Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-34: Enzymatic tools used in Molecular Biology

Hello everybody. Welcome to SWAYAM NPTEL online course on Basics of Crop Breeding and Plant Biotechnology. Today we will start the title of this module, Introduction to Genetic Engineering and Plant Tissue Culture. And this is the lecture we will be discussing about the Enzymes and Vectors used in Genetic Engineering. So, these are the concepts that will be covered in this particular module. First of all, we will be discussing different enzymatic tools used in genetic engineering.

Then different vectors that are commonly used in molecular biology will be discussed. Then what is plasmid will be discussed, and various types of plasmids will be discussed little bit in detail. And finally, we will be discussing about the *Agrobacterium tumefaciens* which is a natural genetic engineer. So, let us start our discussion on different enzymatic tools which are used in molecular biology.

So, different sets of enzymes are needed first of all we will be discussing Ligase. So, ligase enzymes are types of enzymes that play a crucial role in DNA replication, DNA repair, and recombination process. Their primary function is to catalyze the formation of phosphodiester bonds between the nucleotides of adjacent DNA strands and sealing breaks in the DNA backbone. So, if we recall the double-stranded DNA, suppose a DNA sequence is there this is the 5′ to 3′ end and this is the 3′ to 5′ end. So, over here if some breakage has taken place within a particular strand then with the help of this ligase enzyme this break is sealed.

Basically in between 2 nucleotides and 2 dNTPs, the phosphodiester bond formation is taken place by ligase enzyme. So, ligases are used in molecular cloning if we have to seal different sets of genes, promoters, and terminators in a particular construct later on we will be discussing those things. So, ligase enzymes are used over there. Then another type of enzyme which is used in molecular biology i.e., polymerase. So, in polymerase mostly we will be discussing about the DNA polymerase I.

Initially, this DNA polymerase I was isolated from *E coli*. So, this DNA polymerase I enzyme is attached to the short single-stranded region, in mainly double-stranded DNA molecule. So, if you recall the DNA replication process in DNA replication process DNA replication is taken place in 5′ to 3′ direction using a template DNA.

So, a template DNA we need there should be 3′ to 5′, and a small RNA primer is needed for initiation of DNA replication. At the end of that RNA primer different bases are gradually added by the action of DNA polymerase I, during the DNA replication process. So, it is attached to the short single-stranded region i.e., the RNA primer region, and on the double-stranded DNA basically it will start its work. The polymerase activity synthesizes new strand on the single stranded DNA template and the different bases will be added will be added based on the complementarity of the template DNA strand. So, in DNA polymerase I the nucleus activity is also there.

So, 2 types of nuclease activities are there we will be discussing. First, nuclease activity degrades the existing old strand as it proceeds, means some nuclease activity is there that will degrade this part the old part within this nascent DNA. This is the 5′ to 3′ nuclease activity, and 5′ to 3′ exonuclease activity, basically while the nuclease activity is there for proofreading also. Once the DNA synthesis takes place using a template DNA, then sometimes the wrong bases may enter over here. Ok! During this process, if some wrong bases enter over there in the newly synthesized strand by this proofreading activity this base will be removed and a new base will be added over there. Ok!

It is done by 3′ to 5′ exonuclease activity, this is the 3′ end. So, the 3′ to 5′ exonuclease activity is responsible for proofreading the DNA strand, while 5′ to 3′ exonuclease activity is used or is available to remove the RNA primer portion or the old DNA strand based on that the new strands are synthesized. So, its major use is DNA polymerization, the DNA polymerase I. Then another set of enzymes, another polymerase i.e., mostly used in molecular biology i.e., Taq DNA polymerase. So, we have already discussed Taq DNA polymerase, once, we had discussed the PCR reaction, polymerase chain reaction. So, the Taq DNA polymerase was initially isolated from *Thermus aquaticus*. Ok! It is the bacteria available in hot springs and within that bacteria, the replication can be taken place at higher temperatures. So, that polymerase is more stable than the DNA polymerase I, which is available in *E.coli*. So, the Taq DNA polymerase has demerits compared to DNA polymerase I.

Basically the Taq DNA polymerase, it do not have 3′ to 5′ exonuclease activity. This exonuclease activity is not available in Taq DNA polymerase. So, the proofreading is not available, proofreading activity is not available in the Taq DNA polymerase. So, some scientists have isolated new types of DNA polymerase for PCR reactions, like *Pfu*, it is a type of polymerase that is commonly used it has high fidelity and the proofreading activity is also there. So, in spite of *Taq*, *Pfu* enzymes are also used in PCR reactions.

Now, we will be discussing about Klenow fragment. Klenow fragment is a modified DNA polymerase I. So, if you think about the DNA polymerase I structure, 2 subunits are there ok, 1 subunit of 323 amino acids is available and another subunit is there. So, if we remove the 323 amino acids, mostly at it is available in the N-terminal part. If we remove it then we can get the Klenow fragment.

