PROFESSOR: So now I have this. And what I need to do is I need to transform. So I need to take E.coli growing in a test tube. And I need to put into it a whole collection of zillions and zillions of plasmid molecules, plasmid circles that all have different pieces of human DNA.

One of these things might have ARG1, one ARG2, one ARG3, et cetera. Or if it's human DNA, one of them your hemoglobin, one of them your collagen, whatever. I have to throw the DNA on top of the bacteria. And then I need to come up with some engineering trick to make the bacteria take up this DNA across their cell membranes and use it.

Why should I be able to persuade bacteria to take up my DNA?

AUDIENCE: They do it anyway.

PROFESSOR: They do it anyway. Remember, we said they like to exchange DNA. That's kind of their thing, right? They pick up DNA from their environment anyway.

See, as usual, we haven't really invented something. What we've done is we have used the natural property that's out there. The natural properties, bacteria like to slurp up DNA.

Now in fairness, some pretty cool protocols were developed that improve the ability to do it. You treat the DNA, a little nastily. You give it certain ions. You heat shock it in a certain way. And then it encourages it to slurp up DNA better.

But the ability to slurp up DNA was there in the first place. We couldn't have given it the ability to slurp up DNA if it wasn't there. But we can always goose it up, improve it in certain ways. And there are great protocols that were developed in order to be able to increase the efficiency of such transformation.

So now we now have our E.coli. Here's an E.coli. Some of the E.coli have acquired a plasmid containing human DNA or maybe, if we used yeast, yeast DNA. Some of them have it, right?

Because it's kind of a random process. Some slurp it up, some haven't. So I might have, I don't know, maybe one in 100 of my cells will have slurped up DNA. But 99 in 100 won't have slurped up DNA.

I want to plate these out on a Petri plate and grow them up into individual colonies. But I would like it to be the case, please, that the individual colonies that grow up are only the ones that got my plasmid. I really don't want to see all these guys who didn't get my plasmid. So what do I do? Do I go in there with a microscope and try to figure out which ones got my human DNA plasmid?

So I would love to plate it out and only have the guys who got my plasmid grow. How do I arrange that only the guys who got my plasmid will grow? Sorry?

AUDIENCE: [INAUDIBLE].

PROFESSOR: For something. I got to put in a gene into that plasmid so that only the guys who get that will grow. Any ideas for what gene might go in there?

AUDIENCE: [INAUDIBLE].

PROFESSOR: Sorry?

AUDIENCE: [INAUDIBLE].

PROFESSOR: How about a gene conferring resistance to an antibiotic. And then what could I do? Well, I could plate. I'm going to select-- see this E.coli here, it had my plasmid. And it's going to have a drug resistance gene. And it will grow up.

This guy over here, he didn't get a drug resistance gene. He doesn't grow up. The only ones who grow up have a drug resistant-- wait a second. Why doesn't this guy grow up?

AUDIENCE: You add the drug.

PROFESSOR: Oh, I got to add the drug, good point. So what I'm going to do is I add my drug to the plate. I add my drug to the medium, to the Petri plate here. And now my Petri

plate is say a penicillin plate or a streptomycin plate or a ampicillin plate or a kanamycin plate or whatever.

And then the only things that could grow up are the ones that have resistance to whatever antibiotic I used. So I grow them up on that plate. And they grew up just fine. The ones who didn't get-- now how did I get that antibiotic gene into the plasmid?

It actually comes that way in nature, sort of. Now when I get good, I can move them around from one to another. But nature thought of it first. Nature gave us something that had an origin of replication and an antibiotic resistance gene. And we could just add things to it.

It had everything we needed. It had the ability to slurp up DNA. It had the ability to confer a new property on the cells like resistance to antibiotics.

And when we're done, we have a library here. And we call this a library of cells or colonies, each one of which has a distinct piece of human DNA, each one of which has a different piece of human DNA.

Let me stop and ask, any questions? This is like one of the coolest new procedures invented. Yes?

AUDIENCE: Is it possible that the same E.coli takes in two of the things?

PROFESSOR: Is it possible that same E.coli takes in two? Yes, turns out we do this at a dilution in a way so that most take up zero, some take up one, and a couple take up two. And what we say there is, oh well. It's not so common. And we live with it.

What else?

AUDIENCE: You need to make sure the bacteria that doesn't already have a resistance to it?

PROFESSOR: I better use a bacteria that's not already resistant to this antibiotic, good point. That's right. And I can do that.

Yes?

AUDIENCE: When making those vectors, how do you make sure it only cuts once so you can put one piece of human DNA and it doesn't break apart?

PROFESSOR: Oh, so what if the vector had five EcoR1 sites? People have engineered them to just have one. In fact, what they've really done now is they engineer them so that within the vector there's a little region, called a polylinker, that has many different sites for many different restriction enzymes, depending on which one you might want to use. And they're all right over here in the same place. And they go around and they fix the vector. So it doesn't have any of the others.

