Molecular Biology
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Module - 08
Molecular Techniques (Part 1)
Lecture-34 Northern Blotting

Hello everyone. This is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering, IIT, Guwahati. And what we were discussing, we were discussing about the different aspects of the molecular biology in this particular MOOC source. And so far what we have discussed, we have discussed about the cellular structures, we have discussed about the basic structure and functions of the different types of biomolecules.

So we have discussed about the DNA, RNA, we have discussed about the proteins and then lastly we have also very briefly we have discussed about the enzymes. Following that we have also discussed about the central dogma of molecular biology and within the central dogma of molecular biology we have discussed about the replication, transcription and translation. And while we were discussing about all these, we have also discussed about the different variations between the prokaryotic and the eukaryotic system and other kinds of discussions. In the current module, we are discussing about the technical aspects of the molecular biology and how the basic principle of the molecular biology could have allowed to develop the different types of techniques and how these techniques can be exploited to answer the complex biological questions and as well as how they can be utilized for different types of applications.

So in the previous lecture if you recall we have discussed about the southern blotting and southern blotting is being used for detecting the DNA, a particular DNA into the genome. So in that discussion we said that it is a multi-step process where first you have to isolate the genomic DNA followed by the digestion of the genomic DNA so that you are going to have the different fragments and then these fragments are supposed to be separated onto a agarose gel followed by the transfer of these fragments onto the nitrocellulose membrane and then probing with the particular gene fragment which you are interested to identify and then ultimately you are going to develop the blot with the help of the autoradiogram. And the answer what you are going to get at the end of the southern blotting is that it is going to tell you that how many times this particular gene fragment or this particular fragment is been repeated within the genomic DNA and what would be the location of that particular fragment. So now in today's lecture we are going to discuss about the another blotting technique and that is called as the northern blotting. Northern blotting is been used for detecting the particular RNA into the RNA pool.

Mostly it is been done for the messenger RNA so you would like to answer which messenger RNA or which particular type of messenger RNA is been present. Northern blotting can be done to understand the expression of a particular gene. Does not give you the information about the translation but it is going to give you the information about the transcription. So it is understood that once you have the messenger RNA transcribed from the DNA it is going to be translated. So in this particular technique the basic steps remains the same that you are going to isolate the RNA and then you are going to separate the RNA onto the agarose gel and then you are going to transfer that onto the nitrocellulose membrane and then you are going to probe it with the suitable radioactive probe followed by you are going to develop that with the help of the autoradiogram.

So in a northern blotting you are going to have the six steps. So in the step one you are going to isolate the messenger RNA from the so you are going to isolate the total messenger RNA pool from the cell whether it is a prokaryotic cell or the euparyotic cell. And then once you have isolated the messenger RNA then it is all these messenger RNAs are going to be isolated and run onto the agarose onto agarose. So you are going to separate the messenger RNA onto agarose gel so that you are going to get a pattern of the messenger RNA and then these patterns are going to be transferred onto a solid support so that you can be able to process because you know that the agarose is very fragile so you cannot process the messenger RNA fragment what are present onto the agarose gel. Instead you are going to transfer that onto a solid support such as the nitrocellulose membrane and then it is actually going to be easy for you to do it.

And once you transfer that onto the nitrocellulose membrane after that you are going to use the cDNA probe radioactive cDNA probe to probe the particular type of messenger RNA so cDNA will actually go and bind to its complementary sequence or complementary sequence or I will say complementary messenger RNA and that is how you are going to use this and then you are going to do the hybridization and you are going to do the development with the radio available probe and you are going to do a washing and the autoradogram. So in the last step you are going to do the washing and the autoradogram and ultimately what you are going to see is you are going to see the messenger RNA bands onto the onto the x-ray film and the intensity of these bands are actually going to give you the expression level or the transcription of that particular messenger RNA under the different experimental conditions. So what you can see here is that in this particular events you are actually going to have the similar kind of steps what we have discussed in the Southern blotting except that there you are actually the genomic DNA and then you are processing the genomic DNA with the help of the restriction enzymes and so on. Here you do not need to do that because your messenger RNAs are actually going to be individually separated right. So you just have to isolate the pool and then you are going to separate them onto the onto the agarose gel and then

you are going to transfer and all that.

