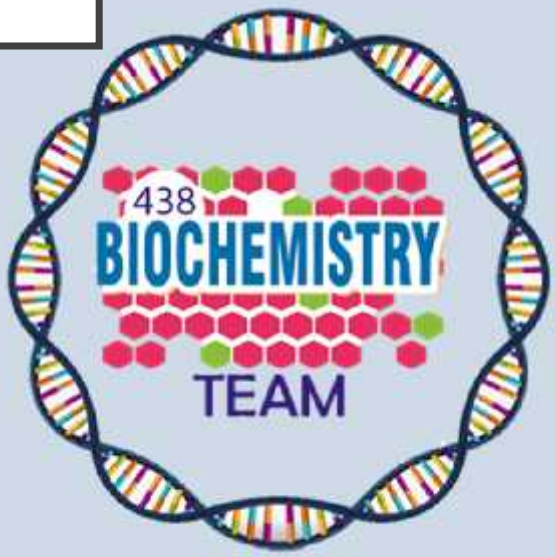
LAB ORIENTATION



Biochemistry team 438

- **Color Index:**
  - **Original slides.**
  - Important.

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:

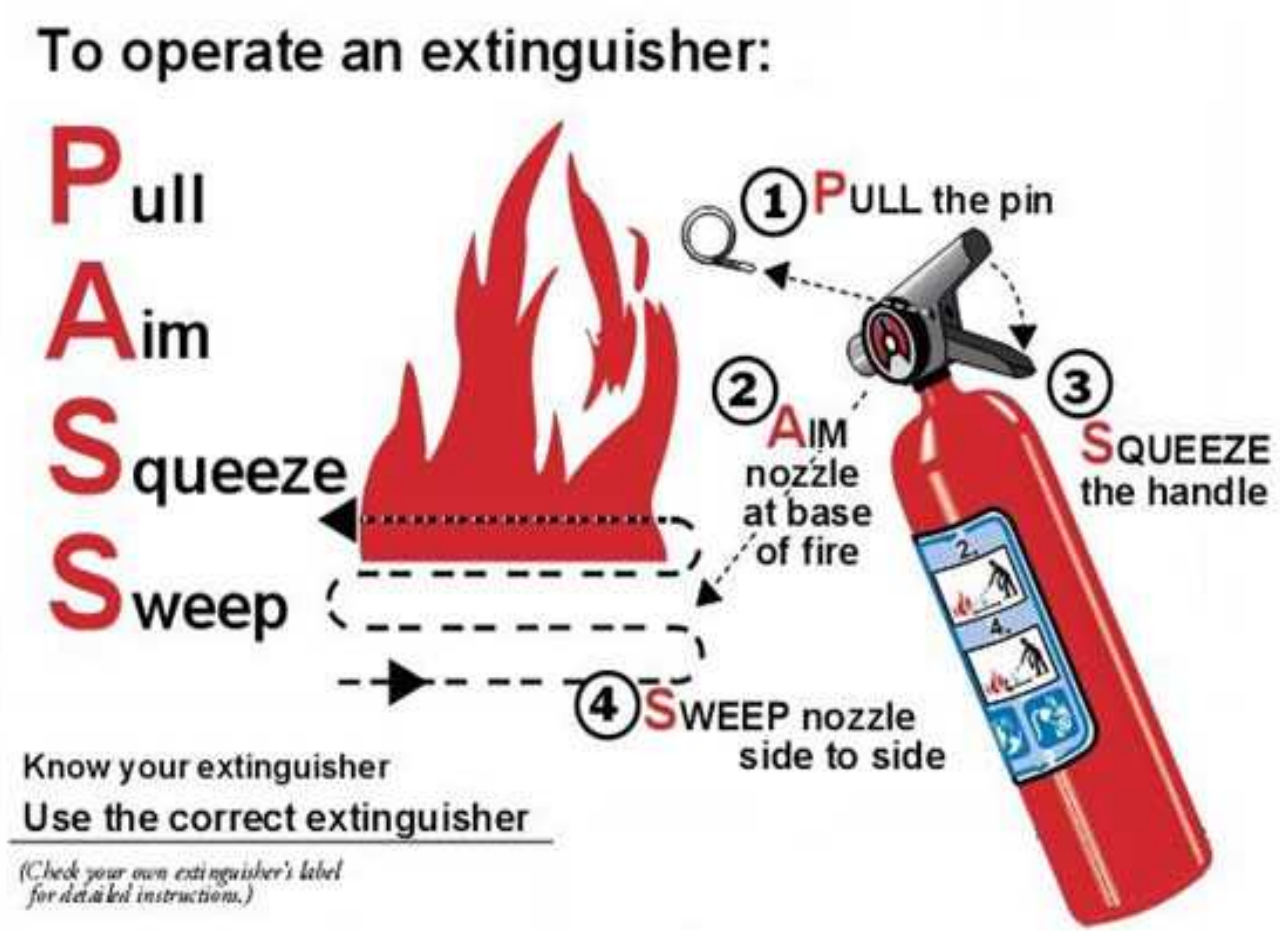
Lay electrical cords where no one can trip on them.

