Enzyme Science and Technology
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Module - III
Enzyme Production (Part 1: Cloning)
Lecture - 15
Enzymes in Molecular Cloning

Hello everyone, this is Dr. Vishal Trivedi from the 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. In this context so far what we have discussed, we have discussed about the development of the field of enzymology, we discuss about the different properties of the enzyme and then in the previous module we have also discussed about the different structures of the enzyme.

So, we have discussed about the primary structure, secondary structure, tertiary structure and quaternary structure, not only the structure we have also discussed about the different methods through which you can be able to determine these structures. And now in the current module we are discussing about how you can be able to produce the enzyme in the large quantities.

And if you recall in the previous few lectures, we have discussed about how you can be able to isolate the gene of the interest. So, we have discussed about the two approaches, we have discussed about the approach when the genomic sequences are not known and in that context, we have discussed we have discussed about the genomic library approach and then we have also discussed about the cDNA library approach.

Not only that when the genomic sequences are known then you can be able to use the technique which is called as polymerase chain reactions. So, with the help of the site specific primers, you can be able to amplify the gene of your interest and that is how you are ultimately you are going to get the gene fragments.

So, either you use the genomic library or cDNA library or the PCR ultimately you are going to get the gene fragments and then these this this particular gene fragment has to

be cloned into a suitable vector, so that you can be able to use that for downstream applications.

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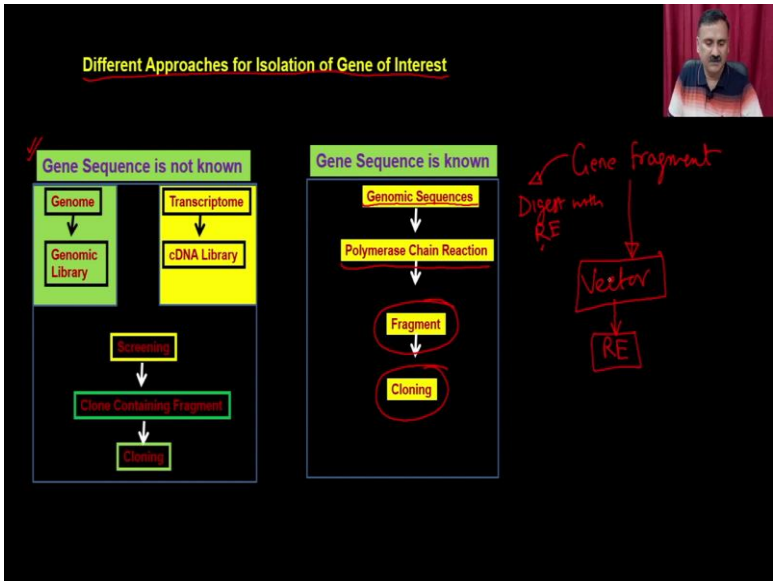

So, this is what we have discussed, we have discussed about the different approaches for isolation of the gene. So, we discuss about the approach when the gene sequence is not known that is the condition when the pregenomic era when the genome sequences are not known even today also for some of the organisms the gene sequences are not known right.

So, then you can actually be able to go with the either the genomic library approach or the cDNA library approach and then the post genomic era we are actually sequencing the genomes of the different organisms the gene sequence is known. So, in those cases you can actually be able to utilize the genomic sequences, you can prepare the site specific primers and that is how you are going to get the gene fragment which you are looking for and that you can actually be able to clone.

So, whether you use this approach or whether you use this approach, you are actually going to get the gene fragment and this gene fragment has to be cloned into a suitable vector, but cloning a gene fragment into a vector is a multi-step process where you are going to take this gene fragment and you are actually going to digest the gene fragment with the help of the restriction enzymes ok and once you are going to get the cut

fragment then you are also going to cut the vector with the same set of restriction enzymes ok. So, this is all we have discussed in this cartoon.

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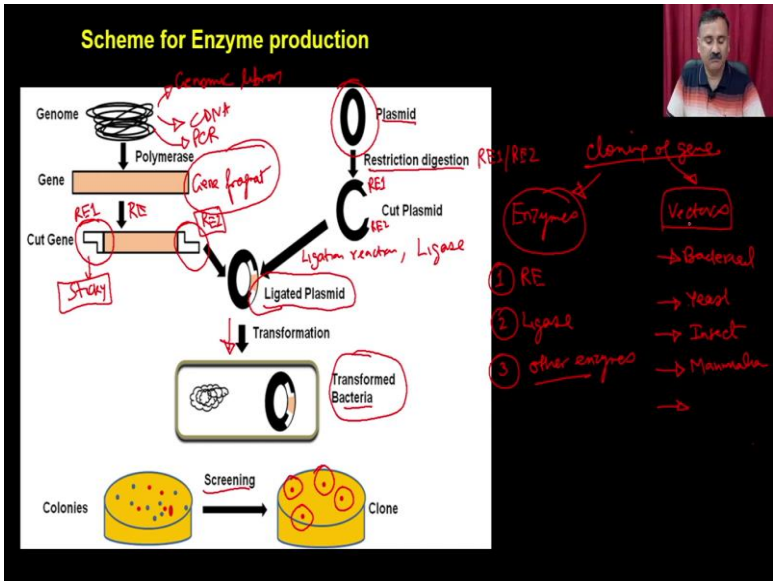

So, what you have is from the genome you have isolated the gene fragment. So, this is the gene fragment you have isolated, this could be either by the genomic library approach or it could be by the cDNA library approach. So, either you use the genomic library or you can use the cDNA library or you can actually be able to do the PCR ultimately what you are going to get is you are going to get the gene fragment.

Now, these gene fragment has to be digested with the restriction enzymes so that you are going to get the sticky ends. So, these are the sticky ends what you are going to generate when you are going to digest the fragments with the restriction enzymes. So, on both the sides so, you can actually be able to have the flexibility of using the same enzyme or you can use the two restriction enzymes so, in that case you are going to have the two fragments.

Similarly, you can use the vector. So, vector could be a plasmids and then plasmid also has to be done has to be performed restriction digestion with the same set of RE 1 and RE 2 and that is how you are going to have the RE 1 and RE 2 on both the ends. So, you are going to have the sticky ends on the plasmids, you are going to have the sticky end on the fragments and then you are going to put that for ligation reactions which is going to be catalyzed by enzyme which is called as ligase.

And then ultimately you are going to get the ligated chimeric plasmid, this ligated chimeric plasmid has to be transformed into a suitable host. So, in this case we have taken the example of the bacteria. So, you can actually be able to do the transformation into the bacteria and that is how you are going to get the transform bacteria and this transform bacteria has to be screened for the positive clones and these are the positive clone which actually contains the plasmid or the chimeric plasmid of interest.

So, what you see here is that in this particular process we are actually utilizing the different types of enzymes and we are also utilizing the different types of vectors. So, what we are using, we are actually utilizing the enzyme and we are also utilizing the different types of vectors because the vectors could be different types.

