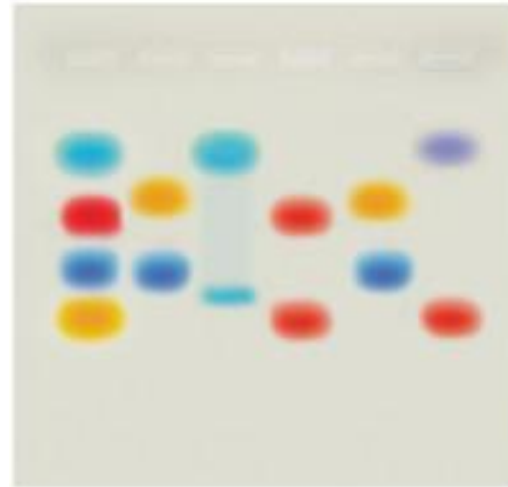
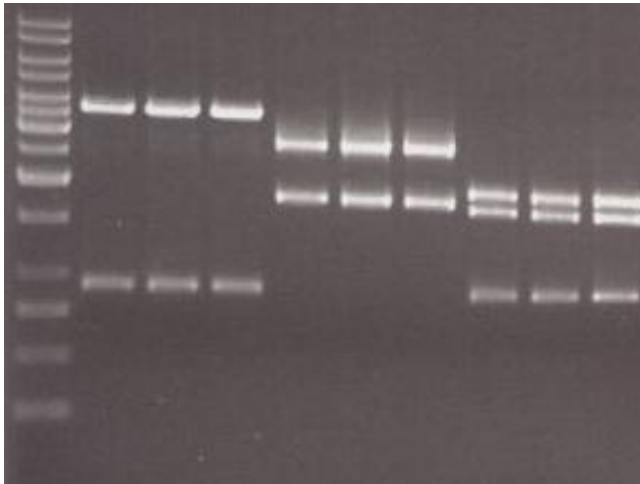


Agarose Gel Electrophoresis



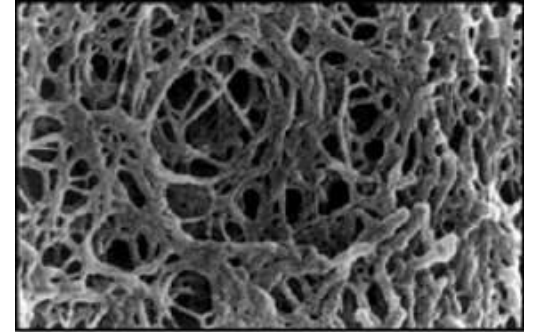
What is agarose gel electrophoresis?

Electro = Electricity

Phoresis = Movement in a (specified) manner or medium

Gel electrophoresis is a laboratory method used to separate mixtures of DNA, RNA, or proteins according to:

- **Electrical charge**
- **Molecular size**



Quora.com

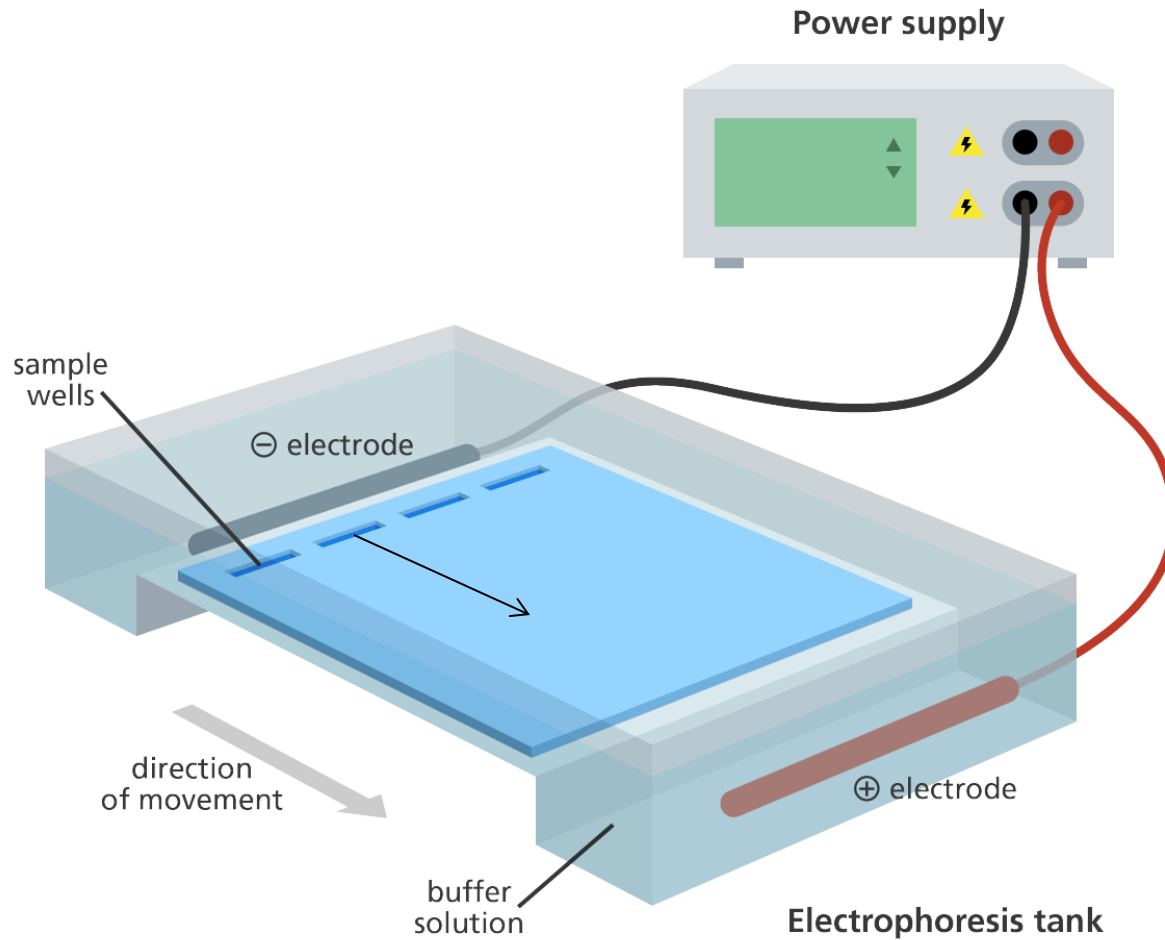
In gel electrophoresis, the molecules to be separated are pushed by an electrical field through an **agarose** gel that contains small pores.

Agarose = A linear polysaccharide polymer generally extracted from seaweed. In powdered form, it can be mixed with gel running buffer (containing salts), melted and poured into a gel mold.

The rate at which a molecule travels through the gel is determined by its size (molecular weight). Small molecules travel more quickly. Larger molecules travel more slowly.

The direction the molecule travels is determined by the molecule's charge.

Gel electrophoresis equipment



Gel electrophoresis equipment



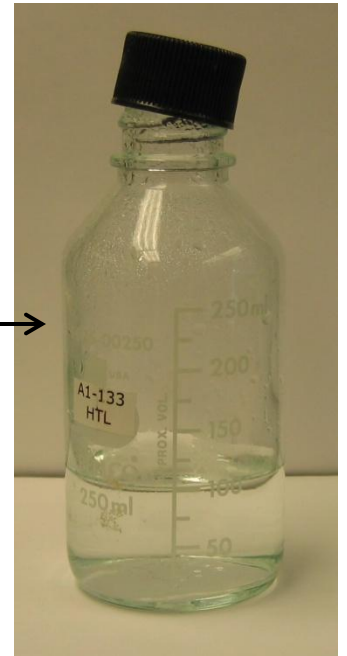
Agarose Gel Electrophoresis

- 1. Make the agarose gel.**
- 2. Prepare your sample.**
- 3. Load your sample on the gel.**
- 4. Run the gel.**
- 5. Visualize the gel.**

Gel Preparation

1. Make the agarose gel.

Agarose powder melted in buffer containing salt and pour in mold. Add **GelGreen** to visualize DNA.

