PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: KYLE BRUNER
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing *(day/month/year)* 26 JUN 2019	
Applicant's or agent's file reference 44158-715601	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2019/027751	International filing date *(day/month/year)* 16 April 2019
Applicant CHROMACODE, INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **22 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver Telephone No. PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2017)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 44158-715601	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2019/027751	International filing date *(day/month/year)* 16 April 2019	(Earliest) Priority Date *(day/month/year)* 17 April 2018
Applicant CHROMACODE, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 3B

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/027751

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 21-33, 38-51, 55-64, 71-78, 82-110
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/027751

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/6851; C12Q 1/686; G01N 21/64; G01N 33/53 (2019.01)
CPC - C12Q 1/6851; C12Q 1/686; G01N 21/63; G01N 33/50 (2019.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/6.12; 435/91.1; 435/91.2 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2017/0145490 A1 (UNIVERSITY OF WASHINGTON THROUGH ITS CENTER FOR COMMERCIALIZATION) 25 May 2017 (25.05.2017) entire document	52, 53 --- 65-70, 79-81
X --- Y	US 2014/0213471 A1 (CALIFORNIA INSTITUTE OF TECHNOLOGY) 31 July 2014 (31.07.2014) entire document	1-6, 9, 10, 13-15, 18-20, 34-37 --- 7, 8, 11, 12, 16, 17
Y	US 2017/0314073 A1 (LIFECODEXX AG) 02 November 2017 (02.11.2017) entire document	7, 8, 79-81
Y	WO 2017/173035 A1 (CHROMACODE INC) 05 October 2017 (05.10.2017) entire document	11, 12
Y	US 2004/0259118 A1 (MACEVICZ) 23 December 2004 (23.12.2004) entire document	16, 17
Y	US 2012/0244534 A1 (CHING et al) 27 September 2012 (27.09.2012) entire document	65-70
Y	MORGAN et al., A commercial line probe assay for the rapid detection of rifampicin resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis, BioMed Central: BMC Infectious Diseases, 28 July 2005 [retrieved on 10 June 2019]. Retrieved from the Internet: <URL: https://bmcinfectdis.biomedcentral.com/track/pdf/10.1186/1471-2334-5-62>. entire document	67-69

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 June 2019	26 JUN 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	RAJAGOPAL et al., Significant Expansion of Real-Time PCR Multiplexing with Traditional Chemistries using Amplitude Modulation, Nature: Scientific Reports, 31 January 2019 [retrieved on 06 June 2019]. Retrieved from the Internet: <URL: https://www.nature.com/articles/s41598-018-37732-y.pdf>. Pgs. 1-8	1-20, 34-37, 52-54, 65-70, 79-81

Form PCT/ISA/210 (continuation of second sheet) (January 2015)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: KYLE BRUNER
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	26 JUN 2019
Applicant's or agent's file reference 44158-715601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2019/027751	16 April 2019	17 April 2018

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/6851; C12Q 1/686; G01N 21/64; G01N 33/53 (2019.01)
CPC - C12Q 1/6851; C12Q 1/686; G01N 21/63; G01N 33/50 (2019.05)

Applicant CHROMACODE, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	10 June 2019	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/027751

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 21-33, 38-51, 55-64, 71-78, 82-110

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. see claims below are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 21-33, 38-51, 55-64, 71-78, and 82-110 are multiple dependent claims not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 21-33, 38-51, 55-64, 71-78, 82-110

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-20, 34-37, 54, 65-70, 79-81	YES
	Claims	52, 53	NO
Inventive step (IS)	Claims	54	YES
	Claims	1-20, 34-37, 52, 53, 65-70, 79-81	NO
Industrial applicability (IA)	Claims	1-20, 34-37, 52-54, 65-70, 79-81	YES
	Claims	None	NO

2. Citations and explanations:

Claims 52 and 53 lack novelty under PCT Article 33(2) as being anticipated by University Of Washington Through Its Center For Commercialization (hereinafter Washington).

Regarding Claim 52, Washington discloses a method of quantifying target nucleic acid molecules in a sample (Paras. [0065]-[0067], methods for using digital measurements to determine a concentration of a sample...a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)), the method comprising: (a) partitioning a plurality of nucleic acid molecules into a plurality of partitions (Paras. [0066]-[0067], droplets of different volumes (diameters) can be generated...a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)); (b) amplifying said plurality of nucleic acid molecules in said plurality of partitions (Paras. [0066]-[0067], amplification of analyte (e.g. digital PCR) can be carried out simultaneously in all droplets of different volumes (sizes)...a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)), thereby generating a plurality of signals (Paras. [0066]-[0067]); (c) detecting said plurality of signals (Para. [0066], presence of amplification product in each droplet is determined based on fluorescence and the size (volume) of each droplet is determined based on the scattering signal from the droplet); (d) based on said detecting, determining for each partition of said plurality of partitions a probability that one or more nucleic acid molecule(s) of said plurality of nucleic acid molecules is present in said partition (Para. [0066], presence of amplification product in each droplet is determined based on fluorescence and the size (volume) of each droplet is determined based on the scattering signal from the droplet; Para. [0075], sample can be distributed into droplets of variable volumes that can be analyzed. The individual volumes of the droplets (all or just some) can be determined by methods provided herein. In addition, using detection methods described herein, droplets can be analyzed for containing a detectable agent or not. For a given sample concentration, some of the variable volume droplets may contain a detectable agent and some may not. For higher sample concentrations, generally more droplets of a plurality may contain detectable agents and vice versa; for low sample concentrations, fewer droplets of a plurality may be occupied by a detectable agent. As further described herein, the probabilities of occupancy by a detectable agent in a particular volume distribution), thereby generating a plurality of probabilities (Para. [0075], probabilities of occupancy by a detectable agent in a particular volume distribution can be defined for a wide range of sample concentrations); and (e) quantifying said target nucleic acid molecules in said sample based on a function of said plurality of probabilities (Para. [0075], probabilities of occupancy by a detectable agent in a particular volume distribution can be defined for a wide range of sample concentrations, which can then be compared to real data to determine the concentration of an unknown sample; Para. [0067], a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)).

Regarding Claim 53, Washington discloses the method of claim 52, wherein said method does not include a step of determining a number of nucleic acid molecules in any individual member of said plurality of partitions (Para. [0100], This example describes a non-limiting theoretical framework that can be used to describe certain aspects of the present invention described herein. It will be understood that the method described in this example is one of many ways to determine concentrations of sample using the present invention. Under this theoretical framework, it is assumed that the target molecules are present in the sample at a concentration Cs in units of molecules/volume. The sample is distributed into digital volumes of variable volumes; the distribution of target molecules into the droplets follows Poisson statistics...the amplification reaction will cause a droplet containing one or more molecules to be distinguishable from empty droplets, e.g. by its fluorescence intensity. In this method we know only whether a droplet is empty or occupied...in order to determine the concentration CS of target molecules, P(n>0, CSVi) needs to be summed over a sufficiently large number of droplets with respective volumes Vi).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Claims 1-6, 9, 10, 13-15, 18-20, and 34-37 lack an inventive step under PCT Article 33(3) as being obvious over California Institute Of Technology (hereinafter California).

Regarding Claim 1, California discloses a method of quantifying (Para. [0036], methods described herein can provide for the direct detection of one or more analytes in a single reaction...allows for more accurate quantification of the analyte) a nucleic acid target in a sample (Para. [0083], analyte is referred to as a nucleic acid analyte), the method comprising: (a) providing a mixture (Para. [0003], combining the sample with a first primer and a first oligonucleotide) comprising: i. a plurality of nucleic acid molecules derived from, and/or corresponding with, said nucleic acid target; and ii. a plurality of oligonucleotide probes (Para. [0154], In some cases, compositions comprise a first and a second primer or probe for the detection of at least one analyte wherein the primers are attached to either a fluorophore or a quencher at the 5' end...In some cases the compositions comprise multiple pairs of first and second primers, wherein each pair of first and second primers comprise either a fluorophore or a quencher at the 5' end. In some cases each pair of first and second primers comprise a different fluorophore and quencher from the remaining set of primers. In some cases the compositions comprise at least 1, 2, 3, 4...or 10000 pairs of first and second primers; Para. [0048], Various combinations of oligonucleotides (e.g. primers and/or probes) can be used to detect an analyte. In some cases, a primer is used with a probe or a plurality of probes. In some cases, a plurality of primers is used with a plurality of probes...In some cases, a primer and a probe is used to detect multiple analytes...a combination of primers and probes is used to detect multiple analytes. In some cases, a probe is used to detect an analyte. In some cases, a probe is used to detect multiple analytes. In some cases, multiple probes are used to detect an analyte; Para. [0083], analyte is referred to as a nucleic acid target; the primers and probes are in a mixture), each of which corresponds to a different region of said nucleic acid target (Para. [0013], the first primer and the second primer are specific for the polynucleotide analyte; Para. [0045], primers to bind to specific regions of the individual template); (c) generating a plurality of signals (Para. [0011], each primer of the primer pair is attached to at least one chromophore; b) measuring a first set of signals generated by the chromophores), wherein said plurality of signals are detectable in one color channel (Para. [0049], In some cases, a single chromophore pair is used to detect an analyte; Para. [0176], Analytes of differing lengths (e.g., 40 bp, 60 bp, and 80 bp) are detected using a single fluorophore/quencher combination...By measuring fluorescence as a function of increasing temperature, multiple analytes in a single color channel are quantified), and wherein at least one signal of said plurality of signals corresponds with a unique combination of two or more of said plurality of nucleic acid molecules (Para. [0011], methods of detecting a plurality of analytes, comprising: a) combining the plurality of analytes with a plurality of primer pairs, wherein each primer pair is specific to a single analyte and each primer of the primer pair is attached to at least one chromophore; b) measuring a first set of signals generated by the chromophores...d) measuring a second set of signals generated by the chromophores; wherein the first and second set of signals are used to detect each analyte of the plurality of analytes...the presence of each analyte of the plurality of analytes is detected by comparing the set of signature profiles to a control set of signature profiles); (d) detecting from said plurality of signals in said color channel (Para. [0049], In some cases, a single chromophore pair is used to detect an analyte; Para. [0176], Analytes of differing lengths (e.g., 40 bp, 60 bp, and 80 bp) are detected using a single fluorophore/quencher combination...By measuring fluorescence as a function of increasing temperature, multiple analytes in a single color channel are quantified); and (e) based on said detecting, quantifying said nucleic acid target in said sample (Para. [0011], measuring a second set of signals generated by the chromophores; wherein the first and second set of signals are used to detect each analyte of the plurality of analytes...the presence of each analyte of the plurality of analytes is detected by comparing the set of signature profiles to a control set of signature profile; Para. [0083], analyte is referred to as a nucleic acid analyte; Para. [0036]), but fails to explicitly disclose (b) partitioning said mixture into a plurality of partitions; (c) generating signals in said plurality of partitions, and signals in a partition of said plurality of partitions; (d) detecting from multiple partitions of said plurality of partitions in the same embodiment. However, since in an alternate embodiment California teaches droplet digital PCR (Para. [0010]), it would have been obvious to one of ordinary skill in the art to modify the method of the primary embodiment of California with the teaching of an alternate embodiment of California so that California's assay using a droplet digital PCR process involves (b) partitioning said mixture into a plurality of partitions; (c) generating signals in said plurality of partitions, and signals in a partition of said plurality of partitions; (d) detecting from multiple partitions of said plurality of partitions, in order to correctly perform the taught method using a droplet digital PCR process.

Regarding Claim 2, modified California discloses the method of claim 1. California further discloses at least a portion of said plurality of signals is generated by said plurality of oligonucleotide probes (Para. [0003], (a) combining the sample with a first primer and a first oligonucleotide, wherein a first chromophore is attached to the first primer, a second chromophore is attached to the first oligonucleotide, the first primer and the first oligonucleotide are specific for a first polynucleotide analyte and the first chromophore is different from the second chromophore; (b) measuring a first signal generated by the first and second chromophores).

Regarding Claim 3, modified California discloses the method of claim 1. California further discloses said sample further comprises (iii) an additional plurality of nucleic acid molecules derived from, and/or corresponding with, an additional nucleic acid target and (iv) an additional plurality of oligonucleotide probes, each of which corresponds to a different region of said additional nucleic acid target (Para. [0007], In some cases, step a) of the method described herein further comprises combining the sample with a third primer and a second oligonucleotide, wherein a third chromophore is attached to the third primer and a fourth chromophore is attached to the second oligonucleotide, and the third primer and the second oligonucleotide are specific for a second polynucleotide analyte; step b) further comprises measuring a fifth signal generated by the third and fourth chromophores; step d) further comprises measuring a sixth signal generated by the third and fourth chromophores; wherein the fifth and sixth signals are used to detect the second polynucleotide analyte. In some cases, the methods disclosed herein further comprising comparing the fifth and sixth signals, wherein a change in the fifth and sixth signals indicates the presence of the second polynucleotide analyte; Paras. [0044]-[0045], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides...primers to bind to specific regions of the individual template).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/027751

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding Claim 4, modified California discloses the method of claim 3. California further discloses at least a portion of said plurality of signals is generated by said plurality of oligonucleotide probes and said additional plurality of oligonucleotide probes (Para. [0007], In some cases, step a) of the method described herein further comprises combining the sample with a third primer and a second oligonucleotide, wherein a third chromophore is attached to the third primer and a fourth chromophore is attached to the second oligonucleotide, and the third primer and the second oligonucleotide are specific for a second polynucleotide analyte; step b) further comprises measuring a fifth signal generated by the third and fourth chromophores; step d) further comprises measuring a sixth signal generated by the third and fourth chromophores; wherein the fifth and sixth signals are used to detect the second polynucleotide analyte. In some cases, the methods disclosed herein further comprising comparing the fifth and sixth signals, wherein a change in the fifth and sixth signals indicates the presence of the second polynucleotide analyte; Para. [0011], methods of detecting a plurality of analytes, comprising: a) combining the plurality of analytes with a plurality of primer pairs, wherein each primer pair is specific to a single analyte and each primer of the primer pair is attached to at least one chromophore; b) measuring a first set of signals generated by the chromophores; Para. [0044], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides).

Regarding Claim 5, modified California discloses the method of claim 1. California further discloses said sample is derived from a biological sample (Para. [0152], sample can be a blood sample).

Regarding Claim 6, modified California discloses the method of claim 5. California further discloses said biological sample is blood (Para. [0152], sample can be a blood sample) or plasma (optionally not included).

Regarding Claim 9, modified California discloses the method of claim 1. California further discloses (c) comprises amplifying said plurality of nucleic acid molecules, thereby generating said plurality of signals (Para. [0036], methods described herein can provide for the direct detection of one or more analytes in a single reaction (such as an amplification reaction...)...once the analyte has been amplified to incorporate two different chromophores, upon excitation of one chromophore, an electron may travel through the pi-bond network of a DNA backbone to interact with a second chromophore, thereby generating a detectable signal; Para. [0003], methods generally involve using oligonucleotides (e.g., primers, probes) attached to chromophores (e.g., fluorophores, quenchers, etc.) in amplification or polymerization reactions in order to detect a polynucleotide analyte. In some embodiments, provided herein are methods of detecting at least one polynucleotide analyte in a sample, comprising: (a) combining the sample with a first primer and a first oligonucleotide, wherein a first chromophore is attached to the first primer...measuring a first signal generated by the first and second chromophores...first and second signals are used to detect the first polynucleotide analyte).

