Basic of Biology
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Module-3 Cells in Biology
Lecture -11
Basics of Cells (Part -1)

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 different properties of the living organisms. And so, far what we have discussed, we have discussed about the classification of the living organism and during the classification, we have discussed different types of properties, what are present in different organisms.

And during the discuss during the classification itself, we understood that the organisms are being evolved from the pre existing lower organisms, and that we have further discussed in when we were discussing about the evolutions and we have discussed how the different types of evidences are being given by the different scientists and how these evidences are helping us to understand that the evolution is happening.

And the lower organisms or the higher organisms are being evolved from the lower organisms. Subsequent to that we have also discussed about the different types of theories and mechanisms, how this evolution could have happened and during that discussion in the last module, we have discussed about the Lamarck's theory. We had discussed about the Darwin's theory, and then at the end we have also discussed about the Hugo de Vries mutation theory as well.

So, so far what we have discussed, we have discussed about the classification and the evolution and that could have given us the clear idea that the organisms are evolving on to the earth from the lower organism to the higher organisms. And if you remember, when we were discussing about the origin of life, the origin of life, and we will discuss when we when we say that okay, these are the potential ways in which the organism could have been evolved or organism could have been originated onto the earth, we talked about the primitive cell.

And that primitive cell is being formed within the primordial oceans. So, that primitive cell is only the existing species which further got evolved into the first the unicellular organisms and

then multicellular organism and then that got further specialized into the organism with the tissue as well as the organism with the organ systems.

So, in today's class, we are going to start discussing about this particular cell and how the cell is actually functioning different types of task. And how, what are the different organelles are present within the cell and what is the structure of the cell. So, when we talk about the cell.

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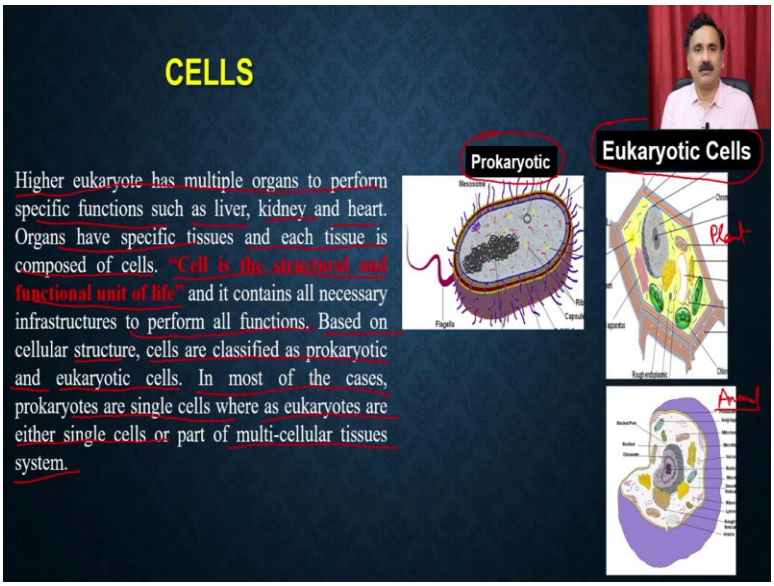

The cell as you can see that the higher eukaryotes have multiple organs to perform the specific functions such as liver, kidney and heart. Whereas in the, and these organs have the specific tissue and each tissue is composed of these cells. So, whatever is function, what is happening in a in a higher organisms like the humans that it has the different types of organs. Like suppose, for example, we have the liver, we have the kidney, we have heart, we have the lungs, and all these organs have their specialized functions.

But these are organs are also made up of tissues and that tissues are made up of the cell. So, function whatever we function we say is actually being performed by the cell what is present in that particular organ, and that is why the cell is considered to be the structural as well as the functional unit of the cell. So, whatever the function you see from that particular organ, could be performed by that particular cell as well, which means a cell is the smallest unit which actually be able to perform all the functions.

For example, in a human body we have different types of organs to perform the different types of task like heart is there for circulating the blood, liver is there for detoxifications, kidney is there for the excretion of the byproducts, lung is there for respirations, but the cell which is actually the structural and functional unit can be able to perform all these function on its own. Because it has all the necessary infrastructure to perform all the functions.

Based on the cellular structures, cells are classified either as the prokaryotic cell or the eukaryotic cell. So, what you see here is a prokaryotic cell which is actually a bacterial cell. And I have taken the two example of the eukaryotic cell, I have taken the example of the plant cell and I have taken an example of the animal cell. So, based on the structure and cellular structure, the cells are classified into the prokaryotic and eukaryotic cell.

In most of the cases prokaryotic cells are the single cell whereas the eukaryotes are either single or the part of the multicellular tissue system. So, before getting into the detail of the structure of the prokaryotic or eukaryotic cells let us discuss about the differences between the prokaryotic as well as the eukaryotic cell, so that you will be understand what could be the differences, what is going to happen and how the eukaryotic cell has evolved from the prokaryotic cell.

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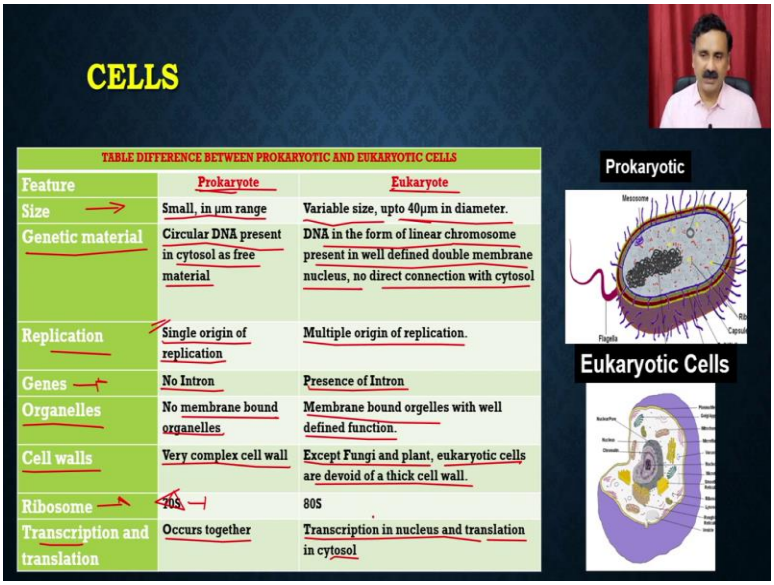

So, what you see here is a table where I have listed the differences. So, this is the properties of the prokaryotic cell and this is a property of the eukaryotic cell. Size, so first criteria is the size and the size is very small. So, prokaryotic cells are mostly in the range of the micrometer range,

whereas the eukaryotic cells, the eukaryotic cells could be of variable size, they could be they could be up to the 40 micrometer in diameter.

