

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. IPR2025-01546

U.S. Patent No. 12,168,797 B2

DECLARATION OF BERNHARD WEIGL, PH.D.

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DECLARATION OF BERNHARD WEIGL, PH.D.

I, Bernhard Weigl, 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 IPR2025-01546, a petition for *Inter Partes* Review of U.S. Patent No. 12,168,797 (“the ’797 Patent”).

2. I am being compensated for my work in these matters at a rate of \$375 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. I am an expert in the field of bioengineering, and particularly in diagnostic assays and technology. This section contains a summary of my educational background, career history, publications, and other relevant qualifications. My full *curriculum vitae* is attached as EX2025.

4. I have over 25 years of experience in the design of diagnostic assays and technology, particularly those involving microfluidic applications. As such, I am familiar with the historical development of the field broadly since the late

1990s. I am also familiar with the historical development of specific technologies within the field during this timeframe, including Polymerase Chain Reaction (PCR) amplification and systems employing droplet digital PCR amplification and technology employed for counting and determining the intensity of droplets.

5. I have several patents and papers in this space that are relevant to the technology described herein. I am a named inventor on over 100 U.S. patents and published patent applications relating to diagnostic technology, including digital droplet assays. I am also an author or co-author on over 170 publications, including book chapters and journal publications relating to diagnostic technology, including digital droplet assays. Many of the patents and publications on which I am a named inventor or author relate to microfluidic applications in diagnostic technology. My work has been published in numerous journals, including *Nature*, *Science*, *Nature Biotechnology*, *Biomedical Microdevices*, *Sensors and Actuators B*, *Lab on a Chip*, *Analytical Chemistry*, *Microchimica Acta*, *Microfluidics*, *BioMEMS*, and *Medical Microsystems*. A full list of these patents and publications is included with my CV. I am a member of the Society of Photo-Optical Instrumentation Engineers (SPIE), the American Society of Tropical Medicine and Hygiene (ASTMH), the American Automatic Control Council (AACC), and the American Association for the Advancement of Science (AAAS), and have served on the editorial board of proceedings and journals published by SPIE and the Institute of Electrical and

Electronics Engineers (IEEE). I have served as a peer-reviewer for academic and technical journals, and in this position I judge the quality of other scientists' work, and determine whether their manuscripts are suitable for publications.

6. I am currently an Affiliate Professor at the University of Washington's Department of Bioengineering, in Seattle, Washington, a position I have held since May 2004. During this same timeframe, from June 2020 to August 2025 I worked as Senior Director of Diagnostics Research and Development at Global Health Labs, where I oversaw the development of diagnostic technology, including ultra-sensitive strip-based immunoassays and molecular assays and droplet-based digital assays. Between April 2015 to June 2020, I served as Principal Investigator for Flow-Based Diagnostics and then Director of In-Vitro Diagnostics at Intellectual Ventures. Before that, from 2004 to April 2015, I led the Diagnostics Group and served as a Portfolio Leader for Non-Communicable Disease Diagnostics, as well as Group Leader for Diagnostics, and Director of the Center for Point of Care Diagnostics for Global Health at nonprofit PATH (Program for Appropriate Technology in Health). Between 1995 and 1999, I was a Post-Doctoral Fellow in Professor Paul Yager's group in the Department of Bioengineering at the University of Washington, and in 1997 a co-founder and until 2003 Director of Microfluidic Applications for the microfluidics-based point-of-care diagnostics company Micronics, Inc. of Redmond, Washington (now

part of Sony America). At Micronics, I led the development of the first Food and Drug Administration (FDA)-approved instrument-free microfluidic test as well as microfluidic flow cytometry-based hematology assays. I also worked at the microfluidic engineering firm MicroPlumbers MicroSciences of St. Paul, Minnesota during this time.

7. I earned my doctoral and master's degrees in Analytical Chemistry from Karl-Franzen-Universität Graz, in Graz, Austria. I started my chemistry studies at Leopold-Franzens-Universität Innsbruck, in Innsbruck Austria, then transferred to Karl-Franzen-Universität where I earned my Mag. rer. nat. (the equivalent of master of science) and Dr. rer. nat. (the equivalent of a Ph.D.) degrees, both in Analytical Chemistry. Thereafter, I completed my post-doctoral studies at the University of Southampton, UK and the University of Washington in Seattle. I also received a Certificate in Technology Management from the University of Washington in 1998.

8. A more detailed listing of my credentials and publications is provided in my CV, EX2025.

9. Based on my experience, I am an expert in the field of diagnostic assays and technology, including in the subject matter described by the asserted patent discussed herein. I have used my years of experience working in the field of diagnostic assays and technology and my understanding of the knowledge,

creativity, and experience of a person of ordinary skill in the art in forming the opinions expressed in this declaration. Additionally, for at least the above reasons, and those described in §IV.A below, I consider myself to possess the qualifications to serve as an expert in the field of diagnostic assays and technology, including in the subject matter described by the asserted patent discussed herein, and possessed such qualifications at the time of the '797 Patent's invention.

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. 12,168,797 “the '797 patent”;
- Exhibit 1002: Declaration of Carl A. Batt, Ph.D.;
- Exhibit 1003: U.S. Patent No. 9,921,154 “Jouvenot”;
- Exhibit 1004: U.S. Patent Provisional Application No. 61/507,082 (filed July 13, 2011) (Serge Saxonov, Simant Dube, John F. Regan, applicants) (“the '082 Provisional”);
- Exhibit 1005: Declaration of Kevin Struhl, Ph.D., *Bio-Rad Labs., Inc. v. Cal. Inst. Tech.*, IPR2024-01451, Exhibit 2001 (Dec. 30, 2024);

- Exhibit 1006: Joseph R. Lakowicz, Principles of Fluorescence Spectroscopy (3d. 2006);
- Exhibit 1007: Petition for Inter Partes Review of U.S. Patent No. 11,827,921, *Bio-Rad Labs., Inc. v. Cal. Inst. Tech.*, IPR2024-01451, Paper 1 (Sept. 16, 2024);
- Exhibit 1008: U.S. Patent No. 8,614,061 “Brabetz”;
- Exhibit 1009: U.S. Patent No. 12,168,797 Infringement Claim Charts from Infringement Contentions, served on July 2, 2025, *In re ChromaCode*, Case No. 5:23-cv-04823, N.D. Cal.;
- Exhibit 1010: 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/>;
- Exhibit 1011: 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 1012: U.S. Patent Application Publication
No. 2008/0003649 A1 “Maltezos”;
- Exhibit 1013: U.S. Patent Application Publication
No. 2011/0250597 A1 “Larson”;
- Exhibit 1014: Complaint for Patent Infringement of U.S. Patent
No. 12,168,797, filed on February, 18 2025, Dkt No. 1, *Cal. Inst.
of Tech. v. Bio-Rad, Laby’s, Inc.*, Case No. 6:25-cv-01701,
consolidated into *In re ChromaCode Litigation*, Case No. 5:23-cv-
04823-EKL (N.D. Cal.);
- Exhibit 1015: Shivaprasad H. Sathyanarayana and Lauren M.
Wainman, “Chapter 2, Laboratory approaches in molecular
pathology: the polymerase chain reaction,” *Diagnostic Molecular
Pathology*, 2024;
- Exhibit 1016: Deposition of Kevin Struhl, Ph.D., *Bio-Rad Labs.,
Inc. v. Cal. Inst. Tech.*, IPR2024-01451 (July 24, 2025);
- Exhibit 1017: U.S. Patent Application Publication
No. 2010/0227386 A1 “Neuzil”
- Exhibit 1018: U.S. Patent Application Publication
No. 2003/0219754 A1 “Oleksy”;

- Exhibit 1019: U.S. Patent Application Publication
No. 2009/0239308 A1 “Dube”;
- Exhibit 1020: U.S. Patent Application Publication
No. 2009/0258414 A1 “Wittwer”;
- Exhibit 1021: U.S. Patent Application Publication
No. 2012/0100600 A1 “Slepnev”;
- Exhibit 1022: European Patent Specification 0,594,763 B1
“Lehnen”;
- Exhibit 1023: Nucleic Acids, <https://www.genome.gov/genetics-glossary/Nucleic-Acids>, updated September 2, 2025;
- Exhibit 1024: Introduction of Molecular Beacons,
https://molecularbeacons.org/MB_introduction.html;
- Exhibit 1025: Notice of Allowance from Prosecution File History
of U.S. Patent No. 12,168,797, filed on November 18, 2024;
- Exhibit 1026: Final Rejection of U.S. Patent No. 12,168,797, filed
on July 13, 2023;
- Exhibit 1027: Information Disclosure Statement noting Saxonov
from Prosecution File History of U.S. Patent No. 10,068,051 filed
on August 5, 2014;

- Exhibit 1028: Information Disclosure Statement from Prosecution
File History of U.S. Patent No. 10,770,170 filed on March 7, 2018;
- Exhibit 1029: Information Disclosure Statement from Prosecution
File History of U.S. Patent No. 11,827,921 filed on May 1, 2020;
- Exhibit 1030: Information Disclosure Statement from Prosecution
File History of U.S. Patent No. 12,168,797 filed on July 13, 2023;
- Exhibit 1031: Declaration of Maria P Garcia, IP Research
Librarian; and
- Bert Vogelstein & Kenneth W. Kinzler, “Digital PCR,”
*Proceedings of the National Academy of Sciences of the United
States of America*, Vol. 96, No. 16 (Aug. 3, 1999) at pp. 9236-41.
DOI: 10.1074/pnas.96.16.9236.

IV. UNDERSTANDING OF THE LAW

12. I am not an attorney. In preparing and expressing my opinions and considering the subject matter of the '797 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 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 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 '797 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 note that the in Petitioner’s description of the POSA, the POSA is a person who would have been familiar with polynucleotide analysis and amplification. The same person would likely not have the same level of knowledge across all areas of medical analysis, and he would not be an immunologist or be

particularly skilled in immunoassays unless he had undergone additional training and/or having related work experiences. This is because the detection and analysis of polynucleotides (typically via PCR) is a different field from immunoassays, which detect antibodies or antigens (typically proteins). Both PCR-based assays (detecting polynucleotides) and immunoassays (detecting proteins) have had numerous recent innovations in their respective fields, and both fields have been evolving rapidly. Both PCR-based assays and immunoassays are sophisticated technologies, and each of them has complexities in its own ways. And as such, two different skillsets are needed. For example, one set of skills is required for designing and preparing hybridization probes for detecting polynucleotides (which require bioinformatic skills) and for running and interpreting PCR assays, while another set of skills is required to prepare and select antibodies (which require biochemical and immunological skills) and for running and interpreting immunoassays. In my opinion, a POSA as Petitioner has defined it is not in possession of the skillset related to immunoassays, unless the person has received cross-training to do both, which cannot be presumed ordinarily. Petitioner does not even mention the distinction between the two different fields of endeavor, let alone explain why the POSA as Petitioner defines it would be expected to possess the skillset related to immunoassays. Petition, 11–12.

20. 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 '797 Patent. I have a Ph.D. in analytical chemistry, and I have worked in biochemistry in my entire scientific career, which includes at least a year of experience in quantitative and/or digital PCR and fluorescence-based analyte detection.

B. Claim Interpretation in *Inter Partes* Review

21. 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.

22. 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

23. 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

24. 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.

25. 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.

26. 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.

