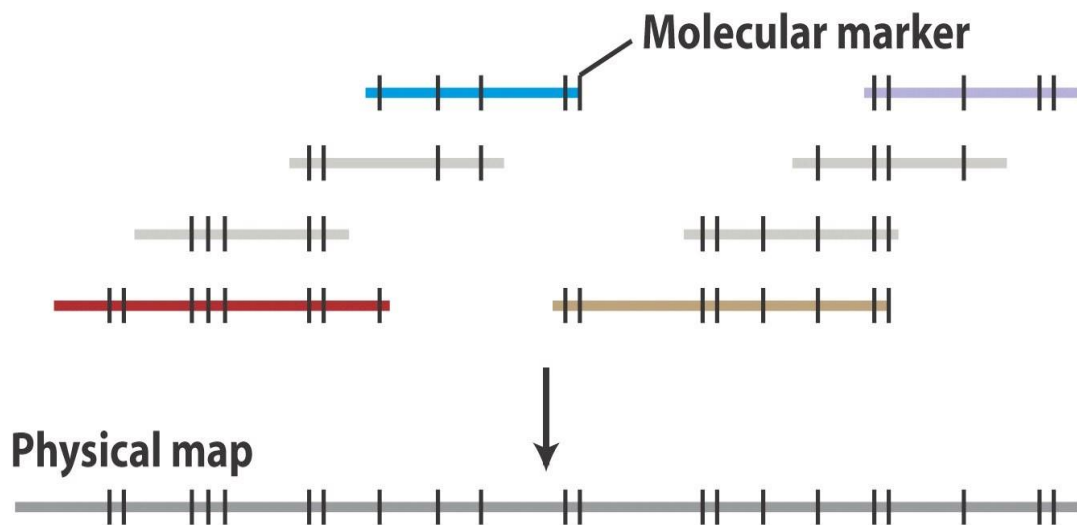


- 4. Shotgun sequencing and assembly (for working draft); subsequent directed finishing (for reference sequence).



Top down mapping

1 Order large-insert clones by overlapping fingerprints to create a physical map.

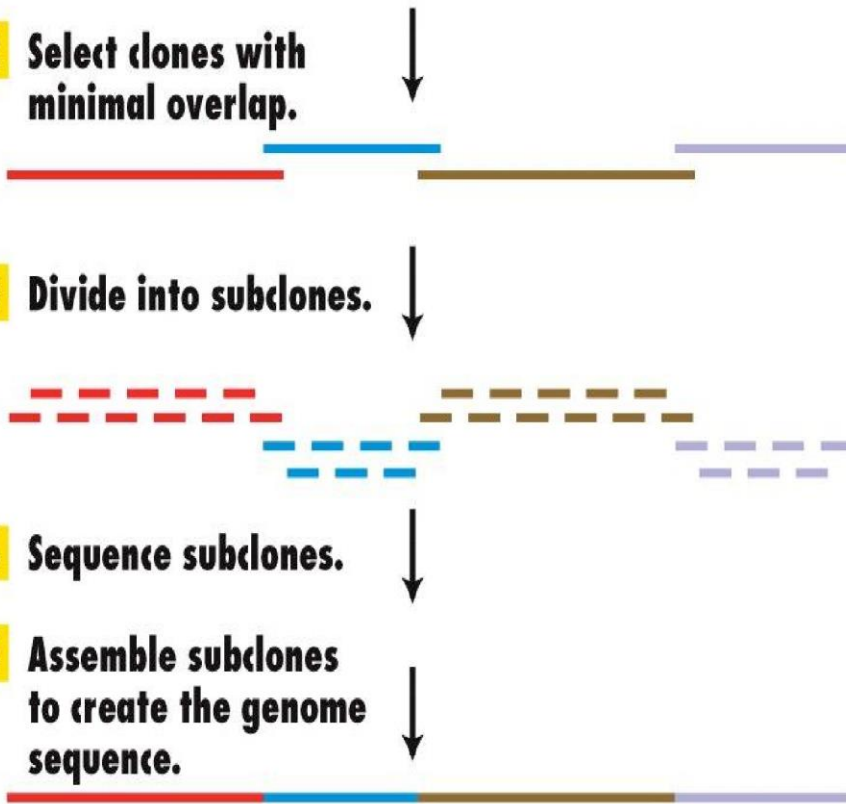


2 Select clones with minimal overlap.

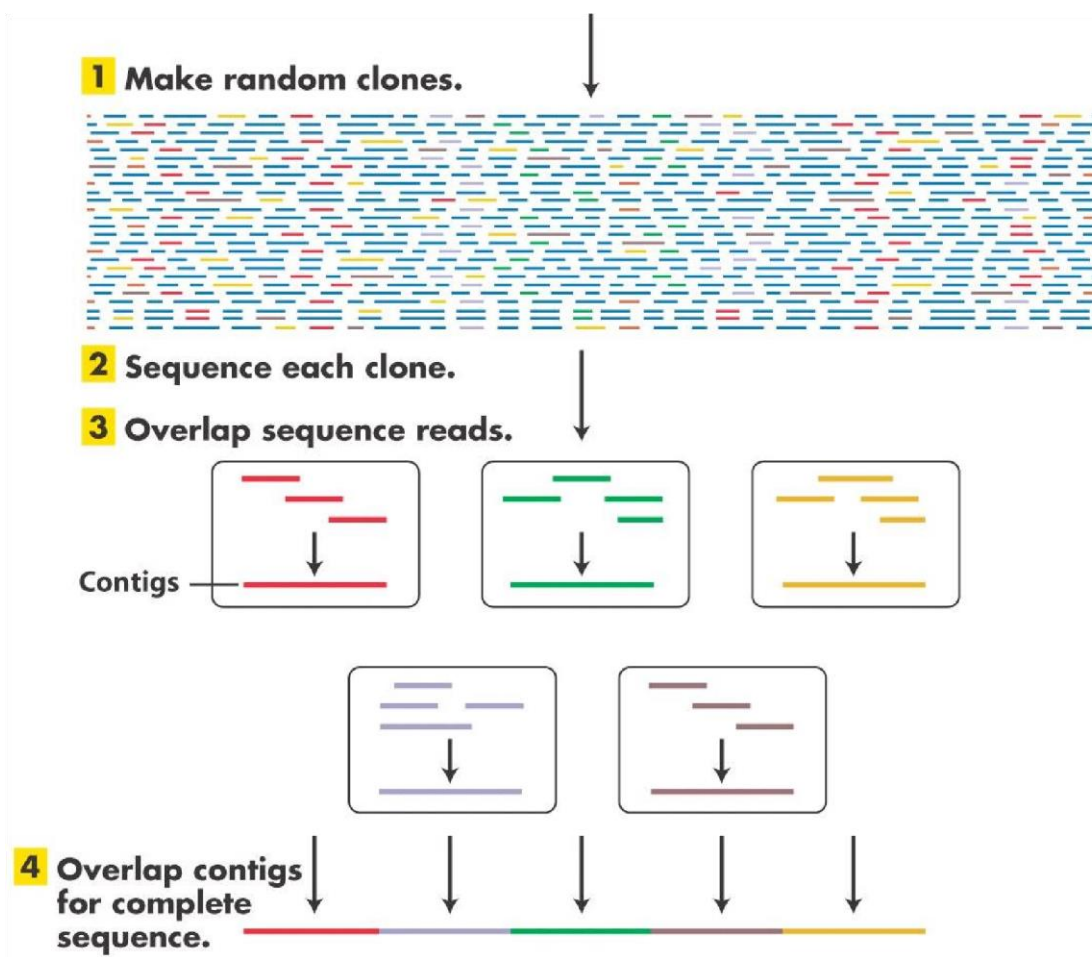
3 Divide into subclones.

4 Sequence subclones.

5 Assemble subclones to create the genome sequence.

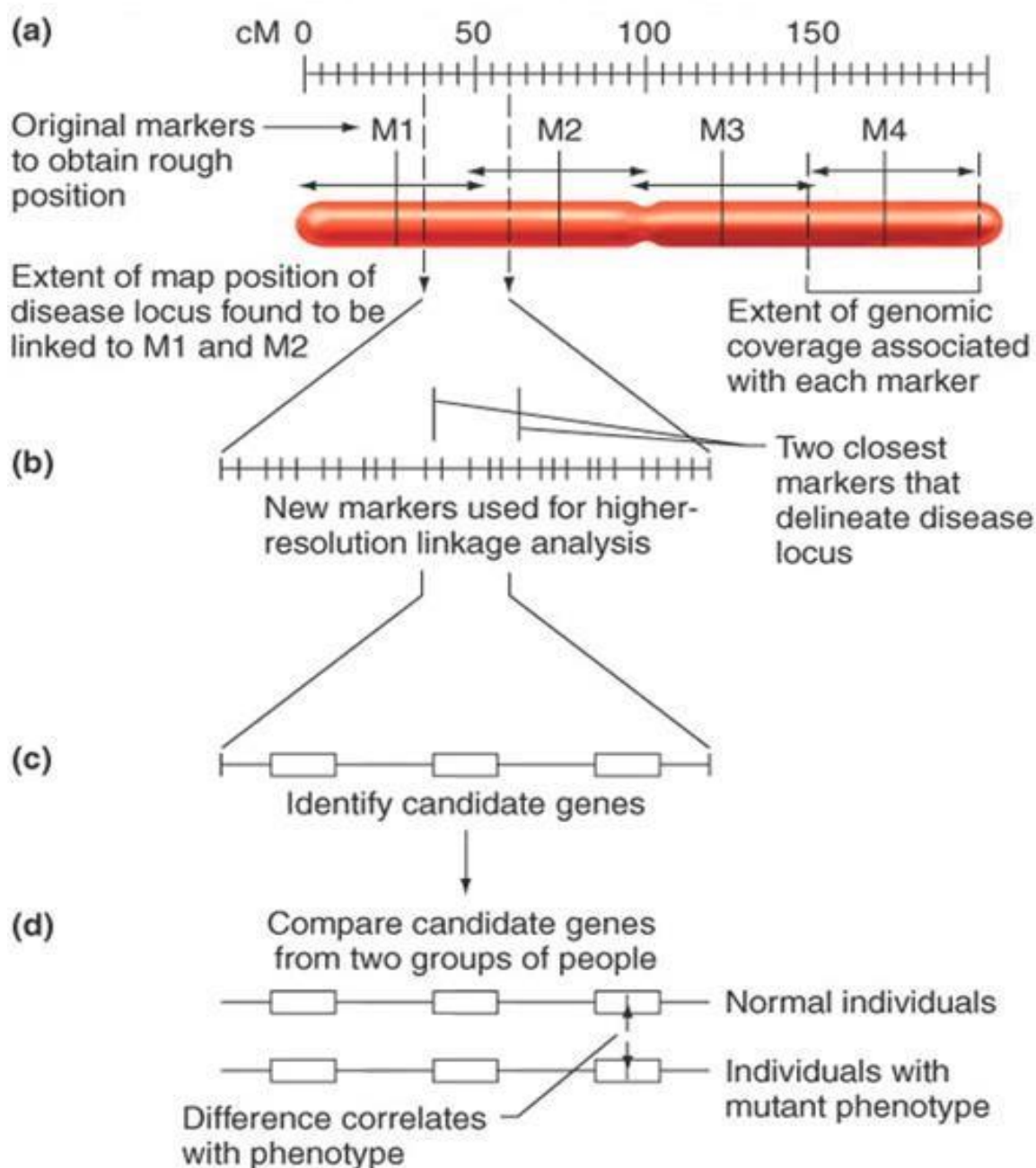


Bottom-up Mapping



Physical Mapping

- Physical maps -locations of the genes on genomes.



91 Mapping genes In human Pedigrees

Mapping diseased genes in Humans

Mapping genes in human pedigrees

- Different methods are used to map human chromosomes and to locate the position of genes.

Mapping human disease gene

- Human pedigrees and DNA typing are used to identify genes.

Polymorphic markers are used

- Polymorphic DNA sequences are used in human genetic mapping.

Several methods, Two are common methods

- Position independent
- Position dependent

Position independent

- Not knowing anything about the location of the gene.
- Starting at the phenotype, determining which protein was involved and then getting to the gene through the protein.

- Forward genetics

Position dependent

- Starting from the approximate location of the gene, to finding the gene itself.
- Translating it to learn about the protein and its function.

Position dependent

- Positional cloning or reverse genetics.
- This is the method most used today.

Oligonucleotide method

- Isolating the protein product of the gene.
- Such genes usually produce large amounts of proteins.
- Gene-specific oligonucleotide are prepared.

Oligonucleotide method

- These probes were used with colony hybridization against a cDNA library.
- Positive clones were re-screened with the secondary probe.

Antibody method

- Antibodies against a known protein are produced.
- A library of expressed cDNAs are then screened using the antibody.

Positional cloning

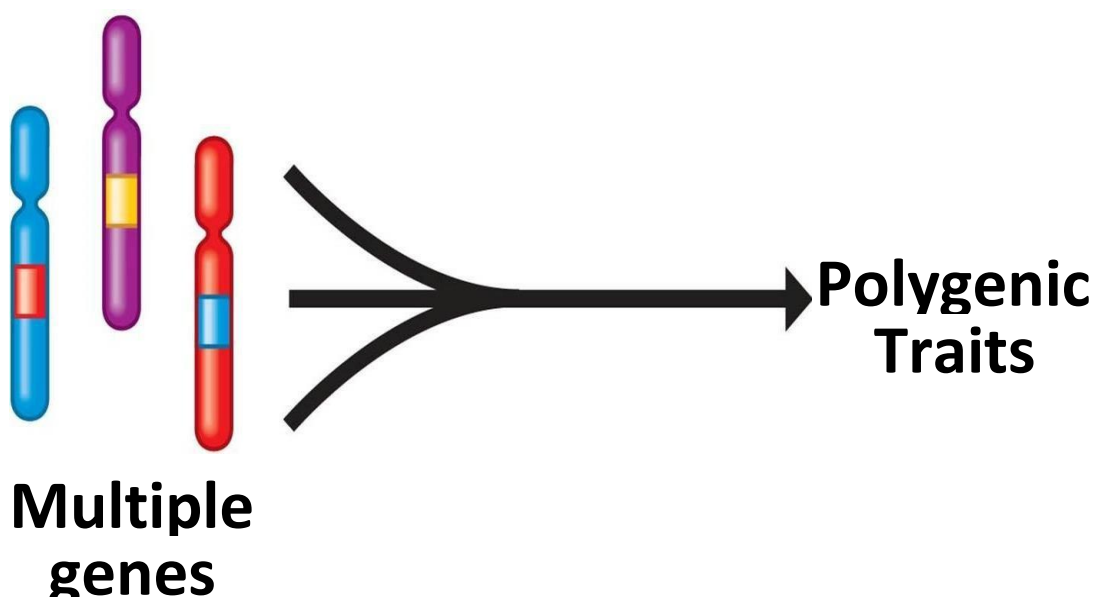
- Disease gene locus is mapped with the help of marker.
- Genes in candidate region are screened by sequencing to identify mutation.
- Protein of identified gene is studied.

Different methods

- Different methods to identify diseased genes

92 Polygenic Inheritance

Polygenic inheritance: Condition when two or more genes influence the expression of one trait. Polygenic inheritance is controlled by multiple genes



Multiple genes produce a spectrum of resulting phenotypes.

- Skin color, height etc.
- Pepper color
- Gene 1: R= red r = yellow
- Gene 2: Y= absence of chlorophyll; y = presence of chlorophyll



Possible genotypes

- R-/Y- : red (red/no chlorophyll)



- R-/yy : brown/orange (red/chlorophyll)



- rr/Y- : yellow (yellow/no chlorophyll)



- rr/yy : green (yellow/chlorophyll)

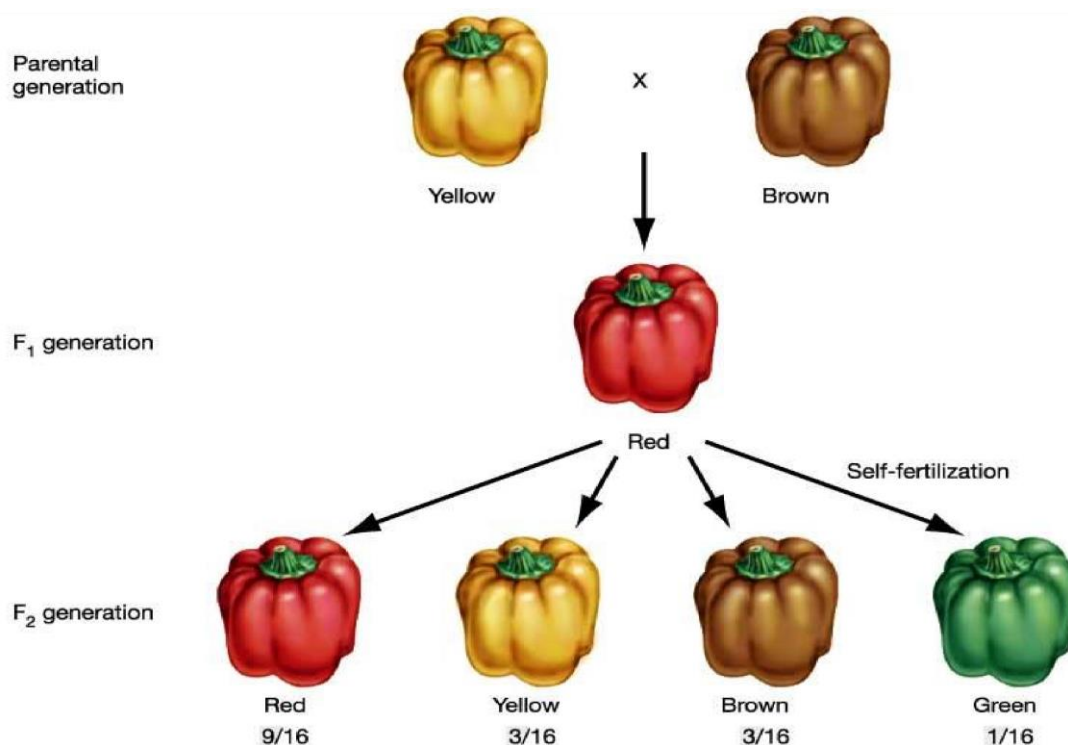


Test cross: Try crossing a brown pepper (RRyy) with a yellow pepper (rrYY). Which trait will your offspring (F1 generation) produce?

What traits are produced when you cross two of the peppers found in the F1 generation?

Brown Pepper RRyy, Yellow pepper rr YY

Polygenic inheritance is a condition when two or more genes influence the expression of one trait.



93 Examples of Polygenic inheritance

Examples of polygenic traits Eye color, height, body shape and intelligence. Many of these traits are also affected by the person's environment, so they are called multifactorial.

Polygenic traits, or continuous traits, are governed by alleles at two or more loci, and each locus has some influence on the phenotype. Hair, eye and skin color are polygenic traits.

Polygenic trait – eye color



Stature, shape of face, finger prints

- Stature
- Shape of face
- Fingerprint patterns

Chicken Combs Four different comb shapes. Comb shape is controlled by two genes found on two different chromosomes.

➤ Gene 1: R

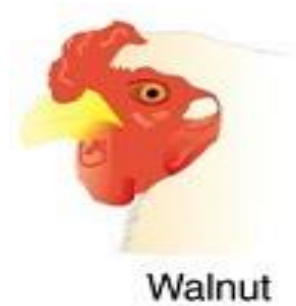
➤ Gene 2: P

Four different phenotypes result

- Rose Combs (R-pp)



- Walnut Combs (R-P-)



- Pea Combs (rrP-)



- Single Combs (rrpp)



Hair color Hair color is controlled by alleles on chromosomes 3, 6, 10, and 18. When more dominant alleles appear in the genotype; the darker will be the hair.



94 What is Epistasis

Epistasis and modifier genes Epistasis is a phenomenon that consists of the effect of one gene being dependent on the presence of one or more modifier genes Type of polygenic inheritance where alleles at one gene locus can hide or prevent expression of alleles at second gene locus.

Positive epistasis – double mutation

- Double mutation has a fitter phenotype than either single mutation.
- Positive epistasis between beneficial mutations generates improvements in function.

Negative epistasis – two mutations have smaller effect

- When two mutations together have a smaller effect than expected from their effects when alone, it is called negative epistasis.

Sign epistasis occurs when one mutation has the opposite effect when in the presence of another mutation.

Reciprocal epistasis occurs when two deleterious genes are beneficial when together.

Epistasis between genes

- Epistasis within the genomes of organisms occurs due to interactions between the genes within the genome.
- This interaction may be direct if the genes encode for proteins.
- Interaction may be indirect, where the genes encode components of a metabolic pathway or network, developmental pathway.

Epistasis within genes when one deleterious mutation can be compensated for by a second mutation within that gene.

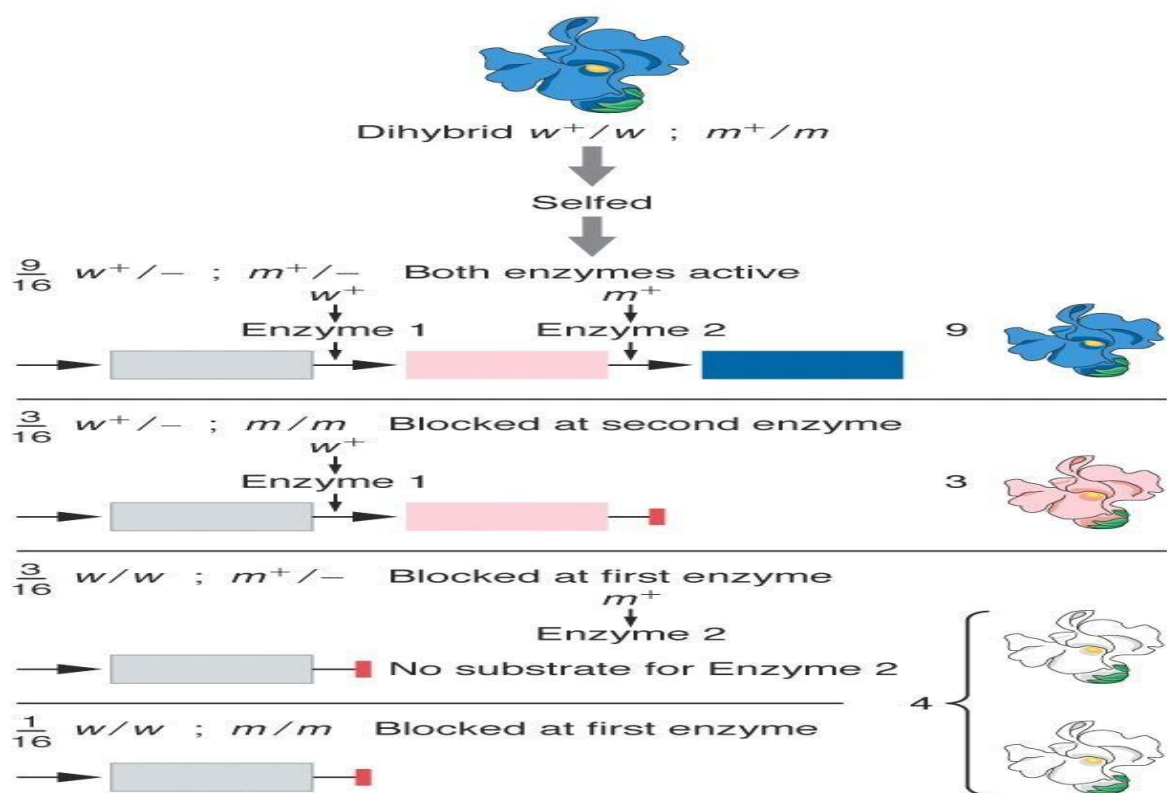
Epistasis Type of polygenic inheritance where alleles at one gene locus can hide or prevent expression of alleles at second gene locus. There are several types of epistasis.

95 Types of Epistasis

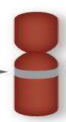
Recessive Epistasis Epistatic gene exerts its affect with homozygous recessive genotype.


Petal color in blue-eyed Mary plants Both enzymes active blue; Enzyme 1 active magenta


Precursor 1 → Precursor 2 → blue anthocyanin
colorless magenta

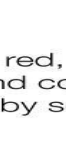


Recessive Epistasis Epistatic gene exerts its affect with homozygous recessive genotype.

Rec. red → 





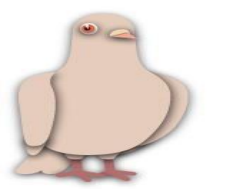



Spread → 

Pattern → 

Color → 

Recessive red, spread, pattern, and color are controlled by separate genes.

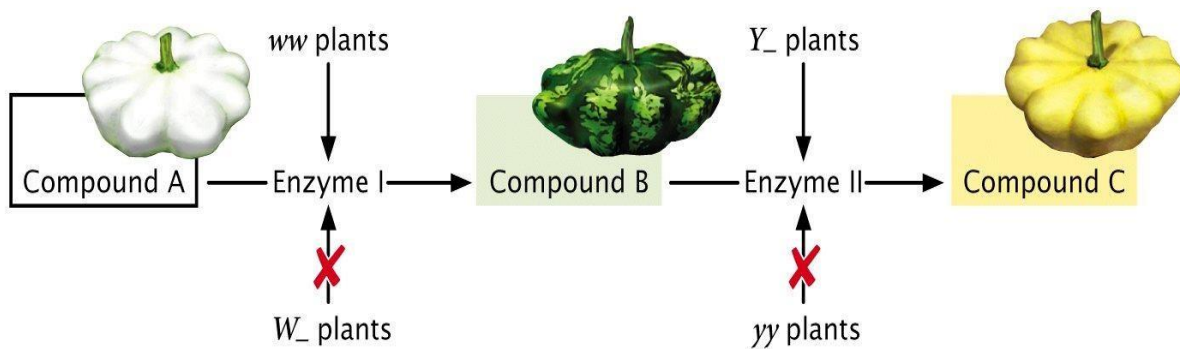
Recessive red is epistatic to spread color, and pattern.
(Spread is epistatic to pattern.)

REC.RED			
COLOR		SPREAD	
		PATTERN	
 (Not recessive red) (Not spread) Ash-red	 (Not recessive red) (Not spread) Blue	 (Not recessive red) (Not spread) Brown	 Recessive red (Not spread) (Any color)
 (Not recessive red) Spread Ash-red	 (Not recessive red) Spread Blue	 (Not recessive red) Spread Brown	 Recessive red Spread (Any color)

96 Types of Epistasis-Dominant

Dominant epistasis

- Epistatic gene exerts its affect with the presence of a dominant allele.
- Dominant epistasis is fruit color in summer squash
- Y = Yellow
- yy = Green
- W inhibits either color = color will be white (dominant)
- w has no effect on color



Dominant Epistasis- ratios

Fruit color in summer squash

$$\begin{array}{rcl}
 WwYy & \times & WwYy \\
 9/16 & W_Y_ & \\
 3/16 & W_yy & 3/16 \quad wwY_ \\
 1/16 & wwyy &
 \end{array}$$

Duplicate dominant Epistasis

Fruit shape in Shepherd's purse

$A_ \text{ or } B_ = \text{heart shape}$ $aa \text{ and } bb = \text{narrow shape}$

Duplicate dominant Epistasis

Fruit shape in Shepherd's purse

$$\begin{array}{rcl}
 A_ \text{ or } B_ = \text{heart} & & aa \text{ and } bb = \text{narrow} \\
 AaBb & \times & AaBb \\
 9/16 & A_B_ & \\
 3/16 & A_bb & \\
 3/16 & aaB_ & \\
 1/16 & aabb &
 \end{array}$$

Epistatic gene exerts its affect with the presence of a dominant allele.

97 Example of Epistasis-Labrador Retrievers

Example of Epistasis

Fur color in dogs Fur color in Labrador Retrievers is controlled by two separate genes.

- Gene 1: Represented by B; which controls color.
- Gene 2: Represented by E which controls expression of gene B.

Epistasis

If a Labrador retriever has a dominant B allele, they will have black fur.

If they have two recessive alleles (bb) they will have brown fur.



- BBEE and BbEe --> Black
- bbEE and bbEe --> Brown
- BBee, Bbee, or bbee --> Golden retrievers



Epistasis-genotypes/phenotype

Epistasis In dogs when a gene in recessive form has effect on expression of other genes.

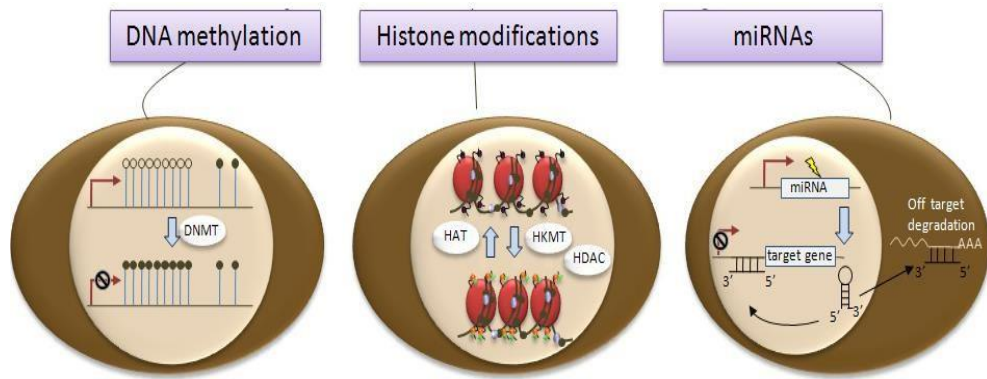
	EE	Ee	ee
BB			
Bb			
bb			



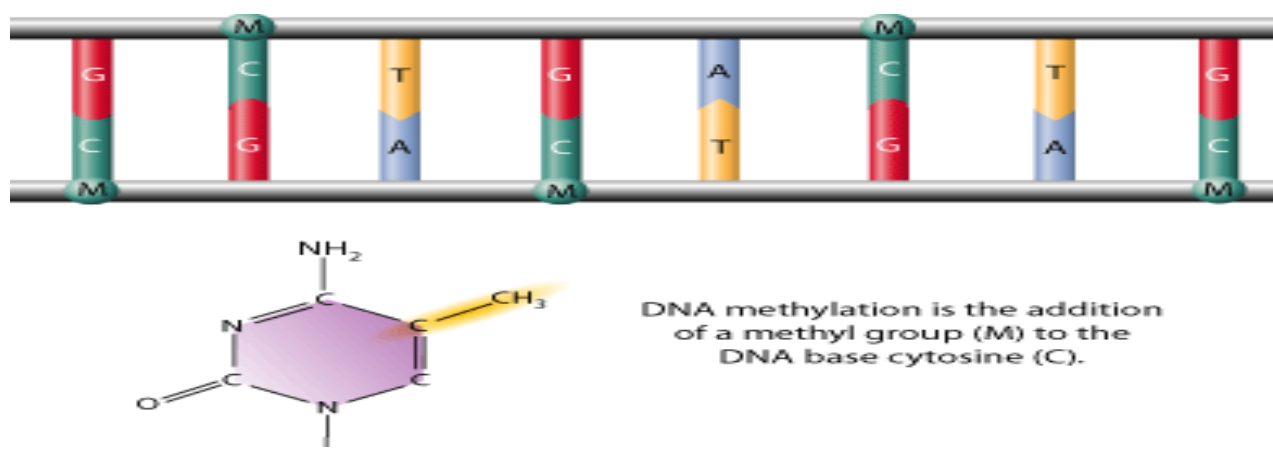
98 What is Epigenetics

Epigenetics literally means ‘above’ the genetics. Epigenetic trait is a stable and heritable phenotype resulting from changes in chromosome without alterations in the DNA sequence.

Causes of Epigenetics



Cytosine methylation: Methyl group to carbon-5 of cytosine residues.

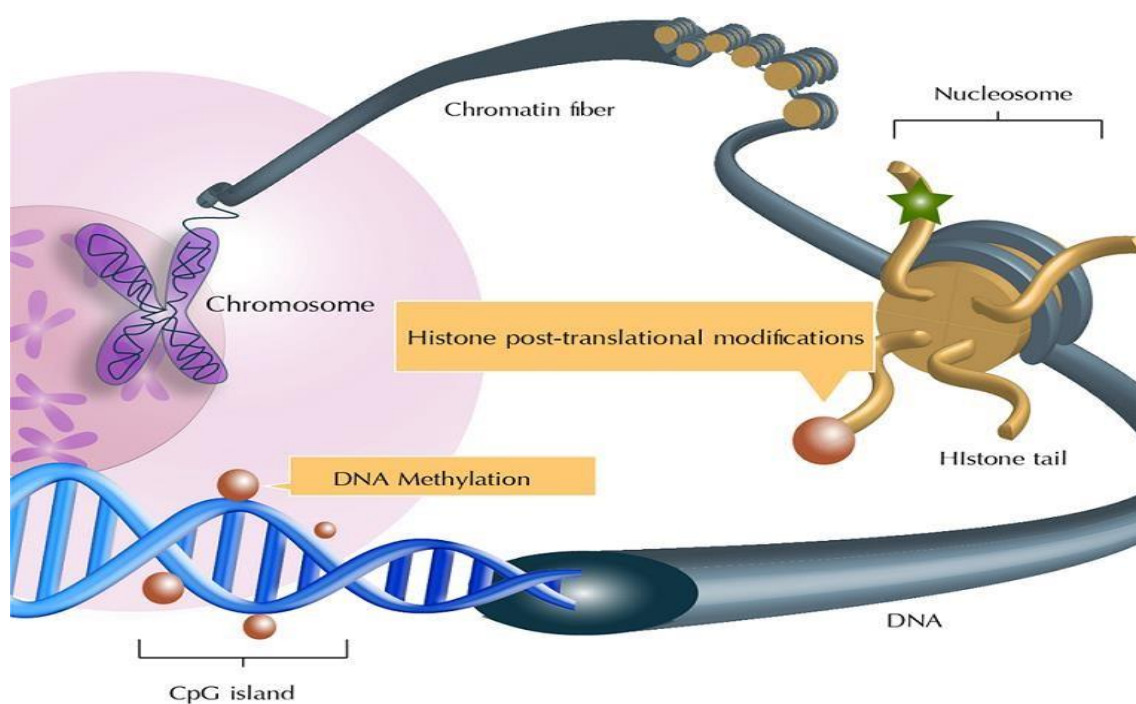


DNA Methylation is epigenetics, not changes in DNA sequence

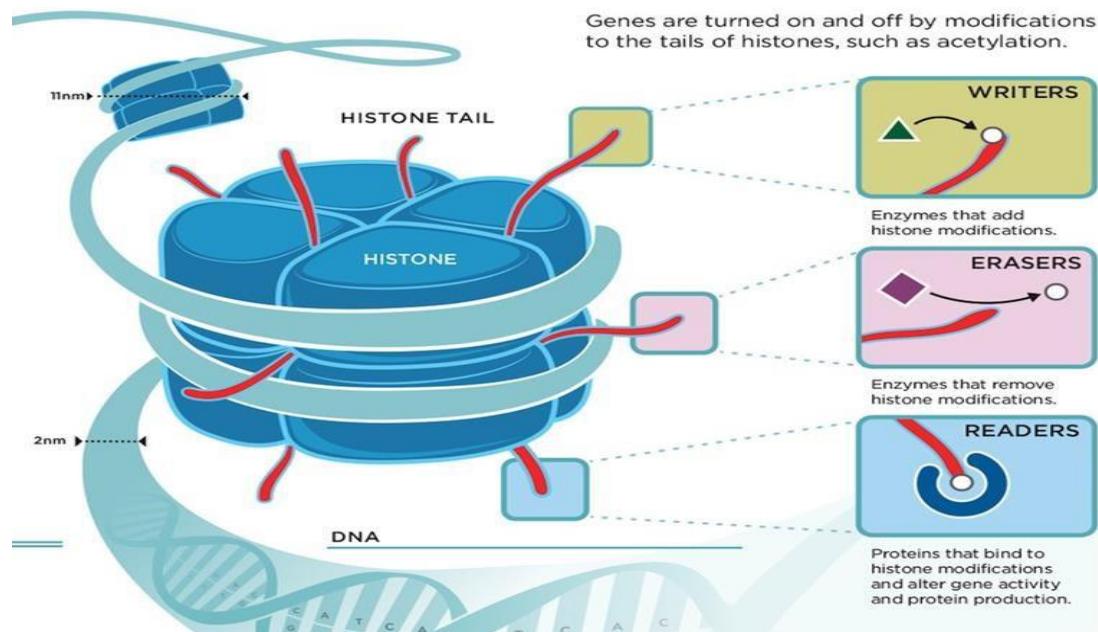
DNA methylations are regarded as epigenetics and not genetic changes in sequence of DNA.

Molecular Mechanism of Epigenetics Two primary mechanisms identified. Methylation of cytosine Posttranslational modification to histone proteins; includes acetylation, phosphorylation etc.

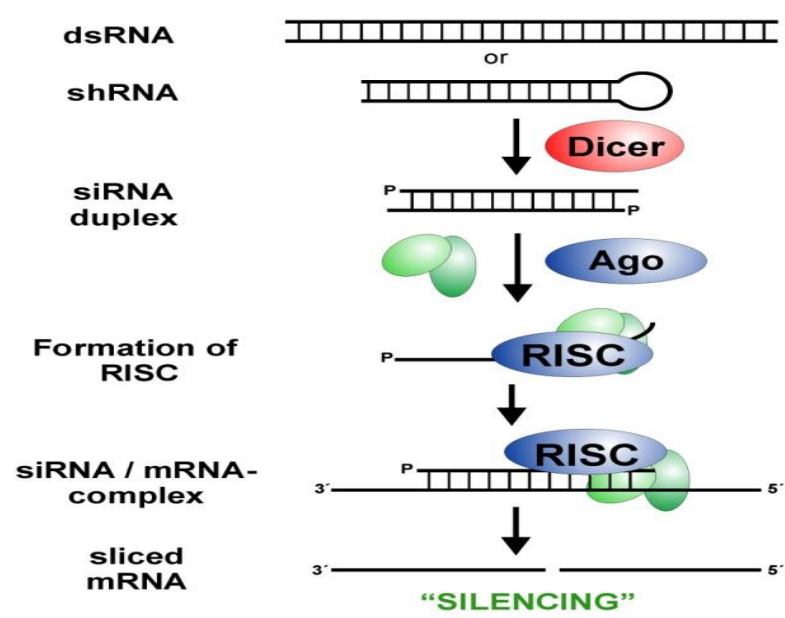
Molecular Mechanisms



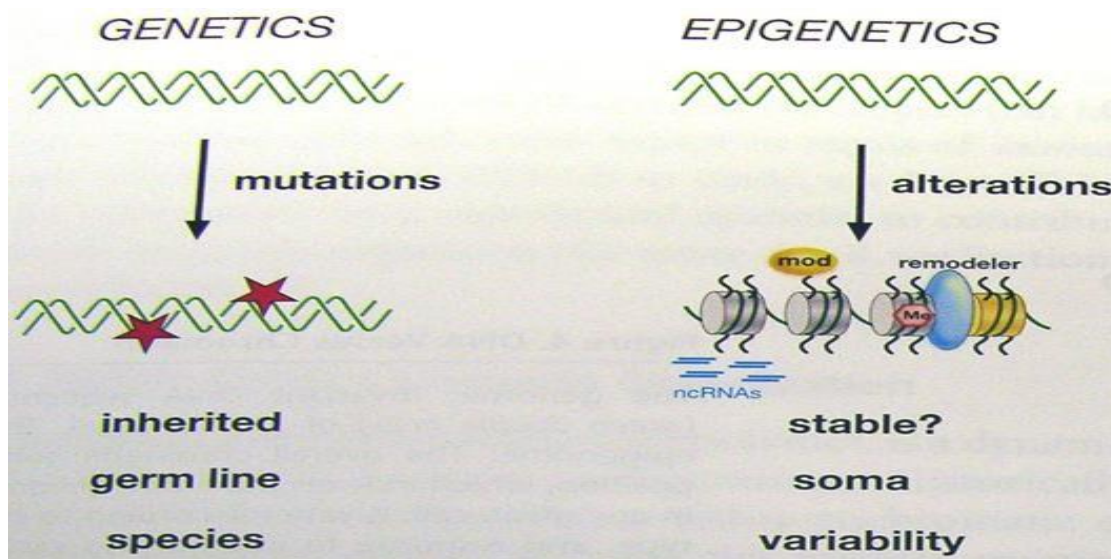
Histones modifications



Small interfering RNA – mechanism causing epigenetics A third mechanism involves expression of small interfering RNAs (siRNA).



Genetics vs Epigenetics



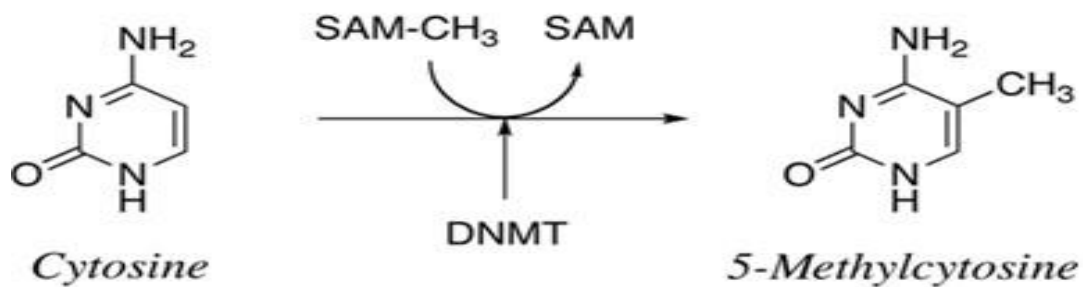
Epigenetics An epigenetic trait is a stable heritable phenotype resulting from changes in chromosome without alterations in the DNA sequence.

99 Epigenetic Modifications

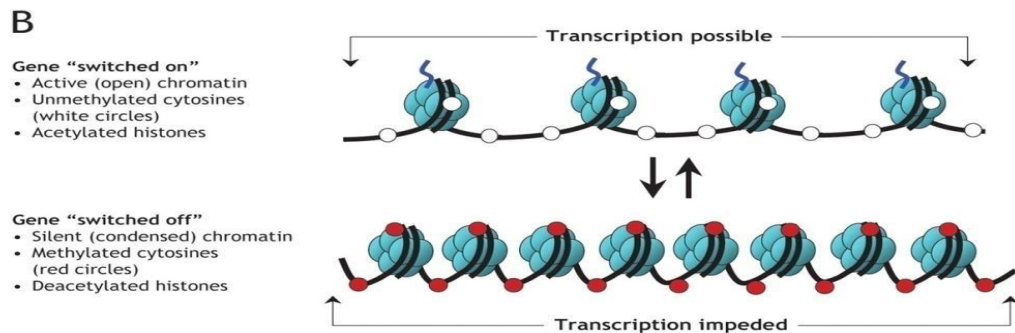
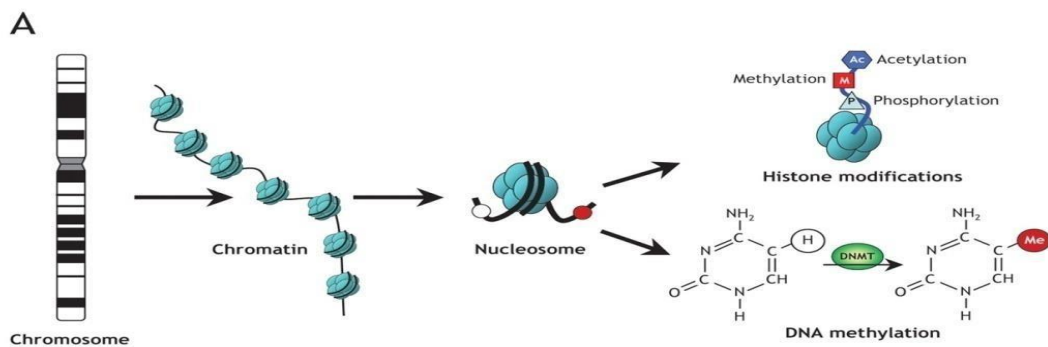
Epigenetic Modifications: Followings are the steps of epigenetics modifications

- Cytosine methylation
- Histones modification
- Non-coding RNAs (siRNAs)
- All above regulate gene expression.

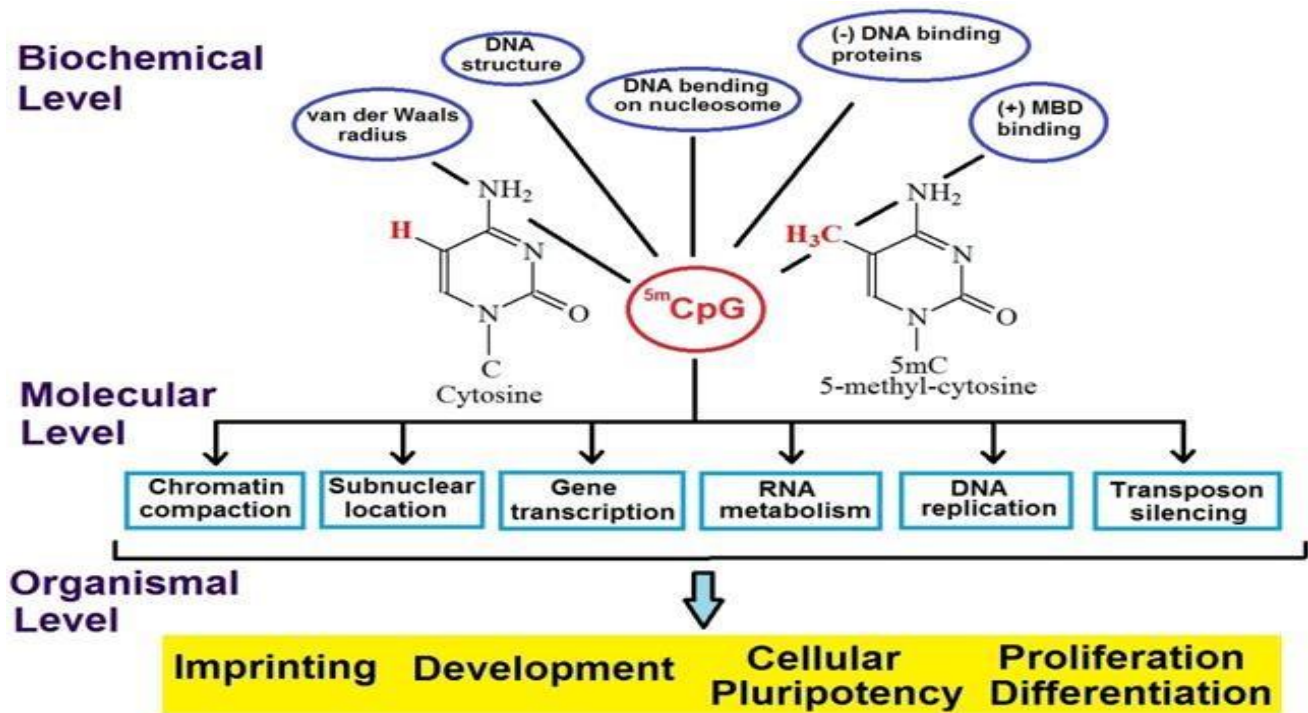
Cytosine Methylation – methyl transferase



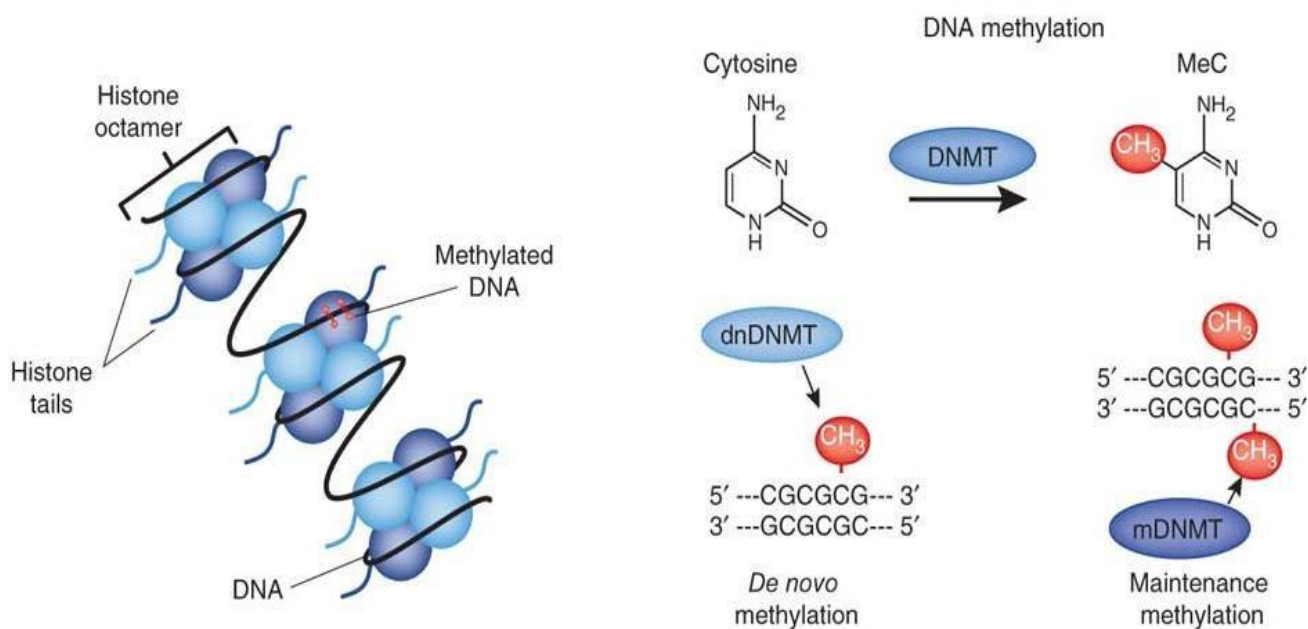
Genes-switched on & off



Methylated cytosine in cPpG



Cytosine Methylation

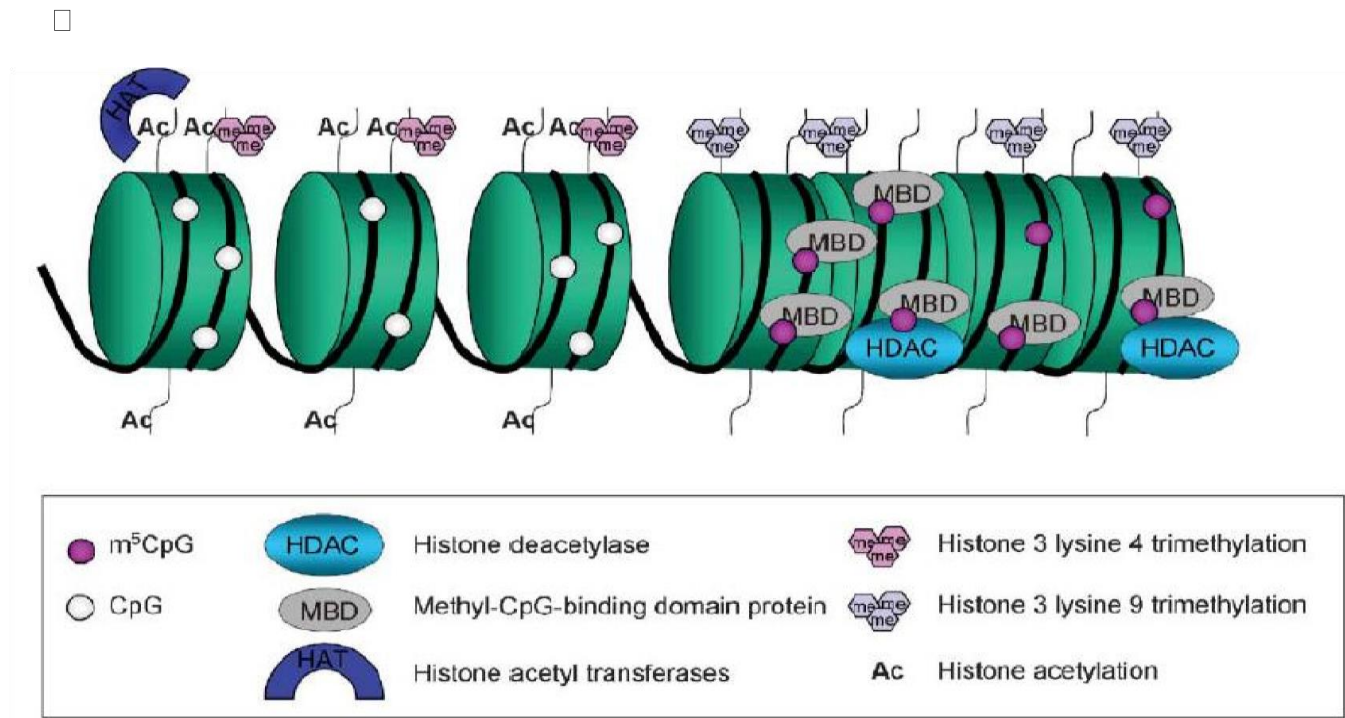


Cytosine Methylation occurs at upstream regions of genes Often located just upstream of genes (promoter regions). Associated with attenuation of expression of nearby genes.

Histones modifications Histones are the proteins that organize the genetic material. Have a high percentage of basic amino acids, which gives histones an overall positive charge, while DNA is negative charged.

Acetylated Histones-genes are active when histones are acetylated, chromatin is open and genes are potentially active. When histones not tagged, deacetylated, the chromatin condenses and genes silenced.

Histones modifications



- HDAC: Histone deacetylase.

Higher acetylation, higher gene expression Chromatin conformations changes to a form which is more open to transcription.

↑ Acetylation = ↑ gene expression

Epigenetic modification - non coding RNA is a new mechanism for gene regulation. RNA which is not used for making proteins can be cleaved and used to inhibit protein-coding RNAs (siRNAs, microRNAs).

Three common types of modification -

- Cytosine methylation
- Histones modifications
- siRNAs

100 Epigenetics regulated Phenomena

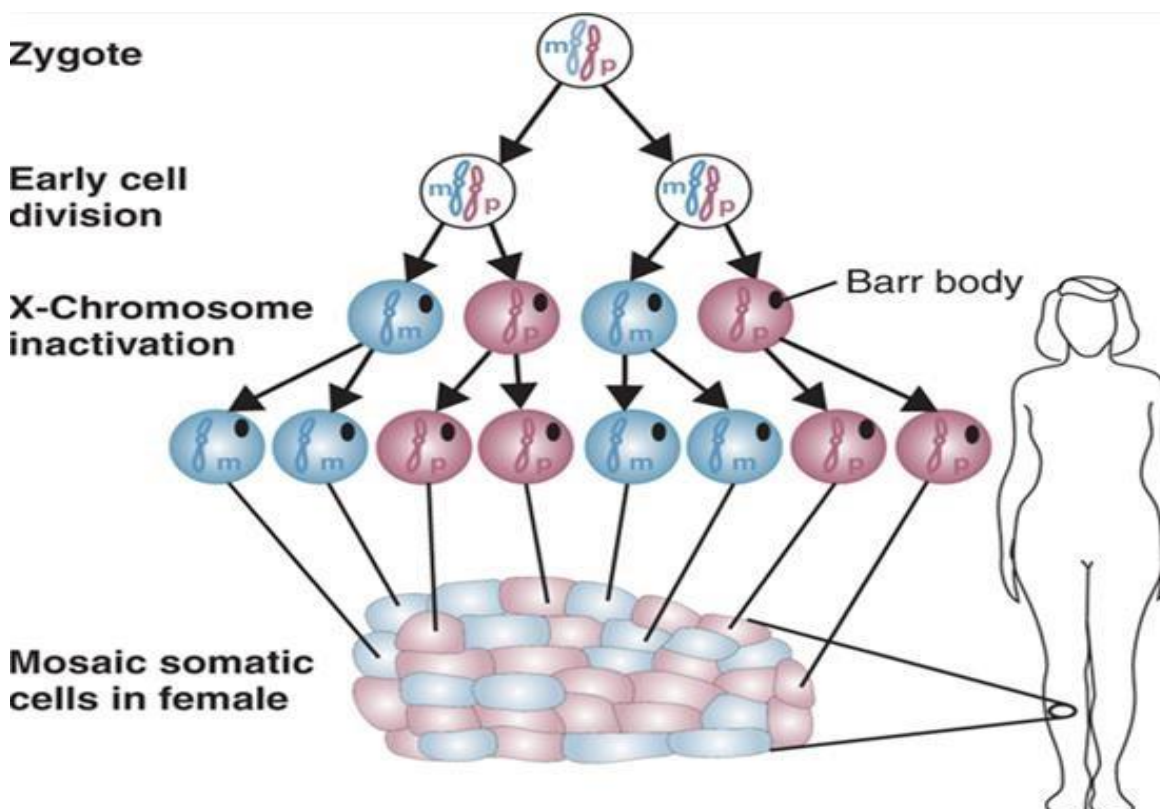
Common Epigenetics regulated phenomena

- Cellular Differentiation
- X Chromosome inactivation
- Genomic imprinting

Cellular differentiation totipotent cells become pluripotent cells of the embryo which differentiate into specific lineages.

