KARPAGAM ACADEMY OF HIGHER EDUCATION

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DEPARTMENT OF BIOCHEMISTRY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Concepts in Genetics-Practical**	**Semester**	**:**	**III**
Subject code	**:**	**17BCU314B**	**Class**	**:**	**II B.Sc Biochemistry**

S. No	TITLE OF THE EXPERIMENTS
1.	Squash preparation of salivary glands of Dipteran larva to observe polytene chromosomes.
2.	Induction of polyploidy in onion roots
3.	Smear technique to demonstrate sex chromatin in buccal epithelial cells.
4.	Monohybrid crosses in Drosophila for studying autosomal and sex-linked inheritance.
5.	PTC testing in a population and calculation of allele and genotype frequencies
6.	Study of abnormal human karyotype and pedigree (dry lab).
7.	Conjugation in bacteria.

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Experiment No: 1

Squash preparation of salivary glands of Dipteran larva to observe polytene chromosomes

Aim:

To study the polytene chromosomes in the salivary glands of Dipteran larva.

Principle:

In the interphase part of mitosis chromosomes of normal cells are not visible in the light microscope. As the chromosome condense in preparation for mitosis. It can easily absorbed then by staining either for DNA or for basis protein. The polytene chromosomes of Drosophila giant cells are partially condensed all the time, but sections of them are more or less extended. By standing for DNA or basic protein we can see bands along the chromosomes dark where there is a high concentration of DNA and proteins, light where there is DNA more extended. These appear to be about 5000-10,000 bands which can be seen in electron microscope. The bands are characteristic in appearance and some have been identified with specific genes using the same techniques that we will use here to identify the bands.

Materials required:

- Chironomous larva
- Glass slide
- Needles
- Acetoceramine stain
- Light microscope etc.,

Procedure:

1. The Dipteran larva was collected and placed on a glass slide.
2. The region between the third and fourth segments is peeled out with the help of two needles.
3. A pair of transparent, circular salivary glands was removed from the body devoid of debris.
4. Salivary gland were transferred to a new slide and stained with acetoceramine for 10 minutes.
5. Then a cover slip was placed on the top of the stained circular salivary gland and the specimen was gently squashed.
6. The stained preparation was then observed under the microscope.

Result:

The polytene chromosome from the given Dipteran larva was observed under the microscope.

Experiment No: 2

Induction of polyploidy in onion root

Aim:

To prepare a slide of onion root tip and identify the different mitotic stages.

Principle:

Acetoceramine is a basic dye used in staining chromosomes and is utilized in viewing the different stages of mitosis in fixed results. Carmine is a basic dye i.e., prepared from the insect Coccus cacti.

Materials required:

- Onion root tips
- Carnoy's fixative*
- Forceps
- Needles
- Slides
- Acetoceramine stain
- 1N HCL
- Cover slip
- Microscope etc.,

*Carnoy's fixative: Absolute alcohol 60 ml, chloroform 30 ml and glacial acetic acid 10 ml.

Procedure:

1. The 1 cm long grown root tips of the onion plant was washed properly with excess amount of distilled water to remove soil attached to the roots.
2. The root tips were fixed in carnoy's fixative for 24 hours (can be stored in 95% alcohol for future use).
3. The root tips were placed in a small beaker containing 1N HCL which is necessary to hydrolyze the tissue.

4. The beaker was warmed to 10°C in a water bath for 10 to 15 minutes.
5. The HCL was rinsed out using fresh water.
6. The meristematic region (1 mm) of the root tips were cut and placed on the slide.
7. A drop of acetoceramine is added to the root tips and the tip were squashed with using a clear glass rod and covered with a cover slip.
8. The squashed, stained preparation was then viewed under the microscope.
9. The meristematic region was located with the 10X objective and then 40X objective to study individual cells.

Result:

The mitotic stage (metaphase) from the onion root tip was observed under the microscope.

