	YILDIZ TECHNICAL UNIVERSITY **BIOMEDICAL ENGINEERING DEPARTMENT** **BME2901- BIOCHEMISTRY COURSE** **2020-2021 FALL SEMESTER**

EXPERIMENT 6

DNA ISOLATION AND GEL ELECTROPHORESIS

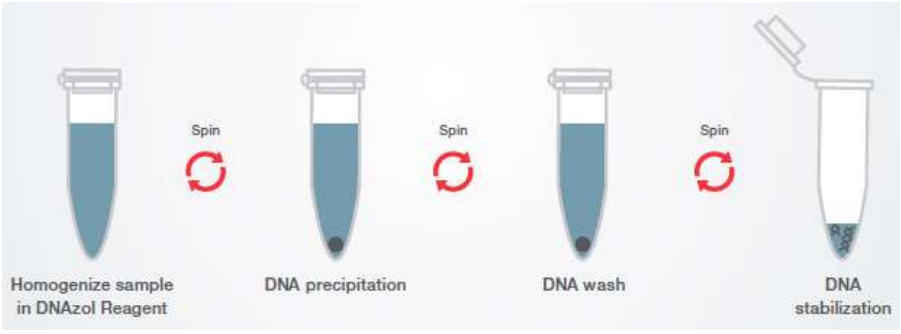
1. THEORETICAL KNOWLEDGE

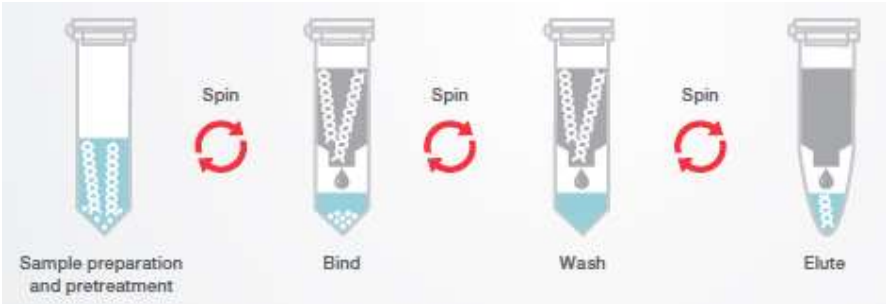
Deoxyribonucleic acid (DNA), "the master molecule," is a natural polymer which encodes the genetic information required for the growth, development, and reproduction of an organism. Found in all cells, it consists of chains of units called nucleotides. Each nucleotide unit contains three components: the sugar deoxyribose, a phosphate group, and a base with single or double ring structure. The base component can be any of four types: adenine, cytosine, guanine or thymine. DNA has a double helical structure with a negatively charged sugar-phosphate backbone and stabilized by hydrogen bonds between the bases attached to the two strands.

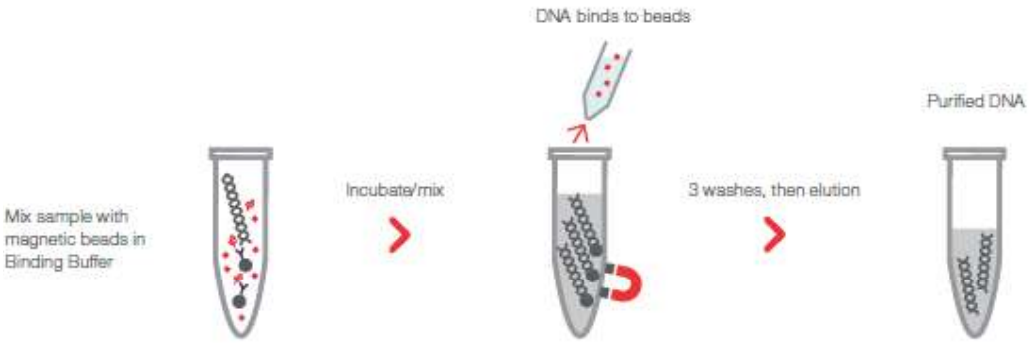
DNA can be isolated from a variety of sample sources including blood, buccal epithelial cells, cryopreserved cells, saliva, urine, hair, plants, fungi, bacteria etc. Choosing the source for DNA is important for the yield and for reduced risk of contamination. Anti-coagulated whole blood is the most convenient source of DNA since the yield is high due to the high number of lymphocytes in the blood. The risk of contamination is also very low. However, it is an invasive method since a syringe is puncturing your skin and vessels. DNA in hair, urine, saliva samples and a few cells that could be left behind by a criminal are generally preferred in forensic science. However DNA yield is generally very low due to the scarcity in the starting material. Buccal epithelial cells are generally used for paternity testing or comparison of the DNA isolated from a crime scene with the suspect's. The yield is high comparatively and the risk of contamination can be reduced by some precautions.

