Experimental Biochemistry

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Lecture - 29
Gel Electrophoresis of DNA and Proteins -Part III

Welcome back, I am Aditya Jyoti Basak and today what I am going to demonstrate is working with Protein Gels. So, I am going to show you how to cast an SDS page gel and later on once it been cast I will show you all the components that are used and will actually use real protein samples and see how it runs.

So, the first thing that we are going to do when we are working with protein gels is to actually cast the gel. Today I am going to cast a 15 percent resolving gel you can cast different strengths of gels. For example, if you are working with a very large protein say greater than 100 kilo Daltons you could cast an 8 percent gel. The protein samples we will be working with today are all less than 25 kilo Daltons.

So, I am choosing to work with a 15 percent gel. So, basically the higher the percentage of the gel matrix the smaller will be the pores. And hence greater will be the sieving effect and you can separate out smaller proteins in this case. If you are working with larger proteins you can use less percentage of acrylamide bis-acrylamide as a result of which the pores will be larger.

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So, the first thing that I am going to show you is; these two glass plates. So, be very careful while working with these glass plates they are very fragile and delicate, they break easily. The point to which I want to draw your attention is that the thicker plate even notice at it is two edges there are these thin basils which act as spaces.

So, once I place this thin plate over this thick plate you will notice that there is still a fine gap in between. It is in the order of maybe 1 millimeter approximately and what happens is the liquid gel preparation that I will be preparing subsequently. Once I added in between these and it polymerizes this solidified gel is what we are going to use.

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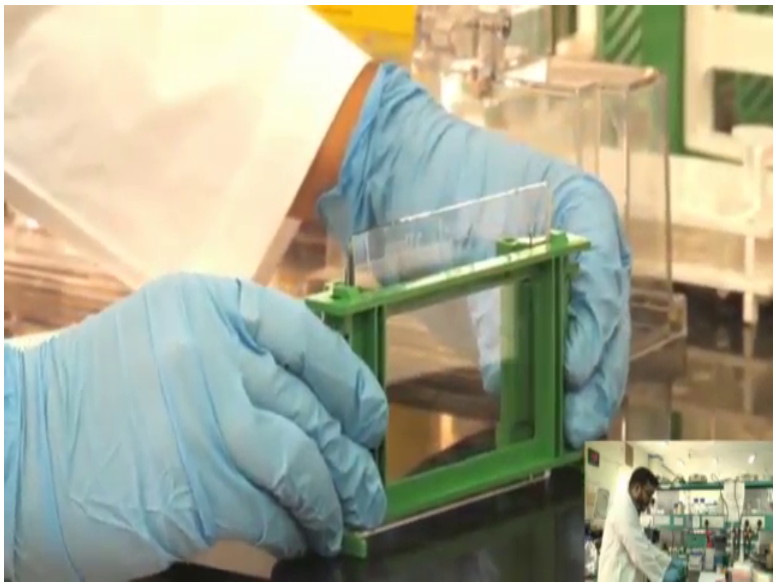

So, what we do is we take these two plates and then we set them up in between these two clamps and then we prepare our gel mixture and add it in between the space which I am going to show you subsequently. So, let us now begin by casting the gel, always ensure when you are working with SDS page gels make sure that the; glass plates are spotlessly cleaned. Yeah, I have cleaned it before hands so I do not need to do it now. But when you are working make sure that the glass plates are clean dry and lint free.

So, the first thing that you need to do is you place them together and very gently there is this clamp, special clamp where you slide them in. Be very gently do not drop the plates because if you drop the plates they will crack, break and it is very expensive do not do that. And very carefully you will notice that these two levers I am applying pressure equally on both sides and simultaneously shutting them down. So, this is the locked position once it is done you will see that it has got hold of the glass plates and they are not slipping out.

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Next you put it on to this stray and fix it in position or bring it closer for you to see. So, now, what happens is at the bottom half there is this rubber gasket which is sealing the bottom gap. On the sides as I had mentioned earlier there are special plates. So, the only opening for the space is at the top through which we are going to add our gel mixture.

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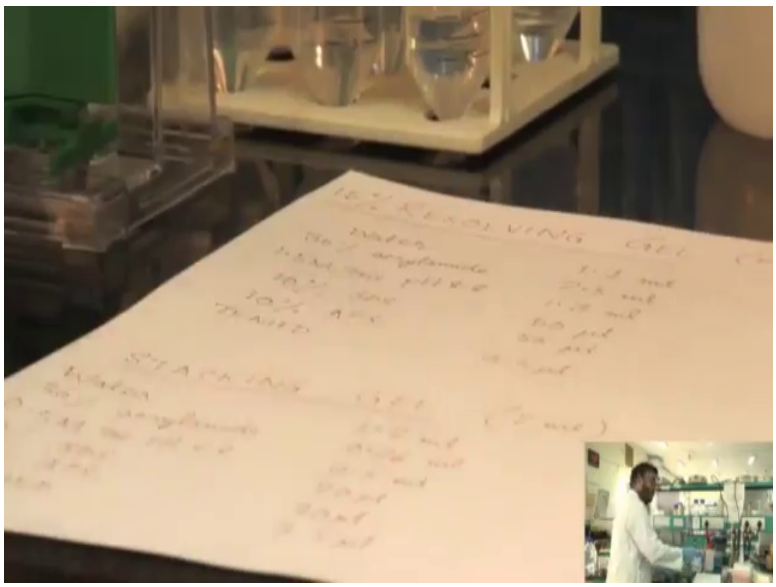

One thing that I always recommend is whenever your casting gels write down the recipe have it written beside you. And each time you add something put a tick, because remember you are all working with colourless liquids and it is very easy to lose track.

