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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 January 2013 (31.01.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/016280 A3

(51) **International Patent Classification**:
C12Q 1/68 (2006.01) ***G01N 33/53*** (2006.01)
C40B 30/04 (2006.01) ***C12N 15/115*** (2010.01)
C40B 40/06 (2006.01)

(21) **International Application Number**:
PCT/US2012/047840

(22) **International Filing Date**:
23 July 2012 (23.07.2012)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/510,796 22 July 2011 (22.07.2011) US

(71) **Applicant** *(for all designated States except US)*: **MEDIOMICS, LLC** [US/US]; 5445 Highland Park Drive, St. Louis, Missouri 63110 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **CHANG, Yie-Hwa** [US/US]; 5445 Highland Park Drive, St. Louis, Missouri 63110 (US). **TIAN, Ling** [CN/US]; 5445 Highland Park Drive, St. Louis, Missouri 63110 (US). **WANG, Rongsheng** [CN/US]; 5445 Highland Park Drive, St. Louis, Missouri 631103 (US).

(74) **Agents**: **RILEY-VARGAS, Rebecca** et al.; Polsinelli Shughart PC, Mark Twain Plaza III, 105 West Vandalia, Suite 400, Edwardsville, IL 62025 (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))*
— *with sequence listing part of description (Rule 5.2(a))*

[Continued on next page]

(54) **Title**: COMPOSITIONS AND METHODS FOR SELECTING APTAMERS

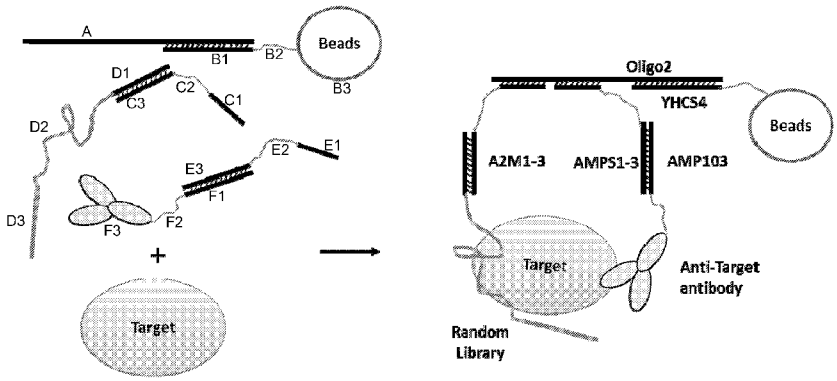

FIG. 1

(57) **Abstract**: The invention encompasses compositions and methods for selecting aptamers.

WO 2013/016280 A3

(88) Date of publication of the international search report:
21 March 2013

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/47840

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68, C40B 30/04, C40B 40/06, G01N 33/53, C12N 15/115 (2012.01)
USPC - 435/6.1, 435/7.1, 506/9, 506/16
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(8) -- C12Q 1/68, C40B 30/04, C40B 40/06, G01N 33/53, C12N 15/115 (2012.01)
USPC -- 435/6.1, 435/7.1, 506/9, 506/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/6.11, 435/6.12, 435/91.2, 536/24.3, 506/2
(Text Search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase; PubWEST (PGPB, USPT, USOC, EPAB, JPAB); Dialog Classic (Files 654, 652, 349, 348, 35, 65, 155) and Google Scholar.
Search Terms: aptamers, bridge, linkers, Spacer 18, specific targeting, primer, ssDNA, triplex, bridge, epitope, solid support, target, single-stranded, primer, bead, plate, oligonucleotide, nucleic acid, analyte, antibody, im

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0021899 A1 (IKEBUKURO et al.) 28 January 2010 (28.01.2010) abstract; para [0013], [0014], [0026], [0028]-[0031], [0035]-[0037], [0062], [0072]	1-12 and 25
Y	US 2005/0009050 A1 (NADEAU et al.) 13 January 2005 (13.01.2005) abstract; para [0020], [0095], [0097], Fig 1	1-12 and 25
Y	US 2005/0089890 A1 (CUBICCIOTTI) 28 April 2005 (28.04.2005) para [0020], [0021], [0032], [0057], [0061], [0083], [0099], [0141], [0144], [0184], [0251], [0379], [0391], [0450], [0507], [0528], [0560], [0565], [0570], [0575], [0578], [0595], [0601], [0603], [0784].	5 and 12

☐ 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
21 December 2012 (21.12.2012)	11 JAN 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/47840

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.

