

PROFESSOR: Hello, and welcome to another help session on recombinant DNA. Today we're going to discuss genomic libraries and cDNA libraries.

So what is a genomic library? Well, with most organisms the genome is really too large to deal with. It's too large to work with or to examine. So oftentimes it's more useful to cut it down into smaller pieces that you can then examine.

How do we make a genomic library? Well, we start off with our cell of interest. And the first thing that we're going to do is we're going to isolate all the DNA from the cell. Once we have the DNA, we're going to use restriction enzymes to cut it into smaller sections.

Now, we have to be very careful when we choose which restriction enzyme we want to use. We want to use an enzyme that has enough restriction sites in the genome so that we'll get small enough pieces to put in the vector, but not so small that all the genes get cut up. Usually what we we'll use for the vector is a plasmid. A plasmid is a very standard and easy way to get foreign DNA into a bacteria. So we're going to cut up our DNA and the plasmid with our restriction enzyme.

Sometimes we might want to even make two different libraries using two different restriction enzymes so that we'll have the cuts in different places. Once we have our cut up DNA and our plasmids, now we need to ligate them together using DNA ligase. Once the cDNA is pasted into the plasmid, now we're ready to actually transform them into bacteria.

It's very easy to transform plasmids into bacteria. There are multiple different ways to do it. One common one is to use heat shock, whereby you heat the bacteria up and then cool it down rapidly, which causes little holes to open up that allows the plasmids to be taken up. What we end up with are thousands of different bacteria cells, all with different sections of the DNA.

So combined, all these different sections form the genome, the original genome that we have, but they're in much smaller sections. They're much easier to work with.

And this combination of all these different cells forms our genomic library. So that's one type of library.

Another type of library is a cDNA library. So the way that the cDNA library is different from a genomic library is that while the genomic library encompasses the entire genome of the cell, the cDNA library just looks at what genes are being expressed in the cell. So let's go over how we would make a cDNA library.

What we're going to start off with is we're going to start off by isolating the mRNA. So the mRNA, clearly, is the RNA that's being expressed in the cell. This is what's ultimately-- the proteins are going to be produced by the cell. We're then going to use reverse transcription to create the DNA version of the RNA. This is what we're referring to, again, as the cDNA.

Once we have the cDNA, we then need to add restriction sites to the ends of them. Remember, before we were just able to use innate restriction sites in the genomes. But for the cDNA library, we really want to keep the cDNA intact. We don't want to cut it up. So we just want to add restriction sites to the ends of the cDNA. Then we can treat the cDNA and the plasmids with restriction enzymes.

Once again, once they've been cut up we can use DNA ligase to combine the cut up plasmids and the cut up cDNA to the complete plasmid plus cDNA. And finally, once again, we can transform our bacteria, and then the combination of all these cells, all with different types of cDNA, form our cDNA library. And cDNA libraries are very useful if you want to compare the difference in protein expressions between two different cells.

Thank you very much. This has been another help session for recombinant DNA.