

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BIO-RAD LABORATORIES, INC.,
Petitioner,

v.

CALIFORNIA INSTITUTE OF TECHNOLOGY,
Patent Owner.

U.S. PATENT NO. 11,827,921 B2

DECLARATION OF CARL A. BATT, PH.D. IN SUPPORT
OF PETITIONER FOR *INTER PARTES* REVIEW

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I. INTRODUCTION

1. My name is Carl Batt, Ph.D. I am over the age of twenty-one (21) and am competent to make this declaration. I reside at 117 Devlen Road, Groton NY 13073.

2. I have been retained as an independent expert witness by Bio-Rad Laboratories, Inc. (“Bio-Rad” or “Petitioner”) in the above-captioned *inter partes* review proceeding to provide my independent evaluation and opinions regarding the validity of all of the claims of U.S. Patent No. 11,827,921 (“the ’921 patent”). I have been asked to provide my opinion regarding whether a person of ordinary skill in the art (“POSA”) in the subject matter of the claims would have found them anticipated or obvious over certain published patent applications and publications.

3. This declaration sets forth my analyses and opinions based on my technical background, skills, knowledge, training, education and experience in the relevant art, and the materials I have considered. As I explain below, it is my opinion that all claims of the ’921 patent are rendered obvious by the combination of prior art references discussed herein. I expect to testify regarding the matters set forth in this declaration if asked to do so.

4. As I explain below, specifically, it is my opinion that all claims of the ’921 patent are invalid under 35 U.S.C. § 102 because claims 1-10, 13-19 are

anticipated by (1) U.S. Patent Publication US 2011/0250597 A1, by Larson et al., entitled “Digital Analyte Analysis” (“Larson”), and claims 11 and 12 are rendered obvious in combination with further references, as discussed below. Further, to the extent Patent Owner contends that claims 1-10, 13-19 are not anticipated by Larson, they are rendered obvious by Larson, alone or in combination with further references, as discussed below.

II. QUALIFICATIONS AND COMPENSATIONS

A. Background and Qualifications

5. I am presently the Liberty Hyde Bailey Professor of Biology at Cornell University in the Department of Food Science and the Director of the Cornell University/Ludwig Institute for Cancer Research Partnership. I am also a founder of Main Street Science and the founder of Nanooze, an on-line science magazine for kids. I am also a co-founder and former co-director of the Nanobiotechnology Center, a National Science Foundation supported Science and Technology Center. I am also appointed as an Adjunct Senior Scientist at the MOTE Marine Laboratory in Sarasota, Florida. My current research interests are a fusion of biology and nanotechnology focusing on important biomedical problems.

6. I received my undergraduate degree in Microbiology from Kansas State University, and Master’s and Ph.D. degrees from Rutgers University, Department of Food Science. From 1981 to 1985, I did postdoctoral work at the Massachusetts

Institute of Technology. From 2002 to 2012, in partnership with the Ludwig Institute for Cancer Research, I established a Good Manufacturing Practices Bioproduction facility in Stocking Hall. See Appendix A (my curriculum vitae).

7. I have published over 290 peer-reviewed articles, book chapters, and reviews. In addition, from 1987-2000, I served as an editor for Food Microbiology, a peer-reviewed journal. I was also an editor for the Encyclopedia of Food Microbiology, published in 2000. In 2010, I undertook a revision of this publication that was released in 2012. I am now currently chief editor for the third revision of the Encyclopedia of Food Microbiology. In 2015-2016, I was subject editor for the Elsevier, Food Science Reference module.

B. Compensation

8. I am being compensated for my time in connection with this IPR. My compensation is not contingent on reaching any particular findings or conclusions, or on any outcome in the case. The opinions contained in this report are mine and are based on my own personal knowledge, experience, training, and study of the materials discussed below in this report.

III. SUMMARY OF OPINIONS

9. It is my opinion that claims 1-10 and 13-19 of the '921 patent was anticipated by Larson. To the extent Patent Owner contends that claims 4, 6-7, and 14-19 of the '921 patent are not anticipated by Larson, the claims would have been

obvious in view of Larson. To the extent Patent Owner contends that claims 1-10 and 13-19 of the '921 patent are not anticipated by Larson, the claims would have been obvious in further view of Saxonov.

10. It is my opinion that claim 12 of the '921 patent is rendered obvious by Larson in further view of Saxonov.

11. It is my opinion that claim 11 of the '921 patent is rendered obvious by Larson in further view of Saxonov and Silverbrook.

IV. LEGAL STANDARDS

12. I am a scientist, principal investigator and instructor by trade, and the opinions I express in this declaration involve the application of my scientific knowledge and experience to the evaluation of the claims of the '921 patent and the impact of certain prior art on the '921 patent. I am not a lawyer, nor do I offer any legal opinion in this declaration. Counsel from Weil, Gotshal & Manges LLP have advised me, however, as to various legal standards that apply to the technical issues I address in this declaration. I have applied those standards in arriving at my conclusions expressed in this declaration.

A. Written Description Support

13. I understand that claims in a U.S. application are only entitled to the benefit of an earlier filing date, such as a foreign priority date or the filing date of a provisional application, if the corresponding application relied on, such as a foreign

application or provisional application, contains sufficient written description of the invention and the manner and process of making it to enable an ordinarily skilled artisan to practice the invention claimed in the non-provisional application.

14. I also understand that a patent or patent publication relied on as a reference is similarly only entitled to the priority benefit of an earlier corresponding application if the corresponding application, such as a foreign application or provisional application, contains sufficient written description for at least one claim.

15. Moreover, where a reference application relies on the priority benefit of an earlier corresponding application to establish the reference as prior art, I understand that the particular disclosure or teaching relied on in challenging any claim must have sufficient written description in an earlier-filed application if that earlier-filed application, and its priority benefit, is relied on to establish the applied reference as prior art.

B. Claim Construction

16. I understand that when interpreting the claims of the '921 patent, I must do so from the perspective of one of ordinary skill in the art at the relevant priority date. I have not independently determined the priority date to which the '921 patent is entitled. For purposes of this proceeding, I have been asked to assume that the '921 patent has a priority date of February 3, 2012, the filing date of provisional

application No. 61/594,480, the earliest-filed application to which the '921 patent claims the benefit of priority. My analysis does not change if the '921 patent is entitled to an earlier priority date falling after July 20, 2011, the filing date of provisional application No. 61/510,013 that one of the applied references relies on for the benefit of priority and incorporates by reference. Likewise, my analysis does not change if the '921 patent is entitled to a later priority date.

17. I understand that in determining whether a patent claim under *inter partes* review before the Patent Trial and Appeal Board ("PTAB") is unpatentable in view of prior art, the words of the claims of a patent are generally given their ordinary and customary meaning from the perspective of and as interpreted by a POSA at the time of the claimed invention. I also understand that patent claims are construed in light of the claim language, the patent specification (including the drawings), and the prosecution history (including the references cited during the prosecution of the patent). Specifically, it is my understanding that in determining whether a patent claim under *inter partes* review before the PTAB is unpatentable in view of prior art, the PTAB uses the *Phillips*¹ standard for claim construction used in civil courts, which is to say that the claims must be given the meaning that the

¹ *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005).

terms would have had to a person of ordinary skill in the art at the time of the priority date of the '921 patent consistent with the specification and file history of the '921 patent.

18. For this declaration, it is my opinion that each claim term can be easily understood by the skilled artisan without further construction, that is, my analysis is based on the plain and ordinary meaning of the claim terms, unless otherwise indicated. I understand that Petitioner has identified and proposed construction of certain terms in related proceedings. EX1003. To the extent any construction is required, I have been asked to apply claim constructions that are consistent with Patent Owner's position in related proceedings. I reserve the right, however, to supplement my opinions if Patent Owner proposes any constructions or the Board adopts any claim constructions.

C. Anticipation

19. I understand that a prior art reference invalidates a claim under 35 U.S.C. § 102 by "anticipation" if all elements of a challenged claim are disclosed in the invalidating prior art reference. I further understand that these disclosed elements must be arranged in the prior art reference in the manner they are arranged in the challenged claim.

20. I also understand that a limitation may be considered to be “inherent” in the disclosure of a prior art reference even if the reference does not expressly disclose the limitation. A claim element is inherent if it is necessarily present in the disclosure, and it not a matter of probability or likelihood. The fact that a limitation might result as part of a given disclosure is insufficient for the limitation to be inherent in the disclosure, i.e., inherently disclosed.

D. Obviousness

21. I understand that a claim is unpatentable under 35 U.S.C. § 103 if the claimed subject matter as a whole would have been obvious to a POSA at the time of the alleged invention, which I have been instructed to treat at present as no earlier than February 3, 2012, the filing date of provisional application No. 61/594,480, the earliest-filed application to which the '921 patent claims the benefit of priority.

22. I understand that a POSA provides a reference point from which the prior art and the alleged invention should be viewed. This reference point prevents a person of ordinary skill from using one's insight or hindsight in deciding whether a claim is obvious. I understand the use of such hindsight is impermissible.

23. I also understand that a claim can be obvious in light of a single reference or a combination of multiple prior art references. I understand that such a combination must meet certain requirements, further described below.

24. I understand that a patent claim composed of several elements is not proved obvious merely by demonstrating that each of its elements was known in the prior art. It has been explained to me that three requirements must be satisfied for a combination of prior art references to render obvious a claimed invention. First, the cited art must teach or suggest all the requirements of the invention as recited in the claims. However, I understand that a claim is obvious if a missing feature would be a natural result of the combination of prior art elements. Second, the cited art, coupled with the knowledge generally available in the art at the time of the invention, must contain some suggestion or incentive that would have motivated the ordinary skilled artisan to modify a reference or to combine references. I understand that the reason to combine prior art references can come from a variety of sources, such as common sense, and not just from the prior art itself. Third, the proposed modification of the cited art must have had a reasonable expectation of success, determined from the vantage point of the ordinary skilled artisan at the time the invention was made. I also understand that a reasonable expectation of success does not require that success is certain.

25. I understand that only analogous art may be considered in an obviousness analysis. Art is considered analogous if it is in the same field of endeavor as the patent or if it is reasonably pertinent to the particular problem with

which the patent is involved. A reference is reasonably pertinent if, though in a different field, it would have commended itself to an inventor's attention in considering the problem the inventor is addressing.

26. I understand that it is impermissible to use hindsight to arrive at the claimed invention. My understanding is that to preclude hindsight in the obviousness analysis, which I understand to be improper, there must be evidence at the time of the invention of some reason to make the variation or combination. Furthermore, I understand that, to render a claim obvious, the prior art cannot be vague, but rather, must collectively guide a person of ordinary skill in the art towards the particular solution claimed. I understand that obviousness does not exist where the prior art discourages or teaches away from the claimed invention.

27. I understand that certain objective factors, sometimes known as objective indicia, must be taken into account in determining whether a claimed invention would have been obvious. I understand that the following is a nonexclusive list of secondary considerations to analyze for this inquiry: (i) a strong showing of commercial success of a product covered by the patent claims that can be attributed to the merits of the invention; (ii) a long felt but unmet need in the prior art that was satisfied by the invention of the patent; (iii) the willingness of industry members to license the patent at issue; (iv) evidence that those of skill in the art were

skeptical as to the merits of the invention, or that prior art references taught away from the invention; (v) evidence that those of skill in the art were surprised by the capabilities of the claimed invention; (vi) praise of the invention by others skilled in the art; and (vii) deliberate copying of the invention. I further understand that contemporaneous and independent invention by others is a secondary consideration supporting an obviousness determination.

28. I understand that any assertion of the above indications must be accompanied by a nexus between the merits of the purported invention and the evidence offered. If there is no such connection between the claimed technology and the secondary evidence, then that evidence does not actually establish the purported invention was nonobvious.

V. TECHNICAL BACKGROUND AND STATE OF THE ART

29. To the extent the following discussion is similar to other declarations, the ideas contained are entirely my own and I agree with the content therein.

A. Polynucleotides: DNA and RNA

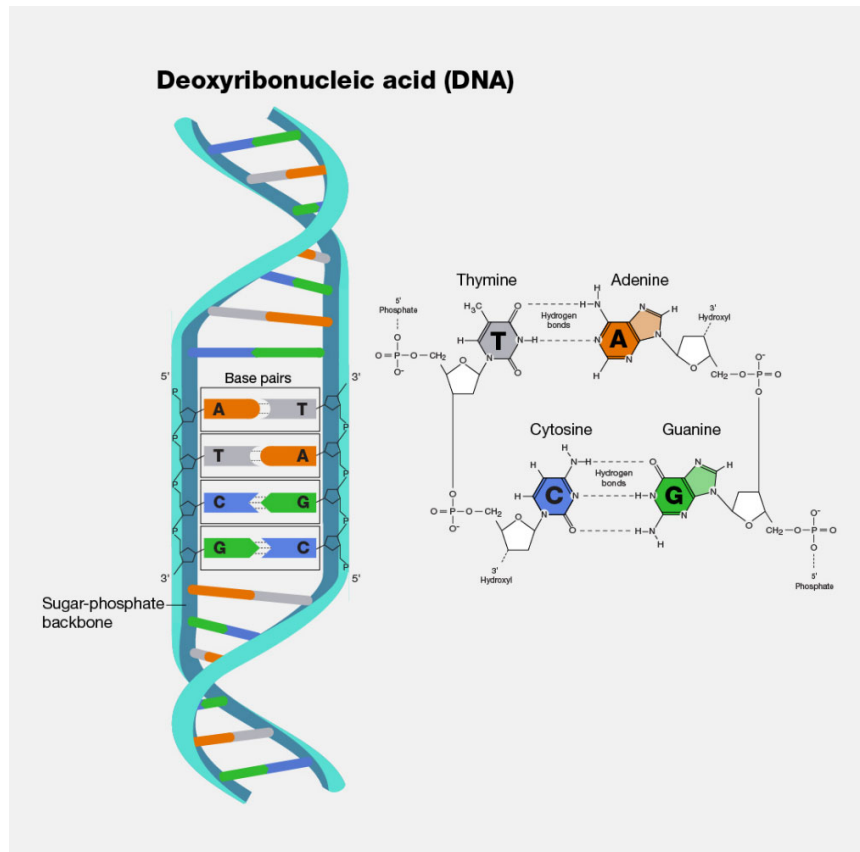
30. Polynucleotides are linear polymers of simpler monomeric units called nucleotides. The two most common types of polynucleotides are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA and RNA contain the information necessary to specify many aspects of development, growth, and maintenance of the cells of organisms. The sequence of the monomeric units, the nucleotide sequence,

in both DNA and RNA encodes or stores information needed for all cellular processes.

31. Each deoxyribonucleotide includes one of four nucleobases (cytosine (“C”), guanine (“G”), adenine (“A”), or thymine (“T”)), a sugar (deoxyribose), and a phosphate group. Each ribonucleotide similarly includes one of four nucleobases (cytosine, guanine, adenine, or uracil (“U”) (that is structurally similar to thymine, lacking a methyl group)), a sugar (ribose), and a phosphate group. Individual nucleotides can form a polynucleotide strand, such that the sequence of the nucleotides can store genetic information.

32. The four nucleotide bases can bind to their complementary base pairs: A with T,² and C with G. As such, two strands with complementary sequences can be base-paired to form a double-stranded polynucleotide:

² In RNA, A pairs with U.



EX1036. Knowledge of the sequence of one strand can be used, using these base pairing rules, to determine the sequence of a complementary strand.

33. Each polynucleotide strand has two ends, on the basis of the orientation of the sugar phosphate backbone. The ends are referred to as 5' and 3', based on the orientation of the deoxyribose or ribose molecule in the backbone, with the 5' end terminating in a 5'-hydroxyl group (with or without phosphate group(s)) and the 3' end terminating in a 3'-hydroxyl group. Both DNA and RNA are synthesized in the 5' to 3' direction. The complementary strands are oriented in opposite directions.

34. There are some key differences between DNA and RNA. Unlike DNA, RNA tends to be single stranded, not double stranded. Also, while both use adenine, guanine, and cytosine as bases, as noted above, RNA contains uracil instead of thymine, but T and U are functionally equivalent because they both form base pairs with adenine, and denote the same genetic information.

35. Determining the sequence of polynucleotides is useful in the context of diagnosis and treatment of patients, as well as for basic scientific research. For example, determining the nucleotide sequence of an individual's genome can provide information regarding whether an individual may be susceptible to or have various disorders. Similarly, determining the polynucleotide sequences of molecules from sources such as tumors or pathogens can provide information regarding a person's current illnesses, such as various cancers or infections. Thus, determining the presence of specific sequences of polynucleotides is useful for diagnostic applications to allow for the monitoring of disease progression and allows for clinicians to tailor an appropriate treatment.

B. Multiplex PCR Amplification

36. Polymerase chain reaction ("PCR") is a method of generating numerous copies of a polynucleotide sequence. This technique relies on the base-pairing principles of nucleotides, such that a single polynucleotide strand is a

template to generate a complementary strand. Targeted sequences are amplified using an enzyme that can replicate DNA *in vitro*, specific oligonucleotide primers, and monomer nucleotides.

37. In general, the first step of PCR, 'denaturation,' is separating the complementary strands of the target DNA. This separation can be accomplished through heating the DNA to its melting temperature, which separates the strands. This is followed by an 'annealing', where primers, short oligonucleotides specific to a region of DNA, are allowed to anneal (or hybridize) to the complementary region in the target DNA strand. The third step is 'extension'. An enzyme, DNA polymerase adds nucleotides to extend off a primer using the target sequence and faithfully copying the sequence. The polymerase binds and extends to create a copy of the targeted DNA sequence with the borders defined by the oligonucleotide primers. These three steps together comprise one PCR cycle. Cycles are repeated to amplify DNA to any extent desired, using the replicated strands as templates for subsequent cycles. Key to the process is the use of a thermostable DNA polymerase and Taq DNA polymerase is among the most common since it can withstand repeated 'denaturation'.

38. In some cases, the target polynucleotide sequence for amplification is RNA. If so, the RNA can be used as a template for generating a strand of

complementary DNA. This is known as reverse transcription, wherein the enzyme known as a reverse transcriptase uses a target strand of RNA as a template to generate a strand of DNA. The DNA can then be used as a template for PCR.

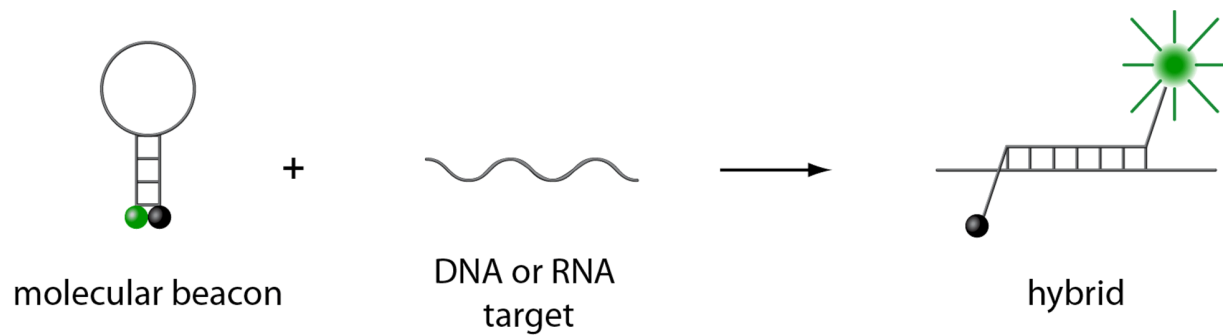
39. PCR can be multiplexed, that is, multiple oligonucleotide primers can be used simultaneously to amplify multiple target sequences in a single PCR at the same time. At least as of 2012, such multiplexed PCR and its use was well known in the art. Multiplex PCR provides numerous advantages, including being faster, less expensive, and more convenient than doing separate PCRs for each target.

C. Fluorescence Signals and Detection of Polynucleotide Analytes

40. Prior to 2012, the use of fluorescence molecules as signals to detect polynucleotide analytes was common. Methods for doing this include, for example, hybridizing fluorescently labeled oligonucleotides to specific, target polynucleotide sequence(s) and detecting and/or measuring the resulting fluorescent signal from the labeled oligonucleotides that bind the target. In some techniques, target polynucleotide sequences with bound labeled-oligonucleotides are isolated or separated from unbound labeled-oligonucleotides and the resulting signal of label is measured. In other techniques, isolation or separation is not required, because a detectable signal is generated only when the labeled-oligonucleotide is bound to the target polynucleotide. Generating a detectable signal without separation can be

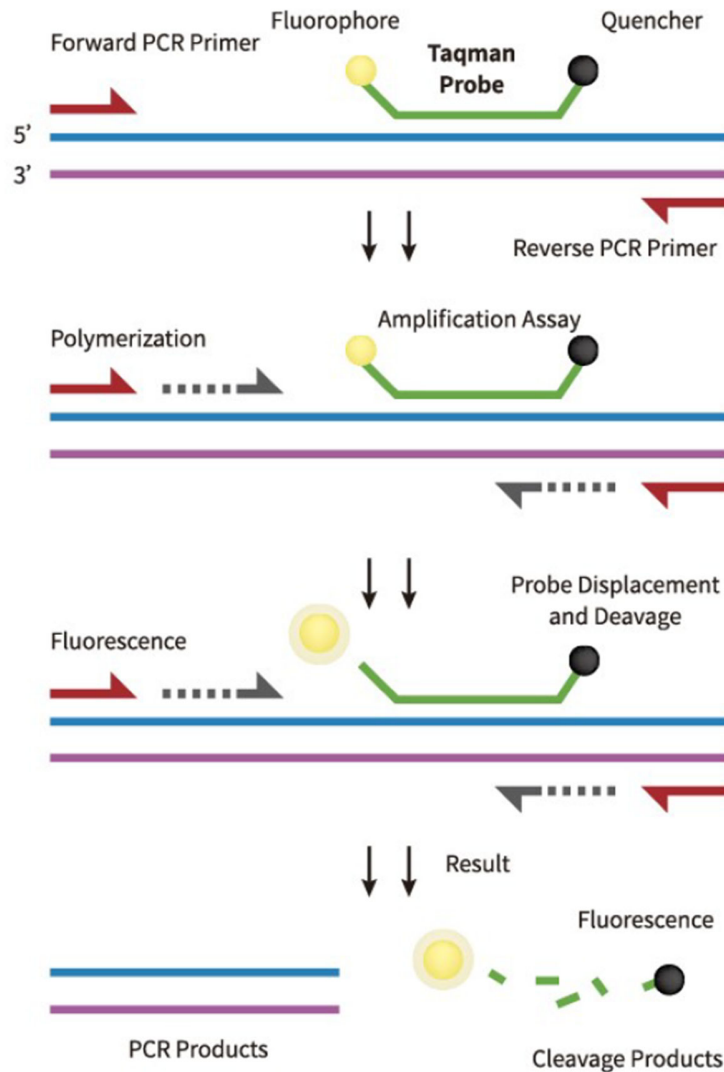
achieved by use of both a fluorophore and a “quencher” where a quencher is a molecule that can “turn off” a fluorescent signal when in close proximity to the fluorophore.

41. Two common approaches are used in conjunction with probes having both a fluorophore and a quencher. One approach is known as a “molecular beacon.” This approach uses an oligonucleotide probe with a fluorophore and a quencher on either end of the probe. The probe sequence encodes a self-complementary sequence that internally anneals, forming a hairpin, and brings the two ends (and thus the quencher and fluorophore) into close proximity with one another. When in close proximity, the quencher suppresses the fluorescent signal of the fluorophore. The probe, however, is designed to include a sequence that binds to the target sequence(s). Polynucleotide analyte(s) having target sequence(s) can be detected by contacting samples that may include the polynucleotide analyte with the probe which will hybridize to the target sequence if it is present or hybridize to itself if no polynucleotide analyte with target sequence is present. Thus, if the probe hybridized to a polynucleotide analyte, the fluorescent label and quencher are separated from one another generating a detectable, unquenched signal. This process is depicted in the figure below:



EX1037.

42. A second, alternative method uses a short probe with a quencher, where the proximity allows the quencher to suppress the signal. This type of probe is generally referred to as a TaqMan probe and was described in literature as early as 1991. EX1014. The TaqMan probe is used with two PCR primers and is designed to bind to a region between the two primers. In this method, a target-bound probe is cleaved during extension by the DNA polymerase, which has an exonuclease activity. The cleavage frees the quencher and fluorophore which both diffuse away and the signal from the fluorophore is no longer quenched, thereby allowing detection of signal emitted from the fluorophore. This process is depicted in the figure below:



EX1013 at 6; *see also* EX1014 (research paper describing assay).

D. Quantitative PCR

43. The basic principles of PCR and fluorescent labeling can be adapted to determine the amount, or number, of target sequences within a sample. Two such adaptations include quantitative PCR (“qPCR”) and digital PCR (“dPCR”).

44. A common form of qPCR relies on the use of an oligonucleotide TaqMan probe that includes both a fluorophore (e.g., a fluorescent molecule) and a quencher, as I describe above. Prior to binding, the fluorophore and quencher are in sufficiently close proximity for the quencher to suppress (quench) the signal from the fluorophore.

45. Taq polymerase, the enzyme used in the extension step of PCR that polymerizes the extended complementary strand, also has an exonuclease function that digests the bound oligonucleotide TaqMan probe during the extension reaction. qPCR relies on this exonuclease activity to cleave the oligonucleotide probe separating the fluorophore from the quencher so as to generate the detectable signal. Specifically, in this method, both probe and primer are permitted to anneal to the polynucleotide analyte (if present) and, during the primer extension step, the Taq polymerase not only extends the primer but also cleaves any oligonucleotide probe bound to the polynucleotide, separating the fluorophore and quencher. Once the fluorophore is freed, the quencher is no longer suppressing the signal, so it generates a detectable signal that can be quantified.

46. The signal generated, i.e. the level of fluorescence intensity, in qPCR, as described above, is proportional to the number of target polynucleotide analyte molecules present in the sample. Therefore, using this technique, the relative

quantity of different polynucleotides, such as the various polynucleotide analytes in a sample, can be determined by comparing the intensity of the generated signals to each other. Thus, by including a known amount of an analyte to serve as an internal standard, the signal from a probe for that standard can be used to compare against the signal from a probe that detects the target analyte to allow quantification of that analyte. For example, where the number of copies of a particular gene or polynucleotide sequence is known, it can be used in an assay as a measure for comparison with the signal generated for a target polynucleotide present in unknown quantities in order to determine the number of copies of the target polynucleotide analyte. EX1017 ¶¶ 94-97.

47. Digital PCR (“dPCR”) is a different form of quantitative PCR. Unlike the comparative nature of qPCR I describe above, relying on standards, dPCR relies on highly dilute samples that are divided into partitions, such as droplets, such that each partition generally has no more than a single copy of the target analyte (if any). Specifically, a sample is subjected to limiting dilution prior to qPCR. A positive result, i.e., generated signal, indicates the presence of at least one copy of the target polynucleotide analyte in the partition. The number of positive partitions, and thereby the proportion of positive partitions, can be translated into the number of copies of the target polynucleotide analyte in the sample using statistical techniques

such as the Poisson distribution. An image showing the workflow for performing ddPCR using Petitioner's droplet-based products is shown below:

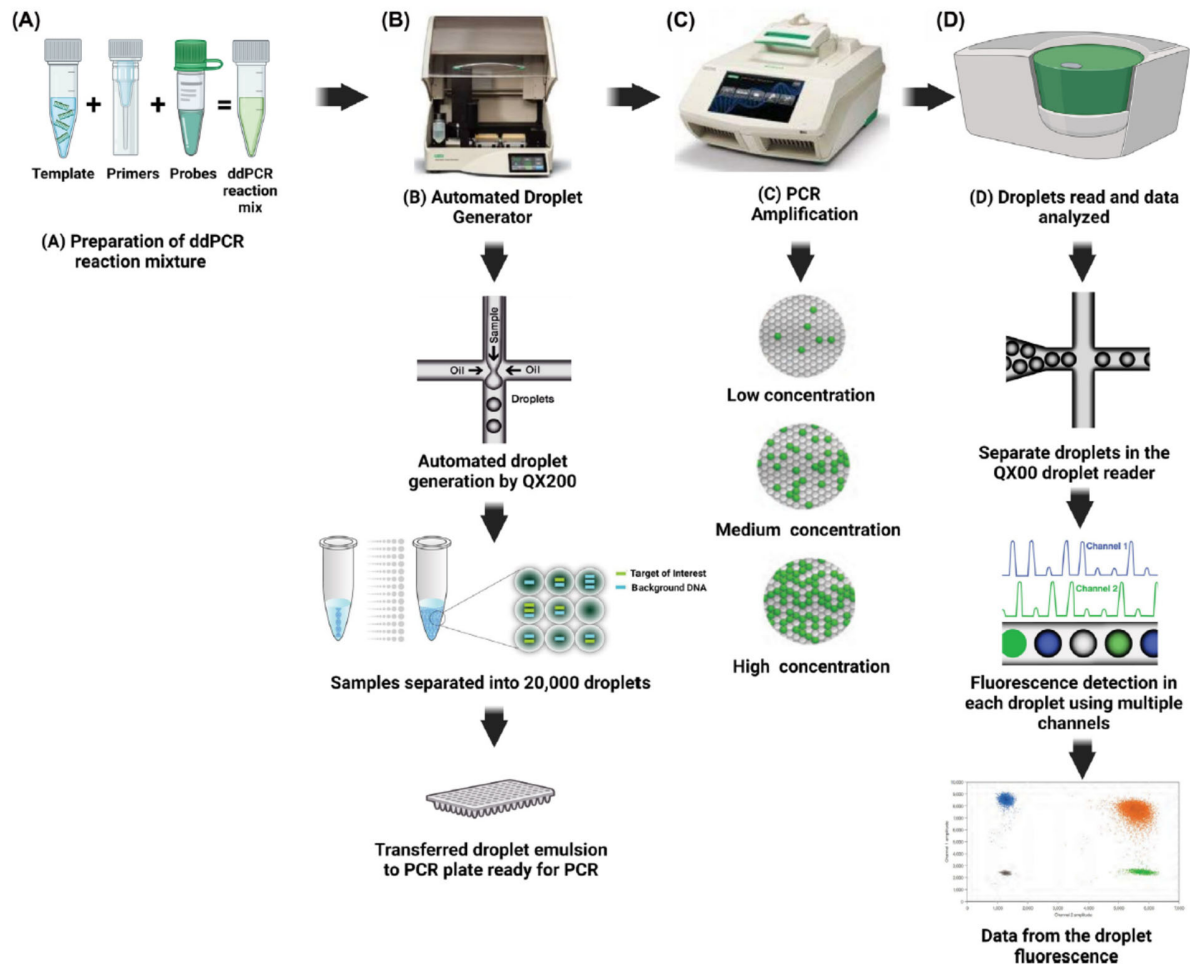


FIGURE 2.7 Overview of droplet digital polymerase chain reaction (PCR) workflow. (A) Standard ddPCR reaction mixtures are prepared in tubes. (B) The reaction mixture is separated into 20,000 droplets using an automated droplet generator, and the droplet emulsion is transferred into a 96-well PCR plate for PCR amplification. (C) PCR amplification is done, and droplets with green were designated as positive reaction and gray color as negative. (D) Using a two- or six-channel digital droplet reader, the fluorescence detection in each droplet is measured and the data is visualized using proprietary software in 1D or 2D plots. Source: Bio-Rad and images created with <http://www.BioRender.com>.

EX1038 at 12.

VI. OVERVIEW OF THE '921 PATENT

48. The '921 patent is titled "Signal Encoding and Decoding in Multiplexed Biochemical Assays." It purports to describe "methods, systems, compositions, and kits for the multiplexed detection of a plurality of analytes in a sample." EX1001 at Abstract. Multiplex detection is the detection or measurement of multiple different analytes in the same sample volume. *Id.* at 1:35-39. As described in the '921 patent, multiplex detection is accomplished by generating signal(s) indicating the presence of the analyte(s) and acquiring cumulative measurement(s) of signal component(s), such as fluorescence, and determining which analytes are present based on the cumulative measurement(s) of the signal(s). *Id.* at Abstract.

