Genetic Engineering: Theory and Applications
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Module 5
Recombinant DNA Technology (Part 1)
Lecture 13: DNA delivery in Host-I

Hello everybody. This is Doctor Vishal Trivedi from department of biosciences and bioengineering, IIT Guwahati. And let us, continue our discussion about to, how to generate genetically modified organism. And, in this discussion, in the previous modules- what we have discussed? We have discussed about the host as well as, in the previous module, we have also about the different types of vectors, which people are using for prokaryotic as well as the eukaryotic system.

During that discussion, we have discussed about the plasmids, which are being used in the bacterial expression system. Then, we have used about the yeast vectors, which are being used for over expression of protein in the yeast. And then, we have also discussed about the mammalian expression vectors as well as the macrophage or the baculovirus vectors.

And, during these discussions, what we have discussed? We have discussed about the many features which these vectors are providing to the user. And, based on these features, people can develop different types of strategies to clone the foreign gene into the, into the vectors and then, they can use these vectors for over expressing the foreign genes into the host.

So, what we have discussed from the host is- that how to isolate the gene. And then, what we have discussed from the vector side is-how to process these vectors and to generate the sticky ends.

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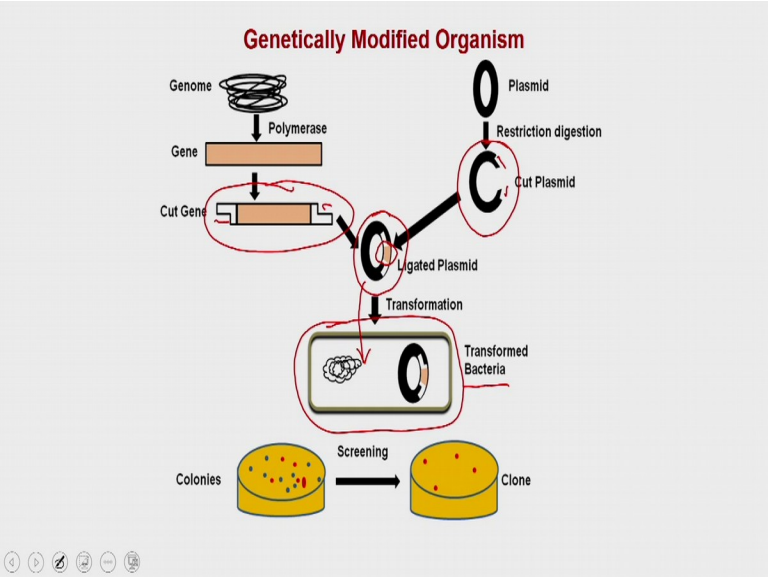

So, you have isolated the gene from vector. You have generated the sticky ends by the help of the restriction enzymes. Similarly, you have isolated the plasmids or the vectors of the different eukaryotic system. You have also generated the similar type sticky ends using the same set of fictitious enzyme. Then, you have put them into the ligation reactions and that is how you have generated a chimeric plasmids or the chimeric DNA.

And, the next step is that you put this chimeric DNA into the host of your choice, either it is the bacteria, which we are depicting in this figure, or it could be the eukaryotic cell, such as yeast or the mammalian cells. Once you insert these into the host cells, then the host cell will take up this particular recombinant DNA and start expressing the, the foreign gene what you have put inside the plasmids.

So, in the today's lecture, what we are going to discuss? We are going to discuss about the different methods of entry of this recombinant DNA into the host species. And when, we will talk about the entry of a foreign DNA into the host. What we have to consider before, to design a delivery method, is that what is the surface chemistry of the host as well as what is the kind of recombinant DNA you are trying to deliver into the host cells.

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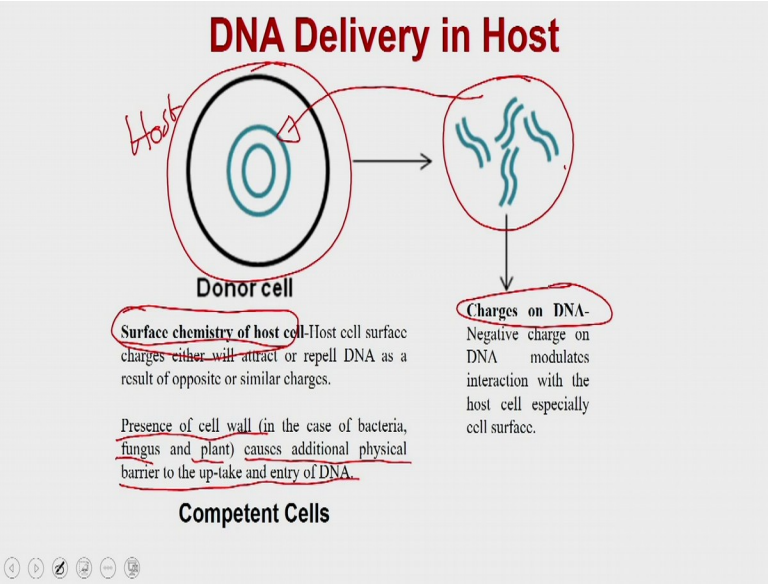

So, as I said you know, you have a host cell which is actually called as the host cell. And, you have the recombinant DNA, which is present in the outside. What is the goal is that, you take this recombinant DNA and put it into the host cells. For that, you have to have the clear knowledge about the surface chemistry of the host cell, because the surface chemistry will allow, or will attract this particular recombinant DNA, for entry into the host cells. And also, the surface chemistry will, will either attract or repel. And accordingly, you have to device or you have to treat the host cells, in such a way, so that it will start express; accepting the, the foreign DNA or the recombinant DNA.

For example, in the case of bacteria you have a very thick cell wall, which is also in the case of yeast as well as the plant. So, when you designed the strategies for these kind of host cells, you have to encounter additional physical barriers, which will going to hinder into the uptake and the entry of DNA.

So, this is the part of the host cells, where you are actually going to work on to the surface chemistry so that, the surface chemistry will facilitate the entry of the recombinant DNA. Whereas on the, on the DNA part the DNA is, as you know, that DNA is negatively charged. So, negatively charged ions are going to… will interact with the host cells. But, you have to modulate these charges also, to facilitate the better entry of the recombinant DNA into the host.

But, the first and the most important question is how the concept of delivery of DNA into the host cell came off? So, this actually came off, from the many observation, where people have found, that the one bacteria or one particular host cell, is exchanging its material, with the, with the neighboring cell. And, with this all, the neighboring organisms, which are falling into the same species and by doing do so, they are actually exchanging the, the good character or they are exchanging the phenotypic; they are also bringing the phenotypic changes into the colony.

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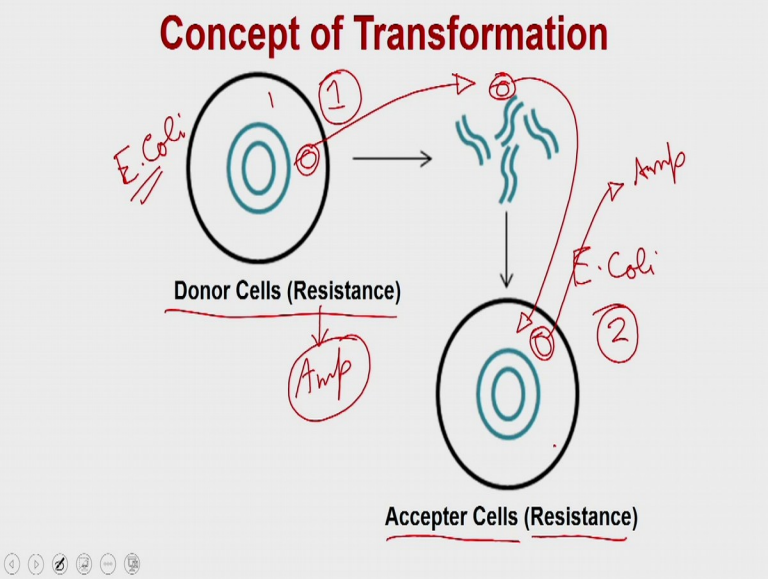

Let us take an example. For example, you have the two bacterial species. One is; this is the first bacteria species, which actually contains a plasmid, which is actually providing the resistance, for the ampicillin, okay, and this particular accepter bacteria, which is actually the bacteria number 2, which is also present in the colony. Both are these bacteria, are belonging to the same species or similar kind of species. For example, this is also E.Coli, and the other one is also E.Coli.

So, whereas it is not very important, that both are belonging to E.Coli. The only thing, which matters actually, between the donor cell as well as accepter cell is that their surface chemistry or the surface chemistry should be identical. And, the DNA what they are going to exchange with them, is going to be compatible.

For example, you might have remember, that we have discussed in the past, about the restriction methylase system. So, the restriction methylase system of this bacteria and the restriction methylase system of this bacteria, should be identical. Otherwise, the DNA what you are putting into the accepter cell, will not going to survive and will be degraded by the restriction methylase system of the accepter DNA, accepter cell.

