**Experimental Biotechnology**
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**Lecture – 44**
**Blotting Techniques (Part-2)**

Hello everybody this is Dr. Vishal Trivedi from Department of bioscience and bioengineering IIT Guwahati. And what we were discussing we were discussing about different types of blotting techniques. So in this particular series in our previous lecture we discuss about the northern blotting. This northern blotting is a technique which is been used to detect the RNA what is present in the cell.

So what we have discussed that you have to first isolate the messenger RNA from the cell and then you have to blot that into the nitrocellulose of membrane and then you incubate that with the radioactive probe. And then eventually you are going to do the washing and then ultimately you are going to take the autoradiogram and that is actually going to give you the signal wherever the gene of your interest is present.

So the southern blotting what we are going to discuss today is very close or very similar to what we have discussed for the northern blotting except that the southern blotting is a technique where you are going to detect the gene instead of the messenger RNA. So let us see what are the different steps we have in the southern blotting.

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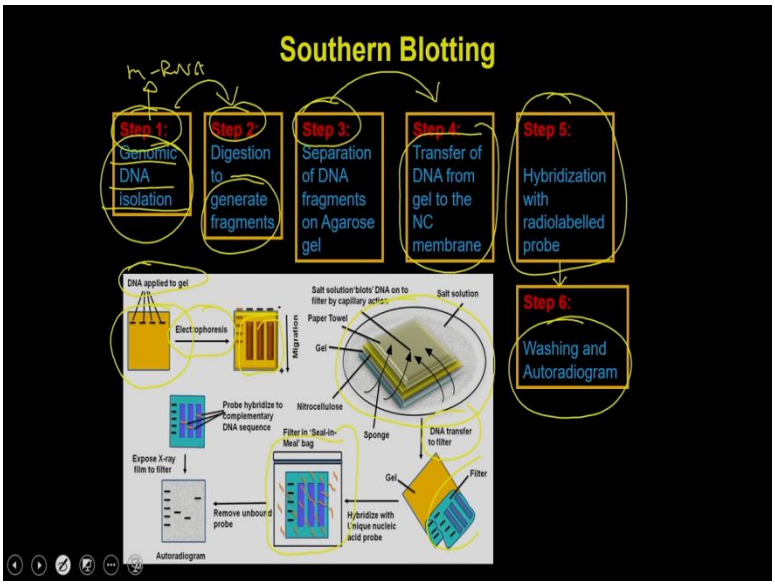


So in the southern blotting you have the 6 steps so in the step 1 you are going to isolate the genomic DNA. Remember when we were doing the northern blotting the step 1 was that you are supposed to isolate the messenger RNA. Whereas in this case since we are more interested with the gene you are going to isolate the genomic DNA from the self. Now once you have done the genomic DNA then in the step 2 you are going to fragment the genomic DNA.

So that it is actually going to give you the different fragments all these different fragments are going to represent one or more genes ideally we make the fragment in such a way so that it is going to represent the single gene. So then the step 2 you are going to do the fragmentation of the genomic DNA. And once you have done the fragmentation of the genomic DNA then in the step 3 you are going to separate the DNA onto agarose gel.

So you are going to apply the DNA into the different wells and then you are going to do the electrophoresis and that is actually going to give you the pattern of the DNA and that is you have to simply use the simple agarose if you remember in the case of the northern blotting we were using the denatured agarose gel or the formamide gels. Now once you are done with the 3 step method then you have to do the transfer of the DNA from the gel to the nitrocellulose membrane.

So in this case also you are going to use the capillary action motion and you are going to use that to transfer the DNA what is present onto the gel to the nitrocellulose membrane. And now in the

step 5 what you are going to do is you are going to incubate the your DNA what has been electro ~~brought~~ blot it onto the membrane with the radio labeled probe and then in the step 6 you are going to do a washing as well as the deployment of the autoradiogram.

So these are the 6 different steps what you have to perform to complete the southern blotting the steps are almost the same as what we have discussed for the northern blotting except that here your target sample is the DNA instead of the messenger RNA.

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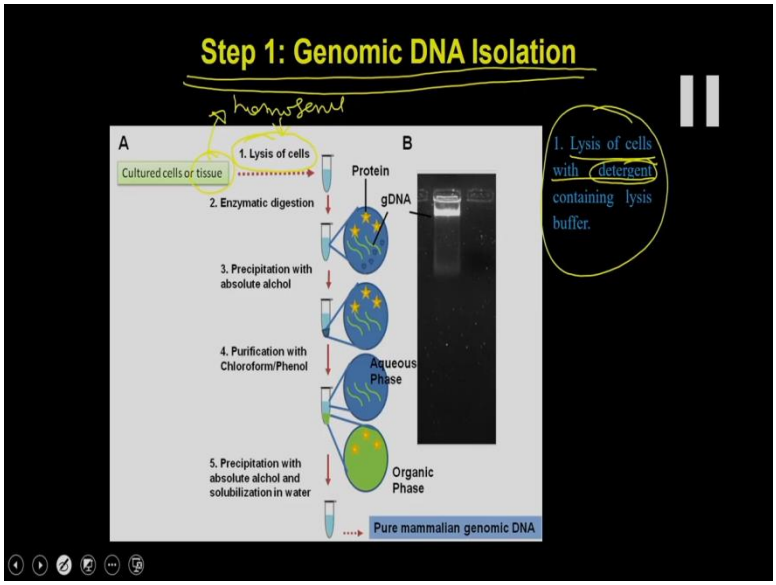


So in the step 1 you have to do the genomic DNA isolations. So in the step 1 when you are want to do a genomic DNA isolations either you will going to do the genomic DNA isolation from the cells or from the tissue irrespective of whether you are doing it from the tissue or the cell. So first method is the first step is going to be that you are going to lysis the cells or in this case if you have a tissue then it is actually going to do the homogenization.

And followed by the homogenization you are actually going to get the individual cells and then in the individual cells has to be incubated with the lysis buffer. So in the step 1 you are going to do the lysis of the cell with the help of the lysis buffer which normally contains the different types of detergents. And these detergents are actually going to disrupt the cell membrane and as a result the cellular content is going to be come out.

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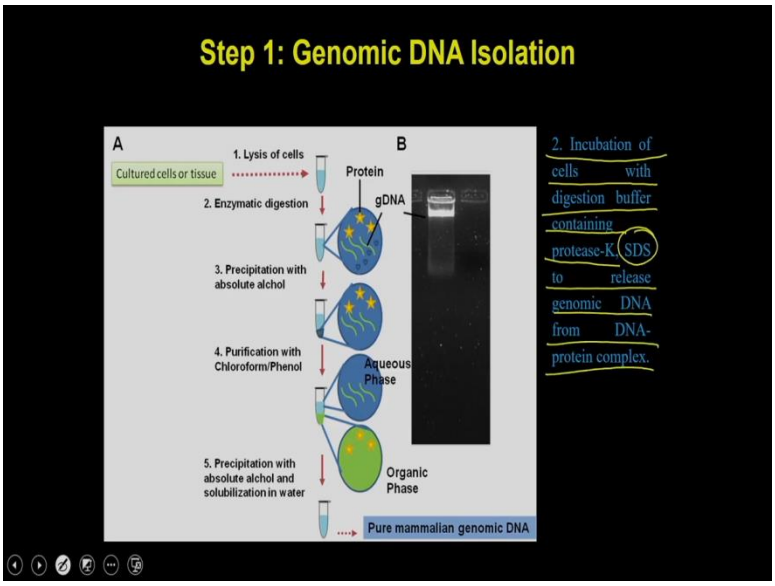


Then in the step 2 you are going to incubate the cells with a digestion buffer. So that digestion buffer normally contains the protease-K, SDS to release the genomic DNA from the DNA protein complex. So if we remember that the genomic DNA and we are mostly talking about the genomic DNA from the eukaryotic cells it is always been packed with the help of the different types of histone proteins.

