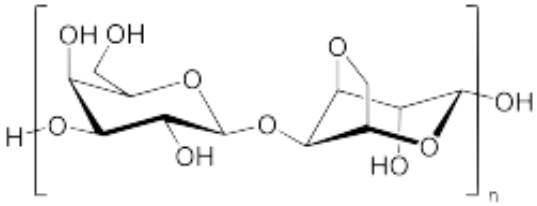
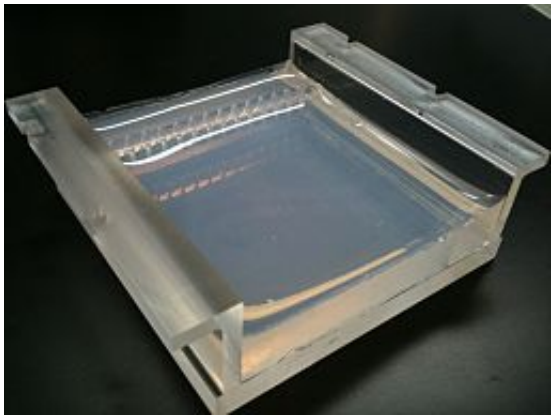
Analyzing DNA

Contents

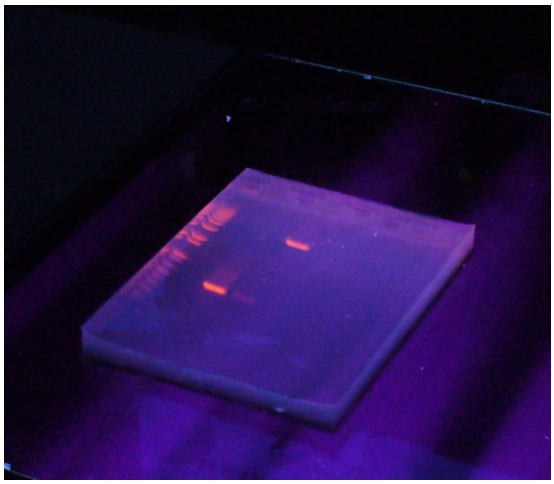
Agarose Gel Electrophoresis



Agarose is a linear carbohydrate polymer purified from the cell walls of certain species of algae. Agar is a combination of the crude extract that contains agarose and the smaller polysaccharide agaropectin. When dissolved and melted in liquid, agarose strands become tangled together to form a netting that holds the fluid in a gel. Reduction of the fluid creates a higher percentage gel that is firmer and contains smaller pores within the netting.



Placing a **comb** within the melted agarose creates spaces that allow for the insertion of samples when the gel is solidified. Molecules can traverse through the pores as they are drawn by electrical currents. Charged compounds will migrate towards the electrode of opposite charge but migration rate will be influenced by the size of the molecules. Smaller compounds can easily traverse through the webbing while larger items are retarded by the pore size. Follow this simulation to get a better idea of how we use **Agarose Gel Electrophoresis** in molecular biology to study DNA fragments.

