Experimental Biotechnology
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Module No # 02
Lecture No # 09
Horizontal Gel Electrophoresis

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(Video Ends: 01:00)

Hello everybody this is Doctor. Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati. And with in the electrophoresis we were discussing about the basic principle of electrophoresis followed by we have also discussed about the vertical gel electrophoresis and how to perform the vertical gel electrophoresis. So in today's lecture we are going to start discussing about the horizontal gel electrophoresis.

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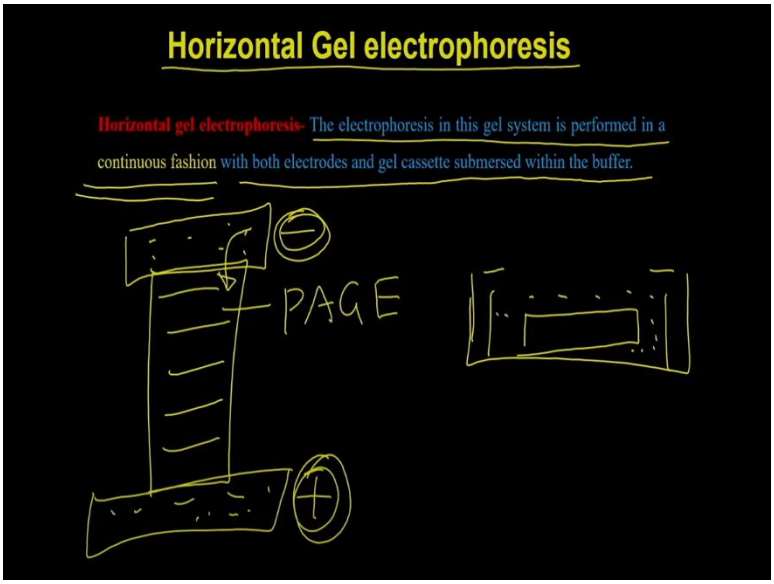

So in the horizontal gel electrophoresis the electrophoresis in this gel system is performed in a continuous passion with the both electrodes and gel cassette are submerged within the buffer. What is mean by the continuous electrophoresis is that the current is not flowing through the gel. Which mean it is actually continuous for example when you are running the vertical gel electrophoresis or if you recall you have the 2 chambers.

One is the top chamber where you are keeping the buffer ok and which is the negative electrode. And then you have the lower chamber where you have the again the buffers ok and then you have the positively charge electrodes ok. And in between these 2 chambers you have the gel actually which is been sandwiched within the glass plates. So here you have the polyacrylamide gel and because and so the current cannot directly go from the negative to positive electrode.

Whereas current has to go through with this gel and then the gel is actually connecting the 2 electrodes chambers. Compared to that in the horizontal gel you have a chamber where you have the negative electrodes and then you have the positive electrode and then a gel slab is been submerged with in the buffer. So the buffer is on the top of the gel, the buffer is on the bottom of the gel and that is why the current can freely move throughout this system.

So the current can flow throughout this buffer solutions and the buffer solution is also continuous. Whereas in this case the buffer 2 buffer chambers are connected through the gel.

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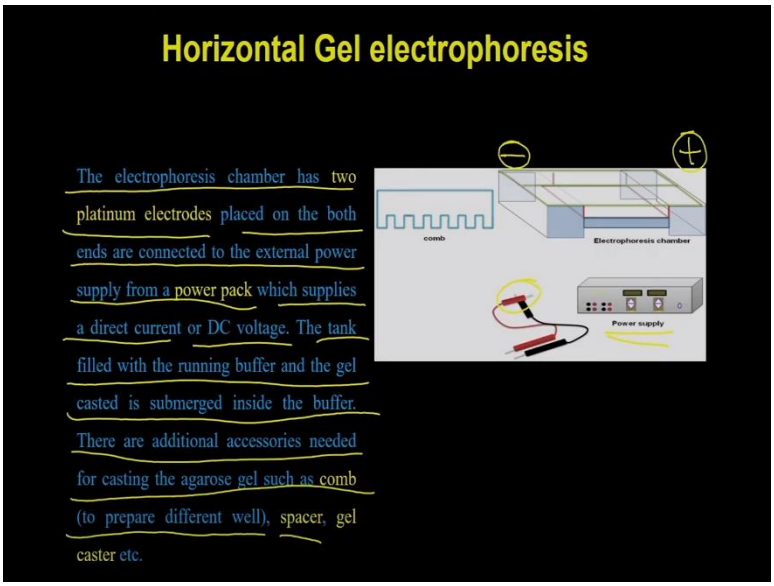

