DNA Analysis (Part Two)

Welcome back! If you do not already know, this is the second on a two part series of podcasts on DNA Analysis. In the first part, we covered the digestion of DNA by endonucleases and some techniques involving micropipetting and gel loading. These techniques, particularly micropipetting and gel loading would come to bear now in this lab. So if you have not already listened to the previous podcast or attended the previous lab, make sure that you check with your instructor to make sure (00'30") that you are competent to do these next sets of exercises because there will be some very technical elements that need to go flawlessly for your lab to work. That said then, what we will be looking at today is DNA gel electrophoresis.

Gel electrophoresis is basically the sieving of particles under the influence of an electrical current. This specifically is useful for DNA in that it is the particle we are going to be sieving or filtering and what we are going to be using is something called agarose which is a sort of molecular filter or sieve. (01'00") Smaller molecules move through this filter or sieve easier than larger molecules do and agarose itself is a polysaccharide derived from kelp which makes it an ideal chemical because it is chemically inert. It does not interact with a lot of other chemicals and essentially represents a very fine filter.

The technology electrophoresis is extremely powerful. It is used in a huge number of technologies, not just in separation of DNA (01'30") or also in the separation of protein. It is fundamental to a number of technologies involving DNA sequencing as well as protein analysis and as we will see today, it is useful for separating pieces of DNA based on their size. Now, what we are going to be looking at then is DNA which is an ideal molecule to separate with electrophoresis because it has a constant charge to mass ratio. To put this another way, a DNA molecule that is two time the size of another molecule (02'00") has twice the negative charge. A molecule that is four times larger has four times the negative charge. This may seem relatively simplistic but is extremely important because DNA itself makes it an ideal molecule for this sort of study because it does have a charge and it is constant as a function of mass. Some proteins have no charge and unless chemically treated, will not move under the influence of an electrical current.

So, what happens is that samples are loaded into a gel. (02'30") They are similar to the gels you folks practiced with just last week. The electrical current is applied and the electrons stream from the negative to the positive pole. They push the DNA towards the positive pole. They are not pulled towards the positive pole but instead a steady stream of electrons pushes the negatively charged DNA closer

and closer. The DNA moves as a function of charge and mass, the smaller molecules move more effectively. A very good analogy is if one looks at species of human (03'00") around the planet. You will notice for instance, that pygmies live in tropical rainforests where there is very dense vegetation. The reason for this is that their small size helps them to move through the dense vegetation, where some of the tallest groups in the world are found in the African plains where there are no vegetation to move through. Being tall or bigger makes it harder to move through a jungle. So there are a lot of analogies, and you can think whatever one you want but essentially, it is the negatively charged phosphate group on the back bone of the DNA (03'30") that helps us to exploit this difference.

Now what we are going to look at are three big factors that affect gel electrophoresis. There are quite a few more, but generally speaking, they are voltage percent agarose and DNA fragment size. As I already mentioned, DNA fragment size is something where the smaller fragments move farther. A really interesting technical note is that it is not a linear relationship. So for you graphing folks out there, this is not a linear axis (04'00"). Let's just say that something that is 2 cm long does not necessarily move twice or one half as far. In fact, it migrates an odd proportion of the distance, fragments that are 4 cm long. In fact, it is a relationship of the distance migrated to the semi

log of the mass. So it is actually a logarithmic iteration; very curious graphs results and if you are interested, ask your instructor about semi log graphing of this phenomena. With that said then, the DNA fragments (04'30") move as a function of the size of the mass, that is to say that smaller fragments move further.

Also, the voltage percent agarose affect the gel. The higher the voltage, essentially the more electrons that are passing and as you might imagine, more electrons, more pushing; more pushing, more movement. So to put this another way, increased voltage increases migration. Another factor is percent agarose. You can make agarose anywhere from about ½ percent all the way to about 4% or 5%. It cannot go much higher than 5% because you simply cannot dissolve (05'00") more agarose in solution. So today we will be using a percent agarose that is around 1 to 1½ percent. You can check your lab for the exact concentration. What happens then is that as you increase the percent agarose, the gel itself becomes denser and denser, and therefore slows migration. So what we can do is researches how we can vary the voltage and we can vary the agarose in order to get the desired results. Well it is not a real primary focus of this lab. (05'30). In the biotech class, we actually do look at the issues of voltage and percent agarose; and ask ourselves the question: "Why don't we simply use as high a voltage as possible or as low a percent agarose