So if you want to perform the Southern blotting you are supposed to have required the following materials. So you require the 1X SSC buffers. So the 1X SSC buffer is having the 5 millimolar sodium citrate pH 8 and the 150 millimolar NaCl. Then you also require the 5X buffers. So 5X XR is actually going to have the 0.

25 normal HCl, Tres-HCl pH 7.5, 0.5% sodium pyrophosphate 1% PVP, 1% BSA, 1% FICOL and 5% DSA. Then you also require the pre hybridization buffer and that will contain the formamide that is 2.5 ml, 5X buffer 1 ml, water 4.

4 ml and the NaCl as 0.292. And then you also require the salmon sperm DNA that is the blocking agent what you are going to use. So you are going to use that for. So this is actually going to be a part of the blocking agent.

Then you also require the Whatman 3mm filters paper and then you also require the labeled RNA or the DNA probes and you also require the UV lamps and the shaking water bath. Apart from that you also require the autoradiogram cassettes and you also require the autoradiogram x-ray films. Now before we discuss about the Southern blotting it is important to recapitulate and the re-review about the RNA structures. So if you see the RNA structure I think we have already discussed that because this is very important to understand then only you will be able to understand the messenger RNA isolations. So what you see here is that it is actually having the three important part.

One is you are going to have the 5 prime cap, the second is you are going to have the 3 prime UTR and the third is you are going to have the coding sequence. So this is the actually the messenger RNA what you are going to get and that is also responsible for the synthesis of the protein. But apart from this you are going to have the 5 prime cap and the 3 prime polyadenylation tail. So this polyadenylation tail is very very handy tool to isolate the total messenger RNA pool from a cell. So messenger RNA has the N-terminal cap structure, the coding sequence and the C-terminal polyA tail.

The nucleotide A form the two hydrogen bonding with the nucleotide T and this pairing is very specific. Exploiting this feature messenger RNA population can be isolated from the RNA pool using the polyT affinity chromatography. Remember that when we were talking about the RNA structures when we discuss about the different types of biomolecules, we discuss about the different types of RNA isolation methods. So we discuss about the trizole method and so on. Apart from that you can also use the affinity chromatography where you can actually be able to exploit this particular part of the messenger RNA and you can actually be able to utilize the fact that the A is actually

going to make the very specific interaction with the T.

So if you have a particular bead which actually contains the you know the T on that right. So if you have a bead which actually contains the T nucleotides then what will happen is that the A will actually go and bind to this and that is how the messenger RNA is going to bind specifically to these beads. Other molecules will not interact with these beads and that is how they can be washed away. So this method is called as the affinity purification of the messenger RNA. So affinity purification of messenger RNA and it can be done with the polyt-agarose beads.

So in a polyt-agarose bead what you are going to do is you are going to have the you are going to have the different steps. So in the first step what you are going to do is you are going to lyse the cells with the help of the lysis buffer. If it is a tissue then you are supposed to homogenize so that you are going to get the single cell suspension and after that you are going to lyse the cells and so that you are going to have the cell lysate. And this cell lysate can be put it into the binding buffer along with the oligo DT beads. So these oligo DT beads are actually going to have a bead on which you are going to have a linker and on this linker you are going to have the T nucleotide attached right.

So you have multiple T nucleotides what is attached to this and this T nucleotides are actually going to bind the A nucleotide very specifically because the A is having the specific base pairing to the T. So this T will interact with the A nucleotides and so it will actually going to capture all the messenger RNA which are actually having the A's right. So that is how you are actually going to have this is actually the messenger RNA and this is going to be the poly poly A tail right and this poly A tail is actually going to interact with the T or the T nucleotide what is present on to the beads. After that you are going to do the wash with the washing buffer and once you wash you are going to remove everything except the these bound messenger RNA. So you can actually be able to discard the supernatant and then you are going to do the elution so you are going to do the elution with the help of the competition and then you can respond this suspend the beads into the elution buffer and you can collect the separate the beads from the eluent with the centrifugation.

So when you separate you are going to have the messenger RNA into the aqueous phase and the beads are going to be get separated and then you can actually be able to get the pure poly A RNA molecule. So you are going to have the RNA species which actually contain the poly A tails actually. So in the steps what you are going to do is release the total RNA by lysis of our containing detergent or by the homogenization in the case of heart tissue then you mix the poly T containing beads with the total RNA species due to the mutual exclusive affinity the messenger RNA bind to the poly T then wash the beads

with the washing buffer to remove the non specific contaminating species and then you elute the messenger RNA from the bead and its purity can be checked on to the poly acrylamide gel. So in the step one you have isolated the messenger RNA pool from the cell after the treatment so you can have the multiple treatment you can have the untreated sample you can have a treated sample and so on. So you can actually be able to collect the messenger RNA from the these species.

