Enzyme Science and Technology

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Module - IV
Enzyme Production (Part 2: Over-expression)
Lecture - 17
DNA Delivery in host (Part-I)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwahati and what we were discussing? We were discussing about the different properties of the enzyme in the course enzyme science and technology. So, far what we have discussed?

We have discussed about the development of the history of the enzyme, history of the enzymology, then subsequent to that in the module 2, we have discussed about the structure of the enzyme. So, while we were discussing about the structure of the enzyme, we discussed about the primary structure, secondary structure, tertiary structure and quaternary structures.

And we have discussed several techniques which you can actually be able to use for you know determining the these structures of the enzyme. In the previous module, we have discussed about how you can be able to isolate a gene from the genome, whether you are aware of the genomic sequences or whether you are unaware of the genomic sequences, you can be able to use multiple approaches to isolate the gene of your interest which is going to code for the enzyme of your interest.

And then once you have got the fragment, we have also discussed in the previous lecture how you can be able to clone that fragment into the vector of your choice. Now, once you have cloned the fragment of your choice into the vector, the vector has to be delivered into the suitable host. So, in the today's lecture, we are going to discuss about the various strategies what you can actually be able to use for delivering the DNA into the suitable host.

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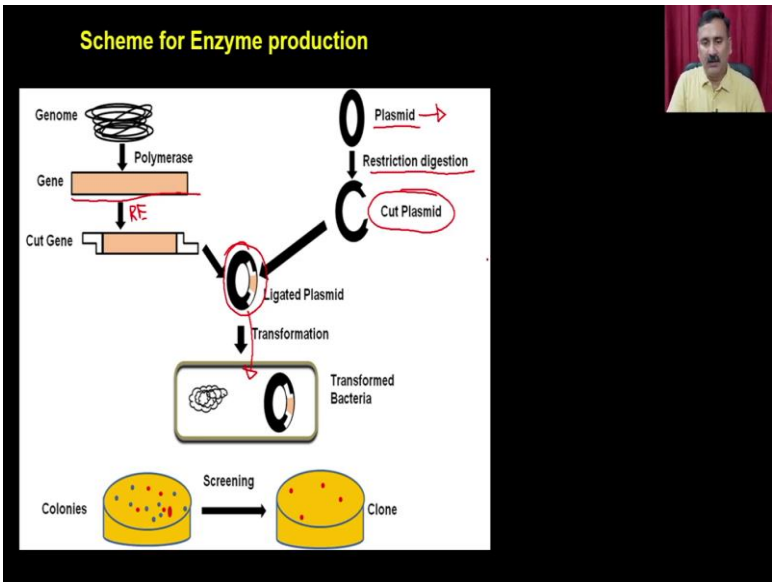

So, what you can see is, this is the scheme for enzyme production right and in this scheme, we have already discussed many aspect of this scheme where we have said that the you are going to isolate the gene fragment from genome, right either you are aware of the genomic sequences or you are unaware of the genomic sequences. This means, either you will use the genomic library approach or the CDNA approach or you are going to use the PCR.

Once you have these genomic sequences, then you are going to perform the restriction digestion of this particular sequence and that is how you are going to get the sticky ends. Same you are going to do for the vectors, right. In this case, we have taken an example of the plasmid which is the bacterial vector.

And then you are going to put the restriction digestion and you are going to have the sticky ends of the plasmid, then you are going to put them for the ligation reaction and that is how you are going to get the recombinant clone. This recombinant clone is has to be delivered into the suitable host. So, that you can be able to use that for protein production. So, when we talk about the DNA delivery in host, we have to understand the many aspects of this particular process.

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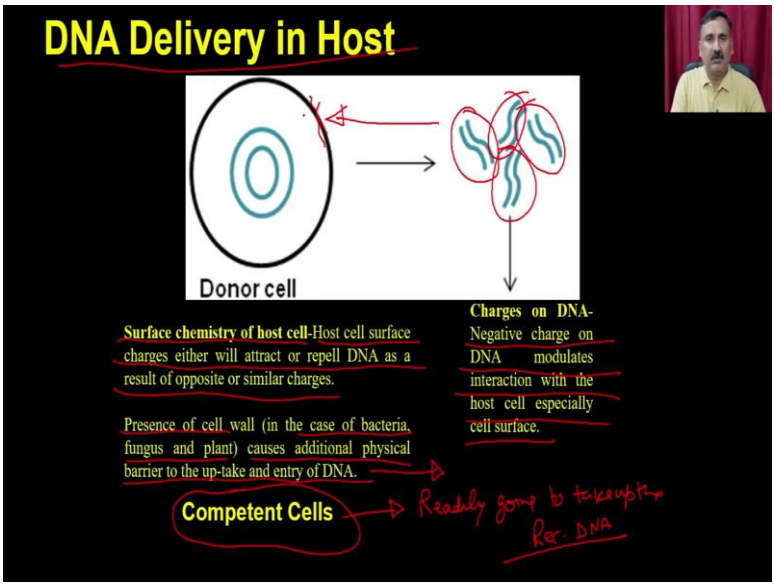

So, DNA delivery in host. So, you can imagine that how the people have discovered or how the people have get to know about the DNA delivery in host. So, you can imagine that you have a donor cell from which you are actually going to have the you know the DNA molecules and these DNA molecules are you know that they are negatively charged.

So, these DNA the charge on the DNA is negative charge on the DNA molecule interacts with the host cell especially with the cell surface which means, if I change the cell surface chemistry of this donor cell in such a way that it is actually going to take up these DNA and they will go and you know stick to this particular you know cell wall or the plasma membrane they will be going to taken up by the cell.

So, the surface chemistry of the host cells, the host cell surface chemistry either will attract or the repell DNA at the result of the opposite or the similar charges which means, the cell surface of the host is either going to be positively charged or the negatively charged. If it is positively charged it is actually going to attract the DNA if it is negatively charged it is going to repell the DNA.

So, presence of the cell wall in case of bacteria or fungus or the plant causes the additional physical barrier to the cellular uptake and the cellular entry. So, actually the entry of the DNA is not a facilitated process, it has it has multiple barriers and multiple hurdles and all these hurdle has to be overcome by preparing a competent cells.

So, what is mean by the competent cell is, the cell which is actually going to be readily be taken up the. So, which is readily going to take up the recombinant DNA ok and you are supposed to prepare their competent cells which means you are not a wild type cells are not competed enough they are not going to take up the DNA.

But you can actually be able to you know treat them in such a way that it is going to change the surface chemistry of the cell surface and that is how it is actually going to take up the cells.

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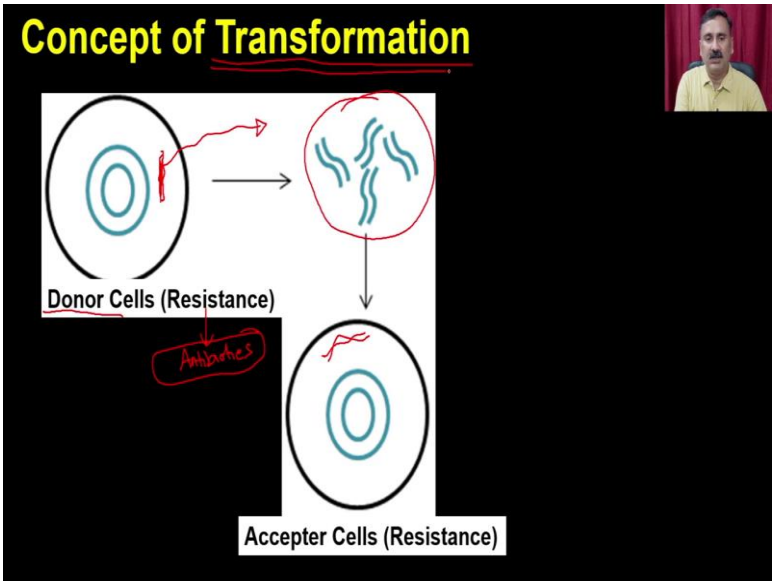

Naturally the this process is called as the transformation which means the process in which the DNA fragment from the one cell is going to be taken up by the other cell. So, you can imagine that if the donor cell is resistance for the antibiotic for example, you have taken an antibiotic and that is been you know some bacteria cells are actually acquired the resistance for that antibiotic cells by many mean because of some presence of some DNA right.