So, they could be in several sizes RBCs, macrophages, Kupffer cells and all those kinds of things, so they will be very different. As far as the genetic material is concerned the genetic material could be that genetic material is circular in the case of the cytosol and it is present as a free material, which means it is not present in the in the bound form whereas the DNA in the form of a linear chromosome present in the well-defined double membrane nucleus.

So, no direct connection with the cytosol, so in the eukaryotes, the DNA is present in the form of a chromosome and that is present in a well-defined structure, which is called as the nucleus and that nucleus is not directly under the contact with the cytosol. Then the replications, as far as the replication is concerned, so replication means, how you are actually going to make another copy of your genome.

So, the replication is done by the single origin of replication what is present in the case of prokaryotes whereas in the case of eukaryotes, it is having the multiple origin of replications. As far as the genes are concerned so the genes are the functional unit, the genes are the functional part of the genome, which are actually be responsible for the production of different types of products or different types of proteins. So, for the gene, there is no intron present.

Whereas in the case of eukaryotes, you have a, introns are present in the eukaryotes. So, do not worry about this particular terminology because these terminologies will be clear when we are going to discuss about the replication transcription and translations in a subsequent modules. Then the organelles, there is no organelle, no membrane bound organelle is present in the prokaryotic system, whereas the membrane bound organelles are with the well-defined functions are present.

So, you have the different types of organelles, you have the nucleus, you have the mitochondria, you have the chloroplast, you have the endoplasmic reticulum and so on. So, that all we are going to discuss in this particular module. Then the cell wall, there is a definite very complex cell wall what is present in the prokaryotic system, whereas in the case of eukaryotes except the fungi and the plant, the eukaryotic cells are devoid of a thick cell wall.

Which means the animal cells are devoid of the cell wall, whereas the fungi and the plants are going to have the cell wall. Then the ribosome, ribosomes are the protein machinery and they are actually going to be 70S. So, this is a kind of a parameter. So, that is 70S and whereas in the case of the eukaryotes, it is the 80S. Then we have the transcription and the translation. So, transcription and translation occurs simultaneously in the case of prokaryotic cell.

Whereas, in the case of transcription in the nucleus, so transcription is happening within the nucleus and the translation is happening within the cytosol as we said already in the beginning that the nucleus is DNA, genome is present in a well-defined nucleus and that is very, very far away from the cytosol that is why the transcription is and the translation is not happening in the same simultaneously, transcription is happening in the nucleus and the translation is happening inside the cytosol. So, before, so now let us start about the discussion about prokaryotic cells.

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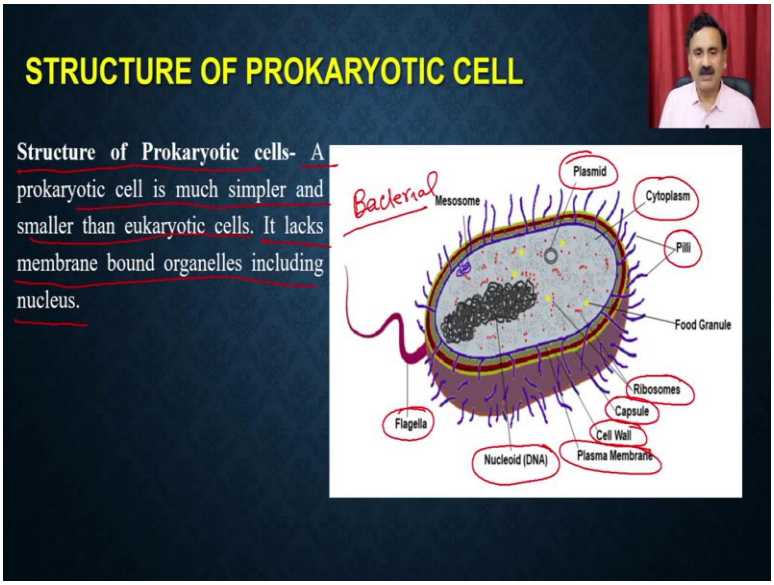

So, the simple prokaryotic cell what is being shown here, this is a bacterial cell. And the structure of the prokaryotic cell are simple, prokaryotic cell is very simple and smaller than the eukaryotic cell as we said prokaryotic cells are in the range of micrometer whereas the eukaryotic cells are very, very big compared to the prokaryotic cell.

One of the classical difference between a prokaryotic cell and a eukaryotic cell that it has a well, it has no membrane bound organelle including the nucleus. So, what you have is a cell where all organelles are present within the cytosol. And what you have here is the different types of

organelle, you have the flagella, you have the genomic DNA or the genome of the bacteria or the prokaryotic cell, then you have the well-defined cell wall, then you have the plasma membrane and then it has a protective capsule which is actually going to give the strength and protection.

And then we it has the ribosomes, which is called as the protein machinery, then it also has the food granules and all other kinds of things and it has a pilli then it has cytoplasm and plasmids. So let us discuss about all these sub-structures what it present in the prokaryotic cell.

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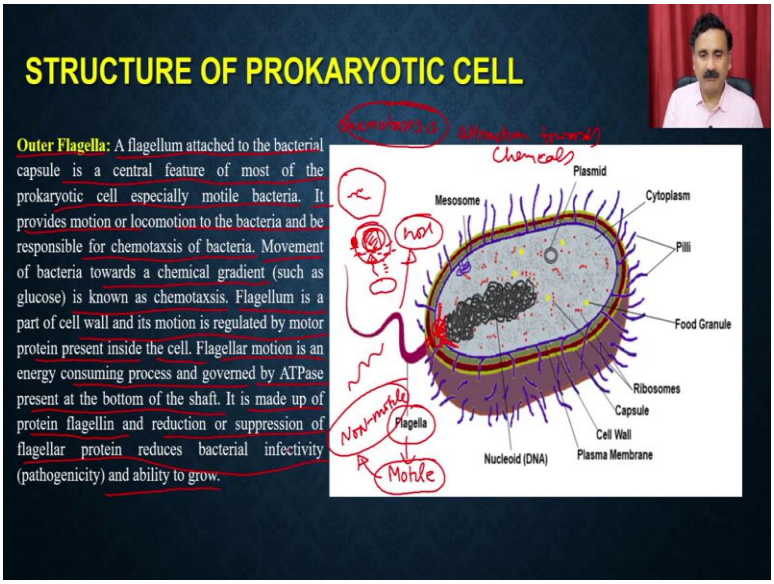

So, the first sub structure is the flagella. So, flagella is present in those bacteria which are actually motile. So, flagella is present in a bacteria and it is required for the motion within the bacteria. So, you can see that if a bacteria is present in a drop it actually can use this flagella to swim around. So, flagella is attached to the bacterial capsule is a central feature of most of the prokaryotic cell especially the motile bacteria.

