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| APPLICATION NO.                                          | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|----------------------------------------------------------|-------------|----------------------|---------------------|------------------|
| 17/330,331                                               | 05/25/2021  | Yuling Luo           | 14582-003-999       | 6997             |
| 20583                                                    | 7590        | 11/09/2021           | EXAMINER            |                  |
| Jones Day<br>250 Vesey Street<br>New York, NY 10281-1047 |             |                      | POHNERT, STEVEN C   |                  |
|                                                          |             |                      | ART UNIT            | PAPER NUMBER     |
|                                                          |             |                      | 1634                |                  |
|                                                          |             |                      | NOTIFICATION DATE   | DELIVERY MODE    |
|                                                          |             |                      | 11/09/2021          | ELECTRONIC       |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

japarker@jonesday.com  
ncgeorge@jonesday.com  
wtokmakidis@jonesday.com

|                              |                                         |                                   |                                 |
|------------------------------|-----------------------------------------|-----------------------------------|---------------------------------|
| <b>Office Action Summary</b> | <b>Application No.</b><br>17/330,331    | <b>Applicant(s)</b><br>Luo et al. |                                 |
|                              | <b>Examiner</b><br>STEVEN C POHNERT PhD | <b>Art Unit</b><br>1634           | <b>AIA (FITF) Status</b><br>Yes |

**-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --**

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

☐ Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.  
☐ If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.  
☐ Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1) ☒ Responsive to communication(s) filed on 10/20/2021.  
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on \_\_\_\_.

2a) ☐ This action is **FINAL**.                      2b) ☒ This action is non-final.

3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on \_\_\_\_; the restriction requirement and election have been incorporated into this action.

4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims\***

5) ☒ Claim(s) 285-314 is/are pending in the application.  
     5a) Of the above claim(s) 285,287-300 and 309-310 is/are withdrawn from consideration.

6) ☐ Claim(s) \_\_\_\_ is/are allowed.

7) ☒ Claim(s) 286,301-308 and 311-314 is/are rejected.

8) ☐ Claim(s) \_\_\_\_ is/are objected to.

9) ☐ Claim(s) \_\_\_\_ are subject to restriction and/or election requirement

\* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see [http://www.uspto.gov/patents/init\\_events/pph/index.jsp](http://www.uspto.gov/patents/init_events/pph/index.jsp) or send an inquiry to [PPHfeedback@uspto.gov](mailto:PPHfeedback@uspto.gov).

**Application Papers**

10) ☒ The specification is objected to by the Examiner.

11) ☒ The drawing(s) filed on 5/25/2021 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.  
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

**Certified copies:**

a) ☐ All      b) ☐ Some\*\*      c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_.

3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\*\* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)  
 Paper No(s)/Mail Date \_\_\_\_.

3) ☐ Interview Summary (PTO-413)  
 Paper No(s)/Mail Date \_\_\_\_.

4) ☐ Other: \_\_\_\_.

## DETAILED ACTION

### *Notice of Pre-AIA or AIA Status*

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

### *Election/Restrictions*

2. Applicant's election without traverse of group II, claims 286, 301-314 in the reply filed on 10/20/2021 is acknowledged.
3. Claims 285, 287-300 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 10/20/2021.

Applicant's election with traverse of (1) paramagnetic beads with poly-T; (2) increasing temperature for releasing (claim 304); (3) (iii) from claim 304; (4) (i) from claim 305; (5) (iii) from claim 307; (6) (iii) from claim 308; (7) codetection of labels (claim 311); and (8) (iii) from claim 314. in the reply filed on 10/20/2021 is acknowledged. The traversal is on the ground(s) that examine the subject matter of the patentably distinct species would not be a serious burden on the Examiner. For example, individual species encompassed within the claims of Group II would likely not require a different field of search, and the prior art applicable to one species would likely be applicable to another species. For example, Applicant asserts that searching poly-A will inherently provide art on poly-T. Similarly, searching salt concentrations will inherently provide art on increasing temperature; searching nucleic acids will inherently

provide art on biotin and streptavidin; searching a first surface as a paramagnetic bead will inherently provide art on a second surface of paramagnetic beads; and searching co-detection will inherently provide art on simultaneous detection. This is not found persuasive because poly A sequence is made of adenines and has a different structure than a polyT which is thymine. Thus searching one nucleotide sequence will not inherently provide art on another nucleotide sequence contrary to the arguments of counsel. Further methods requiring increasing melting temperature is physically different than methods requiring altering salt concentrations to disrupt bonds, contrary to the assertions of counsel. Further biotin and streptavidin and proteins and are not nucleic acids. Thus searching nucleic acids will not inherently provide art on biotin or streptavidin. Further methods requiring the first solid surface paramagnetic bead will not inherently provide art on the second surface being a paramagnetic beads. The response further provides arguments with respect to search burden with respect to the species election, but has not provided any evidence that the species are not patentably distinct or explicitly stated on the record the species are obvious variants.

However, in view of the search the species election with respect to elections 1-6, 8 have been withdrawn.

Claims 309-310 withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected species, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 10/20/2021.

Claims 285, 287-300, 309-310 are withdrawn.

Claims 286, 301-308 and 311-314 are being examined.

***Priority***

The instant application was filed 05/25/2021 is a continuation of PCT/US2020/062791 , filed 12/02/2020 and claims priority from provisional application 62943135, filed 12/03/2019.

***Information Disclosure Statement***

1. The information disclosure statement (IDS) submitted on 5/25/2021 and 8/4/2021 is being considered by the examiner.
2. The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609.04(a) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

***Specification***

The specification on pages 218-227 recites, "SEQ ID NO." However, 37 CFR 1.821 (d) requires , "Where the description or claims of a patent application discuss a sequence that is set forth in the "Sequence Listing " in accordance with paragraph (c) of this section, reference must be made to the sequence by use of the sequence identifier, preceded by "SEQ ID NO: " in the text of the description or claims, even if the sequence is also embedded in the text of the description or claims of the patent application." The claims/specification should be amended to be consistent with 37 CFR 1.821 (d).

***Claim Rejections - 35 USC § 112***

3. The following is a quotation of 35 U.S.C. 112(b):  
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:  
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 286, 301-308, 311-314 rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

Claim 286 in the preamble recites, “in at least two samples.” Step (1) recites, “the sample.” Thus “the sample” of step (1) does not have antecedent basis in the preamble. The claim becomes more confusing and unclear as step (8) recites, “pooling the nucleic acid reporters of at least two samples together.” However the previous limitations are limited to a single sample. The claims should be amended to clarify antecedent basis of step (1) and how at least two samples are pooled when only one sample is required to go to steps (1) to (8).

Claim 301 recites, “An assay method for detecting an analyte in at least two samples, comprising: (1) mixing a first binding moiety comprising a first binder and a first presenting group, a second binding moiety comprising a second binder and a second presenting group, and the samples in a solution” Thus step 1 requires the samples are mixed. However, step 8 recites, “(8) pooling the nucleic acid reporters from the at least two samples.” Thus it is unclear what is required of pooling of samples

as they are already mixed in step 1. The rejection can be overcome by amending the claim to clarify that a single sample is mixed in step 1.

***Claim Rejections - 35 USC § 103***

5. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

6. The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

7. Claim 286, 301-308 and 311-314 is/are rejected under 35 U.S.C. 103 as being unpatentable over Darmanis (PLOS One (2011) volume 6, e25583) (IDS), Nong (Nature Protocols (2013) volume 8, pages 1234-1248) Wan (PLOS one (2018) volume 13, e0191987), Soldo (US20180292394),.

The prior art as exemplified below demonstrates proximity ligation of DNA attached to antibodies with ligation was known for the detection of analytes in a sample by next generation sequencing. Further the use of DNA tags attached to antibodies to identify the analyte being detected was known in the art. The use of a first solid support and second solid support to purify analytes by washing for removal contaminants and background were known. Further the use of samples IDs as part of labels were known.

Further the use of polyA-polyT complexes and streptavidin-biotin were known for the isolation of nucleic acids in assays. Thus the instant claims are no more than the combination of known methods of isolating and detecting analytes in known samples.

