Enzyme Science and Technology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Guwahati

Module - IV
Enzyme Production (Part 2: Over-expression)
Lecture - 18
DNA Delivery in host (Part-II)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bio Engineering IIT Guwahati and we what we are discussing, we are discussing about the different properties of the Enzyme. In the course, enzyme science and technology and so far what we have discussed in this particular module. We have discussed about the delivery of DNA into the host.

And why we are discussing this because in the previous module we have discussed about the how you can be able to utilize the different enzyme and as well as the vector to produce a recombinant DNA.

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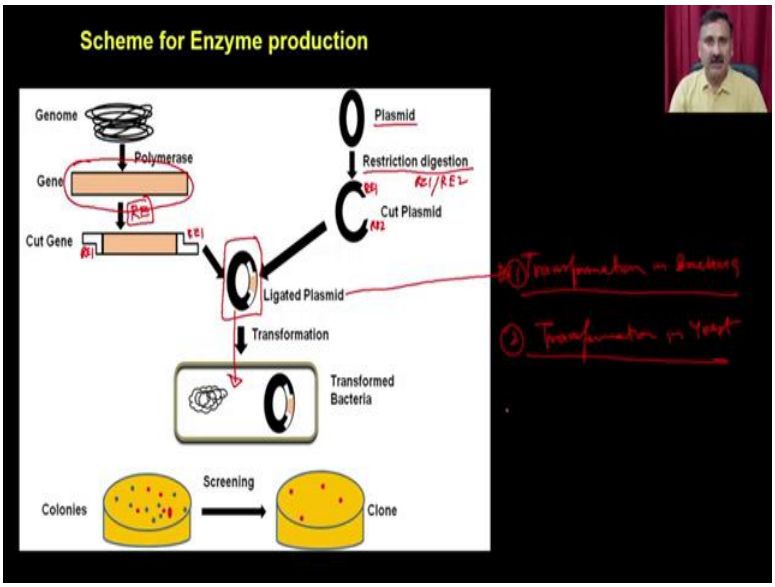

So, what we have discussed in the previous lecture is that you are going to isolate a gene or the gene fragment from the genome, either you are going to aware of the genome sequences or you are not aware of the genome sequences. In both of these cases either

you will use the genomic library or the C DNA library or you will use the side directed primers and you will actually going to use the PCR to get the gene fragment.

Irrespective of the sources, you are going to get the gene fragment. This gene fragment has to be digested with the restriction enzyme and that is why you are going to get the sticky end. So for example, in this case if you are using the 2 restriction enzyme, then you are going to have the two sticky ends.

And the same way you are actually going to treat the vectors. So, in this case the plasmid which is going to be digested with the restriction enzyme RE1 and RE2. So, that is how you are actually going to have the sticky ends like the RE1 and RE2. And once you put them together and you are going to put a ligation reaction with the help of the enzyme T4 DNA ligase, you are going to get the chimeric DNA or the chimeric plasmids.

This chimeric plasmid has to be delivered into the host and if you recall in the previous lecture, we have discussed about the DNA delivery methods utilizing the transformations. So, we have discussed about the transformations in the bacteria and or we have discussed about the transformation in yeast. And in both of these methods, you are going to treat the cells with a chemical agent.

And with the help of the chemical agent, it is actually going to change the surface chemistry and it will also going to make the cells competent enough to take up the exogenous DNA. And once the DNA is been taken up, you are going to put them into the recovery phase. And that is how you are going to get the transformed colonies. Now, in today's lecture, we are going to discuss about the how you can be able to deliver the DNA in the mammalian cells.

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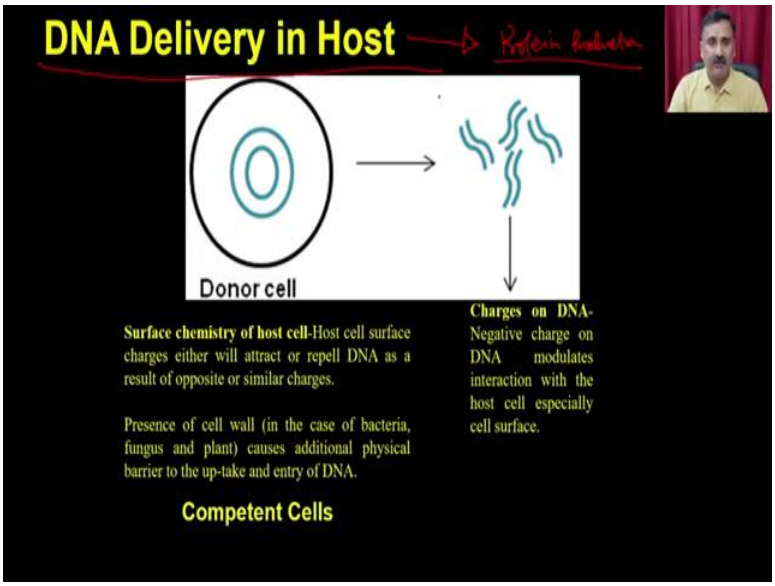

So as we discussed, the DNA delivery in host is very important for the protein production right and when you do the protein production, the and as you can as you can recall when we were discussing about the surface chemistry or the other kinds of proper modulations, which are allowing the cells to take up the DNA.

But that kind of modifications are not possible in the mammalian system, because the first of all, the mammalian cell wall or does not have the cell wall and the mammalian system is the surface chemistry is very, very complicated compared to the bacterial system. So in those cases, we have the alternate approaches to deliver the DNA into the host.

