Introduction to Complex Biological Systems
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Lecture 53
Overview of genetic engineering and its impact on society

Hello everyone. Today, we are going to discuss an overview of genetic engineering and its impact on society. This is particularly for human society, and now, these are the major concepts I am going to cover today. The concept of genetic engineering, and then followed by genetically engineered products for human uses, and a brief overview of tools and techniques required for genetic engineering. So, in this Module 11 if you can remember the last two lectures, mostly I was discussing some techniques which are very important for genetic engineering. In Lecture 51, I was discussing agarose gel electrophoresis, where you can analyze different types of DNA molecules based on their size, and polymerase chain reaction, where you can amplify a particular DNA sample.

In Lecture 52, we were discussing DNA sequencing. Whenever you are doing some kind of genetic engineering or trying to manipulate some genes, after manipulation, we always have to check whether our manipulation is right or wrong. So, that is why DNA sequencing is also very important. That is why I covered those three topics first before the actual discussion about genetic engineering. So, what is genetic engineering?

So, if you see, genetic engineering means manipulation of an organism's genome and this manipulation can be of different types. For example, it can range from changing one base

pair for example, I would say if this is our DNA and you know that we have two strands in our DNA. This is 5′ and 3′. So, if I write some sequence like this, so, some sequence there. I would say this is part of a gene.

The gene might be much longer than this, but this is just a segment of a gene. Now, what I am trying to say here is that we can just change one base pair; that means, if I concentrate here, for example, we have TA. So, we can change it to, in this strand on the top strand, we can make it A. So, the lower strand will be T, or on the top strand, we can put G, and the lower strand should be C based on AT and GC base pairing. So, this is just one base pair change. What we can do is delete a whole region of DNA. So, for example, in E.coli we have just one DNA molecule, one circular DNA, and I would like to make some kind of E.coli where I would like to delete a particular gene for my own interest, whatever research I am doing in my lab; I would like to delete one gene, which is also an example of genetic engineering, and not only deleting, the other thing is what we can do: we can introduce an additional copy of a gene. That is also very common. Sometimes to attribute an additional property, we can provide some extra copy of a gene. So, that is very common and I will discuss it in more detail in today's class itself. If we go a little bit more, we can also think of extracting DNA from an organism's genome and combining it with the DNA of another individual. In the previous point I was talking about the same organism.

But here we can take some genetic information, some gene from a different organism and put it into a different organism. That is also very common and useful in genetic engineering. We do that very often. Here are all those things we are doing because we used to enhance or modify the characteristics of an individual organism. So, that is our goal for this genetic engineering approach. Now first I will briefly discuss genetic engineering and its impact on human well-being. Genetic engineering has been used a lot in agricultural applications for a long time.

Genetic Engineering

❑ Manipulation of an organism's genome

❑ Can range from changing one base pair (A-T or C-G), deleting a whole region of DNA, or introducing an additional copy of a gene

❑ Extracting DNA from an organism's genome and combining it with the DNA of another individual

❑ Used to enhance or modify the characteristics of an individual organism
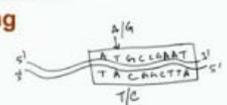
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So, the thing is, in agricultural applications, there are many examples, but I would give you some classic examples of genetic engineering. So, here for example, nowadays whatever cotton we cultivate even in India. Those cottons are called Bt cotton. So, Bt cotton is now available everywhere in the US, China, and India; Bt cotton is being used everywhere.

So, now, if you see this term came from this Bt, it means this is the name of a bacteria. So, this is bacillus thuringiensis. So, this is a bacterium. Now, B and t came from here, that is why this is Bt cotton.

So, what happened is this bacterium produces some kind of toxins. A lot of toxins, almost more than 100 different types of toxins, are available in these bacteria. So, those are commonly called Bt toxins because Bacillus thuringiensis toxin and many of those toxins are actually very toxic against insects. So, what scientists and researchers have done is that they have taken some of these toxin-producing genes.

