

ERIC LANDER: That worked. Let's do it again. Now, number 2. I would like to clone not the Arg1 gene, but let's say the beta globin gene from human. So let's take human beta globin. I want to clone it.

So human beta globin, hemoglobin, it's a tetramer. It has four parts. It's got an alpha, an alpha, a beta, and a beta. Four proteins come together in a protein tetramer. And it's got two alphas, two betas. The beta subunit of hemoglobin is encoded by the human beta globin gene. That's the nomenclature here.

All right. Let's clone beta globin. Same deal. How are we going to do it? Any takers? What should we start with? Yes?

AUDIENCE: Find a restriction enzyme?

ERIC LANDER: Find a restriction enzyme. I'll go to catalogue and try EcoRI today. OK? Now what?

AUDIENCE: Add it to a bunch of [INAUDIBLE].

ERIC LANDER: So I'm going to start with human DNA?

AUDIENCE: Yes.

ERIC LANDER: Yes is a good answer. Yes. I'm going to start with human DNA. Good. Let's get that up there. We're going to start--

AUDIENCE: [INAUDIBLE].

ERIC LANDER: Sorry?

AUDIENCE: [INAUDIBLE].

ERIC LANDER: So we'll start with human DNA. There we go, pieces of human DNA cut with EcoRI. Now, what are we going to do? Takers? Let's attach it to a vector. Actually, wait a second. This vector here, I told you that there were vectors that could grow in E. coli. Did I ever tell you there were vectors that could grow a yeast?

There are. And they're in the catalog. OK. Yes? Now, what am I going to do? I'm going to attach this to a vector. Now, what do I do? What vector do I want to attach this to?

Where is it going to grow? Human cells. That's interesting. So one option would be human cells. So we want to take a vector that grows in human cells. Ha. Yep.

AUDIENCE: [INAUDIBLE].

ERIC LANDER: Then I need a mammalian origin of replication.

AUDIENCE: [INAUDIBLE].

ERIC LANDER: And a mammal-- Well, so it turns out that I can technically do this. There are now vectors that I can use to grow in human cells. It's a lot harder to do. So let's try doing it with a microbe first, but then we'll come back and we'll do it with human. So try doing this in a microbe.

What have you got for a microbe? How do we do this in a microbe? Well, let's try E. coli for the sake of argument. But we could also try, keep in mind your human for a second. We do this, we do this, we get a plate.

One of these guys is going to have alpha globin, one of them will have collagen. That guy here has beta globin. How are we going to recognize it? This is tough. What were you going to do for the human?

AUDIENCE: No idea.

ERIC LANDER: But you were going to get it into a human cell.

AUDIENCE: Yeah.

ERIC LANDER: Were you going to try to do complementation?

AUDIENCE: Maybe.

ERIC LANDER: Yeah. The problem doing complementation is most human cells don't need beta

globin. What's beta globin good for?

AUDIENCE: [INAUDIBLE].

ERIC LANDER: What's hemoglobin good for?

AUDIENCE: [INAUDIBLE].

ERIC LANDER: It's in your red blood cells to transport oxygen around. You think individual cells care? Nah. Your marrow might, but only in C2. The problem with doing it in a human cell initially is, if we tried the same exact trick and we tried a beta globin-deficient human cell, and we're going to try to complement with a beta globin, the problem is we don't have any conditions under which that cell cares.

But we'll come back and even do more because we could do things here. So now what are we going to do? How are we going to see where our beta globin is?

AUDIENCE: [INAUDIBLE].

ERIC LANDER: Make the protein. So we're going to persuade this E. coli to make the protein. And how are we going to recognize the protein?

AUDIENCE: [INAUDIBLE].

ERIC LANDER: So how can we recognize a protein?

AUDIENCE: Function.

ERIC LANDER: Sorry?

AUDIENCE: Function.

ERIC LANDER: Function. The problem is we didn't really have a function to complement. Electrophoresis. We could actually take every colony on the plate, grow it up, purify the proteins, and see if there was a protein band at just the right place for beta globin. We'd have to look at about a million colonies to do it. Maybe a little more, actually more because you know, statistics and all, 10 million colonies. And the

issue there is graduate students tend to, you can check with the TAs, but on the whole, tend to rebel at the thought of prepping protein from 10 million separate cells.

How else can we tell if our cells are making beta globin?

AUDIENCE: You add a marker to it?

ERIC LANDER: Something that sticks to beta globin. Turns out that antibodies are really good for this. If you take beta globin and you inject a mouse with beta globin, it'll make an immune reaction against it. And we'll learn more about the immune system in the course, but it will actually make antibodies that bind to beta globin. So suppose I told you there were antibodies that actually bind to beta globin? Now, what can I do?

AUDIENCE: [INAUDIBLE].