A. Claims

49. All of the claims of the '921 patent are challenged in the Petition, including independent claims 1 and 14. The claims are reproduced below:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,
wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,
wherein said fluorescent signal comprises F wavelength components, and
wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample

volume, based on detection of said F wavelength components, when M is greater than F,

wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and

wherein the analytes are amplified in the single sample volume.

2. The assay of claim 1, wherein each of said at least M analytes is encoded as at least one first value in a first component of said fluorescent signal and at least one second value in a second component of said fluorescent signal.
3. The assay of claim 2, wherein said first value is an intensity or range of intensities.
4. The assay of claim 2, wherein said coding scheme is made nondegenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.
5. The assay of claim 1, wherein said detecting is performed with reagents comprising hybridization probes.
6. The assay of claim 5, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.
7. The assay of claim 5, wherein said fluorescence signal from said hybridization probes are used to construct a chromatogram.
8. The assay of claim 1, wherein said signal is generated during a polymerase chain reaction.
9. The assay of claim 1, wherein at least one of said analytes is encoded by at least one additional value, wherein said at

least one additional value is selected from the group consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal, and combinations thereof.

10. The assay of claim 1, wherein at least one of said analytes comprises a polynucleotide.
11. The assay of claim 1, further comprising transmitting information concerning the presence or absence of at least one of said analytes through a computer network.
12. The assay of claim 1, wherein at least one step of said assay is performed using a computer and wherein said computer is connected to a thermal cycler.
13. The assay of claim 1, wherein said coding scheme is innately nondegenerate or non-degenerate by construction.
14. A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising analyte-specific reagents, wherein:
 - a) each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy; and
 - b) each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the value;

wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single sample volume, in any combination of presence or absence, and wherein the kit does not require the use of

mass spectrometry or immobilization of said M analytes,
and

wherein the analytes are simplified in the single sample volume.

15. The kit of claim 14, wherein said fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, when M is greater than F.
16. The kit of claim 14, wherein said analyte-specific reagents comprise a plurality of hybridization probes.
17. The kit of claim 16, wherein said plurality of hybridization probes comprise a fluorophore, and wherein said plurality of hybridization probes are specific for different analytes.
18. The kit of claim 17, wherein said number of hybridization probes in the kit is greater than M.
19. The kit of claim 14, wherein the analyte-specific reagents are each present at a different amount in the kit.

B. Specification

50. The '921 patent purports to describe multiplex detection of polynucleotide analytes, specifically using fewer fluorophores (colors) in sequence-specific labeled probes than there are polynucleotide analytes. EX1001 at Abstract, 1:51-59, 3:10-21, 8:1-15, 10:31-55, 13:57-14:8.

51. To achieve this multiplex detection, the labeled probes generate signals which are used in a coding scheme that maps the different combinations of signals to the different analytes that may be present in a sample, as detailed below. The

coding schemes required to achieve the multiplex detection vary in complexity depending on the number of analytes to be encoded. The number of analytes the '921 patent claims its methods may be used to detect may be “selected from the group consisting of any 2, 3, 4,...100 or more” and likewise with the corresponding reagents and measured signals for the methods. *Id.* at 26:18-28; *see also id.* at 12:50-62, 30:13-63.

52. The specification also purports to describe encoding each analyte as a value of at least one component of a signal, such as color or intensity. *Id.* at 8:63-9:5 (“In some examples, each analyte to be detected is encoded as a value of a signal (e.g., intensity), where the values are assigned so that the results of the assay unambiguously indicate the presence or absence of the analytes being assayed.”). Probes specific to the analytes are purportedly labeled according to the encoding scheme and contacted with a sample that may contain the corresponding analytes. *Id.* at 8:63-9:32. The signal is quantified, such that the cumulative value of the signal purportedly indicates the presence or absence of the analytes in the sample. *Id.* at 9:33-38.

53. The '921 patent states that the encoding schemes may be degenerate or non-degenerate. A possible result from a given encoding scheme, or a generated signal result from an assay, is referred to as “degenerate” when the result is

ambiguous as to which specific analytes are present, alone or in combination, in a given sample. *Id.* at 9:48-50, 16:47-56. In other words, a signal is degenerate if more than one combination of analytes in a sample can result in that same signal. “Non-degenerate” refers to encoding schemes, or generated signal results from an assay, that are unambiguous as to which specific analytes are present, alone or in combination, in a given sample. *Id.* at 5:4-9, 11:44-48, 20:13-21:16, 22:18-59.

54. The specification discloses the above-described coding schemes employing signals of differing intensities of the same color label to distinguish between different analytes. *Id.* at 22:9-23:67 (using one color and one intensity for each analyte and different specific intensities to distinguish different analytes).

55. An example of the encoding scheme wherein different intensities of the same color are used to differentiate between analytes is described in the '921 patent and exemplified in Table 8, reproduced below:

TABLE 8

Example of encoding method using one color and one intensity per analyte, but different intensities among analytes.					
Tier	Analyte	B	G	Y	R
1	A	1	0	0	0
	B	2	0	0	0
	C	4	0	0	0
	D	8	0	0	0
2	E	0	1	0	0
	F	0	2	0	0
	G	0	4	0	0
	H	0	8	0	0
3	I	0	0	1	0
	J	0	0	2	0
	K	0	0	4	0
	L	0	0	8	0
4	M	0	0	0	1
	N	0	0	0	2
	O	0	0	0	4
	P	0	0	0	8
		15	15	15	15

56. As shown above, each analyte is encoded by only one color, with analytes grouped in tiers with column headings for various color signals: analytes A-D encoded in blue; analytes E-H encoded in green; analytes I-L encoded in yellow; and analytes M-P encoded in red. Here, the tiers (1-4) correspond to the different colors, i.e., blue (B), green (G), yellow (Y), and red (R). The number in the column denotes a unit-less fluorescence intensity for a given color. For example, at Tier 1, Analyte A is assigned an intensity of ‘1’ for the blue color and Analyte B

is assigned an intensity of '2' for the blue color. At Tier 4, analyte P is assigned and intensity of '8' for the red color.

57. The specification purports that the above scheme using different intensities allows each color in an assay to be used to distinguish two, three, or four analytes in a sample volume. *Id.* at 23:1-67. Specifically, because the signals in a given color do not sum up to a greater intensity specific for another analyte, the possible combinations of signals from different analytes or combinations thereof can purportedly be distinguished in any combination of presence or absence. *Id.* at 23:1-5. For example, in the blue fluorophore, individual intensities 1, 2, 4, and 8 for analytes A-D, respectively, ensure that any combination of the targets allows for unambiguous interpretations of the result, with analytes present alone or in any combination. Specifically, an assay that reports an intensity of 5 in blue could only mean analytes A and C present ($1+4 = 5$) and an assay that reports an intensity of 6 in blue could only mean analytes B and C are present ($2+4 = 6$). This unambiguous detection is a byproduct of the signals of each given color not summing up to a greater intensity specific for another analyte. In practice, this lack of summing up can be shown by the sum of the blue signals for targets A and B, which is three, and is less than the signal for target C, which is 4. Similarly, the sum of the signals for targets A, B, and C is 7, which is less than the signal for target D (8).

58. This ability to distinguish analytes, alone and in combination, allows the cumulative signals detected by the assay to be ***non-degenerate*** and purportedly allow unambiguous determination of the presence or absence of polynucleotide analytes. *Id.* at 23:1-39, 23:38-39 (“The coding scheme illustrated in Table 8 is non-degenerate by design.”).

59. The '921 patent further describes coding schemes wherein the approach of encoding employs more than one color per analyte. An example of such an encoding scheme is shown in Table 3, reproduced below:

TABLE 3

Encoding of eight analytes (including control) with four colors and up to four colors per analyte.				
	Blue	Green	Yellow	Red
Ctrl. (p)	1	0	0	0
A	1	0	0	1
B	1	0	1	0
C	1	1	0	0
D	1	0	1	1
E	1	1	0	1

TABLE 3-continued

Encoding of eight analytes (including control) with four colors and up to four colors per analyte.				
	Blue	Green	Yellow	Red
F	1	1	1	0
G	1	1	1	1

60. In the coding scheme in Table 3, each of the seven analytes is encoded by a combination of up to four color signals, with blue as the “control” color. *Id.* at 13:55-14:39. Each of the seven analytes (A-G) is encoded by at least the blue “control” label and one or more other color labels. *Id.* at 13:55-14:9.

61. In this example, three analytes—A, B, and C—are encoded by two colors (blue and either green, yellow or red), three analytes—D, E, and F—are encoded by three colors, and one analyte—G—is encoded by four colors. *Id.* at 14:10-22. The combination of the control color with additional colors provides signals that indicate both that there is a copy of at least one of the target analytes (based on the presence of the control color signal) and its identity (based on the combination of additional color(s) signal(s) detected). *Id.* at 12:63-13:14. Thus, using this encoding scheme, the presence or absence of up to seven different analytes can be detected using probes with four fluorophores.

62. In this approach, however, certain combinations of analytes are, absent some modification to the scheme, necessarily degenerate, i.e., result in ambiguous signals, because different combinations of analytes result in the same cumulative signal measurement. For example, the presence of analytes A and F will yield cumulative signals of 2 in blue, 1 in green, 1 in yellow, and 1 in red. The presence of analytes C and D will similarly yield cumulative signals of 2 in blue, 1 in green,

1 in yellow, and 1 in red. Likewise, a sample with analytes B and G will yield the same signal as a sample with analytes D and F, as both samples will produce 3 blue, 1 green, 2 yellow, and 1 red. To address these potentially degenerate signals, where the signals are ambiguous, the '921 patent suggests nothing more than ignoring one or more analytes, removing them from the coding scheme to reduce or eliminate this degeneracy. *Id.* at 14:35-38, Table 4.

C. Prosecution History of the '921 Patent

63. I have reviewed the prosecution history for the '921 patent and include a brief summary of relevant actions below. I do not purport to be an expert in the process of patent examination, but for the purposes of my opinions I wished to have a general understanding of the history of how the '921 patent came to be issued.

64. The '921 patent issued on November 28, 2023, from U.S. Patent Application No. 16/864,744 (the "'744 Application"), filed May 1, 2020, by applicant California Institute of Technology ("CalTech"). EX1001 at codes (21), (22), (45), (71). The '744 Application was a continuation of U.S. Patent Application No. 15/914,356, filed on March 7, 2018, which was a continuation of U.S. Patent Application No. 14/451,876, filed on August 5, 2014, which was a continuation of U.S. Patent Application No. 13/756,760, filed February 1, 2013 (now Patent No. 8,838,394), which claims the priority benefit of U.S. Provisional Application

Nos. 61/594,480, filed February 3, 2012, and 61/703,093, filed September 19, 2012.

Id. at code (63), (60), 1:6-14.

65. I understand that during prosecution of the '744 Application, the patent examiner identified various bases for rejecting claims, including subject matter ineligibility, anticipation, obviousness, and indefiniteness. Eventually, the examiner allowed amended claims that were issued as '921 patent claims. EX1006.

VII. PERSON OF ORDINARY SKILL IN THE ART

66. I understand that whether a patent is invalid as obvious in light of the prior art is considered from the perspective of a person of ordinary skill in the art ("POSA") at the time of the alleged invention. I am knowledgeable about what the level of ordinary skill in the art would have been at the time of the alleged invention based on my education and experience.

67. In determining the definition of a POSA, I have considered the following factors: (1) the level of education and experience of those in the field; (2) the complexity of the technology at issue; (3) the rate of innovation; (4) the kinds of problems faced by those in the art; and (5) solutions to such problems known in the prior art.

68. In my opinion, a POSA would have been someone with a Ph.D. in a relevant field, such as molecular biology, genetics, biochemistry, or a related

discipline, with at least a year of experience in the use and development of genetics and genomic approaches for detecting and monitoring human diseases, such as multiplex detection of target analytes in a sample via quantitative and digital PCR. Alternatively, a POSA would have been someone with greater relevant experience that has an equivalent understanding of approaches used in the field without the same amount of formal education.

69. A POSA would have a strong understanding and/or experience with the design and application of various assays to detect polynucleotide sequences. Specifically, this includes the following techniques:

- Multiplex PCR and its application in detecting and measuring polynucleotide analytes. *See, e.g.*, EX1017 ¶ 5 (“the ability to perform and quantify multiple amplifications simultaneously within the same reaction volume (multiplexing) is paramount for both PCR and qPCR.”); *id.* ¶ 7 (“The invention generally relates to the manipulation of nucleic acid in droplets, and in particular, nucleic acid amplification and detection.”); *id.* ¶ 25 (“FIG. 5A is a schematic representation of a droplet having 5 sets of primers for PCR amplification of a template sequence and 5 probes, each labeled with a fluorescent dye, that binds specifically to the amplified sequences”).

- The design and use of fluorescent-labeled oligonucleotide probes, including use of quenchers, and use in conjunction with multiplex PCR. *See, e.g., id.* ¶ 93 (“The invention is not limited to the TaqMan assay, as described above, but rather the invention encompasses the use of all fluorogenic DNA hybridization probes, such as molecular beacons, Solaris probes, scorpion probes, and any other probes that function by sequence specific recognition of target DNA by hybridization and result in increased fluorescence on amplification of the target sequence.”); *id.* ¶ 153 (“Adding extra colors would increase the capability even further, however with some diminishing returns because the fluorescence of the empty droplets would continue to rise. The capacity could be yet further increased with better probes yielding larger differential signals, such as hybrid 5'-nuclease/molecular beacon probes that reduce background by contact quenching yet exhibit the bright signals typical of free unquenched fluorophores. With such improvements multiplexing capacity exceeding 50x can be envisioned.”).
- The quantitative detection and measurement of polynucleotide analytes using fluorescent-labeled oligonucleotide probes in multiplex applications. *See, e.g., id.* ¶ 5 (“the need for higher throughput in analyzing multiple

targets in parallel continues to escalate in the fields of genomics and genetics.”); *id.* ¶ 108 (“An example demonstration of droplet-based amplification and detection of multiple target sequences in a single droplet is shown here. Multiple copies of 5 sets of primers (primers for TERT, RNaseP, Ela, SMN1 and SMN2) were encapsulated in a single droplet at various concentrations along with the template DNA and the PCR master mix.”).

- Techniques for labeling probes and detecting multiple targets using fewer differently colored fluorophores than the number of polynucleotide targets. *See, e.g., id.* ¶ 121 (“Methods of the invention involve performing accurate quantitation of multiple different DNA targets by dPCR using probes with the same fluorophore.”); *id.* (“As shown in Panel A of FIG. 9, a template DNA is amplified with three sets of primers: forward primers (F1, F2 and F3) and reverse primers (R1, R2 and R3). Probes (P1, P2 and P3) are labeled with fluorophores (color 1, color 2 and color 1) and bind to the target genetic sequences (target 1, target 2 and target 3) (Panels B and C).”); *id.* ¶ 140 (“a single droplet might contain four different probes for measuring three different targets (say, T1, T2, and T3). Two probes

might be of color A (say A1, and A2), and two probes might be of color B (say, B1 and B2).”).

- Quantitative PCR (qPCR) and digital PCR (dPCR) and their application in detecting and measuring polynucleotide analytes, including in multiplex applications. *See, e.g., id.* ¶ 6 (“The principle advantage of digital compared to qPCR is that it avoids any need to interpret the time dependence of fluorescence intensity-an analog signal-along with the main underlying uncertainty of non-exponential amplification during early cycles.”); *id.* ¶ 129 (“Another advantage of the dPCR approach for multiplexed reactions is that the different reactions do not compete with each other for reagents as they would in a bulk qPCR assay.”).

VIII. THE PARALLEL DISTRICT COURT LITIGATION

70. I have been informed that Patent Owner is asserting the '921 patent, along with related patents, in a district court litigation against Petitioner. *In re ChromaCode Litigation*, Case No. 5:23-cv-04823-EKL (N.D. Cal., Filed October 5, 2023). I have been informed that Patent Owner accuses several of Petitioner’s digital PCR assays and kits of infringement. I have reviewed Patent Owner’s infringement allegations in its infringement contentions. *See* EX1012.

71. The accused assays and kits include simple assays that detect three target sequences using only two fluorophores to generate different color signals. *See, e.g., id.* at 10 (“In the SARS-CoV-2 Kit, N1 is encoded as FAM positive, N2 as FAM and HEX positive, and RP as HEX positive.”), 11 (“In the SARS-CoV-2 Wastewater Quantification Kit, N2 is encoded as FAM positive, E as FAM and HEX positive, and NHV as HEX positive.”), 14, 21, 25, 30-33, 38, 50, 52-53. For example, Patent Owner contends, consistently throughout its infringement contentions, that coding schemes fitting this description and as shown below satisfy the limitations of the ’921 patent to constitute infringement:

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

id. at 10 (depicting coding scheme in SARS-CoV-2 Kit);

Analyte	FAM	HEX
N2	1	0
E	1	1
MHV	0	1

id. at 11 (depicting coding scheme in SARS-CoV-2 Wastewater Quantification Kit (“Wastewater Kit”).

72. As I described above, *see supra* Sections V.C-D, and describe in detail below, *see, e.g., infra* Sections IX.A.2, XI.A.1-4, the accused droplet digital PCR (“ddPCR”) assays allow detection of targeted analytes in a sample solution. This is done by generating droplets from the sample solution, where each droplet may contain one or more of the polynucleotide targets along with the necessary reagents for amplification of each of the target analytes and generating the detectable color signal. This PCR amplification is done within the droplet to generate the detectable signals and, specifically, the probes are labeled as such: probes for target 1 are labeled with color 1; probes for target 2 are labeled with color 2; and probes for target 3 are labeled with colors 1 and 2. During DNA extension, as discussed, the attached probes are cleaved and the fluorescence is detected. Target 3 will have a multi-colored burst, differentiating it from targets 1 and 2. *See supra* Sections V.C-D; *see infra* Sections IX.A.2, XI.A, I, L, XIII.A, E.

73. As an example, one of the accused assays/kits is the SARS-CoV-2 Kit for ddPCR. *See, e.g.,* EX1012 at 10. In the SARS-CoV-2 Kit, there are three targeted DNA sequences (genes): N1, N2 (both are 2019-nCoV genes), and RP (human RNase P gene). *Id.* at 3, 10. The probes for these genes are sequence-specific, and are labeled with the FAM and HEX fluorophores, specifically, probes for N1 are labeled with the FAM fluorophore, probes for RP are labeled with the

HEX fluorophore, and probes for N2 are labeled with both fluorophores. *Id.* at 10.

Patent Owner alleges that “each analyte is encoded as a value of one component of a fluorescence signal (corresponding to a specific wavelength as defined by the fluorophore...)” and creates a coding scheme “as follows”:

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

Id. (depicting coding scheme in SARS-CoV-2 Kit).

74. Based on these contentions, Patent Owner alleges that the '921 patent is infringed by these assays or kits that perform the following: generating droplets from a sample solution, amplifying targets in the droplets, generating signals for detection, and monitoring those signals in order to unambiguously detect three analytes using two fluorophores without mass spectrometry or immobilization techniques. *See id.* at 3, 9-10, 16 (“In the SARS-CoV-2 Kit, the fluorescence signal comprises two components as discussed in [1a] (i.e., FAM channel to detect FAM fluorophores and HEX channel to detect HEX fluorophores). Thus, F=2.”), 21 (“Based on the cumulative intensity measurements and as stated above, the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes”), 25-26, 27-29 (“The analytes are amplified in the single

sample volume. For example, the single sample is partitioned into individual droplets.”).

75. Another example assay/kit alleged to infringe the '921 is the SARS-CoV-2 Wastewater Kit. *See, e.g., id.* at 10-11. This ddPCR assay similarly pairs analytes with fluorophores, analyte N2 with FAM fluorophore, analyte MHV with HEX fluorophore, and analyte E with both FAM and HEX fluorophores. *Id.* at 11 (“In the SARS-CoV-2 Wastewater Quantification Kit, N2 is encoded as FAM positive, E as FAM and HEX positive, and NHV as HEX positive.”). In accusing this assay of infringement, Patent Owner alleges that “each target (*i.e.*, the analyte) is associated with a fluorophore (corresponding to a specific wavelength)....” and creates a coding scheme “as shown below”:

Analyte	FAM	HEX
N2	1	0
E	1	1
MHV	0	1

Id. at 10-11 (depicting coding scheme in Wastewater Kit).

IX. THE PRIOR ART REFERENCES OF THE GROUNDS

A. Larson

1. Larson is § 102(a) Prior Art

76. Larson et al., US 2011/020597 A1, entitled “Digital Analyte Analysis” (“Larson”), is the publication of U.S. Patent Application No. 13/026,120, published on October 13, 2011. EX1017 at codes (10), (21), (43), (54). I have been informed by counsel that because Larson was published prior to the earliest priority date claimed by the ’921 patent, Larson is prior art to the ’921 patent.

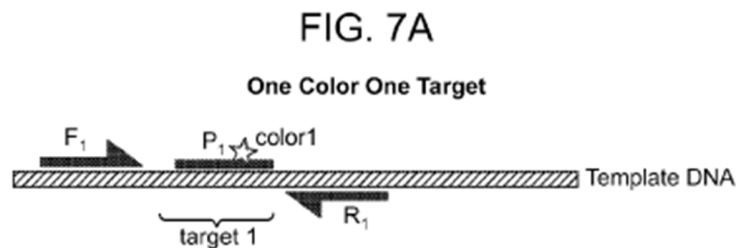
2. Larson Overview

77. Larson teaches a droplet based digital PCR (dPCR) method for analyzing a target nucleic acid. *Id.* at Abstract. The summary describes the invention as generally relating “to the manipulation of nucleic acid in droplets, and in particular, nucleic acid amplification and detection.” *Id.* ¶ 7. The methods disclosed in Larson allow for dPCR detection of greater numbers of polynucleotide analytes than unique color labels are used to perform such detection. *Id.* at Abstract, ¶¶ 119-122, 138-140.

78. Larson discloses forming sample droplets where “some droplets contain zero target nucleic acid molecules, some droplets contain one target nucleic acid molecule, and some droplets may or may not contain multiple nucleic acid molecules.” *Id.* ¶ 52. Larson further describes assays using both one set of droplets

formed from the sample fluid, and a second set of droplets containing reagents for PCR (such as Taq polymerase, nucleotides, primers, and labeled probes, which can be used to generate detectable signals). *Id.* ¶ 68. The two sets of droplets can be merged so that each resulting merged droplet contains both nucleic acid sample and all reagents needed for PCR. *Id.* PCR is then conducted within the merged droplets to generate amplified products. *Id.* ¶ 69; *see also id.* ¶¶ 47, 74, 97, 113, 132-134.

79. Larson teaches examples wherein different color labels are simply used for each polynucleotide analytes. For example, Larson teaches an assay where there is “a single labeled probe specific for an individual target.” *Id.* ¶¶ 119-120, Figs. 7A, 8A. Figure 7 depicts a single-plex assay:



Id. Fig. 7A. As shown, a template DNA is amplified with a forward primer (marked as F1) and a reverse primer (marked as R1). *Id.* ¶ 119. A probe (P1) is labeled with a fluorophore (the star marked “color1”) and binds to the target sequence. *Id.* The fluorophore on probe P1 is released PCR during extension of forward primer F1. *Id.*

A laser is used to excite the released fluorophore and the emission signal is detected accordingly. *Id.*; *id.*, Figs. 7B, 7C.

80. Larson teaches applying this approach to multiple targets wherein each is detected with specific probes labeled with different fluorophores, i.e., different colors, as depicted in the Figure 8A:

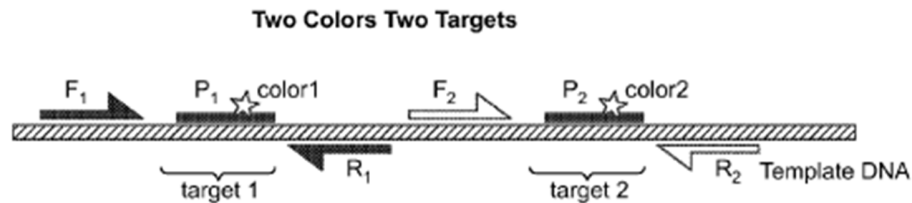
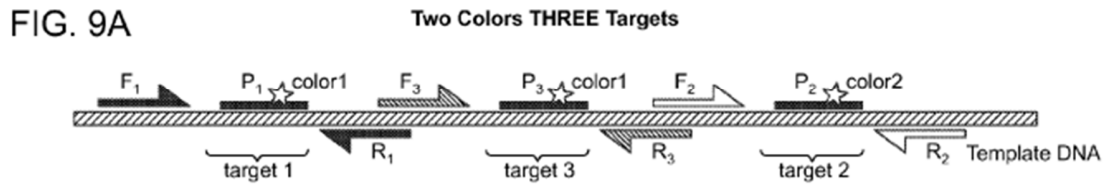


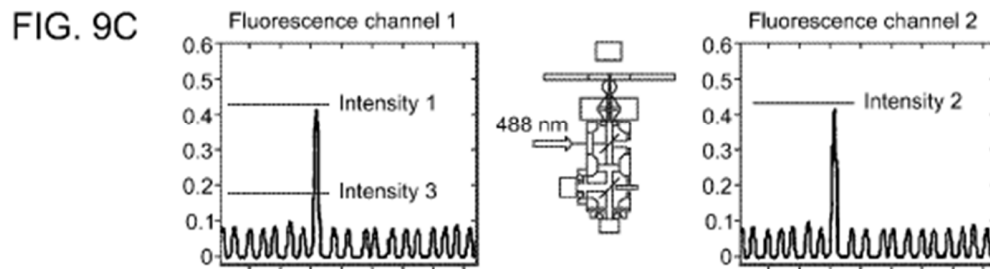
FIG. 8A

Id. Fig. 8A. As depicted, each two sets of primers (F_1/R_1 and F_2/R_2) are used to amplify two targets (target 1 and target 2), which are detected with two different probes (P_1 and P_2) with different colors (color1 and color2). *Id.* ¶ 120.

81. Larson also teaches other approaches to multiplexing. First, different intensities of the same color can be used to identify different targets. *Id.* ¶ 138. This is illustrated in Figure 9A:



Id., Fig. 9A. In the figure, targets 1 and 3 are both detected using probes labeled with the same color, while target 2 is detected using probes or a second color. *Id.* ¶ 121. As shown in the figure below, two separate intensities are detected in one color, allowing determination of the target present in the droplet:



Id., Fig. 9C. According to Larson, this example can be scaled up, such that the maximum number of targets “is related to the resolution of different intensity levels.” *Id.* ¶ 138. Larson states that the number of intensity levels “0, or 1, or 2, or 3, or 4, or up to 10, or up to 20, or up to 50, or up to 100,” or “can be higher than 100.” *Id.*

82. A second approach is that in addition to multiple intensities of a single color, “multiple colors that are spectrally separable can be used simultaneously.” *Id.*

¶ 139. In this approach, multiple targets are “identified based on intensity.” *Id.*

Larson provides an example of how this might work:

For example, a single droplet might contain four different probes for measuring four different targets. Two probes might be of color A with different intensities (say, A1 and A2), and the other two probes of color B with different intensities (say B1 and B2). The corresponding targets are T1, T2, T3, and T4 for A1, A2, B1, and B2 respectively. If a droplet shows an increase in fluoresce in color A, the droplet therefore contained either targets T1 or T2. Then, based on the fluorescence intensity of color A, the target could be identified as T1 or the target could be identified as T2. If, however, a droplet shows an increase in fluorescence in color B, the droplet therefore contained either targets T3 or T4. Then, based on the fluorescence intensity of color B, the target could be identified as T3 or the target could be identified as T4.

Id. The number of colors that can be used can be “1, or 2, or 3, or 4, or up to 10, or up to 20,” and “can be higher than 20.” *Id.*

83. A third approach is to utilize a “unique signature” of “both colors and intensities” to identify each target. *Id.* ¶ 140. Larson likewise provides an example of this approach:

For example, a single droplet might contain four different probes for measuring three different targets (say, T1, T2, and T3). Two probes might be of color A (say, A1, and A2), and two probes might be of color B (say, B1 and B2). T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2. Thus, when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B. However when a droplet contains T3, increased fluorescence appears in both colors A and B.

Id. Thus, multiplexing can be achieved with combinations of intensities and colors.

Id.

B. Saxonov

1. Saxonov is § 102(e) Prior Art

84. Saxonov et al., US 2013/0040841 A1, entitled “Digital Assays With Multiplexed Detection of Two or More Targets in the Same Optical Channel” (“Saxonov”), is the publication of U.S. Patent Application No. 13/548,062 (the “’062 Application”), published on February 14, 2013. EX1004 at codes (10), (21), (43), (54). The ’062 Application was filed on July 12, 2012, and claims priority benefit of Provisional Application No. 61/507,082 (filed July 12, 2011) and 61/510,013 (filed July 20, 2011). *Id.* at codes (22), (60). I understand that Saxonov was identified by Applicant during prosecution of the ’744 Application that resulted in the ’921 patent as well as during the prosecution of related applications that resulted

in U.S. Patent Nos. 10,068,051 and 10,770,170. EX1005 at 1; EX1015 at 2; EX1016 at 11.

