

Cell and Molecular Biology
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Concepts of Genetics (Part 2)
Lecture - 29
Transgenic Organisms

Hello everyone, this is Dr. Vishal Trivedi from the Department of Biosensors and Bioengineering, IIT Guwahati. Now in today's lecture, we are going to discuss the transgenic animals. So, when we talk about transgenic animals, they have foreign DNA deliberately inserted into their genome to express the desired gene product. This gene product could be of multiple types depending on the type of transgenic animal that is going to be produced. They are produced using several common molecular genetic techniques such as DNA pore nuclear microinjections, embryonic stem cell-mediated gene transfer, or retrovirus-mediated gene transfer.

So, there are three methods through which you can generate transgenic animals. Number one is the DNA pore nuclear microinjections, number two is embryonic stem cell-mediated gene transfer, and number three is retrovirus-mediated gene transfer. So, in a particular transgenic animal protocol, what you are going to do is isolate the gene from the organisms, right? So, you are going to actually isolate a DNA sequence containing the human gene, and then the cells from the ear of a goat are isolated. multiplied from a small or the auricular biopsy.

Then a cell transfection procedure is carried out using an electrical discharge. It causes the membrane of the goat cell to open, allowing the integration of the gene into the genome. So that is how you have actually isolated a gene from humans. You have isolated the cells from the goats. Then you did the gene delivery protocol, so you actually did the electroporation, and that is how the gene is going to be inserted into the cell.

Then you are going to screen the transgenic cells. So the best transgenic cells are selected and inserted into the goat oocyte from which the maternal DNA had been removed. So you are actually going to take the oocyst from the goat cells, and then you are going to remove its original nucleus so that it does not express the gene from the original nucleus, and then you are going to insert this recombinant DNA. Then you are going to have another electroporation procedure, and then there are embryos that are going to be generated, okay. Where the human gene is going to be, you know, present, right? And to determine whether the gene is present, the protein can be analyzed with the help of Western blotting.

You can actually use many of the blotting techniques; for example, you can do southern blotting to see whether the gene is being integrated into the genome or not. And then you can do the northern blotting to see whether the protein, you know, the RNA is being synthesized from that particular gene or not. And then you can do the western blotting to see whether that particular gene is being expressed or not. And then ultimately, you are going to have the transgenic goat, which is actually going to express this particular protein, and because of this particular protein, it is actually going to have the different variants of the milk. So, in order to become a medication, the milk protein has to be purified and transported into the injectable product.

From this goat, you are going to get the milk, and that milk is actually going to have the protein. That protein can either be purified from the milk, and that is how you are actually going to have a medicine. So this is just a general protocol for how you are going to create a transgenic animal and what the advantages of transgenic animals are. Now let us move on to the techniques through which you can generate a transgenic animal. So the first technique is the DNA pronuclear microinjections.

So in the DNA pure nuclear microinjections, what you are going to do is use a fine needle to inject the DNA solution into the male pronuclei. This is done with extreme precision to avoid damaging the egg. So, using a fine glass needle, the DNA solution is injected into the male pronuclei, and then the injected DNA might or might not integrate into the genome of the egg, and this is why the selectable markers are included in the construct. The embryos that have been given the transgenic injections are grown in vitro and subsequently implanted into the uterus of the foster mother. So what you are going to do in this is take the egg, okay? And then you are actually going to inject the DNA from the male pronuclei, and that is how they will actually fuse to give you a zygote, and then these zygotes are actually going to be implanted.

Implanted into the uterus of the mother, some of these embryos will probably grow and actually provide you with the DNA. This is one of the easiest strategies employed to produce transgenic animals. Superovulated females are produced by hormone treatment, and they are mated with the males. Just after fertilization, the egg contains a small female pronucleus and a large male pronucleus. In the pro-nuclear microinjection technique, the transgene is directly injected into the large male pronucleus.

