



US007270951B1

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

(10) **Patent No.:** **US 7,270,951 B1**
(45) **Date of Patent:** **Sep. 18, 2007**

(54) **METHOD FOR DIRECT NUCLEIC ACID SEQUENCING**

(75) Inventors: **Derek L. Stemple**, Hertfordshire (GB);
Niall A. Armes, Cambridge (GB)

(73) Assignee: **ASM Scientific, Inc.**, Cambridge, MA (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.: **09/936,095**

(22) PCT Filed: **Mar. 10, 2000**

(86) PCT No.: **PCT/GB00/00873**

§ 371 (c)(1),
(2), (4) Date: **Jun. 6, 2002**

(87) PCT Pub. No.: **WO00/53805**

PCT Pub. Date: **Sep. 14, 2000**

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/266,187, filed on Mar. 10, 1999.

(51) **Int. Cl.**
C12Q 1/68 (2006.01)
C12P 19/34 (2006.01)
C07H 21/02 (2006.01)
C07H 21/04 (2006.01)

(52) **U.S. Cl.** **435/6; 435/91.2; 536/23.1; 536/24.3**

(58) **Field of Classification Search** **435/6, 435/91.2; 536/23.1, 24.3**
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,811,218	A	3/1989	Hunkapiller et al.	
5,171,534	A	12/1992	Smith et al.	
5,302,509	A	4/1994	Cheeseman	
5,405,747	A	4/1995	Jett et al.	
5,547,839	A	8/1996	Dower et al.	
5,716,825	A	2/1998	Hancock et al.	
5,728,529	A	3/1998	Metzker et al.	
5,798,210	A	8/1998	Canard et al.	
5,821,058	A	10/1998	Smith et al.	
5,846,727	A	12/1998	Soper et al.	
5,853,668	A	12/1998	Begg et al.	
5,856,174	A	1/1999	Lipshutz et al.	
5,858,671	A	1/1999	Jones	
5,919,626	A	7/1999	Shi et al.	
6,007,987	A	12/1999	Cantor et al.	435/6
6,136,543	A	10/2000	Anazawa et al.	
6,210,896	B1	4/2001	Chan	
6,255,083	B1*	7/2001	Williams	435/6

FOREIGN PATENT DOCUMENTS

WO	WO90/13666	11/1990
WO	WO 91/06678	5/1991
WO	WO91/06678	* 5/1991
WO	WO93/21340	10/1993
WO	WO94/21822	9/1994
WO	WO96/31622	10/1996
WO	WO98/33939	8/1998
WO	WO 99/05315	2/1999
WO	WO99/05315	* 2/1999
WO	WO 00/36152	6/2000

OTHER PUBLICATIONS

Brandis et al. "Slow Rate of Phosphodiester Bond Formation Accounts for the Strong Bias that Taq DNA Polymerase Shows against 2',3'-Dideoxynucleotide Terminators." *Biochemistry* 35: 2189-2200 (1996).

Burns et al. "An Integrated Nanoliter DNA Analysis Device." *Science* 282: 484-487 (1998).

Chan and Nie "Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection." *Science* 281: 2016-2018 (1998).

Dickson et al. "On/off blinking and Switching Behaviour Of Single Molecules of Green Fluorescent Protein." *Nature* 388: 355-358 (1997).

Dickson et al. "Three-Dimensional Imaging of Single Molecules Solvated in Pores of Poly(acrylamide) Gels." *Science* 274: 966-971 (1996).

Dovich et al. "DNA Sequencing By Capillary Electrophoresis." *Electrophoresis* 18: 2393-2399 (1997).

Gordon et al. "Quantitative Fluorescence Resonance Energy Transfer Measurements Using Fluorescence Microscopy." *Biophys. J.* 74: 2702-2713 (1998).

Ha et al. "Probing The Interaction Between Two Single Molecules: Fluorescence Resonance Energy Transfer Between A Single Donor And A Single Acceptor." *Proc. Natl. Sci. USA* 93: 6264-6268 (1996).

Hacia, "Resequencing and Mutational Analysis Using Oligonucleotide Microarrays." *Nature Genetics Supp.* 21: 42-47 (1999).