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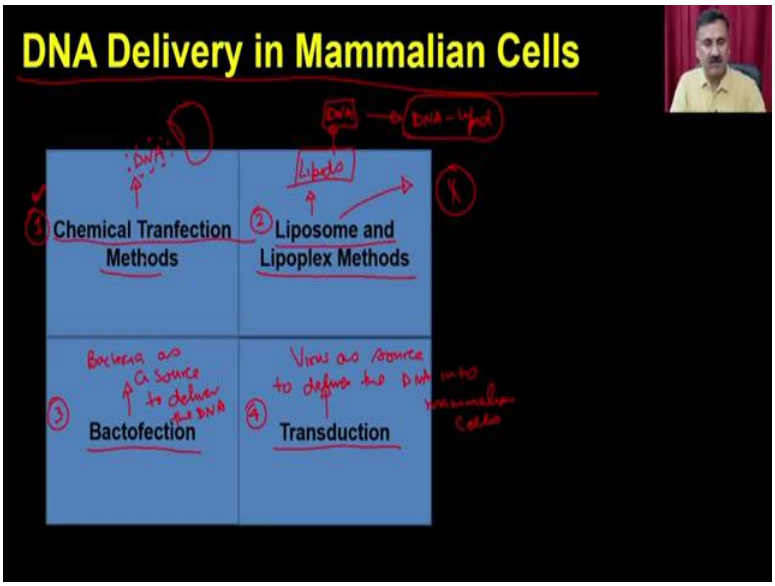

So, we have the 4 approaches, what we can use in the delivering the DNA into the mammalian cells. The 1st approach is called as the chemical transfection method, so 2nd approach is called as the liposome or the lipoplex methods. The 3rd approach is called as the bactofection and the 4th method is called as the transductions.

So in the first method, you are going to use the transfection agents or you are going to use the chemical agents. These chemical agents are actually going to make the complex with DNA in such a way that DNA is going to be taken up by the mammalian cells. So, they will be going to taken up by the mammalian cells.

So, in the liposome or the Lipoplex method, you are going to do the same thing, but instead of using the chemical agents, you are going to use the lipids. And in this case, sometime you are going to use the cationic lipids and in that case, the cationic lipids are actually going to bind the DNA and that is how you are going to have. You are going to get the DNA lipid complex and this DNA lipid complex can be delivered or can be easily readily been taken up by the cells.

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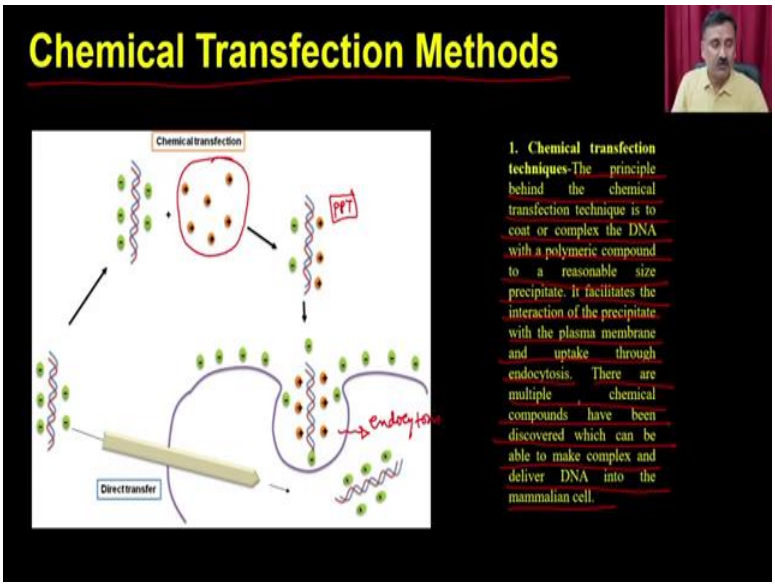

In some cases, people are also trying with the liposome method. So, where you are actually entrapping the DNA into the liposome and that is how you are actually these this liposomes are going to be having the DNA inside and that is how they are going to be taken up by the mammalian cells.

In the Bactofection, you are using the bacteria as a source to deliver the DNA, so bacteria as a source to deliver the DNA. Similarly, in the transductions, you are going to use the virus as a source to deliver the DNA into mammalian cells. So, let us start discussing with the first method and that is called as the chemical transduction method.

So, in the chemical transduction method, you are going to treat the principle, the principle behind the chemical transfection technique is that you coat or complex the DNA with a polymeric compound to a reasonable size precipitate. It facilitate the interaction of the precipitate with the plasma membrane and uptake through the endocytosis.

There are multiple chemical compound have been discovered which can be able to make the complex and deliver the DNA into the mammalian cells. So, in the chemical transfection method, because you know that the DNA is negatively charged. So, you can add the chemical agents which are positively charged.

So, once you are actually going to add the positively charged, they are going to make the complex with DNA and as a result they are actually going to make the visible precipitate and these visible precipitate are actually going to be taken up by the cell with a process just called as the endocytosis.

So, these particles will you know going go and sit on to the plasma membrane and then the plasma membrane is going to be taken up inside and by a process which is called as the endocytosis. There are many methods, many chemicals what you can use. So, one of the popular method is the calcium phosphate method.

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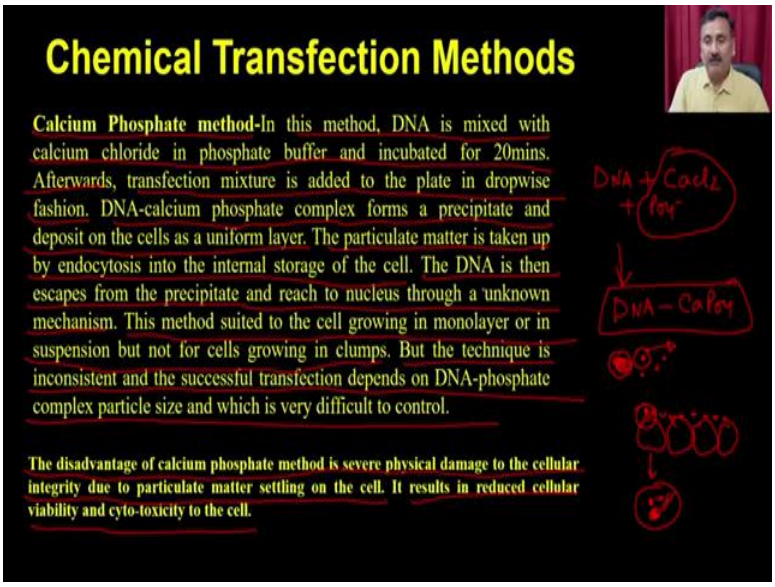

So, in this method, the DNA is mixed with the calcium chloride in a phosphate buffer and incubated for 20 minutes. Afterwards, the transfection mixture is added to the plate in a drop-wise fashion. DNA calcium phosphate complex forms a precipitate and deposited on the cell in a uniform layer.