So, what are the enzymes we are utilizing, we are actually utilizing the restriction enzymes, we are utilizing the ligase and we are also going to utilize the other enzymes which are going to be participate or which are required actually for the cloning reactions right.

So, cloning of a gene requires the information about the enzymes what you are going to use for the cloning and the vectors the different types of vectors you can have the vectors as per the host. So, you can have the vector for the bacterial system, you can have the vectors for the yeast system, you can have the bacteria for the vector for the insect cell lines and you can also have the vectors which are for the mammalian system and you can also have the vectors which is for the bacteriophage.

So, these are the two important thing which we could discuss the how you are going to perform these reactions. So, before we get into the details of the cloning of the gene into a suitable vector we have to have the complete information about these enzymes and as well as the different types of vectors which are available.

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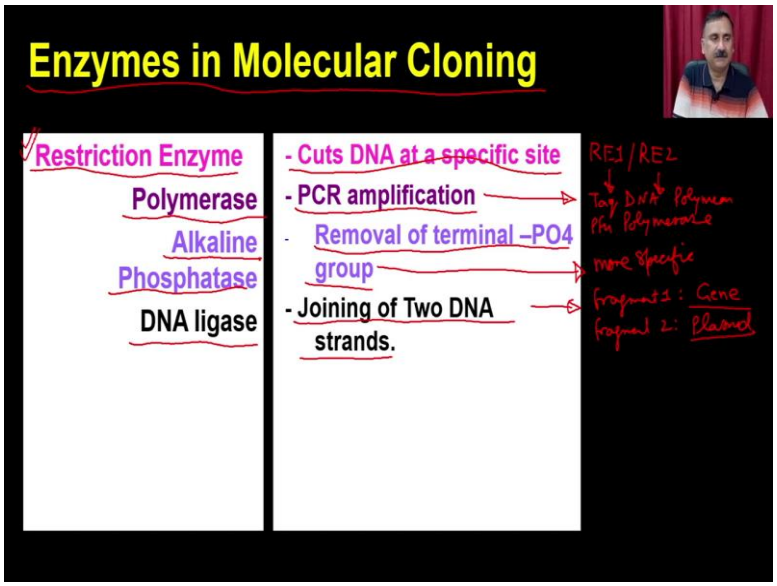

So, the enzymes which are available in the or which are actually going to be used in the molecular cloning are restriction enzymes, the purpose of restriction enzyme is to cut the DNA at the specific site. For example, in our in our previous slide we have used the restriction enzyme like restriction enzyme 1 and restriction enzyme 2. So, both of these restriction enzymes are specific, they are actually going to be specific for their own sequence.

Then you can also use the polymerase. So, polymerase is actually going to use for PCR amplifications, if you recall when we were discussing about the PCR we talk about the Taq DNA polymerase and we have also discussed about the PFU polymerase. So, that we are going to use for you know amplifying the gene fragments.

Then we can also use the alkaline phosphatase, the purpose of the alkaline phosphatase is that it is going to remove the terminal phosphate group and that actually is going to make the reactions or the ligation more specific. And then we are also going to use the DNA ligase. So, DNA ligase is going to join the two DNA fragments.

So, in this case fragment one is going to be the gene amplified gene product, the fragment two is going to be the cut plasmid right. So, both of these you can actually be able to use for the molecular cloning. So, let us start first with the restriction enzymes.

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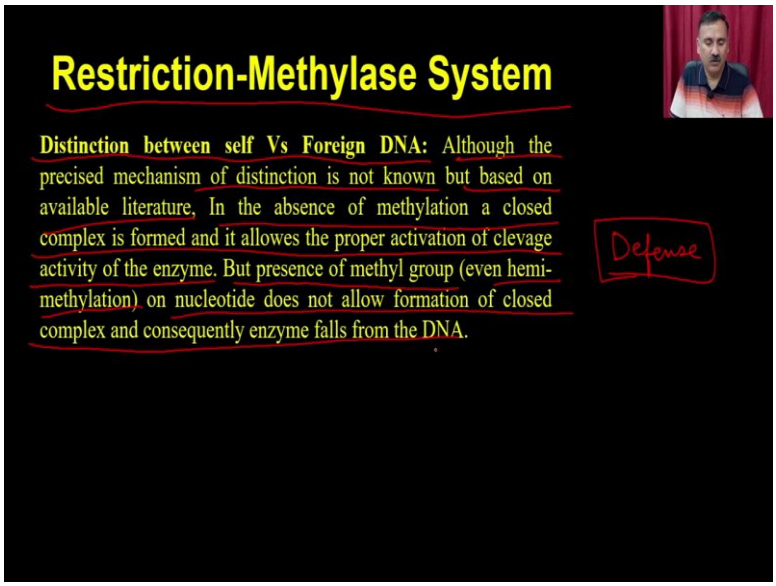

So, restriction enzymes are actually be a part of the restriction- methylase system. A restriction methylase system is a defense system what is being present in the bacterial system. So, it actually allows the bacteria to make a distinction between a self-versus the foreign DNA. Although, the precise mechanism of distinction is not known but based on the available literature, in the absence of the methylation a closed complex is formed and it allows the proper activation of the cleavage activity of the enzyme.

But presence of methyl group even the hemi-methylation on the nucleotide does not allow the formation of the closed complex and consequently enzymes fall from the enzyme.

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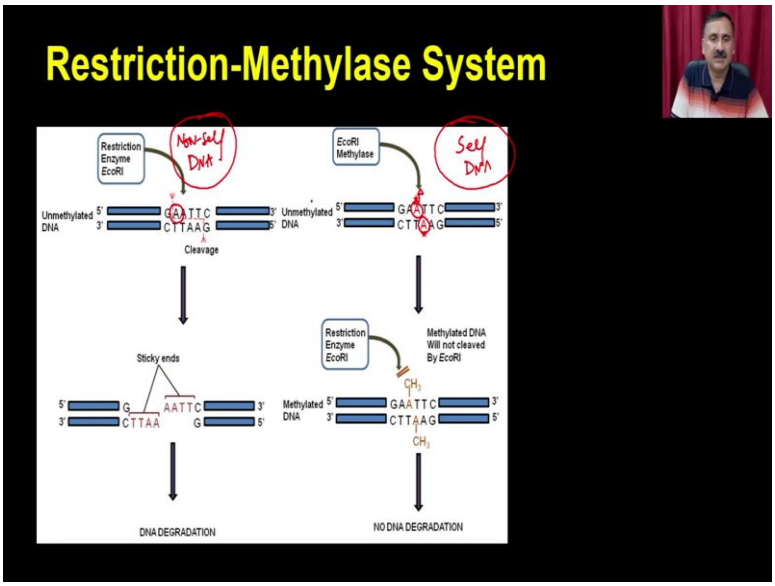

So, what it is mean is that you can have the two different types of enzymes ok and so, for example, you can have the two different types of the DNA one is unmethylated DNA and in unmethylated DNA you what you will happen is that because the methylation is not present when the DNA will go and bind, it is actually going to make when the restriction enzyme will go and bind it is actually going to make the closed complex and that is why it is actually going to cleave the DNA.

