Molecular Biology

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Module - 10

Molecular Cloning

Lecture-44 DNA Delivery (Part 2)

Hello everyone, this is Dr. Vishal Trivedi from Department of Bioscience and bioengineering IIT Guwahati. 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 ctNA library or you will use the psi 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 how you are going to get the sticky ends. So, for example in this case if you are using the two 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 is 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 transform colonies. Now in today's lecture we are going to discuss about the how you can be able to deliver the DNA in mammalian cells. So, as we discussed the DNA delivery in host is very important for the protein production and 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 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. So, we have the four approaches what we can use in the delivering the DNA into the mammalian cells. The first approach is called as the chemical transfection method, the second approach is called as the liposome or the lipoplex methods, the third approach is called as the bacteriofactin and the fourth method is called as the transduction. 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. 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 liposomes are going to be having the DNA inside and that is how it they are going to be taken up by the plasmids by the mammalian cells. In the bacteriophactin you are using the bacteria as a source to deliver the DNA.

So, bacteria as a source to deliver the DNA. Similarly, in the transduction 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 transduction 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 take make the complex and deliver the DNA into the mammalian cells. So, in a chemical transduction method because you know that the DNA is negatively charged. So, you can add the chemical agent 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 which is called as the endocytosis.

So, these particles will you know going go and sit onto 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. 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 complex DNA calcium phosphate complex forms a precipitate and deposited on the cell in a uniform layer.

The particulate matter is taken up by the endocytotic in the into the internal storage of the cell. The DNA is then escapes from the precipitate and reach 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 a 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.

Okay. 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 perform the precipitate. So, imagine that you have a cell, 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 an inherent tendency that it is actually going to eat these cells or these particles. Okay. 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.

Okay. And as a result, what will happen is the this particle is going to be cut inside, then once 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 all 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 due to the particle matter particulate matter sitting settling onto the cell it results in the reduced cellular viability and the cytotoxicity to the cell. So, one of the major disadvantage 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 dozen, then you know the particle size are going to be very big, it is going to cause a physical damage to the cellular integrity and that is how it is actually going to kill the cells.

So, what is the alternative? The ultimate alternative is that you may go with the polypexis method. So, polypexis method is that in a is an alternative method, which was evolved

where the DNA was complexed with the chemical agent to form the soluble precipitate through the electrostatic interaction with the DNA. A number of polycatenic carbohydrates such as DAE dextran, positively charged catenic lipids such as transectin or polyamines etc. are being used for this purpose. The soluble aggregates of the DNA with the polycatenic complex is readily being taken up by the cell and reaches to the nucleus for the expression.

So, what you are going to do in a polypexis method, we are 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 DAE dextran or positively charged lipids or you can use the polyamines. All of these are actually going to make a complex with DNA and 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 polypex method or you are going to take the plasmid in two vials and then you will mix them together. So, the mix the equal volume of transectin and the DNA solution, then you incubate for 20 minutes to form the DNA liposome complex. So, the DNA liposome complex is formed, then you can take the plated cells and then you can actually be able to add this drop wise onto the cells.

So, when you drop add the drop white, 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 into the cell and then the DNA will go to the nucleus for the transfection and as well as translations. 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.

Depression of the liposome and encapsulating DNA was a crucial step to achieve the good transfection efficiency. Liposome prepared with the cationic or the neutral lipid facilitate the DNA binding to form the complex or the lipoplex and allow the uptake of these pluses 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 lipoplex 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 protein come the lipid complexes or you are going to make the liposomes and you are going to entrap the DNA into that. So, once you prepare the liposome, it is going to go and fuse with the cells and that is how it is actually going to give you the DNA, they deliver the DNA. Now, let us go to the next method. 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 agent 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 studies. Now, let us go to the next method and the next method is the bactofectin. So, bactofectin is the method where you are going to use the bacteria for the DNA delivery. So, bactofectin is more common in term in the case of plants.

So, this mode of gene transfer is very popular in the plant where agrobacterium tomyphasia is used. So, in animal cells, the bacteria is actively being taken up by the host cell through a process which is called phagocytosis and the entrapped in a membranous vesicle known as phagosome. Then the bacteria get escaped 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 salmola, shigella, etc.

Most of the strain used to deliver the DNA are attenuated, so they should not harm the host cells. So, in a bactofectin what you are going to do is you are going to take the bacteria. So, this is a bacterium cell and you take the DNA 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 membranous vesicle and from this membranous vesicle, the DNA is going to be released and this DNA will go into the nucleus for the expression studies. Then we come to the last process and that 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 well 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 then 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 integrated into the host and replicate.

Virus has essential component for expression of protein required for 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 functions, then it is fully sufficient to propagate independently. Few virus sustain 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.

Through 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 stain 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, adenovirus or you can have the lentivirus. Okay, so adenovirus can be used very extensively to express the protein where the adenovirus is going to attack to the cell, they will deliver the genome 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 the infect they will be going to when you use the lentivirus, the lentivirus can be used in two cassette. So 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.

Okay. So that is how you can actually be able to control the activity of some of the infectious viral particles such as the lentiviruses. So, in a transaction, what happened is that you are actually going to use the, you know, 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. Then the phage enzyme are going to break down the host DNA. 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, the phage has injected, it will be a part of the host genome. And that is how once the host is replicating, it is also going to replicate the viral genome. So, this is the different method what we have discussed for delivering the DNA into the host system. Now, in the, what we have discussed, we have discussed about the isolation of the gene.

And we have discussed about the isolation of genes. So, we discussed about the two approaches A and B. One is the genomic library approach and the 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 two, so this is the step one, this is the step, sorry, this is the step one, this is the step two, that same you are going to do for the vector also. And then in this, and then there is a step three, step three, you are going to put the ligation reactions. So, that is also a part of the cloning reactions. And the step four, you are going to deliver the DNA into the host.

So, deliver the delivery of DNA, deliver the DNA into host. Then the step five, you are going to screen these transformed clones. So, you are going to do the screening of transformed clones. And the step six, you are going to check the overexpression. So, so far what we have discussed, we have discussed about the step one, we have discussed about the step three, we discussed about step three and step four.

Now, in the subsequent lecture, we are going to discuss about the step five and six, 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, 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, the screening of the clones and as well as the overexpression. Thank you.
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