Harada, et al. "Single-Molecule Imaging of RNA Polymerase-DNA Interactions in Real Time." *Biophys. J.* 76: 709-715 (1999).

Hiratsuka et al. "New Ribose-Modified Fluorescent Analogs of Adenine and Guanine Nucleotides Available as Substrates for Various Enzymes." *Biochim Biophys Acta* 742: 496-508 (1983).

Idury and Waterman, "A New Algorithm for DNA Sequence Assembly." *J. Comput. Biol.* 2: 291-306 (1995).

Ishijima et al. "Simultaneous Observation of Individual ATPase and Mechanical Events by a Single Myosin Molecule During Interaction with Actin." *Cell* 92: 161-171 (1998).

(Continued)

Primary Examiner—Teresa E. Strzelecka
(74) *Attorney, Agent, or Firm*—Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C.

(57) **ABSTRACT**

The present invention provides a novel sequencing apparatus and the methods employed to determine the nucleotide sequence of many single nucleic acid molecules simultaneously, in parallel. The methods and apparatus of the present invention offer a rapid, cost effective, high throughput method by which nucleic acid molecules from any source can be readily sequenced without the need for prior amplification of the sample or prior knowledge of any sequence information.



OTHER PUBLICATIONS

- Iwane et al. "Single Molecular Assay of Individual ATP Turnover by a Myosin-GFP Fusion Protein Expressed In Vitro." *FEBS Lett.* 407: 235-238 (1998).
- Izawa et al. "Recognition Sites of 3'-OH Group by T7 RNA Polymerase and Its Application to Transcriptional Sequencing." *J. Biol. Chem.* 273: 14242-14246 (1998).
- Keller et al. "Single-Molecule Fluorescence Analysis in Solution." *Applied Spectroscopy* 50: 12A-32A 1996.
- Kricka, "Miniaturization of Analytical Systems." *Clinical Chemistry* 44:9: 2008-2014 (1998).
- Luckey et al. "High Speed DNA Sequencing By Capillary Electrophoresis," *Nucleic Acids Res* 18: 4417-4421 (1990).
- Mertz et al. "Single-Molecule Detection By Two-Photon-Excited Fluorescence." *Opt. Lett.* 20:2532-2534 (1995).
- Metzker et al. "Termination Of DNA Synthesis by Novel 3'-Modified-Deoxyribonucleoside 5'-Triphosphates." *Nucleic Acids Res.* 22: 4259-4267 (1994).
- Metzker et al. "Elimination of Residual Natural Nucleotides from 3'-O-Modified-dNTP Syntheses by Enzymatic Mop-Up." *BioTechniques* 25.5: 814-817 (1998).
- Metzker et al. "Quantitation of Mixed-Base Populations of HIV-1 Variants by Automated DNA Sequencing with BODIPY® Dye-Labeled Primers." *Bio Techniques* 25.3: 446-462 (1998).
- Nie et al. "Probing Individual Molecules with Confocal Fluorescence Microscopy." *Science* 266: 1018-1021 (1994).
- Nie and Zare, "Optical Detection of Single Molecules." *Annu. Rev. Biophys. Biomol. Struct.* 26: 567-596 (1997).
- Orita et al. "Rapid and Sensitive Detection of Point Mutations and DNA Polymorphisms Using the Polymearse Chain Reaction." *Genomics* 5: 874-879 (1989).
- Pierce et al. "Imaging Individual Green Fluorescent Proteins." *Nature* 388: 338 (1997).
- Prober et al. "A System for Rapid DNA Sequencing With Fluorescent Chain-Terminating Dideoxynucleotides." *Science* 238: 336-341 (1987).
- Rawlinson et al. "Analysis of the Complete DNA Sequence of Murine Cytomegalovirus." *J. Virol* 70: 8833-8849 (1996).
- Smith et al. "Fluorescence Detection in Automated DNA Sequence Analysis." *Nature* 321: 674-679 (1986).
- Sutton et al. "TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects." *Genome Sci. Technol.* 1: 9-19 (1995).
- Tokunaga et al. "Single Molecule Imaging of Fluorophores and Enzymatic Reactions Achieved by Objective-Type Total Internal Reflection Fluorescence Microscopy." *Biochem. Biophys. Res. Comm.* 235: 47-53 (1997).
- Venter et al. "Shotgun Sequencing of the Human Genome." *Science* 280:1540-1545 (1998).
- Wang et al. "Force and Velocity Measured for Single Molecules of RNA Polymerase." *Science* 282: 902-907 (1998).
- Yin et al. "Transcription Against an Applied Force." *Science* 270: 1653-1657 (1995).
- International Search Report for PCT/GB00/00873 dated Aug. 18, 2000.
- Wang et al. "Discontinuous Movements of DNA and RNA Polymerase Accompany Formation of a Paused Transcription Complex"; *Cell*, 81:341-350 (1995).