So, if you have a presence of DNA which is responsible for providing the antibiotic resistance, this particular donor cell is actually going to be overcome or it is actually going to be overcome from the antibiotic mediated killing. So, in that case what it will do is, it will actually going to throw this particular DNA and this DNA is going to be very very valuable for the other bacterial members right.

So, what they will do is, they will going to accept by the other cells right and as soon as the other cell is going to take up this DNA, it is actually going to also acquire the resistance and this is actually the way in which the transformation is actually going to be you know going to help the single bacteria to acquire the resistance and also to spread the resistance throughout the colony.

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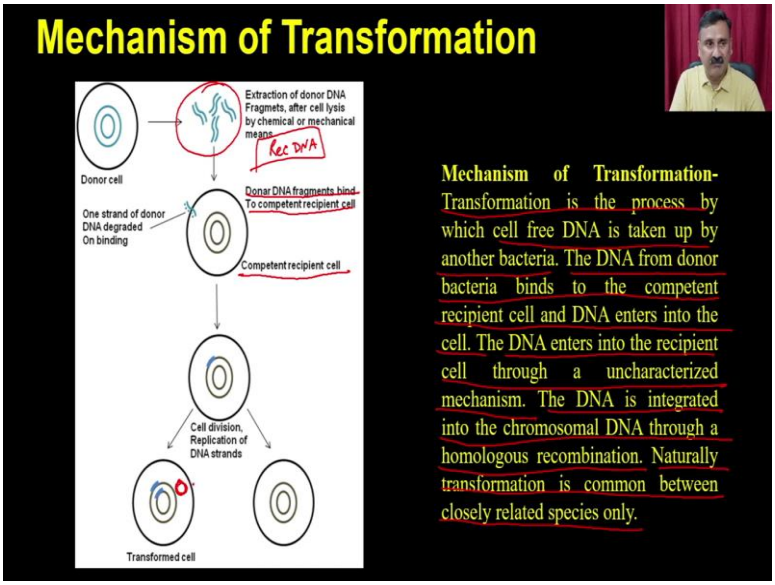

How it happens? It happens that you are actually going to have the donor cell from the donor cell the these the donor DNA or the DNA fragment which is actually going to have the antibiotic resistance genes or any kind of phenotypic gene is actually going to put into the extracellular media and then these extracellular media is actually going to interact with the acceptor cells.

So, you are going to have the competent recipient cell. So, the donor DNA fragment will bind to the competent recipient cells and that is how it is actually going to be taken up. So, mechanism of transformation is the process by which the cell free DNA is taken up by the another bacteria.

The DNA from the donor bacteria binds to the competent recipient cells and DNA enters into the cell. The DNA enters into the recipient cell through a uncharacterized mechanism. The DNA is integrated into the chromosomal DNA through a homologous recombinations.

Naturally transformation is common between the closely related species which means there are you know. So, natural transformation we have already taken an example where you can actually be able to use that for transferring the resistance from the resistance or the phenotype from the one cell one bacterial population to another bacterial populations.

But the in the lab what you are going to do is, you are going to add the extrachromosomal DNA or you are going to add the recombinant clone recombinant DNA and in presence and when you add this recombinant DNA to the competent recipient cells, the DNA is actually going to interact with the competent recipient cells and that is how it is actually going to taken up by the cell.

And once it is going to taken up by the cell it is actually going to go for cell division and that is how you are going to have the transformed cells. So, where the copy is on the both side of the DNA. Sometimes you are actually going to have the integration sites on these DNA. So, they will integrate into the genome otherwise they will remain as the extrachromosomal DNA in the form of the plasmids. Now, how you are going to prepare the competent cells for your laboratory experiments?

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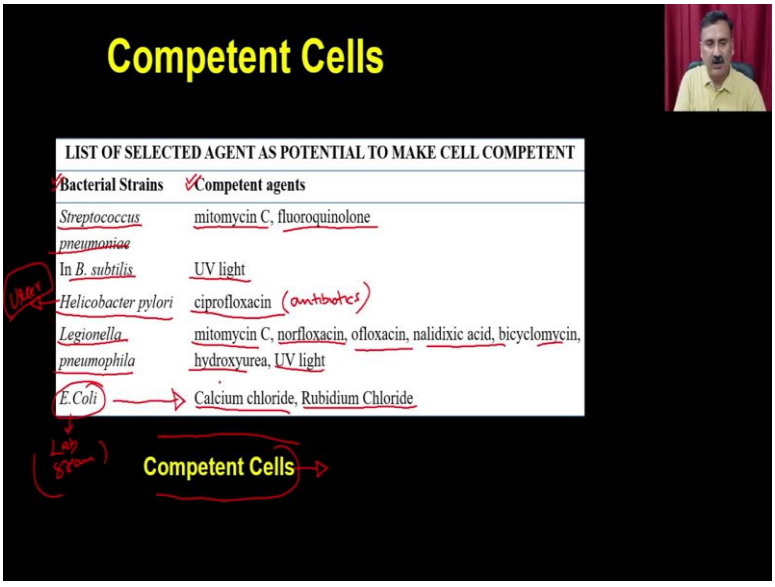

Depending on the bacterial species you can be able to use the different types of chemical reagents which are actually going to change the surface chemistry and will make the cell competent so, that they will take up the new cell. They will take up the extra cellular DNA.

So, for example, in the case of streptococcus pneumonia, you are going to treat the cells with the mitomycin C or fluoroquinolone. So, when you treat the cells with mitomycin C or fluoroquinolone they are going to be competent and they will actually going to take up the extra cellular DNA. Similarly, you have the bacillus subtilis you are going to treat the cells with the UV light and that actually is going to induce or that is going to change the surface chemistry and that is how it is actually going to be competent also.

In the case of helicobacter pylori the bacteria which is responsible for the ulcer right or it is going to be treat with the ciprofloxacin is a antibiotic ok. So, the treatments are different because your surface chemistry is going to be different the physiology of that particular bacteria is going to be different.

So, that is why you are actually going to treat it with the different competent agents. Then we have the legionella pneumophila, then that is actually going to be make the competent by treating with the mitomycin C, norfloxacin, ofloxacin and all these kind of antibiotics and hydroxyurea and as well as UV light.

For example, E.Coli which is the called as laboratory strain. So, this is the lab strain and that can be competent by treating with the covalent chemicals like the calcium chloride and rubidium chloride and the cells are how you going to make the competents also you are going to treat the cells with the different agents. So, all of these agents. So, just for sake of how we can be able to make the competent cells, I am giving you an example of the E.Coli cells.

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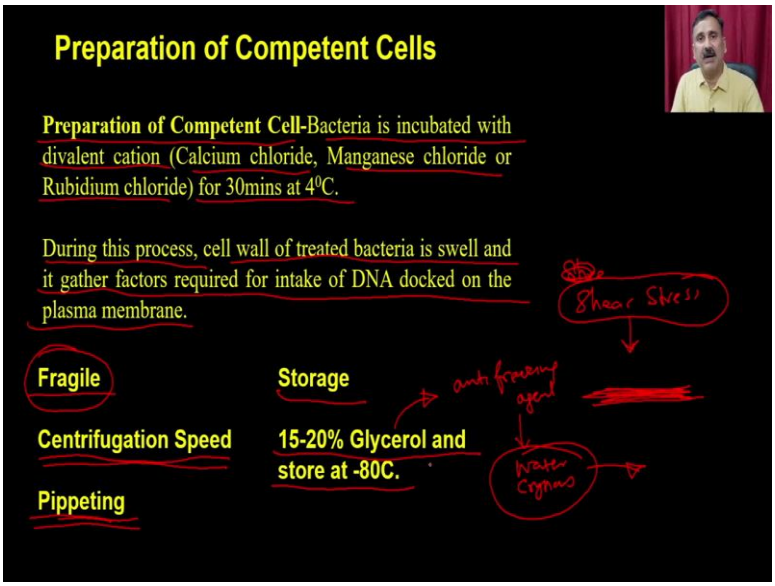

So, in in the E.Coli cells we are going to take an example of how you can be able to use the calcium chloride to prepare the E.Coli competent cells. So, preparation of the competent cells. So, bacteria is incubated with the divalent cation like the calcium chloride, manganese chloride or the rubidium chloride for 30 minute at 4 degree Celsius.