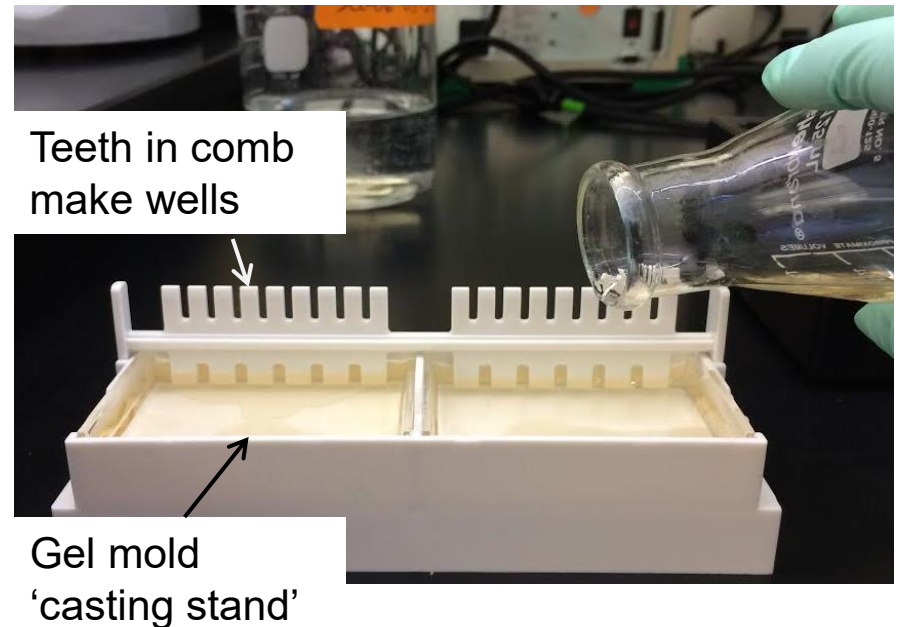


2. Prepare your sample.

3. Load your sample on the gel.

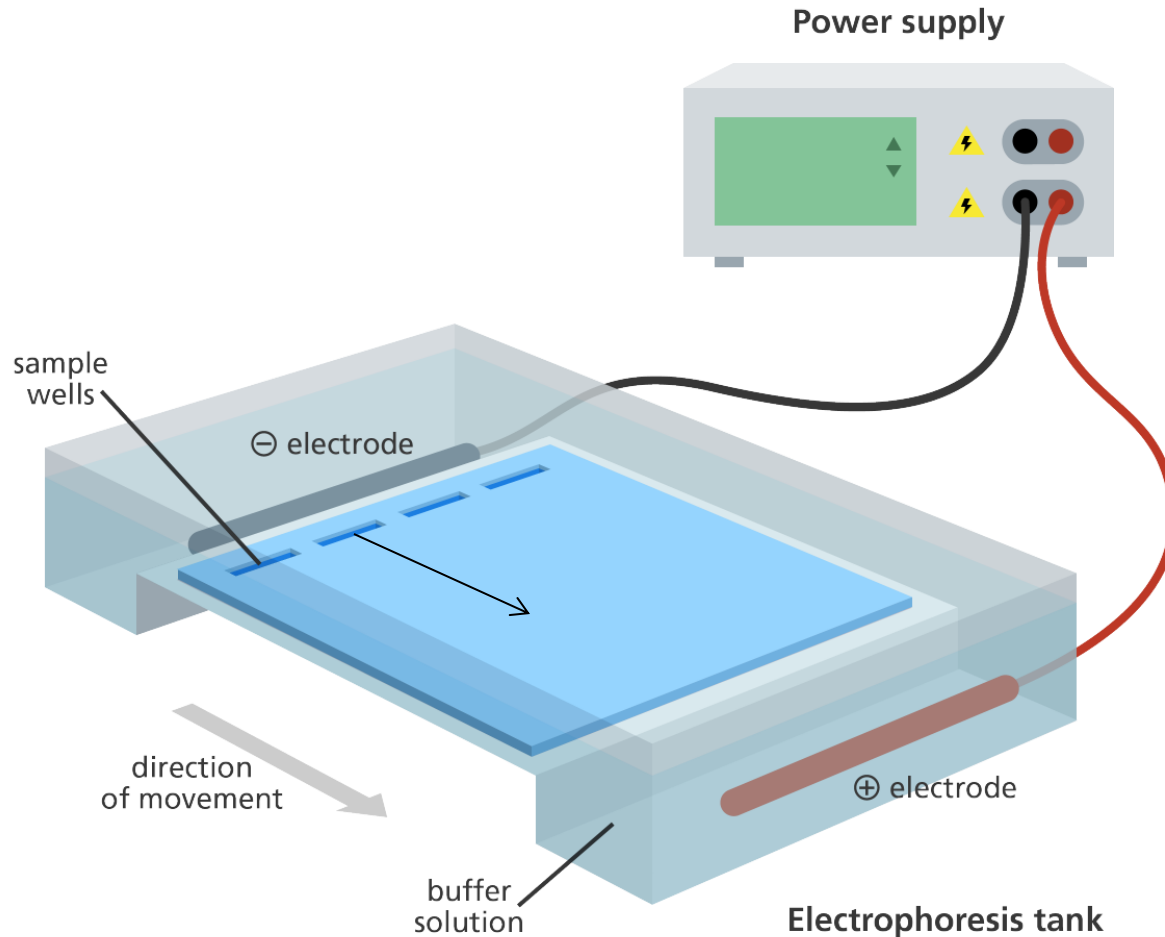
4. Run the gel.