Now the second step is that you are going to do the separation of messenger RNA on to the agarose gel. Now there you have a little complications because messenger RNA is a single standard nucleic acid and these single standard nucleotides are actually having the freedom to move around because if you have a double standard DNA like this then you have a restriction because all these are actually been bound to each other and because of that you cannot have you have lot of restrictions. So because of this you can actually may not be get the very good separation of these messenger RNA molecule on to the regular agarose gel instead you are supposed to run the denaturating agarose gels. Now why there is a need to run the denaturating agarose gel because the RNA gels are performed under the denaturating conditions and why it is so because the presence of secondary structure in the RNA allows the faster migration of RNA on to the agarose gel. So what happen is that if you have a single strand it can actually rotate and it can actually you know form the different types of secondary structures and because of these secondary structure it actually becomes very compact structure and once it becomes compact it runs very fast.

So if it is runs very fast it does not allow the agarose molecules you know to exert any kind of retardation forces and because of which the messenger RNA will not get separated instead of getting the individual bands what will happen is that you are going to get a smear because then you are going to get these no separations and that happens because the messenger RNA is going to form the different types of secondary structures they are going to form the stem they are going to form the hairpin loops they are going to form the pseudo knots bulge and they are also going to have the internal loops and multiple loops. The idea is that once you have these kind of secondary structures they are actually going to make the structure very compact and because of that they will run very fast. So as a result of these secondary structure it gives less time for the molecule to interact with the agarose gel and consequently less resolution within the different RNA species. Destruction of the secondary structure in the RNA structure minimizes these efforts and allow the better separation on to the agarose gel. So suppose you can imagine that you have RNA which is forming the secondary structure like this so if I and it is happening because you have the A here and you have a T here so you are actually going to have the you know the hydrogen bonding formed and because of that it may perform the different types of secondary structures like stems hairpin loop pseudo knots

bulge internal loops multiple loops and all that.

So if I break these bonds like if you add some kind of the for example if you add the strong salt so if you add the strong salt you are actually going to break the hydrogen bonding and because of that it becomes a straight line or it will actually going to adopt extended confirmation. Now if you are actually going to maintain the extended confirmation then they are actually going to follow the electrophoretic mobility principle and they are actually going to get separated based on the mass instead of the hydrodynamic surface area. So they are actually going to be get separated from the mass and then what happen is that when you run these you are actually going to get the separate masses and separate bands on to the agarose gel and that is what is required because in the subsequent step you are going to do the transfer you are going to do the hybridization and that is why it is important that you should know that where my probe is interacting whether it is interacting with this particular band or whether it is interacting with this particular band and so on. So it is important that we should actually destroy the secondary structure into the RNA species that kind of secondary structure is not present in the DNA because DNA is double standard. So we have to run the destructive or formamide gels actually.

So RNA sample and agarose gel contains the formaldehyde to denature the secondary structure present in the RNA and prevent the reformation of double standard region into the RNA structures. Now if I want to perform the formamide gels I require the specific materials and equipments and all the kind of things. So what are the materials you require? You require the agarose, you require the 10x MOPS buffer. So this is the composition of the 10x MOPS buffer and you also require the 37% formaldehyde and you require the RNA molecular weight ladders, you require the 0.5 molar sodium acetate, you require the 0.

5 microgram per ml ethidium bromide so that you can actually be able to use that for staining and you require the RNA free water. If you do not have the RNA free water then you can be able to utilize the standard procedure to prepare the RNA free water although RNA free water is readily available from the different types of vendors but if you do not have then you can actually be able to prepare the RNA free water in your lab. Then you also require the formamide and you also require the formaldehyde loading buffer with the composition of the formaldehyde loading buffer is that 1 millimolar EDTA pH 8, 0.5% bromophenol blue, 0.