X chromosome inactivation Gene expression on one of the female X-chromosomes is down regulated. DNA methylation and histone modifications.

X chromosome inactivation



Genomic Imprinting Epigenetic marking of a locus on the basis of parental origin. Results in monoallelic gene expression.

Genomic Imprinting Some genes are expressed only from the maternal genome and some only from the paternal genome. It is estimated that about 40 genes are imprinted and they can be found on several different chromosomes.

Epigenetics and fragile X chromosome Fragile X syndrome is most commonly caused by a CGG trinucleotide repeat expansion in the 5' region of the FMR1 gene. Unaffected individuals have 6-50 CGG repeats.

Epigenetics and fragile X chromosome

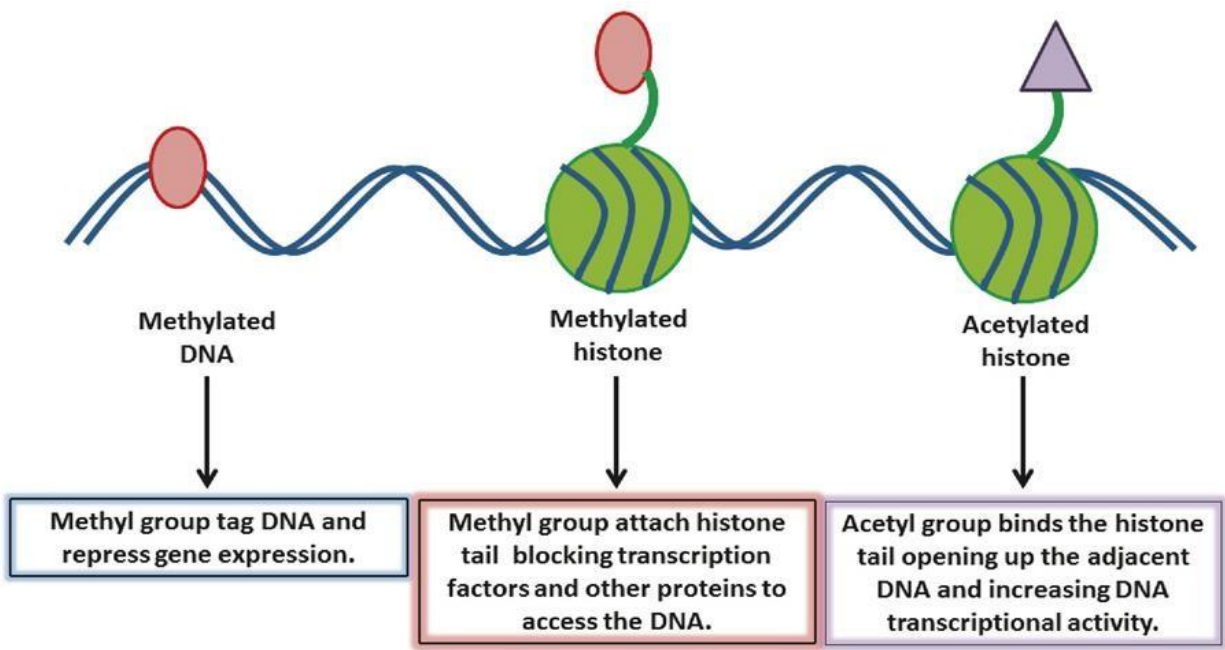
- 200 CGG repeats is seen in individuals with fragile X.
- >200 CGG repeats is correlated with hyper methylation at CpG dinucleotides and silencing.

Identical twins Identical twins are from the same zygote, so they begin life with the same genetic information. While infants they experience the same or very similar environments, so there is little variation in the epigenome. Over time the twins' environments will diverge, resulting in individual epigenetic tags to form for each twin. The difference in the twins' epigenomes is what makes them become different when they are older. The epigenetic tags can have such an effect on the twins that one can develop a disease while the other is fine.

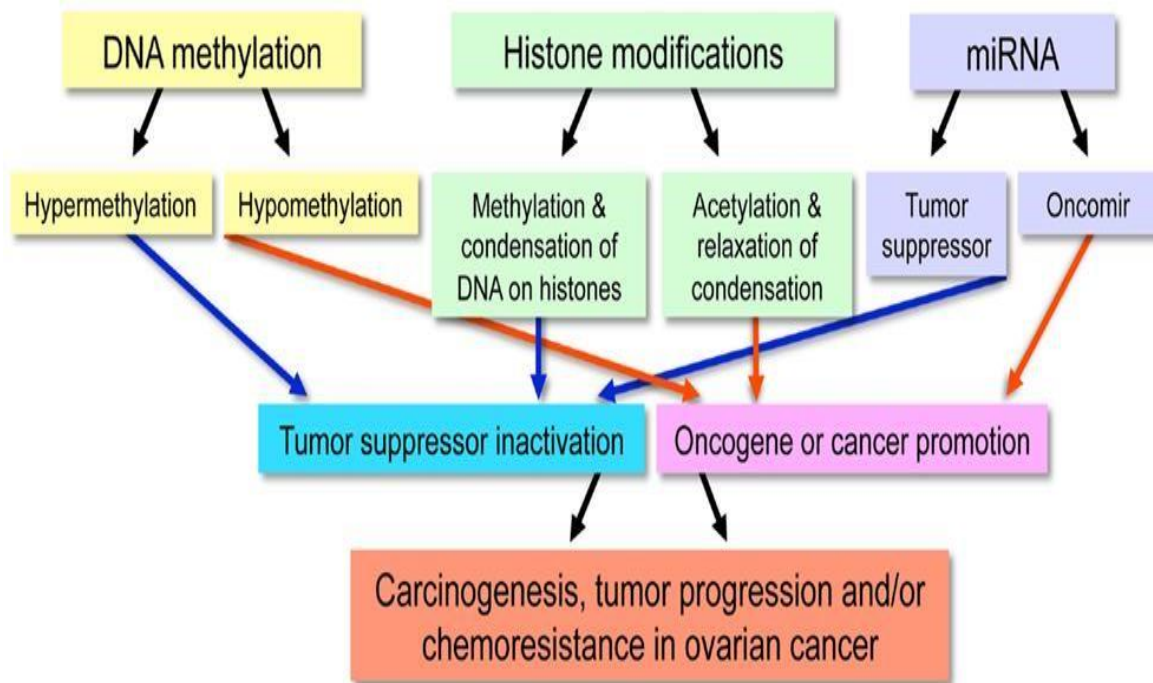
101 Therapies Targeting Epigenetics

Therapies for epigenetics In contrast to mutations, epigenetic changes can be reversed. Are there therapies that influence epigenetic patterns? Yes

Therapies for epigenetics



Epigenetics and diseases



Therapies targeting Epigenetics

- Vorinostat
 - Vorinostat is a histone deacetylase inhibitor.
- ↑ Acetylation = ↑ gene expression.

102 Environmental Factors and Epigenetics

Environmental factors and Epigenetics

Nutrition of mother

- The nutrition of the mother can affect the epigenome of a fetus.

Stress hormones

- Stress hormones also travel from the mother to a fetus to affect the epigenome

Social interactions and physical activities

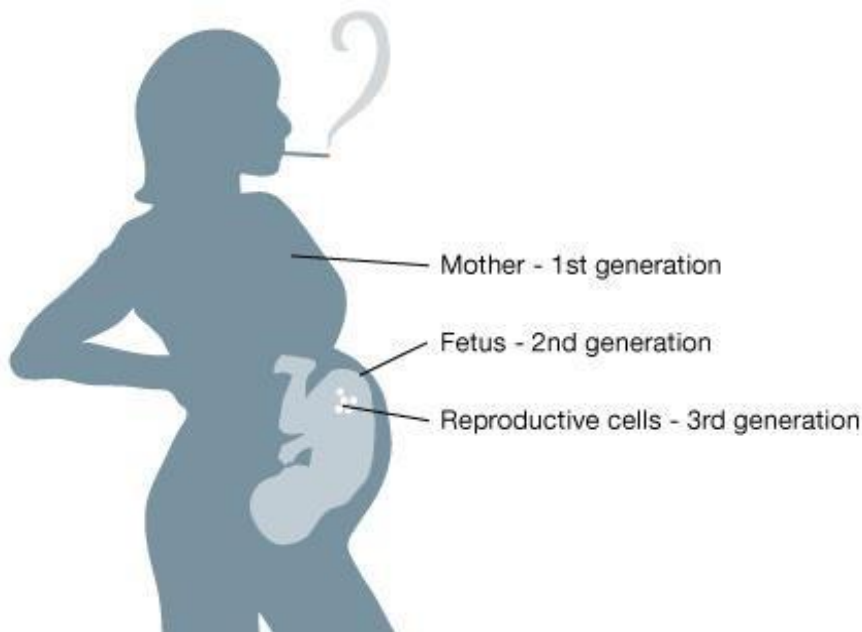
- Social interactions, physical activity, are factors that affect the epigenome

Exposure to toxins and diet

- Exposure to toxins and diet are major factors that affect the epigenome

vs.

generations.



- ☐
- ☐
- ☐
- ☐
- ☐

- Epigenetics has opened a new field in genetic research.
- Muddles the nature nurture argument.
- Personal responsibility towards health for further

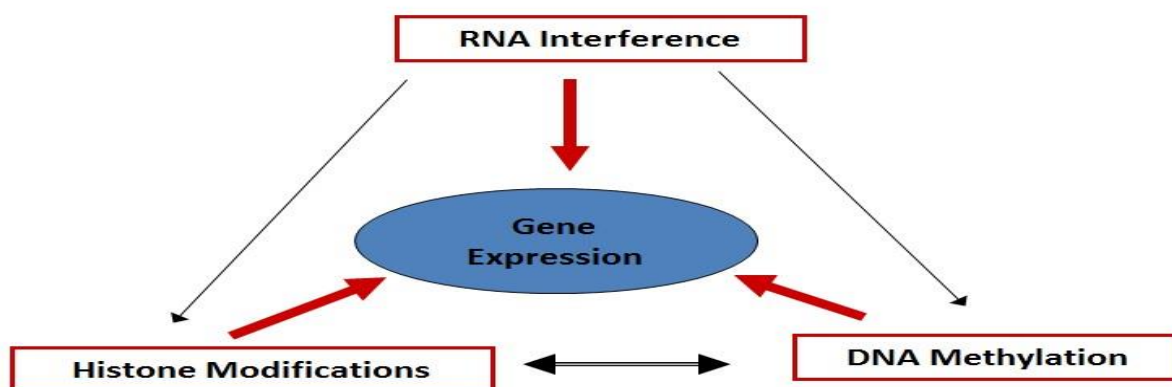
103 Epigenetics- Conclusions

Epigenetics- conclusions: Epigenetic traits are heritable phenotypes resulting from changes in chromosomes that do not involve changes in DNA sequence. Two prominent epigenetic mechanisms involve

- DNA methylation (gene silencing)
- Histone acetylation (gene activation).

Errors in epigenetic patterns can influence human diseases including cancer.

Epigenetics conclusion: The epigenome changes as we age and can be influenced by the environment. Drugs that influence the epigenome represent a major area of current research.



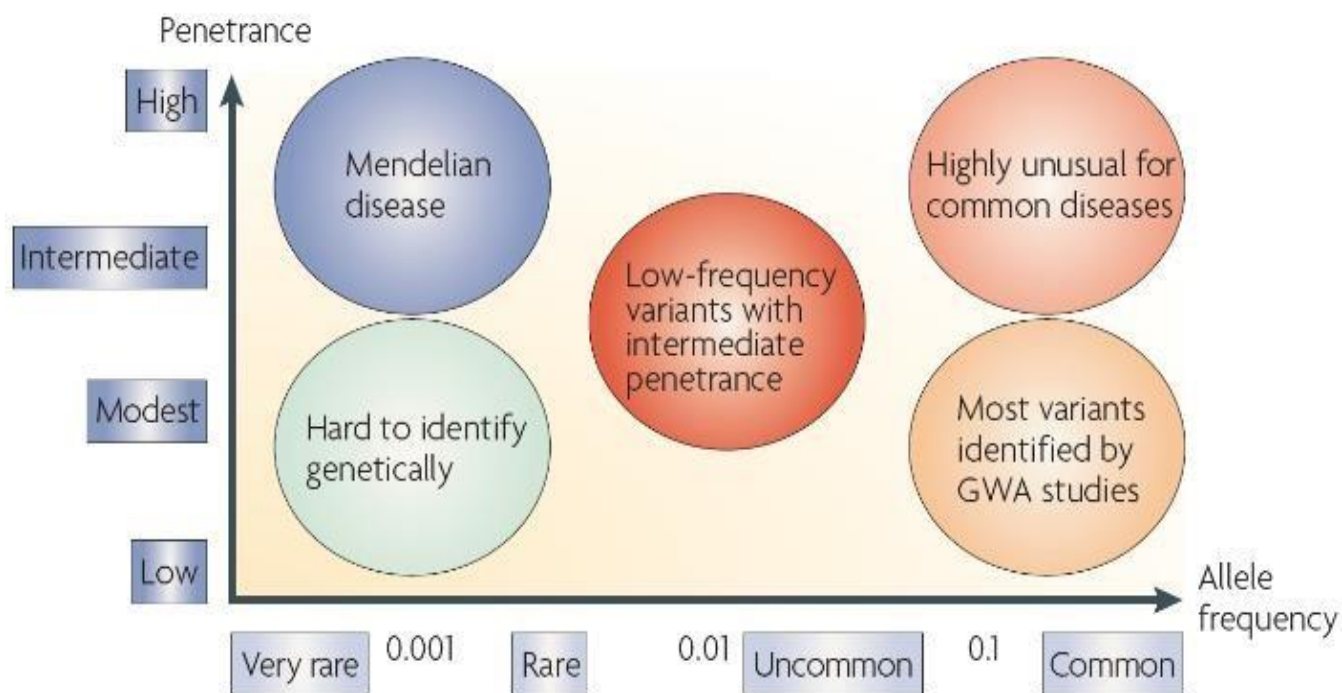
104 Penetrance and Expressivity

Penetrance and Expressivity

Percent individuals with a given allele that show the phenotype of the allele

Proportion of individuals carrying a particular variant of a gene (allele or genotype) that also expresses an associated trait (phenotype)

Less than 100% penetrance is a result of modifiers, epistasis, suppressors, and environmental conditions.



Expressivity

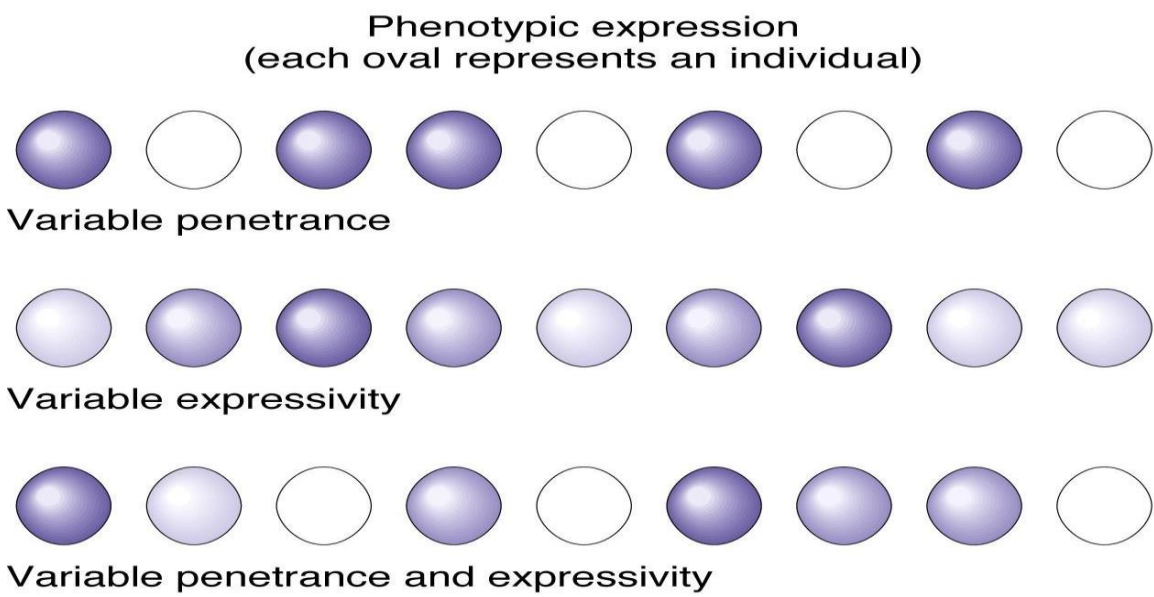
Extent to which allele is expressed at phenotypic level affected by genetic background and environment.

Difference likelihood of the gene generating its associated phenotype. Influence of an expressed gene at the level of particular individuals.

Expressivity

The term is analogous to the severity of a condition in clinical medicine.

Variable expressivity when phenotype is expressed to a different degree among individuals with the same genotype.



Penetrance & expressivity – Conclusion

- Likelihood of the gene generating its associated phenotype
- Influence of an expressed gene at the level of particular individuals

105 Penetrance and Expressivity- Example

Types of Penetrance

Complete Penetrance The allele is said to have complete penetrance if all individuals who have the disease-causing mutation have clinical symptoms of the disease.

Highly Penetrant If an allele is highly penetrant, and then the trait it produces will almost always be apparent in an individual carrying the allele.

Incomplete or reduced Penetrance is said to be reduced or incomplete when some individuals fail to express the trait, even though they carry the allele.

- **Incomplete or reduced Penetrance**



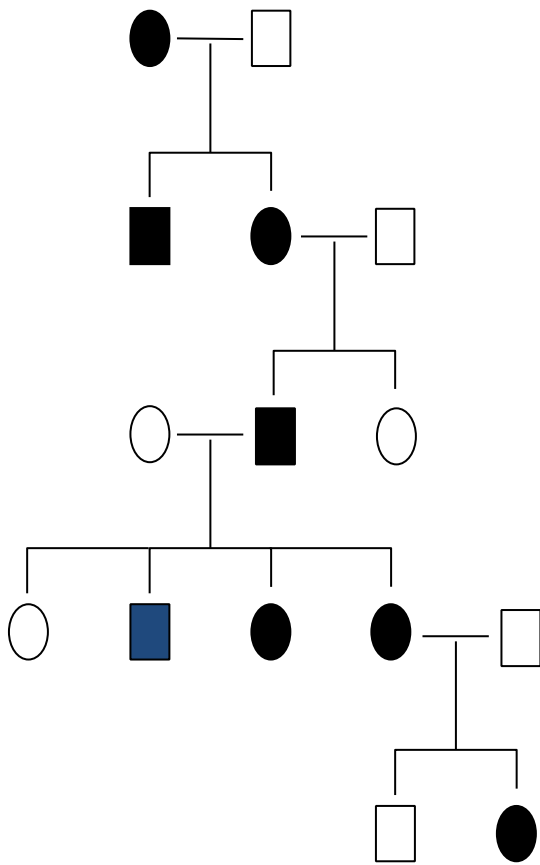
Low Penetrance An allele with low penetrance will only sometimes produce the symptom or trait with which it has been associated at a detectable level. In low penetrance, it is difficult to distinguish environmental from genetic factors.

Determine Penetrance is difficult – if age related Penetrance can be difficult to determine reliably, even for genetic diseases that are caused by a single polymorphic allele.

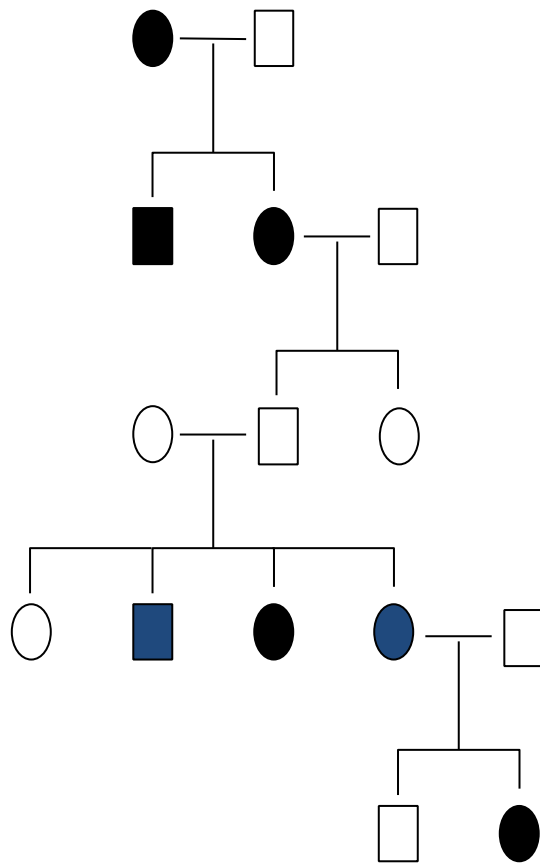
For many hereditary diseases, the onset of symptoms is age related.

- **Penetrance is expressed as frequency**
- Multiple endocrine neoplasia 1 (MEN 1), a hereditary disorder
- Age related – 7% at age 10, 100% at age 60.

Types of Penetrance



Complete Penetrance



Partial Penetrance

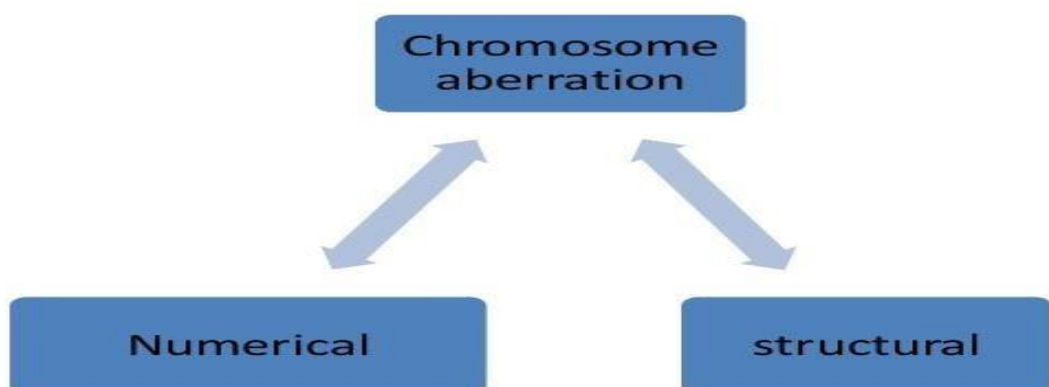
106 Chromosomal Aberrations Closed 1

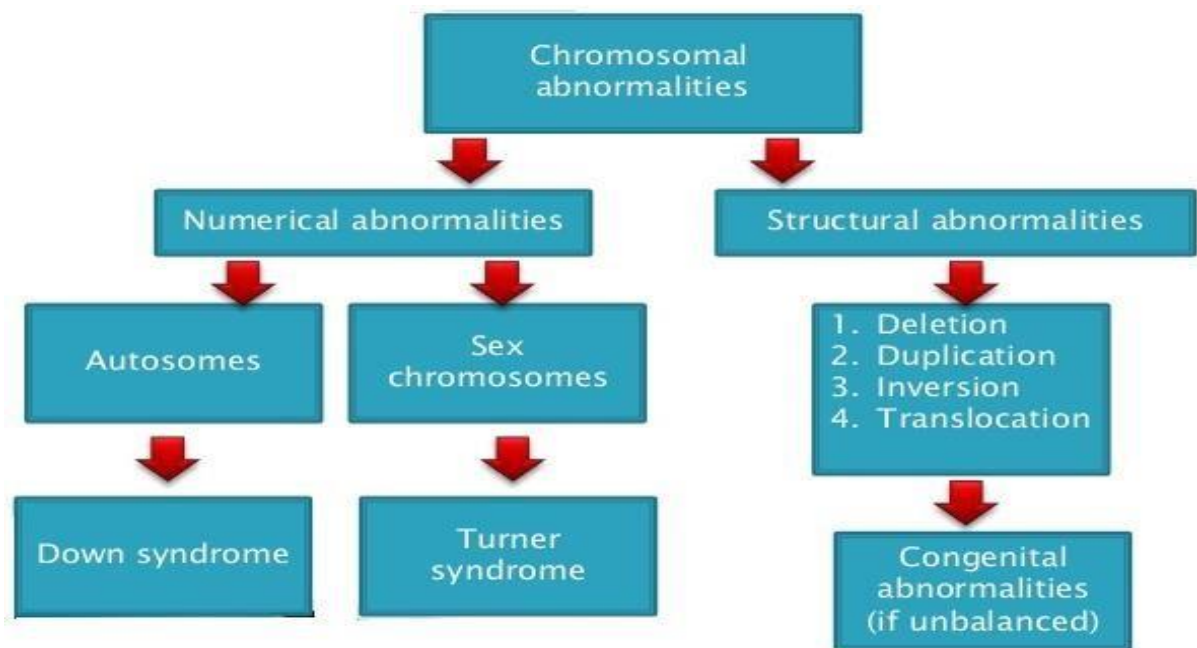
Chromosomal Aberrations: any change in normal number or structure of chromosomes. Affects individuals may be due altering the amounts of products of the genes involved. Altered amounts may cause anomalies directly or may alter the balance of genes

Classification

- Numerical (usually due to de novo error in meiosis).
- Structural (may be due to de novo error in meiosis or inherited).

Different cell lines (occurs post-zygotically).





Numerical changes

- Aneuploidy
 - Hypo ploidy
 - Hyper ploidy
- Euploidy
 - Monoploidy
 - Diploidy
 - Polyploidy

Structural

- Translocations
- Deletions
- Duplications
- Inversions

Different cell lines

- Mosaicism

107 Karyotyping- international description

Karyotyping- Nomenclature

Karyotype A normal male chromosome pattern would be described as: 46, XY.

- 46 = total number of chromosomes
- XY = sex chromosome constitution

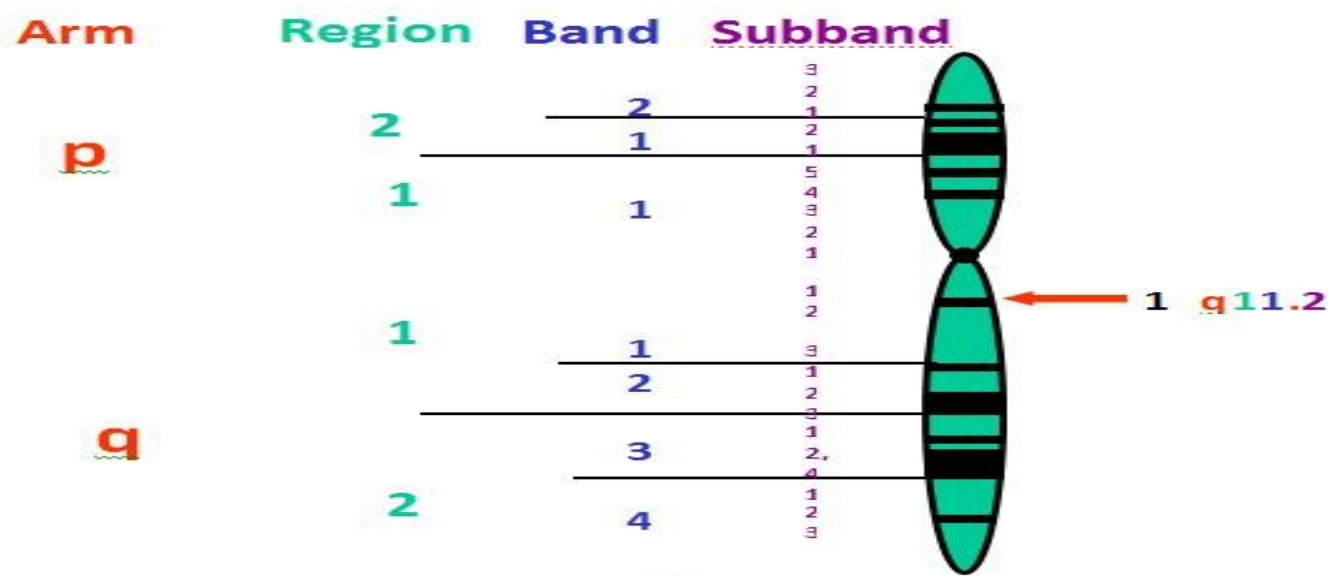
Description of anomaly any further description would refer to any abnormalities or variants found.

- 47,XX,+21
- 47,XXX
- 69,XXY
- 45,XX,der(13;14)(q10;q10)
- 46,XY,t(2;4)(p12;q12)
- 46,XX,del(5)(p25)
- 46,XX,dup(2)(p13p22)
- 46,XY,inv(11)(p15q14)
- 46,XY, fra(X)(q27.3)
- 46,XY/47,XXY

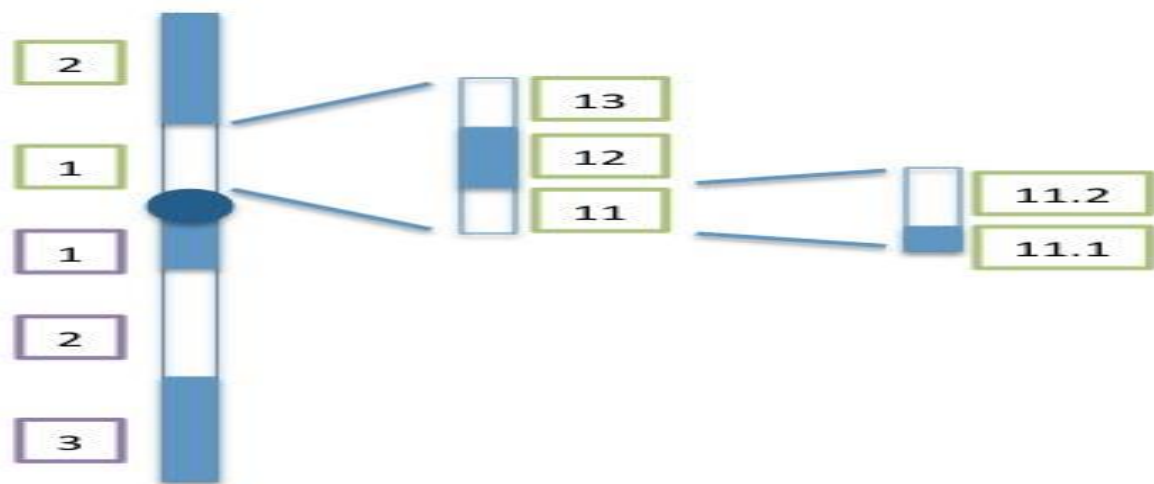
- 46,XY
- 47,XX,+21 Trisomy 21 (Down syndrome)
- 47,XXX Triple X syndrome
- 69,XXY Triploidy
- 45,XX,der(13;14)(p11;q11) Robertsonian translocation
- 46,XY,t(2;4)(p12;q12) Reciprocal translocation
- 46,XX,del(5)(p25) Deletion tip of chromosome 5
- 46,XX,dup(2)(p13p22) Duplication of part of short arm Chr 2
- 46,XY,inv(11)(p15q14) Pericentric inversion chromosome 11
- 46,XY, fra(X)(q27.3) Fragile X syndrome
- 46,XY/47,XXY Mosaicism normal/Klinefelter syndrome

108 Defining Chromosome Location

Defining chromosome regions

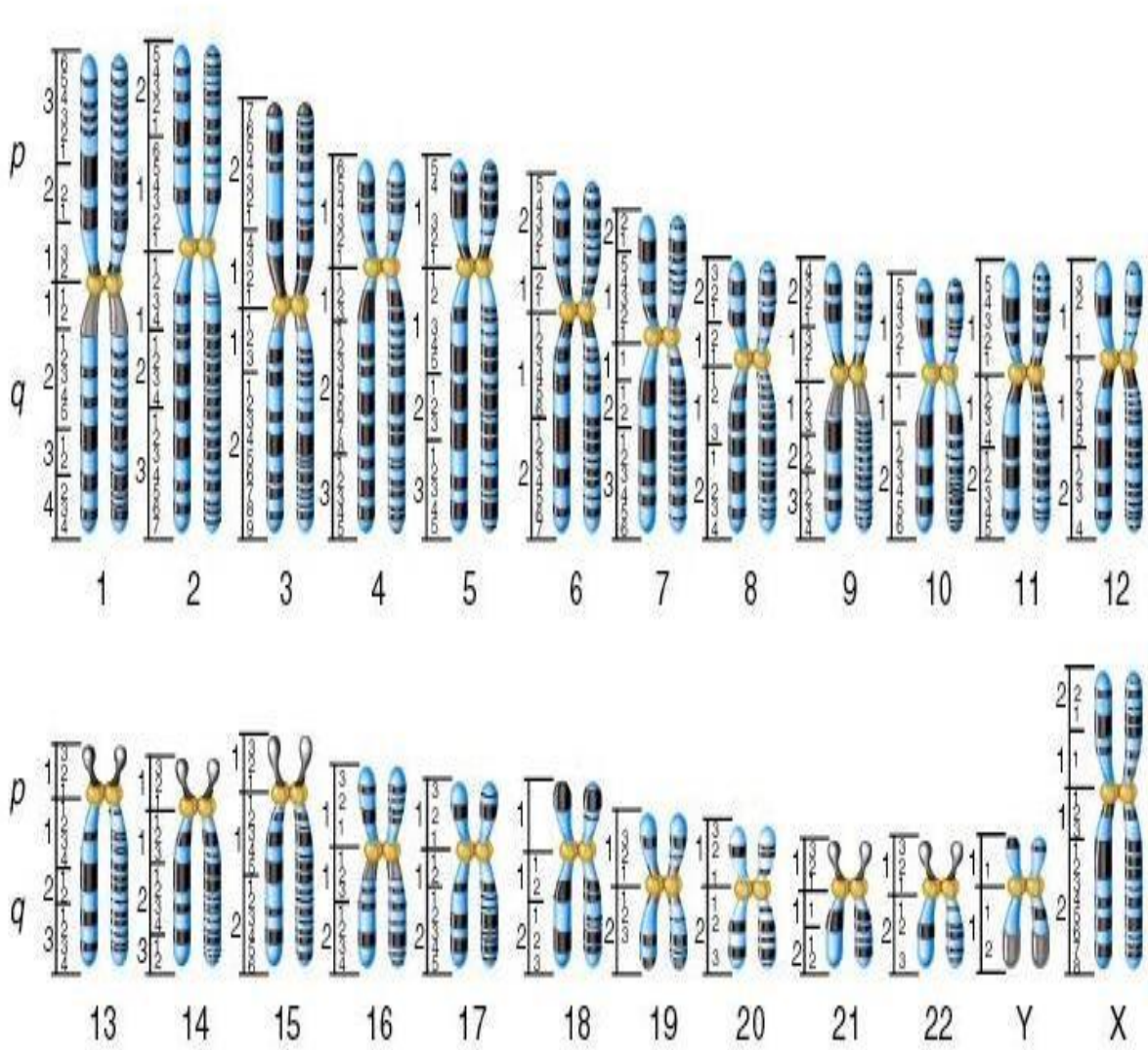


Bands within bands



Chromosome identification Microscopic examination of chromosomes - Karyotype To identify and classify

- 1. Size
- 2. Location of the centromere
- 3. Banding patterns



109 Common Numerical Abnormalities

Common numerical abnormalities

- Down syndrome (trisomy 21: 47,XX,+21)
- Edwards syndrome (trisomy 18: 47,XX,+18)
- Patau syndrome (trisomy 13: 47,XX+13)

Sex chromosome abnormalities

Turner syndrome 45,X

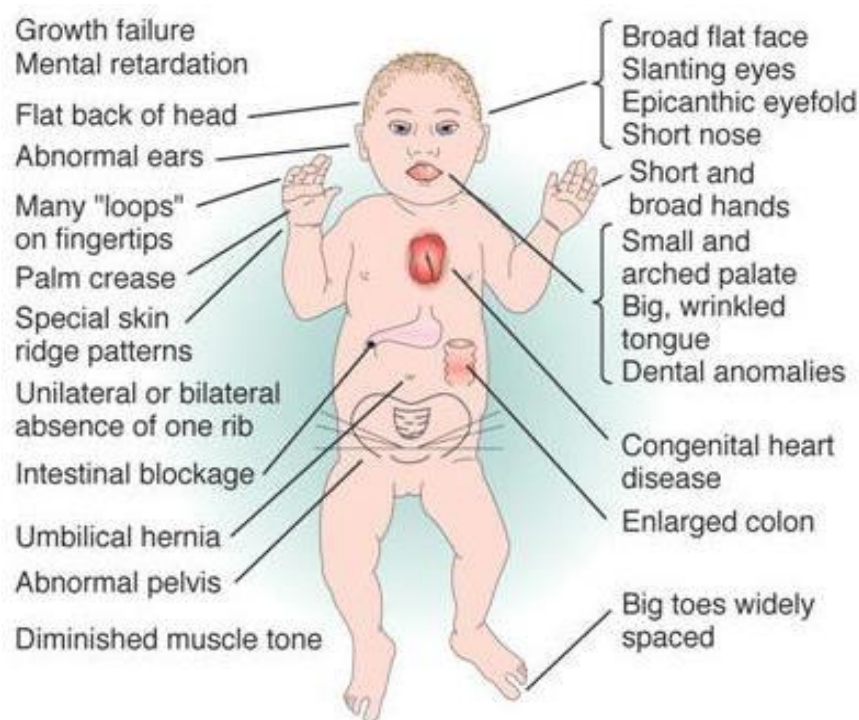
Klinefelter syndrome 47,XXY

All chromosomes abnormalities

Triploidy (69 chromosomes)

Down syndrome this is a Trisomy 21 (47+21). The frequency of trisomy increases with increasing maternal age.

Sometimes, Robertsonian translocation involving chromosome 21 3-4 % not related to maternal age.



Down syndrome clinical features

• Head and neck

Brachycephaly

Epicanthal folds

- Brushfield spots
- Flat nasal bridge
- Folded ears
- Open mouth
- Protruding tongue
- Short ne

Extremities

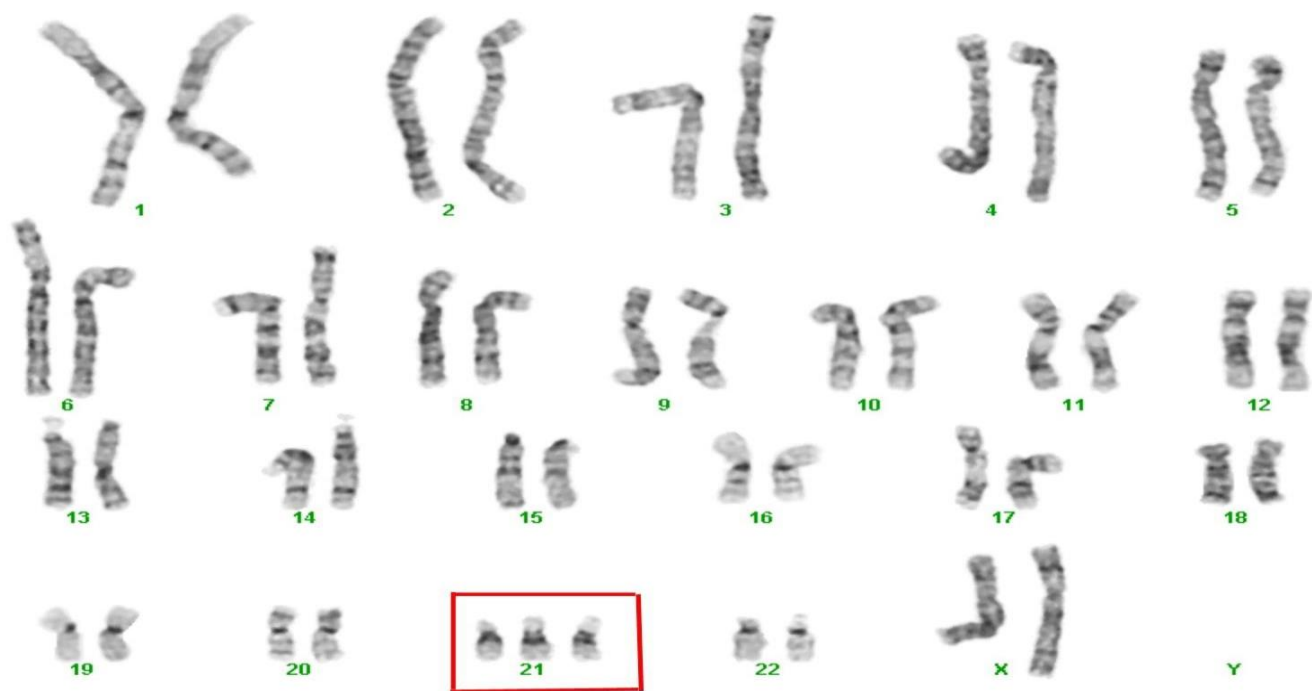
Short fifth finger

Short broad hands

Incurved fifth finger

Space between first and second toe

Hyper flexibility of joints

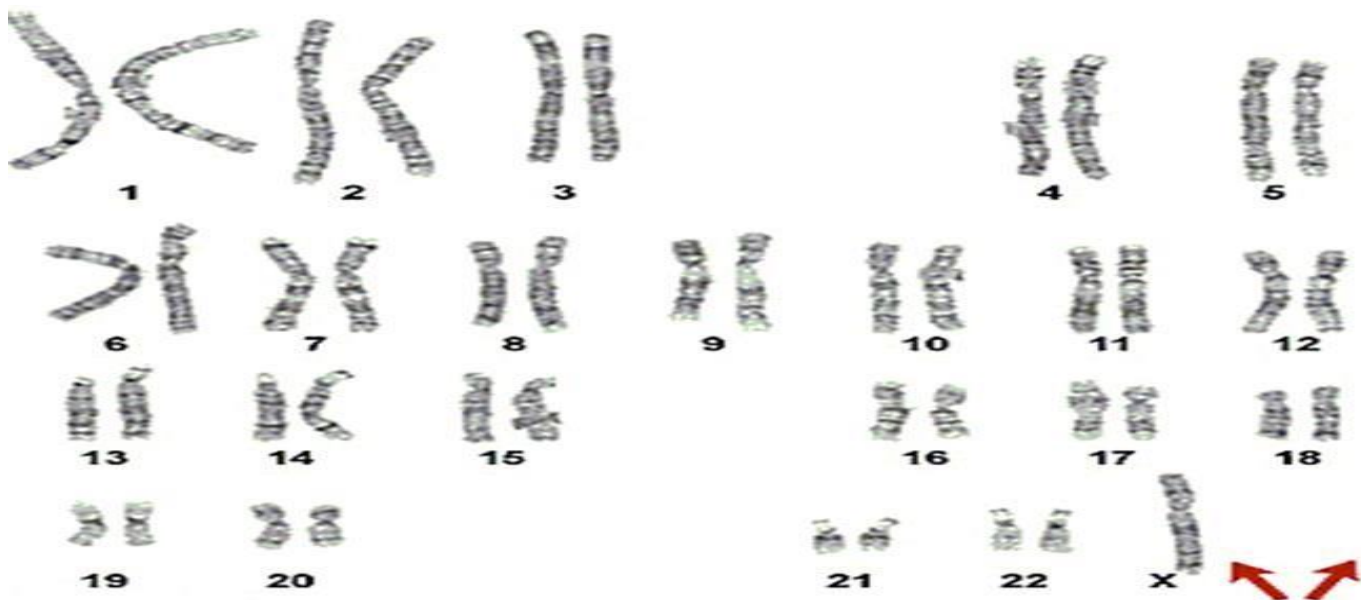
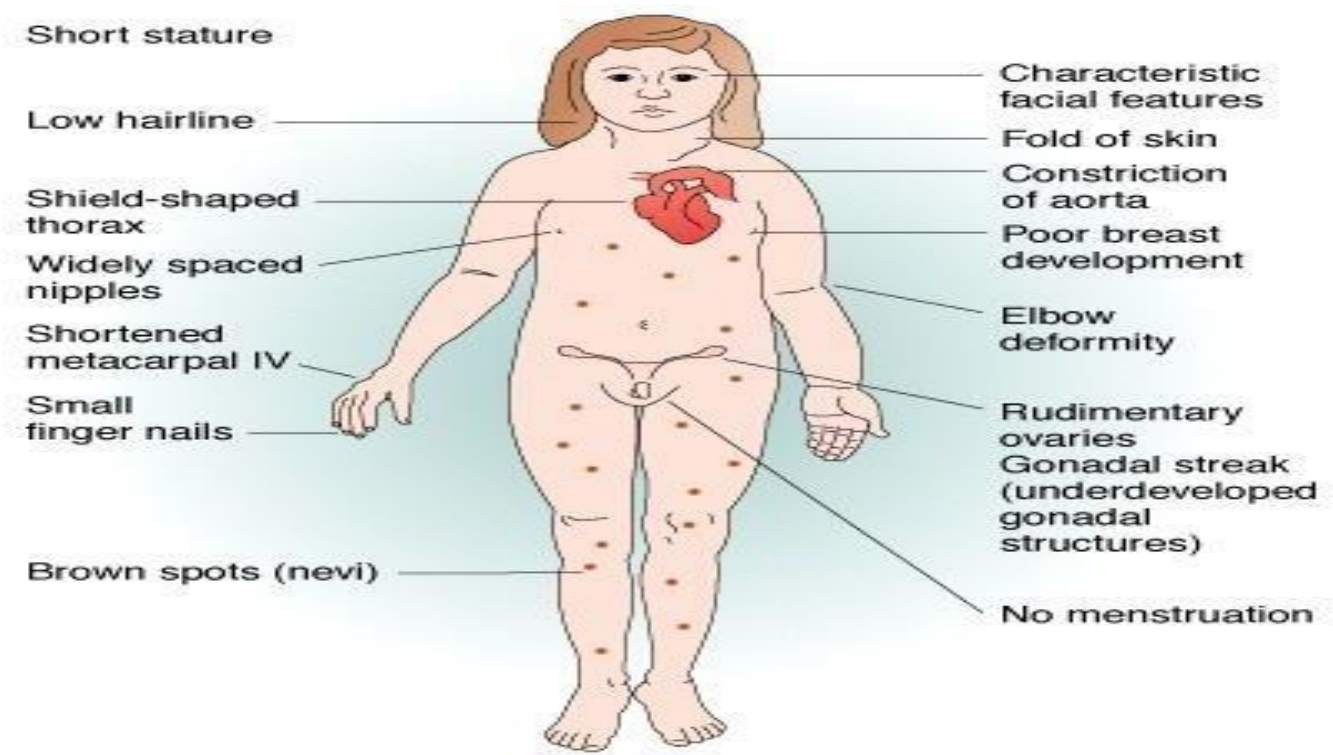


Turner syndrome relatively common disorder caused by the loss of genetic material from one of the sex chromosomes.

Affects only females

Turner syndrome - clinical features

- Short stature (143-145cm tall)
- Loss of ovarian function
- Hormone imbalances(thyroid, diabetes)
- Stress and emotional deprivation
- Diseases affecting the kidneys, heart, lungs or intestines
- Bone diseases
- Learning problems(esp. in math)



Although it is a genetic disorder, it is not usually hereditary.

There are no known environmental causes of the syndrome

Women with Turner Syndrome are usually infertile .

Triploidy- 69 chromosomes

Numerical abnormalities

- Due to chromosomes numbers.



110 Structural Abnormalities

Structural Abnormalities

Chromosome breakage & subsequent reunion in a different configuration

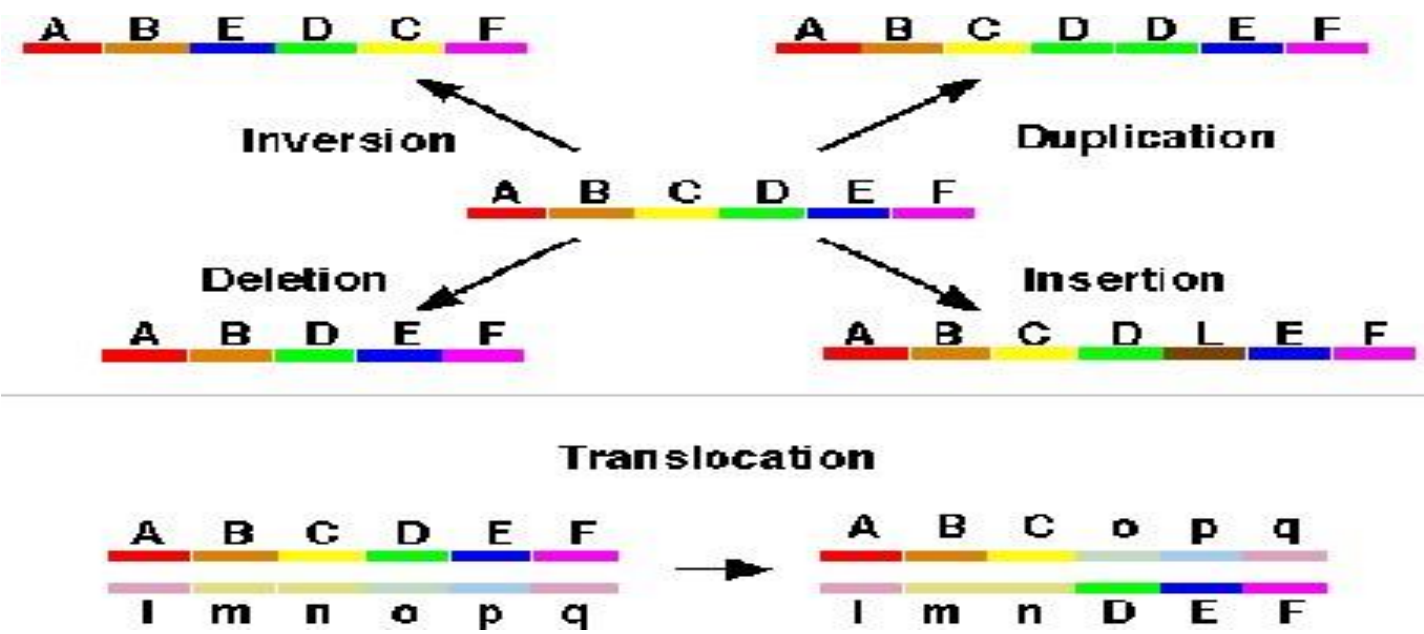
Types based on genetic material

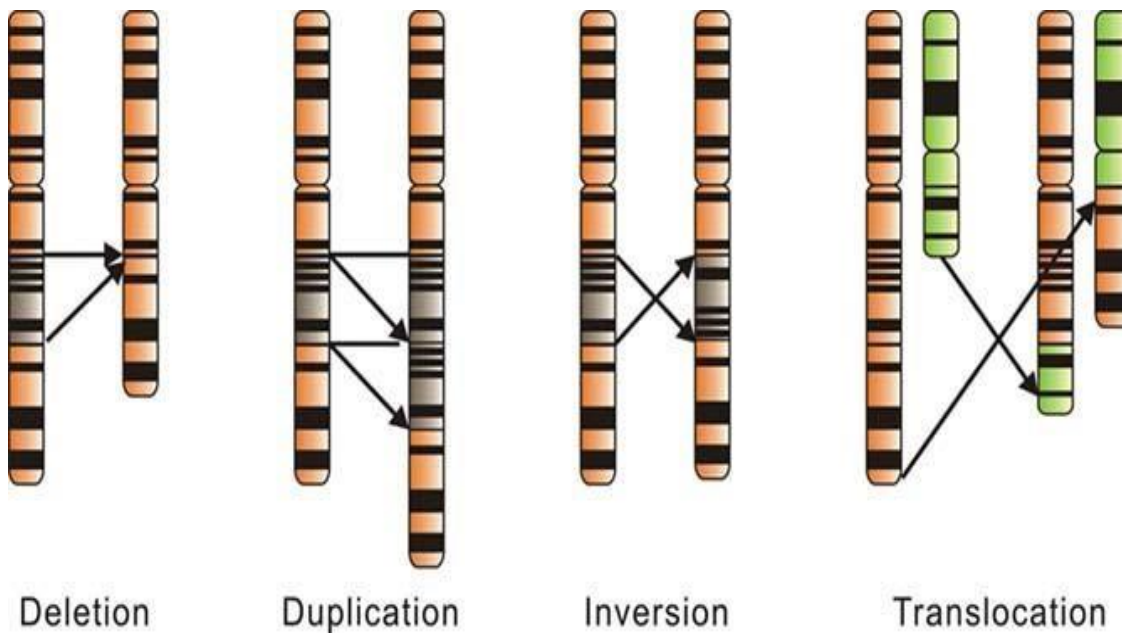
- Balanced – chromosome complement is complete.
- Unbalanced – when there is incorrect amount of genetic material.

Types

- Translocations
- Deletions
- Insertions □ Inversions
- Ring chromosome
- Isochromosomes

Types of structural abnormalities





111 Translocations

Translocations

Definition Transfer of genetic material from one chromosome to another. There are two types of translocations

- Reciprocal translocation
- Robertsonian translocation

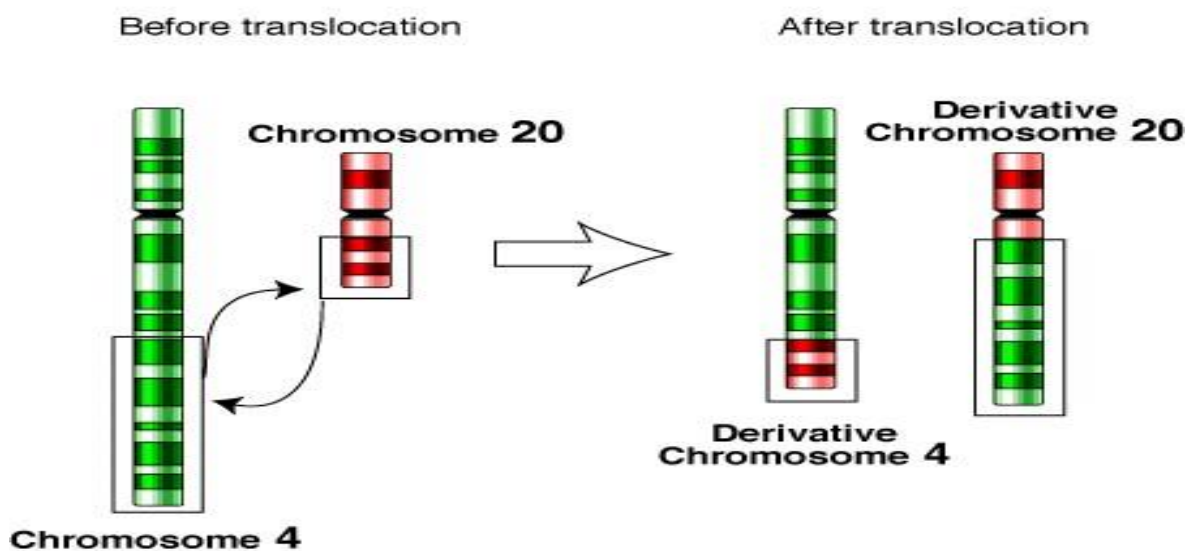
Balanced and unbalanced where no genetic information extra or missing genes.

