Bioengineering: An Interface with Biology and Medicine
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Lecture – 07
DNA Tools: Gene Cloning

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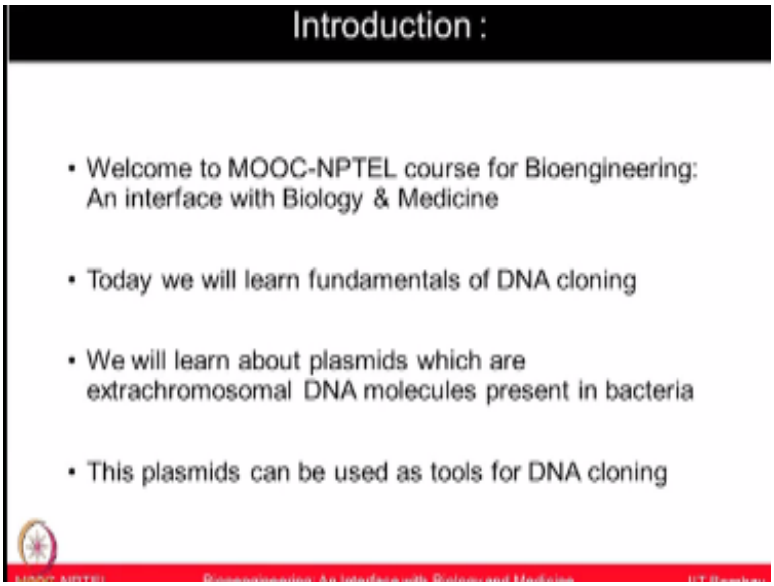

Welcome to MOOC NPTEL course on bioengineering an interface with biology and medicine. Today, we are going to learn the fundamentals of DNA cloning, first we will learn about plasmids which are extrachromosomal DNA present in variety of bacteria, these are used as tools for DNA cloning and genetic engineering, so let us start today's lecture. If you remember from one of the previous discussion contest, I had talked to you about cloning briefly of the DNA cloning.

Although, we talk cloning in many ways, thinking about even plant cloning or even an organism cloning but just you know recall yourself about DNA cloning part and let us kind of refresh on this and talk more about to begin with about plasmid.

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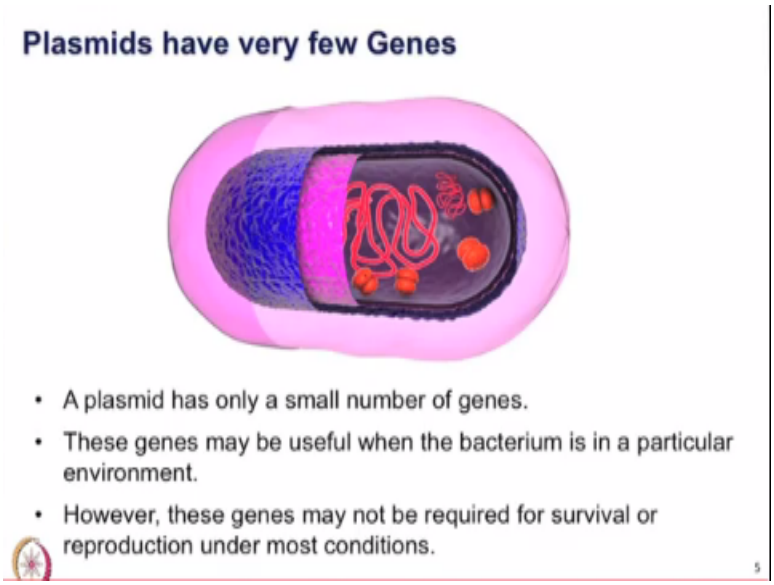

So, what is plasmid? Plasmid is one of the extra chromosomal part of the bacterial, different organelle it may have present, so in addition to the bacterial chromosome it also possess these extra chromosomal component which are known as plasmids. Now, these plasmids unlike the bacterial chromosome which has all the useful genes and which are of course essential for bacteria for its own propagation for its own information.

The plasmid has only very few genes and they are useful but you know they are extra chromosomal, so therefore, they are not so essential as well and many people who are studying in the area of genetic engineering, they have made these observations closely and then they felt that studying plasmids could be very useful as a tool for us to transfer the genes from one organism to the other organism.

So, these plasmids are double standard, covalently closed, circular molecules and they are present in variety of bacterial species, they behave as additional unit or accessory units of bacteria which can replicate and inherit independently of the bacterial chromosome part. So, as I mentioned, they have very few genes and therefore, even if you are removing it out of bacteria or if you are adding a new plasma in the bacteria, it is not changing too much, the overall the gene dynamics for the bacteria.

So, these genes may be useful especially for a particular environment for the bacteria, how would they may not be required for the bacterial survival or its reproduction purpose.

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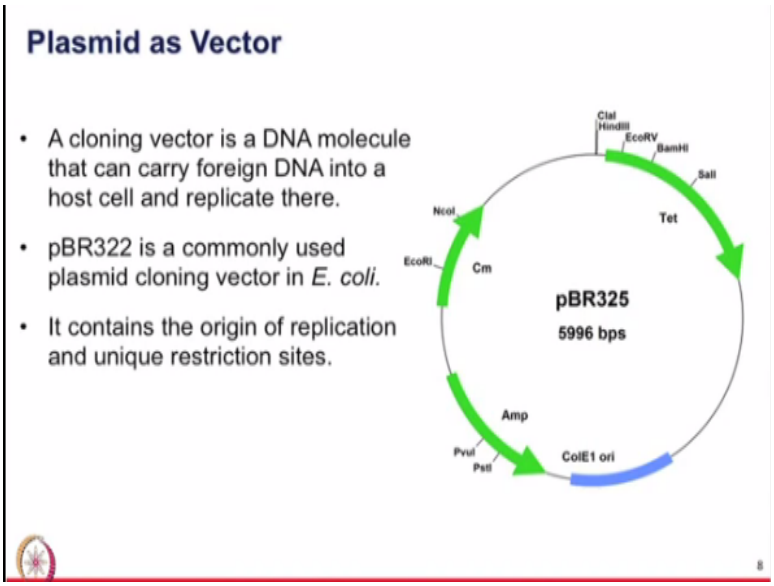