Experiment No: 3

Smear techniques to demonstrate sex chromatin in buccal epithelial cells

Aim:

To observe the inactivated X-chromosome (barr body) in the human buccal epithelial cell.

Material required:

- Glass slides
- Cover Slip
- Methylene blue stain

Procedure:

1. The wall of the buccal cavity is scraped with the help of the clean slide and then the mucus was spread on the slide with the help of another slide.
2. The slide is allowed to dry and the material is stained with the methylene blue stain.
3. The excess stain was washed with water, allowed to dry and observed under the microscope.

Principle and mean behind the procedure:

Normal male (XY) does not have any barr body, whereas normal female (XX) has one barr body. Abnormal males who do not have barr body (Turner's Syndrome-X0), but abnormal males who have an extra X-chromosome (Klinefelter's Syndrome)-XXY, have one barr body.

Female who have an extra chromosome (Super female-XXX) have 2 barr bodies. This reveals the inactivation occurs only in the presence of 2 or more X-chromosome. The number of barr bodies of a cell is one less than the no of X-chromosomes in the original cell. Any one of the two X-chromosomes has the chance of being inactivated. This inactivation occurs in order to compensate the dosage of X-chromosome genes in the both sexes.

Result:

The barr body present in the human buccal epithelial cells was observed under the microscope.

Experiment No: 4

Monohybrid crosses in Drosophila for studying autosomal and sex-linked inheritance.

Aim:

To perform experiment to prove Mendel mono hybrid experiment.

Background information:

A monohybrid cross is a cross between parents who are heterozygous at one locus. Monohybrid inheritance is the inheritance of single character. The different forms of the characteristics are usually controlled by different alleles of the same gene. For example a monohybrid cross between two pure breeding plants, one with yellow seeds and one with green seeds, would be expected to produce an F1 generation with only yellow seeds because the allele for yellow seeds is dominant to that of green. A monohybrid experiment compares only on trait. The simplest form of a cross is a monohybrid cross, which analyses a single and its associated variations. During gamete formation, the members of a pair of alleles are duplicated and then segregated from one cell into four separate gametes so that each contains only one member of the pair (Law of Segregation).

Materials required:

- Wild body Drosophila melanogaster
- Ebony Drosophila melanogaster
- Bottles with standard medium
- Anaesthetic ether
- Etherizer
- Re-etherizer
- Needles
- Brushes
- Yeast granules

- Glass plate

Procedure:

1. Select wild type and ebony type flies.
2. Place male and female flies in fresh media containing Petri plates (PI-Day 1) and allowed to cross.
3. Maintain flies in the plates and observe the plates after 6-8 days for the presence of larvae. This is considered as F1 generation.
4. Discard adult flies.
5. Determine sex and other characters of F1 generation (F1 larvae emerge as fly on 14^{th} – 20^{th} days). In general all F1 flies are identical phenotypically and heterozygous genotypically.
6. Collect F1 males and females (5 each) and add them to a new Petri plate with fresh media. Allow them for crossing (PII- D1).
7. Wait for 7 days (28^{th} day) and clear adult flies and look for larvae.
8. Allow larvae to hatch. F2 offspring emerge on 31^{st} – 40^{th} day. Determine eye color, sex and other characters.
9. Tabulate the results and determine the phenotypic nature.

Cross Diagram:

PI Wild type body (EE) X ebony body (ee) [homozygous patens].

F1 Wild type body (Ee) [heterozygous offspring].

Crossing F1 Males and F1Female

F2 Wild-type body Male (Ee) X Wild type body Female (Ee)

F2 Phenotype ratio 3 wild-type: 11 ebony body Genotype ratio 1 EE: 2 Ee: 1 ee

Result:

Results of this experiment proved Mendel monohybrid ratio of inheritance.

Experiment No: 5

PTC test in a population and calculation of allele and genotype frequencies

Aim:

To calculate allele and genotype frequencies in a population by PTC testing.