V. BACKGROUND ON RELEVANT TECHNOLOGY

A. Technology

27. The detection of target polynucleotides, such as a particular genetic sequence, within a biological sample has been investigated in many biological and chemical fields, and many techniques have been developed over the years.

Polymerase chain reaction (PCR) has been commonly used to detect polynucleotides. In early versions of these assays, a polymerase chain reaction is used to amplify (create more copies of) target polynucleotides, and the amplified products are often separated and identified by gel electrophoresis.

28. More sophisticated PCR assays use “probes” with colorimetric labels (fluorophores). For example, a probe is designed to be complementary to a particular genetic sequence, and the probe and sequence bind via “hybridization.” The fluorophore-labeled probe emits light only if the probe has bound its target within the sample (otherwise it remains “quenched” and does not fluoresce). It is also possible to use multiple different probes that can fluoresce in different colors corresponding to the different analytes. 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. The fluorescence, *i.e.*, colors produced by the probes when the probes bind or “hybridize” to complimentary genetic sequences in the targets, can be detected with appropriate optical channels configured to detect specific wavelength(s) of light. More analytes can be detected in the same assay by increasing the numbers of probe types (each with distinct fluorophore color) and detector channels (one per color); this is, of course, being limited by, among other things, the number of distinct colorimetric probes available and the number of optical channels that can be installed in a detector.

29. In some versions of PCR, color is not used to distinguish the analytes from one another: light in the channel means an analyte is present, but does not identify which analyte is present. Some techniques (*e.g.*, Larson) tries to distinguish the analytes by (i) highly diluting the sample, (ii) physically dividing the diluted sample into droplets, (iii) observing the intensity of light from each droplet, and (iv) drawing conclusions about the analytes based on that intensity.

30. In some versions of PCR, it is also possible to design probes that fluoresce at different intensities, and thus to identify the analyte using intensity in the same color.

31. The term “digital PCR”, or digital assay more broadly, was first used in the late 1990s. In a conventional assay (also called “bulk assay”, to differentiate from “digital assay”), the concentration of an analyte is usually determined by determining a “bulk” parameter (such as fluorescence intensity) that is correlated with the total number of analyte particles in the sample in an analog fashion—*i.e.*, the more molecules, the higher the signal. Often, this means that an analyte molecule reacts with another molecule such that it becomes fluorescent. In the case of PCR amplification, this means that fluorescent molecules continue to be generated during amplification until a plateau is reached (the “end point”). The more fluorescent molecules are in the pathway of the excitation and detection optics, the higher the signal (within the dynamic range of the detection system). *See, e.g.*, Bert Vogelstein & Kenneth W. Kinzler, “Digital PCR,” Proceedings of the National Academy of Sciences of the United States of America, Vol. 96, No. 16 (Aug. 3, 1999) at pp. 9236-41. DOI: 10.1074/pnas.96.16.9236.

32. In a typical digital assay, the sample volume is diluted and then partitioned into many smaller volumes such that most of the individual volumes contain only zero or one analyte particle or molecule (called “terminal dilution”). Then, a signal (again, *e.g.*, fluorescence) is determined in each individual partitioned volume that is correlated to the presence or absence (but not the concentration within each droplet) of an analyte. Then, the “positive” and

“negative” partitioned volumes are counted, and the ratio of positive to negative volumes is a measure of the overall concentration of an analyte. Thus, the analyte concentration can be determined digitally (by “counting” of positives vs. negatives), as opposed to analog (by “measuring a bulk signal” and comparing it to a calibration graph). The primary advantage of this method is that no calibration of signal intensity is needed, and in general the signal to noise ratio of a presence vs absence measurement is higher than that of an analog signal measurement.

33. The method has been applied to PCR. The partitioning can be implemented by the generation of droplets (separated by an immiscible liquid) from a sample volume, in which case it is called digital droplet PCR or ddPCR, invented in the late 2000s. A secondary benefit of this method (in addition to the fact that no calibration is needed for quantitation) was found to be that by diluting the sample, inhibitors of PCR reactions that could depress the sensitivity of the method are also “terminally diluted” and thus not present in some of the droplets. In those droplets the reaction then proceeds unhindered, thus simplifying overall sample preparation needs.

34. The process of performing a ddPCR analysis involves typically four distinct steps. They can be performed individually and manually or by an integrated analyzer machine. They are (A) mixing of sample and reagents, (B) droplet generation, (C) polynucleotide amplification via thermocycling, and

(D) droplet characterization and analyte identification. The following description is intended to reflect the current state of the technology, rather than the state of the technology as it existed at the time of the '797 Patent's priority applications.

35. Step A—Mixing of sample and reagents: The typical constituents that are mixed together in a vial prior to droplet generation are polynucleotide targets from a biological specimen (usually after sample preparation/polynucleotide extraction), as well as, usually, a control target, forward and reverse primers (as many as are needed for the detection of all targets), probes (TAQMAN or equivalent) with fluorophore + quencher (as many as needed, labeled with the desired fluorophores, and present at the desired concentrations). The concentration and fluorophore labeling of probes are defined by the multiplexing strategy that is employed for the particular assay. Further constituents include polymerase, dNTPs (constituents to build up the amplified polynucleotide), and buffer constituents (such as Mg^{2+} , salts, and stabilizers).

36. Step B—Droplet generation: Droplets are generated, typically by injecting about 1 nL of the aqueous sample and reagent mix into oil, resulting in about 100 μm diameter droplets. About 20,000 are typically generated per reaction. The droplet generation involves partitioning: each droplet receives a random number of analyte DNA molecules which is based on the concentration of those molecules in the aqueous sample (*e.g.*, described by the Poisson distribution), PCR

reagents, primers/probes, polymerase, salts, Mg^{2+} , and dNTPs. Each droplet becomes an independent PCR microreactor.

37. Step C—Polynucleotide amplification via thermocycling: PCR proceeds independently inside each droplet during thermal cycling. If multiple different targets are present in a droplet along with the probes and primers to amplify them, then these amplifications also proceed largely independently from each other within each droplet, and result in fluorescent emissions indicating positive signals for each of the targets. However, multiple copies of the same analyte within one droplet will result in the same level of fluorescence after amplification as a single copy of the same analyte.

38. Step D—Droplet detection: Droplets are then fluidically focused and illuminated by multiple LEDs or lasers. The emitted fluorescence from each droplet is collected by the detection optics and directed onto a set of dichroics and emission filters that split the light into several spectral bands, each typically read by its own avalanche photodiode detector. Common wavelength detection channels used are those optimized for the fluorescent dyes FAM, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590 but others can be used also. Droplets that originally contained one or more than one copies of one or several of the target sequences will now, after amplification, and when illuminated by a light source, fluoresce at one or several wavelength channels at a predictable fluorescence intensity level

that can be predetermined using a variety of techniques. Droplets containing no copies of any target DNA remain dark across all fluorescence wavelengths.

Depending on the labeling scheme used for each target polynucleotide and the targets that are present in each droplet, the droplets, when illuminated, can show a vast variety of fluorescence emission intensities and combinations of wavelengths.

If observed with a microscope and excitation filters, the droplets, when illuminated, would appear as liquid-filled spheres having a range of colors and intensities.

39. The mix of colors, and intensities of each of the colors, of these droplets are indicative of the analytes that were initially present in each droplet, as well as of the encoding scheme used to label the analytes. For example, a droplet containing two analytes, one amplified using a probe labeled with a yellow fluorescent dye, and the other labeled with a probe labelled with a red fluorescent dye, and if both probes are used at equal concentration, then the droplet will appear orange. If a third analyte is present that is labeled also with a red fluorescent dye but at higher probe concentration then that droplet will appear almost, but not quite, red.

40. Consequently, a vast number of combinations of analytes, probe concentration, and fluorescent dyes in a single droplet can therefore result in many shades, combinations, and intensities of colors. While in very simple systems—

such as a system that detects only two analytes—it is possible to determine what analytes are present in a droplet simply from observing or detecting the color and intensity, more complex mixtures require a much more complex analysis. Further, it is quite common (even in modern systems) that different combinations of analytes will result in droplet colors and intensities that are indistinguishable from each other, and thus do not allow the unambiguous determination of the presence or absence of analytes in individual droplets. Therefore, prior to the inventions described in the '797 Patent, multiplexing in ddPCR was limited to labeling methods that result in only very simple combinations of colors and intensities. The encoding and decoding algorithms disclosed in the '797 Patent are therefore essential to modern ddPCR multiplexing approaches.

41. As recognized by the '797 Patent and its priority applications, there is often a need to detect more analytes than the number of color channels used for the detection. Around 2010, scientists began developing novel multiplexed digital droplet PCR methods that used various encoding methods to detect multiple specific target polynucleotide sequences present in the sample at the same time. This can refer to multiple target sequences on the same gene (*e.g.*, to determine if there are any variations present on a particular gene, *e.g.*, to see if a cancer marker is present, or if a COVID virus has evolved), or targets on different genes (*e.g.*, to determine the presence of several respiratory viruses such as flu, COVID, and RSV

in a nasal swab (to diagnose a disease) or in waste water (for epidemiological purposes) at the same time). Multiplexed testing may mean that a sample is analyzed for multiple targets at the same time, but usually only one of them is present (such as in clinical testing for infectious diseases) or it may mean that multiple targets are determined and likely to be present in the sample (such as in testing for the presence of polynucleotides resulting from circulating viruses in a community waste water stream).

42. In general, as long as the primers and probes for each of the target sequences that one wants to detect in a single sample are sufficiently different from each other, it is possible to have tens or more different such primers and probes in a single reaction mix, and they will all run largely independently from each other (again, assuming there is an excess of all reaction components compared to what is needed to amplify and detect the analytical targets).

43. However, in order to tell which of the target sequences are actually present and are being amplified, one needs to be able to tell apart the fluorophores that are released by each of the TAQMAN probes that are specific to each target sequence. This had previously been done, to a degree, by using different fluorophores with different emission wavelengths (or “colors”), and detect them each with separate detectors (or “channels”) that are built into the PCR machine.

44. In practice, since often more than one sequence is needed to positively identify a target sequence, and usually a “control,” or reference, sequence also needs to be detected, this means that usually fewer useful targets can be detected in a sample using fluorescence color based multiplexing.

45. At the time the '797 Patent and its priority applications were filed, there were many multiplexing techniques, and they frequently involved some type of immobilization, separation, mass spectrometry, or melting curve analysis. These techniques typically required large amounts of sample materials, long assay time, additional reagents, and/or additional instruments. Therefore, there was a significant need for inexpensive, material-efficient, and time-efficient multiplexing that could non-degenerately identify a higher number of analytes within a given sample, without the need to add another optical channel corresponding to each additional analyte. The '797 Patent's key insights derive from intentional encoding of polynucleotide targets using amplitude modulation (usually fluorescence intensity) and multi-spectral (usually fluorescence emission wavelength) encoding, followed by algorithmic decoding (comprising signal processing using decoding formulas). The solution reached in the '797 Patent 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.

B. The '797 Patent and the Challenged Claims

46. The subject matter of the '797 Patent pertains to an encoding and decoding scheme that allows multiplexed detection of analytes. It is widely applicable across many different types of assays. It is especially applicable in allowing laboratories to measure many genetic targets simultaneously in a single test—far beyond what standard PCR typically allows. Through structured algorithms and mathematical techniques, the '797 Patent teaches encoding and interpreting overlapping fluorescent signals to increase the number of detectable targets for a given piece of PCR hardware, specifically a set of light sources and wavelength detectors (channels). This enables measuring more disease biomarkers without new instruments.