So the electrophoresis chamber has 2 platinum electrodes placed on both the ends and are connected to the external power supply from a power pack which supplies a direct current or the DC voltage. So you have a electrophoresis chamber where you have a negative electrode and the positive electrode on both the side. And then these electrodes are connected to the power cod to the power pack.

And this power pack can supply the DC voltage of the any amount. The tank is filled with the running buffer and the gel casted is submerged inside the buffer. There are additional necessaries needed for casting the agarose gel such as comb. Comb is required to prepare the differ wells then we required the spacer, then we required the gel caster. So, how to run the agarose gel electrophoresis?

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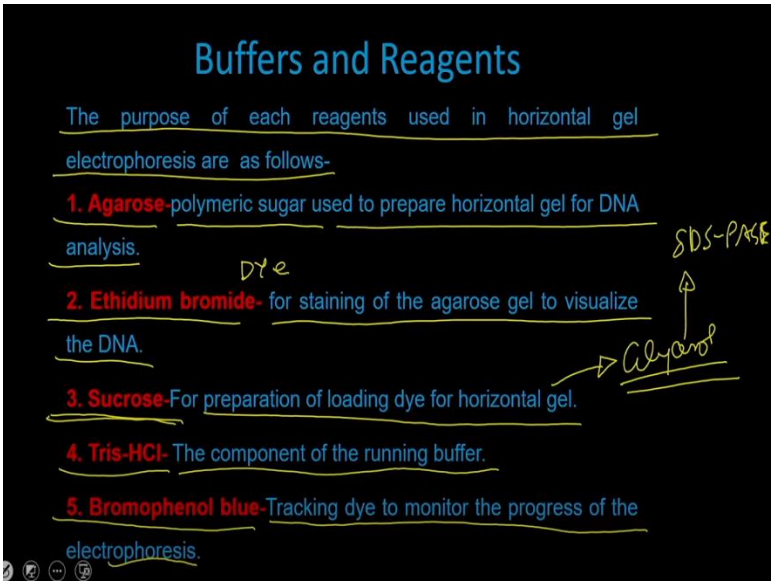

So before discussing about how to run the agarose gel electrophoresis let understand the reagent as well as the instrument what you are required. So for the buffer the purpose of each reagent used in horizontal gel electrophoresis is are as follows. So you require agarose, so agarose is a polymeric sugar which is used to prepare the horizontal gel for the DNA analysis.

Then you required the ethidium bromide, so the ethidium bromide is a dye it is actually going to use to stain the DNA. So see the so ethidium bromide is required for the staining of the agarose gel to visualize the DNA. Then you require the sucrose, sucrose is required for the preparation of the loading dye for the horizontal gel. If you remember we were using the glycerol in the case of the SDS page.

So you have a choice either you use the glycerol or to the sucrose the purpose is that you want to provide a high density solution. So that the, whatever you load in to the gel will remain within the gel. Then you require the tris HCL, the tris HCL is the component of the running buffer and then you will require the bromophenol blue, bromophenol blue is a tracking dye to monitor the

progress of the electrophoresis. Now once you prepare the buffer and the reagents then you can actually perform the horizontal gel agarose electrophoresis.(