Principle:

Allele frequency is a measure of the relative frequency of an allele in a population. Hardy-Weinberg equilibrium to determine the probable gene frequencies in a population and to track the changes in gene frequencies from one generation to another.

$$p^2 + 2pq + q^2 = 1$$

Where p is the frequency of the dominant allele (A) for a trait, q is the frequency of the recessive allele (a) for a trait.

$$P + q = 1$$

Materials required:

- Phenylthiocarbamide (PTC) taste test paper
- Calculator
- Control taste test paper
- Phenylthiocarbamide taste test paper
- A little taste reaction to PTC ("called tasters") is evidence for the presence of the dominant allele is either the homozygous (AA) or heterozygous (Aa) genotype. The inability to taste the chemical (called "non-tasters") depends on the presence of homozygous recessive allele (aa).

Procedure:

1. Record the number of students in the class.
2. Have each student place a piece of control taste test paper on his or her tongue and note the taste of the control paper.

3. Have each student place a piece of PTC taste test paper on his or her tongue. If he or she senses a bitter taste (it will be obvious), then he or she is a taste of PTC. If the test paper tastes like the control paper, then he or she is a non-taster.
4. Record the number of non tasters in the class. These students are homozygous recessive (aa) for the PTC tasting gene.
5. Calculate q^2 by dividing the number of non-tasters by the total number of student's record q^2.
6. Calculate q by taking the square root of q^2.
7. Calculate p by subtracting q from 1.
8. Determine the frequency of homozygous dominant (AA) genotypes in the class by calculating p^2.
9. Determine the frequency of heterozygous genotype in the class by calculating 2pq.

Experiment No: 6

Study of abnormal human karyotype and pedigree (dry lab)

According to the position of centromere and size of the chromosomes, they are classified as following:

Acrocentric:

A Chromosome whose centromere is close to one end.

Sub-metacentric:

A Chromosome whose centromere is close to its centre, but is slightly off the centre.

Metacentric:

A Chromosome whose centromere is at its centre.

Chromosomes are divided into pairs 1 to 23 progressing from largest to smallest and placing the sex chromosome pair at the last.

Chromosome number 1:

- Metacentric-largest.
- There is a secondary constriction (purely stained segment)near the centromere of one of the arms.

Chromosome number 2:

Largest sub-metacentric chromosome.

Chromosome number 3:

- Second largest metacentric.
- Distinguished from pair one only by its slightly shorter length.

Chromosome number 4:

Large sub-metacentric chromosome with band in shoulder region.

Chromosome number 5:

Large sub-metacentric chromosome with a band in middle (belly) region.

Chromosome number 6:

- Sub-metacentric.
- Largest 'C' pair usually.

- It is less metacentric than X chromosome, but more metacentric than chromosome number 7 where the longest is not the more median.
- Light band on p arm.

Chromosome number 7:

- Medium size sub-metacentric.
- Chromosome with distinct 'V' shape, p arm and 3 bands in q arm.

Chromosome number 8:

- Sub-metacentric.
- 'p' arm is not 'v' shaped, 2 bands are present.

Chromosome number 9:

Medium sized sub-metacentric with 'p' arm is shorter and 'v' shaped with 2 bands.

Chromosome number 10:

Medium sized sub-metacentric with up shaped 'p' arm.

Chromosome number 11:

Medium sized sub-metacentric with dark 'q' arm and jar like bottom.

Chromosome number 12:

Medium sized sub-metacentric with dark 'q' arm, bands close to centromere.

X-chromosome:

- Medium sized sub-metacentric.
- Bands are in shoulder region, bands are seen in 'p' arm and 'q' arm.

Chromosome number 13:

Medium sized Acrocentric with satellite with band in 'q' arm away from centromere.

Chromosome number 14:

Medium sized Acrocentric with satellite with 2 prominent bands.