25% xylene cyanol, 3% glycerol. Then you also require the loading buffer can be filter sterile by passing through a 0.22 micron filter and it can be allocated to the small volume and stored at minus degree. Then you also require the equipments such as you require

the autoclave gloves, you require the 50% water bath, you require the horizontal gel electrophoresis system, power supply, RNA-CD container for staining and destaining, you require a shaker, UV chambers, gel dock and flask for preparing the agarose gel. Now let us see what are the procedure. So in the procedure in the step 1 you are going to do the isolation of messenger RNA that we have already done with the help of the affinity chromatography.

In the step 2 you are going to prepare and cast the denaturating agarose gel. So the preparation of the RNA free water. So this is what I was trying to say that if you do not have the RNA free water then you can be able to prepare the RNA free water in your lab. What you are supposed to do is you dissolve the DEPC or diethyl pyrocarbonate in the deionized distilled water to a final concentration of 0.

1%. Remember that DEPC is a very strong inhibitor of the RNA. So if you add the DEPC it is actually going to kill the RNAs whatever RNAs is present into the water. And what is the source of RNAs in the water? The source of RNAs into the water is the different types of bacteria, small organisms and all that. So that has to be removed otherwise you will isolate the RNA and it is going to be degraded by these RNA species. Then you stir this solution in 12 hours at room temperature and then autoclaved to remove the DEPC.

So if you stir this solution for 12 hours in room temperature the DEPC what is present in this solution is going to kill the RNAs what is what is been present and then you can actually autoclave and autoclaving is actually going to deactivate the DEPC and then you can store this at room temperature. Now the third step is that step is the casting of the agarose gel. So in a flask add 1 gram of agarose to 75% RNAs free water. Heat the solution to melt the agarose and observe the disappearance of the agarose flecks. Then you allow the solution to cool down up to the 55 degree Celsius inside of fuming hood add 10 ml of 10X MOF buffer and 18 ml of 37 formaldehyde.

Remember that formaldehyde is a very corrosive liquid. So you should be very careful because it may actually cause the burning and other kind of accidents. Then we set up the casting tray with the comb and pour the gel into the fuming hood. That is why we remember that we are using the fuming hood because it is actually going to form the different very corrosive fumes and they may actually cause the you know irritant to the eyes and other kinds of face actually. So you once you add the 37% formaldehyde and then you are going to pour this solution into the casting tray.

I already written that formaldehyde is very toxic and can be easily absorbed through skin. So you wear the gloves and mask. Then you prepare the sample RNA sample for

running. So take the RNA sample and make up the volume to 6.

5 microlitre with the appropriate quantity. To each sample add 2.5 microlitre of 10X MOF buffer, 4.5 microlitre of 37 formaldehyde and 11.5 microlitre of formamide. Then you mix by vortexing and briefly spin to collect the sample at the bottom.

Then inside the hood add 5 microlitre of RNA loading buffer, mix by vortexing and briefly spin to collect the sample at the bottom. This is required so that you should not waste the sample actually. Then in the step 4 you are going to load the RNA. So fill the agarose denaturing gel prepared with the 10X MOF running buffers, load the RNA sample onto the lid.

So this is what it is showing right. You are going to load it with the help of the RNase free, DNase-RNase free tips right. And then you are going to run it for agarose gel. So in the step 5 you are going to run the denaturing agarose gel. So place the lid onto the buffer chamber and perform the electrophoresis at 5 volt per centimeter until the dye front reaches the two-third length of the gel. And then in the step 6 you are going to stain the agarose gel.

So in a RNA free container agarose gel is dipped into the 0.5 molar sodium acetate for 40 minutes at room temperature. You drain off the solution and dip the solution in 0.5 molar ammonium acetate containing 0.

5 microgram per ml ethidium bromide. Then incubate on room temperature for 30 to 40 minutes if required and the stain is too intense it can be destained by the 0.5 molar ammonium acetate for another 60 minutes. So initially you are going to dip the agarose gel into the 0.5 molar sodium ammonium acetate for 40 minutes at room temperature. Then you are actually going to stain it with the help of the 0.

5 molar ammonium acetate containing 0.5 microgram per ml ethidium bromide. So this is actually going to stain the RNA and then if the stain is too high then you can actually be able to use the destaining solution which is 0.5 molar ammonium acetate and if required the stain is too intense it can be destained by the 0.5 molar ammonium acetate for the 1 hour. Then transfer the gel to the UV chamber and capture the image with the gel documentation system and what you are going to see here is that you are going to if you run it along with the ladder that what you are going to see is the individual bands of the RNA species and you are also going to see the RNA what is being separated from your sample.