**********Continued in Supplemental Box**********

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.:
1-12 and 25

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/47840

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

Group I: Claims 1-12 and 25, drawn to a composition, the composition comprising three components: a bridge construct, an aptamer construct, and an epitope binding agent construct, wherein a) the bridge construct comprises A and B1-B2-B3; b) the aptamer construct comprises C1-C2-C3 and D1-D2-D3; c) the epitope binding agent construct comprises E1-E2-E3, F1-F2-F3; and wherein A is a single-stranded nucleic acid comprising a binding site for B1, C1, and E1, B1 is a single-stranded nucleic acid that binds to a complementary region on A, B2 is optionally a linker that joins B1 to B3, B3 is optionally a solid support, C1 is a single-stranded nucleic acid that binds to a complementary region on A, such that when D2 and F3 bind to a target molecule, C1 stably binds to A, but in the absence of a target molecule, C1 does not stably bind to A, C2 is a linker that joins C1 to C3, C3 is a single-stranded nucleic acid that is complementary to D1, D1 is a single-stranded nucleic acid that is complementary to C3, D2 is a potential aptamer sequence that binds a target, D3 is a primer sequence, E1 is a single-stranded nucleic acid that binds to a complementary region on A, such that when D2 and F3 bind to a target molecule, E1 stably binds to A, but in the absence of a target molecule, E1 does not stably bind to A, E2 is a linker that joins E1 to E3, E3 is a single-stranded nucleic acid that is complementary to F1 . F1 is a single-stranded nucleic acid that is complementary to E3, F2 is a linker that joins F1 and F3, and F3 is an epitope binding agent that binds to a target; AND A composition, the composition comprising three components: a bridge construct, and two aptamer constructs, wherein a) the bridge construct comprises A and B1-B2-B3; b) the first aptamer construct comprises C1/1-C1/2-C1/3 and C 1/1-D1/2-D1/3. , c) the second aptamer construct comprises C2/1-C2/2-C2/3 and D2/1-D2/2-D2/3, wherein A is a single-stranded nucleic acid comprising a binding site for B1, C1/1, and C2/1, B1 is a single-stranded nucleic acid that binds to a complementary region on A, B2 is a linker that joins B1 to B3, B3 is a solid support, C1/1 is a single-stranded nucleic acid that binds to a complementary region on A, such that when D1/2 and D2/2 bind to a target molecule, C1/1 stably binds to A, but in the absence of a target molecule, C1/1 does not stably bind to A, C1/2 is a linker that joins C1/1 to C1/3, C1/3 is a single-stranded nucleic acid that is complementary to D1/1, D1/1 is a single-stranded nucleic acid that is complementary to C1/3, D1/2 is a potential aptamer sequence that binds a target, D1/3 is a primer sequence, C2/1 is a single-stranded nucleic acid that binds to a complementary region on A, such that when D1/2 and D2/2 bind to a target molecule, C2/1 stably binds to A, but in the absence of a target molecule, C2/1 does not stably bind to A, C2/2 is a linker that joins C2/1 to C2/3, C2/3 is a single-stranded nucleic acid that is complementary to D2/1, D2/1 is a single-stranded nucleic acid that is complementary to C2/3, D2/2 is a potential aptamer sequence that binds a target, and D2/3 is a primer sequence.

