28.8: THE POLYMERASE CHAIN REACTION

OBJECTIVES

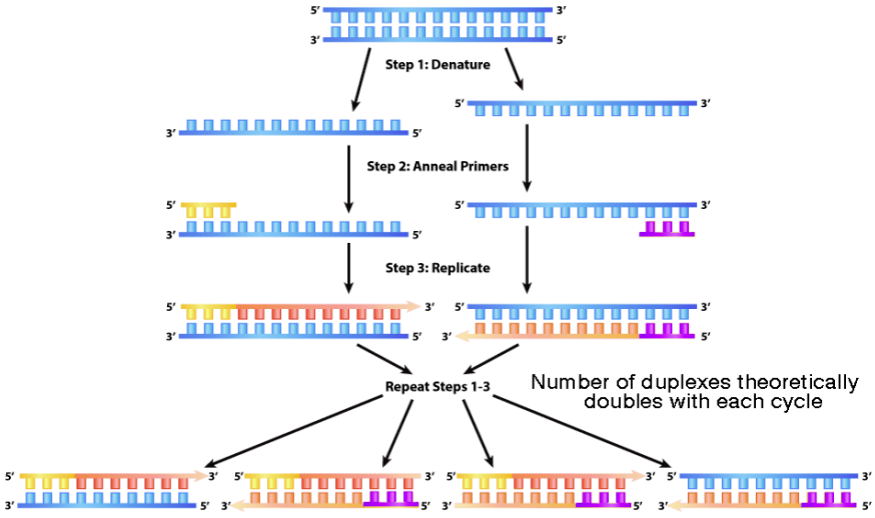
After completing this section, you should be able to

1. describe, briefly the three steps of PCR.

The polymerase chain reaction (PCR), allows one to use the power of DNA replication to amplify DNA enormously in a short period of time. As you know, cells replicate their DNA before they divide, and in doing so, double the amount of the cell's DNA. PCR essentially mimics cellular DNA replication in the test tube, repeatedly copying the target DNA over and over, to produce large quantities of the desired DNA. Kary B. Mullis was awarded a Nobel Prize in 1993 for his development of PCR, which is now the basis of innumerable research studies of gene structure, function and evolution as well as applications in criminal forensics, medical diagnostics and other commercial uses.

PCR requires a DNA fragment, some primers, which are short synthetic oligonucleotides whose sequence matches a region flanking the target sequence. All four deoxynucleotide triphosphates (dATP, dCTP, dGTP, dTTP), are added along with a heat stable DNA polymerase, **Taq**, from the organism *Thermophilus aquaticus* (which lives in hot springs).

PCR is run in a repeating cycle which involves three steps, template denaturing, primer annealing and primer extension.



TEMPLATE DENATURING

This step is the first regular cycling event and consists of heating the reaction to 94-98°C. It causes denaturing of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules. The Taq polymerase is heat-stable so it is not denatured by the high temperature needed to separate the DNA template strands.

PRIMER ANNEALING

Next, the solution is cooled to 50-65°C, a temperature that favors complementary DNA sequences finding each other and making base pairs, a process called **annealing**. Since the primers are present in great excess, the complementary sequences they target are readily found and base-paired to the primers. These primers direct the synthesis of DNA. Only where primer anneals to a DNA strand will replication occur, since DNA polymerases require a primer to begin synthesis of a new strand.

PRIMER EXTENSION

The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75-80°C, and commonly a temperature of 72°C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5′ to 3′ direction, condensing the 5′-phosphate group of the dNTPs with the 3′-hydroxyl group at the end of the nascent (extending) DNA strand. On completion of this step there are two copies of the DNA template. The new DNA then be denatured to start the cycle again. Each cycle doubles the amount of DNA. Using automated equipment, each cycle of replication can be completed in less than 5 minutes. After 30 cycles, what began as a single molecule of DNA has been amplified into more than a billion copies.