47. The inventive encoding and decoding technology enables a very high degree of multiplexing (*i.e.*, detecting multiple analytes in a single sample volume) while also ensuring that the presence or absence of each analyte to be determined can be unambiguously confirmed.

48. The encoding and decoding technology doesn't use a standard binary or color-tag scheme like conventional PCR. Instead, it encodes multiple genetic targets into distinguishable signal patterns (typically fluorescence intensity signatures across one or several fluorescent channels) that can be resolved using the analysis algorithms described in the '797 Patent. Multiple probes are

engineered so that each target produces a distinct fluorescence intensity pattern during PCR amplification—not just a simple “present/absent” signal. By modulating fluorescence intensities for different targets (whether by probe concentration and/or other aspects of the detection chemistry), unique amplitude signatures are created even when multiple targets share the same dye/color channel.

49. One application of this technology lies in digital droplet PCR (ddPCR). EX1001, 36:27–36. The raw intensity data from droplets are analyzed using an algorithm which decodes the patterns back into distinct target calls, to discriminate which combination of targets in a given droplet produced the observed signal by matching amplitude signatures to previously-defined encoded signatures.

50. The Caltech inventors recognized that traditional multi-channel methods, such as those described further above, are limited by the number distinguishable fluorophores available. The '797 Patent states that in such previously known methods “the number of detectable analytes is equal to the number of spectrally resolvable fluorophores, and the number of analytes may only be increased by increasing the number of spectrally resolvable fluorophores.” EX1001, 12:39–45.

51. A fundamental problem that the Caltech inventors have solved with their inventions as described and claimed in the '797 Patent (and its priority applications) is how to ensure that analytes analyzed in complex multiplexed assays can be unambiguously labeled (encoded) and detected (decoded). They developed a technique to non-degenerately identify analytes in a sample, using fewer fluorophores than the number of analytes (in contrast to prior multi-channel techniques). Instead, “each analyte to be detected is encoded as a value of a signal (e.g., intensity),” and by “measuring one component of the signal (e.g. fluorescence intensity)” from a sample, the technique can “determine the presence and absence of certain analytes based on the values used to encode the presence of each analyte and the cumulative value of the signal.” EX1001, 8:66–9:6, 9:35–40.

52. For simple combinations of small numbers of analytes in a bulk solution the analysis is not especially complicated and need not use the '797 Patent's encoding and decoding schemes. For example an Analyte A can be labeled with a red dye, and an Analyte B can be labeled with a yellow dye. If only A is present, the solution appears red, if only B is present the solution appears yellow, and if both are present the solution appears orange.

53. However, this problem quickly becomes more pronounced when many more analytes are to be detected in a single solution, and the Caltech

inventors recognized that additional color and intensity-based labeling must be employed to differentiate analytes from each other.

54. This is especially relevant in digital droplet assays such as digital droplet PCR (ddPCR). In ddPCR, there is a random, typically Poisson, distribution of analyte molecules in the individual droplets. For example, the solution may contain on average 0.3 copies of analyte A per droplet, and 0.7 copies of analyte B. However, for these example concentrations of Analytes A and B this means that some droplets will contain 0, some 1, some 2, fewer 3, and even fewer 4, copies of Analytes A or B, as well as various combinations of A and B. It is therefore important to understand what colors and mixtures of colors will result from any possible combination of Analytes A and B that can be expected to occur in order to positively determine what actually is present in each droplet. While this simple binary mixture is fairly easy to understand, increasing the number of analytes exponentially increases the number of possible outcomes.

55. The Poisson distribution describes the statistics of how many analyte molecules the droplets contain. In the limiting dilution regime, 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. At terminal dilution, the sample is sufficiently diluted that the Poisson distribution basically guarantees that almost all droplets are

either empty or contain only a single analyte molecule, with droplets rarely containing multiple analyte molecules. At terminal dilution, to best avoid droplets containing multiple analyte molecules, most droplets are empty, which wastes material and time.

56. As recognized by the Caltech inventors, for more complex mixtures of analytes, such as 8 or more, the analytes may be labeled with 4 or more different fluorophores, and encoded with several different concentrations of probes to make it possible to determine the presence or absence of analytes, using a specific decoding algorithm.

57. What's more, the '797 Patent's encoding schemes avoid the circumstance where different combinations of analytes, encoded with different probe concentrations and labeled with different fluorophores, would by coincidence result in identical droplet colors and color intensities, thus causing encoding degeneracy and the inability to unambiguously determine the presence or absence of analytes in each droplet. Table 8 of the '797 Patent, below, describes an example in which 16 analytes (A–P) are encoded using fluorescence intensities of four fluorophores (fewer fluorophores than analytes):

TABLE 8

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

58. This example encoding scheme allows the unambiguous detection of combinations of different analytes in a single sample volume. For example, the following table demonstrates a small fraction of the possible combinations of how combinations of analytes A, B, and E can be detected from the cumulative signal intensity from a single sample volume that contains a given combination of analytes. (This table below shows only a fraction of the $2^{16} = 65,536$ possible combinations of the 16 analytes shown in Table 8.)

59.

B	G	Y	R	Analytes(s) Present
1	0	0	0	A
2	0	0	0	B
3	0	0	0	AB
1	1	0	0	AE
2	1	0	0	BE
3	1	0	0	ABE

60. In this example, the presence of analyte A is indicated by a result of 1-0-0-0, the presence of analyte B is indicated by a result of 2-0-0-0, and the presence of analyte E is indicated by a result of 0-1-0-0. The digits express the intensity of the signal in each color: 1-0-0-0 has one unit of signal intensity in the blue channel, and 2-0-0-0 has two units of signal intensity in the blue channel.

61. In a typical example of the use of the '797 Patent's encoding and decoding schemes that illustrates the value of the inventive decoding algorithm, when using 6 colors and 4 intensities each there are 2^{24} possible combinatorial states that would have to be experimentally investigated, or 16,777,216 different states. The algorithm taught in the '797 Patent identifies each combination and how to distinguish it from each other combination.

62. In the particular coding scheme used in Table 8 of the '797 Patent, each analyte is represented by a code in a single color, wherein the value of the code in that single color equal to the sum of all previous values plus one. In effect, this example scheme encodes fluorescence intensities as powers of two. In other words, if the first code contains a 1 in a particular color, the next codes are 2, 4, 8, 16, 32, 64, 128, etc., although the '797 Patent contemplates other progressions as well. EX1001, 22:43–64.

63. The Caltech inventors extended the inventive schemes, such as in Table 8, to describe the measurement capabilities of a system using C colors with the equation $M = C \times \log_2 (F + 1)$, where F is the maximum cumulative intensity, for any color, when all of the analytes are present, and M is the maximum number of analytes that can be unambiguously detected using the system. EX1001, 23:21–27, 77:35–78:51. In claim 1 of the '797 Patent, C is constrained to be equal to 4, 5, or 6, and F+1 is constrained to be a positive integer and a power of 2. EX1001, 78:39–51. Under these constraints, the minimum value of F is 3, based upon which the presence or absence of each of eight analytes can be unambiguously detected using the system, without immobilization, mass spectrometry or melting curve analysis.

64. The Caltech inventors have solved the encoding problem as described in the '797 Patent, both in a general way, and specifically when applied to ddPCR.

65. A core invention of the '797 Patent is a generalized coding and decoding system that enables highly multiplexed biochemical detection without requiring as many optical detection channels as analytes or complex physical separation methods (such as immobilization on arrays or microbeads) or spatially separated multiplexing.

66. The '797 Patent teaches a scheme in which each analyte is assigned a unique code based on a first signal component—typically intensity or a range of intensities, and a second signal component—typically wavelength (*i.e.*, fluorescence color). Instead of each analyte requiring its own fluorophore, many analytes share the same fluorophores but are encoded and thus distinguished by differences in their intensity patterns across the available channels. This allows the system (as described in Claim 1 of the '797 Patent), with various limitations as listed, to encode up to $M = C \times \log_2(F + 1)$ analytes when multiple wavelengths (colors) are used (where F = maximum cumulative detectable intensity in a single color channel when all analytes are present, C = number of channels). A companion part of the encoding process is the decoding process, which translates measured signals back into analyte identities.

67. Prior to the priority date, a POSA would have been able to decode only simple combinations of analytes in reaction mixtures or in droplets based on color or spectroscopic measurements. For example, a mixture of two analytes

labeled with fluorescent dyes will result in visually distinctive colors that can be differentiated quite easily with spectral detectors or through optical filter arrangements. Similarly, a POSA would have been able to differentiate between two analytes labeled with dyes having different concentrations, visually, or at least with good quantitative detectors, as long as those concentrations were sufficiently different from each other. However, this becomes infeasible for highly multiplexed analyses that require more than a very small number of dyes or a very small number of concentrations. Therefore, a POSA at the time before the priority date would have instead typically performed the analysis using separation techniques (such as chromatography, or spatial immobilization based multiplexing (*e.g.*, in an array or on beads)), before detection, in order to reduce the numbers of analytes in each fraction or spatial area to be analyzed down to a manageable number.

68. In fact, I personally, as a POSA, upon seeing the formula as described in Claim 1 of the '797 Patent, was incredulous that there was so many analytes that could be encoded in the claimed system based on fluorescence intensity and color. I therefore set out to test the formula and created tables of possible droplet contents to see what signal they would result in. Over time, I came to realize that the inventors had arrived at a non-obvious solution to eliminate degeneracy through their $M = C \times \log_2(F + 1)$ encoding and decoding methodology.

69. The '797 Patent thus teaches a POSA how to employ an encoding and decoding algorithm that allowed to vastly increase the numbers of different analytes to detect in a single reaction volume, and especially in a ddPCR analysis.

C. The '797 Patent's Prosecution History

70. I understand that the Examiner of the '797 Patent allowed the application on the basis that "[t]he claims are free of the prior art because the prior art does not teach experimentally decoding electromagnetic signals to determine the presence and absence patterns of analytes using the equation and values input into the equation recited in the claims." EX2026, 595. I agree with the Examiner's assessment, for at least the reasons that I will explain in the sections which follow.

D. Overview of Jouvenot

71. U.S. Patent No. 9,921,154 to Jouvenot et al. ("Jouvenot") was filed on December 6, 2013, and issued on March 20, 2018. EX1003, §§(22), (43).

72. Both Jouvenot and the '797 Patent address the core challenge of expanding multiplex detection beyond the number of available fluorescence channels. However, they do so through fundamentally different mechanisms.

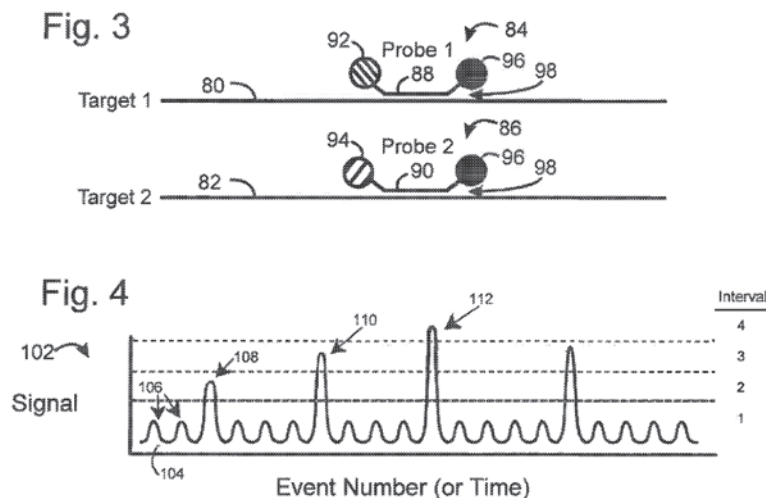
73. The '797 Patent employs an algorithmic approach to encoding and decoding that allows unambiguous and non-degenerate labeling and detection of analytes with essentially no limit to analyte number and complexity other than the limits of detector dynamic range and wavelength discrimination.