5. Visualize the gel.



Gel electrophoresis equipment

DNA has a net negative charge, so it moves towards the positive pole.



Gel Electrophoresis with Dyes



Dye molecules separate based on size and charge

Positively charged dyes move towards the negative pole.

Negatively charged dyes move towards the positive pole.

Dyes with lower molecular weights move through the gel more rapidly than dyes with higher molecular weights.

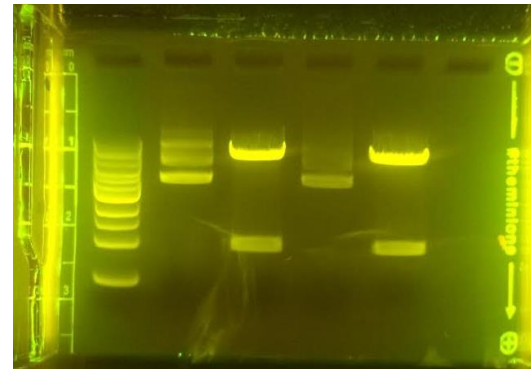
DNA Gels - GelGreen

- To visualize DNA, GelGreen is added to the liquid gel solution.
- GelGreen binds to DNA as the DNA travels through the gel.
- When an LED light shines through the gel, the GelGreen bound to the DNA makes the DNA glow green.



Fisherbiotec.com.au

GelGreen & Blue Light



Sample Preparation

1. Make the agarose gel.

2. Prepare your sample.

3. Load your sample on the gel.

4. Run the gel.

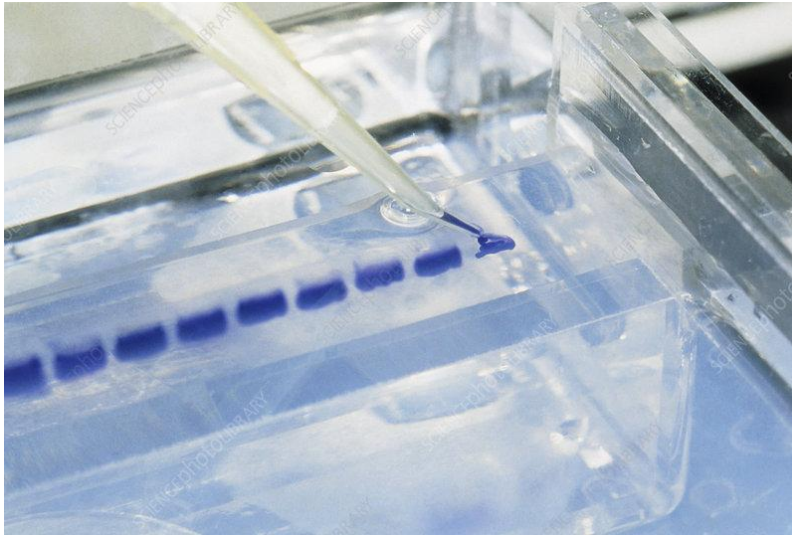
5. Visualize the gel.



