PCR amplification of an *Escherichia coli* gene using mixed primers containing deoxyinosine at ambiguous positions in degenerate amino acid codons

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The polymerase chain reaction (PCR) is a powerful technique widely used for enzymatic amplification of specific sequences from small amounts of DNA; the process is directed by a pair of oligonucleotide primers which flank and hybridize to opposite strands of the DNA segment to be amplified (1). In cases of targeting DNA of unknown sequence, mixed primers representing all codon choices are synthesized using the amino acid sequence of the protein encoded by the DNA. Although PCR with mixed primers is clearly successful under a wide range of conditions, the degree of specific amplification depends critically on the use of sufficient primer and the choice of annealing temperature. The number of possible structures in mixed primers can be very large and, as a consequence, could become ineffective for proteins with numerous degenerate codons. This problem can be solved by synthesizing mixed primers having deoxyinosine at ambiguous positions. We show here successful PCR amplification of the coding region of 2-keto-4-hydroxyglutarate aldolase of *Escherichia coli* (2) using two mixed primers corresponding to the 10 N-terminal and the 10 C-terminal amino acids of the enzyme containing deoxyinosine at ambiguous positions and the EcoRI linker at the 5' ends. The N-terminal and the C-terminal primer contained 4 and 7 deoxyinosine residues, respectively. The primers were used without HPLC purification. PCR products obtained after 30 cycles, which were separated by agarose gel electrophoresis, showed a single major DNA band of the predicted size when primers were annealed at 55°C (two bands were seen when the annealing temperature was below 55°C). The amino acid sequence deduced from sequencing the PCR-amplified DNA subcloned into the EcoRI site of pUC18 plasmid was found to be in complete agreement with the known primary structure of the aldolase (2). This method is useful generally for proteins with highly degenerate codons.

Figure 1 shows the PCR products after separation by agarose gel (0.7%) electrophoresis and stained with ethidium bromide. A 50- μ l PCR reaction mixture contained 0.5 μ g of *E. coli* genomic DNA, 1.0 μ g of each primer, 0.2 mM of each

nucleotide, 0.5 μ l (2.5 U) of Taq DNA polymerase from U.S. Biochemical Corp. in 100 mM Tris·HCl buffer (pH 8.3) containing 500 mM KCl, 15 mM MgCl₂, and 0.1% (w/v) gelatin. The reaction mixture was covered with 100 μ l of mineral oil to avoid evaporation. Each cycle was 1 min at 94°C, 1 min at 55°C, slow rise (in 2 min) to 72°C, and then 2 min at 72°C regulated by a thermal cycler from Perkin-Elmer Cetus. Lane 1: DNA size markers. Lane 2 and 3: PCR products in 2 μ l of reaction mixture after 30 cycles; the arrow indicates the amplified DNA from the aldolase coding region.

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