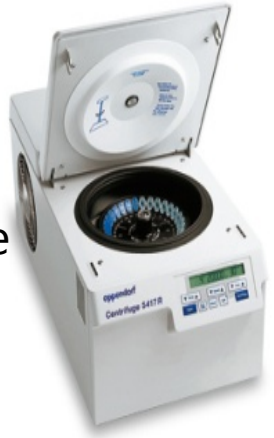
DNA Extraction and Purification

Lab Equipment

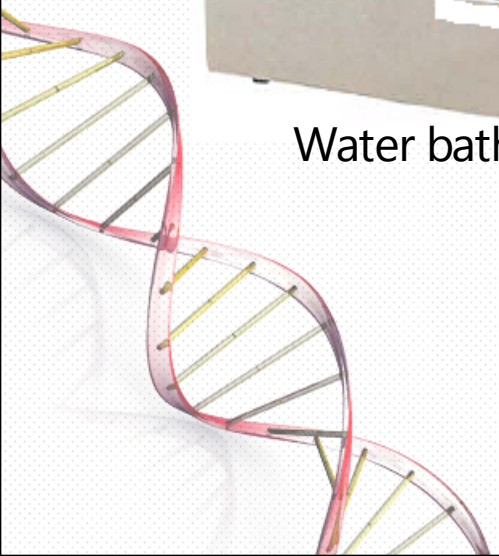
Vortex

Automatic pipettes

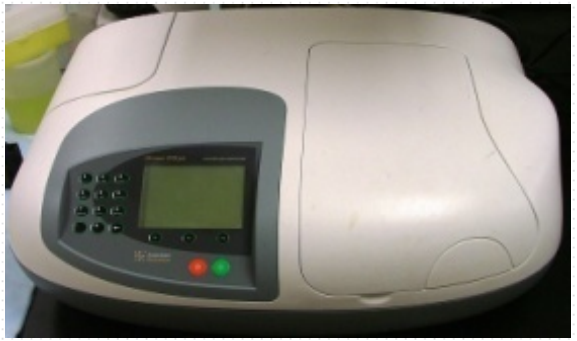
Microcentrifuge



Water bath



UV-spectrophotometer



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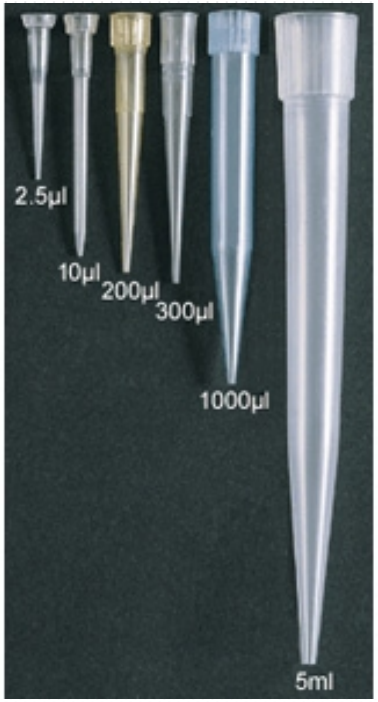
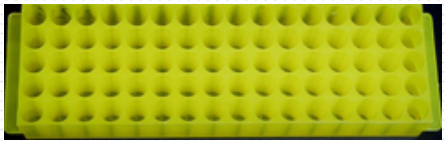
Eppendorf tube