So, they have taken some of these toxin-producing genes from this bacterium. So, they have taken some of these genes and integrated them with the cotton plant. So, as a result of that, this cotton plant produces this toxin everywhere in its cells and tissues. So, when those cotton plants grow, insects, particularly cotton bollworms, if they eat the leaves of this cotton plant, they will not mature; they will die. So, this is a very good pest control mechanism here, in this case, through a genetic engineering approach. So, as a result of that, you do not need to spray insecticide or pesticide from outside; it is already built-in, already present inside the cotton plant itself. So, this is one example: genetically modified

sweet corn produces a huge amount of sugar and starch. So, there are a lot of different types of examples available which we are now using in the agricultural field.

Then the application in dairy and poultry farms, I will not take much time here. So, for example, you know that different types of hormones are routinely used to increase the amount of, for example, cow milk and also to support the growth of poultry; all those things are very much dependent on growth hormones. So, I would say these are growth hormones and these hormones are produced through genetic engineering approach. So we are making those hormones in a lab or in some pharmaceutical industry and then we are actually commercializing this growth hormone for dairy and poultry farms.

Now, the third one is an environmental application. There are many different types of applications. I am just giving you some kind of overview, some important examples that is all. Now, environmental application, naturally some bacteria can metabolize hydrocarbons, but scientists engineered those bacteria in such a way that they are very stable and can metabolize hydrocarbons very nicely; hydrocarbon means they can even metabolize petroleum. So, as a result of that, these are generally commonly called oil eating bacteria for example, this one, if you see Pseudomonas putida.

So, this is one bacterium, and scientists engineered this bacterium in such a way that it is very stable and can metabolize hydrocarbon petroleum very nicely. So, what is the benefit? The thing is these kinds of bacteria we can use for clearing environmental pollutants. For example, in oil spillage, these kinds of bacteria can be used so that they can scavenge the oil and metabolize it. So, as a result of that, it is a much better mechanism to take care of this oil spillage.

So, I would like to particularly mention that this oil-eating bacteria and this Pseudomonas putida, this strain was actually designed and mostly done by one of the Indian scientists, his name is Anand Mahan Chakraborty. So, he contributed a lot in making this oil eating bacteria. Now, therapeutic application, many therapeutic products are being made through a genetic engineering approach, but one of the very important products which we all know is insulin, particularly I would say human insulin was produced through genetic engineering approach in bacteria.

That insulin nowadays we are using to treat diabetic patients is one of the very important examples of therapeutic application. Apart from that, there are different types of growth hormones also available for therapeutic application. So these are some examples of what you can do through a genetic engineering approach. Again some genetically modified products, some interesting products I mentioned here. For example, if you see this tomato, this is called flavor saver tomato. So, as you can see this is a genetically modified tomato and the idea here is this tomato is modified in such a way that their outside wall, this part if you see is much thicker. This is thick and during the ripening process it will not be very thin soon, it will take some time.
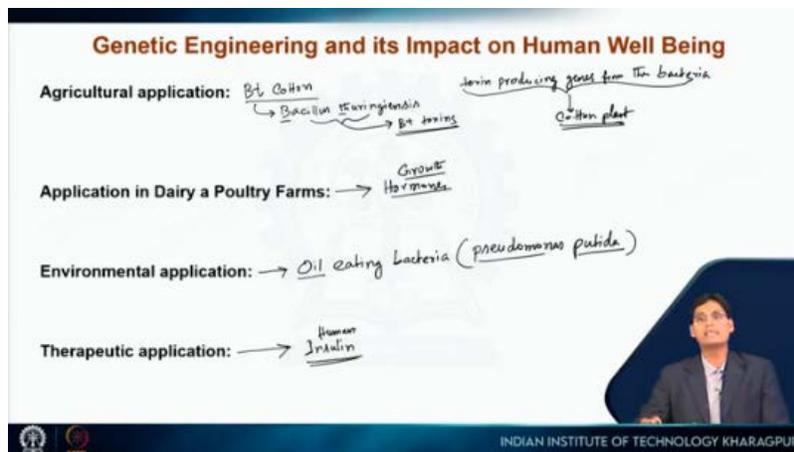

So, the idea is they engineered some enzymes in such a way that this process will be slow. So, as a result, this tomato should not be very soft. So, the shipping is very easy, and for long storage, this is also very good. So, this is a genetically modified tomato. This one is a zebrafish. As you can see, it is called a zebrafish because of these stripes, like a zebra. This zebrafish is very common in Southeast Asia, for example, in India, Pakistan, and Bangladesh.

