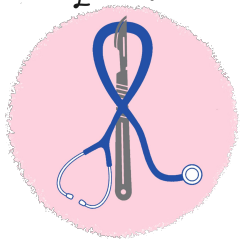
MED441
KING SAUD UNIVERSITY

Biochemistry O.S.P.E.

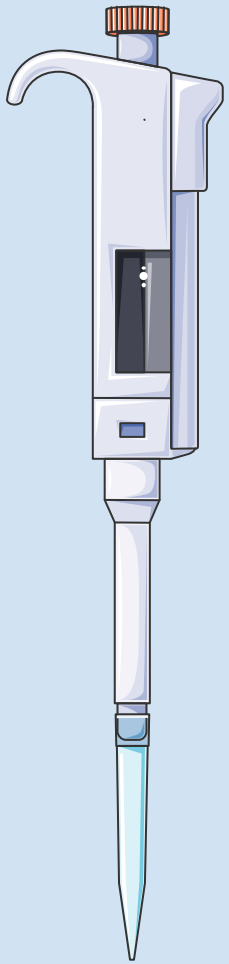
Revised & Reviewed by:
Abdulaziz & Bahammam
Faye Wael Sendi



Color Index:

- Main text
- Important
- Notes
- Boys slides'
- Girls slides'
- Extra

Editing File



Lab orientation

Objectives

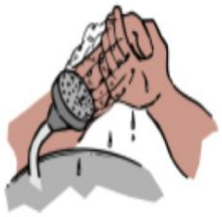
The student should be able to understand and become familiar with:

- General safety rules followed in biochemistry laboratory.
- Safety with laboratory equipment.
- Basic emergency procedure.
- Biological safety and waste disposal.
- The basics of spectrophotometer and general equipment to be used in the lab during biochemistry practical sessions.
- Procedure to follow in the event of a fire emergency.

General safety rules

Always use appropriate clothes & personal protective tools:
- Lab coat.
- Safety goggles.
- Masks.
- Gloves.
- No open shoes.
- No eye lenses.

- After handling chemicals, always wash your hands with soap and water.
- During lab work, keep your hands away from your face.
- Tie back long hair.



- Roll up loose sleeves.
- Know the location of the fire extinguisher, fire blanket, eyewash station, and first aid kit.
- Keep your work area uncluttered.
- Take to the lab station only what is necessary.

- It is suggested that you wear glasses rather than contact lenses.
- Never eat or drink during a lab work.

Safety with laboratory equipment

Never use any laboratory equipment unless you are trained & have been authorised to do so.

As well as injuring yourself you may cause very costly damage.

Electrical safety

Lay electrical cords where no one can trip on them.

Be sure your hands and your lab area are dry before using electrical equipment.

Unplug cords by pulling the plug and not the cord.

Fire safety - R.A.C.E

Procedure to follow in the event of a fire emergency:

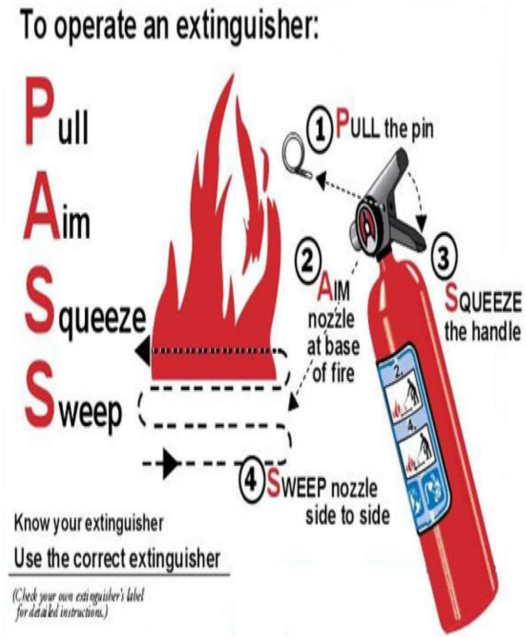
- **R** — Remove or secure individuals in immediate danger.
- **A** — Activate the alarm by pulling a fire pull station located in the corridors and calling 953.
- **C** — Confine the fire by closing windows, vents, and doors.
- **E** — Evacuate to a safe area.

Fire extinguisher

whole slide

To operate extinguisher:

- **P** — **Pull** the pin.
- **A** — **Aim** nozzle at base of fire.
- **S** — **Squeeze** the handle.
- **S** — **Sweep** nozzle side to side.