Basically, this 323 amino acid is responsible for nucleus activity and the 5′ to 3′ exonuclease activity. So, in the Klenow fragment that nucleus activity is not available, but the polymerase activity is there it can retain the polymerase activity. So, once we discuss the probe synthesis in southern hybridization, we need to prepare different radiolabeled probes. So, for those probe preparations, these Klenow fragments are

mostly used. They are based on a particular template DNA the using based on a particular template DNA probe, are synthesized and their radiolabeled DNTPs are added. Ok!

So, their Klenow fragment is used, it is a modified DNA polymerase I. Now we will be discussing reverse transcriptase i.e., another enzyme earlier we have discussed. Reverse transcriptase is a type of enzyme that basically, converts mRNA into complementary DNA. It converts mRNA into complementary DNA. mRNA is messenger RNA i.e., mostly the protein-coding genes i.e., produced from the protein-coding genes and using this particular reverse transcriptase enzyme we can convert the mRNA into cDNA.

And thereafter, those cDNA could be used for library preparation, those cDNA could be used for gene expression study those things. Basically, reverse transcriptase is a type of RNA-dependent DNA polymerase. Ok! Now we will be discussing about different DNA modifying enzymes which are commonly used in different molecular biology laboratories. The first one is the alkaline phosphatase. So, alkaline phosphatase, it removes the 5′ terminal phosphate group from the DNA.

It removes the 5′ terminal phosphate group of the DNA. Suppose if we have a circular double-stranded DNA, suppose we have a circular double-stranded DNA somehow, we have cleaved this DNA. So, this circular double-stranded DNA will be linearized at one end 5′ phosphate will be there another end 3′ OH will be there. In this strand it will be 3′ end, it will be 5′ end. So, if we use alkaline phosphatase then the phosphate group will be removed from the 5′ end.

And if the phosphate group is removed then this DNA cannot be sealed again using normal ligase, but if this phosphate group is attached there and 3′ OH group is also there. If we add ligase over here, this DNA could be sealed in its circular form in its original form. But, if we treat with alkaline phosphatase, once the phosphate group from the 5′ end is removed the DNA cannot be sealed. Ok! Then another enzyme known as poly-nucleotide kinase, basically, add different phosphate groups to the 5′ terminal end. Then another enzyme i.e., terminal deoxynucleotide transferase it basically add one or more

deoxynucleotide at the 3′ end.

In different molecular biology experiments, these enzymes are mostly used like alkaline phosphatase, poly-nucleotide kinase, and terminal deoxynucleotide transferase. Now we will be discussing different nucleases. Ok? Two types of nucleases are there one is exonuclease, another one is endonuclease. So, from the name exonuclease, we can tell that it can cut the DNA from the outside part. Ok! So, an exonuclease is an enzyme that functions by cleaving nucleotides one at a time from the end of the DNA or RNA molecule.

So, there are two main types of exonucleases based on the direction in which they cleave. One is the 5′ to 3′ exonuclease this type of exonuclease, cleaves nucleotides sequentially from the 5′ end of the DNA or RNA molecule. It is involved in the removal of RNA primer in DNA replication. Earlier we discussed about DNA polymerase I, DNA polymerase I has 5′ to 3′ exonuclease activity, and with that activity it can remove the RNA primers i.e., the part of the initiation of DNA replication ok. So, by 5′ to 3′ exonuclease activity DNA could be cleaved from the 5′ end.

While 3′ to 5′ exonuclease activity these exonucleases act by cleaving nucleotides from the 3′ end of the DNA or RNA molecule. They are essential for maintaining the integrity of the genetic material ensuring accurate replication and it participates in DNA repair process also. We have discussed about DNA proofreading during replication, the proofreading is done by this 3′ to 5′ exonuclease activity. If any wrong base enters in the 3′ end it will be removed by this exonuclease activity and new base complementary to the template strand will be added over there.

Now we will come to the endonuclease. So, endonuclease refers to a class of enzymes that cleave phosphodiester bonds within a nucleic acid chain typically within the middle

of the molecule rather than at the ends. Exonuclease starts from the end part 5′ end or 3′ end, while endonuclease cleaves the DNA from the middle part. Endonucleases are crucial in various cellular processes involving DNA and RNA. So, different types of endonucleases are there gradually we will be discussing and what are their involvement. First, in DNA repair, the endonuclease, plays essential roles in the DNA repair mechanism.

Some repair mechanisms are there one is the base excision repair, another type of repair is nucleotide excision repair, then double-strand break repair. Ok! In this repair process, those endonucleases play a major role. They recognize specific DNA damage and create nicks in the DNA strand at or near the damage site and they initiate the repair process. Ok! If any DNA damage is there, some broken DNA is there first by this mechanism, endonuclease cleaves the DNA close to the damaged part, and thereafter repair process is initiated.

Then coming to the restriction enzymes, restriction endonuclease is those are mostly used in different molecular biology in different construct preparations. Ok! So, these enzymes are crucial tools in molecular biology, they are derived from bacteria and are capable of recognizing specific DNA sequences. That sequence is known as restriction site-specific DNA sequence, on which those restriction endonuclease types of enzymes will sit, and they cleave the DNA at or near this restriction site. So, different types of restriction endonucleases are there, later on, we will mention some will cleave directly at that position where it is binding, and some of the restriction endonucleases will cleave the DNA a little bit away from its binding site. So, in this way, different restriction endonuclease works. So, this property is extensively utilized in genetic engineering techniques like DNA cloning gene editing through, CRISPR or Cas9 mediated approach in DNA fingerprinting also different restriction endonuclease is used, in RFLP analysis different restriction endonuclease are used.

