

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

IPR 2024-_____
U.S. PATENT NO. 10,068,051 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 to provide my independent evaluation and opinions regarding the validity of all of the claims of U.S. Patent No. 10,068,051 (“the ’051 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 ’051 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 ’051 patent are invalid under 35 U.S.C. § 103 because claims 1-4 are rendered obvious by (1) U.S. Patent Publication US 2013/0040841 A1, by Saxonov et al.,

entitled “Digital Assays With Multiplexed Detection of Two or More Targets in the Same Optical Channel” (“Saxonov”), alone or in combination with (2) U.S. Patent Publication US 2008/0124705 A1, by Kramer, entitled “Homogeneous Multiplex Screening Assays and Kits” (“Kramer”) and/or (3) U.S. Patent Publication US 2011/0250597 A1, by Larson et al, entitled “Digital Analyte Analysis” and claims 5-9 are rendered obvious 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 1-9 of the '051 Patent would have been rendered obvious by Saxonov, alone or in combination with Kramer and/or Larson.

10. It is my opinion that to the extent claims 5-6 of the '051 Patent are not disclosed or rendered obvious by Saxonov, alone or in combination with Kramer and/or Larson, claims 5-6 are rendered obvious in further view of Silverbrook.

11. It is my opinion that to the extent claim 7 of the '051 Patent is not disclosed or rendered obvious by Saxonov, alone or in combination with Kramer and/or Larson, claim 7 is rendered obvious in further view of Maltezos.

12. It is my opinion that to the extent claims 8-9 of the '051 Patent are not disclosed or rendered obvious by Saxonov, alone or in combination with Kramer and/or Larson, claims 8-9 are rendered obvious in further view of Oh.

IV. LEGAL STANDARDS

13. 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 '051 patent and the impact of certain prior art on the '051 patent. I am not a lawyer, nor do I offer any legal opinion in this declaration. Counsel from Weil, Gotshal & Manges has 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

14. 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.

15. 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.

16. 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

17. I understand that when interpreting the claims of the '051 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 '051 patent is entitled. For purposes of this proceeding, I have been asked to assume that the '051 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 '051 patent claims the benefit of priority. My analysis does not change if the '051 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 '051 patent is entitled to a later priority date.

18. 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 terms would have had to a person of ordinary skill in the art at the time of the priority date of the '051 patent consistent with the specification and file history of the '051 patent.

19. 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. Obviousness

20. 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

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

than February 3, 2012, the filing date of provisional application No. 61/594,480, the earliest-filed application to which the '051 patent claims the benefit of priority.

21. 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.

22. 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.

23. 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.

24. 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.

25. 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.

26. 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.

27. 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

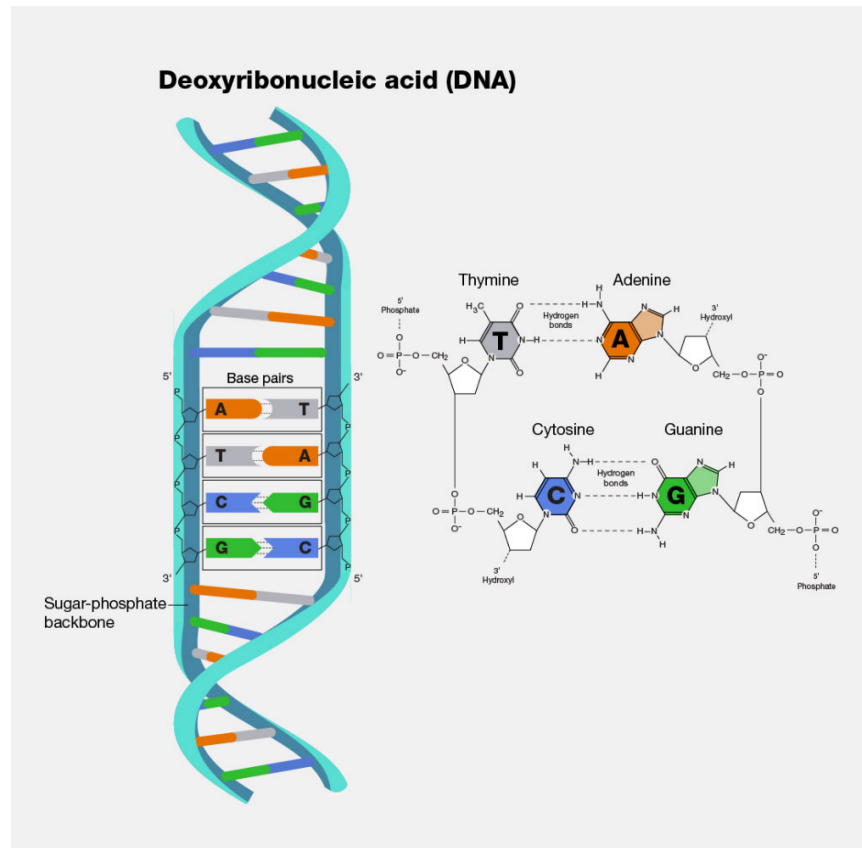
28. To the extent the following discussion is similar to other declarations, the ideas contained are entirely my own and I agree with the content therein.

A. Polynucleotides: DNA and RNA

29. 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.

30. 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 stores genetic information.

31. 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:



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

32. 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

² In RNA, A pairs with U.

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.

33. 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.

34. 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

35. 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.

36. 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'.

37. 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 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.

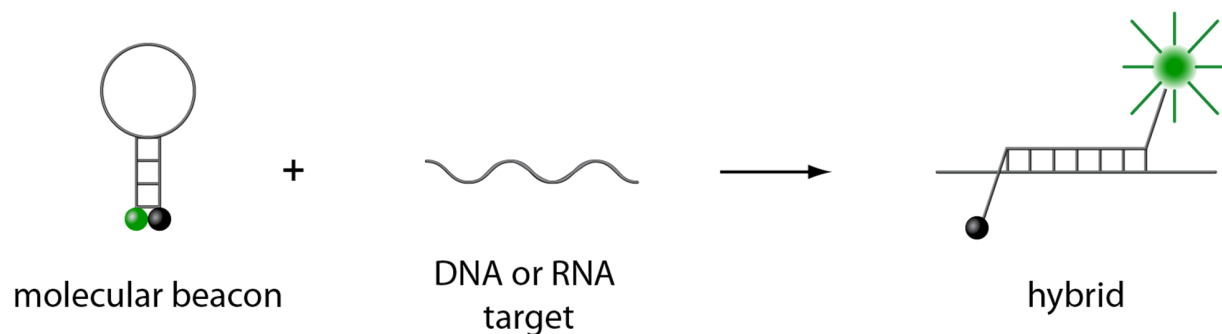
38. PCR can be multiplexed, that is, multiple oligonucleotide primers can be used simultaneously to amplify multiple target sequences in a single PCR at the same time. At least as of 2012, such multiplexed PCR and its use was well known in the art. Multiplex PCR provides numerous advantages, including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

C. Fluorescence Signals and Detection of Polynucleotide Analytes

39. 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

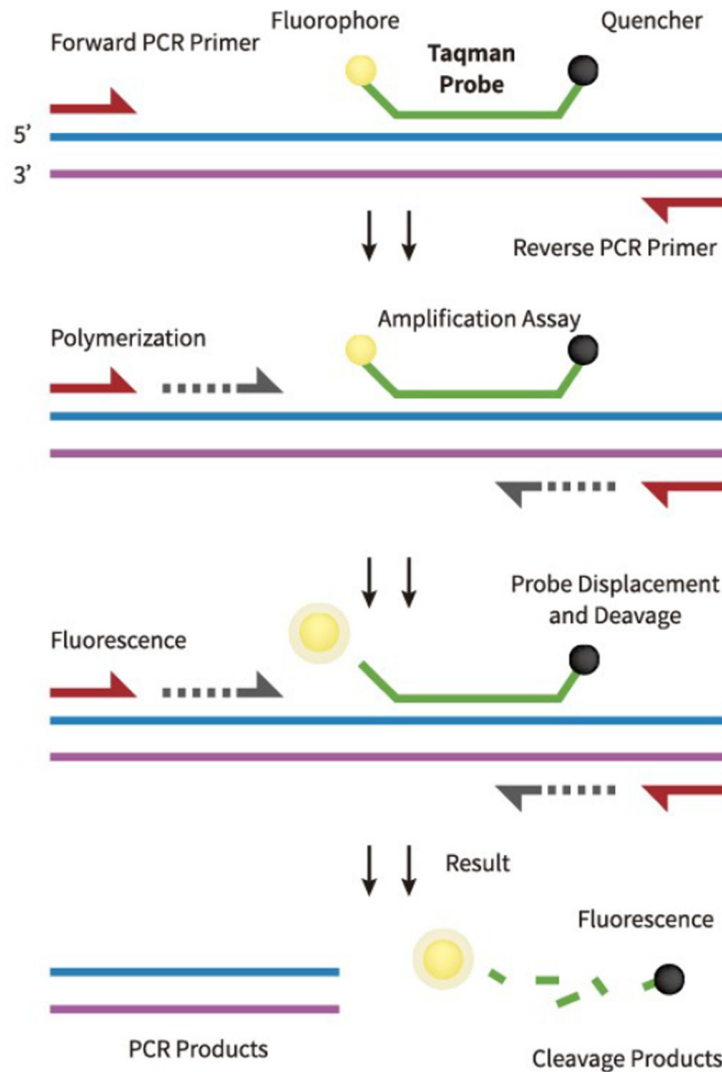
measured. In other techniques, isolation or separation is not required, because a detectable signal is generated only when the labeled-oligonucleotide is bound to the target polynucleotide. Generating a detectable signal without separation can be achieved by use of both a fluorophore and a “quencher” where a quencher is a molecule that can “turn off” a fluorescent signal when in close proximity to the fluorophore.

40. 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 anneal, 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. 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.

41. 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 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; EX1014 Fig. 1.

D. Quantitative PCR

42. 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”).

43. 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.

44. 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.

45. 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 a 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. EX1004 ¶¶ 60-75 (Examples 2-3).

46. 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 dPCR using Petitioner’s droplet-based products is shown below:

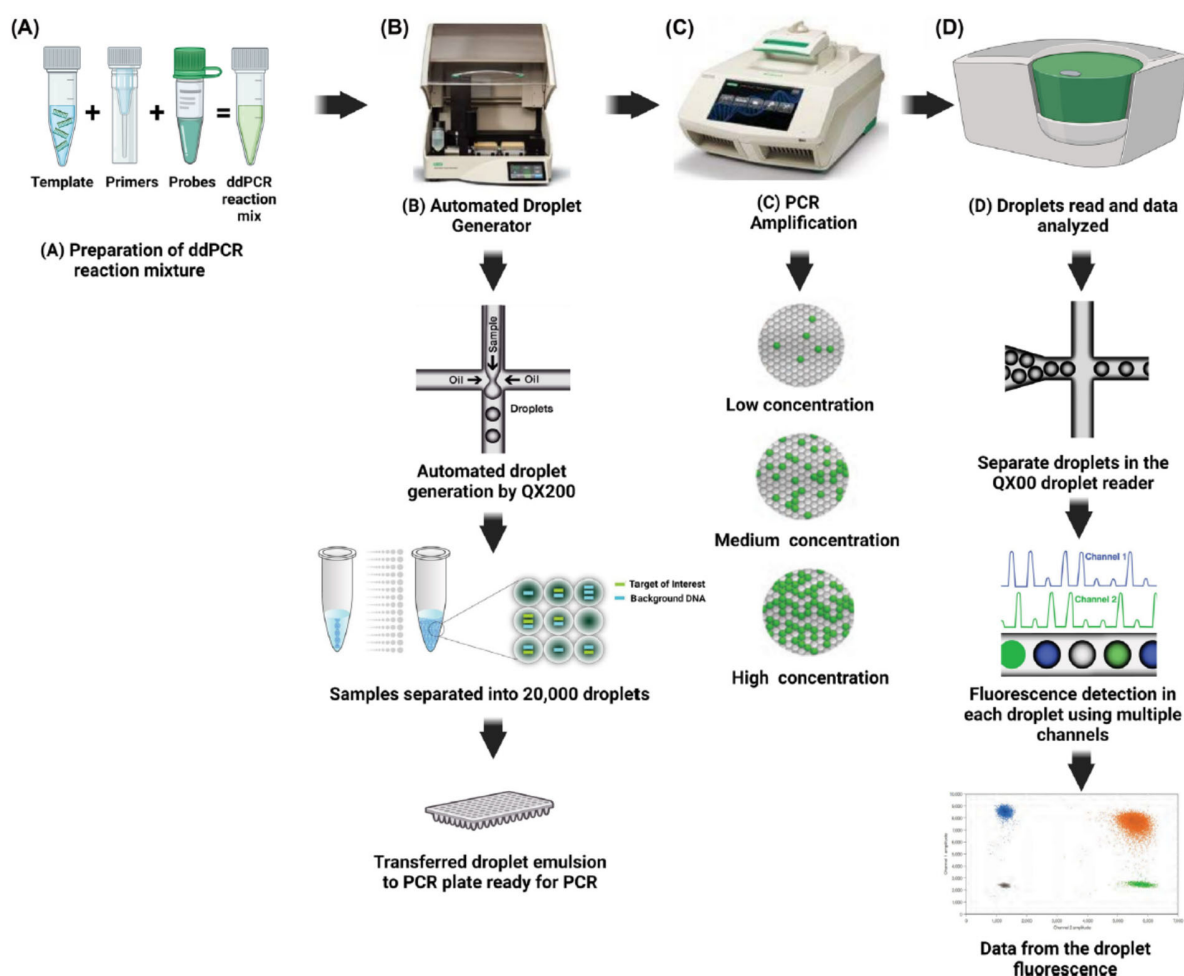


FIGURE 2.7 Overview of droplet digital polymerase chain reaction (PCR) workflow. (A) Standard ddPCR reaction mixtures are prepared in tubes. (B) The reaction mixture is separated into 20,000 droplets using an automated droplet generator, and the droplet emulsion is transferred into a 96-well PCR plate 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 '051 PATENT

47. The '051 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 1:30-34. As described in the ’051 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

48. All of the claims of the ’051 Patent are challenged in the Petition, including independent claim 1. The claims are reproduced below:

1. A method of detecting the presence or absence of at least seven polynucleotide analytes, in any combination of presence or absence, in a single sample solution volume, the method comprising:
 - a) providing a sample solution volume comprising or potentially comprising, at least one of at least seven polynucleotide analytes, and comprising at least seven hybridization probes, each hybridization probe corresponding to one of the at least seven polynucleotide analytes, wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores, and wherein each hybridization probe, when excited and when contacted with its corresponding polynucleotide analyte, generates a cumulative intensity signal, further wherein each generated cumulative intensity signal comprises at least one signal generated by excitement of at least one of the four fluorophores;

- b) contacting said sample solution volume with the plurality of hybridization probes and exciting the hybridization probes to generate the cumulative intensity signal(s) if one or more of the at least seven polynucleotide analytes is present in the sample volume; and
- c) measuring the cumulative intensity signal(s), thereby generating a cumulative signal measurement, wherein the cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence,

wherein said method non-degenerately detects the presence or absence of the at least seven polynucleotide analytes in said single sample solution volume, in any combination of presence or absence, without requiring any step of immobilization of said polynucleotide analytes, physical separation of said polynucleotide analytes, or mass spectrometry.

- 2. The method of claim 1, wherein said hybridization probes comprise an identical fluorophore, and wherein said plurality of hybridization probes are specific for different polynucleotide analytes.
- 3. The method of claim 1, wherein said detecting is performed with a plurality of hybridization probes comprising different fluorophores, wherein said plurality of hybridization probes are specific for different analytes.
- 4. The method of claim 1, wherein said cumulative intensity signal(s) are generated during a polymerase chain reaction.
- 5. The method of claim 1, further comprising transmitting information concerning the presence or absence of said polynucleotide analytes through a computer network.

6. The method of claim 1, further comprising providing information concerning the presence or absence of said polynucleotide analytes to a health care professional, wherein the health care professional makes a clinical decision based on said information.
7. The method of claim 1, wherein at least one step of said method is performed using a computer.
8. The method of claim 7, wherein said computer is located on a remote server.
9. The method of claim 7, wherein said computer is located on a thermal cycler.

B. Specification

49. The '051 patent purports to describe various methods and schemes that can be used for the multiplex detection of analytes, including polynucleotide analytes using labeled sequence-specific probes. EX1001 at 1:46-54, 3:5-16.

50. The specification purports to describe encoding each analyte as a value of at least one component of a signal, such as color or intensity. *Id.* at 8:58-67 (“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 8:58-9:27. 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 9:28-33.

51. These signals, which are generated from the labeled probes, are used to construct a coding scheme that maps the overall set of signals to the combination of analytes that are present. *Id.* at 12:29-14:2 (using more than one color per analyte); *id.* at 19:35-38, 23:31-25:30 (using one color and one intensity for each analyte and different specific intensities to distinguish different analytes); *id.* at code (57).

52. The '051 patent states that the above-described 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 9:43-45, 17:62-18:15. 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 4:66-5:5, 11:37-41, 21:40-22:45, 23:40-24:20.

53. The '051 Patent describes a “traditional encoding method for multiplexed fluorescent assays” wherein each analyte is encoded with a single color.

Id. at 8:40-44. In other words, one target equals one color. Table 1, reproduced below, provides an example of an assay that illustrates a traditional approach:

TABLE 1				
Traditional encoding of four analytes with four probes, each probe having a single color, and one probe per analyte.				
	Blue	Green	Yellow	Red
A	1	0	0	0
B	0	1	0	0
C	0	0	1	0
D	0	0	0	1

In the scheme denoted in Table 1, each analyte (A, B, C, and D) is encoded with a probe with a unique color (blue, green, yellow, and red, respectively), as denoted by a “1.” Thus, using four different fluorescent molecules, the presence or absence of each of four analytes can be determined in a multiplexed fashion by spectrally resolving the output color of the sample. *Id.* at 11:48-63. However, the traditional method of encoding purportedly “suffers from the fact that it is limited by the number of spectrally resolvable fluorophores.” *Id.* at 12:31-33. For this application, the practical limit is 4 or 5 colors due to the spectral resolution in fluorophore dyes used in DNA analysis.

54. The '051 Patent describes three encoding methods to purportedly overcome the limitation of the traditional method: (i) using different intensities of the same color signals to distinguish between the analytes; (ii) using more than one

color and one intensity per analyte; and (iii) using different colors and intensities for individual analytes.

55. An example of the first encoding scheme wherein different intensities of the same color are used to differentiate between analytes is described in the '051 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
TABLE 8-continued					
Example of encoding method using one color and one intensity per analyte, but different intensities among analytes.					
Tier	Analyte	B	G	Y	R
2	C	4	0	0	0
	D	8	0	0	0
	E	0	1	0	0
	F	0	2	0	0
	G	0	4	0	0
3	H	0	8	0	0
	I	0	0	1	0
	J	0	0	2	0
	K	0	0	4	0
4	L	0	0	8	0
	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: 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. The number in the column denotes a

unitless 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 an intensity of '8' for the red color.

57. The coding scheme in Table 8 purportedly has an advantage over the traditional scheme wherein more than one color per analyte is used (discussed below), because, in that scheme, different combinations of analytes can yield the same color signals, i.e., color signals that are “degenerate” or ambiguous. The combinations of analytes that yield the same set of signals are these “degenerate” combinations and require extra steps to eliminate the degeneracy, as discussed below.

58. Conversely, using one color signal with different intensities where the different combinations of intensities do not sum up to the same intensity as another analyte is non-degenerate by design. For example, as shown in Table 8 above, when analytes A and B are in the same sample the cumulative intensity of the blue signal is 3 ($1+2=3$) and there is no other analyte or combination of analytes that have a cumulative intensity of 3 in the blue signal. Therefore, the combination of analytes A and B is unambiguously identifiable. Accordingly, the cumulative signals are non-degenerate and allow unambiguous determination of the polynucleotide analytes present, alone or in combination, without needing to eliminate various

labeled probes (or sets of probes) as is necessary for schemes using multiple colors to encode individual polynucleotide analytes.

59. An example of the second encoding method, where more than one color is sometimes used per analyte to cover the desired number of analytes targeted, 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
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. EX1001 at 14:5-45. 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 14:5-17.

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 14:18-28. 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 12:55-13:5. Thus, using this encoding scheme, the presence or absence of up to seven different analytes can be detected using probes with four fluorophores.

62. The “control” signal purportedly measures the total number of analytes present in a given sample, hence allowing one to distinguish certain distinct combinations of analytes that might yield the same combination of green, yellow, and red signals. *Id.* at 14:5-45. For example, analyte F and the combination of analytes B and C will exhibit the same combination of green, yellow, and red signals, but may be differentiated by the intensity of the signal in the blue “control” signal. Analyte F will produce intensities of 1 in blue, 1 in green, 1 in yellow, and 0 in red while the combination of analytes B and C will produce intensities of 2 in blue, 1 in green, 1 in yellow, and zero in red. *Id.* at Table 4. Thus, the presence of analyte F alone and analytes B and C present in combination are distinguished because in the latter case the intensity of the blue signal will be double owing to the presence of two analytes.

63. In this approach, however, certain combinations of analytes are unavoidably degenerate, i.e., unavoidably providing 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 also yield cumulative signals of 2 in blue, 1 in green, 1 in yellow, and 1 in red. To address these potentially degenerate signals, where the signals are ambiguous, the '051 Patent suggests nothing more than ignoring one or more analytes, removing them from the coding scheme to reduce or eliminate this degeneracy. *Id.* at 14:41-43, Table 4.

64. Specifically, the '051 Patent recites “(i) encoding each potential analyte to be detected as a value of a signal and, optionally, as a value in each of at least two components of a signal; (ii) enumerating every legitimate result that can be obtained from the coding scheme; (iii) identifying each legitimate result that is degenerate; and (iv) eliminating at least one potential analyte (or potential analyte code) from the coding scheme, wherein eliminating the at least one potential analyte reduces or eliminates degeneracy.” *Id.* at 18:19-27.

65. A third method to purportedly overcome the limitation of the traditional method of encoding wherein analytes are encoded in both differing intensities and multiple colors per polynucleotide analyte is exemplified in Table 6, reproduced below:

TABLE 6

Encoding of 15 analytes and one control using
four colors and multiple intensities.

Tier	Analyte	B	G	Y	R	Comments
1	Ctrl. (p)	1	0	0	0	
	A	1	0	0	1	
	B	1	0	1	0	
	C	1	1	0	0	
	Ⓓ	†	⓪	†	†	Eliminated from coding scheme to eliminate degeneracy.