Biological safety

All biological samples are considered potentially infectious.

Should be handled and processed using strict precautions.

Waste disposal

whole slide

For disposal of contaminated waste, use containers with yellow plastic bags.

Regular waste like papers etc go into containers with black/white plastic bags.

All sharp objects such as needles, scalpels and even broken glassware go into yellow-red sharp container.

Clinical biochemistry laboratories

- Routine Biochemistry / STAT Bench Lab
- Endocrinology Lab
- Inherited Metabolic Lab
- Toxicology Lab
- Newborn screening lab
- Receiving Bench

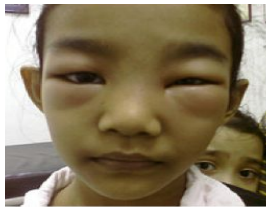
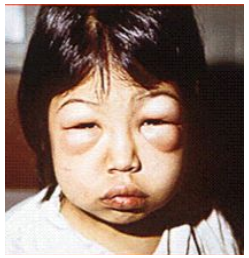
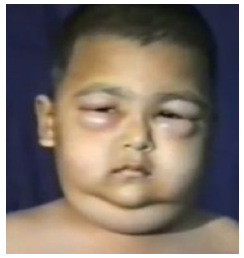
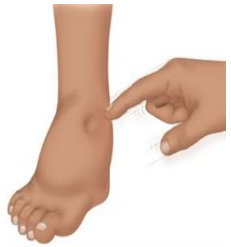
Biochemical test profiles

Cardiac Profile	-S.Creatinine kinase -S.Lactate dehydrogenase -S.Troponin
Renal Profile	-Blood urea -S.Creatinine -S.Electrolytes (Na, K & Cl)
Lipid Profile	-S.Triglycerides -S.Cholesterol -S.HDL-cholesterol -S.LDL- cholesterol
Hepatic Profile	-S.Total proteins -S.Albumin -S.Alanine & Aspartate aminotransferases (ALT & AST)
Bone Profile	-S. Calcium -S. Phosphorus -S. Alkaline Phosphatase -S. Vitamin D
Glucose (diabetic) Profile	-S. Fasting glucose -S. 2 hours postprandial glucose -S. Random glucose -S. Glycosylated hemoglobin

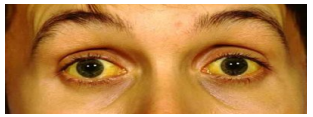
Clinical biochemistry for diagnosis of disease

Biochemical laboratory test are crucial for diagnosis of many human diseases:

Kidney diseases → e.g., nephrotic syndrome

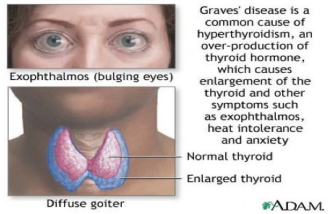
Liver diseases → e.g., hepatitis and jaundice




Metabolic diseases → e.g., diabetes mellitus

Endocrine diseases → e.g., Thyrotoxicosis

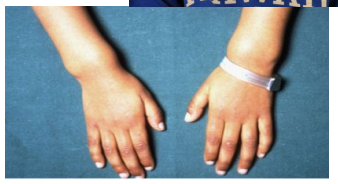
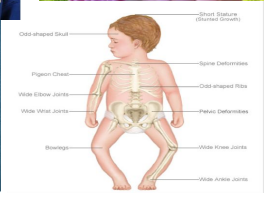




Cancers & malignancy → e.g., Prostate cancer

Inherited diseases → e.g., PKU (phenylketonuria)

Skeletal disorder → e.g., Rickets

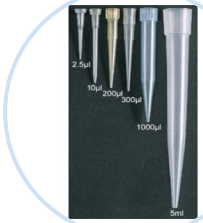



Lab Equipment

whole slide

Automatic pipettes

UV-spectrophotometer



Tips

Rack- eppendorf tube

Vortex

Cuvettes

Water bath

Rack- test tube

Microcentrifuge

Eppendorf tube

Spectrophotometer

whole slide

Most of visible spectrometer are composed

- **Light source** which works with visible wavelengths (400-700 nm).
- **Monochromator filter** for choosing desired wavelength.
- **Sample holder (cuvette).**
- **Detector.**
- **Meter or recorder.**

