King Saud University
College of Medicine
Department of Pathology
Clinical Biochemistry unit

DNA

Extraction and Purification

Biochemistry Practical Class
Foundation Block

By the end of this practical class, the students should be able to:

1. Understand the principle behind DNA extraction and purification.
2. Perform DNA extraction, purification and measurement according to the provided protocol (spin protocol).
3. Interpret the results in terms of quantity, purity and yield.
4. Have a knowledge about some molecular techniques and applications.

Work plan:

1. The presentation about DNA extraction and purification will be given for (20 minutes).
2. Hands-on practical for DNA purification from whole blood using spin protocol will be conducted for about (60 minutes).
3. Measuring the quantity and purity of the purified DNA by UV-spectrophotometer.
4. Students will be requested to record their results in the provided report sheet, commenting on the quantity and purity of the DNA they purified. DNA yield is also requested to be calculated.
5. Sign for attendance.

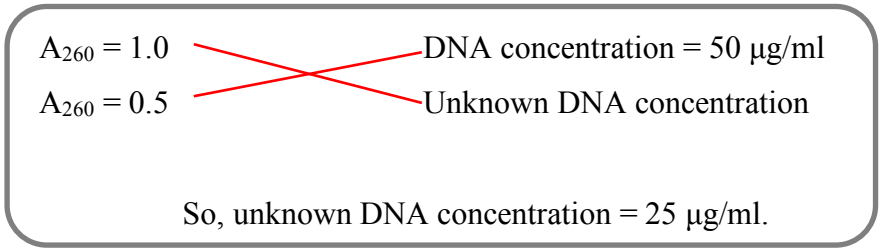
Principle:

Genomic DNA is extracted from peripheral blood samples preserved in EDTA using QIAamp DNA Blood Mini Kit, spin protocol. The principle of the test includes lysis of the nucleated cells using lysis buffer, which has high salt concentration that breaks the cellular membrane; after the lysing step, DNA is allowed to bind to the spin column membrane for separating the DNA from the cell debris; removal of the contaminants with wash buffers; and elution of pure DNA. The measurement of the purified DNA is performed by UV absorbance at 260nm and 280nm. DNA concentration is determined by measuring at 260nm, and the purity of the purified DNA is determined on the bases of 260nm/280nm ratio.

A pure DNA falls in the accepted ratio, which ranges from 1.7 up to 1.9.

Calculations:

DNA concentration (µg/ml) is estimated by measuring the absorbance at 260nm (A_{260}), and using the relationship that an A_{260} of 1.0 = 50 µg/ml of pure DNA, the concentration of unknown sample can be calculated by cross multiplication as in the following example:

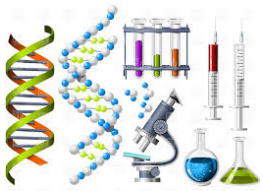


For DNA yield:

DNA Yield = DNA Volume x final DNA Conc.

e.g. provided volume of DNA solution = 200µl (0.2 ml), final DNA concentration = 30 µg/ml, then, the yield (µg) = 0.2 ml x 30 µg/ml

= 6.0 µg



Experimental protocol:

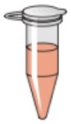
A. Laboratory safety policy and procedures **MUST** be strictly followed.

B. Carefully read the protocol before you proceed to the hands-on practical.

C. Work in groups consist of 5-6 students each and perform the following procedure for the provided blood samples:

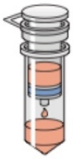
Video

https://www.youtube.com/watch?v=gmNw6CWtN5k

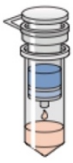


1. Pipette **20µl** protease.
2. Add **200µl** sample.
3. Add **200µl** Buffer **AL**.

4. Mix by pulse-vortex for 15s and incubate at **56˚C** for **10 minutes**.
5. Briefly centrifuge.
6. Add **200µl** 96-100% ethanol and mix by pulse-vortex for 15s.
7. Briefly centrifuge.

