# DNA Analysis (Part One)

DNA Analysis lab, as you already know, is a very detailed lab taking up actually two separate entire labs for good reasons. DNA analysis is one of the major ideas in molecular biology covering about maybe 30% to 40% of the lecture alone. To give in two labs is actually not enough. Let's go ahead and work with what we have. The first part of the lab focuses on micropipetting. (00'30") That is specifically a technique to deliver and transfer small volumes of liquid. It is extremely crucial that you learn how to micropipette accurately. In fact, this is listed by a lot of employer as one of the essential skills that incoming freshman employees, new hires have as far as skills go.

Let me talk a little bit about the micropipetting. There certainly are enough points in your lab. You want to make sure (01'00") that you are comfortable with the volume. If you are a little rusty on the metric system and converting between microliters and milliliters, now is the time to make sure this is absolutely clear in your brain. So take a few minutes to review those units. Also then, when you are actually working with the micropipette itself, this is very much a hands on lab. You do not want to watch somebody working with the micropipette. You actually want to work with the micropipette yourself.

First thing you will notice if you get the micropipette in your hand is that there are two stops. (01'30") What that is, is that there is a plunger at

the end of the unit (a colored plunger or disk and as you push down) you will feel some resistance at a certain point. You will feel what is called the first stop. If you push a little harder, you can actually push all the way down so that the plunger is close to the bottom of the pipette (as far as the plunger position goes). That said then, these are the two stops. Again the first stop is the first bit of resistance you meet when you are pushing down the plunger and the second (02'00") is the second stop which is the final stop as the plunger is as close to the micropipette as you can get. It is very important that you understand how to use theses two stops.

Inaccuracies in these techniques not only waste reagents but they can destroy the experiment. Recognize that, let say you are going to transfer five microliters. If you transfer five microliters appropriately, great, you transfer five microliters. I you do not know what are the stops about and you go all the way down to the second stop when you are sucking up the sample, you might have transferred (02'30") 8, 9, 10 microliters. So you can double the error. Again the first stop is to deliver. So what you do is that you push down until that first stop is felt and then with the micropipette tip in place (remember that there is always a tip), you put it into the sample. Do that with a very slow thumb release making sure that the tip stays in the sample the entire time. Then, pull the pipette out of the sample. You look and inspect the sample to see if it is all there. Then what you do is that when you are delivering it to a new container that is when you use the

second (03'00") stop. You put the micropipette tip into the new microfuge tube. You push to the first stop and you will that most of the sample had been squeezed out. Well, that second stop helps you to squeeze out that little extra volume. Recognize that those two stops are there; become very familiar with their effects on that. Also, in just a minute, I will talk to you about an exercise on how to work with this but you also want to become familiar with the scale.

Micropipettes commercially come available in a wide range of scales. (03'30") Most of them have very specific demarcations on the side. These are lines, dots, or decimals that tell you what the units are. Make sure that you are familiar with your micropipette. For instance, we have some 2 to 20 micropipettes. They have a bright yellow top on them. 2 to 20 mean their range. So if you have 2, 0, red line, 0, that is 20.0 microliters. On these particular pipettes the red line means the decimal point. So again, 2, 0, red line, (04'00") 0 is 20.0 microliters. 2, red line, 0 or to put all the digits in, 0, 2, red line, 0 is 2 microliters. **If you do 0, 0, red line, 2, that is a broken pipette**. Remember, you can only go down to 2 microliters so 0, 0, red line, 2 is 0.2 microliters, an order of magnitude below what the pipette is meant to go to. You will not be able to get down to that ready very easily or you would have to force the unit (04'30") to do so. Again, recognize the scale. Also then, make sure that you spend some time working with basic volume extra transfers.