It provides the motion or the locomotion to the bacteria and it is responsible for the chemotaxis of the bacteria. I am sure you probably are not aware of this terminology, which is called as chemotaxis, what is mean by the chemotaxis. Chemotaxis means the attraction of organism, attraction towards chemicals. For example, if there is a sugar crystal, if there is a sugar crystal, then the what the bacteria is going to see, it is actually going to move towards this sugar crystal because it is looking for that particular sugar crystal, it wants to eat that.

So, that motion, that directed motion of a bacteria towards the particular chemical is known as the chemotaxis and how it will move? It is actually going to use this flagellum, which is attached to the capsule. Moment of a bacteria towards a chemical gradient is known as the chemotaxis, which means, once you have a sugar molecule here, it is actually going to be dissolved into the water and it is actually going to have a gradient.

So, because this bacteria can be able to have the senses, it can be able to sense this particular gradient and that is how it will move towards that particular food source, it could be glucose, it could be any other molecule as well. So, flagellum is a part of cell wall and motion is regulated by the motor protein present inside the cell. So, flagellum is attached to the cell wall and inside it has actually has the motor neurons like just like when, in humans we have the muscles.

It simply has, also attached to the cellular machinery so that it actually can have the motor proteins and that motor protein actually can change the flipping moments. So flagellum is actually going to have the flipping moment. It is actually going to move like that. And the flagellar motion is an energy consuming process and it is governed by the ATP is present at the bottom of that particular shot.

It is made up off of the protein which is called as flagellin. And the reduction or the suppression of the flagellar protein reduces the bacterial infectivity and ability to grow. So, some of the bacteria also uses the flagellum even for accessing the different types of host. And that is how they can be able to use this for reaching to the host. So, they can also use for reaching to the host, and that is how they can be infectious.

So, if you actually reduce the production of this flagellin protein, and if somehow you compromise the flagellar movement, you are actually going to make the bacteria non-motile. And that is how the bacteria is going to lose its ability to infect. And that is how they will be not going to cause the disease.

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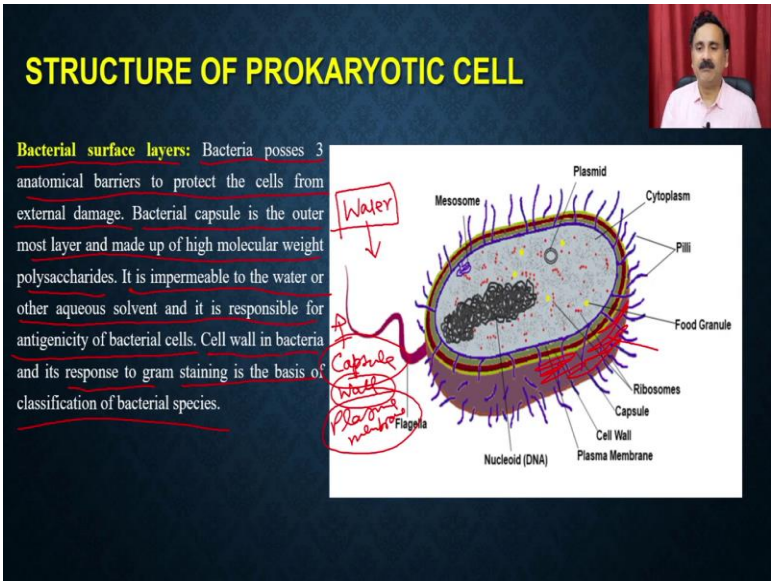

Now, the second is the bacterial surface layer. So, as you can see, the bacteria has a very, very complex surface layer because bacteria processes the three anatomical barrier to protect the cells from the external damages. So, since the bacteria does not have the membrane bound organelles and it is very susceptible for the hypotonic lysis because bacteria is mostly been present in the water or hypertonic solutions.

It has a very well defined anatomical barrier so that it can actually be able to withstand this. So, what are the cap, what are the different layers you have you have a bacterial capsule, which is the outermost layer and it is made up off of the high molecular weight polysaccharide. So, what you see here is this is the bacterial capsule and this capsule is required because it gives the protections and this is the outermost layer.

And then it is impermeable to the water or other aqua solvent and it is responsible for the antigenicity of the bacterial cell. Then you have the cell wall which is present in the middle layer and the cell wall and it is responsible for giving its response to the gram staining and the third is the plasma membrane. So, you have the three layer one is the capsule. So, the outermost layer is capsule then you have the cell wall and then you have the plasma membrane.

And why the bacteria has such a complicated system because bacteria is always been present in a harsh environmental condition. It could be present in the water, it could be present in the strong acid solution, it could be present in a alkali solution, it could be present in a solution where a lot

of chemical toxicants are present. So, because these are things that are there, it actually is protecting themselves by using all these layers. So, capsule is a very, very thick layer which actually is not going to allow these chemical to get inside the cell.

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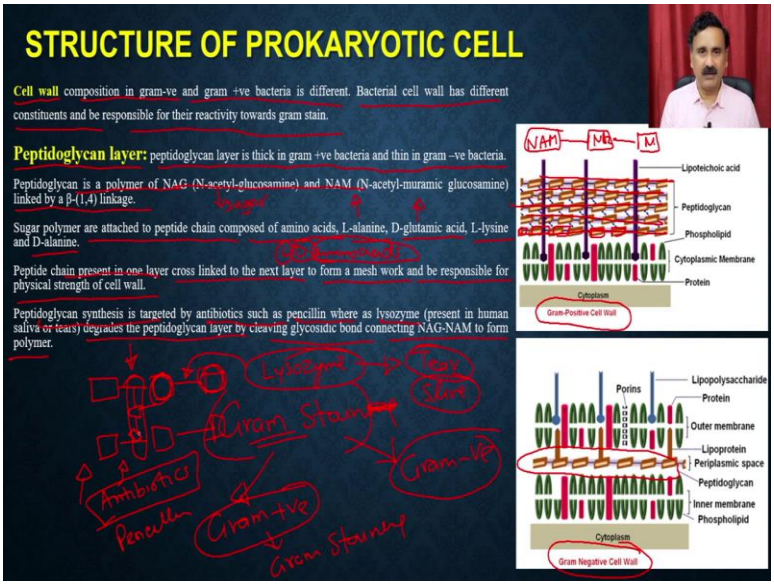

Then we have the cell walls. For cell wall composition in gram negative and gram positive bacteria is different. The cell wall has different constituents and be responsible for the reactivity towards a gram stain. So, we have the two different types of bacteria one is called as the gram positive bacteria and other one is called as the gram negative bacteria.