74. Jouvenot purports to demonstrate the ability to differentiate very small numbers of analytes by signal intensity by visually analyzing peak heights or two-dimensional clusters of signals. For example, Jouvenot discloses “performing digital assays . . . through multiplexed detection of signals from reporters for two or more distinct targets in a common or shared channel.” EX1003, 4:52–55. With respect to digital assays, Jouvenot discloses that such assays rely on detecting individual copies or activity of an analyte within a sample. In an exemplary digital assay, the sample is partitioned into a large number of generally equal-volume partitions, each containing on average fewer than one copy of the analyte. When analytes are randomly distributed, some partitions contain no copies, others contain one copy, and, if sufficient partitions are used, some contain two, three, or more copies. The probability of observing a given number of copies per partition is governed by a Poisson distribution. EX1003, 1:39–51.

75. Jouvenot discloses that “digital assays frequently rely on amplification of a nucleic acid target in partitions to enable detection of a single copy of an analyte,” and that such amplification may be performed using polymerase chain reaction (PCR). EX1003, 2:1–4. Jouvenot further states that amplification can be detected optically using a fluorescent probe in the reaction, where the probe includes a dye that generates a fluorescence signal indicating whether the target has been amplified. EX1003, 2:7–11.

76. Jouvenot identifies limitations of then-existing multiplexing techniques, noting that instruments with more optical channels are more expensive and that increasing the number of distinguishable dyes is costly and becomes impractical beyond a certain point. EX1003, 2:16–18. Accordingly, Jouvenot states that “a new approach is needed to increase the multiplex levels of digital assays.” EX1003, 2:21–22.

77. In Jouvenot, Example 1 is “an exemplary digital PCR assay with multiplexed detection of two targets.” EX1003, 13:24–25. The two targets (Target 1 and Target 2) are detected with two probes (Probe 1 and Probe 2), each of which includes a fluorophore (92 and 94 in figure 3 below):



EX1003, Fig. 3, Fig. 4.

78. Jouvenot discloses that “the intensity associated with one fluorophore, following reaction, may be lower or higher than the intensity(ies),” such that

“fluorophores 92, 94 . . . create detectable but distinguishable signals in the same channel.” EX1003, 13:46–48, 51–53. According to Jouvenot, in the example of Fig. 4, Fluorophore 94 corresponds to Target 2 and produces a higher-intensity signal than Fluorophore 92 for Target 1, such that four states ostensibly may be distinguished: both negative, Target 1 positive, Target 2 positive, and both positive:

Peaks 106 with maxima in Interval 1 correspond to droplets containing no Target (T1–/T2–). The measured signal corresponds to background (e.g., background fluorescence, scattering, etc.) and does not reflect the presence or amplification of either Target.

Peaks 108 with maxima in Interval 2 correspond to droplets containing Target 1 but not containing Target 2 (T1+/T2–). The measured signal corresponds to signal from Target 1 plus background and reflects amplification of Target 1 implying the presence of Target 1.

Peaks 110 with maxima in Interval 3 correspond to droplets containing Target 2 but not containing Target 1 (T1–/T2+). The measured signal corresponds to signal from Target 2 plus background and reflects amplification of Target 2 implying the presence of Target 2.

Peaks 112 with maxima in Interval 4 correspond to droplets containing both Targets 1 and 2 (T1+/T2+). The measured

signal corresponds to signal from both Targets 1 and 2 plus background and reflects amplification of Targets 1 and 2 implying the presence of Targets 1 and 2.

EX1003, 14:36–56.

79. Jouvenot’s Example 1 is simple, involving only two targets and four possible outcomes. Jouvenot further discloses that multiplexing complexity increases exponentially with the number of analytes, noting that for N analytes, “[t]he data including all permutations of positives will generally fall into 2^N populations or clusters, . . . assuming that each population is distinguishable.”

EX1003, 10:23–26. Jouvenot provides tables purporting to show “[e]xemplary results for one, two, and three target systems in which data are collected in a single channel.”

	Target A	Intensity
Population 2	+	Highest
Population 1	–	Lowest

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

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

EX1003, 10:32–55. As may be seen from these tables, the one-analyte system contains two (2^1) populations, the two-analyte system contains four (2^2) populations, and the three-analyte system contains eight (2^3) populations

80. Jouvenot expressed skepticism as to whether the four outcomes in even a simple two-analyte system can be reliably distinguished:

“Specifically, the peaks are assigned based on intervals delineated by values lying between (e.g., half way between) the peak heights for one outcome and the peak heights for adjacent outcomes. In other cases, the peak

heights for each outcome may overlap at their extremes,
so that thresholding may be neither simple nor linear.”
EX1003, 15:2–7.

81. Importantly, Jouvenot teaches and suggests here that the peak height for each outcome has a range of certainty, which may be so large that neighboring ranges of certainty overlap, and thus different outcomes cannot be reliably distinguished. Jouvenot does not teach any example of detecting more than two analytes in the same channel.

82. However, for any analysis of sufficient complexity (*i.e.*, those requiring a priori determination of signal overlap between different populations that could impede unambiguous determination of the analytes) a cluster or peak height analysis is simply not sufficient to decode a signal pattern. Note that the level of complexity arrives much more quickly in ddPCR assays than in conventional bulk PCR assays because of the Poisson distribution of analyte population across droplets. resulting in many more different possible outcomes for a given analyte mixture than in bulk PCR where the analyte concentration is the same throughout the sample.

83. Jouvenot seems to agree and only presents simple examples for multiplexing (*e.g.*, two analytes per detection channel). For such simple assays, the question of degeneracy largely does not come into play, as even with Poisson

droplet distribution it is still ostensibly possible to infer analyte presence and absence from the simple cluster patterns.

E. Overview of Larson

84. Larson discloses “droplet based digital PCR and methods for analyzing a target nucleic acid using the same.” EX1013, ¶45. Larson primarily discloses a droplet-based digital PCR workflow, that is, forming picoliter-scale droplets, each containing on average a single target nucleic acid, then amplifying and analyzing each droplet independently. This is the basis for traditional ddPCR which is normally operated at terminal dilution.

85. Importantly, Larson describes “forming sample droplets containing, on average, a single target nucleic acid, amplifying the target in the droplets.” EX1013, abstract. This is key and importantly does not describe a form of multiplexed ddPCR that is specifically designed to account for, and unambiguously distinguish, different combinations of analytes from one another, let alone in the specific manner the ’797 Patent teaches.

86. Where Larson’s ¶45 states that terminal or limiting dilution is essential for ddPCR in general and ddPCR multiplexing specifically, this clearly refers to a form of multiplexing where the analytes do *not* in fact occur in combinations with one another (as a result of the limiting or terminal dilution). Larson states that the fact that only one or no copy of an analyte is typically

present in a droplet is the reason that positive droplets are uniformly amplified. I believe this is incorrect. The limiting factor for amplification, and resulting fluorescence intensity, for a given analyte in a droplet, is typically probe concentration, not analyte concentration. Regardless, Larson teaches away from multiple occupancy droplets, for example as indicated in Figure 15 and FIGS. 9A-9D and the accompanying text.

87. In so far as Larson does discuss multiplexing (at limiting or terminal dilution) Larson is silent on any notion of degeneracy of signals, or any sort of higher levels of multiplexing as described in the '797 Patent. Larson describes terminal dilution as a condition in which “the vast majority of reactions contain either one or zero target DNA molecules,” and limiting dilution as one where some reactions contain zero molecules, some contain one, and others contain multiple molecules, consistent with a Poisson distribution. EX1013, ¶6. Larson identifies advantages of having one allele per droplet, explaining that the approach relies on the “singular nature of amplifications at terminal or limiting dilution,” because most droplets contain only a single target allele even when multiple primers or probes are present. *Id.*, ¶¶45, 98–99. In other words, the sample is diluted such that most frequently either no analyte or a single analyte is present in a given droplet.

88. Larson discloses digital PCR (dPCR) assays for detecting different numbers of analytes, each analyte being in its own droplet; illustratively, FIG. 15 purports to disclose a “9-plex dPCR assay for spinal muscular atrophy with only two fluorophores, showing the process of optimizing droplet intensities.” EX1013,

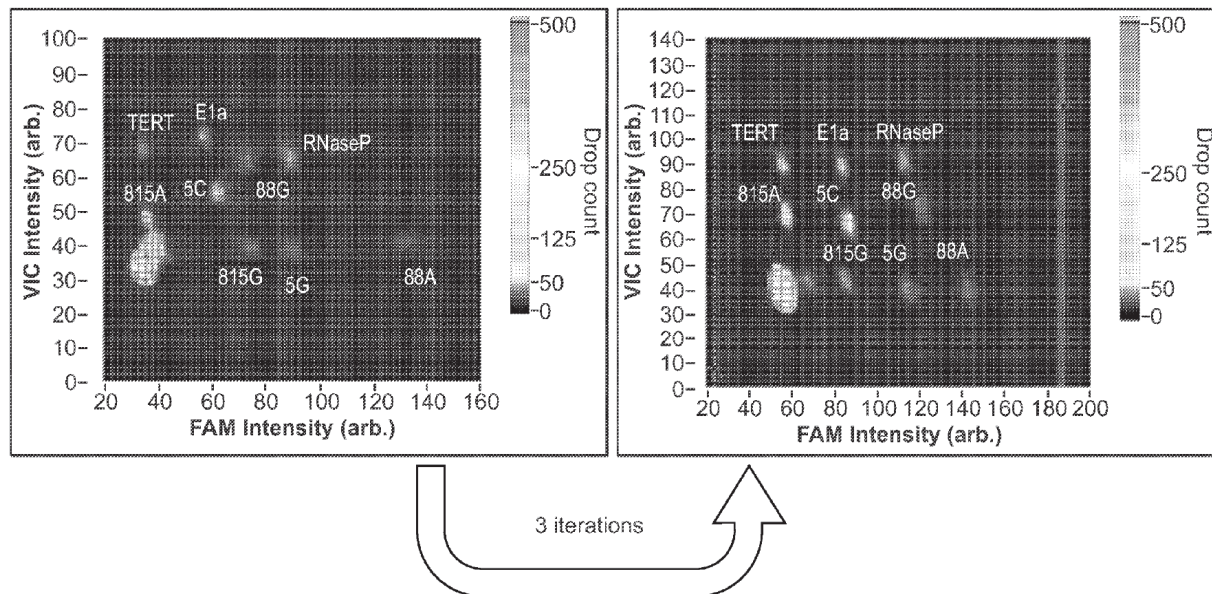


FIG. 15

¶35, FIG. 15.

89. Larson’s FIG. 15 shows only single-analyte clusters (respectively TERT, 815A, E1a, 5C, 815G, RNaseP, 88G, 5G, 88A). EX1013, ¶35, FIG. 15. Other graphs in Larson similarly appear to show measurements from only single-analyte droplets/clusters, and conspicuously omit measurements from multi-analyte

droplets/clusters. *See* EX1013, FIGS. 5B, 5C, 6B, 7C, 8D, 9A-9D, 14A, 18A, 19A, 20A, 21B, 21E, 21H.