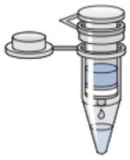


8. Apply the mixture to the Mini spin column.
9. Centrifuge at **8000 rpm** for **1 minute**.



10. Discard the collection tube.
11. Place the column in a new 2ml collection tube.
12. Add **500µl** buffer **AW1**.
13. Centrifuge at **8000 rpm** for **1 minute**.

14. Place the column in a new 2ml collection tube.
15. Add **500µl** buffer **AW2**.
16. Centrifuge at **14000 rpm** for **3 minutes**.
17. Place the column in a new 2ml collection tube.
18. Centrifuge at **14000 rpm** for **1 minute**.



19. Place the column in a new 1.5ml tube.
20. Add **200µl** buffer AE.
21. Incubate at room temperature for **1 minute**.
22. Centrifuge at **8000 rpm** for **1 minute**.

Add 400µl buffer AE to dilute the eluted DNA (3X dilution)

23. Quantify the DNA concentration.

Experimental Report:

Students' Names/ IDs:

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Readings:

260nm: .....

280nm: .....

260nm/280nm ratio: .....

Concentration: .....

Purity: .....

Comments on the purity: .....

DNA yield calculation:

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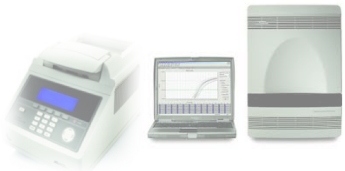
DNA yield = ...

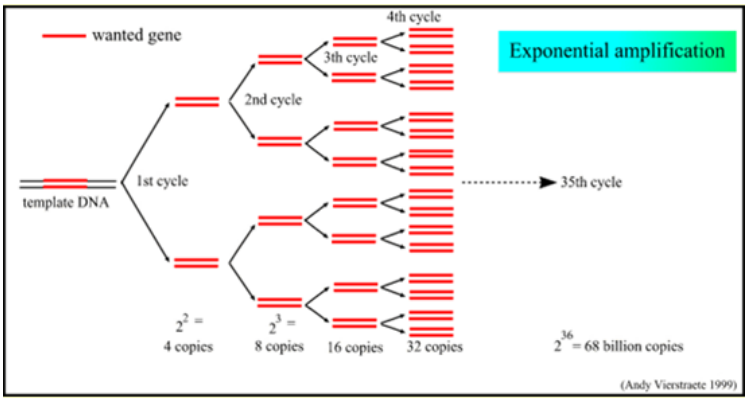
Molecular Techniques and Applications

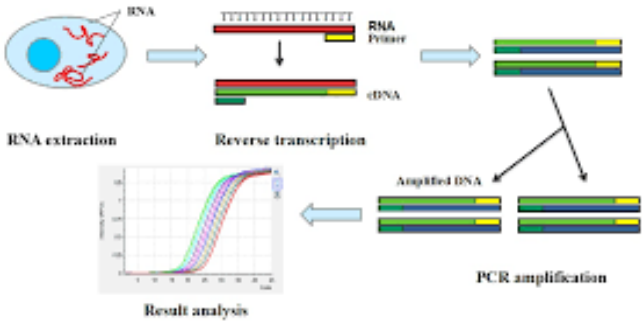


Amplification techniques: ***e.g. Polymerase Chain Reaction (PCR)***

PCR is a technique used to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

There are several types of **PCR** techniques. e.g. conventional PCR and Real-Time PCR.





The major advantages of **PCR** are sensitivity and speed, in comparison to other amplification techniques.

Applications of PCR:

- Comparison of a normal gene with a mutant form of the gene.
- Detection of low-abundance nucleic acid sequences.
- Forensic analysis of DNA samples.
- Prenatal diagnosis.

Other examples molecular techniques:

For more information about the following techniques, please see the links QR codes below.

1. Restriction Fragment length polymorphism (RFLP).

   https://www.ncbi.nlm.nih.gov/probe/docs/techrflp/

2. Southern blotting.

   http://www.onlinebiologynotes.com/southern-blotting-principle-procedure-application/