So, therefore people have used in genetic engineering, these plasmids have been engineered, so people have taken out these plasmids from bacteria and further engineered them in genetic engineering field for our own advantage, just imagine that you want to take some of the human genes or fish gene or a plant gene and now you want to make multiple copies of it, you are doing that in the bacteria, you are using bacteria as a system where you can multiply these genes.

So, in this case let us imagine, this is one of the vector, vector is any of these plasmids which can be used to transfer a foreign gene from one to other place, okay so this is one of the example of pBR 325 vector, where we have shown multiple features which has been added, in addition to the original properties of the plasmid, some new features have been added in this using genetic engineering.

So, a cloning vector is a DNA molecule which can move the DNA into a host cell and it can replicate there, this is one of the example, pBR 325 or 322, which are different type of vectors present in the E. coli, it contains an origin of replication which is shown in the blue colour here, it also contains many of the antibiotic resistant marker and please be attention to this part,

bacteria when you know there are, present everywhere, right so, if you want to grow some bacteria, you will actually automatically have lot of contamination from other bacteria.

So, how to select that you want given type of bacteria which can only have your gene of interest, so if you have this particular vector where you have inserted some of the antibiotic resistance genes, so that can be used for you to select as a selection marker, only those bacteria which can contain your gene of interest only they can grow, so therefore in this vector map itself, these particular genes have been added either ampicillin, chloramphenicol or tetracyclin etc.

Additionally, there are many enzyme names you can see, EcoR1 and co1 etc. these enzymes are known as restriction enzymes and these sites are there, where these essential enzymes are going to work, so some of the restriction enzymes you know, they have very tricky name, difficult name, do not worry too much about what the name is and exactly how they, where they work, so I am not going to ask you exactly where the Hind were.

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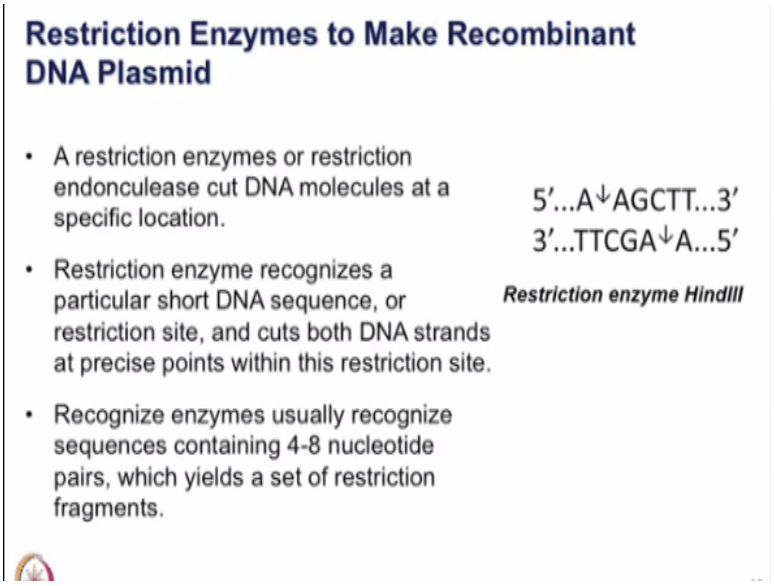

What is important for you to remember that these restriction enzymes, they work very precisely, they only work on a specific DNA sequence and when they see for (()) (05:25) in this case from 5 prime to 3 prime; AAGCTT, so now between 2A's, it is going to have a neck, it is going to cleave only between these 2 A's, so as from the opposite strand, so this particular thing is known

as restriction site because you are able to very precisely put the you know cleave this particular DNA fragment at that particular site.

So, these restriction enzymes are very precise, very specific, so now if we are looking at different properties of restriction enzymes, they are very short stretch of DNA sequences, sometime 4 to 8 nucleotide longs and they can cut both DNA stands at very precise point within the restriction sites.

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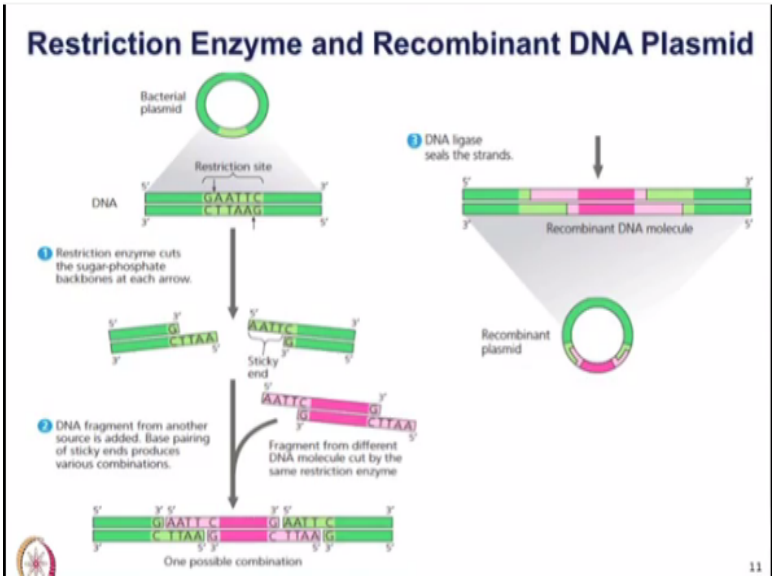

So this is how you can see you know, if you have bacterial plasmids, now this plasmid, if you elaborate this is how the DNA sequence looks like and within DNA sequence, you have some part where you have GAATTC, now this restriction enzymes is going to only work and cleave at sites, so now this circular DNA; the circular plasmid is opened up because of the restriction enzyme cut and now, you are using the same restriction enzyme to also cut open your gene of interest.