So what you are going to do is this: it is a very simple technique where you are actually going to take the superovulated female. So this means you are actually going to inject the female hormones in large quantities so that the females are actually going to produce a lot of eggs, and then you allow these females to go for mating. After mating, there will be,

you know, eggs that are actually going to contain the female pronucleus and a large male nucleus. Now you target this large pro-nucleus, and then with the help of the needle, you inject the transgene directly into this large male nucleus. And as a result, what will happen is that there will be a fusion of this transgene with the male nucleus, and that is why the male nucleus is going to start producing the transgenic gene.

It is going to start expressing the transgene, and that is why you are going to have the transgenic animal. Now the second technique is embryonic stem cell-mediated gene transfer. So embryonic stem cells are the cells that, under the influence of different signals, can differentiate into different types of cells, right? Now this is the definition of embryonic stem cells. So they can actually be if you take the embryonic stem cell, they could be differentiated into macrophages, they can be differentiated into skin cells, and they can be differentiated into the heart, lungs, liver, and pancreas, or any of those cells, right? And that is why this means they are actually going to have the enormous potential of achieving differentiation and de-differentiation. Embryonic stem cell-mediated gene transfer is a sophisticated technique used to create genetically modified animals, especially mice, for studying gene functions, disease mechanisms, and various biotechnological applications.

The embryonic stem cells are collected from the inner cell mass of the blastocyst stage and cultured in a media that contains the leukemia inhibitor factor, or LIF, to maintain their pluripotency. So you do not have to worry about these terms, such as what is meant by pluripotency and all that kind of thing. So what you are going to do is take the embryonic stem cells that you are going to collect from the blastocyst, and then you are going to culture them in the in vitro system. So what you are going to do is take the embryonic stem cells and then culture them in a culture plate, right? While they are culturing, you can actually inject the DNA you would like. So this is going to be a transgene that you can inject with the help of different types of DNA delivery methods.

And then you are going to select the cells that are expressing the desired genes. So, at this stage, you may have to perform the different types of blotting techniques. So you can actually perform the southern blotting, you can also do the northern blotting. and you will be able to do the western blotting, okay? Remember that in the previous lecture we discussed these blotting techniques. So with the southern blotting, it will tell you whether the transgene is integrated into the genome or not.

With the northern gene northern blotting, it will tell you whether this transgene is forming the messenger RNA or not. And with the western blotting, it was actually going to tell you whether the protein has been expressed or not. And that is kind of a confirmation, and based on these properties, you may actually be able to select the cells

as well. There are cells that may contain the transgene, and they will be positive in the Southern blot. They may be positive in the northern blotting, but there are some issues.

Maybe the promoter is not good, or something happened. It may not be expressing a desired protein, or it may be expressing a protein, but the protein is either degraded or something else. So you should not use those cells. You select the cells that are actually going to be where you are going to find the DNA. where you could find the messenger RNA and where you could find the protein product in adequate quantities and your desirable 3D conformations.

Then you inject these transformed embryonic stem cells into the inner cell mass, right? So you are going to inject them back into the blastocyst, right? Then the inner cell mass is going to grow, and there you are actually going to inject these transformed embryonic stem cells. And then they will be implanted into the uterus, right? And once you implant this into the uterus of a foster mother, I am sure you know about the foster mother. Foster mother means the false mother, right? This guy, there are two mothers, right? You are going to have the real mother, who will be getting the embryo through fertilization, and then you are going to have the foster mother, who is actually going to carry your transformed cells, or you are actually going to carry the embryo that you have produced in the other females. So, then you test the offspring for the presence of the gene; again, you will do the same kind of analysis even in the offspring, which means you are actually going to get the mice. And then you can actually test whether the mice cells are showing the integration of the gene into the genome, whether they are forming the messenger RNA, or whether they are forming the protein as well.

Then the male heterozygous offspring will produce the homozygous transgenic strain, which means initially you are actually going to produce a male heterozygous. I am sure you probably know what is meant by heterozygous and what is meant by homozygous. Heterozygous means the gene is only present on one copy of the chromosome, right? You know that everybody has a two sets of chromosomes, right? You have 23 pairs of chromosomes, and then you have XY or XX, actually. So these 23 pairs have the cognate pairs right. So you are going to have a homologous pair.

