

LAB ORIENTATION

Editing File

▣ **Color Index:**

➤ **Original slides.**

➤ **Important.**



Biochemistry team 438

Objectives:

- General safety rules followed in Biochemistry laboratory
- Safety with laboratory equipment
- Basic emergency procedures
- Biological safety and waste disposal
- The basics of spectrophotometer and general equipment to be used in the lab during Biochemistry practical sessions



General safety rules:

- Lab safety is everyone's responsibility
- Lab safety policy and procedures must be strictly followed



Always use appropriate clothes and 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:

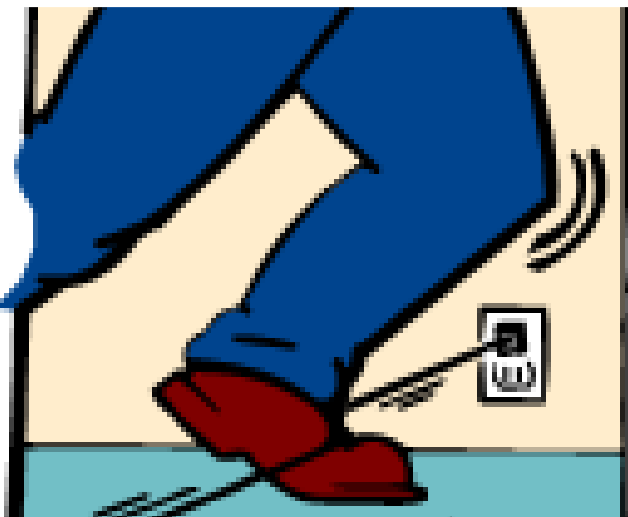
Never use any
laboratory equipment
unless you are trained &
have been authorised to
do so

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:

**IN CASE OF FIRE
REMEMBER RACE**

R 'Rescue'
ANY PERSONS IN
IMMEDIATE DANGER



A 'Alarm'
ALERT OTHERS BY
ACTIVATING ALARM
and calling **953**.



C 'Contain'
THE EMERGENCY BY
CLOSING DOORS
, Windows and vents



E 'Evacuate'
To a safe area



Fire extinguisher:

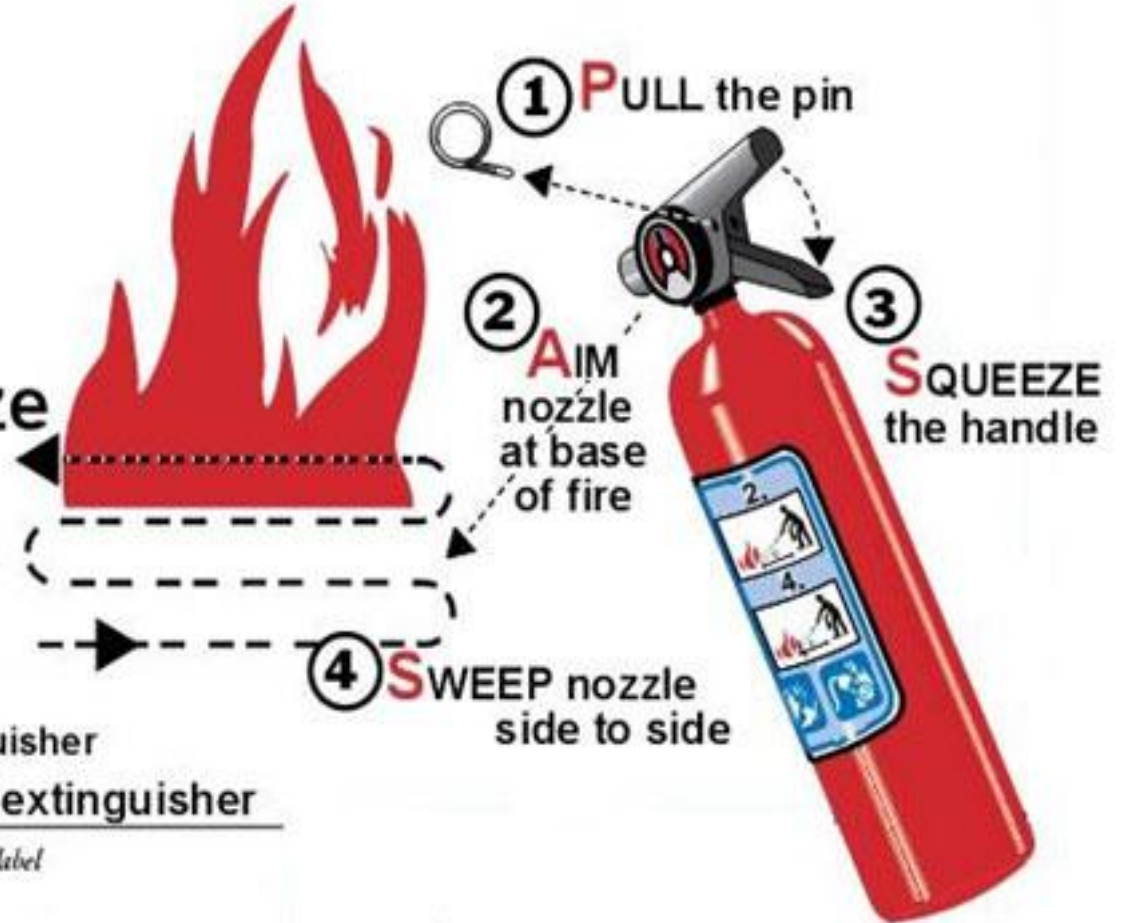
To operate an extinguisher:

Pull

Aim

Squeeze

Sweep



**Know your extinguisher
Use the correct extinguisher**

*(Check your own extinguisher's label
for detailed instructions.)*

Biological safety:

All biological samples are considered **potentially infectious**



Should be handled and processed using **strict precautions**

Waste disposal:



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 sharps container**



A framed sign is mounted on a grey stone wall. The sign has a white border and a black center with white text. To the right of the sign, a light blue DNA double helix is visible, extending from the top right towards the bottom right of the frame.

**SOME SUPERHEROES
DON'T WEAR CAPES...**

**THEY ARE CALLED
DOCTORS.**

Clinical biochemistry laboratories:

Routine
Biochemistry/
STAT Bench
Lab

Endocrinology
Lab

Inherited
Metabolic Lab

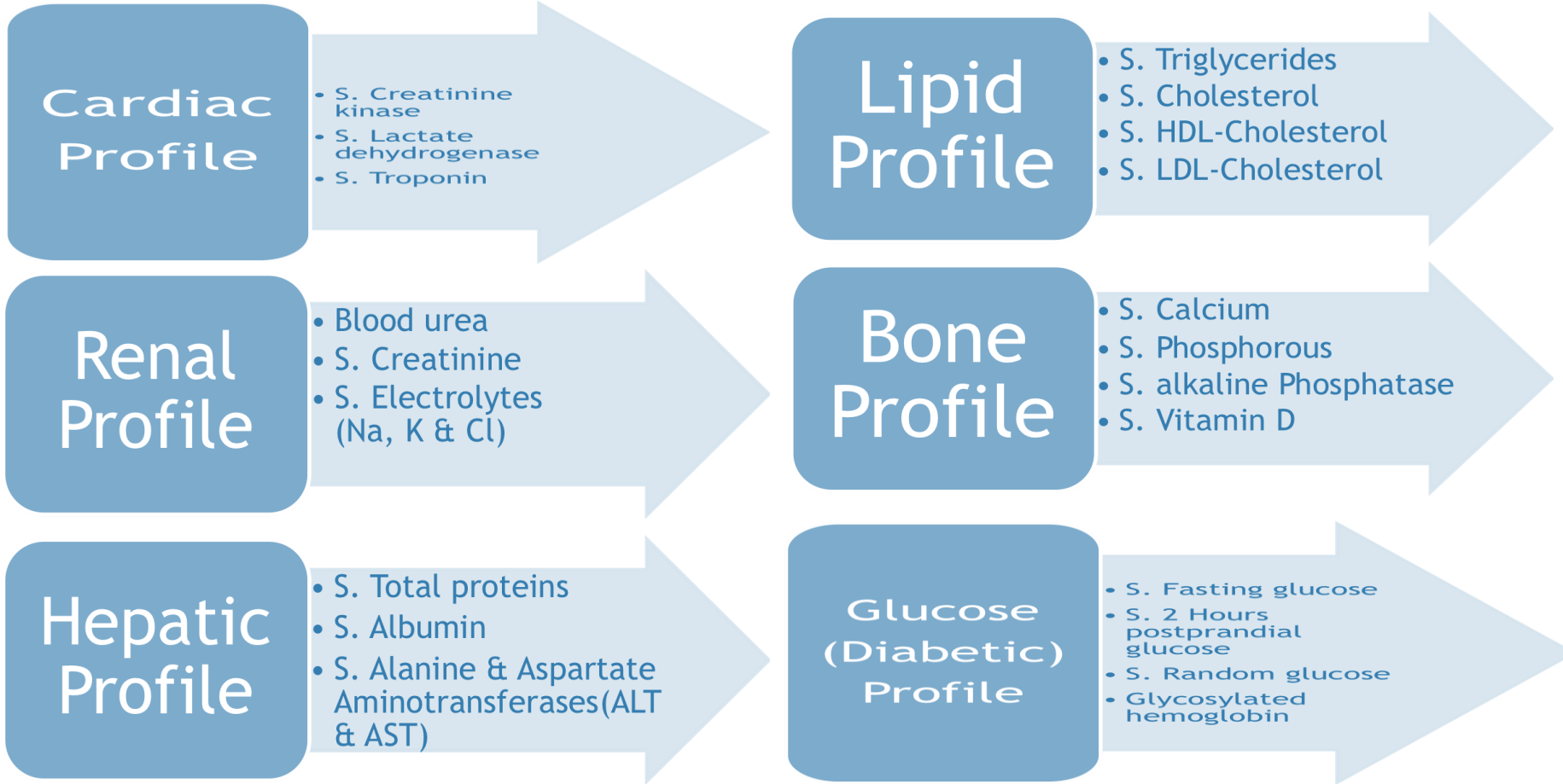
Toxicology Lab

Newborn
Screening Lab

Receiving Bench



biochemical test profiles:



Clinical biochemistry for diagnosis of diseases:

Biochemical laboratory tests are crucial tools for diagnosis of many human diseases:

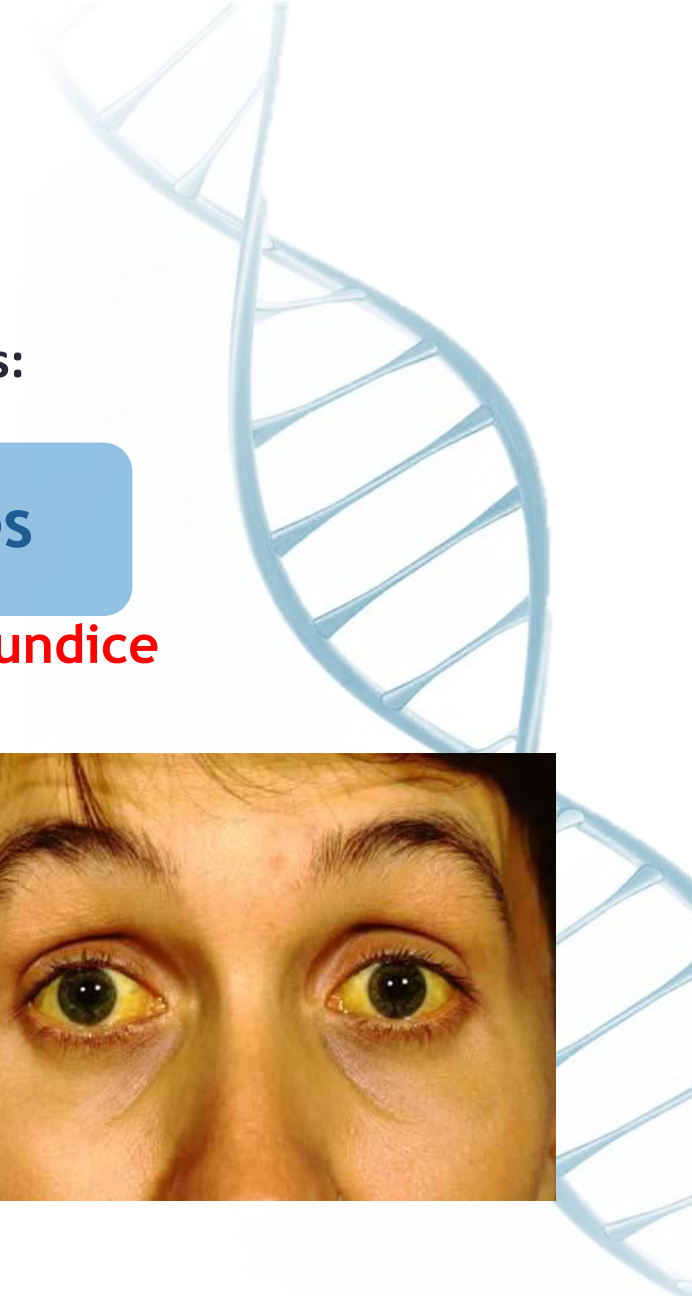
Kidney diseases

e.g., nephrotic syndrome



Liver diseases

e.g., hepatitis and jaundice



Metabolic diseases

e.g., diabetes mellitus

Cancers & malignancy

e.g., prostate cancer

Endocrine diseases

e.g., Thyrotoxicosis

Inherited diseases

e.g., PKU

Phenylketonuria (fen-ul-key-toe-NU-ree-uh), also called PKU, is a rare inherited disorder that causes an amino acid called phenylalanine to build up in the body. PKU is caused by a defect in the gene that helps create the enzyme needed to break down phenylalanine



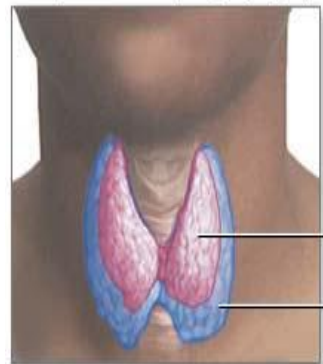
Exophthalmos (bulging eyes)



Graves' disease is a common cause of hyperthyroidism, an over-production of thyroid hormone, which causes enlargement of the thyroid and other symptoms such as exophthalmos, heat intolerance and anxiety

