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A

Gel Electrophoresis Loading

Life on the Edge

B

C

Acknowledgements

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Photography Credits:

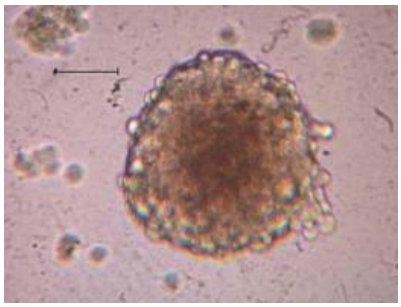
A. Picture of a gel.
B. Picture of a student loading a sample into the gel.
C. Picture of another student loading a sample into the gel.

D. National Standards Correlation

Life Science Content Standard C

The Cell

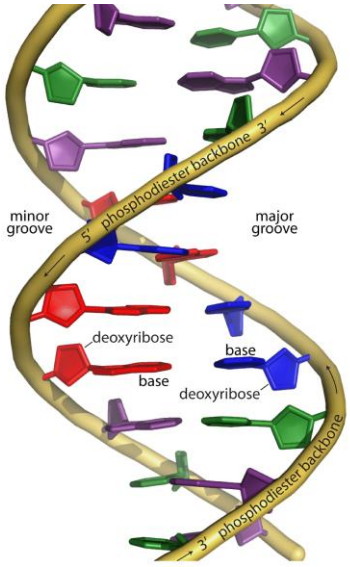
Cells store and use information to guide their functions. The genetic information to guide their functions. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires.



SK-N-SH cells grown in the NASA RCCS.

The Molecular Basis of Heredity

In all organisms, the instructions for specifying the characteristics of the organism are carried in the DNA, a large polymer formed from subunits of four kinds (A, G, C, and T). The chemical and structural properties of DNA explain how the genetic information that underlies heredity is both encoded in genes (as a string of molecular “letters”) and replicated (by a templating mechanism). Each DNA molecule in a cell forms a single chromosome. Changes in DNA (mutations) occur spontaneously at low rates. Some of these changes make no difference to the organism, whereas others can change cells and organisms. Only mutations in germ cells can create the variation that changes an organism’s offspring.



DNA’s double helix.

Prep Time: Approximately 30 minutes
Class Time: Approximately 60 minutes for activity and discussion

Purpose
Students will become familiar with the technique used to analyze DNA through gel electrophoresis and micropipetting.

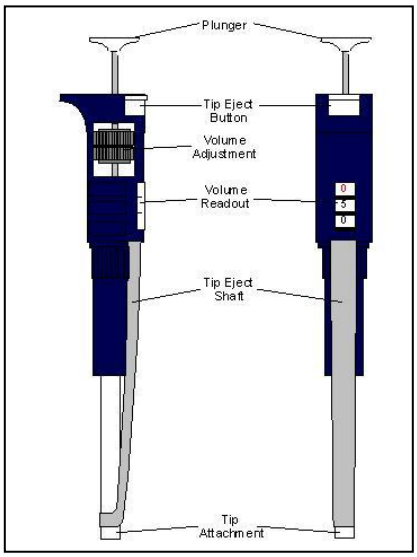
Key Concepts
Students will master the skills necessary to micropipette extremely small volumes associated with molecular biology into agarose gels for DNA analysis.

Common Misconceptions

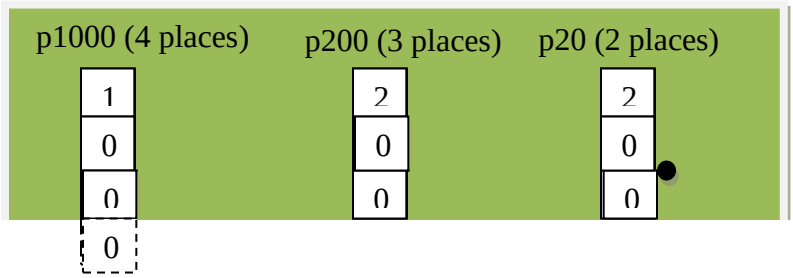
- Each band represents a single DNA fragment of a certain length. However, each band actually consists of many DNA fragments of the same size.
- Students assume a linear relationship between size of the DNA fragment and the distance travelled through the gel. They think that if you have two fragments, the one that is half the size of the other will travel twice as far.