Whereas when it is a methylated which means if a gene is actually going to be methylated it is actually going to not allow the enzyme to form a closed complex and in that case it is going to be get protected from the cleavage, this means this is going to be considered as the self DNA and this is going to be considered as non self DNA and this kind of distinction is very important because the bacteria does not allow or does not want the non self which means the organism the different organisms DNA to be propagated.

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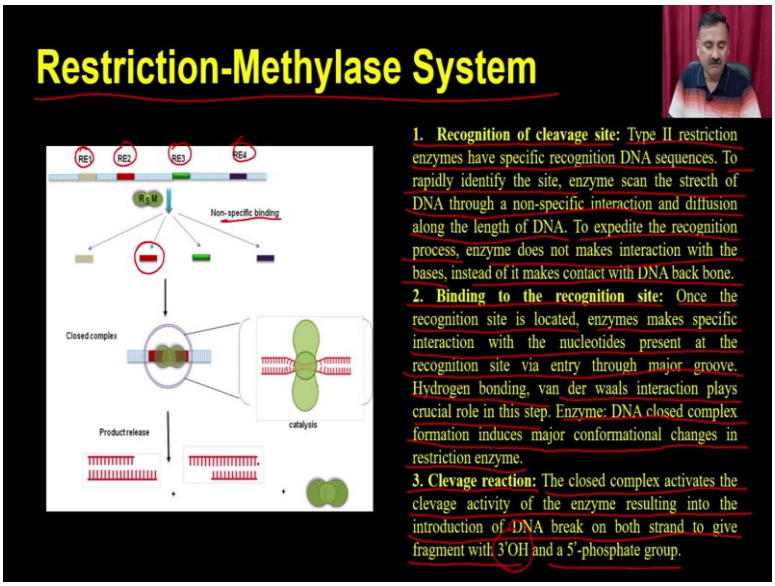

Now, how this actually going to happen is that in the restriction methylase system you have the different types of restriction sites, you can have the RE 1, RE 2, RE 3, RE 4. So, what will happen is that the restriction you know enzymes are actually nonspecifically will go and bind to these sites, but in some of one of the sites it is actually going to form a very tight complex and when it will form the tight complex all these subunits such as the RSM we are actually going to participate into the reactions.

So, how the restriction methylase system is going to work is first they are actually going to recognize the cleavage sites. So, type 2 restriction enzymes have the specific recognition sequences. To rapidly identify the site enzyme scans the stretch of DNA through a non-specific interaction and the diffusion along the length of DNA which means, if you have the 4 restriction sites the types 2 restriction enzyme will actually going to bind all of these very rapidly ok.

So, that they will know which restriction site is having the very very you know high affinity. To expedite the recognition, process the enzyme does not make interaction with the basis instead of it makes a contact with the DNA backbone. So, once the recognition is over then it is actually going to bind the restriction site for example, in this case this is the red one is actually is going to be considered as restriction recognition site.

So, once the recognition site is located, enzymes makes specific interaction with the nucleotide present at the recognition site via entry through the major groove. Hydrogen

bonding van der waal interactions play crucial role in this step. The enzyme DNA closed complex formation induces the major conformational changes in the restriction enzymes and then once the it will go and bind then it is actually going to catalyze the cleavage reaction.

The closed complex activates the cleavage activity of the enzyme resulting into the introduction of the DNA breaks on both the strands to give the fragment with 3 prime hydroxyl and as well as the 5 prime hydroxyl group. So, on one side it is actually going to have the 3 prime hydroxyl and the other side it is actually going to have the 5 prime phosphate.

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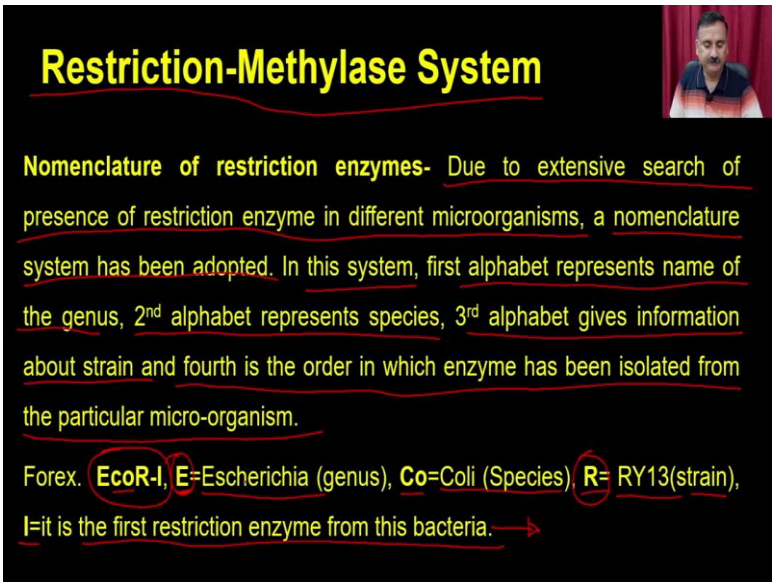

Since, we have isolated so many restriction enzymes type 2 type 1 type. So, we should also have a system so that you can be able to put that the nomenclature of these restriction enzymes. So, due to the extensive search of the presence of restriction enzyme in different micro-organisms, a nomenclature system has been adopted.

In this system first alphabet represent the name of the genus, 2nd alphabet represent the species, 3rd alphabets gives the information about the strain and the fourth is the order in which that enzyme has been isolated from a particular micro-organism. For example, we have a restriction enzyme name as EcoR-1.

So, in the EcoR 1 the first alphabet E is actually corresponds to the genus Escherichia ok. So, this is the genus from which the enzyme is being isolated. Then the second term is called co so, that is the species. So, C o is the coli. So, coli is the species of the E coli. Then we also have the thirds alphabets and third alphabet is R.

So, R is the strain. So, RY 13 strain is the strain of source of this and since and then we have the 1. So, it the first restriction enzyme from this bacteria that is why its name is EcoR 1, similarly you can have the Hind 3, you can have BamH 1 and so on.

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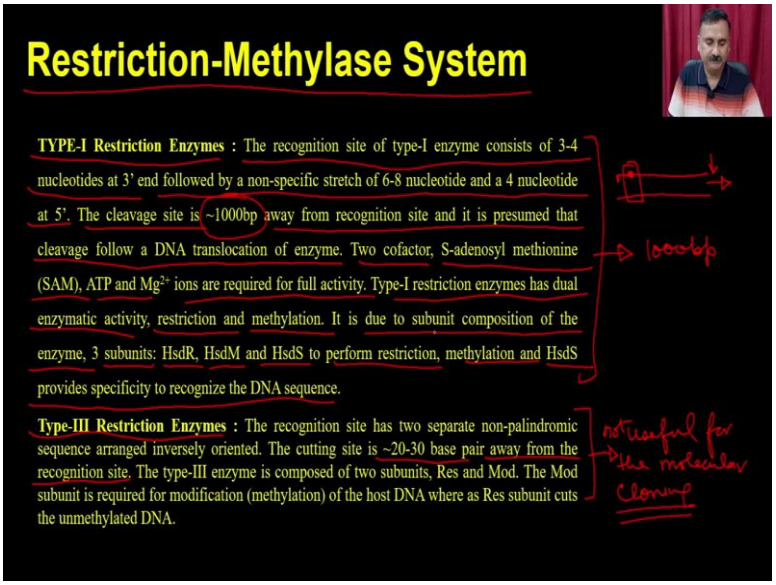

We can have the different types of restriction enzymes. So, we can have the type 1 restriction enzyme, we can have type 3 restriction enzyme and we also can have the type 2 restriction enzymes.