So you know that the DNA is packed in the in the form of the nucleo formes and all other higher organizations and ultimately it is going to form the chromosomes and all these chromosomes are been present within the nucleus. So the first thing what you have to do is you have to this digest all the proteins what has been associated with the DNA so that the DNA is going to be released and then you can actually you can do subsequent steps to purify the DNA.

So, that you are going to do with the help of the digestion buffers. So you are going to incubate the cell lysate in the presence of the digestion buffer which actually contains the protease-K as well as the SDS. So protease-K is a protease and it is going to start chewing the DNA and start chewing the proteins. And that is actually going to continue for another 16 to 20 hours so that all the proteins are going to be chewed up and then the DNA is going to be free into the solutions.

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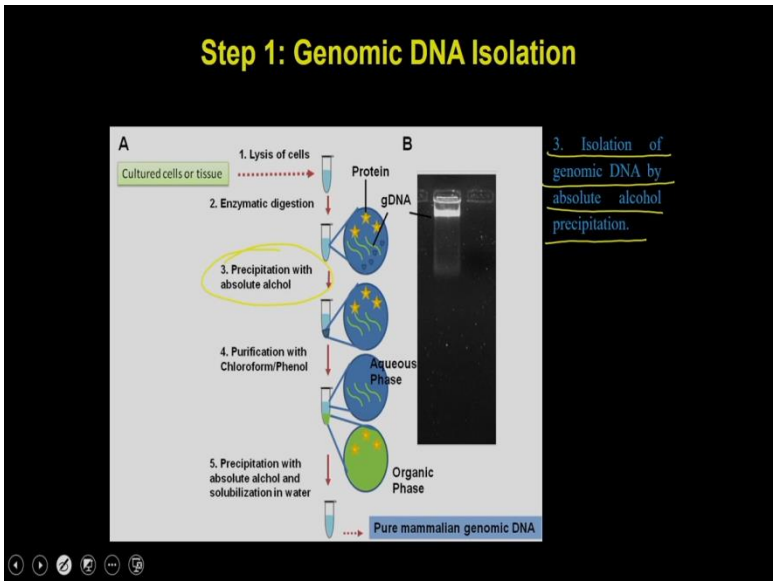


So once the DNA is free then you can do the isolation of genomic DNA simply by the absolute alcohol precipitations. So in the third stage you are going to do the alcohol the precipitation of the genomic DNA with the alcohol or the absolute alcohol. So what will happen is that the DNA is going to be precipitated but it is still the DNA is going to be associated with some of the impurities like the proteins and that has to be removed.

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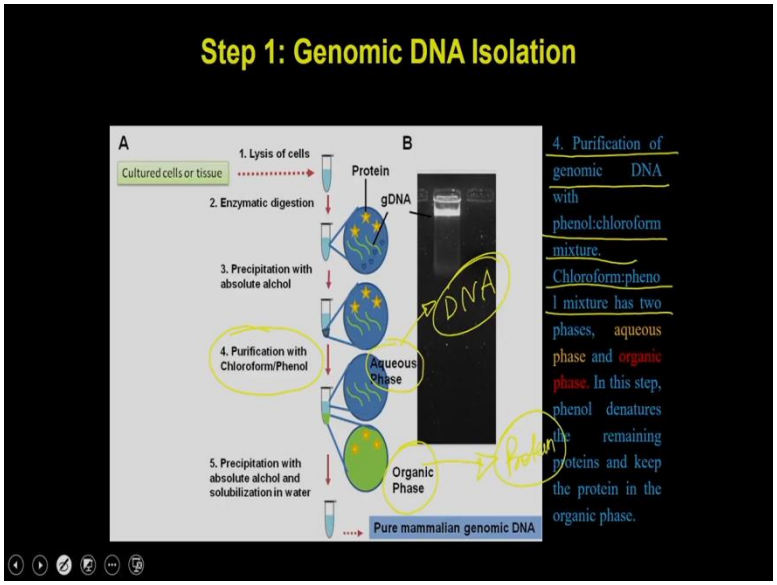


So in the next step you are going to do the purification of the genomic DNA with the help of the phenol chloroform mixture. So the phenol chloroform mixture has the 2 phases. So when you add the phenol chloroform mixture to this precipitated DNA it is actually going to form the 2

layers 1 is called as the aqueous layer and the other one is called as the organic layer. So as you can see here when you are going to add; the chloroform phenol mixture to the precipitated DNA.

It is actually going to form the 2 layer 1 is called as the aqueous phase the other one is called as the organic phase this organic phase is mostly going to have all the protein what is been precipitated by the chloroform phenol mixture and the aqueous phase is going to contain the DNA of your interest which means it is going to contain the genomic DNA. So now what you have to do is you have to remove the aqueous phase without disturbing the organic phase and you can collect that into the separate tubes.

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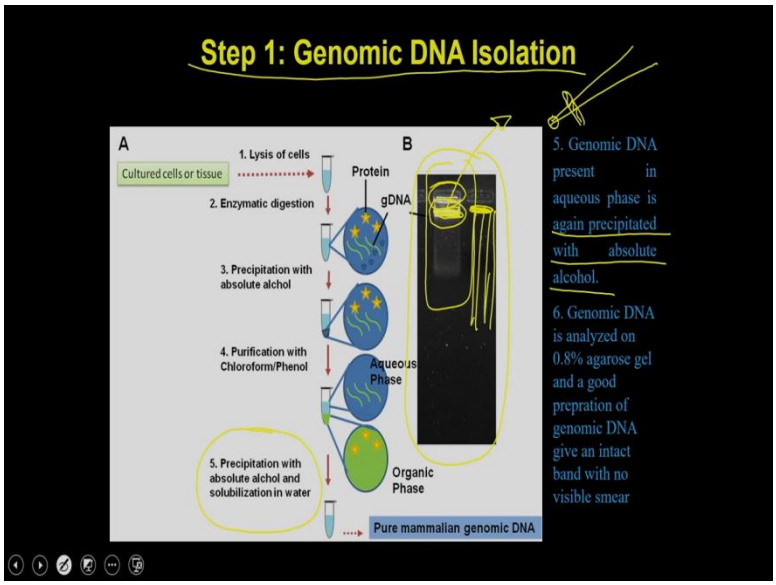


Then in the third step or you are going to precipitate the DNA what is present into the aqueous phase. So DNA so you are going to precipitate the DNA with the help of the absolute alcohol. And at this stage if you are interested to even make the genomic DNA purification more pure then what you can do is you can simply wash this precipitated DNA with 70% alcohol and then again followed by the precipitation with the 100% alcohol.

So at the end you are going to get the genomic DNA and then what you can do is you can simply make a small Ellicott and you can run it on to the agarose gel and that actually should give you the intact band of the genomic DNA. So remember that the genomic DNA is a very very high

molecular weight DNA. So it is actually not going to run very fast it is going to run very close to your well and it is actually going to give you the single band.

