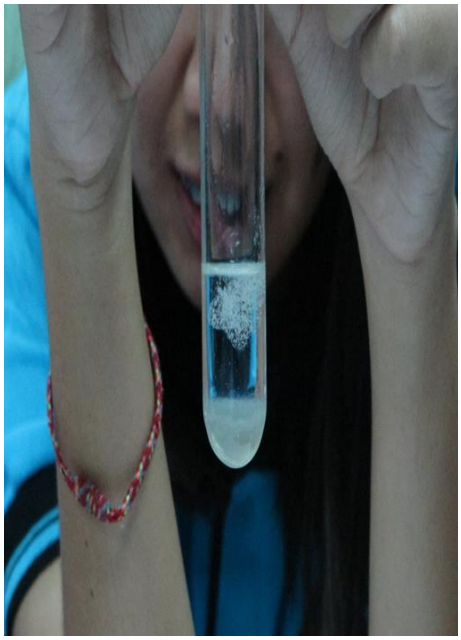
# Molecular Biology

## DNA Extraction



- There are many different methods and techniques available for isolation of genomic DNA from prokaryotes (Bacteria) and Eukaryotes (animals & plants).

**All methods involve:**

Disruption and lysis of cell wall and cell membrane, followed by removal of proteins and other contaminants.

## First: Opening of different cells:

**a) Bacterial Cells**

Method:

1) Suspend the overnight bacterial cells in broth culture, mix well by vortex. Vortex is used to homogenize the suspension of bacterial cells with the broth.
2) Add Sodium Dodecyl Sulfate **(SDS)** to lysis Gram negative bacteria, and **lysozyme + (SDS)** to lysis Gram positive bacteria; then incubate (30-60 min.) at 37 C
   - **Lysozyme:** is capable of breaking the bonds in the peptidoglycan in the cell wall of bacteria which is thick in Gram positive bacteria comparing with Gram negative bacteria; therefor, lysozyme used to open the Gram positive bacteria.
   - **SDS:** anionic detergent used to soluble the cell membrane to release cell contents.
     - ❖ **We add RNase to destroy RNA because we want to extract DNA.**
3) Add (**Perichloric acid)** which dissociate DNA from proteins.

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## Second: Isolation of DNA from other components:

*Method:*

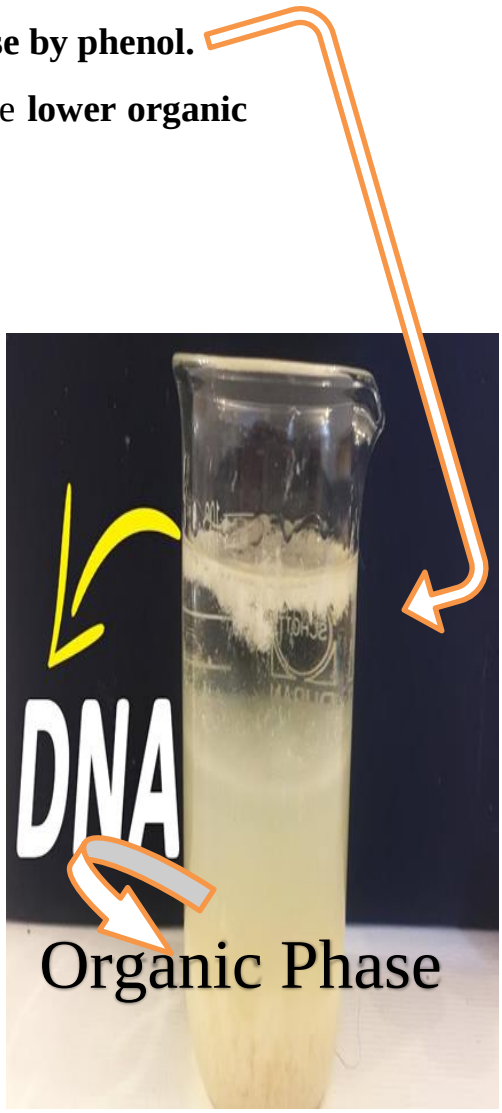
1) Add [Phenol / chloroform / Isoamylalcohol]

   It is an extraction solution that used to remove proteins and other contaminants from nucleic acid sample.

   - Nucleic acids are remained in **the aqueous phase.**
   - Proteins are denature and separated in the **inter phase by phenol.**
   - Most of lipids & polysaccharides are separated in the **lower organic phase by chloroform.**
   - **Isoamylalcohol** acts as an anti-foam.