90. Larson states that “[h]igher degrees of multiplexing would require greater dilution” due to the risk of unintended “cross-reactivity” between analytes within a droplet. EX1013, ¶129. Larson thus expressly relies on increasing sample dilution as the number of analytes increases in order to reduce probe overlap—that is, to lower the probability that any droplet contains two or more analytes.

91. To the extent any droplets contain more than one analyte, Larson teaches excluding those droplets from analysis. Larson discloses that the methods “detect which droplets contain a heterogeneous population of molecules and are able to exclude those droplets from the analysis.” EX1013, ¶172. Droplets producing two signals are classified as containing a heterogeneous population of molecules. EX1013, ¶173. A POSA would understand that such droplets produce two signals because they contain two different analytes, each generating a distinct signal.

92. By contrast, droplets producing only a single signal are classified as containing a homogeneous population of analytes. EX1013, ¶172. A POSA would understand that these droplets contain only a single analyte and therefore generate one signal.

93. Larson further describes excluding droplets that produce two signals in a section entitled “Droplet Sorting,” stating that droplets with heterogeneous populations are sorted away from those with homogeneous populations based on the detected signal. EX1013, ¶181. Thus, Larson teaches detecting droplets by the number of signals produced and excluding droplets that produce two signals.

94. Larson’s reasons for excluding “multiple occupancy” droplets relate to complexities associated with “multiple occupancy”: “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.” EX1013, ¶141.

95. A POSA would have understood, in view of Larson ¶141 and the disclosure as a whole, that “multiple occupancy” droplets contain a heterogeneous population of molecules. Larson’s statement that the methods “can accommodate these complications arising from multiple occupancy” would be understood to mean, first, increasing sample dilution as analyte number increases to reduce the likelihood that a droplet contains more than one analyte, and second, detecting,

sorting, and excluding droplets with heterogeneous populations based on the number of signals they produce.

96. Larson repeatedly emphasizes identifying a single analyte per droplet as a core inventive concept, and a POSA reasonably would have viewed single-analyte identification as Larson's principle of operation. *See* EX1013 abstract, ¶¶7, 8, 11, 13, 18–20, 45, 46, 49, 68, 99–101, 107, and claims 1, 3, 10, 11, and 15.

97. My understanding is that the Examiner considered Larson's disclosures before allowing the claims of the '797 Patent. *See* EX2026, 1333 (IDS, Cite No. 060, "US-9441266," a continuation of Larson).

F. Overview of Lehn

98. Lehn concerns a fluorescent immunoassay in which antibodies are used to immobilize an antigen to a microsphere. EX1022, 2:53–54. The microsphere constitutes a solid matrix, equivalent in function to the nitrocellulose of a covid lateral flow rapid test used in many peoples' homes. Lehn discloses a general procedure for detecting an antibody or antigen in a fluid sample as follows:

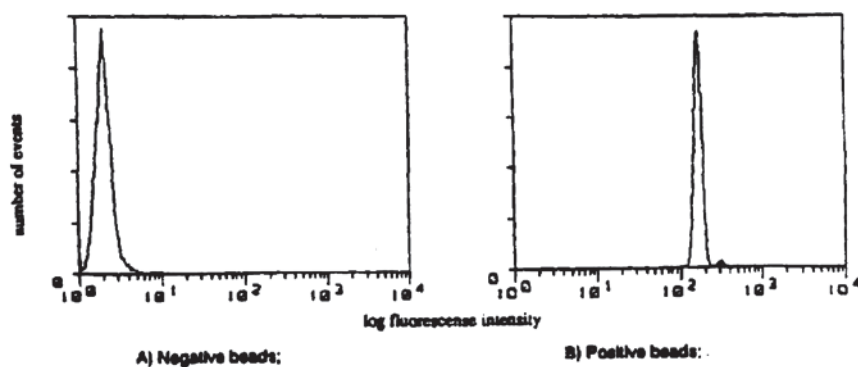
“For example a 10 micron diameter microsphere can be coated with an antigen A. When this coated microsphere is exposed to a fluid which contains antibodies to antigen A, these antibodies will specifically bind to the coated microsphere. Next a reagent which contains a fluorochrome labelled antibody which will specifically

bind to antibody A is added. Thus an antibody “sandwich” arises when antibody A is present in the sample such that the fluorochrome is now bound to the microsphere via the specific antibody antigen pairing. This is an example of an “antibody capture” FIA. Alternatively the microsphere can be coated with certain antibodies and thus selectively capture antigens which might be present in the sample in an “antigen capture” immunoassay. In this manner the effective fluorescence of the 10 micron diameter microsphere is determined by how many specific antigens or antibodies are captured, i.e., are present in the sample.”

EX1022, 2:54–3:4.

99. Lehnert further discloses that the microspheres are to be examined in a flow cytometer. EX1022, 2:54–3:4. Lehnert discloses using a histogram to analyze the fluorescence information collected, and the histogram is a plot of the number of fluorescent microspheres having a certain level of fluorescence intensity on vertical axis against the level of fluorescence intensity on the horizontal axis:

FIGURE THREE Fluorescence histogram of 4 micron microspheres stained with FITC anti-human IgG.



EX1022, 11:26–40, Fig. 3.

100. Lehnert discloses a multiplexed FIA method based on known reagent proportions, “wherein each discrete ligand complex comprises a known, unique proportion of the composition and the sum of any two or more unique proportions also is unique.” EX1022, claim 1. This approach is illustrated in the following example:

If a 3 part reagent were prepared in which the subpopulation proportions were 1:2:3 (16.67% : 33.33 % : 50%), and then was reacted with an unknown sample which was positive for the first two of the three target analytes as listed an indeterminate result would be possible. If the mcf values of the positive peaks were by coincidence the same or nearly so, i.e., overlapping peaks, the histogram would show two peaks of equal size each comprising 50% of the total number of events analyzed. One of these peaks would be in the left hand region, or negative domain, of the histogram, and one would be in the right hand positive region. From this resulting pattern, it would not be possible to discern whether the sample was positive for both the first and second analytes as listed or positive for the third analyte only. However, if the reagent had been designed with proportions 1:2:4 (14.28%, 28.57%, 57.14%), the resulting histogram would be uniquely interpretable. A sample positive for the first two

analytes as listed wherein their peaks overlapped would show a right hand or positive peak of area 42.85% and a left hand or negative peak of 57.14%, thus correctly identifying the respective analytes unambiguously since 42.85% could only be the sum of 14.28% and 28.57%.

EX1022, 13:24–34.

101. Lehn then discloses a general method of devising the reagent proportions:

The algorithm $n: 2n: 4n: 8n \dots : 2^{(m-1)}n$ can be utilized to determine the proportions of unequivocal analytical utility to be used in the preparation of a reagent containing m subpopulations of coated microspheres of similar diameter, and wherein n is any number.

EX1022, 13:41–43.

102. The technologies underlying Lehn—antibody and antigen detection, immunoassays, and flow cytometry—are outside the skillset of a POSA, who, as described by Petitioner, is familiar with detecting and measuring polynucleotide analytes. Petition, 11–12. As a POSA would readily appreciate, Lehn’s fluorescent immunoassays are fundamentally different from the PCR-based assays of the ’797 Patent, Jouvenot, and Larson. PCR assays detect polynucleotides, whereas immunoassays detect antibodies or antigens, typically proteins. Polynucleic acids are a distinct class of biomolecules from antibodies or antigens.

Designing and running PCR assays requires expertise in polynucleotide hybridization, sample preparation, amplification, and data interpretation, while immunoassays require different skills involving antibody preparation, antigen recognition, and assay execution. PCR specificity arises from Watson–Crick base pairing and includes target amplification, yielding high sensitivity; immunoassays rely on antibody–antigen binding and do not amplify targets, making them generally less sensitive. Accordingly, a POSA would have understood that PCR assays and fluorescent immunoassays operate in fundamentally different ways and require different training and skillsets.

103. As such, Lehen is not relevant to PCR, let alone to the '797 Patent, to ddPCR, or to any other digital assay format. While Lehen discloses the microspheres are read one by one in a flow cytometer, that is where this at most superficial similarity to ddPCR assays ends. While Lehen purports to demonstrate the use of microbeads having different fluorescence intensities as labels for analytes that can be detected in the same color channel of a flow cytometer, that fluorescence is not generated by a reaction; instead, it is present a priori on the labelling bead, and functions equivalent to a bead having the same fluorescence but a bigger diameter instead. This is a very different mechanism from fluorescence generated in direct response to the presence or absence of an analyte as described

in the '797 Patent, or Larson and Jouvenot for that matter, and it would not be at all obvious to a POSA how or why to extend Larson or Jouvenot from Lehnén.

VI. CLAIM CONSTRUCTION

104. For purposes of this declaration, I have reviewed the challenged claims of the '797 Patent in view of their common and ordinary usage and in view of the specification of the '797 Patent.

105. 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 in EX2027 and EX2028.

106. 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 in the '797 Patent specification, based on my own understanding as at least a POSA. However, my ultimate opinion is that the references cited in the Petition do not teach the challenged claims of the '797 Patent would remain the same even under the Petitioner's proposed constructions.

VII. GROUNDS OF CHALLENGE

107. Petitioner points primarily to Jouvenot as rendering obvious the '797 Patent claims, either alone or in view of Larson, Lehnén, and/or others. However, for the reasons set forth below, it is my opinion that none of these references, whether alone or in combination, teach the challenged claims of the '797 Patent.

A. Ground 1 (Claims 1, 2, 5, 6, 10, and 18): Obviousness in View of Jouvenot

108. In Ground 1, Jouvenot’s Example 1 is the basis for Petitioner’s obviousness arguments. *See, e.g.*, Petition, 28 *et seq.* Jouvenot’s Example 1 describes detecting two analytes using a single fluorophore by a single-channel detector at one wavelength. In Jouvenot’s Example 1, there are only four possible outcomes with two analytes. *See supra*, ¶78.

109. The ’797 Patent’s claim 1 is directed to a system comprising “multiple fluorophores” and “a multi-channel detector” that detects signals in four, five, or six colors. EX1001, 77:38, 77:39, 77:53. With a minimum value of $F=3$ and $C=4$, at least 8 analytes can be detected, and from which there are at least 2^8 (256) possible outcomes. *See infra*, ¶111. The system of claim 1 can detect more than 8 analytes, and the number of possible outcomes grows exponentially with the number of analytes. While Jouvenot acknowledges an exponential increase in possible outcomes as the number of analytes increases, Jouvenot merely “assum[es] that each population is distinguishable” but does not actually teach or suggest how to unambiguously distinguish eight or more analytes from one another. EX1003, 10:23–26. Jouvenot does not teach or suggest each and every limitation in the challenged claims of the ’797 Patent, and indeed there is a huge conceptual gap between Jouvenot and the invention recited in the ’797 Patent claims.

1. **Claim 1: “apply a decoding matrix to the cumulative signal to unambiguously detect the presence or absence of at least each of M analytes”**

110. Petitioner’s arguments have confused decoding matrix with encoding matrix. Claim 1 requires “applying a decoding matrix to the cumulative signal to unambiguously detect the presence or absence of at least each of M analytes.”

However, Petitioner instead points to *encoding* matrices. *See* Petition, 40–41.