There are at least 6 or 7 components you add something put a tick here and then move on to the next.

Once you have set it up in this position although I mention that it is sealed on all three sides and only this is open, but you need to check if there are any leaks. So, the easiest ways you just add some water in between you will see the water level is rising and you can I usually prefer to fill it up to the brim, wait for a few minutes if you see that the water level is remaining the same; it shows that it is not leaking and you have set up your gel plates perfectly.

So, we just saw for the last 2 minutes there is no change in the water level which shows that the gel has been properly set up.

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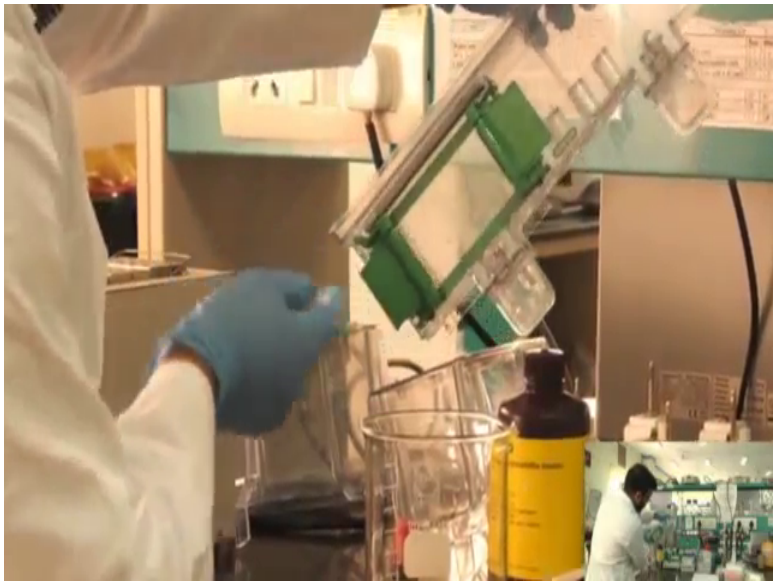

So, now what we do is we just pore out the water, you cannot dismantle this setup because if you do that your defeating the purpose. So, what you do is you just pore out the water you will notice that there is some water left in the corner what I usually do in such cases is just grab some tissue take it to the rim and very gently just soak up the water ok, it is fine do not be afraid these clamps are very hardy. So, they will not fall of make sure you remove the water right away.

So, now we are ready to actually begin preparing the mixture for casting a 15 percent resolving gel. So, let us now begin preparing the 15 percent resolving gel. So, we are

going to add water 30 percent acrylamide bis-acrylamide solution 1.5 molar Tris pH 8.8 10 percent SDS 10 percent APS and then timed. Always be very careful that you add APS and temed in the very end because you do not want your gel to polymerize before your absolutely ready to cast it and pore it into the glass plates.

So, I will begin with water I need to measure out 1.1 ml of water. So, what I will do is I will set my pipette at 650 microlitres 550 microlitres and pipette out water twice so that I have 1.1 ml of water.

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This recipe we will be providing you so you do not need to worry about how much of each component that I am adding, just see how I do it. So, I add some water 550 microlitres and now I am adding another 550 microlitres of deionized water.

Generally I prepare my SDS page mixes in glass beakers sometimes you can use falcons for preparing the gel mix also, but I use glass beakers.

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Next what I am going to add is 30 percent acrylamide bis solution. Be very careful when you are working with acrylamide, in the liquid form it is a potent neurotoxin and a suspected carcinogen. So, make sure that you are wearing gloves and be very careful so that there are no spillages. In case there is any spillage thoroughly wash it with water and report it to your supervisor.

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So, you will notice that I did not touch the tip to the bottom of my beakers. So, it is not in contact with the solution. So, I can actually we use this tip to take out more of

acrylamide. If while you are working at any point you feel that your tip has been contaminated like suppose while mixing you accidentally touch the bottom of the beaker do not worry just discard your tip and use a fresh tip.

Because keep in mind when you are working in the biochemistry lab you do not want to contaminate your stock solutions; so, yes I have added the acrylamide to the water and now I am just mixing it pipe pipetting it up couple of times to ensure that it is uniformly mixed together with the water yes. So, next I am going to add 1.3 ml of 1.5 molar Tris pH 8.8.

So, I will just set it at 650 microlitres and pipette out the solution twice. Something that I always insist on students doing is once they have added components to put a tick marks. So, I have added water and I have added acrylamide put tick marks and then move on to the next solution; because you are working with colourless liquids it is very easy to lose track of what you have added and what you have not.

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So, 1.5 molar Tris pH 8.8 will be used in making the resolving gel. I will take out 650 microlitres now, followed by another 650 microlitres. And before we add SDS, the next component is SDS so SDS is a detergent right. So, if you are not very careful while working with it if you pipette it up in a erroneous manner it will cause bubble. So, in order to avoid that just mix up the solution as it is now.