The particulate matter is taken up by the endocytosis into the internal storage of the cell. The DNA is then escaped from the precipitate and reached to the nucleus through a unknown mechanism. This method suits to the cells growing in a mono layer or in a suspension, but not for the cell growing in the clumps.

But the technique is inconsistent and the successful transfection depends on the DNA phosphate complex particle size and which is very difficult to control. Which means, in

this case you are actually going to take the DNA and then you are actually going to add the calcium chloride along with the phosphate buffer ok.

So, what will happen is the calcium phosphate is going to react and that is how the DNA is going to make a complex with the calcium phosphate. And once it forms the complex, with the calcium phosphate, it is actually going to form the particles like structure. So, it is going to form the precipitate.

So, imagine that you have a cell, right you have a single mono layer of cell right, so all these particles are actually going to sit on top of this. So, once the particle sits on any cell, the cell has a inherent tendency that it is actually going to eat these cells or these particles. Just like as we take the food particles for example.

So, when we take the food particle and it goes inside the you know our body, the cells are actually going to take up this food. And as a result, what will happen is the this particle is going to be cut inside. Then once if the particles are going to be inside, the DNA is going to be released from this and that is how the DNA will actually going to reach to the nucleus.

Now, what is the disadvantage? The disadvantage is that if this particle size are small enough, they will they are not going to cause any damage to the cell. But they are if they are big enough, if they are going to grow, because more and more calcium phosphate, if it reacts with the DNA, the size of this calcium phosphate particle is going to grow up.

So, if the size is very high, then it is actually going to cause the damage to the cell. So, disadvantage of the calcium phosphate method is the severe physical damage to the cellular integrity to the particle matter particulate matter sitting settling on to the cell. It results in the reduced cellular viability and the cytotoxicity to the cell.

So, one of the major disadvantages of the calcium phosphate method is that it is actually going to give you the very low recovery. Because, if you are if you are very good and you are controlling the events in such a way that you are going to make the particle size very small, then it is going to work. If it is does not right then your the particle size are going to be very big, it is going to cause the physical damage to the cellular integrity and that is how it is actually going to kill the cells.

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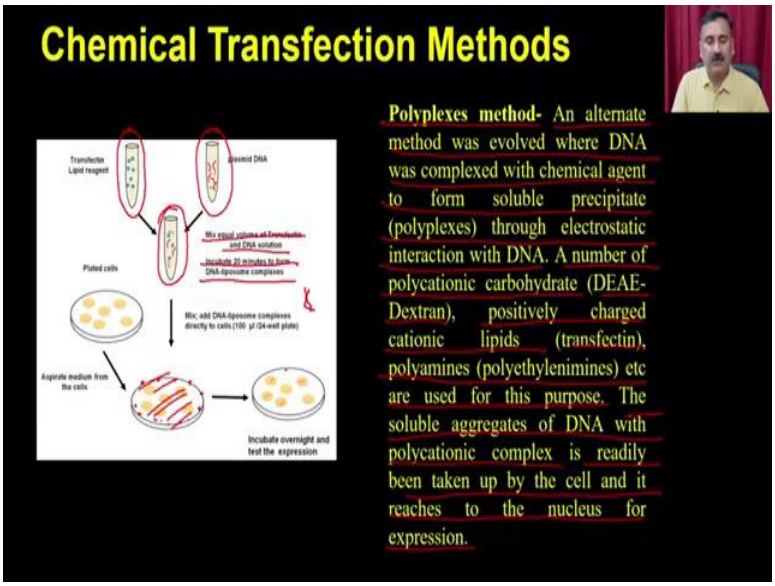

So, what is the alternative? The ultimate alternative is that you may go with the Polyplexes method. So, polyplexes method is that in a it is an alternative method which was involved where the DNA was complexed with the chemical agent to form the soluble precipitate through the electrostatic interaction with the DNA. A number of polycationic carbohydrates such as DEAE Dextran, positively charged cationic lipids such as transfectin or polyamines etcetera are being used for this purpose.

The soluble aggregates of the DNA with the polycationic complex is readily being taken up by the cell and it teaches to the nucleus for the expression. So, what you are going to do in a polyplexes method where instead of using the calcium phosphate, you are going to use the material which is going to make a soluble precipitate with the DNA and in this category you can use the DEAE Dextran or positively charged lipids or you can use the polyamines. All of these are actually going to make a complex with DNA.

These complexes are going to be soluble in nature and that is how they are actually going to be taken up by the cell. So, what you are going to do is you are going to take up the you are going to take this chemical agent polyplex method or you are going to take the plasmid right in 2 vials and then you are mixed them together.

So, the mixed the equal volume of transfectin and the DNA solution, then you incubate for 20 minutes to form the DNA liposome complex. So, the DNA of liposome complex

is formed, then you can take the plated cells and then you can actually be able to add this drop wise on to the cells ok.

So, when you drop add the drop wise it is going to eventually going to spread on this and since it is actually going to be a soluble aggregate, it is going to still form the aggregates. These are going to be taken up by the cell and that is how you are going to get the expression of these cells. So, their DNA will enter inside the cell and then the DNA will go to the nucleus over the transfection and as well as translations.

