B.Sc. ZOOLOGY LAB MANUAL

5th Semester

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PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for UG Zoology fifth Semester. Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers and non teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

C11P: Molecular Biology (Lab) Credits 02

List of Practical

1. Demonstration of polytene and lampbrush chromosome from photograph
2. Isolation and quantification of genomic DNA using spectrophotometer (A260 measurement)
3. Agarose gel electrophoresis for DNA

C12P: Genetics (Lab) Credits 02

List of Practical

1. Chi-square analyses
2. Linkage maps based on conjugation
3. Identification of chromosomal aberration in Drosophila and man from photograph
4. Pedigree analysis of some human inherited traits

DSE1P: Fish and Fisheries (Lab)

List of Practical

1. Morphometric and meristic characters of fishes
2. Study of Petromyzon, Myxine, Pristis, Chimaera, Exocoetus, Hippocampus, Gambusia, Labeo, Heteropneustes, Anabas
3. Study of different types of scales (through permanent slides/ photographs).
4. Study of crafts and gears used in Fisheries
5. Water quality criteria for Aquaculture: Assessment of pH, conductivity, Total solids, Total dissolved solids
6. Study of air breathing organs in Channa, Heteropneustes, Anabas and Clarias
7. Project Report on a visit to any fish farm/ pisciculture unit/Zebrafish rearing Lab.

DSE2P: Animal Biotechnology (Lab) Credits 02
List of Practical

1. Genomic DNA isolation from E. coli
2. Plasmid DNA isolation (pUC 18/19) from E. coli
3. Restriction digestion of plasmid DNA.
4. Construction of circular and linear restriction map from the data provided.
5. Calculation of transformation efficiency from the data provided.
6. To study following techniques through photographs a. Southern Blotting
   b. Northern Blotting
   c. Western Blotting
   d. DNA Sequencing (Sanger's Method)
   e. PCR
   f. DNA fingerprinting
7. Project report on animal cell culture

C11P: Molecular Biology

1. Demonstration of polytene and lampbrush chromosome from photograph

POLYTENE CHROMOSOME

Polytene chromosomes are large chromosomes which have thousands of DNA strands. They provide a high level of function in certain tissues such as salivary glands.

Polytene chromosomes are found in dipteran flies(*Drosophila*, *Chironomus*, *Rhynchosciara etc.).* They are present in another group of arthropods of the class Collembola, a protozoan group Ciliophora, mammalian trophoblasts and antipodal, and suspensor cells in plants. In insects, they are commonly found in the salivary glands when the cells are not dividing. In insects, polytene chromosomes are commonly found in the salivary glands; they are also referred to as "salivary gland chromosomes".

They are produced when repeated rounds of DNA replication without cell division forms a giant chromosome. Thus polytene chromosomes form when multiple rounds of replication produce many sister chromatids *which stay fused together*. The large size of the chromosome is due to the presence of many longitudinal strands called chromonemata; hence the name polytene (many stranded). The chromosomal strands are formed after repeated division of the chromosome in the absence of cytoplasmic division. This type of division is called endomitosis.

STRUCTURE: They are about 0.5 mm in length and 20 μm in diameter. The polytene chromosome contains two types of bands, dark bands and interbands. The dark bands are darkly stained and the inter bands are lightly stained with nuclear stains. The dark bands contain more DNA and less RNA. The interbands contain more RNA and less DNA. The amount of DNA in interbands ranges from 0.8 - 25%.

The bands of polytene chromosomes become enlarged at certain times to form swellings called puffs. The formation of puffs is called puffing. In the regions of puffs, the chromonemata uncoil and open out to form many loops. The puffing is caused by the uncoiling of individual chromomeres in a band. The puffs indicate the site of active genes

where mRNA synthesis takes place. The chromonemata of puffs give out a series of many loops laterally. As these loops appear as rings, they are called Balbiani rings after the name of the researcher who discovered them. They are formed of DNA, RNA and a few proteins. As they are the site of transcription, transcription mechanisms such as RNA polymerase and ribonucleoproteins are present. In protozoans, there is no transcription, since the puff consists only of DNA.

FUNCTIONS: In addition to increasing the volume of the cells' nuclei and causing cell expansion, polytene cells may also have a metabolic advantage as multiple copies of genes permits a high level of gene expression. In *Drosophila melanogaster*, for example, the chromosomes of the larval salivary glands undergo many rounds of endoreduplication to produce large quantities of adhesive mucoprotein ("glue") before pupation. Another example within the fly itself is the tandem duplication of various polytene bands located near the centromereof the X chromosome which results in the Bar phenotype of kidney-shaped eyes.

The interbands are involved in the interaction with the active chromatin proteins, nucleosome remodeling, and origin recognition complexes. Their primary functions are: to act as binding sites for RNA pol II, to initiate replication and, to start nucleosome remodeling of short fragments of DNA.

Polytene chromosomes, at interphase, are seen to have distinct thick and thin banding patterns. These patterns were originally used to help map chromosomes, identify small chromosome mutations, and in taxonomic identification. They are now used to study the function of genes in transcription.

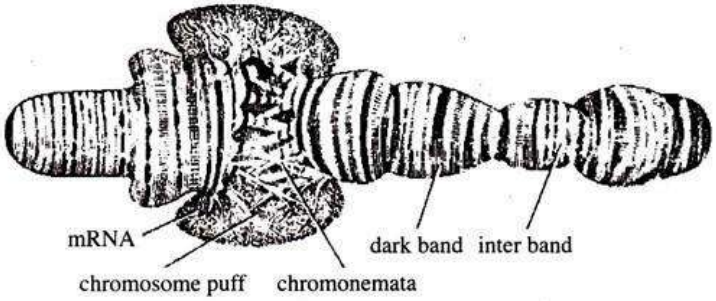
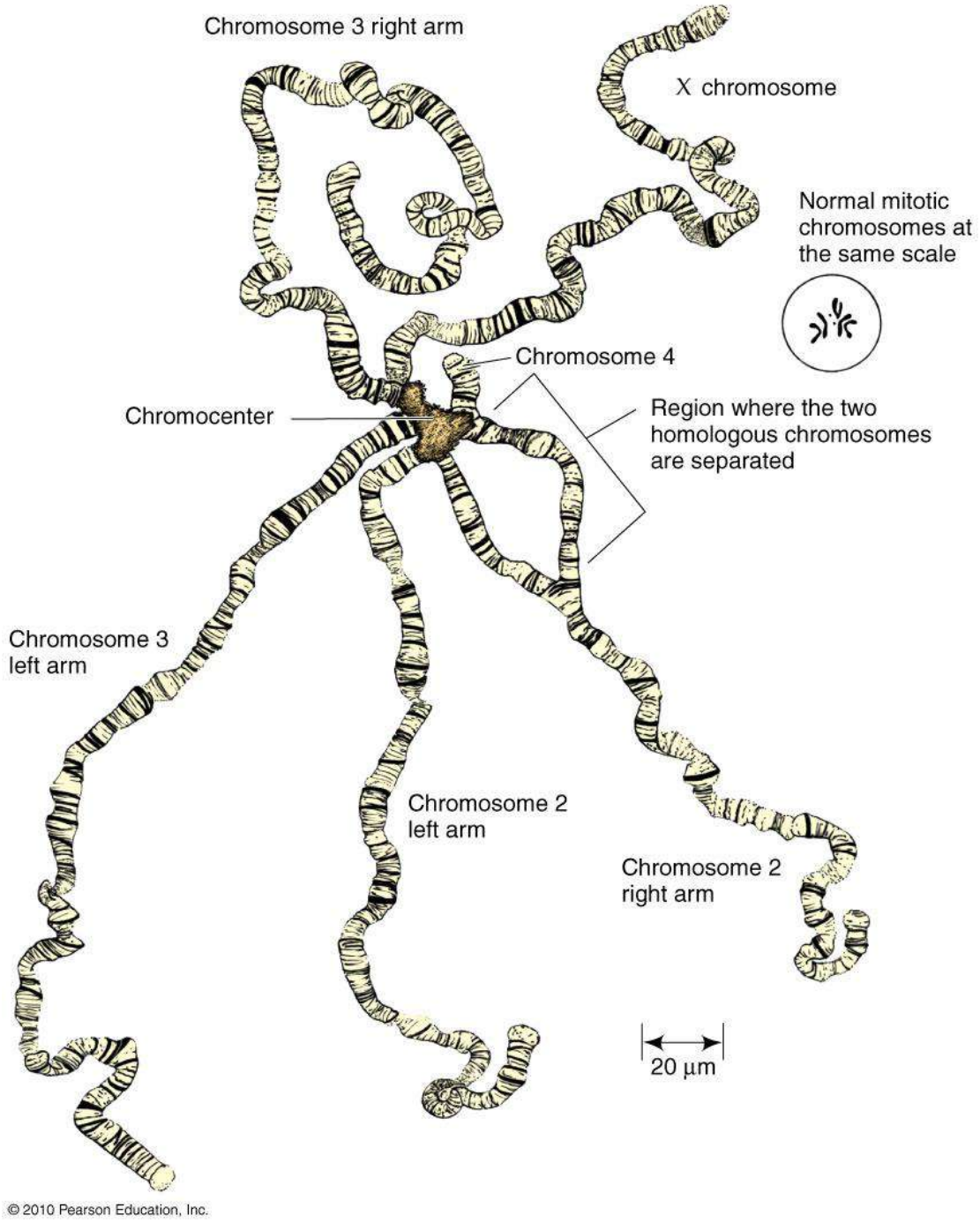


Fig. 3.29 : Polytene chromosome of an insect, showing bands and interbands and a puff or Balbiani ring.



LAMPBRUSH CHROMOSOME

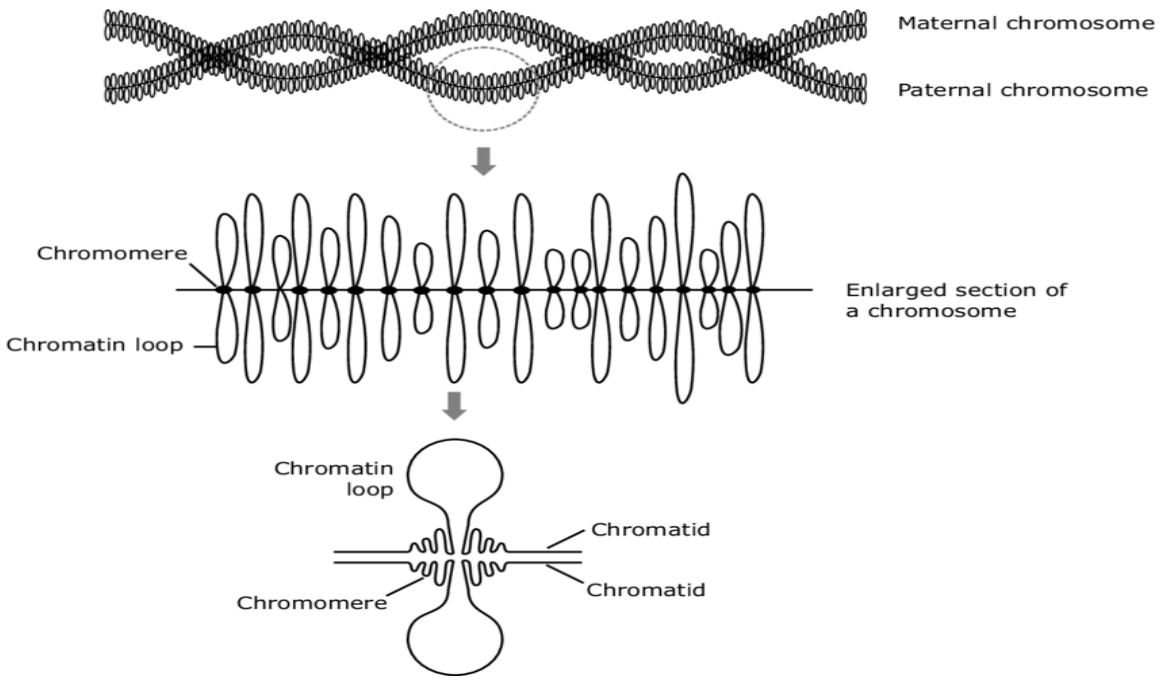
Lampbrush chromosomes are a special form of chromosome found in the growing oocytes (immature eggs) of most animals, except mammals. They were first described by Walther Flemming in 1882. Lampbrush chromosomes of tailed and tailless amphibians, birds and insects are described best of all. Amphibian and avian lampbrush

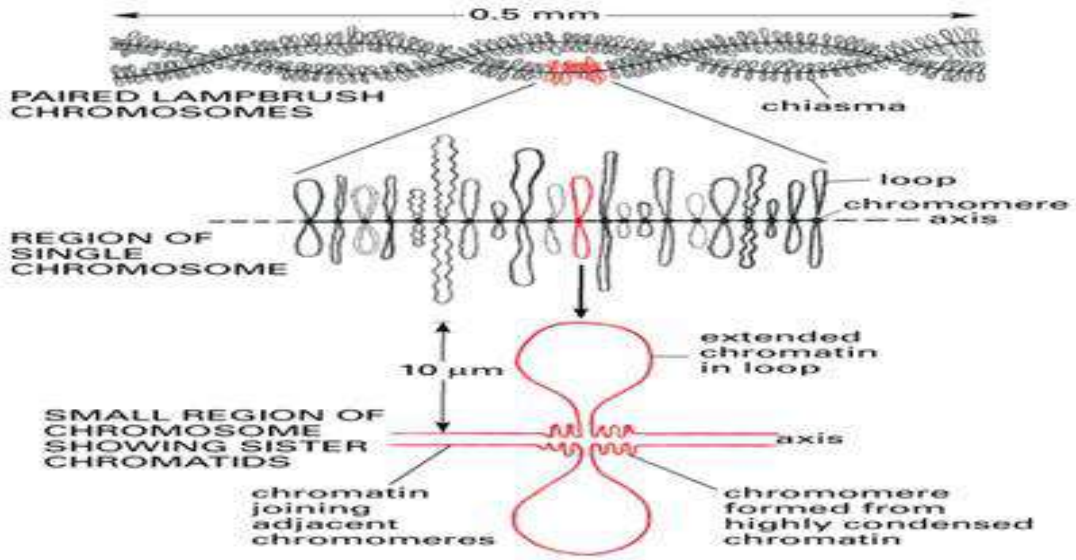
chromosomes can be microsurgically isolated from oocyte nucleus (germinal vesicle) with either forceps or needles.

Chromosomes transform into the lampbrush form during the diplotene stage of meiotic prophase I due to an active transcription of many genes. They are highly extended meiotic half-bivalents, each consisting of 2 sister chromatids.

STRUCTURE: Lampbrush chromosomes are clearly visible even in the light microscope, where they are seen to be organized into a series of chromomeres with large chromatin loops extended laterally. Each lateral loop contains one or several transcription units with polarized RNP-matrix coating the DNA axis of the loop.

FUNCTIONS: Giant chromosomes in the lampbrush form are useful model for studying chromosome organization, genome function and gene expression during meiotic prophase, since they allow the individual transcription units to be visualized.[10] Moreover, lampbrush chromosomes are widely used for high-resolution mapping of DNA sequences and construction of detail cytological maps of individual chromosomes.





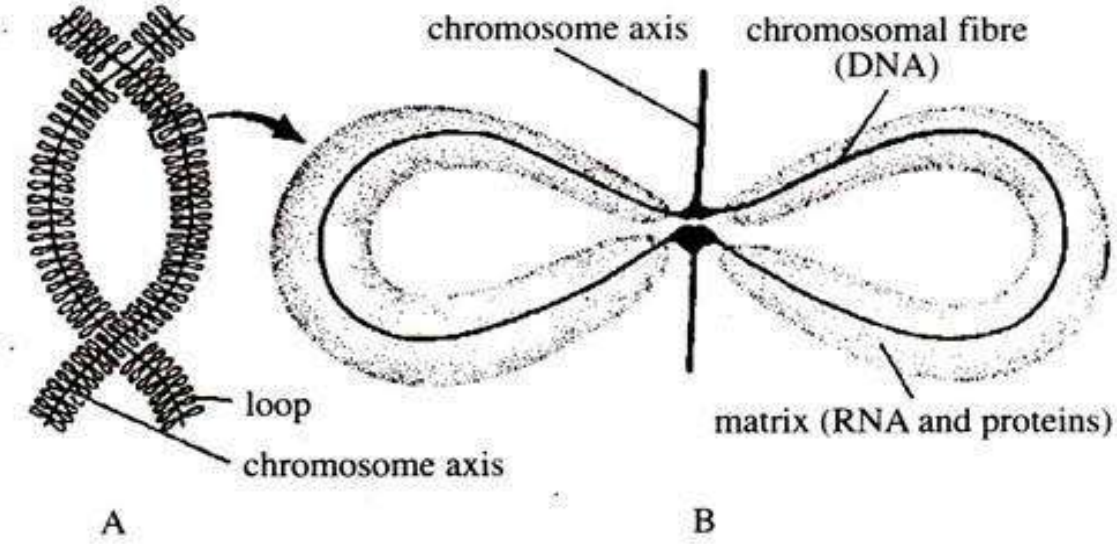


Fig. 3.30 : Lampbrush chromosome. A. At low magnification; B. Loop magnified.

2. Isolation and quantification of genomic DNA using spectrophotometer (A260 measurement)

Principle:

DNA isolation is based on four basic principles

a. Preparation of a cell extract

b. Purification of DNA from cell extract

c. Concentration of DNA samples

d. Measurement of purity and DNA concentration

DNA isolation and concentration involves disruption of the cells followed by removal of the contaminants and then the recovery of the DNA. The cell is homogenized followed by centrifugation to disrupt the cell and separate the contents in a gradient. The soluble material is then mixed with a 1:1 mixture of Phenol and Chloroform, these precipitates the protein but leaves the nucleic acids in the aqueous phase. This aqueous phase is pipette out and the protein and RNA are got rid off with the use of proteases and RNase. After purification, the DNA is concentrated using chilled ethanol.

Reagents Required:

1. 0.1 M EDTA
2. Lysis Buffer (0.1 M Tris – HCl, 0.1 M EDTA, 0.01 M NaCl, 1% SDS, pH 8.0)
3. Proteinase K (mg/ml)
4. RNAse (10mg/ml)
5. Phenol: Chloroform : Isoamyl alcohol (25: 24: 1)
6. Chloroform : Isoamyl Alcohol (24: 1)
7. 5 M NaCl
8. 100% Ethanol
9. TE Buffer (10mM Tris – HCl, 1mM EDTA, pH 8.0)

Method:

1. 1oo mg of goat liver was taken. It was homogenized in lysis buffer (for 200mg take 1.8 ml of lysis buffer) at 4°C.
2. It was centrifuged at 14,000 rpm for 15 min. Supernatant was decanted, and the pellet was

taken.

3. To 500µl of the pellet 4.5 ml of lysis buffer was added.

4. It was then centrifuged at 8000 rpm for 10 min. The supernatant was carefully decanted leaving behind the pellet containing the cells. The pellet was slowly dislodged into the soup and cell lysate was prepared.

5. 1ml of cell lysate was taken into a 2ml micro centrifuge tube and to it 200µl (200 µg/ ml) of proteinase K and 2 µl of RNAse was added. It was kept at 55°C for 2 hours.

6. To it 500 µl of Tris saturated phenol was added followed by gentle mixing by inverting up and down for about 3 min and it was centrifuged at 14, 000 rpm for 5 min

7. Denatured protein is evident from the white precipitate forming at the boundary between organic and aqueous phase. The top aqueous phase is pipetted out into a fresh micro centrifuge tube; care should be taken not to disturb the organic layer.

8. To the collected aqueous layer 500µl of phenol: chloroform: isoamyl alcohol (25:24:1) was added followed by mixing by inverting up and down for about 3 min and it was centrifuged at 14, 000 rpm for 5 min. The top aqueous layer was again pipetted out into a fresh micro centrifuge tube; care should be taken not to disturb the organic layer.

9. To the collected aqueous layer 500 µl of chloroform : isoamyl alcohol (24:1) was added mixing by inverting up and down for about 3 min and it was centrifuged at 14, 000 rpm for 5 min. The top aqueous layer was again pipetted out into a fresh micro centrifuge tube; care should be taken not to disturb the organic layer.

10. If the volume of the aqueous phase is 500 µl, to this 1 ml (twice the volume of aqueous phase) of 100% chilled ethanol and 5 µl of 5 M NaCl were added and mixed well leading to DNA precipitation.

11. White thread like DNA appears and the solution is mix to coalesce the DNA. The tube is kept at -20°C for 20 min. it is then centrifuged at 14,000 rpm for 5 min, DNA forms a pellet at the bottom. Alcohol is decanted and evaporated. DNA was re-suspended in 200 µl of TE buffer by thoroughly mixing it.

Calculation of DNA Concentration:

A DNA concentration is measured by UV absorbance spectrometry. The amount of UV radiation absorbed by a solution of DNA is directly proportional to the amount of DNA sample. Absorbance is measured at 260 nm and DNA content is directly proportional to the content of DNA.

It is generally considered that 1 O.D is equivalent 50µg of double stranded DNA.

Concentraion of DNA: Units (x) mg ml^{-1}/ O.D 260

= 50/1 Units (x) mg ml^{-1}

= 50 * O.D 260 * Dilution factor.

Example: If 5µl of extracted DNA in 1000µl gives an OD260 = 0.14

Then, Dilution factor = 1000/5

= 200 Concentration of DNA

= 0.14x200x50

=1400µg/ml

=1.4mg ml^{-1}.

Critical Notes:

1. OD value should be between 0.1 to 1.0 for an optimal measurement, which are based on the extinction coefficients of nucleic acids in water. Hence, this values differs in different buffers.
2. An A260/A280 < 1.8 indicates contamination with protein and/or phenol.
3. An A260/A280 > 2.0 indicates a possible contamination with RNA.
4. OD does not gives any information about the size of the DNA.

Justification of the Reagents Used:

1. Isoamyl alcohol is added to chloroform to prevent frothing and esterification of lipid.
2. Chloroform improves efficiency of nucleic acid extraction by denaturing protein. It helps in the dissociation of protein from nucleic acid. It also help in the removal of lipid.
3. SDS being a detergent helps by disrupting the phospholipid bilayer of cell membrane and also nuclear membrane.
4. Phenol helps in protein denaturation and precipitation. Saturated phenol cause dehydration of DNA by extracting water.
5. EDTA chelating agent. Other than Ca2+ it also helps in the removal of divalent cation Mg2+ necessary for inactivation of DNAse.
6. Proteinase K helps in the breakdown of peptide bonds.
7. Chilled ethanol and high salt concentration displaces water of hydration required for solubilisation of DNA creating hydrophobic environment and precipitates DNA.

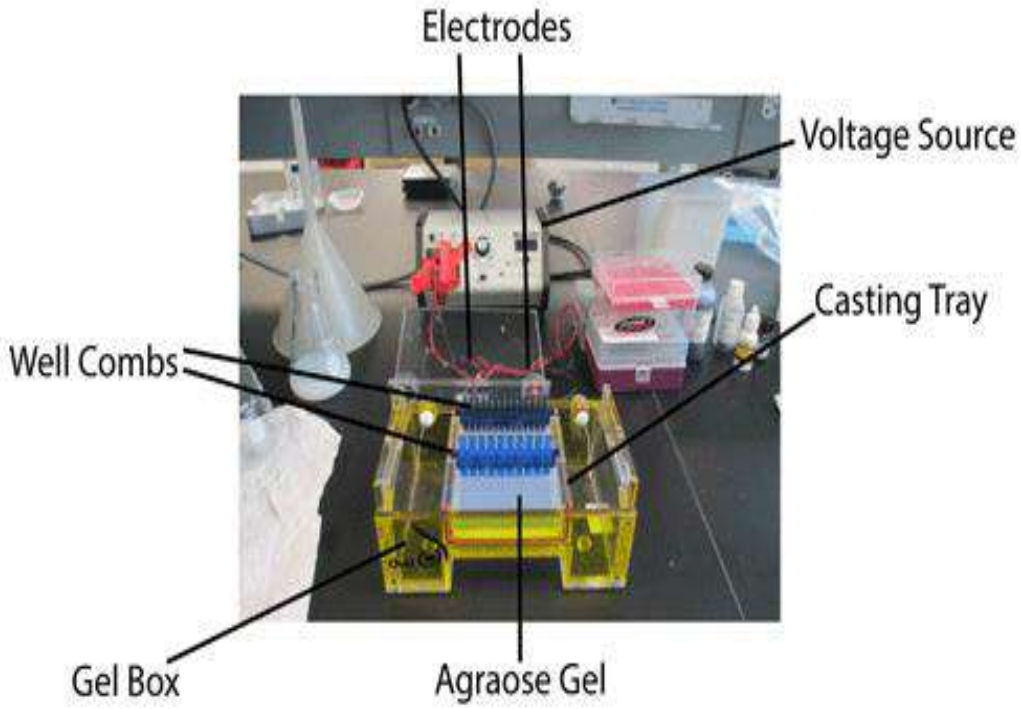
3. Agarose gel electrophoresis for DNA

Introduction

Gel electrophoresis is the standard lab procedure for separating DNA by size (e.g., length in base pairs) for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode. Shorter DNA fragments migrate through the gel more quickly than longer ones. Thus, you can determine the approximate length of a DNA fragment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known lengths).

Equipments

1. Casting tray
2. Well combs
3. Voltage source
4. Gel box
5. UV light source
6. Microwave

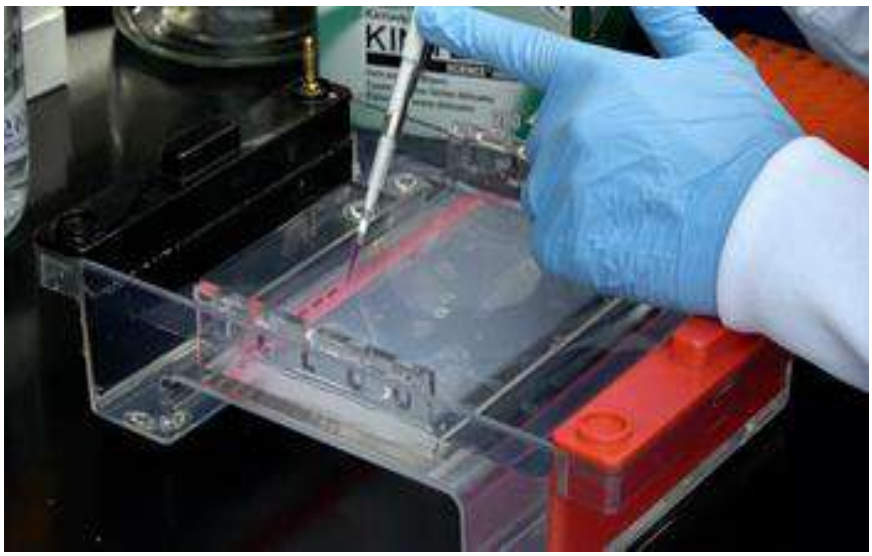


Procedure

Reagents Required

a. 1% Agarose Gel:

1. Measure 1 g of agarose.
2. Mix agarose powder with 100 mL 1xTAE in a microwavable flask.
3. Microwave for 1-3 min until the agarose is completely dissolved (but do not overboil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Many people prefer to microwave in pulses, swirling the flask occasionally as the solution heats up.).
4. Let agarose solution cool down to about 50 °C (about when you can comfortably keep your hand on the flask), about 5 mins.
5. Add ethidium bromide (EtBr) to a final concentration of approximately 0.2-0.5 µg/mL (usually about 2-3 µl of lab stock solution per 100 mL gel). EtBr binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light.
6. Pour the agarose into a gel tray with the well comb in place.
7. Place newly poured gel at 4 °C for 10-15 mins or let sit at room temperature for 20-30 mins, until it has completely solidified.



b. Running Buffer

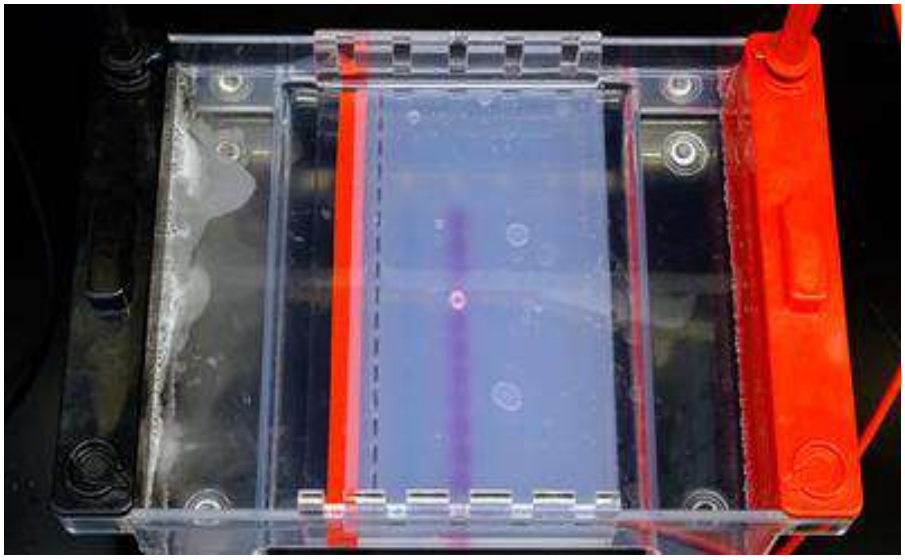
For one liter 50X stock of TAE

1. Tris-base: 242 g
2. Acetate (100% acetic acid): 57.1 ml
3. EDTA: 100 ml 0.5M sodium EDTA
4. Add dH_20 up to one litre.

To make 1x TAE from 50X TAE stock, dilute 20ml of stock into 980 ml of DI water.

Loading Samples and Running an Agarose Gel:

2. Add loading buffer to each of your DNA samples.
3. Once solidified, place the agarose gel into the gel box (electrophoresis unit).
4. Fill gel box with 1xTAE (or TBE) until the gel is covered.
5. Carefully load a molecular weight ladder into the first lane of the gel.
6. Carefully load your samples into the additional wells of the gel.
7. Run the gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel. A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.
8. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.
9. If you did not add EtBr to the gel and buffer, place the gel into a container filled with 100 mL of TAE running buffer and 5 µL of EtBr, place on a rocker for 20-30 mins, replace EtBr solution with water and destain for 5 mins.
10. Using any device that has UV light, visualize your DNA fragments. The fragments of DNA are usually referred to as ‘bands’ due to their appearance on the gel.



Analyzing Your Gel:

Using the DNA ladder in the first lane as a guide (the manufacturer's instruction will tell you the size of each band), you can infer the size of the DNA in your sample lanes. For more details on doing diagnostic digests and how to interpret them please see the Diagnostic Digest page.

Purifying DNA from Your Gel:

If you are conducting certain procedures, such as molecular cloning, you will need to purify the DNA away from the agarose gel. For instructions on how to do this, visit the Gel Purification page.

1. Agarose gels are commonly used in concentrations of 0.7% to 2% depending on the size of bands needed to be separated. Simply adjust the mass of agarose in a given volume to make gels of other agarose concentrations (e.g., 2 g of agarose in 100 mL of TAE will make a 2% gel).
2. TBE can be used instead of TAE, labs usually use one or the other, but there is very little difference between the two.
3. It is a good idea to microwave for 30-45 sec, stop and swirl, and then continue towards a boil. Keep an eye on it the solution has a tendancy to boil over. Placing saran wrap over the top of the flask can help with this, but is not necessary if you pay close attention.

4. Pour slowly to avoid bubbles which will disrupt the gel. Any bubbles can be pushed away from the well comb or towards the sides/edges of the gel with a pipette tip.
5. If you are in a hurry, the gel will set more quickly if you place the gel tray at 4 °C earlier so that it is already cold when the gel is poured into it.
6. Remember, if you added EtBr to your gel, add some to the buffer as well. EtBr is positively charged and will run the opposite direction from the DNA. So if you run the gel without EtBr in the buffer you will reach a point where the DNA will be in the bottom portion of the gel, but all of the EtBr will be in the top portion and your bands will be differentially intense. If this happens, you can just soak the gel in EtBr solution and rinse with water to even out the staining after the gel has been run, just as you would if you had not added EtBr to the gel in the first place.
7. If you will be purifying the DNA for later use, use long-wavelength UV and expose for as little time as possible to minimize damage to the DNA.

Notes :

1. Make sure to use the same buffer as the one in the gel box (do not mix different buffers and do not use water).
2. If you add EtBr to your gel, you will also want to add it to the running buffer when you run the gel. If you do not add EtBr to the gel and running buffer, you will need to soak the gel in EtBr solution and then rinse it in water before you can image the gel.
3. Loading buffer serves two purposes: 1) it provides a visible dye that helps with gel loading and allows you to gauge how far the DNA has migrated; 2) it contains a high percentage of glycerol that increases the density of your DNA sample causing it settle to the bottom of the gel well, instead of diffusing in the buffer.
4. When loading the sample in the well, maintain positive pressure on the sample to prevent bubbles or buffer from entering the tip. Place the very top of the tip of the pipette into the buffer just above the well. Very slowly and steadily, push the sample out and watch as the sample

fills the well. After all of the sample is unloaded, push the pipettor to the second stop and carefully raise the pipette straight out of the buffer.

5. Black is negative, red is positive. The DNA is negatively charged and will run towards the positive electrode. **Always Run to Red.**
6. When using UV light, protect your skin by wearing safety goggles or a face shield, gloves and a lab coat.

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C12P: Genetics (Lab)

Chi-Square Test

This is a statistical test performed to determine how closely the observed ratio fits the This expected ratio. Chi-square ($\chi 2$) test was used in testing statistical hypothesis in the year 1900 by Karl Pearson.

Statistical Hypothesis: Any kind of statement about a statistical population or statistical value are called statistical hypothesis.

Test of Hypothesis: A test of hypothesis is a method which specifies whether to accept or reject the hypothesis under consideration under the preview of a set of rules for decision. Test of hypothesis deals only with the acceptance and rejection of null hypothesis.

Null Hypothesis: According of R. A. Fisher, "Null Hypothesis is the hypothesis which is to be tested for possible rejection under the assumption it is true.

" Null hypothesis is denoted by H_o and is tested against an alternative hypothesis.

Alternative Hypothesis: The negative form of null hypothesis is known as the alternative hypothesis. Alternative hypothesis is denoted by H_a.

Level of significance: The maximum probability with which a null hypothesis is rejected is called the level of significance of the statistical test. Generally, level of significance is considered at 1% or 5% level or any other level depending upon the consequences of statistical decision.