111. Illustratively, Petitioner refers to Table 8 as “the only exemplary decoding matrix for Claim 1 in the ’797 Patent.” Petition, 40. However, Table 8 is as an “Example of encoding method using one color and one intensity per analyte, but different intensities among analytes.” EX1001, Table 8. Encoding matrices and decoding matrices are different. *See* §II.B above. When the number of analytes increases, the size of an encoding matrix increases linearly as a function of the number of analytes, but the size of the corresponding decoding matrix grows exponentially as a function of the number of analytes. *See* §II.B above. For example, Table 8 in the ’797 Patent is an encoding matrix with 16 entries corresponding to 16 analytes (A–P). The corresponding decoding matrix has not 16 entries, but 2^{16} (65,536) entries, where each entry is used for decoding signal intensities for one particular combination of the 16 analytes. *See* §II.B above. Petitioner does not explain why it would have been obvious to expand the two-analyte system in Jouvenot’s Example 1 (having only four possible outcomes)

to using a decoding matrix as recited in claim 1, which is exponentially more complex as illustrated in the following examples:

- **F=3 and C=4:** an 8-analyte 4-color system, with a decoding matrix containing 256 entries (2^8), or
- **F=15 and C=4:** a 16-analyte, 4-colors system, with a decoding matrix containing 65,536 entries (2^{16}), or
- **F=30 and C=6:** a 30-analyte, 6-color system, with a decoding matrix containing 1,073,741,824 entries (2^{30}). *See* §II.B above.

112. Petitioner’s confusion between encoding matrices and decoding matrices is repeated later on page 41. Petitioner points to an encoding matrix and calls it a “decoding matrix,” namely “the same type of decoding matrix as Table 8.” Petition, 41. The table on Petition, 41 is not a decoding matrix: for 12 analytes (“a” to “l”), the corresponding decoding matrix contains 2^{12} (or 4,096) entries.

113. Jouvenot does not teach or suggest any decoding matrix that, when applied to a cumulative signal, allows the “unambiguous detection of the presence or absence of each analyte.” Jouvenot also does not recognize the degeneracy problem, as reflected in the following table in Jouvenot:

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

EX1003, 10:46–55. Here, Jouvenot purports to tabulate eight (2^3) possible outcomes for a hypothetical three-analyte system. EX1003, 10:23–29, 46–55. In this table, only the all-positive (“Population 8”) and the all-negative (“Population 1”) cases can be unambiguously identified as having the “Highest” or the “Lowest” intensity. EX1003, 10:48, 54. The remaining six cases (“Population 2” to “Population 7”) all have “Intermediate” intensities and Jouvenot does not teach how they can be unambiguously distinguished. EX1003, 10:49–53. This table cannot work as a decoding matrix because it cannot be used to “unambiguously detect the presence or absence of at least each of M analytes,” as recited in claim 1 of the ’797 Patent. EX1001, 77:61–62.

114. Jouvenot further contains teachings that would have discouraged a POSA from expanding Example 1 to accommodate more analytes. Jouvenot expresses doubt as to whether the two-analyte system can work reliably. *See* §II.D.1; EX1003, 15:1–7. Specifically, Jouvenot suggests that under some conditions, fluorescent intensities (*i.e.*, “peak heights”) may be ambiguous and

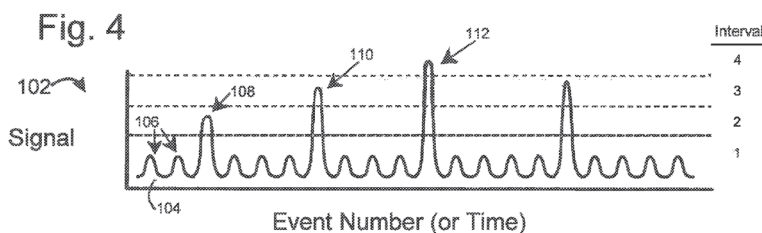
does not point to a definite conclusion as to which analytes are present. A POSA would have expected far worse ambiguities if he were to “increase the plex of Jouvenot Example 1” as Petitioner suggests. §II.D.1; EX1003, 15:1–7. Jouvenot does not explain how to disambiguate the outcomes in any system with three, four, or more analytes—let alone eight or more, as in claim 1 of the ’797 Patent.

2. **Claim 1: “ $M=C \cdot \log_2 (F+1)$. . . wherein $F+1$ is a positive integer and wherein $F+1$ is a power of 2”**

115. Jouvenot is silent on this equation and mathematical conditions.

Petitioner does not contend that Jouvenot contains this equation. *See generally* Petition at 47. Petitioner’s argument is that a hypothetical system (“[t]he obvious four channel version of Jouvenot Example 1 and Larson Fig. 11a”), which Jouvenot does not teach, would have parameters that satisfy the equation. Petition, 47. Petitioner argues that this hypothetical system would have $C=4$, $F=3$, and $M=8$, which would satisfy the equation since $4 \times \log_2 (3 + 1) = 8$. Petition, 47.

116. Jouvenot, however, does not teach or suggest these values. Petitioner picked these particular values to fit the equation in the ’797 Patent. Specifically, Petitioner picked $F=3$ from following Fig. 4 in Jouvenot:



Petition, 42; EX1003, Fig. 4. Petitioner's assertion that $F=3$ comes from its interpretation of the height of peak 112 as "F." Petition, 42–44, 47. However, assigning a peak height of "3" to peak 112 contradicts Jouvenot's express disclosure that "Peaks 112 with maxima in Interval 4 correspond to droplets containing both Targets 1 and 2[.]" EX1003, 14:36–56. The Petition does not explain why a POSA would have considered F to be equal to 3 instead of the value 4 which Jouvenot expressly discloses for peak 112. Petition, 47; EX1003, 14:51.

117. Jouvenot further does not teach or suggest "F" as a concept. In Jouvenot's Example 1, there are only four (2^2) possible outcomes when detecting the presence or absence of two analytes, and the peaks for Target 1, Target 2, and Target 1 + Target 2 can be distinguished for as long as the signals for Target 1 and Target 2 have different intensities. *Supra*, §V.D; EX1003, Fig. 4, 14:36–56. Due to its simplicity, there is no need to "apply a decoding matrix to the cumulative signal" to Jouvenot's two-analyte system in the manner recited in claim 1 of the '797 Patent.

118. Because Jouvenot does not teach or suggest F , it also does not teach or suggest the mathematical constraints on F . EX1001, 22:40–45, 77:60. Moreover, Jouvenot does not disclose any requirement that $F+1$ be an integer and also be power of 2. Petitioner does not appear to contend that Jouvenot contains this requirement.

119. Even assuming, purely for the sake of argument, that the height of Jouvenot's peak 112 corresponds to "F" as Petitioner suggests (it does not), Jouvenot still does not meet the limitation that $F+1$ be an integer and also be power of 2. Jouvenot states that peak 112 has a "maxima in Interval 4," EX1003, 14:51. If $F=4$, then $F+1=5$ which does not satisfy the mathematical requirements of claim 1 that $F+1$ be a power of 2. Petition, 47. The Petition does not explain why a POSA would have considered it obvious that peak 112 satisfy the claimed requirement that $F+1$ be a power of 2 in view of Jouvenot's express disclosure of the value 4 for peak 112.

3. **Claim 1: "C=4, 5, or 6"**

120. Jouvenot does not disclose the claim limitation that "C=4, 5, or 6." Even though Petitioner argues that the limitation is satisfied by "[t]he obvious four channel version of Jouvenot Example 1," Petition, 47, Jouvenot does not disclose a "four channel version." Jouvenot's Example 1 has a single channel ($C=1$), and Petitioner fails to adequately explain why Jouvenot's disclosure of $C=1$ renders obvious the limitation "C=4, 5, or 6," especially when there are significant differences between $C=1$ and $C=4, 5, \text{ or } 6$.

121. For example, when $C=4$ and $F=3$, the claimed system can detect at least 8 analytes. This can be derived from the equation $M=C*\log_2(F+1)$. Substituting $C=4$, the claimed equation becomes $M=4*\log_2(F+1)$. Because claim 1

requires that “F+1 is a positive integer,” “F+1 is a power of 2,” and M is greater than C, the minimum value for F is 3. Therefore the minimum number of analytes (M) is $4 \cdot \log_2(3+1) = 8$. Similarly, when C=5, the minimum number of analytes (M) is $5 \cdot \log_2(3+1) = 10$; and when C=6, the minimum number of analytes (M) is $6 \cdot \log_2(3+1) = 12$. In contrast, Jouvenot’s Example 1 is a single-channel system that detects two analytes. EX1003, 14:29–31; EX1001, 78:45–48.

122. The differences between the claimed system (using the recited values of C=4, 5, or 6) and Jouvenot’s Example 1 (C=1) is much more significant than the apparent numerical difference between detecting at least 8–12 analytes on the one hand, and detecting only 2 analytes on the other. This is because the number of possible outcomes, and the size of the corresponding decoding matrix increases exponentially as a function of the number of analytes. *Supra*, §VII.A.1; EX1003, 10:23–26.

123. In my opinion, the ’797 Patent addresses a far more challenging problem than Jouvenot’s two-analyte system. In light of Jouvenot’s teachings, a POSA would not be motivated to “simply duplicat[e] the number of channels/fluorophores to at least four,” and the POSA would not have a reasonable expectation of success in doing so. In Jouvenot’s Example 1, only four possible outcomes need to be distinguished. EX1003, 14:63–64. And yet Jouvenot teaches that its method is not always reliable because signal peaks can lie “half way

between” or “overlap at their extremes.” EX1003, 15:1–7. A POSA would not expect the ambiguous cases to go away when “duplicating the number of channels/fluorophores to at least four” as Petitioner proposes, Petition, 45; instead the POSA would expect much *more* ambiguities in four channels, where 256 or more outcomes must be distinguished. The Petition does not explain why a POSA would reasonably have believed that there is no meaningful difference between distinguishing 4 outcomes and distinguishing 256 outcomes, particularly in view of the limitations in Jouvenot’s method that Jouvenot discloses. EX1003, 15:1–7. In my opinion, the claimed limitation “C=4, 5, or 6” is not obvious in view of Jouvenot.

4. **Petitioner’s Arguments Are Based on Hindsight**

124. I find the conclusion of obviousness in the Petition and in the declaration by Dr. Batt is strongly influenced by their hindsight bias. For example, the Petition’s hindsight bias is demonstrated by its misidentification of the success of the ’797 Patent as evidence that a POSA would have expected the same success. For example, the Petition argues that a POSA would have had a reasonable expectation of success because the ’797 Patent does not suggest “any impediments to implementing optical detection machinery for analysis in four colors.” Petition, 35–36. As another example, the Petition argues that claim 6’s use of multiple fluorophores is obvious because the ’797 Patent “treats them as well-known prior

art fluorophores that could be used without difficulty.” Petition, 54. The Petition’s logic appears to be that because the ’797 Patent does not describe difficulties, its achievement must be obvious.

125. The declaration of Dr. Batt also suffers from the same hindsight bias. For example, the ’797 Patent explains that its methods of the invention can be readily implemented using existing instruments having four color channels. EX1001 at 35:30–35. Dr. Batt, however, mistakes the fact that the invention can be readily used on existing instruments, *once given knowledge of the invention*, for obviousness at the time of invention. He states:

The ’797 patent, in fact, makes clear that four color instrumentation had previously existed in “many” incarnations. EX1001 at 35:30-35. In view of this, it would have been a no-brainer for a skilled artisan to simply take the scheme disclosed in Figure 4 of Jouvenot and, rather than use it in one color channel, duplicate it in four or more independent color channels to detect more targets. This would have been nothing more than the straightforward idea of, for instance, digging a hole faster and deeper by using multiple workers each with their own shovel rather than having a single worker do the task with just one shovel.