What researchers have done is, they took this fish, which already looks nice, but in addition, scientists added some genes from other organisms, like jellyfish. Jellyfish come in different colors, so they took some genes from jellyfish and incorporated them into the zebrafish so as a result, from the zebrafish, we now have this GloFish, and they are glowing. So, as you can see, people are keeping these GloFish in their aquariums

nowadays. They are very much available commercially. So, you can see here, for example, this fish. So, it is green in color.

So, this zebrafish is expressing a protein called green fluorescent protein, or GFP. Similarly, this fish is expressing YFP, or yellow fluorescent protein, and this one is expressing RFP, or red fluorescent protein. We use these proteins very frequently in the lab for different purposes, but here we are discussing them in a different context: they change the color of the fish, and people are keeping these fish in aquariums. That is all. Now, the production of human insulin is the best example of genetic engineering, which is also called recombinant DNA technology. I would like to briefly summarize how people used human insulin in the past and how we are making insulin in modern times, currently.
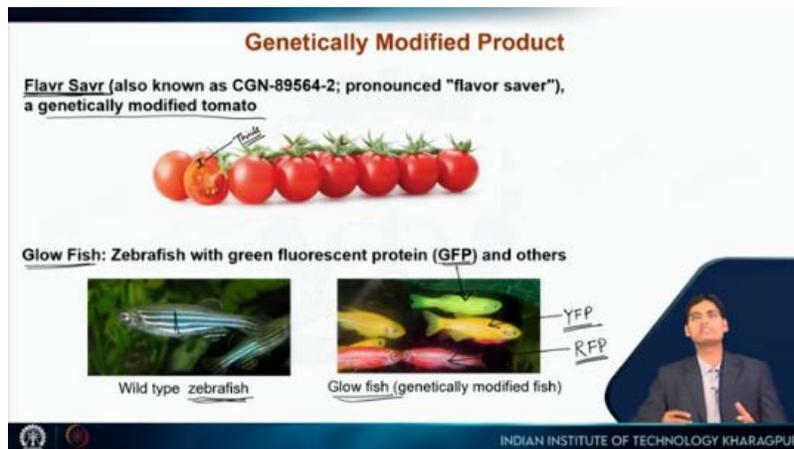
So, here the production of insulin in 1921 Frederick Banting and Charles Best successfully purified insulin from a dog's pancreas. and over the years, scientists made continual improvements in insulin production because insulin is a very important therapeutic reagent for many of us who suffer from diabetes; insulin is absolutely required when diabetes is really difficult to manage by medicine. So now the thing is later on many scientists also purified this insulin from pig from horse different types of animal, but the problem is you have to sacrifice a lot of animals that is one thing and the other thing is although those are insulin of those animals, but still they are foreign protein. So, whenever humans are using this protein, this insulin, insulin is actually a

