



***Alamar Biosciences, Inc. v. Olink
Proteomics, AB***

IPR 2024-01353

Patent 7,883,848

Petitioner's Demonstratives

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

*Corrected Dec. 5, 2025



Instituted Grounds

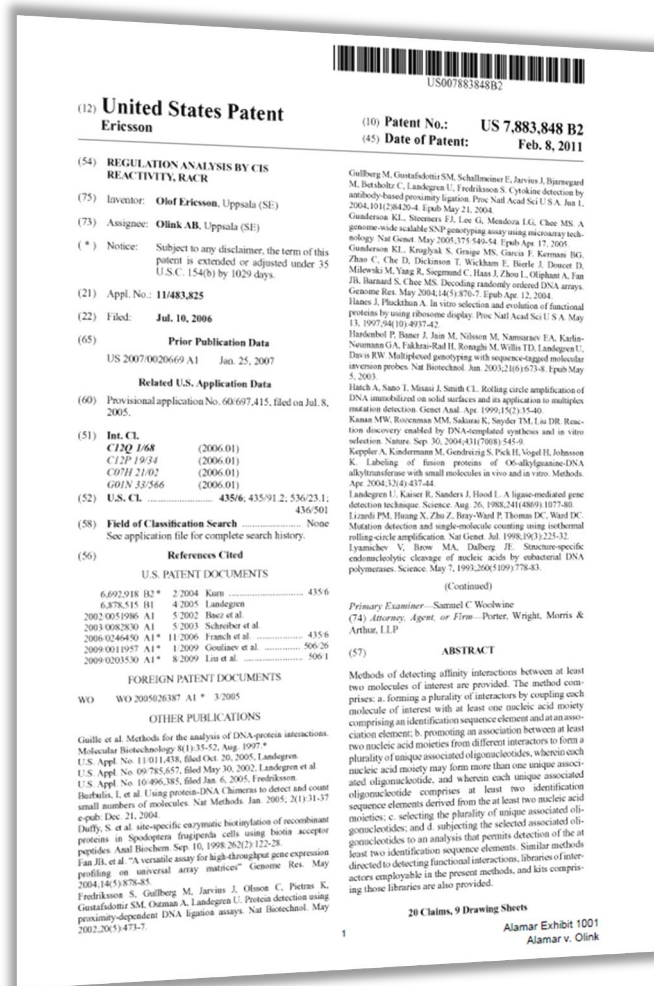


Ground	Claims	35 U.S.C. §	References
1	1-15, 17-20	103(a)	Kanan, Neri
2	16	103(a)	Kanan, Neri, Famulok
3	1-8, 11, 12	103(a)	Baez, Landegren
4	14, 15	103(a)	Baez, Landegren, Freskgard

Institution Decision at 7, 36-37

'848 Patent and Prior Art

'848 Patent



1. 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 oligonucleotide comprises 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.

Earliest possible priority date: July 8, 2005

The '848 Patent's Method



Figure 4(A)

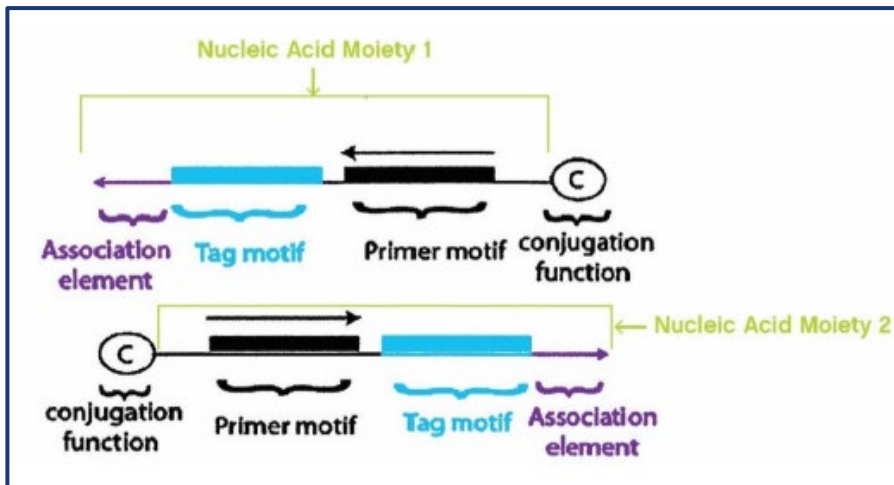
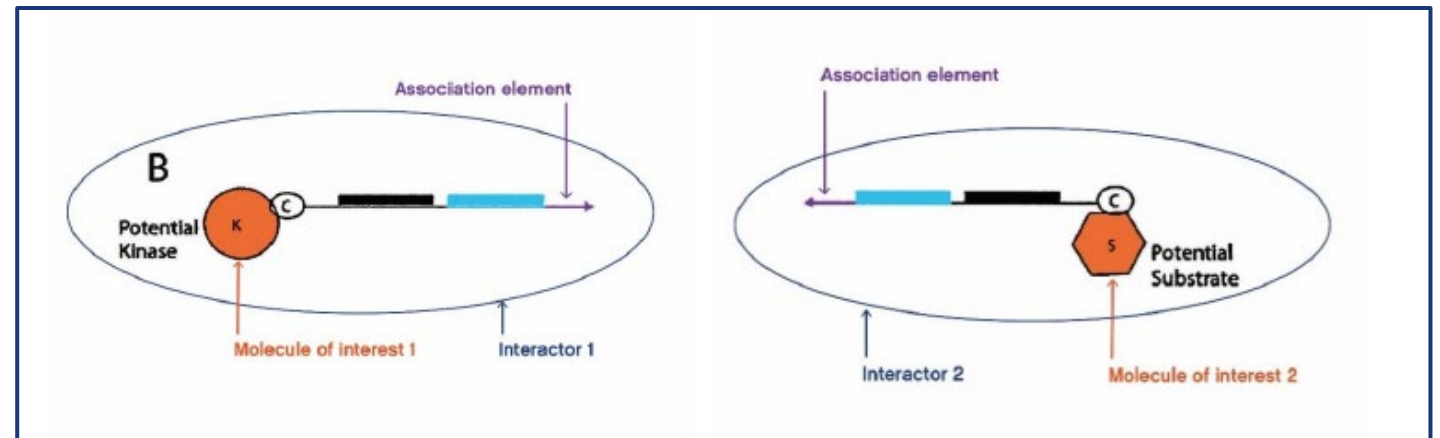


Figure 4(B)

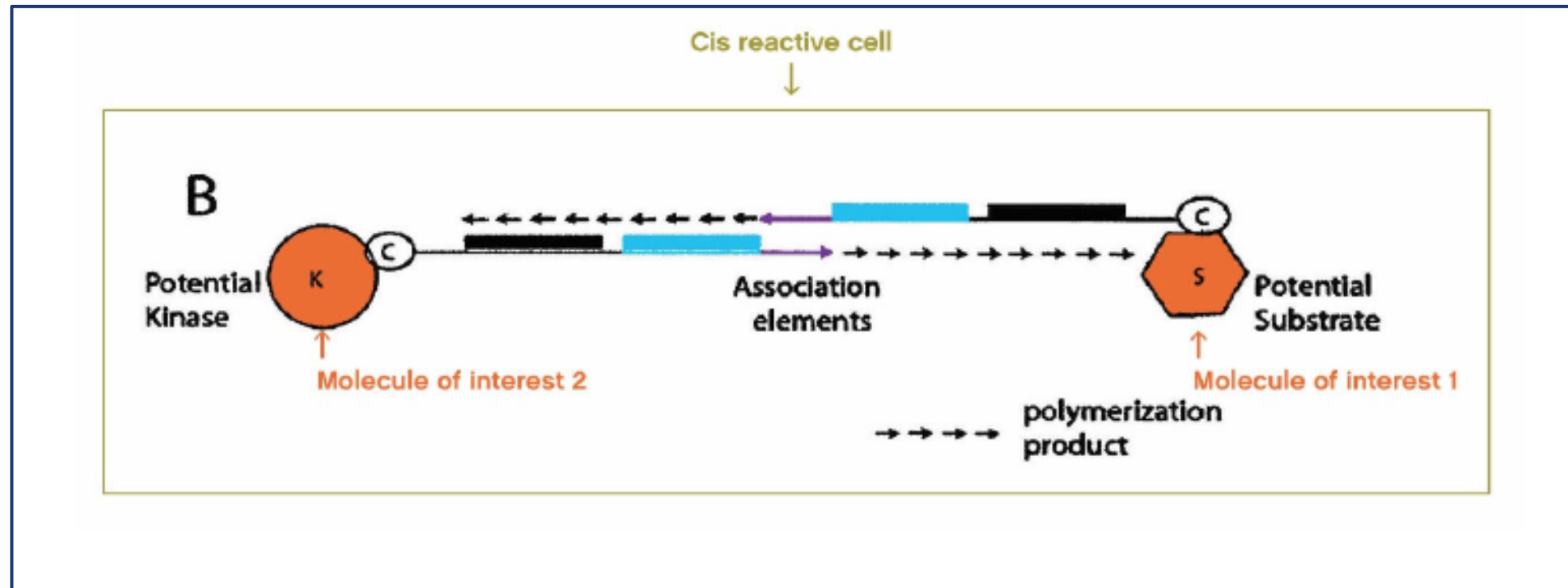


Petition at 3-7; Ex. 1001 at Fig. 4(A) (color added, elements labeled), Fig. 4 (B) (color added, modified)

The '848 Patent's Method



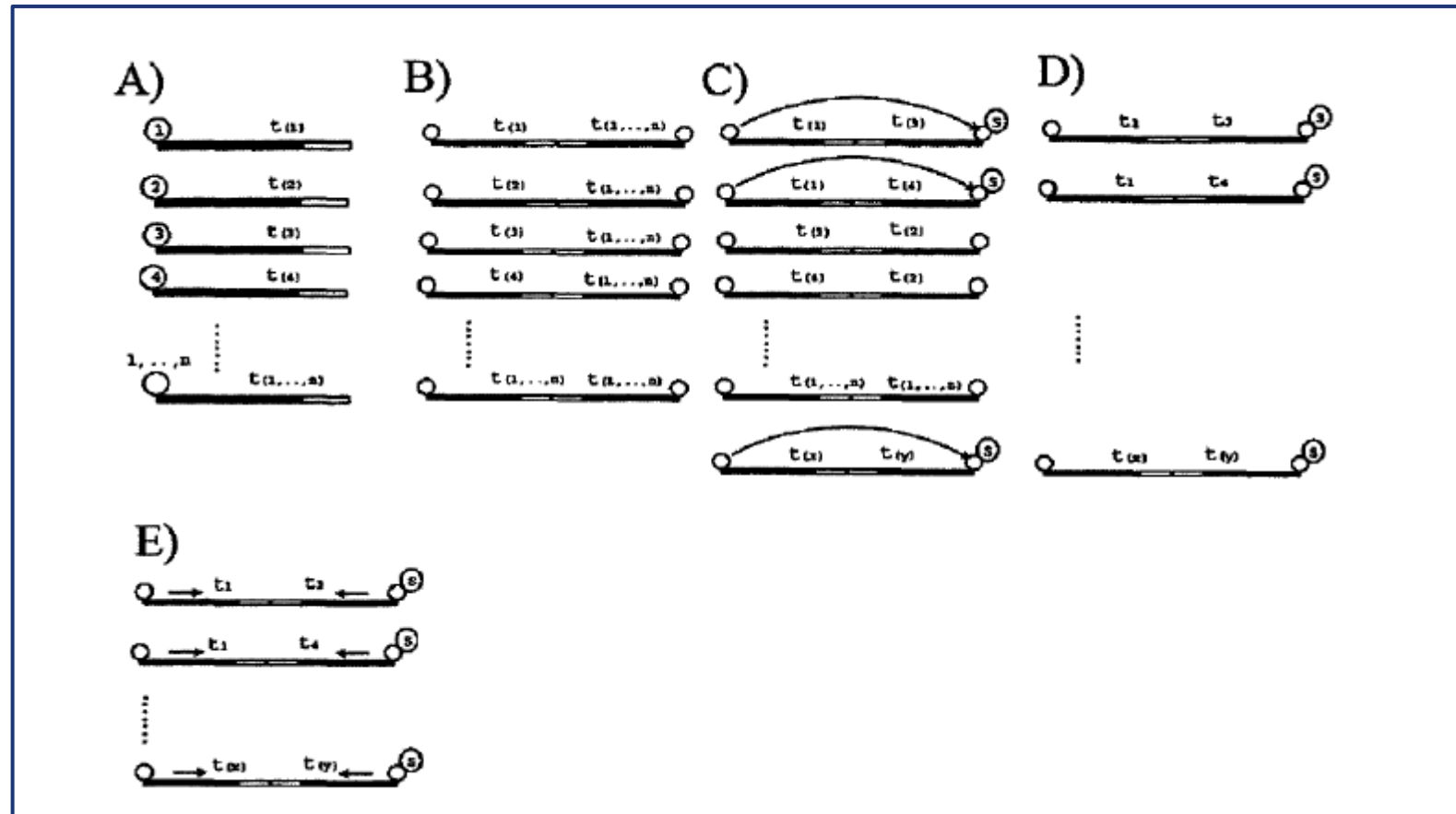
Figure 4(B)



The '848 Patent's Method



Figure 2



Petition at 3-7; Ex. 1001 at Fig. 2 (showing functional interaction in step c)

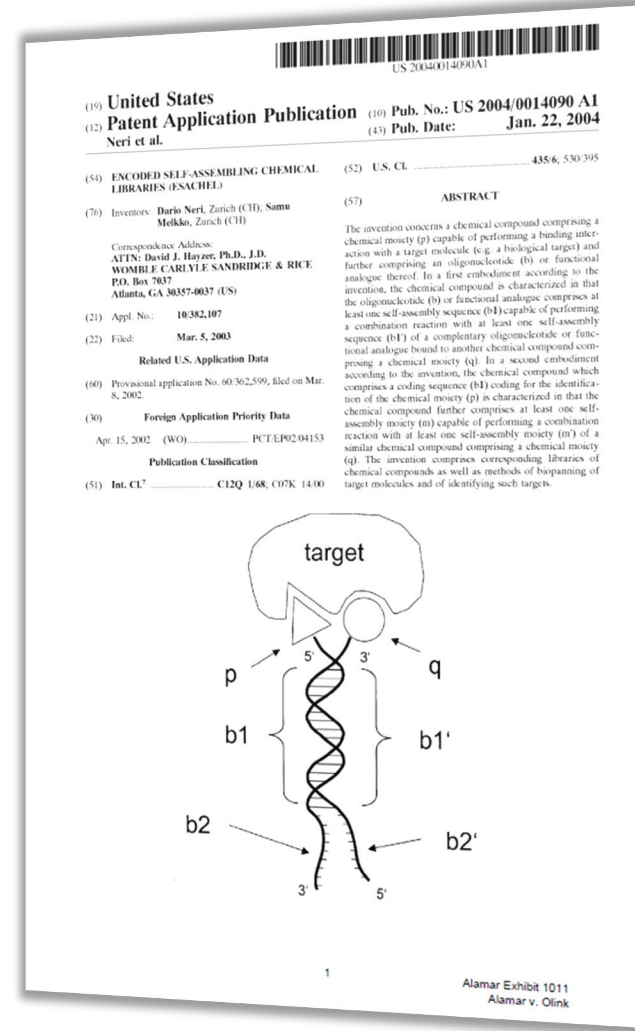


The '848 Patent's Method

Following functional activation the secondary library is filtered for CRCs which contain MOIs that have been altered by the activation. Selection of positive (that is, altered) CRCs can be done by, e.g., affinity purification of the library of CRCs with respect to a modification introduced by an enzymatic reaction. The CRCs are then identified by the sequence motifs in the NAMs, thus both members of a functional interaction can be identified, for example, pairs of novel enzymes and novel substrates.



