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Birch et al.

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[54] **NUCLEIC ACID AMPLIFICATION USING A REERSIBLY INACTIVATED THERMOSTABLE ENZYME**

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[73] Assignee: **Roche Molecular Systems, Inc.**, Branchburg, N.J.

[21] Appl. No.: **684,108**

[22] Filed: **Jul. 19, 1996**

Related U.S. Application Data

[60] Provisional application No. 60/002,673, Aug. 25, 1995.

[51] **Int. Cl.** [6] **C12P 19/34**; C12Q 1/68; C07K 13/00

[52] **U.S. Cl.** **435/91.2**; 435/6; 530/350

[58] **Field of Search** 435/91.2, 6; 530/350

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[57] **ABSTRACT**

The present invention provides methods for the amplification of nucleic acids using a reversibly inactivated thermostable enzyme. The reversibly inactivated enzyme is the result of a chemical modification of the protein which inactivates the enzyme. The activity of the inactivated enzyme is recovered by an incubation of the reaction mixture at an elevated temperature prior to, or as part of, the amplification reaction. Non-specific amplification is reduced because the reaction mixture does not support the formation of extension products prior to the activating incubation.

30 Claims, 5 Drawing Sheets

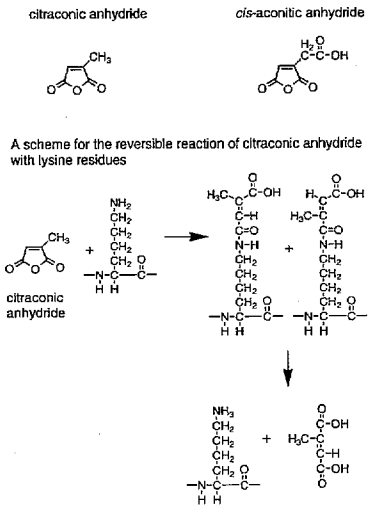

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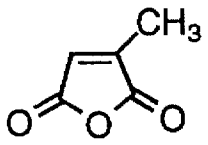
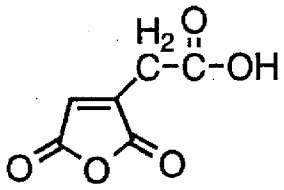
Fig. 1