Many different methods and technologies are available for the isolation of genomic DNA. In general, all methods involve disruption and **lysis** of the starting material followed by the removal of proteins and other contaminants (**wash**) and finally recovery (**elution**) of the DNA.

Removal of proteins is typically achieved by digestion with proteinase K, followed by salting-out, organic extraction, or binding of the DNA to a solid-phase support (either anion-exchange or silica technology). Magnetic bead technology is also developed recently to isolate DNA more rapidly and with a higher yield. In this method,



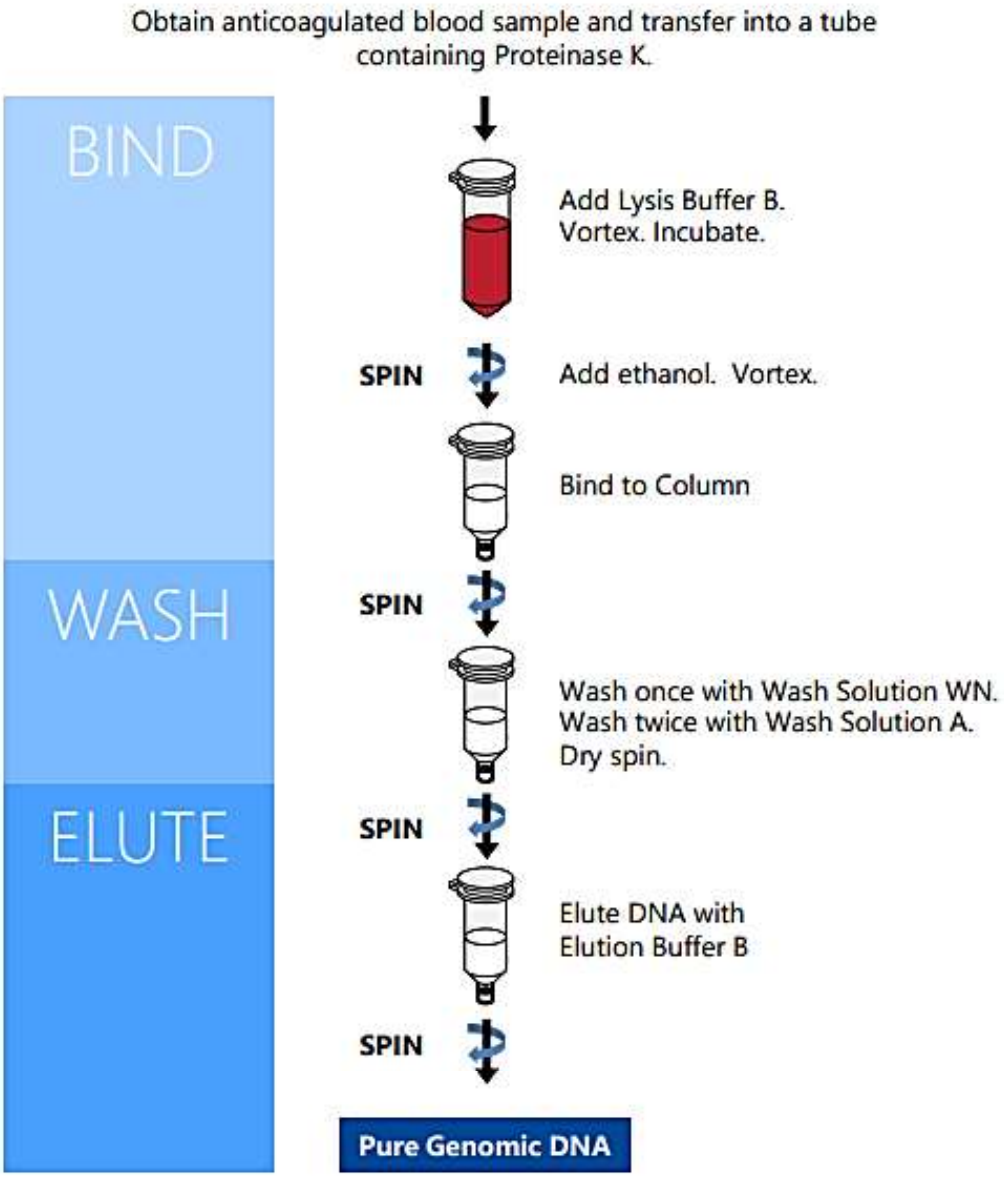




In this experiment DNA is adsorbed onto the silica membrane. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the silica membrane.

DNA is usually recovered from the membrane by precipitation using ethanol or isopropanol and then solubilized in the solution by the elution buffer.

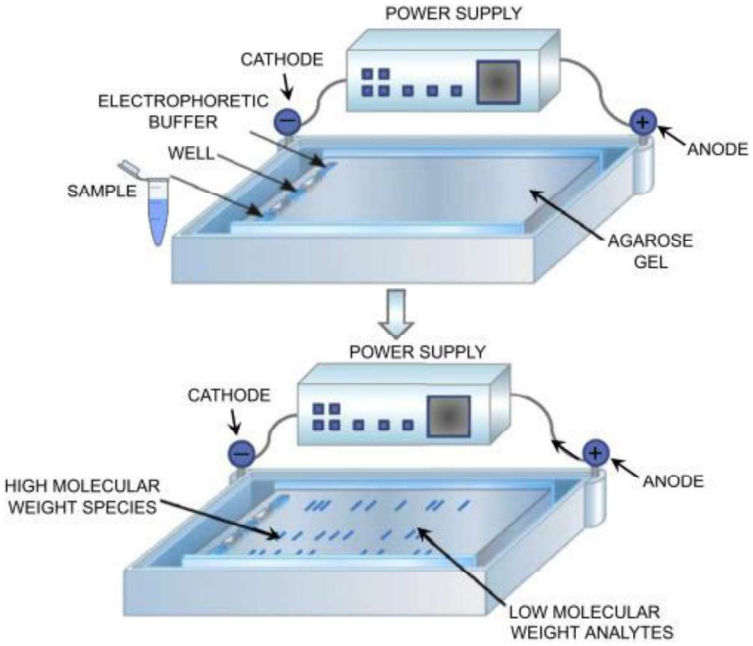
The choice of a method depends on many factors: the required quantity and molecular weight of the DNA, the purity required for downstream applications, and the time and expense.



- There are some necessary precautions for DNA isolation and purification:

1. Blood should be handled with the appropriate precautions to avoid exposure to infectious agents.
2. Anticoagulant should be used. Anticoagulant is a substance that prevents blood from clotting or thickening of blood (e.g. EDTA, heparin, sodium citrate, sodium oxalate). Blood samples older than one week may produce poor yields and/or poor quality DNA unless they have been stored frozen.