2) Centrifuge the mixture in (10000 rpm for 10 min. at 4 C). Three layers are separated; the first aqueous layer contains DNA.

   Centrifugation at 4 C is **to prevent** the generation of heat through centrifugation that lead to denature the DNA and **to increase** the precipitation.




3) Transfer the upper layer to another **plastic tube (**because the DNA has ability to bind to glass tube therefore plastic tube is used**)**, then add twice volume of **cold absolute ethanol**, which makes dehydration and pull out the water molecule from the DNA hence precipitate the DNA.
4) Mix by inversion; after 3 min., DNA will precipitate like spool DNA on a glass rod.
5) Dissolve the DNA in **by using TE buffer.**
6) Do the Electrophoresis.

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## " Modern producer to Extract The DNA from Bacteria"

1- After Isolation and Purification of bacteria, inoculate a broth (10ml) with it and incubate it for 24 hours at 37C.

2- Transfer (1400µl) from the broth using Micropipette to Eppendorf tubes.

3- Discard the Eppendorf tubes in a Centrifuge for 2 minutes at (13000 rpm). After the centrifugation is completed, the upper liquid "**Supernatant**" is removed with Micropipette and the "**pellet**" is preserved.

4- Add (500µl) from **Nuclei lysis solution (responsible for Cell lysis and Destroys the cell wall and cell membrane)** to the Eppendorf tubes that contain the pellet , mix them by Vortex, then transfer the tubes to Water bath at 70C for 30 minutes , then leave them to cool at room temperature.

5- Add (3 µl) of RNase solution to the tubes and mix them well , then incubate them for 10 minutes at 37C , then cool them at room temperature.

6- Add (200µl) of **Protein precipitation solution (responsible for Proteins precipitation)** to the Eppendorf tubes and mix them well by the Vortex, incubate the tubes in ice for 5 minutes.

7- The tubes are Centrifuged at (12000 rpm) for 5 minutes.

8- The supernatant transfer to new Eppendorf tubes, and then add (500 µl) of **Isopropanol** and mix it well by pipetting.

9- The tubes are Centrifuged at (12000 rpm) for 5 minutes, the supernatant Poured and the Pellet was kept.

10 – (500 µl) of **Ethanol** (70%) was added to the Eppendorf tubes and mixed well, then the tubes are centrifuged for 2 minutes at (13000 rpm).

11 – The Ethanol is removed by evaporation and left to dry at room temperature. **(Isopropanol and Ethanol are used to remove water molecule from the DNA and make it Precipitate).**