Ground 1: Kanan & Neri (Claims 1-15, 17-20)

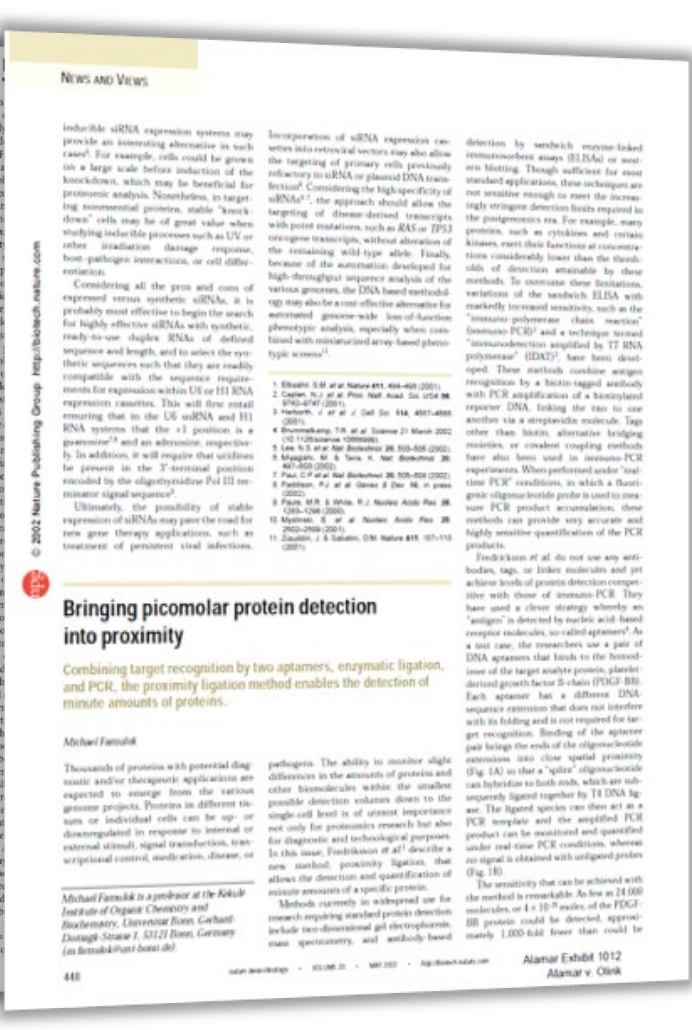
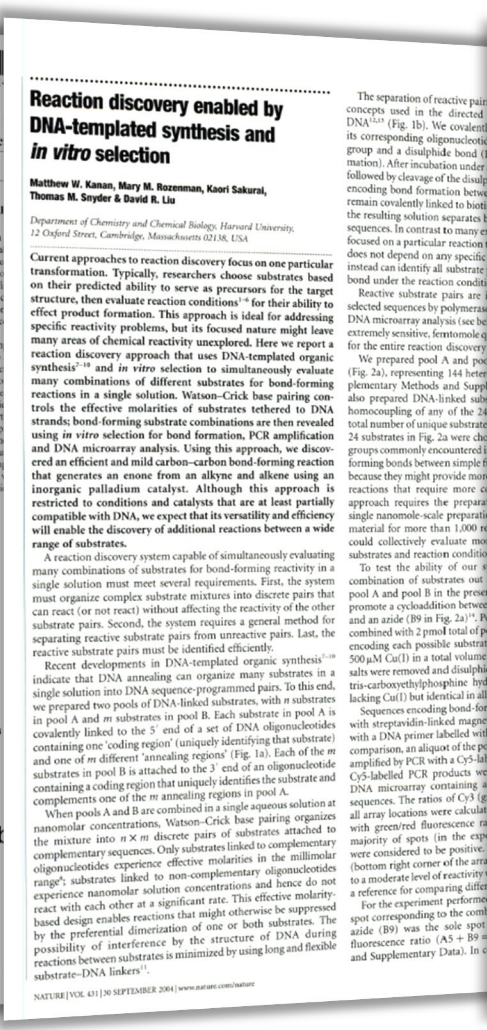
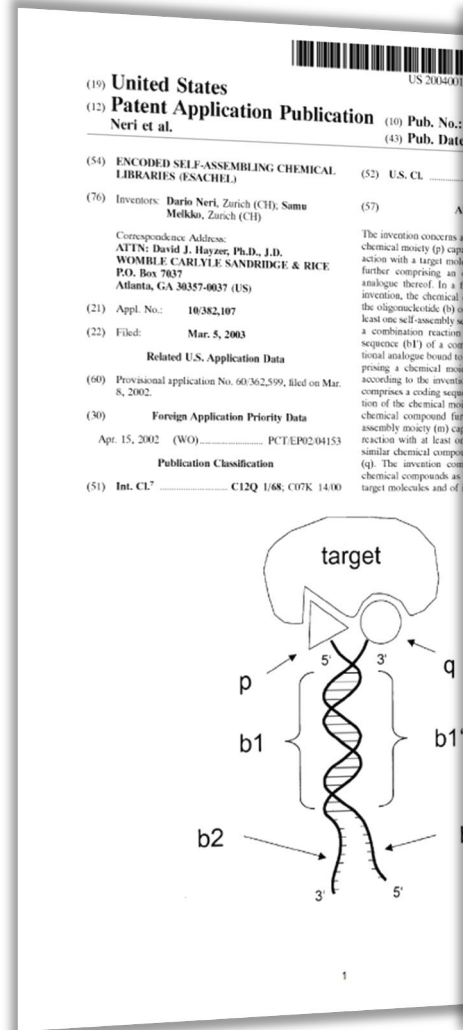


Ex. 1010; Ex. 1011

Published Sept. 30, 2004

Published Jan. 22, 2004

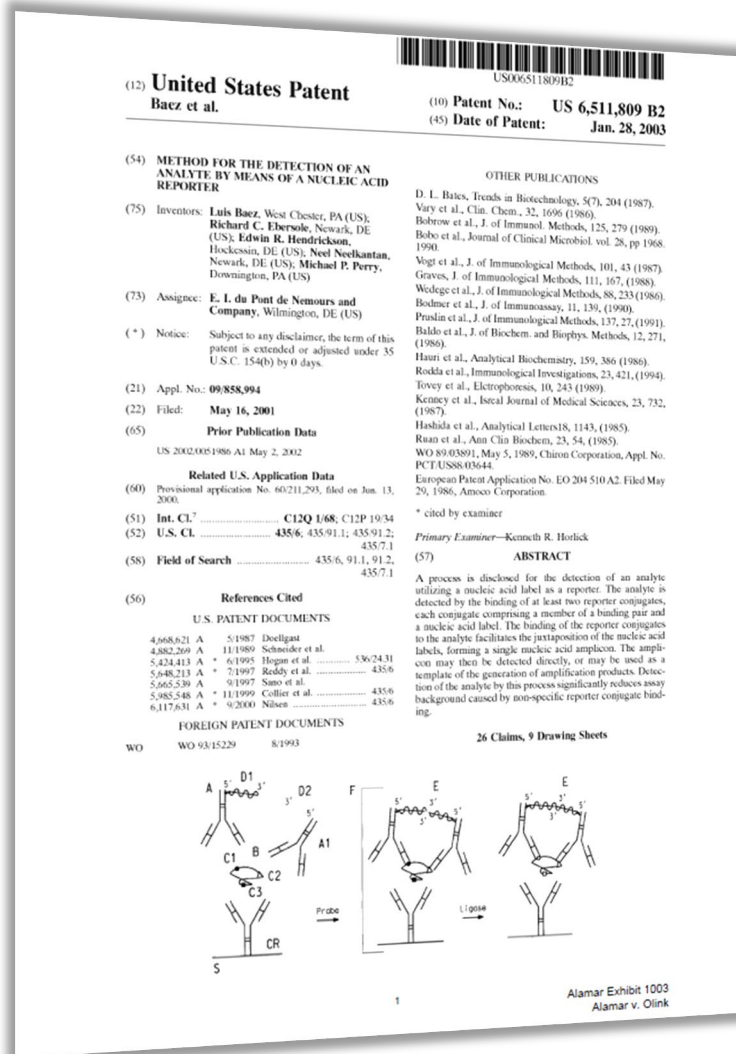
Ground 2: Kanan, Neri, & Famulok (Claim 16)



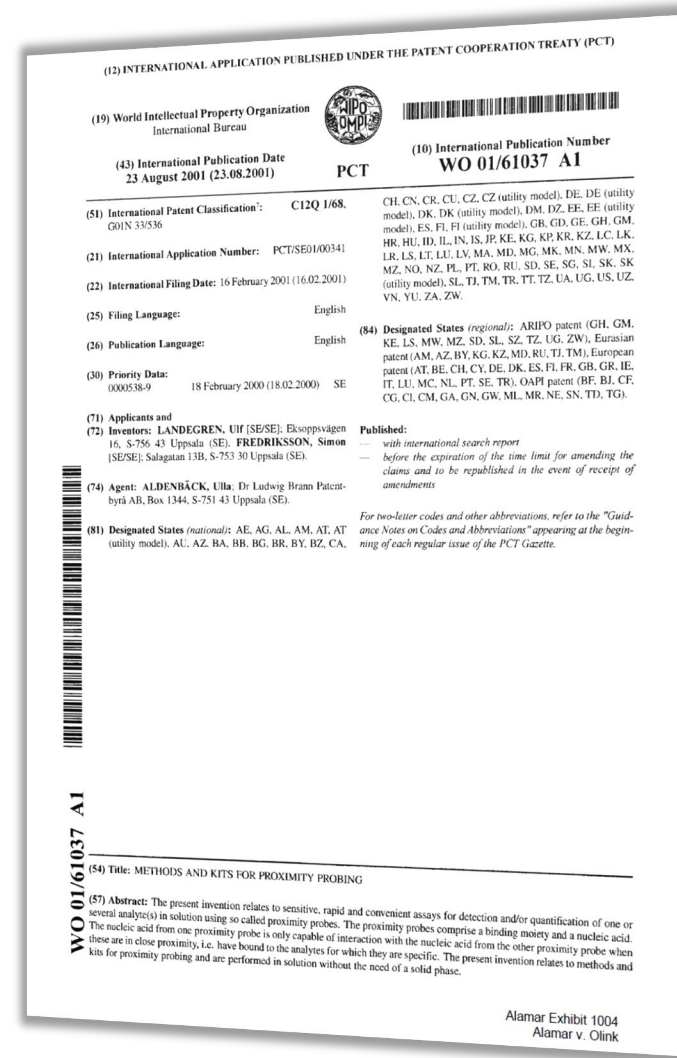
Ex. 1010; Ex. 1011; Ex. 1012

Published May 1, 2002

Ground 3: Baez & Landegren (Claims 1-8, 11, 12)

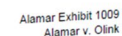


Published Jan. 28, 2003



Published Aug. 31, 2001

Ex. 1003; Ex. 1004



Published Jan. 13, 2005

Person Of Ordinary Skill In The Art



Person Of Ordinary Skill In The Art

Petitioner's Definition

(a) an advanced degree, such as a Ph.D., in organic 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 organic 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 such as nucleic acid sequencing.

Obvious under
↔
either definition

PO's Definition

Before July 8, 2005, a person of ordinary skill in the art (POSA) would have had an advance degree, such as a Ph.D. in chemistry, organic chemistry, biochemistry, biotechnology, molecular biology, or a related discipline, with about 2 years of experience in laboratory research involving small molecule, proteins, nucleic acid chemistry, and hybridization techniques. EX2008, ¶¶79-80. A POSA would have had familiarity with high throughput assays, such as screening and sequencing techniques. *Id.*, ¶79. A POSA may also have had less formal education and more years of technical or professional experience. *Id.*

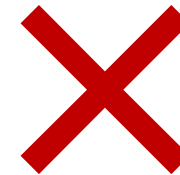


Only Petitioner's Expert Was A POSA In 2005

Dr. Norm Nelson (Petitioner)



Dr. Bradley Pentelute (PO)



Ground 1:
Kanan in View of Neri
Claims 1-15, 17-20
Disputed Limitations 1(d), 1(e)



Ground 1 – Disputed Limitations For Claim 1

Claim	Limitation	Disputed?
1(pre)	A method of detecting functional interactions between at least two molecules of interest	No
1(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	No
1(b)	Forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors bound in proximity to on 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	No
1(c)	Subjecting the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction with a detectable trace	No
1(d)	Selecting all cis-reactive cells exhibiting the detectable trace	Yes
1(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	Yes



Kanan Teaches The Preamble

'848 Patent

1. A method of detecting functional interactions between at least two molecules of interest, the method comprising:

'848 Patent

In DTS, two molecules are forced together by using nucleic acids to allow the molecules to interact to a higher degree than if they were not brought in proximity. For example, in the DTS-based method taught by Kanan et. al (Kanan et. al Nature 2004), 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. To create interactions

Kanan

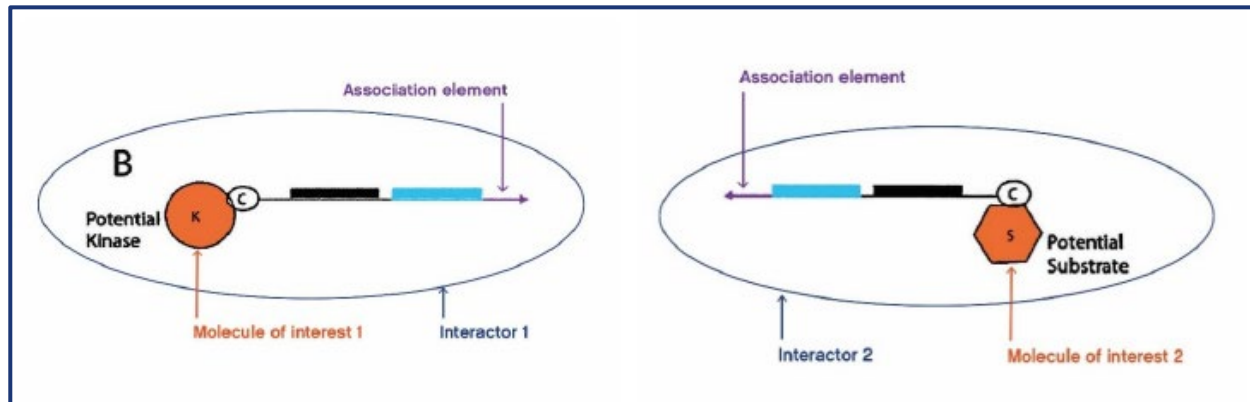
Current approaches to reaction discovery focus on one particular transformation. Typically, researchers choose substrates based on their predicted ability to serve as precursors for the target structure, then evaluate reaction conditions¹⁻⁶ for their ability to effect product formation. This approach is ideal for addressing specific reactivity problems, but its focused nature might leave many areas of chemical reactivity unexplored. Here we report a reaction discovery approach that uses DNA-templated organic synthesis⁷⁻¹⁰ and *in vitro* selection to simultaneously evaluate many combinations of different substrates for bond-forming reactions in a single solution. 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. Using this approach, we discov-

Kanan & Neri Teach Claim 1(a)

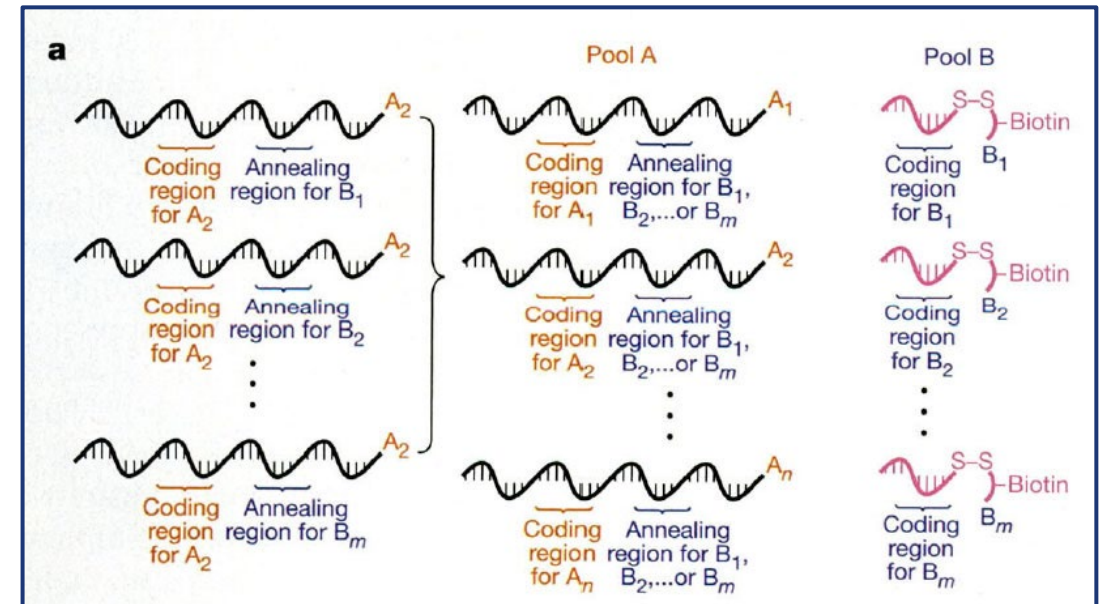
'848 Patent

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;

'848 Patent, Fig. 4(A)



Kanan, Fig. 1.a



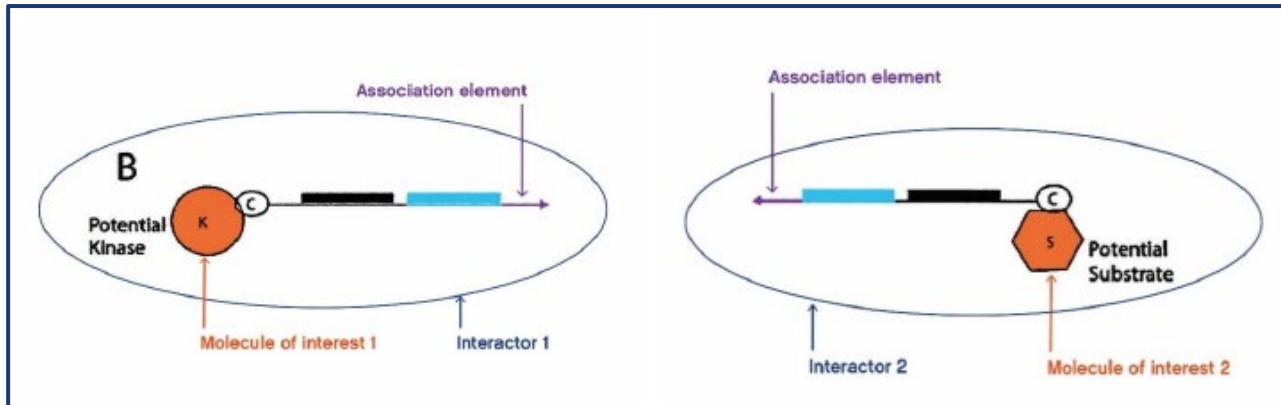


Kanan & Neri Teach Claim 1(a)

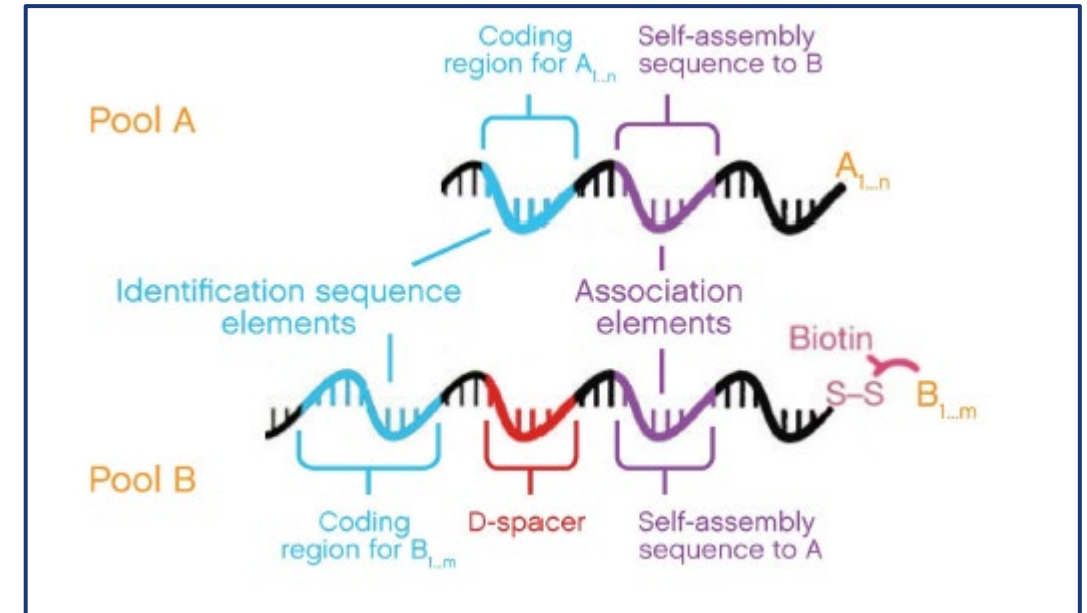
'848 Patent

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;

