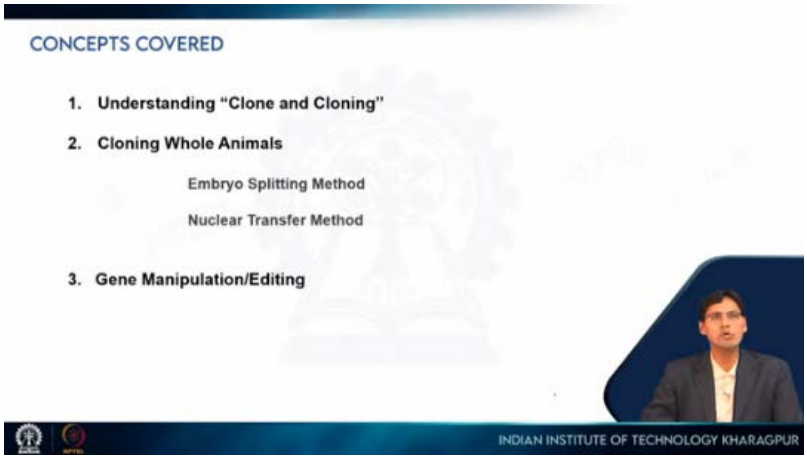
Introduction to Complex Biological Systems
Professor Dibyendu Samanta and Professor Soumya De
Department of Bioscience and Biotechnology
Indian Institute of Technology, Kharagpur

Lecture 55
Cloning and Gene manipulation

Hello everyone, today I am going to discuss cloning and gene manipulation. So, this is the last topic of this module, and here particularly I will be discussing clones and the cloning method, an overall idea about these two terms. Followed by cloning whole animals, and there are two major methods here: one is the embryo splitting method, and the other one is the nuclear transfer method, followed by gene manipulation or gene editing. So, here the first point I am going to discuss is clones and cloning.



So, a clone means organisms carrying identical genes. We will say those two are clones of each other. During the last lecture, what I discussed was mostly about cloning a gene. So, if I summarize that, what I mentioned there is that you have one plasmid.

So, this is a plasmid, and here you have one gene of interest. I would say this is your gene of interest. So, this is the gene of interest. Now, if you digest this gene of interest by two restriction enzymes, for example, I would say EcoR1 and here BamH1.

So, these are restriction enzymes, which I discussed during the last lecture. Similarly, in the plasmid bit, you have many important properties, and you have some portion which is called the multiple cloning site, or MCS. So, you have many different choices of restriction enzymes. So, as a result of that, you can digest this plasmid by the same set of

enzymes, which are EcoR1 and BamH1. So, as a result of that, your plasmid will be in a linearized form.

I would say you might get something like this structure. So, this is now your plasmid after digestion with EcoR1 and BamH1. Similarly, from your gene of interest, you will be getting something like this. So, this is your gene of interest.

Now, since we use the same set of enzymes, this end here and this end can base pair here, and they will be complementary to each other in this region. So, as a result of that, you can actually put your gene of interest here in this plasmid. Now, on the basis of selection and all those things, what do you have to do? You have to transform some host cells, for example, bacteria. You are sending this plasmid, and then when the bacteria divide into many numbers, this plasmid will also grow in number, and the plasmid can replicate by itself. So, as a result, many plasmids will be present in one bacterium.

Now as a result of that, what you are doing is increasing the number of your genes of interest. So, those are exactly the same gene. Those are the cloning of the gene. So, your genes are now cloned. You have multiple copies of the same gene by this method.