as possible if this is the fastest method?" Just to give you a real quick answer, really high voltages can smear the bands and so the DNA does not appear as distinct bands but instead appears as a smear. Not to mention, it can make the gel very hot. Also then, the percent agarose (06'00") you really want to watch really close because agarose is not cheap and if done on a commercial scale, it cost a lab thousands of dollars in agarose related reagents. So again, these are not simple answers. However, let's return to the topic of the lab and that is that we are going to carry out gel electrophoresis and what does that involve in is setting up your gel.

On the last page of the lab, on page 8, there is some information on casting the gel. You want to cast the gel first (06'30") and let that gel solidify much like you guys did with the earlier petri dish practice gel stuff, let it solidify. It takes about 20 minutes. When you put the gates up, those are small plastic partitions that hold the ends up; you want to just barely tighten the end screws. Do not over tighten. If your gel leaks, let your instructor know and do not try to clean it up right the way. Just let it solidify because once it is solidified, it is a lot easier to work with. Once the gel has been solidified, you are going to remove the comb (07'00") and then drop the gates. This involves simply loosening the end screws and dropping the gate. Then you are going to position the gel in such a way that the negative electrode, the

black electrode is near the wells. Again, remember that the electrons start at the negative then move to the positive so they need to push the DNA through most of the gel.

You will also notice that the rig or the device you pour the gel into fits very nicely inside of the gel electrophoresis chamber. Once you got the gel inside of the box and you have everything set to go (07'30"), you want to make sure that there is just enough buffer to cover the gel. Too much buffer and the electrons will actually run around the gel and go through the buffer and not push the DNA as much as we would like. If you do not have enough buffer, you will have a situation where you might actually heat up the gel. It has been known, these gels will actually melt and really degrade in quality. In fact, the electrophoresis chambers that you are working with right now were very safe. There is a ground fault interrupter in the power supply. There is a fuse box, rather; a fuse in the power supply. There are numbers of fail safe devices. Prior to these technologies gel electrophoresis chambers were leaning causes of death in laboratories. If you pause and think about it, you are putting a lots of voltage through a solution of saltwater. That is essentially how we electrocute people. Except that nowadays, the technologies are so sophisticated and there are so many derivative ways of doing things that if you get electrocuted, quite frankly, you probably deserve it. Simply put these

devices (08’30”) have so many fail safe devices in them that you are just not going to hurt yourself.

That said then, you are going to take your samples and you will be preparing your standard which is a little bit of DNA that we will talk about a little more later. Your four samples of digested DNA have already been prepared. Then, you are also going to prepare some unknown DNA for loading. You will then load all of the samples into the gel in such way that (09’00”) you will actually not load samples into the first and last wells. The reason for this has to do with the electrical field that spreads around the gel, typically speaking, the edges of the gel tend to be the least predictable. So by not loading samples in the edges, we can then get the best results. Again, then after running the gel for the prescribed amount of time, you will then stop the gel. You will then transfer the gel only to special containers that the faculty member will use to stain the gel. (09’30”)

To transfer the gel, basically just open up the box. You will then lift the rig and sort of slide the gel only into the special staining boxes. Then you want to label the box with your group’s name, not on the lid but on the under side of the container because lids tend to sort of get tossed around in the lab. Sometimes the wrong lid ends up on the wrong bottom so if you have your tape on the bottom of the tray, you will be in great shape. That said then, you will turn those into

professor. They will then go ahead and stain them (10'00") and then de-stain them. All this will occur next door.

When your professor calls you into the room, you want to make sure that you grab some goggles to make sure that your eyes are shielded from the ultraviolet light and what you are going to go and see then is your DNA that has been stained with a substance called ethidium bromide. Now, ethidium bromide is a powerful mutagen agent and mutagens agent are defined as the ability to mutate or damage DNA. What happen is that the ethidium bromide will actually get in there and stick itself into (10'30") or intercalate (this is a fancy word for it) itself into the DNA helix. So it sort of literally wedges itself into the middle of the DNA and we will illuminate it with ultraviolet light; it now becomes visible.