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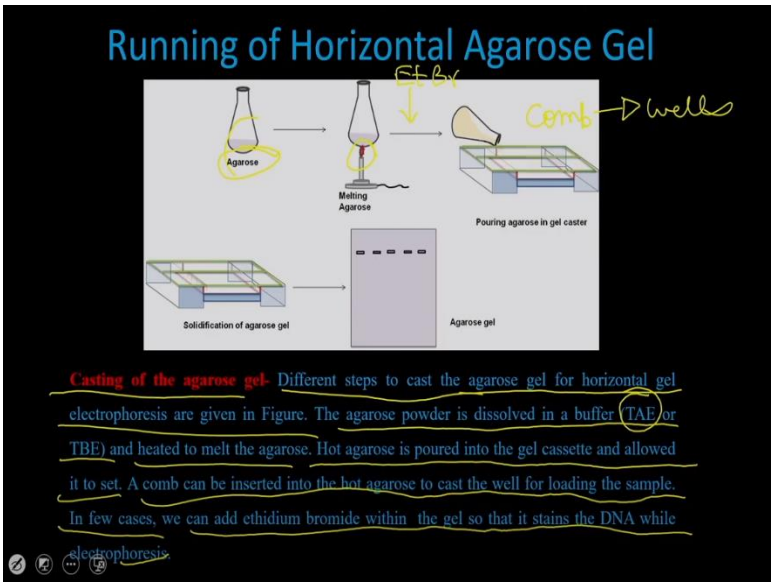

To performing the agarose gel electrophoresis the first you have to cast the gel compare to the acrylamide gel where you are actually going to add the cross linker and then you are going to add the temed and APS to induce the polymerization reactions. Here you are actually not going to do that for casting of the gel. What you are going to do is? You just going to take the agarose. So agarose is the sugar.

So, when you boil this and it is polymer actually when you boil this sugar it actually going to form a jelly like solution. Just like as you might have seen when you cook the rice in your home and if you boil the rice it actually give you some amount of starch. So it is agarose is also similar kind of carbohydrates so where you if you boil the gel it actually forms a jelly like solution. And then if you pour like jelly like solution into a tray it actually contains the comb when the jelly like solution cool down then you are going to have a gel slab.

So for the casting of the gel different step to cast the agarose gel for horizontal gel electrophoresis are given in the figure. The agarose powder is dissolve in a buffer either the test TAE or TBE buffer. And then you heated to melt the agarose. Hot agarose is poured into the gel cassette and allowed it to set. A comb can be inserted into the hot agarose to cast the gel well for loading the samples.

In few cases we can add the ethidium bromide within the gel so that it stains the DNA while the electrophoresis is going on. So what you are going to do is you are going to take the required amount of the agarose into a beaker and then you boil this you can use the burner or you can use the microwave. And then once it get dissolve then at this step you can actually have a flexibility of adding the EtBr.

And then you pour it into the gel cassette and before it gets solidified you can also put the comb so that it is actually going to give you the wells. And once the wells are prepared you can load the samples on to that particular wells and you can able to dissolve the gel DNA to visualize.

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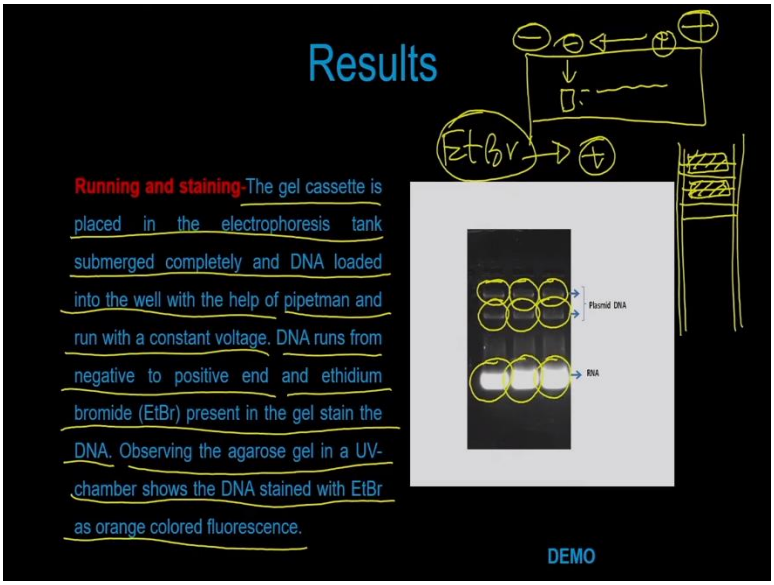

Now the gel cassette is placed in the electrophoresis tank sub submerged completely and the DNA are loaded into the well with the help of a pipetman and run with a constant voltage. So once the gel is prepared then you can just take out the gel from the gel cassette put it into the your your running buffer putting into the electrophoresis chamber and then you connect.