'848 Patent, Fig. 4(B)



Kanan/Neri



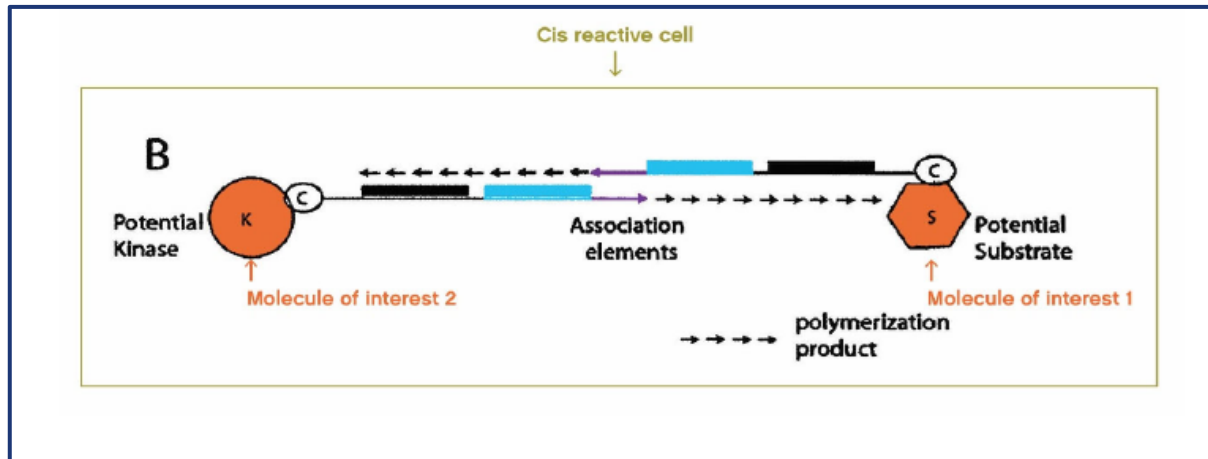


Kanan & Neri Teach Claim 1(b)

'848 Patent

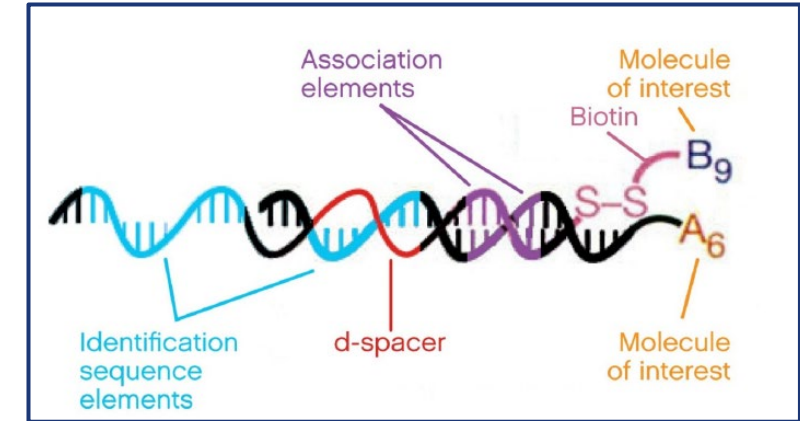
b. forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors bound in proximity to one another by an associated oligonucleotide formed from the association between at least two nucleic acid moieties, wherein the associated oligonucleotide comprises at least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

'848 Patent, Fig. 4(B)

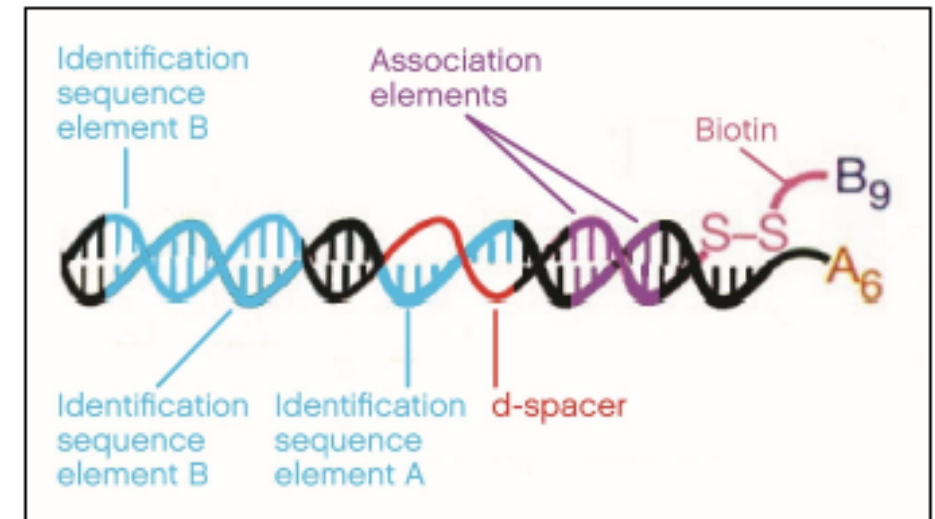


Petition at 6, 23, 31-33; Ex. 1001 at Claim 1(b),
Fig. 4(B) (color added and annotated); Reply at 4; Ex. 1051 ¶¶ 7-19

Kanan/Neri (association)



Kanan/Neri (association via polymerization)





Kanan & Neri Teach Claim 1(b)

- Dr. Pentelute agrees that “polymerization” (described in claim 17) is the association from claim 1(b)



Q. The association via polymerization referenced in claim 17, that is referring to the association taking place in step (b) of claim 1; is that right?

A. . . . So the association appears to be, according to claim 1, the association as described in claim 1(b).



Kanan & Neri Teach Claim 1(b)

- PO's expert agrees that polymerization results in both identification sequence elements

Q. And it's the polymerization that takes place that encodes the second identification sequence onto the other nucleic acid which is being polymerized?

A: In this CRC.

Q: Is that right?

A: Yes.



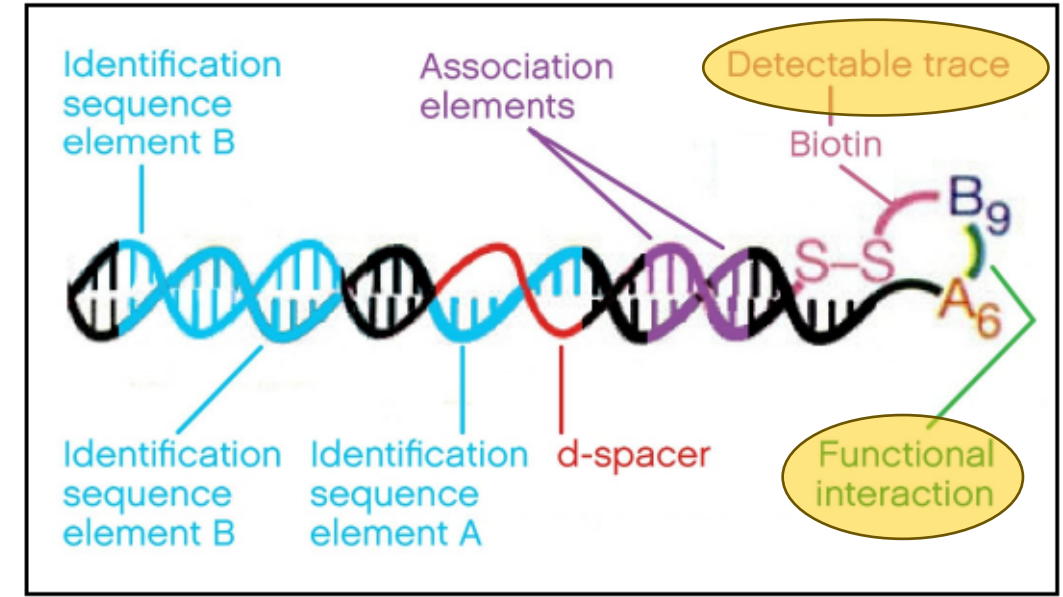
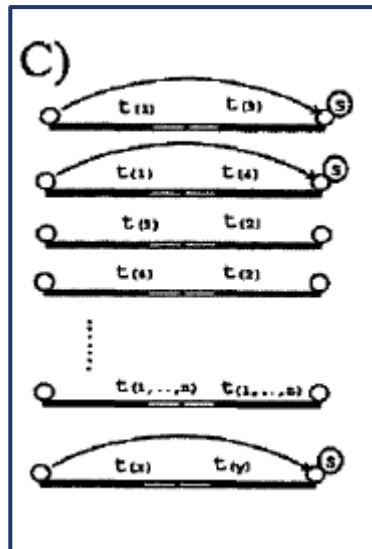
Kanan & Neri Teach Claim 1(c)

Kanan/Neri

'848 Patent

c. subjecting the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction having a detectable trace;

'848 Patent, Fig. 2



Petition

The bond formation between substrates A and B (i.e., the addition of substrate B₉ to A₆, below) would be considered the functional interaction between the substrates. Ex. 1005, ¶119. The biotin linked to the newly bound substrates is the detectable trace. *Id.*

Petition at 33-34; Ex. 1001 at Claim 1(c), Fig. 2 (partial, showing functional interaction); Ex. 1051 at ¶ 27

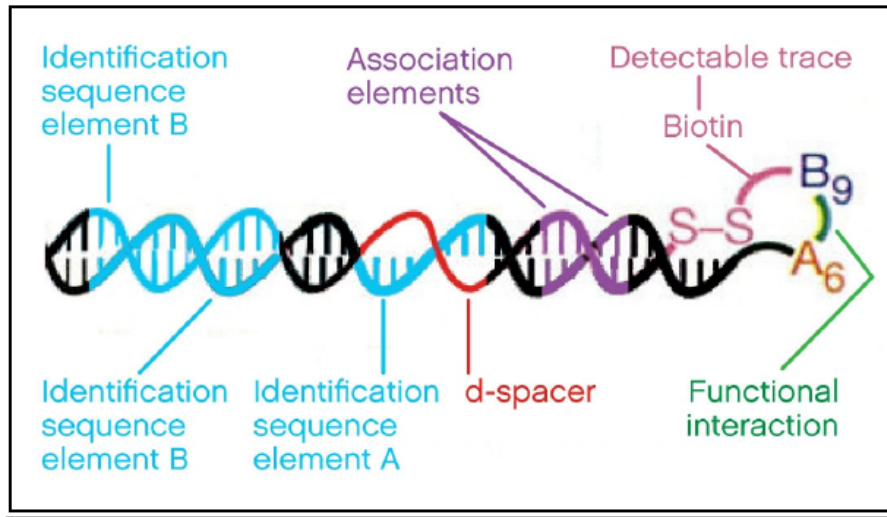


Kanan & Neri Teach Claim 1(d)'s "Selecting" Step

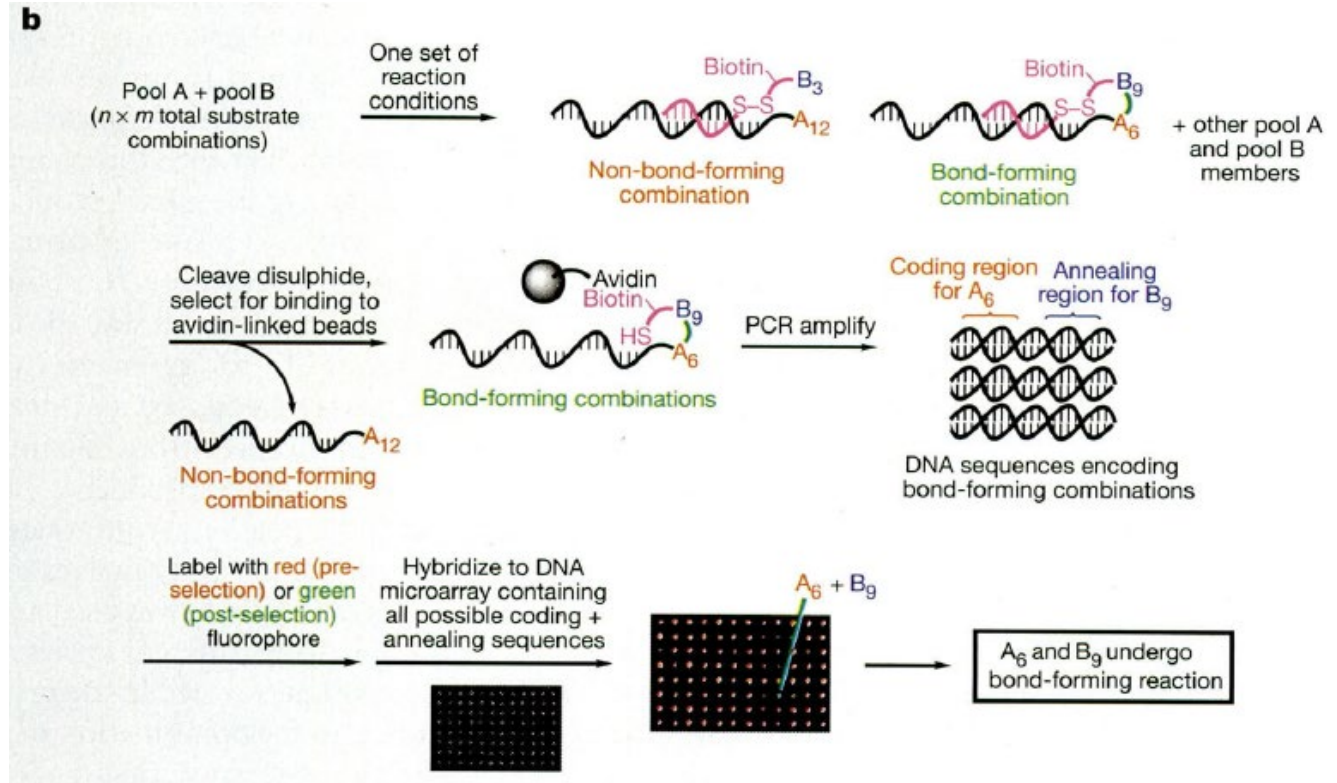
'848 Patent

d. selecting all cis-reactive cells exhibiting the detectable trace; and

Kanan/Neri



Kanan Fig. 1.b

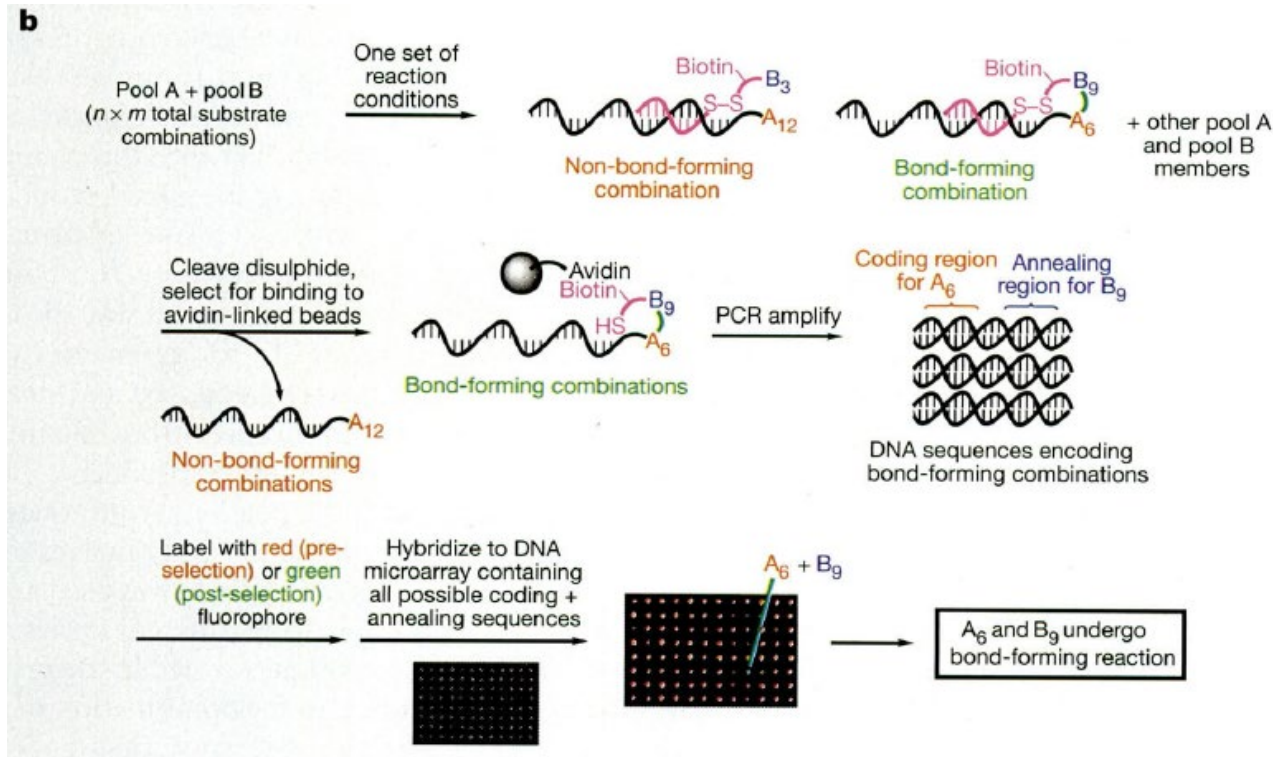


Petition at 20-23, 33-35, 43-44; Reply at 4-10; Ex. 1051 at ¶¶ 7-11, 27; Ex. 1001 at Claim 1(d); Ex. 1010 at Fig. 1.b

