

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

Case No. IPR2024-01451

U.S. Patent No. 11,827,921 B2

SUPPLEMENTAL DECLARATION OF KEVIN STRUHL, PH.D.

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DECLARATION OF DR. KEVIN STRUHL

I, Kevin Struhl, declare as follows:

I. INTRODUCTION

1. I am making this Declaration at the request of California Institute of Technology (“Patent Owner” or “Caltech”) in the matter of IPR2024-01451, a petition for *Inter Partes* Review of U.S. Patent No. 11,827,921 (“the ’921 Patent”).

2. I am being compensated for my work in these matters at a rate of \$1200 per hour for my professional consulting services. I am also reimbursed for my reasonable expenses incurred in connection with my work in this proceeding. My compensation is not in any way contingent upon the outcome of this *Inter Partes* Review. I have no interest in the outcome of this proceeding or any related litigation.

II. EXPERT QUALIFICATIONS AND CREDENTIALS

3. My qualifications for presenting the opinions in this Declaration are set forth below and in my *curriculum vitae*, a copy of which is provided as EX2002.

4. I earned Bachelor’s and Master’s degrees in biology from the Massachusetts Institute of Technology (MIT) in 1974. I earned a Ph.D. in biochemistry from Stanford University in 1979. I have been a professor of

Biological Chemistry and Molecular Pharmacology at Harvard University since 1982.

5. In addition to teaching courses in molecular biology and biochemistry while serving on the faculty at Harvard University for nearly 43 years, I actively researched various topics combining genetic, molecular, biochemical, genomic, and evolutionary approaches to study many aspects of gene regulation in yeast, and the processes of cellular transformation and formation of cancer stem cells. In the course of conducting my research, I have used multiplex polymerase chain reaction (PCR). Indeed, I started using multiplex PCR twenty years ago, which is before real-time PCR machines existed.

6. I have also accrued substantial industry experience. For example, I served as a member of the advisory board for Pharmagenics Inc. from 1991 to 1997. I was also a member of the advisory board for Scriptgen Inc. from 1993 to 2000. I also was a member of the advisory board for Sangamo Inc. from 1999 to 2015. I am currently a member of the advisory board for UTR Therapeutics.

7. I have authored or co-authored over three hundred publications in the fields of biochemistry and molecular biology.

8. Additionally, I was one of the founding editors of *Current Protocols in Molecular Biology*, the manual which publishes the standards for experimental design, methods, and analyses in all areas of molecular biology. I have authored

procedures for quantitative PCR for this journal.

9. I have been qualified as a PCR and/or DNA sequencing expert in multiple litigations, including in proceedings before U.S. District Courts, the International Trade Commission, and Patent Trial and Appeal Board.

III. BASIS FOR OPINIONS, AND MATERIALS REVIEWED

10. The opinions set forth in my Declaration are based on my personal knowledge gained from my education, on my personal experience, and on the review of the documents and information described in this Declaration.

11. In preparation of this Declaration, I have considered:

- Exhibit 1001: U.S. Patent No. 11,827,921 “the ’921 Patent”
- Exhibit 1002: Declaration of Carl A. Batt, Ph.D.;
- Exhibit 1003: Joint Claim Construction Statement, filed on June 18, 2024, Dkt No. 65, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23- cv-06360, N.D. Cal.;
- Exhibit 1004: U.S. Patent Application Publication No. US 2013/0040841 A1 “Saxonov”;
- Exhibit 1005: Information Disclosure Statement noting Saxonov from Prosecution File History of U.S. Patent No. 10,068,051 filed on March 23, 2018;

- Exhibit 1006: Notice of Allowance from Prosecution File History of U.S. Patent No. 11,827,921 filed on August 10, 2023;
- Exhibit 1007: U.S. Patent Provisional Application No. 61/510,013 (filed July 20, 2011) (Serge Saxonov, Simant Dube, John F. Regan, applicants) “Saxonov Provisional”;
- Exhibit 1009: U.S. Patent Application Publication No. US 2011/0312704 A1 “Silverbrook”;
- Exhibit 1012: U.S. Patent No. 11,827,921 Infringement Claim Charts from Infringement Contentions, served on March 21, 2024, *ChromaCode, Inc. et al. v. Bio-Rad Laboratories, Inc.*, Case No. 5:23-cv-06360, N.D. Cal.;
- Exhibit 1013: Internet Archive WayBackMachine, Feb. 22, 2024, archived copy of Synbio Technologies, Precision and Accuracy with TaqMan Probes, <https://synbio-tech.com/precision-and-accuracy-with-taqman-probes/>, <https://web.archive.org/web/20240519044916/https://synbiotech.com/precision-and-accuracy-with-taqman-probes/>, accessed Sept. 12, 2024;
- Exhibit 1014: Pamela M. Holland, et al., “Detection of specific polymerase chain reaction product by utilizing the 5′ → 3′

exonuclease activity of *Thermus aquaticus* DNA polymerase,”

Proc. Natl. Acad. Sci. USA, Vol. 88, pp. 7276-7280, August 1991

Biochemistry “TaqMan Paper”;

- Exhibit 1015: Information Disclosure Statement from Prosecution File History of U.S. Patent No. 10,770,170 filed on March 7, 2018;
- Exhibit 1016: Information Disclosure Statement from Prosecution File History of U.S. Patent No. 11,827,921 filed on March 25, 2021;
- Exhibit 1017: U.S. Patent Application Publication No. US 2011/0250597 A1 “Larson”;
- Exhibit 1022: U.S. Patent No. 9,366,632 “Link”;
- Exhibit 1023: Federal Judicial Caseload Statistics (2023), at <https://www.uscourts.gov/statistics-reports/analysis-reports/federaljudicial-caseload-statistics>;
- Exhibit 1024: Civil Minutes - General, filed on December 7, 2023, Dkt No. 34, *ChromaCode, Inc. et al. v. Bio-Rad Laboratories*, Case No. 2:23-cv-08417, C.D. Cal.;
- Exhibit 1025: Patent Owner’s Response to Non-Final Office Action from Prosecution File History of U.S. Patent No. 11,827,921 filed on March 28, 2023;

- Exhibit 1026: Final Rejection from Prosecution File History of U.S. Patent No. 11,827,921 filed on May 10, 2023;
- Exhibit 1027: Joint Stipulation and Order Regarding Filing of Second Amended Complaint and Amended Case Schedule, filed on March 27, 2024, Dkt No. 53, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360, N.D. Cal.;
- Exhibit 1028: Amended Complaint for Patent Infringement of U.S. Patent Nos. 10,068,051 and 10,770,170, filed on October 13, 2023, Dkt No. 12, *ChromaCode, Inc. et al. v. Bio-Rad Laboratories*, Case No. 2:23-cv-08417, C.D. Cal.;
- Exhibit 1029: Patent Owner's Response to Final Office Action from Prosecution File History of U.S. Patent No. 11,827,921 filed on July 10, 2023;
- Exhibit 1030: Non-Final Rejection from Prosecution File History of U.S. Patent No. 11,827,921 filed on December 28, 2022;
- Exhibit 1033: U.S. Patent No. 9,441,266 "Larson patent";
- Exhibit 1036: Nucleic Acids, <https://www.genome.gov/genetics-glossary/Nucleic-Acids>, updated July 7, 2024;

- Exhibit 1037: Introduction of Molecular Beacons,
https://molecularbeacons.org/MB_introduction.html (downloaded
July 10, 2024)
- Exhibit 1038: Shivaprasad H. Sathyanarayana and Lauren M.
Wainman, “Chapter 2, Laboratory approaches in molecular
pathology: the polymerase chain reaction,” *Diagnostic Molecular
Pathology*, 2024;
- Exhibit 1039: U.S. Patent No. 10,068,051 Infringement Claim
Charts from Infringement Contentions, served on March 21, 2024,
ChromaCode, Inc. et al. v. Bio-Rad Labs., Case No. 5:23-cv-
06360, N.D. Cal.;
- Exhibit 1040: U.S. Patent No. 10,770,170 Infringement Claim
Charts from Infringement Contentions, served on March 21, 2024,
ChromaCode, Inc. et al. v. Bio-Rad Labs., Case No. 5:23-cv-
06360, N.D. Cal.;
- Exhibit 1041: Order of Consolidation filed August 16, 2024, Dkt.
No. 77, *ChromaCode, Inc. v. Bio-Rad Labs., Inc.*, Case No. 5:23-
cv-04823, N.D. Cal.;

- Exhibit 1042: Order Reassigning Case filed August 20, 2024, Dkt. No. 78, *In re: ChromaCode Litig.*, Case No. 5:23-cv-04823, N.D. Cal.;
- Exhibit 2001: Declaration of Kevin Struhl, Ph.D.;
- Exhibit 2002: *Curriculum Vitae* of Kevin Struhl, Ph.D.;
- Exhibit 2003: File History of U.S. Patent No. 11,827,921;
- Exhibit 2004: U.S. Provisional Application No. 61/507,082;
- Exhibit 2005: Patent Scheduling Order dated March 8, 2024, Dkt No. 47, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.);
- Exhibit 2006: Petitioner's Amended Invalidity Contentions, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.)
- Exhibit 2007: Comparison of Saxonov and Saxonov Provisional
- Exhibit 2008: Appendix M to Bio-Rad's Amended Invalidity Contentions;
- Exhibit 2009: Appendix I to Bio-Rad's Amended Invalidity Contentions;

- Exhibit 2010: Docket, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.);
- Exhibit 2011: Plaintiff's Opening Claim Construction Brief, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.);
- Exhibit 2012: Reply Claim Construction Brief, *ChromaCode, Inc. v. Bio-Rad Laboratories et al.*, Case No. 5:23-cv-06360 (N.D. Cal.);
- Paper 8, Patent Owner's Preliminary Response for U.S. Patent No. 11,827,921; and
- Paper 11, Decision Granting Institution of Inter Partes Review of U.S. Patent No. 11,827,921.

IV. UNDERSTANDING OF THE LAW

12. I am not an attorney. In preparing and expressing my opinions and considering the subject matter of the '921 Patent, I am relying on certain basic legal principles that have been explained to me. These principles are discussed below.

A. A Person Having Ordinary Skill in the Relevant Field During the Relevant Time

13. I understand that the disclosures of patents and prior art references are to be viewed from the perspective of a mythical person of ordinary skill in the relevant field (sometimes referred to by lawyers as “the relevant art”) at the relevant timeframe (a “POSA”). I understand that, for purposes of this IPR proceeding, I should interpret the term POSA as referring to a mythical person to whom an expert in the relevant field could have assigned a routine task with reasonable confidence that the task would be successfully carried out.

14. I have been asked to provide my opinion regarding the “level of ordinary skill in the art” at the time of the invention, which I understand is on or before February 3, 2012.

15. I understand that the hypothetical person of ordinary skill in the art is considered to have the normal skills and knowledge of a person in a certain technical field. I understand that factors that may be considered in determining the level of ordinary skill in the art include: (1) the education level of the inventor; (2) the types of problems encountered in the art; (3) the prior art solutions to those problems; (4) rapidity with which innovations are made; (5) the sophistication of the technology; and (6) the education level of active workers in the field. I also understand that “the person of ordinary skill” is a hypothetical person who is presumed to be aware of the universe of available prior art.

16. I also understand the level of ordinary skill in the art can be evidenced by the prior art. Accordingly, I have also considered the prior art discussed herein in determining the level of ordinary skill in the art.

17. Petitioner asserts that a POSA relevant to the '921 Patent as of February 3, 2012 would have a Ph.D. in molecular biology, genetics, biochemistry, or a related discipline, with at least a year of experience in quantitative and/or digital PCR and fluorescence-based analyte detection. Petition, 9-10. Alternatively, the Petitioner asserts that a POSA would have been someone with greater relevant experience sufficient to compensate for having less relevant formal education. *Id.* The Petitioner states that a POSA would have “understood” several technologies and techniques which are enumerated in the Petition. *Id.*

18. I understand that, for purposes of this IPR proceeding, Patent Owner does not contest Petitioner’s description of qualifications for a POSA. Therefore, I adopt Petitioner’s definition of a POSA for purposes of this declaration.

19. I would qualify as at least a POSA at all relevant times for purposes of this proceeding, including at least as early as the priority date of the '921 Patent. I have a Ph.D. in biochemistry with at least a year of experience in quantitative and/or digital PCR and fluorescence-based analyte detection.

