Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 12 : DNA Cloning and Recombinant DNA technology -II

Namaskar. Welcome back to the lecture series of Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis. We were discussing the different tools of molecular diagnostics and gene expression analysis. We are at the second part of DNA cloning and recombinant DNA technology. In the last class, we have discussed cloning, the basic concept, different steps. Then we have discussed one very important enzyme restriction endonuclease, their function, the different types, the different types of ends which have been which can be generated by restriction endonuclease that is tickings and blunt ends.

Different examples of restriction endonucleases we also have discussed. Today we are going to start with the cloning vectors. So, if you remember this diagram, you know that initially the target DNA also the vector DNA are cut by the same restriction endonuclease. So, the very basic concept of vector is that vector is basically a small fragment of DNA of very small DNA sequence into which the foreign DNA our target DNA can be inserted.

And that inserted DNA inserted target DNA along with that small DNA sequence can be transported and introduced in inside the host cell, the cell which we want to infect or where we want to deliver our target DNA. Inside that host cell that vector containing our target DNA can stable recite, can be maintained maintaining their nature and characteristics and can replicate autonomously. That means, when this small DNA sequence the vector DNA sequence replicates along with that the foreign DNA insert will be replicated. So, this small DNA sequence is our vector. So, basically vector is nothing, but a transport vehicle via which we are transporting our insert or target DNA inside the host cell.

Now, when there is this combination of the target DNA along with the vectors DNA, what we get is the recombinant DNA where the transgenes present as well as the vectors own nucleic acid backbone is also present. Now, there are few very common

characteristics of all different types of vectors. There should be one origin of replication. Now, if you remember from our very first weeks class that is regarding the replication that replication start from a specific sequence that is a signal we sometime call it Ori C. Now, that Oris C or the origin of replication must be present in a vector.

As I told you the vector can replicate autonomously inside the host cell. So, their replication system must be complete and adequate and that must not be dependent over the hosts replication system. Then there must be some cloning sites as a point of entry of the foreign DNA. Now, you can see in this picture that this plasmid plasmid is a type of vector. So, plasmid is having this sequence that is the cloning site or the recognition site by a restriction endonuclease.

So, if you remember this is the recognition site or restriction site identified by the restriction endonuclease eco R 1. Why this is required? So, that we can simultaneously treat the vectors DNA as well as our donor or target DNA by the same restriction endonuclease. So, that we get sticky ends which are complementary to each other and can be joined easily. Now, these days as we are I mean we are engineering genetically. So, MCS can be introduced.

What is MCS? MCS is multiple cloning sites. So, vectors are organized in such a way that inside the vectors DNA via genetic engineering a cluster of different restriction sites can be introduced. So, that that sequence can be identified by multiple restriction endonucleases. So, that segment is known as MCS or polylinker and the digestion of the target and as I told you the digestion of the target and vectors must be done by the same restriction endonuclease. The next characteristics is there should be one selectable marker.

Now, what is selectable marker? Selectable markers basically help us to identify the successful cloning. How? Here you can see one such selectable marker is the different antibiotic resistance gene. So, there are genes which imparts resistance to antibiotics for different bacteria. Now, consider if a plasmid is having this antibiotic resistance gene sequence like ampicillin resistance gene sequence, tetracycline resistance gene sequence or chloramphenicol resistance gene sequence. So, if we grow this plasmid over a media containing these ampicillin tetracycline or chloramphenicol antibiotics they will not die.

Now, what is done? If we introduced our target DNA segment in between this gene antibiotic resistance gene. Suppose a genes target gene segment it introduced here we have introduced a cut by restriction endonuclease and inside this we have introduced our target DNA. What will happen? This chloramphenicol resistance gene is broken it will not manifest. So, if we grow this plasmid over a media containing chloramphenicol it will die. It indicates that yes the formation of recombinant DNA is successful or the

target DNA is successfully inserted within the plasmids DNA.