Degrees of freedom: This is the values of a sample which are freely variable without disturbing the mean. This parameter is used to determine whether a chi-square value is statistically significant or not.

If the data is given in the form of a series of variables in a row or column then the degrees of freedom (df) is calculated by the formula.

df = n-1, where 'n' is the number of items in the series. Chi-square value against particular degrees of freedom is obtained from the probability chart published by Yates & Fisher.

Type of Chi-squares: There are three types of Chi-square analysis. These are- (i) Goodness of fit, (ii) Contingency Chi-square, (iii) Homogeneity Chi-square.

Goodness of fit: Common type of Chi-square analysis is known as Pearsonian Chi-square test. This his is also known as test for goodness of fit, because the test is performed to determine whether the observed value is in good agreement with the expected values.

This is determined by the following formula:

$$\chi^2 = \sum \frac{\left[(O-E) - \frac{1}{2}\right]^2}{E}$$

Where O = observed value

E = Expected value

$\frac{1}{2}$ = Yates correction

*Yates correction means reduction of 0.5 from absolute difference between observed and expected (+ or -) frequencies is generally applicable in Monohybrid cross.

Null hypothesis for Goodness of fit: Null hypothesis for Goodness of fit can be studied very well with the ratio obtained from Mendelian monohybrid and dihybrid crosses e.g.

(i) Mendelian monohybrid test cross ratio 1:1

(ii) Mendelian monohybrid F_2, ratio 3:1

(Iii) Mendelian dihybrid test cross ratio 1:1:1:1

(iv) Mendelian dihybrid F_2, ratio 9:3:3: 1

Some Examples of Goodness of Fit:

Example1: In a cross between tall and dwarf garden pea plants 350 tall and 110 dwarf pea plants were obtained in F_2, Test the goodness of fit of these data to a 3: 1 ratio, using the chi-square test considering probability at 5% level.

Observed (O)	Expected (E)	(O–E)	$(O-E)-\frac{1}{2}$	$\{(O-E)-\frac{1}{2}\}^2$	$X^2 = \frac{\left[(O-E)-\frac{1}{2}\right]^2}{E}$
Tall = 350	345	5	5 – 0.5 = 4.5	20.25	$\frac{20.25}{345} = 0.058$
dwarf = 110	115	–5	|–5| –0.5 = 4.5	20.25	$\frac{20.25}{115} = 0.176$
Total = 460					$\sum = 0.234$

Solution : (a) Here Null hypothesis Ho = 3 : 1

(b) Alternative hypothesis Ha = 1 : 1

(c) Formula : $\chi^2 = \Sigma \frac{\left[(O-E) - \frac{1}{2}\right]^2}{E}$

Here degrees of freedom 2 – 1 = 1

At 5% level of significance the table value is 3·84

Inference: Since the calculated value is 0.234 and table value is 3.84, therefore difference between the observed and expected values are insignificant, so the null hypothesis is accepted. In this case the data has good fit to the Mendelian 3:1 ratio.

Example-2: In a cross between long wing and vestigial wing Drosophila, 510 long wing and 490 vestigial wing Drosophila were obtained in F_2. Test goodness of fit of these data to a 1:1 ratio, using the X^2 test considering 5% level of significance.

Solution : (i) Null hypothesis Ho = 1:1

(ii) Alternative hypothesis Ha = 3:1

(iii) Formula : $\chi^2 = \Sigma \frac{\left[(O-E) - \frac{1}{2}\right]^2}{E}$

Here degrees of freedom 2-1=1

At 5% level of significance the table value is 3.84

Observed (O)	Expected (E)	(O–E)	$(O-E) - \frac{1}{2}$	$\{(O-E) - \frac{1}{2}\}^2$	$X^2 = \frac{(O-E)^2}{E}$
Long Wing = 510	half of 1000 = 500	10	$(10) - \frac{1}{2} = 9.5$	90.25	$\frac{90{\cdot}25}{500} = 0.180$
Vestigial Wing = 490	= 500	–10	$(-10) - \frac{1}{2} = 9.5$	90.25	$\frac{90{\cdot}25}{500} = 0.180$
Total = 1000					$\Sigma = 0.360$

Inference: The difference between the observed and expected values are insignificant at 0.05 probability. Hence the Null Hypothesis is accepted.

Example-3: From a cross between Grey body-Long wing *Drosophila melanogaster* and Ebony body-vestigial wing flies, the following progenies were obtained in F_2, Grey long = 380: Ebony 135 Vestigial = 125, Ebony-vestigial = 40. Test the goodness of fit of the data to a 9:3:3:1 ratio using x^2 test considering probability at 5% level.

Observed (O)	Expected (E)	(O – E)	$(O-E)^2$	$\chi^2 = \frac{(O-E)^2}{E}$
Grey long–380	382.5	382.5 – 380 = –2.5	6.25	$\frac{6{\cdot}25}{382{\cdot}5} = 0.016$
Ebony–135	127.5	135–127.5 = 8.5	68.0	$\frac{68{\cdot}0}{127{\cdot}5} = 0.533$
Vestigial–125	127.5	127.5–125 = –2.5	6.25	$\frac{6{\cdot}25}{127{\cdot}5} = 0.049$
Ebony–vestigial–40	42.5	42.5–40 = –2.5	6.25	$\frac{6{\cdot}25}{42{\cdot}5} = 0.147$
Total–680				$\Sigma = 0.745$

Solution: Here, Null Hypothesis H_o 9: 3:3:1

Alternative hypothesis H_a = 1:1:1:1

Here, degrees of freedom = 4-1 = 3

At 5% level of significance the table value is 7.82

Inference: Difference between observed and expected is insignificant at 5% level of significance and hence the Null Hypothesis is accepted.

Example-4: From a cross between heterozygous tall plant with round seed Coat pea plant and homozygous dwarf plant with wrinkled seed the following phenotypes were obtained in F_2. Tal-round = 200, Tall-wrinkled = 190; dwarf-round = 180 and dwarf-wrinkled = 170. Test the goodness of fit considering suitable null hypothesis. Consider the level of significance at 5%.

of fit considering

Observed (O)	Expected (E)	Obs–Exp. (O – E)	$(O-E)^2$	$\chi^2 = \frac{(O-E)^2}{E}$
Tall-round = 200	$\frac{740}{4} = 185$	(200–185) = 15	225	$\frac{225}{185} = 1.216$
Tall-wrinkled = 190	= 185	(190–185) = 5	25	$\frac{25}{185} = 0.135$
Dwarf-wrinkled = 180	= 185	(185–180) = –5	25	$\frac{25}{185} = 0.135$
Dwarf-wrinkled = 170	= 185	(185–170) = –15	225	$\frac{225}{185} = 1.216$
rotal–740				Σ=2.702

Solution : Here Null Hypothesis is 1 : 1 : 1 : 1 and alternate hypothesis is 9 : 3 : 3 : 1

Here degrees of freedom = 4 – 1 = 3

At 5% level of significance the table value is 7·82

Inference : Difference between observed and expected is insignificant at 5% level of significance and hence the Null hypothesis is accepted.

(ii) Contingency Chi-square: In many experiments, it becomes necessary to compare one set of observation recorded under a particular condition to those of identical nature but under different conditions. For this studies it can be identified whether the both the results are dependent (contingent upon) or independent of conditions under which the observation were made. Therefore, the test is called **contingency test**.

Formula of contingency Chi-square: Common formula for contingency Chi-square is as follows -----

$$\chi^2 = \frac{N\left\{(ad-bc)-\frac{N}{2}\right\}^2}{R_1 \times R_2 \times C_1 \times C_2}$$

Where 'a', 'b', 'c' and 'd' are the frequencies for the four cell contingency table, where 'a' &'b' are in one row and 'c' & °d' in the other row. On the other hand, 'a' and 'c', 'b' and 'd' are in two columns.

The R_1 denotes the total of a + b ; R_2 denotes the total of c + d. Likewise a + c is denoted by C_1; b + d is donoted by C_2.

$N = a + b + c + d = R_1 + R_2 = C_1 + C_2$

In this case the degress of freedom = $(R - 1)(C - 1) = 1 \times 1 = 1$

Example : Among 60 males and 50 females, 25 males and 20 females were with attached ear lobes. Statistically probe whether attached ear lobe has any relation with the sex.

Null hypothesis: Attached ear lobe character is not a sex-linked character.
Alternative hypothesis: The character is sex linked.

Calculation :

Ear lobe	Male	Female	Total	
Free ear lobe	35 (a)	30 (b)	65	$(a + b) R_1$
Attached ear lobe	25 (c)	20 (d)	45	$(c + d) R_2$
	a + c = 60	b + d = 50	a + b + c + d = 110	
	C_1	C_2		

$$\chi^2 = \frac{N\left\{|ab - bc| - \frac{N}{2}\right\}^2}{R_1 R_2 C_1 C_2}$$

$$= \frac{110 \times \left\{|700 - 750| - \frac{110}{2}\right\}^2}{65 \times 45 \times 60 \times 50}$$

$$= \frac{110 \times \{|-50| - 55\}^2}{65 \times 45 \times 60 \times 50}$$

$$= \frac{110 \times \{|-5|\}^2}{65 \times 45 \times 60 \times 50} = \frac{^{11}\cancel{55}\cancel{110} \times \cancel{25}}{65 \times 45 \times \cancel{60}_{12} \times \cancel{50}_{\cancel{25}}}$$

$$= \frac{11}{65 \times 45 \times 12} = \frac{11}{35100}$$

$= 0{\cdot}0003$

Calculation :

$ad = 35 \times 20 = 700$

$bc = 30 \times 25 = 750$

$R_1 = a + b = 65$, $C_1 = a + c = 60$

$R_2 = c + d = 45$, $C_2 = b + d = 50$

$N = a + b + c + d = 110$

Degrees of Freedom (df) : $(R-1)(C-1) = (2-1)(2-1) = 1 \times 1 = 1$

Table value at 5% level of significance at df is 3.84

Inference: The relationship between the attached ear lob and sex of human being is insignificant and hence the null hypothesis is accepted.

(iii) **Homogeneity Chi-square**: Homogeneity Chi-square is done when different results are obtained of similar kind of experiments. For example, results obtained from a number of Mendelian monohybrid or dihybrid crosses are taken together for X^2 analysis first individually are between the individual X^2 data and pooled data can be observed.

Working Principle:

(1) Calculate the X^2 of individual experiments.

(2) Make the summation of all X^2 values along with df.

(3) Make the X^2 of the summed individual data.

(4) Draw inference on the basis of null hypothesis considered from the results of homogeneity Chi-square.

Example: From five sets of monohybrid crosses between tall and dwarf pea plants following results were obtained in F_2.

Table : A

	1st set	2nd set	3rd set	4th set	5th set
Tall	55	40	44	41	31
Dwarf	15	10	12	11	9

Solution : Null hypothesis : The results conform 3 : 1 principle.

Alternative hypothesis : The results do not conform 3 : 1 principle.

Table : B

	Observed	Expected	Obs–Exp.	$(O-E)^2$	$\frac{(O-E)^2}{E}$	χ^2	df
1st	55	52·5	2·5	6·25	$\frac{6\cdot25}{52\cdot5}=0\cdot119$	0·476	2–1
set	$\frac{15}{70}$	17·5	–2·5	6·25	$\frac{6\cdot25}{17\cdot5}=0\cdot357$		=1
2nd	40	37·5	2·5	6·25	$\frac{6\cdot25}{37\cdot5}=0\cdot016$	0·516	2–1
set	$\frac{10}{50}$	12·5	–2·5	6·25	$\frac{6\cdot25}{12\cdot5}=0\cdot500$		=1
3rd	44	42	2	4	$\frac{4}{42}=0\cdot095$	0·380	2–1
set	$\frac{12}{56}$	14	–2	4	$\frac{4}{14}=0\cdot285$		=1
4th	41	39	2	4	$\frac{4}{39}=0\cdot102$	0·409	2–1
set	$\frac{11}{52}$	13	–2	4	$\frac{4}{13}=0\cdot307$		=1
5th	31	30	1	1	$\frac{1}{30}=0\cdot033$	0·133	2–1
set	$\frac{9}{40}$	10	–1	1	$\frac{1}{10}=0\cdot100$		=1

Summed X^2 $\Sigma X^2 = 1.914$

Data	χ^2	df	Table value
Total	1.914	4	
Sum Data	1.989	1	
Homogenity	Difference between the two is 0.075	3	at 5% level of significance table value 7.82

Inference: The difference is insignificant and null hypothesis is accepted.

Linkage maps based on conjugation

Introduction

In conjugation experiments, it was observed that the number of genes transmitted from donor to recipient was directly proportional to the time interval for which conjugation was allowed. Based on this feature, **Francois Jacob** and **Ellie Wollman** developed a technique called '**interrupted mating technique**' for mapping the bacterial chromosome. In this technique donor Hfr and recipient F" strains are mixed and allowed to conjugate for a short period of time. Then samples are removed at periodic intervals and subjected to violent agitation to break the conjugation tube. The length of donor chromosome transmitted could then be determined and mapped in terms of time units required for transfer. It is known that 8 minutes are needed for conjugation to begin and then chromosome is transferred slowly in terms of time units (Fig. 1), one time unit being equal to one minute.

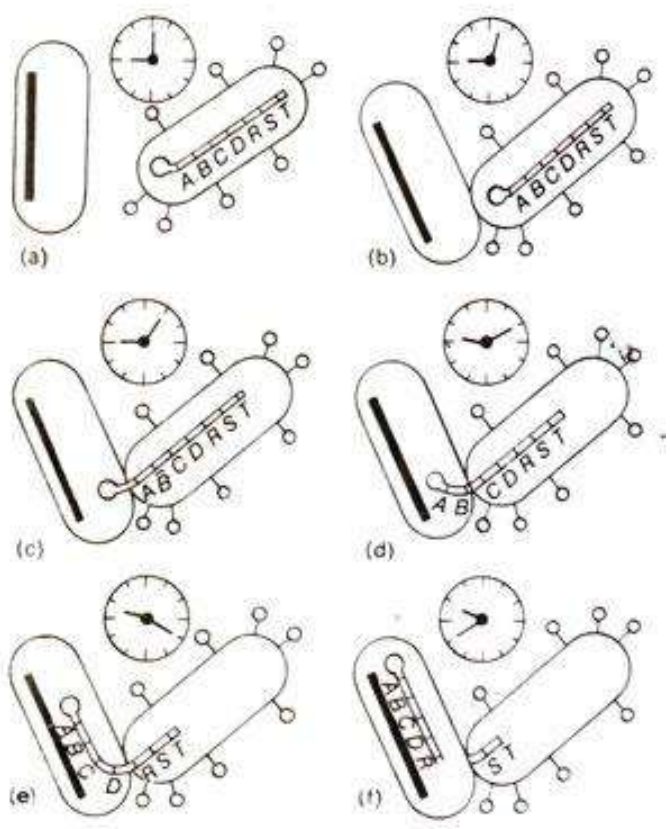


Fig. 1. The transfer of genes in linear order from male bacterium (tagged with virus) to female; the length of transferred segment is dependent upon the time taken for transfer and the chromosome is, therefore, mapped in terms of time units.

The complete chromosome of *E. coli* is transferred in about 89 minutes (Fig. 2) and therefore the bacterial chromosome is 89 time units in length. Genes which are 2-3 time units apart can be precisely mapped by this method. Further mapping within the limits of 1-3 minutes is done by conventional recombination methods.

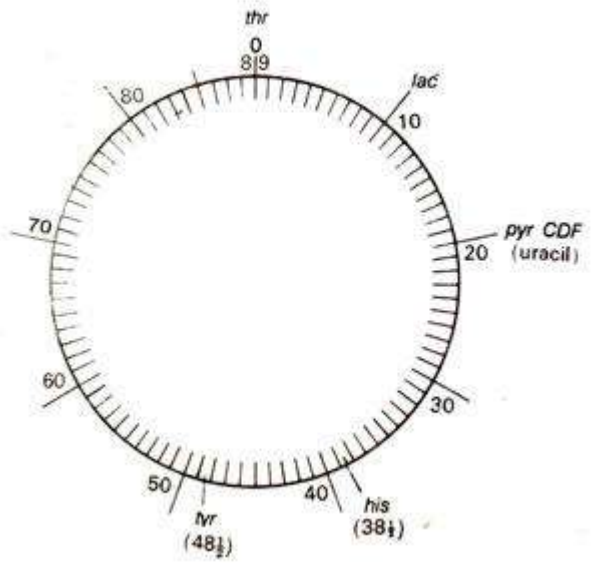


Fig. 2. Circular linkage map of *Escherichia coli* chromosome, the units of recombination shown as minutes required for transfer.

Circular linkage map

When linkage maps in bacteria were prepared using several Hfr strains, these maps differed with respect to the first and the last genes of the map. However, once we know the first gene transferred and the direction of transfer of subsequent genes, the order is predetermined. The only way these results could be explained, was through the assumption of a circular map, which cleaves at any point due to attachment of F factor to form a linear chromosome. This linear chromosome enters the recipient cell from the end away from the site of attachment of F factor. Experimental evidence was also available to prove that Hfr really results due to the insertion of F into the chromosome. Circular nature of linkage group was later confirmed by evidence for physical circular nature of bacterial chromosome.

Linkage information from transformation

Transformation is achieved through the uptake of naked DNA extracted from one strain of bacteria by another strain of bacteria. While extracting this DNA, some breakage into small pieces does take place. If two genes are closer to each other, they have better chance of being carried on the same piece of DNA, thus causing double transformation. On the other hand genes widely separated, will have a better chance of being carried on separate DNA segments and the frequency of double transformation will be the product of single transformation frequencies. Therefore a departure from **product rule** of probability for the observed

frequency of double recombinants will prove close linkage.

Recombination after gene transfer

When a chromosome segment is transferred from a donor to a recipient strain (or through transduction or transformation), this transferred segment must be integrated into host genome by an exchange mechanism to produce a stable recombinant. The recipient cell at this stage is called a merozygote (partial diploid) which has the complete genome of F^-, called **endogenote** and an incomplete genome called exogenote derived from F^+ or Hfr. An even number of crossovers, rather than a single or odd number of crossovers, allows incorporation of a part of the genome from exogenote into the endogenote; one of the two products of exchange (i.e. a linear fragment) is usually lost (Fig. 3).

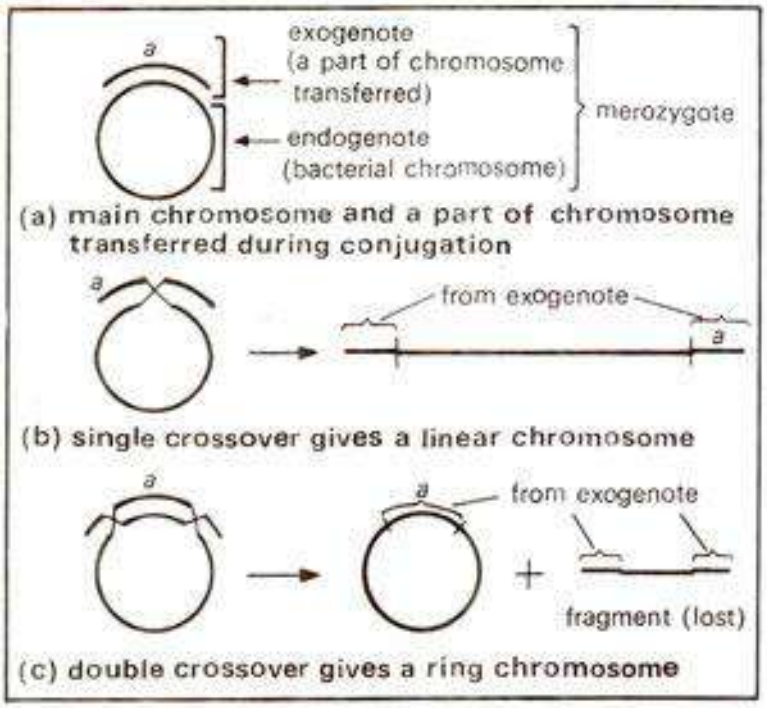


Fig. 3. Recombination in bacteria after gene transfer (single and double crossovers give linear and ring chromosomes respectively)

Identification of chromosomal aberration in Drosophila and man from photograph

Chromosomal abnormalities occur when there is a defect in a chromosome, or in the arrangement of the genetic material on the chromosome. Very often, chromosome abnormalities give rise to specific physical symptoms, however, the severity of these can vary from individual to individual.

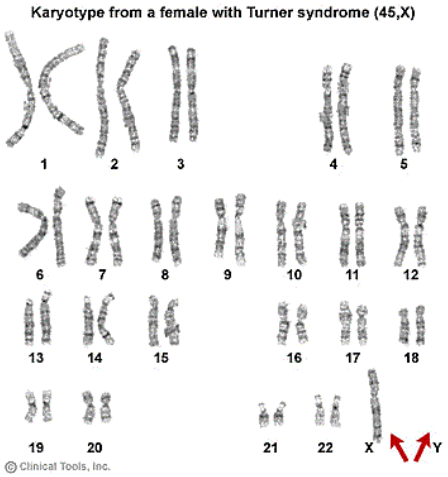
Abnormalities can be in the form of additional material which may be attached to a chromosome, or where part or a whole chromosome is missing, or even in defective formation of a chromosome. Any increases or decreases in chromosomal material interfere with normal development and function.

There are two main types of chromosomal abnormality which can occur during meiosis and fertilization: numerical aberrations and structural aberrations.

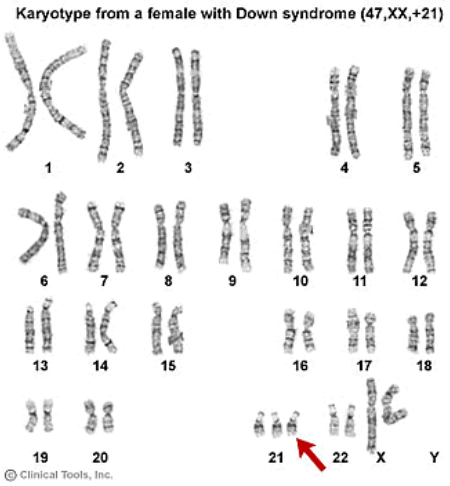
Numerical Aberrations

These are usually caused by a failure of chromosome division, which results in cells with an extra chromosome or a deficiency in chromosomes.

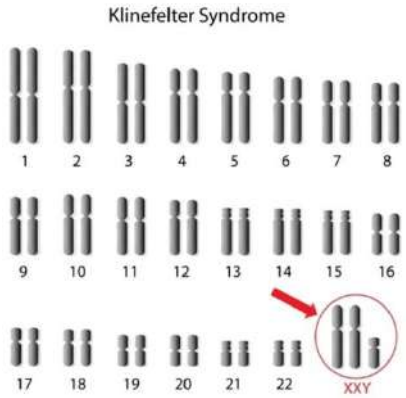
Gametes with these anomalies can result in conditions such as Down syndrome (who have 47 chromosomes instead of 46), or Turner syndrome (45 chromosomes). Common types of numerical aberrations are: triploidy, trisomy, monosomy and mosaicism.



Karyotype of Turner syndrome (45 chromosomes instead of 46)



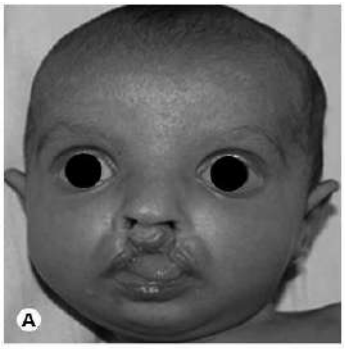
Karyotype of Down Syndrome (47 chromosomes instead of 46)

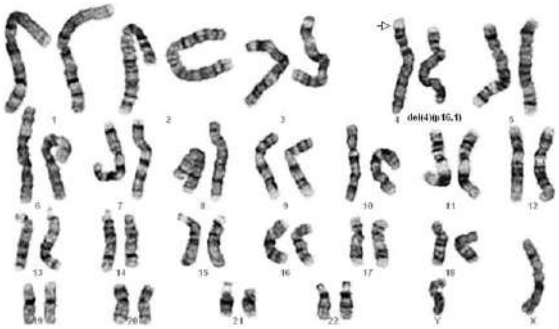


Structural Aberrations

These occur due to a loss or genetic material, or a rearrangement in the location of the genetic material. They include: deletions, duplications, inversions, ring formations, and translocations.

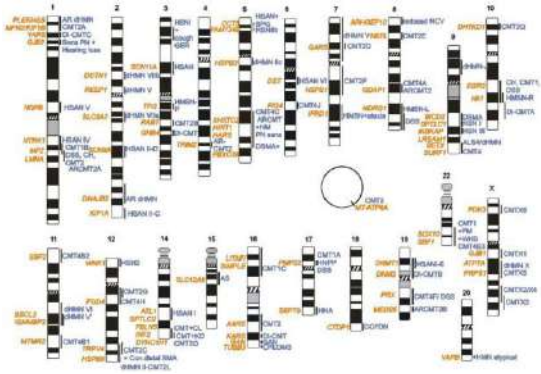
- Deletions: A portion of the chromosome is missing or deleted. Known disorders include Wolf-Hirschhorn syndrome, which is caused by partial deletion of the short arm of chromosome 4; and Jacobsen syndrome, also called the terminal 11q deletion disorder.





Wolf-Hirschhorn syndrome

- Duplications: A portion of the chromosome is duplicated, resulting in extra genetic material. Known disorders include Charcot-Marie-Tooth disease type 1A which may be caused by duplication of the gene encoding peripheral myelin protein 22 (PMP22) on chromosome 17.



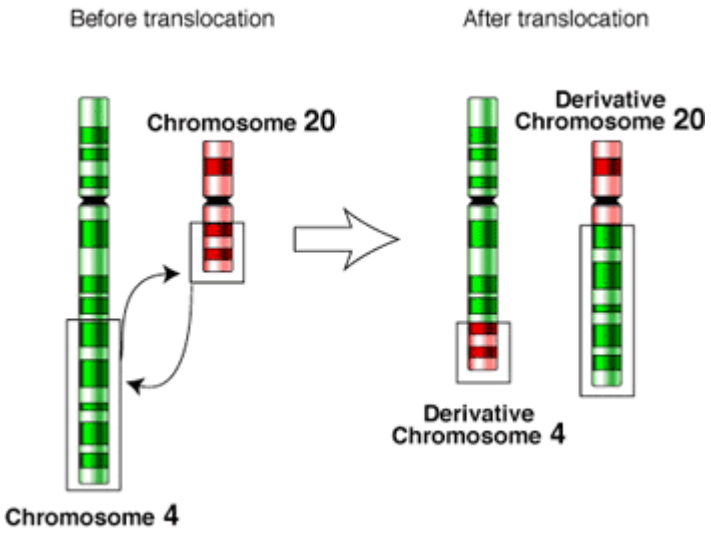
- Translocations: When a portion of one chromosome is transferred to another chromosome. There are two main types of translocations. In a reciprocal translocation, segments from two different chromosomes have been exchanged. In a Robertsonian translocation, an entire chromosome has attached to another at the centromere; these only occur with chromosomes 13, 14, 15, 21 and 22.

- Inversions: A portion of the chromosome has broken off, turned upside down and reattached, therefore the genetic material is inverted.

- Rings: A portion of a chromosome has broken off and formed a circle or ring. This can happen with or without loss of genetic material.
- Isochromosome: Formed by the mirror image copy of a chromosome segment including the centromere.

Structural aberrations also include some disorders which are characterized by chromosomal instability and breakage. One example, is the creation of a fragile site on the X Chromosome - Fragile X syndrome. Boys are worse affected by this because they only have one X-Chromosome but even in girls, Fragile X syndrome can cause learning difficulties.

Most chromosome anomalies occur as an accident in the egg or sperm, and are therefore not inherited. The anomaly is present in every cell of the body. Some anomalies, however, can happen after conception, resulting in mosaicism (where some cells have the anomaly and some do not). Chromosome anomalies can be inherited from a parent or be "de novo". This is why chromosome studies are often performed on parents when a child is found to have an anomaly.



Translocation - showing a portion of one chromosome transferred to another chromosome

4. Pedigree analysis of some human inherited traits

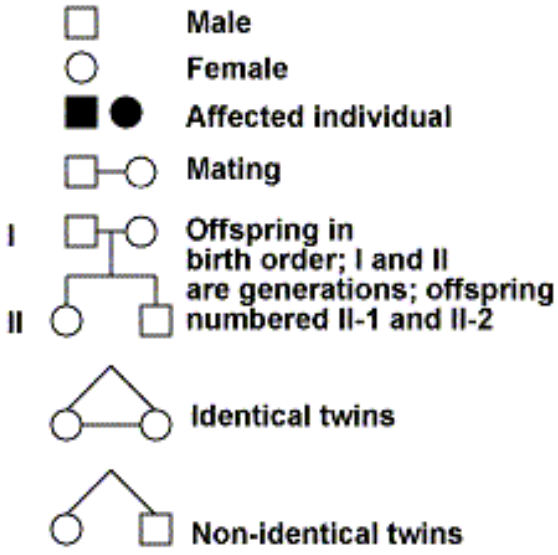
Pedigrees are used to **analyze** the pattern of **inheritance** of a particular trait throughout a family. **Pedigrees** show the presence or absence of a trait as it relates to the relationship

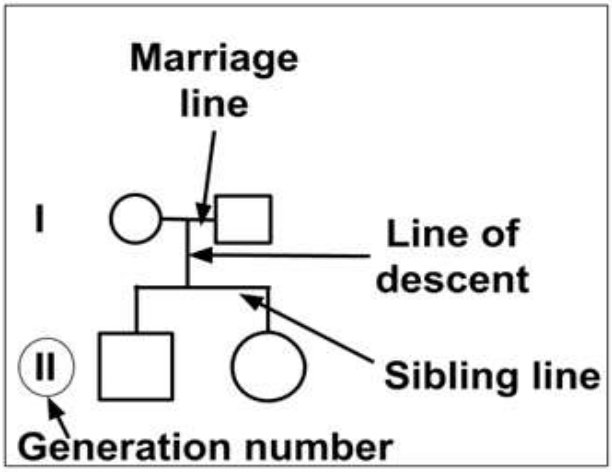
among parents, offspring, and siblings. It is usually undertaken if families are referred to a genetic counsellor following the birth of an affected child. The **pedigree analysis** chart is **used** to show the relationship within an extended family. Males are indicated by the square shape and females are represented by circles. It is very important tool for studying human inherited diseases. These diagrams make it easier to visualize relationships with in families, particularly large extended families. Pedigrees are often used to determine the mode of inheritance (dominant, recessive, etc.) of genetic diseases. **Pedigree** analysis is therefore an **important** tool in both basic research and genetic counseling. Each **pedigree** chart represents all of the available information about the inheritance of a single trait (most often a disease) within a family

Key terms

Term	Meaning
Pedigree:	Chart that shows the presence or absence of a trait within a family across generations
Genotype:	The genetic makeup of an organism (ex: TT)
Phenotype:	The physical characteristics of an organism (ex: tall)
Dominant allele:	Allele that is phenotypically expressed over another allele
Recessive allele:	Allele that is only expressed in absence of a dominant allele
Autosomal trait:	Trait that is located on an autosome (non-sex chromosome)
Sex-linked trait:	Trait that is located on one of the two sex chromosomes
Homozygous:	Having two identical alleles for a particular gene
Heterozygous:	Having two different alleles for a particular gene

Reading a pedigree



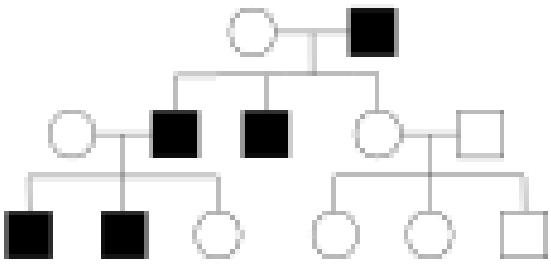


Pedigrees represent family members and relationships using standardized symbols.

By analyzing a pedigree, we can determine **genotypes**, identify **phenotypes**, and predict how a trait will be passed on in the future. The information from a pedigree makes it possible to determine how certain alleles are inherited: whether they are **dominant**, **recessive**, **autosomal**, or **sex-linked**.

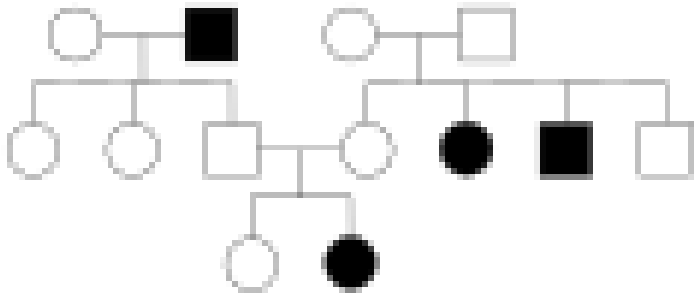
To start reading a pedigree:

1. **Determine whether the trait is dominant or recessive.** If the trait is dominant, one of the parents *must* have the trait. Dominant traits will not skip a generation. If the trait is recessive, neither parent is required to have the trait since they can be heterozygous.
2. **Determine if the chart shows an autosomal or sex-linked (usually X-linked) trait.** For example, in X-linked recessive traits, males are much more commonly affected than females. In autosomal traits, both males and females are equally likely to be affected (usually in equal proportions).

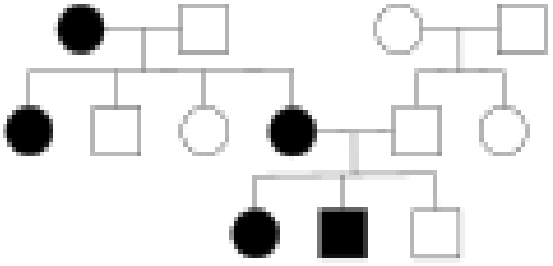


In a Y-linked disorder, only males can be affected. If the father is affected all sons will be

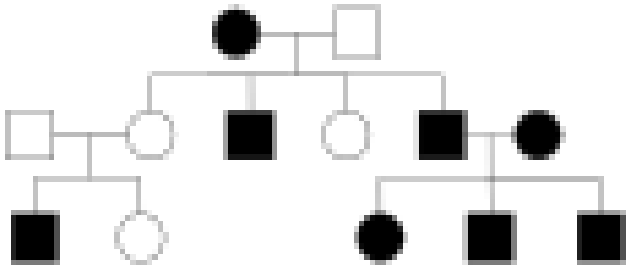
affected. It also does not skip a generation.