Group II: Claims 13-25*, drawn to a composition, the composition comprising three components: a bridge construct, and two aptamer constructs, wherein a) the bridge construct comprises H1-H2-H3; b) the first aptamer construct comprises I1/1-I1/2-I1/3-I1/4, c) the second aptamer comprises I2/1-I2/2-I2/3-I2/4, wherein H1 is a single-stranded nucleic acid comprising a binding site for I1/1 and I2/1 , H2 is a linker that joins H1 H3 is a solid support, I1/1 is a single-stranded nucleic acid that binds to a complementary region on H1, such that when I1/3 and I2/3 bind to a target molecule, I1/1 stably binds to H1, but in the absence of a target molecule, I1/1 does not stably bind to H1, I1/2 is a linker that joins I1/1 to I13, I1/3 is a potential aptamer sequence that binds a target, I1/4 is a primer sequence, I2/1 is a single-stranded nucleic acid that binds to a complementary region on H1, such that when I2/3 and I2/3 bind to a target molecule, 12/1 stably binds to H1, but in the absence of a target molecule, I2/1 does not stably bind to H 1, I2/2 is a linker that joins I2/1 to I2/3, I2/3 is a potential aptamer sequence that binds a target, and I2/4 is a primer sequence AND A composition, the composition comprising three components: a bridge construct, an aptamer construct, and an epitope binding agent construct, wherein a) the bridge construct comprises H1-H2-H3; b) the aptamer construct comprises I1-I2-I3-I4; c) the epitope binding agent construct comprises J1-J2-J3, wherein H1 is a single-stranded nucleic acid comprising a binding site for I1 and J1, H2 is a linker that joins H1 and H3, H3 is a solid support, 1 is a single-stranded nucleic acid that binds to a complementary region on H1, such that when I3 and J3 bind to a target molecule, I1 stably binds to H1, but in the absence of a target molecule, 11 does not stably bind to H1, I2 is a linker that joins I1 to I3, I3 is a potential aptamer sequence that binds a target, I1/4 is a primer sequence, I2/1 is a single-stranded nucleic acid that binds to a complementary region on H1, such that when I2/3 and I2/3 bind to a target molecule, 12/1 stably binds to H1, but in the absence of a target molecule, I2/1 does not stably bind to H 1, I2/2 is a linker that joins I2/1 to I2/3, I2/3 is a potential aptamer sequence that binds a target, and I2/4 is a primer sequence AND A composition, the composition comprising three components: a bridge construct, an aptamer construct, and an epitope binding agent construct, wherein a) the bridge construct comprises H1-H2-H3; b) the aptamer construct comprises I1-I2-I3-I4; c) the epitope binding agent construct comprises J1-J2-J3, wherein H1 is a single-stranded nucleic acid comprising a binding site for I1 and J1, H2 is a linker that joins H1 and H3, H3 is a solid support, 1 is a single-stranded nucleic acid that binds to a complementary region on H1, such that when I3 and J3 bind to a target molecule, I1 stably binds to H1, but in the absence of a target molecule, 11 does not stably bind to H1, I2 is a linker that joins I1 to I3, I3 is a potential aptamer sequence that binds a target, I4 is a primer sequence, J1 is a single-stranded nucleic acid that binds to a complementary region on H1, such that when 13 and J3 bind to a target molecule, J1 stably binds to H1, but in the absence of a target molecule, J1 does not stably bind to H1,J2 is a linker that joins J1 to J3, and J3 is an epitope binding agent that binds to a target.

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 contains a bridge construct, an aptamer construct and an epitope binding agent construct that are different from the bridge construct, aptamer construct and epitope binding agent construct included in the Group II.

Groups I-II share the technical feature of a composition, the composition comprising three components, a bridge construct, a first aptamer construct and a second aptamer construct, wherein the bridge construct comprises a single-stranded nucleic acid (H1) coupled to a solid support via a linker, the first and the second aptamer constructs comprising a single-stranded nucleic acids (I1/1 and I1/2) coupled to a potential aptamer that binds to a target via a linker, and further coupled to a primer, wherein said I1/2 and I1/2 bind to a complementary region on H1, such that when both the aptamers bind to a target molecule, both said I1/1 and I1/2 stably binds to H1, but in the absence of a target molecule, neither I1/1 nor I2/1 stably binds to H1; AND A composition, the composition comprising three components, a bridge construct, an aptamer construct and an epitope binding agent construct, wherein the bridge construct comprises a single-stranded nucleic acid (H1) coupled to a solid support optionally via a link, the aptamer construct comprising a single stranded nucleic acid (I1) coupled to a potential aptamer that binds to a target via a linker, and further coupled to a primer sequence, the epitope binding agent comprising a single stranded nucleic acid (J1) coupled to an epitope binding agent that binds to a target optionally via linker, wherein H1 comprises a binding site for I1 and J1, I1 binds to a complementary region on H1, such that when I3 and J3 bind to a target molecule, I1 and J1 stably bind to H1, but in the absence of a target molecule, neither I1 nor J1 stably binds to H1.

