

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference SML21PCT	FOR FURTHER ACTION		See item 4 below
International application No. PCT/US2008/070386	International filing date (<i>day/month/year</i>) 17 July 2008 (17.07.2008)	Priority date (<i>day/month/year</i>) 17 July 2007 (17.07.2007)	
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237			
Applicant SOMALOGIC, INC.			

<p>1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).</p> <p>2. This REPORT consists of a total of 11 sheets, including this cover sheet.</p> <p>In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.</p>																								
<p>3. This report contains indications relating to the following items:</p> <table> <tr> <td><input checked="" type="checkbox"/></td> <td>Box No. I</td> <td>Basis of the report</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. II</td> <td>Priority</td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </table> <p>4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).</p>	<input checked="" type="checkbox"/>	Box No. I	Basis of the report	<input type="checkbox"/>	Box No. II	Priority	<input checked="" type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	<input type="checkbox"/>	Box No. IV	Lack of unity of invention	<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	<input type="checkbox"/>	Box No. VI	Certain documents cited	<input type="checkbox"/>	Box No. VII	Certain defects in the international application	<input checked="" type="checkbox"/>	Box No. VIII	Certain observations on the international application
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	Date of issuance of this report 19 January 2010 (19.01.2010)
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PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

To:
BARRY J. SWANSON
SWANSON & BRATSCHUN, L.L.C.
8210 SOUTHPARK TERRACE
LITTLETON, CO 80120

Date of mailing
(day/month/year) **16 OCT 2008**

Applicant's or agent's file reference
SML21PCT

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 08/70386

International filing date (day/month/year)

17 July 2008 (17.07.2008)

Priority date (day/month/year)

17 July 2007 (17.07.2007)

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

IPC(8) - C12Q 1/68; C07H 21/04 (2008.04)

USPC - 435/6, 530/24.3

Applicant SOMALOGIC, INC.

1. This opinion contains indications relating to the following items:

- Box No. I Basis of the opinion
- Box No. II Priority
- Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- Box No. IV Lack of unity of invention
- Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability: citations and explanations supporting such statement
- Box No. VI Certain documents cited
- Box No. VII Certain defects in the international application
- Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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

Date of completion of this opinion

9 October 2008 (09.10.2008)

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

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Box No. 1 Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:
- the international application in the language in which it was filed.
- a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. This opinion has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))
3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established 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
4. In addition, in the case that more than one version or copy of a sequence listing and/or table(s) 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.
5. Additional comments:

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

the entire international application

claims Nos. _____

because:

the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 27 are so unclear that no meaningful opinion could be formed (*specify*):

Claim 27 is directed towards chemical modifications "listed in Figure (19)". However, the present application does not contain a "Figure (19)".

the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

no international search report has been established for said claims Nos. _____

a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

See Supplemental Box for further details.

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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	see below	YES
	Claims	1, 4, 17, 23-26, 28-31, 33-35, 37-38, 40, 70, 77, 79	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-26, 28-68a, 68b, 69a, 69b, 70-80	NO
Industrial applicability (IA)	Claims	1-26, 28-68a, 68b, 69a, 69b, 70-80	YES
	Claims	None	NO

2. Citations and explanations:

1. Statement of Novelty (N) Claims 2, 3, 5-16, 18-22, 32, 36, 39, 41-68a, 68b, 69a, 69b, 71-76, 78, 80 YES

Claims 1, 4, 17, 23-26, 28-31, 33-35, 37-38, 40, 70, 77 and 79 lack novelty under PCT Article 33(2) as being anticipated by US 2006/0105341 A1 to Krause et al. (hereinafter 'Krause').