If you have any kind of contamination or if you have any kind of problem with the purifications what will happen is that the genomic DNA is going to give you the intact band and then it is actually going to give you a smear at that if smear formation is directly indicate that the genomic DNA prep what you are have been isolated from the cell is not good. Because what you have is you have deep fragmented or the degraded genomic DNA.

Which means if you use this particular type of prep you are going to lose some of the genes or you are going to lose some information And as a result if you do the southern blotting you may not see the signal or it could be having it be show you some of the artifacts. And that is why it is very important that you once you have isolated the genomic DNA you should run the genomic DNA onto the agarose gel and it should give you an intact band.

The other thing what you have to also remember that the genomic DNA is very fragile because it is a very long string. So it is very very fragile compared to the plasmid because plasmid is a very circular DNA. So it can withstand the mechanical sharing. So when you are doing any kind of procedure with the genomic DNA suppose you are allocating the genomic DNA or you are taking out the genomic DNA from 1 append off to another append off or you are preparing the samples all these has to be done with a tip which is been cut in the front.

So if you have seen that the pipette tips what you have seen is that it had a very narrow bore at the end. So if you use the narrow bore and while the genomic DNA is being sucked through this narrow bore it is actually going to damage the DNA and as a result what you are going to see you are going to see the smears. So instead of that what you can do is you can simply cut this tip so that the lower end of the tip is going to have a higher diameter. And if you have the higher diameter the sharing as well as chances of the damage to the genomic DNA is very very less. So that is step 1 we have isolated the genomic DNA.

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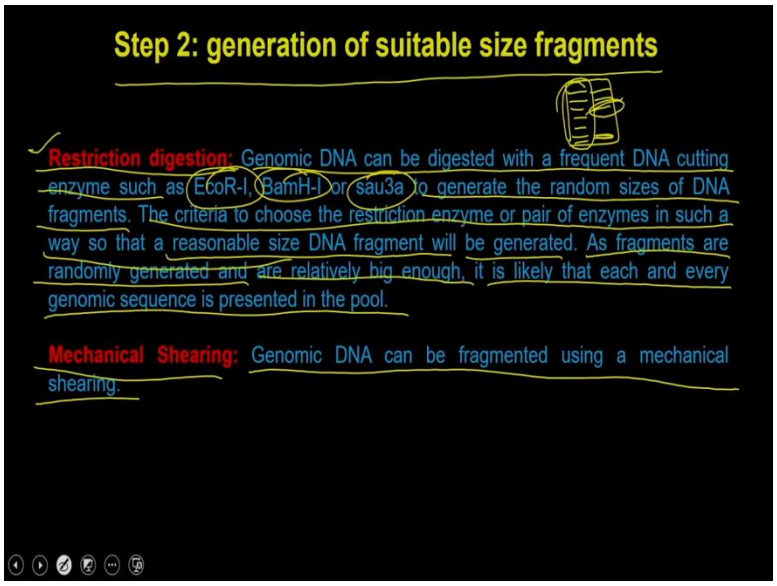


In the step 2 you are going to generate the different types of fragments. So in the step 2 you have to generate the suitable size the fragment what is mean by the suitable size fragment is that you have to generate a size of the fragment which should not be so big that it is actually going to contain 5 or 10 genes or it is not going to be the case that you are generating a fragment which is too low or too less. So it is actually not even containing the 1 gene.

So the visible size fragment means the size of the fragment which only going to be cover 1 gene or 1 or half genes that you are going to do with the restriction digestion. So genomic DNA can be digested with a frequent DNA cutting enzyme for example you can use the EcoR-1, BamH-1 hint-3, sau3a to generate the random sizes of the DNA fragments the criteria to choose the restriction enzymes of pair of enzyme in such a way so that you are going to have the reasonable size DNA fragment will be generated.

As fragments are randomly generated and are relatively big enough it is likely that each and every genomic sequence is represented in the pool which means the purpose of generating the genomic fragment is that each fragment should represent a single gene that is the ideal situation. But in the normal situation what happened is that when you are doing the genomic fragments you have to keep checking the fragments.

So that you know that whether the fragment size is good enough because that you have to optimize when you are doing the digestion with the EcoR-1 or any of these enzymes which have us several cutting sides on to the genomic DNA. So irrespective whether you use any of these or you can use the pair of combinations also like EcoR-1 and BamH-1 or EcoR-1 and hint-3 or the BamH-1 or sau3a only you have to keep a criteria that you should get the bands starting from the end to the top.

So that if you run these fragmented DNA you should see the bands like from the bottom of the gel to the top of the gel it should not be the case that you have some bands here and then you have some bands here which means either you are generating the bigger chunk of the DNA or you are generating the small fragments. So these small fragments even not going to compromise a single gene whereas the bigger fragments are going to have the multiple genes.

And remember at the end of the southern blotting you want to detect your single gene you want to detect your gene of your interest. So if you have the bigger fragments where which contains actually 5 genes then it is actually going to give you the misinterpretation as well as the focusing of the whole sequence is very less because if suppose you have the 3 copy of a genome right and if you suppose you have a 3 copy of a particular gene.

And all these 3 copies are only present on one of the fragment then what will happen is you are going to get the single band whereas in the ideal conditions if you have generated the 3 fragments all these single copy of the gene is going to give you the 3 copies or 3 events that actually is going to tell you that you have the 3 copy of the particular gene present in the genomic DNA.

The other method what you can do also is you can do a mechanical sharing for genomic DNA can be fragmented using a mechanical sharing for example you can simply do the vortexing of the DNA. As I said you know genomic DNA is very long so it is very very susceptible for the mechanical sharing as well as the so if you do have a vortexing of the DNA it is going to get fragmented into the multiple pieces but the most of the people always use an restriction enzyme as a criteria.

Because with the mechanical sharing you have to do a lot of optimization depends on the how long you are going to do the vortexing what would be the power of your vortexing and all that. So that is very difficult to optimize compared to the decision division where you at least assure that restriction enzymes are going to cut. So suppose you have done the setting of the restriction enzymes you are going to keep taking out the aliquot after every 6 hours then you can be able to optimize, how to do that?

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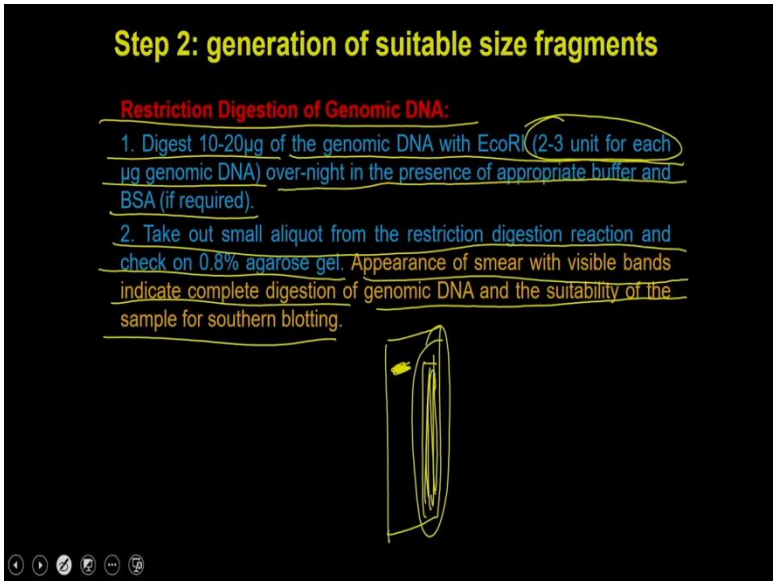


You can do the restriction digestion of the genomic DNA. So what is the recipe you can digest 10 to 20 microgram of the genomic DNA with EcoR-1 and you can take that 2 to 3 units for each microgram of genomic DNA and you can do over night digestion with the appropriate buffer and BSA If required you can take out a small aliguot from the restriction digestion reactions and check it on to the 0.8% agarose appearance of smear with visible band indicate the complete digestion of the genomic DNA and the suitability of the sample for the southern blotting.