Overview
Very tiny amounts of chemical and biological reagents are used in many astrobiology experiments. To measure these minute volumes, scientist use micropipettes that measure microliter (µl) amounts. This activity introduces micropipetting technique. As with all fine motor skills, learning how to use a micropipette takes practice and determination. Students will be required to measure these very tiny volumes with accuracy. See picture below.

Pipette	Max (µl)	Min(µl)
p1000	1000	100
p200	200	20
p20	20	2



Below are pictures of the display windows of the micropipettes.



Prep Time for Teachers: 30 minutes
Class time: 90 minutes

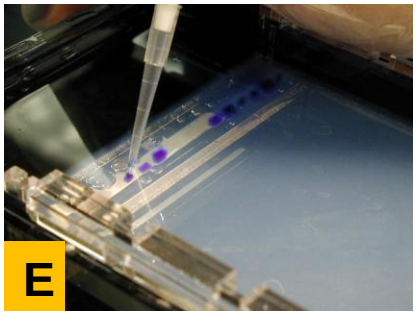
Objectives

- Learn how DNA fragments are sorted by size with gel electrophoresis.
- Work in groups to construct and compare different DNA fingerprints.
- Be able to explain the process of gel electrophoresis.

Skills

1. *Predicting* the outcome of an experiment
2. *Conducting* an experiment
3. *Collecting, recording,* and *graphing* data
4. *Drawing* conclusions and *communicating* them to others

Materials per group



1. 2% agarose gel
2. gel electrophoresis box
3. gel combs
4. gel tray
5. gel blocks
6. Micropipettes and tips (P-1000, P-200, and P-20)
7. Food Coloring (red, blue, yellow and green) 2 drops in 250ml of water for each color
8. 250 ml beaker
9. 250 ml beaker of water for pipette rinsing

Practice Loading Samples into Gels

Step 1- Select your micropipette needed and set the desired volume at 15 µl.
Step 2- Push the end of the pipette into the proper-size tip.
Step 3- Open the cap or lid of the tube from which you are taking fluid. (Or have you lab partner do this.)
Step 4- Hold the micropipette in one hand, at a 45º angle from vertical. In this way contaminants from your hands or the micropipette will not fall into the tube. Hold the tube with the sample in your other hand. Both should be almost eye level.

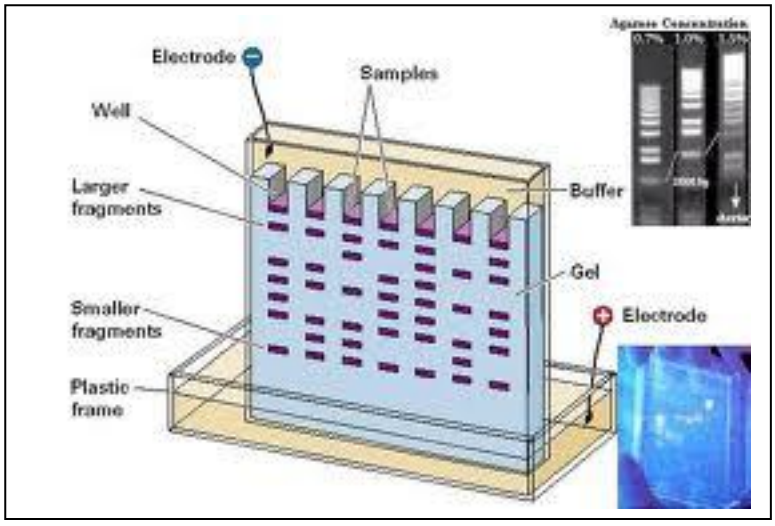
Step 5- Depress the plunger of the micropipette to the first stop and hold it in this position.
Step 6- Place the tip into the solution to be pipetted.
Step 7- Draw the dye into the tip by slowly releasing the plunger.
Step 8- Place the tip of your pipette in Lane 1 of your gel without touching the bottom of the gel. Be careful to not poke a hole in the bottom of your gel.
Step 9- Slowly depress the plunger of the micropipette to the first stop to release your dye into the well. Then continue to the second stop to expel the last bit of fluid, do not release. Hold the plunger in this position.
Step 10- Slowly remove the pipette from the well, keeping the plunger depressed to avoid drawing any liquid back into the tip.
Step 11- Always change tips for each new solution you pipette. To eject a tip depress the ejector button on the top of the micropipette.
Step 12- Repeat steps 2 – 11 for the remaining sequence of dyes and amounts.
Step13- After you have completed steps 1-12, obtain a DNA sample and practice loading that into a gel.