85. I have been asked to opine on whether Provisional Application No. 61/510,013 (the “’013 Provisional Application”) contains adequate written description support of what Saxonov identifies as its invention. In my opinion, the ’013 Provisional Application discloses, in its specification and claims, substantially the same subject matter as Saxonov, with Saxonov amplifying the disclosure, including adding illustrative examples and figures supported by the ’013 Provisional Application. The table below, for example, identifies written description support in the ’013 Provisional Application for each and every element of Saxonov’s claim 1:

Saxonov	’013 Provisional Disclosure
1. A method of performing a digital assay, comprising:	“The present disclosure provides a system, including methods and apparatus, for performing a digital assay with multiplexed detection of two or more distinct targets in the same channel.” EX1007 at 3:6-8; <i>id.</i> at 3:10-14, 4:17-20, Figs. 1-2.
amplifying two or more targets in droplets containing a reporter for each target;	“The sample may be separated into partitions” (<i>id.</i> at 7:11); “preparation of the sample may render the sample (or partitions thereof) capable of amplification of each of one or more targets, if present, in the sample (or a partition thereof)” (<i>id.</i> at 7:3-5) “the partitions may be droplets” (<i>id.</i> at 7:17); “reactions may be performed in

Saxonov	'013 Provisional Disclosure
	the partitions” (<i>id.</i> at 8:11); “the reaction may be an amplification reaction” (<i>id.</i> at 8:19); “[s]ignals may be created for each of the partitions ... from reporters for two or more distinct targets” (<i>id.</i> at 9:4-8)
detecting a signal from the droplets representing combined emission of light from the reporters for the targets, wherein each reporter provides a portion of the signal having an intensity that varies according to whether or not the corresponding target is present in a droplet, and wherein a plurality of the droplets contain more than one target and have a distinguishable intensity of the signal relative to droplets containing only one target;	“[s]ignals may be created for each of the partitions” (<i>id.</i> at 9:4); “the signal may be a composite signal that represents two, three, four, or more reactions and thus two, three, four, or more targets” (<i>id.</i> at 9:14-15); “[t]he signal may be analyzed to determine whether neither Target, Target 1 alone, Target 2 alone, or both Targets 1 and 2 are present in each droplet” (<i>id.</i> at 15:17-19); “assignment of a droplet to a particular outcome (i.e., to one of T1-/T2-, T1+/T2-, T1-/T2+, and T1+/T2+) may be performed using any suitable algorithm” (<i>id.</i> at 16:26-27); “each droplet, whether positive or negative for each target, produces an increase in signal strength above the baseline signal that forms an identifiable peak” (<i>id.</i> at 16:21-23); <i>id.</i> at 12:17-20:22 (Examples 1-4)
determining for each target a number of droplets that are positive for only one of the targets;	“signal is created from light detected over time in a single channel from a fluid stream containing the droplets and flowing through an examination region of the channel. The signal may be analyzed to determine whether neither Target, Target 1 alone, Target 2 alone, or both Targets 1 and 2 are

Saxonov	'013 Provisional Disclosure
	present in each droplet” (<i>id.</i> at 15:15-19); “assignment of a droplet to a particular outcome (i.e., to one of T1-/T2-, T1+/T2-, T1-/T2+, and T1+/T2+) may be performed using any suitable algorithm” (<i>id.</i> at 16:26-27); “thresholding may be neither simple nor linear. In such cases, statistical methods such as expectation maximization algorithms may be used to estimate the number of droplets or peaks associated with each outcome” (<i>id.</i> at 16:32-17:2); <i>see also</i> Example 3 (<i>id.</i> at 17:4-19:23), <i>id.</i> at 18:5-19:1 (disclosing digital PCR assay that includes determining number of droplets positive for only target for both RPP30 and C61 targets, populations 3 and 2, respectively); “droplets that included the RPP30 gene but did not include the C61 gene” (<i>id.</i> at 18:16-17); “droplets that included the C61 gene but did not include the RPP30 gene” (<i>id.</i> at 18:11-12).
determining at least one number of droplets that are positive for more than one target; and	“signal is created from light detected over time in a single channel from a fluid stream containing the droplets and flowing through an examination region of the channel. The signal may be analyzed to determine whether neither Target, Target 1 alone, Target 2 alone, or both Targets 1 and 2 are present in each droplet” (<i>id.</i> at 15:15-19); “assignment of a droplet to a particular outcome (i.e., to one of T1-/T2-, T1+/T2-, T1-/T2+, and T1+/T2+) may be performed using any

Saxonov	'013 Provisional Disclosure
	suitable algorithm” (<i>id.</i> at 16:26-27); “thresholding may be neither simple nor linear. In such cases, statistical methods such as expectation maximization algorithms may be used to estimate the number of droplets or peaks associated with each outcome” (<i>id.</i> at 16:32-17:2); <i>see also</i> Example 3 (<i>id.</i> at 17:4-19:23), <i>id.</i> at 18:5-19:1 (disclosing digital PCR assay that includes determining number of droplets positive for both RPP30 and C61 targets, population 4); “droplets that are positive for RPP30 and C61 (i.e., droplets that included both genes” (<i>id.</i> at 18:19-20).
determining a quantity representative of a level of at least one target.	“<u>Example 3</u> [titled ‘Digital PCR Assay to Assess Copy Number of C61’] ... describes a first exemplary digital PCR assay, in which multiplexing in a single channel is used to assess copy number of the C61 gene” (<i>id.</i> at 17:4-6); “There are 4033 droplets containing RPP30 (i.e., that are positive for RPP30, irrespective of whether they are positive or negative for C61), as determined by adding the number of droplets in Populations 3 and 4 (i.e., by adding 2865 and 1168, respectively). There are 4022 droplets containing C61 (i.e., that are positive for C61, irrespective of whether they are positive or negative for RPP30), as determined by adding the number of droplets in Populations 2 and 4 (i.e., by adding 2854 and 1168, respectively). Thus, the ratio of C61 to RPP30 is $4022/4033 = 0.997 = 1:1$

Saxonov	'013 Provisional Disclosure
	within experimental error. Thus, because RPP30 is known to have two copies per genome, C61 must also have two copies (i.e., the copy number of C61 is two).” (<i>id.</i> at 19:1-9)

86. In sum, each and every limitation of claim 1 of Saxonov is disclosed by the '013 provisional. Thus, the '013 provisional provides written description support for the invention claimed in Saxonov. Accordingly, I understand from counsel that Saxonov is § 102(e) prior art as of the '013 Provisional Application's filing date of July 20, 2011.

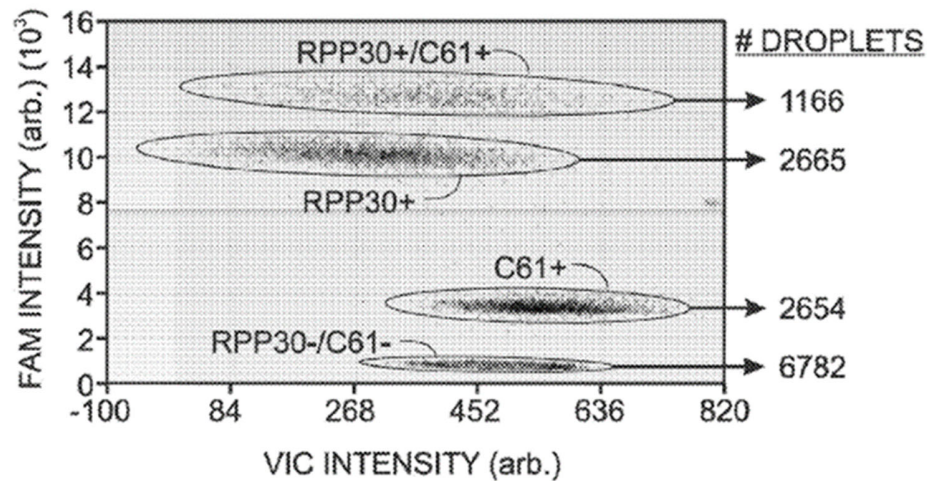
2. Saxonov Overview

87. Saxonov is directed to the performance of assays using multiplexed detection of signals from labeled probes for two or more targets where the different targets can share colors (fluorophores) that are the probe label. EX1004 ¶¶ 19, 20, 37. Saxonov discloses the same types of coding schemes as the '921 patent to enable the multiplexed detection of analytes such that there are more analytes than the number of colors used.

88. For example, Saxonov teaches the multiplexed detection of signals from labeled probes for two or more targets where the labels for different targets can share colors. EX1004 ¶¶ 19, 20, 37 76-79. Specifically, Saxonov discloses assays

wherein two targets are detected in any combination of presence using a single unique color label. *See, e.g., id.* ¶¶ 63-67, Fig. 5.

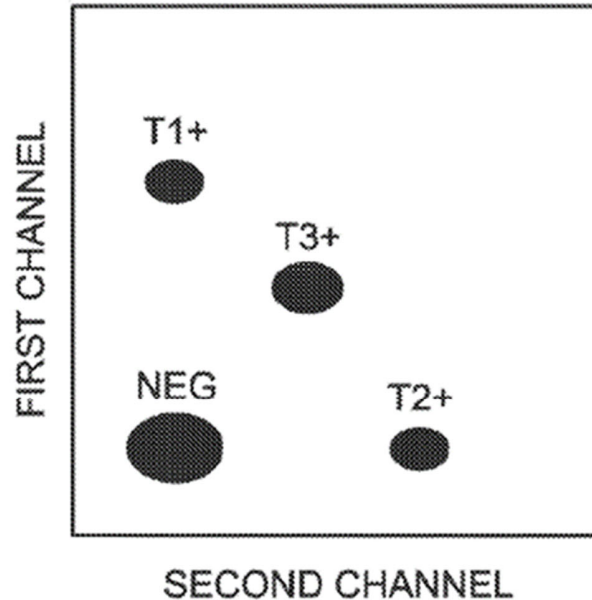
Fig. 5



Id. at Fig. 5.

89. As an additional example, Saxonov further teaches assays wherein three targets are detected in any combination of presence or absence using two unique color labels. *See, e.g., id.* ¶¶ 76-79, Figs. 9-10.

Fig. 10



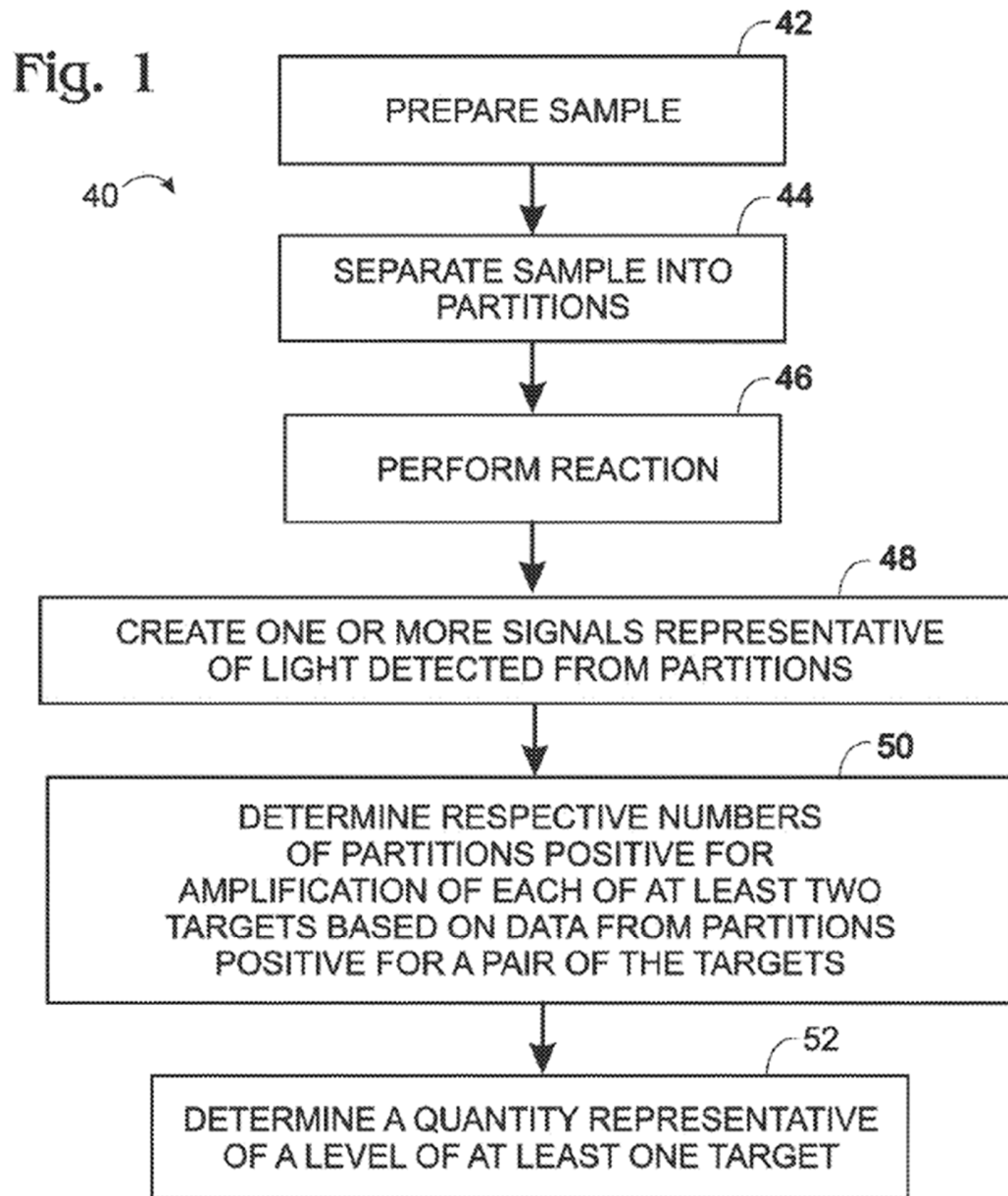
Id. at Fig. 10. The provisional application to which Saxonov claims priority to also teaches this method (the “’013 Provisional”), stating that signals can be “collected in one or more different channels” from “the same” target. EX1007 at 9:8-10. As further described in the ’013 Provisional, “[t]he signals may represent aspects of light, such as the intensity of the light, detected in the same channel (i.e., in the same wavelength range (color regime) such as for FAM or VIC) from reporters for two or more distinct targets. The signals optionally may include data collected in one or more different channels (i.e., in different wavelength ranges (color regimes) from reporters for the same and/or different targets).” *Id.* at 9:5-10. Thus, as Figure 10

shows, signals are detected in two channels—FAM and VIC. In addition, “[t]he signals may be distinguishable because an aspect of the fluorescence is different for one fluorophore than for the other fluorophore(s).” *Id.* at 14:6-10 (“For example, the intensity associated with one fluorophore, following reaction, may be lower or higher than the intensity(ies) associated with the other fluorophore(s).”). Thus, signals from probes for different analytes can be distinguished from each other because of differences in intensity and/or colors, as is the case in Figure 10, where T1 is detected with one color, T2 is detected on another color, and T3 is detected at different intensities than T1 and T2. This allows the detection of three targets in two channels.

90. Saxonov discloses a “system, including methods and apparatus, for performing a digital assay on a potentially greater number of targets through multiplexed detection of signals from reporters for two or more distinct targets in a common or shared channel (‘the same optical channel’).” EX1004 ¶ 19. An optical channel, as used in Saxonov, “represents a particular detection regime with which emitted light is generated and detected.” *Id.* ¶ 37. In other words, two optical channels are different when there are differences in the “wavelength/waveband of excitation light,” “wavelength/waveband of emitted light that is detected,” “the time

interval during which emitted light is detected relative to each pulse of excitation light,” or other differences. *Id.*

91. In Figure 1, Saxonov provides a flowchart depicting the steps of the method:



Id. Fig. 1.

92. Saxonov discloses that the sample may be partitioned; for example, “partitions may be droplets disposed in a continuous phase, such that the droplets

and the continuous phase collectively form an emulsion” (*id.* ¶ 28), where “[e]xemplary partitions...are monodisperse droplets.” (*Id.* ¶ 29).

93. After partitioning, reactions can be performed in the partitions, such as in droplets. *Id.* ¶¶ 32, 28-29. The reaction performed can be a polymerase chain reaction (PCR), including quantitative PCR using a fluorophore. *See id.* ¶¶ 33-34. The reaction may involve target and a binding partner, *e.g.*, a target molecule (that functions as a PCR template) and a probe (that binds the template and generates a signal upon PCR extension). *See id.* ¶ 32.

94. Droplets with a generated signal are marked/scored positive for a given target that corresponds to the signal, while droplets without that signal are marked negative, meaning a given target is not in the droplet. *Id.* ¶ 41. From this information, the number of partitions/droplets that are positive or negative for each target can be determined. *Id.*

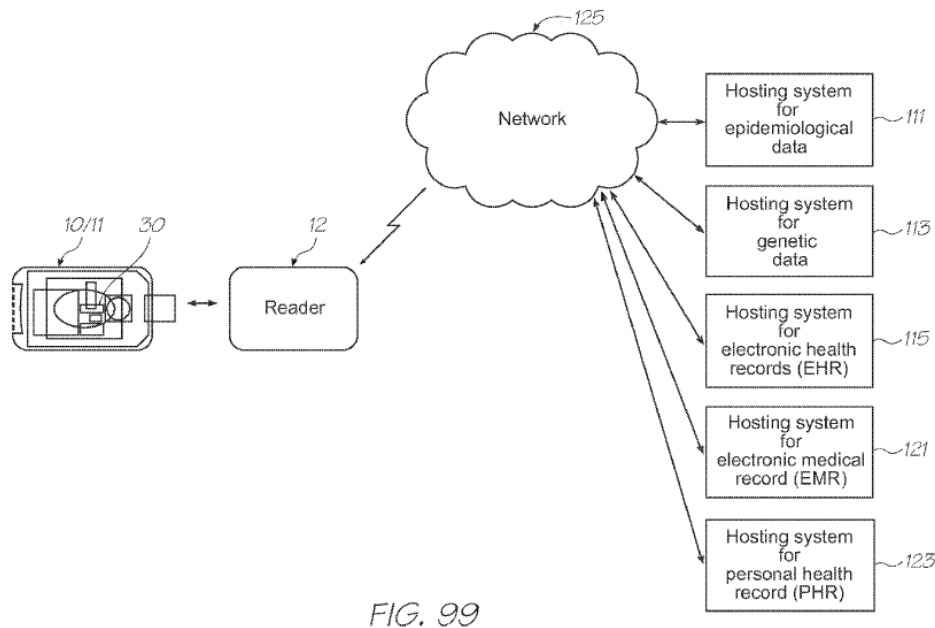
95. In addition to determining the presence or absence of a signal, the quantity of each target may be determined “based on the respective numbers of partitions positive for the target alone and for the target in combination with any other target(s).” *Id.* ¶ 43. This partition data may be used to estimate copy number variation. *Id.* This information may be further used to determine the amount of a given target that is present in the original sample. *Id.*

96. In addition, regardless of the mode of detection (i.e., detection via one color or via multiple colors), Saxonov further teaches that these principles may be used to detect fluorescence intensity with the same sensor, i.e., in the same color or channel, at the same time from different labeled probes and that the composite signal may represent “two, three, four, or more targets.” EX1004 ¶ 36; EX1007 at 9:11-15.

C. Silverbrook

97. Silverbrook et al., US 2011/0312704 A1, entitled “Microfluidic Device for PCR Amplification Using Low PCR Mixture Volume” (“Silverbrook”), is the publication of U.S. Patent Application No. 13/150,037, published December 22, 2011. EX1009 at codes (10), (21), (43), (54). I have been informed by counsel that because Silverbrook was published prior to the earliest priority date claimed by the ’921 patent, Silverbrook is prior art to the ’921 patent.

98. Silverbrook teaches a nucleic acid testing device and methods that employ PCR and hybridization to identify which analytes are present in a given sample and transmitting results of such tests through a computer network. *Id.* ¶¶ 195-201, Figs. 96-99.



Id. Fig. 99.

99. For example, as shown in Figure 99 above, Silverbrook teaches the transmission of “data generated by the test module” to update, for example, “electronic health records hosted on the hosting system for electronic health records.” *Id.* ¶ 201.

100. The data detected by the methods and modules taught by Silverbrook generally relates to biological applications, including the rapid identification of diseases such as influenza, pneumonia, tuberculosis, typhoid, Ebola, and HIV among others. *Id.* ¶¶ 207-243.

101. Relatedly, Silverbrook teaches that quantification of the starting amount of different nucleic acid targets in samples from patients is “particularly

useful in molecular diagnostics where treatment options may differ depending on the pathogen load.” *Id.* ¶ 34. This information may be particularly beneficial when applied to clinical and healthcare decisions. *See id.* ¶¶ 55-62.

X. CLAIM CONSTRUCTION

102. I do not believe any express claim construction is required to determine that the ’921 patent claims are invalid because the prior art teaches and/or discloses the claim limitations under the apparent plain and ordinary meaning of the limitations as according to Patent Owner.

103. In my analysis below, I have applied constructions consistent with those advanced by Petitioner and/or Patent Owner in parallel litigation. *See* EX1003. These terms include the following identified by Petitioner with proposed constructions:

- “degenerate” or “degeneracy”: “a situation where a legitimate result is not definitive, because it can indicate more than one possibility in terms of the presence or absence of an analyte.” *Id.* at 9; ’921 patent, claims 4, 13, 14. Also, conversely, “non-degenerately.” EX1003 at 9.

104. Where there are other terms with constructions advanced by Patent Owner in parallel litigation, including apparent interpretations such as those in

Patent Owner's infringement contentions (*see generally* EX1012), I have applied constructions consistent with them, as noted below where necessary.

105. I reserve the right to provide express construction(s) later in this proceeding in response to any explicit claim constructions or claim limitations that are contrary to a term's reasonable construction. My opinion may change if I am informed that the Court has construed any claim term in the '921 patent.

XI. GROUND 1: CLAIMS 1-10 AND 13-19 ARE ANTICIPATED BY LARSON

106. The '921 patent claims an assay for purportedly detecting the presence or absence of at least M analytes based on detection of F wavelength components of fluorescent signals wherein M is greater than F, and a kit for the same. EX1001 at claims 1, 14. The fluorescent signal has F wavelength components, which can be, for example, color or intensity. *Id.* at claims 1, 2.

107. At its core, the '921 patent relies on nothing more than the concept of detecting a number of targets that exceeds the number of available color signals simply by mixing color signals and/or using different signal intensities to provide composite signals that unambiguously identify which analytes are present in a sample. To achieve this unambiguous identification, as recognized in the art, the coding scheme must be such that the combination of colors and intensities assigned to each analyte is unambiguous. In other words, the coding scheme unambiguously

indicates the presence of specific (targeted) analytes, i.e., it is non-degenerate, because the scheme is such that different combinations of color signals and signal intensities corresponding to different combinations of the polynucleotide analytes can always be resolved without uncertainty as to which analytes are present. As explained below, however, a POSA would have already known about both of these concepts (both alone and in combination) prior to 2012.

108. Larson is similar to the '921 patent, as it is also directed to the multiplex detection of analytes using probes, including in circumstances where there are more analytes to be detected than the total number of colors used to label probes. For example, Larson discloses that “[m]ethods of the invention are able to uniquely detect multiple targets with the same optical color.” EX1017 ¶ 15. Larson’s Figure 9 depicts this analysis, “a template DNA is amplified with three sets of primers: forward primers (F1, F2 and F3) and reverse primers (R1, R2 and R3). Probes (P1, P2 and P3) are labeled with fluorophores (color 1, color 2 and color 1) and bind to the target genetic sequences (target 1, target 2 and target 3) (Panels B and C).” *Id.* ¶ 121.

109. In my opinion, accordingly, Larson is analogous art to the '921 patent because the two are within the same field of endeavor: multiplex detection of polynucleotide analytes using color-labeled sequence-specific probes, including

where there are fewer different colors than analytes, i.e., more analytes than colors. EX1001 at Abstract, 12:36-13:55, 18:10-13, 22:9-23:67; EX1017 ¶¶ 15, 29, 32, 121, 135, 140.

110. It is my opinion claims 1-10 and 13-19 of the '921 patent are anticipated by Larson. As discussed below, Larson discloses the methods and kits in claims 1-10 and 13-19.

A. Claim 1

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes

111. A POSA would have understood, as an initial matter, that “M” can be as small as two. This is because the only restriction that the claim places on the value of “M” is that it be greater than “F,” where “F” is the number of wavelength components in the assay. The value of “F,” in turn, is not restrained by a bound, and may be any positive integer, meaning that the minimum value of “M” is 2.

112. Larson teaches assays, including dPCR assays, where probes are “labeled with a fluorescent dye that binds specifically to the amplified sequences” and the assays are capable of detecting two or more analytes. *See, e.g.*, EX1001 at 26:18-28 (disclosing that the analytes are those “selected from the group consisting of any 2, 3, 4,...100 or more”); EX1017 ¶¶ 26 (describing a multiplex assay detecting five polynucleotide analytes, as shown in Figure 6), 116 (same), 28

(describing the detection of two sequences using two-color detection, as shown in Figure 8), 120 (same), 29 (describing the detection of three sequences using two-color detection, as shown in Figure 9), 121 (same), 32 (describing detecting different sequences using different signal intensity, as shown in Figure 12, including Figure 12B which shows detecting two sequences labeled with one color at different intensities), 135 (same), 140 (“T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2. Thus, when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B. However when a droplet contains T3, increased fluorescence appears in both colors A and B.”), Figs. 6, 8, 9, 12.

113. Specifically, Larson discloses an assay in paragraph 140 that describes detecting three polynucleotide analytes using two colors. This assay makes use of a single-color probes of a first color to detect the first analyte, a second single-color probe of a second color to detect the second analyte, and two probes, one of each color, to detect the third analyte. Assays of this particular format correspond to the exact type of assay that Patent Owner accuses of infringing in the district court proceedings. *See* EX1012.

114. As documented further herein, a POSA would have further understood that Larson discloses or teaches an assay capable of detecting these analytes

“unambiguously.” I detail why this is so below in connection with the additional claim elements that require “unambiguous” detection of multiple targets. *See infra* Sections XI.A.4, XIII.A.4.

2. wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,

115. It is my opinion that Larson discloses an assay “wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,” as recited in Claim 1.

116. As discussed above, Larson teaches assays that use coding schemes to differentiate multiple analytes based on fluorescent signals, wherein the fluorescent probes are specific for identifying two, three, or more different polynucleotide analytes. *See supra* Section XI.A.1; EX1017 ¶¶ 26, 28-29, 32, 116, 120-121, 135, 140, Figs. 6, 8-9, 12. For example, in discussing multi-colored signals, Larson created a coding scheme for three analytes where each analyte was encoded as a value of a wavelength component of a fluorescent signal (i.e., color). *See supra* Section XI.A.1; EX1017 ¶¶ 139-140.

117. As discussed above, this coding scheme is of the same kind that Patent Owner accuses of infringement, including as alleged to be used for the accused

SARS-CoV-2 Kit and SARS-CoV-2 Wastewater Kit products. *See supra* Section VIII; EX1012 at 2-4, 9-11.

118. For example, Patent Owner contends that in “the SARS-CoV-2 Kit, each analyte is encoded as a value of one component of a fluorescence signal (corresponding to a specific wavelength as defined by the fluorophore given below) as represented by the identified fluorophores FAM and HEX” and provides that a “corresponding coding scheme for the SARS-CoV-2 Kit is as follows:”

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

Id.

119. As shown above, in this scheme, the signal from the FAM fluorophore corresponds to target N1, the signal from the HEX fluorophore corresponds to target RP, and signals from both fluorophores corresponds to target N2.

120. As discussed, Larson teaches using two colors to detect three polynucleotide analyte targets wherein two different single-color probes are used separately for a first and a second target, and the two colors are both used together for multi-colored probe(s) for a third target. *See supra* Section XI.A.1; EX1017 ¶ 140 (describing the use of four probes for three polynucleotide analyte targets where

a probe of a first color is specific for the first analyte, a second probe of a second color is specific for the second analyte, and a third and fourth probe, labeled with the first and second color, respectively, are specific for the third analyte, to generate different single color signals for the first and second analytes and a two-color signal for the third analyte). In each of the assays in Larson, each of the fluorophore-labeled sequence-specific probes provides a fluorescent signal corresponding to the polynucleotide analyte that it binds, thus encoding the analyte as a value of the wavelength (i.e., color or a non-zero intensity) of the generated fluorescent signal. This creates a coding scheme that describes which signal(s) show detection of which polynucleotide analyte target. For example, Larson discloses:

[A] single droplet might contain four different probes for measuring three different targets (say, T1, T2, and T3). Two probes might be of color A (say, A1, and A2), and two probes might be of color B (say, B1 and B2). T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2. Thus, when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B. However when a droplet contains T3, increased fluorescence appears in both colors A and B.

Id. at ¶ 140.

121. This coding scheme as described in Larson is exactly the coding scheme that Patent Owner contends infringes the claim in district court litigation as described

above. Patent Owner contends that three analytes, detected in two colors, with one color detected in a first color, a second target detected in a second color, and a third target detected in both colors infringes claim 1 of the '921 patent. In the example, the colors or fluorophores used to detect the targets are FAM and HEX fluorophores. Thus, in the described coding scheme, F (number of colors) is 2 and M (number of targets) is 3.

122. Accordingly, a POSA would have understood that the types of assays in Larson have at least M analytes where those analytes are encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme.

3. wherein said fluorescent signal comprises F wavelength components, and

123. It is my opinion that Larson discloses assays “wherein said fluorescent signal comprises F wavelength components.”

124. A POSA would have understood that a wavelength component could be for example a color or range of colors, i.e., a specific wavelength or range of wavelengths of light. A POSA would have further understood that the “F” in “F wavelength components” could represent any positive integer, as one cannot have a negative number of colors, nor a portion of a distinguishable color, and at least one color must be used to generate any detectable fluorescent signal. Thus, F can be as small as 1.

125. As discussed above, Larson teaches assays using multiple intensities of the same color as signals, multi-color signals, and combinations of multiple intensities and multiple colors as distinguishable signals. *See supra* Section XI.A.1 (wherein I discuss how Larson discloses the use of multi-color signals and multiple intensities of the same color as distinguishable signals); EX1017 ¶¶ 26, 28-29, 32, 116, 120-121, 135, 140, Figs. 6, 8-9, 12. For example, Larson discloses an assay to detect three analytes where each polynucleotide analyte target was encoded as a value of a fluorescent signal, as one of two colors (or both for the third analyte). *See supra* Section XI.A.1; EX1017 ¶ 140.

126. As described above, a POSA would have understood Larson to teach an exemplary assay that makes use of 2 wavelength components, i.e., colors. Specifically, Larson teaches that in an assay for detecting three targets, “Two probes might be of color A (say, A1, and A2), and two probes might be of color B (say, B1 and B2).” *Id.* In the example, target 1 emits a signal in color A, target 2 emits a signal in color B, and target 3 emits a signal in both color A and color B. *Id.* Only two unique colors are used to generate the coding schema and thus, in the example, $F=2$.

127. This is the same mapping that Patent Owner accuses of infringement. Specifically, where each of the different fluorescent labels used in an assay, such as

FAM and HEX, are identified and that number of different types of fluorophores equals the number of wavelength components. EX1012 at 16-17, 9-11. For example, in the SARS-CoV-2 Kit and the Wastewater Kit, the fluorophores used are FAM and HEX, which are both types of fluorophores, so the number of F wavelength components is two (2). *Id.*

128. Accordingly, a POSA would have understood that the types of assays in Larson use fluorescent signals that comprises F wavelength components.

4. **wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,**

129. It is my opinion that Larson discloses assays wherein “said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F.”

130. In the example that I repeatedly discuss above from Larson, the disclosed assay unambiguously detected three targets (M analytes) where each analyte was encoded as one of two colors (F wavelength components), therefore M (3) is greater than F (2). *See supra* Section XI.A.1; EX1017 ¶ 140. Larson discloses conducting the digital PCR assay in paragraph 140 under conditions of terminal

dilution, where the probability of a single target molecule in droplets is much greater than, and overwhelms that of, multiple targets, in paragraph 141. Larson further describes the terminal dilution used in paragraph 6. Under the conditions of terminal dilution, the digital PCR assay in the example described in paragraph 140, the detectable signal generated for each potential analyte is distinct and readily distinguishable and, thus, provides “unambiguous” detection of the presence or absence of targets. EX1017 ¶¶ 140, 141.

131. Larson further discloses detecting whether these analyte targets are present in a “sample volume,” specifically, a portion of a sample solution that is subsequently partitioned into droplets. *Id.* ¶¶ 154 (“Using droplet-based microfluidics, multiple targets can also be measured simultaneously by a different method. According to the alternative method, primers and probes may be loaded individually into droplets along with an optical label to uniquely identify the assay.”); 164 (“The assays (probes and primers) combined with their unique optical labels were first encapsulated into droplets along with the PCR master mix.”), Figs. 1-3.

132. Furthermore, Larson teaches assays that disclose a “single sample” in the exact manner that Patent Owner alleges infringing assays and kits perform the step, by subsequently partitioning a “single sample” containing targets into droplets.

Larson teaches various means for partitioning a portion of a sample mixture into droplets. *See, e.g., id.* ¶¶ 11 (“One or more droplets are formed, each containing a single nucleic acid template and a heterogeneous mixture of primer pairs and probes, each specific for multiple target sties on the template.”), 16 (“Methods of the invention involve forming sample droplets containing target nucleic acid. Ideally, methods of the invention comprise forming droplets for digital PCR. Preferred digital PCR droplets contain one copy of a nucleic acid to be amplified, although they may contain multiple copies of the same nucleic acid sequence.”), 13-15, 17, 21-23 (describing Figs. 1-3), 52-68 (describing “Droplet Formation”), Figs. 1-3.

133. This is the same approach that Patent Owner contends is used in Petitioner’s allegedly infringing assays including, for example, the SARS-CoV-2 Kit and Wastewater Kit. EX1012 at 17-25. Patent Owner contends, for example, that for “all of Bio-Rad’s infringing ddPCR assays and kits, the detection is unambiguous in that it detects the presence of the nucleic acid target sequences based on a coding scheme where the same detection cannot represent two or more analytes.” *Id.* at 19. These accused ddPCR assays include the SARS-CoV-2 Kit and the Wastewater Kit, each include a first color for the first polynucleotide analyte target, a second color for a second analyte, and using both colors for a third analyte. *See supra* Sections XI.A.1-3; EX1012 at 21.

134. Patent Owner alleges the SARS-CoV-2 Kit meets this limitation because “the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme,” which is reproduced below:

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

Id.

135. Patent Owner alleges the Wastewater Kit meets this limitation because “the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme,” which is reproduced below:

Analyte	FAM	HEX
N2	1	0
E	1	1
MHV	0	1

Id.

