Enzyme Science and Technology
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Module - III
Enzyme Production (Part 1: Cloning)
Lecture - 16
Cloning of Enzyme Coding Gene

Hello everyone this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme in the course enzyme science and technology and in this context. So, far what we have discussed we have discussed about the development of the enzymology, we discussed about the different aspects or the of the enzyme properties and then in the previous module, we have also discussed about the structure of the enzymes.

So, we discussed about the primary structure secondary structure tertiary structure and quaternary structures and in this particular module we are trying to understand and discuss how we can be able to produce the enzyme in the bulk quantities. So, that you can be able to use that for the different types of applications or for studying the different aspects of the particular enzyme.

So, in this context so far what we have discussed we have discussed about how you can be able to isolate the enzymes whether the genomic sequences are known or whether the genomic sequences are not known right. So, when the genomic sequences are not known you are actually going to screen the genomic library or the CDNA library and that is why you are going to get the gene fragments of your choice and that you can be able to clone into a suitable expression system.

Similarly, when the genomic sequences are known you can use the polymerase chain reactions and that is how you are going to get the gene fragment and that you can be able to clone into the suitable expression vector and that you can be used for the protein production.

Now, as far as the scheme for the enzyme production is concerned you are going to first take the gene fragment you are going to isolate that from the genome either of these

approaches what we have discussed and then these gene fragments has to be digested with the restriction enzyme.

So, that you are going to generate the cohesive ends and similarly you are going to use the vector for and it is also going to be digested with the similar set of restriction enzymes and that is why you are going to have the cohesive ends on to the insert you are also going to have the cohesive ends on to the vector then you will going to put them together into a legation reactions.

And that is why you are going to get the ligated kinematic DNA and that ligated kinematic DNA you are going to transform into the suitable host and that transformed suitable that transform host can be used for the protein production or the enzyme production.

So, in this particular type of scheme you are going to use the different types of enzymes you are going to use the restriction enzymes you are going to use the polymerases you are going to use the ligases and alkaline phosphatase. So, these are the some of the things what we have discussed in our previous lecture. So, we have discussed about the restriction enzymes different types of restriction enzymes.

So, type 1 restriction enzyme, type 2 restriction enzyme and type 3 restriction enzymes and then we have also discussed about the properties of the type 2 restriction enzyme which can be used for the cloning purposes such as the type 2 restriction enzymes are producing the palindromic sequence, they are recognizing the palindromic sequences and they are actually generating the sticky ends.

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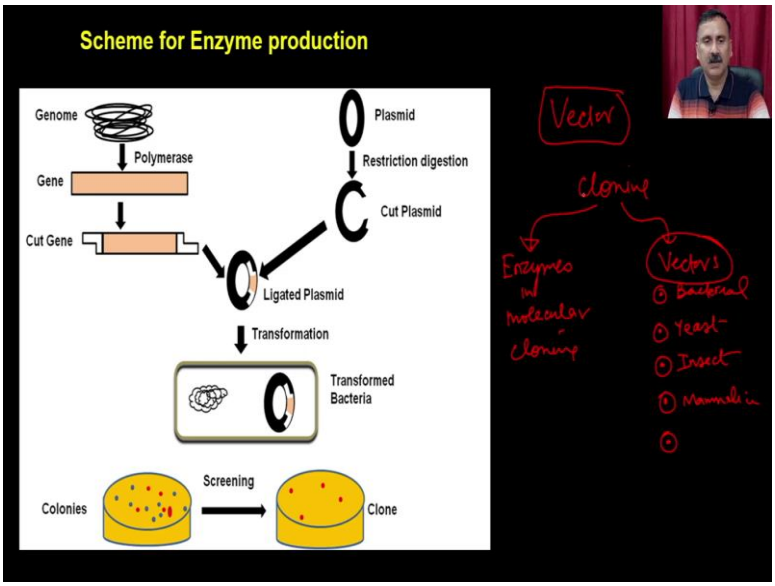

Now, in today's lecture we are going to discuss more about the different types of vector. So, as far as the cloning is concerned right the cloning is require the knowledge of two components one you are actually going to have the proper knowledge of the enzymes which are going to be used in the molecular cloning. So, enzymes in the molecular cloning or you can also be able to use the different types of vectors and vectors are always being specific to the particular host.

So, you can actually have the vector which are for the bacteria bacterial system you can have the vector which is for the eukaryotic system. So, within the eukaryotic system you can have the vectors which are for the yeast you can have the vectors for the insect cell lines or you can also have the vector which are for the mammalian cell lines ok and then also you have the bacteriophage based vector which are also going to be used for different types of expression studies.

So, in today's lecture we are going to discuss about the different types of vectors and how you can be able to use them for the cloning of the particular gene.

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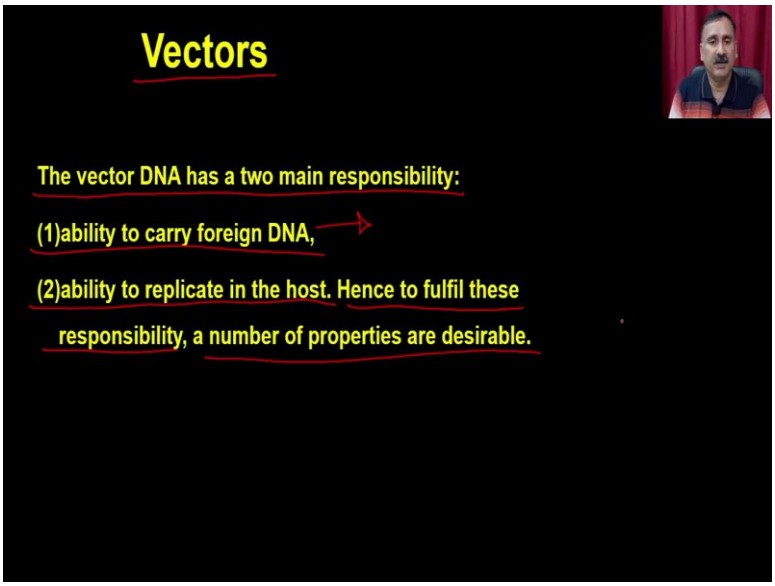

So, when we say about the vector, vector is a DNA which has two responsibilities one it has the ability to carry the foreign DNA, this means it should have the instruments and it should have the region where you can be able to insert the foreign DNA then it also have to have the ability to replicate in the host which means hence to fulfil these responsibility a number of properties are desirable right.

This means if you want to design a vector or if you want to analyze a vector you should follow the certain criteria for checking its suitability and as well as the you can check the different properties.

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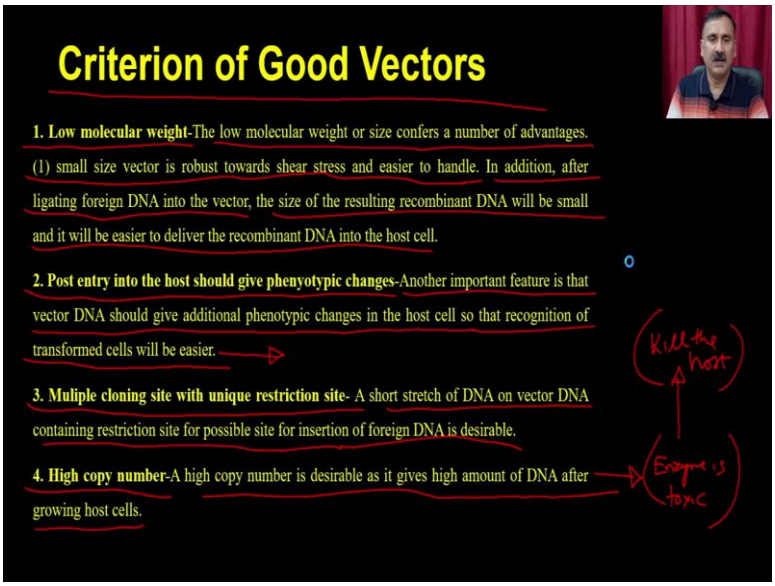

So, what are the different criterias one can use for choosing the good vector? So, good vector should be of low molecular weight, the low molecular weight or the size confers a number of advantages. For example, the small size vector is robust towards the shear stress and it is easier to handle.