This means if an organism is going to be heterozygous when a particular gene is present on one pair but not present on the other pair, that is called heterozygous. And when you have a heterozygous condition, the proteins are only going to be expressed, but they will not be expressed at the full high level, right? And as a result of that, these heterozygous males may actually show you the genotypic changes, but they may not show you the phenotypic changes. And this is very good because you can actually test whether the protein you are expressing may not be lethal for the organism; in some cases, the gene

product may interfere with the animal's normal metabolism, and all those kinds of things are why they are lethal. So when you produce the heterozygous you will see that they are sick but they are not dying. When you produce the homozygous, it means when you crossbreed the two heterozygous species or two heterozygous animals.

they are actually going to give you a homozygous right and as soon as they will produce a homozygous and if this gene product is lethal they will actually going to kill the organisms And that will actually be a warning sign that I should not go with the ectopic expression of this particular gene in this particular system because it is ultimately going to lead to the death of that particular organism. Once you are sure about this, you can implant it into the uterus, and once you have implanted it, it is actually going to give you the animals, which can be used for downstream applications. So, this is the early show I have discussed. The cultured embryonic stem cells are treated with a solution of the DNA construct and taken up by the cells. The transformed cells are injected into the inner cell mass of the blastocyst and implanted in the uterus of a foster mother.

The offspring are then monitored for the expression of these transgenes. A big example of this delivery method is chimeras. These organisms have at least two genetically distinct cell lineages originating from different zygotes. And then we have a third method, and the third method is called retrovirus-mediated gene transfer. So, retrovirus-mediated transgenesis is a technique used to introduce a foreign gene into the host animal genome utilizing retroviruses.

Examples of retroviruses are HIV or human immunodeficiency virus. The genetic material of the retrovirus is RNA, and these vectors stably integrate the foreign DNA into the host genome, specifically in early embryos or the ES cells. The advantage of this method is that the location of the integration is specific, and it reduces the chances of mutation into the host genome. So, what we are doing is producing a transgene, packaging that transgene into a virus, and then this virus binds to the receptor on the cell, delivering the gene of interest, while the double-stranded DNA is also delivered. Then this double standard DNA will enter the genome, and that is how it will actually be integrated into the genome of that cell.

So virus uncoating, reverse transcription, and second strand synthesis—all of these are going to happen by the machinery of the retrovirus, and then ultimately, it is actually going to give you the gene of interest. That gene of interest is going to be integrated into the genome, and that is how it is actually going to give you protein synthesis. It is most important to note that the safety concern regarding retroviruses is extremely high. For this reason, the virus vector must be made replication-incompetent.

It cannot replicate in the host cells. The retroviral vectors are transfected into packaging cells that provide the necessary viral proteins required to produce a complete recombinant retrovirus. The complete virus particles are harvested from the culture media of the packaging cells. So this is very, very important that safety is a very, very high concern when you are using the retrovirus, and that is why you know that the virus has two components. One is the coat proteins, and plus you are actually going to have the genomic DNA; right, you are going to have two components. If you mix them together, it is actually going to give you a functionally active virus particle.

So, when you are actually using the retroviruses, some of these coded proteins are not being added onto the same viral DNA. Where the so suppose a coded protein is required, and suppose you have 5 coded proteins that are required for making the code of the virus. Then what you do is put all five into five different vectors, right? So you are going to have the 1st vector, 2nd vector, 3rd vector, 4th vector, and 5th vector. So when you put them together and add the viral DNA, which actually contains the transgene, it will produce the virus particles, and these virus particles will be taken up by the cell, and that is how it will actually form. But in due course, these plasmids are actually going to be eradicated, or they will not be available to provide their protein product, and as a result, the viral DNA will still be present.

but it will not get packed into a functionally active virus particle and that is why you are keeping control over the self-replication of these retroviruses. Because if there is a self-replication of these retroviruses, they may actually create or cause the disease because they have acquired self-replication, and you are going to treat the animal cells with this particular virus. So, if a person does not even have the disease, it may actually develop the disease because this particular virus is going to be self-replicating, and to avoid that. What you are doing is putting the core protein onto the plasmids, the different DNA, and the viral DNA, which contain the transgenes. So as a result, as long as these two are put together, they will actually form the functional virus particles that you are going to use in the transfection study or that you are going to use for transfections. But as soon as the transaction is over, these plasmids, because every DNA molecule has an age or a lifespan, will degrade over the course of time.