protein. So, when this insulin we are using, which is actually produced in a different type of animal, is foreign protein to us.
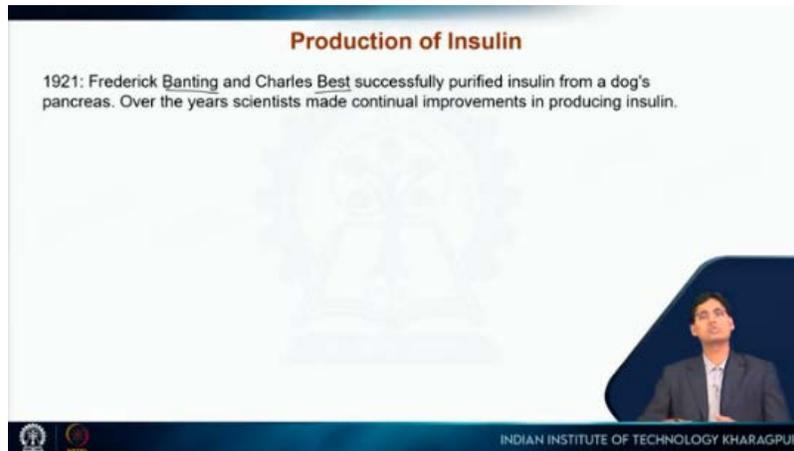
So, as a result of that it can cause some immunogenic reactions. It can vary person to person so it is a problem. So, nowadays, we currently use all insulin, whatever type it is, recombinantly produced human insulin, not the dog or pig insulin which were used much before, a long time back. Now, as you can see here in 1980 researchers used genetic engineering to manufacture a human insulin. This is the first time they use human insulin. Why 1980? Why not long ago? Because when I discuss in much more detail about genetic engineering, you will know that the problem is genetic engineering means you need some kind of tools and techniques where you can actually manipulate our genes or genomes.

But those tools and techniques were discovered mostly in the 1970s. So genetic engineering was not possible much before and in 1982, The Eli Lilly Corporation produced human insulin, which became the first approved genetically engineered pharmaceutical product for human use.

So, this is the first product, and at present, as I already told you, researchers could produce genetically engineered insulin in unlimited supply without depending on animals. How? The answer is through a genetic engineering approach. Even I would say, in India, one pharmaceutical company, Biocon.

They produce a lot of insulin in their company itself. Now, keywords in genetic engineering, I would like to explain a few terms in order to discuss mechanistically how we carry out genetic engineering. The first one is the gene of interest. So, here, the gene of interest means a DNA segment that is to be inserted or deleted. So, for example, if I want to delete some genes from some organism or I would like to add an extra copy of a gene into a tomato plant.

So, for example, I already discussed Bt cotton. That means, we put some genes from bacillus thuringiensis bacteria into the cotton plant. So, that is our gene of interest, the gene which produces this toxin. So, this is the gene of interest and then the next one is the vector. So, vector here means DNA molecule such as plasmid.

So, plasmid is an example of a vector; there could be other types of vector also. I will be mostly discussing plasmid in our discussion. So, plasmids are small circular DNA, small circular double stranded DNA. So, this is plasmid double stranded DNA. There should be a helix also. I am not showing here and this is an extra chromosomal that means this plasmid is present naturally in some bacteria.

So this is present naturally in some bacteria, but they are not part of their chromosome; bacteria have one circular chromosome. In addition to that some bacteria might have this plasmid and these plasmids are very useful for our genetic engineering strategy and I am going to discuss soon. Now, the idea is that this is used as a vehicle to carry the gene of interest into another cell where it can be expressed. For example, this plasmid we modify, this plasmid we are giving some additional genes here; this is our gene of interest.

So, it will make some kind of protein which we are interested in. Now, this plasmid we can send to and transfer into a bacteria. So, this bacterium is a bacterial chromosome. This is a bacteria and this is a bacterial DNA chromosome. Now, we are transferring this plasmid into this cell, and these bacteria will make the product from this gene itself.

So, as a result of that, although this gene of interest was initially not present in this DNA, when we transfer this plasmid here in this bacterial cell, the host cell, we say. Now that particular gene will be expressed. Expressed means I discussed at the beginning that expression means here from gene to RNA and RNA to proteins for the product we will be getting from this bacterium. This is called a vector because sometimes in school days we read that mosquitos are also a vector. Why a mosquito is called a vector? Because a mosquito carries some germs, and due to its bite, someone else gets these germs. So, all of you know that malaria is carried by mosquitoes.

