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W6L31_Bacterial DNA Isolation and PCR - Hands-on (Guest Lecture) Dr. Beena DB(Azim Premji University)

Hi, I am Beena, I am biology faculty at Azim Premji University. Today I am going to show you the DNA isolation process and how to do the PCR. You guys have already studied the cell molecular biology in theory. So this technique what I am going to do today is a part of it. So before getting into the technical part, so I just want to give an overview of how the cell looks and where the DNA will be present.

So this makes you more clear when you carry on with the process. So usually when you draw the cell on the textbook, you do not think that it has a 3D or it has a volume. So you just draw a flat diagram, but actually cell has a volume and it has cell contents like cytoplasm, organelles and nucleus in it and inside the nucleus there will be DNA. So you can see here I just made a clay model just to demonstrate how it looks and what are the contents that have to be removed if I have to get out of the DNA from the cell.

So here is a clay model. So imagine this is a 3D model, it is a cell and there is a hollow space inside filled with a cytoplasm and inside there is a nucleus and DNA. So let me cut open this, the outer membrane. So cell has a cell membrane. Let me remove this membrane, then you can see that when I remove this, you can see that there is a pink color substance.

So all this, imagine this is a, imagine this is a cytoplasm and inside this you have one more round structure, this is a nucleus. So now I am going to break open this nucleus, then we will see what is there inside. So when I break open this, you can see inside the nucleus there is a white color thing that you see is a DNA. It is not exactly DNA, it is just model for you to show how the DNA, where the DNA will be present. Now you have an idea like if I have to isolate DNA from the cell, so I have to remove all these unwanted parts from the cell and then to get this pure DNA.

So the first important thing before starting any lab experiment is that you have to take care of yourself. So you need to wear gloves because you do not want to contaminate or damage your skin cells. So it is mandatory that you have to wear gloves because you will be using harmful chemicals. Either it may be a carcinogen or some other chemicals which is going to affect you. So now as I mentioned, the DNA extraction process involves all the removal of unwanted things from the cells or cell membrane and the protein content present inside to get a pure DNA.

So basically any DNA process, any DNA extraction process, it involves the following steps. The first step is lysing the cell membrane. So use chemicals which is required to break down this cell membrane. The second step is removal of the proteins, unwanted proteins and cell debris where you use the proteinase enzymes and RNA,RNase is the enzyme which removes the RNA content. Third step is now you have removed the cell membrane, you have degraded the protein which is not required. Now you have to centrifuge and take out the debris. So you will be left with a liquid which has a DNA.

Now we need to isolate, take out this DNA from the liquid part that we have. For the first step when you, to break down the cell membrane, use a detergent. You can use a SDS or if you try at home like if you take a soap solution and detergent, diluted detergent solution and you can use it. To remove the proteinases, sorry protein content, you can use the proteases. Again you can use the fruit juice like papaya which has the protease if you are doing it at home. But in lab we will use the purified enzymes. And third step is precipitating the DNA. It will be done by using chilled ethanol.

So when you add ethanol into the DNA solution, so initially the DNA will be dissolved in the water. Now when you add ethanol, so this separates the DNA from the water and it gets precipitated in the ethanol. So these are the basic things, are the basic steps that are involved in the DNA solution. So when you use it, again the protocol that, steps that you follow, it is not same for all the samples. It depends on what sample you are going to take. So if you are taking a complex tissue like plant or animal tissue, then you have multiple steps like you have various purification steps. But when you use a single-celled like bacteria, prokaryotes, you do not need that much purification. So it is a simple process.

Today I am going to show you bacterial DNA isolation process which does not involve this purification steps. But it is a very simple one-step process. Take a colony and use the solution that is required or the chemical that is required to break open this cell and then use this solution as a template for PCR amplification. To do that, so I have the required reagents here. So the first reagent is Tris EDTA. So this helps to protect the DNA from the enzyme degradation and there is a second solution like KOH EDTA. This is actually, this is a protocol that I am going to follow today.