Regarding claim 1, Krause discloses a method for detecting a target molecule that may be present in a test sample, the method comprising:

- (a) preparing a mixture by contacting the test sample with an aptamer comprising a first tag (para [0006] L 'at least two different RNA tags') and having a specific affinity for the target molecule (para [0006]), wherein an aptamer affinity complex is formed if said target molecule is present in said test sample (para [0006]);
- (b) exposing the mixture to a first solid support comprising a first capture element (para [0006] L 'affinity resin'), and allowing the first tag to associate with the first capture element (para [0006]);
- (c) removing any components of the mixture not associated with said first solid support (para [0006]);
- (d) releasing the aptamer affinity complex from said first solid support (para [0006]);
- (e) attaching a second tag to said target molecule in the aptamer affinity complex (para [0006] L 'at least two different RNA tags');
- (f) exposing the released aptamer affinity complex to a second solid support comprising a second capture element (para [0006] L 'affinity resin') and allowing the second tag to associate with said second capture element (para [0006]);
- (g) removing any uncomplexed aptamer from said mixture by partitioning the uncomplexed aptamer from said aptamer affinity complex (para [0006]); and
- (h) detecting said target molecule by detecting the aptamer portion of said aptamer affinity complex (para [0032] and [0105]).

Regarding claim 4, Krause further discloses wherein the first tag is different than the second tag and the second tag is added to the target molecule at any point before (f) (para [0100]).

Regarding claim 17, Krause further discloses wherein said aptamer is detected and optionally quantified using MS (para [0032] L 'Mass Spectrometry').

Regarding claim 23, Krause further discloses wherein said aptamer is a single-stranded nucleic acid or a double-stranded nucleic acid (para [0054]-[0055]).

Regarding claim 24, Krause further discloses wherein said aptamer comprises DNA or RNA (para [0054]-[0055]).

Regarding claim 25, Krause further discloses wherein said aptamer comprises at least one chemical modification (para [0084]-[0085]).

Regarding claim 26, Krause further discloses wherein said at least one chemical modification is a chemical substitution (para [0085]) at one or more positions independently selected from a deoxyribose position (para [0084]).

Regarding claim 28, Krause further discloses wherein said target molecule is selected from the group consisting of a protein, an antibody, a drug (para [0054]).

Regarding claim 29, Krause further discloses wherein said target molecule is a protein or a peptide (para [0054]).

Regarding claim 30, Krause further discloses wherein said test sample is selected from the group consisting of a biological sample (para [0006]).

Regarding claim 31, Krause further discloses wherein said test sample is a biological sample, a cellular extract (para [0006]).

Regarding claim 33, Krause further discloses wherein said first tag and said second tag each comprises a peptide nucleic acid (para [0106] L 'MS2 phage coat protein binding RNA' and 'streptavidin binding aptamer').

Regarding claim 34, Krause further discloses wherein said first capture element and said second capture element each comprises at least one component independently selected from a polypeptide (para [0022] L 'MS2 coat protein') and streptavidin (para [0022]).

-----Please see continuation in supplementalbox-----

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 27 is directed towards chemical modifications "listed in Figure (19)". However, the present application does not contain a "Figure (19)". An opinion therefore was not established for claim 27.

There are two claims labeled .68.. For the purpose of this opinion, the first of these has been labeled .68a. and the second .68b..

There are also two claims labeled .69.. For the purpose of this opinion, the first of these has been labeled .69a. and the second "69b..

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box No. V Citations and Explanations

Regarding claim 35, Krause further discloses wherein the first tag comprises a releasable moiety (para [0032] . 'first tag . . . capable of being capable of being dissociated from its affinity resin').

Regarding claim 37, Krause further discloses wherein said first solid support and second solid support each is an agarose bead (para [0103]).

Regarding claim 38, Krause further discloses further comprising quantifying said target by quantifying said aptamer (para [0032] and [0103]).

Regarding claim 40, Krause discloses a method for detecting a target molecule that may be present in a test sample, the method comprising:

- (a) preparing a mixture by contacting a test sample with an aptamer having a specific affinity for a target molecule, wherein an aptamer affinity complex is formed if said target molecule is present in said test sample (para [0006]);
- (b) at any point prior to (c) adding a tag to said target molecule (para [0006]);
- (c) exposing the mixture to a solid support comprising a capture element, and allowing the tag on the target molecule to associate with the capture element (para [0006]);
- (d) removing any uncomplexed aptamer from said mixture by partitioning the uncomplexed aptamer from the aptamer affinity complex (para [0006]);
- (e) detecting said target molecule by detecting the aptamer portion of said aptamer affinity complex (para [0030] and [0032]).