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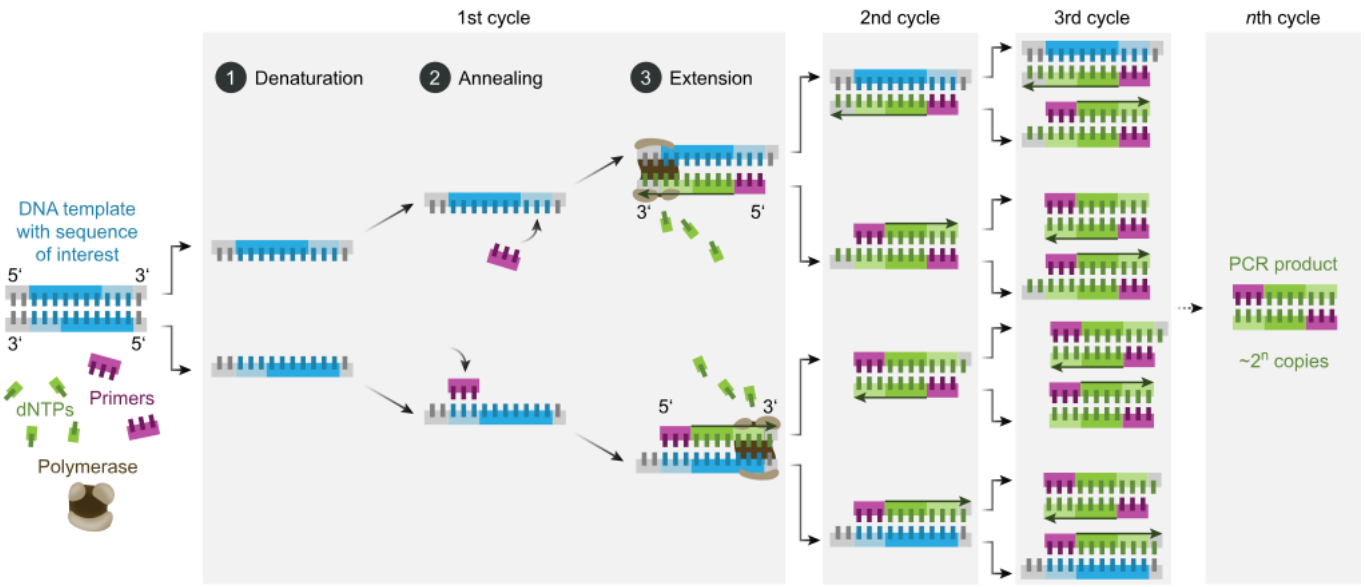
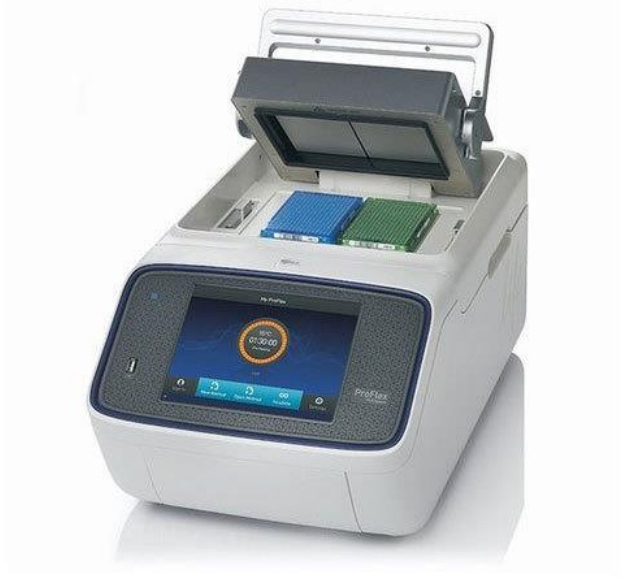
12 – After Evaporation for 2 hours and a half, add (70 µl) of **DNA Rehydrate Solution** to the tubes, and then put in the refrigerator at -4C for 24 hours, until it is used.

13- The DNA extract was carried out to **PCR "Polymerase Chain Reaction"** device and then on an Agarose gel at a concentration of 1% with a voltage of 100 volts for 80 minutes.



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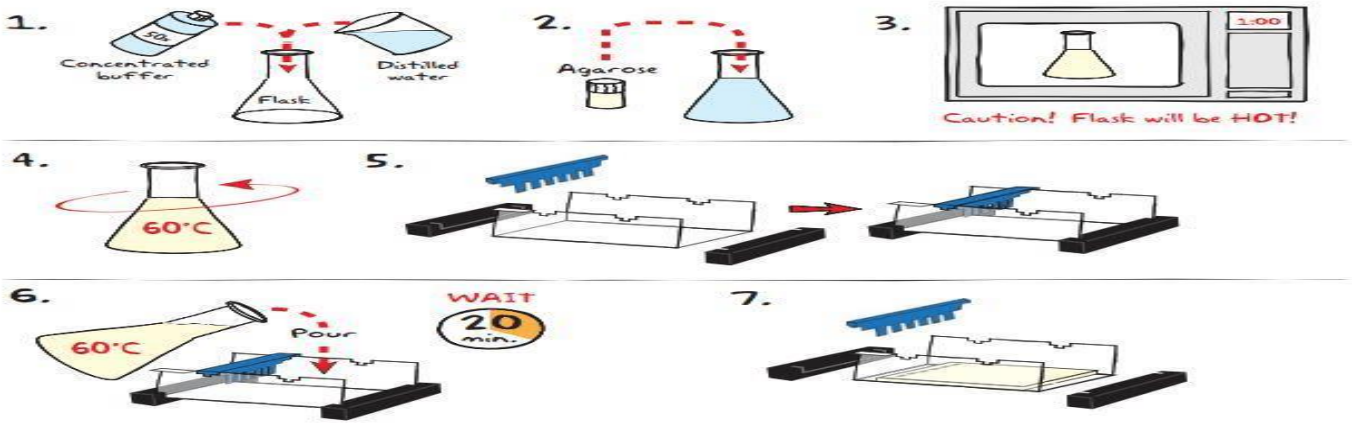
## " Agarose Gel Preparation "




1- Agarose gel was prepared by dissolving (2gm) of agarose in (100ml) of TBE buffer, then the mix was heated to boiling point, and then leave to cool to 50C, (1 µl) of **Ethidium Bromide** with a concentration (0.1%) µg/ml. (**Ethidium Bromide** It is used because upon binding of the molecule to the DNA and illumination of UV light source, the DNA can be visualized).