Then you load your DNA and connect it to the DC power with the help of the power bank you can supply the constant voltage and then you can dissolve the DNA into the agarose. And the DNA runs from the negative to the positive end and the ethidium bromide actually runs present in the gel stain the DNA. Observing the agarose gel in the UV chamber shows that, the DNA stained with the EtBr as the orange color fluorescence.

So what happen in this case is that when you have a negatively charge electrodes and then you a positive charge electrodes and then you have a place where you can actually load the DNA. So what will happen is the DNA because the DNA is negatively charge it actually runs in this direction because it runs towards the positively sized. Whereas the EtBr is a positively charge dye so EtBr is actually runs in this direction.

So while they are running in opposite directions the EtBr is stain the DNA and EtBr is stains the DNA within the major and minor group which means the EtBr actually intercalate within the DNA. So you know that the DNA has 2 strand and if these 2 strands are actually having the basis on the sides. So within the basis the EtBr is getting intercalated and as a result it actually gives the orange fluorescence when you visualize them under the UV.

And what you see is when you keep the agarose gel into the UV chamber that you are going to see the orange color bright DNA glowing within the agarose. So there is no de staining step required because the EtBr only gives the fluorescence when it interact with the when interact with the DNA and intercalate to within the basis. That is why there is no de staining step is required because the EtBr which is not interacting with the DNA does not give the orange fluorescence

And what you can see is for example in this particular representative image what I am what we are showing is that the these are the plasmid DNA which has being resolved on to the agarose gel and they are showing you the intense stain. What you see is this blurry signal from the gel is actually the RNA. So RNA is never being get resolved and never give you the compact band because of that the RNA is going to give you the hissy appearances.

So with this we have given you the complete theoretical information how to perform the agarose gel electrophoresis and how to run the gel electrophoresis. But the theoretical information is not enough that is why I would like to take you to the laboratory where we have prepared a small demo clip where we have shown how to boil the samples what are the precaution you should take when you are boiling the agarose solution and then how to pour it and then how to prepare the gel?

How to prepare the wells? How to load the DNA? What are the precaution you should take while you are loading the DNA in to the chamber in to the wells? And ultimately; how to visualize these gel into the UV chambers? What are the precaution you should take when you visualize the DNA into the UV chambers because as you know that the EtBr UV and all these are dangerous for dangerous for the human being. EtBr is the is a carcinogenic molecules.

So it actually causes the cancer. So that is why you have to always wear the gloves when you are performing the agarose gel electrophoresis. So in this demo students have shown you the how to perform the gel electro for the horizontal gel electrophoresis, the agarose gel electrophoresis.

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Today we are going to give you the demo of the agarose gel electrophoresis which we used to dissolve the DNA. So in a typical agarose gel electrophoresis separators what you need is you have horizontal you have the buffer chamber where you can have the 2 electrodes. You can have the anode which is the black color electrode and then you can have the cathode.

This cathode and anode will go and house into the buffer chamber. Connecting these cords to the power cords you have the 2 different cords one is the red cord and one is the black cords. And apart from these you also need a gel caster where you can be able to cast the gel. So this is the small gel tray which you can use to cause the agarose gels. And apart from that you also have the comb which actually can be used to prepare the well.

And besides this reagent what you need to perform the agarose gel electrophoresis you need the agarose powder which you can buy from the sigma or any other company of very high quality. Then you require the EtBr which is the staining dye and as you can see that the EtBr is being kept in a vial which is covered with the foil because EtBr is light sensitive. And so it should be protected from the light.

And apart from that you also require the running buffer which is the 50 STA and so let us start the casting of the agarose gel and performing the gel electrophoresis. Before you start the preparation of the agarose gel what you have to do is you have to set the tray so that you can able to pour the agarose in to that particular gel in particular tray and then you could cast.