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

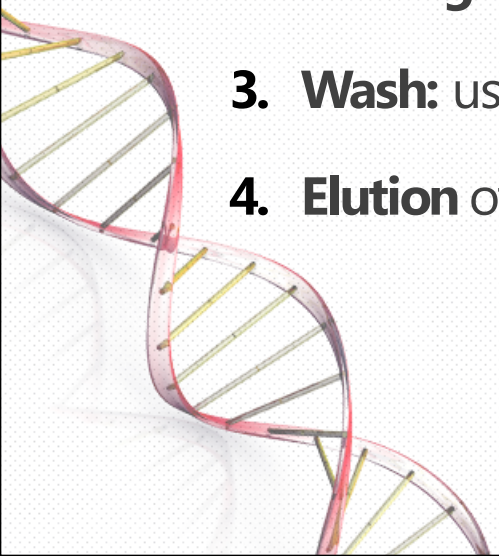
Purity of DNA solution: 260/280 ratio

DNA concentration: Absorbance at 260nm

Continued …

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

Video

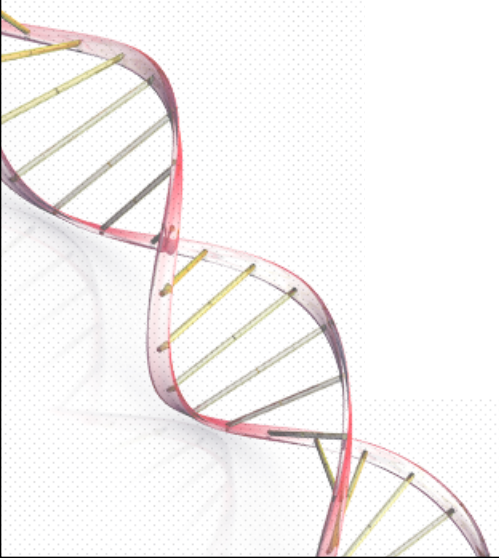
https://www.youtube.com/watch?v=gmNw6CWtN5k

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**.

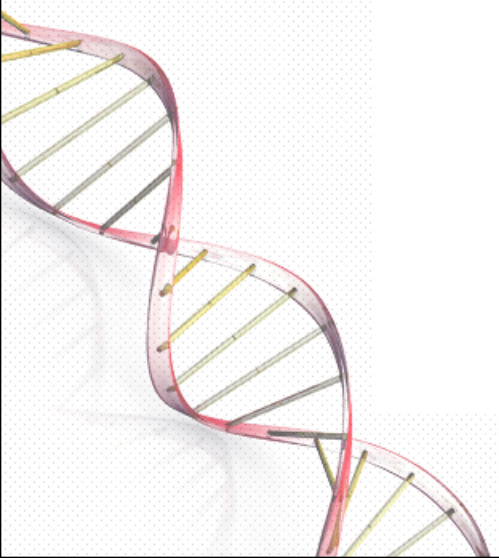


14. Place the column in a new 2ml collection tube.
15. Add **500µl** buffer **AW2**.
16. Centrifuge at **14000 rpm** for **3 minutes**.
17. Place the column in a new 2ml collection tube.
18. Centrifuge at **14000 rpm** for **1 minute**.

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

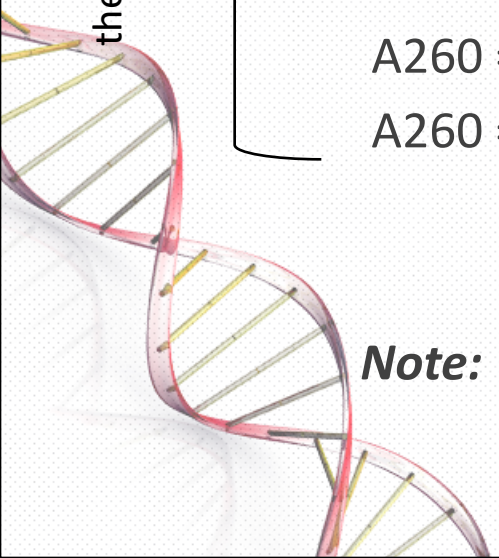
Done by the spectrophotometer

- 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

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

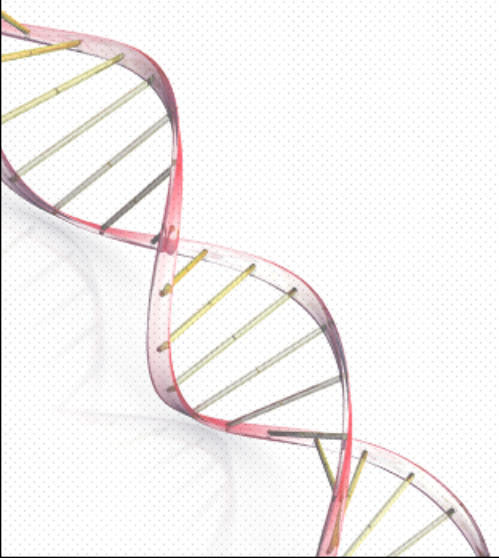
Then, the yield (µg) = 0.2 ml x 30 µg/ml

