## 3.2 From a few copies of DNA to millions

Welcome to a new installment of this exciting subject. Remember that we saw in the last video how to extract DNA from cells. One of the most common applications of the DNA is in diagnosis, using a technique called Polymerase chain reaction, or PCR.

This is a very sensitive technique. Using it we would be able to detect the proverbial "needle in a haystack", and, to paraphrase some scientists "make a haystack from a needle". This is because a few molecules of DNA, as you'll see, will be multiplied millions of times. It is a versatile technique, which has many different applications; and fast, achieving results within a few hours.

Not all are advantages. The main disadvantage is probably the possibility of contamination with other DNAs, giving false positive results.

Let's make a PCR in our virtual laboratory to amplify our DNA. We will first prepare the reagents. Let's see what we need. Ah, yes, we need some sequences 18-25 nucleotides in length called primers, primers or oligonucleotides. These sequences are specific for the DNA that we want to amplify. We need two: one sense and another antisense, each one annealing one of the strands of the target DNA in regions separated between 200 and 1500 nucleotides.

What else do we need? Ah, we need dNTPs, i.e. the Deoxy-ribonucleotides ATP, CTP, GTP, and TTP, that make up DNA.

Also, ah, yes, we need an enzyme, DNA polymerase, that is named Taq, extracted from a very heat-resistant bacterial species, called Thermus aquaticus. Taq synthesizes a strand complementary to the DNA.

Finally, we need a buffer with the appropriate concentration of magnesium; and not to forget to include positive and negative controls.

We combine the precise amounts in small Eppendorf tubes or in multi-well plates, depending on the number of samples. We will use a device called thermocycler that we can program to change temperatures following series of three phases, that are repeated about 25-30 times or cycles.

The first phase is called denaturation. It consists in submitting the DNA sample to 94° or 95°C to separate the two strands of DNA.

Once they are separated it progresses to the next stage, called annealing. It occurs at temperatures between 50 and 65°C, depending on the sequence of primers. In this phase the primers will recognize the specific DNA sequence, if it exists, and they will hybridize with it. Remember that there is one for the positive strand and the other the antisense, for the negative strand.

In the last phase of extension at 72°C, the Taq polymerase will add the dNTPs following where the primers have annealed, extending the DNA strand all the way to the end. Thus, it returns to being a double-stranded DNA. After this, it will start again at 94 or 95°C for denaturing the amplified product, that, as you've noticed, has doubled. The majority of the enzymes would inactivate at this temperature, but as the Taq is obtained from heat-resistant bacteria, it is not damaged by temperature and it doesn't need to be added in each cycle.

The number of DNA molecules is duplicated in each cycle, so in theory, over one million copies of the original fragment are obtained after 20 cycles, delimited by the primers. Do you understand now why scientists say that a haystack can be obtained from a needle? Impressive. Now we only need to visualize the amplification, usually separating the result by electrophoresis on an agarose gel, or by other techniques.

In the following video we will see different versions of the PCR and how to quantify the amount of nucleic acid present.

Thank you very much for your attention.