Normal thyroid

Enlarged thyroid

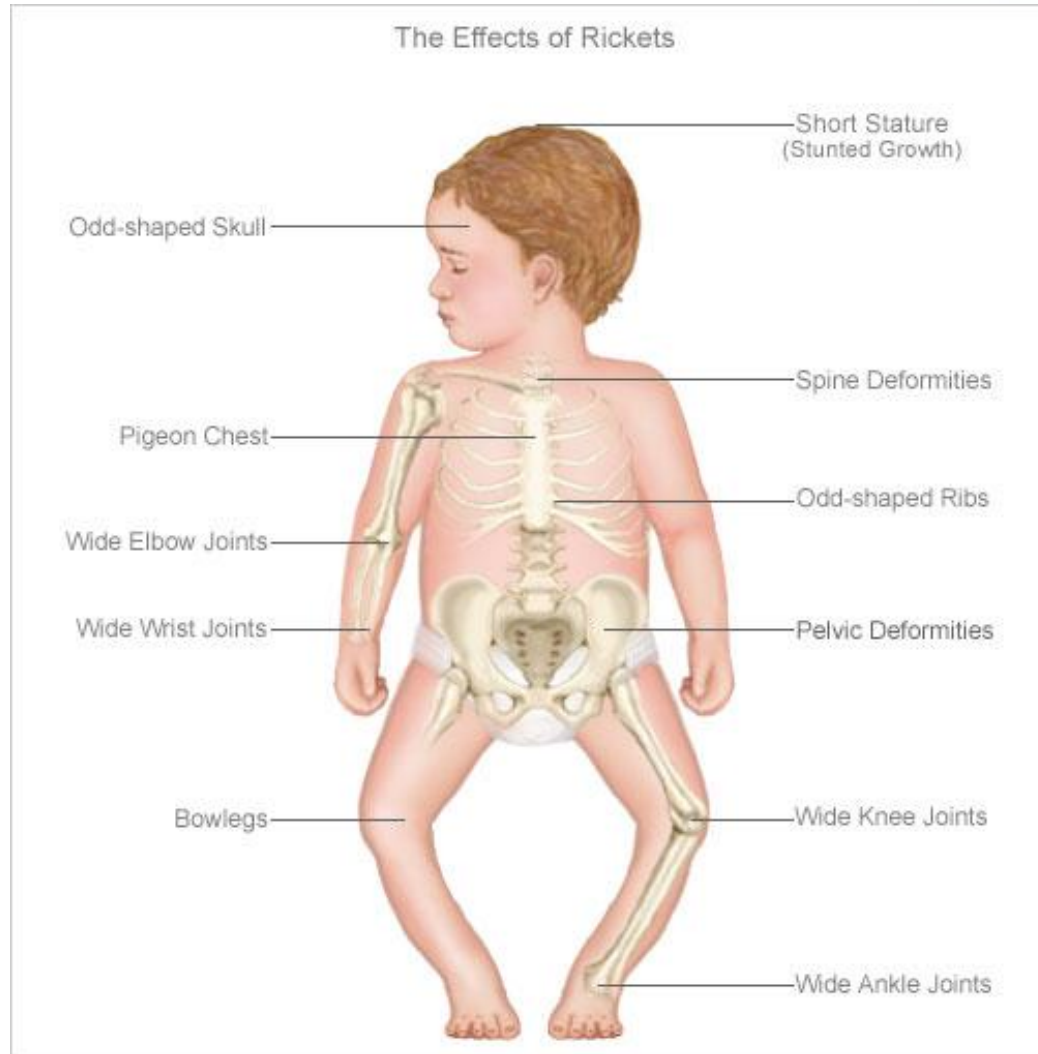


Diffuse goiter

ADAM.



Rickets:



Spectrophotometer: **Very important with spilling*

spectrophotometers are composed of:

Light source which works with visible wavelengths (400-700 nm)

monochromator filter for choosing desired wavelength

Sample holder (cuvette)

Detector

Meter or recorder

UV light

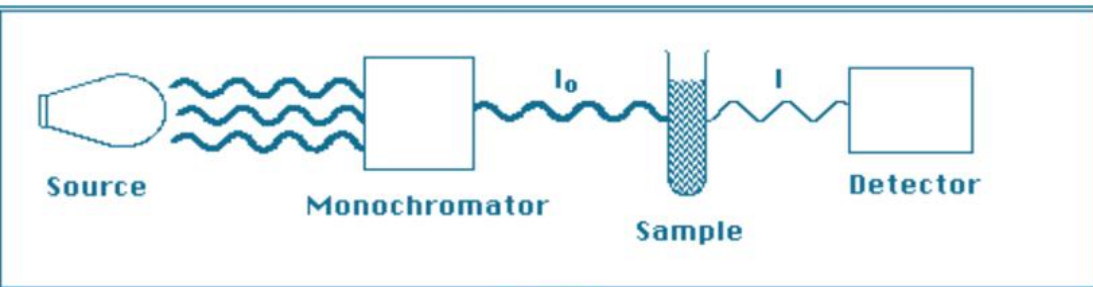
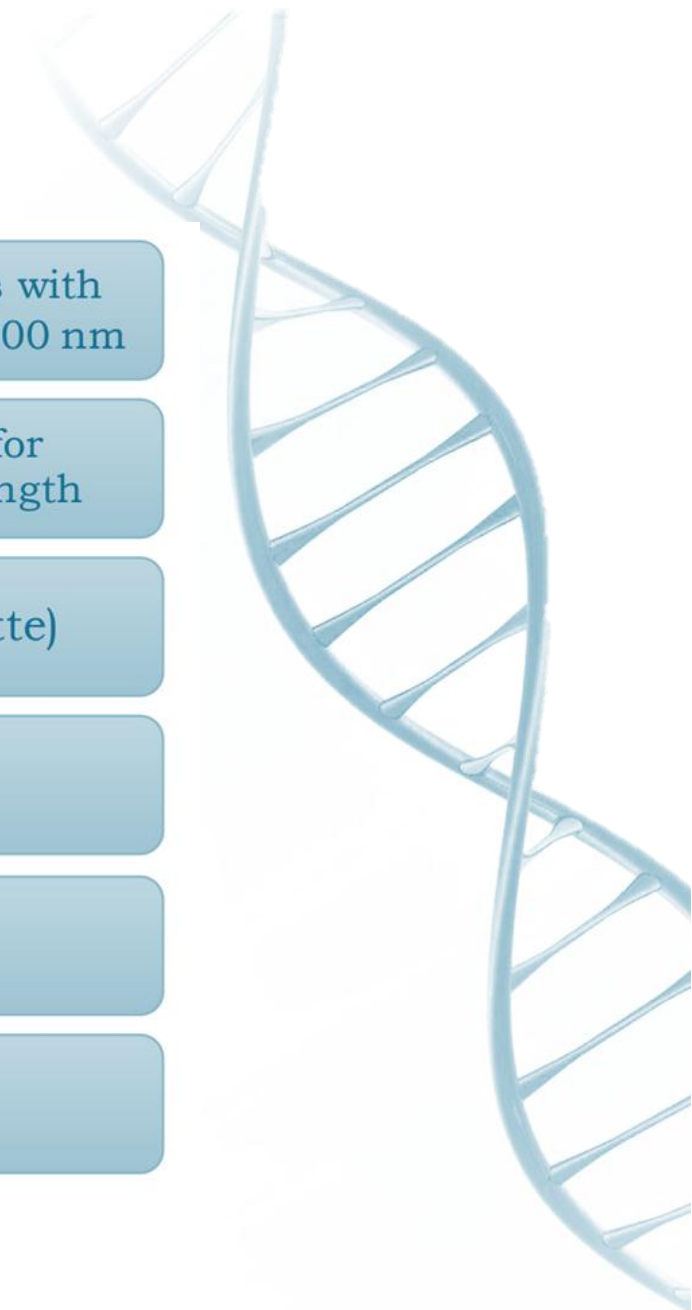