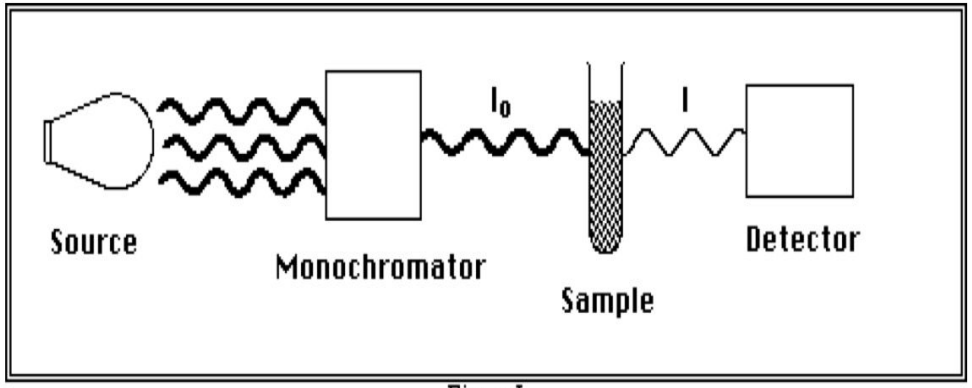
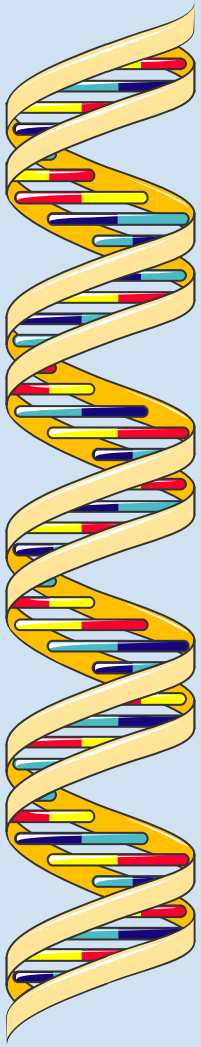


Figure I



DNA Extraction and Purification

Objectives

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

Principle:

Special thanks To MED439

whole slide

Genomic DNA is extracted from peripheral blood samples preserved in EDTA using QIAamp DNA Blood Mini Kit, spin protocol.

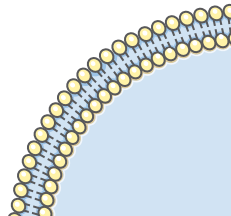
The principle of the test includes **lysis** of the nucleated cells using lysis buffer, which has high salt concentration that breaks the cellular membrane; after the lysing step, DNA is allowed to **bind** to the spin column membrane for separating the DNA from the cell debris; **removal (wash)** of 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.**

1-Lysis of the nucleated cells

2-Removal of contaminants (any other substance other than DNA e.g.,proteins)

3-Measurements



Lab Equipment: (Tools)

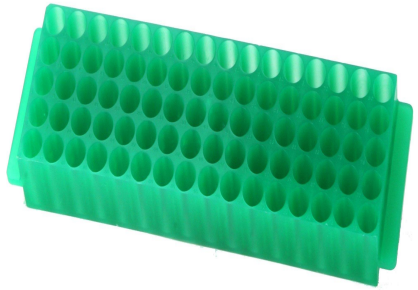
whole slide

Automatic pipettes

Eppendorf tube

Rack-test tube

Tips



Rack- eppendorf tube

Cuvettes

Lab Equipment: (Machines)

names only

هو جهاز يدور الجسم الموضوع داخله حول محور ثابت حيث يتسبب تسارع الجاذبية الناتج عن سرعة الدوران حول محور ثابت في اكتساب المواد ذات الكثافة تسارعا مختلفا عن المواد ذات الكثافة الأقل مما يسبب في فصل المكونات ذات الكثافات المختلفة. إذن، تترسب المواد الأثقل في أسفل الأنبوب تليها المواد الأخف فالأخف.

Microcentrifuge

هو جهاز بسيط يستخدم عادة في المختبرات لخلط قارورة صغيرة من السائل

Vortex

Steps :
1. Lysis of nucleated cells using lysis buffer.
2. Binding of DNA to the membrane of spin column
3. Wash: using wash buffer.
4. Elution of pure DNA.

