Molecular Biology
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Module - 03
Basics of Biomolecules
Lecture-16 Enzymes

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 properties of the different biomolecules which have extensive role in running the cellular metabolisms and they are also been responsible for different types of activities what are being done in the cells or in an organism actually. And majority of these biomolecules are be a part of the different types of activities and it is important for us to understand these structure and the function of these biomolecules so that you can be able to understand the molecular processes what they are actually going to run and regulate. So in this series, so far what we have discussed, we have discussed about the genetic material in the DNA.

Although we are going to discuss in detail about how people have discovered the DNA as the major genetic material what is present in the different types of organisms. But for the time being, you can imagine that the DNA is the major genetic material what is present in the different types of organism. There are exception that there are other kind of biomolecules which is also been responsible for carrying the genetic information from one generation to another generation. Apart from that DNA is also actively participate into replications and that is how the DNA is going to make multiple copies and these multiple copies are going to be given to the daughter cells after the cell division and the mitosis and meiosis.

And then apart from that the DNA is also going to be active in terms of the into another activity which is called as transcription and in the process of transcription the DNA is going to make the RNA and then these RNA are actually going to be responsible for making the proteins. So then subsequent to that we have also discussed about the RNA and the RNA is responsible for production of the proteins and the proteins are responsible for you know the different types of activities. So one of the major thing what we have discussed we have discussed about that the protein is the building block and if you recall in the previous lecture we discussed about the relevance of the proteins. So as a so we have in the previous lecture we discussed about structure and the function of the different protein molecules. So we discussed about the amino acids what are the different types of amino acids are present and then we also discuss about that the proteins is having the four layer of structures.

So it has a primary structure and secondary structures and tertiary structure and quaternary structures and very extensively we discuss how you can be able to study these different types of structures. So you can actually be able to have the different types of reactions what you can perform to sequence the reactions sequence the protein molecules and so on. Now in today's lecture we are going to discuss about the enzymes because enzyme is also proteinaceous in nature in majority the enzymes are proteinaceous in nature and these enzymes are actively being participated into the two different types of activities. First they are actually been responsible for running the metabolic reactions. So if you recall we have discussed about that the enzymes are actually responsible for running the catabolic reactions and the anabolic reactions.

Apart from that the enzymes are also responsible for running the detoxification reaction so that they can be able to produce the urea and then urea is actually going to be secreted out from the body in the form of the acids and the urine also and apart from that the catabolic reactions are responsible for energy production and this energy is actually going to be utilized for different types of activities. It is going to be utilized for growth, reproduction and the synthesis and the synthesis of the new biomolecule. The synthesis part is actually a part of the anabolic reactions. So it is actually going to participate into the anabolic reactions which is nothing but the synthesis and when we were discussing about the anabolic reactions we discussed that the anabolic reactions are so we discuss about the biosynthesis of the different types of amino acids and other things. So in today's lecture we will going to discuss about what are the different types of enzymes and what is the basic property of enzyme and apart from these biogasity role of these enzyme in the biochemistry the enzymes are also having the extensive role in the case of molecular biology protocols or the molecular biology processes.

So that the processes we are actually going to discuss in detail but today we are just going to discuss the different types of enzyme which are actually having the role in the molecular cloning. So what is the enzyme? So the enzymes as the name suggests is also being called as the biological catalyst. Okay so these are the catalyst molecule which are present in all the living cells. They are also present in all the living cells. What is their job? So just like any other chemical catalyst their job is to convert the substrate into the product and the enzymes are mostly been made up of the protein.

There is an exception that enzymes are also been made up of the RNA molecule. These are these enzymes are called as the ribozymes. So there are enzyme which are made up of the RNA molecule and these enzymes are called as ribozymes but this the amount of these enzymes are very very small and they are also being required for a specific function. So but majority of the enzymes are made up of the protein molecules and the as

I said you know enzymes are called as the biological catalyst. So what is the role of the catalyst? The role of the catalyst is that it is actually going to increase the rate of the chemical reaction right.

