Molecular Biology

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Module - 10

Molecular Cloning

Lecture-42 Cloning Vectors

DNA selection marker and the bacterial origin of the applications before vector delivery to yeast it is digested with the unique restriction endonuclease to produce linear DNA to increase the transformation efficiency and the integrations.

In most of the cases integration is done in such a way that the yeast chromosome DNA remain intact and integration may not affect the yeast growth. But in an alternate approach a portion of the yeast chromosome DNA is replaced with the vector DNA through the homologous recombination. These vectors are known as the transplant integration vector and they have the foreign DNA selection marker and homologous DNA into the region of chromosomal DNA to be replaced. Then we can also have the third type of vector which is called as a yeast artificial chromosome or YAC vector.

So, yeast artificial chromosome is the vector of the choice used for cloning very large DNA fragments. Remember that when we were talking about the preparation of the human genomic library we have said that we are going to clone that into the YAC vectors. To prepare the genomic library YAC vector is like a chromosomal and it has the ARS sequences centromere sequences and telomeres at the two end to give the stability. It has an MPC resistance genes for selection in E. coli and an E.

coli in the E. coli in the E. coli in the original replication for the propagation in bacteria. In addition it has the ARS for replication, SEND for the centromere functions and the URA3 and TIP211 for the selection in the yeast. For cloning YAC is digested with the enzyme called SM1 and BAMH1.

So, you are going to digest the YAC with the SM and BAMH1 and you are going to treat that with alkaline phosphatase to generate a linear plasmid. Now foreign DNA is added for the ligation. So, at this stage you are going to add the foreign DNA and that's how it is actually going to get inserted into these two fragments and the recombinant DNA will allow a yeast to grow on a uracil and tryptophan deficient media. So, screening anyway we are going to discuss in our subsequent class. Then we have the eukaryotic plasmid, another eukaryotic plasmid which you can use in the insect cell lines.

So, these are the vector for expressing the protein in the insect cell lines and or the baculovectors right. So, baculovirus is a rod-shaped virus infecting the invertible bait including the insect cells. Post infection the virus is either released as a free viron or many virus particles are trapped in a protein complex known as polyhedron. The protein responsible for this is polyhedron. The protein responsible for trapping the virus into polyhedron is polyhedron and it helps in the transmission of virus from one host to another.

The polyhedron is not important for virus propagation, but it is under very strong promoter to produce the protein in large quantity. So, realizing this fact the replacement of the polyhedron gene with a foreign DNA fragment will allow the expression of the protein in a large quantities. So, the baculovirus autografa california multiple nuclear polyhedron virus or the ACMPNP this is what is used as a vector to express the protein. The transfer vector map of the ACMPNP is given right. This is the vector the map of the ACMNPB where you have the cloning site.

This is the cloning site what you have the gene of interest will be inserted into the cloning site place adjacent to the promoter. So, you have the this is the polyhedron gene promoter right and it has the polyhedron termination sequences downstream to the cloning site to stop the transcription of the clone gene and or more will be discussed in future lectures. So, this is what you have you have the ACMPNP vector where you have the polyhedron promoters and next to the promoter you have the cloning site. So, within this cloning site you can actually be able to insert the gene of your interest right and that's how it is going to start expressing this particular protein instead of the polyhedron polyhedron and it also has the termination sequences. So, that the transcription is going to stop after this and you can actually be able to take this and put it into the insect cell lines and that's how you're going to express.

Then we have the eukaryotic vectors like the mammalian vector. So, large number of excellent mammalian vectors are in circulation to clone the eukaryotic gene for the protein synthesis and to study the transcription mechanisms. It contains a eukaryotic replication of origin from an animal virus such as SV40 from a simian virus a promoter to derive the expression of foreign gene and the selection marker and the other eukaryotic features such as adenylation, transcription termination, etc. So, this is what is the mammalian expression vector where you have the multiple cloning site, you have the promoter and you have the other features of the plasmids. You can also have the origin of replication for the eukaryotic system.

You can also have the origin of replication for the bacterial system. Then you have the antibiotic resistance genes and so on. Then we have the bacteriophage based vectors. So, bacteriophage lambda based vectors are the virus using the bacteria as their host for replication. Bacteriophage lambda is the virus of E.

coli and have been used to develop vector for the genetic recombination. So, what you have is a bacteriophage genome. So, far genome is a linear double standard DNA of 48.5 kB. On both end of the genome, it has a stretch of 12 nucleotides which are complementary to each other.