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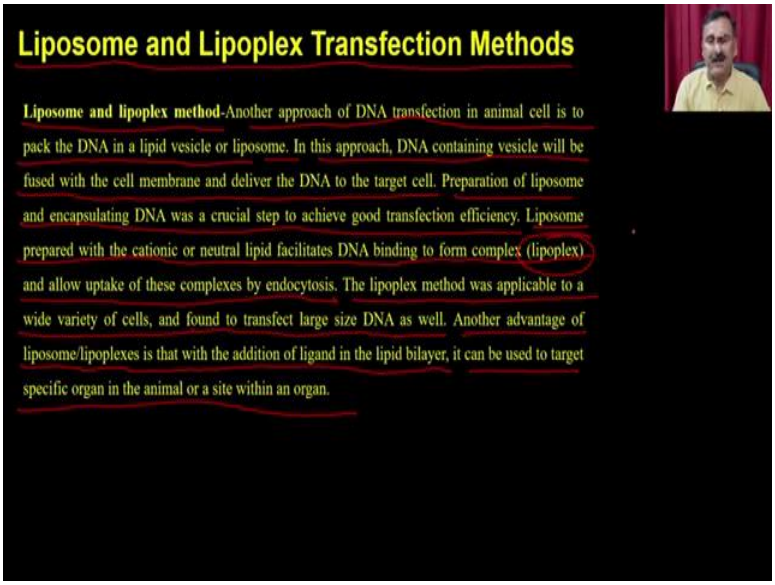

Then we have the second method where you are going to use the liposome and lipoplex transfection method. So, the liposome and the lipoplex method, another approach of DNA transfection in the animal cell is to pack the cell in a lipid vesicle or liposome. In this approach, the DNA containing vesicle will be fused with the cellular membrane and deliver the DNA to the target cell.

Preparation of the liposome and encapsulating DNA was a crucial step to achieve the good transfection efficiency. Liposome prepared with the cationic or the neutral lipids facilitate the DNA binding to form the complex or the lipoplex and allow the uptake of these plexes complexes by the endocytosis.

The lipoplex method was applicable to a wide variety of cells and found to be transfect large size DNA as well. Another advantage of the liposome or lipoplexes is that the

addition of ligand in the lipid bilayer, it can be used to target a specific organ in the animal or a site within the organ. So, in the liposome or the lipoplex method, you are going to either use a cationic lipids and make the DNA lipid complexes or you are going to make the liposomes and you are going to entrap the DNA into that.

So, one once you prepare the liposome, it is going to go and fused with the cells and that is how it is actually going to give you the they deliver the DNA. So, to explain this method we have prepared a small demo clip, where we have actually prepared the you know where we have you know discussed about how what are the different steps you can take and how you can be able to perform the transfections.

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Hello everyone, myself is Sooram Banesh Research Scholar at Department of Biosciences Bioengineering IIT Guwahati. In this video, we will take you to how to transfect any mammalian cell and analyze the results. And during the video, we will also discuss what are the precautions needs to be taken while transfecting and different ways of transfecting like chemical transfection and electroporation methods. And also we will discuss about how to analyze the results.

Like after transfecting what are the ways we can analyze whether the transfection is happened or not like through western blot or through fluorescence microscopy, if it is if you are insert of interest is conjugated to any fluorescent protein. So, let us start the video.

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In this video, we will show how to subculture the cells and count the cells and seed for the transfection studies. First we have to remove the remaining media, then trypsinize the cells then we will count the cells and seed. Now, I will show how to do trypsinization. Now, I am going to add the trypsin to detach the cells. Hello everyone, in this video we will be demonstrating how to deliver plasmid DNA into mammalian cells.

There are two methods available to deliver DNA one is chemical basis method like using transfection reagents and another method is electrophoretic method. Electrophoretic method there are various instruments in which specially designed buffer containing plasmid will be delivered into mammalian cell using pulses. But in chemical transfection method there are wide variety of chances available like using cationic lipids or peptides or polymers.

In this video we are going to show how to transfect the mammalian cells using polyethylenimine based reagent. So, in this method first we are going to mix stop. [FL] in this method we are going to show pi based transfection. For that we have to mix DNA with the incomplete media first. After mixing we have to add polyethylenimine directly to the temperature.

Then we can see a visible 5 precipitant which means the DNA is complex between the PEI and ready to. The ratio between DNA and the transfection reagent should be 1 is to 4. Let us start the demo.

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We have already aliquoted incomplete medium. So, for this we have to add Plasmid DNA. This is the DNA already concentration is divided. The mixing should be proper otherwise there is no complex production. You can tap the tube in order to get mixed. This is the PEI transfection reagent. So, this is 1 mg per ml concentration. We added 10 microgram of DNA, so we have to add 1 is to 4. That means 40 microgram of PEI. So, I am going to add 40 microliter of pi to the cell mix properly.

Incubate the tube for at least 5 minutes to get the complex form. The 5 minutes are over. Then we can see a visible precipitant, white precipitate inside that tube so I am going to add this complex directly to the cells. Here the bells which contains reduced serum medium, reduced serum containing medium So, if serum 10 percent serum if we add 10 percent serum then it may complexed with the PEI DNA complex, then it may not get internalized (Refer Time: 24:14) So, we should take care of them.

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After cells are detached we have taken into clean falcon, then we have to centrifuge these cells. As these cells are very delicate, we have to centrifuge at 1500 rpm for 2 minutes. Now, we have to remove the supernatant and resuspend the cells in fresh media. After resuspension we have to count the cells. So, I am going to take 20 microliter of this cell suspension and mix with the 20 micro liter of trypan blue and count under new bar chamber.