So it is actually going to increase the rate of chemical reaction so that the more number of substrate molecule will get converted into the product but at the end of the reaction it is actually going to be remain unchanged which means it is going to perform the chemical reaction but it itself is not going to be a part of the reaction. That is why at the end of the reaction the enzyme is going to be remain intact and that is how it is actually going to keep running the reaction right. Now the first question come is how the enzymes are working right. So what the enzyme is doing for example if I am taking an example that you have a substrate A it is getting converted into substrate B right. So what it is doing is it is actually you know converting A to B for example but like more precisely so and the molecule A is actually having a phosphate group okay and it is reacting with the molecule C for example and molecule C does not have a phosphate group but it has the groups right.

So and then it is forming the B molecule and B molecule is nothing but the C phosphate. This means it is actually breaking a bond here right and it is transferring this particular group on to the C molecule and that is how it is generating a B molecule which is nothing but the C phosphate. So it is here in the B it is actually making a bond. So at one place it is breaking the bond in the other side it is making bond okay. So how it is going to happen? It is going to happen that you have the substrate A plus B and that A plus B is actually you need to so what is happening here is that you are supposed to break some of the bonds and then you are supposed to make the new bonds right.

So if you want to break the bond you are actually going to infuse the energy into the system right and that is what it is actually going to happen. So if A and B are actually reacting with each other they are supposed to cross a barrier and once they cross the barrier then there will be a change of groups between the A and B and that is how they are actually going to form the PQ. This barrier is actually going to be high when you do not have the enzyme okay. This barrier is going to be on a higher side that means if you do not do this reaction without in the absence of enzyme then you are supposed to supply more amount of energy and as a result it is actually going to be difficult for this reaction to proceed. Whereas in the presence of enzyme what will happen is that it is actually going to lower down the amount of energy what you require to catalyze this reaction and this is what exactly what is going to happen when you have an enzyme.

So enzyme as a catalyst change the rate of chemical reaction but it does not alter the equilibrium okay. This means it is actually going to reduce the activation energy which

means the energy what is required to activate to A plus B to form the P plus Q is actually going to be on a lower side and that is how it is actually going to achieve this value much quicker right and that is how it is actually going to catalyze more number of reaction. So a catalyst function by lowering the activation energy of a reaction the energy barrier for a reactant to become the product. Now this is what exactly what happens right. Now the question comes why we need the enzymes.

So you need an enzyme because of this reason right. So you see there are some examples of the different types of enzyme and I am giving you the value of the enzyme constant right the rate of reactions in the presence and absence of the enzyme. So this is the absence of the enzyme and this is the presence of enzyme. So what you see here is that in the absence of enzyme if you are going to perform the reaction what is being catalyzed by the carbonic anhydrides it is actually going to have a reaction rate of 1.3 into 10 to power minus 1 okay.

Whereas if you are adding the enzyme to this reaction it is actually going to have the rate of reaction as 1 into 10 to power 6 which means you are actually going to have the rate enhancement if you have the enzyme as in the range of 10 to power 6. So 10 to power 6 fold there is a rate in enhancement same is true for the course mate mutase if you have the non enzymatic reaction it is going to be in the range of 10 to power minus 5. But if it is a enzyme catalyzed reaction then the rate of reaction is 50 this means there will be an enhancement of 10 to power 6 folds. Same is true for the triosulfate isobanase in the absence of enzyme it is going to have a rate constant of 10 to power minus 6 per minute whereas in the pace of enzyme it is going to be 4300. So there will be an enhancement of 10 to power 9 folds.

So what it says is that enzyme which is going to increase the rate of reactions but it will not participate into the reactions and it will going to be remain unutilized at the end of the reactions. So there are other example also right. So why we need an enzyme? We need an enzyme to increase the rate of reactions increase the rate of reaction that is one point. The second point is that some of these reactions will not going to proceed at a rapid rate. So to perform these reaction at a rapid rate you are also going to increase the temperature.

So for example if you are to run the carbonic and HEDIS reactions right you are supposed to have a degree angle if you supposed to have the heating of these reactions at 100 degree Celsius and couple of atmospheric pressures. Those kind of conditions are non physiological conditions. So if you are going to perform the same reactions you are actually going to have the non physiological conditions like for example 100 degree Celsius. Most of the organisms will not going to survive at 100 degree Celsius right. So

what is the permissive temperature? Permissive temperature is 37 degree Celsius.