Water bath

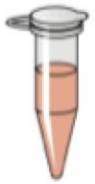
We use it for DNA quantitation

UV-spectrophotometer

Spin Protocol of DNA Purification from Blood

Helpful video

يكون موجود Protease 3 , 2 ,1 بالأنبوب جاهز
نضيف عليه العينة (عينة الدم) ثم Buffer AL



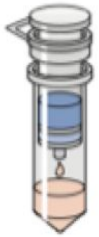
1. Pipette **20µl** protease.
2. Add **200µl** sample.
3. Add **200µl** Buffer **AL**.

in water bath

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.

Use pipettes

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

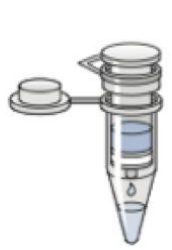


10. Discard the collection tube.
11. Place the column in a new 2ml collection tube.
12. Add **500µl** buffer **AW1**.
13. Centrifuge at **8000 rpm** for **1 minute**.

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**.

skip

we have to check that all filtrate is in the collection tube if there is still some in the spin column just do 17 , 18 steps



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**.

After step 22. remove the upper part then add buffer AE to

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

23. Quantify the DNA concentration.

After adding the buffer AE put the mixture in cuvettes tube by pipettes then into UV-spectrophotometer then do step 23

Cuvettes

Quantification of the purified DNA

whole slide

Measurements:

- Measure the Absorbance at 260nm and at 280nm.
- Assess the DNA purity= 260/280 ratio

  (Accepted ratio: 1.7 - 1.9)

المعطى هذا غالبا ثابت فيحفظ

- Calculate DNA Conc. : Provided A260 = 1.0, DNA is 50 µg/ml, unknown DNA Conc. can be calculated by cross multiplication (Cross multiplication)

A260 = 1.0 — DNA conc. = 50 µg/ml

A260 = 0.5 — DNA conc. ?

DNA Conc.(µg/ml) = (0.5 x 50µg/ml) / 1 = 25 µg/ml

مهم
إذا قال
3 times dilution
اضرب الناتج النهائي ب3 (أو الرقم المحدد)

Note: In case of diluting the eluted sample, multiplies the final concentration by the dilution factor. This can be adjusted by the spectrophotometer.

DNA Yield

whole slide

DNA Yield (µg) = DNA Volume(ml) x final DNA Conc.(µg/ml)

يكون µl ولازم نحوله ل ml
200µl -> 0.2ml
ضروري نسوي التحويلة

Example:

If you have:

1-Volume of DNA solution: 200 µl (0.2 ml)

2- Final DNA Conc.: 30 µg/ml

Then, the yield (µg)

= 0.2 ml x 30 µg/ml

= 6.0 µg

Molecular Techniques and Applications

Note: Almost all molecular biology techniques can be utilized for diagnosis and research

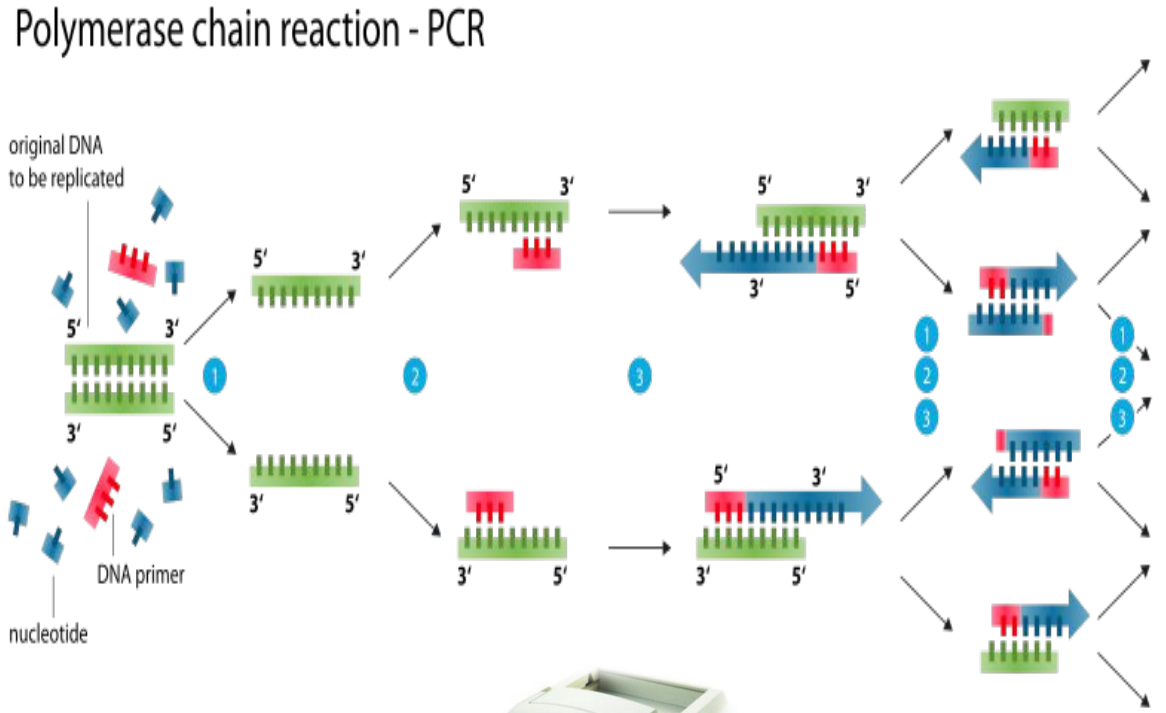
name?
for?
apps?

