

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

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

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

a) Materials cited in my First Declaration (Ex. 1005);

b) The declaration of Patent Owner’s expert, Dr. Bradley Pentelute (Ex. 2008);

c) Deposition transcript of Dr. Pentelute (Ex. 1049); and

d) Materials cited in this Declaration.

I. Professional Background

3. My background and qualifications are provided in paragraphs 4 through 11 in my First Declaration (Ex. 1005). My qualifications are also set forth in my *curriculum vitae* (Ex. 1006).

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

4. I understand that Patent Owner’s expert, Dr. Pentelute, provided his own definition of a POSA.

5. I have reviewed Dr. Pentelute’s definition. Ex. 2008, ¶ 79.

6. Dr. Pentelute's definition is similar, but not exact to my own. Regardless, I would reach the same conclusions provided in this Declaration if I applied either my definition or Dr. Pentelute's.

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

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

1. Dr. Pentelute is wrong that a POSA would perform polymerization after selection, a POSA would perform polymerization during step (b)

7. Dr. Pentelute appears to argue that a POSA (and my opinions) would follow the *timing* of polymerization from Neri when implementing Grounds 1 & 2. *E.g.*, Ex. 2008, ¶ 129. That is incorrect. A POSA would not have followed Neri's *timing* to perform polymerization, which "occurs *after* biopanning," contrary to Dr. Pentelute. Ex. 2008, ¶ 129. Rather, a POSA would have used Neri's *method* of polymerization that is combined with Kanan. *See, e.g.*, Ex. 1005, ¶¶ 180-184. Neri's biopanning relates to screening the "ESACHEL components with the target molecule . . . followed by the physical separation of the resulting complex from the ESACHEL components which have not bound to the target." Ex. 1011, [0084]. In other words, this process allows molecules to bind to a target and then separates the molecules that have not bound to a target. Neri's biopanning does not relate to either Neri's "self-assembly sequence" or method of polymerization. Thus, Neri's biopanning disclosure is not relevant to the Kanan/Neri design.

8. My previous discussion of association “via polymerization” explains that polymerization occurs in claim 1 step (b). Ex, 1005, ¶¶ 180-184. Claim 1 step (b) requires that the “associated oligonucleotide [is] formed *from the association* between at least two nucleic acid moieties[.]” See Ex. 1001, Cl. 1, Fig. 5 (emphasis added). An embodiment of the “association” within claim 1 step (b) is “association via polymerization.” *Id.*, Cl. 17. Moreover, Dr. Pentelute agrees that the association referenced in claim 17 is the association from claim 1 step (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?

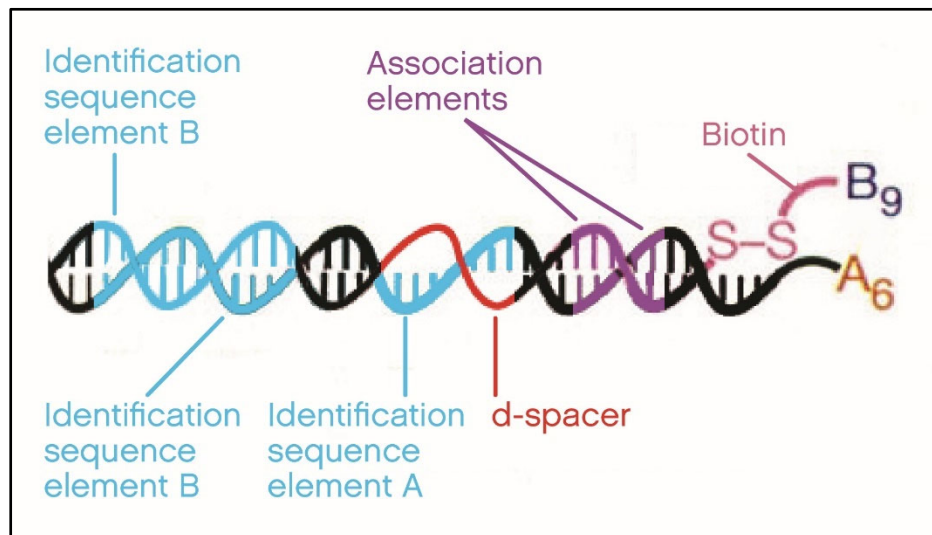
A: . . . So the association appears to be, according to claim 1, the association as described in claim 1(b).

Ex. 1048, 103:5-104:20. Thus, the “association” and polymerization discussed in claim 17 is the association of the NAMs that occurs in claim 1 step (b).

9. As I previously explained, with the polymerization in the Kanan/Neri design, a POSA would have understood that the “[o]ligonucleotides of sub-library B [pool A oligonucleotides in Kanan/Neri design] . . . can work as primers for a DNA polymerase reaction on the template A [pool B oligonucleotide in Kanan/Neri design].” Ex. 1011, [0117]; Ex. 1005, ¶¶ 181-182. A POSA would use “a suitable buffer,” “dNTPs,” and “Taq polymerase” to perform the primer extension. Ex. 1011, [0119]. A POSA would then “incubat[e] . . . the mixture at 40° C for 5 minutes” to

achieve the primer extension reaction. *Id.* A POSA would have understood that a primer extension reaction would be routine, would be accomplished in only a few minutes, and would not affect the functional interaction process described in Kanan (Ex. 1013, 8). Ex. 1005, ¶ 183. As discussed in my First Declaration, this association via polymerization would occur at the time of association, which would be prior to the functional interaction. Ex. 1005, ¶¶ 107-116, 180-184.

10. In discussing polymerization, my First Declaration replicated Figure 5 of Neri, *id.*, ¶¶ 83, 96 and 181. Below is a depiction of how the polymerization process of the Kanan/Neri design, *id.*, ¶¶ 180-184, from my prior analysis would be visualized.



Each component of the above figure is described and/or illustrated by my First Declaration. Ex. 1005, ¶¶ 105-106, 114-115, 180-184; *see also* Ex. 2009, 163:3-22; 165:12-20; 167:1-168:24; 169:8-12 (discussing figures at Ex. 1005, pp. 63 and 67).

As discussed in detail below (paragraphs 54-58), the Kanan/Neri design established in my First Declaration supports polymerization, contrary to Dr. Pentelute.

11. I note that Dr. Pentelute was asked how polymerization would work with the Kanan/Neri design, but he was unable to provide details. Ex. 1048, 181:15-184:7. He claimed that:

I've already mentioned, look at the number of variables here. Right? The first thing you have to think about is: I'm going to add in a primer. What are the primers? Where are they binding? And then you have to denature this whole thing, and then you have to anneal it. But if you look, there's pieces in here that can self-anneal in different directions. And so all of a sudden you're going to have these, I call them, phantom constructs, where it's not clear what's going to happen if you're going to do a polymerization that you're proposing might happen. But as a POSA, I can't -- I don't know what's going to happen. I have to sit down and think about a lot of parameters. There's a lot of parameters here.

Ex. 1048, 183:13-184:7. But with the Kanan/Neri design, it is a primer extension reaction, there is no denaturing and annealing that Dr. Pentelute discusses. And as identified in my First Declaration, the pool B oligonucleotide can work as a primer for the primer extension reaction in the Kanan/Neri design. Ex. 1005, ¶ 181. Thus,

Dr. Pentelute's testimony regarding his understanding of polymerization does not change my opinions.

12. If polymerization is performed during step (b), Dr. Pentelute's declaration argues polymerase buffers "could break the disulphide bond [of Kanan] prematurely[.]" Ex. 2008, ¶ 132. According to him, polymerase buffers require a reducing agent in an amount that would efficiently cleave the disulfide bonds of the Kanan/Neri design. *Id.* Dr. Pentelute offers no evidentiary support for his claim. Nonetheless, as provided by Neri, a POSA would have chosen a "suitable buffer." Ex. 1011, [0119]. As discussed below, a POSA would not be limited to a polymerase buffer containing a reducing agent and would not use a polymerase buffer that contained a reducing agent in a concentration that would meaningfully cleave disulfide bonds.

13. To perform polymerization, a POSA would use a polymerase storage buffer, a polymerization reaction buffer (if needed), and designed reaction conditions where the enzyme in the storage buffer is diluted into the reaction buffer. Identifying and using polymerase, polymerase buffers and reaction buffers are within the routine skill of a POSA at the time of the application. Ex. 1005, ¶ 183. The '848 patent even admits that gap-fill polymerization was known at the time of the application. Ex. 1001, 17:33-37.

14. Dr. Pentelute's arguments are overblown for the simple reason that when designing experiments, a POSA balances various variables so as to design the experiment to optimize the goal of the experiment, which I understand Dr. Pentelute agrees:

Q: And you could design that buffer to be such that it would work best in the reaction that you're doing? Is that right?

A: So every reaction needs the correct conditions. It needs to have the right pH, the right ions, the right amount of salt. Yeah, so a biochemist would set up a set of conditions that allow that enzyme to be functional.

Ex. 1048, 54:12-25.

Q: And if, for example, you had something in your buffer that would cause problems in your reaction, you would adjust your buffer so that it wouldn't cause that.

A: So it's a spectrum. It depends on what the goal is. So you may have an additive or something in a buffer that doesn't allow maximal performance, but it turns out to be important for something else. And so in that case, you may tolerate a condition that not as efficient for the chemistry perhaps, but important for a different variable. Generally speaking, it's multiple variables that have to be understood in order to do, you know, the set of chemistry or the goal of the experiment.

Ex. 1048, 55:23-56:14.

15. With the Kanan/Neri design, if there was a concern that a reducing agent could prematurely break the disulfide bond (I do not believe there was such a concern), a POSA would design the experiment to prevent unintended cleavage. For example, prior to July 2005, a POSA would have several options—(1) making their own polymerase storage buffer and polymerization reaction buffer that do not contain reducing agents; (2) purchasing a polymerase storage buffer and polymerization reaction buffer that do not contain reducing agents; or (3) purchasing and/or making a polymerase and utilizing a polymerization reaction buffer that contains no reducing agents, resulting in such a low concentration of a reducing agent that it would not meaningfully cleave disulfide bonds when used in a polymerase reaction. Dr. Pentelute admitted during his deposition that a POSA could either create their own polymerase and buffer or order a custom buffer. Ex. 1048, 52:20-22, 59:3-9.