So if I supposed to run the reaction at 37 degree Celsius I am supposed to enhance the rate of reactions and that rate enhancement of the rate of reaction is only possible if I have an enzyme into the reactions right. Now what are the different properties of enzyme? So enzymes are made up of the protein except there is an exception of ribosome. So that we have already discussed that it is made up of the RNA. Then due to presence of the amino acids it provides the specific environment for catalyzing the reaction with the different types of substance. You know that the since the enzymes are made up of the proteins and the proteins are made up of the amino acid and you have the 20 different types of amino acids and all these amino acids vary in terms of the different types of property like the charge, polarity, then you also have the hydrophobic amino acids, you have hydrophilic amino acids and so on.

And all these actually provides a local micro environment which is different for the different types of substrate and that's why the enzyme can be able to recognize even the subtle changes into the substrate. That's why they are very precise and they are very specific. The substrate binds to a small pocket within an enzyme. This pocket is known as the active site. The molecule produced by the reaction is called the product right and the enzyme catalyzed reactions are very rapid than the uncatalyzed reactions.

Now they are very specific towards the substrate and the product and the enzyme activity can be modulated by the non substrate molecules such as allosteric control or the covalent enzyme modifications. In a few specific cases, enzyme amount can be modulated by the synthesis or the degradation. So this actually happens within the cell when they are actually either degrading the enzyme so that they can be able to modulate the reactions or they can be able to synthesize the new molecule. So that anyway we are going to discuss when we are going to discuss about the translation and other kinds of molecular events. Now how the enzyme is recognizing the substrate? So it is actually recognizing the substrate due to three important parameters.

One is geometrical complementarity, the second is electronic complementarity and the third is stereospecificity. Geometric completivity means it is actually going to see whether the 3D structure of the substrate is matching with the enzyme or not right. So you can see that this is a substrate and it is matching exactly with the 3D structure of the enzymes or 3D structure of the active site. The third is electronic complementarity. So electronic complementarity means whether the electron donor and electron acceptor groups are being compatible to each other which means wherever the electron donor is present onto the substrate whether the electron acceptor is present on the enzyme or not because you have two pairs, one is enzyme, the second is the substrate.

So if you have the electron donor onto the substrate molecule then you should have the electron acceptor onto the enzyme actually. For example here right this is the hydrogen donor right so you have the hydrogen acceptor on this. So when the enzyme will when the substrate will fit into this cavity the hydrogen donor and the hydrogen acceptor they will actually going to interact with each other and that is how there will be hydrogen bonding formations. Similarly you have the one charge right so you have the for example you have the negative charge. So it is actually going to interact with the positive charge what is present onto the enzyme.

So there will be a salvage interaction between the substrate and product. That is how it is actually going to bind very strongly to the enzyme. And there are other kinds of interaction also for example you have the hydrophobic interaction, hydrophobic molecule, hydrophobic substances what is present onto the substrate and then you also going to have the hydrophobic groups onto present on the enzyme. So these are the some of the things what is responsible for the substrate specificity. Apart from that you also require the stereosensitivity which means that it will actually going to recognize whether the substrate is L type or the D type.

Although this particular in this particular course we are not going to discuss in detail about any of these aspects because we are actually going to discuss more about the molecular biology related stuff. So if you want to know more about these things you can we have another MOOCs course which is called as enzyme science and technology and you can actually be able to follow that. So there is a MOOCs course where you can actually be able to use that. So this is what exactly it says that the geometric complementarity means that enzyme binding side has a structure which is complementary to the substrate it binds. Then electronic complementarity the amino acid that are from the that is forming the enzyme binding side are arranged to specifically interact and attract the substrate molecule and then the stereosensitivity that the binding of chiral substrate and the catalysis of the action is highly specific due to the large part of inherent chirality of the L amino acid that comprising enzyme.

