

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
31 August 2000 (31.08.2000)

PCT

(10) International Publication Number
WO 00/50632 A3

(51) International Patent Classification⁷: **C12Q 1/68**,
C12N 15/10

(21) International Application Number: PCT/US00/04349

(22) International Filing Date: 18 February 2000 (18.02.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/121,023 22 February 1999 (22.02.1999) US
60/158,483 8 October 1999 (08.10.1999) US

(71) Applicant (for all designated States except US): **LYNX
THERAPEUTICS, INC.** [US/US]; 3832 Bay Center
Place, Hayward, CA 94545 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **BRENNER, Sydney**
[GB/GB]; 17B St. Edwards Passage, Cambridge CB2 EPJ
(GB).

(74) Agents: **TRECARTIN, Richard, F.** et al.; Flehr Hohbach
Test Albritton & Herbert LLP, Suite 3400, 4 Embarcadero
Center, San Francisco, CA 94111-4187 (US).

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ,
BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,
DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,
IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent
(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent
(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

(88) Date of publication of the international search report:
29 March 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYMORPHIC DNA FRAGMENTS AND USES THEREOF

(57) **Abstract:** The invention provides methods and materials for generating a reference library of restriction fragments from pooled nucleic acids that contain a sequence polymorphism. Preferably, such a library is formed by digesting genomic DNA from a pool of individuals with a first and a second restriction endonuclease to form a population of restriction fragments; isolating restriction fragments of the population digested by both the first and second restriction endonucleases and forming a first single stranded fragment population therefrom; separately isolating restriction fragments from the population digested by the first restriction endonuclease but not the second restriction endonuclease and forming a second single stranded fragment population therefrom; hybridizing the first and second single stranded fragment populations to form a population of duplexes; and isolating the population of duplexes to form a reference library of restriction fragments that contain sequence polymorphism. An important aspect of the invention is the use of the reference population of restriction fragments to compare the frequencies of polymorphic sequences between different population pools. Such comparisons may be accomplished by competitively hybridizing DNA from the respective pools which has been enriched for the presence of a restriction site polymorphism with DNA from the reference population. Preferably, such competitive hybridization reactions are carried out the reference library attached to one or more solid phase supports. Most preferably, members of the reference library are attached to individual microparticles so that each microparticle has a unique fragment attached. After competitive hybridization, the microparticles may be analyzed and sorted for identifying those microparticles carrying sequences for which the pools being compared exhibit different polymorphic frequencies.

WO 00/50632 A3



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/04349

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12N15/10

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 7 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)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 25538 A (GEN HOSPITAL CORP ;UNIV LELAND STANFORD JUNIOR (US); AUSUBEL FREDE) 28 September 1995 (1995-09-28) the whole document figure 8 ---	1-18
X	WO 98 12352 A (GEN HOSPITAL CORP ;UNIV LELAND STANFORD JUNIOR (US)) 26 March 1998 (1998-03-26) the whole document figure 9 ---	1-18
X	EP 0 735 144 A (JAPAN RES DEV CORP) 2 October 1996 (1996-10-02) the whole document --- -/--	1,13-18

☒ Further documents are listed in the continuation of box C.

☒ 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

2 November 2000

Date of mailing of the international search report

09/11/2000

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

Molina Galan, E

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/04349

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 40515 A (SAMADPOUR MANSOUR) 17 September 1998 (1998-09-17) claim 16 ---	1
A	LISITSYN N ET AL: "CLONING THE DIFFERENCES BETWEEN TWO COMPLEX GENOMES" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 259, no. 5097, 12 February 1993 (1993-02-12), pages 946-951, XP000196441 ISSN: 0036-8075 cited in the application the whole document ---	1-18
A	WO 96 12014 A (LYNX THERAPEUTICS INC) 25 April 1996 (1996-04-25) the whole document ---	2,4,6-8, 10-12
A	WO 96 41011 A (SPECTRAGEN INC) 19 December 1996 (1996-12-19) cited in the application ---	
A	WO 96 12039 A (LYNX THERAPEUTICS INC) 25 April 1996 (1996-04-25) cited in the application ---	
A	QIANG LIU ET AL: "RESTRICTION INDONUCLEASE FINGERPRINTING (REF): A SENSITIVE METHOD FOR SCREENING MUTATIONS IN LONG, CONTIGUOUS SEGMENTS OF DNA" BIOTECHNIQUES,US,EATON PUBLISHING, NATICK, vol. 18, no. 3, 1 March 1995 (1995-03-01), pages 470-477, XP000493933 ISSN: 0736-6205 ---	
P,X	WO 99 23256 A (LUCITO ROBERT ;WIGLER MICHAEL (US); COLD SPRING HARBOR LAB (US)) 14 May 1999 (1999-05-14) the whole document -----	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/04349

