Research

Learning Objectives:
- Learn research methods and techniques commonly used in genetics laboratories
- Apply the above to concepts learned in prior workshops, i.e
- Relate the program to careers in scientific research

Key Vocabulary:
- Solutes/Solvents
- Pipettes
- Recombinant DNA
- PCR (polymerase chain reaction)
- Gel electrophoresis
- chromatography
- Centrifugation

INTRODUCTION (15 MIN. OPEN DISCUSSION)

What do you guys think of when you hear the word "scientist"?
Sample Answers:
- crazy, white-haired man in lab coat
- bubbling beakers
- explosions

Those were all great suggestions, but scientist are actually way more diverse than that. Scientists can be girls and guys, they can be from diverse backgrounds, and they don't just wear lab coats and goggles. Sometimes they wear hardhats or scuba suits or scrubs!

But today, we are going to talk about those lab coats. Any one have any ideas of what they do at their jobs?
Sample Answers:
- science
- Discover things
- Make breakthroughs
- Discover new elements, new organisms etc

Many of these scientists work in labs that study chemistry, biology, genetics, and a variety of other physical scientists. These laboratories are built with high-tech equipment that allow scientist to perform complex experiments, leading to their amazing discoveries.

The reason why we know about how our molecular processes work and why we could piece this whole program together, is because of the all the awesome work a long string of scientists have dedicated their lives to.

Lets talk about a few of those scientists!

15-20 min Background Info.

Barbara McClintock was a geneticist who, in 1931, characterized how genes cross over information for maximum genetic variation. *Who can remember what genes are?* In 1950, she published on mobile genetics, the theory that some genes could turn on and off through a process no one had thought of before.

↳ McClintock developed improved *staining* techniques, which allowed her to see chromosomes under the microscope better than anyone else had before.

Rosalind Franklin, James Watson, and Francis Crick were the three scientist who discovered the structure of DNA in 1953. And back then, they didn't have the tech we have today. ***Raise your hands to tell me a few facts we learned about DNA***.

↳ Rosalind used *x-ray crystallography*, a method of beam diffraction that allows 3D imaging of a crystal. Through this procedure, Rosalind was able to show the double helix and dimension of fiber of DNA while Watson and Crick were only postulating its structure.

In 1969, Klaus Weber and Mary Osborn introduced denaturing agents, like a compound called SDS, for gel electrophoresis experiments.

↳ *Gel electrophoresis* is a powerful technique used to manipulate DNA and as an analytical tool, such as in DNA fingerprinting. *PAGE* (polyacrylamide gel electrophoresis) is used to separate proteins by size and electrical charge; it is also used to separate DNA and RNA fragments by length.

In 1990, the *Human Genome Project* had officially began. The endeavor to sequence our entire genetic makeup took off with a group of dedicated scientists, including **Craig Venter.**

He was passionate about the ability *genomics* had to transform healthcare delivery in the United States. Venter was heavily involved towards the completion of HGP in 2000, and later pursued advancing biofuels and synthetic biology through genetic research.

We've come a long way since these discoveries, and new things are being found everyday. Current research interests involve how our DNA may or may not be responsible for aging, how to use DNA to treat disease, and even the possible presence of a known rat poison in the origin story of our genetic material.

All of this is made possible not just by the scientists, but through the laboratory techniques they use to conduct their experiments. **Today, we're going to be making our own gel electrophoresis tray and running a sample to compare the different compounds in food coloring!**

