DNA Extraction and Purification

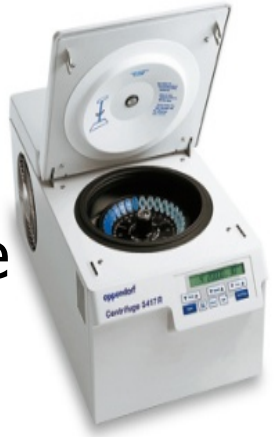
Lab Equipments

Automatic pipettes

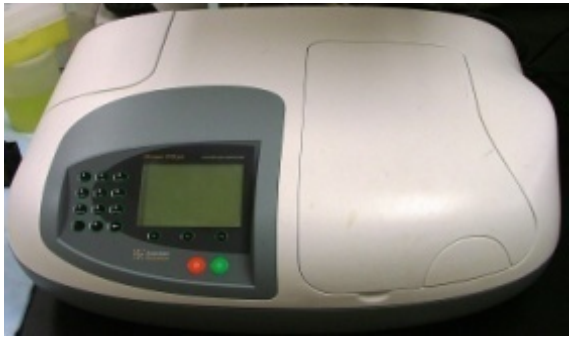
Vortex

Water bath

Microcentrifuge

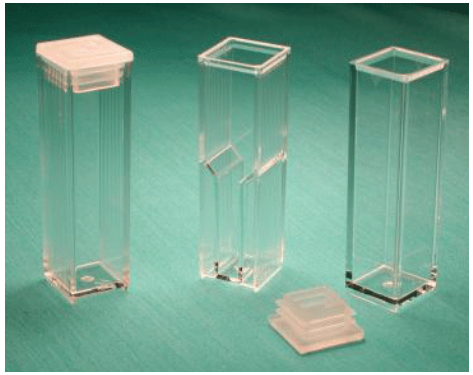


UV-spectrophotometer

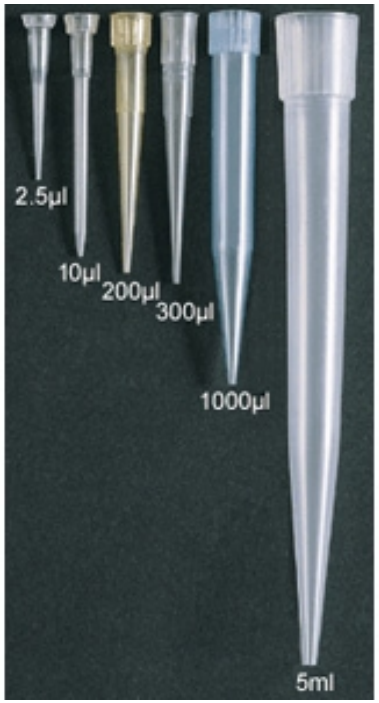


Lab Equipments

Eppendorf tube

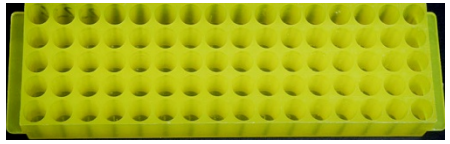


Cuvettes



Tips

Rack- test tube



Rack- eppendorf tube

DNA Extraction

Principle:

1. **Lysis of nucleated cells.**
2. **Removal of contaminants:** Any substance other than DNA, e.g., proteins
3. **Measurements:** UV absorbance at 260nm and 280nm

Purity of DNA solution: 260/280 ratio

DNA concentration: Absorbance at 260nm

DNA Extraction

Steps:

- **Lysis** of nucleated cells using lysis buffer.
- **Binding** of DNA to the membrane of spin column.
- **Wash:** using wash buffer.
- **Elution** of pure DNA

Spin Protocol
of
DNA Purification from Blood

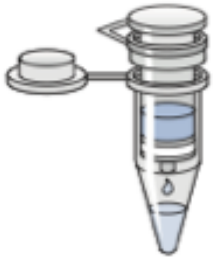
1. Pipette **20µl** protease.
2. Add **200µl** sample.
3. Add **200µl** Buffer **AL**.

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.

Quantification of the purified DNA

Measurements

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

Measurements

Done by the spectrophotometer

- **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

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

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 = DNA Volume x final DNA Conc.

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

DNA Applications

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.**

Molecular techniques using purified DNA:

a. **Amplification techniques: P**olymerase **C**hain **R**eaction (**PCR).**

b. Southern blotting.

c. Restriction Fragment length polymorphism (RFLP).