Molecular Biology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Guwahati
Module - 08
Molecular Techniques (Part 1)
Lecture-35 Western Blotting (Part 1)

Hello everyone, this is Dr. Vishal Trivedi from department of biosciences and bioengineering, IIT Guwahati. So, in that we have discussed about the apoptosis cell divisions and the autophagy. So, subsequent to that we have also discussed about the central dogma of molecular biology and within the central dogma of molecular biology we discussed about the replication, transcription and translations and we have discussed about all of these events in the prokaryotic as well as the eukaryotic system. We have discussed about how these events are being regulated within the cell with the help of different types of modifications and other kinds of regulation at the cellular signaling levels and all that. Subsequent to that we have also discussed about the different types of techniques what is being evolved or what is being developed using the molecular biology principles and in this series we are in the current module we are discussing about the different types of blotting techniques. So, far what we have discussed we have discussed about the southern blotting where and the purpose of the southern blotting is to detect the DNA fragment into genome and then following that we have also discussed about the northern blotting.

So, if you recall in the previous lecture we have discussed about the applications of the northern blotting and how the northern blotting is being performed. So, when you want to perform the northern blotting you are supposed to first isolate the total messenger RNA from the cell and then it has to be separated on to a denaturating agarose gel and following that we have also discussed about the transferring of these RNA molecules on to the nitrocellulose membrane following that you have to hybridize that with the suitable radioactive probe and then you have to develop that with the help of the autoradiogram and then at the end you are going to get the pattern and this pattern is actually going to tell you about the transcriptional activity of that particular gene fragment and it is going to tell you how it is modulating in the different types of response. Now in today's lecture we are going to discuss about the another technique which is called the western blotting and the purpose of the western blotting is to detect the proteins on to the into the cell lysate or into the cellular system and protein detection of protein is very important because it is going to give you the idea about the translational activity of that particular gene or gene product and the detection of western blotting or detection of protein with the help of western blotting is a multi step process where initially you are going to run the you are going to separate the proteins on to a

SDS page following that you are supposed to transfer that on to a nitrocellulose membrane and then you are supposed to treat that with the primary antibody followed by the secondary antibody and then followed by the development of the depending upon the secondary antibody are supposed to use the suitable substrate to give you the bat. So what you are going to see here is that you are going to first run the so this is the step number 1 that you are going to run the proteins on to a SDS page then you are going to transfer that on to a nitrocellulose membrane and then you are going to do so this is the step number 2 and then you are going to do a blocking step followed by the primary and after the primary you are going to have a blocking you are going to have a washing step so that the excess antibody can be removed and then so this is going to be the step number 3 this is going to be step number 4 and then you are going to have the secondary antibody followed by the washing and then you are going to add the substrate or the different types of reagents what is required for developing and then it is actually going to give you a band corresponding to a particular protein. So what you see here is that it is mainly having the 5 different steps step number 1 you are going to resolve the protein mixture on to on SDS page.

In step 2 you are going to transfer the protein band on nitrocellulose membrane just like as we have discussed about the southern as well as northern blotting step 3 you are going to treat this with primary antibody step 4 you are going to do a washing and you are going to treat that with the secondary antibody and in the step 5 you are going to do a washing and you are going to develop that with the help of develop by the substrate or you if some cases you are using the radioactivity then you are going to use the autoradogram. So if you see the technique you can be able to very clearly see that it can be divided into 2 parts one part where you are going to run the protein on to SDS page and then you are actually going to do the subsequent steps by after transferring the protein band on to the nitrocellulose membrane. So just for the convenience of understanding this we are first going to discuss about the protein resolution resolving of the protein mixture on to the SDS page following that we are also going to discuss about the all these steps in a subsequent lecture. So the western blot is western blotting is a popular technique to detect the specific protein present into the crude lysate or the homogenate it uses the separation of the protein different proteins into the gel electrophoresis which is called as the SDS page and then the transfer of the protein on to a solid support such as nitrocellulose membrane a primary antibody direct against the protein of interest and the secondary antibody is used to detect the primary antibody and give either colored or the chemiluminescent substrate. So what we are going to do is we are going to first understand so it is actually having the 2 part one you are going to understand about the electrophoresis so that you can be able to understand how the you can be able to separate the so protein runs on the SDS page so how you can be able to separate the protein on to a SDS page and the second part is the transfer of protein band

and following by the subsequent treatments so that anyway we have discussed.