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:

Fire extinguisher:



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

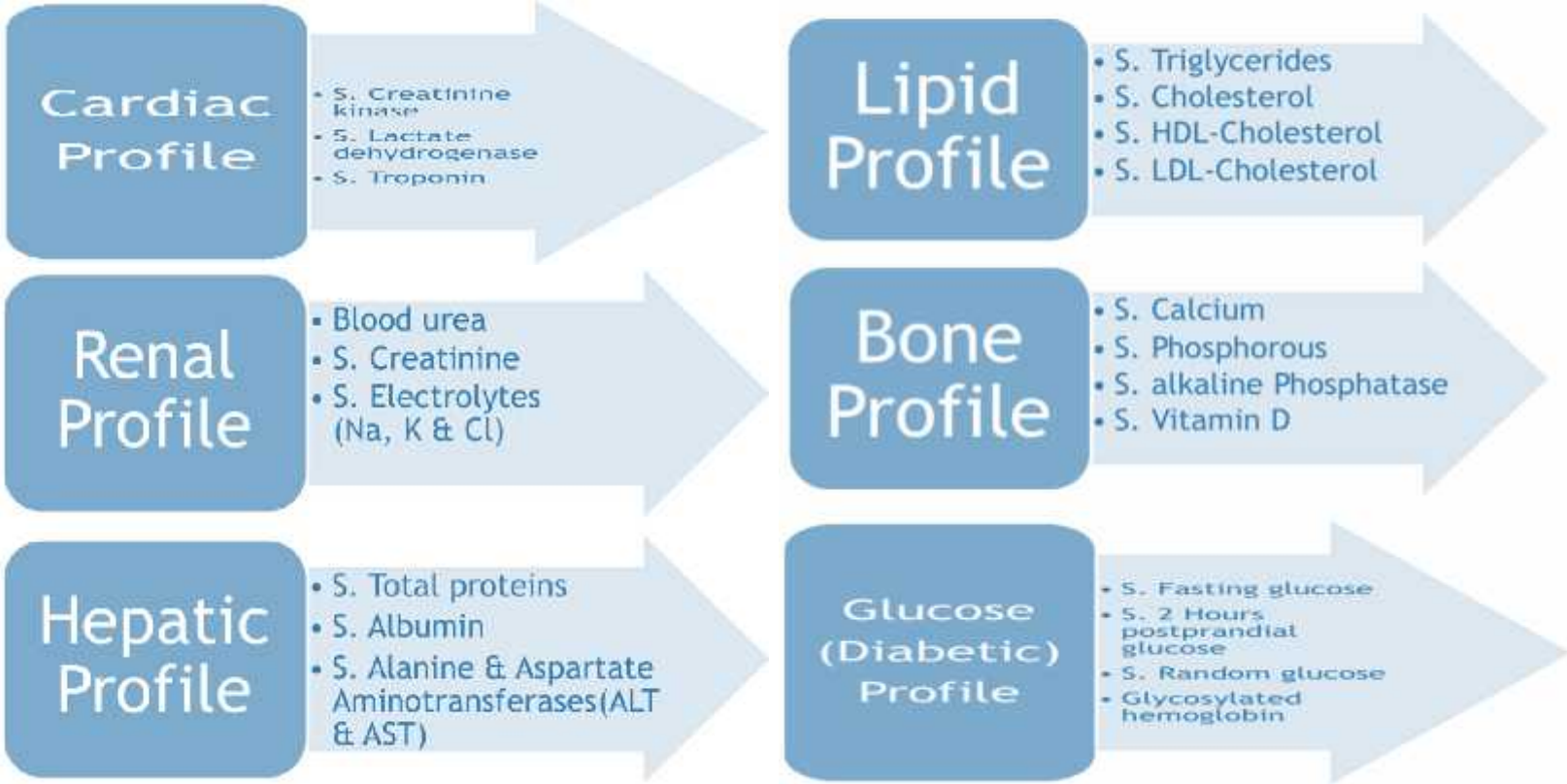
All sharp objects such as needles, scalpels and even broken glassware go into yellow-red sharps container


