PROFESSOR ROBERT DORKIN: Hi, and welcome to a help session on recombinant DNA. Today we will be talking about the polymerase chain reaction as well as DNA sequencing. The polymerase chain reaction, also known as PCR, has many uses. One of the most common uses is to amplify a desired section of DNA. What you need for the reaction is your DNA sequence of interest, DNA polymerase, DNA primers, and then four different nucleotides. You combine all these together, and the first thing that you do is you heat the reaction up. What this does is that by adding heat to the system, you break the hydrogen bonds between the two different DNA strands. This results in two separate DNA sequences.

Now what happens is that you allow the system to cool. As it cools, the DNA primers are able to hybridize to this separate strands. Now as you remember from lecture, when you're synthesizing DNA, you synthesize from the five prime to three prime direction. This means that the primers you design have to match the three prime end of your sequences of interest. So for example, if we were designing primers for these two sequences, one of them would be GGTA and the other one would be AGCT. Now, I've written four here. In actuality, these primers are generally longer, around 16 or so, 16 to 27. However, they can be a whole different variety of lengths dependent on numerous different factors.

The next thing that happens is that the DNA polymerase is going to bind to the DNA sequence with the primer. Once the DNA polymerase is bound, it's going to take some of the free nucleotides in the surrounding area and slowly add them to finish up the strand. And so on and so forth.

Once it's completed, we are going to have now doubled our original DNA sequence. We're going to have two strands that are identical to the first one. As you can see, by repeating the steps, heating it, allowing it to cool, allowing more primers to bond, and then allowing the DNA polymerase to elongate, we can double the number of sequences every round. And you can rapidly get a large amount of the desired sequence.

PCR has other uses though besides simply increasing the total amount of DNA that you have. One of the uses of PCR is to sequence DNA. Now, if we look over here, normally DNA is form of deoxyribonucleic acids. You have the phosphate group on the five prime end. You have a hydroxyl group on the three prime end. This hydroxyl group is very important. That's because when a new nucleotide is added, this hydroxyl group undergoes a covalent bond with the phosphate on the new nucleotide and then adds a new nucleotide that way. So you can see you're adding the five prime the three prime direction.

However, it is possible to create a dideoxyribonucleic acid. The dideoxyribonucleic acid, instead of having a three prime hydroxyl group, has a three prime hydrogen. This three prime hydrogen is no longer capable of forming a covalent bond with a phosphate. That means as soon as the dideoxyribonucleic acid is added to DNA, no further nucleotides can be added in the series.

Let's go back to our example with the primers. What does that mean for here? Well, let's say you have a normal PCR reaction, but in addition to the four deoxyribonucleic acids you have, you also take a little bit of dideoxyribonucleic acids of one of the types. So let's say we add in some ddTTP. Now what happens is that your DNA polymerase will go along adding nucleotides as normal, but if it ever adds a dideoxyribonucleic acid, the polymerase will stop. So if it adds, say a normal T here-- continues down, continues down. If it adds a dideoxyribonucleic acid here, it's going to stop, and we're going to get a truncated sequence. And so you can see that at any position that we have an A, it's going to be possible to have a truncated sequence of that length. What this means that we're now going to, once the PCR is complete, have different DNA sequences of numerous different lengths. But the one thing they're all going to have in common is that they're all going to end with a T. So you can imagine doing this now for each of the four different letters.

Then we can take them and run them out of a gel. Let's go look at such a gel over here. Here we have a gel. Each of these letters represents which dideoxyribonucleic acid was used for that experiment. And then the PCR was run out on the gel. As you remember, the strands close to the bottom are the shorter strands, and the strands

close to the top are the longer strands. So if we look at this gel, we know that the shortest strand ends with a G. The next shortest strand ends in a T. Oh, sorry, ends in an A, excuse me. Then the next short strand ends in a T, then two A's, then a G, then a C, then a T. So as you can see, this is one way to determine the sequence of DNA.

Another way has been devised, which is even faster. Instead of running the sequences all out in different polymerase chain reactions, what they do is they have some of each of the dideoxynucleotides together. But now, they fluorescently label them, such that you have a different fluorescent label on each dideoxyribonucleic acid. Now what happens is that you can run it out all on one column. And then by just looking at the colors, you can determine what the sequence is. So once again, the sequence would be GATAAGCT. And so this way, you can more efficiently, more rapidly, determine what the DNA sequence is.

This has been two examples of polymerase chain reactions and their uses. This has been another help session on recombinant DNA. We hope you join us again next time. Thank you.