* cited by examiner

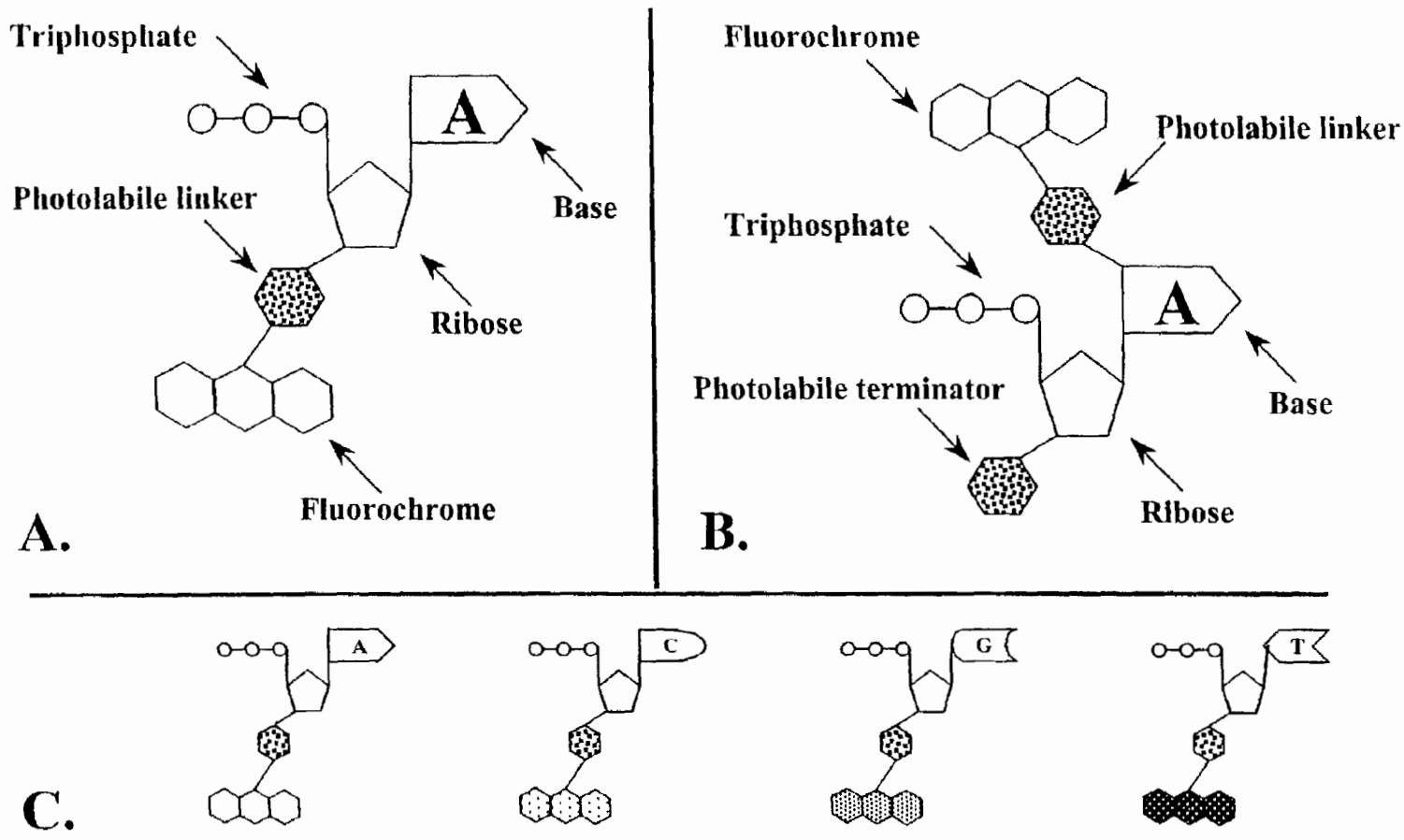


FIG. 1 Example Modified Nucleotides for DNAs