And that is why, the donor cell and the accepter cell, should be of the similar species, so that their restriction methylase system should be very much compatible with each other. And that is how; they will allow the propagation of the external DNA into the cell. So, you can imagine that, you have two bacteria one is E.Coli, which is actually resistance for ampicillin and this ampicillin resistance could be because it has a plasmid which is having, the ampicillin resistance gene.

So, what will happen is this plasmid, it will going to throw into the environment. And then, this plasmid is going to be taken up by the acceptor cell. Although, the mechanism is not known but that is how; now, this bacteria, which was actually sensitive in the beginning, now it has accepted a resistance, ampicillin resistance plasmid. And now, it is; it has also acquired the resistance for the ampicillin. This is just an example, for the resistance against the antibiotics.

It could be something else also. It could be that, this bacteria has the enormous capacity to withstand the high temperature. And, that high temperature withstands is, because it is actually expressing, some of the unique proteins, which are, which are protecting this bacteria under the high temperature conditions. And, those things also; those kind of factor also, maybe being produced, by the extra chromosomal DNA, which is present in this donor cell. And, by doing so, it actually can confer, this bacteria also to a, to withstand the high temperatures.

So, it is not important that, it is always been, for the, for the antibiotic resistance. Or, it is always for the resistance against, some kind of the external agents. It could be for any kind of feature, for example, this bacteria might be, doing the sporulation better and this bacteria may not be. And, this bacteria will provide the factors, which will facilitate this

kind of additional feature, to the acceptor cell. And that is how, the bacterias are actually exchanging their genetic material between the two cell.

And that is how, it is always been advisable you, when you are working in your laboratory, that you should destroy the bacterial, bacteria what you are using, before you discard them, into the sink. Or, before you discard them for when you throw them into the dustbin. Because, all these bacteria are having, the extra chromosomal DNA, which is like, in the form of plasmids.

And, these bacteria are, which we are actually generating genetically modified, could be potential target or could be potential source of spreading that particular type of phenotype into the, the bacteria which is present in the environment. And, that is why it is advisable, that when you discard any of these recombinant DNA containing bacteria or yeast or mammalian cells, you should destroy them.

And, that process you, what is called as the insulation, which means, you have to insulate your bacterial plates, or you have to de-inactivate the bacteria, which is present in the culture media, before you throw them into the sink, for, so that it will go into the environment. But, it should not contaminate the environment, so that the, other bacteria will take up.

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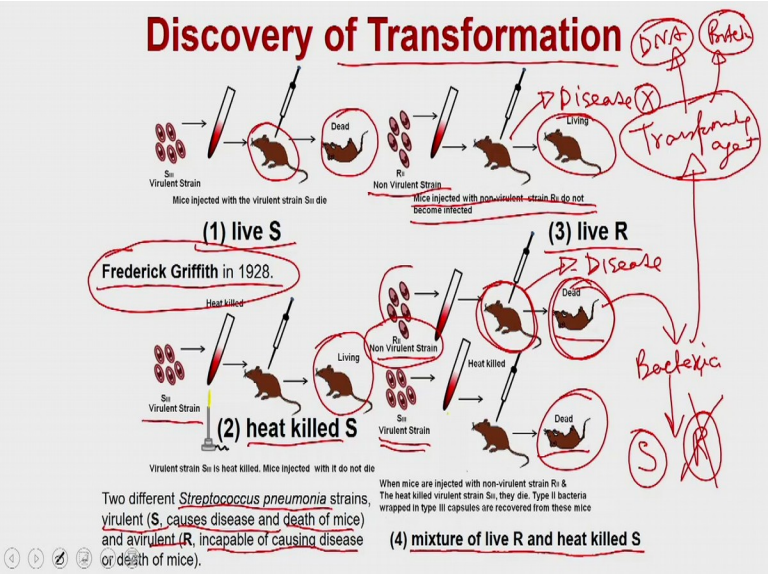

Okay, how this is been discovered is, that there is a history about this. So, this process of this of, this bacteria, receiving the genetic material from the other bacteria, is called as the transformation. And, the process of transformation, is been discovered by the scientist, known as the Frederick Griffith in 1928. What Frederick Griffith is done is, he has taken the four different strains of the bacteria, which is called as the Streptococcus pneumonia. So, Streptococcus pneumonia, he has taken the four strains. One is called the virulent strain or the S-strain.

The virulent strain is causing the disease, and that actually is causing the death of the mice. And then, he has also taken a avirulent strain, avirulent strain or the R-strain. The avirulent strain is incapable of causing the disease or the death of the mice. So, what you can see is that he has done the experiment in four phases. In the first phase, what he has done is- he had taken the live, virulent strain, S-strain. And then, he has injected those live strain in to the mice. And, what you can see is, that mice has developed the disease, and ultimately the mice died.

Now, he has done another experiment. What he has done is, he has taken the same virulent strain, S- strain, and then he has done the heat killed. So, by doing the heat killing, he has inactivated those bacteria. And then, when he has killed, when he has injected those heat killed bacteria, he has; those mice did not developed the disease. And they were actually survived, and they were living.

In the second third, third set of experiment, what he has done is, he has done, he has taken a live avirulent strain or the R-strain. And since, these are the non-virulent strain; it has been injected into the mice. And, what he could find is, that these non-virulent strain, are not developing the disease. And since, they are not developing the disease, these mice are also been survived.

Now, in the fourth set of experiment, what he has done is, he has taken the non-virulent strain. So, non-virulent strains are not killing the mice, or they are not developing the disease. And then, he has mixed the heat killed virulent strain, along with the non-virulent strain.

So, in the fourth set, what he has done is, he has taken a mixture of live avirulent strain and the heat killed virulent strain. And, mixed them together, and injected that to the mice. And, what he could find is, that although he has taken the live non-virulent strain, the mice has developed the disease. And, it has caused the death of that particular mice.

Similarly, so it has caused the death of the disease; the mice. What they have also found is, when we, they have isolated the bacteria from these killed mice, they could not find the non-virulent strain. What they could found is that it actually contains the S-strain instead of R-strain. So, this S-strain was live S-strain, which means the, live non-virulent strain has taken up, the DNA, or the transforming agents, from the virulent strain. And that is how the non-virulent strain becomes the virulent strain. And that is how, it has developed, the disease into the mice. And that is how; it has cause the death of the mice.

So, this factor, what they have made the non-virulent strain to the virulent strain is called as the transforming agent and so, at the time of Griffith's time, in 1928 Griffith has turned these agent, which are converting the non-virulent strain to the virulent strain as the transforming agents. And, he proposed that it could be DNA or the protein. But, later on, people have done the similar kind of experiment and they have figured out, that it is actually the DNA, which is actually being transferred from the virulent strain to the non-virulent strain. And that is how, the mice has developed the disease and he died.

So, by doing this simple experiment, the Frederick Griffith, has postulated the concept of transformations and that is how, he has, he has turned these agents, which are bringing or which are making the non-virulent strain to the virulent strain, as the transforming agents. And, that is how the people have developed the concept, of transformations.

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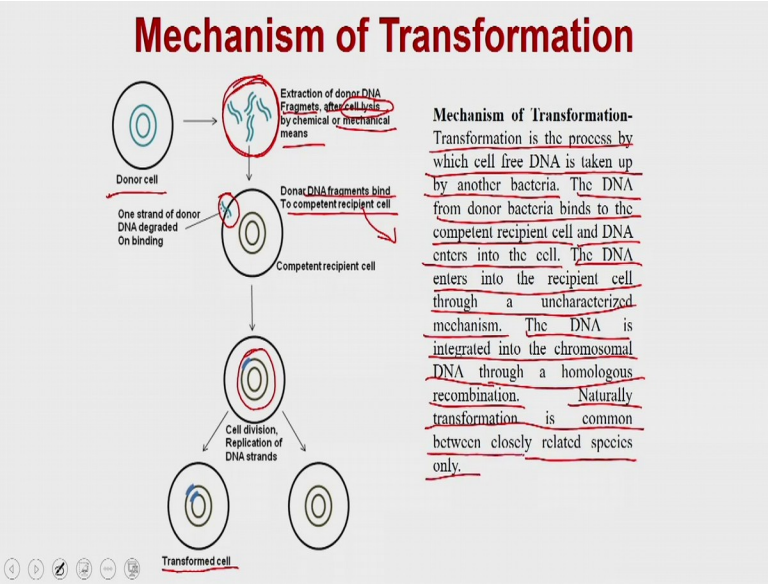

So, in a typical transformation, what is the mechanism of transformation? You have a donor cell, okay. So, donor cell, is actually giving the DNA, or giving the recombinant DNA. This recombinant DNA, are going to the membrane. So, these DNA could be of, fragments or which, which will be coming out, from these donor cells- either by the chemical lyses or by the mechanical lyses or by the cell lyses; either by the chemical or the mechanical means.

