## 3.3 Variations on a theme of PCR

Hello! In the last video we saw how PCR is performed. This is a very useful technique to amplify the desired DNA fragments. After being invented, variants of the technique have been developed that multiply its applications. That's what we'll see in this video.

**RT-PCR**

You will remember that the PCR is a technique for amplifying DNA. But there are many viruses that have RNA genome. Does this mean that we cannot use the technique for them? Yes, it may be used, if we enter a small step. After isolating and purifying RNA, we can use reverse transcriptase, or RT to synthesize a complementary DNA molecule which serves as starting DNA for the conventional PCR. A recombinant polymerase enzyme is currently available. It is derived from *Thermus thermophilus*, and it has has two functions: It synthesizes DNA from RNA, acting as RT, and it synthesizes DNA from DNA as in conventional PCR. It is heat-resistant, so all the reactions may be carried out at high temperature, avoiding the problems of the temperatures below 42°C. This technique is called RT-PCR.

**Nested-PCR**

Although PCR is very sensitive, sometimes there is very little specific DNA (the one that we want to detect) in the sample to be analysed, and a second amplification is needed using the first product of amplification as target. This technique is called nested-PCR and employs two pairs of primers, an external one and another internal to the first. A characteristic of this technique is that after the first amplification (20 cycles), the product is diluted, and can thus eliminate inhibitors present in the original sample. On the other hand, it has the disadvantage that, as it requires more handling, there is an increased risk of contamination with foreign DNA.

**Multiplex PCR**

Another variant of the PCR is called Multiplex. It uses different pairs of primers, each of them specific to a different virus. Different pathogens can thus be detected in the same reaction, for example, different respiratory viruses.

**qPCR o real time (rt)PCR**

The last technique that we will see is quantitative or real-time PCR. It is abbreviated as qPCR or rtPCR, but don't mistake it with RT-PCR that we have already seen. Unlike the conventional PCR, amplification of the sample DNA molecule is monitored during each cycle of amplification and not at the end. This is achieved through fluorescent reagents, called fluorochromes, of which there are two types. Oh, and also because there are thermocyclers that have a sensor that measure the fluorescence for a few brief seconds in a specific time of each cycle.

The first type of flurocrome is sandwiched between the double-stranded DNA. The one most widely used is called SYBR Green. The problem is that it can be also inserted between non-specific structures formed by the primers, interfering with the measurement. It has the advantage that the same reagent, the fluorochrome, can be used with all the pairs of primers (and thus lowering costs), but only one target sequence can be detected by tube.

The second type of fluorochrome is incorporated at the end of a short sequence of DNA or oligonucleotide that anneals specifically with the sequence to be amplified, which we will call "probe". At the other end, the probe has a molecule called "quencher". It is an inhibitor of the fluorescence. When the probe is free or loose it does not emit fluorescence, but when it anneals with the target DNA, as Taq polymerase progresses it cleaves the probe (because Taq has exonuclease activity), separating the quencher from the fluorochrome, and the latter emits fluorescence when it is struck by a laser beam. As the PCR amplifies the number of DNA

molecules, the number of targets that bind the probes increases. This system is more specific than the other, since the probe is specific. In addition, probes can be marked with different colours allowing multiple targets to be detected in the same tube turning it into a multiplex.

Different dilutions of a positive control are usually included, in order to determine by comparison with them the concentration of DNA in the sample. The reported values are usually represented in a Logarithmic chart as fluorescence in each cycle. The cycle number at which the fluorescence exceeds the threshold cycle is called threshold cycle or Ct. A higher Ct means that the sample takes longer to reach that threshold and so it has less initial DNA concentration.

In this video we have seen the most used variants of the PCR: the RT-PCR, the nested PCR, the multiplex and the quantitative PCR. The PCR and all its variants are widely used. Make sure that you understand everything before continuing. See you in the next video!

Thank you for your attention.