

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Alamar Biosciences, Inc.
Petitioner

v.

Olink Proteomics AB
Patent Owner

U.S. Patent No. 7,883,848

DECLARATION OF NORMAN C. NELSON, Ph.D.
UNDER 37 C.F.R. § 1.68

TABLE OF CONTENTS

I.	Professional Background.....	1
II.	Summary Of Opinions.....	3
III.	Relevant Legal Standards	5
IV.	Person Of Ordinary Skill In The Art (“POSA”).....	7
V.	Technology Background.....	9
VI.	Description Of The ’848 Patent.....	15
	A. Background Section	15
	B. Detailed Description Section.....	19
	C. The Claims	24
VII.	Excerpts From The File History	25
VIII.	Claim Construction.....	31
IX.	Grounds 1 & 2: Claims 1-20 Of The ’848 Patent Are Unpatentable	32
	A. Overview Of The Prior Art	32
	1. Kanan	32
	2. Neri.....	36
	3. Famulok	38
	B. Claims 1-20 Are Unpatentable As Obvious Based On Kanan In View Of Neri (Ground 1) And In Further View Of Famulok (Ground 2)	39
	1. Independent Claim 1	43
	2. Dependent Claim 2	73
	3. Dependent Claim 3	74
	4. Dependent Claim 4	74
	5. Dependent Claim 5	75

TABLE OF CONTENTS

6.	Dependent Claim 6	76
7.	Dependent Claim 7	76
8.	Dependent Claim 8	77
9.	Dependent Claim 9	78
10.	Dependent Claim 10	82
11.	Dependent Claim 11	84
12.	Dependent Claim 12	87
13.	Dependent Claim 13	87
14.	Dependent Claim 14	88
15.	Dependent Claim 15	89
16.	Dependent Claim 16	90
17.	Dependent Claim 17	93
18.	Dependent Claim 18	95
19.	Dependent Claim 19	96
20.	Dependent Claim 20	97
C.	Kanan With Neri Is Not Cumulative To Substantively Considered Prior Art.....	98
X.	Grounds 3 & 4: 1-8, 11-12, And 14-15 Of The '848 Patent Are Unpatentable	103
A.	Overview Of Co-Pending Litigation.....	103
B.	Overview Of The Prior Art	108
1.	Baez.....	108
2.	Landegren.....	112
3.	Freskgard.....	115

TABLE OF CONTENTS

C.	Claims 1-8, 11-12, And 14-15 Are Unpatentable As Obvious Based On Baez In View Of Landegren (Ground 3) And In Further View Of Freskgard (Ground 4)	117
1.	Independent Claim 1	117
2.	Dependent Claim 2	130
3.	Dependent Claim 3	132
4.	Dependent Claim 4	132
5.	Dependent Claim 5	134
6.	Dependent Claim 6	135
7.	Dependent Claim 7	135
8.	Dependent Claim 8	136
9.	Dependent Claim 11	137
10.	Dependent Claim 12	138
11.	Dependent Claim 14	140
12.	Dependent Claim 15	143
XI.	Declaration.....	144

I, Norman C. Nelson, Ph.D., do hereby declare:

1. I am making this declaration at the request of counsel for Alamar Biosciences, Inc. (“Alamar”), in the matter of the *Inter Partes* Review of U.S. Patent No. 7,883,848 (“the ’848 Patent”).

2. I am being compensated for my work in this matter at an hourly rate of \$500.00 per hour. My compensation in no way depends on the outcome of this matter.

3. In preparing this Declaration, I considered the following materials:

- a) The ’848 Patent (Ex. 1001) and its file history (Ex. 1002);
- b) Materials cited in this Declaration.

I. Professional Background

4. My qualifications generally are set forth in my *curriculum vitae*, which is submitted as Exhibit 1006.

5. I obtained a Ph.D. in Chemistry, with a focus in Biochemistry, in 1982 from the University of California, San Diego. I also received a Bachelor of Science in Chemistry from the California Institute of Technology in 1976.

6. I have over 35 years of experience in molecular diagnostic and nucleic acid chemistry, particularly nucleic acid-based techniques. I am and was very knowledgeable about conventional techniques for attaching nucleic acids to other moieties like small molecules and proteins. I worked for Gen-Probe Inc. (“Gen-

Probe”) (now acquired by Hologic)—a leading molecular diagnostic company—for 27 years (June 1985 to August 2012). While at Gen-Probe, I co-invented multiple core technologies involving nucleic acids analysis, which are now used in commercial products approved by the U.S. Food and Drug Administration. I also played a key part in the commercialization of those products.

7. I started my career at Gen-Probe as a scientist (1985 to 2005), where I developed and implemented key nucleic acid-based technologies and assays, including nucleic acid capture/immobilization and labeling techniques, hybridization, amplification, and detection of nucleic acids. In 2005, I was promoted to Director of Biochemistry. In this role, I led a multidisciplinary team in the development of multiplexed nucleic acid-based assays. I was again promoted in 2009 to Senior Director of Discovery Research. As Senior Director, I focused on the development and commercialization of various nucleic acid-based diagnostic products.

8. I have been working as a consultant in the field of nucleic acid-based diagnostics, DNA sequencing, and genomics since 2012.

9. My research work has led to 65 issued U.S. patents and over 200 issued or pending patent applications worldwide.

10. I have co-authored over 20 peer-reviewed journal articles and given over 35 technical poster presentations in the field of nucleic acid-based diagnostics.

I have also chaired or served on the program committee of many conferences and symposia related to my field of work.

11. I have extensive experience in the field of protein analysis, including purification, enzymatic assay development and utilization, catalytic site mapping, fragmentation analysis, peptide separation and identification, amino acid analysis, and immuno-PCR as well as in the field of nucleic acid analysis, including purification, hybridization, amplification, detection, and sequencing—the technical field of the '848 Patent.

II. Summary Of Opinions

12. For the reasons discussed more specifically throughout this declaration, a person of ordinary skill at the time of the application would have viewed claims 1-15 and 17-20 of the '848 Patent as unpatentable over Kanan et al. (2004) (Kanan) (Ex. 1010) in view of U.S. Patent Application Publication No. 2004/0014090 (Neri) (Ex. 1011), and claim 16 of the '848 Patent as unpatentable over Kanan et al. (2004) (Kanan) in view of U.S. Patent Application Publication No. 2004/0014090 (Neri) and in further view of Famulok (2002) (Famulok) (Ex. 1012).

13. As explained below in paragraph 79, my opinions for Grounds 1 and 2 reflect the understanding of the claims of the '848 Patent in view of a person of ordinary skill in the art.

14. In addition, for the reasons discussed more specifically throughout this declaration, applying Patent Owner's positions taken in co-pending district court litigation with Alamar where Patent Owner alleges the '848 Patent's scope covers steps of proximity ligation (see Section X.A below), a person of ordinary skill at the time of the application would have viewed claims 1-8 and 11-12 of the '848 Patent as unpatentable over U.S. Patent No. 6,511,809 (Baez) (Ex. 1003) in view of PCT Publication No. WO01/61037 (Landegren) (Ex. 1004) and claims 14-15 as unpatentable over U.S. Patent No. 6,511,809 (Baez) in view of PCT Publication No. WO01/61037 (Landegren) in further view of PCT Publication WO 2005/003778 (Freskgard) (Ex. 1009).

15. As explained below in paragraph 230, my opinions for Grounds 3 and 4 are made by applying Patent Owner's positions taken in the district court litigation with Alamar. In the district court litigation, Patent Owner contends that the claims of the '848 Patent cover the fundamental aspects of proximity ligation, which is a method to detect analytes in a solution utilizing the affinity of antibodies, which are coupled to nucleic acid sequences, to an antigen. Ex. 1007; Ex. 1008. Thus, applying Patent Owner's positions, in my opinion, a person of ordinary skill would have found the proximity ligation art renders the '848 Patent claims obvious.

III. Relevant Legal Standards

16. I have been asked to provide my opinion as to whether claims 1-20 of the '848 Patent would have been obvious to a person of ordinary skill in the art at the time of the application in view of the prior art.

17. In this Declaration, I provide opinions on technical issues that involve the application of my knowledge, experience, and understandings from the perspective of a person of ordinary skill in the art, to the evaluation of certain prior art with respect to the '848 Patent. In addition, I understand that I must apply applicable patent law in this matter. The paragraphs below express my understanding of the current legal principles related to my analysis, as explained to me by Alamar's legal counsel.

18. I am advised that a claim is unpatentable if the claimed subject matter as a whole would have been known or obvious to a person of ordinary skill in the art at the time of the application. I also understand that an obviousness analysis takes into account the scope and content of the prior art, the differences between the claimed subject matter and the prior art, and the level of ordinary skill in the art at the time of the application.

19. In determining the scope and content of the prior art, it is my understanding that a reference is considered analogous prior art if it falls within the field of the inventor's endeavor. In addition, a reference is analogous prior art if it

is reasonably pertinent to the particular problem with which the inventor was involved. A reference is reasonably pertinent if it logically would have commended itself to an inventor's attention in considering his problem. If a reference relates to the same problem as the claimed invention, that supports use of the reference as prior art in an obviousness analysis.

20. I am advised that a patent claim is unpatentable for obviousness if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the alleged invention was made to a person of ordinary skill in the art to which the subject matter pertains. I am further advised that a patent claim is obvious if a person of ordinary skill in the art would have been motivated to combine the elements of the claim in view of the prior art and the state of the art, and would have had a reasonable expectation of success in doing so. I am advised that the law requires only a reasonable expectation of success and does not require absolute certainty of success.

21. I am advised that the combination of known elements according to known methods is likely to be obvious when it does no more than yield predictable results, and that any need or problem known in the art at the time of the application can provide a reason for combining elements. I am further advised that obviousness is assessed via a commonsense approach that is flexible, rather than adherent to rigid application of legal standards or tests.

22. It is my understanding that obviousness is viewed through the eyes of a person of ordinary skill in the art as of the time of the application. I am advised that, for purposes of this proceeding, I should consider only “prior art” in the form of patents and printed publications that were publicly available and were published before the filing date of the earliest application for the patent. Here, the application that issued as the ’848 Patent was filed on July 10, 2006, and the ’848 Patent issued on February 8, 2011. The ’848 Patent claims priority to U.S. Provisional Application No. 60/697,415, which was filed on July 8, 2005. For purposes of this declaration, I have been asked to assume that patents and printed publications that were published before July 8, 2005, constitute prior art to the ’848 Patent and may be considered as part of an obviousness analysis in this proceeding.

IV. Person Of Ordinary Skill In The Art (“POSA”)

23. As discussed above, it is my understanding that when interpreting the claims of the ’848 Patent, I must do so based on the perspective of one of ordinary skill in the art at the relevant priority date. I understand that a person of ordinary skill in the art is a hypothetical person who is presumed to be aware of all the pertinent art, thinks according to conventional wisdom in the art, and is a person of ordinary creativity. The person of ordinary skill in the art may work as part of a multidisciplinary team and draw not only upon his or her own skills, but also take advantage of specialized skills of other team members.

24. I am advised that in defining a person of ordinary skill in the art the following factors may be considered: (1) the educational level of the inventor; (2) the type of problems encountered in the art; (3) prior art solutions to those problems; (4) rapidity with which innovations are made; (5) sophistication of the technology; and (6) educational level of active workers in the field.

25. In my opinion, a person of ordinary skill in the art as of July 2005 would have (a) an advanced degree, such as a Ph.D., in chemistry, molecular biology, biochemistry, or a related field, and at least two years of experience in industry and/or laboratory experience relating to small molecules and proteins and would have been familiar with nucleic acid chemistry and conventional hybridization techniques; and (b) access to one or more team members having an advanced degree, such as a Ph.D., in chemistry, molecular biology, biochemistry, or a related field, and at least two years of experience in industry and/or laboratory experience relating to high throughput screening techniques and/or nucleic acid sequencing. A person could also have qualified as a person of ordinary skill in the art with less formal education and more technical or professional experience.

26. I more than meet this definition because I exceed the requisite education and experience. I provide my opinion, however, from the perspective of what a person of ordinary skill in the art would have understood at the time of the application.

V. Technology Background

27. To properly understand the issues raised by the '848 Patent, it is useful to review some general information about techniques for detecting and analyzing molecular interactions that existed at the time of the application.

28. The human body is complex with networks comprising trillions of molecular interactions and chemical reactions. The study of these interactions between various molecules—such as proteins, peptides, enzymes, nucleic acids, hormones, co-factors, antibodies, small molecules, lipids and carbohydrates—leads to a better understanding of these networks. These studies have resulted in improved health and well-being of people and have aided in reducing the progression of diseases as well as the development of treatments.

29. A highly used assay for the detection of an analyte in a sample is called an immunoassay. An immunoassay uses the specificity of an antigen-antibody molecular interaction to detect and quantify target molecules in a biological sample. Ex. 1016 (Hage 1999).

30. One of the earliest methods of immunoassays is called an enzyme-linked immunosorbent assay (ELISA) and was first developed in the 1970s. It uses the affinity properties of antibodies and the detection properties of enzyme/substrate systems to detect and quantify antigens/haptens (e.g., peptides, proteins, polysaccharides, lipids, steroids, and nucleic acids) and antibodies. For example,

Reen 1994 describes that “[b]ecause of the extreme discriminating power of antibodies to recognize an almost infinite array of antigenic structures, the application of ELISA to analyte measurement is almost unlimited.” Ex. 1017, 461. In the ELISA assay, one of the immunoreactants (antigen or antibody) is immobilized to the solid surface, such as a microtiter well, a magnetic particle, or a plastic bead. *Id.* This attachment facilitates the separation of bound and un-bound analytes. *Id.* In the most common approach to using the ELISA technique, an aliquot of sample containing the antigen (Ag) to be quantified is added to and allowed to bind with a solid-phase antibody (Ab). *Id.* After washing, an enzyme-labeled antibody is added and forms a “sandwich complex” of solid-phase Ab-Ag-Ab enzyme. *Id.* The unbound antibody is then washed away, and enzyme substrate is added. *Id.* The amount of product generated is proportional to the quantity of antigen in the sample.

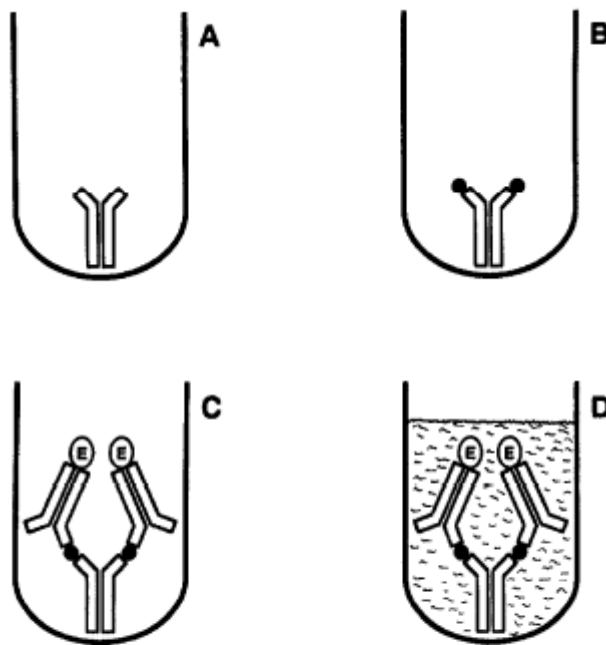


Fig. 1. Schematic representation of ELISA technique (noncompetitive assay). (A) Solid-phase adsorbed antibody on microwell plate. (B) Formation of antigen-antibody complex. (C) Detecting enzyme-labeled second antibody combining with "solid-phase" antigen "sandwich." (D) Addition of substrate results in color development.

Ex. 1017 (Reen), 463, Fig. 1.

31. In the 1990s, scientists built upon ELISA and developed immuno-PCR (IPCR), which combines the basic ELISA format with the nucleic acid amplification power of polymerase chain reaction (PCR) to improve the sensitivity of conventional antigen/antibody detection. Ex. 1018 (Sano 1992). With IPCR, a DNA label is attached to antigen/monoclonal antibody complexes that have been immobilized on microtiter plate wells. *Id.* This DNA label is then amplified by PCR, and the product thereof (amplicon) is detected, yielding a 100,000-fold increase in sensitivity compared to ELISA. *Id.*

32. Next, in the early 2000s, scientists developed new assay formats that did not require the solid support feature of the ELISA format. One such assay format was named “proximity ligation” assay (“PLA”). *See, e.g.*, Ex. 1019 (Gullberg 2004); Ex. 1020 (Fredriksson 2002); Ex. 1003 (Baez). Like IPCR, the assay described in Gullberg utilized antibodies coupled to nucleic acid sequences and relied on the affinity between antigens and antibodies. Ex. 1019. But unlike IPCR, the proximity ligation format described therein requires the binding of two different nucleic acid labeled antibodies to a single target. *Id.*

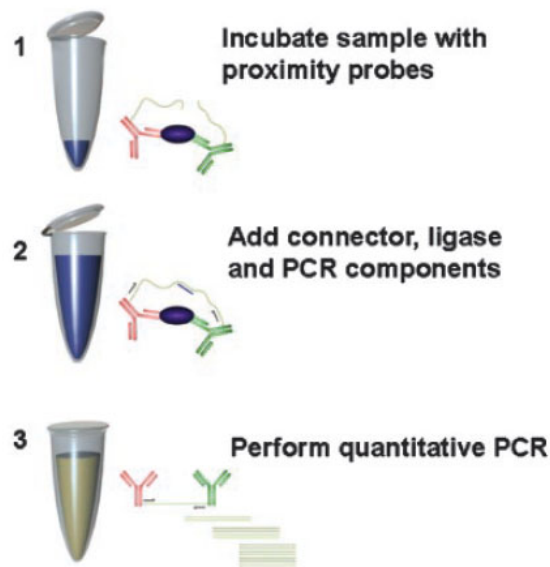


Fig. 1. The principal steps of the proximity ligation assay. Step 1, incubation of sample with proximity probe pair (≈ 1 h); step 2, addition of all components required for ligation and detection by quantitative PCR (≈ 5 min ligation time); step 3, quantitative PCR (≈ 2 h).

Id. at 8421, Fig. 1. The binding of the two antibodies to a target molecule brings the two nucleic acid labels into close proximity, thereby allowing them to be ligated

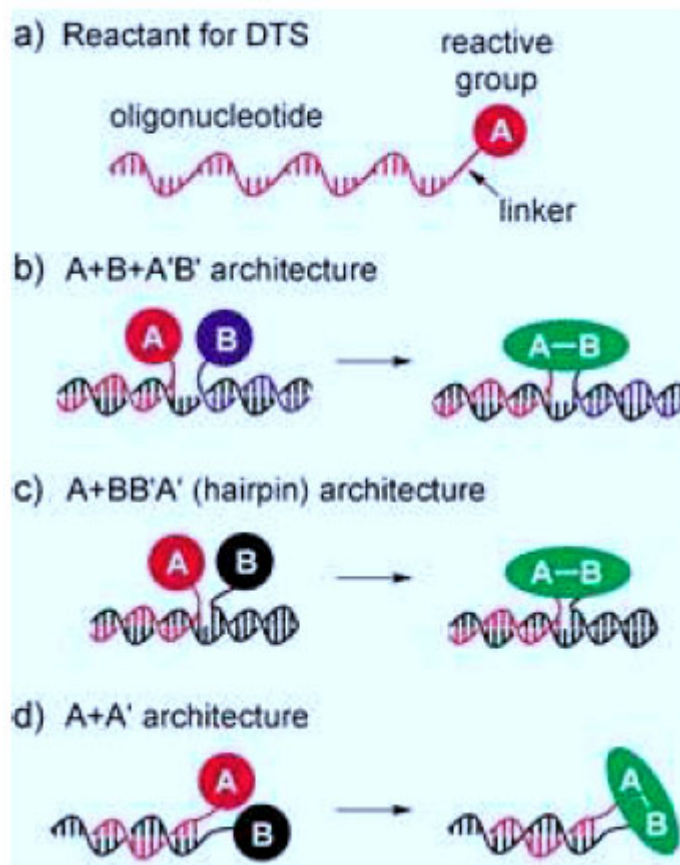
together. *Id.* This ligated product can then be amplified (e.g., via PCR) and detected. *Id.*

33. As with immunoassays that utilize high affinity molecular interactions for detection purposes, there are many other known methods to study the interactions between various molecules, such as nucleic acids, peptides, proteins, and other chemical moieties.

34. One such method, which was developed in the 1990s, is called encoded combinatorial chemistry. This process involves using combinatorial synthesis “to encode individual members of a large library of chemicals with unique nucleotide sequences” and allowing these library members to interact with a target. Ex. 1021 (Brenner 1992), 5381. “After the chemical entity is bound to a target, the genetic tag can be amplified by replication and utilized for enrichment of the bound molecules by serial hybridization to a subset of the library.” *Id.* “The nature of the chemical structure bound to the receptor [i.e., target] is decoded by sequencing the nucleotide tag.” *Id.* By combining the capacity for information storage in oligonucleotide code with the amplification of PCR and decoding of sequencing, this method provides a means for identifying each member in a vast library of molecules. *Id.*; Ex. 1022 (Needels 1993), 10700. This process is used to screen libraries of compounds to discover potential lead compounds in drug discovery.

35. The libraries created by encoded combinatorial chemistry were initially formed from nucleic acids and peptides. Ex. 1021, 5383; Ex. 1022, 10700. But its application is not limited to those entities and “is constrained only by the compatibility of the two alternating syntheses.” Ex. 1021, 5383. Indeed, researchers developed similar DNA-encoded libraries for proteins to study protein-protein interactions. Ex. 1023 (Pelletier 2001).

36. Another method known at the time of the application is DNA-templated synthesis (“DTS”). DTS uses a reactive group linked to DNA oligonucleotides that encodes the reactive group (e.g., small molecules) to create “reactants.” Ex. 1024 (Li 2004), 4850. When the reactants are combined, the “DTS reactants with complementary oligonucleotides undergo DNA hybridization” and “their reactive groups are confined to the same region in space.” *Id.* This confinement increases the effective concentration of the reactive groups and allows them to interact. *Id.* The DTS reactants are then selected for a desired property, amplified, and sequenced to identify desired compounds. *Id.* at 4866-67.



Ex. 1024, 4850, Fig. 3.

VI. Description Of The '848 Patent

37. The '848 Patent is titled "Regulation Analysis by Cis Reactivity, RACR." Ex. 1001, [54]. The abstract states: "Methods of detecting affinity interactions between at least two molecules of interest are provided[,]" and that "methods directed to detecting functional interactions . . . are also provided." Ex. 1001, Abstract.

A. Background Section

38. The Background of the '848 Patent criticizes existing prior art methods because such methods do not "permit high throughput screening and identification

of all partners of an interaction.” Ex. 1001, 1:29-31. As an example, it states that microarray analysis is limited because it is “performed on a solid phase and interaction studies are limited by the number of resolvable detectable functions which also limits the possibility of inter- and intra-library interaction screens.” Ex. 1001, 2:26-29.

39. The Background distinguishes its alleged invention from the prior art DTS method stating, the alleged invention “utilize[es] . . . combinatorial association of different nucleic acid tags instead of pre-synthesis of all different combinations[.]” *Id.* at 4:16-18. The Background further distinguishes its alleged invention from DTS:

- “[T]he present invention provides a method whereby the nucleic acid encoding the tags to identify both members of the interaction are formed upon joining of the two molecules.” Ex. 1001, 3:61-64.
- Unlike DTS where “the number of ‘template’ oligonucleotides which have to be synthesized are in the range of $n^2/2$, since one specific oligonucleotide has to be synthesized for each individual interaction on the library,” with the present invention “the number oligonucleotides required is in the order of n .” *Id.* at 3:55-66.

- “In the current invention the nucleic acid formed by the NAM association encodes the combination of identification sequences. Therefore the link between the identification sequences in the associated nucleic acid molecule and the phenotype of the molecule is more effectively maintained.” *Id.* at 4:11-15.

40. The Background also goes to great lengths to emphasize that “[t]he current inventive method differs from proximity ligation in that it does not detect or quantify a predefined target in a sample.” Ex. 1001, 4:48-50. The Background further distinguishes its alleged invention from PLA and detection of a known target in several ways:

- “The present inventive methods do not utilize molecules with predefined affinities but rather the reverse; the inter-molecular affinities are retrieved as a result of the inventive method.” Ex. 1001, 4:50-53.
- “In the current invention the association of the nucleic acid is combinatorial and the novel nucleic acid produced by this association is then identified to yield information concerning the molecular interactions in the library. This enables interrogation of all intra- or inter-library member interactions. The proximity ligation methods[, however,] always include at least one

molecule which is not attached to a nucleic acid label and this ‘target’ is the molecule which is detected. Thus the co-localization/proximity of the binders and thereby the nucleic acid in the assay arises from the presence of a target molecule.” *Id.* at 4:54-63.

- “Finally, the information gained from the inventions differs significantly. In the present inventive methods, combinations of interacting molecules may be detected and quantified. The proximity ligation art, on the other hand, teaches high throughput screening of inhibitors for a defined binding event.” *Id.* at 4:64-5:2.

41. The Background states that “[t]here is a need in the art for methods capable of investigating affinity and/or functional interactions within libraries of molecules wherein such interactions are not previously established to exist[.]” *Id.* at 5:14-17.

42. The alleged invention disclosed in the ’848 Patent purports to allow “investigation of all possible interactions within or between libraries of molecules, such as proteins, and also permits relative quantification of the detected interactions.” Ex. 1001, 5:26-28.

B. Detailed Description Section

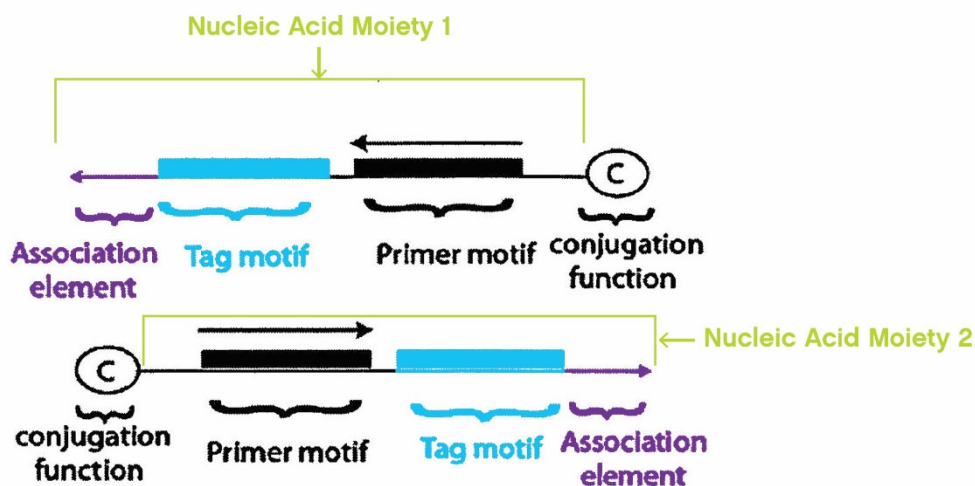
43. The Detailed Description section of the '848 Patent describes the creation of the library of “interactors,” which bind to form “associated oligonucleotides,” and then the selection, amplification, and detection of functional interactions between molecules of interest. *E.g.*, Ex. 1001, Fig. 2. The alleged invention “aims to retrieve information about molecule interactions” by “allow[ing] combinatorial analysis of all potential interactions in a library[.]” Ex. 1001, 9:63-64, 10:34-36.