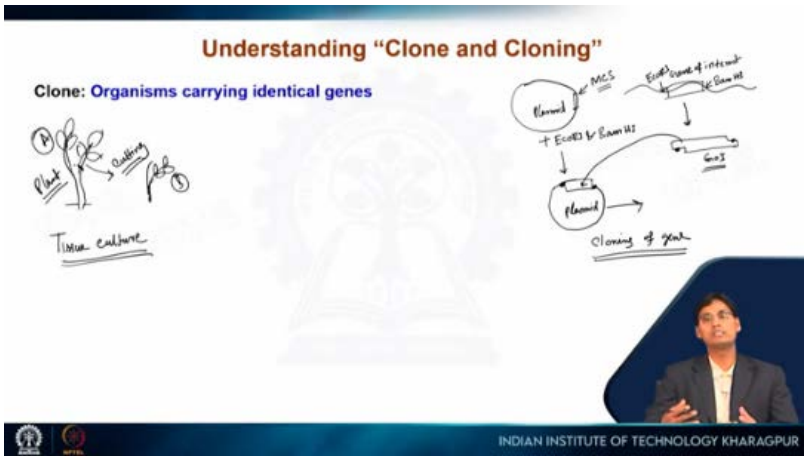
So, this is cloning of a gene. So, this is just a summary of what we discussed before. Here, I mention in the context of some organism that the organism is carrying identical genes. So, this cloning method is very primitive if you think about the plant system. So, all of us know that, for example, if this is a plant and if you cut this portion, one branch or one stem, and put it in a suitable environment in soil and pour water, everything is fine, then you will see that you can have a new plant here. So, a new plant here from this portion only. So, this is the cutting method. So, now, if you see in terms of genetics, the constituents of these two plants, Plant A and Plant B. You will say they have exactly the same genomic material because we are just putting one branch or cutting a little bit of this plant, and this plant is growing again.

So, this is a very basic method of cloning in the case of plants, just by cutting; more advanced methods, for example, tissue culture. From plant cells, particularly plant tissue, you can grow in a laboratory environment. You can grow them in numbers, like plant cells, and then, in a controlled environment with proper nutrients, from that tissue, you

can have multiple identical plants. As a result, those are also clones of each other; they are exactly the same plant. But naturally, what we generally see, for example, if I would say this plant A is a flowering plant, then after the plant matures, flowers will appear, some pollination will occur, and some fruit followed by seeds and new plants will form. That is not a clone because during pollination, meiosis happens to make the ovule as well as the pollen. As a result of that, the exact genetic composition is not the same as the parent plant.

So, as a result, those are not clones. But whatever I discussed right now, by the cutting method or by tissue culture method, you can have plant clones. There are several methods; I am not going to discuss that. So, now, this is the cloning thing in the context of organisms I just mentioned, and also I mentioned cloning of a gene. . Then, cloning in animals is a little bit problematic because, unlike plant tissue, animal tissue will not always grow properly. Only stem cells have this power; they are totipotent.

So, you can probably make a new animal from stem cells themselves. Particularly, I would say, embryonic stem cells when it is a zygote or just at the very early division stage. During that stage, you can do that. Now here, the cloning of animals, I will discuss this point separately. I will emphasize here one method called embryo splitting. So, in this case, what we will see here is, for example, we are taking sheep. So if I consider this is the female sheep and this is the male sheep.

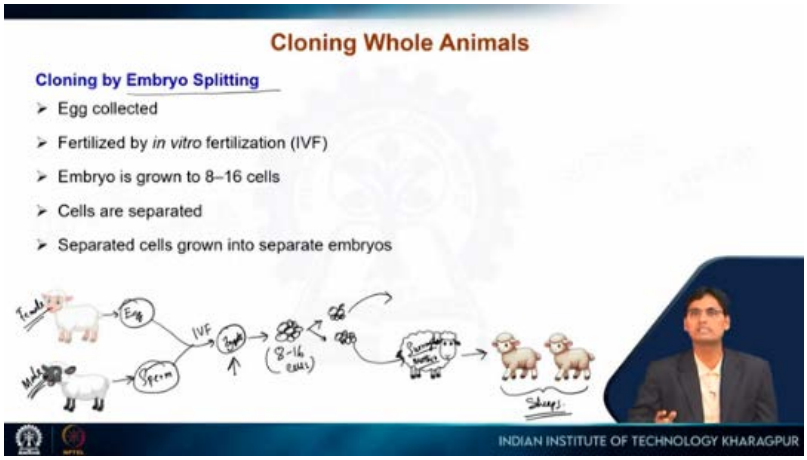


So, now from this female sheep, an egg can be collected. This is an egg cell, and similarly, from this male sheep, sperm can be collected. Now, what will happen if we

conduct IVF, that is, in vitro fertilization? So, as a result of that, you will get a zygote and then in a laboratory environment, because you are doing in vitro fertilization here.