Darmanis teaches a method of detecting proteins by proximity ligation and next generation sequencing (title, abstract). Darmanis teaches antibodies were attached to oligonucleotides via the 5' or 3' end or attached to paramagnetic beads for SP-PLA (page 2, 1<sup>st</sup> column, last paragraph). Darmanis teaches, " We recently developed a solid-phase version of PLA (SP-PLA). In this assay, an antibody immobilized on a solid support acts as a capture reagent for localized enrichment of the antigen from a complex mixture of proteins, such as serum or plasma [19]. After washes, a pair of PLA probes is added followed by washes and ligation of oligonucleotides brought in proximity. The solid support allows washes to remove excess PLA probes and other molecules that could interfere with detection of the protein of interest. The unique requirement that each targeted protein is recognized via three binding events in order to be recorded provides exceptional specificity of detection compared to single-binder or sandwich assays. This, in combination with the use of PCR amplification to detect signals, allows for high specificity and sensitivity of detection, and a broad dynamic range for protein quantification." (page 2, 1<sup>st</sup> column, 2<sup>nd</sup> paragraph).

Darmanis teaches, "Pools of microparticles, each coated with one of the antibodies, are mixed with the sample (I). After washes, pairs of PLA probes directed against each of the proteins are incubated with the microparticles (II), followed by washes and ligation of the attached DNA strands (III). Ligated molecules are first amplified with primers directed against sequences present on all ligated DNA molecules

(IV). This universal pre-amplification is then followed by preparation of the reporter strands for next generation sequencing, or for qPCR (V). Subsequently the abundance of each protein is calculated and multivariable classification of cases and controls is performed (VI).” (figure 1).

Darmanis teaches the use of different PLA tags for use with different antibodies with 3' and 5' overhangs (supplementary figure 1). Darmanias teaches the use of streptavidin-biotin to attach antibodies to beads.(solid support preparation).

Darmanis teaches the use of sample tags in addition to target tags to allow pooling (supplementary figure 3, page 4 1<sup>st</sup> column).

Darmanis teaches detection of proteins by amplification and next generations sequencing (page 4, 1st column).

Nong teaches method of solid phase proximity ligation of individual or protein through PCR or sequencing (tittle). Non teaches detection and overview of spPLA (figures 1 and 2).Nong provides guidance on experimental design including affinity reagents, sample types, detection of ligation products design of oligonucleotides including a sample tag, PLA probe conjugations, etc. (pages 1234-12389)

Darmanis and Nong do not specifically teach the use of a first surface (paramagnetic beads) or a second surface (paramagnetic beads) and use of poly-T/ployA hybridization and biotin/streptavidin to bind to the paramagnetic beads.

However, Wan teaches methods of detection biomarkers in serum by use of pre-purification using photocleavable antibodies to overcome the matrix effect. Wan teaches, “A major limitation of most multiplex assays, such as those based on the Luminex® xMAP® system, is the well-known “matrix effect” [18–25]. This effect is

caused by the presence of non-target constituents in blood such as proteins, cholesterol, lipids, salts and low-specificity heterophile antibodies which interfere with detection of the often less abundant biomarkers. While all assays are impaired by the matrix effect to some degree, the effect is exacerbated in multiplex assays mainly because of the lower binding capacity of the miniaturized assay surfaces (e.g. microspheres and microarrays). Such surfaces quickly saturate with the specific or non-specific binding of unintended matrix components from the sample (which can suppress signal, mediate background and/or cause microsphere aggregation; Fig 1). In addition, high viscosity and undesirable conductance of the sample matrix can interfere with microfluidics [26, 27], which are commonly used in today's multiplex assay platforms." (page 2). Wan teaches preisolation of samples using beads, followed by release and recapture prior to analysis (figure 2). Wan teaches the use attachment of antibodies to beads by the use of streptavidin-biotin (page 6).

Soldo teaches, "capture moieties (streptavidin, neutravidin, avidin, polyA, polyDT, aptamers, antibodies, Fab, F(ab')<sub>2</sub>, antibody fragments, recombinant proteins, enzymes, proteins, biomolecules, polymers." (page 18, 2<sup>nd</sup> column #7).

Soldo teaches, "[0116] A method of measuring the amount, mass, molarity, concentration, or yield of targeted biomarker captured and enriched by the microparticulate binding surface whereby the biomarker is eluted, disassociated or freed from the microparticulate binding surface by disrupting the binding interaction using elution strategies such as pH (e.g. increased pH with a base such as sodium bicarbonate, decreased pH with an acids such as acetic acid, trichloroacetic acid, sulfosalicylic acid, HCl, formic acid, and common pH elution buffers such as 100 mM

glycine.HCl, pH 2.5, 3.0, 100 mM citric acid, pH 3.0, 50, 100 mM triethylamine or triethanolamine, pH 11.5, 150 mM ammonium hydroxide, pH 10.5), a displacer or displacing agent, competitive elution (e.g. >0.1M counter ligand or analog), ionic strength and/or chaotropic effects (e.g. NaCl, KCl, 3.5, 4.0M magnesium chloride pH 7.0 in 10 mM Tris, 5M lithium chloride in 10mM phosphate buffer pH 7.2, 2.5M sodium iodide pH 7.5, 0.2, 3.0M sodium thiocyanate), surfactant, detergent, a concentrated inorganic salt, denaturing (e.g. 2, 6M guanidine.HCl, 2, 8M urea, 1% deoxycholate, 1% SDS), an organic solvent (e.g. alcohol, chloroform, ethanol, methanol, acetonitrile, hexane, DMSO, 10% dioxane, 50% ethylene glycol pH 8, 11.5 (also chaotropic)), radiation or heat (increased temperature), conformational change, disulfide bond reducers (2-mercaptoethanol, dithiothreitol, tris(2-carboxylethyl)phosphine), enzyme inactivation, chaotropic agents (Urea, Guanidinium chloride, Lithium perchlorate), mechanical agitation, sonication, and protein digestive enzymes (pepsin, trypsin), and combinations thereof.”

Therefore it would have been prima facie obvious to one of ordinary skill in the art prior to the effective filing date of the claims to isolate a biomarker in two or more samples by use of an immunocomplex to paramagnetic beads with a first target specific ID via a polyA-polyT capture, wash, release the immunocomplex via heating or ionic strength, recapture with a second antibody to paramagnetic beads via biotin-streptavidin with a second target specific ID and sample ID, wash, generate a reporter by proximity ligation, pool samples and detect via reporter by next generation sequencing. The artisan would be motivated as Wan teaches the capture and recapture of protein biomarkers decreases matrix effect and background. The artisan would be motivated to

use polyT-polyA and streptavidin-biotin as Soldo teaches they are known capture reagents. The artisan would have a reasonable expectation of success as the artisan is using known methods and reagents to capture and recapture protein biomarkers as taught by Wan in the method of Darmanis and Nong.

With regards to claim 302, Nong teaches the affinity reagents should be capable of recognizing non-overlapping epitopes (affinity reagents).

With regards to claim 303, the binding of the antibody to immune complex to the beads is an equilibrium process, thus some of the immunocomplex will be formed prior to binding to the beads.

With regards to claim 304, Soldo teaches, "[0116] A method of measuring the amount, mass, molarity, concentration, or yield of targeted biomarker captured and enriched by the microparticulate binding surface whereby the biomarker is eluted, disassociated or freed from the microparticulate binding surface by disrupting the binding interaction using elution strategies such as pH (e.g. increased pH with a base such as sodium bicarbonate, decreased pH with an acids such as acetic acid, trichloroacetic acid, sulfosalicylic acid, HCl, formic acid, and common pH elution buffers such as 100 mM glycine.HCl, pH 2.5, 3.0, 100 mM citric acid, pH 3.0, 50, 100 mM triethylamine or triethanolamine, pH 11.5, 150 mM ammonium hydroxide, pH 10.5), a displacer or displacing agent, competitive elution (e.g. >0.1M counter ligand or analog), ionic strength and/or chaotropic effects (e.g. NaCl, KCl, 3.5, 4.0M magnesium chloride pH 7.0 in 10 mM Tris, 5M lithium chloride in 10mM phosphate buffer pH 7.2, 2.5M sodium iodide pH 7.5, 0.2, 3.0M sodium thiocyanate), surfactant, detergent, a concentrated inorganic salt, denaturing (e.g. 2, 6M guanidine.HCl, 2, 8M urea, 1%

deoxycholate, 1% SDS), an organic solvent (e.g. alcohol, chloroform, ethanol, methanol, acetonitrile, hexane, DMSO, 10% dioxane, 50% ethylene glycol pH 8, 11.5 (also chaotropic)), radiation or heat (increased temperature), conformational change, disulfide bond reducers (2-mercaptoethanol, dithiothreitol, tris(2-carboxylethyl)phosphine), enzyme inactivation, chaotropic agents (Urea, Guanidinium chloride, Lithium perchlorate), mechanical agitation, sonication, and protein digestive enzymes (pepsin, trypsin), and combinations thereof.”