And both of these bacteria will have the different types of cellular cell wall compositions. And because of that, they will be differentially responsible for a one of the classical stain which is called as the gram stain. And because of the gram stain, they are being classified either as the gram positive which means the gram positive are actually going to give you the staining.

Whereas they are going to be gram negative if they are not going to be give you the gram staining. And based on these only the gram staining the bacteria's are classified as a gram positive or gram negative. So, let us see what are the different components are present in a cell wall. The outermost layer what you have is the peptidoglycan layer. So, this is the peptidoglycan layer what you see here.

So, the peptidoglycan layer is very thick in the case of the gram positive bacteria. So, you see the multiple layers are present in the gram positive bacteria whereas it is very thin in the case of the

gram negative bacteria. And because of this only it is actually having a differential response towards the gram staining. Peptidoglycan is a polymer of the NAG and the NAM, Nag is N–acetyl –glucosamine. And the NAM is the N–acetyl -moramic acid.

So, NAG and Nam are actually the sugar molecules which are present. And these sugar molecules are connected to each other by a peptide by a beta 1, 4 linkage. And the sugar polymers are attached because you see this it has sugar molecules. So, these are the sugar molecules which are been attached to each other by a beta 1, 4 linkage and alternatively you have you have the NAM block then it is connected to a NAG block.

And then it has NAM block like that. So, do you have you see that it has one layer then you have second layer you have third layer, your fourth layer and fifth layer and then these layers are being attached by the peptide chains. Which are composed of the amino acid, l alanine, D glutamic acid, l lysine and D alanine, which means, it is actually a combination of the L and D amino acid.

If you are not very aware very much aware of the L and D form then we are going to discuss that when we are going to talk about the amino acids. But these are the two different types of amino acids. And you know that the L amino acids are more abundant in the nature compared to the D amino acids. So, the peptide chain present in one layer crosslink to the next layer to form a meshwork which is responsible for the physical strength of the cell.

So, what you have is you have the NAM and NAG blocks and then the second layer is also having the same way. And then these layers are actually been connected by the peptide chains and these that is how it is actually giving a tensile strength to the cell wall. And that is how they are very, very, robust or they are very, very rigid in terms of accepting the outside molecules.

And the peptidoglycan synthesis is targeted by the antibiotics such as penicillin whereas the lysozyme actually degrades the peptidoglycan layer by cleaving the glycosidic bond connecting the NAG and NAM to form the polymer. So, you have the two options if you want to destroy the cell wall, what you have is you can actually have the antibiotics.

So, if you put the antibiotics, what antibiotics is going to do is it is actually going to, it is going to target the peptidoglycan synthesis, one of the classical example is the penicillin, the other

option is that you can use an enzyme which is called as the lysozyme. And that lysozyme is actually going to degrade the linkage between the NAM and the NAG.

Lysozyme is a very, very important enzyme what is present in our tears and as well as the saliva and that is how the tear and the saliva are actually protecting the humans from the bacterial infection. Because as soon as the bacteria goes, irrespective of whether it is a gram positive bacteria or the gram negative bacteria, lysozyme is actually cleaving the bond between the NAG and NAM and that is how they are actually destroying the cell wall.

And once they destroy the cell wall they are, these bacterias are very, very susceptible for the osmotic damages. So, they will be very susceptible for the water and or they will be very susceptible for the tear light conditions. And so they were actually going to get lysed and that is how they will die. So, this is one of the strategies and that is how people are trying to develop many antibiotics which are actually going to work on the peptidoglycan synthesis.

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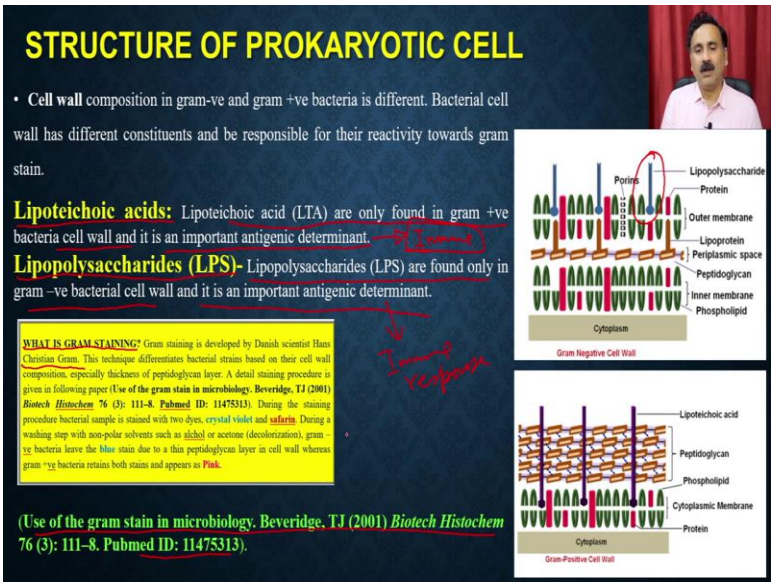

Apart from that the cell wall is also having lipoteichoic acid. So, apart from the peptidoglycan layer, you also have the lipoteichoic acid which is present in the cell wall. So, lipoteichoic acid are only present in the gram positive bacterial cell wall and it is an important antigenic determinants. So, aside the this for reporting uric acid, our immune system is actually going to work and that is how it is actually going to produce the response.

Then we have the lipopolysaccharides, all the LPS. The lipid polysaccharides are only be present in the gram negative cell wall and it is important antigenic determinants. So, compared to the lipoteichoic acid, which is only present in the gram positive bacteria, in the gram negative bacteria you have the lipopolysaccharide and that lipopolysaccharide is a very, very important antigenic determinants because that is actually going to induce the immune response in the humans.

This is I have just given you a write up so that if you are interested you can actually be able to read about the gram staining. So, gram staining is a staining which is been developed by Danish scientists which is called as the Hans Christian Gram. And as I said, gram staining is gram positive bacteria is taking up the Gram stain whereas the gram negative bacteria are not taking up the positive stain.

So, if you want to be more interested about the reading the gram staining you can be able to go through with this publication and as well as I have given you a small write up, so that you can also go to this particular item as well. So, now, let us move on to the beyond the cell wall.