So you are going to have the two different types of samples and you can be able to get

separated. So this could be control sample, this could be treated sample. So that is how you are going to do. Now once you see that there is a very nice separation of the RNA then you can actually go move on to the next step of the northern blotting. So in the next step you are going to transfer the gel to the NC membrane and this step is exactly the same as what we have discussed in the southern blotting.

So place the gel in a RNA free petty dish rinse 4 times with the sufficient DNA's water soak the gel for 20 minutes in 0.05 normal NAOH and keep it in a enough 20X SSC for 30 minutes then you place the two pieces of what meant 1 mm paper of the size of gel on to the glass plate and wet it with the 20X SSC. Place the gel on the filter paper and remove any air bubble trapped between the gel and the filter paper cover slides with the plastic wrap. Once the membrane on the gel avoiding the air bubble flood the surface of the membrane with the 20X SSC and you place 4 sheet of what meant 3 mm filter paper of the same size on top of the membrane then you place a heap of water paper towel on top of the filter and add on the approximately 500 grams weight and leave for overnight. So what we are describing here is actually the capillary action transfer site that we have already discussed in the DNA in the southern blotting.

And then this is what it is going to be the procedure right. Now once the step 3 is done your RNA is going to be transferred on to the nitrocellulose membrane then you are going to have the next step. The next step is preparation of the radiolabelled probe or radiolabelled DNA right radiolabelled cDNA probe right. So preparation of probe DNA so preparation of the complementary DNA or cDNA multiple approaches are been developed to prepare the complementary DNA from the isolated messenger RNA in all approaches the 3 steps are performed. You are going to have the first strand synthesis with the help of reverse transcriptase then you are going to have the removal of RNA template and then you are going to have the synthesis of the second strand synthesis. So there are multiple method what people have used in previous when we were discussing about southern blotting we discussed about the random primer method and now we are going to discuss the another method how you can be able to prepare the cDNA probe corresponding to a messenger RNA and then you how you can be able to use that.

So this method what we are using is Gubber Hoffman method in which you are going to use the standard procedure first you are going to do a first strand synthesis with the help of reverse transcriptase followed by the removal of the RNA template and followed by the synthesis of the second strand. So in the Gubber Hoffman method in this approach after the first strand synthesis using the oligo dt primer in the presence of the reverse transcriptase and dntp's DNA RNA hybrid is treated with the RNA edge to produce the nick at multiple side then the RNA DNA polymerase is used to perform the DNA synthesis using the multiple fragment of the RNA as a primer to synthesize the new

DNA strand and this method produces the blunt and duplex DNA. So what will happen is that suppose this is the messenger RNA which you want to use for preparing the cDNA probe. So what you are going to do is you are going to add the poly dt primers and using these primers the reverse transcriptase is going to make the RNA.

So this is going to be RNA and this is going to be DNA. This means at the end of the reverse transcriptase reactions you are going to have the RNA and DNA as the hybrid form. So you are going to have the hybrid species and these hybrid species are actually been targeted by the one RNA which is called as RNA's edge. So RNA's edge is very specific for those RNA's which are been part of this hybrid species. So when you treat this with the RNA's edge and it is actually going to make the cleavage. So it is going to cleave this fragment into the multiple places and because of that these small fragments are going to be attached but they are actually going to have the nicks.

So these are the nicks what you are going to be present and now what you are going to do is you are going to add the DNA polymerase. So what DNA polymerase is going to do remember that we have already discussed about the replication. So what DNA polymerase is going to do is it is going to utilize this as a primer and then it is actually going to start synthesizing the whole DNA and as a result you are going to get the cDNA of this corresponding messenger RNA. So it is going to have the double standard DNA. And then the second step is the radio labeling of the cDNA probe what you have prepared.

For that you can actually use the terminal transferase method. So in this method a terminal transferase enzyme will be labeled the probe at the end to the last nucleotide of the probe and the probe is incubated with the labeled nucleotide and terminal transferase enzyme will add the labeled nucleotide at the end. A partial purification with gel filtration column will give you the labeled primers. So then this what we have is you have this is the double standard cDNA what you have prepared and then what you are going to do is you are going to treat this with the alpha exonuclease and that is actually going to create the nicks and then what you are going to use you are going to use the terminal transferase with the help of the radioactive nucleotides and then what will happen is that you are actually going to add the nucleotide on both the sides and that is how you are going to have the radio labeled probe and now you can actually be able to use these double standard DNA. So you are going to actually this you know you are going to denature this and as a result you are going to have the two strands you are going to have two strands from this and that is how you when we are able to use this as a probe for subsequent steps of hybridizations.