TABLE 6-continued

Encoding of 15 analytes and one control using four colors and multiple intensities.					
Tier	Analyte	B	G	Y	R Comments
Cumulative Maximum Result 2	E	1	1	0	1
	~~F~~	~~+~~	~~+~~	~~+~~	~~0~~ Eliminated from coding scheme to eliminate degeneracy.
	G	1	1	1	1
		6	3	2	3
	H	1	0	0	4
	I	1	0	3	0
	J	1	4	0	0
	~~K~~	~~+~~	~~0~~	~~3~~	~~4~~ Eliminated from coding scheme to eliminate degeneracy.
	L	1	4	0	4
	~~M~~	~~+~~	~~4~~	~~3~~	~~0~~ Eliminated from coding scheme to eliminate degeneracy.
Cumulative Maximum Result 3	N	1	4	3	4
		11	15	8	15
	O	1	0	0	16
	P	1	0	9	0
	Q	1	16	0	0
	~~R~~	~~+~~	~~0~~	~~9~~	~~16~~ Eliminated from coding scheme to eliminate degeneracy.
	S	1	16	0	16
	~~T~~	~~+~~	~~16~~	~~9~~	~~0~~ Eliminated from coding scheme to eliminate degeneracy.
	U	1	16	9	16
Cumulative Maximum Result for Three Non- Degenerate Tiers		16	63	26	63

Id. at 21:55-22:44, 21:14-17. Under this coding scheme, combinations of intensities and colors are used to encode the analytes. As shown in Table 6, analytes can be encoded in tiers, such that intensity values along with colors are used to encode each

analyte. *Id.* at 19:64-20:46. For example, analyte H is encoded as one level of intensity of blue (as previously blue is the control) and four intensity levels of red. Also shown in Table 6, some codes are eliminated, such as those for analytes R and T, because they may result in ambiguous, degenerate results. *See id.* at 18:19-27. By eliminating some codes, the remaining codes are non-degenerate.

66. The '051 Patent states that “[a]ny suitable reagent may be used with the invention.” *Id.* at 33:35. This includes hybridization probes with “an oligonucleotide probe attached to a fluorophore and a quencher (e.g., a TAQMAN probe.” *Id.* at 33:39-42. As described, TaqMan probes “comprise an oligonucleotide probe attached to a fluorophore and a quencher.” *Id.* at 10:29-31. “So long as the quencher and the fluorophore are in proximity, the quencher quenches the fluorescence emitted by the fluorophore upon excitation by a light source.” *Id.* at 10:31-34. “Upon extension of the primers by a DNA polymerase, the 5’ to 3’ exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium.” *Id.* at 10:40-43. The '051 patent also states that “one of ordinary skill in the art will readily recognize that other forms of probes would work equally well with the invention described in the disclosure.” *Id.* at 36:23-26.

C. PROSECUTION HISTORY OF THE '051 PATENT

67. I have reviewed the prosecution history for the '051 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 '051 Patent came to be issued.

68. The '051 Patent issued on September 4, 2018, from U.S. Patent Application No. 14/451,876 (the "'876 Application"), filed August 5, 2014, by applicant California Institute of Technology ("Caltech"). EX1001 at codes (21), (22), (45), (71). The '876 Application 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 1:6-11.

69. I understand that during prosecution of the '876 Application, the patent examiner raised various objections, including subject matter ineligibility, anticipation, obviousness, and indefiniteness. Eventually, the examiner allowed amended claims that were issued as '051 Patent claims. EX1006.

VII. PERSON OF ORDINARY SKILL IN THE ART

70. 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.

71. 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.

72. 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.

73. 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.*, EX1004 ¶ 19 (“The present disclosure provides 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’).”); *id.* ¶ 49 (“FIG. 3 shows a pair of targets 80, 82 (‘Target 1’ and ‘Target 2’) and corresponding probes 84, 86 (‘Probe 1’ and ‘Probe 2’) that may be used to create a dedicated signal for each target in a digital PCR assay.”)
- The design and use of fluorescent-labeled oligonucleotides, including use of quenchers, and use in conjunction with multiplex PCR. *See, e.g., id.* ¶ 49 (“Each probe may include an oligonucleotide 88, 90, a fluorophore 92, 94, and a quencher 96. Each of the fluorophore and the quencher may (or may not) be conjugated to the oligonucleotide by a covalent bond.”); *id.* ¶ 52 (“Quencher 96 is configured to quench the signal produced by fluorophore 92 or 94 in a proximity-dependent fashion.”).
- The quantitative detection and measurement of polynucleotide analytes using fluorescent-labeled oligonucleotides in multiplex applications. *See, e.g., id.* ¶ 6 (“A digital PCR assay can be multiplexed to permit detection

of two or more different targets within each partition.”); *id.* ¶ 49 (“FIG. 3 shows a pair of targets 80, 82 (‘Target 1’ and ‘Target 2’) and corresponding probes 84, 86 (‘Probe 1’ and ‘Probe 2’) that may be used to create a dedicated signal for each target in a digital PCR assay. Each probe may include an oligonucleotide 88, 90, a fluorophore 92, 94, and a quencher 96.”); *id.* ¶ 53 (“FIG. 4 shows an exemplary graph 102 of data corresponding to an exemplary digital PCR assay for Target 1 and Target 2 performed in droplets. The graph plots a signal 104 that represents light detected from probes 84, 86 (and/or one or more modified (e.g., cleavage) products thereof) (see FIG. 3).”).

- Techniques for labeling probes and detecting multiple targets using fewer differently colored fluorophores than polynucleotide targets. *See, e.g., id.* ¶ 19 (“The present disclosure provides 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’).”); *id.* ¶ 48 (“This example describes an exemplary digital PCR assay with multiplexed detection of two targets, using two probes, analyzed in the same channel. Other assays may involve three or more

targets and three or more probes, where at least two targets are analyzed in the same channel.”).

- Quantitative PCR (qPCR) and digital PCR (dPCR) and their application in detecting and measuring polynucleotide analytes, including in multiplex applications. *See, e.g., id.* ¶ 5 (“Amplification may be conducted via the polymerase chain reaction (PCR), to achieve a digital PCR assay.”); *id.* ¶ 6 (“A digital PCR assay can be multiplexed to permit detection of two or more different targets within each partition.”).

VIII. THE PRIOR ART REFERENCES OF THE GROUNDS

A. Saxonov

1. Saxonov is § 102(e) Prior Art

74. Saxonov et al., US2013/0040841 A1, titled “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 ’876 Application that resulted in the ’051 Patent as well as during the prosecution of related applications that resulted

in U.S. Patent Nos. 10,770,170 and 11,827,921. EX1005 at 1; EX1015 at 2; EX1016 at 11.

75. 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>see also 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” (EX1007 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>see also 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 suitable

Saxonov	'013 Provisional Disclosure
	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 5 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)

76. 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.

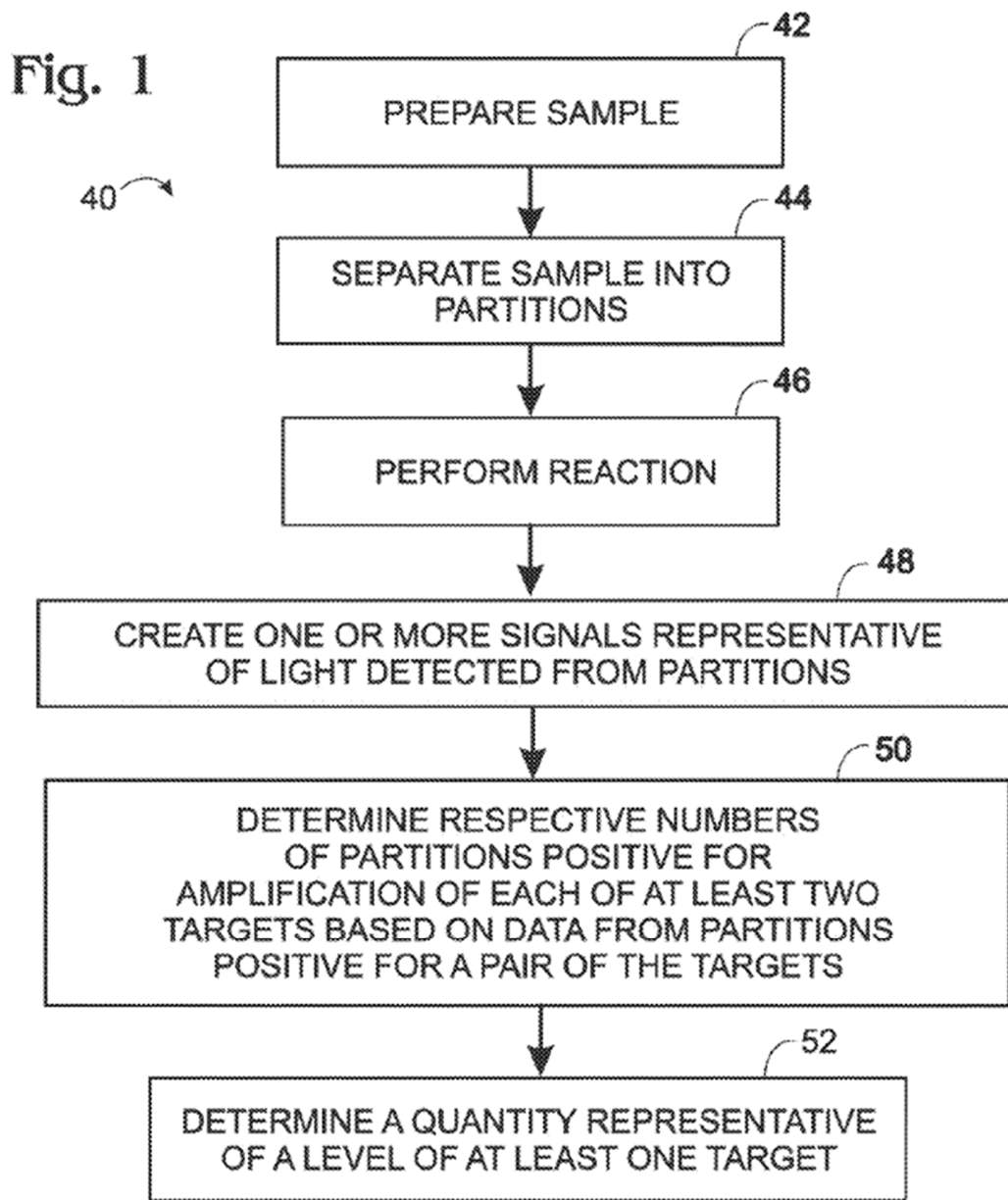
2. Saxonov Overview

77. 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. As described further below, Saxonov discloses the same types of coding schemes as the '051 Patent to enable the multiplexed detection of analytes such that there are more analytes than the number of colors used. This includes, for example, probes with fluorophores of more than one color to detect each analyte and probes with fluorophores of different intensities to detect each analyte.

78. 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 within 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.* This can be used to compute the relative numbers of different targets, which allows determination of “copy number (CN), copy number variation (CNV), and the presence/abundance of single polynucleotide polymorphisms (SNPs), among other quantities.” *Id.* ¶ 19.

79. At Figure 1, Saxonov provides a flowchart depicting the steps of the method:



Id. Fig. 1.

80. The first step of the method is sample preparation. Saxonov describes “any suitable manipulation of the sample, such as collection, dilution, concentration,

purification, lyophilization, freezing, extraction, combination with one or more assay reagents, performance of at least one preliminary reaction to prepare the sample for one or more reactions in the assay, or any combination thereof.” *Id.* ¶ 25.

81. Following sample preparation, 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).

82. 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 template molecule and a probe. *See id.* ¶ 32.

83. Saxonov’s method also includes elements or steps such that light is emitted so that it can be detected from the partitions. *Id.* ¶ 36. The light may be emitted from a fluorophore or combination of fluorophores. *Id.* Signals from one or more fluorophores of each partition can be detected in the same or different optical channel, where signals from different fluorophores may be accumulated and summed together. *Id.* For example, where the luminophore is a fluorophore, it can be quenched prior to the assay reaction where the emitted light of a fluorescent label

is suppressed prior to reactions by being in proximity to a quencher. *Id.* ¶ 52. Upon binding to an amplified target or upon cleavage of the probe during the amplification, the distance between fluorophore and quencher is increased thereby generating a readable signal. *See id.*

84. Signals can be created representing the light detected from the partitions, including representing “an aspect of light, such as the intensity of the light, detected in the same optical channel from reporters for two or more distinct targets.” *Id.* ¶¶ 36, 12 (“signal created by detecting light...from a fluid stream containing the droplets”); *id.* ¶ 53 (“The signal is created from light detected over time in a single channel from a fluid scheme containing the droplets and flowing through an examination region of the channel.”). The signals may also include data collected “in one or more different channels (e.g., in different wavelength ranges (color regimes) from reporters for the same and/or different target.” *Id.* ¶ 36.

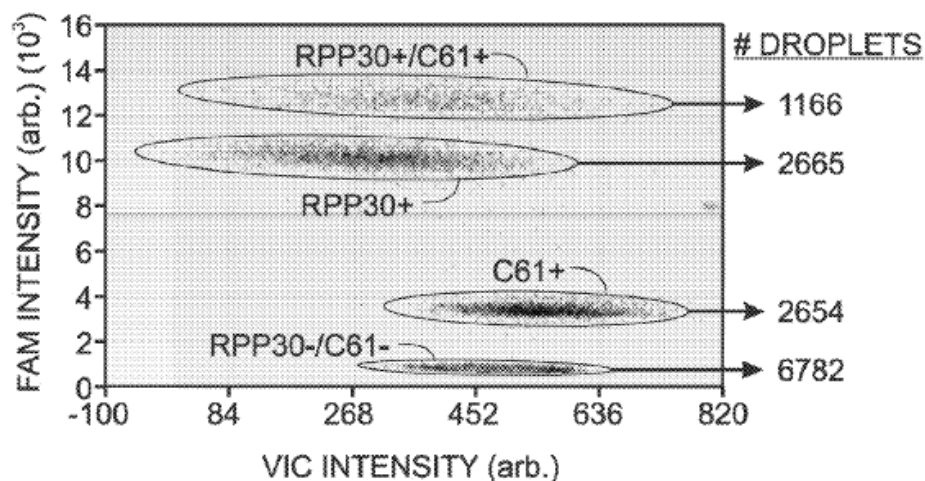
85. Based on the created signals, the number of partitions that are positive or negative for each target can be determined. *Id.* ¶ 41. A positive signal is read as the presence of a target, while a negative signal is read as the absence of a target. *Id.*

86. In addition to determining the presence or absence of a signal, a level 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 and copy number variation. *Id.*

87. Saxonov further describes assays wherein the signal intensity is used to distinguish among different polynucleotide analytes, alone and in combination, in a droplet based on the cumulative signal intensity generated. *Id.* ¶¶ 20, 51, 63-67. For example, Saxonov teaches, as shown by Figure 5 (reproduced below), differentiating two polynucleotide analytes in signals of a single color based on differing intensities:

Fig. 5

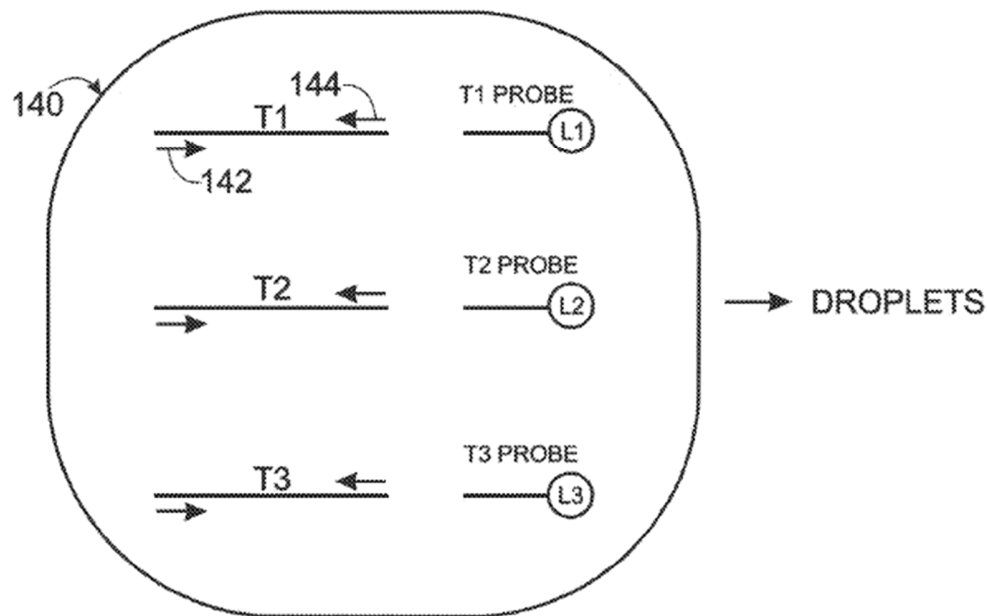


Id. Fig. 5. Combinations of two analytes with four potential droplet populations are depicted in Figure 5, from bottom to top: (1) droplets negative for both targets; (2) droplets containing target “C61”; (3) droplets containing target “RPP30”; and (4) droplets containing both target “C61” and target “RPP30.” *Id.* ¶¶ 60-67.

88. Saxonov also describes an example of using this system for a digital assay where two colors are used to label probes to distinguish between three different analytes, as depicted by Figures 9 and 10. *Id.* ¶ 76, Figs. 9, 10; *see also* EX1007 at 4:17-20, 5:19-6:5.

89. Figure 9 shows a bulk phase mixture (140) being separated into droplets during the performance of a digital assay:

Fig. 9

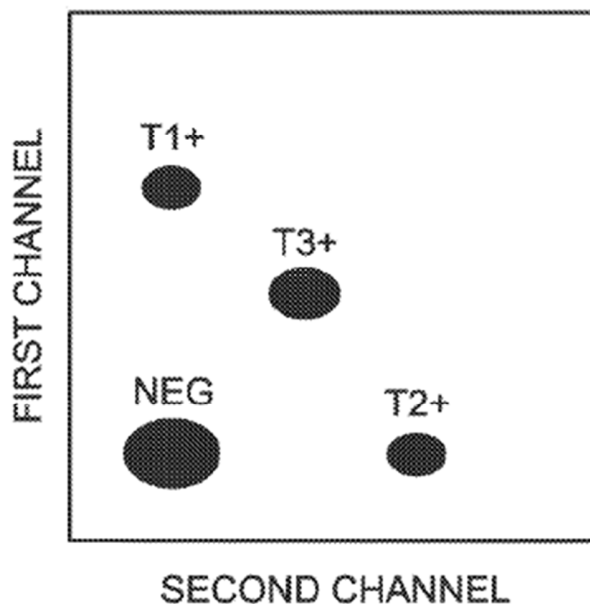


Id. Fig. 9. In this Figure, the mixture can include a forward primer (142) and a reverse primer (144) specific to each target (marked as T1, T2, and T3). *Id.* ¶ 77. The mixture may also include a probe to detect each target. *Id.* This probe may be

labeled with a different fluorophore or set of fluorophores (marked with an L1, L2, and L3, respectively). *Id.* Thus, the fluorescence intensity indicates whether or not each target is present in each individual droplet. *Id.*

90. Figure 10 represents an exemplary graph showing droplet intensities for the three targets in an assay using two channels:

Fig. 10



Id. Fig. 10. The axes are the intensities of the respective color signals generated in the assay and detected in a given channel. The vertical axis represents the intensity detected in the first color (channel) while the horizontal axis represents the intensity detected in the second color (channel). Data are collected measuring signal

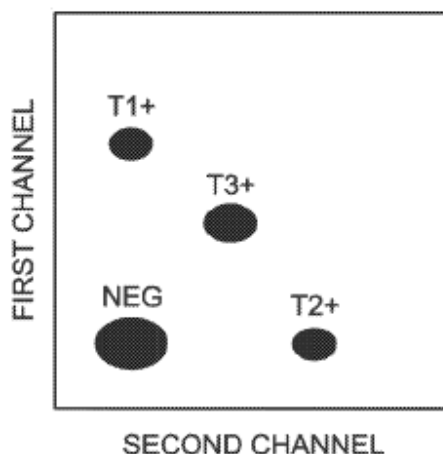
generated from each probe in both a first and second optical channel, and the cumulative intensities are plotted as a function of intensity in the first channel versus intensity in the second. *Id.* ¶ 78. Intensities produced by different populations of droplets are identified by filled ellipses, with the size of each ellipse corresponding to the number of droplets in each population, including those negative for any target and those positive for targets 1, 2, or 3. *Id.* As shown, the probe for T1 generates one color signal, detected in the “first channel,” the probe for T2 generates a second color signal, detected in the “second channel,” while T3 generates a signal in both the first color and second color, detected in both the “first channel” and “second channel.” *Id.* ¶ 78. As taught 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).” EX1007 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 with both colors at different intensities than T1 and T2. This allows the detection of three targets in two channels.

91. Saxonov further discloses increasing the number of analytes detected using more than two fluorophores and multiple fluorophore intensities. Signals generated from labeled probes that are detected may be different colors and intensities “for the same and/or different target(s).” EX1004 ¶ 36; EX1007 at 9:5-11. That is, targets may be differentiated based on both the color(s) emitted by their corresponding probe and the intensity at which those color(s) are emitted.

92. As to using different colors for the same target, Saxonov’s Figure 10 depicts signals of different colors for the same target, as well as of different signal intensities for that target than for other targets, in using label L1 in a first channel for target T1, label L2 in a second channel for target T2, and a label L3 in both the first and second channels at lesser intensity:

Fig. 10



EX1004 Fig. 10, ¶¶ 18, 79.

93. In addition, regardless of whether a target is identified 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.

B. Kramer

94. Kramer, US 2008/0124705 A1, entitled “Homogeneous Multiplex Screening Assays and Kits” (“Kramer”), is the publication of Patent Application No. 10/426,556, published on May 29, 2008. I have been informed by counsel that because Kramer was published prior to the earliest priority date claimed by the '051 Patent, Kramer is prior art to the '051 Patent.

95. Kramer discloses *in vitro* screening assays for detecting numerous possible polynucleotide analytes simultaneously, i.e., multiplexed assays, by using fluorescent hybridization probes that have unique combinations of fluorophores for different polynucleotide analyte target sequences. EX1008, Abstract, ¶ 8 (“Assays...are highly multiplexed...capable of detecting any of at least six, preferably at least 10...possible target sequences”).

96. Kramer performs such multiplexed assays to detect polynucleotide targets in a reaction without separating the targets spatially and without removing unbound hybridization probes. EX1008 ¶ 9.

97. The probes used to detect the analytes in Kramer may be designed such that each hybridization “probe is specific to one target among the multitude of possible targets being screened.” *Id.* ¶ 10. Kramer teaches multiple embodiments and techniques that rely on hybridization probes binding to polynucleotide targets, including with either molecular beacon or TaqMan probes. *Id.* ¶ 31.

98. Such probes may be labeled in different ways to perform the multiplexed detection of polynucleotide analytes taught by Kramer. For example, Kramer teaches the use of combinations of different fluorophore colors corresponding to a single polynucleotide target, which Kramer refers to as “combinatorial coding.” *Id.* ¶¶ 3, 11. Kramer further teaches the use of combinations of different amounts of each fluorophore used in an assay to

differentiate between the presence of analytes alone or in combination with one another, which Kramer refers to as “ratio coding.” *Id.* ¶¶ 3, 14. Kramer teaches that combinatorial coding and ratio coding may be employed in the same assay. *Id.* ¶ 11; *see also id.* ¶ 31.

99. Kramer teaches labeling a single probe with multiple fluorophores or a single fluorophore and dividing a target sequence into smaller portions with fluorophore-labeled probes of the desired code to generate a unique color signal pattern when the probes hybridize to the target analyte. *Id.* ¶¶ 12, 32. The color signal patterns corresponding to polynucleotide analytes can be single colors, multiple colors, or patterns based on both combinatorial, ratio coding, or both combinatorial and ratio coding. *Id.* ¶ 31.