So that now, you can create these particular type of sticky ends, if you are using the same enzymes, so they are going to create the same type of cuts and they are going to have a sticky ends now, if you add enzyme DNA ligases, then they can go and insert and pretty much get ligated, inside the this backbone of plasmid, so your gene of interest can be inserted in like you know the pink one is the gene of interest, the green one is your backbone from the plasmid.

So, now after doing the ligation reaction, you can have a recombinant DNA molecule which can be formed, a recombinant DNA molecule is a plasmid carrying the gene of your interest, right or he had some question. **"Professor – student conversation starts"** So, depends on the origin of replication in which the origin of replication can happen, definitely, yes, alright, **"Professor – student conversation ends."**

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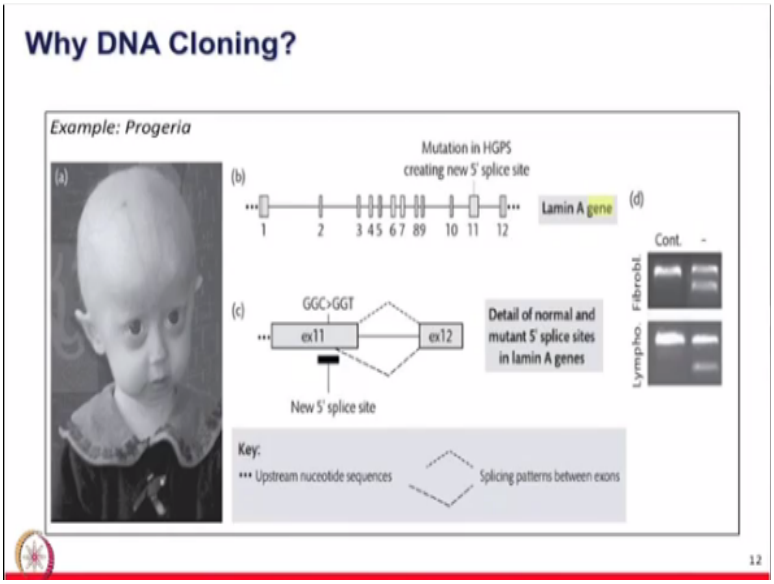

So, let us say that we have been discussing about one of the interesting example of progeria, right and by now we know that there is a gene which is lamin A gene, which is having deficiency in this individual and therefore, you know it is not showing the normal phenotype, to overcome this let us say you want to study about the gene that you want to know that what is the, the you know, malfunctioning happening at the gene level or at the protein level in lamin A.

So, you want to clone this particular gene and you want to also purify the protein format, right, so let us think about our cloning experiment briefly, I think I have talked in the past, let us refresh it now and talk in more detail now.

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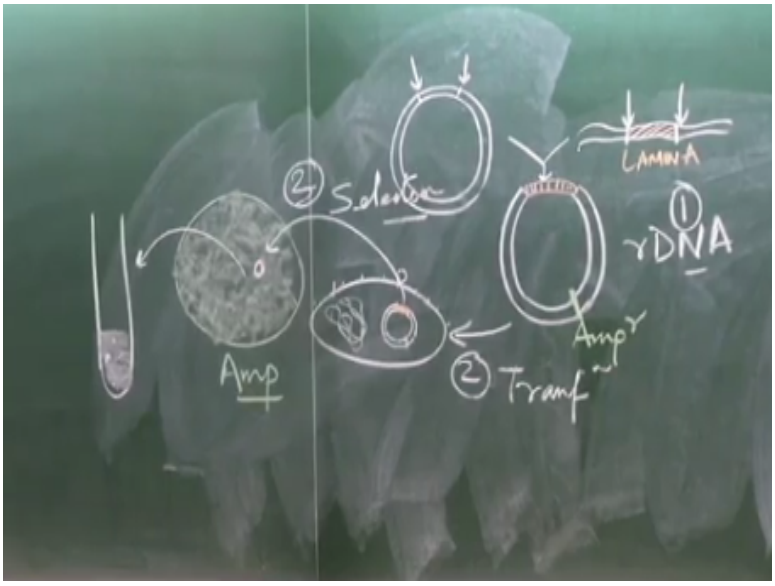

So, you just learned that you know you have this plasmid and you have a gene of interest in this case, let us say, we have lamin A, so now you are using some restriction enzymes, they will work at certain restriction sites and very precisely, they will make cut over there, so now your plasmid is getting open and now, you can have the lamin A gene here inserted, if you can add the DNA ligase enzyme.

So, this particular molecule which is formed that is known as recombinant DNA molecule because it is having a plasmid containing the gene of your interest, this is a recombinant DNA molecule. So, in the cloning part, it is the first step to have the rDNA formation, now this is coming from the bacteria, so you want to make multiple copies of it and your original intention was; can we multiply this inside the bacteria?