So, first you are going to grow the bacteria and then your bacteria is going to incubate it with the divalent cation such as calcium chloride, manganese chloride or rubidium chloride for 30 minutes at 4 degree Celsius. What will happen in this period? During this process the cell wall of the heated bacteria is going to swell and it gathered the factor required for the intake of the DNA docked onto the plasma membrane.

So, you can imagine that when you are doing all this preparation, it is actually going to take up the calcium chloride or the divalent cations and these divalent cation they were what they are going to do is they are actually going to take, where they are going to make the cells little fragile ok. So, in that case you might have to take care of these cells very nicely because they are also get susceptible for any kind of shear stress.

So, shear stress in the is means that you are actually going to suppose when you are swimming into the water right the water whatever the stress you will feel when the water is actually hitting you that is called as a shear stress. Shear stress means you have two layers and these two layers are actually rubbing to each other and that is actually be

responsible for causing a friction and that is actually a called as shear stress. So, they are very susceptible for shear stress.

For example, if you spin them a very high speed you probably will actually going to like the cells. So, that is why these competent cells are very fragile you cannot run that very high speeds ok and you also cannot do the pipetting at a very high pipetting right. For example, if you do a pipetting a lot of you know with the thin more tips it is actually going to destroy the cells because it is going to lice the cells.

How you going to store the competent cells? You can once your competent cell preparation is over then you can actually be able to add the 15 to 20 percent glycerol and you can store it at minus 80 degree Celsius and whenever you require. So, 15 to 20 percent glycerol is actually going to work as anti-freezing agent right. So, it is not going to allow the formation of the water crystal and that is how it is actually going to protect the bacteria from getting the any kind of damages.

So, you have to be very careful that the cells are very fragile they are sensitive for the centrifugations and they are also sensitive for the pipetting that is why you can once you prepare the competent cells you aliquot them in a you know in into a suitable volume and then you can just use the same that volume completely you cannot just pipette it out and prepare the more aliquots.

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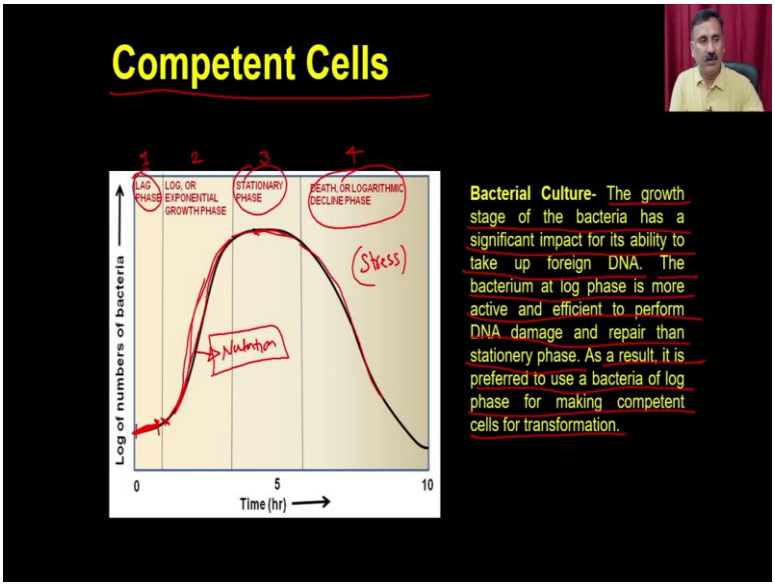

How you going to prepare the competent cells? So, what you can do is first you are going to do grow the bacteria ok. So, if you see the bacterial growth curve what you going to see is, it has the lag phase it is going to have log phase it has the stationary phase and it has the death phase or the decline phase.

So, the cells which are in the log phase, stationary phase or the cells which are in the decline phase are actually under the stream stress which means these three phase of the cell right whether you are under the log phase or the stationary phase or the death phase are not suitable for preparing the competent cells.

So, you have to grow the bacteria in such a way that it should be in a log phase you know in a logarithmic phase. So, that they are very healthy they are actually acquiring the different types of factors and there is a no dearth of nutrition. So, there is no loss of nutrition.

So, they are actually going to have lot of nutrition and they are actually having the ability to produce lot of factors. So, that is actually going to be the best bacterial culture what you are going to use. So, the growth stage of the bacteria has a significant impact for its ability to take up the foreign DNA.

The bacteria at log phase is more active and efficient to perform the DNA damage and repair than the stationary phase. As a result, it is preferred to use a bacteria of log phase for making the competent cells for the transformation. So, once you grow the bacteria bring it to the log phase and then you know collect the log phase bacteria and then you are going to use that for the competent preparation.

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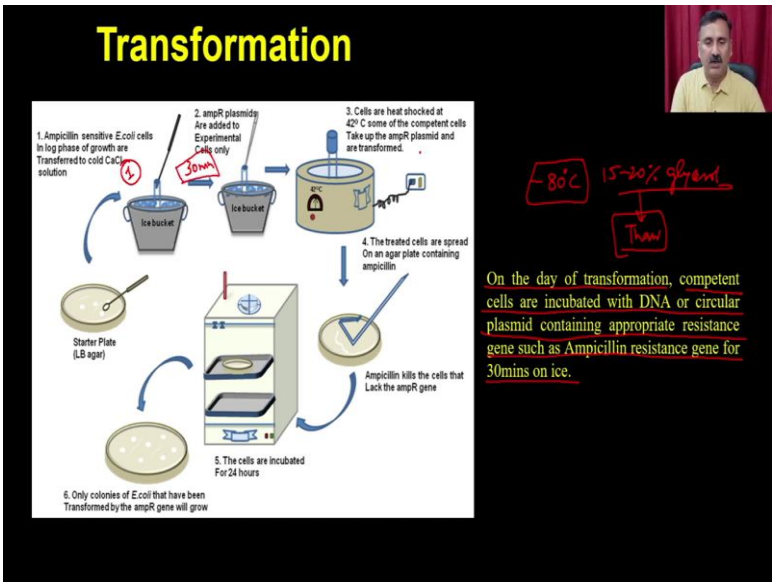

So, how you are going to do the transformation? So, on the day of transformation, competent cells are incubated with the DNA or the circular plasmid containing appropriate resistance gene such as mpicillin resistance genes for the 30 minutes on ice. So, first you what you are going to do is in the this is the step 1 number 1.

So, in the step 1 what you are going to do is you are going to thaw the competent cells right because remember that the competent cells are going to be stored at minus 80 degree Celsius in the 15 to 20 percent glycerol right. So, first what you are going to do is you are going to thaw the cells and you are going to use that for transformations.

Then in the step 1 you are going to incubate the DNA either the linear DNA or the circular plasmid with in the ice right along with the for 30 minutes. So, and then you are going to add the ampicillin resistance ok. And then once this step is over. So, you are going to have the 30 minutes incubation and once the 30 minutes incubation is over, then you are going to give the heat shock.