16. For a polymerization reaction, a polymerase contained in a storage buffer is diluted into a polymerization reaction buffer prior to the reaction. If the polymerase storage buffer contained a reducing agent, such as DTT (dithiothreitol), as Dr. Pentelute alleges, it would be in a very low amount (e.g., ≤ 1.0 mM) used to prevent oxidation and preserve the enzymatic activity of the polymerase and would not be a concern in the Kanan/Neri design. The concentration of the DTT is at such

a low concentration that it would not meaningfully cleave the disulfide bonds during polymerization in the Kanan/Neri design. *See* Ex. 1052, 29 (“Although the disulfide bond can be cleaved with reducing agents, such as DTT, the attachment is stable between room temperature and 37°C under the hybridization conditions described here. No significant detachment of disulfide-bound oligonucleotides was observed when biological samples and reaction mixes, such as *PCR and polymerase extension reaction mixes*, were applied (data not shown).”) (emphasis added).

17. This is because a much higher concentration of DTT is needed for meaningful and measurable reduction of disulfide bonds. For example, in Shimkus 1984, the authors used 50 mM of DTT to cleave a simple disulfide bond between biotin and DNA. *See* Ex. 1053, 2596, Fig. 3. Even at a concentration of 50 mM of DTT, only 87% of the disulfide bonds were cleaved. *Id.* I understand at his deposition, Dr. Pentelute discussed his experience with DTT and cleaving disulfide bonds. According to Dr. Pentelute the lowest concentration he was aware of to meaningfully cleave disulfide bonds was 1 mM of DTT:

Q: What is the lowest concentration of DTT that you can remember that’s reliably and measurably broken disulfide bonds?

A: So I did an experiment with a protein. This is a specific set of conditions. This is what – it’s – every reaction matters. I’m not giving a general answer, but I did reduce a disulfide bond in a protein called EETI-II at pH 7.8 with a

reductant, such as DTT, approximately – I don't remember the exact number, but probably 1 millimolar concentration.

Ex. 1048, 80:10-23

Q: Sitting here today, you can't recall an experiment where a lower concentration of less than 1 millimolar of DTT was used to break disulfide bonds? Is that right?

A: I'm sure I've done experiments where I've – yeah, I can't remember.

Ex. 81:20-82:2.

18. Further, the polymerization reaction buffer would not typically include a reducing agent. Assuming a DTT at a concentration of 1mM in a storage buffer, the final concentration of DTT in reaction conditions is 0.1 mM or less because the storage buffer is typically diluted by at least a factor of 10, which results in reaction conditions with a 10X lower concentration than the lowest concentration of DTT that Dr. Pentelute could remember being able to cleave simple disulfide bonds. A POSA, however, had other options to use buffers that contained no reducing agent or buffers that resulted in a reaction conditions that contained even less than 0.1 mM DTT. As a result, a POSA would have understood that premature cleavage of the disulfide bonds in the Kanan/Neri design was not a concern.

19. Kanan in view of Neri teaches polymerization during formation of the CRCs during step (b) that would encode both identification sequence elements onto the pool A oligonucleotide.

2. Dr. Pentelute is wrong that Kanan/Neri's design does not teach step (d) – selecting all CRCs that exhibit the detectable trace

20. Dr. Pentelute argues that the Kanan/Neri design does not teach step (d) because “streptavidin affinity selection (to select only bond-forming combinations) would take place *after* disulphide bond cleavage[,]” which “*destroys* the alleged CRCs formed in step (b) by uncoupling the B oligonucleotides from the B substrates[,]” so “the alleged CRC formed in step (b) cannot be selected in step (d)[.]” Ex. 2008, ¶ 113. I disagree because Dr. Pentelute (1) improperly interprets “selecting” to be a single capture event and (2) improperly requires that Kanan’s streptavidin must capture CRCs. These positions ignore the claim language and exclude embodiments that are within the scope of the claims as understood by their plain and ordinary meaning.

21. Dr. Pentelute improperly limits “selecting” in step (d) as a single capture event in Kanan—affinity capture. Dr. Pentelute does this by picking out one part of Kanan’s selection procedure: streptavidin affinity capture. But this narrowly applies “selecting” inconsistently with the dependent claims and specification. “Selecting” may occur in different ways, such as those claimed in dependent claims

2-3 and 19-20: “selective separation,” “selective detection,” “affinity separation,” or allowing for “modification [that] permits the selective detection.” Ex. 1001, Cls. 2, 3, 19, 20. Dr. Pentelute’s overly narrow view would also exclude embodiments in the specification disclosing “selecting” as a process. *Id.*, 19:4-20:47 (describing “filtering process” for selection). For example, the specification discloses an embodiment of “selecting” including multiple procedures—first, hybridizing to a DNA oligonucleotide tag array and, second, staining with a labelled affinity reagent. *Id.*, 20:31-39. Specifically, in this embodiment, a microarray is created, which is an array of oligonucleotides representing all possible combinations of identification sequences. After the functional interaction (step 1(c)), a filtering (selection; step 1(d)) process would occur. A typical process would be: 1A) if the NAMs of CRCs are double stranded, the strands are denatured by heating to 95°C (for example) followed by rapid cooling to the annealing temperature to be used for hybridization to the array; the reaction mixture is immediately added to the microarray (typically via a flow cell); 1B) if the NAMs of CRCs are single stranded, the reaction mixture is added to the microarray (typically via a flow cell); 2) the CRCs are hybridized with the oligonucleotides on the surface of the array; 3) the array is washed; 4) an affinity reagent is added to the flow cell and allowed to bind to the CRCs that underwent a functional interaction; 5) the array is washed; 6) the antibody is detected

(directly or indirectly) to “identify which NAM combinations represent a CRC with a positive interaction pair.” *Id.*

22. His narrow application of “selecting” conflicts with his recognition that the specification “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. 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, ¶ 35 (internal quotations omitted) (emphases added); Ex. 1001, 19:41-21:43. Dr. Pentelute also testified in his deposition that he understands selection to be “a number of things that are taking place” and a process that allows determination of “cis-reactive cells that give you a read out”:

Q: So the selection can be a process?

A: Well, in this case, it’s a number of things that are taking place.

Ex. 1048, 73:11-14.

Q: But the point of the process is to be able to select for the CRCs with a detectable trace from the background CRCs?

A: Not just background CRCs, but there’s also many interactors that are present too.

Q: Okay.

A: Yeah. So there are a lot of things in solution that are in that test tube. And you want to be able to get those cis-reactive cells that give you a readout. And the readout is the detectable trace.

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

A: Yes.

Ex. 1048, 145:12-146:3. This is consistent with my understanding of the plain and ordinary meaning of “selecting”: it is not limited to a single event or even a single type of event like affinity capture.

23. The specification further discloses that “selecting” can be a process. *See* Ex. 1001, 19:4-20:47. As one example, the specification states that “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 pairs the array with the CRCs can be stained with a labelled affinity reagent specific for the interaction.” *Id.*, 20:31-39.

24. Here, Kanan's Supplementary Information describes both the disulfide cleavage and the streptavidin capture under the “Selections for Bond-Formation” process of Kanan's workflow. Ex. 1013, 7-8. Dr. Pentelute's declaration, however,

ignores these embodiments and Kanan's teachings and identifies "selecting" to be the single event of streptavidin affinity capture in Kanan. I understand Dr. Pentelute testified that Kanan's selection process "start[s] on page 7 [of Ex. 1013] and "the key part of [Kanan's] selection is the reaction discovery experiments and selection procedure[,]" (Ex. 1048, 164:3-16), which is the process described on page 8 under "Selections for Bond-Formation," *see* Ex. 1013, 8. This confirms that Kanan's selection is a process that begins prior to the cleavage of the disulfide bond of the pool B oligonucleotides. Ex. 1013, 7-8.

25. In the Kanan/Neri design, the CRCs are stimulated to create the functional interaction, as discussed in my First Declaration. Ex. 1005, ¶¶ 117-120. The identity of these CRCs are then determined through the selection process described in Kanan. *See id.*, ¶¶ 121-124; Ex. 1010; Ex. 1013, 7-8. Specifically, Kanan's Supplementary Information details this process. *See* Ex. 1013, 7-8. And, as Dr. Pentelute stated, without a selection process "[y]ou wouldn't be able to measure/understand the cis-reactive cells that have that detectable trace":

Q: Yeah. Why do you need a selection step in this process in claim 1?

A: So if you think about what's being done here, you're doing a MOI-NAM, MOI-NAM. So you have at least two different MOIs and NAMs. Okay. But you have many more than that. So you have X number and Y number. And mix everything together. So let's refer to it as combinatorial nature of this

patent. Now if you were just to skip the detectable trace and selecting, you would have very high background. You wouldn't be able to measure/understand the cis-reactive cells that have that detectable trace.

Ex. 1048, 143:1-17.

26. Given that the Kanan/Neri design discloses "selecting," Dr. Pentelute manufacturers an argument by focusing on a single part of Kanan's selection process to attempt to raise an issue where none exists. But Dr. Pentelute's arguments do not hold up.

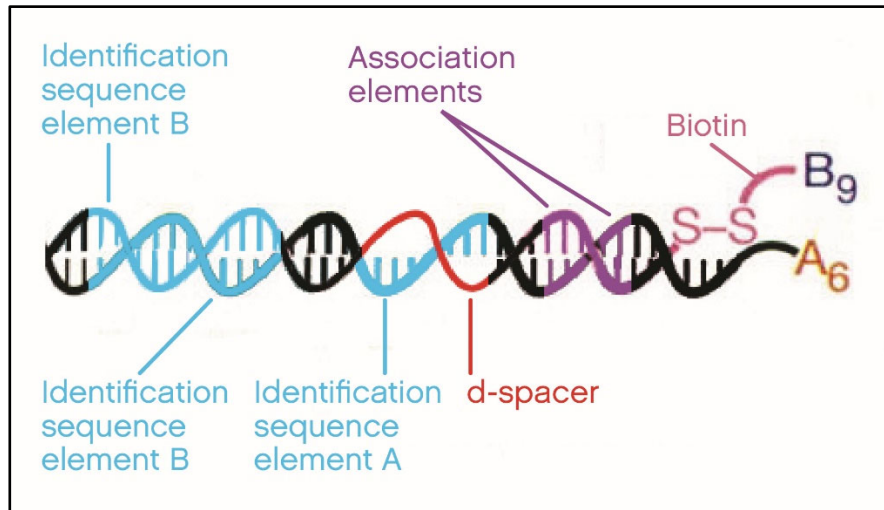
27. After the CRCs are stimulated for the functional interaction, the solution contains CRCs that have participated in a functional interaction and CRCs that have not participated in a functional interaction (i.e., both bond-forming combinations and non-bond-forming combinations). Ex. 1013, 8. Dr. Pentelute also agrees:

Q: But if we look at after the reaction, there are two types of combinations that exist in the pot. There's the non-bond-forming combinations and the bond-forming combinations. Is that right?

A: For the large part, yes.