100. Kramer further discloses in Figure 1 coding schemes that generate “distinguishable combinations obtained using two or three colors per [analyte] and panels of 4-8 distinguishable fluorophores” without requiring ratio coding to distinguish targets on the basis of signal intensity (*id.* ¶ 22):

PANEL OF 4(a, b, c, d), 5(+e), 6(+f), 7(+g) or 8(+h) FLUOROPHORES

ab									} = 6	}	}	}	}
ac	bc								}	}	}	}	}
ad	bd	cd							}	} = 10	}	}	}
									}	}	}	}	}
ae	be	ce	de						}	}	} = 15	}	}
									}	}	}	}	}
af	bf	cf	df	ef					}	}	}	} = 21	}
									}	}	}	}	}
ag	bg	cg	dg	eg	fg				}	}	}	}	} = 28
									}	}	}	}	}
ah	bh	ch	dh	eh	fh	gh			}	}	}	}	}

PANEL OF 5(a, b, c, d, e), 6(+f), 7(+g) or 8(+h) FLUOROPHORES

abc				} =10	}	}
abd				}	}	}
abe				}	}	}
acd	bcd			}	}	}
ace	bce			}	}	}
ade	bde	cde		}	}	}
					}	}
				} =20	}	}
abf				}	}	}
acf	bcf			}	}	}
adf	bdf	cdf		}	}	}
aef	bef	cef	def	}	}	}
					}	}
					} =35	}
abg					}	}
acg	bcg				}	}
adg	bdg	cdg			}	}
avg	beg	ceg	dgg		}	}
afg	bfg	cfg	dfg	efg	}	}
					}	}
abh					}	}
ach	bch				}	}
adh	bdh	cdh			}	}
ahh	bhh	chh	dhh		}	}
afh	bfh	cfh	dfh	efh	}	}
agh	bgh	cgh	dgh	egh	fgh	}
						}

FIGURE 1

Id. Fig. 1.

101. Kramer teaches assays that detect multiple polynucleotide analytes in the same sample simultaneously. *Id.* ¶ 39 (“[T]wo or even more than two targets...in a sample.”). Kramer teaches methods to determine the analyte or combination of analytes present in a sample based on the colors and intensities of colors in the detected signals. *Id.* ¶¶ 39-42. For example, Kramer teaches determining the analytes present in a sample based on the threshold cycle or time, i.e. the

amplification cycle, at which the signals become detectable, as long as the analytes are not each present at a nearly identical concentration, their corresponding signals will appear at a different time, thus allowing different targets to be detected. *Id.* ¶ 39. Further, Kramer teaches that if the codes for two targets share a color, the intensity of that color will be greater than—and will increase faster than—the colors associated with the other targets, identifying the common color. *Id.* ¶ 40. Similarly, when ratio coding is used, the accelerated increase of the fluorescent signal generated may be used to identify the target or targets present in a sample. *Id.* ¶ 41.

102. In instances wherein it is not immediately determinable which target or targets are present in a sample, Kramer teaches using a “repeat assay utilizing an alternative coding scheme for the targets” to “resolve the ambiguity.” *Id.* ¶ 42.

103. Accordingly, Kramer teaches the problem of degeneracy resulting from multiple combinations of targets that include colors in common that generate the same cumulative color and intensity signal, as well as some strategies to resolve such ambiguities. *See id.* ¶¶ 39-42. More generally, a POSA informed by the teachings of Saxonov would have understood the problem of degeneracy and to address the issue.

C. Larson

104. Larson et al., US 2011/020597 A1, entitled “Digital Analyte Analysis” (“Larson”) is the publication of U.S. Patent Application number 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 '051 Patent, Larson is prior art to the '051 Patent.

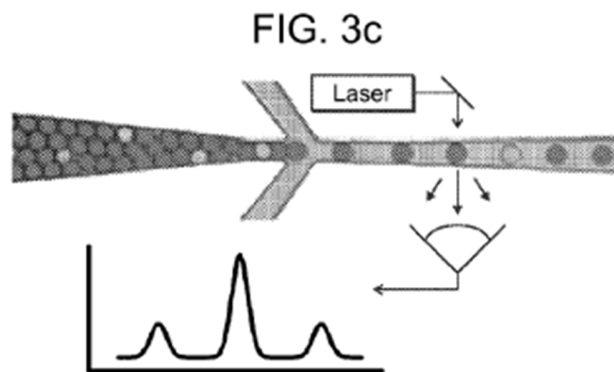
105. Larson teaches a droplet based digital PCR (dPCR) method for analyzing a target nucleic acid. EX1017 at Abstract. The summary describes the invention as generally relating “to the manipulation of nucleic acid in droplets, and in particular, nucleic acid amplification and detection.” *Id.* ¶ 7.

106. 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 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 other reagents). *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.

107. Larson discloses generating a signal during PCR by using a TaqMan assay, which includes an oligonucleotide probe that binds to a target polynucleotide sequence labeled with both a fluorophore and a quencher. *Id.* ¶¶ 47, 97, 132-134. The TaqMan probe acts, accordingly, as a reporter for the presence of the targeted

polynucleotide sequence. *Id.* In the TaqMan assay, the polymerase that extends the complementary strand includes an exonuclease activity that cleaves the TaqMan probe such that the fluorescent signal is no longer quenched thereby generating a detectable signal. *Id.* ¶¶ 126-127.

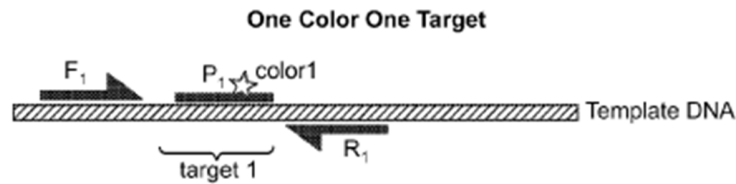
108. Once amplification is complete, droplets can be flowed through a device to detect the amplified products based upon the presence or amount of a reporter. *Id.* ¶ 89. As depicted in Figure 3c, droplets may be analyzed by counting the numbers of droplets positive or negative for fluorescence—in other words, determining the frequency of target-positive droplets in a population (*id.* ¶ 97):



Id. Fig. 3c.

109. In one type of dPCR assay, there is “a single labeled probe specific for an individual target.” *Id.* ¶¶ 119-120, Figs. 7A, 8A. Figure 7 depicts a single-plex assay:

FIG. 7A



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.

110. 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:

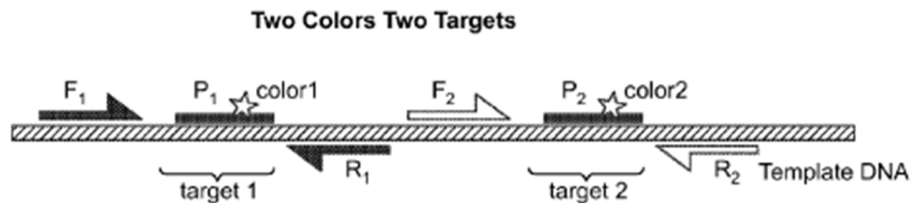
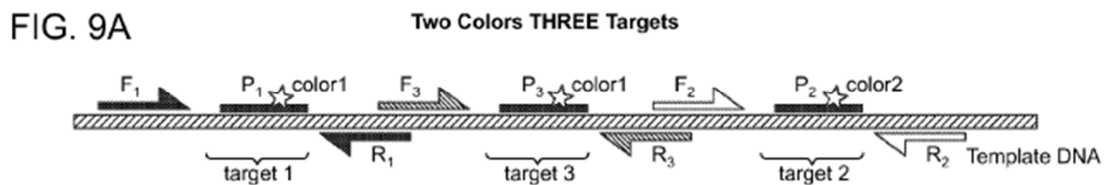


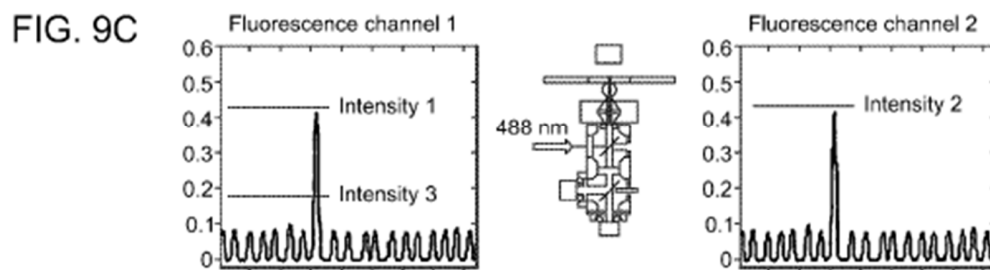
FIG. 8A

Id. Fig. 8A. As depicted, each two sets of primers (F1/R1 and F2/R2) are used to amplify two targets (target 1 and target 2), which are detected with two different probes (P1 and P2) with different colors (color1 and color2). *Id.* ¶ 120.

111. 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.*

112. 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.*

113. 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.*

114. Larson also teaches that “[a]dding extra colors would increase the capability even further,” and that with such improvements “multiplexing capacity exceeding 50x can be envisioned.” *Id.* ¶ 153. Thus, Larson teaches not only the use of different intensities, but also using different colors to perform highly multiplexed digital PCR assays.

115. Larson discloses that “the above three methods for higher-plex dPCR are simplest to implement under conditions of terminal dilution, that is when the probability of multiple different target molecules co-occupying the same droplet is very low compared to the probability of any single target occupying a droplet.” *Id.* ¶ 141. As explained, “terminal dilution” occurs when “the vast majority of reactions contain either one or zero target DNA molecules for practical intents and purposes.” *Id.* ¶ 6. By ensuring that dPCR occurs at terminal dilution, issues that arise with multiple occupancy of a droplet are avoided, which include “the complexity of simultaneous assays competing within the same reaction droplet” and the “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.” *Id.* ¶ 141. Larson explains that the methods disclosed “can accommodate these complications arising from multiple occupancy.” *Id.* Larson also discloses how droplets with multiple occupancy—in other words, multiple DNA molecules—may be identified and excluded from analysis. *Id.* ¶¶ 18, 20, 45, 68, 100.

116. 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 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; varying the thermal cycling program; varying the PCR master mix; incorporating into the assay some fraction of probes that lack fluorophores; or incorporating into the assay any hybridization-based competitive inhibitors to probe binding, such as blocking oligomer nucleotides, peptide nucleic acids, and locked nucleic acids.” *Id.* ¶ 137. These and other methods allow probe intensity to be adjusted. *Id.*

117. Several of these methods for tuning intensity are illustrated in Figures 12A, 12B, and 12C:

FIG. 12A

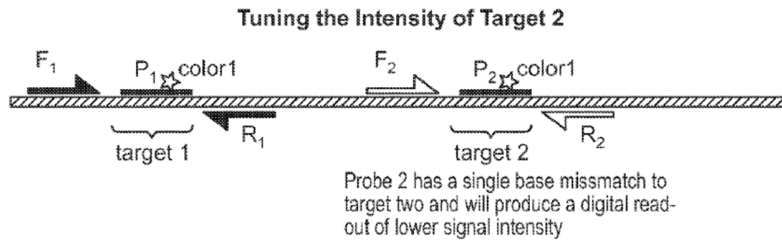


FIG. 12B

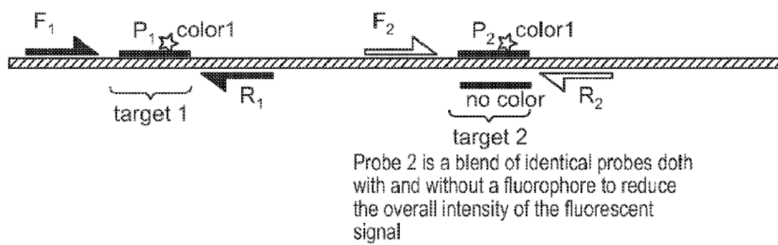
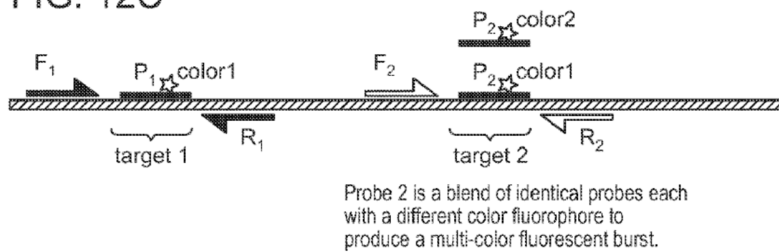


FIG. 12C



Relative intensity of signals from multiple targets of the same color can be generated in a variety of ways. Examples include: FIG.12A) using a single base mismatch of the probe and target to distinguish different targets. FIG.12B) blend identical probes with and without fluorophores. FIG.12C) blend identical probes with two or more different color fluorophores.

Id. Figs 12A, 12B, 12C. In Figure 12A, where two targets are detected using the same color, a mismatch in the probe for target 2 decreases the effective intensity from that probe. *Id.* ¶ 135. In Figure 12B, probes with no fluorophore are used to compete with those with a fluorophore to effectively reduces the intensity associated with target 2. *Id.* In Figure 12C, a competing probe with a different color effectively

reduces the intensity in color 1 associated with target 2, while also allowing target 2 to be detected if both colors 1 and 2 are present. *Id.*

118. Larson provides experimental data applying the methods discussed successfully, in particular a 5-plex and 9-plex assay, the results for which are depicted in Figures 14 and 15:

FIG. 14a

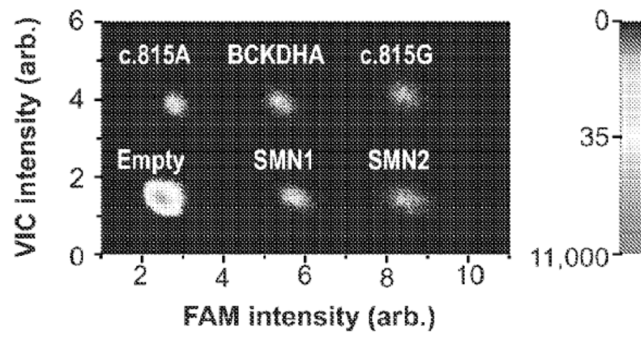
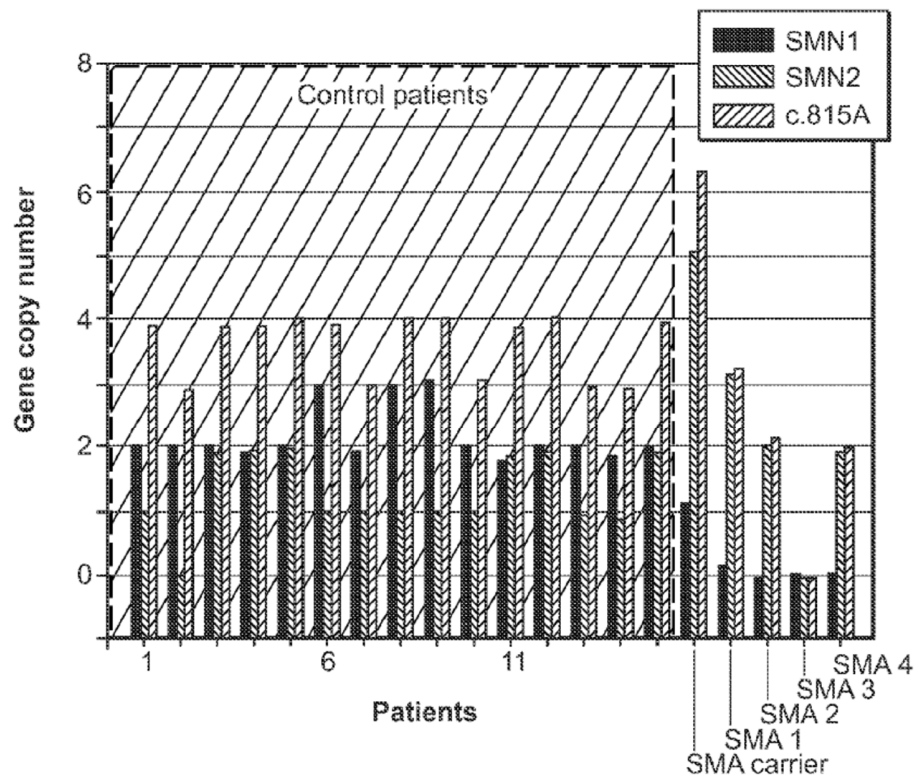


FIG. 14b



Id. Fig. 14a, 14b;

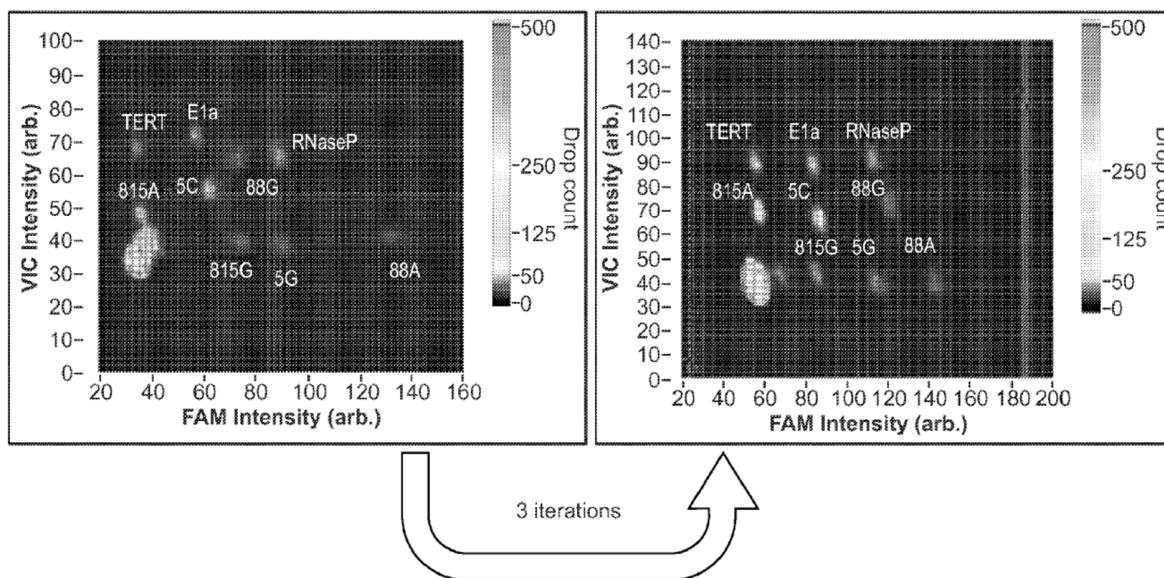


FIG. 15

Id. Fig. 15. Figure 14 depicts a 5-plex dPCR using two colors, with each population of droplet fluorescence shown as a heat map in Figure 14a and counts of each droplet population are determined via integration across the boundaries and depicted in Figure 14b. *Id.* ¶¶ 146-147. Figure 15 depicts the result of a 9-plex assay using two colors, FAM and VIC, and demonstrates three aspects:

- (1) nine DNA targets were uniquely identified in a two-dimensional histogram, far beyond the capabilities of conventional qPCR; (2) target DNA molecules were distinguished on the basis of some combination of both color and intensity arising from one or multiple probes against the same target; and (3) the relative positions of the target molecules within the histogram were adjusted by varying the probe concentrations to optimize the

pattern of colors and intensities for increased resolution amongst the various droplet populations.

Id. ¶ 150; *see also id.* ¶¶ 34, 145-153.

D. Silverbrook

119. 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 ’051 Patent, Silverbrook is prior art to the ’051 Patent.

120. Silverbrook teaches a nucleic acid testing device and methods that employ PCR and hybridization to identify what analytes are present in a given sample and transmitting results of such tests through a computer network. EX1009 ¶¶ 195-201, Figs. 96-99.

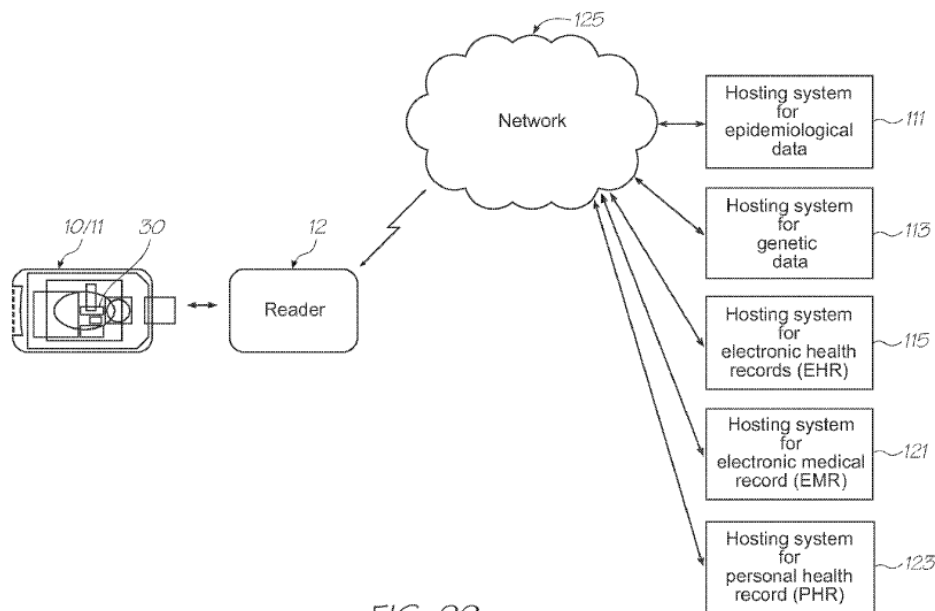


FIG. 99

Id. Fig. 99.

121. For example, as shown by 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.

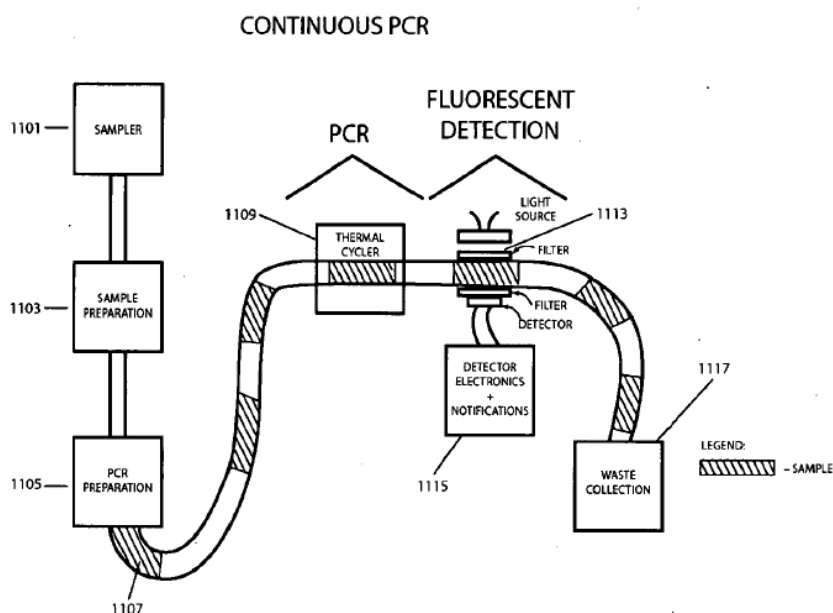
122. The data detected by the methods and modules taught by Silverbrook generally relates to biological applications, including the rapid identification of diseases such as influenza, pneumonia, tuberculosis, typhoid, Ebola, and HIV among others. *Id.* ¶¶ 207-243.

