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United States Patent [19]

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Birch et al.

[45] Date of Patent: ***Jun. 30, 1998**

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

[75] Inventors: **David Edward Birch**, Berkeley; **Walter Joseph Laird**, Pinole; **Michael Anthony Zoccoli**, Moraga, all of Calif.

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

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,677,152.

[21] Appl. No.: **680,283**

[22] Filed: **Jul. 11, 1996**

Related U.S. Application Data

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

[51] Int. Cl.⁶ **C12P 19/34**; C12N 9/00; C12N 9/99

[52] U.S. Cl. **435/91.2**; 435/183; 435/184; 435/810

[58] Field of Search 435/91.2, 810, 435/183, 184

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Primary Examiner—Kenneth R. Horlick

Assistant Examiner—Joyce Tung

Attorney, Agent, or Firm—George W Johnston; Dennis P. Tramaloni; Douglas A. Petry

[57] ABSTRACT

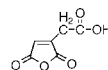
The present invention provides methods for the amplification of nucleic acids using a reversibly inactivated thermostable enzyme. The reversibly inactivated enzyme is the result of a chemical modification of the protein which inactivates the enzyme. The activity of the inactivated enzyme is recovered 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.

13 Claims, 5 Drawing Sheets

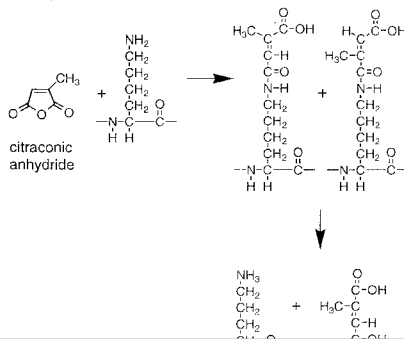
citraconic anhydride



cis-aconitic anhydride



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



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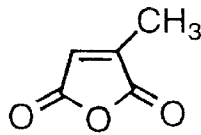
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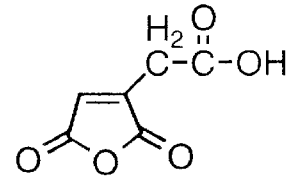
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Fig. 1