With regards to claim 305, Darmanis and Nong teachings of MPLA with target specific IDs renders this obvious.

With regards to claim 306, Soldo teaches polyA and polyT as capture reagents. (page 18, 2<sup>nd</sup> column #7).

With regards to claim 307-308, Darmanis teaches the use of different PLA tags for use with different antibodies with 3' and 5' overhangs (supplementary figure 1).

With regards to claim 311, the next generation sequences of Darmanis and Nong would sequence and thus co-detect both target IDs.

With regards to claim 312, Non teaches the use of positive and negative controls and calibration curve (controls and calibration curves).

With regards to claim 313, Nong and Darmanis teaches the use of next generation sequencing.

With regards to claim 314, Nong and Darmanis teach the use of paramagnetic beads.

***Double Patenting***

1. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit [www.uspto.gov/patent/patents-forms](http://www.uspto.gov/patent/patents-forms). The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to [www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp](http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp).

2. Claim 286, 301-308 and 311-314 provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claim 286, 301-314 of copending Application No. 17/330385. Although the claims at issue are not identical, they are not patentably distinct from each other because they are coextensive in scope.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

The instant claims are to methods of detecting analytes in two or more samples.

The cited claims of '385 are to systems of detecting analytes in two or more samples. The claims of the system are for use in the instantly claimed method. Thus they are obvious variants.

### **Summary**

No claims are allowed.

### **Conclusion**

1. Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN C POHNERT PhD whose telephone number is

(571)272-3803. The examiner can normally be reached Monday- Friday about 6:00 AM- 5:30 PM, every second Friday off.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Steven Pohnert/  
Primary Examiner, Art Unit 1634

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

|                  |                                                          |                      |                   |
|------------------|----------------------------------------------------------|----------------------|-------------------|
| Applicant:       | Alamar Biosciences, Inc.                                 | Confirmation No.:    | 6997              |
| Application No.: | 17/330,331                                               | Art Unit:            | 1634              |
| Filing Date:     | May 25, 2021                                             | Examiner:            | Steven C. Pohnert |
| For:             | NUCLEIC ACID LINKED<br>IMMUNE-SANDWICH ASSAY<br>(NULISA) | Attorney Docket No.: | 133579-5001-US    |

**AMENDMENT AND RESPONSE UNDER 37 C.F.R. § 1.116**

**MAIL STOP AMENDMENT**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Commissioner:

In response to the non-final Office Action mailed October 26, 2022, please enter the following amendments and remarks.

**Amendments to the Claims** are reflected in the listing of claims which begins on page **2** of this paper.

**Remarks** begin on page **5** of this paper.

DB2/ 44998801.3

### AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1-285. (Cancelled)

286. (Currently amended) An assay method for detecting an analyte in at least a first biological sample and a second biological sample, comprising:

(1) forming ~~an tripart~~ immunocomplex in solution by adding, to each of a first assay reaction comprising the first biological sample and a second assay reaction comprising the second biological sample, a first binding moiety and a second binding moiety, wherein:

(i) the first binding moiety comprises (a) a first antibody or a first antibody fragment that specifically binds the analyte, (b) a first nucleic acid tag that ~~comprises a poly-A or poly-T sequence and~~ is conjugated to the first antibody or the first antibody fragment, and (c) a first nucleic acid target label that ~~comprises a first identity barcode ("ID") that is analyte-specific ("first target ID") and~~ is hybridized with the first nucleic acid tag and is not covalently attached to the first antibody or the first antibody fragment, wherein:

the first nucleic acid tag (b) comprises a poly A or poly T sequence,

and

the nucleic acid target label (c) comprises a first identity barcode that is analyte-specific;

(ii) the second binding moiety comprises (d) a second antibody or second antibody fragment that specifically binds the analyte, (e) a second nucleic acid tag that ~~comprises a second nucleic acid target label and~~ is conjugated to the second antibody or the second antibody fragment, ~~wherein the second nucleic acid target label comprises a second identity barcode ID that is analyte specific ("second target ID");~~ and (f) a nucleic acid probe that is ~~conjugated with biotin and~~ is hybridized with the second nucleic acid tag and is not covalently attached to the second antibody or the second antibody fragment, wherein:

the second nucleic acid tag (e) comprises a second identity barcode that is analyte specific, and

the nucleic acid probe (f) is conjugated to biotin; and

(iii) the immunocomplex comprises the first binding moiety ~~antibody or the first antibody fragment~~, the second binding moiety ~~antibody or the second antibody fragment~~, and the analyte;

(2) to each of the first assay reaction and the second assay reaction, introducing first paramagnetic beads coupled with a poly-T or poly-A sequence, and capturing the immunocomplex on the first paramagnetic beads by hybridization between the poly-A sequence from the first binding moiety and the poly-T sequence from the first paramagnetic beads, or between the poly-T sequence from the first binding moiety and the poly-A sequence from the first paramagnetic beads;

(3) washing the first paramagnetic beads, in each of the first assay reaction and the second assay reaction, to remove unbound molecules;

(4) releasing the immunocomplex from the first paramagnetic beads, in each of the first assay reaction and the second assay reaction, by disrupting the hybridization between the poly-A sequence from the first binding moiety and the poly-T sequence from the first paramagnetic beads, or between the poly-T sequence from the first binding moiety and the poly-A sequence from the first paramagnetic beads;

(5) to each of the first assay reaction and the second assay reaction, introducing second paramagnetic beads coupled with streptavidin and recapturing the immunocomplex on the second paramagnetic beads via binding between the biotin from the second binding moiety and the streptavidin from the second paramagnetic beads;

(6) washing the second paramagnetic beads, in each of the first assay reaction and the second assay reaction, to remove unbound molecules;

(7) to each of the first assay reaction and the second assay reaction, introducing a double-stranded nucleic acid sample label that comprises (i) a longer strand (~~“longer strand”~~) with a 5' overhang and a 3' overhang, wherein one of the overhangs hybridizes with the ~~first~~ nucleic acid target label and the other overhang hybridizes with the second nucleic acid tag target label; and (ii) a shorter strand (~~“shorter strand”~~) comprising ~~an ID~~ a third identity barcode that is sample-specific (~~“sample ID”~~), hybridizing the 5' overhang and the 3' overhang of the sample label to the ~~first~~ nucleic acid target label and the second nucleic acid tag target label, and completing proximity-based ligation between (i) the ~~first~~ nucleic acid target label and the shorter strand of the nucleic acid sample label and (ii) the second nucleic acid tag target label and the shorter strand of the nucleic acid sample label, to generate a nucleic acid reporter that is conjugated to the second antibody or second antibody fragment

and is not covalently attached to the first antibody or first antibody fragment, wherein the nucleic acid reporter comprises the nucleic acid target label (c) comprising the first identity barcode, the second nucleic acid tag (d) comprising the second identity barcode, and the third identity barcode positioned between the nucleic acid target label (c) and the second nucleic acid tag (d) first target ID, the sample ID, and the second target ID, wherein the sample ID is between the first target ID and the second target ID;

(8) pooling the nucleic acid reporters from the first assay reaction with the nucleic acid ~~reporters~~ reports from the second assay reaction together to form a pooled sample; and

(9) ~~detecting the nucleic acid reporters in the pooled sample, thereby~~ detecting the analyte in the first biological sample and the second biological sample by detecting the nucleic acid reporters in the pooled sample.

287-314. (Cancelled)

315. (Currently amended) The assay method of claim 286, wherein in step (9), detecting the analyte comprises co-detection of the first identity barcode and the second identity barcode target IDs.

316. (Currently amended) The assay method of claim 286, wherein the nucleic acid reporter is detected by next generation sequencing (~~NGS~~).

317. (Previously presented) The assay method of claim 286, wherein the sample is a serum sample, a plasma sample, or a cerebrospinal fluid sample.

318. (Previously presented) The assay method of claim 286, wherein the sample is derived from a cell sample or a tissue sample.

