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, G01N 23/00 // C07H 19/00

A3

(11) International Publication Number: WO 94/16101

(43) International Publication Date: 21 July 1994 (21.07.94)

(21) International Application Number: PCT/US94/00193

(22) International Filing Date: 6 January 1994 (06.01.94)

(30) Priority Data:
08/001,323 7 January 1993 (07.01.93) US

(71)(72) Applicant and Inventor: KÖSTER, Hubert [DE/US]; 1640 Monument Street, Concord, MA 01742 (US).

(74) Agents: DeCONTI, Giulio, A., Jr. et al.; Lahive & Cockfield, 60 State Street, Boston, MA 02109 (US).

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

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.

(88) Date of publication of the international search report:
24 November 1994 (24.11.94)

(54) Title: DNA SEQUENCING BY MASS SPECTROMETRY

(57) Abstract

The invention describes a new method to sequence DNA. The improvements over the existing DNA sequencing technologies are high speed, high throughput, no electrophoresis and gel reading artifacts due to the complete absence of an electrophoretic step, and no costly reagents involving various substitutions with stable isotopes. The invention utilizes the Sanger sequencing strategy and assembles the sequence information by analysis of the nested fragments obtained by base-specific chain termination via their different molecular masses using mass spectrometry, as for example, MALDI or ES mass spectrometry. A further increase in throughpout can be obtained by introducing mass-modifications in the oligonucleotide primer, chain-terminating nucleoside triphosphates and/or in the chain-elongating nucleoside triphosphates, as well as using integrated tag sequences which allow multiplexing by hybridization of tag specific probes with mass-differentiated molecular weights.

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
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 94/00193

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12Q1/68 C12P19/34 G01N23/00 //C07H19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GENETIC ANALYSIS TECHIQUES AND APPLICATIONS, vol.8, no.8, December 1991, NEW YORK US pages 223 - 229 JACOBSON K ET AL 'Application of mass spectrometry to DNA sequencing' ---	1-34, 66-73
A	WO,A,92 13629 (WAYNE STATE UNIVERSITY) 20 August 1992 see the whole document ---	1-34, 66-73
A	WO,A,89 12694 (GENOMYX INC) 28 December 1989 cited in the application see the whole document --- -/--	1-34, 66-73

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 April 1994	13. 10. 94
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer OSBORNE, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,5 064 754 (MILLS, R.) 12 November 1991 cited in the application see the whole document	1-34, 66-73
A	EP,A,0 360 677 (COMMISSARIAT A L'ENEGIE ATOMIQUE) 28 March 1990 cited in the application see the whole document	1-34, 66-73
E	US,A,5 288 644 (BEAVIS, R ET AL) 22 February 1994 see the whole document	1-32, 66-73
A	NUCLEIC ACIDS SYMPOSIUM SERIES, no.24, June 1991, OXFORD, UK pages 318 - 321 KÖSTER, H ET AL 'oligonucleotide synthesis and multiplex DNA sequencing using chemiluminescent detection' cited in the application see the whole document	33,34, 66-73
A	WO,A,89 09282 (CEMU BIOTEKNIK) 5 October 1989 see claims 1-12	33,34, 66-73

INTERNATIONAL SEARCH REPORT

.nternational application No.
PCT/US94/00193

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.- claims 1-34, 37, 66-73
2.- claims 35, 36: Solid supports insofar as not used in the methods of the invention.
3.- claims 38-65: Sets of mass-modified/differentiated nucleotides used as primers or probes in so far as not used in the methods of the invention.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-34, 37, 66-73

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten. .onal Application No
PCT/US 94/00193

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9213629	20-08-92	US-A-	5210412	11-05-93
WO-A-8912694	28-12-89	US-A-	5003059	26-03-91
		AU-B-	632756	14-01-93
		AU-A-	3847789	12-01-90
		EP-A-	0379548	01-08-90
		JP-T-	3500093	10-01-91
		US-A-	5174962	29-12-92
US-A-5064754	12-11-91	US-A-	5221518	22-06-93
EP-A-0360677	28-03-90	FR-A-	2636739	23-03-90
		DE-D-	68914372	11-05-94
		JP-A-	2176552	09-07-90
US-A-5288644	22-02-94	NONE		
WO-A-8909282	05-10-89	AU-B-	633252	28-01-93
		AU-A-	3360289	16-10-89
		DE-T-	68907407	18-11-93
		EP-A,B	0406296	09-01-91
		JP-T-	4500902	20-02-92