So let us first discuss about the electrophoresis and we are not going to discuss in detail about the electrophoresis we are only going to tell you about how to perform the electrophoresis and then we can use that technique to tell you about how to perform the electrophoresis and how to perform the SDS page so that it will become a complete learning experience. So what is electrophoresis? Electrophoresis is that you are going to run a charged particle into a electric field so you can imagine that if you have a charged particle with Q then it is actually going to have this process is called as the electrophoresis. So electrophoresis is an electrokinetic process which separates the charged particle in a fluid using a field of electric charge it is most often used in the life science to separate the protein molecules or the DNA molecule and can be achieved through the several different procedures depending upon the size and the type and size of the molecule. So you can imagine that if you have a charge Q which is been resolved and it is so it is actually going to experience electric field or electric charge which is electric force which is called as F is equal to Qe and it is also going to experience a retardation forces and these retardation forces are going to be dependent on to the radius of this particular molecule and as well as the viscosity of this particular media and that is the friction forces is going to be called as 6 pi eta RV and where this molecule is going to stop the place where the F is always equal to the friction forces and at that point the electrophoretic mobility is going to be proportional to the Ze by the 6 pi eta R. So Ze is actually and so if you if you subsequent if you further simplify this what you will see is that the electrophoretic mobility is directly proportional to the E by R actually and that means it is actually going to be proportional to the charge by mass because R is directly proportional to the M because for most of the spherical molecules R is proportional to the M and E is proportional to the charge.

So it is going to be Ze is the charge actually. So hence the electrophoretic mobility V is directly proportional to the charge and the inversely proportional to the viscosity of the media size and the shape of the molecule in case of relative mobility it is directly proportional to the charge by radius of the molecule for a globular protein the radius of the molecule is related to the molecular mass of the molecule and that is why the relative electrophoretic mobility is charged by mass. So you can actually be able to run the electrophoresis in two different mode the mode in which the charge and mass both are actually going to be intact and that mode is called as the native page. Other mode is that you if you if you neutralize the charge what is present onto the protein and then it is going to be called as the SDS page and the difference between the SDS page and the native page is that in the native page the electrophoretic mobility is directly proportional to the charge by mass whereas the electrophoretic mobility in the case of SDS page is going to be inversely proportional to the mass because you have already you have

already nullified the charge. So this is very very important because it is actually going to separate out the molecule based on the mass and that is how you can be able to very precisely predict the mass of that particular protein if you look at the pattern. So what we are discussing in this is about the SDS page we are not discussing about the native page because the most of the western blotting procedures does not involve the running of the native page.

Now what are the things you required for running the electrophoresis? So you require the following components you require the gel cassette you require the electrophoretic chamber you require the cords you require the power pack or the instrument which actually can supply the requisite power supply then you also require the electrophoretic tank and then you also require the comb so that you can be able to prepare the wells. What are the chemicals you require? So you require the different types of reagents in preparing the gel electrophoresis you require the buffers and reagent for the electrophoresis you require the NN, NN tetramethyl ethylene diamine which is also in short form it is called as TEMID so it catalyses the acrylamide polymerization then you require the ammonium bar sulfate or APS it is an initiator for the acrylamide polymerizations then you also require the TrisSCL so it is the component of the running and the gel casting buffers then you require the glycine and it is the component of the running buffer then you require the bromophenol blue and it is the tracking dye to monitor the progression of the gel electrophoresis then you require the comasibilian blue R250 it is used to stain the acrylamide gel you require the SDS or sodium dodecyl sulfate it is used to denature and provide negative charge to the protein and it also require the acrylamide and acrylamide is a monomeric unit to prepare the gel so you always have the combination of the acrylamide and the bisacrylamide and you also require the bisacrylamide and that is the cross linker for the polymerization of acrylamide monomer to the form gel how the bisacrylamide and acrylamide come together and form the network or the mesh so that you can be able to have the retard you can you are going to have the frictions and that actually is been responsible for the separation of the different molecules so you are going to have the acrylamide polymerization there is a complete mechanism in which the acrylamide is and bisacrylamide is actually going to be mixed up with the tamid and APS and that is how it is actually going to make the cross link so the bisacrylamide monomers are actually going to make the cross link these fibers and that is how it is actually going to make a network. So ammonium bar sulfate in the presence of tamid form the oxygen free radicals and induce the polymerization of acrylamide monomer to form a linear polymer these linear polymers are interconnected or connected by the cross linking with the bisacrylamide monomer to form the 3d mesh with the pores the size of the pore is controlled by the concentration of the acrylamide and amount of bisacrylamide in the gel in a vertical gel electrophoresis system we cast two different types of gel stacking gel