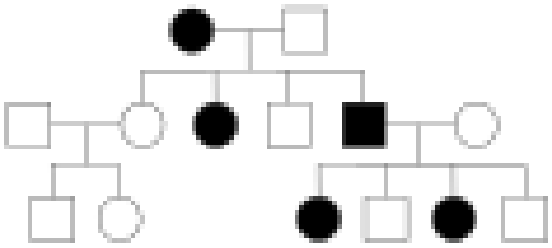
In an Autosomal Recessive Disorder, both parents can not express the trait, however, if both are carriers, their offspring can express the trait. Autosomal recessive disorders typically skip a generation, so affected offspring typically have unaffected parents. With an autosomal recessive disorder, both males and females are equally likely to be affected.



Autosomal Dominant disorders don't skip a generation, so affected offspring have affected parents. One parent must have the disorder for its offspring to be affected. Both males and females are equally likely to be affected, so it is an autosomal disorder.

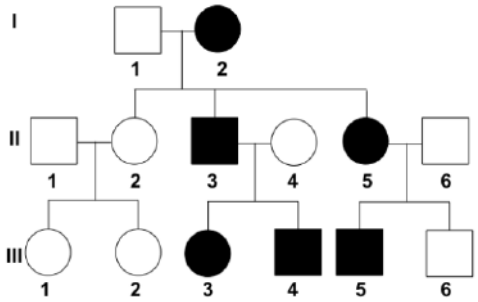


In a X-linked Recessive Disorder, males are more likely to be affected than females. Affected sons typically have unaffected mothers. The father also must be affected for daughter to be affected and the mother must be affected or a carrier for the daughter to be affected. The disorder is also never passed from father to son. Only females can be carriers for the disorders. X-linked recessive disorders also typically skip a generation.



In a X-Linked Dominant disorder, if the father is affected all daughters will be affected and no sons will be affected. It doesn't skip a generation and if the mother is affected she has a 50% chance of passing it onto her offspring

Example: Autosomal dominant trait



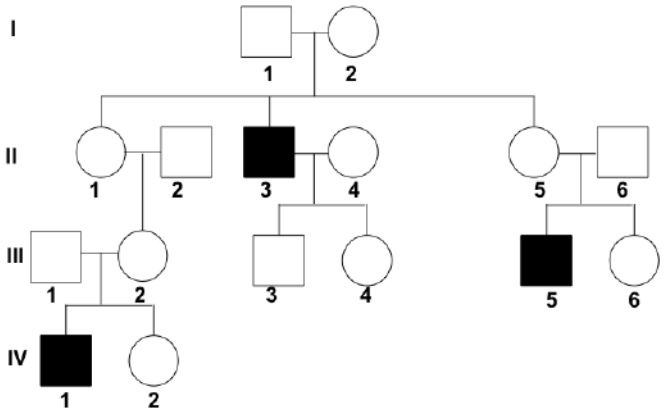
Freckles are small brown spots on your skin, often in areas that get sun exposure. In most cases, **freckles** are harmless. They form as a result of overproduction of melanin, which is responsible for skin and hair color (pigmentation). Overall, **freckles** come from ultraviolet (UV) radiation stimulation. Since freckles are dominant to no freckles, an affected individual such as I-2 must at least have one **F** allele.

The diagram shows the inheritance of freckles in a family. The allele for freckles (**F**) is dominant to the allele for no freckles (**f**). At the top of the pedigree is a grandmother (individual I-2) who has freckles. Two of her three children have the trait (individuals II-3 and II-5) and three of her grandchildren have the trait (individuals III-3, III-4, and III-5).

What is the genotype of 1-2?

The trait shows up in all generations and affects both males and females equally. This suggests that it is an autosomal dominant trait. Unaffected individuals must have two recessive alleles (**ff**) in order to not have freckles. If we notice, I-2 has some children who do *not* have freckles. In order to produce children with a genotype of **ff**, I-2 must be able to donate a **f** allele. We can therefore conclude that her genotype is **Ff**.

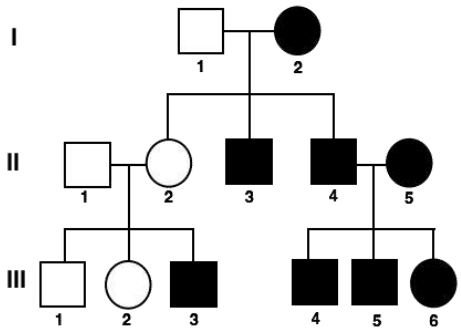
Example: X-linked recessive trait



The diagram shows the inheritance of colorblindness in a family. Colorblindness is a recessive and X-linked trait (X^b). The allele for normal vision is dominant and is represented by X^B. In generation I, neither parent has the trait, but one of their children (II-3) is colorblind. Because there are unaffected parents that have affected offspring, it can be assumed that the trait is recessive. In addition, the trait appears to affect males more than females (in this case, exclusively males are affected), suggesting that the trait may be X-linked

What is the genotype of Generation III - 2?

We can determine the genotype of III-2 by looking at her children. Since she is an unaffected female, she must have at least one normal vision allele X^B. Her two genotype options are then X^BX^B or $X^BX^{b.}$ However, her son (IV-1) is colorblind, meaning that he has a genotype of X^bY. Because males always get their X chromosome from their mothers and Y from their fathers), his colorblind allele must come from III-2. We can then determine that III-2's genotype is X^BX^b, so she can pass the X^b her son.



DSE1P: Fish and Fisheries (Lab)

1. Quantitative Characters Morphometric & Meristics laboratory

Taxonomic Characters

The first step in successfully working with fishes is correct identification. Similar species require in depth examination to discern the few differentiating characteristics. Many times these examinations require accurate measurements and counts of fin ray elements.

Objectives: The purpose of this lab is to introduce students to these characters.

Quantitative Characters
Quantitative characters are usually expressed as numbers, these include a measurable and countable characters.

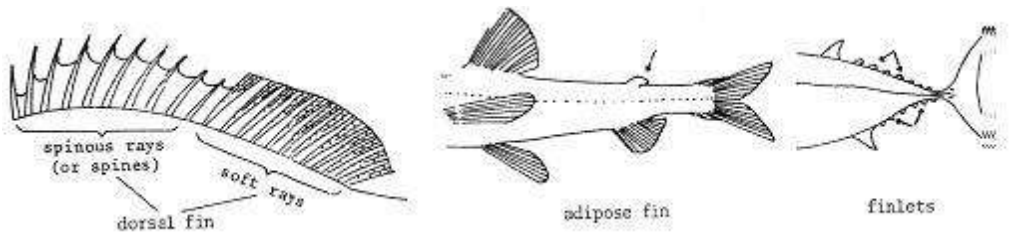
Morphometrics are measurable characters or length-based measures of specific body parts, such as total length of the body or diameter of the eye. These characters are usuallymeasured in the millimeter scale.

Meristics are counts of things which occur more than once, but a variable number of times between species (and sometimes within species).

These include counts of fin elements, i.e.: the number of dorsal fin spines and rays.

NOTICE Spines are hard, pinlike projections, while rays are soft and brush-like. Occasionally, fins will be a mix of both spines and rays; however, spines are ALWAYSthe most forward structures (closest to the head) on the fin.
The table below helps you to differentiate between a spine and a ray

Spines	Rays
Hard and pointed Unsegmented Unbranched Solid	Segmented Sometimes branched Bilateral with left and right halves



Because they are already dimensionless, meristic measurements can be compared directly.

Laboratory exercise

In this exercise we will be making various measurements and counts on several species offish. Record the appropriate information in the attached table.

Procedure—Morphology

- Each group is equipped with:

• Dissecting microscope	• Needle probe
• Dissecting tray	• Scissors
• Petri dish	• Scalpel
• Calipers	• Slides and coverslips
• Forceps (fine tip and blunt tip)	• Latex gloves (optional)

Each team should get a fish.

Some important considerations:

- Take care to not let the specimens dry out. Use the provided trays with a thin layer of water.
- Frozen fish don't bend easily and their fins often get stuck down. If frozen sample is used, the fish must be completely (or almost) thawed.
- All measurements and counts on paired fins and other structures should be made on the left side of the fish
- You will need to **convert all measurements into millimeters** (mm) so that your ratios work.
- Check and recheck your counts and measurements.
- When making counts it is important to examine the base of each fin where each element inserts into the body. Since most fin elements are difficult to discern without magnification, for this exercise you will use a dissection microscope. Transmitted light usually works best. Since rays are often branched, examining fins at the element tips would result in 2 or more counts for what is only a single element.
- As with most things in ichthyology (fish science) meristic counts are written in a strict format. For example, **Spines** are written using **roman** numerals and **soft-rays** are written using **Arabic** numbers, so:

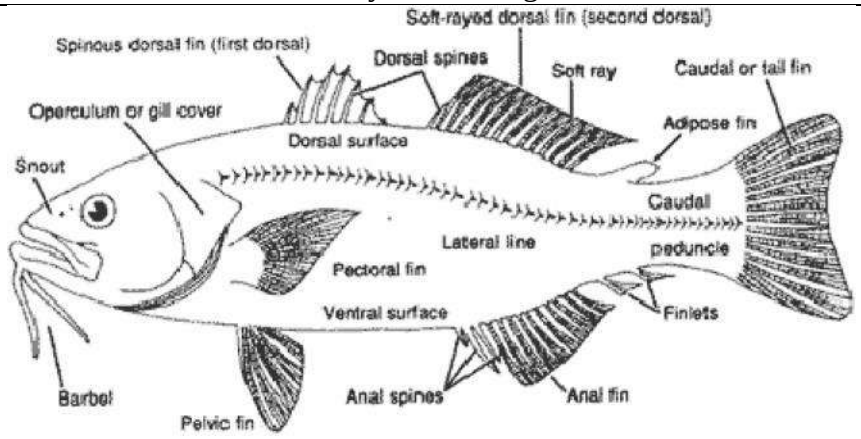
  D: VII, 10 - is a fish that has seven dorsal spines followed by 10 soft raysP$_2$: 8 - is a fish which has 8 pelvic rays and no spines.

It is common to list a range on meristic counts (i.e.: Inshore lizardfish (Synodus foetens) D: 10-13, A: 10-14, P: 12-15). This illustrates the fin element variability inherent in a single species of fish.

Note these abbreviations: Dorsal - D, Pectoral – P1, Anal - A, Pelvic – P2, Caudal – C

Identifying External Characters

Use the diagram below as a guide to identify all of the fins on your fish. Be CAREFUL not to rip the membranes in between the fin rays; they are delicate. Make a drawing of your fish, labeling each fin, in your lab notebook. Identify the caudal (tail) peduncle, the operculum (gill cover), and the lateral line. Label these on your drawing.



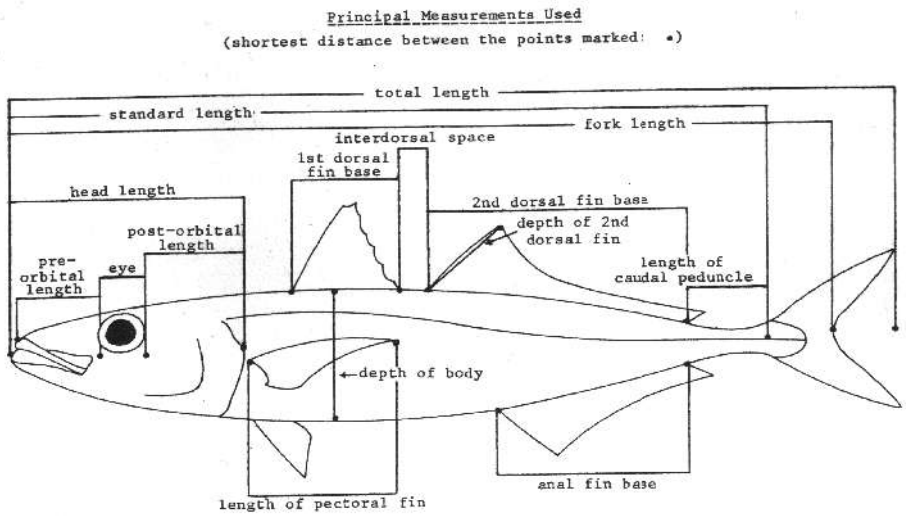
Draw your fish here

Morphometric measurements

Make all morphometric measurements BEFORE meristic measurements because the latter will tend to tear up the fins, making them hard to measure.

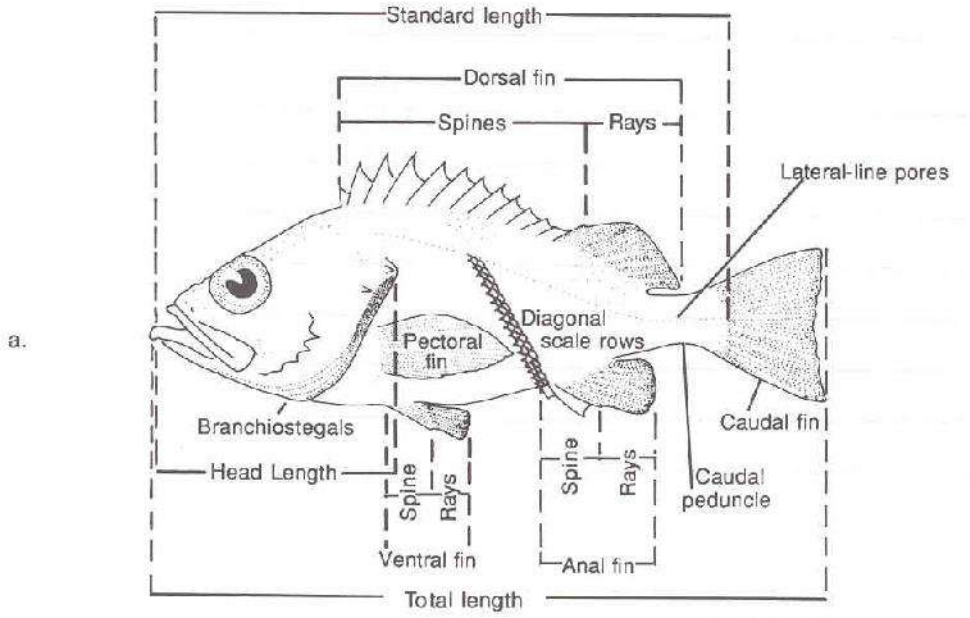
All morphometric measurements can be made with your **calipers**. For large fish, you may need a **ruler**. Using the diagram as a guide, make the morphometric measurements listed in the table below. Copy this table into your lab notebook. Using your calipers, take and record these measurements.

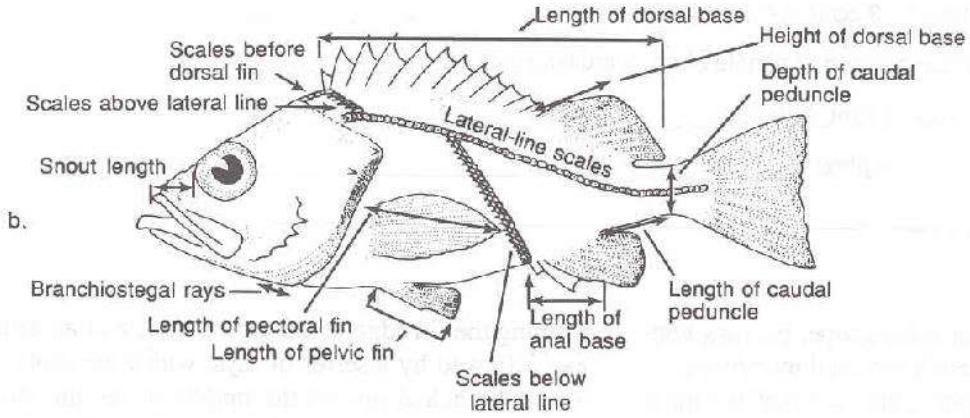
Morphometrics—Refer to diagram to assist you.

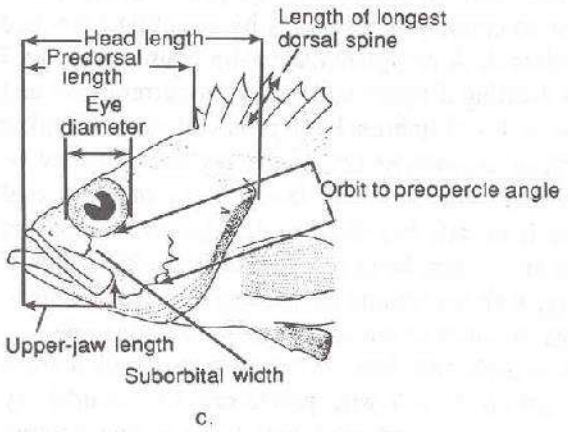


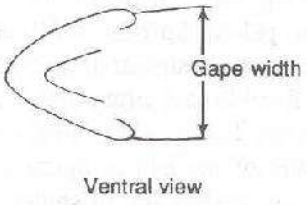
General Nomenclature of the External Morphology

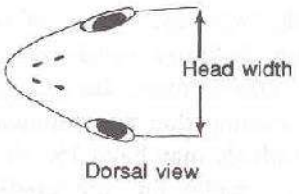
Measurement Type	Length (mm)
Total Length - tip of mouth to tip of tail fin	
Standard Length - tip of mouth to beginning of tail fin	
Fork length: tip of mouth to the tip of the median caudal fin rays.	
Body Depth - The distance from the highest part of the dorsal surface to the ventral surface in a straight, vertical line.	
Body Width - widest measurement side-to-side	
Head Length - tip of mouth to back edge of gill cover	
Eye Diameter - The distance between the margins of the eye.	
Pre-orbital Length = Snout Length – tip of upper jaw to eye anterior	
Post-orbital - The region behind the eye to the rear of the operculum.	
Pectoral Fin Length - upper insertion point of fin to end of longest ray	
Tail Height - top tip to bottom tip of stretched out tail	
Depth of caudal peduncle (refer to diagram)	
Length of caudal peduncle (refer to diagram)	
Head width (refer to diagram)	
Gape width (refer to diagram)	


a.
Standard length
Dorsal fin
Spines
Rays
Lateral-line pores
Pectoral fin
Diagonal scale rows
Branchiostegals
Caudal fin
Head Length
Spine
Rays
Spine
Rays
Caudal peduncle
Ventral fin
Anal fin
Total length


b.
Length of dorsal base
Height of dorsal base
Scales before dorsal fin
Depth of caudal peduncle
Scales above lateral line
Lateral-line scales
Snout length
Branchiostegal rays
Length of caudal peduncle
Length of pectoral fin
Length of anal base
Length of pelvic fin
Scales below lateral line


Head length
Predorsal length
Eye diameter
Length of longest dorsal spine
Orbit to preopercle angle
Upper-jaw length
Suborbital width
c.


Gape width
Ventral view
d.


Head width
Dorsal view

Morphometric Ratios

Although they can be compared to each other directly (for instance, the length of one fish versus another), this does not tell us much because fish change size as they age. However, when compared as ratios (for instance, head length/total length), morphometric measurements can tell us a lot. For instance, cruiser and accelerators might have small head-to-body ratios, whereas maneuverers and especially benthic fish might have large ratios.

Measurement Type	Ratio
Body Width/Standard Length	
Body Depth/Standard Length	
Head Length/Total Length	
Predorsal Length/Total Length	
Eye Diameter/Head Length	
Tail Height^2/Tail Area	

Meristics

After you have labeled and measured the fins, you will need to count the spines and rays. Carefully spread the fins, count the spines and rays, and enter these meristic measurements into your lab notebook.

Scales: TURN THE FISH OVER to the side. Using the dissecting microscope to aid you, count the lateral line scales and enter the number in the meristics table in your lab notebook.

Gill Arch: Gently pull back the gill cover to reveal the gills. Notice that the gills come in sets of four "arches" on each side of the fish. The red material pointing towards the tail are the gill filaments (used for respiration). The spiny projections pointing towards the mouth are the gill rakers (used for feeding).
The size of gill rakers differs based on the type of prey a species consumes. For example, fish that feed on plankton (Planktivorous) have many very fine gill rakers that are long, whereas fish that feed on large prey have few, small gill rakers

Procedure

- Using your scalpel or your scissors, carefully cut the gill cover back to expose the gills. With your scalpel or your scissors, cut out the FIRST gill arch, leaving the others in place.

- Once the gill arch has been removed, place it in a petri dish and GENTLY wash it off. Remove the sticky mucus material which may be coating both the gill filaments and gill rakers.
- Once the arch has been washed, drain the petri dish and place it under the dissecting scope for a better view.
- Now you can count and measure the upper and lower gill rakers.

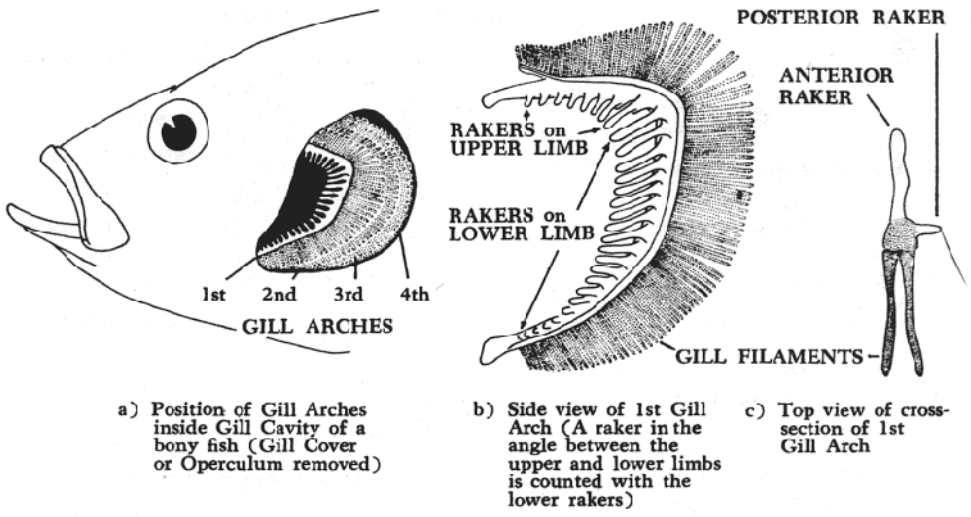
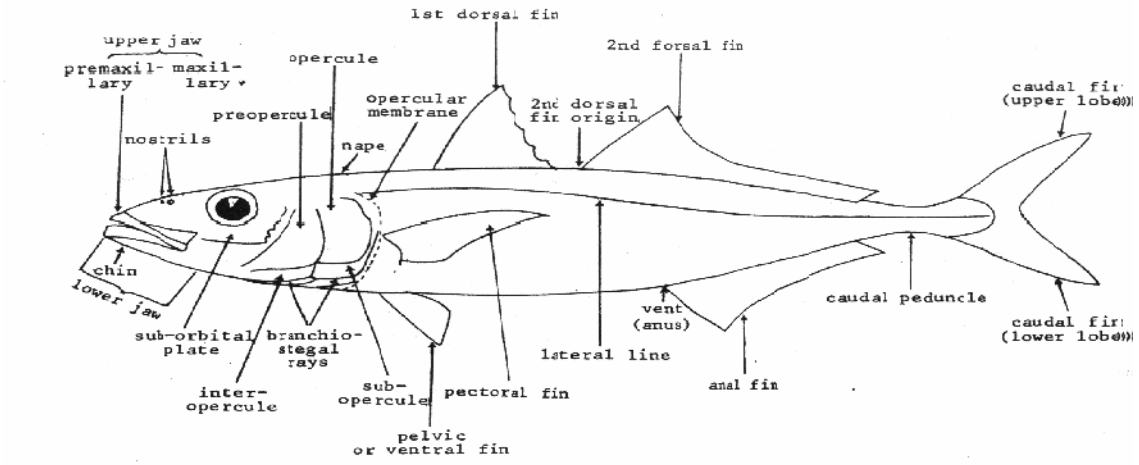
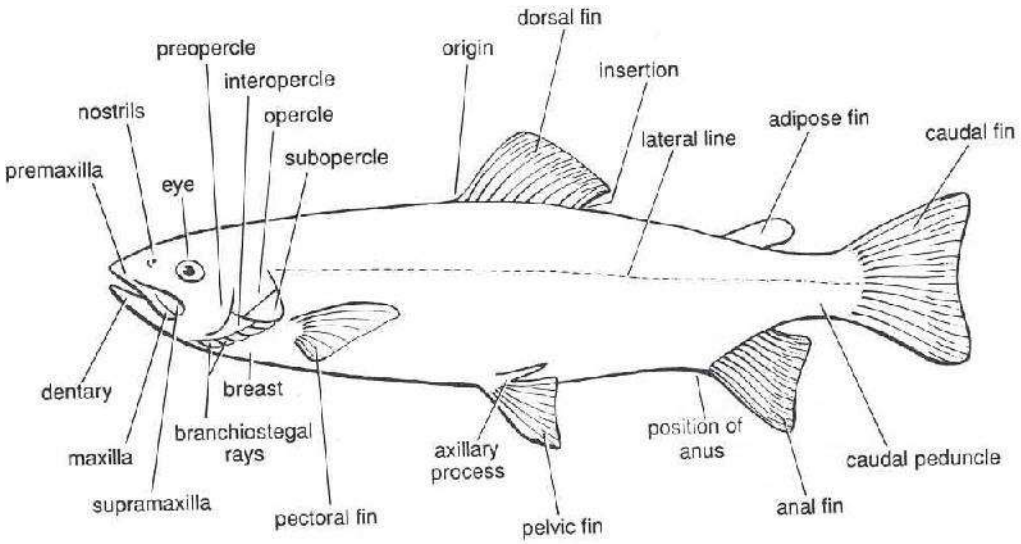


Figure Arrangement and structure of gill rakers and gill arches of a bony fish. (a) location of the gill arches. (b) side view of the first gill arch showing the rakers and filaments. (c) cross section of the first gill arch.

Meristics—Refer to diagrams to assist you

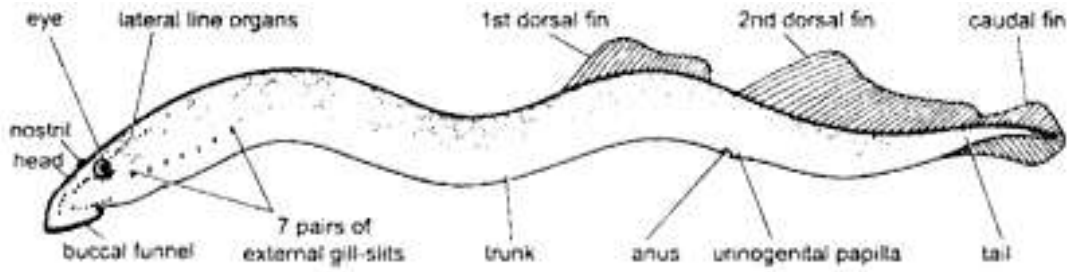


Measurement Type	Number
First dorsal fin - spines	
Second dorsal fin - spines	
Second dorsal fin - rays	
Anal fin - spines	
Anal fin - rays	
Tail fin - rays	
Pectoral fin - spines	
Pectoral fin - rays	
Pelvic fin - spines	
Pelvic fin - rays	
Lateral line scales	
Gill rakers - upper Limb	
Gill rakers - lower Limb	



2. Study of specimens

Petromyzon sp. (Lamprey)



1. Presence of hollow, dorsal, tubular nerve cord.

2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Jaws and paired fins are absent.

Hence, the specimen belongs to the Super Class Agnatha.

1. Mouth is suctorial and circular.

2. Gill slits are 1 to 16 pairs.

3. Nostril is single and median.

Hence, the specimen belongs to the Class Cyclostomata.

1. Presence of seven pairs of round gill openings.

2. Branchial basket is complete.

3. Dorsal fin may be one or two.

Hence, the specimen belongs to the Order Petromyzontia.

1. Body is smooth and eel-like.

2. Head and trunk are cylindrical but tail is laterally compressed.

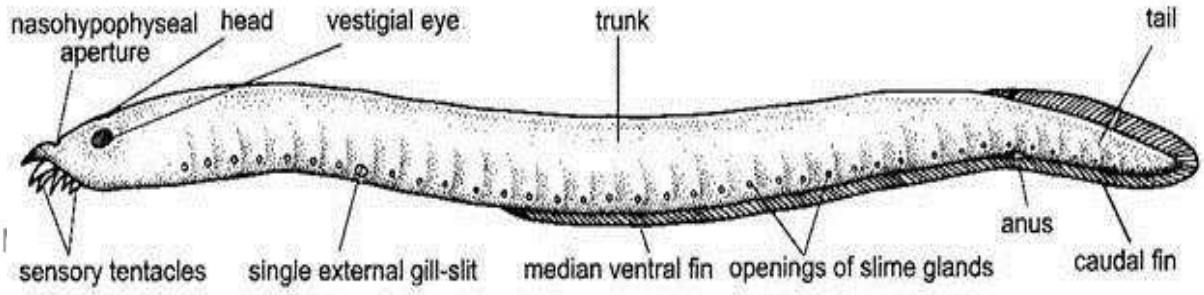
3. Presence of paired eyes covered by transparent skin.

4. Buccal funnel is beset with many horny teeth.

5. Two median dorsal dorsal fins are almost equal in dimensions.

Hence, the specimen seems to be Petromyzon sp.

Myxine sp. (Hag fish)



1. Presence of hollow, dorsal, tubular nerve cord.

2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Jaws and paired fins are absent.

Hence, the specimen belongs to the Super Class Agnatha.

1. Mouth is suctorial and circular.
2. Gill slits are 1 to 16 pairs.
3. Nostril is single and median.

Hence, the specimen belongs to the Class Cyclostomata.

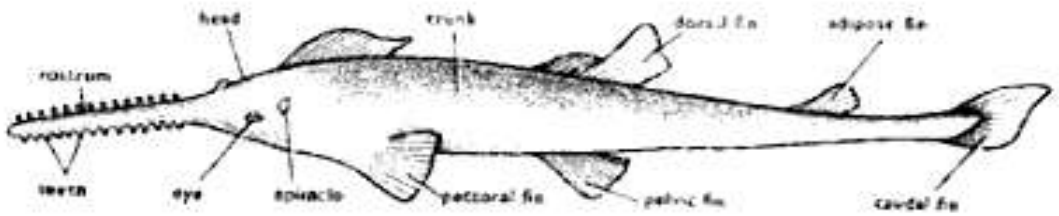
1. Mouth is terminal with four pair of tentacles.
2. Nostril is terminal.
3. Gill slits are 1 to 15 pairs.

Hence, the specimen belongs to the Order Myxinoidea.

1. Scale-less cylindrical body divided into head, trunk and tail.
2. Mouth is surrounded by lips.
3. Eyes are vestigeal.
4. Single external branchial aperture on both sides.
5. Down the sides of the body, mucous pores are present.

Hence, the specimen seems to be Myxine sp.

Pristis sp. (Saw fish)



1. Presence of hollow, dorsal, tubular nerve cord.

2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.
2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Presence of ventrally located subterminal mouth.
2. Tail is **heterocercal.**
3. Presence of calcareous endoskeleton.
4. Presence of 5-7 pairs of gill slits.

Hence, the specimen belongs to the Class Chondrichthyes.

1. Multiple gill-slits are protected by individual skin flap.
2. Male bears **clasper.**

Hence, the specimen belongs to the Subclass Selachii.

1. Gill-slits are on the ventral side of the body.
2. Pectoral fins are enlarged but tail and other fins are reduced.

Hence, the specimen belongs to the Superorder Hypotremata.

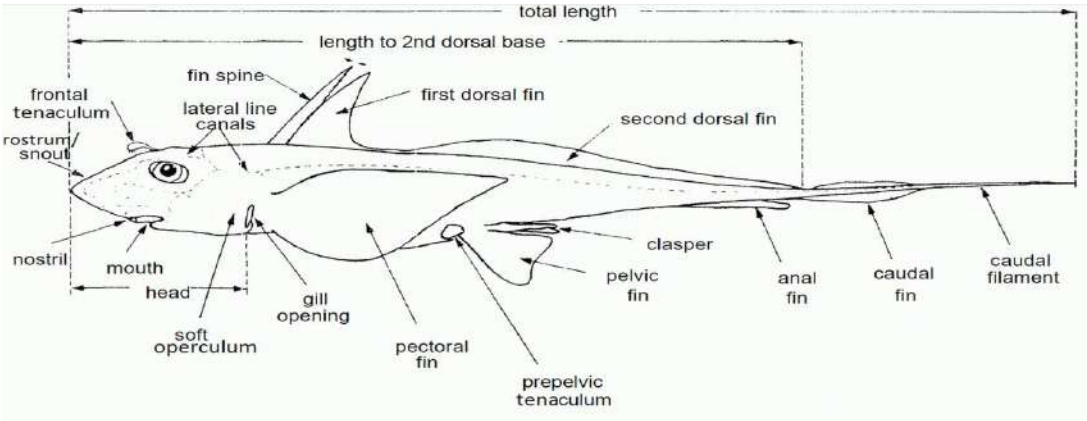
1. Body is fleshy and dorsoventrally compressed.
2. Eyes are on the dorsal surface.

Hence, the specimen belongs to the Order Rajiformes.

1. Anterior part of the body is dorsoventrally flattened while the posterior part is shark like.
2. **Snout** is prolonged into a long **rostrum** with a series of lateral tooth-like **denticles.**

Hence, the specimen seems to be Pristis sp.

Hydrolagus sp. (Ghost shark) (Chimaera)



1. Presence of hollow, dorsal, tubular nerve cord.
2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.
2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Presence of ventrally located subterminal mouth.
2. Tail is **heterocercal.**
3. Presence of calcareous endoskeleton.
4. Presence of 5-7 pairs of gill slits.

Hence, the specimen belongs to the Class Chondrichthyes.

1. The tail is long and thin and they move by sweeping movements of the large pectoral fins.
2. The erectile spine in front of the dorsal fin is sometimes venomous.
3. The mouth is a small aperture surrounded by **lips**, giving the head a **parrot-like** appearance.

Hence, the specimen belongs to the Subclass Holocephali.