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Before counting, we have to see how a counting chamber or hemocytometer look like? This is a typical hemocytometer also called as Neubauer chamber which contains these squares in upper side and lower side, with each square having depth of 0.1 mm and area of 0.0025 millimeter square. Now, I am going to put a cover slip on this chamber then I will add slowly cell suspension through capillary action it will spread all over the squares.

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So, we checked how many cells are there in all the squares. Now, we have to count how to count the cells. So, here a typical Neubauer chamber which contains squares, 5 squares. So, we have to count cells in these squares. So, each square is an area of 0.0025 millimeter square and total small squares 16. So, total area of this whole square is 0.04 millimeter square.

So, the depth of the this each varies 0.1 millimeter. So, what is the volume? 0.04 into 0.1, so that is total 0.004 millimeter cube or 0.004 microliter. So, say we have combinedly cells in each way. Say this is ABCD, here we have 100 here we have 150 here we have 110 here we have 100 again. So, the total cells we have to take average. That means 100 plus 150 plus 110 plus 100 divided by 4 total 4 squares we are counting the average is 115.

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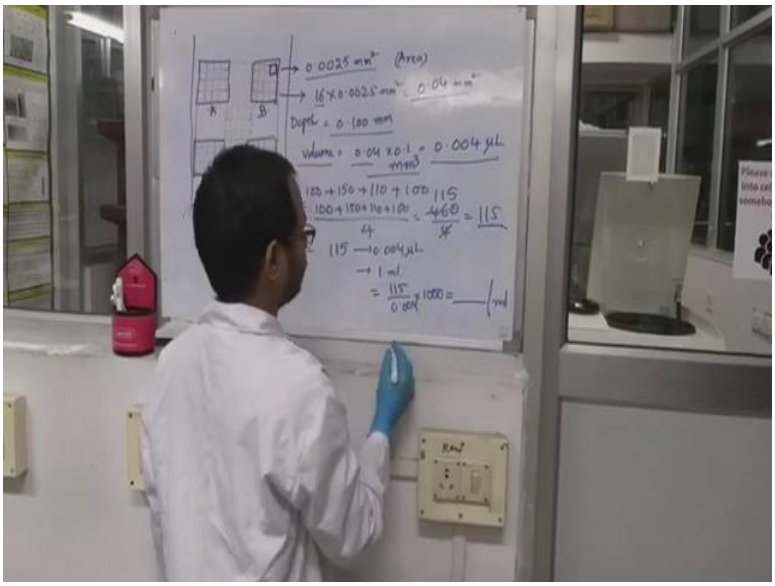

So, 115 cells in 0.004 microliter volume. So, how many cells per 1 ml? So, that we can calculate simply 0.004 into 1000, that will give the value cells per ml.

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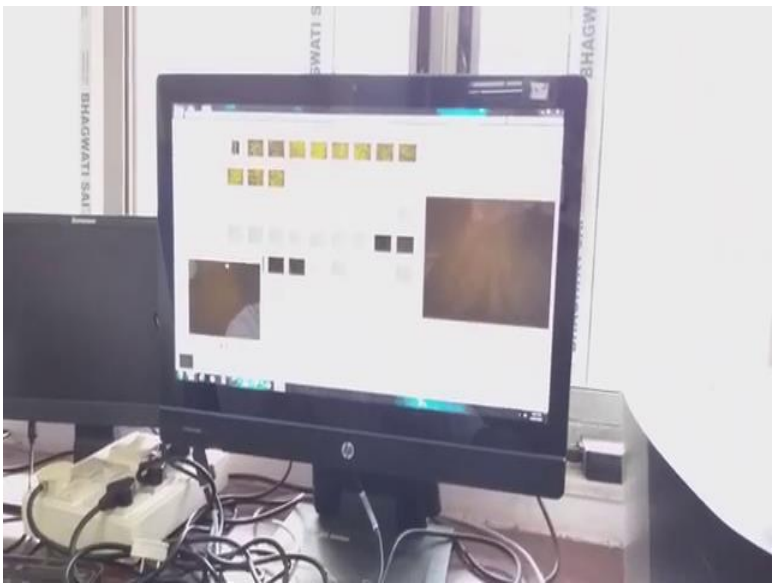

We have successfully transfected the cells. Now, we will try to analyze whether the transfection is successful or not. So, I will show some of the images I have taken after this transfection. So, let us see first of all this is a non-transfected image of non-transfected. So, we can see there is no fluorescence.

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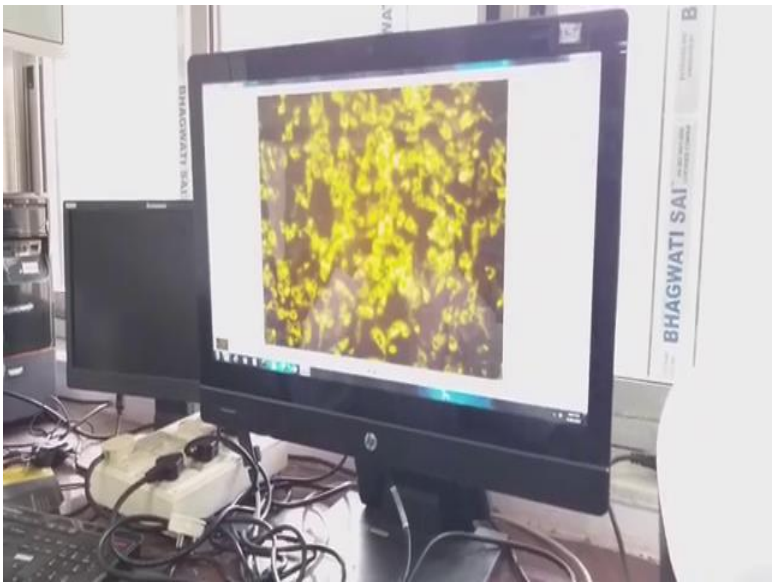

When you see transfected one the cells will blow like this. So that means our transfection is successful. So, far we have learned that how to transfect the cells. We have used polyethylenimine based transfection reagent which is basically a polymer, which conjugates with the DNA and the precipitation we will use for the transfection. The most of the transfection process through endocytosis.