So, now you want to move it inside the bacteria, so bacterial membrane; you try to make it little permeable just by doing some sort of chemical treatment with calcium chloride or using some elctrophiration, some small bit electric shock, so that the bacterial membrane becomes permeable and now this particular plasmid can move inside from this membrane here, inside the bacteria and now, this contains the gene of interest which was lamin A.

And it has of course, its own circular DNA, so now this particular bacteria whether your gene of interest or the plasmid which you have made recombinant DNA, whether it has inserted or not

you have no idea and you only want to select that for your further experiments, so now you are using that property where we said that you can have some of the antibiotic resistant genes, so let us say we have ampicillin resistant gene here; in your plasmid.

So, now in this particular plasmid, this ampicillin resistant marker is there, if you make a plate and on that plate, you add LB medium agar and you add the ampicillin antibiotic on this plate, you have added ampicillin, so only those bacteria which are having this particular plasmid is going to survive here and you will see only those which are having your gene of interest are going to survive on this plate and they will make the colony, this is the way of selection.

This was the first process which is making a recombinant DNA molecule, this is a second process which is the; you are having; this is second process actually, here is transformation and third process is which is known as the selection, you are doing the selection here. So, now once you select that is the right clone, this is the right bacteria which is containing gene of your interest, now you can do multiple things from it.

Because bacteria can multiply very rapidly, so now you can take this bacterial colony and you can grow them in the tube and they are going to keep multiplying and from this one either you can harness the properties of having multiple DNA copies or we will also talk about how best to get the get the same system to make the protein as well, so both of these can be used just by doing this basic experiment.

This is going to be very crucial for many concepts which I am going to ask you subsequently, where you will be asked to you know given some situation of a disease or some hypothetical situation in which way you can overcome that problem and you need to just know how the cloning process works, in which way you can make the recombinant DNA molecule and you can have a precise selection only for the clones which are having gene of your interest.

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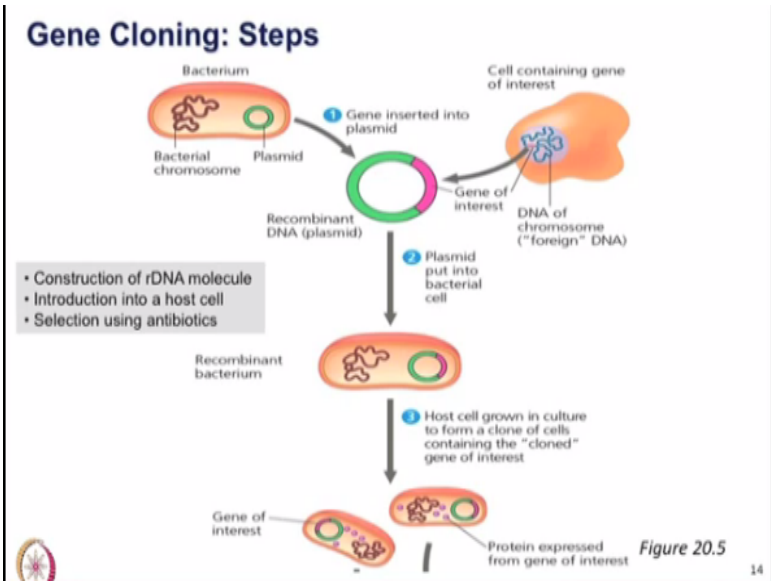

So, these are the; this step, I have already shown you earlier in the previous concept, how biotechnology has made a huge difference that time, I did not explain you in much more detail but today I think it is the place when we can talk about it much more detail, so we have made the recombinant DNA molecule, we have a transformed it and now, we have selected and we are making the multiple copies of the bacteria, which is containing gene of our interest.

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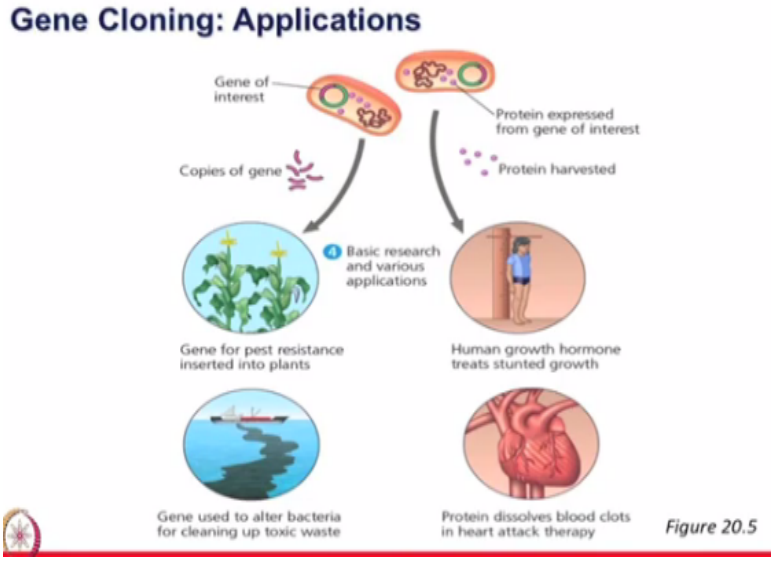

Now, this particular gene of interest could be for multiple application whether you think about having the plants containing the pest resistant properties or plant containing you know certain vitamin increased contents or many type of therapeutic proteins could also be purified and

produce in excess amount based on these experiments. So, this, this has you know lot of application.

