Molecular Biology
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Module - 08
Molecular Techniques (Part 1)
Lecture-33 Southern Blotting

Hello everyone, this is Dr. Vishal Tewedi from Department of Biosciences and Bioengineering IIT, Guwahati and what we were discussing we were discussing about the different aspects of the of the molecules in the course molecular biology. So, so far what we have discussed we have discussed about the cellular structures in the initial modules then followed by we have discussed about the different types of biomolecules. So, we have discussed about the DNA, RNA, proteins and then we also discussed about the enzymes and following that we have also discussed about the different types of cellular processes. So, we have discussed about the central dogma of molecular biology and then we have seen the detailed discussion about the different types of processes what is occurring within the central dogma of molecular biology.

So, we have discussed about the replications, transcription and translations. And with this kind of discussion we understood that the molecular biology has developed the different types of basic principles on which you can be able to develop the different types of techniques and these techniques can be used even to explore the aspects of the molecular biology or they can also be used for different types of applications. So, in today's lecture we are going to discuss about the some of the important molecular techniques and how these molecular techniques can be used for answering some of the basic questions related to the cellular physiology or other aspects related to the molecular biology. So, when we talk about the molecular biology, the molecular biology is all about the molecules right and these molecules interact due to a specific requirements.

I am sure you can recall when we were discussing about the proteins. We said that the protein is interacting with the substrates and protein is interacting with the other proteins due to the recognition of the complementary regions due to the specific three dimensional requirements, geometric constraints, stereo specificity and other kinds of requirements. And because of these kind of specific requirements the molecular biology or the interaction between the molecules can lead to the development of different types of techniques. So, when we talk about molecular technique, molecular technique can be of many types ok. Molecular technique where you are actually going to amplify a particular DNA or other kinds of regions.

So, it can be a amplification technique. Within this amplification technique you can

have the different types of amplification techniques such as the polymerase chain reactions, you can have the real time PCR or you can also have the rd PCR ok. So, this is I will write real time PCR and this is the rd PCR or reverse transcriptase PCR. And all of these amplification techniques are having the utility in terms of the amplifying a specific region of the genome. So, that you can be able to get the genome fragment and these fragments can be used for multiple for the downstream cloning applications and all that.

So, that anyway we are going to discuss in detail when we are going to take up the molecular cloning. Then apart from that you can also have the different types of the blotting techniques. So, within the blotting techniques you can have the techniques which is called as southern blotting. You can have the northern blotting and you can also have the western blotting and you can also have the eastern blotting ok. So, most of these blotting techniques where you are actually going to use the hybridization techniques and you are actually going to blot the molecule of your interest onto a support system and then you are going to detect that with the help of the different types of the analytical reagents.

For example, in the case of southern blotting you are going to use as the DNA as a probe and northern blotting also you are going to use the DNA as a probe and then western blotting you are going to use the different types of antibodies and so on. Apart from that you can also have the molecular techniques like where you are actually going to have the sequencing technique. So, you can actually be able to do the sequencing of the DNA and protein also. So, you can have the sequencing of the DNA or you can also have the sequencing of the proteins and that can be useful in terms of the identification of the that particular region or accuracy of the cloning and other kinds of things. And there are many techniques for example, you can have the RFLP, you can have the RFLP you know you can have the small molecular polymorphisms and all those kind of things.

So, the so molecular technique is a very vast subject and there are there are there could be a MOOCs course on that actually. So, what we are going to do is within this molecular technique topic what we are discussing we are going to discuss about the amplification techniques because they also going to have the downstream applications into the molecular cloning and then we are also going to discuss about the blotting techniques. So, in current module we are discussing about we are going to discuss about the blotting techniques. So, within the blotting technique we are going to discuss about Northern blotting, Northern blotting and Western blotting. And in the next module we are going to discuss about the amplification techniques such as the PCR, RT-PCR and real-time PCR.