You could, for example cut it open and change the sequence a little bit. All these tools are available to us. And rather than you having to do it, they're all in the catalog. The catalog has vectors that have polylinkers, don't have any other cut sites, et cetera.

Yes?

AUDIENCE: How do you isolate the sequence you want if the cutting sequence is so frequent?

PROFESSOR: Oh, so you're asking two questions. One is you're asking how do I find the particular thing I'm looking for on the plate amongst the millions of colonies that might grow up? Or are you asking if the cutting sequence is so frequent, how do I avoid cutting in the middle of the sequence I want?

AUDIENCE: Well both, I guess. If you want to replicate human genes, if you have a bunch of DNA, how can you make it so that you isolate only that gene?

PROFESSOR: OK, so now there are really two questions you're asking. They're both really good questions. One, I take my human DNA. And let's say we're trying to clone the gene for beta-globin. Beta-globin is a part of hemoglobin. It's one of the two proteins in hemoglobin.

And let's say the beta-globin gene is a certain length. And suppose the beta-globin gene actually has several EcoR1 sites. I'm going to chop it up into multiple pieces.

That's bad, right? That's step one bad. How do I avoid that? Sorry?

AUDIENCE: Paste it.

PROFESSOR: Paste it again. Oh no, once I cut it, all the pieces of human DNA are floating around. How do I know what to paste to what?

AUDIENCE: Methylate it.

PROFESSOR: Methylate it. So first I could take my human DNA. And I could methylate it. Then it won't get cut up by the enzyme. But what's the problem with that? None of it will get cut up with the enzyme.

Suppose I did something that was a compromise though. Suppose I added a little methylase and a little restriction enzyme. And the methylase went around randomly and put some methyl groups on. Then these wouldn't get cut. And sometimes it might put on that. And those wouldn't get cut. And it would just get cut here.

I would call such a thing a partial digestion. It can be done one of two ways. I could just add a little bit of restriction enzyme and not give it too long. Or I could add a mixture of a little restriction enzyme, a little methylation enzyme, and let them have at it. And if I had the right balance, I could cut on average every second restriction site or on average every fourth restriction site. Or if I use a different ratio, every tenth restriction site.

So I actually can play a stochastic, a probabilistic game of cutting every second, fourth, tenth site and obviously get a mix. I'll get a mixture of different cuts. But in terms of cutting in the middle of my gene, I no longer care because I can do partial digestions.

Now that means that in my library I might not just have the Eco fragments. But because I did a partial digestion, I might have two consecutive or five consecutive Eco fragments, if I've done a partial digestion. But your question still stands. How do I find my right gene? How do I figure out which is the right gene?

So I've got this. I've got my plate. I've done this amazing purification. Every one of these guys has a chunk of human DNA that might be just one EcoR1 fragment. Or if I happen to have done a partial digestion, it might be two EcoR1 fragments.

But it turns out that this guy here is beta-globin. How do I know? I've purified beta-globin. I get points, right? I purified beta-globin. Somewhere on my plate is a pure beta-globin sitting there.

But the problem is I don't know where it is. How am I going to find it? They all still look the same. They look like bacteria.

AUDIENCE: [INAUDIBLE].

PROFESSOR: To do what?

AUDIENCE: Well, in this case find some way to determine whether or not a certain certain has beta-globin.

PROFESSOR: Whether a certain colony--

AUDIENCE: Has beta-globin.

PROFESSOR: Has the beta-globin gene in it. So I somehow have to test each colony to figure out whether it's got the beta-globin gene in it. How do you propose to do that?

AUDIENCE: There's some observation you can make.

PROFESSOR: Talk loud.

AUDIENCE: There's some observation you can make.

PROFESSOR: And you make that observation. Right, so-- I'm with you.

Yeah?

AUDIENCE: You basically want some ribosomes to create the proteins.

PROFESSOR: Oh, oh, oh, so maybe I can make that thing make beta-globin. Oh, and then look for

the protein. Problem with that is E.coli doesn't read human instructions. So E.coli's polymerase won't read. But it's a great idea. Maybe we can do that.

Any other ideas? How are we going to find our gene? We've made a library. We have a library. It's a bigger library than MIT. It's got more volumes than MIT. How are we're going to find our book?

AUDIENCE: Put it in a medium without beta-globin.

PROFESSOR: Put a medium without beta-globin.

AUDIENCE: See if it grows or not.

PROFESSOR: See if it grows up. E.coli couldn't care less about beta-globin. It doesn't need beta-globin. And in addition, it won't produce beta-globin because it doesn't read the human instructions.

All right, so we've gone to all this trouble. We've made a library with all the possible books in the human genome. And we don't know how to use the library. Friday's lecture let us discuss how to use the library.