Biochemistry Practical Revsion

❖ BEFORE YOU START YOU SHOULD KNOW:

- **We would like to thank 433 team for letting us use their work.**
- **We added some notes that will help you to understand it better .**
- **Don not skip the safety instructions we should know them.**
- **the Good news is : we do not have to know the equipment nor the steps so we did not includ them.**

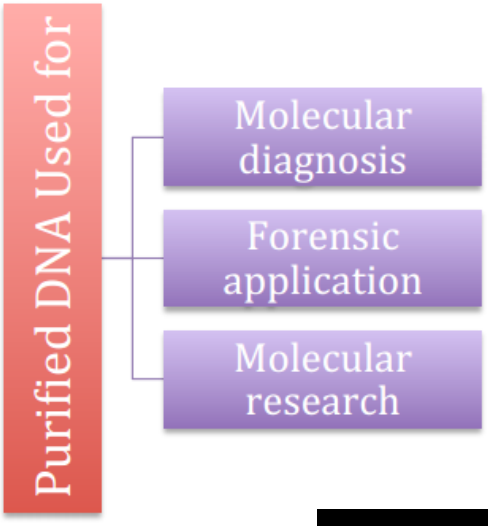
Best of luck every one

Application: 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 elctrophorsis.



Determination of purity and concentration of DNA :

1. **Purity of DNA Solution:** Determine A260/A280 ratio by spectrophotometer: 1.7-1.9 is accepted.
2. **Concentration of DNA (μ g/ml):**

Provided: A260 = 1.0, DNA concentration is 50 µg/ml
So, Unknown DNA conc. can be calculated by cross multiplication

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

(1 × ??) = (50 × 0.5)
DNA conc. = 50 × 0.5 = 25 ml

3. **Yield of DNA :**

DNA concentration × Total volume of DNA

1.DNA purity:

What do we mean by A 260/280 ratio? when we did the practical, the spectrophotometer sends UV light and the DNA absorbs it at 260 nm and the proteins absorb the UV light at 280 nm so let's say that the UV light absorbed at 260 nm is 2.33 and at 280 nm is 1.3 so the ratio is: the absorbance at 260 nm / the absorbance at 280 nm

So 2.33/1.3= 1.79

2. Concentration:

It is known that when A260= 1 then the DNA concentration is 50 µg/ml so when A260 = 2.33 then the concentration is 116.5 µg/ml by cross multiplication.

3. Yield:

So the Concentration is 116.5 µg/ml what if we have 0.2 ml how are we going to get the yield?

So we multiply DNA concentration X the total volume of DNA so the result will be = 23.3 µg

BECAREFUL ABOUT THE UNITS.

LAP Safety

Introduction:

1. A chemical lab is potentially hazardous environment
2. Accident and injury can happen anytime
3. Lab safety is everyone's responsibility
4. Lab safety standards and practices must be strictly followed

General safety rules

1) Listen to or read instructions carefully before attempting to do anything.

2) Always use appropriate personal protective equipments (lab coat, safety goggles, masks, gloves, no open shoes, no eye lenses)

3) After handling chemicals, always wash your hands with soap and water.

4) During lab work, keep your hands away from your face.

5) Tie back long hair.

6) Notify your supervisor if any spills or accidents occur.

7) Roll up loose sleeves.

8) Know the location of the fire extinguisher, fire blanket, eyewash station, and first aid kit.