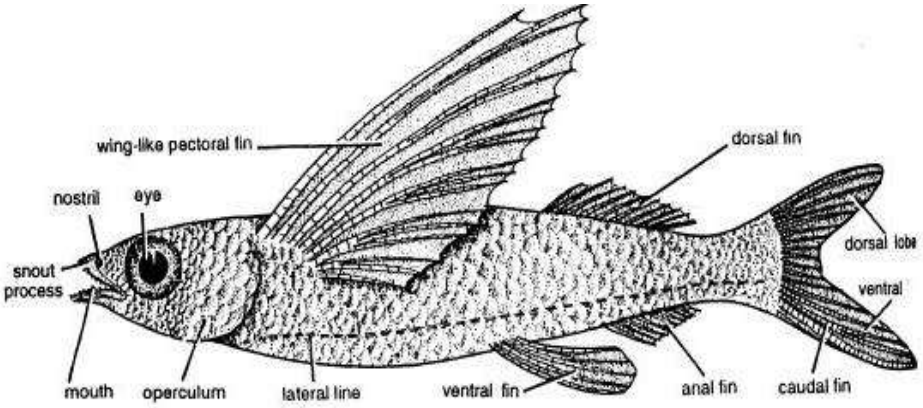
1. Elongated, soft bodies, with a bulky head and a single gill-opening.
2. Have a **venomous spine** in front of the dorsal fin.
3. Male chimaeras have retractable sexual appendages on the forehead and in front of the pelvic fins.
4. Their upper jaws are fused with their skulls having three pairs of large permanent grinding tooth plates.
5. Have gill covers or **opercula** like bony fishes.

Hence, the specimen belongs to the Order Chimaeriformes.

1. Have a smooth and scaleless skin that is a silvery-bronze color, often with sparkling shades of gold, blue, and green.
2. Dark edges outline both the caudal and dorsal fins, whereas the pectoral fins have a transparent outline.
3. Pectoral fins are large and triangular, and extend straight out from the sides of their bodies like airplane wings.
4. It has a duckbill-shaped snout and a rabbit-like face.
5. Have large, emerald green eyes, which are able to reflect light.

Hence, the specimen seems to be Hydrolagus sp.

Exocoetus sp. (Flying fish)



1. Presence of hollow, dorsal, tubular nerve cord.
2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.
2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**
2. Tail is **homocercal.**
3. Presence of bony endoskeleton.
4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.
2. Fins are supported by fin rays.

Hence, the specimen belongs to the Subclass Actinopterygii.

1. Scales are covered by **epidermis** or absent.
2. Gills are fully filamentous.
3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

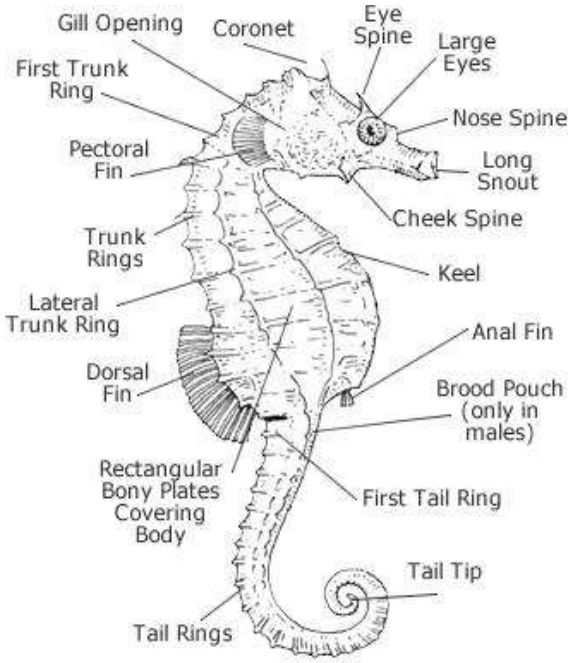
1. Actually spindle like long body.
2. Pectoral fins are very big and modified into flying structure.
3. Pelvic fins are at the abdominal region.

Hence, the specimen belongs to the Order Beloniformes.

1. Pectoral fins are enormously elongated to form wing-like structures. They serve as parachute to sustain the fish in its gliding leap.
2. Lateral line is located low on the body.
3. Pectoral fin with black spots.
4. Tail is hypobatic and ventral lobe of the tail fin is much elongated and help in skipping over the water.

Hence, the specimen seems to be Exocoetus sp.

Hippocampus sp. (Sea horse)



1. Presence of hollow, dorsal, tubular nerve cord.
2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.
2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**
2. Tail is **homocercal.**
3. Presence of bony endoskeleton.
4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.
2. Fins are supported by fin rays.

Hence, the specimen belongs to the Subclass Actinopterygii.

1. Scales are covered by **epidermis** or absent.
2. Gills are fully filamentous.
3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

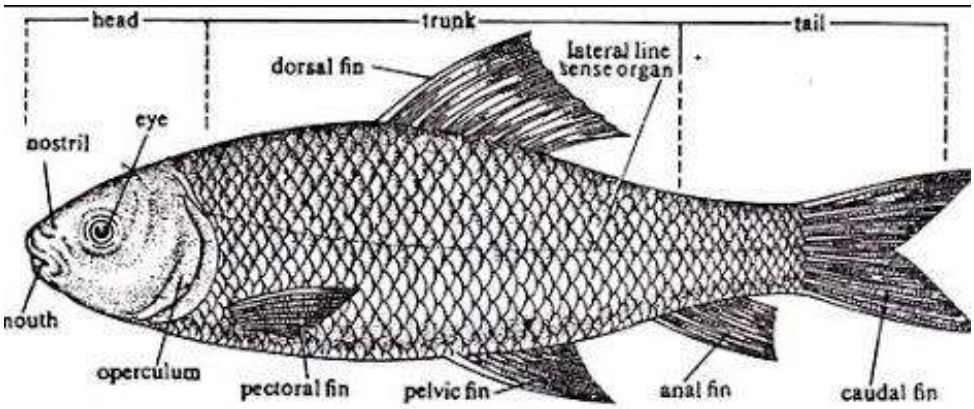
1. Body is covered by ring like bony plates.
2. Snout tubular with suctorial mouth.

Hence, the specimen belongs to the Order Solenichthyes.

1. Head is at right angle to the body axis.
2. Pectoral fin is transparent behind the operculum.
3. Tail is long and **prehensile.**
4. Presence of **brood pouch** on the belly (in male)/an anal fin on the belly (in female).

Hence, the specimen seems to be Hippocampus sp.

Labeo sp. (Rohu)



1. Presence of hollow, dorsal, tubular nerve cord.
2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.
2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**
2. Tail is **homocercal.**
3. Presence of bony endoskeleton.
4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.
2. Fins are supported by fin rays.

Hence, the specimen belongs to the Subclass Actinopterygii.

1. Scales are covered by **epidermis** or absent.
2. Gills are fully filamentous.
3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

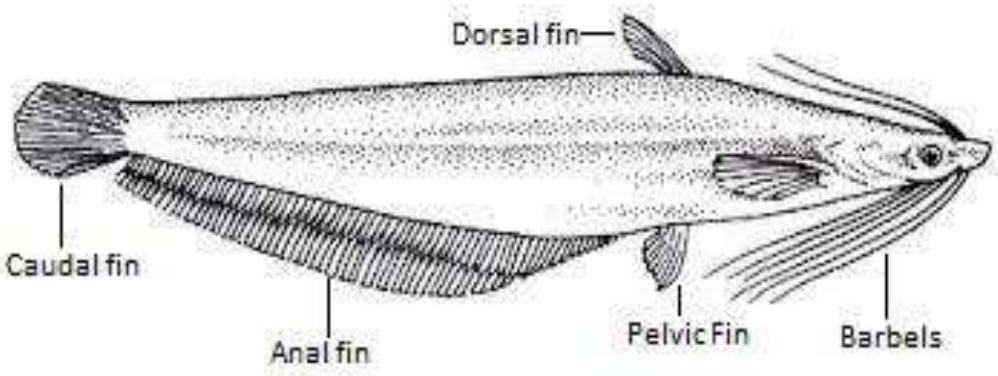
1. No scale on the head, head is covered by plates.
2. No teeth in the jaws.
3. Single large dorsal fin.
4. Fins supported by soft fin rays.
5. Lateral line distinct.

Hence, the specimen belongs to the Order Cypriniformes.

1. Body covered with large cycloid scales, no scale on the head.
2. Head prominent with blunt snout projecting beyond the narrow mouth.
3. Lips thick and horny covering the jaw, having inferior transverse fold.
4. Barbels are very short or absent.
5. Body colour is bluish-brown on the dorsal aspect, and silvery-white with reddish-orange tinge on the ventral aspect.

Hence, the specimen seems to be Labeo sp.

Heteropneustes sp. (Singhi)



1. Presence of hollow, dorsal, tubular nerve cord.
2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.
2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**
2. Tail is **homocercal.**
3. Presence of bony endoskeleton.
4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.
2. Fins are supported by fin rays.

Hence, the specimen belongs to the Subclass Actinopterygii.

1. Scales are covered by **epidermis** or absent.
2. Gills are fully filamentous.
3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

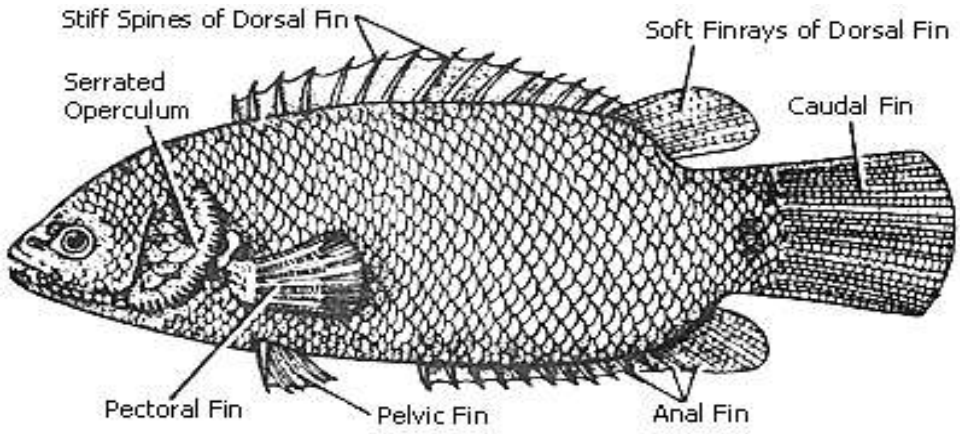
1. Pectoral fin with a spine.
2. Barbels present.
3. Scales absent.

Hence, the specimen belongs to the Order Siluriformes.

1. Skin is scaleless.
2. Head is flat with four pairs of **barbells.**
3. First ray of pelvic fin is modified into a serrated spine.
4. Dorsal fin is very small.
5. Caudal fin is separated by a notch from extended anal fin.

Hence, the specimen seems to be Heteropneustes sp.

Anabus sp. (Koi fish)



1. Presence of hollow, dorsal, tubular nerve cord.
2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.
2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**
2. Tail is **homocercal.**
3. Presence of bony endoskeleton.
4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.
2. Fins are supported by fin rays.

Hence, the specimen belongs to the Subclass Actinopterygii.

1. Scales are covered by **epidermis** or absent.
2. Gills are fully filamentous.
3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

1. Dorsal and anal fins are both with spiny and soft rays.
2. Dorsal fins are two in number.
3. Body is covered by **ctenoid** scales.

Hence, the specimen belongs to the Order Perciformes.

1. Dorsal and anal fins are divided and supported by anterior spiny and posterior soft rays.
2. Small spiny projections are present along the posterior edge of operculum.

Hence, the specimen seems to be Anabus sp.

3. STUDY OF PLACOID, CYCLOID AND CTENOID SCALES THROUGH PERMANENT SLIDES/PHOTOGRAPHS

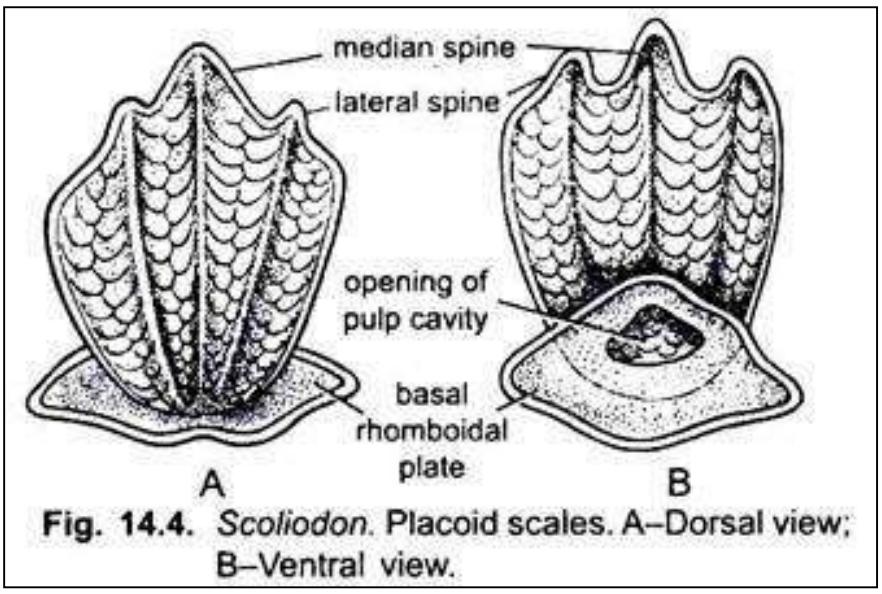
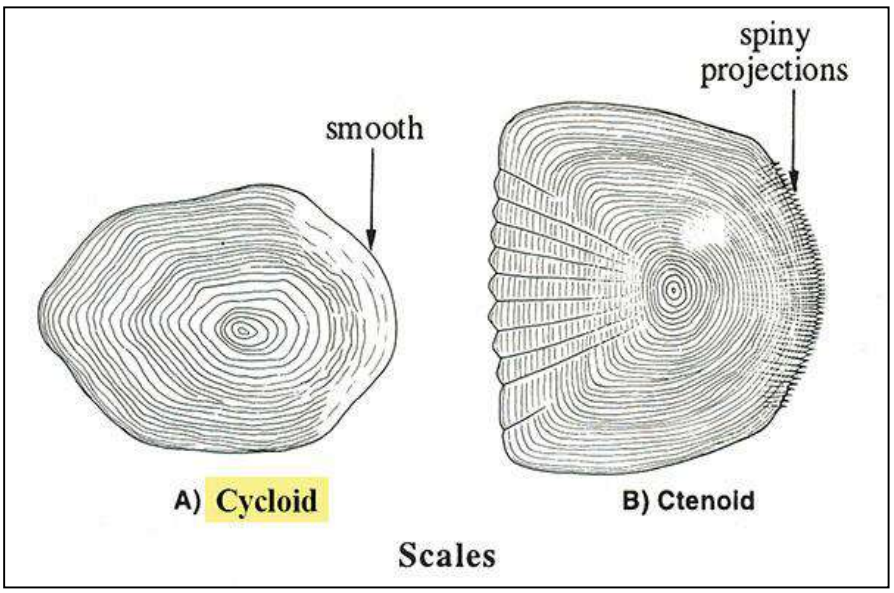


Fig. 14.4. *Scoliodon*. Placoid scales. A–Dorsal view; B–Ventral view.



4. Study of crafts and gears used in Fisheries

Crafts and Gears used for Fishing (With Diagram)

The use of crafts and gears in fishing technology plays very important role and help enhancing the production commercial bases. The success of fishing largely depends on to how and which types of nets are used to capture the fish.

There are two main types of devices used to capture fishes in both marine and inland fisheries:

(1) Nets or gear — these are instruments used for catching fish.

(2) Crafts or Boats — it provides platform for fishing operations, carrying the crew and fishing gears.

There are various types of gears and crafts used in different parts depending upon the nature of water bodies, the age of fish and their species. Some nets are used without craft; however, others are used with the help of crafts. Generally, locally made gears and crafts may be non-mechanized or mechanized.

Crafts and Boats:

There are many types of fishing crafts being successfully made and used for marine and inland fisheries.

A. Marine Fishing Crafts:

Different crafts are used due to different conditions of sea on the east and west coasts.

I. Crafts used on the East Coasts:

(1) Catamaran:

The word catamaran is originated from a Tamil word Kattumaram which means 'lashing timber'. It is used mainly on the east costs of Orissa from Kanyakumari. It is also used on northeast cost of Kerala. It is the most primitive, traditional, economical and efficient craft.

It is made by tying many wood logs in such a way that it takes the shape of a canoe, which consists of two main logs and two side logs cut into boat shape and held together with rope. The logs are held in position by loose rubber called Teppa. Generally the Catamaran is 5-10 meter long, 0.5 meter wide and 0.3 meter deep.

Types of Catamarans:

(i) Orissa and Ganjam type

(ii) Coromandal type

(iii) Andhra type

(iv) The boat-Catamaran

i. Orissa and Ganjam Type:

It is made by five logs pegged with wood. The logs are cut in boat shape and are not tied with rope.

ii. Coromandal Type:

It is used in Tamil Nadu to capture flying fish of Nagapattanam. It is made by 3-5 logs. A modified type of Coromandal type is called Kolamaram, which is made by 7 logs.

iii. Andhra Type:

It is the modified form of Orissa type, larger in size—about 5-7 metres long, hence made by nine heavy side wood logs that are fitted with a median logs.

iv. The Boat-Catamaran:
It is made of three wood logs tied in boat shape. It is used in Mandapam and Mukkun coastal regions.

(2) Masula Boat:
It is a weakly constructed boat of about 8-12 metres long (Fig. 32.1). It is used in clear weather near the shore. The masula boat is keel less and frameless made by mango planks, which are stitched with palm leaf fibres. There are several variations of the musula boat. In Orissa it is called 'Bar boat' and in Andhra called Padava or Padgam.

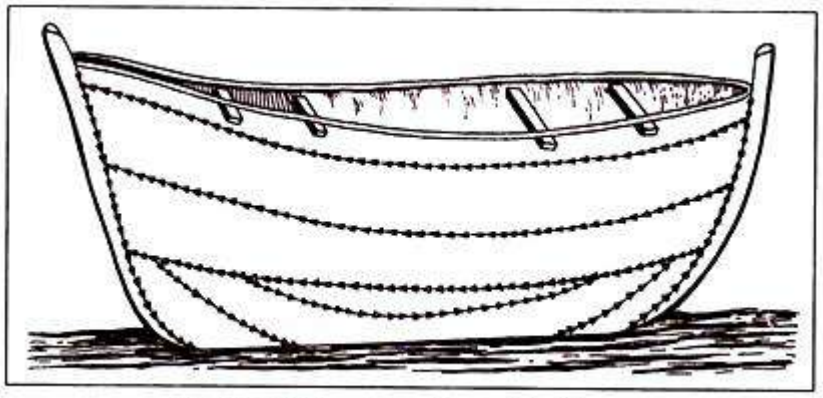


Fig. 32.1 : Padava—Masula boat of Andhra coast

(3) Nauka and Dinghi:
These boats are with carvings and are operated in West Bengal and Orissa. These are well-designed large boats measuring about 11-13 m x 2-3 m x 2 m in size.

(4) Tuticorin Boats or Fishing Luggers:
These are operated in inshore waters and are used as cargo boats. They are carved boats of 11 m x 2m x 1m in size (Fig. 32.2).

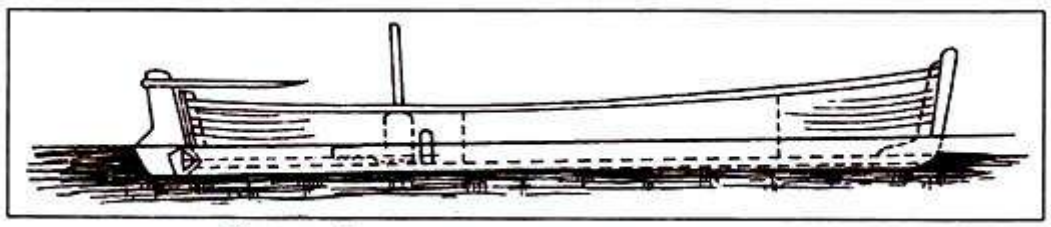


Fig. 32.2 : Tuticorin-type fishing boat (Tamil Nadu State)

II. Crafts used on West Coasts:

(1) Dugout Canoes:
These are constructed from large wood logs (Fig. 32.3). These logs are hollowed by scooping inner part. Their bottom is thicker than sides. They are operated on Kerala and Konkan coasts. The small boats of 5-10 m long are called 'Thonies', which are used for gill nets or drift fishing and for seining.

The large boat of 10-22 m long is called Vanchi or Odam and is used for operating variety of nets on Malabar Coast. Dug-out canoes are also operated on the west coast from Colachal to Kathiawar.

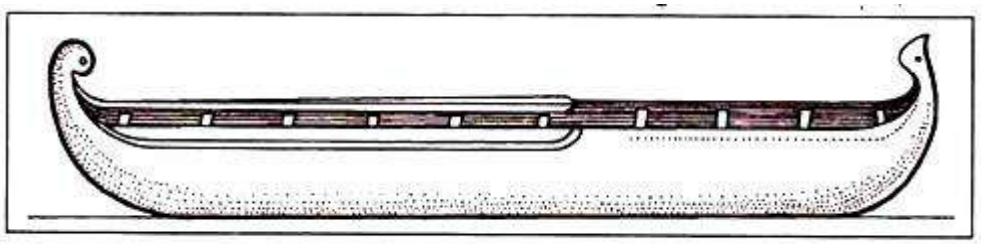


Fig. 32.3 : Odam—A dug-out canoe of Malabar

(2) Plank-Built Canoes:
It is a kind of dug-out canoes. It is extended with planks on sides. They are popularly used on coast of Kerala, Karnataka and North Bombay.

(3) Outrigger Canoes:
These are large size canoes of about 15 m long. It has narrow keel and single outrigger and extended out with planks. They are commonly called as Rampani, because they are used for the casting of the Rampani net for catching mackerels (Fig. 32.4). They are operated on the Kanara and Konkan coasts. Small size outriggers are used between Bhatkal and Majali.

(4) Built-Up boats:
It is highly specialized indigenous fishing craft. They are commonly used along the Bombay coast and north Ratnagiri.

There are small variations according to different places such as:

(a) Ratnagiri type boat, which has pointed bow, straight and narrow keel and low gunwale.

(b) Machwa:
It is provided with broad hull, straight keel and pointed bow. It is popular in Bassein hence called Bassien type.

(c) Satpati or Galbati Type:
It has straight keel, high gunwale, medium pointed bow and broad beam. Satpati can be mechanized with a motor engine without any modification in design.

(d) Broach Type:
It has flat bottom and is widely used in inshore and estuarine water.

(5) Coracle:
It is used in rivers, reservoirs and canals for fishing. One or two fishermen can operate this craft. It is made like a round basin and its frame is made with split bamboos. The outer surface is covered with leather.

(6) Shoe Dhonie:

It is shaped like a shoe. It is used both marine and inland fishery. It is constructed by teak wood with planks grooved with ribs and frames fitted with nails. It is used for fishing with gill nets.

(7) Kakinada Nava:
It is commonly used for inshore fishing. It is made by teak wood. It is keel less but ribs are fitted in frame with nails. It is about 9-10 m in length.

Marine Fishing Gears:
Various types of gears are used for fishing in sea. They may be of different size, shape and designs. These gears may be made by fishermen. They are also manufactured in cottage industries. The most commonly and widely used fishing gear are different types of nets.

They are used for catching large fishes offshore. The main type of nets being used are boat seine, shore seine, bag nets, fixed or stationary nets, drag nets, drift nets and cast nets.

(i) Seines:
These are specially designed and large fishing nets. They are generally used in running water. When they are spread in sea; they collect large numbers of fishes. Seines are rectangular in shape mounted on wire. They are spread vertically in the water. Seines are of two types, boat and shore seines.

(a) Boat Seine:
These nets are conical in shape provided with wings. The mesh of the nets is smaller in center and increase in size towards outer ends of flanks. This seine is operated in sea by catamarans or boats. The seine traps the fishes. Towing is done with the help of coir.

(b) Shore Seine:
It is operated from seashore. It is popularly called as Ber Jal (Fig. 32.5) in Orissa, Pedda or Alivi vala in Andhra coast, Periya vala or Mada valai in coromandel coast and Kara valai in the Gulf of Mannar. It is a conical bag-like with two wings.

One end of net is kept fixed to shore and other end is spread into sea with the help of a boat, in the form of semicircular fashion. When the net is filled up with fishes, the two ends are slowly dragged by group of fisherman.

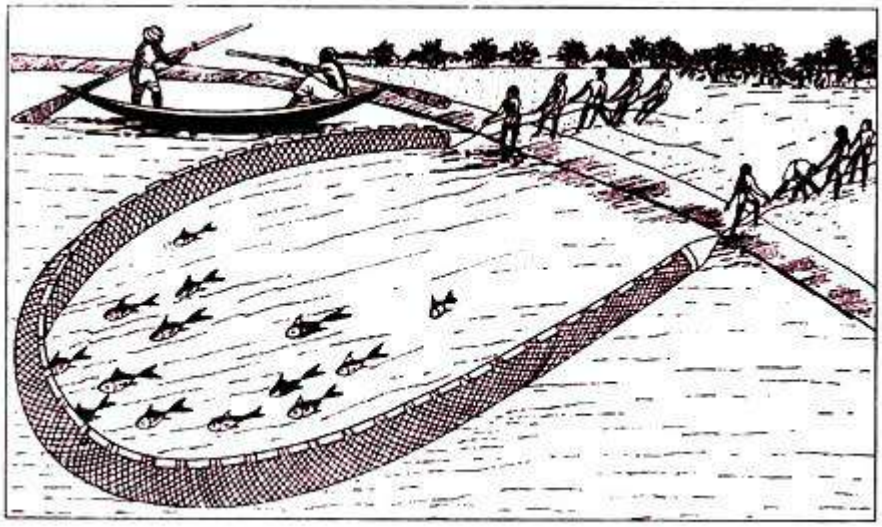


Fig. 32.5 : Ber Jal

(ii) Danish Seine:
It is also called drag seine. It is used in deep waters and do not reach to surface. It has small wings.

(iii) Beach Seine:
It is also called haul seine. It has two wings made by strong twines (Fig. 32.6). Both wings are joined to a central bag. The ends of wings are tapering and connected to the wraps either directly or by means of spreader (brail) of strong pole. The mesh size is smaller in the bag than in the wing. Beach seine has side, i.e., float line and lead line. The float line contains appropriate floats whereas lead lines carry sinkers.

The net is used in such a way that its one wing remains on the beach. The other wing is spread on right angle in such a way that, when it is dragged slowly it encircles that part of water section. Both float and lead line do not allow fish to escape.

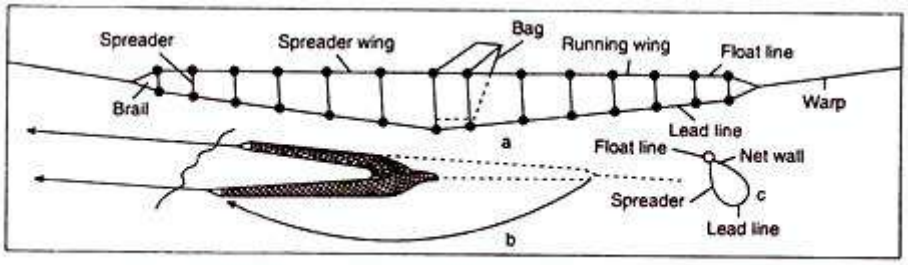


Fig. 32.6a-c : Beach seine : (a) The net, (b) Operation of net, (c) Net wall in cross section

(iv) Purse Seine:
It is used to catch pelagic and migratory fishes. It is purse-like (Fig. 32.7). It has two main lines—float line, which remains on the surface and a lead line, which sinks into the water, but does not touch the bottom. The fishes are trapped and do not escape because net is pursed during the operation.

At the time of use, one end of the net is tucked with a boat and another end is laid down with the help of a cruise making a circle and bringing this end back to the boat. The net then takes the shape of purse.

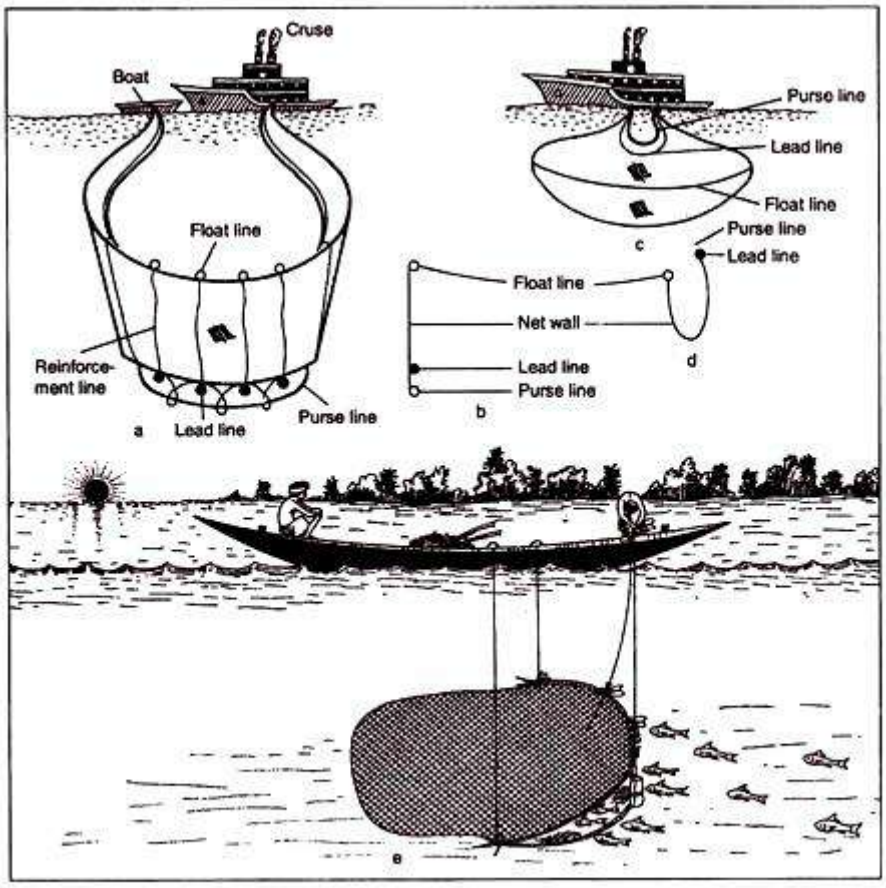


Fig. 32.7a-e : Purse seine : (a) before pursing, (b) net wall at (a), (c) after pursing, (d) net wall at, (e) Shanglo Jal

(v) Trap Nets:
They are generally used for fishing in shallow waters. Trap nets are strong and made in various shapes and sizes. These nets may be stationary or fixed. Its lower part is cylindrical while upper part is conical. Interior region of the net contains one or two cone-shaped necks to prevent escape of fish. Large trap nets are called a pound net, which has a chamber with a wide gate.

(vi) Drop Net:
It is square in shape and mounted with to supple loops at the corners that tied in a cross at the top and is attached to a pole. Drop net is operated with a boat. It is dropped and pulled to catch fishes.

(vii) Cast Net:
It is a circular and cone shaped net. It is spread from the edges of water. Its circumference is attached to lead line while its centre is attached with a rope. The net assumes shape of umbrella when it is spread on the water. When the net sinks to the bottom it is pulled and fishes are collected.

(viii) Drift Nets and Gill Nets:
These types of nets are. made by nylon materials (Fig. 32.8). Gill nets are kept overnight in the water and then dragged. The fishes get entangled in the meshes. There are two types of nets—simple and trammel nets.

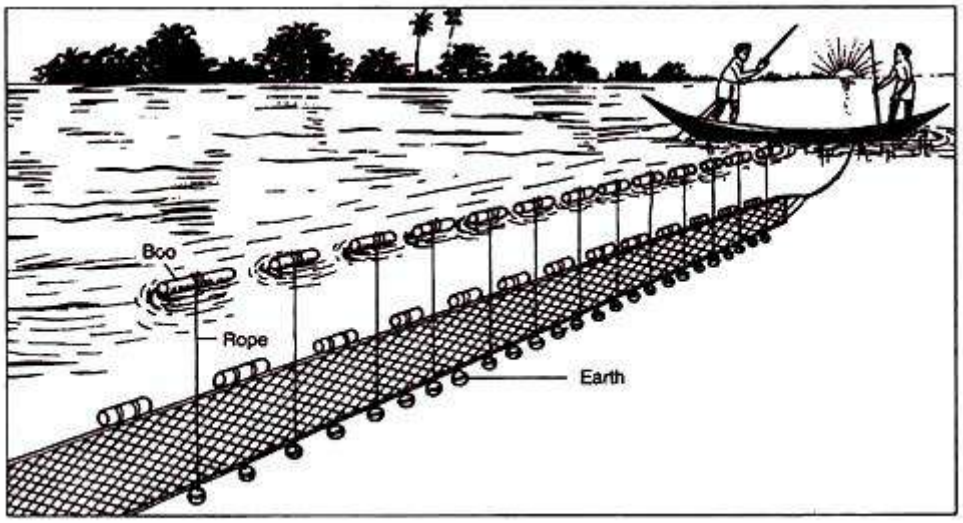


Fig. 32.8 : Chandi Jal

(ix) Simple Gill Net:
These are loosely woven nets. When spread in water, fishes get entangled in mesh. If the fishes try to escape the twine of the net get mingled in the gills of fishes. The fish is said to be gilled (captured by gills) and hence the name given 'Gill Net'.

(x) Trammel Gill Net:
It has a float line at the top and a dead line at the bottom; two walls are attached to these lines. It is generally operated to catch small fishes.

(xi) Fixed or Stationary Net:
These nets are used to catch fish at inshore water during low tides. These nets are kept fixed with the help of floats, sinkers and stakes. It is rectangular or conical in shape. They are available in various sizes.

In West Bengal and Orissa conical fixed nets are used, which are called Ghurni Jal, or behundi, Kathia-kool Jal, Panch-Kathiaber Jal and Panch. However, rectangle nets used are called as Mai Jal in West Bengal, Barnada Jal in Orissa and Kakavalai, Jadi or Mtagh Jal in Tanjore.

(xii) Bag nets:
It is conical in shape without wings (Fig. 32.9a, b). Some commonly used bag nets are iroga in Andhra, thuriwala in Tamil Nadu and koliwala in Kerela. These nets are used with the help of two catamarans or boats. In the coasts of Mumbai and Gujarat a special type of bag net called 'dol' is used. It is conical with wide mouth. The mouth is fixed on a bamboo.

