GENOMIC DNA ISOLATION FROM FLIES

INTRODUCTION

DNA extraction is a routine procedure used to isolate DNA from the nucleus of cells. The basic principle of DNA isolation is disruption of the cell , Cell membrane and nuclear membrane to release the highly intact DNA into solution followed by precipitation of DNA and removal of the contaminating biomolecules such as Proteins, Polysaccharides, lipids etc by chemical method.

DNA purification strategies rely on the chemical properties of DNA that distinguish it from other molecules in the cell, namely that it is a very long, negatively charged molecule. To extract purified DNA from a tissue sample, cells are broken open by grinding or **lysing** in a solution that contains chemicals that protect the DNA while disrupting other components of the cell. These chemicals may include **detergents**, which dissolve lipid membranes and denature proteins. A cation such as Na^+ helps to stabilize the negatively charged DNA and separate it from proteins such as histones. A chelating agent, such as EDTA, is added to protect DNA by sequestering Mg^{2+} ions, which can otherwise serve as a necessary co-factor for **nucleases** (enzymes that digest DNA). As a result, free, double-stranded DNA molecules are released from the chromatin into the extraction buffer, which also contains proteins and all other cellular components.

The free DNA molecules are isolated by one of several methods. Commonly, proteins are removed by adjusting the salt concentration so they precipitate. The **supernatant**, which contains DNA and other, smaller metabolites, is then mixed with ethanol, which causes the DNA to precipitate. A small **pellet** of DNA can be collected by centrifugation, and after removal of the ethanol, the DNA pellet can be dissolved in water (usually with a small amount of EDTA and a pH buffer) for the use in other reactions.

MATERIALS REQUIRED

Homogenizer, Centrifuge tubes, Centrifuge tube holder, Eppendorf Tubes, Eppendorf tube stand, Micropipette, Microtips, Cooling Centrifuge, Beakers, Measuring cylinders

PROCEDURE:

Step 1: Wings and Appendages removed from the flies

Step 2: Homogenized in 500µl Grinding Buffer

Step 3: Added 500µl Grinding Buffer again and Incubated for 10 Min at 65°C

Step 4: Added 20 µl RNase and Incubated for 30 Min at 37°C

Step 5: Added 500 µl of 8M Potassium Acetate Incubated for 45 min – 1Hr at 4°C

Step 6: Centrifuged with 500 µl Phenol Chloroform (1:1)at 15000 rpm for 5to 8 min

Step 7: 500 µl of the supernatant Centrifuged with Chloroform-Isoamyl Alcohol (24:1) at 15000 rpm for 5 to 8 min

Step 8: 250 µl of the supernatant Centrifuged with 1000 µl chilled alcohol at 15000 rpm for 5 to 8 min

Step 9: Supernatant decanted, added 1000 µl 70% Ethanol and Centrifuged at 15000 rpm for 5 to 8 min

Step 10: Air Dried and DNA was re-suspended in TE and stored at -20°C

PRECAUTIONS.

All the steps to be performed carefully to avoid any contamination

All the glassware tips and materials should be autoclaved before use

Wear gloves during all the steps

DNA should be stored at low temperatures.

Amplification of a desired DNA Template using PCR Thermocycler

Object: To amplify a 285 bp fragment of desired Gene using PCR Thermocycler

INTRODUCTION

PCR is a very sensitive technique that allows rapid amplification of a specific segment of DNA. PCR makes billions of copies of a specific DNA fragment or gene, which allows detection and identification of gene sequences using visual techniques based on size and charge. PCR, the reaction is repeatedly cycled through a series of temperature changes, which allow many copies of the target region to be produced.using some components as follows:

Following components are required

DNA will be amplified during PCR. Heat is used to unwind the double stranded DNA, resulting in two complementary single strands. The two single strands now act as templates to generate new double stranded molecules of DNA.

Nucleotides, also called dNTPs (deoxynucleotide triphosphates), bases or DNA bases, are single units of Adenine (A), Thymine (T), Cytosine (C), and Guanine (G). They must be added to the PCR reaction and serve as building blocks for new DNA molecules.

Primers are small lengths of DNA, generally around 20 nucleotides, that are designed to bind and amplify a specific section or gene of the DNA strand. They are needed because DNA polymerase, the enzyme that adds nucleotides to the single stranded DNA, can only add to an existing nucleotide. Because the primers must be specific to the strand, abiding by the typical base pairing rules, you generally need to know the DNA sequence that you wish to amplify before you begin the PCR process. Buffer

Buffer will be added to the PCR mix in order to maintain pH conditions for the entirety of the reaction and promote primer binding.

Taq polymerase acts as a DNA polymerase to add new DNA bases to the end of the primer sequence using the base pairing rules of nucleotides. While most DNA polymerases are temperature sensitive, Taq is able to withstand the high temperatures needed to denature DNA in PCR and is thus used as the primary source of extension in modern PCR reactions.

MATERIALS REQUIRED:

Microtips Micropipette, Sterile water, PCR Tubes, Eppendorf Tubes, Microtip stand PCR tube stand, Eppendorf tube stand.Thermocycler, Reaction Mixture.