And all of these techniques will help you to understand the application part that will also we are going to discuss at the end of this particular course. So, as far as the blotting technique is concerned the blotting techniques are been categorized based on the target molecules. So, for example, as I said to know that we have the four different types of blotting technique we can have the Southern blotting, we can have the Northern blotting, we can have the Western blotting and we can also have the Eastern blotting. And the Southern blotting you are going to use the DNA as the target molecule. So, you are going to identify the DNA if DNA is a molecule then it is going to be called as the Southern blotting.

If you are going to detect the RNA then it is going to be called as the Northern blotting and if it is the protein then you are it is going to be called as the Western blotting and if it is a carbohydrate then it is going to be called as the Eastern blotting. Although Eastern blotting is not very popular, but since they were having the Southern, Northern and Western blotting the people have whatever the technique is using for detecting the carbohydrate onto the nitrocellulose membrane or other kinds of support system that is been called as the Eastern blotting. So, Eastern blotting is not very popular and that is why we are not discussing about Eastern blotting in this particular course. So, we will start with the Southern blotting then we are going to discuss about the Northern blotting, then we are going to discuss about the Western blotting and then lastly and so when we discuss about these techniques we are going to talk about the basic principle of the technique and then we are also going to talk about the underlining mechanisms and we are going to talk about how to perform this particular technique. So, let us start with the Southern blotting.

So, Southern blotting is a six step blotting technique and remember that in all the blotting techniques you are going to blot the material onto a support system such as the nitrocellulose membrane or the PVDF membranes and then you are going to perform the reactions on that particular support system. So, the first step is for example, so Northern blotting is for DNA and the purpose of Northern blotting is to detect a fragment of DNA into a particular genome or a genomic library. So, the first step is that you are going to have the genomic DNA of that particular organisms and you are going to isolate the genomic DNA from that organism. Then in the second step you are actually going to do the digest the genome because genome is very long. So, you cannot analyze that where the actually the probe is binding.

So, what you can do is you can digest the genome so that you are going to have the different types of fragments. And then in the third step you are actually going to use you are going to separate these DNA fragments onto a suitable agarose gel. So, you are going to use that sub suitable agarose percentage of the agarose gel and then you are

actually going to analyze that and that is actually going to give you the separate fragments. Then in the step 4 these DNA fragments are going to be transferred onto the nitrocellulose membrane with the help of the different types of transfer techniques. You can use the manual method or you can use the high vacuum method as well.

Once the it is been done then you are actually going to prepare a probe and you are going to prepare a radio labeled probe. Probe is a small fragment of DNA that you are actually going to use to detect this specific DNA into the genomic DNA. So, you are going to use the hybridization with the radio labeled probe and then in the step 7 you are actually going to do a washing and then you are going to do the autoradiogram. And at the end of the autoradiogram you are actually going to get the result and that result can be interpreted in terms of how many different DNA fragment are present into the genome and how many different copies. So, basically the with the help of the southern blotting you can be able to say whether this particular DNA fragment is present in that particular genome or not.

And the second information what you can also extract is what will be the copy number how many how many fragments or how many different gene of that particular fragment is present in that particular genome. So, it is actually going to tell you the location of that DNA fragment and it also going to tell you the information about the copy number. So, we have so far we have not discussed about the copy number, but I think we will going to discuss when there will be a molecular cloning and other kinds of events. So, the first step is that you are actually going to start with the genomic DNA isolations. So, genomic DNA isolation is also a step 1 in the genomic DNA isolation.

So, genomic DNA isolation is also a multi step process in that multi step process the first step is that you are going to do the lysis of the cell with the help of the detergent containing the lysis buffer. Now at this stage you can have the two different types of sources you can have the either the cell or you can actually have the tissue. For example, let us take an example of the liver. So, for example, if you have a liver from where you want to extract the genomic DNA and then you want to ask the question how many copies of that particular gene is present into the liver genome cell we can take an example of the cell also. So, for example, hepatocyte.