So, a mosquito is called a vector similarly, this kind of small circular DNA which is called plasmid in this case. They are also called vectors for genetic engineering. Now, transformation means the transfer of the gene of interest into a host cell, maybe a bacterium where it can be maintained as well as expressing what I already explained that this plasmid was used to transform this bacterium. So, as a result of that, whatever bacteria were present before now has some additional property. It is transformed. Why? Because some extra copy of genes we have already provided in this bacteria. So, that is why it is transformed and the process is called transformation. The last keyword here is clone, which means organisms carrying identical genes. That is all. This is called a clone. If we talk about organisms, then two organisms are exactly similar by their genetic element. They are carrying exactly the same genetic information, then those two are clones to each other.

Similarly, if we make this plasmid again and again, many copies of those plasmids are exactly the same. So, those plasmids are also cloned to each other. That means, they are exactly the same. That is all. So here I am going to discuss the basic idea of genetic engineering during this lecture. In our next lecture, I will be discussing more technical details about those things. For example, if I say I am interested in some proteins which are present in some bacteria.

Key Words in Genetic Engineering

❑ **Gene of interest:** DNA segment that is to be inserted or deleted

❑ **Vector:** DNA molecule (such as plasmid) used as a vehicle to carry gene of interest (or foreign genetic material) into another cell where it can be expressed

❑ **Transformation:** Transfer of gene of interest in to a host cell (may be bacteria) where it can be maintained as well as expressed.

❑ **Clone:** Organisms carrying identical genes

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For example, nowadays there are many detergent powders which we use for cleaning our clothes. So those detergent powders also contain some enzymes. So for example I would say cellulase, lipase. Those are enzymes Means they are proteins and those proteins, if you add in detergent, they will work better. So for example, if you have some stain from some green grass, you are playing cricket, for example, and you skid for a catch and then some green stain maybe in your trousers. If cellulose is present in detergent it will clean that stain very nicely. As a result, this kind of enzyme nowadays we use in detergent, and this enzyme we can engineer also to make it more stable. Sometimes when we clean our clothes, we use a little bit of hot water.

So, if you engineer those enzymes in such a way that those enzymes will be thermostable. They can tolerate a little bit more heat. So, all those things you can do in the lab. You can engineer those genes which are producing cellulase, lipase, and all those things, and then you can transfer that into the E. coli strain and you can make more and more products. So, I am giving you just one very simple example.

For example, this cellulase is the enzyme you have taken. So, this is a portion of DNA. So, now, this portion is a gene that codes for an enzyme called cellulase. So, what will you do?

You just use some specific primer to amplify this gene. As I already discussed, how is the PCR technique so you amplify this gene. So this gene fragment will have many more copies of this gene, and now this is after PCR reaction. This is primer. Now you use some

plasmid. This is a circular plasmid, but now what you have to do is somehow use some enzymes that will cleave here.

So, it will make a cut, and then you have to put your gene of interest here. So, as a result of that, the new gene of interest you put here. This is, I would say, a cellulase-producing gene. Now you can use this plasmid to transform some bacteria.

So, now this is the bacterial chromosome, and this is the plasmid, and this bacterium will produce cellulase enzymes for you. So, the thing is here when we are sending this plasmid into this host cell bacterium. This is bacteria. We can control the number of plasmids because most of the plasmids we use for genetic engineering have a resistance gene; for example, most of them carry an antibiotic resistance gene. So, as a result of that, after transformation, when you are culturing these bacteria, if you increase the concentration of that particular antibiotic, the antibiotic-resistant gene is already present in the plasmid. So the bacteria will try to survive. So, as a result of that, that plasmid will be copied more and more. So, plasmid replication will happen, and this plasmid replication is independent of chromosome replication. So one bacterium might contain a huge number of plasmids, and remember, all these plasmids also contain your gene of interest.