44. The interactors “comprise at least two distinct parts which are coupled, one part is the molecule of interest (MOI) and one part is the nucleic acid moiety (NAM).” Ex. 1001, 10:45-48.

45. The molecule of interest (MOI) “refers to a molecule about which interactive information is desired.” Ex. 1001, 8:53-55. “Examples of suitable MOIs include, but are not limited to, whole proteins, protein domains, peptides, peptoids, functional enzymes, low molecular weight compounds, small molecules, polymers, non-proteinaceous cellular products, virus particles, cells metabolites, lipids, carbohydrates, nucleic acids, inorganic compounds or combinations thereof.” Ex. 1001, 8:57-63. In addition, each “MOI is coupled with at least one NAM.” Ex. 1001, 8:55.

46. The nucleic acid moiety (NAM) “refers to a nucleic acid molecule which is coupled to at least one molecule of interest (MOI)” and “comprises at least one unique identification nucleic acid sequence [i.e., the identification element] so that detection of that sequence infers involvement of the coupled MOI.” Ex. 1001, 8:41-45, claim 1. The “identification element,” also called the “tag motif,” allows for the downstream detection of the NAM. Ex. 1001, 5:60-61, Fig. 4.

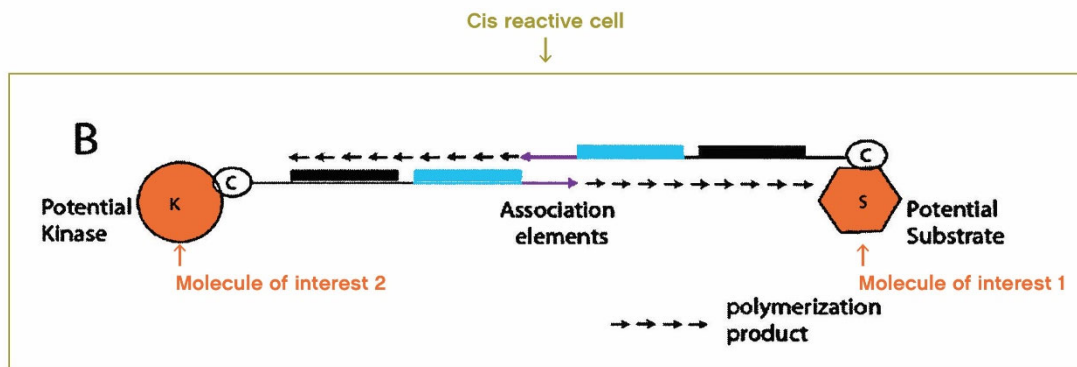
47. “A NAM also comprises at least one element which enables association with other NAMs[,]” i.e., the association element. *Id.* at 8:45-47, claim 1. The “association element” enables “association of the NAMs[,]” “which results in a molecule containing nucleic acid sequences originating from the associated NAM molecules.” *Id.* at 9:44-47. As shown below, an example NAM used “for detecting a functional interaction” comprises a PCR primer, identification element, and an association element. *Id.* at 7:27-40, Fig 4.



Ex. 1001, Fig 4 (annotated and color added).

48. In the disclosed method, a “plurality of interactors [is formed] by coupling each molecule of interest with a nucleic acid moiety” Ex. 1001, 6:6-11; *see also id.* at 11:15-17, Fig. 4.

49. The plurality of interactors is then “permitted to interact and equilibrate, creating a secondary library of ‘cis reactive cells’ (CRCs) wherein each CRC consists of MOI molecules brought into proximity by the affinity between their respective NAMs, and co-localized by the association of their respective NAMs.” Ex. 1001, 6:11-17, 6:28-34, 10:44-48.



Ex. 1001, Fig 4 (annotated and color added). As shown in the figure, the two nucleic acids hybridize due to the complementary association elements. Ex. 1001, 18:41-46. In addition, “[e]ach CRC is joined by addition of a polymerase that stabilizes the hybridization of the respective 3’ ends and incorporates the nucleic acid tags and PCR primers into one associated oligonucleotide by polymerization.” *Id.* at 7:35-40. The creation of CRCs through the “[a]ssociation of interactors is a key feature of the current invention The binding of the interactors by their respective NAM association elements forms novel molecules, herein referred to as associated oligonucleotides, comprised of the nucleic acid tags Analysis of the associated oligonucleotide molecule formed from the combination of these tags permits identification of the interactor MOI components.” *Id.* at 16:63-17:6.

50. “Once the CRC library is constructed the functional interaction being investigated is stimulated. This can be accomplished by, for example, addition of a substrate or a shift in reaction conditions. The MOIs in the CRC will primarily act on each other in cis since they are physically linked, but could potentially act in trans

with MOIs in other CRCs.” Ex. 1001, 16:14-19. The ’848 Patent describes a functional interaction as an interaction “where two or more members affect each other in some detectable way[.]” Ex. 1001, 14:23-28.

51. Next, “the CRCs which display functional interactions must be selected for detection and/or analysis. . . . Nonlimiting examples of filters [i.e., ways to selectively separate the desired CRCs] for functional interactions include: affinity purification of the form which has undergone alterations in the functional interaction; detection of the CRCs by labelling with affinity reagents which detect interactors which are included in the functional interaction; and introduction of a modified molecule which becomes incorporated by the functional interaction where the modification permits selective detection.” Ex. 1001, 19:41-55.

52. Once selected, the nucleic acid sequences of the NAM combinations are then analyzed, for example, using sequencing-based approaches. Ex. 1001, 20:49-52, 23:66-67. “To increase the sensitivity and speed up the analysis, the associated oligonucleotides can be amplified by conventional nucleic acid amplification techniques such as, for example, polymerase chain reaction (PCR), rolling circle amplification (RCA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), RNA transcription or invader assay.” Ex. 1001, 21:6-12.

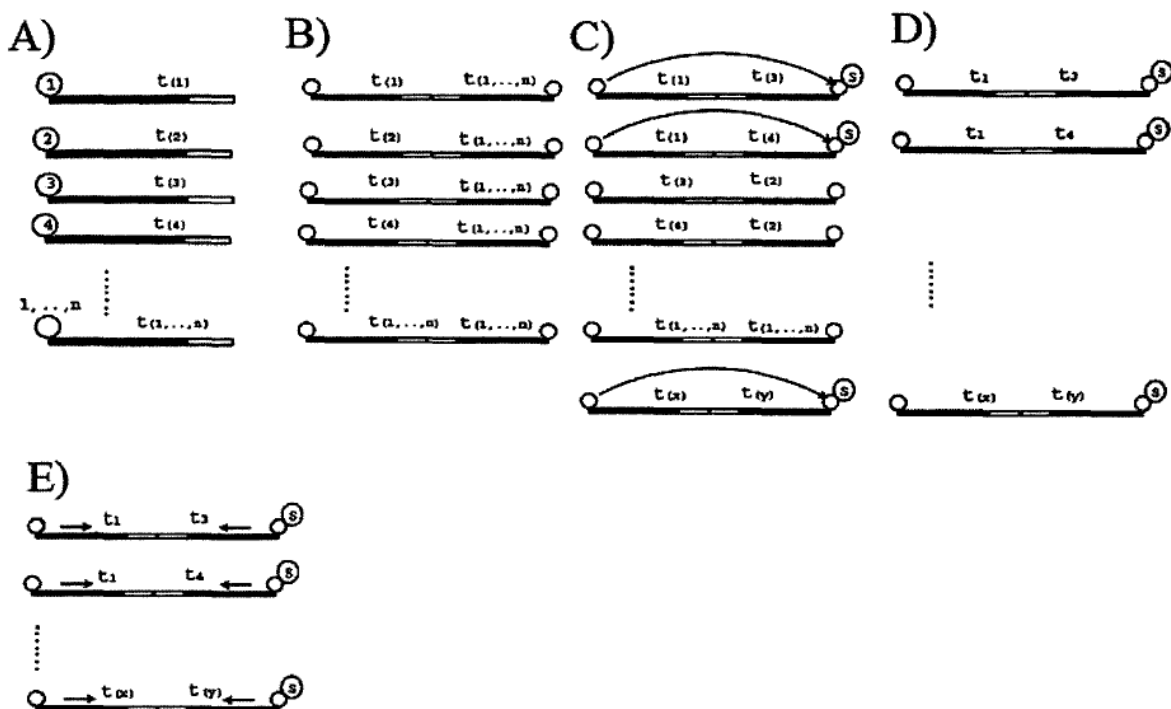
C. The Claims

53. The '848 Patent has 20 claims, of which claim 1 is independent. Claim 1 is directed to a method of detecting functional interactions between at least two molecules of interest, the method comprising:

- a. forming a plurality of interactors by coupling each molecule of interest with at least one nucleic acid moiety, the nucleic acid moiety comprising an identification sequence element and an association element;
- b. forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors bound in proximity to one another by an associated oligonucleotide formed from the association between at least two nucleic acid moieties, wherein the associated oligonucleotides comprise at least two identification elements derived from the at least two nucleic acid moieties;
- c. subjecting the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction having a detectable trace;
- d. selecting all cis-reactive cells exhibiting the detectable trace; and
- e. subjecting the associated oligonucleotides from the cis-reactive cells selected in step (d) to an analysis that permits detection of the at least two identification sequence elements.

Ex. 1001, claim 1. Each of these steps are shown in Figure 2:

Figure 2



Ex. 1001, Fig. 2.

VII. Excerpts From The File History

54. The provisional application was filed with 28 claims relating to a method to detect affinity interactions between molecules (claims 1-14) and a method to detect functional interactions between molecules (claims 15-28). Ex. 1034. The non-provisional application leading to the '848 Patent was originally filed with 43 claims—certain claims related to detecting affinity interactions (claims 1-19), others related to detecting functional interactions (claims 20-37), and additional claims related to compositions for a library of interactors (claims 38-40) and kits for assaying interactions (claims 41-43). Ex. 1002, 63-72.

55. The “affinity interaction” claims are different than the “functional interaction” claims for several reasons, but at least because the “affinity interaction” claims require “promoting an association between at least two nucleic acid moieties from different interactors to form a plurality of unique associated oligonucleotides” (Ex. 1002, 284, Cl. 1). Whereas, the “functional interaction” claims require “forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors *bound in proximity* to one another *by an associated oligonucleotide* formed from the association between at least two nucleic acid moieties” (*id.* at 288, Cl. 20) (emphases added). Thus, a key difference between the types of claims is that the functional interaction claims require the cis-reactive cell to be formed *from* the binding of two interactors to each other by an associated oligonucleotide. *See id.* at 360-363. The affinity interaction claims do not include this requirement.

56. On December 30, 2009, the pending claims received an Office Action rejecting claims 3 and 21 under 35 U.S.C. § 112 for the term “enhancement” being indefinite and rejecting the “affinity interaction” claims (claims 1-12, 15-17, and 19) under 35 U.S.C. §§ 102 and 103. Ex. 1002, 162-173.

57. Claims 1-12, 15-17, and 19 were rejected under 35 U.S.C. § 102(a) as being anticipated by WO 2005/026387 A1 (“Thisted”) (Ex. 1035). Ex. 1002, 164. The Examiner found that Thisted (Ex. 1035), which teaches “a method for identifying from a library a display molecule having affinity towards one or more

molecular targets[.]” anticipated the claims directed toward “a method of detecting affinity interactions between at least two molecules of interest.” Ex. 1002, 164. Claims 13 and 14 were rejected under 35 U.S.C. § 103(a) as being obvious over Thisted in view of Guille 1997 (Ex. 1025). The Examiner found Guille teaches applying the method of Thisted to detecting and quantifying the affinity between a protein and a nucleic acid. Ex. 1002, 170-171. Claim 18 was rejected under 35 U.S.C. § 103(a) as being obvious over Thisted in view of U.S. Patent No. 6,692,918 (“Kurn”) (Ex. 1026). Ex. 1002, 171. The Examiner found Kurn teaches analyzing the method of Thisted using sequence by synthesis. Ex. 1002, 171-172.

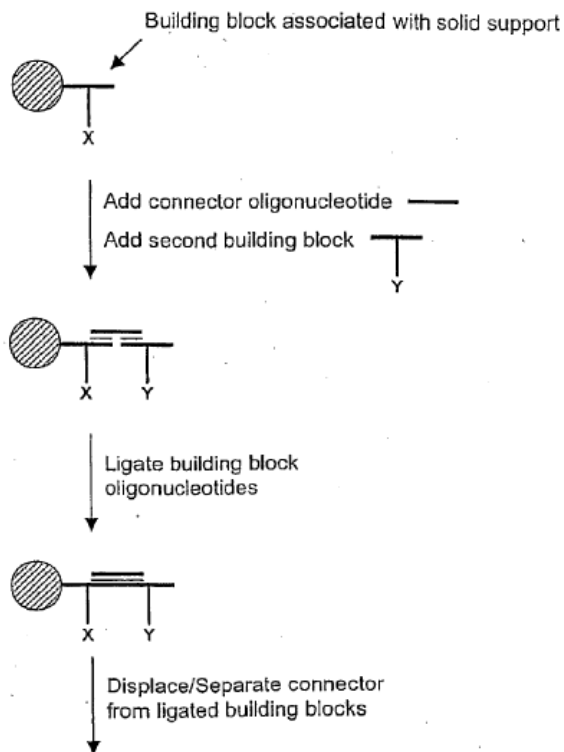
58. The Office Action allowed claims 20 and 22-37, which are the claims directed to detecting “functional interactions.” Ex. 1002, 172, 362. The Examiner stated that “US 2009/0011957 [“Goulaev” (Ex. 1036)] or US 2006/0246450 [“Franch” (Ex. 1037)]” is “[t]he closest prior art” to the functional interaction claims. Ex. 1002, 172. The Examiner found that these references “teach the aspects of steps a-c of claim 20 [’848 Patent claim 1] except for the limitation that the desired interaction produces a detectable trace[.]” Ex. 1002, 172.

59. Goulaev “relates to a method for synthesizing a bifunctional complex comprising a molecule and an identifier polynucleotide identifying at least some of the chemical entities which have participated in the synthesis of the molecule[.]” Ex. 1036, Abstract. Goulaev synthesizes the bifunctional complex by using

“building blocks” linked to chemical entities that are “brought into reactive proximity by sequential or simultaneous hybridisation of building, [sic] block identifier nucleotides to connector oligonucleotides[.]” *Id.* at [0014].

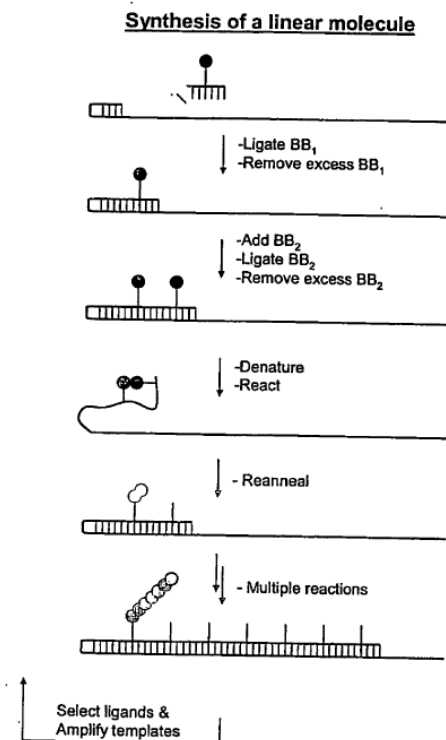
“Neighbouring building block anti-codons can be ligated by chemical ligation or by enzymatic ligation, thereby covalently linking said neighbouring building block anti-codons[.]” “generating an identifier polynucleotide.” *Id.* at [0024], [0081] The “chemical entities” “attached to the identifier polynucleotide are reacted, and the reaction product . . . is formed [i.e., the bifunctional complex].” *Id.* at [0061], Cl. 1.

Figure 1A



Ex. 1036, Fig. 1A.

60. Like Goulaev, Franch “relates to a method for synthesizing a bifunctional complex comprising an encoded molecule and a template coding for chemical entities which have participated in the synthesis of the encoded molecule.” Ex. 1037, [0002]. Franch teaches “building blocks each comprising an anti-codon associated with one or more chemical entities” and a “template comprising one or more codons capable of hybridizing to an anti-codon[.]” *Id.* at [0026]-[0027]. The building block hybridizes to the template “generating an identifier polynucleotide[.]” *Id.* at [0028]-[0029]. The “chemical entities associated with the identifier polynucleotide” are then reacted, generating “an encoded molecule.” *Id.* at [0031]-[0032].

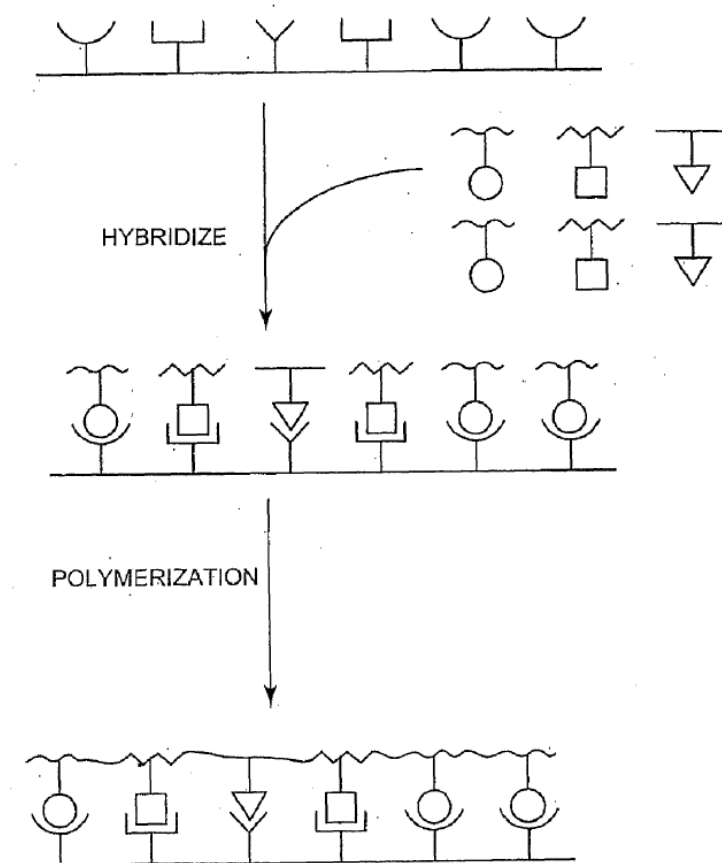


Ex. 1037, Fig. 1.

61. On June 29, 2010, in response to the Office Action, Applicant canceled claims 1-19 and 38-43. Applicant also submitted an amendment to claim 21 to replace “enhancement” with “selective separation or selective detection of the cis-reactive cells” to traverse the indefiniteness rejection and added two new dependent claims. Ex. 1002, 337-341. Applicant stated that “[b]oth selective separation and selective detection are techniques well known in the art. . . .” Ex. 1002, 505.

62. On September 17, 2010, the Examiner issued a Notice of Allowance. Ex. 1002 at 360-363. There, the Examiner stated that the prior art “did not teach or suggest the formation of an ‘associated oligonucleotide’ comprising the nucleic acid moieties attached to two interacting molecules . . . or analyzing such an ‘associated oligonucleotide.’” *Id.* at 361. The Examiner also stated that Liu (Ex. 1038) was a close prior art reference but that Liu’s “template is not ‘formed from the association between at least two nucleic acid moieties [of at least two interactors]’ and so cannot be considered as the claimed ‘associated oligonucleotide.’” *Id.* Liu relates to “polymer synthesis, and more specifically relate[s] to evolutionary polymer synthesis by nucleic acid-mediated chemistry.” Ex. 1038, [0002]. To create the polymers, Liu relies on a “nucleic acid template” that “typically comprises a plurality of coding regions which anneal to complementary anti-codon sequences associated with reactive units, thereby bringing the reactive units together in a

sequence-specific manner to create a reaction product [(i.e., polymer)].” *Id.* at [0027].



Ex. 1038, Fig. 1.

VIII. Claim Construction

63. I understand that the issue as to whether a claim is unpatentable is decided after first interpreting the meaning of the wording and terminology found in the claim. I understand that the interpretation should be made from the perspective of a person of ordinary skill in the art, at the time of the application.

64. For my analysis, I have reviewed and considered the '848 Patent's specification, claims, and prosecution history, along with various pieces of relevant extrinsic evidence. Based on this evidence, I have applied these terms as they would have been understood by a POSA as of the time of the '848 Patent's earliest priority date.

65. For my analysis in Grounds 1 and 2, it is my opinion that the claim terms of the '848 Patent do not require construction beyond their ordinary and customary meaning at this time.

66. For my analysis in Grounds 3 and 4, the scope of the '848 Patent is informed by Patent Owner's position on the scope of the '848 Patent as applied in its complaint, claim chart, and opposition to Alamar's motion to dismiss in the co-pending litigation with Alamar (Ex. 1007; Ex. 1008; Ex. 1015). For purposes of my analysis in Grounds 3 and 4, I presume that a POSA at the time of the application would apply the claims consistent with Patent Owner's positions in the district court litigation.

IX. Grounds 1 & 2: Claims 1-20 Of The '848 Patent Are Unpatentable

A. Overview Of The Prior Art

1. Kanan

67. Matthew W. Kanan et al., "Reaction discovery enabled by DNA-templated synthesis and in vitro selection," (Ex. 1010) ("Kanan") was published on September 30, 2004, in Volume 431 in the journal *Nature*. *Nature* is one of the

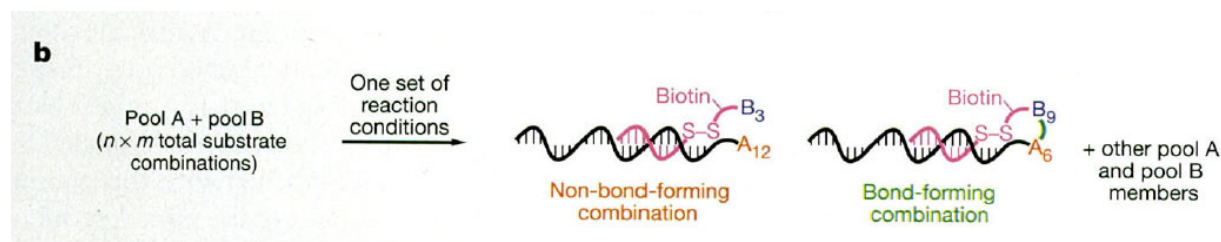
leading journals in the field of multidisciplinary sciences and would have been available and widely distributed to scientists with backgrounds in chemistry, molecular biology, or biochemistry interested in nucleic-acid chemistry. I am informed that Kanan qualifies as prior art under at least pre-AIA 35 U.S.C. § 102(a) because it was published before the earliest possible priority date for the '848 Patent.

68. Kanan is discussed in the '848 Patent in columns 3 and 14. Ex. 1001, 3:47-60, 14:6-12. During prosecution, the Examiner did not apply Kanan against the pending claims.

69. Kanan teaches “a reaction discovery approach that uses DNA-templated organic synthesis and *in vitro* selection to simultaneously evaluate many combinations of different substrates for bond-forming reactions in a single solution.” Ex. 1010, 545. The substrates in Kanan are linked to DNA with “ n substrates in pool A and m substrates in pool B. Each substrate in pool A is covalently linked to the 5' end of a set of DNA oligonucleotides containing one ‘coding region’ (uniquely identifying that substrate) and one of m different ‘annealing regions’ (Fig. 1a). Each of the m substrates in pool B is attached to the 3' end of an oligonucleotide containing a coding region that uniquely identifies the substrate and complements one of the m annealing regions in pool A.” *Id.* The pool B oligonucleotides, unlike the pool A oligonucleotides, do not contain a separate coding region and an annealing region. Rather it has only one region, called the “coding region” that

anneals to a complementary code-specific annealing region of the pool A oligonucleotides. *See* Ex. 1010, Fig. 1.a.

70. “When pools A and B are combined in a single aqueous solution at nanomolar concentrations, Watson-Crick base pairing organizes the mixture into $n \times m$ discrete pairs of substrates attached to complementary sequences.” Ex. 1010, 545.



Ex. 1010, 546, Fig. 1.b. “Only substrates linked to complementary oligonucleotides experience effective molarities in the millimolar range,” and “[t]his effective molarity-based design enabled reactions that might otherwise be suppressed by the preferential dimerization of one or both substrates.” Ex. 1010, 545.

71. Kanan also discloses that members of pool B are linked to biotin that aids in the selection of the bond-forming pairs. *Id.* More specifically, Kanan teaches that “[a]fter incubation under a set of chosen reaction conditions, followed by cleavage of the disulphide bonds, only pool A sequences encoding bond formation between a pool A and pool B substrate remain covalently linked to biotin. Streptavidin affinity selection of the resulting solution separates biotinylated from non-biotinylated sequences.” *Id.* After selection, the “[r]eactive substrate pairs are

identified by amplification of the selected sequences by polymerase chain reaction (PCR) followed by DNA microarray analysis[.]” *Id.* In sum, Kanan discloses a method to “organize complex substrate mixtures into discrete pairs,” “separat[e] reactive substrate pairs from unreactive pairs,” and “identify all substrate pairs capable of forming a covalent bond under the reaction conditions.” *Id.*

72. The ’848 Patent distinguished Kanan because “one of the molecules forced together is attached to a ‘template’ nucleic acid which specifically directs the second compound by direct hybridization of the nucleic acid attached to the second molecule” wherein “the ‘template’ nucleic acid contains both tags used to identify the [two] compounds which are forced together.” Ex. 1001, 3:46-4:10. The ’848 Patent further contrasts Kanan because “the present invention provides a method whereby the nucleic acid encoding the tags to identify both members of the interaction are formed upon joining of the two molecules[.]” and because the present invention “utilize[es] . . . combinatorial association of different nucleic acid tags instead of pre-synthesis of all different combinations[.]” Ex. 1001, 3:61-64, 4:16-18.

73. Kanan’s Supplementary Information contains 20 pages of additional material related to the experiments, including materials and studies referenced, but not described in detail, in Kanan. Ex. 1013. Kanan’s Supplementary Information was not disclosed to or considered by the Examiner during prosecution and was not

applied by the Examiner against the pending claims. *See* Ex. 1002. And the '848 Patent's incorporation by reference of Kanan was specific to pages 545-549 of "Kanan et. al Nature 431, 545-9," which had been submitted during prosecution but without the Supplementary Information. Ex. 1002, 213-218. Kanan's Supplementary Information provides crucial details relating to Kanan—the goals the authors of Kanan were trying to achieve, the process used by the authors to achieve those goals, and the issues faced by the authors in achieving those goals. Unlike Kanan itself, Kanan's Supplementary Information provides a detailed description on the process used to create pool A and pool B substrates. Ex. 1013, 2-7. Specifically, it details the complex six-step process to create pool A substrates and the two-step process to create pool B substrates. Ex. 1013, 2-7. The Supplementary Information also provides missing details related to selecting bond-forming substrates and analyzing the selected substrates (e.g., microarray analysis). Ex. 1013, 7-9. If a POSA was evaluating the experiments described in Kanan, a POSA would have relied heavily on Kanan's Supplementary Information relating to the preparation of the pools, selection of the bond-forming substrates, and analysis of the selections.

