Experimental Biochemistry
Prof. Soumya De
School of Bioscience
Indian Institute of Technology, Kharagpur

Lecture - 28
Gel Electrophoresis of DNA and Proteins Part – II

Hello, today I am going to talk about the second part of Gel Electrophoresis

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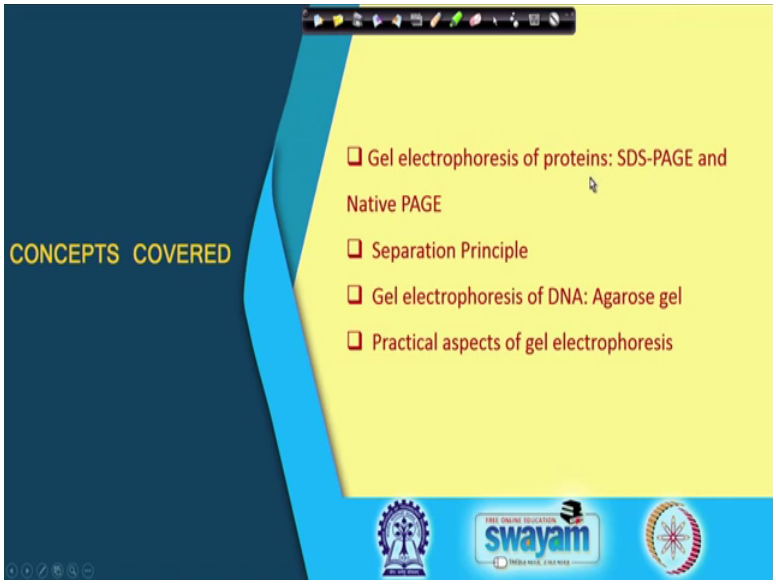

So, in the last lecture I introduced the basics of gel electrophoresis. And I specifically talked about polyacrylamide gel electrophoresis. And I also introduced SDS-PAGE gel electrophoresis which is done under denaturing condition. In today's lecture I will talk about two particular examples where SDS-PAGE is used to determine structural information about proteins. And then I will talk about DNA agarose gel and also some practical aspects of gel electrophoresis.

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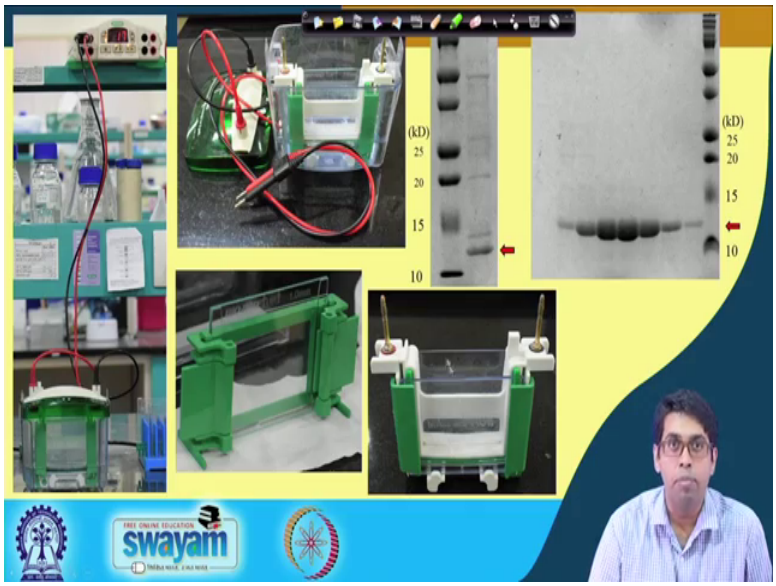

So, in the last lecture we saw this total setup of SDS-PAGE gel electrophoresis where the polyacrylamide gel is cast between these two glass plates. And this glass plate is assembled here and this whole setup goes into this buffer chamber. So, the running buffer that is used is poured between these two plates and also at the bottom of this buffer chamber. And then this electrodes are connected, which looks something like this and then this whole thing is collected to the power source.