As a result, active virus production will be stopped. The stable integration of embryos or stem cells is finally implanted in the uterus of a foster mother and allowed to grow. The progeny are screened and selected for the desired gene products. So we have discussed the three different methods, right? We are utilizing these three different methods. You can produce the transgenic animals, right? Now once you produce the transgenic animals, there will first of all be a diversity in terms of what kind of transgenic animals you have produced, and then depending on the diversity, you will also be able to have different

types of applications from these transgenic animals.

So let us take a few examples of these transgenic animals and their applications. So, the first transgenic animals, we have taken the class of fish, right? Because fish is very, very important economically, it is a stable product for consumption. So the first transgenic animal is the super fish, right? Super fish are bigger in size; you see that this is a normal fish and this is the transgenic fish, right? So you see how big it actually is. So it is an edible fish, super fish; it has increased the growth and the size, and why it is so is because the growth hormone gene has been integrated into the genome. Because if you produce a very high quantity of growth hormone, it is actually going to give you an increase in length and an increase in body mass.

For example, the transgenic salmon grow about 10 to 11 times faster than normal fish, and this is very important because if the fish grow very big, they will have a very high volume and will also be good for consumption. Then the second is glowfish. So glowfish are transgenic fish that express fluorescent proteins, including different types of fluorescent proteins such as green fluorescent protein, cyan fluorescent protein, red fluorescent protein, and combinations of these proteins, and so on. So that is why you see these fish are actually going to give you fluorescence in the aquarium, and that is why they are of different colors, right? So you can have yellow or golden color, red color, blue color, and green color. So, these glow fish are very, very popular, and they are very, very high in demand for aquarium enthusiasts.

So, these are the ornamental fish, and they are actually expressing fluorescent colored genes, and what they are doing is expressing the fluorescent protein, and people are using the glow fish. Glowfish is a general term. People are developing the glowfish into goldfish. People are developing goldfish into the different types of fish that are available in the aquarium.

So, there are normal fish, and there are glow fish. The glow fish are going to give you fluorescence at night; the normal fish are not. Then we also have the zebrafish. So, zebrafishes are, you know, having the optically clear shell, and they are actually being used for the study and the cure of different disease models, and the globe zebrafishes are also being used in aquariums sometimes. So that is the advantage of having a zebrafish.

Then we also have the grass crops. So grass crop is being used, and this is a transgenic grass crop where you are actually putting the human lactoferrin gene. So it is actually going to give you lactoferrin production, and that is why it is actually going to give you milk proteins. Then we have the enviro pig, which is a pig variety that uses better digestion against phytate. How are you doing this? You are actually integrating the

phytase gene from *E. coli*, so because of this phytase gene, it is actually going to digest some of the plants.

They are also being used for organ transplants. Remember that the pigs are very closely related to humans, not only in terms of physiology but also in terms of the size of the organs, and that is why there are trials where you can actually use the organ from pigs for transplants. Then we have the transgenic cows, right? Transgenic cows are being developed for having higher nutritional values, where you are actually overexpressing the human lactoferrin gene. So when you are overexpressing the human lactoferrin genes, these transgenic cows give milk with lysozymes, lactoalbumins, and clotting factors 8 and 9, etc. So, this is actually going to increase the therapeutic value of that particular milk, and it is actually going to be in very high demand in the European countries. And then we also have the transgenic bulls, right? So in the year 1990, the first transgenic bull, Herman, was created, and the cow's milk that actually contains lactoferrin is also being supplemented because of these transgenic cows.