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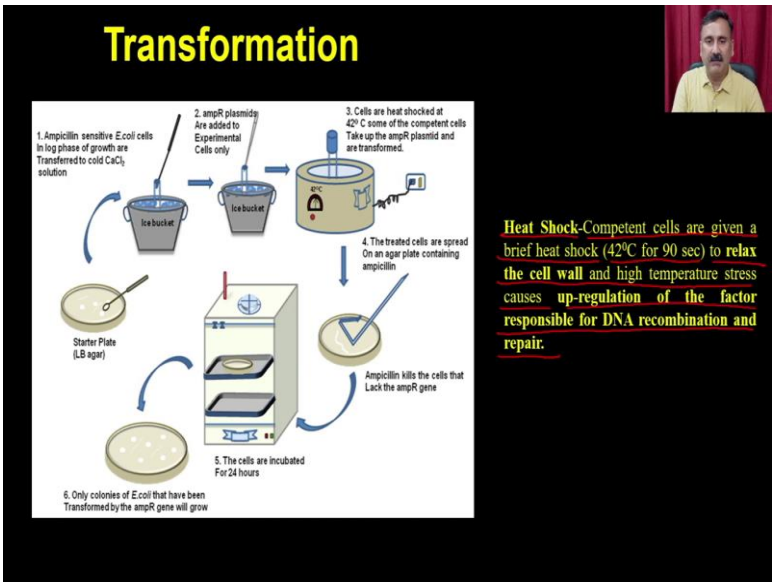

So, in the step 2 you are going to do a heat shock. So, competent cells are given a brief heat shock such as 42 degree Celsius for 90 seconds to relax the cell wall and high temperatures stress causes up regulation of the factor responsible for DNA recombination and repair. So, then what you are going to do is you are going to give the heat shock.

So, in the heat shock step what will happen is that because you are giving the heat shock the cell is actually going to swell and it is actually going to take up the DNA into that. And because you are providing the heat shock it also going to induce the production of the cellular machinery which is a sample for DNA recombination and repair; because that is very, very important event because if the even if the it has taken up the DNA and it cannot actually be able to recover from the damages then the cell is actually going to die.

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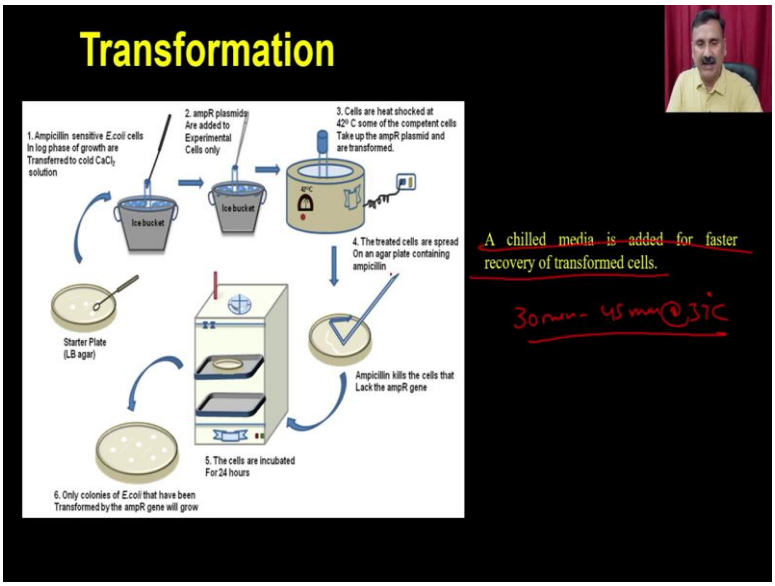

Then in the next step what you are going to do is, you are going to add once the heat shock is over right, then you are going to add a chilled media and that is actually going to bring the rigidity of the cell wall or the plasma membrane. And that is how it is actually going to seal the pores what is going to be prepared into the plasma membrane and that is how it is actually going to help in terms of the faster recovery of the transformed cells.

So, once you add the chilled media, you can actually allow them to recover for 30 to 45 minutes at 37 degree Celsius. And after this recovery you are actually going to plate the cells onto the agarose plates right.

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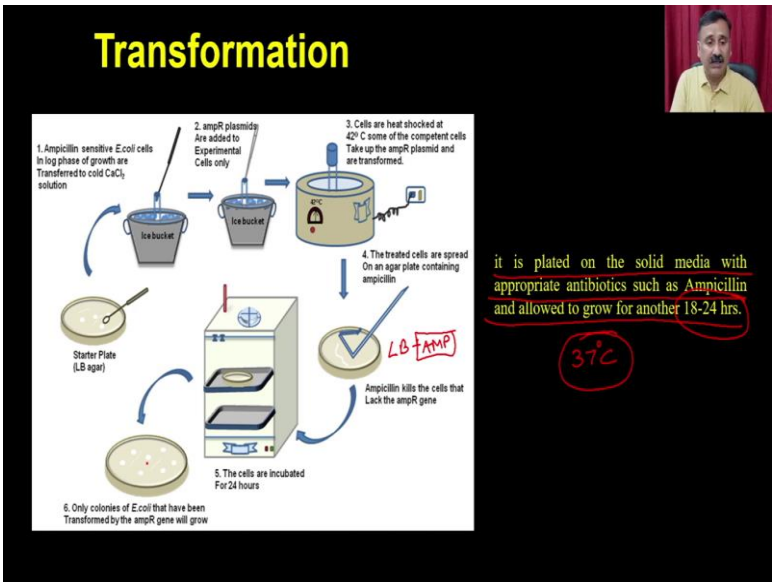

So, after this it is plated onto the solid media with the appropriate antibiotics such as ampicillin and allowed to grow for another 18 to 24 degree Celsius at 37-degree Celsius incubator. So, when you are done with the you know chilled media you added the chilled media you let them to you know remain into the 37 for some time and then you are actually going to plate them onto the LB-ampicillin plate.

So, for example, in this case we have taken the ampicillin resistance gene. So, when you do the ampicillin plating which is actually and then you incubate into the incubator for 18 to 24 hours then it is actually going to form the colonies. And now these colonies are the transform colonies which has taken up the DNA ok. Then untransformed colonies will actually not going to have the ampicillin resistance and that is what they will actually going to die in this LB-ampicillin plate.

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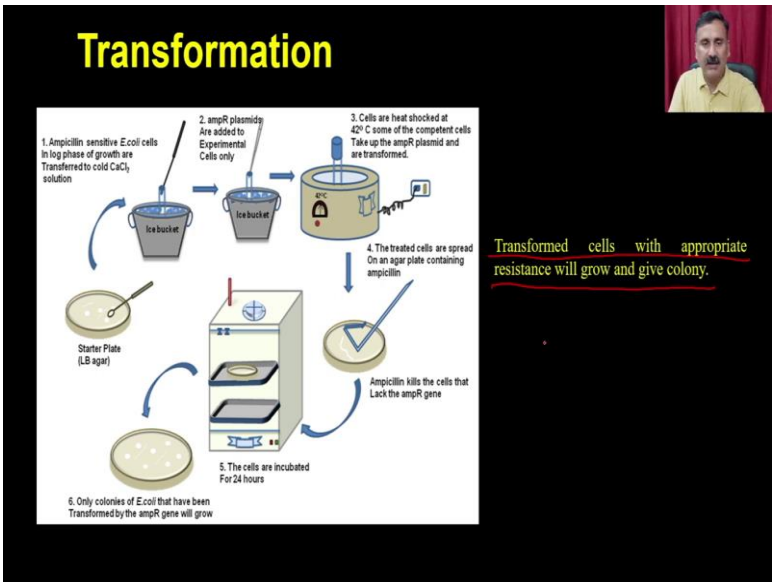

So, transform cells with appropriate resistance will grow and it will give you the colonies. So, once you are done with the transformations how the how the bacterial plates will look like.