And then let us move on to SDS, so these three components that I have added till now namely water acrylamide and Tris we use large amounts in the order of ml. But SDS and APS we will add only 50 microlitres each. So, it is a very small amount, I will just set my pipette to 50 microlitres.

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And I am going to take 10 percent SDS. Yes, I have 10 percent SDS which I now add to this solution yeah. Next so once you have done this after this what I am going to add is ammonium persulfate and temed. Once these have been added you should not really stop just mix it thoroughly once.

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And then pore it in between the gel plates because as you know from your theory classes A P S and temed are responsible for creating the gel polymer for the cross linking. So, yes I have added 50 microlitres of A P S release your pipette, do not keep it in unreleased condition. And now I am going to finally, add 3.5 microlitres of temed it is ticked up A P S has been added.

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So, the last component that I I am left to add is temed, it need very small amounts 3.5 microlitres is sufficient for. So, when you are pipetting it out very small volumes always

see that you have taken up something in the tip there are chances that you might make a mistake. And you think you have added something, but you have not so be careful. So, now I have to be very fast just pipette it once or twice to ensure uniform mixing try to avoid bubbles and then see what I am doing.

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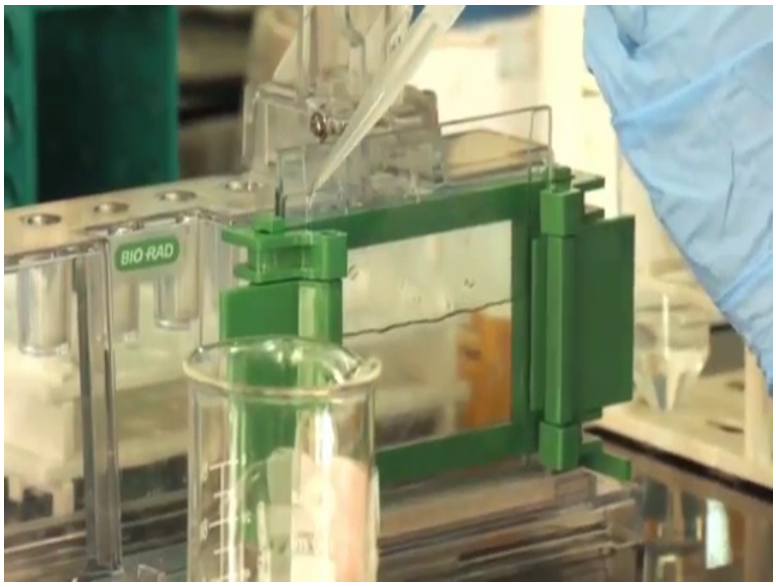

I have taken up 1 ml you put it at the junction of the two plates. And you gently keep pipetting you will notice that the space between the two gels. The two plates is gradually getting filled up with the liquid mixture. In around 15 minutes time it is going to polymerize and from the resolving gel, 15 percent resolving gel in this case.

Keep on going, keep adding do not panic just keep doing it at a steady pace and you will be fine. So, always remember when you are adding resolving gels do not put it up till the very top because then will not be left with enough space for setting up your stacking gel.

So, water usually go with is you will see this green border, I just go up till the lower level anything beyond that I end up risk in not having enough space for the stacking gel which we are going to cast subsequently. So, after waiting for around 15 minutes in order to allow the gel to solidify; what we are going to do is; pore of the layer of isopropanol that we had added on top.

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So, what you do is you just pour it away and soak up whatever excess is there using tissue paper excellent. So, now if you can see here, there is this area is the resolving gel it is solidified how do you know I am shifting it had it been liquid it would have changed it is level and spilled over, but we will see it still solid so it is solidified. Now what I am going to do is I am going to prepare the stacking gel.

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So, again I have written the recipe over here and I start off by adding 1.2 ml of deionized water.

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I will added in two steps of 600 microlitres each. So, I have added the water so 1.2 ml. Now I need to add 260 microlitres of the acrylamide that I have. So, I will just set it at 260, take the bottle to be very careful while working with acrylamide I cannot stress this enough it is very important always keep this in mind.

So, yes it is added just mix it up uniformly. So, next what I am going to add I have added acrylamide. Now I am going to add 500 microlitres of 0.5 molar Tris pH 6.8. In your theory classes you must have been taught as to why we use different pHs when we are casting resolving gels and stacking gels.

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In case you do not remember just referred to that because this is pretty important to this principle, so 500 microlitres of 0.5 molar Tris pH 6.8. It is just added, mix it gently try to be careful and do your best to avoid bubbles. So, once you have added a solution always release your pipette, this is a general good lab practice do not leave your pipette in unreleased condition.

It will take you just 5 seconds extra, but it will increase the lifespan of your instrument. Now, what I am going to add is APS no, first I will add 10 percent of SDS and after that I will add APS. So, once you start adding these final components it is best to not pause just keep on adding them one after the other 10 percent SDS, I will add 20 microlitres here we go yes.

