Welcome students, this is unit number 3 and the title of the unit is molecular techniques, enzymes and vectors in gene manipulation. The name of the module is lambda bacteriophage. I am Shahnaz Shaikh, assistant professor in the Department of Zoology, Government College of Arts, Science and Commerce Quepem. So, in today's topic we will be studying about the Lambda bacteriophage, the characteristic features of the Lambda bacteriophage, the phage infection cycle, cloning in Lambda bacteriophage, and classes of Lambda bacteriophage vectors. At the end of the session, students will be able to define Lambda bacteriophage vectors, describe the structure of lambda bacteriophage vectors, explain the construction of lambda bacteriophage vectors, enlist types of lambda bacteriophage vectors. Let us begin.

So what are bacteriophages? Bacteriophages are the viruses that infect bacterial cells and after injecting the genetic material they kill them. The viral DNA or the RNA it replicates and it expresses inside the bacterial cell and it produces a number of phage particles which can be released after bursting the bacterial cell. Bacteriophages are used as a phage vector in gene cloning. Phages are very simple in structure and they consist merely of a DNA or sometimes RNA molecule carrying a number of genes surrounded by a protective coat or you can also call it as ‘capsid’ which is made up of protein molecules. They undergo two modes of life cycle, the Lytic cycle and the lysogenic cycle.

Now, why bacteriophages are used as a vector, it can accept very large pieces of foreign DNA. Genetic engineer they have constructed numerous derivatives of vectors that contain one or more than one sites for variety of restriction enzymes. Some of the examples of bacteriophage vectors are lambda phage, M13 Phage, T4, T7 phage, P1 phage, etc.

So, lambda phage is a DNA virus. It has origin of replication which allows it to replicate. Lambda genome size is 48.5 kilobase pairs. It contains proteinaceous head and a long tail which is attached to the head. There is a gene for required enzymes for DNA replication. In this picture you can see the structure of a lambda bacteriophage. So you can see it has a icosahedral head which contains DNA and it has a long filamentous tail. The characteristic features of lambda bacteriophage. It merely has 50 nanometer diameter and it has icosahedral head a flexible tubular protein tail. There is a connector which serves as a site for attachment of preformed head to tail. It has a double stranded linear DNA molecule and because of presence of cos sites which are present at the end of a DNA strand, it allows circularization of a DNA after injection into the host cell. There are two modes of phage infection cycle. One is lytic and the other one is lysogenic. Now in the lytic mode, there is a general pattern of infection which is same for all types of lytic phages. It has three steps. First step is the phage particle attaches to the outside of a bacterium and it injects its DNA into the cell. In the second step, the phage DNA molecule replicates, usually by specific phage enzymes which are coded by genes in the phage chromosome. And after replication the phage genes direct synthesis of the protein component that is capsid and the tail, and then there is assembly and

release of phages from the bacterium. So here in the lytic cycle you can see the phage inserts its DNA inside the host and after that it starts replicating and it directs the synthesis of phage particles that is capsid and the tail and once these particles are ready they will assemble and make smaller many copies of phage particles and this will release out by lysing and killing the bacterial cell.

The next mode of infection is lysogenic cycle. Now, in contrast to Lytic cycle, lysogenic infection is characterized by retention of a phage DNA molecule in the host bacterium. With many lysogenic phages the phage DNA, which is now called as a prophage, is inserted into the bacterial genome. However, the prophage it eventually releases out from the host genome and phage reverts to the lytic mode and lyses the cell. So here you can see the phage it inserts its DNA and Lambda DNA, which is a phage DNA it gets inserted and integrates with the bacterial DNA and now it is called as a prophage. OK, so after cell division along with the bacterial DNA the phage DNA will also be transferred to the daughter cells. But after several generations the Lambda phage will revert back to the lytic mode of infection.
Lambda bacteriophage as a cloning vehicle. Why lambda bacteriophage is preferred as a cloning vector? Because of its large genome size and low insert capacity, head can accommodate certain amount of a DNA that is up to 48.5 kilobase pairs. There is presence of multiple sites for commonly used restriction enzymes. It has a smaller size and increase cloning capacity. Only 50% of genes of wild type phage are essential for its replication and lysis of host cell so you can remove out 50 remaining 50% of genes and insert the gene of interest.

Now how to carry out cloning in Lambda vectors?
The steps are the first step is restriction digestion of Lambda vector as well as its genomic DNA. Then after digestion there is a ligation of DNA fragments into Lambda vector to form the recombinants. So here the new gene is inserted inside. Introduction into host by transfection of competent E.coli host or in vitro packaging of recombinant DNA by addition of packaging extract followed by natural infection of the host. And the last step is selection and the screening of recombinants. Classes of Lambda vectors. There are two classes of Lambda vectors. The first one is the insertional vector and the second type is replacement vectors. So insertional vectors are the vectors that contain at least one unique restriction enzyme site for the insertion of foreign DNA are insertional vectors. The size of a insertional vector should not be below 37 Kbp and that of a foreign DNA should be such that it should not increase the size of vector above 52K base pairs. Some of the examples are Lambda GT 10, Lambda GT 11, lambda GT 18, Lambda GT 23. So in this picture you can see how the construction of Lambda insertional vectors takes place. So as you can see in a normal Lambda DNA it has a nonessential region, so this region will be cleared out and in place of that add foreign DNA, which is a gene of interest, will be inserted. OK so example of lambda gt 10 you can see after deletion it has a specific restriction site for Eco R1 here the gene of interest can be inserted. Similarly, in lambda ZAP2 also has a specific site for restriction enzyme. Then you can insert the gene of interest. Here they have inserted lac Z gene. The next class is replacement or the substitution vector. Now the

replacement vector. They have a nonessential part which is called as stuffer fragment, so this fragment can be removed out and in place of this gene of interest can be inserted. Lowest possible size of replacement vector should be 37 kilobase pairs. Example includes Charon series, Lambda EMBL series, Lambda GT, Lambda C, Lambda GEM 11 Level, GEM 12, lambda DASH, Lambda FIX. So here you can see a normal lambda replacement vector. It has a stuffer fragment so this fragment can be removed out and in place of this add DNA which has a gene of interest can be inserted so you can see in lambda EMBL4 it has a restriction site for Eco R1, BamHI, SalI. So here this can be removed and the gene of interest which can be up to 23 KB can be inserted. Cloning experiments with lambda insertion or replacement vector. And how to carry out cloning in circular lambda DNA and linear Lambda DNA. When it is a circular Lambda DNA it has specific cos sites and because of this cos sites, the lambda DNA circularizes. So here you can see there is a cos site and there is a restriction site for Eco R1 so you can insert a DNA fragment which has EcoR1 site. So this new DNA fragments will get inserted where there is Eco R1 site which is a recombinant phage DNA. Now when there is linear Lambda DNA, so here there are many fragments, many small small linear fragments having cos sites and also Eco R1 sites and you have to insert your specific gene of interest that has Eco R1 site. So after ligation since there are cos sites which are present because of this cos sites, the DNA will likely ligate the fragments of this DNA. They're going to ligate, they're going to join to one another and form a linear DNA molecule. And this DNA molecule can be packaged or it can be inserted inside the lambda phage, making it recombinant lambda and then this lambda recombinant DNA can be used to infect E. Coli.

You can refer this books for this topic.

Thank you.