Figure 1



DNA EXTRACTION AND PURIFICATION

Color Index:

- Original slides.
- Important.



Biochemistry team 438

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:

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.



Lysis of the
nucleated cells

Removal of
contaminants
(any other substance other than
DNA)

measurements

Lab Equipment:

Automatic pipettes



Vortex



Microcentrifuge



Water bath

UV-spectrophotometer



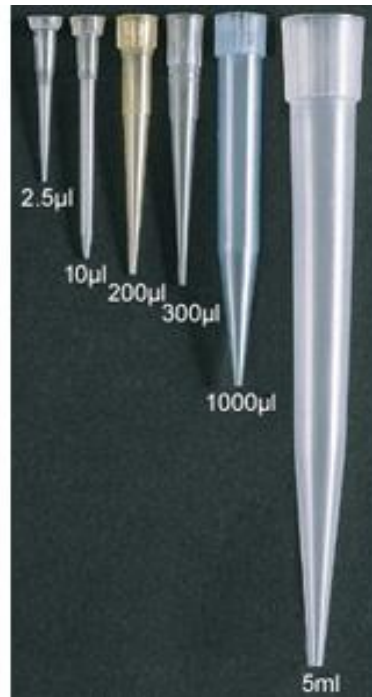
Continued...



Eppendorf tube



Cuvettes



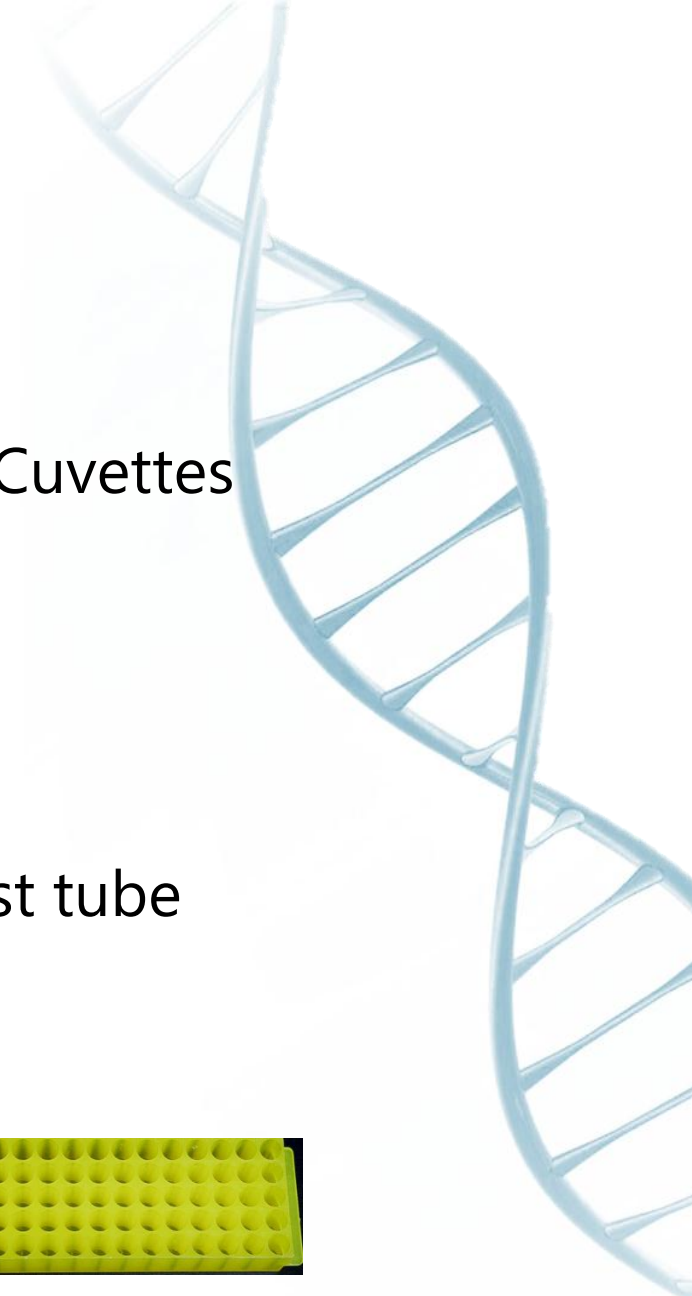
Tips



Rack- test tube



Rack- eppendorf tube



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.



Spin Protocol of DNA Purification from Blood

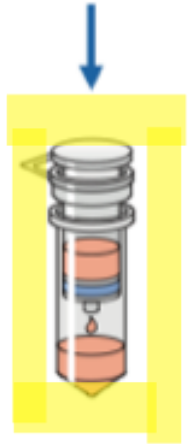




1. Pipette **20µl** protease.
2. Add **200µl** sample.
3. Add **200µl** Buffer **AL**.

1,2,3. Protease يكون موجود
بالأنبوب جاهز نضيف
عليه عينه الدم ثم
buffer AL

Water path



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**.



Video

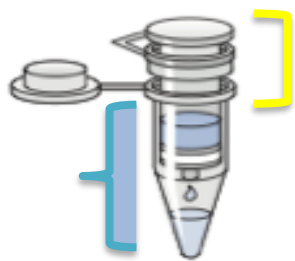


<https://www.youtube.com/watch?v=gmNw6CWtN5k>