Lane	Color	Amount
1	Blue	15 µl
2	Red	15 µl
3	Green	15 µl
4	Yellow	15 µl
5	Red and Blue	20 µl
6	Green and Yellow	20 µl

Data:

Complete the diagram of the gel below to show the samples and volumes loaded into each well

Example of Gel.

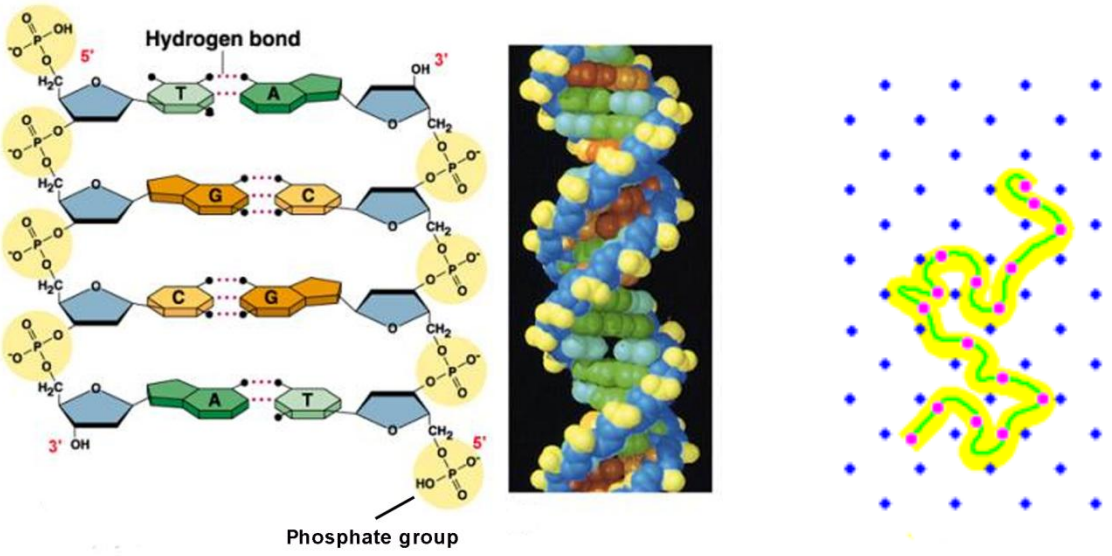


Student Handout

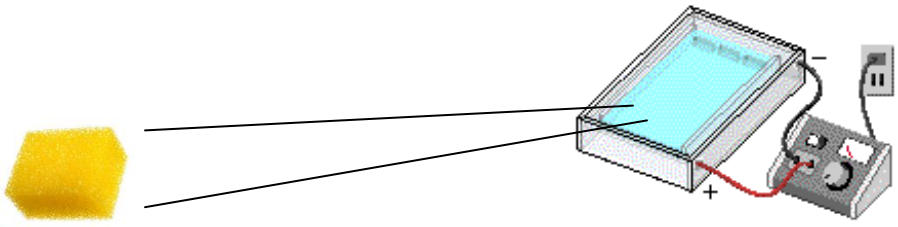
Basics of Gel Electrophoresis Gel electrophoresis is among the most common molecular biology tools. It can separate DNAs of different lengths. It can also separate different lengths of RNA or protein molecules.