So, this DNA is present in the environment. Now, this DNA being; so, one strand of this DNA is going to be degraded. And then, the DNA will be taken up by; will bind into the competent recipients cells. So, we have only discuss about, what could be the competent cells; competent recipient cells. Competent recipient cells are those cells, which are not going to degrade this DNA. And by doing so, this DNA will enter into the, into the competent cells, and then it will integrate, along with the genome.

And, that is how, that the recipient cells also will confer, the similar kind of features, what this DNA is encoding for, and that is how, you are going to get the transformed cells. So, that is, what is given here-that transformation is the process by which the cell free DNA is taken up by another bacteria. The DNA from the donor bacteria binds to the competent recipient cell and the DNA enters into the cell. The DNA enters into the recipient cells through a uncharacterized mechanism.

So, the mechanism by, how this DNA enters into the competent recipient cell, is still not known. And, the DNA which, which is present as a single, single DNA or single strand, is get integrated into the chromosomal DNA of the recipe, recipient bacteria, through a homologous recombinations. And, it is always been observed, that the natural transformation which occurs between the donor as the recipient cells, is always been between the related species.

And, we have already discuss about why it is so; because, the closely related species, are going to have the similar kind of restriction modification system. So, as soon as the DNA will enter into the recipient's cell, it is not going to be degraded. And, that is how; it is going to get a chance, to go to the chromosome. And then, it will integrate into the chromosome by a homologous recombinations.

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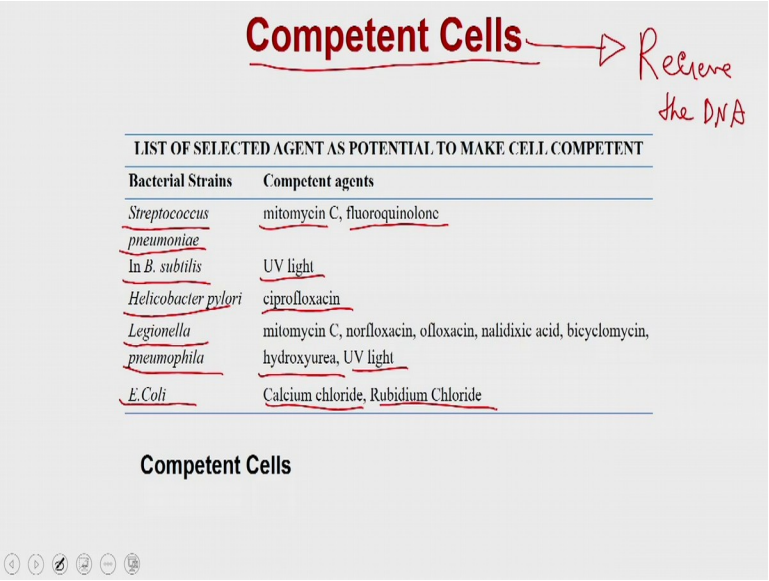

Now, as we discussed in the past, that you have to prepare a, the competent recipient cells. So, what is mean by competent recipient cell is the cell, which is ready to receive the DNA. And, this is always been done, by treating the cell, bacterial cell or treating the bacterial cell, with different types of agents. These agents could be the chemical agents or the physical processes.

So, you can see a small list, where I have enlisted the different type of bacteria. For example, in the case of Streptococcus pneumonia, you have to treat these cells, with the mitomycin C or fluoroquinolones. Similarly, in the bacillus subtilis, you have to treat them, with the UV light, and that actually induces, or that actually makes these cells competent to receive the DNA. Similarly, Helicobacter pylori, the bacteria which is responsible for ulcer, is you have to treat it with the chemical called ciprofloxacin.

And, the other kind of bacteria, and pneumophila, which you have the chemical treatment as well as the physical treatment, such as UV light or hydroxyurea. And, in the case of E.Coli, you can use the calcium chloride, rubidium chloride or the magnesium chloride, for making them competents.

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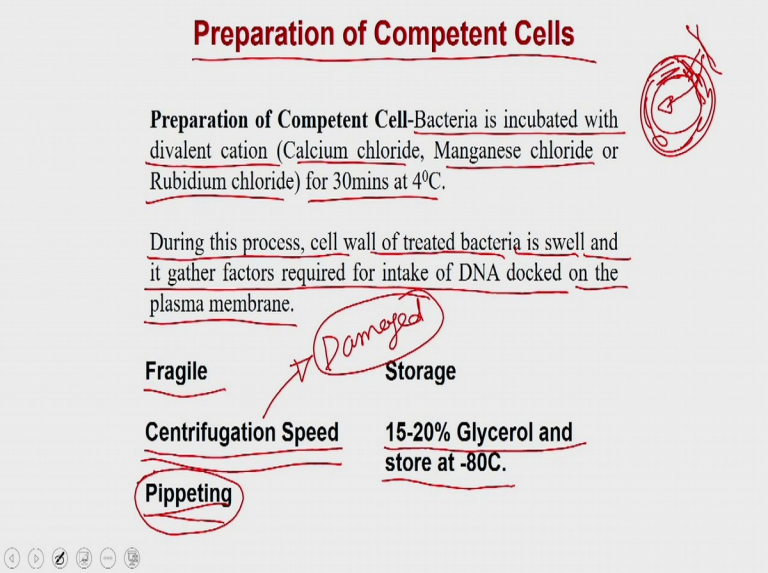

And, so how to prepare a competent cells? Competent cell is very simple. So, in this case, we have taken an example of E.Coli. How you are going to prepare a computer cell is, that you take a bacteria and incubate with the divalent cation, such as calcium chloride, rubidium Chloride for 30 minutes at 4 degree Celsius. What will happen during, when you treat the cells with a divalent cation is, that this treatment is actually making the cell wall to swell.

So, it actually bring the some kind of flexibility, in the cell wall. And, on the other hand, it also gathers the factor, which is required for the intake of DNA, which is docked onto the plasma membrane. So, it actually doing two things. One, it is actually loosening the cell wall, which is present in the outside. So, once the cell wall is getting loose, you have a DNA, which is actually getting a chance to docked onto the plasma membrane.

And, once it is docking, then you have actually, you have put many factors. Or, by doing the treatment with the divalent cation, these factors are being more. So, that is how the DNA, will enter into the bacterial cell. And, but, what will, is important is, that when you are making these E.Coli cells for the competent cells. Or, when you are treating them with the divalent cations, they actually, and as we discussed, the cell wall is going to be loose or swell.

So, because of doing so, it actually becomes very, very fragile. So, you have to take care of these cells, after you make them a competent; very, very sophisticated. Or, you have to treat them very gently, which means, you can not allow to centrifuge these cells, at a very, very high speed. Because, if you centrifuge or if you spin them, at a very, very high speed actually that these cells, will may get damaged. Because, the cell wall is already compromised, okay. So, cell wall is already swollen.

So, that is how, the protection what cell wall provides, for the bacteria to withstand the different types of shear stress and different types of harsh treatment, is already gone. Or, it is actually swelled. So, you are swelled to such an extent, that the plasma membrane, the DNA is getting access to the plasma membrane. So, because of that, if you spin it at very high speed, the high-speed spinning is going to give them, the shear stress. And, that actually will eventually, going to lies the bacteria.

Similarly, when you are prepared these competent cells; these competent cells are going to be pippeted into the small aliquot, because you have to store them, for long term storage. Or for, you have to store them, because you have to use them in a future. So, for that, you have to aliquot them, okay. That pippeting also, has to be done, in a gentle manner, so that; you should not give them the shear stress. The same thing is, centrifugation is also, is going to give them the shear stress. Pippeting is also, going to

give them the shear stress. And, these are the feature, factors which are eventually, going to damage these cells and eventually, going to reduce the ability of these cells to take up DNA.

Once, you prepare these; once, you incubate these cells, with the divalent cations, they become competent. But, you cannot use them, all the cells in a one goal, because you are going to prepare, large quantity of competent cells in single day. So, what you have to do is, you have to store these cells in a buffer, which contains the 15 to 20 percent glycerol. And then, you can store them in a minus 80 degree Celsius. And now, whenever you are required, you can, you can take out these cells, and use them for the transformations.