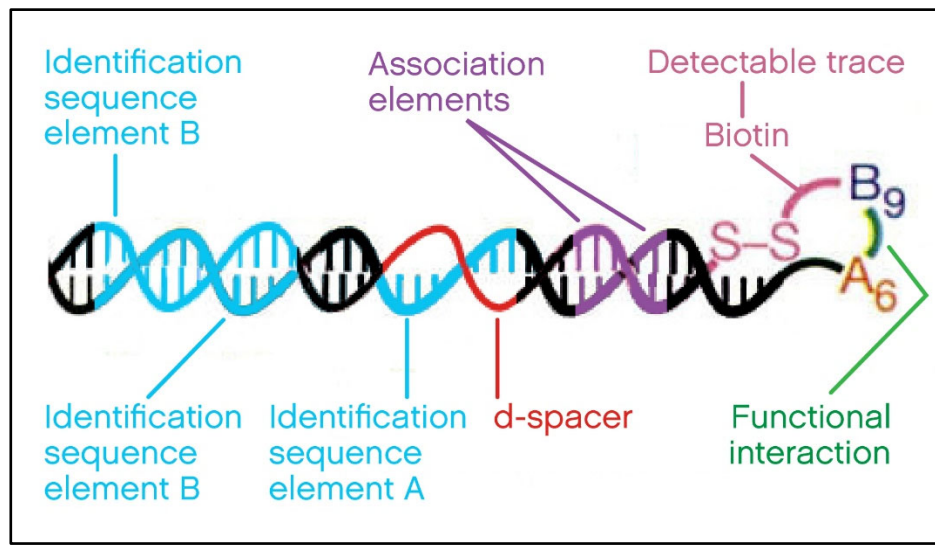
Ex. 1048, 152:17-153:16.

Non-bond-forming combination



Each component of the above figure is described and/or illustrated by my First Declaration. Ex. 1005, ¶¶105-106, 114-115, 119, 180-184; *see also* Ex. 2009, 163:3-22; 165:12-20; 167:1-168:24; 169:8-12 (discussing figures at Ex. 1005, pp. 63 and 67).

Bond-forming combination

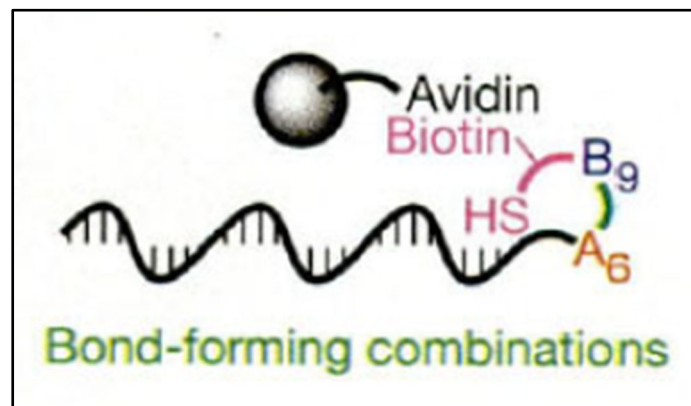


Each component of the above figure is described and/or illustrated by my First Declaration. Ex. 1005, ¶¶105-106, 114-115, 119, 180-184; *see also* Ex. 2009, 163:3-22; 165:12-20; 167:1-168:24; 169:8-12 (discussing figures at Ex. 1005, pp. 63 and 67).

28. After a gel filtration step, a reducing agent (tris(2-carboxyethyl)phosphine hydrochloride in 1.0 M sodium phosphate) is then added to the solution that contains the CRCs to cleave the disulfide bond between each MOI B (bound to biotin) and the attached pool B oligonucleotide. Ex. 1013, 8. This cleavage permits the selection of all CRCs with a detectable trace because those oligonucleotides that were attached to CRCs not involved in a functional interaction (i.e., non-bond-forming combinations) are no longer bound to biotin and can be washed away after addition of streptavidin-linked magnetic particles. *Id.* Without

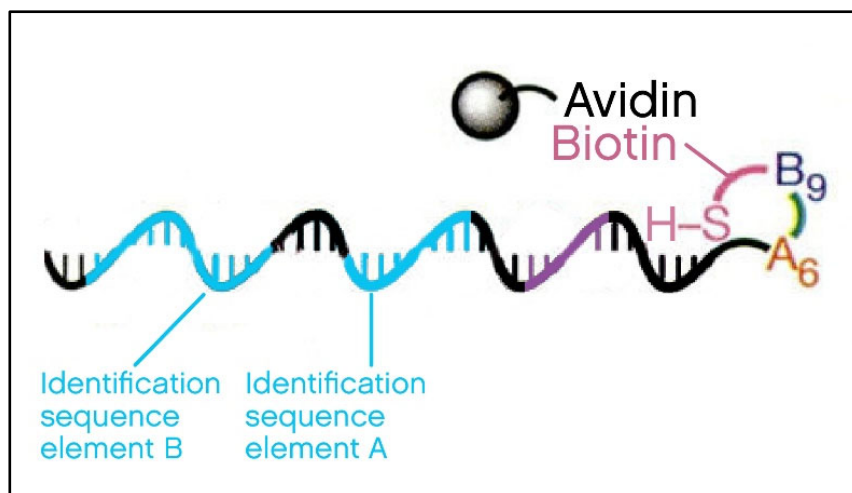
this part of the selecting process, the streptavidin would bind to all CRCs, regardless if there was a functional interaction between their MOIs because “everything with biotin . . . will be captured if enough streptavidin or avidin beads are added.” Ex. 1048, 154:13-155:5. As a result of the cleavage and washes following the addition of streptavidin-linked magnetic particles, the Kanan process selects all CRCs exhibiting a detectable trace such that the “associated oligonucleotides from the cis-reactive cells selected in step (d) [can be subjected] to an analysis that permits detection of the at least two identification sequence elements.” Ex. 1010, 545, Fig. 1.b.; Ex. 1005, ¶ 122.

29. My First Declaration replicated Figure 1.b. of Kanan, Ex. 1005, ¶ 122 (see excerpt depicted below).



Ex. 1005, 545, Fig. 1.b. (excerpt).

30. A depiction of how the bond-forming combination of the Kanan/Neri design after polymerization, *id.*, ¶¶ 181-182, and the selection process, *id.*, ¶ 122; Ex. 1013, 8, as described in my First Declaration, would be visualized as follows:



Each component of the above figure is described and/or illustrated in my First Declaration. Ex. 1005, ¶¶ 105-106, 114-116, 119, 122, 182; *see also* Ex. 2009, 163:3-22; 165:12-20; 167:1-168:24; 169:8-12 (discussing figures at Ex. 1005, pp. 63 and 67).

31. Dr. Pentelute also argues that the CRCs “are further destroyed once the oligonucleotide of pool A and the oligonucleotide of pool B are separated in Kanan’s method.” Ex. 2008, ¶ 118. But Dr. Pentelute is wrong that step (d) requires the CRC to remain intact or cannot be modified through the entire process of “selecting all cis-reactive cells exhibiting the detectable trace” because this excludes embodiments in the specification that are within the scope of step (d) under its plain and ordinary meaning.

32. For instance, Example 5 in the specification provides an example of “encoding interactors with unique tags and detecting the combinations formed therefrom on a combinatorial array may resolve all interactions within a library.”

Ex. 1001, 29:66-30:2. This example details an assay falling within the scope of claim 1. *Id.*, 29:66-32:39. Example 5 discloses using PCR for “selective amplification” for selecting all cis-reactive cells exhibiting the detectable trace. *Id.*, 30:56-67.

33. During the PCR process “[t]he mixture is incubated at 95 C for 2 min and then cycled 30 times between 60 C and 95 C.” Ex. 1001, 30:66-67. During this incubation and cycling process, the solution would be heated and cooled, resulting in denaturing the double-stranded nucleic acid and separating the interactors of the CRC.

34. Thus, the specification discloses an example of “selecting” a CRC that does not maintain a CRC throughout the entire selection process. The oligonucleotides that represent that CRC that participated in the functional interaction are then detected and analyzed using microarray analysis. *Id.*, 31:1-32:28.

35. Step (d) can be a process that selects all CRCs with a detectable trace, which allows for the associated oligonucleotides to be analyzed in step (e). Step (e) can include single-stranded oligonucleotides. Dr. Pentelute agrees that methods of analysis in step (e) can be “microarray, PCR, or sequencing.” Ex. 2008, ¶ 35. Dr. Pentelute also agrees that these methods analyze and/or involve single-stranded DNA:

Q: Let me ask it this way. Can PCR work with single-stranded DNA and appropriate primers?

A: So in the context as I walked through the PCR reaction, you take double-stranded DNA, you heat it up. And if you do that in the presence of primers, you actually – when you cool it down, it will anneal with those primers, now forming that double-stranded DNA at one region. And another region of that PC DNA will be single-stranded.

Q: Could you start that process with a single-stranded DNA, along with the appropriate primers and polymerase and other reaction conditions?

A: Yes.

Ex. 1048, 61:13-62:6.

Q: And does a microarray analyze single-stranded DNA?

A: It can.

Ex. 1048, 62:22-24.

36. Thus, a POSA would have understood that at some point during step (d), the double-stranded DNA could be separated into single-stranded DNA that can be analyzed in step (e).

37. The result of selecting all CRCs that exhibit the detectable trace is being able to subject the “associated oligonucleotides” from the CRCs for analysis to

detect the identification sequence elements of the molecules involved in the functional interaction (step (e)).

38. Kanan in view of Neri teaches that even when the pool B oligonucleotides are removed in Kanan's workflow the remaining pool A oligonucleotides reflect (1) that a functional interaction occurred between pool A and pool B molecules of interest (bond-forming combination) and (2) the identity of the pool A molecule and pool B molecules that were involved in the interaction (through the pool A oligonucleotide that contains both identification sequence elements after polymerization). Ex. 1005, ¶¶ 125-133, 180-184.

39. Dr. Pentelute also argues that Kanan in view of Neri does not teach step (d) because "a POSA would have understood that Kanan's water and formamide wash steps would remove the pool B DNA segment that is no longer coupled to its substrate after the disulphide cleavage step." Ex. 2008, ¶ 116. But Dr. Pentelute's argument seems to ignore those wash steps are part of the process of "selecting," which does not require that CRCs must be maintained through the entire selection process in step (d). *See* Ex. 1013, 8.

40. For the reasons discussed above, a POSA would have understood Kanan in view of Neri as teaching "selecting all cis-reactive cells exhibiting the detectable trace."

3. Kanan/Neri's design permits detection of the two identification sequence elements as required in step (e)

41. Dr. Pentelute argues that Kanan in view of Neri does not disclose “subjecting the associated oligonucleotides from the cis-reactive cells selected in step (d) to an analysis” because no CRCs were selected in step (d). Ex. 2008, ¶ 120. For the reasons discussed above, and the additional reasons provided below, I disagree.