2. Neri

74. U.S. Patent Application Publication No. 2004/0014090 ("Neri") is titled "Encoded Self-Assembling Chemical Libraries (ESACHEL)" (Ex. 1011). Neri published on January 22, 2004. I am informed that Neri qualifies as prior art

under at least pre-AIA 35 U.S.C. § 102(b) because it was published more than one year before the earliest possible priority date for the '848 Patent. During prosecution, Neri was not disclosed, and the Examiner did not consider or apply Neri against the pending claims.

75. Neri teaches “self-assembling libraries of organic molecules (typically forming dimers, trimers or tetramers), in which the organic molecules are linked to an oligonucleotide which mediates the self-assembly of the library and/or provides a code associated to each binding moiety.” Ex. 1011, [0005]. The “self-assembly sequence (b1)” is “capable of performing a combination reaction with at least one self-assembly sequence (b1’) of a complementary oligonucleotide . . . bound to another chemical compound comprising a chemical moiety.” *Id.* at [0016]. Neri relates to “chemical compounds, comprising an oligonucleotidic moiety (typically, a DNA sequence) linked to an oligomerization domain [capable of mediating the (homo- or hetero-) dimerization, trimerization or tetramerization of the chemical compounds], linked to a chemical entity, which may be involved in a specific binding interaction with a target molecule (FIG. 2). Part of the sequences of the oligonucleotidic moiety will be uniquely associated with the chemical entity (therefore acting as a ‘code’).” Ex. 1011, [0040] (brackets in original). In other words, each oligonucleotidic moiety includes discrete identification sequences separate from the self-assembly sequence. Once self-assembled, “[t]he resulting

DNA segment, carrying both code A and code B, can be amplified (typically by PCR), using primers which hybridize at the constant extremities of the DNA segment.” *Id.* at [0030].

76. Neri also discloses that “[a]fter the capture of the desired binding specificities on the target of interest, the ‘binding code’ can be ‘decoded’ by a number of experimental techniques (e.g., hybridization on DNA chips or by a modified polymerase chain reaction (PCR) technique followed by sequencing).” Ex. 1011, [0005].

3. Famulok

77. Michael Famulok, “Bringing picomolar protein detection into proximity: Combining target recognition by two aptamers, enzymatic ligation, and PCR, the proximity ligation method enables the detection of minute amounts of proteins” (Ex. 1012) (“Famulok”) was published in May 2002 in Volume 20 in the journal *Nature Biotechnology*. I am informed that Famulok qualifies as prior art under at least pre-AIA 35 U.S.C. § 102(b) because it was published more than one year before the earliest possible priority date for the ’848 Patent. During prosecution, Famulok was not disclosed, and the Examiner did not consider or apply Famulok against the pending claims.

78. Famulok teaches detecting an antigen “by nucleic acid-based receptor molecules, so-called aptamers.” Ex. 1012, 448. “Each aptamer has a different DNA-

sequence extension that does not interfere with its folding and is not required for target recognition.” *Id.* The “[b]inding of the aptamer pair brings the ends of the oligonucleotide extensions into close spatial proximity . . . so that a ‘splint’ oligonucleotide can hybridize to both ends, which are subsequently ligated together by T4 DNA ligase.” *Id.* “The ligated species can then act as a PCR template and the amplified PCR product can be monitored and quantified under real-time PCR conditions[.]” *Id.*

B. Claims 1-20 Are Unpatentable As Obvious Based On Kanan In View Of Neri (Ground 1) And In Further View Of Famulok (Ground 2)

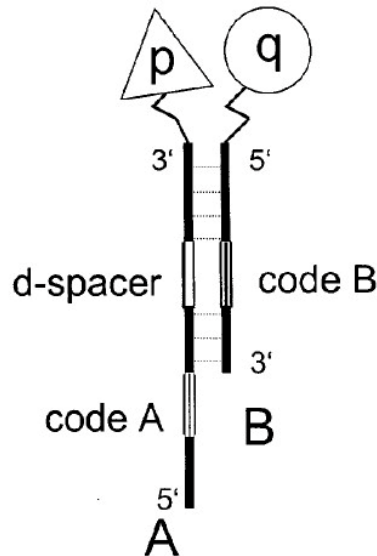
79. My opinions for Grounds 1 and 2, as detailed below, are based on what a POSA would have understood regarding the scope of the claims of the ’848 Patent. As such, my opinions for Grounds 1 and 2 do not apply Patent Owner’s positions taken in the district court litigation with Alamar. *See* Section X.A below. Instead, it is my opinion that a person of ordinary skill in the art would have understood that the specification of the ’848 Patent clearly distinguished the alleged invention claimed in the ’848 Patent from proximity ligation such that the alleged invention claimed in the ’848 Patent was intended to discover new, unknown interactions between molecules and not detecting a known analyte. And as discussed below, the alleged invention in the ’848 Patent was not new but rather an obvious combination of known methods.

80. For my opinions on Grounds 1 and 2, I rely on Kanan, which was disclosed during prosecution and discussed in the '848 Patent's specification (e.g., Ex. 1001, 3:46-53), and Neri, which was not disclosed during prosecution. In the '848 Patent, Patent Owner distinguished the alleged invention claimed in the '848 Patent from Kanan by describing that in Kanan, "one of the molecules forced together is attached to a 'template' nucleic acid which specifically directs the second compound by direct hybridization of the nucleic acid attached to the second molecule. Thus, the 'template' nucleic acid contains both tags used to identify the compounds which are forced together." Ex. 1001, 3:46-4:10. The '848 Patent further explains that Kanan's approach requires "the number of 'template' oligonucleotides which have to be synthesized are in the range of $n^2/2$ " in order to "create interactions between all members of a library with n members." Ex. 1001, 3:55-60. This is because with Kanan's template approach, the annealing region does not promote self-assembly between all members of the library and instead codes for each interaction with a molecule, which requires "one specific oligonucleotide . . . to be synthesized for each individual interaction on the library." Ex. 1001, 3:55-60. In other words, Kanan does not teach a common association element (i.e., self-assembly sequence) and only one of the two interactors having a nucleic acid moiety that contains both a discrete association element and a discrete identification sequence element.

81. The '848 Patent states that the alleged invention improves upon Kanan because the use of “combinatorial association” allows all library members to interact and allows “the number of oligonucleotides required [to be synthesized to be] in the order of n ” for a library of n members. Ex. 1001, 3:54-4:1. The '848 Patent also states it improves upon Kanan because the use of two discrete identification sequences “provides a method whereby the nucleic acid encod[es] . . . tags to identify both members of the interaction” (i.e., one identification sequence element on each interactor). Ex. 1001, 3:46-66. In the alleged invention, “the NAM association encodes the combination of identification sequences.” Ex. 1001, 4:11-13.

82. While Kanan does not teach self-assembly of all library members or the use of discrete identification sequences separate from the association region on each interactor, it would have been obvious to a POSA to create interactors with a self-assembly sequence and separate identification sequence elements in view of Neri, a reference not considered during prosecution. As detailed below, a POSA would have been motivated to combine Kanan with Neri as Neri addresses ways to improve the method of Kanan, such as a way to reduce the time required to synthesize the library members (substrates) of Kanan, a way to improve specificity of the binding between pool members in Kanan, and a way to more efficiently identify the bond-forming combinations in Kanan.

83. Neri discloses a method whereby the members self-assemble and the nucleic acid encodes separate tags to identify both members of the interaction.



Ex. 1011, Fig. 5. First, Neri discloses a self-assembly sequence; as shown above, the 3' ends of the oligonucleotides of sub-library A have a “DNA sequence [that] is designed to hybridize to the DNA sequences at the 5' extremity of oligonucleotides of sub-library B.” Ex. 1011, [0029]. Second, Neri discloses two discrete identification sequences; as shown above, the moiety p is attached to a nucleic acid sequence that has a unique sequence (Code A) that codes for “p,” and the moiety q is attached to a nucleic acid sequence that has a unique sequence (Code B) that codes for “q.” *Id.* Once self-assembled, “[t]he resulting DNA segment, carrying both code A and code B, can be amplified (typically by PCR), using primers which hybridize at the constant extremities of the DNA segment.” *Id.* at [0030]. Thus, the approach taught in Neri would allow interaction between

all members of sub-library A and sub-library B and would result in DNA segments (i.e., NAMs) that code for both members of the interaction.

84. My opinions regarding how the claims of the '848 Patent would have been obvious in view of Kanan and Neri (and Famulok for claim 16) are described below.

1. Independent Claim 1

(a) Preamble: “A method of detecting functional interactions between at least two molecules of interests, the method comprising:”

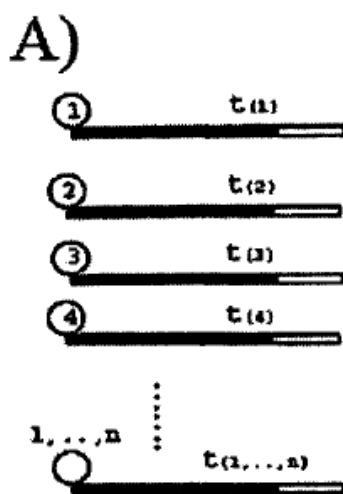
85. The '848 Patent describes that “[i]n the case of detecting functional interactions the MOI association activity is controlled and the interactors in the library are forced together by association of their respective NAMs, NOT primarily between any affinities between the MOIs.” Ex. 1001, 15:57-61 (emphasis in original). The '848 Patent further describes that “[f]unctional interactions are defined as interactions between molecules where the nature of at least one of the partners which participate in the interaction deviates from the nature of that partner when the other participant(s) of the functional interaction is/are present in conditions permitting the functional interaction.” Ex. 1001, 15:31-36. The molecule of interest in the '848 Patent “refers to a molecule about which interactive information is desired.” Ex. 1001, 8:53-55.

86. Kanan describes detecting a functional interaction between two molecules of interest. Kanan discloses “a reaction discovery approach that uses DNA-templated organic synthesis and *in vitro* selection to simultaneously evaluate many combinations of different substrates for bond-forming reactions in a single solution.” Ex. 1010, 545. The molecules of interests described in Kanan include, among other things, small molecules. *Id.* at 547, 548, Fig. 3. These small molecules, which Kanan refers to as substrates, are “tethered to DNA strands” and use “Watson-Crick base pairing [to] control[] the effective molarities of substrates.” *Id.* at 545. Once in proximity, the substrates create “mild carbon-carbon bond-forming reaction[s],” which are the functional interactions. *Id.* The “bond-forming substrate combinations are then revealed using *in vitro* selection for bond formation” and analyzed using “PCR amplification and DNA microarray analysis.” *Id.* In fact, the ’848 Patent recognizes that the DTS-approach described in Kanan discloses “two molecules [that] are forced together by using nucleic acids to allow the molecules to interact[.]” Ex. 1001, 3:46-48.

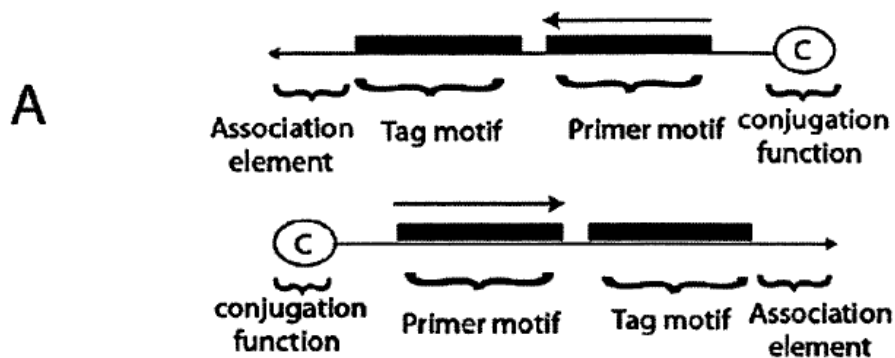
87. A POSA would have understood that detection of bond formation between substrates, as disclosed in Kanan, represents a method of detecting functional interactions between two molecules of interest. Ex. 1001, 10:51-56. Therefore, it is my opinion that Kanan discloses the claim 1 preamble.

- (b) **Step a: “forming a plurality of interactors by coupling each molecule of interest with at least one nucleic acid moiety, the nucleic acid moiety comprising an identification sequence element and an association element;”**

88. Step a of claim 1 of the '848 Patent reflects forming a plurality of interactors, an embodiment of which is depicted in Figure 2(A). Figure 2(A) reflects “a library with 1, 2, . . . , n species of MOI, each MOI species is associated with a defined NAM with nucleic acid tags 1, 2, . . . , n.” Ex. 1001, 7:3-5, Fig. 2(A) (below).

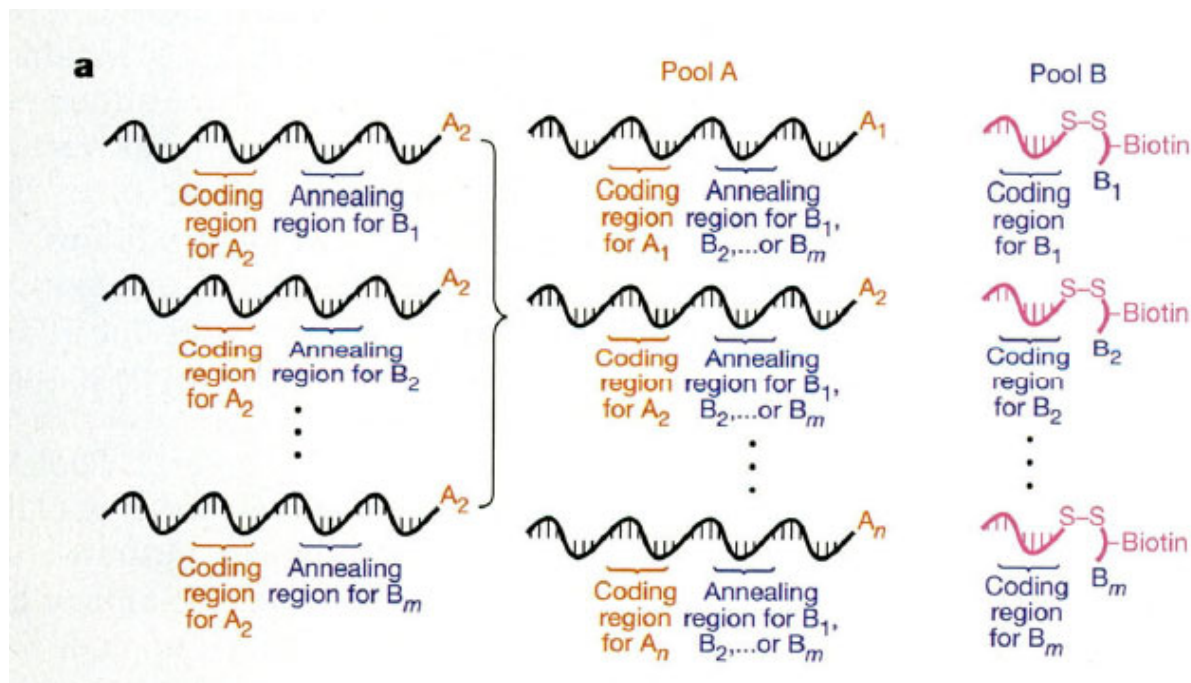


89. In Figure 4(A) of the '848 Patent, “NAMs before conjugation to the MOI are depicted, including two separate sub-populations of NAMs comprising potential kinases and substrates respectively, one with the forward primer and one with the reverse primer. Each NAM comprises a PCR primer, identification element [the “Tag motif”] and an association element.” Ex. 1001, 7:30-35. The molecule of interest, not shown in Figure 4(A), attaches to nucleic acid moiety at the conjugation function.



Ex. 1001, Fig. 4(A).

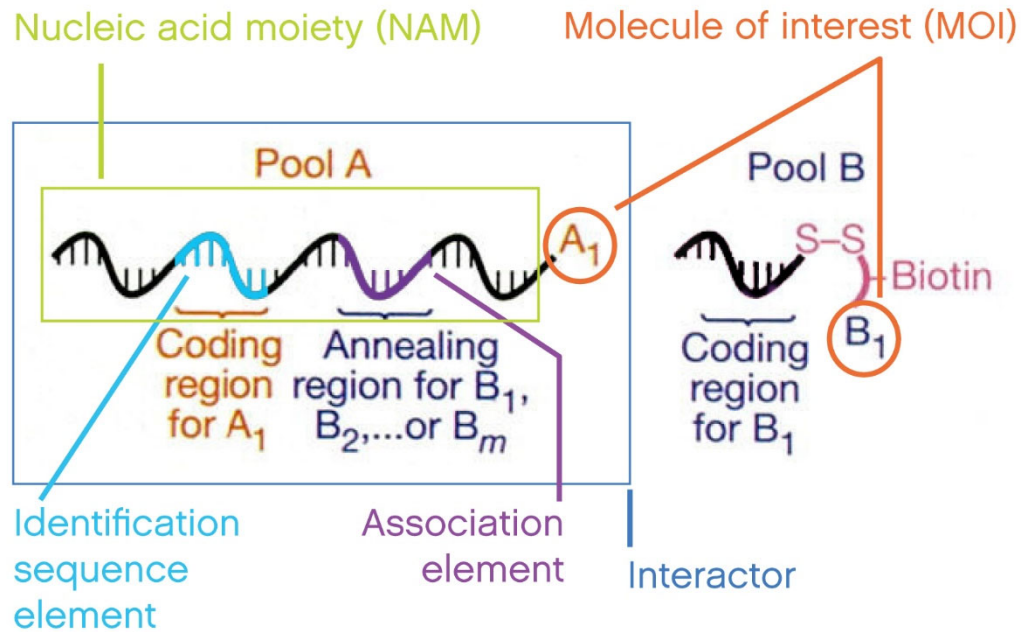
90. Kanan discloses creating interactors by coupling molecules of interest with a nucleic acid moiety comprising an identification sequence element and an association element by coupling the substrates to DNA with specific coding and annealing regions. Ex. 1010, 545, 546, Fig 1.a. Kanan further discloses molecules of interest in that the substrates involved in the interactions are “small molecules.” *Id.* at 547, 548. As disclosed in Kanan, the “two pools of DNA-linked substrates” were prepared “with n substrates in pool A and m substrates in pool B. Each substrate in pool A is covalently linked to the 5’ end of a set of DNA oligonucleotides containing one ‘coding region’ (uniquely identifying that substrate) and one of m different ‘annealing regions’ (Fig. 1a). Each of the m substrates in pool B is attached to the 3’ end of an oligonucleotide containing a coding region that uniquely identifies the substrate and complements one of the m annealing regions in pool A.” *Id.* at 545.



Ex. 1010, 546. In addition, the '848 Patent concedes that DTS approaches, like Kanan, are used to build libraries of interactors: “This approach has also been utilized for multi step reactions, where libraries of compounds joined to nucleic acid tags are directed to serially react with a compound attached to a template nucleic acid. The synthesized library can then be screened with respect to a desired property and selected compounds can then be identified.” Ex. 1001, 3:37-42. Specifically, with regards to Kanan, the '848 Patent also concedes that “*DTS [DNA-templated synthesis], described in Kanan . . . may be utilized to synthesize libraries encoded by nucleic acids and these library members can subsequently be utilized as interactors in the current invention.*” Ex. 1001, 14:6-12 (emphases added).

91. As shown below, I have annotated Kanan to identify that the pool A and pool B substrates are molecules of interest; the pool A DNA oligonucleotide

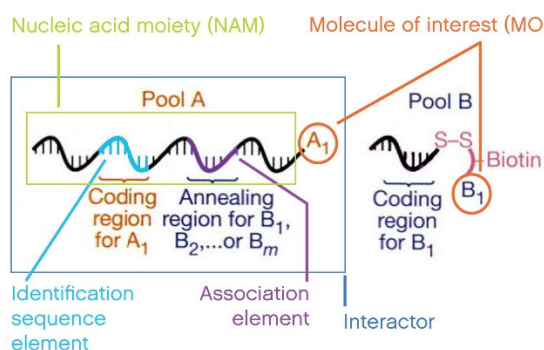
contains an association element, an identification sequence element for A₁, and an association element specific for B₁; the pool B DNA oligonucleotide contains a coding region for B₁ complementary to the B₁-specific association element in Pool A; and the pool A DNA-linked substrates are interactors.



Ex. 1010, 546, Fig 1 (annotated and colored added).

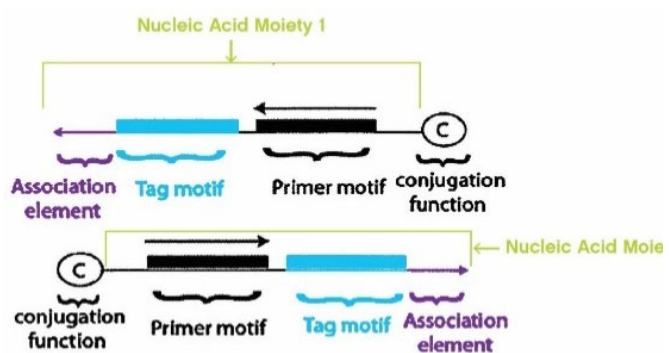
92. As discussed above in paragraph 80, the '848 Patent distinguished the alleged invention from Kanan because pool A's annealing region specifically codes to bind with a single pool B member. *See* Ex. 1001, 3:48-66. That is, while Kanan teaches a technique where "nucleic acids can be attached to low molecular compounds in order to establish identification of the compound after selection for a desired property[,]" Kanan only teaches a "template" method where "one specific oligonucleotide has to be synthesized for each individual interaction on [sic] the

library” and “one nucleic acid [pool A] contains both tags used to identify the compounds which are forced together.” Ex. 1001, 3:26-31, 48-60. Instead, the alleged invention discloses a method that allows “combinatorial association” and “the nucleic acid encoding the tags to identify both members of the interaction are formed upon joining of the two molecules.” Ex. 1001, 3:60-64, 4:16-18.



Ex. 1010, 546, Fig 1

(color added and annotated).



Ex. 1001, Fig. 4(A)

(color added and annotated).

93. As shown above in the '848 Patent's Figure 4(A), the interactors of the alleged invention both have an association element and an identification sequence element (tag motif). But in Kanan, shown above, pool A interactors are the only library members with both an association element and an identification sequence

element, and pool A's annealing region is specific for the specific pool B member it is intended to interact with.¹

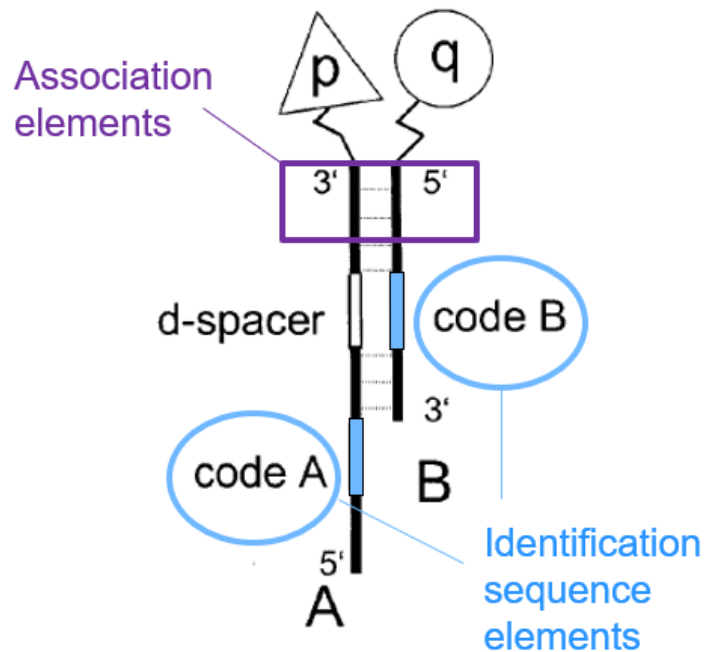
94. Although Kanan does not disclose pool A and pool B interactors with an association element that allows all library members to interact, Neri does. Neri teaches “self-assembling libraries of organic molecules . . . , in which the organic molecules are linked to an oligonucleotide which mediates the self-assembly of the library and/or provides a code associated to each binding moiety.” Ex. 1011 [0005]. Neri teaches the library members can be self-assembled by the 3' ends of the oligonucleotides of sub-library A having a “DNA sequence [that] is designed to hybridize to the DNA sequences at the 5' extremity of oligonucleotides of sub-library B.” *Id.* at [0029].

95. In addition, although Kanan does not disclose the pool B members with a nucleic acid moiety comprising a separate identification sequence element and an

¹ Pool B oligonucleotides contain “a coding region that uniquely identifies the substrate and complements one of the *m* annealing regions in pool A.” Ex. 1010, 545. To the extent that the claims allow for the single coding region to satisfy both the “sequence identification element” and the “association element,” the Examiner erred by not rejecting the claims based on Kanan alone because the coding region in pool B could be both an association element and sequence identification element. However, I note that the pool B code-specific annealing regions in pool A were the primary distinguishing feature in Kanan, a shortcoming that the '848 Patent's alleged invention purportedly overcame through its use of an “identification sequence element” and “association element” on each “nucleic acid moiety” coupled to a “molecule of interest.”

association element, Neri does. Neri discloses chemical libraries of chemical moieties “which carry distinctive ‘sequence tags’ (associated with [and therefore ‘coding for’] the chemical moiety attached to their extremity).” *Id.* at [0020] (brackets in original).

96. In sum, Neri teaches both pool A and pool B members that have a self-assembly region (association element) and unique identification sequence (identification sequence element.) In Neri, “[t]he oligonucleotides of pool A are designed to have: one portion of the DNA sequence which can hybridize to compounds of pool B[;] a distinctive DNA sequence for each of the n members of pool A[;] [and] additional portions of the DNA sequence judiciously designed for ‘decoding’ binding combinations[.]” Ex. 1011, [0048]-[0051]. Likewise, the “oligonucleotides of pool B are designed to have: one portion of the DNA sequence which can hybridize to compounds of pool A[;] a distinctive DNA sequence for each of the m members of pool B[;] [and] additional portions of the DNA sequence judiciously designed for ‘decoding’ binding combinations[.]” *Id.* at [0053]-[0056]. When pool A and pool B are mixed, “the resulting self-assembled $m \times n$ combinations will carry unique DNA codes, corresponding to the non-covalent but stable association (heterodimerization) of the DNA code of the member of Pool A with the DNA code of the member of Pool B.” *Id.* at [0057].



Ex. 1011, Fig. 5 (color added and annotated). As taught in Neri, a POSA would have understood that Neri's "self-assembly sequence" provides a complementary sequence between library members that allows library members to interact and would constitute association elements because the sequences allow association of pool A and pool B library members, resulting in self-assembled $m \times n$ combinations. A POSA would have also understood that Neri's "sequence tags," such as "code A" and "code B" in the figure above, provide a unique nucleic acid identification sequence for each conjugate that can be identified downstream.

97. It would have been obvious to a POSA to add the "self-assembly sequence" and "sequence tags" of Neri to the DNA regions of Kanan because the inclusion of self-assembly sequences and sequence tags (coding regions in Kanan)

for the members of pool A and pool B would allow for a self-assembly of pool A members with pool B members, reducing time required to synthesize the library and mis-hybridization.