A very common exercise for a new kid on the block is to standardize all the pipettes, to check to make sure that they are working accurately. The way to do this is to transfer 2, 3, and 5 microliters into a new container. Centrifuge it real quickly to make sure all the samples are at the bottom. You set the pipette to 10 and then you suck the samples back out. (05'00") If there is nothing left in the microfuge tube and the pipette tip is full, that means there is a full 10 microliters in there. That suggests that your technique is good. Again the protocol in the lab actually has you doing exactly this. They work with slightly different volumes than what is on page 5 and you will notice that all the volumes come close to being 10 microliters. The idea then is that as you transfer these different amounts, if you are going to the right stop and you are transferring the right way, (05'30") at the end of each transfer, there should be 10 microliters. If you suck it up and there is more than 10 microliters, like there is still some left in the microfuge tube that suggests that you are probably going on the second stop on some of your transfers. So check that out. If when you suck it up, you notice that there is not 10 microliters, the chances are that you may have pulled out the pipette tip during one of the transfer and actually sucked up a little bit of air instead of fluid during the process. Again, make sure that you and yourself personally (06'00") know how to use the micropipette and that you are very comfortable with the procedures. This is not a spectator sport, not to be taken lightly.

The other activity is to load the gel. The practice gels are actually petri dishes. You will take the petri dish and you will put a little bit of water into it and so this water actually stimulates or recreates what the gel would look like in the DNA chamber. The gel will actually be under a little bit of buffer which for all kind of purposes looks like water. You would then practice loading (06'30") a small sample of 10 to 15 microliters of loading dye into the well. The tip of the pipette should be just below the fluid level, just underneath the water and should be right above the gel. So you don't want it actually poke into the well like stabbing. You want it just kind of sitting above it. Ideally, if you do this right, the pipette tip does not even need to touch the gel. You just want to make sure that all the DNA gets transferred as well as to ensure that the gel itself is not damaged.

So again, those two core techniques (07'00"), micropipette and practicing in volume metrics, transfer, scale, stops, so on and so forth but then also, you want to make sure that you are comfortable with the gel loading. Next week, you will have one chance to get it right. So do not hesitate to practice these techniques until you are very comfortable with them.

The other big aspect of the lab is the restriction endonuclease part. The restriction endonuclease ends in letters A, S, E (07'30") so you may note that it is an enzyme. It is an enzyme that cuts DNA at what are called palindromes. Palindromes are simply words that read the same way

forwards as they do backwards. For instance, “RACECAR”. “RACECAR” spelled forward is R-A-C-E-C-A-R. “RACECAR” spelled backwards is R-A-C-E-C-A-R. So that is a palindrome. Now DNA actually has an alphabet that runs in two directions. One strands is going AATT, the other strand below is going AATT in the opposite direction. (08’00”)

So take a look in your lab, there are actually some example (restriction endonucleases free) to look at on page 2 show that the endonucleases EcoRI, HindIII, and BamHI. Now, these endonucleases can be cut in what is called a blunt cut that means you just sort of literally cut straight across. (08’30”) A lot of time they are cut in such way that they are staggered. So there are actually some nucleotides hanging over and if you look on page 2 of the DNA digestion lab, the restriction analysis of Lambda DNA, there is a cut of EcoRI and AATT actually over hangs. These are what we call sticky ends. As you might imagine, it might stick back together with another piece of DNA. Where things get really interesting is that they will stick back together within (09’00”) piece of DNA. So you can cut a bacteria or cut a human piece of DNA with the same endonuclease and then knit them back together or glue them back together with these sticky ends and a little bit of extra chemistry. Some very interesting sciences recombinant is that we are actually learning a little bit more about when you look at DNA or combined engineering a little later on in lecture. In back to the lab however,

you want to make sure that you set up your digestion accurately and quickly.

The solution order is important. (09'30") The buffer, DNA, and water can be added in any particular order, however, do pay attention to the procedure in your notes. However, the restriction endonuclease needs to be added at last. The buffer is quite wimpy and should not be added for instance with just water by itself or just buffer by itself which might happen if you do not add them in the correct order. Again, make sure that you add the restriction endonuclease last. Also, spin everything to make sure it is all down at the bottom of the tube. If you add the restriction endonuclease but it is a drop of liquid stock (10'00") at the top of the tube, it is never going to get down at the bottom and to be able to cut up that DNA. What you are also going to be doing is that you are going to be digesting these for about half an hour maybe an hour and you can look at the protocol to be sure on that. However, while that is happening, take a look at the lab pages 2 through 4 and get a little bit of a handle on that theory.