In addition, the after ligation the foreign DNA into the vector the size of the resulting recombinant DNA will be small and it will be easier to deliver the recombinant DNA into the host cells. Because once you generate the recombinant DNA the recombinant DNA has to be transformed or delivered into the host and then only you can be use that for a that recombinant DNA for the protein production or the enzyme production.

So, if the size of the vector itself is going to be very high then the when you put the recombinant DNA the resulting size is also going to be even bigger. So, in that case sometime people also face the you know the delivery issues like you cannot deliver that big DNA ok then the post entry in the host should give the phenotypic change that is very important right.

Another important feature is that the vector DNA should give you the additional phenotypic changes in the host cell. So, that the recognition of the transformed cells will be easier which means it should show you some phenotypic changes like the cell should turn like green. So, if you imagine that if I could easily be able to recognize the cells which are getting the DNA or which are not getting the DNA simply by the fact that the

DNA containing cells are going to be green in color right or blue in color or white in color

So, in that kind of phenotype is going to help me in terms of identifying the transform host then it should have the multiple cloning site with a unique restriction site. So, a small stretch of DNA on a vector contain the restriction site for possible site for insertion of the foreign DNA and that is desirable because that is the place where you are going to cut the vector and you are going to insert the foreign DNA.

Then it should be a high copy number. A high copy number is desirable at gives the high amount of DNA after growing the host cells. There are stances when you can avoid the high copy number like for example, if the enzyme is going to be enzyme is toxic for example, to the cell.

So, in those cases if you take the high copy number it is actually going to give you very high quantity of protein and that may actually going to kill the host right. So, in those cases only you are going to go with the low copy number plasmid or low copy number vector, but in all other cases the high copy number is desirable.

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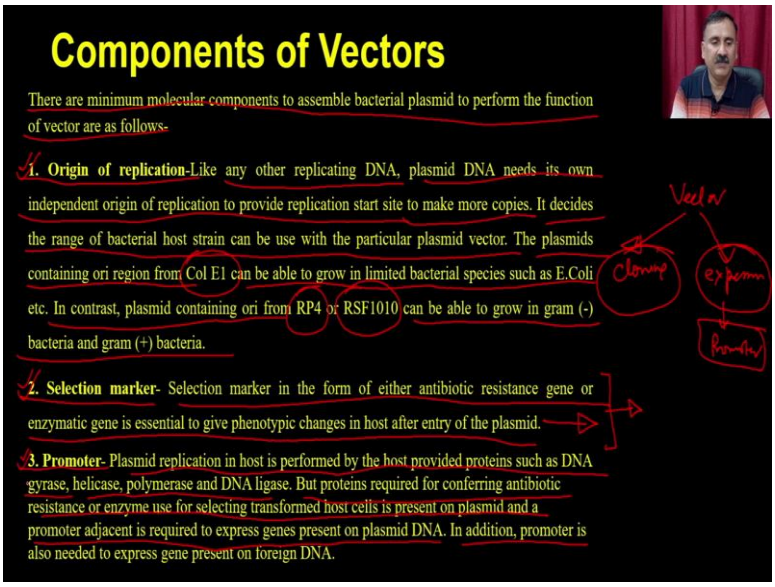

Now, if I have to design a vector what are the different components I should use? So, there are minimum molecular component which are required to assemble the vector to perform the function as a vector for example, it should have the origin of replication.

Like other like any other replicating DNA, plasmid DNA needs its own independent origin of replication to provide the replication start site to make the more copies. It decides the range of the bacterial host strain can be used with the particular plasmid vector.

The plasmid containing the ori region from the Col E 1 can be able to grow in limited bacterial species such as E Coli in contrast the plasmid containing the origin of replications from the RP4 or RSF11010 can be able to grow in gram negative as well as the gram positive bacteria which means depending on the origin of replications you can be able to decide the range of the host what you can actually be able to use.

Then you also require the selection marker selection markers in the form of either antibiotic resistance genes or the enzymatic gene is giving the phenotypic changes in the host after the entry of the host. So, this is the we are going to take a very you know many examples when we are going to talk about the screening of these transformed clones. So, that time you will understand how the different types of selection marker is actually helpful in you know in selecting the transformed host.

Then we also require the promoter. So, plasmid replication is host performed by the host provided protein such as the DNA gyrase helicase and all that, but protein required for conferring antibiotic resistance or the enzyme used for selection transformed is present on plasmid and as a promoter adjacent is required to express gene present on the plasmid DNA. In addition, promoter is also needed to express the gene present on the foreign DNA.

So, when we talk about the vector, vector could be of two types vector could be of cloning vectors and vector could be of expression vector ok. So, if you are talking about the cloning vector you only require the origin of replication and the selection marker ok. But when you talk about the expression vector because you also require the promoter because the promoter is the region where the all the transcription factors and the transcription machinery is actually going to bind then you also require the promoter.

So, if you want to make a expression vector then it also should have the promoter also.

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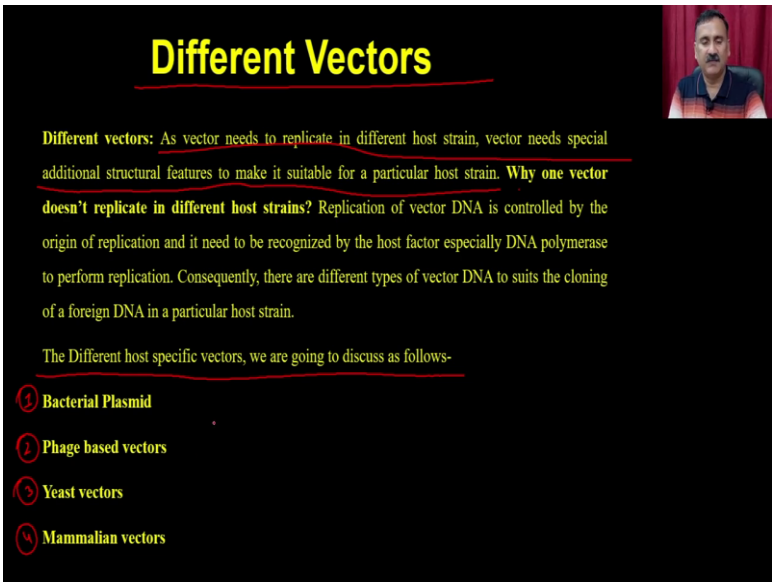

We have different types of vectors. So, different types of vectors as vector need to be replicate in different host strain vector need the additional features to make it suitable for a particular host strain. So, the different host strain we are going to discuss is as follows like you are going to discuss about the bacterial plasmid, phage based vectors yeast vectors and then we are also going to talk about the mammalian vectors.

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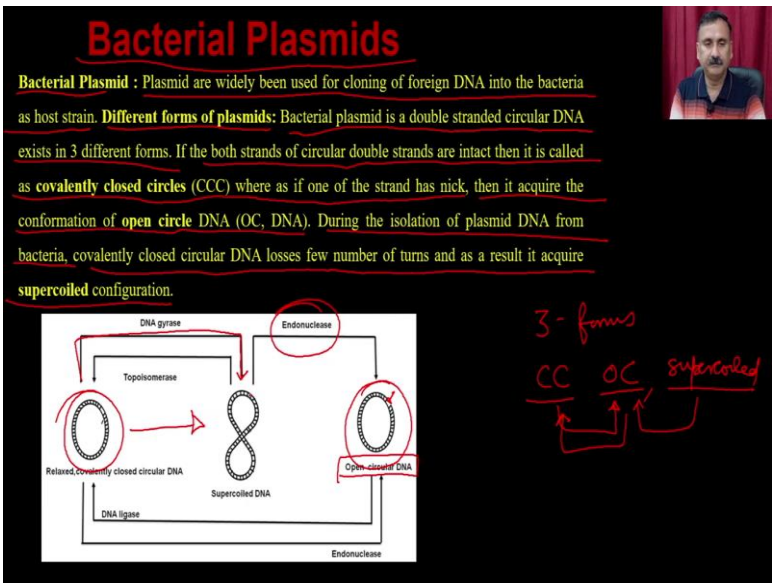

So, let us talk about first the bacterial vector or bacterial plasmid. So, bacterial vector is also called as the bacterial plasmid. So, bacterial plasmid or plasmids are widely been

used for cloning of foreign DNA into the bacteria as a host strain different forms of the plasmid bacterial plasmid is a double stranded circular DNA exists in three different forms if both the strands of the circular DNA are intact.