So, whether you use the tissue or whether you use the cell the ultimately is that if you are trying with the tissue then it has to be fragmented and into such a case that it should reach to a cellular level. So, tissue is made up of the different types of cells. So, what you are going to do is you are going to use the enzymes which actually can dismantle the inter space inter cellular spaces. For example, you can use an enzyme which is called as collagenase. So, if you treat the liver with collagenase collagenase is an enzyme which

actually degrades the collagen fibers and you know that the most of the cells are stick to each other because they have a matrix in between and this matrix is made up of the collagen or the collagen like material.

So, in the liver you have the collagen like material which is actually sticking these cells. So, if you use treat them with the collagenase what you are going to get is you are going to get the cellular suspension or cell single cell suspension and this single cell suspension can be used for further downstream. So, single suspension means from the liver you got the single suspension and now you can actually be able to treat them with the help of the detergent. So, whether you are using with the single cell or whether you are using the tissue at the end you are going to get the cells and then you are actually going to treat them with the detergent and once you do with the detergent treatment the cell is actually going to break open then it is actually going to release the cellular content. And, what is there in the cellular content? The cellular content is actually going to contain the cellular proteins, it is going to have the RNA's, it is actually going to have the lipids and it also going to have the proteins and it also going to have the genomic DNA.

So, now from the cellular content your job is that you want to isolate the DNA, but you want to get rid of you do not want that. So, you want to get rid of the protein, you want to get rid of the lipids, you want to get rid of the other materials right. So, that is what you are going to achieve in the subsequent purification steps. Now, in the step 2 you are actually going to incubate the cells with the digestion buffer containing the protein SK, SDS to release the genomic DNA from the DNA protein complexes. Remember when we were discussing about the genetic material we said that in the eukaryotic system the genetic material is associated with the protein in the form of the nucleosome and these nucleosomes are even forming the higher order organizations right such as the chromosomes and other kinds of things.

So, the first step is that you want to isolate or you want to dislodge the protein part. So, that the DNA would be free and then you can be able to get the purification of the DNA from the cellular material. So, that for that what you are going to do is you are going to incubate the cellular content or the live cells with the digestion buffer which contains the protein SK and SDS. So, in the presence of SDS the protein is actually going to get denatured right and because the protein is going to denature it is actually going to lose its affinity for the DNA. On the other hand the protein SK is a protease right.

So, it is actually going to degrade the protein into the protein into the peptide or peptide fragments right. So, ultimately what you are going to get is you are going to get and peptide fragment can further can be digested. So, if you continue the digestion it is actually going to get converted into amino acids right because the protein SK is a non

specific protease. So, it is actually going to start chewing up the proteins and ultimately what you are going to get is you are going to get the DNA which is separated from the DNA protein complexes plus you are going to have the protein which you are going to get from the cellular content and then you are also going to have the lipids right because lipid is also present in the cellular material and some minor component of like for example, you can also have the RNA right. So, now what you have is you have a mixture of DNA protein lipid and RNA and your target or target molecule is the DNA.

So, you can actually devise a strategy so that you can be able to purify the DNA, but get rid of the protein lipid and RNA species. So, that you can do sequentially by removing the protein lipid and RNA and that is how you are going to get at the end you are going to get the purified DNA. So, in the step 3 you are actually going to isolate the genomic DNA by the absolute alcohol precipitation. So, therefore, what you are going to do is you are going to add the absolute alcohol to the thing and it is actually going to precipitate the genomic DNA, but when it is going to precipitate the genomic DNA is it is actually so it is going to give you the genomic DNA, but it also going to have the small amount of proteins it is also going to have the small amount of lipids and it also going to have the small amount of RNA specially the messenger RNA. So, these has to be get rid of right this has to be get rid of.

