



# DNA Extraction and Purification

# Lab Equipment

Automatic  
pipettes



Vortex



Water bath

Microcentrifug  
e



UV-spectrophotometer



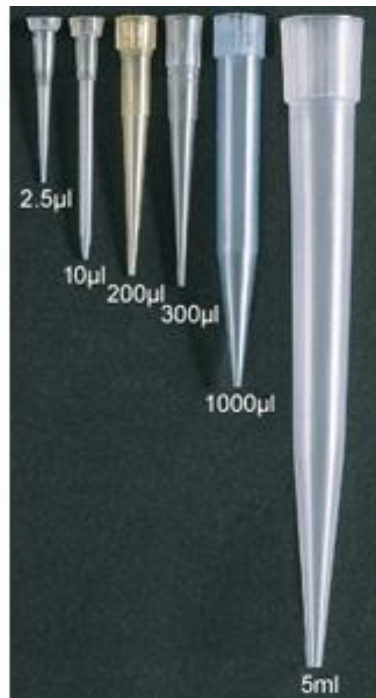
# Lab Equipment



Eppendorf tube



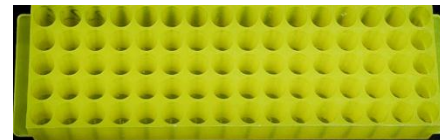
Cuvettes



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 **20 $\mu$ l** protease.
2. Add **200 $\mu$ l** sample.
3. Add **200 $\mu$ l** Buffer **AL**.



4. Mix by pulse-vortex for 15s and incubate at **56°C** for **10 minutes**.
5. Briefly centrifuge.
6. Add **200 $\mu$ l** 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**.

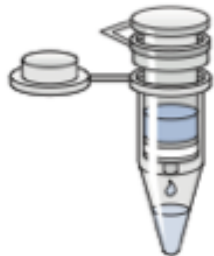


10. Discard the collection tube.
11. Place the column in a new 2ml collection tube.
12. Add **500 $\mu$ l** buffer **AW1**.
13. Centrifuge at **8000 rpm** for **1 minute**.





14. Place the column in a new 2ml collection tube.
15. Add **500 $\mu$ l** 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 **200 $\mu$ l** buffer **AE**.
21. Incubate at room temperature for **1 minute**.
22. Centrifuge at **8000 rpm** for **1 minute**.



**Add 400 $\mu$ l 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  $A_{260} = 1.0$ , DNA is  $50 \mu\text{g/ml}$ , unknown DNA Conc. can be calculated by cross multiplication

$$A_{260} = 1.0$$

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

$$A_{260} = 0.5$$

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

*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:** 200 $\mu$ l (0.2 ml)

**Final DNA Conc.:** 30  $\mu$ g/ml

**Then, the yield ( $\mu$ g) = 0.2 ml x 30  $\mu$ g/ml  
= 6.0  $\mu$ g**

# 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: Polymerase Chain Reaction (PCR).**

**b. Southern blotting.**

**c. Restriction Fragment length polymorphism (RFLP).**