Amplification techniques:

e.g. Polymerase Chain Reaction (PCR)

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 of molecular techniques:

1- Restriction Fragment length polymorphism (RFLP).

2- Southern blotting.

Summary

How to Calculate DNA Purity?

A260/A280

Example:
If you have:
A260 = 0.50 A280 = 0.35
Then
Answer: 0.50/0.35 = 1.428 (not pure because DNA purity should be between 1.7 and 1.9)

How to Calculate DNA Conc.?

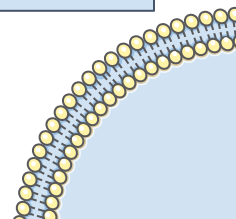
260nm x 50µg/ml

Example:
If you have:
A260 = 0.50
Then
Answer: 0.50 x 50 µg/ml = 25 µg/ml

How to Calculate DNA Yield?

0.2 x Final DNA Conc. = Yield

Example:
We will use the conc. from the previous question
Conc = 25 µg/ml
Then
Answer: 0.2 ml x 25 µg/ml = 5 µg



Quiz

Q1: What does RACE stand for?

Q2: Identify these devices.

Q3: Calculate the DNA conc. & the purity ?

If A260= 0.52 , A280= 0.29

Q4: Calculate the DNA yield.

-If the volume of DNA solution 200µl (0.2ml)
-final DNA Conc. 20µg/ml

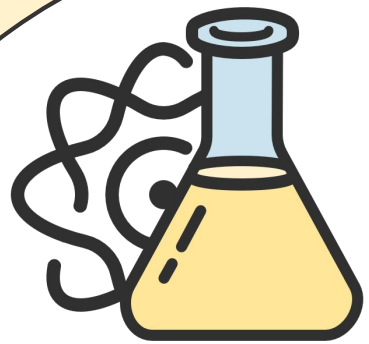
Quiz answers:

Q1: R: Remove. A: Activate. C: Confine. E: Evacuate.

Q2:  Eppendorf tube.  Vortex.

Q3: -DNA Conc. = (0.52 x 50 µg/ml) / 1 = 26 µg/ml
-DNA purity = 260nm / 280nm ratio = 0.52/0.29 = 1.79, it is pure because it is between 1.7-1.9

Q4: DNA yield (µg) = DNA volume x final DNA conc.
-The yield = 0.2 ml x 20 µg/ml = 4 µg



Biochemistry 441

Girls

Boys

Ghadah Alarify - Leader

Yara Almufleh
Reema Alrashedi
Wareef Almousa
Joud Alangari
Fay Alluhaidan
Sarah Alhamlan
Arwa Almobeirek
Jumana AL-qahtani
Latifa Alkhdiri
Alanoud Alhaider
Futoon Almotairi
Manal Aldhirgham
Raaoum Jabor
Norah alawlah
Shahad Helmi
Rand Aldajani

Khalid Alhamdi - Leader

Ahmed Alayban
Sultan Alosaimi
Abdullah Alomran
Bassam Alghizzi
Ibrahim Aljurayyan
Mohammed Almutairi
Turki Alkhalifa
Malik Alshaya
Faisal Alhmoud
Abdulrahman Alnoshan
Ahmed Alqahtani
Hamad Alshaalan
Anas Alharbi
Mohammed Alwahibi
Saad Alghadir
Firas Alqahtani

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