So, the type 1 the recognition site of the type 1 restriction enzyme consist of 3 to 4 nucleotide at the 3 prime and followed by a non-specific stretch of 6 to 8 nucleotide and a 4 nucleotide at the 5 prime. The cleavage site is approximately 1000 base pair away from the restriction site and it is presumed to cleavage follow a DNA translocation enzyme.

Two cofactor, S- adenosyl methionine SAM, ATP and the Magnesium ions are required for the full activity. Type 1 restriction enzyme has dual enzymatic activity which means it is going to have the restriction and as well as the methylation. It is due to the subunit

composition of the enzyme. So, it has 3 subunits: HsdR, HsdM and HsdS to perform the restriction, methylation and HsdS provide the specificity to the recognition of the DNA.

But as you can see that the type 1 restriction enzyme is actually not going to have a cleavage site within the recognition site. So, it is going to have a cleavage site which is 1000 base pair away from the recognition site. So, it is you can imagine that if the enzyme is recognizing this particular site as restriction site, it is actually going to cleave somewhere here this means the cleavage and as well as recognition is going to be different. So, that is why these type 1 restriction enzymes are not useful for the molecular cloning.

Similarly, the type 3 restriction enzyme; the type 3 restriction enzyme has two separate palindromic sequences. So, the recognition site has two separate palindromic sequences arranged inversely oriented. The cutting site is 20 to 30 base pair away from the recognition site which means these are also not going to be useful for the molecular cloning because they are not useful for the molecular cloning because their recognition site and their cutting site is different.

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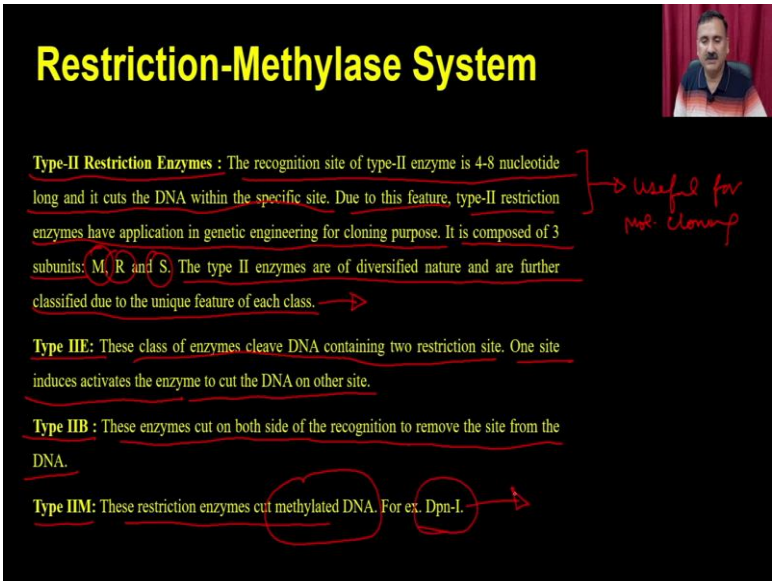

Then we have type 2 restriction enzymes. So, the recognition site of type 2 enzyme is 4 to 8 nucleotide long and it cuts the DNA within the restriction site which means they are actually going to be useful for the molecular cloning because they are cutting within the restriction site. So, with their recognition site and their cutting site are within that.

So, due to this feature the type 2 restriction enzymes have a specific application in the genetic engineering for the cloning purposes. It is composed of 3 subunits M, R and S. The type 2 restriction enzymes are of diversified nature and are further classified due to the unique feature of each class which means the type 2 E, type 2 B and type 2 M. Type 2 E these class of enzyme cleave DNA containing two restriction sites. One site induced activates the enzyme to cut the DNA on the other site.

Similarly, you can have type 2 B these enzyme cuts on both side of the restriction site to remove the site from the DNA and then you have type 2 M these restriction cut methylated DNA for example, DPN. So, this is a specific class of enzyme which actually are restriction enzymes, but they only cut the methylated DNA rather than the unmethylated DNA.

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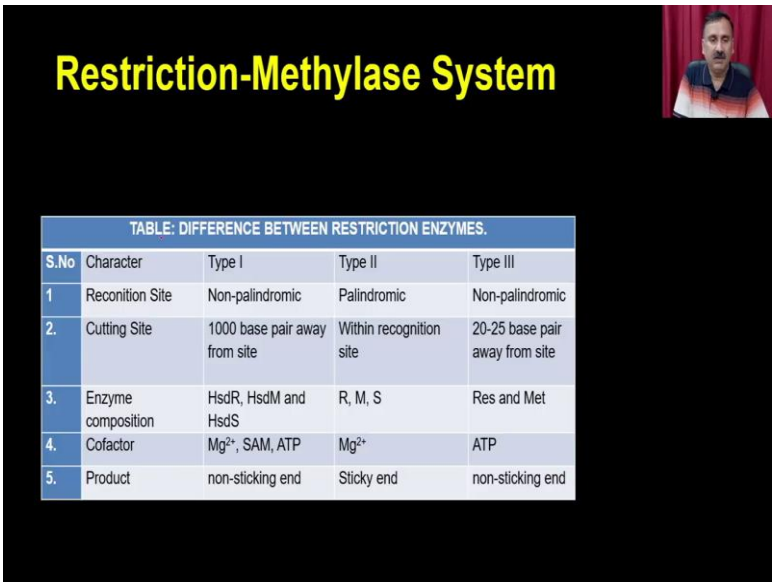

So, then I have given you a table where I have compared the properties of the restriction enzymes you can have the characteristics of the type 1, type 2 and type 3 and type 2 restriction enzymes are useful for molecular cloning only with the reason that they are actually the recognition site is palindromic in nature.

They are actually going to recognize the site and they are actually going to cut the site within the restriction site. So, it means wherever they are actually going to recognize they are actually going to cut the DNA as well.

