**PROFESSOR:** Recombinant DNA, often referred to also as genetic engineering. This is a series of techniques, series of methods that allow us to manipulate DNA for a variety of reasons. Now, we take it for granted. It's very much part of our everyday life in the laboratory. It's made a huge impact on the biotechnology and pharmaceutical industries as well.

It wasn't always so uncontroversial. In fact, in the 1960s and '70s, when this technology was first being developed, it was great concern about scientists manipulating DNA, manipulating genetic material. In the city of Cambridge, in fact, had a moratorium for a while on the practice of genetic engineering or recombinant DNA technology, which fortunately, ultimately was overcome with good practices and I think good education about what the real limits of risk and benefit were so that now it's used very widely and, I would also say, very safely.

The bottom line for what we're going to talk about today and for this section of the class is the ability to isolate and to amplify specific DNA sequences. They might be particular genes of interest to us. They might be whole genomes. They might be other regions of DNA, but we need to be able to isolate them from the cells of the organisms of interest, amplify them up into large quantities in order to be able to study them in detail, and to make modifications in them.

This is done in a variety of organisms for a variety of purposes. One of them, and this is by no means the only one, but one that sort of strikes home is the ability to make therapeutic proteins, to be able to manufacture in the laboratory or in a company proteins they could have benefit for patients who are, for example, lacking the function of a particular protein or enzyme leading to a disease state. One can use these methods to produce that protein in the laboratory, and treat the individual.

This can be done in bacteria such as E. coli. E. coli, which is the common gut bacterium that we all carry, a very useful organism. We use it in the lab. We're going to use in our demonstrations today. This is the standard vehicle in which we'd grow up, recombinant DNA, amplify recombinant DNA for further uses, but we can also

make therapeutic proteins using the manufacturing capabilities of the bacterium.

Plants, likewise, can be a source of therapeutic proteins. We can modify the genomes of plants so they will produce in large quantities therapeutic proteins of interest, which then can be ingested by the individual, which can reduce production costs significantly, and animals, likewise.

We can manipulate the genes of animals so that they will express a protein of interest and secrete that protein, for example, into the milk. So there are so-called transgenic cows and transgenic goats that produce therapeutic proteins in the mammary gland and secrete those therapeutic proteins into the milk. So the individual just needs to drink the milk and receive the relevant dose.

So there are lots of ways, that this technology can be helpful including in the context of medicine. We can also engineer organisms. I've already given you examples of that, but that was really for the purposes of using those organisms as a factory to make something of interest to us. But we can manipulate the genes of plants, for example, to make them resistant to various pests to make them more robust, to give them a longer shelf life. We can manipulate them for the better production of things that are valuable to us.

Again, this is a bit of a controversial area, genetically modified foods, not always well-accepted by everyone because again, the thought is, this might be disrupting the food chain in important ways, and this might be ultimately not so beneficial. Personally don't agree with that, but lots of people do feel that way. We're going to teach you the methods that we use to allow us to do this.

And again, in work that I've alluded to from my own lab, we use these methods to manipulate the genes of animals, in our case it's mice, but there are lots of animal species that one can use in order to create, for example, disease models. We talked to you about, Professor Brown talked to you about genetic diseases. They have specific alterations in genes. We can use these methods to make similar alterations in the genes of mice and other animal species and then study the disease process in those animals, develop new treatments in those animals in ways that are hard to

do in people.

So there's lots and lots, and these are just a few examples, lots and lots of important uses for genetic engineering and recombinant DNA technology. One that we'll emphasize in just a few lectures and is becoming extremely common and popular nowadays is DNA sequencing.

You need to isolate the DNA from the organism of interest and then have it in such a fashion that you can sequence it's nucleotides. This is a very popular activity now. And specifically with respect to human health, DNA sequencing, But some of the other methods that we're going to talk about as well, allow us to characterize disease-causing mutations at molecular detail.