So, once you start running current through your gel the proteins start getting separated according to their size. Because in case of SDS-PAGE the proteins are denatured and the hydrophobic parts of the protein are bound by the detergent SDS giving them and overall negative charge and which gives which results in a linear rod type molecule with a constant charge to mass ratio. So, all this molecules are then separated based only on their size. So, this is one example of polyacrylamide gel where this lane has the molecular weight marker.

So, the molecular weight marker is a mixture of non proteins with non molecular weights some of the molecular weights are listed here. So, this is 10 kilo Dalton this is fifteen kilo Dalton so on and so forth. And this lane has the sample of our interest. So, what we see in this particular gel is that our protein is here it has the right molecular mass which is around 12 kilo Daltons. So, it shows a between this 10 and 15 kilo Dalton bands and we also see that there are some impurities.

So, from this gel we can confirm that we indeed have our protein of interest and it is not hundred percent pure there are some impurities which are shown here. So, this sample was can taken an run through a size exclusion chromatography and that resulting sample that came out from the SCC column was run on another page gel. So, here you can see that we get again bands in the same region.

So, it is exactly the same protein, but now no impurities are present. So, now, we can tell that our protein sample is almost 100 percent pure. So, this is the typical use of polyacrylamide gel electrophoresis where we can determine the purity of a sample. And also we can estimate the molecular weight of a sample if it is not known.

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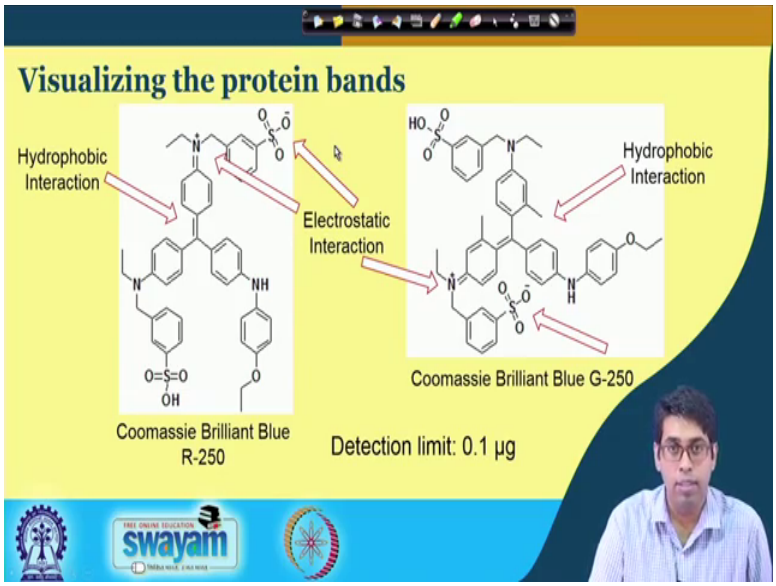

So, I briefly discuss this in the last lecture that to visualize this protein bands which were shown in the previous slide we use a particular type of dye the most common dye that is used is Coomassie Blue, Coomassie Brilliant Blue there are two types of coomassie brilliant blue R-250 and g-250 and the typical structures are given here.

The detection limit of this dye is in the range of point one microgram. So, the way this dyes bind our protein is remember the protein is denatured. So, all the hydrophobic parts are actually out. So, this hydrophobic part of the dye can actually intact with the hydrophobic part of the hydrophobic side chains such as tryptophan tyrosine phenylalanine and these charged groups can interact with the charged side chains of the protein.

So, this positively charged can interact with the negative charged side chain such as aspartic acid and glutamic acid whereas, this negative charge can interact with the positively charged side chains of lysine and arginine. So, using this dye we can visualize the bands of the protein in the polyacrylamide gel electrophoresis.