So as I said you know when you are checking the genomic DNA the intact genomic DNA is going to give you the single band whereas when you are done with the restriction digestion it is going to give you a single band for the leftover the genomic DNA whereas it is actually going to give you a smear that smear means it is actually mean completely digested and it has a small

small small small fragments. So that is the indication that now your sample is ready for performing the southern blotting. So once you are done with this you can go to the step 3.

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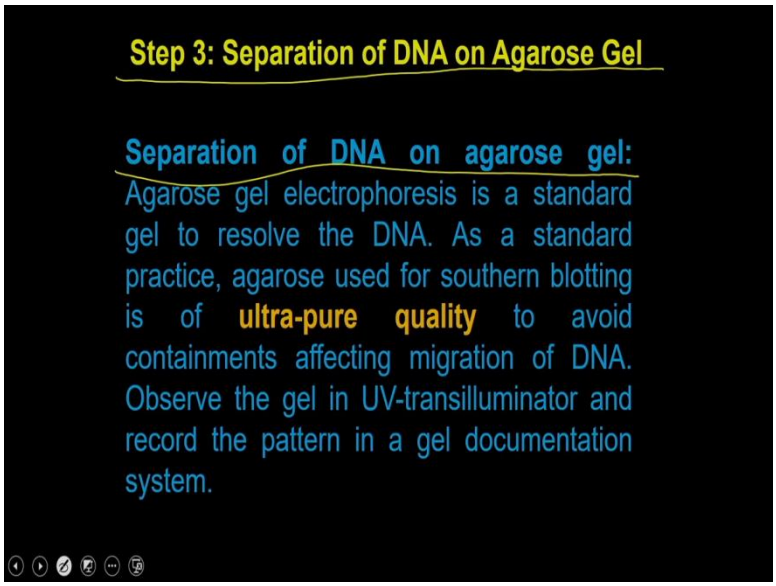


And you can resolve this genomic DNA onto the agarose. So the step 3 is the separation of DNA onto the agarose gel. So separation of the DNA on the agarose gel is a standard agarose gel what you are going to use in your lab except with the exception that the agarose what you are going to use the buffer what you are going to use are have to be very very high quality which means first of all they are supposed to pre of DNA sets and as well as the RNA sets and the agarose what you use should be of a very high quality so that it should give you proper separation of all the DNA fragments.

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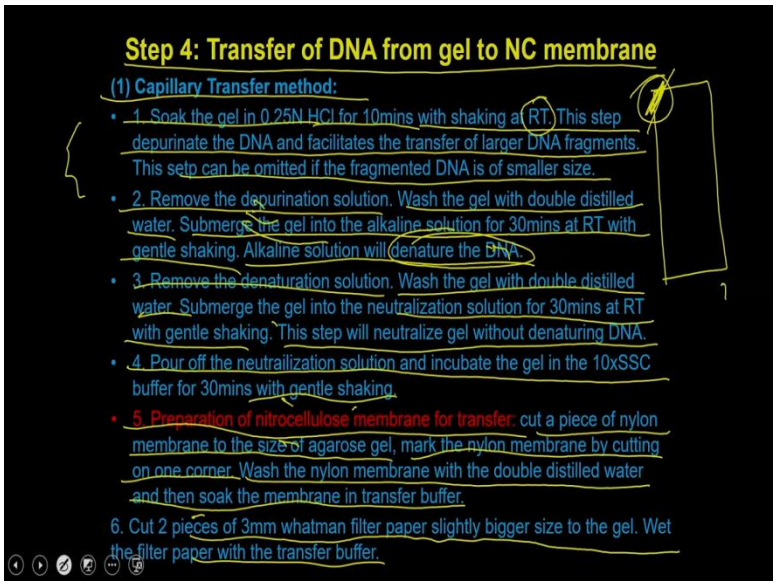


Now once you are done with the separation then the step 4 you can do the transfer of the DNA from the agarose gel to the nitrocellulose membrane can be done in 2 methods one is called as the capillary transfer method the other one is called as the vacuum manifold method. So in the capillary transfer method you can soak the gel in 0.2 normal HCL for 10 minutes with shaking at room temperature.

This is step depurinate the DNA and facilitate the transfer of larger DNA fragment this step can be omitted a fragment is of smaller size then you remove the depurination solution wash the gel with double distilled water submerge the gel into the alkaline solution for 30 minutes at room temperature with the gentle shaking alkaline solution will actually going to denature the DNA remember that we have discussed the same way in when we were doing the northern blotting also that the denaturation of your DNA or the your messenger RNA is important.

So that it should get completely transferred onto the nitrocellulose membrane then you remove the denaturing solution wash the gel with the double distilled water. Submerge much the gel into the neutralization solution for 30 minutes at room temperature with gentle shaking this is step will neutralize the gel without denaturing DNA. Now you pour off the neutralizing solution and incubated the gel in a 10 x SSC buffer for 30 minutes with the gentle shaking.

While you are doing all these procedures you can also prepare the nitrocellulose membrane for transfer. So you can cut a piece of nylon membrane to the size of the agarose gel mark the nylon membrane by cutting on one of the corner. So what you have to do is you have to cut the nylon sheets of the same size at the size of your agarose and then what you can do is you can simply chop off one corner of this agarose gel.

So that you know that this is the size what you have taken on the left corner of the gel that is just an indication that you know to give a kind of orientation because once you take out the membrane it is very difficult to say what is left what is right because so that so you have to keep a mark so that later on you can be able to identify how I have actually placed the membrane for transfer. Wash the nylon membrane with the double distilled water. And then you soak the membrane in transfer buffer. You can cut the 2 pieces of 3mm whatman filter papers slightly bigger to the gel wet the filter with the transfer buffer.

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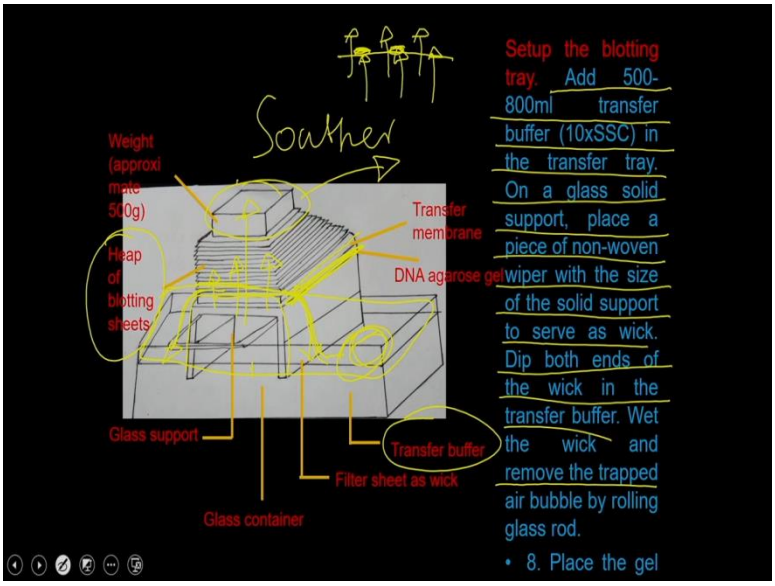


Now you can just put it into the transfer operators. So this is a typical transfer operator what people use for southern blotting. So what you have here is you have a glass tray and which glass tray you have the transfer buffer and then this transfer buffer you have a glass support. So on this glass support what you are supposed to do is your first what you do is you put a sheet of the whatman filter paper which is going to be slightly bigger and it should actually submerge within this buffer.