319. (New) The assay method of claim 286, wherein in step (9), detecting the analyte comprises PCR amplification of the nucleic acid reporters in the pooled sample.

**REMARKS**

Claims 286, 315-318 were previously pending in the application.

With this Response, claim 286 has been amended to improve clarity and/or subject-antecedent agreement. Specifically, claim 286 has been amended to indicate the asymmetry between the first binding moiety recited in step (1)(i) and the second binding moiety recited in step (1)(ii) more clearly.

Claim 286 is also amended to recite that the nucleic acid target label is not covalently attached to the first antibody or the first antibody fragment, and that the nucleic acid probe is not covalently attached to the second antibody or the second antibody fragment. Support for the amendment can be found throughout the application, for example, at paragraphs [0069] and [0074], as well as in Figure 22, as originally filed.

Claim 286 is also amended to remove the parenthetical terms “(ID),” “(first target ID),” “(second target),” “(longer strand),” “(shorter strand),” and “(sample ID).”

Claims 286 is also amended to clearly indicate the product formed in the ligation reaction of step (7). Support for the amendment can be found throughout the application, for example, at paragraphs [00294] and [00299], as well as in Figures 16 and 21H, as originally filed.

Claim 286 is also amended to improve more positively recite that the analyte is detected, in step (9) by detecting the nucleic acid reporters.

With this Response, claims 315 and 316 are amended to improve clarity and/or subject-antecedent agreement.

With this Response, claim 319 is newly presented to recite that the detecting in step (9) comprises PCR amplification of the nucleic acid reporters in the pooled sample. Support for this amendment is found throughout the application, for example, at paragraphs [0022], [00299] and in Figures 16 and 21I, as originally filed.

Upon entry of the claim amendments, claims 286 and 315-319 will be pending for consideration on the merits.

Accordingly, Applicant respectfully request entry of the amendments to the claims.

**Examiner Interview**

Applicant thanks Examiner Pohnert for his helpful comments during a telephonic interview on January 23, 2023, with Applicant's representative, Andrew Antczak. During the interview, the outstanding rejection under 35 U.S.C. § 103 was discussed in view of the amended claims. Specifically, referring to Figures 22 and 21H of the application as filed, Applicant's representative explained that the binding moieties recited in claim 286 had an asymmetric design in which one identity barcode is present on a polynucleotide covalently conjugated to an antibody or fragment thereof and another identity barcode is present on a different polynucleotide that is not conjugated to an antibody or fragment thereof. As such, following the ligation in step 7 of the claimed method, the resulting reporter polynucleotide is conjugated on one end to an antibody and is not conjugated to anything on the other end. The Examiner suggested clarifying these structures in claim 286. The Examiner indicated that the art of record did not appear to teach or suggest these orientations, but that a further search would be necessary.

**I. The rejection under 35 U.S.C. § 112(a) should be withdrawn**

Claims 286, and 315-318 stand rejected under 35 U.S.C. § 112(a) as allegedly failing to comply with the written description requirement. Specifically, the Office Action states that the amendment to Claim 286, "forming a tripart immunocomplex" introduces new matter. *See*, Office Action, page 4.

Applicant respectfully disagrees. However, in an earnest attempt to expedite prosecution and without acquiescing to the merits of the rejection, claim 286 has been amended to remove recital of "tripart," rendering the rejection moot.

As such, Applicant respectfully requests that the above rejection be reconsidered and withdrawn.

**II. The first rejection under 35 U.S.C. § 112(b) should be withdrawn**

Claims 286, and 315-318 also stand rejected under 35 U.S.C. § 112(b) as allegedly indefinite. Specifically, the Office Action alleges that a search of the specification did not reveal antecedent basis for the recited limitation "forming a tripart immunocomplex."

Applicant respectfully disagrees. However, in an earnest attempt to expedite prosecution and without acquiescing to the merits of the rejection, claim 286 has been amended to remove recital of “tripart,” rendering the rejection moot.

As such, Applicant respectfully requests that the above rejection be reconsidered and withdrawn.

**III. The second rejection under 35 U.S.C. § 112(b) should be withdrawn**

Claim 286 stands rejected under 35 U.S.C. § 112(b) as allegedly being indefinite for reciting the following parentheticals:

- (i) The term “(ID)” from claim 286;
- (ii) The term “(first target ID)” from claim 286;
- (iii) The term “(second target)” from claim 286;
- (iv) The term “(longer strand)” from claim 286;
- (v) The term “(shorter strand)” from claim 286; and
- (vi) The term “(sample ID)” from claim 286.

Applicant respectfully disagrees. However, in an earnest attempt to expedite prosecution and without acquiescing to the merits of the rejection, claim 286 has been amended to remove these parentheticals.

As such, Applicant respectfully requests that the above rejection be reconsidered and withdrawn.

**IV. Claim Rejections - 35 U.S.C. § 103**

Claims 286, and 315-318 stand rejected under 35 U.S.C. § 103 as allegedly obvious over the combination of Jalili (Proceedings National Academy of Sciences USA (2018), e925-33), Lundberg (Molecular & Cellular Proteomics 10.4 (2011)), Persson (WO 2018/160397, published 9/7/2018), Nong (Nature Protocols (2013) volume 8, pages 1234-1248) Wan (PLOS one (2018) volume 13, e0191987), Soldo (US 2018/0292394), Cuppens (US 2010/0227329), Bystrykh (Hematopoietic Stem Cell Protocols, Methods in Molecular Biology, vol. 1185, pages 345-360), and Fields (US 2017/0335369). Applicant respectfully

disagrees because the combination of references does not teach or suggest all the claim elements. Further, the claimed methods provide unexpectedly improved results, evidencing the non-obviousness of the claims.

For any rejection under 35 U.S.C. § 103, a clear articulation of the reason(s) why the claimed invention would have been obvious is required. The U.S. Supreme Court has noted that the analysis supporting a rejection under 35 U.S.C. § 103 should be made explicit, and that “rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.” *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) (quoting *In re Kahn*, 441 F. 3d 977, 988 (2006)).

Indeed, finding all the claim elements in the prior art is necessary, but not sufficient, as a claim requiring “several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art.” *Id.* Rather, it must be determined “whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue.” *Id.* “If [a] proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification.” MPEP § 2143.01(V) (citing *In re Gordon*, 733 F.2d 900 (Fed. Cir. 1984)).

Furthermore, the Applicant can rebut a *prima facie* case of obviousness by presenting evidence of surprisingly effective results. The Supreme Court has affirmed the analysis set forth in *Graham* for the determination of obviousness. See, *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007). A greater than expected result is evidence of nonobviousness that can rebut a *prima facie* case. MPEP § 716.02(a). Here, the pending claims are not obvious because the claimed invention yielded surprisingly effective results.

Here, a *prima facie* case of obviousness does not exist because the combination of references fails to teach or suggest all the claim elements, let alone teach or suggest assembling such claim elements and using them in a fashion approximating the presently claimed invention. Specifically, the combination of cited references fails to teach or suggest (I) use of a first binding moiety as recited in element (1)(i) of claim 286, (II) use of a second binding moiety as recited in element (1)(ii) of claim 286, (III) use of a double-

stranded nucleic acid, comprising a longer strand and a shorter strand, which comprises a third sample-specific identity barcode, wherein one strand of the double-stranded nucleic acid binds, and the other strand is ligated, to a nucleic acid target label that is not covalently attached to an antibody or fragment thereof and to a nucleic acid tag that is covalently attached to an antibody or fragment thereof, as recited in element (7) of claim 286 and (IV) the resulting ligation product, as recited in element (7) of claim 286, which is conjugated on one end to an antibody or fragment thereof and is not conjugated on the other end to an antibody or fragment thereof. As discussed during the Examiner interview, the asymmetric design of the binding reagents and ligation product in the claimed method is not taught or suggested by the combination of cited references. Further, the non-obviousness of Applicant's claimed method is evidenced by the surprising results it provides, namely a 200 to nearly 100,000-fold improvement in the level of detection relative to the detection assays described in the cited art. Thus, no *prima facie* case of obviousness exists.