Regarding claim 70, Krause discloses a method of detecting the presence of, or determining the amount of, a target molecule in a sample, the method comprising:

- (i) providing a plurality of aptamers to a target molecule, wherein the aptamers have a cleavable capture tag (para [0006]);
- (ii) contacting the aptamers with a sample containing target molecules to form a mixture containing aptamer-target molecule complexes (para [0006]);
- (iii) providing a solid support having probes adhered to the surface of the support, wherein the probes are capable of binding to the cleavable capture tag; (para [0006])
- (iv) contacting the mixture with the solid support such that aptamer-target molecule complexes become bound to the support through binding of the cleavable capture tag and probe (para [0006]);
- (v) partitioning aptamer-target molecule complexes bound to the solid support from the remainder of the mixture (para [0006] . 'substances not bound to the affinity resin have been removed');
- (vi) introducing a second capture tag to the target molecule component of the aptamer-target molecule complexes (para [0006]);
- (vii) dissociating the aptamer-target molecule complexes from the surface of the solid support by cleaving the cleavable capture tags (para [0006]);
- (viii) providing a solid support having probes adhered to the surface of the support, wherein the probes are capable of binding to the second capture tag on target molecules (para [0006]);
- (ix) contacting the dissociated aptamer-target molecule complexes with the solid support from (viii) such that the aptamer-target molecule complexes become bound to the support through binding of the second capture tag and probe (para [0006]);
- (x) dissociating the aptamer-target molecule complexes to yield free aptamers and target molecules bound to the support (para [0006]);
- (xi) detecting the free aptamers (para [0032] and [0105]).

Regarding claim 77, Krause discloses a kit (para [0105]) comprising:

- a) one or more aptamers specific to one or more targets of interest (para [0054]-[0055]); and
- b) one or more solid supports (para [0130]); and
- c) one or more partitioning reagents (para [0123]); and
- d) one or more reagents for the release of an aptamer from the affinity complex (para [0097] - 'biotin').

Regarding claim 79, Krause further discloses a reagent to cleave a cleavable moiety in said one or more aptamers (para [0022] . 'streptavidin protein, eluting a first eluate with the addition of biotin').

Claims 3, 13-16, 36, 39 and 50-53 lack an inventive step under PCT Article 33(3) as being obvious over Krause.

Regarding claim 3, Krause discloses the method of claim 1, but does not disclose wherein the first tag is the same as the second tag and the second tag is added to the target molecule at any point after (b) and before (f), and comprising blocking the first capture agent prior to the addition of the second tag. However, this element would have been obvious to one of ordinary skill in the art because Krause suggests that the tag may be repeated (para [0011]), and it would have been obvious to one of ordinary skill in the art that use of the same tag for both first and second affinity purification steps would require the blocking of the first capture agent prior to the addition of the second tag in order to prevent capture of the second tag by the first capture agent.

Regarding claims 13-16, Krause does not expressly disclose wherein said aptamer affinity complex has a slow rate of dissociation (t .) expressed in terms of minutes. However, this would have been obvious to one of ordinary skill in the art because Krause does teach that hybridization of the aptamer to the sample is formed under stringent conditions, such that the stability of the complex is high (para [0096]). Therefore, one of ordinary skill in the art would have recognized that the rate of dissociation of a highly stable complex would be slow.

-----Please see continuation in next supplementalbox-----

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box No. V Citations and Explanations

Regarding claim 36, Krause discloses the method of claim 35, but does not expressly disclose wherein the releasable moiety comprises a photocleavable moiety. However, this element would have been obvious to one of ordinary skill in the art since Krause teaches that the releasable moiety must be one that releases the first tag under conditions that do not disrupt the RNA-protein complex (para [0032]), such as a moiety that is photocleavable.