During transfection also we have to make sure the ratio between the transfection reagent and DNA should be optimized. The result what we have shown you is the optimized one. So, you have to according to your requirement you have to optimize the transfection reagent versus DNA. And also the plasmid DNA need to be contamination free, otherwise you can see bacteria count before the transfection happens.

We can do the same transfection through electroporating method also. But in that case we should not use any cells while preparing plasmid DNA, that should be taken care of otherwise there should be some conduction inside the incubate. So, it will give you all the cells, so these precautions need to be taken. With this I will conclude the video and thanks for watching.

So, in this demo the students have discussed how you can be able to pay the cells, how you can be able to count the cells and then how you can be able to treat the cells. Prepare the you know the lipoplex complexes and how you can be able to treat the cells with these complexes and then how you can be able to check the transfection efficiency.

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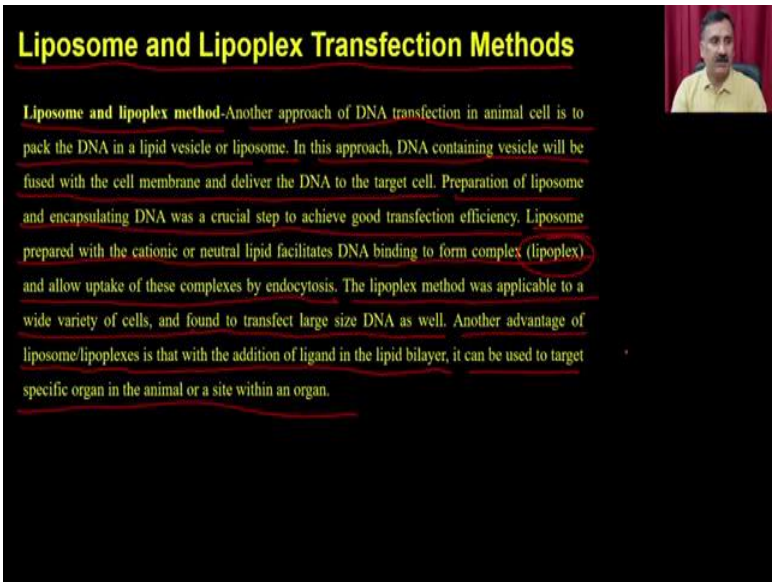

Now, let us go to the next method.

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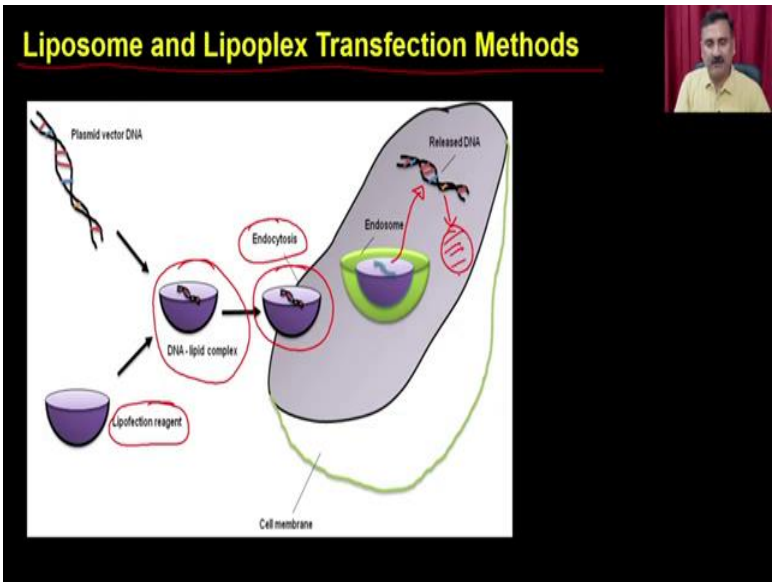

And the next method is the liposome and the lipoplex transfection method that is anyway we have discussed. So, this is the basic principle that you have the lipofectamine reagent and that is actually going to make the complex with the DNA. So, DNA complex is going to be taken up by the cell by a process which is called as endocytosis and from the endosome this DNA is going to be released and this DNA will go to the nucleus for the expression status.

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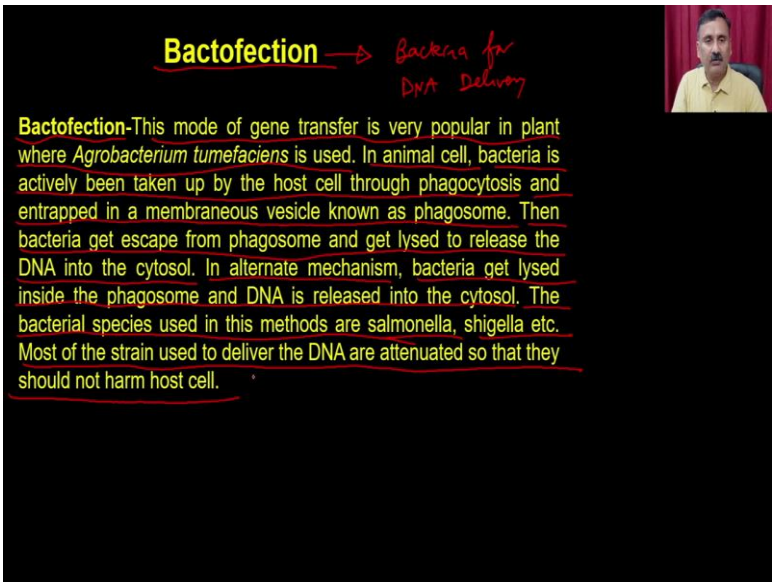

Now, let us go to the next method and the next method is the bactofactin. So, bactofactin is the method where you are going to use the bacteria for the DNA delivery ok. So, bactofactin is more common in the case of plants. So, this mode of gene transfer is very popular in the plant where agrobacterium tumefaciens is used. So, in animal cells the bacteria is actively been taken up by the host cell through a process which is called phagocytosis and the entrapped in a membraneous vesicle known as phagosome.