98. For the reasons discussed below, a POSA would have been motivated to search for a way to reduce the time required to synthesize the library members (substrates) of Kanan in order to study more combinations in less time. Kanan discusses a desire to search “large number[s] of substrate combinations for unexpected reactions.” Ex. 1010, 548. Although Kanan describes that “a single researcher can evaluate thousands of combinations of substrates and reaction conditions in a two-day experiment,” that efficiency only occurs “once DNA-linked substrate pools are prepared,” which is time intensive and costly. *Id.* at 549; Ex. 1013 (Supplementary Information) (detailing multi-step process to create the substrate pools). As a result, a POSA would have understood that synthesizing the library members is the rate limiting factor in evaluating interactions between substrates. As described in Kanan’s Supplementary Information, pool A members are created through a six-step process to create both the coding region for each A substrate and the annealing region for each B substrate. Ex. 1013. The process to create pool A members is as follows: the substrate is attached to an oligonucleotide; the annealing region for a specific B substrate is hybridized to the coding region for a specific A substrate; the resulting annealing region of the oligonucleotide is

extended by DNA polymerase; the substrate region is hybridized to the coding/annealing region through a splint oligonucleotide; the substrate region and coding/annealing region are then ligated together by T4 DNA ligase; and finally the oligonucleotide is purified by PAGE purification to create single-stranded library pool members. The process to create pool B members is as follows: synthesizing coding region for B substrate linked to biotin by a disulphide bond and then attaching the coding region to the substrate through an amide linkage. Ex. 1013, Fig. S1.

99. This process to create pool A members must be repeated for each and every different A substrate (A_{1-n}) and must be repeated so each A substrate can anneal to all B substrates (B_{1-m}). *Id.* With Kanan's method, this lengthy, cumbersome process is the only way to evaluate all possible interactions ($n \times m$). Ex. 1010, 546, Fig. 1. To increase efficiency (ability to screen larger libraries faster), a POSA would have been motivated to look to combine Kanan with a reference that teaches how to promote and detect interactions between members of a library but uses a common hybridization sequence to reduce the time required to synthesize the library members. Neri's use of a common "self-assembly sequence" provides the teaching needed to promote interactions between all members of the library.

100. A POSA would have been motivated to add the self-assembly sequence of Neri to the annealing region of Kanan because Neri's self-assembly sequence requires that the synthesis of pool A members be repeated only for the number of A

substrates (A_{1-n}). Unlike Kanan alone, with Neri, the process to create pool A library members does not need to be repeated for each B substrate as the self-assembly sequence promotes binding of all pool A members with all pool B members. And the self-assembly sequence allows interactions between all library members (i.e., combinatorial), so would yield the same desired information sought in Kanan.

101. As an example, in Kanan, to analyze the interaction between 100 A substrates and 100 B substrates, the POSA would have been required to synthesize 10,000 (100^2) pool A members and 100 pool B members. But with Neri's self-assembly sequence, a POSA would only have to synthesize 100 pool A members and 100 pool B members because all pool A members can now interact with all pool B members. This results in significant time savings to create the library as now the number of pool A library members needed to be synthesized has reduced by approximately 98%.

102. A POSA would also have been motivated to combine other various aspects disclosed in Neri with the method disclosed in Kanan. First, a POSA would have been motivated to improve specificity of the binding between pool members in Kanan. As Kanan's Supplementary Information describes, it needed ways "to minimize undesired reactivity arising from the mismatched hybridization of a pool B member with a non-complementary pool A member." Ex. 1013, 10. The self-assembly sequence of Neri reduces the concern of mismatched hybridization

because the pool A members are no longer designed to bind to only a specific pool B member.

103. A POSA would have been also motivated to maintain the identification of the pool B members involved in the interaction. To do so, a POSA would be motivated to add the additional orthogonal tail and “d-spacer” disclosed in Neri to the members of pool B to create identification sequences in addition to the self-assembly region on all library members. One of the goals of Kanan states “the reactive substrate pairs must be identified efficiently.” Ex. 1010, 545. By creating a coding region of each library member as taught in Neri, the goal of Kanan is improved upon.

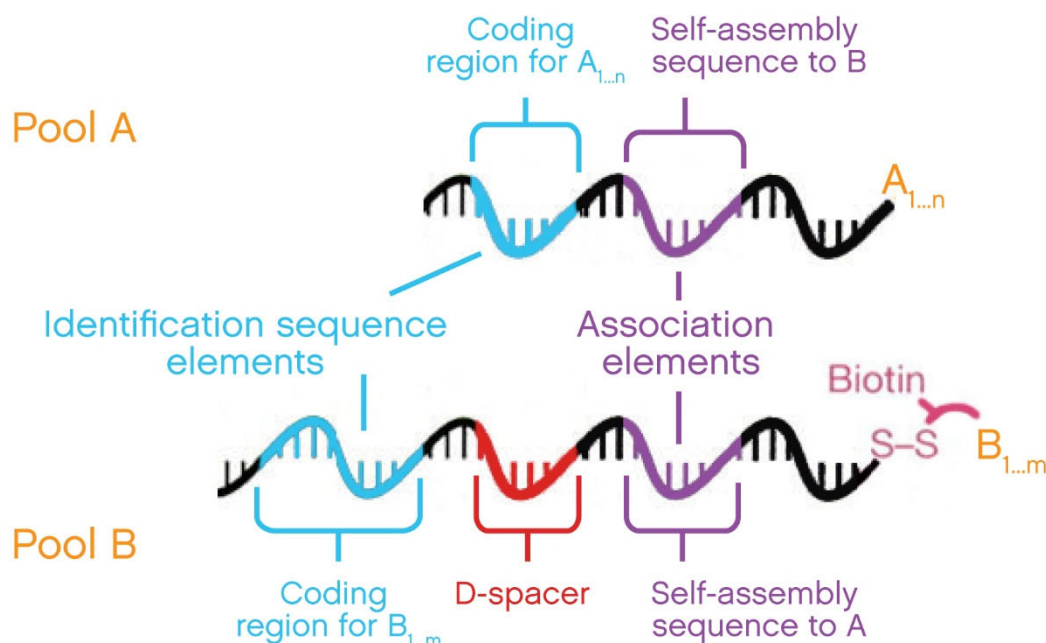
104. A POSA would have a reasonable expectation of success in combining Kanan and Neri. A POSA would review Neri and determine that it teaches a method of preparing organic molecules, peptides, and oligomeric proteins linked to an oligonucleotide that results in stable self-assembled associated oligonucleotides. Ex. 1011, [0082]. As such, the teachings of Neri directly include the “small molecules” of Kanan. Thus, a POSA would have known that the difference between the interactors disclosed in Kanan and Neri are minimal.

105. A POSA would have known that the self-assembly sequence, “d-spacer,” and identification elements as taught in Neri could have been readily applied to oligonucleotides taught in Kanan because the formation of specific

oligonucleotide sequences is a well-known technique requiring only simple modifications. First, a POSA would have readily incorporated the self-assembly sequence taught in Neri by modifying the annealing regions of pool A and pool B library members to share common, complementary sequences to direct the hybridization of pool A members with pool B members. Second, a POSA would have readily incorporated the second identification sequence element taught in Neri to the pool B library members in Kanan by adding the “d-spacer” and identification sequence taught in Neri. The d-spacer is a “small segment . . . composed of [a] phosphodiester backbone without bases.” Ex. 1011, [0029]. The purpose of the d-spacer is “to prevent any undesired pairing.” *Id.* at [0115].

```
typeA_oligo (d=d-Spacer element)
5'-CGT CAG CTC GAA TTC TCC ATA TAT GCA GCG ATA GCG ATC DDD DDD CTG
GGA ATT CCG GTA TGC-3'-chemical entity A
```

Id. at [0118] (annotated). A POSA would have modified pool A and pool B members of Kanan with the teachings of Neri to create two sub-libraries of interactors that allow self assembly and retain identification sequence elements for each attached substrate. Below I have included a figure I have created to represent the library members in Kanan with the additional elements of Neri.

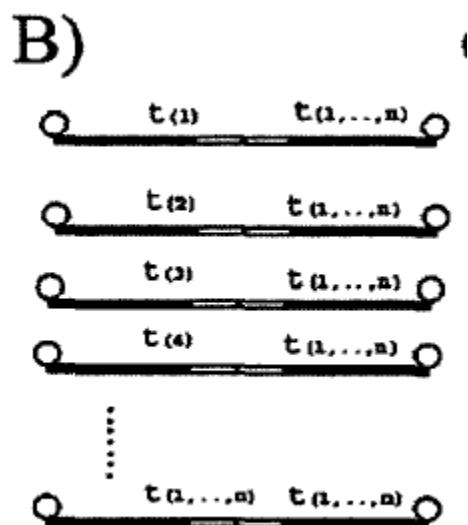


106. Therefore, in my opinion, Kanan in view of Neri discloses forming a plurality of interactors by coupling each molecule of interest with at least one nucleic acid moiety, the nucleic acid moiety comprising an identification sequence element and an association element.

- (c) **Step b: “forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors bound in proximity to one another by an associated oligonucleotide formed from the association between at least two nucleic acid moieties, wherein the associated oligonucleotides comprise at least two identification elements derived from the at least two nucleic acid moieties;”**

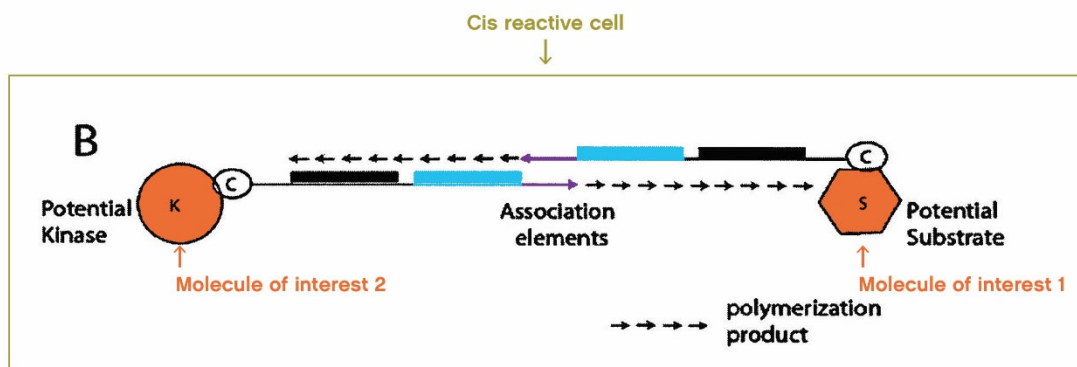
107. In the '848 Patent, the cis-reactive cells (CRCs) refer “to an association of at least two interactor moieties, joined by an associated oligonucleotide.” Ex. 1001, 9:35-37. The '848 Patent's Figure 2(B) depicts “[c]onstruction of CRCs”

where “the association function is designed to co-localize the library members in a combinatorial fashion to create all possible CRC variants between the n library members ((n-n²)/2 unique combinations).” Ex. 1001, 7:6-9.



Ex. 1001, Fig. 2(B).

108. In Figure 4(B) of the '848 Patent, “[l]ibrary morphology after association of the interactors to construct CRCs is illustrated.” Ex. 1001, 4:35-36.

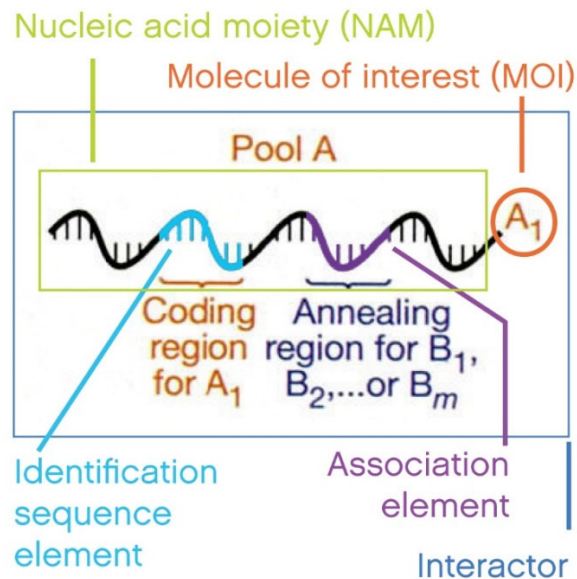


Ex. 1001, Fig. 4.B (annotated and color added).

109. Kanan in view of Neri discloses forming a plurality of cis-reactive cells. Kanan teaches that “[w]hen pools A and B are combined in a single aqueous solution at nanomolar concentrations, Watson-Crick base pairing organizes the mixture into $n \times m$ discrete pairs of substrates attached to complementary sequences.” Ex. 1010, 545.

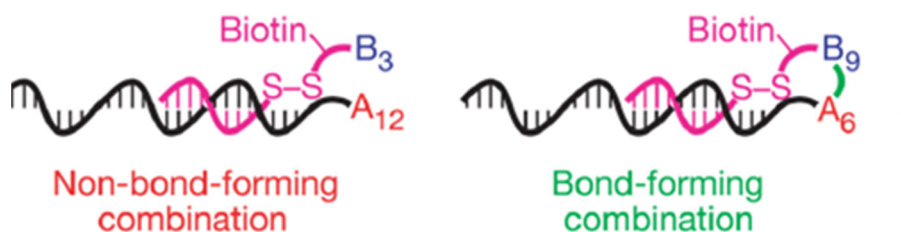
110. Indeed, the '848 Patent concedes that the DTS approach, as taught in Kanan, allows for hybridization of complementary nucleic acids attached to compounds, which “will force the two compounds in proximity[.]” Ex. 1001, 3:31-35.

111. As described above in paragraph 96, Kanan in view of Neri discloses interactors, which are the DNA-linked substrates in pool A and pool B. Pool A interactors are shown below:



Ex. 1010, 546, Fig. 1 (annotated).

112. The interactors (pool A and pool B) in Kanan in view of Neri are then bound in proximity to one another through their respective annealing regions, as described in the '848 Patent.

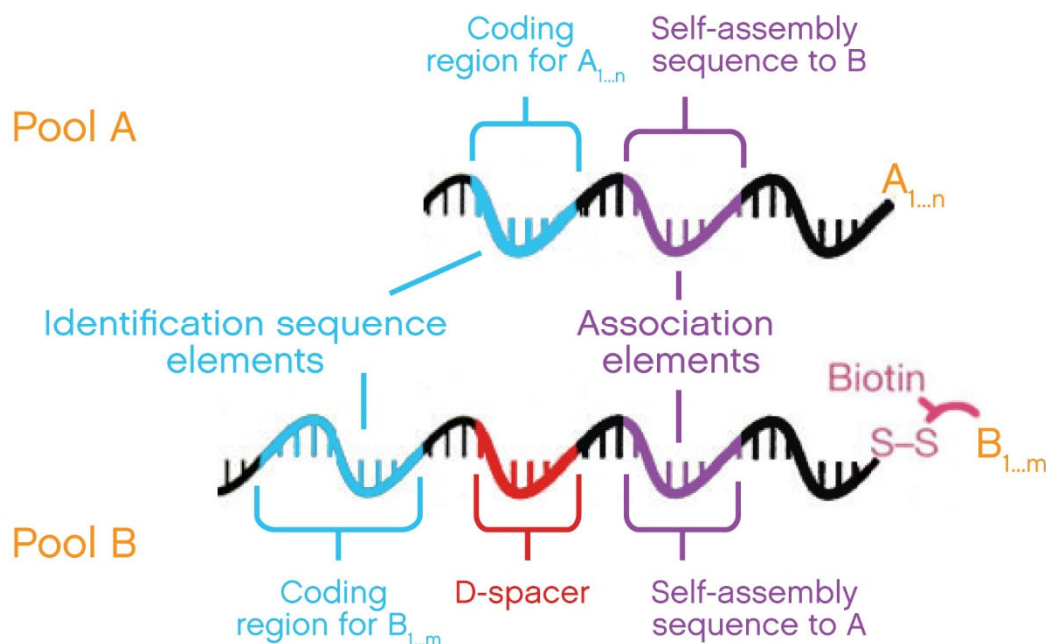


Ex. 1010, 546, Fig. 1 (partial). As shown above in Kanan Fig. 1, substrate A₁₂ and B₃ are bound in proximity via the annealing region for B₃. Likewise, substrate A₆ and B₉ are bound in proximity via the annealing region for B₉. Based on the annealing regions (association elements), the DNA linked to the substrates (A and B) bind regardless of the interaction between substrates themselves. This is demonstrated above in the fact that the DNA of A₁₂ and B₃ bind despite that there is no bond formation between substrates A₁₂ and B₃. The labels in Kanan of “Non-bond-forming combination” and “Bond-forming combination” refer to binding of substrates, not binding via the annealing region.

113. Further, as shown above (paragraphs 97-106), interactors (pool A and pool B) in Kanan in view of Neri contain a self-assembly sequence (i.e., association element) “designed to co-localize the library members in a combinatorial fashion.” Ex. 1001, 7:6-9. The DNA regions in Kanan with Neri’s self-assembly sequence

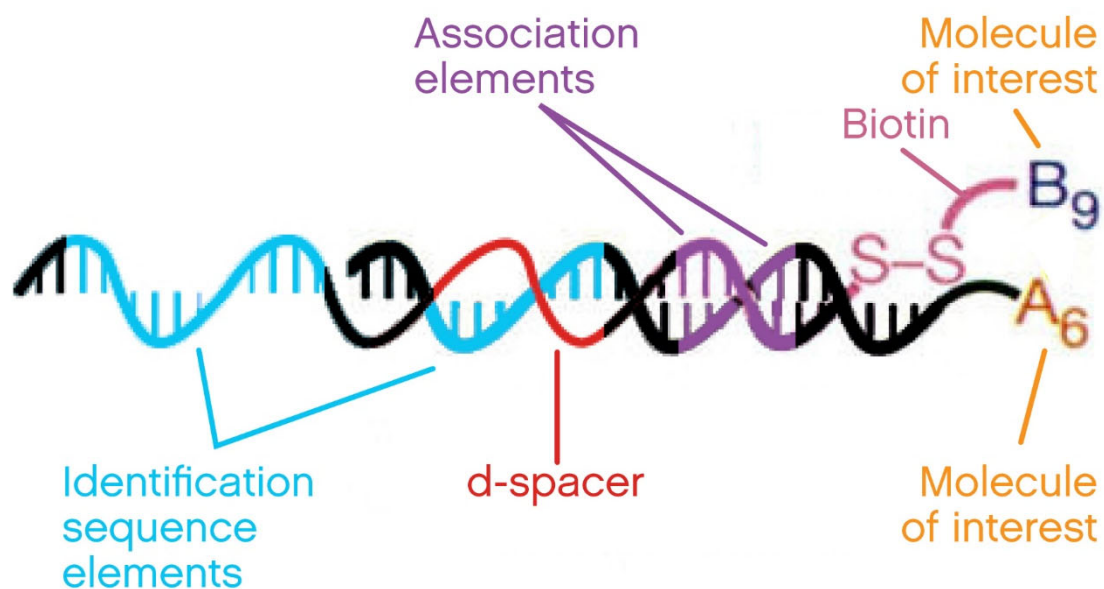
creates interactors that can be self-assembled, that is they can interact combinatorially, by the 3' ends of the oligonucleotides of sub-library A having a “DNA sequence [that] is designed to hybridize to the DNA sequences at the 5' extremity of oligonucleotides of sub-library B.” Ex. 1011, [0029].

114. In addition, as shown above (paragraphs 97-106), the DNA regions of the DNA-linked substrates in Kanan together with two “sequence tags” of Neri create two pools of interactors where each NAM contains a self-assembly sequence and a unique identification sequence element. Below I have included a figure I have created to represent the library members in Kanan with the additional elements of Neri.



115. In other words, both pool A and pool B interactors in Kanan in view of Neri would comprise association elements (self-assembly sequences) that would

allow all interactors to interact and unique identification sequence elements for each interactor. I have created the below figure to represent a cis-reactive cell formed by interactors from the pool A and pool B library members in Kanan combined with the additional elements of Neri (here: A₆ and B₉).

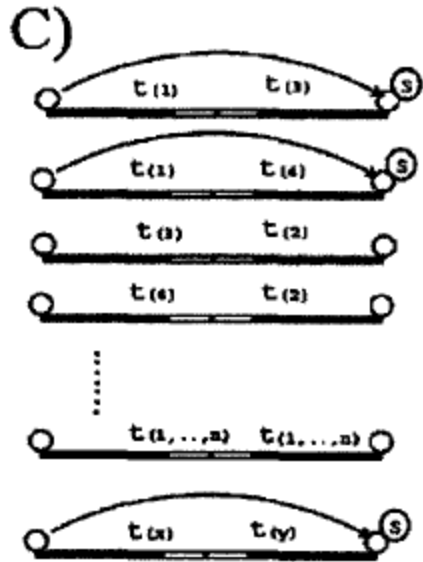


Thus, a POSA would have understood that the DNA regions linked to the substrates in both pool A and pool B in Kanan in view of Neri would contain both a self-assembly sequence and identification sequence element on each interactor that codes for each substrate. And because Kanan with Neri allows for all library members to interact from two sub-libraries (without pre-synthesizing A library members to a certain B library member), there would be two or more cis-reactive cells formed of any A_{1-n} interactor with any B_{1-m} interactor, such as A₁ with B₁, A₁ with B₂, and so on.

116. Therefore, Kanan in view of Neri discloses forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors bound in proximity to one another by an associated oligonucleotide formed from the association between at least two nucleic acid moieties, wherein the associated oligonucleotide comprises at least two identification elements derived from the at least two nucleic acid moieties.

(d) Step c: “subjecting the plurality of cis-reactive cells to conditions which stimulate desired functional interaction having a detectable trace;”

117. The '848 Patent describes that the “conditions which stimulate” “can be accomplished by, for example, addition of a substrate or a shift in reaction conditions.” Ex. 1001, 16:13-17. In addition, the '848 Patent states that a functional interaction includes “addition of a molecular group.” Ex. 1001, 16:33-36; *see also id.* at 15:31-37. The '848 Patent's Figure 2(C) depicts that “[t]he functional interaction to be detected is activated and reaction conditions are controlled to restrict the functional interactions to substantially within the CRCs.” Ex. 1001, 7:9-12.

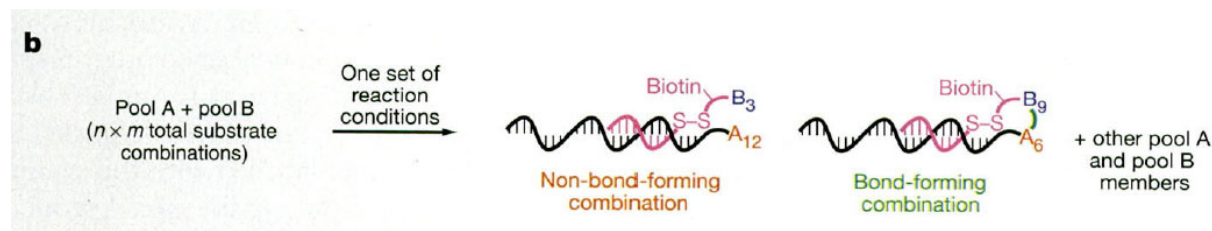


Ex. 1001, Fig. 2(C).

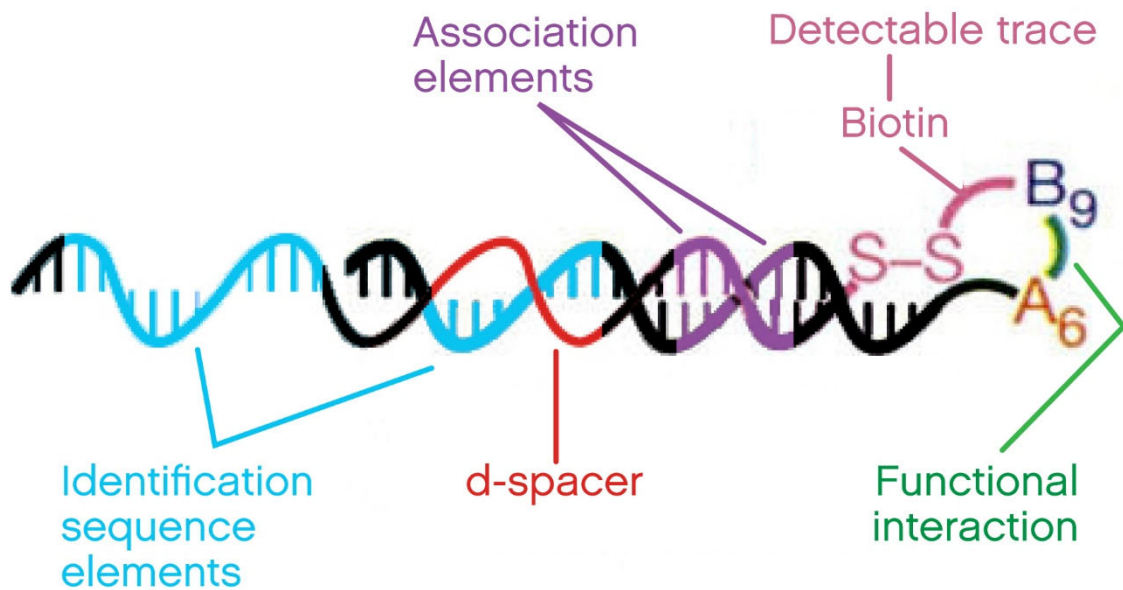
118. The '848 Patent describes that the “detectable trace” includes “detection of the CRCs by labelling with affinity reagents which detect interactors which are included in the functional interaction; and introduction of a modified molecule which becomes incorporated by the functional interaction where the modified molecule permits selective detection.” Ex. 1001, 19:48-55; *see also id.* at 20:17-25.

119. Kanan teaches subjecting the CRCs, such as those generated from the combination of Kanan and Neri, to conditions that stimulate a desired functional interaction with a detectable trace. Kanan teaches each pool B substrate is linked “to its corresponding oligonucleotide by a linker containing a biotin group and a disulphide bond.” Ex. 1010, 545. “After incubations under a set of chosen reaction

conditions, followed by cleavage of the disulphide bonds, only pool A sequences encoding bond formation between a pool A and pool B substrate remain covalently linked to biotin.” Ex. 1010, 545; Fig. 1.b (below).



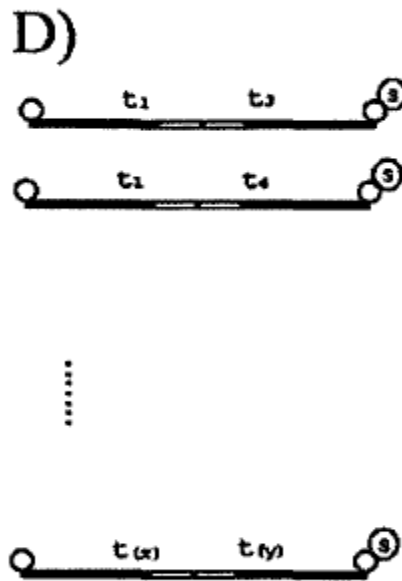
A POSA would have understood that the bond formation between substrate A and substrate B (i.e., the addition of substrate B to A) would be the functional interaction between the substrates. Further, a POSA would have understood that the bond formation between a pool A substrate and a pool B substrate covalently linked to biotin would be the detectable trace, as only the bond-forming pairs contain the biotin that is subsequently selected using streptavidin or avidin for analysis. A POSA would understand that the CRCs made from Kanan and Neri, consisting of interactors of any A_{1-n} with any B_{1-m}, would be subjected to conditions to stimulate a desired functional interaction having a detectable trace as shown, for example, in the CRC below:



120. Therefore, it is my opinion that Kanan discloses subjecting the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction having a detectable trace.