136. Larson teaches assays that use a scheme with the same approach as the ones alleged to be infringing by Patent Owner. For example, Larson discloses the detection of three targets in a sample with an assay using two colors where a first target is detected using a first color, a second target is detected using a second color, and a third target is detected using both the first and second colors. EX1017 ¶ 140. Accordingly, this assay detects the presence of three targets ($M = 3$), in two colors ($F = 2$), thus M is greater than F . *Id.*

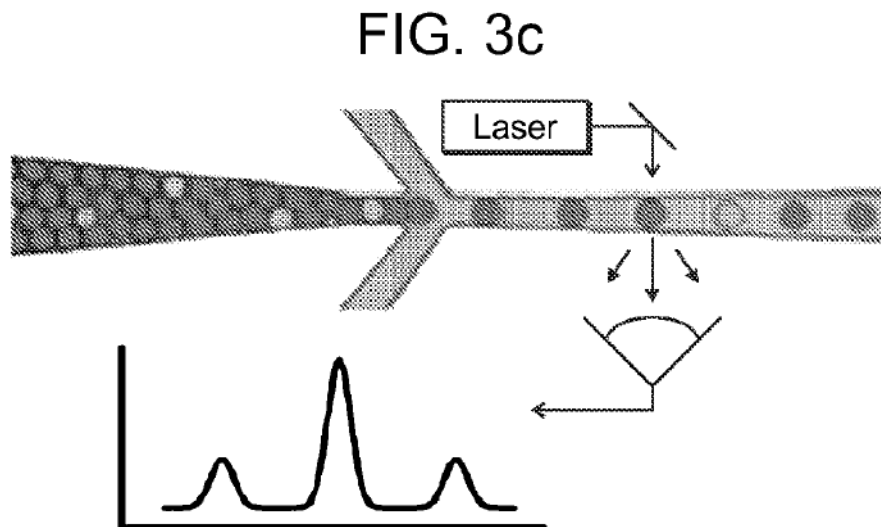
137. Patent Owner alleges that the Bio-Rad ddPCR assays and kits perform detection in a single sample volume that is partitioned into droplets. Specifically, “the detection is performed in a single *sample volume* that is *subsequently* partitioned into multiple droplets.” EX1012 at 20. Patent Owner also explains that the SARS-CoV-2 Kit and the Wastewater Kit are each “capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme.” *Id.* at 21.

138. Accordingly, a POSA would have understood that the assays in Larson unambiguously detect the presence or absence of M analytes in any combination of presence or absence in a single sample volume, relying on detection of F wavelength

components (i.e., colors) wherein the number of analytes detected is greater than the number of colors used for such detection.

5. wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and

139. It is my opinion that Larson discloses assays “wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes,” as recited in Claim 1. As discussed above, Larson teaches assays for detection of multiple polynucleotide analytes and does not require mass spectrometry or immobilization of the analytes. *See supra* ¶¶ 130-132. Specifically, Larson teaches amplification of targets, generation of color signals representative of targets, and detection of the analytes in droplets. EX1017 ¶¶ 47, 52-54, 69, 70-72, 83, 89, Fig. 1-3. This is shown best in Figure 3C, reproduced here:



Id. Fig. 3C.

140. As shown, the droplets containing the analytes with their colored probes pass through a channel illuminated by a laser, which causes the excitation and fluorescence of the probes. *Id.* This fluorescence is then detected on a droplet-by-droplet basis with no need for the sample to be immobilized nor is mass spectrometry required. *Id.* ¶¶ 47, 52-54, 69, 70-72, 83, 89, Fig. 1-3.

141. Larson's assays perform exactly the sort of amplification Patent Owner contends infringes the '921 patent. Larson's assays rely on PCR performed in droplets, which are individual partitions of the single sample volume, and include techniques which were well-known in the art at the time of the alleged invention, including the TaqMan assay. *Id.* ¶¶ 2, 9, 11, 13, 14, 16, 17, 20, 47, 49 ("Methods of the invention involve forming sample droplets containing a single target nucleic acid, ***amplifying the target in the droplets***"), 91 ("In certain aspects, the droplets of the invention contain a plurality of detectable probes that hybridize to amplicons ***produced in the droplets.***"), 93 ("The invention is not limited to the TaqMan assay, as described above, but rather the invention encompasses the use of all fluorogenic DNA hybridization probes"), 119-121, 150, 195, Figs. 1-4, 7-11, 15.

142. This is the same mapping that Patent Owner uses in its infringement contentions. Patent Owner relies on the amplification and detection of target

sequences in droplets, specifically describing the accused ddPCR assays as preparing samples, including sample with necessary reagents, then generating droplets by partitioning or “fractioninat[ing]” that sample, and then running the droplets through a thermal cycler to perform PCR amplification. EX1012 at 26. Patent Owner explains that then the droplets are read to determine the fraction of positive droplets (droplets containing a target) in the original sample. *Id.*

143. Accordingly, a POSA would have understood that the types of assays in Larson do not require the use of mass spectrometry or immobilization of any analytes.

6. wherein the analytes are amplified in the single sample volume.

144. It is my opinion that Larson discloses assays “wherein the analytes are amplified in the single sample volume,” as recited in Claim 1. *See supra* Section XI.A.5.

145. As discussed in detail above, Patent Owner contends that Petitioner’s ddPCR assays are infringing and asserts that the claimed “sample volume” is a portion of the sample mixture that is *subsequently* partitioned into droplets. *See supra* ¶ 136; EX1012 at 20. Patent Owner Asserts that Petitioner’s ddPCR products perform methods wherein “the detection is performed in a single sample volume that is subsequently partitioned into multiple droplets.” *Id.*; *see also id.* at 27 (“For

example, the single sample is partitioned into individual droplets”). Patent Owner goes on to contend the “analytes are amplified in the single sample volume” where “the single sample is partitioned into individual droplets” and that “[f]ollowing droplet generation, the sample is amplified in a thermal cycler” prior to reading “each droplet to determine the fraction of positive droplets in the original sample and us[ing] Poisson statistical formulas to determine the absolute quantity.” *Id.* at 27-29; *see also supra* ¶ 142.

146. Larson’s assays perform exactly the sort of amplification Patent Owner contends infringes the ’921 patent. Larson’s assays rely on PCR performed in droplets, which are individual partitions of the single sample volume, and include techniques which were well-known in the art at the time of the alleged invention, including the TaqMan assay. EX1017 ¶¶ 2, 9, 11, 13, 14, 16, 17, 20, 47, 49 (“Methods of the invention involve forming sample droplets containing a single target nucleic acid, *amplifying the target in the droplets*”), 91 (“In certain aspects, the droplets of the invention contain a plurality of detectable probes that hybridize to amplicons *produced in the droplets*.”), 93 (“The invention is not limited to the TaqMan assay, as described above, but rather the invention encompasses the use of all fluorogenic DNA hybridization probes”), 119-121, 150, 195, Figs. 1-4, 7-11, 15.

147. Accordingly, a POSA would have understood that the types of assays in Larson are those where the analytes are amplified in the single sample volume.

B. Claim 2: The assay of claim 1, wherein each of said at least M analytes is encoded as at least one first value in a first component of said fluorescent signal and at least one second value in a second component of said fluorescent signal.

148. As described above, Larson discloses the method of claim 1 by disclosure of an assay unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A.

149. It is further my opinion that Larson discloses the further limitations of claim 2.

150. As discussed, Patent Owner alleges that the SARS-CoV-2 Kit and the Wastewater Kit infringe the '921 patent. *See* Section XI.A; EX1012 at 30. Specifically, Patent Owner alleges that the Bio-Rad ddPCR assays infringe this claim because “in each coding scheme for each exemplary accused assay or kit, each analyte for that assay/kit is encoded a 0 or 1 value for each of at least two component [*sic*] of the fluorescent signal (i.e. the fluorophore),” that is, FAM and HEX fluorophores. *Id.* In the accused ddPCR assays, including the SARS-CoV-2 Kit and the Wastewater Kit, a first target is coded as a 1 in color 1 and a 0 in color two, a second target is coded as a 0 in color 1 and a 1 in color 2, and a third target is coded as a 1 in color 1 and a 1 in color 2 as shown below:

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

Analyte	FAM	HEX
N2	1	0
E	1	1
MHV	0	1

Id. (depicting coding scheme for: SARS-CoV-2 Kit with Analytes N1, N2, and RP; and Wastewater Kit with Analytes N2, E, and MHV, respectively).

151. Larson teaches assays wherein probes correspond to polynucleotide analytes and are encoded to detect and distinguish between the analytes using one or more colors (wavelength regimes). *See supra* Sections X.A.1-3; EX1017 ¶¶ 26, 28-29, 32, 47, 116, 121, 135, 140, Figs. 9A-C, 12B-C. Specifically for color, and as discussed above, Larson teaches encoding the presence of a fluorophore's signal as a positive value and the absence of a fluorophore's signal as a null value. *See supra* Sections IX.A.2, XI.A.1; EX1017 ¶ 140 ("Thus when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B.").

152. Larson teaches assays consistent with Patent Owner's urged construction. As examples, and as detailed above, the assays according to Larson using: (i) one color at two different intensities and a second color at one intensity to detect and identify three different polynucleotide analytes; (ii) a single color with two different intensities to detect and identify two different polynucleotide analytes; and (iii) two colors with a first color at an intensity for one analyte, a second color at the same intensity for a second analyte, and both colors at a lesser intensity for a third analyte, each encode M analytes as a first value in a first component of a fluorescent signal and a second value in a second component. *See supra* Section XI.A.1.

153. For example, Larson specifically teaches the above-described three analyte, two color assay. Larson recites "a single droplet might contain four different probes for measuring three different targets (say T1, T2, and T3). Two probes might be of color A (say A1, and A2), and two probes might be of color B (say, B1 and B2). T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2. Thus when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B. However when a droplet contains T3, increased fluorescence appears in both colors A and B." Thus, for clarity, the coding scheme

Larson describes, where a sufficiently significant increase in fluorescence is marked as 1 and no such fluorescence, or a nominal increase over background, is marked as 0, is depicted below in tabular form:

Target	Color A	Color B
T1	1	0
T2	0	1
T3	1	1

154. Accordingly, a POSA would have understood that the types of assays in Larson are those where each of said at least M analytes is encoded as at least one first value in a first component of said fluorescent signal and at least one second value in a second component of said fluorescent signal.

C. Claim 3: The assay of claim 2, wherein said first value is an intensity or range of intensities.

155. As described above, Larson discloses the method of claim 2 that each of said M analytes is encoded as at least one first value in a first component of a fluorescent signal and at least one second value in a second component of said fluorescent signal. *See supra* Sections XI.A, B.

156. It is further my opinion that Larson discloses the further limitations of claim 3, wherein the first value is an intensity or a range of intensities.

157. As I discuss above, Larson teaches assays wherein the first value for which an analyte is encoded may be an intensity or range of intensities. EX1017 ¶¶ 34-42, 47, 52, 121, 139-141, 145, 150, 153-154, 162, Figs. 14-15. For example, Larson discloses assays wherein “for each color probe, multiple targets can be identified based on intensity.” *Id.* ¶ 139. As a further example, Larson teaches assays where the particular intensity of the fluorescent signal is a first value of a first component of that signal. *See supra* Sections XI.A.1, XI.B; *see, e.g.*, EX1017 ¶ 140 (a single target may be identified by multiple probes that constitute a unique signature of both colors and intensities).

158. Patent Owner contends Petitioner’s ddPCR assays infringe this claim by stating, after incorporating its analysis from claim 2, “for each infringing Bio-Rad assay or kit, the value is an intensity based on fluorescent signal and its wavelength.” EX1012 at 32.

159. As explained above, Patent Owner argues that the SARS-CoV-2 Kit and the Wastewater Kit infringe the ’921 patent because the analytes for these kits are allegedly encoded as a 0 or a 1 value for the FAM and HEX fluorophore signals. *See supra* Sections XI.A.1, X.B. These values of 1 in the accused products correspond to increased fluorescence, (i.e., intensity). This is as shown below:

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

Id. at 10.

160. Larson discloses assays using the same approach as this. Specifically, Larson discloses an assay wherein “when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B. However when a droplet contains T3, increased fluorescence appears in both colors A and B.” EX1017 ¶ 140. A POSA would have understood this “increased fluorescence” to be using values that are fluorescence intensities or ranges of fluorescence intensities. *Id.*

161. Accordingly, a POSA would have understood that the types of assays in Larson are those where the first value can be an intensity or range of intensities.

D. Claim 4: The assay of claim 2, wherein said coding scheme is made nondegenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.

162. As described above, Larson discloses the method of claim 2 that each of said M analytes is encoded as at least one first value in a first component of a

fluorescent signal and at least one second value in a second component of said fluorescent signal. *See supra* Sections XI.A-B.

163. It is further my opinion that Larson discloses the further limitations of claim 4. For example, Larson discloses adjusting the intensity values of signals emitted from color labels to avoid potential results that are ambiguous, particularly to avoid overlap between signals for droplet populations containing two different target analytes. EX1017 ¶¶ 35 (describing Figure 15's depiction of optimizing droplet intensities), 146 (describing optimizing intensities to adjust positions of signals in histograms), 150 (describing optimization of intensities depicted in Figure 15 to separate populations within the histogram), Fig. 15.

164. Accordingly, a POSA would have understood that Larson discloses identifying the possible combinations of signals that may arise from the different droplets and, where signals for individual analytes result in ambiguity, i.e., create degenerate results, then adjusting the intensity of signals so that the results are no longer degenerate.

165. In light of the above disclosures in Larson, A POSA would have understood that this adjustment is effectively no different than eliminating a potential analyte code from the coding scheme to eliminate degeneracy, as a color signal (defined by both color and intensity) is removed when the intensity is

adjusted. For example, if an assay were performed, and two targets emitted indistinguishable signals when present, i.e., degenerate, they would effectively have the same analyte code. Thus, when this disclosed adjustment or optimization occurs, at least one of the analyte codes will necessarily be changed to generate a set of signals that are distinguishable.

166. Accordingly, a POSA would have understood that the types of assays in Larson are those where a coding scheme is made nondegenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.

E. Claim 5: The assay of claim 1, wherein said detecting is performed with reagents comprising hybridization probes.

167. As described above, Larson discloses the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A.

168. It is further my opinion that Larson discloses the further limitations of claim 5.

169. As discussed, Larson teaches assays using hybridization probes, i.e., probes that hybridize/bind to the target sequence. EX1017 ¶¶ 91 (“In certain aspects, the droplets of the invention contain a plurality of detectable probes that hybridize

to amplicons produced in the droplets.”), 68 (“The second sample fluid contains reagents...includ[ing] detectably labeled probes for detection of the amplified target nucleic acid, the details of which are discussed below.”), 74 (“In embodiments involving merging of droplets, two droplet formation modules are used...The second droplet also includes detectably labeled probes for detection of the amplified target nucleic acid, the details of which are discussed below.”), 121 (“Probes (P1, P2 and P3) are labeled with fluorophores (color 1, color 2 and color 1) and bind to the target genetic sequences (target 1, target 2 and target 3) (Panels B and C).”). Larson details a variety of assays that use probes to perform the step of detecting, including, as discussed above, in Figure 9 three probes for binding “to the target genetic sequences” with fluorophores of two colors. *Id.* ¶ 121, Fig. 9; *see also id.* ¶¶ 25-26, 34-35, 108-115, 146-150, 189 (Table 2), Figs. 5-6, 14-15. As a further example, Larson discloses the use of probes specific for those sequences present in the same sample solution used to form droplets. *Id.* ¶¶ 114-116 (“The five different probes TERT, RNaseP, Ela, 815A and 815G were also encapsulated in the droplets containing the primers.”), 154. As yet another example, Larson discloses the use of TaqMan assays to perform the detection methods described in the disclosure, which make use of hybridization probes. *Id.* ¶¶ 93, 145, 156, Fig. 5.

170. For example, Larson discloses an assay that is performed using four hybridization probes to detect three analytes. *Id.* at ¶ 140. In disclosing this exemplary assay, Larson teaches that a “single droplet might contain four different probes for measuring three different targets (say, T1, T2, and T3).” A POSA would have understood this disclosure to constitute an assay performed with reagents comprising hybridization probes.

171. Patent Owner contends infringement based on the allegation that Petitioner’s assays and kits use a process where “samples...are prepared using primer, probes dye, and supermixes,” which include use of labeled hybridization probes. EX1012 at 40-42. These are the same kinds of reagents and probes as used in Larson discussed above.

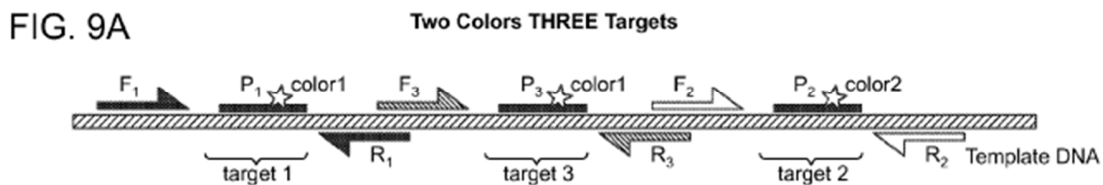
172. Accordingly, a POSA would have understood that the types of assays in Larson are those where detection is performed with reagents comprising hybridization probes.

F. Claim 6: The assay of claim 5, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.

173. As described above, Larson discloses the method of claim 5 where detecting is performed with reagents comprising hybridization probes. *See supra* Sections XI.A, XI.E.

174. It is further my opinion that Larson discloses the further limitations of claim 6.

175. As discussed, Larson teaches using hybridization probes, where those probes are specific for different analytes, and teaches that the probes may comprise identical fluorophores or combinations of fluorophores. Specifically, as discussed, Larson teaches using the same fluorophore (color) for different analytes. *See supra id.*; EX1017 ¶¶ 121 (“Methods...involve...quantitation of multiple different DNA targets...using probes with the same fluorophore.”), 135, 140, Figs. 9A, 12B. Additionally, Larson depicts, in Figure 9A, a multiplex PCR that detects three targets using only two fluorophores:

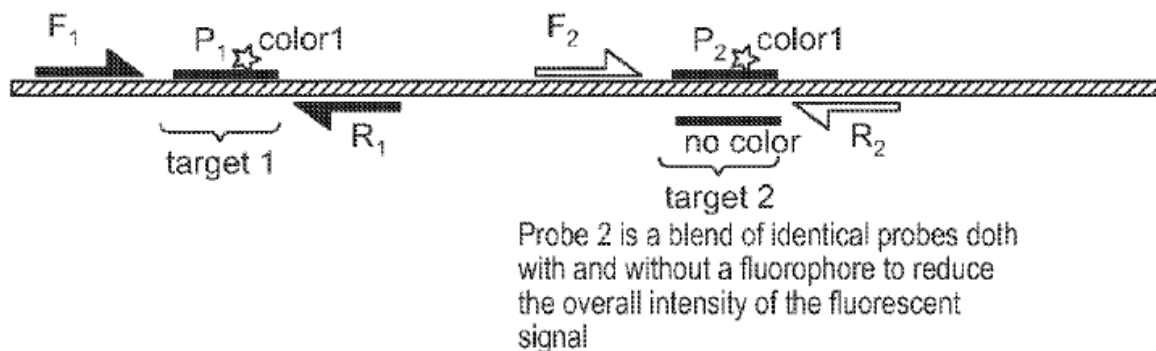


Id. Fig. 9A, ¶ 121; *see supra* Section XI.A. As shown above, above, two probes P₁ and P₃ each comprise “color1,” i.e., the same fluorophore for target 1 and target 3.

176. Larson teaches, as depicted in Figure 12B, using a combination of fluorophores for different analytes. EX1017 ¶ 135, Fig. 12B. As shown below,

multiple colors are used for probes where each probe is specific for a target, which provides a combination of color signals:

FIG. 12B



Id. Fig. 12B, ¶¶ 32, 135, 140. As shown above, above, two probes P₁ and P₂ each comprise “color1,” i.e., the same fluorophore for target 1 and target 2.

177. Larson further discloses, as in the two-color, three-target assay detailed above, using one color for one target, a second color for a second target, and both colors for the third target. In this example, hybridization probes specific for the first and third targets comprise the same fluorophore (i.e., the fluorophore that emits the color A), as do the probes specific for the second and third targets (i.e., the fluorophore that emits the color B). *Id.* ¶ 140. Specifically, the described assay makes use of “four different probes for measuring three different targets.” *Id.* In this assay, two “probes might be of color A (say, A1, and A2), and two probes might

be of color B (say, B1 and B2). *Id.* Probe A1 detects target 1, probe B1 detects target 2 and probes A2 and B2 each detect target 3. *Id.* A POSA would have understood Larson to thus disclose hybridization probes specific for different targets that comprise an identical fluorophore as probes A1 and A2 both comprise a fluorophore of color A and are specific for targets 1 and 3, respectively while probes B1 and B2 both comprise a fluorophore of color B and are specific for targets 2 and 3, respectively.

178. Patent Owner urges that this claim should be interpreted in such a manner that assays that use the same coding scheme as described in paragraph 140 of Larson should be deemed infringing. Patent owner contends assays that use encoding schemes having two or more probes labeled using the same fluorophore that are specific for different analytes infringe the '921 patent. EX1012 at 43-44. Specifically, and for example, Patent Owner contends an assay that uses an encoding scheme with two probes labeled with the same FAM fluorophore with a low intensity signal for one analyte and a high intensity signal for a second analyte. *Id.* Specifically, for example, Patent Owner accuses Petitioner of infringing this claim for two reasons.

179. For example, Patent Owner contends that one of Petitioner's assays practices this claim because it allegedly includes two hybridization probes singly-

labeled with the same FAM fluorophore, generating different intensity signals (probes for the gene PLAU which fluoresce at a low intensity and for the gene CCND1 which fluoresce at a high intensity), along with additional singly-labeled probes using different fluorophore labels for different polynucleotide analytes. EX1012 at 43-44 (citing claim 3 of Appendix B1 (EX1040 at 66-69)) (citing element [1a.2] from Appendix A1 (EX1039 at 15-21)). Patent Owner lays out its reasoning as reproduced below:

From the above, it appears the following coding scheme was selected to correspond to the presence of a unique combination of presence or absence of eight targets. The x and y axes of the two 2D amplitude plots indicate the targets and their corresponding fluorophore components (e.g., “HEX amplitude: low VCL, high REN” indicates VCL target corresponds to low HEX signal, REN target corresponds to high HEX signal). Low is represented by 1 (e.g., low HEX for VCL indicated as 1), and high is represented by 2 (e.g., high HEX for REN indicated as 2).

Analyte	FAM	HEX	Cy5	Cy5.5
PLAU	1	0	0	0
CCND1	2	0	0	0
VCL	0	1	0	0
REN	0	2	0	0
BRCA1	0	0	1	0
KLF8	0	0	2	0
SORL1	0	0	0	1

EX1040 at 68; *see also* EX1039 at 20 (showing a FAM Hi and FAM Lo in detection channel 1).

180. Just as in Larson, as detailed above, the same fluorophore is used to unambiguously detect two analytes, using different intensities. *See* EX1017 ¶ 121 (“Methods...involve...quantitation of multiple different DNA targets...using probes with the same fluorophore. FIG. 9...depict[s] two-color detection of three

genetic sequences...Probes (P1, P2 and P3) are labeled with fluorophores (color 1, color 2 and color 1) and bind to the target genetic sequences (target 1, target 2 and target 3) (Panels B and C).”).

181. Accordingly, a POSA would have understood that the types of assays in Larson are those where the hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.

G. Claim 7: The assay of claim 5, wherein said fluorescence signal from said hybridization probes are used to construct a chromatogram.

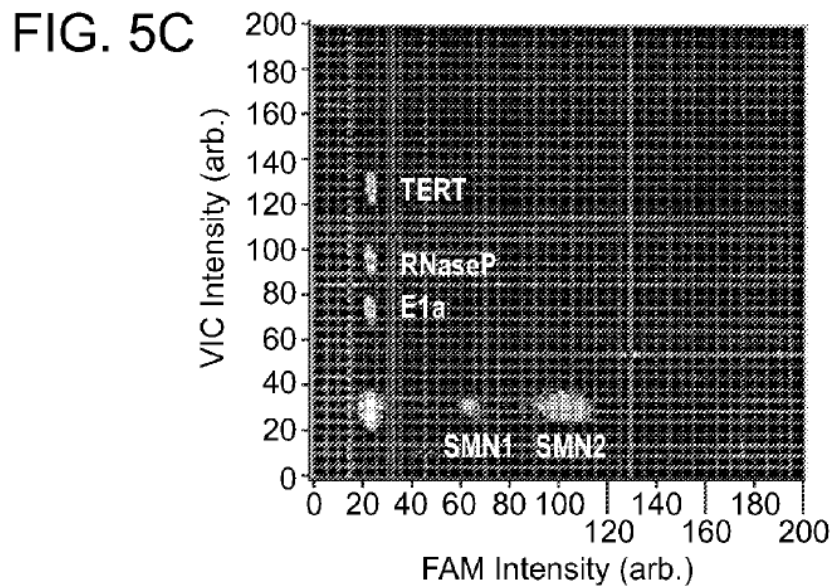
182. As described above, Larson discloses the method of claim 5 where detection is performed with reagents comprising hybridization probes. *See supra* Sections XI.A, XI.E.

183. It is further my opinion that Larson discloses the further limitations of claim 7.

184. As discussed, Larson teaches using hybridization probes. *See supra* Sections XI.A.1, XI.E. Larson also teaches using the signal(s) detected from the hybridization probes to create a chromatogram.

185. Larson discloses a “scattered plot.” Larson discusses constructing these scattered plots: “the fluorescence emission from each droplet was determined and

plotted on a scattered plot based on its wavelength and intensity.” EX1017 ¶¶ 113, 116. Exemplary scattered plots depicted in the figures show each of the potential signal clusters for each analyte. *Id.* Figs. 5C, 6B, 9, 10, 14a, 15. Figure 5C, reproduced below, is exemplary:



Id. Fig. 5C, ¶ 113.

186. Further, Larson discloses assays wherein each droplet may contain more than one analyte. *See, e.g.*, EX1017 ¶ 68 (In “a preferred embodiment, the first sample fluid contains nucleic acid templates. Droplets of the first sample fluid are formed....Those droplets will include nucleic acid templates.”). Also, Larson explains that “certain embodiments” will include droplets with “only a single nucleic

acid template.” *Id.* Necessarily, this means that droplets in other embodiments will include multiple nucleic acid templates.

187. Additionally, Larson teaches a Poisson distribution of analytes in droplets, meaning some droplets have multiple analytes:

The distribution of target DNA molecules among the reactions follows Poisson statistics, and at so called “terminal dilution” the vast majority of reactions contain either one or zero target DNA molecules for practical intents and purposes. In another case, at so called “*limiting dilution*” some reactions contain zero DNA molecules, some reactions contain one molecule, and *frequently some other reactions contain multiple molecules*, following the Poisson distribution.

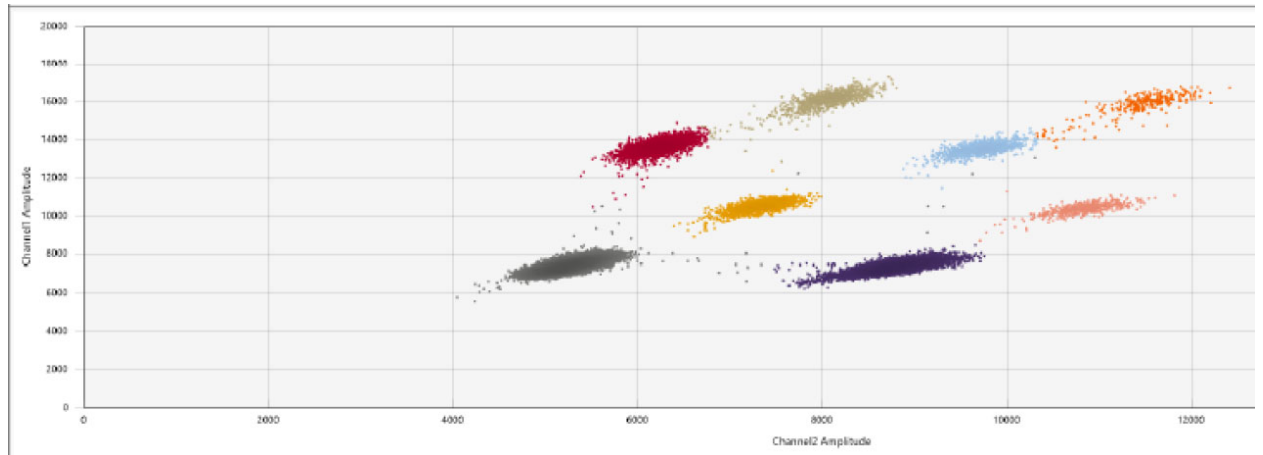
Id. ¶ 6. Larson also specifically teaches using its assays at limiting dilution. *Id.* ¶ 45. This means that sets of reactions must be occurring in droplets that contain multiple target molecules. Larson also discloses, in describing droplet formation, “the invention is compatible with all distributions of DNA loading that conform to limiting or terminal dilution.” *Id.* ¶ 52. With these disclosures, a POSA would expect to have droplets that contain different numbers of polynucleotide targets at a Poisson distribution, including droplets with all analytes in a single droplet when the number of analytes is a low number, such as two or three, where the frequently

occurring droplets with multiple molecules necessarily would include those with all of the analytes. *See id.* ¶¶ 96-97.

188. As discussed above, Larson discloses how its assays work to detect multiple analytes in a single droplet. Specifically, in Figure 9, Larson discloses an assay using two probes P_1 and P_3 labeled with the same color labels, but of different intensity, and probe P_2 is labeled with a different color label, in order to generate three distinguishable signals to identify three target analytes; wherein a POSA would have understood that the various combinations of analytes would result in distinguishable signals for the three analytes because the signals from P_1 , P_2 , P_3 , P_1+P_2 , P_1+P_3 , P_2+P_3 , and $P_1+P_2+P_3$ have different intensities as the combinations are additive and would each sum to unique values in each of the two colors used. *Id.* ¶ 121, Fig. 9C. As discussed, this unique sum of values for the different analytes provides a method for a POSA to use the assays in Larson to detect multiple analytes in one droplet.

189. Accordingly, the scattered plots for these assays and the droplets used in those assays would have included the combined signals for every combination of the analyte targets as present in the single sample solution. This would provide a chromatogram according to the '921 patent's claim 7.