Unbalanced translocations where exchange of genetic information is extra or missing some of the genes.

Reciprocal an exchange of material between two different chromosomes is called a reciprocal translocation. When this exchange involves no loss or gain of chromosomal material.

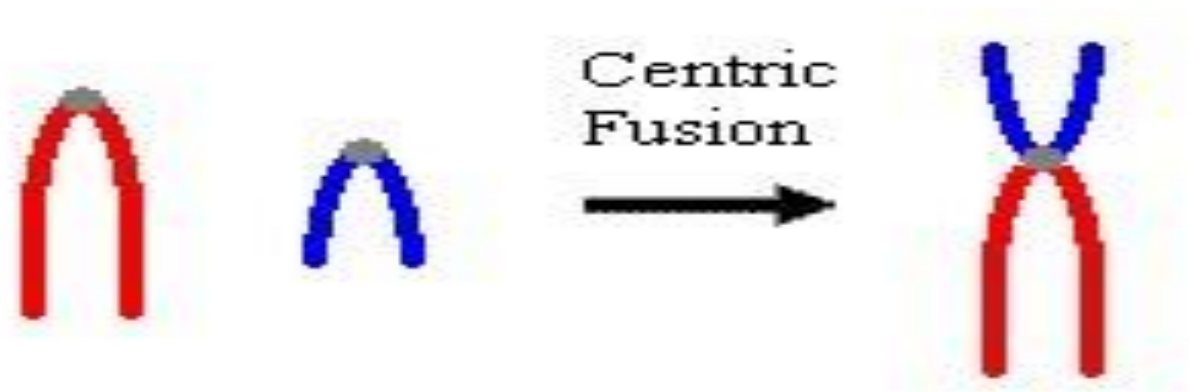
Reciprocal in non-homologous chromosomes Reciprocal translocations are usually an exchange of material between non-homologous chromosomes. One in each of 600 births in humans.

Reciprocal translocations

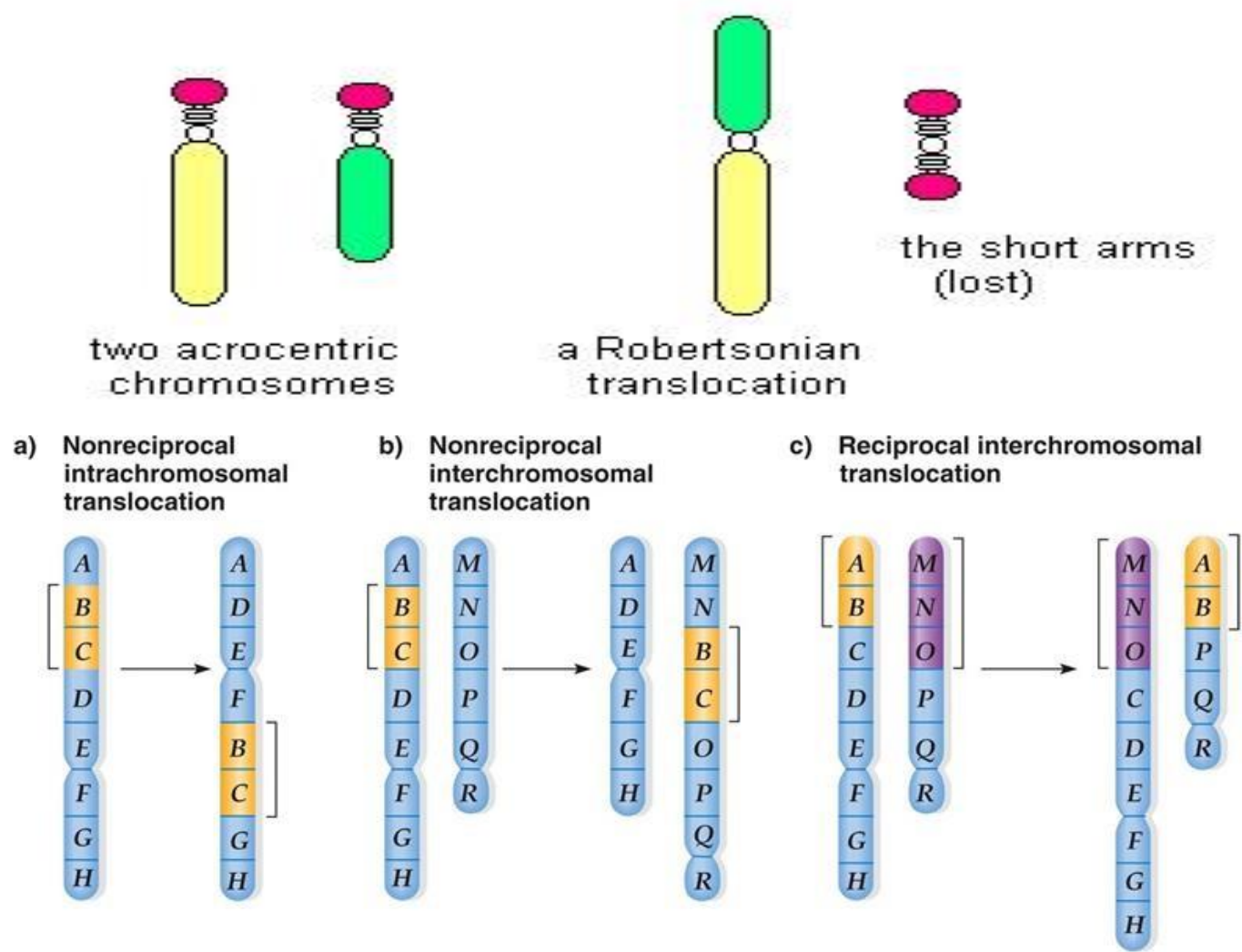


Robertsonian Translocations

Breaks occur at the extreme ends of the short arms of two non-homologous acrocentric chromosomes. The small acentric fragments are lost. The larger fragments fuse at their centromere regions to form a single chromosome. The most common translocation in humans involves chromosomes 13 and 14 and is seen in about 0.97 / 1000 newborns.



Types of translocation

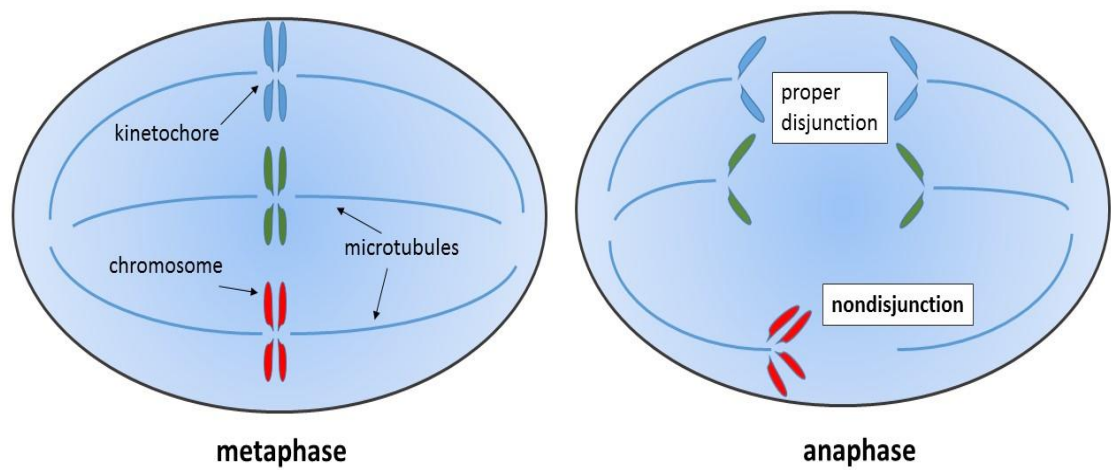


Common human diseases caused by translocation

- Cancer
- Infertility
- Down syndrome
- Leukemia
- XX male syndrome

Non-disjunction:

When two homologous chromosomes or sister chromatids fail to separate during cell division, such a phenomenon is called as Non-disjunction



Types of Non-disjunction

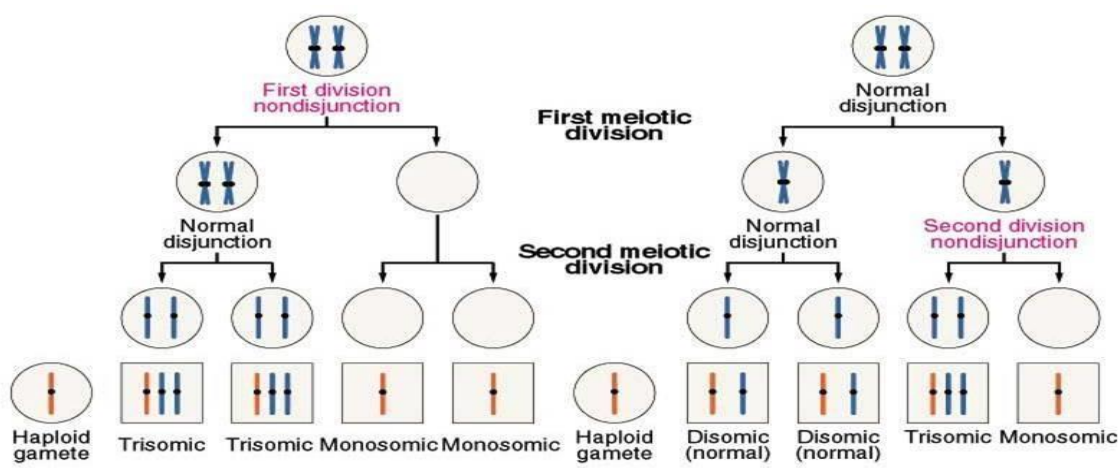
- Meiotic non-disjunction
- Mitotic non-disjunction

Meiotic Non-disjunction If non-disjunction occurs during 1st meiotic division then all the sperms/ova derived from primary spermatocyte/oocytes will be abnormal.

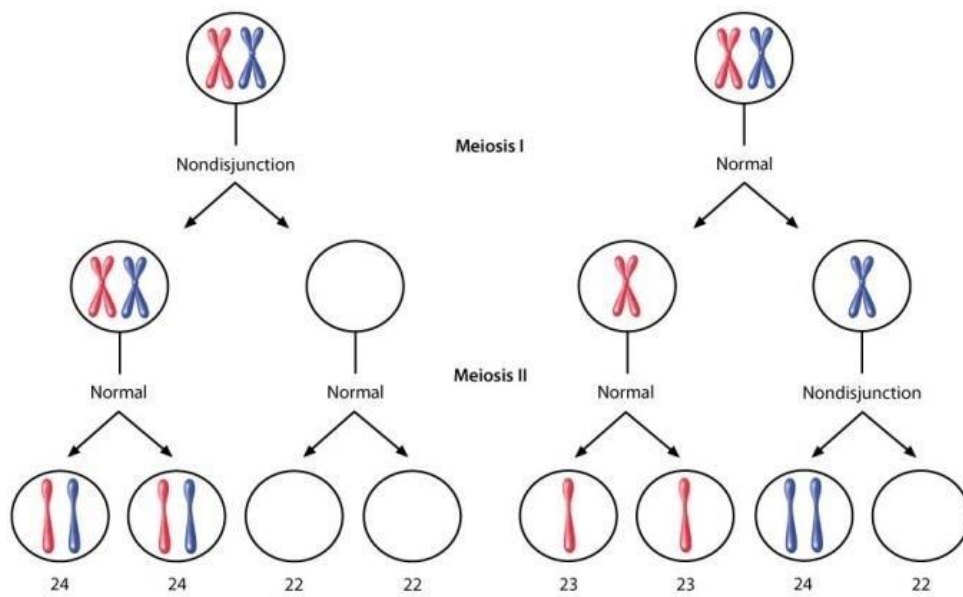
Failure of chromosomes separation

- Failure of a pair of homologous chromosomes to separate in meiosis I.
- Failure of sister chromatids to separate during meiosis II.
- Failure of sister chromatids to separate during mitosis.

Mitotic Non-disjunction Mitotic non-disjunction results in cells with different number of chromosomes. Breaking of the spindle fibers during metaphase or anaphase.If spindle fibers do not form for one of the chromosomes, then during anaphase it will not separate into two chromatids and one cell will have an extra chromatid and the other will be lacking one. Mitotic nondisjunction causes trisomic and monosomic daughter cells to be formed.



Cell with 46 chromosomes



Causes of non-disjunction

- Aging effect on primary oocyte
- Radiation
- Delayed fertilization after ovulation

113 Trisomy

Trisomy is a type of polysomy in which there are three copies of a particular chromosome, instead of the normal two. Trisomy is a type of aneuploidy (an abnormal number of chromosomes).

Trisomy -Autosomes and sex chromosome

- Autosomal trisomy
- Sex-chromosome trisomy
- Trisomy of chromosome 21, which is found in Down syndrome, is called trisomy 21.

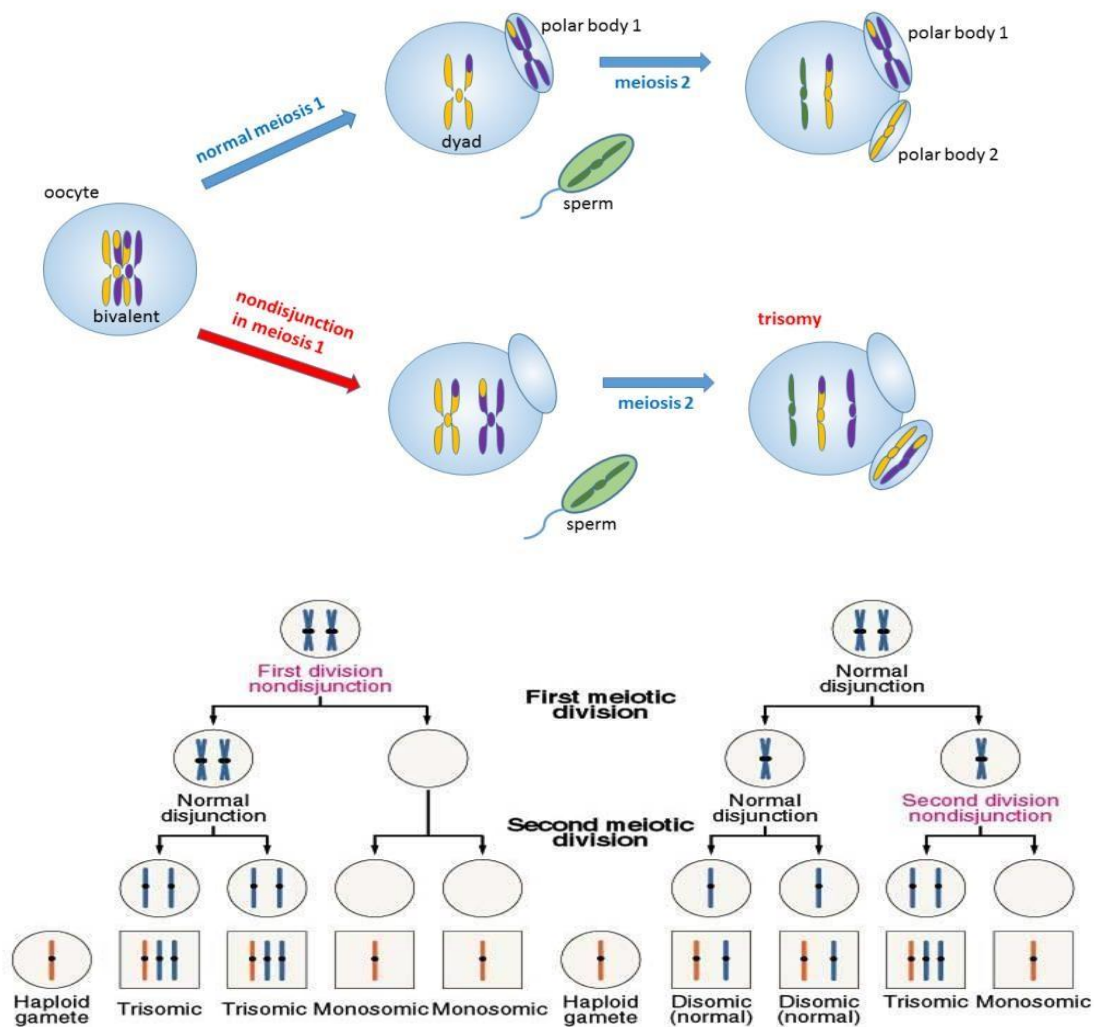
Trisomy Types

- Primary trisomy - an entire extra chromosome has been copied.
- Partial trisomy - an extra copy of part of a chromosome.
- Secondary trisomy - the extra chromosome has duplicated arms.
- Tertiary trisomy - extra chromosome is made up of copies of arms from two other chromosomes.

Common trisomy in humans

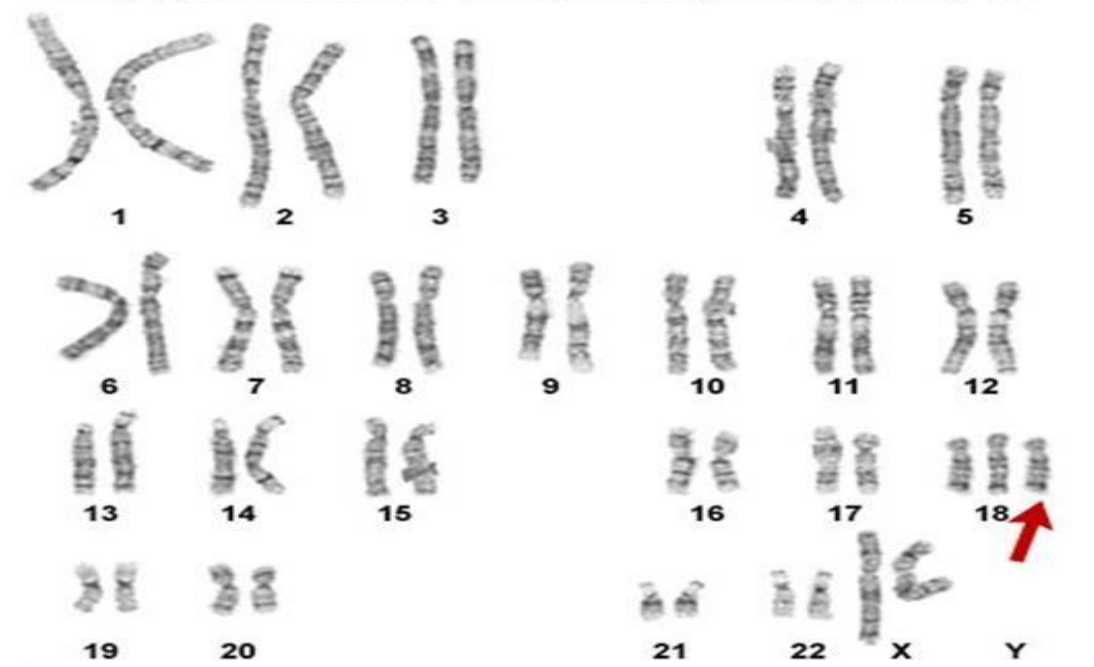
- Trisomy 21
- Trisomy 18
- Trisomy 13
- Klinefelter's syndrome (47 XXY)

Trisomy 21 Down syndrome Trisomy 21 1 in 700 live births Associated with increased maternal age

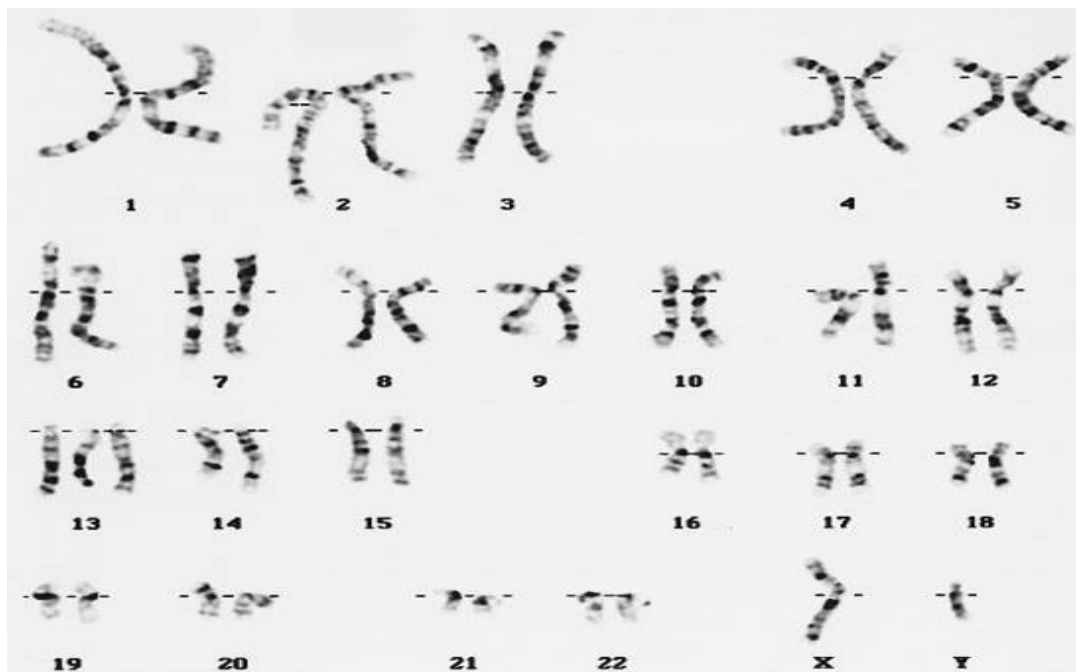


Trisomy 18 Edwards s' syndrome

Karyotype from a female with Edwards syndrome (47,XX,+18)



Trisomy 13 Patau s' syndrome



Trisomy is a type of polysomy in which there are three copies of a particular chromosome, instead of the normal two.

114 Monosomy

Monosomy: Loss of a single chromosome

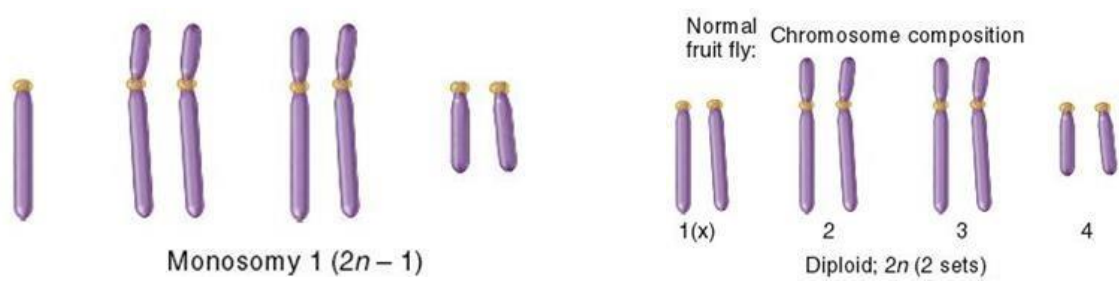
Monosomy of autosomes is lethal

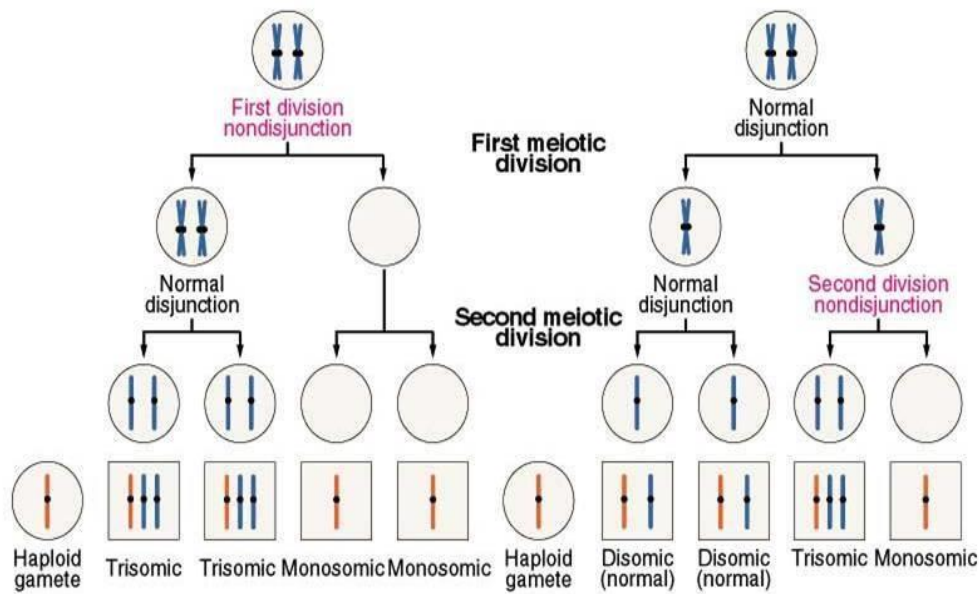
Turner syndrome XO i.e. loss of sex chromosome.

Causes of monosomy: followings are the causes of monosomy

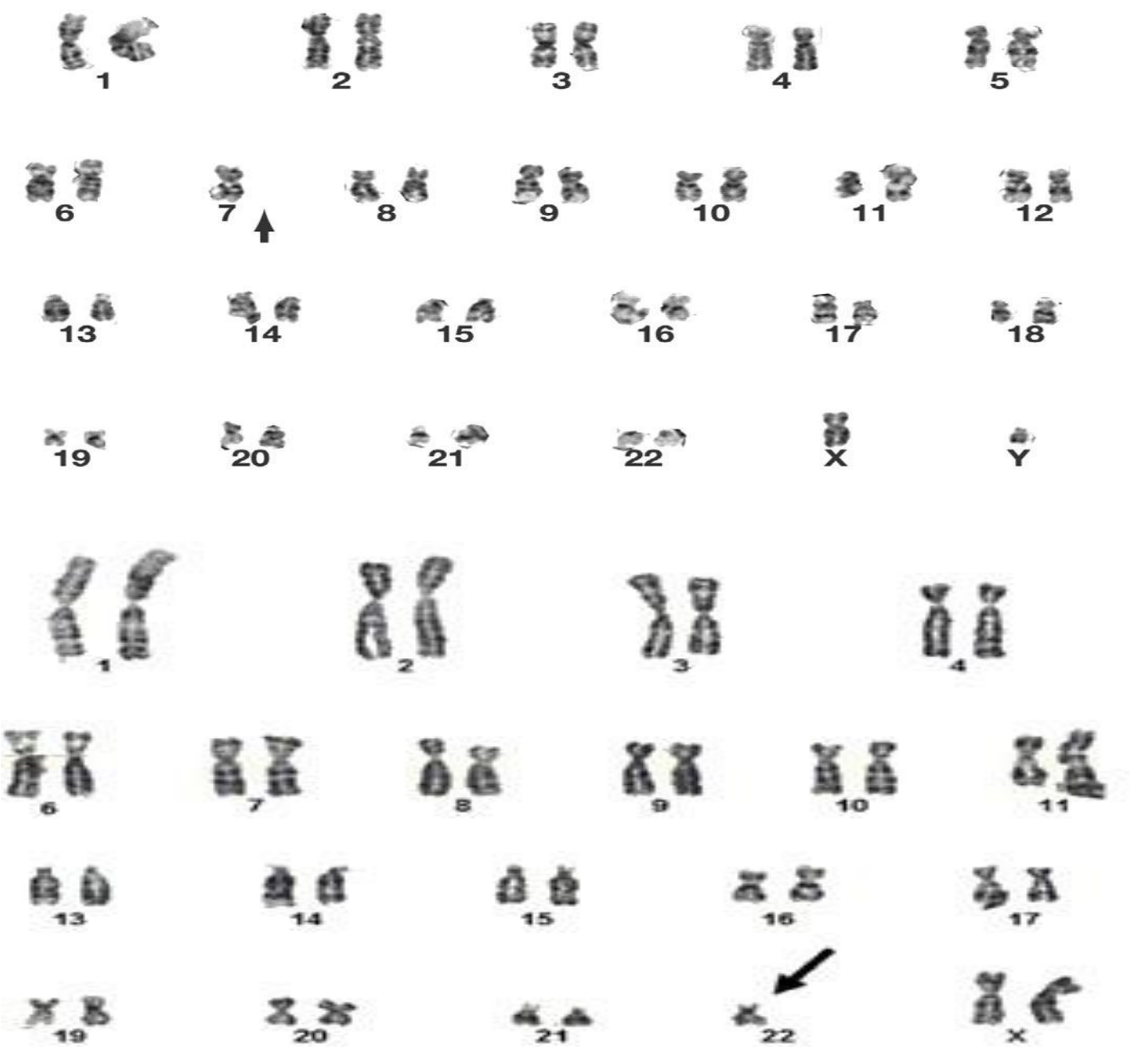
- Non disjunction – one gamete receives two copies of homologous chromosomes and other will have no copy.
- Loss of chromosome. As it move towards pole of cell during anaphase.

Fruit fly





Monosomy – Chromosome 7



Monosomy – chromosome 22

Diseases due to Monosomy

- Turner syndrome
- Cri du chat syndrome

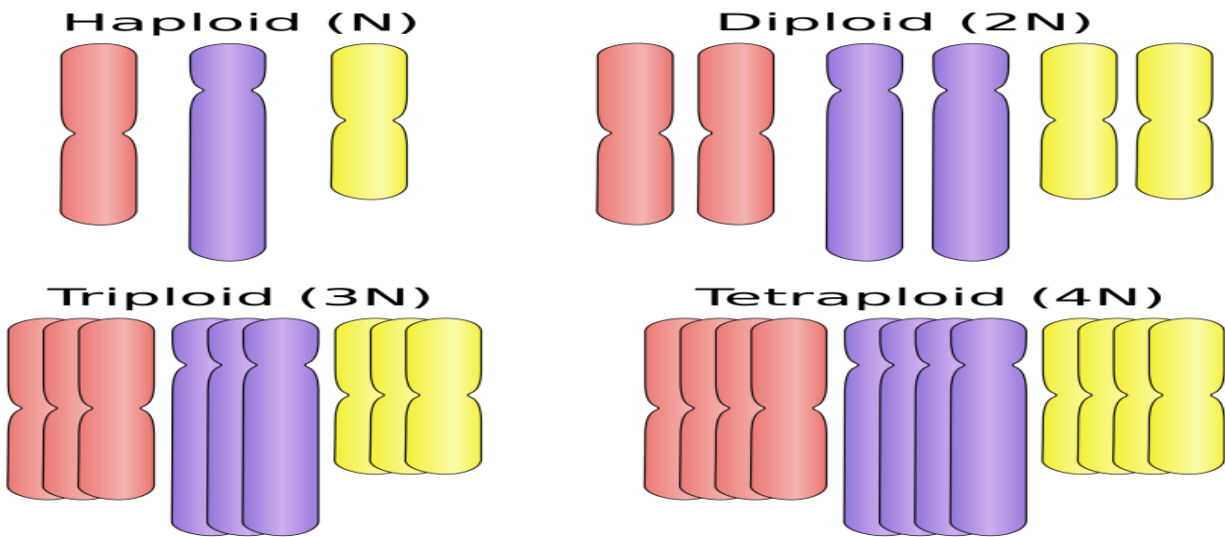
Polyploidy: Multiples of haploid number

- Triploidy or tetraploidy etc
- Fetus does not survive

Two major types

- Autopolyploidy
- Allopolyploidy

Types of polyploidy



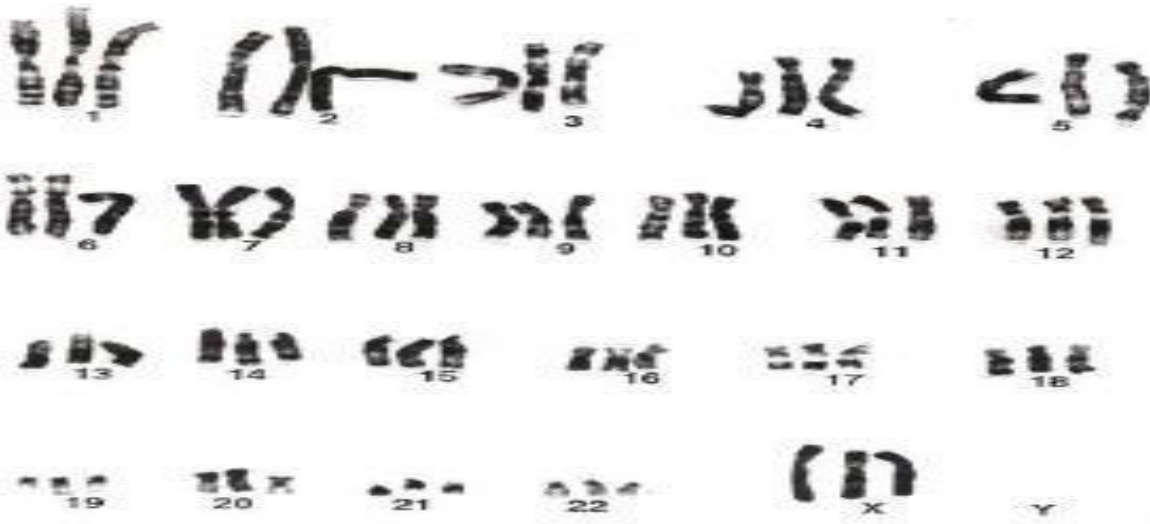
- Triploid (3x) watermelon
- Tetraploid (4x) - cotton
- Pentaploid (5x) - Kenai Birch
- Hexaploid (6x) - wheat



Causes of polyploidy

- Retention of polar body.
- Formation of diploid sperms.
- Di-spermy – fertilization by two sperms.

Triploidy



Polyploidy in Plants

- Triploid: apple, banana, citrus, ginger, watermelon
- Tetraploid: cotton, potato, tobacco, peanut
- Hexaploid: oat, kiwifruit
- Octaploid: strawberry
- Decaploid: some sugar cane hybrids

116 Allopolyploidy

Allopolyploidy

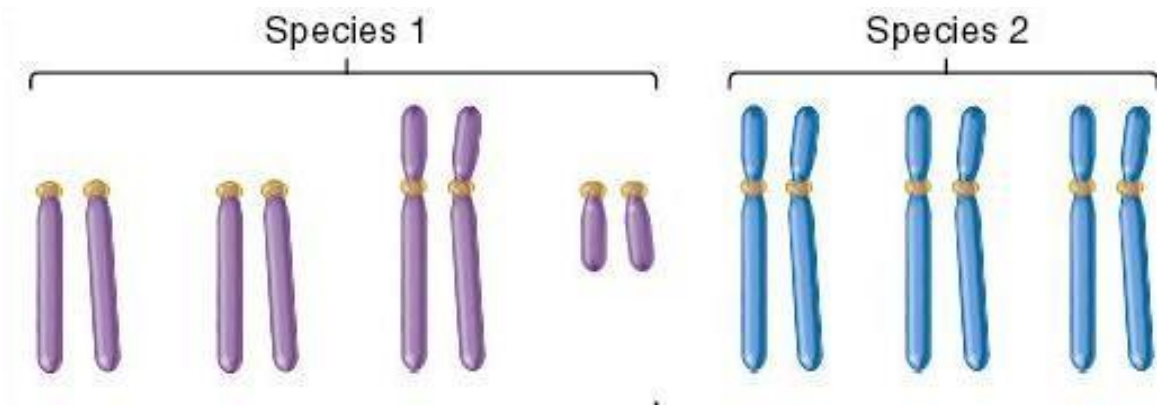
Types of Polyploidy:

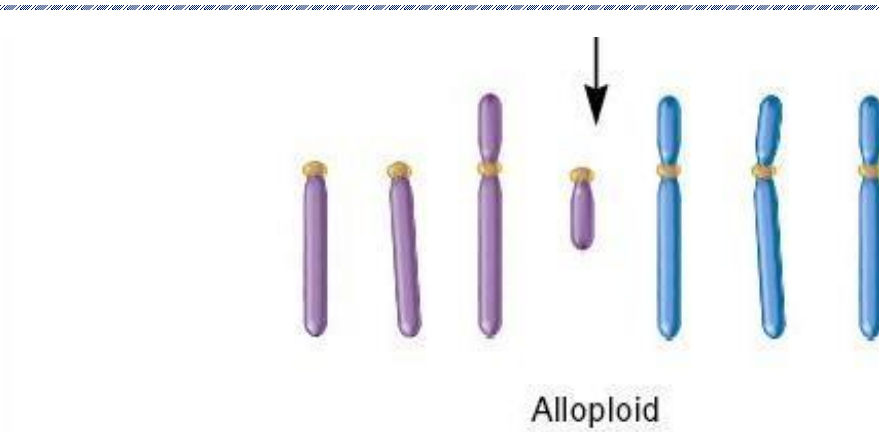
- Autopolyploidy
- Allopolyploidy

Autopolyploidy : Autopolyploids are polyploids with multiple chromosome sets derived from a single species.

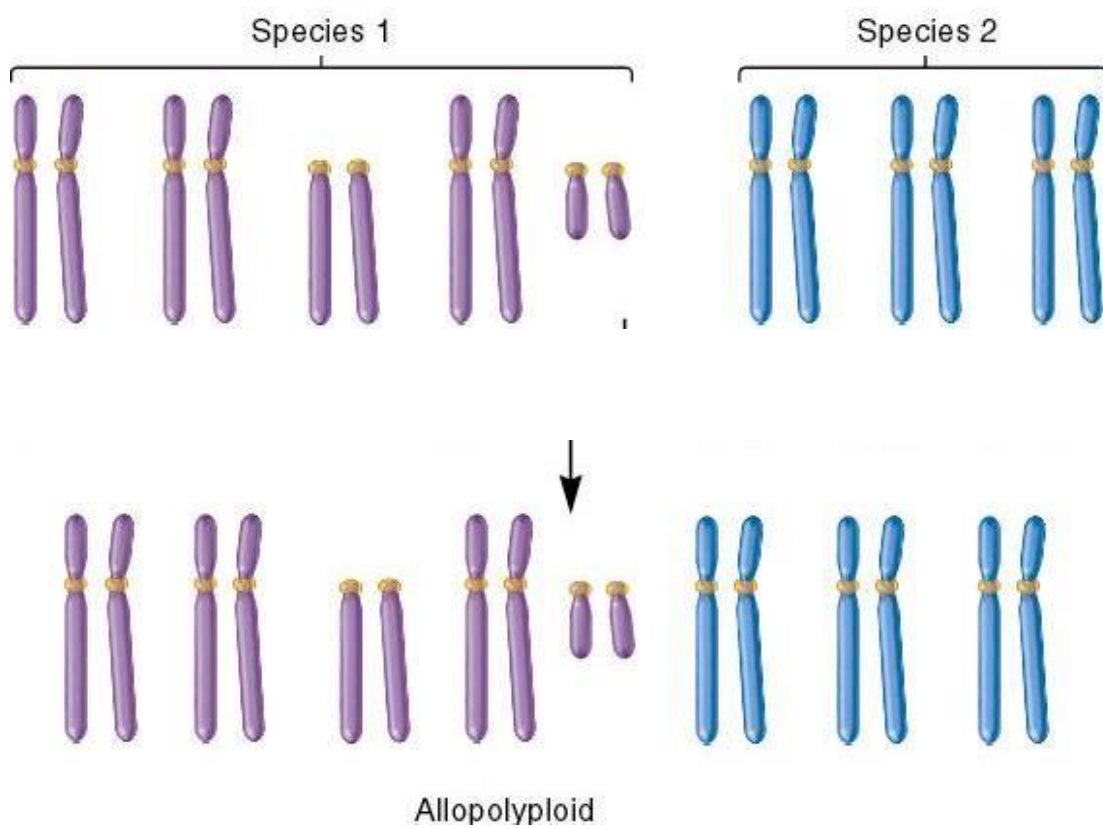
Allopolyploidy: Allopolyploids are polyploids with chromosomes derived from different species. Allopolyploidy is inter-species cross Interspecies crosses can generate allopolyploids. Offspring are generally sterile.

Allopolyploid



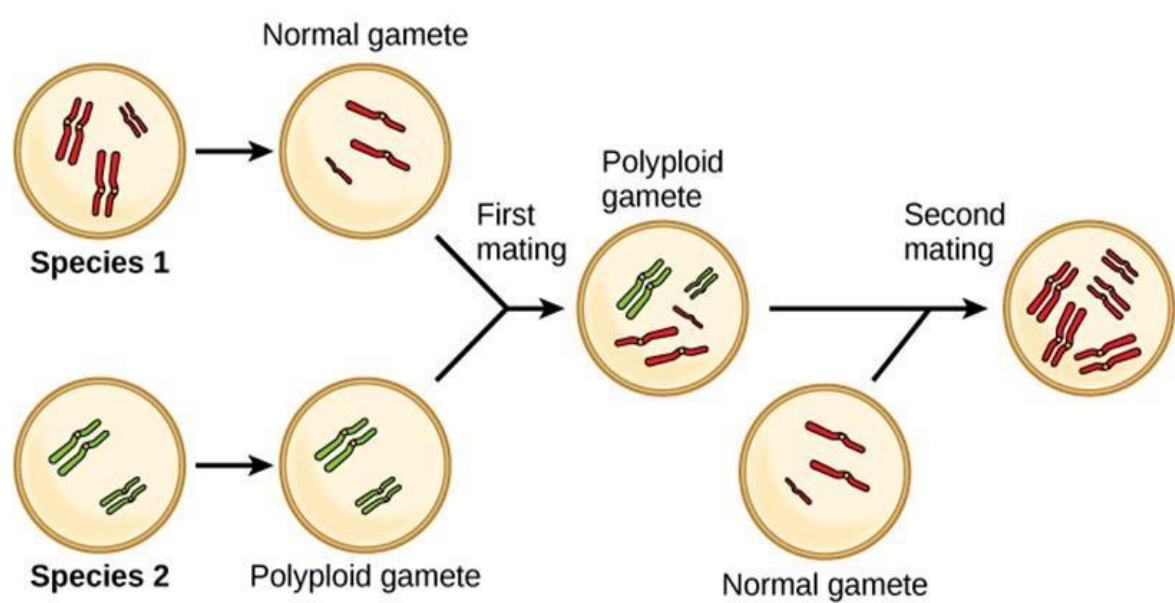


Inter-species cross can results in Allopolyploid



Colchicine to promote polyploidy: Polyploidy and allopolyploid plants often exhibit desirable traits. Colchicine is used to promote polyploidy Colchicine binds to tubulin, disrupting microtubule formation and blocks chromosome segregation.

Allopolyploidy Resulting from Viable Matings between Two Species



Allopolyploidy

An interspecies cross results in allopolyploidy.

Mother donkey

+

Father horse:

Offspring is a hinny (sterile)

Domestic mare

+

Father zebra:

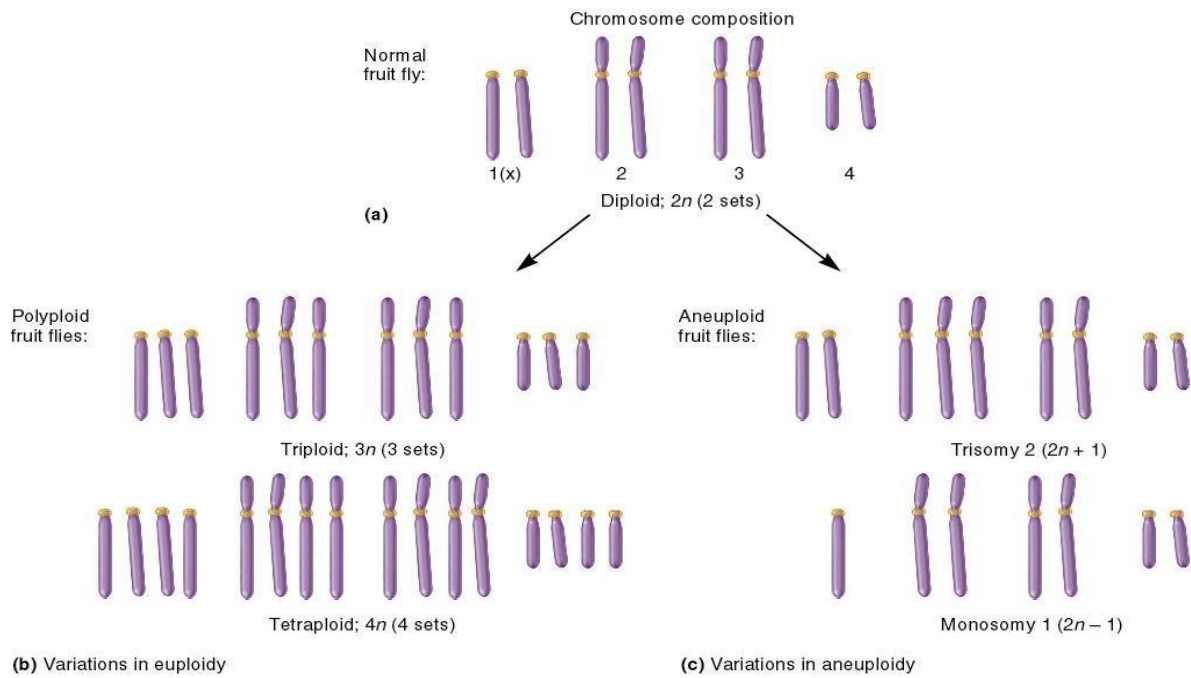
Offspring is a zebra hinny (sterile)



117 Polyploidy and Aneuploidy

Aneuploidy: Change in chromosomes number can occur by the addition/deletion of chromosome or part of a chromosome.

Euploidy: Gain of one or more complete sets of chromosomes



Aneuploidy

- Nullisomy
- Monosomy
- Trisomy
- Tetrasomy

Euploidy

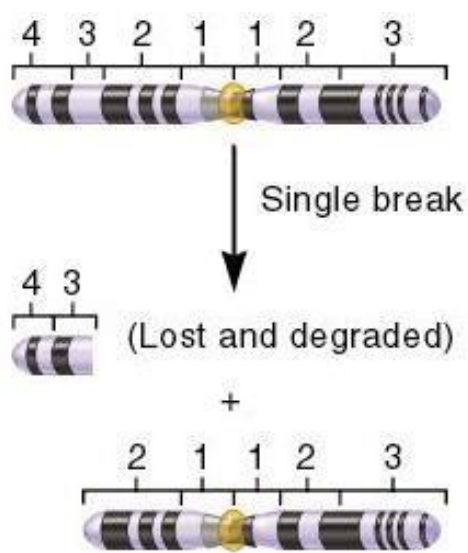
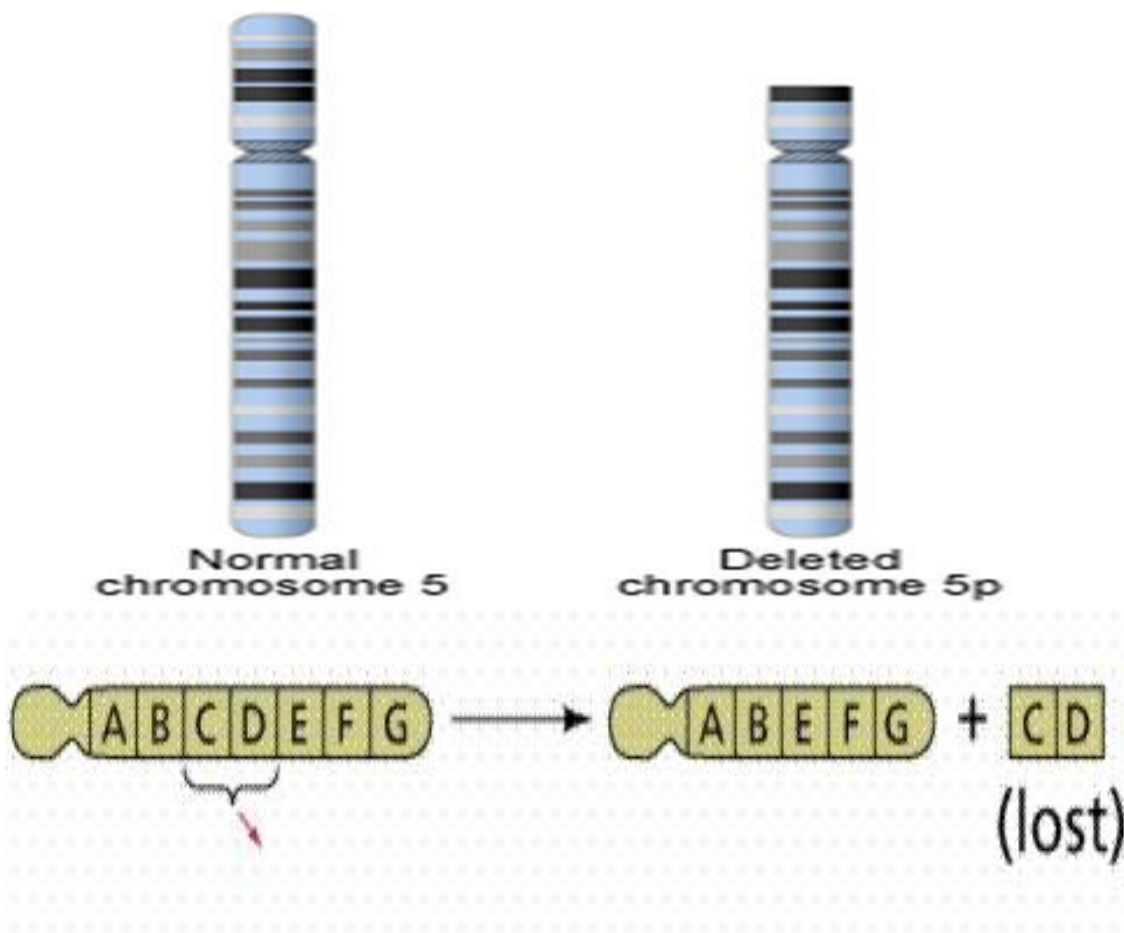
- Triploid
- Tetraploid
- Pentaploid

Comparison

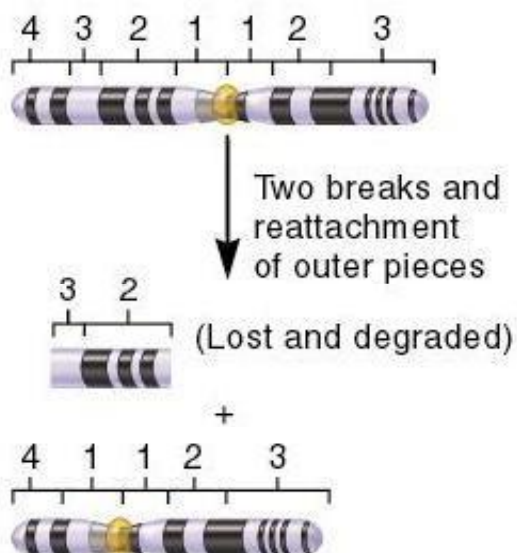
- Aneuploidy:** change in chromosomes number.
- Euploidy:** gain of one or more complete sets of chromosomes

118 Chromosomal Deletions

Chromosomal deletions: Deletions of larger portions are usually incompatible with life. 1015% is due to balanced translocations in one parent. 85-90% are true deletions.



(a) Terminal deficiency



(b) Interstitial deficiency

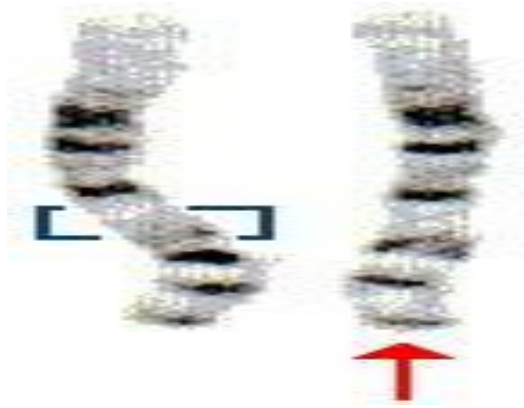
Phenotypic consequences of deficiency depends on

- Size of the deletion
- Functions of genes

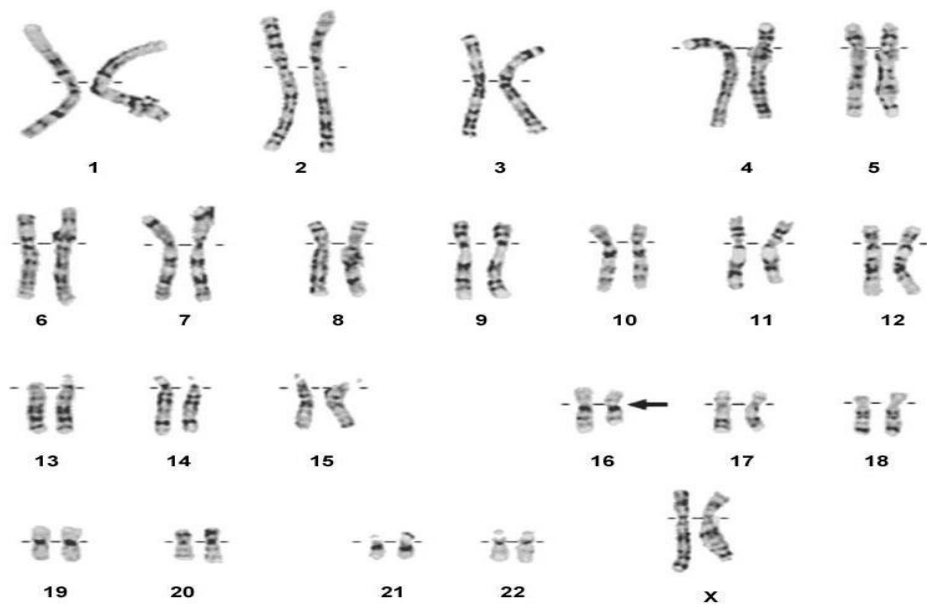
Deleted Phenotypic consequences of deficiency depends on

- Phenotypic effect of deletions usually detrimental.