Chromosome number 15:

Medium sized Acrocentric with satellite with band in 'q' arm near centromere.

Chromosome number 16:

Small metacentric with band near centromere.

Chromosome number 17:

Small sub-metacentric with band near tail region of 'q' arm.

Chromosome number 18:

Small sub-metacentric with square shaped 'q' arms within 2 hours.

Chromosome number 19:

Small metacentric with band on centromere.

Chromosome number 20:

Small setacentric with band on 'p' arm.

Chromosome number 21:

Small Acrocentric with satellite showing broad band.

Chromosome number 22:

Small Acrocentric with satellite showing short band in centromere.

Y-chromosome:

- Small Acrocentric without satellite.
- Denser staining arms are closer together.

Basic principles

If more than one individual in a family is afflicted with a disease, it is a clue that the disease may be inherited. A doctor needs to look at the family history to determine whether the disease is indeed inherited and, if it is, to establish the mode of inheritance. This information can then be used to predict recurrence risk in future generations.

A basic method for determining the pattern of inheritance of any trait (which may be a physical attribute like eye color or a serious disease like Marfan syndrome) is to look at its occurrence in several individuals within a family, spanning as many generations as possible. For a disease trait, a doctor has to examine existing family members to determine who is affected and who is not. The same information may be difficult to obtain about more distant relatives, and is often incomplete.

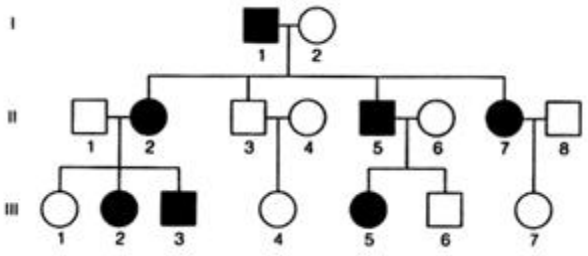
Once family history is determined, the doctor will draw up the information in the form of a special chart or family tree that uses a particular set of standardized symbols. This is referred to as a pedigree. In a pedigree, males are represented by squares □ and females by circles ○. An individual who exhibits the trait in question, for example, someone who suffers from Marfan syndrome, is represented by a filled symbol ■ or ●. A horizontal line between two symbols represents a mating □—○. The offspring are connected to each

other by a horizontal line above the symbols and to the parents by vertical lines. Roman numerals (I, II, III, etc.) symbolize generations. Arabic numerals (1,2,3, etc.) symbolize birth order within each generation. In this way, any individual within the pedigree can be identified by the combination of two numbers (i.e., individual II3).

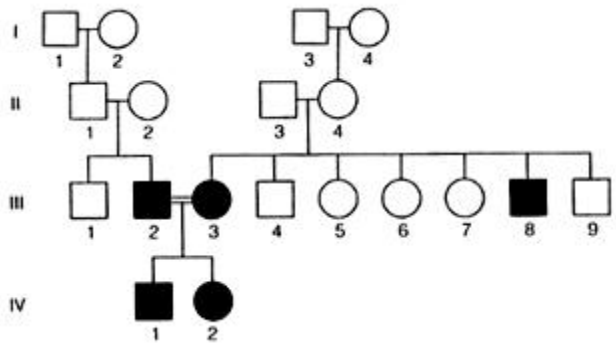
Dominant and recessive traits

Using genetic principles, the information presented in a pedigree can be analyzed to determine whether a given physical trait is inherited or not and what the pattern of inheritance is. In simple terms, traits can be either dominant or recessive.

A dominant trait is passed on to a son or daughter from only one parent. Characteristics of a dominant pedigree are: 1) Every affected individual has at least one affected parent; 2) Affected individuals who mate with unaffected individuals have a 50% chance of transmitting the trait to each child; and 3) Two affected individuals may have unaffected children.