Once the RNA processing takes place, initially in the case of mRNA formation the pre mRNA is formed thereafter finally, the mature mRNA is formed by different processes the splicing of RNA, then 5′ capping 3′ polyadenylation those things are taken place. So,

in the case of tRNA also initially a pre-tRNA kind of thing is formed, thereafter the actual tRNA is formed due to the action of some enzymes some endonucleases. Ok! So, this type of endonucleases are involved in RNA processing and maturation, where they cleave the precursor RNA molecule to generate mature functional RNAs, in case of mRNA from the pre mRNA the mature mRNA is formed then in case of tRNA in case of RNA formation also some source of RNA processing is involved, and in that RNA processing different endonucleases are involved. Now let us discuss about the specificity of endonuclease, some the endonucleases often recognize specific sequences or structures within DNA or RNA molecules, and for their binding to the DNA or RNA, some specific sequences are needed based on that specific sequence those restriction enzymes or endonuclease will attack the DNA molecule or RNA molecule. The specificity of different endonucleases varies widely allowing researchers to use them for precise manipulation of genetic material.

So, generally, 3 types of restriction endonucleases are there one is type I, second is type II and third one is type III. So, in the case of type I restriction endonuclease, suppose it is a DNA sequence if the type I restriction endonuclease, binds at this specific region it causes cleavage approximately at around 1000 base pairs away or 1000 bases away. In the case of type III restriction enzymes if they recognize some part of the DNA, in most of the cases their cleavage is taken place approximately 20 to 30 bases away its cleavage is taken place. For the type II restriction enzymes, for those specific restriction enzymes, the binding site and cleavage site are the same wherever they are binding, and in which sequence they are binding they cause cleavage over there. So, this type II type restriction enzyme or restriction endonucleases are mostly used in molecular biology in different types of cloning.

So, we will have, about some examples of type 2 restriction enzymes, like *Eco*RI, it is a type II restriction enzyme that basically, recognizes GAATTC, this particular sequence from the 5′ to 3′ end. So, if you think about another strand of this DNA, it will be CTTAG from 3′ to 5′ if you read in this way. So, if you carefully see this type of sequence which is recognized by type II restriction enzymes they are known as

palindromic sequences. So, those sequences are the same if you read the I mean if you read this sequence from 5′ to 3′ direction in both the strands here from GAATTC here from GAATTC it is a palindromic sequence with some restriction enzymes. So, *Eco*RI types of restriction enzymes it can cause staggered cut means it can cause cleavage over here in one strand and cleavage over here in another strand. And after cleavage, we will see this will be one DNA and it will be another DNA. So, in this way basically it can cause cleavage in the DNA. The sticky end is generated by *Eco*RI, *Bam*HI, *Hin*dIII those types of restriction endonuclease while some restriction endonuclease belonging to type II is also there that can cause a blunt end. Like, let us take an example of *Sma*I this enzyme recognizes, the region CCCGGG from 5′ to 3′ detection and this is 5′ to 3′ direction. So, this restriction enzyme basically, causes cleavage in between and thereafter it will generate 2 fragments, one fragment will be CCC and another fragment will be GGG. In this way the DNA will be cleaved this is known as the blunt end restriction enzyme these 2 types of restriction enzymes under the type II category are mostly used in molecular biology.

Now, we will be discussing about, vectors first what is a vector in molecular biology different types of vectors are used. So, vectors refer to a carrier or delivery system for transferring genetic materials like genes or DNA, from one organism to another organism. This serves as an important tool in the biotechnology field or in the molecular biology field. So, basically vectors are a carrier molecule through which we can deliver some target gene or some target DNA molecule from one organism to another one.

So, these are the different functions of vectors. So, first of all, they are involved in the study of the role of different genes, if we have to understand the role of different genes available in a particular organism, we can use vectors, we can clone each and every gene separately and we can express it in a heterologous system, to know what type of proteins are being produced from that particular gene. Then vectors could be used for creating genetically modified organisms, means the transgenic plants could be generated the transgenic yeast could be generated by using different vectors then the modified or recombinant bacterial strains could be generated using vectors. Ok! We can produce some specific proteins, using vectors if we clone a particular gene and in some expression

vector, and from that vector if the gene is expressed properly then within a bacterial system or in the future in a plant system, we can produce a specific protein using different sets of vectors. Then introducing the desired genes, in our target-oriented manner suppose we would like to edit a particular gene in a rice crop then through vector we can do the CRISPR Cas9 mediated genome editing. So, the desired gene could be introduced, or desired genes could be modified. Ok!

Then manipulating the expression of the desired gene, that thing also could be done using vectors we can play with different promoters, which means we can use different types of activators using specific vectors. So, the gene expression could be regulated the gene expression could be up-regulated, or down-regulated by using different types of regulatory molecules using vectors.