Deletions – interstitial



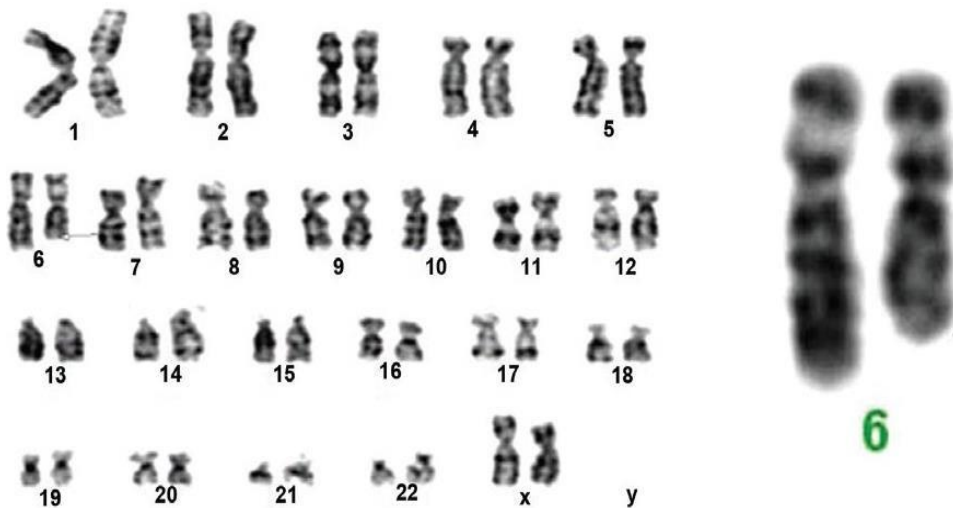
Interstitial deletion at 16



Deletion – terminal in Cri-du-chat Syndrome



Terminal deletion in chromosome 6



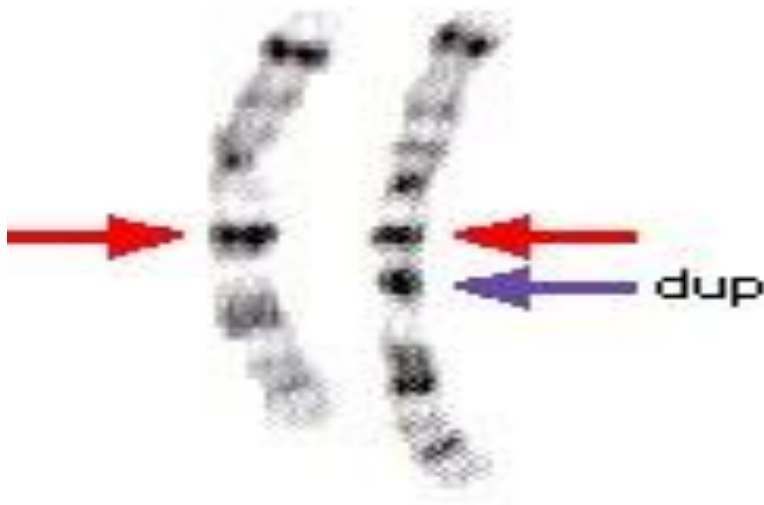
Type of Deletions

- Terminal – from one end
- Interstitial – two breaks and middle part is lost
- Micro deletions

119 Chromosomal Insertions

Chromosomal duplication: Gene duplication (or chromosomal duplication) is a major mechanism through which new genetic material is generated during molecular evolution

Chromosomal Duplications



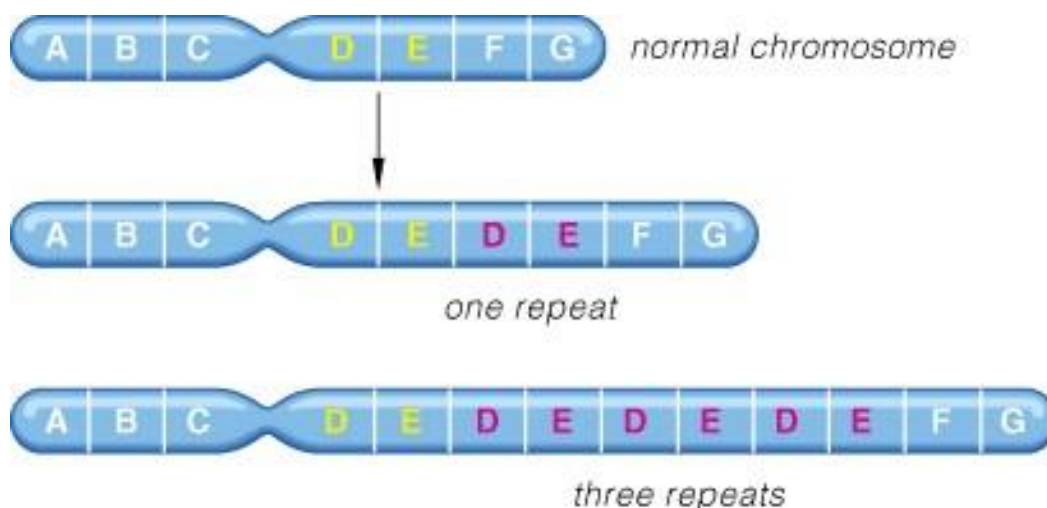
Causes of Duplications – DNA replication: Gene duplications can arise as products of several types of errors in DNA replication and repair machinery. Common sources of gene duplications include ectopic homologous recombination.

Ectopic recombination - misaligned homologous Duplications arise from an event termed unequal crossing-over that occurs during meiosis between misaligned homologous chromosomes.

Other Causes of Duplications Retro-transposition event, aneuploidy, polyploidy, and replication slippage are also cause of duplications.

Replication slippage: Replication slippage is an error in DNA replication that can produce duplications of short genetic sequences. During replication DNA polymerase begins to copy the DNA.

Duplications



Dispersed and tandem duplications: Some duplication is “dispersed”, found in different locations from each other. Tandem duplications found next to each other.

Tandem duplications become pseudo-genes: These extra copies can then mutate to take on altered roles in the cell, or they can become pseudogenes, inactive forms of the gene, by mutation.

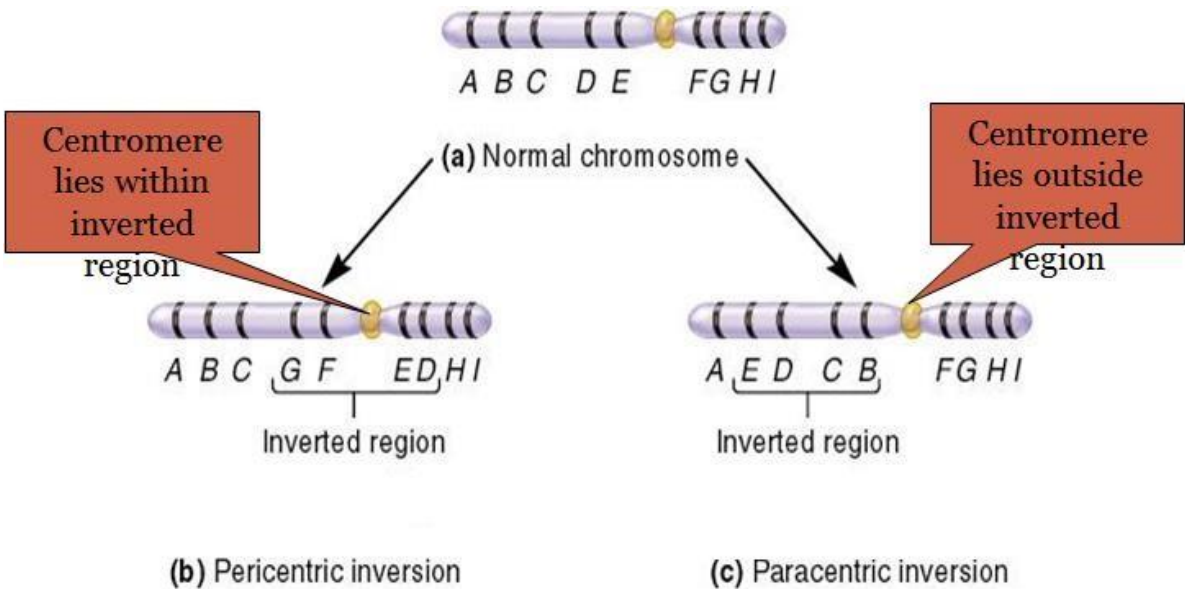
120 Chromosomal Inversions

Chromosomal Inversions

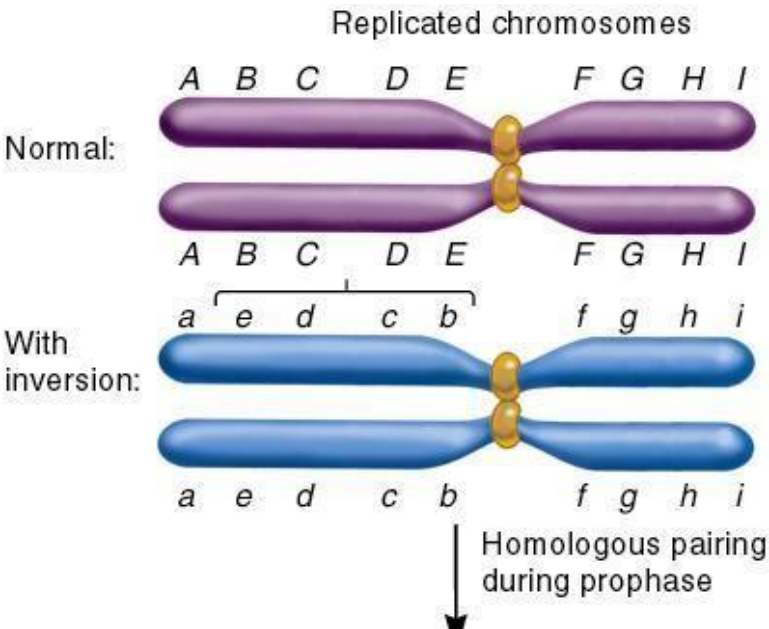
- Two breaks re-arrangement
- Segment is reversed
- Pericentric – when centromere is involved
- Paracentric – only one arm is involved

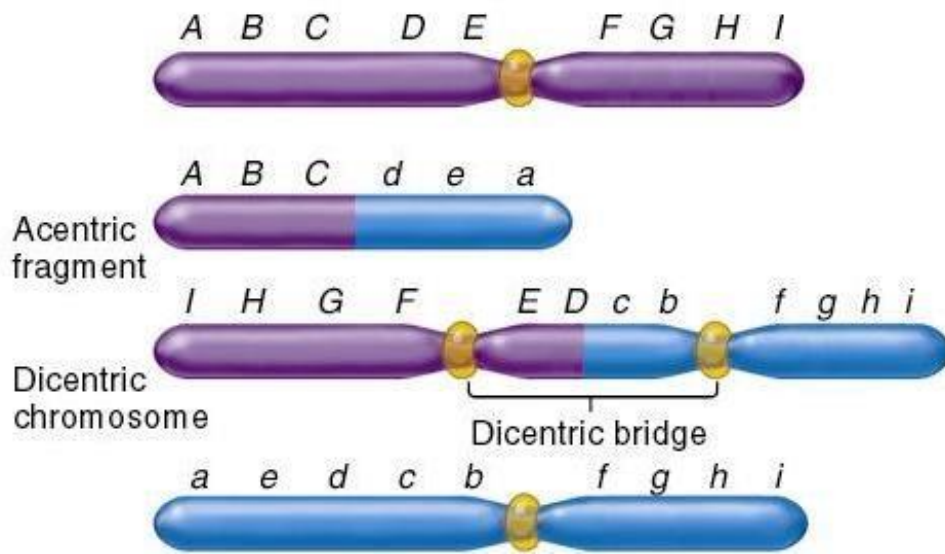
There is no loss of genetic information. Many inversions have no phenotypic consequences. Sometimes break point effects are within regulator or structural portion of a gene.

Chromosomal inversions affects gene expression Gene is re-positioned in a way that alters its gene expression. ~ 2% of the human population carries karyotypically detectable inversions.



Inversion Heterozygotes: Individuals with one copy of a normal chromosome and one copy of an inverted chromosome. Usually phenotypically normal





Chromosomal Inversion: The most common inversion seen in humans is on chromosome 9, at inv (9)(p12q13). This inversion is generally considered to have no harmful effects, but increased risk for miscarriage or infertility.

121 Ring ChromosomeClosed

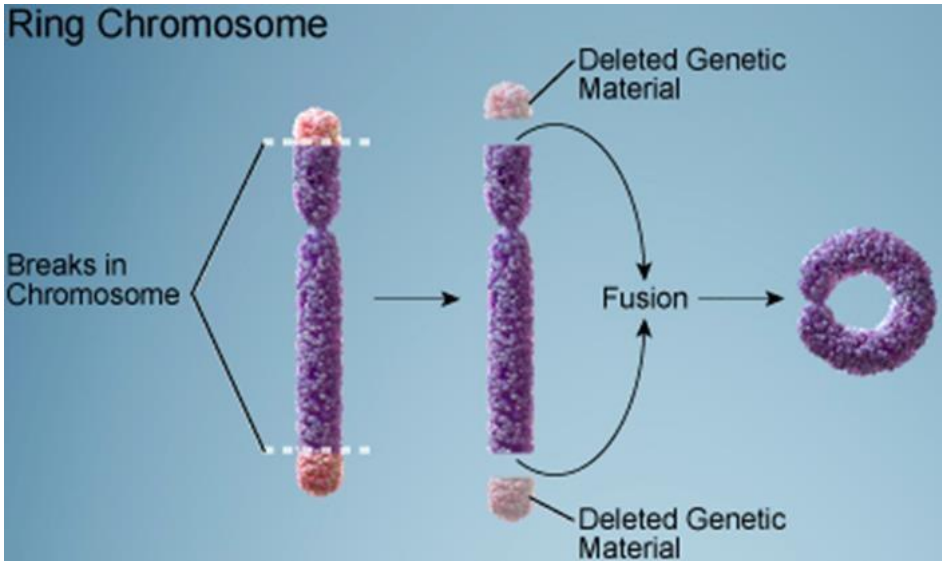
Ring chromosome: A ring chromosome is a chromosome whose arms have fused together to form a ring. A ring chromosome is denoted by the symbol r in human genetics or R in drosophila genetics.

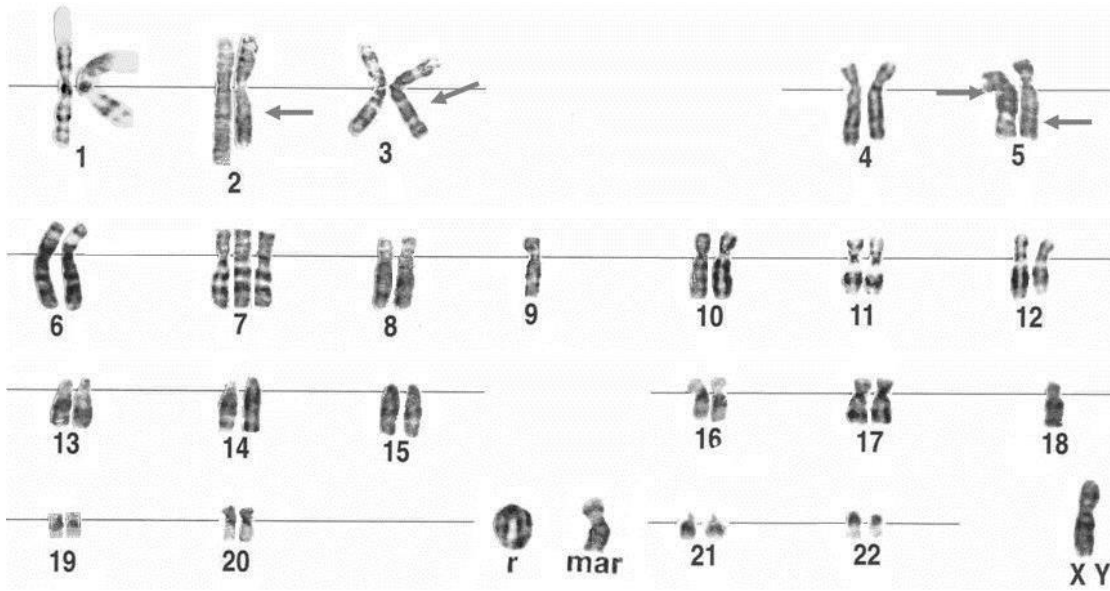
Ring chromosome due to mutagens: Ring chromosomes may form in cells following genetic damage by mutagens like radiation.

Ring chromosome formation – lost arms: both ends of the chromosome must usually be lost, enabling the arms to fuse together. Ring formation can also occur with only one end being lost.

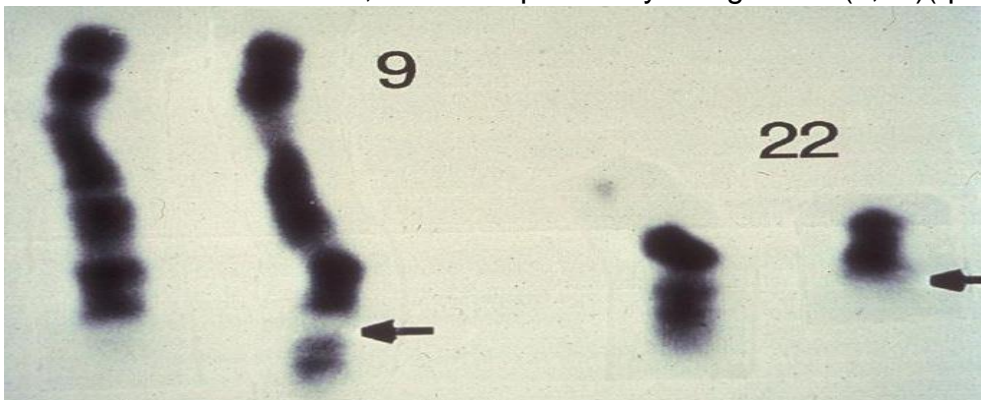
Telomeres of chromosomes fuse to form ring: In rare cases, the telomeres at the ends of a chromosome fuse without any disappearance of genetic material.

Ring chromosome 20% cases of Turner’s syndrome are due to ring chromosome.

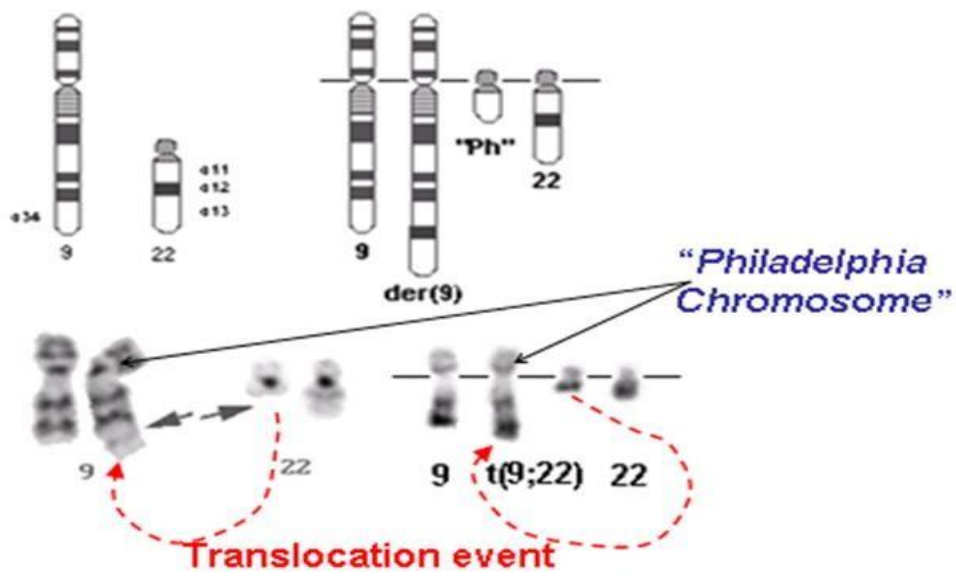




Philadelphia chromosome: Philadelphia chromosome is a specific chromosomal abnormality that is associated with chronic myelogenous leukemia (CML). Reciprocal translocation between chromosome 9 and chromosome 22, which is specifically designated t(9;22)(q34;q11).



Formation of Philadelphia



Ring and Philadelphia chromosome Fusion of ends of chromosomes to form a ring is ring chromosome. Fusion of segments of chromosomes 9 and 22 is Philadelphia chromosome.

122 What is Mutation

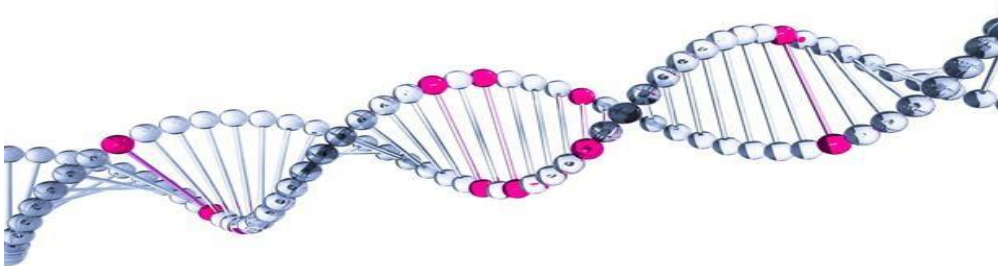
Mutation: In some disciplines the term "mutation" is used to indicate "a change" while in other disciplines it is used to indicate "a disease-causing change".

Sequence/ allelic variant some geneticists do not use term mutation. They use neutral terms like "sequence variant", "alteration" and "allelic variant" "Mutations occurs at a frequency of about 1 in every 1 billion base pairs. Every individual has about 6 mutations in each cell in their body.

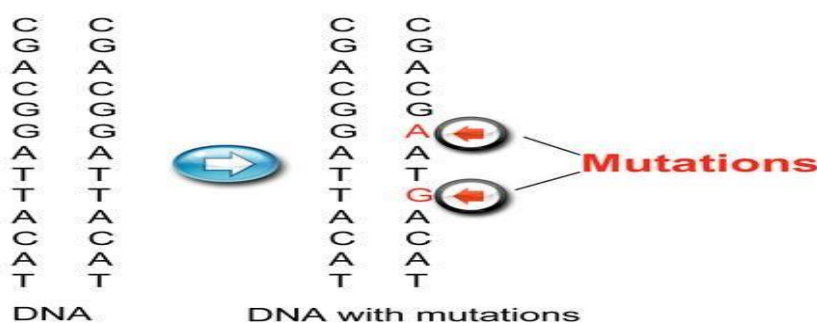
Effect of mutation:

- Mutations may be harmful.
- Mutations may be beneficial.
- Mutations may have no effect on the organism.

Mutation at DNA level



Mutation and variation Mutations are a major source of genetic variation in a population increasing biodiversity. Some variations may help to survive better and longer. Mutations in gametes (egg & sperm) are passed onto offspring. Mutations in body cells only affect the organism in which they occur and are not passed onto offspring.



123 Types of Mutation Closed 1

Types of mutation: Followings are the types of mutations.

- Points Mutations
- Variations in chromosomes number
- Variations in chromosomes structure
- **Point mutation** Base Pair Substitutions Silent

Missense

Nonsense

Base pair insertions/deletions

Triplet Repeats

Frameshift

➤ **Variation in Chromosome Number** Aneuploidy

Hyperploidy, Hypoploidy

Euploidy

Monoploidy, Diploidy, Polyploidy

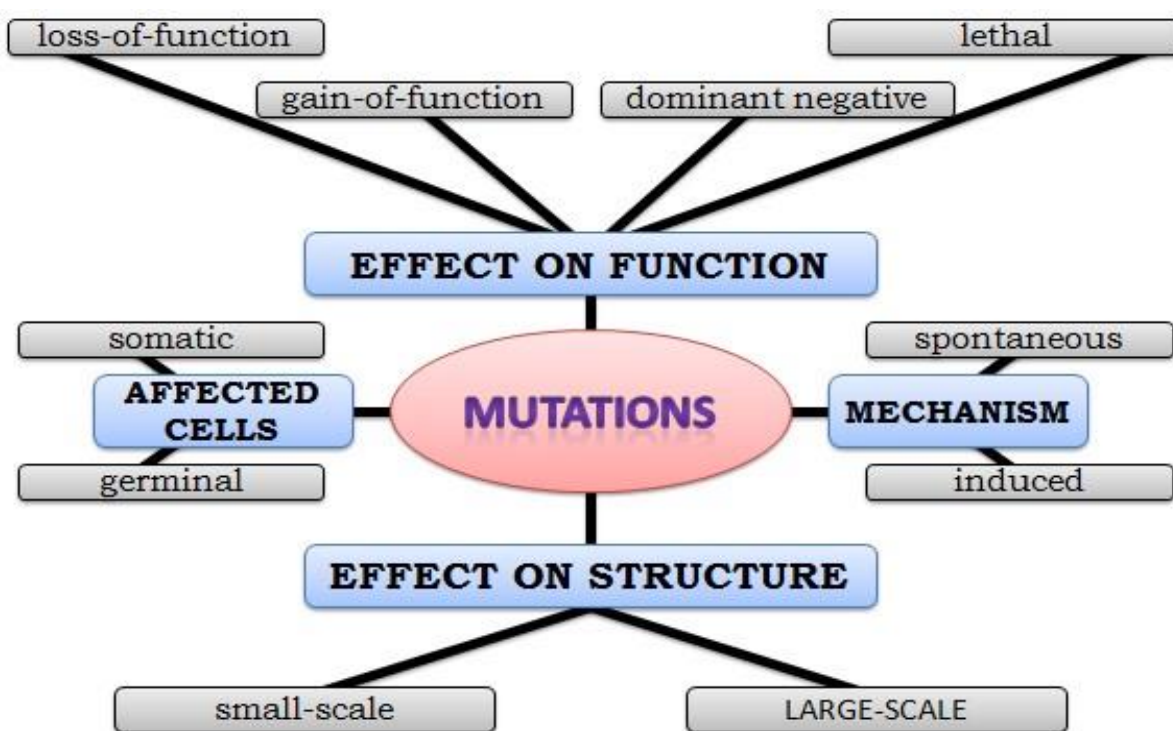
➤ **Variation in chromosome structure** Deletions

Translocations

Duplications

Inversions

Mutations and effects



Major types of mutations

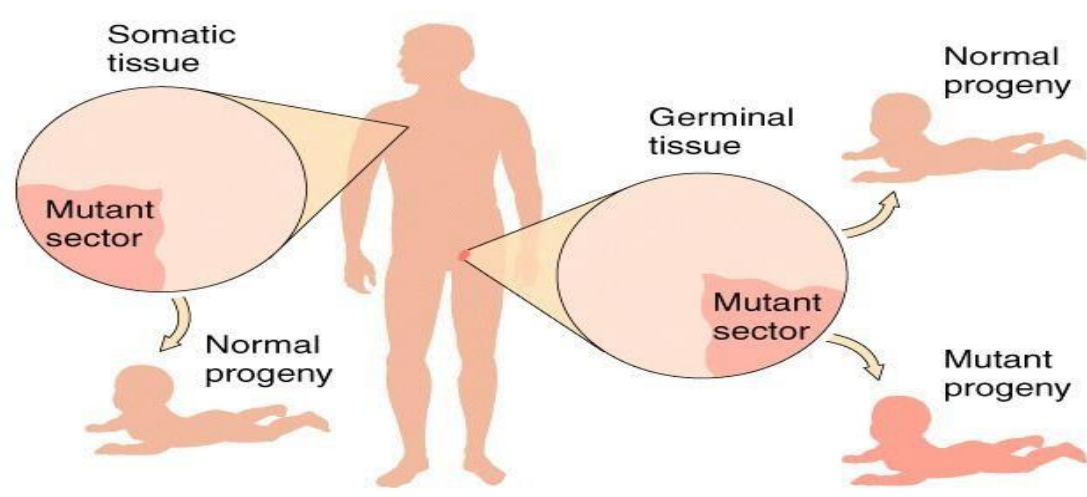
- Points Mutations
- Variations in chromosomes number
- Variations in chromosomes structure

124 Somatic and Germline Mutation

Somatic and Germline mutation

Somatic mutation: Arise in the somatic cells. Passed on to other cells through the process of mitosis.Effect of these mutations depends on the type of the cell in which they occur.

Germline mutation: They occur in the cells that produce gametes. This type of mutation usually passed on to future generation. In multicellular organisms, the term mutation is generally used for germ line mutations.



Methods to classify mutations:

Followings are the methods to classify mutations.

- On the basis of the molecular nature of the defect
- On the nature of the phenotypic effect □ On basis of the causative agent

125 Forward, Reverse and Neutral Mutation

Mutation - Forward, Reverse and Neutral

Wild type most common phenotype in natural populations of the organism is called as wild type phenotype. The effect of mutation is considered with reference to wild type phenotype.

Forward mutation: Mutation that alters the wild type phenotype into mutant phenotype.

Reverse mutation: Mutation that changes a mutant phenotype back into the wild type.

Neutral mutation: Mutation that alters the amino acid sequence of the protein but does not change its function as replaced amino acid is chemically similar or the affected AA has little influence on protein function.

126 Loss of function, Gain of function

Loss of function, Gain of function

Loss of function: Complete or partial loss of the normal function. Structure of protein is so altered that it is no longer works correctly. Mutation can occur in regulatory regions that affect transcription, translation or splicing of the protein. Frequently recessive

Gain of function: Produces an entirely new trait. Such a mutation causes a trait to appear in inappropriate tissues or at inappropriate times in development. It **is** frequently dominant.



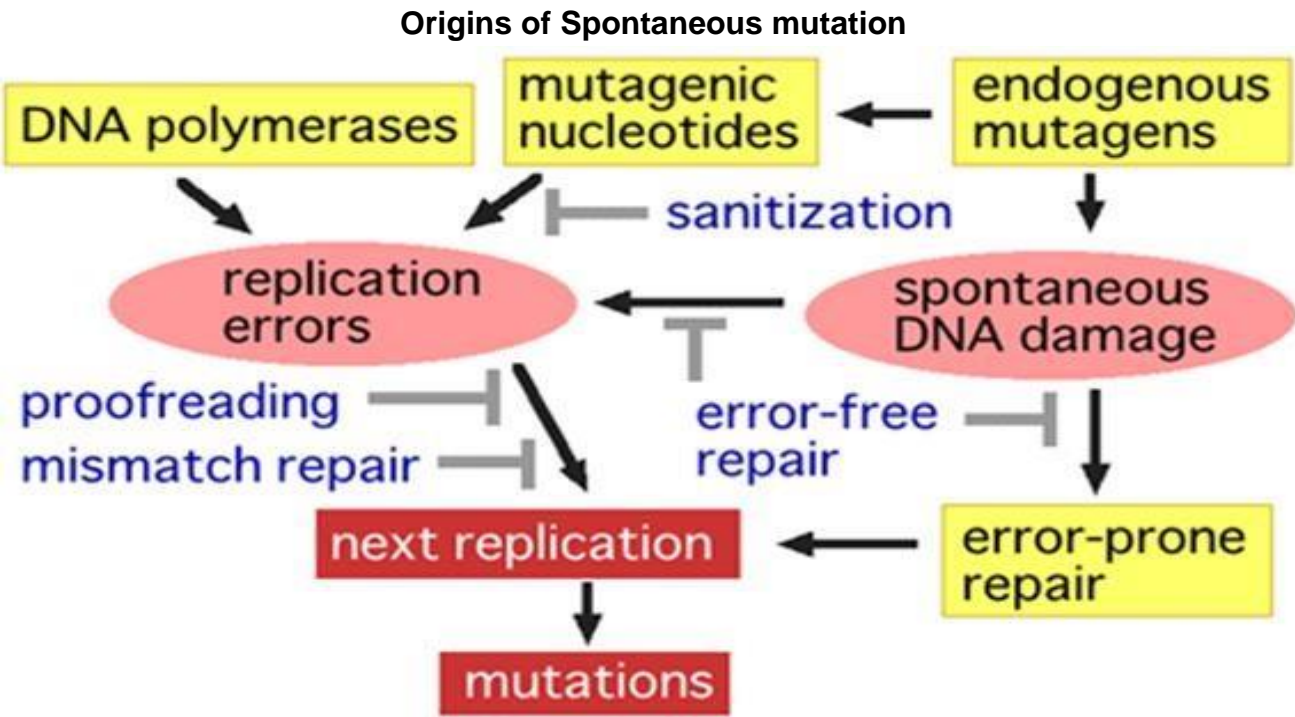
Conditional mutation: Expressed only under certain conditions. E.g. A temperature-sensitive mutation can cause cell death at high temperature, but might have no deleterious effects at a lower temperature.

Lethal mutation: Causes the death of the organism. Death does not have to occur immediately, it may take several months or even years. Longevity of an individual is significantly reduced.

127 Spontaneous and Induced Mutation Closed

Spontaneous and Induced mutation

Spontaneous mutation: Mutations that result from natural changes in DNA

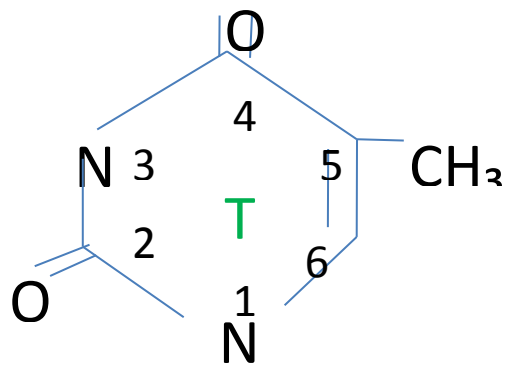
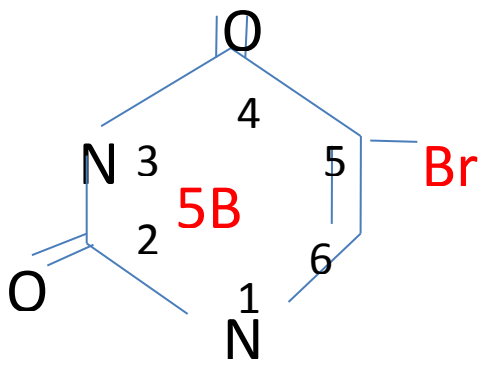


Induced mutation results from changes caused by environmental, chemicals and radiations.

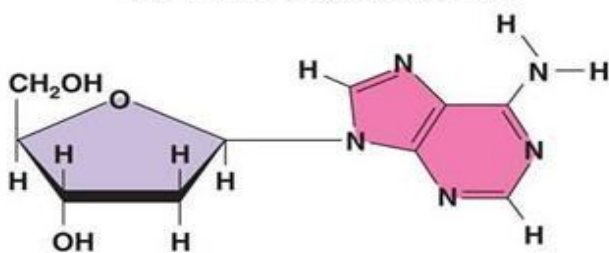
Mutagen: Any environmental agent that increases the rate of mutation above the spontaneous is called a mutagen such as chemicals & radiations, X-rays, Mustard gas etc

Base analogs: Chemicals with structures similar to that of any of the four standard bases of DNA. DNA polymerases cannot distinguish these analogs. They may be incorporated into newly synthesized DNA molecules

Base analogs: 5 bromouracil is an analog of thymine

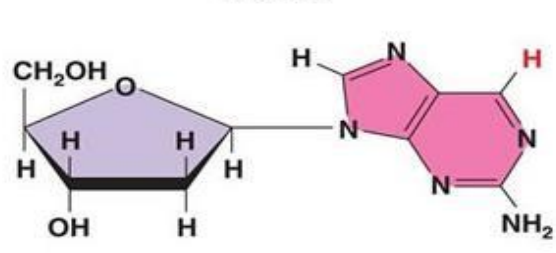


Normal nitrogenous base



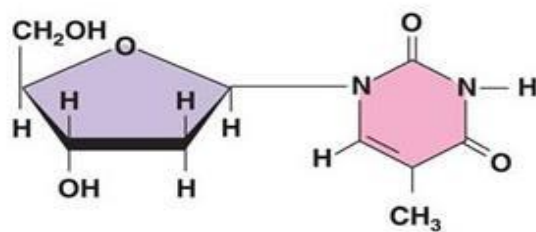
Adenine nucleoside

Analog

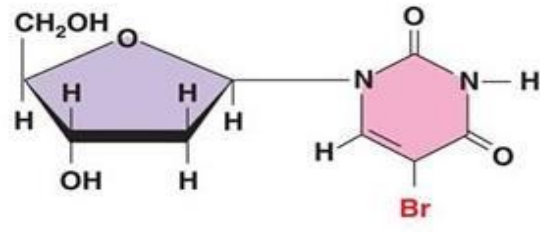


2-Aminopurine nucleoside

(a) The 2-aminopurine is incorporated into DNA in place of adenine but can pair with cytosine, so an AT pair becomes a CG pair.



Thymine nucleoside



5-Bromouracil nucleoside

(b) The 5-bromouracil is used as an anticancer drug because it is mistaken for thymine by cellular enzymes but pairs with cytosine. In the next DNA replication, an AT pair becomes a GC pair.

128 Suppressor Mutation

Suppressor mutation: Suppresses the effect of other mutation. Suppressor mutation occurs at a site different from the site of original mutation. Organism with a suppressor mutation is a double mutant but exhibits the phenotype of an unmutated wild type. Different from reverse mutation in which mutated site is reverted back into the wild type sequence. Genetic suppression therefore restores the phenotype seen prior to the original background mutation. Suppressor mutations are useful for identifying new genetic sites which affect a biological process of interest.

Intragenic suppression: Intragenic suppression results from suppressor mutations that occur in the same gene as the original mutation. Intergenic (extragenic) suppression relieves the effects of a mutation in one gene by a mutation somewhere else within the genome. Second mutation is not on same gene as original mutation

129 Point Mutations

Point mutations:

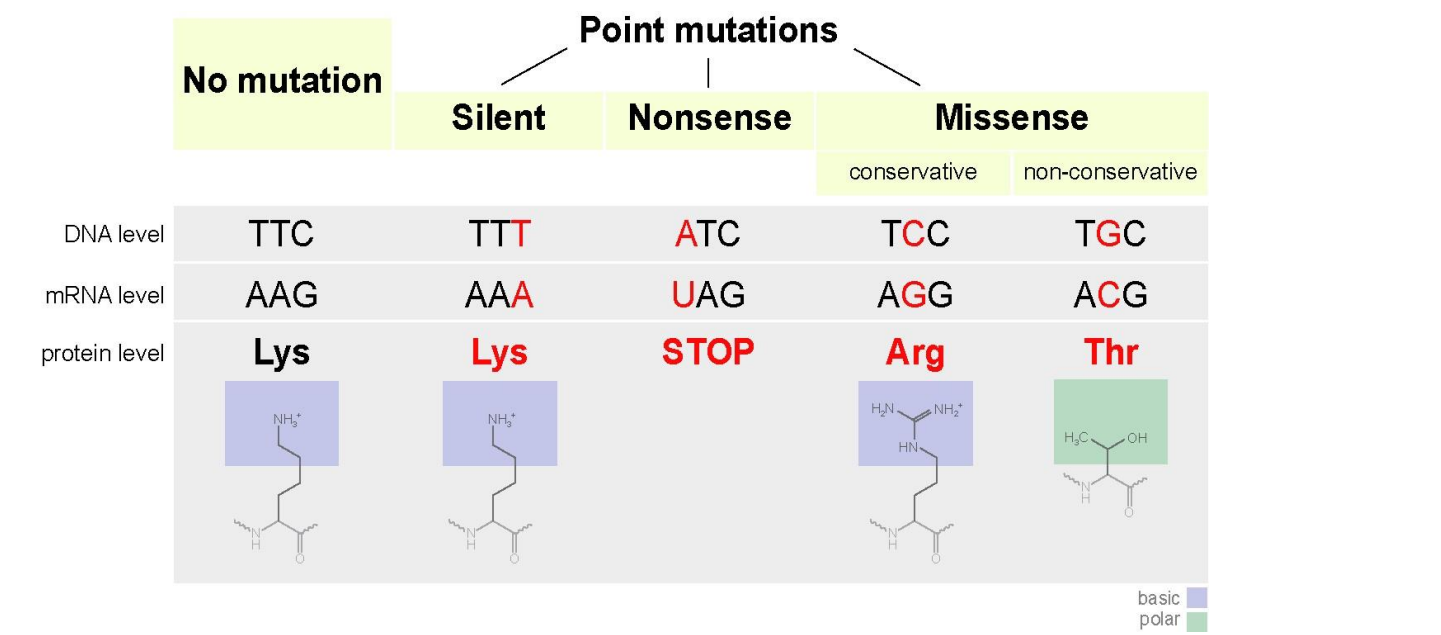
- Point mutations affect single sites on DNA.
- Substitution of one base for another.
- Deletion/addition of a single base or small number of bases.

Transition: If purine (A/G) or pyrimidine (T/C) substitutes for itself = transition substitution

Transversion: If purine substitutes for pyrimidine or vice versa = transversion substitution

Results of point mutations

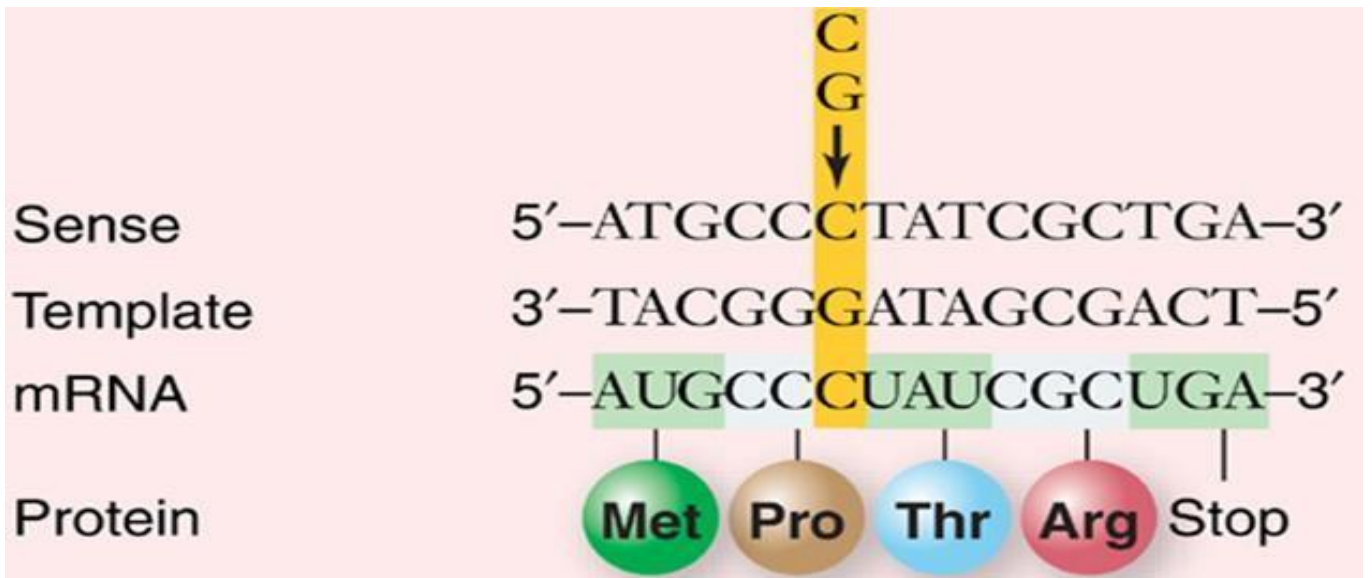
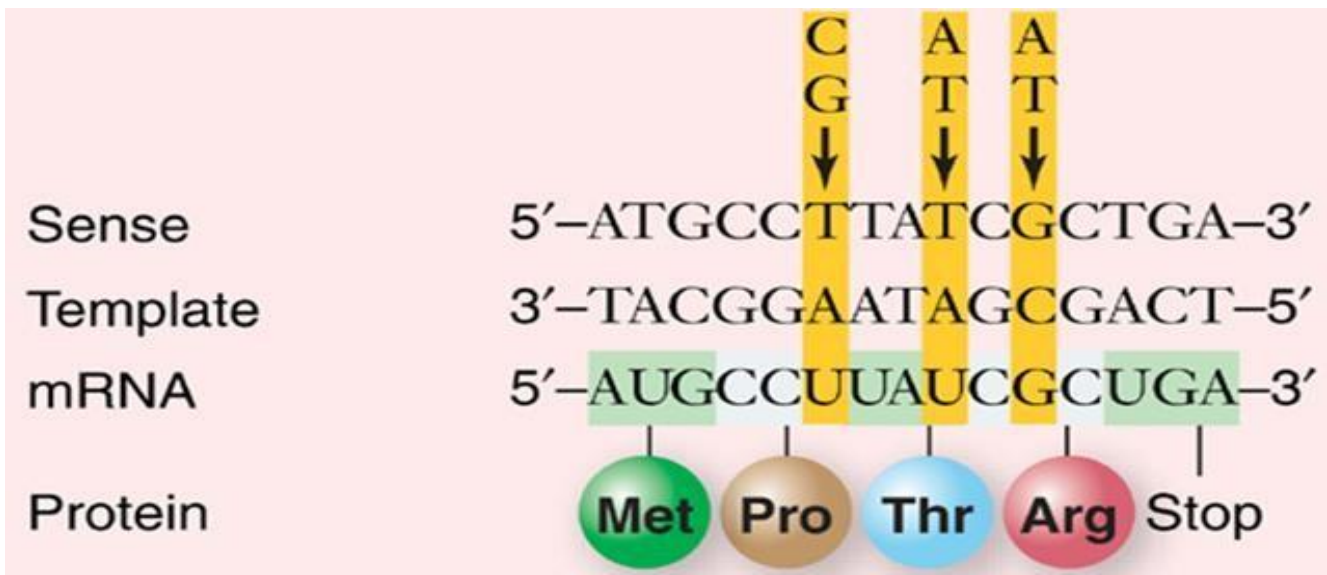
- Silent mutations
- Missense mutations
- Nonsense mutations



130 Silent Mutation

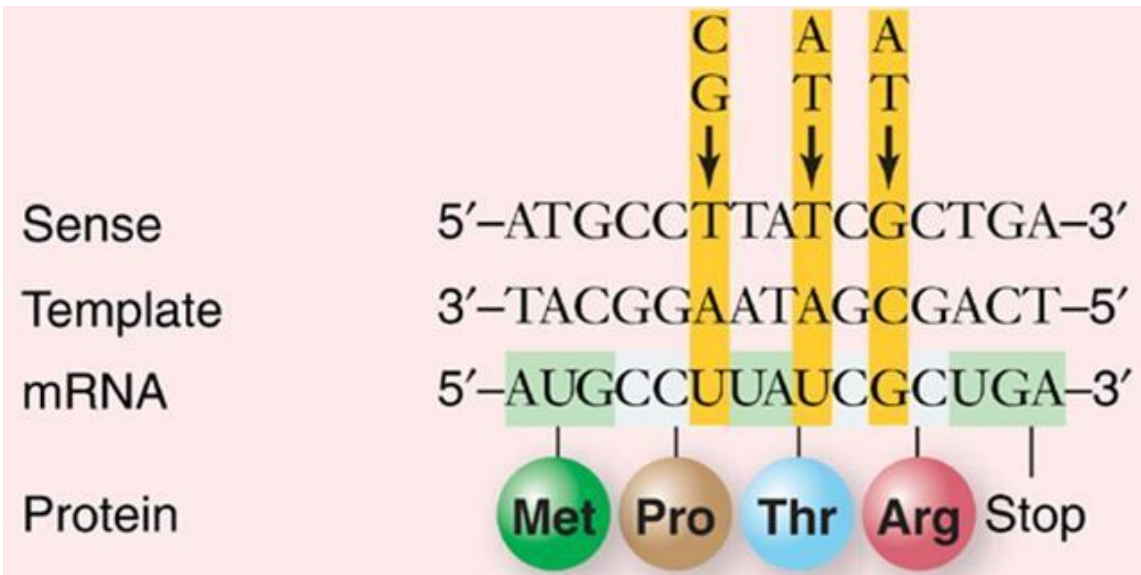
Silent Mutation: These types of mutations have affected the DNA sequence but not the protein. They have no effect on the organism’s phenotype. Since the genetic code is degenerate, several codons produce the same amino acid. Third base changes often have no effect on the amino acid sequence of the protein.

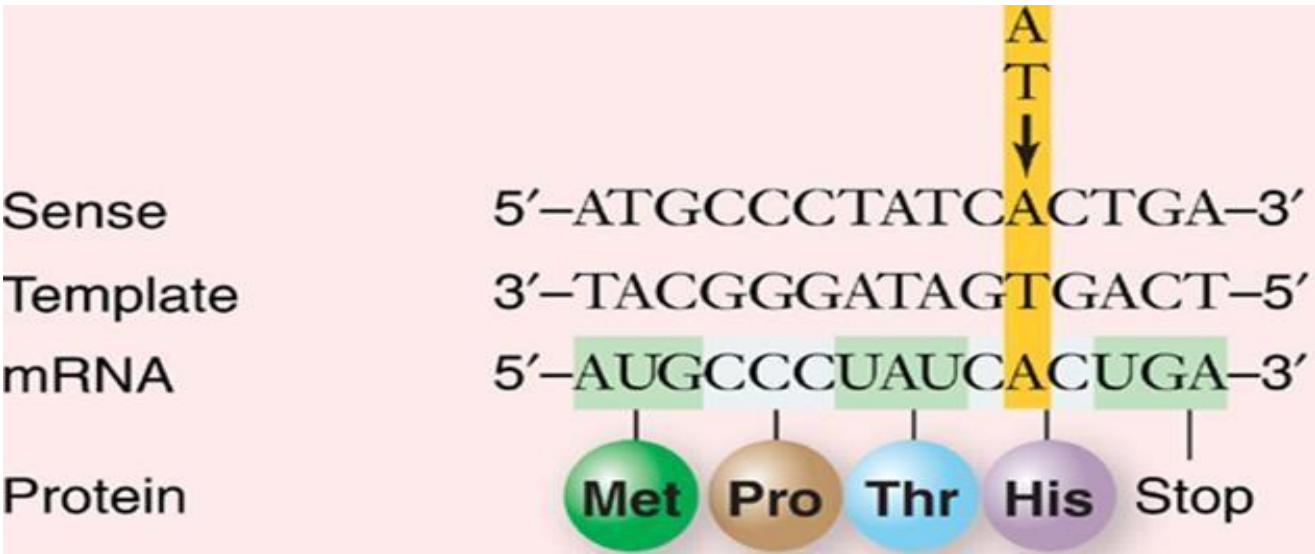
		second base in codon					
		U	C	A	G		
first base in codon	U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA stop UAG stop	UGU Cys UGC Cys UGA stop UGG Trp	U	third base in codon
	C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U	
	A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U	
	G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U	



131 Missense Mutation

Missense mutation: Missense mutations substitute one amino acid for another. Some missense mutations have very large effects. Some have minimal or no effect. It depends on where the mutation occurs in the protein's structure and how big a change in the type of amino acid it is. Missense mutation produces a change in amino acid sequence in protein product (e.g. Histidine in for Arginine). May change function of protein or may not!





132 Nonsense Mutation

Nonsense mutation: Nonsense mutations convert an amino acid into a stop codon. The effect is to shorten the resulting protein. Sometimes this has only a little effect, as the ends of proteins are often relatively un-important to function. However, often nonsense mutations result in completely non-functional proteins.

GGTCTCCTCACGCCA

↓

CCAGAGGAGUGCGGU

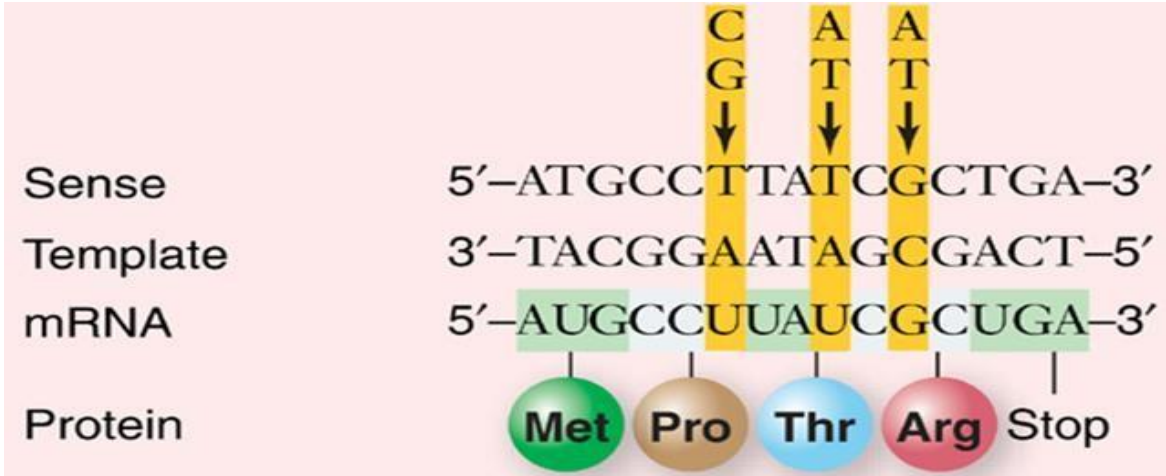
Pro-Glu-Glu-Cys-Gly

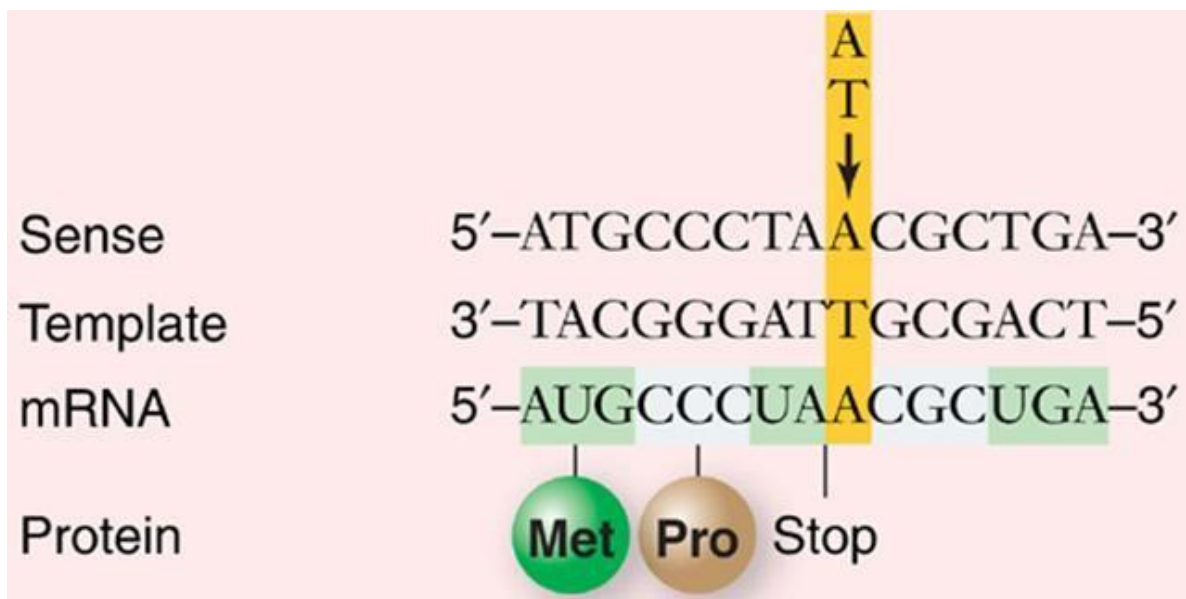
GGTCTCCTCACTCCA

↓

CCAGAAGAGUGAGGU

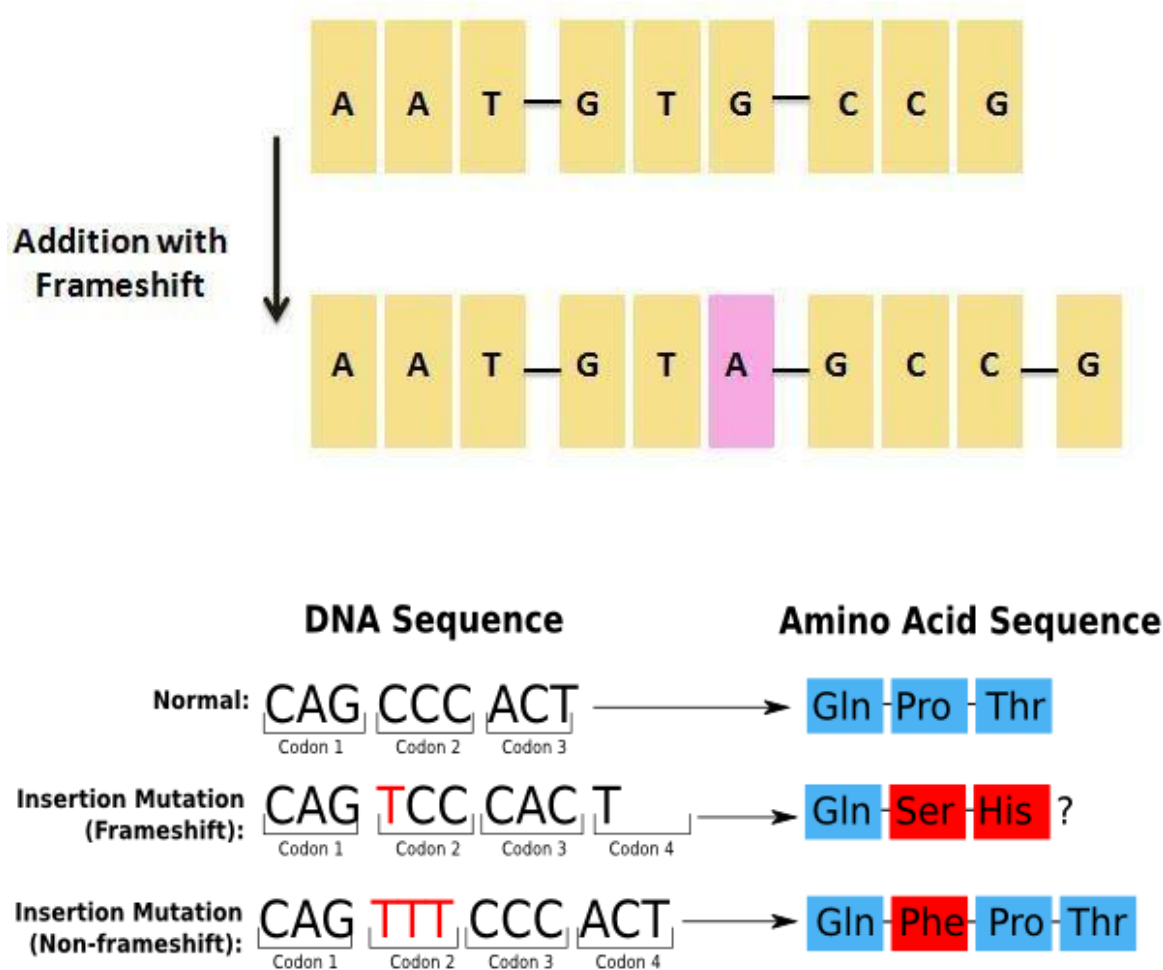
Pro-Glu-Glu-STOP





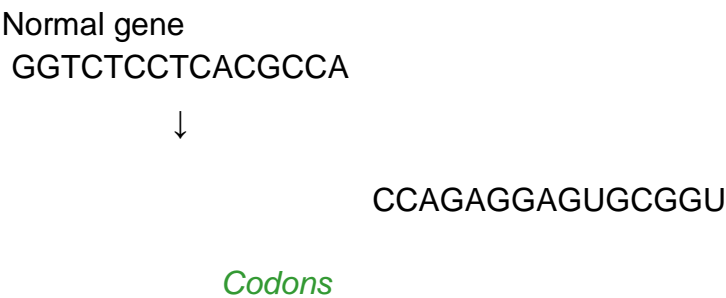
133 Additions Mutation

Additions mutation: Addition mutation is actually results in a frame shift mutation. Often, it has severe effects on protein function. It is also called as insertions.



