

Experiment :

Date _____
Page No. _____

Experiment :- 1

- * Aim: Isolation of Genomic DNA from E-Coli.
- * Theory: Isolation of genomic DNA is one of the most important and common experiment that is carried out in molecular biology and includes the transition from cell biology to molecular biology. The most common method of isolating genomic DNA without the use of commercial kit is by Phenol / Chloroform method. So, the basic objective of this is to study the method of isolation genomic DNA from E-Coli.

Requirements:-

Reagents & Chemicals:- Tris base, Proteinase K, Phenol / Chloroform (1:1), 200 proof ethanol, SDS, EDTA, Tryptone, yeast extract, NaCl, LB medium, 1% Tryptone, 0.5% yeast extract, 200 mM NaCl), TE buffer (10 mM Tris - Cl [PH 8.0], 1 mM EDTA [PH 8.0]), Lysis buffer (10ml) (9.34 ml TE buffer, 600 µl of 10% SDS, 60 µl of Proteinase K.

Equipment:- Tabletop Centrifuge (Eppendorf), 1.5ml Eppendorf tube, Incubator, Gloves,

Teacher's Signature : _____

Experiment :

Date _____
Page No. _____

Procedure :-

At first take about 2.5 ml of E. coli overnight culture that was grown in LB medium and transfer it to a 1.5 ml of Eppendorf tube and then centrifuge it at maximum possible speed for about 1 min for extracting the cell pellet. Then discard the supernatant and resuspend the pellet in about 600 microliter of lysis buffer and completely vortex it for mixing it properly. Incubate it for about 1 hour at 37°C. To it add a equal volume of Phenol/Chloroborm and properly mix it till they are mixed properly. Now at max speed spin for about 15 min which will lead to formation of a white layer in the aqueous Phenol Chloroborm interface.

Now very carefully transfer the aq. Phase to a new tube through a 1 ml Pipette. You can repeat above two steps till the white layer disappears for removing the Phenol take equal volume chloroborm and add to the aq. layer.

Mix it properly and spin at max. speed for about 5 min. Now transfer the aq. layer to a new tube. For ppt the DNA add about 2.5 ml of cold ethanol and mix it properly.

Teacher's Signature : _____

PPT may diffuse for that you can keep the tube 20°C for about 30 min and then spin it. You will be able to see DNA Pellet. Now spin for about 15 min at 4000. Discard the supernatant and rinse the DNA Pellet with 1 ml of 70% ethanol.

Now again spin at maximum speed with 1 ml of 70% ethanol. Now again spin at max. speed. Now resuspend the DNA in TE buffer. Check DNA on a agar gel.

Result :-

Chromic DNA is isolated without any impurity.

Precautions

- i) pH of buffer should be constant.
- ii) In this method DNA should be taken out very carefully.
- iii) Cell should be live.

Experiment :

Date _____

Page No. _____

Experiment :-2

⇒ Aim : To extract plasmid DNA from E-Coli.

⇒ Theory : Plasmid is double stranded, circular extrachromosomal DNA of bacteria. Plasmids can be transferred b/w same species or b/w different species. They are part of mobilomes like transposons or prophages and are associated with Conjugation.

- * Base of functions, there are 5 types of plasmids.
 1. Fertility Plasmid for Conjugation.
 2. Resistance Plasmids which contain genes which provide resistance to antibiotics.
 3. Col Plasmids which contain genes that code for bacteriocin.
 4. Degradoative Plasmids which help in digestion of unusual substances like toluene.
 5. Virulence Plasmids which is responsible for Pathogenicity. Relaxed Plasmids have high Copy no. Strangely Plasmid have low Copy no.

Teacher's Signature : _____

Experiment :

Date _____
Page No. _____

→ Plasmids are imp. tools in genetics and biotechnology & are commonly used to multiply or express genes.

They may also be used large amount of proteins.

→ Requirements :-

Equipment

- 1) Micro Centrifuge
- 2) Water bath (37°)
- (3) Automatic micro pipette with tips
- (4) 95-100% isopropanol ice.

