Bioengineering: An Interface with Biology and Medicine

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Lecture – 08
DNA Tools: Gene Cloning-II

Welcome to today's lecture, we are going to talk about DNA tools and gene cloning, so in the previous lecture, you have learnt about the theoretical concepts of DNA cloning, today, you will get familiar with the various steps, which are involved in performing DNA cloning in laboratory setup, you will also see that how a gene of interest is inserted in a vector then it is selected based on the presence of an antibiotic gene.

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So, let us start the lab demonstration session now, hello everybody today we are going to discuss about the workflow of molecular cloning.

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Molecular cloning has some basic steps which we are going to go one by one, so firstly what we are trying to do is; introduce a foreign gene into an organism which we normally referred to as an insert, so this is my insert sample.

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So, what we do is; we take our insert DNA, we introduce into a carrier molecule normally known as vector, so most commonly use vectors includes plasmids, sot in this case we are using a plasmid DNA in which we are going to introduce a foreign gene; our insert, so there are as we may know plasmids, they occur naturally in bacteria's and over the year's studies have been done, and you have seen that these plasmids also have some antibiotic resistance gene.

So, which confers a resistance against particular antibiotic to the bacteria, so if a bacteria is grown under that antibiotic condition, it will, it will; its growth will not be affected and it will grow perfectly normal. So, here we have a vector DNA, we have our insert, so in order to introduce my insert into the vector, so let us begin with the experiment, we have taken a vector DNA also, we have an insert DNA.

What we are going to do is; we are going to treat them with restriction enzymes, restriction enzymes are going to cut these DNA and they will create compatible ends, so that the insert and the vector they can join and form a perfectly circular structure. So, we are going to carry out the restriction digestion separately for insert a vector, so what we need to do is; first we take a vector, then we add the restriction enzyme buffer which is needed by the enzyme to carry out the digestion.

We also add water to make up the volume and in the last step, we add the enzyme, so enzyme usually is added in a very small quantity, so I will use a different pipette for that so, in this case we are using EcoR1 enzyme, so throughout the process we need to make sure that it is carried out using aseptic conditions, so the tip should we autoclaved, tubes also should be sterile, at the same time we should carry out the entire reaction on ice.

So, right now I have made the cocktail; restriction digestion cocktail for the vector, I am going to do the same thing for the insert and then we will keep the tubes at 37 degree which is the optimum temperature for the enzymes to act. So, right now I am carrying a restriction digestion for the insert, so this is the insert, I am going to add the restriction buffer, so I am adding different volumes for each of the components.

And accordingly, I am adjusting the pipette as well, now I am adding water, so the last thing is the enzyme, so I am going to vortex the tubes, so that the components are mixed properly, then I am going to give them a short spin, so that whatever has stuck to the walls will come down. Now, we will incubate the tubes at 37 degree for 1 hour.

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Now we will incubate the tubes at 37 degree.

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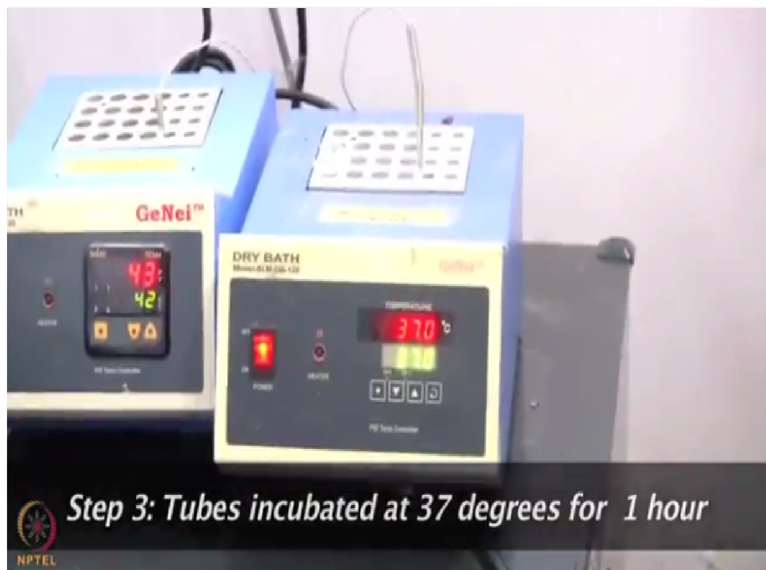

So, the incubation will be done for 1 hour, so after restriction, digestion has been performed.

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The next step is ligation of the 2 DNA's, so we have our vector DNA as well as the insert DNA which has been cleaved by the restriction enzyme, so now we will join the 2 compatible ends using the ligase enzyme.

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So, here we have all the components of the reaction, first we will take rDNA, vector DNA, then we will add the insert, so once we have added vector as well as insert, we will add ligation buffer, so before adding anything, so we just need to pipette it a little, you need to mix it a little bit.