and the resolving gel for the resolving gel solution is prepared and poured into the gel cassette for polymerization a thin layer of organic solvent is attached or layered to the top the entry of oxygen and this is been done so that you can actually be about avoid the entry of oxygen and as well as to make the top surface smooth oxygen is actually inhibitor of the free radicals and if you allow the oxygen entry then it is actually going to destroy or it is actually going to inhibit the acrylamide polymerization. So it is actually going to inhibit the acrylamide cross linking or polymerization because acrylamide polymerization is associated with the free radical formation and that is can be stopped after the polymerization of the resolving gel a stacking gel is poured and the comb is fitted into the gel for construction of the different lanes for the samples. How you are going to run the gel electrophoresis? So gel electrophoresis you are going to have the two different types of gels you are going to have the resolving gel and you are going to have a stacking gel the purpose of both the gels are very different the stacking gel is required for the stacking of the sample whereas, the resolving gel is required for the separation of the molecules.

So first the resolving gel is prepared and poured into the gel cassette for polymerization and thin layer of organic solvent is layered and all that and then you are actually going to have the separating the pouring of the stacking gel. So there is a recipe available to which you can be able to prepare the solution for the resolving and as well as the stacking gels and these are the different components what is required for running the electrophoresis system. Now the question comes how the stacking gel and what is the relevance of the stacking gel into the gel electrophoresis. So the sample is prepared in the loading dye containing the SDS, beta mercaptoethanol, the glycerol to denature the sample and preference of the itself loading. So you can imagine that you have a well and in which the samples are filled like this.

So if you fill the sample like this some samples are going to be present here and some samples are going to be present here just like in a race some runners are present here and another runner is present here. So there is a difference between these two runners and that is why you see very clearly that this runner is actually running in a circle which is of a larger diameter whereas this guy is running in a smaller diameter. This means the distance what they are going to run is actually be the same. So same is the concept when you are want to do the stacking because what you have is you have a well and this well is standing like this. So you are actually going to have a sample here and you are going to have a sample here.

This means these two guys are even if they are of similar molecular weight they are separated from each other to this distance and that is why if you do not do the stacking this guy which is like the number 1 and this guy is number p. Number 1 guy is always

going to be remain on the behind side. So number 1 will come here and the p will come here because even if they are of the same molecular weight this means you are supposed to device a mechanism so that the 1 is also going to come here and then the 1 and p also should come together and they will run together so that they are actually going to show you the real separation and real separation would depend on to the molecular weight. So as the samples are filled vertically there is a distance drift between the molecule at the top versus the bottom lane and the problem is taken care once the problem runs to the stacking gel. So stacking is and that is why you are actually going to have a specific composition of the stacking gel.

So what is the composition of a stacking gel? In the stacking gel what you are having is they are actually going to have the this SCL of pH 6.8 and that is a very very important to take care of this problem. So what you are going to do is the problem is been taken care once the sample runs to the stacking gel the pH of the stacking gel is 6 point at and this pH the glycine is moving slowly in the front whereas the tris glycine is moving fast as a result the sample get sandwiched between the glycine tris and get stacked in the form of a thin band. As the sample enters the resolving gel with the pH 8.8 the glycine is now charged it moves fast and now sample run as well as their molecular weight.

After tracking dye reaches to the bottom of the gel is taken out from the glass plate with the help of the spatula and it is stained with the chromatic blue and blue R250 the dals type present on to the gel. So this is what is actually going to happen. So this is actually going to be the well and you are going to have the sample which is on the top and you are also going to have a sample at the bottom.

So this is and you are actually going to have a pH of 6.8 in the stacking gel. So at pH 6.8 the glycine which is also been present in the running buffer is actually going to work as a block. So it is not going to allow these molecules to run beyond this. And on the top you are actually going to have tris ions and these tris ions are actually going to run from the top. So this is actually going to put a pressure on to these molecules and that is how it they will come they will they are free to move but these guys are not free to move because there is a block there is a glycine block at the front.