Then the bacteria get escape from the phagosome and get lysed to release the DNA into the cytosol. In alternate mechanism the bacteria get lysed inside the phagosome and the DNA is released into the cytosol. The bacteria species used in methods are salmonella, shigella, etcetera. Most of the strain used to deliver the DNA are attenuated, so they should not harm the host cells.

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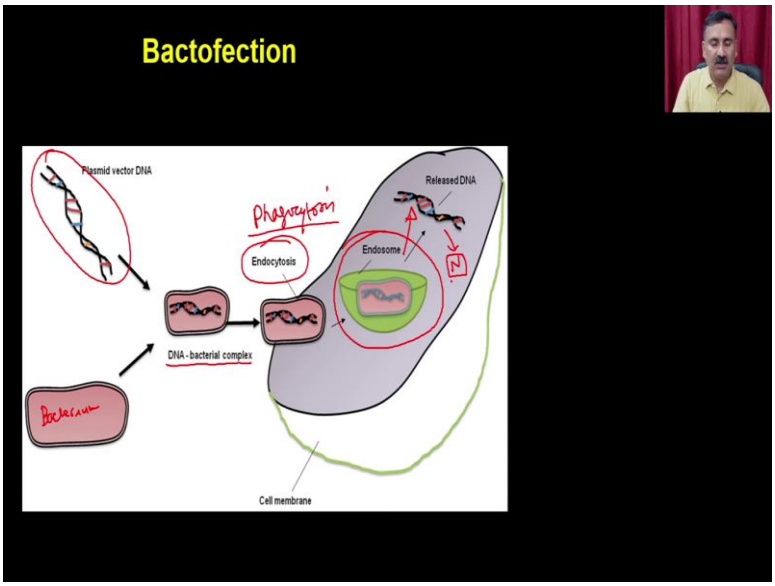

So, in a bactofactin what you are going to do is you are going to take the bacteria right. So, this is the bacterium cell right and you take the DNA right and then you mix them together. So, bacteria will take up this DNA and that is how it is actually going to form the DNA bacterial complex.

Once the DNA bacterial complex is formed it is going to be taken up by the endocytosis or the phagocytosis. And once it is going to be taken up by the cell entry process it is going to be present in the membraneous vesicle and from this membraneous vesicle the DNA is going to be released and this DNA will go into the nucleus for the expression studies.

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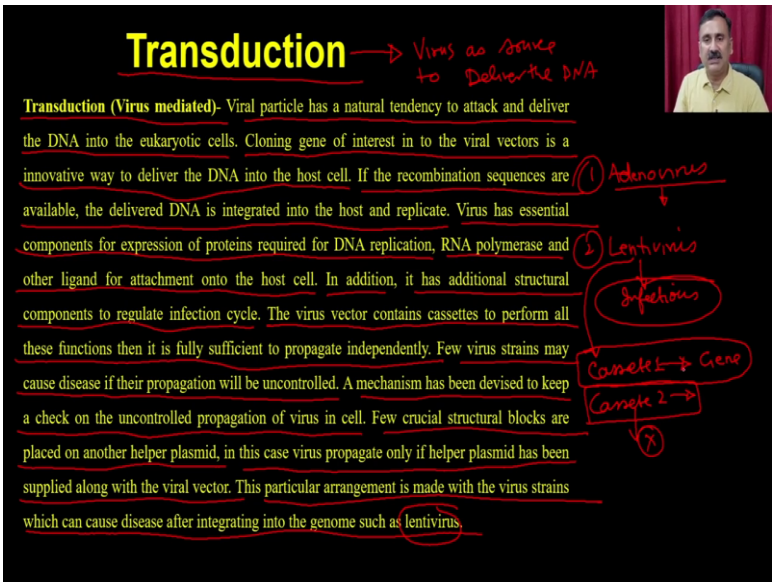

Then we come to the last process and last process is called as the transduction. So, in the transduction you are going to use the virus as a source to deliver the DNA. So, transduction or the virus mediated DNA delivery into the mammalian cells. So, virus particle has a natural tendency to attack and deliver the DNA into the eukaryotic cell.

Most of the viruses they do not have their own cellular machine treat for replication and that is why they have the inherent tendency, that if you add them to the mammalian cells they will go and attach to the mammalian cell and that is how they will actually going to inject the DNA into the cell.

And this DNA will go directly to the nucleus and it is actually going to recombine with the genome and that is how it is going to be a part of the genome. And then once the genome is going to replicate it is actually going to make the multiple copies of the virus and that is how your the virus is going to spread throughout the body.

So, utilizing or exploiting that mechanism we can actually be able to deliver the DNA into the eukaryotic cell. So, cloning the gene of interest into a viral vector is a innovative way to deliver the DNA into the host cell. If the recombination sequences are available the delivered DNA is indicated into the host and replicate. Virus has essential component for expression of protein required for the DNA replication, RNA polymerase and the other ligand for the attachment onto the cell.

In addition it has additional structural component to regulate the infection cycle. The viral vectors containing cassette to perform all these function, then it is fully sufficient to propagate independently. Few virus strain may cause disease if their propagation will be uncontrolled.

A mechanism has been devised to keep a check on the uncontrolled proliferation of the virus in a cell. Few crucial structural blocks are placed on another helper virus. In this case the virus propagate only if the helper virus has been supplied along with the viral vector.

