KING SAUD UNIVERSITY
COLLEGE OF MEDICINE

PATHOLOGY DEPARTMENT
MEDICAL BIOCHEMISTRY UNIT

FIRST YEAR

FOUNDATION BLOCK

DNA EXTRACTION AND PURIFICATION

(PRACTICAL SESSION)

Foundation Block (1st Year)

Practical Biochemistry: DNA Extraction and Purification

Objectives:

It is the first practical class in Biochemistry for medical students in Foundation Block. It will allow orientation of students about commonly used devices and equipments in biochemistry lab. It will also give the students the chance to be exposed to lab safety procedure, a prerequisite for working in laboratories.

It is expected by the end of this practical class, students should be able to:

1. Understand and follow the policy and procedure for Lab safety
2. Understand the principle for extraction and purification of DNA
3. Determine the purity and concentration of the isolated DNA
4. Identify different applications and uses of purified DNA

DNA isolation and purification:

Sample: Nucleated cells
Principle: Lysis of cells, removal of contaminants and isolation of pure DNA
Measurement: UV absorbance at 260nm and 260/280 ratio for the determination of concentration and purity of DNA, respectively

Applications:

Purified DNA can be used for:

1. **Molecular diagnosis of diseases** (e.g., sickle cell anemia)
2. **Forensic applications** (e.g., paternity testing)
3. **Molecular biology research:**
   a. **Amplification techniques:** polymerase chain reaction (**PCR**).
   b. **Southern blotting:** Detection of specific DNA (gene) by hybridization between target DNA sequence and the labeled probe.
   c. **Restriction Fragment length polymorphism (RFLP):** Digestion of DNA by specific restriction enzymes and separation of digestion products (DNA fragments) by electrophoresis.

Protocol for DNA extraction and purification (QIAamp DNA blood Kit, Qiagen)

Sample: Whole blood sample (mononuclear blood cells).

Reagents (Qiagen Kit):

1. Protease stock solution
2. Lysis buffer: Buffer AL
3. Wash buffer 1: AW1
4. Wash buffer 2: AW2
5. Elution buffer: AE
6. Spin columns and Collection tubes

Other reagents:

Absolute ethanol (96 – 100 %)

Equipments:

- Automatic pipettes
- Microcentrifuge
- Vortex
- Water bath
- UV-spectrophotometer

Preparation:

1. Allow samples and reagents to equilibrate at room temperature (15–25°C)
2. Adjust a water bath to 56 °C.
3. Ensure that protease, AW1 and AW2 are prepared according to manual.
4. If precipitate has formed in buffer (AL), dissolve by incubating at 56 °C.

Steps for DNA Extraction:

A. Lysis

1. Pipette 20 µl Qiagen protease into the bottom of tube.
2. Add 200 µl whole blood sample.
3. Add 200 µl buffer AL and mix by pulse-vortex for 15 seconds.
4. Incubate at 56 °C for 10 minutes.
5. Brief centrifuge to remove drops from the inside of the lid.
6. Add 200 µl ethanol (96 –100 %), mix by pulse-vortex for 15 seconds and brief centrifuge to remove drops from the inside of the lid.

B. Binding

7. **Carefully apply the mixture from step 6 to the QIAamp spin column** (in a 2 ml collection tube) without wetting the rim, close the cap.
8. Centrifuge at 6000 x g (8000 rpm) for 1 minute.
9. Place the spin column in a clean 2 ml collection tube and discard the tube containing the filtrate.

C. Wash

10. Carefully open the column and add 500 µl buffer AW1 close the cap.
11. Centrifuge at 6000 x g (8000 rpm) for 1 minute.
12. Place the spin column in a clean 2 ml tube and discard the tube containing the filtrate.
13. Carefully open the column and add 500 µl buffer AW2 without wetting the rim, close the cap.
14. Centrifuge at 20,000 x g (14,000 rpm) for 3 minutes.
15. Place the spin column in a clean 1.5 ml microcentrifuge tube and discard the tube containing the filtrate.

D. Elution of pure DNA

16. Add 200 µl elution buffer AE and incubate at room temperature for 5 minutes.
17. Centrifuge at 6000 x g (8000 rpm) for 1 minute.
18. Save the eluant (pure DNA) and discard the column.

Determination of Purity and concentration of DNA:

Purity of DNA solution:
Determine A260/A280 ratio by spectrophotometer: 1.7 – 1.9 is accepted

Concentration of DNA (µg/ml):
Measure A260
Calculate the DNA concentration (µg/ml) provided
DNA is 50 µg/ml when A260 = 1.0

Yield of DNA: DNA concentration x Total volume of DNA