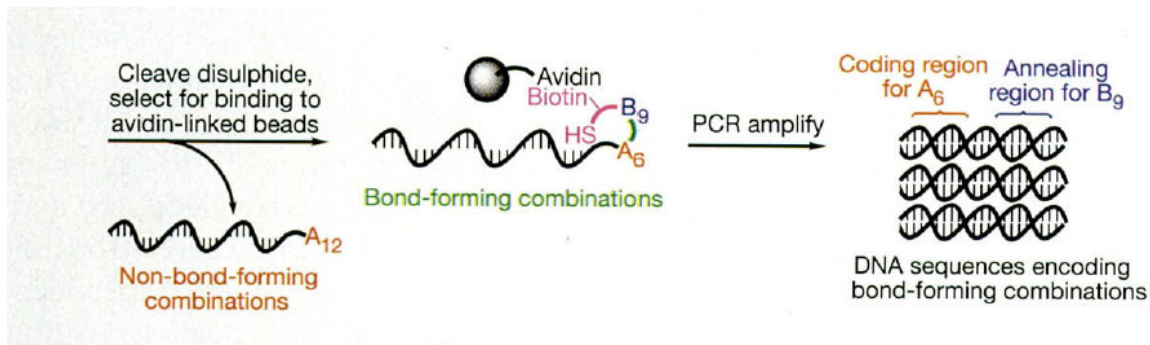
(e) Step d: “selecting all cis-reactive cells exhibiting the detectable trace; and”

121. The '848 Patent describes that “[s]election is carried out with respect to at least one detectable alteration induced by the functional interaction[,]” as depicted in Figure 2(D). Ex. 1001, 7:12-14.



Ex. 1001, Fig. 2(D).

122. Kanan teaches selecting the CRCs, such as those generated from the combination of Kanan and Neri, that exhibit a detectable trace. Kanan discloses that after bond formation, “[s]treptavidin affinity selection of the resulting solution separates biotinylated from non-biotinylated sequences.” Ex. 1010, 545, Fig. 1.b. (below).

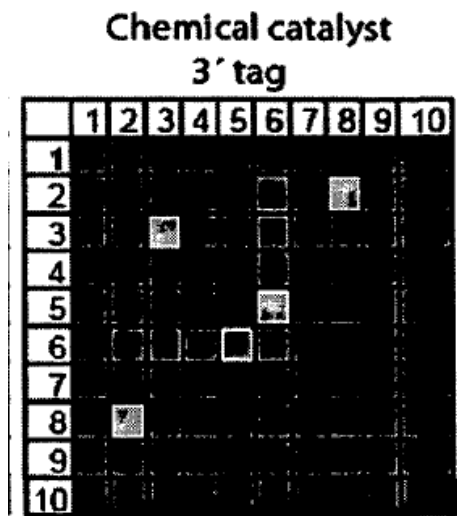


123. The '848 Patent concedes that the DTS approach, as taught in Kanan, allows for the “synthesized library . . . [to] be screened with respect to a desired property and selected compounds can then be identified.” Ex. 1001, 3:40-42.

124. A POSA would have understood that the use of the biotin-streptavidin capture would allow selection of only the CRCs with bond-forming combinations, i.e., demonstrate the detectable trace, because only those combinations would have the linked biotin with affinity for streptavidin-linked magnetic particles, as shown above in Fig. 1.b. Therefore, in my opinion, Kanan discloses selecting all cis-reactive cells exhibiting the detectable trace.

(f) Step e: “subjecting the associated oligonucleotides from the cis-reactive cells selected in step (d) to an analysis that permits detection of the at least two identification sequence elements.”

125. The '848 Patent describes that “the associated NAMs (the associated oligonucleotide) can optionally be amplified and subsequently detected, by, for example, microarray analysis.” Ex. 1001, 14:67-15:3. In Example 3 of the '848 Patent, the PCR products were detected using microarray. Ex. 1001, 28:12-15. Figure 7 of the '848 Patent depicts the result, which “schematically illustrates a microarray comprising tags complementary to combinations of identification sequence elements.” Ex. 1001, 28:40-42, Fig. 7.



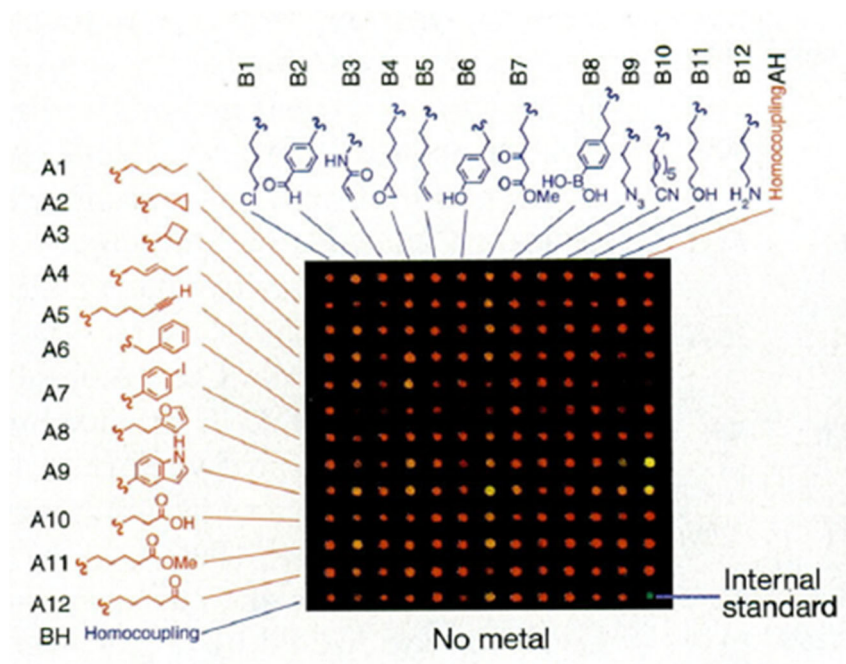
Ex. 1001, Fig. 7 (middle image).

126. Kanan in view of Neri teaches subjecting the selected CRCs from step (d) to an analysis that permits detection of the at least two identification sequence elements.

127. Kanan teaches that the bond-forming pairs are amplified by PCR and analyzed with microarray analysis through the incorporation of fluorophores to identify the sequences encoding the bond-forming substrates. Ex. 1010, 545-546. A POSA would have understood that the detection of the bond-forming substrate products permits the detection of the at least two identification sequence elements in the combination of Kanan with Neri because it is the resulting nucleic acid, which includes the two identification sequence elements of Neri, that is detected and corresponds to each unique substrate. For example, “[t]he ratios of Cy3 (green) to Cy5 (red) fluorescence for all array locations were calculated and ordered by rank,

and spots with green/red fluorescence ratios significantly higher than the majority of spots (in the experiments below, ratios above 1.5) were considered to be positive.”

Ex. 1010, 545. In this experiment, Kanan discloses “the array spot corresponding to the combination of the alkyne (A5) and the azide (B9) was the sole spot that had a significant green/red fluorescence ratio ($A5 + B9 = 8.5$, standard = 2.8; see Fig. 2.b and Supplementary Data). In contrast, the experiment performed in the absence of any added metal yielded no green array spots other than the standard (Fig. 2b).” *Id.* at 545-546.



Ex. 1010, Fig. 2.b.

128. The '848 Patent concedes that the DTS approach, as taught in Kanan, allows for “the identity of compounds synthesized by DTS . . . [to] be identified by

microarray hybridization of the nucleic acid template which directed the synthesis and to which the product is also attached.” Ex. 1001, 3:42-45.

129. In addition, Neri teaches an analysis of the selected CRCs that permits detection of the identification sequences because Neri teaches sequencing of the identification sequences. Ex. 1011, [0117]-[0119]. A POSA would have found it obvious to combine the sequencing of the identification sequences as taught in Neri with the analysis of the bond-forming combinations in Kanan because sequencing was a well-known method to identify the sequences encoding the bond-forming substrates. *See* Ex. 1028 (Wilton 2002); *see also* Ex. 1030 (Heather 2016).

130. Neri discloses that the pool A and pool B DNA-linked chemical entities are allowed to bond and then after bonding are analyzed by PCR followed by sequencing. Ex. 1011, [0111]-[0119]. A POSA would have understood that Neri’s “sequencing” of the coding regions allows identification of the chemical entity in which the “code” identifies. *Id.* at [0112].

131. A POSA would have been motivated to efficiently identify the reactive pairs in Kanan. A POSA would have been motivated to add sequencing of the bond-forming substrates, as taught in Neri, as another method to detect and analyze the bond-forming substrates because sequencing is a well-known method to efficiently determine the nucleic acid sequences of the identification sequence elements of the substrates involved in the bond formation. As discussed above in paragraphs 104-

106, a POSA would have a reasonable expectation of success in combining Kanan and Neri because sequencing is a routine method that would yield expected results in obtaining the desired information from the oligonucleotide sequences.

132. Therefore, in my opinion, both Kanan and Neri disclose subjecting the associated oligonucleotides from the cis-reactive cells selected in step (d) to an analysis that permits detection of the at least two identification sequence elements.

133. As shown above, in my opinion, claim 1 is obvious in view of Kanan and Neri.

2. Dependent Claim 2

- (a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein selecting all cis-reactive cells exhibiting the detectable trace is accomplished via selective separation or selective detection of the cis-reactive cells.”**

134. During prosecution, Patent Owner admitted that “[b]oth selective separation and selective detection are techniques well known in the art[.]” Ex. 1002, 505. I agree.

135. Kanan teaches selective detection of the cis-reactive cells. As discussed above in paragraphs 121 through 124, Kanan discloses that the bond-forming pairs can be selectively detected using streptavidin affinity selection. Ex. 1010, 545.

136. A POSA would have understood that the use of the biotin-streptavidin selection would allow selective detection of only the bond-forming combinations

because only those combinations would have the affinity for streptavidin-linked magnetic particles or immobilized streptavidin.

137. Therefore, in my opinion, claim 2 is obvious in view of Kanan and Neri.

3. Dependent Claim 3

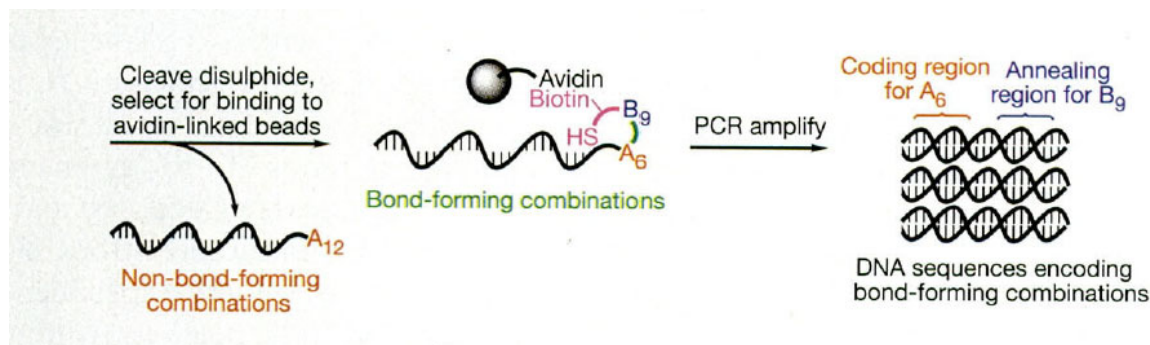
- (a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 2, wherein selecting all cis-reactive cells exhibiting the detectable trace is accomplished by affinity purification.”**

138. A POSA would have understood streptavidin affinity selection as a form of affinity purification. As described above with respect to dependent claim 2 (paragraphs 134-137), it is my opinion that claim 3 is obvious in view of Kanan and Neri.

4. Dependent Claim 4

- (a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, further comprising enhancing the selected associated oligonucleotides by PCR amplification.”**

139. Kanan teaches enhancing the associated oligonucleotides by PCR amplification. Kanan teaches the bond-forming pairs are amplified using PCR. Ex. 1010, 545-546; Fig. 1.b (below).



140. Therefore, in my opinion, claim 4 is obvious in view of Kanan and Neri.

5. Dependent Claim 5

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein coupling is covalent or noncovalent.”**

141. Kanan teaches “wherein the coupling is covalent.” Kanan discloses that “[e]ach substrate in pool A is covalently linked to the 5’ end of a set of DNA oligonucleotides containing one ‘coding region’ (uniquely identifying that substrate) and one of m different ‘annealing regions’ (Fig. 1a). Each of the m substrates in pool B is attached to the 3’ end of an oligonucleotide containing a coding region that uniquely identifies the substrate and complements one of the m annealing regions in pool A.” Ex. 1010, 545. A POSA would have understood that both the substrates in pool A and pool B of Kanan were covalently linked to the DNA oligonucleotides.

142. Therefore, in my opinion, claim 5 is obvious in view of Kanan and Neri.

6. Dependent Claim 6

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein coupling is covalent.”**

143. As described above with respect to dependent claim 5 (paragraphs 141-142), each substrate in Kanan is covalently linked to a DNA oligonucleotide. Thus, it is my opinion that claim 6 is obvious in view of Kanan and Neri.

7. Dependent Claim 7

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein at least one molecule of interest comprises a protein, a protein domain, a peptide, a peptoid, a functional enzyme, a small molecule, a polymer, a non-proteinaceous cellular product, a virus particle, a cell metabolite, a lipid, a carbohydrate, a nucleic acid, an inorganic compound, a reactive compound, or any combination thereof, however, wherein not all molecules of interest are nucleic acids.”**

144. Kanan teaches a molecule of interest being a small molecule. As discussed above in paragraph 86, Kanan discloses that the substrates involved in the interactions are “small molecules.” Ex. 1010, 547-548. A POSA would have understood the organic functional groups disclosed in Kanan (*see, e.g.*, Ex. 1010, Fig. 2.a) as being small molecules. A POSA would have also understood that a small molecule would meet this claim limitation because it only requires the disclosed molecule of interest to be one of the items recited in the claimed list.

145. Therefore, in my opinion, claim 7 is obvious in view of Kanan and Neri.

8. Dependent Claim 8

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 7, wherein at least one molecule of interest comprises at least one protein.”**

146. Neri teaches a molecule of interest being a protein. A POSA would have understood that it would have been obvious to combine the analysis of proteins as taught in Neri with the method described in Kanan. Kanan states “we expect that [the described approach’s] versatility and efficiency will enable the discovery of additional reactions between a wide range of substrates.” Ex. 1010, 545. A POSA would not have understood the method described in Kanan as being limited to only small organic molecules.

147. Neri discloses that “[t]he methodologies described in this Patent work not only for small organic molecules, but also for peptides and oligomeric proteins [e.g. antibody Fv fragments, consisting of a VH and VL domain; see Example 1].” Ex. 1011, [0082] (brackets in original). Neri also states that “one strength of ESACHEL technology is its compatibility with a variety of different chemical moieties, including peptides and globular proteins (e.g., antibody domains).” *Id.* at [0094].

148. It would have been obvious to a POSA to use a protein as one of the substrates taught in Kanan. A POSA would have been motivated to study protein interactions because it was well-known at the time of the application to create

peptide or protein libraries to understand the role and properties of proteins. Ex. 1023. Thus, a POSA would have been motivated to combine Kanan with Neri because of the known ease to create protein libraries. *See, e.g.*, Ex. 1011, [0010]; Ex. 1023.

149. In addition to the reasons discussed above in paragraphs 104-106, a POSA would have a reasonable expectation of success in combining Kanan and Neri because a POSA would have known that the method disclosed in Kanan could be altered based on the teachings of Neri to incorporate coupling a protein or nucleic acid to the nucleic acid labels because not only was it taught in Neri but it was also well-known at the time of the application to create peptide or protein libraries to understand the role and properties of proteins. *See, e.g.*, Ex. 1011, [0010], [0082]; Ex. 1023.

150. Therefore, in my opinion, claim 8 is obvious in view of Kanan and Neri.

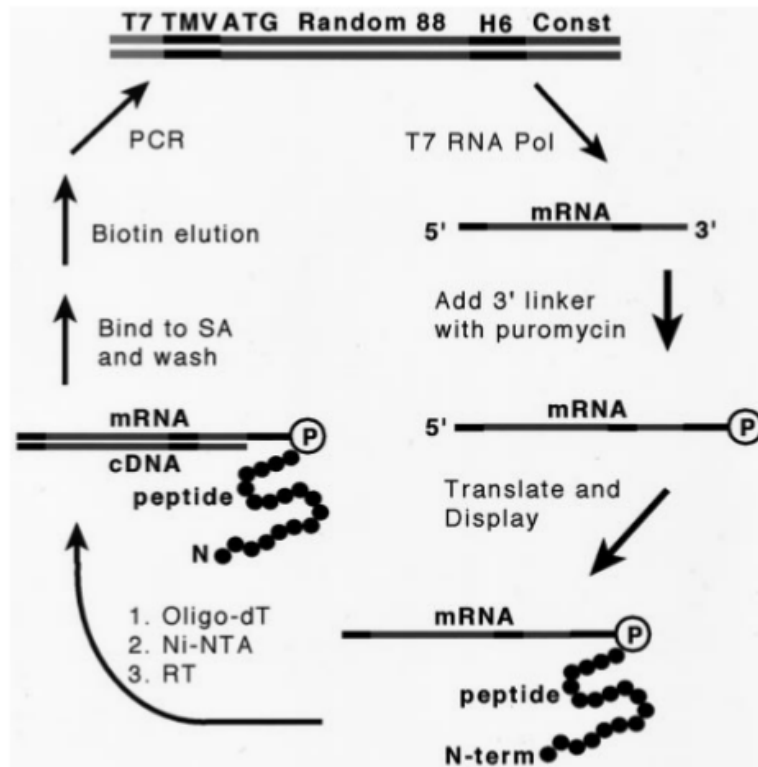
9. Dependent Claim 9

- (a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 8, wherein the protein is an expression product of a nucleic acid molecule, and further wherein at least some of the plurality of interactors are formed by coupling the protein with a nucleic acid moiety comprising an identification sequence element derived from the nucleic acid molecule.”**

151. Neri teaches a molecule of interest being a protein that is an expression product because a POSA would have understood the limitations of claim 9 as

describing phage display or mRNA display. Ex 1001, 13:5-14, 13:43:14:5. For example, the '848 Patent describes that the “cis coupling of a nucleic acid to a desired protein could also be mediated by in vivo expression of, for example, plasmids, phages or viruses.” Ex. 1001, 13:52-54.

152. A POSA would have known that in both phage display and mRNA display, the protein is coupled to a nucleic acid sequence that encodes for that specific protein. Ex. 1032 (Winter 1994); Ex. 1033 (Wilson 2001). Specifically, with mRNA display, “a pool of proteins is produced from a pool of mRNA templates and the protein products are coupled with the mRNA they were produced from The protein then serves as the MOI and the coupled mRNA as the NAM.” Ex. 1001, 13:7-12. As shown in Wilson 2001, “the puromycin-containing linker is ligated onto the 3' end of the mRNA,” and “[w]hen this template is translated *in vitro*, the nascent peptide forms a covalent bond to the puromycin moiety.” Ex. 1033, 3751. This creates an “mRNA-peptide covalent fusion molecule[.]”



Id. A POSA would have understood the “protein is an expression product of a nucleic acid molecule” limitation as describing a protein coupled to nucleic acid that encodes for the protein, as described in phage display and mRNA display. Further, using phage display and mRNA display, a POSA would have understood that the identification sequence element is “derived from the nucleic acid molecule” because the identification sequence element is the same sequence that encodes for the displayed protein coupled to the NAM. This is described in the ’848 Patent as well when it states “[i]f the nucleic acid encoding the protein product is utilized as the NAM, constant motifs can be used for amplification purposes and MOI encoding motifs for identification.” Ex. 1001, 13:54-57.

153. A POSA would have understood that it would have been obvious to combine the analysis of phage display proteins as taught in Neri with the method described in Kanan. Kanan states “we expect that [the described approach’s] versatility and efficiency will enable the discovery of additional reactions between a wide range of substrates.” Ex. 1010, 545. A POSA would not have understood the method described in Kanan as being limited to only small organic molecules.

154. Neri discloses that “[t]he methodologies described in this Patent work not only for small organic molecules, but also for peptides and oligomeric proteins [e.g. antibody Fv fragments, consisting of a VH and VL domain; see Example 1].” Ex. 1011, [0082]. Neri also states that “one strength of ESACHEL technology is its compatibility with a variety of different chemical moieties, including peptides and globular proteins (e.g., antibody domains).” *Id.* at [0094]. Further, Neri discloses that it was known at the time of the application to create protein libraries using phage display. *Id.* at [0010] (“[M]ethods such as phage display produce particles in which a phenotype (typically the binding properties of a protein, displayed on the surface of filamentous phage) is physically coupled to the corresponding genotype (i.e., the gene coding for the protein displayed on phage) [Winter, 1994], allowing the facile amplification and identification of library binding members with the desired binding specificity.”) (brackets in original); Ex. 1032 (Winter 1994).

155. It would have been obvious to a POSA to use a phage display protein as one of the substrates taught in Kanan. A POSA would have been motivated to study protein interactions because it was well-known at the time of the application to create protein libraries to understand the role and properties of proteins and the use of phage display allows identification of the attached protein. *See, e.g.*, Ex. 1011, [0010]; Ex. 1032. Thus, a POSA would have been motivated to combine Kanan with Neri because of the known ease to create and use phage display. *See, e.g.*, Ex. 1011, [0010]; Ex. 1032.

156. In addition to the reasons discussed above in paragraphs 104-106, a POSA would have a reasonable expectation of success in combining Kanan and Neri because coupling a protein to the nucleic acid labels and the use of phage display was well-known at the time of the application. *See, e.g.*, Ex. 1011, [0010], [0082], [0117]-[0119]; Ex. 1023; Ex. 1032.

157. Therefore, in my opinion, claim 9 is obvious in view of Kanan and Neri.

10. Dependent Claim 10

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 7, wherein at least one molecule of interest comprises at least one protein and at least one nucleic acid.”**

158. Neri teaches a molecule of interest being a protein and a nucleic acid. A POSA would have understood that it would have been obvious to combine the

analysis of proteins and nucleic acid as taught in Neri with the method described in Kanan. Kanan states “we expect that [the described approach’s] versatility and efficiency will enable the discovery of additional reactions between a wide range of substrates.” Ex. 1010, 545. A POSA would not have understood the method described in Kanan as being limited to only small organic molecules.

159. Neri discloses that it “is therefore an object of the present invention to provide a chemical compound comprising a chemical moiety of any kind capable of performing a binding interaction with a target molecule (e.g. a biological target)[.]” Ex. 1011, [0015]. As discussed above regarding claim 8, Neri discloses that “[t]he methodologies described in this Patent work not only for small organic molecules, but also for peptides and oligomeric proteins [e.g. antibody Fv fragments, consisting of a VH and VL domain; see Example 1].” Ex. 1011, [0082] (brackets in original). Neri also discloses that the prior art taught “generation of libraries of genes,” which a POSA would understand as meaning nucleic acid. *Id.* at [0010]; Ex. 1032.

160. It would have been obvious to a POSA to use a protein as a substrate and nucleic acid as a substrate in the substrate pairs taught in Kanan. A POSA would have been motivated to study the interaction of DNA molecules with DNA-binding proteins, for example, in the use of DNA-binding proteins to positively or negatively regulate transcription initiation by the RNA polymerase. Studying the interaction of

DNA molecules with DNA-binding proteins was well-known to a POSA at the time of the application. Ex. 1025 (Guille 1997).

161. As discussed above in paragraph 149, a POSA would have a reasonable expectation of success in combining Kanan and Neri because it was well-known at the time of the application to create peptide or protein libraries to understand the role and properties of proteins. *See, e.g.*, Ex. 1011, [0010], [0082]; Ex. 1023.

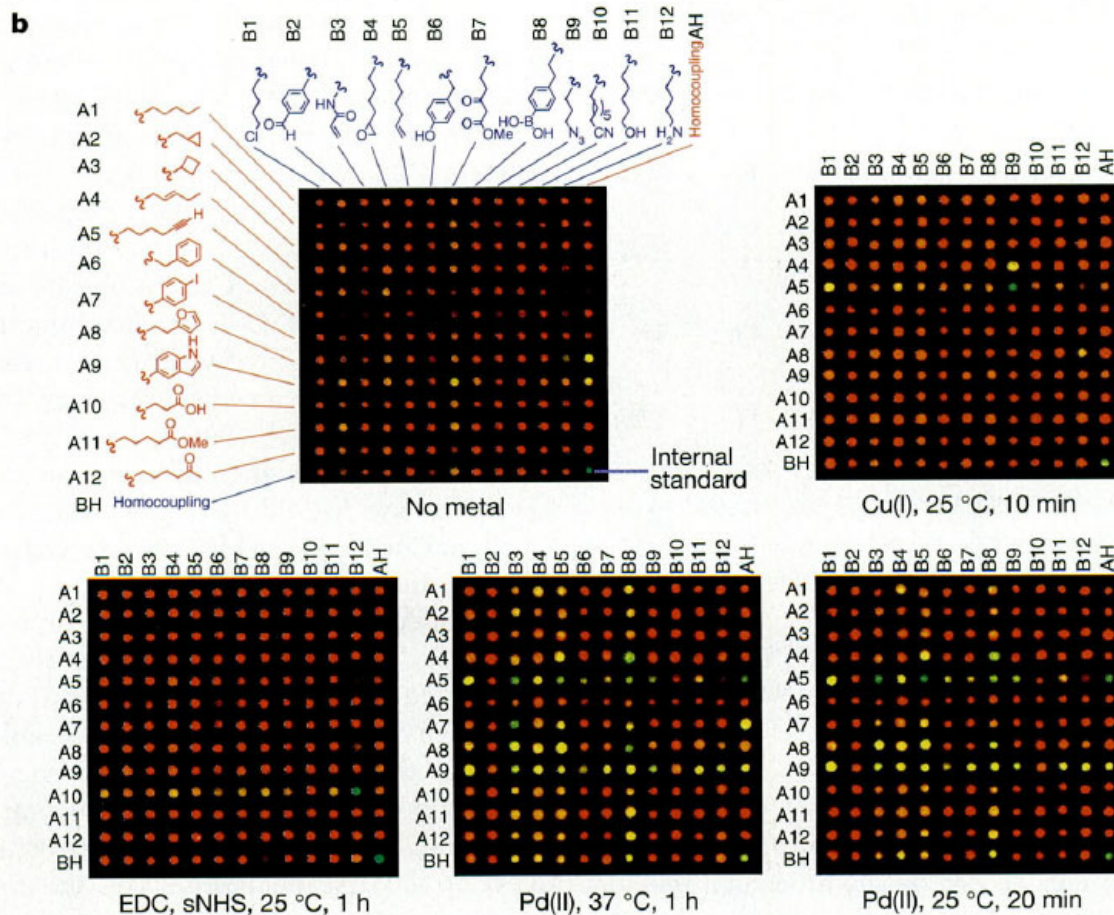
162. Therefore, in my opinion, claim 10 is obvious in view of Kanan and Neri.

