Genetic Engineering: Theory & Applications
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Module 4
Basics of Cloning Part 2
Lec 12 Cloning Vectors (Part - 1)

Hello everybody, this is Dr. Vishal Trivedi from the department of Biosciences and Bioengineering IIT Guwahati and what we were discussing, we were discussing about how to produce or how to generate the genetically modified organism utilizing the biotechnology-related principles and in this particular discussion so far what we have discussed. We have discussed about different properties of the host whether it is the structure of the host which we have discussed in the module 1 or whether it is the procedure or the approaches how to isolate a particular gene from the host. That we have discussed in the module 2.

And following that we were also discussing about the different genetic tools what we have what are available to perform the biotechnology-related recombinant technology and in this context today we are going to discuss about the vector or the transforming agents.

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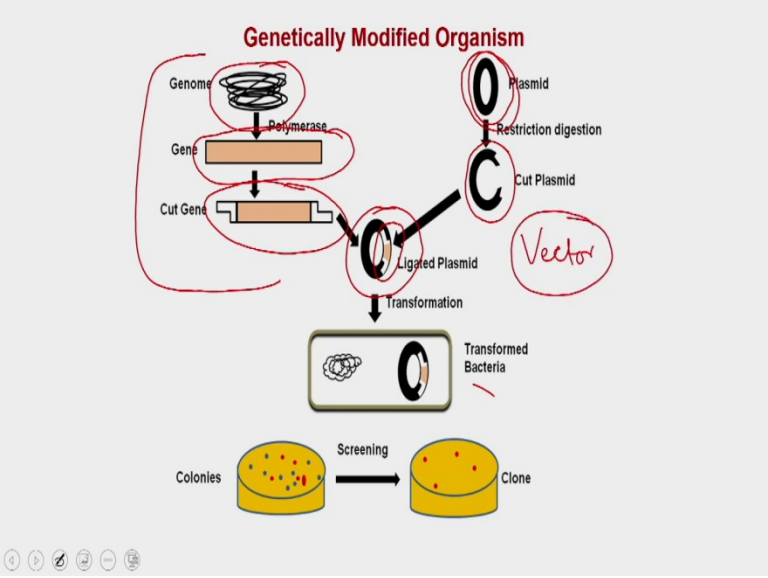

So, if you have, what we have discussed so far we have discussed about how to produce the genetically modified plasmid or the recombinant plasmids and for producing this recombinant

plasmid we need the gene from the particular host which you can isolate either by the PCR or you can isolate that from the genomic library or the cDNA library.

Once you got this gene, you have to digest the gene with the particular set of restriction enzymes and similarly you also have to digest the transforming agents with the same set of restriction enzymes and after that you have to put these two components together in a ligation reaction and then you have to transform that into the bacteria to produce genetically modified organisms.

So, so far we have discussed the many aspects related to the host such as we have discussed about the structure of the prokaryotic or the eukaryotic genes, eukaryotic organisms, we have also discussed about the downstream metabolism. We have also discussed what are the different media or the media components, what are required to propagate the prokaryotic as well as the eukaryotic cell and when we also have discussed how to propagate or how to monitor the growth of a bacteria in a particular media and following that we have also discussed about how to isolate a gene from the host so that you can use these genes into the downstream recombinant technology to regenerate the recombinant DNA plasmid.

Now, following this discussion, today we are going to discuss about the transforming agents so the transforming agents which are actually performing to carry the foreign gene is called as the vector. So what is the purpose of a vector is?

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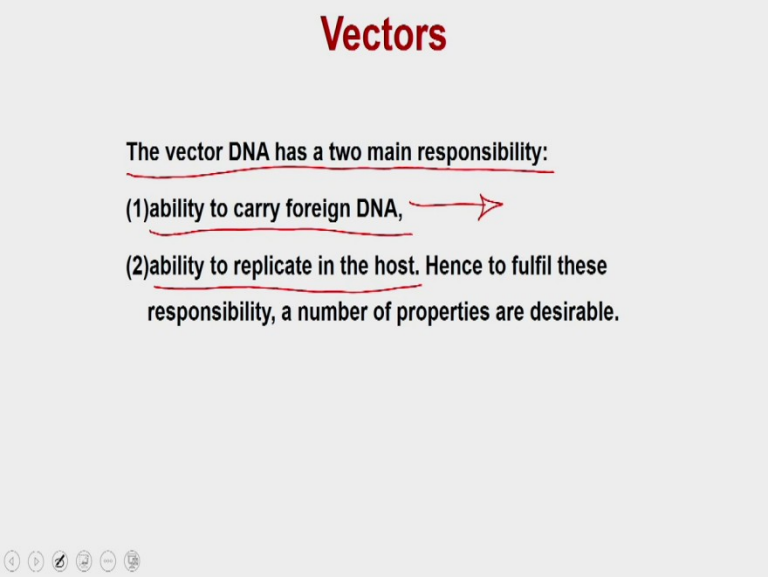

The vector is a DNA which actually has two responsibility. It should be have the ability to carry a foreign DNA and it should be have the ability to replicate in the host. So, to carry a plasmid DNA or to carry the foreign DNA and the ability to replicate in the host, the vector should have the desirable features or the properties. So what are these properties?

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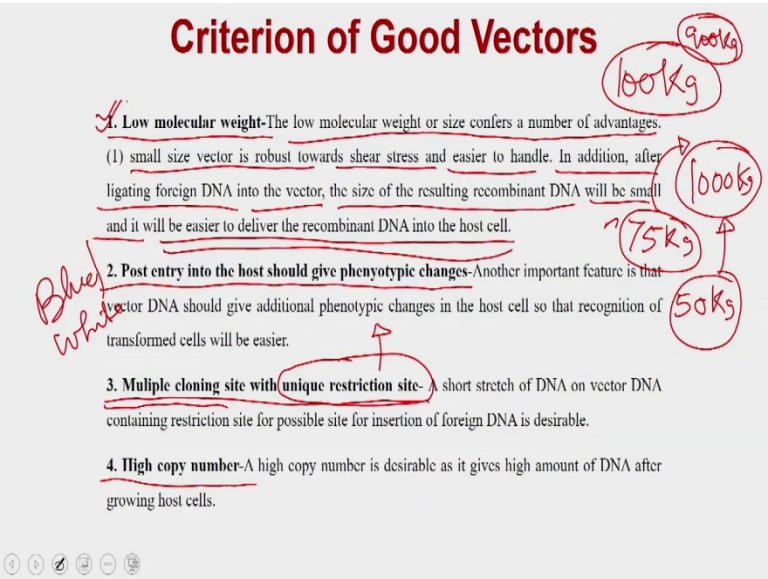

The property is the number one is that a good vector should be of a low molecular weight. The vector you can easily understood this simply by that a vector is nothing but a carrier which actually going to carry the DNA from the one host to another host where it is actually going to provide this DNA for its expression. Now you can imagine that suppose you are would like to carry the goods from your home to the another place and for that if you are looking for a carrier you are also looking for a carrier which will be of low molecular weight or which will be very light so that you could be able to fill as many as material as you want.

For example, if we have a vehicle which actually can run 14 km in 1 liter but it depends on the what is the weight you are going to put into this vehicle. So, if the vehicle itself is going to be of 100 kg and the total carrying capacity of this vehicle is only 1000 kg then you will be able to put only 900 kg as the weight which it can carry whereas you can imagine a situation where if suppose we make a vehicle which is of 75kg.

This means you are actually can be able to carry more material into this. Forget about this, if you have a vehicle of 50 kg you will be going carrying more number of material into this. Similarly, you can understand in the same way that you need to be looking for a vector which is going to be of low molecular weight because the higher level of molecular weight of the vector will reduce its tendency or capacity to carry the bigger foreign DNA.

So, as you remember when we were discussing about the preparation of genomic DNA in the cDNA library we have given you a table which says that what is the carrying capacity of different vectors and you might remember that for the bigger genome you might have to use the carrier which can carry the bigger piece of DNA. So, the low molecular weight is having many advantages.

One is the small size of the vector is robust towards the shear stress and easy to handle because you can imagine that if you have a larger DNA and if you have a large size of DNA it is actually been prone for shear stresses and also the isolation of these kind of genomic these kind of vector is a time consuming as well as it is not easy to perform.

In addition to that, ligating the foreign DNA into the vector, the size of the resulting recombinant DNA will be of small right and because it is small it can be delivered very easily into the host

cell and because it is small so that is what we are saying that if you are actually can… if the total amount what it can go into the host cell is only 1000 kg you can actually increase the number of insert or increase the size of the foreign DNA automatically simply by reducing the size of the vector and that has an additional advantage that isolating this particular type of vector is easy, transforming the resulting recombinant DNA is easy because the resulting recombinant DNA is also going to be of smaller in size and at the end the transformation is going to be very very easy.

So, and on the other hand, the manipulation of these smaller DNA is going to be easier compared to the manipulation of the larger DNA.

The second phenotype which actually also be very important for a vector is that it should give you a phenotypic changes in the host when it enters into the host cell it should give you the phenotypic changes. How it is important is that when you put the vector DNA into the host it should actually once it starts giving you the phenotypic changes then it is easier for us to isolate that particular host cell which is actually having the vector which you have put compared to the untransformed host cells.

One of the classical example is that the blue-white screening where the colony color itself will tell you whether a particular type of host cell is having the vector or is not having the vector. Similarly, you have some of the vector which actually contains the different types of reporter genes and that reporter gene will actually give the phenotypic changes in the host cell once the vector enter into the host cell and that actually facilitate the isolation as well as identification of the host cells which have taken up the vector.

The third which is very important is that the good vector should have the multiple cloning sites with the unique restriction site. When it says a unique restriction site is that these are the restriction sites which are not going to repeat within the body of a particular vector and the multiple cloning site is going to be the site where you can choose these restriction sites to clone your or to insert your foreign DNA.

Then the fourth is the high copy number. So if the vector is of high copy number or a smaller low copy number, the high copy number has always been desirable because it is actually going to give you the more amount of DNA from the same bacteria. What is mean by the copy number is

that the particular vector is going to have those many copies within the bacterial cell. You know that the plasmid is the extrachromosomal DNA which is present inside the bacterial cell. This we have discussed when we were discussing about the structure of the prokaryotic cell.

So, it could be 1 number, it could be 2 number, it could be 10, it could be 20, it could be 500, and it could be 1000s. So as the number will go up, the final product what you are going to get from these vectors is also going to be higher because the every plasmid or every vector is going to carry your foreign DNA and that is how every vector is going to produce those number of molecules.

This means a high copy number of vector is going to lead you to isolate the very large quantity of that particular DNA or correspondingly it also can lead you to produce the large quantities of proteins. But when you talk about the high copy number vectors it also has disadvantage in terms of that suppose you are overexpressing a protein which is toxic or which is non-desirable for the or which is actually going to disturb the cellular metabolism of the bacteria.

In those cases, the high copy number of the vector is not desirable because the high copy number actually gives very high quantity of that particular protein and such a large quantity causes disturbance in the metabolism sometimes actually does not allow bacteria to survive whereas if you work with the low copy number of vectors it actually going in, it still creates a disturbance to the bacterial metabolism but that actually is so slow or it actually happens so slowly that bacteria get adapted to that particular type of changes and in those cases we normally use the low copy number of plasmids or the low copy number of vectors.

But, in general if you are using the vectors for carrying the foreign DNA which means you are just using the vectors for cloning purposes, not for the overexpression or production of the proteins purposes then it is always been desirable that you use the vector which should have the high copy number because at the end it is going to give you the more number of DNA.

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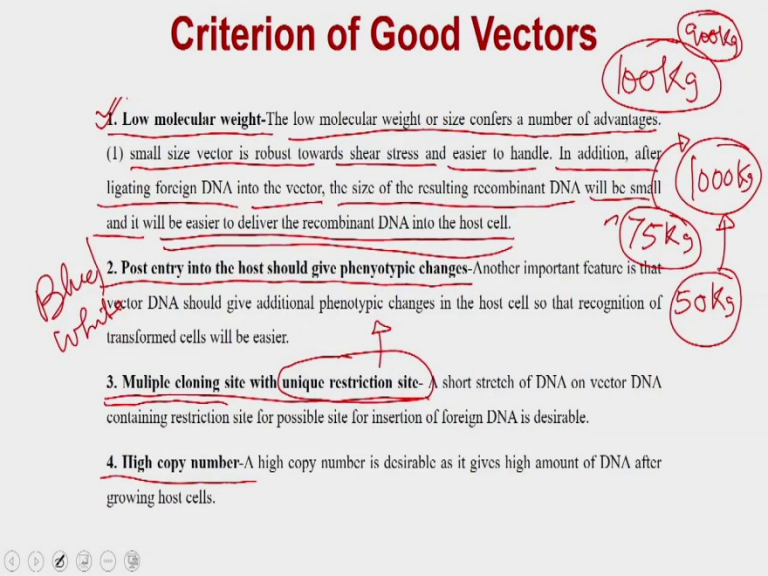

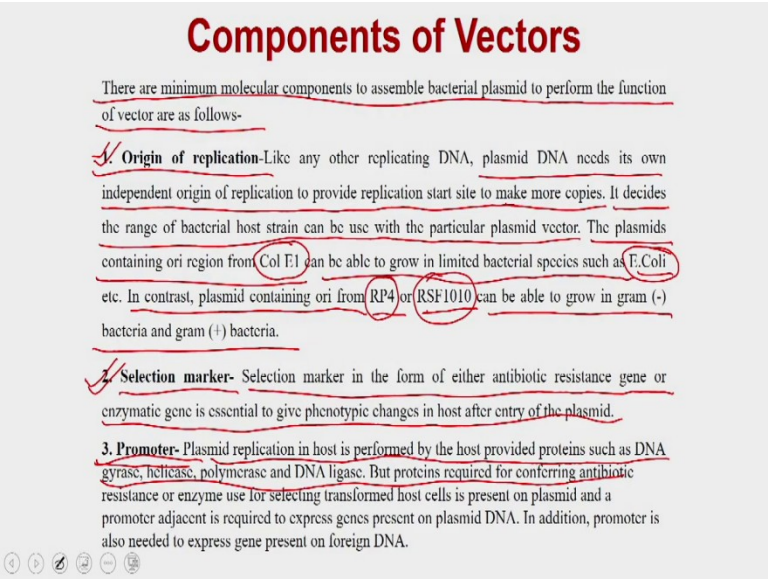

So taking into these criteria we have the you have to have these many components of the vector. So, the minimum number of components which are required to perform the function of the vector which we have just discussed that it should have the criteria of a good vector and these are the molecular component of a particular vector.

The first is the origin of replication. Ok. As you have seen in the first slide itself that the property of a vector is that it should be able to replicate within the host. Ok. So that replication is going to

be decided by the origin of replication. So, every vector should have its own origin of replication and it depends on the type of host it is going to it is going to work. For example, if you have the bacterial plasmids or the bacterial vector then it should have the origin of replication for the bacterial system. If it is for the mammalian system, then it should have the origin of replication for the mammalian system.

So the bacterial plasmid needs to have its own independent origin of replication to provide replication start site to make more copies. It decides the range of bacterial host strain can be used with the particular vector. For example, the plasmids carrying origin of replication from Col E1 can be able to grow in a limited number of bacteria such as Ecoli. In contrast, the plasmid containing ori from the RP4 or RSF1010 can be able to grow in the gram-negative as well as the gram-positive.

So depending on the origin of replication what you use to prepare a vector decides whether this particular type of vector is going to replicate in the particular host strain or not. For example, in this case if you are having the origin of replication from Col E1 it is only going to replicate within the E.coli whereas if you have taken the origin of replication from RP4 or RSF1010 that is actually will allow you to generate a vector which will actually will grow in gram-negative as well as the gram-positive bacteria.

Apart from the origin of replication, the vector should have the selection marker which means that you need to have some kind of selection pressure which allows you to select the transformed host cells from the non-transformed host cells which means the host cell which have taken up your vector in comparison to the host cell which has not taken up your vector. These selection marker could be in the form of antibiotic resistance gene or it could be an enzymatic gene which is essential for giving the phenotypic changes into the host after entry of the vector into the host. Ok.

So, there is no such hard and fast rule that you always have to use the antibiotic resistance gene as the selection pressure. You can use any other selection pressure, for example as we have discussed in the previous slide, you can use the blue-white screening as well where an enzyme is been used to distinguish between the host which has taken up the vector or the host which has not taken up the vector.

The third is the promoter. So, every vector which you are going to design is going to either should have the vector. If it is an overexpression vector the plasmid replication is performed by the DNA of all these enzymes, but the promoter is smoething which actually allows the transcription as well as the translation of that particular gene. So the promoter you will put in those vectors which you are going to use for the overexpression of that particular protein or overexpression of the recombinant DNA whereas the promoter can be or can be removed or can be excluded from the vector if you are simply designing a vector for the cloning purposes which means if you are just using the vector for carrying the foreign DNA from one host to another host but your purpose is not to overexpress that particular foreign gene. In those cases, the promoter can be excluded from the vector design.

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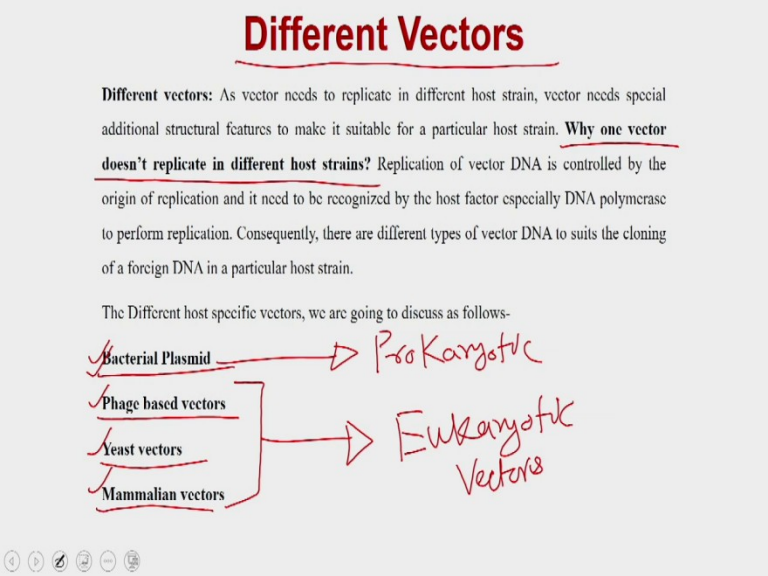

So, depending upon these people have designed different types of vectors. These vectors are could be are the bacterial plasmids, it could be the phage-based vectors, it could be yeast vector, or it could be the mammalian vector. This is the example of the prokaryotic vector whereas all these three are in the category of eukaryotic vector and as we have discussed the vectors are actually contain its own origin of replication that is why it is not that one vector will replicate in different host strains as you have seen in the previous slide itself. If you have the origin of replication from the Col E1that is going to replicate only in the Ecoli whereas if you have the

origin of replication from RS1 then it is going to replicate in the gram-negative or the gram-positive bacteria.

So that is what is not true only for prokaryotic system that is also true for the prokaryotic as well as the eukaryotic system. So if you that is why it says that one vector will not replicate in different strains as long as if it does not contain the origin of replication. That is why most of the eukaryotic plasmids are also contain the origin of replication for the bacterial system. Means if they also have the origin of replication from prokaryotic system as well.

So considering these we, the people have developed the different types of vectors. We are going to discuss only the following set of vector which are bacterial plasmid, phage, yeast, and mammalian type of vectors. What we are not going to discuss is not the extensive discussion about the different types of plasmids or different types of vectors found under these categories. What we are going to discuss is the probably one or two representatives of each class and what we are going to discuss is only the features.

There is no end of the plasmids or there is no end of other kinds of vectors which people have developed for these host strains and it is almost impossible to discuss all of them. So, let us start with the bacterial plasmids.

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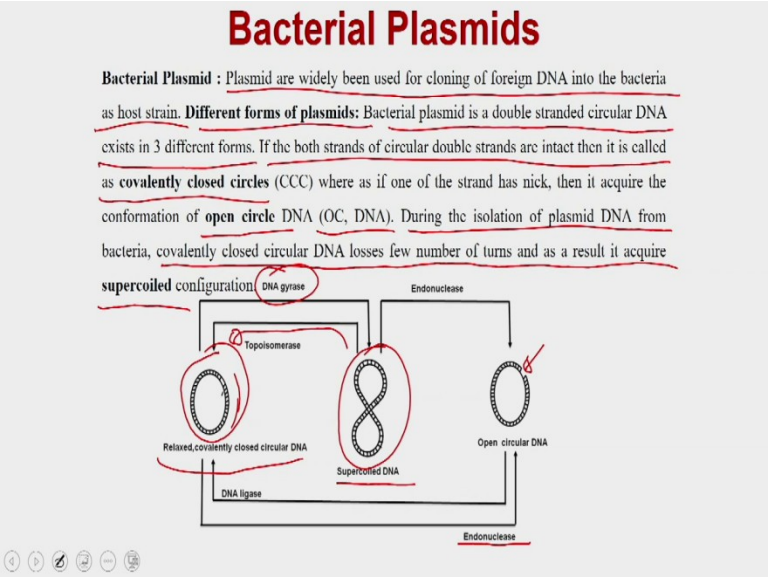

So, bacterial plasmids is widely been used for foreign DNA into bacteria as host strain. If you take a bacterial plasmid it can be exist in 3 different forms. What are these different forms? So, you can have the bacterial plasmid which is as you know that the bacterial plasmid is a double-stranded DNA but it can exist in 3 different forms.

If the both strands are intact and circular then it is called as the covalently circular model or covalently circular closed circles which is called triple C whereas if you expose this particular DNA to the endonucleus what will happen is, the endonucleus is going to create the nick on one of the strands and that is how it is actually going to create the opening of the outer circle and that is how it is going to create another form of plasmid which is going to called as the open circle or the OC DNA whereas when you isolate the plasmid from the bacteria, the covalently closed circular loses few of its turns so what happen is that it actually twists around its own and because of that it actually creates a supercoiled configuration which is this and you can actually convert a supercoiled DNA from a supercoiled DNA to the closed circular DNA simply by adding the topo-isomerase.

Topoisomerase is going to make destroy the supercoiled DNA to make it to the relaxed covalently closed circular DNA or if you put the DNA gyrase to the triple C DNA that actually will convert the triple C DNA to the supercoiled DNA.

Similarly, so all these three forms are existing at the same time and all these three forms are mutually and exclusively could be exchanged to each other simply either by the enzymatic changes or by the some kind of physical changes as well and if you isolate the plasmid, you will see that all these 3 forms exist at the same time.

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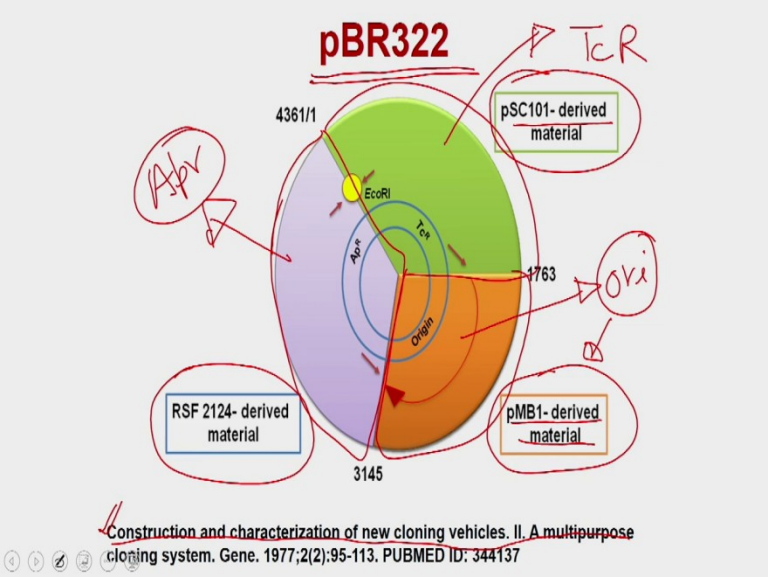

So, when the people have started the molecular cloning they were, in the beginning, they were using the plasmids or the plasmids which are available into the nature, natural plasmids which are present inside the bacterial cells and these were like either the pSC101 or pMB1 or the RSF 2124, ok. But, when they realize that some of these plasmids are not good enough to do the molecular cloning because of the disadvantages of one or the other because of that what they have decided is that they decided to design a unique as well as the robust plasmids which can be used for molecular cloning purposes and that is how they have taken the good area or good components of all these 3 plasmids and that is how they have designed a new plasmid which is called as the pBR322.

So, you can see that they have taken this part of the DNA from the RSF 2124, this part they have taken from the pSC101 and this part what they have taken is from the pMB1 derived material. What you could see is that from the RSF 2124 they have taken the ampicillin resistance gene and from the pSC101 they have taken the tetracycline resistance gene so that is how they have introduced two selection markers into the into the into the plasmids and then they have taken the origin of replication from the pMB1.

So that is how they have taken the origin of replication from this particular plasmids and that is how what they have realized that this particular newly designed plasmid which they called as the pBR322 is robust enough to perform different steps of the molecular cloning and it was having the better utility of perform of intigating the inserting the foreign DNA into the plasmid as well as the selection of this particular newly designed plasmid was much easier compared to the selection of either of these plasmids and they have published this particular work into this particular article.

So if you are interested to know how they have designed and how they have constructed this particular plasmid I strongly encourage you to go through this particular such article and read. That actually not only going to give you the idea how to design the new plasmids but also that will tell you that how much efforts these people could have put to design a new plasmid taking up the material from the 3 different individual plasmids.

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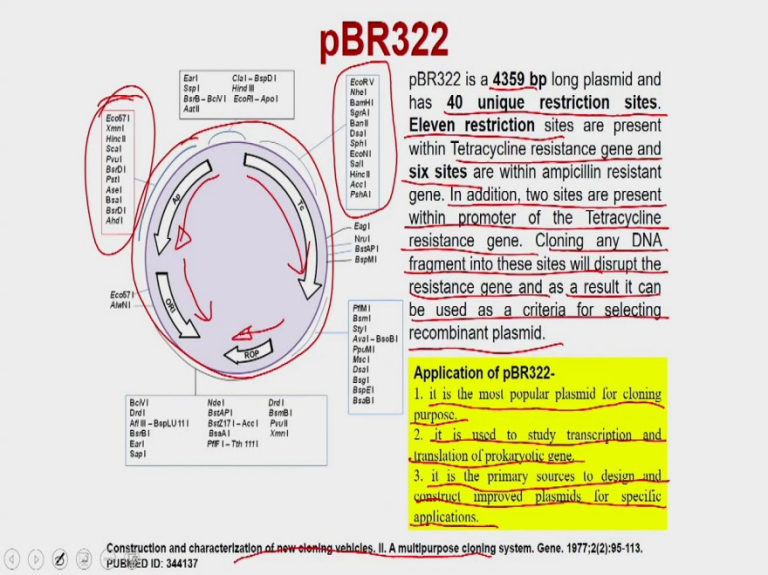

So, this is the vector map of pBR322. What you will see is this is the ampicillin resistance gene, this is the tetracycline resistance gene and this is the origin of replication and this is the recombinant gene ok. What you will see is that pBR322 is a 4359 base pair long plasmid and it has the 40 unique restriction sites. These restriction sites are widely distributed among the ampicillin resistance gene or the tetracycline resistance genes.

So, you have the 11 restriction sites which are present in the tetracycline resistance genes. So you have actually the 11 restriction enzymes which are present in the tetracycline resistance genes. Then you have the 6 sites which are present in the ampicillin resistance genes and the 2 sites are present within the promoter of the tetracycline resistance genes and cloning of any DNA fragment into these sites will disrupt the resistance gene and as a result it can be used as a criteria for selecting the recombinant plasmid which means if you use any of these restriction sites or any of these restriction sites you could be able to disrupt the gene of that particular resistance gene and that could be one of the criteria which you can use to screen the newly formed recombinant DNA.

What is the application of pBR322? pBR322 was the first or the initial plasmid which people have developed for molecular cloning purposes. So, it was very very popular for cloning purposes. It is used to study the transcription as well as the translation of the prokaryotic genes and it is the primary source to design and construct improved plasmid for specific applications and because of this third point only I am encouraging all the students to read this particular article to know that how to design a new vector or new plasmids so that if required they could be able to redesign their own plasmids for their own purposes and that is how they can be able to circumvent or that is how the can be able to overcome from the problem what they were facing with the existing plasmids which they were having in their own labs.

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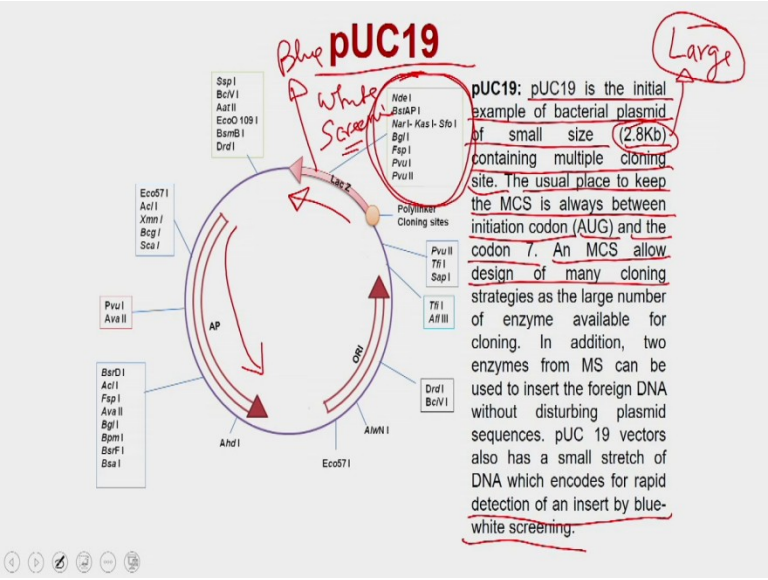

So, the second plasmid which have been derived from the pBR322 is called as pUC19. pUC19 is the initial example of bacterial plasmids of a small size. It is 2.8 kb plasmid containing the multiple cloning sites. The multiple cloning sites is been kept in this between the initiation code that is AUG and the codon number 7. The MCS design of these many cloning strategies are that the large number of enzymes are available within the Lac Z ok or you have the multiple cloning sites which you can use and because it also has a restriction sites within the Lac Z you can actually use for the rapid detection by the blue-white screen which we are going to discuss in the subsequent lecture and apart from, if you see apart from the blue-white screening criteria you also have the ampicillin resistance gene which it got from the pBR322 and so that is how this is actually much more advance compared to pBR322 simply because it is smaller in size.

So, you can see that the first thing is people have done is they have removed the unwanted junk DNA from pBR322 to reduce the size and that actually will allow you to produce and put more larger DNA into the plasmid and then what they have done is. They have introduced the Lac Z gene and the Lac Z will actually will allow you to do the blue-white screening. So, that is an additional screening criteria or screening method what has been introduced by deriving the pUC 19 from the pBR322 and the multiple cloning sites has also been put in a better way compared to the pBR322.

So, with this, we would like to conclude our lecture here. Thank you.