EX1002, ¶133. The statements here do not explain to me how a POSA would duplicate Figure 4 of Jouvenot in four or more independent color channels, how the modified Jouvenot would operate to detect more targets, or how the modified

Jouvenot would render claim limitations obvious. The hole-digging analogy is also not a helpful aid to my understanding of Dr. Batt’s conclusion of obviousness.

126. The logic in Dr. Batt’s testimony is circular at places. For example, in EX1002, ¶129, Dr. Batt’s argues that Jouvenot’s Example 1, when combined with the encoding scheme in Table 8 of the ’797 Patent, “reflect[s] the same scheme” as Table 8. In another example, Dr. Batt argues that the equation $M=C*\log_2(F+1)$ is obvious because it is “an intuitive mathematical formula” that reflects Table 8 of the ’797 Patent. EX1002, ¶¶142–143. In yet another example in paragraph 146, Dr. Batt argues that the claimed limitation “F is a positive integer” is obvious in view of the explanation in the specification of the 797 ’Patent.

127. The following table lists occurrences of hindsight bias and circular reasoning in the Petitioner and in Dr. Batt’s declaration.

Citations to the ’797 Patent in Petition or Dr. Batt’s Declaration	Summary of Cited Text in the ’797 Patent	Erroneous Argument
Ground 1: Claim 1		
Petition, 34–35, <i>citing</i> EX1001, 35:30–35, 34–35	The cited text explains that the methods of the invention can be readily applied using existing instruments having four channels.	Petitioner uses the ’797 Patent’s own disclosure as ostensible evidence of how a POSA would have been motivated to expand Jouvenot to four channels, and as evidence of a reasonable expectation of success

		because “[n]owhere does the ’797 patent suggest that . . . there were any impediments to implementing optical detection machinery for analysis in four colors.”
Batt Declaration, ¶113, citing EX1001, 35:30–35	<i>same as above</i>	Dr. Batt uses the ’797 Patent’s own disclosure as evidence of “pre-existing motivation” and a reasonable expectation of success for using four or more channels.
Batt Declaration, ¶133, citing EX1001, 35:30–35	<i>same as above</i>	Because the ’797 Patent discloses that four-channel instrumentation had existed, Dr. Batt argues that it would be a “no-brainer” to expand the single-channel example in Jouvenot to four channels. “This would have been nothing more than the straightforward idea of, for instance, digging a hole faster and deeper by using multiple workers.”
Petition, 40, citing EX1001, 22:9–29	The cited text describes an exemplary encoding matrix.	Petitioner uses the ’797 Patent’s own disclosure as ostensible evidence that Jouvenot somehow

		teaches the claimed decoding matrix.
Batt Declaration, ¶129, <i>citing</i> EX1001, 21:65–67	The cited text explains that encoding based on intensities 1, 2, 4, and 8 results in a code scheme without degeneracy.	In his circular reasoning, Dr. Batt applies the '797 Patent's teaching to expand Jouvenot, which supposedly shows how Jouvenot renders the '797 Patent obvious.
Batt Declaration, ¶143, <i>citing</i> EX1001, 22:10–30	The cited text describes an exemplary encoding matrix.	Dr. Batt argues that Jouvenot renders the claimed equation $M=C*\log_2(F+1)$ obvious because the equation is “intuitive” in view of the '797 Patent's own embodiment.
Batt Declaration, ¶146, <i>citing</i> EX1001, 2:1–5	The cited text explains the equation $M=C*\log_2(F+1)$.	Dr. Batt argues that the claimed limitation “F is a positive integer” is obvious because of the explanation in the specification of the '797 Patent itself.
Ground 1(a): Claim 6		
Petition, 54 <i>citing</i> EX1001, 34:57–61	The cited text explains that fluorophores not specifically named in the specification can also be used in the invention.	Petitioner argues that the '797 Patent's use of the fluorophores is not novel or inventive because the '797 Patent “treats them as well-known prior art fluorophores that could be used without difficulty.”

Ground 1(a): Claim 7		
Petition, 56, citing EX1001, 45:19– 35, 33:67, 34:8, 45:29– 30, 48:50, 49:10, 49:14, 49:33.	The cited text describes fluorophores used in exemplary embodiments (including Examples 1 and 2).	Petitioner argues that the '797 Patent's use of the fluorophores is not novel or inventive because they are well-known in the prior art, and the '797 Patent does not describe any difficulty in using them.
Ground 1(b): Claim 11		
Petition, 60 <i>citing</i> EX1001, 28:11– 13, 35:30–33.	The cited text explains that the measurements of wavelength and intensity in the invention can be accomplished using known components (band pass filters).	Petitioner uses the '797 Patent's own disclosure as evidence that a POSA would have been motivated to use band pass filters with a reasonable expectation of success, because the '797 Patent supposedly “acknowledges” that band pass filters are commonly employed.
Ground 1(c): Claim 1 ☐		
Petition, 62, <i>citing</i> EX1001 at 35:59– 62	The cited text explains that the measurements of wavelength and intensity in the invention can be accomplished using known components (photodetector and band pass filters).	Petitioner uses the '797 Patent's own disclosure as evidence of “a reasonable expectation of success because these are standard optical components well-known in the art[.]”
Batt Declaration, ¶146,	<i>same as above</i>	Dr. Batt uses Patent's own disclosure as

<i>citing</i> EX1001 at 35:59–62		ostensible evidence of “a reasonable expectation of success.
Ground 1(d): Claim 1 ☐		
Petition, 64–65, <i>citing</i> EX1001, 35:26–33, 35:34–35	The cited text explains how the methods of the invention can be readily applied using existing instruments.	Petitioner uses the ’797 Patent’s own disclosure as ostensible evidence of “motivation and reasonable expectation of success.”
Batt Declaration, ¶203, <i>citing</i> EX1001 at 35:26–33, 35:34–35	<i>same as above</i>	Dr. Batt uses Patent’s own disclosure as ostensible evidence of motivation and a reasonable expectation of success.
Ground 1(e): Claim 15		
Petition, 65, <i>citing</i> EX1001, 45:19–35	The cited text describes an exemplary embodiment (Example 1).	Petitioner uses the ’797 Patent’s own disclosure as ostensible evidence that “it would have been obvious to use fluorophores having maximum emission wavelengths selected from the group consisting of about 518 nm, about 565 nm, about 591 nm, and about 670 nm[.]”

128. For the above reasons, it is my conclusion that the challenged claims of the ’797 Patent are not obvious on Ground 1.

**B. Grounds 1 and 1(a)–1(f) (Claims 1, 2, 5–11, 13–15, and 18):
Obviousness in View of Jouvenot, Larson, and Other References**

129. In Grounds 1(a)–1(f), Petitioner argues that Jouvenot in various combinations with Larson and other references renders claims 6–9, 11, 13–15, and 18 obvious. Petition, 25–26. Petitioner’s Ground 1 also includes proposed combination of Jouvenot and Larson. Petition, 31, 33–36, 38, 39, 44–47.

130. For the following reasons, however, I find the proposed combination of Jouvenot and Larson to be untenable.

1. Larson Attempts to Eliminate the Need to Detect Multiple Analytes in the Same Sample

131. Jouvenot’s method is purportedly motivated by a desire to multiplex a digital PCR assay to “permit detection of two or more different targets in each partition.” EX1003, 2:12–13. Because “instruments with more detection channels, to detect more colors, are more expensive than those with fewer detection channels,” Jouvenot disclosed “a new approach . . . to increase the multiplex levels of digital assays,” *id.*, 2:16–22.

132. Although Larson discusses “multiplexing,” *e.g.*, EX1013, ¶45, its actual method is incompatible with Jouvenot. A POSA reading Larson as a whole would have understood that Larson’s “multiplexing” refers to a totally different strategy. First, Larson increases sample dilution as the number of analytes increases, so as to reduce the probability that any droplet contains even two

analytes (and consequently the probability for any droplet containing more than two analytes is even less). EX1013, ¶129. Second, Larson detects, sorts away, and excludes droplets containing a heterogeneous population of analyte molecules. EX1013, ¶172; *supra* §V.E.

133. Therefore, Larson would have motivated a POSA to dilute the sample in order to reduce the probability of having two or more analytes in the same sample partition, and/or to exclude partitions that do contain two or more analytes. Larson would not have motivated a POSA to do the opposite: try to increase the probability of having multiple analytes in the same partition. Therefore, I disagree with Petitioner’s argument that Larson would have motivated a POSA to “expand Jouvenot Example 1 to use four or more different color channels and fluorophores.” *E.g.*, Petition, 33–36. The *raison d’être* for expanding to four or more color channels is to detect four or more analytes, but without any partition actually having at least four analytes, such expansion would serve no purpose.

2. **Larson Teaches Away from Claim 1 of the ’797 Patent**

134. Claim 1 of the ’797 Patent contains the equation $M = C * \log_2 (F + 1)$. In Petitioner’s analysis, this equation is “a restatement of the use of signal intensities for analytes that follow the geometric progression 1, 2, 4, 8, 16, and so on[.]” Petition, 10. Petitioner states that the ’797 Patent makes use of this geometric progression’s arithmetic properties:

For example, for the color blue in Table 8, intensities of 1, 2, 4, or 8 mean that analytes A, B, C, or D are present alone. *Id.* But an intensity of 3 means that A and B are present ($1+2=3$), an intensity of 7 means that A, B, and C are present ($1+2+4=7$), and an intensity of 15 means that all analytes are present ($1+2+4+8=15$). *Id.* This progressive pattern prevents overlap of intensities—each value is “equal to the sum of all previous values plus one.”

Petition, 9.

135. Petitioner’s analysis above is based on an unstated, but critical assumption that fluorescent intensities are additive. For example, in order for the encoding scheme to work as Petitioner describes, two analytes that respectively have a fluorescent intensity of 1 and 2 must have a cumulative intensity of 3 (*i.e.*, $1 + 2$) when both analytes are present. In Petitioner’s logic, if an observed cumulative intensity of 3 is not necessarily the result of $1 + 2$, then it does not lead to the positive identification of A and B. In other words, it would be fatal to the whole encoding scheme (as Petitioner has outlined above) if any cumulative intensity is not a sum of the fluorescent intensities of the individual analytes.

136. Yet Larson (as well as Jouvenot—*see* ¶80 above) questions whether fluorescence intensities are additive. Larson discloses that one way of “performing higher-plex assays with a single probe color (*i.e.* fluorophore)” is to have “each intensity level uniquely identifies a DNA target.” EX1013, ¶138. “For example, targets T1, T2, T3, and T4 might be uniquely identified by intensity levels I1, I2, I3,

and I4.” *Id.* But Larson then says there is “complexity” in this method when determining what observed cumulative intensity represents, because fluorescent intensities are not necessarily additive:

With multiple occupancy arises the . . . complexity of assigning the resulting fluorescence intensity that involves a combination of fluorescence from two different reaction products that *may or may not be equal to the sum of the two fluorescence intensities* of the individual reaction products.

EX1013, ¶141.

