Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 11 : DNA Cloning and Recombinant DNA technology -I

Namaskar. Welcome back to the NPTEL lecture series of Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis. So, we were discussing the different tools of molecular diagnostics and gene expression analysis since last few weeks. Again we are into another new technique that is DNA cloning and recombinant DNA technology. So, in this class the concepts we are going to cover are the basic concept of recombinant DNA technology, cloning, their steps, different types of restriction endonuclease are very special enzyme and what these class of enzyme is doing and then different cloning vectors another very important feature rather requirement of DNA cloning, what are the different types of vectors and their special characteristics. So, let us start.

So, we are discussing cloning. Now just assume that let us give an example. So, you love mango very much, but throughout the season mango is not available you have banana tree. So, you want to introduce the flavor of mango inside your banana is it possible? It is possible by recombinant DNA technology where the scientists are actually introducing the gene which is responsible for the test of mango and that gene is introduced in the gene of banana.

So, while you are eating banana you are getting the flavor of mango. So, that is what restriction sorry recombinant DNA technology is doing. Now coming to cloning, cloning you already know. So, it is the process of producing large number of identical copies from one single original DNA molecule or fragment. You have read such one method that is polymerase chain reaction, but polymerase chain reaction is the cell free method.

Here we are going to discuss the cell based method polymerase chain reaction we do in the laboratory system in the PCR tubes whereas, that similar multiplication of the same DNA molecule we are going to conduct that experiment inside a cell and that is recombinant DNA technology. So, why this cell based cloning is required? So, the problem with PCR is basically contamination, whenever you are extracting the cellular

contents from within the cell there is a chance that the environmental contaminants can be present there though rare, but can be. Whereas, if you just multiply that DNA inside a cell there is no chance of contamination. So, we can obtain a purified form of gene in sufficient amount and this cloning is extensively used while studying sequencing, while checking the expression of different genes under certain condition imposed condition, environmental condition, drugs. So, we can study the level of expression.

Now recombinant DNA technology is as I told you the concept it is a sort of genetic engineering. If I give you one real life experience that the patients of type 1 diabetes mellitus they are suffering from the deficiency of insulin. So, they need insulin externally is insulin basically is the treatment. Now, where from we will get the insulin? Initially what has been done insulins were extracted from different types of animal like pigs, cows, but the problem is those insulin inside our cell human body are causing used to cause different types of allergic reaction different as adverse reaction. So, what we wanted is our insulin only.

So, what the scientist again did they extracted or rather purified the gene of insulin of human insulin from a human body and they incorporated that gene inside a bacterial gene they let the bacteria multiply. So, along with the bacterial gene the insulin human insulin gene was also multiplying after that a huge number of human insulin from that gene is expressed and purified. So, the huge amount of human insulin can be formed via the recombinant DNA technology. So, what is it? It is a direct manipulation of DNA to alter an organism's characteristics that is phenotypical characteristics in a particular way. Remember the E. coli does not have human insulin gene, but we changed its phenotype we introduced the human insulin gene inside it. So, that is a phenotypical change and recombinant DNA technology is that method where a living organism or its part or the part of its genetic material can be incorporated in another living organism. So, that is the recombinant gene technology or genetic engineering. So, let us see the basic growth steps what we need to do. So, here you can see we have one set of DNA and we want a particular sequence of these DNA to be incorporated in this genetic material that is a second genetic material.

Now, what we are going to do? We are going to cut the specific sequence. So, you can see this part we have cut. Now, we want to introduce this part inside the blue genetic material. Again we are cutting that blue genetic material with the same enzyme, we need an enzyme to cut this and then we are incorporating this cut part of DNA inside this genetic material. So, we are getting this is our final product.

So, we are ligating this cut part over this blue DNA sequence. Now, what we need? We need two very specific enzymes which those two lie at the core of this recombinant DNA in this application. Number one is ligase. Ligase DNA ligase you know it helps to

join the double strand DNA to another. So, here you can see these double strand DNA by DNA ligase can be ligated or joined to the blue DNA.

Now, another enzyme is very important which causes the cut of double strand DNA and that is restriction endonuclease. That restriction endonuclease is the very typical endonuclease we are going to discuss. So, restriction endonuclease is a an endonuclease that it cuts the DNA strands from within not the terminal parts rather this is the exonuclease when there is a double stranded DNA. Exonuclease they cut the peripheral part whereas, endonuclease can cut inside a DNA. So, this segment can be cut out by endonuclease.

So, restriction endonuclease is one such endonuclease which selectively cut the gene from the parent DNA. Now, what is that selective cutting? I am going to discuss and these restriction endonuclease because they cut out a specific segment from the parent DNA they are also called molecular seizures. Now as I have told you there is selective cut that means, there is some selective area which are recognized by the specific restriction endonuclease. There are multiple types of restriction endonuclease endonuclease and they have their own specific recognition site also known as restriction site. Sometimes restriction site and recognition site can be different.