Regarding claim 39, Krause discloses the method of claim 1, but does not expressly teach wherein detection of the aptamer comprises hybridizing the aptamer to a third solid support wherein the third solid support comprises a plurality of addressable features and wherein at least one of said features comprises at least capture element disposed thereon that is complementary to any sequence contained within the aptamer. However, this element would have been obvious to one of ordinary skill in the art since Krause teaches that the method comprises at least two different affinity purification steps (para [0006]), therefore suggesting that the method may comprise a third solid support herein the third solid support comprises a plurality of addressable features and wherein at least one of said features comprises at least capture element disposed thereon that is complementary to any sequence contained within the aptamer.

Regarding claims 50-53, Krause does not expressly disclose wherein said aptamer affinity complex has a slow rate of dissociation (t_{1/2}) expressed in terms of minutes. However, this would have been obvious to one of ordinary skill in the art because Krause does teach that hybridization of the aptamer to the sample is formed under stringent conditions, such that the stability of the complex is high (para [0096]). Therefore, one of ordinary skill in the art would have recognized that the rate of dissociation of a highly stable complex would be slow.

Claims 2, 19-22, 32 and 41 lack an inventive step under PCT Article 33(3) as being obvious over Krause in view of US 2006/0057573 A1 to Gold et al. (hereinafter 'Gold').

Regarding claims 2 and 41, Krause discloses the methods of claims 1 and 40, but does not disclose dissociating the aptamer from said aptamer affinity complex before detecting the aptamer. This element is suggested by Gold (para [0003]). One of ordinary skill in the art would have found it obvious to include the step of dissociating the aptamer, as taught by Gold, with the method of Krause because Krause discloses a method of capturing a ligand using nucleic acid binding (Krause para [0001]), and Gold suggests that the step of dissociating the aptamer (Gold para [0003]) may be used in methods of capturing a ligand on a solid support (Gold para [0015]).

Regarding claim 19, Krause discloses the method of claim 1, but does not disclose adding a detectable moiety to the aptamer. This element is taught by Gold (para [0047]). One of ordinary skill in the art would have found it obvious to combine the detectable moiety of Gold with the method of Krause because Krause discloses a method of capturing a ligand on a solid support (Krause para [0001]), and Gold suggests that a detectable moiety (Gold para [0023]) may be used in methods of capturing a ligand on a solid support (Gold para [0015]).

Regarding claim 20, Gold further discloses wherein said detectable moiety is selected from the group consisting of a dye, a quantum dot, a radiolabel, an enzyme, and an enzyme substrate (para [0128]).

Regarding claim 21, Gold further discloses wherein said dye is a fluorescent dye (para [0128] - 'fluorophore').

Regarding claim 22, Gold further discloses wherein said enzyme is alkaline phosphatase or horseradish peroxidase (para [0023]).

Regarding claim 32, Krause discloses the method of claim 1, but does not disclose wherein said test sample is serum. This element is taught by Gold (para [0096]). One of ordinary skill in the art would have found it obvious to apply the method of Krause to the test sample of Gold because Krause discloses a method of capturing a ligand on a solid support (Krause para [0001]), and Gold suggests that a serum (Gold para [0096]) may provide the sample in methods of capturing a ligand on a solid support (Gold para [0015]).

Claims 5-12, 42-49, 71-76 and 80 lack an inventive step under PCT Article 33(3) as being obvious over Krause in view of US 2004/0235053 A1 to Lam et al. (hereinafter 'Lam').

Regarding claim 5, Krause discloses the method of claim 1, but does not disclose introducing a kinetic challenge at any point after (a) and before (d). This element is taught by Lam (para [0157]). One of ordinary skill in the art would have found it obvious to include the kinetic challenge step of Lam in the method of Krause because Krause discloses a method of capturing a ligand on a solid support (Krause para [0001]), and Lam suggests that including a kinetic challenge in such methods provides the benefit of identifying ligands with the greatest binding affinity (Lam para [0157]).