Regarding Claim 10, modified California discloses the method of claim 9. California further discloses said amplifying comprises polymerase chain reaction (Para. [0036], amplification reaction (such as a PCR reaction or PCR reaction process)).

Regarding Claim 13, modified California discloses the method of claim 1. California further discloses said plurality of signals is a plurality of fluorescent signals (Para. [0005], change in the first and second signals is a decrease in fluorescent intensity) or a plurality of chemiluminescent signals (optionally not included).

Regarding Claim 14, California discloses the method of claim 1. California further discloses said plurality of oligonucleotide probes each bind to a different region of said nucleic acid target (Para. [0013], the first primer and the second primer are specific for the polynucleotide analyte; Paras. [0044]-[0045], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides...primers to bind to specific regions of the individual template).

Regarding Claim 15, modified California discloses the method of claim 1. California further discloses said at least one of said plurality of signals uniquely corresponds with a presence of exactly two of said plurality of nucleic acid molecules (Para. [0011], c) performing at least one polymerization reaction with the plurality of primer pairs using the plurality of analytes as templates; and d) measuring a second set of signals generated by the chromophores; wherein the first and second set of signals are used to detect each analyte of the plurality of analytes. In some cases, the method described herein further comprises: e) repeating step c and d at least once; and f) generating a set of signature profiles; Para. [0080], A signature profile is unique for each nucleotide mismatch, UU), but fails to explicitly disclose in said partition in the same embodiment. However, since in an alternate embodiment California teaches droplet digital PCR (Para. [0010]), it would have been obvious to one of ordinary skill in the art to modify the method of the primary embodiment of California with the teaching of an alternate embodiment of California so that California's assay using a droplet digital PCR process involves in said partition, in order to correctly perform the taught method using a droplet digital PCR process.

Regarding Claim 18, modified California discloses the method of claim 3. California further discloses at least a portion of said plurality of signals is generated by said plurality of oligonucleotide probes (Para. [0003], (a) combining the sample with a first primer and a first oligonucleotide, wherein a first chromophore is attached to the first primer, a second chromophore is attached to the first oligonucleotide, the first primer and the first oligonucleotide are specific for a first polynucleotide analyte and the first chromophore is different from the second chromophore; (b) measuring a first signal generated by the first and second chromophores).

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Regarding Claim 19, modified California discloses the method of claim 18. California further discloses said sample further comprises (iii) an additional plurality of nucleic acid molecules derived from, and/or corresponding with, an additional nucleic acid target and (iv) an additional plurality of oligonucleotide probes, each of which corresponds to a different region of said additional nucleic acid target (Para. [0007], In some cases, step a) of the method described herein further comprises combining the sample with a third primer and a second oligonucleotide, wherein a third chromophore is attached to the third primer and a fourth chromophore is attached to the second oligonucleotide, and the third primer and the second oligonucleotide are specific for a second polynucleotide analyte; step b) further comprises measuring a fifth signal generated by the third and fourth chromophores; step d) further comprises measuring a sixth signal generated by the third and fourth chromophores; wherein the fifth and sixth signals are used to detect the second polynucleotide analyte. In some cases, the methods disclosed herein further comprising comparing the fifth and sixth signals, wherein a change in the fifth and sixth signals indicates the presence of the second polynucleotide analyte; Paras. [0044]-[0045], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides...primers to bind to specific regions of the individual template).

Regarding Claim 20, modified California discloses the method of claim 19. California further discloses at least a portion of said plurality of signals is generated by said plurality of oligonucleotide probes and said additional plurality of oligonucleotide probes (Para. [0007], In some cases, step a) of the method described herein further comprises combining the sample with a third primer and a second oligonucleotide, wherein a third chromophore is attached to the third primer and a fourth chromophore is attached to the second oligonucleotide, and the third primer and the second oligonucleotide are specific for a second polynucleotide analyte; step b) further comprises measuring a fifth signal generated by the third and fourth chromophores; step d) further comprises measuring a sixth signal generated by the third and fourth chromophores; wherein the fifth and sixth signals are used to detect the second polynucleotide analyte. In some cases, the methods disclosed herein further comprising comparing the fifth and sixth signals, wherein a change in the fifth and sixth signals indicates the presence of the second polynucleotide analyte; Para. [0011], methods of detecting a plurality of analytes, comprising: a) combining the plurality of analytes with a plurality of primer pairs, wherein each primer pair is specific to a single analyte and each primer of the primer pair is attached to at least one chromophore; b) measuring a first set of signals generated by the chromophores; Para. [0044], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides).

Regarding Claim 34, California discloses a method of quantifying (Para. [0036], methods described herein can provide for the direct detection of one or more analytes in a single reaction...allows for more accurate quantification of the analyte) a first nucleic acid target and a second nucleic acid target (Para. [0011], wherein the first and second set of signals are used to detect each analyte of the plurality of analytes; Para. [0044], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides) in a sample (Para. [0003], the sample), the method comprising: (a) providing a mixture comprising: i. a first plurality of nucleic acid molecules derived from, and/or corresponding with, said first nucleic acid target; ii. a second plurality of nucleic acid molecules derived from, and/or corresponding with, said second nucleic acid target (Para. [0011], a) combining the plurality of analytes with a plurality of primer pairs; Para. [0044], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides); iii. a first plurality of oligonucleotide probes, each of which corresponds to a different region of said first nucleic acid target (Para. [0011], the presence of each analyte of the plurality of analyte; Para. [0019], first primer or probe is a primer...In some cases, the second primer or probe comprises an oligonucleotide sequence complementary to the sequence of a target analyte); and iv. a second plurality of oligonucleotide probes, each of which corresponds to a different region of said second nucleic acid target (Para. [0011], the presence of each analyte of the plurality of analyte; Para. [0019], the second primer or probe is a primer...In some cases, the second primer or probe comprises an oligonucleotide sequence complementary to the sequence of a target analyte); (c) generating a plurality of signals (Para. [0011], each primer of the primer pair is attached to at least one chromophore; b) measuring a first set of signals generated by the chromophores), wherein said plurality of signals are detectable in one color channel (Para. [0049], In some cases, a single chromophore pair is used to detect an analyte; Para. [0176], Analytes of differing lengths (e.g., 40 bp, 60 bp, and 80 bp) are detected using a single fluorophore/quencher combination...By measuring fluorescence as a function of increasing temperature, multiple analytes in a single color channel are quantified); (d) detecting said plurality of signals in said color channel (Para. [0049]; Para. [0176]); and (e) based on said detecting, quantifying said first nucleic acid target and said second nucleic acid target in said sample (Para. [0011], measuring a second set of signals generated by the chromophores; wherein the first and second set of signals are used to detect each analyte of the plurality of analytes...the presence of each analyte of the plurality of analytes is detected by comparing the set of signature profiles to a control set of signature profile; Para. [0044], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides; Para. [0036]), but fails to explicitly disclose (b) partitioning said mixture into a plurality of partitions; (c) signals in said plurality of partitions; (d) detecting from multiple partitions of said plurality of partitions in the same embodiment. However, since in an alternate embodiment California teaches droplet digital PCR (Para. [0010]), it would have been obvious to one of ordinary skill in the art to modify the method of the primary embodiment of California with the teaching of an alternate embodiment of California so that California's assay using a droplet digital PCR process involves (b) partitioning said mixture into a plurality of partitions; (c) signals in said plurality of partitions; (d) detecting from multiple partitions of said plurality of partitions, in order to correctly perform the taught method using a droplet digital PCR process.

Regarding Claim 35, modified California discloses the method of claim 34. California further discloses said plurality of signals is generated by said first plurality of oligonucleotide probes and said second plurality of oligonucleotide probes (Para. [0011], measuring a first set of signals generated by the chromophores...and d) measuring a second set of signals generated by the chromophores; Para. [0019], first primer or probe is a primer...In some cases, the second primer or probe comprises an oligonucleotide sequence complementary to the sequence of a target analyte).

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Regarding Claim 36, modified California discloses the method of claim 34. California further discloses at least one of said plurality of signals corresponds with a unique combination of two or more of said plurality of first nucleic acid molecules and/or said plurality of second nucleic acid molecules (Para. [0011], the first and second set of signals are used to detect each analyte of the plurality of analytes. In some cases, the method described herein further comprises: e) repeating step c and d at least once; and f) generating a set of signature profiles; wherein the presence of each analyte of the plurality of analytes is detected by comparing the set of signature profiles to a control set of signature profiles; Para. [0044], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides; Para. [0080]), but fails to explicitly disclose in a partition of said plurality of partitions in the same embodiment. However, since in an alternate embodiment California teaches droplet digital PCR (Para. [0010]), it would have been obvious to one of ordinary skill in the art to modify the method of the primary embodiment of California with the teaching of an alternate embodiment of California so that California's assay using a droplet digital PCR process involves in a partition of said plurality of partitions, in order to correctly perform the taught method using a droplet digital PCR process.

Regarding Claim 37, modified California discloses the method of claim 36. California further discloses said at least one of said plurality of signals uniquely corresponds with a presence of exactly two of said first plurality of nucleic acid molecules or said second plurality of nucleic acid molecules (Para. [0044], terms "polynucleotide," "oligonucleotide," or "nucleic acid," as used herein, are used herein to refer to biological molecules comprising a plurality of nucleotides; Para. [0011], c) performing at least one polymerization reaction with the plurality of primer pairs using the plurality of analytes as templates; and d) measuring a second set of signals generated by the chromophores; wherein the first and second set of signals are used to detect each analyte of the plurality of analytes. In some cases, the method described herein further comprises: e) repeating step c and d at least once; and f) generating a set of signature profiles; Para. [0080]. A signature profile is unique for each nucleotide mismatch, UU), but fails to explicitly disclose molecules in said partition in the same embodiment. However, since in an alternate embodiment California teaches droplet digital PCR (Para. [0010]), it would have been obvious to one of ordinary skill in the art to modify the method of the primary embodiment of California with the teaching of an alternate embodiment of California so that California's assay using a droplet digital PCR process involves molecules in said partition, in order to correctly perform the taught method using a droplet digital PCR process.

Claims 7 and 8 lack an inventive step under PCT Article 33(3) as being obvious over California Institute Of Technology (hereinafter California) in view of Lifecodexx AG (hereinafter Lifecodexx).

Regarding Claim 7, modified California discloses the method of claim 1. California fails to explicitly disclose said nucleic acid target is a chromosome. Lifecodexx is in the field of DNA detection (Lifecodexx Abstract) including with amplification (Lifecodexx Para. [0065]) and teaches said nucleic acid target is a chromosome (Lifecodexx Para. [0121], detection (or amount) of the first species of DNA (for example, from a chromosome, or part thereof, related to a chromosomal aneuploidy such as human chromosome 21)). It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify California with the teaching of Lifecodexx to detail said nucleic acid target is a chromosome. The motivation for doing so would have been to identify and quantify chromosomes and thereby identify if a fetus has Downs Syndrome prior to birth (presence of an extra chromosome 21) during non-invasive pre-natal testing (Lifecodexx Para. [0081]) and thereby allow families to prepare additional resources if needed prior to birth.

Regarding Claim 8, modified California discloses the method of claim 7. California fails to explicitly disclose said chromosome is chromosome 21, chromosome 18, chromosome 15, chromosome 13, an X chromosome, or a Y chromosome. Lifecodexx teaches said chromosome is chromosome 21 (Lifecodexx Para. [0121], human chromosome 21). It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify California with the teaching of Lifecodexx to detail said chromosome is chromosome 21. The motivation for doing so would have been to identify if a fetus has Downs Syndrome prior to birth (presence of an extra chromosome 21) during non-invasive pre-natal testing (Lifecodexx Para. [0081]) and thereby allow families to prepare additional resources if needed prior to birth.

Claims 11 and 12 lack an inventive step under PCT Article 33(3) as being obvious over California Institute Of Technology (hereinafter California) in view of Chromacode Inc. (hereinafter Chromacode).

Regarding Claim 11, modified California discloses the method of claim 9. California fails to explicitly disclose said amplifying degrades said plurality of oligonucleotide probes, thereby generating said plurality of signals. Chromacode is in the field of identifying nucleic acid targets with oligonucleotides by generating signals (Chromacode Abstract) and teaches said amplifying degrades said plurality of oligonucleotide probes, thereby generating said plurality of signals (Chromacode Para. [008], amplifying the up to thirty target nucleic acid sequences with the polymerase chain reaction, thereby degrading the signal oligonucleotides and permitting generation of up to thirty signals, generating up to thirty signals). It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify California with the teaching of Chromacode to detail said amplifying degrades said plurality of oligonucleotide probes, thereby generating said plurality of signals. The motivation for doing so would have been to amplify the nucleic acid sequences (Chromacode Para. [008]) and thereby provide measurable signal intensities for the nucleic acid target identification (Chromacode Abstract).

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Regarding Claim 12, modified California discloses the method of claim 1. California fails to explicitly disclose said plurality of oligonucleotide probes are degraded by a nucleic acid enzyme, thereby releasing a signal tag from each of said plurality of oligonucleotide probes and generating said plurality of signals. Chromacode is in the field of identifying nucleic acid targets with oligonucleotides by generating signals (Chromacode Abstract) and teaches said plurality of oligonucleotide probes are degraded by a nucleic acid enzyme, thereby releasing a signal tag from each of said plurality of oligonucleotide probes and generating said plurality of signals (Chromacode Claims 8-10, signal oligonucleotides comprise a signal tag and a signal quenching tag...signal quenching tag is separated from the signal tag by oligonucleotide degradation during the polymerase chain reaction, thereby permitting the signal to be generated; Para. [008], degrading the signal oligonucleotides and permitting generation of up to thirty signals, generating up to thirty signals; polymerase is an enzyme that degrades nucleic acids). It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify California with the teaching of Chromacode to detail said plurality of oligonucleotide probes are degraded by a nucleic acid enzyme, thereby releasing a signal tag from each of said plurality of oligonucleotide probes and generating said plurality of signals. The motivation for doing so would have been to define a pathway for the signal generation (Chromacode Para. [008]) and thereby provide measurable signal intensities for the nucleic acid target identification (Chromacode Abstract).

Claims 16 and 17 lack an inventive step under PCT Article 33(3) as being obvious over California Institute Of Technology (hereinafter California) in view of Macevicz.

Regarding Claim 16, modified California discloses the method of claim 1. California further discloses at least one signal of said plurality of signals corresponds with combinations of said plurality of nucleic acid molecules (Para. [0011], c) performing at least one polymerization reaction with the plurality of primer pairs using the plurality of analytes as templates; and d) measuring a second set of signals generated by the chromophores; wherein the first and second set of signals are used to detect each analyte of the plurality of analytes. In some cases, the method described herein further comprises: e) repeating step c and d at least once; and f) generating a set of signature profiles), but fails to explicitly disclose at least one signal corresponds with two or more unique combinations. Macevicz is in the field of signature sequences of polynucleotides determined by signals (Macevicz Abstract) and teaches at least one signal corresponds with two or more unique combinations (Macevicz Para. [0047], An important aspect of the present invention is based on the observation that when oligonucleotide tags representing different events, e.g. different nucleotides at the same locus of a template, have distinguishable labels, then the occurrence of so-called "doubles" (i.e., two different polynucleotides having the same oligonucleotide tag) can be detected by the presence of two distinct labels at the same hybridization site. Thus, the sample size may be much larger than that taught in the above references because "doubles" can simply be discarded or ignored during a detection step). It would have been obvious to one of ordinary skill in the art to further modify California with the teaching of Macevicz to detail at least one signal corresponds with two or more unique combinations. The motivation for doing so would have been to increase the sample size and thereby increase the sequencing efficiency (Macevicz Para. [0047]).