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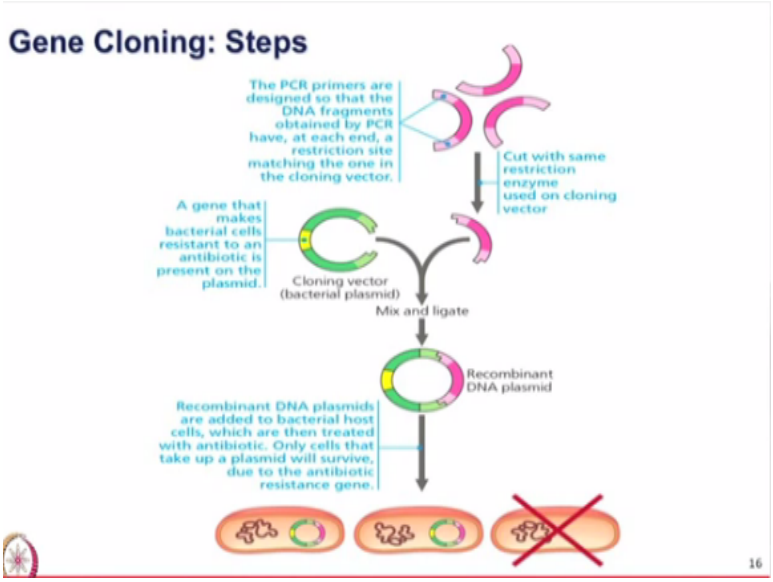

And just to another view of understanding the cloning experiment, where you can think about the gene of your interest and the vector, you are ligating it here making the recombinant DNA plasmid and from here those which are containing your gene of interest are only getting selected on the selection medium but the ones which does not have any plasmid or do not contain the, the plasmid of your interest are not getting selected.

So, therefore only selectively you are selecting those plasmids which are having your gene of interest because you have engineered the plasmid in such a way that they have certain antibiotic resistant properties, so those properties you are using for the selection, alright, so in general, you know these are all being done by the biotechnologists, by the genetic engineering people however we can understand some of these things in much simpler words.

And especially, in your, in your words thinking more about the just numbers, right, so do not worry too much about the name, whether name of a you know vector or name of an enzyme, these 2 just for the time being you can ignore that.

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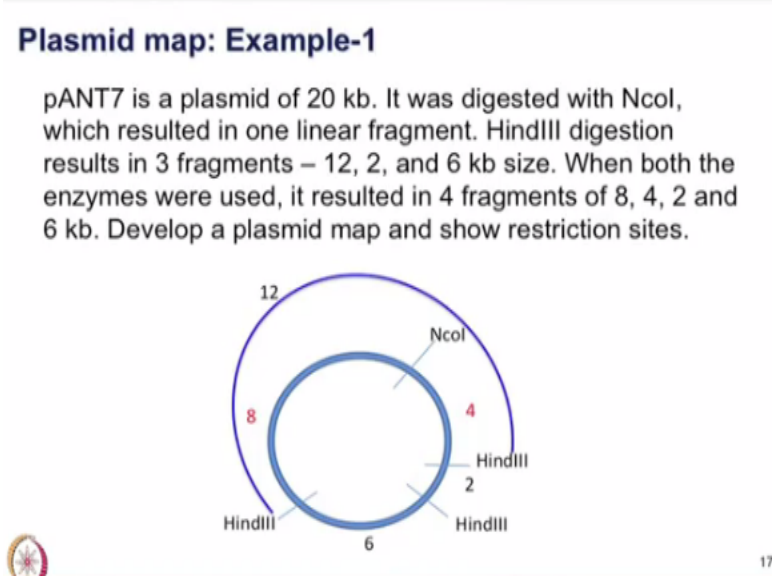

Imagine you have a vector or a plasmid which is 20 kilo base per long and now you are using 2; restriction enzymes; one enzyme is Nco1, second enzyme is Hind 3, by doing this restriction digestion, now you are generating certain fragments, so it is good idea to take your notebooks and start doing few examples here.

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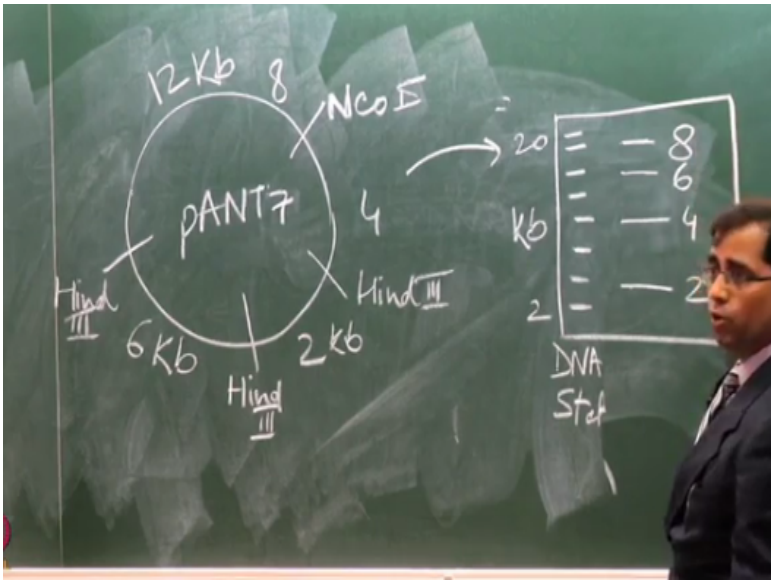

Alright, we have pANT 7 could be GST or some other vector here, now what it says that if you are using Hind 3 digestion, you have 3 fragments generated, the fragments sizes are 12 Kb, 2 Kb and 6Kb, if you use only one enzyme, you are making 3 cuts, which is 2 Kb, 6 Kb and 12 Kb, now if we have used another enzyme which is Nco1, total you you are seeing 4 size of the this plasmid; 4 fragments.