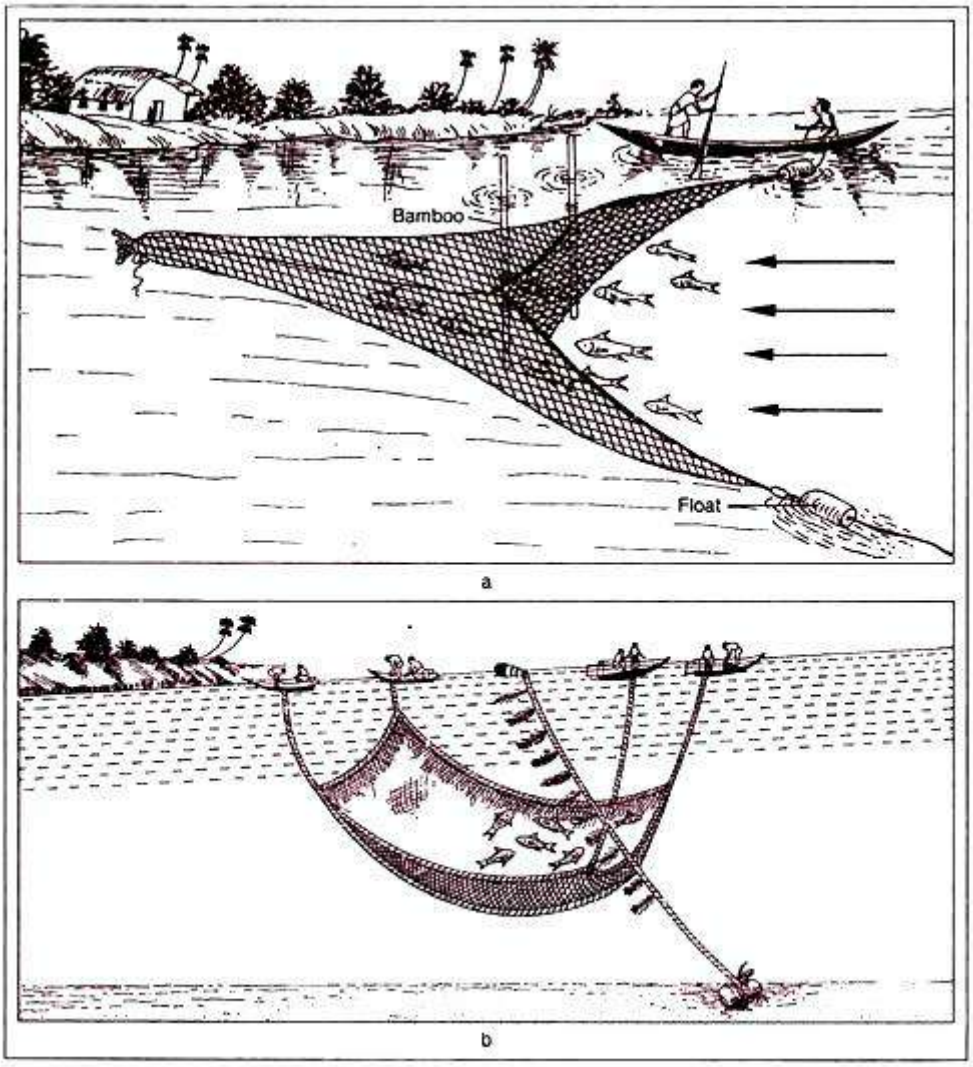


Fig. 32.9a, b : (a) Been Jal, (b) Mada Valai — A bag net of Coromandel coast (after Hernell).

(xiii) Scoop Net or Dip Net:
It is round in shape and is used to capture delicate fishes. It is like a finger bowl and can be moved swiftly in a scooping manner, collecting the fish.

(xiv) Hooks and Lines:
It consists of two types of hand lines and long lines. Various types of hooks are used, such as chain hooks, baited hooks, revolving and non-revolving hooks for capture of larger fishes.

(xv) Trawls:
These are large dragging type nets (Fig. 32.10). There may be two types of trawls with beam called beam trawls and otter trawls.

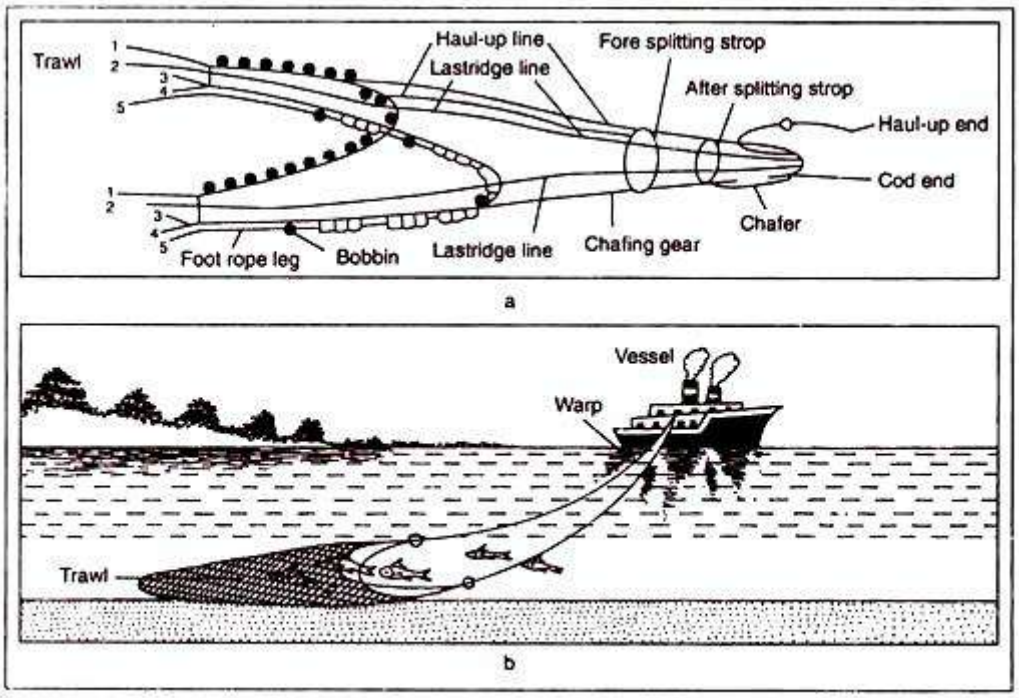


Fig. 32.10a, b : Trwa : (a) The Gear, (b) The net in operation

B. Inland Fishing Crafts and Gears:

Inland Fishing Crafts:
Rafts and Dongas are age-old inland fishing crafts used in steady waters.

Rafts:
Rafts are traditional crafts made by different types of materials. In Bihar earthen pots are tied together to provide support to a high bamboo platform. In West Bengal and Tamil Nadu stems of banana trees are held together to construct a floating platform.

In olden days buffalo skins are tied together to make a crude raft. In West Bengal a simple types of dugout canoe called Dongas are used. It is constructed by hollowing the stem of palm tree. It is commonly used for fishing in shallow waters. Vellum is stronger dugout canoe used in brackish waters of Kerala. In Tanjore and Tiruchirapalli chatty rafts made by earthenware are commonly used.

Boats:

Following types of boats are used for fishing in rivers:

i. Plank-Built Boat:
These boats are sturdy and are used for fishing in rivers with strong tides and currents. In different regions different types of plank boats are operated. One of common types is ‘Dinghis’ used in west Bengal. Dinghi is narrow and it has a tapering bow and stern. It has no Keel.

Dinghi is generally used to operate purse nets and dip nets. Another type is Chandi nauka, which is used to operate drift nets. It is 18 m long and 3 m wide.

In Calcutta (Kolkata) and the other parts of West Bengal a medium sized boat called Mechho bachari is used to transport live fishes. However, in Chilka lake (brackish water lagoon) another type of plank boat called Nava is used.

ii. Kulnawa:
It is a specialized boat used in the river Ganges for fishing of minnows. Kulnawa means open wale boat. This boat is used in calm water during night from February to April. It is brought from Tarai parts of Nepal.

Kulnawa is made up of 3 parts, viz., a frilled pole, screen platform and boat with one of the walls open. It is made by Sal wood or Kathal wood. It is 7 m long and is dinghy type of boat without any keel. The boat is painted by coaltar to keep it dark in colour.

The screen platform is made by 5 m x 0.5 m bamboo splinters, which are woven interlacing plastic cords. Screen is painted with enamel paint. The free margin of screen is always immersed in water. The frilled pole is 4-6 m long bamboo pole kept drooping as frill. 6% dry grass bunches are tied to these poles.

Inland Fishing Gears:
India has a wide variety of diversified water bodies, hence the nets used are also diversified.

Nets used in hill streams:
Cast nets are used in small pockets of hilly regions. Different types of traps and cast nets are fixed in narrow gaps of streams. It catches fish during breeding seasons.

Nets used in Ponds and Lakes:
These are most commonly used for commercial fishing. Seines are large nets operated from boats. In big lakes and the Ganges the most commonly used seine is Jagat Ber Mahajal. Simple drag nets are also employed in ponds for commercial fishing.

Rangoon Nets and Uduvalai:
They are used in those lakes where use of seines and dragnets are not easy. Rangoon net is made by fine cotton rectangular pieces of net. These pieces are tied in such a way so as to form a big wall. It is then spread in water with the help of floats.

The fish gets entangled in the net. Rangoon net is generally used for fishing in less deep water. However, in deep water bodies the use of uduvalai is preferred. It has small sinkers with footrope.

Gears used in rivers:
The following gears/ nets of different dimensions are used for fishing in rivers.

Seine and Drag Nets:
They are most commonly used nets for fishing in rivers. Seines can be operated from one or more boats.

Kuriar Nets:
It is used in shallow water for fishing of carps and herring (Hilsa ilisha). This net is very easy to operate. Kurian net is umbrella like and kept in inverted position and dipped in water for

some times. Then it is pulled out with capture.

Kona Jal or Bhasa Gulli:
It is a special type of large cotton seine net used for fishing of Hilsa.

Kona Jal:
It is a special type of seine net of about 90 m x 9 m in size. It has conical pockets of small mesh size. These pockets are fixed at a distance of 8-10 m all along the net. It is made by cotton, fish once caught, cannot escape because of a valve-like flap present in the pocket. It is also called Bhasa Gulli.

Moi or Moia Jal:
It is a simple net commonly used in shallow waters for fishing.

Jagat Ber or Maha Jal:
It is a simple net used all around in rivers.

Chunti Jal:
It is a type of drag net used in rivers of Bihar. Two fishermen operate it.

Kharra Jal:
Hela jal, bhesal jal and firki jal are also commonly used for fishing of carps and Catla in the river Narmada.

Khorsula Jal or Koila Jal:
It is a special type of dip net. Bhil tribal fishermen use this net in Narmada river for fishing of migratory Hilsa.

Jhanda Net:
It is like an open bag operated in shallow waters. It is rectangular in shape with small mesh. This net is stretched on a bamboo frame.

Suti Jal:
It is a long-tube like stationary bag net. It has a long wing.

Bada Jal:
It is a modified type of Suti jal, which has a wide opening, being kept open with the help of a rod.

Purse Net:
Special types of purse nets used for fishing in rivers are kharki jal and shanglo jal or sharki jal. In shanglo jal the mouth of purse can be opened or closed with a vertical cord. However, in kharki jal a vertical bamboo rod is fixed to the lower part of mouth of the purse to open or close it. These nets are operated from a dug-out canoe.

C. Mechanized Craft:

Selection of Gear:
Selection of appropriate gear for catching fish is of utmost importance in order to increase catch.

Correct gear may be chosen taking into consideration of the following points:
1. For catching the big and strong fish, the gear should also be strong and sturdy with proper mash size.

2. To catch fish at various level of the water body, different nets should be used, viz. surface gill nets,' column gill nets and bottom gill nets.

3. Fishes which swim in shoals may be caught by using encircling nets like drag nets, purse nets, etc. Similarly using hooks and lines can catch the individual fish.

Maintenance of Gears:
Proper care and handling of fishing gears after their use is as important as their use. Proper maintenance increases the durability of the gears.

Following care is necessary:
1. The gear should be washed thoroughly with the clean water and weeds and mud, etc. should be removed carefully.

2. Then dip the net in dilute $KMnO_4$ or $CuSO_4$ or common salt solution to get rid of harmful bacteria.
3. Wash again with clean water and then spread in shade for drying.

4. To increase durability and strength of the fibre of gear, it may be kept immersed for 10-15 min in hot tar diluted with kerosene.

5. Water quality criteria for Aquaculture: Assessment of pH, conductivity, Total solids, Total dissolved solids

Water Quality Criteria and Standards for Freshwater and Marine Aquaculture

INTRODUCTION

In 2004, capture fisheries and aquaculture have supplied the world with 106 million tons of food fish (FAO, 2006). Of this, 43% came from aquaculture. Within the growing aquaculture industry, it is accepted that good water quality is needed for maintaining viable aquaculture production. Poor water quality can result in low profit, low product quality and potential human health risks. Production is reduced when the water contain contaminants that can impair development, growth, reproduction, or even cause mortality to the cultured species. Some contaminants can accumulate to the point where it threatens human health even in low quantities and cause no obvious adverse effects. The fish perform all its physiological activities in the water – breathing, excretion of waste, feeding, maintaining salt balance and reproduction. Thus, water quality is the determining factor on the success or failure of an aquaculture operation. The continued degradation of water resources due to anthropogenic sources necessitates a guideline in selecting sites for aquaculture using water quality as a basis.

pH determination of water

Principle

Hydrogen ion concentration of water is very important chemical constituent of water. It both affects the biological as well as chemical of water.

pH meter is regulated using a freshly made buffer solutions (pH 4, 7 and 9) and the slope of electrode adjusted against the respective strengths of solutions. Temperature compensation is adjusted manually according to the ambient sample temperature. The electrode is thoroughly rinsed with distilled water before each measurement.

Materials required

a. pH meter
b. Distilled water
c. Sample water
d. Buffer tablets (pH 4, 7 and 9) and corresponding buffer solutions.

Procedure

1. Buffer solution of pH 4.0, 7.0 and 9.0 are prepared by using standard buffer tablets.
2. Electrodes are connected to pH meter.
3. Electrodes are dipped in a buffer solution of pH 7.0.
4. Temperature control of the pH meter is set as per the temperature of the sample water.
5. pH is recorded. The meter gives a reading near 7.0.
6. The electrodes are then removed from buffer solutions and washed with distilled water and dried. The electrodes are dipped to buffer of pH 4.0 and 9.0 consecutively. The meter should correspondingly show a reading of 4.0 and 9.0 respectively.
7. Next the electrodes are dipped in the water sample (test sample). The sample is gently swirled twice and pH is seen in the digital display or in the meter of the instrument. Three such replicates are taken.
8. Mean pH value is taken.

Observation

No. of Observations	pH value	Mean pH value

Inference

Depending upon the pH of the water one can state whether the sample water is acidic, neutral or alkaline.

The pH of sample water is _____. Thus it has _____ pH.

Assessment of Conductivity of Water

Introduction

Conductivity of water allows measuring ionic constituents of all types of water including surface waters, process waters in water supply and treatment plants.

Principle

Conductivity is the ability of a solution, a metal or a gas - in brief all materials - to pass an electric current. In solutions the current is carried by cations and anions whereas in metals it is carried by electrons. How well a solution conducts electricity depends on a number of factors:

- Concentration of ions
- Mobility of ions
- Valence of ions
- Temperature

All substances possess some degree of conductivity. In aqueous solutions the level of ionic strength varies from the low conductivity of ultrapure water to the high conductivity of concentrated chemical samples.

Conductivity may be measured by applying an alternating electrical current (I) to two electrodes immersed in a solution and measuring the resulting voltage (U). During this process, the cations migrate to the negative electrode, the anions to the positive electrode and the solution acts as an electrical conductor.

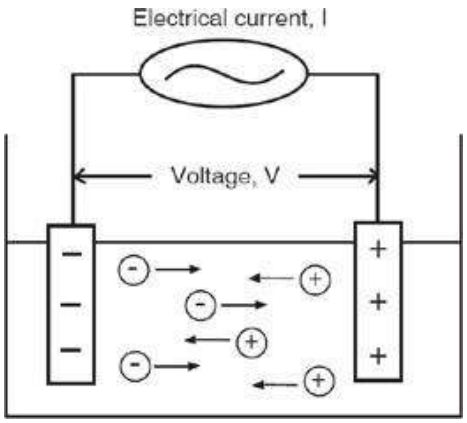


Figure 1: Migration of ions in solution

The resistance of the solution (R) can be calculated using Ohm's law as shown below. The resistance unit is [Ohms] or [Ω].

Where:

U = voltage [V] I = current [A]
R = resistance of the solution [Ω]

$$R = \frac{U}{I}\ [\Omega] \qquad \textbf{(Eq. 1)}$$

The conductance (G) is defined as the reciprocal of the electrical resistance (R) of a solution between two

electrodes. It is measured in Siemens [S] which equals [Ω^{-1}].

$$G = \frac{1}{R}\ [S] \qquad \textbf{(Eq. 2)}$$

The conductivity meter measures the conductance, and displays the reading converted into conductivity. The cell constant (K) is the ratio of the distance (d) between the electrodes to the area (a) of the electrodes.

Where:

K= cell constant

[m^{-1}] d **K = [m^{-1}]** **(Eq.3)** –

a = effective area of the electrodes [m^2] d = distance between the electrodes [m]

Commonly, K is expressed in [cm^{-1}] instead of [m^{-1}], because the cell dimension is expressed in [cm^{-1}].

Electricity is the flow of electrons. This indicates that ions in solution will conduct electricity. Conductivity is the ability of a solution to pass current. The conductivity reading of a sample will change with temperature. The conductivity (κ) is defined as:

$$\kappa = G \times K \quad [S/m] \quad \textbf{(Eq. 4)}$$

Where:
G = conductance [S] K = cell constant [m^{-1}]

Usually the cell constant K is expressed in [cm^{-1}], the conductivity κ is expressed in [S/cm^{-1}], or even in a more convenient unit such as [mS/cm^{-1}] or [$\mu S/cm^{-1}$].

Definitions:

- **Calibration:** Determination of the cell constant required converting conductance readings intoconductivity results
- **Standard solution:** A solution of known conductivity that is used to calibrate the conductivitymeasuring chain
- **Reference temperature:** Conductivity readings are often referenced to a specific temperature(typically 20 °C or 25 °C) for comparative purposes
- **Automatic temperature correction:** Algorithms for automatic conversion of sample conductivity to areference temperature

Electrode

Conductivity sensor: Intellical™ CDC401. The range of conductivity for this electrode is 0.01 µS/cm - 200mS/cm, see section **6 Results** for additional specifications.

The temperature compensation law is valid for a conductivity range from 60 to 1000 µS/cm.

Application default settings

This application has been optimized with the following settings:

Name	Default parameter	Unit
Application		
Name	Water Conductivity	
Sample		
Name	Water ?[1]	
Amount	100	[mL]
QC		
Name	QC Sample	
Electrode		
Type	Conductivity	

Recommended electrode	CDC401	
Temp. compensation	Natural water 25 °C	
Method: Conductivity		
Active	Yes	
Max. stability time	150	[s]
Stability criterion	1	[%/min]
Stirring speed	20	[%]
Result 1 (R1) name	Conductivity	
R1 hide	No	
R1 QC min.	0	[uS/cm]
R1 QC max.	9999	[uS/cm]

[1] "?" in the name, indicates that the sample name will be automatically incremented with a number for each analysis

Remarks on settings:

Sample amount

The sample amount of 100 mL is given as an indication. The sample volume must be sufficient to allow theconductivity sensor to be immersed in the solution being measured.

Temperature compensation

For **Natural Water**, the instrument is set to compensate the temperature at 25 °C in a measurement range between 0 to 35 °C, using the "Natural Water Standards IS0/DIS_7888". The conductivity range is about 60 to 1000 µS/cm.

For other types of temperature compensation such as **German drinking water regulation**, **Linear temperature compensation** or **No temperature compensation**, contact a Hach/Hach Lange application support engineer.

Procedure:

Conductivity electrode calibration

If an electrode calibration is required, refer to section **8 Appendix: conductivity electrode calibration**.

Sample analysis

To avoid sample contamination, take a sample in a polyethylene bottle (not glass) and proceed to measurement as soon as possible to avoid gas exchange, such as CO_2, or biological activity. You can reduce biological activity by keeping the sample below 4 °C and in obscurity.

In **Direct measurement** (called by the application) the Natural Water temperature compensation is applied. For
Live measurement there is no temperature compensation applied.

Electrode cleaning

For electrode cleaning recommendations, refer to the CDC401 conductivity probe user manual (DOC022.53.80022).

Results

Performance is directly linked to that of the CDC401 conductivity sensor:

	CDC401
Type of sensor	Standard Intellical™ Conductivity Probe - CDC401 Graphite, 4-pole conductivity probe
Cell constant	0.40 cm^{-1} ± 10%
Conductivity Range	0.01 µS/cm to 200.0 mS/cm
Accuracy	± 0.5% of the reading
Conductivity resolution	0.0 to 19.99 µS/cm: 0.01 µS/cm 20.0 to 199.9 µS/cm: 0.1 µS/cm 200 to 1999 µS/cm: 1 µS/cm 2.00 to 19.99 mS/cm: 0.01 mS/cm 20.0 to 200.0 mS/cm: 0.1 mS/cm
Operating Temperature range	-10 to 110 °C (14 to 230 °F)
Temperature accuracy	± 0.3 °C (± 0.54 °F)
Minimum sample depth	45 mm (1.77 in.)
Conductivity response time	> 99% in 30 s between KCl 0,01 D and KCl 1D
Temperature response time	95% of the response in 30 s between 5 and 40 °C

Assessment of Total Solids

Total solids are dissolved solids plus suspended and settleable solids in water. In stream water, dissolved solids consist of calcium, chlorides, nitrate, phosphorus, iron, sulfur, and other ions particles that will pass through a filter with pores of around 2 microns (0.002 cm) in size. Suspended solids include silt and clay particles, plankton, algae, fine organic debris, and other particulate matter. These are particles that will not pass through a 2-micron filter.

The concentration of total dissolved solids affects the water balance in the cells of aquatic organisms. An organism placed in water with a very low level of solids, such as distilled water, will swell up because water will tend to move into its cells, which have a higher concentration of solids. An organism placed in water with a high concentration of solids will shrink somewhat because the water in its cells will tend to move out. This will in turn affect the organism's ability to maintain the proper cell density, making it difficult to keep its position in the water column. It might float up or sink down to a depth to which it is not adapted, and it might not survive.

Higher concentrations of suspended solids can serve as carriers of toxics, which readily cling to suspended particles. This is particularly a concern where pesticides are being used on

irrigated crops. Where solids are high, pesticide concentrations may increase well beyond those of the original application as the irrigation water travels down irrigation ditches. Higher levels of solids can also clog irrigation devices and might become so high that irrigated plant roots will lose water rather than gain it.

A high concentration of total solids will make drinking water unpalatable and might have an adverse effect on people who are not used to drinking such water. Levels of total solids that are too high or too low can also reduce the efficiency of wastewater treatment plants, as well as the operation of industrial processes that use raw water.

Total solids also affect water clarity. Higher solids decrease the passage of light through water, thereby slowing photosynthesis by aquatic plants. Water will heat up more rapidly and hold more heat; this, in turn, might adversely affect aquatic life that has adapted to a lower temperature regime.

Sources of total solids include industrial discharges, sewage, fertilizers, road runoff, and soil erosion. Total solids are measured in milligrams per liter (mg/L).

Sampling and equipment considerations

Total solids are important to measure in areas where there are discharges from sewage treatment plants, industrial plants, or extensive crop irrigation. In particular, streams and rivers in arid regions where water is scarce and evaporation is high tend to have higher concentrations of solids and are more readily affected by human introduction of solids from land use activities.

Total solids measurements can be useful as an indicator of the effects of runoff from construction, agricultural practices, logging activities, sewage treatment plant discharges, and other sources. As with turbidity, concentrations often increase sharply during rainfall, especially in developed watersheds. They can also rise sharply during dry weather if earth-disturbing activities are occurring in or near the stream without erosion control practices in place. Regular monitoring of total solids can help detect trends that might indicate increasing erosion in developing watersheds. Total solids are related closely to stream flow and velocity and should be correlated with these factors. Any change in total solids over time should be measured at the same site at the same flow.

Total solids are measured by weighing the amount of solids present in a known volume of sample. This is done by weighing a beaker, filling it with a known volume, evaporating the water in an oven and completely drying the residue, and then weighing the beaker with the residue. The total solids concentration is equal to the difference between the weight of the beaker with the residue and the weight of the beaker without it. Since the residue is so light in weight, the lab will need a balance that is sensitive to weights in the range of 0.0001 gram. Balances of this type are called analytical or Mettler balances, and they are expensive (around $3,000). The technique requires that the beakers be kept in a desiccator, which is a sealed glass container that contains material that absorbs moisture and ensures that the weighing is not biased by water condensing on the beaker. Some desiccants change color to indicate moisture content.

The measurement of total solids cannot be done in the field. Samples must be collected using clean glass or plastic bottles or Whirl-pak® bags and taken to a laboratory where the test can be run.

Assessment of Total dissolved solids (TDS)

Water is a good solvent and picks up impurities easily. Pure water -- tasteless, colorless, and odorless -- is often called the universal solvent. Dissolved solids" refer to any minerals, salts, metals, cations or anions dissolved in water. Total dissolved solids (TDS) comprise inorganic salts (principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides, and sulfates) and some small amounts of organic matter that are dissolved in water.

TDS in drinking-water originate from natural sources, sewage, urban run-off, industrial wastewater, and chemicals used in the water treatment process, and the nature of the piping or hardware used to convey the water, i.e., the plumbing. In the United States, elevated TDS has been due to natural environmental features such as mineral springs, carbonate deposits, salt deposits, and sea water intrusion, but other sources may include: salts used for road de-icing, anti-skid materials, drinking water treatment chemicals, stormwater, and agricultural runoff, and point/non-point wastewater discharges.

In general, the total dissolved solids concentration is the sum of the cations (positively charged) and anions (negatively charged) ions in the water. Therefore, the total dissolved

solids test provides a qualitative measure of the amount of dissolved ions but does not tell us the nature or ion relationships. In addition, the test does not provide us insight into the specific water quality issues, such as Elevated Hardness, Salty Taste, or Corrosiveness. Therefore, the total dissolved solids test is used as an indicator test to determine the general quality of the water. The sources of total dissolved solids can include all of the dissolved cations and anions, but the following table can be used as a generalization of the relationship of TDS to water quality problems.

Cations combined with Carbonates CaCO3, MgCO3 etc	Associated with hardness, scale formation, bitter taste
Cations combined with Chloride NaCl, KCl	Salty or brackish taste, increase corrosivity

An elevated total dissolved solids (TDS) concentration is not a health hazard. The TDS concentration is a secondary drinking water standard and, therefore, is regulated because it is more of an aesthetic rather than a health hazard. An elevated TDS indicates the following:

1)The concentration of the dissolved ions may cause the water to be corrosive, salty or brackish taste, result in scale formation, and interfere and decrease efficiency of hot water heaters; and

2)Many contain elevated levels of ions that are above the Primary or Secondary Drinking Water Standards, such as an elevated level of nitrate, arsenic, aluminum, copper, lead, etc.

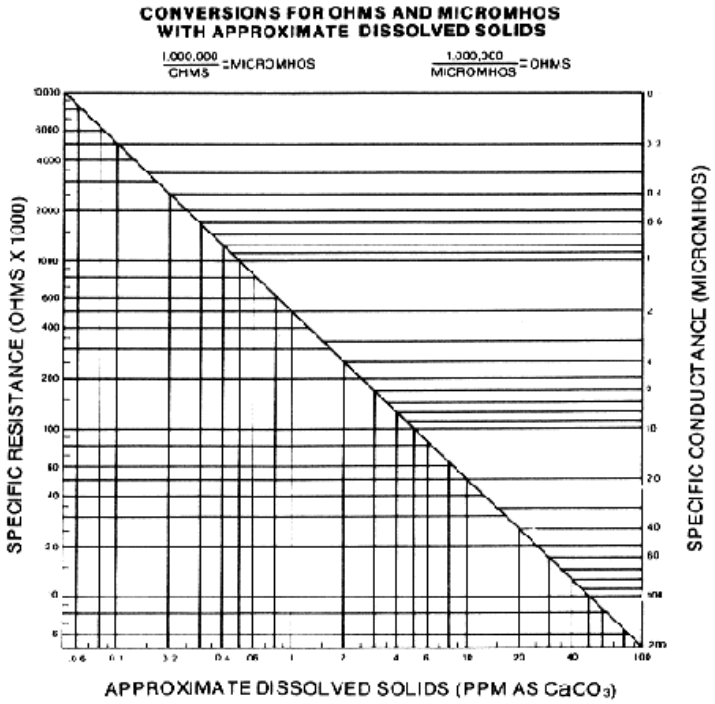
Total Dissolved Solids (TDS)**:** In a laboratory setting, the total dissolved solids is determined by filtering a measured volume of sample through a standard glass fiber filter. The filtrate (i.e., filtered liquid) is then added to a preweighed ceramic dish that is placed in a drying oven at a temperature of 103 C. After the sample dries, the temperature is increased to 180 C to remove an occluded water, i.e., water molecules trapped in mineral matrix. Total dissolved solids means the total dissolved (filterable) solids as determined by use of the method specified in Title 40 of the Code of Federal Regulations (40 CFR) Part 136.

High total dissolved solids may affect the aesthetic quality of the water, interfere with washing clothes and corroding plumbing fixtures. For aesthetic reasons, a limit of 500 mg/l (milligrams per liter) has been established as part of the Secondary Drinking Water Standards.

An approximation of the Total Dissolved Solids:

A. The total dissolved solids concentration can be related to the conductivity of the water, but the relationship is not a constant. The relationship between total dissolved solids and conductivity is a function of the type and nature of the dissolved cations and anions in the water and possible the nature of any suspended materials. For example, a NaCl solution and KCl solution with a conductivity of 10000 umhos/cm will not have the sample concentration

of NaCl or KCl and they will have different total dissolved solids concentration. Conductivity is measured through the use of a meter and is usually about 100 times the total cations or anions expressed as equivalents and the total dissolved solids (TDS) in ppm usually ranges from 0.5 to 1.0 times the electrical conductivity.



Total Dissolved Solids can be measured in the field using an electronic pen. Many of these devices actually measure the conductivity of the water, i.e., the ability of the water to carry a charge, and not the actual total dissolved solids. These devices then calculate the total dissolved solids assuming that the primary dissolved minerals are either a combination of NaCl or KCl. Therefore, the measurement of total dissolved solids by these devices are not an accurate measure, but an approximation. If you are thinking of using these devices for a project, I would recommend purchasing a conductivity pen which measures the conductivity of the water.

B. Student Total Dissolved Solids Test (Student Use Only - Not for Regulatory Use !)

1. Filter your water sample through a rinsed and dried glass fiber filter. Collect the filtrate (liquid) and rinse water in a flask. The minimum sample volume should be 100 ml and you should use at least 3 rinses of 20 to 30 ml volumes. (Recording your data)- Record weight of container and volume of filtrate - do not include the volume of the rinse water). The rinse water should be deionized water. Do not touch container with bare hands.

2.Transfer the filtrate to a ceramic or glass Pyrex container. The container should be weighed to the nearest 0.0000 g and place the container in the drying oven, which is set at 103 C. Add the filtrate to the container and allow the sample to stay in the oven at 103 C for 24 hours. If possible, increase the temperature of the drying oven to 180 C and allow the sample to dry for up to 8 hours. Remove the container Assessment of 3.After the container cools, reweigh the container at least three times to the nearest 0.0000 g (Recording your data)

4.Subtract the initial weight (in grams) of the empty container from the weight of the container with the dried residue to obtain the increase in weight. Then do the following:

A- Weight of clean dried container (0.0000 grams)

B- Weight of container and residue (0.0000 grams)

C- Volume of Sample (do not include rinse water) (100 mls)

Concentration (mg/L) = ((B - A)/ C)* (1000 mg/g) * (1000 ml/L)

Example

A= 100.0001g

B = 100.0020 g

C = 100 mls

Concentration (mg/L) = ((100.0220 - 100.0001)/ 100) * 1000 * 1000 = 219 mg/L

7. Air-breathing organs in Channa, Heteropneustes, Anabas and Clarias

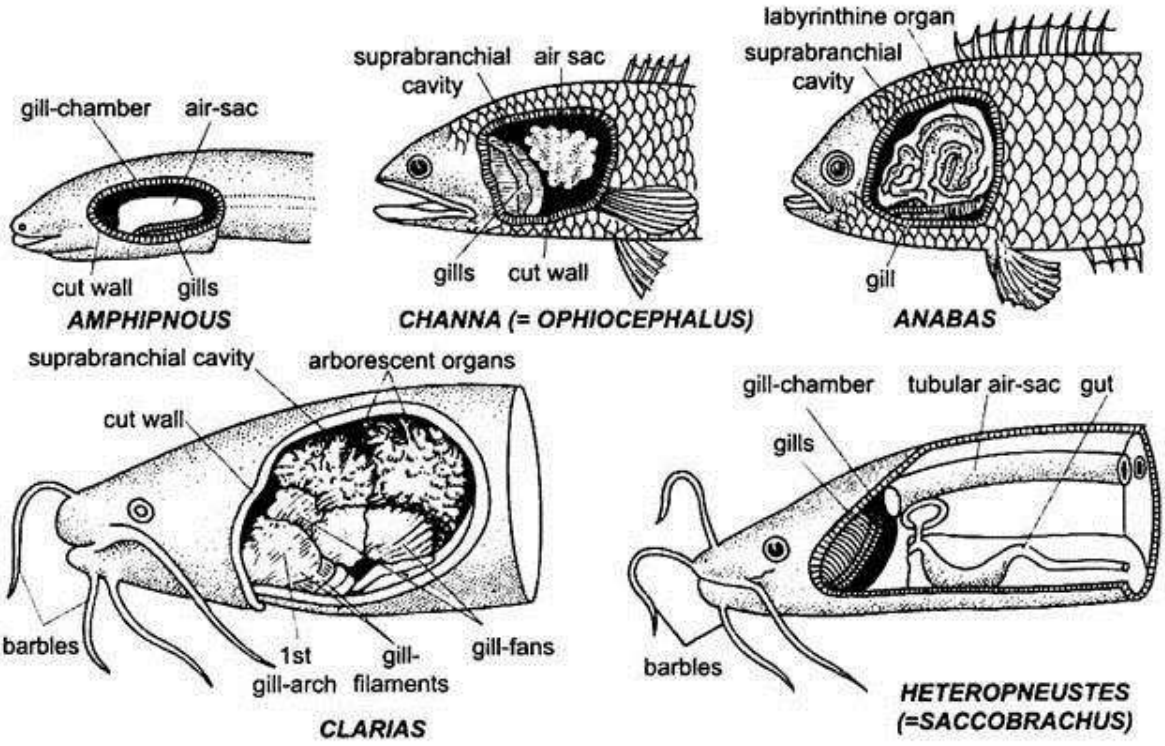


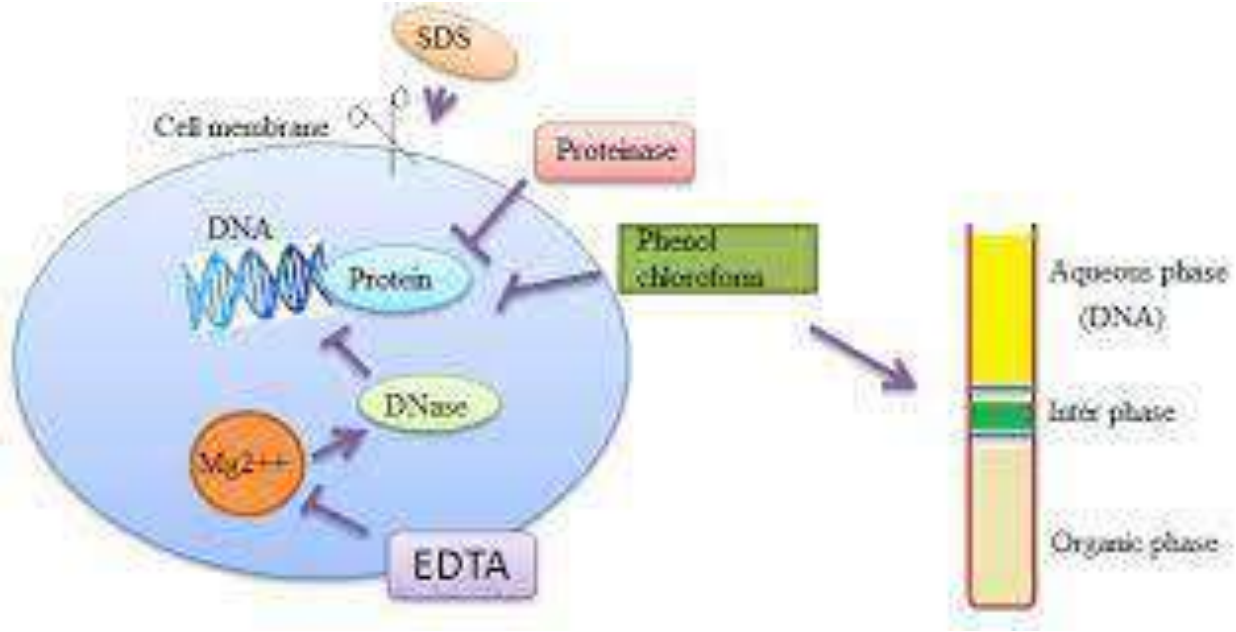
Fig. 17.8. Accessory respiratory organs dissected on the left side in some air-breathing teleost fishes.