42. As stated in my First Declaration, a POSA would have understood that the bond-forming pairs from Kanan in view of Neri would have “associated oligonucleotides.” Ex. 1005, ¶¶ 125-133. A POSA would have understood the pool A oligonucleotides from the Kanan/Neri design to be “associated oligonucleotides” because these oligonucleotides “comprise[the] at least two identification elements derived from the at least two nucleic acid moieties.” Ex. 1001, Cl. 1. The pool A oligonucleotides being analyzed in step (e) are the product of a pool A oligonucleotide hybridizing with a pool B oligonucleotide, and the pool B identification sequence element being transferred onto the pool A oligonucleotide using polymerization after the formation of the CRC, creating a novel oligonucleotide comprised of the identification elements of the component interactors. *See* Ex. 1001, 16:66-17:3; Ex. 1005, ¶¶ 180-184.

43. Further, Dr. Pentelute cannot argue that “associated oligonucleotide” must be double-stranded nucleic acid because “associated oligonucleotide” in step

(e) includes both single- and double-stranded nucleic acid. Indeed, he testified that the scope of the claims includes “a lot of possibilities” and does not exclude single-stranded associated oligonucleotides.:

Q: So the question is: Can you have a single-stranded associated oligonucleotide that comprises at least two identification sequence elements derived from the at least two nucleic acid moieties?

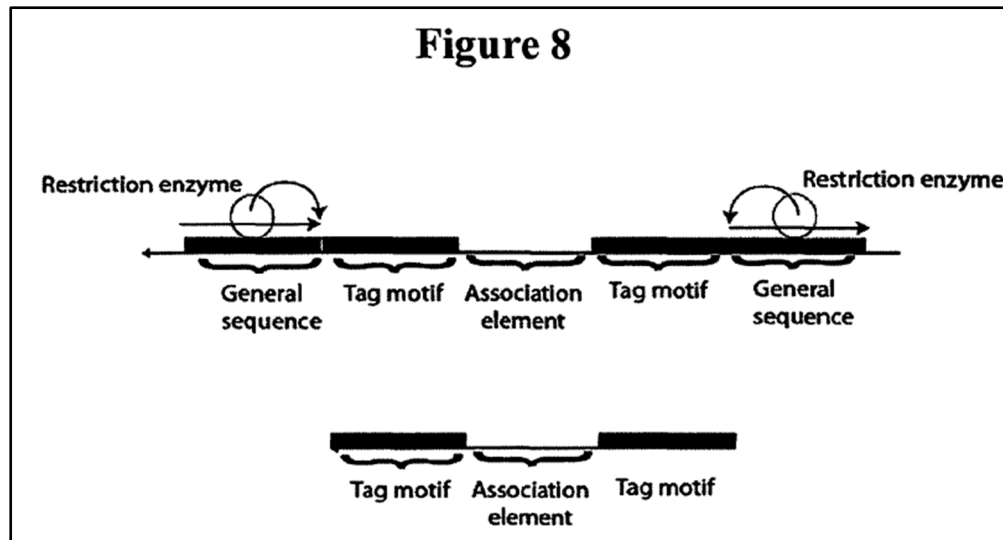
A: I mean, as the patent walks through, there’s a lot of possibilities.

Ex. 1048, 117:10-18.

44. In addition, as an example, claim 13 states 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 microarray analysis.” Ex. 1001, Cl. 13. As a dependent claim¹, I understand that this means that microarray analysis is within the scope of the methods of detecting associated oligonucleotides in step (e). A POSA would have understood that microarray analysis analyzes single-stranded DNA. *See* Ex. 1001, 28:62-29:54, Fig. 8; Ex. 1048, 62:22-24.

45. In addition, Figure 8 shows an “Illustration of a single stranded associated oligonucleotide,” which shows two identification sequence elements (tag motifs, below) derived from the at least two nucleic acid moieties:

¹ Claim 13 depends from claim 12 which further depends from claim 1.



Thus, the specification discloses a single-stranded associated oligonucleotide. Ex. 1001, Fig. 8, 8:17-25.

46. Thus, Kanan in view of Neri discloses “subjecting the associated oligonucleotides from the cis-reactive cells selected in step (d) to an analysis.”

47. Dr. Pentelute also argues that Kanan in view of Neri does not disclose “detection of the at least two identification sequence elements” because the identification sequence element of Kanan’s pool B oligonucleotide is cleaved and removed before the detection step of step (e). Ex. 2008, ¶¶ 124-126. While Dr. Pentelute references polymerization (*id.*, ¶¶ 127-133), he is incorrect that polymerization would not permit detection of the two identification sequence elements.

48. As discussed above (paragraphs 10, 19, 31), using the combination of Kanan and Neri’s method of polymerization, when pool A oligonucleotides are

analyzed in step (e), they contain the identification sequence element of MOI A as well as the identification sequence element of MOI B that was added to the pool A oligonucleotide during polymerization, which Dr. Pentelute agrees:

Q: And then what takes place with the polymerization?

A: End up elongating the remaining single-stranded pieces of the NAMs.

Ex. 1048, 106:10-13.

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.

Ex. 1048, 108:14-20.

49. As a result, when Kanan's workflow removes the pool B oligonucleotides, this removal does not "remove" the second identification sequence element because the pool A oligonucleotides contain both identification sequence elements that can be detected, including with microarray analysis and sequencing.

Ex. 1005, ¶¶ 127-130, 182.

50. Thus, Kanan in view in Neri discloses claim 1 step (e) because in the Kanan/Neri design both identification sequence elements can be detected. Ex. 1005, ¶¶ 182-184.

4. A POSA would have been motivated to combine Kanan and Neri

51. Dr. Pentelute provides several arguments for why a POSA would not have been motivated to combine Kanan and Neri, but I disagree with all of his reasons.

52. First, Dr. Pentelute argues “a POSA would not have been motivated to modify Kanan with the disclosure of Neri” because “a POSA would know that the proposed method would not yield information allowing the determination of which two substrates had a functional interaction.” Ex. 2008, ¶ 134. This argument is based on an incorrect assumption that the Kanan/Neri design prevents the detection of both identification sequence elements. But as I discussed above (paragraphs 9-10, 31, 37), when the pool A oligonucleotides are analyzed and detected in step (e) based on Kanan and Neri’s methods, the identification sequence elements for MOI A and the identification sequence elements for MOI B are both detected. Thus, a POSA would have understood that the use of a self-assembly sequence and polymerization as disclosed in Kanan in view of Neri would reduce time required to synthesize the library, improve the specificity of the assay, and provide a way to

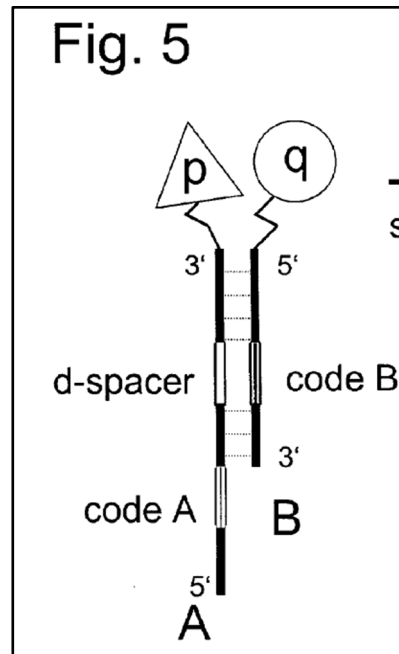
more efficiently identify the bond-forming combinations over the methods of Kanan alone. *See* Ex. 1005, ¶¶ 82, 97-103, 182.

53. Second, Dr. Pentelute argues “a POSA would not have been motivated to modify Kanan with the disclosure of Neri” because “a POSA would have known of the false positive and false negative results that would arise from Dr. Nelson’s proposed combination of Kanan and Neri.” Ex. 2008, ¶ 154. This argument is based on an incorrect assumption that the Kanan/Neri design contains a fundamental technical issue. Also, Dr. Pentelute ignores that it is within the skill of a POSA to design assay conditions to reduce concerns of unintended hybridization.

54. In addition to arguing that a POSA would have performed polymerization after selection (which I disagree), Dr. Pentelute also argues that the prior art’s Kanan/Neri combination “makes polymerase extension impractical.” Ex. 2008, ¶ 136. Despite allegedly reviewing my entire declaration and deposition testimony (*see* Ex. 2009, 161:23-164:6) in preparing his declaration (Ex. 2008, ¶ 20 (listing Ex. 2009 as materials considered), Dr. Pentelute’s arguments do not consider my analysis and are instead based on a disingenuous misunderstanding of a single illustrative figure in my First Declaration to contend that there would not be hybridization after the d-spacer. Ex. 2008, ¶¶ 139-141.

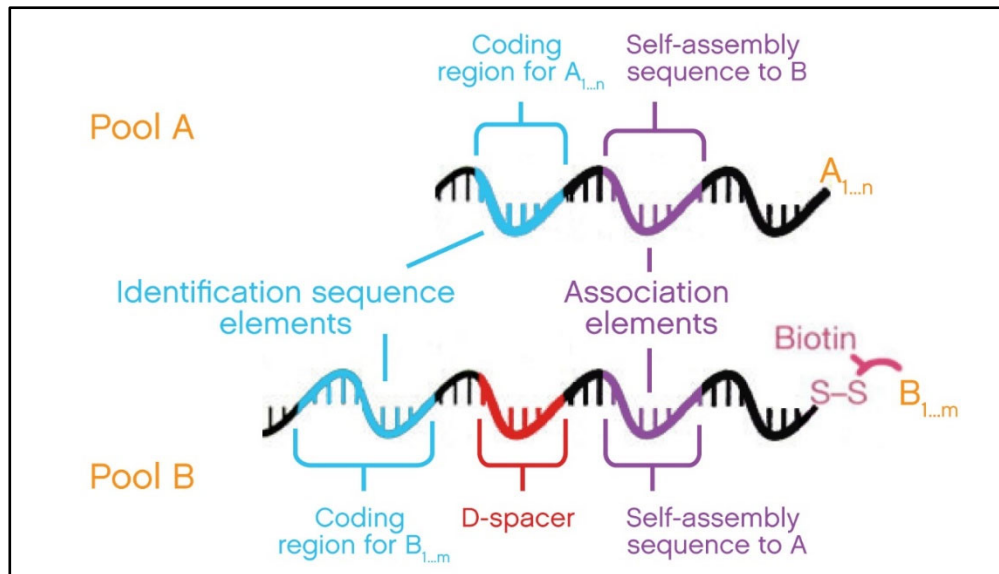
55. My First Declaration established that Kanan/Neri design allows for polymerization. As I explained in my First Declaration, the Kanan/Neri design relies

on Neri's Figure 5 to create the Kanan/Neri interactors. Ex. 1005, ¶ 96. Neri's Figure 5 depicts hybridization between the two oligonucleotides after the d-spacer region.