- 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



- 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

Quantification of the purified DNA



Measurements:

- Measure the Absorbance at **260nm** and at **280nm**.

الجهاز يحسبها لك

- **Assess the DNA purity = 260/280 ratio**

(Accepted ratio: **1.7 - 1.9**)

- **Calculate DNA Conc.:** Provided $A_{260} = 1.0$, DNA is $50 \mu\text{g/ml}$, unknown DNA Conc. can be calculated by cross multiplication

Done by
the spectrophotometer

$$A_{260} = 1.0$$

$$\text{DNA conc.} = 50 \mu\text{g/ml}$$

$$A_{260} = 0.5$$

$$\text{DNA conc. ?}$$

القاعده باختصار و ثابتته: $\times 3 \times 50 \mu\text{g/ml}$ القراءة عند **260nm** = DNA concentration

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:

$$\text{DNA Yield } \mu\text{g} = \text{DNA Volume} \times \text{final DNA Conc.}$$

Example:

If you have

Volume of DNA solution: 200 μ l (0.2 ml)

Final DNA Conc.: 30 μ g/ml

$$\begin{aligned} \text{Then, the yield } (\mu\text{g}) &= 0.2 \text{ ml} \times 30 \mu\text{g/ml} \\ &= 6.0 \mu\text{g} \end{aligned}$$