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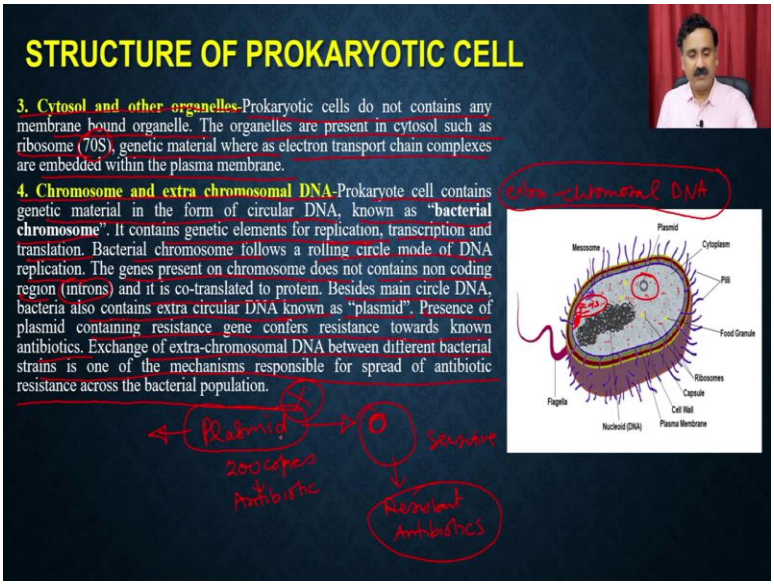

So, apart from the cell wall, you have, they have the cytosol and the other organelles. So, prokaryotic cells do not contain any membrane bound organelles, the organelles are present in the cytosol such as the ribosome, which is a 70S ribosomes and the genetic material whereas

electron transport chain and complexes are embedded within the plasma membrane. So, within the plasma membrane, you have the electron transport chain.

You will see the description about the electron transport chain when we are going to talk about the mitochondria. Apart from that genomic material is present in the chromosome and as well as the extra chromosomal DNA. So, prokaryotic cells contain the genetic material in the form of a circular DNA known as the bacterial chromosome. So, but bacterial chromosome is different from the eukaryotic chromosome but is present in the eukaryotic cells.

It contains the genetic elements for the replications, transcription and the translations. Bacterial chromosomes follow a rolling circle model of the DNA replications. The genes present on the chromosome does not contain the non-coding region which is called as introns and it is co-translated to the protein. Besides main circular DNA bacteria also contains the extra chromosomal or extra circular DNA known as the plasmids.

So, what you see here is actually a plasmid these plasmids are called as the extra chromosomal DNA, which means, they are actually we important for the bacteria, but they are being present as extra chromosomal DNA. Presence of plasmid containing resistant genes confer the resistance towards the known antibiotic exchange of extracurricular extra chromosomal DNA between the different bacterial strain is one of the mechanism responsible for the spread of antibiotic resistance across a bacterial populations.

So, the plasmid is very important because the plasmid is the only genetic material which actually been exchanged between the different bacterial species and that is how they can be able to exchange their properties with the neighboring (())(27:45). For example, if you have, if a bacteria has supposed 200 copies of a plasmid, which is actually giving our conference the resistance against the antibiotic, for example, antibiotic penicillin.

Then what it will do is it will actually going to give some of these plasmids to the another bacteria, which is actually sensitive bacteria. So, once it will, the sensitive bacteria are actually going to receive these plasmids, they will also going to be resistant for the antibiotics. That is why it is important that when people are working in the laboratories or when people are working in biopharma industries or something these plasmids has to be the plasmid bearing bacteria.

Which we people are generating while they are doing the recombinant DNA technology, has to be destroyed very nicely, so that the genetic pool of this plasmid should not go into the environment and that is how if there will be an exchange of the genetic material or exchange of the plasmid between the two bacteria, it is actually going to spread the antibiotic resistance even in the natural bacteria.

And that is a one of the mechanism through which the bacteria's are acquiring resistance and they acquired the resistance very fast because the exchange of the plasmid material and that is why it is important to study about the plasmids.

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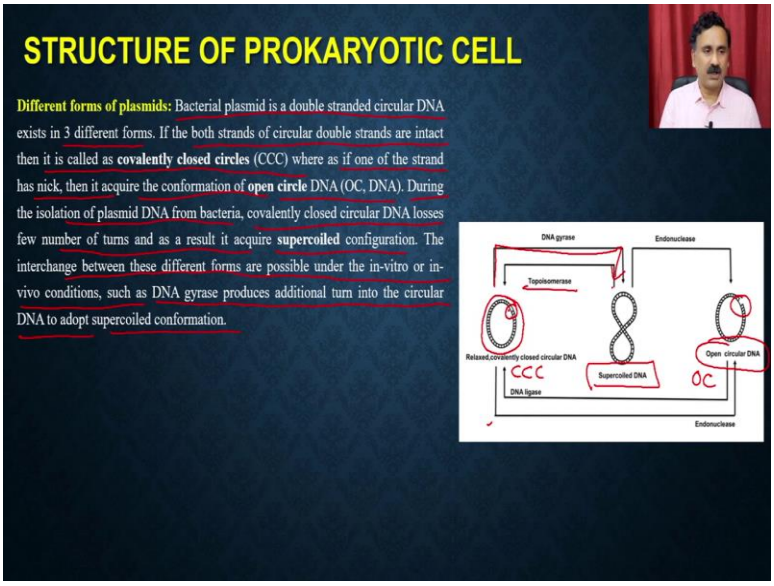

We have the, so plasmid is a circular DNA and there are different forms of plasmids what is present when you are going to do the plasmids for the different types of treatment. For example, if you take the, so you have the bacterial plasmid is a double circular DNA molecule and it exists in the three different forms. If the both strands of the circular DNA are intact, it is called as covalently circular DNA.

So, this is what you see here is a covalently circular DNA, whereas if one of the strand has nick, then it is acquired the confirmation of the open circular DNA. So, if you are actually going to put the nick in one of the strands like, for example, here, then it is actually going to acquire another conformation which is called as the open circular DNA or the OC form. This is called as the Triple C form, this is called the OC form and the third is called as a super coiled form.

During the isolation of the plasmid DNA from the bacteria covalently circular DNA loses few numbers of turn and as a result it acquire the super coiled confirmations. The interchange between these forms are possible under the in-vitro or the in-vivo conditions such as the DNA gyrase produces the additional terms into the circular DNA to adopt the circular confirmations.

So, bacteria plasmid is actually acquiring all the three confirmations under the in-vitro or the in-vivo conditions and that is how they can be, different enzymes are working. For example, if you take the circular DNA, and if you put the DNA gyrase it is actually going to create the turns into this and so, it will going to generate the supercoiled DNA.

But if you take the supercoiled DNA and treat it with the topoisomerase, it is actually going to reduce the turns and that is how it is going to be turned into the (())(31:18) covalently close circular DNA. Let us see one of the plasmids.