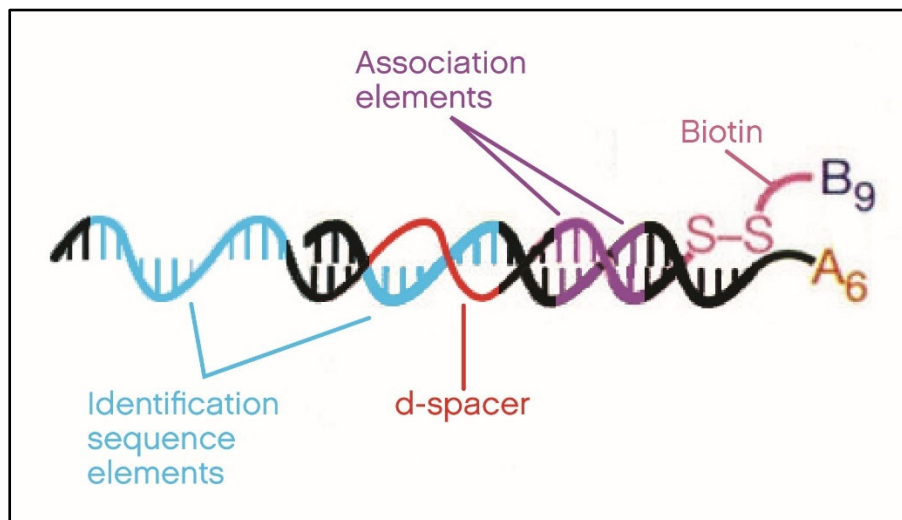
Dr. Pentelute agrees that Neri's Figure 5 shows hybridization "except for region in the d-spacer." Ex. 1048, 169:3-171:5.

56. I then created Kanan/Neri "interactors" as shown in paragraph 114 of my First Declaration based on the combination of Kanan and Neri.



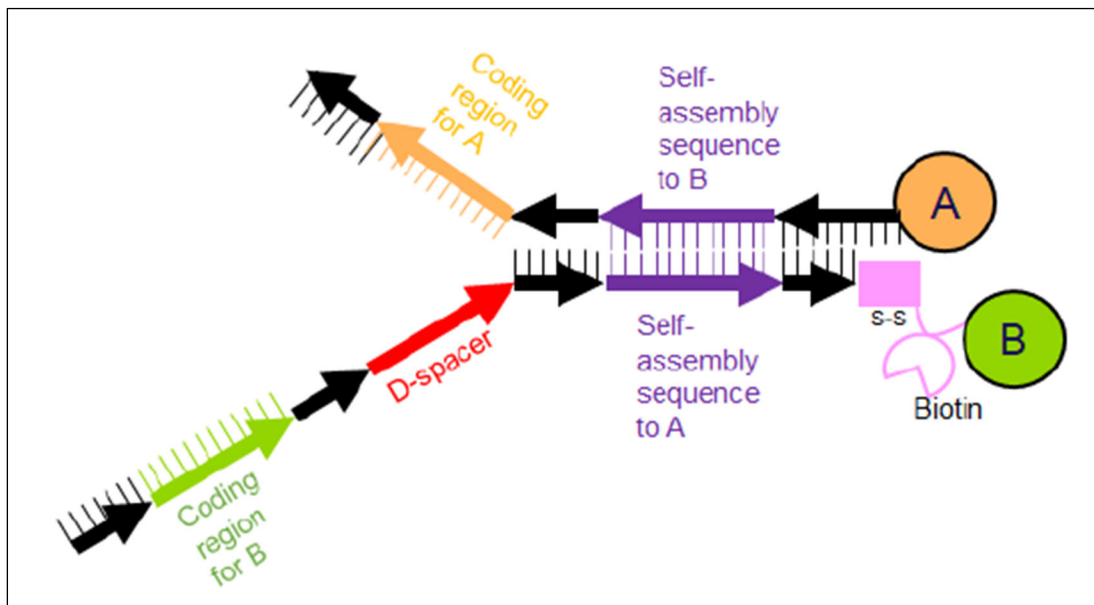
Ex. 1005, ¶ 114. The interactors depict vertical lines that represent the ability to hybridize after the d-spacer, consistent with Neri's Figure 5.

57. When the Pool A and Pool B members above are hybridized or self-assembled, they can be visualized with the following figure based on my description in my First Declaration:



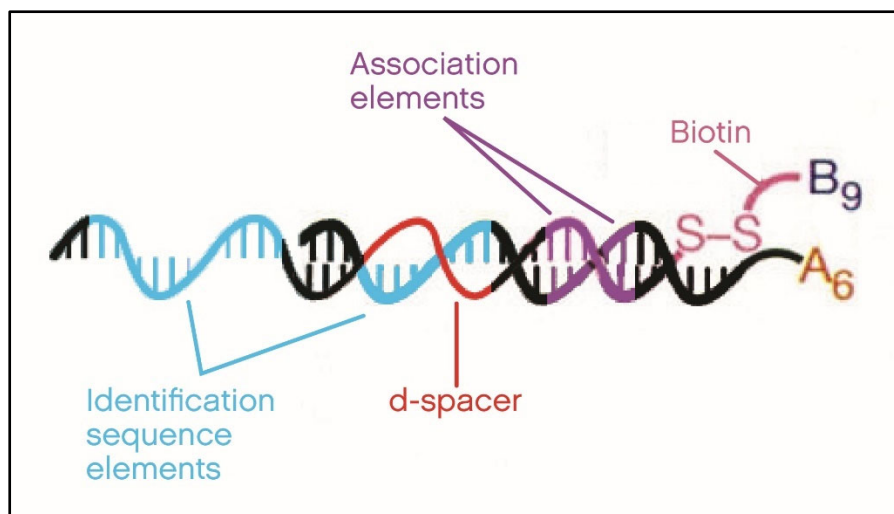
Each component of the above figure is described and/or illustrated in my First Declaration. Ex. 1005, ¶¶105-106, 114-115; *see also* Ex. 2009, 163:3-22; 165:12-20; 167:1-168:24; 169:8-12(discussing figures at Ex. 1005, pp. 63 and 67).

58. Because of hybridization after the d-spacer, I disagree that the Kanan/Neri design would take the “Y-shaped structure” Dr. Pentelute depicts in paragraph 142.



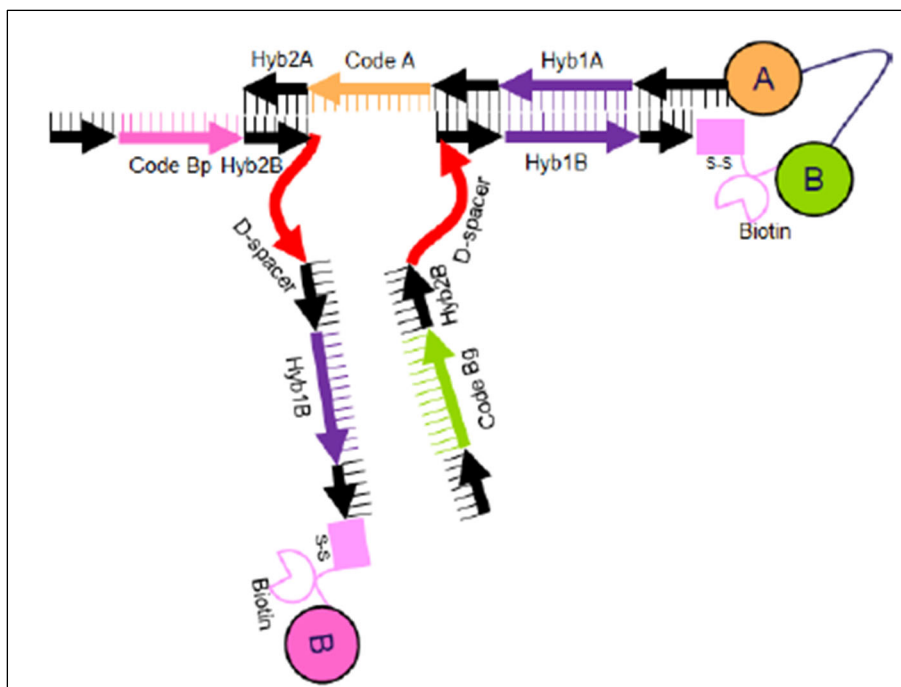
Ex. 2008, ¶ 142.

59. The hybridized oligonucleotides after the d-spacer, pool A oligonucleotides “can work as primers” for polymerization (Ex. 1011, [0117]; Ex. 1005, ¶ 181-182), as shown in the figure below.



Each component of the above figure is described and/or illustrated by my First Declaration. Ex. 1005, ¶¶105-106, 114-115; *see also* Ex. 2009, 163:3-22; 165:12-20; 167:1-168:24; 169:8-12 (discussing figures at Ex. 1005, pp. 63 and 67).

60. Dr. Pentelute recognizes this—because he then offers an alternative argument that even if there is hybridization after the d-spacer, claiming that the Kanan/Neri design “is still technically flawed” because it “would be expected to yield false negative and false positive results[.]” Ex. 2008, ¶¶ 146-148. He bases his opinion on the design having a universal hybridization region after the d-spacer that would allow “*two* regions where a pool A member can bind two *any* pool B member.” Ex. 2008, ¶ 149. This design is shown in paragraph 150 of Dr. Pentelute’s declaration and replicated below.



61. Dr. Pentelute's concerns regarding an increase in false negatives and false positives are unfounded, and he completely ignores the details of the design and skills of a POSA. Reducing unintended hybridization is a common issue faced with all nucleic acid chemistry and well within the skill on a POSA to control and minimize.

62. Additionally, Dr. Pentelute's bare concerns regarding increased false positives and false negatives are overblown. Dr. Pentelute's arguments assume that a POSA, who is skilled in nucleic acid chemistry, would not be able to control assay conditions or the design of the oligonucleotides, and could not minimize undesired hybridization. That is wrong. A POSA would have the skills and desire to produce reliable results. Dr. Pentelute also cites no scientific references in support of his position. Moreover, the cited prior art describes working examples and does not

raise undesired hybridization as a concern. For example, the basic structure of the Kanan/Neri design is based on Neri's Figure 5 (*see, e.g.*, Ex. 1005, ¶ 96), yet Neri does not discuss unintended hybridization after the d-spacer being a concern. *See generally* Ex. 1011. Specifically, Neri's Example 3 details creating a library of pool A and pool B interactors to interact for analysis and does not describe any issues with unintended hybridization in the region after the d-spacer. Ex. 1011, [0111]-[0119].