Next I am going to add ammonium persulfate solution 10 percent ammonium persulfate this is also 20 microlitres, release the pipette quickly the final component that needs to be added is temed. I am going to add 3.5 microlitres or 4 microlitres it is fine of temed. Since it is such a small volume always visually check that you actually have solution in your pipette tip yeah.

Now that we have added that a very quickly release your pipette and move on to fully thoroughly very gently mixing the solution yes. Sincere working with SDS some bubbles might be formed try to avoid bubble formation and then layer it on top of your already solidified resolving gel. So, in this case what I usually do is take up the liquid and fill it

to the brim completely to the brim ok. There is a reason why we going to do that which you are going to see just about now.

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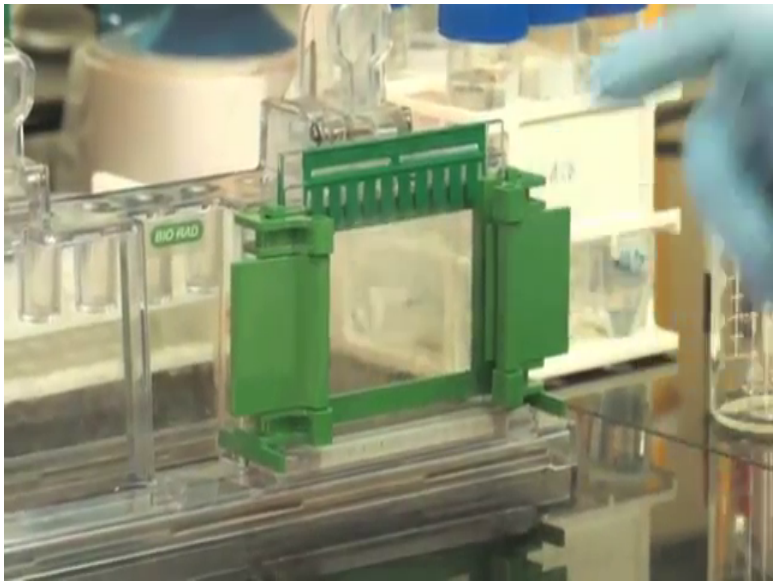

So, once you have layered on your stacking gel mixture on top of your solidified resolving gel you take a comb, you will notice that this comb has different teeth shaped protrusions which we are going to now place into the in between the two glass plates. So, there is going to be some spillage, but that is absolutely fine; do not worry about that just soak up whatever spilled over and your good to go.

So, what will happen is the comb that I inserted these plastic protrusions that I now placed inside the stacking gel. Once the stacking gel solidifies there will be gaps in these protrusion regions which will form the wells into which we are going to subsequently load our samples. So, after 15 minutes your stacking gel has also solidified.

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So, now what we are going to do is we are going to take it out from here very gently we take it out; put equal pressure on both the levers. Do not open one side and leave the other one closed because then that could result in your glass plates cracking open them together uniformly and gently just pull it out. It is easy ones you do it, but be very careful and make sure that you do not drop or break things.

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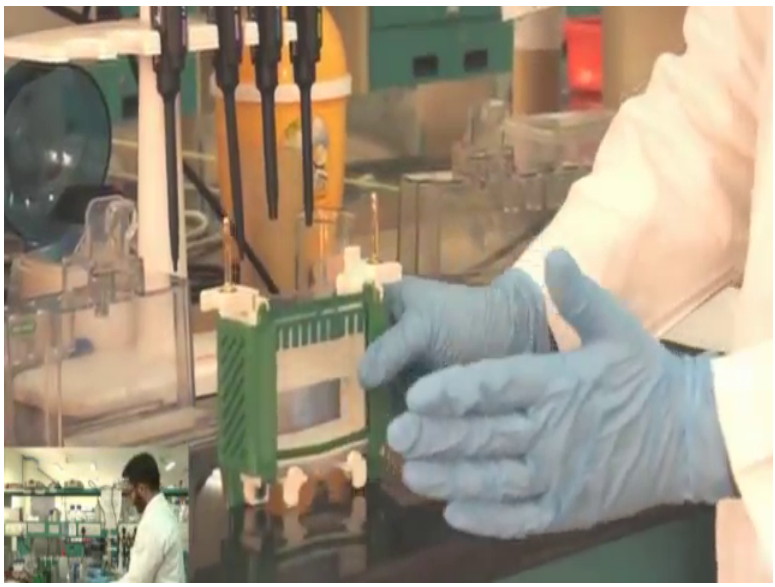

So, next what we are going to do is we are going to put it into this gel cassette, there is a particular orientation. So, the thin glass plate will face away from me on the inner side.

And on the opposite sides you put in a buffer dam which is something similar, but made of plastic. And then you fit both of them together and then you have to do this uniformly take these two clamps and just slight them in. And you will see that it has become an assembled gel cassette.

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Then what we are going to do is we are going to put it into the buffer tank. So, basically your gel running buffer will be present here.

So, we will just take the gel running buffer, all these compositions will be provided to you. So, you do not need to worry about that at the moment just noticed what I am doing and we pour it inside here to the brim and also outside. So, the sort of setup that I am using at a time you can actually run four different gels. Right now I am running only one, but you can actually run four different gel simultaneously ok. I need some more buffer which I have here and I will just show this to you.