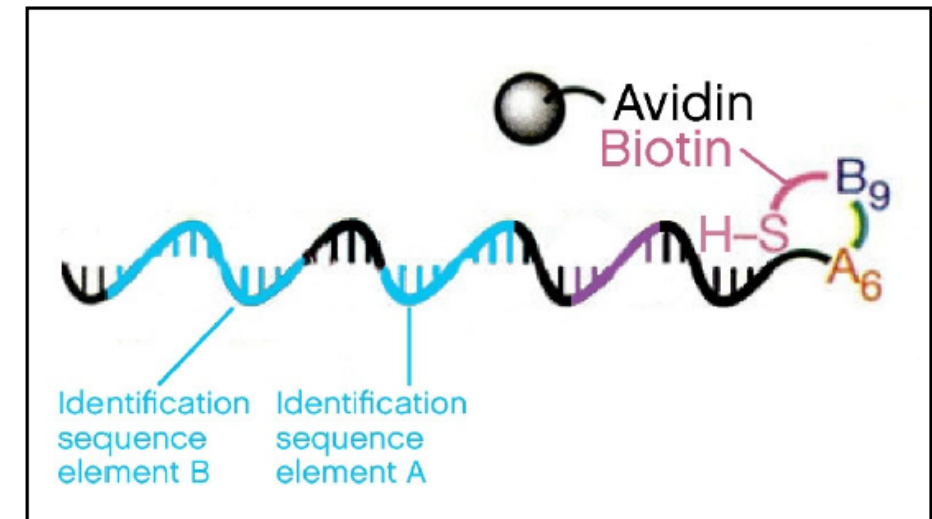


Kanan & Neri Teach Claim 1(d)'s "Selecting" Step

Kanan Fig. 1.b



Kanan/Neri





PO's Narrow Construction Of "Selecting" As A Single Capture Event Excludes Embodiments

- PO: "because Kanan teaches performing the disulfide cleavage step *before* its streptavidin affinity selection . . . Kanan does not and cannot teach selecting '*all cis-reactive cells* exhibiting the detectable trace'"
- But the specification discloses that "selecting all cis-reactive cells exhibiting the detectable trace" can be a process:

'848 Patent at 20:31-39

Another example of a suitable filtering process is conditional detection. The CRCs can be hybridized to a DNA oligonucleotide tag array where all possible combinations of identification sequences in the library are present. The different combinations of associated NAMs are spatially separated by the hybridization. To identify which NAM combinations represent a CRC with a positive interaction pair the array with the CRCs can be stained with a labelled affinity reagent specific for the interaction. However this approach requires that the MOIs are present during detection and thus no ampli-

'848 Patent at 19:40-57

Functional Interactions

To detect functional interactions in a pool of CRCs the CRCs which display functional interactions must be selected for detection and/or analysis. It will be appreciated by one of ordinary skill in the art that this step will be modified according to the nature of the functional interaction that is being investigated. As used herein, a "filter" or a "filtering process" is the means employed to selectively separate the desired CRCs. Nonlimiting examples of filters for functional interactions include: affinity purification of the form which has undergone alteration 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. Downstream of the filtering process of CRCs the compositions of associated NAMs are detected and/or quantified.

Dependent Claims Include Various Methods of Selecting



'848 Patent

2. 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.

'848 Patent

3. 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**.

'848 Patent

19. The method of detecting functional interactions between at least two molecules of interest as recited in claim 2, wherein the selective detection comprises **labeling the cis-reactive cells with an affinity reagent**.

'848 Patent

20. 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**.

Reply at 6; Ex. 1001 at cls. 2-3, 19-20 (reflecting certificate of correction for claim 19)



PO's Expert Agrees "Selecting" Not So Limited

- PO's expert agrees that "selecting" can be a process

"Filtering **processes**, including affinity purification, are employed during the **process to selectively separate** the desired CRCs. This selection step can be **modified** according to the nature of the functional interaction that is being investigated. Downstream of the filtering **process** of CRCs the compositions of associated NAMs are detected and/or quantified."

Ex. 2008 at ¶ 35



Q. And that's the **process of selection** that you want to do?

A. Yes.

Ex. 1048 at 146:1-3

"But the key part of the selection is the reaction discovery experiments and selection **procedure**, and that's the section that we're discussing right now."

Ex. 1048 at 164:3-16

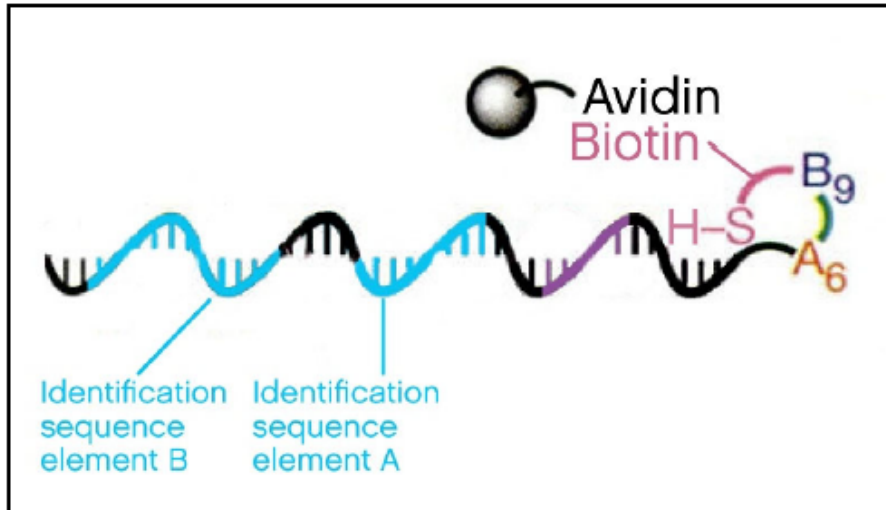
Reply at 6-7; Ex. 2008 at ¶ 35; Ex. 1048 at 146:1-3, 164:3-16. All emphasis is added unless noted.

Kanan & Neri Teach Claim 1(e)'s “detection of the at least two identification sequence elements”

'848 Patent

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.

Kanan/Neri



Petition

polymerization creates double-coding code on the extended nucleic acid sequence to increase the specificity of the assay. Ex. 1005, ¶182. Further, encoding the anticodon

the bond-forming substrates. *Id.*, ¶127; Ex. 1010, 545-546. A POSA would have understood Kanan in view of Neri that the detection of the bond-forming substrates product permits the detection of the at least two identification sequence elements 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. Ex. 1005, ¶127.

A POSA Would Have Been Motivated To Modify Kanan With Neri



Kanan requires that “DNA-linked substrate pools are prepared,” “which is *time intensive and costly*. . . . As a result, a POSA would have understood that *synthesizing the library members is the rate limiting factor*,” and needed “to minimize undesired reactivity arising from the mismatched hybridization of a pool B member with a non-complementary pool A member.”

“[I]n Kanan, to analyze the interaction between 100 A substrates and 100 B substrates, the POSA would have been required to synthesize 10,000 . . . 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***



Significant time savings

A POSA Would Have Been Motivated To Modify Kanan With Neri



“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.”



Efficient identification of reactive pairs

A POSA Would Have Been Motivated To Modify Kanan With Neri



Petition, Motivation to Combine Section (Claim 1)

A POSA further would have been motivated to combine the polymerization of the substrate pair disclosed in Neri with the method disclosed in Kanan because the polymerization creates double-coding code on the extended nucleic acid sequence to increase the specificity of the assay. Ex. 1005, ¶182. Further, encoding the anticodon of the interactor would allow identification of a multistep interaction, if desired. *Id.*



Polymerization encodes both identification sequence elements



Increased test specificity

A POSA Would Have Been Motivated To Modify Kanan With Neri



“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.”



Polymerization encodes both identification sequence elements



Increased test specificity



A POSA Would Have Been Motivated To Modify Kanan With Neri



“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. . . . Thus, a POSA would have been motivated to combine Kanan with Neri because of the known ease to create protein libraries.”



Motivated to create libraries to study proteins

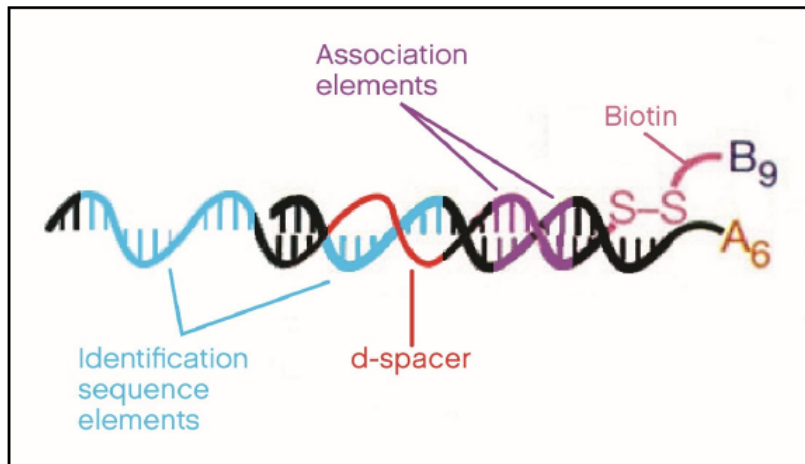


The Petition & Declaration Show Hybridization

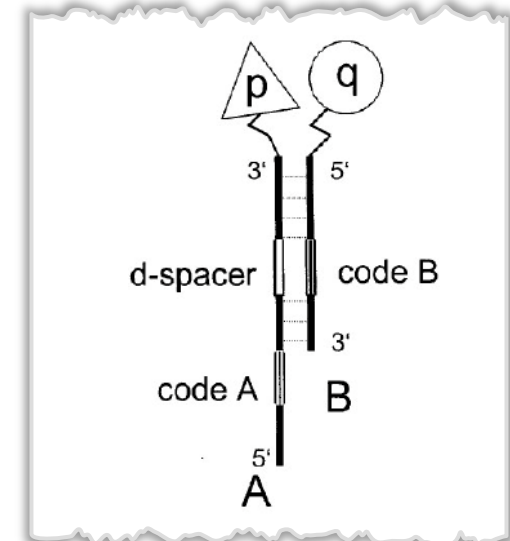
Nelson Declaration

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

Reply



Petition



Petition

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;

PO's Concerns About Mis-Hybridization Are Unsupported



✗ Pentelute's "Say So"
No scientific references supporting opinions on unintended hybridization

Kanan Supplementary Information

Annealing and coding sequences were generated by computer algorithms to minimize undesired reactivity arising from the mismatched hybridization of a pool B member with a non-complementary pool A member. The overall GC/AT ratio of these sequences was kept constant to minimize differences in melting temperatures between different annealing and coding sequences.

Fredriksson

20 pM proximity probes in a preincubation volume of 5 μ l. The very low probe concentration during the ligation phase of the assay keeps target-independent ligation events to a minimum, and requires a mere 100 attomoles of proximity probes per assay.

PO's Mis-Hybridization Concerns Would Not Defeat The POSA's Motivation To Combine



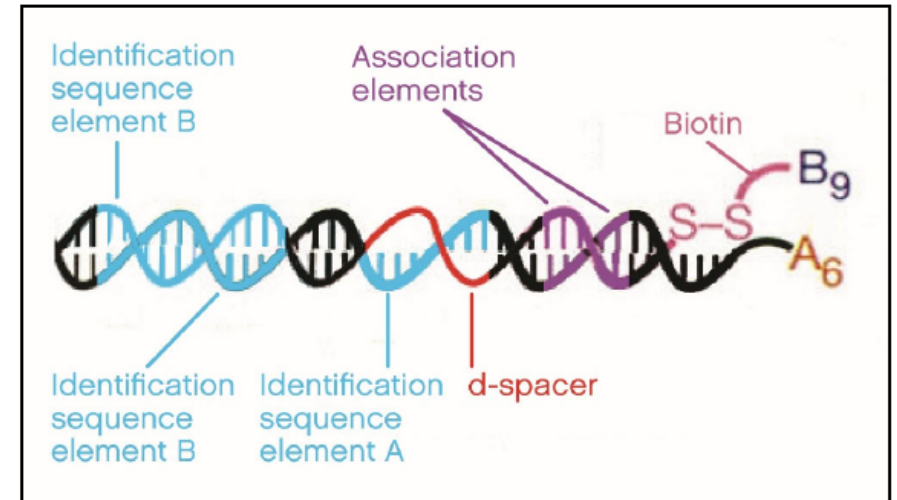
“[I]t is within the skill of a POSA to design assay conditions to reduce concerns of unintended hybridization.”

“Reducing unintended hybridization is a common issue faced with all nucleic acid chemistry and well within the skill o[f] a POSA to control and minimize.”

Petition



Kanan/Neri



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;

A POSA Would Have A Reasonable Expectation Of Success

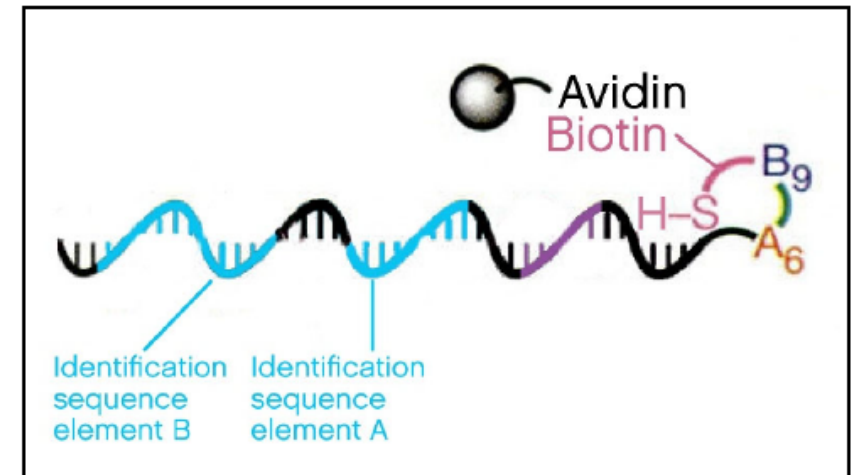


Petition

standard practice when the application was filed. Ex. 1005, ¶171. A POSA would have known that polymerization of oligonucleotides as taught in Neri could have been readily applied to oligonucleotides taught in Kanan because the use of primers and polymerase enzymes to extend oligonucleotides was a standard practice when the application was filed. Ex. 1005, ¶183.

- ✓ Kanan/Neri allow polymerization
- ✓ Polymerization was well known and routine

Kanan/Neri



A POSA Would Have A Reasonable Expectation Of Success

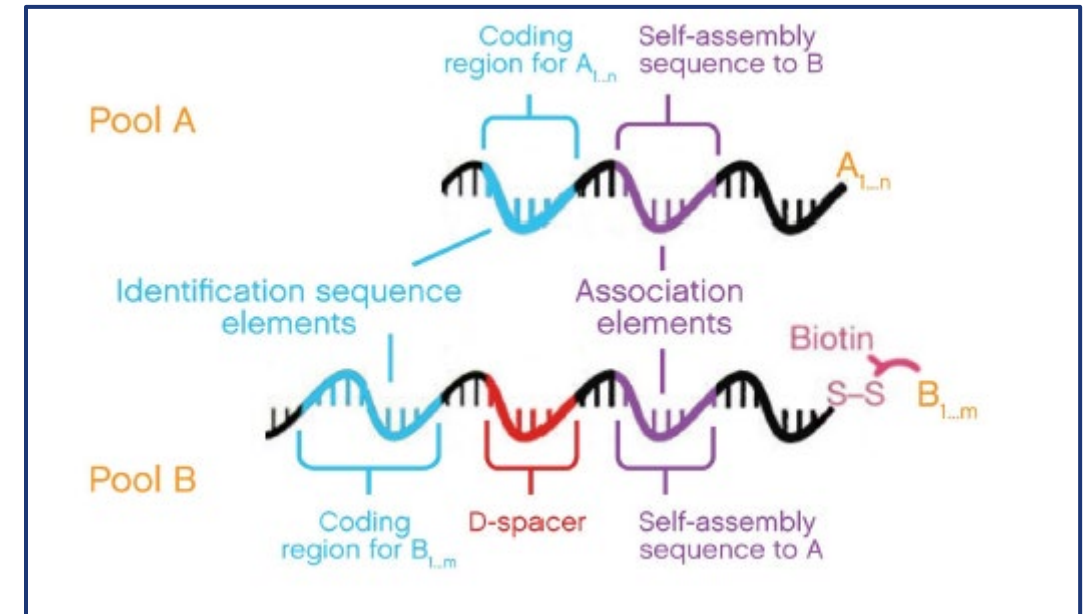


“A POSA would have known that *the self-assembly sequence, ‘d-spacer,’ and identification elements as taught in Neri could have been readily applied to oligonucleotides taught in Kanan* because the formation of specific oligonucleotide sequences is a *well-known technique requiring only simple modifications.*”



Petition at 21-23; Ex. 1005 at ¶ 105

Kanan/Neri



Including identification sequence element, d-spacer, and self-assembly sequence could be “readily applied”

A POSA Would Have A Reasonable Expectation Of Success



Petition

of success need only be reasonable, not absolute”). First, a POSA would have known that the difference between the interactors disclosed in Kanan and Neri are minimal, as both references teach libraries of oligonucleotides attached to a substrate that are designed to associate and interact with other members of the library. Ex. 1005, ¶104.



Minimal differences between Kanan/Neri’s interactors

Nelson Declaration



Creating protein libraries was well known

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];

Kanan's Method Allows Detection Of The At Least Two Identification Sequence Elements



Petition, Claim 1(e)

the bond-forming substrates. *Id.*, ¶127; Ex. 1010, 545-546. A POSA would have understood Kanan in view of Neri that the detection of the bond-forming substrates product permits the detection of the at least two identification sequence elements 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. Ex. 1005, ¶127.