B. Claim Interpretation in *Inter Partes* Review

20. I understand that the first step in determining the validity of a claim is for the claim to be properly construed. I understand that “claim construction” is the process of determining a patent claim’s meaning. I also understand that the proper construction of a claim term is the meaning that a POSA would have given to that term during the relevant timeframe.

21. For purposes of *Inter Partes Review*, I understand that the Board applies the *Phillips* standard, under which claims are to be given their ordinary and customary meaning as understood by a POSA at the time the application was filed. I understand that this analysis is performed by primarily looking to the patent’s claims, specification (including drawings), and prosecution history (the “intrinsic” evidence). I understand that if the claim is unclear in view of the intrinsic evidence, then outside (“extrinsic”) evidence also may be considered.

C. Anticipation

22. I understand that a patent claim is unpatentable as anticipated if a POSA during the relevant timeframe would have understood a single prior art reference to disclose an embodiment that expressly or inherently teaches every limitation contained in the claim. The disclosure in a reference does not have to be in the same words as the claim, but all of the requirements of the claim must be described in enough detail, or necessarily inherent in an embodiment disclosed in

the reference, to enable a POSA looking at the reference to practice the claimed invention.

D. Obviousness

23. I understand that a patent claim also, or alternatively, is unpatentable if it would have been obvious to a POSA during the relevant timeframe. I understand that the following factors must be evaluated to determine whether the claimed subject matter was obvious: (1) the scope and content of the prior art; (2) the difference or differences, if any, between the subject matter defined by the claim of the patent under consideration and what is disclosed in the prior art; (3) the level of ordinary skill in the relevant field at the time of the patent's earliest priority date or, possibly, at an earlier time; and (4) so-called "objective evidence of non-obviousness." However, I understand that, for the present, I can ignore both the possibility that obviousness is to be determined at a date earlier than the patent's earliest priority date and the possibility that Patent Owner will submit objective evidence of non-obviousness.

24. I understand that prior art references can be combined to support a finding that a claim is unpatentable for obviousness when there was an apparent reason for one of ordinary skill in the art, at the time of the invention, to combine the references and that such apparent reasons include: (A) identifying a teaching, suggestion, or motivation to combine prior art references; (B) combining prior art

methods according to known methods to yield predictable results; (C) substituting one known element for another to obtain predictable results; (D) using a known technique to improve a similar device in the same way; (E) applying a known technique to a known device ready for improvement to yield predictable results; (F) trying a small number of identified, predictable potential solutions, with a reasonable expectation of success; and (G) identifying that known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the relevant field.

25. Moreover, I understand that so-called “objective indicia of non-obviousness” (also known as “secondary considerations”) like the following can be considered when assessing obviousness: (1) commercial success; (2) long-felt but unresolved needs; (3) copying of the invention by others in the field; (4) initial expressions of disbelief by experts in the field; (5) failure of others to solve the problem that the inventor solved; and (6) unexpected results. I also understand that evidence of objective indicia of non-obviousness must be commensurate in scope with the claimed subject matter.

E. Priority Date

26. I understand that to be entitled a priority date of an earlier-filed application, the earlier-filed application must provide support for the invention

claimed in the later-filed application as required by the “written description” requirements of 35 U.S.C. § 112. I understand that an adequate written description requires showing that the inventor was in possession of the invention at the time of the patent application’s filing.

27. I understand that in order to show the possession of the claimed invention, a POSA must discern the claimed invention with all of its limitations based on disclosure of the patent or patent application at issue, including the originally filed claims, specifications, and/or the drawings.

V. BACKGROUND ON RELEVANT TECHNOLOGY

A. Technology

28. A variety of techniques, including polymerase chain reaction (PCR), have been developed to detect a target polynucleotide analyte, such as a particular genetic sequence, within a biological sample. *See* EX1001(’921 Patent), 11:12-17, 11:55-12:35; *see also, e.g.*, EX1017(Larson), ¶¶3-6 (“Assays have been developed that rely on analyzing nucleic acid molecules from bodily fluids for the presence of mutations The advent of PCR and real-time PCR methodologies has greatly improved the analysis of nucleic acids from both throughput and quantitative perspectives.”).

29. Caltech’s innovations described and claimed in the ’921 Patent and its priority applications addressed a long-standing problem in the field: namely, as the

number of analytes in a biological sample increases, it quickly becomes significantly more complicated and difficult to uniquely identify those analytes. EX1001('921 Patent), 1:35-47. The term “multiplexing” refers to a broad class of techniques for detecting multiple analytes in a biological sample under a single test (also referred to as an “assay”). EX1001('921 Patent), 1:35-47; *see also* EX1017(Larson), ¶45. Early assays used PCR to amplify (create copies of) each of the target polynucleotide analytes, and often involved gel electrophoresis to separate the various PCR products that distinguished the analytes.

30. A subsequent multiplex PCR approach uses “probes” to bind to target analytes and fluoresce with different colorimetric labels (fluorophores) corresponding to the different analytes, with results being detected with appropriate optical channels. EX1001('921 Patent), 11:18-51, 8:33-62; *see also* EX1017(Larson), ¶¶4-5 (“qPCR reactions are monitored either using a variety of highly sequence specific fluorescent probe technologies[.]”). Optical channels can be configured to detect specific wavelength(s) of light, *i.e.*, colors the probes respectively produce when the probes bind or hybridize to complementary genetic sequences in the target analytes, such that each optical channel corresponds to a probe color (wavelength). EX1001('921 Patent), 28:27-45. For example, a probe for a particular genetic sequence is designed to be complementary to that sequence, and they bind via hybridization. *See* EX1002(Batt Decl.), ¶¶36-37, 39-40, 42;

EX1001('921 Patent), 11:18-51. The fluorophore emits light only if the probe has bound its target within the sample. EX1001('921 Patent), 9:20-32, 11:18-51. A TaqMan probe is a particular type of probe that functions by releasing a fluorescent reporter molecule upon the probe binding to its target DNA sequence and subsequent PCR amplification, wherein the 5' exonuclease activity of the extending DNA polymerase cleaves the probe, separating the fluorescent reporter from a quencher molecule, resulting in an increase in fluorescence. EX1001('921 Patent), 10:32-34 (“‘TAQMAN probe’ generally refer[s] to hydrolysis probes used to detect the presence of an analyte in a polynucleotide amplification assay.”); EX1017(Larson), ¶47; *see also* EX1002(Batt Decl.), ¶¶36-37, 39-40, 42. Such techniques to detect a target polynucleotide analyte in a biological sample were generally known, and commercially available TAQMAN probes were, and still are, commonly used to detect specific genetic sequences in this manner.

31. For example, a probe with a blue fluorophore binds analyte A and its light is observed in a blue channel; and a probe with a red fluorophore binds analyte B and its light is observed in a red channel. EX1001('921 Patent), 11:55-12:35. Of course, increasing the numbers of probe types (each with distinct fluorophore color) and channels (one per color) increases the number detectable analytes. EX1001('921 Patent), 12:39-45. However, this has the limitation of the

number of distinct colorimetric probes and optical channels that could be assayed simultaneously. *Id.*

32. Some multiplexing techniques use probes with colorimetric labels (fluorophores) of the same color. *See* EX1017(Larson), ¶47; EX1001('921 Patent), 8:33-44. Here, color alone does not distinguish the analytes from one another: light in the channel means an analyte is present, but does not identify *which* analyte is present. *Id.* Analytes that are indistinguishable from one another in a given assay may be referred to as “degenerate.” *See* EX1001('921 Patent), 16:46-56. Some techniques (*e.g.*, Larson) tried to avoid this problem by (i) highly diluting the sample, (ii) physically dividing the diluted sample into droplets, (iii) performing PCR in each droplet to amplify the respective contents of the droplets, (iv) observing the intensity of light from each droplet, and (v) drawing conclusions about the single-analyte droplets based on that intensity. *See, e.g.*, EX1017(Larson), ¶6. For example, probes with the same color could be designed to fluoresce at different intensities, so as to identify the analyte using the intensity. *See* EX1017(Larson), ¶12.

33. When a dilute sample is divided into droplets before PCR, different droplets may contain different numbers of unique analyte molecules. EX1017(Larson), ¶6. The “Poisson distribution” describes the statistics of how many molecules the droplets contain. *See id.* In the “limiting dilution” regime,

before PCR the majority of droplets contain zero analyte molecules, fewer droplets contain exactly one analyte molecule, even fewer contain exactly two analyte molecules, still fewer contain exactly three analyte molecules, and so on. *Id.* At terminal dilution, the sample is sufficiently diluted that the Poisson distribution basically guarantees that before PCR, almost all droplets are either empty or contain only a single analyte molecule, with droplets rarely containing multiple analyte molecules. *Id.* At terminal dilution, to best avoid droplets containing multiple analyte molecules before PCR, most droplets are empty both before and after PCR, which wastes material and time.

34. The Poisson distribution describes the statistics of loading individual analyte molecules into the droplets of a collection of droplets before PCR. Because the analyte molecules are physically independent of one another, the droplets of the collection can contain any combination of presence or absence of such molecules. In comparison, if a given analyte molecule contains more than one target, the statistics for those targets relative to each other are not described by the Poisson distribution. For example, if a given polynucleotide analyte molecule (sometimes called a template) contains first and second targets (that is, contains first and second sequences it is desired to detect), the first and second targets are physically linked to each other. Accordingly, the first and second targets are not physically independent of one another, and indeed will always co-occur with one another in

the same droplet so long as they are linked by the same polynucleotide analyte molecule.

35. Using a well-known technique called fragmentation, which may naturally occur or be performed intentionally, the first and second targets may be physically unlinked from one another, *e.g.*, by fragmenting the polynucleotide analyte molecule on which these targets formerly resided. This process results in two physically independent molecules—one containing the first target, and one containing the second target. In this case, the Poisson distribution would describe the loading of the resultant molecules into droplets of a collection of droplets. That is, the unlinking process (fragmentation) would allow the two resultant molecules to be independently loaded into droplets before PCR, and the Poisson distribution would describe the statistics of the combinations of presence or absence of those two resultant molecules in the droplets. Thus, at limiting dilution, the co-occurrence of the two resultant molecules (and thus the co-occurrence of the two targets which had previously been linked on the same analyte molecule) would be low, as described by the Poisson distribution. And at terminal dilution, the co-occurrence of those two resultant molecules (and thus the co-occurrence of the two targets which had previously been linked on the same analyte molecule) would be rare, again as described by the Poisson distribution.

36. The '921 Patent refers to analytes that are indistinguishable from one another in a given assay as being “degenerate.” EX1001('921 Patent), 16:47-50. At the time the '921 Patent and its priority applications were filed, a significant need remained for inexpensive, material-efficient, and time-efficient multiplexing that could non-degenerately identify a higher number of targets within a given sample, without the need to add another optical channel corresponding to each additional analyte. EX1001('921 Patent), 1:35-47, 8:45-62.

B. The '921 Patent and the Challenged Claims

37. The '921 Patent inventors conceived of a solution—described and claimed in the '921 Patent and its priority applications—that enables non-degenerate detection of more polynucleotide analytes in a single sample solution volume, in any combination of presence or absence, than the number of wavelength components used to detect them. The application that issued as the '921 Patent was filed on May 1, 2020 as a continuation of application no. 15/914,356, filed March 7, 2018, which was a continuation of application no. 14/451,876, filed August 5, 2014 as a continuation of Application No. 13/756,760, filed February 1, 2013, now U.S. Patent No. 8,838,394. EX1001('921 Patent), §§(22), (63). The '921 Patent claims priority to Provisional Application Nos. 61/594,480, filed February 3, 2012 and 61/703,093, filed September 19, 2012. EX1001('921 Patent), §(60).

38. The '921 Patent inventors recognized that in traditional multi-channel methods such as described above, “the number of detectable analytes is equal to the number of spectrally resolvable fluorophores. Therefore, the number of analytes may only be increased by increasing the number of spectrally resolvable fluorophores.” EX1001('921 Patent), 12:39-45; *see also id.*, 8:58-62. The inventors developed a technique to non-degenerately identify analytes in a sample, using *fewer* fluorophores than the number of analytes. *Id.*, 8:63-9:19. Such scheme is distinctive from prior multi-channel techniques or terminal dilution-based techniques described above. *Id.*; *see* ¶¶28-33 of this Declaration. Instead, “each analyte to be detected is encoded as a value in each of two components of a signal (*e.g.*, intensity and wavelength),” these components are cumulatively measured for the sample, and the “presence or absence of an analyte may then be determined based on the values used to encode the presence of the analyte (*i.e.*, those values in the coding scheme).” *Id.*, 8:66-9:5, 9:41-47.