So this simple protocol which was developed by Zhibin et al. in 2014. So this is a solution

actually which breaks down the cell membrane and then releases the DNA. And then you have a Tris HCL solution which actually is used to balance or to maintain the pH of the solution. This is just three-step protocol where I am going to add three different reagents and keep it in the water bath and take out and directly use this light set for PCR amplification.

So let us start with the pipetting, the first reagent. So I will set my micropipette. So I am going to pipette 27 microliter of this into a clean Eppendorf tube. And into that solution, I am going to add the bacterial cells. So you can see here, so I have a bacterial plate here. You can see the individual colonies. From backside, it is very clear to observe the colonies. So I am going to take a sterile toothpick and transfer a colony into the solution which I have taken there and mix it thoroughly.

So now I am going to add the second solution, that is KOH EDTA-3 microliter of it. So after this, actually you have to keep the tube or incubate the tube in water bath at 70 degrees. So I have already turned on this water bath, so which is set to 70 degrees. So we have to keep these things ready beforehand, before starting the experiment. So I am going to transfer 3 microliter of KOH EDTA, that is potassium hydroxide, into the bacterial suspension that I have already taken in a tube and mix it thoroughly. Now close the lid and incubate in the water bath for 5 minutes. So I am going to turn on the timer now.

It is going to be 4 minute now, so I have to add the next reagent immediately after the 5 minute is done. So I am keeping my next reagent ready that is 3 microliter of Tris-HCL. So you have to be careful that you cannot start pipetting. Once the 5 minutes is done, you have to keep your reagents ready before 5 minutes, in the 4th minute and immediately after 5th minute like you have to take out and add this reagent and mix thoroughly. Now your sample is ready for the PCR amplification.

So till now you have seen how to isolate DNA from the bacterial cells. So you could not see the precipitate because it is a one-step process where everything is there in one solution. So when you actually do with other materials like plant or animal tissues and when you add ethanol you can see the white color structure that is coming out when you add the ethanol. So that is actually the DNA. So maybe I can show you in the next video.

So the next step is a PCR process. PCR is nothing but polymerase chain reaction. You would have heard during the COVID everywhere RT-PCR, RT-PCR, so PCR is a common term. But RT-PCR is different, this is different. RT-PCR is where you use the RNA as a genetic material but here I am going to use DNA as a genetic material for amplification.

So what exactly happens in PCR is you have a genomic DNA. Now the whole mixture what we have. So once I took out from the water bath, I kept the DNA sample in the ice. So throughout your PCR process whatever the reagents you are going to use for the PCR process you have to

keep it in the ice. So I have stored my DNA sample in ice.

So you will be taking the DNA and then so this is a genomic DNA now. Genomic DNA is a total DNA that is extracted from the cell. Now in the PCR what I am going to do is so you have a whole lot of DNA, it is a big stretch of DNA, genomic DNA. I am going to amplify that is making the copies of a particular stretch of DNA from the whole stretch of genomic DNA. In order to do that, so I have to provide an artificial condition where all the resources or the raw material that is required for the synthesis of new DNA strand has to be provided in artificial condition.

Say when the replication normally happens inside the cells, you know that all these material is required. So DNTPs, enzymes and the optimum condition like the pH and the temperature has to be balanced. So same condition we are going to provide in the outside artificially so that the DNA replication happens. The first thing is I said like I mentioned I am going to amplify only particular stretch of DNA not the whole genomic DNA. So in order to do that I need something called primer.

Primers are nothing but a small stretch of DNA you would have studied in theory. So it is a few nucleotide sequences which is complementary to the known sequence of genomic DNA exactly like the which piece of the genomic DNA I want to be replicated. So this piece of DNA or the primers will be complementary to that. So I am going to use those primers, forward primer and a reverse primer which binds in two different directions in the DNA strand and then they help in the amplification. So along with that you have to provide the nucleotides.