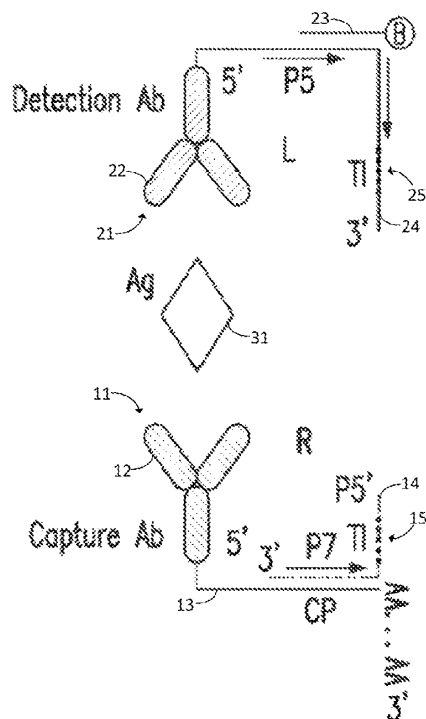
**i) The claims are not obvious because the combination of cited references does not teach or suggest the first binding moiety as recited in claim 286.**

As an initial matter, Wan, Soldo, Cuppens, Bystrykh, and Fields do not disclose a binding reagent with an antibody or antigen binding fragment thereof that is associated with a polynucleotide.

Jalili, Persson, Lundberg, and Nong teach variations of a proximity ligation assay ("PLA assay") that use antibodies associated with a polynucleotide. However, none of these references teach or suggest capturing an analyte with an antibody or fragment thereof associated with two polynucleotide strands, let alone the binding reagent recited in claim 289 where the antibody or fragment thereof is covalently associated with a first polynucleotide having a capture affinity moiety and the second polynucleotide has an analyte-specific barcode and is hybridized to the first polynucleotide—rather than being covalently conjugated to the antibody.

Specifically, as exemplified in Figure 22 of the present application, amended claim 286 recites, in step (1)(i), using a first binding moiety 11 comprising a first antibody or a first antibody fragment 12 that specifically binds the analyte 31, a first nucleic acid tag 13 that comprises a poly-A or poly-T sequence and is conjugated to the first antibody or the first

antibody fragment, and a nucleic acid target label 14 that comprises a first identity barcode 15 that is analyte-specific and is hybridized with the first nucleic acid tag.



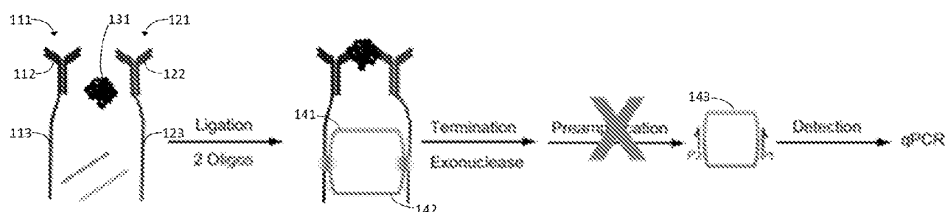
Present Application at Figure 22, annotations added.

Jalili and Persson

Jalili and Persson describe the same PLA variation, referred to as circular proximity ligation assay (cPLA). In fact, all four of the inventors of the Persson PCT application are listed as authors of the Jalili paper. Accordingly, as the references disclose the same method, they will be discussed together below.

As illustrated in Figure 1 of Jalili, and described in the Methods section on page E932), cPLA involves capturing an analyte 131 using two detection moieties 111 and 121. Both detection moieties of Jalili consist of an antibody 112 and 122 conjugated to a single polynucleotide 113 and 123. After capture of the analyte, two connector oligos 141 and 142

are added to the reaction.<sup>1</sup> The connector oligos 141 and 142 each hybridize to a portion of both conjugated polynucleotides 113 and 123 at adjacent regions. The connector oligos 141 and 142 are then ligated together to form a circular single stranded polynucleotide 143. Exonuclease is then added to the reaction to degrade all nucleic acids with a free 3' or 5' end, namely the polynucleotides 113 and 123 conjugated to the antibody and any non-ligated connector oligos. The free circular single stranded polynucleotide 143 can then be detected to indicate the presence of the analyte.



Jalili at Figure 1, annotations added.

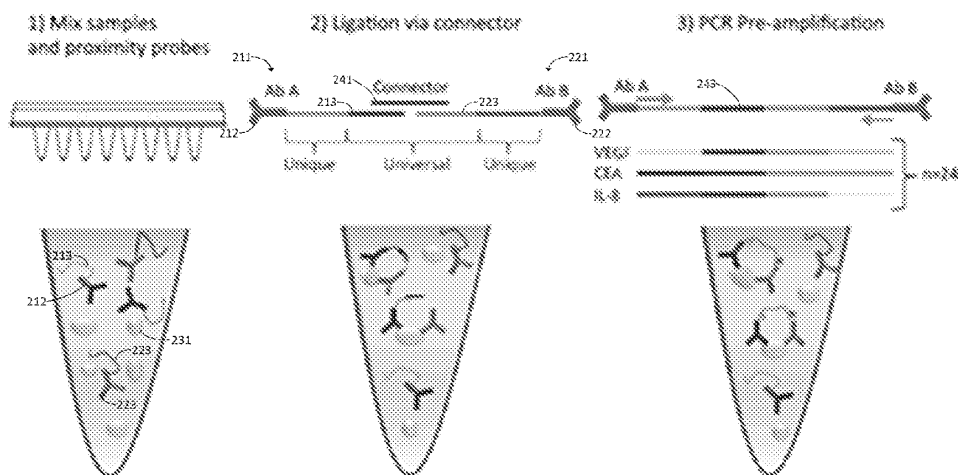
As can be seen in Figure 1 of Jalili, the detection moieties 111 and 121 used in cPLA do not include (i) any affinity tag, let alone a nucleic acid tag comprising a poly-A or poly-T sequence or (ii) any nucleic acid hybridized to the polynucleotide 113 or 123 that is conjugated to the detection antibody 112 or 122, both of which are recited in amended claim 286.

#### Lundberg

Lundberg describes a multiplexed version of a PLA assay, as shown in Figure 1 of the reference. In this version, an analyte 231 is captured using two detection moieties 211 and 221. Both detection moieties consist of an antibody 212 and 222 conjugated to a single polynucleotide 213 and 223. After capture of the analyte, a connector oligo 241 is used to place polynucleotides 213 and 223 in proximity to each other for ligation of the two

<sup>1</sup> Although Figure 1 shows connector oligos 141 and 142 in the same panel as the detection moieties and unbound analyte, the Methods section of Jalili makes clear that the detection reagents are mixed with the analyte and incubated for two hours “to establish complex formation between the target analyte and antibodies in proximity probes” prior to addition of the connector oligos as part of the ligation mixture.

polynucleotides to form a single polynucleotide 243 covalently attached to both detection antibodies 212 and 222.

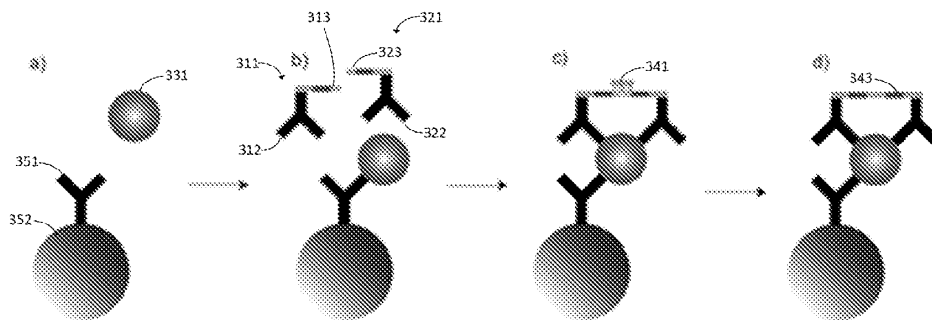


Lundberg at Figure 1, annotations added.

As can be seen in Figure 1 of Lundberg, the detection moieties 211 and 221 used in the multiplex PLA assay do not include (i) any affinity tag, let alone a nucleic acid tag comprising a poly-A or poly-T sequence or (ii) any nucleic acid hybridized to the polynucleotide 213 or 223 that is conjugated to the detection antibody 212 or 222, both of which are recited in amended claim 286.