Then it is called as covalently closed circle whereas, if one of the strands has nick then it acquire the conformation of the open circle DNA. During the isolation of the plasmid DNA from the bacteria covalently circular DNA may lose few of few number of turn as a result it acquire the super coiled conformation.

Which means bacterial plasmid can be present in the three different forms it can be closely circular DNA. So, it can be closely covalently closed circular DNA which means this one, it can be open circular DNA which is this one. So, it can be OC form or it can be super coiled form ok.

So, in the super coiled form this closely circular DNA is also having the getting the additional turns and that is how it is actually going to have the super coiled DNA. And all these DNA forms can be interchangeable to each other. For example, if you have the covalently circular DNA and if you incubate that with the DNA gyrase the DNA gyrase is actually going to induce the additional turns and that is how it is actually going to be converted into the super coiled DNA.

But if you take the super coiled DNA and if you incubate that with the endonuclease it is going to you know cause the nick and that is how it is actually going to be get converted on to the super coiled DNA and so on. So, all these three forms like the covalently closed circular DNA, open circular DNA or the super coiled DNA are interchangeable to each other ok which means all of these can be interchangeable with the help of the different types of enzymes.

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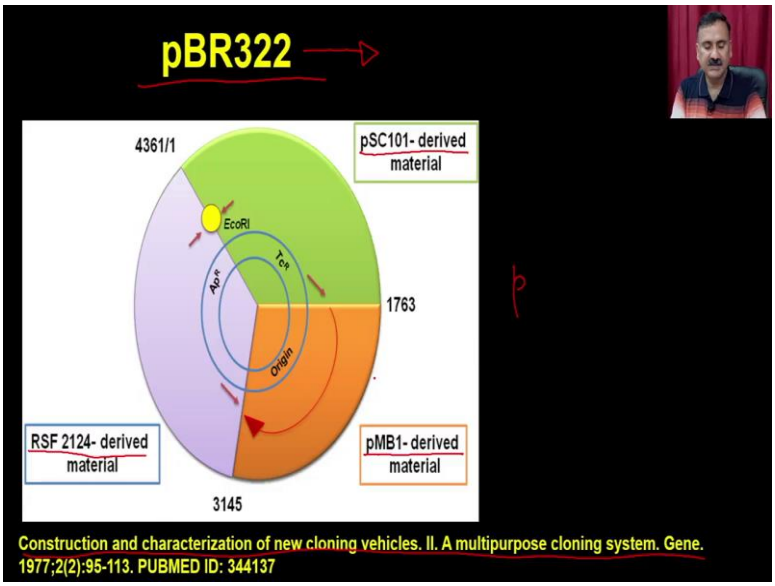

Now, this is the first bacterial plasmid which is been designed. So, it is called as pBR322 and pBR322 is the first bacterial plasmid which has been developed by taking the different DNA fragments from the three different naturally occurring plasmids. So, the what are the different naturally occurring plasmid they have taken?

So, they have taken the pSC101 they have taken the fragments from the pMB1 and they have also taken the plasma the region from the RSF 2124 and that is how they will put together and that is how it is actually going to give you a vector which is called as pBR322.

And this is I have given you the link right with the old paper where they have actually developed this particular plasmid and that you can go through to understand how the people are actually designing the or developing the new plasmids.

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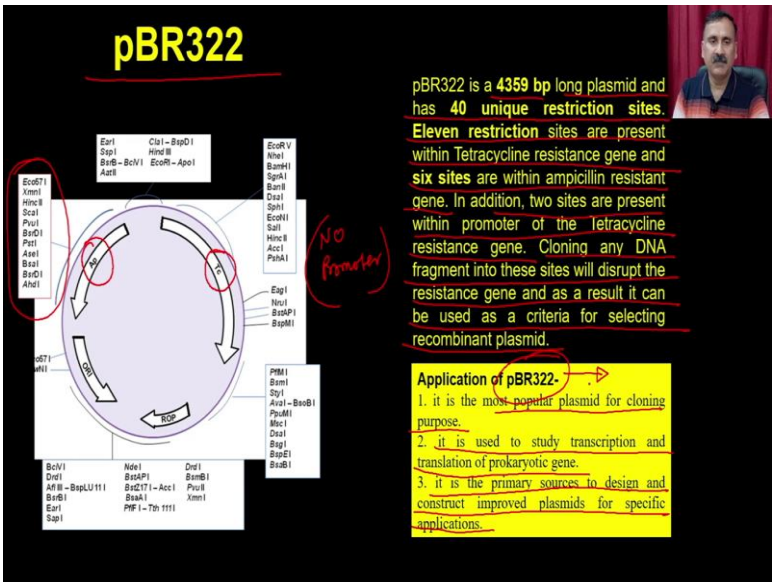

So, pBR322 is a 4359 base pair long plasmid and it has the 40 unique restriction sites. Eleven restriction sites are present within the tetra site resistance genes and six sites are present within the ampicillin resistant genes. So, it has actually two antibiotic resistant genes, tetracycline resistant genes and as well as the ampicillin resistant genes and both of these restriction ampicillin resistant genes or the antibiotic resistant genes have the multiple cloning sites.

In addition, the two sites are present within the promoter of the tetracycline resistance genes cloning of any DNA fragment into the site will disrupt the resistant genes and as a result it can be used as a criteria for selecting the recombinant plasmids. What is the application of pBR322 it is the most popular plasmid for the cloning purpose? So, you can see that there is there is no promoter. So, there is no promoters it means it is actually going to be a cloning vector.

And it is used for studying the transcription as well as the translation of the prokaryotic gene it is primary source to design and construct the improved plasmid for the specific actuation which means pBR22 is a basic plasmid and that was very popular and it was very extensively being used for cloning reactions and it is also been a source for designing the further advanced plasmids such as pUC19 ok.

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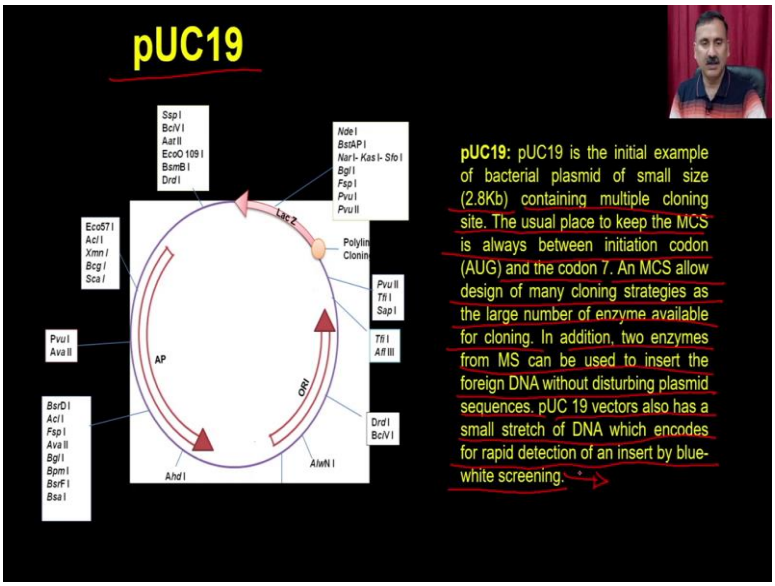

So, pUc19 is a initial example of the bacterial plasmid of a small size. So, 2.8 Kb, you remember that the pBR322 was 4000 base pair. So, containing the multiple cloning site the usual place to keep the MCS is always been between the initiation codon and the codon 7 and MCS allow design of many cloning strategies as a large number of enzyme available for cloning.

In addition, the two enzyme from the multiple cloning site can be used to insert the foreign DNA without disturbing the plasmid sequences. pUC19 vectors also has a small stretch of DNA which encodes for the rapid detection of an insert by the blue white screening. So, do not worry about the blue white screening that anyway we are going to discuss in our subsequent reaction or subsequent lectures.

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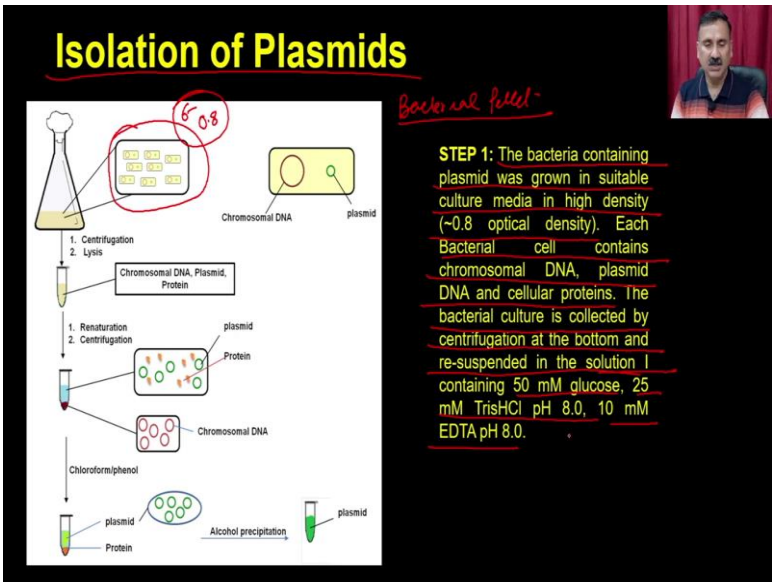

Now, how you can be able to isolate the plasmids. So, first you are going to do is what you are going to do is you are going to transform the you know the bacteria with your plasmid which you want to isolate. So, they will and then you will grow them at ODE of 0.6 to 0.8. So, once you have this culture right you can actually be able to centrifuge the culture and collect the bacterial pallet.

So, first you are going to collect the bacterial pellet and then in the step 1 what you are going to do is the bacterial containing pellet was grown in suitable culture media in a high intensity like 0.6 to 0.8 each bacterial cell contains the chromosomal DNA, plasmid DNA and the cellular protein the bacterial culture is collected by the centrifugation at the bottom and resuspended in the solution 1 containing the 50 millimolar glucose, 25 millimolar tris pH 8 and 10 millimolar EDTA.

So, in the first step what you are going to do is you are going to pellet down the bacteria remove the media supernatant and then (Refer Time: 19:01) this bacterial pallet into solution 1 which contains the 50 millimolar glucose, 25 millimolar tris and 10 millimolar pH 8 EDTA pH 8.

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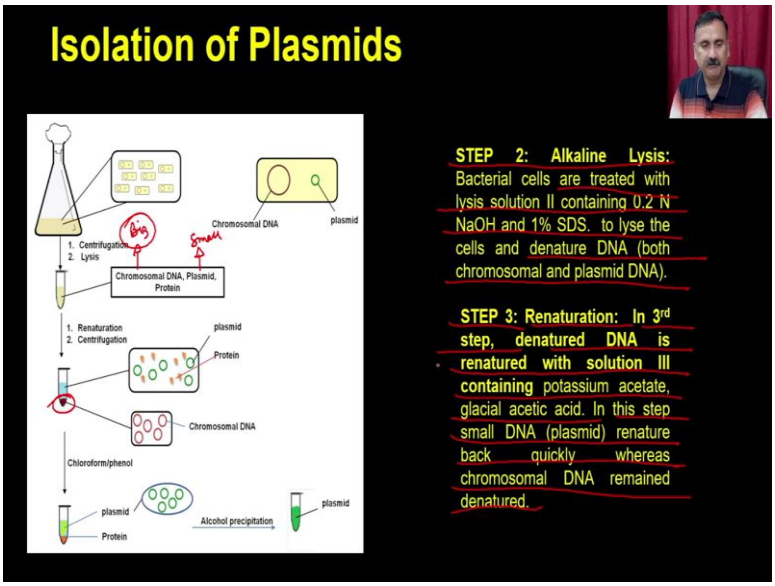

Then in the step 2 you are going to do the alkaline lysis. So, bacterial cells are treated with the solution 2 containing 0.2 normal NaOH and 1 percent SDS. So, what will happen is that the NaOH is actually going to denature the DNA whereas, the SDS is actually going to break up on the cells. And so, it is actually going to lysis the cells and its going to denature the DNA both the chromosomal and as well as the plasmid DNA. Then in the step 3 you are going to do renaturation.

So, in the 3rd step the denatured DNA is renatured with the solution 3 containing the potassium acetate, glacial acetic acid and in this step small DNA renature back quickly whereas, the chromosomal DNA remain denatured. So, this means when you are actually going to have the chromosomal DNA and the plasmid DNA into the alkaline lysis method and when you are going to renature the chromosomal DNA is big right.

So, it is actually not going to be renatured whereas, the plasmid DNA is going to be small compared to the chromosomal DNA. So, it is actually going to renatured back and that is why it is going to be present into the solution whereas, the chromosomal DNA is going to be present in the pellet.

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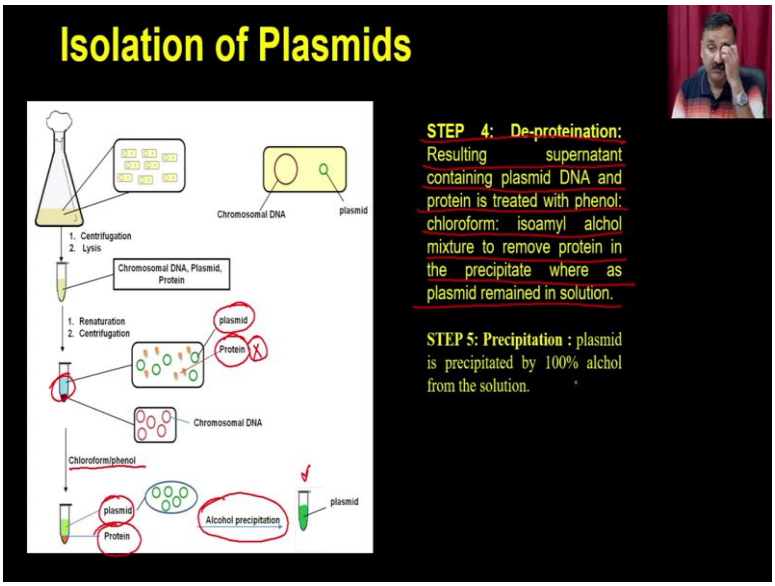

Now, what we have to do is, we have to do we have to remove the protein because at this stage what we have is we have the plasmid DNA and we have also have the small amount of protein. So, what you are going to do is, you are going to treat this supernatant with the chloroform and phenol mixture, right.

So, when you do the chloroform and phenol mixture the chloroform and phenol are going to denatured the proteins and that is why you are going to remove the protein from this ok. So, protein is going to be present in the pellet and the plasmid is going to be present in the supernatant.

So, in the step 4 you are going to do the deproteinations. So, resulting supernatant containing plasmid DNA and the protein is treated with the phenol chloroform isoamyl alcohol mixture to remove the protein in the precipitate whereas, the plasmid remain in the solution. And in the step 5 you are going to do the alcohol precipitation. So, you add alcohol and that is how the plasmid is going to be precipitated by the 100 percent alcohol.

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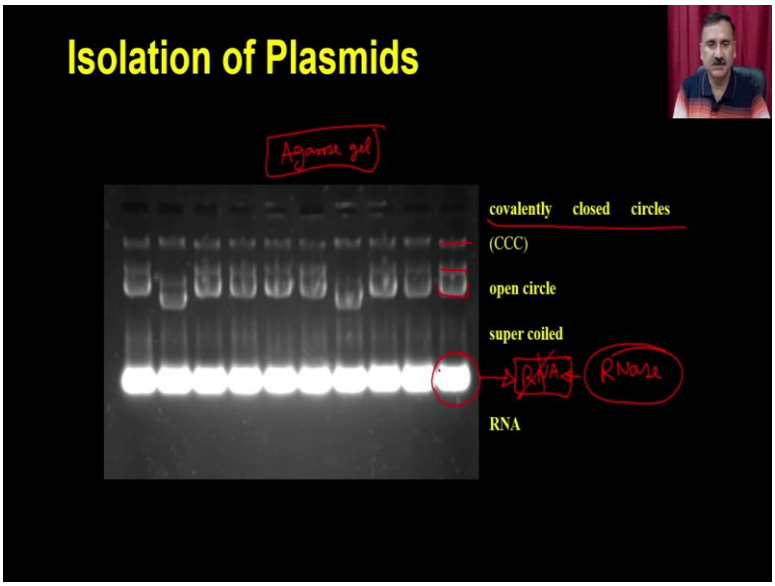

And once you isolate the plasmid you are going to see the classical three different forms you are going to see the covalently circular DNA and if you analyze them onto a agarose gel. So, when you isolate the plasmid and you analyze them onto a agarose gel what you are going to see is, you are going to see all the three forms onto the plasmid.

So, you are going to see the closed circular DNA you are going to see the open circular DNA and then you are also going to see the super coiled DNA. And what you see here this is actually the RNA what is also present. Because when we done this experiment we have not added or we have not removed the RNA otherwise what you can do is you can treat this whole reaction with the RNAs and that is actually going to remove the RNA from the reactions.

So, this is all about the plasmid isolations and we have prepared a small demo clip. So, that you can be able to more familiar with the different processes and in this particular demo clip we have taken care of the manual method and we also have discussed about the kit method as well.

Hello everyone, myself Sooram Banesh, research scholar at Department of Biosciences Bioengineering IIT, Guwahati. In this video we will be demonstrating how to isolate plasmid using manual method. And how to extract the plasmid DNA using phenyl chloroform extraction method or isothermal method both the methods we will be

demonstrating in this video. And also, we will show how to analyze the results like what are the different bands you will get after lasting running analyzing the gel.

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Hello everyone, in this video we will show you how to isolate plasmid DNA using alkaline lysis method. For preparation of plasmid DNA, we need resuspension buffer, lysis buffer and neutralization buffer. In addition to that we need isopropanol, RNase and ethanol. Resuspension buffer contains 25 millimolar tris and 10 millimolar EDTA and we have to add RNase at a final concentration of 100 micro gram (Refer Time: 24:20)

Lysis buffer contains pi 2 normal sodium hydroxide and 1 percentage STS. Neutralization buffer contains 3 molar potassium acetate H 6.4. For isolation of plasmid DNA, we need at least overnight grown culture with ODF 3.0. So, this is already a cultured one we have to harvest the cells by centrifugation.

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These files we have to centrifuge 1000 rpm for at least 1 minute to get the cells precipitated Siddarth.

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Now we got the cell pellet we can proceed for alkaline lysis method to isolate plasmid DNA. In first step we are going to add resuspension buffer which contains RNase.

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Mix thoroughly until all the cells suspended in resuspension solution. After the cells got suspended completely now we have to lyse the cells using strong alkaline condition that is 0.2 normal sodium hydroxide and also 1 percentage sodium dodecyl sulphate.

Now, we have to gently flip the tube in order to lyse the cells completely. We can keep in this condition for up to 5 minutes, but not more than 5 minutes which will degrade the plasmid DNA and also genomic DNA will come out and it will interfere with the (Refer Time: 29:01) In next step we have to neutralize the sodium hydroxide using neutralization buffer to prevent any further degradation.

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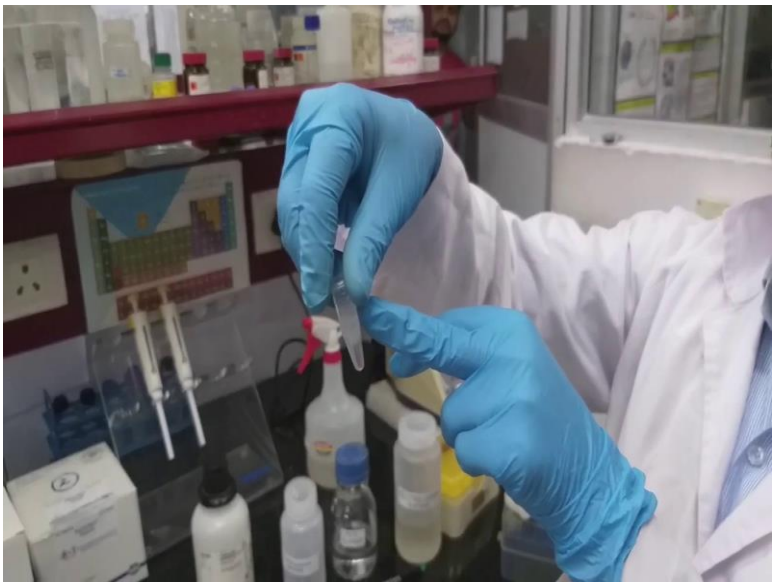

After adding neutralization buffer, you can see there is a white precipitate; that means, all the proteins precipitated by neutralization buffer. You can flip the tube 2-3 times completely precipitate all the remaining proteins. Now, the solution contains solution part contains or plasmid DNA and the all the precipitated one contains genomic DNA and also the proteins from bacteria.

Now we have two centrifuge this lysit for 10 minutes at 11000 g. The precipitate guard settled now we have to transfer the white clear supernatant to another tube this clear supernatant contains plasmid DNA. Now, we have to precipitate this plasmid DNA with the isopropanol followed by washing with the 70 percent ethanol we can see white precipitate in the solution.

Now we have to centrifuge it collect the that white precipitate and wash with the 70 percent ethanol after precipitating plasmid DNA with the isopropanol we will get a pellet of plasmid DNA. Now, we have to wash that pellet. We wash this pellet with the 70 percent ethanol.

Again, centrifuge the pellet. Now we got the pellet we have to air dry the pellet and dissolve it in (Refer Time: 34:35) water or TE buffer. We will keep leave at room temperature till the till the ethanol got evaporated next we will add (Refer Time: 34:53) To easy the process of manual alkali lysis method there are several kits available from commercial vendors.

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The basic difference between alkali lysis method and the kit basis method is kit based method contains silca based columns where lysis lysed which containing plasmid DNA bites through these beads and after washing whatever the unwanted components are there they will elute out and we will elute the plasmid DNA in TE buffer or water.

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The composition of the lysis buffer is same as previous method and also neutralization buffer raises a power every buffer contains same composition, but in commercial case

we have one extra wash buffer which will remove any unwanted contamination and give pure DNA.

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We have already seen isopropanol precipitation of plasmid DNA, but there is another method which we can utilize for isolation of plasmid DNA. This is called phenyl chloroform extraction method. In this method once recovering this open (Refer Time: 36:42) which contains plasmid DNA we have to mix with the equal portion of phenyl chloroform nature which is readily available.

Once we mix with the phenyl chloroform that since the DNA is more tends to solubilize in water then the DNA remains soluble in water and whatever the other content protein or other materials they go into phenyl based medium.

So, once we will centrifuge that one and we will connect the water based thing and again we will go for silica based method purification or we can use isopropanol precipitation by washing with the followed by washing with the 70 percent ethanol. Then again re solubilizing or resuspending it (Refer Time: 37:40) nuclease pure water.

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As we can see phenyl chloroform those mixture is high density component. So, it will set in bottom portion and the water containing plasmid DNA separated at top one. We will just take out this top portion which is containing plasmid DNA then we will precipitate with the isopropanol and use for other applications.

This is the overall procedure for isolating plasmid DNA from bacterial cells, but we have to remember all the methods having their advantages, but we will prefer isopropanol method in that method we can directly take the plasmid DNA precipitate it, but it may contain some of the other contaminants for that we need extra silica based method. In this phenyl chloroform based method here we will precipitating the other than plasmid DNA components. So, we will get pure DNA.

During this procedure we have to remember if we do not add proper amount of RNase then we can see a band which correspond to RNA in our other (Refer Time: 39:41) and comes with the plasmid DNA. So, post purification also we can add RNase and utilize for the application.

In this video we have discussed how to isolate the plasmid using alkaline lysis method. What are the components are required like chemicals and what are the steps like resuspension lysis and deneutralization and there and is put behind the lysis neutralization and these things. And also, two different methods for extracting plasmid

like phenyl chloroform extraction and isopropanol extraction we just discussed. So, with this we conclude the video and thanks for watching.

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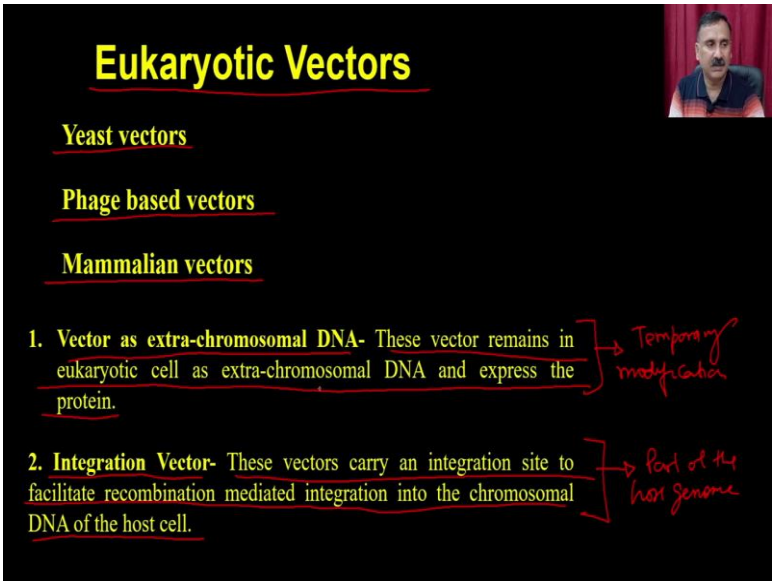

So, after the bacterial plasmid we are going to discuss about the eukaryotic vectors. So, within the eukaryotic vectors we are going to have the yeast vectors, we are going to have phase base vector and we are going to have the mammalian vector. So, vector as a extra chromosomal DNA. So, these vectors remains in the eukaryotic cell as extra chromosomal DNA and they are going to express the protein.

So, in the eukaryotic vector you can have the two different types of vectors, vectors which are going to be present as the extra chromosomal DNA. So, these vectors will remain in the eukaryotic cell as a extra chromosomal DNA and they will express the protein whereas, you can also have the integration vector.

So, these vectors carry an integration site to facilitate the recombination mediated integration into the chromosomal DNA of the cell this means they are actually going to be a part of the host part of the host genome. It means if you use the integration vectors they were actually going to modify the organisms whereas, this is actually going to be a temporary modification right.

So, that as long as the vector is present in the extrachromosomal DNA it will going to express the protein, but once the vector is out of the cell it is not going to express

whereas, in this case since you have introduced the integration sites it will actually go through the recombination process and during utilizing these integration site it can be a part of the chromosomal DNA of the host cells.

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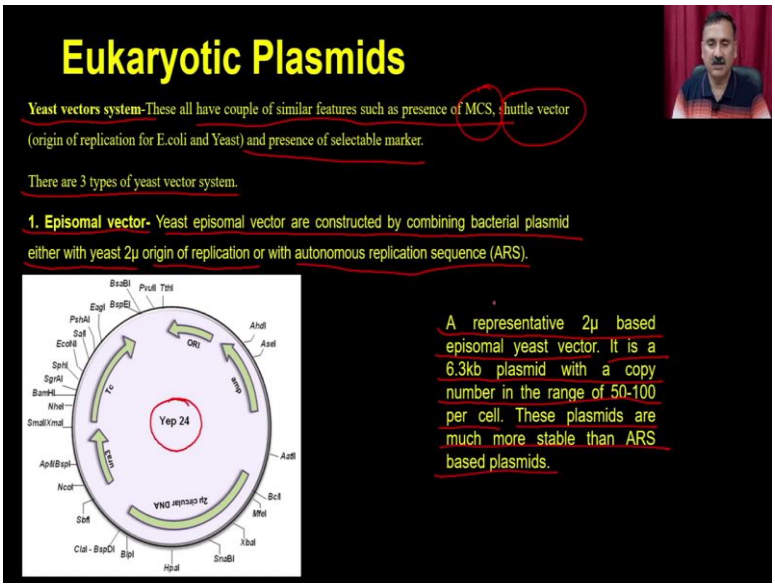

So, first we will talk about the yeast vectors. So, these all have the couple of similar features as like MCS, shuttle vectors and all that and the presence of the selection marker. So, there are three different types of yeast vectors which are present you have the episomal vector.

So, yeast episomal vectors are constructed by the combining the bacterial plasmid either with the yeast 2 micron origin of replication or with the autonomous replication sequences one of the such example is the web 24. So, yeast episomal plasmid or vector 24 where they have taken the bacterial plasmid and as well as the original replication from the yeast.

A representative 2 micron based episomal vector it is a 6.3 Kb plasmid with a copy number in the range of 5200 per cell and these plasmids are much more stable than the autonomous replicating sequences based plasmids.

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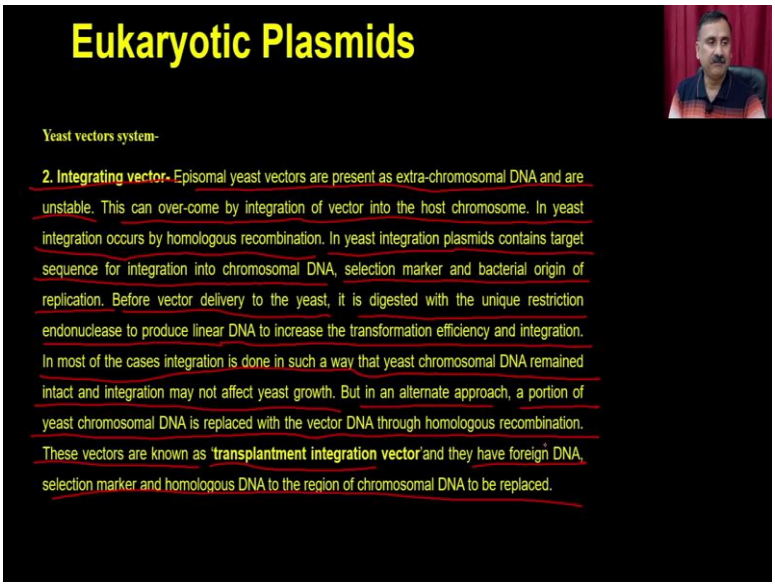

Then you can also have the integrating vectors. So, episomal vectors are present as the extra chromosomal DNA and are unstable. This can overcome by the integration of vector into the host chromosome and in the east integration occurs by the homologous recombinations.

So, in the east integration plasmid contains the target sequence for the integration into the chromosomal DNA, selection marker and the bacterial origin of the replications. 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 chromosomal DNA remain intact and integration may not affect the yeast growth.

But in an alternate approach a portion of the yeast chromosomal 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.