Recessive traits are passed on to children from both parents, although the parents may seem perfectly "normal." Characteristics of recessive pedigrees are: 1) An individual who is affected may have parents who are not affected; 2) All the children of two affected individuals are affected; and 3) In pedigrees involving rare traits, the unaffected parents of an affected individual may be related to each other.



The reason for the two distinct patterns of inheritance has to do with the genes that predispose an individual to a given disease. Genes exist in different forms known as alleles, usually distinguished one from the other by the traits they specify.

Individuals carrying identical alleles of a given gene are said to be homozygous for the gene in question. Similarly, when two different alleles are present in a gene pair, the individual is said to be heterozygous. Dominant traits are expressed in the heterozygous condition (in other words, you only need to inherit one disease-causing allele from one parent to have the disease). Recessive traits are only expressed in the homozygous condition (in other words, you need to inherit the same disease-causing allele from both parents to have the disease).

Penetrance and expressivity

Penetrance is the probability that a disease will appear in an individual when a disease-allele is present. For example, if all the individuals who have the disease-causing allele for a dominant disorder have the disease, the allele is said to have 100% penetrance. If only a quarter of individuals carrying the disease-causing allele show symptoms of the disease, the penetrance is 25%. Expressivity, on the other hand, refers to the range of symptoms that are possible for a given disease. For example, an inherited disease like Marfan syndrome can have either severe or mild symptoms, making it difficult to diagnose.

Non-inherited traits

Not all diseases that occur in families are inherited. Other factors that can cause diseases to cluster within a family are viral infections or exposure to disease-causing agents (for example, asbestos). The first clue that a disease is not inherited is that it does

not show a pattern of inheritance that is consistent with genetic principles (in other words, it does not look anything like a dominant or recessive pedigree).

Human Karyotyping

Aim:

To observe the human karyotype (23 pairs of chromosome) from the human peripheral blood.

Principle:

Chromosomes which are in almost every cell of our body contain the genetic material inherited from the parents. When a cell divides it needs to pass on a complete set of genetic instructions to each new cell it forms. Normally when a cell is not in the process of divisions the chromosomes are arranged in a diffuse, unorganized way. However during divisions the chromosomes in these new cells live up in pairs. In a karyotype test which examines dividing cells, these pairs are arranged by their size and appearance to determine if any chromosomes are missing or damaged.

Materials required:

- RPMI medium
- Fetal calf serum
- Phytohaemagglutinin (PHH)
- Colchicine
- Hypotonic solution KCl (Potassium chloride)
- Glass Slides
- Gimsa stain

Procedure:

- Blood collection with cell culture.
- Prepare a sterile 5 ml syringe with 21 gauge needle.
- Wipe the top of the green top tube with an isopropyl alcohol.

- Insert the needle on the syringe into the green top and withdraw few ml of blood.
- Open the bottle of chromosomal medium/5 ml of growth medium (RPMI & 10% fetal calf serum + PHA), and add 5-10 drops of blood into the medium under sterilization (laminar flow).
- The cells are grown for 60 hours at 38°C (with gentle inversion twice a day during incubation).

Stopping the cell division at metaphase

- Add 0.5 ml of pre-warmed 37°C colcemid to the culture. Mix gently and put the culture back into the incubator.
- Incubate for 30-60 minutes.
- Put the blood and colcemid solution into a conical centrifuge tube and centrifuge at 500-900 rpm for 6 minutes.
- Remove the supernatant with a Pasteur pipette.
- Hypotonic treatment of red and white blood cells.
- Add 1 ml of warmed 37°C hypotonic solution/KCl solution, 0.075 M to the tube. Mix by flicking the tube then add 9 ml of hypotonic solution.
- The hypotonic solution should not be in contact with the cells for more than a total of 27 minutes. Excess exposure may cause rupture of the WBC.
- Place the mixed solution into 37°C incubator for 17 minutes.
- Prepare the fixative solution: add 3 parts chilled absolute methanol to 1 part glacial acetic acid.
- After 9 minutes centrifuge for 6 minutes at 500-900 rpm.
- Remove the supernatant.