**********Continued on Next Page**********

Continuation of Previous page:

However, these shared technical feature does not represent a contribution over the prior part, specifically, US 2010/0021899 A1 to Ikebukuro et al. (hereinafter 'Ikebukuro) teaches a composition, the composition comprising two components, a bridge construct and an aptamer construct (abstract-'An aptamer capable of hybridizing with an oligonucleotide when it is bound to a test substance, but is incapable of hybridizing with the oligonucleotide when it is not bound to the test substance, is utilized. The aptamer is brought into contact with a sample, and the aptamer bound to the test substance is brought into contact with an immobilized oligonucleotide which hybridizes with the aptamer', [said 'immobilized oligonucleotide' being the bridge construct]), wherein:
--a) the bridge construct comprising: H1-H2-H3 (abstract; para [0072]-'by covalently bonding biotin to one end of an oligonucleotide while immobilizing avidin on a carrier, it is possible to bind the oligonucleotide to the carrier via the avidin-biotin bond... Further, the oligonucleotide may also be bound to the carrier via a spacer. For example, a longer oligonucleotide comprising in its one end the oligonucleotide to be immobilized may also be prepared, the end, which is opposite to the oligonucleotide to be immobilized, being bound to the carrier via the above described biotin-avidin bond or the like. In this case, in the nucleic acid immobilized on the carrier, the polynucleotide moiety other than the oligonucleotide to be immobilized simply acts as a spacer');
--b) the aptamer construct comprises I1-I3-I4 (para [0014]-'by adding or inserting a hybridization region which hybridizes with an oligonucleotide to or into an aptamer molecule capable of binding to a target substance and of hybridizing with an oligonucleotide when it is bound to the target substance', [said 'hybridization region' being I1], para [0062]-'This hybridization region can be added to, for example, the 5'-end of the aptamer', [0026]-'the both end regions of the nucleic acid may also be known base sequences, to make PCR simple when SELEX is carried out. In this case, PCR primers can be hybridized to the regions of these known sequences, respectively') wherein
--H1 is a single stranded nucleic acid comprising a binding site for I1 (abstract-'an immobilized oligonucleotide which hybridizes with the aptamer')';
--H2 is a linker that joins H1 to H3 (abstract; para [0072]-'spacer');
--H3 is a solid support (abstract; para [0072]);
--I1 is a single stranded nucleic acid that binds to a complementary region on H1, such that when I3 bind to a target molecule, I1 stably binds to H1, but in the absence of a target molecule, I1 does not stably bind to H1 (abstract-'An aptamer capable of hybridizing with an oligonucleotide when it is bound to a test substance, but is incapable of hybridizing with the oligonucleotide when it is not bound to the test substance, is utilized', [0014]-'by adding or inserting a hybridization region which hybridizes with an oligonucleotide to or into an aptamer molecule capable of binding to a target substance and of hybridizing with an oligonucleotide when it is bound to the target substance');
--I3 is a potential aptamer sequence that bins a target (abstract; para [0014], [0062]);
--I4 is a primer sequence (para [0026]).
--I4 is a primer sequence (para [0026]).
Ikebukuro does not expressly disclose I2 between I1 and I3, wherein the I2 is a linker that joins I1 to I3, however, such a linker between two oligonucleotides was routinely used in the art.
Further Ikebukuro does not expressly disclose that the composition further comprises an epitope binding agent construct comprising, J1-J2-J3, wherein J1 is a single-stranded nucleic acid that binds to a complementary region on H1, such that when i3 and J3 bind to a target molecule, J1 stably binds to H1, but in the absence of a target molecule, J1 does not stably binds to H1, J2 is a linker that joins J1 to J3; and J3 is an epitope binding agent that binds to a target.