Remember restriction endonuclease can identify when the restriction site and recognition site are same it happens like there are the sequence is a 4 to 8 nucleotide long sequence this area is identified and in this region only restriction endonuclease cut the double strand DNA. Now, what is the feature of this restriction site? It is palindromic in nature in complementary strand. So, if you read the nucleotide sequence it is same from 5 prime to 3 prime direction. Now restriction site and recognition site are sometimes different like one sequence is identified by the restriction endonuclease and based on the identification nearby sequence where the restriction site is present and there the restriction endonuclease cut it. Now why this is so named why the name is restriction endonuclease? Initially 1960's Warner Arber discovered this restriction endonuclease enzyme and it is identified as the first DNA which can infect bacteria.

Now those first DNA are called to be restricted why because those first DNA to protect the bacterial DNA from the first DNA, bacterial DNA used to get methylated which causes restriction of this first DNA to infect the bacterial DNA and it is helped by their own restriction E. coli's own restriction endonucleases. So, those groups because they restrict the infection of the E. coli by the first DNA this group of enzyme were named restriction endonuclease. Now there are multiple types of restriction endonuclease and they are named following a specific pattern.

The naming system is based on bacterial, genus, species and strain from which the

restriction endonuclease is isolated. Let us see one example. So, EcoR1 is one example one very common example of restriction endonuclease. Now this EcoR1 restriction endonuclease is obtained from the bacteria Escherechia this is the genus.

Escherechia coli or E. coli co stands for coli which is the specific species of the bacteria. Then R, R is the specific strain of the bacteria RY13 that is the specific strain and R is the sequence of identification or order of identification. So, this is the first restriction endonuclease which has been isolated from E. coli RY13 strain. So, it is such named as EcoR1.

So, this is how different types of restriction endonucleases are named. Now restriction endonucleases cut the double strand DNA. So, there are the ends which are generated specific ends. Now those ends can be of two different types one is sticky end another is blunt end. What happens few restriction enzymes suppose you have a double stranded DNA molecule.

Now few restriction endonucleases cut the two strands of DNA in such way that there is overhanging of 2 to 4 nucleotides and those nucleotides are unpaired and they are complementary to each other let us see. So, it happens like if the restriction endonuclease cut and this is our restriction site. So, what they do they cut here and here. So, we are getting this or if you draw it like a diagram it is like this. So, this is our sticky end or staggered ends where you can see this is the overhanging which is 4 2 2 to 4 nucleotide long and these two are complementary to each other.

Now as I told you here you can see the example as I told you the sequences are the recognition sites are of palindromic nature or we also call it inverted palindromic. So, you can see this is one recognition site or restriction site where the sequence is like this GAATTC. So, if this is our 5 prime to 3 prime sequence in the complementary strand it will be CTT GAATC in this strand if you read it from the 5 prime to 3 prime direction you can see GAATTC. So, it is the palindromic when you read the sequence only from 5 prime to 3 prime direction in both the strands. Now this restriction site when treated by eco r 1 gives rise to sticky ends.

Here you can see the sequence is cut here and here. So, the sequence is like that in one strand this in the other strand this. So, this is our sticky end and of course, they are complementary to each other. So, when the sticky ends come to each other they once again can form the hydrogen bond and form the double stranded DNA. Another type of end which is generated by restriction end zone nucleus is the blunt end where there is no overhanging the DNA is cut just the opposing phosphodiester bond leaving no unpaired bases.

So, here you can see another example one example of blunt end. So, you can see CTGTAGC and if you this is the 5 prime to 3 prime direction rate and in the opposite strand if you read it from the 5 prime to 3 prime direction it is the same that is GCT here this is the read GCTACAG it is like this fine. Now here this is our cut. So, we are getting one strand like this another strand like this. This is one strand and this is another strand.

So, this is our blunt end generated. So, there is no overhanging. Now the if you treat one DNA fragment with one specific restriction endonuclease if the restriction site is present for multiple times in that DNA fragment there will be multiple DNA fragment generated when treated with the restriction specific restriction endonuclease. Now how you can assume how many fragments will be there? It depends on the average size of the average size of the DNA fragments based mostly depends on the frequency of the restriction site, how frequently the restriction site is present over the DNA also the size of the recognition sequence. Now hypothetically if the restriction site is 6 base pair long in the DNA where all the 4 nucleotides are present in equal amount in those cases considering there is 50 percent GC content the average size will be 4 number of the fragments will be 4 to the power 6 base pair.

At this frequency the restriction site will be present. So, again if the restriction site is 4 base pair long the frequency will be like this. So, this is how in a standard equally distributed all the 4 nucleotides containing DNA we can assume the size of the DNA fragments, but that is not natural. So, these are the examples of different types of restriction endonucleases. You need to remember name of a few also like give 2 examples or 3 examples of restriction endonucleases which give rise to sticky ends, one or two examples of the restriction endonucleases which gives rise to blunt end.