And what you are doing is putting the human lactoferrin gene, which is produced by a method called pro-nuclear microinjections. Remember that we discussed the three methods. One method we are using is pro-nuclear microinjection, the second method is stem cell, and the third method is retroviruses. Then we also have the transgenic rabbits, right? In the year 1985, the first transgenic rabbit was produced using nuclear microinjection to carry the transgene, and it is a model for cardiovascular diseases (CVD), AIDS, and cancer. And the human arthropoietin alpha glucosidase and the human AAT are the genes that are being used for producing the transgenic rabbit.

And depending on the different types of protein, you are actually going to develop the transgenic rabbit for the various types of applications. Then we also have the transgenic chicken, right? So this is produced by infecting embryos with a viral vector carrying the transgenes, and you can actually put any human protein into the transgene, and that is how it is actually going to increase both the economic and therapeutic value of the egg. So, it is actually going to increase the additional value of 0.1 grams of human protein in each egg. So, it is actually going to produce a very high quantity of human proteins.

An additional advantage of human proteins is that when you produce the human protein, it will actually be produced with the correct sugar glycosylation pattern. Which means if the glycosylation pattern is very important for using these proteins in subsequent steps, for example, if you would like to use this protein as a vaccine material or whether you use these proteins for therapeutic supplements. The glycosylation is very, very important, right? So, this is the transgenic chicken. Then we also have the super mouse, which has been developed in the year 1982. So you see, this is a super mouse, and this is the normal

mouse, right? So, the super mouse has better digestion than the phyti, and the human growth hormone is being overexpressed in the super mouse.

They are large in size and are being used for the mouse model of different diseases. Then we have a transgenic monkey; in the year 1990, the first transgenic monkey, ND, was developed. This transgenic monkey has the GFP protein from the jellyfish, so it expresses the green fluorescent protein produced by the pronuclear microinjection method, and this is a disease model for cancer, AIDS, and Alzheimer's disease, making it very popular. Now, what is the application of transgenic animals? So, there are multiple areas where transgenic animals could be used. So, first is the understanding of the disease mechanism, the second is the understanding of normal physiology, the third is the biological product, number four is the ability to ensure vaccine safety, and number five is the use of these transgenic animals for toxicity testing.

Now, as far as the transgenic animal is concerned, you can actually have two different varieties of transgenic animals. The transgenic animals where you are actually going to overexpress a protein or where you are actually going to remove a protein. So in overexpression of protein, these are always used in cases where you are either overexpressing a protein so that the therapeutic or nutritional value of that product from the animal is going to be increased, or in general, the animal itself is actually going to carry that particular gene. So that is one advantage. In a knockout, you are actually going to remove the gene expressing a functional gene, and that is useful for understanding the role of that particular protein in any kind of disease mechanism.

I am sure you might have noticed when we were discussing the transgenic animals that we said these transgenic animals are a very good model for understanding the disease mechanisms related to cardiovascular diseases, Alzheimer's, cancer, AIDS, and so on. So, for any of these diseases, you are actually going to produce transgenic animals and then you are actually going to create. The particular disease conditions and in those disease conditions because we already know that this particular protein is actually going to have a role. These transgenic animals are actually going to be helpful in terms of not only understanding the disease mechanism, but also for screening the therapeutic molecules or screening the inhibitors, for example. There are many examples, such as proteins, that are very good for the Alzheimer model or atherosclerosis model, and you can actually use them for screening different types of inhibitors to see whether a particular inhibitor can be used for that specific disease.

Similarly, you can actually use these for normal physiology. So you can actually be able to, for example, use knockout mice and say what the role of actin in muscle contractions will be. So I can remove the actin, or I can generate the conditioned knockout. All these

are way beyond your syllabus. But suppose I generate an actin knockout; then that will actually impair muscular contractions and other kinds of events, and that is how you will be able to study the role of actin. Or you can actually generate the mutants and replace the wild type with the mutant, and that is how you can ask these kinds of questions.

Then you can also use these knockout mites for the different types of products. For example, you can use these for the production of erythropoietin, you can use these for the production of lactoferrin, you can use these for developing different types of vaccines, and so on. And then, vaccine safety—right? So when you are developing a vaccine, you actually have two choices: one, you can inject this vaccine into a human. And then you can ask whether it is providing the protection or not; in this process, you are actually sacrificing or putting the lives of these humans in danger because, in case you are testing a vaccine for HIV, for example. Now, if you are testing only on real human beings, then you are actually going to inject the HIV virus, and suppose you injected the vaccine.