Also there are different types of reporter genes reporter genes are just like the reporter they are reporting these genes are reporting that yes there is successful cloning. Now, one such example is blue white screening. Now, what is blue white screening? Blue white screening is basically where the plasmid is having a gene that is lacZ. Now, this lacZ is synthesizing an enzyme which is known as beta galactosidase. Beta galactosidase is the enzyme which is responsible for breaking the beta glycoside bond.

Beta glycosidic bond is present in lactose lactose is broken down to galactose and glucose via this enzyme. Now, consider there is a gene this is our this blue region is our lacZ gene. Now, if this plasmid vector is grown over a media containing a lactose analog. What is that lactose analog? That is X gal. Now, X gal is the analog of lactose where is the this X is basically representing a dye that is 5 bromo, 4 chloro, 3 indole derivative.

Now, what happens if this plasmid is grown over a media containing this X gal? It will express this beta galactosidase enzyme. This beta galactosidase will break this bond. So, this BCIG dye is released in the media and this they will dimerize and will form an insoluble dimer of this BCIG which is of blue color. The monomer or this X gal is basically colorless. So, wherever there is the growth of this plasmid there will be blue color due to the presence of or the expression of beta galactosidase.

Now, if we insert one foreign DNA within the lacZ gene definitely you can see the lacZ gene is broken here within this lacZ gene the foreign DNA is introduced. So, they will not be able to express the beta galactosidase. So, what will happen? There will be white colonies because X this beta galactosidase bond will not be broken. So, the the colony will remain colorless. Whereas, if the insertion is somewhere else not within lacZ or if there is no insertion in those cases there will be blue colony.

So, if we see the media you can see these are the white colony. So, these are the successful colony where the foreign DNA is inserted in the right target place by breaking the lacZ gene. So, this is known as insertional inactivation. Due to the insertion of the foreign DNA there is inactivation of the lacZ gene. Similarly, there are other reporter genes as well like some fluorescent, green fluorescent protein they are manifested like green fluorescent protein in presence of the enzyme luciferase and a chemical luciferin produces green fluorescent colony.

And if we insert a foreign DNA within that green within the gene of green fluorescent protein in that case the fluorescent will not be there. So, these are the reporter genes. Now, we are coming to one very special vector that is expression vector. Now, what is

expression? We call a gene is expressed when the genes target protein is synthesized. That means, for a successful expression of a gene there should be a successful mRNA formation that is a successful transcription after that there should be successful translation that is the expression of protein.

So, an expression vector must contain all the all the characteristics of a common vector we discussed just now. Along with that there should be the machineries which are responsible for transcription like promoters, promoter termination sequence also the required machinery for the successful translation like translation initiation sequence top codons like that. So, these type of vectors is responsible for the final expression of our target protein. And that expression can be either constitutive or can be induced. Now, in case of induced expression these expression vectors contains the sequence of inducers or enhancers as well.

So, that we can express the or the induce the expression of our target proteins. So, this is expression vector. Now, we are coming to the different types of vectors the vectors are of different types based on the size of the target DNA we want to insert. So, there are different types of vectors we are going to discuss all these 4 types of vectors.

So, the first one is plasmid. Now, plasmid this is one this is a type of extra chromosomal double stranded circular small DNA molecule which is found commonly in different types of bacteria. Sometimes it is also found in archaea and eukaryotic organisms as well. Now, this plasmid DNA is basically physically separable from the chromosomal DNA of the bacteria and it can replicate independently and autonomously from the bacterial DNA or the host DNA. Now, the size of trans gene which can be inserted into the plasmid vectors DNA is up to 10 kilobytes. So, basically we based on the sizes we are going to discriminate or categorize different types of vectors.

Now, these are the examples of few vectors now PBR 322, PUC 18, F plus these are the different types of vectors. Now, one very important thing regarding this PBR 322 is that this is one very initial vector which has been identified and designed. Now, this is our PBR 322. So, you can see in these PBR 322 there are multiple types of segments. So, basically from different naturally occurring plasmid this PBR 322 is genetically engineered in such a way that different segments of different naturally occurring plasmids are joined via restriction digestion or by treating different restriction enzyme.