DSE2P: Animal Biotechnology

Isolation of Genomic DNA from E. coli

PRINCIPLE:

The isolation and purification of DNA from cells is one of the most common procedures in contemporary molecular biology and embodies a transition from cell biology to the molecular biology (from in vivo to in vitro). The isolation of DNA from bacteria is a relatively simple process. The organism to be used should be grown in a favorable medium at an optimal temperature, and should be harvested in late log to early stationary phase for maximum yield. The genomic DNA isolation needs to separate total DNA from RNA, protein, lipid, etc. Initially the cell membranes must be disrupted in order to release the DNA in the extraction buffer. SDS (sodium dodecyl sulphate) is used to disrupt the cell membrane. Once cell is disrupted, the endogenous nucleases tend to cause extensive hydrolysis. Nucleases apparently present on human fingertips are notorious for causing spurious degradation of nucleic acids during purification. DNA can be protected from endogenous nucleases by chelating Mg2++ ions using EDTA. Mg2++ ion is considered as a necessary cofactor for action of most of the nucleases. Nucleoprotein interactions are disrupted with SDS, phenol or proteinase K. Proteinase enzyme is used to degrade the proteins in the disrupted cell soup. Phenol and chloroform are used to denature and separate proteins from DNA. Chloroform is also a protein denaturant, which stabilizes the rather unstable boundary between an aqueous phase and pure phenol layer. The denatured proteins form a layer at the interface between the aqueous and the organic phases which are removed by centrifugation. DNA released from disrupted cells is precipitated by cold absolute ethanol or isopropanol.



MATERIALS REQUIRED:

i. LB Broth
ii. E. coli DH5α cells

REAGENTS

i. TE buffer (pH 8.0)
ii. 10% SDS
iii. Proteinase K
iv. Phenol-chloroform mixture
v. 5M Sodium Acetate (pH 5.2)
vi. Isopropanol
vii. 70% ethanol
viii. Autoclaved Distilled Water
ix. Eppendorf tubes 2 ml
x. Micropipette
xi. Microtips
xii. Microfuge

PREPARATION OF REAGENTS:

1. TE BUFFER (pH 8.0): 10 mm Tris HCl (pH 8.0), 1 mm EDTA (pH 8.0)

2. 10% SDS: Dissolve 10 g of SDS in 100 ml autoclaved distilled water.

3. PROTEINASE K: Dissolve 10 mg of Proteinase K in 1 ml autoclaved distilled water.

4. PHENOL – CHLOROFORM: The pH is very important. For RNA purification, the pH is kept around pH 4, which retains RNA in the aqueous phase preferentially. For DNA purification, the pH is usually 7 to 8, at which point all nucleic acids are found in the aqueous phase. Mix equal volume of phenol with chloroform. Keep the mixture on ice and add 20 ml TE buffer, extract by shaking for 15 minutes. Remove the dust on the surface layer using a pipette. Repeat 4-5 times. Add 30-40 ml of TE buffer and store it on ice.

5. 5M SODIUM ACETATE: Dissolve 41 g of sodium acetate in 100 ml distilled water and adjust pH with dilute acetic acid (pH 5.2).

6. ISOPROPANOL

7. 70% ETHANOL

PROCEDURE:

i. 2 ml overnight culture is taken and the cells are harvested by centrifugation for 10 minutes

ii. 875 µl of TE buffer is added to the cell pellet and the cells are resuspended in the buffer by gentle mixing.

iii. 100 µl of 10% SDS and 5 µl of Proteinase K are added to the cells.

iv. The above mixture is mixed well and incubated at 37º C for an hour in an incubator

v. 1 ml of phenol-chloroform mixture is added to the contents, mixed well by inverting and incubated at room temperature for 5 minutes.

vi. The contents are centrifuged at 10,000 rpm for 10 minutes at 4º C.

vii. The highly viscous jelly like supernatant is collected using cut tips and is transferred to a fresh tube.

viii. The process is repeated once again with phenol-chloroform mixture and the supernatant is collected in a fresh tube.

ix. 100 µl of 5M sodium acetate is added to the contents and is mixed gently.

x. 2 ml of isopropanol is added and mixed gently by inversion till white strands of DNA precipitates out.

xi. The contents are centrifuged at 5,000 rpm for 10 minutes.

xii. The supernatant is removed and 1ml 70% ethanol is added.

xiii. The above contents are centrifuged at 5,000 rpm for 10 minutes.

xiv. After air drying for 5 minutes 200 µl of TE buffer or distilled water is added.

xv. 10 µl of DNA sample is taken and is diluted to 1 or 2 ml with distilled water.

xvi. The concentration of DNA is determined using a spectrophotometer at 260/280 nm.

xvii. The remaining samples are stored for further experiments.

xviii. PRECAUTIONS:

xix. Cut tips should be used so that the DNA is not subjected to mechanical disruption.

xx. Depending on the source of DNA the incubation period of Proteinase K should extended.

xxi. The phenol chloroform extraction should be repeated depending on the source of DNA to obtain pure DNA.

xxii. DNase free plastic wares and reagents should be used.

OBSERVATIONS

Genomic DNA from E. coli

Plasmid DNA isolation (pUC 18/19) from E. coli

Principle:

Plasmid is an extra chromosomal circular self-replicating double stranded DNA molecule found in bacterial cell. For plasmid isolation bacterial culture should be grown to late logarithmic on stationary phase. This buffer is the typical buffering substance for DNA because it is slightly alkaline and DNA also be stored best pH 7.5-8.2. EDTA is an important substance in plasmid isolation because it inhibits nuclease activity.

Requirements:

1. Microfuse tubes
2. Micropipette and tips
3. Reagents:
    i. TE buffer- 1 M Tris buffer 0.5 M EDTA Solution

ii. 10mM EDTA solution
iii. Solution I (pH 8) : 50 M glucose + 10mMEDTA +25mM Tris buffer stored at 2-8 ^{0}C
iv. Solution II (pH 7) : 0.2N NaOH +1% SDS stored at RT
v. Solution III (pH 6) : 3M Potassium acetate & stored at 2-8 ^{0}C
vi. DNA loading Dye: 0.05% Bromophenol blue& 10% glycerol.
vii. Ethidium bromide; (EtBr); 10mg/ml
viii. Running Buffer (pH-8) 48gm Tris +0.115gm Acetic acid + 0.5 M EDTA

Procedure:

i. At first LB media was Prepared and autoclaved after that 40µg/ml of antibody was added .
ii. 500 µl of sample (Plasmid containing Bacteria) is transferre into newly prepared LB media & allowed for incubation overnight at 37 ^{0}C in stable condition.

Isolation of Plasmid:

i. Firstly newly prepared bacterial culture was taken in eppendroff and centrifuge at 8000 rpm for 8 min.
ii. Discard the supernatant then 100 µl of Solution I (ice cold) was poured into tube to get uniform suspension.
iii. Incubate in ice for 30 minutes.
iv. After incubation 200 µl of Solution II was added and inverted microfuse tube several times and mixed all reagents properly.
v. Incubate in ice for 30 minutes.
vi. Then 150µ l of Solution III is added and kept in ice for 5 minutes.
vii. The solution should be mixed in proper way and centrifuse at 10000 Rpm for 10 minutes.
viii. Supernatant is transferred to a fresh microfuse.
ix. After that equal volume of isopropyl alcohol was mixed and incubate at 20 ^{0}C.

x. Then the complete mixture was allowed for centrifugation at 10000 rpm for 10 mins.

xi. Discard the supernatant and further work with pellet.

xii. Next pellet was washed with 70% ethanol.

xiii. Then the pellet was dried at 40^0C for completely evaporation of the alcohol.

xiv. After that the pellet was dissolved in TE and load in 1.2% Agarose Gel.

Observation:

Orange colour DNA bands were observed when gel slab was exposed on UV light. Two distinct bands were found. The closer bands indicate released plasmid DNA bands and the farthest was super oil plasmid DNA.

Comment:

The DNA is separated by their molecular weight and bases. The plasmid is observed in gel which indicates that the bacterial cell culture is contained Plasmid.

Restriction digestion of plasmid DNA.

Introduction

Restriction enzyme digestion takes advantage of naturally occurring enzymes that cleave DNA at specific sequences. There are hundreds of different restriction enzymes, allowing scientists to target a wide variety of recognition sequences.

Restriction enzyme digestion is commonly used in molecular cloning techniques, such as PCR or restriction cloning. It is also used to quickly check the identity of a plasmid by diagnostic digest.

Equipment

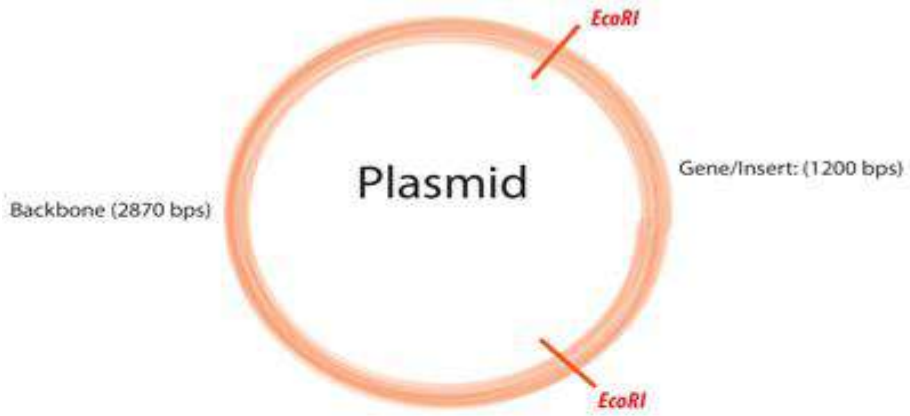
1. Electrophoresis chamber
2. Pipetman

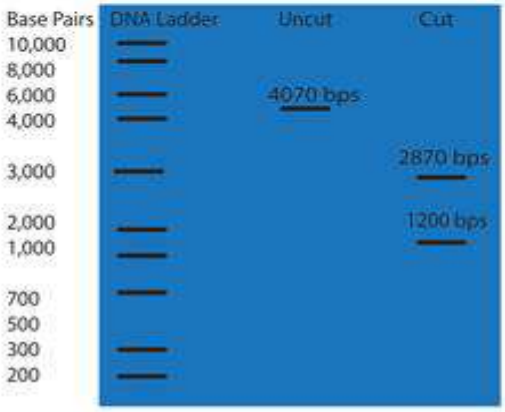
Reagents

1. Liquid DNA aliquot of your plasmid of interest (see below for recommend amounts)
2. Appropriate restriction enzyme (see manufacturer's instructions for proper ammount)
3. Approrpriate restriction digest buffer (see manufacturer's instructions)
4. Gel loading dye
5. Electrophoresis buffer
6. Pipet tips

Procedure

1. Select restriction enzymes to digest your plasmid.
2. Determine an appropriate reaction buffer by reading the instructions for your enzyme.
3. In a 1.5mL tube combine the following:
    a. DNA
    b. Restriction Enzyme(s)
    c. Buffer
    d. BSA (if recommended by manufacturer)
    e. dH2O up to total volume





4. Mix gently by pipetting.

5. Incubate tube at appropriate temperature (usually 37 °C) for 1 hour. Always follow the manufacturer's instructions.

6. To visualize the results of your digest, conduct gel electrophoresis.

Tips :

1. To determine which restriction enzymes will cut your DNA sequence (and where they will cut), use a sequence analysis program such as Addgene's Sequence Analyzer.

2. If you are conducting a double digest (digesting with two enzymes at the same time), you will need to determine the best buffer that works for both of your enzymes. Most companies will have a compatibility chart, such as the double digest finder tool from NEB.

3. The amount of DNA that you cut depends on your application. A diagnostic digest typically involves ~500 ng of DNA, while molecular cloning often requires 1 µg of DNA. The total reaction volume usually varies from 10-50 µL depending on application and is largely determined by the volume of DNA to be cut.

4. A typical restriction digestion reaction could look like this:

a. 1 µg DNA

b. 1 µL of each Restriction Enzyme

c. 3 µL 10x Buffer

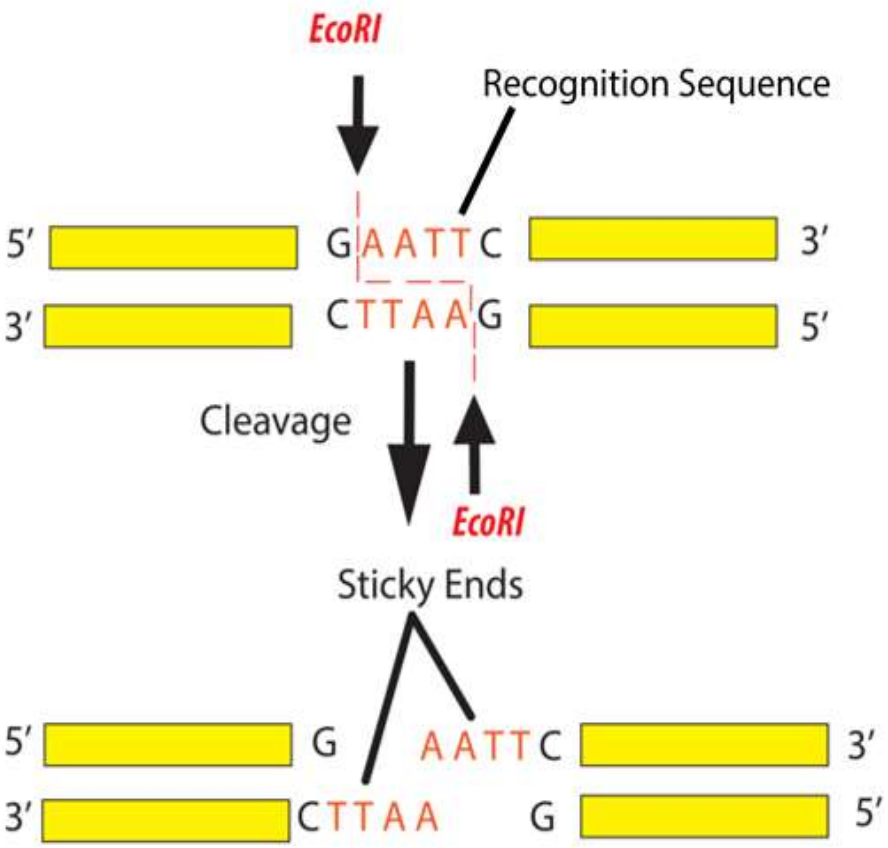
d. 3 µL 10x BSA (if recommended)

e. x µL dH2O (to bring total volume to 30µL)

5. The amount of restriction enzyme you use for a given digestion will depend on the amount of DNA you want to cut. By definition: one unit of enzyme will cut 1 µg of DNA in a 50 µL reaction in 1 hour. Using this ratio, you can calculate the minimal amount of enzyme for your reaction. However, keep in mind that restriction enzyme activity is determined under ideal conditions with very clean DNA, so using a little more enzyme is advisable. Reactions are often performed with 0.2-0.5 µL of enzyme because it is difficult to pipette less volume than this; 0.2-0.5 µL will likely be more enzyme than you will need, but that's okay because a little more enzyme is usually better.

6. Depending on the application and the amount of DNA in the reaction, incubation time can range from 45 mins to overnight. For diagnostic digests, 1-2 hours is often sufficient. For digests with >1 µg of DNA used for cloning, it is recommended that you digest for at least 4 hours.

7. If you will be using the digested DNA for another application (such as a digestion with another enzyme in a different buffer), but will not be gel purifying it, you may need to inactivate the enzyme(s) following the digestion reaction. This may involve incubating the reaction at 70 °C for 15 mins, or purifying the DNA via a purification kit, such as a QIAGEN DNA cleanup kit. See the enzyme manufacturer's instructions for more details.



Notes :

1. Restriction enzymes MUST be placed in an ice bucket immediately after removal from the -20 °C freezer because heat can cause the enzymes to denature and lose their function.

2. If you are having difficulty finding an enzyme that cuts your vector's multiple cloning site (MCS), but does not cut your insert, you could try using two different enzymes that have compatible sticky ends.

3. If you cannot find compatible sticky ends, you will need to fill in the overhangs and conduct a blunt end ligation. Use T4 DNA Polymerase or Klenow DNA Polymerase I for 3′ overhang removal and 5′ overhang fill-in.

4. If you are using blunt ends or a single enzyme to cut the vector, you will need to use a phosphatase to prevent re-circularization of the vector if you are cloning in an insert. CIP (calf alkaline phosphatase) or SAP (shrimp alkaline phosphatase) are commonly used. Follow the manufacturer’s instructions.

5. If your enzyme did not cut, check to make sure that it isn't methylation sensitive. Plasmids grown in Dam or Dcm methylase positive strains will be resistant to cleavage at certain restriction sites.

6. Sometimes enzymes cut sequences which are similar, but not identical, to their recognition sites. This is due to "Star Activity" and can happen for a variety of reasons, including high glycerol concentration.

7. If you are digesting a large number of plasmids with the same enzyme(s) (for instance, in a diagnostic digest), you can create a "Master Mix" consisting of all of the reaction components except for the DNA. Aliquot your DNA into individual tubes and then add the appropriate amount of Master Mix to each tube. This will save you time and ensure consistency across the reactions.

Restriction Mapping:

Introduction:

DNA mapping refers to the variety of different methods that can be used to describe the positions of genes. DNA maps can show different levels of detail, to indicate how far two genes are located from one another. At a low resolution, DNA can be mapped at the level of banding patterns that roughly show the distance between two genes after a chromosome has been stained with dye. Genetic mapping or linkage mapping can be used to indicate the relative order of genes on a chromosome. A restriction map is another type of DNA map that roughly describes the relative positions of genes by breaking apart sections of DNA at locations known as restriction sites. A physical DNA map describes the absolute position of genes on a chromosome. Physical maps can be constructed by breaking a section of DNA, a chromosome, or an entire genome into smaller fragments. These overlapping DNA fragments can be cloned, or copied. Then, sections of the DNA can be used to obtain nucleotide sequences and establish the precise locations of genes.

Restriction Mapping:

Restriction map is the physical map of a DNA which shows the relative positions of restriction enzyme cleavage sites. One common method for constructing a restriction map involves digesting the unknown DNA sample in three ways. Here, two portions of the DNA sample are individually digested with different restriction enzymes, and a third portion of the DNA sample is double-digested with both restriction enzymes at the same time. Next, each digestion sample is separated using gel electrophoresis, and the sizes of the DNA fragments are recorded. The total length of the fragments in each digestion will be equal. However,

because the length of each individual DNA fragment depends upon the positions of its restriction sites, each restriction site can be mapped according to the lengths of the fragments. The final drawing of the DNA segment that shows the positions of the restriction sites is called a restriction map.

Principle:

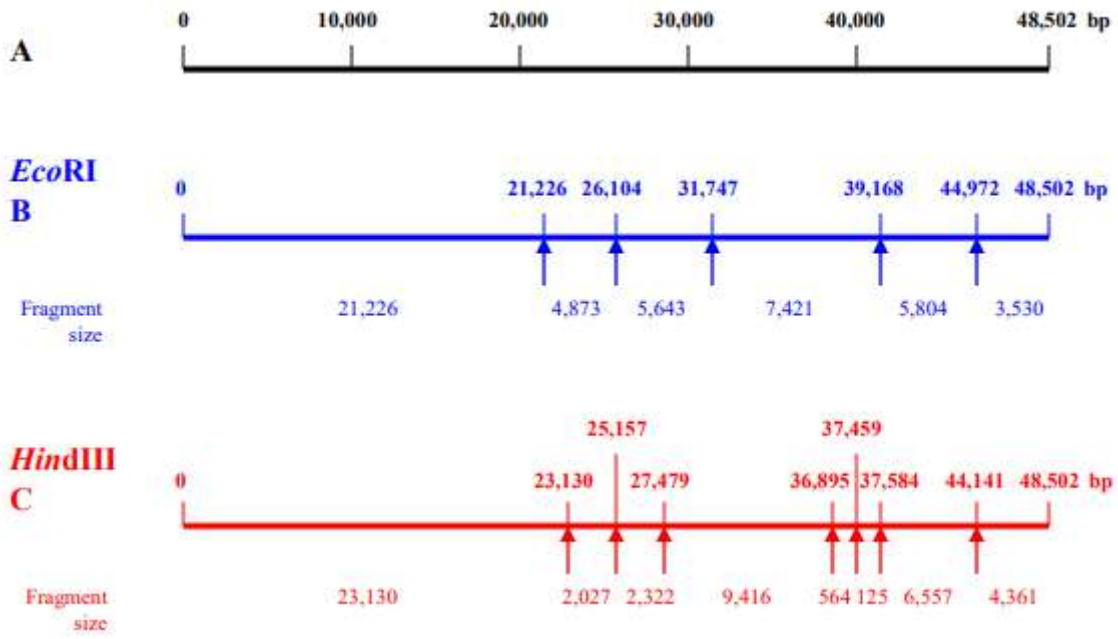
Restriction mapping is a method used to map an unknown segment of DNA by breaking it into pieces and then identifying the locations of the breakpoints. This method relies upon the use of proteins called restriction enzymes, which can cut, or digest, DNA molecules at short, specific sequences called restriction sites. After a DNA segment has been digested using a restriction enzyme, the resulting fragments can be examined using a laboratory method called gel electrophoresis, which is used to separate pieces of DNA according to their size.

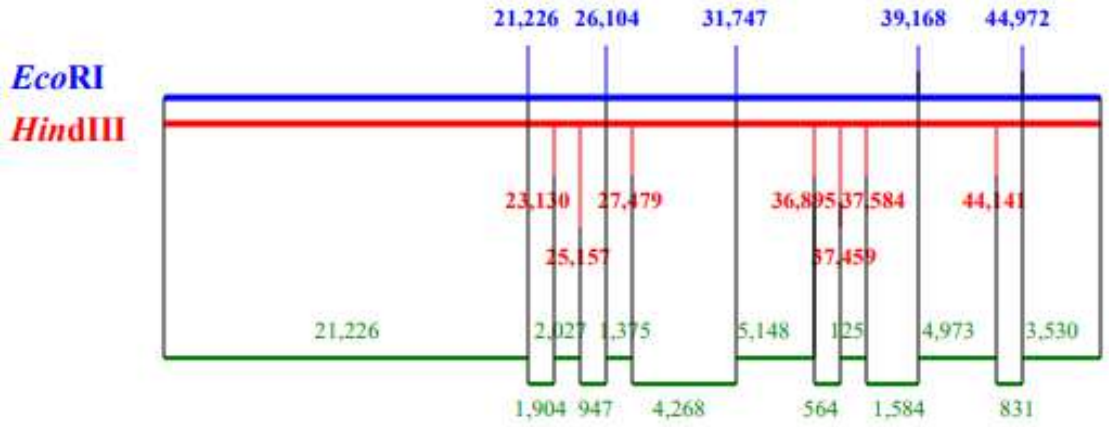
Objectives:

a) To cut the DNA into pieces

b) To construct a restriction map for the DNA of interest

The linear map of phage λ (lambda) DNA:




21,226
26,104
31,747
39,168
44,972
EcoRI
HindIII
23,130
27,479
36,895
37,584
44,141
25,157
37,459
21,226
2,027
1,375
5,148
125
4,973
3,530
1,904
947
4,268
564
1,584
831

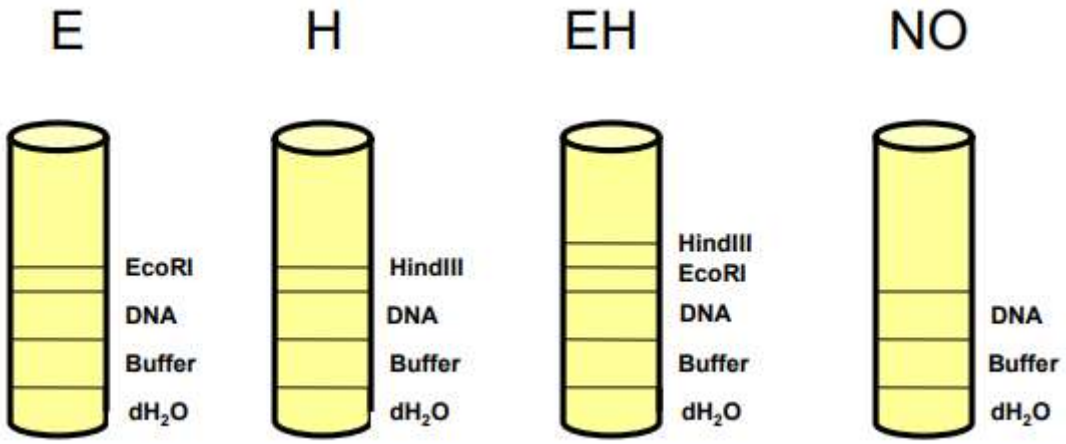

21,226
26,104
31,747
39,168
44,972
EcoRI
HindIII
23,130
27,479
36,895
37,584
44,141
25,157
37,459
21,226
2,027
1,375
5,148
125
4,973
3,530
1,904
947
4,268
564
1,584
831

EcoRI	*HindIII*
21226	23130
7421	9416
5804/5643	6557
4873	4361
3530	2322
	2027

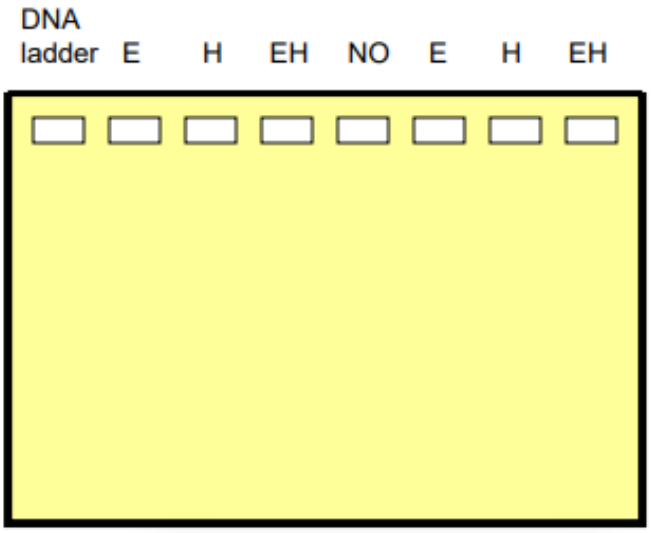
EcoRI + HindIII	No enzyme control
21226	48502
5148/4973	
4268	
3530	
2027	
1904	
1584	
1373	
947	
831	

Procedure:

1. Prepare digestion reactions as described in the Lab Manual.



2. Incubate at 37°C for 45 min.
3. Add 5 µL of gel loading dye to each tube; mix well and centrifuge briefly.
4. Load on gel.



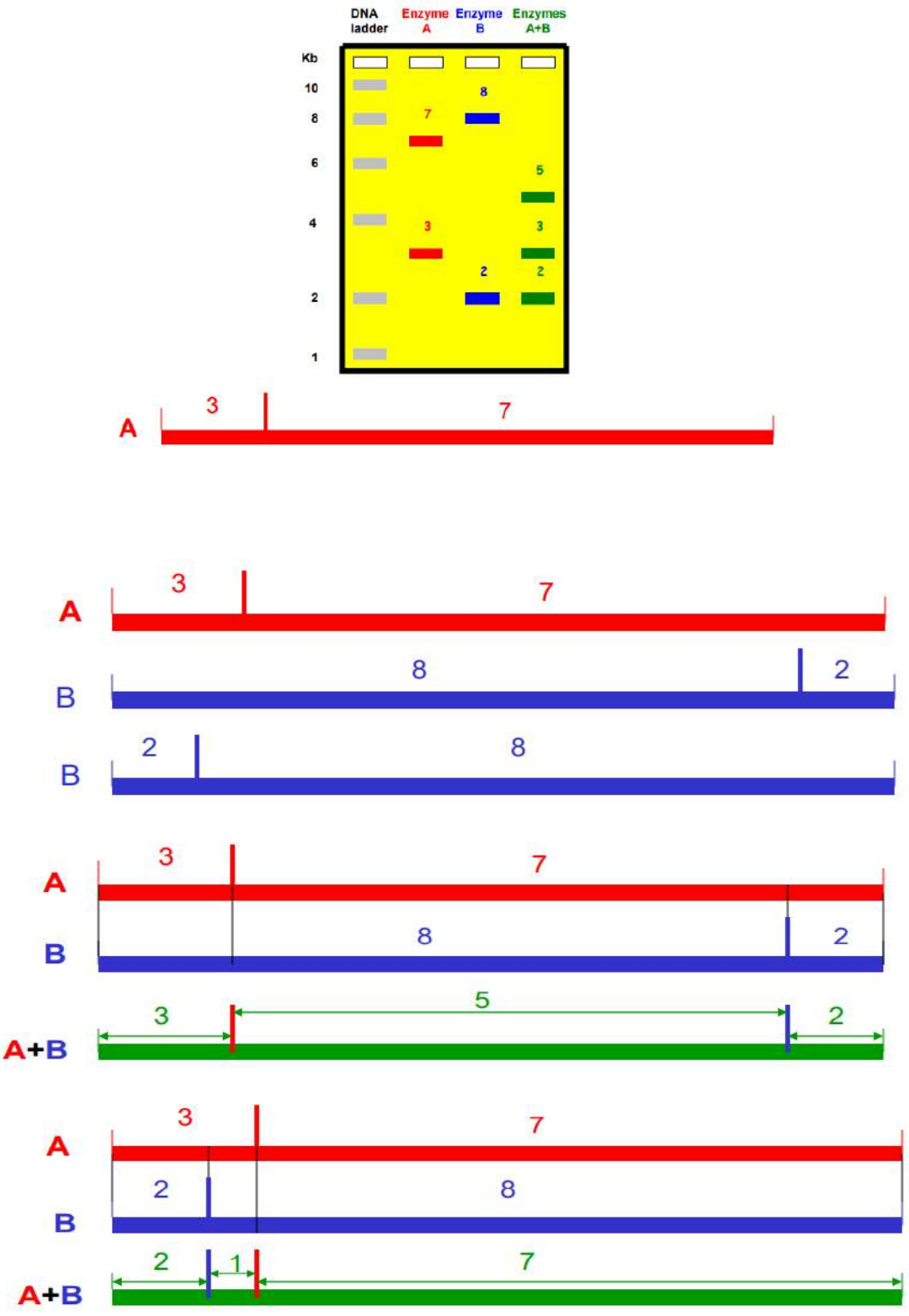
5. Electrophorese for 40 min at 90 volts.