And then what you are going to do is you are going to keep the agrose gels on top of this cluster you are going to keep the agarose gel and then on top of that you are going to keep the nitrocellulose sheets. And then on top of that you are going to keep the blotting sheets so you going to have like 50, 60 blotting sheets and then on top of that you are going to keep a big weight so that this weight is actually going to press these blotting sheets.

And these since these blotting sheets are dry they will start sucking the buffer or they are start sucking the buffer from the nitrocellulose membrane that they are start going to suck the buffer from the agrose gels. And once the agarose gel is going to be dried up it is actually going to start sucking the buffer from the whatman filter papers and then whatman filter paper are actually been submerged into this tray and which has the transfer buffer.

So what will happen is the transfer buffer is going to be suck by the whatman filter paper they will pass through DD agarose gel and then they pass through to the nitrocellulose membrane and as a result what will happen is there will be a current of water from bottom to top and because they are the current of water and the DNA is soluble in water it is actually going to be transferred and it will start running along with the water.

So as a result what will happen is wherever the DNA is present it will start running towards the upper side. And as a result what will happen is the DNA is going to run towards the nitrocellulose membrane but you know that a nitrocellulose membrane is permeable for the buffer but it is not permeable for the DNA. So what will happen that DNA is going to hit the places wherever it was present into the agarose gel and it is going to be stopped here.

So as a result it is going to be transferred onto the nitrocellulose membrane. Whereas the buffer whatever is buffer is present it is going to come out so that is how it is actually going to continue or it is going to maintain a current of the water from the bottom to top and you can keep changing these blotting sheets. So that once the blotting sheets are going to be wet this current is going to be stopped.

So that time you can just remove some of some of the blotting sheets and you can just put the new blotting sheets like dry blotting sheets and then it will again continue the movement of the water and that is how you continue it for 16 to 24 hours and that is actually good enough to transfer the DNA what is present on to the agarose gel to the nitrocellulose membrane. So in the setup of you add the 500 to 800 level transfer buffer in the transfer tray.

So here you add the 500 to 800 ml of transfer tray on a glass solid support piece of non woven wiper with the size of the solid support to serve as a wick dip both the end of the wick in the transfer buffer right so you can just dip the wick into the transfer so that it will start sucking the liquid and then you are going to put the agarose gel and on top of that you are going to keep the nitrocellulose membrane.

You have to remove air bubble as what we have discussed when we are discussing about the northern blotting and then you are going to go with this setup and that is how it is actually going to transfer the DNA what is present onto the agarose gel onto the nitrocellulose membrane.

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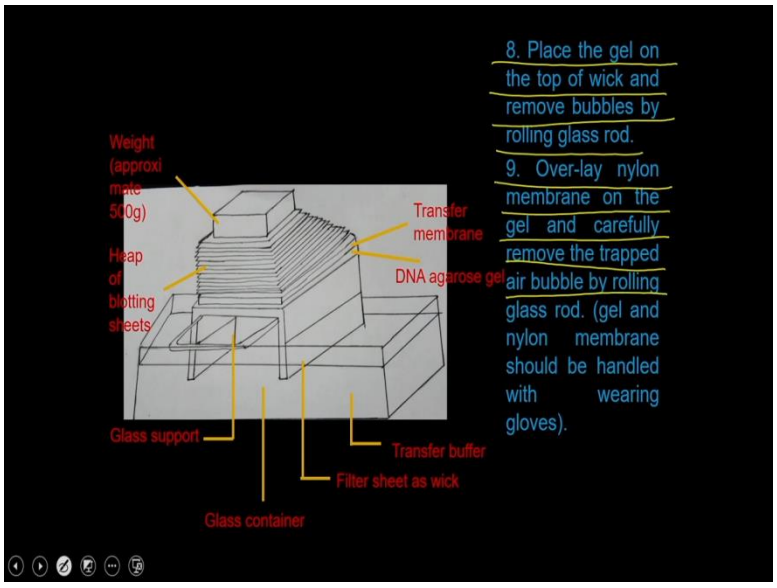


You can place that gel on top of the wick and remove the bubbles by the rolling glass rod. ~~overlay~~ Overlay membrane onto the gel and carefully remove the trapped air bubble by rolling the glass rod.

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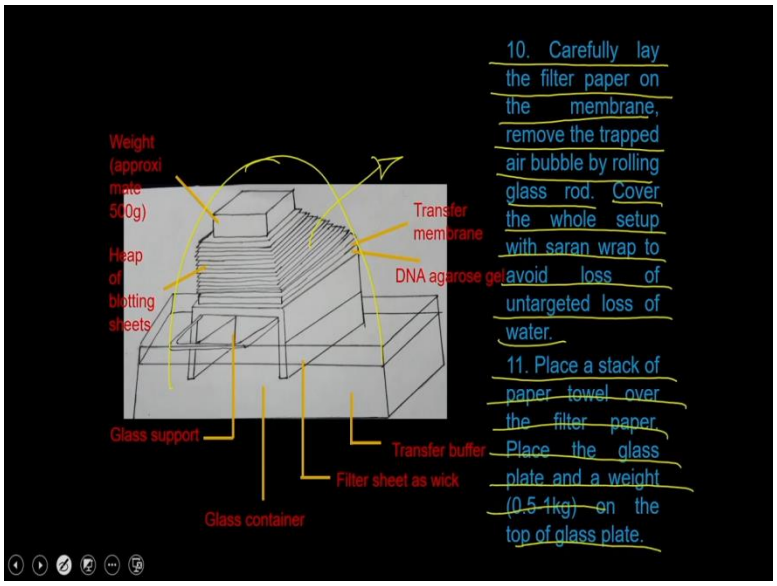


And then after that carefully lay the filter paper onto the membrane remove the trapped air bubbles by rolling the glass rod cover the whole setup with saran wrap to avoid the loss of the untargeted loss of water. So that means you have to cover this whole thing into a saran wrap so that the water should not be evaporate from this system. So it should go up to the top and then you can keep changing this filter paper. So that there will be always a current of water which goes from the bottom to top dip place the stack of the paper towel over the filter paper place the glass plate and a way of 0.5 to 1kg onto the top of glass plates.

