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PREPARATION OF GENOMIC DNA FROM BLOOD

AIM

To isolate pure DNA from blood.

Introduction

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 permeabilisation, cell lysis (using hypotonic buffers), removal of nucleases, protein degradation, protein precipitation, solubilisation of nucleic acids and finally various washing steps. Cell permeabilisation may be achieved with the help of non-ionic (non DNA-binding) detergents such as SDS and Triton.

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.

It is very important to extract pure DNA to give a good and reliable results when using it in different experiments. Extraction of DNA from cells is a relatively straight-forward and simple process. It is routinely carried out in laboratories and has numerous downstream applications, including sequencing and PCR.

Literally hundreds of protocols for DNA preparation from various sources of tissue have been published over the last few decades, There are different protocols and several commercially available

kits that can be used for the extraction of DNA from whole blood. The choice of a method depends on many factors: the tissue type, the concentration of DNA, sample Number, safety of the experiment and coast.
This procedure is one routinely used both in research and clinical service provision and is cheap and robust. It can also be applied to cell pellets from dispersed tissues or cell cultures (omitting the red blood lysis step).

Principle of DNA Extraction:

Blood Collection:

Draw blood in EDTA-containing Vacutainer tube by venipuncture. Store at room temperature and extract within the same working day

1. Removing Red Blood cells:

The hypotonic solution lyses red blood cells, but leaves WBCs intact. WBCs are separated by centrifugation, forming a WBC "pellet" in the bottom of the centrifuge tube. In this case the Supernatant contains…….....

2. Preparation of a cell extract

The cell membranes of WBC have to be disrupted by using "Cell lysis buffer".
Examples of chemicals used in this step and their purposes
…….
…….

In addition to maintain the integrity of DNA and preventing nucleases from breaking down DNA
……..must be added which act as a chelating agent that will…….....

3. Purification of DNA from cell extract

In addition to DNA the cell extract will contain significant quantities of protein and RNA. A variety of procedures can be used to remove these contaminants, leaving the DNA in a pure form.
The standard way to deproteinize a cell extract is to add phenol or a 1:1 mixture of phenol:chloroform. These organic solvents precipitate proteins but leave the nucleic acids in aqueous solutions.

4. Collecting DNA

The most frequently used method of concentration is ethanol precipitation. In the presence of salt and at a temperature of -20 °C or less, absolute ethanol will efficiently precipitate polymeric nucleic acids. With a concentrated solution of DNA one can use a glass rod to spool the adhering DNA strands. For dilution purposes the precipitated DNA can be collected by centrifugation and redissolving in an appropriate volume of water.

5. Measurement of purity and DNA concentration

Materials:

2.4.1 Chemicals 1 EDTA 2 NaOH 3 Tris-HCl 4 Sucrose 5 MgCl2 6 Triton X 7 Sodium dodecyl sulphate. 8 NaCl 9 Sodium perchlorate 10 TE Buffer 11 Chloroform 12 Ethanol

2.4.2 Equipments 1. Waterbath set at 65°C. 2. Centrifuge tubes (15 mL; Falcon). 3. Microfuge (1.5 mL) tubes

Method:

1. Place 3 mL of whole blood in a 15-mL falcon 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, cover it by tissue before you rotate by hand)
4. Centrifuge at 3000g for 5 min at room temperature.
5. Discard supernatant without disturbing cell pellet using pasture pipette. Remove remaining moisture by inverting the tube and blotting onto tissue paper.
6. Add 1 mL of reagent B (cell lysis solution) and vortex briefly to resuspend the cell pellet.
7. Add 250 µL of 5 M sodium perchlorate and mix by inverting tube several times.
8. Place tube in water bath for 15 to 20 min at 37°C.
9. Add 2 mL of ice-cold chloroform.
10. Mix on shaker for 20 min, **do not mix it by hand after this step removing it**!
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 freshly prepared flamed Pasteur pipette spool the DNA onto the hooked end.
15. Transfer to a 1.5-mL Eppendorf tube and allow to air dry.
16. Resuspend in 200 µL of TE buffer or sterilized water.
17. As a final step in nucleic acid isolation, the yield and purity of the extracted nucleic acid may need to be determined.

Results:

Add a picture of your extracted DNA.

Discussion:

Discuss the purpose of each step that you done.

Questions:

1. What do you think is the purpose of the cell lysis solution?
2. What is the purpose of ethanol?
3. Isolated DNA should be free from contaminating protein, heme and other cellular macromolecule, what precautions did you take to solve this situation?
4. Heme, the non-protein iron component of hemoglobin, is a primary contaminant of DNA from blood preparations, how can you detect this type of contaminant in the isolated DNA?
5. In this procedure, if you didn't have sodium perchlorate, what other chemical can you use instead?