Regarding Claim 17, modified California discloses the method of claim 16. California further discloses said at least one signal of said plurality of signals (A) corresponds with a presence of one of said plurality of nucleic acid molecules (Para. [0011], methods of detecting a plurality of analytes, comprising: a) combining the plurality of analytes with a plurality of primer pairs, wherein each primer pair is specific to a single analyte and each primer of the primer pair is attached to at least one chromophore; b) measuring a first set of signals generated by the chromophores...d) measuring a second set of signals generated by the chromophores; wherein the first and second set of signals are used to detect each analyte of the plurality of analytes) and (B) corresponds with a presence of two of said plurality of nucleic acid molecules (Para. [0011], c) performing at least one polymerization reaction with the plurality of primer pairs using the plurality of analytes as templates; and d) measuring a second set of signals generated by the chromophores; wherein the first and second set of signals are used to detect each analyte of the plurality of analytes. In some cases, the method described herein further comprises: e) repeating step c and d at least once; and f) generating a set of signature profiles; Para. [0080], A signature profile is unique for each nucleotide mismatch, UU).

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Claims 65, 66, and 70 lack an inventive step under PCT Article 33(3) as being obvious over Ching et al. (hereinafter Ching) in view of University Of Washington Through Its Center For Commercialization (hereinafter Washington).

Regarding Claim 65, Ching discloses a method of quantifying target nucleic acid molecules in a sample (Para. [0012], a method for determining relative amounts of one or more target polynucleotides in a sample), the method comprising: (b) amplifying said plurality of nucleic acid molecules (Para. [0012], amplifying in the sample the one or more target polynucleotides), thereby generating a plurality of signals (Para. [0016], reading values of an optical signal from a first-stage amplification reaction, the optical signal being monotonically related to a concentration of an amplicon in the first-stage amplification reaction); (c) detecting said plurality of signals; (Para. [0016], reading values of an optical signal from a first-stage amplification reaction) (d) comparing members of said plurality of signals to one another (Para. [0016], computing a predetermined level from the values of the optical signal; (d) comparing the predetermined value with the most recent value of the optical signal; Para. [0012], (iii) comparing second amplicons of the second amplification reaction to amplicons of the at least one reference sequence in the first amplification reaction to determine relative amounts of the one or more target polynucleotides in the sample); and (e) quantifying said target nucleic acid molecules in said sample based on said comparing (Para. [0012], (iii) comparing second amplicons of the second amplification reaction to amplicons of the at least one reference sequence in the first amplification reaction to determine relative amounts of the one or more target polynucleotides in the sample), but fails to explicitly disclose (a) partitioning a plurality of nucleic acid molecules into a plurality of partitions; (b) amplifying in said plurality of partitions, said method not including a step of quantifying said plurality of nucleic acid molecules in any individual member of said plurality of partitions. Washington is in the field of digital measurements of nucleic acids (Washington Para. [0013]) and teaches (a) partitioning a plurality of nucleic acid molecules into a plurality of partitions (Washington Paras. [0066]-[0067], droplets of different volumes (diameters) can be generated...a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)); (b) amplifying in said plurality of partitions (Washington Paras. [0066]-[0067], amplification of analyte (e.g. digital PCR) can be carried out simultaneously in all droplets of different volumes (sizes)...a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)), and a method not including a step of quantifying a plurality of nucleic acid molecules in any individual member of said plurality of partitions (Washington Para. [0100], This example describes a non-limiting theoretical framework that can be used to describe certain aspects of the present invention described herein. It will be understood that the method described in this example is one of many ways to determine concentrations of sample using the present invention. Under this theoretical framework, it is assumed that the target molecules are present in the sample at a concentration Cs in units of molecules/volume. The sample is distributed into digital volumes of variable volumes; the distribution of target molecules into the droplets follows Poisson statistics...the amplification reaction will cause a droplet containing one or more molecules to be distinguishable from empty droplets, e.g. by its fluorescence intensity. In this method we know only whether a droplet is empty or occupied...in order to determine the concentration CS of target molecules, P(n>0, CSVi) needs to be summed over a sufficiently large number of droplets with respective volumes Vi; Para. [0067], a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Ching with the teaching of Washington to detail (a) partitioning a plurality of nucleic acid molecules into a plurality of partitions; (b) amplifying in said plurality of partitions, said method not including a step of quantifying said plurality of nucleic acid molecules in any individual member of said plurality of partitions. The motivation for doing so would have been to divide the sample up and allow for repetitive tests for comparison and thereby ensure testing accuracy.

Regarding Claim 66, modified Ching discloses the method of claim 65. Ching further discloses said comparing comprises generating one or more signal distribution curves from said plurality of signals (Para. [0056], FIGS. 1A and 1B, curves (1000) and (1002) represent accumulated amplicon of for example, a reference sequence and target polynucleotide, respectively, as determined by two different fluorescent signals generated by amplicon-specific probes) and analyzing said one or more signal distribution curves (Para. [0056] FIGS. 1A and 1B, a second-stage reaction is initiated when curve (1002) of the target amplicon reaches or exceeds a predetermined level (1006)).

Regarding Claim 70, modified Ching discloses the method of claim 65. Ching further discloses said plurality of nucleic acid molecules are derived from, correspond with, or are members of, said target nucleic acid molecules (Para. [0012], a method for determining relative amounts of one or more target polynucleotides in a sample).

Claims 67-69 lack an inventive step under PCT Article 33(3) as being obvious over Ching et al. (hereinafter Ching) in view of University Of Washington Through Its Center For Commercialization (hereinafter Washington) and Morgan et al. (hereinafter Morgan).

Regarding Claim 67, modified Ching discloses the method of claim 66. Ching further discloses said analyzing comprises for each of said one or more signal distribution curves (Para. [0056] FIGS. 1A and 1B, a second-stage reaction is initiated when curve (1002) of the target amplicon reaches or exceeds a predetermined level (1006)), but fails to explicitly disclose measuring an area under the curve (AUC). Morgan is in the field of molecular detection (Morgan Abstract) and teaches measuring an area under the curve (AUC) (Morgan Pg. 3 Col. 2 Para. [1] Lns. 4-5, area under the curve (AUC) represents an overall summary measure of the curve). It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify Ching with the teaching of Morgan to detail measuring an area under the curve (AUC). The motivation for doing so would have been to detail the diagnostic performance of the test (Morgan Pg. 3 Col. 2 Para. [1] Lns. 1-5) and thereby best identify a target nucleic acid for treatment and/or diagnosis of a subject (Morgan Abstract).

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Regarding Claim 68, modified Ching discloses the method of claim 67. Ching further discloses (d) comprises comparing to a reference value (Para. [0059] FIG. 1B, closed-loop control, microprocessor (1080) may calculate values of characteristics of curves, such as (1013) or (1015) of FIG. 1B, at predetermined intervals so that they may be compared to a predetermined level), but fails to explicitly disclose comparing said AUC. Morgan teaches comparing said AUC (Morgan Pg. 3 Col. 2 Para. [1] Lns. 4-13, The area under the curve (AUC) represents an overall summary measure of the curve and the test's overall ability to accurately distinguish cases from non-cases. The Q* index, the highest point on the SROC curve that intersects the anti-diagonal, represents a summarization of test performance where sensitivity and specificity are equal (so the probability of an incorrect test result is the same for cases and non-cases). An AUC of one represents perfect discriminatory ability, while a Q* index of one represents perfect accuracy). It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify Ching with the teaching of Morgan to detail comparing said AUC. The motivation for doing so would have been to detail the diagnostic performance of the test (Morgan Pg. 3 Col. 2 Para. [1] Lns. 1-5) and thereby best identify a target nucleic acid for treatment and/or diagnosis of a subject (Morgan Abstract).

Regarding Claim 69, modified Ching discloses the method of claim 67. Ching further discloses said one or more signal distribution curves comprises a plurality of signal distribution curves (Para. [0057] FIGS. 1A and 1B, "amplicon growth curve' means a curve, such as curves (1000), (1002), (1013), or (1015)), wherein (d) comprises comparing for each of said one or more signal distribution curves to one another (Para. [0057] FIGS. 1A and 1B, "amplicon growth curve' means a curve, such as curves (1000), (1002), (1013), or (1015); Para. [0075], it is desirable to compare readouts from different assays), but fails to explicitly disclose comparing an AUC to one another. Morgan teaches comparing an AUC to one another (Morgan Pg. 3 Col. 2 Para. [1] Lns. 4-13, The area under the curve (AUC) represents an overall summary measure of the curve and the test's overall ability to accurately distinguish cases from non-cases. The Q* index, the highest point on the SROC curve that intersects the anti-diagonal, represents a summarization of test performance where sensitivity and specificity are equal (so the probability of an incorrect test result is the same for cases and non-cases). An AUC of one represents perfect discriminatory ability, while a Q* index of one represents perfect accuracy). It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify Ching with the teaching of Morgan to detail comparing an AUC to one another. The motivation for doing so would have been to detail the diagnostic performance of the test (Morgan Pg. 3 Col. 2 Para. [1] Lns. 1-5) and thereby best identify a target nucleic acid for treatment and/or diagnosis of a subject (Morgan Abstract).

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Claims 79-81 lack an inventive step under PCT Article 33(3) as being obvious over Lifecodexx AG (hereinafter Lifecodexx) in view of University Of Washington Through Its Center For Commercialization (hereinafter Washington).

Regarding Claim 79, Lifecodexx discloses a method of determining a quantity of a first target nucleic acid relative to a quantity of a second target nucleic acid in a sample (Paras. [0124]-[0125], detect a plurality (such as two or more) species of DNA in the sample, such methods can further comprise the steps of: determining the relative amount, preferably a ratio, of: (x) said first species of DNA detected with the first DMR (or first set of two or more DMR); and (y) said second species of DNA detected with the second DMR (or first set of two or more DMRs)), the method comprising: (a) providing a mixture comprising: i. a first plurality of nucleic acid molecules derived from, and/or corresponding with, said first target nucleic acid; and ii. a second plurality of nucleic acid molecules derived from, and/or corresponding with, said second target nucleic acid (Paras. [0016]-[0017], a first aspect, and as may be further described, defined, claimed or otherwise disclosed herein, the invention relates to a method for detecting in a sample from an individual an amount of a species of DNA originating from cells of a given type, which sample comprises said species of DNA in admixture with differently methylated DNA not originating from cells of said type; said method comprising the steps: (a) treating the DNA present in said sample with a reagent that differentially modifies methylated and nonmethylated DNA); (c) amplifying said first plurality of nucleic acid molecules and said second plurality of nucleic acid molecules (Para. [0065], In certain embodiments, prior to or as part of the detection that occurs as part of a step (b) and/or a step (c) of any method of present invention, each DNA region comprising said DMR(s) and/or said other region(s), respectively, is(are) amplified), thereby generating a plurality of signals; (d) detecting said plurality of signals (Para. [0123], said detected amount of each of said species of DNA is expressed as a relative concentration of said species of DNA to the respective amount of total DNA detected in said sample from the respective other region. In further of such embodiments, the methods of the present invention can further comprise the steps of detecting an amount of total DNA in a standard sample of DNA of known amount using the same other regions as used in step (c); and comparing the signal detected from said standard sample of DNA to the respective signal detected in step (c) for each of the other regions); and (e) based on said detecting, determining a ratio representative of a quantity of said first target nucleic acid relative to a quantity of said second target nucleic acid in said sample (Paras. [0124]-[0125], In those embodiments of the present invention that quantitatively detect a plurality (such as two or more) species of DNA in the sample, such methods can further comprise the steps of: determining the relative amount, preferably a ratio, of: (x) said first species of DNA detected with the first DMR (or first set of two or more DMR); and (y) said second species of DNA detected with the second DMR (or first set of two or more DMRs)), but fails to explicitly disclose (b) partitioning said mixture into a plurality of partitions; (c) amplifying in said plurality of partitions, or said method not including a step of quantifying, in any individual member of said plurality of partitions, said first plurality of nucleic acid molecules or said second plurality of nucleic acid molecules. Washington is in the field of digital measurements of nucleic acids (Washington Para. [0013]) and teaches (b) partitioning said mixture into a plurality of partitions (Washington Paras. [0066]-[0067], droplets of different volumes (diameters) can be generated...a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)); (c) amplifying in said plurality of partitions (Washington Paras. [0066]-[0067], amplification of analyte (e.g. digital PCR) can be carried out simultaneously in all droplets of different volumes (sizes)...a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)), and a method not including a step of quantifying, in any individual member of said plurality of partitions, a first plurality of nucleic acid molecules or a second plurality of nucleic acid molecules (Washington Para. [0100], This example describes a non-limiting theoretical framework that can be used to describe certain aspects of the present invention described herein. It will be understood that the method described in this example is one of many ways to determine concentrations of sample using the present invention. Under this theoretical framework, it is assumed that the target molecules are present in the sample at a concentration Cs in units of molecules/volume. The sample is distributed into digital volumes of variable volumes; the distribution of target molecules into the droplets follows Poisson statistics...the amplification reaction will cause a droplet containing one or more molecules to be distinguishable from empty droplets, e.g. by its fluorescence intensity. In this method we know only whether a droplet is empty or occupied...in order to determine the concentration CS of target molecules, P(n>0, CSVi) needs to be summed over a sufficiently large number of droplets with respective volumes Vi; Para. [0067], a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Lifecodexx with the teaching of Washington to detail (b) partitioning said mixture into a plurality of partitions; (c) amplifying in said plurality of partitions, and said method not including a step of quantifying, in any individual member of said plurality of partitions, said first plurality of nucleic acid molecules or said second plurality of nucleic acid molecules. The motivation for doing so would have been to divide the sample up and allow for repetitive tests for comparison and thereby ensure testing accuracy.

Regarding Claim 80, modified Lifecodexx discloses the method of claim 79. Lifecodexx further discloses further comprising, based on said ratio, quantifying said first target nucleic acid and said second target nucleic acid in said sample (Paras. [0123]-[0125], In alterative certain embodiments, one or more (preferably each) of said detection steps comprises quantitative detection and said detected amount of said species of DNA is expressed as an absolute amount of each or said species of DNA in said sample. In those embodiments of the present invention that quantitatively detect a plurality (such as two or more) species of DNA in the sample, such methods can further comprise the steps of: determining the relative amount, preferably a ratio, of: (x) said first species of DNA detected with the first DMR (or first set of two or more DMR); and (y) said second species of DNA detected with the second DMR (or first set of two or more DMRs); Para. [0198], a relative and/or absolute amount of foetal cfDNA and total cfDNA present in the plasma is determined).

Regarding Claim 81, modified Lifecodexx discloses the method of claim 79. Lifecodexx further discloses said ratio is representative of a fetal fraction (Para. [0106], cfDNA fraction is...of total cfDNA present in maternal plasma; Para. [0198]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/027751

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Claim 54 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest:

Regarding claim 54, the prior art of record, individually or in combination, does not teach or fairly suggest the method of claim 52, wherein said method does not include a step of determining a number of partitions comprising a nucleic acid sequence corresponding to said target nucleic acid molecules.