Buffers & Solutions :-

- 1) Alkaline Lysis Sol. I, (2) Alkaline Lysis Solution II,
- 3) Alkaline Lysis solution III.
- 4) Antibiotic for plasmid selection
- 5) Ethanol
- 6) Phenol : Chloroborm (1:1 v/v), (7) STE
- 8) TE Buffer [pH 8.0, containing 20 mg/ml RNAse A]

→ Alkaline Lysis solution I :- 50 mM glucose, 25 mM Tris-HCl
 10 mM EDTA (pH 8.0), Pre-boiled
 brom stcl. stocks, sterilize by autoclaving & store at
 4°C.

Teacher's Signature : _____

Experiment :

Date _____

Page No. _____

- Alkaline Lysis solution II :- 0.2 N NaOH (freshly diluted from a 1M stock).
1% (w/v) SDS.
- Alkaline Lysis solution III :- 5M potassium acetate 6ml, glacial acetic acid 11.5 ml, H₂O, H₂O 28.5 ml.
- Procedure :-
- 1) Incubate 2 ml of rich medium containing appropriate antibiotic with a single colony of transformed bacteria. Incubate the culture overnight at 37°C with vigorous shaking.
 - 2) Pour 1.5 ml of culture into microtube tube centrifuge at max. speed for 30 sec. at 4°C.
 - 3) Remove the medium by aspiration, leaving the pellet as dry as possible.
 - 4) Resuspend the bacterial pellet in 1ml of ice-cold Alkaline Lysis solution I by vigorous vortexing.
 - 5) Add 200 μl of freshly prepared Alkaline Lysis solution II to each bacterial suspension. Close the tube slightly.

Teacher's Signature : _____

Experiment :

Date _____

Page No. _____

mix the contents well by inverting the tube. Don't vortex store the tube in ice.

- 5) Add 150 μl of ice-Cold lysis solution III Close the tube and disperse the solution through bacterial lysate by inverting the tube several times. Store the tube in ice Box 3-5 min.
- 6) Centrifuge the bacterial lysate Box 5 min at max speed at 4°C in a microfuge. Collect of Phenol: the Et_h supernatant in a fresh tube.
- 7) Add equal volume of phenol: chloroform mix the organic & aq. Phases by vortexing and then centrifuge the emulsion at max. speed for 2 min at 4°C in a microfuge. Transfer the qd. Upper layer to a fresh tube.
- 8) Pipet nucleic acids from the supernatant by adding 2 volume of ethanol at room temp. mixed the solution by vortexing and then allow the mix to stand for 2 min at room temp.
- 9) Collect the precipitated nucleic acid by centrifugation at maximum speed for 5 min at 4°C.

Teacher's Signature : _____

Experiment :

Date _____

Page No. _____

- 1) Discard the supernatant by aspiration stand the tube in an inverted position on a paper towel to allow all the fluid to drain away. Use a Kim wipe or disposable pipette tip to remove any drops of fluid adhering to the walls of the tube.
- 2) Add 1 ml of 70% ethanol to the pellet and invert the closed tube several times. Recover the DNA by centrifugation at max. speed for 2 min at 4°C in a microtube.
- 3) Remove all the supernatant by aspiration. Take care with this step, as the pellet sometimes does not adhere tightly to the tube.
- 4) Remove any beads of ethanol from the tube. Store the open tube at room temp. until the ethanol has evaporated & no fluid is visible in tube.
- 5) Dissolve the nucleic acids in 50 μl of TE containing 20 μg/ml DNase free RNase A. Vortex the solution gently for a few sec. and store the DNA 20°C.

Teacher's Signature : _____

Experiment :-

Date _____
Page No. _____

Result :-

Plasmid DNA is isolated efficiently.

Precautions

- 1) Lyse your cells completely.
- 2) Do not use too many cells.
- 3) Don't vortex your cell after lysis.
- 4) Don't let the tip of column touch the blow through in collection tube after washing.

Experiment :

Date _____
Page No. _____Experiment :- 3

Aim:- To separate DNA fragments using Gel Electrophoresis.