63. For the reasons discussed above, a POSA would disagree that the Kanan/Neri design contained fundamental technical issues incompatible with polymerization and that it would increase mis-hybridization between the interactors.

64. Thus, contrary to Dr. Pentelute, a POSA would have been motivated to combine Kanan with Neri as I previously explained. Ex. 1005, ¶¶ 82, 99-103, 182.

5. A POSA would have had a reasonable expectation of success in combining Kanan and Neri

65. Dr. Pentelute makes three arguments for why a POSA would not have had a reasonable expectation of success in combining Kanan and Neri, but each of these three arguments ignore modifications a POSA would make to Kanan to incorporate Neri's disclosure.

66. Dr. Pentelute first argues that a POSA would not have had a reasonable expectation of success in combining Kanan and Neri because Kanan/Neri's methods "would not allow detection of both identification sequence elements." Ex. 2008,

¶ 156. This is incorrect, as shown above. Based on Kanan/Neri's methods, the pool A oligonucleotides that are analyzed and detected in step (e) contains both identification sequence elements, allowing identification of the molecules involved in the functional interaction.

67. Dr. Pentelute next argues that a POSA would not have had a reasonable expectation of success in combining Kanan and Neri because "the Kanan and Neri combination [is] structurally incapable of any polymerization due to the lack of a priming region hybridized to initiate polymerase extension." Ex. 2008, ¶ 157. Again, this argument is incorrect, as shown above, because hybridization after the d-spacer permits polymerization.

68. Dr. Pentelute finally argues that a POSA would not have had a reasonable expectation of success in "detecting functional interactions between two MOIs because two separate regions of the Pool A strand could hybridize to two distinct Pool B sequences and result in unreliable false positive and false negative results." Ex. 2008, ¶ 158. I disagree because this argument ignores that the Kanan/Neri design reduces unintended hybridization, as I discussed above. Dr. Pentelute also ignores that a POSA would have a reasonable expectation of success in preventing unintended hybridization because a POSA would have the skills to design oligonucleotides and the assay conditions to promote hybridization between the intended hybridizing regions.

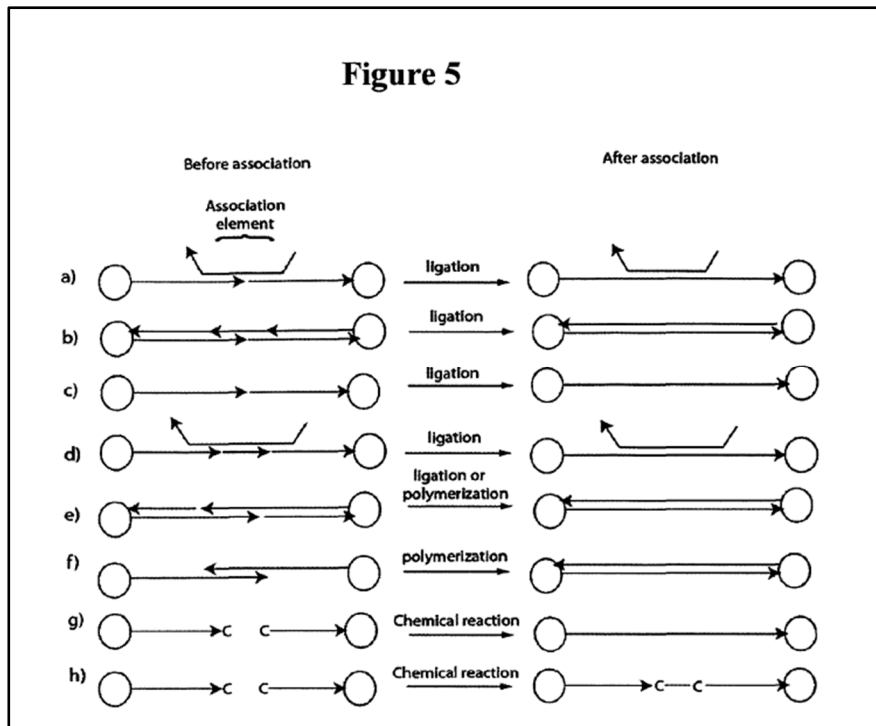
69. Dr. Pentelute's arguments do not change the fact that a POSA would have a reasonable expectation of success in combining Kanan and Neri for the reasons provided in my First Declaration. Ex. 1005, ¶¶ 104-105, 183.

6. Dr. Pentelute is wrong regarding the teachings of Famulok

70. Dr. Pentelute first argues that I failed to articulate how to combine Kanan, Neri, and Famulok in my First Declaration. This is incorrect. I discussed that a POSA could use a Kanan/Neri design that contains an overhang region that can then be ligated as taught by Famulok. *See* Ex. 1005, ¶¶ 175-179.

71. Dr. Pentelute next argues that Kanan in view of Neri and in further view of Famulok teaches "CRCs made from interactors *associated by hybridization* of the self-assembly region in the oligonucleotides, *not by ligation*, as required by claim 16." Ex. 2008, ¶ 163. Dr. Pentelute's argument requires that there is no hybridization between the interactors prior to the ligation. But this interpretation would exclude embodiments in the '848 Patent.

72. For example, Figure 5 provides "[n]on-limiting examples of association of NAMs." Ex. 1001, 7:42.



Id., Fig. 5. As shown in Figure 5, in the ligation examples a, b, d, and e, there is some prior association (i.e., hybridization) between the interactors before the ligation. This is because the interactors must be in close spatial proximity to each other for the ligation to occur.

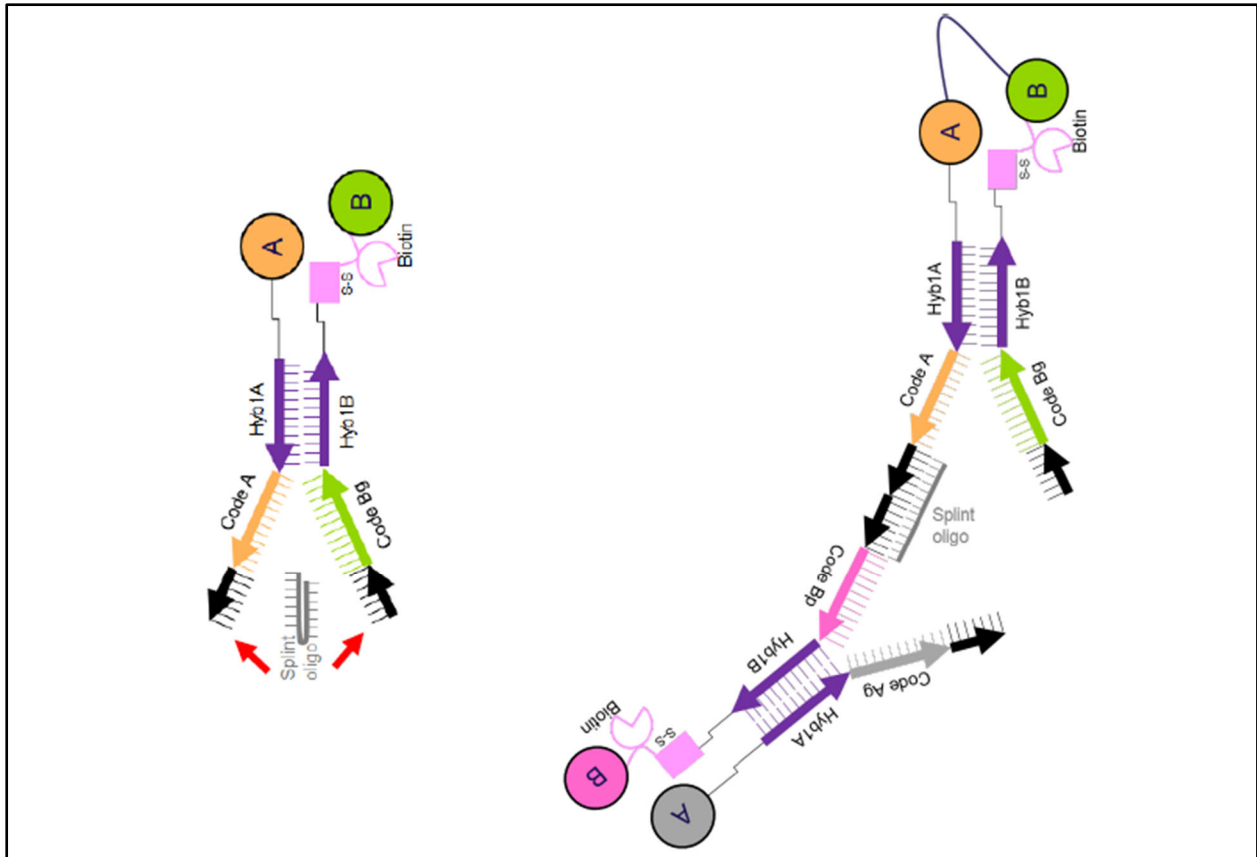
73. Dr. Pentelute also argues Ground 2 is flawed because of its reliance on Kanan's process, which does not allegedly disclose claim 1 step (d) or step (e). Ex. 2008, ¶ 164. But as discussed above, Kanan in view of Neri discloses claim 1 step (d) and step (e), rendering claim 1 obvious.

74. Next, Dr. Pentelute argues that a POSA would not have motivation or a reasonable expectation of success in combining Kanan, Neri, and Famulok "due to the inability to detect more than one identification sequence element and determine

MOIs that functionally interact” and “the expectation of false negatives and false positives.” Ex. 2008, ¶ 165. A POSA would have understood neither argument to be true for the reasons provided below.

75. As explained in my First Declaration (Ex. 1005, ¶¶ 176-178), a POSA would have understood that ligation of the overhang region in Neri’s Figure 7 would create a nucleic acid sequence that contains both identification sequence elements that can later be detected and analyzed. Dr. Pentelute disagrees but presents no scientific references supporting the argument that Kanan, Neri, and Famulok would prevent detection of more than one identification sequence element.

76. Next, using a universal splint, Dr. Pentelute argues that “false positives and false negatives [would arise] from binding of multiple strands[,]” as shown in the below diagram. Ex. 2008, ¶ 167.



77. But, a POSA would not share Dr. Pentelute's concerns regarding unintended hybridization (shown in right figure). A POSA, who is skilled in nucleic acid chemistry, understands controlling assay conditions and the design of oligonucleotides and splint to minimize trans reactivity. A POSA would have understood that the design would allow the splint to stably bind only when the two tails are held in proximity to each other, as exemplified in Neri's Figure 7 (Ex. 1011, Fig. 7). 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, as claimed by Dr. Pentelute, is minimal as the likelihood that two separate interactors and a splint are all in close enough proximity to hybridize is low.

