Course Name: I Think Biology

Professor Name: Dr. Kaustubh Rau

Department Name: Biology

Institute Name: Azim Premji University

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W6L30_Molecular Biology Techniques

Hello and welcome to this lecture on molecular biology techniques. This is the I think biology NPTEL course. Most of the material that I will use for this lecture is from the primer called molecular biology techniques, which is found in the I think biology textbook online. I've also used certain concepts from a very standard reference molecular biology of the cell, which I have referred to earlier. And this can also be found online on the PubMed website. The particular chapter I have referred to is called isolating, cloning and sequencing DNA.

Before we talk about molecular cloning, let's try and get some definitions out of the way. The image shown here encapsulates pretty much everything we need to know about a molecular cloning experiment. So on this petri dish, we see certain spots and each spot is a bacterial colony. Now you also see highlighted by the black marker, a colony which has not grown on the petri dish.

and This is the sign of a successful experiment. So these researchers were trying to clone a gene from the herpes simplex virus in a particular bacterium. And so along with this gene, they also destroyed the resistance of this bacterium to the antibiotic tetracycline, which meant that the successful incorporation of these herpes simplex virus gene also destroyed the ability of this bacterium to grow in media which can contain the antibiotic tetracycline and which is what is shown here. So this is normally what you would do in any molecular cloning experiment is express a gene from a foreign organism within a host organism and then try and isolate that organism. So let's look at these definitions.

So recombinant DNA is basically DNA from two different sources and so they could be two different species or the DNA could also be chemically synthesized now. And molecular cloning is just the production of multiple copies of this recombinant DNA in

different host organisms. And a transgenic or genetically modified organism is an organism that contains one or more foreign genes. So in this case, you could say that the bacteria which has been transformed with a gene from the herpes simplex virus is a transgenic organism.

We need to know two other things. One category is biomolecules. So two other kinds of biomolecules we need to know are restriction enzymes and plasmids. And then we also need to know the techniques of gel electrophoresis and the polymerase chain reaction. So let's start with the first biomolecule which are restriction enzymes. So restriction enzymes are endonucleases that can cleave DNA at a particular site and they're called endonucleases because they cleave DNA within the strand.

You can also have exonucleases that cleave the ends of the strand. Restriction enzymes were first isolated in bacteria and they are used to protect bacteria from viral invasions. So restriction enzymes will recognize a particular sequence on the viral DNA, produce breaks in the viral DNA to degrade it. And bacteria also contain another set of enzymes called methyltransferases. So these add particular groups to the bacterial DNA in order to protect the bacterial DNA from the action of the restriction enzymes.

So we need to think of restriction enzymes and methyltransferases as acting together within a particular organism. There are many types of restriction enzymes so we are only concerned with type 2 in molecular cloning. And this is the nomenclature by which one refers to a particular restriction enzyme. So EcoR1 is a restriction enzyme which has been isolated from E. coli, the strain R, and this was the first such molecule isolated, so hence the one.

BamH1 is another restriction enzyme isolated from Bacillus amyloliquefaciens. The strain is H, again the first such molecule isolated, so BamH1. And now you have over 3600 nucleosides which have been identified. They can cleave DNA at greater than 250 specific sites. This is a schematic shown for HindⅢ which is another restriction enzyme.

And so this recognizes the sequence AAGCTT. And as students of biology we now know that we always refer to a particular DNA sequence from the 5′ end to 3′ end. And HindⅢ cleaves this sequence between the two AA molecules. This is more about restriction enzymes showing the structure of the restriction enzyme or the model of a restriction enzyme when it is latched on to the DNA molecule. And so you can see that it almost looks like a clamp when it adheres to the DNA molecule.

And then on the right is shown the cleaving of a DNA molecule. So this restriction enzyme which is EcoR1 recognizes the sequence GAATTC and then it produces a cut between the G and the A to produce these kind of overhanging DNA ends. So you can

have two kinds of cuts which restriction enzymes can produce. A sticky end digestion will produce these overhangs and a blunt end digestion produces cuts which are without overhangs. Another thing to note is that restriction enzymes recognize what is called as a palindromic sequence.

So going from the 5′ end to 3′ end. it reads the same on both strands. Then we need to look at the second molecule which are plasmids. So plasmids are small DNA molecules which are extra-chromosomal which means they do not occur on the main chromosome. They are circular, double stranded and they can either be coiled or linear and that is shown here in the schematic. A bacterial plasmid has been shown.

They have their own replication machinery and they are found in bacteria, archaea and in some yeast and they can have a large size range from 1 to 1000 kilobase pairs. And they typically confer some advantage to the host organism. So whether it is genes for antibiotic resistance or genes for toxins or genes for virulence that they carry. And nowadays we can get plasmids by ordering them. So Addgene is one such place that we can order plasmids from and at last count they contained almost 1 lakh plasmids which people have deposited in their repository.

So this is a closer look at a plasmid and it contains particular sites. So first you have the origin of replication site and then a plasmid can have different selection markers which will allow us to select a bacterial colony after successful incorporation of the gene of interest. So you can also have a gene on the plasmid to confer antibiotic resistance to that bacterium or you can have a different kind of selection marker which could provide a particular color. So one such known one is the blue-white screening which is used in bacterium. So successful incorporation, the bacterial colony becomes blue and the unsuccessful colonies are in white.