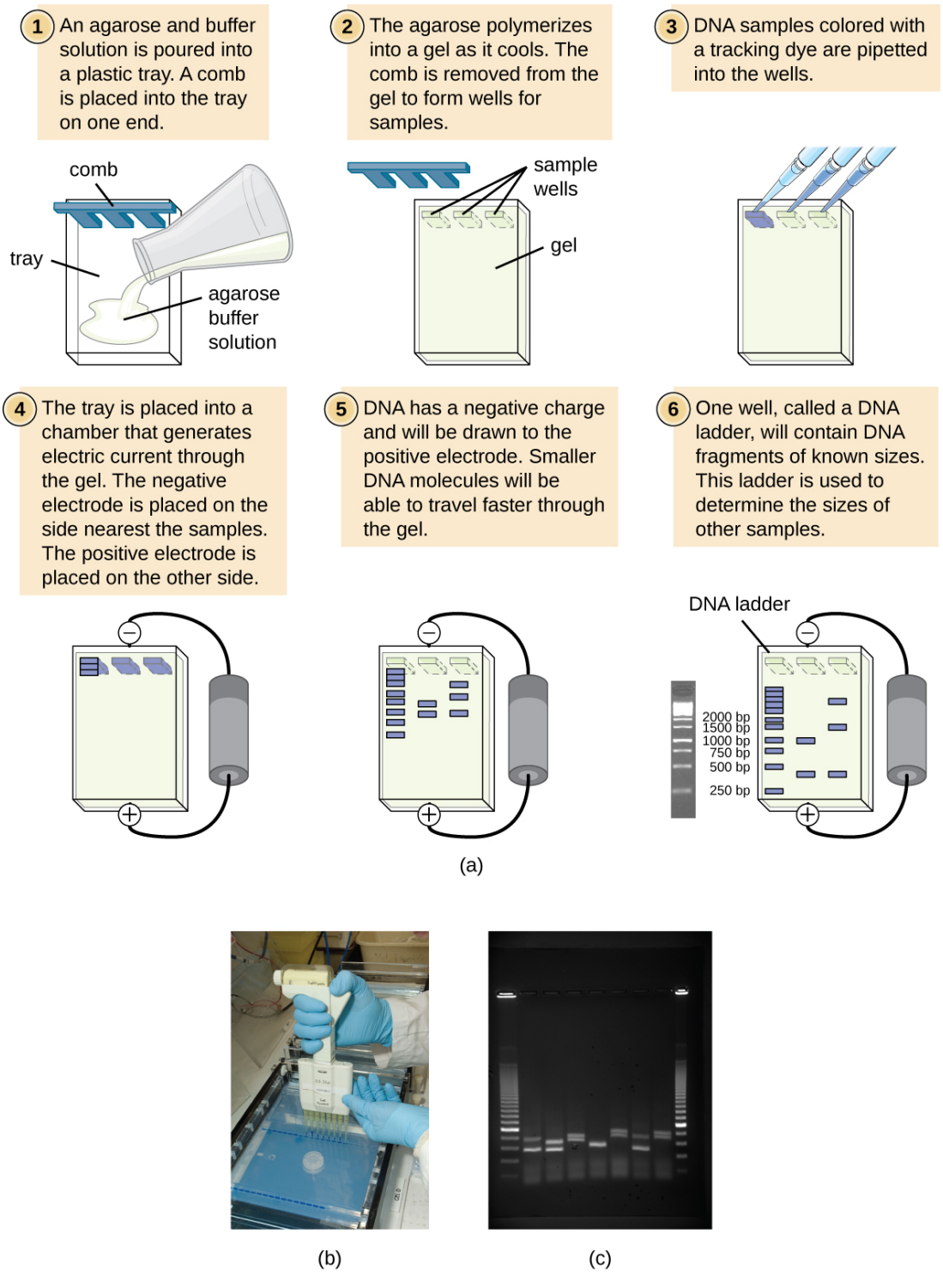


DNA molecules are not readily visible when **resolved** (separated) on an agarose gel. In order to visualize the molecules, a DNA dye must be administered to the gel. In research labs, a **DNA intercalating agent** called Ethidium Bromide is added to the molten gel and will bind to the DNA of the samples when run. Ethidium Bromide can then be visualized on a UV box that will fluoresce the compound and reveal bands where DNA is accumulated. Since Ethidium Bromide is known as a carcinogen, teaching labs will use a safer DNA intercalating agent known as Sybr Green. This can be visualized in a similar fashion, but will fluoresce a green color instead.

Agarose gels are made of and bathed in a buffered solution, usually of Tris-Borate-EDTA (**TBE**) or Tris-Acetate-EDTA (**TAE**). Regardless of buffer solution, the buffer provides necessary electrolytes for the current to pass through and maintain the pH of the solution.

DNA samples are prepared in a buffer similar to the solution that it will be run in to ensure that the phosphate backbone of the DNA remains deprotonated and moves to the positive electrode. Additionally, **glycerol** or another compound is added to this buffer in order for the solution to sink into the wells without spreading out. A dye is often included in this loading buffer in order to visualize the loading in the wells and to track the relative progression of gel.

Agarose Gel Set-up



https://upload.wikimedia.org/wikipedia/commons/8/84/Agarose_Gel_Electrophoresis_-_Assembling_the_Rig_and_Loading-Running_the_Gel.webm

External Resources

- Gel Electrophoresis Simulation

- The Structure of DNA http://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397
- http://learn.genetics.utah.edu/content/science/forensics/

Electrophoresis of Dyes (activity)

1. Prepare a 1% agarose gel by adding 60ml Tris-Borate-EDTA buffer (TBE) to 0.6g agarose in an erlenmeyer flask
2. Place flask in microwave or on heat until agarose is melted
    - stop periodically and swirl solution and do not permit to boil over
3. Assemble the casting tray by blocking the ends with tape or plastic gaskets
4. Place the comb into the center of the casting tray
5. You may place the casting trays inside a refrigerator and pour the solution into the tray
6. Wait until the gel is solidified
7. Carefully separate the gaskets from the tray
8. Remove the comb and place the casting tray into an electrophoresis chamber
9. Cover the gel with TBE buffer
10. Using a micropipettor, load 40-50?l dye samples sequentially into the wells
11. Cover the electrophoresis chamber with the lid and ensure good contact between electrodes
    - It is conventional that the **POSITIVE** side of the tank is nearest to you
    - With the **POSITIVE** side nearest to you, load the samples from left to right
12. Set the power supply to 100-120V and press the Run button (you should see bubbles at each electrode) and allow to run for at least 40 minutes
13. After 40 minutes, stop the current and remove the gel in casting tray
14. Place tray on a white background and document your gel

Activity Follow-up

1. What colors were the dyes originally before loading into the wells?
2. How many separate bands of dye are in each well following the run?
3. What does it mean that there are multiple bands in a lane? What does it mean that there is only one band in a lane?
4. What does the length of migration illustrate to us about the properties of the dye molecules?
5. In which direction did the dye molecules migrate? What does the direction of migration indicate about the analytes?
6. Are there lanes where there are multiple bands of the SAME color?

Tags: analysis, visual communication