And as a result what will happen is that when they are running they are actually going to come closer closer closer like that and at the end what will happen is that they all will be at one place they all will be at one place and this is going to be called as the stacking. And as soon as this is been done you are actually going to have the resolving gel. So this is going to be a resolving gel and they will enter into the resolving gel and then the resolving gel has a pH 8.

8. So once the pH 8.8 is there this glycine block is actually going to be removed and then the molecule will run as per their molecular weight. So they are actually going to be and then the V is inversely proportional to the 1 by m. And that is how the larger molecule will run slower and the smaller molecule will run faster. So just to and then once you are done with the once and how long the gel is running for that purpose you are actually adding a tracking dye which is and then the when the tracking dye is going to reach at the bottom of the gel then you can actually be able to remove this particular thing and then you can if you are doing the western blotting then you can just keep it as such. But if you are doing it for the just for to looking at the pattern and then you can actually be able to stain it with the comasibilian blue and that is how you are going to get the pattern.

So you are going to see a pattern of the proteins after the staining. So just to explain you all these steps and how to make you familiarize with the whole process we have prepared a demo video where we have taken we have shown you how to assemble the acids, how to cast the resolving gel, how to cast the stacking gel and so on and it will help you to understand the whole process. Hi everyone myself Suram Banish research scholar at department of biosciences and bioengineering at IIT, Guwahati. In this video we will demonstrate you how to run a SDSS gel and how to prepare various reagents required for the running of SDSS gel and what are the different instruments we can use. So here this is the gel casting stand so where we can use this glassware to prepare the gel. In between there is a space where we can pour our gel solution then we will keep for some time at least 20 to 30 minutes let it solidify then we will prepare stacking gel then we will load the protein solution.

So here before doing that we need some reagents. So what are those reagents? The first reagent we need for this experiment is acrylamide. So generally we will prepare acrylamide 30 percentage. 30 percentage means 29 grams of acrylamide and 1 gram of bisacrylamide. This both we can use 29 is to 1 ratio in 100 ml of water to get 30 percentage of acrylamide.

So both these are neurotoxic so we have to wear gloves always. After this we have to prepare resolving gel. For resolving gel we need 1.5 molar Tris HCL pH 8.8. In addition to that we also need 10 percentage SDSS prepared in double distilled water and also 10 percentage ammonium per sulphate and also TEMED.

The role of ammonium per sulphate and TEMED we can see during preparation of gel. They acts as a catalyst. After solidifying we have to use we have to prepare stacking gel. So stacking gel is nothing but composition is same but we can say it is diluted.

It contains pH 6.8 Tris HCL and remaining components same but in less quantities. So after preparing the gel we load the marker and the protein which is denatured at 100 degree Celsius for 3 minutes. After that we will fix this gel into this one. We will keep in this reservoir then we will connect to the power pack and run the gel. So this is the overall introduction of how to prepare a SDSS prepared gel.

So let us start with preparing gel. We will learn more things while preparing the gel. Before preparing the resolving gel we have to prepare the casting set up the casting gel.

So this is the glass plate. This is very thin one. So this is the main glass plate. This is 1.5 mm glass plate. It is available in 1 mm glass plate also. If your loading solution is less like you want to load only 20 micro litre, 30 micro litre then 1 mm gel is good enough.

But if you have extended volumes like 70 micro litre you can use 1.5 mm. You have to arrange like this short plates on this plate and the bottoms should be equal. Then we have to put in this one, this tray. Then we are going to keep like this. So we have to check if we perfectly set up this one then there should not be any leakage.

But if there is any leakage your resolving gel may leak out and you will get nothing. So in that case we have to check it prior to pouring the gel. So whether it is okay or not. So I am going to use milk water. After checking the gel if there are any leakages or not.

So we move forward for preparing the resolving gel. So the composition is given in this slide. Please go through that slide.

This is just water. First I used water. I am going to add sequentially 4 ml of water. Now I have to add 3.3 ml of already prepared 30 percent acrylamide. Already in introduction I explained how much percentage we have to prepare and how much quantities of acrylamide and bisacrylamide need to take.

So here we have to add 3.3 ml of acrylamide solution 30 percentage.