137. In my view, this teaching in Larson would have motivated a POSA to avoid any encoding scheme based on fluorescent intensities because it would be very difficult, if not impossible, to interpret any cumulative intensity reading when there is no guarantee that the cumulative intensity is a sum of individual fluorescence intensities. And without encoding in fluorescent intensities (let alone in powers of two), a POSA would not have been able to arrive at the equation $M = C * \log_2 (F + 1)$.

138. For the above reasons, it is my conclusion that the challenged claims of the ’797 Patent are not obvious on any ground involving Larson, including Grounds 1 and 1(a)–1(f).

**C. Grounds 2 and 2(a)–2(f) (Claims 1, 2, 5–11, 13–15, and 18):
Obviousness in View of Jouvenot, Lehnert, and Other References**

139. Petitioner evidently thinks that its Grounds 1 and 1(a)–1(f) are probably insufficient because Jouvenot and Larson may be “deemed to not disclose encoding analytes by the geometric progression of 1, 2, 4, 8, etc. because the signals in Jouvenot Example 1 and Larson Figure 11 do not exactly match this progression.” Petition, 69. Therefore, Petitioner repeats the same grounds as Grounds 2 and 2(a)–2(f), and “[t]he sole change is the addition of Lehnert.” *Id.*, 69.

140. Because Grounds 2 and 2(a)–2(f) “are based on the same logic and rationale as grounds 1–1(f),” Petition, 68, they have the same defects as explained above in §§V.A.–B. In addition, Lehnert cannot remedy the defects in Petitioner’s other grounds because it is about an entirely different technology that is not relevant to the system recited in the ’797 Patent claims.

1. Lehnert is not in the same technical field as the ’797 Patent

141. I do not agree with Petitioner’s broad characterization that all the secondary references cited in its Petition are analogous art “because they [belong] to the same field of endeavor as the ’797 patent, specifically, the detection of target analytes (including nucleic acids) in a sample.” Petition, 22. “[T]he detection of target analytes” is such a broad overgeneralization that it includes a large range of

diverse and distinct scientific endeavors in chemistry, medicine, and geology all the way from several centuries ago to modern times.

142. Judging from the explanations in the '797 Patent itself, I think the technical field of the '797 Patent should be fairly characterized as the detection of polynucleotides, to which all disclosed examples are all directed. EX1001, 40:31 (Example 1 describing preparation of analytes: “[f]ive nucleic acids from organisms of clinical relevance”), 45:36–37 (Example 2 entitled “Simultaneous Detection of Polynucleotide Markers”), 50:1–2 (Example 3 similarly entitled). The claims of the '797 Patent are also directed to the detection of polynucleotides because all independent claims recite “hybridization probes” (which detects polynucleotides), “PCR” (which amplifies polynucleotide), and/or “polynucleotide analytes.” *See*, EX1001, 77:38 (independent claim 1), 79:57–61 (independent claim 19), 80:44–46 (independent claim 22).

143. Lehnert is in the field of detecting antibodies and antigens. Lehnert describes detecting “antibodies to recombinant HIV gp4, recombinant HIV p24, Hepatitis B core protein, and recombinant HTLV re-5.” *See* EX1022, 2:8–10 (“Technical Field”). Lehnert discloses 12 examples, and none of them is about detecting polynucleotides. The claims in Lehnert also do not recite anything like DNA, RNA, or polynucleotides, but claims 4, 6, 7, and 10 are specifically directed to detecting antibodies and antigens.

144. A POSA would have known, as a matter of basic scientific knowledge, that detecting polynucleotides and detecting antibody/antigen are fundamentally different. For example, detecting antigens with antibody-based reagents does not involve target amplification, which is the key step in PCR. Out of common sense understanding of science, a POSA would not have regarded the '797 Patent and Lehnert to be in the same field.

2. **Lehnert is not reasonably pertinent to the problem the Caltech inventors of the '797 Patent faced**

145. Lehnert is not reasonably pertinent to the problem the Caltech inventors of the '797 Patent faced because a POSA would not reasonably have consulted Lehnert in seeking a solution to the problem.

146. The starting point for Lehnert is fluorescent immunoassays using immobilized reagents. From there, Lehnert wanted to find ways to do immunoassays in a multiplexed manner without being limited by “particle size resolution.” EX1022, 2:53–3:16. Lehnert’s method was to use different types of immobilized reagents in known ratios. *Id.*, 5:14–21. For example, a two-analyte system can contain two types of microspheres—each type carrying immobilized reagents for detecting one analyte—present at a 1:2 ratio, where the ratio is in the number of the microspheres of each type; a three-analyte system can contain three types of microspheres present at a 1:2:4 ratio. EX1022, 13:37–38. After reacting

with microspheres with a sample, a flow cytometer is used to measure the microspheres one at a time, *see* EX1022, Fig. 4 (showing apparatus for detecting “fluorescence characteristics of individual particles moving in flow path from the sample source”).

147. For the ’797 Patent, the Caltech inventors wanted to find ways to detect polynucleotides with “the ability to extract rich information from a sample in a fast and efficient manner,” but without complex mechanisms involving spectrally resolved fluorescence or chemiluminescence, spatially resolved signals, or temporally resolved signals. EX1001, 1:37–49. The Caltech inventors’ efforts ultimately resulted in the claimed systems, in which “the determination is made without immobilization, mass spectrometry or melting curve analysis.” *Id.*, 78:36–37.

148. Lehnert’s microsphere-based approach is a technique to resolve signals spatially and temporally because each microsphere is a spatially discrete unit of reaction and each microsphere is examined sequentially at different times. Therefore, a POSA seeking to improve PCR multiplexing would not have consulted Lehnert because its microsphere-based approach did not fit the POSA’s problem and the problem’s requirements (*e.g.*, without “complex reporting mechanisms” like “spatially resolved signals” or “temporally resolved signals,” EX1001, 1:43–46). Furthermore, Lehnert’s method involves the sequential

examination of hundreds of thousands microspheres (*e.g.*, EX1022, 16:34), and the Caltech inventors would have regarded this method as slow and inefficient (not in “a fast and efficient manner,” EX1001, 1:41). Therefore, it is my opinion that Lehnert is not reasonably pertinent to the problem the Caltech inventors of the ’797 Patent faced.

3. **Lehnert Does Not Teach Any Encoding in the Cumulative Intensity of Signals Because There Is No Cumulative Intensity in Lehnert**

149. The ’797 Patent teaches encoding and decoding information in the cumulative intensity of signals. Because the cumulative intensity is a sum of the individual fluorescence intensities of all the different analytes present, *see* EX1001, 78:41–44, it is possible to deduce information about the different analytes even though their fluorescence signals are on the same wavelength. *See above* §§V.B.

150. Lehnert does not make use of any cumulative intensity because fluorescence signals from different analytes are examined separately without any accumulation or summation. Lehnert uses microspheres on which different reagents are immobilized. For example, one set of microspheres maybe coated with a reagent to detect human antibody IgG, another set of microspheres maybe coated with another reagent to detect HIV. EX1022, 12:44–46. The microspheres are then used to react with a sample, and the analytes present in the sample will bind to the reagents on the microspheres. After reaction, a flow cytometer is used to examine

every microsphere, which emits a fluorescence signal when a positive binding between the reagent on the microsphere and the analyte the reagent is designed to detect. The important fact to note is that a flow cytometer measures the microspheres one at a time, *see* EX1022, Fig. 4 (showing apparatus for detecting “fluorescence characteristics of *individual* particles moving in flow path from the sample source”). Because of the individual and sequential measurement of each microsphere in the flow cytometer, fluorescence signals from different analytes are never combined to form a signal of cumulative intensity. In other words, there is no cumulative intensity in Lehn, and it therefore does not teach or suggest encoding in cumulative intensity.

151. I emphasize here that the individual examination of the microspheres in Lehn is not due to any limitation of the instrument. Lehn’s method must do so in order to obtain a count of the number of microspheres at different levels of fluorescence. As I will explain below, Lehn extracts information about analytes from microsphere populations (*i.e.*, the numbers of microspheres having different levels of fluorescence).

4. **Lehn Discloses Encoding in Microsphere Populations, Which Cannot Be Applied to Jouvenot**

152. Lehn’s method works in a way that is radically different from Jouvenot, Larson, or the ’797 Patent. Lehn applies encoding in the populations

of the microspheres that detect different analytes. For example, a two-analyte system can contain two types of microspheres present at a 1:2 ratio, where the ratio is in the number of the microspheres of each type; a three-analyte system can contain three types of microspheres present at a 1:2:4 ratio. EX1022, 13:37–38. In general, Lehnert discloses that “the algorithm $n: 2n: 4n: 8n \dots : 2^{(m)}n$ can be utilized to determine the proportions.” *Id.*, 13:41. Lehnert means that there should be n microspheres with immobilized reagent for detecting a first analyte, and $2n$ microspheres with a different immobilized reagent for detecting a second analyte, etc. Any POSA would have been able to understand that this ratio of “ $n: 2n: 4n: 8n \dots$ ” is not about the intensity of fluorescent signals. They are about the number of microspheres of each different type.

153. Lehnert’s method is not compatible with Jouvenot. First, there are not any microspheres, particles, or solid supports, or any discrete units of solids that can be counted in Jouvenot. Second, it would be impractical and unrealistic to apply Lehnert’s method in the context of digital PCR, in which at least tens of thousands of partitions (droplets) must be examined. If the analysis of each partition would require reacting it with 200,000 microspheres (*e.g.*, EX1022, 16:34) and then examining each microsphere one-by-one, the whole digital PCR process would be very time consuming and wasteful of materials because the same method

involving 200,000 microspheres must be repeated for each one of the thousands of partitions. A POSA would have viewed this hypothetical method as irrational.

5. **Lehnen Teaches the Opposite of What Claim 1 Expressly Requires**

154. Claim 1 of the '797 Patent requires that “the determination is made without immobilization[.]” EX1001, 78:36–37. Lehnen’s method, however, requires combining a sample with a “mixture containing the known proportions of multiple subpopulations of immobilized complementary binding moieties.” EX1022, 7:42–43. By “immobilized,” Lehnen means that the complementary binding moieties are attached to a solid support, such as particles of microspheres. EX1022, 6:49–50, 7:15–16.

155. Lehnen discusses microspheres, solid supports, and particulate supports throughout the reference. *E.g.*, EX1022, 2:43 (“Relevant Literature”), 3:24 (“Summary of the Invention”), 3:51 (“Brief Description of the Drawings”), 4:13 (“Brief Description of the Specific Embodiments”), 10:33 (“Examples”), 23:51 (claim 1). These terms all refer to solid support materials on which reagent molecules can be attached (*i.e.*, immobilized). Immobilization is an integral part of Lehnen’s method, which relies on counting the number of microspheres by a flow cytometer. Without the microspheres, there would not be any discrete units to count, no ratio of different populations could be observed, and no information

could be decoded from observed population ratios. Lehnert's method would not work at all without immobilizing reagents on microspheres.

156. Therefore, even if Lehnert could be combined with Jouvenot and/or Larson (it cannot), the resulting combination would have required the use of immobilized reagents, and that is the opposite of what claim 1 of the '797 Patent requires.

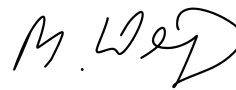
157. For the above reasons, it is my conclusion that the challenged claims of the '797 Patent are not obvious on Grounds 2 and 2(a)–2(f).

VIII. DECLARATION

158. 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 in Seattle, Washington.

Dated: December, 23 2025

By

A handwritten signature in black ink, appearing to read 'B. Weigl', written over a horizontal line.

Bernhard Weigl