ERIC LANDER: I'll make it a fluorescent antibody, sure. You can have a fluorescent antibody if you want. Fluorescent antibody that knows how to bind to beta globin. Now, how do I find my cell?

AUDIENCE: Colonies.

ERIC LANDER: Take my colonies. Do what to them?

AUDIENCE: Add antibodies. And look for the colony that had antibodies--

ERIC LANDER: Stuck to it. I just wash antibodies over my colonies and see where it sticks. Now, the only details are this is on an agar plate, and it's really messy to do that. So just technical details of pulling that off are I actually grew these on a little piece of filter paper. So I put a piece of filter paper down. I grow the colonies on filter paper getting their little nutrients through there.

I take off my piece of filter paper that has my colonies growing on it. I need to crack open all of those cells, and it turns out I can do that by a chemical treatment that will crack open all the cells and leave their protein contents just spewed out. They spew out their guts right here, each cell. And then I wash my antibody over it, and my

fluorescently-labeled antibody sticks to that guy telling me that beta globin was right there. Any questions?

I did it. You did it. Except there's a problem. What was your problem?

AUDIENCE: Is it possible that the antibody won't stick?

ERIC LANDER: It's a wonderful antibody. It's a perfectly specific antibody. The antibody is totally perfect. It only sticks to beta globin. Does the bacteria want to make beta globin? You're throwing this stuff in and if any bacteria actually did make you beta globin, this would be working perfectly. The little problem is, will a piece of human DNA thrown into E. coli make beta globin?

No. Why not? Well, let's go back to our understanding of how genes work. The beta globin gene is a locus that has a human promoter which then goes and makes a transcript. That transcript has multiple exons. Those exons go into the transcript and are spliced together to make a mature message. Here is the mature message, which gets translated.

We have splicing, and then this gets translated. The spliced product gets translated. And what's the problem asking E. coli to do this?

AUDIENCE: It doesn't do splicing.

ERIC LANDER: It doesn't do splicing. And in fact, if you told me we'll use yeast instead, it turns out yeast does a very bad job in splicing human introns also. And E. coli doesn't recognize human promoters. So this doesn't work very well. Any takers on what we're going to do about this? How do we fix this problem?

AUDIENCE: [INAUDIBLE].

ERIC LANDER: Ah. So one solution would be teach E. coli how to splice DNA. That's hard. Start with cDNA. That's nice. So let's take our messenger RNA, mRNA, and let's now convert it into cDNA. What is cDNA? cDNA remember, we can copy RNA into DNA. What enzyme copies RNA into DNA? We learned about it when we studied replication and all that.

AUDIENCE: Reverse transcriptase.

ERIC LANDER: Reverse transcriptase. So if I can get my hands on some reverse transcriptase, I would be able to add reverse transcriptase to mRNAs from a human cell and make cDNA. And where do I get reversed transcriptase? It's in the catalog. So I now make cDNA and instead, I put the cDNA in the vector.

I do this, of course, for many, many, many different RNAs. I take total RNA from the human cell, I convert all the total RNA all at once into different cDNAs. Those different cDNAs get put into the vector, and I now have what's called a cDNA library. So now I have a cDNA library.

And now each of these guys has not a piece of human genomic DNA, but a piece of human cDNA copied back from a message. One of those guys has beta globin. Now will E. coli produce beta globin? No. Because

AUDIENCE: Promoter.

ERIC LANDER: Promoter. It doesn't have a promoter that it recognizes. What are we going to do we do about that?

AUDIENCE: Add one in the vector.

ERIC LANDER: Add one in the vector. Give it a promoter in the vector. Good. You guys are getting into the engineering of this. Let's say that the vector has an E. coli promoter. Now, each of these cells will actually make that mRNA. That mRNA is spliced, and it will, in fact, produce an mRNA. The cell will translate it if we do this right, and is the genetic code the same in E. coli in human? Yup. So we can actually make, you could make beta globin.

Now, we wash over our antibody, it recognizes which cell is making beta globin, and it sticks. There's beta globin. It works. Oh, by the way, what have we also just invented here? We've invented a bacterial cell that's able to produce a human protein.

What if instead of beta globin, we put in the gene for insulin? What would we have invented? A way to produce insulin without having to purify it from the pancreases of animals, which is how we used to have to treat juvenile diabetics.

But instead, if we put the insulin gene in, and we have an E. coli promoter, let's say, in theory, we can make us insulin. And what would we have invented? The biotechnology industry. That's basically the start to the biotechnology industry. One of the first things that was done was cloning the insulin gene to make recombinantly-produced insulin by just the method you guys invented today.

It's just unfortunately others invented them first, so you don't get a patent on it or anything, but all right. So now we got two ways to clone genes so far, and there are a lot of ways to clone genes. I've given you two, later in the course, I'll give you a third.