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:

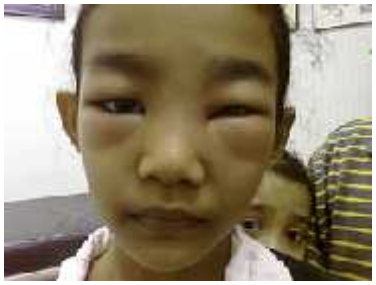


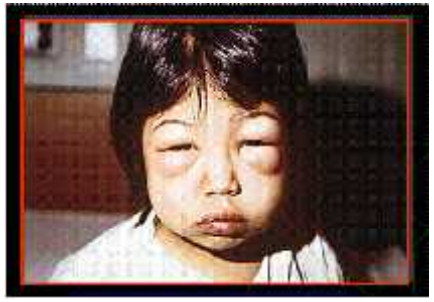
Clinical biochemistry for diagnosis of diseases:

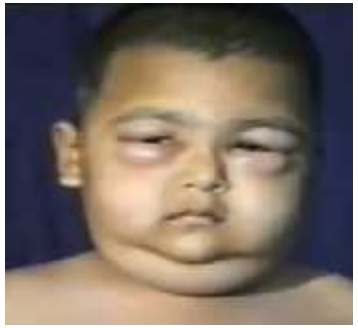
Biochemical laboratory tests are crucial tools for diagnosis of many human diseases:

Kidney diseases

e.g., nephrotic syndrome

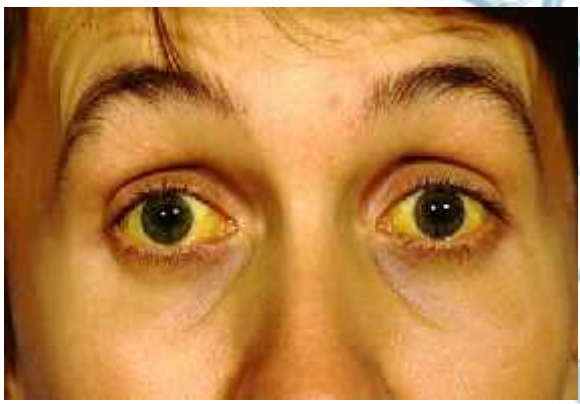






Liver diseases

e.g., hepatitis and jaundice



Metabolic diseases

e.g., diabetes mellitus

Cancers & malignancy

e.g., prostate cancer

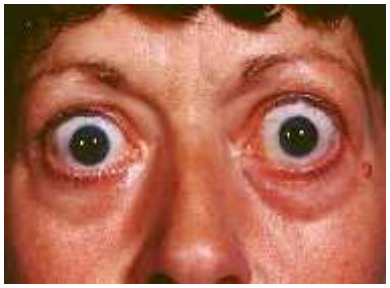
Endocrine diseases

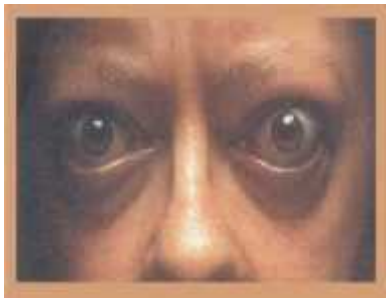
e.g., Thyrotoxicosis

Exophthalmos (bulging eyes)