#### Nong

Nong describes a solid-phase version of a PLA assay (spPLA), as shown in Figure 1 of the reference. In this version, an analyte 331 is pre-captured by an antibody 351 conjugated to a solid phase 352. Two detection moieties 311 and 321 are then added to the reaction to further bind the analyte 331. Both detection moieties consist of an antibody 312 and 322 conjugated to a single polynucleotide 313 and 323. After the detection moieties bind to the immobilized analyte, a connector oligo 341 is used to place polynucleotides 313 and 323 in proximity to each other for ligation of the two polynucleotides to form a single polynucleotide 343 covalently attached to both detection antibodies 312 and 322.



Nong at Figure 1, annotations added.

As can be seen in Figure 1 of Lundberg, the detection moieties 311 and 321 used in the multiplex PLA assay do not include (i) any affinity tag, let alone a nucleic acid tag comprising a poly-A or poly-T sequence or (ii) any nucleic acid hybridized to the polynucleotide 313 or 323 that is conjugated to the detection antibody 312 or 322, both of which are recited in amended claim 286.

No motivation exists to modify any of the detection moieties in Jalili, Persson, Lundberg, or Nong to hybridize a nucleic acid target label to a nucleic acid affinity tag conjugated to an antibody.

As described above, none of the cited reference teaches or suggests a binding moiety configuration in which a first polynucleotide (nucleic acid tag 13) that is directly conjugated to the antibody or antibody fragment 12 is hybridized with a second polynucleotide comprising an analyte-specific barcode (nucleic acid target label 14), as recited in amended claim 286. In fact, none of the cited references teach or suggest use of any detection reagents comprising two polynucleotide strands, where one strand is covalently conjugated to an antibody and the other is not covalently conjugated to the antibody.

Further, the Office Action does not address how or why the combination of cited references could be combined to arrive at the specific configuration of the first binding moiety as recited in claim 286. Rather, the Office Action alleges that the claim does not recite that the nucleic acid target label 14 is not covalently attached to the antibody or fragment thereof. Applicant respectfully disagrees, as it is implicit that when a first

polynucleotide is conjugated to an antibody, a second polynucleotide that is hybridized to the first polynucleotide is not covalently attached to the antibody. However, in an earnest attempt to expedite prosecution, and without acquiescing on the merits of the rejection, Applicant has amended claim 1 to expressly recite that the nucleic acid target label 14 is not covalently attached to the first antibody or the first antibody fragment.

Accordingly, as the combination of references does not teach or suggest use of an identity barcode (“ID”) that is analyte-specific (“first target ID”) and is hybridized with a nucleic acid tag, and not directly conjugated to a binding moiety, the combination of references cannot render the amended claims obvious.

**ii) The claims are not obvious because the combination of cited references does not teach or suggest the second binding moiety as recited in claim 286.**

As described above, Jalili, Persson, Lundberg, and Nong teach variations of a PLA assay that use antibodies associated with a polynucleotide. However, none of these references teach or suggest capturing an analyte with an antibody or fragment thereof associated with two polynucleotide strands, let alone the binding reagent recited in claim 289 where the antibody or fragment thereof is covalently associated with a first polynucleotide having an analyte-specific barcode and the second polynucleotide is attached to a capture affinity moiety and ***is not covalently conjugated*** to the antibody, as recited in amended claim 286. This orientation is shown in Figure 22, reproduced above.

As described above, the binding moieties disclosed in Jalili, Persson, Lundberg, and Nong include a single polynucleotide strand. There is simply no teaching or suggestion in any of the references, and no motivation provided in the Office Action, to modify these binding moieties to include two polynucleotides, where one polynucleotide is covalently attached to the antibody and the other polynucleotide is hybridized to the first polynucleotide and not covalently attached to the antibody. Nor is there any commonsense reason for the PHOSITA to modify the binding moieties disclosed in Jalili, Persson, Lundberg, or Nong to arrive at the specific configuration recited in amended claim 286.

Accordingly, as the combination of references does not teach or suggest the second binding moiety as recited in amended claim 286, the combination of references cannot render the amended claims obvious.

**iii) The claims are not obvious because the combination of cited references does not teach or suggest proximity ligation adaptors with a sample-specific barcode.**

Amended claim 286 recites introducing a double-stranded nucleic acid sample label that comprises (i) a longer strand with a 5' overhang and a 3' overhang, wherein one of the overhangs hybridizes with the nucleic acid target label and the other overhang hybridizes with the second nucleic acid tag; and (ii) a shorter strand comprising a third identity barcode that is sample-specific. Advantageously, by incorporating the sample ID in the proximity ligation adaptor, ligation products generated across a plurality of samples can be pooled together prior to amplification and/or direct sequencing of the ligation products. This not only saves time and resources by performing amplification and/or sequencing in a single reaction, but also eliminates artifacts and/or biases that may be introduced by performing amplification and/or sequencing of each sample in a separate reaction. In contrast, none of the references cited in the Office Action teach or suggest integrating a sample specific barcode into a proximity ligation adaptor. Rather, each of the references directly ligates a first reporter oligo to a second reporter oligo.

The Office Action recognizes that none of the references disclosing a PLA assay “teach the use of a sample ID barcode that is used to bridge or link the unique sequences attached to each antibody by use of an adaptor or bridge with a short strand and a long strand with a 3' and 5' overhang.”<sup>2</sup> However, the Office Action alleges that Cuppens, Bystrykh, and/or Fields supplements this deficiency. Applicant respectfully disagrees.

First, Cuppens teaches incorporation of a barcode using a 2-step PCR protocol. This, however, would defeat the purpose of Applicant's nucleic acid sample label. As outlined above, Applicant's incorporation of the sample ID in the proximity ligation adaptor allows ligation products generated across a plurality of samples to be pooled together *prior* to amplification. In contrast, the contemplated modification of the PLA assays described in the art by use of the barcoding method of Cuppens is performed **by PCR amplification**, necessitating a separate PCR reaction be performed for each sample. This is not Applicant's claimed method and, in fact, would frustrate the purpose of Applicant's design which saves

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<sup>2</sup> Office Action at page 14.

time and resources by performing amplification and/or sequencing of a plurality of samples in a single reaction, as well as eliminates artifacts and/or biases that may be introduced by performing amplification and/or sequencing of each sample in a separate reaction. Thus, Cuppens cannot supplement the disclosure of Jalili, Persson, Lundberg, and Nong.

The Office Action alleges that Bystrykh provides linkers with adaptor barcodes with 3' and 5' overhangs. This is not true. The linkers disclosed in Bystrykh contain two 5' overhangs and no 3' overhangs, as illustrated in Figure 1, reproduced below. Applicant notes that because of the antiparallel nature of nucleic acids, the top strand of the linkers shown in Figure 1 is written 5' to 3', while the bottom strand is written 3' to 5'. As such, these linkers could not bridge the nucleic acid target label and second nucleic acid tag, as recited in amended claim 286. Thus, Bystrykh cannot supplement the disclosure of Jalili, Persson, Lundberg, and Nong.

```
A  GTACAAGTAAATCATCCTGATSSAAANNNGGTNNAACNN TGTAAAACGACGGCCAGTGA  
   TTCATTNNTAGNNCTASCTTTNCCAGNTTGTACATTCTTGCTGCCGGTCACCTCTAG  
B  GTACAAGTAAAGGATCCTGATSSAAANNNGGTNNAACNN TGTAAAACGACGGCCAGTGA  
   TTCATTCTCTAGNNCTASCTTTNCCAGNTTGTACATTCTTGCTGCCGGTCACCTCTAG  
C  GTACAAGTAAAGGATCCTGATSSAAANNNGGTNNAACNN TGTAAAACGACGGCCAGTGA  
   TTCATTCTCTAGNNCTASCTTTNCCAGNTTGTACATTCTTGCTGCCGGTCACCTCTAG
```

**Fig. 1** Structure of barcode linkers. Details of linker design are described in the text

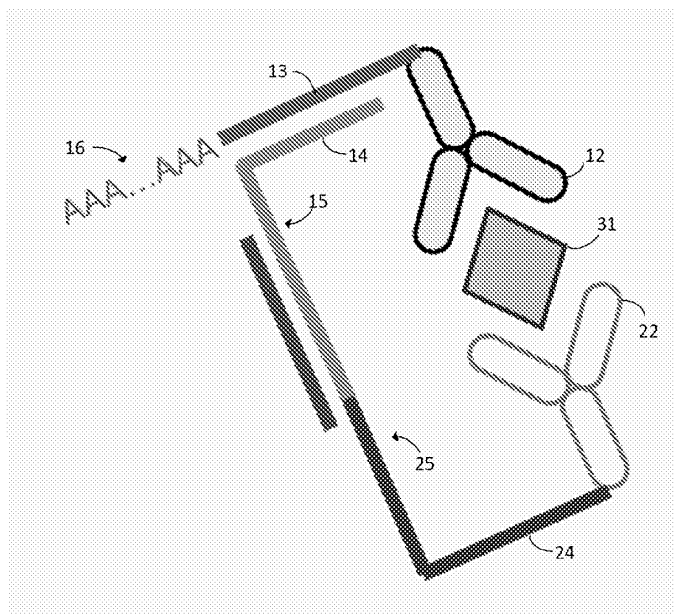
Bystrykh at Figure 1.