Theory:-

- 1) Gel electrophoresis is a technique commonly used in laboratories to separate charged molecules like DNA, RNA & Protein according to their size.
- 2) DNA being -ve. charged, migrates towards anode. Shorter strands move faster and cover longer distances compared to longer strands.
- 3) The use of dyes, fluorescent dyes or radioactive labels enables the DNA on gel to be seen after separation. They appear as bands on gel.
- 4) Agarose gel matrix is used for DNA separation and ethidium bromide is commonly added for visualization. Most agarose gels are made b/w 0.7% & 2% of agarose. 0.7% gel shows good separation for large fragments whereas at 2%, gel shows higher resolution for small fragments.
- 5) Factors affecting movement of DNA:
 - (a) Voltage applied :- Greater Voltage, greater speed of DNA

Teacher's Signature : _____

Experiment :

Date _____

Page No. _____

- 2) EtBr - Ethidium Bromide is added for visualisation of separated DNA bands. Binding of EtBr to DNA alters its mass and rigidity and hence by its mobility.
- 3) Buffer :- Different buffers provide difference strength of ions that are meant to support conductivity.
- 4) Conformation of DNA :- slowest to fastest nicked or open circular linear supercoiled.

Material Required :- I Buffers and Solutions

- 1) Agarose solution
- 2) Ethidium Bromide
- 3) Electrophoresis buffer
- 4) DNA ladder
- 5) DNA samples
- 6) Loading Buffer.

II Equipment :-

- 1) Electrophoresis Chamber and Power Supply
- 2) Gel Casting tray (3) T

Teacher's Signature : _____

Experiment :

Date _____
Page No. _____# Procedure :-

- 1) Preparation of gel : - weight appropriate mass of agarose into an E.T.H.L. mayer flask. The concentration of agarose in a gel will depend on the size of DNA fragments to be separated. The volume of buffer should not be greater than 1/3 capacity of flask.
- 2) Add remaining buffer to agarose - containing flask. Sufce to mix. The most common gel running buffer are TAE or TBE.
- 3) Melt the agar agarose / buffer mixture. This is most commonly done by heating in microwave or over a Bunsen burner flame. At 30s of intervals remove the flask and suface to mix until agarose dissolve completely.
- 4) Add EtBr to a conc of 0.5 μ g/ml. Alternatively, the gel may also be stained after electrophoresis in running buffer containing 0.5 μ g/ml. EtBr for 15-30 min followed by destaining in running buffer for equal amount in 65°C.
- 5) Allow the agarose to cool either on benchtop or by incubation in 65°C water bath. Failure to do so will result the gel dry.

Teacher's Signature : _____

Experiment :

Date _____

Page No. _____

- 6) Pour Place the gel tray into the Casting apparatus. Alternatively one may also take the open edges of a gel tray to create a mold. place an appropriate comb into the gel mold to create the well.
- 7) Pour The melted agarose into the gel mold. Allow the agarose to set at room temp. Remove the comb and play the gel in the gel box. Alternatively the gel can also be wrap wrapped in parafilm wrap & stored at 4°C until use.

II Setting up of gel apparatus and separation of DNA fragments.

- 1) Add loading dyes to the DNA samples to be separated. Gel loading dye is typically made of G×Conc. bonding dye. helps to take how far your DNA sample has traversed and allow the sample to sink into gel.
- 2) Program the power supply to desired voltage (1.5 V/cm)
- 3) Add enough running buffer to cover the surface of the gel. It is imp. to use running buffer as the one used to prepare the gel.
- 4) Attach the leads of the gel box to the power supply.

Teacher's Signature : _____

Experiment :

Date _____
Page No. _____

Turn on the Power supply and verify that both gel box & Power supply are working.

- 5) Remove the lid slowly & carefully load the DNA sample into gel. An appropriate DNA size marker should always be loaded along with experimental samples.
- 6) Replace the lid to the gel box. The Cathode should be closer to the wells than anode. Double check the electrodes, if they are plugged into the correct slots in the Power supply.
- 7) Turn on the Power Run the gel until the dye has migrated to an appropriate distance.