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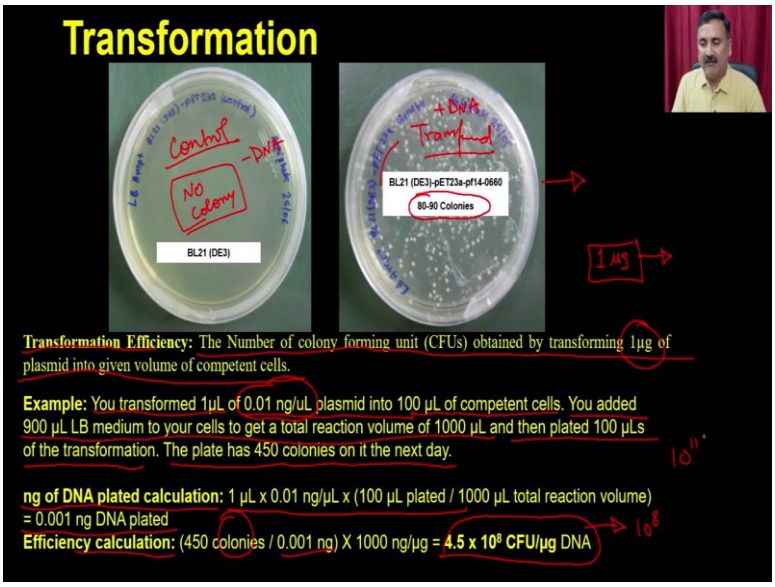

So, this is actually the untransformed or the control plate ok and this is your transformed plates. So, what you see here is, I have we have transformed a plasmid into the LB-ampicillin and if you count the number of colonies it is 80 to 90 colonies what we got

into this particular plate whereas, there is no colony in the control plate which means, the plate where we have not done any plating we its only contains the these cells.

So, this is without DNA and this actually has plus DNA ok. Now, using this in this number right how many colonies you got you can be able to calculate the transformation efficiency. What is mean by transformation efficiency? The number of colony forming unit obtained by transforming one microgram of DNA into a given volume of competent cells ok.

So, the number of colonies what you got from the one microgram of DNA is called as the transformation efficiency and it is a very very important parameter to judge how good your competent cells are because if they are very bad you cannot use them for the cloning reactions.

For example, if you transformed the one microliter of 0.09 microgram per microliter plasmid into 100 microliter of competent cells, you added 900 microliter of LB to your cell to get a total reaction volume of 1000 microliter and then plated 100 microliter of the transformation the plate has 450 colony on its next day.

Which means, you have actually added the this much amount of DNA and this much amount was the reaction volume. So, what you are going to do is, you are first going to calculate the amount of DNA what is you have used.

So, if you calculate this your what you are going to know is that you have plated 0.001 nanogram of DNA. Now, if you want to calculate the efficiency 450 colonies from 0.005 nanograms into 1000 because this is what the dilution you have done and that will actually going to tell you that you have a transformation efficiency that is 4.5 into 10 to the power 8.

So, that is very good actually ok. So, 10 to the power 8 is a very good efficiency sometime you may get even more 10 to the power 11 and so on. So, that will be going to be very very good actually. So, any number which is above to 10 to the power 4 or 5 is reasonably ok. Apart from the transformation by the chemical method you can also use you can also do the electroporation.

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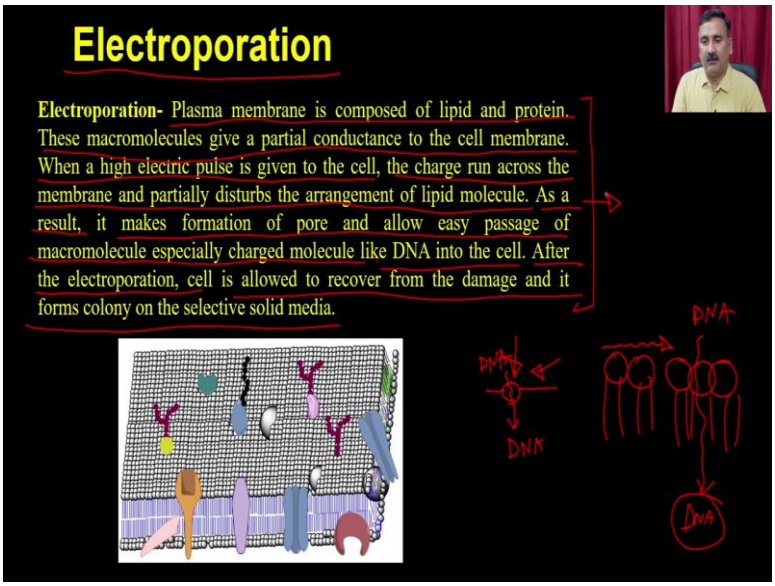

So, electroporation is a method where you are going to use the electrical pulses into the cell. So, what will happen is that when you put the electrical pulses you are actually going to make the hole into the plasma membrane and utilize and since the DNA is already out it is actually going to enter into the cell and that is all. And after that you are going to put the chilled media and it is actually going to resell this pore and that is how it is actually going to be taken up the DNA.

So, the plasma membrane is composed of the lipid and protein. These macromolecule gives a partial conductance to the cell membrane. So, when a high electrical pulse is given to the cell the charge run across the membrane and partially disturb the arrangement of the lipid molecule. As a result it makes the formation of pore and allow easy passage of the macromolecule especially the charge molecule like DNA into the cell.

After the electroporation the cell is allowed to recover from the damage and it forms a colony on the selective solid media. So, you can see that this is a plasma membrane. So, plasma membrane is made up of the lipids right. So, lipids are arranged like this right and lipids are partially been charged. So, when the pass plasma when there is a very high pulse which goes on top of this these lipid molecules are getting rearranged and because of that it actually allows the passage of the DNA into the cell.

And this DNA is. So, this is actually very momentarily. So, once you add the media this pulse is going to be over and that is how it is actually going to seal this particular thing and that is how the DNA is going to be delivered into the host. And then later on you can actually allow the cells to recover and that is how you are going to have the you are going to have the transformation done.

The advantage of the electroporation is that, it does not depends on the surface chemistry of the o cell ok. It depends on the. So, so that is why it is very very robust compared to the chemical transformations. The disadvantage is that the electroporation is required a specialized instrument like the electroporator and it also requires the electro cubits.

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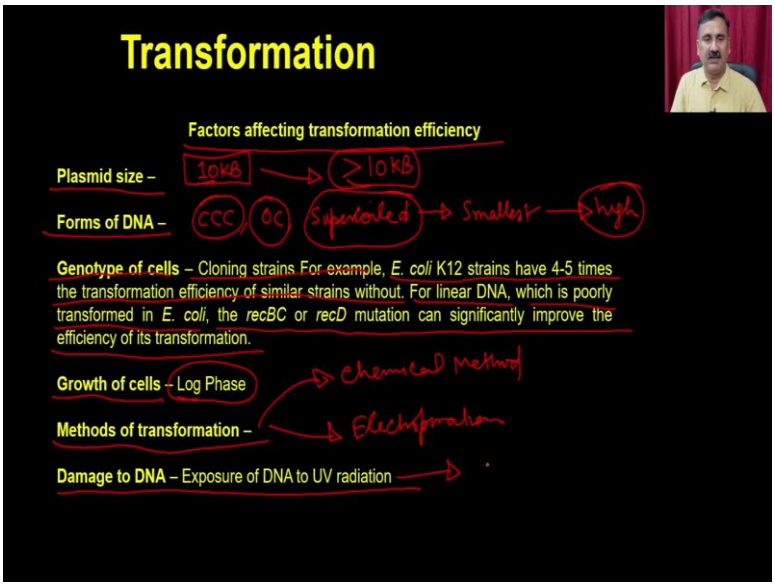

So, what are the factors affecting the transformation efficiency? The plasmid size then the form of DNA right you can you know that when we were discussing about the plasmids, there are three different forms either the triple C forms, O C forms and the super coiled form ok.

So, the transformation efficiency depends on the surface area of the molecule. So, surface area of the molecule the super coiled is smaller right or smallest whereas, the other two forms are actually having the larger surface area. So, that is why if you use the super coiled DNA the transformation efficiency is actually going to be very high compared to the closed circular DNA or the open circular DNA.