However, US 2005/0009050 A1 to Nadeau et al. (hereinafter 'Nadeua') teaches a composition comprising two component: an aptamer construct and an epitope binding agent binding construct, wherein the composition is used to detect an analyte (abstract-'two proximity members that each comprise an analyte-specific binding component conjugated to an oligonucleotide. Binding an analyte brings the oligonucleotide moieties of the proximity members in sufficiently close contact that the oligonucleotides form an amplicon'),
wherein the epitope binding construct comprises: J1-J2-J3 (para [0095]-'Ab1 and Ab2 are antibodies that recognize adjacent epitopes 1 and 2 and that are conjugated to oligonucleotide probes P1 and P2, respectively (FIG. 1A). The antibodies are representative, but not limiting examples, of the analyte-specific binding components that are useful in the present invention... Other examples of analyte-specific binding components include aptamers', [0097]-'conjugate Ab1-P1 is formed through a linkage located at or near the 3' terminus of P1'), wherein
--J1 is a single-stranded nucleic acid that binds to a complementary region on the aptamer construct para [0095]-'Ab1 and Ab2 are antibodies that recognize adjacent epitopes 1 and 2 and that are conjugated to oligonucleotide probes P1 and P2, respectively (FIG. 1A). The antibodies are representative, but not limiting examples, of the analyte-specific binding components that are useful in the present invention... Other examples of analyte-specific binding components include aptamers', 'The 3' ends of P1 and P2 hybridize to one another when the two antibodies to which they are linked are held in close proximity by binding to their respective epitopes (FIG. 1B)'),
--J2 is a linker that joins J1 to J3 (para [0097]-'linkage'); and
--J3 is an epitope binding agent that binds to a target (para [0095]-'analyte-specific binding component', 'antibodies').
Based on this disclosure, one of ordinary skill in the art would have found it obvious to include said epitope binding agent construct used in conjunction with an aptamer construct to detect a target analyte into the two-component composition comprising the bridge component and the aptamer component disclosed by Ikebukuro so that when i3 and J3 bind to a target molecule, both j1 and I1 stably binds to H1, but in the absence of a target molecule, neither I1 nor J1 stably binds to H1 based on the hybridization domain inserted in the aptamer construct disclosed by Ikebukuro (abstract; para [0014]), because such a dual-hybridization composition could improve sensitivity and specificity of the assay.
Further Ikebukuro teaches teaches a composition, the composition comprising two components, a bridge construct and an aptamer construct (abstract-'An aptamer capable of hybridizing with an oligonucleotide when it is bound to a test substance, but is incapable of hybridizing with the oligonucleotide when it is not bound to the test substance, is utilized. The aptamer is brought into contact with a sample, and the aptamer bound to the test substance is brought into contact with an immobilized oligonucleotide which hybridizes with the aptamer', [said 'immobilized oligonucleotide' being the bridge construct]), wherein:
--a) the bridge construct comprises: H1-H2-H3 (abstract; para [0072]-'by covalently bonding biotin to one end of an oligonucleotide while immobilizing avidin on a carrier, it is possible to bind the oligonucleotide to the carrier via the avidin-biotin bond... Further, the oligonucleotide may also be bound to the carrier via a spacer. For example, a longer oligonucleotide comprising in its one end the oligonucleotide to be immobilized may also be prepared, the end, which is opposite to the oligonucleotide to be immobilized, being bound to the carrier via the above described biotin-avidin bond or the like. In this case, in the nucleic acid immobilized on the carrier, the