So, in the next step what you are going to do is you are going to do the purification of the genomic DNA with the help of the phenol chloroform isomer mixture. And when you mix the phenol chloroform one mixture has two phases right you are going to have the aqueous phase and you are going to have the organic phase. See here you have the two phases you have the chloroform phase which is going to be the part of the organic phase right. And you know that when we are going to do a fractionation with the solvents the molecules will actually go to their respective phase right. For example, if there is a organic material then it actually going to prefer to the organic phase and if it is a polar molecules then it will go to the polar environment right.

And as a result what will happen is that in a append off you are going to have the two phases you are going to have the organic phase and you are going to have the aqueous phase. So, this is going to be aqueous phase and this is going to be organic phase. So, all the molecule which are soluble into the organic solvents such as lipids such as the other kinds of molecules they will be present in this whereas, the DNA and RNA is going to be present into the aqueous phase. So, in this step the phenol is actually going to denatured the remaining proteins and keep the protein into the organic phase. So, protein organic phase is actually going to take care of the lipids.

So, it is actually going to remove the lipids it is going to remove the protein because

protein is going to be denatured by the phenol and as a result the protein is also going to be present into the organic phase. Whereas in the aqueous phase you are going to have the DNA and you are also going to have the RNA because RNA is also going to be polar in nature. Now, next step is that you want the DNA, but you do not want the RNA. So, then you are going to do a further purification. So, first you are going to do a isolation of the genomic DNA what is present in the aqueous phase and it will again precipitated with the absolute alcohol.

So, if you do the reprecipitations it is actually going to precipitate the DNA and when it is also going to have the small amount of RNA and that you can actually be get rid of by putting the things into the RNA. So, if you incubate this mixture with the RNAs then it RNAs is actually going to chew up the RNA and it is actually going to destroy the RNA and then ultimately your genomic DNA is going to be isolated. So, this is what exactly is being depicted in this particular picture. First step you are going to do the lysis, second step you are going to do the enzymatic digestion, the third step you are going to precipitate the genomic DNA with the help of the absolute alcohol, but when you are going to precipitate along with the genomic DNA you are also going to get the lipids and the protein part and that you are supposed to get rid of with the help of a purification step. So, in that purification step you are going to add the phenol chloroform mixture and when you add the phenol chloroform mixture the phenol is actually going to denature the proteins whereas, the chloroform is actually going to remove the lipid part.

And when the phenol is going to denature the protein they also will be come into the organic phase. So, when you collect the aqueous phase this aqueous phase is actually going to contain the DNA and as well as RNA and then from this aqueous phase you can precipitate the alcohol with the help of absolute alcohol you can again precipitate the DNA and then you can treat this DNA with the small amount of RNAs. So, that you can get rid of the RNA part and then ultimately you are going to get the pure mammalian genomic DNA. Now when you run the mammalian genomic DNA onto agarose gel what you are going to see is that you are going to see a intact band which is going to be of high molecular weight. So, it is going to be of high molecular weight rather than a low molecular weight and this is going to be indication that you have isolated the genomic DNA.

If it is not a genomic DNA or if it is not an intact genomic DNA then in that case you are going to see a band, but below to that band you are going to see a streak or you are going to see a smear. That smear is actually an indication that you have broken the genomic DNA and you are actually going to generate the or you have degraded the genomic DNA. So, that is not good for the further analysis of into the sudden blotting. Now the next step is next step is step 2 you are going to have the generation of the

suitable size fragments because genomic DNA is very long. For example, when we were talking about genetic material in the eukaryotes we said that in the humans the length of the genomic DNA is approximately around 1 meter right.

So, if this this kind of lengthy genomic DNA cannot be analyzed for you know for probing purposes. What you want is that you do digest that genomic DNA so that you are going to have the small small small small fragment and then depending upon the binding of the probe it will actually going to say that if the probe is binding to the multiple location that means that many number of copies are of that particular fragment is present into the genomic DNA. So, for that purpose the genomic DNA has to be fragmented with a suitable system. So that you going to have not very big fragments not very small fragment, but should be a suitable size fragments and that you can be able to achieve by the two different methods. First you are going to do a restriction digestion.