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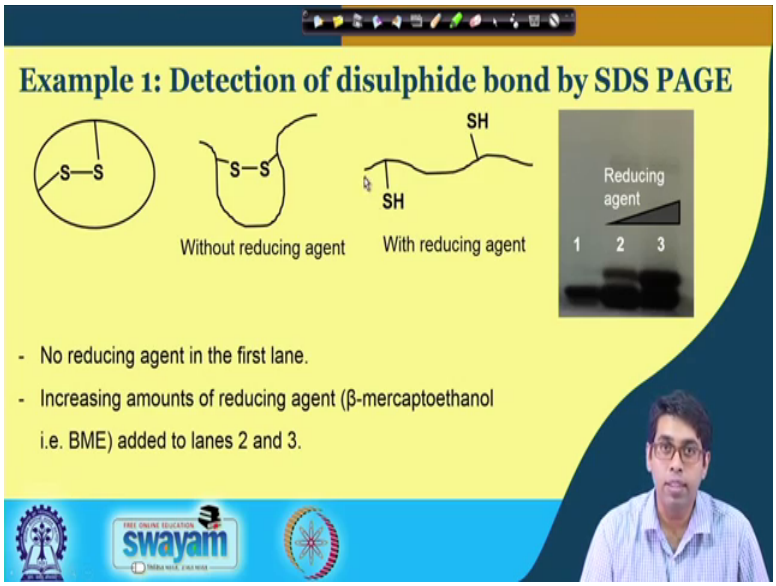

So, as I mentioned before I will talk about two examples of SDS-PAGE gel electrophoresis where it can be used to determine some information about the structure of a protein. So, this first example talks about the detection of a disulfide bond by SDS-PAGE suppose we have a protein and the structure of this protein is not known. But what we know is that this protein has 216 residues.

Now this is a question or a possibility that whether this 216 residues pharma disulfide linkage like this or maybe they are there. But they do not they are not close enough to pharma disulfide bond. So, how can we answer this question using a simple experiment of a poly of this SDS-PAGE. So, in this particular example what was done a protein which has 216 residues was taken. And three samples were made in three different eppendorf tubes the loading dye which typically contains SDS and beta mercaptoethanol was used, but in this particular case beta mercaptoethanol which is a reducing agent was left out.

So, in the loading dye we only have SDS and that loading dye was used to was added in all three samples, which have the same protein and in case of sample two and three we

also added increasing amount of beta mercaptoethanol. So, no BME in the first sample little bit of BME in the second sample and a higher concentration of BME in the third sample. Then this three samples were boiled and loaded on the top of the gel in this three different lanes and it was run and it was dyed using coomassie blue. In case of the first lane what we see is a single band ok.

So, this was a very highly pure protein sample was used otherwise your conclusions will not be very clear. So, in case of the first lane which has no BME only one band is visible what is happening here. So, remember in this case we do not have the reducing agent. So, when this protein is denatured it will look something like this, but since there is no BME the disulfide bond is still intact. So, it will still be present their resulting in sort of a compact structure of a protein it is not linear rot like structure and this is the only species present.

So, we get a single band here now in case of a lane two and lane three we have some BME. So, some of the molecules will show up like this where the disulfide bond is now reduced as the disulfide bond is reduced these two residues are not held together in close proximity. That will result in this linear or rot like shape of the denatured protein. So, in case of lane two now we have two species one this is a more compact structure and this a more open extended linear structure the compact structure will run faster.

So, we see a band which is in the same place as lane one and the extended structure will run slower. So, we see a new band on the top in lane three we have more BME which means that we will get more of this reduced structure or the extended structure. So, you can see that this upper band in lane three is now more darker and bigger compared to lane two.

So, all of this together point to the conclusion that in the folded protein these two 16 residues indeed form a disulfide bond which is broken by the addition of more and more beta mercaptoethanol which results in this more extended structure and the appearance of this band on the top. And the more compact structure runs faster as this lower band. So, by this simple experiment we can actually get a very conclusive structural information of a protein.

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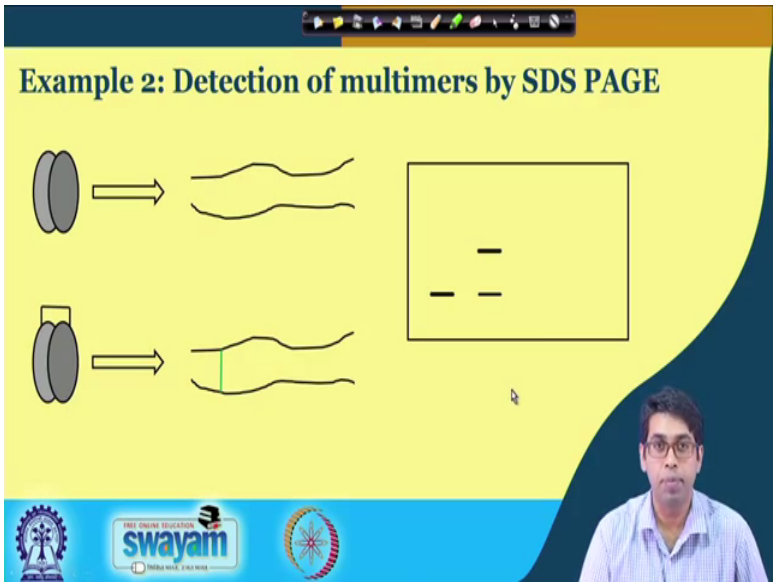