The following prior art is made of record to support and further define the reasons for meeting the criteria set out in PCT Article 33(2)-(3) for claim 54:

(i) Washington discloses determining a number of partitions comprising a nucleic acid sequence corresponding to said target nucleic acid molecules (Paras. [0103]-[0108], The total number of occupied droplets in a simulation, NS, is counted...Results for a typical set of simulations are shown in FIG. 4. Simulations were performed with 10,000 droplets and six different concentrations...The droplet sizes were drawn from a uniform distribution of diameters between 4.0 and 190 microns. For each simulation, the droplets were sorted by volume, the droplet with the smallest volume was number 1 and the droplet with the largest volume was number 10,000. FIG. 4 plots the cumulative number of occupied droplets. The cumulative number occupied for the ith droplet is the sum of the occupied droplets for all droplet; Para. [0067], a sample can include an aqueous solution that includes a molecule of interest (e.g., a nucleic acid molecule)). Washington fails to fairly teach or suggest, either alone or in combination with the art, a method does not include a step of determining a number of partitions comprising a nucleic acid sequence corresponding to said target nucleic acid molecules.

Claims 1-20, 34-37, 52-54, 65-70, and 79-81 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference		FOR FURTHER ACTION
44158-708.601		see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US19/35129	03 June 2019 (03.06.2019)	04 June 2018 (04.06.2018)
Applicant		
CHROMACODE, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 3

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/35129

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Continued Within the Next Supplemental Box-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/35129

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07H 21/00, 21/04; C12Q 1/68; G01N 21/64, 33/533, 33/542 (2019.01)

CPC - C12Q 1/68, 1/686, 1/6818, 1/6804, 1/6816, 1/6827, 1/6888

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2017/173035 A1 (CHROMACODE, INC.) 05 October 2017; paragraphs [008], [018], [037]	1 --- 2-6
Y	US 2010/0041092 A1 (LIN et al.) 18 February 2010; paragraph [0017], [0096], [0112]	2-4
Y	(MYERS et al.) A Handheld Point-of-Care Genomic Diagnostic System. PLoS ONE. 01 August 2013, Vol. 8 No. 8: e70266, pages 1-9; page 6, second column; page 7, first column, second paragraph. DOI:10.1371/journal.pone.0070266	5-6
A	US 2009/0042735 A1 (BLAIR et al.) 12 February 2009; entire document	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 October 2019 (02.10.2019)	01 NOV 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US19/35129

-***-Continued from Box No. III Observations where unity of invention is lacking: -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-6 are directed toward a method of verifying the synthesis of a chemical mixture.

Group II, Claims 7-17 are directed toward a method of tracking a batch of chemical mixture.

Group III, Claims 18-67 and 95-114 are directed toward a method and composition for watermarking a chemical composition, comprising a watermarking agent configured to produce a detectable signal upon application of reaction conditions.

Group IV, Claims 68-94 are directed toward a method of identifying a chemical composition, comprising calculating a ratio of a first signal to a second signal and comparing the ratio to a reference ratio.

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include verifying the synthesis of a chemical mixture, not present in any other Group; the special technical features of Group II include tracking a batch of chemical mixture, not present in any other Group; the special technical features of Group III include a watermarking agent configured to produce a detectable signal upon application of reaction conditions, not present in any other Group; the special technical features of Group IV include comparing a ratio of signals to a reference ratio to identify a chemical composition, not present in any other Group.

Groups I-IV share the technical features including: a method associated with a chemical mixture, said method comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture; and ii) a first watermarking agent configured to emit a first detectable signal; and b) detecting said first detectable signal. Groups I and IV share the further technical features including: ii) a second watermarking agent configured to emit a second detectable signal; b) and detecting said second detectable signal. Groups II and IV further share the technical features including: comparing a signal or ratio of signals to a reference.

However, these shared technical features are previously disclosed by WO 2017/173035 A1 to Chromacode, Inc. (hereinafter 'Chromacode').

Chromacode discloses a method associated with a chemical mixture (a method associated with a polymerase chain reaction chemical mixture; paragraphs [008], [018]), said method comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture (comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture; paragraphs [008], [018]); and ii) a first watermarking agent configured to emit a first detectable signal (a first signal oligonucleotide (watermarking agent) configured to emit a first detectable signal; paragraph [008]); and b) detecting said first detectable signal (detecting said first detectable signal; paragraphs [008], [032]); ii) a second watermarking agent configured to emit a second detectable signal (a second signal oligonucleotide (watermarking agent) configured to emit a second detectable signal; paragraph [008]); detecting said second detectable signal (detecting said second detectable signal; paragraphs [008], [032]); and comparing a signal or ratio of signals to a reference (correlating the first signal to the first ratio (comparing a signal or ratio of signals to a reference); abstract, paragraph [008]).

Since none of the special technical features of the Groups I-IV inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Chromacode reference, unity of invention is lacking.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Lee A. Johnson
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	01 NOV 2019
Applicant's or agent's file reference 44158-708.601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US19/35129	03 June 2019 (03.06.2019)	04 June 2018 (04.06.2018)

International Patent Classification (IPC) or both national classification and IPC
IPC - C07H 21/00, 21/04; C12Q 1/68; G01N 21/64, 33/533, 33/542 (2019.01)
CPC - C12Q 1/68, 1/686, 1/6818, 1/6804, 1/6816, 1/6827, 1/6888

Applicant
CHROMACODE, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	02 October 2019 (02.10.2019)	Shane Thomas PCT Help Desk Telephone No. 571-272-4300

Form PCT/ISA/237 (cover sheet) (revised January 2019)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US19/35129

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(b)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US19/35129

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-6 are directed toward a method of verifying the synthesis of a chemical mixture.

Group II, Claims 7-17 are directed toward a method of tracking a batch of chemical mixture.

Group III, Claims 18-67 and 95-114 are directed toward a method and composition for watermarking a chemical composition, comprising a watermarking agent configured to produce a detectable signal upon application of reaction conditions.

Group IV, Claims 68-94 are directed toward a method of identifying a chemical composition, comprising calculating a ratio of a first signal to a second signal and comparing the ratio to a reference ratio.

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include verifying the synthesis of a chemical mixture, not present in any other Group; the special technical features of Group II include tracking a batch of chemical mixture, not present in any other Group; the special technical features of Group III include a watermarking agent configured to produce a detectable signal upon application of reaction conditions, not present in any other Group; the special technical features of Group IV include comparing a ratio of signals to a reference ratio to identify a chemical composition, not present in any other Group.

Groups I-IV share the technical features including: a method associated with a chemical mixture, said method comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture; and ii) a first watermarking agent configured to emit a first detectable signal; and b) detecting said first detectable signal. Groups I and IV share the further technical features including: ii) a second watermarking agent configured to emit a second detectable signal; b) and detecting said second detectable signal. Groups II and IV further share the technical features including: comparing a signal or ratio of signals to a reference.

However, these shared technical features are previously disclosed by WO 2017/173035 A1 to Chromacode, Inc. (hereinafter 'Chromacode').

Chromacode discloses a method associated with a chemical mixture (a method associated with a polymerase chain reaction chemical mixture; paragraphs [008], [018]), said method comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture (comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture; paragraphs [008], [018]); and ii) a first watermarking agent configured to emit a first detectable signal (a first signal oligonucleotide (watermarking agent) configured to emit a first detectable signal; paragraph [008]); and b) detecting said first detectable signal (detecting said first detectable signal; paragraphs [008], [032]); ii) a second watermarking agent configured to emit a second detectable signal (a second signal oligonucleotide (watermarking agent) configured to emit a second detectable signal; paragraph [008]); detecting said second detectable signal (detecting said second detectable signal; paragraphs [008], [032]); and comparing a signal or ratio of signals to a reference (correlating the first signal to the first ratio (comparing a signal or ratio of signals to a reference); abstract, paragraph [008]).

Since none of the special technical features of the Groups I-IV inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Chromacode reference, unity of invention is lacking.

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-6

Form PCT/ISA/237 (Box No. IV) (revised January 2019)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US19/35129

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2-6	YES
	Claims	1	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-6	NO
Industrial applicability (IA)	Claims	1-6	YES
	Claims	NONE	NO

2. Citations and explanations:

Claim 1 lacks novelty under PCT Article 33(2) as being anticipated by WO 2017/173035 A1 to Chromacode, Inc. (hereinafter 'Chromacode').

As per claim 1, Chromacode discloses a method of verifying the synthesis of a chemical mixture (a method of detecting the presence of a first and a second target nucleic acid in a chemical mixture (verifying the synthesis of a chemical mixture); paragraphs [008], [018]), said method comprising a) providing a sample comprising: i) a chemical mixture (comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture; paragraphs [008], [018]), comprising a first chemical component and a second chemical component (comprising a first and a second target nucleic acid; paragraph [008]); ii) a first watermarking agent configured to emit a first detectable signal (a first signal oligonucleotide comprising a first signal tag (watermarking agent) configured to emit a first detectable fluorescent signal; paragraph [008]), wherein said first watermarking agent indicates the presence of said first chemical component (wherein said first signal oligonucleotide generates signal for detection in the presence of the first target nucleic acid; paragraphs [008], [038]); and iii) a second watermarking agent configured to emit a second detectable signal (a second signal oligonucleotide comprising a second signal tag (watermarking agent) configured to emit a second detectable fluorescent signal; paragraph [008]), wherein said second watermarking agent indicates the presence of said second chemical component (wherein said second signal oligonucleotide generates signal for detection in the presence of the second target nucleic acid; paragraphs [008], [038]); and b) detecting the presence of said first detectable signal and said second detectable signal (measuring the intensity of said first and second signal tag; paragraphs [008], [038]), thereby verifying the synthesis of said chemical composition (thereby detecting the presence of a first and a second target nucleic acid in a chemical mixture (verifying the synthesis of a chemical mixture); paragraphs [008], [018]).

Claims 2-4 lack an inventive step under PCT Article 33(3) as being obvious over Chromacode in view of US 2010/0041092 A1 to Lin et al. (hereinafter 'Lin').

As per claim 2, Chromacode discloses the method of claim 1, but Chromacode does not disclose obtaining a time stamp. Lin discloses obtaining a time stamp (tagging of newly synthesized fluorescently tagged compounds with a timestamp tag that gradually shifts from green to red over time, which allows selective labelling of proteins synthesized after performing global photoconversion at a time of interest; paragraphs [0017], [0018], [0031]). It would have been obvious to a person of ordinary skill in the art, at the time the invention was made, to have modified the disclosure of Chromacode, to include obtaining a timestamp, as previously disclosed by Lin, in order to provide a superior method for monitoring the synthesis of a reaction mixture.

As per claim 3, Chromacode and Lin, in combination, disclose the method of claim 2, but Chromacode does not disclose wherein said time stamp indicates a synthesis time. Lin discloses wherein said time stamp indicates a synthesis time (tagging of newly synthesized fluorescently tagged compounds with a timestamp tag that gradually shifts from green to red over time, which allows selective labelling of proteins synthesized after performing global photoconversion at a time of interest; paragraphs [0017], [0018], [0031]). It would have been obvious to a person of ordinary skill in the art, at the time the invention was made, to have modified the disclosure of Chromacode, to include wherein said time stamp indicates a synthesis time, as previously disclosed by Lin, in order to provide a superior method for monitoring the synthesis of a reaction mixture.

As per claim 4, Chromacode and Lin, in combination, disclose the method of claim 3, but Chromacode does not disclose wherein outputting a report with a value of said first detectable signal, a value of said detectable signal (outputting a report with a value of said first detectable signal, a value of said detectable signal; paragraph [024]; figure 1). Chromacode does not disclose and said time stamp. Lin discloses outputting a value for a time stamp (outputting a value for a time stamp; figure 2B; paragraph [0112]). It would have been obvious to a person of ordinary skill in the art, at the time the invention was made, to have modified the disclosure of Chromacode, to include outputting a value for a time stamp, as previously disclosed by Lin, in order to provide a superior method for monitoring the synthesis of a reaction mixture.

-***-Continued Within the Next Supplemental Box-***-

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4280

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US19/35129

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

-***-Continued from Box V: Citations and Explanations-***-

Claims 5-6 lack an inventive step under PCT Article 33(3) as being obvious over Chromacode in view of the publication entitled 'A Handheld Point-of-Care Genomic Diagnostic System' by Myers, et al. (hereinafter 'Myers').

As per claim 5, Chromacode discloses the method of claim 1, but Chromacode does not disclose obtaining a geo-tag. Myers discloses obtaining a geo-tag (capability to geotag genetic assay results and transmit these results over cell phone networks; page 7, first column, second paragraph). It would have been obvious to a person of ordinary skill in the art, at the time the invention was made, to have modified the disclosure of Chromacode, to include obtaining a geo-tag, as previously disclosed by Myers, in order to provide a superior method for monitoring the synthesis of a reaction mixture.

As per claim 6, Chromacode and Myers, in combination, disclose the method of claim 5, but Chromacode does not disclose wherein said geo-tag indicates a location of verification of synthesis or a location of synthesis. Lin discloses wherein said geo-tag indicates a location of verification of synthesis or a location of synthesis (capability to geotag genetic assay results (location of synthesis) and transmit these results over cell phone networks; page 6, second column; page 7, first column, second paragraph). It would have been obvious to a person of ordinary skill in the art, at the time the invention was made, to have modified the disclosure of Chromacode, to include wherein said geo-tag indicates a location of verification of synthesis or a location of synthesis, as previously disclosed by Myers, in order to provide a superior method for monitoring the synthesis of a reaction mixture.

Claim 1-6 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: Lee A. Johnson
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
United States of America

PCT

INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

(PCT Article 17(3)(a) and Rules 40.1 and 40.2(e))

Date of mailing *(day/month/year)*	19 AUG 2019
Applicant's or agent's file reference: 44158-708.601	**PAYMENT DUE** within **ONE MONTH** from the above date of mailing
International application No.: PCT/US19/35129	International filing date *(day/month/year)*: 03 June 2019 (03.06.2019)
Applicant: CHROMACODE, INC.	

1. This International Searching Authority

   (i) considers that there are 4 *(number of)* inventions claimed in the international application covered by the claims indicated below/on an extra sheet:

   -***-Please See Supplemental Page-***-

   (ii) therefore considers that **the international application does not comply with the requirement of unity of invention** (Rules 13.1, 13.2 and 13.3) for the reasons indicated below/on an extra sheet:

   -***-Please See Supplemental Page-***-

   (iii) ☐ has carried out a partial international search (see Annex) ☒ will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

   Group I, Claims 1-6

   (iv) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid.

2. Consequently, the applicant is hereby **invited to pay**, within the time limit indicated above, additional fees in the amount indicated below:

   $2080.00 (Fee per additional invention) x 3 (number of additional inventions) = $6,240.00 (See Item 2 of annex) (total amount of additional fees/currency)

3. The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fees may be made under protest**, that is, a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fees is excessive, where applicable, subject to the payment of a protest fee.

   Where the applicant pays additional fees under protest, the applicant is hereby invited, within the time limit indicated above, to pay a protest fee (Rule 40.2(e)) in the amount of _____ *(amount/currency)*

   Where the applicant has not, within the time limit indicated above, paid the required protest fee, the protest will be considered not to have been made and the International Searching Authority will so declare.