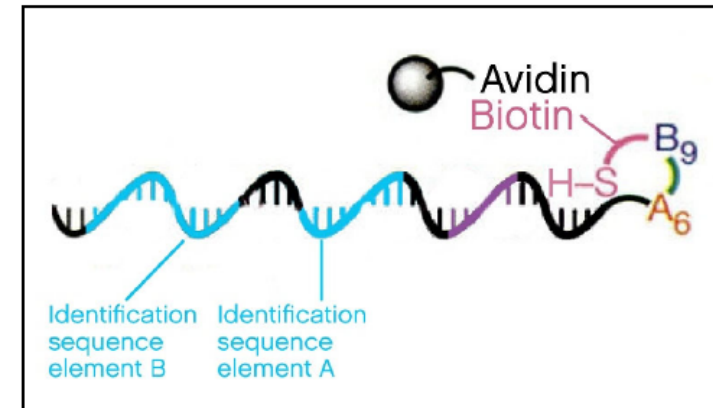
Useful; provides both identification sequences

Kanan's Method Allows Detection Of The At Least Two Identification Sequence Elements



"[D]etection of the bond-forming substrate products permits the detection of the at least two identification sequence elements . . . 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."

Kanan/Neri



Petition

the bond-forming substrates. *Id.*, ¶127; Ex. 1010, 545-546. A POSA would have understood Kanan in view of Neri that the detection of the bond-forming substrates product permits the detection of the at least two identification sequence elements 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.** Ex. 1005, ¶127.



✓ Useful; provides both identification sequences

Petition at 17-21, 35-36; Reply at 9, 14-17; Ex. 1005 at ¶¶ 125-133

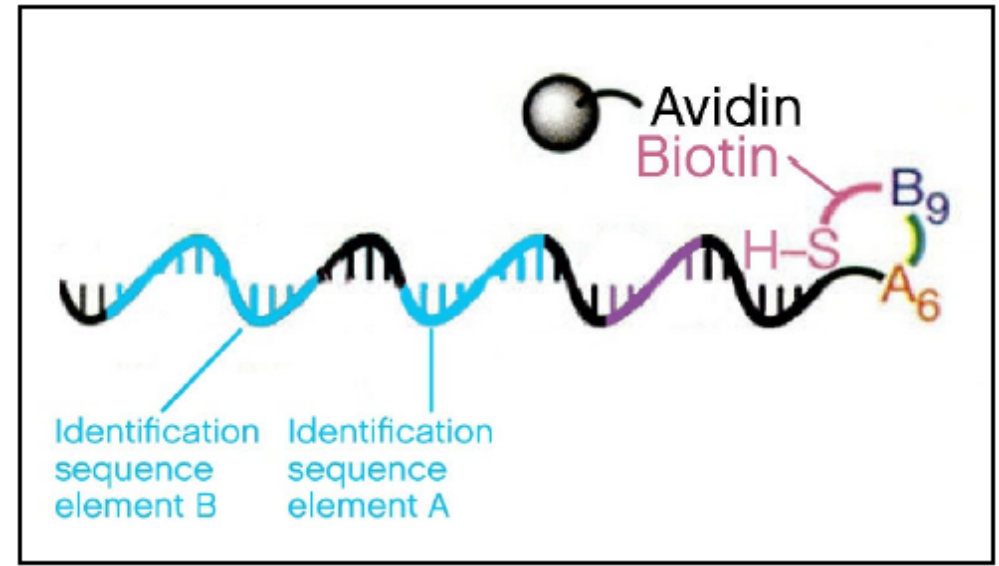
PO's Concerns Would Not Defeat The POSA's Reasonable Expectation Of Success



- Kanan/Neri provide both identification sequence elements
- Polymerization was well-known & standard practice
- POSA could control unintended hybridization



Kanan/Neri

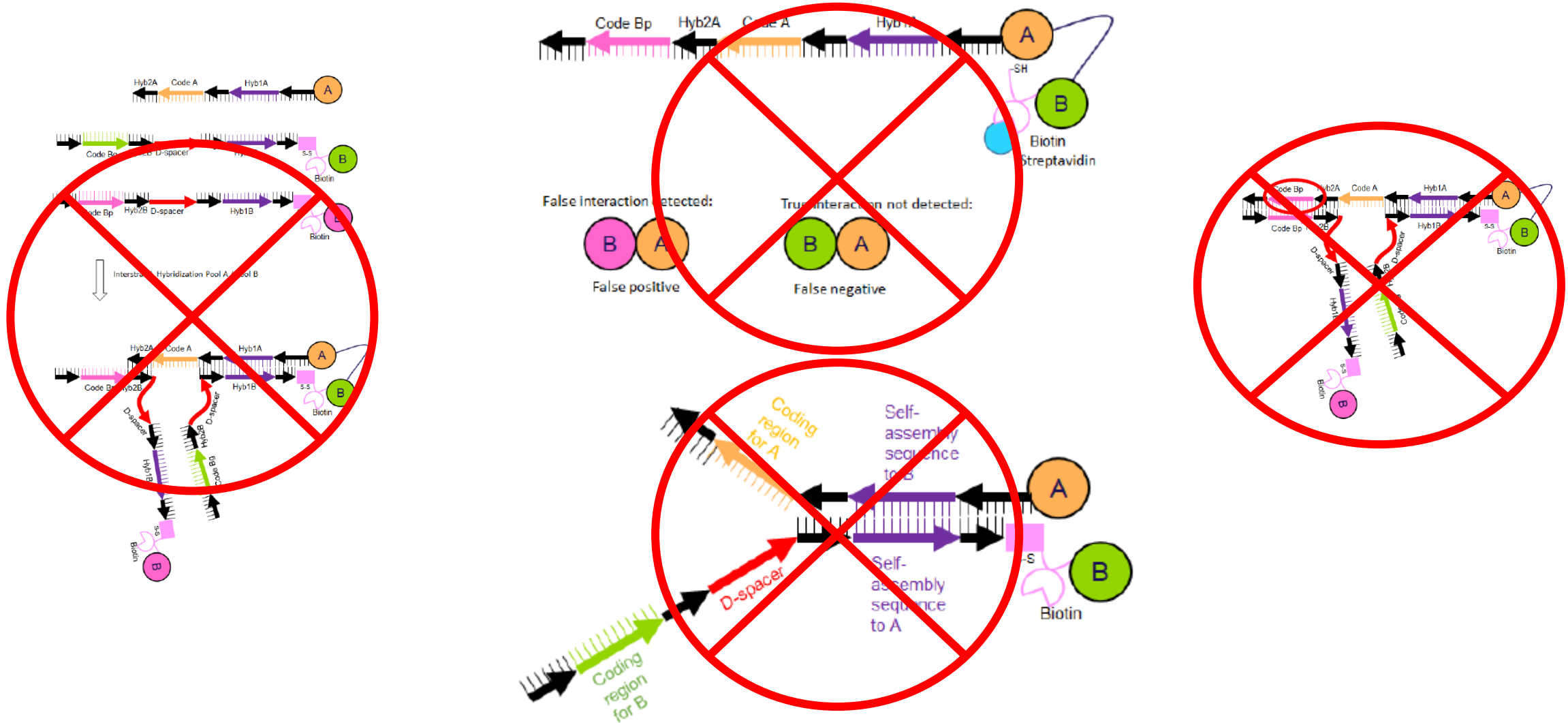


Provides both identification sequences



PO's unsupported concern for mis-hybridization, false positives, or false negatives

Dr. Pentelute's Speculative And Unsupported Designs Do Not Negate Expectation of Success



Response at 40-45; Reply at 14-19



Ground 1 – Independent Claim 1 Is Obvious

Claim	Limitation	Obvious?
1(pre)	A method of detecting functional interactions between at least two molecules of interest	Yes
1(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	Yes
1(b)	Forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors bound in proximity to on 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	Yes
1(c)	Subjecting the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction with a detectable trace	Yes
1(d)	Selecting all cis-reactive cells exhibiting the detectable trace	Yes
1(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	Yes



Ground 1 - Dependent Claims

Claim	Disputed?*	Claim	Disputed?*	Claim	Disputed?*
2	No	9	No	17	No
3	No	10	No	18	No
3	No	11	No	19	No
4	No	12	No	20	No
5	No	13	No		
6	No	14	No		
7	No	15	No		
8	No	16	N/A		

*Patent Owner disputes that claims 2-20 are obvious only due to their direct or indirect dependence on claim 1. Response at 20.



Kanan & Neri Teach Dependent Claim 2

'848 Patent

2. 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.

Kanan

remain covalently linked to biotin. **Streptavidin affinity selection of the resulting solution separates biotinylated from non-biotinylated sequences.** In contrast to many existing reaction discovery screens^{1,5} focused on a particular reaction type, our selection-based approach does not depend on any specific substrate or product property but instead can identify all substrate pairs capable of forming a covalent bond under the reaction conditions.

'848 Prosecution History

cells. **Both selective separation and selective detection are techniques well known in the art,** whereby claim 21 is definite and clear to one of ordinary skill in the art. Accordingly, the



Kanan & Neri Teach Dependent Claim 3

'848 Patent

3. 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**.

Kanan

remain covalently linked to biotin. **Streptavidin affinity selection of the resulting solution separates biotinylated from non-biotinylated sequences.** In contrast to many existing reaction discovery screens^{1,5} focused on a particular reaction type, our selection-based approach does not depend on any specific substrate or product property but instead can identify all substrate pairs capable of forming a covalent bond under the reaction conditions.



“A POSA would have understood streptavidin affinity selection as a form of affinity purification.”

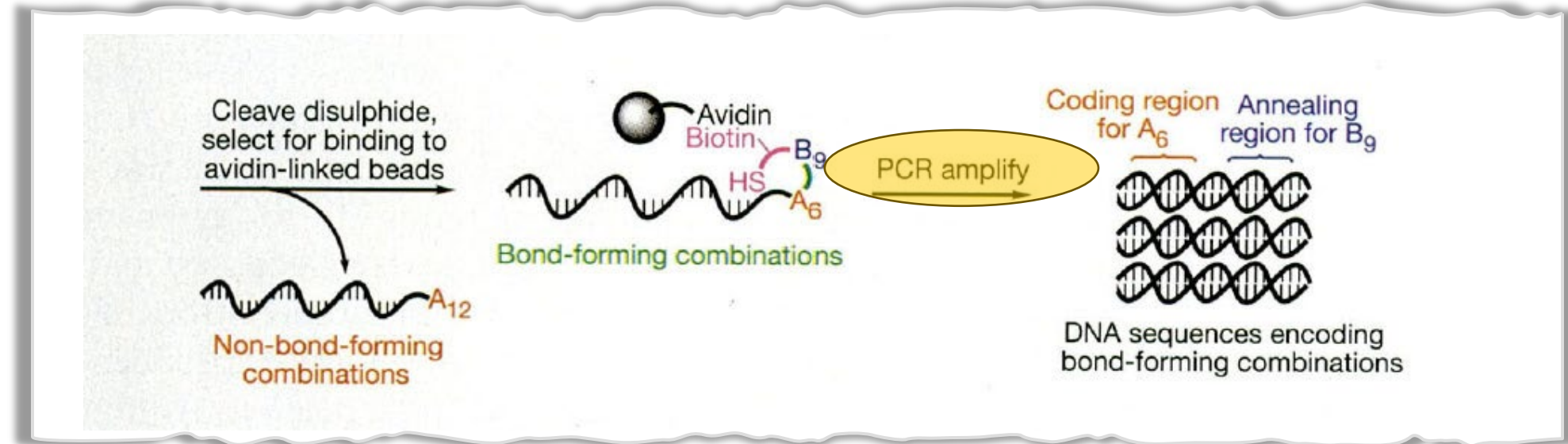


Kanan & Neri Teach Dependent Claim 4

'848 Patent

4. A 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**.

Kanan





Kanan & Neri Teach Dependent Claim 5 & 6

'848 Patent

5. A method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein coupling is **covalent or noncovalent**.

6. A method of detecting functional interactions between at least two molecules of interest as recited in claim 5, wherein coupling is **covalent**.

Kanan

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.



Kanan & Neri Teach Dependent Claim 7

'848 Patent

7. A 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.

Kanan

gation of this reaction. We synthesized a small-molecule substrate (1) to study a non-DNA-templated, intramolecular version of this alkyne–alkene coupling reaction on a multi-milligram scale (Fig. 4).



Kanan & Neri Teach Dependent Claim 8

'848 Patent

8. A 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.

Neri

[0082] The 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]. Indeed, the attachment of a DNA heteroduplex at the C-terminus of cysteine-tagged VH and VL domains will provide an extra stabilization to the Fv heterodimer.



Kanan & Neri Teach Dependent Claim 9

'848 Patent

9. 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.

“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.***”



Neri

[0094] As mentioned in previous sections, one strength of ESACHEL technology is its compatibility with a variety of different chemical moieties, including peptides and globular proteins (e.g., antibody domains).

Neri

[0082] The 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]. Indeed, the attachment of a DNA heteroduplex at the C-terminus of cysteine-tagged VH and VL domains will provide an extra stabilization to the Fv heterodimer.



Kanan & Neri Teach Dependent Claim 10

'848 Patent

10. A method of detecting functional interactions between at least two molecules of interest, as recited in claim 7, wherein the at least two molecules of interest comprises at least one protein and at least one nucleic acid.

“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 as taught in Kanan Studying the interaction of DNA molecules with DNA-binding proteins was well-known to a POSA at the time of the application.”



Neri

[0015] 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) and further comprising an oligonucleotide or functional analogue thereof which chemical compound does not need to be individually synthesized in order to build up a chemical library.

Neri

[0082] The 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]. Indeed, the attachment of a DNA heteroduplex at the C-terminus of cysteine-tagged VH and VL domains will provide an extra stabilization to the Fv heterodimer.



Kanan & Neri Teach Dependent Claim 11

'848 Patent

11. A 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.

“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.***”

Kanan

Sequences encoding bond-forming substrate pairs were captured with streptavidin-linked magnetic particles and amplified by PCR with a DNA primer labelled with the cyanine fluorophore Cy3. For





Kanan & Neri Teach Dependent Claims 12 & 13

'848 Patent

12. A method of detecting functional interactions between at least two molecules of interest, as recited in claim 1, wherein the analysis comprises high throughput screening.

13. The method of detecting functional interactions between at least two molecules of interest as recited in claim 12, wherein the high throughput screening comprises a micro array analysis.

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.”

Kanan

reactions in a single solution. 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**. Using this approach, we discov-



Petition at 41; Ex. 1001 at Claims 12-13; Ex. 1010 at 545; Ex. 1005 at ¶ 165 (quoting Ex. 1001 at 1:47-49)



Kanan & Neri Teach Dependent Claims 14 & 15

'848 Patent

14. 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.

15. 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.

“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.”

“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. . . . A POSA would have understood that Neri’s ‘sequencing’ of the coding region allows identification of the chemical entity in which the ‘code’ identifies.”





Kanan & Neri Teach Dependent Claim 17

'848 Patent

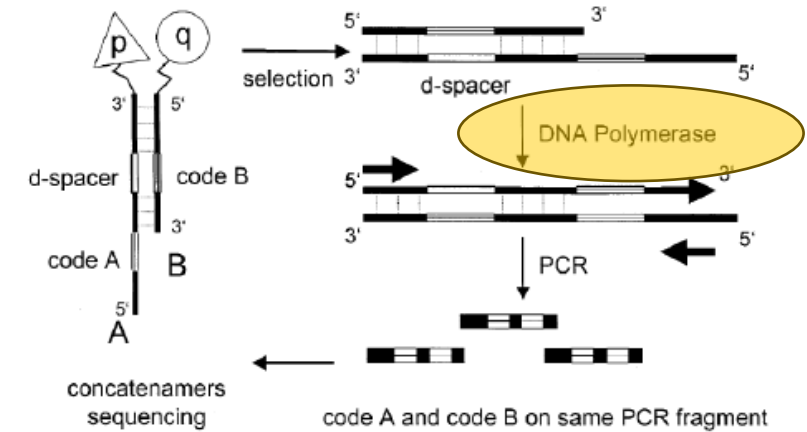
17. The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein association is via polymerization.

Neri

[0117] Oligonucleotides 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).

Neri (Fig. 5)

Fig. 5





Kanan & Neri Teach Dependent Claim 18

'848 Patent

18. The method of detecting functional interactions between the 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 comprises manipulating conditions to maximize cis reactivity and to minimize trans reactivity between the at least two molecules of interest.

Kanan

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. Only substrates linked to complementary oligonucleotides experience effective molarities in the millimolar range⁸; substrates linked to non-complementary oligonucleotides experience nanomolar solution concentrations and hence do not react with each other at a significant rate. This effective molarity-

“A POSA would have understood that the disclosed organizational method . . . 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).”





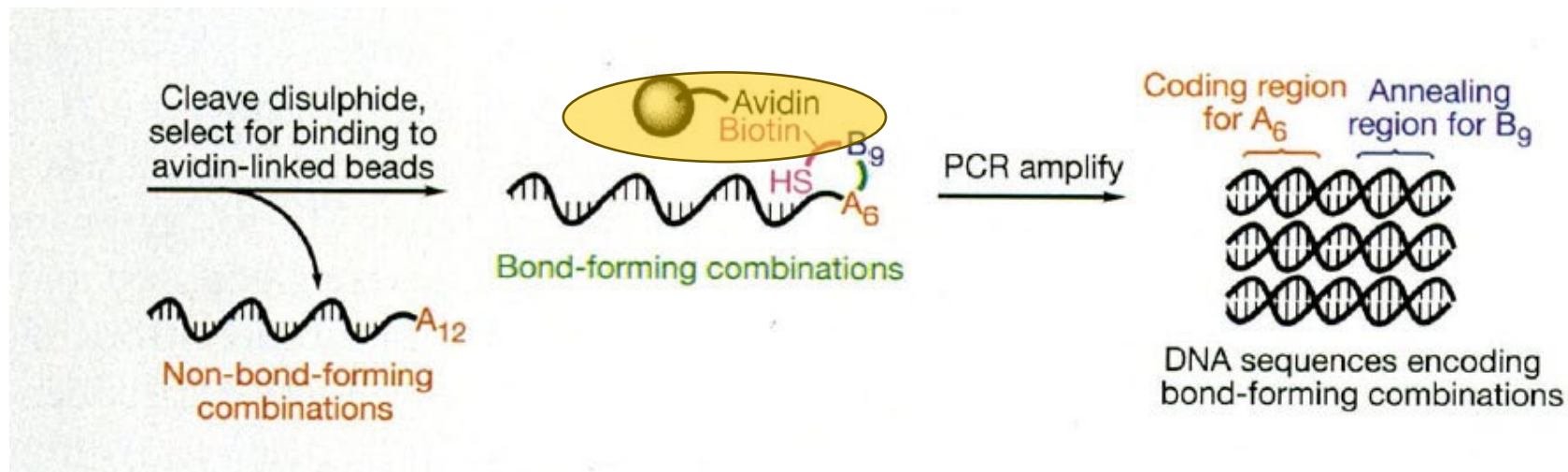
Kanan & Neri Teach Dependent Claim 19

'848 Patent

19. The method of detecting functional interactions between at least two molecules of interest as recited in claim 2, wherein the selective detection comprises labeling the cis-reactive cells with an affinity reagent.