123. 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 pathogen load.” *Id.* ¶ 34. This information may be particularly beneficial when applied to clinical and healthcare decisions. *See id.* ¶¶ 55-62.

E. Maltezos

124. Maltezos et al., US 2008/0003649 A1 entitled “Thermal Cycling System,” (“Maltezos”), is the publication of U.S. Patent Application No. 11/750,326, published January 3, 2008. EX1010 at codes (10), (21), (43), (54). I have been informed by counsel that because Maltezos was published prior to the earliest priority date claimed by the ’051 Patent, Maltezos is prior art to the ’051 Patent. Maltezos teaches apparatus and methods for performing PCR analysis including “a control assembly that controls...operation of the optical assembly” and describes detection electronics to evaluate the signals detected in the detection region of the apparatus from which fluorescence measurements from the PCR are detected. *Id.* ¶¶ 12, 60, Fig. 11.



Id. Fig. 11.

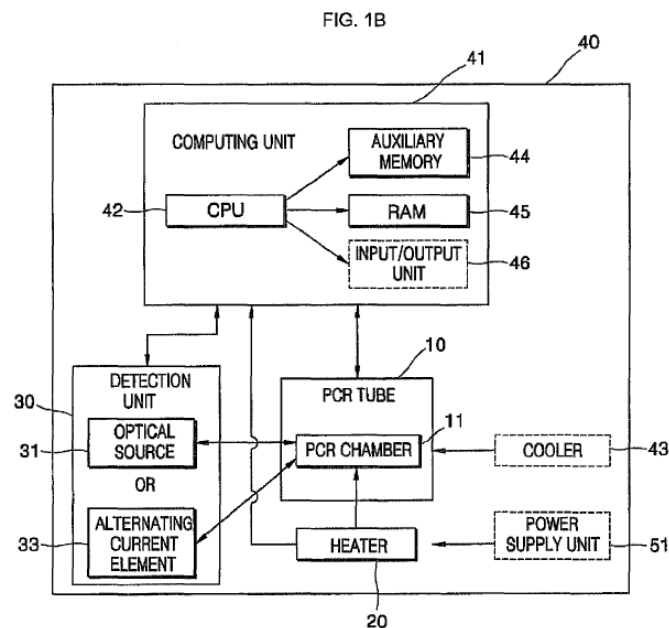
125. As shown by Figure 11 above, aliquots (or slugs) of the sample, represented by the shaded regions, is subject to PCR amplification in a thermal cycler (1109), before it is passed through the “detection region” (1113) where the fluorescent signals are measured and subsequently evaluated by the “detector electronics” (1115). *Id.* ¶ 60. Maltezos teaches that the signals to be detected may be generated by the excitation of fluorophores by a light source. *Id.*

F. Oh

126. Oh et al., US 2010/0311070 A1 entitled “Polymerase Chain Reaction (PCR) Module and Multiple PCR System Using the Same” (“Oh”), is the publication of U.S. Patent Application No. 12/843,552, published December 9, 2010. EX1011

at codes (10), (21), (43), (54). I have been informed by counsel that because Oh was published prior to the earliest priority date claimed by the '051 Patent, Oh is prior art to the '051 Patent. Oh teaches a PCR module and a multiple PCR system using the same with a combined PCR thermal cycler, a PCR product detector, and a multiple PCR system using the same module. EX1011 at Abstract, ¶ 17.

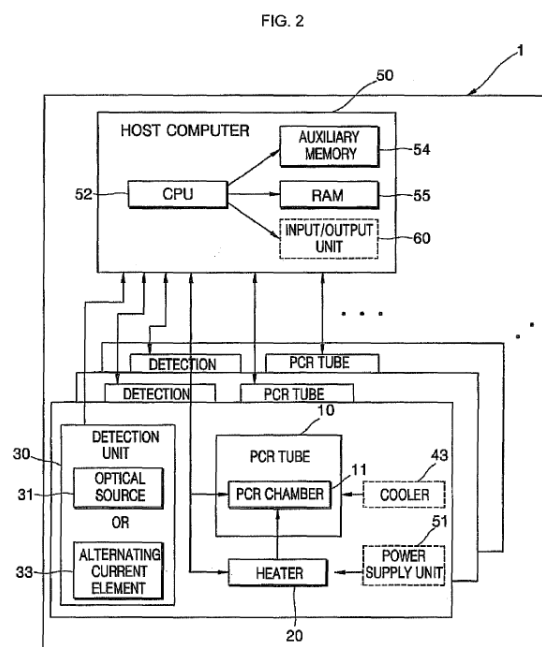
127. In its disclosure of the PCR module, Oh teaches methods of using the module, including those where the module contains a computing unit that performs computations on data received from sensors. EX1011 ¶ 58, Fig. 1B (reproduced below).



Id. Fig. 1B.

128. The “computing unit” described by Oh is depicted in Figure 1B above (41) and it is connected to the “detection unit” (30), the “PCR tube” (10), and the heater (20), all connected to a single power supply unit (51). *Id.* ¶¶ 58-63, Fig. 1B.

129. Oh alternatively considers a “host computer” depicted by 50 in Figure 2 (reproduced below) that performs the functions of the computing unit if the computing unit is not included in a given embodiment. *Id.* ¶¶ 66, 71, Fig. 2.



Id. Fig. 2.

IX. CLAIM CONSTRUCTION

130. I do not believe any express claim construction is required to determine the '051 Patent claims are invalid because the prior art teaches and/or discloses the claim limitations under any reasonable construction.

131. 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; ’051 patent, claim 1. Also, conversely, “non-degenerately.” EX1003 at 9.
- “each hybridization probe”: “each collection of hybridization probes specific for a polynucleotide analyte.” *Id.* at 11; ’051 patent, claims 1-3.
- “cumulative intensity signal(s)”: “the signal or collection of signals from the probes that contact a given analyte.” EX1003 at 14; ’051 patent, claims 1, 4.
- “cumulative signal measurement”: “the signal or collection of signals from the probes that contact the present analytes.” EX1003 at 16; ’051 patent, claim 1.
- “single sample solution volume” is non-limiting. EX1003 at 19; ’051 patent, claim 1.
- “wherein said method non-degenerately detects the presence or absence of the at least seven polynucleotide analytes in said single sample solution volume,

in any combination of presence or absence” is non-limiting. EX1003 at 23; ’051 patent, claim 1.

132. 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.

133. 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 ’051 Patent.

X. GROUND 1: CLAIMS 1-4 WOULD HAVE BEEN OBVIOUS IN VIEW OF SAXONOV AND KRAMER

134. The ’051 Patent claims a method of purportedly detecting the presence or absence of at least seven polynucleotide analytes using at least seven hybridization probes that are each labeled with at least one of four fluorophores. EX1001 at claim 1. When a hybridization probe specific for at least one of the seven polynucleotide analytes contacts the analyte in a sample, a color signal is purportedly generated. *Id.* The cumulative signal detected from the sample is purportedly used to determine whether any of the at least seven polynucleotide analytes are present. *Id.*

135. At its core, the '051 patent relies on nothing more than the concept where the total number of different polynucleotide targets detected exceeds the number of available colors (fluorophores) simply by employing mixed color signals and/or using different signal intensities to provide composite signals that unambiguously identify which analytes are present in a sample. More specifically, the main concept of the '051 patent's claims is that four different colors provided by the four different fluorophores can be used with a coding scheme in order that more than four polynucleotide analytes can be detected. Another concept is that 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 what 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.

136. Saxonov is similar to the '051 patent, as it is also directed to the multiplex detection of polynucleotide analytes using probes specific for each analyte, including in circumstances where there are more analytes to be detected than the total number of colors used to label probes. For example, Saxonov discloses

“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,” that is, using the same color. EX1004 ¶ 19. As one example, Saxonov discloses “an assay for three targets [that] may generate eight clusters or populations of data, separated by intensity” as a way to detect and identify a greater number of polynucleotide analytes in a single sample. *Id.* ¶ 20.

137. In my opinion, accordingly, Saxonov is analogous art to the '051 patent because they 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 code (57), 12:29-14:2, 19:35-38, 23:31-25:30; EX1004 ¶¶ 19, 20, 37.

138. It is my opinion claims 1-4 of the '051 Patent would have been obvious to a POSA in view of Saxonov. As discussed below, Saxonov discloses or suggests the methods in claims 1-4 to a POSA.

A. Claim 1

- 1. A method of detecting the presence or absence of at least seven polynucleotide analytes, in any combination of presence or absence, in a single sample solution volume, the method comprising:**

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

140. To the extent that the preamble is deemed limiting, it is my opinion that Saxonov discloses and at minimum renders obvious the claimed methods for detecting the presence or absence of at least seven polynucleotide analytes, in any combination of presence or absence, in a single sample solution volume. EX1001 at Claim 1.

141. Saxonov teaches methods for “the multiplexed detection of two or more distinct targets in the same optical channel,” that is, using the same color signals. EX1004 ¶ 8; *see also id.* ¶¶ 20, 36, Fig. 5; EX1007 at 13:10-13, 17:4-19:23 (Example 3). A POSA would have understood the use of the term “optical channel” as meaning “color,” with the terms being effectively interchangeable to the extent that an optical channel means an emitted light signal of a certain frequency or wavelength that is detected or perceived.

142. Regarding detecting two or more polynucleotide analytes, for example, Saxonov teaches the detection of two polynucleotide analytes using signals of a single color. Specifically, Saxonov teaches differentiating the two polynucleotide analytes, alone and in combination, based on the signal intensity of a single fluorophore (FAM). EX1004 ¶¶ 8, 20, 36, Fig. 5; EX1007 at 13:10-13 17:4-19:23 (Example 3), 4:17-20, 5:19-6:5, 9:4-15, 2:15-3:3, 12:21-13:8, Fig. 5.

143. Saxonov further discloses the use of more than one color, for example, describing that each analyte “may be at least predominantly or substantially

exclusively detectable in only one [color], or may be substantially detectable in two or more [colors].” EX1004 ¶ 79; *see also id.* ¶¶ 76-78, Fig. 10; EX1007 at 13:10-13, 17:4-19:23 (Example 3), 4:17-20, 5:19-6:5, 9:4-15, 2:15-3:3, 12:21-13:8, Fig. 5. As shown in Figure 10, Saxonov illustrates the detection of three analytes on two channels, where analytes T1 and T2 each have the same intensity in each of two different colors and analyte T3 has a lower intensity for both colors. EX1004, Fig. 10; *see also id.* ¶¶ 76-80. As Saxonov does not limit the number of analytes that the method describes can be detected, a POSA would have understood that the method disclosed in Saxonov can be used detect the presence or absence of at least seven analytes.

144. In my opinion, in view of Saxonov, it would have been obvious to a POSA to use an assay, as performed in Saxonov, to detect the presence or absence of at least seven polynucleotide analytes in a single sample volume. While I have provided a brief description here, I will provide a more complete description of each recited element set forth in the preamble in addressing them in the body of the claim as set forth below.

145. In addition, it is my opinion that the preamble is wholly redundant of what is set forth below in the claim elements. Accordingly, to the extent the preamble is found to be limiting, all of the claim elements in the preamble are fully addressed in the corresponding elements in the subsequent claim steps.

2. a) providing a sample solution volume

146. It is my opinion that Saxonov discloses “providing a sample solution volume,” as recited in Claim 1. Saxonov discloses that “preparation of the sample may include combining the sample with reagents for amplification.” EX1004 ¶ 26; EX1007 at 6:21-23. Saxonov further discloses that sample preparation methods may include “any suitable manipulation of the sample, such as collection, dilution, concentration, purification, lyophilization, freezing, extracting,” among others. EX1004 ¶ 25; EX1007 at 6:13-15. In my opinion, a POSA would have understood “any suitable manipulation...such as dilution [or] concentration” of a sample to include the recited claim limitation of “providing a sample solution volume.” Figure 1 in Saxonov further shows that the method described includes providing a sample solution volume that contains the analytes and other reagents for the assay. EX1004 Fig. 1, ¶ 25.

147. It is further my opinion, in particular, that Saxonov discloses the preparation of a sample solution into tiny aqueous droplets that may contain small numbers of target polynucleotide(s). EX1004 ¶ 28 (“In some embodiments, the partitions may be droplets disposed in a continuous phase, such that the droplets and the continuous phase collectively form an emulsion.”). Such droplets are precisely what Patent Owner contends correspond to the claimed “sample solution volumes” in parallel litigation. *See* EX1012 at 9, 12. Specifically, in that parallel litigation,

Patent Owner has applied a construction such that droplets correspond to the claimed “sample solution volume.” *Id.* For example, Patent Owner specifically alleges that “[t]he QX600 system requires dividing each sample into a sample solution volume (e.g., a water-in-oil droplet) with target and background DNA randomly distributed into the droplets and allowing for quantification of at least seven targets in a single well.” *Id.* at 12, 89 (“The QX One system requires dividing each sample into a sample solution volume (e.g., a water-in-oil droplet) with target and background DNA randomly distributed into the droplets and allowing for quantification of up to 8 targets in a single well.”); *id.* at 9 (“Samples may be further provided using a droplet generator such as the AutoDG or QX200 Droplet Generator.”). Thus, Saxonov discloses the preparation of droplets that Patent Owner, in the parallel litigation, currently contends is the claimed “sample solution volume.”

148. Accordingly, a POSA would have understood that the types of assays in Saxonov are conducted in solution, wherein aqueous reagents are combined with a sample prior to a detection step. EX1004 ¶ 26; EX1007 at 6:21-23. A POSA would further have understood that the assays in Saxonov involve the detection of analytes in aqueous solutions, i.e., in a sample solution volume.

3. comprising or potentially comprising, at least one of at least seven polynucleotide analytes, and

149. It is my opinion that Saxonov discloses including multiple polynucleotide analytes within the same sample solution volume. At the outset, a POSA would have understood that the use of seven target polynucleotides as claimed in the '051 patent is not inventive, as it was well known and routine within the relevant art. In my opinion, this is confirmed in the '051 patent itself, as it never identifies assays including seven target polynucleotides as novel or non-obvious, but rather buries the possibility of seven polynucleotide targets among a range of possible target numbers, including in disclosing that “at least 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7...48, 49, or 50 analytes may be detected per fluorophore.” EX1001 at 12:42-45, 37:44-48. Nonetheless, Saxonov teaches the detection of seven targets and, at a minimum, would have rendered this obvious to a POSA.

150. First, a POSA would have understood that Saxonov teaches multiplexed detection of polynucleotides—that is, the detection of multiple polynucleotides in an assay at the same time. EX1004 ¶ 5 (describing digital PCR to detect “nucleic acid target[s]”); *id.* ¶ 6 (“A digital PCR assay can be multiplexed to permit detect of two or more different targets within each partition.”); *id.* Fig. 10 (depicting signals for detection of three or more analytes); *id.* ¶ 77, Fig. 5; EX1007 at 13:9-13, 15:12-22, 3:6-8, 17:4-10, Fig. 5. Further, Saxonov specifically teaches

the use of forward primers, reverse primers, and oligonucleotide probes that a POSA would have known are used in assays that rely on PCR for amplifying target polynucleotides. EX1004 ¶ 77.

151. Second, a POSA would have understood that Saxonov contemplates not just multiplexed detection, but also multiplexed detection of seven or more targets such that it teaches the analysis of samples with seven or more targets. Specifically, Saxonov teaches detecting multiple targets using different intensities of a single color along with the use of multiple colors. For example, Saxonov teaches “performing a digital assay with multiplexed detection of two or more distinct targets in the same optical channel” including “three or more distinct targets.” EX1004 ¶¶ 8, 17. Saxonov also teaches that this “analysis of more than two targets in the same channel” is achieved by using different intensities of the same color. *id.* ¶ 19 (disclosing an assay for the multiplexed detecting of “three or more distinct targets”); *id.* ¶ 20 (“In some embodiments, the assays may involve analysis of more than two targets in the same channel.”); *id.* ¶ 36 (“the signal may be a composite signal that represents two, three, four or more targets of the reactions”); *id.* ¶ 47 (“two or more targets in the same channel”); *id.* ¶ 48 (“[o]ther assays may involve three or more targets and three or more probes, where at least two targets are analyzed in the same channel”); *id.* ¶ 62 (“principles described here also may be extended to...two or three

or more [analytes]”); *id.* ¶ 77 (“multiplexed detection of three or more distinct targets”); *id.* ¶¶ 60-67; EX1007 at 5:19-6:3, 10:18-11:3.

152. For example, for two targets, Saxonov teaches that two intensities of the same color results in four possible populations of intensities: droplets with no target, target 1 alone, target 2 alone, and both target 1 and 2. EX1004 ¶¶ 41, 53-59, 63-68, Figs. 4-7. Similarly, for three targets, three different intensities of one color can be used to “generate eight clusters or populations of data, separated by intensity.” *Id.* ¶ 20. As detailed in Saxonov, in general, “all permutations of positives will generally fall into 2^N populations or clusters, where N is the number of targets,” which is also the number of distinguishable intensities that must be detected. *Id.* ¶ 41.

153. Specifically, Saxonov teaches that two or three targets can be detected with a single color by varying the intensity of assigned to each analyte:

	Target A	Target B	Intensity
Population 4	+	+	Highest
Population 3	+	–	Intermediate
Population 2	–	+	Intermediate
Population 1	–	–	Lowest

	Target A	Target B	Target C	Intensity
Population 8	+	+	+	Highest
Population 7	+	+	–	Intermediate
Population 6	+	–	+	Intermediate
Population 5	–	+	+	Intermediate
Population 4	+	–	–	Intermediate
Population 3	–	+	–	Intermediate
Population 2	–	–	+	Intermediate
Population 1	–	–	–	Lowest

Id. ¶ 41 (“Exemplary results for...two, and three target systems in which data are collected in a single channel are shown in the following tables.”); *see also id.* ¶ 20 (“For example, an assay for three targets may generate eight clusters or populations of data, separated by intensity.”).

154. As shown in the first table, the different combinations for the presence or absence of two analytes results in four possible intensity signals, as a POSA would understand that the two intermediate intensity signals be selected so as to be distinguishable from each other. Similarly, the second table shows that different combinations of three analytes with a different signal intensity for each results in eight possible intensity signals. Although the tables above do not explicitly assign different intensity levels to the intermediate values, Saxonov teaches to select intensity values in a manner that allows one to distinguish the different possible combinations. *Id.* ¶ 20. Thus, a POSA would have understood that the ordering of the rows depicts a step-wise difference (gradation) in intensity levels such that each level represents a unique intensity level that can be used to determine the

combination of analytes in a sample. For example, Population 4 has a higher intensity than Population 3, which has a higher intensity than Population 2, etc. In other words, a POSA would have understood that what is depicted is an assay in which the different populations can be distinguished, that is, that the intensity represented by each row is different, such that the overall intensity detected can be used to distinguish which analytes are present in the sample. Again, Saxonov teaches that the different intensity levels are “distinguishable” from each other. *Id.* ¶ 41; *see also id.* ¶ 20 (“three targets may generate eight clusters or populations of data, separated by intensity.”). Thus, a POSA would have understood that each combination of analytes detected using labels of a single color and of different intensities for each analyte produces a different cumulative intensity signal.

155. Saxonov also teaches the use of at least three colors, wherein multiple analytes are detected with different intensities of each color. This is evident from its teaching throughout, first of using multiple colors and then of using multiple intensities, as discussed above, with each of those multiple colors.

156. Specifically, Saxonov teaches the use of “one channel” plus “one or more other channels,” which is an express disclosure of at least three channels or colors. EX1004 Fig. 5, ¶¶ 6, 8, 20 (“some targets may be analyzed in one channel (e.g., a FAM channel), and one or more other targets may be analyzed in one or more other channels (e.g., a VIC channel).”).

157. Thus, a POSA would have understood from Saxonov’s disclosure of at least three colors **and** two or three intensities corresponding to the targeted analytes per color that Saxonov contemplates the analysis of seven or more targets at the same time. For example, three colors and three intensities per color would allow the non-degenerate identification of **nine** different analytes:

	Color 1	Color 2	Color 3
Intensity 1	Analyte 1	Analyte 4	Analyte 7
Intensity 2	Analyte 2	Analyte 5	Analyte 8
Intensity 3	Analyte 3	Analyte 6	Analyte 9

As shown in the table, analytes 1-3 can each be detected with three different intensities of color 1. The presence of 1-3 can be detected because, as taught by Saxonov, eight possible intensity levels allow the unique identification of the combination present. This holds similarly for analytes 4-6 using color 2 and analytes 7-9 using color 3. A POSA would have understood that the intensity of each color is independent of each other.

158. A POSA would have further understood Saxonov’s disclosure of “one or more other channels” in addition to a first channel would necessarily also encompass using four colors, and that this would have been wholly consistent with

selecting fluorophores from Saxonov’s listing of at least five fluorophores, which states that “[e]xemplary fluorophores that may be suitable include FAM, VIC, ROX, TAMRA, JOE, etc., among others.” *Id.* ¶¶ 20, 51.

159. A POSA would also have understood that using four colors is preferable, due to the pre-existing availability of four-color instrumentation, and the lessened requirements to distinguish two different intensities versus three or more. *See infra* Section X.A.5 (discussing more extensively the numbers and selection of different fluorophores). Thus, a POSA would have understood that by using four colors, with two intensities per color, ***eight*** analytes can be unambiguously detected:

	Color 1	Color 2	Color 3	Color 4
Intensity 1	Analyte 1	Analyte 3	Analyte 5	Analyte 7
Intensity 2	Analyte 2	Analyte 4	Analyte 6	Analyte 8

160. A POSA would have understood that resolving two intensities per color is preferable to resolving three intensities per color, which Saxonov expressly discloses in teaching to use an additional color rather than an additional intensity level so that the data is “more easily resolvable.” *Id.* ¶ 20. Further, a POSA would have simply known that reducing the number of different intensity signals per color would have necessarily simplified the resolution of different signals. As discussed,

detecting two analytes in one color results in four possible intensity levels, while detecting three analytes in one color results in eight possible intensity levels of that color. Thus, detecting two rather than three analytes with one color results in having to resolve fewer intensity levels. Regardless, both schemes described—three colors at three intensities and four colors at two intensities—allow for the analysis of samples that have at least seven analytes and a POSA would have had a reasonable expectation of success in using either scheme contemplated in Saxonov.

161. Due to the need and the advantages of multiplexing, a POSA would have been motivated to detect more analytes in the same assay, such as detecting at least seven polynucleotide analytes. *Id.* Accordingly, it is my opinion that a POSA would have understood the benefits of a highly multiplexed assay, including one that assays at least seven polynucleotide analytes, and would have been motivated to increase the number of analytes that can multiplexed in one assay.

162. In my opinion, thus, for the reasons above, it would been obvious, in view of Saxonov to detect at least seven polynucleotide analytes in a multiplexed fashion.