=0.2 ml × concentration μ g/ml: باختصار وثابت



Molecular Techniques and Applications

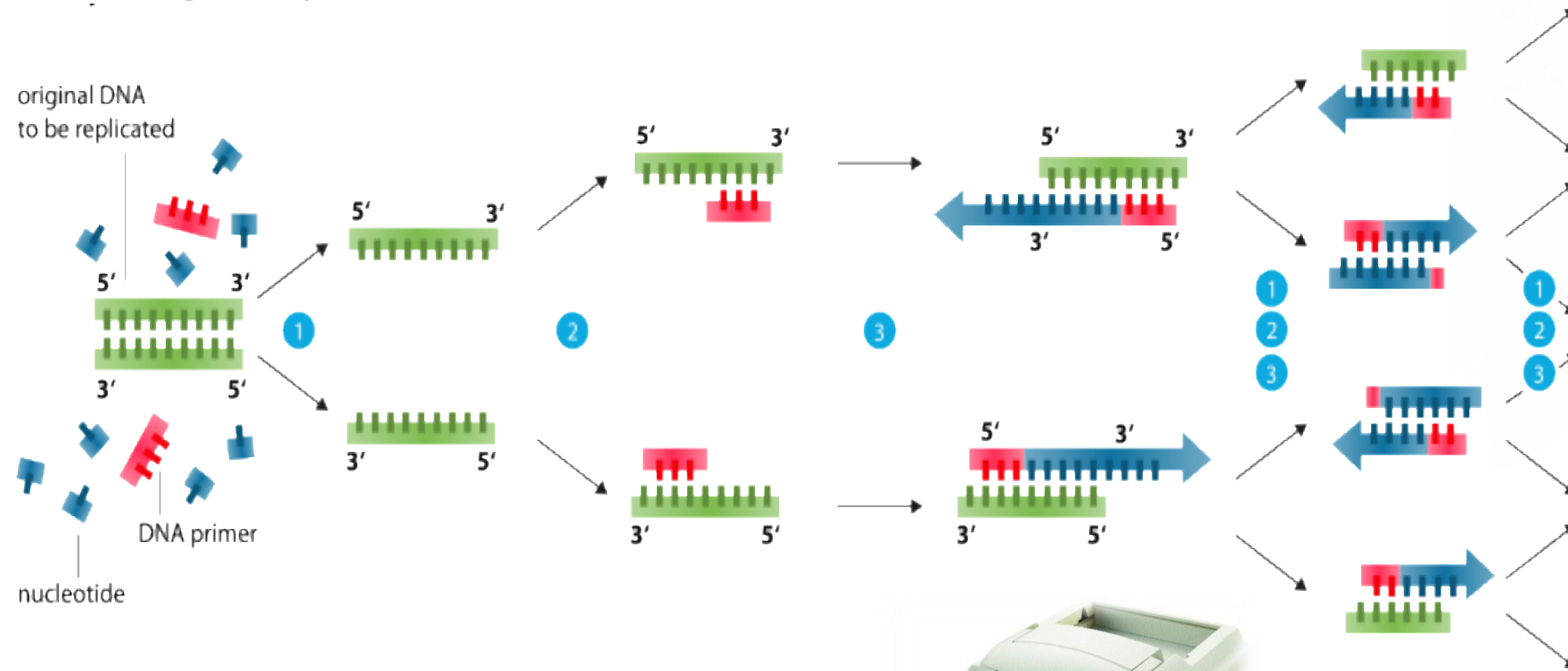
Note:

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



a. Amplification techniques:

e.g. Polymerase Chain Reaction (PCR)



- 1 **Denaturation** at 94-96°C
- 2 **Annealing** at ~68°C
- 3 **Elongation** at ca. 72 °C



Continued...

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).

<https://www.ncbi.nlm.nih.gov/probe/docs/techrfp/>



2. Southern blotting.



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



Review

DNA concentration = 260nm القراءة عند $\times 50 \mu\text{g/ml} \times 3$

DNA Yield μg = DNA Volume x final DNA Conc.



0.2 ml \times concentration $\mu\text{g/ml}$: باختصار وثابت

DNA purity = 260/280 ratio

(Accepted ratio: 1.7 - 1.9)





Readings:

260nm: 0.386
280nm: 0.207
260nm/280nm ratio: 1.86
Concentration: 57.9 µg/ml
Purity: pure
Comments on the purity: Pure because it is on the range 1.7-1.9

DNA yield calculation:

$$\text{DNA yield} = \text{DNA conc.} (\mu\text{g/ml}) \times \text{DNA volume} (0.2) (\text{ml})$$

$$\text{DNA yield} = 57.9 \mu\text{g/ml} \times 0.2 \text{ ml}$$

$$= 11.58 \mu\text{g}$$

$$\text{DNA yield} = 11.58 \mu\text{g}$$

❖ Girls team:

- أجدد آل رشود
- الوئين البلوي
- إيلاف المسيدل
- جود الخليفة
- جود العتيبي
- ريم القرني
- سارة الهلال
- شهد السلامه
- طيف العتيبي
- عبير الخضير
- غيداء البرين
- لينا العصيمي
- نورة التركي
- نورة المزروع
- نوف الحميضي
- هيفاء الوايلي

❖ Boys team:

- بدر الشهري
- حميد حميد
- سهيل باسهيل
- عمر الغامدي
- مهند القرني
- نايف السبر

❖ Team leaders:

ديما المزيد
رائد العجيري



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➤ Special thanks to:



BIO TEAM



Biochemistry Team⁴³⁵



Biochemistry team 436