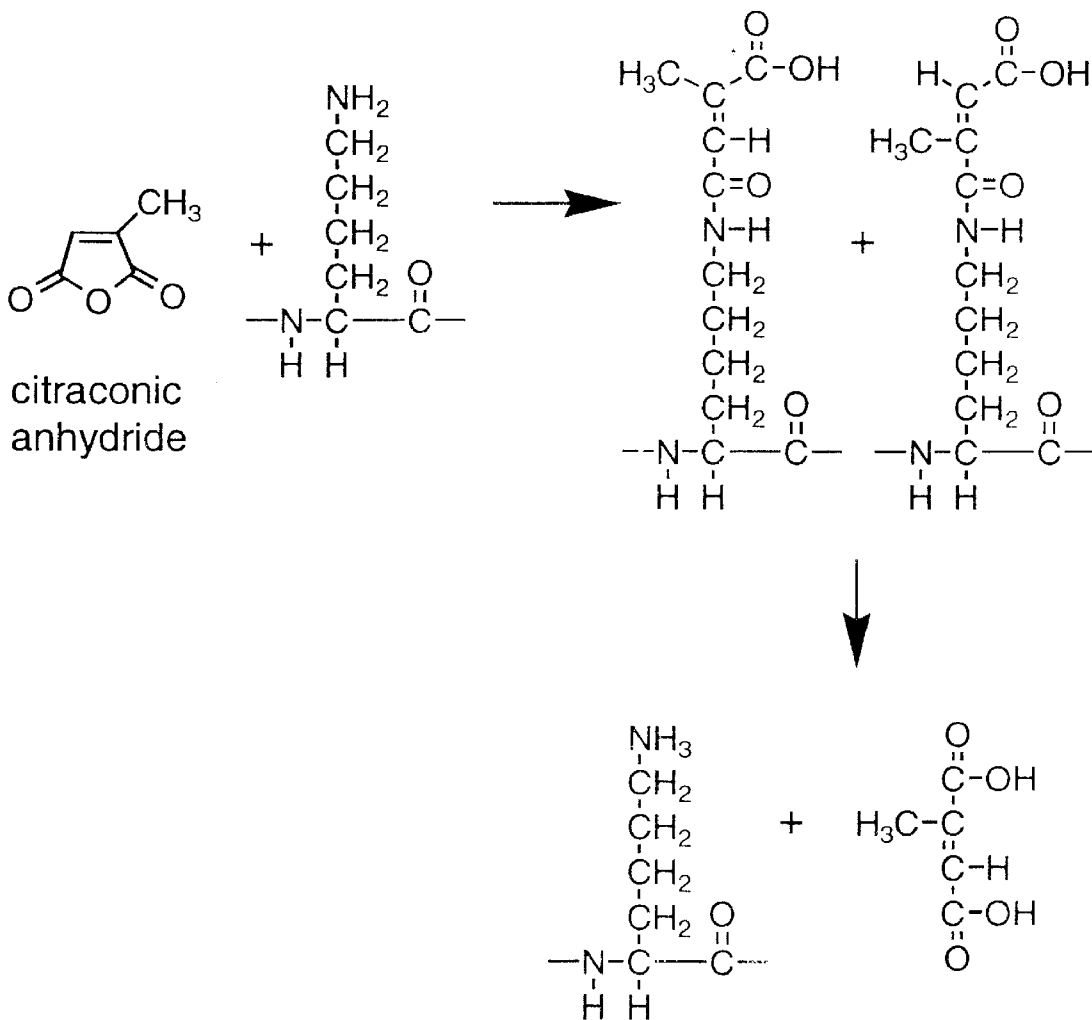
citraconic anhydride



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



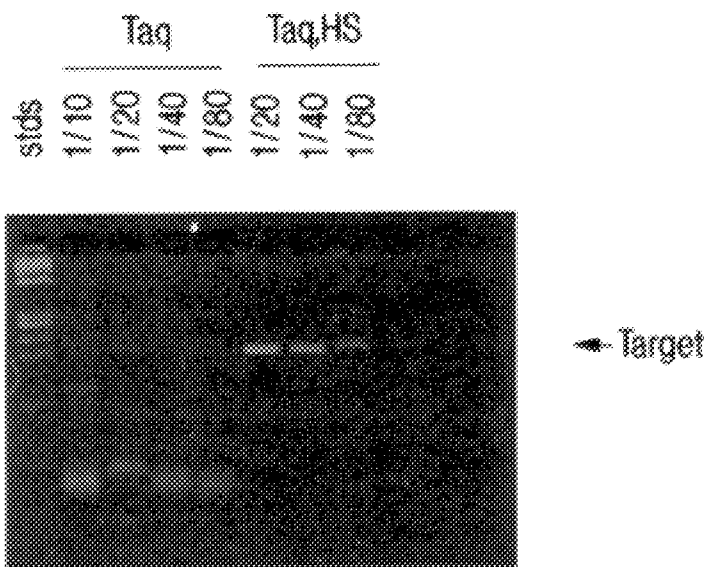


FIG. 2

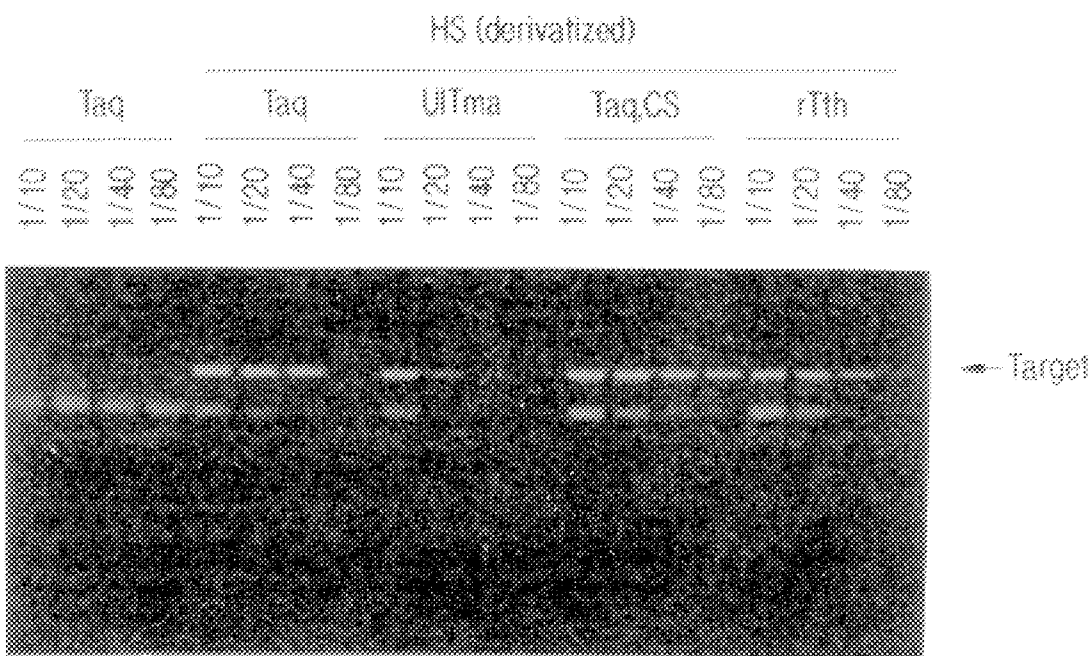


FIG. 3

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