United States Patent [19]

Blanco et al.

[11] **Patent Number: 5,001,050**

[45] **Date of Patent: Mar. 19, 1991**

[54] **PHϕ29 DNA POLYMERASE**

[75] Inventors: **Luis Blanco; Antonio Bernad; Margarita Salas,** all of Madrid, Spain

[73] Assignee: **Consejo Superior Investigaciones Cientificas,** Madrid, Spain

[21] Appl. No.: **328,462**

[22] Filed: **Mar. 24, 1989**

[51] **Int. Cl.**[5] **C12Q 1/70;** C12N 9/12; G01N 33/566; C12P 19/34

[52] **U.S. Cl.** **435/5;** 435/6; 435/91; 435/19.9; 435/183; 435/172.3; 436/501; 436/93

[58] **Field of Search** 435/6, 91, 194, 810, 435/5; 436/501; 935/77, 78

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,795,699	1/1989	Tabor et al.	435/5
4,921,794	5/1990	Tabor et al.	435/194
4,942,130	7/1990	Tabor et al.	435/172.3
4,946,786	8/1990	Tabor et al.	435/194

OTHER PUBLICATIONS

Pastrana et al., "Overproduction and Purification of Protein P6 of *Bacillus subtilis* Phage ϕ29; Role in the Initiation of DNA Replication", *Nucleic Acids Research,* vol. 13, No. 9, 1985, p. 3083.

Watabe et al., "A Novel DNA Polymerase Induced by *Bacillus subtilis* Phage ϕ29", Nucleic Acids Research, vol. 11, No. 23, 1983, p. 8333.

Zaballos, "Initiation of Phage ϕ29 DNA Replication by Mutants With Deletions at the Amino End of the Terminal Protein", *Gene,* vol. 63, pp. 113–121, 1988.

Carrascosa et al., "Synthesis in Vitro of ϕ29-Specific Early Proteins Directed by Phage DNA", Eur. J. Biochem., 51, (1975), p. 587.

Talavera et al., "Temperature-Sensitive Mutants Affected in DNA Synthesis in Phage ϕ29 of *Bacillus subtilis*", *Eur. J. Biochem.,* 31, 367, 371, (1972).

Watabe et al., "A 3′ to 5′ Exonuclease Activity is Associated with Phage ϕ29 DNA Polymerase", *Biochemical and Biophysical Research Communications,* vol. 123, No. 3, 1984, pp. 1019–1926.

Zaballos et al., "Initiation of Phage ϕ29 DNA Replication by Mutants with Deletions at the Carboxyl End of the Terminal Protein", *Gene,* 43, (1986), 103–110.

Bianco et al., "Cloning and Expression of Gene 2, Required for the Protein Primed Initiation of the *Bacillus subtilis* Phage ϕ29 DNA Replication", *Gene,* 29, (1984), 33–40.

Prieto et al., "Interaction of the Bacteriophage ϕ29 Protein p6 with Double-Stranded DNA", Proc. Natl. Acad. Sci., U.S.A., vol. 85, pp. 314–318, (1988).

Gutierrez et al., "Signals in the ϕ29 DNA-Terminal Protein Template for the Initiation of Phage ϕ29 Replication", *Virology,* 155, 474–483, (1986).

Matsumoto et al., "Aphidicolin-Resistant Mutants of Bacteriophage ϕ29: Genetic Evidence for Altered DNA Polymerase", *Virology,* 152, 32–38, (1986).

Inciarte et al., "Physical Map of Bacteriophage ϕ29 DNA", Virology, 74, 314–323, (1976).

Kuzmin et al., "S1 Plasmid from CMS-S-Maize Mitochondria Encodes a Viral Type DNA-Polymerase", *Nucleic Acids Research,* vol. 15, No. 16, 1987, p. 6758.

(List continued on next page.)

Primary Examiner—Robert A. Wax
Assistant Examiner—Stephanie W. Zitomer
Attorney, Agent, or Firm—Fish & Richardson

[57] **ABSTRACT**

An improved method for determining the nucleotide base sequence of a DNA molecule. The method includes annealing the DNA molecule with a primer molecule able to hybridize to the DNA molecule; incubating the annealed mixture in a vessel containing four different deoxynucleoside triphosphates, a DNA polymerase, and one or more DNA synthesis terminating agents which terminate DNA synthesis at a specific nucleotide base, wherein each the agent terminates DNA synthesis at a different nucleotide base; and separating the DNA products of the incubating reaction according to size, whereby at least a part of the nucleotide base sequence of the DNA can be determined. The improvement is provision of a DNA-polymerase which is a ϕ29-type DNA polymerase.

20 Claims, 2 Drawing Sheets