Now these are vaccinated people, but once you inject them with HIV, they may develop the disease or they may not. If they develop the disease, they are actually going to be HIV patients, and ultimately they may die because the disease does not have any permanent cure. But apart from this, you can actually be able to do the same experiment in a transgenic animal. So, what you can do is actually be able to develop humanized mice. So, where this mouse is actually going to have the immune system from behaving like a human system.

So, you can actually take the humanized mice, and their immune system belongs to humans. And then you can actually infect this with HIV. So you can actually administer the vaccine; you can actually inject a human. So ultimately, if the experiment does not go as per your plan, at least these mice will only get HIV, not the humans.

And there are other cases where the testing itself is problematic. So is the same for the toxicity testing. When you are testing the toxicity of drug molecules, you have a choice: either you use real humans, or you can actually use transgenic animals. And that is why you are going to use the transgenic animals, because these animals are meant for doing these kinds of experiments. So, I have also listed some applications of transgenic organisms.

So, this actually includes both the animals and the plants. So transgenic plants and animals have various applications across several fields. It has helped us advance in several areas such as agriculture, medicine, the medicinal industry, and environmental management. The progress of transgenic organisms through time has helped to enhance the nutritional gain from both plants and animals. It has also produced more resistant

plants and animals so that both farmers and breeders have lower losses and abundant gains at the end of each season. The generation of transgenic organisms has also paved the way to create a newer breed with a specific social role.

For example, the more muscular dogs that help in the maintenance of security along with the police as well as the military forces have been produced. And all these advantages have paved the way for the immiserable growth in the transgenic organisms. One major goal of transgenics is to increase crop yield. So what is the role of transgenic plants in agriculture? So, one of the major areas where the transgenic plants are effective is that they are actually increasing the nutritional quality of crops to cater to the needs of the growing population. Remember when we were discussing the introduction, we said how you are actually going to overcome the food shortage for the 9.

7 billion people, right? This is one of the ways you are actually going to use recombinant DNA technology to produce a transgenic plant, and that transgenic plant is actually going to overcome the shortage of food. Because the same plant that was producing 1 kg of rice, the transgenic plant may be resistant to disease conditions and may also yield 2.5 kg. So, pathogens like bacteria, fungi, viruses, and insects, along with several abiotic stresses, have flawed the growth of the crop for several decades. With the advancement in the production of genetically modified organisms, cultivars with increased resistance to both biotic and abiotic stress have been developed.

This will allow the farmers to grow crops in large quantities with increased resistance to pathogens and harsh environmental conditions. A common example of such a cross is the bitty cotton. It has increased resistance to the insects, right? I mean, Bt cotton is very, very popular, and it has also been involved in many controversies, but these insect-resistant crops have been produced by introducing the Bt gene, which is also known as the cry gene, isolated from *Bacillus thuringiensis*. And that is how these *Bacillus* gene products are actually toxic to the insects and how they provide resistance in the plant for insect resistance.

The Bt cotton encodes a toxin that is effective against specific insects. Several classes of Bt genes are effective for different insects and ineffective for others. For example, the cries A C and 1 C, 1 A C, and 2 S B. Code for a toxin that controls the cotton bollworms, whereas the cry 1 AB encodes for a toxin that controls the corn borers. Several Bt crops have been produced, such as rice, maize, tomato, and brinjal. Many of these transgenic crops are grown commercially, resulting in a significant yield increase.

Another example is golden rice. which has an increased expression of vitamin A, resulting in the golden color of the rice and hence the name. Several transgenic crops

have been produced in the past two decades with desirable traits, better yields, and nutritional qualities. The table in the following slide shows the different running crops that have been produced in the past decade, as well as their desirable traits. So this is the simple table that actually shows what transgenic plants we have growing, what the source of the transgenes is, and what their applications are.