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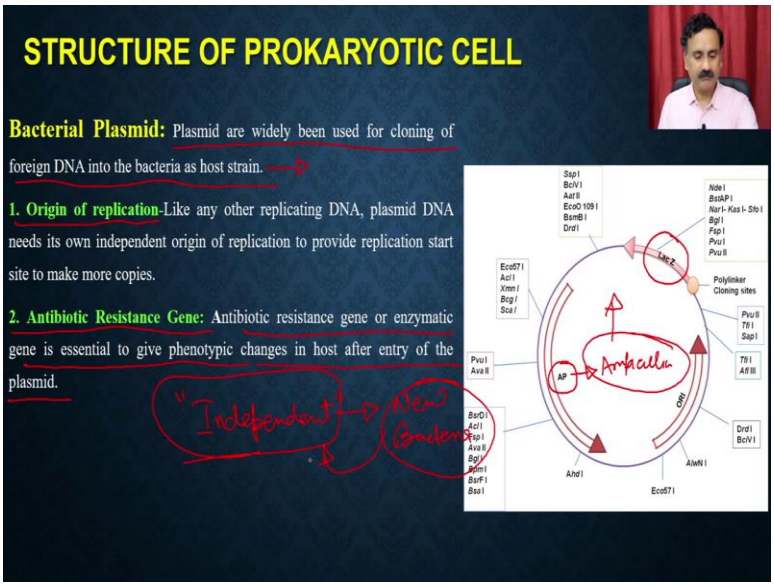

These are the bacterial plasmids, which are very commercially been available or very been used in the recombinant DNA technologies. So, plasmids are widely been used for the cloning of foreign DNA into the bacterial system as a host of strain. And this is the plasmid which is, which has the different types of components, one of the thing but you have here is the origin of applications.

So, origin of application is a place from where the bacteria is actually going to start its applications. Then need to have so has the antibiotic resistance genes. For example, here you

have, you see it has the antibiotic resistant genes, which is for the ampicillin. So, if this bacteria, if this plasmid will go to any bacteria it is actually going to give the resistance against the ampicillin, the antibiotics.

So, the antibiotic resistance genes or the enzymatic gene is responsible for giving the phenotypic changes in the host after the entry of the plasmid. Apart from that you, what you see here is an enzyme which is also been present within the plasmids and because the plasmid has the origin of replication, it has the antibiotic resistance and it has all these components, they are independent.

And that is how they are very, very dangerous because they can be independent, they do not be dependent on the nucleus for its replications or (())(32:54) activities. And that is why they can independently go to the new bacteria and the new bacteria is also going to have the additional features whatever is this plasmid is actually acquiring. Let us see, how you we can be able to isolate the plasmid from the prokaryotic cell.

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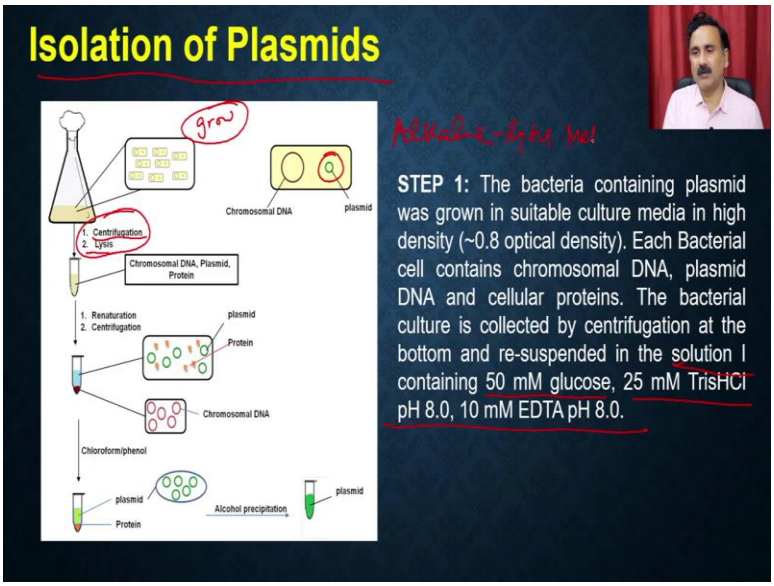

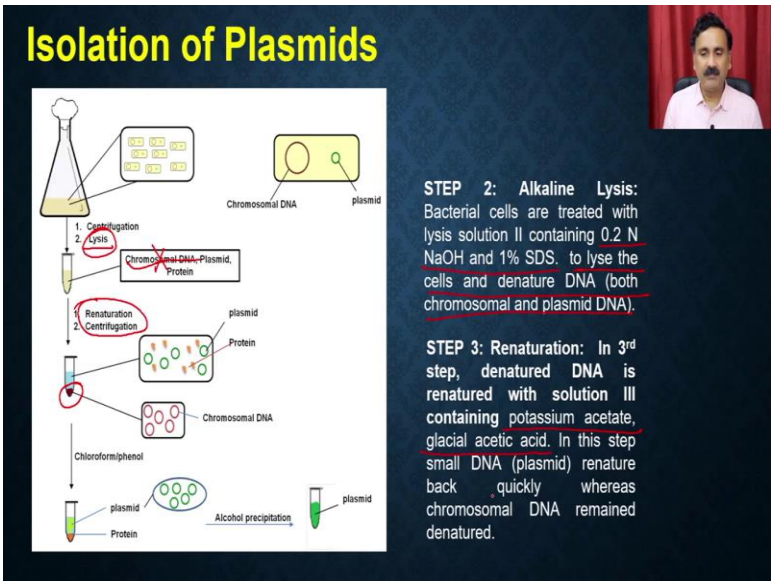

So, the plasmid isolation is a multiple step process, it is having the many steps. So, in the step one what you have to do is you have to collect the bacterial. So, first you have to do is you have to take the bacteria you have to transform that bacteria with the plasmid or suppose the bacterial plasmid is present in the bacterial cell, first thing is what you have to do is you have to grow the cells so that you have a sufficient number of bacteria.

Then you will have to do, what you have to do is you have to in the step one you have to do a centrifugation and then you have to re-suspend these bacterial cell in a solution one. The solution one is actually containing 50 milli molar glucose, 35 milli molar trace Sal and 10 milli molar EDTA. So, the method what we are discussing is called as the alkaline lysis method. In the step two, you are going to do the alkaline lysis.

So, in step two you are going to do the alkaline lysis that alkaline lysis you are going to do with the help of T 0.2 to normal NaOH and 1 percent SDS. And that is actually going to lyse the cells and it is going to renature the DNA ultimately you are going to do the third step which is called as renaturation. So, in the renaturation is going to be performed by the potassium acetate solutions and the classical glacial acetic acid solution.