Diffuse goiter

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



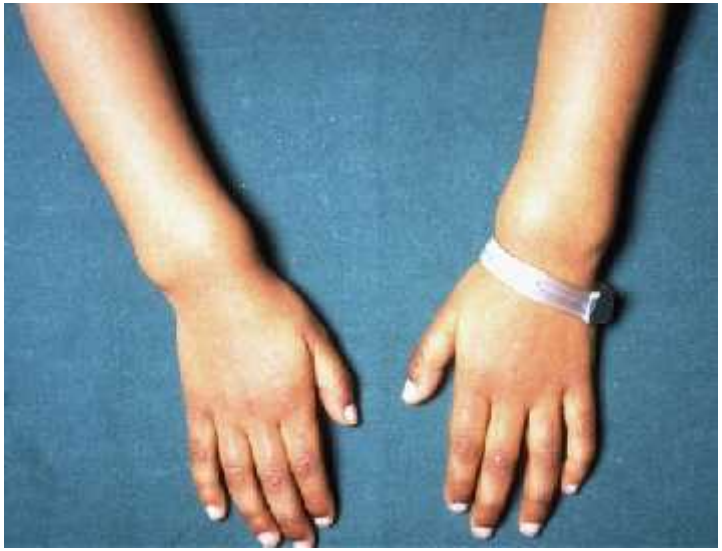


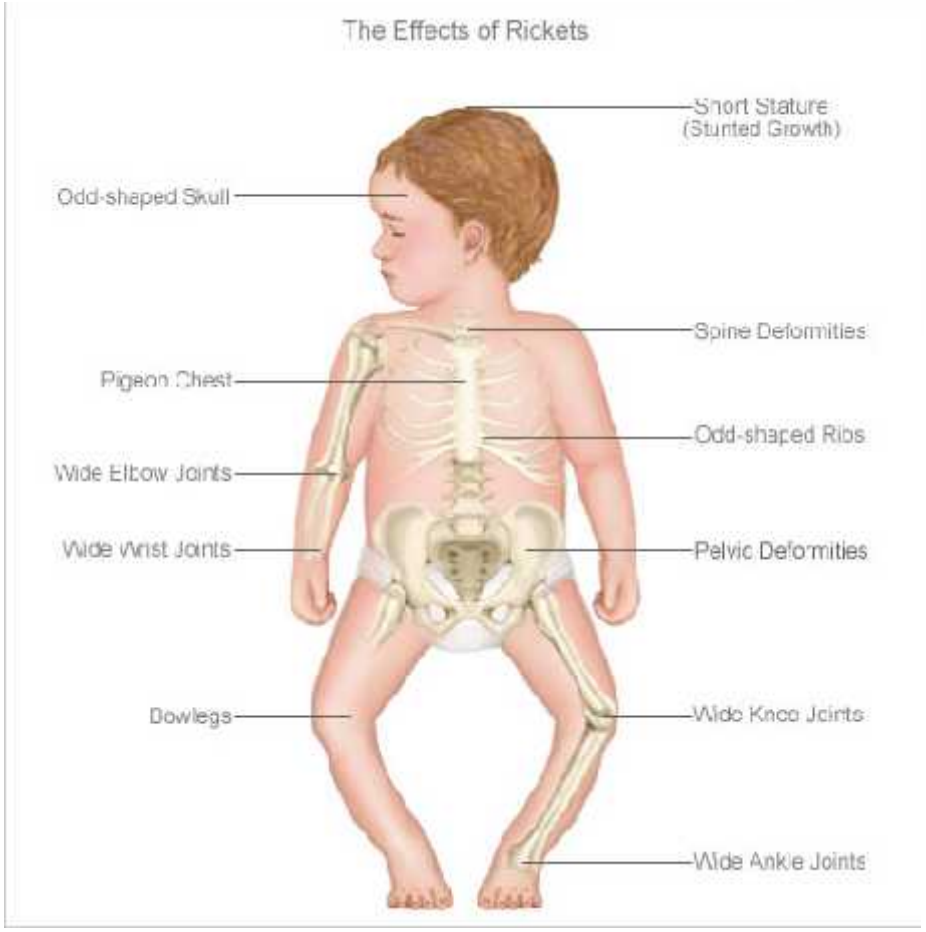
Inherited diseases

e.g., PKU

Phenylketonuria (fen-ul-key-toe-NU-ree-uh), also calledPKU, 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

Rickets:




The Effects of Rickets
Short Stature
(Stunted Growth)
Odd-shaped Skull
Spine Deformities
Pigeon Chest
Odd-shaped Ribs
Wide Elbow Joints
Wide Wrist Joints
Pelvic Deformities
Bowlegs
Wide Knee Joints
Wide Ankle Joints

Spectrophotometer: *Very important with spilling

spectrophotometers are composed of:

- Light source which works with visible wavelengths (400-700 nm
- momochromatorfilter for choosing desired wavelength
- Sample holder (cuvette)
- Detector
- Meter or recorder
- UV light