So, you actually have a corn. So, corn actually has a source of genes from bacteria or other species of corn, and because of this transgenic corn, these transgenic corns have resistance to insects. They are tolerant of the herbicides. They are male corn sterility, alpha amylase expressions, increased lysine levels for use in animal feeds, and the reduction of yield loss under water-limited conditions. Then we have cotton, so we have taken out the bacteria, such as *Bacillus thuringiensis*, like in BT cotton.

It has a tolerance to the herbicides and is resistant to the insects. Then we have a soybean; soybean, you are expressing the transgene from the bacteria, corn, oats, and soybean, and because of this, it is actually going to have different types of applications. So it is going to be tolerant to the herbicides, it is going to have high oleic acid soybean oil, and it is resistant to the insects. Then we have canola; canola is an oil. It is going to have the transgenes from the bacteria, mustard, and fungus, and it is also tolerant to the herbicides. It involves fertility restoration, male canola sterility, and degradation of phytoestrogens in animal food.

Then we have the transgenic plants and their application in industrial production. So, lipid organisms are factories on their own. The advancement of biotechnology has enabled all to utilize this power and convert plants into a manufacturing unit for their own. Several biomolecules are generated by this method, and it has been famously dubbed molecular farming. So molecular farming is where you are actually putting the transgene inside a particular plant, and then the plant is utilizing that transgene to produce that product, and that is how it is actually going to require no additional support from outside. This involves the genetic modification of the plant by either incorporating the gene of interest into the plant or multiplying the number of genes in the plants.

These foreign genes are incorporated into the nuclear genome or the chloroplast DNA; as chloroplast DNA is present in several copies, a gene inserted in the chloroplast A gives a higher yield of the target biomolecules. The products are generally produced by the plant in its leaves, stems, or tubers. Some of the common products that have been produced using this method include vaccine antigens, therapeutic proteins, diagnostic reagents, nutritional products, bioplastics, or industrial enzymes. We have a big list of the vaccine antigens, therapeutic proteins, diagnostic reagents, nutritional products, bioplastics, and industrial enzymes that are being produced in the plant and are being successfully utilized

in the industry.

Then we have the transgenic plants for industrial production. The most common advantages of molecule formation are the safety and purity of the obtained molecule. Plants are complex organisms that can provide a robust framework for post-translational modification, which is often very important for the functionality of the proteins. Along with this, using plants to produce complex biomolecules is a sustainable method with renewable sources. The first human-made biologist accepted by the FDA as a viable treatment for Gaucher disease is Eliso.

The enzyme was initially produced in the carrot cell culture. So, these are some examples that are being used in industrial production. So, you can have a transgenic plant; this is the therapeutic protein that you are going to use, and this is the disease that is actually going to be cured. So, if you have transgenic lettuce, it is actually going to express the hepatitis B antigen, and then it is going to provide you with protection against hepatitis B. Then we also have transgenic tobacco, which is actually going to have a cancer vaccine, and then it is going to give you protection against non-Hodgkin's lymphoma. Then we have a transgenic carrot that is actually going to express human glucocerebrosidase, and it is going to provide the cure for Gaucher disease, and this is what we were talking about here, right? Then we have this safflower; safflower is going to express the insulin.

And it is actually going to give you the cure or management of diabetes. Then we also have the rice, where you are actually going to express the human lysozymes, and it is actually going to be anti-infection or anti-inflammatory. Then the transgenic plants can also be used in the environment while taking care of the environmental issues. Remember that the plants are going to be used not only for managing the different types of environmental byproducts produced by the animals or the industry. They are also being used for sensing environmental factors.

So, with the evolution of civilization, the amount of pollution has also increased over time. Plastics contribute to a sizable pollutant in modern society. One of the most common inventions of our time is the production of bioplastics that can be degraded by nature over time. Transgenic plants are now exploited to produce the polyhydroxyalkanooids that are an attractive and degradable alternative to plastics. An example of PHA production is the production of bacterial polyhydroxybutyrate or PHB.