So, as a result of that, they will produce a huge amount of cellulose for you. This is the overall idea of how we make the protein of interest in a bacterial system. So, I just gave an example of how to use cellulase or how to produce cellulase or lipase, some proteins for human use, for example. Sometimes in a research lab, we also use this system for a slightly different purpose. For example, if I am interested in some proteins which are present in MTB, MTB means mycobacterium tuberculosis.

It is a pathogen. So mycobacterium tuberculosis, this strain of bacteria, cannot be used in a lab here and there; you should have a proper facility that is called, biosafety level, a very high biosafety level facility should be there. If I am interested in that protein, then how can I grow mycobacterium tuberculosis in my lab to get the protein? It is not possible if I do not have that facility. Rather, what I can do is I can clone a gene from this bacterium, whatever I am interested in, that particular protein. So you can take some

mycobacterium tuberculosis genome, just the DNA and then continue the PCR reaction depending on. You have to use gene-specific primers for that particular gene you are interested in. On the basis of that, you have to design your primers, then you amplify that. So, as a result of that, you will get the gene of interest, which is coming from MTB or mycobacterium tuberculosis. Then you just amplify that means nothing but the PCR reaction, and now you have many copies of this gene. These genes you have to again insert into a plasmid, and that plasmid should be transferred into a laboratory strain of E. coli. So, as a result of that you can make that mycobacterium tuberculosis protein in E. coli itself. So, that is why this is called heterologous protein expression. That means you are making this protein in E. coli, which is a non-pathogenic laboratory strain that you can use in almost all labs. But you are actually purifying the protein from mycobacterium; you are getting the protein that actually originated from mycobacterium tuberculosis, but without culturing mycobacterium tuberculosis itself. So, very commonly, we do this kind of work in the lab.

So, all those things are because of the genetic engineering approach. So, here, genetic engineering means in bacteria, we are adding an extra copy of a gene that is producing some MTB protein. That is all. Now, this will be a little bit different when we talk about eukaryotic proteins, particularly. That is why I intentionally provided some examples of MTB proteins.
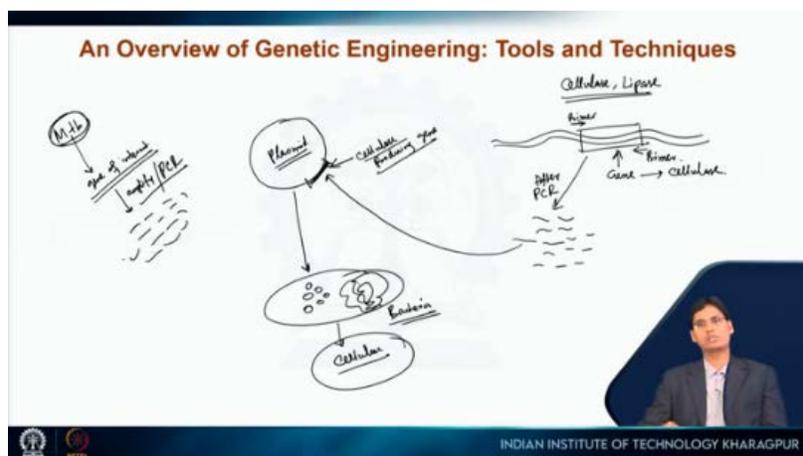
But, for example, sometimes during the cell adhesion process, I discussed E-cadherin a lot, which forms adherens junctions in our body. This is a membrane protein present in

our body; mostly on all epithelial cells this protein is present. Now, if I am interested in working on E-cadherin, then we have to clone this because it is very difficult to get pure protein in high amounts from original tissues. You could say that we will take this protein from some epithelial tissues, but where will you get that much epithelial tissue? And again, you have to purify it. It is a very challenging job. Much before the genetic engineering approach, scientists and researchers used to do that. But now it has completely changed; we are relying on the genetic engineering approach.

So, what do we have to do? We have to clone this E-cadherin gene into some plasmid. So, this is our E-cadherin, this is the plasmid, and this is the E-cadherin gene. So, here now this E-cadherin gene containing plasmid can be expressed in a suitable host cell, not the vector.