4. ☐ Claim(s) Nos. _____ have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300

Form PCT/ISA/206 (April 2005)

INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

International application No.
PCT/US19/35129

Item 2 (continued). For International Applications filed on or after 01 January 2014, Applicant is reminded that the search fee per additional invention indicated in item 2 is the undiscounted fee per additional invention. An Applicant may pay the search fee per additional invention fee reduced by 50% (small entity assertion) or 75% (micro entity certification), as appropriate. See 37 CFR 1.27 and 1.29.

-Continued from 1. (i):

Group I, Claims 1-6
Group II, Claims 7-17
Group III, Claims 18-67 and 95-114
Group IV, Claims 68-94

-Continued from 1. (ii):

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-6 are directed toward a method of verifying the synthesis of a chemical mixture.

Group II, Claims 7-17 are directed toward a method of tracking a batch of chemical mixture.

Group III, Claims 18-67 and 95-114 are directed toward a method and composition for watermarking a chemical composition, comprising a watermarking agent configured to produce a detectable signal upon application of reaction conditions.

Group IV, Claims 68-94 are directed toward a method of identifying a chemical composition, comprising calculating a ratio of a first signal to a second signal and comparing the ratio to a reference ratio.

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include verifying the synthesis of a chemical mixture, not present in any other Group; the special technical features of Group II include tracking a batch of chemical mixture, not present in any other Group; the special technical features of Group III include a watermarking agent configured to produce a detectable signal upon application of reaction conditions, not present in any other Group; the special technical features of Group IV include comparing a ratio of signals to a reference ratio to identify a chemical composition, not present in any other Group.

Groups I-IV share the technical features including: a method associated with a chemical mixture, said method comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture; and ii) a first watermarking agent configured to emit a first detectable signal; and b) detecting said first detectable signal. Groups I and IV share the further technical features including: ii) a second watermarking agent configured to emit a second detectable signal; b) and detecting said second detectable signal. Groups II and IV further share the technical features including: comparing a signal or ratio of signals to a reference.

However, these shared technical features are previously disclosed by WO 2017/173035 A1 to Chromacode, Inc. (hereinafter 'Chromacode').

Chromacode discloses a method associated with a chemical mixture (a method associated with a polymerase chain reaction chemical mixture; paragraphs [008], [018]), said method comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture (comprising a) providing or obtaining a chemical mixture comprising: i) a chemical mixture; paragraphs [008], [018]); and ii) a first watermarking agent configured to emit a first detectable signal (a first signal oligonucleotide (watermarking agent) configured to emit a first detectable signal; paragraph [008]); and b) detecting said first detectable signal (detecting said first detectable signal; paragraphs [008], [032]); ii) a second watermarking agent configured to emit a second detectable signal (a second signal oligonucleotide (watermarking agent) configured to emit a second detectable signal; paragraph [008]); detecting said second detectable signal (detecting said second detectable signal; paragraphs [008], [032]); and comparing a signal or ratio of signals to a reference (correlating the first signal to the first ratio (comparing a signal or ratio of signals to a reference); abstract, paragraph [008]).

Since none of the special technical features of the Groups I-IV inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Chromacode reference, unity of invention is lacking.

Form PCT/ISA/206 (extra sheet) (April 2005)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 44158-710601	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2019/040392	International filing date *(day/month/year)* 02 July 2019 (02.07.2019)	Priority date *(day/month/year)* 03 July 2018 (03.07.2018)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant CHROMACODE, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 18 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 05 January 2021 (05.01.2021)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Sun Hwa Lee e-mail: pct.team1@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: NICHOLAS TIEE
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	06 NOV 2019
Applicant's or agent's file reference 44158-710601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 19/40392	02 July 2019 (02.07.2019)	03 July 2018 (03.07.2018)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68, G01N 21/64, G06F 19/20 (2019.01)
CPC - C12Q 1/686, C12N 9/22, C12Q 1/6806, C12Q 1/6828

Applicant CHROMACODE, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	25 October 2019	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 19/40392

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 19/40392

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 53-63, 67-77, 83-92, 96, 100-101

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. ---please see below--- are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 53-63, 67-77, 83-92, 96, 100-101 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 53-63, 67-77, 83-92, 96, 100-101

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

Form PCT/ISA/237 (Box No. III) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 19/40392

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: claims 1-52, 64-66, 97-99, drawn to a method of detecting the presence or absence of at least three polynucleotide analytes in a sample.

Group II: claims 78-82, 93-95, drawn to an assay or a composition for detecting a plurality of polynucleotide analytes.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I includes the special technical feature of a method which differs from the special technical feature of a composition, as disclosed by Group II.

Common Technical Features

The inventions of Groups I and II share the technical feature of detecting the presence or absence of at least three polynucleotide analytes in a sample.

However, these shared technical features do not represent a contribution over prior art in view of US 2013/0040841 A1 to Saxonov et al. (hereinafter 'Saxonov').

Saxonov discloses (instant claim 1) a method of detecting the presence or absence of at least three polynucleotide analytes in a sample, in any combination of presence or absence (Abstract - "System, including methods and apparatus, for performing a digital assay with multiplexed detection of two or more distinct targets in the same optical channel."; para [0019] - "Concentrations for each target may be estimated based on the number of droplets positive for each target and the total number of droplets, for example, using Poisson statistics. Moreover, the relative numbers of different targets (including reference targets) may be estimated, allowing determination of copy number (CN), copy number variation (CNV), and the presence/abundance of single nucleotide polymorphisms (SNPs), among other quantities"), the method comprising:

-----please see continuation in first supplemental box-----

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-52, 64-66, 97-99

Form PCT/ISA/237 (Box No. IV) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 19/40392

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2-4, 11, 13, 18, 21, 22, 31-33, 37-39, 43, 45, 46, 49, 52, 97-99	YES
	Claims	1,5-10,12,14-17,19,20,23-30,34-36,40-42,44,47,48,50,51,64-66	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-52, 64-66, 97-99	NO
Industrial applicability (IA)	Claims	1-52, 64-66, 97-99	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1, 5-10, 12, 14-17, 19, 20, 23-30, 34-36, 40-42, 44, 47, 48, 50, 51 lack novelty under PCT Article 33(2) as being anticipated by US 2004/0191794 A1 to Weindel et al. (hereinafter 'Weindel').

Regarding claim 1, Weindel discloses a method of detecting the presence or absence of at least three polynucleotide analytes in a sample, in any combination of presence or absence (Abstract - "The present invention is directed to a method for the determination of at least 3 analytes using specific binding partners"; para [0039] - "The analytes are bound by specific binding partners. Depending on the analyte one has to choose specific binding partners...If a nucleic acid analyte should be determined one can use a nucleic acid sequence being complementary to the analyte as specific probe."), the method comprising:
(a) contacting a first subset of the sample with a first plurality of hybridization probes to generate a first cumulative signal measurement comprising one or more signals generated from the first plurality of hybridization probes (para [0016]-[0022] - "Therefore the present invention is related to a method for the determination of at least 3 analytes comprising the steps:...a) Providing a mixture of a sample containing said at least 3 analytes or suspected of containing one or more of said analytes and at least 3 different binding partners under conditions allowing the specific binding of said binding partners to said analytes...a first of said at least 3 binding partners is specific for a first of said at least 3 analytes and is coupled to a first label...a second of said at least 3 binding partners is specific for a second of said at least 3 analytes and is coupled to a second label, which label is separately detectable from the label coupled to said first binding partner, and...a third of said at least 3 binding partners specific for a third of said at least 3 analytes whereby a first amount of said third binding partner is coupled to the same label as coupled to said first binding partner and a second amount of said third binding partner is coupled to the same label as the second binding partner and said first amount of said third binding partner is not coupled to the same label as coupled to the second binding partner and said second amount of said third binding partner is not coupled to the same label as coupled to the first binding partner... b) Detecting the signal intensities indicative for said first and second label"), wherein the first cumulative signal measurement fails to non-degenerately identify the presence or absence of any combination of the at least three polynucleotide analytes (para [0051] - "three labels combined in different combinations can be coupled with up to seven different specific binding partners allowing the distinct determination of six different analytes...P stands for Parameter=analyte...The labeling of the different binding partners are: type P1.Label1 (signal in channel 1, P2.Label 2 (signal in channel 2), P3.label 3(signal in channel 3), P4.half amount label 1, half amount label 2, P5.half amount label 1, half amount label 3, P6.half amount label 2, half amount label 3 and P7.. amount label 1, . amount label 2, . amount label 3"; para [0053] - "A 50:50 signal ratio might also occur in case of the presence of two analytes...Thus, co-infections of 2 parameters indicated by single labeled probe, respectively, e.g. P1 and P2, should result in an uneven distribution of >>50: <<50:0 or <<50: >>50:0 and so be distinguishable from e.g. P4 indicated by an even distribution of 50:50:0. However such results could also derived from samples co-infected with two agents for example P4 and P1.");
(b) contacting one or more additional subsets of the sample with one or more additional pluralities of hybridization probes to generate one or more additional cumulative signal measurements each comprising one or more additional signals generated from the one or more additional plurality of hybridization probes, wherein each of the one or more additional cumulative signal measurements fails to non-degenerately identify the presence or absence of any combination of the at least three polynucleotide analytes (para [0054] - "In addition one can run an additional assay especially testing for the remaining possible analyte distributions which may be present in the sample. Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)"); and
(c) comparing the first cumulative signal measurement to the one or more additional cumulative signal measurements, wherein the comparing uniquely identifies any combination of the polynucleotide analytes in the sample, thereby detecting the presence or absence of the least three polynucleotide analytes in the sample, in any combination of presence or absence (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)").

Regarding claim 5, Weindel discloses the method of claim 1, wherein the first subset and the one or more additional subsets are each provided in a well (para [0005] - "As an alternative one can separate each binding reaction by conducting sequential reactions or using parallel reaction formats. Suitable formats are for example microtiterplate- or dot-blot-assays.").

Regarding claim 6, Weindel discloses the method of claim 1, wherein the number of unique hybridization probes in the first plurality of hybridization probes is less than or equal to the number of unique polynucleotide analytes in the at least three polynucleotide analytes (para [0011] - "The main aspect of the present invention is related to a method for determination of multiple analytes using less labels than analytes determined.")
-----please see continuation in third supplemental box-----

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 19/40392

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
Note: Claim 24, 25, 27, 29-30, 33, 35, 36, 39, 41-45, 47, 49, which depends from "The method of claim 14" is objected to. For the purposes of this application, claim 24, 25, 27, 29-30, 33, 35, 36, 39, 41-45, 47, 49 are construed as though depending from "The method of claim 23".

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 19/40392

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. IV Lack of unity of invention

(a) contacting a first subset of the sample with a first plurality of hybridization probes to generate a first cumulative signal measurement comprising one or more signals generated from the first plurality of hybridization probes (para [0023] - "FIG. 1 shows a flowchart of an exemplary method 40 of performing a digital assay."; para [0026] - "preparation of the sample may include combining the sample with reagents for amplification and for reporting whether or not amplification occurred....Accordingly, preparation of the sample may render the sample (or partitions thereof) capable of amplification of each of one or more targets, if present, in the sample (or a partition thereof)...The reporters each may be a labeled probe that includes a nucleic acid (e.g., an oligonucleotide) labeled with a luminophore, such as a fluorophore."; para [0028] - "The sample may be separated into partitions, indicated at 44."; para [0032]-[0033] - "One or more reactions may be performed in the partitions, indicated at 46...the reaction may be an amplification reaction, such as a polymerase chain reaction and/or ligase chain reaction."; para [0036] - "One or more signals may be created that are representative of light detected from the partitions, indicated at 48. The signal may represent an aspect of light, such as the intensity of the light"; para [0041] - "A number of partitions that are positive (or negative) for each target may be determined for the signal, indicated at 50. The signal detected from each partition, and the partition itself, may be classified as being positive or negative for each of the reactions/targets contributing to the signal. Classification may be based on the strength (and/or other suitable aspect) of the signal...Exemplary results for one, two, and three target systems in which data are collected in a single channel are shown in the following tables:...Population 8...Target A...+...Target B...+...Target C...+...Intensity...Highest...Population 7...Target A...+...Target B...+...Target C...-...Intensity...Intermediate...Population 1...Target A...-...Target B...-...Target C...-...Intensity...Lowest"), wherein the first cumulative signal measurement fails to non-degenerately identify the presence or absence of any combination of the at least three polynucleotide analytes (para [0041] - "The signal detected from each partition, and the partition itself, may be classified as being positive or negative for each of the reactions/targets contributing to the signal. Classification may be based on the strength (and/or other suitable aspect) of the signal...Exemplary results for one, two, and three target systems in which data are collected in a single channel are shown in the following tables:...Population 7...Target A...+...Target B...+...Target C...-...Intensity...Intermediate...Population 6...Target A...+...Target B...-...Target C...+...Intensity...Intermediate");
(b) contacting one or more additional subsets of the sample with one or more additional pluralities of hybridization probes to generate one or more additional cumulative signal measurements each comprising one or more additional signals generated from the one or more additional plurality of hybridization probes (para [0026] - "preparation of the sample may include combining the sample with reagents for amplification and for reporting whether or not amplification occurred....Accordingly, preparation of the sample may render the sample (or partitions thereof) capable of amplification of each of one or more targets, if present, in the sample (or a partition thereof)...The reporters each may be a labeled probe that includes a nucleic acid (e.g., an oligonucleotide) labeled with a luminophore, such as a fluorophore."; para [0028] - "The sample may be separated into partitions, indicated at 44."; para [0032]-[0033] - "One or more reactions may be performed in the partitions, indicated at 46...the reaction may be an amplification reaction, such as a polymerase chain reaction and/or ligase chain reaction."; para [0036] - "One or more signals may be created that are representative of light detected from the partitions, indicated at 48. The signal may represent an aspect of light, such as the intensity of the light"; para [0041] - "The signal detected from each partition, and the partition itself, may be classified as being positive or negative for each of the reactions/targets contributing to the signal. Classification may be based on the strength (and/or other suitable aspect) of the signal...Exemplary results for one, two, and three target systems in which data are collected in a single channel are shown in the following tables:...Population 8...Target A...+...Target B...+...Target C...+...Intensity...Highest...Population 7...Target A...+...Target B...+...Target C...-...Intensity...Intermediate...Population 1...Target A...-...Target B...-...Target C...-...Intensity...Lowest"), wherein each of the one or more additional cumulative signal measurements fails to non-degenerately identify the presence or absence of any combination of the at least three polynucleotide analytes (para [0041] - "The signal detected from each partition, and the partition itself, may be classified as being positive or negative for each of the reactions/targets contributing to the signal. Classification may be based on the strength (and/or other suitable aspect) of the signal...Exemplary results for one, two, and three target systems in which data are collected in a single channel are shown in the following tables:...Population 7...Target A...+...Target B...+...Target C...-...Intensity...Intermediate...Population 6...Target A...+...Target B...-...Target C...+...Intensity...Intermediate"); and
(c) comparing the first cumulative signal measurement to the one or more additional cumulative signal measurements, wherein the comparing uniquely identifies any combination of the polynucleotide analytes in the sample, thereby detecting the presence or absence of the least three polynucleotide analytes in the sample, in any combination of presence or absence (para [0043] - "A level of each target may be determined, indicated at 52. The level of each target may be determined based on the respective numbers of partitions positive for the target alone and for the target in combination with any other target(s)").

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

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Regarding claim 7, Weindel discloses the method of claim 1, wherein the number of unique hybridization probes in each of the one or more additional plurality of hybridization probes is less than or equal to the number of unique polynucleotide analytes in the at least three polynucleotide analytes (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible).").