2- The agarose was poured into a support plate after gently dampening the comb into the plate and then allowed to harden for 30 minutes.

3- The comb was lift quietly from the hardened agarose plate, then fixing the plate inside the electrical relay unit represented by the electrical relay basin. After that, the transfer basin was filled with TBE with an amount covering the entire surface of the agarose gel.

4- (6 µl) of the product of the PCR reaction was transferred to the wells designated for them and also transferring 3 microliters of the DNA Ladder solution (100-1500) the user base pair **to determine the sizes of the DNA segments**, then pass an electrical current with a voltage of 100 volts for a period of 80 minutes.

5- Examination of the gel-agarose using a UV-Transilluminator.

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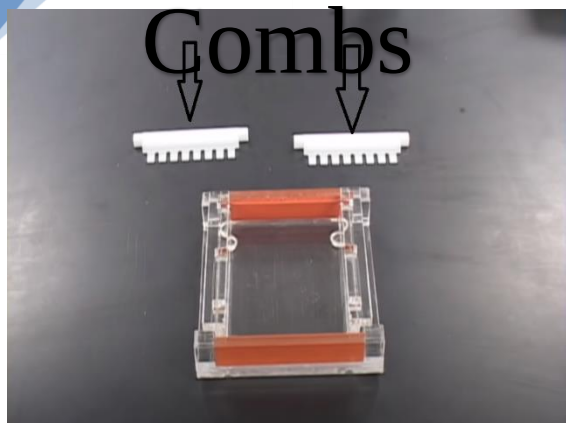
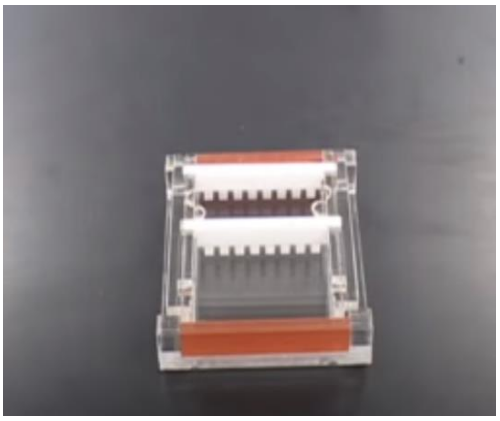
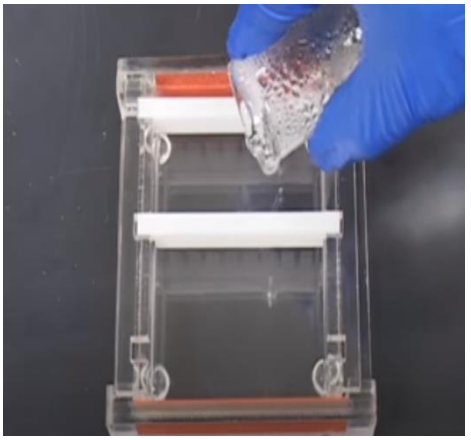
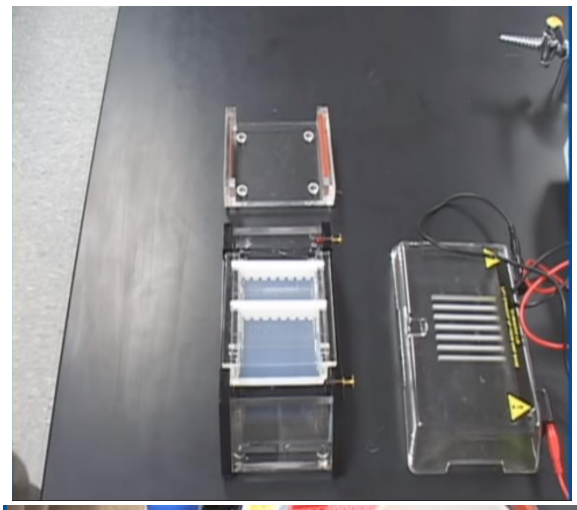
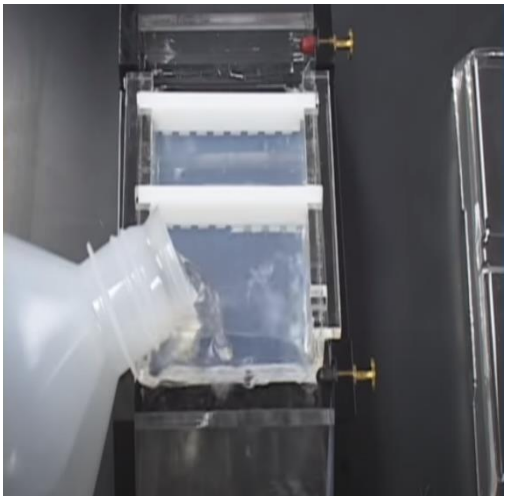
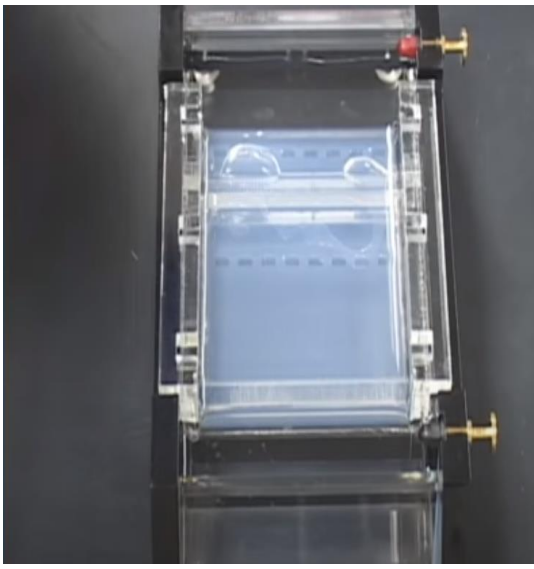