11. Dependent Claim 11

- (a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein detecting further comprises quantifying the functional interactions.”**

163. Kanan teaches quantifying the functional interactions. Kanan teaches that after the DNA-linked substrates are allowed to bond, the “[s]equences encoding bond-forming substrates pairs were captured with streptavidin-linked magnetic particles and amplified by PCR with a DNA primer labelled with the cyanine fluorophore Cy3. For comparison, an aliquot of the pool A sequences before selection was amplified by PCR with a Cy5-labelled primer. The Cy3-labelled and Cy5-labelled PCR products were combined and hybridized to a DNA microarray containing all 168 possible reaction-encoding sequences. The ratios of Cy3 (green)

to Cy5 (red) fluorescence for all array locations were calculated and ordered by rank, and spots with green/red fluorescence ratios significantly higher than the majority of spots (in the experiments below, ratios above 1.5) were considered to be positive.” Ex. 1010, 545. In this experiment, Kanan discloses “the array spot corresponding to the combination of the alkyne (A5) and the azide (B9) was the sole spot that had a significant green/red fluorescence ratio ($A5 + B9 = 8.5$, standard = 2.8; see Fig. 2.b and Supplementary Data). In contrast, the experiment performed in the absence of any added metal yielded no green array spots other than the standard (Fig. 2b).” *Id.* at 545-546.



Ex. 1010, Fig. 2.b.

164. A POSA would have understood that the use of fluorophores and the detection allowing calculations and ranks to determine positive bond formations as quantifying the bond forming interactions. Therefore, in my opinion, claim 11 is obvious in view of Kanan and Neri.

12. Dependent Claim 12

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein the analysis comprises high throughput screening.”**

165. Kanan teaches the analysis comprising high throughput screening. Kanan discloses that the bond-forming combinations are analyzed by DNA microarray analysis. Ex. 1010, 545. More specifically, Kanan discloses the “Watson-Crick base pairing controls the effective molarities of substrates tethered to DNA strands; bond-forming substrate combinations are then revealed using *in vitro* selection for bond formation, PCR amplification and DNA microarray analysis.” *Id.* A POSA would have understood that “DNA microarray” method of detection allows for high throughput screening. The ’848 Patent itself states that “[m]icroarray analysis is a conventional platform which enables cost effective data generation in a high throughput manner.” Ex. 1001, 1:47-49.

166. Therefore, in my opinion, claim 12 is obvious in view of Kanan and Neri.

13. Dependent Claim 13

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein the high throughput screening comprises micro array analysis.”**

167. As described above with respect to dependent claim 12 (paragraphs 165-166), Kanan discloses microarray analysis of the bond-forming substrates. Ex.

1010, 545. Thus, it is my opinion that claim 13 is obvious in view of Kanan and Neri.

14. Dependent Claim 14

- (a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein the analysis comprises nucleic acid sequencing.”**

168. Neri teaches the analysis comprising nucleic acid sequencing. A POSA would have understood that it would have been obvious to combine the sequencing of the DNA oligonucleotides as taught in Neri with the analysis of the bond-forming combinations in Kanan.

169. Neri discloses that the pool A and pool B DNA-linked chemical entities are allowed to bond and then after bonding are analyzed by PCR followed by sequencing. Ex. 1011, [0111]-[0119]. A POSA would have understood that Neri’s “sequencing” of the coding region allows identification of the chemical entity in which the “code” identifies. *Id.* at [0112].

170. It would have been obvious to a POSA to include the “sequencing” of Neri to the detection and analysis of the bond-forming substrates taught in Kanan. A POSA would have been motivated to efficiently identify the reactive pairs in Kanan. Sequencing, as disclosed in Neri, is a well-known method to determine the identification sequence of the substrates involved in the bond formation. Ex. 1024, 4866-67. Thus, a POSA would have been motivated to combine the method of

Kanan with sequencing as a method to efficiently detect and analyze the bond-forming substrates.

171. In addition to the reasons discussed above in paragraphs 104-106, a POSA would have a reasonable expectation of success in combining Kanan and Neri because it was well-known and standard practice at the time of the application to do sequencing. Ex. 1011, [0117]-[0119].

172. Therefore, in my opinion, claim 14 is obvious in view of Kanan and Neri.

15. Dependent Claim 15

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 14, wherein the analysis comprises sequencing by synthesis.”**

173. As described above with respect to dependent claim 14 (paragraphs 168-172), Kanan in view of Neri discloses sequencing of the oligonucleotides in Kanan. A POSA would have understood Neri as disclosing Sanger sequencing, which is a form of sequencing by synthesis, because Sanger sequencing was a well-known form of sequencing at the time of the application. *See* Ex. 1028 (Wilton 2002); *see also* Ex. 1030 (Heather 2016).

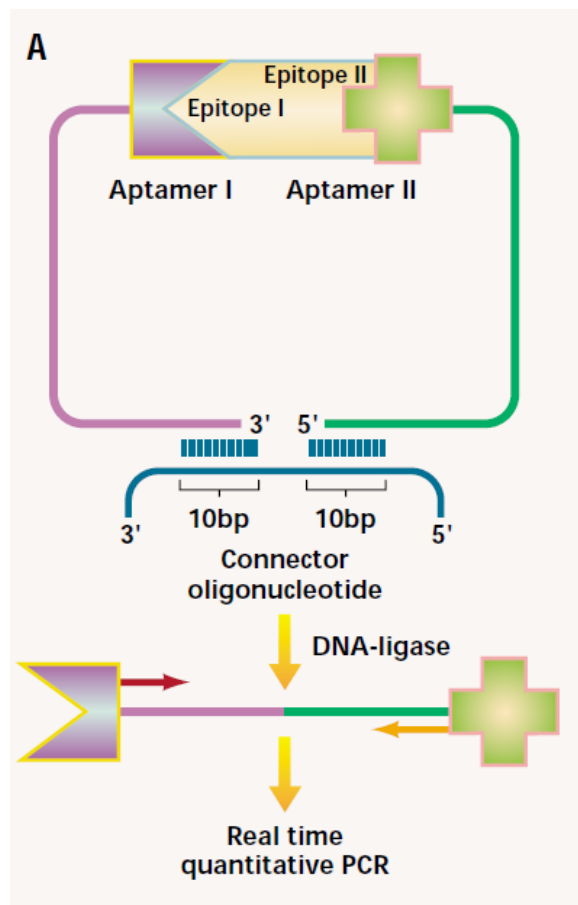
174. Therefore, it is my opinion that claim 15 is obvious in view of Kanan and Neri.

16. Dependent Claim 16

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein association is via ligation.”**

175. The '848 Patent admits ligation as a technique known in the art at the time of the application when it describes that “examples of NAM association approaches which could be used are gap fill polymerization and subsequent ligation and gap ligation as described in Lizardi et al. *Nat Genet*, 19, 225, 232 (1998)[.]” Ex. 1001, 17:32-40. In addition, Famulok teaches associating oligonucleotides via ligation. Ex. 1012.

176. Famulok teaches associating the oligonucleotide extensions of the aptamers by hybridizing the end of the two oligonucleotides to a third “connector” or “splint” oligonucleotide and then ligating the ends together using DNA ligase. Ex. 1012.



Ex. 1012, Fig. 1A.

177. A POSA would have understood that it would have been obvious to use the method of ligation of the oligonucleotides as taught in Famulok to ligate together the ends of the DNA oligonucleotides of the paired substrates in Kanan. A POSA would have been motivated to reduce false negatives produced by errors in the polymerization step—for example, matched pairs not being properly encoded during polymerization. Famulok teaches preparing the matched library members for amplification through ligation instead of polymerization. Ex. 1012, Fig. 1A. A POSA would have been motivated to combine the teachings of Kanan and Neri with

the teachings of Famulok because the splint oligonucleotide disclosed in Famulok would create a DNA strand that does not rely on polymerization to encode the anti-codon of the shorter library member to the sequence of the longer library member. With the ligation approach taught in Famulok, the oligonucleotide would not create false negatives based on errors in polymerization.

178. A POSA would have a reasonable expectation of success in combining Kanan, Neri, Famulok because Neri teaches how to design interactors that contain an overhang region (see Ex. 1011, Fig. 7) that can be ligated together using the method taught in Famulok, and the use of a splint and DNA ligase to connect oligonucleotides was well-known at the time of the application. For example, Kanan's Supplementary Information describes using a splint and DNA ligase to connect the substrate-linked oligonucleotide to the coding/annealing oligonucleotide. Ex. 1013, Fig. S1. In addition, a POSA would have known that these references all relate to encoding a molecule with a unique nucleotide sequence, allowing the encoded molecules to interact, and then detecting and analyzing the identification of the members involved in the interaction. Ex. 1010, 545; Ex. 1011, [0117]-[0119]; Ex. 1012.

179. Therefore, in my opinion, claim 16 is obvious in view of Kanan and Neri and in further view of Famulok.

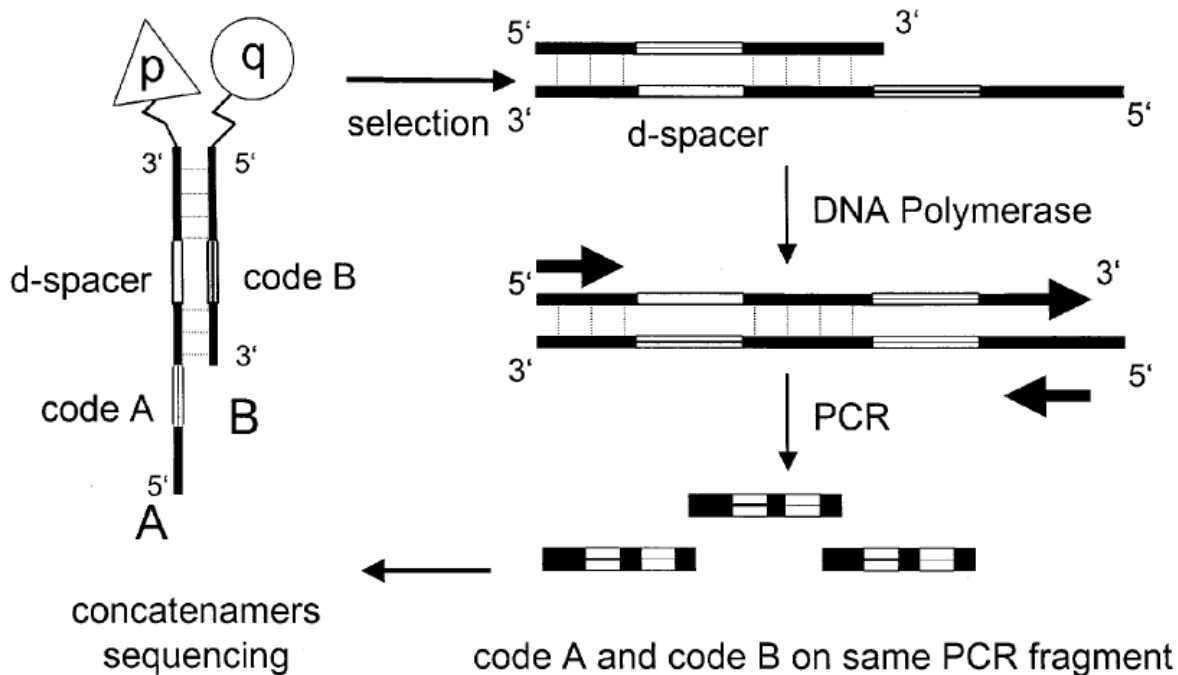
17. Dependent Claim 17

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein association is via polymerization.”**

180. The '848 Patent admits polymerization as a technique known in the art at the time of the application when it describes that “examples of NAM association approaches which could be used are gap fill polymerization and subsequent ligation and gap ligation as described in Lizardi et al. *Nat Genet*, 19, 225, 232 (1998)[.]” Ex. 1001, 17:32-40. In addition, Neri teaches associating the oligonucleotides of the library via polymerization.

181. Neri discloses in Example 3 that the “[o]ligonucleotides of sub-library B remain stably annealed to oligonucleotides of sub-library A, and can work as primers for a DNA polymerase reaction on the template A. The resulting DNA segment, carrying both code A and code B, can be amplified (typically by PCR), using primers which hybridize at the constant extremities of the DNA segment (Fig 5).” Ex. 1011, [0117]; Fig. 5 (below).

Fig. 5



182. A POSA would have understood that it would have been obvious to use the polymerization of the oligonucleotides as taught in Neri with the creation of the paired substrates in Kanan. A POSA would have been motivated to create this double-coding code taught in Neri to increase the specificity of the test. With polymerization, one of the interactors will not only contain its coding region but will also contain the anticodon of the other interactor. Further, encoding the anticodon of the interactor would allow identification of a multistep interaction, if desired. A POSA would have also known it would have been obvious to modify the linkage of the DNA to the substrate so that pool A is attached by the 3' end and pool B is attached by the 5', or vice versa, as desired.

183. In addition to the reasons discussed above in paragraphs 104-106, a POSA would have a reasonable expectation of success in combining Kanan and Neri because the use of primers and polymerase enzymes to extend oligonucleotides was well-known and a standard practice when the application was filed. Ex. 1010, 545; Ex. 1013, Fig. S1; Ex. 1011, Fig. 5, [0117]-[0119].

184. Therefore, in my opinion, claim 17 is obvious in view of Kanan and Neri.

18. Dependent Claim 18

- (a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein the conditions for stimulating the functional interaction between the at least two molecules of interest comprise manipulating conditions to maximize cis reactivity and to minimize trans reactivity between the at least two molecules of interest.”**

185. Kanan teaches manipulating conditions to maximize cis reactivity and to minimize trans reactivity. Kanan discloses “the system must organize complex substrate mixtures into discrete pairs that can react (or not react) without affecting the reactivity of other substrate pairs.” Ex. 1010, 545. To achieve this, Kanan discloses the “Watson-Crick base pairing organizes the mixture into $n \times m$ discrete pairs of substrates attached to complementary sequences. Only substrates linked to complementary oligonucleotides experience effective molarities in the millimolar range[.]” *Id.* Whereas “substrates linked to non-complementary oligonucleotides

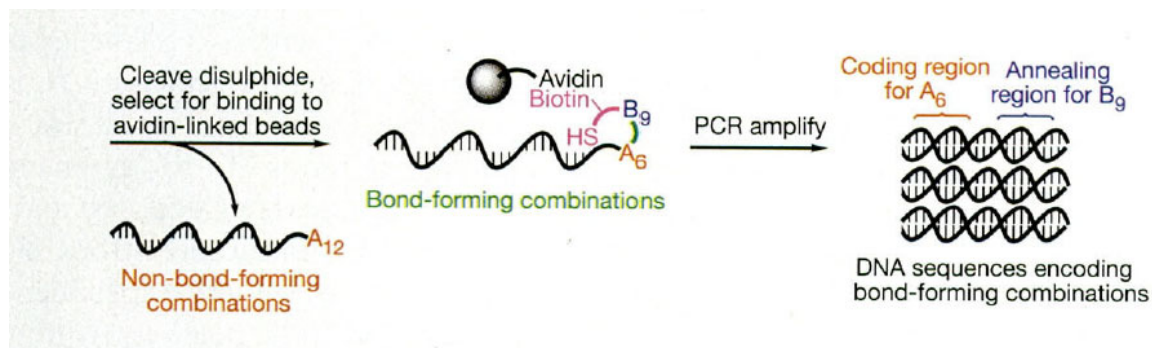
experience nanomolar solution concentrations and . . . do not react with each other at a significant rate.” *Id.* A POSA would have understood that the disclosed organizational method of the substrate pairs would promote formation between pool A and pool B (maximizing cis reactivity) while minimizing formation of bonds between pairs that do not share complementary sequences (minimizing trans reactivity).

186. Therefore, in my opinion, claim 18 is obvious in view of Kanan and Neri.

19. Dependent Claim 19

- (a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 12, wherein the selective detection comprises labeling the cis-reactive cells with an affinity reagent.”**

187. Kanan teaches labeling the CRCs with an affinity reagent. Kanan discloses that members of pool B are labeled with biotin and “[a]fter incubation under a set of chosen reaction conditions, followed by cleavage of the disulphide bonds, only pool A sequences encoding bond formation between a pool A and pool B substrate remain covalently linked to biotin.” Ex. 1010, 545, Fig. 1.b (below).



188. A POSA would have understood biotin as an affinity reagent because the biotin allows for streptavidin affinity selection for the bond-formation combinations. Ex. 1010, 545. Therefore, in my opinion, claim 19 is obvious in view of Kanan and Neri.

20. Dependent Claim 20

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 2, wherein the selective detection comprises introducing a modified molecule wherein the modification permits the selective detection.”**

189. Kanan teaches introducing a modified molecule for selective detection. As discussed above with regards to claim 2 (paragraphs 134-137), Kanan discloses using biotin and streptavidin affinity selection to select the bond-forming pairs. Ex. 1010, 545. The biotin is introduced to the bond-forming pair because the substrate in pool B is linked to biotin. *Id.* at 546, Fig. 1.a. A POSA would have understood the substrate linked to biotin as a modified molecule because the substrate would not contain biotin without modification.

190. Therefore, in my opinion, claim 20 is obvious in view of Kanan and Neri.

C. Kanan With Neri Is Not Cumulative To Substantively Considered Prior Art

191. As discussed above in Section VII, during prosecution, the Examiner considered three references to be close prior art to the then-pending functional claims—Goulaev (Ex. 1036), Franch (Ex. 1037), and Liu (Ex. 1038). According to the Examiner, these references did not teach all of the limitations of the claims. These references also do not provide the same teachings as Neri when combined with Kanan.

192. In the December 30, 2009 Office Action, the Examiner stated Goulaev and Franch teach “steps a-c” but that “[t]hese disclosures . . . fail to teach or suggest step d of claim 20 [now claim 1].” Ex. 1002, 316. I agree with the Examiner that these references do not teach a “detectable trace,” including, the functional interactions creating the detectable trace, selecting CRCs exhibiting a detectable trace, or detecting the identification of both members of the selected CRCs that were involved in the interaction as found in steps d and e of claim 1.

193. Goulaev teaches that its interactors (i.e., building blocks) “are initially brought into reactive proximity by sequential or simultaneous hybridisation of building, [sic] block identifier nucleotides to connector oligonucleotides[.]” Ex. 1036, [0014], Figs. 1A-C. Next, “[a]fter separation of [the] identifier polynucleotide

and the connector oligonucleotide, chemical entities attached to the identifier polynucleotide are reacted, and the reaction product . . . is formed.” *Id.* at [0061]. This reaction between chemical entities is the functional interaction. However, Goulaev does not teach this functional interaction creating a detectable trace. As noted by the Examiner, “[w]hile these references teach that the ‘identifier polynucleotide’ . . . may comprise flanking regions that encompass a signal group, such as a fluorophore (e.g. paragraph [0272] of US 2009/0011957 [Goulaev]), the references do not teach that such a signal is the result of the functional interaction. As taught in these disclosures, the ‘signal’ would be present in the identifier polynucleotides whether any functional interaction had occurred or not.” Ex. 1002, 316. I agree. In addition, because Goulaev does not teach a detectable trace, it cannot teach selecting a CRC exhibiting a detectable trace or detecting the identification elements of the selected CRCs as required by step d in claim 1.

194. The Examiner, however, erred in not recognizing that Kanan teaches creating and selecting a detectable trace using biotin. *See* Section IX.B.1(e), *supra*. As detailed above, unlike Goulaev and Franch, the biotin in Kanan is only present if a functional interaction occurs. *Id.*

195. Goulaev discloses an identification sequence element in that it teaches that the “nucleotide part of a CPN [connector] or a CCPN [building block] harbours information as to the identity of the corresponding reactant or chemical entity linked

to the nucleotide part of the CPN [connector] or the CCPN [building block].” Ex. 1036, [0041]. But such a disclosure does not add to the teachings of Kanan because Goulaev does not teach a reason to modify the current coding regions of Kanan as a POSA would not be motivated to turn to Goulaev to improve upon Kanan, as discussed below.

196. The combination of Kanan with Goulaev also would not yield the same teachings as Kanan with Neri because Goulaev does not teach Neri’s self-assembly sequence and is directed toward synthesizing a molecule. Ex. 1036, Abstract. Like Kanan, Goulaev does not teach a way for all interactors to interact without synthesizing oligonucleotides directed to each interaction (i.e., not combinatorial). Goulaev teaches directed synthesis of a molecule in that “[b]uilding blocks of the present invention are initially brought into reactive proximity by sequential or simultaneous hybridisation of building, [sic] block identifier nucleotides to connector oligonucleotides[.]” *Id.* at [0014]. In other words, “[o]ne or more CPN’s [connectors] guide the synthesis of a molecule by ‘calling’ for CCPNs [building blocks] capable of hybridizing to a CPN [connector].” *Id.* at [0040].

197. In addition, although the Examiner found Goulaev teaches step b, I note that in Goulaev’s preferred embodiment, it does not teach bringing two interactors into proximity through an associated oligonucleotide. Goulaev teaches “it is preferred that the CPNs [connector] comprise only a nucleotide part (connector

oligonucleotides), and no reactant (or chemical entity) or reactive group(s)[,]” and thus in this preferred embodiment, the connector cannot be considered an interactor because it lacks a coupled chemical entity or reactant. *Id.* at [0040].

198. The Examiner did not apply the combination of Kanan and Goulaev to the pending claims, but even if the Examiner had, a POSA would not have been motivated to combine Goulaev with Kanan because Goulaev’s directed synthesis does not teach a way to overcome Kanan’s need to pre-synthesize all combinations of interactors—a problem solved by Neri’s self-assembly sequences. In addition, Goulaev, like Kanan, relies on correct hybridization of the building block to the connector to properly form the identifier polynucleotide. *See* Ex. 1036, [0061], Fig. 1. As a result, a POSA would not have been motivated to modify Kanan’s annealing regions and coding regions to create two discrete regions on each interactor in view of Goulaev, which is instead taught in Neri through its self-assembly sequence and unique identification codes on each interactor. Thus, in my opinion, the combination of Neri and Kanan is not cumulative to the combination of Kanan and Goulaev.

199. As noted by the Examiner, Franch has similar disclosure as Goulaev. Like Goulaev, Franch does not teach a detectable trace because there is no signal generated once the chemical entities react or a way to isolate only the reacted entities. Franch also lacks Goulaev’s flanking regions discussed in Goulaev that contain signal groups. As a result, Franch does not teach or suggest step d or e of claim 1.

200. Franch is also directed toward synthesizing a molecule and does not teach Neri's self-assembly sequence. Ex. 1037, Abstract. Like Kanan and Goulaev, Franch does not teach a way for all interactors to interact in a combinatorial manner. Franch teaches "methods for the synthesis of molecules such as encoded molecules resulting from template directed synthesis involving a plurality of building blocks." Ex. 1037, [0008]. Franch relies on a "template" to "bring[] building block chemical entities into reactive contact with one another." *Id.* at [0014], Fig. 1.

201. The Examiner did not apply the combination of Kanan and Franch to the pending claims, but even if the Examiner had, a POSA would not have been motivated to combine Franch with Kanan because, like Goulaev, Franch's directed synthesis does not teach a way to overcome Kanan's need to pre-synthesize all combinations of interactors—again, a problem solved by Neri. In addition, Franch, like Kanan, relies on correct hybridization of the building block to the template to properly form the identifier polynucleotide. *See* Ex. 1037, Fig. 1. As a result, a POSA would not have been motivated to modify Kanan's annealing regions and coding regions to create two discrete regions on each interactor in view of Franch. Thus, in my opinion, the combination of Neri and Kanan is not cumulative to the combination of Kanan and Franch.

202. Lastly, in the Notice of Allowance, the Examiner found that "Liu did not teach or suggest the formation of an 'associated oligonucleotide[.]'" Ex. 1002,

523. Like Franch, Liu relies on a template to bring the entities, here polymers, into proximity. Ex. 1038, [0006], Fig. 1. I agree with the Examiner that Liu’s template method does not form polymers *from* the association of each polymer subunit (i.e., associated oligonucleotide), and instead forms polymers through affinity to an oligonucleotide sequence template. Ex. 1002, 523. I also agree with the Examiner that “[e]ven if one considers the original nucleic acid template to correspond to the claimed ‘nucleic acid moiety’ of one of the claimed ‘interactors,’ this template is not ‘formed from the association between at least two nucleic acid moieties [of at least two interactors]’ and so cannot be considered as the claimed ‘associated oligonucleotide.’” Ex. 1002, 523 (brackets in original). Thus, in my opinion, Neri is not cumulative to Liu because Neri teaches the claimed “associated oligonucleotide” as discussed in Section IX.B.1(c).

X. Grounds 3 & 4: 1-8, 11-12, And 14-15 Of The ’848 Patent Are Unpatentable

A. Overview Of Co-Pending Litigation

203. On November 15, 2023, Patent Owner, Olink Proteomics AB, filed a patent infringement claim against Alamar asserting that Alamar’s NULISA platform infringes the ’848 Patent. Ex. 1007. The complaint alleges that Alamar’s NULISA platform—“a biomarker detection and quantification assay for proteins” “is based on the proximity litigation [sic] assay (PLA) originally commercialized by Olink Bioscience, the forerunner of Olink.” Ex. 1007, ¶¶ 18-20, 35-39 (quotation

omitted). Patent Owner alleges that NULISA, “[l]ike Olink’s PLA . . . platforms,” “aims to achieve superior sensitivity for detecting low-abundance proteins and ultra-high sensitivity enabled the detection of previously difficult-to-detect but biologically important, low-abundance biomarkers.” *Id.* at ¶ 19 (quotation omitted).

204. Patent Owner alleges Alamar’s PLA-based NULISA product infringes the ’848 Patent despite the specification’s clear statements that its alleged invention is not PLA. *See supra*, paragraph 40. In applying claim 1 of the ’848 Patent to NULISA, the complaint’s allegations map the fundamental aspects of PLA to the elements of the claim. Specifically, the complaint alleges that NULISA’s use of “probes consisting of an antibody and a nucleic acid” to detect a target is detecting a functional interaction. Ex. 1007, ¶¶ 21, 26. These antibody probes are “matched antibody pairs conjugated with oligonucleotides” where one antibody conjugate is a “capture antibody” and the other is a “detection antibody,” which allegedly meet the “identification sequence element” of limitation 1(a). *Id.* at ¶¶ 22-23. The complaint alleges these “probes” “form ‘a plurality of interactors’” found in limitation 1(a). *Id.* at ¶ 21. The complaint also alleges that the “nucleic acid moieties in the NULISA platform . . . also satisfy the claimed ‘association element’ that facilitates association between the DNA probe arms.” *Id.* at ¶ 24 (quoting Ex. 1001 at claim 1(a)).

205. Further, the complaint alleges that a “highly specific immunocomplex forms when a specific protein target is present in the sample while using the

NULISA platform – that is, the two NULISA probes are bound to an analyte.” *Id.* at ¶ 25 (quotation omitted). Patent Owner further alleges that “[a]fter the immunocomplex is formed,” the “ligation of the proximal ends of DNA attached to the paired antibodies[,]” generating “a new DNA reporter molecule containing unique target-specific barcodes” creates an “associated nucleotide.” *Id.* at ¶ 26 (quotation omitted). The complaint also alleges that NULISA practices the limitations related to “detectable trace” because the “highly specific immunocomplex that forms when the target protein is present in the sample” is selected by “NULISA’s dual capture and release mechanism[,]” and “the levels of the DNA reporter can be quantified.” *Id.* at ¶ 27 (quotation omitted).