The main site here is the cloning site. So this contains DNA which has the restriction site for the particular enzyme that you want to use. And nowadays you have a multiple cloning sites so you can use different restriction enzymes to insert the gene of interest at that cloning site. And then we need to look at two techniques. So first is the technique of gel electrophoresis.

This is a way to separate a mixture of biomolecules by applying an electric field and allowing them to migrate in a gel matrix. And so typically you need a power source, you need a gel and you also need a UV light source. So what you do is you mix a dye with your sample, you add the sample to the gel in these wells which can be seen at the top in the image and then you turn on the electric field so the molecules start to migrate. And then after you stop the electric fields, you expose your gel to a UV light source and

because of the fluorescence you are able to visualize your biomolecules. This is a schematic of a typical gel.

So you have these wells at the top into which you add your sample. And so the first well is typically reserved for the marker. So this is a mixture of DNA molecules with known size. And so you produce what is called as a ladder in your gel. And then to the other wells you add the DNA of interest.

And after looking at it under the UV lamp, you will see at what location has your DNA molecule stopped and then you can read off the size based on the ladder. Of course it is an obvious question, how do you produce a DNA ladder? You should look up certain techniques which are used to produce these DNA ladders online. The other thing to note is shown on the graph is the linear relationship between the size of the molecule and the distance it migrates. So the smaller the size of the molecule, the faster it migrates and it will travel a longer distance for a particular run of the gel electrophoresis. And the other technique we need to know is that of the polymerase chain reaction which is used for amplifying DNA.

Shown in the image is Professor Kary Mullis who was the inventor of the PCR. And so he developed this when he was working at the Cetus Corporation. And he realized that he could use small primers and which could be used to bind to the ends of the DNA molecules of interest and then by using DNA polymerase, they could be replicated and then you could repeat this cycle. I should mention that this idea had been thought of before but due to technology not developing when this idea was thought of in the late 1960s and early 1970s, by the time of the 1980s, you had certain developments in machinery and molecules which could allow the successful generation of this amplification reaction.

And after the first demonstration of the proof of principle by Dr. Mullis, other chemists at Cetus Corporation made a major improvement by using the DNA polymerase from the bacterium Thermus aquaticus. And so this enzyme could withstand higher temperatures and it also had a faster run so it could amplify DNA in a much faster time scale. And you can watch this video for an introduction to PCR. So this is what a typical PCR run would look like. You start on the left with your DNA template which is the sequence that you want to amplify and to that you add nucleotides shown here as dNTPs.

You add your primers. These are actually chemically synthesized and they're typically between 18 to 22 nucleotides long and then you also add your DNA polymerase. And then if you look at the first cycle in detail, it has three steps, denaturation, annealing and extension. So in the first denaturation part, you raise the temperature in your reaction. So the double helix falls apart. Then in the annealing step, you reduce the temperature and it

allows the primers to bind to your DNA strands and then you run the extension part of the reaction.

So DNA polymerase will bind to these strands and then it will start adding nucleotides from the 5' end to the 3' end. And so at the end of the first cycle, you start with two molecules of DNA and you end up with four molecules. And then you can just repeat the cycle. So in the second cycle, you will go from four to eight molecules and so on. Of course, we are all very familiar with PCR because of the COVID-19 pandemic.

So I thought I would show you the results of one such PCR test, which I had to take. And you will see here that they tried to amplify two genes from the COVID-19 virus, which is ORF1AB gene and the N gene. and the result is that they weren't detected even after the amplification reaction. On other test reports, you sometimes see the number of cycles they run during the reaction. So now that we know the biomolecules which are used and the techniques which are also used, we can think about actual molecular cloning experiments.

So what you do is that you run restriction digest on your sample to isolate the gene of interest from a particular organism. And you also choose a plasmid and you run a restriction enzyme digest on that plasmid. So now you have two DNA molecules, the plasmid and your gene of interest. And because you have used the same restriction enzyme, they have the same overhangs or sticky ends on them. when you run a ligation reaction where you combine these two DNA molecules.

And so you produce your recombinant DNA plasmid. Then you use that to transform it, a host. This can be E. coli or some other host. And then you try and produce more copies of your plasmid within the host.

So you basically allow it to grow. And then you, because you have a selection marker, so you can try and grow it on media which contains an antibiotic in this case. And so only bacteria which have the successful incorporation of your recombinant plasmid will grow on that media. And then you can isolate your molecules of interest from these bacterial colonies. Or you can also store these bacteria so you can access the plasmid later on if you want. So this is what is known as classical or traditional molecular cloning.

This is typically what you would do in the lab. Currently, people are also using other kinds of cloning methods. So there's one called Gibson assembly or there's Golden Gate assembly. And basically this allows you to multiplex which means you can clone multiple genes in the same experiment. The other technology which is quite advanced is chemical synthesis of genes because a very large number of organisms have now been,

their genome has been sequenced.

So you can actually try and chemically synthesize genes and this technology is also advancing quite rapidly. The other thing is the rise of the field of synthetic biology and that is mainly due to the iGEM competition. GEM here stands for Genetically Engineered Machines. So the idea is that because cloning technology is now quite advanced, we can think of bacteria almost like little machines in which we can change or adjust parts and by parts we mean little reactions that will run within the bacteria and do it in a way that will produce results which are of interest to us. And so the iGEM competition is organized every year and college teams from around the world participate in it and it has been growing by leaps and bounds.

And University teams from India have also been competing in it with great success and I urge you to look up this competition website. With that short introduction to molecular cloning, I will end my lecture and we can talk some more about it in the tutorial. Thank you.