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9525538 A	28-09-1995	AU 2187295 A CA 2185902 A EP 0812211 A US 6004783 A US 6110709 A	09-10-1995 28-09-1995 17-12-1997 21-12-1999 29-08-2000
WO 9812352 A	26-03-1998	US 6004783 A EP 0948645 A US 6110709 A	21-12-1999 13-10-1999 29-08-2000
EP 0735144 A	02-10-1996	JP 2763277 B JP 9028399 A JP 2763278 B JP 8322598 A AU 692685 B AU 5031196 A US 5707807 A	11-06-1998 04-02-1997 11-06-1998 10-12-1996 11-06-1998 10-10-1996 13-01-1998
WO 9840515 A	17-09-1998	AU 6455198 A EP 0970245 A	29-09-1998 12-01-2000
WO 9612014 A	25-04-1996	US 5604097 A AU 3946195 A AU 712929 B AU 4277896 A AU 5266399 A CA 2202167 A CZ 9700866 A DE 69513997 D DE 69513997 T EP 0786014 A EP 0793718 A EP 0952216 A FI 971473 A HU 77916 A JP 10507357 T NO 971644 A WO 9612039 A US 5695934 A US 5635400 A US 5654413 A US 5863722 A US 5846719 A	18-02-1997 06-05-1996 18-11-1999 06-05-1996 09-12-1999 25-04-1996 17-09-1997 20-01-2000 27-07-2000 30-07-1997 10-09-1997 27-10-1999 04-06-1997 28-10-1998 21-07-1998 02-06-1997 25-04-1996 09-12-1997 03-06-1997 05-08-1997 26-01-1999 08-12-1998
WO 9641011 A	19-12-1996	US 5635400 A AU 718357 B AU 6102096 A CA 2222581 A CN 1193357 A CZ 9703926 A EP 0832287 A HU 9900910 A JP 11507528 T NO 975744 A PL 324000 A AU 712929 B AU 4277896 A AU 7717596 A	03-06-1997 13-04-2000 30-12-1996 19-12-1996 16-09-1998 17-06-1998 01-04-1998 28-07-1999 06-07-1999 05-02-1998 27-04-1998 18-11-1999 06-05-1996 30-04-1997

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/04349

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9641011 A		CZ 9700866 A EP 0793718 A EP 0931165 A FI 971473 A JP 10507357 T NO 971644 A WO 9713877 A	17-09-1997 10-09-1997 28-07-1999 04-06-1997 21-07-1998 02-06-1997 17-04-1997
WO 9612039 A	25-04-1996	US 5695934 A AU 3946195 A AU 712929 B AU 4277896 A AU 5266399 A CA 2202167 A CZ 9700866 A DE 69513997 D DE 69513997 T EP 0786014 A EP 0793718 A EP 0952216 A FI 971473 A HU 77916 A JP 10507357 T NO 971644 A WO 9612014 A US 5604097 A US 5635400 A US 5654413 A US 5863722 A US 5846719 A	09-12-1997 06-05-1996 18-11-1999 06-05-1996 09-12-1999 25-04-1996 17-09-1997 20-01-2000 27-07-2000 30-07-1997 10-09-1997 27-10-1999 04-06-1997 28-10-1998 21-07-1998 02-06-1997 25-04-1996 18-02-1997 03-06-1997 05-08-1997 26-01-1999 08-12-1998
WO 9923256 A	14-05-1999	AU 1449799 A EP 1032705 A	24-05-1999 06-09-2000