This is another example; so in many cases it happens that proteins are multimeric. So, they are not just monomeric they can be a dimer they can also pharma tetramer and so on. So, the example that I am showing here is of a particular protein which forms a dimer. So, if we do the standard treatment that I have discussed before where we take this sample at our loading dye or loading solution which has SDS and BME.

And then boil it then both protomers in this dimer will denature. So, that we will get this linear extended change bound by SDS. So, if we run this on an SDS-PAGE gel we will get only one band because there is only one polypeptide in another experiment, what we can do is; we can use cross linking reagents. So, there are many crosslinking reagents which can react with this certain side chains. For example, the NH 2 groups of lysine or some other groups on other amino acids.

So, let us say we have a cross linking agent which can react with the lysine side chains. So, we take this protein and add our crosslinking reagent it can it will randomly interact or react with two different licence side chains. This two licence can happen can be present on the same protomer or they can be present on the two different protomers. So, if that is the case then what we will get is a covalent linkage on a lysine on one protomer and another lysine on another protomer.

Now if this sample is denatured we will get again two denatured polypeptides, but now these polypeptides are linked by a covalent bond. If we run this sample on to the same

polyacrylamide gel in this case we will see two bands because not all of them will be cross linked. So, we will get bands which correspond to these linear polypeptides which are the monomers. So, that band will show up here like the previous case and we will get another band which corresponds to this two polypeptide chains connected by a covalent bond.

And since the molecular weight will be almost twice it will run slower at a much higher position. So, from this experiment and if we have a molecular weight marker we can also check the molecular weight of these two bands. And this molecular weight should correspond to twice of this molecular weight approximately and from that we can confirm that our protein forms a dimer. So, again it is a very simple experiment that can be done to get a very unique structural information about a protein.

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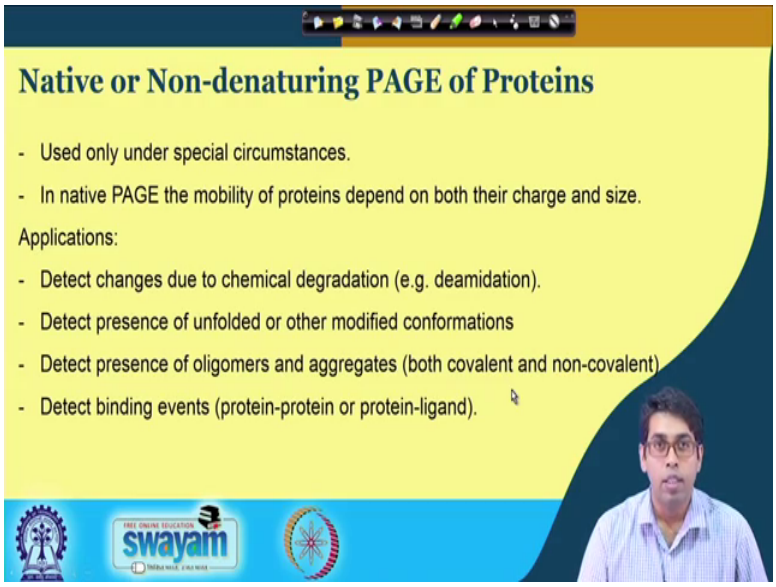

So, polyacrylamide gel electrophoresis can also be used under non denaturing conditions. All the examples that have talked now where under denaturing conditions where we used SDS to uniformly quote a negative charge on the protein. But a native page or a non denaturing page can be used where you leave out SDS from your experiment.