So, now one more size which appears is 8 and 4, so let us say if Nco cut was made here, this 12 could be split into 8 and 4, right, so in this case, this will be the map with a different restriction enzymes, now if you want to test this out that how many fragments have been generated is a theoretical possibilities, now people do this experimentally using a technique known as DNA electrophoresis or agarose electrophoresis in which this is these are the DNA standards.

So, let us say we you know 1 to 20 kilo base per markers are there and now we are having we have 8, 6, 4 and 2, so just imagine that you have run certain standard of the DNA and then based on those DNA standards, you are now trying to our see this plasmid fragments, how many fragments you can resolve on that agarose gel, looking at them you can see there are 4 gel piece, gel bands are appearing on the gel, one is 8, 6, 4 and 2 kilo base pair.

It is possible in certain cases that you have 2, 6 Kb fragments and both of them in that case are going to separate on the same place because in the electrophoresis, it is only going to separate based on the size, so that may create some confusion but in this situation, it was only one fragment you had, so it was much more straightforward.

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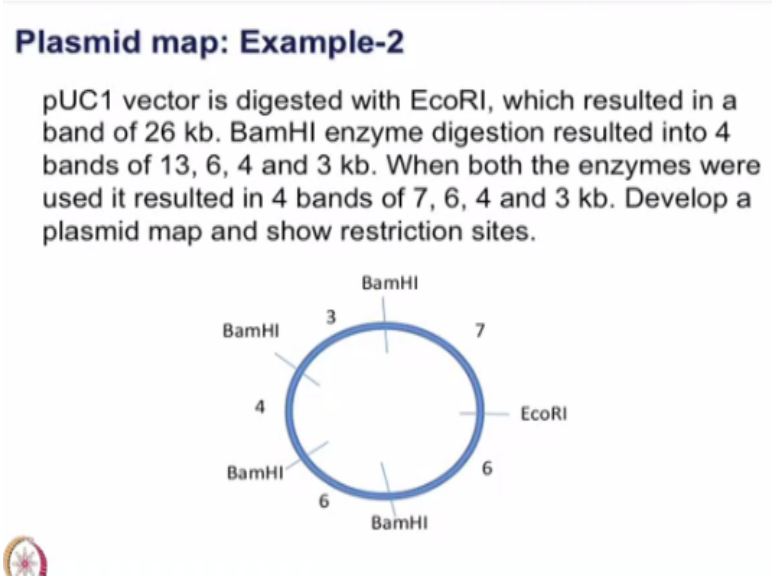

Alright, so this is much more a straightforward example, where we have 2 enzymes; Hind 3 and Nco1 which created different fragments of 6, 2 and 12, after adding Nco1, you got 8 and 4. Now,

let us take another example, where we have another vector which is pUC1 vector and now, you are using enzyme EcoR1, which resulted into a band of 26 kilo base pair, so if you have use BamH1 enzyme, it made 4 fragments; 13, 6, 4 and 3 kilo base pairs.

Now, when both the enzymes were used, you still see 4 bands; 7, 6, 4 and 3 Kb, so please develop a plasmid map and show restriction sites; restriction sites are the places where restriction enzymes are going to work, they are going to cleave, so do not need to know anything about how these enzymes work, what are the name of these enzymes or the vector, just first draw a circle used you know for 1 enzyme, what are the possible fragments?

Now, if you add another enzyme where those fragments will fitted, so I am sure something which you can easily do, you have to just try out multiple possibilities that where these fragments can give you the right size fragments from the both the enzymes, right. Now, let us one more situation, when we have multiple enzymes being used, pleases try this now.

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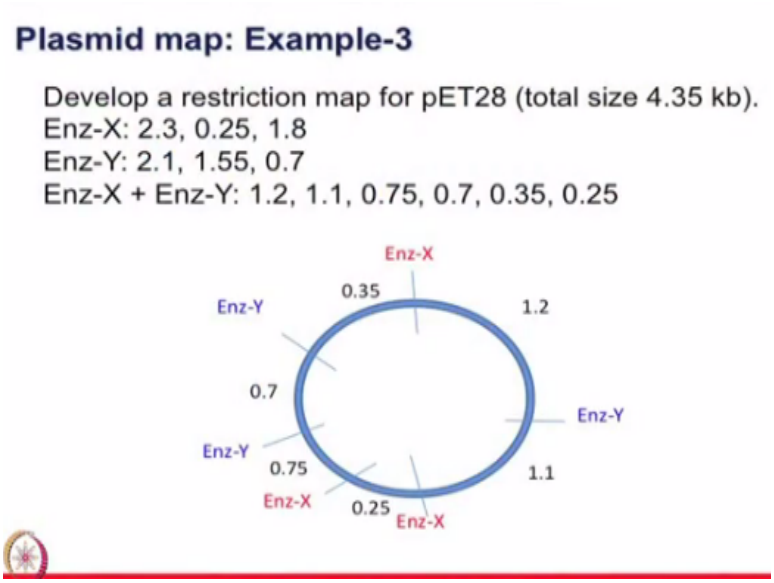

You have used the enzyme X and enzyme Y and also enzyme X + Y for a vector which is pET 28, which gave different sizes of restriction, different bands which you can see of 2.3, 0.25 and 1.8, in another case, you have 2.1, 1.55 and 0.7 and with both the enzymes used, you have multiple fragments from 1.2, 1.1, 0.75, 0.7, 0.35 and 0.25, so now you have do 2 things; one is draw the vector map, where these bands are going to be placed on the vector map.

