27.7: POLYMERASE CHAIN REACTIONS

In the mid 80's a new method was developed to copy (amplify) DNA in a test tube. It doesn't require a plasmid or a virus. It just requires a DNA fragment, some primers (small polynucleotides complementary to sections of DNA on each strand and straddling the section of DNA to be amplified. Just add to this mixture dATP, dCTP, dGTP, dTTP, and a heat stable DNA polymerase from the organism Thermophilus aquaticus (which lives in hot springs), and off you go. The mixture is first heated to a temperature which will cause the DsDNA strands to separate. The temperature is cooled allowing a large stoichimetric excess of the primers to anneal to the ssDNA. The heat stable Taq polymerase (from Thermophilus aquaticus) polymerizes DNA from the primers. The temperature is raised again, allowing dsDNA strand separation. On cooling the primers anneal again to the original and newly synthesized DNA from the last cycle and synthesis of DNA occurs again. This cycle is repeated as shown in the diagram. This chain reaction is called the polymerase chain reaction (PCR). The target DNA synthesized is amplified a million times in 20 cycles, or a billion times in 30 cycles, which can be done in a few hours.

***Figure**: Copying DNA in the test tube - the polymerase chain reaction (PCR)*

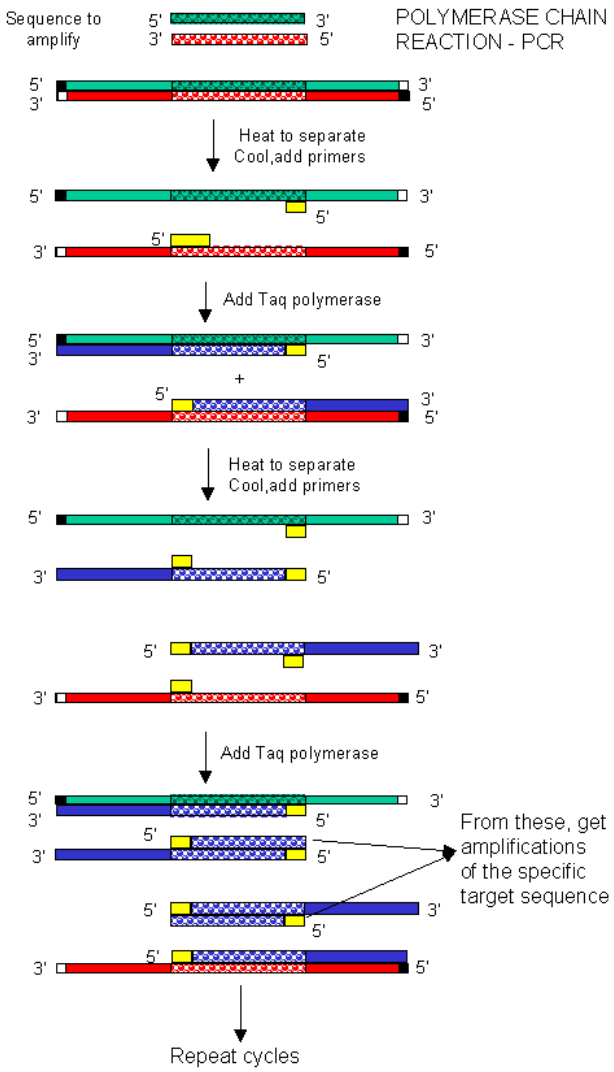

Figure: Another View of PCR