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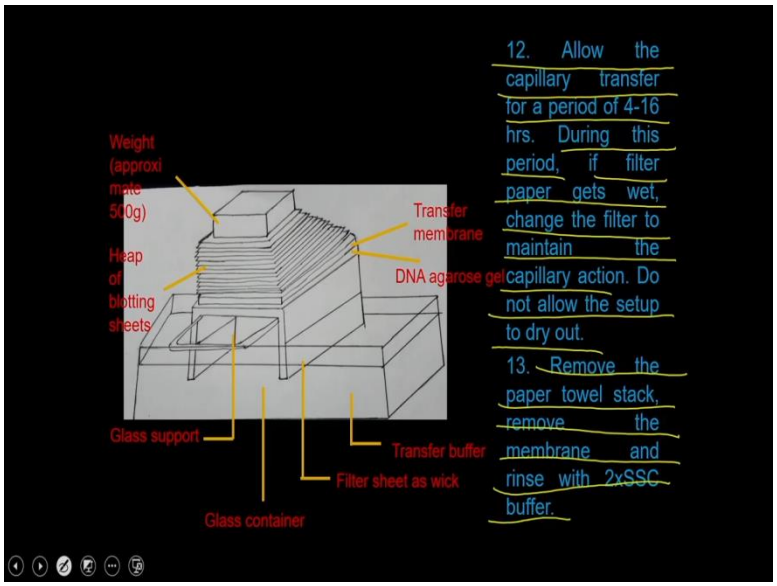


Then you allow the capillary transfer for a period of 4 to 16 hours during this period. If the filter papers get wet change the filters to maintain the capillary actions do not allow the setup to dry

out. Then once the transfer is over you can remove the paper towel stack the remove the membrane and rinse with it the 2xSSC buffer. So once the transfer is over you can remove all the paper towel you can just take out the membrane wash it with the 2xSSC buffers.

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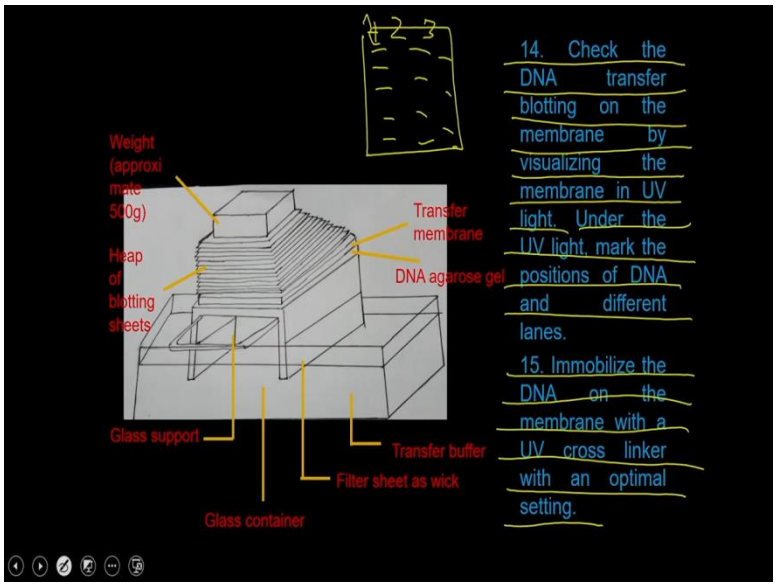


And then you can check the DNA transfer blotted onto the membrane by visualizing the membrane in a UV light. So that actually is going to tell you where whether the DNA is been transferred and under the UV light you can mark the position of the DNA and different lanes which means once you can see the your nitrocellulose membrane and you will see the all the bands then only you can be able to make out that this is the lane.

So what you can do is you can simply write 123 or whatever the identification you want to write for these lanes. And that is how you can be able to identify all the each enemy lane once you are going to develop this nitrocellulose membrane the immobilize you immobilize the DNA onto the membrane with a UV crosslinker within optimal settings. So that is very important because once a DNA is present onto the nitrocellulose membrane.

You are going to expose that DNA to the UV and it is actually going to crosslink the DNA to the nitrocellulose membrane because all the subsequent stebly where you are going to add the probe or you are going to do all these hybridization reactions are actually going to use all the harsh conditions for example the high temperature conditions and all that. So that is should not remove

the DNA from the nitrocellulose membrane. So for that purpose only you are going to do the you know the cross linking UV cross linking.

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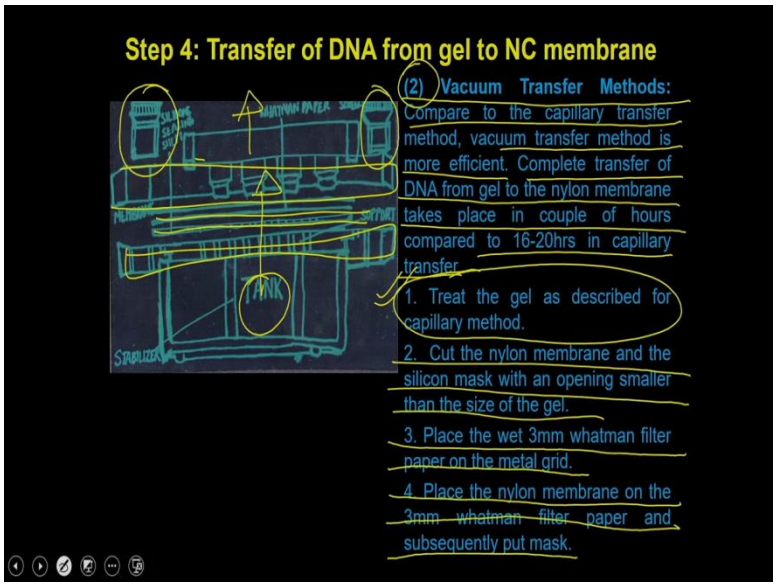


Apart from the capillary actions you can also use the vacuum transfer method. So in a vacuum transfer method instead of using the capillary actions you can simply use the vacuum pumps. So vacuum pumps you are going to place in such a way so that you are going to suck the liquid from the bottom to top and in that process the DNA is going to be you know again going to be the same mechanism DNA is going to be dissolved into the buffer and is going to be run through in the current of the water.

But as the what current of the nitrocellulose membrane is impermeable for the DNA it is going to get immobilized onto the nitrocellulose membrane compared to the water which is going to come out. So the setup is slightly complicated where you have the 2 you know plates and in these 2 plates in between you are going to have the your agarose gel as well as the nitrocellulose membrane and then you are going to have the filter papers or whatman filter papers.

And then you can tie up these 2 plates with the help of the different types of screws. And ultimately you are going to maintain a vacuum and because of that the water what is present in this particular tank is going to run towards this side and the DNA what is present onto the agarose gel is going to be transferred. So there is no difference in terms of the capillary action

transfer or the vacuum transfer method except that the vacuum transfer method is more efficient or it will take less amount of time.

So compared to the capillary transfer method the vacuum transfer method is more efficient new it completes the DNA transfer from the gel to the nylon membrane in a couple of hours compared to the 16 to 24 hour main coupled transfer method. So first of all the initial experiment or initial treatment is remained the same that where you are going to you know treat the gel with depurinater solutions or neutralization solutions and all that.

That remains the same whether it is a vacuum transfer or the capacity transfer then you can cut the nylon membrane and the silicone mask with size of the gel. Then you place the 3mm water filter paper onto the metal grid to place the nylon membrane onto the 3mm whatman filter paper and the subsequently you put the mask.