Regarding claim 6, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]). While Lam does not expressly specify a time for incubating the mixture containing the aptamer affinity complex, it does teach that the mixture should be incubated for a time sufficient to allow binding to occur (para [0148]). Therefore, one of ordinary skill in the art would have found it obvious to determine an appropriate length of time to use.

Regarding claim 7, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]) and incubating the mixture containing the aptamer affinity complex (para [0148]) for a time such that the ratio of the measured level of aptamer affinity complex to the measured level of the non-specific complex is increased (para [0157] - 'ligand(s) with the greatest binding affinity can be identified').

-----Please see continuation in next supplementalbox-----

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box No. V Citations and Explanations

Regarding claim 8, Lam further discloses wherein said kinetic challenge comprises adding a competitor to the mixture containing the aptamer affinity complex (para [0157]). While Lam does not expressly specify a time for incubating the mixture containing the aptamer affinity complex, it does teach that the mixture should be incubated for a time sufficient to allow binding to occur (para [0148]). Therefore, one of ordinary skill in the art would have found it obvious to determine an appropriate length of time to use.

Regarding claim 9, Lam further discloses wherein said kinetic challenge comprises adding a competitor to the mixture containing the aptamer affinity complex (para [0157]) and incubating the mixture containing the aptamer affinity complex (para [0148]) for a time such that the ratio of the measured level of aptamer affinity complex to the measured level of the non-specific complex is increased (para [0157] . 'ligand(s) with the greatest binding affinity can be identified').

Regarding claim 10, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]), adding a competitor to the mixture containing the aptamer affinity complex (para [0157]). While Lam does not expressly specify a time for incubating the mixture containing the aptamer affinity complex, it does teach that the mixture should be incubated for a time sufficient to allow binding to occur (para [0148]). Therefore, one of ordinary skill in the art would have found it obvious to determine an appropriate length of time to use.

Regarding claim 11, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]), adding a competitor to the mixture containing the aptamer affinity complex (para [0157]) and incubating the mixture containing the aptamer affinity complex (para [0148]) for a time such that the ratio of the measured level of aptamer affinity complex to the measured level of the non-specific complex is increased (para [0157] . 'ligand(s) with the greatest binding affinity can be identified').

Regarding claim 12, Lam further discloses wherein the kinetic challenge comprises the introduction of a competitor molecule (para [0157]), but does not specify wherein said competitor molecule is an oligonucleotide. However, Lam teaches that any known competitor may be used (para [0157]), and Krause further teaches that other known binding moieties include oligonucleotides (para [0100] . 'binding sequence'). Therefore, it would have been obvious to one of ordinary skill in the art wherein said competitor molecule is an oligonucleotide.

Regarding claim 42, Krause discloses the method of claim 40, but does not disclose introducing a kinetic challenge at any point after (a) and before (d). This element is taught by Lam (para [0157]). One of ordinary skill in the art would have found it obvious to include the kinetic challenge step of Lam in the method of Krause because Krause discloses a method of capturing a ligand on a solid support (Krause para [0001]), and Lam suggests that including a kinetic challenge in such methods provides the benefit of identifying ligands with the greatest binding affinity (Lam para [0157]).

Regarding claim 43, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]). While Lam does not expressly specify a time for incubating the mixture containing the aptamer affinity complex, it does teach that the mixture should be incubated for a time sufficient to allow binding to occur (para [0148]). Therefore, one of ordinary skill in the art would have found it obvious to determine an appropriate length of time to use.

Regarding claim 44, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]) and incubating the mixture containing the aptamer affinity complex (para [0148]) for a time such that the ratio of the measured level of aptamer affinity complex to the measured level of the non-specific complex is increased (para [0157] . 'ligand(s) with the greatest binding affinity can be identified').

Regarding claim 45, Lam further discloses wherein said kinetic challenge comprises adding a competitor to the mixture containing the aptamer affinity complex (para [0157]). While Lam does not expressly specify a time for incubating the mixture containing the aptamer affinity complex, it does teach that the mixture should be incubated for a time sufficient to allow binding to occur (para [0148]). Therefore, one of ordinary skill in the art would have found it obvious to determine an appropriate length of time to use.