The gene responsible for producing the PHB, NLB, PHA, and PHC has been identified and isolated. These genes are incorporated into the sugarcane to produce PHBs in transgenic sugarcane. Another important use of the animal is phytoremediation. So,

phytoremediation means the removal of the toxins, right? It is the use of plants to remove various pollutants from the environment. So, there are several types of bioremediation.

For example, phytostabilization involves the immobilization of pollutants in soil using plants. Then we have phytoextractions, so plants absorb the contaminants, particularly heavy metals, from the soil, and examples of this are producing transgenic plants containing metal detoxifying genes. Then we also have phytodegradation; this involves the uptake of organic pollutants and degradation. Then we have rhizofiltration; this involves the absorption, concentration, and precipitation of the organic pollutant from the aqueous environment. All of these have been made possible by the production of the transgenic plant that contains a specialized gene to produce a sustainable method to clean our environment.

Then, we have transgenic animals. So transgenic animals also have applications in the agricultural field. So, like transgenic plants, transgenic animals also have a wide variety of applications in agriculture. The increase in the quality of livestock, which was previously achieved through selective breeding, can now be easily accomplished by transgenic methods. Engineered transgenic animals such as pigs, sheep, and cattle grow faster and more efficiently. For example, transgenic salmon have been developed to grow to market size in half the time of conventional salmon. Genetic modification makes livestock resistant to certain diseases, thus reducing the need for medicine and improving animal welfare.

An example of such modification is the transgenic chickens developed to resist avian flu. Then the transgenic animals are also being used in molecular farming. Remember that the molecular formation means you are expressing a particular product in the transgenic organisms. Transgenic animals are used as small bioreactor units to produce both normal and recombinant proteins in large quantities. The first example of such an animal was Tracy.

A transgenic that produced a large amount of alpha-1-antitrypsin in its milk. Deficiency of this protein in humans caused lung disease, and thus the use of recombinant protein was thought to cure some of the symptoms of these diseases. In 1997, the first transgenic cow, Rosie, was created, which produced alpha-lactoalbumin-rich milk. This dairy was very balanced for human infants. than normal cow's milk.

So this is some of the animals where you are actually expressing the different types of proteins. So you are expressing the albumin into a cow, you are expressing the alpha-fetoprotein which is from a goat, then you are expressing the growth hormone into the goat, and you are also expressing the tissue plasminogen activator which is from the goat.

Then you have a coagulation factor 9, which is in the mouse, and you also have a coagulation factor 8, which is in the rabbit. Remember that all these transgenic animals are very, very important for the many types of applications. Nowadays, transgenic animals are engineered to produce pharmaceutical proteins in their milk, blood, and eggs.

Goats have been modified to produce antithrombin, a protein used to prevent blood clots. Additionally, the transgenic mice and other animals are engineered to produce the humanized antibodies that are critical for the development of treatments for various diseases, including cancer and autoimmune disorders. Then the transgenic animals are also being used in the research, right? So transgenic animals are also used as models for the study of different physiological disorders. This gives the researcher a scope to identify the cause and cure of certain diseases which are otherwise important to study in vitro. Even the application of certain drugs is initially performed on this transgenic animal model to check for any adverse outcomes before progressing to the clinical trial or human trials. For example, the transgenic mice have been engineered to develop conditions like Alzheimer's disease, diabetes, and cancer.

This model helps researchers understand the disease mechanism and test potential treatments. Let's summarize what we have discussed so far. We have discussed the transgenic animals. We have discussed how you can be able to produce the transgenic animals.

So we have discussed how you can have the three different options. You will be able to use the microinjections. You can use stem cell technology, and you can also use retrovirus methods. Once you develop the transgenic animals, you will be able to use these transgenic animals for various types of applications, and we have discussed various types of transgenic animals that are being produced, whether they are glowfish, salmon, transgenic rabbits, sheep, goats, cows, buffaloes, and so on. Once you develop the transgenic animals, you will be able to use these transgenic organisms in different types of applications. So, you can use this for bioremediation, you can use it for environmental monitoring, you can use this for the production of protein products, and so on. So, with this kind of elaborate discussion about transgenic organisms and the application of combined air technology to produce them, I would like to conclude my lecture here. Thank you.