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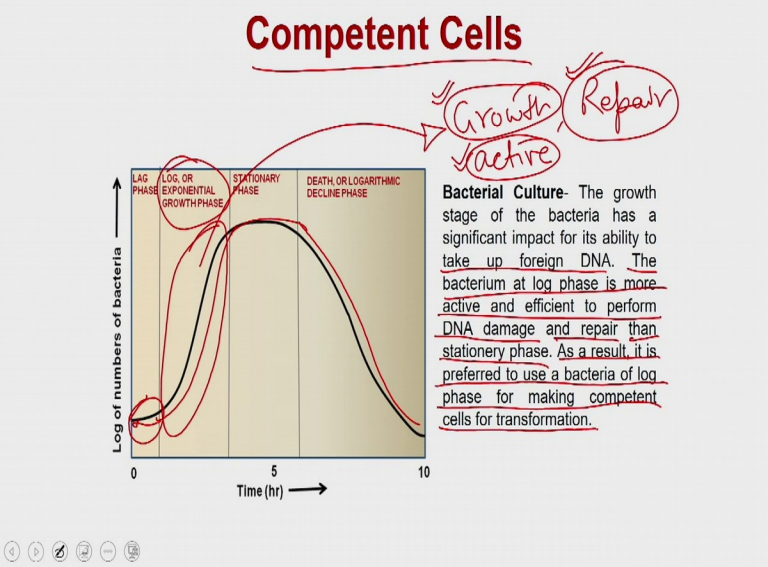

So, while you are preparing the competent cells, you have to be little careful, that what kind of the bacteria you should use. So it is, the ability of a bacteria changes, while it is going through the different phases of the growth, in the media. And that is why, it is important to choose a phase, which is very, very efficient in take up the DNA as well as it is going to give you the better transformation. So, if you see a bacterial growth curve, what you will see is, that you have a lag phase. Then, you have a log phase. Then, you have a stationary phase. And, then you have a death phase.

And, all these phases, we have discussed in a previous lectures and their significance. So, the log phase bacteria, is the bacteria which is actually very active, in terms of metabolism, in terms of growth, in terms of the repair mechanism. So, these bacteria are actually, very good in terms of growth, repair, and they are metabolically very active. So, these are the phase, which you can use, for making the competent cells.

You cannot take the lag phase, because the lag phase bacteria are still under the stress. And, it is actually adjusting to the new media conditions. You cannot take the stationary phase, because these stationary phase bacteria are not growing or not very good in terms of growth as well as the repair mechanisms. Death phase, anyway you cannot take, because those bacteria are not growing and they are in the death phase.

So, the lock phase bacteria is good, because they are showing very high growth. They are metabolically very active. And, the most important thing is, that if you give them some kind of stress or some kind of damages, they will be very good in terms of their repair mechanism. And that is why; they are very good in take, in terms of taking up the foreign DNA. The bacteria in lock phase, is more active and efficient to perform the DNA damage and repair than a stationary phase bacteria. As a result, it is preferred to use a bacteria of lock for making a competent cell for transformation.

And if you remember, when we were discussing about the bacterial growth kinetics, we have said, that you can actually use the different types of growth measurement, measuring methods, to know when the bacteria will be in the lock phase. And that is how; you can choose that particular time point or that particular growth period. And, that is how, you can choose the bacteria, for the making the competent cells.

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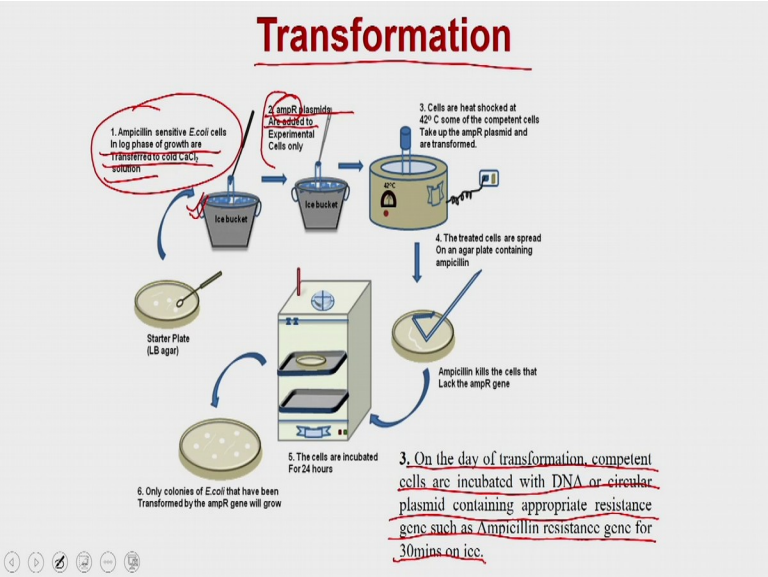

So, transformation is a multi step process. In the, and in the first step, we have already discussed, that you actually will take up the log phase of bacteria. And, transfer to, and added to the cold calcium chloride solution, into a ice pack. And, in the second step, what you are going to do is, once you are ready to take up, once you are ready to do the transformations; so the step 2 onwards, whatever you do is. So, in the step 1, you are going to make the competent cells. And then, you store them in minus 80, under the 15 to 20 percent glycerol.

On the day of transformation, what you are going to do is, you take out one vial, and put it into the ice buckets. And then, you are going to incubate the plasmid, which you want to transform. So, in this case we have taken a plasmid, which is containing the ampicillin resistance. And, then you incubate that with the, with that particular plasmids in ice, for 30 minutes. So, on the day of transformation, the competent cells are incubated with DNA or the circular plasmids, containing resistance genes, such as ampicillin, for 30 minutes on ice.

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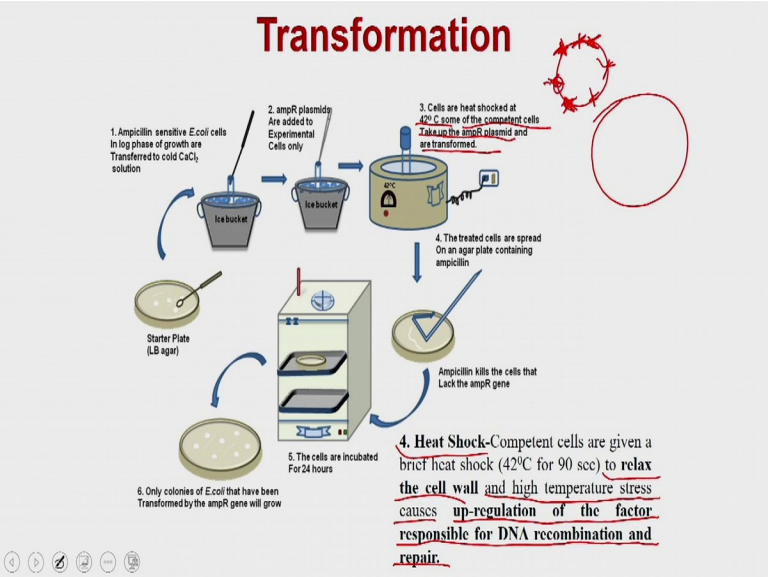

Then, what you do is, you go to the next step and that is called as the heat shock. So, what you do is, then you will give a heat shock, at 42 degree Celsius for 90 seconds. And, that actually will induce the uptake of the plasmid. And, what happen when you do the heat shock is, that it actually relax the cell wall. So, it actually loosens the cell wall further. And, because you are putting, giving a high temperature stress, that actually up-regulates the different types of factors, which are responsible for DNA recombination and repair.

So, you are actually going to give them, a very short period heat shock. And, because of that, the bacteria will respond. The first thing, what will happen is, the cell wall is going to be further relaxed. And, that actually will allow the plasmid to interact with the plasma membrane. In the, in the heat shock process, sometime the plasma membrane, also becomes slightly leaky, or slightly compromised. And, that is how, the plasmid which are been present on to the plasma membrane, they get internalized into the cytosol.

On the other hand, it actually up-regulates; because heat shock is giving some kind of stress. And, these stress, are always been associated with the downstream signaling processes. And, that actually up-regulates many type of factor, to take care of this particular stress. And, these factors are required for DNA recombination, as well as for repair mechanism. Because, you can imagine that once, you have created the, you know,

some kind of compromised cell wall, you also have to seal these things. You have to repair these, so that, the bacteria is going to close these pores or close these compromised cell wall as well as compromised plasma membrane.

Otherwise, the cytosol will leak out from those pores; those places. So, as soon as you have done the heat shock, you are going to start, these cascades of events. And, that actually, will up-regulate different types of factors. So, that these damages which you have done, into the bacterial cell will going to be repaired. And, it also going to induce, the a DNA recombination. So, that once the plasmid is inside, it has a high chances of getting integrated into the genome.