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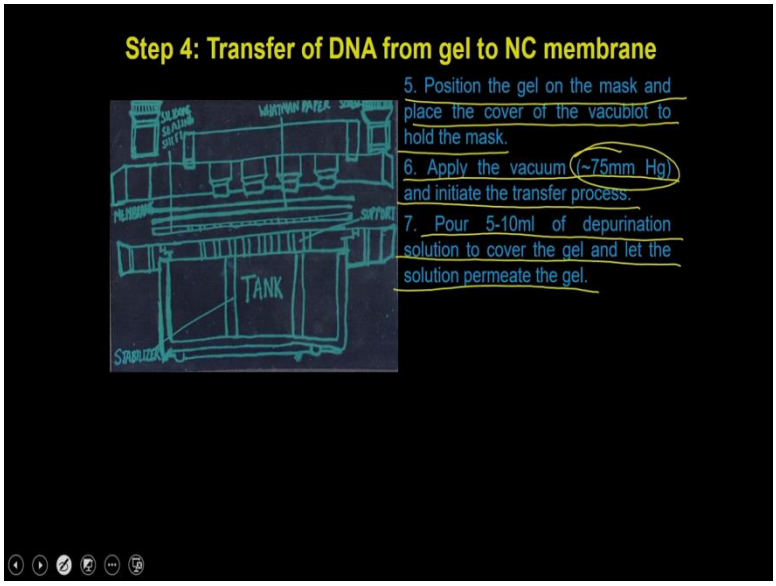


And then you are going to position the gel onto the mask and place the cover onto the vacuum blot to hold the mask then you apply the vacuum so 75 mm mercury pressure and to initiate the transfer process you pour 5 to 10 ml of depurination solution to cover the gel and let the solution permeate the gel.

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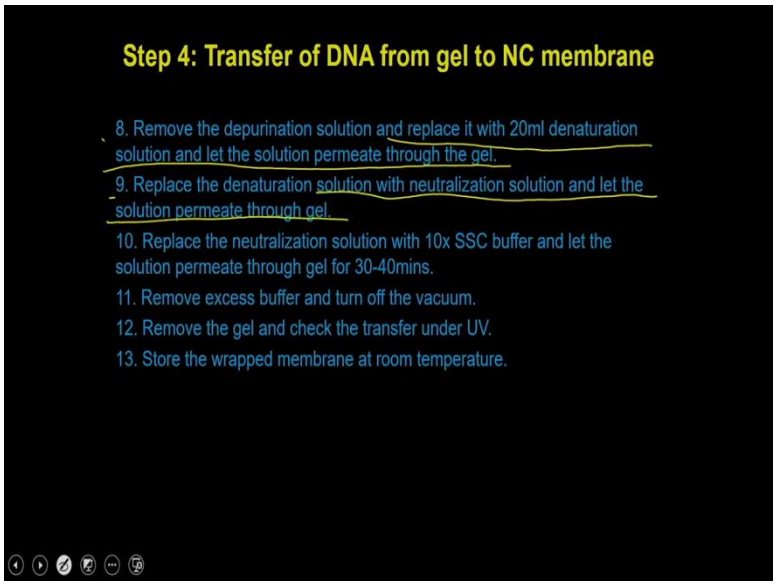


And then you remove the depurination solutions and replace it with that 20 Ml denaturing solution and let the solution permeate to the gel replace the depurinating solution with the neutralization and let the solution permeate through the gel. And by doing so ultimately you are going to see a transfer of the DNA from the gel to the nitrocellulose membrane and then exactly the same. As what we discussed before you can visualize this under the UV light you can mark lanes and you can also cross link the DNA to the nitrocellulose membranes so that it is actually going to withstand the harsh treatment but we are going to do in the next step.

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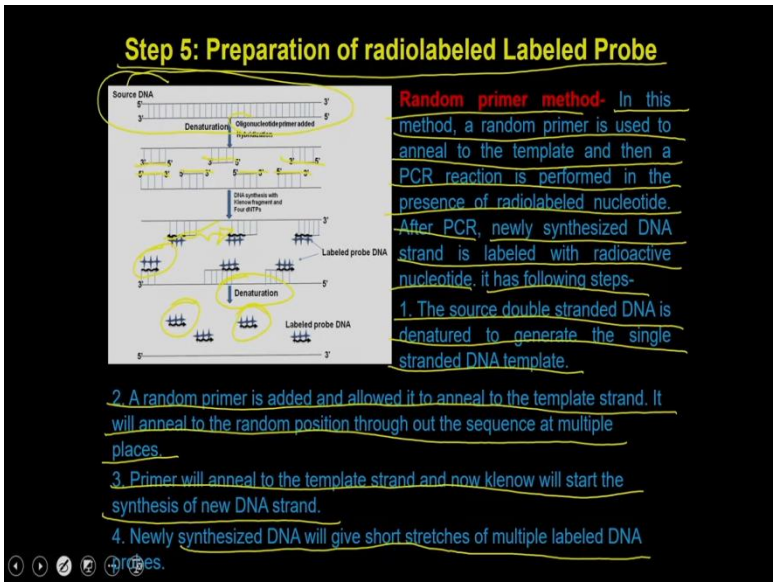


So in the step 5, you are going to prepare the radio labeled probes. So if you remember when we were discussing about the northern blotting that time we have discussed about one of the

methods of radio labeling that is that terminal transference method here we are going to use the different method. So you have the choice whether you use the terminal transfer method or the random primer method.

So in the random primer method in this method a random primer is used to anneal to the template and then a PCR reaction is performed in the presence of the radio labeled nucleotide. After the PCR the newly synthesized DNA strand is labeled with a radioactive nucleotide. So it has following steps. So in this process what you are going to do is first to take the source DNA then what you are going to do is you just take the random oligonucleotide you add that.

And then you are going to do a PCR. So you can imagine that that particular random primer is going to attach to the multiple places within the sense as well as the antisense strands and then it is actually going to generate the DNA. So once it is going to do a DNA synthesis it is actually going to utilize dntps what you are going to add So among the dntps one of the dntps you can add as a radioactive dntps.

So what will happen when the DNA is going to be synthesized it is going to incorporate the radioactive ATP or radioactive GTP. And as a result the DNA what is going to be synthesized is going to be labeled and then what you are going to do you are just going to do a denaturation. And that actually is going to remove these small stretches of the DNA from the template and that is actually going to give you the labeled probe.

The source double standard DNA is denature to generate the single standard DNA template then a random primer is added and allowed to anneal to the template strand it will anneal to the random position out of the sequence at the multiple places then the primer will anneal to the template strand and now klenow will start the synthesis of the new DNA strands the newly synthesized DNA will have the short stretches of the multiple labeled DNA.

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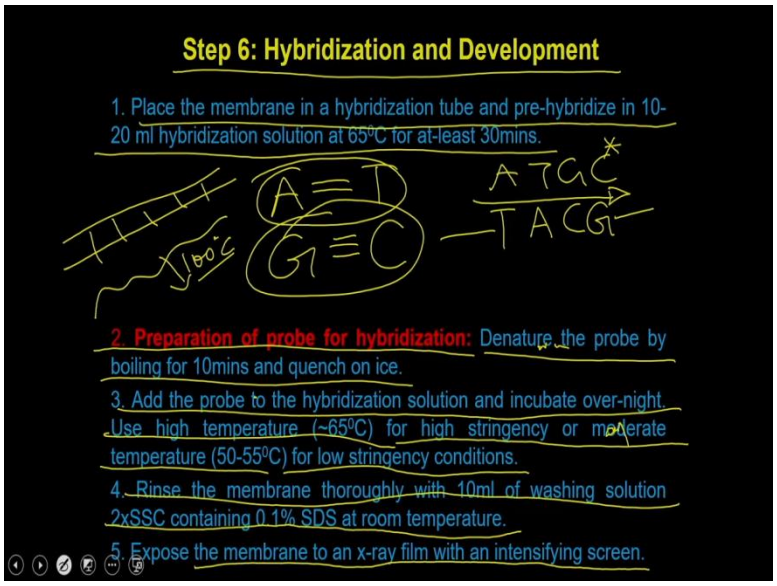


Then the step 6 is going to be the hybridization. So you place the membrane in a hybridization tube and it is going to be in a 10 to 20 ml hybridization solution is 65 degrees Celsius for 30 minutes. So what is mean by hybridization is that you A is going to hybridize with T and G is actually going to hybridize with C. So hybridization is a reaction where the probe is actually going to look for the complementary sequence.