So if I summarize the properties of the enzyme the enzymes are actually going to have the different types of groups what are present and enzymes are actually going to be very specific for their substrate. Apart from that the enzymes are also requiring the metallic and as well as the other kinds of small groups which are actually being a part of the cofactor. So if it is a metal then it is going to be called as cofactor and if it is a small molecules then it is going to be called as coenzyme. So these are just some examples of the cofactors for example we have the copper, iron, potassium, magnesium, manganese, nickel, selenium, zinc and for example the copper is a cofactor in the cytochrome C

cytochrome C oxidase. Then for iron it is actually present as a cofactor in catalase and peroxidase, potassium is present in pyruvate kinase, magnesium is present in hexokinase, glucose 6 phosphate, manganese is present in the arginase, dibonucleotidase and urease, nickel is present in urease and so on.

Similarly we have the co-enzymes. So coenzymes are mostly the vitamins or the other kinds of molecules. So it is a small organic molecule for example you have the biocytidine so that will be a coenzyme for the carbon dioxide right. So it is actually going to bind the carbon dioxide then we have coenzyme A, it is actually going to have the acyl group. Then we have coenzyme B12 so it is going to have the hydrogen and alkyl groups. Then we have FAD which is going to have the electron and so on.

So these are the some of the things and since the enzyme require these molecules for their optimal activity if these molecules are not present then they will be responsible for different types of disease. For example if you have the deficiency of the iron then you are going to have the parenthesis anemia and so on. And then pellagra is being caused by some of the vitamins deficiency and so on. Now we will focus more on the enzyme which is responsible or which are going to participate actively into the molecular cloning. So these are the general you know the scheme of the molecular cloning where from the genome you are actually going to identify the gene and you are going to amplify this gene with the help of the polymerase.

This process is called as the polymerase chain reaction and these are the things we are actually going to discuss in this particular course. So just for you know this is summary of what we are going to discuss in molecular cloning. Then you are going to digest this with the restriction enzymes and that is actually going to generate the cohesive ends in both the sides. The same is true for the plasmid also and then you are going to have the cohesive ends of the plasmid and then you are actually going to do the ligation reactions. And once you do the ligation reaction you are going to have the ligated plasmids.

So these are the recombinant plasmids and then you are going to transform this and that is how you are actually going to have the you know the organisms the transformer organism and that can be used for protein production. So this is the general scheme and in this particular scheme what you see here is that you are first using the polymerase. So this is the enzyme 1 what you require. Then you also require the restriction enzyme. So this is the enzyme 2 and then you also require the ligation reaction.

So you also require a third enzyme which is called as ligase. So these are the three enzyme which are very very crucial for the different types of but these are the some of the enzyme which is actively participating into the different types of activities within the

molecular biology. So the first is polymerase right which is required for the PCR amplifications. Then you require the repetition enzyme that is for the cutting the DNA at a specific site. Then you also require the alkaline phosphate that is required for the removal of terminal phosphate group and then you also require the DNA ligase which is joining of the two DNA strands.

So we will start first with the restriction enzyme then we are going we are not going to discuss about the polymerase chain reaction polymerases because that we are going to cover when we are going to discuss about the PCR and then we are going to discuss about the alkaline phosphatase and at the end we are going to discuss about DNA polymerase DNA like research. So the first is restriction methylase system. Substition methylase system is immune system which is present in the prokaryotic system ok and it does not allow the propagation of propagation of foreign DNA ok. So this it distincts with self versus foreign DNA. So foreign DNA is the DNA from the infectious organisms, self will be that the DNA from your own right.

So although the precise mechanism or distinction is not known but based on the available literature in the absence of methylation a closed complex is formed and allow the proper activation of the cleavage activity of the enzyme. The presence of methyl group on nucleotide does not allow the formation of the closed complex and consequently the enzyme fall from the DNA. So this is what exactly happens. So restriction enzyme or restriction methylase system what it is doing is that it is checking the you know the DNA for the presence of the methylation on to the adenine groups.

So you have the methyl group right. So methyl group either would be unmethylated, hemi methylated or the fully methylated. So for example in this case this is the one DNA is unmethylated the other DNA is methylated right. So this is unmethylated DNA. Now if I add the station enzyme what will happen is that it is actually going to recognize that okay there is no methylation and that is how it is actually going to cleave. So it is actually going to cleave this band more and that DNA and that is how it is actually going to generate the sticky ends okay.