3. Yield is dependent on the white cell count of the sample. One ml whole blood has about 10 million white cells and yields ~100 μg DNA suitable for ~200 amplification reactions.
4. Polypropylene tubes and tips should be used for isolating DNA; other plastic products may absorb DNA.
5. The need to store prepared DNA for long times should be considered in the choice of a protocol. For example, reference DNA used to monitor the specificity of primers and probes may be utilized over a long period of time. Therefore, a protocol that produces a more purified DNA preparation should be selected.

Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA or proteins in a matrix of agarose, one of the two main components of agar. The proteins may be separated by charge and/or size (isoelectric focusing agarose electrophoresis is essentially size independent), and the DNA and RNA fragments by length. Biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix, and the biomolecules are separated by size in the agarose gel matrix. DNA as well as RNA are normally visualized by staining with ethidium bromide, which intercalates into the major grooves of the DNA and fluoresces under UV light. The intercalation depends on the concentration of DNA and thus, a band with high intensity will indicate a higher amount of DNA compared to a band of less intensity



2. MATERIALS

I. Norgen Blood DNA Isolation Kit

This system provices a rapid extraction and purification of DNA. DNA is extracted from the cell nucleus by lysis of the cell and nuclear membranes, occurring by a chemical reaction. A proteinase enzyme degrades proteins bound to the DNA. The extracted DNA is separated and trapped in an affinity column by osmotic selection. The DNA is released by washing with an eluant that solubilizes the DNA.