“A POSA would have understood biotin as an affinity reagent because the biotin allows for streptavidin affinity selection for the bond-formation combinations.”

Kanan





Kanan & Neri Teach Dependent Claim 20

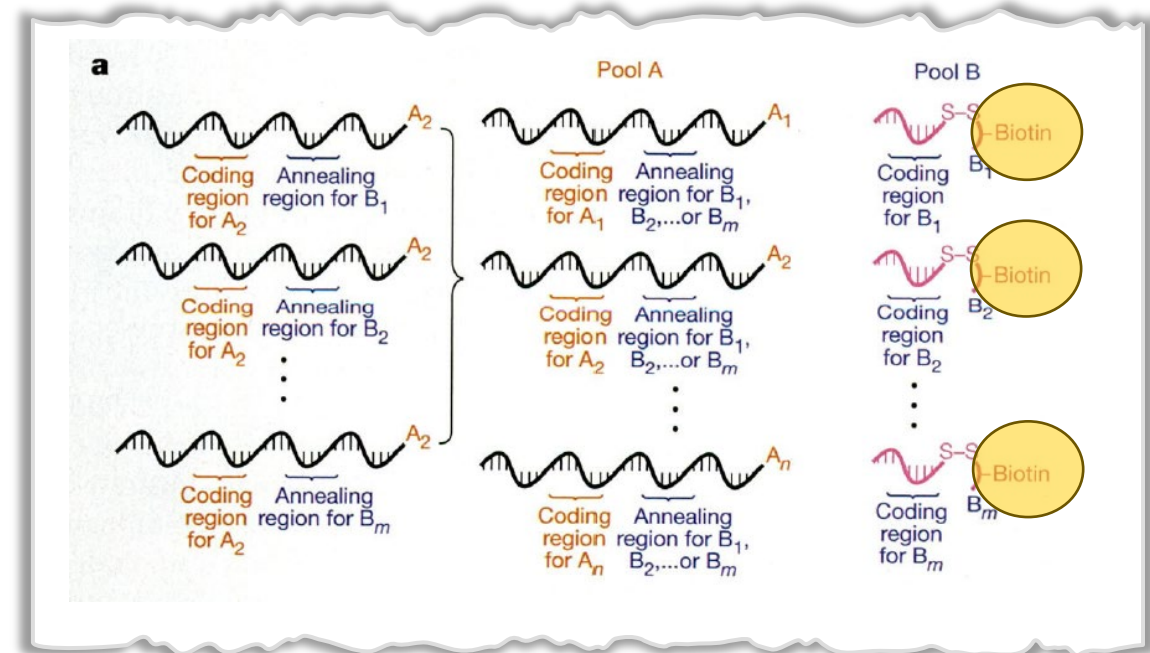
'848 Patent

20. 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.

“A POSA would have understood the substrate linked to biotin as a modified molecule because the substrate would not contain biotin without modification.”



Kanan



Petition at 45-46; Ex. 1001 at Claim 20; Ex. 1010 at 545-546, Fig. 1.a; Ex. 1005 at

Ground 1 – The Challenged Dependent Claims Are Obvious



Claim	Obvious?	Claim	Obvious?	Claim	Obvious?
2	Yes	9	Yes	17	Yes
3	Yes	10	Yes	18	Yes
3	Yes	11	Yes	19	Yes
4	Yes	12	Yes	20	Yes
5	Yes	13	Yes		
6	Yes	14	Yes		
7	Yes	15	Yes		
8	Yes	16	N/A		

Ground 2: Kanan in View of Neri & Famulok Claim 16

Claim 16

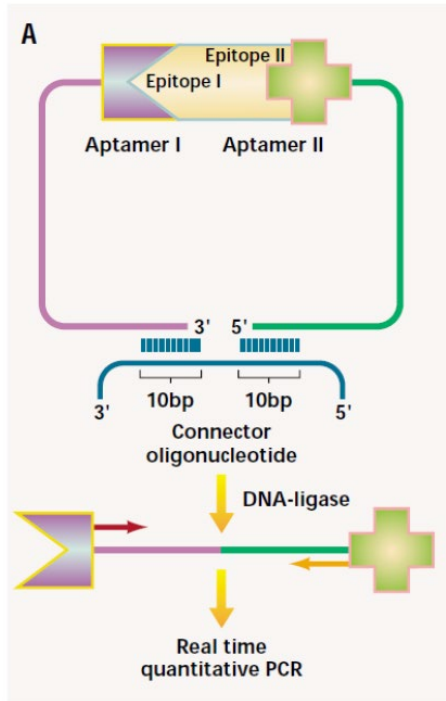


Claim	Disputed?
16	Yes

Kanan, Neri, & Famulok Teach Claim 16

'848 Patent

16. The method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein association is via ligation.



Famulok

Each aptamer has a different DNA-sequence extension that does not interfere with its folding and is not required for target recognition. Binding of the aptamer pair brings the ends of the oligonucleotide extensions into close spatial proximity (Fig. 1A) so that a "splint" oligonucleotide can hybridize to both ends, which are subsequently ligated together by T4 DNA ligase. 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, whereas no signal is obtained with unligated probes (Fig. 1B).



Kanan, Neri, & Famulok Teach Claim 16

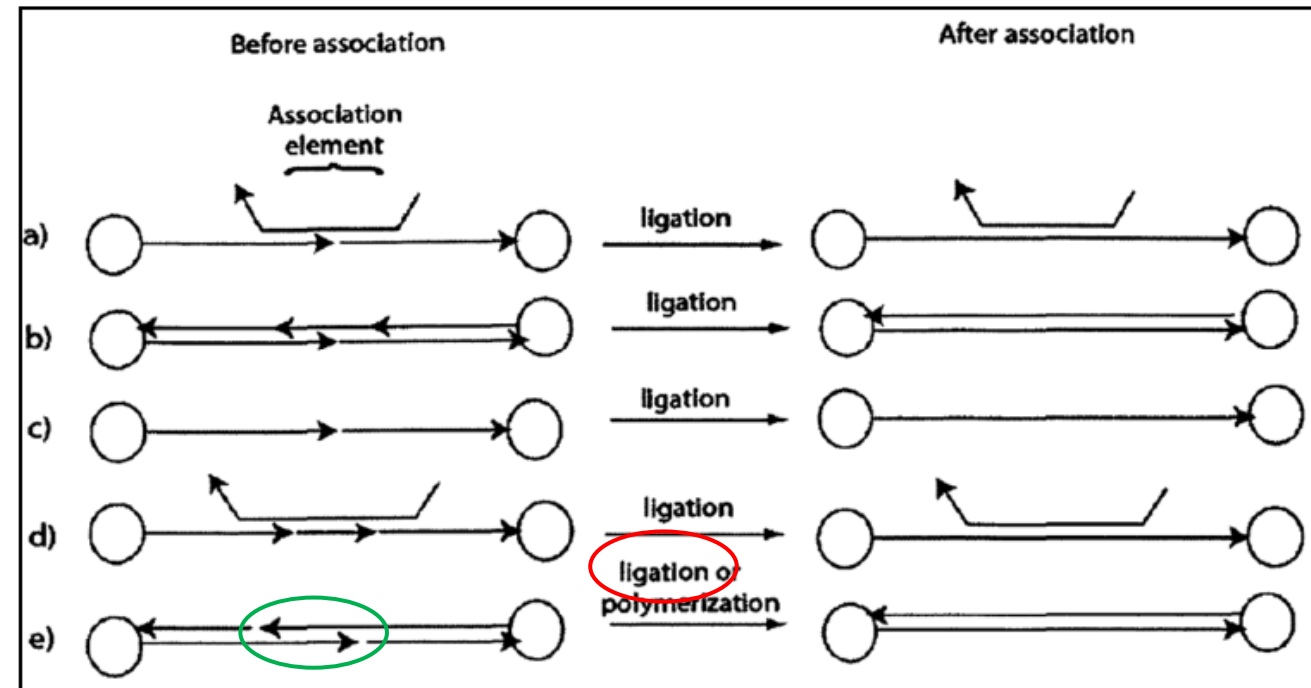
- PO's argument that association "by **ligation**" for claim 16 cannot be preceded by **hybridization** as in Kanan/Neri/Famulok disregards embodiment from the specification:

Nelson Second Deposition

THE WITNESS: So figure 5 of the '848 Patent, 5E shows overlap of the two 3 prime ends of the NAMs, which indicate hybridization and, therefore, prior to ligation and, therefore, Dr. Pentelute's argument that requires there is no hybridization between interactors is wrong. And that's a clear example.

THE WITNESS: Figure 4B shows the interactors overlapping. And in this particular figure, it says polymerization. And in figure 5E, there's the additional oligomers that are hybridized that show then the ligation in those spots next to the hybridization of the 3 prime ends of the interactors.

'848 Patent



Ex.1001, Fig. 5(a)-(e).

A POSA Would Have Been Motivated to Combine Kanan, Neri, & Famulok



“[T]he 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.”

“But a POSA would have understood that polymerization errors occur. They would have understood that not doing polymerization would be one possible method to avoid those issues and that Famulok, which was – would have – the ligation method of Famulok combined with Kanan and Neri would have presented an obvious alternative to them.”



Reduce errors

A POSA Would Have Been Motivated to Combine Kanan, Neri, & Famulok



- PO's concern of "technical design flaws" for cross-ligation to separate interactors at Neri's "overhang" region is unfounded



"Without the initial hybridization of the interactors to bring the strands in close spatial proximity, the risk of the splint ligating to another separate interactor in solution, is minimal"

Petition

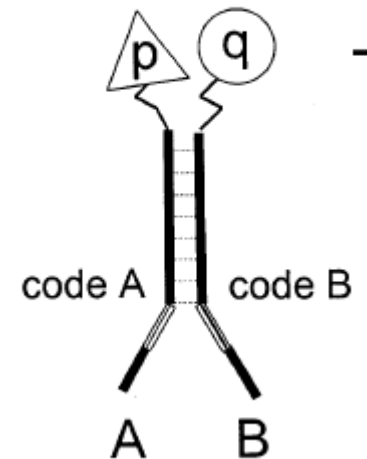
recognition." *Id.* The "[b]inding of the aptamer pair brings the ends of the oligonucleotide extensions into close spatial proximity . . . so that a 'splint' oligonucleotide can hybridize to both ends, which are subsequently ligated together by T4 DNA ligase." *Id.* "The ligated species can then act as a PCR template and the



No concern of cross-hybridization or mis-ligation

Neri

Fig. 7



Claim 16 Is Obvious



Claim	Obvious?
16	Yes

Ground 3:
Baez in view of Landegren
Claims 1-8, 11-12
Disputed Limitations 1(a), 1(d), 1(e)



Ground 3 – Disputed Limitations For Claim 1

Claim	Limitation	Disputed?
1(pre)	A method of detecting functional interactions between at least two molecules of interest	No
1(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	Yes
1(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 oligonucleotide comprises at least two identification elements derived from the at least two nucleic acid moieties	No
1(c)	Subjecting the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction with a detectable trace	No
1(d)	Selecting all cis-reactive cells exhibiting the detectable trace	Yes
1(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	Yes



Grounds 3 & 4 Adopt PO's District Court Positions

Petition

Grounds 3 and 4 apply PO's positions taken in the district court litigation as if

a POSA would have applied the claims in the same way at the time of the application.

See Section V.B.1. Petitioner's application of PO's positions is not an admission that the claims cover Petitioner's NULISA platform.

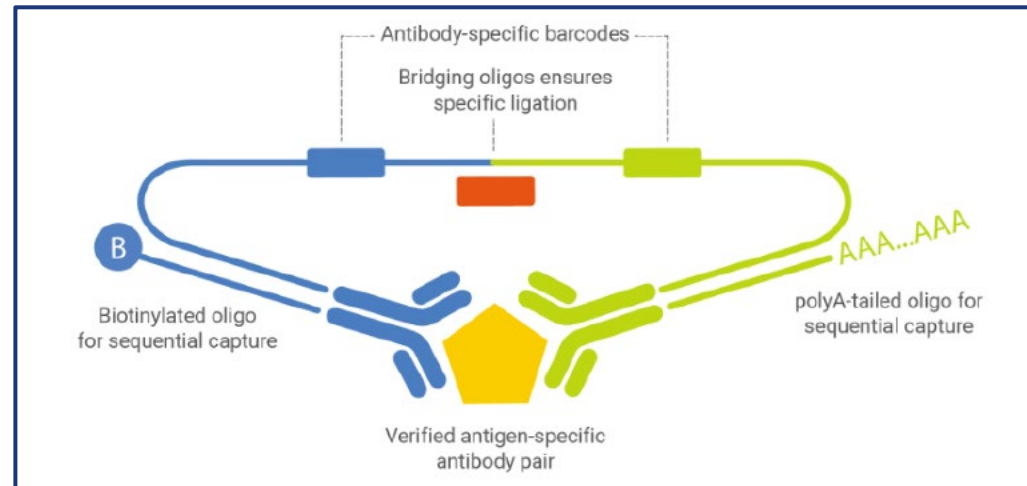
PO's Infringement Claim Chart

Infringement Analysis of U.S. Patent 7,883,848	
Claim	Alamar's NULISA Platform
1. 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;	The 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. Specifically, 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 one 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). See, e.g., Feng¹ at 2 ("The capture antibody is conjugated with a partially double-stranded DNA containing a poly-A tail and a target-specific molecular identifier (TMI), whereas the detection antibody is conjugated with another partially double-stranded DNA containing a biotin group and a matching TMI (Fig. 1a-1)."); see also Feng at 10 ("Antibody-oligonucleotide conjugation was performed based on amine-to-sulphydryl cross-linking chemistry."); https://alamarbio.com/technology/nulisa-platform/. 

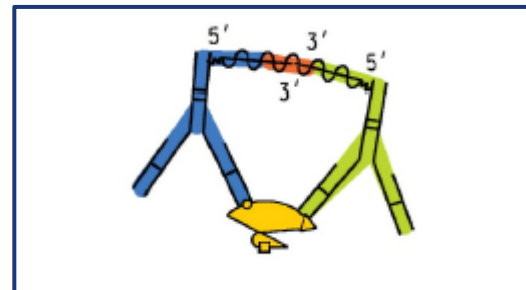
Baez & Landegren Teach Claim 1 of the '848 Patent



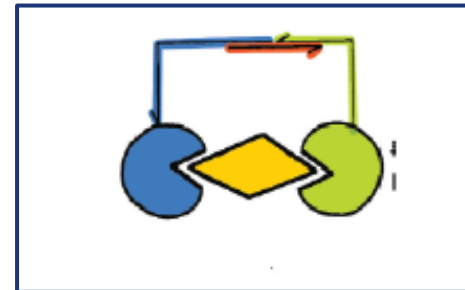
PO's Infringement Claim Chart



Baez



Landegren



Petition at 57-58; Ex. 1001 at Claim 1(b); Ex. 1008 at 2; Ex. 1003 at Fig. 2 (partial and color added); Ex. 1004 at Fig. 1 (partial and color added)



Baez & Landegren Teach the Preamble

'848 Patent

1. A method of detecting functional interactions between at least two molecules of interest, the method comprising:

Petition

PO contends that MOIs include “matched antibody pairs.” Ex. 1008, 1.



“Applying Patent Owner’s position, a POSA would have understood the ligation of the conjugate disclosed in Baez as a functional interaction.”



Baez & Landegren Teach Claim 1(a)

- Antibodies are the molecules of interest as set forth in the Petition, expert declaration of Dr. Nelson (Ex. 1005), and PO's own infringement claim chart (Ex. 1008)

Petition

PO contends that MOIs include “matched antibody pairs.” Ex. 1008, 1.

Petition

Baez in view of Landegren discloses this step. Ex. 1005, ¶¶234-240. Baez teaches that the plurality of conjugates, which can be “an antibody, lectin, receptor or binding protein,” are tagged with a nucleic acid moiety, creating “reporter conjugates.” Ex. 1003, 7:54-57, 7:48-53. PO contends that antibodies coupled to NAMs are the “interactors.” Ex. 1008, 1. Thus, the interactors include Baez’s “reporter conjugates.” Ex. 1003, 7:53-56, 10:51-56; Ex. 1005, ¶235.

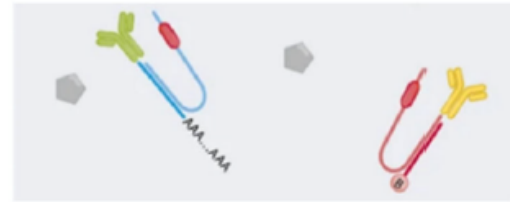
Petition at 55-56; Reply at 22-23



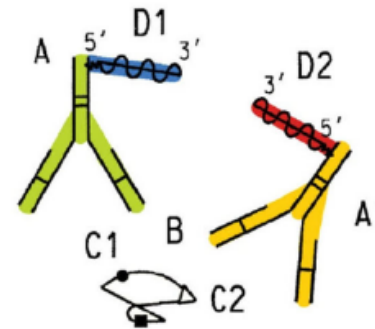
PO Feigns Ignorance of Baez's MOIs & Interactors

- PO's only dispute regarding 1(a) is on claim mapping, not the merits

Reply



Ex. 1008, 1.