134 Inversion Mutation

Inversion mutation: Inversion mutations only affect a small part of the gene.



↓
Pro-Glu-Glu-Cys-Gly
Amino acids

Inversion mutation

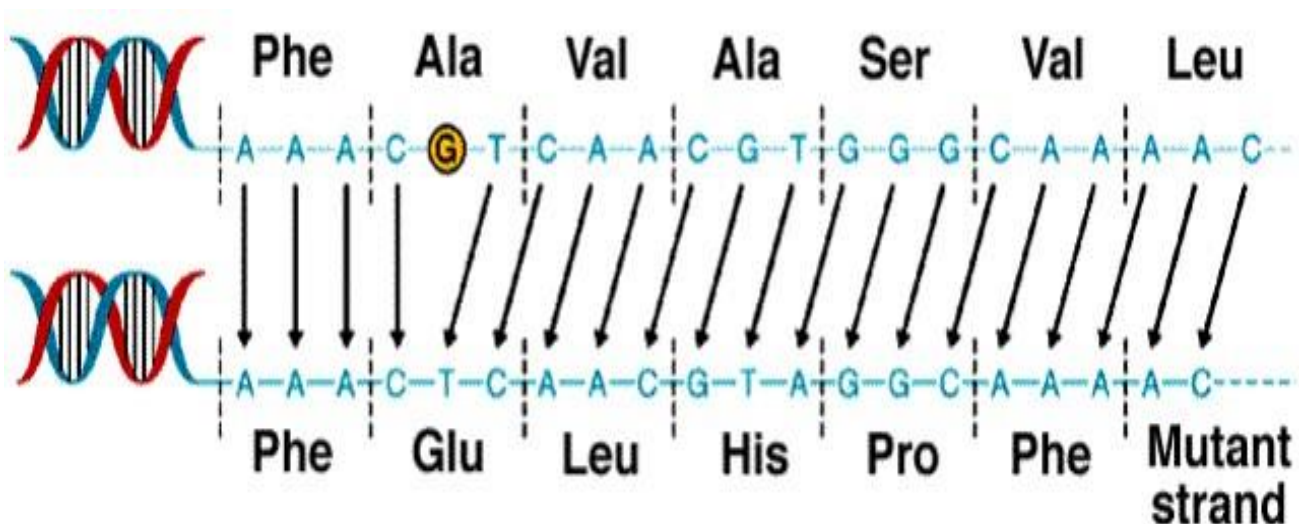
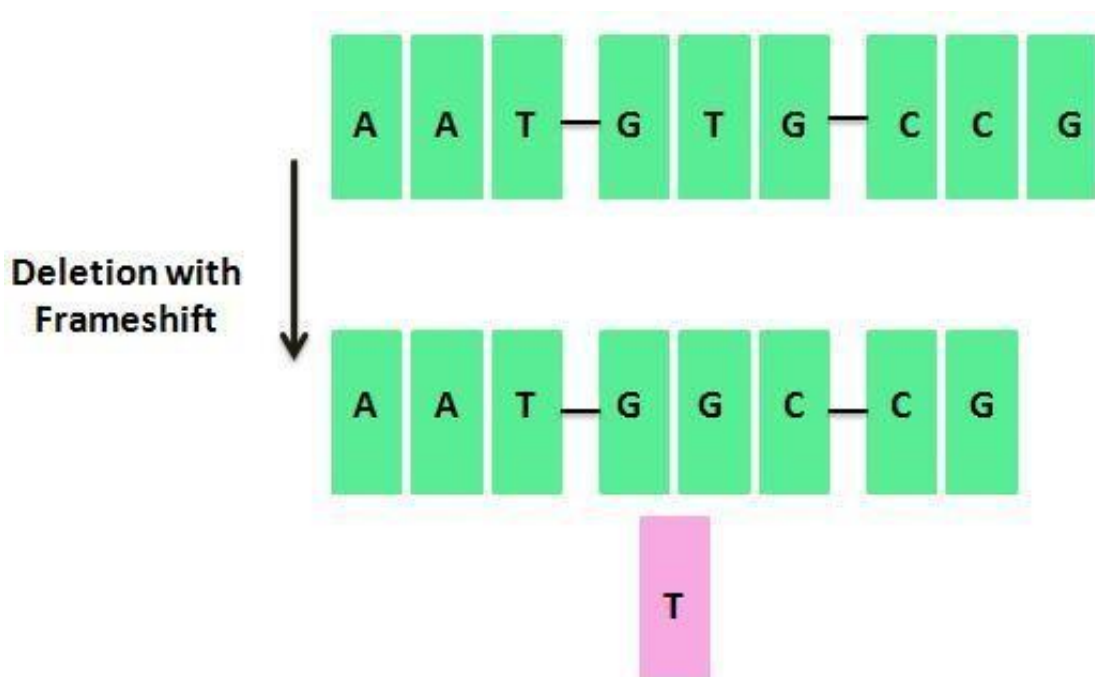
GGTCCTCTCAGCCA

↓
CCAGGAGAGUGCGGU

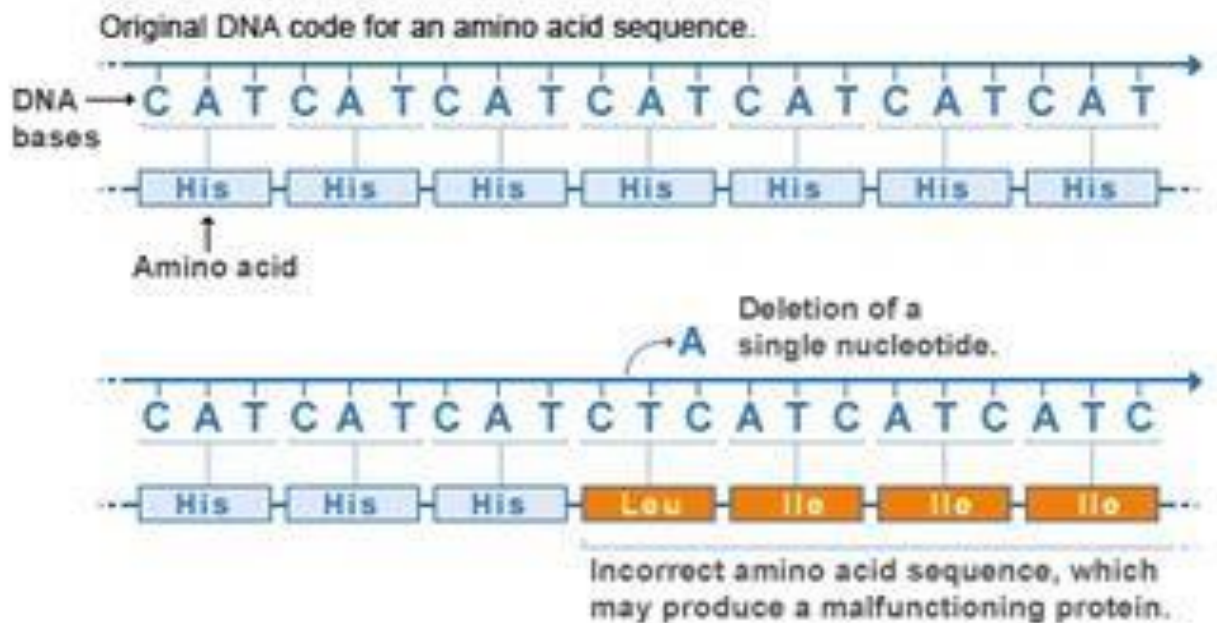
↓
Pro-Gly-Glu-Cys-Gly

135 Deletions Mutation

Deletions Mutation: Frameshift mutations result in all amino acids downstream from the mutation site being completely different from wild type. These proteins are generally nonfunctional.



Deletion mutation



U.S. National Library of Medicine

Deletions - are designated by "del" after the first and last amino acid(s) deleted; p.Lys2del in the sequence MKMGHQQQCC denotes a deletion of amino acid Lysine-2 (Lys, K).

As another example: p.Cys28_Met30del denotes a deletion of three amino acids, from Cysteine28 to Methionine-30

136 How Mutations alter Gene Function

How mutations alter gene function

Non-coding mutations: Promoter/enhancer element trinucleotide repeats in 5' and 3'UTRs Splice sites

Effect of mutation on gene function

- Null allele (loss of function) - no gene product.
- Hypomorph – decreased activity.
- Gain of function - increased activity.
- Dominant negative-antagonizes normal product
- Neomorph - novel activity

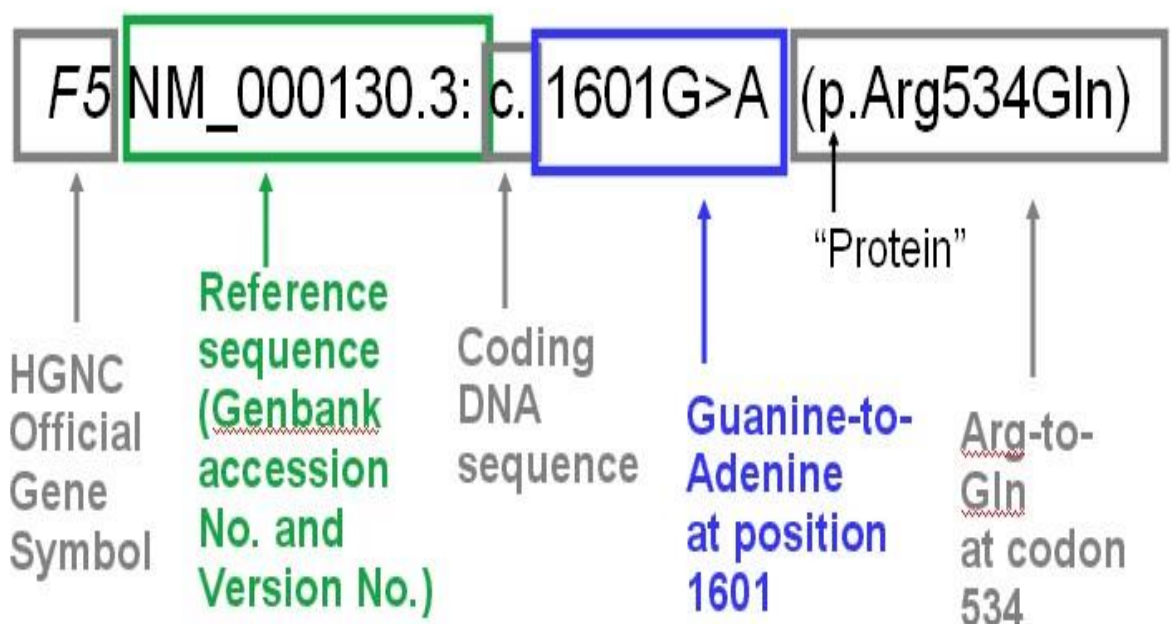
Predicting that a gene product won't do the job

- Deletion, nonsense, frameshift of sequence is deleterious.
- Mutation in splice site usually has severe effects.
- Missense mutations depend on location in protein.
- Is it non-conservative
- Is AA conserved in evolution

137 Mutation Nomenclature

Mutation nomenclature

- All variants should be described at the most basic level, i.e. DNA level.
- Descriptions should always be in relation to a reference sequence, either a genomic or a coding DNA sequence.
- Although theoretically a genomic reference sequence seems best, in practice a coding DNA reference sequence is preferred.
- Good for promoters, alternative splicing etc.
- When the entire genomic sequence is not known, a cDNA reference sequence should be used.
- To avoid confusion in the description of a variant it should be preceded by a letter indicating the type of reference sequence used.
- Several different reference sequences can be used.
- "c." for a coding DNA sequence (like c.76A>T)
- "g." for a genomic sequence (like g.476A>T)
- "m." for a mitochondrial sequence (like m.8993T>C,)
- "r." for an RNA sequence (like r.76a>u)
- "p." for a protein sequence (like p.Lys76Asn)
- Describing genes / proteins, only official HGNC (HUGO gene nomenclature committee) gene symbols should be used (www.genenames.org)
- DNA reference sequence used should preferably be from the RefSeq database, listing both database accession and version number (like NM_004006.2)



138 Salient feature of Mutation Nomenclature

Salient feature of Mutation nomenclature

- ☐ There is no nucleotide 0 (zero).
- Nucleotide 1 is A of the ATG-translation initiation codon.

- The nucleotide 5' of the ATG-translation initiation codon is -1, the previous -2, etc. e.g. in 5' UTR c.-12G>A
- The nucleotide 3' of the translation stop codon is *1, the next *2, etc. e.g. in 3' UTR c.*70T>A
- Nucleotide numbering is purely arbitrary and starts with 1 at the first nucleotide of the database reference file.
- No +, - or other signs are used.
- The sequence should include all nucleotides covering the sequence (gene) of interest and should start well 5' of the promoter of a gene.

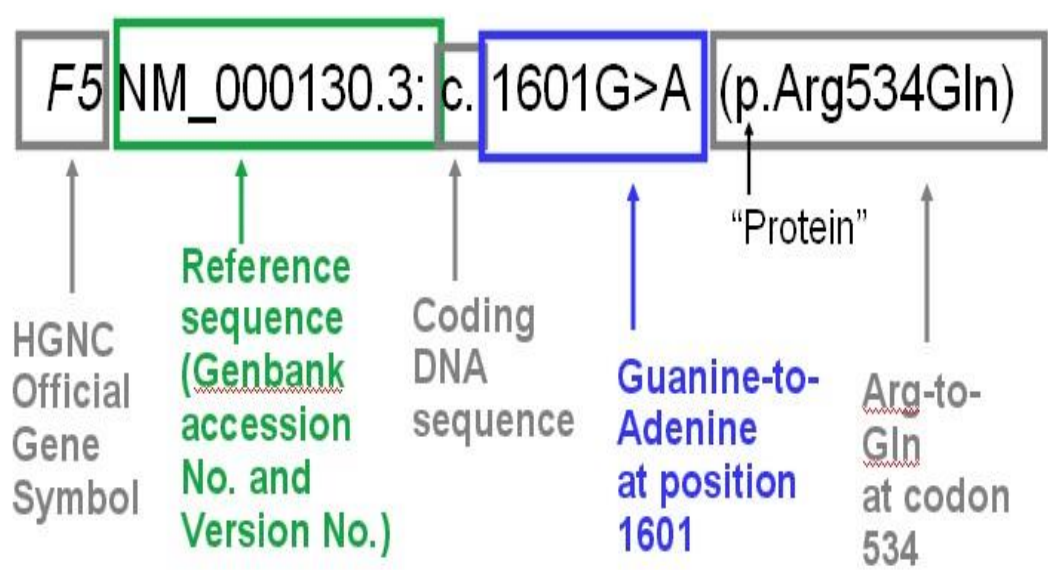
139 Mutation at DNA, RNA and Protein level

Mutation at DNA, RNA and protein level

Mutation at DNA Level

- DNA-level
in capitals, starting with a number referring to the first nucleotide affected (like c.76A>T or g.476A>T)
- Mutation at RNA Level
- RNA-level
in lower-case, starting with a number referring to the first nucleotide affected (like r.76a>u)
- Mutation at Protein Level
- Protein level in capitals, starting with the letters referring to first the amino acid affected (like p.Lys76Asn)

Nomenclature



140 Mutation in Intronic Sequence

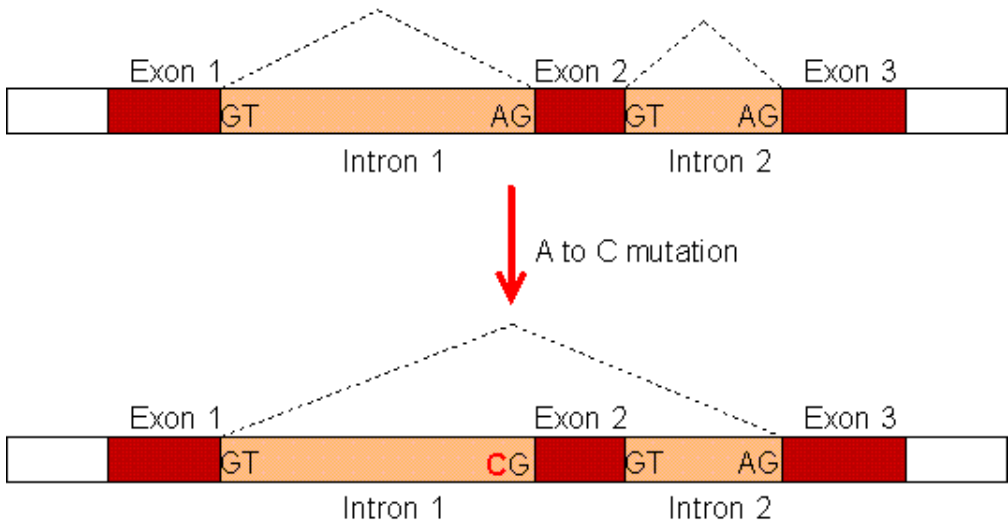
Mutation in intronic sequence

Beginning of intron

- The number of the last nucleotide of the preceding exon, a plus sign and the position in the intron, like c.88+2T>G

End of intron

- The number of the first nucleotide of the following exon, a minus sign and the position upstream in the intron, like c.89-1G>T
- **Splicing boundaries**



Exon and intron



Middle of intron

- In the middle of the intron, numbering changes from "c.77+.." to "c.78-.."; for introns with an uneven number of nucleotides the central nucleotide is the last described with a "+"

141 Specific changes in Sequence

Specific changes in sequence

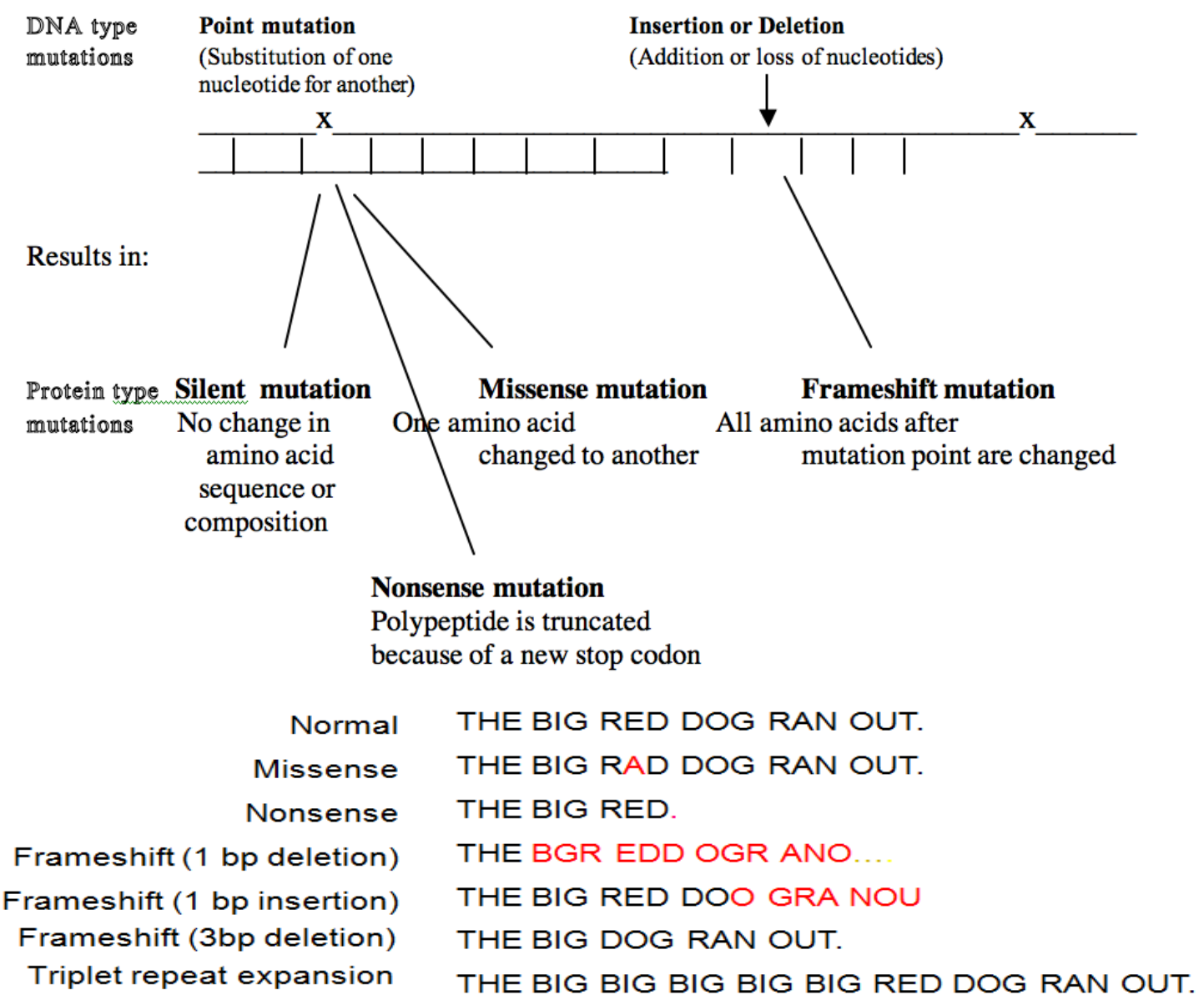
- ">" indicates a substitution at DNA level (like c.76A>T)
- "_" (underscore) indicates a range of affected residues, separating the first and last residue affected (like c.76_78delACT).
- "del" indicates a deletion (like c.76delA).
- "dup" indicates a duplication (like c.76dupA); duplicating insertions are described as duplications, not as insertions.
- "ins" indicates a insertion (like c.76_77insG)
- "inv" indicates an inversion (like c.76_83inv) .

Nomenclature

- While describing sequence variants, it should be written according to HGNC rules.

142

Mutations-Summary



Summary to understand

- Point mutations affect single sites on DNA.

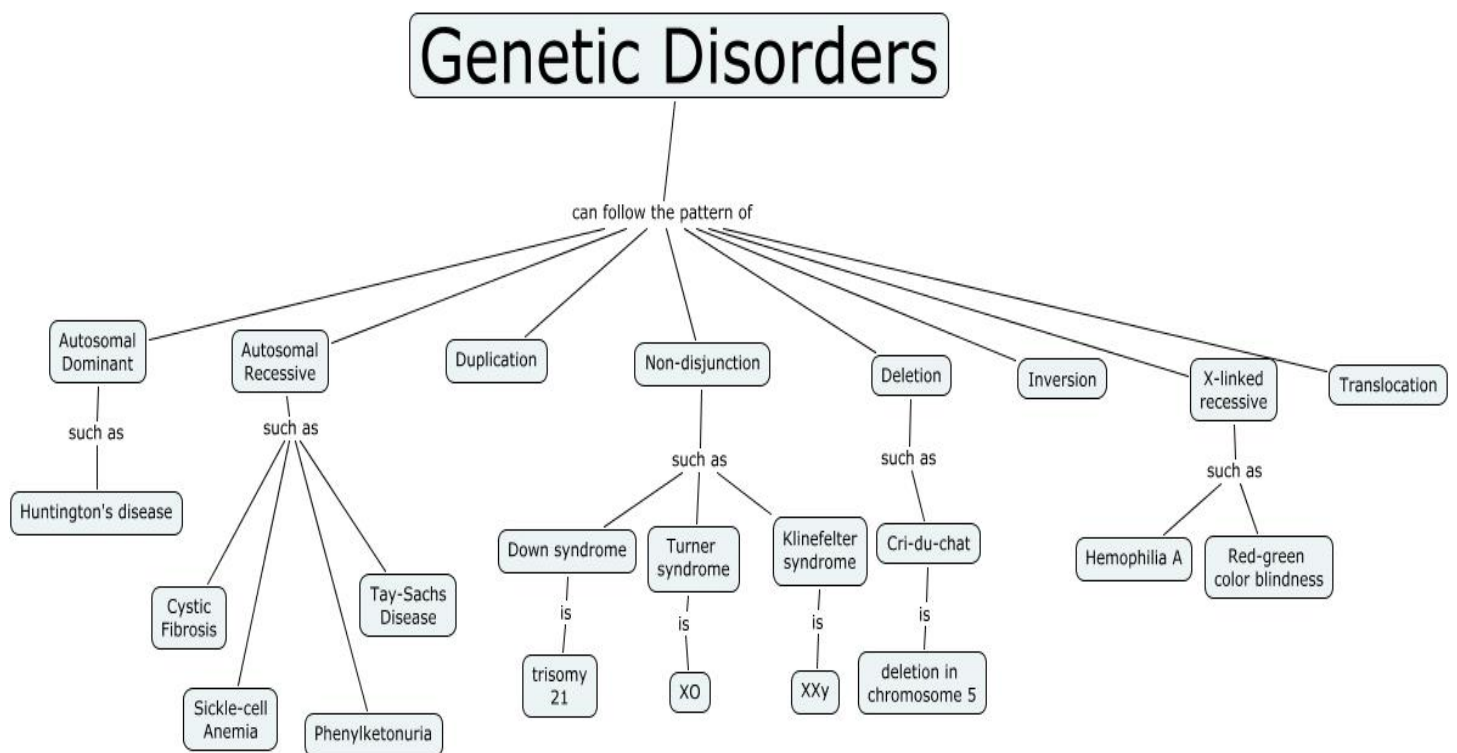
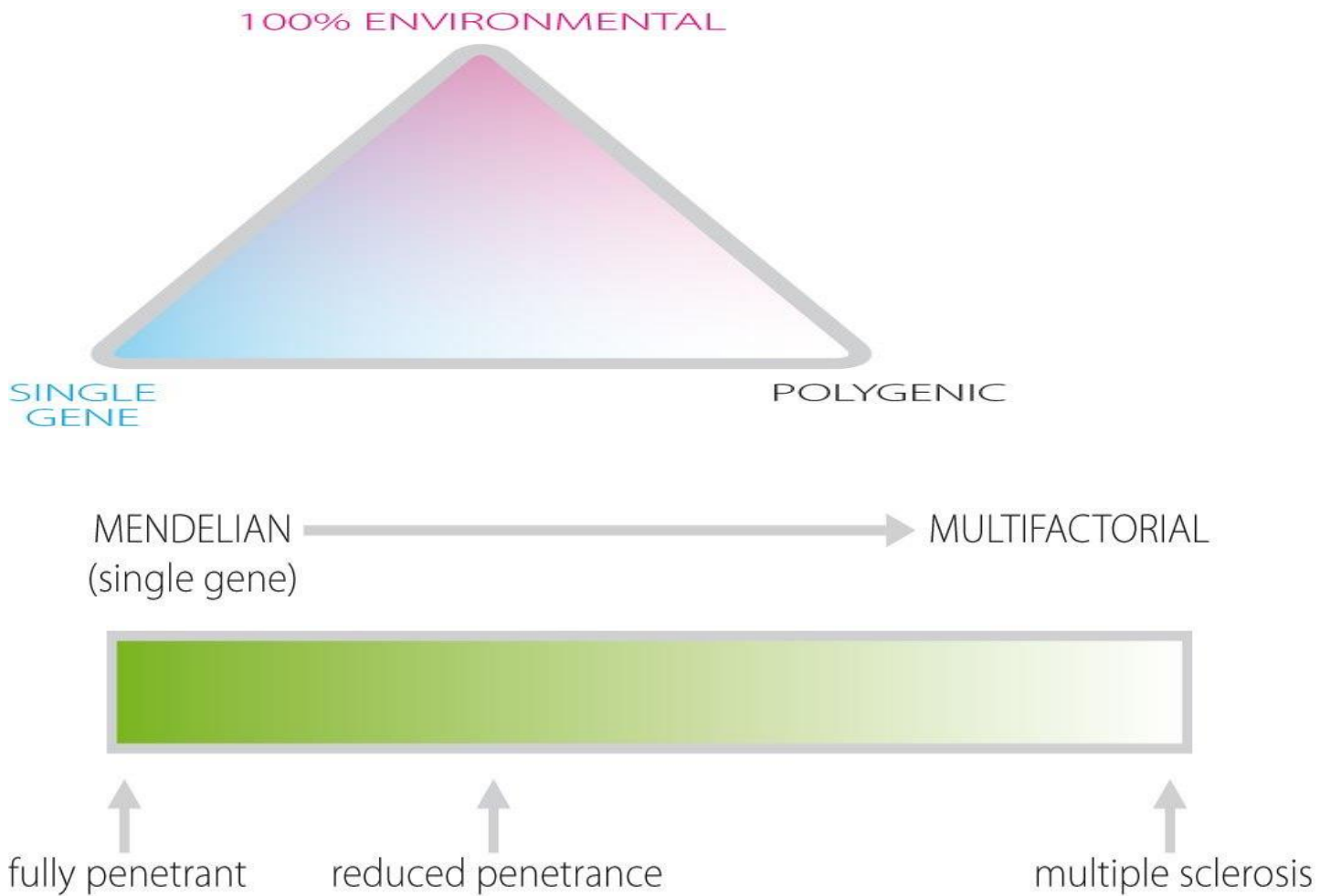
143

What are Genetic Disorders

Genetic disorders

- A genetic disease or disorder appear as a result of mutations in an individual's DNA.
- Change in DNA sequence may be small or large like deletions/addition of entire chromosome.

Classification



Fully penetrant and low penetrant

- Fully penetrant, where other genes and environmental factors have no effect.
- Low-penetrance, genes play a small part, along with other environmental factors.

Classification of genetic disorders

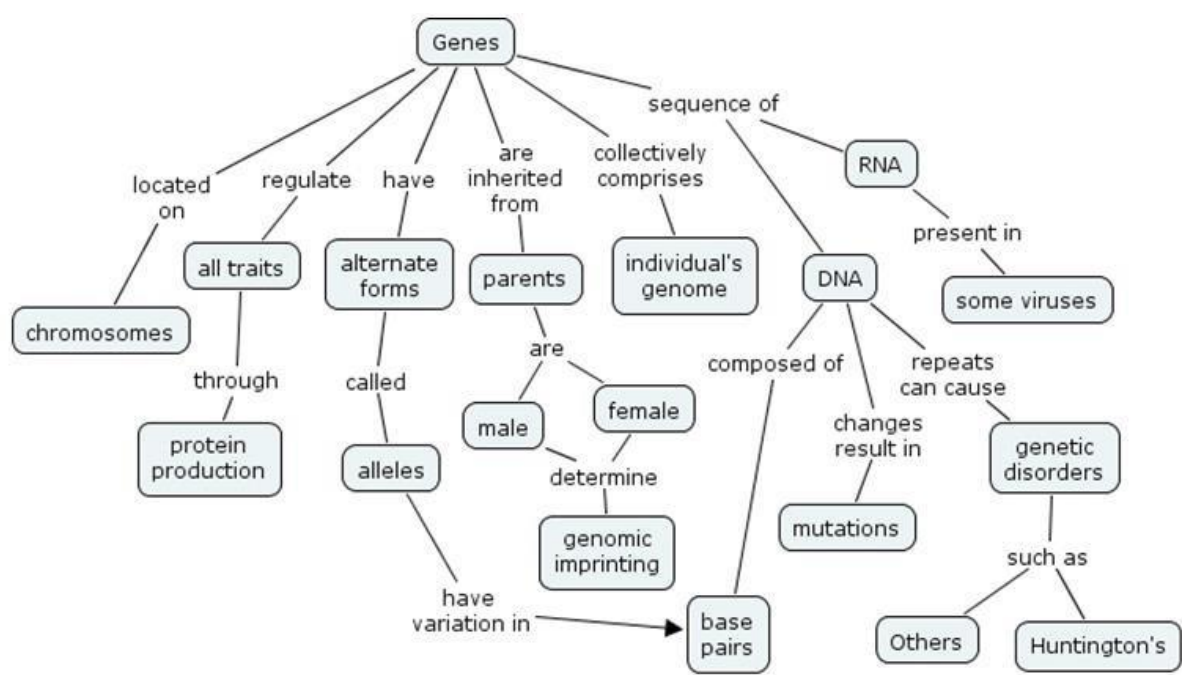
- Multifactorial disorders
- Single gene disorders
- Chromosomal disorders
- Mitochondrial disorders
- Somatic mutations (cancers)

144 Inheritance Patterns of Genetic Disorders

Inheritance patterns of genetic disorders

- Autosomal Dominant:** Affected individuals are heterozygote. One allele is mutated, while other one is normal.
- Autosomal recessive:** Homozygous, when both alleles of the gene are mutated.
- X-linked recessive:** Mutated alleles are present on either of the sex chromosomes.
- Multifactorial:** Genes and environment both contribute.
- Multifactorial frequent, single gene disorders less frequent:** Multifactorial are common. Single gene less common like dominant/recessive pedigree patterns

Genes and genetic disorders



Chromosomal disorders

- Thousands of genes may be involved.

Multiple organ systems affected at multiple stages in gestation

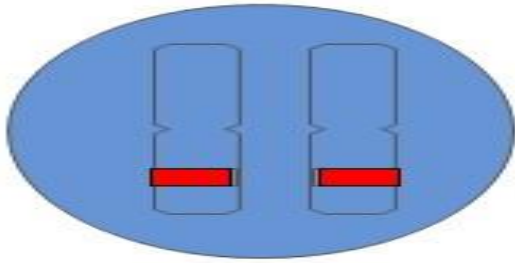
145 Autosomal Recessive Inheritance

Autosomal recessive inheritance / diseases

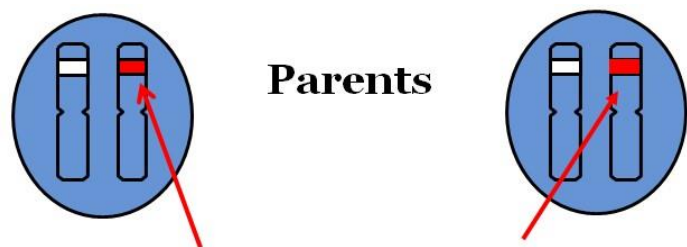
Autosomal Recessive diseases: Diseases occurs in individuals with two mutant alleles.

- In general, an individual inherit one mutant allele from each of the parent.

Recessive diseases: Homozygotes with two copies of the altered gene

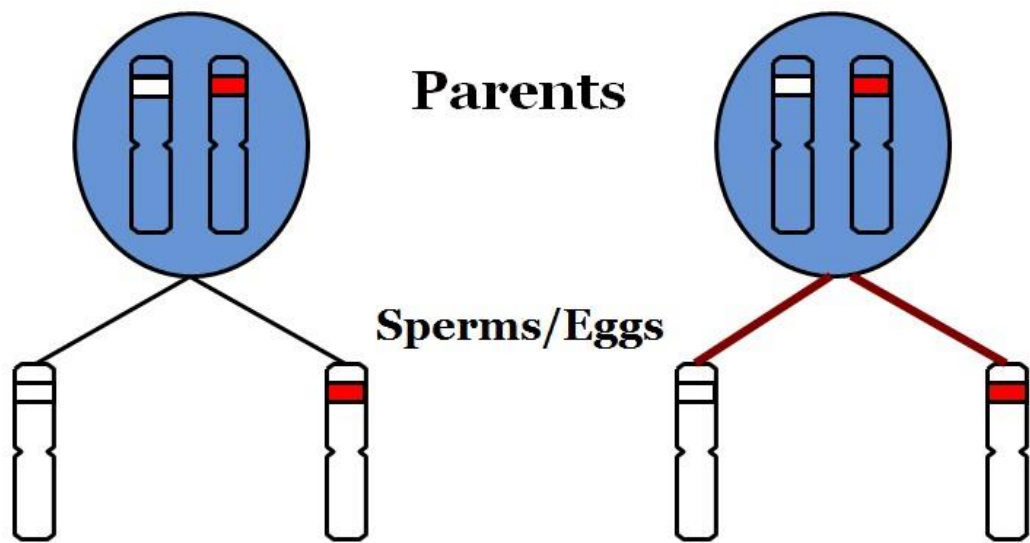


Recessive diseases: Since each parent has two alleles. Chance of inheriting a mutant allele from one parent is 50% and for the other parent is also 50%. Net chance of inheritance of two mutant alleles is 25%.



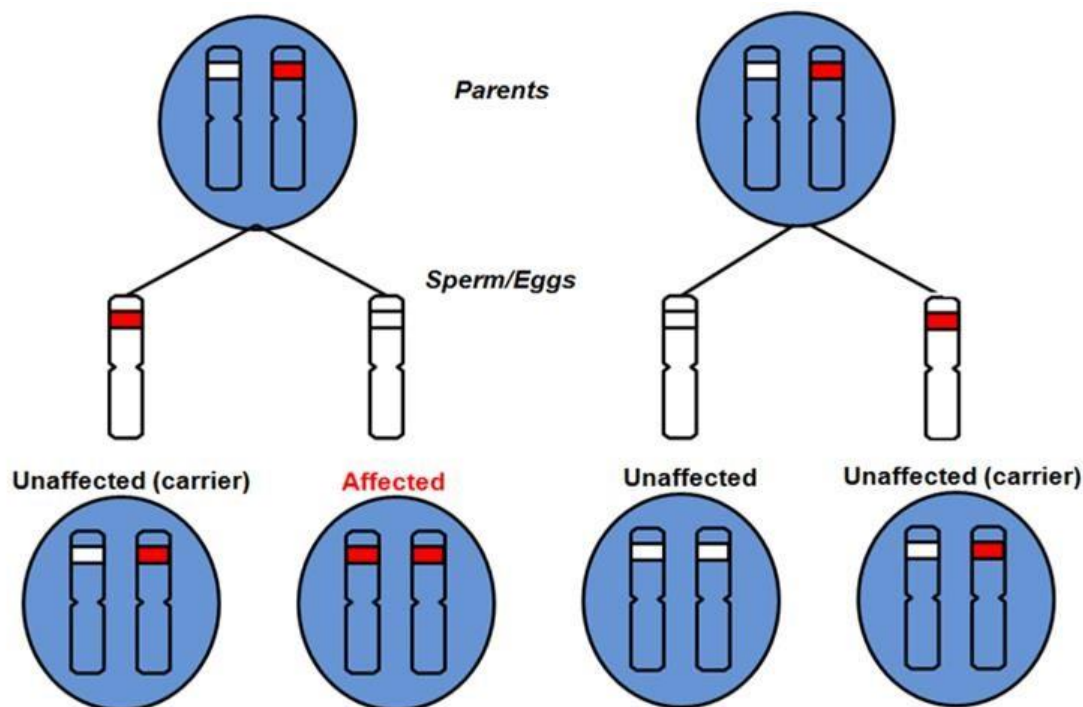
Parents

Parent who are carriers for the same autosomal recessive condition have one copy of the normal form of the gene and one copy of an altered form of gene



Parents

Sperms/Eggs



Generally, the disease appears in the progeny of unaffected parents.

Affected progeny include both males and females equally.

Phenotypic proportions are equal – autosomal recessive: When we know that both male and female phenotypic proportions are equal, we can assume that we are dealing with autosomal inheritance.

Common recessive disorders

- Bloom syndrome
- Carpenter syndrome
- Cystic fibrosis
- Thalassemia
- Many forms of mental retardation
- Gaucher's disease
- Glycogen storage diseases
- Rotor syndrome
- Many of eye diseases

146 Characteristics of Autosomal Recessive Inheritance

Properties of Autosomal recessive inheritance

Consanguinity: These diseases appear where parents have common ancestors.

Same allele increases chances of inheriting disorders: The chance of inheriting identical alleles by an individual from both parents increases the chance of inheriting a recessive disorder.

Common genetic background: Small populations of individuals with a common genetic background may have increased risk of recessive diseases.

Ashkenazi: Gaucher disease

Phenotype found more likely in siblings of proband than in other relatives. Males and females are equally affected. In most of the cases, parents of affected individuals are asymptomatic.

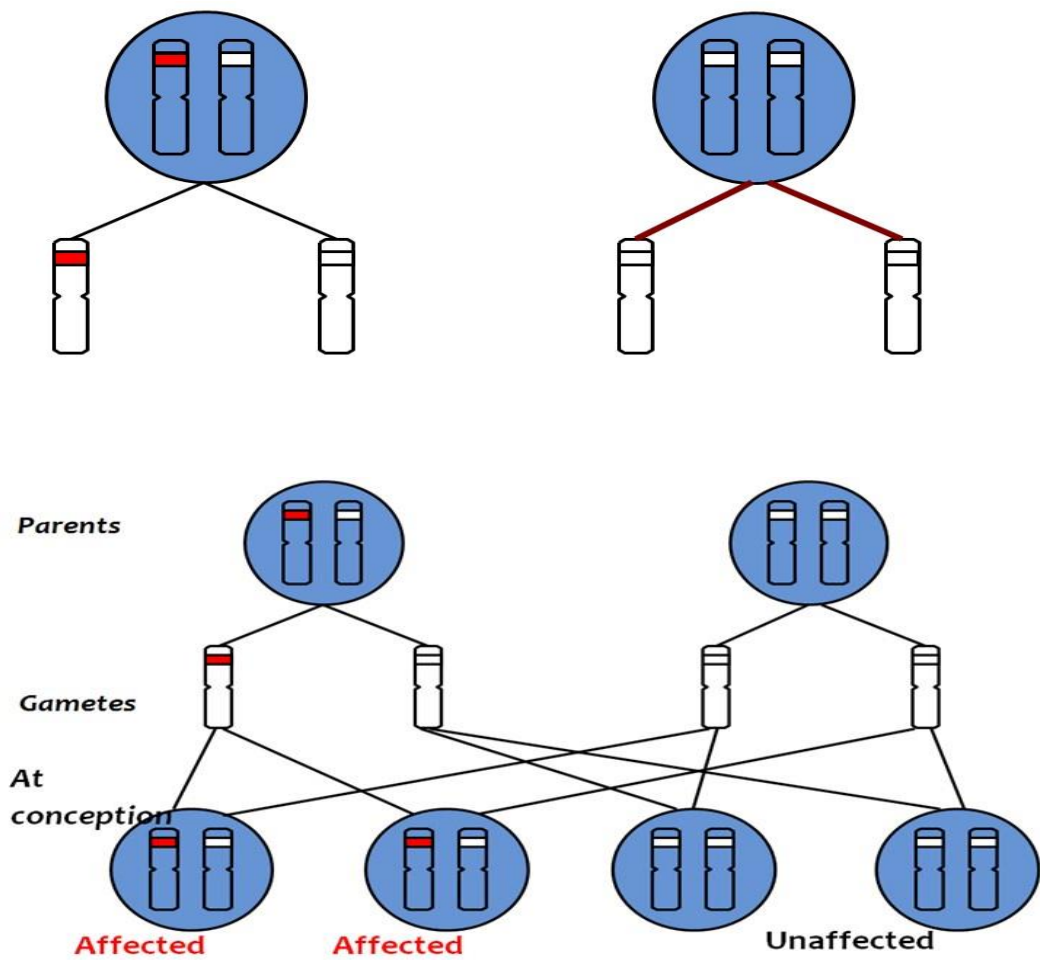
Risk for each of the sibling being affected .

Risk of disease for each sib is 25 %

Autosomal dominant inheritance/ disorders

Autosomal dominant disorders: In autosomal dominant disorders, the normal allele is recessive and the abnormal allele is dominant. In a typical pedigree, every affected person has one affected parent. Equal numbers of affected females/males are expected. Male to male transmission is possible. One-half of the children of an affected individual are expected to have inherited the dominant allele.

Autosomal Dominant disorders



Pedigree analysis, main clues for identifying a dominant disorder is that the phenotype tends to appear in every generation of the pedigree. Affected fathers/mothers transmit the phenotype to both sons and daughters. Trait is common in the pedigree. Trait is found in every generation.

Autosomal Dominant diseases

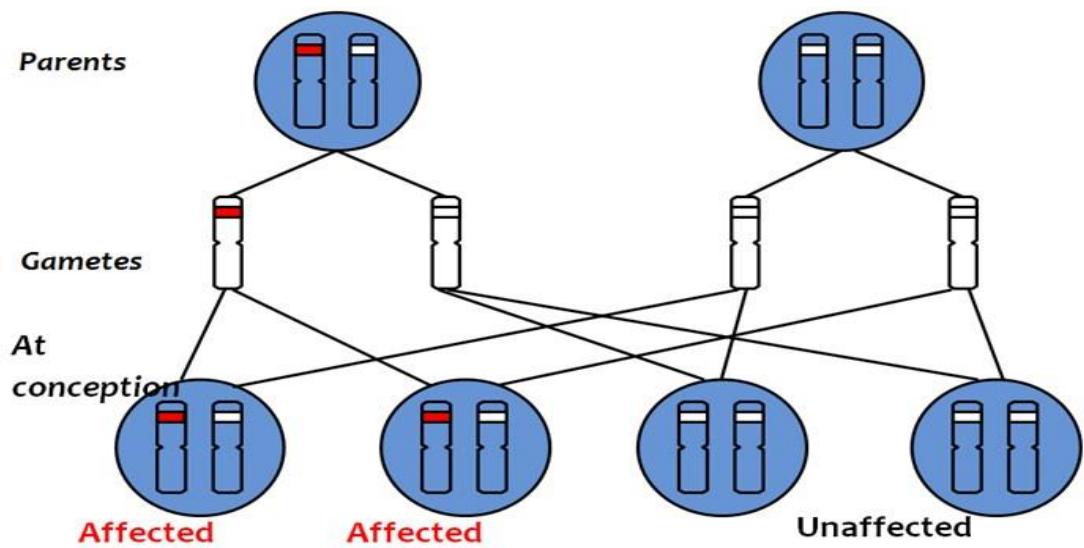
- Marfan syndrome
- Huntington's disease
- Retinoblastoma
- Waardenburg syndrome
- Myotonic dystrophy
- Polycystic kidney disease
- Achondroplasia
- Polydactyly

Heredity hearing loss

Variations in Autosomal dominant inheritance

Autosomal dominant disorders: In autosomal dominant disorders, the normal allele is recessive and the abnormal allele has dominant effect. In a typical pedigree, every affected person has one affected parent.

Autosomal dominant disorders



Penetrance - variation to A.D inheritance

Some people with an appropriate genotype fail to express the phenotype called as incomplete or reduced penetrance.

Expressivity - variation to autosomal dominant inheritance

Severity of the phenotype: When phenotypic severity varies among those with same genotypes

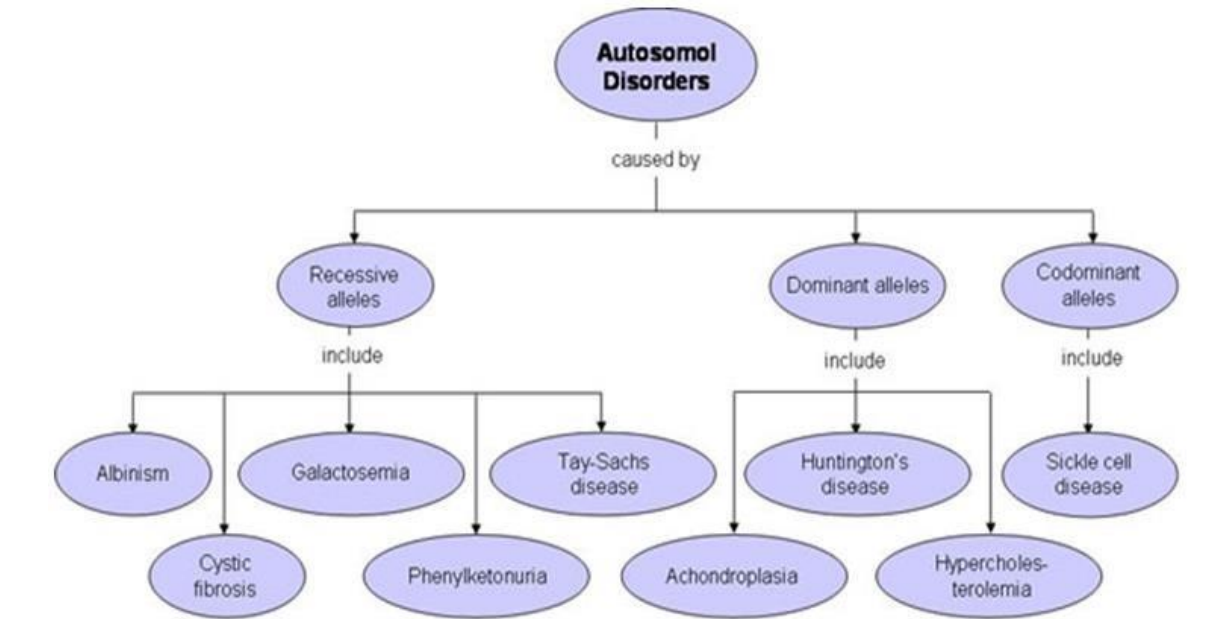
Pleiotropy - variation to autosomal dominant inheritance Pleiotropy: Multiple phenotypic effects of a single gene.

149 Dominance and Recessiveness

Dominance and Recessiveness Dominance and recessiveness are the properties of characters/traits, not genes. A character is dominant if it is expressed in the heterozygote and recessive if not.

Recessiveness: Cystic fibrosis is recessive because only homozygotes manifest it, whereas heterozygotes show the normal phenotype.

Hemizygous: Males are hemizygous for loci on the X and Y chromosomes, where they have only a single



Traits	Dominant	Recessive
Eye color	brown eyes	grey, green, hazel, blue eyes
Hair	dark hair	blonde, light, red hair
	non-red hair	red hair
	curly hair	straight hair
Facial features	dimples	No dimples
	unattached earlobes	attached earlobes
	broad lips	thin lips
Appendages	extra digits	normal number
	clubbed thumb	normal thumb

Dominance and recessiveness

Dominance and recessiveness are the properties of characters/traits, not the genes.

150 X-linked recessive inheritance

linked recessive inheritance and disorders

X-linked recessive: Traits which are due to the genes which are present on either of the sex chromosomes. Many of the genes have a disease phenotype.

Males are hemizygous and express the disease: Males are hemizygous, will express the disease phenotype if one mutation is present.