So all of you know that you have the DNA strand has 4 nucleotides ATGC that is nothing but adenine, cytosine, guanine and thymine. So this has to be provided in the mixture when I make a PCR mix. So I am going to show you now in this chart like these are the different chemicals that I am going to use and what is the volume of it. So for this PCR what I am going to do now I am setting it for 50 microliter reaction, that is when I add all these reagents the total volume will make up to 50 microliter, that is including DNA. The first reagent that is DNTP that consists all 4 nucleotides, 2.5 micromolar concentration of DNTP is used and the volume pipetted will be 4 microliter and then forward primers and reverse primers. I already explained what is forward primer, what is reverse primer. So each will be added 2.5 microliters, then DMSO 5 microliter followed by Taq polymerase. Taq polymerase is an enzyme so normally in the cell also we need DNA polymerase to carry out the replication process.

So here also we have to provide Taq polymerase in the artificial condition. Then we use the PCR grade water. So this is not the normal, this is a distilled water but it is much purified water which you can you will use it for PCR and any kind of molecular biology work. Then the template DNA. So this is a whole set of chemicals which are provided in the reaction mixture

for PCR.

You can actually have a look into the volumes what exactly I pipetted in this chart. So I am going to pipette out all these and take it in a small tube this is 0.5 ml tube, you call it as PCR tubes. I am going to pipette all these reagent into this and then mix it. So all these I have pipetted out into this PCR tube and I am going to spin it now to mix the reagents because the reagents what we have taken all these are in microliters. So there may be chances that these drops are getting stuck on the wall.

So even 1 microliter of whatever the reagents you have taken if it is missing so you do not get a proper result. So mixing is very important step. So once this is ready now we have to keep it in the PCR machine.So before keeping it in the PCR machine I am going to mix it and as I mentioned mixing or vortexing is important process. So I spin my material now. I am going to keep it inside the PCR.

So now after vortexing I am going to keep it in the PCR machine. So I already set the PCR cycles. So you can see here for the bacterial DNA so I have set the cycle the first cycle denaturation at 95 degrees for 1 minute, followed by 95 degrees at 30 seconds and primer annealing at 52 degrees for 30 seconds and primer extension for 74 degrees for 1 or 30 minutes and then the whole steps will be repeated for 29 cycles and then hold at infinity. So once amplification is done if you are not around the machine will hold it in the cold temperature. So now I am going to turn it on, press run cycle and set the reaction volume.

So I have set it for 50 micro liters so I am going to change it to 50, that is it you do not have to do anything else you can see here now it takes around 1 hour 58 minutes 59 seconds it is approximately 2 hours. So after 2 hours I have to come back and take out the sample then load it on the gel. By the time when our PCR cycle is going on let us prepare the gel which is needed for the next step. So what I am going to do is I have taken agarose in this.

So I am going to prepare 0.1% agarose gel, usually you can use like 0.8% also but if you want the bands to be separated even the small bands to be separated clearly, then you have to use increase the concentration of the agar.

So I am going to use 1% agarose gel to prepare that so I have to weigh agarose required quantity and then pour 1X TAE buffer 1X is 1X usually we prepare in 50X and keep from that you are going to dilute whenever you need, Tris acetate EDTA buffer-TAEs. So then mix it thoroughly. So I am going to keep this in the microwave oven now to boil it till it becomes completely clear okay.

So I am going to keep it in the microwave oven and then turn it on. So you have to keep watching. It should not spill when it starts boiling till it comes up and then spills so you should

be very careful while doing it. So now you can see as it starts boiling agar starts melting and it becomes more clear and clear but I can still see the crystals here so I am going to keep it back. You can see here now it has become much more clearer but still I can see the agarose sticking to the beaker so I have to heat it little more till it becomes very clear. Now you can see the difference so there are no more agarose crystals you can see here, are particles so it is very clear that means the agarose has completely melt.

Now I am going to allow it to cool till it becomes around 40 degrees and then add Ethidium bromide into it. So ethidium bromide it is a carcinogen you should handle it with care so should not touch it so always wear the gloves when you are using the ethidium bromide and discard the tips micro tips what we have used and the containers carefully otherwise there may be chances that people who do not know that you have used these tips as a container for this and they will come and touch and then they may get contaminated. So be very careful when you use the ethidium bromide. It has cooled down so I am going to add ethidium bromide-3 microliter of it. Now mix it thoroughly and pour into a gel casting tray so this is a gel casting tray so I have made taped it and put the end so that when I pour the gel it will not leak and come out and there is a comb here which has wells which will make the wells when you pour the gel pour the melted agarose into it.

