

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
2 April 2009 (02.04.2009)

PCT

(10) International Publication Number
WO 2009/041776 A3

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/KR2008/005696

(22) International Filing Date:
25 September 2008 (25.09.2008)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
10-2007-0097352
27 September 2007 (27.09.2007) KR

(71) Applicant (for all designated States except US): **KOREA UNIVERSITY INDUSTRIAL & ACADEMIC COLLABORATION FOUNDATION** [KR/KR]; Korea University, 5-1 Anam-dong, Seongbuk-gu, Seoul 136-701 (KR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **GU, Man Bock** [KR/KR]; 202-602 Samsung Raemian Apt., Imun 2-dong, Dongdaemun-gu, Seoul 130-080 (KR). **K.M., Javed Hossain Niazi** [IN/KR]; 543 Division of Life Sciences, Korea University, 5-1 Anam-dong, Seongbuk-gu, Seoul 136-701 (KR). **KIM, Yeon Seok** [KR/KR]; 514 Seungwon

Apt., 1028-20 Bisan 3-dong, Dongan-gu, Anyang-si, Gyeonggi-do 431-050 (KR). **KIM, Yoonjin** [KR/KR]; 202Ho 12-51 Anam-dong 5-ga, Seongbuk-gu, Seoul 136-075 (KR). **LEE, Su Jin** [KR/KR]; 2-301 Nulbom village Villa, Bupyeong 2-dong, Bupyeong-gu, Incheon 403-012 (KR).

(74) Agent: **LEE, Moon-Sup**; 4th Fl. Oksan Bldg., 1627-10, Bongcheon-7-dong, Gwanak-gu, Seoul 151-818 (KR).

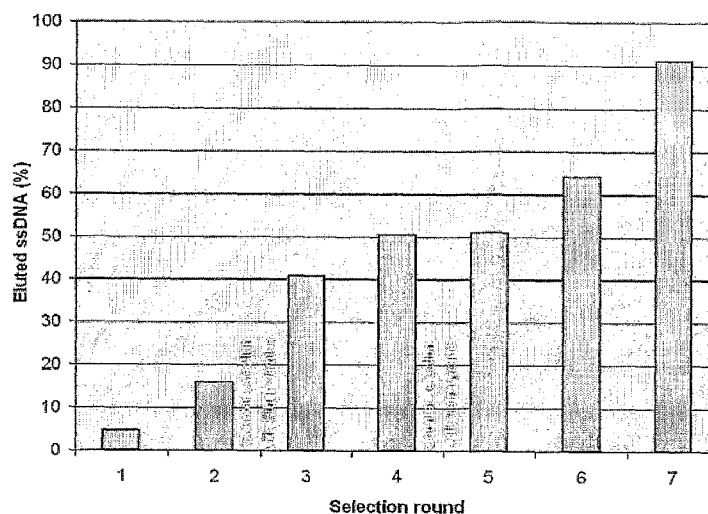
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL,

[Continued on next page]

(54) Title: DNA APTAMER BINDING TO OXYTETRACYCLINE WITH SPECIFICITY AND PRODUCTION METHOD THEREOF

[Fig. 3]



(57) Abstract: Provided is a DNA aptamer which binds specifically to Oxytetracycline (OTC). More particularly, there are provided a DNA aptamer which can bind specifically to OTC selected from a random DNA library, a method for producing the same, and a method for detecting or removing OTC using the same. DNA aptamers showing specifically high affinity for the antibiotics OTC were selected from a random DNA pool using the FluMag-SELEX process, a modification of the SELEX process. The selected DNA aptamers were confirmed to have strong binding affinity for OTC. Accordingly, the DNA aptamers provided herein may be used to effectively detect antibiotics remaining in drinking water sources, sewage, foods, human bodies, etc. Further, the DNA aptamers may be used to selectively remove a target substance from a sample containing a trace amount of antibiotics.

WO 2009/041776 A3



NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

Published:

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) **Date of publication of the international search report:**
25 June 2009

A. CLASSIFICATION OF SUBJECT MATTER*C12Q 1/68(2006.01)i*

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 C12Q 1/68

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 practicable, search terms used)

NCBI PubMed, eKIPASS, "aptamer, SELEX, oxytetracycline, DNA, etc."

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STOLTENBURG R et al. 'FluMag-SELEX as an advantageous method for DNA aptamer selection' Analytical and Bioanalytical Chemistry, September 2005, Vol.383(1), pp83-91. - see the whole document, especially Figure 1.	2-7
A	BERENS C et al., 'A tetracycline-binding RNA aptamer' Bioorganic & Medicinal Chemistry, October 2001, Vol.9(10), pp2549-2556. - see the whole documents.	2-7
A	NITSCHKE A et al., 'One-step selection of vaccinia virus-binding DNA aptamers by MonoLEX' BMC Biotechnology, August 2007, Vol.7(48), pp1-12. - see the whole documents.	2-7

☐ Further documents are listed in the continuation of Box C.☐ See patent family 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 application or patent 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 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

12 MAY 2009 (12.05.2009)

Date of mailing of the international search report

13 MAY 2009 (13.05.2009)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, 139 Seonsa-ro, Seo-
gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

Lee, Jun Hyuk

Telephone No. 82-42-481-8115



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2008/005696

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of :

a. type of material



a sequence listing



table(s) related to the sequence listing

b. format of material



on paper



in electronic form

c. time of filing/furnishing



contained in the international application as filed



filed together with the international application in electronic form



furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2008/005696**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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.: 1, 8-11
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:

The claims do not meet the requirements of PCT Article 6 in that the matter for which protection is sought is not clearly defined. The constitution of "a DNA aptamer which is capable of binding specifically to OTC" is so unclear and too functional to make meaningful search. It is necessary to define the specific nucleotide sequence.
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 No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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 searched 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.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.