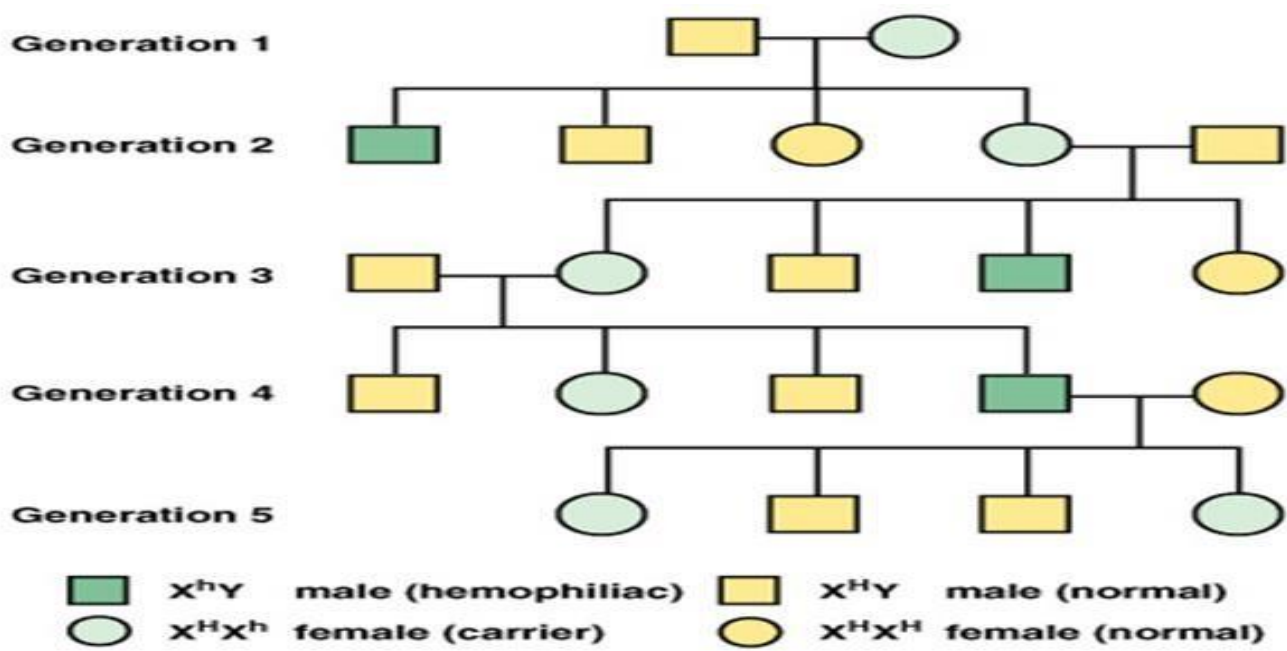
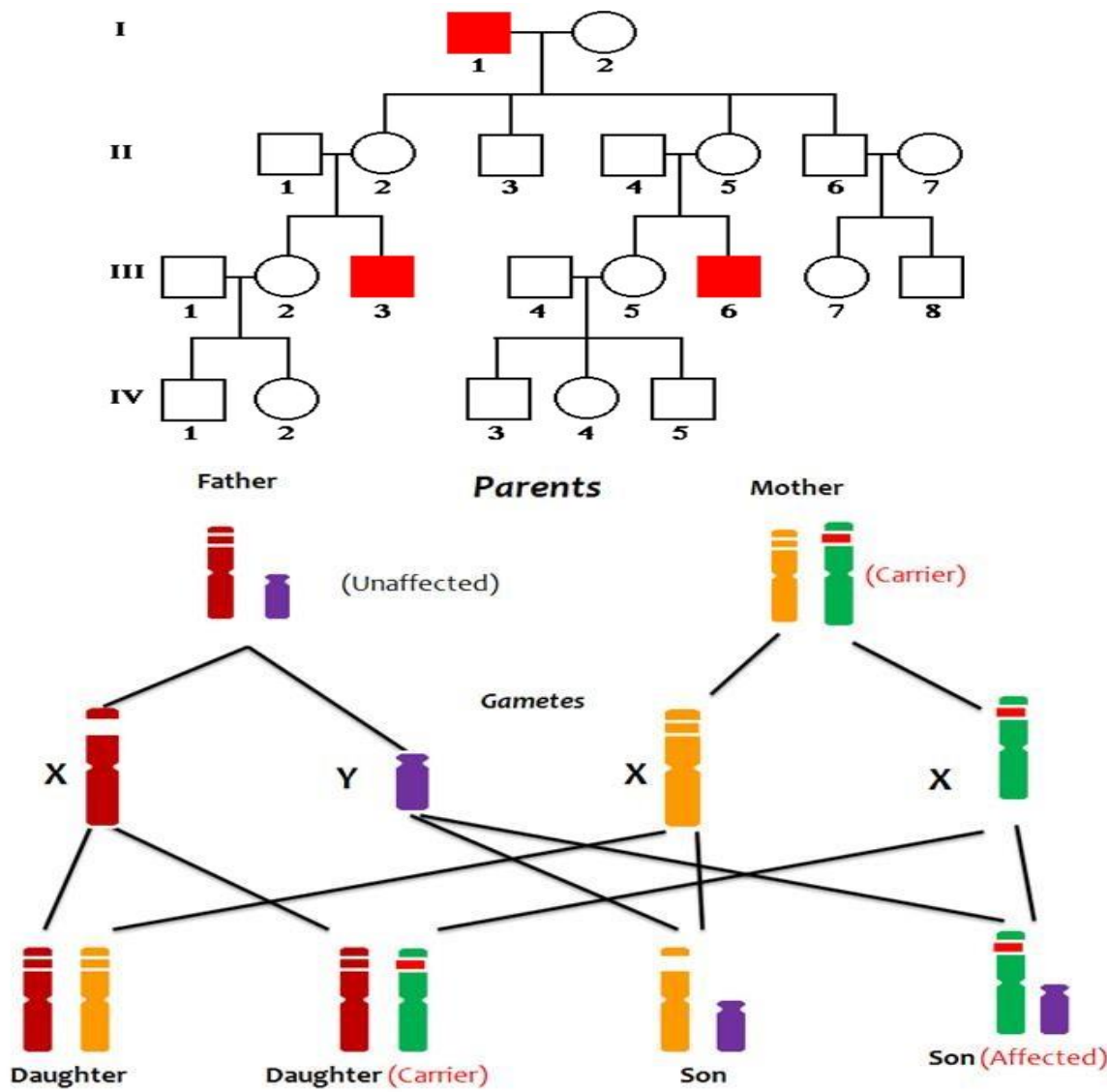
Homozygous Females manifest disease: Females may be homozygous or heterozygous. Homozygous may manifest the disease.

General characteristic ..

- Trait is rare in pedigree.
- Trait skips generations.
- Affected fathers do not pass to their sons.

- Males are more often affected than females.

X-linked recessive disorders



linked recessive: X-linked recessives expressed in all males but only in homozygous females.

X-linked color blindness

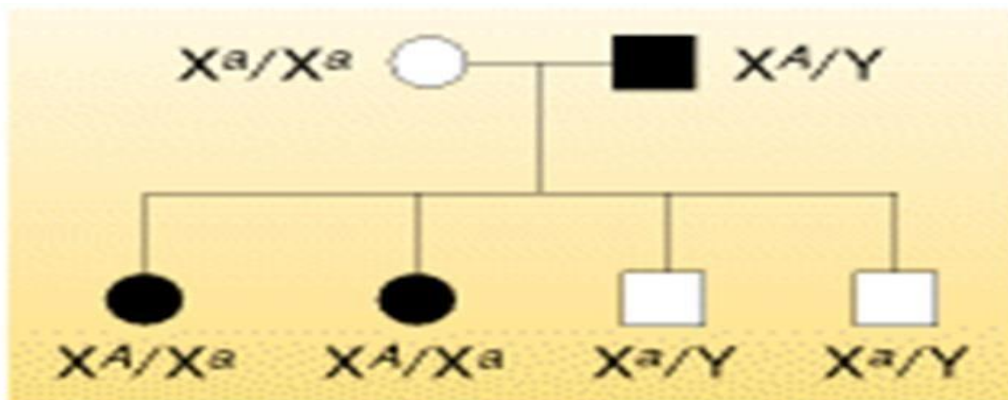
Common X-linked disorders

- Duchene Muscular Dystrophy
- Hunter's Disease
- Menkes Disease
- Hemophilia A and B
- Color Blindness

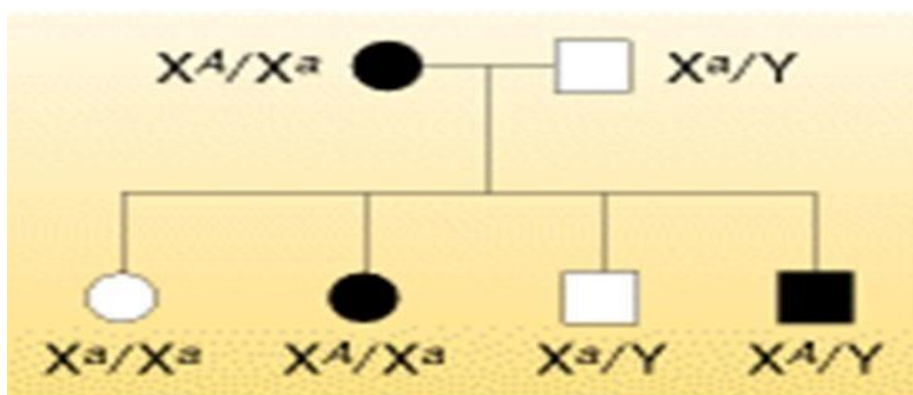
151 X-linked dominant inheritance

linked dominant inheritance and disorders

Affected males transmit disease to all daughters but none of sons

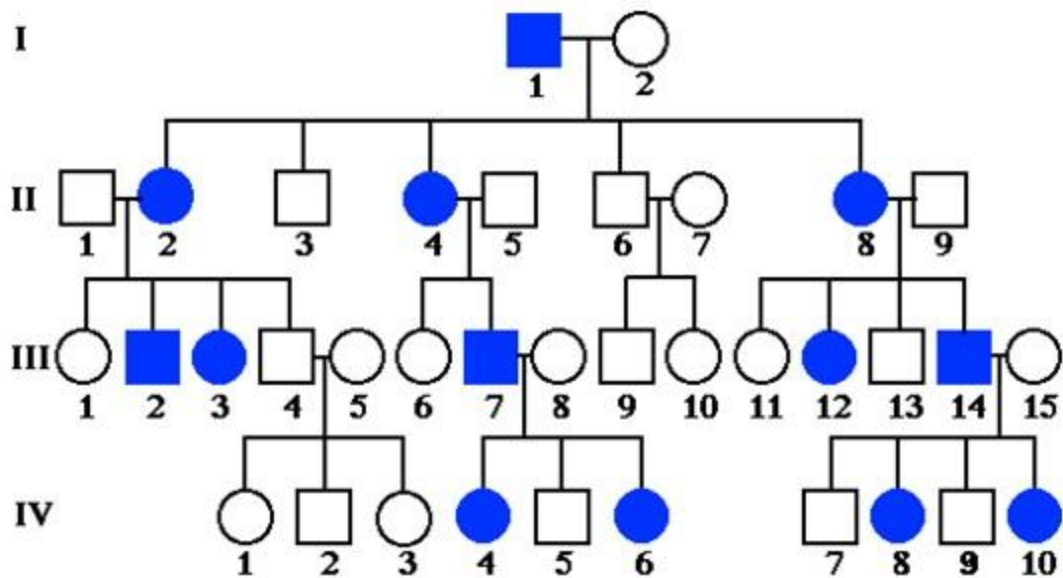


Affected heterozygous females and un-affected males pass the disease to half their sons and daughters



linked dominant diseases: Affected fathers pass to all of their daughters.

Males and females are equally likely to be affected.



Common X-linked dominant diseases

- Rett syndrome
- Alport syndrome
- Goltz syndrome
- Fragile-X Syndrome

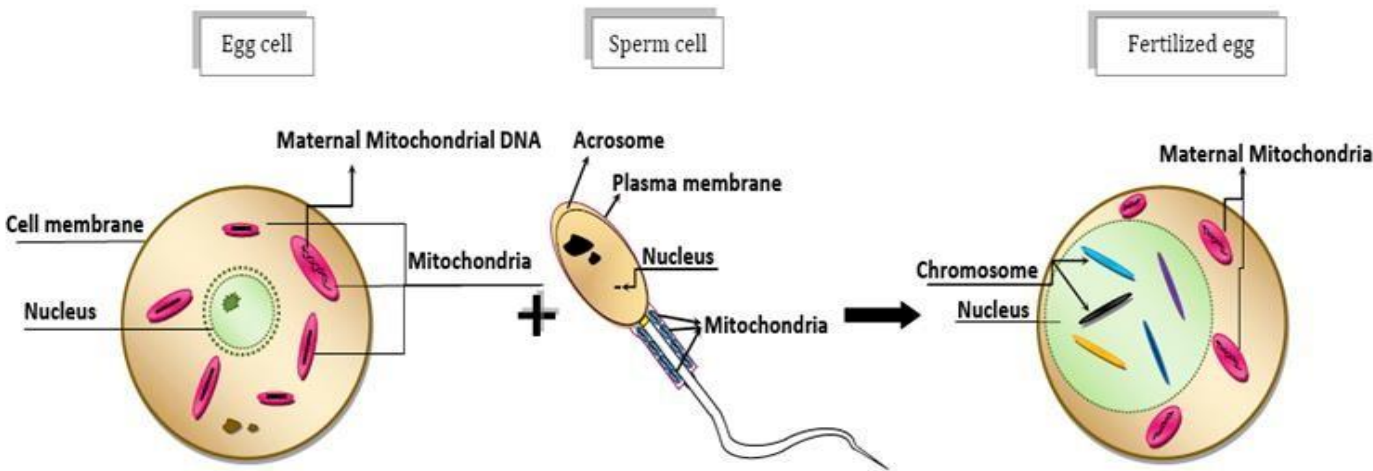
152 Maternal inheritance of mt DNA

Maternal inheritance of mitochondrial DNA

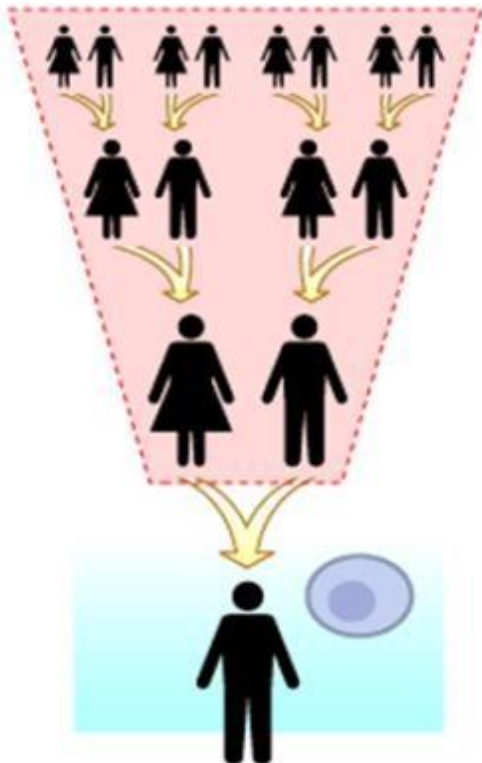
- Mitochondrial DNA (MTDNA) is inherited through ovum not sperm □
- Mother could pass it to all children.
- Father will pass it to none of the children.

Mitochondrial DNA

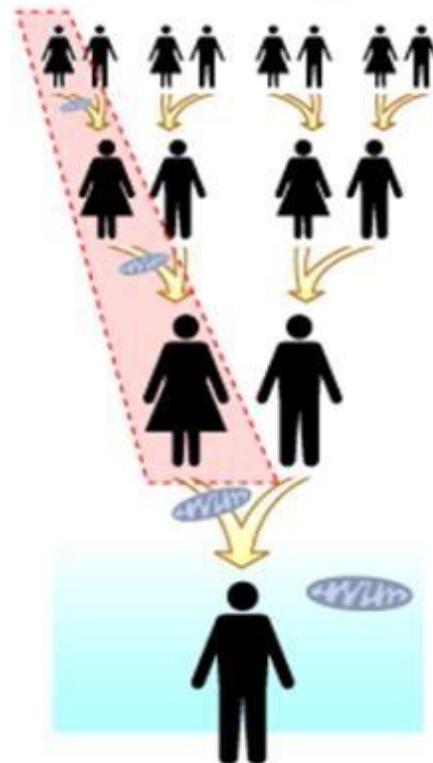
- More than one copy of mtDNA is passed.
- Mutations are common.
- More than one type of mtDNA genome is passed.



Nuclear DNA is inherited from all ancestors.



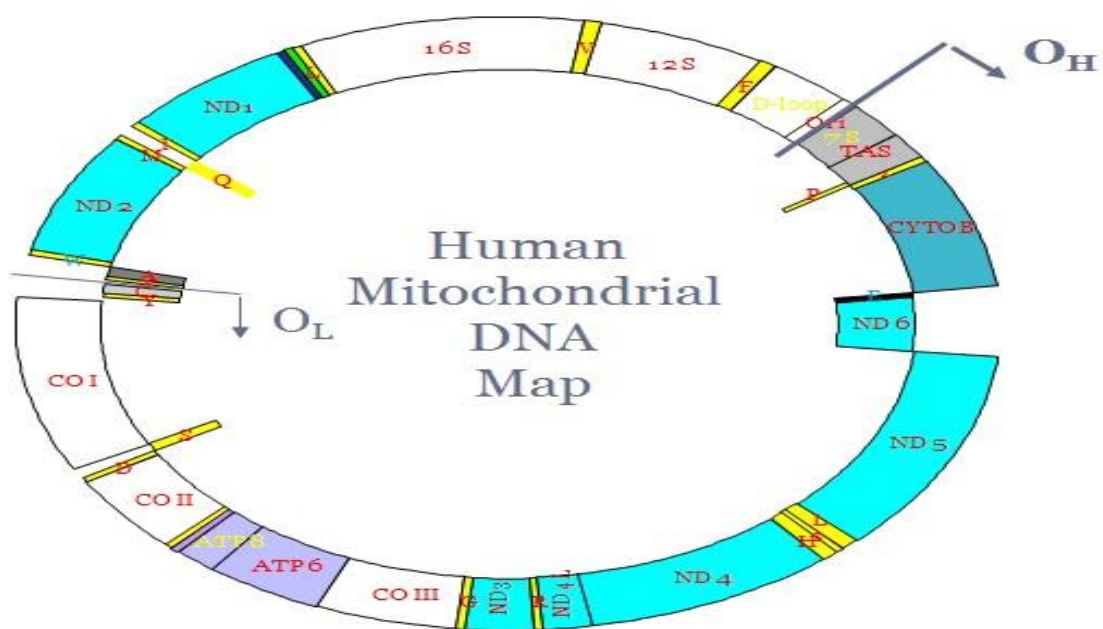
Mitochondrial DNA is inherited from a single lineage.



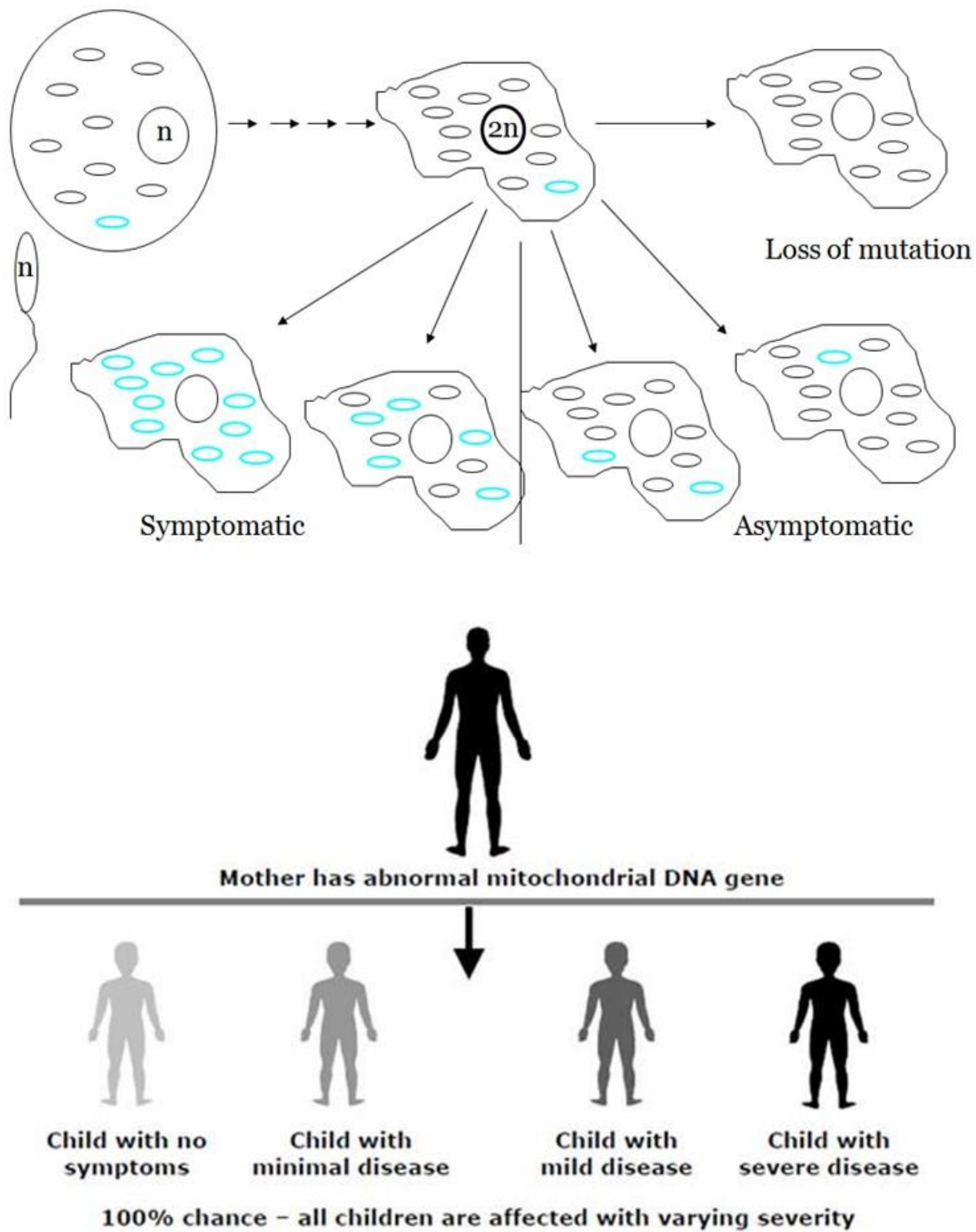
Properties of Mitochondrial inheritance

- Heteroplasmy
- Variable expression
- Pleiotropy
- Reduced penetrance
- Functional somatic mosaic

Mitochondrial DNA



Mitochondrial DNA – Severity



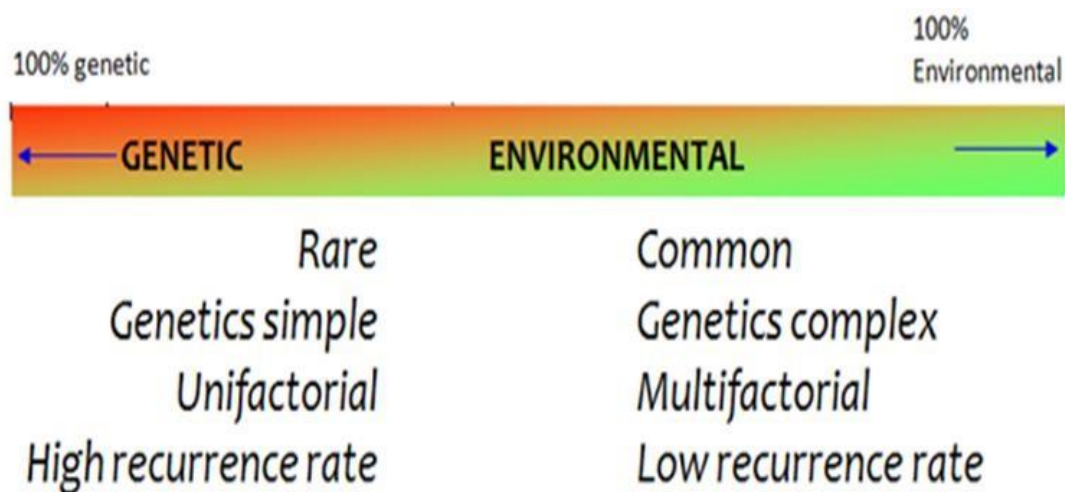
Common mitochondrial diseases

- Myoclonic epilepsy
- Mitochondrial recessive ataxia
- Leber hereditary optic neuropathy
- Sensory ataxia neuropathy

153 Multifactorial inheritance

Multifactorial inheritance and disorders

Multifactorial diseases: Genetic disorders controlled by many genes plus the effects of the environment.



Multifactorial diseases

- Asthma
- Cancers
- Ciliopathies
- Cleft palate
- Diabetes
- Heart diseases
- hypertension
- inflammatory bowel disease
- intellectual disability
- mood disorder
- Obesity
- infertility

154 Frequency of genetic disorders

Frequency of genetic disorders

Frequency of autosomal dominant disorders

Frequency of autosomal dominant disorders varies between 3 – 9.5 per 1000 individuals

Frequency of autosomal recessive disorders

Frequency of autosomal recessive disorders is between 2-2.5 per 1000 individuals.

Frequency of x-linked disorders

Frequency of X-linked disorders is between 0.5 – 2 per 1000 individuals.

Frequency of chromosomal disorders

Frequency of chromosomal disorders is between 6-9 per 1000 individuals.

Frequency of multifactorial disorders

Frequency of multifactorial disorders is between 20 – 50 per 1000 individuals.

Most common multifactorial disorders are cancers, diabetes, heart diseases.

155 Diagnostic tests for genetic disorders

Diagnostic tests for genetic disorders

Physical analysis: Physical analysis for affected individual.

Ultrasonography of fetus: Ultrasonography of fetus determines malformations of head, internal organs, and extremities.

Analysis of amniotic fluids: Amniotic fluid analysis to determine genetic and chromosomal disorders after 14 wks gestation. More than 200 various genetic diseases easily be detected.

Maternal blood analysis: Maternal blood analysis to observe abnormal fetus.

156 Genetic counseling

Genetic Counseling

Genetic counseling and background history of parents

A genetic counselor usually begins with a complete family history of both prospective parents.

Genetic counseling and pedigree drawing/ analysis

A complete, detailed family history is called a pedigree. Pedigrees are used to determine the pattern of inheritance of a genetic disease within a family.

Genetic counseling Genetic counselor can inform prospective parents of the possibility of having genetically abnormal offspring, and they can make an informed decision.

Genetic counseling and molecular tests Genetic counselor advise them to perform molecular tests for relevant disease in the parents.

Gene therapy A procedure that involves identification, manipulation, and transference of genetic segments into a host to replace defective genes and to perform desired genetic activities.

157 Complications to basic inheritance pattern

Complications in basic inheritance pattern

Single genes -Non-mendelian inheritance

Differences in gene actions can generate more complex inheritance patterns.

Single gene can give codominance, incomplete dominance, overdominance.

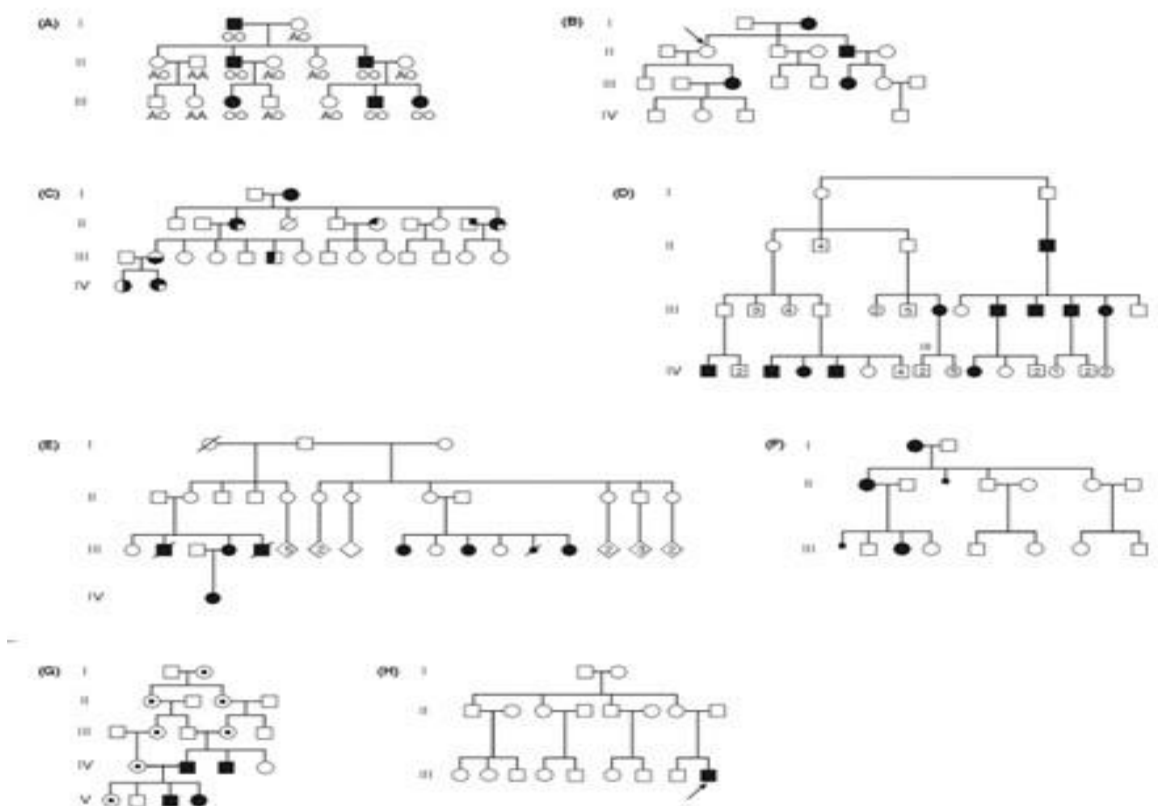
Complications to inheritance patterns

Multiple genes - epistasis, polygenic traits Genes & the environment - sex-influenced traits, incomplete penetrance

Complications

- A common recessive inheritnace can give dominant pattern
- Autosomal dominant inheritance with non-penetrance
- Autosomal dominant inheritance with variable expression.

Pedigrees with complicated inheritance pattern



Complications

- A common recessive can give dominant pattern
- Autosomal dominant inheritance with non-penetrance
- Autosomal dominant inheritance with variable expression

158 Drawing pedigree

Drawing pedigree

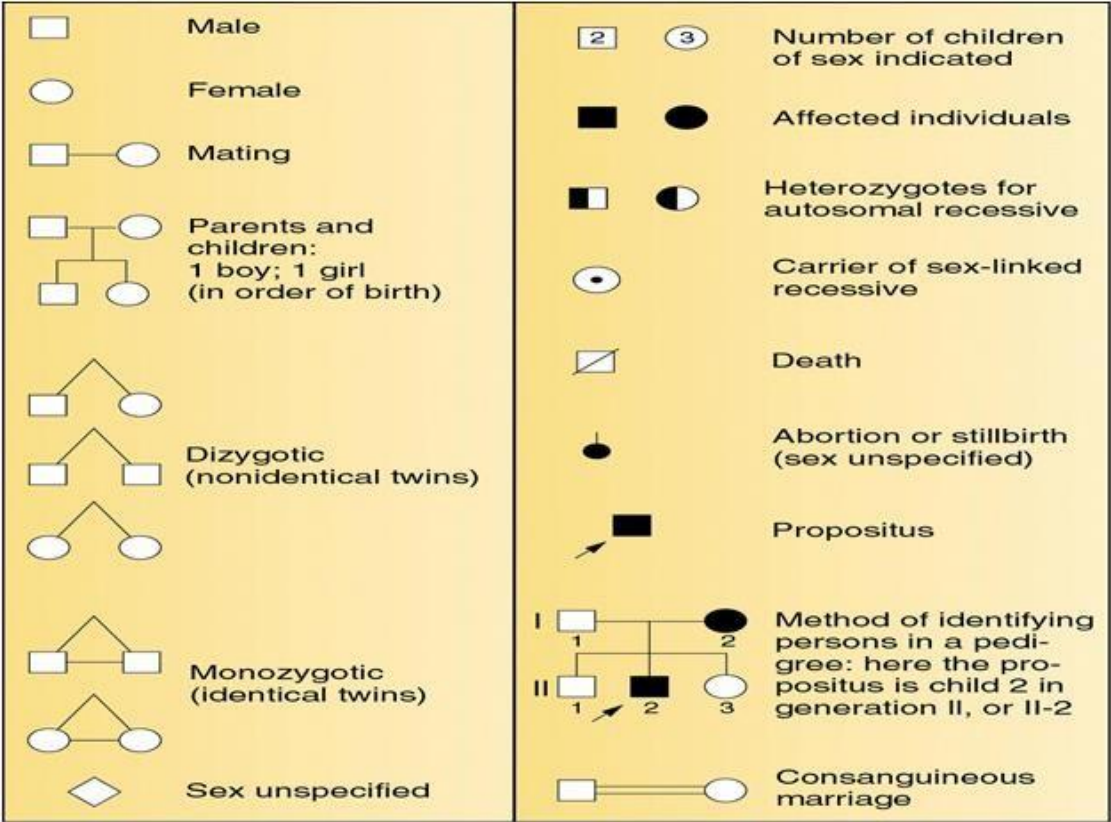
Generations of a pedigree as roman numerals

Generations in a pedigree diagram are numbered, by using Roman numerals, starting with the parental generation, at the top of the diagram as generation I.

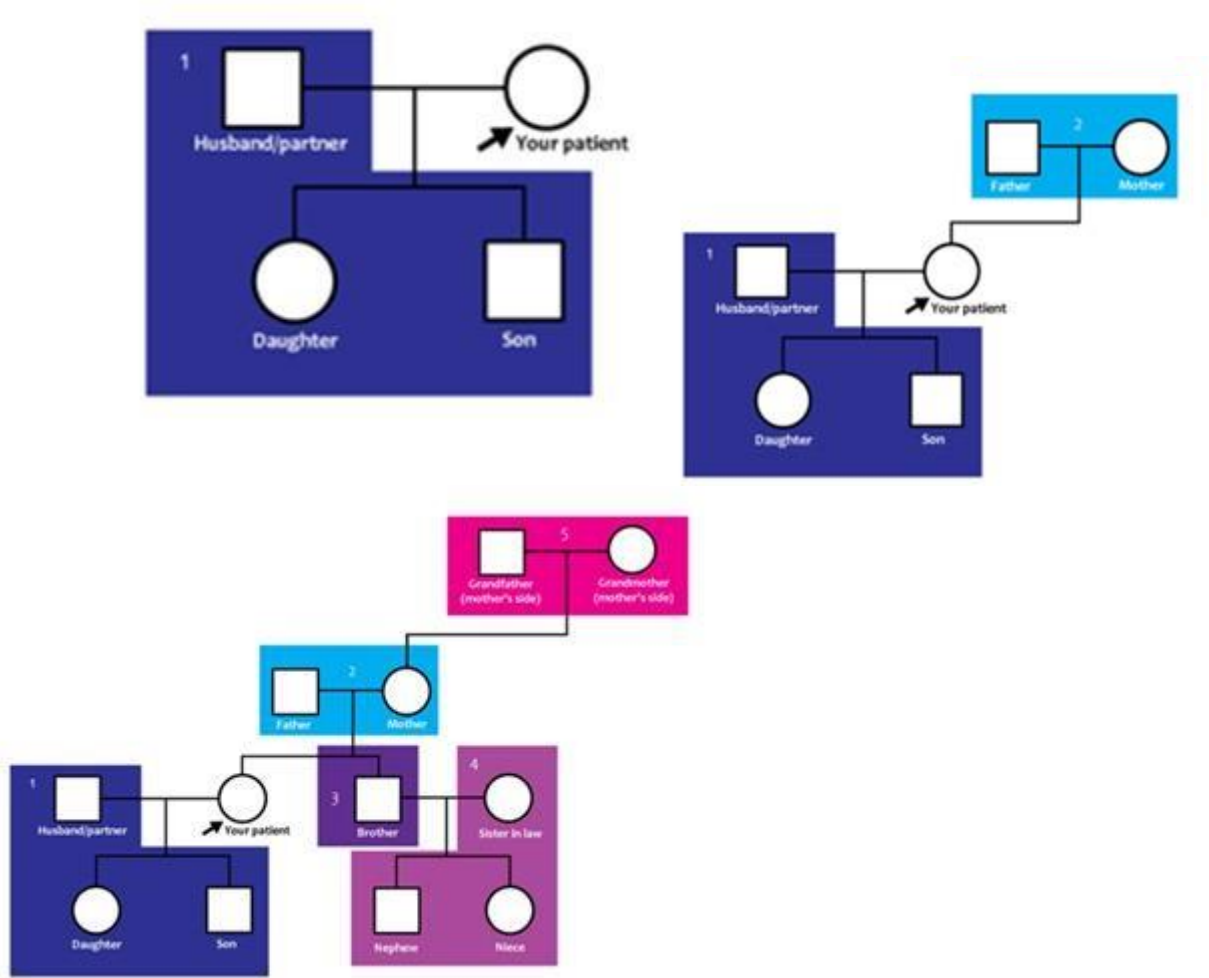
Members of each generation are numbers from left to right

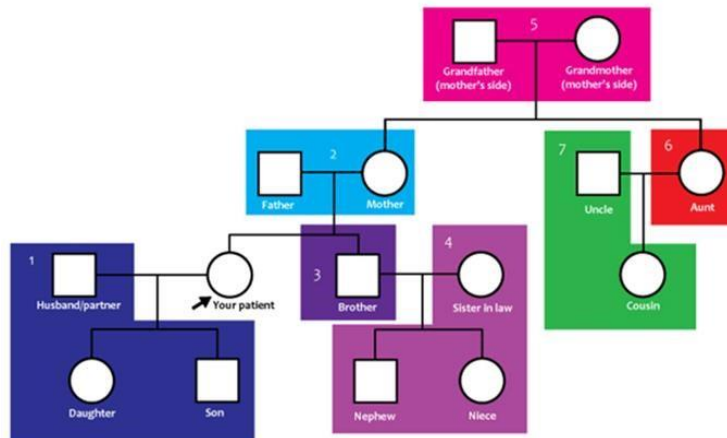
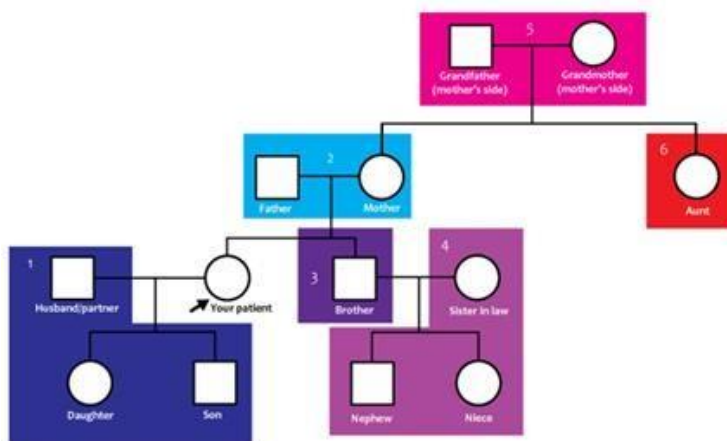
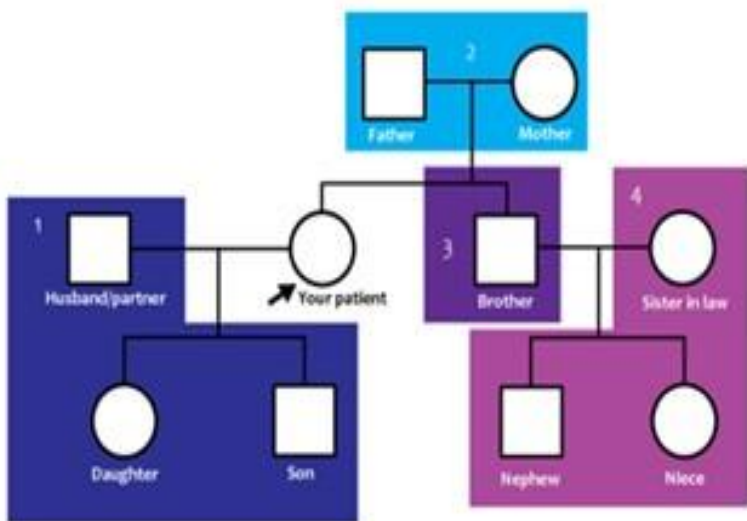
For convenience, the members of each generation are numbered across the line, from left to right, using normal numerals.

Drawing pedigree



Steps for drawing pedigree





Drawing pedigree

Pedigree drawing helps in understand the pattern of inheritance of the disease.

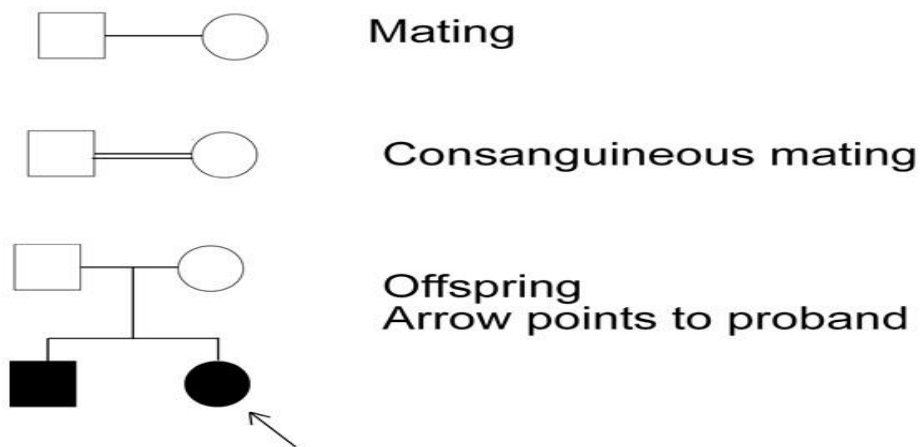
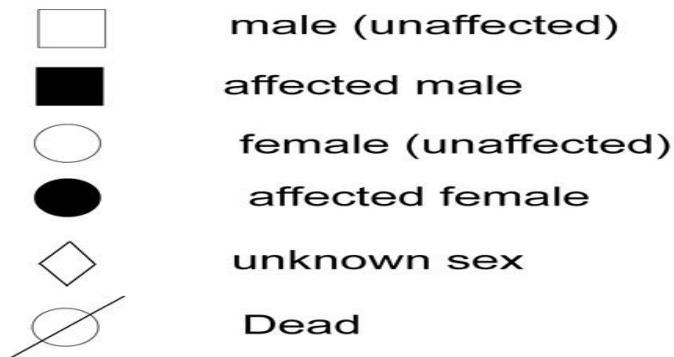
159 Pedigree analysis

Pedigree analysis

Goals of pedigree analysis

- Determine the mode of inheritance: dominant, recessive, sex-linked.
- Determine the probability of an affected offspring for a given cross.

Basic symbols



Pedigree analysis: Pedigree analysis helps to determine mode of inheritance.

160 What is polymorphism

DNA polymorphism

- DNA polymorphism is a sequence difference compared to a reference standard present in at least 1–2% of a population.

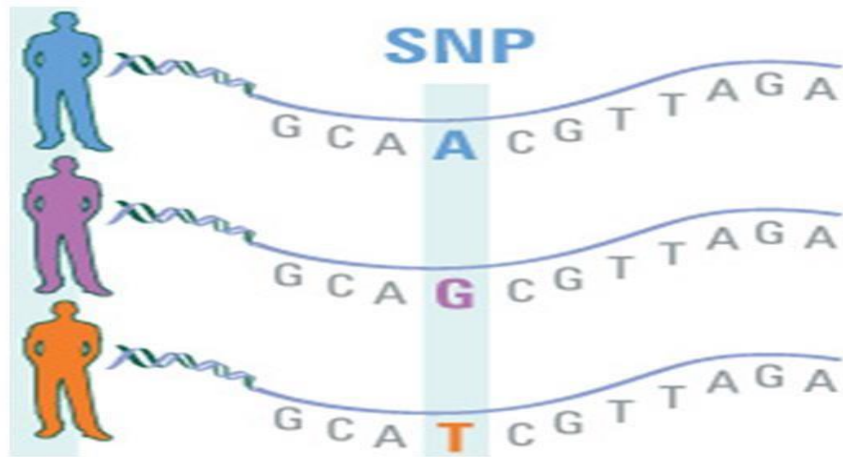
DNA Polymorphism may be single nucleotide or thousands of BASES □

Polymorphisms can be due to a single base or thousands of bases.

DNA Polymorphism may or may not have phenotypic effects □

Polymorphisms may or may not have phenotypic effects.

DNA POLYMORPHISM - SNP



DNA Polymorphism are found throughout the genomes □

Polymorphisms are found throughout the genome.

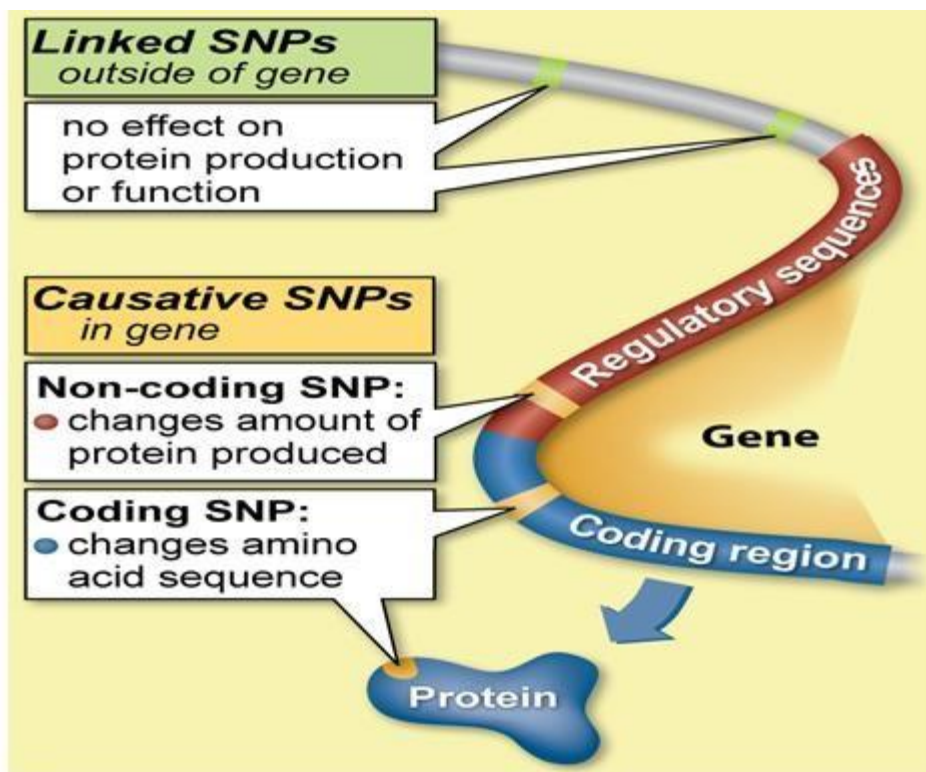
Polymorphic sequence can serve as marker

- If the location of a polymorphic sequence is known, it can serve as a marker for locating other genes or genetic regions.

Polymorphic markers have different versions

- Each polymorphic marker has different versions or alleles.

Polymorphisms can be silent, or be exhibited at levels of Proteins



Polymorphism - why to study

Essential to study inheritance patterns. Map phenotypes and anchor genes to the genetic map by co-segregation analysis. Determine change in function: resistant/sensitive populations genetically determined variability among humans is due to a difference in 0.1% of the genomic sequence.

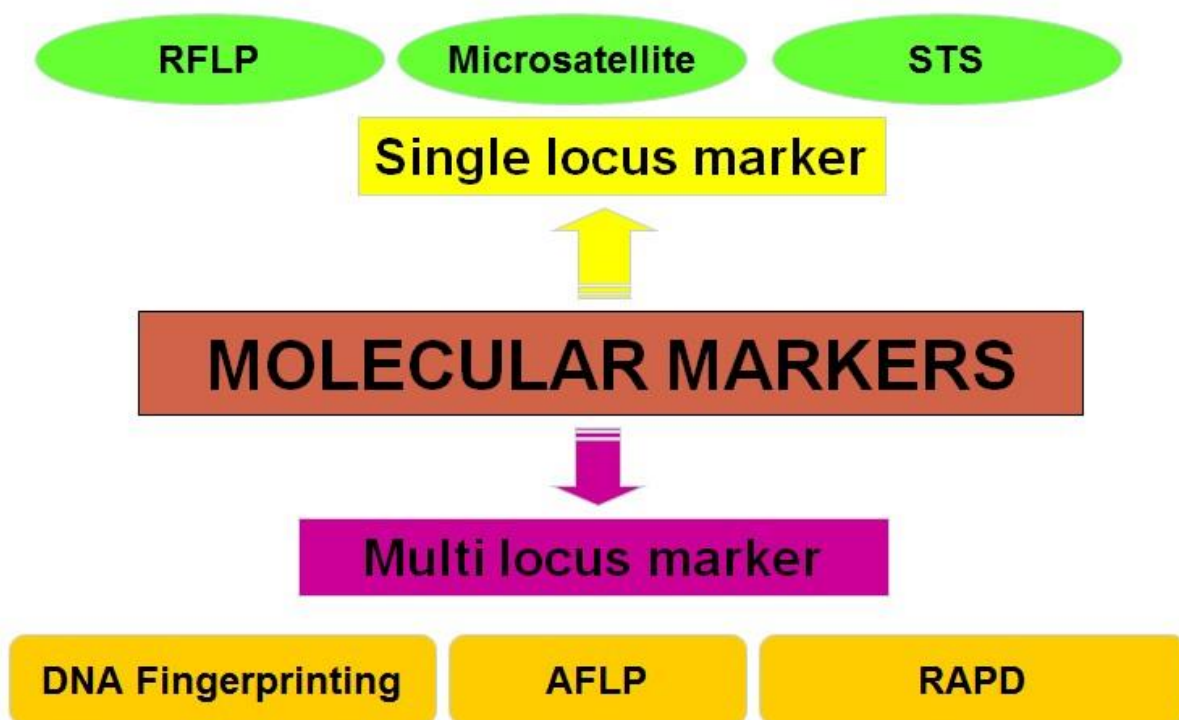
161 Types of polymorphism

Types of Polymorphic DNA Sequences

- RFLP: Restriction Fragment Length Polymorphism
- VNTR: Variable Number Tandem Repeats (8 to > 50 base pairs)

Types of Polymorphic DNA Sequences

- STR: Short Tandem Repeats (1– 8 base pairs).
- SNP: Single Nucleotide Polymorphisms
- AFLP: Amplified Fragment Length Polymorphism



Applications of molecular markers – short range

- Parentage determination
- Genetic distance estimation
- Determination of twin zygosity
- Sexing of pre-implanted embryos
- Identification of disease carriers

Applications of Molecular Markers – Long Range

- Gene mapping & mapping of QTL by linkage
- Markers assisted selection of breeds

Polymorphic DNA sequences in humans □ In

humans, 99.9 percent bases are same.

- Remaining 0.1 percent makes a person unique.
- These variations can be harmless and harmful.

162 Restriction fragment length polymorphism

Restriction Fragment Length Polymorphism

What is RFLP: RFLP is a difference in homologous DNA sequences that can be detected as fragments of different lengths after digestion of the DNA samples.

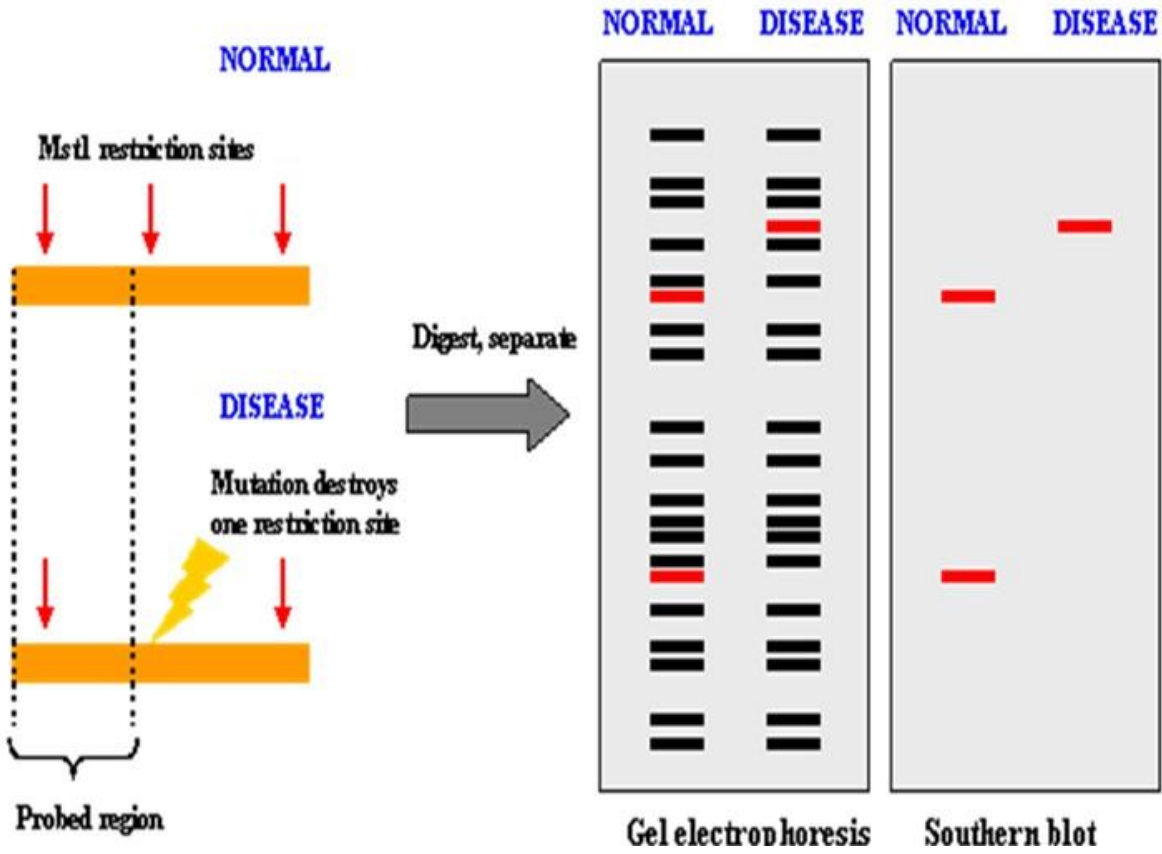
Most RFLP markers are heterozygous and highly locus-specific.

RFLP probe is a labeled DNA sequence that hybridizes with one or more fragments of the digested DNA sample after they were separated by gel electrophoresis.

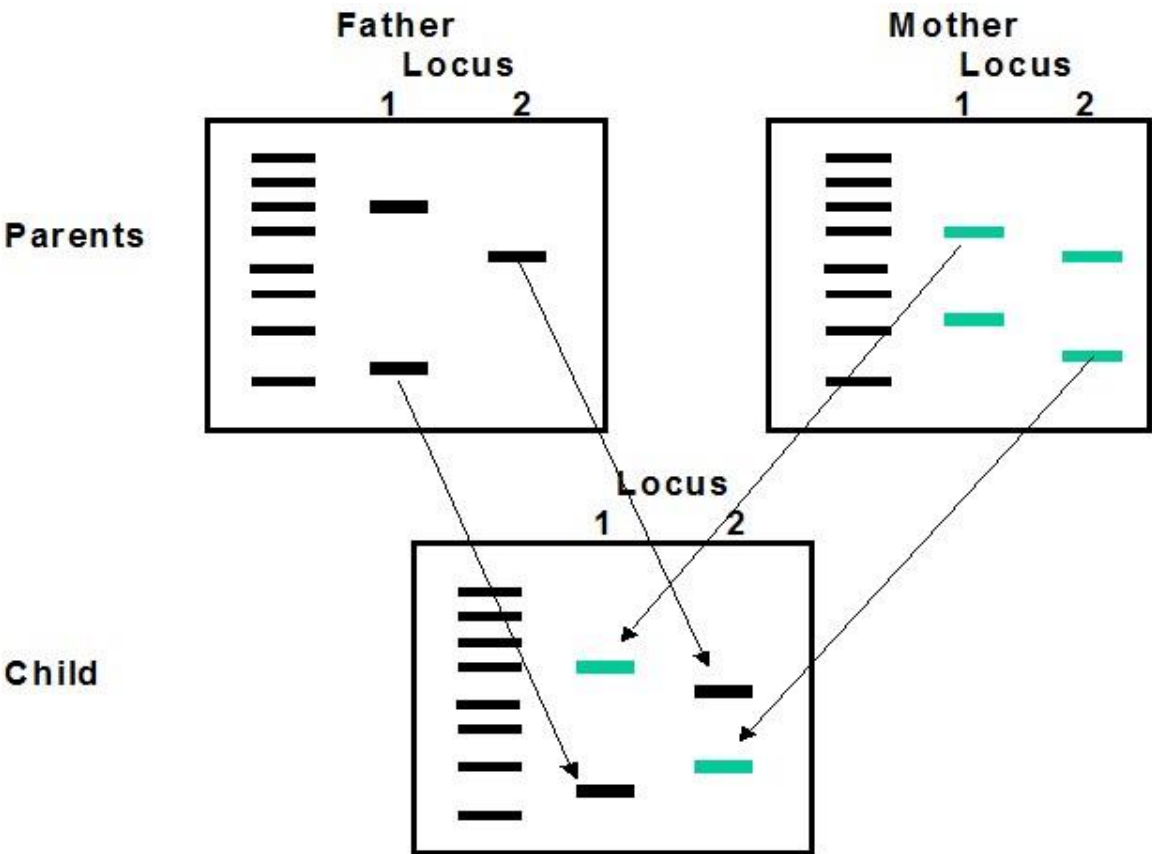
Methodology

- Genomic DNA digested with restriction enzymes.
- DNA fragments separated via electrophoresis and transfer to nylon membrane.
- Membranes exposed to probes labeled with P32.