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

Id. Dr. Nelson colored Baez's *antibodies* in *green* and *yellow* as the same *molecules of interest* identified by PO—and colored the *DNA labels*, shown in *blue* and *red*, as the same *nucleic acid moieties*. *Id.*



PO Feigns Ignorance of Baez's MOIs & Interactors

- PO's only dispute regarding 1(a) is on claim mapping, not the merits

Petition

PO contends that MOIs include “matched antibody pairs.” Ex. 1008, 1.

Baez in view of Landegren discloses this step. Ex. 1005, ¶¶234-240. Baez teaches that the plurality of conjugates, which can be “an antibody, lectin, receptor or binding protein,” are tagged with a nucleic acid moiety, creating “reporter conjugates.” Ex. 1003, 7:54-57, 7:48-53. PO contends that antibodies coupled to NAMs are the “interactors.” Ex. 1008, 1. Thus, the interactors include Baez’s “reporter conjugates.” Ex. 1003, 7:53-56, 10:51-56; Ex. 1005, ¶235.

Baez teaches a “reporter conjugate,” which can be “an antibody, lectin, receptor, or binding protein” that is coupled to a short “nucleic acid-label.” Ex. 1003, 7:48-57, Fig. 1. The conjugate is designed to bind to a predefined analyte. *Id.*, 7:53-57.



PO Feigns Ignorance of Baez's MOIs & Interactors

- PO's only dispute regarding 1(a) is on claim mapping, not the merits

Petition

274. PO contends that the “coupling of the molecule of interest (antibody) with [a] nucleic acid moiety (oligonucleotide) is covalent.” Ex. 1008, 7. Baez discloses that the “DNA labels” “were covalently attached to their respective antibodies through 5’ terminal amine groups,” and thus, this limitation includes a covalent bond between the antibody and oligonucleotide as taught in Baez. Ex. 1003, 26:57-59; Ex. 1005, ¶¶271-272, 274.



PO Feigns Ignorance of Baez's MOIs & Interactors

- PO's only dispute regarding 1(a) is on claim mapping, not the merits

Dr. Nelson

278), Patent Owner contends that the antibodies are protein molecules of interest, and Baez discloses antibodies as molecules of interest. Thus, relying on Patent

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.

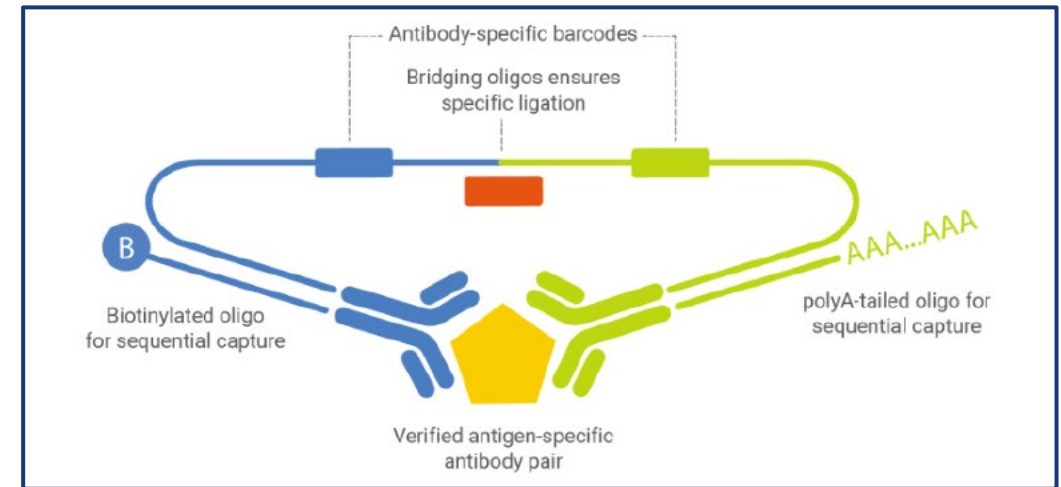


Baez & Landegren Teach Claim 1(b)

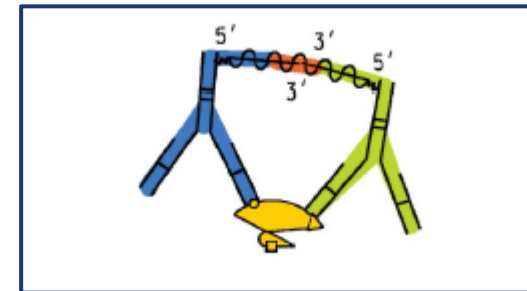
'848 Patent

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 oligonucleotide comprises at least two identification elements derived from the at least two nucleic acid moieties;

PO's Infringement Claim Chart



Baez



Landegren



Petition at 57-58; Ex. 1001 at Claim 1(b); Ex. 1008 at 2; Ex. 1003 at Fig. 2 (partial and color added); Ex. 1004 at Fig. 1 (partial and color added)



Baez & Landegren Teach Claim 1(c)

'848 Patent

c. subjecting the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction having a detectable trace;

Infringement Claim Chart

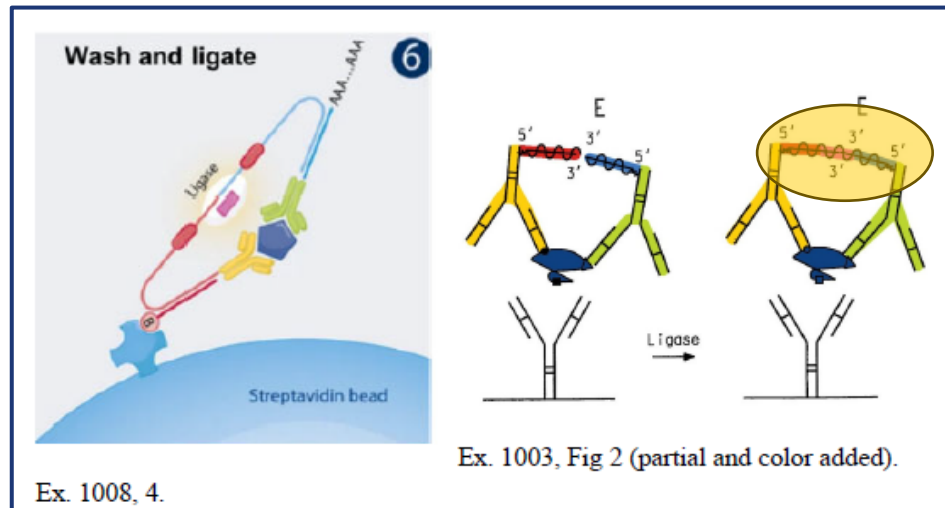
<https://alamarbio.com/technology/nulisa-platform/>. This step of introducing the ligation mix stimulates a desired functional interaction (ligation) having a detectable trace. *See, e.g.,* Feng, Figs. 1a-7a.

Baez & Landegren Teach Claim 1(c)

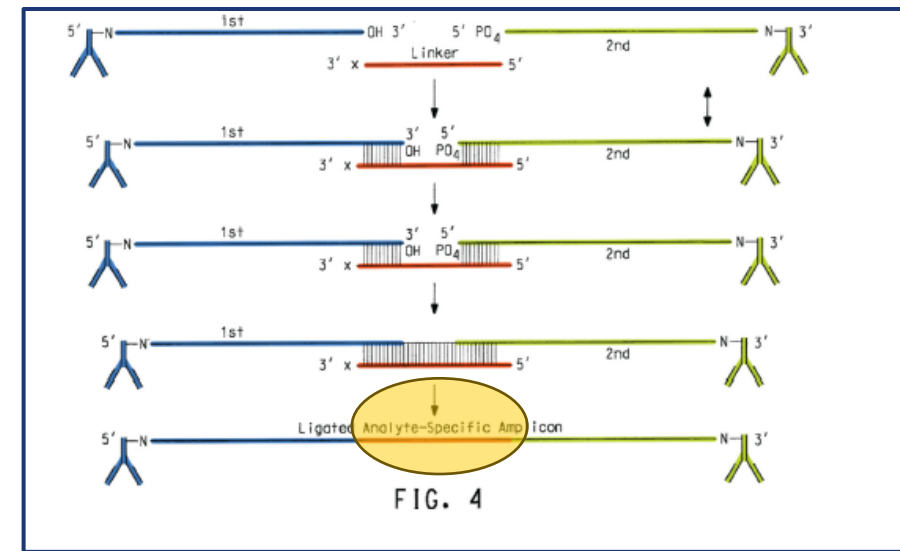
Infringement Claim Chart

<https://alamarbio.com/technology/nulisa-platform/>. This step of introducing the ligation mix stimulates a desired functional interaction (ligation) having a detectable trace. *See, e.g., Feng, Figs. 1a-7a.*

Petition



Baez





Baez & Landegren Teach Limitations 1(d) & 1(e)

'848 Patent

d. selecting all cis-reactive cells exhibiting the detectable trace; and

'848 Patent

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.



Baez & Landegren Teach Claim 1(d)

Infringement Claim Chart

d. selecting all cis-reactive cells exhibiting the detectable trace; and

The NULISA and NULISAseq Platforms can be used to select all cis-reactive cells exhibiting the detectable trace.

Specifically, the use of the NULISA and NULISAseq Platforms includes a step wherein “[a]fter 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.” Feng at 10.

Baez

rated into the amplified product sequences. These approaches afford several detection strategies. For example, the incorporation of biotinylated or ligand modified bases provides means of isolating the amplified nucleic acid products from solution onto a immobilized streptavidin or avidin. The addition of an avidin-signal-generating conjugate then facilitates detection. The amplified sequences may



Baez & Landegren Teach Claim 1(d)

- The '848 Patent identifies affinity purification as a type of “selecting” and Dr. Pentelute describes the same

'848 Patent

investigated. As used herein, a “filter” or a “filtering process” is the means employed to selectively separate the desired CRCs. Nonlimiting examples of filters for functional interactions include: affinity purification of the form which has undergone alteration in the functional interaction; detection

“The '848 patent teaches that CRCs which display functional interactions must be selected for detection and/or analysis. . . . Filtering processes, including ***affinity purification***, are employed during the process to selectively separate the desired CRCs.”





Baez & Landegren Teach Claim 1(d)



“Baez discloses that the ASAs can be selected using affinity purification 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., demonstrative the detectable trace, because only those ASAs would have the affinity for the immobilized streptavidin.”

Q. Can that also be used for the – you know, biotin and streptavidin used for affinity purification?

A. So the biotin-streptavidin complex is ***driven by affinity***. And you do capture it because of the high affinity. And the molecules that have biotin will be captured to the resin, and the other molecules that don't have biotin will not be captured. So there is an enrichment or ***purification*** step.

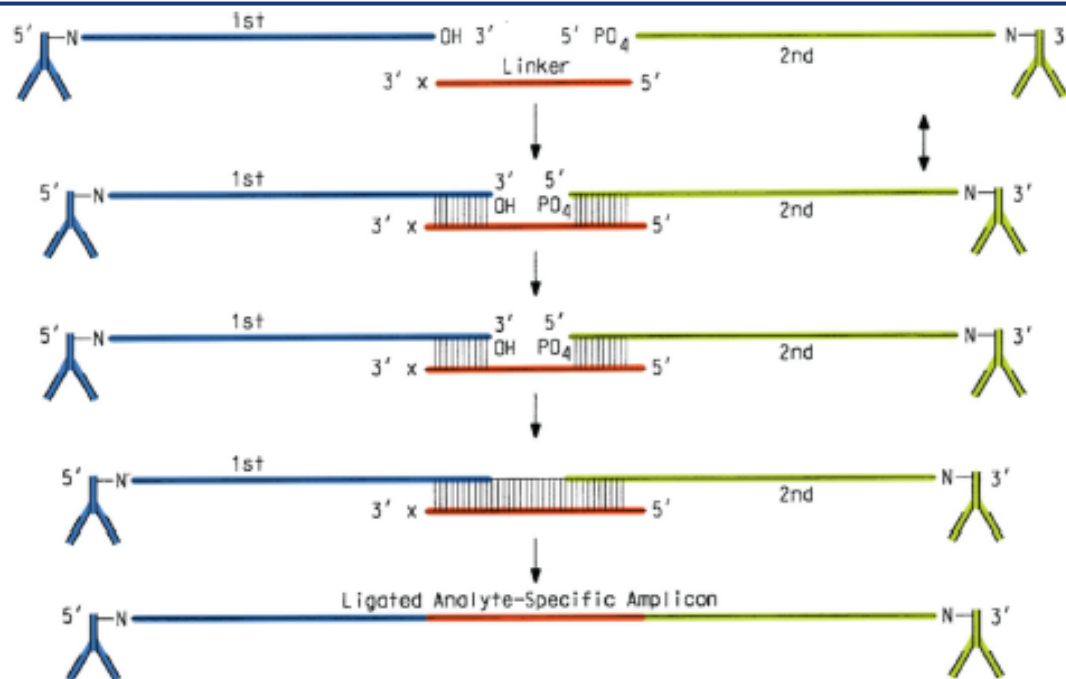


Baez & Landegren Teach Claim 1(d)'s "Selecting" Limitation

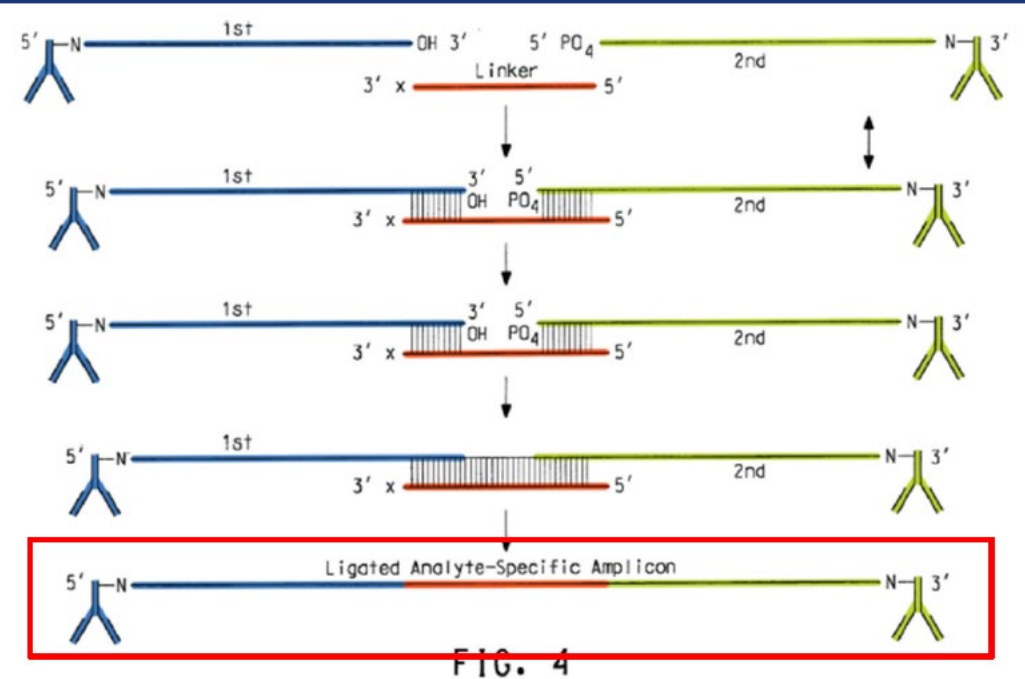


- Baez & Landegren would select Baez's ASA, which would include antibodies bound to their nucleic acid labels, to select "only the **ASAs** that have been ligated together"

Petition



Reply



Ex.1003, Fig. 4 (color added, showing ASA); Ex.1005, ¶217.



Baez & Landegren Teach Claim 1(e)

Infringement Claim Chart

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.	The NULISA and NULISAseq 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. Specifically, the use of the NULISA and NULISAseq Platforms includes the following steps: The final eluate was collected for quantification by qPCR for single-plex NULISA and by NGS for NULISAseq.
--	--

Baez

analyte and two are used for reporter conjugates. Third, the amplification by PCR is used to detect the product of two joined nucleic acid-label in contrast to the amplification product of a single reporter label. Fourth, the random



Baez & Landegren Teach Limitations 1(d) & 1(e)

- **Claim 1(d):** First, Petitioner's Baez & Landegren combination would select the CRCs (Baez's ASA, which includes the interactors) with affinity purification:

1008, 5. Thus, selecting the CRCs includes Baez's isolating CRCs (ASAs in Baez) comprising a biotin label using immobilized streptavidin. Ex. 1005, ¶251.

Baez discloses that the ASAs can be selected using affinity purification. This involves detecting the ASA "through the linker oligonucleotide itself." Ex. 1003,

- **Claim 1(e):** Then, the ASA's ligated nucleic acid (comprising the identification sequence elements of Landegren's tags) is analyzed with PCR:

Further, it would have been obvious to perform the PCR amplification after the selection of the ASA using the biotin linker. Ex. 1005, ¶257.

A POSA Would Have Been Motivated To Modify Baez With Landegren



“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. . . . A POSA would have been motivated to create a multiplex assay in order to detect and analyze multiple analytes in a single assay.”



Multiple analytes in a single assay



A POSA Would Have Been Motivated To Modify Baez With Landegren



- Petitioner's Baez & Landegren combination only takes identification tags from Landegren to modify Baez's nucleic acid labels



"A POSA would have understood this combination to be only altering the nucleic acid labels of Baez. Thus, a POSA's incorporation of Landegren's identification tags does not require the assay to be performed in solution."

X Landegren's tags don't require solution phase

A POSA Would Expect Success In Modifying Baez With 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 any analyte Moreover, both Baez and Landegren disclose that the analyte is detected by the interaction of the nucleic acid sequences couples to each member of a binding pair (e.g., antibodies).”



Landegren

Baez

The term “reporter conjugate” refers to a conjugate comprising a “nucleic acid-label” coupled to one member of a binding-pair such as an antibody, lectin, receptor or binding protein or other moiety which can bind to an analyte.

The binding moieties of the proximity-probes may be proteins such as monoclonal and polyclonal antibodies, peptides, lectins, nucleic acids, carbohydrates, soluble cell surface receptors, combinatorially derived protein from phage display or ribosome display, or any other type of binding moiety. Combinations of binding moieties may also be used.