Regarding claim 46, Lam further discloses wherein said kinetic challenge comprises adding a competitor to the mixture containing the aptamer affinity complex (para [0157]) and incubating the mixture containing the aptamer affinity complex (para [0148]) for a time such that the ratio of the measured level of aptamer affinity complex to the measured level of the non-specific complex is increased (para [0157] . 'ligand(s) with the greatest binding affinity can be identified').

Regarding claim 47 Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]), adding a competitor to the mixture containing the aptamer affinity complex (para [0157]). While Lam does not expressly specify a time for incubating the mixture containing the aptamer affinity complex, it does teach that the mixture should be incubated for a time sufficient to allow binding to occur (para [0148]). Therefore, one of ordinary skill in the art would have found it obvious to determine an appropriate length of time to use.

Regarding claim 48, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]) , adding a competitor to the mixture containing the aptamer affinity complex (para [0157]) and incubating the mixture containing the aptamer affinity complex (para [0148]) for a time such that the ratio of the measured level of aptamer affinity complex to the measured level of the non-specific complex is increased (para [0157] . 'ligand(s) with the greatest binding affinity can be identified').

Regarding claim 49, Lam further discloses wherein the kinetic challenge comprises the introduction of a competitor molecule (para [0157]), but does not specify wherein said competitor molecule is an oligonucleotide. However, Lam teaches that any known competitor may be used (para [0157]), and Krause further teaches that other known binding moieties include oligonucleotides (para [0100] . 'binding sequence'). Therefore, it would have been obvious to one of ordinary skill in the art wherein said competitor molecule is an oligonucleotide.

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

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Supplemental Box

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Regarding claim 71, Krause discloses the method of claim 70, but does not disclose wherein the dissociated aptamer-target molecule complexes are contacted with an excess of competitor molecule. This element is taught by Lam (para [0157]). One of ordinary skill in the art would have found it obvious to include the kinetic challenge step of Lam in the method of Krause because Krause discloses a method of capturing a ligand on a solid support (Krause para [0001]), and Lam suggests that including a kinetic challenge in such methods provides the benefit of identifying ligands with the greatest binding affinity (Lam para [0157]).

Regarding claim 72, Lam further discloses wherein the dissociated aptamer-target molecule complexes are diluted (para [0157]).

Regarding claim 73, Lam further discloses wherein the dissociated aptamer-target molecule complexes are diluted (para [0157]).

Regarding claims 74-76, Krause further discloses the method, further comprising the step of measuring the amount of free aptamer detected (para [0032] and [0105]).

Regarding claim 80, Krause discloses the kit of claims 77, but does not disclose wherein the kit further comprises a reagent for use in a kinetic challenge. However, this element is suggested by Lam (para [0157]). One of ordinary skill in the art would have found it obvious to include reagents for carrying out the kinetic challenge, as taught by Lam, in the kit as taught by Krause, because the kit of Krause is used to perform a method of capturing a ligand on a solid support (Krause para [0001]), and Lam suggests that including a kinetic challenge in such methods provides the benefit of identifying ligands with the greatest binding affinity (Lam para [0157]).

Claim 18 lacks an inventive step under PCT Article 33(3) as being obvious over Krause in view of US 20070151020 A1 to Luo et al. (hereinafter 'Luo').

Regarding claim 18, Krause discloses the method of claim 17, but does not teach Q-PCR, and wherein the Q-PCR is performed using TaqManL PCR, an intercalating fluorescent dye during the PCR process, or a molecular beacon during the PCR process. However, Luo does disclose Q-PCR (para [0093]) and further discloses wherein the Q-PCR is performed using TaqManL PCR, an intercalating fluorescent dye during the PCR process, or a molecular beacon during the PCR process (para [0093]). One of ordinary skill in the art would have found it obvious to combine the Q-PCR of Luo because Krause teaches that PCR may be used to assess eluates from its process (Krause para [0032]), and Luo suggests that Q-PCR offers a number of advantages over other traditional PRC techniques (Luo para [0093]).