III Observing Separated DNA Fragments

- 1) When electrophoresis has completed turn off the power & remove the lid of the gel box.
- 2) Remove gel from the gel box. Drain off excess buffer from the surface of the gel.
- 3) Remove the gel from gel tray and expose the gel to UV light. This is most commonly done using a gel documentation system.

Teacher's Signature : _____

Experiment :

Date _____

Page No. _____

- 4) Properly dispose off the gel & running buffer per institution regulation.

Result :-

After separation the resulting DNA fragments are visible as clearly defined bands. The DNA ladder should be separated to a degree that allows for useful determination of size of sample bands.

Precaution :-

- 1) Handle ETBr carefully as it is carcinogenic.
- 2) Inspect the buffer tank for cracks or leaks, expand connectors or missing cover correct them.
- 3) If electrophoresis buffer is spilled or leaks from gel box, stop the run and clean up the bench top immediately.

Teacher's Signature : _____

Experiment :

Date _____

Page No. _____

Experiment -4

Aim:- To understand process of isolation of RNA from plant sample.

Theory:-

- RNA is a Polymeric substance present in living cells and many viruses, consisting of a long ~~sig~~ single - stranded chain of phosphate and ribose units with the nitrogen bases adenine, guanine, Cytosine and uracil, which are bonded to ribose sugar.
- RNA is used in all the steps of protein synthesis it is all required and carries the genetic information for many viruses.
- The isolation of RNA with high quality is a crucial step required to perform various molecular biology experiments.
- Trizol reagent is a ready to use reagent used for RNA isolation from cells and tissues. It works by maintaining RNA integrity during homogenization, while at the same time disrupting and breaking down cells and cell components.
- RNA extracted can be used in Northern blot analysis, in vitro translation, play, selection, RNase protection assay and molecular cloning.

Teacher's Signature : _____

Experiment :

Date _____
Page No. _____Requirement :-

- 1) Chloroform
- (2) Isobutyl alcohol
- 3) 75% ethanol
- (4) RNase, water or 0.5% SDS solution.

Procedure :-

I : Homogenization :- Growth medium on cells was discarded and cells were washed with ice cold 1x PBS. The monolayer was then covered with 1 ml of Trizol & cells were lysed and homogenized by repeated pipetting.

II Phase Separation :-

- 1) The homogenized samples were incubated for 5 min at 15 to 30°C for complete dissolution of nucleoprotein complexes.
- 2) 0.2 ml of Chloroform after 0.75 ml of Trizol is reagent was added. The tubes were shaken vigorously by hand for 15 sec & incubated them at 15 to 30°C for 2 min.
- 3) The sample were centrifuged for 15 min at no more than 12,000 g (4°C)
- 4) The cf. Phase was transferred to other tube following

Teacher's Signature : _____

Experiment :

Date _____

Page No. _____

Centrifugation, the mix separates into a lower red, Phenol Chlorobrom Phase an interphase and a colourless upper aq. Phase. RNA remains only in aq. Phase.

III RNA Precipitation (1) RNA was precipitated from the aq. Phase by mixing with 3 mL of glycogen and 500 mL of isopropanol alcohol.

2) The mixture was centrifuged for 30 min at 12000 xg. RNA precipitate forms a pellet on the side of tube at bottom.

IV RNA Wash : (1) The supernatant was removed the RNA Pellet was washed once with 75% ethanol, adding 400 mL of 75% ethanol per 0.75 mL of Trizol is reagent used for initial homogenization.

2) The sample were inverted and mixed and Centrifuged at 12,000 rpm for 30 min at 4°C.

v) Redissolving RNA : (1) The RNA Pellet was dried.

2) RNA was dissolved in RNase - free water by passing through the Pipette tip box a few times, and incubating for 10 min at 55 to 60°C.

Teacher's Signature : _____

Experiment :

Date _____
Page No. _____# Result :-

RNA is Precipitated El obtained

Precaution :-

- 1) Avoid even accidental introduction of RNase into the apparatus.
- 2) Use sterile, disposable Plasticware and automatic Pipettes to prevent cross-contamination.