And what will happen is that in this step, there will be the renaturation. So, that renaturation is actually going to renature the plasmid DNA but it will not renature the chromosomal DNA. And because of that the chromosomal DNA is actually going to be discarded in the next step. So, when you are going to do the centrifugation, the chromosomal DNA since it is not been

renatured, it is going to be precipitate. And it will going to be present in the form of pellet, whereas the supernatant is going to contain the plasmid DNA.

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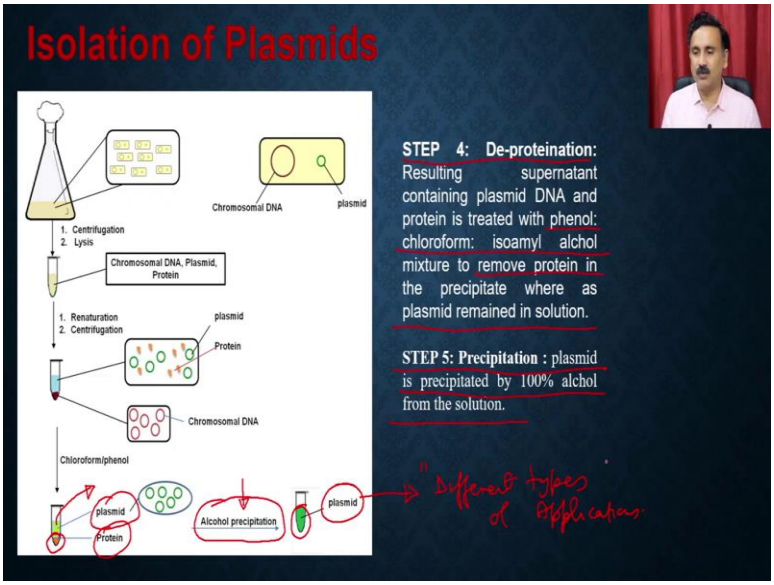

Then in the step protein, step four, you are going to just do the purification of this plasmid. So, you are going to do the de-proteination and that will result into the isolation of the plasmids. That de-proteination you are going to do with the help of the chemical which is called as the phenol chloroform isoamyl solutions. And that is actually going to remove the proteins, so that you can be able to make the very highly qualified high purity plasmid DNA.

And in the step five, you are going to, you are going to re-suspend that plasmid into alcohol from the solutions. So that is what you are, you are going to get in the plan, you are in the step four, you are actually going to have the plasmid as well as the protein and then what you are going to do is protein will be present in the precipitate that supernatant you are going to collect and then that supernatant is going to add the alcohol.

And once you add the alcohol the plasmid is going to be precipitate and that is how you are going to isolate the pure plasmid. And that pure plasmid can be used for the different types of applications, like different types of application which we are not going to discuss. So, let me give you a very real experience how you can be able to isolate the plasmids from the bacterial cell.

So, I will take you to my lab where my student is actually going to show you a very small demo and how you can be able to isolate and the plasmids from the bacterial cell.

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TARSONS


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Lab Student: Hello everyone. In this video, we will show you how to isolate lasting DNA using alkaline lysis. For preparation of plasmid DNA we need resuspension buffer, lysis buffer and neutralization buffer. In addition to that we need isopropanol, RNAs and ethanol. Resuspension buffer contains 25 milli molar trace and 10 milli molar EDTA.

We have to add RNAs at a final concentration of 100 microgram (())(37:47). Lysis buffer contains 0.2 normal sodium hydroxide and 1 percentage SDS.

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TARSONS


TARSONS

Neutralization buffer contains 3 molar potassium state, PH 6.4. For isolation of plasmid DNA we need at least (())(38:10) grown culture with ODF 3.0. So, this is already a cultured one. We have to harvest the cells by centrifugation. These (())(38:36) we have to centrifuge (())(38:38) 1000 RPM for at least 1 minute to get the cells precipitate. Now we got the cell pellet.

We can proceed for alkaline lysis method to isolate plasmid DNA. In first step we are going to add resuspension buffer which contains RNAs.

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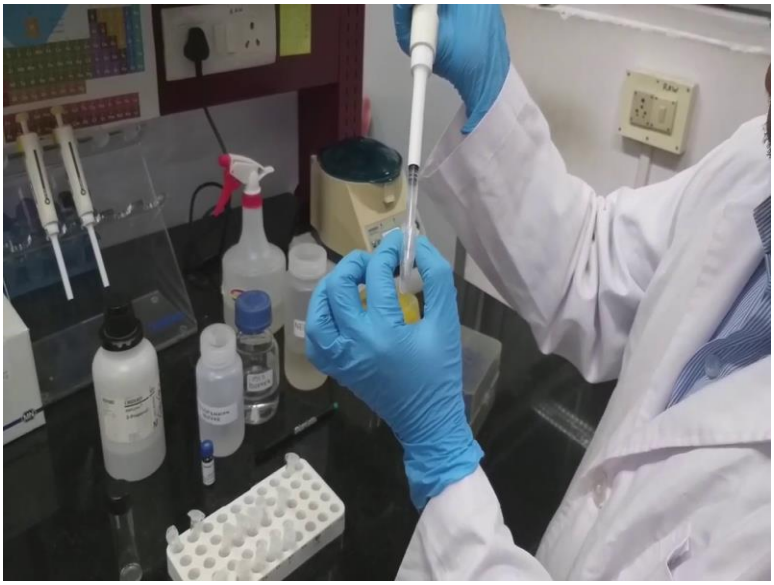

Mix thoroughly until all the cells suspended in resuspension solution. After the cells got suspended completely now we have to lyse the cells using strong alkaline condition that is 0.2 normal sodium hydroxide. And also 1 percent is sodium dodecyl sulfate. Now we have to gently flip the tube in order to lyse the cells completely. We can keep in this condition for up to 5 minutes but not more than 5 minutes.

Which will degrade the plasmid DNA and also genomic DNA will come out and it will interfere with the (())(41:19). In next step we have to neutralize the sodium hydroxide using neutralization buffer to prevent any further degradation. After adding neutralization buffer you can see there is

a white precipitate that means all the proteins precipitated by (())(42:08) buffer. You can flip the tube 2 - 3 times completely precipitate all the remaining proteins.

Now, the solution contains, solution part contains our plasmid DNA and all the precipitated one contains genomic DNA and also the proteins from bacteria. Now, we have to centrifuge this lysed for 10 minutes at 11000 G. The precipitate got settled. Now we have to transfer the white clear supernatant to another tube. This clear supernatant contains plasmid DNA.

Now, we have to precipitate this plasmid DNA with the isopropanol followed by washing with the 70 percent ethanol.