39. Table 3 of the '921 Patent, reproduced below, describes an example in which seven analytes (A-G), plus control, are encoded using combinations of four fluorophores (fewer fluorophores than analytes):

TABLE 3

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

TABLE 3-continued

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

EX1001('921 Patent), 13:44-14:9.

40. As the '921 Patent explains,

The presence of the control and analyte A is indicated by a result of 2001. The presence of the control and analyte G is indicated by a result of 2111. These results express the cumulative intensity of the signal in each color. For example, the result 2111 has a 2× signal intensity in the

blue channel, while the result 1000 has only a $1\times$ signal intensity in the blue channel.

Id., 14:14-21.

41. While these example results are for a single analyte, the '921 Patent also explains how the inventive coding method of Table 3 may be used to detect ***combinations*** of different analytes in a single sample volume. For example, the following excerpt from Table 4 demonstrates that the cumulative signal intensity from the combination of analytes A-F in a single sample volume is different, and measurably distinguishable from, that from the combination of analytes A-G in a single sample volume:

7	3	3	3	pABCDEF
8	4	4	4	pABCDEFGG

EX1001('921 Patent), 15:60-61.

42. The '921 Patent inventors recognized that in some circumstances, a result may be degenerate “because it can indicate more than one possibility in terms of the presence or absence of an analyte. For example, with reference to Table 4, result 5233 is degenerate because it can be decoded as either pADFG or pBDEG.” *Id.*, 16:47-52. The inventors solved this problem by reducing or eliminating degeneracy from the coding scheme, “thereby increasing the confidence with which an analyte is detected.” *Id.*, 16:57-59. The '921 Patent describes different ways to eliminate degeneracy such that every possible

combination of analyte molecules has a unique cumulative intensity. *Id.*, 22:11-23:56.

43. One example method of eliminating degeneracy according to the '921 Patent encodes the individual analyte molecule states using intensity levels that are separated by integer $\log_2$ differences. *Id.* This design is non-degenerate because for N analyte molecules, there are 2^N states, and with each analyte molecule uniquely encoded by a $\log_2$ encoding intensity value (1,2,4,8,16, etc.). The cumulative sums of analyte intensities for any combinatorial state using this $\log_2$ encoding method produce a unique cumulative intensity value for each state. This is because any N digit combinatorial state can be represented as a binary number with N digits. The presence or absence of eight different analyte molecules in an assay can be encoded using a binary representation, such as (00100110). In this system, each analyte molecule is assigned an intensity based on its presence and its binary position. For example, the first position corresponds to $2^0 = 1$, the second is $2^1 = 2$, the third is $2^2 = 4$, and so on. The base-10 equivalent of the binary number represents the cumulative intensity signal, with each value being unique due to the distinct binary representation of each combinatorial state. This ensures that every possible combination of analyte molecules has a unique cumulative intensity, allowing for precise detection and differentiation. *See id.*, 18:10-25; 23:38-56.

44. Another example method of eliminating degeneracy according to the '921 Patent is to enumerate every legitimate result that can be obtained from the coding scheme, identify at least one legitimate result that is degenerate, and eliminate at least one potential analyte code from the coding scheme to eliminate degeneracy. EX1001('921 Patent), 17:60-18:9, 18:26-20:27, Table 6. Eliminating a given analyte molecule code from the coding scheme does not mean that a particular analyte molecule in an assay would be undetectable or ignored; instead, a different code would be selected to encode that analyte molecule.

45. The '921 Patent describes detecting a variety of different analyte molecules. See, e.g., EX1001('921 Patent), 9:10-12, 24:59-26:29. However, the primary examples of analyte molecules are polynucleotides, which the '921 Patent defines as follows:

The terms “polynucleotide,” “oligonucleotide,” or “nucleic acid,” as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides. Exemplary polynucleotides include deoxyribonucleic acids, ribonucleic acids, and synthetic analogues thereof, including peptide nucleic acids.

EX1001('921 Patent), 11:12-17, *see also id.*, 24:14-58.

46. The '921 Patent claims are directed to the innovations providing assays and kits that can unambiguously detect the presence or absence of each of M analytes, in any combination of presence or absence, in a single sample volume, based on detection of F wavelength components, when M is greater than F. *See, e.g., id.*, 67:9-20. For example, claim 1 of the '921 Patent recites:

1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes, wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises F wavelength components, and wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F, wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and

wherein the analytes are amplified in the single
sample volume.

EX1001('921 Patent), 67:9-26.

Claim 14 of the '921 Patent recites:

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

a. each of said at least M analytes is encoded as a
value of one component of a fluorescent signal, thereby
generating a coding scheme, wherein each of said at least
M analytes is represented in said coding scheme by said
value, and wherein said encoding is performed in a
manner that eliminates degeneracy; and

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

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

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

wherein the analytes are simplified [*sic*: amplified]

in the single sample volume.

EX1001('921 Patent), 68:19-38.

47. It is clear from the language of the claims and the original specification that the '921 Patent can detect three or more analyte molecules ($M \geq 3$) using multiple colors ($F \geq 2$). For example, the '921 Patent states that “by utilizing at least two components of a signal during encoding, the methods described herein may be used to detect more than one analyte per fluorophore,” and the lowest example number of analytes that the '921 Patent discloses detecting per fluorophore is “1.5” analytes. *See* EX1001('921 Patent), 12:46-62. For this lower bound of $M=1.5$ analytes per fluorophore disclosed in the '921 Patent, the smallest value of F that yields an integer number of analytes (and thus the smallest value of F that yields a physical result) is $F=2$, for which $M=3$. *See* EX1001('921 Patent), 12:46-62.

48. Other sections of the '921 Patent confirm that in all cases, $F \geq 2$ and $M \geq 3$, such as the following:

The right-hand side of FIG. 1 shows one exemplary embodiment of the method described above. More specifically, the right-hand side of FIG. 1 shows an encoding method where nine or more analytes (Seq 1-Seq 9, etc.) are each encoded by at least two colors, with varying intensities within each color.

EX1001('921 Patent), 21:18-23.

In Table 2, the presence of the control (p) is indicated by a result of 1000. The presence of the control and analyte A is indicated by a result of 2001. The presence of the control and all three other analytes is indicated by a result of 4111. The control color (blue) provides an indication that the assay is functioning properly...The coding scheme shown in Table 2 encodes each analyte using one control color and one additional color (up to 2 probes per analyte). However, as shown below, the number of analytes that can be encoded increases when up to four colors are used per analyte.

EX1001('921 Patent), 13:27-32, 13:41-47.

In Table 3, the presence of the control and all seven other analytes is indicated by a result of 8444. Three analytes (A, B, and C) are encoded by two colors. Three analytes (D, E, and F) are encoded by three colors, and one analyte (G) is encoded by four colors.

EX1001('921 Patent), 14:10-14.

49. Using the encoding techniques provided and claimed by the '921 Patent, a single sample volume (such as a vial or droplet) can be loaded with any combination of analyte molecules before PCR is performed in that volume, and the '921 Patent's assays and kits can successfully analyze the contents of that volume after performing PCR.

C. The '921 Patent's Prosecution History

50. I understand that the Examiner of the '921 Patent allowed the application on the basis that "[t]he claims recite an empirical assay for detecting the presence and absence of M analytes with F wavelength components wherein M is greater than F without using mass spectrometry or immobilization." EX2003, 4525-4526. I agree with the Examiner's assessment, for at least the reasons that I will explain in the sections which follow.

D. Overview of Larson

51. U.S. Patent Application Publication No. US 2011/0250597 to Larson et al. (“Larson”) was filed on February 11, 2011, and issued on October 13, 2011. EX1017(Larson), §§(22), (43).

52. Larson discloses “droplet based digital PCR and methods for analyzing a target nucleic acid using the same.” EX1017(Larson), Abstract. Larson states that:

Methods of the invention involve novel strategies for performing multiple different amplification reactions on the same sample simultaneously to quantify the abundance of multiple different DNA targets, commonly known to those familiar with the art as “multiplexing”. Methods of the invention for multiplexing dPCR assays promise greater plexity—the number of simultaneous reactions—than possible with existing qPCR or dPCR techniques. It is based on the singular nature of amplifications at terminal or limiting dilution that arises because most often only a single target allele is ever present in any one droplet even when multiple primers/probes targeting different alleles are present. This alleviates the complications that otherwise plague

simultaneous competing reactions, such as varying arrival time into the exponential stage and unintended interactions between primers.

EX1017(Larson), ¶45; *see also id.*, ¶¶98-99.

53. Larson describes terminal dilution as “the vast majority of reactions contain either one or zero target DNA molecules for practical intents and purposes,” and limiting dilution as “some reactions contain zero DNA molecules, some reactions contain one molecule, and frequently some other reactions contain multiple molecules, following the Poisson distribution.” EX1017(Larson), ¶6.

54. Larson asserts various advantages of having one allele per droplet: Having *only one* haploid genome (i.e., one allele) per droplet gives droplet PCR advantages over standard singleplex or multiplex PCR in tubes or microwells. For example, in traditional PCR, both alleles are present in the reaction mix so if there is a difference in the PCR efficiency between alleles, the allele with the highest efficiency will be over represented. Additionally, there can be variances in the sequence to which the PCR primers hybridize, despite careful primer design. A variance in the primer hybridization sequence can cause

that primer to have a lower efficiency for hybridization for the allele that has the variance compared to the allele that has the wild type sequence. This can also cause one allele to be amplified preferentially over the other allele if both alleles are present in the same reaction mix.

These issues are avoided in droplet based PCR because there is only one template molecule per droplet, and thus one allele per droplet. Thus, even if primer variance exists that reduces the PCR efficiency for one allele, there is no competition between alleles because the alleles are separated and thus uniformly amplified.

EX1017(Larson), ¶¶99-100.¹

55. Accordingly, Larson’s attempt to obtain “greater plexity” in ¶45 clearly is meant to be performed at terminal or limiting dilution, in which the sample is sufficiently diluted before PCR that most often a single analyte molecule is located within a droplet, to “alleviate[]” the complications that come with multiple occupancy droplets. Larson discloses using PCR to amplify the single analyte molecule within its individual droplet, thus “alleviat[ing]” complications

¹ Emphasis added throughout except where noted.

such as “varying arrival time into the exponential stage and unintended interactions between primers.” Larson, ¶¶44-45. Larson discloses analyzing the droplets one droplet at a time. Larson, ¶172, FIG. 9B.

56. Larson discloses digital PCR (dPCR) assays for detecting different numbers of analytes. For example, FIG. 15 (reproduced below) discloses a “9-plex dPCR assay for spinal muscular atrophy with only two fluorophores, showing the process of optimizing droplet intensities.” EX1017(Larson), ¶35, FIG. 15.