Claims 54-55, 64-68a, 68b, 69a, 69b, and 78 lack an inventive step under PCT Article 33(3) as being obvious over Krause in view of the article entitled 'Photoaptamer arrays applied to multiplexed proteomic analysis' by Bock et al. (hereinafter 'Bock').

Regarding claim 54, Krause discloses a method for detecting a target molecule that may be present in a test sample, the method comprising:

(a) preparing a mixture by contacting a test sample with an aptamer comprising a first tag and having a specific affinity for a target molecule, wherein an aptamer affinity complex is formed if said target molecule is present in said test sample (para [0006]);

(c) exposing the mixture to a first solid support comprising a first capture element, and allowing the first tag to associate with the first capture element (para [0006]);

(d) removing any components of the mixture not associated with said first solid support (para [0006]);

(e) releasing the aptamer covalent complex from said first solid support (para [0006]);

(f) attaching a second tag to said target molecule in the aptamer covalent complex (para [0006]);

(g) exposing the released aptamer covalent complex to a second solid support comprising a second capture element and allowing the second tag to associate with said second capture element (para [0006]);

(h) removing any uncomplexed aptamer from said mixture by partitioning the uncomplexed aptamer from said aptamer covalent complex (para [0006]); and

(i) detecting said target molecule by detecting the aptamer portion of said aptamer covalent complex (para [0032] and [0105]).

Krause does not teach wherein the aptamer is a photoaptamer. This element is taught by Bock (p 610, col 1, para 3). Krause also does not expressly teach converting said aptamer affinity complex into an aptamer covalent complex. This element is also taught by Bock (p 610, col 1, para 3). One of ordinary skill in the art would have found it obvious to one of ordinary skill in the art to use the photoaptamer of Bock as the aptamer in the method of Krause because Bock suggests that use of photoaptamers permits vigorous washing to remove background proteins, thus providing the benefit of superior signal-to-noise ratios and lower limits of quantification in biological matrices (p 609, abstract).

Regarding claim 55, the combination of Krause and Bock disclose the method of claim 54, but does not expressly disclose wherein (i) further comprises releasing the photoaptamer from said aptamer covalent complex, before detecting the photoaptamer. However, this element would have been obvious to one of ordinary skill in the art since Krause does teach that the aptamer comprising a tag must be capable of being dissociated from the solid support (para [0032]), thus release of the aptamer would have been obvious to an ordinarily skilled artisan.

Regarding claims 64-67, Krause does not expressly disclose wherein said aptamer affinity complex has a slow rate of dissociation (t L) expressed in terms of minutes. However, this would have been obvious to one of ordinary skill in the art because Krause does teach that hybridization of the aptamer to the sample is formed under stringent conditions, such that the stability of the complex is high (para [0096]). Therefore, one of ordinary skill in the art would have recognized that the rate of dissociation of a highly stable complex would be slow.

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Regarding claim 68a, Krause further discloses wherein the first tag comprises a releasable moiety (para [0032] L 'first tag . . . capable of being capable of being dissociated from its affinity resin').

Regarding claim 68b, Krause further discloses attachment of the aptamer via a cleavable moiety (para [0029] L 'streptavidin . . . biotin').

Regarding claim 69a, Krause does not expressly disclose wherein the releasable moiety comprises a nucleic acid sequence that is complementary to a region of the photoaptamer, wherein the complementary sequences can be separated by chemical or thermal conditions that destabilize nucleic acid duplexes. However, this element would have been obvious to one of ordinary skill in the art because Krause does disclose that complexes may be eluted using RNase, high salt, glutathione or denaturants (para [0045]).

Regarding claim 69b, Krause discloses the method of claim 68a, but does not expressly disclose wherein the releasable moiety comprises a photocleavable moiety. However, this element would have been obvious to one of ordinary skill in the art since Krause teaches that the releasable moiety must be one that releases the first tag under conditions that do not disrupt the RNA-protein complex (para [0032]), such as a moiety that is photocleavable.