And second; draw an electrophoresis gel; electrophoretic gel, agarose gel and draw that you know, how the bands will appear if you have used only enzyme X and Y and when you have used only enzyme X or only enzyme Y, where the bands will be appearing on the gel, 2 things we will try out, now this is a pattern which will look for the vector map, can you now draw how your electrophoretic pattern will look like for the agarose gel.

This was the gel right, so just imagine you can use some theoretical numbers here as the standard, then use one lane for running X and lane X based fragments, another lane for a lane Y based fragments and third lane for a lane X + Y, how the patterns are look like, alright, so we cannot have so much time right now, please take this question and do it back in your room, when you reach back and try out this thing yourself.

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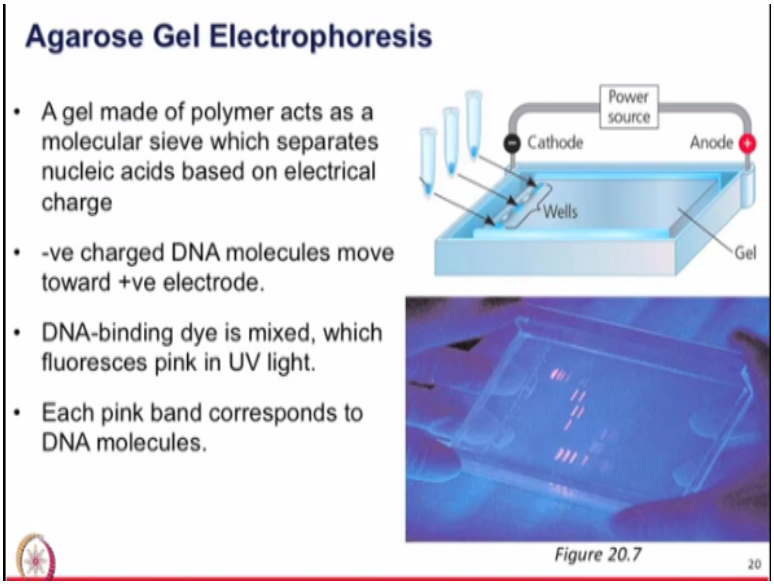

So a technique which is known as agarose gel electrophoresis is useful for separating DNA on the gel mattresses, where the DNA is negatively charged, so it can move towards the positive electrode and this property is being used to separate DNA in this particular type of gel electrophoresis. Now, to visualise DNA you can add certain dye, which bind to DNA or DNA can also be visualised with the ultraviolet.

So, one could used even that to visualise the DNA and sometime if you are visualising on the transilluminator, you will see these kind of pink DNA gel, the DNA pattern will appear on the gel in fact, today we will have some demonstration at the end, how the agarose gel electrophoresis and how you can visualise the DNA on the gel because we have been discussing about many times about gene and DNA, how people when actually do the experiment how they look on these gels.

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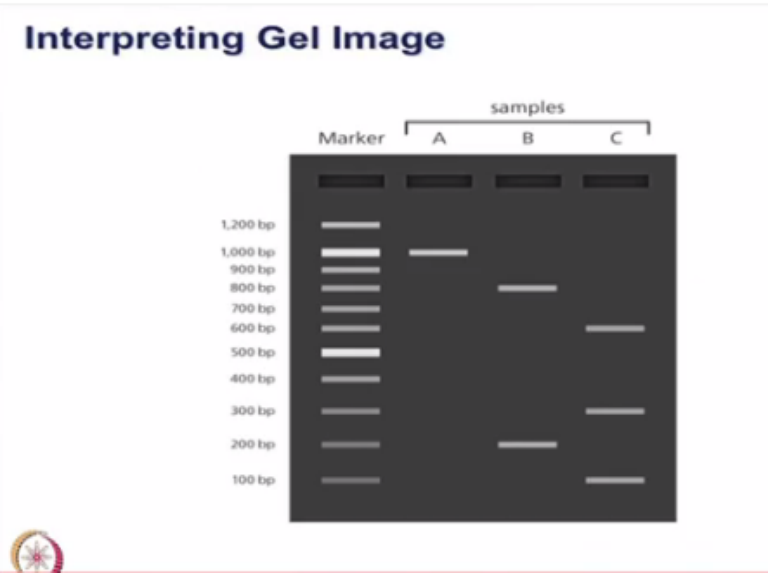

We will have some little bit demo of that today, so just imagine the previous question, if you are talking about X, Y and X + Z, right, so in this case let us say we have you know only with one enzyme this is a pattern emerging, when we have used 2 enzymes, this is the kind of pattern is there and when we use both the enzymes, this additional pattern is emerging, so this is how you have to first draw different lanes and then try to see what can be the overlap image from all of these lanes, right, this is how you have to derive the electrophoretic based questions based on the vector maps.

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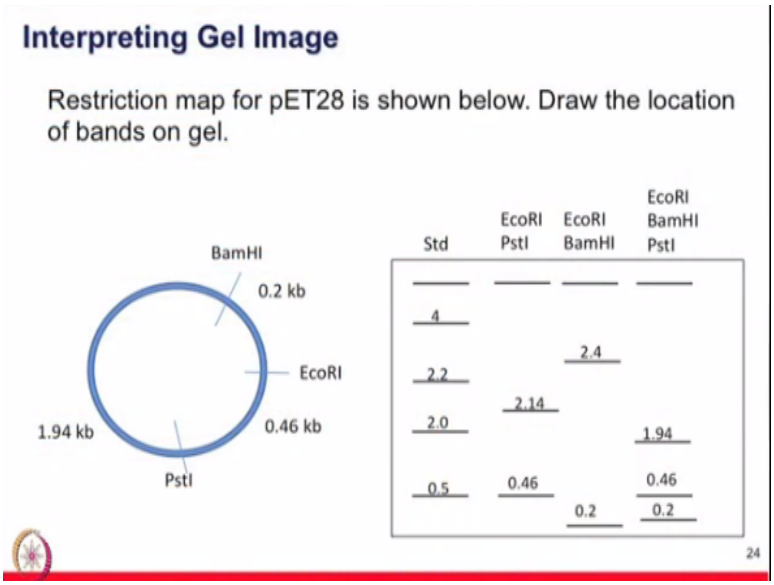