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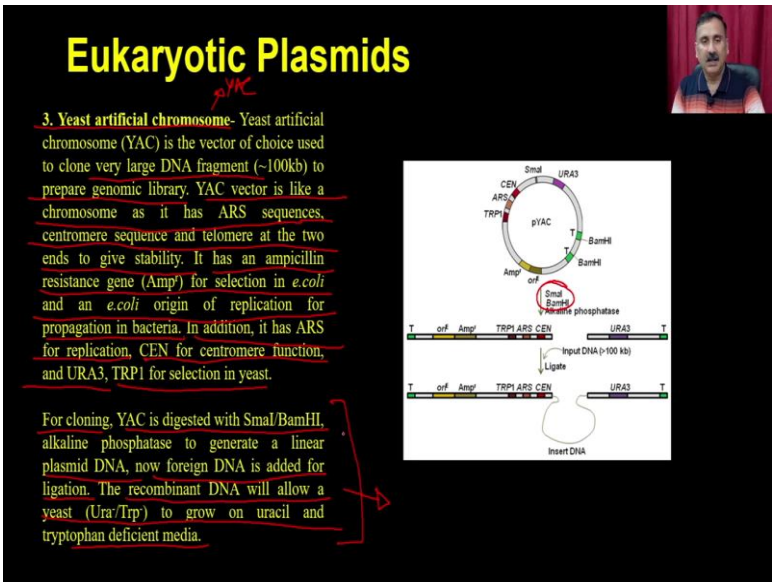

Then we can also have the 3rd type of vector which is called as a east artificial chromosome or YAC vector. So, yeast artificial chromosome is the vector of the choice used for cloning very large DNA fragments right. Remember that when we were talking about the preparation of the human genomic library 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 telomere at the two end to give the stability it has an ampicillin resistance genes for selection in e. coli and an e. coli origin of replication for the propagation in bacteria in addition it has the ARS for replication, send for the centromere functions and URA3 and tryptophan 1 for the selection in the yeast.

For cloning YAC is digested with the enzyme called SMA1 and BamH 1. So, you are going to digest the YAC with the SMA1 and BamH 1and you are going to treat that with the 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 is how it is actually going to get inserted into the these two fragments and the recombinant DNA will allow a yeast to grow on a uracil and tryptophan deficient media right. So, screening anyway we are going to discuss in our subsequent class.

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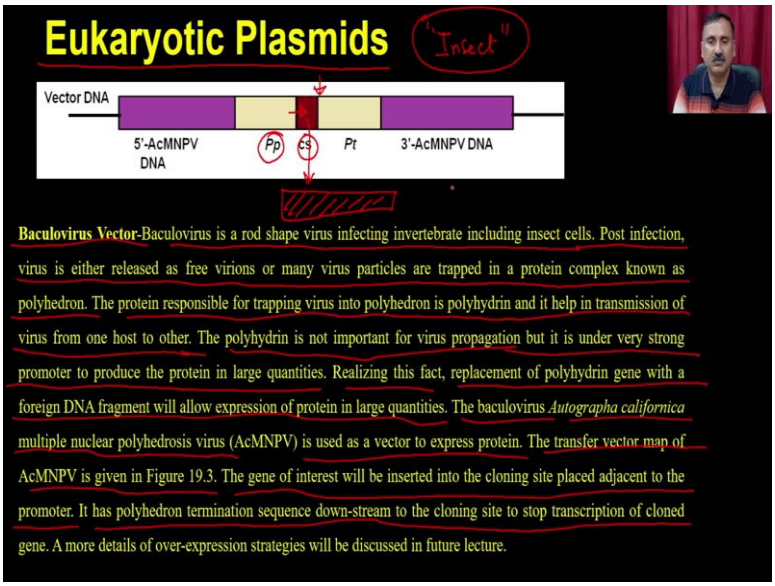

And then we have eukaryotic plasmid another eukaryotic plasmid where you which you can use in the insect cell lines. So, these are the vector for the for expressing the protein in the insect cell lines and or the baculo vectors right. So, baculovirus is a rod shape virus infecting the invertebrate including 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 trapping the virus into polyhedron is polyhydrin and it helps in the transmission of virus from one host to another ok the polyhydrin 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 polyhydrin gene with a foreign DNA fragment will allow the expression of the protein in a large quantities.

So, the baculovirus autographa california multiple nuclear polyhedron virus or the AcMNPV this is what is used as a vector to express the protein the transfer vector map of the acpnpv is given right this is the vector map of the Ac AcMNPV 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 acpnpv vector where you have the polyhydrin 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 is how it is going to start expressing this particular protein instead of the polyhydrin 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 is how you are going to express.

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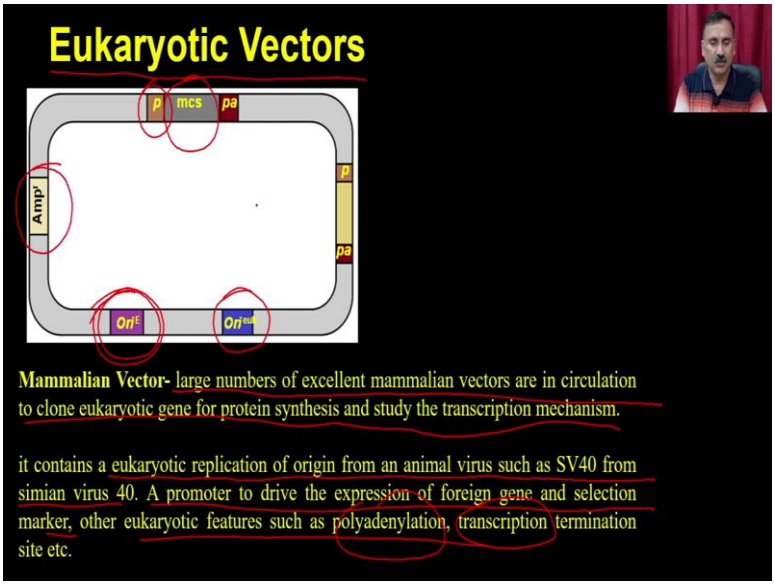

Then we have the eukaryotic vectors like the mammalian vectors. So, large number of excellent mammalian vectors are in circulation to clone the eukaryotic gene for the protein synthesis and to study the transcription mechanisms it contains a eukaryotic replication of origin from a animal virus such as 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 polyadenylation, transcription termination etcetera etcetera.

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 resistant genes and so on.

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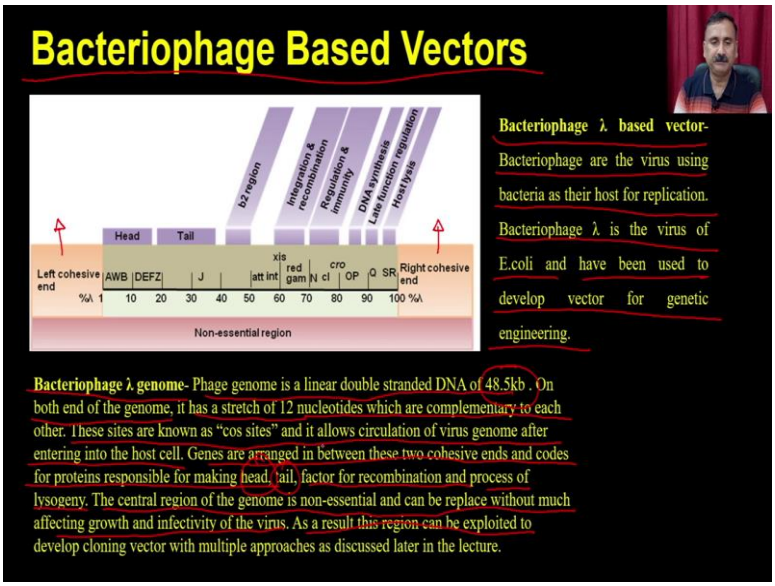

Then we have the bacteriophage based vectors. So, bacteriophage are the virus using the bacteria as their host for replication bacteriophage lambda is a virus of e. coli have been used to develop vector for the genetic recombinations. So, what you have is a bacteriophage genome?