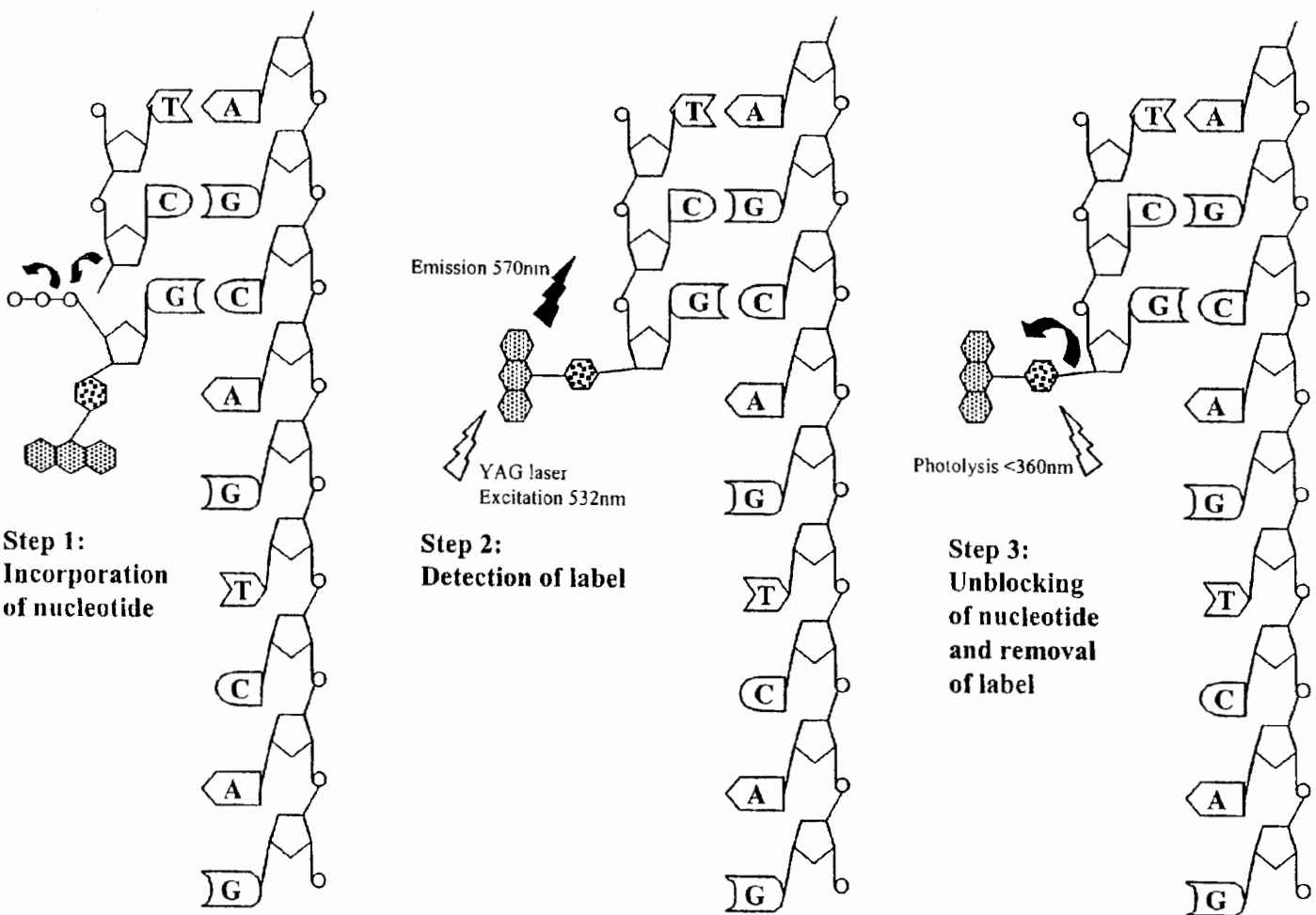


FIG. 2 The DNAS Reaction Cycle

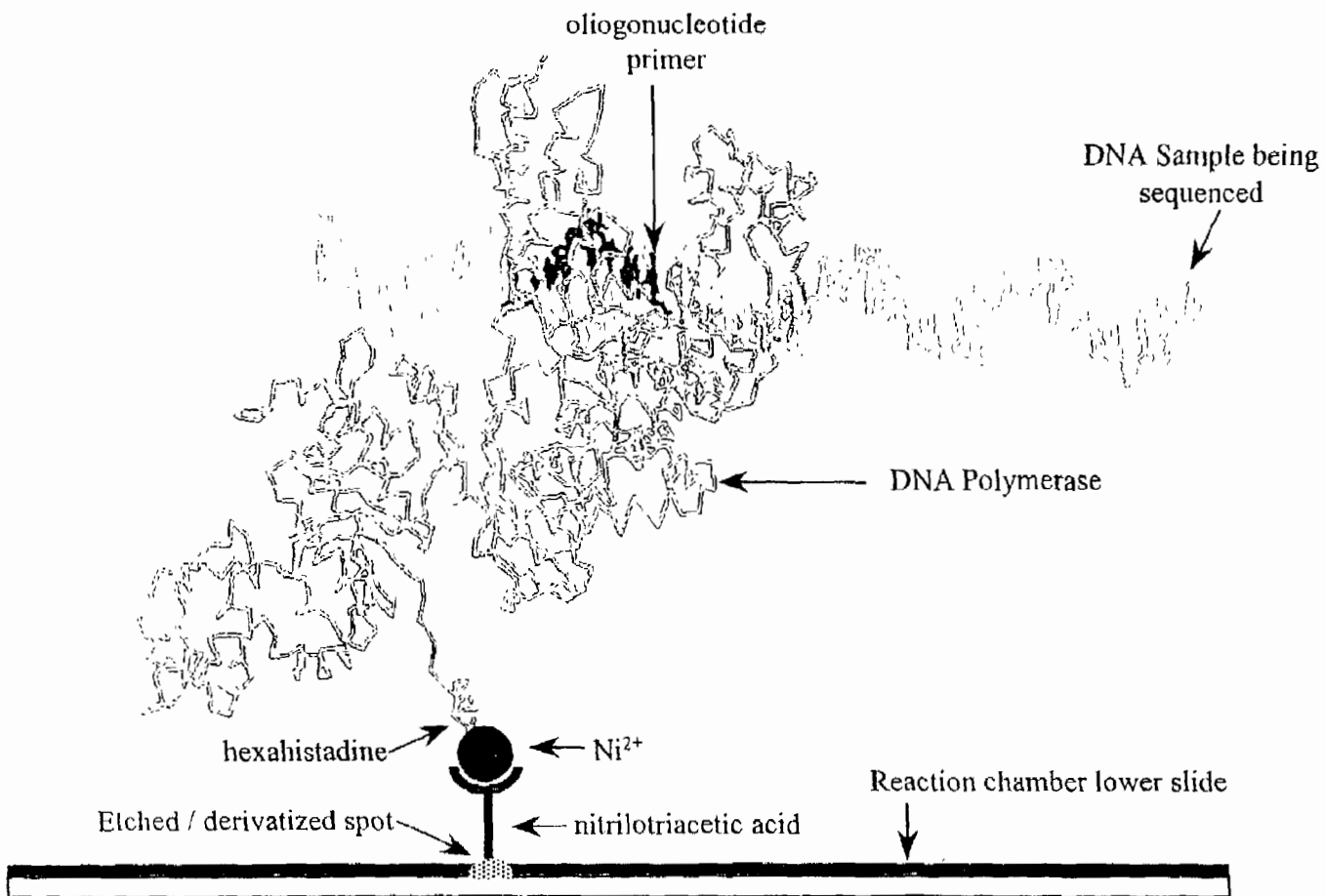


FIG. 3 Example of a DNAS Reaction Center

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.