So, in case you are running four different gels then in the outer region of the tank you need to pour approximately this much amount of the buffer, but since we are only running one or two up to two if you are running then even this much amount of buffer is sufficient for carrying out your experiment. So, yes will just add the running buffer yeah, a little bit of extra no issues. A practical issue that you might sometimes face is that the inside region might start leaking and you will lose your buffer, if it is leaking very badly

then what you need to do is just take it out dismantle it again and remantle it hopefully that will solve your problem, yes I think that is enough for now.

So, now once everything is ready will notice I have not yet removed the comb, only once my samples are ready for loading will I gently remove the comb do not remove the comb until and unless you have filled up the inner part of the cassette with your running buffer. Because that that is a good way of doing it, because otherwise there are chances that you might break up at the wells and damage your entire preparation.

Now that we have successfully cast a protein gel we are going to load our protein samples into it. But before I do that I have already prepared my samples four different samples which I have kept here, but before I load them into the gel I am going to spend a few moments telling you how we actually process our protein samples and get them ready for loading.

So, the major component that this will have is; obviously, your protein of interest, but along with that you will have to add a gel loading dye. So, usually I use a concentrated stock solution of 4 x dye which after I mix with my protein sample and some additional water comes down to 1 x. One of the main components of the gel loading dye will as the name suggests be a dye which you can visualize so we use bromophenol blue. Along with that we also add glycerol to the gel loading dye because that will ensure that once you load your sample into the well it uniformly sinks to the bottom it does not keep floating within the well and behaviorally.

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So, what I am going to do now is first I am going to remove the comb, very gently your put your hands inside and uniformly putting the same amount of force on both sides. You just pull it up it is not a very difficult thing to do, but maybe you need to do it a couple of times before you have fully comfortable. And probably you will not be able to see it, but there are wells well formed.

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Initially what you can do is you can use a loading guide. So, this is something that is provided for your benefit in the initial few runs when you are not experienced enough.

So, what happens is sometimes it is very difficult for beginners to see which is the well and which is the gel.

So, in that case what you can do is use this well guys. So, the gap here correspondence with your well and you can just load your sample accordingly. However, with practice you will not be needing this aids and I will not use it today for today's demonstration I am just going to use the pipette alone alright.

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I will just set up my pipette to 10 microlitres because if you noticed my comb here it will have 10 wells. So, I have actually cast of 10 well gel. Other forms of combs are also available which I can show right now.

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So, if I had used this type of comb while casting my static gel I would have ended up with more number of wells 15 approximately. But as you will see the volume of the wells would be much less. So, I can load less amount of sample if I use this, but I can load more amount of heat sample if I use this sort of comb. What I am going to do is I am going to load 10 microlitres of sample in each well, generally I prefer not to use the corner most lanes.

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So, one advise that I would like to give his do not leave your wells empty.

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In case you do not have enough samples just load your well with some loading dye do not leave it blank just add some loading dye. So, as you can see this is the well and it is filled up with loading dye. You should always write down what sample you are loading in which well. So, I will begin with this sample, I will just take 10 microlitres of this sample. So, I have already process the sample.

But in case you want to see how it is done or you want to know how it is done basically I took 8 microlitres of my protein added 4 microlitres of 4 x dye. So, now, it is 12 microlitres and your dye has been diluted to 1 x and then, always remember to add beta mercaptoethanol because that will remove the disulfide bonds that are present in your sample and then boil the sample in boiling water for around 10 minutes.

So, that everything gets properly denatured and after that spin it in a centrifuge and in the end you will have something like this. I have not shown it here because I did that beforehand. So, I am now loading my first protein sample into the well and here we go yes 10 microlitres be slow and gentle there is no need to rush change tips. After that I am going to do load my second sample I am going to load a little less amount of this sample it is very concentrated yes.

So, this goes into my third well yes. Another thing that you should remember loading is always load a protein marker. So, protein markers are basically mixture of proteins of non molecular weights. So, they can help you estimate the molecular weight of the

sample that you have loaded. So, I am going to load a small amount of the protein marker, it is basically a mixture of different proteins having different sizes and the interesting part is the marker that I am loading right now is a priest 10 marker.

So, basically once your samples have been loaded and they start migrating within the gel you will not be able to visualize the individual proteins or protein that is present in your sample. But the priest 10 marker you can actually physically see it, and you will notice that as time progresses it will separate out into it is components.

Yes, in goes my third sample, it might be a bit daunting while your loading your samples into the well initially because some people might find it difficult to understand which region is the well and which is the gel part, but with a couple of rounds of practice it is going to be very easy; do not worry about it, it is no big deal. So, I have four samples so I have I am now going to load my final protein sample in into goes.

So, always write down in which lane you have loaded what sample and what I am going to mention is so this has 10 wells. So, always try to load your protein marker do not load it in the middle because once you dismantle the gel you will forget what is the left side what is the right side what is sample 1 what is sample 10. So, what I do is asymmetric like I loaded it in the third or fourth lane.

So, even if the gel gets flipped over I will know on which side the ladder is and using that as a reference if I write down in which lane I have loaded what sample I can easily track back even after a week; so always do that. Load your ladder in an asymmetric fashion as in a asymmetric well so that you can track the relative orientation of the wells.