So, it is still in the lab. Now, this zygote will divide into a few cells. I would say an 8 to 16 cell stage. So, every cell has the power to make anything in the body. They are properly embryonic stem cells. Now, if surgically we split these cells into two groups, we are separating this cell mass. So, the cells are separated, and now these separated cells can be put into a surrogate mother. So, it is another, actually, sheep surrogate mother. So, you can put this one into another surrogate mother or the same surrogate mother; it does not matter. But finally, what you will be getting here is that this surrogate mother will give birth to these sheep. Now, these two sheep are identical to each other.

They are identical because both of them actually originated from this zygote, the same zygote. So they are not identical to their parents. Here, female sheep or male sheep are not like that. But these two are clones of each other because they originated from the same zygote. So, this is the embryo splitting method and by which you can get this identical sheep and these two are clones. These things sometimes happen naturally in our human population, also not in this way, but naturally. So, as a result of that, what I would say is after fertilization, the zygote, when it is getting divided at a very early stage, accidentally, by some other reason, if it divided into two, and both of those two sets of very early embryos, if they are implanted in the uterus then that mother will give birth to identical twin. So, they will be exactly identical. So, identical twins are coming from the same zygote, but in the case of non-identical twins, that is a completely different scenario where two different egg cells are fertilized by two different sperm. So, in the human population you will be seeing both sometimes identical twins and sometimes non-identical twins, but I hope that you understand the basic difference between these two sets of twins. We will be discussing the next technique here, the cloning by nuclear transfer.



So, this nuclear transfer, particularly this is also known as somatic cell nuclear transfer or SCNT. So here, for example, many of us know that the first cloned mammal is Dolly, one sheep actually. The same way in which I am going to discuss Dolly was generated in the same way.

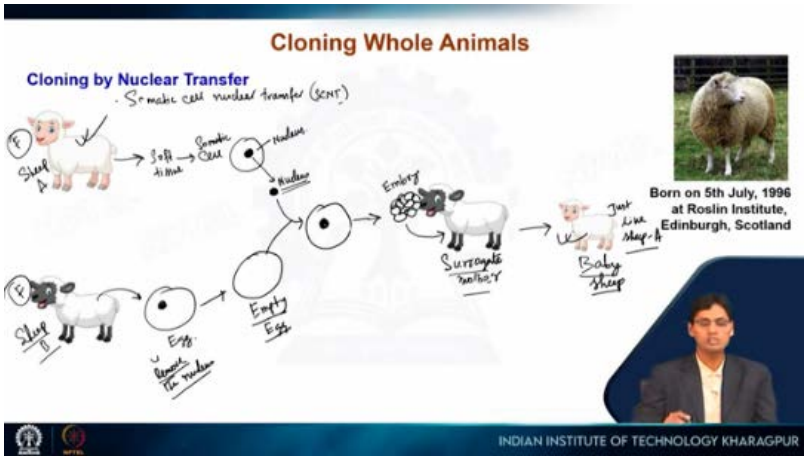
So, here what we do? So, as you can see this one, this is I would say Sheep A and this is sheep B. In this case both sheep can be female, no problem. It can be one male one female that is also OK, but one female is required because we will take one egg cell. I will be discussing why. So, now, the thing is if I say sheep A and sheep B both are female sheep for example, now we are taking some soft tissue from this sheep so that we can quickly take the cells and properly handle them just for experimental reasons, some soft tissue, and from there you will get a cell. What kind of cell? Not an egg cell, not a sperm cell. This is a somatic cell that means, anybody cell. So, therefore, it will contain the whole genetic information, as you know that sperm and ovum egg have half of the information, but in this case, the total set of chromosomes should be there, like the diploid number of chromosomes should be there in this somatic cell. So if this is the somatic cell here and at the center, in black color, whatever I am showing is the nucleus.

So, I would say this is the nucleus. We will take this nucleus out. So, the nucleus was removed and it will be used in this method, and the cells without the nucleus will be discarded; no requirement. Similarly, from sheep B as I already mentioned this is the female sheep.