So, you can see in this PBR 322 there is a MCS site or multiple cloning site where is where you can see a cluster of restriction sites present as well as there are this antibiotic resistance gene as selectable markers. Now, why the selectable markers are required? You see there are some production of unwanted product. So, the successful one is where in a plasmid suppose this is our target DNA. So, it is properly introduced. So, this is the

successful or the wanted product.

What are the unwanted product? Because as we told the restriction enzymes are giving rise to the sticky ends which are complementary to each other. So, what can happen this target DNA? Two target DNA's can join with each other. So, that is a dimer formation transgenes or inserts dimer formation. Similarly, the plasmid two plasmid DNA can itself join and can form a plasmid dimer. Without this insert the plasmid can rejoin or the direction can be opposite as well.

So, these all are unwanted products. So, and those unwanted products are can be identified by agarose gel electrophoresis differently the sizes will be different or by selectable markers. Now, how this selectable markers help? We have discussed the blue white screening as well as the antibiotic resistance gene how they are helping. So, consider we have treated the target DNA and the plasmid both with the BAMH1 restriction enzyme which is giving rise to sticky ends which are complementary to each other. I am repeating this because this is very important and you must remember. Then what will happen because there is a cut this tetracycline resistance gene will be broken fine.

Now, if we grow this plasmid over a media containing ampicillin antibiotic definitely they will grow because the ampicillin resistance gene is intact. Now, it is intact for the wanted product as well as all these unwanted product. So, by this we cannot differentiate. So, what we will do? We will form another plate with the same replica. So, these colonies are same and here in the media we are incorporating the antibiotic tetracycline.

Now, because the tetracycline gene is broken in our unwanted product, sorry in our wanted product what will happen? The cells which are successfully growing they will be the successful clone. So, by selecting this so, these colonies are unsuccessful because they are growing over the in presence of tetracycline which are absent. So, you can see this is absent. Similarly, this is absent. So, what we how we can identify? Here in these colony we are identifying the successful clones.

So, these are the colonies which are absent here. So, they are the successful wanted product. So, the blue white screening also can be done in case of PBR 322. Now, we are coming to the viral vector. So, one such virus is bacteriophage, bacteriophage which can be which is grown inside E.

coli and they are having these features head, tail and body. This is the capsid which which actually encapsulates this genetic material of the bacteriophage. Now, in the bacteriophage the insert which can be introduced that their size can be up to 53 k B. The

very common bacteriophage which are used are lambda phage, M 13 phage. Now, what happens in these bacteriophages they are having two different cycle lytic cycle and lysogenic cycle.

What happens? When introduced suppose the common very common bacteria is E. coli within which the lambda phage is introduced. So, this is our lambda phage which is introduced in the E. coli's in the bacterium. Now, in case of phage the bacteria are having lysogenic path what happens the DNA phage DNA is incorporated or integrated with the bacterial DNA and remain dormant.

Till there is some trigger which causes the replication or amplification of the phage DNA. Finally, the phage is forming the capsid head and tail and causes host cell lysis that is the lysogenic path. In contrary lytic path is where the introduced phage DNA starts amplifying immediately causing the lysis of the cell. Now, why I am saying this because there are two types of phage vectors one is insertion vector another is replacement vector. Now, in case of insertion vector insertion vector must be digested by one restriction endonuclease and they are cutting and opening up the DNA.

They are creating space where the target sequence can be introduced. So, we cannot introduce larger fragments or larger segment of DNA in case of insert vector because much the size increases the transfer the replication of the vector goes low. In case of insertion of replacement vector we replace a segment of DNA what are those segment the lysogenic phases that is genes which are causing the lysogenic cycle those are unwanted they just help to remain dormant. So, we are removing this part where the lysogenic where a part of lysogenic gene segments are present and the phage DNA is is digested by the different two different restriction enzyme causing two different restriction sites where the insert is incorporated and then it is allowed to replicate that is the importance of lytic cycle and lysogenic cycle. Then these phages are having cost sites or cohesive sites.