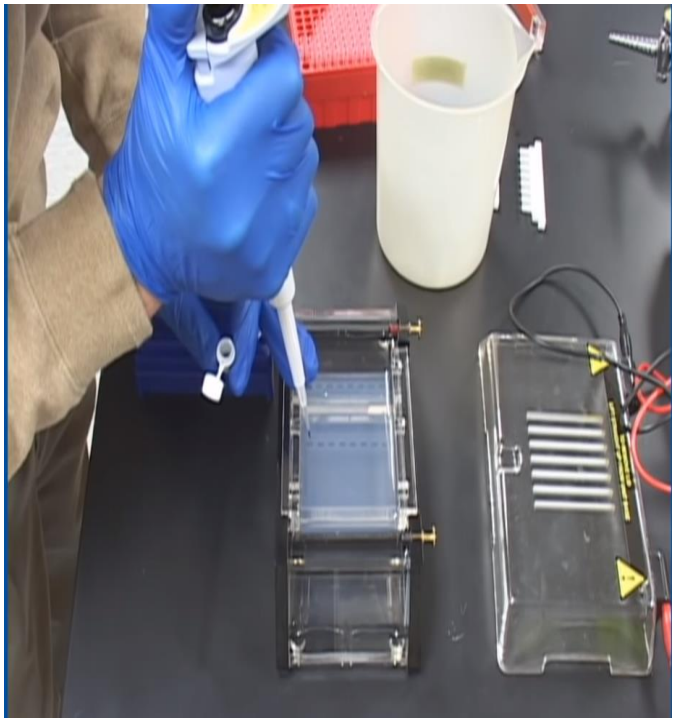



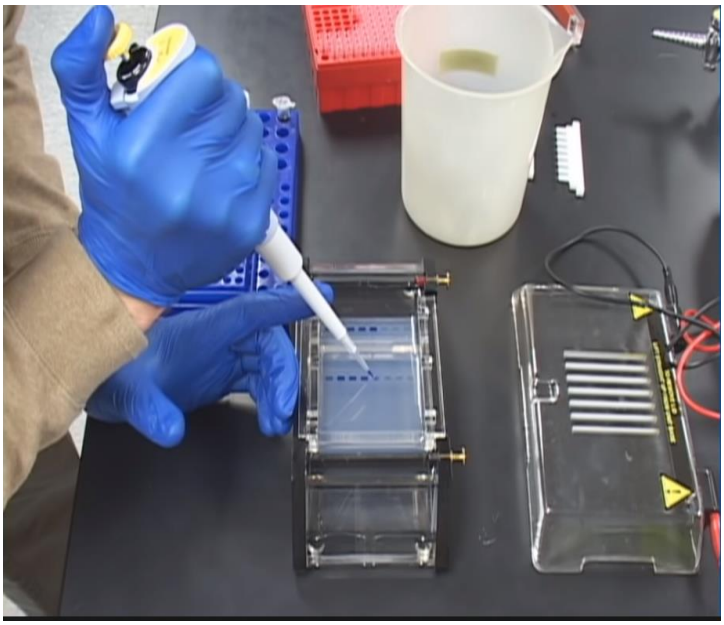




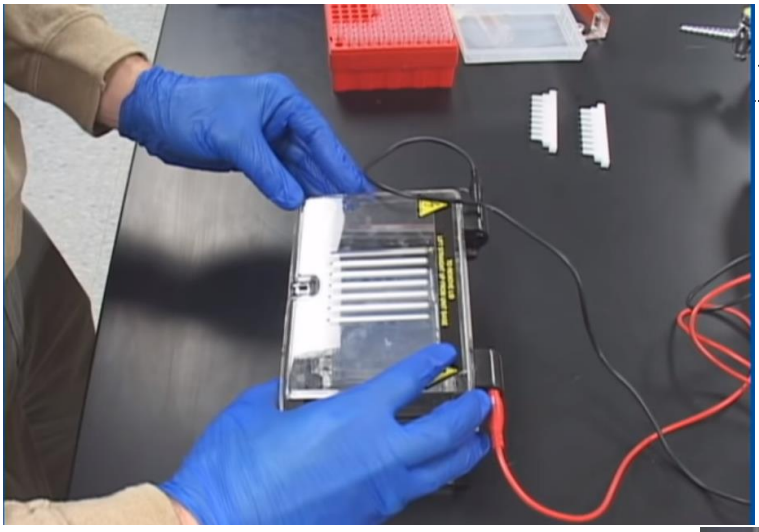


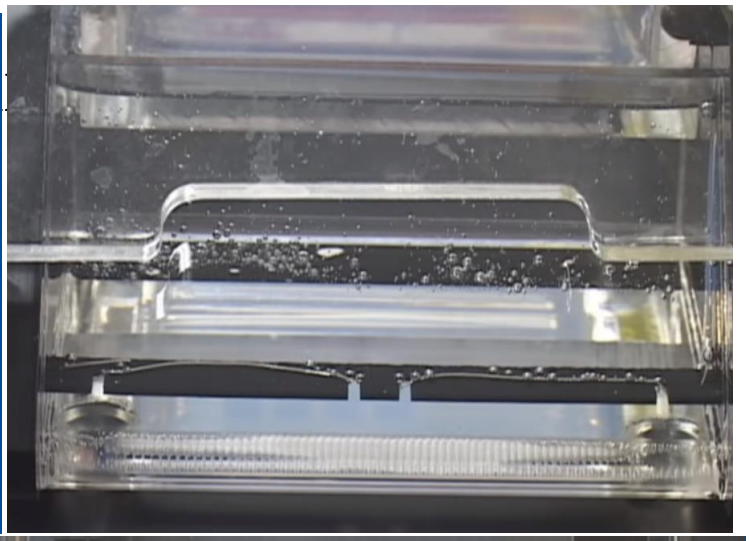