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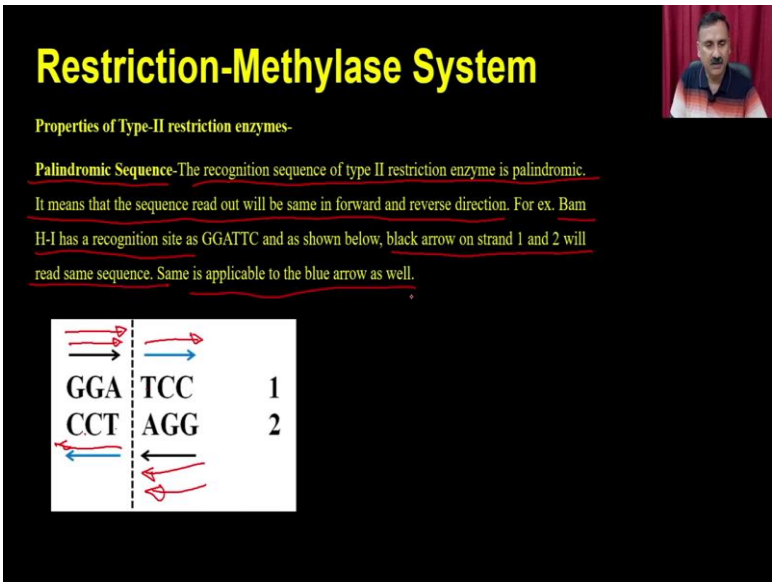

Now, what is mean by the palindromic sequences? So, pandemic sequence are the sequences which actually can read the same either if you read from the forward direction to reverse direction or from the reverse direction to forward direction.

For example, you have a sequence called GGATTC. So, if you read from this side it will say GGA, similarly if you read this from the reverse orientation it will say GGA this means these two are palindromic to each other. Similarly, you can read from this side to this side it will say TCC if you read from this side to this side it will say TCC. So, this means if you read in the forward direction or if you read in the reverse direction it is actually going to give you the same sequence.

So, the recognition sequence of type 2 restriction enzyme is palindromic. It means that the sequence read out will be same in forward and the reverse direction. For example, the Bam H-1 has a restriction site as GGATTC as shown below and the black arrow on strand 1 and strand 2 will read the same sequence. Same is applicable to the blue arrow as well ok.

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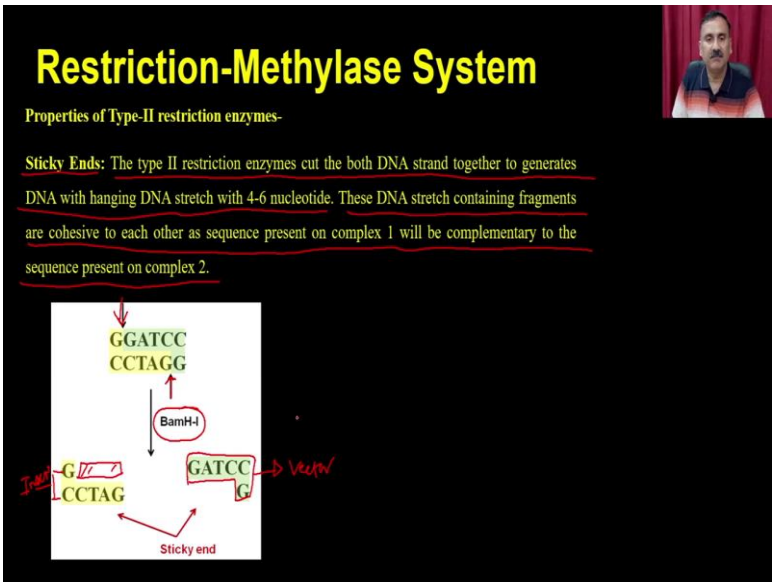

Then it also generates the sticky end. So, what is mean by the sticky end? The type 2 restriction enzyme cut the both DNA strand together to generate the DNA with the hanging DNA stretch with 4 to 6 nucleotide. These DNA stretch containing the fragments are cohesive to each other as sequence present on complex 1 will be complementary to the sequence present on the complex 2.

For example, this is the site for the Bam H-1. So, what it is going to cut is it is going to cut here just after the G right. So, it is a GGATTC. So, when you cut it with the BamH-1 it is going to generate these sequences. So, it is going to generate G and then it is going to generate this overhang.

So, this overhang region is actually going to have the affinity for this overhang which is actually going to be present in the other sequence. So, that is why if you see so, these two are actually going to have the sticky end. So, you can imagine that if this is actually from the vector and this is from the insert and if you put them together they will actually come together and they will actually going to make the bonds together.

So, that is why these sticky ends are very useful in terms of the molecular cloning. Now the question comes, how you are going to set up the restriction reactions?

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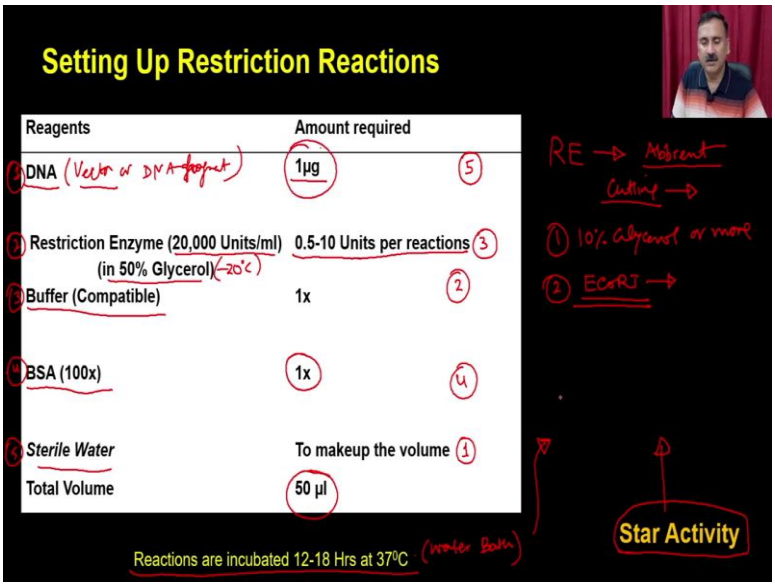

So, for restriction reactions you require the following components you can require the DNA. So, DNA means either the vector or DNA fragment what you have you know amplified from the PCR. So, you require the DNA number 1, you require the DNA, then you require the recitation enzymes, you require the buffer, you require the BSA, you require the sterile water and the total volume.

So, in a total volume of 50 micro liter you are going to perform the restriction enzymes reactions. So, what you are going to do is you are going to take the DNA. So, for example, I have taken a DNA of 1 micro gram then I am going to put the reactions in the range of 0.5 to 10 units per reactions.

So, normally the restriction enzymes what you are going to get from the vendors are in the range of 20000 units per ml and they are in the 50 percent glycerol, because mostly the restriction enzymes are being stored at minus 20 degree Celsius. So, they will be always be kept in the 50 percent glycerol so that they should not get freeze ok.

Because if you do a repeated freezing towing of your enzyme it is actually going to deactivate or it is going to reduce its activity. Then you also require the buffer in which the activity of these enzymes are actually going to be very high. These buffers are mostly contains the ATP and all of the kinds of you know molecules so that and they also have the specific you know buffer and pH so, that you can be able to give you the 100 percent activity.

Then you also require the BSA in some cases where the enzymes are not compatible to each other. So, you are actually going to put the BSA and then you also ultimately going to make up the volume with the water ok. So, the order in which you are going to put the reactions are you are going to first take the water.