This is one of the reasons why you need to be very careful when working with this stuff because it does not know the difference between the DNA in the gel and human DNA. It could easily go and wedges itself into your DNA. That said then, while it is a dangerous chemical compound, (11'00") curiously it is not anymore toxic than other mutagen substances such as moldy peanuts. Believe it or not, aflatoxins or chemicals found in moldy peanuts are just as toxic as ethidium bromide. Long term exposure can cause mutations.

Well that said then, you are going to have your images photographed and you are going to be using those photographic images to look at your samples of DNA. Now what you have is the earlier data you did (11'30") on predicting the number of fragments and the size of fragments can now be used to look at the length and see if they agree with your results. So for instance, are there six pieces of DNA where you predicted the six pieces of DNA? Are there eight pieces of DNA where you predicted the eight pieces of DNA? Are the DNA the size that you looked at? A lot of times, especially if you are just starting out in this field, you can say: "Well, this person has exactly that piece of DNA" but instead you can you can eliminate such facts. (12'00") In fact, this is how most of the DNA technology works. We do not use it to identify the guilty. We use it instead to eliminate suspects until really the only person left standing has to be that person because we have systematically eliminated every other suspect on the field.

Now what is going to happen is that after your samples have been stained, you are going to photograph and look at the gel image and try to say: "OK this fragment looks like it is bigger than that fragment", so on and so forth. If you are inclined, you can then use (12'30") the standard. The standard is a piece of DNA and we put that in there for a few reasons. One is that is a piece of DNA that we know

that it is going to be in the gel. Remember earlier when you added DNA and you were just beginning to do this. Maybe you messed up and maybe no DNA got into your gel. Well, how would we know this unless we put some sort of standard in there. So the standard helps us to verify that yes there is DNA. Furthermore, the standard has known molecular weights and so by comparing the standard to your unknowns (13'00") you can estimate the weight of the different fragments.

Now you can do this very casually just by looking at a band. If there is a band in your standard that you know is 3000 and in your unknown a band is right next to it, it is pretty simple that that band too is 3000. However, if you really wanted to, you can do a semi log graph. On one axis, the linear axis, you have the distance migrated. It simply measures in millimeters how far the different fragments moved from the well. Then after those different fragments have (13'30") moved so many millimeters, on the other axis, you put their molecular weight. Now this is where the semi log axis comes in. The semi log covers fragments that might be 200, 300, all the way up to 20,000. As you might imagine, you cannot graph that on linear axis because there is very few pieces of graph paper that will allow you to go from say 200 to 20,000 on the same single piece of paper. So semi log paper (14'00") helps you to do this by allowing you to graph very

large and very small masses on the same axis. I really encourage you to do this because it shows you a semi log phenomenal which is extremely common in biology and is actually another example of standard curve which if you recall, your protein standard curve was an example.

You will also notice your onion DNA. Usually it looks like a piece or smear (14'30") rather than bands of DNA. There are so many fragments of DNA and the genome is so large that you do not see individual bands but instead you see a relatively large smear. The other thing that you might notice is that this technology is about thirty years old. As I said before, it is a relatively old technology. The science is still sound but now we are about five or six generations ahead. In fact, if you are a fan of those forensics channels, you might have heard that this technology for instance is old (15'00") and break through in forensic science has allowed them to go back and look at cold cases as they are called.

This essentially is because technologies have emerged where we cannot only cut up the DNA and look at pieces of it but we can make copies of the DNA if there is not very much left at the crime scene using a technology called PCR. We can also use technologies that not only allows to copy the DNA but as we copy the DNA, we can separate the DNA and so there is a really broad range (15'30") of emerging and

established technologies that allow some of the basics you have learned in lab today to be fully understood in forensics lab settings. So if you are interested in this science, you find this intriguing, you might want to consider taking a bio-technology class because we do DNA fingerprinting and analysis, and look at complex topics such as modern day DNA fingerprinting technology. Last but not least, you know where I am going with this. You want to do a little bit of lab clean up. Make sure that your lab is spotless because remember (16'00") that happy clean labs make happy technicians which make the happy faculty members and make happy exams. Thanks again for your attention and I will talk to you again.