**********Continued on Next Page**********

Continuation of Previous page:

polynucleotide moiety other than the oligonucleotide to be immobilized simply acts as a spacer');
--b) the first aptamer construct comprises I1/1-I1/3-I1/4 (para [0014]-'by adding or inserting a hybridization region which hybridizes with an oligonucleotide to or into an aptamer molecule capable of binding to a target substance and of hybridizing with an oligonucleotide when it is bound to the target substance', [said 'hybridization region' being I1/1], para [0062]-'This hybridization region can be added to, for example, the 5'-end of the aptamer', [0026]-'the both end regions of the nucleic acid may also be known base sequences, to make PCR simple when SELEX is carried out. In this case, PCR primers can be hybridized to the regions of these known sequences, respectively')
wherein
--H1 is a single stranded nucleic acid comprising a binding site for I1/1 (abstract-'an immobilized oligonucleotide which hybridizes with the aptamer')';
--H2 is a linker that joins H1 to H3 (abstract; para [0072]-'spacer');
--H3 is a solid support (abstract; para [0072]);
--I1/1 is a single stranded nucleic acid that binds to a complementary region on H1, such that when I1/3 bind to a target molecule, I1/1 stably binds to H1, but in the absence of a target molecule, I1/1 does not stably bind to H1 (abstract-'An aptamer capable of hybridizing with an oligonucleotide when it is bound to a test substance, but is incapable of hybridizing with the oligonucleotide when it is not bound to the test substance, is utilized', [0014]-'by adding or inserting a hybridization region which hybridizes with an oligonucleotide to or into an aptamer molecule capable of binding to a target substance and of hybridizing with an oligonucleotide when it is bound to the target substance');
--I1/3 is a potential aptamer sequence that bins a target (abstract; para [0014], [0062]);
--I1/4 is a primer sequence (para [0026]).
Ikebukuro does not expressly disclose I1/2 between I1/1 and I1/3, wherein the I2 is a linker that joins I1 to I3, however, such a linker between two oligonucleotides was routinely used in the art.
Further Ikebukuro does not expressly disclose that the composition further comprises a second aptamer construct comprising I2/1-I2/2-I2/3-I2/4, wherein said second aptamer construct has the same structure as the first aptamer.
However, Nadeau teaches a composition for detecting a target analyte comprising two analyte-specific binding component constructs comprising I1-I2-I3, wherein the analyte-specific binding components are aptamers , and wherein the construct may further comprise I4 coupled to said construct, and wherein the binding of both constructs to said target is required to generate a signal (abstract-'two proximity members that each comprise an analyte-specific binding component conjugated to an oligonucleotide. Binding an analyte brings the oligonucleotide moieties of the proximity members in sufficiently close contact that the oligonucleotides form an amplicon'; para [0095]-'Ab1 and Ab2 are antibodies that recognize adjacent epitopes 1 and 2 and that are conjugated to oligonucleotide probes P1 and P2, respectively (FIG. 1A). The antibodies are representative, but not limiting examples, of the analyte-specific binding components that are useful in the present invention... Other examples of analyte-specific binding components include aptamers', 'The 3' ends of P1 and P2 hybridize to one another when the two antibodies to which they are linked are held in close proximity by binding to their respective epitopes (FIG. 1B)', [0097]-'conjugate Ab1-P1 is formed through a linkage located at or near the 3' terminus of P1', [0020]-'the capture oligonucleotide or the oligonucleotide moiety of the proximity member or analyte comprises a complementary sequence to a primer' [said 'P1' being I1, said 'linkage' being I2, said 'aptamers' as the analyte specific binding components being I3, said 'complementary sequence to a primer' being I4.]).
Thus one of ordinary skill in the art would have found it obvious to incorporate said dual analyte-specific binding component constructs which could comprise aptamers used to detect a target analyte into the two-component composition comprising the bridge component and the aptamer component disclosed by Ikebukuro so that when both I1/3 and I2/3 bind to a target molecule, both I1/1 and I2/1 stably binds to H1, but in the absence of a target molecule, neither I1/1 nor I2/1 stably binds to H1 based on the hybridization domain inserted in the aptamer construct disclosed by Ikebukuro (abstract; para [0014]), because such a dual-hybridization composition could improve sensitivity and specificity of the assay.

As said shared technical features would have been obvious to one of ordinary skill in the art, these 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.

*NOTE:
In the context of the claims, Claim 17 is construed to a dependent claim of Claim 13 rather than Claim 11 (the term "I11 and I12" is only disclosed in Claim 13, but not in Claim 11.).

In the context of the claims, Claims 21-24 are construed to be dependent claims of Claim 18 rather than Claim 1 or 4 (the terms 'I2', 'J2' and 'J3' are only disclosed in Claim 19 but not in Claim 1 or 4.).