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So, this is the ligation buffer, I will give it a small vortex, I will add this to the ligation makes now, the last thing to be added is the ligase enzyme again, we are using a small pipette because we require very little quantity of the enzyme, I will give it a short vortex again to the enzyme, now our ligation mix is ready, we will vortex it a little, followed by a short spin.

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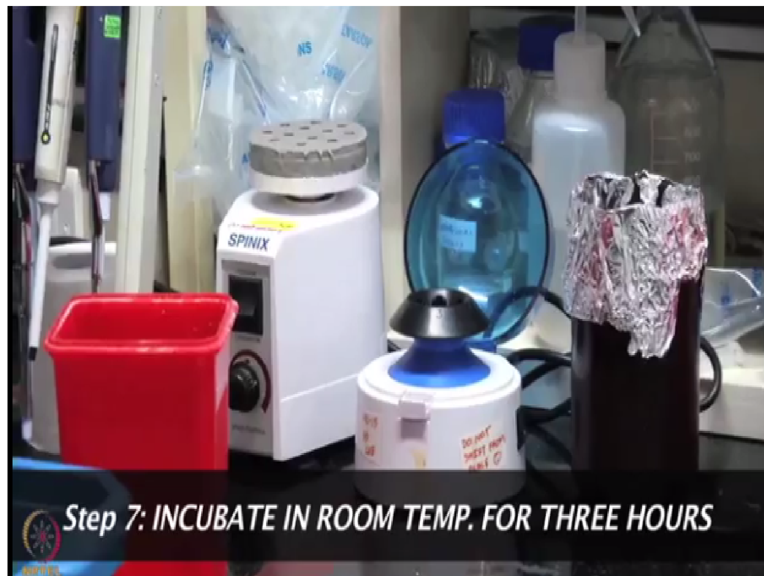

Now, we will incubate this at room temperature for 3 hours.

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After ligation is done, the next step is transformation, so after ligation the vector and the insert they form a covalently closed structure and this DNA molecule, the combined DNA molecule we introduce into a host cell, so in this case you have taken DH5 alpha host cell, which does not have any restriction enzyme, which will chop of any foreign DNA, so we will introduce our DNA into this host cell, so that it can propagate further.

So, this step transformation is carried out inside the laminar hood under a sterile conditions, so inside the hood, we have sterile filters, which may keep the inside air clean.

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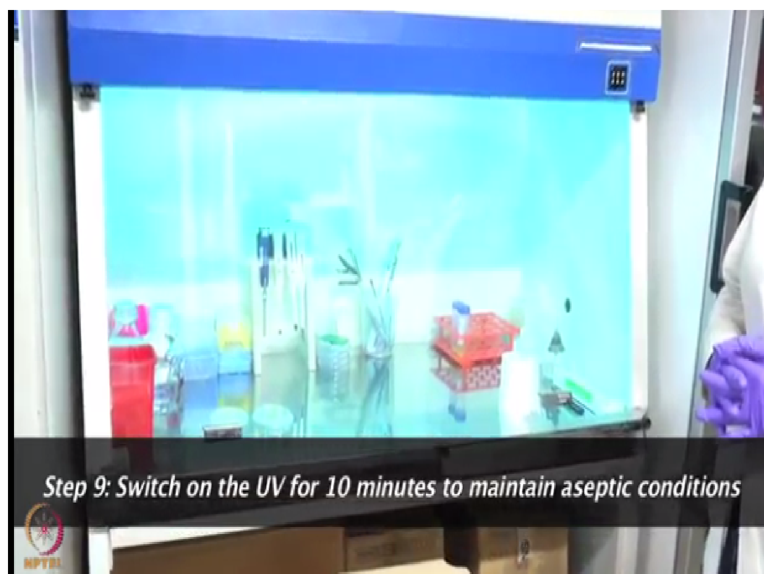

So, before we start with the experiment, first we switch on the UV for 10 minutes, this step ensures that inside the conditions are extremely sterile, this step ensures that inside the laminar hood conditions are extremely sterile under good for a work. So, after this we will switch off the UV and we switch on the fan and the light button.

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Now, we will carry out the transmission experiment, so we have our ligated sample, we will also keep a negative control water, so in all the experiments, we normally keep a negative control, so we have a competent cells which are the host cells in which we will introduce our recombinant molecule, in one of the tubes, we will add our recombinant molecule, in the other tube, we will add a negative control, water.

This is our ligation mix which we will add in the competence cells, so now we will incubate the tubes on ice for 30 minutes.

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Now, we will give the samples, heat shock treatment, so we will keep tubes at 42 degree Celsius for 2 minutes. This heat shock treatment helps the host cell to get the foreign DNA; this heat shock treatment helps the foreign DNA to get introduced inside the host cell.