Fixing the cells

- Add 5 ml of fixative solution to the centrifuge tube.

- Place this solution of cells and fixative into a refrigerator for 30 minutes. Then centrifuge the tube for 6 minutes at 500-900 rpm.
- Remove the supernatant and add another 6 ml of cold fixative and mix.
- Centrifuge the tube for 6 minutes at 500-900 rpm.
- Repeat the above 2 steps.
- Remove the supernatant.

Making the chromosome slides

- The slide should be cold.
- Lay 5 or 6 slides next to each other on paper covering with no separation between them.
- Withdraw the entire condense of the centrifuge tube into a Pasteur pipette.
- From a height of about 18 inches drop 2 or 3 drops of fluid into each side.
- Allow the slides to dry thoroughly. Infact the best way to cover the slides or to place them in the incubator (37°C), overnight.
- Stain the slide by immersion in fresh Gimsa stain for 7 to 10 minutes.
- Remove the slides from the stain and rinse in distilled H20 until all the excess stain is removed and viewed under the microscope.

Result:

23 pairs of chromosomes from the human peripheral blood was observed.

Experiment No: 7

Conjugation in bacteria

Aim:

To compare conjugation frequencies with distances between the markers by gradient mating using an Hfr donor.

Principle:

In a Hfr-X-F-mating, chromosome transfer proceeds unidirectional commencing from the given point of origin which is determined by the site and orientation of the integration of the F plasmid. Since DNA transfer has a tendency to break off spontaneously (unlike the mating pairs) there is a gradient of transfer of markers, while most of the recipient cells will receive markers located close to the origin of transfer only a few will receive, one's located at the terminus. Thus, the number of recombinants obtained for a marker when an F-crossed with a given Hfr will be inversely proportional to the distance of the marker from the origin of transfer. This principle has been extremely useful in genetic mapping. In this experiment, chromosomal transfer between the mated cultures is artificially interrupted by agitation and the transfer gradient is recorded.

Materials required:

- Cultures used: Donor-Escherichia coli Hfr Str/tet/Kan,
- Recipient- E.coli CSH57 str/tet/Kan, ile, met, arg, ieu

Glassware's

- Conical flask
- Screw cap tubes
- Test tubes
- Petri plates
- Pipettes
- Vortex mixer

Media:

- LB borth
- Minimal medium

Stock solutions:

- Amino acids
- Streptomycin
- Vitamin B1
- Saline

Procedure:

- Prepare Donor and recipient, overnight culture in LB (don't shake).
- Subculture – 1:50 LB and incubate 2-21/2 hours (don't shake).
- Mix 4.5 mL R + 0.5mL D (9:1).
- Incubate at 37°C without shaking.
- Interrupt at different time till 100 minutes.
- Take 0.1 mL culture in sterile stopper tube and vortex for 5 minutes.
- Dilute up to 10^{-5} with saline plate 10^{-3}, 10^{-4} in selective plates (minimal agar with all required amino acids except one).
- Incubate at 37°C for 24 hours.
- Patch on other selective plates for C-conjugant's.

Observation and result:

Experiment of interrupted conjugation is performed at regular interval. Different time growth is noted on different medium with or with amino acids. Different time interval related growth indicates conjugation and passage of different gene at different time interval through conjugation canol.

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Viva-Questions

S. NO	Questions
1.	Who discovered conjugation?
2.	Define conjugation, F pili, Hfr.
3.	Have you construct genetic map through conjugation?
4.	Name donor bacterium and its characters.
5.	Why root tip is taken to study mitosis?
6.	Define Mitosis, mitotic index.
7.	What are the different stages of cell division?
8.	Define cell cycle, cytokinensis.
9.	What is karyotyping and karyotype.
10.	Mention the importance of karyotyping.