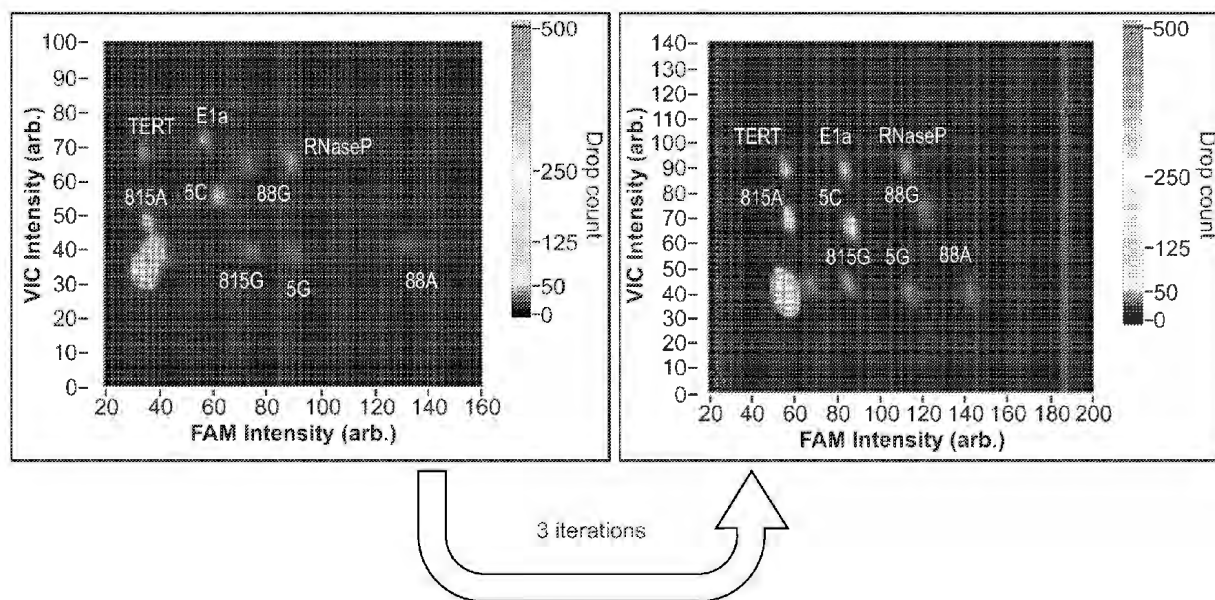


FIG. 15

57. Larson’s FIG. 15 shows only single-analyte clusters (respectively, TERT, 815A, E1a, 5C, 815G, RNaseP, 88G, 5G, 88A). EX1017(Larson), ¶35, FIG. 15. Other graphs in Larson similarly appear to show measurements from only single-analyte droplets, or single-analyte clusters. *See, e.g.*, EX1017(Larson) at

FIG. 5B (showing peaks for “one molecule SMN2” and “one molecule E1a” and omitting any double-positive peaks), FIG. 5C (showing clusters for TERT, RnaseP, E1A, SMN1, and SMN2 and omitting any double-positive clusters), FIG. 6B (showing clusters for TERT, 815A, RNaseP, E1a, and 815G and omitting any double-positive clusters), FIG. 7C (showing peak for “droplet containing a single molecule of target 1” and omitting any double-positive peaks), FIG. 8D (“[h]istogram showing three populations of droplets; with target1, with target2, without target1 or target2” and omitting any clusters containing both target1 and target2), FIG. 9D (“[h]istogram showing four populations of droplets; with target1, with target2, with target3 and without any of the three targets” and omitting any clusters containing both target1 and target2, any clusters containing both target 1 and target3, any clusters containing both target2 and target3, and any clusters containing all three targets), FIG. 14a (showing clusters for c.815A, BCKDHA, c.815G, SMN1, and SMN2 and omitting any double-positive clusters), FIG. 18A (showing clusters for TERT, SMN1, and SMN2 and omitting any double-positive clusters), FIG. 19A (showing clusters for TERT, 5C, and E1a and omitting any double-positive clusters), FIG. 20A (showing clusters for TERT, 88G, and RNaseP and omitting any double-positive clusters), FIG. 21B (showing clusters for TERT, 88G, and RN5CoP and omitting any double-positive clusters), FIG. 21E (showing clusters for TERT, 6C, and E1b and omitting any double-positive clusters); and

FIG. 21H (showing clusters for TERT, SMN1, and SMN2 and omitting any double-positive clusters).

58. Larson teaches “[h]igher degrees of multiplexing would require greater dilution,” because of the possibility for unintended “cross-reactivity” between analytes in a given droplet. EX1017(Larson), ¶129. Larson describes the possibility for unintended “cross-reactivity” between analytes in a given droplet as follows:

A multiplexes (*sic*) assay can require a more dilute sample. For instance, at 10% occupancy a duplex reaction would have double occupancy 1% of the time. Hence 1 in 10 PCR+ droplets would be doubles, resulting in a final intensity at least as high and possibly higher than the brighter of the two probes. For a simple duplex system the contribution from each probe could be recovered. In this example the total number of PCR+ droplets for probe 1 would be (Probe1)+(Probe1+Probe2). Higher degrees of multiplexing would require greater dilution. For example, for a 4-plex at 1% occupancy the probability of one probe overlapping any of the other 3 is ~3%, and that error may be too high for

some applications. The need for large dilutions strongly favors the large number of dPCR reactions.

EX1017(Larson), ¶129.

59. According to the excerpt above, Larson teaches increasing the sample dilution as the number of analytes increases, in order to reduce the probability that any droplet contains even two analyte molecules, let alone more than two, before and during PCR.

60. Larson uses the term “occupancy” to describe the number of analytes in droplets:

In a serial dilution the average number of target DNA molecules per droplet—called the “occupancy” from this point forward—should decrease in direct proportion to the DNA concentration. The occupancy was calculated

$$\text{occupancy} = \ln\left(\frac{P + N}{N}\right).$$

from Poisson statistics using the following equation well known to those experienced in the art:

where P and N are the numbers of PCR(+) and PCR(−) droplets respectively.

EX1017(Larson), ¶96.

61. According to Larson’s apparent definition of “occupancy,” the term “10% occupancy” refers to a dilution at which 90% of droplets are empty, 10% of droplets contain analyte, and 10% of the droplets that are not empty (that is, 1% of all the droplets) contain exactly two analytes. *See id.*, ¶129. In this situation, in which the original sample must be very highly diluted, Larson teaches that in a duplex system (an assay for two analytes), the contribution from each probe can be recovered from a droplet that contains both of the analytes, based on the increased intensity from such a droplet relative to droplets containing only a single analyte. *See id.* And, in any event, the information obtained from the single-analyte droplets is sufficient for Larson’s purposes; indeed, this is why Larson expressly teaches to exclude analyzing multi-analyte droplets, as discussed below.

62. And, for “higher degrees of multiplexing,” that is, more than two analytes, Larson teaches that even “greater dilution” is needed. *Id.* Larson gives the example of a “4-plex” (four analyte assay) at a “1% occupancy,” referring to a dilution at which 99% of droplets are empty and 1% of droplets contain an analyte. *Id.* Even at this high dilution, Larson states that the “probability of one probe overlapping any of the other 3” may be “too high for some applications,” so the “need for large dilutions strongly favors the large number of dPCR reactions.” *Id.*

63. Accordingly, Larson expressly teaches to increase the sample dilution before PCR as the number of analytes increases, in order to reduce the probability

that one probe overlaps any of the other probes before and during PCR—that is, to reduce the probability that any droplet contains even two analyte molecules before and during PCR, let alone more than two.

64. To the extent that any droplets nonetheless contain more than one analyte molecule before and during PCR, Larson expressly teaches excluding any such droplets from analysis after PCR, and before analyzing the droplets. More specifically, Larson states that “[m]ethods of the invention are able to detect which droplets contain a *heterogeneous population of molecules* and are able to *exclude* those droplets from the analysis.” EX1017(Larson), ¶172. Below, I will address each of the emphasized terms in turn.

65. Larson describes a *heterogeneous population of molecules* as follows: “[d]roplets that produce two signals are classified as droplets that contain a heterogeneous population of molecules.” EX1017(Larson), ¶173. In my view, a POSA considering Larson as a whole would have readily understood that droplets containing a heterogeneous population of molecules produce *two signals* because such droplets contain *two different analytes*, each analyte producing a different signal. Indeed, Larson’s descriptions of “heterogeneous population of molecules” are wholly consistent with my interpretation, especially in the context of the highly diluted regime in which Larson operates.

66. As one example, Larson discloses that “probes that hybridize to the wild-type of the target have a different fluorophore attached than probes that hybridize to a variant of the wild-type of the target.” EX1017(Larson), ¶172. Here, a POSA would have readily understood that the “wild-type of the target” corresponds to a first analyte molecule to which a first probe type hybridizes, while the “variant of the wild-type of the target” corresponds to a second analyte molecule to which a second probe type hybridizes. A POSA would have readily understood that the wild-type of the target and the variant of that wild-type would never co-occur on the same molecule. The POSA therefore would have readily understood that each droplet in this scenario produces *two signals*: one corresponding to a first analyte molecule containing the wild-type target, and one corresponding to a second analyte molecule containing the variant of the wild-type target. The POSA therefore would have readily understood each droplet in this multiple-occupancy scenario contains a *heterogeneous* population of molecules as described by Larson.

67. As another example, Larson discloses that “certain droplets will contain a heterogeneous mixture of both target and target variant due to polymerase errors during the PCR reaction.” EX1017(Larson), ¶169. Here, a POSA would have readily understood that the “target” corresponds to a first analyte molecule to which a first probe type hybridizes, while the “target variant”

corresponds to a second analyte molecule to which a second probe type hybridizes, because the “target” and the “target variant” would never co-occur on the same molecule. The POSA therefore would have readily understood that each droplet in this scenario produces *two signals*: one corresponding to the target, and one corresponding to the target variant produced through PCR error. The POSA therefore would have readily understood each droplet in this scenario contains a *heterogeneous* population of molecules as described by Larson.

68. In contrast to heterogeneous populations that produce two signals, Larson describes a “homogeneous” population as follows: “[d]roplets that produce only a *single* signal are classified as droplets that contain a homogeneous population of target.” EX1017(Larson), ¶172. In my view, a POSA considering Larson as a whole would have readily understood that droplets containing a homogeneous population of molecules after PCR produce *one signal* because they contained only *a single analyte molecule* before PCR, which produces a single signal after PCR. Indeed, Larson’s descriptions of “homogeneous population of molecules” are wholly consistent with my interpretation, especially in the context of the highly diluted regime in which Larson operates.

69. Illustratively, continuing with the example of wild-type targets, Larson discloses that “[a]nalyze (*sic*) is then performed on only the droplets that contain a homogeneous population of molecules. The analysis may be based on

counting, i.e., determining a number of droplets that contain only wild-type target, and determining a number of droplets that contain only a variant of the target.”

EX1017(Larson), ¶174. Here, a POSA would have readily understood that the droplets containing “*only* wild-type target” correspond to a first analyte molecule to which a first probe type hybridizes, while the droplets containing “*only* a variant of the target” correspond to a second analyte molecule to which a second probe type hybridizes, again because the “target” and the “target variant” would never co-occur on the same molecule. The POSA therefore would have readily understood that each droplet in this scenario produces *only one signal*: either a signal corresponding to the wild-type target, *or* a signal corresponding to the variant of the wild-type target. The POSA therefore would have readily understood each droplet in this scenario contains a *homogeneous* population of analyte molecules as described by Larson.

70. Larson expressly discloses that droplets containing a heterogeneous population of molecules—that is, “[d]roplets that produce two signals” are “detected and excluded from analysis.” EX1017(Larson), ¶173. I will now address, in turn, Larson’s express teachings regarding *how* and *why* to exclude such droplets from analysis.

71. Regarding *how* to exclude “[d]roplets that produce two signals” from analysis, Larson’s ¶¶178-181 form an entire section entitled “Droplet Sorting”

which begins as follows: “Methods of the invention may further include sorting the droplets based upon whether the droplets contain a homogeneous population of molecules or a heterogeneous population of molecules.” EX1017(Larson), ¶178.

Larson further discloses in this section that:

A sorting module may be a junction of a channel where the flow of droplets can change direction to enter one or more other channels, *e.g.*, a branch channel, depending on a signal received in connection with a droplet interrogation in the detection module. Typically, a sorting module is monitored and/or under the control of the detection module, and therefore a sorting module may correspond to the detection module. The sorting region is in communication with and is influenced by one or more sorting apparatuses.

EX1017(Larson), ¶178. Larson’s “Droplet Sorting” section concludes with the statement that: “[b]ased upon the detected signal at the detection module, droplets containing a heterogeneous population of molecules are sorted away from droplets that contain a homogeneous population of molecules.”

EX1017(Larson), ¶181.