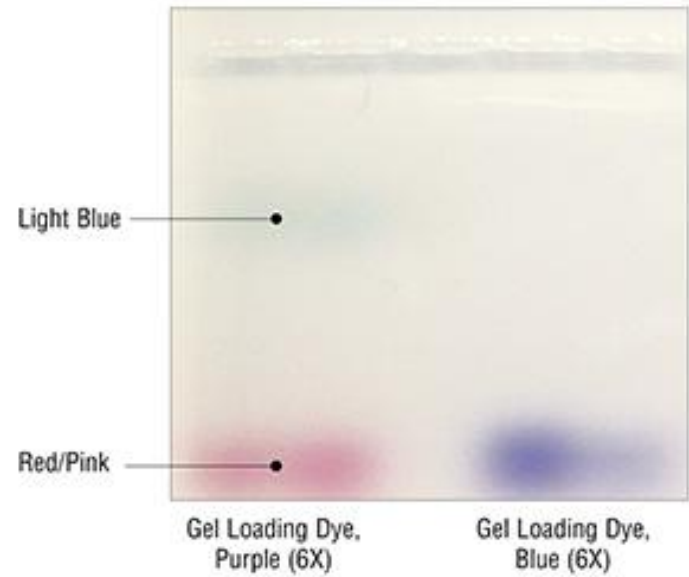
www.life.illinois.edu

- Add loading dye to your sample as described in your protocol
- Loading Dye contains
 - Dye – to see the sample as it runs through the gel
 - Glycerol – to help sample sink into the well

Loading Dye



Sciencephoto.com



neb.com

Gel Loading

1. Make the agarose gel.

2. Prepare your sample.

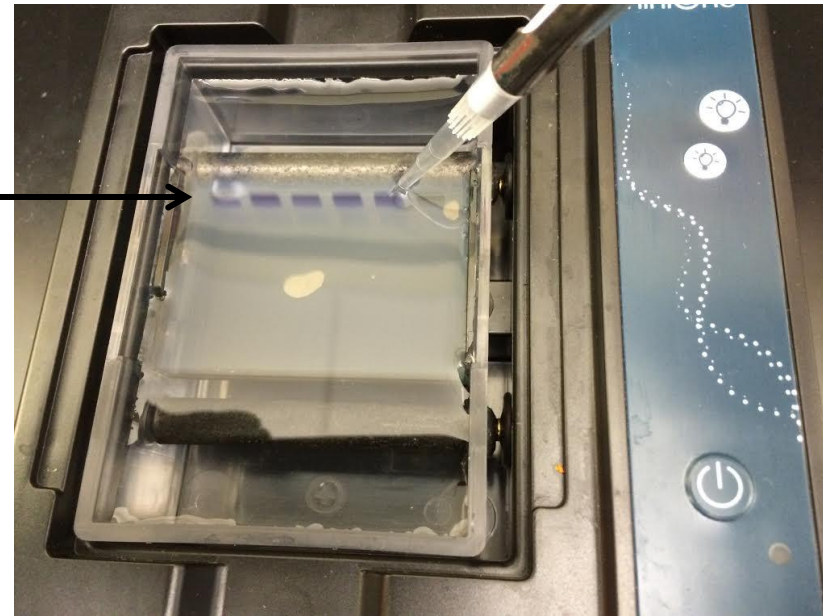
3. Load your sample on the gel.