Then you are actually going to take the buffer then you are going to take the enzymes and then you are actually going to add the BSA if there is a need to add the BSA and then ultimately you are going to add the DNA and that is how you are actually going to set up the reactions.

Why we do actually add the BSA, because we want to reduce the star activity. So, what is meant by the star activity? Star activity is that ideally the restriction enzyme should recognize the particular DNA stretch and then they are actually going to make a cleavage within that site, but what happened is that because you have too much enzymes or you have too many DNA restriction enzymes are actually going to show aberrant cutting site ok.

So, aberrant cutting and that happens sometime even with the glycerol itself. So, if you have the 10 percent or more glycerol. So, if you have the 10 percent or more it is actually going to induce the star activity. Number 2, if you are taking a you know combinations of some enzymes like for example, if you take the Eco R 1. So, Eco R 1 is known to provide or show the star activity. So, if you take the Eco R 1 versus other enzyme like x bar 1. So, in that case the Eco R 1 there is chance that Eco R 1 will show you the star activity.

So, what is mean by the star activity? That it is going to give you the aberrant cutting. So, wherever the enzyme supposed to cut it will not cut to that site it will cut to somewhere else. So, in that case you will not going to get the specific you know sticky ends and if you do not get the sticky specific sticky ends it will actually going to create trouble in terms of the you know the ligations.

So, once you set up the restriction enzymes reactions you are actually going to incubate the reactions at 12 to 18 hours for at 37 degree Celsius mostly in a water bath rather than the dry bath. Because water bath is actually soft in terms of providing the temperature or incubating the temperature.

Sometime when you are you know not sure about the activity of these enzymes to the DNA then you can actually be able to do a time curve. For example, you can do the reaction for 1 hour 4 hours like that to so, to need to know that whether the enzymes are cutting the DNA or not.

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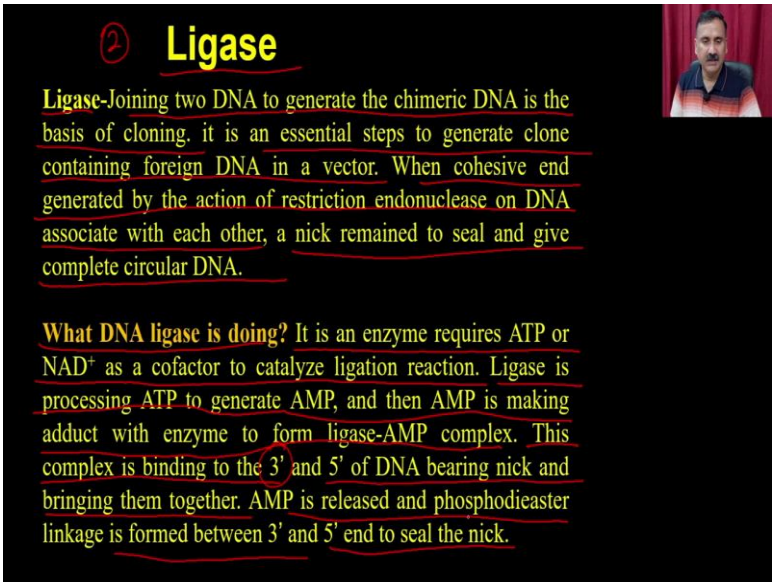

Then we will talk about the second enzyme. So, second enzyme is the ligase. So, ligase the joining the two DNA to generate the chimeric DNA is the basis of cloning its essential step to generate the clones containing foreign DNA in a vector. When cohesive ends generated by the action of restriction endonuclease on the DNA associate with each other, a nick remain to seal and give complete circular DNA ok.

So, what the DNA ligase is doing? It is an enzyme which requires ATP or NAD plus as Acofactor to catalyze ligation reaction. Ligase is processing ATP to generate AMP and then AMP is making an adduct with the enzyme to form the ligase- AMP complex. This complex is binding to the 3 prime and the 5 prime of the DNA bearing nicks and bringing them together.

AMP is released and the phosphodieaster linkage is formed between the 3 prime and 5 prime end of the seal ok. And that is how it is actually going to seal the nick. So, this is what is going to happen ok.

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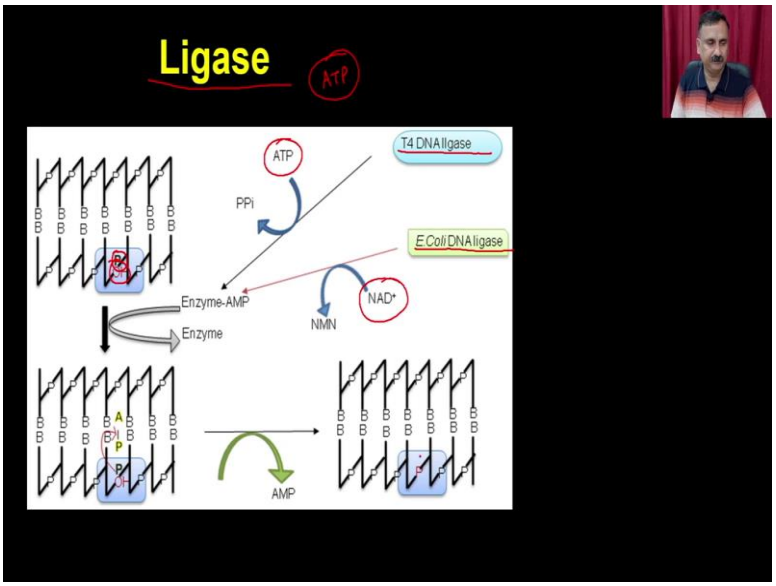

When you are going to add the ligase along with ATP what will happen is that we have taken an example of two different enzymes we have taken the enzyme of T 4 DNA ligase or the E Coli DNA ligase, in the case of T 4 DNA ligase it is actually going utilize the ATP as the source of the phosphate.

So, it is actually going to bring the nicks closer to each other and that is how the AMP is going to bind and ultimately the AMP is actually going to bring the 3 prime and 5 prime together and the ultimately there will be a bond which is going to be formed between the 3 prime hydroxyl and the 5 prime phosphate.

And that is how and the AMP is actually going to be released in the case of in the case of the E Coli DNA ligase you are going to use the NAD plus as a cofactor and that is how the reactions remain the same except that the NAD plus is actually going to provide the same kind of support to bring the 3 prime hydroxyl to the 5 prime phosphate and that is why it is actually going to make a bond and it will actually going to seal the nick.