4. comprising at least seven hybridization probes, each hybridization probe corresponding to one of the at least seven polynucleotide analytes,

163. It is my opinion that Saxonov discloses a sample solution volume that comprises at least seven hybridization probes, where each hybridization probe

corresponds to the at least seven polynucleotide analytes, that is, each is capable of binding to one of the at least seven polynucleotide analytes, as recited in claim 1.

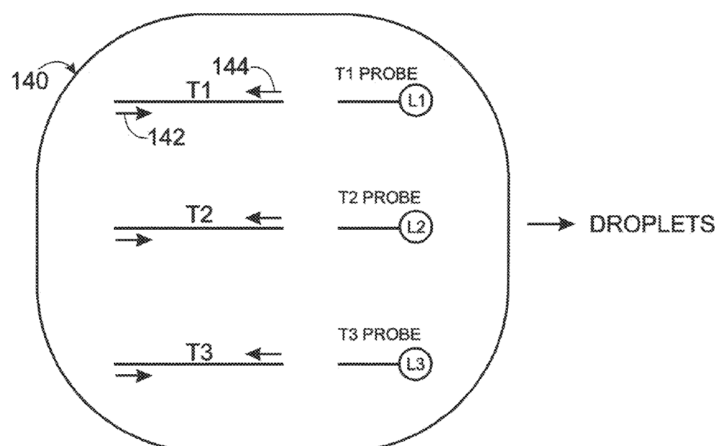
164. In my opinion, a POSA would have known to use at least as many hybridization probes as different polynucleotide analytes, because one probe is needed for each analyte, as taught by Saxonov and as commonsense dictates.

165. A POSA would have known that there is a specific probe for each polynucleotide analyte. Under techniques commonly known in the art, such as qPCR and dPCR, multiplex assays include at least one probe that specifically binds to each polynucleotide analyte. This is so because the probe must contain sequences that are complementary to a specific sequence in the polynucleotide analyte. In addition, probes specific to each analyte allows the determination of the presence or absence of each of the analytes in a sample. For example, the '051 Patent states that a "traditional encoding method for multiplexed fluorescent assay" involves pairing each analyte with a unique color, thereby requiring a hybridization probe specific to each analyte. EX1001 at 8:40-57. As a further example, a commonly used probe, a TaqMan probe, is "an oligonucleotide probe attached to a fluorophore and a quencher," where the "sequence of the oligonucleotide is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte." *Id.* at 10:30-37. The probes disclosed in the '051 patent are among those known, or

certainly within categories of those known, in the art. *See id.* at 11:25-27 (“Any suitable probe may be used with the methods presented in this disclosure, so long as the probe generates a quantifiable signal in the presence of an analyte.”). Accordingly, a POSA would have known that each hybridization probe contains sequences that are specifically complementary to a targeted polynucleotide analyte, thereby requiring at least one hybridization probe for each analyte.

166. Saxonov also provides ample examples of probes that are each specific for an analyte to be detected. For example, Saxonov discloses using hybridization probes specific for each polynucleotide analyte. EX1004 ¶ 50. As another further example, Saxonov discloses using “corresponding probes 84, 86 (‘Probe 1’ and ‘Probe 2’)” specific for “targets 80, 82 (‘Target 1’ and ‘Target 2’).” *Id.* ¶ 49, Fig. 3; EX1007 at 13:14-17; *see also* EX1004 ¶¶ 50, 63-67, Fig. 5. Even though there is a specific probe for each analyte, some of the probes may have a fluorophore in common, such as disclosed by Saxonov wherein multiple analytes are encoded using a FAM fluorophore. *See, e.g.*, EX1004 ¶¶ 13, 14, 63-67, Fig. 5; EX1007 at 4:1-7, 17:20-19:9, Fig. 5. As a further sample, Saxonov depicts in Figure 9 a probe specific for each target:

Fig. 9



EX1004 Fig. 9; *id.* ¶ 77 (describing “an exemplary bulk phase mixture 140” that “may include a reporter for amplification of each target (such as a T1 probe, a T2 probe, and a T3 probe)”; *id.* ¶ 49 (disclosing “corresponding probes” specific for each target).

167. Thus, as a POSA would have understood that Saxonov discloses, but at a minimum renders obvious, detection of at least seven polynucleotides (*see supra* Section X.A.3), or at a minimum renders such obvious (*id.*), a POSA would likewise have understood that Saxonov teaches using at least seven corresponding hybridization probes.

5. wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores, and

168. It is my opinion that Saxonov discloses using at least seven hybridization probes with each of the probes including at least one of four fluorophores as a label. As I detailed above, using at least seven hybridization

probes that are each labeled with at least one of four fluorophores is sufficient to detect seven targets. *See supra* Sections X.A.3, X.A.4.

169. Saxonov discloses multiple fluorophores suitable for labeling hybridization probes, including FAM, VIC, ROX, TAMRA, and JOE. EX1004 ¶¶ 49, 51, Fig. 3; EX1007 at 14:20-21, 4:20-23. Saxonov also teaches labeling each probe with one or more of these fluorophores, thereby using one or more colors to detect each target. *See, e.g.*, EX1004 ¶ 26 (“The reporters each may be a labeled probe that includes a nucleic acid (e.g., an oligonucleotide) labeled with a luminophore, such as a fluorophore.”); *id.* ¶ 20 (describing targets analyzed in one color and additional targets in “one or more other [colors]”); *id.* ¶ 79 (target “detectable in two or more [colors]”); *see also id.* ¶¶ 13, 36, 63-67, 76-78, Figs. 5, 9-10.

170. In my opinion, a POSA would have understood Saxonov’s teaching of using “two or more” colors to be a broad disclosure that one can use any one of a range of a different number of fluorophores, including up to four. In particular, it would have been clear to a POSA that Saxonov teaches up using one or more of at least four fluorophores because Saxonov would have just stated “up to three,” or something similar, if meant to teach using no more than three fluorophores. Thus, Saxonov does not disclose any limits on the number of fluorophores to be used with each probe.

171. To reiterate, as discussed above, three fluorophores or colors with three distinguishable levels of intensity is sufficient to detect nine polynucleotide analytes. *See supra* Section X.A.3. Meanwhile, four fluorophores or colors with two levels of intensity allows the detection of eight polynucleotide analytes. *See id.*

172. While Saxonov provides a broad disclosure on the range of fluorophores to be used in an assay, a POSA would likely have selected from four fluorophores. Specifically, a POSA would have considered the practical considerations of an available instrument, the number of colors it can detect, and the range of intensities it can reliably detect.

173. It is my opinion that labeling probes with at least one fluorophore selected from four different fluorophores would have been an obvious design choice for a POSA in light of the instruments available at the time. Nowhere does the '051 patent specification suggest that four is a novel or inventive number of fluorophores. Rather, it does just the opposite, as the '051 patent acknowledges that four is the natural number of different fluorophores to select from because this was the number of colors pre-packaged on “instruments widely used in the art.”

Many real-time PCR and quantitative PCR instruments comprise an excitation light source and band pass filters that enable the detection of fluorescent signals in four colors (e.g., blue, green, yellow, and red). Therefore, the methods of the invention can be readily applied using instruments widely used in the art.

EX1001 at 37:27-32. This limitation of instruments that can only detect four colors alone would have motivated a POSA to label probes with no more than four fluorophores—one for each color. The specification does not suggest that it would be novel or inventive to use the four colors that were available on such “instruments widely used in the art” or that four is a novel or inventive number of fluorophores.

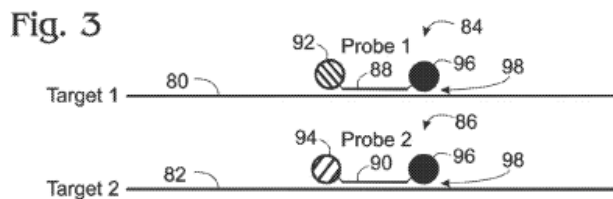
174. Given these considerations, a POSA would have been motivated to use four fluorophores in a multiplexed assay. First, as explained above, detecting two analytes with the same color requires differentiating between four possible intensity levels, rather than eight levels with three analytes per color. *See supra* Section X.A.3. Thus, using four colors with two intensities each to detect eight analytes is more straightforward since it is easier to distinguish four intensity levels (from two analytes) as compared to eight or more (from three or more analytes). Second, a POSA would be motivated to use the widely available instruments, which have four optical channels that can detect four colors. As such, a POSA would further have understood that the claimed use of four fluorophores in hybridization probes is neither inventive nor special and would have been motivated to use four colors. Further, given that “widely used” instruments already employed four colors to detect targets, a POSA would have had a reasonable expectation of being able to successfully use four fluorophores (one associated with each color) for multiplex detection of biological targets. As explained above, when using four colors with

each at two intensities, there are only four resulting clusters or populations of signal data for the possible combinations for each of the colors used that must be distinguished, rather than eight when using three intensities. *See supra* Section X.A.3; EX1004 ¶¶ 20, 41 (explaining that data with all permutations of positives for each color fall into 2^N populations where N is the number of targets); *id.* ¶ 60-67. A POSA would have understood that it is easier to use systems to detect signals and measure analytes when there are fewer different possible combinations for each color that is used. It is my opinion, accordingly, that a POSA would have been motivated to use four colors rather than three for the benefit of more straightforward distinction among the clusters or populations of data for each color used.

6. wherein each hybridization probe, when excited and when contacted with its corresponding polynucleotide analyte, generates a cumulative intensity signal,

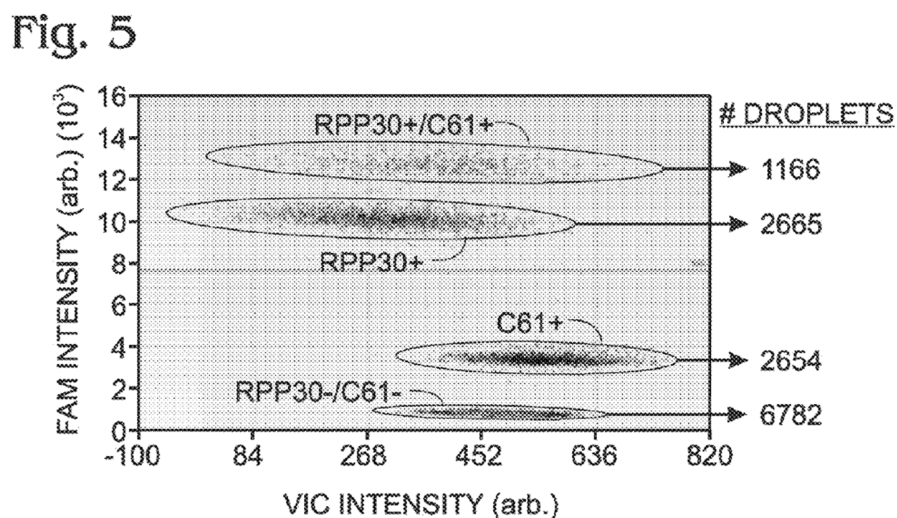
175. It is my opinion that Saxonov discloses the excitation of each hybridization probe when contacted with its corresponding polynucleotide analyte to generate a cumulative intensity signal, as recited in claim 1.

176. Saxonov discloses that each hybridization probe, when excited and when contacted with its corresponding polynucleotide analyte, generates a cumulative intensity signal. *Id.* ¶¶ 49-52. For instance, Saxonov discloses hybridization probes that hybridize to their corresponding polynucleotide analyte targets and that include fluorescent labels detectable upon excitation:



Id. Fig. 3. Specifically, Saxonov teaches that “light detected from the fluorophore may increase when the associated oligonucleotide 88 or 90 binds to the amplified target...or when the probe is cleaved and the fluorophore and quencher become uncoupled during target amplification, among others.” *Id.* ¶ 52; *see also id.* ¶ 37. Moreover, the probes in Figure 3 of Saxonov wherein “the probe is cleaved” are the same types of probes, essentially TaqMan probes, that Patent Owner accuses of infringing in parallel litigation. *See* EX1012.

177. Saxonov discloses numerous examples of signals generated from excitation of fluorophores present in droplets. For example, Figure 5 depicts the FAM signal from droplets with C61+, RPP30+, or both:



EX1004 Fig. 5; *id.* ¶¶ 63-67, Fig. 5; EX1007 at 17:4-19:9, 18:5-21; 5:19-3. A POSA would have understood that a signal generated using a probe comprising fluorophores would include at least one color signal generated by exciting at least one fluorophore. Specifically, as would have been and is well known by a POSA, fluorophores are excited in order to generate, i.e., emit, a detectable light signal. A POSA would understand that this is how fluorescent probes are used in qPCR and dPCR. *See supra* Section V. Further, for example, Saxonov specifically teaches using optical channels to detect emitted light signals that are generated upon excitation of fluorophores. EX1004 ¶ 37.

178. Saxonov also teaches that light emitted from different probes that use the same fluorophore may be detected together in a cumulative fashion, thereby detecting a cumulative intensity signal. *Id.* ¶ 37 (“The light detected in a given channel may be detected with the same sensor at the same time such that light detected from different reporters is summed or accumulated without attribution to a particular reporter. Thus, the signal may be a composite signal that represents two, three, four, or more reactions and thus two, three, four, or more targets of the reactions.”). Thus, the cumulative intensity signals generated in the assays taught by Saxonov are a result of exciting at least one of the fluorophores employed by the assay.

179. Saxonov provides multiple examples of cumulative intensity signal generation as a result of fluorophore excitation. For example, Figure 5 depicts assay results for detecting two target polynucleotides, C61 and RPP30. EX1004 ¶¶ 13, 63-67, Fig. 5; EX1007 9:5-21. First, droplets positive for analyte “C61” have a generated cumulative intensity in the FAM channel of ~3,000 in arbitrary units and a negligible VIC intensity. *Id.* Second, droplets containing analyte “RPP30” have a generated cumulative intensity of ~10,000 in the FAM channel in arbitrary units with a negligible VIC intensity. *Id.* Finally, droplets containing both “RPP30” and “C61” exhibit a generated cumulative intensity of ~13,000 in the FAM channel in arbitrary units and a negligible intensity in the VIC channel. *Id.*

7. further wherein each generated cumulative intensity signal comprises at least one signal generated by excitement of at least one of the four fluorophores;

180. It is my opinion that Saxonov discloses generating a cumulative intensity signal that includes at least one signal generate by excitement of at least of four fluorophores, as recited in claim 1.

181. Saxonov discloses that each generated cumulative intensity signal comprises signal generated by exciting at least one fluorophore label of the hybridization probes. EX1004 ¶ 37. For example, each positive droplet (C61+, RPP30+, and RPP30+/C61+) in Figure 5 includes signal from the FAM fluorophore label. EX1004 ¶¶ 63-67, Fig. 5; EX1007 at 17:4-19:9, 18:5-21; 5:19-3.

182. In my opinion, a POSA would have understood that signals from fluorescent probes, such as TaqMan probes, are generated by excitation of the fluorophores attached to the hybridization probes, as that is how such probes are understood to function and have been commonly used in the field, including techniques such as qPCR and dPCR. *See supra* ¶ Section V.C.

183. Saxonov discloses the exact type of fluorophore-labeled hybridization probes specific for target polynucleotide analytes that Patent Owner contends infringe the claims. *See, e.g.*, EX1013. A POSA would have understood that the signals from these fluorophores that are included in the hybridization probes are generated by excitation and that they lead to the claimed cumulative intensity signals.

184. Likewise, Saxonov teaches generating cumulative intensity signals comprising signals from the different fluorophores used to label the sequence-specific hybridization probes for the same target. Figure 10, for instance, illustrates results for an assay and shows cumulative intensity signals comprising signals from two different fluorophores used to label sequence-specific probes for three different targets. EX1004 ¶¶ 77, 79, Fig. 10; EX1007 at 2:15-3:3, 3:15-4:15, 9:4-10:3. The cumulative signal for analyte 1 is detectable in one color, the cumulative signal for analyte 2 in a second color, and the cumulative signal for analyte 3 in both colors. EX1004 ¶ 79, Fig. 10.

185. A POSA would have understood, thus, that a cumulative intensity signal generated using a probe comprising fluorophores would include at least one color signal generated by excitement of at least one fluorophore of the four fluorophores.

8. b) contacting said sample solution volume with the plurality of hybridization probes and

186. It is my opinion that Saxonov discloses contacting the sample solution volume with the plurality of hybridization probes in disclosing a sample having a volume comprising polynucleotide analytes and probes that hybridize to them. EX1004 ¶¶ 17-18, 77, Fig. 9; EX1007 at 6:21-7:10, 12:21-13:8.

187. As previously discussed, Saxonov teaches contacting analytes in the sample solution volume with hybridization probes. *See supra* Sections X.A.2-X.A.4.

188. Further, Patent Owner accuses Petitioner of infringing claim 1 wherein the sample solution is contacted with the various hybridization probes through the process described above for providing the sample solution. EX1012 at 28-42. Specifically, in parallel litigation, Patent Owner contends that the contacting step is met by pointing to steps of the probes hybridizing to the analytes and being excited. *See id.* at 28-42 (“When the process described above is performed using a QX600, the sample solution described above in step 1a will be contacted with the various

hybridization probes and excited through the process described in Steps 1a.4 and 1a.5.”).

189. Here, as explained above, Saxonov likewise teaches hybridizing probes to their targets and exciting the probes to generate a signal. *See supra* Sections X.A.6-X.A.7. Accordingly, Saxonov teaches contacting the sample solution with the plurality of hybridization probes.

9. exciting the hybridization probes to generate the cumulative intensity signal(s) if one or more of the at least seven polynucleotide analytes is present in the sample volume; and

190. It is my opinion that Saxonov discloses exciting labels of hybridization probes to generate cumulative intensity signal(s) if one or more polynucleotide analytes are present in the sample, as recited in claim 1, thus, signaling the presence of polynucleotide analytes in the sample volume. EX1004 ¶¶ 5, 20, 37, 79, Figs. 4-8, 10; EX1007 at 12:21-13:8.

191. As discussed previously, Saxonov discloses generating cumulative intensity signals from fluorophores on probes that are excited to emit a signal if the corresponding polynucleotide analyte is present. *See supra* Section X.A.6.

192. A POSA would have further understood from Saxonov that hybridization probes for unambiguous detection of at least seven polynucleotide analytes, as discussed above in Sections X.A.3 to X.A.7, would generate cumulative

intensity signal(s) if the corresponding polynucleotides were present. As an example, Saxonov teaches a series of target specific probes that bind to polynucleotide analyte sequences and are labeled with fluorophores so that the presence of such analytes is detectable via excitation of the corresponding fluorophores. EX1004 ¶¶ 5, 20, 37, 76-79, Figs. 4-8, 9, 10; EX1007 at 12:21-13:8.

193. Thus, a POSA would have been motivated to excite hybridization probes to generate the cumulative intensity signals, which allows for detection of the presence or absence of one or more polynucleotide analytes. *See supra* Sections X.A.3-X.A.7.

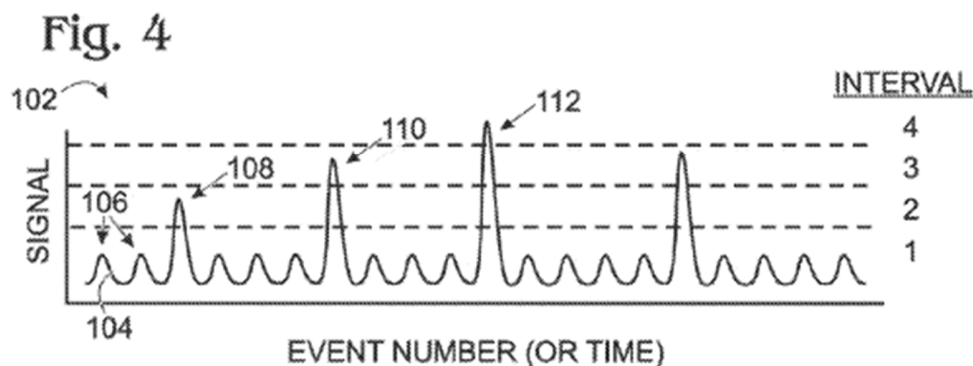
194. A POSA further would have had a reasonable expectation of success in exciting such hybridization probes because the probes comprise fluorophores, as discussed above, and a POSA would have understood that fluorophores may be excited by, for example, excitation light. *See, e.g.*, EX1004 ¶ 37; *see also supra* Section V.C. Such generated signals of fluorophores would result in cumulative intensity signals that are measured by the instrument. *See supra* Sections X.A.3-X.A.7.

- 10. c) measuring the cumulative intensity signal(s), thereby generating a cumulative signal measurement,**

195. It is my opinion that Saxonov teaches measuring cumulative intensity signal(s), thereby generating a cumulative signal measurement, as recited in claim 1. *See* EX1004 ¶¶ 5, 20, 37, 79, Figs. 4-8, 10; EX1007 at 12:21-13:8.

196. As discussed above, Saxonov discloses detecting the signal from the fluorophores on hybridization probes. *See supra* Section X.A.6-X.A.7. A POSA would have known to use a detection instrument to detect the light emitted from corresponding fluorophores. *See supra* Section X.A.5.

197. For example, Saxonov Figure 4, reproduced below, depicts measuring signals in its graphical trace of light signal(s) from probes in a digital PCR assay and Figure 5 graphs measured intensities of different signals in droplets, including the intensities for C61+, RPP30+, and RPP30+/C61+, which constitutes cumulative signal measurement. EX1004 ¶¶ 12-13, 20, 41, 53, 63-67, Figs. 4-5; *see also* EX1007 at 13:10-13, 17:4-19:23 (Example 3), 4:17-20, 5:19-6:5, 9:4-15, 10:8-11:3, 2:15-3:3, 12:21-13:8, Fig. 5.



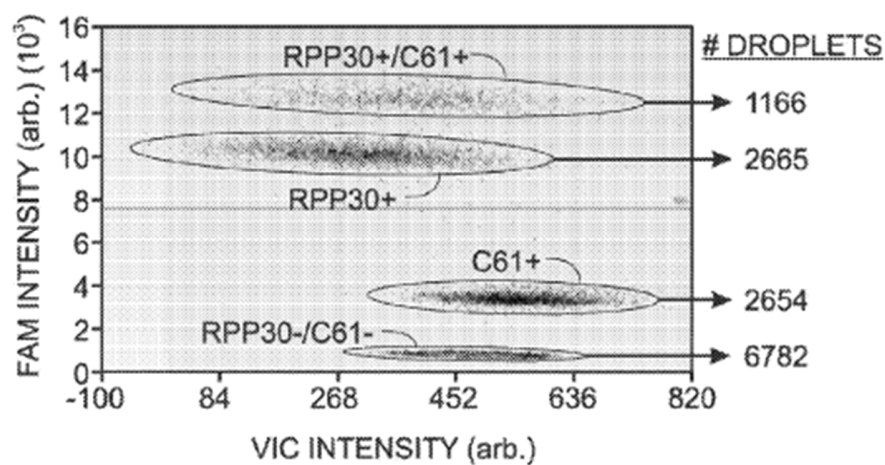
EX1004 Fig. 4.