So, host means you can express in a bacterial system. Sometimes, some proteins cannot be expressed in a bacterial system, so you might need to rely on a eukaryotic expression system, for example, yeast cells or a mammalian expression system, which we often use. But I started this discussion because here you have to use slightly different tricks based on how you clone this thing. For example, if you isolate DNA from some human cells, like a human cell sample, so now, from this human cell, you can get DNA, and now if you use this DNA as a template to carry out a PCR reaction using some primer DNA, this is the template, and then do the PCR reaction to amplify the E-cadherin gene, and then you transfer that into this plasmid, but there will be a problem if we want to express this in a bacterial system because, as we discussed during the transcription lecture, eukaryotic genes have introns. So, they have exons and introns, but introns should not be present in mature mRNA. So, as a result, you have to remove introns from your construct, from the gene of interest, which you are inserting into your plasmid. So, as a result, you cannot rely on this thing.

This is not possible in this way rather; we use a slightly different technique. What we do is we isolate RNA from a human cell sample, for example, some cell line you can use for that, also a human cell line. So, you have RNA but we are interested in E-cadherin, E-cadherin is a protein, like a cell surface protein. So that means this E-cadherin has been produced from some mRNA or messenger RNA, and then messenger RNA is transcribed

from the DNA itself, but we are relying on RNA. Particularly now, the major RNA present in our body is rRNA, tRNA, and mRNA. Apart from that, many other small RNAs are also present, but for the time being, I am ignoring that. It would be great if we could purify this messenger RNA because that would actually enrich our initial sample. So how can you purify it? Because mature mRNA in eukaryotic systems should have a poly-A tail at the 3 prime ends.

So, as a result of that, whatever total RNA we have isolated, this total RNA again we can purify through one more step using some oligo-dT. That means in the purification system, if you use some beads which are attached with poly-dT, that means multiple T residues are attached with some beads, and then your poly-A containing mRNA will be attached there. So, the major idea here is that through this method, you are purifying mRNA or messenger RNA. From this messenger RNA, you can perform reverse transcription, and then what will you get?

You will get cDNA, and this cDNA you have to use for the PCR reaction, then you can put that in some plasmid. So now, this is your E-cadherin gene present in the plasmid, but this E-cadherin gene does not have any introns; it has just exons. Why? Because we have isolated mature mRNA. It does not have any introns, and now that mature mRNA was converted into cDNA, and from there, we made the PCR. So, this is again a very important step in the case of eukaryotic proteins. When you are working with eukaryotic proteins, it is better to start with RNA and then make cDNA so that you can ignore the introns part.
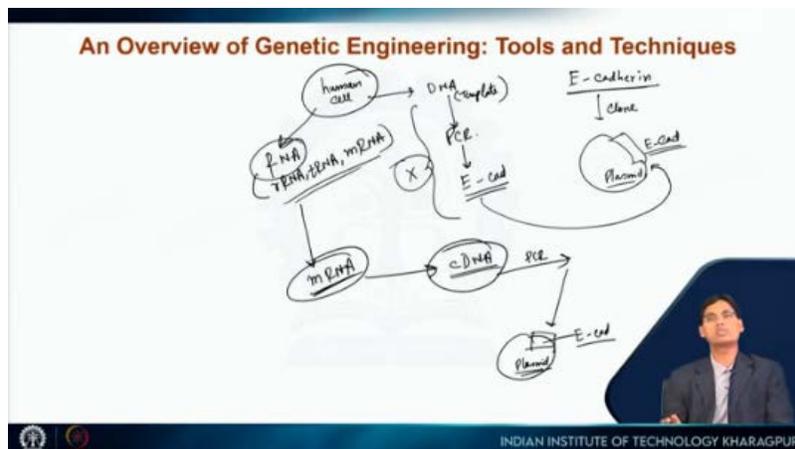
So, this is just a little bit of discussion. In the next class, we will discuss in more detail about genetic engineering. So, that is all. You can follow any of these textbooks. Thank you very much.

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