And in the step 5 you are going to have the hybridization. So rinse the membrane in 5x

SSC then place it on the sheet of the Whatman filter paper heat it for 2 minutes in full power in a microwave oven and further cross linked with the UV rays for a appropriate time. So you can use the UV rays of 250 nanometer wavelength. So why it is important that you are going to do the cross linking because you do not want the disturbance of that appearance. Then for pre hybridization of the membrane wet it into the 5x SSC next place it in a pre hybridization solution kept in a tube and incubate in an incubator with the rotation at 60 degree without probe. Remember that all most of these steps we already discussed that you are actually requiring a hybridization chamber and hybridization chamber has a tube like this and in this tube this tube will go into a hybridization chamber.

And then in this you can actually be able to place your membrane and then you can just fill this solution and put it into the hybridization chamber and that will maintain a temperature of 60 degree Celsius and then it is actually going to keep rotating. And because of that the hybridization buffers and everything will keep interacting with the membrane. So for hybridization denature a double standard probe by heating it into a water bath for 10 minutes at 100 degree Celsius and then by quick chilling. Then pipetting the desired volume of the probe into the hybridization tube and incubate overnight with rotation at 42 degree Celsius for DNA probe.

The membrane was washed 5 minutes in 1x SSC 0.1% SDS at room temperature and washed for 5 minutes at 68 degree Celsius in 0.5% SSC and 0.1% SDS. Air dry the membrane exposed to the sensitive x-ray film at minus 80 degree Celsius with intensify the screen prepare an autoradiogram after developing a film.

Now the next step is the autoradiogram. So you are going to do the air dry the membrane exposed to the sensitive x-ray film at minus 80 degree Celsius with intensify the screen prepare an autoradiogram after developing a film. So why we do at low temperature because we just do not want the spreading of the radio activity. You just want to keep the signal very intensified so that when you are doing this actually it is going to give you a band instead of giving you a blob. If you do it on room temperature or any other temperature then the radioactive will scatter actually and it is going to give you the blob rather than a band actually. So just to reduce the activity and that is why we are doing this like 48 hours or 72 hours exposure because you are increasing the exposure time but you are reducing the activity of those beta particle meeting atoms so that the band what you are going to see will be very sharp.

Now the intensity of the band on the developing pale which is a measure of the specific messenger RNA in the sample can be compared in the different samples. So what it is actually going to tell you it is actually going to tell you the expression of a gene or I will

say transcriptional activity. Transcriptional activity of the gene or the target gene whatever you are probing. If you compare this if you have two samples suppose you have a control sample you have a treated sample and suppose this is the band what you get from the control sample and this is the band what you get in the treated sample or vice versa then it will going to say you that there is a enhanced production of the messenger RNA or of that particular gene under this particular treatment. That indirectly mean that it is actually enhancing the expression of the gene although you have to verify that with the help of the other techniques such as western blotting and other kind of techniques to verify that whether the transcriptional activity what you observe for that particular gene is also been reflected in terms of the translation or not.

Because sometime it happens that you have the RNA species but it may not be get completely been translated. So sometime you may see a increase in transcriptional activity but that may not be into the translation. So that kind of variation could be possible and that is why you are supposed to do the further verification with the help of a translation into the translation with the help of another technique which is called as the western blotting. So this is what we have discussed so far we have discussed about southern blotting we have discussed about the northern blotting and as you recall while we were discussing about the northern blotting it is actually going to tell you about the transcriptional activity of a gene. But if you want to confirm whether that transcriptional activity is also been resulted into the translation of that particular gene fragment and giving the protein or not then you are supposed to do the next blotting technique and that first blotting technique is called as the western blotting and that we are going to discuss in our subsequent lecture.

So in our today's lecture what we have discussed we have discussed about the northern blotting we have discussed about the different steps of the northern blotting and how you can be able to separate the messenger RNA how you can be able to isolate the messenger RNA with the help of the affinity chromatography how you can be able to separate the messenger RNA onto the denaturating formamide gels.