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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 November 2012 (15.11.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/154689 A3

(51) **International Patent Classification**:
C12Q 1/68 (2006.01) *C12P 19/34* (2006.01)

(21) **International Application Number**:
PCT/US2012/036816

(22) **International Filing Date**:
7 May 2012 (07.05.2012)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/483,323 6 May 2011 (06.05.2011) US

(71) **Applicants** *(for all designated States except US)*: **RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY** [US/US]; Office of Corporate Liaison & Technology, Transfer, Asb III, 3 Rutgers Plaza, 3rd Floor, New Brunswick, NJ 08901-8559 (US). **RATIONAL AFFINITY DEVICES, LLC** [US/US]; 246 Brattle Street, #41, Cambridge, MA 02138 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **BRESLAUER, Kenneth, J.** [US/US]; 308 North Eighth Avenue, Edison, NJ 08817 (US). **BRAUNLIN, William, H.** [US/US]; 246 Brattle Street, #41, Cambridge, MA 02138 (US). **BEADLING, Leslie, C.** [US/US]; 416 Margaret Court, South Plainfield, NJ 07080 (US). **VOLKER, Jens** [DE/US]; Dept. of Chemistry & Chemical Biology, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854 (US).

(74) **Agents**: **NETTER, JR., Robert, C.** et al.; Dann, Dorfman, Herrell & Skillman, 1601 Market Street, Suite 2400, Philadelphia, PA 19103-2307 (US).

(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, CL, 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, KR, 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, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) **Date of publication of the international search report**:
3 January 2013

WO 2012/154689 A3

(54) **Title**: MOLECULAR CONSTRUCTS FOR DIFFERENTIATING A TARGET MOLECULE FROM A OFF-TARGET MOLECULE

(57) **Abstract**: Molecular constructs, populations thereof, arrays, compositions, methods and kits for differentiating a target molecule from an off-target molecule are provided.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/36816

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C12P 19/34 (2012.01)
USPC - 435/6.1, 6.11, 6.12, 91.2
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)
USPC: 435/6.1, 6.11, 6.12, 91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.1, 6.11, 6.12, 91.2
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google; PubMed and PatBase.
Search terms: biosensor, nucleic acid, duplex, free energy, displacement, SNP, target, off-target, equilibrium

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/0176032 A1 (BRESLAUER et al.) 11 August 2005 (11.08.2005) para [0007], [0041], [0019], [0061], [0064], [0101]-[0104], [0108]	1-17 and 28-54
A	US 2010/0204461 A1 (BEADLING et al.) 12 August 2010 (12.08.2010) entire document	1-17 and 28-54
A	MIR et al. Towards a target label-free suboptimum oligonucleotide displacement-based detection system. Anal. Bioanal. Chem. 03 May 2008, Vol. 391, pages 2145-2152, entire document	1-17 and 28-54
A	HAN et al. Design strategies for aptamer-based biosensors. Sensors. 04 May 2010, Vol. 10, pages 4541-4557, entire document.	1-17 and 28-54

☐ Further documents are listed in the continuation of Box C. ☐

* 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 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
19 October 2012 (19.10.2012)	26 OCT 2012

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/36816

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.:
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 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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-17 and 28-54, drawn to a molecular construct for differentiating a target molecule from an off-target molecule, said target molecule having a target domain and said off-target molecule having an off-target domain that differs from said target domain, said molecular construct comprising a) a first domain that is capable of binding to said target domain, and b) a second domain that is at least partially hybridizable with said first domain, said first domain is capable of binding to said off-target domain; wherein a hybrid of said first domain with said second domain is in a state of predetermined stable equilibrium in the absence of said target domain and in a state of predetermined metastable equilibrium in the presence of said target domain; and wherein the free energy of displacement of said second domain by said target domain from said hybrid of said first domain with said second domain is more favored than the free energy of displacement of said second domain by said off-target domain from said hybrid of said first domain with said second domain;
----------(Continued on extra sheet)----------

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 additional fees, this Authority did not invite payment of additional fees.

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.:
1-17 and 28-54

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.

Continuation of Box III - observations where unity of invention is lacking:

AND a population of molecular constructs for differentiating a target molecule from an off-target molecule, said target molecule having a target domain and said off-target molecule having an off-target domain that differs from said target domain, each molecular construct comprising a) a first domain that is capable of binding to said target domain, and b) a second domain that is at least partially hybridizable to said first domain, wherein said first domain is capable of binding to said off-target domain; wherein a hybrid of said first domain with said second domain is in a state of predetermined stable equilibrium in the absence of said target domain and in a state of predetermined metastable equilibrium in the presence of said target domain; and wherein the free energy of displacement of said second domain by said target domain from said hybrid of said first domain with said second domain is energetically more favored than the free energy of displacement of said second domain by said off-target domain from said hybrid of said first domain with said second domain.