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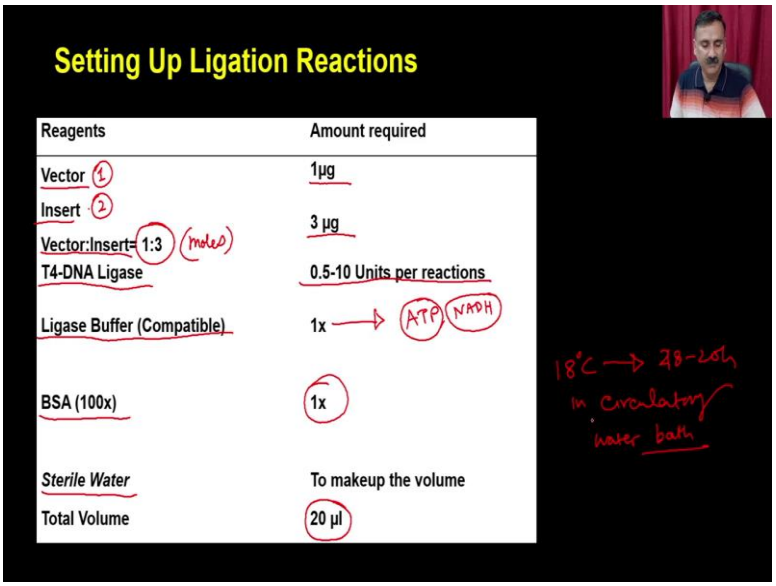

Now, how you are going to set up the ligation reactions? So, you are going to take the two DNA right DNA vector or the insert right. So, you are going to take the two DNA source then you are always going to maintain the vector versus insert ratio 1 is to 3 this means and this is in terms of the moles ok it is not in terms of micrograms ok.

So, vector you are going to take 1 microgram, the insert you are going to take 3 micrograms then you are going to add the enzyme which is 0.5 to 10 units per reactions then you are going to add the ligase buffer. So, ligase buffer will actually going to have the buffer and then ATP or NADH whichever you are actually going to use so, depending on the source of this ligase.

So, for example, if you are using the T 4 DNA ligase, it is going to have the ATP, but if you are using the E Coli ligase it is actually going to have the NADH. Then it also going to have the BSA so, 1 x BSA and then you are going to have the sterile water. Remember that the ligase reaction has to be kept in a small volume like 20 micro liter compared to that restriction enzyme reactions are going to be done in a large volume such as 50 micro liter because you require large quantity of water to catalyze the restriction ligation reactions.

And in the case of ligations, you require the reactions to be small so that the vector and the insert which have the cohesive ends should interact with each other and that is how they are actually going to be you know form the double stranded DNA and the nick is

going to be sealed by the ligase. Once you have done this this you are going to incubate this at 18 degree Celsius for 18 to 20 hours in in circulating water bath and ok.

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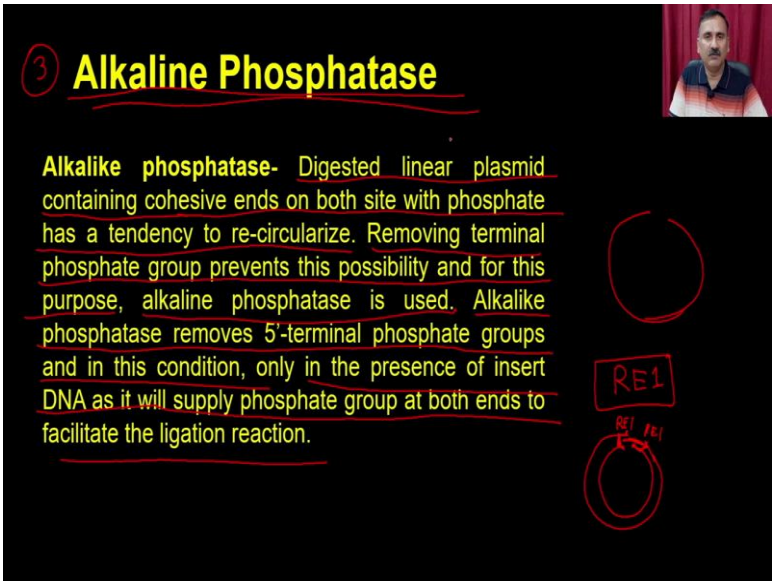

Now, the third enzyme which is called as alkaline phosphatase so, alkaline phosphatase is it is digested linear plasmids containing. Why we use the alkaline phosphatase is because the digested linear plasmid containing cohesive ends on both the site with phosphate has a tendency to re-circularize ok.

So, removing the terminal phosphate group prevents this possibility and for this purpose the alkaline prophatase is used. Alkaline phosphatase remove the 5 prime terminal phosphate group and in this condition, only in the presence of insert DNA it will supply the phosphate group at both the end to facilitate the ligation reaction.

So, alkaline phosphatase is being used in those places when you are actually only using the single restriction enzyme you are not using the two restriction enzyme. So, in that case both the ends of the DNA is actually going to have the cohesive ends which is going to have the RE 1 on both the sites. So, if you have RE 1 on both the sites and one site you are actually going to have the phosphate, they will actually re-circularize themselves in the absence of even in without insert also.

And because this guy has a phosphate and this guy has a phosphate, this guy has a hydroxyl group, this guy has a hydroxyl group. So, they will actually going to have the

compatibility with each other and that is why they will re-circularize. If you have the RE 1 versus RE 2 in that case this particular sequence and this particular sequence will not be identical.

So, they will not going to have the affinity for each other. So, if you are doing the single digestion or single enzyme digestion then you are going to use the alkaline ligase alkaline phosphatase to remove the phosphate from the vector.

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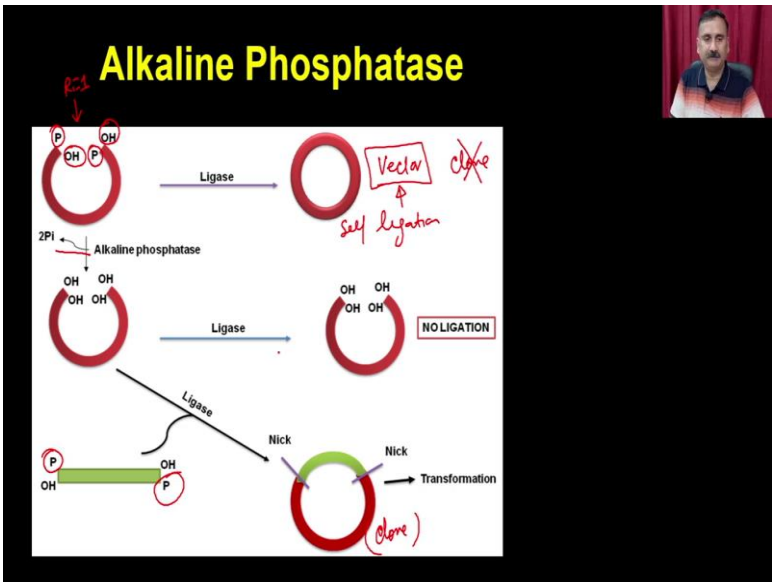

Now, what will happen is that in the case of vector when you are digesting it with the single restriction enzyme like for example, RE 1 you are going to have the phosphate and hydroxyl overhangs and you are going to have hydroxyl on that side and phosphate on this.

So, if you put the ligase reaction they will actually re-circularize without taking the insert and that is how you are actually going to get the vector back instead of the clone ok. So, you are not going to get the clone, insert the vector is actually going to re circularized and that is how it is actually going to make the background very high.