So, it is a 10 well plate, I only have four samples I have added a ladder and I have added dye in the corner most so only 6 wells I have been filled up. I need to add loading dye to the remaining 4 wells try not to leave them blank because you will get good results if all your wells are occupied. Yes, so just load some here Snigdha dye the (Refer Slide Time: 34:20). So, just load some small amount of sample in each of the wells yes, release the pipette.

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Now, what I am going to do is I am going to setup the whole tank I am going to attach the electrodes on top. So, positive electrode negative electrode, always make sure that they are in the correct orientation.

So, this is red this is black this is red this is black do not flip it because in that case you are going to affect the migration of your protein. Instead of travelling downwards in this vertical gel it will just float out flow out of the well and you will not get results.

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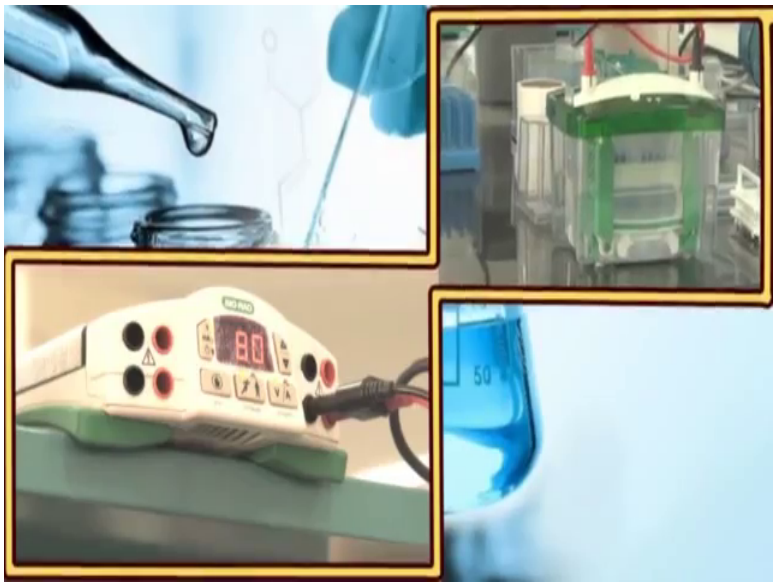

Once you have done that connected to the power pack. So, this is the main power pack from where we control the voltage. Switch it on you will see it is at 0 volt what I am going to do is I am going to increase it and set it up at 80 volts.

Once it is done I just press run and you will notice that small bubbles will rise from the bottom of the plate. And; that means, current is flowing and your samples will start migrating right now. So, always ensure that you have connected the electrodes in the proper orientation positive negative as per the clamps that you have seen here.

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So, this is another gel cassette. So, this is the positive electrode this is the negative electrode the lead electrode should fit on directly onto this do not flip that. Also when you are connecting to the power pack make sure that you have connector the plug in the correct orientation, if you flip it what will happen is your proteins in migrate in the opposite direction.

Normally what you have in SDS page is your proteins all have negative charge and hence they migrate towards the positive electrode. However, if you flip this the orientation will change and the samples that you have. So, painstakingly loaded will start migrating out of the well into solution and no resolution will be observed. So, this is a point where many mistakes do occur, but it can be rectified by just paying attention.

So, today I am going to prepare 1 percent agarose gel, the DNA agarose gel can be used to visualize and to check the different sizes of DNA.

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For making agarose gel the first thing you need is the agarose powder. So, we are using molecular get grade agarose powder and as I am preparing 1 percent agarose gel. So, I will measure that agarose powder accordingly. So, if you have bigger molecular size of DNA like the genomic DNA you can reduce the percentage of the agarose like 0.8 percent to 0.5 percent. But if you have smaller DNA sizes like the PCR products or few nucleotide base pair then you can increase the agarose percentage, like 1.5 percent and so on. So, today I am going to prepare 1 percent agarose gel.

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And for my gel setup I am going to make 35 ml volume of agarose gel. So, for that I have measured 0.35 grams of agarose powder and this will be dissolved in the buffer. So, for normal agarose gel we make usually TAE or TBE buffer. So, the TAE actually contains stress acetic acid and EDTA or the TB contains that is boric acid and EDTA. So, in our lab we prepare TAE buffer and we usually prepare the TAE buffer in higher concentration like 50 x.

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But before running your experiment you should always prepare the buffer as 1x.

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So, basically you dilute your 50x buffer to make it 1x for your own experiment. So, suppose you are making 100 number of 1x TAE buffer you take 2 ml of 50x TAE buffer and make up the volume up to 100 ml.

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So, here I am going to measure 35 ml of 1x TAE buffer because I am making 35 ml of gel.

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And I m going to pour this in my measured agarose powder; now, I have to heat up this solution to dissolve the agarose powder, and I am going to use microwave. So, you put the microwave in medium heat condition and after 30 second you check if the agarose powder is dissolved or not. Before that I am going to also need a gel setup.

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So, in the gel setup what we have is a gel castor.

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And a casting tray or gel tray where I am going to pour the gel and comb.

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This comb size or the number of wells depends on how many samples you have and also labeling bubble. So, labeling bubble will ensure that while you are making the gel or where you are pouring your gel that surface should be at the proper level. Otherwise it may happen that one part of the gel is thicker. And the other part of the gel is thinner, and the run the migration of the DNA will be hampered.