Group II: Claims 18-27 and 55, drawn to a method for differentiating a target molecule from an off-target molecule, said method comprising contacting a molecular construct with an unknown molecule; AND A method for detecting the presence of a nucleic acid sequence of interest, said method comprising: a) contacting a population of probe complexes with a sample, wherein said population of probe complexes comprises at least i) a first subpopulation of probe complexes comprising a probe strand and a competitor strand, wherein said competitor strand comprises at least one hybridization domain and at least one domain which does not hybridize with said probe strand, wherein said hybridization domain is at least partially complementary to said probe strand, and ii) a second subpopulation of probe complexes comprising a probe strand and a competitor strand, wherein said competitor strand comprises at least one hybridization domain and at least one domain which does not hybridize with said probe strand, wherein said hybridization domain is at least partially complementary of said probe strand, wherein the non-hybridization domain of the second subpopulation differs from the non hybridization domain of the first subpopulation; and b) detecting the formation of complexes between the probe strand and a nucleic acid molecule from the sample, wherein the presence of such complexes is indicative of the presence of the nucleic acid sequence of interest; AND A method for detecting the presence of a nucleic acid sequence of interest, said method comprising: a) contacting a population of molecular constructs with a sample may comprise said nucleic acid sequence, wherein said population of molecular constructs comprises at least i) a first subpopulation of molecular constructs, each molecular construct comprising a probe strand and a competitor strand, wherein said probe strand comprises at least one hybridization domain and at least one other domain which does not hybridize with said competitor strand, wherein said at least one hybridization domain is at least partially complementary to said competitor strand, and ii) a second subpopulation of molecular constructs, each molecular construct comprising a probe strand and a competitor strand, wherein said probe strand comprises at least one hybridization domain and at least one other domain which does not hybridize with said competitor strand, wherein said at least one hybridization domain is at least partially complementary to said competitor strand, wherein the non-hybridization domain of the second subpopulation differs from the non-hybridization domain of the first subpopulation; and b) detecting the formation of complexes between the probe strand and a nucleic acid sequence from the sample, wherein the presence of such complexes is indicative of the presence of the nucleic acid sequence of interest.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I does not include the inventive concept of a method for differentiating a target molecule from an off-target molecule, said method comprising contacting a molecular construct with an unknown molecule; and A method for detecting the presence of a nucleic acid sequence of interest, as required by Group II.

Group II does not include the inventive concept of that a first domain (probe strand) of the molecular construct (probe complex) is capable of binding to an off-target domain; wherein a hybrid of said first domain with said second domain (competitor strand) is in a state of predetermined stable equilibrium in the absence of said target domain and in a state of predetermined metastable equilibrium in the presence of said target domain; and wherein the free energy of displacement of said second domain by said target domain from said hybrid of said first domain with said second domain is energetically more favored than the free energy of displacement of said second domain by said off-target domain from said hybrid of said first domain with said second domain, as required by Group I.

----------(Continued on Next Page)----------

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/36816

Continuation of Box III - observations where unity of invention is lacking (previous page):

Groups I-II share the technical feature of a population of molecular constructs (probe complexes) for detecting a target molecule in a sample, said target molecule having a target domain, each molecular construct comprising a) a first domain (probe strand) that is capable of binding to said target domain, and b) a second domain (competitor strand) that is at least partially hybridizable to said first domain, wherein the first domain (probe strand) is capable of forming complex between said first domain and the target domain, wherein the presence of said complex is indicative of the presence of the target molecule.
However, this shared technical feature does not represent a contribution over the prior art, specifically, US 2005/0176032 A1 to Breslauer et al. (hereinafter 'Breslauer') teaches a population of molecular constructs (para [0064] - "an array of donor/acceptor duplexes of differing stability") for detecting the presence of a target molecule (nucleic acid of interest) in a sample and differentiating a target molecule from an off-target molecule (para [0102]-[0103] - "SNP screening is based upon two segments of DNA, the first being the "major allele" base (the common base or so-called "wild type" base), and the second being the "minor allele"", 'the presence or absence of wild-type sequence'), said target molecule having a target domain and said off-target molecule having an off-target domain that differs from said target domain (para [0102]-[0103] - SNP major vs. minor alleles), each molecular construct comprising:
(a) a first domain that is capable of binding to said target domain (para [0007] - "The initial duplex is comprised of...a second nucleic acid strand having a sequence, wholly or in part, complementary to the target strand" - note that the "second" strand taught by Breslauer corresponds to the first claimed domain),
(b) a second domain that is at least partially hybridizable to said first domain (para [0007] - "The initial duplex is comprised of a first nucleic acid strand having a sequence, wholly or in part, homologous to a target strand" - note that the "first" strand taught by Breslauer corresponds to the second claimed domain),
wherein the first domain is capable of binding to said off-target domain (para [0103]),
wherein a hybrid of said first domain with said second domain is in a state of predetermined stable equilibrium in the absence of said target domain and in a state of predetermined metastable equilibrium in the presence of said target domain (para [0007] - competitive equilibria of initial duplex vs. binding of target with "first" nucleic acid strand - first" as taught by Breslauer, corresponding to "second" as claimed); and
wherein the free energy of displacement of said second domain by said target domain from said hybrid of said first domain with said second domain is energetically more favored than the free energy of displacement of said second domain by said off-target domain from said hybrid of said first domain with said second domain (para [0107] - "Thus the actual measurement is the ratio of the two equilibrium constants, and thus the difference in free energies for formation of these alternative duplexes. The difference in free energy caused by a defect, such as a single base pair mismatch (i.e. not a canonical Watson-Crick pairing), is the same whether the defect is within a very long duplex or a short one, so long as the bulk of the duplex generally remains stable."); and
Wherein the first domain (probe strand) is capable of forming complex between said first domain and the target domain, wherein the presence of said complex is indicative of the presence of the target molecule.(claims 7 and 9; para [0103]-[0107], [0064]).

As said shared technical feature was known at the time of the invention, this cannot be considered special technical feature that would otherwise unify the Groups.

Groups I-II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.