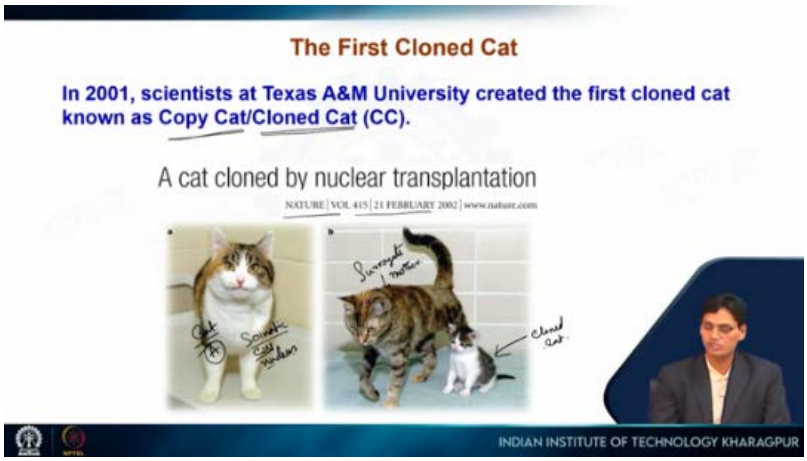
So, from this sheep we will be getting the egg cell. So, this is an egg cell. here you have half of the chromosome, but that does not matter because we are not relying on the egg chromosome here so we will be removing this nucleus so just remove the nucleus; nucleus is not required here just take the empty egg and now we will be fusing this together. This nucleus is from the somatic cell which contains the whole information and the empty egg together. So, see this is not the fertilization like sperm and egg are getting united. We are putting the full nucleus from the somatic cell inside the egg, that is all, and then here you need a little bit of strategy, some little bit of pulse and all those things, and everything is happening in the lab, and then this one will start dividing and it will make early embryo. A few more cells will be there as you can see the early embryonic stage here, and this one we can transfer into a surrogate mother. So, this is here, the surrogate mother. So, now as you can see, this is an embryo, and if we put it in a surrogate mother, this embryo will then give birth to a baby sheep. So, this is a baby sheep.

So, now if you see this baby sheep exactly looking like neither sheep B nor its surrogate mother, the sheep is exactly similar to sheep A. Why? Because of the nucleus we have taken from sheep A, this process is called somatic cell nuclear transfer for the cloning of the whole animal. Now, this empty egg is absolutely required because inside the egg, apart from the nucleus, there are many important ingredients, particularly many mRNA and proteins which are absolutely required, and that is why we have to take the empty egg and fuse it with the nucleus, finally getting this cloned sheep. Here this baby sheep and sheep A are clones of each other; they are the same thing. They have exactly the same genetic composition.

So now, by this method, Dolly was created. As I already mentioned, Dolly is the first cloned mammal and it was born on July 5th, 1996, at the Roslin Institute in Scotland. The sheep was overall healthy and had multiple babies. At around six and a half years of age, it showed some kind of problem and finally passed away. So, this is what I discussed, the two different methods of whole animal cloning. Now, apart from those cloning methods I just discussed, some interesting facts are there, and I am just mentioning those for fun. so, if you see here, in 2001, scientists at Texas A&M University created the first clone cat by the same method, somatic cell nuclear transfer.



So, this is known as a clone cat, but they also call it a copycat because it's the same thing and here, if you see, I am taking this image from this issue of the Nature journal. So, here, from this cat, a somatic cell nucleus was taken, and this is the surrogate mother, and this is the cloned cat. It looks like cat A here.



So, this is similar to cat A because it is a clone cat and if we move a little bit further, whatever I mentioned about some examples of cloning, Dolly is very famous. Everyone knows about it, but the cat we might not know. But this one is a bit more interesting because this is the first cloned primate. So, a primate means it is very close to humans. This is a monkey, particularly called a crab-eating monkey, and these are small in size. Their names are Zhong Zhong and Hua Hua. They were born in November 2017 at the Institute of Neuroscience of the Chinese Academy of Science in Shanghai. So, the method is the same, the somatic cell nuclear transfer, and they are also healthy and doing well. So if you see the overall advancement in technology and understanding in science,

those are working fine. But now the question is coming about ethical issues that what we should do and what we should not do. That is the more important question here and also I would like to mention here that whenever I was discussing right now about the cloning of a whole animal whether it is by embryo splitting or nuclear transfer. We will be noticing that here we are not really cleaving some particular DNA, and then again, we are integrating DNA not like that. This is like I would say we are not relying on any of those kinds of enzymes like ligase or restriction enzyme, not like that. This is a kind of natural process we are trying to mimic.