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So, now we will perform the plating, so as we can see, we already have plates; LB agar plates.

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These plates, they have a lot of components, which will help the bacteria to grow additionally, we have added the antibiotic ampicillin because a vector, the plasmid DNA has ampicillin resistant gene, so that when we will plate our ligated product onto this, only those colonies will show up which have our vector. So, for 1 plate, I will be using the ligated sample which has been transformed into the DH5 alpha host cell.

For the other plate, I will use the negative control which has been given the same treatment as our ligated product, so I will take the sample, so I will add the sample in drops, so this is the spreader which I am going to use for plating, so this spreader is already dipped in ethanol, so that all the microorganisms if any will get killed, so I will put it to flame, so that the alcohol evaporates, I will wait for the spreader, spreader's temperature to get down.

Because if it is too hot, it may kill my DNA sample as well, I can touch it here, so that it becomes cool now, I will start plating, so I will be doing the plating in circular motions, so that the sample is spreaded uniformly, I will do it till a point where I feel that the entire sample is spread uniformly and when the agar seems to look a little dry and there is some friction at that point I will stop.

One needs to ensure that we do not presser too hard else we may end up breaking the agar plate as well, I will show the lid some flame and I will keep it back, I will label my plate. Next, I will

plate the negative control sample which is nothing but water, so normally we keep the plates this way upside down otherwise, if we keep them like this, they sometimes because of the loose ends, we may end up having some sort of contamination, so usually we keep upside down.

Right now, I have done plating, so for some time I will keep it like this, straight way, later I will keep it upside down as well. Now, I will take my; now I will take the negative control sample again, I will add in the form of little drops, spread it uniformly, I will show my spreader flame, I will wait for few seconds for the spreader, for the spreader's temperature to come down, this is how my plate looks like.

Now, I have started feeling some friction on the plate, so if I poke it further, it may break, so at this point, I will stop, now I will take my plates and I will keep them in the incubator.

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So, these are the plates which I will keep in the incubator for overnight incubation, so as you can see the temperature ranges from 37 to 38 which is ideal for the bacteria to grow.

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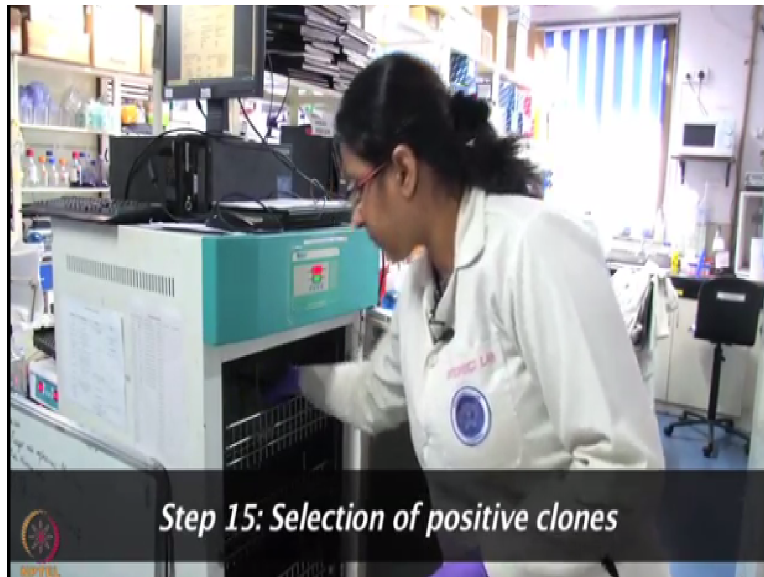

I can show you another plate, which we have already done transformation yesterday and how a transform plate looks like.

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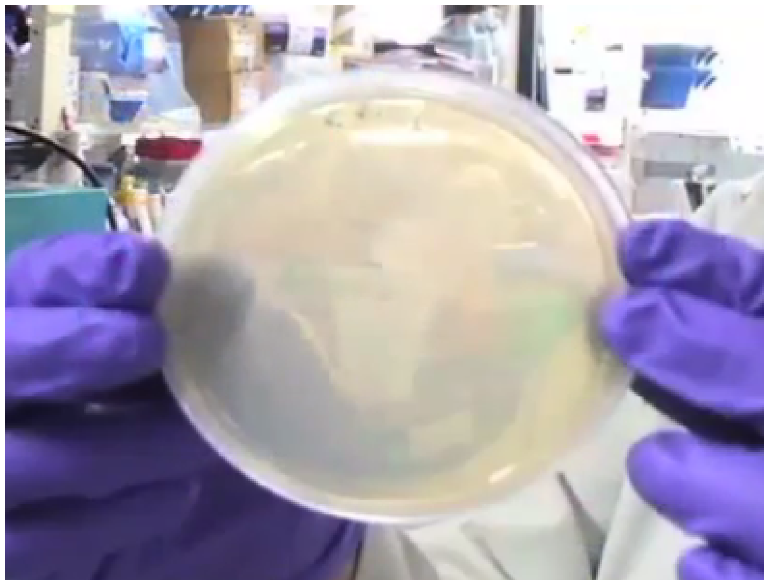

So, this is a transform plate which has a lot of colonies inside, so this in this case of transformation efficiency has been very good.