206. In addition to the complaint (Ex. 1007), I understand from counsel that Patent Owner provided Alamar with an infringement chart (Ex. 1008). In this chart, Patent Owner provided Alamar with its application of how Alamar’s NULISA’s platform allegedly meets each limitation for claims 1-8, 11-12, 14, and 15 of the ’848 Patent.

207. Like the allegations in the complaint, the claim chart (Ex. 1008) maps the claim elements to the foundational steps of PLA. For example, the claim chart alleges that NULISA’s “functional interaction” is the “ligation” between the “cis-reactive cells” and that “[t]he ligation reaction produces a ligated nucleic acid as a detectable trace.” Ex. 1008, 4. For claim limitation 1.a., the chart alleges that “the

matched antibody pairs of the NULISA and the NULISAseq Platforms” “are molecules of interest” and that the oligonucleotides attached to the antibody pairs are the claim’s “nucleic acid moiety.” *Id.* at 1. Further, the claim chart alleges that the “target-specific molecular identifier” on the “double-stranded DNA” attached to the antibodies is the “identification sequence element” and the oligonucleotide’s “capab[ility] of hybridizing to the ligator sequence” is the “association element.” *Id.*

208. For claim limitation 1.b., the chart alleges that a “cis-reactive cell” is formed when “at least two interactors . . . are bound in proximity to one another by the ligator sequence hybridized to the nucleic acid moieties . . .”, indicating that the ligation product is both the “associated oligonucleotide” and the “desired functional interaction having a detectable trace.” *Compare* Ex. 1007, ¶ 26 *with* Ex. 1008, 2. For claim limitation 1.c., the claim chart alleges that “the ligation of the proximal ends of the DNA attached to the paired antibodies” is the “functional interaction” and “the nucleic acid” is the “detectable trace.” Ex. 1008, 4. For claim limitation 1.d., the claim chart alleges that eluting the reporter molecules from streptavidin beads is considered “selecting all cis-reactive cells exhibiting the detectable trace.” *Id.* at 5. Finally, for claim limitation 1.e., the claim chart alleges that “quantification [of the final eluate] by qPCR for single-plex NULISA and by NGS for NULISAseq” is “subjecting the associated oligonucleotides . . . to an analysis that permits detection of the at least two identification sequence elements.” *Id.*

209. I understand from counsel that on April 1, 2024, Alamar moved to dismiss Patent Owner's complaint because (1) it fails to state a claim for infringement and (2) the claim scope cannot cover NULISA's use of proximity ligation to detect a known analyte based on Patent Owner's disclaimer and disavowal of proximity ligation in the '848 Patent. Ex. 1014 (Opening brief at 3-6). In response to Alamar's motion to dismiss, Patent Owner claimed it did not disavow proximity ligation, and thus proximity ligation (i.e., detecting a known analyte) is properly within the scope of the claims of the '848 Patent. Ex. 1015 (Opposition brief at 15-17). I understand from counsel that to date, the district court has not heard or decided Alamar's motion.

210. For my analysis in this declaration related to Grounds 3 and 4, I have relied on Patent Owner's positions provided in its complaint, infringement chart, and opposition to Alamar's motion to dismiss and apply those positions to how a POSA would have understood the claims as if Patent Owner's positions were correct at the time of the application. For clarity, my analysis for Grounds 3 and 4 do not reflect my opinion on what a POSA's ordinary understanding of the scope of the '848 Patent but instead applies the scope used by the Patent Owner in the co-pending district court litigation against Petitioner.

B. Overview Of The Prior Art

1. Baez

211. U.S. Patent No. 6,511,809 (“Baez”) is titled “Method for the Detection of an Analyte by Means of a Nucleic Acid Reporter” (Ex. 1003). Baez published on January 28, 2003. I am informed that Baez qualifies as prior art under at least pre-AIA 35 U.S.C. § 102(b) because it was published more than one year before the earliest possible priority date for the ’848 Patent.

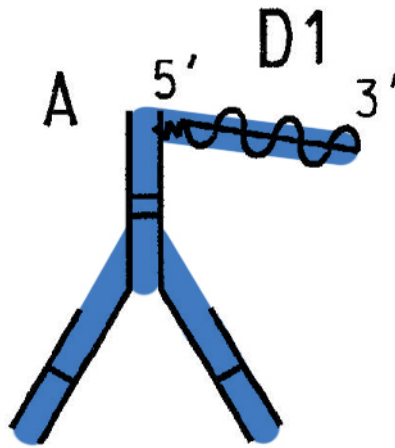
212. Although Baez itself was not disclosed during prosecution of the ’848 Patent, Baez is the published patent of the U.S. application publication identified in the ’848 Patent in column 4 (US2002051986). Ex. 1001, 4:26-29. The ’848 Patent states, “[t]he current inventive method differs from [US2002051986] in that it does not detect or quantify a predefined target in a sample.” Ex. 1001, 4:48-50. During prosecution, the Examiner did not apply Baez or its published patent application against the pending claims.

213. The ’848 Patent describes Baez as teaching proximity ligation. Ex. 1001, 4:26-29. Baez teaches a method “for the detection of an analyte utilizing a nucleic acid label as a reporter.” Ex. 1003, Abstract. This method is the foundational elements of proximity ligation.

214. It is my opinion that based on the clear distinction of the alleged invention from proximity ligation and its detection of a known analyte (*see*

paragraph 40), a POSA would initially not view the claims as being described by Baez. However, based on Patent Owner's assertion that the '848 Patent covers Alamar's NULISA product based on its use of proximity ligation as described in Baez (Ex. 1007; Ex. 1008), a POSA would consider Baez as relevant prior art in light of Patent Owner's position.

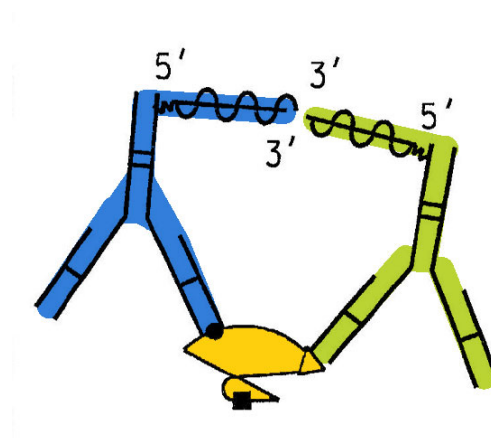
215. Baez teaches a "reporter conjugate," which can be "an antibody, lectin, receptor, or binding protein" that is coupled to a short "nucleic acid-label" and "can bind to an analyte." Ex. 1003, 7:48-57. "The 'nucleic acid-label' may be single-stranded or double-stranded." Ex. 1003, 7:52-53.



Ex. 1003 at Fig. 1 (partial and color added). The "analyte" "may include, but [is] not limited to proteins, peptides, nucleic acid segments, molecules, cells, microorganisms and fragments and products thereof, or any substance for which attachment sites, binding members or receptors (such as antibodies) can be

developed.” Ex. 1003, 7:4-10. These “attachment sites” allow at least two conjugates to bind to a single analyte. *Id.* at 3:34-37.

216. The binding of the reporter conjugates to the attachment sites forms an “analyte-dependent-reporter-complex [ADRC].” Ex. 1003, 11:18-20; *id.* at 9:21-26, 12:59-61.



Ex. 1003, Fig. 1 (partial and color added). The creation of the ADRC brings the two nucleic acid labels of each individual conjugate “in molecular proximity” to each other, as shown above. *Id.* at 11:18-24. “The spatial proximity being close enough to enable the labels to be joined.” *Id.* at 11:23-24.

217. “This joining process is accomplished enzymatically using ligase or nucleic acid polymerase enzymes.” *Id.* at 12:36-38. With ligase, there is “a ligase enzyme that will catalyze the formation of a phosphodiester bond between 3’ hydroxyl and 5’ phosphoryl termini of adjacent oligonucleotides-labels, which are a hydrogen bonded to the ligation linker. The ligation reaction joins the two labels to

form the ASA [analyte specific amplicon].” *Id.* at 14:41-46. This is shown in the figure below.

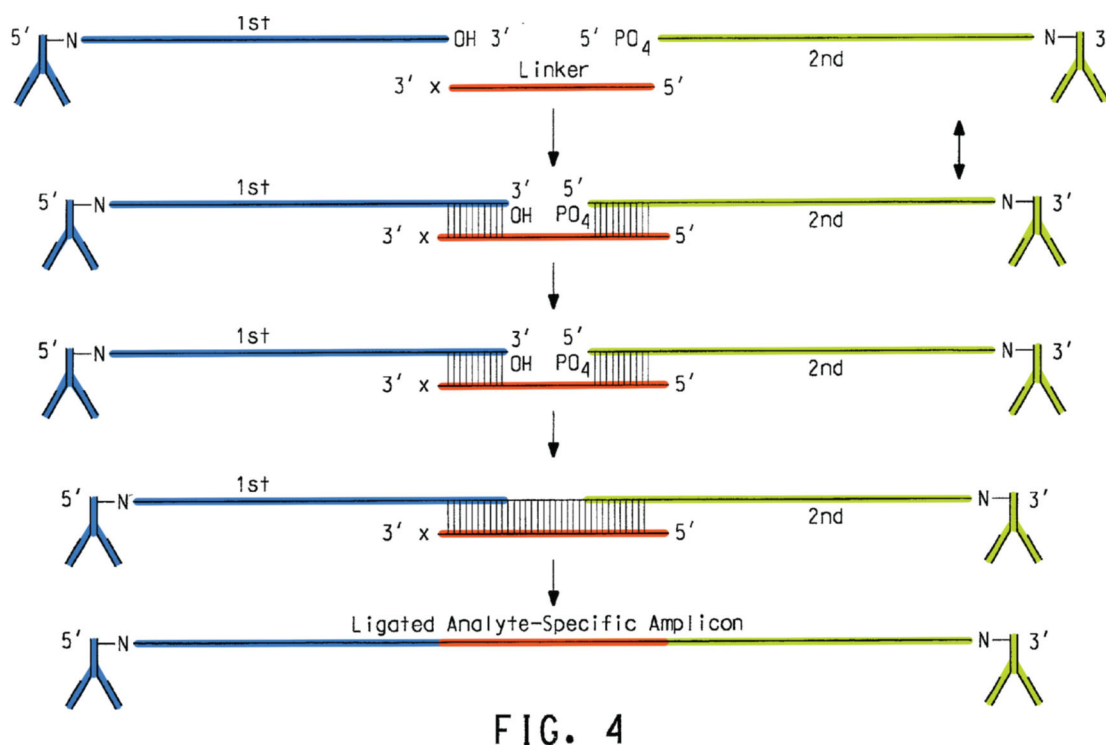


FIG. 4

Ex. 1003, Fig 4. (color added).

218. The ASA (i.e., the ligated product) “is comprised of sequence elements from both nucleic acid-labels. The analyte-specific amplicon [ASA] thus functions as a molecule surrogate for the analyte. Once joined, the analyte-specific amplicon can then be amplified using appropriate primers and polymerase enzyme.” Ex. 1003, 11:30-35. This produces “a detectable nucleic acid product.” *Id.* at 13:9-12.

219. After amplification, the “reaction mixture [can be] analyzed for the ASA amplified product[.]” Ex. 1003, 16:62-64. The amplified product is

“characteristic of the analyte and proportional to the amount of analyte present [in a sample].” *Id.* at 12:49-53.

2. Landegren

220. PCT Publication No. WO01/61037 (“Landegren”) is titled “Methods and Kits for Proximity Probing” (Ex. 1004). Landegren published on August 23, 2001. I am informed that Landegren qualifies as prior art under at least pre-AIA 35 U.S.C. § 102(b) because it was published more than one year before the earliest possible priority date for the ’848 Patent.

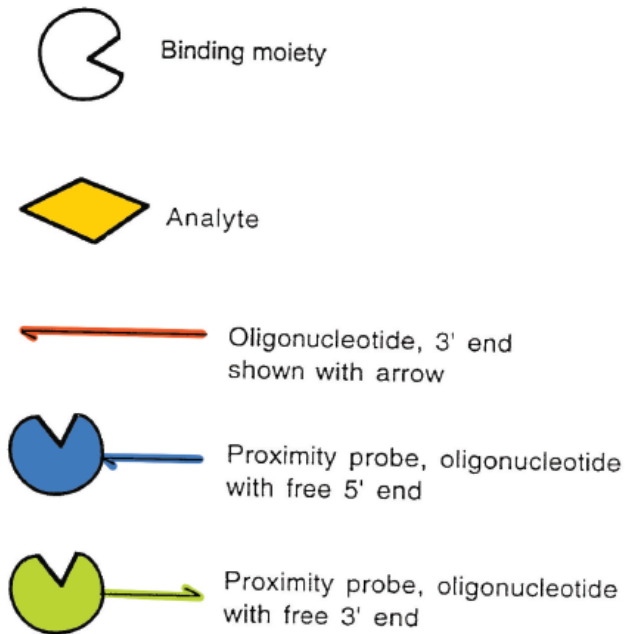
221. Landegren is identified in the ’848 Patent in column 4. Ex. 1001, 4:26-29. The ’848 Patent states, “[t]he current inventive method differs from [Landegren] in that it does not detect or quantify a predefined target in a sample.” Ex. 1001, 4:48-50. During prosecution, the Examiner did not apply Landegren against the pending claims.

222. Landegren discloses “sensitive, rapid and convenient assays for detection and/or quantification of one or several analyte(s) in solution” using proximity probes (called “binders” in the ’848 Patent). Ex. 1004, Abstract; Ex. 1001, 4:29-31.

223. Landegren is the same reference identified in the ’848 Patent in column 4. Ex. 1001, 4:26-29. The ’848 Patent describes Landegren as teaching proximity ligation. *Id.* Like Baez, it is my opinion that based on the clear distinction of the

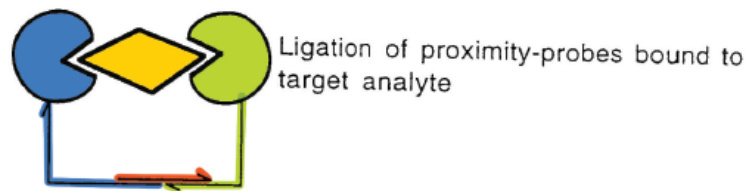
alleged invention from proximity ligation and its detection of a known analyte (*see* paragraph 40), a POSA would initially not view the claims as being described by Landegren. However, based on Patent Owner's assertion that the '848 Patent covers Alamar's NULISA product based on its use of proximity ligation as described in Landegren (Ex. 1007; Ex. 1008), a POSA would consider Landegren as relevant prior art in light of Patent Owner's position.

224. The proximity probes in Landegren "comprise a binding moiety and a nucleic acid." Ex. 1001, 4:26-29. Landegren teaches that the proximity probes contain binding moieties that "may be proteins such as monoclonal and polyclonal antibodies, peptides, lectins, nucleic acids, carbohydrates, soluble cell surface receptors, combinatorially derived protein from phase display or ribosome display, or any other type of binding moiety." Ex. 1004, 3. Landegren also teaches that the proximity probe comprises a "nucleic acid coupled to the binding moiety." *Id.* at 3.



Ex. 1004, Fig. 1 (color added).

225. The binding moiety is designed “with affinity for the analyte.” Ex. 1004, 2. The analyte “may be proteins, nucleic acids or any other molecule of interest capable of specifically binding two or more proximity probes.” *Id.* at 2. The binding of two proximity probes to an analyte “acts in a way analogous to a catalyst by bringing the reactive probes close to one another and increasing their ability to interact through an increased local concentration.” *Id.* at 2. Once in proximity, two probes can hybridize to a linker oligonucleotide and be coupled through ligation. *Id.* at 7.



Id. at Figure 1 (partial and color added). “This mechanism serves to promote ligation in the presence of analyte, and to inactive proximity-probes in the absence of antigens, improving the signal to noise ratio.” *Id.* at 7.

226. “The interaction between the coupled [probes] results in a detectable signal[.]” *Id.* at 2. And “[t]he resulting ligation between these nucleic acids is readily amplifiable by several reactions, such as polymerase chain reaction (PCR) These amplification reactions are thus designed only to give a product when the two nucleic acids have been crosslinked by ligation.” *Id.* at 4. “The resulting product can also be quantified by electrophoresis in gels.” *Id.*

3. Freskgard

227. PCT Publication No. WO 2005/003778 (“Freskgard”) is titled “A Method For Identifying A Synthetic Molecule Having Affinity Towards A Target” (Ex. 1009). Freskgard published on January 13, 2005. I am informed that Freskgard qualifies as prior art under at least pre-AIA 35 U.S.C. § 102(a) because it was published before the earliest possible priority date for the ’848 Patent. During

prosecution, Freskgard was not disclosed, and the Examiner did not consider or apply Freskgard against the pending claims.

228. Freskgard teaches “a novel method for enrichment of libraries of bifunctional complexes.” Ex. 1009, 4:5-6. Freskgard “relates to a method for identifying from a library a synthetic molecule having affinity towards a target. The synthetic molecule has initially been a part of a complex also comprising an identifier that codes for said synthetic molecule.” *Id.* at 1. The identifiers are used to identify the molecule uniquely and “it is preferred that the identifier is a sequence of nucleotides.” *Id.* at 5-6.

229. Freskgard also discloses that libraries of bifunctional complexes can be used to select lead compounds that demonstrate a desired biological activity on a target. *Id.* at 1-2. In one embodiment of Freskgard, “[e]ach bifunctional complex in the library comprises a potential lead compound coupled to an identifier sequence. The identifier sequence is suitably a nucleic acid which uniquely identifies the potential lead compound. When a library of bifunctional complexes is screened against a target, one or more of the potential leads may bind to the target. After removal of the remainder of the library, the binding bifunctional complexes can be eluated and the lead compound identified by sequencing the identifier.” *Id.* at 2.

C. Claims 1-8, 11-12, And 14-15 Are Unpatentable As Obvious Based On Baez In View Of Landegren (Ground 3) And In Further View Of Freskgard (Ground 4)

230. My opinions for Grounds 3 and 4, as detailed below, are reflective of my application of Patent Owner's positions taken in the district court litigation with Alamar. As such, I apply Patent Owner's positions found in its complaint, claim chart, and opposition to Alamar's motion to dismiss to the claims of the '848 Patent in view of the proximity ligation prior art.

1. Independent Claim 1

(a) Preamble: "A method of detecting functional interactions between at least two molecules of interests, the method comprising:"

231. Relying on Patent Owner's positions regarding the scope of the '848 Patent, a POSA would have understood Baez as disclosing the preamble of claim 1. Baez discloses detecting nucleic acid sequences generated by the bonding of two reporter conjugates once they are brought into proximity to each other through affinity to a specific analyte. Ex. 1003, 11:10-35, 11:55-67.

232. Patent Owner contends a molecule of interest includes "the matched antibody pairs of the NULISA and NULISaseq Platforms." Ex. 1008, 1. Thus, applying Patent Owner's position, a POSA would have understood the antibody conjugates disclosed in Baez as a molecule of interest. Ex. 1001, 10:51-56. Patent Owner also contends that the "functional interaction" in the '848 claims is the "ligation" of the NULISA antibody pairs. Ex. 1001, 14:23-27; Ex. 1008, 4. Thus,

applying Patent Owner's position, a POSA would have understood the ligation of the conjugates disclosed in Baez as a functional interaction. Ex. 1001, 9:27-31; Figs. 2, 4.

233. Therefore, relying on Patent Owner's positions, it is my opinion that Baez discloses the claim 1 preamble.

(b) Step a: “forming a plurality of interactors by coupling each molecule of interest with at least one nucleic acid moiety, the nucleic acid moiety comprising an identification sequence element and an association element;”

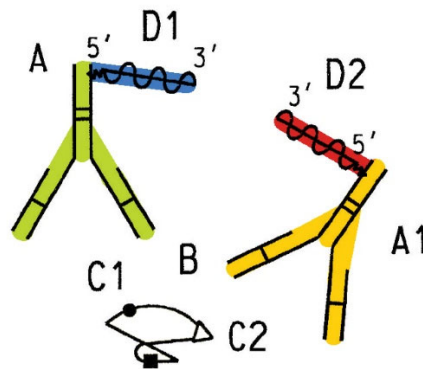
234. Relying on Patent Owner's positions regarding the scope of the '848 Patent, a POSA would have understood Baez as teaching “forming a plurality of interactors.” Baez teaches that the binding partner, which can be “an antibody, lectin, receptor or binding protein,” is tagged with a nucleic acid sequence, creating a reporter conjugate. Ex. 1003, 7:54-57, 7:48-53.

235. Patent Owner contends antibodies coupled to nucleic acid moieties are the “interactors” in NULISA. Ex. 1008, 1. Specifically, Patent Owner states, “[t]he NULISA and NULISAseq Platforms can be used to carry out a method of detecting functional interactions between at least two molecules of interest by *forming a plurality of interactors by coupling each molecule of interest with at least one nucleic acid moiety.*” Ex. 1008, 1 (emphasis added). Patent Owner further states that NULISA meets this limitation because “the matched antibody pairs of the

NULISA and NULISaseq Platforms (shown in green and yellow in the figure below) are molecules of interest as the user of the platform is seeking to obtain information regarding these antibodies, and the matched antibody pairs are conjugated with at least once nucleic acid moiety (oligonucleotides, shown in blue and red in the figure below), *comprising an identification sequence element (target-specific molecular identifier or TMI) and an association element (capable of hybridizing to the ligator sequence).*” Ex. 1008, 1 (emphasis added). Thus, applying Patent Owner’s positions, a POSA would have understood the “reporter conjugates” disclosed in Baez are “interactors.” Ex. 1003, 7:53-56, 10:51-56.



Ex. 1008, 1.



Ex. 1003, Fig 1 (partial and color added).

236. Baez also discloses the nucleic acid moiety comprising an association element. Baez discloses that the nucleic acid sequences of two reporter conjugates can have either (1) two 3’ ends or (2) a 3’ end and a 5’ end. Ex. 1003, Fig. 3, Fig. 4. In the first case, the 3’ ends are designed to overlap within the ADRC, wherein overlap “refers to the annealing of the 3’ ends of the two proximal positioned DNA

labels bound to antibodies to form an overlapped duplex. Once formed, both 3' OH ends of the overlapped duplex serve as primers for a nucleic acid polymerase extension reaction.” *Id.* at 9:61-65, 18:56-19:11. In the second case, the two nucleic sequences are complementary to a “ligation linker” oligonucleotide such that annealing of both sequences to the ligation linker holds “the 3' hydroxyl group of one oligonucleotide in adjacent position to the 5' phosphorylated terminus of a second oligonucleotide so that a ligase can catalyze the formation of a phosphodiester bond between the two oligonucleotides” (i.e., nucleic acid sequences). *Id.* at 10:16-26. Thus, a POSA would have understood that the ability of the two nucleic acid labels to be joined as described in either format is considered the association element.

237. Landegren discloses a nucleic acid moiety comprising an identification sequence, i.e., the identification element. Landegren teaches that the nucleic acid coupled to the proximity probe (i.e., conjugate) has a sequence identification element or “tag.” Ex. 1004, 11. These tags generate a positive signal “when the two matching sequence identification tags (ID:A and ID:B) are united.” *Id.* at 11. A POSA would have understood that Landegren’s “sequence identification tags” provide a unique nucleic acid sequence to each proximity probe (or conjugate) that can be identified downstream.

238. Applying Patent Owner's position that the '848 Patent covers the steps of PLA, it would have been obvious to a POSA to add the "sequence identification tags" of Landegren to the nucleic acid labels of Baez because the identification element would allow analysis of a multiplex assay. *Id.* at 15. A POSA would have been motivated to create a multiplex assay in order to detect and analyze multiple analytes in a single assay. A POSA would also have been motivated to search for a way to reduce background signal in the assay. As Baez explains, "[m]ethods for the reduction of background noise are continually being sought." Ex. 1003, 1:32-33. The strategies employed in Landegren such as use of unique identifier sequences allow for the selection of only true positives. Thus, a POSA would have been motivated to combine Baez with Landegren as a way to reduce background signal in the assay.

239. A POSA would have a reasonable expectation of success in combining Baez and Landegren. Both references relate to using "an antibody, lectin, receptor or binding protein or other moiety which can bind to an analyte" coupled to nucleic acid to detect an analyte. Ex. 1003, Abstract, 7:53-56; Ex. 1004, 1, 3. Moreover, both Baez and Landegren disclose that the analyte is detected by the interaction of the nucleic acid sequences coupled to each member of a binding pair (e.g., antibodies). Ex. 1003, Abstract; Ex. 1004, 3.

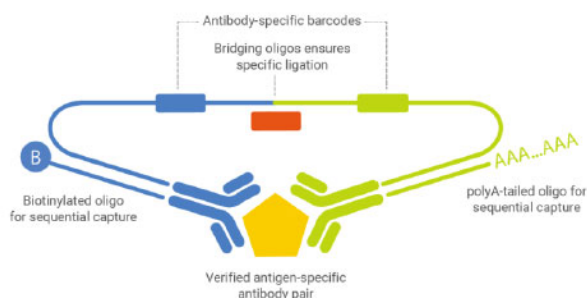
240. Therefore, relying on Patent Owner's positions, it is my opinion, Baez in view of Landegren discloses forming a plurality of interactors by coupling each molecule of interest with at least one nucleic acid moiety, the nucleic acid moiety comprising an identification sequence element and an association element.

(c) **Step b: "forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors bound in proximity to one another by an associated oligonucleotide formed from the association between at least two nucleic acid moieties, wherein the associated oligonucleotides comprise at least two identification elements derived from the at least two nucleic acid moieties;"**

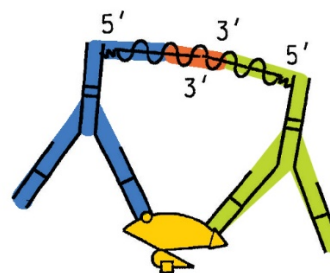
241. Relying on Patent Owner's positions regarding the scope of the '848 Patent, a POSA would have understood Baez as teaching "forming a plurality of cis-reactive cells."

242. Patent Owner contends that the "immunocomplex with ligator" in NULISA is a cis-reactive cell that comprises the interactors. Ex. 1008, 2. Patent Owner contends the interactors are "the NULISA probes, comprised of the antibodies and nucleic acid moieties," and that when they are "interacting with an analyte" and "are bound in proximity to one another by the ligator sequence hybridized to the nucleic acid moieties" that forms a cis-reactive cell. *Id.* at 2. Patent Owner further claims that the "cis-reactive cell (immunocomplex with ligator) comprises at least two interactors (two matched antibodies conjugated with at least one nucleic acid moiety, shown in blue and green in the figure [below]) bound in

proximity to one another by an associated oligonucleotide formed from the association between the at least two nucleic acid moieties and the ligator sequence, so that the associated oligonucleotide comprises at least two identification elements derived from the at least two nucleic acid moieties.” *Id.* Thus, applying Patent Owner’s positions, a POSA would have understood that “cis-reactive cell” includes the reporter strands of antibody conjugates hybridized to an oligonucleotide linker after the conjugates are bound to an analyte or target (i.e., the ASA disclosed in Baez).



Ex. 1008, 2.