Similarly, the plasmid size if you are working till 10 K B the transformation efficiency is going to be very high. But if you go beyond that right if you go above to the 10 KB fragments the transformation efficiency is going to be very very low and in those cases you might have to use the electroporation or the other methods you cannot rely on the chemical methods.

Then the genotype of the cell cloning strains for example, E. Coli K 12 strain have 4 to 5 times the transformation efficiency of the similar strain. For linear DNA which is poorly transformed in E. Coli the recB or recD mutation can significantly improve the efficiency of its transformation.

Then the third is growth of the cell. So, log phase cells are best for preparing the competents house then the method of transformation. So, we already have discussed that the chemical methods are actually going to give you the less transformation efficiency compared to the electroporations. And then we have to also have the damage. So, exposure to DNA to UV radiation is actually going to be another factor which is also affect the transformation efficiency.

So, we have we have prepared a small demo clip only to show you the different steps of the transformations and there the student have discussed the many aspects of the you know practical considerations which you can use or which you can consider while you are performing the transformations. So, let me take you to my lab and they will actually going to show you a demo where they are going to show you how to transform the competent cells.

Myself Sooram Banesh, Research Scholar at Department of Biosciences and Bioengineering at IIT Guwahati. In this video, we will show you how to prepare competent cells and how to transform plasmid DNA. In addition to that while preparing comp cells, I will show you what are the purpose we needed and how to prepare this comp cells step by step and also how to transform plasmid DNA using electroporation method.

So, let us learn how to do these things. Hello everyone, in this video, we will show you how to prepare competent cells and transform the plasmid and play with the cells. So, let us start the procedure. Before preparing the comp cells, we need some inoculum. This is

the DH file for inoculum. I am going to inoculate in a new oil for comp cells preparation. So, I will show you how to inoculate.

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So, now I am going to inoculate the culture, then we will incubate in a shading incubator, to get (Refer Time: 29:14) that is the large phase of bacteria where we can prepare the comp cells. That is the good stage to transformed any plasmid. After inoculation, now we have to keep it in incubator shaker till we get required OD.

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So, it should be 37 degree Celsius and 150 RPM. Now, we can see some growth in here. Now, we have to check what is the OD of this culture. We can measure it in spectrophotometer at 600 nanometer Now, how to take out one mm from this culture and plank against the media and at the (Refer Time: 31:29) you have to measure the cultures. I am going to use spectrophotometer to measure what is the OD of the bacteria. Now, before doing that, I have to plank against the media. So, this is the plain media.

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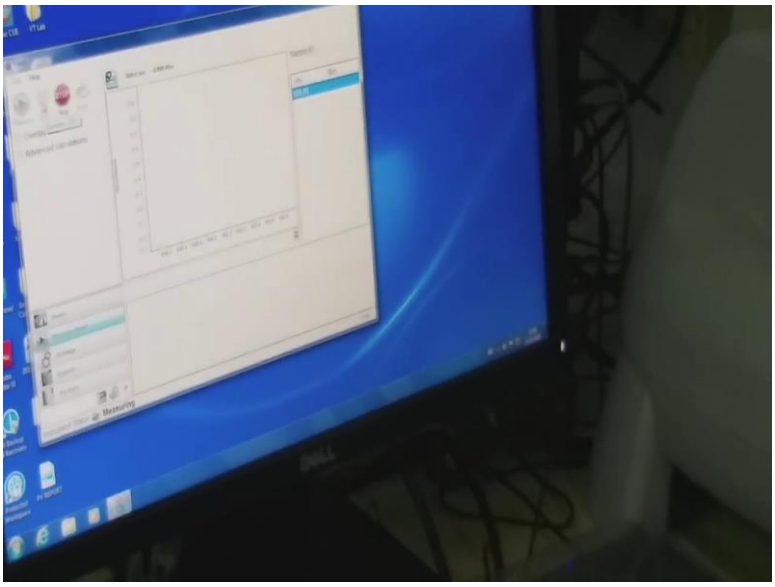

I am going to keep it and say, measure 0. Now, it is 0. So, this is our, inoculated culture. And if we put this, it is ampli folder.

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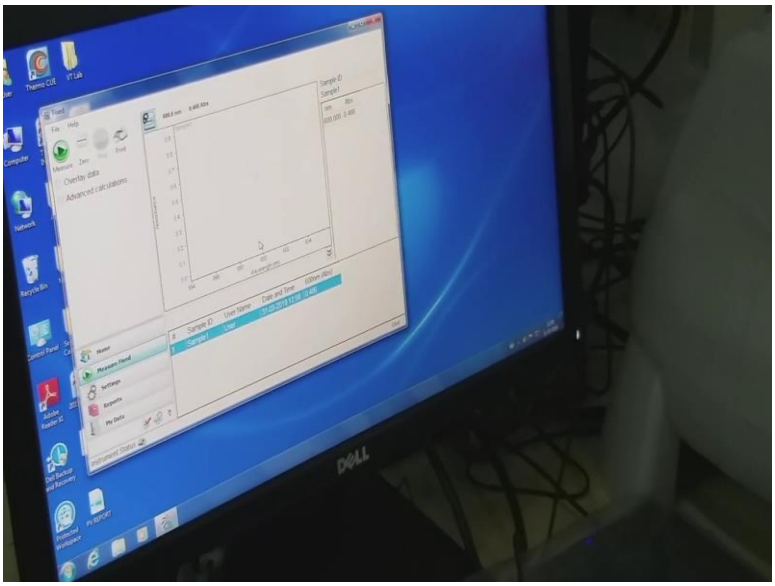

Then ask, we will find that. So, that OD is 0.466 OD is the sufficient for our comp cells definition. So, we will use this culture for comp cells preparation, then transfer. So, we got sufficient OD. Next, we have to set use the culture and pellet out the cells. After that, we will build a comp cells using point one molar calcium chloride.

So, I am going to transfer into new centrifuge to and set using we get the Now, I am going to centrifuge the culture at 4000 RPM for 10 minutes. So, in order to get the pellet. (Refer Time: 34:14) After mixing the competent cells cell pellet with 0.1 molar calcium chloride. We have to keep the cell suspension for half an hour.

Then, we have subsequent steps how to centrifuge and wash with the 0.1 molar calcium chloride for another two times. After the final step, we have to add 0.1 molar calcium chloride 2 to 3 ml re suspend gently and aliquot in to pick and rub used for storage. We have watched in final step so, we have to mix the cell pellet with the 0.1 molar calcium chloride. Then, we will aliquot into pick and rub used for storage.

We have aliquoted 100 ml micro litre now we have to store this comp cells in minus 80 degree Celsius till further use. Any mechanical disturbances or freeze time will subsequently destroy the comp cells. So, there is no transformation.

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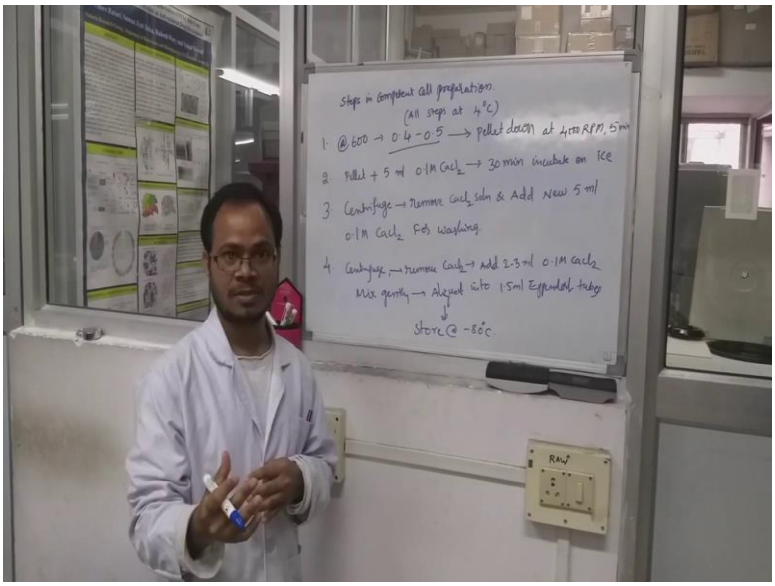

For preparing comp cells there must be some stepwise procedure which contains checking the OD like what is the observance of the cell culture for 0.4 to 0.5 observation at 600 nanometer is preferable because at that point the sensor in exponential phase. Exponential phase sets are good for transformation and we have to pellet down at 4000 RPM for 5 minutes the resulting pellet with resuspending 0.1 molar calcium chloride.