So we know what causes x disease or y disease. We can understand the consequences for the individual-encoded proteins for the pathways that they regulate, and we can come up with better medicines. I think about this with respect to cancer, but it's really changing the treatment of all diseases as we understand more the molecular basis, the genetic basis of those diseases. And these techniques have been essential to allow us to do that.

I want to cover a little bit of history so that you know from whence this came. Things really began to pick up in this field in the late 1960s. And the critical advance at this stage, was the discovery of a method to cleave DNA into defined fragments, to start with a genome or chromosome and to be able to cut it particular places reproducibly so that one could isolate fragments of DNA away from the mass of DNA, isolate a particular region of the DNA away from everything else using this method.

It wasn't enough just to cut the DNA up. You had to amplify it up. In order to amplify it up, you needed a vessel in which to do the amplification, and the vessel of choice as I mentioned was bacteria. And this relied on a method that actually was known for decades before but actually was not used for this purpose until the early 1970s, and it's called bacterial transformation. You can transform bacteria by adding a new DNA sequence, and the bacteria will take up that new DNA sequence and begin to express the genes that are present on that DNA sequence as if it was one of their

own.

So the transfer of DNA a was critical, this process of bacterial transformation. And then the final thing which also occurred in the 1970s was the identification of methods to amplify DNA sequences once they got inside of bacteria. It wasn't enough to actually get them in there. You needed some special way to cause the bacterium to amplify, that is, to replicate the DNA that was present within them. And these three events, which all came together in about a five to ten year period, really initiated, launched what we now call the recombinant DNA revolution and initiated the biotechnology industry, which started in the mid 1970s.

And an individual above all others who is credited with launching the biotechnology industry was Robert Swanson who in 1966 or so was sitting where you are as an MIT freshman. Bob Swanson was class of '69, actually. And at the age of 28, in 1976, founded the company Genentech with a scientist, Herb Boyer, who was very instrumental in some of those breakthroughs that I just mentioned to you.

And I mention Bob to you now because of your connections through MIT. But he was a wonderful guy and sadly passed away from glioblastoma at the age of 52. So he didn't really get to fully realize the benefits of what he had started, but he did, in fact, start a great deal. And this is a famous statue at Genentech which shows Herb Boyer and Bob Swanson talking about the idea for the first time and you might notice over a beer, very important part of science, discussions over a beer.

In addition to this connection to MIT and really in honor of Bob's connections to MIT, and a great pride that Bob had actually in MIT, credited MIT greatly with him learning about science, the importance of science, and also business. He got a degree in chemistry as well as a degree from the Sloan School.

We decided when we launched the Koch Institute to name a very large space in the Koch Institute for Bob Swanson. It's called the Swanson Biotechnology Center. You can visit it. So it's a series of core facilities that support all of our researchers. And indeed, researchers across the MIT campus, and this is a nice quote from Bob's widow, Judy Swanson, who has been very supportive of this effort.

So Bob Swanson and MIT, in many ways, actually, have a lot to do with the technology and the revolution that we're going to talk about now. All right. So as I mentioned, we are going to do a demo. We're going to teach you a real-life example now of how this work is done.

And our goal is to clone a gene. You can actually wait right there, Anna. Thanks. We're going to clone a gene, and we're going to clone a particular gene. It's a toxin gene. It's a gene from a pathogenic bacterium. And you might ask the question, a reasonable question, why the heck would you do that? Why would you clone a toxin gene?

So what do you think? What's the purpose in the laboratory of cloning out a toxin-encoding gene from a pathogenic bacterium? So several people have suggested the obvious was that you want to engage in global terror, which we actually don't support here at MIT, so we're going to take that down. But maybe somebody else is going to do that.

So perhaps it would be good if we got one step ahead of the game, isolated the gene, manufactured the protein, and then made a vaccine against that toxin so that we could prevent the bad consequences of exposure to the toxin. Or maybe that thing is actually very interesting independent of its bad uses. We could learn stuff, which might be helpful ultimately in related activities. So for the general purpose of biomedical research, we often study how these organisms work because it can teach us things, sometimes surprising things that are useful down the road.

