

Today, we'll be talking about gel electrophoresis. What is gel electrophoresis, you might ask. Well, it's a lab technique usually used in the biochemistry lab for separating out DNA or proteins based on their size-

And let's talk about how it works.

So first, you need to have the gel -This can be made out of different kinds of substances, such as agarose and polyacrylamide, both of which I'll discuss later.

And the electrophoresis part of it means that you need to have an electrical field passing through the gel to get the bands to move. So, to create an electrical field, we have to have a cathode and an anode. The cathode is on this side.

Remember from general chemistry that at the cathode, reduction takes place, so you'll have a negative charge. But on the other side, where the anode is, you'll have a positive charge, because this is where oxidation is taking place.

And to power this, you'll need some kind of battery to connect them. And these are both usually also connected to a big box that contains the gel electrophoresis apparatus. In order for charge to flow across, you need something that will conduct electricity, so you have a buffer that's usually composed of different ions.

So, this is completely covering this entire gel. And remember that it's always there, but I'm going to erase it just because it's going to get a little messy for what I need to show you next.

So next, you'll load a sample of DNA. In order to load your sample, you'll need to take a pipette and put it into one of these wells. So usually you'll want to make sure that you have a dye mixed with sample so that you can see it as it's running. So I'll show that as a pink line here, so I've filled that well, followed by yellow here, and green here. So that's just the color of the dye. It can really be any color you want. This will just help you track the movement of the bands later on.

Once you have these bands, what will happen once you turn on the electrical field?

VERY IMPORTANT:

***Remember that DNA has a negatively charged backbone because of all the phosphate groups so what you'll actually observe is that they'll travel towards the positive end

So, maybe after a short period of time, what you'll see is that-- it'll look something like this. Maybe the pink will have split up into a few bands, indicating that there's a few different size fragments in there. With the yellow, you might only see one band. And with

the green, you might actually have 2 bands, but they're so close together still that it's kind of hard to tell.

So how can you really see what's going on?

**The solution is to let it run for longer period of time

And eventually, what you'll see is shown here at the bottom. You'll see that whatever sized fragments were in your original samples have effectively split up by their size. And note that in pink, you'll always want to load something known as a DNA ladder. A DNA ladder is like a standard. This is something that you buy from a company, and they tell you exactly what sizes their fragments so that you can match them up to your unknown samples.

So, this could be 400 base pairs, 200 base pairs, and 100 base pairs. And as you'll note, the smallest fragments travel the furthest.

This is because the smallest things are really easy to push with the electrical field. But when you have such a big molecule, or rather a big DNA fragment, it can be hard to move.

So, you can see that the 400 base pair doesn't move too fast or too far.

And what's this tell us about our unknown yellow and green samples?

This shows us that the yellow sample has a band that's 200 base pairs long.

And the green sample actually was composed of 3 different fragments, one that was 100 base pairs and another that was 200 base pairs.

So, what would we do with this information?

If you needed a particular size of DNA, say for the next step of your experiment, if you wanted to insert it into a plasmid or a vector, you could cut this out of the gel and use it for that.

Now let's talk about the two kinds of gels that are most commonly used. The first is agarose, and the second is SDS-PAGE.

So, agarose is a gel that's usually used for separating thick pieces of DNA.

So, if you think about the pore size in the agarose, it has pretty big pores, so imagine it looking kind of like this.

The gel is pretty big. There's big holes here, so that you'll be able to separate out the big pieces of DNA that come through. However, if you're trying to separate out little pieces, it won't be that obvious, because they'll all just race through these giant holes.

So, remember that this is for **big DNA fragments**. Usually, this is for DNA that's **bigger than 50** base pairs.

SDS PAGE, on the other hand, can be used for very **small** things.

So, imagine that being a much finer weaving with **smaller** pores. Although this can be used for small pieces of DNA, it can also be used for **proteins**.

You might be wondering, what does SDS-PAGE even stand for. The SDS part is Sodium Dodecyl Sulfate. This is a chemical agent that **DNA of proteins** disrupting any non-covalent interactions they may have.

This makes it so that the **charge of the protein** isn't a factor when they're separating out onto the gel, and they're only being separated strictly by **size**. The PAGE part is Polyacrylamide Gel Electrophoresis, or we'll just leave it at GE.

So, **polyacrylamide** is the substance that gel's made out.

So, how can we remember the difference between these two types of gels? Remember that SDS-PAGE is for **small DNA of proteins**. S for **small**, and S for **SDS**.

And **agarose** is for **bigger** fragments of DNA.

So, today we've talked about how you would setup a gel electrophoresis, why it works, and how you would want to pick the **substance** that your gel's made of. If you were really doing this in the lab, now that you have your fragments of known size of DNA or protein, you could either sequence them or use them in other molecular techniques.