198. The low peaks in Figure 4 represent droplets with no polynucleotide analytes present, which have minimal fluorescence resulting from noise from the unbound probes present in the droplets. *Id.* ¶ 53, Fig. 4. The peak 108 over the first signal threshold but below the second signal threshold depicted by the dotted lines corresponds to the cumulative intensity signal generated by droplets containing target analyte one. *Id.* The peak 110 between the second signal threshold and third signal threshold corresponds to the cumulative intensity signal generated by droplets containing target analyte two. *Id.* The peak 112 above the third signal threshold corresponds to the cumulative intensity signal generated by droplets containing both target analytes one and two. *Id.*

199. Figure 5, reproduced below, depicts measured cumulative intensity signals of each droplet, including measured intensities of signals for droplets that are

C61+, RPP30+, and RPP30+/C61+, which constitutes a cumulative signal measurement. *Id.* ¶¶ 63-67, Fig. 5; EX1007 at 13:10-13, 17:4-19:23 (Example 3), 4:17-20, 5:19-6:5, 9:4-15, 2:15-3:3, 12:21-13:8, Fig. 5. First, droplets positive for analyte “C61” have a generated cumulative intensity in the FAM channel of ~3,000 in arbitrary units and a negligible VIC intensity. *Id.* Second, droplets containing analyte “RPP30” have a generated cumulative intensity of ~10,000 in the FAM channel in arbitrary units with a negligible VIC intensity. *Id.* Finally, droplets containing both “RPP30” and “C61” have a generated cumulative intensity of ~13,000 in the FAM channel in arbitrary units and a negligible intensity in the VIC channel. *Id.*

Fig. 5



Id. Fig. 5.

200. Saxonov further discloses measuring cumulative intensities for signals from three probes of different intensity in the same color, and for two probes in the same color and one in a second color. *Id.* ¶¶ 20, 41; EX1007 at 5:19-6:5, 10:8-11:3. In my opinion, a POSA would have understood that the measured signals include both different colors and intensities, as also taught by Saxonov. *Id.* ¶¶ 12, 20, 53, 76-78, Figs. 4-5, 10; EX1007 at 9:4-15. Thus, a POSA would have understood Saxonov to teach obtaining a cumulative signal measurement including each signal of the potential signals from the included probes to identify and quantify the polynucleotide analyte(s) present.

11. wherein the cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence,

201. It is my opinion that Saxonov discloses and at a minimum renders obvious the cumulative signal measurement non-degenerately indicating the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence, as recited in claim 1.

202. In my opinion, this claim element merely reflects the results of analyzing the claimed “at least seven” polynucleotides using the components in the steps of the method that I have discussed above. *See supra* Sections X.A.3-X.A.5.

The basis for why this element would have been obvious to a POSA is, accordingly, already amply set forth. *See id.*

203. As discussed above, Saxonov discloses the unambiguous detection of multiple analytes using multiple different colors and signal intensities to distinguish among polynucleotides. *See supra* Section X.A.3; EX1004 ¶¶ 13, 17-18, 20, 63-67, 76-79, Figs. 5, 9-10; EX1007 at 2:15-3:3; 3:15-4:20, 5:19-6:5, 9:4-10:3, 12:21-19:29, Fig. 5. Saxonov, for instance, teaches using target-specific probes that are each of a single color and emit one of two or three different intensities to distinguish the presence or absence of any combination of eight or nine targets. *See supra* Section X.A.3; EX1004 ¶ 13, 20, 60-67, Fig. 5; EX1007 at 5:19-6:3.

204. In my opinion, also discussed above, a POSA would have understood Saxonov to teach, but at a minimum renders obvious, selecting color labels from four different colors. Saxonov also teaches that two or three different intensities per color provides more than seven distinct labels. *See supra* Sections X.A.3, X.A.5. Further, Saxonov teaches selecting intensity values for labels such that the combined intensity for each color for any combination of targets is “separated by intensity” so that it can be uniquely mapped to the combination of targets. *See supra* Sections X.A.3, X.A.5; EX1004 ¶ 20 (describing that “an assay for three targets may generate eight clusters...separated by intensity”).

205. Saxonov thus teaches assays that are non-degenerate by design in the same manner, and for the same reasons, as the analogous assays using single color labels of varying intensity disclosed in the '051 patent. EX1001 at 25:1-2, 23:41-43, 23:59-24:19 (Table 8).

206. Specifically, the use of multiple intensities per color to multiplex the detection of analytes as discussed is analogous to the assays described in the '051 Patent as non-degenerate by design:

For example, given the 16 analytes provided in Table 8 (A-P), a single color coding scheme encoding all 16 analytes may assign the values 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192, and 16384 to analytes A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, and P, respectively. Such a coding scheme is non-degenerate by design and the cumulative intensity result can be unambiguously decoded to indicate the presence or absence of analytes A-P.

Id. at 25:5-13; *see also id.* 25:1-2, 23:41-43, 23:59-24:19 (Table 8). In my opinion, the design described in the '051 Patent is analogous and the equivalent to that disclosed in Saxonov that utilize the same color and different intensity for analytes.

207. A POSA informed by Saxonov would have been motivated to use single color probes of different intensities that can be distinguished from one another, selecting from four colors and two or three intensities, because the resulting assay would allow non-degenerate determination of useful numbers of polynucleotide

analytes while also providing the benefits of a multiplex assay. *See supra* Section X.A.3. A POSA would have had a reasonable expectation of success because using multiple single color probes of varying intensities was well-known.

12. wherein said method non-degenerately detects the presence or absence of the at least seven polynucleotide analytes in said single sample solution volume, in any combination of presence or absence,

208. In my opinion, Saxonov discloses and at minimum renders obvious a method that non-degenerately detects the presence or absence of at least seven polynucleotide analytes in a single sample solution volume, as recited in claim 1. This is clearly set forth in the preceding section. *See supra* Section X.A.11 (and sections cited therein). Specifically, it is my opinion that a POSA would not have understood this claim limitation to require any additional feature not already present in the prior claim limitation at Section X.A.11. The claim requires non-degenerately detecting the presence or absence of at least seven polynucleotide analytes in a single sample volume, which occurs from decoding a cumulative signal measurement taken of the sample volume. As such, a POSA would not have understood the two to be practically different. Accordingly, it is my opinion that Saxonov discloses this step for the reasons expressed above.

209. Briefly, Saxonov discloses techniques wherein one can non-degenerately detect the presence of, for instance, eight different targets in a droplet

by associating the targets with different intensity/color combinations, by using four different colors and two intensities for each. *See supra* Sections X.A.3, X.A.11.

210. In addition, the methods and examples disclosed in Saxonov are the exact same types of assays detecting the presence of polynucleotide analytes in droplets that Patent Owner accuses of infringing in the parallel litigation. Both employ the use of dPCR involving droplets and are directed to determining the presence or absence of analytes in each droplet. *See generally* EX1012. In my opinion, the assays in Saxonov are wholly consistent with those identified in Patent Owner's infringement contentions, which contend that the determination of polynucleotide analytes in droplets are infringing. *See generally* EX1012.

211. Further, it is my opinion that, to the extent Patent Owner contends that "single sample solution volume" in this clause does not encompass a droplet and instead refers only to a "sample volume" that is used to generate droplets, such as an entire volume including sample provided to a droplet generator, the claim remains invalid because Saxonov teaches using the total number of droplets that are positive and negative for the target with Poisson statistics to "determine the concentration" of each analyte in the sample. EX1004 ¶ 19 ("Concentrations for each target may be estimated based on the number of droplets positive for each target and the total number of droplets, for example, using Poisson statistics."). A POSA would have understood that this allows one to non-degenerately detect the presence or absence

of any number of polynucleotide analytes in the single sample solution volume, in any combination of presence or absence. In addition, this is how Patent Owner contends the products that it accuses of infringing operate. *See generally* EX1012.

13. without requiring any step of immobilization of said polynucleotide analytes, physical separation of said polynucleotide analytes, or mass spectrometry.

212. It is my opinion that Saxonov teaches the detection of multiple polynucleotide analytes without immobilization of the polynucleotide analytes or mass spectrometry, as recited in claim 1.

213. As an initial matter, the polynucleotide analytes in Saxonov are not immobilized nor is there use of mass spectrometry. The polynucleotide analytes detected in the assays in Saxonov are not immobilized. The exemplary assays in Saxonov do not make use of mass spectrometry.

214. Regarding physical separation of the polynucleotide analytes, Saxonov discloses forming droplets. *See* EX1004 ¶ 28 (“The sample may be separated into partitions [and] the partitions may be droplets.”); *id.* ¶ 29 (teaching that droplets “may be formed with a fluid dispenser, such as a pipette, with a droplet generator, by agitation of the sample (e.g., shaking, stirring, sonication, etc.), and/or the like.”).

215. Under Patent Owner’s construction in parallel litigation, however, the formation of droplets wherein reactions take place does not prevent the claim from being rendered obvious. There, the products Patent Owner contends are infringing

are ones that specifically include droplet formation. *See generally* EX1012. It is my opinion that Patent Owner cannot credibly contend that the use of droplets in Saxonov prevents it from rendering the claims obvious in light of Patent Owner's position in parallel litigation. *Id.*

216. Further, Saxonov expressly teaches detecting and measuring multiple polynucleotide analytes and multiple copies of the same polynucleotide analytes in the same droplet. EX1004 ¶¶ 78-79, Figs. 9-10 EX1007 at 13:10-13, 17:4-19:23 (Example 3), 4:17-20, 5:19-6:5, 9:4-15, 2:15-3:3, 12:21-13:8, Examples 2-4, Fig. 5; *see supra* Section X.A.12.

217. Accordingly, I apply Patent Owner's construction and find that droplet formation does not prevent Saxonov from rendering the '051 patent claims obvious.

B. Claim 2: The method of claim 1, wherein said hybridization probes comprise an identical fluorophore, and wherein said plurality of hybridization probes are specific for different polynucleotide analytes.

218. As described above, Saxonov renders obvious the method of claim 1 that non-degenerately indicates the presence or absence of the polynucleotide analytes. *See supra* Section X.A.

219. It is further my opinion that Saxonov discloses the further limitations of claim 2. Saxonov teaches using hybridization probes that include an identical

fluorophore where the hybridization probes are specific for different polynucleotide analytes. *See supra* Sections X.A.4, X.A.11.

220. For example, as I describe above, Saxonov teaches assays using sets of hybridization probes specific for different polynucleotide analytes that include single color labels of different intensities. *See supra* Sections X.A.3, X.A.A.5.

221. In my opinion, a POSA would have also understood that generating signals with the same colors using the same fluorophores would have been straightforward relative to methods using different fluorophores, even if they were similar, to minimize differences in the generated signals. *See, e.g.*, EX1004 ¶ 51 (discussing that in some cases different fluorophores can be used, but only so long as each of the different fluorophores can be detected in the same channel).

222. As such, a POSA would have understood Saxonov to teach that “an identical fluorophore” is present in the disclosed assays, in which multiple polynucleotide analyte targets are detected using different target specific probes that include the same fluorophore, albeit with the probes generating different signal intensities. *Id.*; *see supra* Section X.A.3, X.A.A.5.

223. Saxonov further teaches that the different intensities of the same color used to differentiate between different polynucleotide analytes can be achieved by using different number of fluorophores on the target-specific hybridization probes or different concentrations of the probes:

In some embodiments, one probe may be labeled with a different number of fluorophores than the other probe, and/or the probes may be located in slightly different local environments, creating a different level of fluorescence for each probe following reaction. Alternatively, or in addition, both probes may be labeled with the same number of fluorophores (e.g., one fluorophore), but there may be more or less of one probe than the other in the sample, so that a greater or smaller signal is created when the reactions have occurred.

EX1004 ¶ 51. Accordingly, Saxonov discloses using the same fluorophore across probes targeted at different polynucleotide analytes.

224. A POSA would have understood generating signals with the same colors by using the same fluorophores would have been straightforward relative to methods using different fluorophores, because any difficulty that might arise if using a collection of similar, but not identical, fluorophores would be minimized. *Id.* Saxonov expressly teaches, in its examples, the use of the same fluorophore to identify and detect different targets, such as in Figure 5, where both targets are detected using a FAM fluorophore. *Id.* ¶¶ 60-67, Fig. 5. A POSA would have understood that using the same fluorophore for each particular color used would be more straightforward than using a mixture of fluorophores for at least the reason that there is only one type of fluorophore for each color, but also because a single excitation and emission wavelength are used for that particular color signal.

225. In a parallel proceeding, Patent Owner contends that an assay is infringing where two probes that are specific for two different polynucleotide analytes are labeled with the same fluorophore at different intensities. *See* EX1012, at 60-61. There, Patent Owner contends that Petitioner infringes claim 2 because two probes carry the same FAM fluorophore, where probes for one analyte, PLAU, is at low intensity and probes for a second analyte, CCND1, is at high intensity. *Id.* In that same assay, other probes with single fluorophores that are not FAM are used to detect other analytes. Accordingly, Saxonov discloses and at minimum renders obvious the claim limitation in claim 2 also under the construction as set forth by Patent Owner.

C. Claim 3: The method of claim 1, wherein said detecting is performed with a plurality of hybridization probes comprising different fluorophores, wherein said plurality of hybridization probes are specific for different analytes.

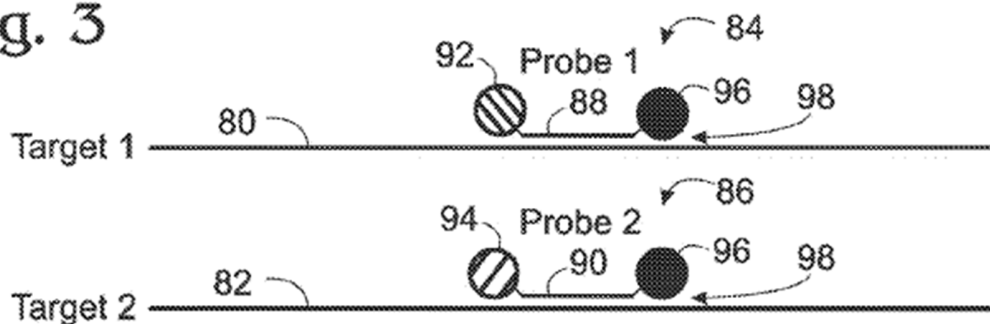
226. As described above, Saxonov renders obvious the method of claim 1 that non-degenerately indicates the presence or absence of the polynucleotide analytes. *See supra* Section X.A.

227. It is my opinion that Saxonov discloses the limitations of claim 3. As discussed above, Saxonov discloses using a plurality of hybridization probes that comprise different fluorophores where the hybridization probes are specific for

different polynucleotide analytes. *See supra* Section X.A.5; *see also* EX1004 ¶¶ 20, 51; EX1007 at 2:11-17, 3:15-20, 7:9-10, 9:5-15; 13:14-16, Fig. 3.

228. For example, Saxonov Figure 3 shows a plurality of hybridization probes comprising different fluorophores, wherein said plurality of hybridization probes are specific for different analytes:

Fig. 3



Id. Fig. 3. In Figure 3, two different targets (80 and 82) are hybridized to corresponding probes (88 and 90). *Id.* ¶ 49. Each of the probes provide target specificity by hybridizing exclusively to only one of the two targets. *Id.* ¶ 50. The fluorophores (92 and 94) may be different to create distinguishable signal, because an aspect of the fluorescence is different between the two. *Id.* ¶ 51.

229. A POSA would have understood that it was well known to use a plurality of hybridization probes comprising multiple different fluorophores where the different probes are specific for different polynucleotide analytes in order to detect and identify the different analytes. As discussed above, the '051 patent states

that using probes with different fluorophores to detect different targets is well known. *See supra* Section VI.B; *see also* EX1001 at 8:40-44 (“The traditional encoding method for multiplexed fluorescent assays has been to encode each analyte with a single color; i.e., $M=N$, where M is the number of analytes that can be detected and N is the number of spectrally resolved fluorescent probes.”).

D. Claim 4: The method of claim 1, wherein said cumulative intensity signal(s) are generated during a polymerase chain reaction.

230. As described above, Saxonov renders obvious the method of claim 1 that non-degenerately indicates the presence or absence of the polynucleotide analytes. *See supra* Section X.A.

231. It is my opinion that Saxonov discloses generating the cumulative intensity signal(s) during a polymerase chain reaction, as required by claim 4. Saxonov discloses detecting the amplification of a target polynucleotide by use of a fluorescent probe included in the reaction, that is, by including a dye that provides a fluorescent signal for detecting if a specific target has been amplified. EX1004 ¶ 5 (“digital assays frequently rely on amplification of a nucleic acid target in partitions to enable detection of a single copy of an analyte” and that amplification “may be conducted via the polymerase chain reaction (PCR), to achieve a digital PCR assay.”); EX1007 at 2:7-9, Figs. 3-4.

232. Saxonov further discloses the use of hybridization probes that include both fluorophore(s) and a quencher and detecting the resulting fluorescent signal when the fluorophore(s) and quencher are uncoupled. EX1004 ¶¶ 49, 52-53, Figs. 3-4. A POSA would have understood this uncoupling occurs during the polymerase chain reaction, where bound hybridization probes are cleaved during the extension of the primer, separating the fluorophore(s) and quencher(s), generating color signal(s). *Id.* ¶¶ 52-53, Figs. 3-4; *see supra* Section V.C (discussing use of TaqMan probes).

233. Saxonov discloses probes including both fluorophore(s) and quencher(s). EX1004 ¶¶ 52-53, Figs. 3-4. A POSA would have understood the benefit of fluorophores to detect polynucleotide analytes in a sample solution, and would have understood the benefit of quenchers to reduce the noise emitted from intact fluorescent probes, thus increasing the signal to noise ratio. *Id.* ¶¶ 52-53, Figs. 3-4

234. A POSA would have used probes where the fluorophore(s) and quencher(s) were separated by the polymerase extension during PCR to generate fluorescent signal(s) for the advantages of eliminating post-amplification steps to detect the amplified products and for the improvement in signal relative to background fluorescence. *Id.* ¶¶ 52-53, Figs. 3-4.

235. A POSA would have further understood that the techniques above as described in Saxonov are well-known and widely-used and include the TaqMan assay that is routinely used successfully and well known in the art. As described above, the '051 Patent likewise mentions the use of TaqMan probes in the art.

XI. GROUND 2: OBVIOUSNESS OF CLAIMS 5-6 IN VIEW OF SAXONOV AND SILVERBROOK

A. Claim 5: The method of claim 1, further comprising transmitting information concerning the presence or absence of said polynucleotide analytes through a computer network.

236. As described above, Saxonov discloses, but at a minimum renders obvious, the method of claim 1 that non-degenerately indicates the presence or absence of the polynucleotide analytes. *See supra* Section X.A.

237. It is my opinion that, to the extent Patent Owner contends Saxonov does not disclose or render claim 5 obvious, as described above, that Saxonov and Silverbrook in combination render claim 5 obvious.

238. A POSA informed by Saxonov would have understood to transmit information concerning the presence or absence of polynucleotide analytes through a computer network. For example, a POSA would have understood that the images depicting results in Saxonov would have been routinely produced by the use of computer(s) and that it would have been obvious to transmit the information necessary to generate the images on a computer network, in addition to the results

of the analysis. A POSA would have understood that results of an assay would be transmitted to a computer through a computer network so that they can be visualized and further analyzed.

239. It is also my opinion that a POSA would have known that the images representing collected data were generated by a computer and so the information necessary to generate those images would have been transmitted through a computer network. For example, Saxonov Figures 4-8 all graphically depict data in a manner that would have been computer generated:

Fig. 4

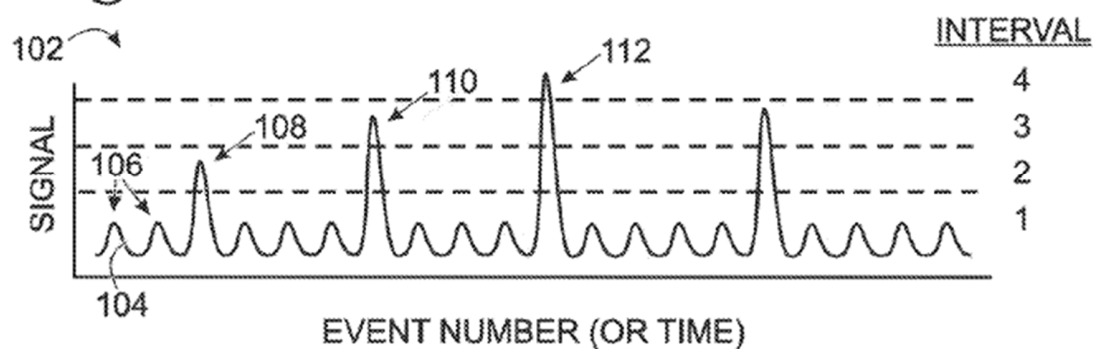
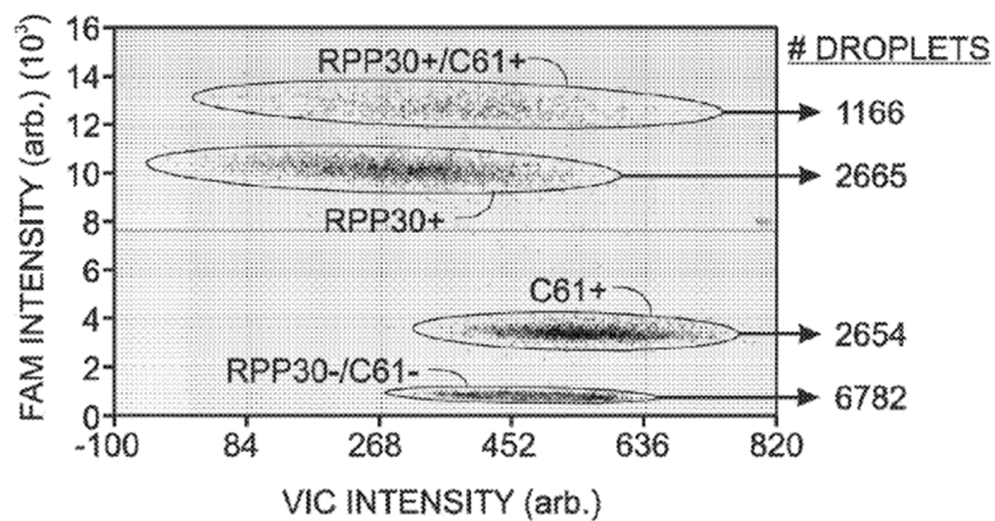


Fig. 5



EX1004 Figs. 4-5;