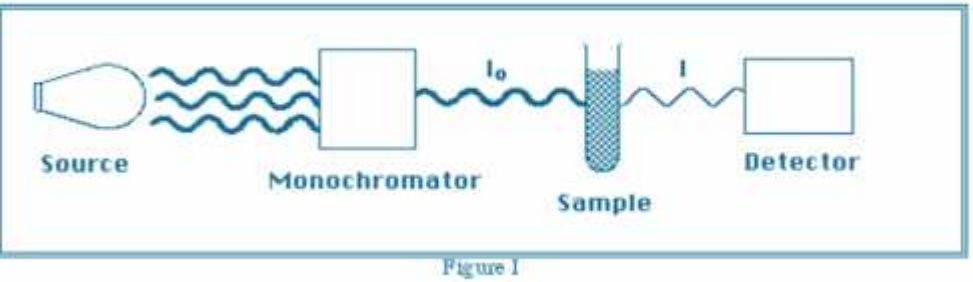


Figure 1

DNA EXTRACTION AND PURIFICATION

- **Color Index:**
  - **Original slides.**
  - Important.

Biochemistry team 438

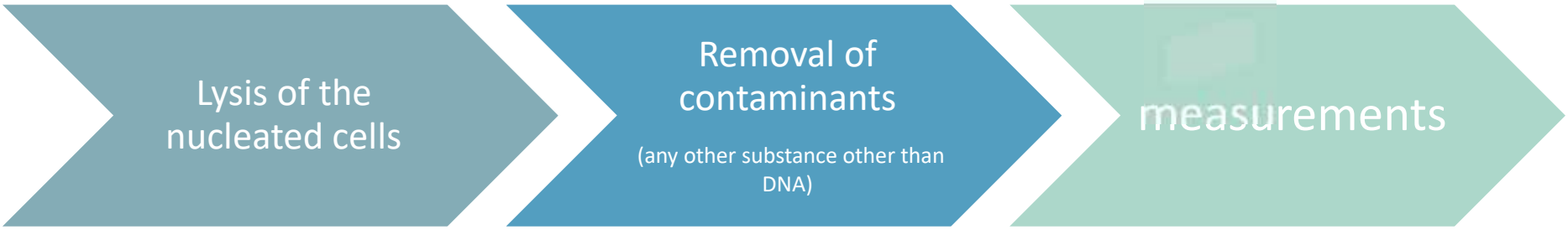
Objectives:

- 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.



Lab Equipment:

Automatic pipettes

Vortex

Microcentrifuge

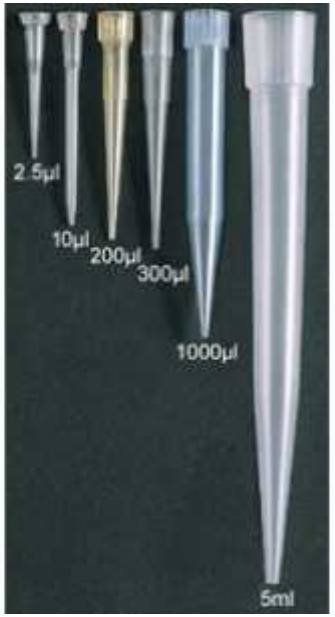
Water bath

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**.

يكون موجود 1,2,3.Protease
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**.

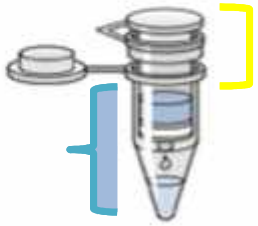
10. Discard the collection tube.
11. Place the column in a new 2ml collection tube.
12. Add **500µl** buffer **AW1**.
13. Centrifuge at **8000 rpm** for **1 minute**.

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:

الجهاز يحسبها لك

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

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

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

Done by the spectrophotometer

A260 = 1.0	DNA conc. = 50 µg/ml
A260 = 0.5	DNA conc. ?

DNA concentration =**260nm** القراءة عند ×50 µg/ml×3 :القاعده باختصار و ثابته

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:

DNA Yield µg = DNA Volume x final DNA Conc.