Teacher's Signature : _____

Experiment : 05

Date _____

Page No. _____

Aim:- To estimate the conc. of given DNA sample by UV Spectrophotometer.

Principle :-

As a result of resonance all the bases present in the nucleic acid absorb U.V light. Nucleic acids are characterised by a maximum absorption of U.V light at wavelength near the 260nm in spectrophotometer. The amount of U.V radiation absorbed by solution containing DNA is directly proportional to the amount of DNA in the sample.

Reagent :- DNA Sample and Distilled water.

Procedure :-

- 1) Ten μ l of given DNA sample was pipetted into a 1ml quartz cuvette.
- 2) 99 μ l of distilled water was added to it.
- 3) The contents were mixed well by inverting the cuvette.
- 4) The cuvette was placed under U.V beam in Sep Spectrophotometer against the black cuvette. 1 ml of distilled water.
- 5) Absorbance of the DNA sample was taken at 260 nm.

Teacher's Signature : _____

Experiment :

Date _____
Page No. _____Conclusion :-

- DNA absorbs 1.87 times light at 260 nm than 230, 280.
- Chemicals absorb 230nm.
- Protein absorb 280nm.
- The values of 260/230 and 260/280 near 1.87 is considered as good quality DNA devoid of protein and salt.

Hence 1 OD is equivalent to different concentration levels for different types of nucleic acid such as 50 μg /ml dsDNA, 33 μg /ml ssDNA, ~ 30 μg /ml oligonucleotides or 40 μg /ml of RNA.

Precautions :-

- i) The DNA sample should be analyzed against the reagent blank.
- ii) The transparent side of the cuvette should be placed in front of the light path.
- iii) The cuvette should be clean, there should not be any dust or finger impression.
- iv) The Cuvette should never be held from its transparent side.
- v) Pipetting should be accurately done.

Teacher's Signature : _____

Aim :- To estimate the concentration of DNA by diphenylamine reaction.

Principle :- This is a general reaction given by deoxy-Pentoses. The 2-deoxyribose of DNA, in the presence of acid, is converted to α -hydroxylevulinic aldehyde, which reacts with diphenylamine to form a blue coloured complex, which can be read at 595 nm.

Requirements :-

1. Standard DNA Solution :- Dissolve calf thymus DNA (200 μ g/ml) in 1N perchloric acid.
2. Diphenylamine solution :- Dissolve 1g of diphenylamine in 100 ml of glacial acetic acid and 2.5 ml of conc. H_2SO_4 . This solution must be prepared fresh.
3. Bubbled Saline :- 0.5 mol/litre NaCl; 0.015 mol/litre sodium citrate, pH 7.

Procedure :-

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working std into the series of labeled test tubes.

2. Pipette out 1ml of the given sample in another test tube.
3. Make up the volume to 1ml in all the test tubes. A tube with 1ml of distilled water serves as the blank.
4. Now add 2 ml of DIA reagent to all the test tubes including the test tubes labeled 'blank' and 'Unknown'.
5. Mix the contents of the test tubes by vortexing/shaking the tubes and incubate on a boiling water bath for 10 min.
6. Then cool the contents and record the absorbance at 545 nm against blank.
7. Then plot the std. curve by taking concentration of DNA along x-axis and absorbance at 545 nm along y-axis.
8. Then form this std. curve calculate the conc. of DNA in the given sample.

Conclusion:- The test is very specific to determine the concentration of DNA and is widely used.

Precautions :-

- 1) Wear eye protection and use a flame cupboard when preparing this reagent.
- 2) DIA is harmful if ingested or inhaled and may irritate skin or eyes if it comes into contact with them.

Experiment : 7

Date _____
Page No. _____

Aim

To estimate the concentration of RNA by orcinol method.

Principle :- This is general reaction for Pentose and depends on the formation of Furfural. When Pentose is heated with concentrated HCl, orcinol reacts in presence of Furfural in presence of ferric chloride as a catalyst Purine to produce green color only the Purine nucleotide.

Materials

- 1) RNA Commercial sample, 10mg
- 2) RNA solution 0.2 mg/ml
- 3) Orcinol reagent: Dissolve 10gm of ferric chloride $\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O}$ in 1L of 1M
- 4) Buffered saline.
- 5) Boiling water bath.