So, these are very common example like you can remember BamH1 which gives rise to sticky end you can see EcoR1 I gave the example already this is also giving the sticky end whereas, PVU3 is the blunt end again the EcoRv it is giving the blunt end. So, one or two examples very common these are very common restriction endonucleases. So, you need to remember their names and if possible you can remember the restriction site sequence. So, we are coming to the step specific steps of recombinant DNA technology. Now recombinant DNA technology apart from the restriction endonuclease there are another two thing which are very important one is a vector another is a host organism.

So, once you cut your intended DNA sequence you need to carry that sequence inside the host chromosome or the target genetic material. So, you need one transport system who will carry that genetic material genetic material or naked material which cannot be directly carried in environment. So, there is a transport vehicle that vehicle is the vector. So, based on the choice of your host organism to whom you want to introduce the

recombinant DNA you need to choose the vector you need to choose the restriction endonuclease. Now here you can see in the recombinant DNA technology you need to treat the target DNA with one specific restriction endonuclease sorry this is your target DNA which is treated with restriction endonuclease specific that specific restriction endonuclease must be applied over your vector.

So, your vector is one genetic material in an organism which will carry the target DNA to the host. So, one such vector example of vector is plasmid we we were going to discuss the vectors in details. Now this plasmid DNA is treated with the same restriction enzyme. So, what will be the effect? This restriction enzyme is having one sticky end the same sticky end is formed in your vectors DNA as well and they are complementary to each other. So, when you mix these two types of DNA they can form the hydrogen bond and form this recombinant DNA the DNA which have the feature of the vector as well as the feature of your target.

Now this genetic material is present in the vector that vector is introduced in one host organism consider that host organism is one bacteria. Inside that bacteria we need multiple clones of such recombinant DNA. So, we let these bacterial cells grow and form multiple copies. After forming this multiple copies we need to identify those bacteria which are having your target recombinant DNA. Remember very few bacteria will accept this recombinant DNA and will form this multiple clones.

So, what will be the effect? Few bacteria will be having normal their own DNA where recombinant DNA is not present and few bacteria will be having the recombinant DNA. So, we need to identify these colony where the recombinant DNA is present. So, there are some method to select the target colonies successful recombinant DNA containing host colonies also we can insert some reporter genes or some markers by which we can say yes the insertion of the target DNA or the formation of the recombinant DNA is successful. Now we are constantly discussing about the sticky ends. What happens if there is blunt ends? Now in the blunt ends if we get the target DNA treated with the restriction enzyme which gives rise to blunt end also the host DNA treated with the same enzyme and causing the blunt end they can be ligated directly with the DNA ligase.

But the problem is sometimes this direction can be just the opposite or sometimes suppose your host organism does not have that specific restriction site which is present in your target DNA. So, here is a type of restriction site and here is another type of restriction site. So, when the problem is of having suitable or common restriction sites which when not available both in case of vector or host organisms in those cases we take the help of linkers adapters also homopolymer tailing. Here I am going to discuss about the linkers and adapters because they are very much used in recombinant DNA technology. Now linkers and adapters are nothing, but short double stranded sequence of

DNA which are chemically synthesized.

So, the common between linkers and adapters are they both are double stranded DNA double stranded oligonucleotides which are chemically synthesized. What are the differences? The major difference between linker and adapter is linker is having blunt end in both the sides whereas, adapters are having one sticky end and one blunt end. Now in case of linkers, linkers do have restriction sites within it those restriction sites can be identified by restriction endonuclease. Now suppose we have one target DNA which is having blunt end and this target DNA and the vector suppose this is our vector they do not have common restriction sites.

So, in that case we use linkers. So, here we have joined the linker. Now this linker is having a restriction site which is commonly identified by restriction endonuclease here as well as in the vector. So, here we can digest and create one restriction site suppose we have created this sticky end here after restriction digestion by restriction endonuclease. Similarly here also by digesting this vector we are creating another sticky end here.

So, these two now can be joined. In case of adapter, adapter is already having one blunt end and one sticky end. So, this sticky end can be complementary with the vector we can choose such adapters also this adapters are having restriction sites within it. So, new sticky ends can also be created. So, by this way even if there is no common restriction sites are available for matching target and vector we can match them by adding linkers and adapters. So, in this class we have discussed the very basic concepts of recombinant DNA technology, there are steps components which are required for recombinant DNA technology like one very important enzyme restriction endonuclease, how the restriction endonuclease act.

So, for the target sequences different types of restriction sites, how they generate sticky and blunt ends also how the blunt ends can be converted to sticky ends via joining adapters or linkers or by the method of homopolymer tailing. In the next class we are going to discuss the remaining of the recombinant DNA technology. These are my references. Thank you, see you in the next class.