=0.2 ml ×concentration µg/ml :باختصار وثابت

Example:

If you have

Volume of DNA solution: 200µl (0.2 ml)

Final DNA Conc.: 30 µg/ml

Then, the yield (µg) = 0.2 ml x 30 µg/ml

= 6.0 µg

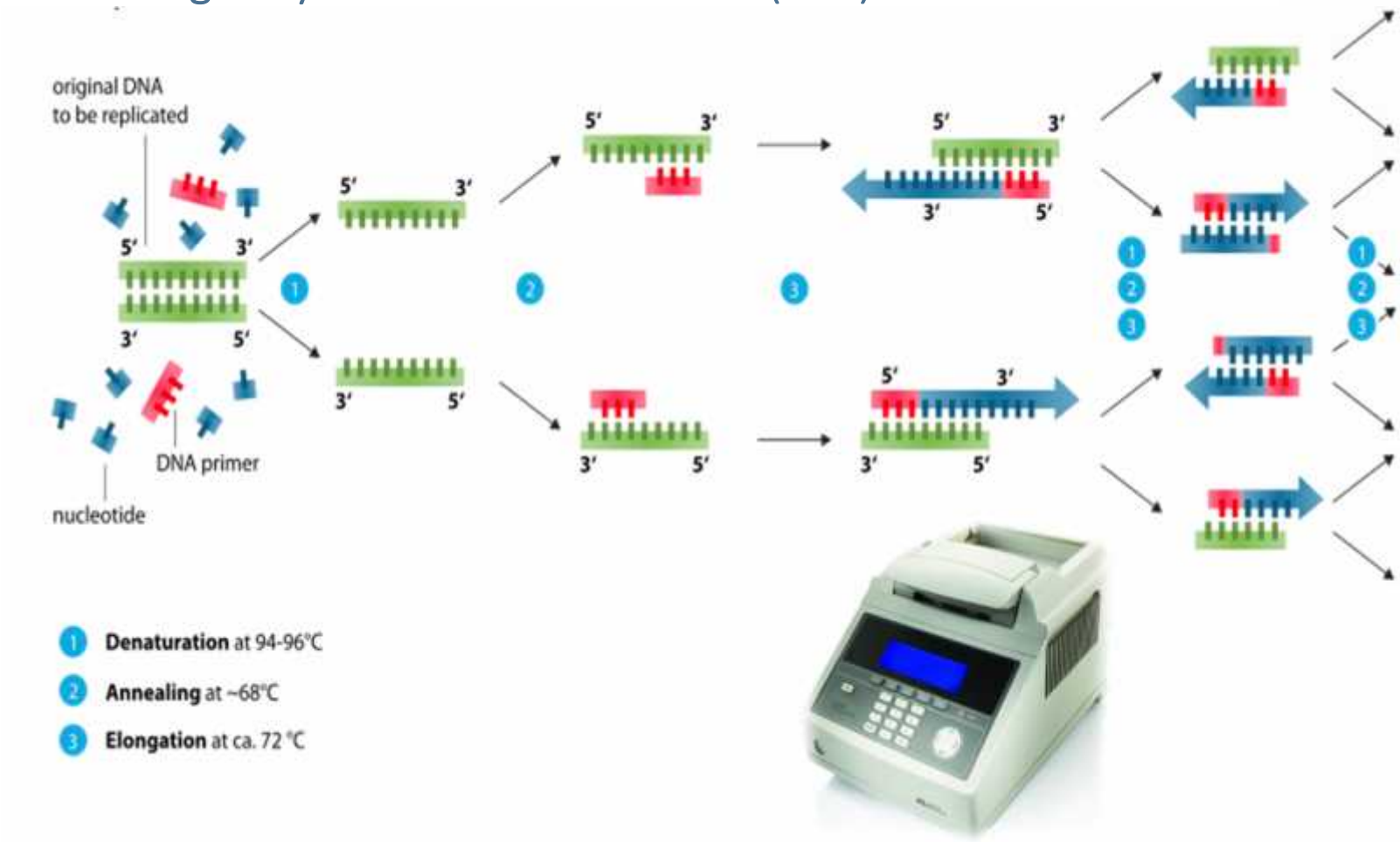
Molecular Techniques and Applications

Note:

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

a. Amplification techniques:

e.g. Polymerase Chain Reaction (PCR)



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/techrflp/

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

Review

DNA concentration =260nm القراءة عند ×50 µg/ml×3

DNA Yield µg = DNA Volume x final DNA Conc.

باختصار وثابت: 0.2 ml ×concentration µg/ml

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:

DNA yield= DNA conc.(µg /ml) X DNA volume (0.2) (ml)

DNA yield= 57.9 µg /ml X 0.2 ml

= 11.58 µg

DNA yield = 11.58 µg

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- جود الخليفة
- جود العتيبي
- ريم القرني
- سارة الهلال
- شهد السلامه
- طيف العتيبي
- عبير الخضير
- غيداء البريثن
- لينا العصيمي
- نورة التركي
- نورة المزروع
- نوف الحميضي
- هيفاء الوايلي

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