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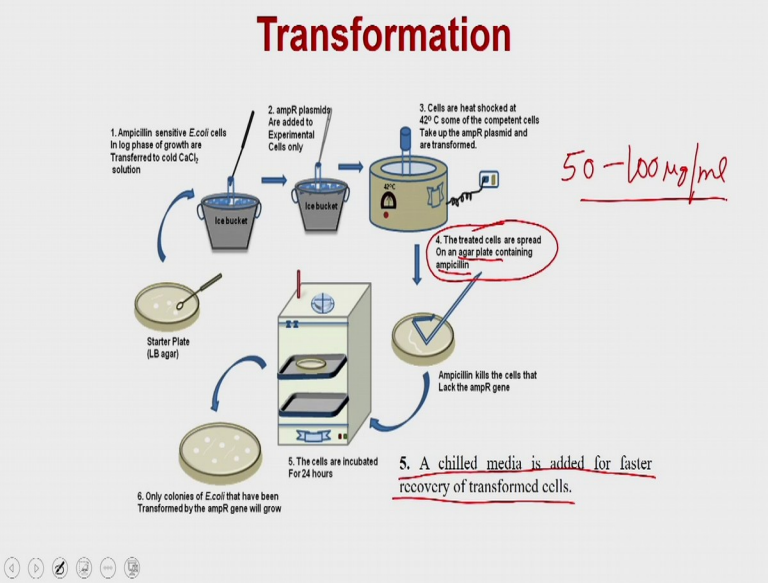

Now, after the heat shock, what you are going to do; because you do not want the; because if you keep the heat shock very long, this stress bacteria cannot survive, cannot sustain. And, then the bacteria is going to die. So, you have to reverse the things. So, you have made cell wall leaky. You have made the plasma membrane compromised, plus plasmid has gone inside. Then, what you have to do is, you have to reverse the things, by adding a chilled media.

And, once you add the chilled media that actually is going to make, the recovery of bacteria very fast. And, then that process, all those pores are going to be sealed. And, that

is how, the plasmid which is present inside, is going to be remain intact, or remain inside. Then, what you do it is, you, you take these cells and plate it onto a agar plate, containing the appropriate ampicillin concentration. Normally, we use, somewhere around 50 to 100 microgram ampicillin per ml. So, that you have to use onto this plate.

And, then you use a spreader and you spread those cells, or spread the competent cells on to this place. If, these cells have taken up the bacteria, they will acquire the resistance against the ampicillin. And, that is how, they are going and then, you incubate them in 37 degree Celsius, into the incubator. And, that is how; they are going to give you the colonies. The bacteria will grow and it will give you the colonies.

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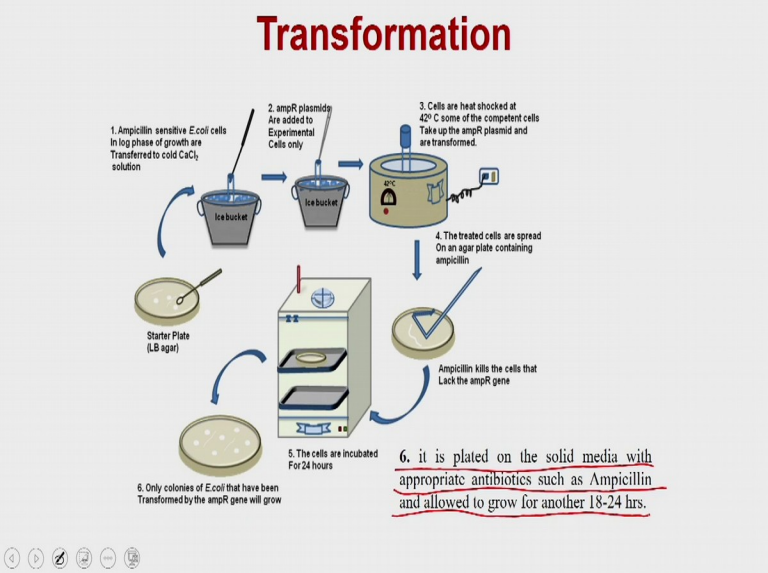

So, in the last step, you are going to plate the transform cells onto the solid media with appropriate antibiotic, such as ampicillin, in this case. And then, you allow them to grow for 18 to 24 hours. And, that is good enough for the bacteria to recover from the stresses, recover from the damages, and then it will start growing and give you the colony.

And, for practical purposes, we have also going to show you, a small movie clip from my lab.

Hello everyone. In this video, we will show you, how to prepare competent cells and transform the plasmid, and plate the cells. So, let us start the procedure. Before, before

preparing the comp-cells, we need some inoculum. This is the, DHI vial inoculum. I am going to inoculate in a new vial, for comp-cell preparation. So, I will show you, how to inoculate.

So, now I am going to inoculate the culture. Let me incubate in a shaking incubator, to get (34:22) that is the log phase of bacteria, where you can prepare the comp-cells. That is the good stage to transform (34:31).

After inoculation, now we have to keep it, in the incubator shaker, till we get required body. So, it should be 37 degree Celsius and 150 ampere. Now, you can see some growth in here. Now, we have to check what is the value of this culture. We can measure it in spectrophotometer at 600 nano meter. How to take out 1 ml from this culture. And, (36:32) the media. And the value will be observance.

I am going to spectrophotometer to measure, what is the value of the bacteria. Now, before what we will do is the, I have to blanch at NH3. So, this is the plate in the. I am going to keep it and set as a zero. Now, it is zero. Now, this is our inoculated culture. And, now I put this, in the sampling holder. Then, start. So, that value is 0.466. It is the exception for our comp-cells preparation. So, we use this culture for comp-cell preparation and transfer.

So, we got sufficient body. Next we have to, centrifuge the culture and palette out the cells. After that, we will prepare comp-cell using point 1 calcium chloride. So, I am going to transfer into this centrifuge tube, and centrifuge it to get the. Now, I am going to centrifuge the culture at 4000 rpm for 10 minutes. So, in order to get, cell palette.

After mixing the competent cells, cell palette, we find 1 molar calcium chloride. You have to keep the cell suspension for half an hour. And, then in subsequent steps, you have to centrifuge and wash with the 0.1 molar calcium chloride for another two times. After final step, we have to add 0.1 molar calcium chloride, 2-3 ml. We suspend gently and I put into pick and drop tubes for storage.

We have blanched the, in final step. So, we have to mix this cell palette, into 0.1 molar calcium chloride. And, we will put into (41:49). Here, I put in 100 ml (42:56). Now we

have to store these comp-cells, in minus 80 degree Celsius till 5 more minutes. Any mechanical disturbances at this time will subsequently disturb the comp-cells. So, there is no transformation.

We have to add plasmids in aseptic conditions. So, you get this comp-cells and plasmid DNA inside. So, now I am going to add plasmid DNA. We kept that competent cells with plasmid DNA, for 30 minutes in ice. Now, we will give heat-shock at 42 degree Celsius.

We gave heat-shock to the competent cells at 42 degree Celsius. And, we have to keep, 10 minutes on ice. Then, we will add, AFB media, cold ABF media. And, the keep it in a incubator shaker. I am going to add AFB media to comp-cell.

After adding media, to the transformed cells, we will keep in incubator shaker, at 37 degree Celsius, with a rotational speed of 150 rpm.

We just transformed the plasmid DNA into comp-cell. But, during this procedure, we have to be cautious. Because, any mechanical disturbance to the comp-cell, will lead to, decrease in transformation efficiency. So, while handling the comp-cell, we are not going to keep outside, like normal temperature. Always you keep comp-cell on an ice. So, otherwise the transformation efficiency will go down.

And, another thing is that, we should not add plasmid, in normal environment. We have always, keep in aseptic condition. And, also during transformation. Now, we got sufficient growth for transformation. We will centrifuge these cells, and plate on solid.

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After centrifugation, we will remove all the media. But, we will keep 100 micro litre, re-suspend the pin let. And, take 20 micro litre and plate on, other plate. But, if you are expecting low transformation efficiency, you can use all 100 micro litre, for plating. This is the best way, to increase chances of transformation.

We got a pin let. And, now we will take out the media, resuspend and re-plate. Now, we have plated the cells. We will keep in the incubator, for overnight. And, we will observe, how many colonies. Now, we can see, there is colonies appeared on the transformed plate but there is no colonies, on only comp-cell plated one. So, you can see colonies here.

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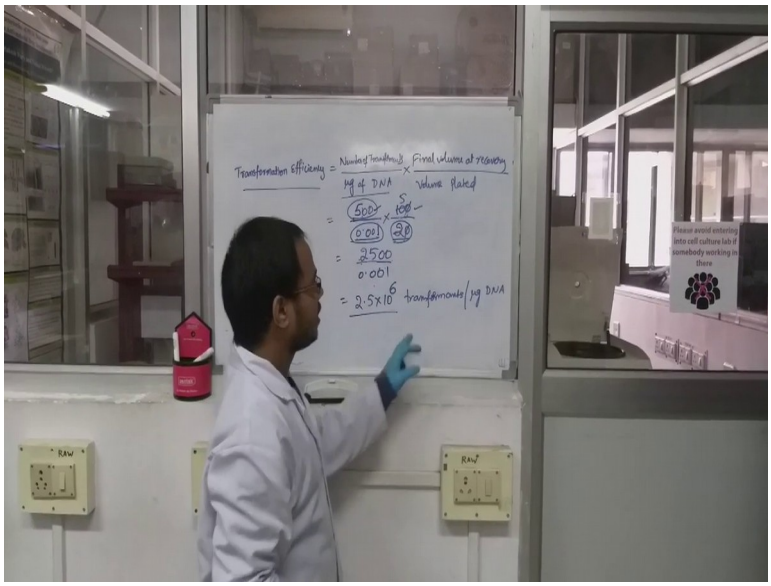

Now, we will calculate the transformation efficiency. So, there is a formula for transformation efficiency- number of colonies you got on that plate, and that microgram of DNA you used, multiplied by final volume at recovery. How much volume you recovered, that is 100 micro litre, for preparation your zest. And, how much volume plated.

