US008097405B1

(12) **United States Patent**
Engelhardt et al.

(10) **Patent No.: US 8,097,405 B1**
(45) **Date of Patent: Jan. 17, 2012**

(54) **NUCLEIC ACID SEQUENCING PROCESSES USING NON-RADIOACTIVE DETECTABLE MODIFIED OR LABELED NUCLEOTIDES OR NUCLEOTIDE ANALOGS, AND OTHER PROCESSES FOR NUCLEIC ACID DETECTION AND CHROMOSOMAL CHARACTERIZATION USING SUCH NON-RADIOACTIVE DETECTABLE MODIFIED OR LABELED NUCLEOTIDES OR NUCLEOTIDE ANALOGS**

(75) Inventors: **Dean Engelhardt**, New York, NY (US); **Elazar Rabbani**, New York, NY (US); **Stanley Kline**, Brooklyn, NY (US); **Jannis G. Stavrianopoulos**, New York, NY (US); **Dollie Kirtikar**, Elmhurst, NY (US)

(73) Assignee: **Enzo Biochem, Inc.**, Farmingdale, NY (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/486,069**

(22) Filed: **Jun. 7, 1995**

Related U.S. Application Data

(60) Continuation of application No. 07/954,772, filed on Sep. 30, 1992, now abandoned, which is a continuation of application No. 07/548,348, filed on Jul. 2, 1990, now abandoned, which is a division of application No. 07/140,980, filed on Jan. 5, 1988, now abandoned, which is a continuation of application No. 06/674,352, filed on Nov. 21, 1984, now abandoned, which is a continuation of application No. 06/391,440, filed on Jun. 23, 1982, now abandoned.

(51) **Int. Cl.**
C12Q 1/68 (2006.01)
C12N 15/11 (2006.01)

(52) **U.S. Cl.** **435/6**; 536/24.3

(58) **Field of Classification Search** 435/5, 6, 435/7.1, 91.1, 91.2, 810; 436/501, 63; 536/22.1, 536/23.1, 24.1, 24.3–24.33, 25.3; 735/77, 735/78

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,906,031	A		9/1975	Carpino
3,931,397	A		1/1976	Harnden 424/85
3,960,840	A		6/1976	Secrist, III et al. 260/211.5
4,038,480	A		7/1977	Robins et al. 536/27
4,119,521	A		10/1978	Chirikjian
4,124,702	A		11/1978	Lampson et al. 424/85
4,151,065	A		4/1979	Kaplan
4,213,893	A		7/1980	Carrico et al. ..... 260/112.5 R
4,230,797	A		10/1980	Boguslaski et al. 435/7
4,255,566	A		3/1981	Carrico et al. 536/27
4,260,737	A		4/1981	Scherberg 536/25
4,293,310	A		10/1981	Weber 436/536
4,310,662	A		1/1982	Crea 536/27
4,313,938	A		2/1982	Arimura et al. 424/180
4,318,846	A		3/1982	Khanna
4,358,535	A	*	11/1982	Falkow et al. 435/5
4,373,071	A		2/1983	Itakura
4,375,401	A		3/1983	Catsimpoolas
4,378,458	A		3/1983	Gohlke et al. 536/29
4,401,796	A		8/1983	Itakura
4,415,732	A		11/1983	Caruthers
4,443,594	A		4/1984	Buckmann 536/27
4,460,772	A		7/1984	Benovic et al. 514/311
4,474,948	A		10/1984	Hudson
4,483,964	A		11/1984	Urdea
4,500,707	A		2/1985	Caruthers
4,517,338	A		5/1985	Urdea
4,534,647	A		8/1985	Gross
4,581,333	A	*	4/1986	Kourilsky et al. 435/6
4,598,049	A		7/1986	Zelinka
4,605,735	A		8/1986	Miyoshi et al. 536/27
4,667,025	A		5/1987	Miyoshi
4,668,777	A		5/1987	Caruthers
4,707,440	A		11/1987	Stavrianopoulos 435/6
4,708,935	A		11/1987	Suhadolnik et al. 435/91
4,711,955	A	*	12/1987	Ward et al. 536/29
4,757,141	A		7/1988	Fung
4,828,979	A		5/1989	Klevan et al. 435/6
4,833,251	A		5/1989	Musso et al. 548/303
4,847,240	A		7/1989	Ryser

(Continued)

FOREIGN PATENT DOCUMENTS

DE A1814134 9/1970

(Continued)

OTHER PUBLICATIONS

Hartman et al., Biopolymers, vol. 20, No. 12, pp. 2635-2648, 1981.*
Biochemistry by Lehniger [Published by Worth Publishers, Inc., 70 Fifth Avenue, New York, NY 10011] pp. 638-639, 1970.*
Dunn et al., Cell vol. 12, pp. 23-26, 1977.*
Becker et al., "Irreversible Inhibition of Biotin Transport in Yeast by Biotinyl-p-nitrophenyl Ester," *Proc. Nat'l. Acad. Sci.* (USA) 68:2604-2607 (1971).

(Continued)

Primary Examiner — John S Brusca
(74) *Attorney, Agent, or Firm* — Natalie Bogdanos, Esq.; Elie Gendloff, Esq.

(57) **ABSTRACT**

A process for determining the sequence of nucleic acids of interest employs nucleotides or nucleotide analogs that have been made detectable by non-radioactive modifying or labeling. Such nucleotides or nucleotide analogs are modified on the sugar moieties, the phosphate moieties or the base moieties, including base analogs. Modified nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA. The modified or labeled nucleotides or nucleotide analogs are also useful in processes for detecting the presence of nucleic acids of interest and for characterizing chromosomal sequences. Detection processes using the modified or labeled nucleotides or nucleotide analogs extend to the use of a gel for separating or resolving hybrids formed between non-radioactively labeled oligonucleotides or polynucleotides and such nucleic acids of interest.

198 Claims, 4 Drawing Sheets