The reactive functionality of a proximity-probe is comprised of a nucleic acid coupled to the binding moiety. This coupling may be a covalent crosslinking or non-covalent, as

A POSA Would Expect Success In Modifying Baez With Landegren



- Baez + Landegren uses Landegren's identification tags
- Modifying Baez's nucleic acid labels to include identification tags as in Landegren would be routine for a POSA



“[T]he structure of the reporter conjugates in Baez and the proximity-probes in Landegren are similar, both are antibodies coupled to nucleic acid”

“[M]odifying nucleic acid is within the skillset of a POSA”

A POSA Would Have Been Motivated To Modify Baez With Landegren



- A POSA would still be motivated to improve Baez

Baez

The development of immunoassays and advances in methods of nucleic acid amplification have significantly advanced the art of the detection of biological analytes. In spite of these advances, nonspecific binding of the analyte to be detected and general assay noise has remained a problem that has limited the application and sensitivity of such assays. Methods for the reduction of background noise are continually being sought.

“The strategies employed in Landegren such as use of unique identifier sequence 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.”



Continue to reduce errors



A POSA Would Have Been Motivated To Modify Baez With Landegren



- PO argues that Landegren teaches away from Baez because Landegren disparages solid-phase supports in favor of solution assays, but Baez teaches both solution assays and solid-phase assays:

Baez

Both the ligation and overlap methods may be conducted in either a heterogeneous format where the analyte is immobilized, of in a homogeneous format where the analyte is free in solution. It should be noted that, the present

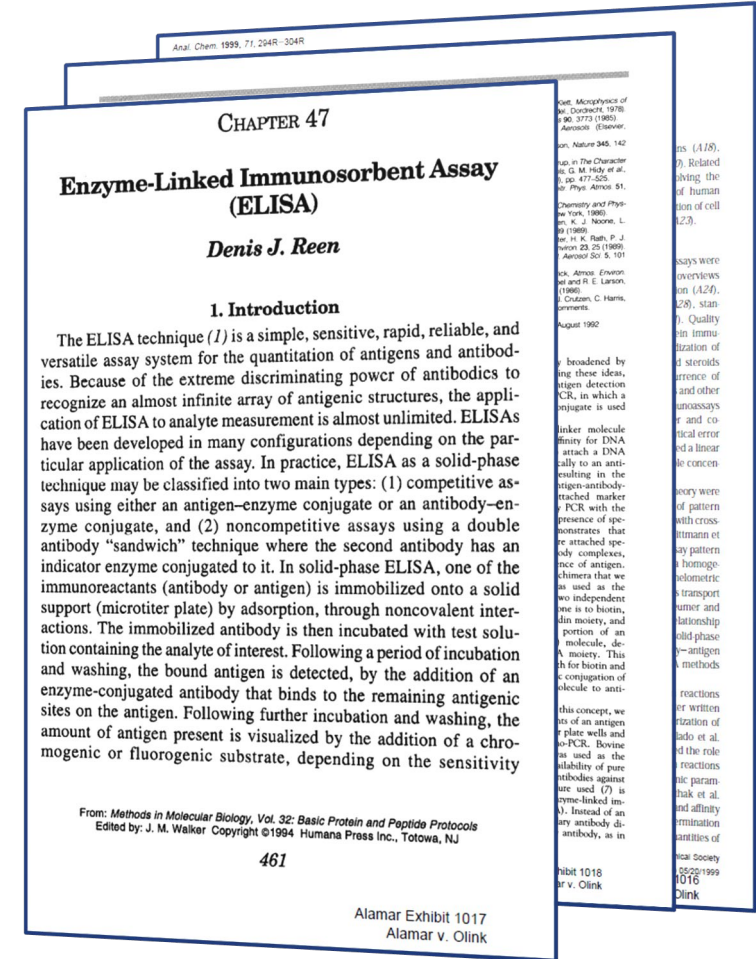


Baez allows solution assays

A POSA Would Have Been Motivated To Modify Baez With Landegren



“[A]t the time of the invention solid-phase capture was a well-known and popular technique. See, e.g., Ex. 1016, 1017, 1018.”



Reply at 28; Ex. 1051 at ¶ 111; Ex. 1016, 1017, 1018



Ground 3 – Independent Claim 1 Is Obvious

Claim	Limitation	Obvious?
1(pre)	A method of detecting functional interactions between at least two molecules of interest	Yes
1(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	Yes
1(b)	Forming a plurality of cis-reactive cells wherein a cis-reactive cell comprises at least two interactors bound in proximity to on 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	Yes
1(c)	Subjecting the plurality of cis-reactive cells to conditions which stimulate a desired functional interaction with a detectable trace	Yes
1(d)	Selecting all cis-reactive cells exhibiting the detectable trace	Yes
1(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	Yes



Ground 3 - Dependent Claims

Claim	Disputed?*	Claim	Disputed?*	Claim	Disputed?*
2	No	9	N/A	17	N/A
3	No	10	N/A	18	N/A
3	No	11	No	19	N/A
4	No	12	No	20	N/A
5	No	13	N/A		
6	No	14	N/A		
7	No	15	N/A		
8	No	16	N/A		

*Patent Owner disputes that claims 2-20 are obvious only due to their direct or indirect dependence on claim 1. Response at 56.



Baez & Landegren Teach Dependent Claim 2

'848 Patent

2. 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.

'848 Prosecution History

cells. Both selective separation and selective detection are techniques well known in the art, whereby claim 21 is definite and clear to one of ordinary skill in the art. Accordingly, the



Baez & Landegren Teach Dependent Claim 2

Infringement Claim Chart

2. 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.	See above re: claim 1. When 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. <i>See, e.g.,</i> Feng at 10 (“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.”).
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Baez

approaches afford several detection strategies. For example, the incorporation of biotinylated or ligand modified bases provides means of isolating the amplified nucleic acid products from solution onto a **immobilized streptavidin** or avidin. The addition of an avidin-signal-generating conju-



Baez & Landegren Teach Dependent Claim 3

Infringement Claim Chart

3. 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.	See above re: claim 2. When using the NULISA and NULISaseq Platforms, the wash and elution steps are selection steps that constitute affinity purification. <i>See, e.g.,</i> Feng at 10 (“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.”).
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Baez

approaches afford several detection strategies. For example, the incorporation of biotinylated or ligand modified bases provides means of isolating the amplified nucleic acid products from solution onto a **immobilized streptavidin** or avidin. The addition of an avidin-signal-generating conju-



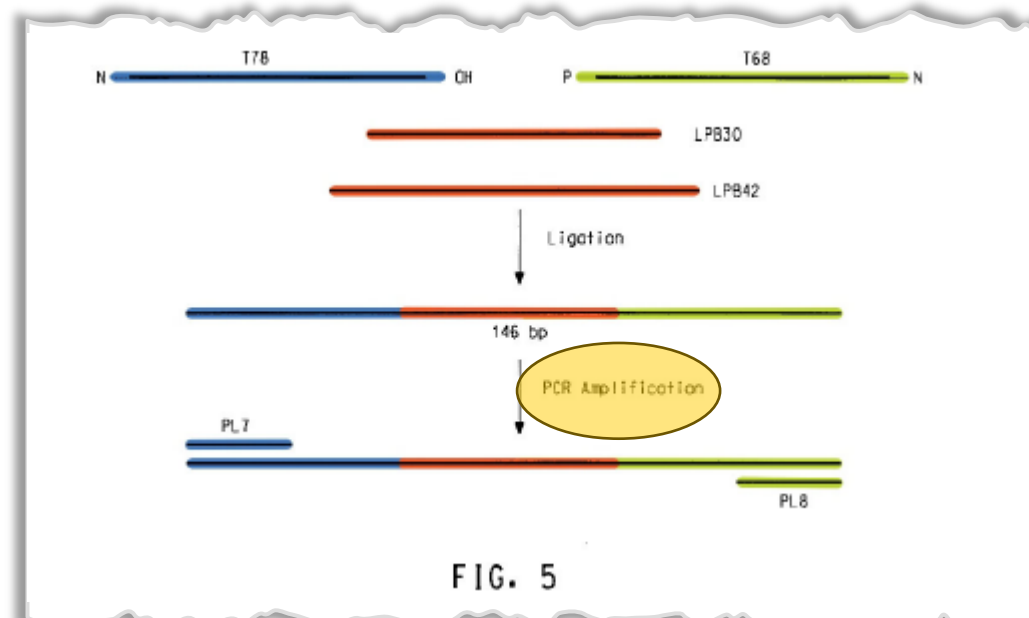
Baez & Landegren Teach Dependent Claim 4

Infringement Claim Chart

4. A 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.

See above re: claim 1.

When using the NULISA and NULISaseq Platforms, the detection of the selected associated oligonucleotides (i.e. the reporter molecules) is enhanced by PCR amplification. *See, e.g.,* Feng at 2 (“The DNA reporter can then be quantified by quantitative PCR (qPCR) for single-plex analysis.”); Feng at 10 (“For multiplexed assays, a DNA strand containing a unique sample barcode is ligated between the two oligos. The ligated products are then pooled, amplified by PCR to create a library ready for Next Gen Sequencing”); Feng at 10 (“For NGS analysis, a library was prepared by pooling the reporter molecules from each NULISaseq reaction and then amplifying the products by 16 cycles of PCR.”); Feng, Figs. 1a-7a and 1a-7b.



Baez

Petition at 63-64; Ex. 1008 at 7; Ex. 1003 at Fig. 5 (color added)



Baez & Landegren Teach Dependent Claims 5 & 6

Infringement Claim Chart

5. A method of detecting functional interactions between at least two molecules of interest as recited in claim 1, wherein coupling is covalent or noncovalent.	See above re: claim 1. When using the NULISA and NULISaseq Platforms, the coupling of the molecule of interest (antibody) with the nucleic acid moiety (oligonucleotide) is covalent. <i>See, e.g.,</i> Feng at 2 (“The capture antibody is conjugated with a partially double-stranded DNA containing a poly-A tail and a target-specific molecular identifier (TMI), whereas the detection antibody is conjugated with another partially double-stranded DNA containing a biotin group and a matching TMI (Fig. 1a–1).”); Feng at 10 (“Antibody-oligonucleotide conjugation was performed based on amine-to-sulfhydryl cross-linking chemistry.”).
6. A method of detecting functional interactions between at least two molecules of interest as recited in claim 5, wherein coupling is covalent.	See above re: claim 5.

Baez

For the overlap format (Examples 1, 2, 3 and 5), the DNA labels, T66 and T68 (Table 1), were **covalently attached** to their respective antibodies through 5' terminal amine groups.



Baez & Landegren Teach Dependent Claims 7 & 8

'848 Patent

7. A 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.

8. A 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.

Baez

The term “reporter conjugate” refers to a conjugate comprising a “nucleic acid-label” coupled to one member of a binding-pair such as an **antibody**, lectin, receptor or binding protein or other moiety which can bind to an analyte.

Infringement Claim Chart

When using the NULISA and NULISAsq Platforms, at least one molecule of interest (**e.g. capture antibody or detection antibody**) is a protein.



Baez & Landegren Teach Dependent Claim 11

Infringement Claim Chart

11. A 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.

See above re: claim 1.

When using the NULISA and NULISAseq Platforms, the method of detecting includes quantifying the functional interactions. *See, e.g.*, Feng at 2 (“After ligation, the ligation products are pooled, amplified, and purified to generate a sequencing library that is compatible with multiple Illumina sequencing instruments. Raw sequencing reads are processed to generate target- and sample-specific counts.”); *see also* Feng, Figs. 1a–7a and Fig. 1a-7b (specifying “Quantify by PCR” and “Quantify by NGS”).

Baez

During 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. In this way, analytes can be specifically detected and quantified.



“[W]ell within the knowledge and experience of a POSA to use the well-known method of quantitative PCR . . . For quantification of the ASA product in Baez.”



Baez & Landegren Teach Dependent Claim 12

Infringement Claim Chart

12. A method of detecting functional interactions between at least two molecules of interest, as recited in claim 1, wherein the analysis comprises high throughput screening.	See above re: claim 1. When using the NULISA and NULISaseq Platforms, the method of detecting includes high throughput screening. See, e.g., https://alamarbio.com/alamar-biosciences-announces-seminal-publication-in-nature-communications-demonstrating-superior-performance-of-the-nulisa-platform/ (“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. The ARGO HT System enables a fully automated workflow for the NULISA Assay, with less than 30 minutes of hands-on time from sample to data, and highly reproducible results, with less than 10% coefficient of variation.”).
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Landegren

amplified. These different PCR products may be detected by any of several methods, such as **DNA microarrays**, mass spectrometry, gel electrophoresis (different lengths of products), or other. A true positive ligation must contain both sequence identification

'848 Patent

amenable to analysis with microarrays. **Microarray analysis** is a conventional platform which enables cost effective data generation in a **high throughput manner**. A method of protein

Ground 3 – The Challenged Dependent Claims Are Obvious



Claim	Obvious?	Claim	Obvious?	Claim	Obvious?
2	Yes	9	N/A	17	N/A
3	Yes	10	N/A	18	N/A
3	Yes	11	Yes	19	N/A
4	Yes	12	Yes	20	N/A
5	Yes	13	N/A		
6	Yes	14	N/A		
7	Yes	15	N/A		
8	Yes	16	N/A		

Ground 4: Baez in view of Landegren & Freskgard Claims 14-15



Ground 4 – Claims 14 & 15

Claim	Disputed?	Claim	Disputed?	Claim	Disputed?
2	N/A	9	N/A	17	N/A
3	N/A	10	N/A	18	N/A
3	N/A	11	N/A	19	N/A
4	N/A	12	N/A	20	N/A
5	N/A	13	N/A		
6	N/A	14	Yes		
7	N/A	15	Yes		
8	N/A	16	N/A		



Baez, Landegren, & Freskgard Teach Claim 14

Infringement Claim Chart

14. 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.	See above re: claim 1. When using the NULISaseq Platform, the method of detecting includes nucleic acid sequencing. <i>See, e.g.</i>, Feng at 2 (“For NGS analysis of multiple samples, sample-specific molecular identifiers (SMI) are introduced into the reporter DNA using double-stranded ligators containing SMI sequences (Fig. 1a–7b), which enables pooling of a full 96-well plate of samples into one sequencing reaction. After ligation, the ligation products are pooled, amplified, and purified to generate a sequencing library that is compatible with multiple Illumina sequencing instruments.”); Feng at 10 (“The library was then quantified using the Qubit 1X dsDNA HS assay kit (Thermo Fisher, Waltham, MA) before being loaded on a NextSeq 1000/2000 instrument (Illumina, San Diego, CA). For the NULISaseq 200-plex inflammation panel, a P2 reagent kit (100 cycles) (Illumina) was used for NGS.”).
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Freskgard

Thus 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. This is typically accomplished by am-



“A POSA would have understood that Freskgard’s ‘sequencing’ is used to identify the nucleic acid sequence of the identifier oligonucleotides.”



Baez, Landegren, & Freskgard Teach Claim 15

Infringement Claim Chart

15. 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.

See above re: claim 14.

When using the NULISaseq Platform and running the NGS analysis, the method of detecting includes sequencing by synthesis. *See, e.g.,* <https://www.illumina.com/science/technology/next-generation-sequencing.html> (“Illumina NGS technology utilizes a fundamentally different approach from the classic Sanger chain-termination method. It leverages sequencing by synthesis (SBS) technology – tracking the addition of labeled nucleotides as the DNA chain is copied – in a massively parallel fashion.”); <https://www.illumina.com/science/technology/next-generation-sequencing/sequencing-technology.html> (“Illumina sequencing technology, sequencing by synthesis (SBS), is a widely adopted next-generation sequencing (NGS) technology worldwide. Illumina sequencing instruments and reagents support massively parallel sequencing using a proprietary method that detects single bases as they are incorporated into growing DNA strands.”).

ward and reverse primers according to the manufacturer’s instructions. A sample of each PCR product was then treated with Exonuclease I (USB) and Shrimp Alkaline Phosphatase (USB) to remove degrade 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). Sequence outputs



“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.”



A POSA Would Have A Reasonable Expectation of Success Combining Freskgard, Baez & Landegren

Freskgard

(57) Abstract: The present invention relates to a method for identifying a synthetic molecule having affinity towards a target. The method includes the steps of providing a library of bifunctional complexes, wherein each complex of the library comprises a synthetic molecule attached to an identifier, which codes for said molecule; subjecting, under binding conditions, the library of bifunctional complexes to a target; removing the non-binding members of the library; separating the identifiers of complexes comprising synthetic molecules having affinity towards the target, and decoding the identifiers to establish the identity of the molecule.

Baez

The term “reporter conjugate” refers to a conjugate comprising a “nucleic acid-label” coupled to one member of a binding-pair such as an antibody, lectin, receptor or binding protein or other moiety which can bind to an analyte.

Landegren

The binding moieties of the proximity-probes may be proteins such as monoclonal and polyclonal antibodies, peptides, lectins, nucleic acids, carbohydrates, soluble cell surface receptors, combinatorially derived protein from phage display or ribosome display, or any other type of binding moiety. Combinations of binding moieties may also be used.

The reactive functionality of a proximity-probe is comprised of a nucleic acid coupled to the binding moiety. This coupling may be a covalent crosslinking or non-covalent, as



Ground 4 – Claims 14 & 15 Are Obvious

Claim	Obvious?	Claim	Obvious?	Claim	Obvious?
2	N/A	9	N/A	17	N/A
3	N/A	10	N/A	18	N/A
3	N/A	11	N/A	19	N/A
4	N/A	12	N/A	20	N/A
5	N/A	13	N/A		
6	N/A	14	Yes		
7	N/A	15	Yes		
8	N/A	16	N/A		

PO's Motion to Exclude

PO's Motion To Exclude Fails To Allege Impeachment



- PO's moves to exclude its infringement claim chart, Ex. 1008, arguing that it is improper "impeachment" evidence under Rule 408



Motion does not identify how PO is impeached (no inconsistent statement or contradictions)



Chart not offered for impeachment purpose



PO has never disavowed any claim chart positions



Claim chart offered to show POSA's understanding of PO's application of the claims

