Experiment (1): Extraction of Genomic DNA from Rat Blood

Aim:

- To isolate pure genomic DNA from rat blood sample.

Introduction:

Genomic DNA constitutes the total genetic information of an organism. The genomes of almost all organisms are DNA, the only exceptions being some viruses that have RNA genomes. Genomic DNA molecules are generally large, and in most organisms are organized into DNA–protein complexes called chromosomes. The size, number of chromosomes, and nature of genomic DNA varies between different organisms. Genomic DNA contains genes, discrete regions that encode a protein or RNA. A gene comprises the coding DNA sequence, as well as the associated regulatory elements that control gene expression. Nuclear eukaryotic genes also contain noncoding regions called introns. The number of genes varies widely between different organisms.

DNA isolation is an essential technique in molecular biology; it is the first step in the study of specific DNA sequences, genomic structure, DNA fingerprinting, restriction fragment length polymorphism (RFLP), and PCR analysis. The quantity, quality and integrity of the isolated DNA will directly affect these results.

Sources for DNA extraction are very diverse, practically DNA can be isolated from any part of human body such as semen, saliva, hair roots, mouth swabs and even from several skin cells left on the surface after it has been touched. However, the most common sources are soft tissue or blood samples. There are many different methods which can be used to perform DNA extraction on such samples such as organic extraction, salting out, magnetic separation and silica based technology. The choice of a method depends on many factors: the tissue type, the concentration of DNA, sample number, safety of the experiment and coast. Regardless of the used methods, they happen to follow some common procedures aimed to achieve effective **cell lysis, proteins and RNA removal, and lastly DNA precipitation**. Resulting in a homogeneous DNA preparation that represent the entire genetic information contained within the cell.

PAUSE AND THINK ➔ Can we obtain DNA from mature RBC? Why?

Principle:

Successful nucleic acid isolation protocols have been published for nearly all biological materials. They involve the physical and chemical processes of tissue homogenisation (to increase the number of cells or the surface area available for lysis), cell permeabilization, cell lysis (using hypotonic buffers), removal of nucleases, protein degradation, protein precipitation, solubilisation of nucleic acids and finally various washing steps. Cell permeabilization may be achieved with the help of non-ionic (non DNA-binding) detergents such as Triton.

Materials:

Chemical

Ethylene diamine tetra acetate (EDTA), NaOH, Tris-HCl, sucrose, $MgCl_2$, Triton X100, Sodium dodecyl sulphate (SDS), NaCl, Sodium perchlorate, TE buffer or double distilled water, cold chloroform, cold ethanol.

Preparation of solutions

1) 0.5 M EDTA, pH 8.0

Add 146.1 g of anhydrous EDTA to 800 ml of distilled water. Adjust pH to 8.0 with NaOH (about 20 g). Make up the volume to 1 L with distilled water.

2) 1 M Tris-HCl, pH 7.6

Dissolve 121.1 g of Tris base in 800 ml of distilled water. Adjust pH with concentrated HCl (about 60 ml). Make up the volume to 1 L with distilled water.

3) Reagent A (Red Blood Cell Lysis Solution)

Containing: 0.01M Tris-HCl (pH 7.4), 320 mM Sucrose, 5 mM $MgCl_2$, and 1% Triton X100.

Add 10 ml of 1 M Tris to 109.54 g of sucrose, 0.47 g MgCl2 and 10 ml Triton X100 to 800 of distilled water. Adjust pH to 8.0; make up the volume to 1 L with distilled water.

4) Reagent B (White blood Cell Lysis Solution)

Containing: 0.4 M Tris-HCl, 150 mM NaCl, 0.06 M EDTA, 1% SDS, pH 8.0.

Take 400 ml of 1 M Tris (pH 7.6), 120 ml of 0.5 M EDTA (pH 8.0), 8.75 g of NaCl, adjust pH to 8.0 with NaOH. Make up the volume to 1 L with distilled water. Autoclave at 15 p.s.i. for 15 min. After autoclaving the mixture, add 10 g of SDS.

Protocol:

1. Place 3 mL of whole blood in a 15-mL falcon tube (centrifuge tube).
2. Add 12 mL of reagent A.
3. Mix on a rolling or rotating blood mixer for 4 min at room temperature (to prevent leakage, close the lid tightly).
4. Centrifuge at 3000g for 5 min at room temperature.
5. Discard supernatant without disturbing cell pellet. Remove remaining moisture by inverting the tube and blotting onto tissue paper.
6. Add 1 mL of reagent B and vortex briefly to re-suspend the cell pellet.
7. Add 250 µL of 5 M NaCl and mix by inverting tube several times.
8. Place tube in water bath for 15 to 20 min at 65 °C.
9. Add 2 mL of ice-cold chloroform.
10. Mix on shaker for 20 min.
11. Centrifuge at 2400g for 2 min.
12. Transfer upper phase into a clean falcon tube using a sterile pipette.
13. Add 2 to 3 ml of ice-cold ethanol and invert gently to allow DNA to precipitate (if a cloudy did not form, add more ethanol).
14. Using a clean Pasteur pipette spool the DNA onto the hooked end.
15. Immediately transfer to a 1.5-mL microcentrifuge tube.
16. Spin the microcentrifuge tube at 6000 rpm for about 5 minutes.
17. Gently remove the supernatant (ethanol layer) without disrupting the DNA pellets, and leave it to dry.
18. Re-suspend in 200 µL of TE buffer or doubled distal water water and label the tube.
19. As a final step in nucleic acid isolation, the yield and purity of the extracted nucleic acid may need to be determined (Lab No. 3).

Results:

Cloudy precipitation can be seen by the naked eye, and it represents the isolated genomic DNA.

References:

1. Surzycki S. Basic techniques in molecular biology. Springer. (2000).