This particular arrangement is made with the virus strain which can cause a disease after integrating into the genome such as the lentivirus. So, in the transduction species you can have the two different types of viruses adeno virus or you can have the lentivirus ok. So, adeno virus can be used very extensively to express the protein, where the adeno virus is going to attack to the cell. They will deliver the gene of your interest to the nucleus and that is how you are going to work.

In the lentivirus, lentivirus are more infectious and self-replicating, that is why when you use the lentivirus, the lentivirus can be used in two cassettes. The cassette 1 which will actually going to have the your gene of interest and the cassette 2, so cassette 1 and then the cassette 2 you can keep all the essential genes.

So, once you supply both of them they will actually going to replicate and they will actually going to supply your gene. But as soon as you do not add the cassette 2 or you remove the cassette 2, it will still be able to deliver the gene, but it will not be able to cause the infection ok. So, that is why you can actually be able to control the activity of some of the infectious viral particles such as the lentiviruses.

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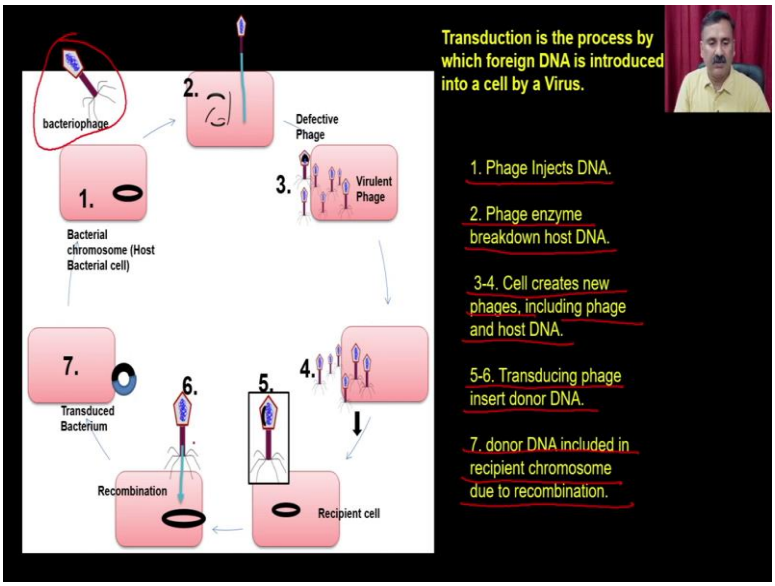

So, in a transduction what happen is that you are actually going to use the you know the for example, in this case we have taken an example of bacteriophage. So, in the step 1 the phage is going to inject the DNA into the host right. Then the phage enzyme are going to break down the host DNA ok. So, it is going to break down the host DNA and the in the step 3 to 4 the cell creates no phage including the phage and the host DNA.

And in the 5 to 6 the transducing phage insert the donor DNA and the donor DNA included in the recipient chromosome due to the recombination. So, in the last step it is going to recombine and that is how whatever the DNA you have in the phage has injected which will be a part of the host genome. And that is how when the host is replicating it is also going to replicate the viral genome. So, this is the different methods what we have discussed for delivering the DNA into the host system.

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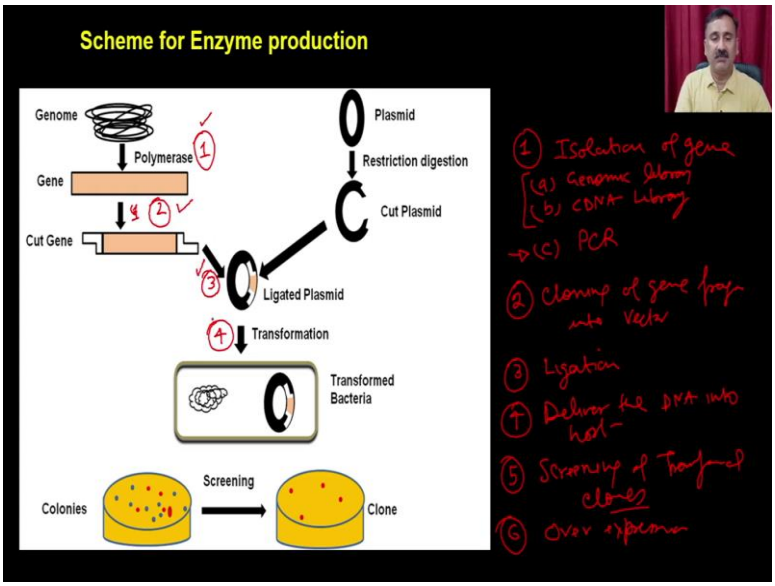

Now, in the what we have discussed we have discussed about the isolation of the gene right and we have discussed about the isolation of genes. So, we discussed about the 2 approaches A and B one is the genomic library approach and other is CDNA library approach and these are the approaches when you can use when the genomic sequences are not known. Whereas, you can use the C approach where you can use the PCR with the site specific primer and that you can do if your genomic sequences are known.

Then once you got the gene fragment like this gene fragment you can actually be able to do the cloning of this gene fragment into cloning of the gene fragment into vector. So, in the step 2 this is the step 1 this is the step 2 right that same you are going to do for the vector also and then in the and then there is a step 3 you are going to put the ligation reactions right.

So, that is also a part of the cloning reactions and the step 4 you are going to deliver the DNA into the host right. So, we will deliver the DNA into host, then the step 5 you are going to screen these transformed clones. So, you are going to do the screening of transformed clones and the step 6 you are going to check the over expression.

So, so far what we have discussed we have discussed about the step 1, we have discussed about the step 2, we discussed about the step 3 and step 4. Now, in the subsequent lecture we are going to discuss about the step 5 and 6 where we are going to first discuss how

you can be able to screen the transformed clones or the clone where the DNA is being delivered.

And the lastly we are going to discuss about how you can be able to use the different method to induce the protein production. So, with this I would like to conclude my lecture here. In our subsequent lecture we are going to discuss about the screening of the clones and as well as the over expression.

Thank you.