Regarding claim 8, Weindel discloses the method of claim 1, wherein the first plurality of hybridization probes and the one or more additional plurality of hybridization probes are oligonucleotide probes (para [0039] - "The analytes are bound by specific binding partners. Depending on the analyte one has to choose specific binding partners...If a nucleic acid analyte should be determined one can use a nucleic acid sequence being complementary to the analyte as specific probe.").

Regarding claim 9, Weindel discloses the method of claim 1, wherein the first plurality of hybridization probes and the one or more additional plurality of hybridization probes each comprise a fluorophore (para [0045]- "Labels are generally known to those skilled in the art as being a group which is detectable or can be made detectable for determination the presence of an analyte. Well-known labels are fluorescent labels").

Regarding claim 10, Weindel discloses the method of claim 9, but does not specifically teach generating the first cumulative signal measurement and the one or more additional cumulative signal measurements comprises exciting the fluorophores and detecting the signals generated from each fluorophore (para [0103] - "detection probes with 2 particular chemical modification are added to the PCR mastermix. One of these modifications is a fluorogenic reporter group (R, for instance a derivative of 6-carboxy-fluorescein) covalently attached to the backbone of the probe, the other one is a dye (for instance a polymethine-cyanine derivative) capable of absorbing the fluorescent light of the reporter and to quench it (quencher, Q)...As soon as primer has annealed to the target and DNA-polymerase gets bound to the primer: target hybrid, elongation starts. Due to the 5'-nuclease activity of the enzyme, simultaneously with copy strand synthesis the probe is cleaved as soon as the polymerase reaches the probe binding site, reporter and quencher get separated, and the fluorescent signal becomes measurable. This process is repeated with every cycle, and more and more fluorescent reporter is accumulated in solution until reagent depletion at the end of the reaction"; note the detection of fluorescence from fluorophores requires excitation of the fluorophore").

Regarding claim 12, Weindel discloses the method of claim 1, wherein each hybridization probe of the first plurality of hybridization probes and the one or more additional plurality of hybridization probes comprises the same fluorophore (para [0016]-[0020] - "Therefore the present invention is related to a method for the determination of at least 3 analytes comprising the steps: a) Providing a mixture of a sample containing said at least 3 analytes or suspected of containing one or more of said analytes and at least 3 different binding partners under conditions allowing the specific binding of said binding partners to said analytes, wherein...a first of said at least 3 binding partners is specific for a first of said at least 3 analytes and is coupled to a first label...a second of said at least 3 binding partners is specific for a second of said at least 3 analytes and is coupled to a second label, which label is separately detectable from the label coupled to said first binding partner, and a third of said at least 3 binding partners specific for a third of said at least 3 analytes whereby a first amount of said third binding partner is coupled to the same label as coupled to said first binding partner and a second amount of said third binding partner is coupled to the same label as the second binding partner and said first amount of said third binding partner is not coupled to the same label as coupled to the second binding partner and said second amount of said third binding partner is not coupled to the same label as coupled to the first binding partner").

Regarding claim 14, Weindel discloses the method of claim 1, wherein the at least three polynucleotide analytes are at least seven polynucleotide analytes (para [0015] - "It should be noted that also an increased number of analytes may be determined using three or even more labels which can be combined in the same way as shown for the two labels. When using three labels up to seven analytes may be determined. Using four different labels even 15 analytes can be detected if applied singular as well as in dual, triplicate and quadruplicate combinations.").

Regarding claim 15, Weindel discloses the method of claim 1, wherein generating the first cumulative signal measurement and the one or more additional cumulative signal measurements comprises polymerase chain reaction (PCR) (para [0011] - "The main aspect of the present invention is related to a method for determination of multiple analytes using less labels than analytes determined...Preferably by performing homogeneous (solution-phase) real-time amplification assays (for example by PCR or TMA)").

Regarding claim 16, Weindel discloses the method of claim 1, wherein first cumulative signal measurement and the one or more additional cumulative signal measurements comprise a signal intensity (para [0021] - "b) Detecting the signal intensities indicative for said first and second label").

Regarding claim 17, Weindel discloses the method of claim 1, wherein the first cumulative signal measurement comprises an ambiguity, and wherein the one or more additional cumulative signal measurements resolves the ambiguity (para [0054] - "In addition one can run an additional assay especially testing for the remaining possible analyte distributions which may be present in the sample. Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible).").

Regarding claim 19, Weindel discloses the method of claim 1, wherein each of the first plurality of hybridization probes and the one or more additional pluralities of hybridization probes corresponds to one of the at least three polynucleotide analytes (para [0001] - "This invention is directed to methods for the determination of multiple analytes using analyte specific binding partners wherein the binding partners are labeled with less different labels than different binding partners used.").

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Regarding claim 20, Weindel discloses the method of claim 1, wherein each of the first plurality of hybridization probes and the one or more additional pluralities of hybridization probes has complementarity to one of the at least three polynucleotide analytes (para [0039] - "The analytes are bound by specific binding partners...If a nucleic acid analyte should be determined one can use a nucleic acid sequence being complementary to the analyte as specific probe.").

Regarding claim 23, Weindel discloses a method of detecting the presence or absence of at least three polynucleotide analytes in a sample, in any combination of presence or absence (Abstract - "The present invention is directed to a method for the determination of at least 3 analytes using specific binding partners"; para [0039] - "The analytes are bound by specific binding partners. Depending on the analyte one has to choose specific binding partners...If a nucleic acid analyte should be determined one can use a nucleic acid sequence being complementary to the analyte as specific probe."), the method comprising:
(a) providing a first and second sample solution volume, each comprising, or potentially comprising, the at least three polynucleotide analytes (para [0016]-[0022]);
(b) contacting the first sample solution volume with a first plurality of hybridization probes, each corresponding to one of the polynucleotide analytes, to generate a first cumulative signal measurement comprising one or more signals generated from the first plurality of hybridization probes (para [0016]-[0022] - "Therefore the present invention is related to a method for the determination of at least 3 analytes comprising the steps:...a) Providing a mixture of a sample containing said at least 3 analytes or suspected of containing one or more of said analytes and at least 3 different binding partners under conditions allowing the specific binding of said binding partners to said analytes...a first of said at least 3 binding partners is specific for a first of said at least 3 analytes and is coupled to a first label...a second of said at least 3 binding partners is specific for a second of said at least 3 analytes and is coupled to a second label, which label is separately detectable from the label coupled to said first binding partner, and...a third of said at least 3 binding partners specific for a third of said at least 3 analytes whereby a first amount of said third binding partner is coupled to the same label as coupled to said first binding partner and a second amount of said third binding partner is coupled to the same label as the second binding partner and said first amount of said third binding partner is not coupled to the same label as coupled to the second binding partner and said second amount of said third binding partner is not coupled to the same label as coupled to the first binding partner... b) Detecting the signal intensities indicative for said first and second label"), wherein the first cumulative signal measurement fails to non-degenerately identify the presence or absence of any combination of the at least three polynucleotide analytes (para [0051] - "three labels combined in different combinations can be coupled with up to seven different specific binding partners allowing the distinct determination of six different analytes...P stands for Parameter=analyte...The labeling of the different binding partners are: type P1.Label1 (signal in channel 1, P2.Label 2 (signal in channel 2), P3.label 3(signal in channel 3), P4.half amount label 1, half amount label 2, P5.half amount label 1, half amount label 3, P6.half amount label 2, half amount label 3 and P7.1/3 amount label 1, 1/3 amount label 2, 1/3 amount label 3"; para [0053] - "A 50:50 signal ratio might also occur in case of the presence of two analytes...Thus, co-infections of 2 parameters indicated by single labeled probe, respectively, e.g. P1 and P2, should result in an uneven distribution of >>50: <<50:0 or <<50: >>50:0 and so be distinguishable from e.g. P4 indicated by an even distribution of 50:50:0. However such results could also derived from samples co-infected with two agents for example P4 and P1.");
(c) contacting the second sample solution volume with a second plurality of hybridization probes, each corresponding to one of the polynucleotide analytes, to generate a second cumulative signal measurement comprising one or more signals generated from the second plurality of hybridization probes, wherein the second cumulative signal measurement fails to non-degenerately identify the presence or absence of any combination of the at least three polynucleotide analytes (para [0054] - "In addition one can run an additional assay especially testing for the remaining possible analyte distributions which may be present in the sample. Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)"); and
(d) comparing the first cumulative signal measurement to the second cumulative signal measurement, wherein the comparing uniquely identifies any combination of polynucleotide analytes in the sample, thereby detecting the presence or absence of at least three polynucleotide analytes in a sample, in any combination of presence or absence (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)").

Regarding claim 24, Weindel discloses the method of claim 23, wherein the first and second sample solution volume are derived from the sample (para [0054] - "In addition one can run an additional assay especially testing for the remaining possible analyte distributions which may be present in the sample. Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)").

Regarding claim 25, Weindel discloses the method of claim 23, wherein the number of unique hybridization probes in the first plurality of hybridization probes is less than or equal to the number of unique polynucleotide analytes in the at least three polynucleotide analytes (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)").

Regarding claim 26, Weindel discloses the method of claim 25, wherein the number of unique hybridization probes in the first plurality of hybridization probes is equal to the number of unique polynucleotide analytes in the at least three polynucleotide analytes (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)").

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Regarding claim 27, Weindel discloses the method of claim 23, wherein the number of unique hybridization probes in the second plurality of hybridization probes is less than or equal to the number of unique polynucleotide analytes in the at least three polynucleotide analytes (para [0011] - "The main aspect of the present invention is related to a method for determination of multiple analytes using less labels than analytes determined.").

Regarding claim 28, Weindel discloses the method of claim 27, wherein the number of unique hybridization probes in the second plurality of hybridization probes is less than the number of unique polynucleotide analytes in the at least three polynucleotide analytes (para [0011] - "The main aspect of the present invention is related to a method for determination of multiple analytes using less labels than analytes determined.").

Regarding claim 29, Weindel discloses the method of claim 23, wherein the first plurality of hybridization probes are oligonucleotide probes (para [0039] - "The analytes are bound by specific binding partners. Depending on the analyte one has to choose specific binding partners...If a nucleic acid analyte should be determined one can use a nucleic acid sequence being complementary to the analyte as specific probe.").

Regarding claim 30, Weindel discloses the method of claim 30, wherein the first plurality of hybridization probes each comprise a fluorophore (para [0045]- "Labels are generally known to those skilled in the art as being a group which is detectable or can be made detectable for determination the presence of an analyte. Well-known labels are fluorescent labels").

Regarding claim 34, Weindel discloses the method of claim 30, wherein generating the first cumulative signal measurement comprises exciting the fluorophores and detecting the signals generated from each fluorophore fluorophore (para [0103] - "detection probes with 2 particular chemical modification are added to the PCR mastermix. One of these modifications is a fluorogenic reporter group (R, for instance a derivative of 6-carboxy-fluorescein) covalently attached to the backbone of the probe, the other one is a dye (for instance a polymethine-cyanine derivative) capable of absorbing the fluorescent light of the reporter and to quench it (quencher, Q)...As soon as primer has annealed to the target and DNA-polymerase gets bound to the primer: target hybrid, elongation starts. Due to the 5'-nuclease activity of the enzyme, simultaneously with copy strand synthesis the probe is cleaved as soon as the polymerase reaches the probe binding site, reporter and quencher get separated, and the fluorescent signal becomes measurable. This process is repeated with every cycle, and more and more fluorescent reporter is accumulated in solution until reagent depletion at the end of the reaction"; note the detection of fluorescence from fluorophores requires excitation of the fluorophore").

Regarding claim 35, Weindel discloses the method of claim 23, wherein the second plurality of hybridization probes are oligonucleotide probes (para [0039] - "The analytes are bound by specific binding partners. Depending on the analyte one has to choose specific binding partners...If a nucleic acid analyte should be determined one can use a nucleic acid sequence being complementary to the analyte as specific probe.").

Regarding claim 36, Weindel discloses the method of claim 23, wherein the second plurality of hybridization probes each comprise a fluorophore (para [0045]- "Labels are generally known to those skilled in the art as being a group which is detectable or can be made detectable for determination the presence of an analyte. Well-known labels are fluorescent labels").

Regarding claim 40, Weindel discloses the method of claim 36, wherein generating the second cumulative signal measurement comprises exciting the fluorophores and detecting the signals generated from each fluorophore fluorophore (para [0103] - "detection probes with 2 particular chemical modification are added to the PCR mastermix. One of these modifications is a fluorogenic reporter group (R, for instance a derivative of 6-carboxy-fluorescein) covalently attached to the backbone of the probe, the other one is a dye (for instance a polymethine-cyanine derivative) capable of absorbing the fluorescent light of the reporter and to quench it (quencher, Q)...As soon as primer has annealed to the target and DNA-polymerase gets bound to the primer: target hybrid, elongation starts. Due to the 5'-nuclease activity of the enzyme, simultaneously with copy strand synthesis the probe is cleaved as soon as the polymerase reaches the probe binding site, reporter and quencher get separated, and the fluorescent signal becomes measurable. This process is repeated with every cycle, and more and more fluorescent reporter is accumulated in solution until reagent depletion at the end of the reaction"; note the detection of fluorescence from fluorophores requires excitation of the fluorophore).

Regarding claim 41, Weindel discloses the method of claim 23, wherein the at least three polynucleotide analytes are at least five polynucleotide analytes (para [0015] - "It should be noted that also an increased number of analytes may be determined using three or even more labels which can be combined in the same way as shown for the two labels. When using three labels up to seven analytes may be determined. Using four different labels even 15 analytes can be detected if applied singular as well as in dual, triplicate and quadruplicate combinations.").

Regarding claim 42, Weindel discloses the method of claim 23, wherein the at least three polynucleotide analytes are at least seven polynucleotide analytes (para [0015] - "It should be noted that also an increased number of analytes may be determined using three or even more labels which can be combined in the same way as shown for the two labels. When using three labels up to seven analytes may be determined. Using four different labels even 15 analytes can be detected if applied singular as well as in dual, triplicate and quadruplicate combinations.").

Regarding claim 44, Weindel discloses the method of claim 23, wherein the first plurality of hybridization probes and the second plurality of hybridization probes are different (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)").

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Regarding claim 47, Weindel discloses the method of claim 23, wherein the first cumulative signal measurement comprises an ambiguity, and wherein the second cumulative signal measurement resolves the ambiguity of the first cumulative signal measurement (para [0054] - "In addition one can run an additional assay especially testing for the remaining possible analyte distributions which may be present in the sample. Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible).").

Regarding claim 48, Weindel discloses the method of claim 47, wherein the ambiguity is a signal that fails to unambiguously identify a single combination of polynucleotide analytes of the at least three polynucleotide analytes (para [0053] - "A 50:50 signal ratio might also occur in case of the presence of two analytes...Thus, co-infections of 2 parameters indicated by single labeled probe, respectively, e.g. P1 and P2, should result in an uneven distribution of >>50: <<50:0 or <<50: >>50:0 and so be distinguishable from e.g. P4 indicated by an even distribution of 50:50:0. However such results could also derived from samples co-infected with two agents for example P4 and P1.").