So, suppose you got 500 colonies on that plate. And, you used point 001 microgram of DNA into 100 micro litre is total recovery. But, you plated only 20 micro litre. So, that means, you 2500 divided by 0.001. So, that will give you, 2.5 into 10 power 6, transformates per micro gram of DNA.

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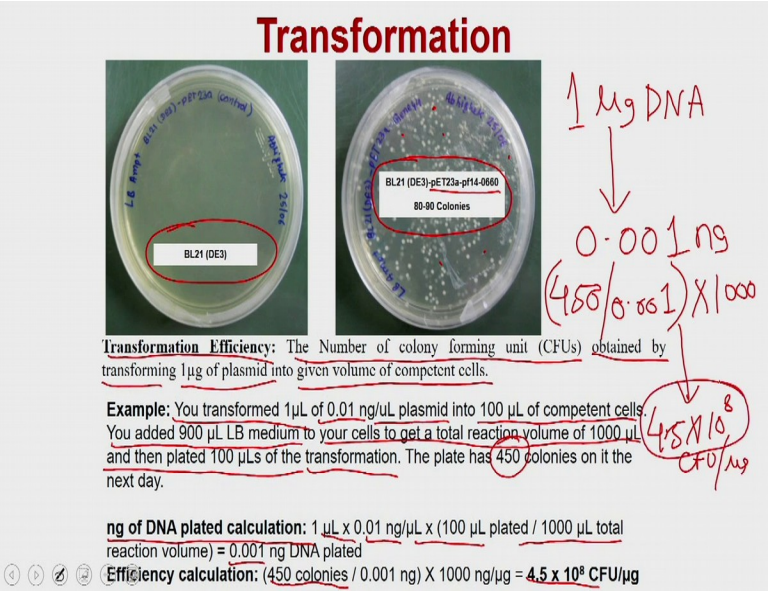

And, this is what you are going to see, when you are going to do a transformation, you are also going to do a control transformations, where you are only going to take the competent cells alone. So, if you if you only plate the competent cells alone, without the plasmid, you are not going to see any colonies. Whereas, if you take the competent cells. for example, in this case we have taken a E.Coli cell, which is called as BL21(DE3) and then, we have transformed a recombinant DNA, which is called as pet 23a-pf14 underscore 06060. And, that has given the bacterial colonies, like the white color spots, what you see is all the bacterial colonies.

And, if you count these colonies, you could be able to calculate, the transformation efficiency or the efficiency of this particular processes. And, this is called as the transformation efficiency. So, what is mean by the transformation efficiency, is that the number of colony forming unit, is number of colonies what are being formed, obtained by transforming 1 microgram of plasmid, into the given volume of competent cells; which means, the number of colonies what you are getting, if you are doing a 1 microgram of transformations into the cell. And, that is called as the transformation efficiency.

Let us take you, a simple data, how you can calculate the transformation efficiency from this after your transformation because, that is very important that, that you are using a

competent cell, which is of a very high confirmation efficiency. So, let us see, that you have transformed, for example, you have transformed 1 micro liter of 0.01 nano gram per micro liter plasmid, into a hundred micro litre of competent cells, okay.

So, this is what you have done. And then, after the heat shock, you have added 900 micro liters of chilled LB media, to these cells. And, the total reaction volume is going to be 1000 micro liters, okay. Now, from this 1000 micro liters, you have taken only 100 micro litre, and that you have plated onto the cell. By doing though, the plate, then you have plate, then you have taken the plate, and put it into the colony counter.

If you remember, we have discuss about the colony counter, even when we were talking about the microbial growth kinetics, and the number of colonies what you got in the colony counter, is 450 colonies, okay. So now, let us see, how to calculate the transformation efficiency.

So, the nanograms of DNA, what you have plated is 1 micro liter into point 01 nanograms, into 100 micro litre, into divided by 1000, okay. Which means, you have actually play, you have transformed point 001 nanogram of DNA. Which is very, very small DNA, into the, into the competent cells. And, the number of colonies, what you got is, 450 colonies, okay. So, 450 now divide that by this number, point 001, okay. And, the number what you are going to get, then you convert that into the microgram.

So, to converting that into the microgram, you have to multiply this number by 1000. And, the number what you are going to get, is 4.5 into 10 to power 8 CFU per microgram, which means, this is 4.5 into 10 to 8, is the transformation efficiency of this particular competent cell preparation, what you have prepared, okay.

But, the transformation efficiency is going to vary, from batch to batch, from cell to cell. And for, and there are many factors, which are affecting your transformation efficiency. It is not important, that you are going to get the transformation efficiency, every time identical.

That is why, it is important, that whenever you prepare the competent cells, in your lab, for any kind of molecular biology or a recombinant DNA technology applications, from

the batch, you should take out one vial, and calculate the transformation efficiency. So, that when you are going to do the transformation of the ligation products, you should be able to, you should be sure that the, you are using the transformation, you are using the competent cells, which are of the very high efficiency.

Because, when you do the ligation products, you may not be even ligating 1 nanograms or less than 1 nanogram DNA. And, in that case, the transformation efficiency of, 8 10 power 8 to 10 power 10 CFUs per microgram, is good enough to give you few colonies of recombinant DNA.

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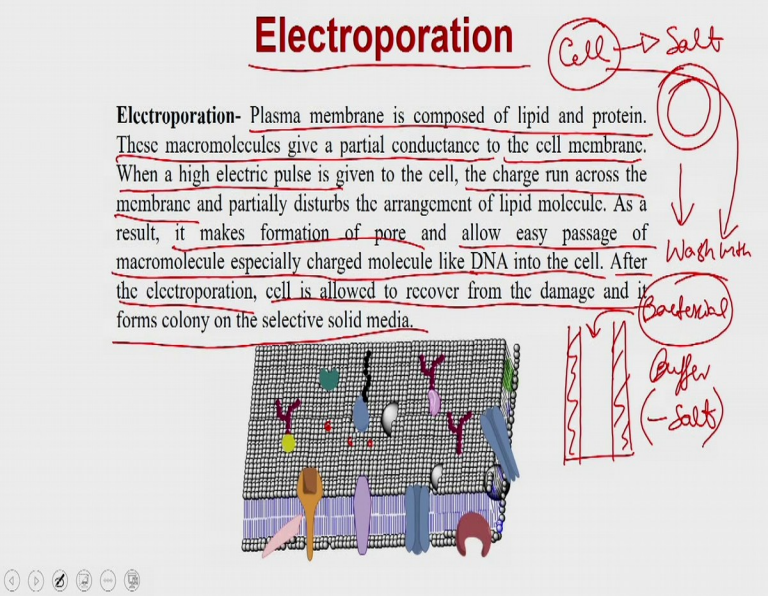

So, apart from the, the transformation by the chemical method, you can also use the electroporation. The electroporation is, is a simple method, where you know that the plasma membrane is composed of lipids and proteins. And, these macromolecules give the charge onto the surface, or charge to the plasma membrane or the cell membrane. So, if you provide a very high electric pulse, if you provide a very high electric pulse to the cell, the charged actually runs across the member.

And because, the charge is running and the you have a partial charge present on the membrane that actually disturbs the arrangement of the charge onto the membrane. And, that actually brings lot of force, onto the membrane. And, that formation of the pore onto

the membrane, allow the easy passage of the macromolecule, especially the charge molecules like DNA into the cell.

So, what you are doing is. This is a plasma membrane, what you have, and when you are putting, so this is actually having the protein and lipids. And the protein, because the protein and lipid, it has some charge onto the membrane. But, when you provide the charge to this, it actually swells or it actually provides the some kind of force. So, you can imagine that, some pores is going to be formed. And those pores, are good enough for the entry of the DNA, especially the charged particles.

And, after the electroporation, the cell is allowed to recover from the damage, and it will form the colony simply like the, what we have discussed in the case of transformations. So, in a typical electroporation you, what you have to have is, you have to provide the electroporation quibit. And, the typical electroporation qubit, what you see is look like as the, a glass qubits, okay. And, in a glass quibit, what you have is, like the metal plates on both sides, okay. And, you will provide the bacterial cell here, between these, between the plates. But, the processing of the bacterial plate is going to be different from the processing what we have done for the competent cells by the chemical method.

In these cases, as we said is, that we are looking for the disturbance of the charge, which is present on the plasma membrane. That is why, when you do a preparation of competent cells for the electro operations the cell should be of, free of salts. So, what you are going to do is, that you take up the cell. You take these cells and wash with buffer, without salt. So, that is the condition, that you wash these E.Coli or some bacterial cell, with a buffer which does not contain the salts.