4. Run the gel.

5. Visualize the gel.

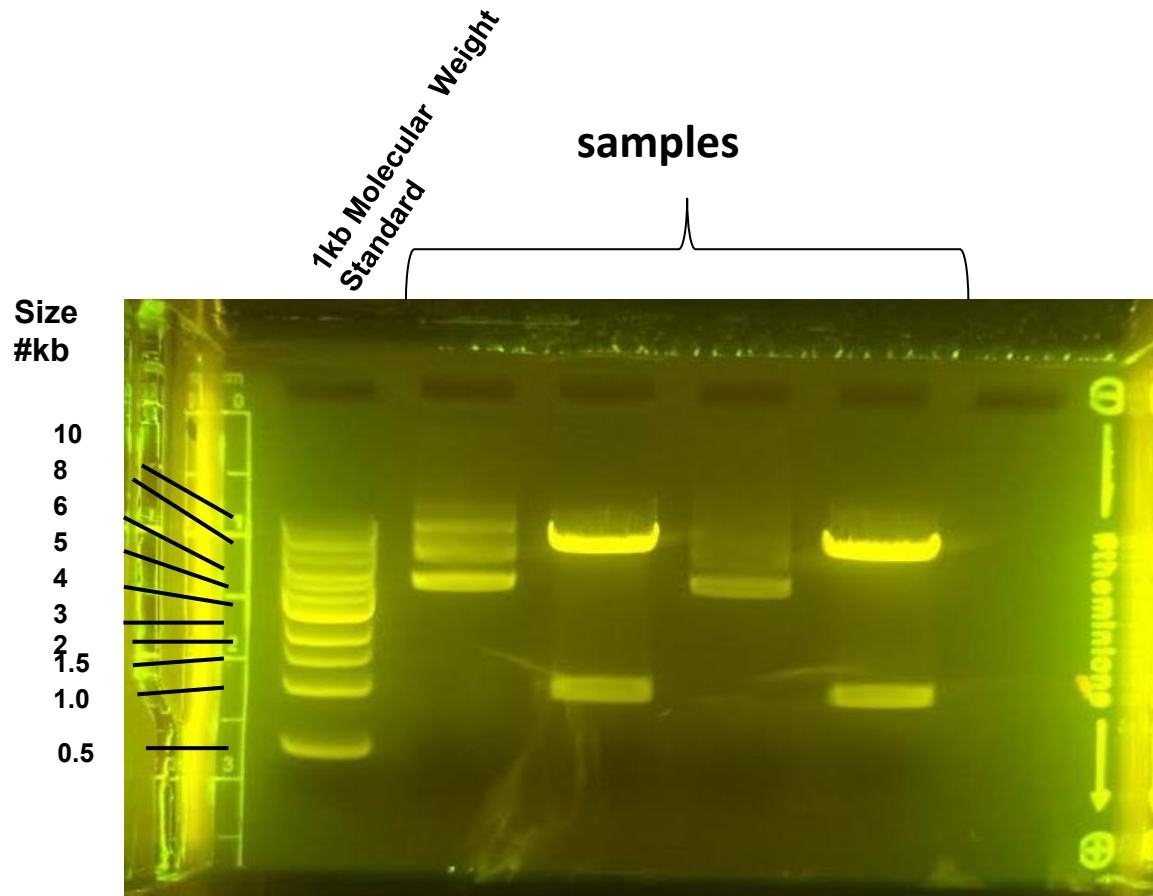
Samples are loaded into gel wells with a micropipette

Top: Negative Electrode



Bottom: Positive Electrode

Molecular Weight Standard



Running the Gel

1. Make the agarose gel.

2. Prepare your sample.

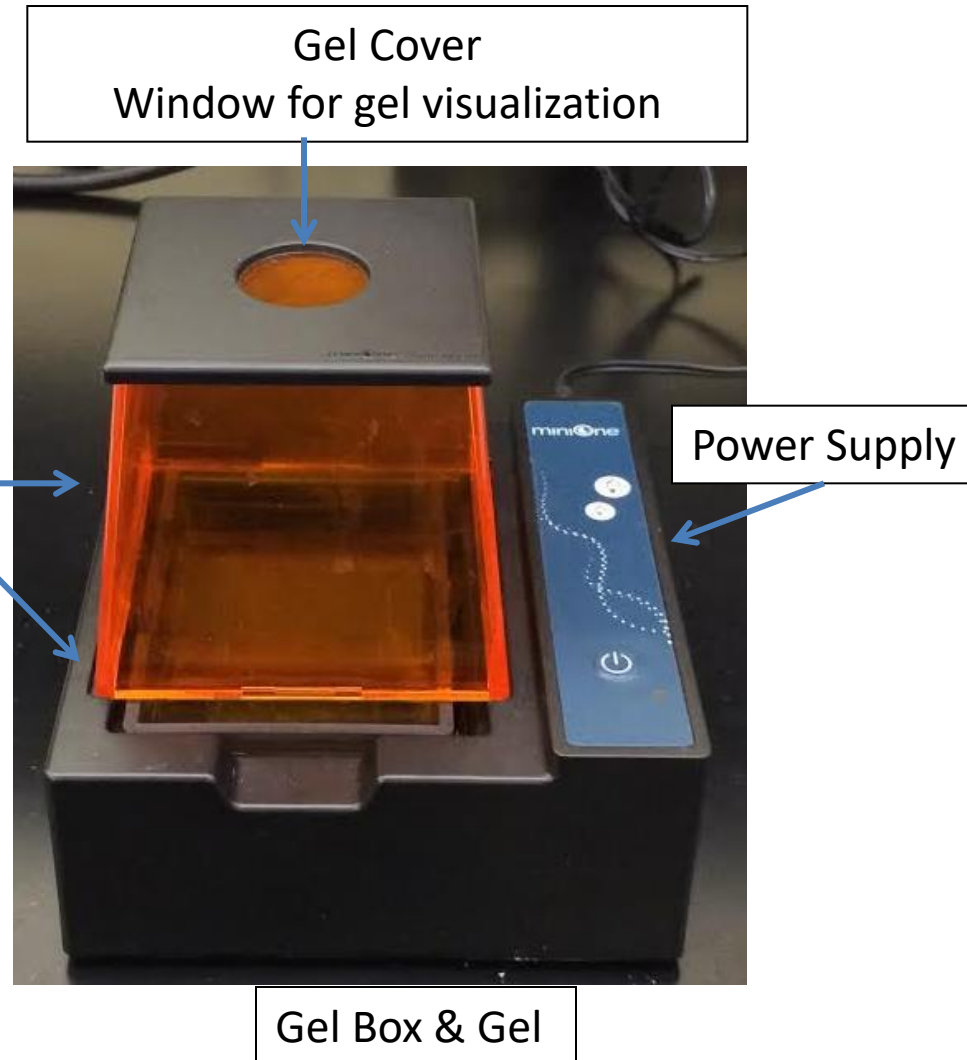
3. Load your sample
on the gel.