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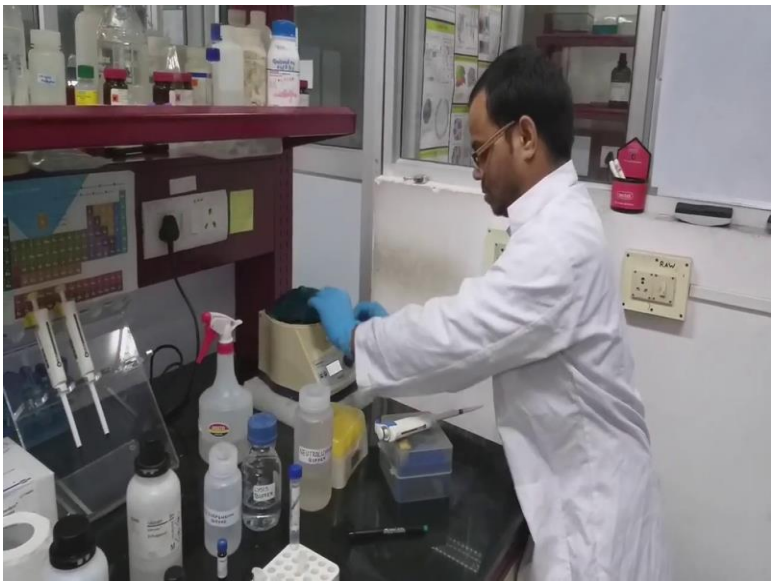


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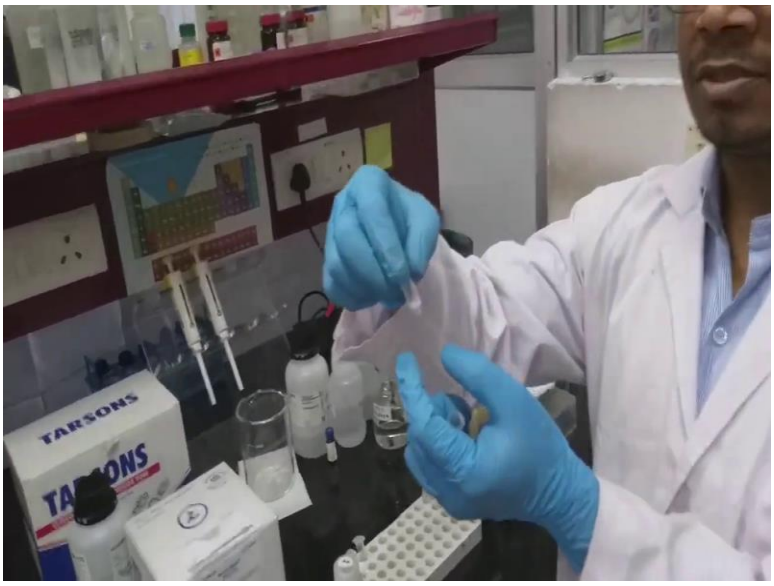

We can see white precipitate in the solution. Now we have to centrifuge it, collect the white precipitate and wash with the 70 percent ethanol. After precipitating plasmid DNA with the isopropanol we will get a pellet of plasmid DNA. Now, we have to wash that pellet. We wash this pellet with the 70 percent ethanol. Again centrifuge the pellet. Now we got the pellet, we have to air dry the pellet and dissolve it (())(45:28) water or TE (())(45:30).

We will keep, leave at room temperature till the ethanol got operated, next we will add (())(45:40). Too easy the process of manual alkaline lysis method there are several kits available from commercial vendors, the basic difference between alkaline lysis method and the kit based

method is, kit based method contains silica based columns where lysis, lysed, which containing plasmid DNA bides through this (())(46:11).

After washing whatever the unwanted components are, they will elute out and we will elute the plasmid DNA in TE buffer or water. The composition of cell lysis buffer is same as previous method. And also neutralization buffer, (())(46:39) buffer, every buffer contains same composition but in commercial kits we have one extra wash buffer which will remove any unwanted contamination and to give pure DNA.

Professor: So, when you see this demo, what you could see is that the students who are discussing about the all the four or five steps what we have just discussed.

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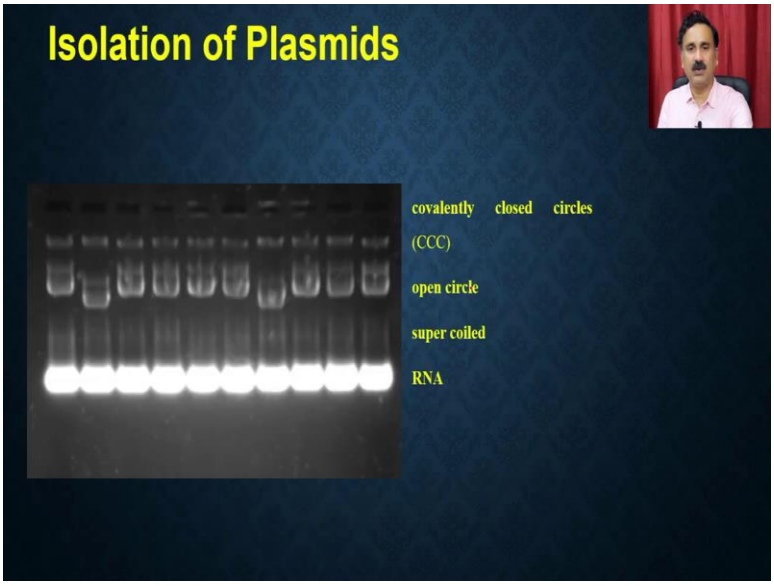

And after these steps, what you are going to get you are actually going to get the plasmid like this. So, what you see here is the three forms of the plasmid, you have the covalently close circular DNA, which means the triple C forms, you are going to have the OC forms and then you also going to have the super coiled form. So, what you see here is this is actually the closed circular, covalently closed circular DNA.

This is the open circular DNA and this is the super coiled DNA. And whereas, since we have not used the RNAs, you are also going to see the some amount of RNA, because RNA is also present in the bacterial cell. So, what we have discussed so far? We have discussed about the bacterial prokaryotic cells, we have different we have discuss about the structure of the prokaryotic cell,

we have discussed about how the cell wall is. Cell wall is thick in the case of the gram positive bacteria, whereas it is thin in the case of the gram negative bacteria.

Apart from the cell wall you also have the capsule and as well as the plasma membrane. And all these barriers, anatomical barriers are making the bacterial cell very, very resistance for the environmental changes or the chemical water present in the environments. So, with this I would like to conclude my lecture here. In our subsequent lecture we are going to discuss about the eukaryotic cells. Thank you.