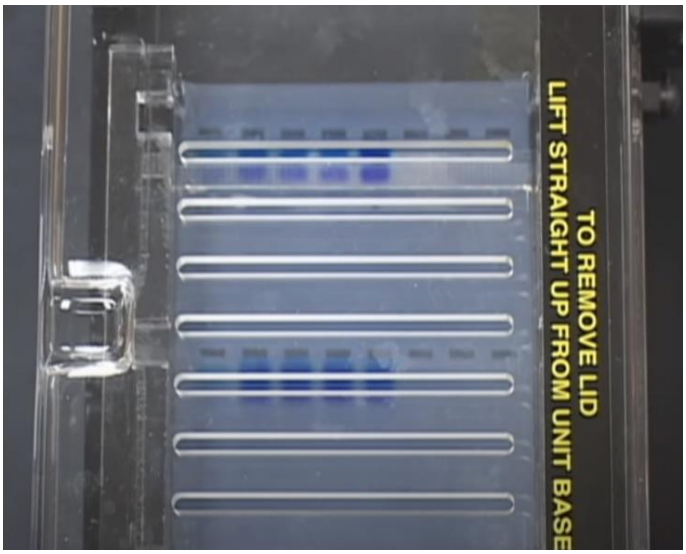


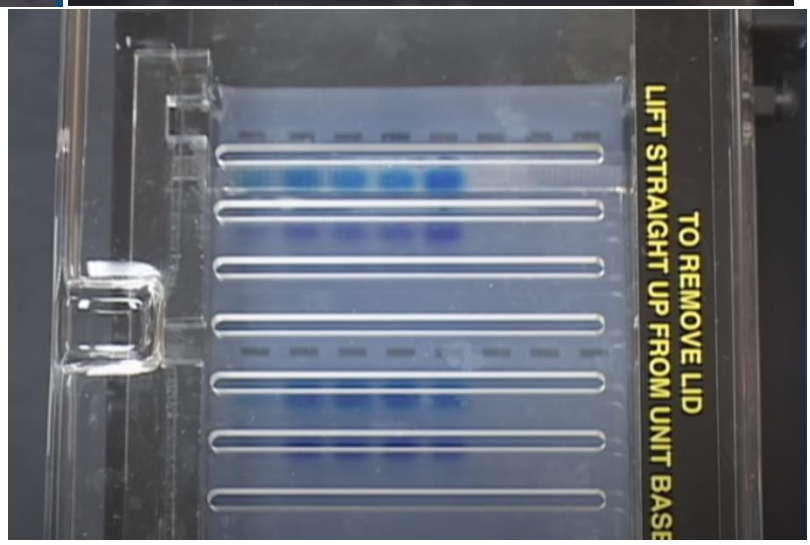


Ladder first and then the DNA samples






TO REMOVE LID
LIFT STRAIGHT UP FROM UNIT BASE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