We will go a little further into the development of what is happening now, so this one, particularly, is again something interesting here. So if you see, this is one hollywood movie, and the name of the movie here is GATTACA. So now There is no language, no word like GATTACA here, so this is nothing but nucleotide sequence now all of you know. It is ATGC sequence present here, so the name of the movie itself is GATTACA. This is just a nucleotide sequence, and this is a science fiction movie. This is mostly talking about selecting the best possible features from parents, and this is a very enjoyable movie, particularly if you have a little bit of knowledge about genes and genetic engineering and all those things, which you already have, so you can enjoy this.

Here is a little bit of a controversial topic here that Lulu and Nana. So, these two are actually human babies, but this is gene edited human baby. That is why a few minutes ago I was saying that we have to be very careful about what is ethical and what is unethical. So in this case, what we came to know, for example, is from BBC news that gene editing babies called to pause human altering research, and here particularly I have taken this from science magazine here. Did CRISPR help-or harm-the first-ever gene-edited babies? So, as a result of that there are a lot of concerns also. So, CRISPR means some kind of particular technique which makes it very easy to edit genes or genomes even in humans, as you can see here. So, I will be discussing CRISPR in the next slide.

But the problem here is what happened with this scientist He in China. So, He edited the genomes of these two babies, Lulu and Nana. So, that is why all these concerns are coming. So, whether it is good or bad, it has some long-term effects and some ethical issues also. So, as I mentioned, the scientific community should be very careful about this thing, which they are rightly doing.

Now, if you see what CRISPR-Cas technology is, it is very important, and this is a very robust technique. Nowadays, many of us use it on a day-to-day basis in the lab, particularly this technique, CRISPR, which was used here as well in this case. So, particularly, I would like to mention that this technique is a very new technique. So, this was only discovered around 2012 to 2015 during that time, and for this discovery, Emmanuelle Charpentier and Jennifer Doudna received the Nobel Prize in 2020 in

chemistry for their gene editing technique based on the CRISPR-based system, the CRISPR-Cas-based system.

So, what is the CRISPR-Cas system that I would like to highlight? I would not go into much more detail but will introduce the idea. The thing is, the CRISPR-Cas system here is a very big name, CRISPR. So, if you see here, what is the meaning of CRISPR? It stands for clustered regularly interspaced short palindromic repeats, a very big name. If you forget, nothing will happen. I will discuss and give you the idea of what CRISPR and the CRISPR-Cas system are.

So, Cas means CRISPR- associated protein, so this is the Cas system So, together, this CRISPR-Cas can be utilized in a very robust method, and it is very useful nowadays, even for academic research. Very frequently and effectively, we can edit genes for our academic research, for example, to knock out or delete some genes from a genome or to introduce some new segment of a gene by this technique. So, let us see what this technique is. The idea is that this is coming from some natural observation. During the last class, I mentioned restriction enzymes, and I particularly mentioned that restriction enzymes were isolated and purified from bacterial systems.

Bacteria use restriction enzymes for their defense mechanism. They use them to degrade or destroy phage virus DNA or bacterial phage DNA. So, this is some kind of immune system, but that is an innate immune system. Here, in this CRISPR-based system, this is also a kind of bacterial immune system, but it is an adaptive immune system. Emmanuelle, particularly, learned a lot about the CRISPR system from bacterial systems,

and together they modified the system so that it can be more applicable for our own purposes. But the bottom line is, you can see these bacteria naturally evolved and generated this system by themselves to gain protection against phage viruses.

As I already mentioned in the first lecture, Lecture 1, in living systems, because of evolution, most organisms solve most of their problems. So, as a result, you can see bacteria solving the problem of their bacteriophage infection. Sometimes they have restriction enzymes to degrade foreign DNA, but they protect their own DNA. Similarly, in the CRISPR system, this is also against phage virus DNA, but this is a kind of adaptive immune system. Let us see what is happening here. So, I would say, if this is bacteria, inside the bacteria I am showing here. Now, the phage virus you can think about T2 phage for example. This is the phage virus. So, what they do is inject their genomic material, phage DNA; I would say they are injecting it inside bacteria So that more phage virus can be generated and the bacteria will be lysed and more and more phage particles will be generated. But, the thing is bacteria are trying to protect themselves by some kind of method.