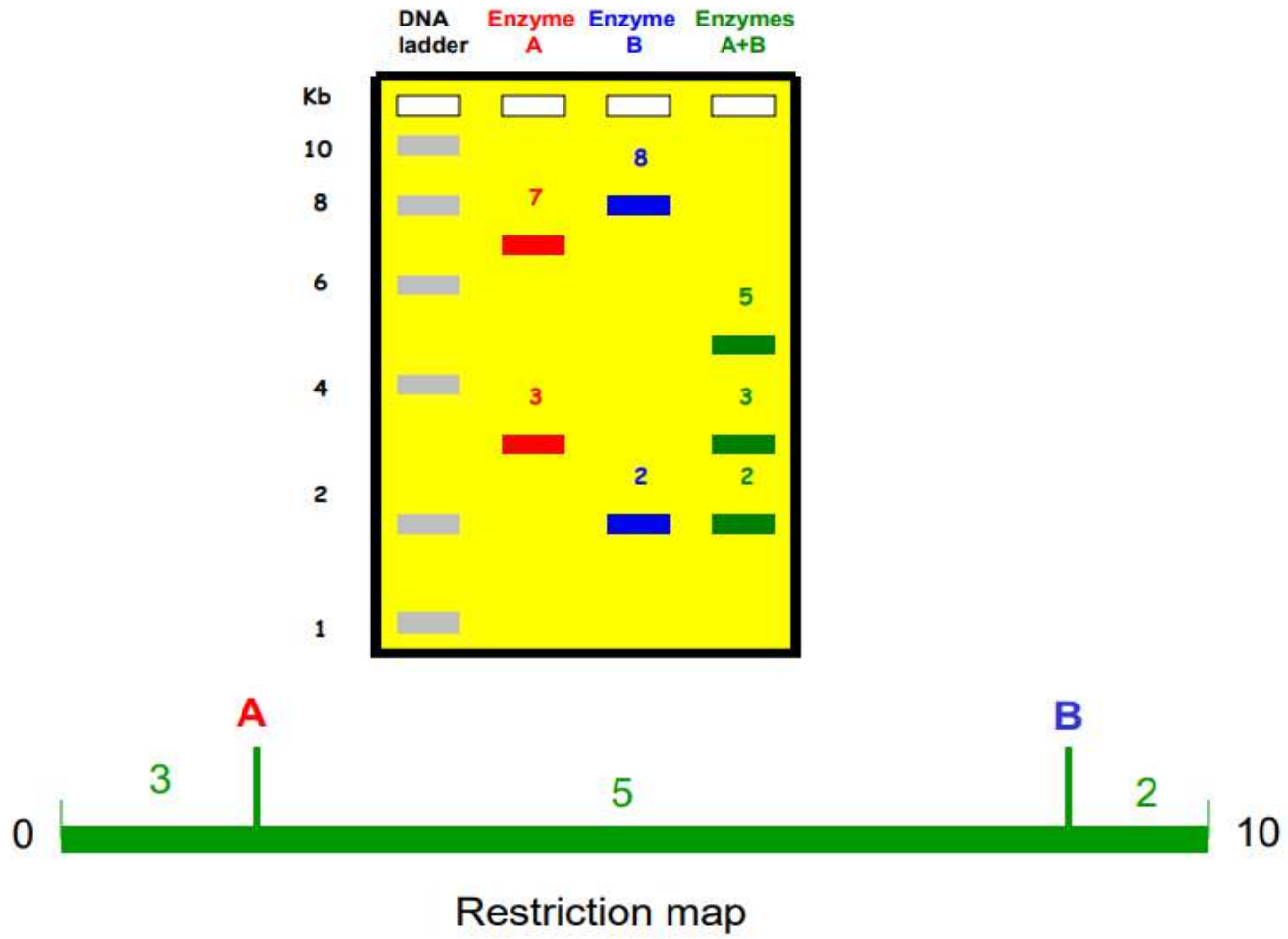
6. Photograph the gel and identify the bands; the results must be consistent with the figure shown on right.

Construction of Restriction Map:

We have a piece of linear DNA of unknown size. We need to digest the DNA once with enzyme A, once with enzyme B, and once with enzymes A and B and draw the restriction

map of the DNA.





STUDY OF MOLECULAR TECHNIQUES

1. Southern Blotting Hybridization :

Principle:

A Southern blot is a method used in molecular biology for the detection of a specific DNA sequence in a mixture of DNA samples. Southern blotting combines restriction digestion of DNA sample, gel electrophoresis, transfer of electrophoresis-separated DNA fragments to a filter membrane, denaturation in order to obtain single stranded DNA and subsequent fragment detection by radio-labeled probe hybridization.

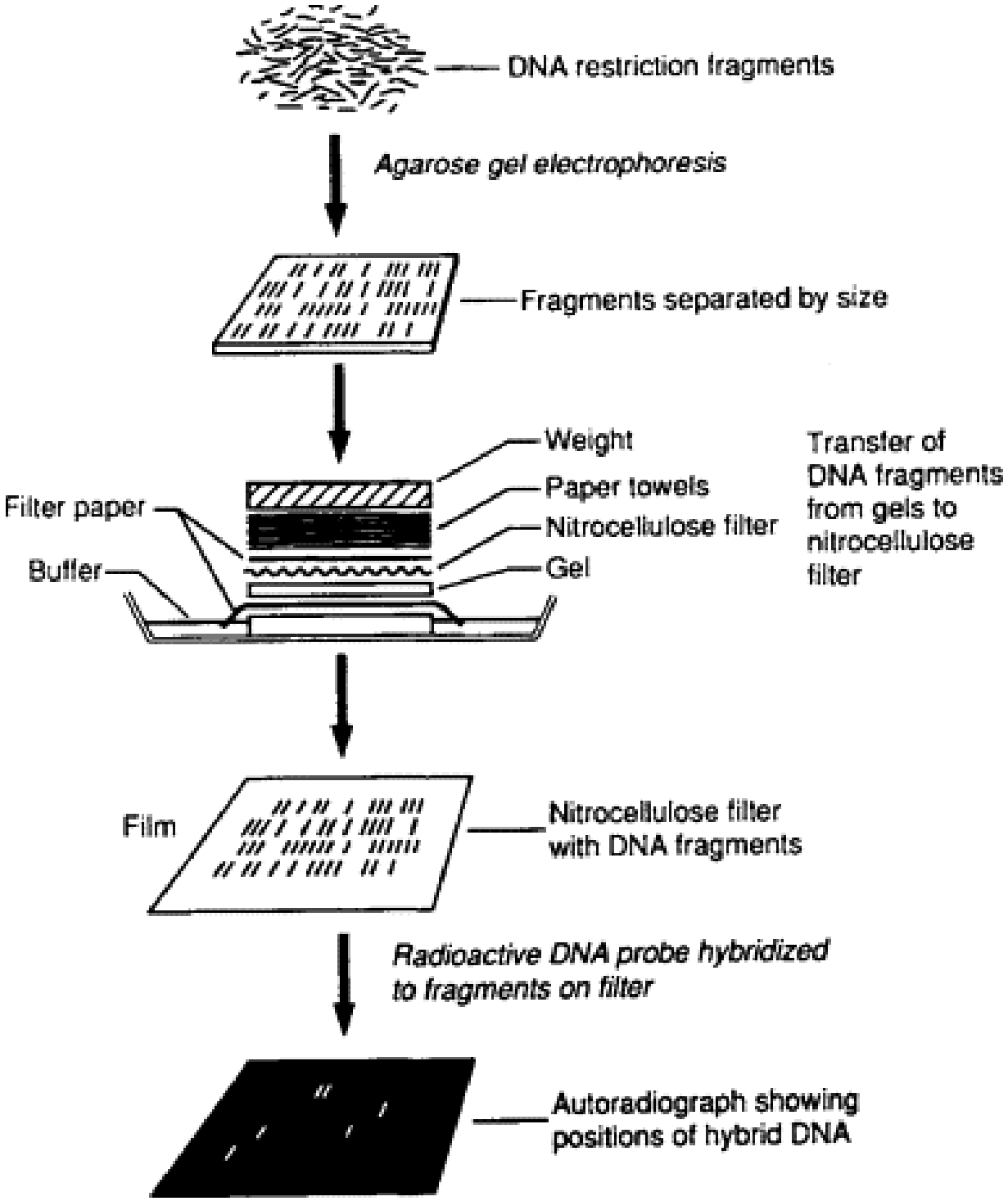
Procedure :

1. Restriction endonucleases are used to cut high-molecular-weight DNA strands into smaller fragments.

2. The DNA fragments are then electrophoresed on an agarose gel to separate them by size.
{If some of the DNA fragments are larger than 15 kb, then prior to blotting, the gel may be treated with an acid, such as dilute HCl. This depurinates the DNA fragments, breaking the DNA into smaller pieces, thereby allowing more efficient transfer from the gel to membrane.}

3. The DNA gel is placed into an alkaline solution (typically containing sodium hydroxide) to denature the double-stranded DNA.
The denaturation in an alkaline environment may improve binding of the negatively charged thymine residues of DNA to a positively charged amino groups of membrane, separating it into single DNA strands for later hybridization to the probe (see below), and destroys any residual RNA that may still be present in the DNA.

4. A sheet of nitrocellulose (or, alternatively, nylon) membrane is placed on top of the gel. Pressure is applied evenly to the gel (either using suction, or by placing a stack of paper towels and a weight on top of the membrane and gel).
{If transferring by suction, 20X SSC buffer is used to ensure a seal and prevent drying of the gel. Buffer transfer by capillary action from a region of high water potential to a region of low water potential (usually filter paper and paper tissues) is then used to move the DNA from the gel onto the membrane; ion exchange interactions bind the DNA to the membrane due to the negative charge of the DNA and positive charge of the membrane.}



5. The membrane is then baked in a vacuum or regular oven at 80 °C for 2 hours or exposed to ultraviolet radiation to permanently attach the transferred DNA to the membrane.

6. The membrane is then exposed to a radio-labeled hybridization probe—a single DNA fragment with a specific sequence whose presence in the target DNA is to be determined.

7. After hybridization, excess probe is washed from the membrane (typically using SSC buffer), and the pattern of hybridization is visualized on X-ray film by autoradiography in the case of a radioactive or fluorescent probe.

Result :

Hybridization of the probe to a specific DNA fragment on the filter membrane indicates that this fragment contains DNA sequence that is complementary to the probe. The transfer step of the DNA from the electrophoresis gel to a membrane permits easy binding of the labeled hybridization probe to the size-fractionated DNA. It also allows for the fixation of the target-probe hybrids, required for analysis by autoradiography.

Application :

1. SBH is used to screen a DNA library, or other collections of cloned DNA fragments.
2. Sequences that hybridize with the hybridization probe are further analysed, for example, to obtain the full length sequence of the targeted gene.
3. Southern blotting can also be used to identify methylated sites in particular genes.
4. SBH is used in DNA fingerprinting.
5. SBH is used in disease detection.

2. Northern Blotting Hybridization :

Principle:

The northern blot, or RNA blot, is a technique used in molecular biology research to study gene expression by detection of RNA (or isolated mRNA) in a sample. Northern blotting involves the use of electrophoresis to separate RNA samples by size, capillary transfer of RNA from the electrophoresis gel to the blotting membrane and detection with a hybridization probe complementary to part of or the entire target sequence.
Northern blotting takes its name from its similarity to the first blotting technique, the Southern blot, named for biologist Edwin Southern. The major difference is that RNA, rather than DNA, is analyzed in the northern blot.

Procedure :

1. A general blotting procedure starts with extraction of total RNA from a homogenized tissue sample or from cells.
2. RNA samples are then separated according to their size by agarose gel electrophoresis with gels containing formaldehyde as a denaturing agent for the RNA to limit secondary structure. {The gels can be stained with ethidium bromide (EtBr) and viewed under UV light to observe the quality and quantity of RNA before blotting. Polyacrylamide gel electrophoresis with urea can also be used in RNA separation but it is most commonly used for fragmented RNA or microRNAs.
3. The RNA samples, now separated by size, are transferred to a nylon membrane through a capillary or vacuum blotting system.
4. Once the RNA has been transferred to the membrane, it is immobilized through covalent linkage to the membrane by UV light or heat.
5. A labeled probe is then hybridized to the RNA on the membrane.
6. The membrane is washed to ensure that the probe has bound specifically to the target RNA.
7. The hybrid signals are then detected by X-ray film and can be quantified by densitometry.

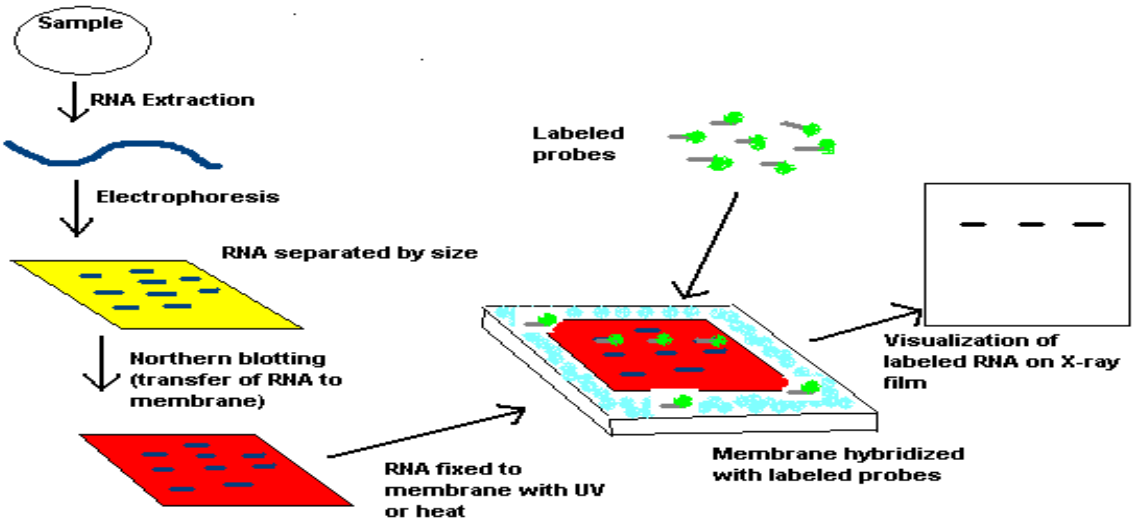


Figure : Flow diagram outlining the general procedure for RNA detection by northern blotting.

Application :

1. NBH is used to observe a particular gene's expression pattern between tissues, organs, developmental stages, environmental stress levels, pathogen infection, and over the course of treatment.
2. NBH is used to show overexpression of oncogenes and downregulation of tumor-suppressor genes in cancerous cells when compared to 'normal' tissue.
3. Since the RNA is first separated by size, the variance in size of a gene product can also indicate deletions or errors in transcript processing.
4. By altering the probe target used along the known sequence it is possible to determine which region of the RNA is missing.

3. Western Blotting Hybridization :

Principle:

The western blot (sometimes called the protein immunoblot), or western blotting, is a widely used technique in molecular biology and immunogenetics to detect specific proteins in a sample of tissue homogenate or extract. The western blot method is composed of a gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide, followed by an electrophoretic transfer onto a membrane (mostly PVDF or Nitrocellulose) and an immunostaining procedure to visualize a certain protein on the blot membrane.

{The sample undergoes protein denaturation, followed by gel electrophoresis. A synthetic or animal-derived antibody (known as the primary antibody) is created that recognises and binds to a specific target protein. The electrophoresis membrane is washed in a solution containing the primary antibody. Excess antibody is washed off. A secondary antibody is added which recognises and binds to the primary antibody. The secondary antibody is visualised through various methods such as staining, immunofluorescence, and radioactivity, allowing indirect detection of the specific target protein.}

Procedure :

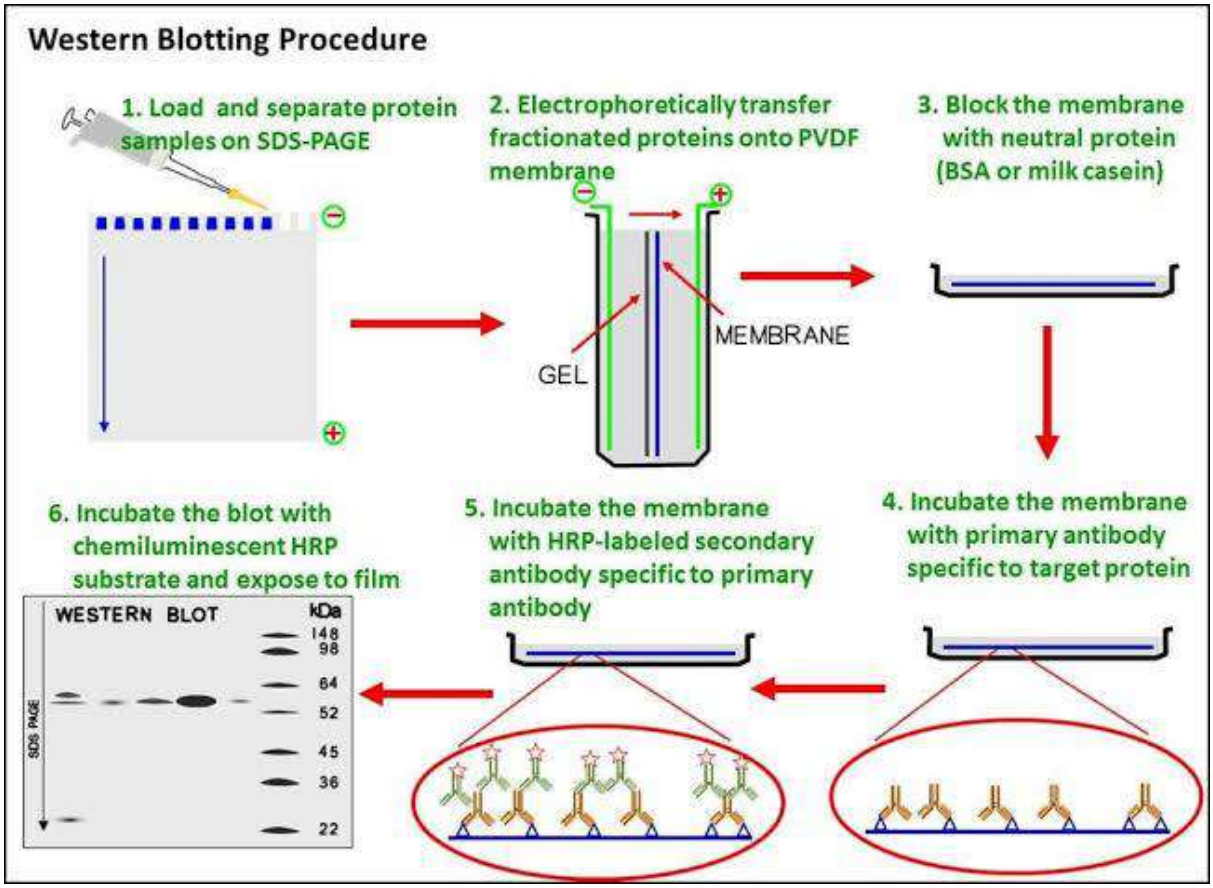
1. Protein samples are often boiled to denature the proteins present. This ensures that proteins

are separated based on size and prevents proteases (enzymes that break down proteins) from degrading samples.

2. Protein samples are then electrophoresed to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide. SDS-PAGE is generally used for the denaturing electrophoretic separation of proteins.
{SDS is generally used as a buffer (as well as in the gel) in order to give all proteins present a uniform negative charge, since proteins can be positively, negatively, or neutrally charged.

3. Following electrophoretic separation, the proteins are transferred to a membrane (typically nitrocellulose or PVDF), where they are blocked with milk (or other blocking agents) to prevent non-specific antibody binding.

4. After blocking, a solution of primary antibody (generally between 0.5 and 5 micrograms/mL) diluted in either PBS or TBST wash buffer is incubated with the membrane for typically an hour at room temperature, or overnight at 4°C. The antibody solution is incubated with the membrane for anywhere from 30 minutes to overnight.



5. After rinsing the membrane to remove unbound primary antibody, the membrane is exposed to another antibody known as the secondary antibody.

{To allow detection of the target protein, the secondary antibody is commonly linked to biotin or a reporter enzyme such as alkaline phosphatase or horseradish peroxidase. Horseradish peroxidase (HRP) is commonly linked to secondary antibodies to allow the detection of the target protein by chemiluminescence.}

6. After the unbound probes are washed away, the western blot is ready for detection of the probes that are labeled and bound to the protein of interest.

{The colorimetric detection method depends on incubation of the western blot with a substrate that reacts with the reporter enzyme (such as peroxidase) that is bound to the secondary antibody. This converts the soluble dye into an insoluble form of a different color that precipitates next to the enzyme and thereby stains the membrane.

Chemiluminescent detection methods depend on incubation of the western blot with a substrate that will luminesce when exposed to the reporter on the secondary antibody. The light is then detected by CCD cameras which capture a digital image of the western blot or

photographic film.}

Application :

1. The western blot is extensively used in biochemistry for the qualitative detection of single proteins and protein-modifications (such as post-translational modifications).
2. It is used as a general method to identify the presence of a specific single protein within a complex mixture of proteins.
3. A semi-quantitative estimation of a protein can be derived from the size and color intensity of a protein band on the blot membrane.
4. The western blot is routinely used for verification of protein production after cloning.
5. It is also used in medical diagnostics, e.g., in the HIV test or BSE-Test.

DNA Sequencing (Sanger's Method)

DNA sequencing is the process of determining the sequence of nucleotide bases (As, Ts, Cs, and Gs) in a piece of DNA. Today, with the right equipment and materials, sequencing a short piece of DNA is relatively straightforward.
Sequencing an entire genome (all of an organism’s DNA) remains a complex task. It requires breaking the DNA of the genome into many smaller pieces, sequencing the pieces, and assembling the sequences into a single long "consensus." However, genome sequencing is now much faster and less expensive than it was during the Human Genome Project.

Introduction:

Regions of DNA up to about 900 base pairs in length are routinely sequenced using a method called **Sanger sequencing** or the **chain termination method**. Sanger sequencing was developed by the British biochemist Fred Sanger and his colleagues in 1977.

In the Human Genome Project, Sanger sequencing was used to determine the sequences of many relatively small fragments of human DNA. (These fragments weren't necessarily 900 bp or less, but researchers were able to "walk" along each fragment using multiple rounds of Sanger sequencing.) The fragments were aligned based on overlapping portions to assemble the sequences of larger regions of DNA and, eventually, entire chromosomes.

Although genomes are now typically sequenced using other methods that are faster and less expensive, Sanger sequencing is still in wide use for the sequencing of individual pieces of DNA, such as fragments used in DNA cloning or generated through polymerase chain reaction (PCR).

Principle:

Sanger sequencing, also known as the “chain termination method”, is a method for determining the nucleotide sequence of DNA. The DNA sequence of interest is used as a template for a special type of PCR called chain-termination PCR. Chain-termination PCR works just like standard PCR, but with one major difference : the addition of modified nucleotides (dNTPs) called dideoxyribonucleotides (ddNTPs).
In chain-termination PCR, a low ratio of chain-terminating ddNTPs is mixed with the normal dNTPs in the PCR reaction. ddNTPs lack the 3'-OH group required for phosphodiester bond formation; therefore, when DNA polymerase incorporates a ddNTP at random, extension ceases. The result of chain-termination PCR is millions to billions of oligonucleotide copies of the DNA sequence of interest, terminated at a random lengths (n) by 5’-ddNTPs.

Ingredients for Sanger sequencing:

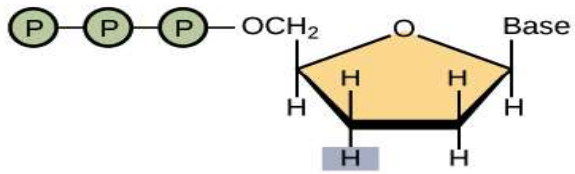
Sanger sequencing involves making many copies of a target DNA region. Its ingredients are similar to those needed for DNA replication in an organism, or for polymerase chain reaction (PCR), which copies DNA *in vitro*.
They include:

1. A DNA polymerase enzyme:
2. A **primer**, which is a short piece of single-stranded DNA that binds to the template DNA and acts as a "starter" for the polymerase

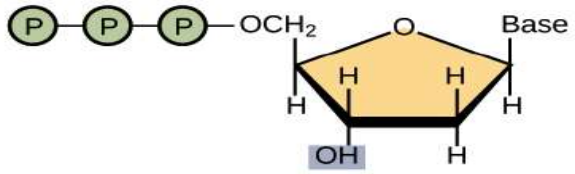
3. The four DNA nucleotides (dATP, dTTP, dCTP, dGTP)
The template DNA to be sequenced

However, a Sanger sequencing reaction also contains a unique ingredient:

4. Dideoxy, or **chain-terminating**, versions of all four nucleotides (ddATP, ddTTP, ddCTP, ddGTP), each labeled with a different color of dye



Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)

Dideoxy nucleotides are similar to regular, or deoxy, nucleotides, but with one key difference: they lack a hydroxyl group on the 3’ carbon of the sugar ring. In a regular nucleotide, the 3’ hydroxyl group acts as a “hook," allowing a new nucleotide to be added to an existing chain.

Once a dideoxy nucleotide has been added to the chain, there is no hydroxyl available and no further nucleotides can be added. The chain ends with the dideoxy nucleotide, which is marked with a particular color of dye depending on the base (A, T, C or G) that it carries.

Method of Sanger sequencing

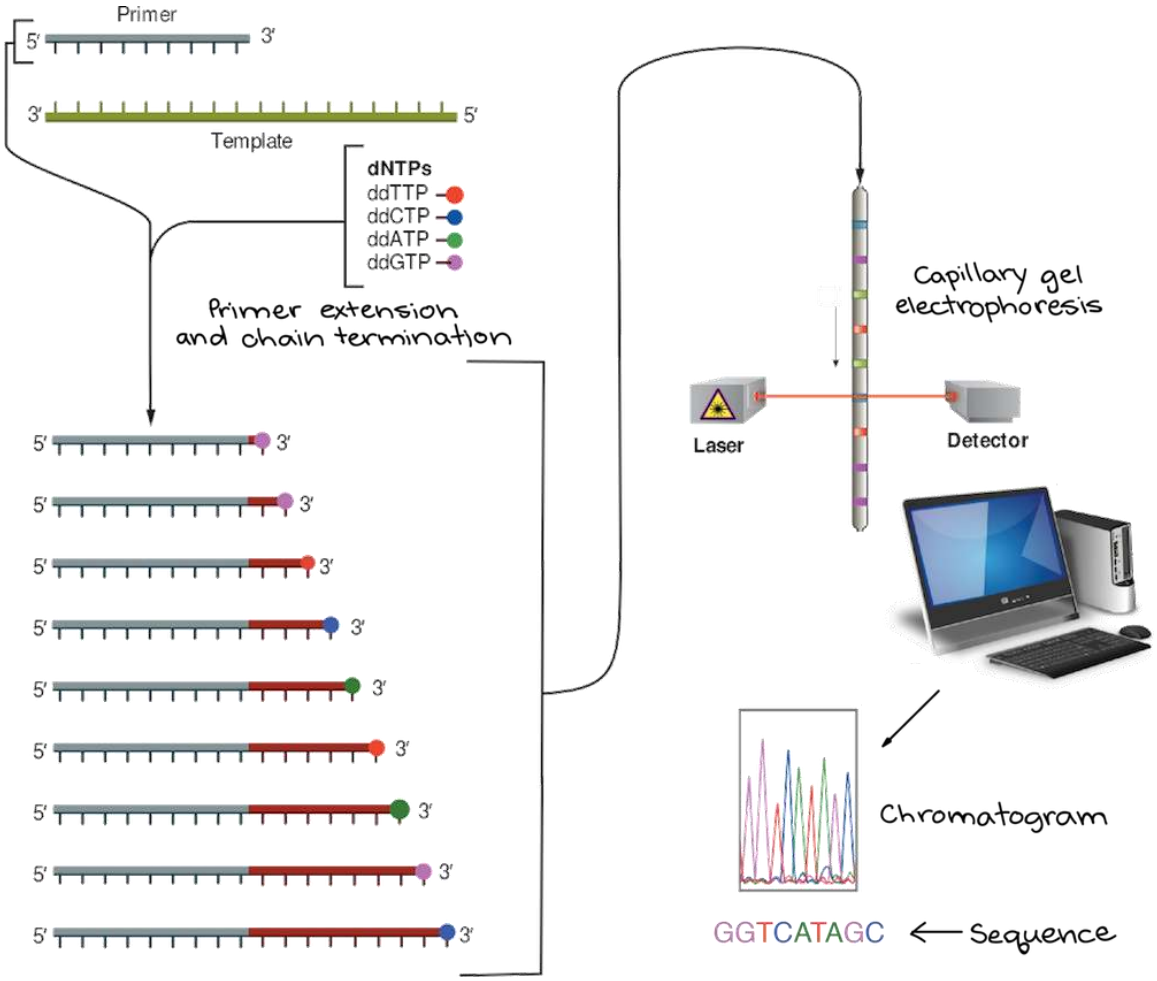
The DNA sample to be sequenced is combined in a tube with primer, DNA polymerase, and DNA nucleotides (dATP, dTTP, dGTP, and dCTP). The four dye-labeled, chain-terminating dideoxy nucleotides are added as well, but in much smaller amounts than the ordinary nucleotides.

The mixture is first heated to denature the template DNA (separate the strands), then cooled so that the primer can bind to the single-stranded template. Once the primer has bound, the temperature is raised again, allowing DNA polymerase to synthesize new DNA starting from

the primer. DNA polymerase will continue adding nucleotides to the chain until it happens to add a dideoxy nucleotide instead of a normal one. At that point, no further nucleotides can be added, so the strand will end with the dideoxy nucleotide.

This process is repeated in a number of cycles. By the time the cycling is complete, it's virtually guaranteed that a dideoxy nucleotide will have been incorporated at every single position of the target DNA in at least one reaction. That is, the tube will contain fragments of different lengths, ending at each of the nucleotide positions in the original DNA (see figure below). The ends of the fragments will be labeled with dyes that indicate their final nucleotide.

After the reaction is done, the fragments are run through a long, thin tube containing a gel matrix in a process called **capillary gel electrophoresis**. Short fragments move quickly through the pores of the gel, while long fragments move more slowly. As each fragment crosses the "finish line" at the end of the tube, it's illuminated by a laser, allowing the attached dye to be detected.



The smallest fragment (ending just one nucleotide after the primer) crosses the finish line first, followed by the next-smallest fragment (ending two nucleotides after the primer), and so forth. Thus, from the colors of dyes registered one after another on the detector, the sequence of the original piece of DNA can be built up one nucleotide at a time. The data recorded by the detector consist of a series of peaks in fluorescence intensity, as shown in the **chromatogram** above. The DNA sequence is read from the peaks in the chromatogram.

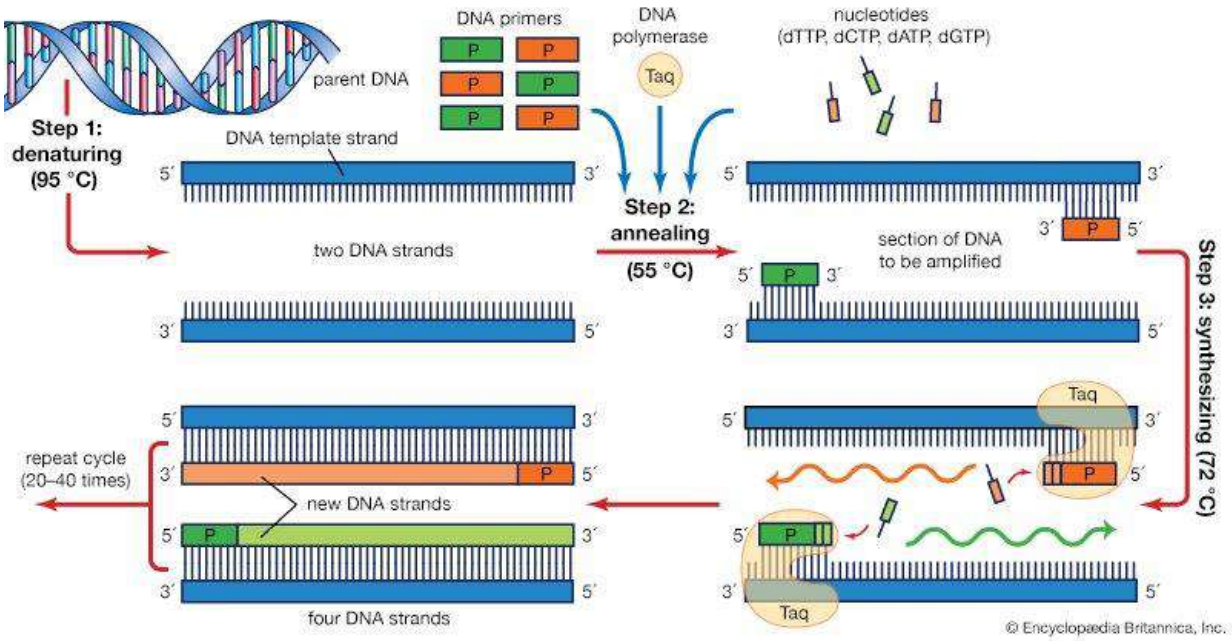
Uses and limitations:

Sanger sequencing gives high-quality sequence for relatively long stretches of DNA (up to about 900 base pairs). It's typically used to sequence individual pieces of DNA, such as bacterial plasmids or DNA copied in PCR.
However, Sanger sequencing is expensive and inefficient for larger-scale projects, such as the sequencing of an entire genome or metagenome (the “collective genome” of a microbial community). For tasks such as these, new, large-scale sequencing techniques are faster and less expensive.

Polymerase Chain Reaction (PCR) :

Principle:

PCR is an artificial technique used in the lab to make multiple copies of a particular section of DNA quickly and accurately. The principle of PCR technique is based on the natural processes by which a cell replicate a new DNA strand. PCR is carried out in repeated cycles of a three-step process -
1. Denaturing – when the double-stranded template DNA is heated to separate it into two single strands.
2. Annealing – when the temperature is lowered to enable the DNA primers to attach to the template DNA.
3. Extending – when the temperature is raised and the new strand of DNA is made by the Taq polymerase enzyme.



PCR involves a process of heating and cooling called thermal cycling which is carried out by machine. Only a few biological ingredients are needed for PCR. These are:

1. DNA template : The DNA segment to be copied.

2. Primers : Short stretches of DNA that initiate the PCR reaction, designed to bind to either side of the section of DNA we want to copy

3. DNA nucleotide precursors (dNTPs) : DNA bases (A, C, G and T) are the building blocks of DNA and are needed to construct the new strand of DNA

4. Taq polymerase enzyme : To add the new DNA bases at the 3' ends of the primers.

5. Buffer : To ensure the right conditions for the reaction.

Procedure :

1. Denaturation :

The initial step is the denaturation, or separation, of the two strands of the DNA molecule. This is accomplished by heating the starting material to temperatures of about 95 °C (203 °F). Each strand is a template on which a new strand is built.

{A. During this stage the cocktail containing the template DNA and all the other core ingredients is heated to 94-95ºC.

B. The high temperature causes the hydrogen bonds between the bases in two strands of template DNA to break and the two strands to separate.

C. This results in two single strands of DNA, which will act as templates for the production of the new strands of DNA.

D. It is important that the temperature is maintained at this stage for long enough to ensure that the DNA strands have separated completely.

E. This usually takes between 15-30 seconds.}

2. Annealing :

In the second step the temperature is reduced to about 55 °C (131 °F) so that the primers can anneal to the template.

{A. During this stage the reaction is cooled to 50-65ºC. This enables the primers to attach to a specific location on the single-stranded template DNA by way of hydrogen bonding (the exact temperature depends on the melting temperature of the primers we are using).

B. Primers are single strands of DNA or RNA sequence that are around 20 to 30 bases in length.

C. The primers are designed to be complementary in sequence to short sections of DNA on each end of the sequence to be copied.