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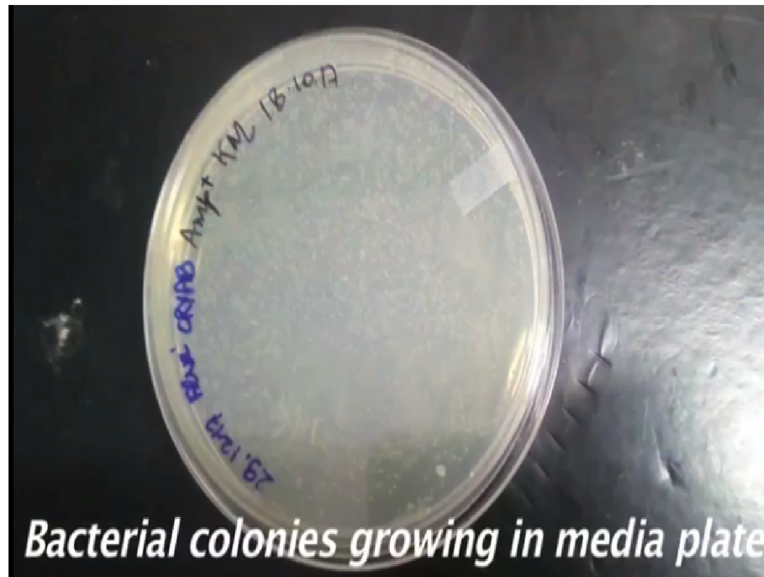
Bacterial colonies growing in media plate

In some cases, we do not get as many colonies, so right now, we have kept 2 plates; in one, we hope that we have a good transformation efficiency and in the second plate, we should expected to be blind because at it is just water to ensure that our experiments are absolutely fine and there is no contamination. So, once you have obtained the colonies we need to be sure whether these colonies reflected to recombinant molecule because as you know once we have done restriction digestion, we can get different kinds of products.

It may happen that during ligation, the vector itself ligates and have a self-ligated molecule or it may happen that there is only the insert that has transformed into the host cell, so we need to be sure whether these colonies actually have a recombinant molecule, so one thing we are sure that there is no insert here because the vector has antibiotic resistance seen and here, in the media we have an antibiotic ampicillin.

So, only those colonies which have the vector will grow on these, now those colonies can be the self-ligated vector or they can be the recombinant molecule having the insert, for that we will take these colonies, so for that we will take these colonies, we will grow them, we will isolate the plasmid and we will check their size, so this is the LB media, this is the same media which has been, which is already you know in the solidified form in this agar plate.

So, this LB media has all the components, which is required by the bacteria to grow, so we will pick up a colony, we will added in these LB media, we will also add our antibiotic, the antibiotic for which the resistant gene is already present on the vector, so that only our vector grows, no other contamination is seen.

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So, we will first add the antibiotic; this is our antibiotic ampicillin, everything we are doing near the flame, so that there is no contamination, there is no chance of any contamination now, I will pick up a colony, this again we will incubate at 37 for overnight almost 16 hours.

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So, this is the shaking incubator in which I will keep this tube for overnight incubation. So, you can see the temperature set is 37 which are ideal for the bacterial growth.

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So, this is how a bacteria culture looks like, you can see it is very turbid and after this is a sign that the bacteria has grown properly overnight, so this is the tube which we have just kept for the overnight incubation, you case see the colour of the tube, it is, it is not so turbid, it is transparent but after the grow this is how it looks like. So, in conclusion, I hope now your concepts for the gene cloning is much better and much clearer now.

As I have just seen the lab demonstration session with the advent of technological advancement, the genetic engineering can be performed in a laboratory set up with great ease now, why it might you know look a straightforward but the cloning a gene often involves you know lot of thought process and the whole parameter optimisation may take several months' time to really obtain the correct clone.

And then you have to ensure that you know the sequence is correct and it is not self-ligated vector, what you obtain is the right gene of interest which you started with, so of course, the process is straightforward and much simpler but to actually obtain a right clone in frame does need you know good listener operating protocols and sometime you know good experience of knowing the concepts involved in genetic engineering.

So, I hope you enjoyed the lab session and we will continue our interactions and discussion about gene cloning in the next lecture as well, thank you.