But if there is a methylation for example in this case right this is the fully methylated then the enzyme will not be able to bind and as a result there will be no degradation of the enzyme. So this DNA is going to be considered as self-DNA and this is actually going to be considered as non-self DNA. And this is a kind of a defense response what is present in the lower into the prokaryotic system but this system has a very unique feature that it actually generates the sticky ends. So you have different types of restriction enzymes and they actually generate some of these restriction enzymes are actually generating the sticky ends and that can be used into the molecular cloning. Now

the question comes how the restriction methylase system actually recognizes the cleavage site.

So for this for example we have the four restriction sites, four restriction site RE1, RE2, RE3, RE4 and now what we have done is we have added one restriction enzyme right. So what will happen is that the restriction enzyme will actually go and non-specifically will go and bind the DNA right at multiple places okay. So it will go and bind a non-specific binding to the restriction sites. So it is going to bind to site 1, site 2, site 3, site 4 and every sublase what it actually going to do is it is actually going to look for the binding cause or binding right. So it can have two different types of binding either it can have the loose binding or it can actually have the tight binding.

If it is having the loose binding then the DNA will not be able to cleave. So it is naturally going to no cut okay. If it is a tight binding or then it is actually going to cut the DNA okay. So what happened is that if there is a closed complex or the tight binding then the enzyme will sit and then it is actually going to catalyze the cleavage reactions and as a result it is actually going to generate the degraded DNA and then the enzyme is actually going to be released. However, the estrogen enzymes are also an enzyme made up of a protein.

So they are not going to utilize into the reactions and ultimately it is actually going to come out from the reactions. Now once the people have started discovering the different types of restriction enzyme they also put rules and regulation for putting the name of these enzymes. So the nomenclature of a restriction enzyme due to the extensive search of the presence of restriction enzyme in the different molecular organism a nomenclature system has been adopted. In this system the first alphabet represent the name of the genus, the second alphabet represent the species, third alphabet gives the information about the strain and the fourth is the order in which the enzyme has been isolated from the particular microorganism.

For example, this is an enzyme name EcoR1. So this is the restriction enzyme. So here the first alphabet is E, the second alphabet is CO, the third is R and the fourth is 1. So for the E it stands for Eschercia.

So this means EcoR1 is being discovered from the E. coli. Then CO which is going to be the species, so coli species. And then R is for the strain. So it is actually been isolated from a strain called RY13. So this is the strain and the one is that it is the first restriction enzyme from there which has been discovered from this particular enzyme.

Now so different types of restriction enzymes. So restriction enzyme vary in restriction

cutting site and cofactor requirement. So you can have the type 1 restriction enzyme, you can have type 2 restriction enzyme and you can have the type 3 restriction enzyme. So for the type 1 restriction enzyme, the restriction site of type 1 restriction enzyme consists of 3 to 4 nucleotides at 3 prime and followed by a non-specific stretch of 6 to 8 nucleotides and a 4 nucleotides at 4 prime. And for type 3 restriction enzyme, the restriction site has two separate non-pilomatomic sequence arranged, inversely arranged and the cutting site is 20 to 30 base pair away from restriction site. So the type 2 restriction enzyme is composed of two subunit, RES and MOD.

The MOD subunit is required for the modification whereas the RES is required for the cutting the unmethylated DNA. Then we have the type 2 restriction enzyme. So the type 2 restriction enzymes are very useful for monotonic cloning because they generate the sticky ends. So the recognition site of type 2 restriction enzyme is 4 to 8 nucleotide long and it cuts the DNA within the specific site. Due to this feature, the type 2 restriction enzymes have an application in genetic engineering for cloning purpose.

It is composed of three different types of subunits, M subunit, R subunit and S subunit where the M is required for methylation, R is required for cutting the DNA and S is recognizing the sequence which it actually going to be specific. And type 2 restriction enzymes are further being classified into type 2E, type 2B and all that that we are not going to discuss in detail. So this is just a summary of the different types of restriction enzymes. So we have the type 1 restriction enzyme, type 2 restriction enzyme and type 3 restriction enzyme and this is just a summary of different properties which you are actually going to use. Now we are going to have the properties of the restriction enzyme because these are the restriction enzyme which are actually going to be having an extensive role in molecular cloning.