So, you have the two sites on both end of the genome. One is called as the left cohesive site, the other one is called as the right cohesive site and within this you have the different region of the genome which is expressing for the different part of the body. So, these sites are called as cosites and it allows the circulation of the viral genome after entering into the host cells. Genes are arranged between these two cohesive sites of the code for the protein responsible for making the head, tail, factor for recombination and the process of lysosin. The central region of the genome is non-essential and can be replaced without much affecting the growth and the infectivity of the virus.

As a result, this region can be exploited to develop a cloning vector with multiple approaches. So, how it is actually going to pack the genome? So, the far genome is replicated by a rolling circuit model to produce a long genome whereas cosites are present on the regular interval, right? So, when it is actually going to produce the far genome into the outside, right? And it is actually going to start producing the cosites. So, you can imagine that you have one cosite, you can have another cosite and once these two cosites are going to be out, they are actually going to come together and they will get circle. The two flanking cosites and the DNA between them constitute the viral genome or the monomeric unit. In the presence of head precursor, the long genome is cleaved into the monomeric unit and encapsulated.

Nicks are introduced on both the strand of the genome to generate the linear strand to serve as a cohesive site to facilitate the circularization in the host. Okay, so this is what. The bacteriophage lambda cloning vector has a middle segment responsible for the insertion or excision and this region can be replaced with a foreign DNA with the help of the two BMH1 sites present on the either side of the insertion or excision regions. Lysosin cycle, lytic cycle and it will form the plaques. So, bacteriophage vector are the EMBL-3 and EMBL-4.

So, this is what exactly going to happen, how you are going to insert the foreign DNA into a IE site. Okay, so this is what all about the different types of vectors what are available for the cloning of the foreign DNA or cloning of the enzyme into the suitable expression vector and depending on the production, depending on the origin of that particular gene, you can be able to have the flexibility to choose the different types of vectors, right. You can use the mammalian expression vector, you can use the E. coli expression vectors and so on. Now, once you have chosen the vector, so that is one thing.

The second is you have only know the enzyme, then you are actually going to use them together to generate the recombinant DNA. Now, how you are going to do that is you are

going to run the parallel multiple reactions. So, what you are going to do is first from the genome, you are actually going to generate or you are going to produce the fragment. So, you are going to produce the fragment, right. Similarly, from the vector you are going to have the circular vector.

Okay. This circular vector will have the multiple cloning site and within the multiple cloning site, you are going to have the, you are going to introduce your insert. Now, for this fragment you are going to generate or you are going to produce the restriction enzyme. So, for example, you can treat it with the two restriction enzyme, restriction 1, 2. So, it is actually going to produce the you know the fragments with the cohesive ends and these two cohesive ends like for example, this is for the restriction enzyme 2, this is for restriction enzyme 2. Same is true for the vector also.

So, vector also you are going to digest with the help of this and that, right. And, okay, so these are the restriction enzymes. So, again the same set of restriction enzyme you are going to use for cutting the vector also. Then you both took for the ligation reaction.

Okay. Just now as we have discussed in the previous lecture, right, and once you put the ligation reaction, you are going to have the recombinant DNA with your insert into the multiple cloning site. Okay. So, this is going to be your chimeric DNA or recombinant DNA. Now, this chimeric DNA you are going to transform into the host.

Okay. And the host is going to have this, right. So, since this restriction site this plasmid is going to have the origin of replication, it is actually going to replicate. So, depending on the genome, depending on the origin organisms from which you are isolating this gene, you can be able to choose the different types of vector. And depending on what kind of applications, if you want the protein or the enzyme in milligram range, you might be good to use the equalized system. If you want to use the higher level, then you can use the yeast expression vectors.

Similarly, if the genome is or the organism is very close to mammalian system, then you should use the mammalian expression system or the yeast expression system because that will give you the properly folded proteins. So, this is all about this, you know, the cloning of the particular enzyme into the suitable vectors. And now you have, what you have done is you have generated a recombinant DNA, right. So, once you have, so this is the recombinant DNA what you have generated, right. Now, once you generated the recombinant DNA, it has to be transformed into the host and then you are going to use that for the protein production or the enzyme production.

So, in our subsequent lecture, we are going to first discuss about how you are going to insert or how you are going to, you know, devise the different types of strategies to deliver the DNA, recombinant DNA into the host of your choice and subsequent to that, we are also

going to discuss about the protein production. So, with this, I would like to conclude my lecture here. Thank you. .