D. Primers serve as the starting point for DNA synthesis. The polymerase enzyme can only add DNA bases to a double strand of DNA. Only once the primer has bound can the polymerase enzyme attach and start making the new complementary strand of DNA from the loose DNA bases.

E. The two separated strands of DNA are complementary and run in opposite directions (from one end - the 5’ end – to the other - the 3’ end); as a result, there are two primers – a forward primer and a reverse primer.

F. This step usually takes about 10-30 seconds.}

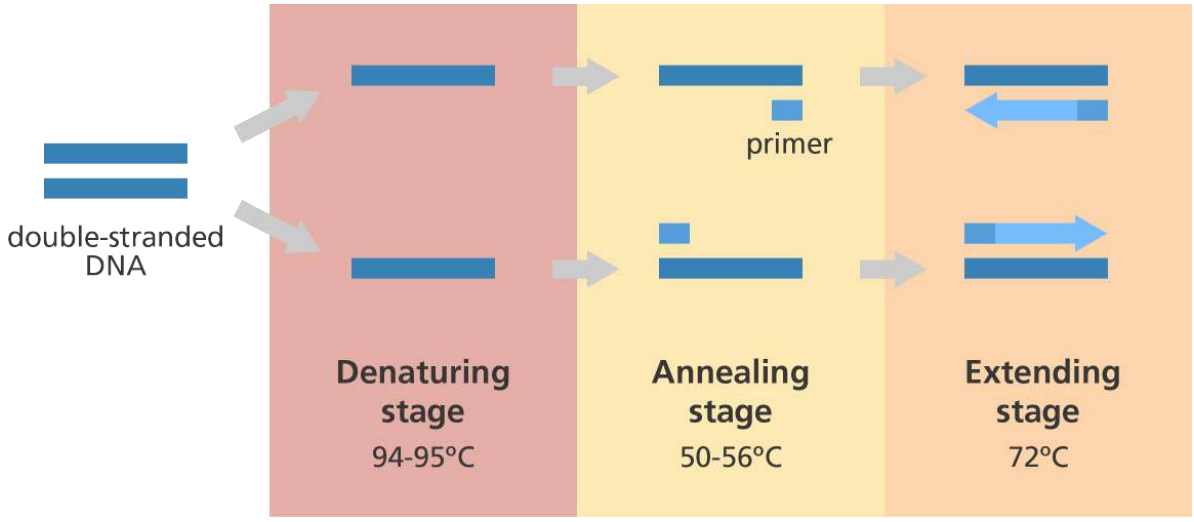


Figure : Figure : Illustration showing the main steps in the polymerase chain reaction (PCR).

3. Chain Extension :

In the third step the temperature is raised to about 72 °C (162 °F), and the DNA polymerase begins adding nucleotides onto the ends of the annealed primers.

A. During this final step, the heat is increased to 72ºC to enable the new DNA to be made by a special Taq DNA polymerase enzyme which adds DNA bases.

B. Taq DNA polymerase is an enzyme taken from the heat-loving bacteriaThermus aquaticus (This bacteria normally lives in hot springs so can tolerate temperatures above 80ºC).

C. 72ºC is the optimum temperature for the Taq polymerase to build the complementary strand. It attaches to the primer and then adds DNA bases to the single strand one-by-one in the 5’ to 3’ direction.

D. The result is a brand new strand of DNA and a double-stranded molecule of DNA.

E. The duration of this step depends on the length of DNA sequence being amplified but usually takes around one minute to copy 1,000 DNA bases (1Kb).

These three processes of thermal cycling are repeated 20-40 times to produce lots of copies of the DNA sequence of interest. The new fragments of DNA that are made during PCR also serve as templates to which the DNA polymerase enzyme can attach and start making DNA. The result is a huge number of copies of the specific DNA segment produced in a relatively

short period of time. The polymerase chain reaction enables investigators to obtain the large quantities of DNA that are required for various experiments and procedures in molecular biology, forensic analysis, evolutionary biology, and medical diagnostics.

In the original PCR procedure, one problem was that the DNA polymerase had to be replenished after every cycle because it is not stable at the high temperatures needed for denaturation. This problem was solved in 1987 with the discovery of a heat-stable DNA polymerase called Taq, an enzyme isolated from the thermophilic bacterium Thermus aquaticus, which inhabits hot springs. Taq polymerase also led to the invention of the PCR machine.

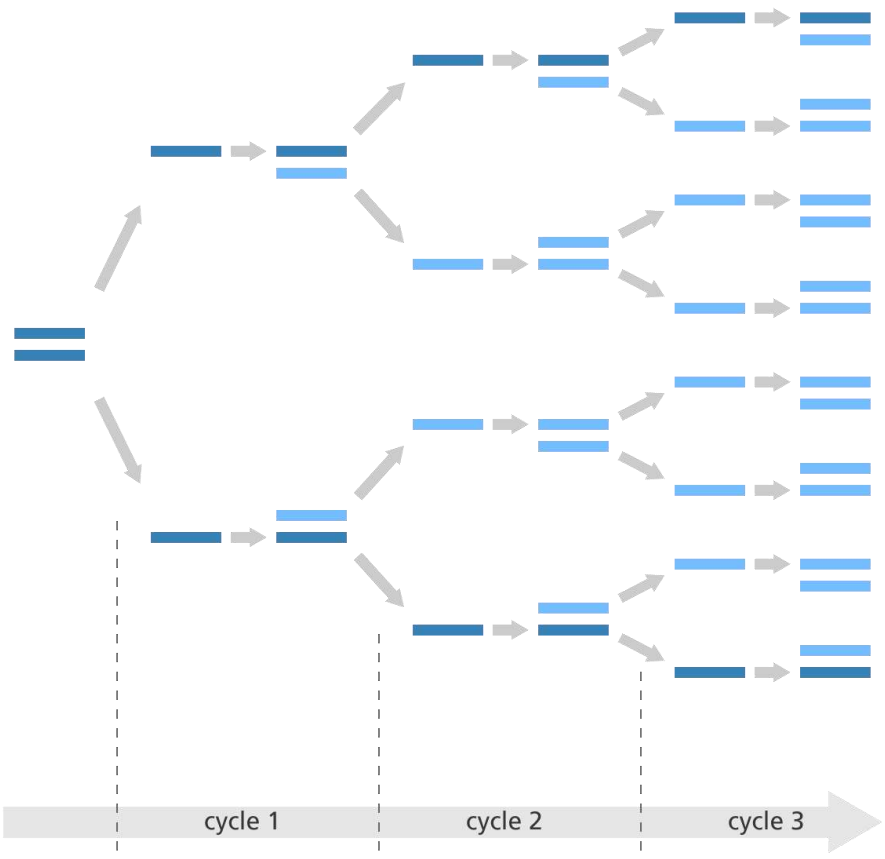


Figure : Illustration showing how the polymerase chain reaction (PCR) produces lots of copies of DNA.

Applications :

1. Once amplified, the DNA produced by PCR can be used in many different laboratory

procedures. For example, most mapping techniques in the Human Genome Project (HGP) relied on PCR.

2. PCR is also valuable in a number of laboratory and clinical techniques, including DNA fingerprinting, detection of bacteria or viruses (particularly AIDS), and diagnosis of genetic disorders.

3. PCR is a common tool used in medical and biological research labs. It is used in the early stages of processing DNA for sequencing.

4. PCR is used for detecting the presence or absence of a gene to help identify pathogens during infection, and when generating forensic DNA profiles from tiny samples of DNA.

DNA Fingerprinting- Principle, Methods, Applications

DNA fingerprinting or DNA profiling is a process used to determine the nucleotide sequence at a certain part of the DNA that is unique in all human beings. The process of DNA fingerprinting was invented by Sir Alec Jeffrey at the University of Leicester in 1985.

Principle of DNA Fingerprinting :

i. The DNA of every human being on the planet is 99.9% same. However, about 0.1% of DNA is unique in every individual.
ii. Human genome possesses numerous small non-coding but inheritable sequences of bases which are repeated many times. They do not code for proteins but make-up 95% of our genetic DNA and therefore called the ―junk DNA.
iii. They can be separated as satellite from the bulk DNA during density gradient centrifugation and hence called satellite DNA.
iv. In satellite DNA, repetition of bases is in tandem. Depending upon length, base composition and numbers of tandemly re-petitive units, satellite DNAs have subcat-egories like microsatellites and mini-satellites.
v. Satellite DNAs show polymorphism. The term polymorphism is used when a variant at a locus is present with a frequency of more than 0.01 population.
vi. Variations occur due to mutations. These mutations in the non-coding sequences have piled up with time and form the basis of DNA polymorphism (variation at genetic level arises due to mutations).
vii. The junk DNA regions are thus made-up of length polymorphisms, which show variations in the physical length of the DNA molecule.
viii. At specific loci on the chromosome the number of tandem repeats varies between individuals. There will be a certain number of repeats for any specific loci on the

chromosome.

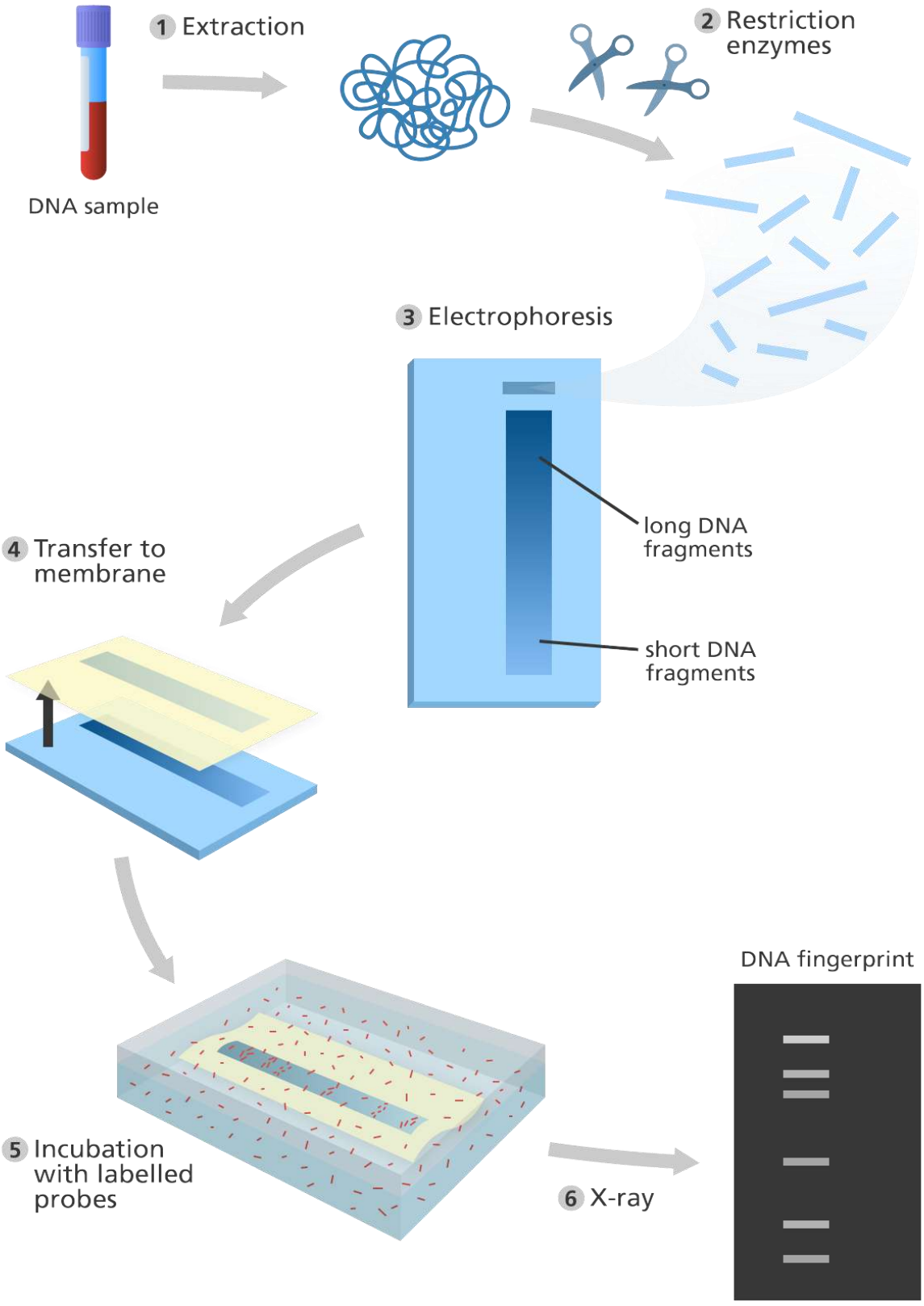
ix. Depending on the size of the repeat, the repeat regions are classified into two groups. Short tandem repeats (STRs) contain 2-5 base pair repeats and variable number of tandem repeats (VNTRs) have repeats of 9-80 base pairs.

x. Since a child receive 50% of the DNA from its father and the other 50% from his mother, so the number VNTRs at a particular area of the DNA of the child will be different may be due to insertion, deletion or mutation in the base pairs.

xi. As a result, every individual has a distinct composition of VNTRs and this is the main principle of DNA fingerprinting.

xii. As single change in nucleotide may make a few more cleavage site of a given nucleotide or might abolish some existing cleavage site.

xiii. Thus, if DNA of any individual is digested with a restriction enzyme, fragments pattern (sizes) will be produced and will be different in cleavage site position. This is the basics of DNA fingerprinting.



Methods of DNA Fingerprinting :

Restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) amplification of short tandem repeats (STRs) are two main DNA tests widely used for DNA fingerprinting.

A. Restriction fragment length polymorphism (RFLP) :

1. The first step in this process is to isolate the DNA from the sample material to be tested. The sample size for RFLP test must be large enough to get the proper result.
2. Once the required size of the sample is available, the DNA is isolated from the sample and is subjected to restriction digestion using restriction enzymes.
3. The digested DNA sample is then separated by agarose gel electrophoresis, in which the DNA is separated based on the size.

4. The next step is transfer of separated DNA from gel slab onto the nitrocellulose membrane to hybridize with a labeled probe that is specific for one VNTR region (radio activity labeled complimentary sequence for VNTR region nucleotide sequence).
5. This technique of transferring and hybridizing DNA onto nitrocellulose membrane is known as southern blotting, a most widely used DNA detection technique by molecular biologists.
6. After the hybridization with the radioactive probes, the X- ray film is developed form the southern blotting and only the areas where the radioactive probe binds will show up on the film.
7. Now these bands when compared with the other known samples, will give the final result of the DNA fingerprinting.

Advantages :
1. The RFLP is considered to be more accurate than the PCR, mainly because the size of the sample used more, use of a fresh DNA sample, and no amplification contamination.

Limitation :
1. The RFLP, however, require longer time period to complete the analysis and is costly.

B. Polymerase Chain Reaction (PCR) amplification of short tandem repeats (STRs) :

1. Thousands of copies of a particular variable region are amplified by PCR which forms the basis of this detection.
2. STR with a known repeat sequence is amplified and separated using gel-electrophoresis.
3. The distance migrated by the STR is examined.
4. For the amplification of STRs using PCR, a short synthetic DNA, called primers are specially designed to attach to a highly conserved common nonvariable region of DNA that flanks the variable region of the DNA.
5. By comparing the STR sequence size amplified by PCR with the other known samples, will give the final result of the DNA fingerprinting.

Advantages :
1. Small amount of specimen is sufficient for the test.
2. Takes a shorter time to complete.
3. Less costly.

Limitation :
Less accurate than RFLP.
Possibility of amplification contamination.

Applications of DNA Fingerprinting :

DNA Fingerprinting is used by scientists to distinguish between individuals of the same species using only samples of their DNA. It is a primary method for identifying an individual.

1. Forensic Science:
Biological materials used for DNA profiling are: Blood, Hair, Saliva, Semen, Body tissue cells etc. DNA isolated from the evidence sample can be compared through VNTR (Variable number of tandem repeats) prototype. It is useful in solving crimes like murder and rape.

2. Paternity and Maternity Determination:

A Person accedes to his or her VNTRs from his or her parents. Parent-child VNTR prototype analysis has been used to solve disputed cases. This information can also be used in inheritance cases, immigration cases.

3. Personal Identification:

It utilizes the concept of using DNA fingerprints as a sort of genetic bar code to pinpoint individuals.

4. Diagnosis of Inherited Disorders:

It is also useful in diagnosing inherited disorders in both prenatal and newborn babies. These disorders may include cystic fibrosis, hemophilia, Huntington's disease, familial Alzheimer's, sickle cell anemia, thalassemia, and many others.

5. Development of Cures for Inherited Disorders:

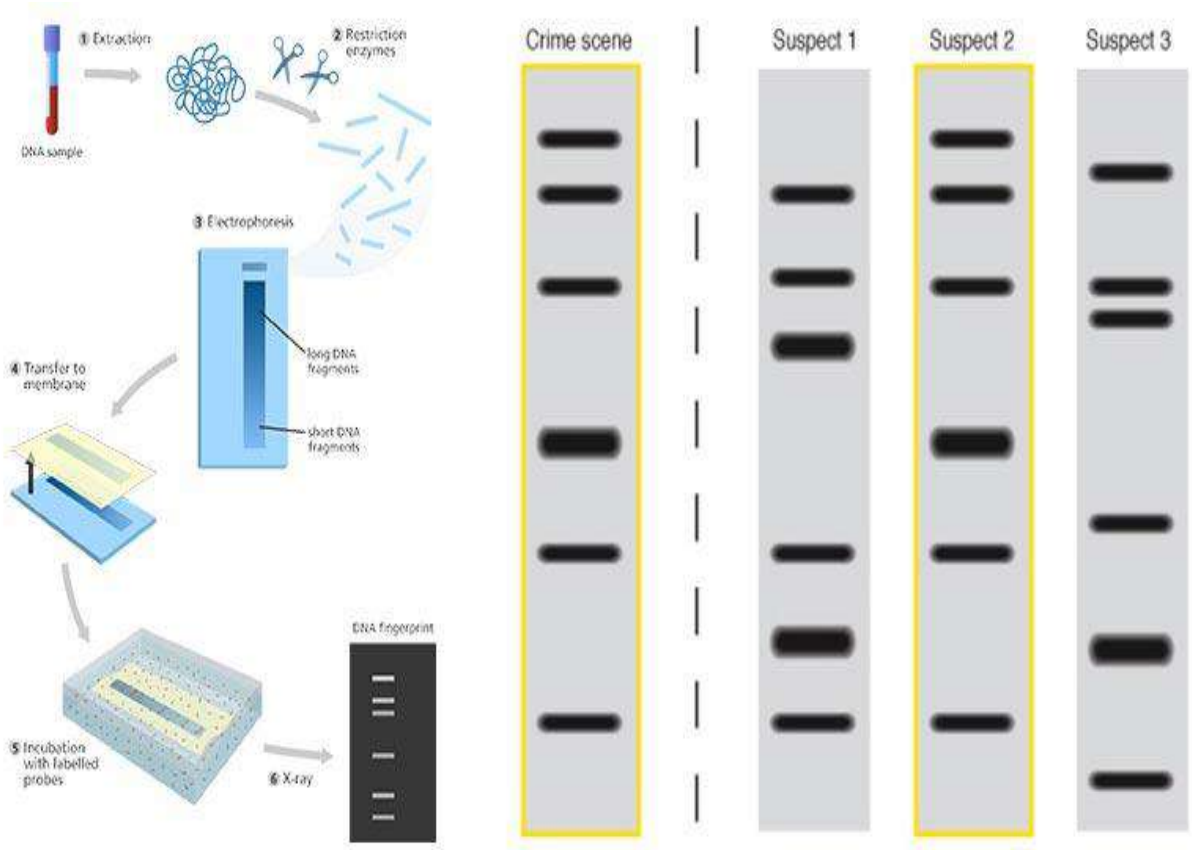
By studying the DNA fingerprints of relatives who have a history of some particular disorder, DNA prototypes associated with the disease can be ascertained.

6. Detection of AIDS:

By comparing the band of HIV "RNA" (converted to DNA using RTPCR) with the bands form by the man's blood, person suffering with AIDS can be identified.

7. Breeding Program:

Breeders conventionally use the phenotype to evaluate the genotype of a plant or an animal.As it is difficult to make out homozygous or heterozygous dominance from appearance, the DNA fingerprinting allows a fastidious and precise determination of genotype. It is basically useful in breeding race horses and hunting dogs.



Animal Cell Culture: Introduction, Types, Methods and Applications

Cell culture refers to the process by which cells are grown in a controlled artificial environment. Cells can be maintained in vitro outside of their original body by this process which is quite simple compared to organ and tissue culture.

In a cell culture technique, cells are removed from an animal or a plant and grown subsequently in a favorable environment. For animal cell culture the cells are taken from the organ of an experimental animal. The cells may be removed directly or by mechanical or enzymatic action. The cells can also be obtained by previously made cell line or cell strain. Examples of cells used to culture are fibroblast, lymphocytes, cells from cardiac and skeletal tissues, cells from liver, breast, skin, and kidney and different types of tumor cells.

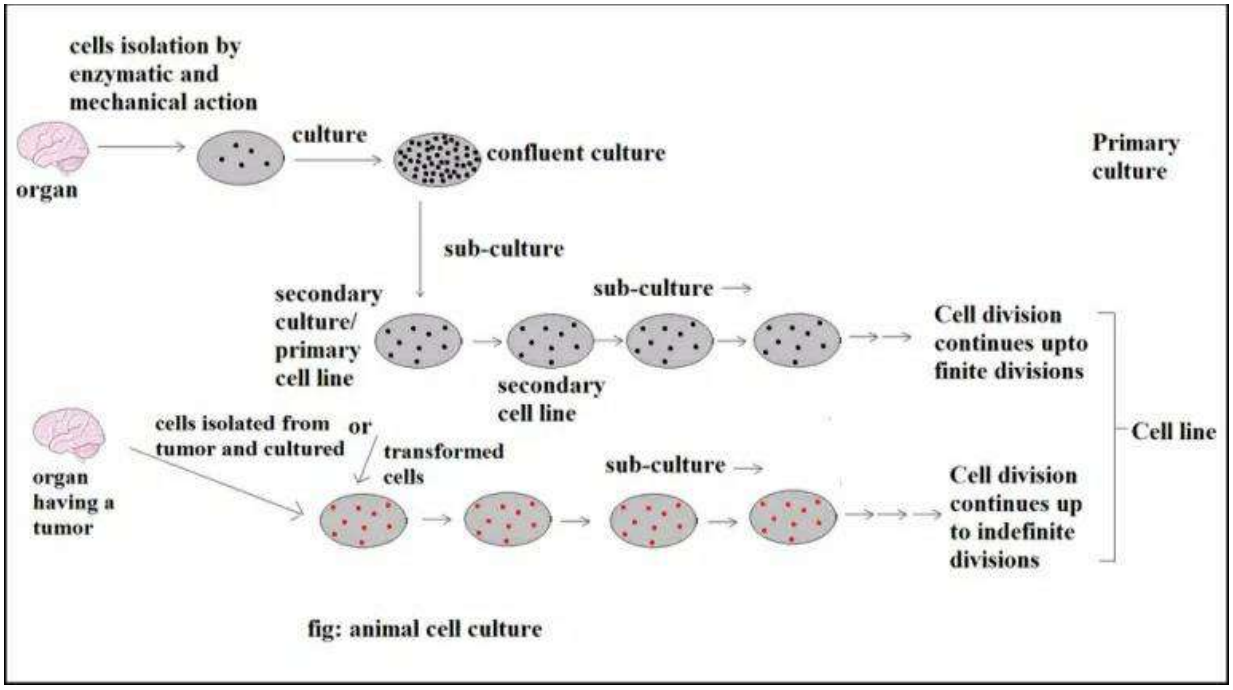


fig: animal cell culture

Types of animal cell culture :

Based on the number of cell division, cell culture can be classified as primary cell culture and cell lines. Cell lines can undergo finite or infinite cell divisions.

Animal cell culture :

A. Primary cell culture :

This is the cell culture obtained straight from the cells of a host tissue. The cells dissociated from the parental tissue are grown on a suitable container and the culture thus obtained is called primary cell culture. Such culture comprises mostly heterogeneous cells and most of the cells divide only for a limited time. However, these cells are much similar to their parents. Depending on their origin, primary cells grow either as an adherent monolayer or in a suspension.

1. Adherent cells :

These cells are anchorage dependent and propagate as a monolayer. These cells need to be attached to a solid or semi-solid substrate for proliferation. These adhere to the culture vessel with the use of an extracellular matrix which is generally derived from tissues of organs that are immobile and embedded in a network of connective tissue. Fibroblasts and epithelial cells are of such types.

When the bottom of the culture vessel is covered with a continuous layer of cells, usually one cell in thickness, these are known as monolayer cultures. Majority of continuous cell lines grow as monolayers. As being single layers, such cells can be transferred directly to a cover slip to examine under microscope.

2. Suspension cells :

Suspension cells do not attach to the surface of the culture vessels. These cells are also called anchorage independent or non-adherent cells which can be grown floating in the culture medium. Hematopoietic stem cells (derived from blood, spleen and bone marrow) and tumor cells can be grown in suspension. These cells grow much faster which do not require the frequent replacement of the medium and can be easily maintained. These are of homogeneous types and enzyme treatment is not required for the dissociation of cells; similarly these cultures have short lag period.

Confluent culture and the necessity of sub-culture :

After the cells are isolated from the tissue and proliferated under the appropriate conditions, they occupy all of the available substrate i.e. reach confluence. For a few days, it can become too crowded for their container and this can be detrimental to their growth, generally leading to cell death if left for a long time. The cells thus have to be subculture i.e. a portion of cells is transferred to a new vessel with fresh growth medium which provides more space and nutrients for the continual growth of both portions of cells.

Hence subculture keeps cells healthy and in a growing state.

A passage number refers specifically to how many times a cell line has been sub-cultured. In contrast with the population doubling level in that the specific number of cells involved is not relevant. It simply gives a general indication of how old the cells may be for various assays.

B. Secondary cell culture and cell line

When a primary culture is sub-cultured, it is known as secondary culture or cell line or sub-clone. The process involves removing the growth media and disassociating the adhered cells (usually enzymatically).

Sub-culturing of primary cells to different divisions leads to the generation of cell lines. During the passage, cells with the highest growth capacity predominate, resulting in a degree

of genotypic and phenotypic uniformity in the population. However, as they are sub-cultured serially, they become different from the original cell.

C. On the basis of the life span of culture, the cell lines are categorized into two types :

1. Finite cell lines :

The cell lines which go through a limited number of cell division having a limited life span are known as finite cell lines. The cells passage several times and then lose their ability to proliferate, which is a genetically determined event known as senescence. Cell lines derived from primary cultures of normal cells are finite cell lines.

2. Continuous cell lines

When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line. Such transformation/mutation can occur spontaneously or can be chemically or virally induced or from the establishment of cell cultures from malignant tissue. Cell cultures prepared in this way can be sub-cultured and grown indefinitely as permanent cell lines and are immortal.

These cells are less adherent, fast growing, less fastidious in their nutritional requirements, able to grow up to higher cell density and different in phenotypes from the original tissue. Such cells grow more in suspension. They also have a tendency to grow on top of each other in multilayers on culture-vessel surfaces.

Common cell lines :

Human cell lines:

1. MCF-7 (breast cancer)
2. HL 60 (Leukemia)
3. HeLa (Human cervical cancer cells)

Primates cell lines: Vero (African green monkey kidney epithelial cells)

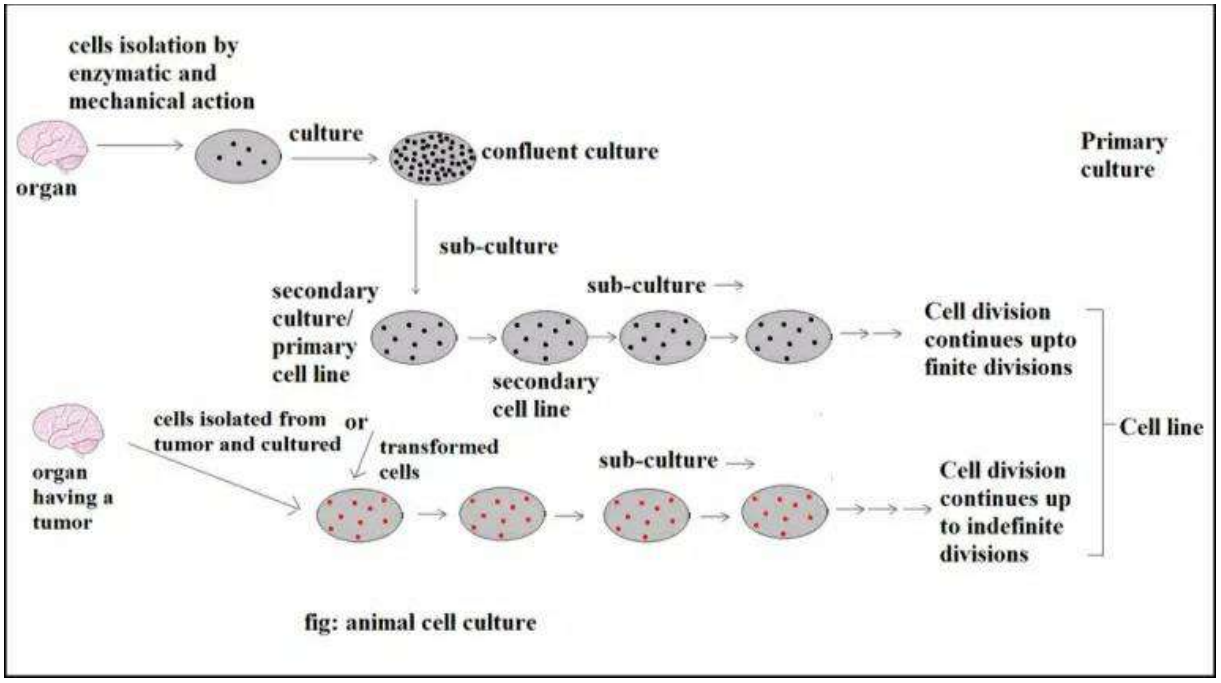


fig: animal cell culture

Cell strain :

Lineage of cells originated from the primary culture is called strain. These are either derived from a primary culture or a cell line by the positive selection or cloning of cells having specific properties or characteristics. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line.

Methods :

Growth Requirements :

The culture media used for cell cultures are generally quite complex, and culture condition widely varies for each cell type. However, media generally include amino acids, vitamins, salts (maintain osmotic pressure), glucose, a bicarbonate buffer system (maintains a pH between 7.2 and 7.4), growth factors, hormones, O2 and CO2. To obtain best growth, addition of a small amount of blood serum is usually necessary, and several antibiotics, like penicillin and streptomycin are added to prevent bacterial contamination.

Temperature varies on the type of host cell. Most mammalian cells are maintained at 37oC for optimal growth, while cells derived from cold-blooded animals tolerate a wider temperature range (i.e. 15oC to 26oC). Actively growing cells of log phage should be used which divide rapidly during culture.

Process to obtain primary cell culture :

Primary cell cultures are prepared from fresh tissues. Pieces of tissues from the organ are removed aseptically; which are usually minced with a sharp sterile razor and dissociated by proteolytic enzymes (such as trypsin) that break apart the intercellular cement. The obtained cell suspension is then washed with a physiological buffer (to remove the proteolytic enzymes used). The cell suspension is spread out on the bottom of a flat surface, such as a bottle or a Petri dish. This thin layer of cells adhering to the glass or plastic dish is overlaid with a suitable culture medium and is incubated at a suitable temperature.

Aseptic techniques :

Bacterial infections, like Mycoplasma and fungal infections, commonly occur in cell culture creating a problem to identify and eliminate. Thus, all cell culture work is done in a sterile environment with proper aseptic techniques. Work should be done in laminar flow with the constant unidirectional flow of HEPA filtered air over the work area. All the material, solutions and the whole atmosphere should be of contamination-free.

Cryopreservation :

If a surplus of cells is available from sub-culturing, they should be treated with the appropriate protective agent (e.g., DMSO or glycerol) and stored at temperatures below –130°C until they are needed. This stores cell stocks and prevents original cell from being lost due to unexpected equipment failure or biological contaminations. It also prevents finite cells from reaching senescense and minimizes risks of changes in long term cultures.

When thawing the cells, the frozen tube of cells is warmed quickly in warm water, rinsed with medium and serum and then added into culture containers once suspended in the appropriate media.

Applications of Cell Line :

A. Vaccines Production :

One of the most important uses of cell culture is in research and production of vaccines. The ability to grow large amounts of virus in cell culture eventually led to the creation of the polio vaccine, and cells are still used today on a large scale to produce vaccines for many other diseases, like rabies, chickenpox, hepatitis B, and measles. In early times, researchers had to

use live animals to grow poliovirus, but due to the development of cell culture techniques, they were able to achieve much greater control over virus production and on a much larger scale which eventually develop vaccines and various treatments. However, continuous cell lines are not used in virus production for human vaccines as these are derived from malignant tissue or possess malignant characteristics.

B. Virus cultivation and study :

Cell culture is widely used for the propagation of viruses as it is convenient, economic, easy to handle compared to other animals. It is easy to observe cytopathic effects and easy to select particular cells on which the virus grow as well as to study the infectious cycle. Cell lines are convenient for virus research because cell material is continuously available. Continuous cell lines have been extremely useful in cultivating many viruses previously difficult or impossible to grow.

C. Cellular and molecular biology :

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of different toxic compounds on the cells, and mutagenesis and carcinogenesis. The major advantage of using cell culture for any of these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells.

D. In Cancer Research :

Normal cells can be transformed into cancer cells by methods including radiation, chemicals, and viruses. These cells can then be used to study cancer more closely and to test potential new treatments.

E. Gene therapy :

Cells having a functional gene can be replaced to cells which are having non-functional gene, and for which the cell culture technique is used.

F. Immunological studies:

Cell culture techniques are used to know the working of various immune cells, cytokines,

lymphoid cells, and interaction between disease-causing agents and the host cells.

G. Others :

Cell lines are also used in in-vitro fertilization (IVF) technology, recombinant protein, and drug selection and improvement.

	Media Type	Examples
Natural media	Biological Fluids	plasma, serum, lymph, human placental cord serum, amniotic fluid
	Tissue Extracts	Extract of liver, spleen, tumors, leucocytes and bone marrow, extract of bovine embryo and chick embryo
	Clots	plasma clots
Artificial media	Balanced salt solutions	PBS, DPBS, HBSS, EBSS
	Basal media	MEM DMEM
	Complex media	RPMI-1640, IMDM