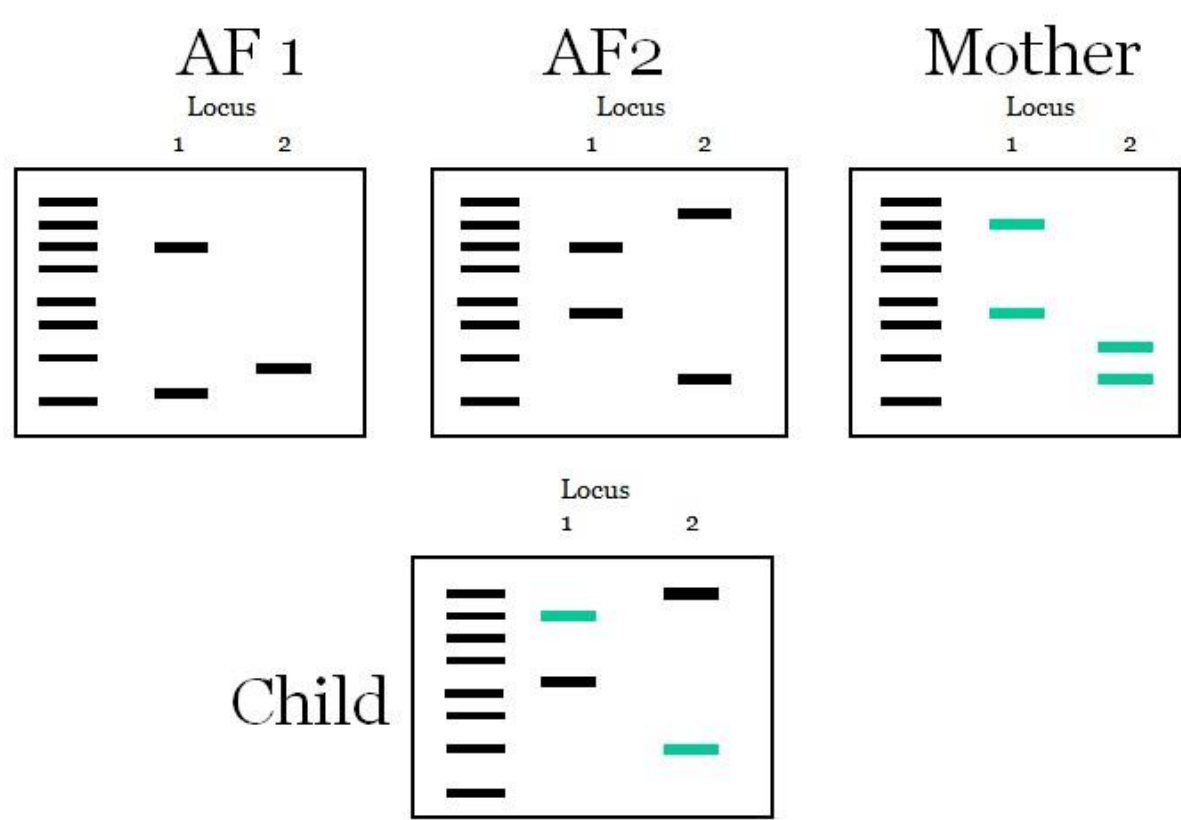
- Film exposed to X-Ray.



Paternity analysis - RFLP



Who is father of child?



Paternity Cases – DNA forensic applications

- RFLPs can be used in paternity cases or criminal cases to determine the source of a DNA sample. (i.e. it has forensic applications).

RFLP used for constructing genetic maps

- RFLPs can be used to measure recombination rates which can lead to a genetic map with the distance between RFLP loci.

Disease resistant genes may be analyzed by RFLP

- Direct method for selecting desirable genes such as disease resistance genes.

RFLP used to determine disease status

- Determine disease status of an individual, i.e.
- Huntington
- Cystic fibrosis
- Sickle cell anemia

Mutation detection

- RFLPs can be used to determine the disease status of an individual; (e.g. it can be used in the detection of particular mutations).

163 VNTR

Variable Number Tandem Repeats

VNTR

- VNTR are short nucleotide sequences organized as a tandem repeats on genomes.
- Found on many chromosomes
- Show variations in length between individuals.

Size of VNTR

- The size of repeat is about 7 to few tens of base pairs long.

Each variant acts as inherited allele

- Each variant acts as an inherited allele, allowing them to be used for personal or parental identification.

VNTR

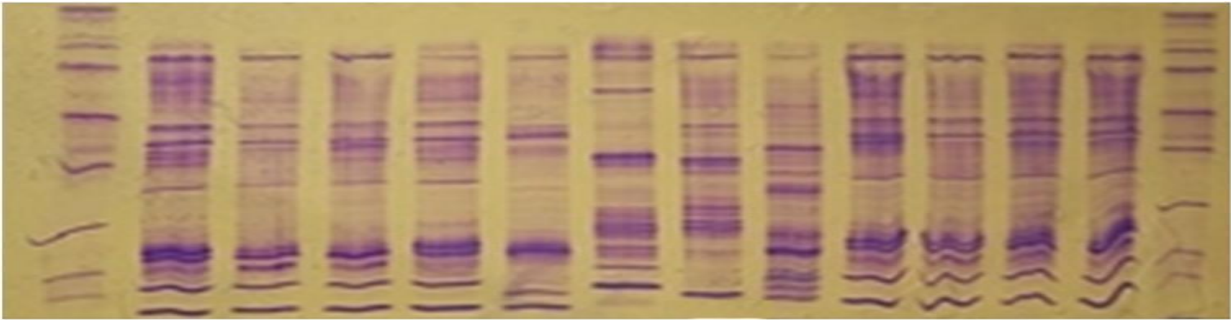
- The rectangular blocks represent each of the repeated DNA sequences.
- The repeats are tandem - they are clustered together and oriented in the same direction.



VNTR - Methodology

- Restriction enzymes are used to cut the VNTR blocks from its specific sites and analyzed by RFLP.
- Blocks (VNTR) are amplified by polymerase chain reaction.
- Amplified products are run on gel electrophoresis which also determined its length.

VNTR – variations in patterns of banding



Importance of VNTR

VNTRs are an important source of RFLP genetic markers used in linkage analysis (mapping) of genomes. When removed from surrounding DNA by the PCR or RFLP methods, and their size determined by gel electrophoresis or Southern blotting. As a result, they produce a pattern of bands unique to each individual.

Basic genetic principles during VNTR data analysis

Identity Matching: VNTR alleles from a specific location must match. If two samples are from the same individual - same allele patter

Inheritance Matching: Matching an individual with his/her parents or children, a person must have an allele that matches one from each parent.

Applications of VNTR

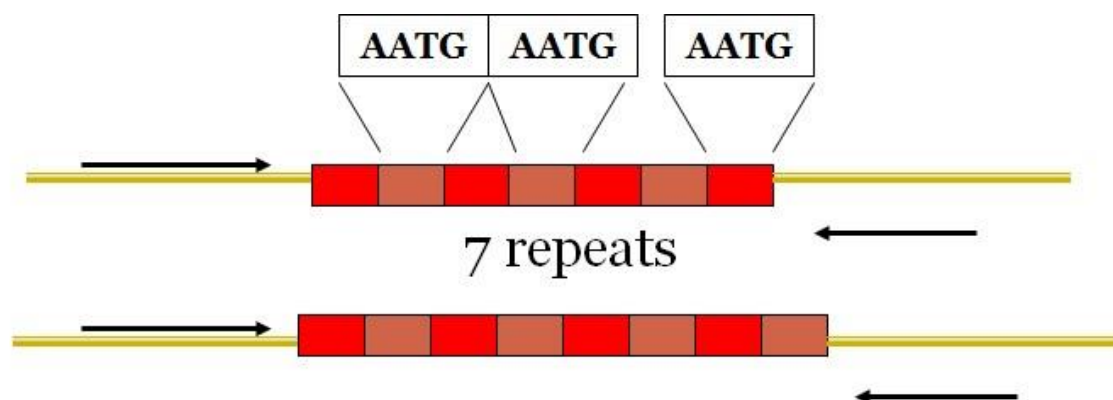
- Microbiology
- DNA fingerprinting
- Genetic diversity
- Forensics
- Mapping of genomes
- Breeding patterns of wild or domesticated animals

164 Short tandem repeat polymorphism

Short Tandem Repeat Polymorphism

Short tandem repeat sequences

- STR are repeats of nucleotide sequences.
- AAAAAA... mononucleotide
- ATATAT... dinucleotide
- TAGTAGTAG...trinucleotide
- TAGTTAGTTAGT... tetranucleotide
- TAGGCTAGGCTAGGC... pentanucleotide
- Different alleles contain different numbers of repeats.

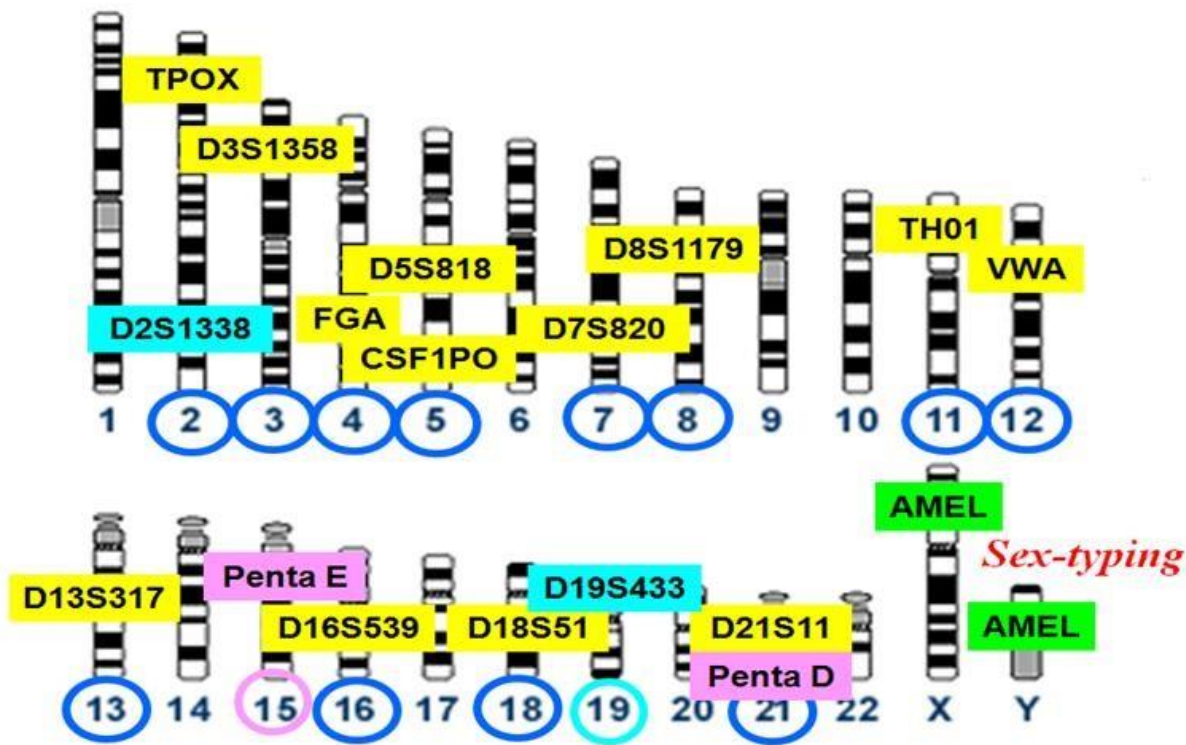


Repeat region is variable between samples while the flanking regions where PCR primers bind are constant

Homozygote = both alleles are the same length

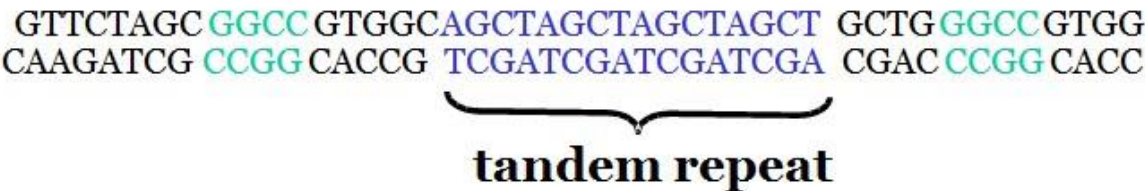
Heterozygote = alleles differ and can be resolved from one another

STR markers - DNA forensics



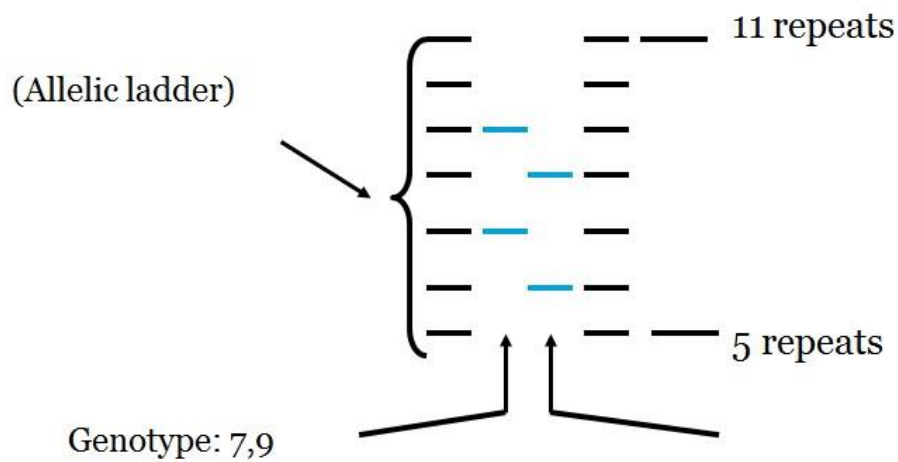
Short tandem repeat sequence

Allele 1	STR Marker	#Alleles	repeat sequence
Short tandem	CSF1PO	11	
	FGA	19	
	TH01	7	
	TPOX	7	
	VWA	10	
	D3S1358	10	
	D5S818	10	
	D7S820	11	
	D8S1179	10	
	D13S317	8	
	D16S539	8	
	D18S51	15	
	D21S11	20	



Allele 2





Short tandem repeat sequences

- STR is simple repeats of nucleotide sequences.
- Mononucleotide, dinucleotide
- Used in linkage analysis □ Used in DNA forensics.

165 RAPD and AFLP

Random Amplified Polymorphic DNA

&

Amplified Fragment Length Polymorphism

Random Amplified Polymorphic DNA

- PCR based marker with 10-12 base pairs
- Random amplification of several fragments
- Amplified fragments run in agarose gel detected by ethidium bromide.
- Unstable amplification leads to poor repeatability

Amplified Fragment Length Polymorphism

- Restriction endonuclease digestion of DNA
- Ligation of adaptors
- Amplification of ligated fragments
- Separation of the amplified fragments via electrophoresis and visualization
- AFLPs have stable amplification and good repeatability

Comparison

Features	RFLP	RAPD	STR	SNP
Detection method	Hybridization	PCR	PCR	PCR
Type of probe/primer used	g DNA/ cDNA sequence of structural genes	Arbitrarily designed primer	Sequence specific primers	Sequence specific primers
Requirement of radioactivity	Yes	No/Yes	No/Yes	No/Yes
Degree of polymorphisms	Low	Medium to High	High	High

Molecular Markers

- Different types of markers used to study diseases
- DNA forensics

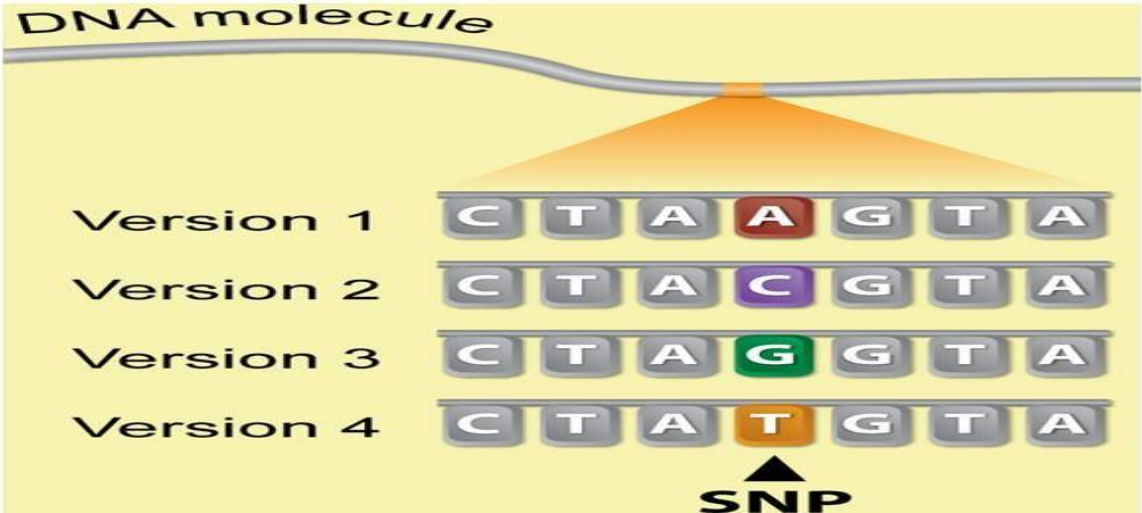
QTL traits in animals and plants

166 Distribution of SNPs

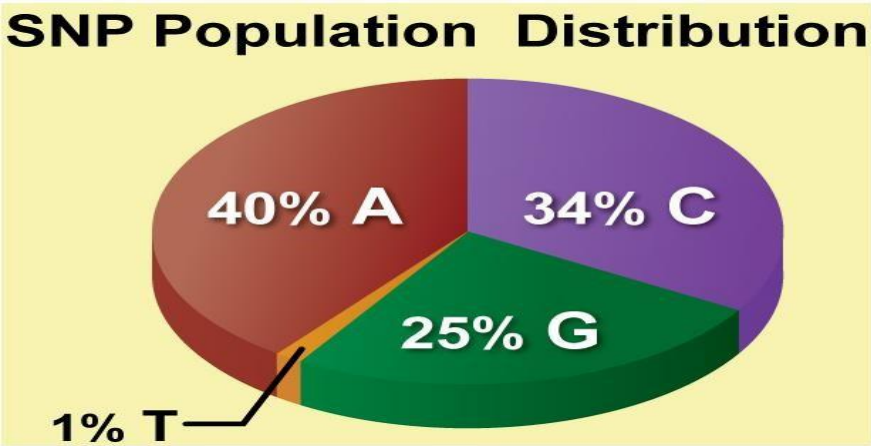
Distribution of SNP

SNP: Genetic variations in a DNA sequence when a single nucleotide in a genome is altered.

Single Nucleotide Polymorphism



SNP



HUMANs SNP DISTRIBUTION

- 25,000 Non-synonymous
- 50,000 Synonymous
- 25,000 Regulatory region
- 50,000 Intragenic
- 50,000 Distributed intergenic SNPs

Human SNP Distribution

Most common changes

Transitions:

Purines to Purines

Pyrimines to Pyrimidines

Transversion:

Purines to Pyrimidines

Pyrimidines to Purinces

Single-base insertions & deletion (indel)

How SNP are indicated

IUPAC code	Meaning
A	A
C	C
G	G
T	T
M	A or C
R	A or G
W	A or T
S	C or G
Y	C or T
K	G or T
V	A or C or G
H	A or C or T
D	A or G or T
B	C or G or T
N	G or A or T or C

SNP Related Databases

- Androgen Receptor Mutation Database

- Ataxia-Telangiectasia Mutation Database
- Breast Cancer Mutation Database
- Cystic Fibrosis Mutation Database
- Cytokine Polymorphism Database
- Fanconi Anemia Mutation Database
- GM2 Gangliosidoses Database
- Human Ornithine Transcarbamylase Database
- Human Type I and Type III Collagen Mutation Database
- Hypertension Candidate Gene SNP Database

SNP are found throughout the genomes.

167 Screening and importance of SNPs

SNP- Screening and importance

People can be grouped based on SNP profile

- Genome of each individual contains distinct SNP pattern.
- People can be grouped based on the SNP profile.

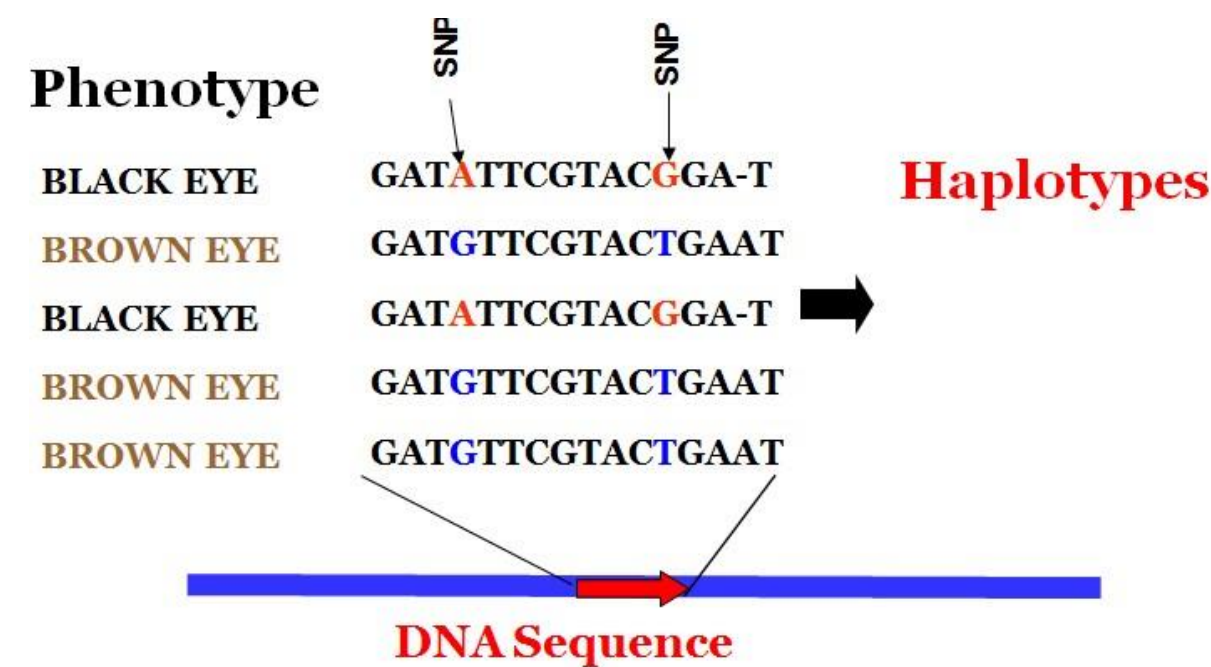
SNP - found in coding and noncoding regions

- SNPs are found in coding and (mostly) noncoding regions.
- Occur with a very high frequency about 1 in 1000 bases to 1 in 100 to 300 bases.

SNP SCREENING methods: Two different screening strategies

- Many SNPs in a few individuals
- A few SNPs in many individuals

SNP Screening- Different strategies will require different tools.



SNP Importance

- In disease diagnosis
- In finding predisposition to diseases
- In drug discovery and development
- In drug responses
- Investigation of migration patterns
- Medication and diagnosis at individual level

168 Genetic bases Problem 1

statement

- Diabetes has been found to be inherited (in many cases) through a recessive allele (d).
- Two parents who are non-diabetic have a diabetic child.
- What would be the genotypes of all three persons?

Answer to Problem

- Both parents are heterozygous (Dd)
- Child is homozygous recessive (dd)

Solution of the problem

Possible genotypes and phenotypes?

Genotype	Phenotype
DD	Normal
Dd	Normal
dd	Diabetic

Answer

- Since diabetes is inherited as a recessive allele, in order to be afflicted with diabetes an individual must be genotypically homozygous recessive (dd).

Parents are heterozygous while affected individual is homozygous.

169 Genetic bases Problem 2

statement

- In corn, there are two alleles for the gene for sugar contents.
- High sugar content (su) is recessive to low sugar content (Su).

Problem

Give the phenotypes for each of the following genotype:

Susu
susu

SuSu

answer

- SuSu low sugar
- Susu low sugar
- susu high sugar

Solution

- **SuSu low sugar (Su, dominant)**
- **Susu low sugar (Su, dominant)**
- **susu high sugar (su, recessive)**
- **When both are recessive alleles (susu), there would be high sugar contents.**

Answer

- **When both are recessive alleles (susu), there would be high sugar contents.**

170 Genetic bases Problem 3

statement

□ A man with blood group A, marries a woman with blood group B.
Their child has group O blood.

What are the genotypes of parents? What other genotypes and in what frequencies would you expect in offspring from this marriage?

solution

- We know, that the ABO blood type exhibits both a multiple allele phenomenon and codominance

explanation

Genotype	Phenotype
$I^A I^A$	A
$I^A i$	A
$I^B I^B$	B
$I^B i$	B

$I^A I^B$ AB $i i$ O

explanation

- In order for someone to have blood group type O, genotypically individual must be $i i$

answer

- Child must have received one i allele from one parent and the other i allele from the other parent.
- Father must be $I^B i$ and the mother must be $I^A i$ or vice versa

Answer

Father must be $I^B i$ and the mother must be $I^A i$ or they would be vice versa

171 Genetic bases Problem 4

statement

- A patient came to a hospital with following clinical symptoms-
- Hypotonia
- Increased sleepiness
- Mental retardation
- Small stature

Craniofacial findings

- Upward slanting eyes
- Protruding tongue
- Low set ears
- Flat nose

Laboratory finding

- Laboratory findings show Trisomy 21
- Increased maternal age

disease

Relating to above features what are the possible syndromes

- ☐ Turner Syndrome
- ☐ Down Syndrome
- ☐ Edward's Syndrome

answer

- **Down Syndrome**
- **Down syndrome is due to trisomy of chromosome 21**

172 Genetic bases Problem 5

statement

- ☐ A patient came to an OPD with monosomy having clinical features as follows

statement

- ☐ Short stature of the hands and feet
- ☐ Broad chest
- ☐ Widely spaced nipples
- ☐ Low hairline
- ☐ Sterility
- ☐ Monosomy (45 X)

statement

- What would be possibility of syndrome ?

Turner syndrome

Down syndrome

Klinefelter syndrome

Answer

- **Turner Syndrome**
- **In Turner syndrome, there is monosomy with 45X chromosome.**

173 Genetic bases Problem 6

statement

- What pattern of inheritance would lead a geneticist to suspect that an inherited disorder of cell metabolism is due to a defective mitochondrial gene ?
- The disorder would always be inherited from the mother because the mother's mitochondrial gene is the only one that survives when the zygote is formed.

soluton

- The gamete from the mother contains all the information.
- The head of the father's sperm is the only part that survives during fertilization.

solution

- The tail of sperm containing male's mitochondria is lost when zygote begins development.
- It is only from mother that disorder can be inherited.

Answer

If gene is present on mitochondrial DNA.
It is only mother that disorder can inherit to children.

174 Genetic bases Problem 7

statement

- Skin color in a fish is inherited via a single gene with four different alleles.
- How many different genotypes would be possible in this system? List them ?
- 10 possible genotypes

Gene designation = C

Alleles = C^A, C^B, C^C, C^D □

All possible genotypes

answer

C^A C^A C^B C^B
C^C C^C C^D C^D
C^A C^B C^B C^C
C^C C^D C^A C^C

C^B C^D C^A C^D

Answer

- 10 genotypes for a gene that has four alleles.

175 Genetic bases Problem 8

statement

- A man has six fingers on each hand and six toes on each foot.
- His wife and their daughter have the normal number of digits (five fingers).
- Extra digits is a dominant trait.
- What fraction of this couple's children would be expected to have extra digits?

explanation

- ☐ Because the daughter is normal the man's genotype must be heterozygous for the trait so:

X = extra digits (six digits)

x = normal digits (five

digits)

Explanation

x x

X Xx Xx x xx xx

50% of the offspring will be polydactyl

Answer

50% of the offsprings will be polydactyl

176 Genetic bases Problem 9

statement

A man came to a hospital with following:

less facial hair

less body hair

broader hips

Gynecomastia

statement

- Learning or reading impairment
- On Karyotyping the patient shows

47,XXY

statement

What would be most likely the syndrome affecting this person

Down Syndrome

Klinefelter Syndrome

Turner Syndrome

Answer

Klinefelter Syndrome

177 Genetic bases Problem 10

statement

- An aneuploid person is female, but her cells have two Barr bodies.
- What is the probable complement of sex chromosomes in this individual?

answer

- This individual is XXX.
- The individual is a female. Nondisjunction of sex chromosomes produces a variety of aneuploid conditions in humans.
- Extra copies of the X chromosome are deactivated as Barr bodies in the somatic cells.
- Two Barr bodies are due to deactivation of two X chromosomes

178 Genetic bases Problem 11

statement

A patient presented to an OPD having following symptoms

- Poor growth
- Poor weight gain
- Infertility
- Thick, sticky mucus

Statement

- What will be likely the disease pattern in this individual while by Karyotyping/sequencing has shown that there is deletion of three nucleotides ?

Statement

- Down Syndrome
- Sickle cell anemia
- Cystic fibrosis

answer

Cystic fibrosis

179 Genetic bases Problem 12

statement

Male pattern baldness is a recessive sex-linked trait on the X chromosome. A woman, whose father had male pattern baldness, marries a man with this trait.

What is the probability that any son born will have pattern baldness?

solution

Genotype Phenotype

X^BX^B	Normal, female
X^BX^b	Normal, female
X^bX^b	Baldness, female
X^BY	Normal, male
X^bY	Baldness, male

solution

The male in this question is X^bY

The woman has at least one X^b from her father and the homozygous recessive condition is rare, therefore she is most likely X^BX^b .

ANSWER

	X^B	X^b
X^b	X^bX^B	X^bX^b
Y	X^BY	X^bY

The probability that a son will be born with pattern baldness is 50 %

ANSWER

The probability that a son will be born with pattern baldness is 50 %

180 Genetic bases Problem 13

statement

Achondroplasia, a form of dwarfism, is inherited as a dominant condition.

Two dwarfs, both of whom had one parent of normal height, marry and plan to have a child.

Their first child is born and is a dwarf. What is the probability that this child is heterozygous?

Solution

Since achondroplasia is due to a dominant allele, the two dwarf parents can either be homozygous dominant (DD) or heterozygous (Dd).

However, each of these dwarfs had a parent that had normal height, (meaning that parents' genotype must have been dd).

They could have only contributed d to the dwarfs in question, dwarfs genotype must be Dd.

ANSWER

The answer to the question is 2/3.

Setting up a Punnett square will predict the potential offspring of these two dwarfs:

ANSWER (2/3 heterozygous)

	D	d
D	DD	Dd
d	Dd	dd

ANSWER

3:1 ratio of dwarf to normal.

Three dwarf individuals

2/3 are predicted to be heterozygous

181 Genetic bases Problem 14

Statement

In humans, normal skin color (A) is dominant over albino (a).

A diabetic albino man marries a normal woman whose mother was an albino and whose father was diabetic.

What are the genotypes of the man and the woman?

What proportion of their children would be expected to be both non-diabetic and have normal color?

Solution

We are looking at two traits in this question.

Man genotype would be aadd (albino, diabetic).

The woman was normal (for both traits) so she must have at least one dominant allele for each trait: A__D__

A__D__ solution

She has a mother that is albino, so her mother could have only supplied her with the recessive allele, a.

Her father was diabetic, so he could only provide her with the recessive allele, d.

Therefore her genotype, with respect to these two traits is AaDd

Solution (1/4 children normal color and non-diabetic)

	ad
AD	AaDd
Ad	Aadd
aD	aaDd
ad	aadd

ANSWER

1/4 of their children will be expected to be non-diabetic and have normal color.

182 Genetic bases Problem 15

statement

Both a husband and wife have normal vision but their son is color blind.

What are the likely genotypes of the boy's parents?

EXPLANATION

Father = X^AY (normal)

Mother = X^AX^a (recessive = colorblind allele; mother has normal vision due to X^A)

answer

Boy = X^aY (recessive from mother = colorblind son)

answer

Boy = X^aY (got recessive allele from mother) He will be color blind.

183 Genetic bases Problem 16

statement

Two babies in a maternity ward have lost their identity bands and there is some confusion about their footprint records.

EXPLANATION

Baby # 1 is Blood group type A

Baby # 2 is Blood group type B

If one of the mothers has blood group type is O, which one of the following statements applies to her ?

EXPLANATION

- a. Neither baby could be yours.
- b. The type A baby is yours.
- c. The type B baby is yours.
- d. Either baby could be yours.

Her blood group is O, so either baby belong to her.

ANSWER

Either of the baby belongs to her.

184 Genetic bases Problem 17

statement

A woman is heterozygous for Tay-Sachs (a recessive disorder).

What are the chances of her giving birth to a child with Tay-Sachs; if the father is normal (homozygous dominant?)

What are the chances of giving birth to a carrier?

answer

Woman: Tt

Man: TT

No chance of child with disease

50% chance of carrier

ANSWER

No chance of child with disease

185 Source and recovery of DNA

SOURCE of DNA

- 1. Blood, Semen
- 2. Saliva, Urine
- 3. Hair root
- 4. Teeth
- 5. Bone
- 6. Tissue
- 7. Cigarette Butts
- 8. Envelope & Stamps
- 9. Fingernail
- 10. Chewing Gum
- 11. Bite Marks

12. Feces

SOURCE of DNA – Blood

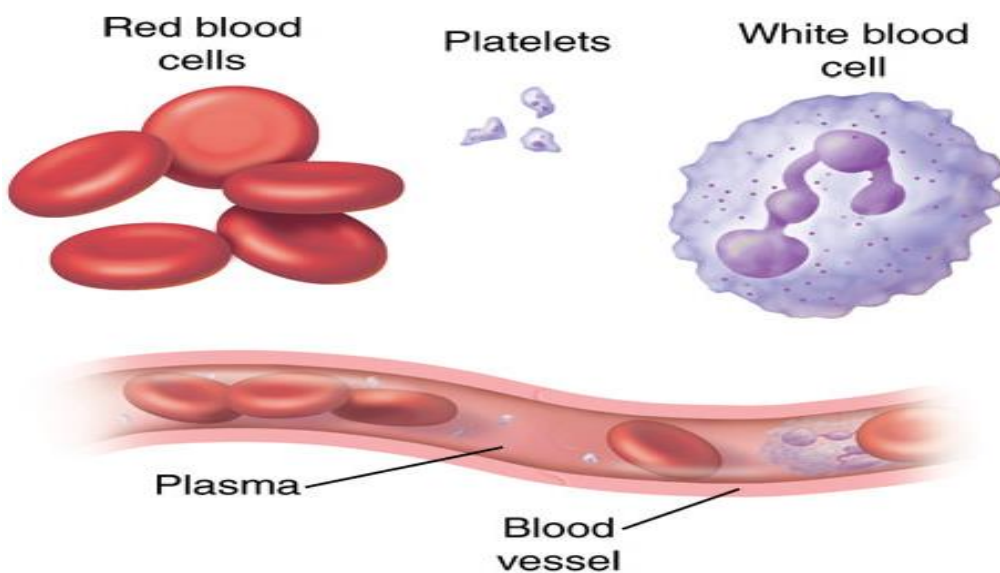
- Red blood cells
- White blood cells
- Platelets

Red blood cells

- Known as Erythrocytes
- Have no nucleus
- Not useful for DNA analysis

WHITE BLOOD CELLS

- Also known as leukocytes
- WBCs have a nucleus
- Useful for DNA analysis



A Diploid cell contains 6 PG OF dna

- A diploid cell contains approximately 6 pg of DNA

A sperm cell contains 3 PG OF dna

- Sperm contains approximately 3 pg of DNA

Recovery of dna

- Theoretical recovery of DNA per ul of blood is 30 - 60 ng.

186 DNA amount for molecular techniques

DNA FOR rflp- 50 ng

RFLP procedure on requires a minimum of 50 ng of high molecular weight double stranded DNA.

DNA FOR rflp

- This is the equivalent of approximately 2 ul of blood.
- The number of intact sperm (3 pg/sperm) is approximately 20,000.

DNA FOR pcr - 1 ng

- PCR reactions – range average 1 - 25 ng of DNA (single or double stranded).
- DNA of high quality 1 ng is sufficient.

DNA FOR pcr

- This is the equivalent of 1/20 of 1 ul of blood, or 350 sperms.
- Many of the commercially available kits are sensitive below 1 ng of DNA (100250 pg).

187 Methods of DNA purification

Different METHODS used

- **Organic (Phenol-Chloroform) Extraction**
- **Non-organic (Proteinase K and Salting out)**
- □ **Chelex (Ion Exchange Resin) Extraction.**

Different METHODS used

- **FTA Paper (Collection, Storage, and Isolation)**
- **Silica Based (Silica exchange resin- Qiagen)**

METHODs utilized

- **The method utilized may be**
- **Sample dependant**
- **Technique dependant**
- **Analyst preference**

188 Organic method reagents

Cell lysis buffer IS USED TO BREAK CELL MEMBRANE

- **Cell lysis buffer designed to lyse outer cell membrane of blood cells and epithelial cells, but will not break down nuclear membrane.**

Cell lysis buffer IS USED TO BREAK CELL MEMBRANE

- **Most lysis buffers contain salts**
- **Tris-HCl**
- **EDTA to regulate the acidity and osmolarity of the lysate,**
- **while detergents (such as Triton X-100 or SDS) are added to break up membrane structures.**

Removal of proteins -Phenol chloroform

- **The key step is the removal of proteins, can often be carried out simply by extracting aqueous solutions of nucleic acids with phenol and/or chloroform.**

Phenol chloroform procedure

- **Phenol–chloroform extraction is a liquid-liquid extraction.**
- **Aqueous samples are mixed with equal volumes of a phenol:chloroform mixture and centrifuged.**
- **Two distinct phases are formed**
- **Aqueous phase is on top (has DNA) because it is less dense than the organic phase (phenol:chloroform). Phenol/ chloroform/isoamyl alcohol**
- **Phenol/Chloroform/Isoamyl Alcohol ratio is 25:24:1 Proteinase k**
- **Proteins are digested by enzymes like proteinase K, which are active against a broad spectrum of native proteins, before extracting with organic solvents.**

Proteinase k and SDS

- **Proteinase K is approximately 10 times more active on denatured protein.**
- **Proteins can be denatured by SDS or by heat.**

Ethanol/ISOPROPANOL

- **Washing of DNA with ethanol**

189 Organic method summary

Cell lysis buffer used to lyse the cell membranes

- Cell Lysis Buffer: lyse cell membrane, nuclei are intact, pellet nuclei.
- Re-suspend nuclei, add SDS, Proteinase K.
- Lyse nuclear membrane and digest protein. Phenol chloroform
- DNA released into solution is extracted with phenol-chloroform to remove proteinaceous material.
- DNA is precipitated from aqueous layer by addition of ice cold alcohol.

Precipitation by ethanol/ISOPROPANOL

- Precipitated DNA is washed with 70% ethanol □ Dried and re-suspended in TE buffer.

190 Organic method procedure (I)

Step 1: Cell lysis and washing

- Cell Lysis Buffer is non-ionic detergent, designed to lyse outer cell membrane of blood and epithelial cells, but will not break down nuclear membrane.
- Washing of blood samples with TE buffer.
- 2-3 washing to get pellets of nuclei (WBCs)
- Step 2: Proteinase K
- It is usual to remove most of the protein by digesting with proteolytic enzymes such as Pronase or proteinase K, which are active against a broad spectrum of native proteins.

prOTEINASE K

- Proteinase K is approximately 10 fold more active on denatured protein. Proteins can be denatured by SDS or by heat.

191 Organic method procedure (II)

Step 3: Phenol chloroform

- The standard way to remove proteins from nucleic acids.
- To extract once with phenol.
- Once with a 1:1 mixture of phenol and chloroform.
- Once with chloroform. Phenol chloroform
- This procedure takes advantage of the fact that deproteinization is more efficient when two different organic solvents are used instead of one.

Phenol chloroform

- Final extraction with chloroform removes any lingering traces of phenol from the nucleic acid preparation.
- Phenol is highly corrosive and can cause severe burns. Organic
- method
- In organic method, phenol chloroform is used to remove the proteins.

192 Phenol chloroform in organic method

What is phenol

- Often means phenol equilibrated with buffer and containing 0.1% hydroxyquinoline and 0.2% b-mercaptoethanol.
- The hydroxyquinoline also gives phenol yellow color, making it easier to identify the layers. What is chloroform
- Chloroform - often means a 24:1 (v/v) mixture of chloroform and isoamyl alcohol.
- The isoamyl alcohol is added to help prevent foaming.
- Phenol/Chloroform/Isoamyl Alcohol ratio is 25:24:1 Precipitation by alcohols
- The most widely used method for concentrating DNA is precipitation with ethanol.
- The precipitate of nucleic acid is recovered by centrifugation.

Organic method

- The technique is rapid and is quantitative even with nanogram amounts of DNA.

193 Role of ethanol and isopropanol

Variables in DNA EXTRACTONS

- The four critical variables are

Purity of DNA

Molecular weight

Concentration

Speed

- Major role of alcohols in DNA precipitations.

ethanol

- DNA concentrations as low as 20 ng/ml will form a precipitate.
- Typically 2 volumes of ice cold ethanol are added to precipitate the DNA.

Difference – Ethanol and Isopropanol precipitation

- DNA is less soluble in isopropanol - fall out of solution faster.
- With ethanol, DNA needs to be at a higher concentration to precipitate but the salt tends to stay soluble, even at cold temperatures.
- Isopropanol useful for precipitations where you have a large sample volumes.

centrifuge

- The optimum pelleting conditions depend on the DNA concentration.

Washing samples with 70 % Ethanol

Solutes that may be trapped in the precipitate may be removed by washing the DNA pellet with a solution of 70% ethanol.

isopropanol

- Isopropanol (1 volume) may be used in place of ethanol (2 volumes) to precipitate DNA.
- Precipitation with isopropanol has the advantage that the volume of liquid to be centrifuged is smaller.

isopropanol

- Isopropanol is less volatile than ethanol and it is more difficult to remove the last traces of solutes such as NaCl.

194 Suspension and storage of DNA

suspension

- TE Buffer - 10 mM Tris-HCl pH 8.0, 1 mM EDTA, or □ TE - 10 mM Tris, 0.1 mM EDTA.
- DNA is resuspended and stored in T.E. buffer.

Suspension

- DNA must be stored in a slightly basic buffer to prevent depurination, and the EDTA chelates any Mg²⁺ helping to inactivate DNases. Storage of dna
- DNA can be stored at 4 °C for extended periods, however for long term storage, - 20 °C is usually utilized.
- Avoid repetitive freeze thawing of DNA, since this can cause degradation.

Storage

- The storage at room temp after drying the DNA.

195 Organic method properties

Properties

- Yields relatively pure, high molecular weight DNA.
- DNA is double stranded – good for RFLP and other molecular techniques.

Drawbacks

- Time consuming.
- Requires sample to be transferred to multiple tubes.
- Increases risk of contamination
- Involves use of hazardous and smelly chemicals.

196 DNA extraction- non organic method

Non-organic method

- No use of organic reagents such as phenol or chloroform.

- **Digested proteins are removed by salting out with high concentrations of LiCl or NaCl.**

Salts should be removed

However, if salts are not adequately removed, problems could occur with the RFLP/PCR procedures due to alteration of DNA mobility.

procedure

- **Step 1: Cell Lysis Buffer - lyse cell membrane, nuclei are intact, pellet nuclei.**
- **Step 2: Resuspend nuclei in protein lysis buffer containing a high concentration of proteinase K.**
- **Step 3: Lyse nuclear membrane and digest protein at 65 °C for 2-3 hours or 37 °C for over night.**
- **Step 4: Temperature helps denature proteins, and digestion of proteins by Proteinase K.**

procedure

- **Step 5: To remove proteinaceous material, LiCl or NaCl is added, and incubated on ice.**
- **Step 6: Proteins precipitate out and are pelleted by centrifugation.**
- **Step 7: DNA remains in solution. Transfer supernatant to a new tube, care must be taken not to take any of protein pellet to next tube.**

procedure

- **Step 8: DNA is precipitated by the addition of room temperature isopropanol. LiCl or NaCl will not precipitate with DNA.**
- **Step 9: Precipitated DNA is washed with 70% ethanol, dried under vacuum and re-suspended in TE buffer.**

Non-organic method

- **No use of organic reagents.**
- **Digested proteins are removed by non organic salts.**

197 Total cellular RNA

mrna

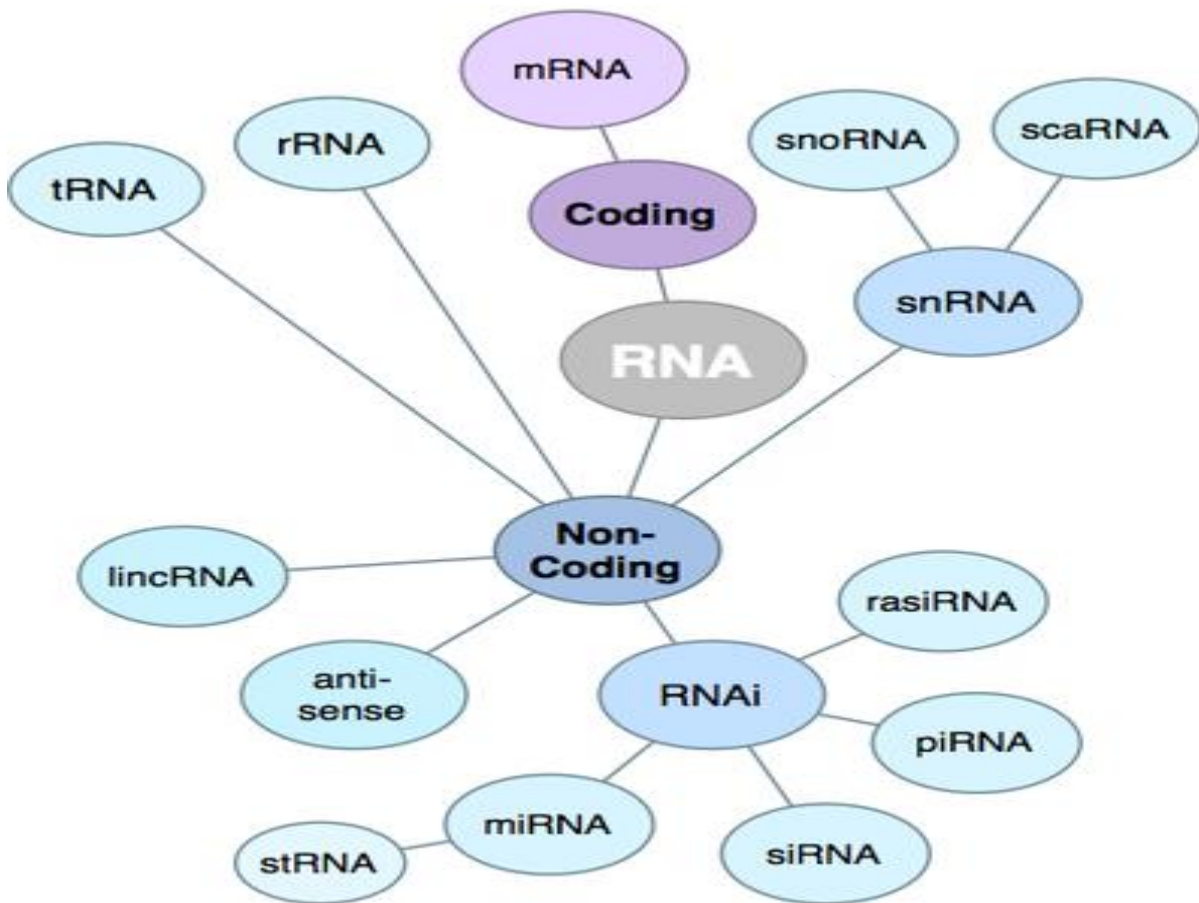
- **Messenger RNA (mRNA): 1-5%.**
- **Serves as a template for protein synthesis.**

rrna

- **Ribosomal RNA (rRNA) ~80%**
- **Structural component of ribosomes**

trna

- **Transfer RNA (tRNA): 10-15%.**
- **Helps for translation of mRNA information into the appropriate amino acid**
- **Small RNAs**
- **Different types of rna**



Rna purification

- To isolate RNA from cellular components
- Inactivate RNases

Rn-ASES

- RNases are naturally occurring enzymes that degrade RNA
- Common laboratory contaminant (from bacterial and human sources)

198 RNA purification- samples

Samples for RNA PURIFICATION

- Tissues
- Cultured cells
- Body fluids like urine, CSF, semen
- Whole blood

Collection and storage OF SAMPLES

- Samples can be stored at -70°C in lysis buffer after disruption and homogenization.
- Frozen samples are stable for months.

Cells and tissues

- Cultured cells are lysed and homogenized directly in the lysis buffer.
- Tissues can be disrupted using rotor–stator homogenizers, Tissue Lyser system, Mortar and Pestle.

Rotor-stator homogenizer

- In the presence of lysis buffer, disrupt and simultaneously homogenizes tissues in 5 –100 s, depending on the toughness of the sample.

Bead-milling

- In bead-milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells.

Mortar and pestle

- Freeze the sample immediately in liquid nitrogen, and grind to a fine powder under liquid nitrogen.
- Allow the liquid nitrogen to evaporate.
- Add lysis buffer, and continue homogenization.
- Transfer the suspension into an RNase-free tube.

Samples for RNA PURIFICATION

- Tissues
- Cultured cells
- Body fluids like urine, CSF, semen
- Whole blood

199 RNA purification -blood

ERYTHROCYTES AND LEUKOCYTES LYSIS

- Blood cells are lysed in two separate procedures, erythrocytes lysis and leukocytes lysis.

ERYTHROCYTES LYSIS

- Erythrocytes (red blood cells) do not contain nuclei □ They neither synthesize nor contain RNA.

RNA IS PURIFIED FROM LEUKOCYTES

- RNA isolation from whole blood is targeted to leukocytes (white blood cells).
- Leukocytes are nucleated and contain RNA.

ERYTHROCYTES LYSIS BY HYPOTONIC BUFFER

- Erythrocyte lysis by hypotonic buffer.
- Intact leukocytes are then recovered by centrifugation. Leukocytes LYSIS
- Leukocytes lysis by denaturing buffer that immediately, inactivate RNases, allowing isolation of intact RNA.

200 RNA purification- organic method

Types OF rna purification

- RNA isolation by salt precipitation
- Total RNA extraction by organic method
- Affinity purification using spin columns
- Selective mRNA isolation Procedure – organic method
- Step 1: Lyse/homogenize cells using trizol reagent/phenol chloroform and centrifuge.

- Step 2: Organic phase separates from aqueous phase organic solvents at bottom.
- Step 3: Aqueous phase on top (contains total RNA).

Step 4: Cellular debris and genomic DNA appears as a “film” of debris at the interface of the two solutions.

Procedure of Rna extraction

- Step 5: Remove RNA solution to a clean tube.
- Step 6: Precipitate RNA and wash with ethanol, then resuspend RNA in water.

Advantages

- **Compatible with a variety of sample types**
- **Can process small and large samples**
- **Established protocol**
- **Inexpensive**

Disadvantages

- **Organic solvents**
- **RNA may contain contaminations of genomic DNA**

201 RNA purification- affinity method

Procedure - AFFINITY Method

- Step 1: Lyse cells, and spin to remove large particulates/cell debris.
- Step 2: Apply lysate (containing nucleic acids and cellular contaminants) to column with glass membrane.
- Step 3: Wash with alcohol to remove contaminants; nucleic acids stick to glass membrane while contaminants wash off.

Procedure - AFFINITY Method

- Step 4: Treat with DNase enzymes to remove contaminating DNA.
- Step 5: Apply water to the column; purified RNA washes off the glass membrane and is collected.

Advantages

- **No use of organic solvents**
- **Variety of sample types**
- **Tissue**
- **Tissue culture cells**
- **White blood cells**
- **Plant cells**
- **Bacteria and yeast Advantages**
- **DNase eliminates contaminating genomic DNA** □ **Excellent RNA purity and integrity**

202 RNA purification- human blood (I)

Rna PURIFICATION

- 1 ml of human blood yield approximately 3 ug of RNA.
- RNA is easily degraded by ever-present RNAses.

All of the tubes and solutions must be RNase-free.

Rna purification – human blood

- Step 1: Add fresh human blood in polypropylene conical centrifuge tube.
- Step 2: Add RBC Lysis Buffer. Let stand at room temperature for 10 minutes .
- Step 3: Pellet the cells by centrifugation at approx 14,000 rpm for 10 minutes at 4 °C. Carefully decant supernatant. PURIFICATION
- Step 4: Gently re-suspend the pellet in RBC lysis buffer and transfer to a 1.5 ml microcentrifuge tube. Let stand for 2 minutes.
- Step 5: Pellet the cells for 2 minutes by centrifuging in a microfuge. Carefully aspirate the supernatant.
- Step 6: Process cells (WBC) for RNA if desired (or store cells at –80°C until needed). Rna PURIFICATION
- **During purification of RNA from blood sample, first step is to prepare pellet of nuclei of WBC.**

203 RNA purification - human blood (II)

homogenization

- Add TRIzol solution to each tube and re-suspend the cells.
- Homogenize and lyse the cells. Incubate for 10 minutes on ice. Phase separation
- Add chloroform according to amount of blood and vigorously shake or vortex each tube for 15 seconds. Let samples stand on ice for 10 minutes.
- Centrifuge the samples at 14,000 rpm for 15 minutes at 4°C. RNA PRECIPITATION
- Remove the upper phase and transfer to a clean microcentrifuge tube to which an equivalent volume of ice-cold isopropanol is added.
- Be careful not to remove any of the white interface when collecting the upper phase of the extraction.

RNA PRECIPITATION

- Immediately vortex and place on ice for 10 minutes.
- Samples are centrifuged at 14,000 -17,000 rpm for 10 minutes at 4°C.
- **Homogenization, phase separation and RNA precipitation are the steps once pellets of WBC were prepared.**

204 RNA purification- human blood (III)

RNA WASH

- Rinse the pellet with ice-cold 75-80% ethanol.
- Centrifuge the samples at 13,000- 17,000 rpm for 2 - 5 minutes.
- Decant the supernatant. Wash again.

Spin the samples to drive the remaining fluid to the bottom of the tube.

- Carefully remove all of the remaining liquid in the bottom of the tube.
 - Allow the pellet to dry for 5 - 10 minutes to remove any remaining ethanol.
- DISSOLVE RNA
- **Dissolve RNA pellet by adding RNase-free H₂O to each sample.**

205 Quantitation of RNA

Quantitation

- Nucleic acids absorb UV light maximally at 260nm
- For RNA: 1 OD₂₆₀ Unit = 40 µg/ml of ssRNA
- The concentration in the sample is calculated by

$$A_{260} \times \text{dilution} \times 40 = [\text{RNA}] \mu\text{g/ml}$$

Rna purity

- Pure RNA will exhibit an A₂₆₀/A₂₈₀ ratio within the range of 1.8 - 2.0.
- If the RNA exhibits a ratio lower than 1.7, this indicates protein contamination in your sample.

Rna integrity

- Two major ribosomal subunits: 28S and 18S.
- Approximately 80% of total RNA is associated with these two subunits.
- **Integrity of the total RNA sample can be determined by evaluating the 28S (5 kb) and 18S (2 kb) rRNA.**
- **28S and 18S rRNA should appear as distinct bands.**

206 Chromosomes staining

CHROMoSOMES Banding

- Chromosomes banding is based on the presence of heterochromatin and euchromatin.
- Heterochromatin are dark stained regions while euchromatin are light stained regions.

CHROMoSOMES are Stained with various dyes

- Chromosomes are stained with various dyes enabling the chromosomes segments to be identified.