Fig. 6

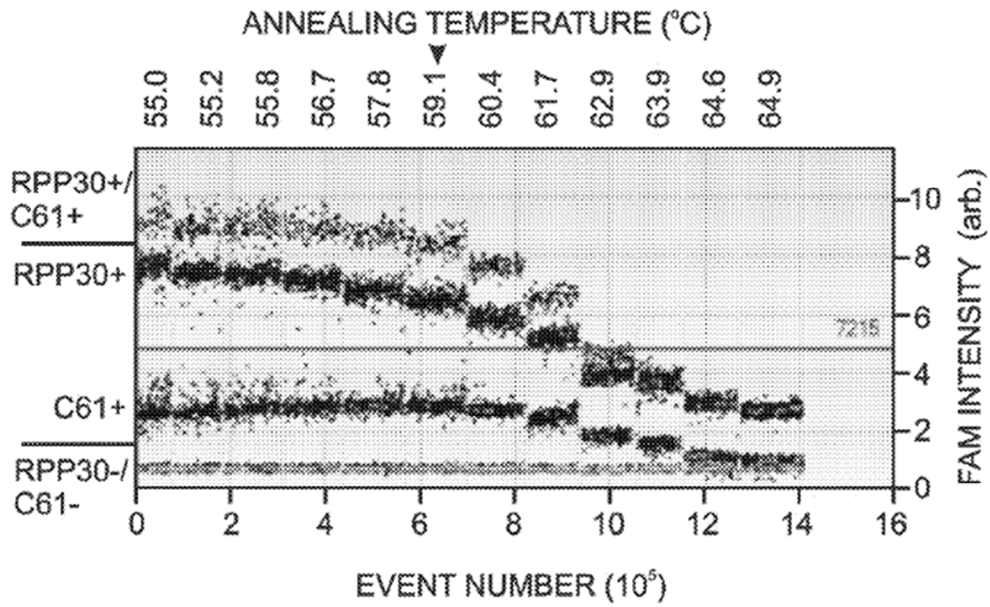
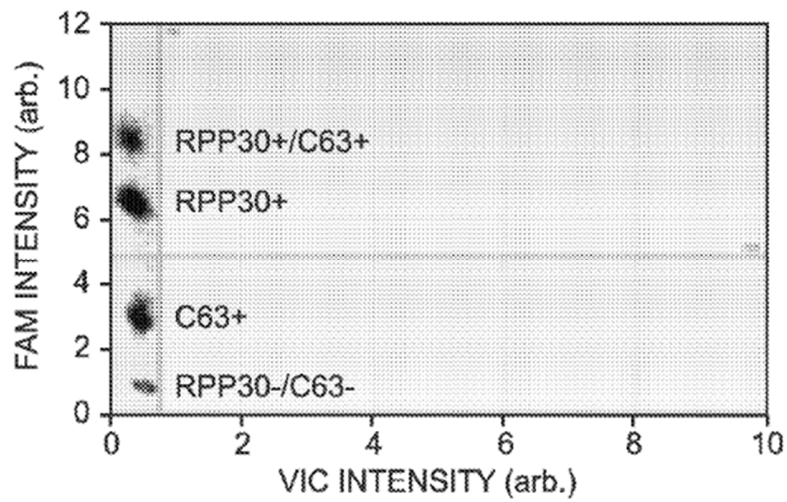


Fig. 7



Id. Figs. 6-7;

Fig. 8A

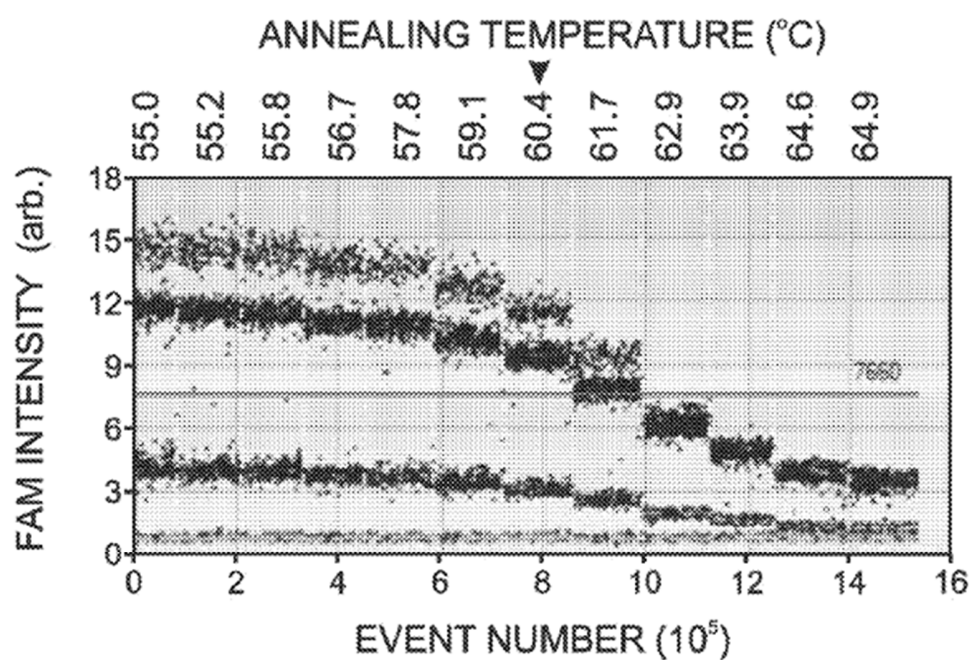
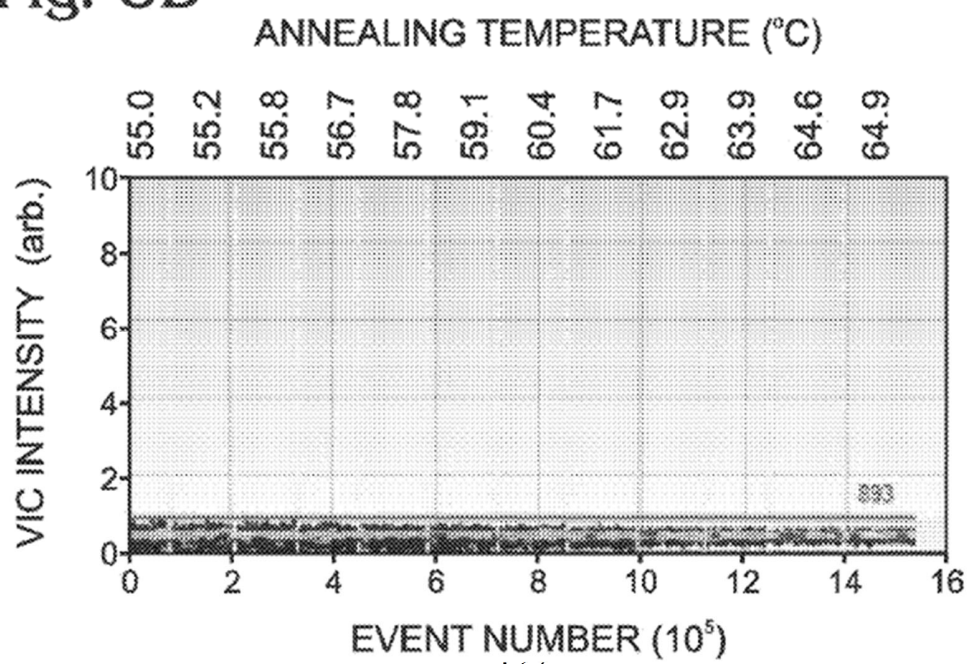


Fig. 8B



Id. Fig. 8.

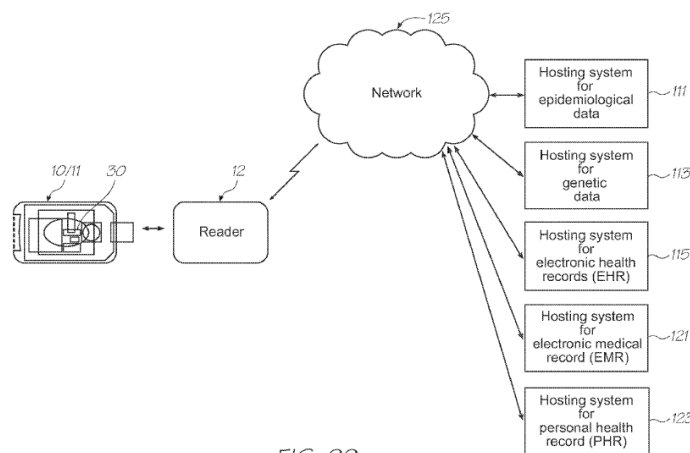
240. It is my opinion that a POSA would have been motivated to transmit information obtained concerning the presence or absence of polynucleotide analytes in a sample through a computer network because it provides relevant, useful information to those who could use the information, such as a physician 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.

241. 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.

242. To the extent that the combination of Saxonov does not disclose the further limitations of claim 5, it would have been obvious in further view of Silverbrook's teaching of a nucleic acid testing device and method that employs PCR and hybridization to identify what analytes are present in a sample and transmitting results of the testing through a computer network. 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. Thus, Silverbrook specifically teaches transmission of clinically relevant assay data through a computer network.

243. Further, a POSA would have understood that information regarding an assay can be transmitted through a computer network from Silverbrook's explicit disclosure regarding "Test module 10... a fluorescence-based detection technique to identify target molecules." *Id.* ¶ 195. Test module 10 amplifies specific nucleic acid analytes and generates fluorescent signals for detection. *Id.* ¶ 198. In addition, "data generated by test module 10 can be used to update, via...network 125,...electronic medical records." *Id.* ¶ 201.

244. Further, Silverbrook's Figure 99, reproduced below, depicts a network configured to host and transmit information including "electronic health records," "electronic medical records," among others.



EX1009 Fig. 99. A POSA would have understood these records to include assay results.

245. Accordingly, Silverbrook teaches transmitting information concerning the results of an assay for detecting polynucleotide analytes through a computer network.

246. In my opinion, a POSA would have been motivated to transmit information through a computer network as taught by Silverbrook, as a POSA would have understood the benefit of providing information remotely to databases of medical records to allow physicians to receive and review assay results efficiently and expeditiously.

247. As such computer networks and transmission were well known in the art, a POSA would have had a reasonable expectation of success in performing the further limitations of claim 5.

B. Claim 6: The method of claim 1, further comprising providing information concerning the presence or absence of said polynucleotide analytes to a health care professional, wherein the health care professional makes a clinical decision based on said information.

248. As described above, Saxonov discloses, but at a minimum renders obvious, the method of claim 1 that non-degenerately detects the presence or absence of the polynucleotide analytes. *See supra* Section X.A.

249. It is my opinion that a POSA would have understood that patient samples are routinely sent to a hospital laboratory or centralized clinical laboratory, such that clinical tests may be performed on the sample and the results transmitted to a physician to inform the physician on a clinical decision based on the result. For example, this includes routine clinical tests, such as the level of glucose, potassium, or creatinine in a patient's serum or plasma. A POSA would further have understood that tests of genetic markers are also routinely ordered and performed, which informs physicians on the proper treatment.

250. It is my opinion that, to the extent Patent Owner contends Saxonov does not disclose or render claim 6 obvious, as described above, that Saxonov and Silverbrook render claim 6 obvious.

251. Silverbrook teaches that quantifying the starting amount of different nucleic acids in samples from patients is “particularly useful in molecular diagnostics where treatment options may differ depending on the pathogen load in the sample.” EX1009 ¶ 34.

252. Silverbrook further teaches the availability of such molecular diagnostic data at various locations including, for example, a healthcare facility or a patient’s home, to facilitate “clinical management decisions such as infection control and antibiotic use.” *Id.* ¶¶ 56-62.

253. Thus, a POSA would have been motivated to provide information concerning the presence or absence of polynucleotide analytes to a healthcare professional to facilitate clinical decisions for improved treatment. It is further my opinion that a POSA would have had a reasonable expectation of doing so, as it was common practice to transmit information regarding the result of an assay, such as the presence or absence of analytes in a patient sample, to a health care professional, such as the patient’s physician, to facilitate a clinical decision such as the appropriate treatment or modification of a treatment.

XII. GROUND 3: OBVIOUSNESS OF CLAIM 7 IN VIEW OF SAXONOV, KRAMER, AND MALTEZOS

A. Claim 7: The method of claim 1, wherein at least one step of said method is performed using a computer.

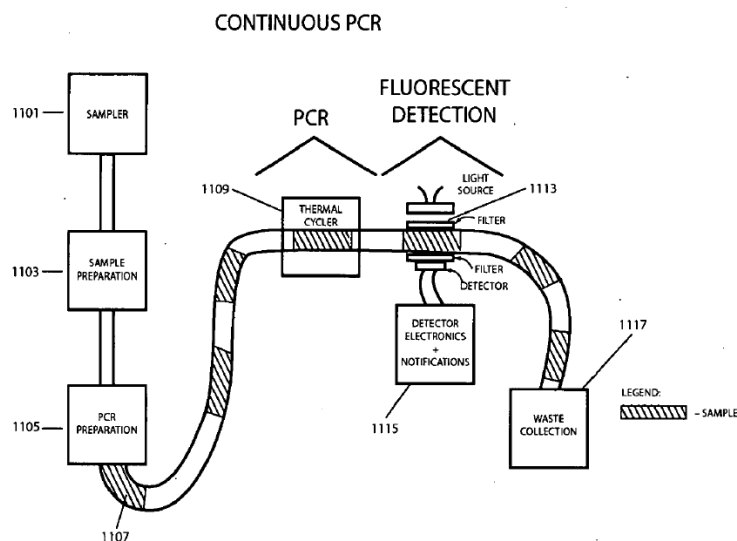
254. As described above, Saxonov discloses, but at a minimum renders obvious, the method of claim 1 that non-degenerately indicates the presence or absence of the polynucleotide analytes. *See supra* Section X.A.

255. Saxonov discloses that at least one step of the method is performed using a computer. Saxonov discloses that an apparatus for performing its method includes a “data processing assembly (a processor),” which a POSA would have understood to be a computer that is used to perform calculations required for steps of the method, including measuring and thereby generating cumulative signal measurement. EX1004 ¶ 45 (“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.”); EX1007 at 12:21-13:8 (citing US 2010/0173394, incorporated by reference).

256. A POSA would have understood that a computer is routinely used to control a droplet generator and fluorescence detection instrument, as well as collect and analyze the data, and would have understood this data processing assembly to be a computer.

257. It is my opinion that, to the extent Patent Owner contends Saxonov does not disclose or renders claim 7 obvious, as described above, that Saxonov and Maltezos render claim 7 obvious.

258. Maltezos discloses an apparatus and method for performing PCR analysis that includes “a control assembly that controls...operation of the optical assembly” and describes detection electronics 1115 to evaluate the signal from the detection region 1113 of the apparatus in which fluorescence measurements from the PCR are detected. EX1010 ¶¶ 12, 60, Fig. 11. This apparatus is shown in Figure 11 of Maltezos, reproduced below:



Id. Fig. 11.

259. A POSA would have understood Maltezos’ control assembly to include a computer based on the disclosure of the control assembly and detection electronics

and in light of Figure 11 shown above. A POSA would have further understood Maltezos to teach that the computer controls the detecting and/or measuring step of the PCR analysis. *See, e.g.,* EX1010 ¶¶ 130-134 (describing that the optical assembly operatively performs the measurement and detection of detectable labels in the PCR analysis).

260. Given the teachings of Saxonov and Maltezos, a POSA would have found it obvious to use a computer to perform the detecting (and/or measuring) step, which involves PCR as described above.

261. A POSA would have been motivated to use a computer to facilitate the operations and calculations necessary to measure cumulative intensity signal(s) and determine the presence of seven polynucleotide analytes.

262. A POSA would have further had a reasonable expectation of success as the performance of such steps using a computer was well known in the art as described above.

XIII. GROUND 4: OBVIOUSNESS OF CLAIMS 8-9 IN VIEW OF SAXONOV MALTEZOS, AND OH

A. Claim 8: The method of claim 7, wherein said computer is located on a remote server.

263. As described above, Saxonov discloses, but at a minimum renders obvious, the method of claim 1 that non-degenerately indicates the presence or absence of the polynucleotide analytes. *See supra* Section X.A.

264. Saxonov discloses that at least one step of the method is performed using a computer, wherein the computer is located on a remote server. For example, Saxonov teaches that the computer is located on a remote server in disclosing “a data processing assembly (a processor) 68 (“PROC”),...[that] may be, or may be included in, a controller that communicates with and controls operation of any suitable combination of the assemblies....[where] [a]ny suitable combination of the assemblies may be operatively connected to one another.” EX1004 ¶ 45 (describing Fig. 2); EX1007 at 12:2-9.

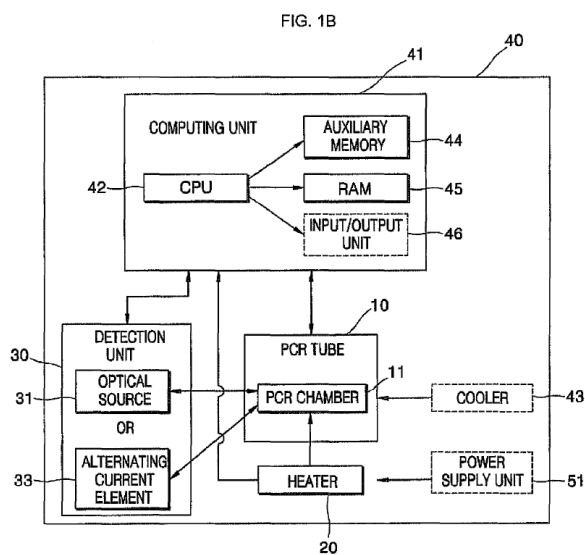
265. A POSA would have understood “operatively connected” to include any type of connection, including via remote connection over a network such that the computer is located on a remote server.

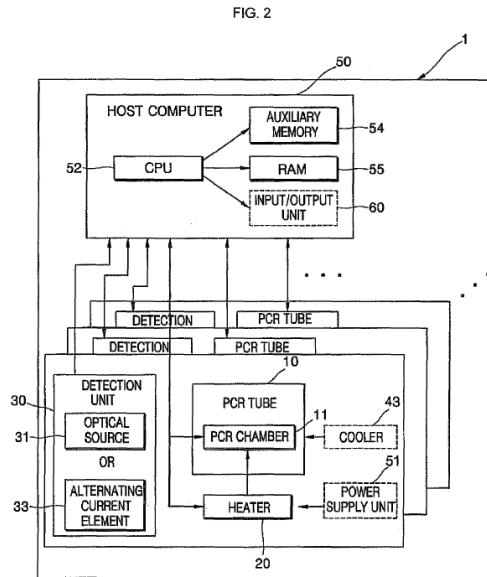
266. As described above, Saxonov and Maltezos render obvious the method of claim 7 that non-degenerately indicates the presence or absence of the polynucleotide analytes, wherein at least one step is performed using a computer. *See supra* Section XII.A.

267. It is my opinion that, to the extent Patent Owner contends that Saxonov, alone or with Maltezos (as applied to claim 7), does not disclose or render obvious locating the computer on a remote server, as described above, that Saxonov, Maltezos, and Oh render locating the computer on a remote server obvious.

268. Oh discloses a PCR module and method using the module, where the module, depicted in reproduced Figures 1B and 2 below, includes a computing unit 41 that “performs appropriate computation based on the data received from [] sensors.” EX1011 ¶¶ 17, 58, 66, 71. Oh also contemplates that if computing unit 41 is not included in the module, a remote host computer 50 performs that function.

Id.;





Id. Figs. 1B, 2.

269. A POSA would have understood the “appropriate computation” that remote host computer 50 performs to include computations for the detecting and/or measuring steps, as computers are routinely used for such purposes.

270. Given the teachings of Saxonov and Oh, a POSA would have found it obvious to use a remote computer to perform the measuring (and calculating) step.

271. A POSA would have been motivated to use a remote computer as they would have understood that computers used to perform analytical processes can be, and routinely are, located remotely from analytical equipment for the numerous benefits of conducting analysis at a remote location, such as at a central location to perform analyses for a number of analytical devices.

272. A POSA further would have had a reasonable expectation of success in performing such methods as such methods were well known in the art as described above.

B. Claim 9: The method of claim 7, wherein said computer is located on a thermal cyclor.

273. As described above, Saxonov discloses, but at a minimum renders obvious, the method of claim 1 that non-degenerately indicates the presence or absence of the polynucleotide analytes and Saxonov and Maltezos render obvious claim 7 wherein at least one step of the method of claim 1 is performed using a computer. *See supra* Sections X.A, XII.A.

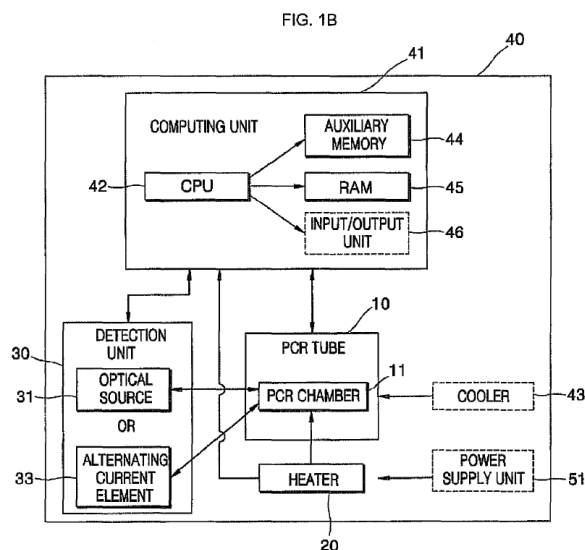
274. Saxonov discloses that at least one step of the method is performed using a computer, wherein the computer is located on a thermal cyclor. For example, Saxonov discloses an exemplary apparatus for the assay that includes a “thermal incubation assembly, such as a thermocyclor” and “a processor.” EX1004 ¶ 45; EX1007 at 11:14-12:11.

275. Saxonov further discloses that the processor “may be, or may be included in, a controller that communicates with and controls operation of any suitable combination of assemblies.” EX1004 ¶ 45. Saxonov teaches that this described combination of assemblies “may be operatively connected to one another and/or...unconnected to the other assemblies.” *Id.*

276. In light of the above, a POSA would have understood Saxonov to disclose or teach the use of systems with the computer physically connected to the thermal cycler, i.e., where “the computer is located on a thermal cycler,” as such devices were well known and in widespread use for their benefits of improved control and ease of use at the time of the alleged invention.

277. It is my opinion that, to the extent Saxonov and Maltezos do not render claim 9 obvious, as described above, it is my opinion that Saxonov, Maltezos, and Oh render claim 9 obvious.

278. Oh discloses a PCR module and method of using the module, where the module is “a combined PCR thermal cycler and PCR product detector” that includes “heater 20...to apply heat to the PCR tube[s]” and a computing unit 41 that “performs appropriate computation based on the data received from attachment sensors”:



EX1011 Fig. 1B , ¶¶ 17, 58, Abstract.

279. A POSA would have understood the included computing unit 41 to be located on the thermal cycler as it is part of the same combined apparatus, i.e., the PCR module. As further illustration, power supply unit 51 powers both the thermal cycler and the computing unit and a POSA would have understood that the devices, including the thermal cycler and computer, are combined together as they all require power and there is only one power source in the diagram.

280. A POSA would have been motivated to locate the computer on the thermal cycler for the benefits discussed above, including that such devices were well known and in widespread use for their benefits of improved control and ease of use at the time of the alleged invention.

281. A POSA would have had a reasonable expectation of success in performing the method of claim 1 wherein a computer to perform at least one step of the method is located on a thermal cycler because such computers and apparatuses were well known and used at the time of the alleged invention.

XIV. GROUND 5: OBVIOUSNESS OF CLAIMS 1-4 IN VIEW OF SAXONOV IN VIEW OF KRAMER AND/OR LARSON

282. Saxonov alone discloses, but at a minimum renders obvious, claims 1-4. *See supra* Sections X.A-X.D. It is my opinion that claims 1-4 would also have been obvious in further view of Kramer and/or Larson. This combination is identical in all respects to Ground 1 with the exception of three specific claim elements that pertain to the claimed number of seven polynucleotide analytes and four fluorophores:

- “comprising or potentially comprising, at least one of at least seven polynucleotide analytes”;
- “wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores”; and
- “wherein the cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence.”

