PROFESSOR: Now having cut DNA, we then need to do something else. What's the next thing we have to do?

AUDIENCE: Paste it.

PROFESSOR: Paste it right. Oh, I put past. It should be paste. There we go. We should paste it.

So, how are we going to paste our DNA? So let's take some human DNA. We'll take your human DNA. We'll add EcoR1 to it. And your human DNA is going to get cut up in lots of little pieces of length about 4,000. And I'm writing R1 at the ends because these pieces of DNA have EcoR1 sites.

Now, I can take that human DNA and I can combine it with other pieces of DNA. Here's a piece of DNA. Remember it had this overhang like that. T-T-A-A was this piece here of human DNA. And I could take another piece of DNA that matched it a A-A-T-T.

And it doesn't have to be human. It could be something else. It could be zebra. Some human DNA, some zebra DNA, and by that base pairing of those four bases, they'll sort of stick. It's not that strong. It's four bases of base pairing, but they'll stick.

Now I'd like to glue them together. What's the word for attaching together DNA strands? We talked about it-- Ligate, we want to ligate them together.

So now we go back to our MIT engineers and say, please invent me a protein that's able to ligate together pieces of DNA. But actually, who invented it first? Bacteria invented it first. And it's called? Ligase.

So all we have to do is add ligase. So it was kind of useful to know how DNA replication worked. We add ligase.

And what does ligase do for a living? For a living, ligase is paid to go around and find pieces of DNA that have nicks and seal them back up by repairing the sugar

phosphate backbone of DNA.

And again, ancient molecular biologists would prove their mettle by purifying ligase and using it in their reactions. And today, where do we get ligase? It's in the catalog. Ligase is in the catalog, reasonably cheap.

So, when we add ligase we could now get a hybrid piece of DNA that was half human and half zebra. I got to say, this freaks some people out. This doesn't freak me out because it's just a sequence of nucleotides. If I give you a sequence of nucleotides, to my mind, there's nothing human or zebra about it. It's a chemical. And you can make bonds between chemicals and all that.

So, we now pasted our DNA using ligase. Ligase from the catalog. Now the question is, what are we going to paste our DNA to? What do we paste our DNA to?

We're going to paste our DNA to a fascinating other piece of DNA like this. I'll make that a little bigger here. That has an EcoR1 site, has an EcoR1 site. And we're going to take our human DNA and paste it in such a way that it makes a circle.

This piece of DNA is a vector. Vector means it travels around. It carries things with it. So I want this vector to be able to replicate if I transfer it to E Coli. So that's the trick. I would like to be able to attachment my human DNA to this vector DNA, and when I transfer it to E coli, have this thing be able to grow.

So that means we need to invent a piece of DNA, a vector, that is capable of causing E. coli to replicate itself. It's got to have all the instructions to cause E. coli to replicate this piece of DNA. So this is another amazing bit of engineering. How do we invent just the right sequence of letters that would allow E. coli to replicate this thing?

AUDIENCE: Already been invented.

PROFESSOR: Sorry.

AUDIENCE: It's already been invented by E. coli.

PROFESSOR: It's already been invented by E. coli, hasn't it? So you're getting the theme here, is that we don't actually do anything in molecular biology. Now look, in fairness, life's had 3.5 billion years. We've been at this a decade or two, on the whole, maybe three. On the whole, life's had a lot more time to work this stuff out. And usually the best solution is to look in nature to see where nature has already done it for you.

So E. coli, it turns out, replicates its own chromosome. You could use the machinery from its own chromosome. But it turns out even better than that. E. coli has the following. Here's its big chromosome, four million letters of E. coli chromosome.

E. Coli also has within it little circles of DNA called plasmids. These little circles of DNA are able to autonomously replicate. They can copy themselves. Or that is to say, E. coli copies them. These little plasmids have the full replication instructions encoded in them.

Now why does E. coli have these plasmids? What's in these plasmids? What's going on?

Well let's blow up one of these plasmids. It's a big circle. And it has an instruction here, a sequence, called an origin of replication - or amongst friends, just ORI. It's called ORI. You'll find on maps, origin of replication. That sequence alone is enough to cause E. coli to open it up and start doing its DNA replication from the origin.

But what tells you why this is so important is that these plasmids typically have one or two genes. And the one or two genes that they typically have are genes that encode a protein that gives them resistance to an antibiotic, like say, penicillin. There could be a gene that gives you penicillin resistance. That's kind of cool. E. coli, or other bacteria-- not E. coli necessarily for penicillin-- carry around often little circles. And these little circles encode genes that give them resistance to streptomycin, penicillin, ampicillin, all sorts of things. This allows it to grow, even if you're taking penicillin.

Now, that's pretty clever. How did E. Coli come up? How is it so smart to know to have come up with a gene able to break down penicillin, given that we've only been

using penicillin since the 1940s? Pretty fast for E. coli to have figured out how to do that, isn't it?

Oh yeah, who invented penicillin? Nature invented penicillin. It's produced by fungi. Fungi have been fighting E. Coli with penicillin for millions of years. E. coli didn't invent it for us. E. Coli invented it because it's in a war down there at the single cell level with fungi. Fungi make antibi--

You see, we think we're so cool. We make antibiotics. Well we've been making antibiotics only since the 1940s. Nature's been making antibiotics forever.

Just like the viruses are infecting E. coli and E. coli needs an immune system against the viruses. Well the fungi are throwing antibiotics at the bacteria and it needs a defense mechanism. And the defense mechanism are these genes that can break down those antibiotics. And as usual, we just come along and we say, oh look at that. It's already been invented. Kind of cool. We'll use that one too.

Now why are these on these little circles? They're on the little circles because when this bacteria has lived a long and happy life and it dies and it spews its guts out, neighboring bacteria suck up the DNA. Sounds a little cannibalistic or something. But that's how it is down there at the single cell level. The neighboring bacteria will suck up DNA.

And why is that cool? It's cool because they can acquire these resistances. That's pretty impressive. They can acquire the resistance. And in fact, it even works across species. That's why you don't put it on the chromosome here. Because E. coli could pick it up from another species that's not E. coli, but related enough that it can use that plasmid. And you can transfer antibiotic resistance across many species of bacteria. So the bacteria like to suck up DNA and see what they find. Pretty cool.

By the way, this is also why indiscriminate use of antibiotics, for example, in animal feed, or when you were given antibiotics by the MIT health service and are told to take them for two weeks and you take them for five days, you're not doing anything very good. You're just selecting for bacteria that can grow in the presence of

antibiotics and selecting for multidrug resistance in the spread of antibiotics. We have to be pretty careful. Because all these mechanisms of swapping antibiotic resistance are pretty impressive tricks.

Anyway, so these things exist. And originally, molecular biologists discovered these plasmids. They purified these plasmids. And the deal was this, they cut the plasmid with EcoR1, ideally. So I'm going to put this up here, vectors. They cut the plasmid with EcoR1. They ligate human DNA into it. And they get these circles. And there you go.

Now today, obviously, if I wanted to do this experiment, would I purify the plasmid myself? No. Where would I get the plasmid from?

AUDIENCE: Catalog.

PROFESSOR: It's in the catalog. There's a whole section a plasmids here. They have vectors of all sorts. So you get it from the catalog. All right.