And, by doing so, what you are going to do is, you are actually removing the cationic as well as the anionic ions, which are present onto the, either onto the cell wall or onto plasma membrane. So that, you are not, these ions should not participate into conductance of electric fields or whatever you are going to provide. Because, that is not going to be, we want to disturb.

What we want to disturb, is the charge, which is present on the, present on the plasma membrane and which is being impart by the lipids as well as the protein. Because, if you allow these small ions to be present, then these small ions are going to take up all the charge, and they will not going to pass on these charge onto the plasma membrane. And, as a result, there will be no expansion of plasma membrane. Or, there will be no pore formations.

So, you have to wash these cells, until they are free of salts. And then, these cells are ready for transformation. Then, what you have to do is, you have to open these qubits. And then, you have to put your cells, between these two electrodes. And then, you have to put it into a electroporators. These electroporators are the specialized instruments, which actually give the current, from one electrode to another electrode, for a very small period of time. And that is how; they are actually going to give you the charge onto the bacterial cells.

And, that is how, they are going to create the pores. And, once the pores are been created, that will take up the DNA. And, for practical purposes, we have also, going to show you a small, movie clip from my lab. How does students are performing the transformation into the E.Coli.

In previous video, we shown that, how to transform plasmid DNA, using calcium chloride method. In this one, we will show, how to use a electroporator for transforming DNA. So, this is the quibit, we use for electroporation. So, there are two electrodes placed and this is the shock part. So, we will connect here like this, and all the adjustments- pulse, how much time we have to give the pulse, all these things, we can set here. So, once the setting is over, we can keep cells, along with our plasmid DNA inside.

And, then hold and we will press. So, what are the pulse generated? We have given the pulse, that is generated inside. And, through the electrodes, through the electrodes, it will passes through, for a minute fraction of time. Because, of this electric pulse, the pores of, there are small pores found in bacteria. If any plasmid, adjust into that cell happens, then it will enter inside the cell, and quickly the pores are sealed. Those cells we will use for plating.

So, this is all about the electroporation, the transformations by the electroporation, as well as the transformation by the chemical methods. Both of these methods have different types of transformation efficiency. And, the transformation efficiency, whether it is by the chemical method or the electroporation method, depend mostly onto the different factors, which we are going to discuss in the next slide.

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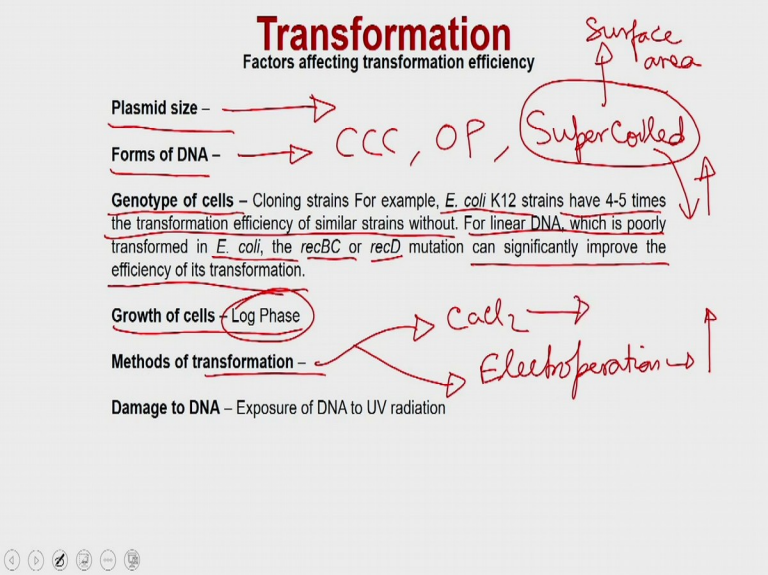

So, one of the crucial point is, that the size of the plasmids. So, as the size of the plasmid will go up, the transformation efficiency will go down. So, up to a certain size, the transformation efficiency is unaffected. But, if you take a plasmid of 10 KB or even more than, then the transformation efficiency will, will drastically going to go down. Then, the form of DNA. If you remember, we have discussed, we have three different forms of DNA closed circular DNA, the open circular DNA and the super coiled DNA.

The super coiled DNA, is having the least surface area, or it actually the, the most compact DNA. And, that is why, the transformation efficiency is very high, for the super coiled DNA compared to the closed circular DNA or an open circular DNA. Then, there are the different; there are bacterial strains, which are competent enough to take up the bacteria, competent enough to take up the particular type of plasmid.

For example, in the case of E.Coli K12 strain. It has the 4 to 5 times more efficiency, than to the similar strain, without some kind of genetic background. For example, for linear DNA, which is poorly transformed. So, compared to the closed circular DNA you, the linear DNA is not getting transformed very efficiently in E.Coli. But, if you have the E.Coli strain, which have the mutations for these two genes- recB C or recD, then it actually improves the transformation efficiency very significantly.

Then, this is already we have discussed. If you are preparing a competent cells, you always have to try to use the log phase cells. Then, the methods of transformations. You have what we have discussed? We have to discuss about the calcium chloride method, and we have discussed, about the electroporation.

So, electroporation is, if you electroporation, it will give you more efficieny compared to the calcium chloride method. The only difference, is that calcium chloride method does not require, a very high end and costly electroporators. And, it also does not require, the transformation quibit. But in the case of electroporation, you require these materials to perform the transformations. But, the transformation efficiency, what you are going to get, in the case of electroporation is going to be way more compared to the calcium chloride method.

Then, the damage to DNA. If, you expose the DNA to UV, it actually going to generate the different types of damages into the DNA. And these DNA, damaged DNA, is having the very high efficiency or a very high transformation efficiency. So, with this we have discussed about the transformation of DNA into the bacteria.

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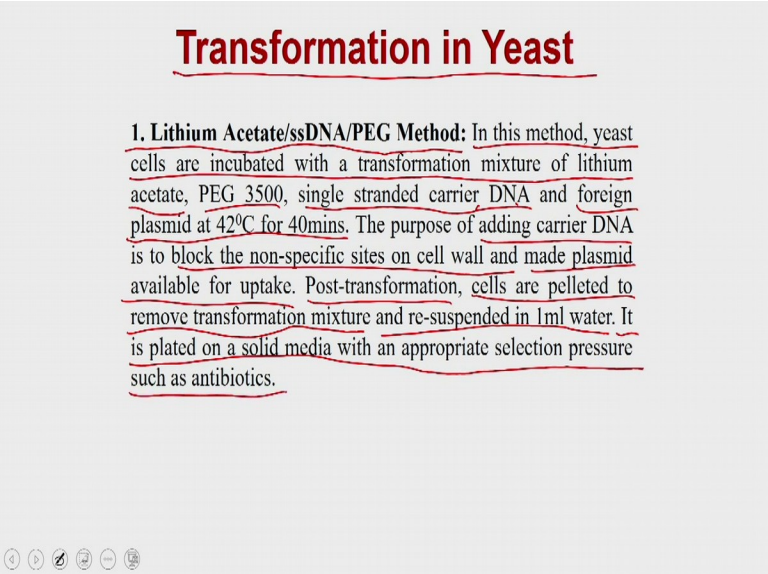

Now, we are going to start with the transformation in yeast. So, yeast you know is much more, is a eukaryotic cell. And, yeast is much more complicated compared to the bacterial cell. The bacterial cell, in the bacterial cell, you only have the cell wall and then the plasma membrane. Once that, you insert the DNA inside the plasma membrane, then your job is done, whereas in the case of yeast- it is very, very complicated, because you also, you have a cell wall at the first place. Then, you also have the plasma membrane. And, inside the plasma membrane, you also have the nucleus.

And, a transformation will be considered only, when you take up the DNA and put it inside the nucleus, so that it will integrate into the genome and that is how, it is going to express, along with that genome, along with the other genes, which are present in the genome.

So, there are many methods what people are using. One of the, one of the classical method is called Lithium Acetate Peg method. And in this method, the yeast cells are incubated with their transformating agent, which is called Lithium Acetate, Peg 3500 and single stranded carrier DNA. So, this single-stranded carrier DNA is required, so that it actually will block, the non specific sites on the cell wall, and made the plasmid available for uptake.

So, which means, that you adding the carrier DNA, simply because, you do not want, your foreign DNA or the foreign plasmids, what you are adding for the transformation, should be consumed. So, what you do is, you incubate the yeast cells into a transformation mixture, which contains Lithium Acetate, Peg 3500, single stranded carrier DNA, foreign DNA, at 42 degree Celsius for 40 minutes, okay. And, that actually will allow, the uptake of these plasmids into the cell.

And, then once transformation is over, the cells are paletted, to remove the transformation mixture and resuspended in water. It is plated onto the solid media, with the appropriate selection pressure such as antibiotics, or some other kinds of selection pressure, which you, according to the plasmid what you have using for, for the, after the transformations.