Regarding claim 78, Krause discloses the kit of claim 77, but does not teach wherein the kit further comprises a reagent for derivatizing the one or more targets of interest. However, this element is suggested by Bock (p 610, col 1, para 3 L 'cross-linking event'). One of ordinary skill in the art would have found it obvious to include the reagent of Bock in the kit of Krause since Bock suggests that use of photoaptamers permits vigorous washing to remove background proteins, thus providing the benefit of superior signal-to-noise ratios and lower limits of quantification in biological matrices (p 609, abstract).

Claims 56-63 lack an inventive step under PCT Article 33(3) as being obvious over Krause in view of Bock as applied above, and further in view of Lam.

Regarding claim 56, the combination of Krause and Bock discloses the method of claim 54, but does not disclose introducing a kinetic challenge at any point after (a) and before (b). This element is taught by Lam (para [0157]). One of ordinary skill in the art would have found it obvious to include the kinetic challenge step of Lam in the method of Krause and Bock because Krause discloses a method of capturing a ligand on a solid support (Krause para [0001]), and Lam suggests that including a kinetic challenge in such methods provides the benefit of identifying ligands with the greatest binding affinity (Lam para [0157]).

Regarding claim 57, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]). While Lam does not expressly specify a time for incubating the mixture containing the aptamer affinity complex, it does teach that the mixture should be incubated for a time sufficient to allow binding to occur (para [0148]). Therefore, one of ordinary skill in the art would have found it obvious to determine an appropriate length of time to use.

Regarding claim 58, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]) and incubating the mixture containing the aptamer affinity complex (para [0148]) for a time such that the ratio of the measured level of aptamer affinity complex to the measured level of the non-specific complex is increased (para [0157] L 'ligand(s) with the greatest binding affinity can be identified').

Regarding claim 59, Lam further discloses wherein said kinetic challenge comprises adding a competitor to the mixture containing the aptamer affinity complex (para [0157]). While Lam does not expressly specify a time for incubating the mixture containing the aptamer affinity complex, it does teach that the mixture should be incubated for a time sufficient to allow binding to occur (para [0148]). Therefore, one of ordinary skill in the art would have found it obvious to determine an appropriate length of time to use.

Regarding claim 60, Lam further discloses wherein said kinetic challenge comprises adding a competitor to the mixture containing the aptamer affinity complex (para [0157]) and incubating the mixture containing the aptamer affinity complex (para [0148]) for a time such that the ratio of the measured level of aptamer affinity complex to the measured level of the non-specific complex is increased (para [0157] L 'ligand(s) with the greatest binding affinity can be identified').

Regarding claim 61, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]), adding a competitor to the mixture containing the aptamer affinity complex (para [0157]). While Lam does not expressly specify a time for incubating the mixture containing the aptamer affinity complex, it does teach that the mixture should be incubated for a time sufficient to allow binding to occur (para [0148]). Therefore, one of ordinary skill in the art would have found it obvious to determine an appropriate length of time to use.

Regarding claim 62, Lam further discloses wherein said kinetic challenge comprises diluting the mixture containing the aptamer affinity complex (para [0157]), adding a competitor to the mixture containing the aptamer affinity complex (para [0157]) and incubating the mixture containing the aptamer affinity complex (para [0148]) for a time such that the ratio of the measured level of aptamer affinity complex to the measured level of the non-specific complex is increased (para [0157] L 'ligand(s) with the greatest binding affinity can be identified').

Regarding claim 63, Lam further discloses wherein the kinetic challenge comprises the introduction of a competitor molecule (para [0157]), but does not specify wherein said competitor molecule is an oligonucleotide. However, Lam teaches that any known competitor may be used (para [0157]), and Krause further teaches that other known binding moieties include oligonucleotides (para [0100] L 'binding sequence'). Therefore, it would have been obvious to one of ordinary skill in the art wherein said competitor molecule is an oligonucleotide.

Claims 1-26, 28-68a, 68b, 69a, 69b, 70-80 have industrial applicability as defined in PCT Article 33(4) because the subject matter can be made or used in industry.