So, we have to keep at an ice for 30 minutes again we have to centrifuge remove the resulting calcium chloride solution and add fresh calcium chloride solution again 5 minutes. Again keep for 20 minutes and centrifuge again whatever the resulting pellet you got we have to resuspend in 0.1 molar calcium chloride and aliquot into 1.5 ml Eppendorf tubes.

So, they can be stored at minus 80 degree Celsius for up to 6 months. We have to add plasmid in aseptic condition. So, we kept 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 on 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 lb media cold lb media and keep it in a incubator shaker. I am going to add lb media to the comp cells. After adding media to the transformed cells, we will keep on incubator shaker at 37 degree Celsius with a rotation speed of 150 RPM.

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We just transformed the plasmid DNA into comp cells. But, during this procedure, we have to be cautious because any mechanical disturbance to the comp cells will lead to decreasing transformation efficiency. So, while handling the comp cells, we are not going to keep outside like normal temperature.

Always we will keep comp cells on in ice. So, otherwise, the transformation efficiency will go down. And another thing is that we should not add plasmid in normal incubator. We have to always keep it in aseptic condition. And also during transformation. Now, we got sufficient growth for transformation. We will centrifuge the cells and plate on suitability.

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After centrifugation we will remove all the media, but we will keep 100 micro litre re suspend the pellet and take 20 micro litre and plate on other players. But, if you are expecting low transformation efficiency, you can use all 100 micro litre for rating. This is the best way to increase chances of transformation we got a pellet. Now, we will take out the media and keep 100 micro litre resuspend and leave back.

Now, we have plated the cells, we will keep in the incubator for overnight then we will observe what is the how many colonies. Now, we can see there is a colonies appeared on the transformed plate, [FL], but there is no colonies on only comp cells plated one. So, you can see colonies here.

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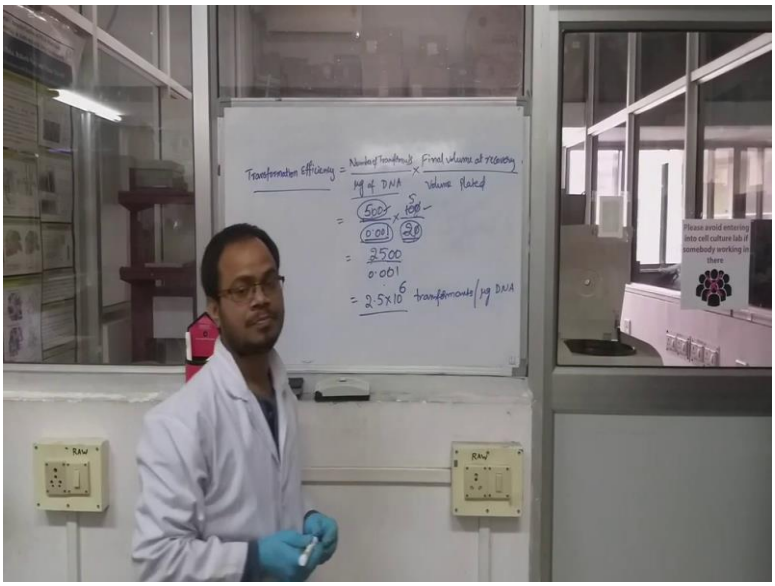

Now, we will calculate the transformation efficiency. So, there is a formula for transformation efficiency number of colonies you got on that plate and that my program of DNA you used multiplied by final volume and recovery how much volume you recovered that is 100 micro liter or it is not your chest and how much value plated.

So, suppose you got 500 colonies on that plate you used 0.001 microgram of DNA into 100 micro liter is total recovery, but you plated only 20 micro liter. So, that means, you have 2500 divided by 0.00. So, that will give 2.5 into 10 power 6 transformants for microgram of DNA.

In this video we showed you how to transform, plasmid DNA. First of all we will take in 0.4 OD that is log phase bacteria for preparing comp cells. Then we prepared this comp cells in 0.1 molar calcium chloride solution and washed and those competent cells we used for the transformation.

And also for electroporation method we discussed what are the things need to be done right. What are the pulse how much pulse we have to give and what time. So, all these things we discussed very nicely. Hello 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 an electroporator for transforming DNA.

So, this is the cuet for we use for electroporation. So, there are two electrodes placed and this is the sharp 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 pulse.

So, whatever the pulse generated we have given the pulse that is generated inside and through the electrodes through the electrodes it will pass us through. For a minute fraction of time because of these electric pulse the pores of there are small pores formed in bacteria. If any plasmid adjacent to 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 transformation of the E. Coli cells and I am sure you could have got the better practical experience of how to how to perform the competent cells and how to you know perform the transformations. Now, let us move on to the next step.

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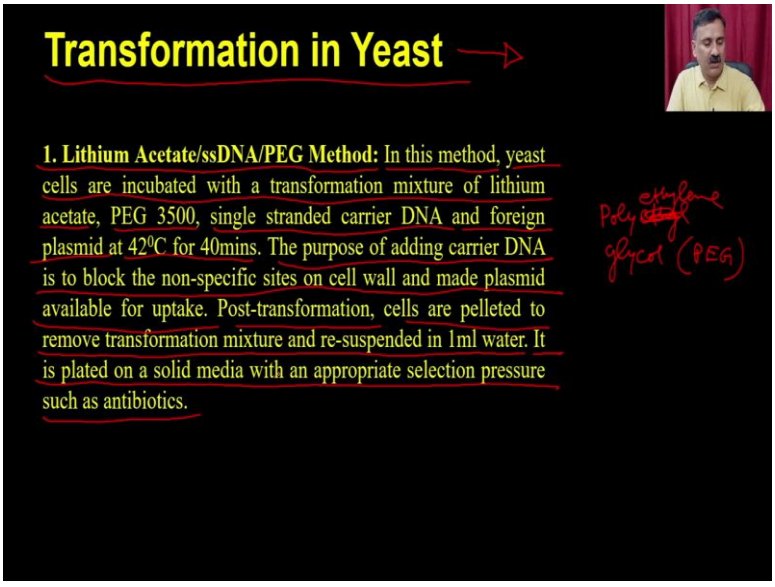

And the next step is transformation in the yeast right. So, yeast is also another host which you can use for the over expression purpose and there are many method which are available in the yeast. The first method is lead acetate single stranded DNA PEG method. So, in this method the yeast cells are incubated with a transformation mixture of the lithium acetate PEG 3500 PEG means, polyethylene glycol PEG ok.

So, PEG is polyethylene glycol polyethylene glycol ok single stranded carrier DNA and the foreign DNA or the foreign plasmid that is the recombinant DNA at 42 degree Celsius for 40 minutes, the purpose of adding the carrier DNA is to block the non-specific site on the cell wall and made the plasmid available for the uptake.

Post transformation the cells are pelleted to remove the transformation mixture and resuspended in 1 ml of water. It is plated onto a solid media with an appropriate selection pressure such as antibiotics.