283. To the extent Patent Owner contends or the Board finds that that the above claim elements are not taught or rendered obvious by Saxonov alone, a POSA would have found it obvious to modify Saxonov's methods based on the teachings of Kramer and/or Larson to achieve a method that includes these elements, as set forth in detail below.

284. Similar to Saxonov and the '051 patent, Kramer and Larson are directed to the multiplex detection of polynucleotide analytes using color-labeled sequence-specific probes, including where there are fewer different colors than analytes. EX1008 ¶¶ 8-12, 14; EX1017 ¶¶ 34, 35, 138-153; EX1004 Figs. 9-10; EX1001 at code (57), 12:29-14:2, 19:35-38, 23:31-25:30. Accordingly, in my opinion, Kramer and Larson are analogous art to the '051 patent because they are within the same field of endeavor.

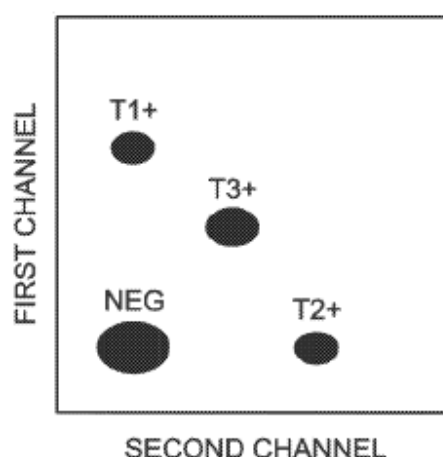
A. Claim 1: comprising or potentially comprising, at least one of at least seven polynucleotide analytes, and

285. As discussed above, Saxonov alone discloses but at a minimum renders obvious this claim element. *See supra* X.A.3. To the extent Patent Owner contends otherwise, this claim element to analyze at least seven analytes would have nonetheless been obvious in further view of Kramer and/or Larson.

286. In addition to the disclosure of distinguishing targets with different intensities of the same color, *see supra* Section X.A.3, Saxonov also teaches

multiplexing using multiple colors to distinguish each target. This is evident in Figure 10, where three targets are detected using two colors, with a first target (T1) identified by a first color (at a certain intensity), a second target (T2) identified by a second color (at a certain intensity), and a third target (T3) identified by both colors (at different intensities than the first and second targets):

Fig. 10



EX1004 Fig. 10; *see also id.* ¶¶ 18, 78-79. This approach of distinguishing targets using combinations of colors is also taught in the '013 provisional, which teaches that signals can be “collected in one or more different channels” from “the same” target. *See* EX1007 at 9:8-10.

287. Importantly, Saxonov contemplates a situation wherein multiple targets are present in a droplet, as it explicitly states regarding Figure 10: “To simplify the presentation, double-positive populations (T1 +/T2+, T1 +/T3+, and T2+/T3+) and the triple-positive population (T1 +/T2+/T3+) are not shown.” EX1004 ¶ 78. Thus,

if are generated that have combinations of multiple targets, Saxonov contemplates distinguishing the different combinations of targets in a droplet.

288. Kramer teaches that the use of different combinations of colors to distinguish targets, as described in Saxonov, can be extended to even larger numbers of targets. This is evident as Kramer discloses assays that are “highly multiplexed” and are “capable of detecting any of at least six, preferably at least 10, and in certain embodiments 30-60 and even more possible target sequences.” EX1008 ¶ 8. As another example, Kramer discloses “highly multiplexed homogeneous assays for screening samples for the presence of a target nucleic acid sequence from among at least ten, and as many as 60 or more, possible targets, utilizing conventional fluorescence detection equipment and techniques, and fluorescently labeled hybridization probes.” *Id.* ¶ 5. As another example, Kramer teaches that “as many as 20 differently coded probes can be combined in the same assay mixture.” *Id.* ¶ 30 (“For example, probes may be subdivided into three portions for use with a panel of five fluorophores, providing up to ten differently coded probes, as outlined in the bottom portion of the table, and additional probes may be subdivided into two portions for use with a panel of five fluorophores, providing up to ten additional differently coded probes, as outlined in the top portion of the table.”). Kramer further illustrates in Figure 1 an example of how different combinations of large numbers of colors can be used to distinguish large numbers of targets:

PANEL OF 4(a, b, c, d), 5(+e), 6(+f), 7(+g) or 8(+h) FLUOROPHORES

[illegible]

PANEL OF 5(a, b, c, d, e), 6(+f), 7(+g) or 8(+h) FLUOROPHORES

[illegible]

FIGURE 1

Id. Fig. 1; *see also supra* Section VIII.B. As shown in Figure 1, Kramer teaches that targets can be distinguished using one, two, three, or more colors. *Id.* Fig. 1, ¶¶ 30-31.

289. Larson, like Kramer and Saxonov, teaches multiplex detection of at least seven polynucleotide analytes. As an example, Larson discloses experimental results of an assay for nine analytes using “just two colors (probes containing FAM and VIC fluorophores).” EX1017 ¶ 150. The results are depicted in Figure 15:

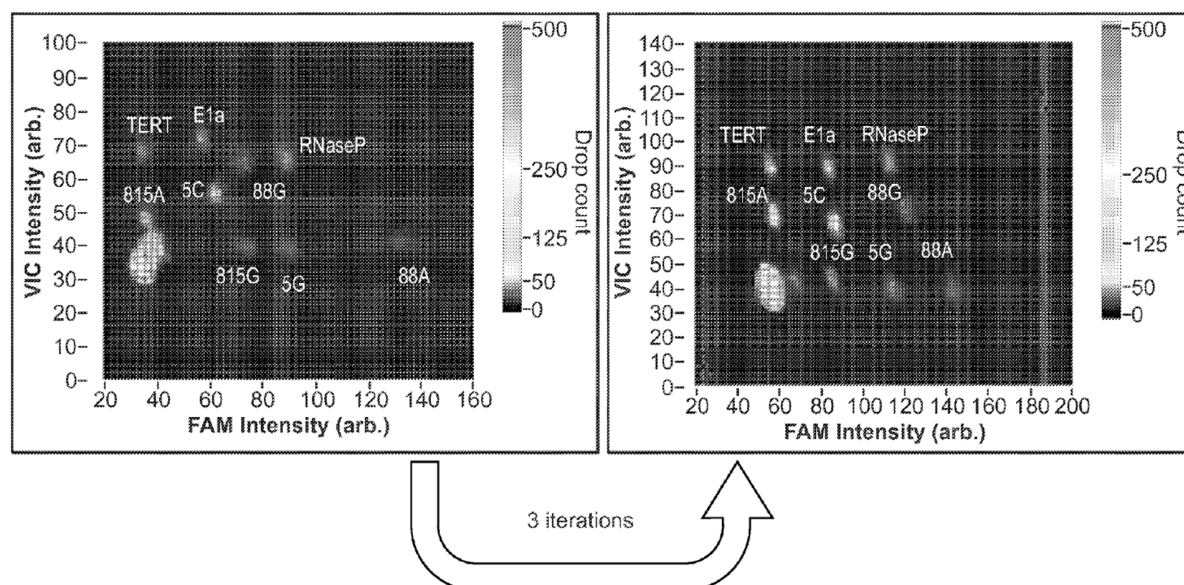


FIG. 15

Id. Fig. 15; *id.* ¶ 35 (“FIG. 15 depicts a 9-plex dPCR assay for spinal muscular atrophy with only two fluorophores, showing the process of optimizing droplet intensities.”). This 9-plex assay was “demonstrated with just two colors (probes containing FAM and VIC fluorophores).” *Id.* ¶ 150.

290. In addition, Larson also teaches the use of additional colors to expand the level of multiplexing, where “multiplexing capacity exceeding 50x can be envisioned.” *Id.* ¶ 153. Specifically, Larson teaches that “adding multiple colors

increases the number of possible reactions geometrically, rather than linearly as with qPCR, because individual reactions can be labeled with multiple fluorophores.” *Id.* ¶ 47; *see also id.* ¶ 139 (“The maximum number of colors can be 1, or 2, or 3, or 4, or up to 10, or up to 20. The maximum number of colors can be higher than 20.”).

291. Larson likewise teaches that additional levels of intensity can achieve higher levels of multiplexing. For example, Larson teaches that “a single target may be identified by multiple probes that constitute a unique signature of both colors and intensities.” *Id.* ¶ 140; *see also id.* ¶ 138 (“The number of intensity levels can be 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. The number of intensity levels can be higher than 100.”). As shown in Figure 15 above, nine targets can be detected using multiple colors and multiple intensities, showing that differences in intensity can be used to distinguish droplets with different target types. *Id.* Fig. 15, ¶¶ 34, 145-153.

292. In my opinion, it would have been obvious to a POSA to modify the two-color approach in Saxonov’s Figure 10 from distinguishing three targets to seven targets. A POSA would have done this by increasing the number of colors and used intensity levels as needed to distinguish different droplets, as taught by Kramer and Larson. For example, the use of two additional colors would allow distinguishing droplets with up to six targets, and a seventh can be distinguished using three colors. *See infra* Section XIV.C.

293. In my opinion, a POSA would have been highly motivated to rely on the related teachings of Kramer and/or Larson, if necessary, to modify Saxonov to detect at least seven polynucleotide analytes. First, a POSA would have understood the benefits a higher-plex assay and thus would have been motivated to increase the number of targets that can be multiplexed in one assay. For example, in light of Kramer, a POSA would have understood that treatment of the HIV-1 assay requires detection of up to 30 targets to determine which mutations are present, which is necessary to determine the proper treatment. EX1008 ¶ 4. Moreover, a POSA would have understood that a highly multiplexed assay allows one “to screen for an early indication of one of numerous possible infectious agents that may be the cause of the patient’s symptoms.” *Id.* Similarly, a POSA would have been motivated to perform higher-plex assays, as “the need for higher throughput in analyzing multiple targets in parallel continues to escalate in the fields of genomics and genetics.” EX1017 ¶ 5. A POSA would also have understood another benefit of multiplexing, which is that “the simultaneous increase in the number of PCR droplets while keeping the amount of input DNA the same or lower” allows detecting more analytes “without the consumption of more DNA.” *Id.* ¶¶ 8, 104. This may be especially beneficial in scenarios of limited starting sample material. For at least these reasons, a POSA would have been motivated to modify Saxonov, if necessary, to detect at least seven polynucleotide analytes.

294. In my opinion, a POSA would have had a reasonable expectation of success in modifying Saxonov because such higher-plex assays are in the prior art. As described above, both Kramer and Larson teach higher multiplex assays for greater numbers of targets, including for at least seven targets. *See* EX1008 ¶¶ 8, 22, 31, Fig. 1; EX1017 ¶¶ 108, 138-43, 150-53, Fig. 15. In addition, both Kramer and Larson teach techniques analogous with those in Saxonov to enable higher-plex assay. For example, Kramer teaches combinations of colors mapped to targets (EX1008 Fig. 1), colors chosen from a panel of four fluorophores (*id.* ¶ 11), types of probes and fluorophores (*id.* ¶¶ 11, 31), and techniques to avoid degeneracy (*id.* ¶ 42). Larson, for instance, teaches using two colors and varying intensities to detect nine targets in a sample using droplets. EX1017 ¶¶ 134-138, 150-151, Figs. 12, 15. Accordingly, a POSA would have reasonably expected success in so modifying Saxonov with Kramer and/or Larson.

B. Claim 1: wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores, and

295. As discussed, Saxonov discloses multiple fluorophore types and, in Figure 10, specifically teaches an approach that uses two fluorophores to detect and distinguish among three targets and droplets containing combinations of the three targets. *See supra* Section XIV.A; *see also supra* Section V.A.2; EX1004 ¶¶ 18, 20, 36, 76-79, Fig. 10. To the extent Patent Owner contends Saxonov does not disclose

using four fluorophores, it would have been obvious to a POSA to modify Saxonov in view of Kramer and/or Larson.

296. For instance, Kramer teaches using four fluorophores. For example, Kramer teaches that “[m]ultiplex capability is achieved by combinatorial coding, with or preferably without added ratio coding, utilizing a panel of distinguishable fluorophores,” including where the “different fluorophores are from a panel of at least four distinguishable fluorophores.” EX1008 ¶ 11. As another example, Kramer teaches that “[c]oding is achieved...from a panel of at least four...distinguishable fluorophores.” *Id.* ¶ 31; *see also id.* ¶¶ 22, 30-31, Fig. 1. A POSA would have understood Kramer to teach using four fluorophores to label probes.

297. Larson also teaches the use of four fluorophores. For example, Larson teaches that “a single target may be identified by multiple probes that constitute a unique signature of both colors and intensities” and teaching that the “maximum number of colors can be 1, or 2, or 3, or 4....” EX1017 ¶¶ 139, 140; *see also id.* ¶¶ 5, 35, 37, 47, 90, 145, 156, 164, Figs. 14-15, 17. Larson also notes that the use of “extra colors would increase the capability even further” to detect multiple analytes. *Id.* ¶ 153. Larson also describes using two colors such that signals from both colors indicate the presence of a target. *Id.* Fig. 12C, ¶ 134; *see also supra* Section VIII.C.

298. Due to the disclosures in Saxonov, Kramer, and Larson, a POSA would have understood that four fluorophores is sufficient to create combinations of colors and/or intensities for detecting at least seven polynucleotide analytes.

299. A POSA would have been motivated to label hybridization probes with at least one of four fluorophores, as taught in Kramer and Larson. As discussed, Kramer and Larson proposed using four fluorophores. Also as discussed, increasing the number of fluorophores used, or colors, increases the level of multiplexing, such that more targets can be detected. *See supra* Section XIV.A; *see also* EX1008 ¶ 20; EX1017 ¶¶ 5, 153. In addition, as discussed above, instruments available at the relevant time are limited to detecting four colors, such that the use of four fluorophores would be an obvious design choice, as the '051 patent acknowledges that widely available instruments were pre-packaged to detect four colors. *See supra* Section X.A.5.

300. A POSA would have expected the use of fluorophores to succeed, including for the reasons discussed in the preceding section that at least seven analytes can be detected. *See supra* Section XIV.A. Importantly, Kramer and Larson expressly teaches the use of four fluorophores, which confirms that a POSA's belief that using four fluorophores would be successful. Saxonov already discloses at least five commonly used fluorophores for labeling hybridization probes, such as FAM, VIC, ROX, TAMRA, and JOE. EX1004 ¶¶ 49, 51, Fig. 3; EX1007 at 14:20-

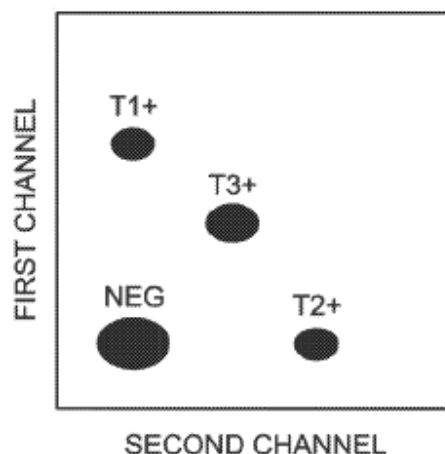
21, 4:20-23. A POSA would have been able to easily select four fluorophores in order to detect at least seven targets. As discussed, and confirmed by the '051 patent, instruments pre-packaged and capable of detecting four colors were widely available.

C. Claim 1: wherein the cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence,

301. As discussed above, Saxonov alone discloses but at a minimum renders obvious this claim element. *See supra* Section X.A.11. To the extent Patent Owner contends otherwise, a POSA would have been motivated to modify Saxonov's Figure 10 based on the teachings of Kramer and/or Larson to include an additional two colors so as to allow for the non-degenerate detection of at least seven targets in any combination of presence or absence. A POSA would have further had a reasonable expectation of success in making such a modification.

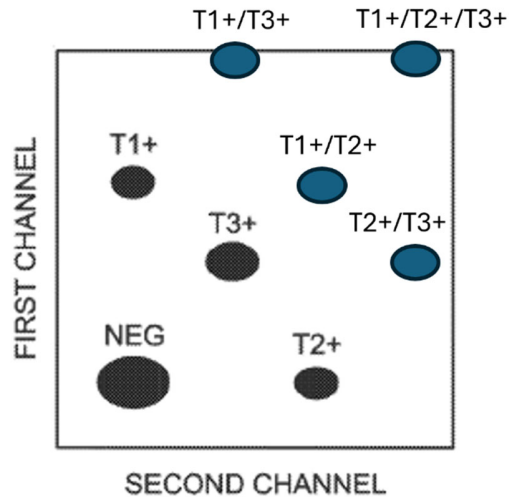
302. As discussed, Saxonov's Figure 10 discloses the distinguishing three targets using two colors. *See supra* Section XIV.A; *see also* Section XIII.A.2. When multiple targets are present in a droplet, Saxonov contemplates cumulative intensity signals that can be distinguished from one another for all combinations of the targets. This is shown in Figure 10, where droplets with different targets yields different signals:

Fig. 10



EX1004 Fig. 10, ¶¶ 18, 78-79. Saxonov specifically states that droplets with multiple targets are omitted from the figure but can be distinguished for any combination of targets. *See, e.g., id.* ¶ 78 (“To simplify the presentation, double-positive populations (T1+/T2+, T1+/T3+, and T2+/T3+) and the triple-positive population (T1+/T2+/T3+) are not shown.”). Thus, Figure 10 can be modified to include populations with different combinations of targets based on the intensities of the populations included as shown:

Fig. 10



Id. Fig. 10 (modified as described). As shown, droplets with any combinations of the three targets can be identified. This follows Saxonov’s teaching that “three targets may generate eight clusters or populations of data, separated by intensity.”

Id. ¶ 20. A POSA would have understood the modified figure to be what is taught by Saxonov, with eight distinguishable droplet populations that unique identifies the targets in those droplets. Thus, employing an additional two colors would allow six targets to be distinguishable. A seventh target can be easily identified using three colors at an intensity different from those already employed.

303. As discussed, a POSA would have been motivated to identify seven or more targets in any combination of presence or absence. *See supra* Section XIV.A. In my opinion, a POSA would have been motivated to do this through a straightforward modification of Saxonov based on the teachings of Kramer. As discussed, Kramer teaches using greater numbers of colors and distinguishable

combinations using two or three colors per set of probes for detecting polynucleotide analytes using four colors. *See supra* Sections XIV.A, XIV.B; EX1008 ¶¶ 22, 30, Fig. 1. In addition to four or less colors, Kramer also teaches using more than four colors. *Id.* Fig. 1.

304. A POSA would have expected the detection of seven targets using four colors, as taught in Kramer, to be straightforward and reasonably have expected success. For example, a POSA would reasonably have expected that the approach in Saxonov's Figure 10 can be used as described above, wherein two additional distinct colors would allow the detection of three additional targets, with a seventh target detected by three colors at a third level of intensity.

305. A POSA would likewise have understood that signals from the targets, or combinations of targets, could be easily distinguished based on intensity as shown above in the modified Figure 10. Targets detected with one color, two colors, and three colors would use intensity levels that are different from each other. Larson's teaching of tuning the intensity of probes can be applied in such a scenario to optimize their detection, similar to that in Saxonov. EX1017 ¶¶ 134-137 (describing methods to adjust probe intensity); EX1004 ¶¶ 51-52; *see supra* Section VIII.C.

306. To the extent overlap of signals arises from different combinations of targets, thus creating degeneracy, a POSA would have followed the teachings of Kramer to address such a situation that may occur when modifying Saxonov.

307. Kramer teaches that potential degeneracy may occur and how that can be overcome. For example, Kramer teaches:

When two different target species are present in the sample at the same concentration, the colors encoding each probe will appear at the same time. In some instances of this situation it may not be possible to unambiguously identify each code.

Id. ¶ 42; *id.* Fig. 1. Kramer teaches that this can be overcome: “In such an instance a repeat assay utilizing an alternative coding scheme for the targets, should in almost all cases resolve the ambiguity.” *Id.* ¶ 42. For example, in Figure 1, the same cumulative signal may be detected between color combinations ab and cd, two combinations used to code two analytes, and color combinations ac and bd, two combinations used to code two different analytes, if the intensity was the same across all colors. Thus, if an ambiguous result occurred, Kramer teaches to repeat the assay with an alternative coding scheme to distinguish between the possible combinations of analytes. *Id.* Accordingly, a POSA would have understood Kramer to disclose the potential for degeneracy and a possible solution to resolve it.

308. In my opinion, a POSA would have been aware of the potential for degeneracy from a coding scheme, because the prior art, such as Kramer, teaches such a potential. Thus, a POSA, aware of the potential for degeneracy, would have also known that if necessary the coding scheme could be modified to eliminate the degeneracy so that a repeat assay would not be needed. For example, a POSA would

have recognized the potential for degeneracy in Kramer's labeling scheme that relies on rely on two colors selected from four, as set forth in Figure 1, and that eliminating either probe ad or probe bc would eliminate the degeneracy. EX1008 Fig. 1.

309. Indeed, a POSA would have understood that Kramer's teachings to this effect are little different from those in the '051 patent, which proposes to address potential degeneracies simply by removing the problematic targets from the experiment. *See* EX1001 at 14:41-43, 19:28-34, 41:56-58. This is because, as in Kramer, this would require modifying the coding scheme by removing degenerate potential signals and performing an additional assay(s) to detect any targets that could not otherwise be distinguished due to degeneracy. Thus, the two are similar in modifying a coding scheme to avoid potentially degenerate signals.

310. In my opinion, a POSA would have understood overcoming the potential problem of degeneracy as obvious. This is confirmed by Patent Owner itself in parallel litigation, where Patent Owner contends that Larson can be combined with references detailing statistical approaches to overcome degeneracy from multiple analytes in the same droplet. EX1019 at 4-8; EX1020; EX1021.

XV. GROUNDS 6-8: OBVIOUSNESS OF CLAIMS 5-9

311. As described above, Saxonov and Silverbrook render obvious, claims 5-6 (Ground 2); Saxonov and Maltezos render obvious claim 7 (Ground 3); and Saxonov, Maltezos, and Oh render obvious claims 8-9 (Ground 4). *See supra*

Sections XI, XII, XIII. Moreover, Saxonov, alone or in combination with Kramer and/or Larson, discloses, but at a minimum renders obvious, claim 1. *See supra* Sections X.A, XIV.

312. In my opinion, claims 5-9, for the same reasons discussed above, are also rendered obvious by the combination of Saxonov and Kramer and/or Larson, which render independent claim 1 obvious. For instance, claims 5-6 are rendered obvious Saxonov and Kramer and/or Larson in further view of Silverbrook, claims 7 is rendered obvious by Saxonov and Kramer and/or Larson in further view of Malteozs, and claims 8-9 are rendered obvious by Saxonov and Kramer and/or Larson in further of Maltezos and Oh. In my opinion, Silverbrook, Malteozs, and Oh disclose but at minimum render obvious the additional limitations in claims 5-9.

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: July 15, 2024 _____

By: *Carl Batt* _____

APPENDIX A

Carl A. Batt

Liberty Hyde Bailey Professor

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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.
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- 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.
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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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