So first is they are actually going to have the palindromic sequence. The restriction sequence of type 2 restriction enzyme is palindromic in nature. It means that the sequence read out would be same in forward and reverse direction. For example, the Bamach one had an restriction site which is GGATTC. So let us see what is mean by the palindromic sequence.

So GGATTC, so this is 5 prime end, this is the 3 prime end. Now if I write the reverse sequence, what is the reverse sequence? CCTAAG. So now we see that if I read this in this direction say GGA, if I read this sequence in this, it is going to be say that GGA. So GGA, GGA, TTC, TTC. So if I read this sequence either in this direction or either in this direction, it is actually going to be same and that is why these kind of sequences are going to be called as palindromic sequence. Now what is the advantage of this? That advantage is that it is actually going to be recognized by the restriction enzyme.

The second is it is actually going to generate the sticky end. The type 2 restriction enzyme cut both DNA strand together to generate the DNA with the hanging DNA stretch with the 4 to 8 nucleotide. This DNA stretch contain fragments are cohesive to each other as sequence present on complex 1 will be complementary to the sequence present on the complex 2. So for example, this is the DNA. So this is the DNA.

Now this is 5 prime, this is 3 prime, this is 3 prime, this is 5 prime. And if this is the restriction, this is the recognition sequence. Now if I cut this DNA, what I will generate is this. What I will generate into this DNA, with this DNA this portion will go. And whereas with this DNA, it is actually going to go with this.

So what you see here is this is actually sticky to each other. This is going to be sticky to each other. This is what it is actually going to happen. This is the 5 prime, this is 3 prime, and this is 3 prime, this is 5 prime. So if I put this together again, they will actually go and stick like this and then this small gap will be filled and that gap is actually going to be filled by the ligase enzymes.

If I want to set up the restriction reactions, then I can use this. So I can have the DNA, restriction enzymes, buffer, BSA and esterile water. And the restriction reactions are supposed to be put in a larger volume so that you should have the free access of the enzyme. So you can do the in esterile water. So in esterile water, what you are going to do is first you are going to add the buffer. So first you are going to take the water, then second you are going to take the buffer, then you are going to add the enzyme.

Okay. And that and then you are actually going to make the buffer. So that is actually going to have the restriction enzyme master mix. Now to this master mix, if I suppose have 5 different types of DNA, right, so I can add the DNA, right. So I can add the vector, I can add the template and all that. And that's how I can set up the different reactions.

And then I incubate that on 37 degrees Celsius for 12 to 18 hours, right. And at the end, it is going to cleave all the, from the restriction sites and that's how I am going to get the cohesivants. And then I can just put the vector and the fragments together and then it is going to be done with the ligation reactions. Now the second enzyme what we are going to discuss is the ligase. So ligase is joining the two DNA fragments to generate the chimeric DNA is the basis of the cloning.

It is an essential step to generate the clone containing foreign DNA in a vector. When the cohesivant generate by the action of the precision endonuclease on a DNA associated

with each other, a nick remain to seal and give complete circular DNA. So what DNA ligase is doing? It is an enzyme which requires the ATP or the NAD plus as a cofactor to catalyze the ligation reactions. 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 5 prime of the DNA bearing nicks and bringing them together. AMP is released and phosphodiester linkage is formed between the 3 prime and 5 prime end to seal the nick.

So this is what exactly it is going to do, right. So when you have the enzyme, right, you have the T4 DNA ligase which utilizes the ATP or you can have E. coli DNA ligase which utilizes the NAD plus as a cofactor. And either of these cases, suppose this is the DNA and you have a nick here, right. So you have a nick here, right. And then what will happen is that the enzyme is actually going to make a complex with the ligand or like with the cofactor.

So it is going to form the enzyme AMP complex and then enzyme is actually going to bind this particular sequence and it is actually going to supply the AMP, right. And then AMP is actually going to come off and then there will be a bond which is going to be formed between the phosphate and the OH which is present. And that is how it is actually going to seal this particular nick. Now how we can be able to set up the ligation reactions? So for ligation reaction what you require, you require the vector or DNA 1, then you require the insert which is DNA 2, then you require the ligase buffer, then you require the BSA and then you also require this trial water.