Procedure :-

1. Pipette out standard RNA solution in amounts of 0.2 ml into a series of test tube and make up the volume of each tube 2ml with distilled water.
2. Add 0.3ml of orcinol reagent to each tube for standard test.
3. For test solution: Take 2ml of nucleic acid sodium.

Teacher's Signature : _____

Experiment :

Date _____

Page No. _____

4. Add 3mL of orcinol reagent to each tube, and heat the tube on boiling water bath for 20 minutes.
- 5) Cool and take the optical density at 665nm against the orcinol blank.

Result

Concentration of RNA Present in the given sample is
----- $\mu\text{g}/2\text{mL}$.

Aim:-

To Perform Restriction Digestion of DNA

Principle:-

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. For a list of many commonly used restriction used restriction enzymes,

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:-

- Electrophoresis Chambers
- Pipetman

Reagents:-

- Liquid DNA aliquot of your plasmid of interest.
- Appropriate restriction enzyme.
- Appropriate restriction digest buffer.
- Gel Loading dye.
- Electrophoresis buffer
- Pipet tips.

Procedure :-

1. Select restriction enzymes to digest your plasmid.
2. Determine an appropriate reaction buffer by reading the instruction of your enzyme.
3. In a 1.5 ml tube Combine the following.
 - DNA
 - Restriction Enzyme(s)
 - Buffer
 - BSA
 - dH₂O up to total volume
4. Mix gently by Pipetting.
5. Incubate tube at appropriate temperature for 1 hour.
6. To visualize the results of your digest, Conduct gel electrophoresis.

Results :-

This method was validated against 16 species of blood-borne helminths and Protozoa. Enzyme digestion prior to PCR enrichment and Illumina amplicon deep sequencing led to a substantial reduction in human reads and a corresponding 5-to 10-fold increase in parasite reads relative to undigested samples. This method allowed for discrimination of all common parasitic agents.

Experiment :

Date _____

Page No. _____

ASSIGNMENTGel Electrophoresis

- ⇒ Gel electrophoresis is a technique used to separate DNA fragments according to their size.
- ⇒ DNA samples are loaded into wells at one end of a gel, and an electric current is applied to pull them through the gel.
- ⇒ DNA fragments are negatively charged, so they move towards the positive electrode. Because all DNA fragment have the same amount of charge per mass, small fragments move through the gel faster than large ones.
- ⇒ When a gel is stained with a DNA-binding dye, the DNA fragments can be seen as bands, each representing a group of same-sized DNA fragments.
- ⇒ Gel electrophoresis involves a gel: a slab of jello-like material. Gels for DNA separation are often made out of a polysaccharide called agarose, which comes as dry, powdered flakes.

Teacher's Signature : _____

How does it work ?

The gel used in gel electrophoresis is usually made a material called agarose, which is a gelatinous substance extracted from seaweed.

- Charged molecules moved through a gel when current is applied.
- One end of gel connected to positive electrode & other to negative ele.
- Molecules migrate towards opposite charge.
- Gel consist of permeable matrix through which molecules can travel.
- Smaller molecules move through gel quickly & travel farther than large fragments. As a result molecules are separated by size.

What is important importance

- It is used in DNA finger printing.
- Example :- Paternity testing, Forensic study, molecular biology, dendic etc.

Experiment :

Date _____

Page No. _____

Gel Electrophoresis strengths

- Inexpensive
- Simple
- Detection flexibility and sensitivity
- Speed

Gel Electrophoresis Weaknesses

- Limited to macromolecules
- Resolving Power
- Separation options
- Precision and reproducibility
- 2-D gel electrophoresis
- Mass Spectrometry compatibility

What's the Future of gel Electrophoresis

I think these techniques have a future as I see them as more complimentary than competing techniques. People need a quick, simple and inexpensive method of visualising macromolecules. Also it removes the requirement for expensive and time consuming mass Spectrometry for many applications.

We also work on weaknesses of electrophoresis and more the exception.

Teacher's Signature : _____