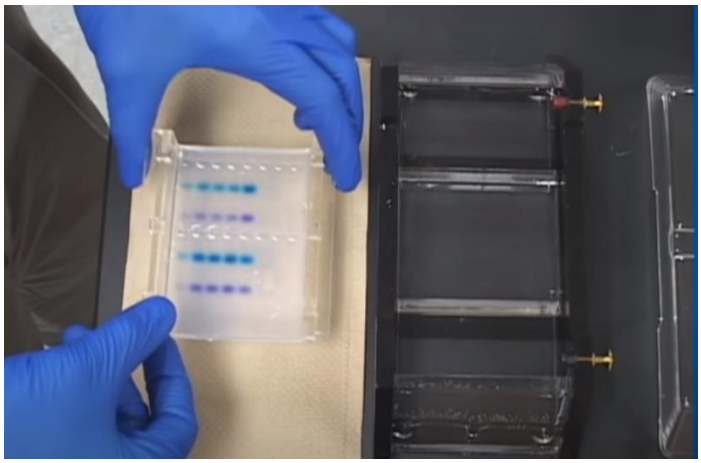
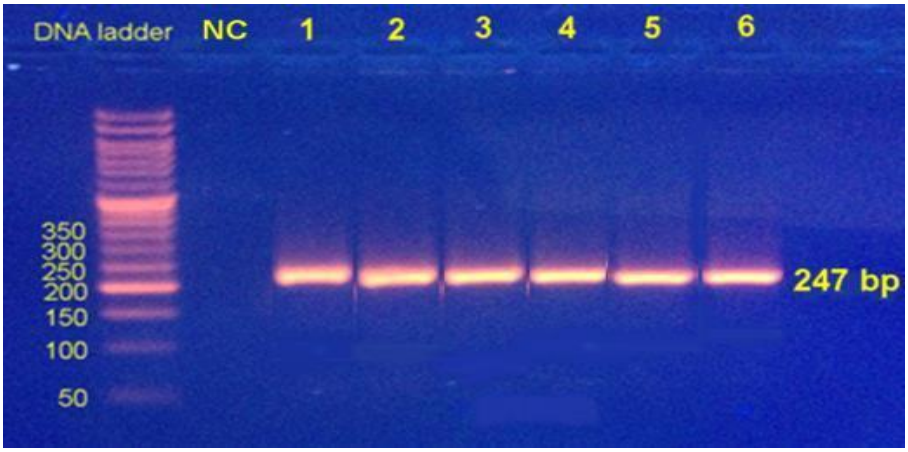



InGenius
TRANSILLUMINATOR


DNA ladder
NC
1
2
3
4
5
6
350
300
250
200
150
100
50
247 bp