So suppose you have a sequence of ATGC. And it is the C is what is the radio labeled then he got it will happen is it is actually going to bind a sequence which is like this. So if you have a template DNA if you have the target DNA where you have this particular type of sequence present then your probe is going to bind to this particular sequence it is going to be very very specific because the A is always going to make the pair with T.

And G is always going to make the pair whisk C. So for that you have to prepare the probe for hybridizations. So denature the probe by boiling for 10 minutes and then you quench it onto the ice. So remember that the probe is a double standard DNA. And that probe has to be denatured. Because when if you want to have this interaction of the probe with ~~the~~ your gene of interest then you are going to first denature the DNA.

So that you are going to have the single stranded DNA and that single stranded DNA is only going to hybridize with the template. So for that what you are supposed to do you have to

suppose to de heat this at 100 degrees Celsius. So when you hit it at 100 degrees Celsius both the strands of the probe is going to be separated. And then abruptly if you cool down they will not get a chance to you know align back to the it is a complementary sequence.

And that is how you are going to have the single standard DNA at the end at the probe to the hybridization solution and incubate overnight use the highest temperatures like 65 degrees Celsius for high stringency or moderate temperature like 50 to 55 day for a low stringency conditions. So depending on what kind of hybridization you are looking for whether the sequence is very very specific.

You can use the height stringency conditions like you can keep the temperature of 65 degree Celsius or above or you can keep the moderate stringency level like 50 to 55 degree Celsius. You rinse the membrane roughly with 10 ml of washing solutions and 2xSSC at room temperatures expose the membrane to an X-ray film with an intensifying screen. So once you have hybridization is over you can wash the membrane with the washing solution 2xSSC.

And that is going to remove all the non specifically bound ropes. And then you can actually expose that to the x-ray firms with the intensifying screens. And that is actually going to give you a signal wherever the probe is binding and it is going to give you the signal in the form of bands.

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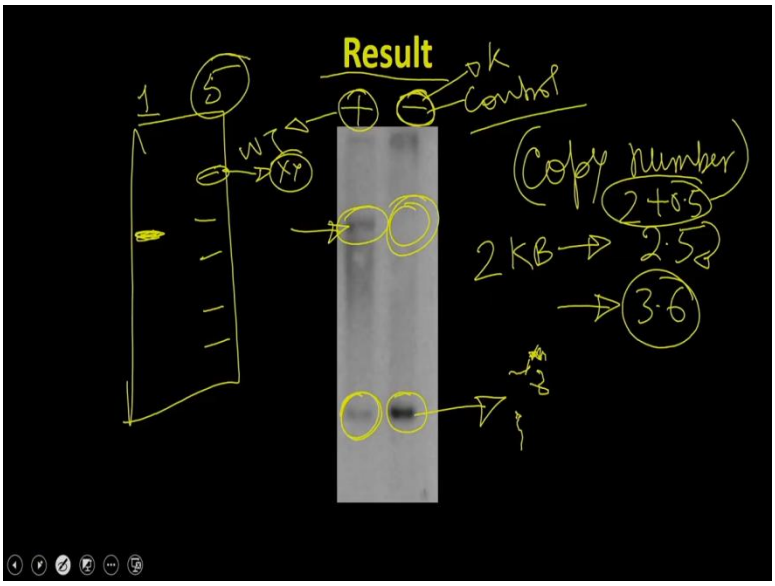


So the; result what you are going to see. So for example this is the Southern blot where what you see here is actually the probe what you have used in the reaction so it is actually been bind to present at the bottom of the gel and then what you see here is actually this is the band of your interest which is completely absent this means this particular is the positive signal and this is the negative signal which means this is a control signal where you have not done anything or this particular gene is absent.

So for example this is a gel which where people were looking for whether a particular knockout is been successful or not. So what they have done is they have taken a wild type and a knockout strain they have done the southern blotting and then they will look for with the help of the probe whether that particular gene was present or not. So this one is the wild type and this is the knockout. So if you this is a knockout it should not have this particular band.

So this is one of the applications of the Southern blotting where you are looking for a particular band appearance or the disappearance. So if you are doing a knockout of particular gene that teen is not going to be present into the genomic DNA. So that is going to be absent when you do into southern blotting. The other thing where the southern blotting is also been used is called as the copy number. So you can also be able to determine the copy number of a particular gene.

So you know that we are actually not going to have a single gene we are also going to have the multiple genes in multiple chromosomes. So for example you know that the humans have the 23 pairs of chromosomes which means the 46 chromosomes but a single gene is not present on the single chromosome. So there are genes which are present in a single copy and a single gene. But there are genes which are of very high demand like most of the genes have the basic metabolic pathways.

Like the enzymes like glycolysis enzyme the Krebs cycle enzymes and all those kinds of enzymes which are participating in the basic reactions are actually going to be having the multiple copies and they will be going to be present onto the multiple chromosomes. So how you can be able to known whether the gene is of a single copy or do the multiple copy. So in that case what you can do is you can simply do a southern blotting and then you run the DNA.

So what will happen is suppose you have the single copy or 5 copy of this particular gene. So in a single copy you are going to see a single band what is going to be present because you only have one gene which is present in the genome but if you have the multiple copies then what will happen for example in this case I am talking about a gene which has the 5 copy so it has copy number is 5. So in what you are going to see is you are going to see 5 bands.

What it is been present in the different fragments because wide you are going to have the different bands because all these genes might be associated with x y or z because it is not that you are going to get the gene off your fragment is fragment what you are generating is only going to have the fragment of your gene size because if that is the case you should get the single band irrespective of whether the copy number is 1 or 5.

But that is not the case when you are generating the random fragments. In some fragments you might have the 100 KB separate in like for example if I have a gene which is off 2 KB then what will happen is some places it is going to have the 2.5 KB some places it is going to be 3.6 KB because the 2 KB + 0.5 is the extra DNA what is present in this one. Similarly here you can have the 1.6 KB of extra DNA.

Because when you generated the fragment you could have the extra gene. So that is how you will expect a multiple bands but that is only possible when you have multiple copies. If you have single copy then you are going to get the single band. So this is the major applications of the Southern blotting either you can look for the presence or absence of particular gene if you are doing a knockout experiment or you can look for the copy numbers.

Like; you can look for the number of copies of that particular gene present in the genome. Apart from this you can also do the purification of the different chromosomes and then you can do a southern blotting of that particular chromosomes to know whether the gene is present on chromosome 5 or 6 or 10 or you know 23. So that is how the people have mapped the different types of gene present on the different chromosomes.

And that is how they have been you know be able to identify the what kind of imitation are happening in this particular gene and what could be the phenotype you expect and all that. So this is all about the blotting technique what we have discussed so far we have discussed about the northern blotting we have discussed about the southern blotting and if you remember when we were discussing about the enological technique that time we have discussed about the western blotting as well.

So with this I would like to conclude my lecture here in our subsequent lecture we are going to start discussing about the different types of experiment we have what we have discussed So far. And in our subsequent module we are going to discuss the experiments or the questions and we are going to solve these questions with the multiple approaches so that you can be able to get the answers of the single questions with the use of the multiple techniques. So with this I would like to conclude my lecture here. Thank you.