PROCEDURE:

In PCR protocol, reaction components are assembled as described below. The final volume should be 50 µL.

- Thaw all reagents on ice.
- Assemble reaction mix into 50 µL volume in a thin walled 0.2 mL PCR tubes.
- Add reagents in following order: water, buffer, dNTPs, template DNA, Forward and Reverse primers, Taq polymerase.
- Gently mix by tapping tube. Briefly centrifuge to settle tube contents.
- Prepare negative control reaction without template DNA.
- Prepare positive control reaction with template of known size and appropriate primers.

PRECAUTIONS

- All the steps to be performed carefully to avoid any contamination
- All the glassware tips and materials should be autoclaved before use
- Wear gloves during all the steps
- All the components of a reaction mixture should be handled at low temperatures.

DNA Visualization by Agarose Gel Electrophoresis

MATERIALS REQUIRED: 1. Agarose , 2. Ethidium bromide, 3. Electrophoresis buffer, 4. 6x gel buffer, 5. DNA sample, 6. DNA ladder.

PRINCIPLE: Agarose gel electrophoresis is a procedure used to separate DNA fragments based on their molecular weight. Agarose is a linear polymer extracted from seaweeds Purified agarose is a powder and insoluble in water or buffer at room temperature but dissolves on boiling. Molten solution of agarose when placed in a tray solidifies and sets on cooling due to polymerization of agarose

Electrophoresis is a technique used to separate charged molecules. DNA is negatively charged at neutral pH and when electric field is applied across the gel, DNA migrates towards the anode. Matrix of agarose acts as a molecular sieve through which DNA fragments move on application of electric current. By changing amount of agarose which is purified from agar, the gel concentration and pore size can be altered. Higher the concentration of Agarose smaller the pore size and vice versa. As the length of DNA increases it becomes harder for the DNA to pass through the spaces. Progress of gel electrophoresis can be monitored by a tracking dye (Bromophenol Blue) which migrates with the same speed with that of DNA.

Since DNA is not a coloured molecule it is not visible on the gel. Hence in molten agarose an intercalating dye Ethidium Bromide is added and when the gel is kept under UV light, the DNA can be visualized as fluorescent orange bands.

PROCEDURE:

Preparation of agarose solution for casting the gel

Dissolve Agarose by placing the flasks in boiling water or microwave.

Placed the comb about 1 cm from one end of the tray.

Poured Agarose solution without making any bubles, cooled it for 20 mins and took off the combs

The DNA sample (100 to 200 ng) is mixed with the loading dye (for 5 µl of DNA sample 1µof 6x dye is used) and loaded in to the well carefully, using a pipette.

Once the sample is loaded in to the well, the cathode (Black negative terminal) is connected towards the top end of the gel and the anode (Red positive terminal is connected towards the bottom end of the gel.

The electrophoresis is started by switching on the D. C. Powerpack. The gel is run at 5v/cm. As the bromophenol blue(the tracking dye) has moved 1 cm above the bottom end, the current is switched off, the power supply is disconnected

The gel is placeded on UV Transilluminator. Now the UV light is switched on and the DNA bands are seen

OBSERVATION: After electrophoresis DNA bands can be visualized under UV light and they appeared as fluorescent orange bands.

PRECAUTIONS

Gloves have to be used while performing the whole experiment.

Handling of Et Br should be done carefully as it is a known mutagen.

Dilution of the stock solution should be done prior to start of the experiment.

Samples should be thawed before loading them on agarose gel

As UV rays are dangerous for the eye, eyes should be protected by wearing a UV face shield, goggles or using glass plate

To count total number of RBCs from your own blood using Haemocytometer

Introduction: RBCs are the most abundant type of blood cell in the human body, making up about 40-45% of the blood volume in adults. RBCs are important because they carry oxygen from the lungs to the rest of the body and transport carbon dioxide back to the lungs to be exhaled. The purpose of performing a total Red Blood Cell (RBC) count is to measure the number of red blood cells in a given blood volume. Measuring RBCs is therefore an essential part of diagnosing and monitoring many medical conditions. A low RBC count can indicate anaemia, a condition where there are not enough red blood cells to transport oxygen throughout the body adequately. An elevated RBC count may indicate polycythemia, a disease with too many red blood cells, leading to the thickening of the blood and an increased risk of blood clots.

Requirements: Needle, Cotton, Hayem's Solution, Coverslips, Alcohol, Haemocytometer

Procedure:

1. Cleaned the slide and the finger with alcohol and let it air dry
2. Pricked the needle in the finger to take out blood and the initial drop was wiped off.
3. The blood was sucked up by using the pipette provided in the haemocytometer for RBC count upto 0.5 mark
4. The blood was diluted using Hayem's solution upto 101 mark thus diluting it upto 200 times
5. Discarded the first two drops of the blood and next two drops were put on the neubaur's chambers meant for counting of cells in the slide.
6. The slide was covered with the coverslip and visualised under high magnification of microscope.