1. Materials

- Whole Blood
- Norgen Kit (Proteinase K, RNAase A, lysis/binding buffer, washing buffers, elution buffer, spin column and 2 mL collection tubes are provided in this kit)
- 1.5 mL microcentrifuge tubes
- Micropipettes and pipette tips
- Minicentrifuge
- Heat block
- Vortex
- 1X Tris/Borate/EDTA buffer (TBE)
- Agarose

- Beaker
- Microwave
- Ethidium Bromide
- Gel Tank
- Power supply
- DNA loading dye
- UV camera

3. METHOD

DNA Isolation

1. Take 200 µl of the fresh whole blood in a 2.0 ml collection tube. Ensure that the blood sample is at room temperature (15-25℃) before beginning the protocol.
2. Add 20 µl of Proteinase K solution into the above collection tube containing blood. Vortex (10-15 seconds) to ensure thorough mixing of the enzyme.
3. Add 20 µl of RNase A solution. Vortex (10-15 seconds) to ensure thorough mixing of the enzyme and incubate for 2 minutes at room temperature.
   - NOTE: This step helps in getting RNA-free genomic DNA.
4. Add 200 µl of Lysis Solution to the sample, vortex thoroughly for few seconds to obtain a homogenous mixture. Incubate at 55 ℃ for 10 minutes.
   - NOTE: If cell clumps are visible, pipette the sample gently to obtain a homogenous mixture.
5. Add 200 µl of ethanol to the lysate obtained from step 4 and mix thoroughly by gentle pipetting for 5-10 seconds.
   - NOTE: A homogenous solution should be obtained after addition of ethanol.
6. Load lysate spin column. Transfer the entire lysate obtained from step 5 into spin column. Centrifuge at 10,000 rpm for 1 minute. Discard the flow-through liquid and place the column in a collection tube.
- NOTE: Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents onto the column.
7. Add 500 µl of wash solution to spin column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through liquid and re-use the same collection tube along with the column.

8. Add 500 µl of wash solution to the column and centrifuge at 14,000 rpm for 3 minutes. Discard the flow through. Place the column in the same collection tube and centrifuge it for an additional one minute at 14,000 rpm to remove the traces of wash solution. Discard the collection tube and place the column in collection tube.
9. Add 200 µl of elution buffer directly into the column without spilling on to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at 10,000 rpm for 1 minute to elute the DNA.

- NOTE: Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short term storage (24- 48 hours) of the DNA, 2-8 ℃ is recommended. For long-term storage, -20 ℃ or lower temperature (-80 ℃) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures

- NOTE: Yield of genomic DNA depends on the number of cells in the sample. A 200 µl sample of whole blood from a healthy donor ($5x10e^6$ leukocytes/ml) will yield approximately 6 µg of DNA.

Agarose Gel Electrophoresis

1. Preparation of 1X TBE: To prepare 500 ml of 1X TBE buffer add 10 ml of 50X TBE buffer to 490 ml of sterile distilled water. Mix well before use.
2. Preparation of agarose gel: To prepare 100 ml of agarose gel, add 1g agarose to 100 ml 1X TBE buffer in a glass beaker. Heat the mixture on a microwave by swirling the glass beaker occasionally, until agarose dissolves completely. Allow the solution to cool down to about 55-60 ℃. Add 5 µl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

- NOTE: Ethidium bromide is a powerful mutagen and is very toxic. Appropriate safety precautions should be taken by wearing latex gloves; however, use of nitrile gloves is recommended.

3. Loading of the DNA samples: To prepare sample for electrophoresis, add 2 µl of 6X gel loading buffer to 10 µl of DNA sample. Mix well by pipetting and load the sample onto the well. Load the Control DNA after extracting the DNA sample.

4. Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red -Anode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized