

The Polymerase Chain Reaction

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The polymerase chain reaction is summarized.

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The polymerase chain reaction exploits the natural ability of DNA to self-replicate. In DNA replication, a separated strand of original DNA is "read" by a primase to produce an RNA primer that is complementary to the original DNA strand. After the RNA primer is created, DNA nucleotides can be added to one-by-one to the primer, creating a growing strand of DNA. The addition of DNA nucleotides to the growing strand is catalyzed by the DNA polymerase III enzyme.

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DNA polymerase III is the enzyme responsible for speeding up the process of adding DNA nucleotides (based on complementarity rules) to a growing strand. The raw materials for building a strand of DNA are nucleoside triphosphates. Each nucleoside triphosphate is really a nucleotide with two additional phosphates attached. When two of those phosphates are removed, the resulting nucleotide can be added to the growing DNA strand. The energy released when the phosphates are removed powers the reaction, and DNA polymerase III speeds up the reaction.

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A nucleoside consists of a sugar attached to a nitrogenous base (i.e., a nucleoside is a nucleotide without its phosphate). If a nucleoside contains ribose as its sugar, it is specifically called a ribonucleotide (or simply a nucleoside). A nucleoside that contains deoxyribose as its sugar is specifically called a deoxyribonucleoside (or simply a deoxynucleoside). Since the polymerase chain reaction (PCR) is most often used to produce DNA (rather than RNA), PCR usually requires deoxyribonucleoside triphosphates as raw materials to use as building blocks.

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The polymerase chain reaction occurs in repeated cycles. Each cycle consists of three processes:

- In the denaturation step, the two complementary strands of the sample DNA are separated. This involves breaking the hydrogen bonds that hold complementary nucleotides (from opposite strands) together. This is done by heating the sample to about 95°C. That elevated temperature is enough to break the hydrogen bonds (which are weak), but the covalent (phosphodiester) bonds that hold nucleotides together within a strand are too strong to be broken at this temperature. Therefore, the two strands are separated from each other, but each strand remains intact along its length.
- For the annealing step, the temperature is reduced to about 65°C. Annealing refers to the binding of primers (that are included in the reaction solution) to complementary sequences of DNA nucleotides in the sample DNA. This is similar to what happens naturally in a cell during DNA replication. In a cell, the primers are made of RNA, and they are created, on the spot, to be complementary to the DNA. In PCR, the primers are made of DNA beforehand, then those primers "look" for a complementary sequence in the DNA sample.
- In the elongation step, DNA polymerase catalyzes the addition of DNA nucleotides to the growing strand, using deoxyribonucleoside triphosphates as raw materials. This step takes place at about 72°C, and it occurs just as it does in cells. However, a special DNA polymerase enzyme (called *Taq* polymerase and discovered in thermophilic bacteria) is used, because it can withstand the high temperature of the denaturation step. The DNA polymerase enzyme naturally occurring in human cells would be destroyed by such high temperature, so it cannot be used for PCR.

Annealing and elongation occur for each of the two strands of the sample DNA, so each cycle creates two copies of the DNA molecules that were present when that cycle began. In other words, each cycle doubles the amount of DNA. After 20 cycles, for instance, the original DNA is copied 2^{20} times, which is roughly one million. Each cycle requires three different temperatures, so the device used for PCR is called a thermal cycler.

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PCR is always used to amplify a very small quantity of sample DNA into a large enough quantity to be able to be used for some purpose. For instance, PCR can be used to amplify sample DNA to be used in electrophoresis. Only if enough copies of DNA are present will bands be visible in the gel.