Observation:

Cells were counted in any 5 squares containing 16 chambers in each And total number of RBCS may be calculated using following Formula

$$\mathbf{NRBC=\frac{N}{N_{sq} X\ Volume\ X\ Dilution}}$$

N= Number of Cells in 5 squares

Nsq= Number of Squares

Volume= Area X Height

To count total number of WBCs from your own blood using Haemocytometer

Introduction: The WBC or leukocyte count method estimates white blood cells per microlitres of blood. By enumerating the total number or concentration of leukocytes, we can determine the condition of our **immune health**. The average WBC count is between 4000 to 11000 cells/µL of blood.An increase in the concentration of leukocytes leads to a medical condition called "**Leukocytosis**". Oppositely, a decrease in the concentration of leukocytes eventually leads to "**Leukopenia**". Therefore, white blood cells make a significant difference to health, as immunity depends on it.

Requirements: Needle, Cotton, Turk's Solution, Coverslips, Alcohol, Haemocytometer

Procedure:

1. Cleaned the slide and the finger with alcohol and let it air dry
2. Pricked the needle in the finger to take out blood and the initial drop was wiped off.
3. The blood was sucked up by using the pipette provided in the haemocytometer for WBC count upto 0.5 mark
4. The blood was diluted using Turk's solution upto 11 mark thus diluting it upto 20 times
5. Discarded the first two drops of the blood and next two drops were put on the neubaur's chambers meant for counting of cells in the slide.
6. The slide was covered with the coverslip and visualised under high magnification of microscope.

Observation:

Cells were counted in any 4 squares containing 16 chambers in each And total number of WBCs may be calculated using following Formula

WBC per cubic mm = N X Average number of chambers (4)XDilution (20)

Volume (0.4)

N= Number of Cells in 4squares

Volume= Area X Height

dth x length x height)= 0.1

Separation Of Amino Acids By Paper Chromatography

AIM- To perform Ascending paper chromatography for the separation of amino acids present in the given sample.

INTRODUCTION-

Chromatography is used to separate mixtures of substances into their individual components. All forms of chromatography work on the same principle. They all have basic requirements of stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates based on their affinities toward stationary phase and mobile phase. In paper chromatography, the stationary phase is a very uniform adsorbent paper. The mobile phase is a suitable liquid solvent or mixture of solvents. Retention (or) retardation factor (Rf)- Retention factor is defined the ratio of the distance travelled by the solute to the distance travelled by solvent. The distance travelled relative to the solvent is called the Rf value.

REQUIREMENTS- Chromatography jars, Whatmann filter paper, Petridishes Measuring cylinder, Developing chamber and capillary tubes etc. Chemicals: n-butanol Glacial acetic acid Distilled water (4:1:5) Amino acids (Proline and Lysine) Ninhydrin reagents

Solvents system and its preparation methods 1. n-butanol and water are taken in 4:5 ratios in a conical flask and allow it to saturate for 24 hours. By using separating funnel separate nbutanol and water. The saturated n-butanol and Glacial acetic acid are taken in the ratio of 4:1 which can be used as a solvent system (or) mobile phase. Ascending paper chromatography

The chromatography paper is cut into rectangular strips and marks a line on the paper with pencil at about 2 cm from the bottom.

With the help of capillary tube, the samples are applied at different points on the starting line.

Now, place the chromatography paper in the developing chamber, which contains the mobile phase. While placing the paper, it is important that the solvent level should not reach the starting line or the sample spots and paper shouldn’t touch the walls of the developing chamber. After sometime the solvent rises up the paper or the stationary phase by capillary action and dissolves the sample.

The components of the sample move along with the solvent in upward direction.

Check if the solvent has reached near the top level of chromatography paper.

Then the paper is removed when it reaches the top and marked the level with pencil. This level (or) height is called the “solvent front”. By using UV light, ninhydrin or iodine vapors examined the different spots of varied colors. Each spot represents a specific component of the sample.

OBSERVATION:

Coloured spots may be seen at different heights of the paper

For ascending paper chromatography The distance moved by tryptophan and threonine is …… cm and ….. cm respectively, and the solvent is ……cm Rf value of tryptophan is …… Rf value of threonine is …….. Rf value of unknown mixture is ….. & ….. By performing the Ascending paper chromatography, the distance moved by the sample and unknown mixture is noted and by substituting these values in the given Rf formula, the Rf values of tryptophan, threonine and unknown samples are known. By performing the Ascending paper chromatography Rf values of both tryptophan and threonine are found to be …… & …… respectively, and the unknown samples are found to be tryptophan and threonine. By performing the radial paper chromatography Rf values of both tryptophan and threonine was found to be ….. & ….. respectively, and the unknown samples were found to be tryptophan and threonine.

PRECAUTIONS 1. The chromatogram should remain in the stretched position on the rim of the petri dish. 2. Do not disturb the system while the chromatogram develops. 3. The chromatogram should be dried properly before introducing it in the chromatography chamber. 4. The solvent mark should be marked immediately with a pencil after removing it from the chromatography chamber.

was able to travel really fast.