6.6 RT-PCR

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The polymerase chain reaction, which was discovered in 1983, has become one of the central methods of molecular biology and analyses DNA and RNA of all forms of life. Its inventor, Kary B. Mullis (California) was awarded the Nobel prize in Chemistry in 1993. Since then, the method has been steadily improved. The RT-TaqMan-PCR (Reverse transcriptase -TaqMan PCR) makes available a method that is not only highly specific but also extremely sensitive. In addition it is also suitable for mass diagnosis. In principle, PCR is based on copying a defined DNA sequence present in the sample. In a first step, primers are made: two oligonucleotides whose sequence (according to the rules of the pairing of bases) is complementary to the start sequences of the two DNA strands to be multiplied. Slow heating of the mixture to a temperature of 94° C causes the DNA double strands to fall apart into two individual strands (denaturation). A subsequent lowering of the temperature (to 40-65°) leads to hybridization of the primers with the DNA start sequences (annealing). Starting with these primers, the added polymerase copies the DNA strands. The ideal working temperature of polymerase is around 72° C. At the end of a cycle there is an exact copy of the original DNA. When this process is repeated over and over again, it is possible to get millions of copies out of a very small quantity of DNA within a short period of time. These copies can then be separated from other DNA molecules in a gel and be made visible by staining. In a suitably modified form, this method can also be used for the multiplication of RNA. This is where reverse transcriptase enters the game. This enzyme converts the RNA into DNA, or rather into cDNA (complimentary DNA) prior to actual PCR reaction.