Finally, the Office Action alleges that Fields demonstrates that DNA Ligase is used to ligate an adapter by TA mediated ligation. However, the ligation reaction contemplated in Fields is performed between the linker and double stranded DNA that has been cut with a restriction enzyme. Fields does not contemplate bridging single-stranded polynucleotides, as recited in amended claim 286. Thus, Fields also cannot supplement the disclosure of Jalili, Persson, Lundberg, and Nong.

Accordingly, as the combination of references does not teach or suggest use of a double-stranded nucleic acid sample label as recited in amended claim 286, the combination of references cannot render the amended claims obvious.

**iv) The claims are not obvious because the combination of cited references  
does not teach or suggest the ligation product formed by the claimed method.**

Amended claim 286 clarifies that the nucleic acid ligation product formed in step (7) is conjugated on one end to an antibody or fragment thereof and is not conjugated on the other end to an antibody or fragment thereof. This is illustrated, for example, in Figure 21H of the application as filed, shown below.



Present Application at Figure 22, annotations added. As discussed during the Examiner interview, Applicant has amended element (7) claim 286 to clarify the asymmetric nature of the ligated product. Specifically, amended claim 286 recites, in pertinent part:

(7) to each of the first assay reaction and the second assay reaction, introducing a double-stranded nucleic acid sample label that comprises (i) a longer strand with a 5' overhang and a 3' overhang, wherein one of the overhangs hybridizes with the ~~first~~ nucleic acid target label and the other overhang hybridizes with the second nucleic acid tag; and (ii) a shorter strand comprising a third identity barcode that is sample-specific, hybridizing the 5' overhang and the 3' overhang of the sample label to the nucleic acid target label and the second nucleic acid tag, and completing proximity-based ligation between (i) the

nucleic acid target label and the shorter strand of the nucleic acid sample label and (ii) the second nucleic acid tag and the shorter strand of the nucleic acid sample label, to generate a nucleic acid reporter *that is conjugated to the second antibody or second antibody fragment 22 and is not covalently attached to the first antibody or first antibody fragment 12*, wherein the nucleic acid reporter comprises the nucleic acid target label 14 comprising the first identity barcode 15, the second nucleic acid tag 24 comprising the second identity barcode 25, and the third identity barcode positioned between the nucleic acid target label (c) and the second nucleic acid tag (d);

Amended claim 286, emphasis and reference numbers added. The combination of cited references does not teach or suggest the recited ligation product.

Accordingly, as the combination of references does not teach or suggest formation of the ligation product as recited in amended claim 286, the combination of references cannot render the amended claims obvious.

**v) The unexpectedly improved results provided by the claimed invention are secondary indicia of the non-obviousness of the claims.**

A prima facie case of obviousness—which Applicant contends has not been presented—can be rebutted by presenting evidence of surprisingly effective results. The Supreme Court has affirmed the analysis set forth in *Graham* for the determination of obviousness. See, *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007). Specifically, the Supreme Court, quoting from *Graham*, stated:

Under §103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background the obviousness or nonobviousness of the subject is determined. Such secondary considerations as commercial success, long felt but unresolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented. *Id.* at 1734.

A greater than expected result is evidence of nonobviousness that can rebut a prima facie case. MPEP 716.02(a). Here, the pending claims are not obvious because the claimed method yields surprisingly effective results.

Specifically, as described in the specification as filed, the method described in the claims results in protein detection sensitivities at the attomolar level. This represents from 100 to 10,000-fold greater sensitivity than is reported for the spPLA assay described in Darmanis and Nong.

For example, as described in section 6.4 of the specification as originally filed, detection of five different analytes were titrated in a multiplexed assay according to the claimed methods. It is reported that “[s]ensitivity of protein detection at attomolar (aM) level was achieved for all five assays, with three of them below 50aM.” Applicant’s specification at paragraph [00731]. Quantitated detection levels for the five analytes are reported in Table 2, reproduced below.

**Table 2**

| Assay        | LOB (aM)* | LOD (aM)* |
|--------------|-----------|-----------|
| IL-4         | 6.6       | 20.3      |
| IL-6         | 4.0       | 16.7      |
| IL-10        | 64.8      | 299.7     |
| TNF $\alpha$ | 28.3      | 95.1      |
| P24          | 8.5       | 40.7      |

Curving fitting: 4P-Logistic model.

LoB = meanblank + 1.645(SDblank)

LoD = LoB + 1.645(SD low concentration sample)

From Clinical and Laboratory Standards Institute (CLSI)

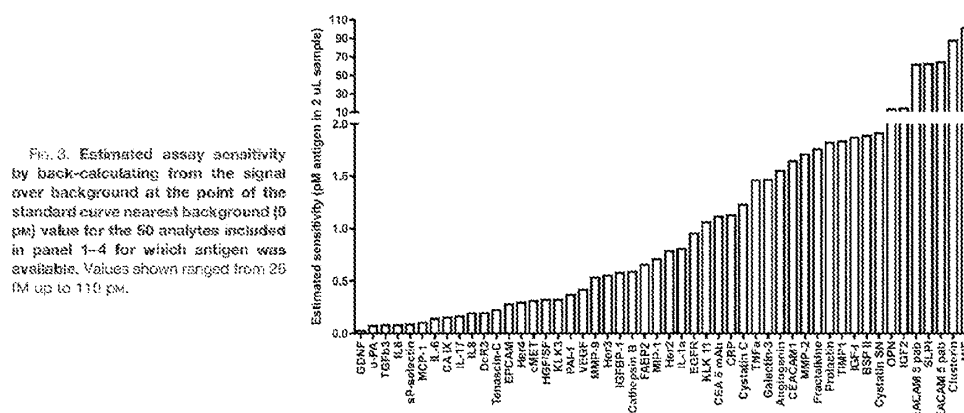
By contrast, the proximity ligation assays described in Jalili, Persson, Lundberg, and Nong provide detection limits in the femtomolar to picomolar range. For example, the cPLA assay described in Jalili and Persson achieves analyte detection down to the low-to-mid femto molar range, as reported in Table 1 of Jalili, reproduced below. Jalili also reports that traditional PLA (tPLA) assays perform slightly worse than the cPLA assay.

**Table 1. Comparison of limit of detection and dynamic range for six biomarkers measured by c-PLA and t-PLA**

| Analyte       | Function        | Data in figure | c-PLA              |               |        | t-PLA              |               |        |
|---------------|-----------------|----------------|--------------------|---------------|--------|--------------------|---------------|--------|
|               |                 |                | Limit of detection | Dynamic range | CVs, % | Limit of detection | Dynamic range | CVs, % |
| VEGF          | Growth factor   | Fig. 2A        | <10 fM             | <5            | 15.6   | 10 fM              | <5            | 29.3   |
| GDNF          | Cell survival   | Fig. 2B        | 15 fM              | <5            | 12.4   | 10 fM              | 5             | 30.8   |
| IL-6          | Inflammation    | Fig. 2C        | 100 fM             | <4            | 28.2   | 150 fM             | <4            | 48.5   |
| MIF           | Innate immunity | Fig. 2D        | 3 pM               | 3             | 10.4   | 5 pM               | 3             | 39.9   |
| TNF- $\alpha$ | Cell signaling  | Fig. 2E        | 20 pM              | 3             | 19.4   | 70 pM              | <3            | 44.2   |
| IGF-1R        | Inflammation    | Fig. 2F        | 1 nM               | 2             | 17.0   | 3 nM               | <2            | 24.3   |

In fact, Jalili tested two of the same analytes as reported in Applicant's specification, IL-6 and TNF- $\alpha$ . Significantly, Applicant's claimed method detected TNF- $\alpha$  at 200-fold greater sensitivity than achieved by the cPLA assay (LOD of 95 aM vs. 20 pM) and at 700-fold greater sensitivity than the tPLA assay (LOD of 95 aM vs. 70 pM) in Jalili. Even more striking, Applicant's claimed method detected IL-6 at 6000-fold greater sensitivity than achieved by the cPLA assay (LOD of 16.7 aM vs. 100 fM) and at 9000-fold greater sensitivity than the tPLA assay (LOD of 16.7 aM vs. 100 fM) in Jalili.