The organism in question is Streptococcus pyogenes. Streptococcus pyogenes, which causes in certain cases, in certain individuals a disease called necrotizing fasciitis. Necrotizing fasciitis, which is otherwise more commonly called the flesh-eating disease. And you might think I'm joking, but I'm not. This is a true thing.

This is a true thing, and some of you might be squeamish, and if you are, and I'm being serious here. If you're squeamish looking at ugly, nasty, disgusting pictures, close your eyes for a second. I'll tell you when you can open them. But this is an

individual who was exposed to this bacterium and developed necrotizing fasciitis. That's a real-world case, so it is really pretty bad. You can open your eyes now if you closed them. I hesitate to show that slide because in the past, I've had a few boys throw up when I showed that slide.

So what are we going to do? Well, we're going to isolate this gene. And in order to isolate this gene, we need to be able to separate it, this gene, from the chromosome in which it is contained. And the chromosome from which it is contained is the chromosome of S. pyogenes. So this is the S. pyogenes chromosome. It's about four million base pairs and it has about 1,000 genes. So spread throughout this circular chromosome, there are lots of genes. We're interested in one of them.

Now, this chromosome also has another thing on it, which I hope you all know about from the material that Professor Sive just covered for you. What is the one piece of DNA material that all chromosomes need in order to replicate?

**AUDIENCE:** Origin of replication.

**PROFESSOR:** An origin of replication, very good. An origin of replication. It has an origin of replication, and then it has a bunch of genes. It has gene A. I'm just making this up. It has gene B, and it has a whole bunch of other genes as well. And then it has -- and imagine that this orange chalk was red because it's more effective if it's red. It has the T gene, and that's the toxin gene.

So our goal is to transfer the T gene and not the rest of this stuff, because we don't actually care about the rest of the stuff. We just care about the T gene -- into the E. coli cells for the reasons that I mentioned up there. And in order to do that, we have to grow large amounts of the organism that's going to in a sense donate the DNA.

We then isolate the chromosomal DNA, and we'll show you how. We're then going to use this method to fragment the DNA not randomly, but in specific places. And then we're going to transfer the DNA of interest to E. coli using this method of transformation. We're going to take this fragment and move it through the

membrane of the E. coli so that it becomes resident inside the E. coli cell.

So now on to our demonstration and my lab assistant, Anna Deconinck, will help me here. So what we've done is to, in the laboratory, isolate S. pyogenes as well as E. coli, grow them up in large quantities. You've got your gloves, right? So I'll take the buffers. So we have various solutions and buffers that will allow us to sort of wash the stuff we don't want away from the bacterial cells, lice the bacterial membrane, isolate the nucleic acid away from all the other stuff that's inside the cells, and then we'll purify the chromosomal DNA.

So Anna has grown up E. coli and S. pyogenes, taken that suspension of cells, and used a centrifuge to spin those cells down to the bottom of these tubes here-- you can show them, Anna-- these tubes here. And the first thing we need to do is get rid of the supernate, the broth that the cells grew in. So first, we're going to decant. Here, you can decant the pyogenes, but be careful with it. So we're going to decant this in order to grow up the amounts of bacteria that we need.

Now we're doing this in a very small quantities. In fact, in industrial scale, you do it in huge--

**ANNA:** Sorry.

**PROFESSOR:** Ah, that's a problem. It's actually a bit more of a problem.

**ANNA:** I just have a buffer to wash.

**PROFESSOR:** I don't think that's going to do it, Anna. Dude, we may need to actually skip, we may need to cancel. This is a little more serious. Wait a minute. Well, I don't know. Maybe. Let's just see if it's safe or not. I think it'll be okay. All right. That was a joke. She did very well though, don't you think? She did very well. That was outstanding.

Anybody want some apple juice? You're welcome to it.

**ANNA:** It needs ice.

**PROFESSOR:** Of course we would never bring pathogenic bacterium to class.