72. Recall Larson’s teachings (explained above at ¶¶64-71 of this Declaration) that “[d]roplets that produce **two signals** are classified as droplets that contain a heterogeneous population of molecules” and that “[d]roplets that produce only a **single** signal are classified as droplets that contain a homogeneous population of target.” EX1017(Larson), ¶¶172-173. In my view, based on a fair reading of all of these teachings, a POSA readily would have understood that where Larson’s “Droplet Sorting” section describes sorting droplets “[b]ased upon the detected signal at the detection module,” Larson means sorting droplets after PCR based on the **number** of signals that the “detection module” detects from respective droplets. Specifically, **two signals** are detected from droplets containing a **heterogeneous** population of molecules after PCR, and **one signal** is detected from droplets containing a **homogeneous** population of molecules after PCR. In my view, the only reasonable way to interpret Larson’s teachings of how to **detect** and **sort** droplets containing a heterogeneous population of molecules after PCR is based on the number of signals (one signal or two signals) detected from each droplet; the droplets with two signals then are **excluded** from analysis before the remaining droplets are individually analyzed to determine which single analyte molecule (if any) is in each such remaining droplet.

73. Larson’s **why** of excluding “[d]roplets that produce two signals” from analysis relates to Larson’s concept of “occupancy” discussed above at ¶¶50-55 of

this Declaration. More specifically, Larson describes “multiple occupancy,” and complexities associated with “multiple occupancy,” as follows:

Generally, without wishing to be constrained by theory, 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. With multiple occupancy arises the complexity of simultaneous assays competing within the same reaction droplet, and also complexity of assigning the resulting fluorescence intensity that involves a combination of fluorescence from two different reaction products that may or may not be equal to the sum of the two fluorescence intensities of the individual reaction products. *However, methods of the invention can accommodate these complications arising from multiple occupancy.*

EX1017(Larson), ¶141.

74. I believe that a POSA, considering the above excerpt from Larson’s ¶141 in view of Larson as a whole, readily would have understood that when Larson refers to “multiple occupancy” droplets, such droplets contain “a heterogeneous population of molecules” after PCR as discussed above at ¶¶64-71 of this Declaration. Illustratively, Larson’s statement that droplets with “multiple occupancy” produce a “combination of fluorescence from two different reaction products,” is entirely consistent with Larson’s statement that “[d]roplets that produce two signals are classified as droplets that contain a heterogeneous population of molecules.” *Compare* EX1017(Larson), ¶141 *with id.*, ¶173.

75. I further believe a POSA, considering the above excerpt from Larson’s ¶141 in view of Larson as a whole, readily would have understood that Larson’s statement “methods of the invention can accommodate these complications arising from multiple occupancy” refers to Larson’s strategies discussed at length herein. **First**, increasing the sample dilution before PCR as the number of analytes increases, in order to reduce the probability that one probe overlaps any of the other probes—that is, to reduce the probability that any droplet contains even two analyte molecules before and during PCR, let alone more than two, as discussed at ¶¶52-63 of this Declaration. **Second**, after PCR, using a dedicated detection module to detect, sort away, and exclude droplets containing a heterogeneous population of molecules based on the number of signals (one signal

or two signals) that each droplet produces, as discussed at ¶¶64-74 of this Declaration. *Third*, after excluding such droplets, analyzing the contents of the remaining droplets, as discussed at ¶¶64-74 of this Declaration.

76. Larson is replete with examples of how to identify a *single* analyte molecule within a droplet, repeats this as a key inventive concept throughout the reference, and indeed I believe that a POSA reasonably would have considered identifying a single analyte molecule within a droplet to be Larson’s principle of operation. *See, e.g.*, EX1017(Larson), Abstract (“single target nucleic acid”), ¶7 (“the invention provides a droplet that contains a single nucleic acid template”), ¶8 (“amplify and detect single nucleic acids in droplets . . .there is only one template molecule per droplet”), ¶11 (“[o]ne or more droplets are formed, each containing a single nucleic acid template”), ¶13 (“single droplets containing the single nucleic acid template”), ¶18 (“each droplet contains only one template strand . . . single droplet that contains a single intact nucleic acid strand”), ¶19 (“compartmentalizing a first fluid into portions, each portion containing a single target nucleic acid”), ¶20 (“forming sample droplets containing a single target nucleic acid derived from a patient sample”), ¶45 (“It is based on the singular nature of amplifications at terminal or limiting dilution that arises because most often only a single target allele is ever present in any one droplet even when

multiple primers/probes targeting different alleles are present”), ¶46 (“the invention provides droplets containing a single nucleic acid template”), ¶49 (“[m]ethods of the invention involve forming sample droplets containing a single target nucleic acid”), ¶68 (“[i]n certain embodiments, the droplets will include only a single nucleic acid template”), ¶99 (“only one haploid genome (i.e., one allele) per droplet”), ¶100 (“[t]hese issues are avoided in droplet based PCR because there is only one template molecule per droplet, and thus one allele per droplet”), ¶101 (“droplet based digital PCR utilizes only one template molecule per droplet...only one amplicon is being generated per droplet”), ¶107 (“there is still only one template molecule per droplet”). *See also* EX1017(Larson) at claim 1 (“[a] microdroplet comprising a single nucleic acid template”), claim 3 (“the single nucleic acid template is a DNA or an RNA”), claim 10 (“forming one or more microdroplets each comprising a single nucleic acid template”), claim 11 (“merging the first and second fluids to form a droplet comprising the single nucleic acid template”), and claim 15 (“the single nucleic acid template is a DNA or an RNA”).

E. Overview of Saxonov

77. U.S. Patent Application Publication No. 2013/0040841 to Saxonov et al. (“Saxonov”) was filed on July 12, 2012 and issued on February 14, 2013. EX1004(Saxonov), §§(22), (43). I understand that the Board found that Petitioner

did not establish Saxonov is §102(e) prior art against the '921 Patent for Grounds 3 and 4 in the Petition. *See* Paper 11, 26-29. Accordingly, I do not address this matter further herein.

F. Overview of Silverbrook

78. U.S. Patent Application Publication No. 2011/0312704 to Silverbrook et al. (“Silverbrook”) was filed on June 1, 2011, and published on December 22, 2011. EX1009(Silverbrook), §§(22), (43). Silverbrook discloses “a fluorescence-based detection technique to identify target molecules.” *See, e.g., id.*, ¶195. A test module using fluorescence-based detection is controlled by a test module reader, which may be a computer. *Id.*, ¶¶198, 200-201, FIGS. 1, 3, 96, 98. The data generated by the module can also be updated using the test module reader and network. *Id.* Petitioner merely argues Silverbrook teaches certain features from dependent claim 11 relating to transmitting PCR test results through a computer network, not that Silverbrook teaches features of any other claim. Petition at 66. Accordingly, my separate analysis herein of claims other than claim 11 does not directly address Silverbrook.

VI. CLAIM CONSTRUCTION

79. For purposes of this declaration, I have reviewed the challenged claims of the '921 Patent in view of their common and ordinary usage and in view of the specification of the '921 Patent.

80. I understand that Patent Owner's construction of some claim terms differs from that of Petitioner. My analysis below is based on, and consistent with, Patent Owner's proposed claim constructions set forth at EX1003, EX2011, and EX2012. In particular, I understand that Patent Owner proposes that "degenerate" and "degeneracy" generally describe 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. EX1003, 9-11; EX2011, 27-28; EX2012, 18-19.

81. I agree with the Patent Owner's position because this position is consistent with the common and ordinary usage of these terms, and in particular how these terms are used the '921 Patent specification, based on my own understanding as at least a POSA. However, my ultimate opinion that the references cited in the Petition do not teach the challenged claims of the '921 Patent would remain the same even under the Petitioner's proposed constructions.

VII. GROUNDS OF CHALLENGE

82. Petitioner points primarily to Larson as anticipating or rendering obvious the '921 Patent claims, either alone or in view of Saxonov and/or Silverbrook. It is my understanding that the Board found that the Petition fails to establish Saxonov qualifies as prior art. Paper 11, 28-29. Accordingly, and for the reasons Patent Owner discussed in its POPR (Paper 8, 29-42) the portions of Grounds 3 and 4 that rely upon Saxonov necessarily fail and I does not further

address them herein. Additionally, for the reasons set forth below, it is my opinion that Larson, whether alone or in combination with Silverbrook, does not disclose or teach the challenged claims of the '921 Patent.

A. Ground 1 (Claims 1-10 and 13-19): Anticipation by Larson

1. Challenged Claim 1 and Larson Differ in Kind

83. While the '921 Patent and Larson both aim to extend the number of analytes that can be examined simultaneously, they solve these problems in significantly different ways, and indeed take opposite approaches.

84. For example, while the '921 Patent is directed to detecting multiple analyte molecules in a single sample solution, as noted above at ¶52 of this Declaration, Larson is expressly “based on the singular nature of amplifications at terminal or limiting dilution that arises because most often only a single target allele is ever present in any one droplet even when multiple primers/probes targeting different alleles are present.” EX1017(Larson), ¶45; *see also id.*, ¶¶98, 99. That is, in Larson, the samples are sufficiently diluted before PCR that for purposes of detecting analytes, the vast majority of droplets before PCR contain either no analyte molecules or only a single analyte molecule.

85. To detect different analyte molecules, Larson extensively dilutes and partitions the sample into numerous droplets before PCR such that “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 (corresponding to limiting or terminal dilution, respectively, as defined above).” EX1017(Larson), ¶¶6, 52; *see* ¶¶33, 52-63 of this Declaration. Droplets with zero analyte molecules provide no information, but inevitably arise by the procedure. EX1017(Larson), ¶¶6, 52; *see* ¶33 of this Declaration. This reduces the mean number of analyte molecules in a droplet before, during, and after PCR, making it easier to identify the analytes after PCR, with the easiest case being that of a single analyte per droplet before, during, and after PCR. EX1017(Larson), ¶141 (“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”).

86. For the rare cases (if any) in Larson that some droplets may have “multiple occupancy” before and during PCR or contain a “heterogeneous population of molecules” after PCR, Larson expressly teaches *excluding* any such droplets from analysis, and explains how to do so and the reasons why to do so. *see, e.g.*, EX1017(Larson), ¶172 (“Methods of the invention are able to detect which droplets contain a *heterogeneous population of molecules* and are able to *exclude* those droplets from analysis”); *see* ¶¶64-76 of this Declaration.

87. Larson not only is replete with examples of how to identify a *single* analyte molecule within a droplet, but Larson repeats this as a key inventive concept, and in view of Larson's many teachings I believe that a POSA reasonably would have considered this to be Larson's principle of operation. *Supra*, §V.D.; EX1017(Larson), abstract, ¶¶7, 8, 11, 13, 18, 19, 20, 45, 46, 49, 68, 99, 100, 101, 107, 141, and claims 1, 3, 10, 11, and 15.

88. Larson's approach has a number of disadvantages. For example, as compared with the '921 Patent's approach, Larson's principle of operation requires partitioning a highly dilute sample into a significant number of droplets before PCR, a large proportion of which provide no information after PCR (because the droplets are empty—that is, do not contain even a single analyte molecule). *See* ¶¶33, 52-63 of this Declaration. Aside from wasting reagents, this is cumbersome to perform and get the optical readouts, because each droplet has to be assessed. As a consequence, the significant number of empty droplets before, during, and after PCR reduces the number of samples that can be analyzed in a given period of time, and the amount of information that can be gathered during that time. This wastes instrument time and PCR reagents. Additionally, diluting down to a single analyte before PCR is complicated by the possibility that different analytes in the sample may be present at very different concentrations than each other, and this is not known before performing the dilution, or the assays. Thus, the optimal dilution

before PCR for analyte A in the sample can be very different from the optimal dilution before PCR for analyte B in the same sample. This requires a very broad dilution series, meaning the vast majority of drops are uninformative, *e.g.*, contain either no molecules or too many molecules. Moreover, even different samples containing the same analyte as each other may have different concentrations of that analyte than each other, meaning that before PCR, the dilution needed to analyze any given one of those samples may be significantly different than the dilution needed to analyze any of the other samples, and none of these dilutions may be knowable in advance (that is, before performing PCR). If those samples contain multiple analytes, the problem becomes even more complicated for the reasons stated herein.

89. Excluding any droplets containing multiple analytes after PCR, as taught by Larson, adds further complexity and time to the assay, requires additional hardware components to perform the sorting, and is wasteful because droplets containing otherwise useful information about multiple analytes in the sample are simply discarded. But Larson did not recognize these aspects of its methods as drawbacks, indeed expressing that the benefits of these heavy dilutions are worth it to avoid the multiple occupancy problem: “The need for large dilutions *strongly favors* the large number of dPCR reactions.” EX1017(Larson), ¶128.