So, phage genome is a linear double stranded DNA of 48.5 Kb on both end of the genome it has a stretch of twelve nucleotide which are complementary to each other right. So, you have t two strand two sides right two sides 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 cos sites and it allows the circulation of the viral genome after entering into the host cells. Genes are arranged between these two cohesive site of the code for the protein responsible for making the head tail factor for recombination and the process of lysogeny.

The central region of the genome is non essential and can be replaced with 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.

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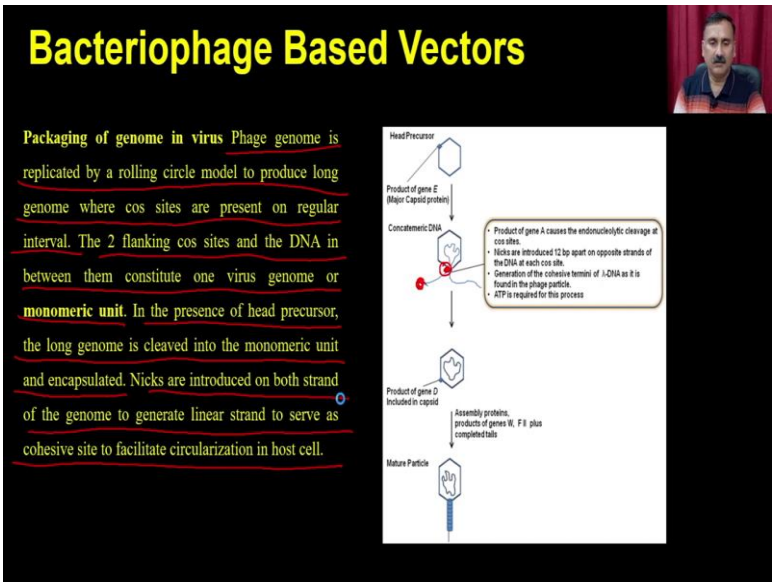

So, how it is actually going to pack the genome. So, the phage genome is replicated by a rolling circuit model to produce the long genome whereas, cos sites are present on the regular interval right. So, when it is actually going to produce the phage genome into the outside right and it is actually going to start producing the cos sites.

So, you can imagine that you have one cos site you can have another cost site and once this cos two cos sites are going to be out they are actually going to come together and it will they will get circularize. The two flanking cos sites and the DNA between them constitute the one 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 ok.

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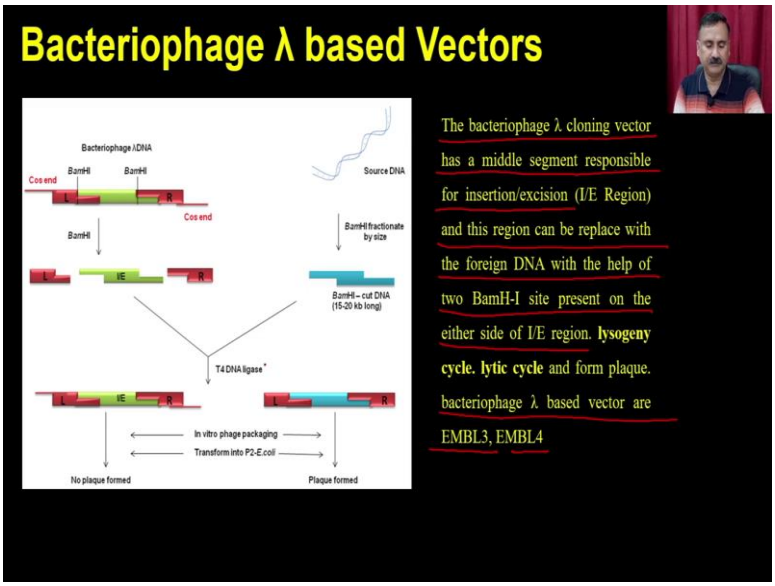

So, this is what the bacteriophage lambda cloning vector has a middle segment responsible for the insertion or excision and this region can be replaced with a foreign DNA with the help of the two BamH 1 site present on the either side of the insertion or excision regions lysogeny cycle lytic cycle and it will form the plaques.

So, bacteriophage vector are the EMBL3and EMBL3. So, this is what exactly going to happen how you are going to insert the foreign DNA into a IE site ok.

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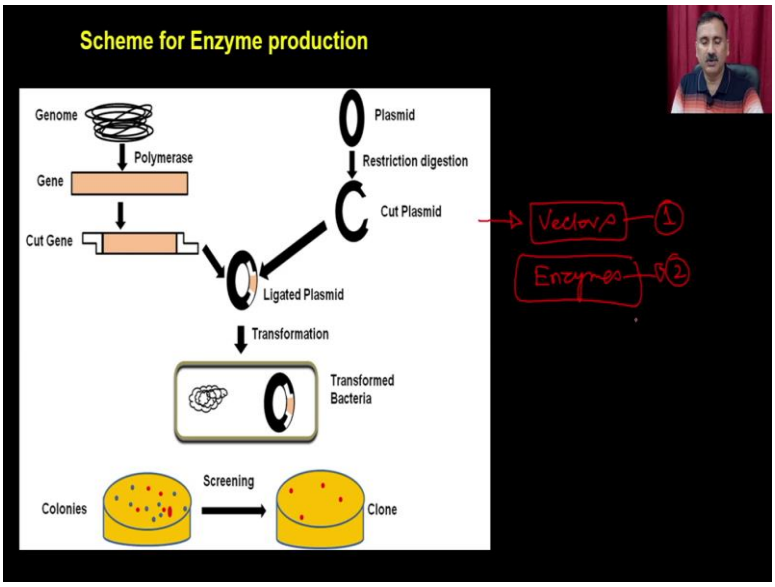

So, this is what all about the different types of vectors what are available for the cloning of the foreign DNA or cloning of the enzyme into the suitable expression vector and depending on the production depending on the origin of that particular gene you can be able to have the flexibility to choose 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.

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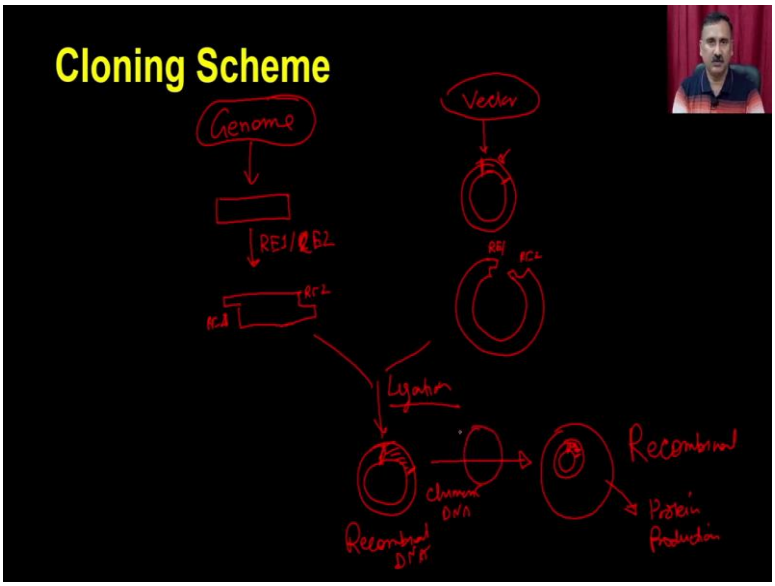

So, what you are going to do is first from the genome you are actually going to generate or you are going to produce the fragment. So, you are going to produce the fragment right. Similarly, from the vector you are going to have the circular vector ok. This circular vector will have the multiple cloning site and within the multiple cloning site 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 one and two. 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 two this is for restriction enzyme two one same is true for the vector also. So, vector also you are going to digest with the help of this and that right and ok. 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 ok.

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 ok. So, this is going to be your chimeric DNA or recombinant DNA. Now, this chimeric DNA you are going to transform into the host ok and the host is going to have this right.

So, since the 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 you built good to use the e. coli 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.