

WORKING KNOWLEDGE

POLYMERASE CHAIN REACTION

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STEVEN SENNE AP Photo

CRIME SCENE CLUES, such as a single hair or a drop of blood, can be analyzed using PCR.

Polymerase chain reaction (PCR) is a technique that mimics nature's way of replicating DNA. First described in 1985, PCR has been adopted as an essential research tool because it can take a minute sample of genetic material and duplicate enough of it for study. PCR has been used to identify the remains of Desert Storm casualties, to analyze prehistoric DNA, to diagnose diseases and to help make identifications in police investigations.

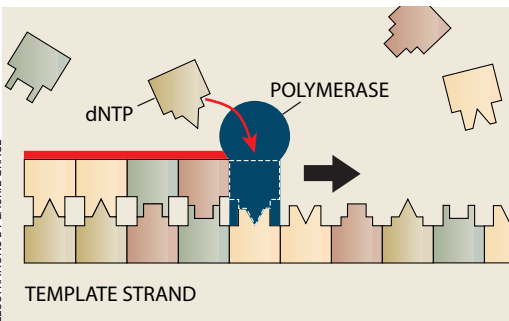
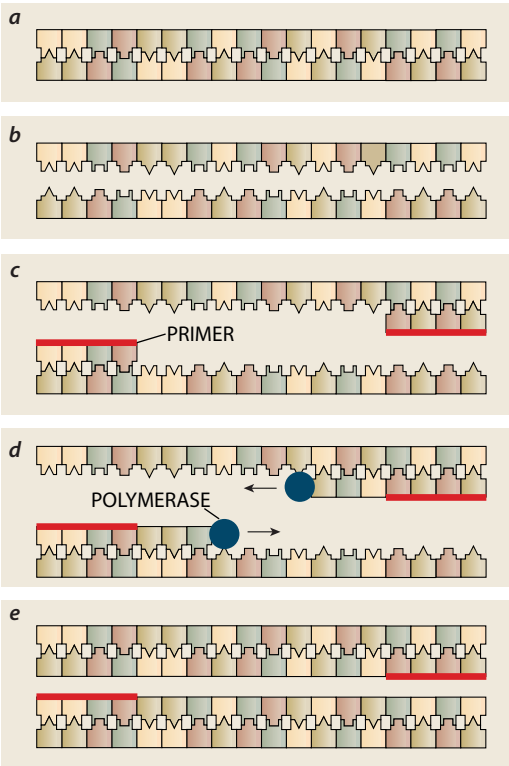
DNA is most often found as a double-stranded molecule, twisted as a helix, in which each strand complements the other. PCR starts with the DNA sample, which is put in a reaction tube along with primers (short, synthetic pieces of single-stranded DNA that exactly match and flank the stretch of DNA to be amplified), deoxynucleotide triphosphates (dNTPs, the building blocks of DNA), buffers and a heat-resistant enzyme (polymerase). Heating the mixture separates the "template" strands of DNA. Then, at varying temperatures, the rest of the components in the mixture spontaneously organize themselves, building a new complementary strand for each original.

At the end of each cycle the DNA count has doubled. If you start with one DNA molecule, at the end of 30 cycles (only a few hours later) there will be about a billion copies. Thus, if you are looking for a single gene among thousands, the game changes from "searching for a needle in a haystack" to "making a haystack of needles."

DUPLICATING DNA begins with a double-stranded stretch of DNA to be amplified, or copied (a). In a solution heated to 95 degrees Celsius (203 degrees Fahrenheit), hydrogen bonds between the strands break, leaving two single strands (b). When the mixture is cooled to between 50 and 65 degrees C, specially manufactured DNA primers bind complementarily to each strand at points flanking the region to be copied (c). At 72 degrees C, polymerase enzymes extend the bound primers in one direction, using the original DNA as a template (d). The products are two new double strands of DNA, both identical to the original (e). This cyclic reaction takes only minutes or less and can be repeated indefinitely.

POLYMERASE ENZYME extends a bound primer. From the surrounding medium, it extracts a free-floating deoxynucleotide triphosphate (dNTP) that will complement the next unpaired position in the template strand of DNA. The enzyme then joins the dNTP to the end of the primer and moves on to the next position.

EXPONENTIAL GROWTH of the DNA target occurs because the products of each cycle become the templates for the next cycle.



ILLUSTRATIONS BY LAURIE GRACE