190. Patent Owner contends that chromatograms plotting all intensity clusters of the analytes, infringe this claim:



EX1012 at 45. These chromatograms are the same as Larson discloses, such as in Figure 15, plotting all intensity clusters:

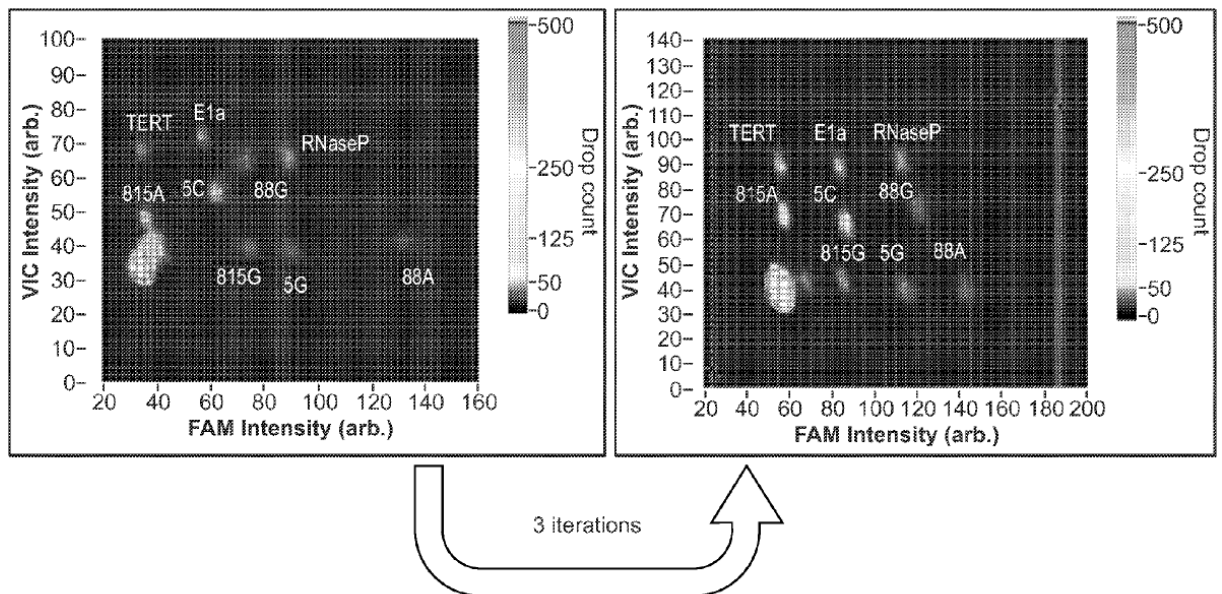


FIG. 15

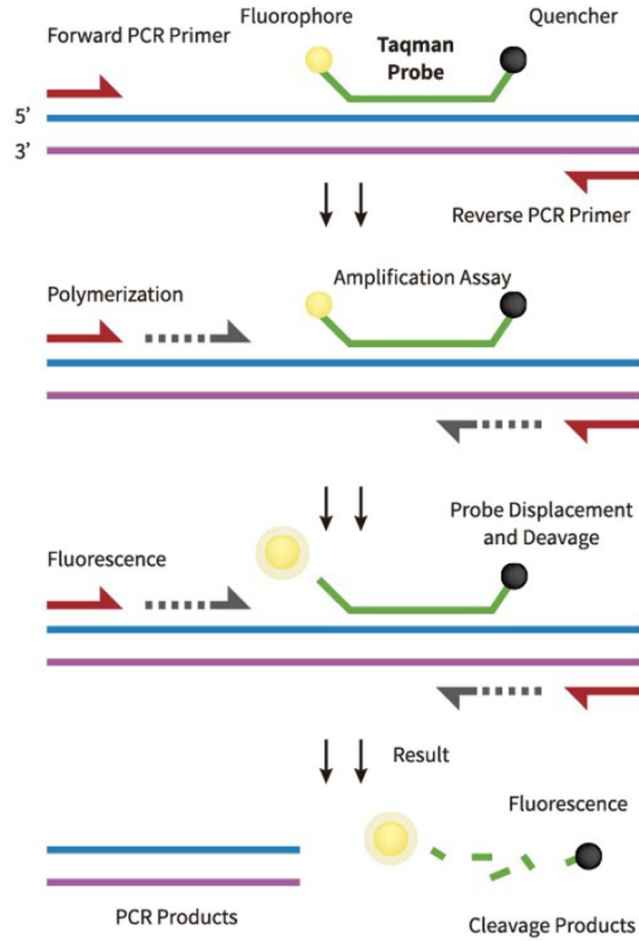
EX1017 at Fig. 15.

191. Accordingly, a POSA would have understood that Larson teaches assays using the fluorescence signal from the hybridization probes to construct a chromatogram.

H. Claim 8: The assay of claim 1, wherein said signal is generated during a polymerase chain reaction.

192. As described above, Larson discloses the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A.

193. It is further my opinion that Larson discloses the further limitations of claim 8. Larson teaches methods for generating fluorescence signals during PCR amplification that were well-known to a POSA. Specifically, Larson teaches the TaqMan assay. As explained above, and depicted in the figure below, this assay generates signals when the labeled sequence-specific probe, bound to the target sequence, is cleaved during PCR amplification (specifically during extension of the primer through the binding site). *See supra* Sections V.C., XI.A; EX1017 ¶ 47, Fig. 5.



EX1013 at 6; EX1014 (research paper describing assay).

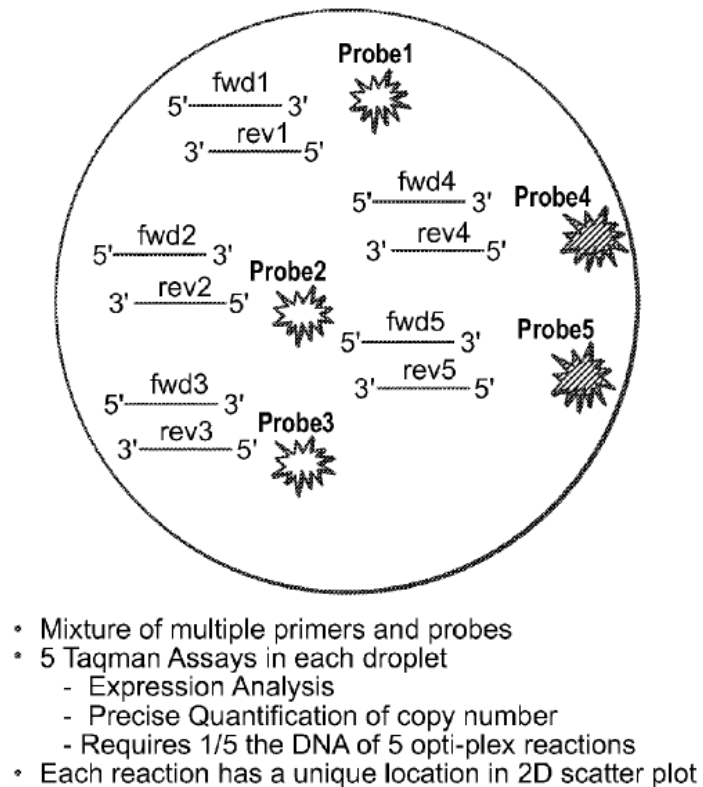


FIG. 5A

EX1017 at Fig. 5. The final step, cleavage of the probe during extension, causes the probe and quencher to separate enough for fluorescence to occur.

194. Patent Owner alleges that the accused assays infringe because they allegedly use “reagents and workflows similar to those used for most standard Taqman probe-based assays.” EX1012 at 49-50. As discussed, in the TaqMan assay the signal is generated during primer extension through the probe binding site, as is disclosed in Larson. EX1017 ¶ 47.

195. Accordingly, a POSA would have understood that the types of assays in Larson are those where the signal is generated during a polymerase chain reaction.

- I. Claim 9: The assay of claim 1, wherein at least one of said analytes is encoded by at least one additional value, wherein said at least one additional value is selected from the group consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal, and combinations thereof.**

196. As described above, Larson discloses the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A.

197. It is further my opinion that Larson discloses the further limitations of claim 9.

198. As discussed, Larson teaches methods wherein an analyte is encoded using a color signal or signals, as is done in the '921 patent. *See supra* Sections XI.A.1-2, X.B; EX1001 at 1:66-2:8, claims 1, 9. As discussed above, Larson discloses such two-color, three-analyte assays. *See supra* Sections XI.A-B; EX1017 ¶ 140. For these assays, the first analyte is encoded by one color, the second analyte is encoded by a second color, and the third analyte is encoded by both colors. That third analyte is encoded by “at least one additional value” “from at least one component of an additional signal” because the second color is an additional signal. *Id.*

199. Patent Owner alleges infringement based on encoding this same two-color, three-analyte assay in the same manner. As examples, the SARS-CoV-2 Kit allegedly has the “N2 Target” analyte “encoded by a positive signal of both FAM and HEX” and the Wastewater Kit includes the same, “the E Target” analyte is “encoded by a positive signal of both FAM and HEX.” EX1012 at 50-51. As to the SARS-CoV-2 Kit, Patent Owner contends the below encoding scheme infringes the ’921 patent:

4. In the **Plate Editor** tab, select all the wells to be analyzed. With **Experiment Type** as **Direct Quantification (DQ)**, select **Probe Mix Triplex** under **Assay Information** and then click **Apply**. Enter Target information (Table 10) and click **Apply**.

Table 10. QuantaSoft Analysis Pro Target Information

Target Name	Signal Ch1	Signal Ch2
N1	FAM	None
N2	FAM	HEX
RP	None	HEX

Id. at 50.

200. Larson, as discussed above discloses assays akin to the kind accused of infringement by Patent Owner. In describing an equivalent two-color, three analyte assay, Larson discloses an assay wherein “a single droplet might contain four different probes for measuring three different targets (say, T1, T2, and T3). Two probes might be of color A (say, A1, and A2), and two probes might be of color B (say, B1 and B2).” A POSA thus would have understood Larson to disclose a target encoded by one additional value in the form of an additional color as target 3 is

encoded in both colors A and B, compared to the encoding for targets 1 and 2, each in a single color, A or B. *Id.*

201. Accordingly, a POSA would have understood that the types of assays in Larson are those where at least one of said analytes is encoded by at least one additional value, wherein the value is selected from the group consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal, and combinations thereof.

J. Claim 10: The assay of claim 1, wherein at least one of said analytes comprises a polynucleotide.

202. As described above, Larson anticipates the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A.

203. It is further my opinion that Larson anticipates the further limitations of claim 10.

204. As discussed, Larson teaches using polynucleotides as the analytes. *See supra* Section XI.A.1; EX1017 at Abstract (“The invention generally relates to droplet based digital PCR and methods for analyzing *a target nucleic acid* using the same.”) (emphasis added). As discussed, nucleic acid strands are polynucleotides. *See supra* Section V.A.

205. Accordingly, a POSA would have understood that the types of analytes in Larson are those polynucleotides.

K. Claim 13: The assay of claim 1, wherein said coding scheme is innately nondegenerate or non-degenerate by construction.

206. As described above, Larson anticipates the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A.

207. It is further my opinion that Larson anticipates the further limitations of claim 13.

208. As discussed, Larson teaches formulating coding schemes in assays that are inherently non-degenerate. *See supra* Sections XI.A-D. Specifically, by using intensities, fine-tuning intensities, and using multiple fluorophores, each analyte is inherently non-degenerate. *See supra* Sections XI.A-D; *see e.g.*, EX1017 Figs. 9, 12B, 15, ¶¶ 29, 32, 121, 135, 140.

209. Patent Owner relies on the same reasoning as described in claims 1.2, 1.4, 2, and 4, which is disclosed in Larson. EX1012 at 64 (citing *id.* at 9-16, 17-25, 32-40); *see supra* Sections XI.A.2, 4, XI.B, D.

210. For example, as described above, Patent Owner contends that in “the SARS-CoV-2 Kit, each analyte is encoded as a value of one component of a fluorescence signal (corresponding to a specific wavelength as defined by the

fluorophore given below) as represented by the identified fluorophores FAM and HEX” and provides that a “corresponding coding scheme for the SARS-CoV-2 Kit is as follows:”

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

EX1012 at 10.

211. Patent Owner further contends the Wastewater Kit infringes the claims of the '921 patent by employing the same coding scheme on the basis that “N2 is encoded as FAM positive, E as FAM and HEX Positive, and NHV as HEX positive shown below”:

Analyte	FAM	HEX
N2	1	0
E	1	1
MHV	0	1

Id. at 11.

212. As discussed, Larson teaches using two colors to detect three polynucleotide analyte targets wherein: two different single-color probes are used separately for a first and a second target, and the two colors are both used together

for a multi-colored probe(s) for a third target. *See, e.g., supra* Section XI.A.1; EX1017 ¶ 140 (describing the use of four probes for three polynucleotide analyte targets where a probe of a first color is specific for the first analyte, a second probe of a second color is specific for the second analyte, and a third and fourth probe, labeled with the first and second color, respectively, are specific for the third analyte). In each of the assays in Larson, each of the fluorophore-labeled sequence-specific probes provides a fluorescent signal for the polynucleotide analyte that it binds, thus encoding the analyte as a value of the wavelength (i.e., color or a non-zero intensity) of the corresponding generated fluorescent signal. Specifically, to be clear, the corresponding fluorescent signal(s) for each analyte shows what is present by what the signal is, i.e., a particular wavelength/color and that it is present, i.e., a non-zero intensity. This creates a coding scheme, describing which signal shows detection of which polynucleotide analyte target. For example, Larson discloses:

[A] single droplet might contain four different probes for measuring three different targets (say, T1, T2, and T3). Two probes might be of color A (say, A1, and A2), and two probes might be of color B (say, B1 and B2). T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2. Thus, when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence

appears in color B. However when a droplet contains T3, increased fluorescence appears in both colors A and B.

Id. at ¶ 140.

213. Accordingly, a POSA would have understood that the types of assays in Larson are those that create an innately nondegenerate or non-degenerate-by-construction coding scheme.

L. Claim 14:

214. It is my opinion that Larson anticipates claim 14.

1. A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising

215. It is my opinion that the preamble is not limiting.

216. It is my opinion that the preamble merely states an intended use, and the claim recites a “structurally complete” invention, which counsel has informed me is part of the test for determining whether a preamble is limiting. It is my opinion that the preamble does not recite an essential structure or steps, nor is it necessary to give the claim meaning to one skilled in the art.

217. To the extent that the preamble is deemed limiting, it is my opinion that Larson discloses the claimed kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement.

218. Specifically, as set forth above in Sections XI.A.1-4 for claim 1, Larson discloses an assay that unambiguously detects the presence or absence of each of at least M analytes in any combination of presence or absence. For example, Larson specifically teaches unambiguously detecting multiple polynucleotide analytes. *See, e.g.*, EX1017 ¶¶ 26 (describing Figure 6's depiction of a multiplex assay detecting five polynucleotide analytes), 116 (same), 28 (describing Figure 8's depiction of two-color detection of two sequences), 120 (same), 29 (describing Figure 9's depiction of two-color detection of three sequences), 121 (same), 32 (describing Figure 12's depiction of using different signal intensity to detect different sequences), 135 (same), Figs. 6, 8, 9, 12. By disclosing assays that perform these limitations, Larson likewise discloses and teaches a kit for performing these limitations, as a POSA would have understood the teaching of the assay including the recited components to also be a teaching the components for carrying out the assay, i.e., a kit.

219. It is further my opinion that the preamble as recited is wholly redundant to the specific elements set forth in the claim body and is, accordingly, fully addressed below in setting forth the ground as to each limitation.

2. analyte-specific reagents, wherein:

220. It is my opinion, as discussed above, that the preamble is not limiting.

221. To the extent that the preamble is deemed limiting, it is my opinion that Larson discloses the claimed kit comprising analyte-specific reagents.

222. Larson discloses the use of analyte-specific reagents. Specifically, Larson discloses the use of hybridization probes that are specific to a given analyte to perform the step of detecting as described above in Sections XI.A, XI.E; *see also* EX1017 ¶¶ 26, 28-29, 32, 116, 120-121, 135, 140, Figs. 6, 8-9, 12. For example, Figure 9 depicts analyte-specific hybridization probes, disclosing “Probes (P1, P2 and P3) are labelled with fluorophores (color 1, color 2 and color 1) and bind to the target genetic sequences (target 1, target 2 and target 3) (Panels B and C).” EX1017 ¶ 121, Fig. 9. As an additional example, Larson discloses the use of analyte-specific reagents in Fig. 12 by depicting exemplary “Probes (P1 and P2) [that] are labelled with fluorophore of color 1 and bind to target 1 and target 2 respectively.” *Id.* ¶ 135, Fig. 12.

223. Larson further teaches that these analyte-specific reagents may be hybridization probes contained in an aqueous buffer. For example, Larson discloses “The second sample fluid also includes detectably labeled probes for detection of the amplified target nucleic acid.” *Id.* ¶ 68.

224. In addition to hybridization probes, Larson also discloses analyte-specific reagents in the form of forward and reverse PCR primers. For example,

Larson teaches a sample fluid containing reagents for PCR reaction, the reagents including “forward and reverse primers, all suspended within an aqueous buffer.” *Id.* ¶ 74; *see also id.* ¶¶ 68, 74, 121, 135, Figs. 6, 8-9, 12.

225. Larson, in the example I often describe, teaches the use of analyte-specific reagents. In paragraph 140 Larson discloses an assay, and thus the components for a kit to perform the assay, making use of 4 probes; one specific to a first target, one specific to a second target and two specific to a third target: “T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2.” *Id.*

226. Thus, Larson discloses kits that use analyte-specific reagents, including analyte-specific hybridization probes and forward and reverse PCR primers.

- a. **a) each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value, and**

227. It is my opinion that Larson discloses this limitation. Larson teaches kits wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value as described above in Sections XI.A.1, 2, XI.B, XI.E, XI. F.

228. The disclosures cited above in Sections XI.A.1, 2 XI.B, XI.E, XI. F, are sufficient to disclose the limitations described in this claim as these sections provide evidence that Larson discloses an assay “wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating an encoding scheme.” Specifically, Larson discloses and teaches generating a coding scheme for multiple analytes based on fluorescent signals by using different color fluorophore-labeled probes that are specific for identifying two, three, or more different analytes. *See, e.g.*, EX1017 ¶¶ 26, 28-29, 32, 116, 120-121, 135, 140, Figs. 6, 8-9, 12. In disclosing this step performed by an assay, Larson likewise discloses and teaches a kit for performing this step, as a POSA would have understood the teaching of the assay included the recited components to also be a teaching of the components for carrying out the assay, i.e., a kit.

229. As discussed above, this coding scheme is of the same kind that Patent Owner accuses of infringement, including as alleged to be used by the SARS-CoV-2 Kit and the Wastewater Kit products. *See supra* Sections VIII, XI.A.1; EX1012 at 10.

230. For example, Patent Owner contends that in “the SARS-CoV-2 Kit, each analyte is encoded as a value of one component of a fluorescence signal (corresponding to a specific wavelength as defined by the fluorophore given below)

as represented by the identified fluorophores FAM and HEX” and provides that a
“corresponding coding scheme for the SARS-CoV-2 Kit is as follows:”

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

Id.

231. As discussed, Larson teaches assays using the same approach. For example, Larson discloses:

[A] single droplet might contain four different probes for measuring three different targets (say, T1, T2, and T3). Two probes might be of color A (say, A1, and A2), and two probes might be of color B (say, B1 and B2). T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2. Thus, when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B. However when a droplet contains T3, increased fluorescence appears in both colors A and B.

EX1017 at ¶ 140.

232. Thus, Larson discloses kits wherein each of at least M analytes is encoded as a value of one component of a fluorescent signal, generating an encoding scheme and the analytes are each represented in the coding scheme by their value.

b. wherein said encoding is performed in a manner that eliminates degeneracy; and

233. It is my opinion that Larson discloses this limitation. Larson teaches encoding performed in a manner that eliminates degeneracy as described above in Sections XI.A.1-4, XI.B, D.

234. The above sections describe how Larson teaches assays wherein the presence or absence of targets is detected “unambiguously” based on a generated encoding scheme that is designed to be nondegenerate or is made nondegenerate by eliminating one or more potential codes from a coding scheme to make the scheme nondegenerate. For example, Larson teaches distinguishable signals, i.e., nondegenerate signals, generated representing three analytes, each distinguishably identified using two unique color labels. *See, e.g.*, EX1017 ¶¶ 5, 121, 131, 135, 140, Figs. 9, 12B.

235. Patent Owner alleges the SARS-CoV-2 Kit meets this limitation because “the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme,” which is reproduced below:

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

EX1012 at 21.

236. Patent Owner alleges the Wastewater Kit meets this limitation because “the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme,” which is reproduced below:

Analyte	FAM	HEX
N2	1	0
E	1	1
MHV	0	1

Id.

237. Larson teaches assays that use a scheme under the same approach as the ones alleged to be infringing by Patent Owner. For example, Larson discloses the detection of three targets in a sample by using two colors where a first target is detected using a first color, a second target is detected using a second color, and a third target is detected using both the first and second colors. EX1017 ¶ 140. Thus,

Larson discloses kits wherein analytes are unambiguously identified by encoding each analyte in one or two colors in a two-color, three-target assay.

238. As described above, this disclosure teaching assays to perform such steps would disclose to a POSA the physical components to perform such an assay, i.e., kits.

239. Thus, Larson discloses kits for detecting at least M analytes wherein encoding is performed in a manner that eliminates degeneracy.

- c. b) each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the value;**

240. It is my opinion that Larson discloses this limitation. Larson teaches assays wherein each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates a value as described above in Sections XI.A, XI.E, XI.H, XI.L.2.

241. The above sections describe how Larson teaches assays that use forward and reverse primers and sequence-specific probes that generate a fluorescent signal during the polymerase chain reaction, i.e., a TaqMan assay if the polynucleotide target is present. Because the analyte-specific reagents generate the fluorescent signal, they also generate the value of one component of a fluorescent signal. *See, e.g.*, EX1017 ¶¶ 47, 52, 68-69, 74, 132-134.

242. The example I often describe further illustrates this disclosure by Larson to a POSA. Larson paragraph 140 recites that a first target is measured by a first probe comprising a first color, a second target is measured by a second probe comprising a second color, and a third target is measured by third and fourth probes comprising the first and second color, respectively. *Id.* Thus, a POSA would have understood Larson to disclose kits wherein each of the targets to be detected is represented in the coding scheme by the fluorescent value in which it is encoded, i.e. is detected by the colored probes it is assigned.

243. As described above, this disclosure teaching assays to perform such steps would disclose to a POSA the physical components to perform such an assay, i.e., kits.

244. These reagents are precisely those that Patent Owner relies on in its infringement contentions for the '921 patent. Patent Owner contends infringement based on the allegation that Petitioner's assays and kits use a process where "samples...are prepared using primer, probes dye, and supermixes," which include use of labeled hybridization probes. EX1012 at 40-44. The included analyte-specific reagents are the same kinds of reagents and probes as used in Larson discussed above.

245. Thus, Larson discloses kits for detecting M analytes using analyte-specific reagents wherein each analyte-specific reagent, when contacted with a sample comprising its corresponding analyte, generates the value.

3. **wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single sample volume, in any combination of presence or absence, and wherein the kit does not require the use of mass spectrometry or immobilization of said M analytes, and**

246. It is my opinion that Larson discloses this limitation. Larson teaches assays that unambiguously detect the presence or absence of multiple analytes in any combination of present or absence in a single sample volume as described above in Sections XI.A.

247. The above sections describe how Larson teaches assays that detect, for example, three analytes in a single sample volume. *See, e.g.*, EX1017 ¶ 120-121, 135, 140 (describing that target 1 is identified by a signal in color 1, target 2 is identified by a signal in color 2, and target three is identified by a signal in colors 1 and 2), Figs. 9, 12. Further, as discussed above, this detection is performed without the use of mass spectrometry or immobilization. EX1017 ¶¶ 47, 52-54, 69, 70-72, 83, 89, Fig. 1-3. As described above, the disclosure teaching assays to perform such steps would disclose to a POSA the physical components to perform such an assay, i.e., kits, and those kits would be capable of performing the same steps.

248. Patent Owner alleges the SARS-CoV-2 Kit meets this limitation because “the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme,” which is reproduced below:

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

EX1012 at 21.

249. Patent Owner alleges the Wastewater Kit meets this limitation because “the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme,” which is reproduced below:

Analyte	FAM	HEX
N2	1	0
E	1	1
MHV	0	1

Id.

250. Larson teaches assays that use a scheme with the same approach as the ones alleged to be infringing by Patent Owner. For example, Larson discloses the detection of three targets in by using two colors where a first target is detected in a first color, a second target is detected in a second color, and a third target is detected in both the first and second colors. EX1017 ¶ 140.

251. Thus, Larson discloses kits for detecting M analytes using analyte-specific reagents wherein the kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single sample volume, in any combination of presence or absence, and wherein the kit does not require the use of mass spectrometry or immobilization of said M analytes

4. wherein the analytes are [amplified] in the single sample volume.

252. It is my opinion that Larson discloses, or at a minimum renders obvious, this limitation. Larson teaches assays that perform amplification of the analytes in the single sample volume in Sections XI.A.1, 5, 6. The above sections describe how Larson teaches assays that amplify analytes in a single sample volume. For example, Larson discloses kits that rely on PCR performed in droplets, which are individual partitions of the single sample volume, and include techniques which were well-known in the art at the time of the alleged invention, including the TaqMan assay. EX1017 ¶¶ 2, 9, 11, 13, 14, 16, 17, 20, 47, 49 (“Methods of the invention involve

forming sample droplets containing a single target nucleic acid, amplifying the target in the droplets”), 52-54, 69-72, 83, 89, 91 (“In certain aspects, the droplets the droplets of the invention contain a plurality of detectable probes that hybridize to amplicons produced in the droplets.”), 93 (“The invention is not limited to the TaqMan assay, as described above, but rather the invention encompasses the use of all fluorogenic DNA hybridization probes”), 96-97, 119-121, 150, 195, Figs. 1-4, 7-11, 15.

253. As described above, this disclosure teaching assays to perform such steps would disclose to a POSA the physical components to perform such an assay, i.e., kits, and those kits would be capable of performing the same steps.

254. To the extent this limitation is not disclosed by Larson, it is my opinion that a POSA informed by the same disclosures would have been motivated to combine the teachings to obtain the kit as set forth and had a reasonable expectation of doing so.

M. Claim 15: The kit of claim 14, wherein said fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes,

in any combination of presence or absence, when M is greater than F.

255. As described above, Larson discloses the kit of claim 14 that detects the presence or absence of M analytes by generating a cumulative measurement. *See supra* Section XI.L.

256. It is further my opinion that Larson discloses the further limitations of claim 15.

257. Patent Owner alleges the SARS-CoV-2 Kit meets this limitation because “the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme,” which is reproduced below:

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

EX1012 at 21.

258. Patent Owner alleges the Wastewater Kit meets this limitation because “the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as

displayed by the 2D amplitude plots, cluster assignments, and the coding scheme,”
which is reproduced below:

Analyte	FAM	HEX
N2	1	0
E	1	1
MHV	0	1

Id.

259. As discussed, Larson teaches assays that use fluorescent signals in F wavelength components to unambiguously detect M analytes in any combination of presence or absence when M is greater than F, exactly the same as Patent Owner describes in its infringement contentions. *See supra* Sections XI.A.3-4. As discussed above, Larson teaches assays using multiple intensities of the same color as signals, multi-color signals, and combinations of multiple intensities and multiple colors as distinguishable signals. *See supra* Section XI.A.1 (wherein I discuss how Larson discloses the use of multi-color signals and multiple intensities of the same color as distinguishable signals); EX1017 ¶¶ 26, 28-29, 32, 116, 120-121, 135, 140, Figs. 6, 8-9, 12. For example, Larson discloses an assay to detect three analytes where each polynucleotide analyte target was encoded as a value of a fluorescent signal, as one of two colors (or both for the third analyte). *See supra* Section XI.A.1; EX1017 ¶ 140.

260. As described above, a POSA would have understood Larson to teach an exemplary assay that makes use of 2 wavelength components, i.e., colors. Specifically, Larson teaches that in an assay for detecting three targets, “Two probes might be of color A (say, A1, and A2), and two probes might be of color B (say, B1 and B2).” EX1017 ¶ 140.

261. Accordingly, a POSA would have understood that the types of assays described in Larson are those that would use a kit where the fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, when M is greater than F.

N. Claim 16: The kit of claim 14, wherein said analyte-specific reagents comprise a plurality of hybridization probes.

262. As described above, Larson discloses the kit of claim 14 that detects the presence or absence of M analytes by generating a cumulative measurement. *See supra* Section XI.L.

263. It is further my opinion that Larson discloses the further limitations of claim 16.

264. Larson teaches assays that make use of analyte-specific reagents comprising a plurality of hybridization probes. *See supra* Sections XI.L.2, XI.A.1-4, XI.E. As discussed, Larson teaches assays performed by the use of kits wherein

the analyte-specific reagents comprise a plurality of hybridization probes by disclosure of the TaqMan assay with probes specific to each of the multiple analytes. *See, e.g.*, EX1017 ¶¶ 47, 52, 68-69, 74, 132-134, *see also id.* ¶¶ 121, 140, Fig. 9. As described above, this disclosure teaching assays to perform such steps would disclose to a POSA the physical components to perform such an assay, i.e., kits, and those kits would be capable of performing the same steps. As discussed, this is the same mapping that Patent Owner accuses of infringing the '921 patent. *See supra* Sections XI.A, E-G; *see also* EX1012 at 67.

265. Further, Larson, in the example I often describe, specifically teaches the use of hybridization probes. In paragraph 140 of Larson, an assay, and thus the components for a kit, makes use of 4 probes; one specific to a first target, one specific to a second target and two specific to a third target is disclosed reciting “T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2.” *Id.*

266. Accordingly, a POSA would have understood that the types of assays in Larson are those that would use a kit wherein the analyte-specific reagents comprise a plurality of hybridization probes.

O. Claim 17: The kit of claim 16, wherein said plurality of hybridization probes comprise a fluorophore, and wherein said

plurality of hybridization probes are specific for different analytes.

267. As described above, Larson discloses the kit of claim 16 that detects the presence or absence of M analytes by generating a cumulative measurement wherein said analyte-specific reagents comprise a plurality of hybridization probes. *See supra* Section XI.N.

268. It is further my opinion that Larson discloses the further limitations of claim 17.

269. Larson teaches assays that make use of hybridization probes comprising a fluorophore, where the hybridization probes are specific for different analytes. *See supra* Sections XI.A., XI.E., XI.F., XI.H., XI.L.2. The above sections describe how Larson teaches assays wherein the analyte-specific reagent comprises a plurality of hybridization probes by disclosure of the TaqMan assay. *See, e.g.*, EX1017 ¶¶ 47, 52, 68-69, 74, 132-134. Further, these hybridization probes taught by Larson comprise one or more fluorophores. *See, e.g.*, EX1017 ¶¶ 26, 28-29, 32, 116, 120-121, 135, 140, Figs. 6, 8-9, 12.

270. Specifically, Larson teaches kits wherein the probes used are described as being “of” a certain color. *Id.* ¶ 140. Larson recites that in an exemplary kit two probes might be “of color A” and two different probes might be “of color B.” *Id.* A POSA would have understood Larson’s description of probes being “of” a specific

color as probes comprising the color they are “of,” which, as I have previously discussed, a POSA would have understood a color to be a fluorophore. *See id.* at ¶ 138 (“a single probe color (i.e. fluorophore).”).

271. As described above, this disclosure teaching assays to perform such steps would disclose to a POSA the physical components to perform such an assay, i.e., kits, and those kits would be capable of performing the same steps. As discussed, this is the same mapping that Patent Owner accuses of infringing the '921 patent. *See supra* Sections XI.A, E-G; *see also* EX1012 at 67.

272. Accordingly, a POSA would have understood that the types of assays in Larson are those that would use a kit wherein the plurality of hybridization probes comprise a fluorophore, and wherein the plurality of hybridization probes are specific for different analytes.

P. Claim 18: The kit of claim 17, wherein said number of hybridization probes in the kit is greater than M.

273. As described above, Larson discloses the kit of claim 17 that detects the presence or absence of M analytes by generating a cumulative measurement wherein said plurality of hybridization probes comprise a fluorophore, and wherein said plurality of hybridization probes are specific for different analytes. *See supra* Section XI.O.

274. It is further my opinion that Larson discloses the further limitations of claim 18.

275. Larson teaches assays that make use of hybridization probes in greater quantities than the number of targets to be detected. *See supra* Section XI.A.4. For example, Larson discloses the detection of three analytes using four probes. *See, e.g.,* EX1017 ¶ 140 (“a single target may be identified by multiple probes that constitute a unique signature of both colors and intensities.”).

276. For example, in disclosing a method where “a single target may be identified by multiple probes that constitute a unique signature of both colors and intensities,” Larson explains that:

a single droplet might contain four different probes for measuring three different targets (say, T1, T2, and T3). Two probes might be of color A (say, A1, and A2), and two probes might be of color B (say, B1 and B2). T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2. Thus, when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B. However when a droplet contains T3, increased fluorescence appears in both colors A and B.

Id. ¶ 140. This teaching that “a single droplet might contain four different probes for measuring three different targets” discloses an assay using a greater number of hybridization probes (4) than the number of “M analytes” (3) to be detected.