90. The '921 Patent solves the problem of detecting any and all combinations of different analytes, in the single sample volume in which amplification is performed, by measuring cumulative optical signals from that volume using a coding scheme. According to embodiments of the '921 Patent's method, PCR reactions go to completion such that all the original colorimetric probe molecules (whose fluorophores are quenched) are hybridized, thereby liberating the colorimetric signal. For example, if two probes that are read in the same optical channel have 1000 units of fluorescence for analyte A and 500 units of fluorescence for analyte B, then one can distinguish between 500, 1000, and 1500 units in that channel and hence determine whether the sample contains A, B, or both A and B analytes. Similarly, with appropriate choices of fluorescence units for probes with multiple colors, one can make the same distinction between different analytes. An advantage of PCR reactions going to completion is that the starting concentrations of the analytes before performing PCR does not matter, in stark comparison to the importance of concentration to Larson's method as discussed at paragraph ¶88 of this Declaration. Moreover, the '921 Patent does not require excluding any single sample volume(s) that contain multiple analyte molecules after PCR, unlike Larson.

91. Indeed, an important feature of the '921 Patent is how to choose probe colors and intensities that uniquely determine a given analyte molecule, even when

that analyte molecule is mixed with other analyte molecules at the same time, in the same single sample volume, before, during, and after PCR, and at the time of detection. This avoids a degeneracy problem, which arises when the same intensity values are consistent for two different combinations of analyte molecules in the same single sample volume, when detection is performed after amplifying the analyte molecules in that same single sample volume. For example, if intensity values 1, 2, 4, 8, 16, 32 are chosen for probes read in the same optical channel, every combination of present/absent analytes is unique. This method of decoding cumulative signals is different in kind (not just degree) from Larson, and makes it much easier, quicker, and more efficient to analyze multiplexed samples than possible with Larson's limiting or terminal dilution.

92. Claim 1 of the '921 Patent, reproduced above in ¶46 of this Declaration, recites an assay that is "capable of *unambiguously* detecting the presence or absence of each of said at least M analytes, *in any combination of presence or absence, in a single sample volume*, based on detection of said F wavelength components, when M is greater than F, . . . wherein the analytes are amplified in the single sample volume." EX1001('921 Patent), 67:16-25. Claim 14 of the '921 Patent, also reproduced above in ¶46 of this Declaration, recites a kit with similar limitations. EX1001('921 Patent), 68:31-38. While the single sample volume may not necessarily contain *all* analyte molecules at the same time, the

power of the '921 Patent lies in its flexibility to detect *any possible combination* of the analyte molecules within the single sample volume within which that combination of analyte molecules is amplified, in a manner that each analyte may be uniquely distinguished from each other analyte. *See* ¶¶37-49 of this Declaration. At the time of the '921 Patent and its priority applications, no such solution was available to solve the problem of how to increase the number of analytes which could be non-degenerately detected in a single sample volume in any combination in a single sample volume (such as a vial or droplet) within which amplification is performed. *See* ¶¶37-49 of this Declaration; *see also* EX1001('921 Patent), 1:46-47 (“There is a need for multiplexed reactions that can be carried out *in a single solution.*”)

1. Larson Does Not Teach How to Unambiguously Identify Three or More Analytes Within a Single Sample Volume

93. As described above at ¶¶52-76 of this Declaration, Larson teaches various complexities that could arise if two or more analyte molecules are in a droplet before and during, and expressly teaches how to avoid that situation: *diluting* and partitioning the sample into numerous droplets before PCR to reduce the likelihood of any droplets having “multiple occupancy,” or containing a “heterogeneous population of molecules” after PCR, and *excluding* such droplets

before individually analyzing the remaining droplets (which are either empty or contain a single analyte molecule).

94. Moreover, Larson is *silent* as to how to analyze droplets having three or more analyte molecules. This is because Larson had no need to try to solve that problem: the single-analyte droplets yielded the information that Larson apparently sought—an identification of whether an analyte was present in the sample—and without having to resolve any of the complications caused by “multiple occupancy.” Larson did not contemplate the solution provided by the ’921 Patent, because there was no need in Larson to solve the problem of how to *unambiguously detect* multiple analytes in a single sample volume after performing PCR in that single sample volume. Larson’s limiting or terminal dilution before PCR and sorting/exclusion after PCR entirely avoided this issue, and Larson refers to the possibility of probes overlapping one another in multiple occupancy as an “error” which is alleviated by “large dilutions” which “strongly favors the large number of dPCR reactions. EX1017(Larson), ¶129. Indeed, the Petition itself states that “under *these* conditions”—that is, under the conditions of “terminal dilution, where the probability of a single target molecule in droplets is much greater than, and overwhelms that of multiple targets”—the detectable signal generated for each potential analyte in Larson is “distinct and readily distinguishable, providing ‘unambiguous’ detection of the analytes.” Petition, 29.

95. The Petition appears to argue that the '921 Patent claims are invalid because they are being asserted against assays and kits that purportedly resemble those described in ¶140 of Larson. *See, e.g.*, Petition at 23-67. However, for at least the reasons given at paragraphs ¶¶83-94 of this Declaration, claims 1 and 14 of the '921 Patent, and claims respectively depending therefrom, differ from Larson in several material aspects which go to the very core of Larson's express teachings, inventive concepts, and principle of operation.

B. Ground 2 (Claims 2, 4, 6-7, and 14-19): Obviousness in View of Larson

96. The Petition identifies dependent claims 2, 4, 6-7, and 14-19 as allegedly being obvious over Larson. Petition at 44-51. However, my analysis and views set forth above in ¶¶83-95 of this Declaration regarding Ground 1, describing the many material differences between Larson and claim 1, apply equally (if not more) to claims 2, 4, and 6-7 which depend from claim 1, to independent claim 14 which recites similar limitations as discussed above for claim 1, and to claims 15-19 which depend from claim 14.

97. In any event, the Petitioner's remarks regarding claim 14 apparently amount to an argument that the word "kit" is not limiting, and that "it would have been obvious to combine the components disclosed in Larson together in a 'kit.'" Petition, 50-51. I believe that such limited argumentation does not adequately

explain why a POSA would have found it obvious to attempt to obtain the kit which is recited in claim 14, and in claims depending therefrom, based on the teachings of Larson considered *as a whole* (that is, including Larson's express teachings regarding diluting and partitioning the sample before PCR and excluding droplets containing more than one analyte after PCR), for at least the reasons set forth above in ¶¶83-110 of this Declaration.

C. Ground 3 (Claims 1-10 and 12-19): Obviousness In View of Larson and Saxonov

98. Whereas Petitioner's arguments in Grounds 1 and 2 appear to be based on Patent Owner's positions purportedly taken in the parallel district court litigation (*see* ¶¶83-97 of this Declaration), some (but not all) of Petitioner's arguments in Ground 3 instead appear to be based on the allegation that "Larson, alone or in combination with Saxonov, at a minimum renders obvious the 'unambiguous' detection of the 'presence or absence' of multiple analytes in such individual sub-volumes." Petition, 51. However, Petitioner's errors in Ground 3 are even more amplified than in Grounds 1 and 2, because Larson teaches something very different from the language recited in the '921 Patent claims. I understand that Petitioner failed to show that Saxonov is prior art to the '921 Patent, so I will not further address Petitioner's theories based on Saxonov. I will first touch on the purported teachings of Larson (I have already discussed Larson's

actual teachings at length in other parts of this Declaration); then I will explain why modifying Larson falls far short what is recited in the '921 Patent's claims 1 and 14, and claims respectively depending therefrom; then I will explain the many deficiencies in Petitioner's and Dr. Batt's explanations about why a POSA allegedly would have been motivated to modify Larson to obtain the '921 Patent claims.

1. Larson Teaches Eliminating the Very "Multiple Occupancy"

Situations to Which Petitioner Points

99. In Ground 3, the Petition cites Larson as allegedly teaching "that there may be multiple targets in a single individual droplet and that its methods accommodate such 'multiple occupancy' situations" and states "Larson's assays use conditions and parameters that do precisely this." Petition, 52, citing EX1017(Larson) ¶¶129, 141 and EX1002(Batt Decl.) ¶321. But Larson's *actual* disclosure about accommodating "'multiple occupancy' situations" is *incompatible* with an assay or kit that is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in the single sample volume in which the analytes are amplified. *See* EX1001('921 Patent), 67:16-20, 68:31-34. Petitioner's invalidity arguments hinge on Larson's ¶¶ 140-141, citing ¶140 well in excess of fifty times and quoting it repeatedly. *See, e.g.*, Petition, 12, 13, 23, 24 ("Most important for the purposes of

the present petition, in paragraph 140, Larson describes...”), 25, 28, 29, 30, 31, 32, 33, 34, 35, 38, 39, 41, 42, 43, 44, 45, 47, 49, 52, 69. For example, Petitioner points to Larson’s ¶140 as disclosing “using two colors to identify three target analytes, relying on the combination of two colors as the multi-color label for one analyte, with each of the two colors individually as a label for the other two targets.” Petition, 28-29. The Board cited this very analysis in its decision to institute IPR of the ’921 Patent claims. Paper 11, 30-31. To better appreciate the discrepancies between Larson’s express disclosure and Petitioner’s representations regarding such disclosure as pertain to the ’921 Patent claims, Larson’s ¶¶ 140-141 bear a closer look.

100. Both by its own terms, and properly read in the context of Larson as a whole, ¶140 of Larson relates to analyzing droplets that contain only a *single analyte both before and after PCR*:

Another method of the invention involves performing higher-plex assays using multiple different probe colors (i.e. fluorophores), however unlike the strategy above where each target is identified by single type of probe with a unique color and intensity, instead in this method a single target may be identified by multiple probes that constitute a unique signature of both colors and

intensities. 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.

EX1017(Larson), ¶140.

101. Larson's ¶140, which is reproduced at ¶100 of this Declaration in its entirety, describes analyzing only three types of droplets after PCR: (1) droplets containing only the T1 molecule (detected with fluorescence in color A); (2) droplets containing only the T2 molecule (detected with fluorescence in color B); and (3) droplets containing only the T3 molecule (detected with fluorescence in colors A and B). *See* EX1017(Larson), ¶140. Larson's ¶140 is silent on how to unambiguously distinguish *any* combination of presence or absence of the T1, T2,

and/or T3 molecules (let alone all possible combinations) in respective droplets after PCR. *See* EX1017(Larson), ¶140.

102. For example, Larson's ¶140 is silent on the possibility of a first droplet containing both T1 and T2, let alone how to differentiate the first droplet's fluorescence in colors A and B after PCR from the fluorescence in colors A and B from a second droplet containing T3 after PCR in such a manner that the first droplet can be unambiguously identified as containing T1 and T2 and the second droplet can be unambiguously identified as containing T3. *See* EX1017(Larson), ¶140. Larson's ¶140 further is silent on the possibility of a first droplet containing both T1 and T3, let alone how to differentiate the first droplet's fluorescence in colors A and B after PCR from the fluorescence in colors A and B from a second droplet containing T3 after PCR in such a manner that the first droplet can be unambiguously identified as containing T1 and T3 and the second droplet can be unambiguously identified as containing T3. *See* EX1017(Larson), ¶140. Larson's ¶140 likewise is silent on the possibility of a first droplet containing both T2 and T3, let alone how to differentiate the first droplet's fluorescence in colors A and B after PCR from the fluorescence in colors A and B from a second droplet containing T3 after PCR in such a manner that the first droplet can be unambiguously identified as containing T2 and T3 and the second droplet can be unambiguously identified as containing T3. *See* EX1017(Larson), ¶140.

103. Similarly, Larson's ¶140 is silent on the possibility of a first droplet containing T1, T2, and T3, let alone how to differentiate the first droplet's fluorescence in colors A and B after PCR from the fluorescence in colors A and B from a second droplet containing T3 after PCR in such a manner that the first droplet can be unambiguously identified as containing T1, T2, and T3, and the second droplet can be unambiguously identified as containing T3. *See* EX1017(Larson), ¶140. Indeed, ¶140 of Larson is silent on the possibility of a given droplet even *containing* any combination of T1, T2, and/or T3 after PCR, let alone how to uniquely differentiate the analytes in a first droplet, which may contain any combination of T1, T2, and/or T3 after PCR, from the analytes in a second droplet, which also may contain any combination of T1, T2, and/or T3 after PCR, using the fluorescence in colors A and B from the droplets. *See* EX1017(Larson), ¶140.