Gel Electrophoresis Trays

Materials:	Procedure:
For the electrophoresis chamber:	1. First, you'll need to make a comb to create wells in the gel that will eventually hold your samples. Rest a regular-sized craft stick on top of the small plastic box.
• Small, shallow plastic box measuring approximately 3 x 5 inches	2. Cut the narrow craft sticks into several shorter lengths as follows: when taped to the side of a regular-sized craft stick, each segment should hang down so it sits just above the bottom of the small plastic box. Cut **five** of these "teeth" and tape them to one side of the regular-sized craft stick. ***Ensure that the teeth are evenly spaced, hang down to the same level, and all fit inside the box.***
• Two regular-sized craft sticks	3. Tape the other regular-sized craft stick parallel to the first one, so that it creates a sandwich for the teeth, then lay the comb across the short side of the box. ***Check that the teeth hang evenly and don't touch the bottom.***
• Two narrow craft sticks or wooden coffee stirrers	4. A buffer will be prepared for you. You will need approximately 100 milliliters per set up—half to make the gel and half to run your samples.
• Scissors	5. Make a 1% gel solution by adding 0.5 g of agar-agar powder to 50 mL of sodium bicarbonate buffer. You will need 40–50 mL of gel solution per set up. To dissolve the agar-agar powder, heat the gel solution in the microwave, stopping every so often to stir it. Watch the solution carefully, as it will quickly boil over when hot enough. When you see bubbles, stop the microwave and stir the solution until the agar-agar particles completely dissolve.
• Masking tape	6. Once the solution is cool enough to pour, add just enough to the box so that the comb teeth are submerged a little further up that half their length. Position the comb across the narrow side of the box, closer towards one edge and leaving about a quarter inch of space between the tray wall and the comb. ***Thinner gels will yield better separations.***
• Two 5-in pieces of stainless steel wire	7. Once the gel sets (approximately 5–10 minutes), you need to make space to place an electrode on either end. Pick up two electrodes from a team leader.
• Two electrical leads with alligator clips	8. The actual gel only needs to be half the length of the box, so you can use a knife to cut away the bottom half of the gel that doesn't include the comb. Being careful not to disturb the comb, also cut away a thin strip from the very top of the gel to make space for an electrode at the top of the box. ***DO NOT* cut any width from the gel, only from the top and bottom of the tray!** Your gel should be the full width of your box. Place one electrode along the top end of the gel and one at the bottom end, using tape on the outside of the box if needed to secure them in place. These will be the positive and negative electrodes.
• Five 9-volt, super heavy duty batteries	9. Make a high-voltage power supply by connecting the five 9-volt batteries. Clip two batteries together by inserting the positive terminal of one into the negative terminal of another. Attach the remaining batteries one by one in this way until you have a five-battery pack.
For the gel and buffer:	10. Clip an electrical lead to each of the exposed terminals of the pack. You should now be able to use the battery pack to power something by attaching the other ends of the electrical leads.
• Water	11. Prepare five different samples by mixing 1–2 drops of food coloring with 1 mL glycerin and 1 mL water in a small tube. We use blue, red, green, yellow, and purple (made by mixing blue and red food coloring).
• Baking soda	12. Once you have set up your gel, pour in just enough sodium bicarbonate buffer to cover the solidified gel. Make sure you fill up the spaces left from where you cut away the gel—the gel and stainless steel wires should be completely submerged.

Materials:	Procedure:
• Agar-agar powder	13. Gently remove the comb by pulling straight up without tearing the gel. The wells left by the comb should fill with buffer. Use the needle-tip pipette to transfer approximately 10 microliters (μL) of each sample to an empty well. Submerge the tip very slight into the well or directly above the well and gently squeeze the sample into the well. It should fall into the well since it is denser than the surrounding buffer. Be sure to use a new pipette for each sample to prevent contamination between samples.
• Mat knife or razor blade	14. Once all the samples are loaded, connect the leads from the power supply to the stainless-steel wire electrodes attached to the box. Connect the negative terminal to the electrode at the top of the gel (near the wells) and the positive terminal to the electrode at the bottom of the gel.
For the samples:	15. Leave the power connected for 15–20 minutes and observe what happens to each sample.
• Water	
• Food coloring	
• Glycerin	
• Needle-tip disposable pipette (for example, Flinn Scientific #FB1260 variety)	
• Optional: beaker of water for rinsing tips between samples	

Reinforcement. Once completed, talk to the students about what their observations were. Which colors moved the fastest? Why? Which brands moved the fastest and what color chemicals did they use that made their dyes move so quickly in our gel? Compare their final observations to what their initial expectations of the experiments were and reinforce the concept of a hypothesis.

Wrap-Up! Gel electrophoresis is an essential technique for experimentation in sciences like microbiology, genetics, and clinical chemistry. Without it, much of the understanding we have about our bodies today would not be common knowledge. So many other techniques are used, just like SDS-PAGE, to manipulate samples and extrapolate information from natural phenomena to help us human understand the world we live in a little better each day.