Ex. 1003, Fig 2 (partial and color added).

243. As described above in paragraphs 216 and 217, Baez teaches the formation of an “analyte-specific amplicon” (ASA) that refers to the joining of the nucleic acid labels from the two reporter conjugates in the presence of a ligation linker once the labels are brought into proximity to each other. Ex. 1003, 9:26-30, 10:16-18.

244. Further, as shown above (paragraphs 237 and 238), the nucleic acid labels comprise a unique identification sequence. Thus, a POSA would have understood that the nucleic acid labels within the ASA would contain the two identification sequences, one from each label.

245. Therefore, relying on Patent Owner's positions, it is my opinion that Baez in view of Landegren discloses forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors bound in proximity to one another by an associated oligonucleotide formed from the association between at least two nucleic acid moieties, wherein the associated oligonucleotides comprise at least two identification elements derived from the at least two nucleic acid moieties.

(d) Step c: “subjecting the plurality of cis-reactive cells to conditions which stimulate desired functional interaction having a detectable trace;”

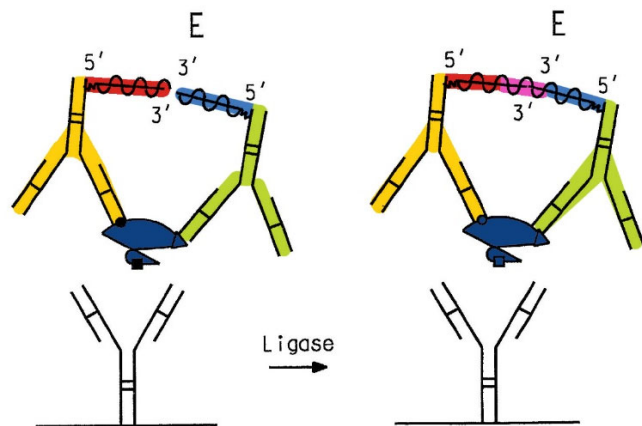
246. Relying on Patent Owner's positions regarding the scope of the '848 Patent, a POSA would have understood Baez as teaching subjecting the CRCs to conditions that stimulate a desired functional interaction with a detectable trace.

247. Patent Owner contends “ligation” of the reporters in NULISA is the required detectable trace. Ex. 1008, 4. In the claim chart, Patent Owner states that “[t]he NULISA and NULISaseq Platforms can be used to subject the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction (ligation) having a detectable trace. The ligation reaction produces a ligated nucleic

acid as a detectable trace.” *Id.* Patent Owner further states that “the use of the NULISA and NULISAseq Platforms includes a step wherein a ligation mix containing T4 DNA ligase and a specific DNA ligator sequence is added to the streptavidin beads, allowing the ligation of the proximal ends of the DNA attached to the paired antibodies and thus generating a DNA reporter molecule containing unique pairs of TMIs (Fig. 1a–6).” *Id.* (internal quotation omitted). “This step of introducing the ligation mix stimulates a desired functional interaction (ligation) having a detectable trace.” *Id.* Thus, applying Patent Owner’s positions, a POSA would have understood that the nucleic acid sequence generated after the ligation of labels coupled to the antibodies in Baez is a “detectable trace.”



Ex. 1008, 4.



Ex. 1003, Fig 2 (partial and color added).

248. Baez teaches, in the ligation approach, that the “3’ and 5’ ends of two proximal positioned antibody bound DNA labels” are ligated together “by the

enzyme ligase in the presence of a ‘ligation linker.’” Ex. 1003, 10:16-18. The ligation linker “is an oligonucleotide that has the complementary strand hydrogen bonded to two oligonucleotides such that it is holding the 3’ hydroxyl group of one oligonucleotide in adjacent position to the 5’ phosphorylated terminus of the a [sic] second oligonucleotide so that a ligase can catalyze the formation of a phosphodiester bond between the two oligonucleotides.” *Id.* at 10:20-26.

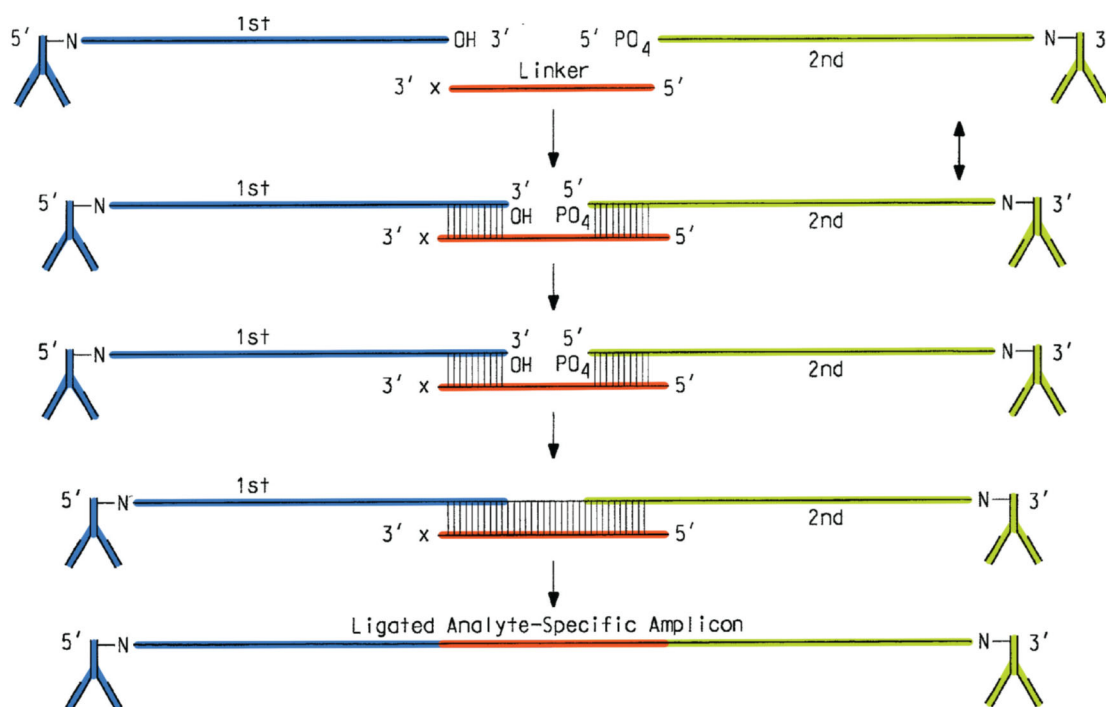


FIG. 4

Id. at Fig. 4 (color added and annotated) (creating analyte-specific amplicon when bound to analyte (analyte not shown)).

249. Therefore, relying on Patent Owner’s positions, it is my opinion that Baez discloses subjecting the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction having a detectable trace.

(e) Step d: “selecting all cis-reactive cells exhibiting the detectable trace; and”

250. Relying on Patent Owner’s positions regarding the scope of the ’848 Patent, a POSA would have understood Baez as teaching selecting the CRCs that exhibit a detectable trace.

251. Patent Owner contends “selecting” the CRCs with a detectable trace is the biotin-streptavidin additional wash (after ligation) and elution in NULISA. Ex. 1008, 5. Specifically, Patent Owner contends that “the use of the NULISA and NULISAseq Platforms includes a step wherein after ligation and one additional wash, reporter molecules were eluted from the SA beads using 50 µL EB2 buffer for 10 min at room temperature. The final eluate was collected for quantification by qPCR for single-plex NULISA and by NGS for NULISAseq.” *Id.* (internal quotation omitted). Thus, applying Patent Owner’s positions, a POSA would have understood that isolating the CRCs (ASAs) comprising a biotin label using a streptavidin solid support structure as taught in Baez is “selecting” the CRCs.

252. Baez discloses that the ASAs can be selected using affinity purification. This involves detecting the ASA “through the linker oligonucleotide itself.” Ex. 1003, 17:52-53. In one example, the linker oligonucleotide is “engineered to contain a label such as biotin[.]” *Id.* at 17:53-54. Once the linker is bound in the ASA, the incorporation of biotinylated bases provides means of isolating the ASA from the solution onto an immobilized streptavidin or avidin. *E.g.*, Ex. 1003, 17:25-28. A

POSA would have understood that the use of the biotin-streptavidin capture and wash would allow selection of only the ASAs that have been ligated together, i.e., demonstrate the detectable trace, because only those ASAs would have the affinity for the immobilized streptavidin.

253. Therefore, relying on Patent Owner's positions, in my opinion, Baez discloses selecting all cis-reactive cells exhibiting the detectable trace.

(f) Step e: “subjecting the associated oligonucleotides from the cis-reactive cells selected in step (d) to an analysis that permits detection of the at least two identification sequence elements.”

254. Relying on Patent Owner's positions regarding the scope of the '848 Patent, a POSA would have understood Baez as teaching subjecting the selected CRCs from step (d) to an analysis that permits detection of the at least two identification sequence elements.

255. Patent Owner contends detecting the identification sequence elements includes PCR analysis of the final eluate in NULISA. Ex. 1008, 5. Patent Owner contends NULISA meets this claim limitation because the “NULISA and NULISAsseq Platforms can be used to subject the associated oligonucleotides (i.e. the two interactors bound in proximity to one another by the ligator sequence hybridized to the nucleic acid moieties and subsequently ligated in step c to form reporter molecules) from the cis-reactive cells to an analysis that permits detection of the at least two identification sequence elements.” *Id.* Specifically, “the use of

the NULISA and NULISAseq Platforms includes the following steps of “[t]he final eluate was collected for quantification by qPCR for single-plex NULISA and by NGS for NULISAseq.” *Id.* Thus, applying Patent Owner’s contentions, a POSA would have understood that amplifying the ASA with PCR and subsequent detection as taught in Baez is “detecting the identification sequence elements.”

256. Baez teaches that the ASA product is amplified by PCR and the “PCR amplification product can be detected by various methods known in the art, such as, gel electrophoresis, hybridization of sequence-specific probes or detected indirectly through incorporation of fluorophores, ligands or haptens.” Ex. 1003, 5:26-29, 5:38-42, Fig. 5. A POSA would have understood that detection of the amplified ASA product permits the detection of the at least two identification sequence elements because it is the resulting nucleic acid, which includes the identification sequence elements, that is amplified with PCR.

257. Further, it would have been obvious to a POSA to perform the PCR amplification after the selection of the ASA using the biotin linker discussed in paragraph 252. Baez teaches that “the labels can be designed to facilitate replication and amplification of the analyte specific amplification[.]” *Id.* at 11:60-63. A POSA would have known the purpose of incorporating biotin to the label is to assist with isolation of the ASA. *E.g.*, Ex. 1003, 17:24-29. Baez also teaches that the “[biotin] labeled ASA would then be detected by any number of detection strategies.” *Id.* at

18:7-12. A POSA would have been motivated to perform the amplification after the selection of the ASAs as a technique to remove non-analyte bound reporters and increase the sensitivity of the assay. *See id.* at 2:51-53 (“It is the objective of the art to increase the sensitivity of analyte detection through the use of various novel signal generating reporter conjugates and amplification strategies.”)

258. Therefore, relying on Patent Owner’s positions, in my opinion, Baez discloses subjecting the associated oligonucleotides from the cis-reactive cells selected in step (d) to an analysis that permits detection of the at least two identification sequence elements.

259. As shown above, relying on Patent Owner’s positions, in my opinion, claim 1 is obvious in view of Baez and Landegren.

2. Dependent Claim 2

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein selecting all cis-reactive cells exhibiting the detectable trace is accomplished via selective separation or selective detection of the cis-reactive cells.”**

260. During prosecution, Patent Owner admitted that “[b]oth selective separation and selective detection are techniques well known in the art[.]” Ex. 1002, 505.

261. Further, relying on Patent Owner's positions regarding the scope of the '848 Patent, a POSA would have understood Baez as teaching selective detection of the cis-reactive cells.

262. Patent Owner contends selective separation or selective detection of the cis-reactive cells is the biotin-streptavidin wash and elution in NULISA. Ex. 1008, 6. Specifically, Patent Owner contends that "[w]hen using the NULISA and NULISAseq Platforms, the wash and elution steps are selection steps that constitute selective separation or selective detection of the cis-reactive cells." *Id.* Thus, applying Patent Owner's positions, a POSA would have understood that the affinity interactions between the CRCs (ASAs) and a solid support structure as taught in Baez is "selective detection." A POSA would have understood that the use of the biotin-streptavidin wash taught in Baez would allow selective detection of only the ASAs that have been ligated together because only those ASAs would have the affinity for the immobilized streptavidin.

263. As discussed above in paragraph 252, Baez discloses that the ASAs can be selectively detected using a linker oligonucleotide "engineered to contain a label such as biotin[.]" Ex. 1003, 17:53-54. Once the linker is bound in the ASA, the incorporation of the biotin label provides means of isolating the ASA from the solution onto an immobilized streptavidin or avidin. *E.g.*, Ex. 1003, 17:25-28.

264. Therefore, relying on Patent Owner's positions, in my opinion, claim 2 is obvious in view of Baez and Landegren.

3. Dependent Claim 3

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 2, wherein selecting all cis-reactive cells exhibiting the detectable trace is accomplished by affinity purification.”**

265. Patent Owner contends that “[w]hen using the NULISA and NULISAsseq Platforms, the wash and elution steps are selection steps that constitute affinity purification.” Ex. 1008, 6. As described above with respect to dependent claim 2 (paragraph 263), Baez discloses that the ASAs can be selected using a biotin label that allows affinity purification to isolate the ASA in the solution using an immobilized streptavidin or avidin. *E.g.*, Ex. 1003, 17:25-28. Thus, relying on Patent Owner's positions, it is my opinion that claim 3 is obvious in view of Baez and Landegren. *See also* paragraphs 250-253.

4. Dependent Claim 4

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, further comprising enhancing the selected associated oligonucleotides by PCR amplification.”**

266. Relying on Patent Owner's positions regarding the scope of the '848 Patent, a POSA would have understood Baez as teaching enhancing the associated oligonucleotides by PCR amplification.

267. Patent Owner contends that “[w]hen using the NULISA and NULISAsseq Platforms, the detection of the selected associated oligonucleotides (i.e., the reporter molecules) is enhanced by PCR amplification.” Ex. 1008, 7. Patent Owner contends, as an example, this limitation is met because the “DNA reporter can then be quantified by quantitative PCR (qPCR) for single-plex analysis” with NULISA. *Id.* Thus, applying Patent Owner’s positions, a POSA would have understood that the PCR amplification of the ASA product as taught in Baez is PCR amplification of the associated oligonucleotides.

268. Baez teaches that the nucleic acid sequence of the ASA product can be amplified using PCR. Ex. 1003, 8:17-24, 22:41-42, Fig. 5. As shown in Figure 5 below, the two nucleic acid labels are ligated together and amplified using PCR.

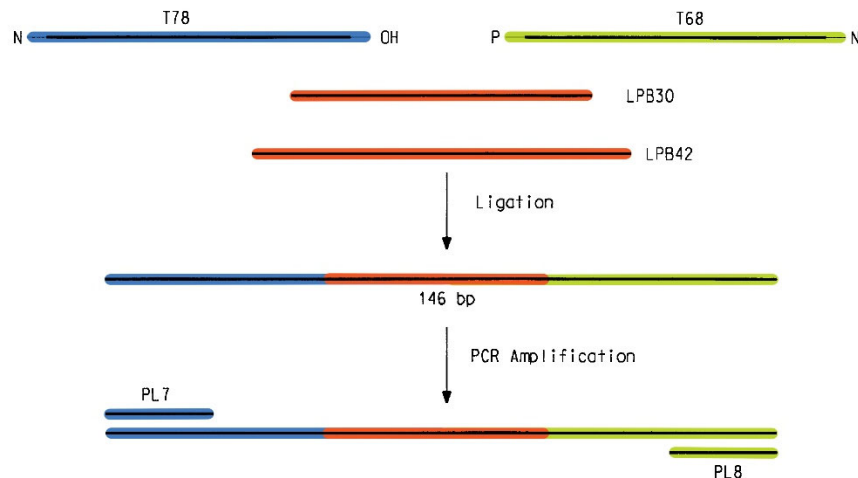


FIG. 5

Ex. 1003, Fig. 5 (color added).

269. Therefore, relying on Patent Owner's positions, in my opinion, claim 4 is obvious in view of Baez and Landegren.

5. Dependent Claim 5

(a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein coupling is covalent or noncovalent.”

270. Relying on Patent Owner's positions regarding the scope of the '848 Patent, a POSA would have understood Baez as teaching “wherein the coupling is covalent.”

271. Patent Owner contends that “[w]hen using the NULISA and NULISAsseq Platforms, the coupling of the molecule of interest (antibody) with the nucleic acid moiety (oligonucleotide) is covalent.” Ex. 1008, 7. As an example, Patent Owner contends the “amine-to-sulfhydryl cross-linking chemistry” utilized for the “[a]ntibody-oligonucleotide conjugation” in NULISA is covalent. *Id.* Thus, applying Patent Owner's positions, a POSA would have understood that the covalent bond between the antibody and oligonucleotide as taught in Baez is covalent coupling.

272. Baez discloses that the “DNA labels” “were covalently attached to their respective antibodies through 5' terminal amine groups.” Ex. 1003, 26:57-59.

273. Therefore, relying on Patent Owner's positions, in my opinion, claim 5 is obvious in view of Baez and Landegren.

6. Dependent Claim 6

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein coupling is covalent.”**

274. As described above with respect to dependent claim 5 (paragraphs 270-273), Patent Owner contends covalent coupling includes a covalent bond between the antibody and oligonucleotide, and Baez discloses that the “DNA labels” “were covalently attached to their respective antibodies through 5’ terminal amine groups.” Ex. 1003, 26:57-59. Thus, relying on Patent Owner’s positions, it is my opinion that claim 6 is obvious in view of Baez and Landegren.

7. Dependent Claim 7

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein at least one molecule of interest comprises a protein, a protein domain, a peptide, a peptoid, a non-proteinaceous cellular product, a virus particle, a cell metabolite, a lipid, a carbohydrate, a nucleic acid, an inorganic compound, a reactive compound, or any combination thereof, however, wherein not all molecules of interest are nucleic acids.”**

275. Relying on Patent Owner’s positions regarding the scope of the ’848 Patent, a POSA would have understood Baez as teaching a molecule of interest being a protein.

276. Patent Owner contends that this limitation is met because “[w]hen using the NULISA and NULISaseq Platforms, at least one molecule of interest (e.g.

capture antibody or detection antibody) is a protein.” Ex. 1008, 8; *see also* paragraph 232. Thus, applying Patent Owner’s positions, a POSA would have understood that the antibodies taught in Baez are molecules of interest being a protein.

277. Baez teaches that the conjugates disclosed in Baez can be an antibody or a binding protein. Ex. 1003, 7:54-57.

278. Therefore, relying on Patent Owner’s positions, in my opinion, claim 7 is obvious in view of Baez and Landegren.

8. Dependent Claim 8

- (a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 7, wherein at least one molecule of interest comprises at least one protein.”**

279. As described above with respect to dependent claim 7 (paragraphs 275-278), Patent Owner contends that the antibodies are protein molecules of interest, and Baez discloses antibodies as molecules of interest. Thus, relying on Patent Owner’s positions, it is my opinion that claim 8 is obvious in view of Baez and Landegren.

9. Dependent Claim 11

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein detecting further comprises quantifying the functional interactions.”**

280. Relying on Patent Owner’s positions regarding the scope of the ’848 Patent, a POSA would have understood Baez as teaching quantifying the functional interactions.

281. Patent Owner contends that quantifying the functional interactions is satisfied by quantitative PCR (qPCR) of the ligation product in NULISA. Ex. 1008, 9. Specifically, Patent Owner contends that “[w]hen using the NULISA and NULISAsseq Platforms, the method of detecting includes quantifying the functional interactions” because NULISA includes “Quantify by PCR” and “Quantify by NGS.” *Id.* Thus, applying Patent Owner’s positions, a POSA would have understood that quantifying the ASA product as taught in Baez is quantifying the functional interactions.

282. Baez discloses that its assay “provides means for both qualitative and quantitative analyte detection.” Ex. 1003, 12:2-3. Baez further discloses that “[d]uring the assay, both the type of analyte-specific amplicon formed and the amount of the nucleic acid amplification product produced are characteristic of the analyte and proportional to the amount of analyte present[,]” allowing the analytes to be “detected and quantified.” *Id.*, 12:49-53; *see also id.*, 7:41-43, 11:60-12:3,

13:13-17. It would have been within the knowledge and experience of a POSA to use the well-known method of quantitative PCR (Ex. 1027 (Reischl 1995)) for quantification of the ASA product in Baez.

283. Therefore, relying on Patent Owner's positions, in my opinion, claim 11 is obvious in view of Baez and Landegren.

10. Dependent Claim 12

(a) “The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein the analysis comprises high throughput screening.”

284. Relying on Patent Owner's positions regarding the scope of the '848 Patent, a POSA would have understood Landegren as teaching the analysis comprising high throughput screening.

285. Patent Owner contends that high throughput screening of the ligation product using the “ARGO HT System” in NULISA satisfies this limitation. Ex. 1008, 9. Specifically, Patent Owner states that “[w]hen using the NULISA and NULISaseq Platforms, the method of detecting includes high throughput screening,” relying on Alamar's website, which states that the “ARGO HT System is a fully automated, high-throughput, precision proteomics platform for ultra-high sensitivity analysis of proteins across a range of multiplex levels to support broad biomarker profiling and translation into clinical use.” *Id.* Thus, applying Patent Owner's positions, a POSA would have understood that high throughput screening

of the ASA product as taught in Landegren is high throughput screening of the detectable trace.

286. Landegren teaches that the ligation product (i.e., ASA) and its identification tags “may be detected by any of several methods, such as DNA microarrays, mass spectrometry, gel electrophoresis (different lengths of products), or other.” Ex. 1004, 15. A POSA would have understood that the “DNA microarray” method of detection allows for high throughput screening, as the ’848 Patent itself states that “[m]icroarray analysis is a conventional platform which enables cost effective data generation in a high throughput manner.” Ex. 1001, 1:47-49.

287. Applying Patent Owner’s positions taken in the district court litigation, it would have been obvious to a POSA to combine the high throughput screening of the ligation product taught in Landegren with the analysis of the ASA product in Baez because high throughput screening allows for quicker assay results. A POSA would have been motivated to include high throughput screening to increase the number of assays that can be run in a certain time period. As discussed above in paragraph 239, a POSA would have a reasonable expectation of success in combining Baez and Landegren.

288. Therefore, relying on Patent Owner’s positions, in my opinion, claim 12 is obvious in view of Baez and Landegren.

11. Dependent Claim 14

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein the analysis comprises nucleic acid sequencing.”**

289. Relying on Patent Owner’s positions regarding the scope of the ’848 Patent, a POSA would have understood Freskgard as teaching the analysis comprising nucleic acid sequencing, including the method of sequencing by synthesis, which includes Sanger sequencing.

290. Patent Owner contends that sequencing of the ligation product using “Illumina NGS technology” in NULISA satisfies this limitation. Ex. 1008, 9. Specifically, Patent Owner states that “[w]hen using the NULISAseq Platform, the method of detecting includes nucleic acid sequencing,” pointing to a statement that “[a]fter ligation, the ligation products are pooled, amplified, and purified to generate a sequencing library that is compatible with multiple Illumina sequencing instruments.” *Id.* Thus, applying Patent Owner’s positions, a POSA would have understood that it would have been obvious to combine the sequencing of the identifiers as taught in Freskgard with the analysis of the ligation product in Baez and Landegren.

291. Freskgard discloses that the identifier (i.e., the nucleic acid tag) is identified by sequencing. Ex. 1009, 2. In one embodiment, “the different identifier sequences of the isolated bifunctional complexes are cloned into separate sequencing

vectors prior to determining their sequence by DNA sequencing methods.” *Id.* at 36. Freskgard states, “[t]he cloning and sequencing of the amplified fragments then is a routine procedure that can be carried out by any of a number of molecular biological methods known in the art.” *Id.* A POSA would have understood that Freskgard’s “sequencing” is used to identify the nucleic acid sequence of the identifier oligonucleotides.

292. Freskgard also discloses that the identifier (i.e., the nucleic acid tag) is identified by sequencing by synthesis. Ex. 1009, 69-70. In one example, “[a] sample of each PCR product was then treated with Exonuclease I (USB) and Shrimp Alkaline Phosphatase (USB) to remove degrade[d] single stranded DNA and dNTPs and sequenced using the DYEnamic ET cycle sequencing kit (Amersham Biosciences) according to the manufacturer’s instructions and the reactions were analyzed on a MegaBace 4000 capillary sequencer (Amersham Biosciences).” *Id.* A POSA would have understood Freskgard as disclosing Sanger sequencing, which is a form of sequencing by synthesis well known in the art to POSAs. *See* Ex. 1028 (Wilton 2002); Ex. 1029 (MegaBace Manual); *see also* Ex. 1030; Ex. 1031 (Evans 2019).

293. Applying Patent Owner’s positions, it would have been obvious to a POSA to include the “sequencing” of Freskgard to the detection and analysis of ligation product (i.e., ASA) and its identification tags, as taught in Landegren and

Baez. Ex. 1004, 15; Ex. 1003, 5:38-42, Figs. 5-7. A POSA would have been motivated to sequence the ligation product (i.e., ASA) as another method to detect and analyze the ASA because it is a well-known method to determine the nucleic acid labels joined in the creation of the ASA. A POSA would have also been motivated to combine Freskgard with Baez because Baez teaches that its nucleic acid products are detected “by sequence.” Ex. 1003, 3:63-64. The sequencing of the nucleic acid taught in Freskgard allows for the detection of the amplified nucleic acid products by sequence.

294. A POSA would have a reasonable expectation of success in combining Baez, Landegren, and Freskgard. All three references relate to tagging a molecule with nucleic acid and analyzing the molecule’s affinity toward a target by way of the nucleic acid label. Ex. 1003, Abstract, 7:53-56; Ex. 1004, 1, 3; Ex. 1009, Abstract. A POSA would have known that sequencing as taught in Freskgard was a known technique that could have been readily applied to the nucleic acid labels taught in Baez and Landgren in a reasonable way, as there would be no significant challenge to sequence the nucleic acid sequences taught in Baez and Landegren.

295. Therefore, relying on Patent Owner’s positions, in my opinion, claim 14 is obvious in view of Baez and Landegren and in further view of Freskgard.

12. Dependent Claim 15

- (a) **“The method of detecting functional interactions between at least two molecules of interest as recited in claim 14, wherein the analysis comprises sequencing by synthesis.”**

296. As described above with respect to dependent claim 14 (paragraphs 289-295), Patent Owner contends that sequencing of the ligation product using “Illumina NGS technology” in NULISA satisfies this limitation (Ex. 1008, 10), and Freskgard discloses that the identifier is sequenced by Sanger sequencing, a form of sequencing by synthesis (Ex. 1009, 69-70). A POSA would have been motivated to sequence the ligation product (i.e., ASA) as another method to detect and analyze the ASA because it is a well-known method to determine the nucleic acid labels joined in the creation of the ASA. Thus, relying on Patent Owner’s positions, it is my opinion that claim 15 is obvious in view of Baez and Landegren and in further view of Freskgard.

XI. Declaration

297. I declare that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: August 23, 2024

By: Norman Nelson
Norman Nelson Ph.D.