104. Indeed, Larson does not disclose how to analyze such multiple occupancy droplets because Larson seeks to avoid such droplets due to the many problems and complexities that "multiple occupancy" causes, e.g., as expressed in Larson's ¶141 which immediately follows ¶140:

Generally, without wishing to be constrained by theory,
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. With multiple occupancy arises the complexity of simultaneous assays competing within the same reaction droplet, and also complexity of assigning the resulting fluorescence intensity that involves a combination of fluorescence from two different reaction products that may or may not be equal to the sum of the two fluorescence intensities of the individual reaction products. However, methods of the invention can accommodate these complications arising from multiple occupancy.

EX1017(Larson), ¶141.

105. Petitioner and the Board each focus on Larson’s statement in ¶141 that “methods of the invention can accommodate these complications arising from multiple occupancy.” *See, e.g.,* Paper 11, 31 (*citing* Petition, 55) (*internal citations omitted*). But Larson’s ¶141 does not actually explain how the methods of Larson’s ostensible invention “accommodate these complications arising from multiple occupancy.” *See* EX1017(Larson), ¶141. Not only does Larson teach away from

having multiple target molecules in a droplet due to numerous associated downsides and complexities, Larson also teaches that it is advantageous to have a single target molecule per droplet. EX1017(Larson), ¶¶99-101, 107, 141

106. As such, and as discussed above at ¶¶73-75 of this Declaration, a POSA considering Larson's ¶141 (cited in the Petition), in view of Larson as a whole, readily would have understood that Larson's statement "methods of the invention can accommodate these complications arising from multiple occupancy" refers to the strategies Larson actually discloses, discussed at length herein. As such, the POSA would have understood Larson's statement refers to increasing the sample dilution before PCR as the number of analytes increases, in order to reduce the probability that one probe overlaps any of the other probes—that is, to reduce the probability that any droplet contains even two analytes before PCR, let alone more than two, as discussed at ¶¶52-63 of this Declaration and described in Larson's ¶129 (cited in the Petition). The POSA also would have understood Larson's statement refers to detecting, sorting away, and excluding droplets containing a heterogeneous population of molecules after PCR and before analysis, based on the number of signals (one signal or two signals) that each droplet produces, as discussed at ¶¶64-76 of this Declaration.

107. For at least the reasons described in the preceding paragraph ¶106 and elsewhere in this Declaration, it is my view that if a POSA wished to

“accommodate” multiple occupancy situations, the full teachings of Larson would, at most, have motivated that POSA to try to *eliminate* multiple occupancy situations by *diluting* the sample to limiting or terminal dilution before PCR to reduce the probability that any droplet contains two or more analytes, and *excluding* droplets containing two or more analytes after PCR and before analyzing the remaining droplets. In my view, these teachings of Larson do not render it at all obvious to instead *try to obtain, and analyze, droplets that may obtain all possible different combinations of analytes both before, during, and after PCR.*

108. Despite Larson’s express disclosure on the subject of “accommodat[ing] these complications arising from multiple occupancy,” (*supra* ¶75) Petitioner and Dr. Batt aver that “Larson teaches an assay that would do precisely this in Figure 9.” EX1017 ¶ 121, Fig. 9A; EX1002 ¶ 333; Petition, 55. But, for the reasons discussed below, Larson’s Figure 9, and its accompanying text at Larson’s ¶¶29 and 121, do not actually disclose how to “accommodate these complications arising from multiple occupancy” in the manner that Petitioner and Dr. Batt so specifically allege.

109. First, contrary to what Petitioner implies, Larson itself does not remotely represent that Figure 9 “accommodate[s] these complications arising

from multiple occupancy” to which Larson’s ¶141 refers (*see supra* ¶75); instead, the only two paragraphs in Larson that mention Figure 9 are the following:

FIG. 9 is a schematic depicting two-color detection of three genetic sequences with a microfluidic device.

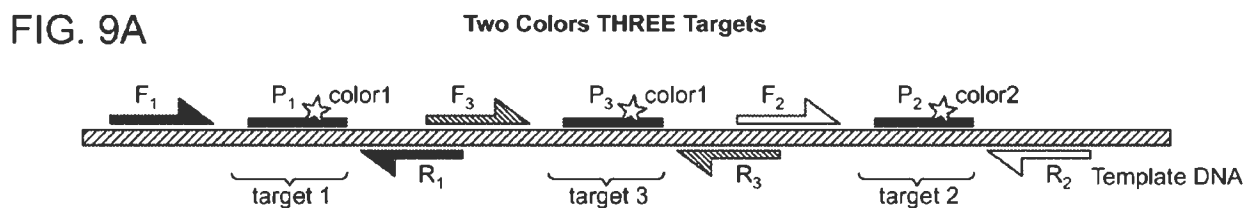
EX1017(Larson), ¶29.

Methods of the invention involve performing accurate quantitation of multiple different DNA targets by dPCR using probes with the same fluorophore. FIG. 9 is a schematic depicting two-color detection of three genetic sequences with a microfluidic device. As shown in Panel A of FIG. 9, a template DNA is amplified with three sets of primers: forward primers (F1, F2 and F3) and reverse primers (R1, R2 and R3). Probes (P1, P2 and P3) are labeled with fluorophores (color 1, color 2 and color 1) and bind to the target genetic sequences (target 1, target 2 and target 3) (Panels B and C). Microdroplets are made of diluted solution of template DNA under conditions of limiting or terminal dilution. Microdroplets containing target sequence 1 or 3 emit fluorescence of color 1 at two different intensities; and microdroplets containing target

sequence 2 emit fluorescence of color 2. The number of microdroplets containing target 1, 2 or 3 is shown by histogram in Panel D.

EX1017(Larson), ¶121.

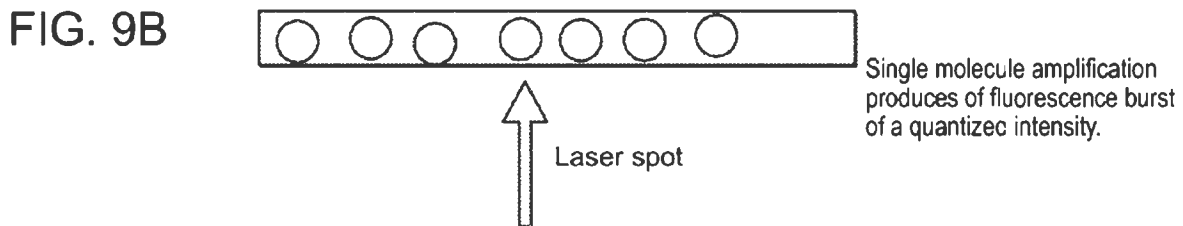
110. Turning now to Larson’s FIG. 9A, reproduced below, Dr. Batt states that “Figure 9 shows one such way to accommodate these complications. Larson depicts, in Figure 9A, a multiplex PCR which detects three targets using only two fluorophores.” EX1002(Batt Decl.), ¶333.



111. Petitioner and Dr. Batt argue that Larson’s Figure 9 accommodates the “complications” which come with multiple occupancy. These arguments are simply wrong. Indeed, FIG. 9A does not even *show* multiple occupancy at all. Instead, FIG. 9A plainly illustrates a ***single template DNA molecule*** which includes three targets, consistent with Larson’s description of this figure as illustrating “A template DNA is amplified with three sets of primers...Probes (P1, P2 and P3) are labeled with fluorophores (color 1, color 2 and color 1) and bind to the target genetic sequences (target 1, target 2 and target 3) (Panels B and C).” EX1017(Larson), ¶121.

112. Because the three targets shown in Larson's FIG. 9A are linked on the same molecule, they are not physically independent of one another, and indeed will always co-occur with one another in the same droplet so long as they are linked by the same single template DNA molecule. Accordingly, a POSA would have readily understood that Larson's FIG. 9A, and the section of Larson's ¶121 describing FIG. 9A, do not teach or suggest "multiple occupancy," let alone how to accommodate the "complications" which come with multiple occupancy as Petitioner and Dr. Batt allege that it does. Moreover, a POSA would have readily understood that the type of analysis shown in Larson's FIG. 9A describes a specific, narrow methodology for analyzing three alleles on a single molecule, in a single droplet. This type of single-molecule methodology is of a very different nature to the methodologies that the '921 Patent teaches and claims, where an ensemble of any combination of analytes may occur in a droplet and nonetheless can be identified using the inventive coding schemes.

113. Petitioner and Dr. Batt do not include or discuss Larson’s FIG. 9B, reproduced below. *See, e.g.*, Petition, 54-57. *See, e.g.*, EX1002(Batt Decl.), ¶¶108-147, 169, 175, 188, 218-222, 295, 299, 313, 325-334, 371.



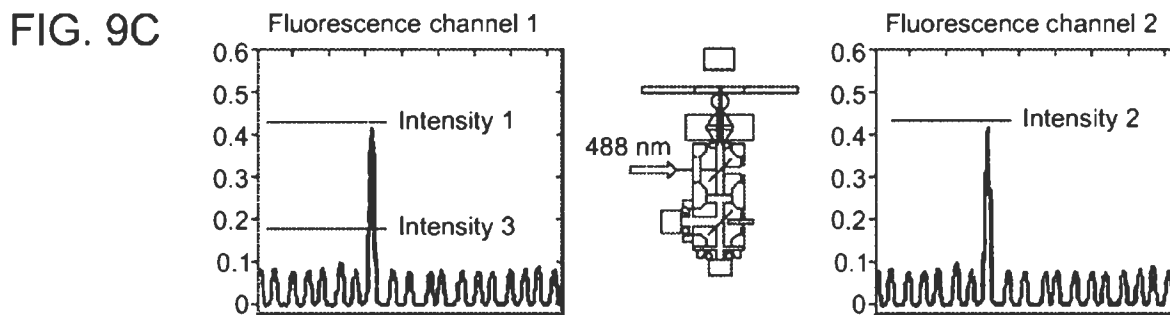
114. Larson’s FIG. 9B is expressly directed to “*single molecule* amplification,” which directly contradicts the “multiple occupancy” story that Petitioner and Dr. Batt are trying to build. EX1017(Larson), FIG. 9B. *See, e.g.*, Petition, 60-61. *See, e.g.*, EX1002(Batt Decl.), ¶321 *et seq.*

115. The Declaration of Dr. Carl Batt (EX1002), Petitioner’s proffered expert, does not change my view on this subject. Indeed, ¶321 of the Batt Declaration, to which the Petitioner points as allegedly supporting the position that Larson’s “methods accommodate . . . ‘multiple occupancy’ situations,” merely points to Larson’s ¶¶129, 141 and rehashes Petitioner’s conclusion regarding obviousness.

116. FIG. 9C of Larson, reproduced below, features prominently in Petitioner’s arguments and Dr. Batt’s analysis. For example, the Petitioner alleged that from Larson’s FIG. 9C, a POSA:

would have understood that the various combinations of analytes would result in distinguishable signals for the three analytes because the signals from P_1 , P_2 , P_3 , P_1+P_2 , P_1+P_3 , P_2+P_3 , $P_1+P_2+P_3$, and so on have different intensities as the combinations are additive and would each sum to unique values in each of the two colors used.

Petition, 57, citing EX1002(Batt Decl.), ¶336; EX1017(Larson), FIG. 9C.²



117. Here, the Petitioner and Dr. Batt hypothesize what scenarios “would result in distinguishable signals” from droplets containing different combinations of three analytes, and allege that the “foregoing coding scheme” is “[c]ritically, consistent with Larson’s teaching that its assays can accommodate ‘multiple occupancy.’” Petition, ¶56, citing EX1002(Batt Decl.), ¶¶335-337. But contrary to

² The “and so on” in this quote is nonsensical because there are only three analytes, and all possible combinations of presence or absence of those three analytes are already enumerated.

what is alleged, the scheme is not at all “consistent” with Larson’s teachings regarding “multiple occupancy,” and indeed Larson’s express disclosure at ¶121 states that *all* of FIGS. 9A, 9B, and 9C are directed to the “single molecule” situation shown in FIG. 9A: “As shown in *Panel A* of FIG. 9, a template DNA is amplified with three sets of primers...Probes (P1, P2 and P3) are labeled with fluorophores (color 1, color 2 and color 1) and bind to the target genetic sequences (target 1, target 2 and target 3) (*Panels B and C*).” EX1017(Larson), ¶121. As such, contrary to what Petitioner alleged, the scheme shown in FIG. 9C is only “consistent with Larson’s teaching that its assays can accommodate ‘multiple occupancy’ to the extent that this figure teaches detecting a *single molecule* in a droplet, that is, Larson’s solution to the “multiple occupancy” problem. *See* Petition, ¶56, citing EX1002(Batt Decl.), ¶¶335-337.