= 6.0 µg

Molecular Techniques and Applications

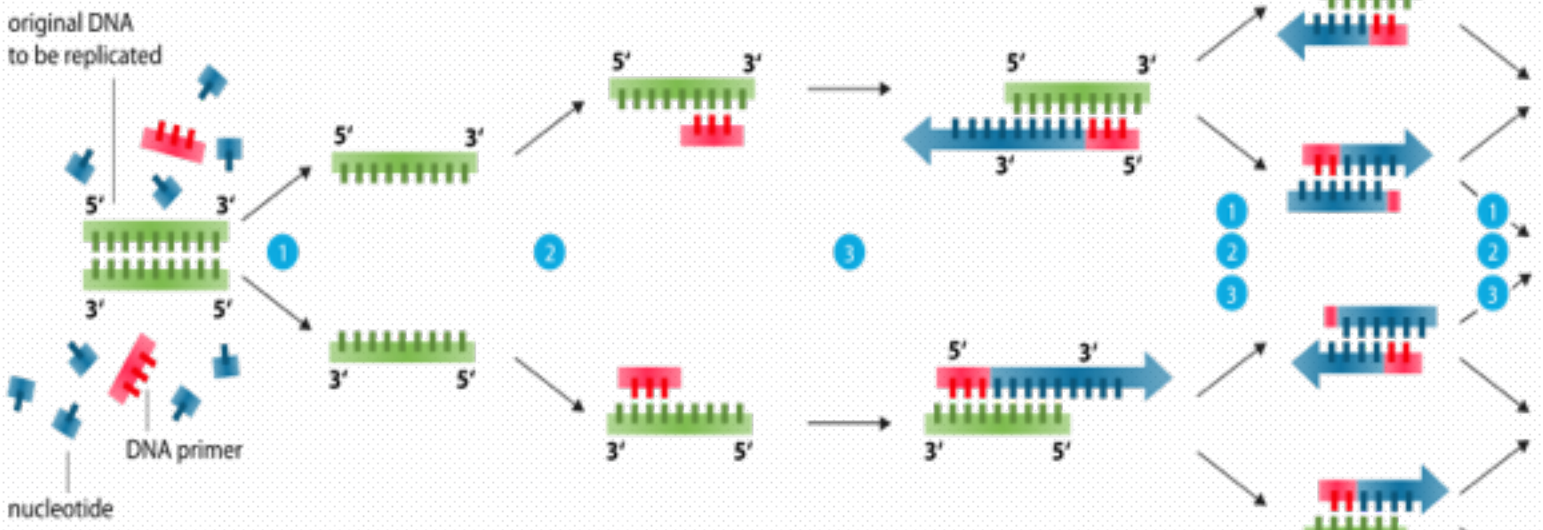
Note:

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



a. Amplification techniques:

e.g. Polymerase Chain Reaction (PCR)

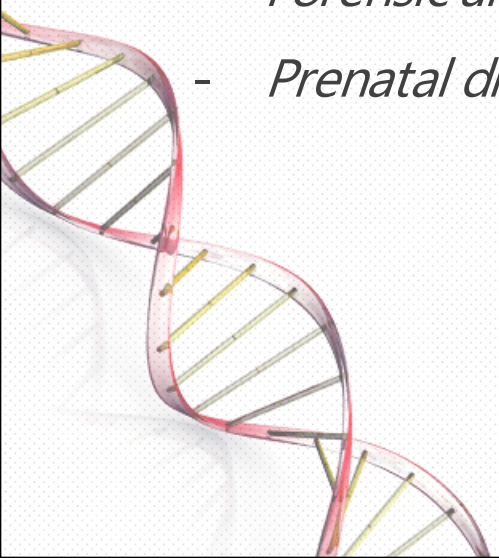


1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

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/techrflp/

2. Southern blotting.

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