277. As described above, this disclosure teaching assays to perform such steps would disclose to a POSA the physical components to perform such an assay, i.e., kits, and those kits would be capable of performing the same steps.

278. Accordingly, a POSA would have understood that the types of assays in Larson are those that would use a kit where the number of hybridization probes in the kit is greater than M.

Q. Claim 19: The kit of claim 14, wherein the analyte-specific reagents are each present at a different amount in the kit.

279. As described above, Larson discloses the kit of claim 14 that detects the presence or absence of M analytes by generating a cumulative measurement. *See supra* Section XI.L.

280. It is further my opinion that Larson discloses the further limitations of claim 19. Larson teaches assays that provide analyte-specific reagents at different amounts. As described above, Larson teaches analyte-specific reagents including probes and primers. *See supra* Section X.L.2; EX1017 ¶¶ 9, 68, 74, 121, 135 Figs. 9, 12.

281. For example, Larson teaches an assay wherein primers are present in a quantity of “1x” and probes are present in a quantity of “0.5x” relative to the concentration of a reference gene. *See, e.g.*, EX1017 ¶ 130. As an additional example, Larson discloses an assay in which “1x assay concentration is defined as

0.2 μ M probes with 0.9 μ M primers,” meaning the probes and primers (the analyte-specific reagents) are provided in different molarities, i.e., in different concentrations. *See, e.g., id.* ¶ 193.

282. It is my opinion that this construction of the claim language is consistent with the construction urged by Patent Owner in the related district court proceedings. Therein, Patent Owner contends that assays with final concentrations of primers/probes of 900 nM/250 nM infringe claim 19. EX1012 at 67-68. Moreover, it is apparent that Patent Owner does not contend the claim requires different probe/primer concentrations for specific target analytes, as the accused instrumentality includes at least two target primers/probe sets sharing the final concentration of 900nM/250nM, identified by their corresponding fluorescent labels being “FAM, Cy5, or ROX” and “HEX/VIC, Cy5.5, or ATTO 590.” EX1012 at 68 (reproducing “Table. Preparation of the reaction mix.”).

283. Further, to the extent the claim is deemed to require different concentrations of probes specific for different analytes, Larson teaches the use of primers and labeled sequence-specific probes at differing concentrations. *See, e.g.,* EX1017 ¶ 137 (disclosing “varying the probe, forward, and reverse primers concentrations in any way”); *id.* ¶ 9 (“The plurality of [sequence-specific] probes can include one or more groups of probes at varying concentrations. The one or

more groups of probes can include the same detectable label which will vary in intensity upon detection, due to the varying probe concentrations.”); *id.* ¶ 12 (“Certain members of the plurality of probes include a detectable label....The plurality of probes can include one or more groups of probes at varying concentrations. The one or more groups of probes can include the same detectable label which varies in intensity upon detection, due to the varying probe concentrations.”); *see also id.* ¶¶ 91, 94, 130, claim 6 (“the plurality of probes comprise one or more groups of probes at varying concentrations.”); *id.* ¶ 146 (disclosing “tuning the probe concentration”); *id.* ¶ 150 (disclosing “varying the probe concentrations to optimize the pattern of colors and intensities for increased resolution”); *id.* ¶ 151 (disclosing “changing the probe concentration alone” or “changing the primer concentrations alone”); *id.* Figs. 9, 12C.

284. As described above, this disclosure teaching assays to perform such steps would disclose to a POSA the physical components to perform such an assay, i.e., kits, and those kits would be capable of performing the same steps.

285. Accordingly, a POSA would have understood that the types of assays in Larson are those that would use a kit where the analyte-specific reagents are each present at a different amount in the kit.

XII. GROUND 2: OBVIOUSNESS OF CLAIMS 4, 7, 14-19 IN VIEW OF LARSON

- A. Claim 4: The assay of claim 2, wherein said coding scheme is made nondegenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.**

286. As described above, Larson discloses the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A. As described above, Larson alone, or in combination with Saxonov, renders obvious the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See infra* Section XIII.A.

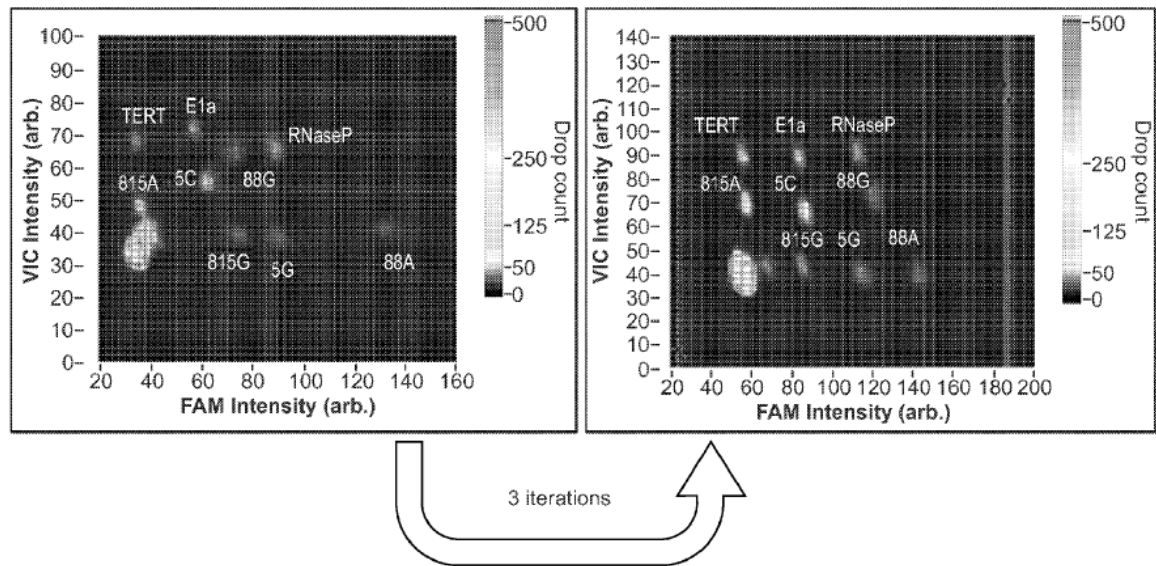
287. Larson further discloses the method of claim 2, *see supra* Section XI.B, and to the extent Patent Owner contends it does not, Larson, in further view of Saxonov renders obvious the method of claim 2. *See infra* Section XIII.B.

288. Larson discloses the method of claim 4 wherein the coding scheme is made non degenerate. *See supra* Section XI.D. To the extent Patent Owner contends it does not, It is my opinion that Larson renders obvious the additional limitation of claim 4 wherein assays that practice the limitation: “said coding scheme is made nondegenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating

at least one potential analyte code from said coding scheme to eliminate degeneracy.”

289. A POSA, in view of Larson would have been motivated to eliminate degeneracy in an assay. A POSA would have understood that Larson, in teaching higher-plex assays, i.e., those that detect multiple analytes, that it would have been desirable to detect multiple analytes in a sample. *See, e.g.*, EX1017 ¶¶ 139-145. Further, Larson specifically notes that the “need for higher throughput in analyzing multiple targets in parallel continues to escalate.” *Id.* ¶ 5. Thus, a POSA would have been motivated to eliminate results that would cause ambiguity or degeneracy in the results of an assay.

290. A POSA would have further had a reasonable expectation of success in eliminating such degenerate codes in view of Larson. Larson teaches, for example, a process of optimizing an assay to generate definite results. *Id.* ¶¶ 35, 146, Fig. 15. For example, Figure 15 depicts, as shown below, results before and after optimization of a nine-plex assay:



Id. Fig. 15.

291. As described in Figure 15 above, over multiple iterations, the clusters representing every legitimate result of the presence of different targets became more readily apparent and separated. It is apparent that every result that is present on the optimized scattered plot, is present on the left as well, which a POSA would have understood to constitute “enumerating every legitimate result that can be obtained from said coding scheme” as required by the claim. Over the optimization process, the codes of different analytes are shifted to achieve the benefit of making the assay nondegenerate. For example, on the left of Figure 15 above, target “E1a” is at a VIC intensity of ~75 and a FAM intensity of ~50, while on the right it has moved to a VIC intensity of ~90 and a FAM intensity of ~80, allowing for easier differentiation

between the targets. Also, as is apparent, every result that is present on the optimized scattered plot, is present on the left as well, which a POSA would have understood to constitute “enumerating every legitimate result that can be obtained from said coding scheme” as required by the claim.

292. A POSA would have understood that this optimization process is functionally no different than eliminating an analyte code that is degenerate to remove the degeneracy. They would have understood that in the process of optimization, the optimized analyte codes are necessarily eliminated, and subsequently replaced, to eliminate degeneracy and detect the signals representing the targets unambiguously.

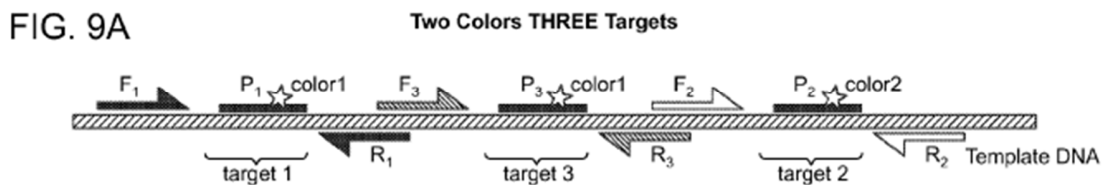
293. Thus, it would have been obvious to a POSA in view of Larson to enumerate every legitimate result, identify those that are degenerate, and eliminate at least one potential analyte code from the coding scheme to eliminate such degeneracy.

B. Claim 6: The assay of claim 5, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.

294. As documented above, Larson anticipated claims 1 and 5. *See supra* Sections XI.A, XI.E. To the extent Patent Owner contends claim 6 is not anticipated, it is rendered obvious in view of Larson, which explicitly teaches using sets of

hybridization probes including one or more probes specific for different analytes that include an identical fluorophore. *See supra* XI.F.

295. As discussed, Larson teaches using hybridization probes, where those probes are specific for different analytes, and teaches that the probes may comprise identical fluorophores or combinations of fluorophores. Specifically, as discussed, Larson teaches using the same fluorophore (color) for different analytes. *See supra id.*; EX1017 ¶¶ 121 (“Methods...involve...quantitation of multiple different DNA targets...using probes with the same fluorophore.”), 135, 140, Figs. 9A, 12B. Additionally, Larson depicts, in Figure 9A, a multiplex PCR that detects three targets using only two fluorophores:

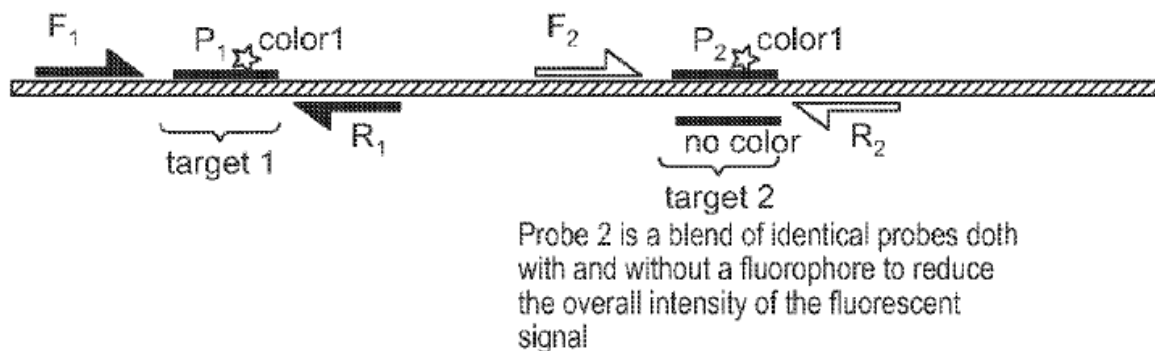


Id. Fig. 9A, ¶ 121; *see supra* Section XI.A. As shown above, above, two probes P₁ and P₃ each comprise “color1,” i.e., the same fluorophore for target 1 and target 3.

296. Larson teaches, as depicted in Figure 12B, using a combination of fluorophores for different analytes. EX1017 ¶ 135, Fig. 12B. As shown below,

multiple colors are used for probes where each probe is specific for a target, which provides a combination of color signals:

FIG. 12B



Id. Fig. 12B, ¶¶ 32, 135, 140. As shown above, above, two probes P₁ and P₂ each comprise “color1,” i.e., the same fluorophore for target 1 and target 2.

297. Larson further discloses, as in the two-color, three-target assay detailed above, using one color for one target, a second color for a second target, and both colors for the third target. In this example, hybridization probes specific for the first and third targets comprise the same fluorophore (i.e., the fluorophore that emits the color A), as do the probes specific for the second and third targets (i.e., the fluorophore that emits the color B). *Id.* ¶ 140. Specifically, the described assay makes use of “four different probes for measuring three different targets.” *Id.* In this assay, two “probes might be of color A (say, A1, and A2), and two probes might

be of color B (say, B1 and B2). *Id.* Probe A1 detects target 1, probe B1 detects target 2 and probes A2 and B2 each detect target 3. *Id.* A POSA would have understood Larson to thus disclose hybridization probes specific for different targets that comprise an identical fluorophore as probes A1 and A2 both comprise a fluorophore of color A and are specific for targets 1 and 3, respectively while probes B1 and B2 both comprise a fluorophore of color B and are specific for targets 2 and 3, respectively.

298. Given this explicit teaching in Larson as described above, it would have been obvious for a POSA to have used an identical fluorophore on different probes specific for different analytes in obtaining same-color signals, such as taught in paragraph 140.

299. A POSA would have been motivated to use the same fluorophore in such an assay. A POSA would have understood the relative ease of obtaining the same color signals when using the same fluorophore, and the utility in expanding the number of targets that can be detected in an assay. A POSA would have had a reasonable expectation of success in performing this kind of assay as Larson discloses this very technique. EX1017 ¶ 121, Figs. 9A, 9C.

C. Claim 7: The assay of claim 5, wherein said fluorescence signal from said hybridization probes are used to construct a chromatogram.

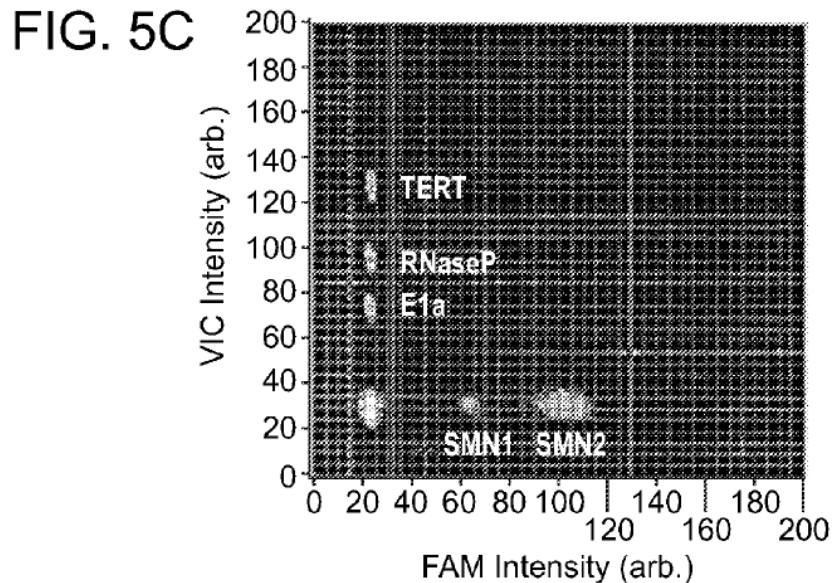
300. As described above, Larson discloses the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A. As described above, Larson alone, or in combination with Saxonov, renders obvious the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See infra* Section XIII.A.

301. Larson further discloses the method of claim 5, *see supra* Section XI.E, and to the extent Patent Owner contends it does not, Larson, in further view of Saxonov renders obvious the method of claim 2. *See infra* Section XIII.B.

302. It is my opinion that to the extent Patent Owner contends Larson does not anticipate the additional limitation of this claim as described above in Section XI.G, that Larson renders obvious the additional limitation of claim 7: “wherein said fluorescence signal from said hybridization probes are used to construct a chromatogram.”

303. As discussed above Larson discloses a “scattered plot.” Larson discusses constructing these scattered plots: “the fluorescence emission from each droplet was determined and plotted on a scattered plot based on its wavelength and intensity.” EX1017 ¶¶ 113, 116. Exemplary scattered plots depicted in the figures

show each of the potential signal clusters for each analyte. EX1017 Figs. 5C, 6B, 9, 10, 14a, 15. Figure 5C, reproduced below, is exemplary:



Id. Fig. 5C, ¶ 113.

304. These “scattered plots” are nothing more than a representation of the colors and the intensities in those colors that analyte-specific labeled-probes emit when given analytes are present in an assay. These are of the type relied on by Patent Owner in its infringement contentions. EX1012 at 42.

305. As I discuss throughout, Larson teaches in paragraph 140 assays involving three targets and two colors of the type that Patent Owner contends infringe the claims. To the extent Patent Owner contends that Larson does not teach generating a chromatogram in connection with such assays, a POSA would have

nonetheless been motivated, in view of Larson to generate a chromatogram using the fluorescent signals of the assay. In fact, Larson teaches the value of visual representations of fluorescence data collected from hybridization probes, because, as Larson's figures show, the scattered plots allow one to quickly ascertain which targets are present and the relative amounts at which the targets are present. Additionally, the plots allow assessment of the fidelity with which the assay distinguishes among targets as reflected in the spacing between clusters. *See, e.g.*, EX1017 Figs. 5, 10, 14, 15, 17.

306. Further, in view of Larson, a POSA would have had a reasonable expectation of success in generating the claimed chromatograms, including in the form Larson depicts.

307. This depiction is a result of a straightforward and routine matter to plot data obtained from a PCR experiment. First, Larson teaches measuring and differentiating fluorescence signals to represent the presence or absence of specific polynucleotide analytes as a function of color and intensity in the same assay. *See, e.g.*, EX1017 ¶¶ 138 (targets "might be uniquely identified by intensity levels"), 139 ("multiple colors that are spectrally separable can be used simultaneously"). Further, Larson teaches representing these detected signals visually and in differentiable signal groups. *See, e.g., id.* Figs. 5, 10, 14, 15, 17. Thus, a POSA

would have had a reasonable expectation of success in plotting the potential detected fluorescent signal in each color detected as a function of intensity in that color, i.e., in performing each of the steps required to construct a chromatogram as described by the '921 patent. Further, a POSA would have understood the value of obtaining and using such a chromatogram in representing potential signals and in determining the presence or absence of analytes in particular assays.

308. Accordingly, a POSA would have understood that Larson, to the extent it does not disclose the limitation, renders obvious assays using the fluorescence signal from the hybridization probes to construct a chromatogram.

D. Claims 14-19:

309. It is my opinion, as described above, that the requirement in claims 14-19 for a “kit” is not limiting. *See supra* ¶¶ 217-218. To the extent Patent Owner contends that Larson does not disclose a “kit” it would have been obvious to combine the components disclosed in Larson together in a “kit.”

310. A POSA would have been motivated to combine the components of a kit disclosed in Larson together. A POSA would have understood that it would have been beneficial and appropriate to include the analyte-specific reagents, *e.g.*, primers and probes together in a package. A POSA would have understood through the disclosure of Larson, that probes and primers are used together in an assay. EX 1017

¶ 164. Further, a POSA would have understood that it would be advantageous to package the reagents in a manner that generates already optimized signals to generate distinguished targets as the process is described in Larson. *Id.* ¶¶ 146 (“relative positions of the different populations in the histogram were then adjusted into a regularly spaced rectangular array by tuning the probe concentration”); 150, Fig. 15. Providing kits comprising these reagents would provide the advantage of not needing to perform the described optimization every time one runs an assay, as the probe concentrations would be known to produce distinct populations to allow for the unambiguous detection of targets.

311. A POSA would have had a reasonable expectation of success in combining the reagents into a kit. A POSA would have understood that both probes and primers can be placed together in a solution as Larson teaches assays that do this and there is no reason to expect that there would be any negative reaction of the reagents. *Id.* Further, a POSA would have understood that once the signals were optimized by tuning the probe concentration, that performing an assay using these probe concentrations would produce distinguished population results, allowing for the unambiguous detection of targets.

312. Claim 19 recites that “the analyte-specific reagents are each present at a different amount in the kit.” It is my opinion that Larson discloses the additional

limitation of this claim. *See supra* Section XI.Q. To the extent Patent Owner contends that this limitation is not disclosed, it would have been obvious to a POSA in view of Larson.

313. Larson teaches the use of primers and labeled sequence-specific probes at differing concentrations. *See, e.g.*, EX1017 ¶ 137 (disclosing “varying the probe, forward, and reverse primers concentrations in any way”); *id.* ¶ 9 (“The plurality of [sequence-specific] probes can include one or more groups of probes at varying concentrations. The one or more groups of probes can include the same detectable label which will vary in intensity upon detection, due to the varying probe concentrations.”); *id.* ¶ 12 (“Certain members of the plurality of probes include a detectable label....The plurality of probes can include one or more groups of probes at varying concentrations. The one or more groups of probes can include the same detectable label which varies in intensity upon detection, due to the varying probe concentrations.”); *see also id.* ¶¶ 91, 94, 130, claim 6 (“the plurality of probes comprise one or more groups of probes at varying concentrations.”); *id.* ¶ 146 (disclosing “tuning the probe concentration”); *id.* ¶ 150 (disclosing “varying the probe concentrations to optimize the pattern of colors and intensities for increased resolution”); *id.* ¶ 151 (disclosing “changing the probe concentration alone” or “changing the primer concentrations alone”); *id.* Figs. 9, 12C.

314. A POSA would have been motivated to change the probe and primer concentrations, i.e., amounts, to enhance the differentiation between analytes as “varying the probe and primer concentrations together [is] a very simple technique to optimize relative intensities in higher-plex reactions.” *Id.* ¶ 134. Thus, a POSA would have understood to achieve the unambiguous detection as described by the kit in claim 14 it would be advantageous to provide the analyte-specific reagents at different amounts to optimize the difference in their intensities, and thus in the coding scheme. *See id.* ¶ 137 (“the example above for adjusting probe fluorescence intensities ... is not limited to this method alone for varying probe intensity.”). Thus, a POSA would have understood that it was possible to, and expected reasonable success in varying any of the analyte-specific reagents in the kit to achieve the claimed invention.

315. I have been informed of the case law from *E.I. DuPont de Nemours & Co. v. Synvina CV*, 904 F.3d 996, 1006 (Fed. Cir. 2018). I have been informed that this case stands for the proposition that if “the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation” and that a variable is result-effective when the prior art recognizes that “the variable affects the relevant property or result.” *E.I. DuPont de Nemours & Co. v. Synvina CV*, at 1006, 1009.

316. I have been asked to opine on whether the concentrations of the PCR primers and the fluorophore-labeled sequence-specific probes are known to be result-effective variables for PCR amplification and generation of detectable signal(s) when conducting TaqMan assays. The short answer is: yes. I have already discussed one such instance, which is described in Larson: Larson's disclosed techniques for adjusting/optimizing the intensity of a fluorophore's signal. *See supra* Sections IX.A.2, XI.A, D, G. Larson explains how to adjust, or tune, the fluorescence intensity of TaqMan probes, as "[i]dentifying probes by fluorescence intensity often requires adjusting the brightness of the probes." *Id.* ¶ 134. Some methods that Larson discusses include "varying probe and primer concentrations together by the same factor," "varying just the probe concentration; varying just the primer concentrations; varying just the forward primer concentration; varying just the reverse primer concentration; varying the probe, forward, and reverse primers concentrations in any way;" and "incorporating into the assay some fraction of probes that lack fluorophores." *Id.* ¶ 137.

317. Thus, in view of Larson, this claim is rendered obvious.

XIII. GROUND 3: OBVIOUSNESS OF CLAIMS 1-10 AND 12-19 IN VIEW OF LARSON AND SAXONOV

318. As discussed, the claims require "unambiguously detecting the presence or absence" of analyte targets in a "single sample volume." As Patent

Owner alleges in its infringement contentions, the claimed “single sample volume” is a portion of an original sample solution which may be partitioned into sub-volumes (droplets) later for amplification. *See* EX1012 at 19-20 (“the detection is performed in a single sample volume that is **subsequently** partitioned into multiple droplets.”). As discussed above, that interpretation of “single sample volume” is the same as disclosed in Larson. *See supra* Sections XI.A-Q.

319. I have been informed that Patent Owner might change its interpretation of “single sample volume.” If that occurs, and Patent Owner construes “single sample volume” as limited to a single partition from the original sample solution (e.g., a single droplet), then Larson alone or in combination with Saxonov obviates that interpretation. This is because Larson alone or in combination with Saxonov at a minimum renders obvious the “unambiguous” detection of the “presence or absence” of multiple analytes in such individual sub-volumes.

320. Saxonov and Larson are analogous art to the ’921 patent—and to each other—because they are within the same field of endeavor: multiplex detection of polynucleotides using color-labeled sequence-specific probes, including where there are fewer colors than analytes. EX1001 at Abstract, 12:28-14:3, 17:57-60, 21:39-23:30; EX1004 ¶¶ 19, 20, 37; EX1017 ¶¶ 34, 35, 138-153.

321. Larson expressly teaches that its methods accommodate “multiple occupancy” situations, i.e., when an individual droplet contains multiple droplets. *See* EX1017 ¶¶ 129, 141. The assays in Larson use conditions and parameters that contain this kind of multiple occupancy. To a POSA, Larson at least renders obvious the claims of the ’921 patent that contain the “single sample volume” term. Saxonov also teaches this kind of multiple occupancy situation and can easily distinguish between droplets having differing combinations of targets. As discussed below, Saxonov does this by differentiating the targets’ intensity in the same optical (color) channels. A POSA would have at least found it obvious to incorporate Saxonov’s teachings into Larson to perform assays with this ability.

A. Claim 1

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes

322. As discussed, Larson teaches assays including PCR where probes are “labeled with a fluorescent dye that binds specifically to the amplified sequences,” i.e., unambiguously detecting M analytes. *See supra* Section XI.A; EX1017 ¶¶ 26; *see, e.g.*, EX1001 at 26:18-28 (disclosing that the analytes are those “selected from the group consisting of any 2, 3, 4, ... 100 or more”).

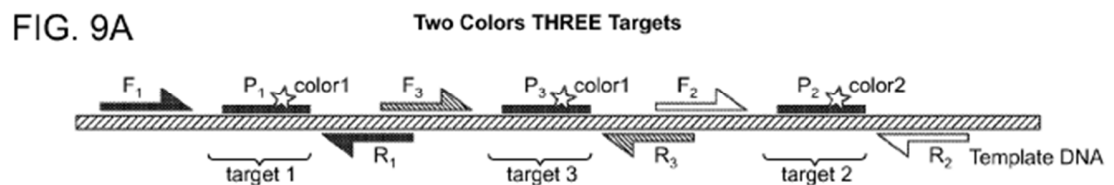
323. As discussed above and below, the way in which Larson “unambiguously” detects the M analytes alone or in combination is explained. *See*

supra Sections XI.A; *see infra* Sections XIII.A.2-6. The way in which Larson in combination with Saxonov “unambiguously” detects the M analytes is explained below. *See infra* Sections XIII.A.2-6.

2. wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,

324. As discussed, Larson teaches a coding scheme for its assays, one of which is a three-analyte assay that is encoded by an intensity of light in one or two colors for each analyte. *See supra* Sections XI.A.1-4; *see, e.g.*, EX1017 ¶¶ 32, 135 (describing detecting different sequences using different signal intensity).

325. Larson also teaches the alternative coding scheme, discussed above, which is shown in Figure 9:



EX1017 Fig. 9A. Figure 9 depicts coding three targets using only two colors. As shown, targets 1-3 are labeled with probes P₁₋₃ via corresponding forward and reverse primer pairs F₁₋₃ and R₁₋₃. *Id.* ¶ 121. The first color, color1, is used in probes

P₁ and P₃ while the second color, color2, is used in P₂. *Id.* ¶ 121. Probes P₁ and P₃ are differentiable because they fluoresce at a different intensity. *Id.* The difference in intensity for the color1 signal from targets 1 and 3 is used to distinguish the signals; while the difference in color between the color1 targets 1 and 3 and the color2 target 2 is used to distinguish those signals. This scheme is an example of an approach that differentiates three analytes by encoding them in one of two wavelengths of an intensity of fluorescent light (a part of the detecting signal) and allows for unambiguous detection of the three targets. EX1017 ¶ 121, Fig. 9C.

326. As an additional example, Larson teaches assays wherein three analytes are encoded in two colors, by the use of two fluorophores. For example, Larson discloses:

[A] single droplet might contain four different probes for measuring three different targets (say, T1, T2, and T3). Two probes might be of color A (say, A1, and A2), and two probes might be of color B (say, B1 and B2). T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2. Thus, when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B. However when a droplet contains T3, increased fluorescence appears in both colors A and B.

EX1017 ¶ 140.

327. As also explained above, this is the coding scheme exactly described as infringing claim 1 of the '921 patent by Patent Owner in the parallel district court litigation.

3. wherein said fluorescent signal comprises F wavelength components, and

328. As just discussed, Larson teaches assays that include fluorescent signals that comprise two different wavelength components. *See supra* Sections XI.A.1-2, 4 (discussing Larson paragraph 140 (“T1 is measured by probe A1, T2 is measured by probe B1, but T3 is measured by both probes A2 and B2. Thus, when a droplet contains T1 only increased fluorescence appears in color A. When a droplet contains T2 only increased fluorescence appears in color B. However when a droplet contains T3, there is an increased fluorescence that appears in both colors A and B.”)), XIII.A.2.

329. Also, as described above, a POSA would have understood Larson to teach an exemplary assay that makes use of 2 wavelength components, i.e., colors. Specifically, Larson teaches that in an assay for detecting three targets, “Two probes might be of color A (say, A1, and A1), and two probes might be of color B (say, B1 and B2).” EX1017 ¶ 140. In the example, target 1 emits a signal in color A, target 2 emits a signal in color B, and target 3 emits a signal in both color A and color B. *Id.* Thus, in this example, F=2.

4. **wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,**

330. In Ground 1, this section defined “single sample volume” the way Patent Owner has described it, that a “single sample volume” is the portion of the original sample mixture prior to partitioning into droplets. *See supra* Section XI.A.4, 6, XI.G. In this Ground 2, “single sample volume” is defined as an individual droplet that has been generated as a result of the partitioning of a sample mixture. With this mapping, the claims are rendered obvious in view of Larson alone or in a further combination with Saxonov.

a. Larson Alone Renders Obvious Unambiguously Detecting Multiple Targets In A Single Droplet

331. Larson focuses on assays using droplets that contain only one target analyte, but does teach scenarios where individual droplets contain multiple target droplets because this eventuality is inevitable when using a Poisson distribution, as discussed above. *See supra* Section XI.A.4, XI.G; EX1017 ¶ 6.