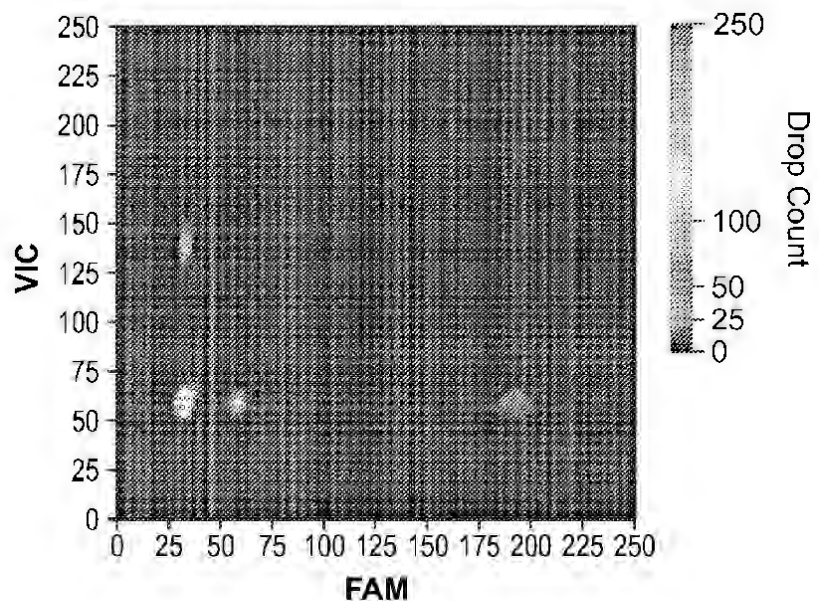
118. As such, contrary to Dr. Batt’s testimony, Larson’s Figure 9C does not “exemplify ‘an approach that differentiates three analytes,’” but instead exemplifies the vastly simpler situation in which three targets are all part of, and linked to one another by, the same analyte molecule. The former situation, where three different analyte molecules would be contained in one droplet, corresponds to the very “multiple occupancy” situation that Larson expressly teaches against and that the ’921 Patent claims expressly recite. The latter situation, where a single analyte molecule is contained in one droplet, corresponds to the very “single

molecule” situation that Larson expressly discloses in FIGS. 9A-9C and propounds throughout its disclosure, for example as discussed at ¶¶57-59 of this Declaration.

119. And, even while Petitioner and Dr. Batt point to Larson’s FIG. 9C, they ignore the related figure FIG. 9D, reproduced below:

FIG. 9D

Histogram showing four populations of droplets; with target1, with target2, with target3 and without any of the three targets.



EX1017(Larson), FIG. 9D. According to Larson, FIG. 9D illustrates a “[h]istogram showing four populations of droplets; with target1, with target2, with target3 and without any of the three targets.” EX1017(Larson), ¶121. FIG. 9D does not disclose, and indeed clearly *omits*, any clusters containing *both* target1 and target2, any clusters containing *both* target 1 and target3, any clusters containing *both* target2 and target3, and any clusters containing all *three* targets. And even if FIG. 9D did include such clusters, Larson’s other disclosure regarding Figure 9 clearly indicates that such clusters come from the *single molecule* embodiment described

with reference to FIGS. 9A-9C. Accordingly, what ostensibly is *actual* data from Larson is entirely consistent with my interpretation of the reference as set forth above at ¶¶52-76 of this Declaration, and not at all consistent with Petitioner's conjectures about what "would result" *if* Larson's teachings were *not actually followed*. I do not believe that anything in Larson would have led a POSA to ignore Larson's express teachings—and indeed, to modify Larson's principle of operation—to try to obtain the scheme Petitioner proposes.

120. For the reasons that follow, it is my view that Petitioner wholly fails show why it would have been obvious to modify Larson to arrive at the recited claims.

121. Petitioner's invalidity theory primarily relies on keeping Larson at limiting or terminal dilution while additionally analyzing the rare multiple occupancy droplets. Petition, 52. For example, Petitioner states that:

Larson expressly teaches that there may be multiple targets in a single individual droplet and that its methods accommodate such 'multiple occupancy' situations. See EX1017 ¶¶ 129, 141; EX1002; ¶ 321. Larson's assays use conditions and parameters that do precisely this.

EX1002 ¶321. Petition, 52. The sections of Larson to which Petitioner points (¶¶129 and 141) teach increasing dilution, rather than decreasing dilution, so it

seems that Petitioner's reference to Larson's "conditions and parameters" can only reasonably be interpreted as those which Larson actually discloses, namely terminal or limiting dilution as described elsewhere herein.

122. In my view, the reasons Dr. Batt offers as allegedly motivating this modification are irrational and inconsistent with what a POSA reasonably would have believed in view of Larson's express teachings.

123. For example, Dr. Batt states "a POSA would have been motivated to use this strategy because limiting an experiment to only single-target droplets severely impacts the total data collected in that experiment." EX1002(Batt Decl.), ¶338. This purported motivation is irrational because multiple occupancy droplets are quite rare at limiting dilution, and extremely rare at terminal dilution. Dr. Batt fails to explain why the POSA would have thought that additionally analyzing the rare multiple occupancy droplets would have any meaningful "impact" on the "total data collected in that experiment." *See* EX1002(Batt Decl.), ¶338.

124. Furthermore, a POSA readily would have recognized that in Larson's method performed at terminal or limiting dilution, the single-analyte droplets already provide all the information required to identify the analytes present in the sample. Dr. Batt fails to explain why, given how rarely the multiple occupancy droplets occur and that such droplets would not actually provide any information beyond that obtained from the single analyte droplets, the POSA would have been

rationally motivated to expend *any* additional effort to analyze multiple occupancy droplets. *See* EX1002(Batt Decl.), ¶338.

125. As an additional purported motivation for modifying Larson, Dr. Batt states that:

These assays, especially at the time of the '921 patent, were quite costly to set up and run, for example, as discussed in Larson, many reagents are required. *See, e.g.,* EX1017 ¶68. The ability to acquire data not just from a fraction of the droplets, but from all droplets in the experiment would thus be extremely cost effective.

EX1002(Batt Decl.), ¶138. But again, it would be nonsensical for a POSA to expect this situation to magically provide “the ability to acquire data not just from a fraction of the droplets, but from *all* droplets in the experiment,” as Dr. Batt hypothesizes. Instead, as amply established elsewhere herein and as disclosed by Larson, at terminal or limiting dilution, a large proportion of the droplets before PCR either contain no analyte molecules or only a single analyte molecule.

EX1017(Larson), ¶6. If Dr. Batt is proposing not to change the dilution (or concentration) in Larson, then the number of empty droplets simply would not change. Dr. Batt does not explain why the POSA would rationally expect that adding analysis of multiple occupancy droplets would provide the ability to acquire

data “from all droplets” in the experiment. Moreover, Dr. Batt does not explain why the POSA would rationally expect that adding analysis of multiple occupancy droplets would meaningfully impact the cost of the experiment—let alone be “extremely cost effective,” as Dr. Batt proposes.

126. As yet another purported motivation for modifying Larson, Dr. Batt states that:

Additionally, by allowing more DNA into each droplet, the dilution factor for the sample decreases, meaning less sample exists and less run-time on the machine is necessary.

EX1002(Batt Decl.), ¶138. But again, assuming that Petitioner’s proposed modification to Larson involves using terminal or limiting dilution, it would be nonsensical for a POSA to expect this situation to magically “allow[] more DNA into each droplet” or decrease “the dilution factor for the sample” as Dr. Batt posits. Dr. Batt does not explain why the POSA would rationally expect that adding analysis of multiple occupancy droplets would “allow[] more DNA into each droplet” or decrease “the dilution factor for the sample.”

127. While Petitioner’s apparent theory of obviousness involves maintaining Larson’s conditions of terminal or limiting dilution, Dr. Batt apparently has an alternate theory of obviousness requiring reducing dilution in

Larson (thus increasing concentration) to obtain more multiple occupancy droplets for analysis. *See* EX1002(Batt Decl.), ¶338.

128. For example, Dr. Batt avers “a POSA would have been motivated to use this strategy because limiting an experiment to only single-target droplets severely impacts the total data collected in that experiment.” EX1002(Batt Decl.), ¶338.

129. As an additional purported motivation for modifying Larson, Dr. Batt states that:

These assays, especially at the time of the '921 patent, were quite costly to set up and run, for example, as discussed in Larson, many reagents are required. *See, e.g.,* EX1017 ¶68. The ability to acquire data not just from a fraction of the droplets, but from all droplets in the experiment would thus be extremely cost effective.

EX1002(Batt Decl.), ¶138.

130. As yet another purported motivation for modifying Larson, Dr. Batt states that:

Additionally, by allowing more DNA into each droplet, the dilution factor for the sample decreases, meaning less

sample exists and less run-time on the machine is
necessary.

EX1002(Batt Decl.), ¶138.

131. Each of these purported motivations evidence that Dr. Batt’s theory is to modify Larson by increasing the number of multiple occupancy droplets, and decreasing the number of empty droplets, by reducing dilution (increasing concentration), ostensibly to “impact[] the total data collected in that experiment,” “acquire data . . . from all droplets in the experiment” and “allow more DNA into each droplet” such that “the dilution factor for the sample decreases.” *See* EX1002(Batt Decl.), ¶338. But each of these changes goes against Larson’s express teachings, for the reasons described elsewhere herein.

132. For example, *despite* the limits that heavy dilution imposes on the data collected, Larson asserts that the benefits of heavy dilutions are nonetheless worth it to avoid the “error” caused by the possibility of probes overlapping one another in multiple occupancy: “The need for large dilutions *strongly favors* the large number of dPCR reactions.” EX1017(Larson), ¶128; *see supra*, ¶¶58-63, 89, 94. Indeed, attempting to “acquire data . . . from all droplets in the experiment” and “allow more DNA into each droplet,” and decreasing the “dilution factor,” as Dr. Batt proposes, would all *aggravate* (if not make certain) the possibility of

unintended “cross-reactivity” between analytes in a given droplet—which Larson teaches *must* be avoided: “[h]igher degrees of multiplexing would *require* greater dilution.” EX1017(Larson), ¶129; *see also id.*, ¶¶6, 96. *See, e.g., supra*, ¶¶33-34, 53, 58-63, 89, 94.

133. Therefore, in my view, claims 1 and 14 of the ’921 Patent, and claims depending therefrom, are not obvious over Larson despite Petitioner’s and Dr. Batt’s unsubstantiated and irrational assertions that contradict what a POSA would have understood to be Larson’s express disclosure and teachings.

D. Ground 4 (Claim 11): Obviousness In View Of Larson, Saxonov, and Silverbrook

134. The Petition alleges that claim 11 of the ’921 Patent is obvious in view of Larson and Saxonov in view of Silverbrook. Petition at 66-67. Claim 11 depends on and adds limitations to claim 1. The Petitioner argues that Silverbrook teaches “PCR and hybridization to identify which analytes are present in a sample and transmitting results of the testing through a computer network.” Petition at 66. Even assuming that Silverbrook teaches that for which it is cited, such features do not cure the many deficiencies of Larson and Saxonov as detailed elsewhere herein. *See supra* §§VII.A.-C.

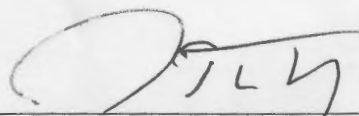
VIII. DECLARATION

135. This Declaration and my opinions herein are made to the best of my

knowledge and understanding, and based on the material available to me, at the time of signing this Declaration. I declare under penalty of perjury under the laws of the United States that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 or Title 18 of the United States Code. I executed this Declaration at Weston, Massachusetts.

Dated: June 19, 2025

By: _____

A handwritten signature in black ink, appearing to read 'K. Struhl', is written over a horizontal line.

KEVIN STRUHL