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So, before melting your DNA agarose gel you should set up this thing. So, you put the casting tray in the caster and then you tighten it up. So, basically it will ensure that your gel is at the proper place and the two side the two open side will be covered.

If you do not have a castor you can also put tape over these two side and pour your gel and you should always check the level of your gel so, that it ensures that the leveling is proper.

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So, I have dissolve the agarose powder in 1x TAE buffer and the solution will become clear. So, you should wait a few minutes after dissolving it because the before pouring the agarose powder or agarose gel solution it should be cool down to 50 to 60 degree centigrade. And now here I am going to add ETBR solution for the staining purpose. So, usually people use 0.5 microgram per ml of ETBR solution. So, you should measure the ETBR accordingly.

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Now, ETBR is a potent carcinogen gel.

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So, while handling you should be careful. You mix the ETBR in the gel and while it is around 50 to 60 degree centigrade temperature you pour the gel from one side.

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Try not to make any bubbles in the gel, if you see any bubbles you can remove the bubble using a tip because if there is a bubble the migration of the DNA will be hampered.

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Now, after pouring the gel or solution you should put the comb which will make the well in the gel. And now you have to wait around 20 to 30 minutes before the gel solidifies properly.

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After removing the gel from the caster you have to place the gel inside the buffered dam. So, in the buffered dam it is properly marked which one is towards the negatives electrode and which one is towards the positive electrode. The comb should be towards the negative electrode because DNA or RNA is highly negative in nature and they will

run from negative side to positive side. So, you will load the DNA inside the well. So, you the DNA should be towards the negative side.

After you put the gel inside the buffer dam again you need to fill the dam with the 1x TAE buffer which was used to prepare the gel. So, in the buffer dam there will be a marking up to which you need to fill your buffer. You should always be careful that your well should be properly dipped inside the buffer.

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Now, remove the comb carefully and do not distort the well. If you ever feel like the gel is not properly set or the gel is thicker at one side or if you are not happy with your agarose gel you can again redisolve this agarose gel in put it in the beaker and resolve it and pour it again.

In case of polyacrylamide gel you cannot do that, if your gel is not properly made you need to through away the gel and again you should make it freshly. So, my gel is in the buffer dam and now I will load my samples.

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So, for sample I already have three samples along with the DNA marker or DNA latter. So, your DNA is in a solution and you need to add the loading dye. So, loading dye basically has glycerol and some kind of tracking dye usually a bromophenol blue or xylene cyanol.

So, you will see how much the dry has moved and accordingly you will stop your gel and the glycerol will make the your sample heavier so that it can goes inside the well. So, I have already prepared my sample and the loading dye was 6 x. So, make sure that you make it 1x before loading to loading into the gel.

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Take your sample in a micropipette.

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And from the above a top of your gel you should be able to see the wells and just go above the well and release your sample.

Since the glyceryl is present in your sample it will push to sample into your well. So, I have loaded my samples inside the well initially for the first few times it may happen that you cannot see the well properly or you might poke your gel with the tips. But it is fine you will get better after you do for sometimes.

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So, unlike DNA gel you do not need to fill the empty lanes with anything else. And now you have to put the lead, again you should follow the colour marking so the black should be with always with the black and the red should be always with the red. If you inverse it basically the orientation or the direction of the movement will differ.

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Now, I am going to put it in a power pack and set the voltage around 90 and start the run. So, after I start the run the gel should be moving towards the sample will move from the

negative side towards the positive side. And you should not increase the voltage too much otherwise the gel might heat up and the migration will be hampered.

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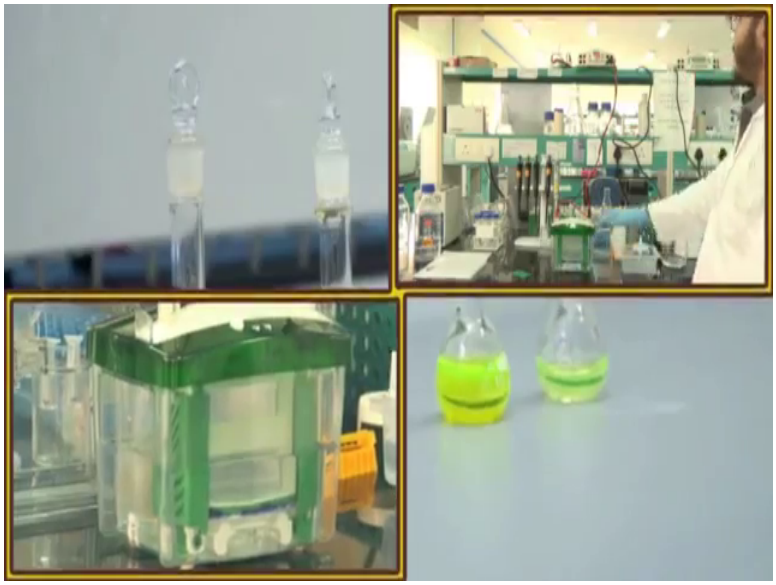

So, as you can see now we have reached the end of our protein gel run, the dye front has reached the end of the gel plates. So, we are going to stop and the ladders since it was prescient it is visible here it is sort of scient. But it is visible I can see it and maybe even if you can skin it you might be able to see it, but the dye front is coming out. So that means, the gel has been reached the end of it is course I am going to now stop this here.