These endonucleases cut DNA in very predictable location, and as a result have given scientists (10'30") enormous tools to work with. For instance, if you cut a piece of linear DNA seven times, you have eight fragments. You have a linear piece of DNA and you cut it nine times, you have ten fragments. Just imagine cutting a piece of string. You cut a piece of string twice and you have three pieces of strings. The same idea works

with DNA. Now if you go a little further as you cut up those pieces of strings, all the individual pieces of string should add up to the original size. If we have a piece of DNA that has a certain size after cutting it up, all the little pieces being cut up (11'00") should add up to the original size of the DNA. That is what the exercises on page 2 and 3 are designed to have you look at. Once you get really comfortable with that, you might see if you want to predict what this pattern would look like on the gel. How many fragments of DNA would you see? How big would they be? Would they all be in the same location? If you notice on page 3, there is an exercise on getting the restriction endonuclease sizes for HindIII. Then on page 4 is an actual image of the digestion HindIII. (11'30")

See if you predicted the number of fragments to the size of the fragments you predict. Are they there in the gel? An important thing for scientists to know is to kind of have a feeling as to how their experiment will turn out. It is very rare that a scientist will be surprised by an experiment. We should know what is going to happen; we should know the order in which it is going to happen. Restriction endonuclease digestion is no exception especially in the area of forensic science where you have samples of DNA that you should have control of. (12'00") You should know what is going on. You should have results that are not unexpected or strange. You certainly would not want to send somebody to jail on a set of results that do

not look like as expected. With that said then, go a head and predict the lab.

As you might imagine, this lab is largely a practice lab with micropipetting, gel loading, and getting your digestions set up. You should be able to work through it fairly quickly. However, I want to give you a couple of extra hints. Do not be a spectator on this. Work personally with the micropipette (12'30") and gel loading of samples. Also, you might note that small samples fall very quickly. Some of the procedures say to put it in the water bath but if the sample looks thawed, do not worry about it, it is ready to go. That might save you a few minutes. Also, look at all the samples while you are loading them especially important, the clear sample. You want to actually see a droplet in the tip of the pipette. You want to see that little bit of pipette solution go into the next while you are actually going to watch it transfers as it occurs. This will give you more confidence (13'00") that you indeed transferred everything. Also, when you are setting up the tubes for digestion, if you for instance add all the same thing to the different tubes at once, you can go through it very quickly. So you have one person that adds all of the water, one person adds the amount of buffer, one person adds the amount of Lambda DNA, and then the enzyme, as it was said before gets added last. By doing that, you can get an assembly line fashion where each person sort of does there drops. Each person gets practice transferring a number of solutions. (13'30")

Last couple of things I want to mention is to make sure that you do the homework on this. You have plenty of time in this lab so do not screw around and leave early. Get the DNA digestion exercises on pages 2, 3, and 4 done. Also remember that this is the first of two labs. The second lab we will talk about next is electrophoresis and looking at gels. It is important that you have a little of an ides of what your DNA bending patterns should look like. This is the tip of an enormous iceberg (14'00") as far as science goes. Current DNA analysis, on at least five generations is past what you are doing right now. The digestion of Lambda DNA is useful for finger printing Lambda DNA. Like to fingerprint human DNA, there are they say four or five new technologies they have come out within the past 25 years that are really engaging and useful. If you are interested, these technologies are talked about in biotechnology to give you a little bit better handle on how we work with DNA and modern lab setting. (14'30") That is it then. This lab is extremely useful giving you a good insight into micropipette practicing as well as working with restriction endonucleases. Last minute comment, make sure that you take a few minutes to clean up your lab, as a habit, in the way it was originally. Put all the materials back. Make sure all of the tips and miscellaneous tubes are returned. Some will go in the garbage; some will be collected by the instructor. Again remember, a clean lab means happy technicians which means happy faculty which means happy exams. Thank you.