What you have to do is you have to take this tray you have to very carefully clean the trays and with the water as well as, so that it will be free of any kind of contaminations and any kind of because you know that the DNA is very sensitive for the DNA's for the other kind of enzymes. Then what you have to do is you have to keep this tray in this way into this gel caster and with the help of this screws you have to screw the gel tray.

And you have to tight it and as you can see that I am tightening it with a both the screws simultaneously so that it will be completely sealed. And then now once this tray got sealed and you are ready to pour the agarose what you have to do is you have to first put the comb. And when you put the comb what you have to do is you have to ensure that there is a enough gap between the comb as well as the lower end of the comb as well as the tray that space the agarose is going to fill and that is how it is actually going to help you to prepare the well.

So for the typical gel agarose gel electrophoresis what you have to do is you have to first prepare the 1X TAE buffer for a typical kind of small chamber. You required somewhere around 300 to 400 ml buffer and for the gel also you require at least 30 to 40 ml. But so for a safer side what we can do is we can just prepare the 50 ml agarose gel and for a DNA of around 1 KB to around 1 KB you can actually run gel of 0.8% because as the DNA size will lower down you have to keep increasing the size of the agarose percentage.

So for example if you are instructed to explore the you know composes or direct segmentation in those cases the gel band or the DNA band what you are expecting is from 200 base weight to higher molecular weight. So in those cases we normally run the agarose of 2%. But in the most of the cases what we do is we run around 0.8% agarose gel and that is good enough to resolve most of the DNA sizes.

So for preparing a 50 ml 0.8% agarose what you need is you have to just wear the 0.4 grams of the agarose and then you have to boil it into the microwave and then you can prepare. So let us understand how to do that. Now what we have done we have weight the required amount of agarose and then we have prepared the 1X TI buffer and some of the buffer we have poured into the chamber itself and the for preparing the agarose gel what we have done is we have taken almost 70 ml of the buffer because when you boil there will be always a loss of some buffers.

So what we have calculated? We have calculated of 50 ml and now we are keeping 10 to 15 ml of more water because when you boil this you are going to buffer it the water is going to buffer it and that is why you are going to loose some water. So once you are you going to pour the agarose in to the buffer and then you are going to do the boiling. So what you can do is you can use a microwave and you just turn on the microwave and what you have to ensure in the microwave is actually boiling the agarose and melting it.

So in this process what will happen is that the water will going to warm up and the agarose is going to melt. And once the agarose is going to melt it is actually going to swell and it is going to take up the water. And in that process it is actually going to make a viscous material or the viscous jelly like material and that is what is in the agarose gel. So but before because the it is going to boil it is actually can also come out from the from the beaker. So you have to keep opening and keep checking that is something that is not happening.

And in between you have to always mix because the agarose is the made up of sugar. So it also can get charred if it get localized heating. And once your agarose is going to heat up then you can be able to use that and you have to ensure that you boil it very cuddly. So that all the granules what is present in the you know the agarose should be get melted. Let us check whether those agarose got melted or not.

For checking what we do is we have different kinds of the gloves which the gloves like is made up of rubber and these are the gloves what you can use for touching the hot solutions because the our normal gloves what I am wearing right now is not good enough. So we have to heat up little more because still I can see some of the agarose granules. So once this step is over then you have to just take out the agarose what you have to see that is the water is boiling right now ok and vapor are coming out.

So this is actually rough estimate that you are going to loose somewhere around 10 to 15 ml of water in the process of boiling. Now what you have to do is you have to just let it get cooled for sometime and then you have to add the dye. So in this case we are adding the EtBr so once it gets cool down you can just add the 3 microliter of EtBr and remember EtBr is a cast agent dye.

So you have to very careful you always have to wear the gloves and you have to discard these tip very thoroughly or very because you do not want to contaminate the environment. So this tip as well as all the phosphor has to be discarded in a in a way so that it is not to out. So you add the EtBr and then you keep this tip in a secured space so that you will be able to throw this in a trash bag which is meant for collecting the carpentering material.