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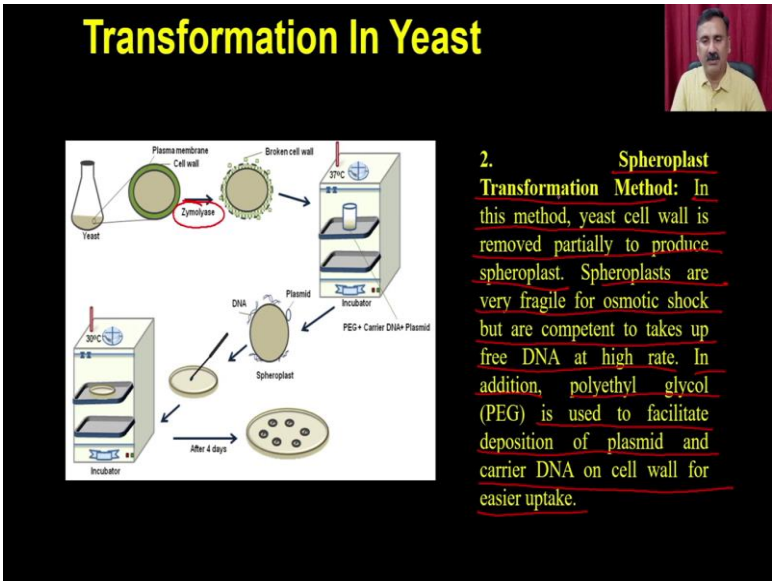

The second method is called as the spheroplast transformation method ok and spheroplast transformation method has multiple steps. So, in this method the yeast cell wall is removed partially to produce the spheroplast and that you are going to do with the help of enzyme which is called as zymolyase.

So, a spheroplasts are very fragile for osmotic shock, but are competent to take up the free DNA at a very high rate. In addition, the polyethylene glycol PEG is used to facilitate the deposition of the plasmid and the carrier DNA on the cell wall for the easier uptake.

So, how you are going to perform the transformation of the yeast with the help of the spheroplast transformation method.

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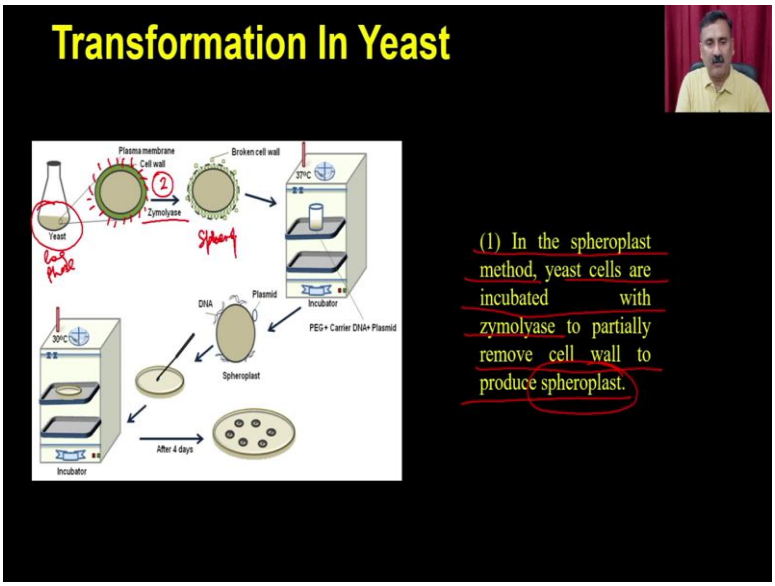

So, in the step 1, in the spheroplast method the yeast cells are incubated with the zymolyase the enzyme. So, first you are going to grow the cells at log phase and then the step 2 you are going to incubate the cells with the enzyme which is called as zymolyase. So, what the zymolyase is going to do is, it is actually going to chew up the cell wall at a very discrete step ok.

So, you if you are not going to do a complete hydrolysis or complete removal of the cell wall you are going to make the partial removal of the cell wall to produce the spheroplasts. So, the species or the cell what is going to be generated once you have removed the partial cell wall then it is called as spheroplast ok.

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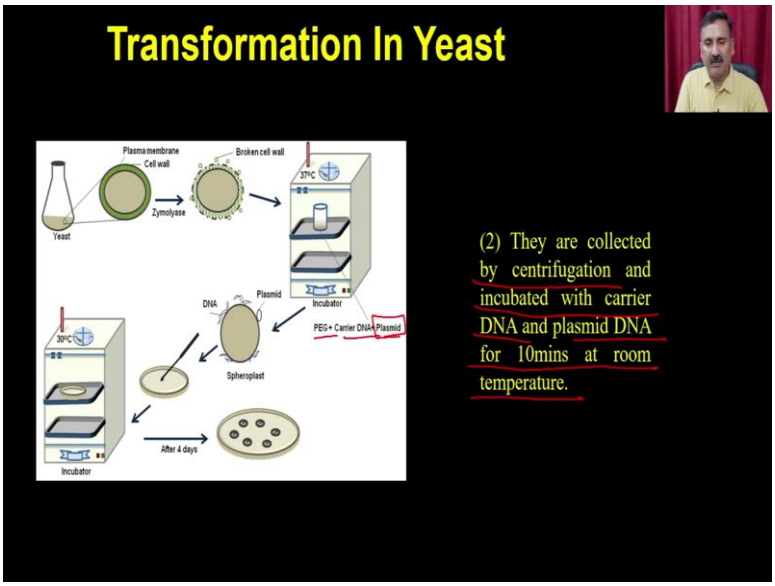

Now, what you are going to do is, you are going the next step this spheroplasts are going to be collected by the certification and incubated with the carrier DNA and the plasmid DNA for 10 minutes at room temperature. So, take the spheroplasts you collect the spheroplasts by centrifugation and then you are incubating the spheroplasts with the PEG carrier DNA and as well as the recombinant plasmids.

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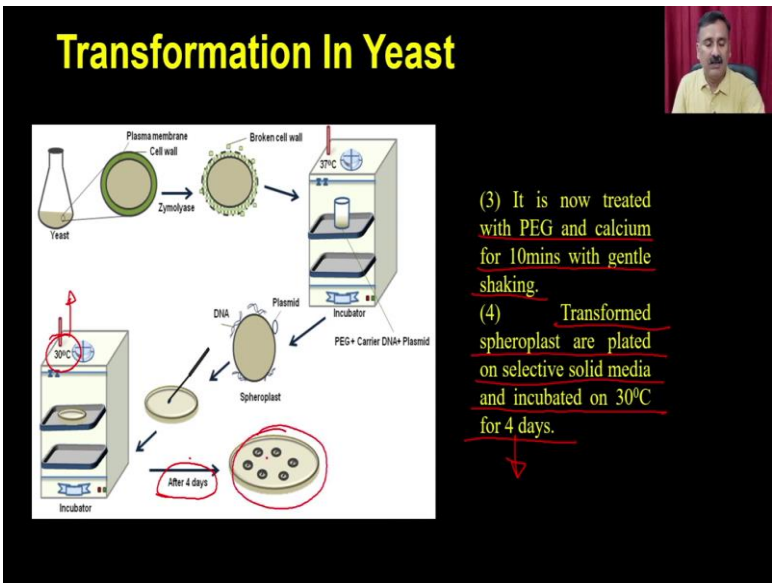

Then in the step 3, it is now treated with the PEG and the calcium for 10 minutes with the gentle shaking. So, you are going to you know keep the cells like the spheroplasts the

carrier DNA and plasmid and then you are slowly slowly you are going to add the PEG and as well as the calcium the transformed spheroplasts are plated onto a selective solid media and incubated onto the 30 degree Celsius for 10 4 days because it takes that much amount of time for getting the transformations.

So, once you have got the you know transformed spheroplasts, you are going to plate them onto a selective media and then you incubate that into a 30-degree Celsius incubator remember that the yeast is growing optimally in 30 degree compared to the 37 degree Celsius and then you incubate that 4 days, after 4 days you are going to get the plate with colonies and all these colonies are a distance for that particular antibiotic.

So, this is all about these the methods the transformation methods what you can use to deliver the DNA into the bacteria or the yeast. In this particular lecture what we have discussed? We have discussed about how you can be able to use the different chemical agents to prepare the you know chemically competent cells and how you can be able to use them for the transforming the term with the transformations.

Apart from that we have also discussed about the electroporations and so, with this I would like to conclude our lecture here in our subsequent lecture we are going to discuss about the DNA delivery into the mammalian cells.

Thank you.