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PURIFIED THERMOSTABLE ENZYME AND PROCESS FOR AMPLIFYING, DETECTING, AND/OR CLONING NUCLEIC ACID SEQUENCES USING SAID ENZYME

Abstract

A purified thermostable enzyme is obtained that has unique characteristics. Preferably the enzyme is isolated from the Thermus aquaticus species and has a molecular weight of about 86,000-90,000 daltons. The thermostable enzyme may be native or recombinant and may be used in a temperature-cycling chain reaction wherein at least one nucleic acid sequence is amplified in quantity from an existing sequence with the aid of selected primers and nucleotide triphosphates. The amplification process comprises treating separate complementary strands of the nucleic acid with a molar excess of two oligonucleotide primers, extending the primers with a thermostable enzyme to form complementary primer extension products which act as templates for synthesizing the desired nucleic acid sequence, and detecting the sequence so amplified. The steps of the reaction can be repeated as often as desired and involve temperature cycling to effect hybridization, promotion of activity of the enzyme, and denaturation of the hybrids formed. The enzyme is preferably stored in a buffer of non-ionic detergents that lends stability to the enzyme.

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