Remember that the reticence enzyme reactions are supposed to be done in larger volumes. That is why we have set up that reaction in 50 microliter whereas the ligation reaction supposed to be done in a smaller volume so that there will be higher probability of these fragments interacting with each other and making the ligations. So in a ligation reaction what you are going to do is you are going to take the trial water first. So you are going to take the trial water first, then you are going to add the ligase buffer, then you are going to add the BSA and then you are going to add the ligase, T4 DNA ligase, 5 to 10 units per reactions and then you are going to add the DNA. If it is a vector and insert then it has to be added in a 1 is to 3 ratio so that there will be higher probability that the vector will interact with the insert and it is going to form the chimeric DNA. Once you set up the reaction, then these reactions have to be set up incubated at lower temperature, 16 degrees Celsius so that it is actually going to help in forming the hydrogen bonding and that helps in sealing the leaks.

And then you incubate that on 60 to 24 hours after that you are going to transform this ligation reactions into the suitable host and that is how you are going to get the

recombinant DNA. The third enzyme what we are going to talk about is the alkaline phosphatase. So alkaline phosphatase is required when you want to do the directional cloning. So the digested linear plasmid containing the cohesive end on both the side with the phosphate has a tendency to recirculate which means if you have a vector, if you have a vector and suppose you have the Eco R1 on this side and you also have an Eco R1 with this side and suppose you have insert that also being digested with Eco R1, then you have a two probability either this vector will go, this insert will go and sit here or this vector itself is actually going to surprise with each other.

So you are actually going to have the two possibilities. One that it is actually going to take up the insert and it is going to form the recombinant DNA. The second that the vector itself is actually going to be get sealed with each other and if that happens, then it is actually going to be recircularization and it is not going to give you the recombinant DNA whereas if the insert comes here, then you are going to get the recombinant clone. So removing the terminal phosphate group prevents this possibility and for this purpose, the alkaline phosphatase is used. So if I want to avoid this, what I can do is I can just remove this particular phosphate group because on this side it has a phosphate group, on this side it has OH group. So if I remove the phosphate group, it is actually going to have the OH on this side, OH on this side and if that happens, then this particular fragment will not be able to be surprise on its own.

It actually requires the supply of this phosphate group and it also requires the help of the ligase reactions. So alkaline phosphatase removes the 5 prime terminal phosphate groups and in that condition only in the presence of insert DNA as its supply phosphate group HN to provide the ligation reactions. So this is what exactly I was trying to explain. You have the vector which has a phosphate group and you have OH group on this side and then you have OH group on this side and phosphate group on this side.

So if you put the ligation reaction, it is actually going to circularize. So you are going to get the plasmid back. So instead of, but you do not want the plasmid back. You want a recombinant clone. So in that case what you do is you treat this with alkaline phosphatase. So if you treat the alkaline phosphatase, it is actually going to chew up all the phosphate what is present on the termini.

So as a result, it is actually going to have the OH on the other side, OH on all the sides. Now this cannot recircularize even if you put it onto the ligation reactions. So there will be no ligation. Now if I put this along with the insert, insert has the phosphate group which is present. So insert will sit here and then it is actually going to have the nicks and these nicks are actually going to be sealed by the ligase and that is how it is actually going to give you the ligation reaction and these ligated product can be transformed into

the bacteria or the other force and that will going to give you the recombinant or the recombinant flows.

So these are the some of the enzymes what we have discussed. So we have discussed about the enzymes what is in general. So what are the different properties of an enzyme and how the enzyme actually works in the biological system and then at the end we have also discussed about the different types of enzymes and their properties which are actively participating into the different types of molecular cloning reactions. So in this particular module what we have discussed, we had discussed about the structure and function of the different biomolecules which are actively participating and regulating the different types of biological pathways and different types of biological properties or biological actually pathways. And so with this I would like to conclude my lecture here.

In our subsequent lecture we are going to discuss some more aspects of related to molecular biology. Thank you. 1