So, genomic DNA can be digested with the frequent DNA cutting enzymes such as EcoR1, BamH1 or SAW3A to generate the random sizes of their DNA fragments. The criteria to choose a restriction enzyme or pair of restriction enzyme in such a way so that 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 into the pool. So, the purpose is that you should not have very small fragments you should not have very big fragment it should be a relatively reasonable size. So that each and every genomic sequence should be presented which means it should be of such a size that a single gene can be present into that each fragment.

And because of that each fragment is actually going to represent the individual genes ok. And because of that if the you are going to see that the probe is binding at multiple location that means, your gene is having the that many copies present within the genome. Then the second method is the mechanical sharing. So, mechanical sharing genomic DNA can be fragmented using the mechanical sharing. Mechanical sharing method you can use the vertex you can use the sonication and so on.

But all these mechanical methods are need to be optimized very nicely so that you should not have very large fragments. Because large fragment had no use because it is going to have the representation of the multiple genes right. So, because of that it may actually going to give you the misguide. Now in this how you are going to perform the restriction digestion right. So, in the step 2 restriction digestion can be done with the help of the restriction enzyme.

So, what you need to do is you digest the 10 to 20 microgram of genomic DNA with the EcoR1 overnight in the presence of appropriate buffer and BSA. So, when you are going

to use any enzyme you require a suitable buffer and the other kinds of additives such as BSA and magnesium and all of the kind of things. Then take out the small aliquot from restriction digestion reaction and check it on to the 0.8 percent agarose gel. The presence of the smear with a small band visible band indicate the complete digestion of the genomic DNA and the suitability of the sample for the sudden blotting.

This means if I am going to run the genomic ah the restriction digestion in the control sample where I have not added a restriction enzyme I am going to see a intact band of the genomic DNA. Just now what I have shown you into the agarose gel. But if the cut fragment or restriction enzyme wherever restriction I am I have I am going to see a fragment and then I am going to see a complete smear kind of situation which means I am going to see a white band with the discrete band also. That means this is actually been done correctly and all these fragments are suitable for the sudden blotting and the step 2 is already been done. Now you can move on to the step 3 that means you are actually going to separate these DNA on to a suitable ah gel system.

So, the step 3 is the separation of DNA on to the agarose gel. So, separation of the DNA on to the agarose gel. Agarose gel electrophoresis is a standard gel to resolve the DNA. As a standard practice the agarose used for the sudden blotting is of ultra pure quality to avoid the contaminants affecting the migration of DNA. Observe the gel in the trans-culimulator and record the pattern in a gel documentation system.

So, this is very easy to say, but it is not easy. You are supposed to prepare an agarose gel then you are supposed to prepare the sample and then you are supposed to run it on to the agarose gel and then only you are going to see the pattern. So, for demo purposes I would like to take you to my lab where the students are actually going to show you the how to perform the agarose gel electrophoresis and how you can be able to observe the gel under the trans-culimulator and how you can be able to do the documentation. We have to analyze the results for amplification. For that we need agarose and TAE buffer.

First we have to weigh agarose and mix with the TAE buffer. It will not dissolve easily. So, we have to heat it in microwave oven until it get dissolved. Now agarose got dissolved in TAE buffer. We have to let it cool down up to 50 degree Celsius. Now before pouring we have to add ethereum bromide for detection purpose.

Now the gel got solidified. We have to take out the gel and keep it in the electroporotic apparatus. We have to gently remove the comb. Loose the knobs and keep the gel in the apparatus. Make sure that the buffer is submerged in the gel.

We have to fill the remaining part with 1X TAE buffer. Generally for analyzing the

DNA samples we will use agarose gel electrophoresis. This is the power pack and this is the electroporotic apparatus. This is the negative electrode and this is the positive electrode. We can change the voltage from here. For loading of sampling we have to mix TCR reaction mixture with 5X loading time. This is the TCR reaction mixture.