Now, if you treat this with the alkaline phosphatase you are actually going to remove this phosphate and you are going to have the hydroxyl on both the ends. Now, if you do the ligation reaction these hydroxyl groups are not going to get ligated because you require a phosphodiester linkag so that phosphodiester linkage requires the phosphate group.

Now, if I add these the insert, which actually contains a phosphate group right, this phosphate group will go and sit to the hydroxyl group very nicely and that is why you are going to have the two nicks on both the ends and that is why you are going to get the clone. So, this is actually going to give you the clone.

So, alkaline phosphatase is always being done to reduce the background of the self-ligation. So, this is called self-ligation where so that you are actually going to get the vector back. Now, apart from these enzymes some of the tools also are very popular when you are doing the molecular cloning.

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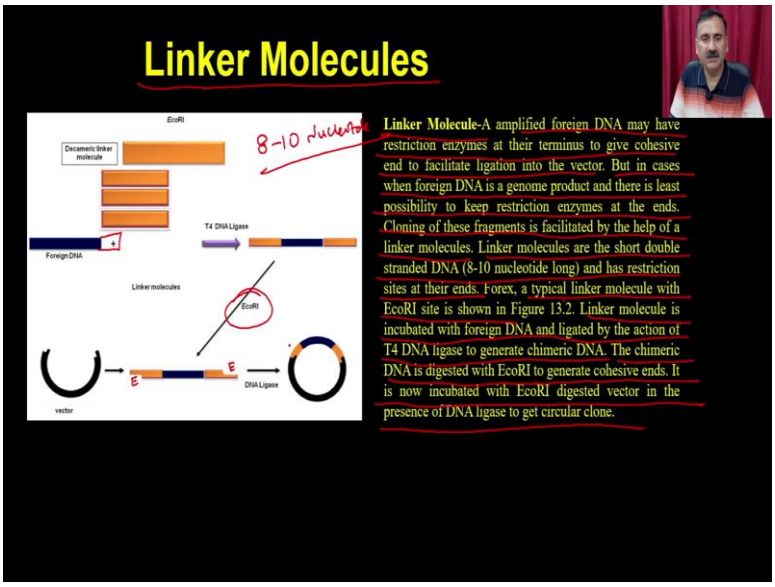

So, one of the tool is the linker molecules. A linker molecule, A amplified for a DNA may have a restriction enzyme at their terminus to give the cohesive ends to facilitate the ligation into the vector. But in case where the foreign DNA is genomic product and it is least possible to keep restriction enzymes at the end. Cloning of these fragment is facilitated by the help of a linker molecule.

Linker molecules are the short double stranded DNA and has the restriction site at their end. For example, a typical linker molecule with Eco R-1 site is shown in this figure ok. So, this is what you are going to see. Linker molecule is incubated with the foreign DNA and ligated by the action of T 4 DNA ligase to generate the chimeric DNA.

The chimeric DNA is digested with Eco R 1 to generate the cohesive end and it is now incubated with Eco R 1 digested vector in the presence of DNA ligase to get the circular clone. So, what happen is when you are producing a foreign DNA and there are restriction that you cannot be able to put the particular enzyme or some of the enzymes are already been present on your foreign DNA right in.

So, in those cases your foreign DNA is going to be the blunt end ok. So, it does not have the cohesive ends on both side. So, in those cases what you are going to do is you are going to put the linker molecule and linker molecule is a 8 to 10 nucleotide long DNA which actually going to be you know. So, you can use the linker DNA for example, in this case this is the Eco R 1 linker DNA.

So, you what you are did is you put the linker DNA on both sides ok with the help of the T 4 DNA ligase and that is what when you do the digestion of this DNA with the help of the Eco R 1. It is actually going to have the Eco R 1 site on both the ends because and that is how it is actually going to be get inserted into the vector and this is the way in which the blunt and dean fragment can be inserted into the vector.

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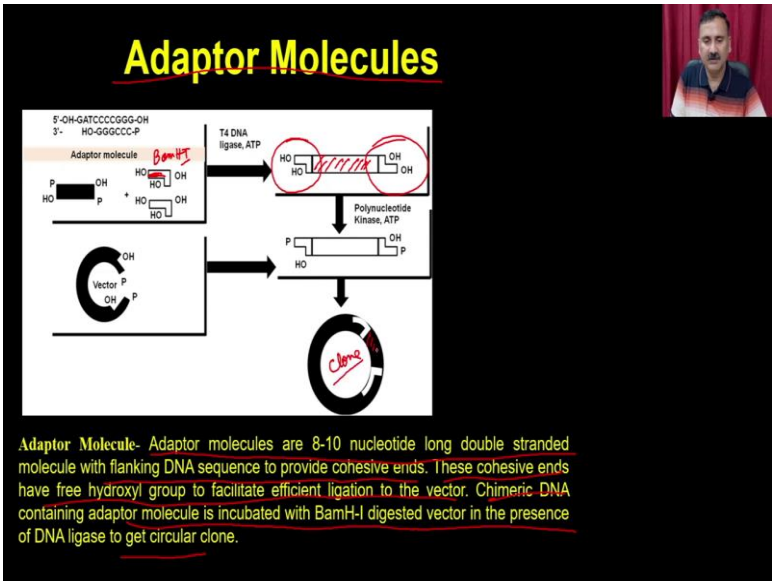

The second molecule which also can be used is a adaptor molecule. So, these adaptor molecules are molecules are 8 to 10 nucleotide long double stranded molecule with a flanking DNA sequence to provide the cohesive ends. So, in the case of linker DNA you

are supposed to provide the restriction enzyme you are going to you know digest the linker molecules with the restriction enzymes.

But what if your that particular restriction site is already present in the fragment right. So, in those cases you are not going to use the linker molecule and then you can use the adaptor molecule. So, adaptor molecules are 8 to 10 nucleotide and they are actually going to have the you know specific cohesive ends on both the ends ok.

And so what you can do is you can take the gene of your interest you put the cohesive adaptor molecules. So, they will actually going to fix on both the sides right both ends and that is how you are actually going to use that for with the vector in the early ligation reaction and it will actually going to give you the clone.

So, those these cohesive ends have the free hydroxyl group to facilitate the efficient ligation into the vector. Chimeric DNA containing adaptor molecule is incubated with bam h one digested vector in the presence of DNA ligase to give the circular DNAs. For example, these are the cohesive adaptor molecule which has the BamH 1 ok. So, the both are this flanking sequence what you see is actually a BamH 1.

So, it will come and stick to both end of the your fragment right, this is the fragment what you have right and then you can just put the legation reaction into the digested vector and it is actually going to be ligated into the vector and that is why you are going to get the clone where you are this is the insert you have actually.

So, this is all about the different types of enzymes and tools what we are actually going to use for molecular cloning and in our subsequent lecture we are actually going to discuss more about the vectors which you are going to use also for the molecular cloning. So, with this I would like to conclude my lecture here.

Thank you.