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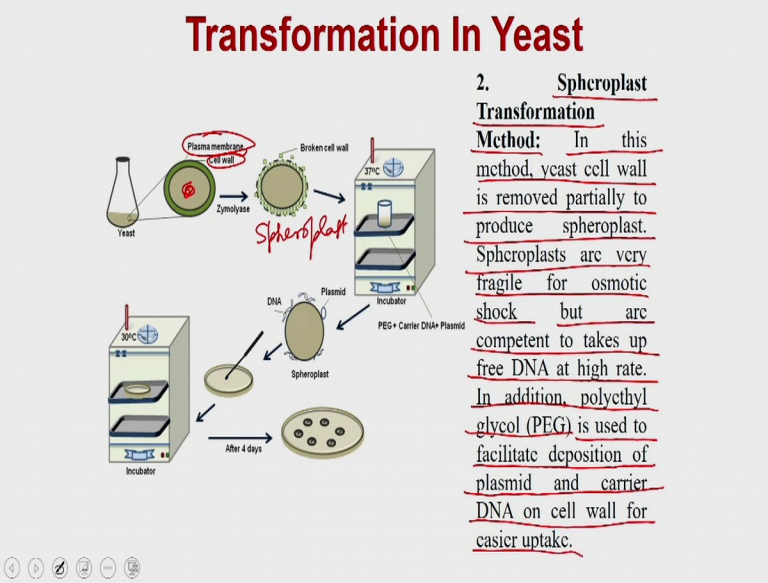

Now, the next method is called as the, the Spheroplast transformation methods. So, as we discussed, the yeast have the cell wall. And, then it has a plasma membrane. Below that, you, it has a nucleus also. So, in this method, the cell wall is removed partially, to produce spheroplast. So, when you remove the cell wall, by the treatments it is actually going to create a, some, some kind of product. And, that is called as the spheroplast.

Once the spheroplast, spheroplast as we discussed, once you do any such kind of treatment, and you remove the cell wall, the remaining cells is going to be very, very

fragile. And that is why, it is very, very fragile for the osmotic shock, but these cells are ready to take up the, free DNA at a very high rate. In addition, you also adding the polyethylene glycol. Polyethylene glycol, is a, is a substance, which actually allows the, facilitate the entry of DNA as well as the carrier into the, into the cell. And, it allows the easier uptake of these products.

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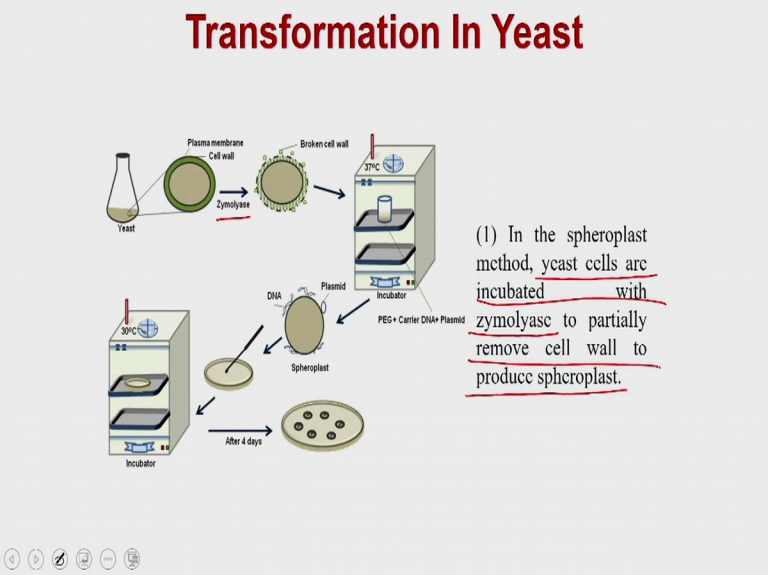

So, how to do these transformations? In the first step, what you are going to generate? You are going to produce the spheroplast. So, what you do is, so first grow the yeast cell into the appropriate culture media. The yeast media we have discussed YPD media, and other kind of media, what we have discussed in the past. So, you first, grow the yeast in those media. Then, you did, what you do is, you incubate the yeast cells with an enzyme, known as zymolase. So, when you treat them with the zymolase, zymolase is going to remove the cell wall components. And that is how, it is actually going to create the, partially degraded cell wall and it will going to produce the spheroplasts.

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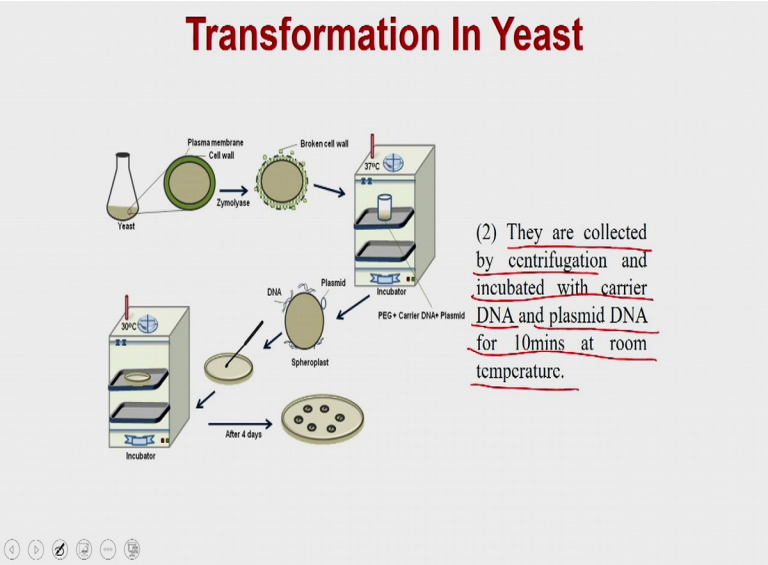

Once the spheroplasts are been generated, then what you do is, you incubate them, and you collect these spheroplasts by centrifugations. And, then you incubate these spheroplasts with carrier DNA. So, that you will block the sites on the cell wall as well as well on the plasma membrane, and as well with the plasmids for 10 minutes at room temperature; that you will do in the incubator.

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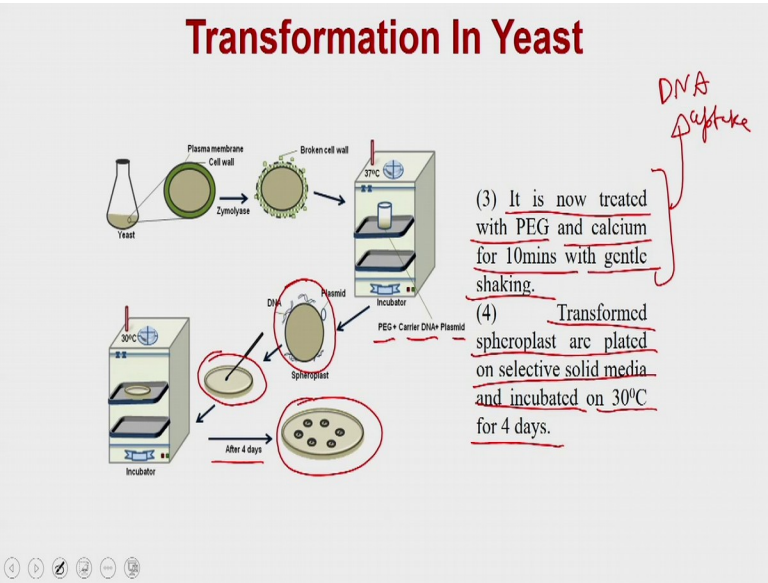

Once you have done that, then in the third step, now you treat this particular mixture with a peg and the calcium, for 10 minutes with gentle shaking. And, this treatment actually, will allow the uptake of DNA.

Now your spheroplasts are transformed, okay. And now, you what you do is, you put it them, into the solid media and incubate on 30, 30 degree Celsius, for 4 days. So, now the spheroplasts are being transformed, along with the peg, carrier, DNA and plasmids. Then, you transform, then you plate them plate them, onto a appropriate plates, which are containing the selection pressures. So, spheroplasts will grow, to these plates. And, you grow them into the 30 degree Celsius, for 4 days. Once you are grow them for 4 days, you are going to get the colonies.

So, so far, what we have discussed? We have discussed about the entry of the DNA into the prokaryotic system as well as the eukaryotic system. In the eukaryotic system, we have taken only the examples of the yeast. And, in the, in the subsequent lecture, we are also going to discuss, about entry of DNA into the higher eukaryotic cells, such as the mammalian cells. How you, what are the different strategies, you have to use for getting the DNA, into the mammalian cells. And, with this we would like to conclude our lecture here. And, in the next lecture, we are going to discuss about the entry of DNA, into the higher eukaryotes, such as the mammalian cells. Thank you.