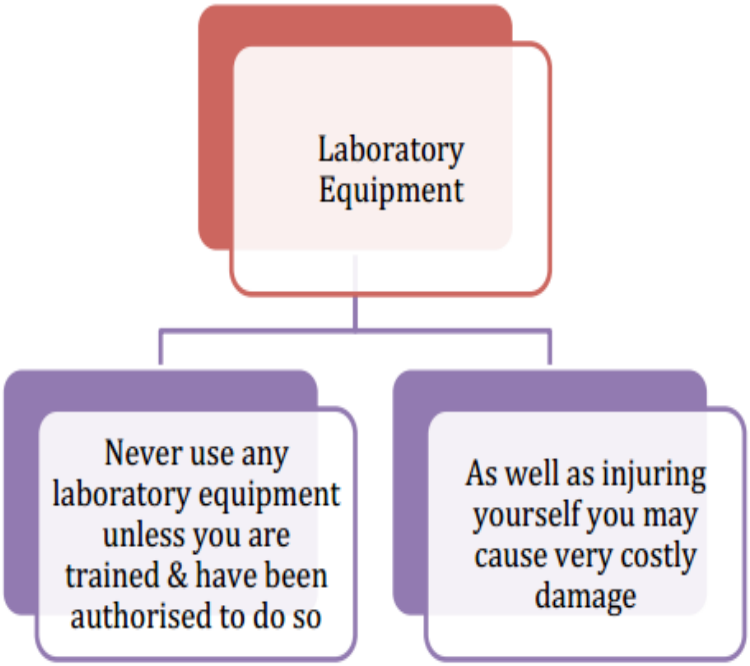
9) Keep your work area uncluttered. Take to the lab station only what is necessary.

10) It is suggested that you wear glasses rather than contact lenses.

11) Never put anything into your mouth during a lab experiment.

12) Clean up your lab area at the conclusion of the laboratory period.

13) Never "horse around" or play practical jokes in the laboratory.



Chemical safe:

1) Wear protective goggles and a lab apron whenever heating or pouring hazardous chemicals.

2) Never mix chemicals together unless you are told to do so (and then only in the manner specified).

3) Never taste any chemicals (you should never taste anything in the lab).

4) Follow the instructions of your teacher when disposing of all chemicals.

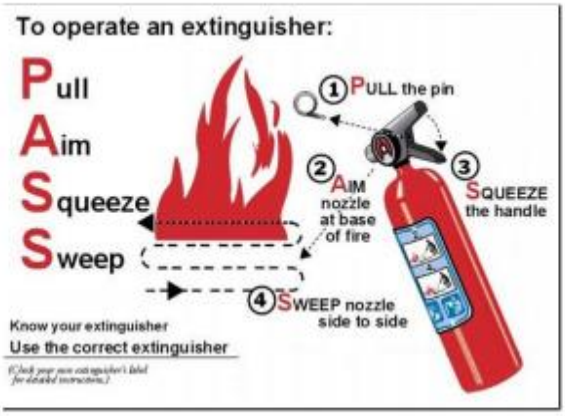
5) Wash your hands after handling hazardous chemicals.

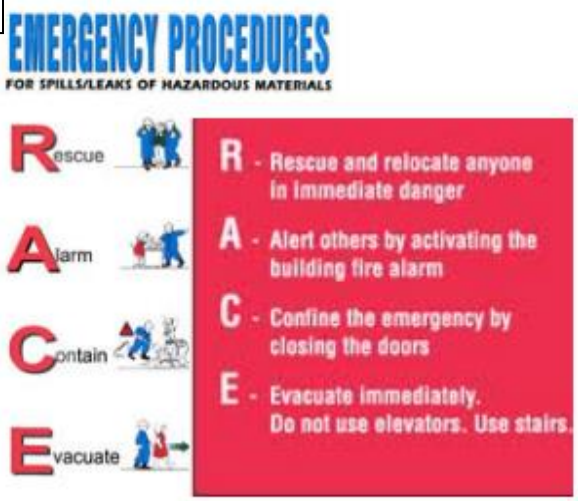
6) Never mouth pippette

Electrical Safety:

1) Lay electrical cords where no one can trip on them or get caught in them.
2) Be sure your hands and your lab area are dry before using electrical equipment.
3) Never poke anything into electrical outlets.
4) Unplug cords by pulling the plug and not the cord.
5) Unplug all electrical equipment at the end of the lab period.

IMPORTANT





Learn how to be always safe:

1. Learn emergency procedures, and be familiar with the location of fire exits, fire extinguishers, blankets, water showers, eye fountains and first aid
2. Report all accidents, injuries and spills to your supervisor
3. Report any and all signs and symptoms of exposure to your supervisor

Biological safe:

1. All biological samples are considered potentially infectious
2. Should be handled and processed using strict precautions

Waste disposal:

1. For disposal of contaminated waste, use containers with with yellow plastic garbage bags.
2. Regular waste like paper etc go in the containers with black/white plastic bags.
3. All sharp objects such as needles, scalpels and even broken glassware go in the yellow-red sharps container.