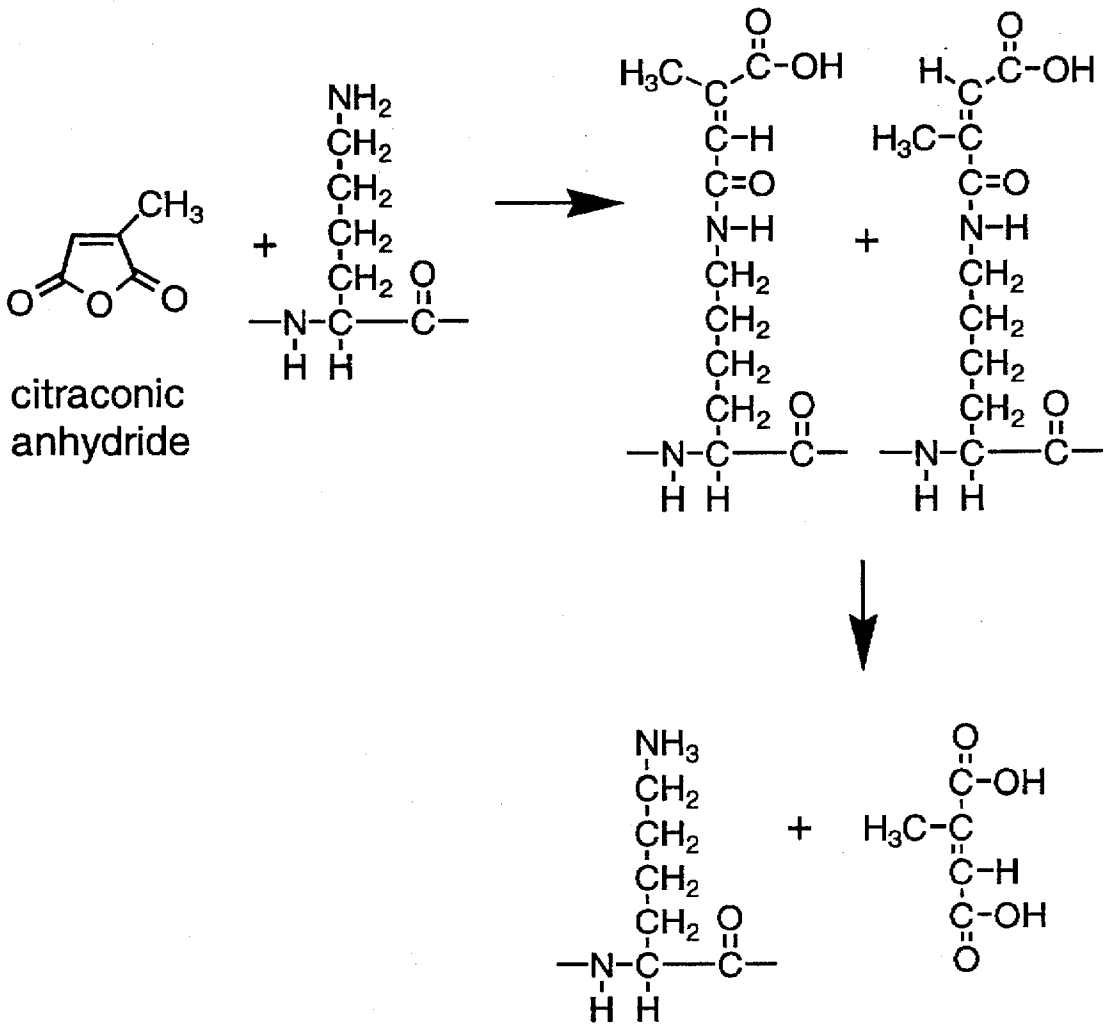
citraconic anhydride



cis-aconitic anhydride



A scheme for the reversible reaction of citraconic anhydride with lysine residues



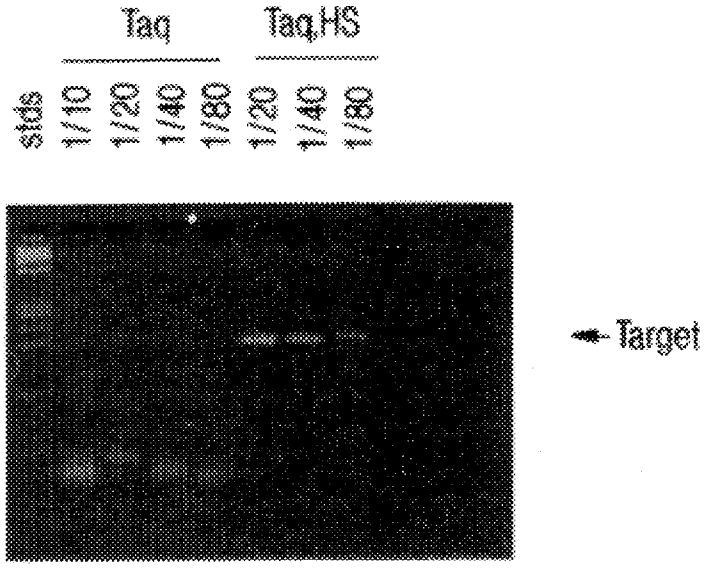


FIG. 2

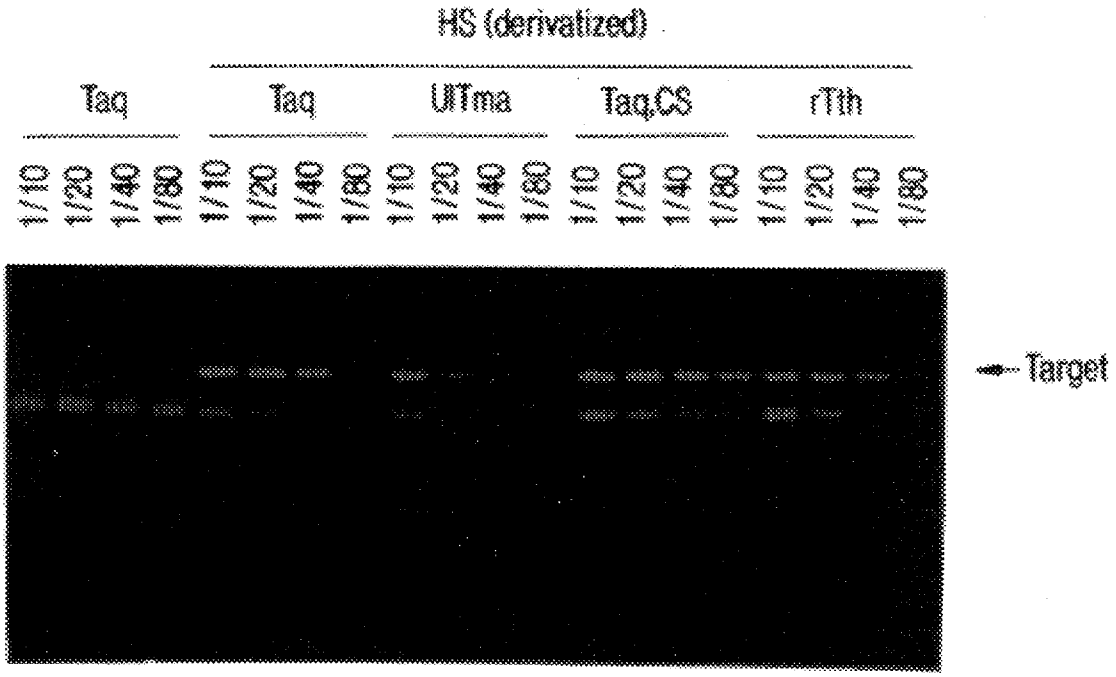


FIG. 3

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