Regarding claim 50, Weindel discloses a method of detecting the presence or absence of a plurality of polynucleotide analytes in a sample, in any combination of presence or absence (Abstract - "The present invention is directed to a method for the determination of at least 3 analytes using specific binding partners"; para [0039] - "The analytes are bound by specific binding partners. Depending on the analyte one has to choose specific binding partners...If a nucleic acid analyte should be determined one can use a nucleic acid sequence being complementary to the analyte as specific probe."), the method comprising:
(a) providing a first and second sample solution volume, each comprising, or potentially comprising, the plurality of polynucleotide analytes (para [0016]-[0022]);
(b) contacting the first sample solution volume with a first plurality of hybridization probes to generate a first cumulative signal measurement that fails to nondegenerately indicate the presence or absence of any combination of the plurality of analytes (para [0016]-[0022] - "Therefore the present invention is related to a method for the determination of at least 3 analytes comprising the steps:...a) Providing a mixture of a sample containing said at least 3 analytes or suspected of containing one or more of said analytes and at least 3 different binding partners under conditions allowing the specific binding of said binding partners to said analytes...a first of said at least 3 binding partners is specific for a first of said at least 3 analytes and is coupled to a first label...a second of said at least 3 binding partners is specific for a second of said at least 3 analytes and is coupled to a second label, which label is separately detectable from the label coupled to said first binding partner, and...a third of said at least 3 binding partners specific for a third of said at least 3 analytes whereby a first amount of said third binding partner is coupled to the same label as coupled to said first binding partner and a second amount of said third binding partner is coupled to the same label as the second binding partner and said first amount of said third binding partner is not coupled to the same label as coupled to the second binding partner and said second amount of said third binding partner is not coupled to the same label as coupled to the first binding partner... b) Detecting the signal intensities indicative for said first and second label"; para [0051] - "three labels combined in different combinations can be coupled with up to seven different specific binding partners allowing the distinct determination of six different analytes...P stands for Parameter=analyte...The labeling of the different binding partners are: type P1.Label1 (signal in channel 1, P2.Label 2 (signal in channel 2), P3.label 3(signal in channel 3), P4.half amount label 1, half amount label 2, P5.half amount label 1, half amount label 3, P6.half amount label 2, half amount label 3 and P7.1/3 amount label 1, 1/3 amount label 2, 1/3 amount label 3"; para [0053] - "A 50:50 signal ratio might also occur in case of the presence of two analytes...Thus, co-infections of 2 parameters indicated by single labeled probe, respectively, e.g. P1 and P2, should result in an uneven distribution of >>50: <<50:0 or <<50: >>50:0 and so be distinguishable from e.g. P4 indicated by an even distribution of 50:50:0. However such results could also derived from samples co-infected with two agents for example P4 and P1.");
(c) contacting the second sample solution volume with a second plurality of hybridization probes to generate a second cumulative signal measurement that fails to non-degenerately indicate the presence or absence of any combination of the plurality of analytes (para [0054] - "In addition one can run an additional assay especially testing for the remaining possible analyte distributions which may be present in the sample. Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)"); and
(d) comparing the first cumulative signal measurement to the second cumulative signal measurement, thereby non-degenerately indicating the presence or absence of the plurality of analytes, in any combination of presence or absence (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)").

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Regarding claim 51, Weindel discloses a method of detecting the presence or absence of a plurality of polynucleotide analytes in a sample solution volume, in any combination of presence or absence, the method comprising:
(a) contacting the sample solution volume with a first plurality of hybridization probes to generate a first cumulative signal measurement that fails to nondegenerately indicate the presence or absence of any combination of the plurality of analytes (para [0016]-[0022] - "Therefore the present invention is related to a method for the determination of at least 3 analytes comprising the steps:...a) Providing a mixture of a sample containing said at least 3 analytes or suspected of containing one or more of said analytes and at least 3 different binding partners under conditions allowing the specific binding of said binding partners to said analytes...a first of said at least 3 binding partners is specific for a first of said at least 3 analytes and is coupled to a first label...a second of said at least 3 binding partners is specific for a second of said at least 3 analytes and is coupled to a second label, which label is separately detectable from the label coupled to said first binding partner, and...a third of said at least 3 binding partners specific for a third of said at least 3 analytes whereby a first amount of said third binding partner is coupled to the same label as coupled to said first binding partner and a second amount of said third binding partner is coupled to the same label as the second binding partner and said first amount of said third binding partner is not coupled to the same label as coupled to the second binding partner and said second amount of said third binding partner is not coupled to the same label as coupled to the first binding partner... b) Detecting the signal intensities indicative for said first and second label"; para [0051] - "three labels combined in different combinations can be coupled with up to seven different specific binding partners allowing the distinct determination of six different analytes...P stands for Parameter=analyte...The labeling of the different binding partners are: type P1.Label1 (signal in channel 1, P2.Label 2 (signal in channel 2), P3.label 3(signal in channel 3), P4.half amount label 1, half amount label 2, P5.half amount label 1, half amount label 3, P6.half amount label 2, half amount label 3 and P7.1/3 amount label 1, 1/3 amount label 2, 1/3 amount label 3"; para [0053] - "A 50:50 signal ratio might also occur in case of the presence of two analytes...Thus, co-infections of 2 parameters indicated by single labeled probe, respectively, e.g. P1 and P2, should result in an uneven distribution of >>50: <<50:0 or <<50: >>50:0 and so be distinguishable from e.g. P4 indicated by an even distribution of 50:50:0. However such results could also derived from samples co-infected with two agents for example P4 and P1."), wherein the first plurality of hybridization probes are attached to fluorophores capable of being detected in a first signal channel (para [0051] - "three labels combined in different combinations can be coupled with up to seven different specific binding partners allowing the distinct determination of six different analytes...P stands for Parameter=analyte, a channel corresponds to an optical tract as part of a detector optimised to capture the signal of a particular detectable label.");
(b) contacting the sample solution volume with a second plurality of hybridization probes to generate a second cumulative signal measurement that fails to nondegenerately indicate the presence or absence of any combination of the plurality of analytes wherein the second plurality of hybridization probes are attached to fluorophores capable of being detected in a second signal channel (para [0054] - "In addition one can run an additional assay especially testing for the remaining possible analyte distributions which may be present in the sample. Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)"); and
(c) comparing the first cumulative signal measurement to the second cumulative signal measurement, thereby non-degenerately indicating the presence or absence of the plurality of analytes, in any combination of presence or absence (para [0054] - "In addition one can run an additional assay especially testing for the remaining possible analyte distributions which may be present in the sample. Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible").

Claims 64-66 lack novelty under PCT Article 33(2) as being anticipated by US 2015/0057178 A1 to California Institute of Technology (hereinafter 'Caltech').

Regarding claim 64, Caltech discloses a method of detecting the presence or absence of a plurality of polynucleotide analytes in a sample solution volume (Abstract - "this disclosure provides methods, systems, compositions, and kits wherein multiple analytes may be detected in a single sample volume by acquiring a cumulative measurement or measurements of at least one quantifiable component of a signal...Each signal or component of a signal may be used to construct a coding scheme which can then be used to determine the presence or absence of any analyte."), the method comprising:
(a) providing the sample solution volume comprising, or potentially comprising, the plurality of polynucleotide analytes (para [0034] - "this disclosure provides systems for detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy; (b) providing a sample comprising, or potentially comprising, at least one of said analytes");
(b) contacting the sample solution volume with a plurality of hybridization probes and exciting the hybridization probes to generate a cumulative signal measurement if one or more of the plurality of polynucleotide analytes is present in the sample solution volume (para [0034] - "(c) contacting said sample with analyte-specific reagents that generate said at least one first value and said at least one second value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement");
(c) receiving a set of information regarding the polynucleotide analytes; and
(d) comparing the cumulative signal measurement to the set of information, wherein the results of the comparing resolve the ambiguity, thereby detecting the presence or absence of the plurality of polynucleotide analytes (para [0034] - "(e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme, wherein, when degeneracy is eliminated, said system is capable of unambiguously detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence, when each of said reagents generates only one second value."; Fig. 3; Fig. 8).

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Regarding claim 65, Caltech discloses the method of claim 64, wherein the method does not require any step of immobilization of the polynucleotide analytes, microscopy, flow cytometry, physical separation of the polynucleotide analytes, mass spectrometry, or melting curve analysis (para [0003] - "this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis.").

Regarding claim 66, Caltech discloses the method of claim 64 or 65, wherein the plurality of polynucleotide analytes comprises at least three polynucleotide analytes (para [0003] - "This disclosure provides methods, compositions, systems, and kits for the multiplexed detection of analytes. In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes"; para [0107] - "In some cases, an analyte may be a polynucleotide").

Claims 2, 3, 13, 33, 39, 43, 45, 46 lack an inventive step under PCT Article 33(3) as being obvious over Weindel.

Regarding claim 2, Weindel discloses the method of claim 1, but does not teach the first subset and the one or more additional subsets are each in the same solution volume. Since it was commonly known in the art the starting volume of a reaction depends on the concentration of the target molecules, it would have been obvious to one of ordinary skill in the art the solution volumes can be the same because the solution volume depends on the concentrations of target molecules within each subset.

Regarding claim 3, Weindel discloses the method of claim 1, wherein the first subset and the one or more additional subsets are each in different solution volumes. Since it was commonly known in the art the starting volume of a reaction depends on the concentration of the target molecules, it would have been obvious to one of ordinary skill in the art the solution volumes can be different because the solution volume depends on the concentrations of target molecules within each subset.

Regarding claim 13, Weindel discloses the method of claim 1, but does not specifically teach hybridization probe of the first plurality of hybridization probes and the one or more additional plurality of hybridization probes is provided at a different concentration. However, Weindel teaches the second subset of the sample is exposed to a different set of probes (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)."). It would have been obvious to one of ordinary skill in the art the one or more additional plurality of hybridization probes can be different from the first plurality of hybridization probes because the composition of probes is different.

Regarding claim 33, Weindel discloses the method of claim 23, but does not teach each hybridization probe of the first plurality of hybridization probes is provided at a different concentration. Since it was commonly known in the art of molecular biology to adjust the concentration of probes comprising fluorophores to attain desirable fluorescent intensity, it would have been obvious to one of ordinary skill in the art each hybridization probe of the first plurality of hybridization probes can have a different concentration to achieve a desirable fluorescent intensity.

Regarding claim 39, Weindel discloses the method of claim 23, but does not teach each hybridization probe of the second plurality of hybridization probes is provided at a different concentration. Since it was commonly known in the art of molecular biology to adjust the concentration of probes comprising fluorophores to attain desirable fluorescent intensity, it would have been obvious to one of ordinary skill in the art each hybridization probe of the first plurality of hybridization probes can have a different concentration to achieve a desirable fluorescent intensity.

Regarding claim 43, Weindel discloses the method of claim 23, but does not teach the first plurality of hybridization probes and the second plurality of hybridization probes are the same. However, Weindel teaches the second reaction is used for a re-run to resolve the ambiguity of the first reaction (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)"). It would have been obvious one of ordinary skill in the art the first and second hybridization probes can be the same because the choice of combinations of probes used in the first and second reactions depends on the results of the first reaction for resolving ambiguity.

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Regarding claim 45, Weindel discloses the method of claim 23, wherein the first plurality of hybridization probes comprises (i) a first hybridization probe corresponding to a first polynucleotide analyte of the at least three polynucleotide analytes and (ii) a second hybridization probe corresponding to a second polynucleotide analyte of the at least three polynucleotide analytes (para [0051] - "Considered is the case that of the analytes P1 to P7 only one at a time would be present in a sample. The labeling of the different binding partners are: type P1.Label1 (signal in channel 1, P2.Label 2 (signal in channel 2)"), but does not teach the second plurality of hybridization probes comprises (1) a third hybridization probe corresponding to the first polynucleotide analyte of the at least three polynucleotide analytes and (2) a fourth hybridization probe corresponding to a third polynucleotide analyte. However, Weindel teaches the second reaction is used for a re-run to resolve the ambiguity of the first reaction (para [0054] - "Such a sequential method is very economical as it allows to determine a distinct analyte distribution in most samples in a first run, whereas ambiguous samples can be re-evaluated in a second run using for example a multiple analyte assay with a reduced number of analytes (for which an unambiguous one label--one binding partner combination should be possible)"). It would have been obvious one of ordinary skill in the art the second plurality of hybridization probes comprises (1) a third hybridization probe corresponding to the first polynucleotide analyte of the at least three polynucleotide analytes and (2) a fourth hybridization probe corresponding to a third polynucleotide analyte because the choice of combinations of probes used in the first and second reactions depends on the results of the first reaction for resolving ambiguity.

Regarding claim 46, Weindel makes obvious the method of claim 45, but does not teach the third hybridization probe and the first hybridization probe are provided at different concentrations. Since it was commonly known in the art of molecular biology to adjust the concentration of probes comprising fluorophores to attain desirable fluorescent intensity, it would have been obvious to one of ordinary skill in the art the third hybridization probe and the first hybridization probe are provided at different concentrations to achieve a desirable fluorescent intensity.

Claims 97-99 lack an inventive step under PCT Article 33(3) as being obvious over Caltech.

Regarding claim 97, Caltech discloses a method of non-degenerately detecting the presence or absence of a plurality of polynucleotide analytes, in any combination of presence or absence (Abstract - "this disclosure provides methods, systems, compositions, and kits wherein multiple analytes may be detected in a single sample volume by acquiring a cumulative measurement or measurements of at least one quantifiable component of a signal...Each signal or component of a signal may be used to construct a coding scheme which can then be used to determine the presence or absence of any analyte."), comprising:
(a) providing a sample solution volume comprising, or potentially comprising, at least one of the plurality of polynucleotide analytes (para [0034] - "this disclosure provides systems for detecting the presence or absence of each analyte of a plurality of analytes, comprising: (a) encoding each of said analytes as at least one first value and at least one second value, wherein said first value is a value from a first component of a signal and said second value is a value from a second component of said signal, thereby generating a coding scheme, wherein each of said analytes is represented in said coding scheme by said at least one first value and said at least one second value, wherein said encoding is performed in a manner that reduces or eliminates degeneracy; (b) providing a sample comprising, or potentially comprising, at least one of said analytes");
(b) providing a plurality of hybridization probes, each comprising at least one fluorophore, wherein each hybridization probe generates a cumulative intensity signal (para [0034]); and
(c) contacting the sample solution volume with the plurality of hybridization probes to generate the cumulative signal measurement, thereby non-degenerately detecting the presence or absence of the plurality of analytes, in any combination of presence or absence (para [0034] - "(c) contacting said sample with analyte-specific reagents that generate said at least one first value and said at least one second value, as represented in said coding scheme, when each of said analytes is present; (d) cumulatively measuring said first values and said second values within said sample, thereby providing a cumulative measurement; e) determining whether each of said analytes is present or absent based on said cumulative measurement and said coding scheme, wherein, when degeneracy is eliminated, said system is capable of unambiguously detecting the presence or absence of each of at least six analytes in a single volume, in any combination of presence or absence."),
but does not teach wherein a cumulative signal measurement generated from the cumulative intensity signals from the plurality of hybridization probes forms a Sidon sequence. Since it was commonly known in the art Sidon sequence is a sequence of integer in which the pairwise sums of the sequence are distinct, it would have been obvious to one of ordinary skill in the art to make use of Sidon sequence in the method of Caltech in order to generate an unambiguous coding scheme for the potential analyte combinations.

Regarding claim 98, Caltech makes obvious the method of claim 97, and Caltech teaches the method does not require any step of immobilization of the polynucleotide analytes, physical separation of the polynucleotide analytes, mass spectrometry, or melting curve analysis (para [0003] - "this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis.").