This is also true for Dr. Pentelute's claims that different CRCs would also be ligated together (*see* Ex. 2008, ¶ 169).

78. As another example, Famulok does not raise any issue or concern regarding an increase of the potential of false negatives or false positives with its ligation approach. *See* Ex. 1012.

79. Because a POSA would not be concerned of an uncontrolled risk of increased false positives or false negatives, a POSA would still be motivated and would have a reasonable expectation of success in combining Kanan, Neri, and Famulok. Ex. 1005, ¶¶ 177-178.

80. Lastly, Dr. Pentelute states that using the Famulok's ligation approach with Neri's Figure 5 would not be useful. Ex. 2008, ¶ 171. Dr. Pentelute misunderstands my cross-examination testimony discussing Neri's Figure 5 in relation to Famulok and claim 16. *Id.*; Ex. 2009, 137:3-8, 138:11-20. My comments regarding Neri's Figure 5 embodiment were not a new argument that the design in Figure 5 must be used to disclose claim 16. Instead, my comments were merely reflecting that a POSA is not limited to Neri's Figure 7 design. The skillset of a POSA includes the ability to modify Neri's Figure 7 as desired and still achieve a structure that is compatible with ligation.

7. Remaining dependent claims

81. Dr. Pentelute does not specifically address claims 2-15 and 18-20 besides a passing reference that his arguments “for claim 1 discussed in Ground[] 1 . . . also apply to dependent claims 2-20.” Ex. 2008, ¶ 208. Thus, because Dr. Pentelute fails to establish that the claims are not obvious over Kanan in view of Neri, the remaining dependent claims are also rendered obvious by Kanan in view of Neri as provided in my First Declaration.

B. 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)

82. Like my opinions in my First Declaration, 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. Ex. 1005, ¶ 230. As such, I apply Patent Owner’s positions found in its complaint, infringement claim chart, and opposition to Alamar’s motion to dismiss to the claims of the ’848 Patent in view of the proximity ligation assay prior art.

1. Baez in view of Landegren discloses claim 1

(a) Baez discloses both MOIs and Interactors as distinct elements

83. Dr. Pentelute states that “according to Dr. Nelson, the same antibody-nucleic acid conjugate in Baez meets *both* the MOI *and* interactor claim limitations

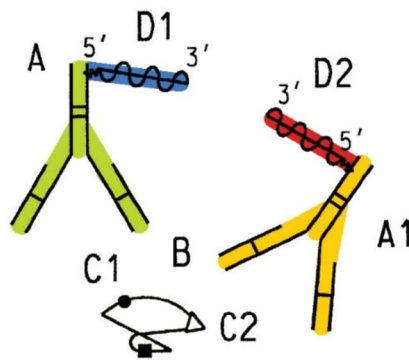
recited in claim 1.” Ex. 2008, ¶ 175. I disagree with Dr. Pentelute, and he does not consider my opinions in full that I provided in my First Declaration.

84. Dr. Pentelute’s opinions are based on an incorrect assumption that in Baez “antibody conjugates” are the same as “reporter conjugates.” Ex. 2008, ¶ 175 (“Baez discloses that the ‘antibody conjugates’ (referred to as ‘reporter conjugates’) are comprised of an antibody probe conjugated to a nucleic acid label.”). This is not what Baez discloses nor what I identified in my First Declaration.

85. In paragraph 232 of my First Declaration, I stated: “a POSA would have understood the antibody conjugates disclosed in Baez as a molecule of interest.” Ex. 1005, ¶ 232. In my mapping of Patent Owner’s claim chart in my First Declaration, I mapped the molecules of interest Patent Owner identified in its claim chart (Ex. 1008) to the disclosure of Baez (Ex. 1003). Ex. 1005, ¶¶ 232, 235. I based my understanding of molecule of interest on both Patent Owner’s claim chart and Patent Owner’s complaint. Ex. 1005, ¶ 230. In Patent Owner’s complaint, it referred to a “capture antibody” and a “detection antibody” and identified these as an “antibody conjugate,” which is the term I used in paragraph 232 to identify the antibodies disclosed in Baez. Ex. 1005, ¶ 204 (discussing Complaint, Ex. 1007, ¶¶ 22-23). As further support, in my mapping, I color coded the antibodies in Baez to match the antibodies (i.e, molecules of interest) from Patent Owner’s claim chart. *See* Ex.

1005, ¶ 235 (“matched antibody pairs . . . shown in green and yellow . . . are molecules of interest”).

86. As shown in the figure below, the molecules of interest that I identified are the matched pairs of antibodies (colored in green and yellow below), and do not include any coupled nucleic acid.



Ex. 1005, ¶ 235.

87. Further, Dr. Pentelute recognized that in my First Declaration, I colored green the antibody in the left figure and yellow the antibody in the right figure:

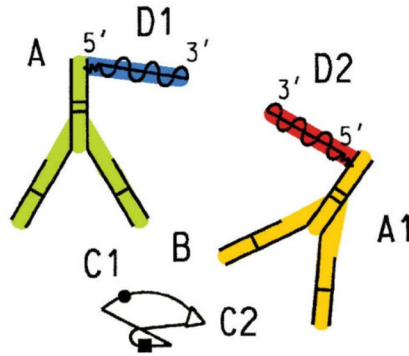
Q: And one antibody is green and the other antibody was yellow; correct?

A: Yes.

Ex. 1048, 195:2-4.

88. I also identified the molecules of interest in Baez as separate from the interactor, which I identified as a “reporter conjugate”—as stated in paragraph 235 of my First Declaration “a POSA would have understood the ‘reporter conjugates’ disclosed in Baez are ‘interactors.’” Ex. 1005, ¶ 235; Ex. 1003, 7:53-56. These are

the antibody conjugates (antibodies colored in green/yellow below) coupled to nucleic acid (colored in blue/red below).



Ex. 1005, ¶ 235.

89. Further, I understand Dr. Pentelute testified he agreed that in my First Declaration that the additional nucleic acid labels attached to the antibodies were identified in blue and red:

Q: And then there are these reporter tags that are shown under where it says D1, and that's colored blue. Do you see that?

A: I do.

Q: And then on the other antibody, there's a reporter tag labeled D2 that's colored red. Do you see that?

A: Yeah, I do. – did you say left? You were talking about the right panel; correct?

Q: Yes, to be clear, for the whole – for the record, we're talking about – there are two items shown in the right-hand panel.

Ex. 1048, 195:5-19.

90. My analysis of the dependent claims further confirms that Baez's antibodies are the molecules of interest, the DNA labels are the nucleic acid moieties, which together make up the interactors. Ex. 1005, ¶¶ 270-279.

91. For dependent claims 5 and 6, which require that the “coupling” in the “forming a plurality of interactors by coupling each molecule of interest with at least one nucleic acid moiety” is “covalent” (claims 5 and 6) or “noncovalent” (claim 5), I explained that:

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.*

Ex. 1005, ¶ 271.

92. Dependent claims 7 and 8 require “at least one molecule of interest comprises a [one] protein.” In discussing claim 7, I explained “applying Patent Owner's positions, a POSA would have understood that the *antibodies taught in*

Baez are molecules of interest being a protein.” Ex. 1005, ¶ 276. Further, I found claim 8 satisfied because “Patent Owner contends that the antibodies are protein *molecules of interest*, and *Baez discloses antibodies as molecules of interest.*” Ex. 1005, ¶ 279. Thus, as set forth in my First Declaration, the antibodies disclosed in Baez are the molecules of interest and the antibodies coupled to nucleic acid labels are interactors. Ex. 1005, ¶¶ 232, 235, 270-279. This claim mapping is consistent with Patent Owner’s positions regarding the scope of the ’848 Patent as I described in my First Declaration. *Id.*

(b) Baez’s affinity purification process meets claim 1 step (d)

93. Dr. Pentelute fails to establish that a POSA would not understand Baez’s affinity purification process to be “selecting” as used in claim 1 step (d). Of course, the claims also allow for “selecting” to include “selective detection.” Ex. 1001, Cl. 2.

94. Dr. Pentelute opines that “a POSA would have understood that Baez’s disclosure refers to a method of *detection*, not selection, as required in ’848 claim 1 element (d).” Ex. 2008, ¶ 181. I disagree.

95. To support his argument, Dr. Pentelute incorrectly limits his analysis to the literal words and headings in Baez and ignores its teachings. For example, he notes that portions of the disclosure I rely upon in Baez is under the header “Detection of Nucleic Acid Products” and that Baez states “[t]he ASA can also be

detected through the linker oligonucleotide itself.” Ex. 2008, ¶ 182 (emphasis removed). From this, Dr. Pentelute then argues that “a POSA would have understood that being able to isolate amplified nucleic acid products from solution provides a means *to detect* an ASA resulting from ligation[.]” Ex. 2008, ¶ 182. Dr. Pentelute, however, is mistaken because I do not rely on amplification in my analysis for isolating the ASA. See Ex. 1005, ¶¶ 252, 257. In addition, Dr. Pentelute focuses on an isolated disclosure of Baez, and not its full teachings. See Ex. 2008, ¶ 181.

96. Dr. Pentelute’s argument is based on the false premise that Baez only discloses a solid-phase assay. However, Baez also discloses that: “Both the ligation and overlap methods may be conducted in either a heterogenous format where the analyte is immobilized, or if in a homogeneous format ***where the analyte is free in solution.***” Ex. 1003, 11:52-55 (emphasis added). When the ASA is free in solution, biotin-streptavidin capture is a method of isolating the ASAs that participated in the functional interaction (i.e., ligation) in the solution. Ex. 1005, ¶¶ 250-253.

97. For the reasons above and those stated in my First Declaration (Ex. 1005, ¶ 252), Baez’s disclosure of using biotin incorporated to the linker as a means to isolate the ASAs from solution is a form of affinity purification that satisfies claim 1 step (d). See Ex. 2008, ¶ 35. In addition, Dr. Pentelute agrees that biotin-streptavidin capture is a form of affinity purification:

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 step or purification step.