So, if this was a question which is something similar to what we talked earlier but just simplified here, you are having this particular electrophoretic patterns to make, so imagine in this case, we have 3 enzymes; BamH1, EcoR1 and Pst 1, so if you are using one enzyme, let us say EcoR1as well as Pst1 for example, this one, so this fragment is (()) (23:27) 0.46 and remaining one which is 1.94 + 0.2, so you will see here 2 bands appearing for this particular restriction enzymes.

If you have used EcoR1 and Pst1, 2.14 and 0.46, now let us say you have used now 2 enzymes; EcoR1 and BamH1, these 2, so one piece is 0.2 Kb and then remaining one is this whole thing, 1.94 + 0.46, this is going to make 2.4 KB band, so you will see 2 bands; 2.4 Kb and 0.2 Kb. In third situation when you have added all the 3 enzymes; EcoR1, BamH1 and Pst1, you have multiple fragments of 0.46, 0.2, 1.94 and they are being mapped here.

These are the standard which are known markers for DNA which can be used to detect that what can be the exact molecular weight or the size of these particular bands, so by looking at this gel pattern, now you can actually extrapolate these information and also draw the vector map, so I am sure by giving you this information understanding, it will be very justified to ask you questions in either way.

If I show you a gel pattern and tell you different restriction enzyme being used, then you have to give me information for how the vector map will look like or if we give you vector map, you can

actually draw the information how it will look like in the electrophoretic band patterns, so these 2 information can be easily deduce from these kind of questions.

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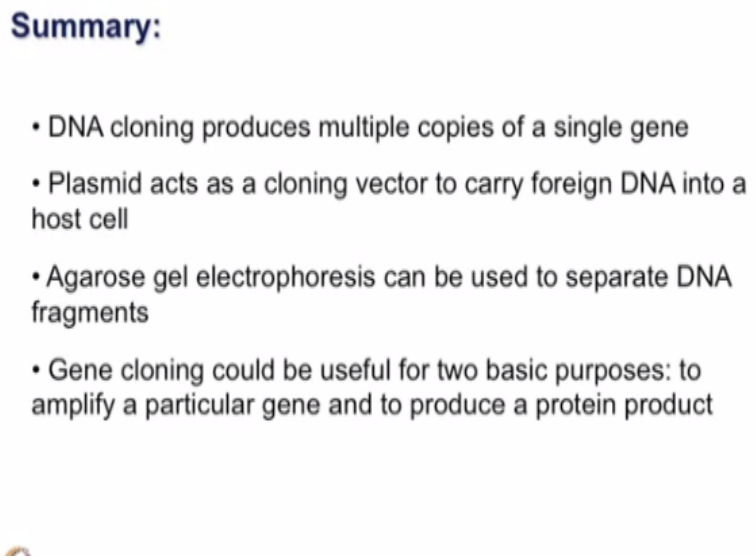

So, in summary so far we have studied that the cloning is very important phenomenon, the process in which multiple copies of a DNA or the single gene can be produced, plasmids are used as the vector to carry the gene of your interest, the foreign DNA and they can be moved into a host cell especially, bacterial cell. Agarose gel electrophoresis could be used to separate these DNA molecules and gene cloning can have many applications, the majorly you can see the 2 broad purpose; one is to multiply the gene copies and second; to make various therapeutic protein production, recombinant protein production.

So, these 2 can be the broad applications which has you know n number of already known application which we can see coming out of the biotechnology field, so just by understanding cloning and how the vector's work, lot of things can be done.

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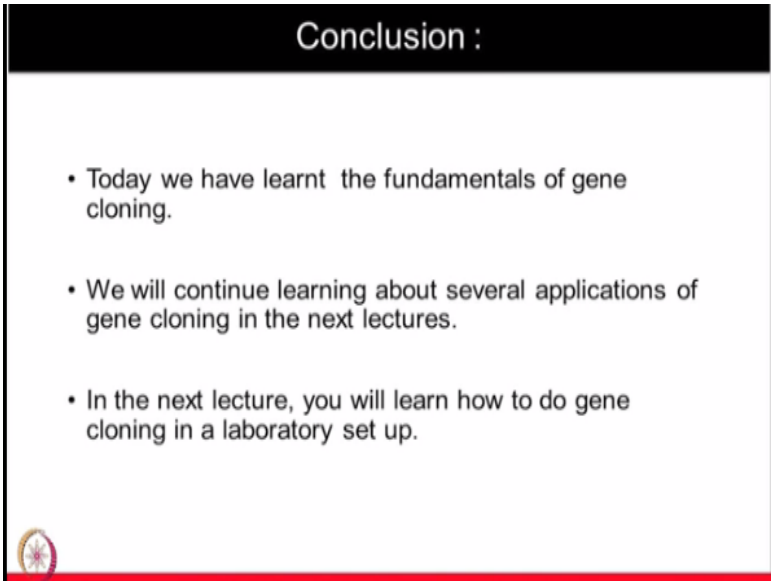

Today, we have learnt the fundamentals of gene cloning; we will continue our discussion on practical application of genetic engineering and DNA cloning in our next lecture. In the next lecture, you will also see how gene cloning could be done in a laboratory set up, thank you.

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