So I have to adjust 300 micro litre. The next component is 1.5 molar Tris PH 8.8. We have to add 2.5 ml. Next component is SDS. Here SDS functions as place as dual role. Like one thing is that it gives negative charge gross negative charge on the polypeptide chain. The next component we have to add is SDS 10 percentage SDS.

We have to add 100 micro litre of SDS to resolving gel. It plays very crucial role in polyacrylamide gel electrophoresis. Like it imparts negative charge on the polypeptide

chain so that despite of their charge they will move based on the molecular weight. So I am going to add SDS. The other important thing is that 10 percentage ammonium persulfate. Ammonium persulfate which is catalyzed by the timid provides free radical species which accelerate the forming mesh like say in acrylamide gel.

Like it will catalyze forming the mesh. So this is the 10 percentage APS. I just add 100 micro litre of 10 percentage APS to resolving gel. In final step we have to add timid. Timid after finishing after adding all the components at the end of the gel we have to add timid.

Because if you add earlier it will quickly facilitate the polymerization. So you cannot take out with the pipette. So it completely solidifies. So that is why you have to add at the end of the cell. So I am going to add 5 micro litre of this timid which catalyzes the ammonium persulfate. Ammonium persulfate in turn provides free radical species and free radical species accelerate the polymerization.

This is the overall principle of this resolving gel. So I will add timid. We have to mix properly. Then add slowly at one corner. So after this we have to overlay with on the top layer we have to overlay with some solvent like 2 butanol or isopropanol or with water. So why we are doing this? Because if the gel is exposed to air then the oxygen from the air will interfere in the polymerization of the gel.

So we have to add either water or 2 butanol for this purpose. Now we have to check whether it is solidified or not. So it is solidified. Now we have to remove the overlay layer like we have used water. So no need to remove. If you are using isopropanol or butanol you have to remove that and wash with the milky water.

So now we will start preparing the stacking gel. The compositions are given in the video. We have to add 3.4 ml of water first. Next 830 microlitre of acrylamide.

630 microlitre of Tris-HCL pH 6.8. 50 microlitre of imide and 50 microlitre of SDS we have to add. At the end we have to add 5 microlitre of Tb. We have to mix properly after adding the Tb. Now we will wait until the gel gets solidified. Then we shift to the buffer tank and then we will run the gel.

While the stacking gel is solidifying we have to prepare sample for loading the SDS-plane gel. So for that we have to prepare loading gel 10x or 6x loading gel. It mainly contains 250 ml of millimolar Tris-HCL pH 6.8, 30 percentage glycerol, 10 percentage SDS and 0.

005 percentage of bromophenol. So here we can add 10 millimolar of DTT as a reducing agent. SDS mainly works as imparting negative charge on the polypeptide chain and DTT raised on the disulfide parts. If you have dimer which you can see as a monomer in SDS-plane gel. Suppose you have 20kDa, 20kDa that means 40kDa protein which is a dimer actually. You can see only 20kDa band corresponding to that protein because DTT raised on the disulfide part and you can see only single band.

If you want to see actual molecular weight you have to run it on native phase where there is no reducing agent or no detergent. The other thing is glycerol. While loading the gel since the protein solution is not that much dense it may come out from well. So in order to prevent this thing we have to load with the denser solution like glycerol. So 30 to 50 percentage glycerol is sufficient for keeping the protein solution intact in the bottom of the well.

So other thing bromophenol blue. Bromophenol blue we use for just tracking the how much gel completed. So this is the loading time. So we have to take the protein solution. Here we already prepared 10 percentage of loading time. So that means this is 10x loading time.

So we have to prepare 1x to mix with the protein solution. So this is 100 ml of solution, loading solution. We mix 10 microlitre of loading time to this protein solution. You can tap down or pipette this protein solution. Then we have to heat it for 3 minutes at 100 degree Celsius so that all the polypeptide chains, I mean dimers are present. If any multimers are present they will break down and you can see nice band.

So I am going to heat this at 100 degree Celsius for 3 minutes. This is the remaining of stacking gel solution. So we can see it is solidified. So that means the stacking gel also got solidified. We have to remove that gel and fix it into this one and we have to keep inside the tank.

So just take out the gel. So inside this tank we only have this side one. You have to cover other side also. So for that we use dummy plate. Just hold it tight and close this thing.