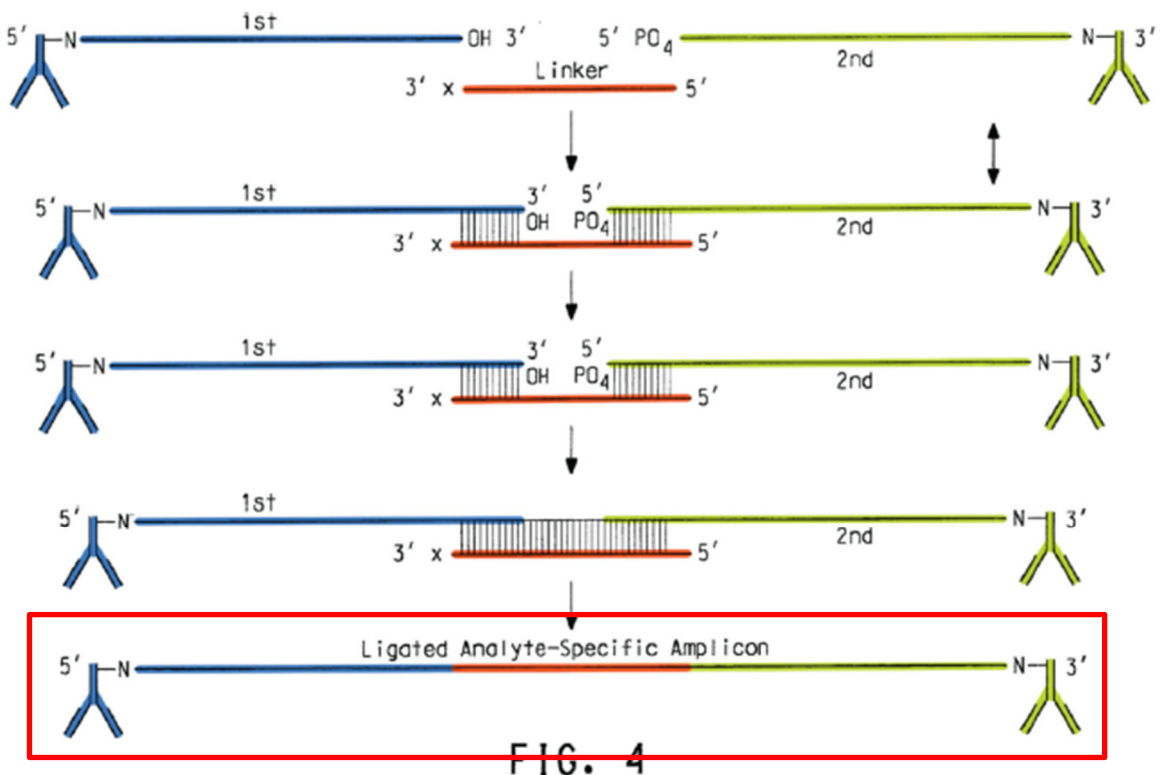
Ex. 1048, 140:1-12.

98. Dr. Pentelute also argues that “Baez’s method of detecting the biotinylated amplicons, would not contain both a MOI and a NAM for each of the two interactors associated by association elements, and thus does not teach selecting all cis-reactive cells exhibiting a detectable trace as required by ’848 patent claim 1 step (d).” Ex. 2008, ¶ 183 (emphasis removed). I disagree with Dr. Pentelute for several reasons.

99. First, I did not opine that Baez teaches only incorporating biotin during the amplification process, and thus “selecting” would be of only the amplified nucleic acid (i.e., Dr. Pentelute’s “biotinylated amplicon”). Ex. 1005, ¶¶ 250-253.

100. Second, I did not opine that selecting “amplified nucleic acid products” would involve selecting CRCs. As provided in my First Declaration, Baez’s ASA’s—not amplified nucleic acid products—are selected through the biotin incorporated in the linker that is attached to the ASA. Ex. 1005, ¶ 252. To be clear,

the ASA in Baez includes the complex containing two antibodies connected via the ligation of their respective nucleic acid labels, as shown in Figure 4 of Baez. Ex. 1005, ¶ 217.



Ex. 1005, ¶ 217 (annotated).

101. In addition, Baez's ASA's contain both a MOI (antibody) and a NAM (DNA label) for each interactor. *See id.*, ¶¶ 241-245. Baez's ASA contains two interactors, which are formed from the joining of two reporter conjugates. *Id.*, ¶ 243. These reporter conjugates (interactors) contain antibodies (MOIs) coupled to nucleic acid. Ex. 1003, 7:53-56. Thus, Baez discloses selecting all cis-reactive cells exhibiting a detectable trace.

102. Based on Dr. Pentelute's incorrect arguments regarding step (d), Dr. Pentelute also argues that Baez and Landegren fail to teach step (e) "because there would have been no CRCs selected in Baez's method." Ex. 2008, ¶ 187. For the reasons discussed above, I disagree with Dr. Pentelute's opinion because Baez's methods would select CRCs that can be analyzed in step (e).

(c) Baez and Landegren disclose "detection of the at least two identification sequence elements" in claim 1 step (e)

103. Claim 1 step (e) requires "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, Cl. 1.

104. Dr. Pentelute argues that Baez in view of Landegren fails to teach this step because "a POSA would have understood that Baez's disclosure of detecting the 'amplified ASA product' would at most detect the presence of a *ligation product*, and would not have determined whether or not Landegren's tags . . . are indeed present in the detected ASA." Ex. 2008, ¶ 190. But Baez in view of Landegren discloses this step. Ex. 1005, ¶¶ 254-259.

105. As stated in my First Declaration, it would have been obvious to a POSA to add Landegren's identification sequence elements (tags) to the nucleic acid labels of Baez. *Id.*, ¶ 238. The nucleic acid sequence that is then ligated together in step (c) includes the identification sequence elements, thus the identification

sequence elements are also part of the ASA that is selected in step (d). *Id.*, ¶¶ 246-253. As a result, when the associated oligonucleotides (i.e., nucleic acid) are analyzed in step (e), the two identification sequence elements can be detected. *Id.*, ¶¶ 254-259.

106. While not clear to me, it appears Dr. Pentelute also argues that Baez's disclosure of PCR does not allow detection of the two identification sequence elements. Ex. 2008, ¶ 189-190. If so, Dr. Pentelute failed to consider the full scope of my previous opinions relating to step (e) wherein I also rely on Baez's disclosure that "Baez also teaches that the '[biotin] labeled ASA would then be detected by *any number of detection strategies*.'" Ex. 1005, ¶ 257 (quoting Ex. 1003, 18:7-12) (emphasis added). Various detection strategies, such as sequencing, were well-known in the art at the time of the application. *See, e.g.*, Ex. 1009, 69-70. A POSA could have readily incorporated sequencing to detect the amplified ASA in Baez and detect the two identification sequence elements.

107. Baez in view of Landegren discloses step (e).

2. A POSA would have been motivated to combine Baez and Landegren despite Dr. Pentelute's concerns

(a) A POSA would have been motivated to further improve Baez

108. Dr. Pentelute argues that "a POSA would not have been motivated to combine Baez with Landegren based on Dr. Nelson's stated rationales, because Baez

itself discloses that its methods already satisfy those goals.” Ex. 2008, ¶ 196. Although Dr. Pentelute is correct that Baez touts its invention’s improvements, I disagree that a POSA would not be motivated to further improve Baez. Ex. 1005, ¶ 238.

109. As Baez explains, “[m]ethods for the reduction of background noise are continually being sought.” Ex. 1003, 1:32-33; Ex. 1005, ¶ 238. The incorporation of Landegren’s tags into Baez’s methods would further reduce background signal because the identification tags allow the user to more readily distinguish true positives (analyte-specific binding of two reporter conjugates, each equipped with an identification sequence element) from false positives. Ex. 1005, ¶ 238. Thus, a POSA would still have been motivated to combine Baez with Landegren to further improve Baez’s methods.

(b) Landegren does not teach away from use of solid-phase assays, contrary to Dr. Pentelute

110. Dr. Pentelute also argues that a POSA would not be motivated to combine Baez with Landegren because “the POSA would not have been motivated to combine Landegren’s solution-phase methods with Baez’s methods that use solid-phase substrates.” Ex. 2008, ¶ 201. While I agree that Landegren discloses solution-phase methods, I disagree that such disclosure would “expressly teach[] against” combining Baez and Landegren. *Id.*

111. Dr. Pentelute relies on Landegren's disclosure that "assays that use a solid phase require considerable time and effort to analyze." *Id.*, ¶ 198 (citing Ex. 1004, 1). But Dr. Pentelute ignores that Baez also discloses solution-phase methods (*e.g.*, Ex. 1003, 11:52-55, 12:54-13:17). He also ignores that at the time of the invention solid-phase capture was a well-known and popular technique (*see, e.g.*, Ex. 1016, 1017, 1018 (and references therein)).

112. In addition, Dr. Pentelute's opinions do not consider how a POSA would have combined Baez and Landegren. As stated in my First Declaration, a POSA would be motivated to combine the sequence identification tags of Landegren to the nucleic acid labels of Baez. Ex. 1005, ¶ 238. 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. As a result, a POSA would disagree that Landegren teaches away from being combined with Baez.

3. A POSA would have a reasonable expectation of success in combining Baez and Landegren

113. Dr. Pentelute argues a "POSA would not have reasonably expected success because in view of Landegren, the POSA would have expected Baez's solid-phase assays to 'require considerable time and effort' and be a 'time consuming process.'" Ex. 2008, ¶ 203 (quoting Ex. 1004, 1). I disagree with Dr. Pentelute's arguments.

114. A POSA would have a reasonable expectation of success in modifying Baez's nucleic acid labels to incorporate the tags of Landegren because modifying nucleic acid is within the skillset of a POSA. Ex. 1005, ¶¶ 25, 239. Specifically, the structure of the reporter conjugates in Baez and the proximity-probes in Landegren are similar, both are antibodies coupled to nucleic acid, the modification of the nucleic acid in Baez to include the identification sequence taught in Landegren would be routine for a POSA. *Id.*, ¶ 239.

4. A POSA would not be concerned by Freskgard's use of solid-phase assays

115. Dr. Pentelute argues a POSA would have the "same concerns over combining Freskgard with Landegren and Baez in view of Landegren's caution against using solid-phase assays." Ex. 2008, ¶ 207. I disagree. For the same reasons discussed above regarding combining Baez and Landegren, a POSA would not have any concerns or issues with combining Freskgard with Landegren and Baez.

5. Remaining dependent claims

116. Dr. Pentelute does not specifically address claims 2-8 and 11-12 besides a passing reference that his arguments "for claim 1 discussed in Ground[] 3 . . . also appl[ies] to dependent claims 2-20." Ex. 2008, ¶ 208. Thus, because Dr. Pentelute fails to establish that the claims are not obvious over Baez in view of Landegren, the remaining dependent claims are also rendered obvious by Baez in view of Landegren as provided in my First Declaration.

IV. Declaration

117. 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: September 22, 2025

By: Norman Nelson, Ph.D.
Norman Nelson Ph.D.