Now, these cohesive sites are presents just at the terminal part of the phage DNA where the terminal 12 base pairs of these nucleotides are basically representing the restriction sites. Those two sites might be asymmetric, but they are restriction sites similar restriction features are similar they are complementary to each other can be joined. So, what happens the linear phage DNA can be circularized can join inside the bacteria and can reside peacefully and that is helpful because different types of phages DNA can be joined then can be incorporate incorporated in one phages capsid and that is known as in vitro packaging. Now, this in vitro packaging and the cost site is exploited in some other vector as well what is that that is cosmid. So, cosmid is just the variant of plasmid where the phages cos site is incorporated.

So, cosmit are the plasmids with the cos sites which helps in packaging in vitro packaging of the DNA in the lambda particles. Definitely the cosmids because they are more modified version here we can incorporate a larger size of the target genome DNA that is 28 to 45 k B. Now, for the mapping of the genome or for the sequencing purpose we need some larger segments to be incorporated because if we want to locate some unknown segment what we need to do the whole genome we need to scan or all the segments we need to scan. Now, imagine if the if the whole chromosome is the whole genome is segmented in 100 segments 100 small segments scanning 100 segments for 100 times is a difficult thing instead of that if we can divide or fragment this within suppose 5 segments. So, the scanning is very easy for any sequence, but who will incorporate those larger segments for that we have designed artificial chromosomes.

Now, there are multiple types of artificial chromosome, bacterial artificial chromosome, yeast artificial chromosome, mammalian artificial chromosome, but amongst those much in use are basically yeast artificial chromosome and bacterial artificial chromosome where by the name you can assume or rather guess that the artificial chromosome is basically derived from yeast and ligated in bacterial plasmid that is why it is yeast artificial chromosome. Similarly, one artificial chromosome where the chromosome is extracted or created in E. coli that is bacterial artificial chromosome the transgene or the insert size definitely varies in case of bacterial artificial chromosome up to 300 k B bacterial the transgene can be introduced whereas, in yeast artificial chromosome up to 100 kilo base pair of DNA insert can be introduced. Now, there are different types of introduction process after forming this forming this what I can say what after forming this recombinant DNA those needs to be incorporated inside the host organism. So, there are multiple techniques like transformation, transduction, transfection.

Now, transformation is basically the direct uptake by the host and here basically non viral DNA are transfer into the bacteria. The very common method you all know conjugation where two bacteria connect with each other and exchange their genetic material. So, in that way or in some other different ways directly the bacteria can take up the exogenous genetic material. Whereas, a foreign DNA when introduced by a virus or a viral vector that is known as transduction. Now, transduction can be done in non touch technique and when this introduction of recombinant DNA is done inside then eukaryotic cells via creating transient pores in cell membrane that is known as transfection.

So, transfection is basically deliberate introduction of the genetic material. Now, deliberate introduction definitely can be done must be done by deliberately giving some external force. What are those forces that can be physical force like electroporation, sonoporation, micro injection, electroporation you know by creating electrical impulse we are creating hole over the cell membrane. Similarly, high frequency sonic waves can

be given micro injection there are different micro needles through which the genetic material can be inserted then chemicals.

So, one very common chemical is calcium phosphate. What happens the media containing calcium chloride a very common media is hepatitis media containing calcium chloride is mixed with sorry media containing phosphate and the calcium chloride which contains the DNA or the target DNA they are mixed together. So, what happens the phosphate and calcium chloride they react and form a calcium phosphate which is precipitate precipitated taking the target DNA and then it is incorporated inside the host. Liposome based transfection can be done liposome is a variant or synthesize synthetic variant of the cell membrane that is a phospholipid bilayer similar to the to our cell membrane. So, and it can easily incorporated and pass through the cell membrane and enter the organ organism cell. So, via liposome we can deliver the recombinant DNA also that can be delivered by nano particle.

Apart from this physical or chemical forces there are different viral mode of introduction. So, different virus like adenovirus lentivirus they are also used to introduce recombinant DNA via transfecting the cell with this type of virus. So, in this class we have discussed different types of vectors like plasmid viral vectors, fudge vectors, cosmid, artificial chromosomes and how the recombinant DNA is introduced in the host cell that is the method of transfection which is done by different chemical and physical methods and also via some viral vectors. These are my references see you in the next class. Thank you.