After that gradually adjust the gel length. So just we have to fix like this. Once fixing here we have to add this running buffer. The running buffer contains 15 grams of tris, 72 grams of glycine and 5 grams of SDS for 2 liters of solution, 1X solution. So this is 1X I have already prepared. I am going to add. We added in this tank but the main tank surrounding to this one we have to add up to the mark.

So for reference you can see here for 4 gels we have to add till here the buffer we have to load outside this gel. So for 2 gels here for 1 gel we can add like this. This is the power pack where we can adjust the how many volts we want to run.

The protein samples are ready. We heated sufficient time. Now we have to load this. So we have to remove the column carefully. First I am going to load marker or protein ladle. Next I will load sand.

Once the loading goes over we have to fix this gas tank. I am going to set it at 170 volts. This thing has Time supply point 0, lightAST. So, we will try to heat it with the staining solution which contains pumasi brilliant blue and along with methanol and water. So, then we will try to de-stain with the water by heating. But in another way, the simplest way is we will just stain the engine for 2 hours, then we will de-stain overnight. So, I am going to show the simplest way.

First we will stain in pumasi brilliant blue staining solution, then we will de-stain in methanol water containing salt. So, I am going to start the children, then I will remove it and show you how to remove the gel. Take out the glass piece. And here we have to be very careful while taking out gel, otherwise the short plates may grow. On a corner we have to take and lift the gel like this.

So, keep the gel in a staining box which is more or less a plastic one but it can sustain the gel. So, then I am going to add staining solution. I will keep it for a rotation on a shaker for at least 2 hours, then we will de-stain the solution. So, once the time is over, after 2 hours we will de-stain the solution. Now, we kept 2 hours in staining solution.

As we can see the staining is over, like we can see the gel completely turned into blue. So, we remove the solution. Then I am going to add de-staining solution. And I will keep this on a rocker for 2 hours for de-staining. So, the composition contains for 100 ml of de-staining solution, 40 ml of water, double distilled water and 40 ml of methanol and 10 ml of glacial acid.

So, I am going to keep this on a rocker. We have run the gel and stained and de-stained. Now, we will capture the gel image. So, you can see manually also, but for record purpose, we have to capture it through gel dark. So, this is the gel dark imaging system from Bio-RAD.

So, I will show you how to take the capture the images. So, here we will use white tray. There is another one, grey or UV tray is also there. So, there you can see any fluorescent one or stained with the ethidium bromide or blots, chemiluminescent blots, you can use

that. But for normal protein imaging, we can use this white tray.

So, you have to open properly. This is very important step, you have to align the tray in a proper way. So, otherwise it will show error. So, once it is over, you just push it back. So, we have to log on to account. So, this is SDSS gel, you can select the application whatever you want. So, here nucleic acids, protein gels, blots, three different categories are there.

So, we are observing here protein gels, protein gels stained with the kumasi blue or white tray, we are using white tray. So, this is the right tray. You can use kumasi blue stained one grey tray also, but we are using as we are using white tray, so we will use kumasi blue. So, auto optimal, then I will ask for capture. So, it will take one to three minutes based on the signal intensity.

So as we can see, it is optimizing the signal intensity, you can minimize this one also. So that you can see the gel image. So, now it is over. If you want to do any modifications to images, for suppose you want to decrease or increase the signal intensity. So, this kind of changes you can do. So if you want to send this gel, you can have send itself. If you have any drive connected to this one, you can send directly to that one, that thing.

So for image analysis part, we will show in the upcoming video, how to analyze the what this band of interest correspond to which molecular weight. So we already loaded the molecular weight one, so we can easily find out using image lab software. In this video, we have learned that how to prepare a SDSS gel and how to run it, what are the precautions need to be taken while preparing the gel and how to record the gel using gel documentation system. So I hope this will give you a list of how to prepare and run a SDSS gel and analyze the protein sample. So this is the part one of the western blotting where we have discussed about the how to resolve the sample on to the SDSS page. And what we have discussed, we have discussed about the basic principle of the electrophoresis, we have discussed about the different component what is required for the running the electrophoresis and then we also taken the crucial mechanistic and as well as the technical steps what is required for running the SDSS page.

And at the end, we have also shown you a demo video how to prepare the how to prepare how to cast the gel, how to run the gel and how to stain the gels. So with this, I would like to conclude my lecture here in our subsequent lecture we are going to discuss some more aspects related to western blotting. Thank you.