4. Run the gel.

5. Visualize the gel.

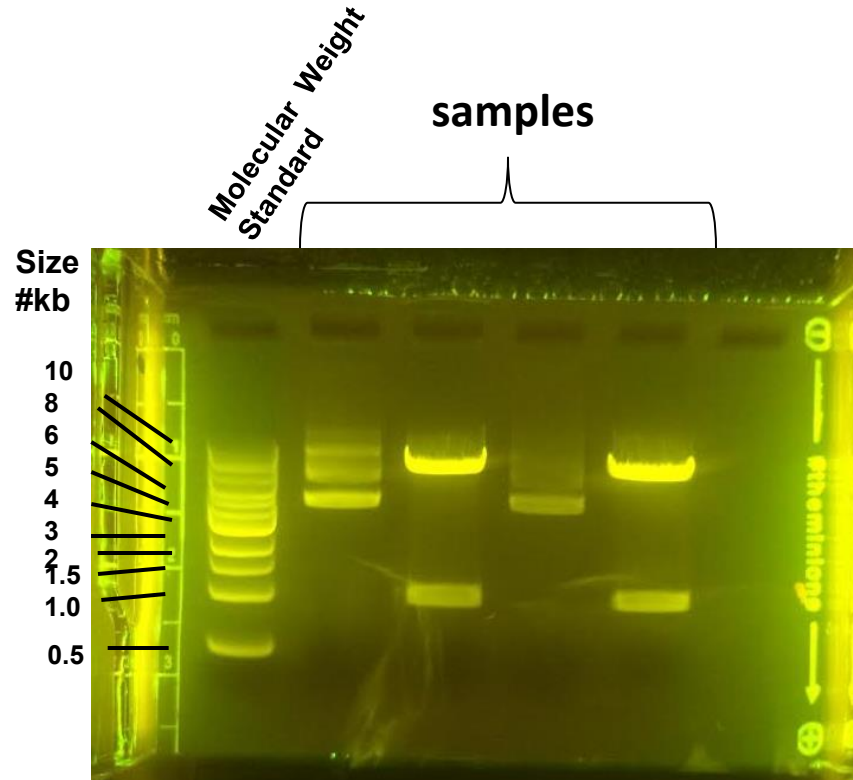
Electrodes

Negative
Positive



Visualization

1. Make the agarose gel.
2. Prepare your sample.
3. Load your sample on the gel.
4. Run the gel.
5. Visualize the gel.

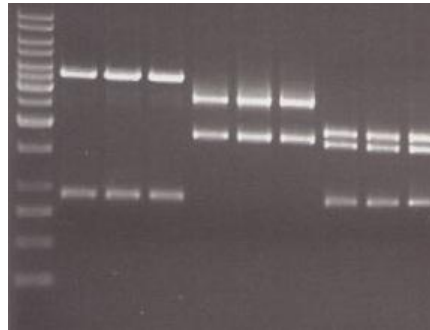


*Separating pieces of DNA
based on charge and size.*

Different Methods of Visualization

1. Make the agarose gel.
2. Prepare your sample.
3. Load your sample on the gel.
4. Run the gel.
5. Visualize the gel.

Ethidium Bromide & UV Light



Fast Blast™ Stain & Visible
(White) Light



GelGreen & Blue Light