So, in this case the protein is not uniformly negative. So, the overall charge of the protein will depend on the Pi of the protein and the pH of the buffer that is used to conduct the experiment. So, this is a very special experiment and it is only used under special circumstances. But it is a very useful experiment and it can be applied for this various

conditions. So, if we want to check whether our protein is absolutely pure or it has undergone some chemical degradation a native page can be run because the structure of the protein is now intact.

And if there are some chemical degradations which can change the structure we might detect that in a native page. Similarly, if a protein is a mixture of folded and unfolded states then they can also be detected by running a native page a protein which forms higher. Oligomers or aggregates can also be run on a native page to see whether we see a one single band or we see a smear that indicates presence of higher oligomeric structures.

And one of the most important use of native page is the determination or the detection of protein ligand interaction where the ligand can be another protein or it can be a DNA or RNA or it can also be a small molecule. If the binding of the ligand considerably changes the molecular mass of the protein ligand complex then we will see two different or two distinct bands and sometimes if the confirmation is changed then also we see a change in the mobility of the protein which indicates binding of the ligand.

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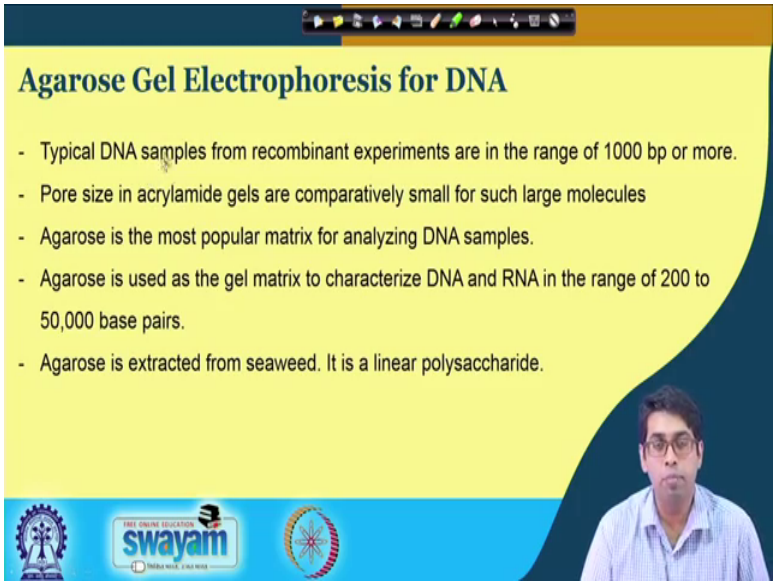

So, all of these gel electrophoresis where done using polyacrylamide. Another very popular gel metrics that is used is the agarose gel. So, agarose gel is typically used for nucleic acid when the size of the nucleic acid is much larger. So, for example, DNA samples when you have a cloning when you do some cloning experiment the plasmids or

even the PCR products from recombinant experiments are in the range of 1000 base pairs or more plasmids are typically in the range of 5000 to 1200 base pair.

So, this molecules are very large for a polyacrylamide gel because in a page gel the pore sizes are very small for this large molecules. So, in this case agarose is used as the gel matrix. So, agarose gel matrix can be used to characterize DNA and RNA molecules in the range of 200 to 5000 base pairs. Agarose is extracted from a seaweed and it is a linear polysaccharide.

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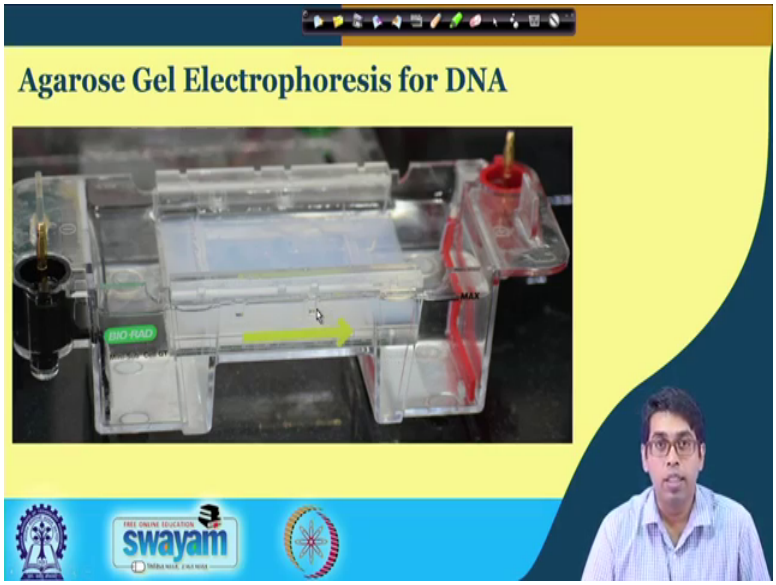