What is happening here? So, in the bacterial chromosome, what scientists observed is that they have some kind of repeat. This CRISPR-Cas system is present inside the bacterial chromosome itself. What is that? So, I would say if this is a bacterial chromosome. So, this is bacterial DNA or bacterial chromosome same thing and I am just zooming a little bit of this portion where all these things are happening. This is part of the bacterial chromosome itself. What is present here is called Cas genes. So, Cas Genes, this is already present in bacterial DNA or bacterial chromosome, followed by you having a CRISPR array. What is a CRISPR array? So, you have some repeats followed by some spacers. So, I will explain that. So, I would say here, particularly if I say this is repeat, repeat, and repeat.

So, this is some kind of short palindromic repeat, and in between, you have, for example, this one particular DNA sequence present here. So, now the thing is, those are actually acquired by bacteria during phage virus infection; they acquired those things. So, this is nothing but phage DNA. Not the whole DNA segment of phage DNA. Why? As I already mentioned, this is just an adaptive immune system.

What is happening? For example, this phage virus, when infecting, has some specific region. I am not going into that much detail, but during this infection cycle, this should be incorporated here. So, for example, this is the same thing here. The phage virus DNA is getting incorporated in this small segment. If I say this is phage A, a particular name here, and this is the A region.

So, it is integrated here and together I would say this is a CRISPR array. Now what will happen is that this CRISPR array will be transcribed into mRNA called pre-CRISPR RNA and some more modification and there are many enzymes involved also then you will get CRISPR RNA. So, why am I making small small fragments? Because you are getting one, this is 1 2 3, for example, from this portion 1 2 and 3 and Cas genes after transcription translation it will make Cas proteins and Cas9 is one of those important proteins. Cas1 and Cas2 are also very important, but Cas9 is also very important. So, now Cas9 will form a complex with this CRISPR RNA. For example, I would say if they form some kind of complex like Cas9 and this CRISPR RNA particularly CRISPR RNA 1. So, this is some kind of complex, the Cas9 protein and RNA itself. So, what will happen? This complex will help to prevent this bacterium from further infection by the same virus. So, what will happen?

Whenever the same virus infects this bacterium, the same DNA will be received. As a result, you have this CRISPR RNA, which I labeled as 1. Now, this CRISPR RNA will recognize the complementary sequence coming from the viral DNA. So, as a result, this CRISPR RNA will bind to the viral DNA. Now, this Cas9 protein will act on it, and as a result, the viral DNA will be destroyed or degraded, and a double-stranded break will happen. In this way, they are actually preventing themselves from phage virus infection.

Initially, they will be infected, and during the infection, they acquire this short segment of DNA. But if a new virus infects this bacterium, it has no signature motif in its DNA itself. So, during that time, it will go through the infection cycle, and then it will acquire a small segment, and it will be integrated with the CRISPR array. But next time, if the same virus infects again, the CRISPR RNA will be generated, and along with the Cas9 protein, the viral DNA will be destroyed. This is the overall technique of how bacteria prevent

themselves. This is very similar to the mammalian adaptive immune system, but in their own way. This was extensively characterized by Emmanuelle and also by Jennifer. Particularly, they made it more convenient instead of CRISPR RNA, they designed guide RNA. So, that it is more programmable, it can be used in a very easy way, and now we are using Cas9 and some guide RNA. Together, you can put that in a plasmid and then send it to a particular cell where you want to modify the genome.

So, it is very common nowadays to delete some portion of a gene or to add some extra segments. You can do a lot of those kinds of gene editing techniques. So, here you can see this is the guide RNA, and this is the Cas9 protein, all in blue color. This is the Cas9 protein, and this is the genomic DNA where you are actually trying to edit it. So, this is a brief introduction to the CRISPR-Cas system, but as I already mentioned, this is very useful and very quickly they received the Nobel Prize in 2020 because of their very important and interesting work and that is all. Thank you very much.