Now your solution is ready and I can pour this in to the your tray. So you can just pour this into the tray and we have to weight for sometime so that it should get solidify and then you can load the DNA and we can run the agarose. Now you can see that the agarose gel is being solidified and you can see that it is clear. So there is no granules or the granulate or the precipitate material present. And now what we have to do is we have to remove this form and we have to keep in to the chamber and then we it is going to be ready to use.

So before you do that you have to add some amount of buffer into this tray. So what you have to do is you have to add some amount of buffer ok. And then you have to very carefully without making any movement in the longitutional direction you have to vertically uplift the comb into the top chamber like this ok. And that actually is going to prepare the well. So what you can see now is the wells in this particular agarose block ok.

And once you sure that there is no leakage and there is all these wells are good enough and then what you can do is? You can unscrew this tray or the cassette and we are going to remove the comb. So what we are going to do is? We are going to remove this into this chamber and then we are going to remove the comb very carefully you remove the comb ok. And then you are going to fill the chamber with the buffer.

So when you fill it with a full buffer the gel is going to be submerged and that is why this particular type of gel electrophoresis is called as the continuous gel electrophoresis. Now what you can see is that we can see the wells in the gel. So if you see the gel you can see the well actually and now we are going to load the sample into those well and then we are going to connect the chamber to the electrophoresis power supply.

And then it is going to perform the electrophoresis. To prepare the DNA sample what we have done is we have to add actually 10X loading dye. So accordingly you have just take the DNA you add this small amount of the buffer and then you add the loading buffer in such a way so that it going to be 1X and then you are going to load the sample. So in this case we are loading the 25 micro liter of the sample.

So you are going to take the sample into the pipette ok make sure that there is no air bubble. And then you are going to visualize the gel and what you can see actually or sometime if there is a problem in looking at a wells or there is a problem of visibility of the wells because sometime the lower chamber or the lower background is also going to be transparent. In that case you can just put a black paper at that at that place where you have the wells.

And that actually is going to allow you to see the well properly. So let see how to load this so you hold your pipette ok and then you bring your tip next to the well ok and then you load the sample. And what you will see is that the DNA is getting built into the well. So now see here how I am loading the sample ok. So I have taken the 20 microliter and first I will do is I will take my tip into the well and then I will load what you will see is when I am loading I will very carefully slowly I am keeping my tip out ok.

So that the whole well is going to fill with that tip but what you have to remember is that while your tip is or while your tip is inside the well you should not leave your plunger. So that it should

not create any air bubble because it that happen the DNA will come out ok. For now what we will do is we will connect this to the cord so the black will go with the black and red will go with the red. So before doing so we have to put the lid so that there will be no evaporation of the buffer.

So you just connect the black to black and red to red and now we have to turn on the power bank. So in a power bank you have a switch here which actually allows the turning on. Connected the chamber or the electrophoresis chamber with a power supply unit we are set it at 80 volt and now it is running. And what you will see is once the DNA (()) (26:39) dye will reach to the end of the gel. Then we are actually going to remove the gel from the apparatus.

And then, since we have only added the EtBr into the gel. The EtBr is going to run from the negative side to positive side. Whereas the DNA is going to run from the towards the positive side because the DNA is negatively charged and the EtBr is the positively charged. So they will run in a opposite side while they are running the EtBr is going to intercalate within the basis of the DNA. And that is how it is actually going to stay in the gel at the DNA into the present in the gel.

So that after that what you have to do is you have to take out this tray and put it into the gel doc machine and that actually will going to visualize. Then close the thing application nucleic acid ethidium bromide exposure, optimal exposure or we can select manual also then we will acquire the images. Now you can find here this is the DNA ladder this is the PCR amplified product.
(Video Ends: 28:29)

So in the demo which I have explained every aspects related to the agarose gel electrophoresis how to perform the gel electrophoresis? And with this I would like to conclude our lecture here and in a subsequent lecture we are also going to discuss different variants of the electrophoresis gel electrophoresis and how you can be able to utilize them for exploring the answer or for solving the experimental questions thank you.