CHROMoSOMES STAINING

- Most methods can distinguish 550 bands / haploid set.

Higher resolution methods

- Higher resolution methods can distinguish up to 850 bands / haploid set.
- Identification of small interstitial deletions.

Three features help to identify CHROMoSOMES

- Size
- Banding pattern
- Centromere position

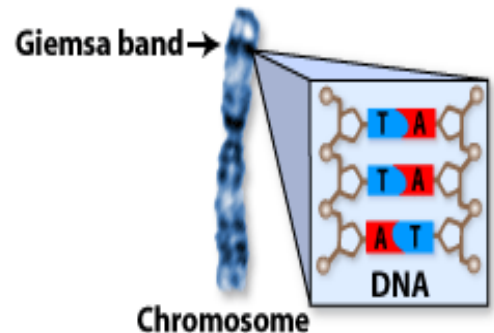
Chromosomes Staining and banding

- Chromosomes are stained with various dyes.
- Based on centromere position, different types of chromosomes exist.

207 Giemsa Staining

Giemsa STAINING gives striped appearance to chromosomes

- Dye gives chromosomes, a striped appearance because it stains the regions of DNA that are rich in Adenine and Thymine.



Dark bands (dark G bands) replicate late during cell cycle

- Regions that stain as dark G bands replicate late in S phase of the cell cycle and contain more condensed chromatin.

light bands (light G bands) replicate early during cell cycle

- While light G bands generally replicate early in S phase, and have less condensed chromatin.

208 Giemsa Staining procedure

Giemsa STAINING

- G-banding is obtained with Giemsa stain following digestion of chromosomes with enzyme trypsin.
- It is a mixture of methylene blue, eosin and Azure B.
- It is specific for the phosphate groups of DNA and attaches itself to regions of DNA where there are high amounts of Adenine-Thymine bonding.
- It produces a series of light and dark bands.
- Dark regions tend to be heterochromatic, late-replicating and A-T rich.
- Light regions tend to be euchromatic, early-replicating and G-C rich .
- **Collection of blood**
- **Cell culture**
- **Stopping the cell division at metaphase**
- **Hypotonic treatment of red & white blood cells**
- **Fixation, slide preparation**
- **Slide dehydration**
- **Treatment with enzyme**
- **Staining**
- **In the case of peripheral (venous) blood.**
- **Sample is added to a small volume of nutrient medium containing phytohemagglutinin, which stimulates T lymphocytes to divide.**

Culturing of cells

- Cells are cultured under sterile conditions at 37 °C for about 3 days, during which they divide, and colchicine is then added to each culture.
- Colchicine has the extremely useful property of preventing formation of the spindle, thereby arresting cell division during metaphase, the time when the chromosomes are maximally condensed and therefore most visible.

Giemsa STAINING

- Hypotonic saline is then added, which causes the cells to lyse and results in spreading of the chromosomes, which are then fixed , mounted on a slide and stained ready for analysis.
- Produces a series of light and dark bands.
- Dark regions tend to be heterochromatic, late-replicating and AT rich.
- Light regions tend to be euchromatic, early-replicating and GC rich .

209 C banding of chromosomes

C banding

- C-banding stains the constitutive heterochromatin, which usually lies near the centromere.

C banding - Procedure

- Treat the slides in 0.2 N HCl for one hour at room temperature.
- Rinse in de-ionized water.
- Immerse in 1% barium hydroxide at 50°C for 5-15 min.
- Rinse in deionized water.
- Incubate at 60°C in 2XSSC (Salt, Sodium Citrate) buffer for one hour.
- Rinse in de-ionized water and stain in 4% Giemsa stain for 90 min.
- Rinse in de-ionized water, dry and examine under oil immersion.
- C-banding stains the constitutive heterochromatin, which usually lies near the centromere.

210 Q banding of chromosomes

Q banding

- Q-banding is a fluorescent pattern obtained using quinacrine for staining.
- The pattern of bands is very similar to that seen in G-banding.

Q banding Procedure

- Dehydrate the slides by dipping in alcohol with decreasing concentration 90%, 70% and 50% one minute each.
- Rinse in distilled water.
- Wash the slide in phosphate buffer at pH 6.8
- Stain the slides in quinacrine mustard (5 mg in 100 ml) or in 5% quinacrine dihydrochloride for 20 min.
- Rinse in phosphate buffer and mount in the same buffer.
- Examine under fluorescent microscope.

Q banding

- Q-banding is a fluorescent pattern obtained using quinacrine for staining.
- The pattern of bands is very similar to that seen in G-banding.

211 R banding of chromosomes

r banding

- Reverse of G-banding (R stands for "reverse").
- Dark regions are euchromatic (guanine-cytosine rich regions) and the light regions are heterochromatic (thymine-adenine rich regions).

r banding - Procedure

- Age the slides for 7 -10 days.
- Place the slides in phosphate buffer of pH 6.5 at 85°C and incubate for 20-25 min.
- Stain the slides in 0.01% acridine orange in the phosphate buffer pH 6.5 for 4-6 min. Rinse in phosphate buffer and mount in the same buffer.
- Examine under fluorescent microscope.

r banding

- Reverse of G-banding (the R stands for "reverse").
- Dark regions are euchromatic (guanine-cytosine rich regions) and the bright regions are heterochromatic (thymine-adenine rich regions).

212 T banding of chromosomes

T banding

- Identifies a subset of the R bands which are especially concentrated at the telomeres.

T banding - Procedure

- Age the slide for 7 days.
- Place the slides in PBS pH 5.0 for 20-60 min at 87°C.
- Rinse in PBS.
- Stain in 3% Giemsa in phosphate buffer pH 6.8 at 87°C, leave for 5-30 min and rinse.
- Slides are stained in Hoechst 33258 stain for 10 min (Hoechst stain 0.5 µg/ml of phosphate buffer).
- Rinse in phosphate buffer and examine in fluorescent microscope.
- Alternatively, the stained slides are covered with a cover slip and placed in a wet chamber under UV lamp for 2 to 3 hours or under direct sunlight for 2 hours.
- Remove the cover slip and stain in Giemsa stain for 10 min.
- Rinse in buffer, dry and mount in DPX.

T banding

- Identifies a subset of the R bands which are especially concentrated at the telomeres.

213 Polymerase chain reaction

pcr

- A technique widely used in Molecular Biology and Biotechnology.
- Its name is from one of its key component - DNA polymerase.

pcr

- As PCR progresses, DNA generated is itself used as template for replication.

Pcr- a chain reaction

- This sets in motion of a chain reaction in which the DNA template is exponentially amplified.

- **heat stable DNA POLYMERASE**

- During PCR, a heat-stable DNA polymerase, such as Taq polymerase - an enzyme derived from the bacterium *Thermus aquaticus*.

conclusion

- PCR can be performed to amplify DNA.
- It can be extensively modified to perform a wide array of genetic manipulations.

214 Steps of PCR

THREE STEPS

- Denaturation
- Annealing
- Extension

Initialization step

- This step consists of heating the reaction to a temperature of 93°C - 96°C, which is held for 1-10 minutes.

Denaturation

- First regular cycling event and consists of heating the reaction to 93°C - 98°C for 20-45 seconds.
- It causes melting of DNA template yielding single strands of DNA.

Annealing

- The reaction temperature is lowered to 40-65°C for 20-40 seconds.
- Annealing of the primers to the single-stranded DNA template.

Extension

- The temperature at this step depends on the type of DNA polymerase used.
- Taq polymerase has its optimum activity temperature at 70-80°C.
- Commonly 72°C is used.

Extension

- At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTP's.

Final elongation

- Single step is performed at a temperature of 70-74°C for 5-15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Final hold temperature

- This step at 4-15°C for an indefinite time may be employed for short-term storage of the reaction.

215 Ingredients of PCR

Major ingredients

- Microfuge tube

- Thermal cycler
- DNA template
- Primers
- Buffer
- MgCl₂

Major ingredients

- Distilled water
- Deoxynucleotide triphosphates
- DNA polymerase

Microfuge tubeS

- These are small cylindrical plastic tubes with conical bottoms.

Thermocycler

- The thermocycler works on the principle of Peltier effect, which raises and lowers the temperature of the block in a pre-programmed manner.

Dna polymerases

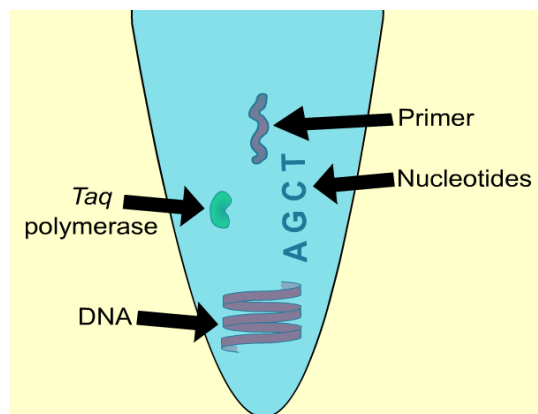
- Polymerases

Taq polymerase

Pfu polymerase

Vent polymerase

INGREDIENTS IN pcr tube



Template CAN BE dna or RNA

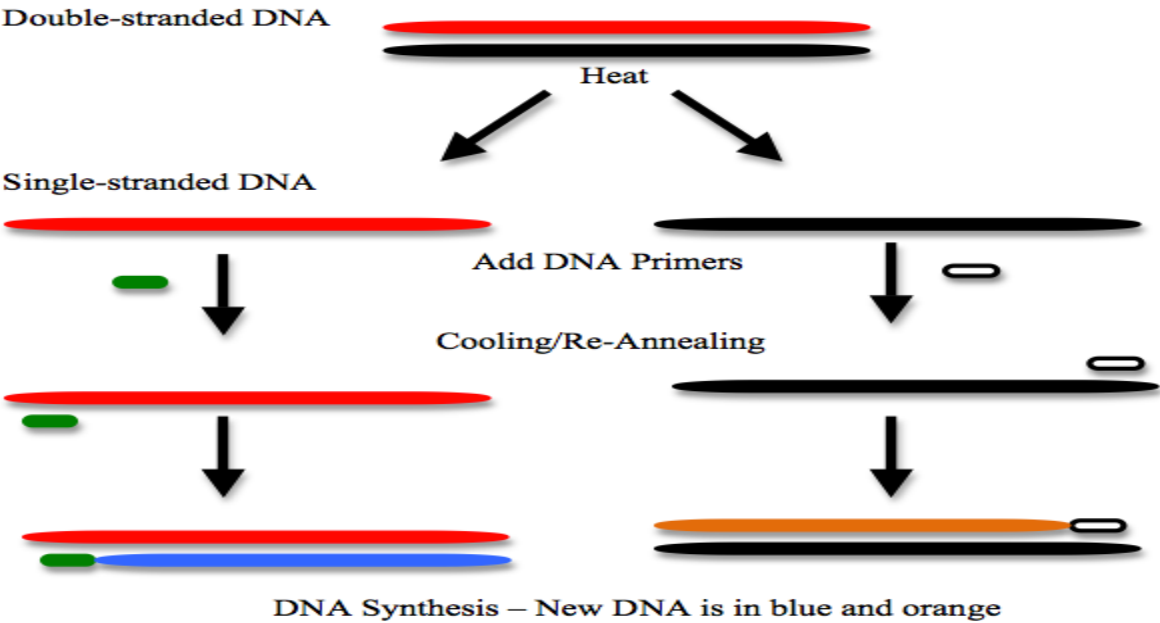
- Template DNA
- RNA in case of reverse transcriptase

216 Primers for PCR

PRIMERS

- Primers are single-stranded 18–30 bp long DNA fragments
- Complementary to sequences flanking the region to be amplified.





PRIMERS can be specific or random

- Primers determine the specificity of the PCR reaction.
- Distance between the primers binding sites will determine the size of PCR product.

FEATURES of primers

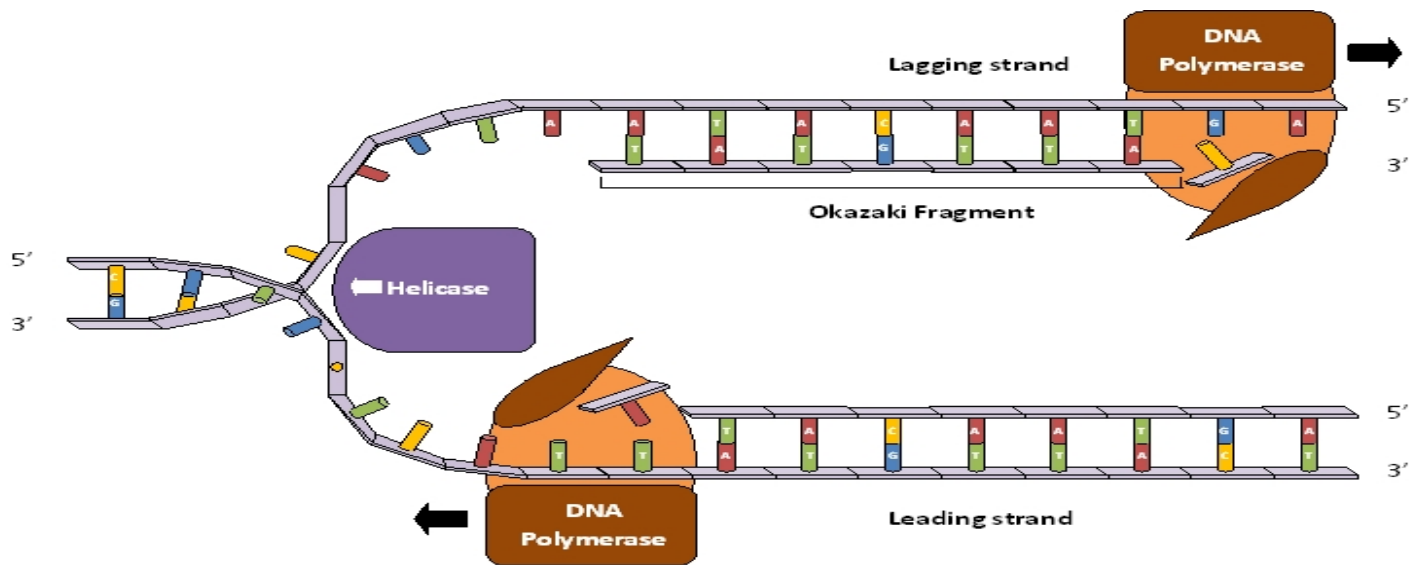
- Types of primers-random or specific
- Primer length
- Annealing temperature
- Specificity
- Nucleotide composition
- Avoid inter-strand homologies
- Avoid intra-strand homologies
- Tm of forward primer = Tm of reverse primer
- G/C content of 20–80%; avoid longer than GGGG
- Product size (100–700 bp)

FORMULA for calculating melting temperature of primers

- $T_m = 4(G+C) + 2(A+T)$

217 DNA polymerases

Polymerase	Extension Rate (nt/sec)	Source
<i>Taq pol</i>	75	<i>T. aquaticus</i>
Amplitaq (Stoffel fragment)	>50	<i>T. aquaticus</i>
Vent	>80	<i>Thermococcus litoralis</i>
Pfu	60	<i>Pyrococcus furiosus</i>
Tth (RT activity)	>33	<i>T. thermophilus</i>



- *Taq*: *Thermus aquaticus* (most commonly used)
- *Tfl*: *T. flavus*
- *Tth*: *T. thermophilus*
- *Tli*: *Thermococcus litoralis*
- *Pfu*: *Pyrococcus furiosus* (fidelity)

Dna polymerases

- Polymerases
- Taq polymerase
- Pfu polymerase
- Vent polymerase

218 Standard PCR reaction

0.25 mM each primer

0.2 mM each dATP, dCTP, dGTP, dTTP

50 mM KCl

10 mM Tris

1.5 mM MgCl₂

2.5 units polymerase

10² - 10⁵ copies of template

50 ul reaction volume

PCR TEMPERATURES

- **Denaturation temperature**
 - Reduces double stranded molecules to single stranded
 - 90–96°C, 20-45 seconds
- **Annealing temperature**
 - Controls specificity of hybridization
 - 40–68°C, 20-30 seconds
- **Extension temperature**
 - Optimized for individual polymerases
 - 70–75°C, 30-45 seconds

• TEMPERATURES

Temp	For	Comments
94-60-72	Perfect, long primers	Higher temp can be used; maximum annealing temp
94-55-72	Good or perfectly matched primers between 19-24 nt	Standard conditions
94-50-72	Adequate primers	Allows 1-3 mismatches/20 nt
94-48-68	Poorly matched primers	Allows 4-5 mismatches/20 nt
94-45-65	Unknown match, likely poor	Primers of questionable quality

TEMPERATURES

- Amplification takes place as the reaction mix is subject to an amplification program and temperatures.
- The amplification program consists of a series of 20–50 PCR cycles.

219 Interpretation of results

interpretation

- The PCR product should be of the expected size.
- Misprimers may occur due to non-specific hybridization of primers.

Primers dimers may reduce amplification

- Primer dimers may occur due to hybridization of primers to each other.

Blank reaction

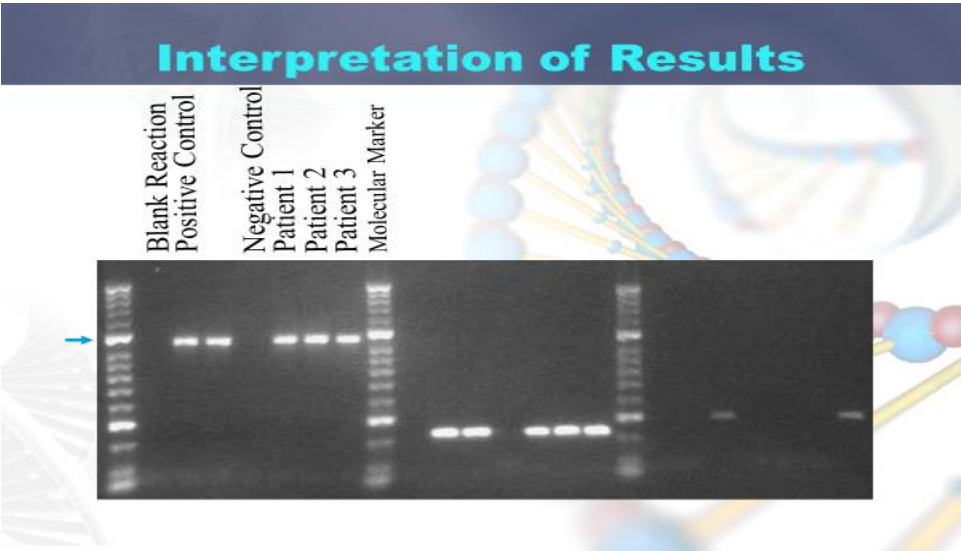
- Controls for contamination

contains all reagents except DNA template.

Negative control

- Controls for specificity of the amplification reaction

contains all reagents and a DNA template lacking the target sequence.



conclusion

- The PCR product should be of the expected size.

220 Types of PCR

types

1. Nested PCR
2. Multiplex PCR
3. Touchdown PCR
4. Sequence-specific PCR
5. Reverse-transcriptase PCR
6. Long-range PCR
7. Whole-genome amplification
8. RAPD PCR (AP-PCR)
9. Quantitative real-time PCR
10. Long-range PCR
11. Whole-genome amplification
12. RAPD PCR (AP-PCR)
13. Quantitative real-time PCR

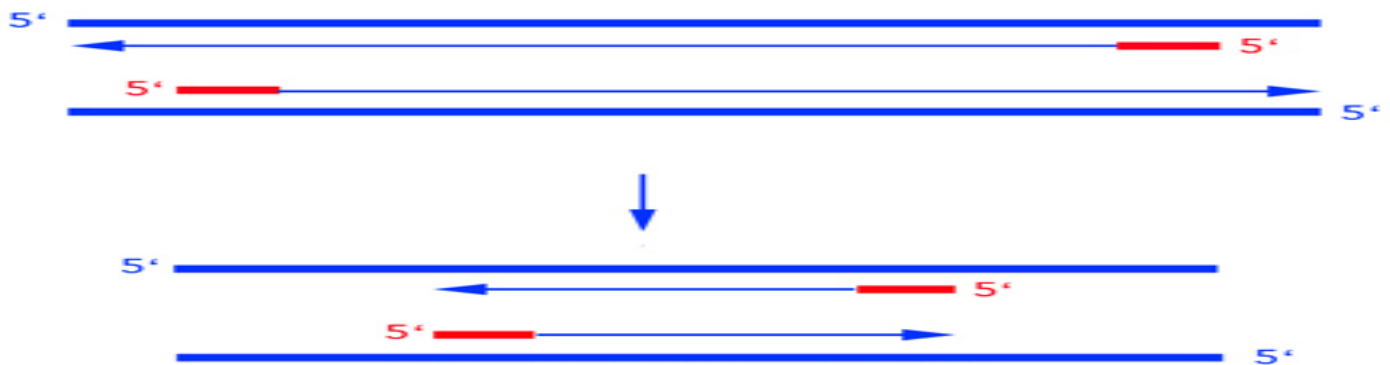
221 Nested PCR

Nested pcr

- Two pairs (instead of one pair) of PCR primers are used to amplify a fragment.
- First pair amplify a fragment similar to a standard PCR.

Nested pcr – second pair of primers

- Second pair of primers - nested primers (as they lie / are nested within the first fragment).



Second pcr product is shorter than first one

- Second pair of primers bind inside the first PCR product fragment to allow amplification of a second PCR product.

Second pcr product is specific

- increases the specificity of DNA amplification, by reducing background due to non-specific amplification of DNA.

Advantage of Nested pcr

- Very low probability of nonspecific amplification.

222 Multiplex PCR

multiplex pcr

- Multiplex PCR is a variant of PCR which enables simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers.

Amplicons of different sizes are produced

- Multiplex-PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences.

Single reaction, many amplicons

- By targeting multiple sequences at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform.

Annealing temperatures be optimized

- Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction.

Amplicon size should be different or be labeled

- Amplicon sizes should be different enough to form distinct bands when visualized by gel electrophoresis.

Labeled primers

- If amplicon sizes overlap, the different amplicons may be differentiated and visualised using primers that have been dyed with different color fluorescent dyes.

Primers pairs be optimized

- The primer design for all primers pairs has to be optimized so that all primer pairs can work at the same annealing temperature during PCR.

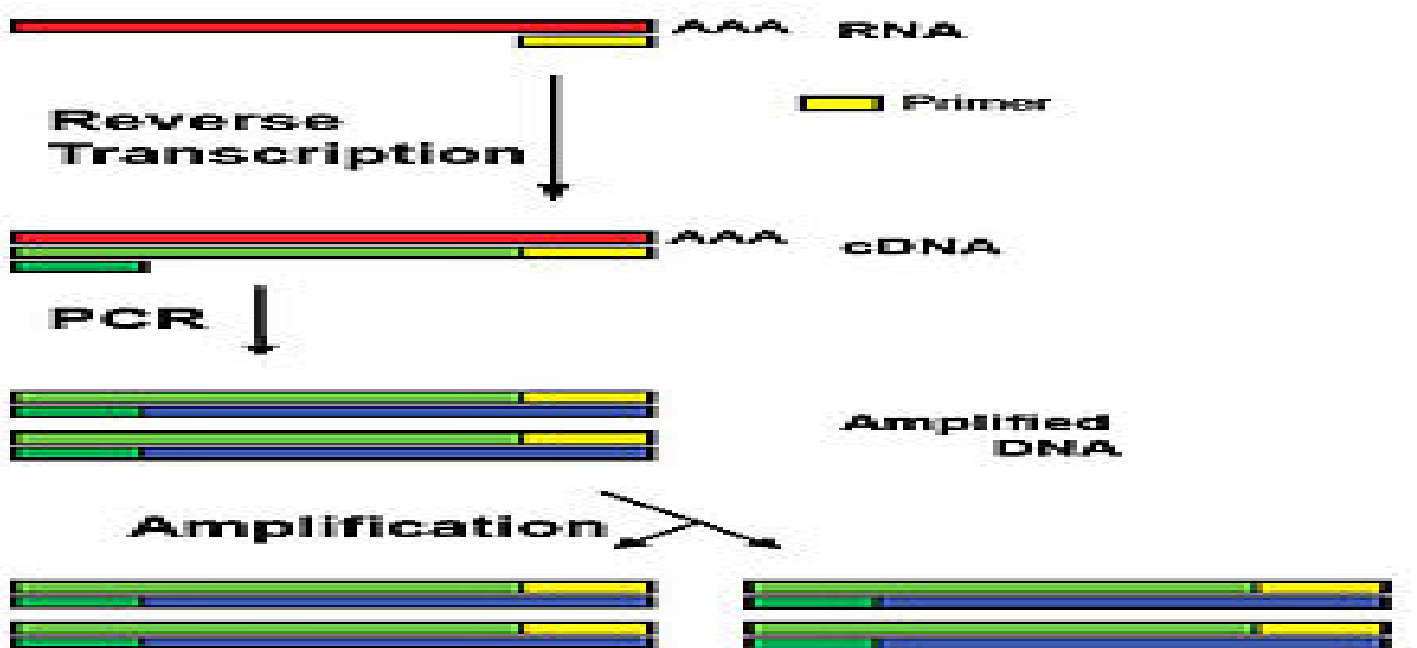
multiplex pcr

- The use of multiple, unique primer sets within a single PCR reaction to produce amplicons of varying sizes specific to different DNA sequences.

223 Reverse transcriptase PCR

Reverse transcriptase pcr

- Based on the process of reverse transcription, which reverse transcribes RNA into DNA.
- First step of RT-PCR - first strand reaction
- Synthesis of cDNA using oligo dT primers (37°C) one hour.
- Second strand reaction - digestion of cDNA:RNA hybrid (RNaseH)-
- Standard PCR with DNA oligo primers .



Enzyme Reverse transcriptase

- The PCR is preceded by a reaction using reverse transcriptase to convert RNA to cDNA.

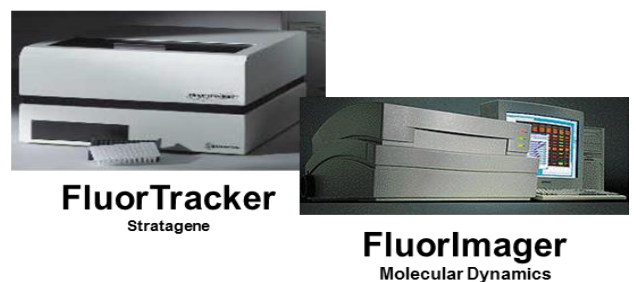
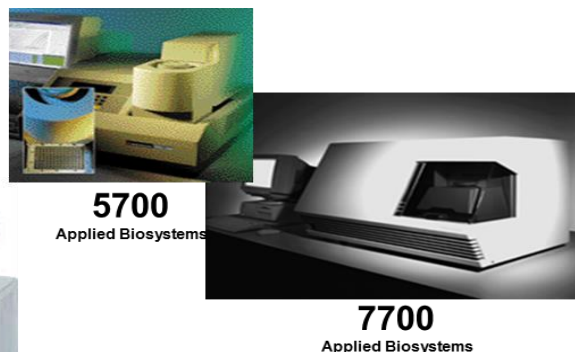
Reverse transcriptase pcr

- RT-PCR is widely used in expression profiling, to determine the expression of a gene
- To identify the sequence of an RNA transcript.

224 Real time PCR

Real time pcr

- Real Time PCR is a technique in which fluoroprobes bind to specific target regions of amplicons to produce fluorescence during PCR.
- Real Time PCR is used to measure the quantity of a PCR product.
- The fluorescence, measured in Real Time, is detected in a PCR cycler with an inbuilt filter flurometer.
- It is the method of choice to quantitatively measure starting amounts of DNA, cDNA or RNA.
- Quantitative real-time PCR is often confusingly known as RT-PCR (Real Time PCR) or RQ-PCR.
- QRT-PCR or RTQ-PCR are more appropriate contractions.
- QRT-PCR methods use fluorescent dyes, such as Sybr Green, or fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time.
- Q-PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample.



225 Hot start PCR

Hot start pcr

- This is a technique that reduces non-specific amplification during the initial set up stages of the PCR.
- The technique may be performed manually by heating the reaction components upto the melting temperature (e.g, 95°C).
- Before adding the polymerase, specialized enzyme systems have been developed that inhibit the polymerase's activity at ambient temperature.
- This is done either by the binding of an antibody or by the presence of covalently bound inhibitors that only dissociate after a high-temperature activation step.
- DNA Polymerase- Eubacterial type I DNA polymerase, Pfu.
- These thermophilic DNA polymerases show a very small polymerase activity at room temperature

226 Asymmetric PCR

Asymmetric pcr

- Asymmetric PCR is used to amplify one strand of the original DNA more than the other.
- It is used in some types of sequencing and hybridization probing where having only one of the two complementary strands is ideal.
- PCR is carried out as usual, but with a great excess of one primers for the chosen strand.
- Due to the slow amplification later in the reaction after the limiting primer has been used up, extra cycles of PCR are required.
- It finds use in some types of sequencing and hybridization probing where having only one of the two complementary strands is required.

227 Long PCR

LONG pcr

- Extended or longer than standard PCR, meaning over 5 kilobases (frequently over 10 kb).
- Long PCR is useful only if it is accurate.

mixtures of polymerases

- Special mixtures of proficient polymerases along with accurate polymerases such as Pfu are often mixed together.

LONG pcr

- Application - to clone large genes not possible with conventional PCR.

228 Allelic specific PCR

Allele specific pcr

- Allele-specific PCR used for identifying of SNPs.
- It requires prior knowledge of a DNA sequence, including differences between alleles.

Allele specific pcr

- Uses primers whose 3' ends encompass the SNP.
- PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer.

Amplification with SNP SPECIFIC PRIMER

- Successful amplification with an SNP-specific primer signals presence of the specific SNP in a sequence.

Allele specific pcr

- This diagnostic or cloning technique is used to identify or utilize single-nucleotide polymorphisms (SNPs).

229 Colony PCR

Colony pcr

- The screening of bacterial or yeast clones for correct ligation or plasmid products.

Colony pcr Methodology

- The screening of bacterial or yeast clones for correct ligation or plasmid products.
- Pick a bacterial colony with an autoclaved toothpick, swirl it into 25 µl of TE autoclaved dH2O in an microfuge tube.
- Heat the mix in a boiling water bath (90-100°C) for 2 minutes
- Spin sample for 2 minutes high speed in centrifuge.

Colony pcr

- Transfer 20 µl of the supernatant into a new microfuge tube.
- Take 1-2 µl of the supernatant as template in a 25 µl PCR standard PCR reaction.
- The screening of bacterial or yeast clones for correct ligation or plasmid products.

230 In situ PCR

In situ pcr

- In Situ PCR (ISH) is a polymerase chain reaction that actually takes place inside the cell on a slide.
- In situ PCR amplification can be performed on fixed tissue or cells .
- Applies the methodology of hybridization of the nucleic acids.
- Allows identification of cellular markers
- Limited to detection of non-genomic material such as RNA.

231 Inverse PCR

Inverse pcr

- Inverse PCR uses standard PCR primers oriented in the reverse direction of the usual orientation.
- The template for the reverse primers is a restriction fragment that has been self-ligated
- Inverse PCR functions to clone sequences flanking a known sequence.
- Flanking DNA sequences are digested and then ligated to generate circular DNA.

Identification of sequences flanking transposable elements

- Amplification and identification of sequences flanking transposable elements, and the identification of genomic inserts.

Inverse pcr

- A method used to allow PCR when only one internal sequence is known.
- Especially useful in identifying flanking sequences to various genomic inserts.

232 AFLP PCR

AFLP pcr - methodology

- Genomic DNA is digested with one or more restriction enzymes. tetracutter (MseI) and a hexacutter (EcoRI).
- Ligation of linkers to all restriction fragments. Pre-selective PCR is performed using primers which match the linkers and restriction site specific sequences.
- Electrophoretic separation and amplicons on a gel matrix, followed by visualization of the band pattern.

AFLP pcr

- AFLP is a highly sensitive PCR-based method for detecting polymorphisms in DNA.
- AFLP can be also used for genotyping individuals for a large number of loci.

233 Assembly PCR

Assembly pcr

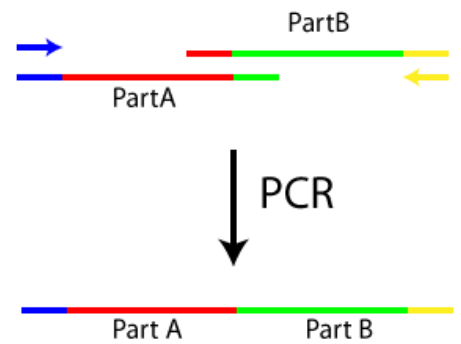
- Assembly PCR used to assemble two or more pieces of DNA into one piece.

Assembly pcr - Principle

- Assembly PCR is the synthesis of long DNA structures by performing PCR on a pool of long oligonucleotides with short overlapping segments, to assemble two or more pieces of DNA into one piece.

Assembly pcr - methodology

- It involves an initial PCR with primers that have an overlap and a second PCR using the products as the template that generates the final full-length product.



Assembly pcr

- Assembly PCR used to assemble two or more pieces of DNA into one piece.

234 Suicide PCR

suicide pcr - Principle

- Suicide PCR is typically used in paleogenetics or other studies where avoiding false positives and ensuring the specificity of the amplified fragment is the highest priority.
- The method prescribes the use of any primer combination only once in a PCR, which should never have been used in any positive control PCR reaction.

suicide pcr

- Primers should always target a genomic region never amplified before in the lab using this or any other set of primers.
- This ensures that no contaminating DNA from previous PCR reactions is present in the lab, which could otherwise generate false positives.
- Use of any primers combination only once in a PCR.

235 Methylation specific PCR

Methylation specific pcr

- Methylation-specific PCR is used to identify patterns of DNA methylation at CpG islands in genomic DNA.

Methylation specific pcr

- Target DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil, which is complementary to adenosine in PCR primers.
- Two amplifications are then carried out on the bisulfite-treated DNA: One primer set anneals to DNA with cytosines (corresponding to methylated cytosine), and the other set anneals to DNA with uracil (corresponding to unmethylated cytosine).

msp

- MSP used in quantitative PCR provides information about methylation state of a given CpG island.

236 Inter sequence specific PCR

Inter sequence specific pcr

- A method for DNA fingerprinting that uses primers selected from segments repeated throughout a genome to produce a unique fingerprint of amplified product lengths.

- The use of primers from a commonly repeated segment is called Alu-PCR, and can help to amplify sequences adjacent (or between) these repeats.

pcr

- PCR used to produce a unique fingerprints of amplified product lengths.

237 Ligation mediated PCR

methodology

- Uses small DNA oligonucleotide 'linkers' (or adaptors) that are first ligated to fragments of the target DNA.
- PCR primers that anneal to the linker sequences are then used to amplify the target fragments.

pcr

- DNA sequencing
- Genome walking
- DNA footprinting

238 Whole genome amplification PCR

Whole genome amplification pcr

- Primers can be designed to be 'degenerate'
- able to initiate replication from a large number of target locations.
- Whole genome amplification (WGA) is a group of procedures that allow amplification to occur at many locations in a genome.

pcr

Whole genomes can be amplified by WGA.

239 Mini primer PCR

Mini primer pcr

- Mini Primer PCR uses a thermostable polymerase (S-Tbr) that can extend from short primers as short as 9 or 10 nucleotides.
- This method permits PCR targeting to smaller primer binding regions, and is used to amplify conserved DNA sequences, such as the 16S (or eukaryotic 18S) rRNA gene.
- PCR that can extend from short primers.

240 Advantages and limitations of PCR

advantages	limitations
<ul style="list-style-type: none">• Specific• Simple, rapid, relatively inexpensive• Amplifies from low quantities• Works on damaged DNA	<ul style="list-style-type: none">• Contamination risk• Primer complexities• Primer-binding site complexities• Amplifies rare species

<ul style="list-style-type: none"> • Sensitive • Flexible 	<ul style="list-style-type: none"> • Detection methods
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241 Applications of PCR

applications

- Detection of Infectious diseases: AIDS, TB, CMV, H1N1, etc Viral, Bacterial and fungal infections,
- Diagnosis of latent viruses.
- Forensic applications: DNA finger printing
- Detection of Mutations: Inherited disorders & carriers Track DNA abnormalities
- Prenatal diagnosis of genetic disorders.
- Detection of pathogens Pre-natal diagnosis DNA fingerprinting Gene therapy Mutation screening
- Drug discovery
- Classification of organisms Genotyping
- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- Bioinformatics
- Genomic cloning
- Site-directed mutagenesis
- Gene expression studies

CLINICAL MICROBIOLY

- Identification of bacteria:
- Slow growing bacteria
- Bacterial antibiotic resistance genes.
- VIRUS: almost all viruses identification and viral load.

FUNGUS:

- | | | |
|-------------------|-------------------|------------------|
| • Aspergillus spp | • Plasmodium spp | • Leishmania spp |
| • Candida spp | • Trypanosoma spp | • Babesia spp |

242 Southern blotting

Southern blotting

- The Southern blot is used to detect the presence of a particular piece of DNA in a sample by a molecular probe.
- Southern Blotting is named after its inventor, the British Biologist Edwin Southern (1975).

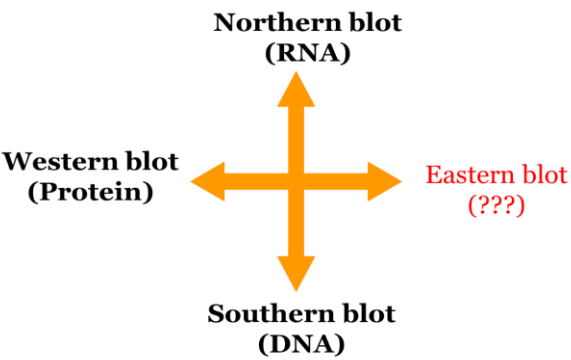
Other blotting methods

- Other blotting methods with similar principles, but using protein or RNA, have been named in reference to Edwin Southern's name.

• BACKGROUND

Southern blotting

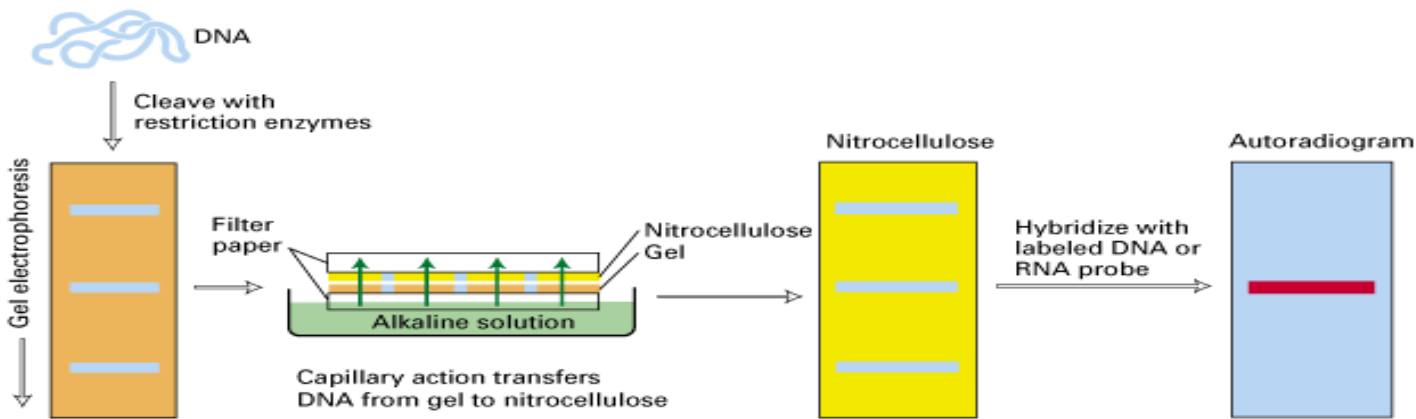
- Identify DNA sequence (gene) of interest.
- Identified DNA may be a small piece of DNA or a mutation.



243 Southern blotting procedure

procedure

- DNA is extracted from cells, leukocytes.
- DNA is cleaved into many fragments by restriction enzyme (e.g, BamH1, EcoR1 etc)
- The resulting fragments are separated on the basis of size by electrophoresis.
- The DNA fragments are denatured and transferred to nitrocellulose membrane for analysis.
- The labeled probe is added to the blocked membrane in buffer and incubated for several hours to allow the probe molecules to find their targets.
- Blot is incubated with wash buffers containing NaCl and detergent to wash away excess probe.
- Radioactive probes enable autoradiographic detection.



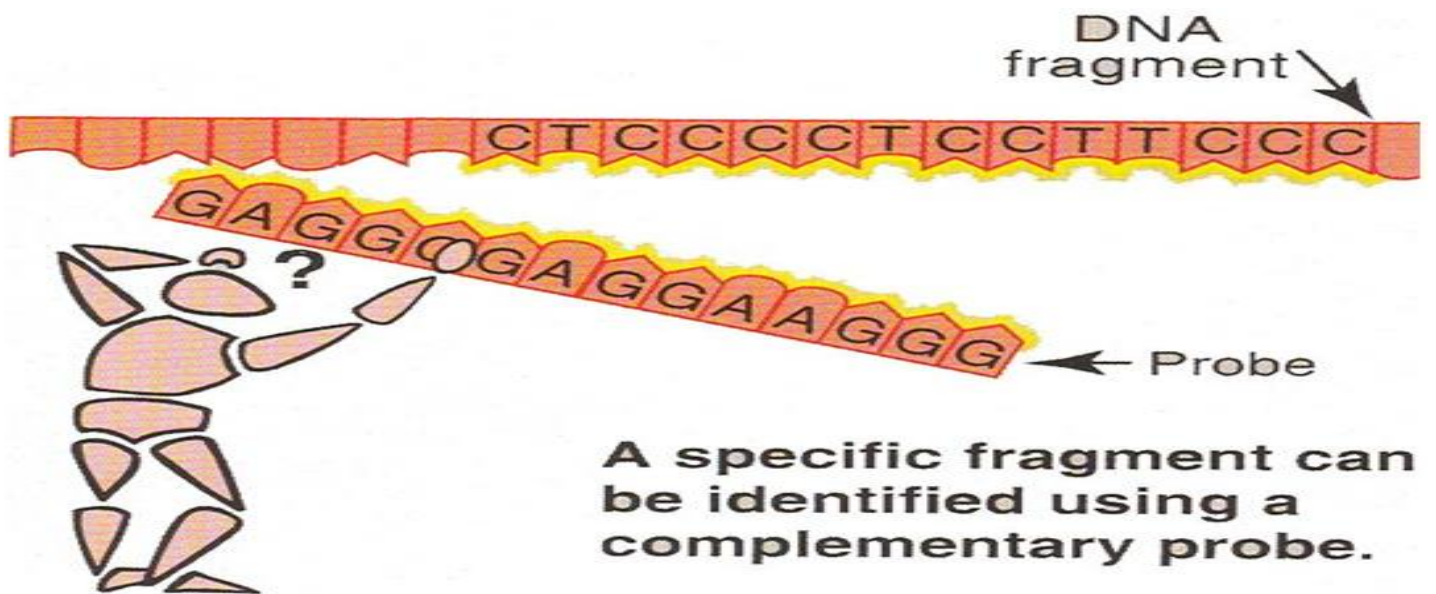
conclusion

- Southern blot is used to detect the presence of a particular piece of DNA in a sample by a molecular probe.

244 Southern blotting probes

probes

- Labeled material to detect a target.
- For DNA: 20-30 nucleotides, complementary to a region in the gene or DNA.



Radioactive Probe - p32

- Sensitive
- Relatively cheap
- Hazardous
- Radioactive waste disposal regulations should be followed

Non-Radioactive Probe - biotin

- Sensitive
- Relatively expensive

Hybridization of probes

- The binding between single stranded labeled probe to a complementary nucleotide sequence on the target DNA.

probes

- Labeled material to detect complementary region in the gene or DNA.

245 Transfer methods in southern blotting

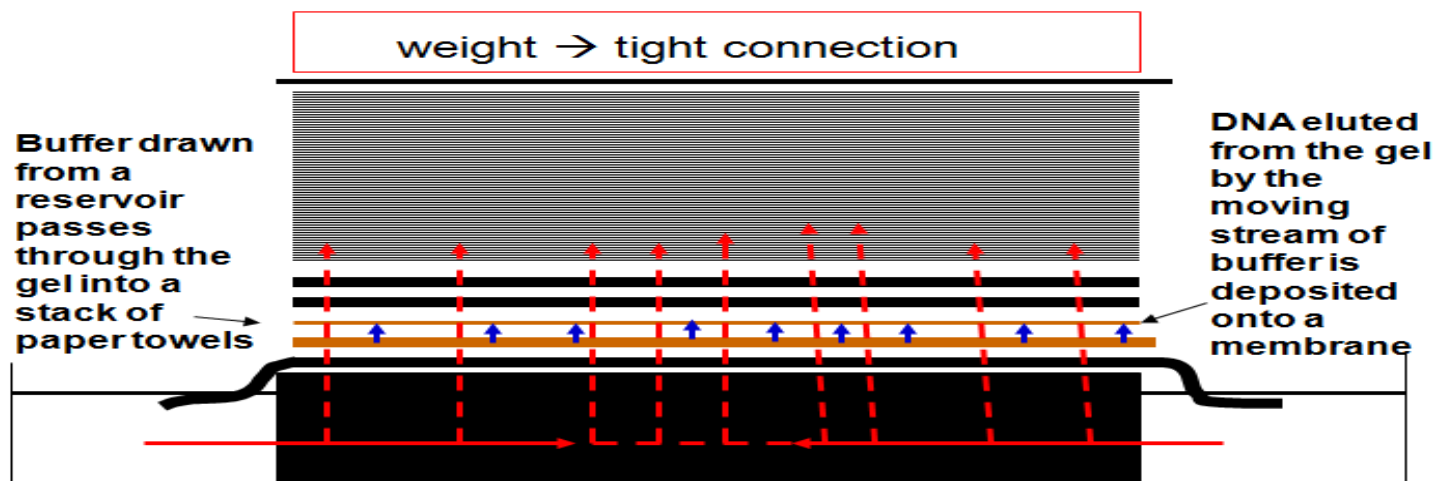
Transfer methods of DNA TO MEMBRANE

- Upward capillary transfer
- Downward capillary transfer
- Simultaneous transfer to two membranes
- Electrophoretic transfer
- Vacuum transfer

Upward Transfer



Upward Transfer of DNA TO MEMBRANE



Transfer methods

- Upward capillary transfer
- Downward capillary transfer
- Simultaneous transfer to two membranes
- Electrophoretic transfer
- Vacuum transfer

246 Mutations identification by southern blotting

Mutation identification

- The presence of a mutation affecting a restriction site causes the pattern of bands to differ from those seen in a normal gene.
- A change in one nucleotide may alter the nucleotide sequence so that the restriction endonuclease fails to recognize and cleave at that site.
- Digestion of genomic DNA to DNA fragments.
- Size-separation of the fragments.
- In situ denaturation of the DNA fragments.
- Transfer of denatured DNA fragments into a solid support (nylon or nitrocellulose).
- Hybridization of the immobilized DNA to a labeled probe (DNA, RNA).
- Detection of the bands complementary to the probe (e.g. by autoradiography).
- Estimation of the size & number of the bands generated after digestion of the genomic DNA will be different.
- Mutations can be identified by Southern blotting.

247 Northern blotting

Northern blotting

- Northern blot is a method used for detection of a specific RNA sequence in RNA samples.

procedure

- Isolation of intact mRNA.
- Separation of RNA according to size (through a denaturing agarose gel).
- Transfer of the RNA to a solid support.
- Fixation of the RNA.
- Hybridization of the immobilized RNA to probes complementary to the sequences of interest.
- Removal of probe molecules that are nonspecifically bound to the solid matrix.
- Detection, capture and analysis of an image of the specifically bound probe molecules.

Northern blotting

- Northern blot is a method used for detection of a specific RNA sequence in RNA samples.

248 Northern blot applications

Applications

- Study of gene expression in eukaryotic cells.
- To measure the amount & size of RNAs transcribed from eukaryotic genes.
- To estimate the abundance of RNAs.
- To equalize the amounts of RNA loaded into lanes of gels.
- Use of housekeeping gene (endogenous constitutively-expressed gene).
- Normalizing samples according to their content of mRNAs of the housekeeping gene.
- Northern blot is used in many ways while studying RNAs.

249 Western blotting

Western blotting

- A technique used to detect the presence of a specific protein in a complex protein mixture.
- To determine the molecular weight of a protein.
- To measure relative amounts (quantitation) of the protein present in complex mixtures of proteins that are not radiolabeled.
- Western blots have become one of the most common analytical tools for the detection of viral proteins.
- Characterization of monoclonal and polyclonal antibody preparations and in determining the specificity of the immune response to viral antigens.
- A technique used to detect the presence of a specific protein in a complex protein mixture.

250 Western blotting procedure

Procedure

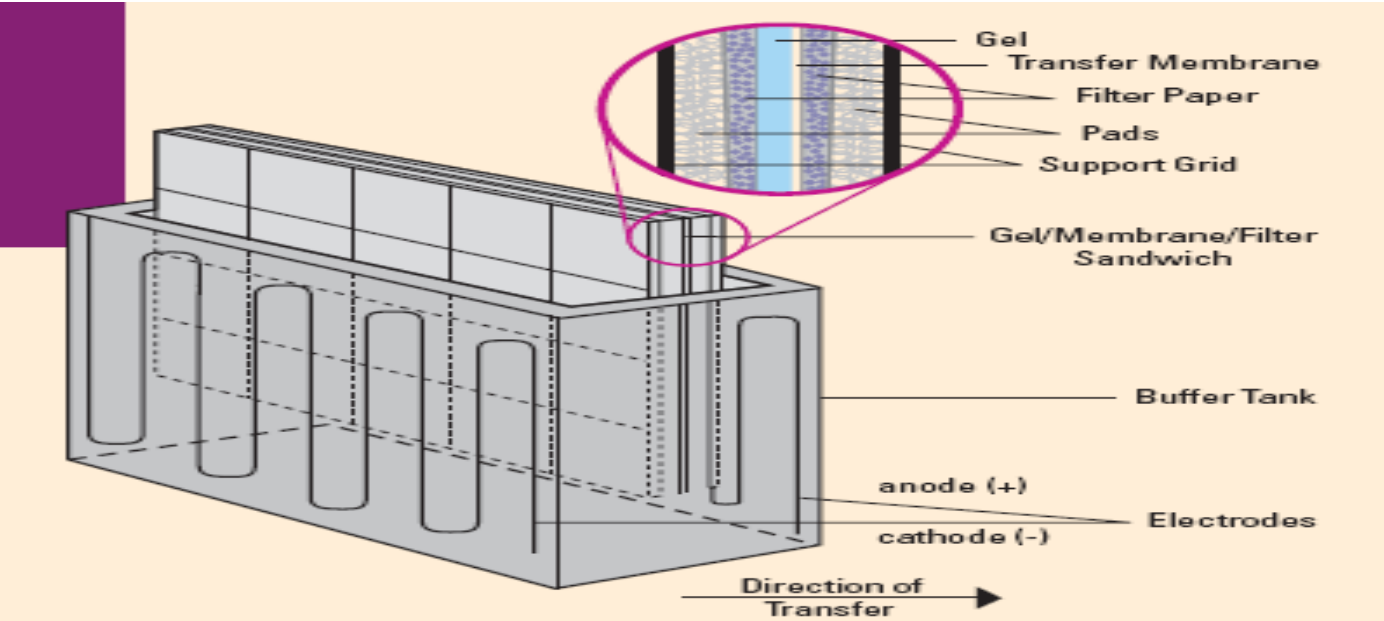
- 1- Sample preparation
- 2- Gel Electrophoresis
- 3- Blotting (or transfer)
- 4- Blocking
- 5- Antibody probing
- 6- Detection

Procedure

- 1- The choice of extraction method depends primarily on the sample and whether the analysis is targeting all the proteins in a cell or only a component from a particular subcellular fraction.
- 2- Samples are loaded into separate wells. A protein marker is also loaded. The separated protein mixtures are transferred to a solid support for further analysis.

- 3- Transfer can be done in wet or semi-dry conditions. Semi-dry transfer is generally faster. Wet transfer is recommended for large proteins, >100 kD.
- 4- Blocking is a very important step in the immunodetection phase of Western blotting because it prevents non-specific binding of antibody to the blotting membrane.

Electrophoretic transfer

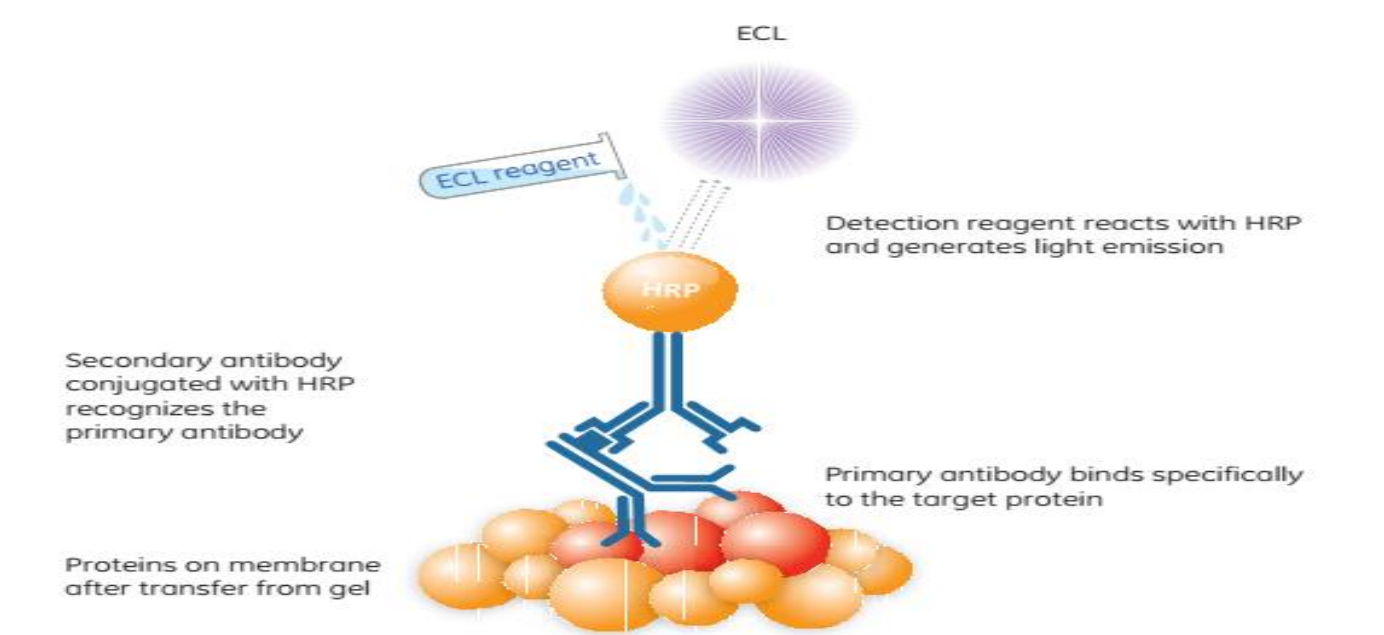


Procedure

Protein of interest is detected and localized using a specific antibody. Western blotting protocols utilize a non-labeled primary antibody directed against the target protein.

A species-specific, labeled secondary antibody directed against the constant region of the primary antibody is then used. The most common antibody label used in Western blots is HRP.

The signal is detected when HRP is exposed to a substrate solution in the final step of the immunodetection procedure



Western blotting

A molecular technique to study proteins.