So, here what we see is a typical setup of a agarose gel electrophoresis for DNA. So, I will point out several differences between agarose gel electrophoresis and SDS-PAGE gel electrophoresis page the setup was vertical in this case the setup is linear. And also if you see the thickness of the gel here is much more compared to the thickness of a polyacrylamide gel. So, polyacrylamide gels are typically 0.5 millimeter in thickness and these gels can be up to 1 centimeter in thickness 5 millimeter to 1 centimeter in thickness.

So, the gel is here sample is applied in one end the electrodes go in this two ends now DNA is naturally negatively charged molecule because of the negatively charged phosphate backbone. So, we do not have to add anything like SDS to make it uniformly negatively charged. So, the DNA samples are simply added here and they once the electric field is applied they start migrating towards the positive terminal.

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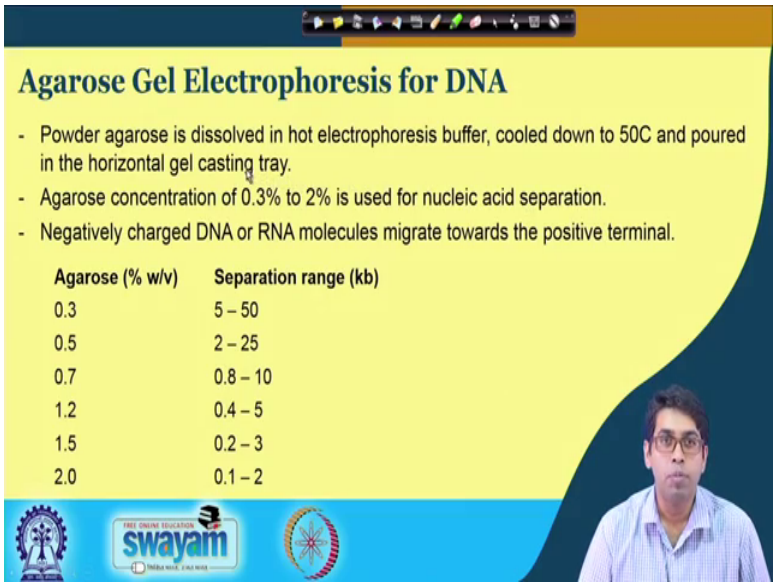

To form the agarose gel powdered agarose is dissolved in the hot electrophoresis buffer and it is cool down to roughly 50 degree centigrade and poured into this horizontal gel set up the casting tray and it is allowed to cool. Once it is once it cools down it polymerization. So, another difference between polyacrylamide and agarose gel is that we do not need any initiator or catalyst to polymerized the agarose gel. Simply heating it and cooling it results in the formation of the gel.

The typical concentrations of agarose is in the range of 0.3 percent to 2 percent. So, higher percentage gels are used for smaller nucleic acids and lower percentage gels are used for larger nucleic acid. So, I have provided you with a table which gives you the percentage of agarose and the typical separation range for that percentage. So, you can see that for 2 percent agarose gel is used to separate DNA in the range of hundred base pair to 2000 base pair. Whereas a lower concentration 0.3 percent agarose gel is used to separate DNA in the range of 5000 base pairs to 5000 base pairs.

