PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12Q 1/68, C12P 19/34
// C07H 21/00, 21/04, G01N 35/08

A1

(11) International Publication Number: WO 91/06678

(43) International Publication Date: 16 May 1991 (16.05.91)

(21) International Application Number: PCT/US90/06178

(22) International Filing Date: 26 October 1990 (26.10.90)

(30) Priority data:
427,321 26 October 1989 (26.10.89) US

(71) Applicant: SRI INTERNATIONAL [US/US]; 333 Ravenswood Avenue, Menlo Park, CA 94025-3493 (US).

(71)(72) Applicant and Inventor: TSIEN, Roger, Y. [US/US]; 8535 Nottingham Place, La Jolla, CA 92037-2125 (US).

(72) Inventors: ROSS, Pepi ; 745 Contra Costa Avenue, Berkeley, CA 94707 (US). FAHNESTOCK, Margaret ; 2724 Gamble Court, Hayward, CA 94542 (US). JOHNSTON, Allan, J. ; 909 North California Avenue, Palo Alto, CA 94303 (US).

(74) Agents: CHEN, John, Y.; SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025-3493 (US) et al.

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DNA SEQUENCING

(57) Abstract

The present invention relates to an instrument and a method to determine the nucleotide sequence in a DNA molecule without the use of a gel electrophoresis step. The method employs an unknown primed single stranded DNA sequence which is immobilized or entrapped within a chamber with a polymerase so that the sequentially formed cDNA can be monitored at each addition of a blocked nucleotide by measurement of the presence of an innocuous marker on specified deoxyribonucleotides. The invention also relates to a method of determining the unknown DNA nucleotide sequence using blocked deoxynucleotides. The blocked dNTP has an innocuous marker so that its identity can be easily determined. The present instrument and method provide a rapid accurate determination of a DNA nucleotide sequence without the use of gel electrophoresis.

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Faso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	SD	Sudan
CF	Central African Republic	KP	Democratic People's Republic of Korea	SE	Sweden
CG	Congo			SN	Senegal
CH	Switzerland	KR	Republic of Korea	SU	Soviet Union
CI	Côte d'Ivoire	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

DNA SEQUENCING

Background Of The Invention

Field of the Invention

This invention relates to DNA sequencing. More particularly, it relates to methods and apparatus for determining the sequence of deoxyribonucleotides within DNA molecules.

Description of Background Art

DNA sequencing is an important tool. A current goal of the biological community in general is the determination of the complete structure of the DNA of a number of organisms, including man. This information will aid in the understanding, diagnosis, prevention and treatment of disease.

Current DNA sequencing methods employ either chemical or enzymatic procedures to produce labeled fragments of DNA molecules. In the chemical method, reactions are performed that specifically modify certain of the nucleotide bases present in the end-labeled DNA. These reactions are carried out only partially to completion so that only a portion of the bases present in the molecules are reacted. These modified bases are then treated with piperidine, to cleave the DNA chains at the modified bases producing four sets of nested fragments. These fragments are then separated from one another according to size by electrophoresis in polyacrylamide gels. The fragments can then be visualized in the gels by

means of radioactive labels. The position of the fragments in the gel indicates the identity of the last nucleotide in each fragment so that on the gel a "ladder" of fragments, with each step identified, is assembled to provide the overall sequence.

In the enzymatic method, the DNA to be sequenced is enzymatically copied by the Klenow fragment of DNA polymerase I or by a similar polymerase enzyme such as Taq polymerase or Sequenase™. The enzymatic copying is carried out in quadruplicate. In each of the four reactions a low concentration of a chain terminating dideoxynucleotide is present, a different dideoxynucleotide being present in each of the four reactions (ddATP, ddCTP, ddGTP and ddTTP). Whenever a dideoxynucleotide is incorporated, the polymerase reaction is terminated, again producing sets of nested fragments. Again, the nested fragments have to be separated from one another by electrophoresis to determine the sequence.

Recently, new advances in sequencing technology have introduced automated methods. Applied Biosystems has developed an instrument based on the use of fluorescent labels and a laser-and computer-based detection system (Smith et al., 1986; Smith, 1987). An automated system developed by E.E. du Pont de Nemours & Company, Inc. (Prober et al., 1987) is similar to the Applied Biosystems instrument but uses fluorescently labeled ddNTPs to terminate the reaction instead of fluorescent primers. Hitachi (Japan) and EMBL (West Germany) have developed similar systems (Ansorge et al., 1986). Other approaches involve multiplexing technology (Church and Kieffer-Higgins, 1988), detection of radioactively labeled DNA fragments by sensitive Beta-detectors (EG&G), automated gel readers (BioRad), and automated liquid handlers (Beckman Instruments; Seiko; Goodenow, University of California, Berkeley).

The need to rely on electrophoresis and a separation according to size as part of the analytical scheme is a severe limitation. The gel electrophoresis is a time-consuming step and requires very highly trained skilled personnel to carry it out correctly. The present invention provides methods and apparatus for sequencing DNA which do not require electrophoresis or similar separation according to size as part of their methodology.

References of Interest

The following articles and patents relate to the general field of DNA sequencing and are provided as a general summary of the background art. From time to time reference will be made to these items for their teaching of synthetic methods, coupling and detection methodologies, and the like. In these cases, they will generally be referred to by author and year.

W.B. Ansorge, et al., (1987) Nucleic Acid Research, 15:4593-4602.

W.B. Ansorge, et al., (1986) Journal of Biochemical and Biophysical Methods, 13:325-323.

J. T. Arndt-Jovin, et al., (1975) European Journal of Biochemistry, 54:411-413.

H. Bunemann, et al. (1982) Nucleic Acids Research, 10:7163-7180.,

L.D. Cama, et al., (1978) Journal of the American Chemical Society, 100:8006.

G. M. Church, et al., (1988) Science 240:185-188.

S.A. Chuvpilo, et al., (1984) "A Simple and Rapid Method for Sequencing DNA," FEBS 179:34-36.

L.F. Clerici, et al., (1979) Nucleic Acids Research, 6:247-258.

L.A. Cohen, et al., (1966) Journal of Organic Chemistry, 31:2333.

DOCKET ALARM

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.