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Take off the lid keep it there and now what I am going to do is I am take out the cassette pour back the buffer here into the tank will deal with it later on. For now our goal is to take out the gel plate from this situation.

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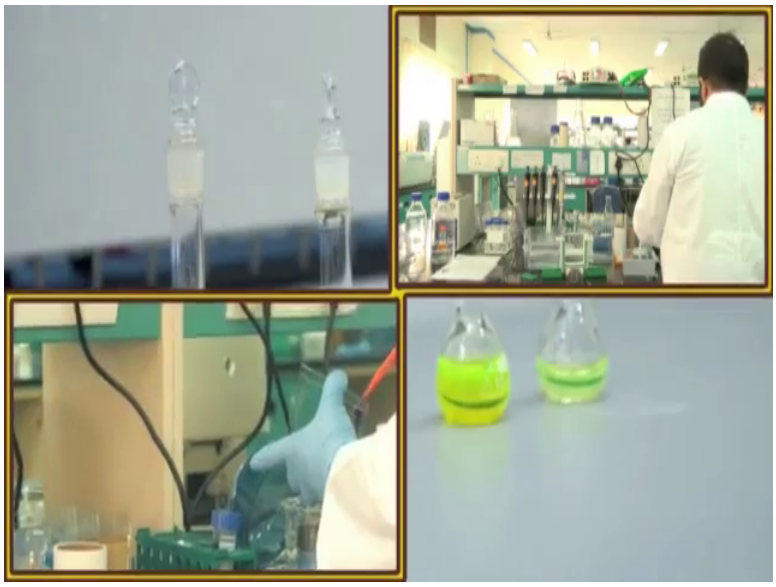

So, now we dismantle it apply equal pressure on both arms and open them simultaneously. This prevents the plates from facing unduced stress and cracking just remove it like this. And what you can do is just rinse it with water to get rid of the SDS that still adherent to the plate this came from the running buffer.

So, once it is relatively you could do this in a washbasin right. Now since we do not have a washbasin nearby, I am just using this squeeze bottle to rain drenched in water to collect the trash here. But you can carefully take this.

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So, next what I do is; very gently using this separator be careful do not apply too much force. So, we will end up breaking the plate, uniformly apply pressure.

Student: (Refer Time: 49:55).

And separate the two plates the once it is separate do not remove the gel like that it will tear, just put it in to this gel tray which has water the water will enter the space. And gradually as you lift out the glass plates you will see that your gel is now inside the tray, I will just show it to you in a moment yes.

So, I have remove the glass plates I will just keep it here for safe keeping, make sure that it does not break. So, now, your gel is in water maybe you can see it from here. So, the gel is now kept in water solution and it is just washed. Quickly what we do is I will just pour out the water.

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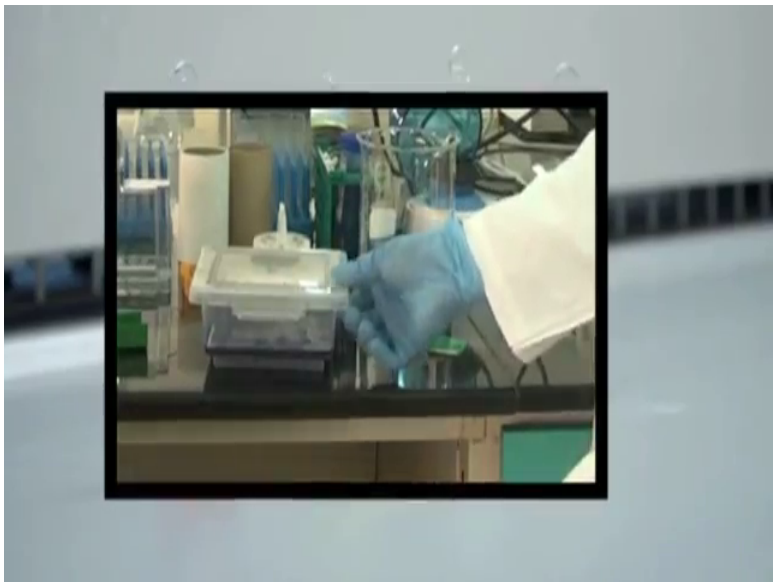

Make sure that you do not dry out your gel. The gel should not get dried out and now I will take the staining solution and pour it over the gel such that it is completely submerged in the stain. And then we are going to put on the lid to this gel tray and leave it for staining overnight.

Sometimes what you can do is instead of if you are in a hurry or you need to visualize your results quickly for routine work. What you can do is you can heat up this stain in the microwave for 30 seconds, do not overheat it, but if you heat it for around 30 seconds and then let it stand for some time you can expedite and speed up the process.

Generally we keep this on a gel rocker so it is a rocking platform which ensures that the staining is uniform and proper. One point that are like to make is in case your heating up your gels do not over heated. And once you have heated it, once you take it out of the microwave do not immediately open the lid because they knew risk inhaling fumes of acetic acid which is present in the gel stain.