332. Larson teaches a Poisson distribution using “limiting dilution” wherein “some reactions contain zero DNA molecules, some reactions contain one molecule, and frequently some other reactions contain multiple molecules.” EX1017 ¶ 6. Larson also discusses “terminal dilution” wherein “the vast majority of reactions

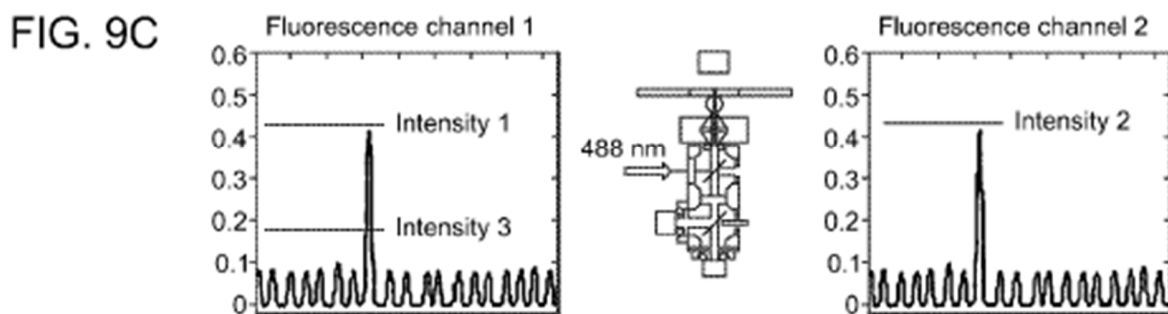
contain either one or zero target DNA molecules for practical intents and purposes,” but that terminal and limiting dilutions are not “necessarily mutually exclusive.” *Id.* This means that sets of reactions must be occurring in droplets that contain multiple target molecules. Larson also discloses, in describing droplet formation, “the invention is compatible with all distributions of DNA loading that conform to limiting or terminal dilution.” *Id.* ¶ 52. With these disclosures, Larson is explicitly teaching that its assays work when a single droplet contains multiple analyte targets. *See also id.* ¶ 141 (“With multiple occupancy arises the complexity of simultaneous assays competing within the same reaction droplet, and also complexity of assigning the resulting fluorescence intensity that involves a combination of fluorescence from two different reaction products that may or may not be equal to the sum of the two fluorescence intensities of the individual reaction products. However, methods of the invention can accommodate these complications arising from multiple occupancy.”).

333. Figure 9 shows one such way to accommodate these complications. Larson depicts, in Figure 9A, a multiplex PCR which detects three targets using only two fluorophores:



Id. Fig. 9A.

334. As shown, targets 1-3 are detected with labeled probes P₁₋₃ via corresponding forward and reverse primer pairs F₁₋₃ and R₁₋₃. *Id.* ¶ 121. The first color, color1, is used in probes P₁ and P₃ while the second color, color2, is used in P₂. *Id.* ¶ 121. Probes P₁ and P₃ are differentiable because they fluoresce at a different intensity. *Id.* The difference in intensity for the color1 signal from targets 1 and 3 is used to distinguish the signals; while the difference in color between the color1 targets 1 and 3 and the color2 target 2 is used to distinguish those signals. This differentiation allows for unambiguous detection of the three targets. EX1017 ¶ 121, Fig. 9C. Figure 9C explains and shows the intensity of the signals, which allows for the differentiation between the signals:



Id. Fig. 9C. As shown, the signal from target 1 has an intensity of slightly greater than 0.4, while the signal from target 3 has an intensity of slightly less than 0.2. *Id.*

335. For clarity, below are the signatures of the signals for the targets as represented in tabular format:

	Color 1	Color 2
Intensity slightly less than 0.2	Target 3	NA
Intensity slightly greater than 0.4	Target 1	Target 2

As made clear, this table shows that each target has its own individual, and completely unique, color and intensity signature. This signature does not overlap with any other analyte's signature.

336. A POSA would have readily understood, detected, and distinguished these signals from one another. Specifically, because the slightly less than 0.2-intensity signal is not multipliable into the slightly more than 0.4-intensity signal,

even a multiple of intensity 3 could be distinguished from intensity 1 because the various combinations of analytes would result in distinguishable signals for the three analytes. The signals from P1, P2, P3, P1+P2, P1+P3, P2+P3, and P1+P2+P3 have different intensities as the combinations are additive and would each sum to unique values in each of the two colors used. EX1017 ¶ 121, Fig. 9C. As discussed, this unique sum of values for the different analytes provides a method for a POSA to use the assays in Larson to detect multiple analytes in one droplet.

337. Accordingly, Larson itself discloses to a POSA an assay that determines without ambiguity the presence or absence of three different targets (and combinations thereof) in individual droplets. A POSA would have had a reasonable expectation of success in handling “multiple occupancy” in a single droplet because Larson explicitly discusses this eventuality when using Poisson distribution of DNA.

338. Additionally, a POSA would have been motivated to use this strategy because limiting an experiment to only single-target droplets severely impacts the total data collected in that experiment. These assays, especially at the time of the '921 patent, were quite costly to set up and run, for example, as discussed in Larson, many reagents are required. *See, e.g.*, EX1017 ¶ 68. The ability to acquire data not just from a fraction of the droplets, but from all droplets in the experiment would thus be extremely cost effective. Additionally, by allowing more DNA into each

droplet, the dilution factor for the sample decreases, meaning less sample exists and less run-time on the machine is necessary. *See* EX1017 ¶ 96.

b. Larson In Combination With Saxonov Renders Obvious Unambiguously Detecting Multiple Targets In A Single Droplet

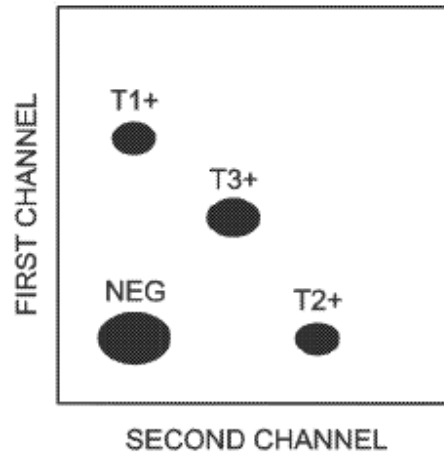
339. If Patent Owner disagrees with the above, that Larson renders obvious the unambiguous detection of multiple targets in a single droplet, the combination of Larson in further view of Saxonov renders obvious this unambiguous, multiple-occupancy detection.

340. As discussed, Saxonov also teaches a dPCR assay. *See supra* Section IX.B.2. As also discussed, Saxonov teaches loading droplets using a Poisson distribution. *Id.*; EX1004 ¶¶ 3 (the “probability of finding exactly 0, 1, 2, 3, or more copies in a partition, based on a given average concentration of analyte in the partitions, is described by a Poisson distribution”), 43 (“The calculation may be based on each target having a Poisson distribution among the droplets.”).

341. Specifically important here, Saxonov teaches the method with results depicted in Figure 10. This method was used to detect three targets using just two fluorophores: T1 is identified by a first color signal, T2 by a second color signal, and T3 is identified by the presence of both colors’ signals. EX1004 ¶¶ 18, 78-79; *see also* EX1007 at 9:8-10 (the provisional application, which Saxonov claims priority

to, also teaches this method, stating that signals can be “collected in one or more different channels” from “the same” target).

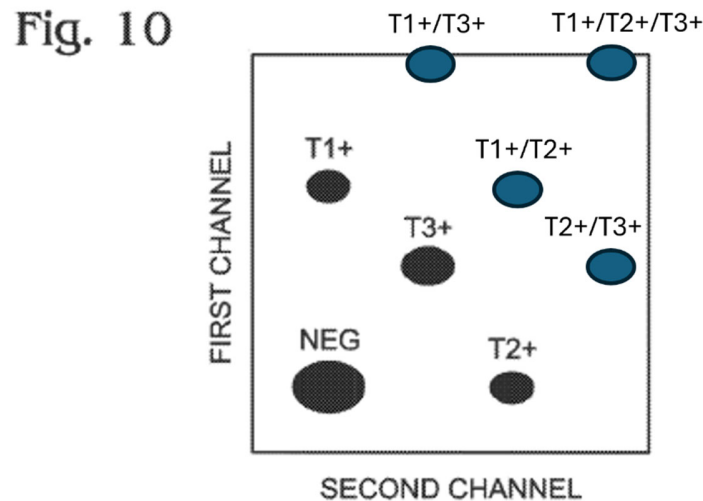
Fig. 10



EX1004 Fig. 10. As discussed above, this is the same exact approach that Larson teaches, that Ground 1 is based upon, and that Patent Owner accuses of infringement. *See supra* Section XI.A.

342. “To simplify the presentation,” Figure 10 only displays the signals from droplets that contain a single target, highlighting how T3 positive droplets fluoresce in both channels. EX1004 ¶ 78. However, Saxonov explains that “double-positive populations (T1+/T2+, T1+/T3+, and T2+/T3+) and the triple-positive population (T1+/T2+/T3+)” could be displayed. *See, e.g., id.* Such extrapolation is not difficult either, given the consistencies in fluorescence intensities that these fluorophores have. A modified version of Figure 10 is produced below, to show how these

double-positive and the triple-positive droplets would look if included. While a touch more complicated looking, as discussed, this is not particularly difficult to measure or convert into a display:



343. Saxonov states that “three targets may generate eight clusters or populations of data, *separated by intensity*,” which is what has been done in this modified figure. EX1004 ¶ 20. As can be seen, this representation shows how easily each of three targets, however they may be distributed in droplets, are to identify.

344. As discussed above, Larson teaches this same technique, the unambiguous detection of analyte targets distributed in various combinations within single droplets using the intensity of the signals as the distinguishing marker. See *supra* Sections XI.A, G.

345. I have been informed that Patent Owner may argue otherwise though, that Larson does not disclose this. If so, then this concept, as explained, is still disclosed through the combination of Larson and Saxonov. Specifically, Larson teaches how to identify three different targets with two colors, based on their difference in intensity, and teaches that multiple targets can be in a single droplet, and which “multiple occupancy” its methods could handle. *See supra* Section XI.A; EX1017 ¶¶ 129, 140-141.

346. Saxonov similarly teaches using two colors to identify three analytes based on differences in the intensities of the signals. EX1004 ¶ 20. A POSA would have already known from Larson that analyte targets could be differentiated based on intensity, so separating out the targets “by intensity,” as Saxonov teaches, would have been the logical next step. *Id.* A POSA would have been highly motivated to do this as well, as discussed, to acquire the maximum amount of information from this kind of costly experiment, *see supra* Section XII.A, but also to adequately deal with the “multiple occupancy” situations that Larson discusses arise when loading targets into droplets with a Poisson distribution. *See, e.g.*, EX1017 ¶¶ 129, 140-141.

347. Perhaps this goes without saying, but a POSA would have had a reasonable expectation of success in achieving these results, since Larson and Saxonov teach the same assay. But, more specifically, Larson teaches, as discussed,

tuning the intensity of the probes that correspond to a target in order to optimize the probe's detection. *See supra* Sections IX.A.2, XI.A, K; EX1017 ¶¶ 134-137, 146. This was done in the same manner as Saxonov teaches differentiating combinations of targets based on color signals generated and the intensities in those colors. For example, as discussed with regards to Figure 10 above, where the intensity of any unique combination of single occupancy, double occupancy, and triple occupancy droplets all differ from each other based on their unique color and intensity signature. EX1004 ¶¶ 51-52 (“The signals may be distinguishable because an aspect of the fluorescence is different for one fluorophore than for the other fluorophore(s). For example, the intensity associated with one fluorophore, following reaction, may be lower or higher than the intensity(ies) associated with the other fluorophore(s).”). Using this technique, Larson discloses distinguishing between nine types of droplets, as shown in Figure 15, reproduced below.

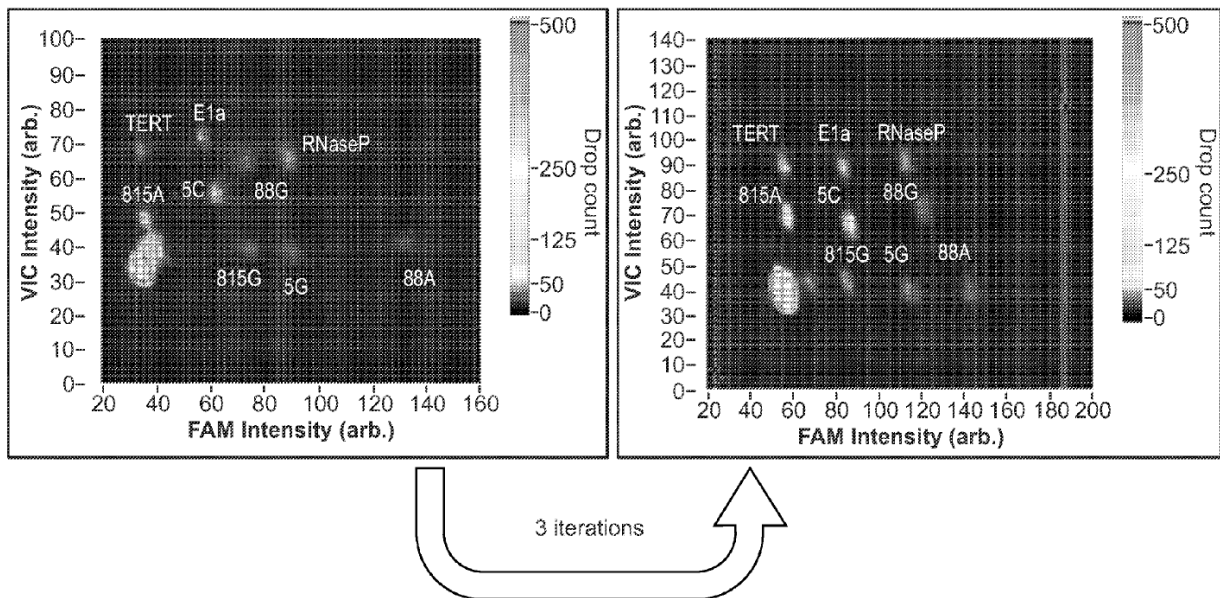


FIG. 15

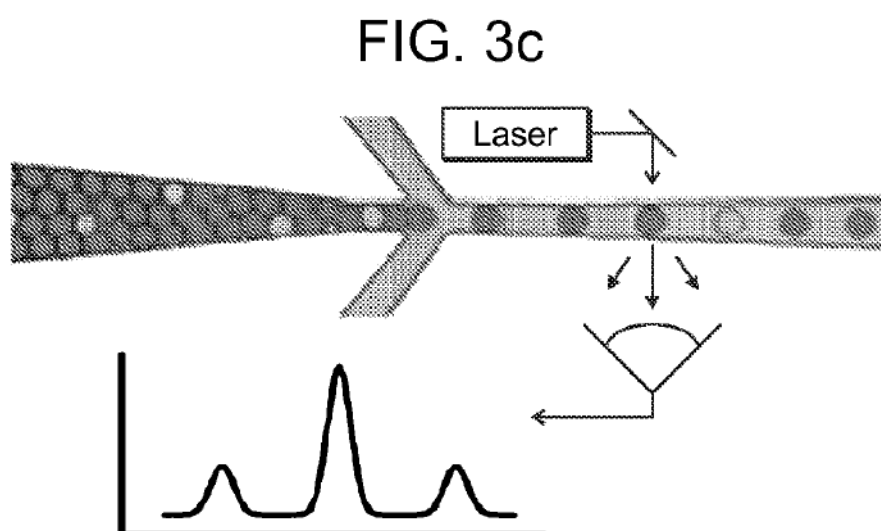
EX1017 Fig. 15. Each label in the figure is a population of droplets containing the specified target. *Id.* ¶¶ 35, 150-151. After three iterations of using Larson's disclosed intensity adjusting technique, the populations are each clearly identifiable and regular. *Id.*

348. Given that Larson had already taught easily adjusting intensity to distinguish among different droplet types, a POSA would have easily been able to, and expected to, implement Saxonov's approach, shown in Figure 10, with Larson.

5. **wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and**

349. As discussed, Larson teaches assays that do not require mass spectrometry or immobilization of the analytes. *See supra* Section XI.A.5.

350. This is shown best in Figure 3C, reproduced here:



EX1017 Fig. 3C.

351. As shown, the droplets containing the analytes with their colored probes pass through a channel with a laser, which causes the fluorescence of the probes. *Id.* This fluorescence is then detected with no need for the sample to be immobilized nor for mass spectrometry to be used. EX1017 ¶¶ 47, 52-54, 69, 70-72, 83, 89, Fig. 1-3.

6. wherein the analytes are amplified in the single sample volume.

352. As discussed above, Larson teaches assays that use PCR amplification of target analytes in droplets. *See supra* Sections XI.A.1-4, 6, XII.A.4.a. As explained above, and as Patent Owner contends, an individual droplet is a “single sample volume.” *See supra* Sections XI.A.1-4, 6, XIII.A, XIII.A.4.

B. Claims 2-10.

353. As discussed Larson discloses these claims. *See supra* Sections XI.B-J. Additionally, Larson at least renders obvious claims 2-10. *See supra* discussion in Sections XI.B-J, XII.A, B, C.

C. Claim 12: The assay of claim 1, wherein at least one step of said assay is performed using a computer and wherein said computer is connected to a thermal cycler.

354. As described above, Larson discloses the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A. Additionally, as described above, Larson alone, or in combination with Saxonov, renders obvious the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XIII.A.

355. It is further my opinion that Larson discloses the further limitations of claim 12. Larson states that an apparatus for performing its assays can include

“processors (e.g., computers and software).” EX1017 ¶ 89 (“[S]uitable detection apparatuses include optical waveguides, microscopes, diodes, light stimulating devices, (e.g., lasers), photo multiplier tubes, and processors (e.g., computers and software), and combinations thereof, which cooperate to detect a signal representative of a characteristic, marker, or reporter, and to determine and direct the measurement.”).

356. A POSA would have understood Larson to disclose that this computer would be used to perform calculations, including calculations “to detect a signal” and “direct the measurement” that would be required for the steps of the method. *Id.* A POSA would have also known that this computer would be used to control the thermal cycler which is required for PCR, *see supra* Section V.B, because Larson discloses “varying the thermal cycling program,” which is and was done by a computer, which would have required that the computer be at least operatively connected to the thermal cycler. EX1017 ¶ 137; *see also id.* ¶¶ 5 (discussing “commercial real-time thermal cyclers” in the context of multiplex PCR using colored fluorophores), 78-79 (“Once final droplets have been produced by any of the droplet forming embodiments above, or by any other embodiments, the droplets are thermal cycled, resulting in amplification of the target nucleic acid in each droplet...Temperature profiles for thermal cycling can be adjusted and optimized as

with any conventional DNA amplification by PCR.”), 82-85 (discussing the same thermal cycling used for amplification and optimization strategies), 137 (discussing “varying the thermal cycling program” used as part of the method of the alleged invention).

357. To the extent Patent Owner contends otherwise, that Larson, as explained, does not disclose or render obvious performing a step of claim 1 using a computer, it would have been obvious in view of Saxonov.

358. Saxonov discloses an apparatus 60 for performing its assay that includes:

[A] partitioning assembly, such as a droplet generator 62 (“DG”), a thermal incubation assembly, such as a thermocycler 64 (“TC”), a detection assembly (a detector) 66 (“DET”), and a data processing assembly (a processor) 68 (“PROC”), or any combination thereof, among others. *The data processing assembly may be*, or may be included in, *a controller that* communicates with and *controls operation of any suitable combination of the assemblies....*Any suitable combination of the assemblies may be operatively connected to one another.

EX1004 ¶ 45, Fig. 2. This data processing unit is a computer.

359. Further, Saxonov discusses that signals “may be detected from the droplets with detector 66. The signals may be processed by processor 68 to determine numbers of droplets and/or target levels,” among other data. *Id.* ¶ 46.

Additionally, as Saxonov describes in the quote above, any “suitable combination of the assemblies may be operatively connected to one another.” *Id.* ¶ 45. This means that the processor/computer can be and is connected to the thermocycler/thermal cycler. *Id.* ¶¶ 45-46 .

360. With these teachings from Larson and Saxonov, a POSA would have found it obvious to use a computer to perform the droplet generation, thermal incubation, detection, and data processing steps of such an assay. The use of a computer facilitates and allows for the necessary operations and calculations required to measure all intensity signals in order to determine whether any analytes are present. A computer also allows for improved control and convenience, as opposed to taking measurements manually. The claims require detection steps and data processing steps (*see, e.g.*, claim 1.4, *supra* Sections XI.A.4, XIII.A.4) and having control of the detection apparatus while it detects signals meets these required steps. A POSA would have had a reasonable expectation of success in using a computer for this purpose. Additionally, as discussed, a POSA would have understood that, since the computer controlled the thermal cycler, the thermal cycler and computer must be connected, and this is disclosed in Saxonov. EX1004 ¶¶ 45-46. This kind of connection and control between the computer and thermal cycler is more convenient than, as Saxonov discloses, “one or more of the assemblies [being]

unconnected to the other assemblies, such that, for example, material/data is transferred manually.” *Id.* ¶ 45. Given Saxonov discloses and shows how to connect the apparatus, a POSA would have had a reasonable expectation of success in doing so.

361. Accordingly, a POSA would have had a reasonable expectation of success in performing the method of claim 1 wherein a computer performs at least one step of the method and is connected to a thermal cyclor.

D. Claim 13: The assay of claim 1, wherein said coding scheme is innately nondegenerate or non-degenerate by construction.

362. As discussed above Larson discloses claim 1. *See supra* Section XI.A. Further, as additionally discussed above, to the extent Patent Owner contends Larson does not anticipate claim 1, Larson in further view of Saxonov would have rendered obvious claim 1. *See supra* Section XIII.A.

363. As discussed, Larson discloses this claim. *See supra* Section XI.K. Additionally, if Patent Owner disputes that, then Larson at least renders obvious claim 13. *See supra* discussion in Sections XI.K.

E. Claim 14: A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising analyte-specific reagents, wherein:

364. It is my opinion that Larson anticipates claim 14. To the extent Patent Owner contends that Larson alone does not anticipate a kit for the detection of the

presence or absence of at least M targets as described above in Section XI.L., or render it obvious as described in Section XII.C, it is my opinion that it would have been rendered obvious by Larson in further view of Saxonov.

1. A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising

365. It is my opinion that the preamble is not limiting.

366. To the extent that the preamble is deemed limiting, it is my opinion that Larson discloses or alone renders it obvious the claimed kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement as described above in Sections XI.L.1, XII.C.

2. analyte-specific reagents, wherein: each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy; and each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the value:

367. It is my opinion that Larson discloses the claimed kit comprising analyte-specific reagents as described above in Section XI.L.2.

3. wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single sample volume, in any combination of presence or absence,

and wherein the kit does not require the use of mass spectrometry or immobilization of said M analytes, and

368. It is my opinion, as described above in Section XI.L.3 that Larson discloses this claim limitation. To the extent Patent Owner contends that Larson does not disclose this limitation, the limitation would have been obvious to a POSA in view of Larson alone or in further view of Saxonov as described above in Sections XIII.A.4, 5.

369. To the extent this ground differs from Ground 1, it is my opinion that it primarily differs as to how “single sample volume” is disclosed. In Ground 1, the “single sample volume” is taken to be a portion of the original sample prior to partitioning, whereas here, it is taken to be an individual droplet generated by partitioning.

a. Larson Alone Renders Obvious Unambiguously Detecting Multiple Targets In A Single Droplet

370. It is my opinion that Larson alone renders obvious the detection of multiple targets in a single droplet. For example, Larson teaches the targets present in droplets will follow a Poisson distribution. EX1017 ¶ 6. Larson further teaches the possibility of “multiple occupancy” situations, and, while noting that complications may arise, says that “methods of the invention can accommodate these complications arising from multiple occupancy.” *Id.* ¶ 141.

371. Larson teaches example assays wherein more targets than colors are identified. For example, in Figure 9, Larson detects three targets using two colors, wherein one target is used to identify two of the targets and a second color is used to identify the third. EX1017 ¶ 121, Fig. 9C. The targets identified in a single color are differentiated from one another by the intensity of the signal generated in that color. *Id.*

372. Consistent with this teaching of differentiation based on intensity, a POSA would have understood that it could be determined precisely which targets are present in a droplet because each different possible combination would generate a different color and intensity combination.

373. As such, in view of Larson, a POSA would have found it obvious to perform an assay that would unambiguously determine the presence or absence of three targets in individual droplets using two colors. A POSA would have further understood the teachings of Larson could be applied to successfully determine the targets or combinations of targets present within a single droplet.

**b. Larson In Combination With Saxonov Renders
Obvious Unambiguously Detecting Multiple
Targets In A Single Droplet**

374. To the extent Patent Owner contends that Larson alone does not anticipate or render obvious the ambiguous detection of multiple targets in a single droplet, Larson in further view of Saxonov would render obvious this detection.

375. Saxonov discloses droplets with targets contained in such droplets according to a Poisson distribution, the same as Larson, stating the “probability of finding exactly 0, 1, 2, 3, or more copies in a partition, based on a given average concentration of analyte in the partitions, is described by a Poisson distribution.” EX1004 ¶ 3; *see also id.* ¶ 43.

376. For example, in Figure 10, Saxonov teaches detecting three targets based on two colors. A first target (T1) is identified by a first color signal, a second target (T2) is identified by a second color signal, and a third target (T3) is identified by the presence of both color signals. EX1004 Fig. 10, ¶¶ 18, 78-79.

377. Saxonov teaches that these targets in the example assay may be identified in any possible combination. EX1004 ¶ 78. This is consistent with Saxonov’s teaching that “three targets may generate eight clusters or populations of data, separated by intensity.” *Id.* ¶ 20. Applying this teaching, a POSA would understand that the eight clusters disclosed would correspond to droplets containing

the following combinations: (1) no targets; (2) target 1; (3) target 2; (4) target 3; (5) targets 1 and 2; (6) targets 1 and 3; (7) targets 2 and 3; (8) targets 1, 2, and 3.

378. It is my opinion that a POSA would have been motivated to combine this teaching of Saxonov with Larson to address the “multiple occupancy” situations as described in Larson that result from a Poisson distribution of targets in droplets.

379. A POSA would have further had a reasonable expectation of success in combining these teachings. A POSA would have understood that the signals from droplets can be distinguished easily based on the colors of the signals emitted, and the intensities of those colors. In fact, Larson teaches optimization of the intensities generated when different targets are present to optimize detection in the same way as Saxonov. EX1017 ¶¶ 134-137; EX1004 ¶¶ 51-52.

4. wherein the analytes are [amplified] in the single sample volume.

380. It is my opinion, as described above in Section XI.L.4 that Larson discloses this claim limitation. To the extent Patent Owner contends that Larson does not disclose this limitation, the limitation would have been obvious to a POSA based on Larson alone and/or in further view of Larson as disclosed in Sections XI.L, XII.C, XIII.A..6.

381. As described above, Patent Owner relies on dPCR assays as infringing and contends that the “analytes are amplified in the single sample volume” where

“the single sample is partitioned into individual droplets,” and that “[f]ollowing droplet generation, the sample is amplified in a thermal cycler” prior to reading “each droplet to determine the fraction of positive droplets in the original sample and us[ing] Poisson statistical formulas to determine the absolute quantity.” EX1012 at 27-29.

382. Larson teaches amplification and detection of polynucleotide analyte sequences in droplets, including the generation of color signal, without mass spectrometry or immobilization of the analytes. EX1017 ¶¶ 47, 52-54, 69, 70-72, 83, 89, 96-97, Fig. 1-3.

383. Larson’s assays perform exactly the sort of amplification which Patent Owner contends infringes the ’921 patent. These assays rely on PCR performed in droplets, which Patent Owner may later contend are each single sample volumes, and include techniques which were well-known in the art at the time of the alleged invention, including the TaqMan assay. EX1017 ¶¶ 2, 9, 11, 13, 14, 16, 17, 20, 47, 49 (“Methods of the invention involve forming sample droplets containing a single target nucleic acid, *amplifying the target in the droplets*”), 91 (“In certain aspects, the droplets of the invention contain a plurality of detectable probes that hybridize to amplicons *produced in the droplets*.”), 93 (“The invention is not limited to the

TaqMan assay, as described above, but rather the invention encompasses the use of all fluorogenic DNA hybridization probes”), 119-121, 150, 195, Figs. 1-4, 7-11, 15.

384. To the extent Patent Owner disagrees with the above, Saxonov also discloses this limitation. Saxonov teaches that targets may be amplified “in the droplets.” EX1004 ¶ 46; *see also, id.* ¶¶ 5-8, 16-18, 78, Fig. 9.

385. A POSA would have had a reasonable expectation of success in performing the described amplification. Such amplification of polynucleotide targets was well-known in the art in the form of digital PCR and droplet-based PCR applications, including those based on TaqMan assays and other well-known amplification-based methods to allow for detection in a droplet. *See, e.g.,* EX1017 ¶ 93.

F. Claims 15-18.

386. As discussed Larson discloses these claims. *See supra* Sections XI.M-P. Additionally, if Patent Owner disputes that, then Larson at least renders obvious claims 15-18. *See supra* discussion in Sections XI.M-P, XII.C.

G. Claim 19: The kit of claim 14, wherein the analyte-specific reagents are each present at a different amount in the kit.

387. As discussed Larson discloses this claim. *See supra* Section XI.Q. Additionally, if Patent Owner disputes that, then Larson at least renders obvious claim 19. *See supra* discussion in Section XII.D.

**XIV. GROUND 4: CLAIM 11 WOULD HAVE BEEN OBVIOUS IN VIEW
OF LARSON, SAXONOV, AND SILVERBROOK**

A. Claim 11: The assay of claim 1, further comprising transmitting information concerning the presence or absence of at least one of said analytes through a computer network.

388. As described above, Larson discloses the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XI.A. As described above, Larson alone, or in combination with Saxonov, renders obvious the method of claim 1 that unambiguously detects the presence or absence of polynucleotide analytes. *See supra* Section XII.A.

389. Silverbrook is analogous art to Larson, Saxonov, and the '921 patent because it also relates to detecting nucleic acid analyte detection/testing employing PCR and hybridization. EX1009 at Abstract, ¶¶ 195-243, Fig. 99.

390. It is further my opinion that claim 11 would have been obvious in further view of Silverbrook. Silverbrook discloses the further limitations of claim 11. Silverbrook teaches assays for nucleic acid testing that uses a device and method which employs PCR, as well as hybridization of sequence-specific probes, to identify the analytes present in a sample. *See supra* Section VIII.B. For example, Silverbrook states that its device, “Test module 10,” “uses a fluorescence-based detection technique to identify target molecules.” EX1009 ¶ 195. Test module 10 amplifies specific nucleic acid analytes and generates fluorescent signals for

detection. *Id.* ¶ 198. Then, Silverbrook sends the information about the analytes that its device receives from the fluorescence emission to, e.g., update medical records. *Id.* ¶ 201 (“data generated by the test module 10 can be used to update, via...network 125,...electronic medical records”), Fig. 99. A POSA would have understood that information regarding an assay can be transmitted through a computer network from this explicit disclosure. This network is depicted in Figure 99, reproduced below:

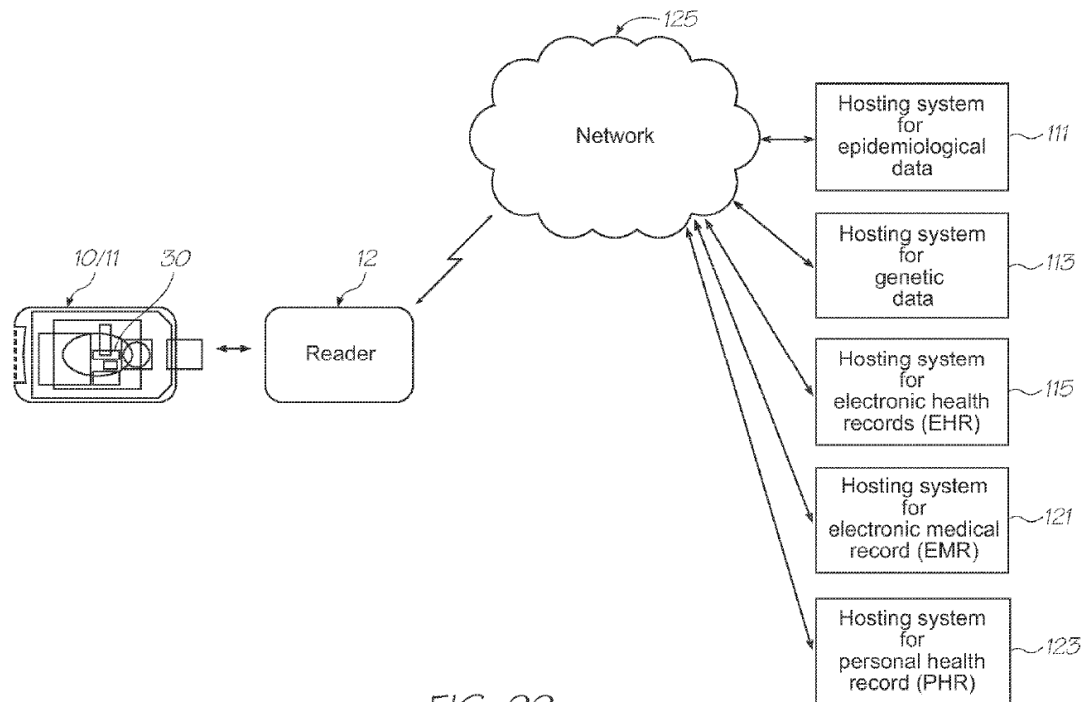


FIG. 99

Id. Fig. 99.