Similarly, the multiplexed PLA assay described in Lundberg achieves levels of detection ranging from 26 fM to 110 pM, as reported in Figure 3, reproduced below.



Lundberg also tested, IL-6 and TNF- $\alpha$ , which were tested by Jalili. Significantly, Applicant's claimed method detected TNF- $\alpha$  at approximately 15,000-fold greater sensitivity than achieved by the PLA assay (LOD of 95 aM vs. 1.5 pM) in Lundberg. Applicant's claimed method detected IL-6 at 6000-fold greater sensitivity than achieved by the cPLA assay (LOD of 16.7 aM vs. 100 fM) in Jalili.

Finally, while Nong does not report levels of detection for the described spPLA assay, Darmanis et al., PLOS One (2011) volume 6, e25583, published by the same group, reports that spPLA assays achieve levels of detection ranging from the low fM to the low pM, as shown in Supplementary Table 2, reproduced below.

| Protein name | LOD (pM)         |                      | LOD (pg/ml)      |                      |
|--------------|------------------|----------------------|------------------|----------------------|
|              | Multiplex SP-PLA | Sandwich immunoassay | Multiplex SP-PLA | Sandwich immunoassay |
| Artemin      | 0.054            | 4.335                | 1.264            | 104.059              |
| Cathepsin B  | 0.218            | 0.685                | 7.305            | 22.938               |
| Cathepsin S  | 1.584            | 0.525                | 50.197           | 20.937               |
| CCL2         | 0.048            | 0.443                | 0.414            | 1.851                |
| CCL4         | 0.025            | 0.259                | 0.195            | 1.839                |
| CCL5         | 0.275            | 0.455                | 2.140            | 3.553                |
| CD40 ligand  | 2.096            | 1.076                | 35.535           | 19.294               |
| CF3          | 0.381            | 0.684                | 9.382            | 19.186               |
| CXCL5        | 0.496            | 0.670                | 5.642            | 5.030                |
| Cystatin B   | 0.493            | 0.570                | 0.317            | 0.641                |
| Cystatin C   | 1.573            | 2.567                | 23.588           | 38.607               |
| E-selectin   | 0.007            | 0.040                | 0.420            | 2.359                |
| EGF          | 0.148            | 0.359                | 0.885            | 38.156               |
| Fas/TNFRSF6  | 0.064            | 0.106                | 0.383            | 29.337               |
| Follistatin  | 0.229            | 0.572                | 7.031            | 17.932               |
| GDF-15       | 0.025            | 0.185                | 0.639            | 4.684                |
| HCC40COL     |                  |                      |                  |                      |
| IG           | 0.231            | 0.591                | 2.540            | 6.489                |
| HGM          | 0.155            | 0.089                | 3.415            | 19.555               |
| ICAM         | 0.068            | 0.060                | 0.373            | 2.515                |
| IL-17A       | 6.788            | 8.661                | 126.295          | 159.240              |
| IL1 alpha    | 0.272            | 0.054                | 0.692            | 1.743                |
| IL16         | 1.641            | 49.589               | 22.971           | 834.117              |
| IL4          | 1.744            | 0.485                | 35.403           | 9.842                |
| IL6          | 0.072            | 0.095                | 1.220            | 1.809                |
| IL7          | 0.066            | 0.389                | 0.044            | 3.109                |
| IL8          | 0.017            | 0.281                | 0.302            | 1.827                |
| Kallikrein 6 | 1.543            | 0.833                | 42.713           | 21.671               |
| NGF beta     | 0.870            | 4.291                | 25.619           | 113.270              |
| p53          | 0.091            | 0.735                | 0.879            | 68.811               |
| PSA          | 0.047            | 0.285                | 2.495            | 15.182               |
| Prolectin    | 0.145            | 0.385                | 4.049            | 10.818               |
| TGF-1        | 0.238            | 0.609                | 0.007            | 12.786               |
| Timp4        | 0.222            | 0.793                | 4.935            | 17.438               |
| TNFA         | 2.082            | 0.452                | 52.356           | 7.903                |
| VEGF         | 0.009            | 0.427                | 0.824            | 11.961               |

**Supplementary Table 2. Detection limits for each protein included in the panel.** The limits of detection for each protein included in the panel for both multiplex SP-PLA and sandwich immunoassays are presented in pg/ml.

Darmanis tested four of the same analytes as reported in Applicant's specification, IL-10, IL-4, IL-6, and TNF- $\alpha$ . Significantly, Applicant's claimed method detected IL-10 at 5000-fold greater sensitivity than achieved by the spPLA assay (LOD of 300 aM vs. 1.6 pM) in Nong. Similarly, Applicants claimed method detected IL-4, IL-6, and TNF- $\alpha$  at 85,000-fold (20 aM vs. 1.7 pM), 4000-fold (16.7 aM vs. 72 fM), and 30,000-fold (95 aM vs. 3 pM) greater sensitivity than achieved by the spPLA assay in Nong.

The Office Action alleges that these significantly superior results do not evidence the non-obviousness of the claimed method because (i) the examples use PCR in the detection and PCR is not recited in the claims and (ii) regardless, it was known in the art that PCR allows for detection of much lower concentrations. Applicant respectfully disagrees because

each of the PLA assays described in Jalili, Persson, Lundberg, and Nong, having levels of detection orders of magnitude higher also include a PCR amplification step. That is, the results reported for the claimed detection method in Example 2 of the present application can be directly compared to the results reported for the Jalili, Persson, Lundberg, and Nong methods because every method includes a PCR amplification step.

Because the methods described in Jalili, Persson, Lundberg, and Nong all include a PCR amplification step, the data above necessarily evidences that the method recited in amended claim 286 provides a superior result in its ability to generate sufficient nucleic acid reports, which can be detected using PCR amplification, from a sample containing orders of magnitude less analyte. However, in an earnest attempt to expedite prosecution, and without acquiescing on the merits of the rejection, claim 319 is newly presented to recite that the detecting in step (9) comprises PCR amplification of the nucleic acid reporters in the pooled sample.

As described above, the combination of references cited in the Office Action fails to teach or suggest many of the steps of the amended claims, as well as many of the features of the reagents used in these steps. Even if the person of ordinary skill would have been motivated to combine these references—and Applicant believes they would not have for the reasons provided before—the combination of references would still not have arrived at the claimed methods. Furthermore, the methods recited in the amended claims provide 200 to nearly 100,000-fold greater sensitivity than the PLA assays described in the cited art, evidencing the non-obviousness nature of the claimed methods.

Accordingly, Applicant respectfully request that the above rejection be reconsidered and withdrawn.

**CONCLUSION**

Applicant respectfully requests the above amendment and remarks be entered and made of record in the above-identified patent application. In light of the amendments and remarks herein, Applicant submits that the claims are now in condition for allowance and respectfully requests a notice to this effect.

No fees are believed due in connection with the filing of this response. However, the Commissioner is hereby authorized to charge any required fee, or credit any overpayment, to Morgan, Lewis & Bockius LLP Deposit Account No. 50-0310 (File No. 133579-5001-US).

Please direct any calls in connection with this application to the undersigned at (415) 442-1000.

Prompt and favorable consideration of this Response is respectfully requested.

Respectfully submitted,

MORGAN, LEWIS & BOCKIUS LLP

Dated: January 26, 2023  
**MORGAN, LEWIS & BOCKIUS LLP**  
One Market, Spear Street Tower  
San Francisco, CA 94105  
Telephone: 415.442.1000  
Facsimile: 415.442.1001

/ Andrew J. Antczak /  
Andrew J. Antczak, Reg. No. 70,871  
*for*  
Brett Lovejoy, Reg. No. 42,813