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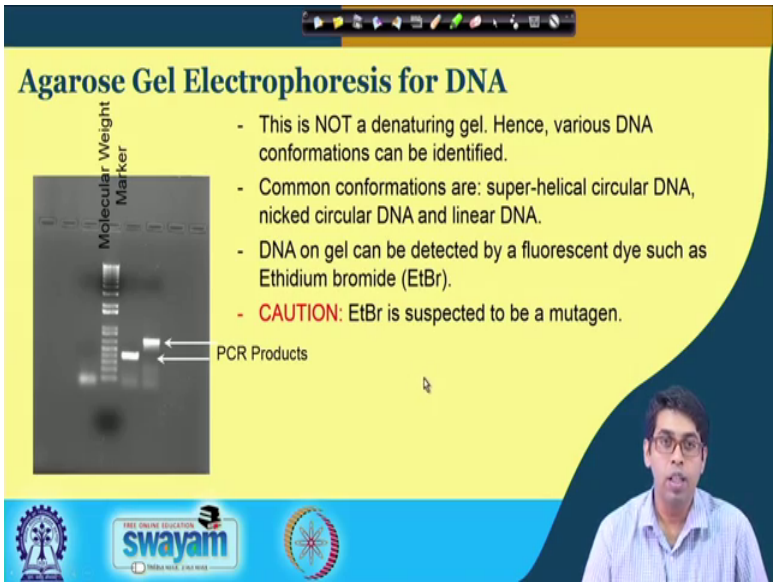

So, a typical agarose gel will look something like this. So, if you can see up here these are the lanes where the samples are added. So, what we have here is a DNA ladder. So, again this is a mixture of DNA molecules of various length, but known lanes and from this bands we can determine where a particular DNA size. DNA of a particular size will appear these two are PCR samples which were collected from two different cloning experiments.

So, the smaller PCR sample runs faster the larger PCR sample runs slower. So, again just a reminder that the agarose gel is not a denaturing gel which means that various conformations of DNA can be identified on this gel so, the common confirmations that are used in that can be identified in an agarose gel are super helical circular DNA. So, these are from plasmids because plasmids are circular DNA nicked circular DNS. So, this is a circular DNA, but it is relaxed and linear DNA.

The super helical circular DNA has the most compact structure and it runs the fastest which means that; it will end up at the bottom of the gel linear DNA are rot like structures and they run slower comparative to this. So, they are the ones to appear next and then nicked circular DNA have the most relaxed structure. So, they are the larger ones and they run the slowest. So, they show up at the top. So, from the positions of this different DNA one can determine the structure or the conformation of that DNA molecule.

So, once this gel is run the DNA on the gel again these are all transparent. So, we need to detect them somehow a very common method is to use some fluorescent dye the most popular fluorescent dye is ethidium bromide. So, one can add ethidium bromide in the agarose solution itself.

And then run the DNA or after running the experiment the gel can be soaked in ethidium bromide solution and then visualized on a UV transilluminator. So, what of caution here ethidium bromide is suspected to be a mutagen. So, one has to be extremely careful when handling ethidium bromide or even the agarose gels which are soaked in ethidium bromide and they also have to be discarded in a very careful manner

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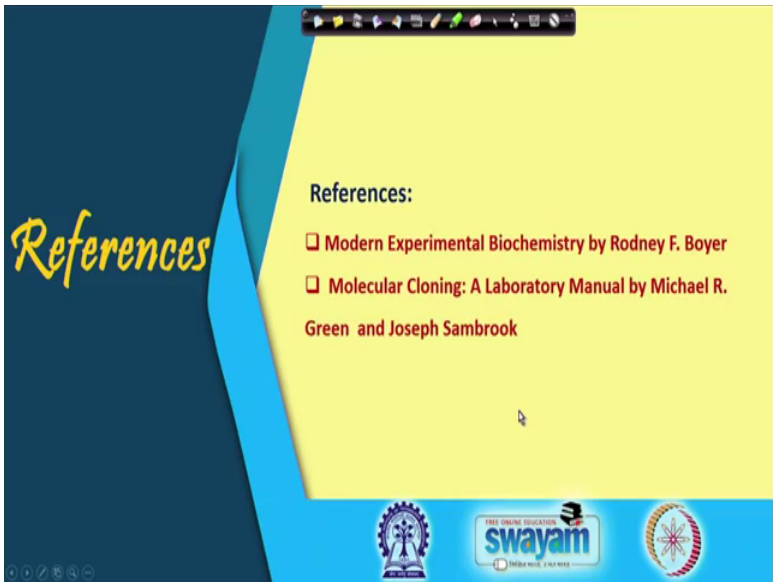

So, that is all for now.

Thank you.