391. Additionally, Silverbrook teaches that “molecular diagnostic tests have the potential to reduce the occurrence of ineffective health care services, enhance

patient outcomes, improve disease management and individualize patient care” and further teaches that after obtaining results of such molecular diagnostic test the “data may be saved locally and/or uploaded to a network server containing patient records.” EX1009 ¶¶ 9, 199.

392. It is my opinion that a POSA would have been motivated to transmit information obtained concerning the presence or absence of polynucleotide analytes in a sample through a computer network because it provides relevant, useful information to those who could use the information, such as a physician making a healthcare decision based on the presence or absence of a disease marker. In order to communicate the results of an assay, a POSA would have sought to transmit the results through a computer network, as a POSA would have understood that that is the practice for transmitting data. For example, a POSA would have understood that the use of centralized information systems to manage and store data, including the result of assays, is commonly used in clinical laboratories and may be referred to as LIMS (Laboratory Information Management System). A POSA would have understood that these systems operate using computer networks to transmit information. Thus, for information such as the presence or absence of a disease marker, a POSA would have been motivated to transmit such information to a physician through a computer network as soon as the assay produced a result.

393. A POSA would have further had a reasonable expectation of success in transmitting such information over a computer network as transmitting information over a computer network was well known in the art at the time of the alleged invention. Indeed, Larson already disclosed using a computer in its methods, which would only need to be attached to a network to send out signals as Silverbrook did. EX1017 ¶ 89 (“Examples of suitable detection apparatuses include optical waveguides, microscopes, diodes, light stimulating devices, (e.g., lasers), photo multiplier tubes, and processors (e.g., computers and software), and combinations thereof, which cooperate to detect a signal representative of a characteristic, marker, or reporter, and to determine and direct the measurement or the sorting action at a sorting module.”).

DECLARATION

I declare that all statements made herein of 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 16, 2024

By: *Carl Batt*

APPENDIX A

Carl A. Batt

Liberty Hyde Bailey Professor

312 Stocking Hall (Food Science)
Cornell University
Ithaca, NY 14853

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E-mail cab10@cornell.edu

EDUCATION:

October 1981 - Ph.D. Rutgers University, Department of Food Science

October 1979 - M.S. Rutgers University, Department of Food Science

December 1975 - B.S. Kansas State University, Microbiology

ACADEMIC EXPERIENCE:

1998-present **Professor**, *Department of Food Science*

Cornell University

1991-1998 **Associate Professor**, *Department of Food Science*

Cornell University

1985-1991 **Assistant Professor**, *Department of Food Science*

Cornell University

1984-1985 **Research Scientist**, *Department of Applied Biological Sciences*

Massachusetts Institute of Technology

1982-1984 **Postdoctoral Research Associate**, *Department of Nutrition and Food Science*

Massachusetts Institute of Technology

OTHER EXPERIENCE:

2021-present **Managing Partner, Empire Syrups**

Empire Syrups is restarting cultivation of sweet sorghum in New York State and establishing an eco-friendly process to produce a unique and versatile sweetener.

that have their roots in traditional fermentations but take advantage of modern science.

2020-2021 **Food Safety Expert, Ample Hills Creamery**

Food safety expert for the largest ice cream plant in New York City. Served as interim food safety manager and hired permanent food safety manager. Member of the food safety team

2018-2024 **Managing Partner, Salvatore BKLYN, Brooklyn, NY**

Created business plan and product pipeline for line extensions of the fresh cheese business.

Interfaced with all customers on sales, quality and safety of products. Acquired production and distribution rights to Tin Foods LLC. Established a vinegar production process and product line to expand Tin Foods LLC

2018-2024 **Food Safety and Quality Assurance Manager, Salvatore BKLYN, Brooklyn, NY**

Established HACCP and GMP program for fresh cheese operation. Developed a new cream cheese product that has no stabilizers.

2010-present **Adjunct Senior Scientist, MOTE Marine Laboratory, Sarasota, FL**

The appointment was for a sabbatical year and continued on as a consultant to the current President of the MOTE.

2000-present **Principal consultant, Stone Fence Collaborative. Groton, NY**

An organization that is focused on strategic advising and presubmission grant development advice for colleges and universities.

1999-2011 **Director, Cornell University/Ludwig Institute for Cancer Research Partnership Cornell University.**

The Partnership was a multiyear \$20M+ enterprise funded by the LICR that included the design, construction and operation of Good Manufacturing Practice facility on the Cornell Ithaca campus. The CU/LICR Partnership produced Phase I recombinant proteins that were used in clinical trials in the US and throughout the world.

2005-2009 **Founder and President, Illuminaria LLC, Ithaca, NY.**

Illuminaria was an early stage start-up company that sought to commercialize microfluidic technology developed in the Batt Laboratory. Early-stage funding was obtained via the SBIR program.

2004-2010 **Co-Founder, Main Street Science Inc. (not for profit) Ithaca, NY**

Main Street Science was a not-for-profit established to help develop and distribute learning materials for K-12 with an emphasis on STEM.

2001-2004 **Founder, Project Leader, Alliance for Nanomedical Technologies Cornell University**

The Alliance for Nanomedical Technology was a NYS Center for Advanced Technology. The \$3M in funding represented the highest level of support given by the NYS CAT at the time. Its membership included 26 NYS companies and supported collaborative research efforts between the Cornell faculty and these member companies.

1999-2004 **Co-Director, Co-Founder Nanobiotechnology Center Cornell University**

The NBTC was a National Science Foundation supported Science and Technology Center which is one of the largest center-funding mechanisms of the NSF with annual funding of \$4M. The NBTC was also supported by a grant from NYS on the order of approximately \$7M for the 10 years the center held its STC status.

1999-2016 **Co-Founder and Science Advisor, Agave BioSystems, Ithaca NY**

Agave BioSystems is a research and development organization that has conducted research for a number of federal agencies including DOD, NASA, and DOE. Its core activities are in the area of nanofabrication and molecular biology.

1996-2001 **Founder and Director, Laboratory for Molecular Typing, Cornell University**

The Laboratory for Molecular Typing was established as a fee-for-service operation offering customers molecular typing based upon the Dupont Riboprinter. The LMT has as its clients a number of large food, pharmaceutical and specialty chemical companies.

PROFESSIONAL ACTIVITIES:

Graduate Fields:	Food Science, Microbiology, Biomedical and Biological Sciences, Biomedical Engineering, Materials Science, Electrical Engineering
2023-	Chief-Editor , Encyclopedia of Food Microbiology (3 rd Edition)
2005-	Editor , Nanooze (www.nanooze.org)
2015-2016	Subject-Editor , Food Microbiology, Food Science Reference Module
2009-2015	Chief-Editor , Encyclopedia of Food Microbiology (2 nd Edition)
2007-2009	Editorial Advisory Board , Wiley Encyclopedia of Chemical Biology
2003-2008	Advisory Board , Sciencenter (Ithaca, NY)
2001-2004	Editorial Board , Protein Expression and Purification
2001-2005	Editorial Board , Journal of Food Science
1987-2000	Chief-Editor , Food Microbiology
1996-1999	Editor , Encyclopedia of Food Microbiology
1987-1992	Editorial Board , Food Biotechnology
1987-1991	Consultant USAID -NIAB, Faisalabad, Pakistan
1988-1989	Chairman , Central New York IFT

AWARDS:

1988	Yoplait Institute Research Award
1990	Samuel Cate Prescott Award (Institute of Food Technologists)
1997	Teaching Excellence Award (Department of Food Science)
2000	Liberty Hyde Bailey Professor (College of Agriculture and Life Sciences)
2001	Faculty Fellows in Service (Cornell University)
2003	Kaplan Family Distinguished Faculty Fellowship in Service-Learning (Cornell University)
2005	Rutgers Alumni Research Award
2007	NSF Discovery Corps Fellow
2008	CALS Promoting Diversity Award (College of Agriculture and Life Sciences)

ACTIVITIES:

Public engagement media

It's a Nanoworld. Traveling museum exhibition (2003) co-developer. Served as co-PI, developer on tour in the US, 1M+ visitors, supported by the National Science Foundation. On permanent tour in the US.

Too Small to See. Traveling museum exhibition (2008) co-developer. Served as PI, developer, on tour in the US, 1M+ visitors, supported by the National Science Foundation. On permanent tour in the US.

Nanooze. Web-site, print magazine (2005-present). Founder and editor. Each edition is distributed to more than 100,000 students in the U.S., supported by the National Science Foundation.

Nanooze radio series. (2009) Co-developer. Produced in collaboration with Earth Sky, a series of six radio episodes aired 2009 with a total listener audience in excess of 12M. Supported by the USDA.

Science Chronicles. (2009) Co-developer. A series of six pod-casts that tracked the activities of a postdoctoral associate over the course of a year. Produced as a podcast series in collaboration with Earth Sky.

Take a Nanooze Break. (2010-2020). Permanent exhibition at Walt Disney World, Lake Buena Vista, FL. Developer, 3M+ visitors each year, supported by the National Science Foundation.

Nanooze Labs. (2011-2014) A long-term exhibition at Disneyland, Anaheim CA. Developer, 5M+ visitors each year, supported by the National Science Foundation

Technical advisor "Mysteries of the Unseen World" an IMAX film produced by Day's End Pictures Inc. and National Geographic. 2012

University committees

Undergraduate Honors Program, CALS-1990-1991

NIH Biotechnology Training Program Grant Executive Committee 1990-1995

Life Safety, Food Science Department, 1992-present

Planning, A Food Science Department committee that serves to advise the department chairperson, 1993-present

Scientific Administrative Board Cornell Biotechnology Program, Center for Advanced Technology, 1993-1996.

Graduate Admissions-Graduate Field of Food Science and Technology, 1993-1995.

The Future of the Geneva Food Science and Technology Department CALS committee initiated at the request of Dean David Call to help chart the future of the Food Science and Technology Department at the Agricultural Experiment Station in Geneva. 1995

Graduate Admissions-Graduate Field of Microbiology, 1996-2005.

Laboratory for Molecular Typing. Founder and managing director. A facility associated with Biotechnology Institute BioResource Center dedicated to providing genetic typing of bacteria. 1995-2000

Nanotechnology: Frontiers in Biology, Chairman. A workshop sponsored by NSF, National Nanofabrication Users Network and the Cornell Nanofabrication Facility November 20-21, 1996.

Nanofabrication Committee. A committee initiated at the Dean's request on future directions for the College of Engineering in nanotechnology. December 1, 1996-1997

Office of Economic Development. A university committee whose mission is to foster economic development in the community. 1997-1999

Stocking Hall Renovation Committee, 1997-1999

Cornell University Genomics Taskforce. A university committee to explore our future directions in genomics. 1998-2003

Executive Committee Nanobiotechnology Center -Advisory committee to Nanobiotechnology Center. 2000-2004

Life Sciences Building Committee. A university committee to design a new building which will be the cornerstone for genomics at Cornell University. 2000-2002

Life Sciences Advisory Council. A Provost-appointed committee to provide guidance to the Provost in the area of Cornell's investment in the Life Sciences. 2000-2002

Johnson Graduate School of Management Nanotech Club. Faculty advisor 2004-2009

Cornell Nanofabrication Facility User Committee. 2008-2013

IGEM Cornell University Chapter. Faculty advisor 2008-2010

Outside committees

Board of Directors- Ithaca Montessori School. 1998-2002; President 1999-2002, Interim Director, summer 1999

Business Innovation Center. A Tompkins County-Cornell joint venture in business assistance. 1998-2001

Advisory Board. Department of the Army, Research and Technology Directorate. 2000-2002

Bacteriology Advisory Board. American Type Culture Collection 2000-2002

Advisory Board. Sciencenter, Ithaca NY 2003-2007

User Proposal Review Committee Center for Integrative Nanotechnologies, 2008- present (Chair 2012-2019)

External Advisory Board. Center for Catalytic Bioscavenger Medical Defense Research Center of Excellence (U-54), NIH 2009- present (Chair 2012-2019)

Advisory Committee. Active Polymer Center for Pattern Integration. ERC, MEST, Korea 2009-present
WV EPSCoR RII External Technical Advisory Board 2009-2015

*Monitoring and Assessment Panel-*University of Hawaii IMUAIII NSF EPSCoR 2010-2013

External Advisory Board United Tribes Technical College, NSF TCUP 2017-Present

External Advisory Board Intertribal Research & Resource Center NSF 2019-Present

(<https://uttc.edu/irrc/>)

Assignments

*Scientific Advisory Committee-*University of Minnesota-Symposium on Application of Biotechnology in the Food Processing Industry. October 8, 1984. St. Paul, MN.

Biotechnology: Microbial principles and processes for fuels, chemicals and biologicals Lecturer-Summer course Massachusetts Institute of Technology, Cambridge, MA. 1984-1994.

*Zone supervisor-*Environmental Health, Radioisotopes, Stocking Hall 1987-1999

Safety Issues of Transgenic Plants Invited-Rapporteur, Boyce Thompson Institute, Cornell University, Ithaca, NY, May 1-2, 1989

Second Symposium on the Genetic Engineering of Animals Organizing committee-, Cornell University, July 25-28, 1989
Safety of foods of animal origin: Charting the course Invited Participant. College of Veterinary Medicine, Texas A&M University, College Station, TX June 25-28, 1991 (see Acuff, G.R., Albanese, R.A., Batt, C.A. et al. [1991]).
NSF panel member-NSF ERC review team September 29, 1999.
Committee on Science witness-US House of Representatives H.R. 766 Nanotechnology Research and Development Act of 2003. March 19, 2003
NIH-NIAID panel member-SBIR Infectious Diseases and Microbiology 2000-2008.
NIH panel member-SBIR Biodefense Bacterial Agents 2004-2009
NCI Centers of Cancer Nanotechnology Excellence (CCNEs) Review Panel 2005, 2014
Earth and Sky Nanotechnology Advisor 2005-present
DEPSCoR review panel 2007- present
NIH-nano review panel 2007- 2009
Air Force Research Laboratory-Wright Patterson, Advisory Panel- Nano-Bio Institute for Aerospace. 2008
NSF-CREST review panel 2009-present
NSF-SBIR review panel 2008-present
Canadian grant review panel 2007- present
Austrian Science Fund grant review panel 2007- present
Israel Science Foundation grant review panel 2007- present
Republic of Georgia Shota Rustaveli National Science Foundation review panel 2009-present
Singapore National Research Foundation 2009-present
NIH F31 Fellowship review panel 2009-2019
NIH RC review panel 2009
Research Corporation for Science Advancement review panel 2009-present
American Chemical Society-Petroleum Research Fund reviewer 2009-present
Department of Energy reviewer 2010-present
NIH SEPA review panel 2010
USDA-Nanotechnology for Agricultural and Food Systems- Panel manager 2011-2012
International Review Committee for the Biotechnology and Food Engineering Faculty, Technion, Haifa Israel 2011
Reviewer, North Dakota EPSCoR 2011-present
DOE- Center for Integrated Nanotechnology-review panel chair 2011-
NSF ERC review panel 2011
Reviewer, Rhode Island EPSCoR 2011-present
Sitting Bull College, advisor/evaluator Research Experience for Undergraduates Summer program, 2011
Science Foundation Ireland review panel 2011-present
Reviewer, South Dakota EPSCoR 2011-present
Israeli Council for Higher Education International Assessment Committee for Biotechnology and Biotechnology Engineering. Chair, 2012.
Reviewer, North Dakota EPSCoR 2011-present
Strategic advisor, Rochester Institute of Technology 2012-present
Science Foundation Ireland site visit review panel-Trinity College, Dublin. Chair, 2012
Strategic advisor, George Mason University, 2012-2018
MITACS Canada reviewer 2013-present
Reviewer, Guam EPSCoR 2011-2015

Reviewer, West Virginia EPSCoR 2011-present
 Technical advisor "Unseen Worlds" an IMAX film produced by Day's End Pictures Inc. 2012-2014.
 NSF NanoBio Hub site visit review team, 2013-2016
 Technical reviewer, National Geographic, 2013-2018
 NSF I/UCRC review panel 2014
 Strategic advisor, George Washington University, 2014-present
 Reviewer, Alaska EPSCoR 2011-present
 NSF SEES CBET review panel 2014
 NCI Small Grants Program for Cancer Research (R03/R21) 2014
 United Tribes Technical College, advisor/program evaluator Research Experience for Undergraduates
 Summer program, 2014-
 Reviewer, New Hampshire EPSCoR 2011-present
 Reviewer, Nevada EPSCoR 2013-present
 Advisor, cGMP facility planning, National Institutes of Health 2015-
 Strategic Advisor, Missouri EPSCoR program 2016-2019
 NSF CBET Career review panel 2016
 Reviewer, New Zealand MBIE fund 2018-present
 NIH NCI panel 2018
 NSF Engineering Research Center review panel 2019
 NSF Research Training program review panel 2021
 NSF SBIR review panel 2022
 NSF Engines review panel 2023
 International Review Committee for the Biotechnology and Food Engineering Faculty, Technion, Haifa
 Israel 2023

Extension

(I have just begun to account for extension activities that I have not captured since 1985, some activities are reflected in 'Public engagement media')

Advisor, Crown Finish Caves, 2017-present. Helped to navigate challenges in supply chain and preventative controls for *Listeria* contamination.

Advisor, Ample Hill Creamery, 2018-present. Assisted production manager in evaluating safety concerns and mitigating risk. Helped students network to gain internships.

Advisor, Shaker and Spoon. 2020-present. Assisted in food science and food safety challenges in the subscription-box business.

Advisor, Brooklyn Larder. Provided advice on food safety plans and expansion of current product lines.

PUBLICATIONS

Peer reviewed publications:

Batt, C. and Solberg, M. (1982) Asexual developmental markers in an aflatoxigenic strain of *Aspergillus parasiticus*. Can. J. Microbiol. 28:1206-1209.

Batt, C. and Solberg, M. (1983) Effect of volatile components of carrot seed oil in growth and aflatoxin production by *Aspergillus parasiticus*. J. Food Sci. 48:762-764.

Batt, C. and Solberg, M. (1985) Association of lactase activity with conidiation in an aflatoxigenic strain of *Aspergillus parasiticus*. FEMS Letters 27:277-280.

Batt, C.A., Claps, M.C., Bodis, M.S., Jamas, S. and Sinskey, A.J. (1985) Analysis of xylose operon regulation by Mud-lac fusion: Trans effect of plasmid coded xylose operon. Can. J. Microbiol. 31:930-933.

Batt, C.A., Carvallo, S., Easson, D.D., Akedo, M. and Sinskey, A.J. (1985) Evidence for a xylose metabolic pathway in *Saccharomyces cerevisiae*. Biotechnol. Bioeng. 28:549-543.

- Batt, C.A., Shanabruch, W. and Sinskey, A.J. (1985) Expression of pAM β I tetracycline resistance in *Corynebacterium glutamicum*: Segregation of antibiotic resistance due to intramolecular recombination. *Biotech. Lett.* 7:717-722.
- Batt, C.A., Novak, S.R., O'Neill, E.O., Ko, J. and Sinskey, A.J. (1986) Hyperexpression of *Escherichia coli* xylose isomerase. *Biotechnol. Prog.* 2:140-144.
- Jamieson, A.C., Vandeyar, M.A., Kang, Y.C., Kinsella, J.E. and Batt, C.A. (1987) Cloning and nucleotide sequence of the bovine β -lactoglobulin gene. *Gene* 61: 85-88.
- Chow, J.J., Batt, C.A. and Sinskey, A.J. (1988) Characterization of *Lactobacillus bulgaricus* bacteriophage χ 2. *Appl. Environ. Microbiol.* 54: 1138-42.
- Hahm, Y.T. and Batt, C.A. (1988) Genetic transformation of an *argB*⁻ strain of *Aspergillus oryzae*. *Appl. Environ. Microbiol.* 54, 1610-1611.
- Vandeyar, M.A., Weiner, M.P., Hutton, C.J. and Batt, C.A. (1988) A simple and rapid method for the selection of oligonucleotide-directed mutants. *Gene* 65, 129-133.
- Kim, S.G. and Batt, C.A. (1988) Heterologous expression of the *Escherichia coli* β -galactosidase gene in *Streptococcus lactis* by translational fusion. *Food Microbiol.* 5, 59-73
- Wong, D.W.S., Yee, L.N.H. and Batt, C.A. (1989) Thermal inducible expression of xylose isomerase and its performance in a hollow fiber bioreactor. *J. Ind. Microbiol.* 4, 1-5.
- Wong, D.W.S., Batt, C.A. and Kinsella, J.E. (1990) Purification and characterization of rat liver transglutaminase. *Inter. J. Biochem.* 22, 53-59.
- Batt, C.A., Jamieson, A.J. and Vandeyar, M.A. (1990) Identification of essential histidine residues in the active site of the *Escherichia coli* xylose (glucose) isomerase. *Proc. Natl. Acad. Sci. USA* 87, 618-622
- Batt, C.A. (1990) A simple procedure for the *in vitro* selection of oligonucleotide primed site-directed mutations. *DNA Prot. Eng. Tech.* 2, 48-50.
- Batt, C.A., Rabson, L.D., Wong, D.W.S. and Kinsella, J.E. (1990) Expression of recombinant bovine β -lactoglobulin in *Escherichia coli*. *Agr. Biol. Chem.* 54, 949-955.
- Silva, M., Wong, D.W.S. and Batt, C.A. (1990) Cloning and sequencing of the genomic bovine β -lactoglobulin gene. *Nucl. Acid. Res.* 18, 3051.
- Hahm, Y.T. and Batt, C.A. (1990) Expression and secretion of thaumatin from *Aspergillus oryzae*. *Agr. Biol. Chem.* 54, 2513-2520.
- Batt, C.A., Webb, J., Oren, P. and Flick, P. (1990) An improved method for oligonucleotide mediated site-directed mutagenesis. *Biotechniques* 9, 555-556.
- Kim, J.H. and Batt, C.A. (1991) Molecular characterization of a *Lactococcus lactis* bacteriophage F4-1. *Food Microbiol.* 8, 15-26.
- Kim, J.H. and Batt, C.A. (1991) Nucleotide sequence and deletion analysis of a gene coding for a structural protein of *Lactococcus lactis* bacteriophage F4-1. *Food Microbiol.* 8, 27-32.
- Kim, S.G. and Batt, C.A. (1991) Identification of a nucleotide sequence conserved in *Lactococcus lactis* bacteriophages. *Gene* 98, 95-100.
- Kim, S.G. and Batt, C.A. (1991) Antisense mRNA mediated bacteriophage resistance in *Lactococcus lactis*. *Appl. Environ. Micro.* 57, 1109-1113.
- Wong, D.W.S., Batt, C.A. and Kinsella, J.E. (1991) Expression of the transglutaminase gene in *Escherichia coli*. *Inter. J. Biochem.* 23, 947-953.
- Chung, D.K., Kim, J.H. and Batt, C.A. (1991) Molecular cloning and nucleotide sequence of the major capsid protein of *Lactococcus lactis* ssp. *cremoris* bacteriophage F4-1. *Gene* 101, 121-125.
- Tortorello, M.L., Billota, S., Woolf, H.D., Bender, J. and Batt, C.A. (1991) Extending the shelf-life of cottage cheese: Identification of spoilage flora and their control using food grade preservatives. *Amer. Cult. Dairy Prod. J.* 26:8-12.

- Whitaker, R.D. and Batt, C.A. (1991) Characterization of the heat shock response in *Lactococcus lactis* ssp. *lactis*. Appl. Environ. Micro. **57**:1408-1412.
- Gavalchin, J., Tortorello, M.L., Malek, R., Landers, M. and Batt, C.A. (1991) Isolation of monoclonal antibodies that react preferentially with *Listeria monocytogenes*. Food Microbiol. **8**, 325-330.
- Winters, D.A. and Batt, C.A. (1991) Irreversible denaturation of *Lactobacillus bulgaricus* β -galactosidase. Milchwissenschaft **46**, 753-757.
- Bor, Y.-C., Moraes, C., Lee, S.-P., Crosby, W., Sinskey, A.J. and Batt, C.A. (1992) Cloning and nucleotide sequence of the *Lactobacillus brevis* xylose isomerase. Gene **114**, 127-131.
- Kim, J.K., Kim, S.G., Chung, D.K., Bor Y.C. and Batt, C.A. (1992) Use of antisense mRNA to engineer a bacteriophage resistant lactic acid starter culture. J. Ind. Microbiol **10**, 71-78.
- Chung, D.C. Chung, S.K. and Batt, C.A. (1992) Antisense RNA directed against the major capsid protein of *Lactococcus lactis* subsp. *cremoris* confers resistance to the host strain. Appl. Microbiol. Biotechnol. **37**, 235-240.
- Jamieson, A.C. and Batt, C.A. (1992) Fluorescent properties of the *Escherichia coli* D-xylose isomerase active site. Prot. Eng. **5**, 235-240.
- Kim, S.G., Bor, Y.-C. and Batt, C.A. (1992) Bacteriophage resistance engineered into a strain of *Lactococcus lactis* subsp. *lactis* using antisense ribonucleic acid. J. Dairy Sci. **75**, 1761-1767.
- Wiedmann, M., Czajka, J., Barany, F. and Batt, C.A. (1992) Discrimination of *Listeria monocytogenes* from other *Listeria* species by ligase chain reaction. Appl. Environ. Microbiol. **58**, 3443-3447.
- Batt, C.A., Cho, Y. and Jamieson, A.C. (1993) A simple and rapid method for the selection of oligodeoxynucleotide-directed mutants. Meth. Enzymol. **217**, 280-286.
- Czajka, J., Bsat, N., Piani, M., Russ, W., Sultana, K., Wiedmann, M., Whitaker, R. and Batt, C.A. (1993) Differentiation of *Listeria monocytogenes* and *Listeria innocua* by 16S rDNA and intraspecies discrimination of *Listeria monocytogenes* strains by random amplified polymorphic DNA polymorphisms. Appl. Environ. Microbiol. **59**, 304-308.
- Herrick, J.B., Madsen, E.J., Batt, C.A. and Ghiorse, W.C. (1993) Polymerase chain reaction amplification of naphthalene catabolic and 16S rRNA gene sequences from indigenous sediment bacteria. Appl. Environ. Microbiol. **59**, 687-694.
- Kim, S. G. and Batt, C. A. (1993) Cloning and sequencing the *Lactococcus lactis* subsp. *lactis* *groESL* operon. Gene **127**, 121-126.
- Lee, S.P. and Batt, C.A. (1993) A micro-scale method for measuring the hardness of heat induced protein gels. Food Texture **24**, 73-79.
- Winn-Deen, E., Batt, C.A. and Wiedmann, M. (1993) Non-radioactive detection of *Mycobacterium tuberculosis* LCR products in a microtitreplate format. Mol. Cell Probes **7**, 179-186.
- Bsat, N. and Batt, C.A. (1993) A combined modified reverse dot blot and nested PCR assay for the specific nonradioactive detection of *Listeria monocytogenes* Mol. Cell. Probes **7**, 199-207.
- Lee, S.P., Cho, Y. and Batt, C.A. (1993) Enhancing the gelation of β -lactoglobulin. J. Agr. Food Chem. **41**, 1343-1348.
- Wiedmann, M., Barany, F. and Batt, C.A. (1993) Detection of *Listeria monocytogenes* using a nonisotopic polymerase chain reaction (PCR)-couple ligase chain reaction (LCR) assay. Appl. Environ. Microbiol. **59**, 2743-2745.
- Wiedmann, M., Brandon, R., Wagner, P., Dubovi, E. and Batt, C.A. (1993) Detection of Bovine Herpesvirus-1 in bovine semen by a nested PCR assay. J. Virol. Method. **44**, 129-140.
- Lee, S.P., Kim, D.S., Watkins, S. and C.A. Batt. (1994) Reducing whey syneresis in yogurt by the addition of a thermolabile variant of β -lactoglobulin. Biosci. Biotechnol. Biochem. **58**, 309-313.
- Cho, Y., Batt, C.A. and Sawyer, L. (1994) Probing the retinol binding site of bovine β -lactoglobulin. J. Biol. Chem. **269**, 11102-11107.

- Cho, Y. Gu, W., Watkins, S., Lee, S.P., Brady, J.W. and Batt, C.A. (1994) Thermostable variants of bovine β -lactoglobulin. *Prot. Eng.* **7**, 263-270.
- Wilson, W.J., Wiedmann, M., Dillard, H.R. and Batt, C.A. (1994) Identification of *Erwinia stewartii* by a ligase chain reaction. *Appl. Environ. Microbiol.* **60**, 278-284
- Batt, C.A., Wagner, P., Wiedmann, M., Luo, J., Gilbert, R. (1994) Detection of bovine leukocyte adhesion deficiency by nonisotopic ligase chain reaction. *Anim. Genet.* **25**, 95-98
- Cha, J., Cho, Y., Whitaker, R.D., Carrell, H.L., Glusker, J.P., Karplus, P.A., and Batt, C.A. (1994) Perturbing the metal site in D-xylose isomerase: The effect of mutation of His-220 on enzyme stability. *J. Biol. Chem.* **269**, 2687-2694.
- Barril, M.J.S., Kim, S.G. and Batt, C.A. (1994) Cloning and sequencing of the *Lactococcus lactis* subsp. *lactis* LM0230 *dnaK* gene using a PCR-based approach. *Gene* **142**, 91-96.
- Czajka, J. and Batt, C.A. (1994) Verification of causal relationships between *Listeria monocytogenes* isolates implicated in food-borne outbreaks of listeriosis by randomly amplified polymorphic DNA patterns. *J. Clin. Microbiol.* **32**, 1280-1287
- Wiedmann, M., Czajka, J., Bsat, N., Bodis, M., Smith, M.C. Divers, T.J. and Batt, C.A. (1994) Diagnosis and epidemiological association of *Listeria monocytogenes* strains in two outbreaks of listerial encephalitis in small ruminants. *J. Clin. Microbiol.* **32**, 991-996.
- Silva, M.C., Moré, M.I. and Batt, C.A. (1995) Development of a molecular detection method for naphthalene degrading *Pseudomonads*. *FEMS Microbiol. Ecol.* **18**: 225-235
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Batt, C.A. Jobs, Nanooze **21**: 1-18 (2024)

Clinical trials:

The following clinical trials have been carried out using material produced by the Cornell University/Ludwig Institute for Cancer Research Partnership.

NCT01154049- Study to Evaluate the Safety of the Vaccine Prepared SM14 Against Schistosomiasis

NCT00112957 Vaccine Therapy in Treating Patients With Stage II, Stage III, or Stage IV Ovarian Epithelial, Fallopian Tube, or Peritoneal Cancer

NCT00299728 NY-ESO-1 Protein with Montanide and CpG 7909 as Cancer Vaccine in Several Tumors

Patents:

Monoclonal antibody-based assay for *Listeria monocytogenes* U.S. Pat. #5,294,537 issued March 15, 1994.

Methods of detecting bovine Herpesvirus 1 (BHV-1) in semen by nucleic acid amplification. U.S. Pat. #5,545,523 issued August 15, 1996.

Enhanced on-chip SERS based biomolecular detection using electrokinetically active microwells. U.S. Pat. # 9,057,099 issued June 16, 2015.

Expert witness work (served as a consultant for party that is underlined, only cases that involved deposition or other hearings are included):

Applera Corp. v. Bio-Rad Lab. Inc., et al., No. 3:04-cv-01881-RNC (D. Conn.)

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