Now you just have to wait till it cools once it cools carefully have to remove the comb and that is the well where you actually you are going to add your DNA sample. Now gel is cooled and you can see that I have removed the boundary that I made with the tape cellophane tape and removed the comb, so you can see the wells here so once this is done so I am going to place it in a electrophoresis tank with 1X TAE buffer which is already filled inside it. So when you keep it inside the tank you should be very careful that the side with the combs should be towards the negative end or negative electrode because DNA is negatively charged when you run this gel so the DNA starts moving towards the positive end so loading should be always in the negative side. So once I place it I have to fill the tank again if the gel is not completely immersed in 1X TAE buffer I have to pour little more buffer.

Now your gel is ready to load the DNA sample. To load the DNA sample I am going to take cellophane paper and then I am going to use gel loading dye, this is 5X sucrose gel loading dye you can get it get the readymade ones. So I am going to take 2 microliters of the gel loading dye on the cellophane paper and then add the DNA into it. Here is a PCR amplified DNA we just kept it in the machine if you remember and it took around 3 hours to complete the cycle and this is now our amplified DNA. I am going to add this DNA sample into the dye which I have taken and mix it and then transfer it into the gel. Already I have taken 2 microliter of dye so I am going to add around 8 microliter of the amplified DNA sample into it. Remember that you have to store the DNA sample in ice and then I am going to mix it.

Now I have to release the pipette and make it to 10 microliter because I have taken 8 microliter of DNA and then 2 microliter of dye. The total volume is 10 microliter and pipette back. So you can see the wells clearly here and the gel so I am going to load so you have to hold the pipette in this way and then grip your finger. It will be easy for you to load. You can see the well also clearly and slowly release the sample into the well. In another well I am going to load 2 microliters of DNA ladder, so DNA ladder has a mixture of DNA different lengths of DNA strands but known length so that when you compare with your amplified product you know that what band you are looking for and say like whether you got amplified band or not. So for this as I mentioned before I used 2 primers so these are the primers for bacterial 16S rRNA genome so they usually amplify the stretch of DNA which is around 1000 approximately around 1400 base pairs.

I am going to load the DNA ladder here in the first well. Now after running, so in the well where I have loaded the ladder you get a bands of different fragments of known length and in the other one where I have loaded the PCR sample you may get a amplified band but you will not know the length but by comparing with the known DNA band in the other well you can say that yes this is your amplified product.

So now I completed my loading the DNA sample and the ladder I am going to connect the electrodes and turn on the power so that it starts running since I have loaded in the negative side so it starts running towards the positive side. So actually the dye in the sample which I previously mentioned 5x gel loading dye helps you to identify till where the DNA has run so you can see actually clearly when it starts running you can see the color on the gel so based on that you can decide like okay till here the DNA has run. So once it runs like almost three fourth of the gel then you turn it off and then take the gel to the UV illuminator and check for the band.

So we have added, remember we have added ethidium bromide into the gel so ethidium bromide actually intercalates with the DNA and when you expose the gel to the, gel to UV light so they glow pink wherever there is a DNA so this ethidium bromide will bind to the DNA and since ethidium bromide glows when you expose to UV light you will know that this is your DNA band.

So that is the purpose of using ethidium bromide in the gel. So initially when you run you have to set it to lower volt otherwise if you increase the temperature it may run fast but the gel may melt or sometimes the bands may forms smears. You will not get clear distinct band so it makes a smear. So it is always better to run in a lower volt.

Okay so my gel is ready now so we have run the gel till three fourths and I have taken it. I place it on a UV illuminator. So you can see now what happens when I turn on the UV light. So you can see this pink color bands these are the amplified products and so while loading I also loaded genomic DNA without amplification, without PCR. So you can see the pink color well, band, very close to the well that is the genomic DNA and the two bands here are the amplified products

and this is a DNA ladder.

Hope you enjoyed the whole process and learnt how to isolate DNA, prepare gel and then load the amplifier and load the DNA samples and how to identify the amplified product from the band from the gel. Thank you, see you all.