2. Gel Electrophoresis uses an **electric field** to separate molecules based on the molecules' **SIZE**, **CHARGE** and **SHAPE**, and their frictional properties in the **GEL**.

3. For DNA molecules:
DNA molecules have a negative charge in solution since its phosphate backbone has oxygen with net negative charges.



Electrophoresis uses an electric field to move DNA molecules through the gel. Negatively charged DNA moves away from **–** electrode (cathode), and toward **+** electrode (anode). Need ions in the gel and the solution. Allows a current to flow when apply voltage. Lack of enough current decreases electric field in the gel and DNA will not move.



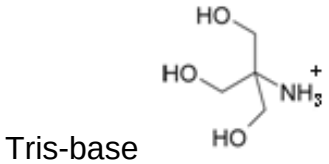
The gel forms a porous sponge-like matrix. DNA duplex fragments separate by length. Smaller molecules move easier through the matrix than large molecules. A Youtube video on DNA electrophoresis in agarose gels

http://www.youtube.com/watch?v=QEG8dz7cbnY&feature=related

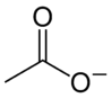
For DNA duplex fragments of different lengths.

1. Migration distance of DNA in gel depends on the voltage (electric field strength in gel), time of run, and percentage of agarose (density of gel).

Generally use 80-120 volts for about ~ 60 minutes with a 1% agarose gel (1 gm/100mls) using a Tris-acetate buffer. Stain the DNA in the gel to visualize it. Often use a dye called ethidium bromide. Ethidium bromide binds to DNA. When it is bound it fluoresces a red color when one shines UV light on it.

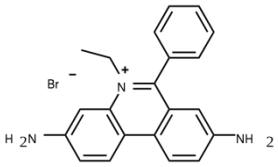


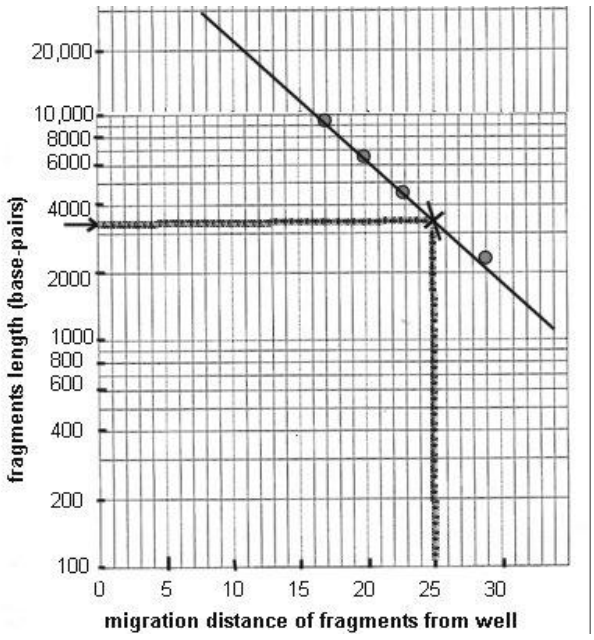
Tris-base



acetate

ethidium bromide





Picture Citations:

A-C- Pictures taken by Aakanksha Angra
D-Map of Georgia-
E-https://sites.google.com/a/luther.edu/genetics/students/andrew-bhagyam/gel-preparation
F-G-Pictures taken by Jamila Cola
Picture of Gel-
http://biotech.biology.arizona.edu/labs/Electrophoresis_dyes_stude.html
Student Handout- Made by Dr. Roger Wartell