Regarding claim 99, Caltech makes obvious the method of claim 97 or 98, and Caltech teaches the plurality of polynucleotide analytes is at least seven polynucleotide analytes (para [0003] - "This disclosure provides methods, compositions, systems, and kits for the multiplexed detection of analytes. In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes"; para [0107] - "In some cases, an analyte may be a polynucleotide").

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Claims 4, 18, 21, 22, 49, 52 lack an inventive step under PCT Article 33(3) as being obvious over Weindel, in view of Caltech.

Regarding claim 4, Weindel discloses the method of claim 1, but does not teach the first subset and the one or more additional subsets are each provided in a plurality of partitions.
Weindel teaches the presence or absence of analytes is detected through PCR (para [0011] - "The main aspect of the present invention is related to a method for determination of multiple analytes using less labels than analytes determined...Preferably by performing homogeneous (solution-phase) real-time amplification assays (for example by PCR or TMA)").
Caltech teaches a method of determining the presence or absence of at least three analytes with the use of droplet digital PCR (Abstract - "This disclosure provides methods, systems, compositions, and kits for the multiplexed detection of a plurality of analytes in a sample... this disclosure provides methods, systems, compositions, and kits wherein multiple analytes may be detected in a single sample volume by acquiring a cumulative measurement or measurements of at least one quantifiable component of a signal...Each signal or component of a signal may be used to construct a coding scheme which can then be used to determine the presence or absence of any analyte."; para [0156] - "The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, end point PCR methods, reverse transcriptase PCR, and digital PCR methods. Digital PCR methods (e.g., DROPLET DIGITAL (BIORAD) and DYNAMIC ARRAY (FLUIDIGM)) produce highly sensitive quantification of polynucleotide copy numbers. The methods provided herein can be easily integrated into these systems to significantly expand their throughput by allowing multiplexing in a droplet or a dynamic array."). It would have been obvious to one of ordinary skill in the art the subsets can be provided in a plurality of partitions in form of droplets because Caltech teaches a method of detecting multiple analytes through droplet digital PCR.

Regarding claim 18, Weindel discloses the method of claim 1, but does not specifically teach the method does not require any step of immobilization of the polynucleotide analytes, microscopy, flow cytometry, physical separation of the polynucleotide analytes, mass spectrometry, or melting curve analysis. However, Weindel teaches the method determines the presence of multiple analytes by detecting the combinations of labels (Abstract - "The present invention is directed to a method for the determination of at least 3 analytes using specific binding partners. Combinations of detectable labels are coupled to the binding partners assuring that each binding partner molecule is coupled with only one detectable label.").
Caltech teaches a method of detecting the presence or absence of a plurality of analytes through cumulative measurements of signals and the method does not require immobilization of the polynucleotide analytes, microscopy, flow cytometry, physical separation of the polynucleotide analytes, mass spectrometry, or melting curve analysis (para [0003] - "this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis"). It would have been obvious to one of ordinary skill in the art the method of Weindel does not require immobilization, separation, mass spectrometry, or melting curve analysis because the method of Caltech which identifies the presence of targets by detecting the corresponding signals is performed without immobilization, separation, mass spectrometry, or melting curve analysis.

Regarding claim 21, Weindel discloses the method of claim 15, but does not teach the PCR is digital PCR. Caltech teaches a method of determining the presence or absence of at least three analytes with the use of digital PCR (Abstract - "This disclosure provides methods, systems, compositions, and kits for the multiplexed detection of a plurality of analytes in a sample... this disclosure provides methods, systems, compositions, and kits wherein multiple analytes may be detected in a single sample volume by acquiring a cumulative measurement or measurements of at least one quantifiable component of a signal...Each signal or component of a signal may be used to construct a coding scheme which can then be used to determine the presence or absence of any analyte."; para [0156] - "The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, end point PCR methods, reverse transcriptase PCR, and digital PCR methods"). It would have been obvious to one of ordinary skill in the art the PCR can be digital PCR, because Caltech teaches a method of identify a plurality of analytes through digital PCR.

Regarding claim 22, Weindel discloses the method of claim 15, wherein the PCR is quantitative PCR. Caltech teaches a method of determining the presence or absence of at least three analytes with the use of quantitative PCR (Abstract - "This disclosure provides methods, systems, compositions, and kits for the multiplexed detection of a plurality of analytes in a sample... this disclosure provides methods, systems, compositions, and kits wherein multiple analytes may be detected in a single sample volume by acquiring a cumulative measurement or measurements of at least one quantifiable component of a signal...Each signal or component of a signal may be used to construct a coding scheme which can then be used to determine the presence or absence of any analyte."; para [0156] - "The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods"). It would have been obvious to one of ordinary skill in the art the PCR can be quantitative PCR, because Caltech teaches a method of identify a plurality of analytes through quantitative PCR.

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Regarding claim 49, Weindel discloses the method of claim 14, but does not teach the method does not require any step of immobilization of the polynucleotide analytes, microscopy, flow cytometry, physical separation of the polynucleotide analytes, mass spectrometry, or melting curve analysis. However, Weindel teaches the method determines the presence of multiple analytes by detecting the combinations of labels (Abstract - "The present invention is directed to a method for the determination of at least 3 analytes using specific binding partners. Combinations of detectable labels are coupled to the binding partners assuring that each binding partner molecule is coupled with only one detectable label.").
Caltech teaches a method of detecting the presence or absence of a plurality of analytes through cumulative measurements of signals and the method does not require immobilization of the polynucleotide analytes, microscopy, flow cytometry, physical separation of the polynucleotide analytes, mass spectrometry, or melting curve analysis (para [0003] - "this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis"). It would have been obvious to one of ordinary skill in the art the method of Weindel does not require immobilization, separation, mass spectrometry, or melting curve analysis because the method of Caltech which identifies the presence of targets by detecting the corresponding signals is performed without immobilization, separation, mass spectrometry, or melting curve analysis.

Regarding claim 52, Weindel discloses the method of claim 50 or 51, but does not specifically teach the method does not require any step of immobilization of the polynucleotide analytes, microscopy, flow cytometry, mass spectrometry, or melting curve analysis. However, Weindel teaches the method determines the presence of multiple analytes by detecting the combinations of labels (Abstract - "The present invention is directed to a method for the determination of at least 3 analytes using specific binding partners. Combinations of detectable labels are coupled to the binding partners assuring that each binding partner molecule is coupled with only one detectable label.").
Caltech teaches a method of detecting the presence or absence of a plurality of analytes through cumulative measurements of signals and the method does not require immobilization of the polynucleotide analytes, microscopy, flow cytometry, physical separation of the polynucleotide analytes, mass spectrometry, or melting curve analysis (para [0003] - "this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis"). It would have been obvious to one of ordinary skill in the art the method of Weindel does not require immobilization, separation, mass spectrometry, or melting curve analysis because the method of Caltech which identifies the presence of targets by detecting the corresponding signals is performed without immobilization, separation, mass spectrometry, or melting curve analysis.

Claims 11, 31, 32, 37, 38 lack an inventive step under PCT Article 33(3) as being obvious over Weindel, in view of US US 2013/0040841 A1 to Saxonov et al. (hereinafter 'Saxonov').

Regarding claim 11, Weindel discloses the method of claim 1, but does not teach each hybridization probe of the first plurality of hybridization probes and the one or more additional plurality of hybridization probes comprises a fluorophore capable of being detected in the same optical channel.
Saxonov teaches the detection of presence of multiple targets using distinguishable fluorophores in the same optical channel (Abstract - "System, including methods and apparatus, for performing a digital assay with multiplexed detection of two or more distinct targets in the same optical channel."; para [0019] - "The present disclosure provides a system, including methods and apparatus, for performing a digital assay on a potentially greater number of targets through multiplexed detection of signals from reporters for two or more distinct targets in a common or shared channel ("the same optical channel"). The reporters may include the same fluorophore, such as FAM or VIC, or different fluorophores with similar spectral characteristics, so that light from two or more reporters may be collected simultaneously in the same optical channel. The assays may be constructed so that data for each target are distinguishable"). It would have been obvious to one of ordinary skill in the art each hybridization probe of the first plurality of hybridization probes can comprise a fluorophore capable of being detected in the same optical channel with the use of the method of Saxonov because Saxonov teaches the use of the same fluorophore or difference fluorophores with similar spectral features to allow the detection of presence of multiple analytes in a common optical channel.

Regarding claim 31, Weindel discloses the method of claim 30, but does not teach each hybridization probe of the first plurality of hybridization probes comprises a fluorophore capable of being detected in the same optical channel. Saxonov teaches the detection of presence of multiple targets using distinguishable fluorophores in the same optical channel (Abstract - "System, including methods and apparatus, for performing a digital assay with multiplexed detection of two or more distinct targets in the same optical channel."; para [0019] - "The present disclosure provides a system, including methods and apparatus, for performing a digital assay on a potentially greater number of targets through multiplexed detection of signals from reporters for two or more distinct targets in a common or shared channel ("the same optical channel"). The reporters may include the same fluorophore, such as FAM or VIC, or different fluorophores with similar spectral characteristics, so that light from two or more reporters may be collected simultaneously in the same optical channel. The assays may be constructed so that data for each target are distinguishable"). It would have been obvious to one of ordinary skill in the art each hybridization probe of the first plurality of hybridization probes can comprise a fluorophore capable of being detected in the same optical channel with the use of the method of Saxonov because Saxonov teaches the use of the same fluorophore or difference fluorophores with similar spectral features to allow the detection of presence of multiple analytes in a common optical channel.

Regarding claim 32, Weindel, in view of Saxonov, makes obvious the method of claim 31, and Saxonov teaches each hybridization probe of the first plurality of hybridization probes comprises the same fluorophore (para [0019] - "The reporters may include the same fluorophore, such as FAM or VIC").

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Regarding claim 37, Weindel discloses the method of claim 36, but does not teach each hybridization probe of the second plurality of hybridization probes comprises a fluorophore capable of being detected in the same optical channel.
Saxonov teaches the detection of presence of multiple targets using distinguishable fluorophores in the same optical channel (Abstract - "System, including methods and apparatus, for performing a digital assay with multiplexed detection of two or more distinct targets in the same optical channel."; para [0019] - "The present disclosure provides a system, including methods and apparatus, for performing a digital assay on a potentially greater number of targets through multiplexed detection of signals from reporters for two or more distinct targets in a common or shared channel ("the same optical channel"). The reporters may include the same fluorophore, such as FAM or VIC, or different fluorophores with similar spectral characteristics, so that light from two or more reporters may be collected simultaneously in the same optical channel. The assays may be constructed so that data for each target are distinguishable"). It would have been obvious to one of ordinary skill in the art the hybridization probe of the second plurality of hybridization probes can comprise a fluorophore capable of being detected in the same optical channel with the use of the method of Saxonov because Saxonov teaches the use of the same fluorophore or difference fluorophores with similar spectral features to allow the detection of presence of multiple analytes in a common optical channel.

Regarding claim 38, Weindel, in view of Saxonov, makes obvious the method of claim 37, and Saxonov teaches each hybridization probe of the first plurality of hybridization probes comprises the same fluorophore (para [0019] - "The reporters may include the same fluorophore, such as FAM or VIC").

Claims 1-52, 64-66, 97-99 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)
(PCT Rule 44bis.1(c))

To:

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Date of mailing *(day/month/year)* 21 January 2021 (21.01.2021)	
Applicant's or agent's file reference 44158-719601	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2019/041239	10 July 2019 (10.07.2019)	11 July 2018 (11.07.2018)

Applicant
CHROMACODE, INC.

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty).

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Form PCT/IB/326 (January 2004)

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 44158-719601	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2019/041239	International filing date *(day/month/year)* 10 July 2019 (10.07.2019)	Priority date *(day/month/year)* 11 July 2018 (11.07.2018)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant CHROMACODE, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 22 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 12 January 2021 (12.01.2021)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Agnès Wittmann-Regis e-mail: pct.team6@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: NICHOLAS TIEE
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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* 30 SEP 2019

Applicant's or agent's file reference	FOR FURTHER ACTION
44158-719601	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2019/041239	10 July 2019	11 July 2018

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/6851; G01N 21/64 (2019.01)
CPC - C12Q 1/6851; G01N 21/6486; G16B 25/20; G16B 40/10 (2019.08)

Applicant CHROMACODE, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	11 September 2019	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
   - [x] the international application in the language in which it was filed.
   - [ ] a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [ ] This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. [ ] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:
   - a. [ ] forming part of the international application as filed:
     - [ ] in the form of an Annex C/ST.25 text file.
     - [ ] on paper or in the form of an image file.
   - b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   - c. [ ] furnished subsequent to the international filing date for the purposes of international search only:
     - [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
     - [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-164	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-164	NO
Industrial applicability (IA)	Claims	1-164	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-23, 25-28, 30-33, 35-69, 71-74, 76-79, 81-108, 110-113, 115-118, 120-146, 148-151, 153-156, and 158-164 lack an inventive step under PCT Article 33(3) as being obvious over California Institute of Technology (hereinafter Caltech) in view of Loeffert et al. (hereinafter Loeffert).

Regarding Claim 1, Caltech discloses a method of quantifying at least a first and a second nucleic acid in a sample (This disclosure provides methods, systems, compositions, and kits for the detection of multiple analytes in a sample.... The analyte may be any suitable analyte, such as a polynucleotide, Para. [0055]; In some cases, an assay provided herein is quantitative, Para. [0026]), the method comprising: a. providing a mixture comprising: i. said first nucleic acid and said second nucleic acid; ii. a first detection probe configured to generate a first signal when in the presence of said first nucleic acid and when subjected to reaction conditions; iii. a second detection probe configured to generate a second signal when in the presence of said second nucleic acid and when subjected to reaction conditions (The compositions may comprise any... reaction mixture... described herein, Para. [0161]; This disclosure provides... compositions,... for the multiplexed detection of analytes, Para. [0003]; In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores, Para. [0012]; In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]); b. subjecting said mixture to said reaction conditions thereby generating said first and second signal (A series of reactions may be performed to label probes with at least one fluorophore and the reaction products may be mixed to generate a mixture of probes with different fluorophores, Para. [0143]; In some examples, the signal that is measured is generated during a polymerase chain reaction, Para. [0013]); c. measuring (i) an intensity of said first signal in first range of wavelengths, (ii) an intensity of said first signal in said second range of wavelengths, (iii) an intensity of said second signal in a third range of wavelengths (For example, building on the traditional methods described above, one color (e.g., blue) may be used to encode the presence of a control analyte that is always present in the sample. The control analyte may be added to the sample, or may be inherently present in the sample. The remaining colors (e.g., green, yellow, red) may be used to encode the presence of additional analytes. Table 2 shows an example of one such method, Para. [0076]; Table 2 discloses analytes A and B, where A has a first color blue and a second color red, and B has a first color blue and and second color yellow); d. generating a first data set derived from said intensity (i) and said intensity (ii) measured in c) and generating a second data set derived from said intensity (iii) measured in c) (In some cases, at least two components of a signal (e.g., intensity and wavelength) are cumulatively measured for the sample. This measurement can be performed, for example, by measuring the intensity at a particular wavelength or the intensity within a particular range of wavelengths, Para. [0058]; The terms "dimension" and "component," as used herein when referring to a signal, generally refer to an aspect of the signal that may be quantified. For example, if a signal is generated by a fluorescent molecule, it may be quantified in terms of its wavelength (e.g., a first dimension or component) and its intensity (e.g., a second dimension or component), Para. [0063]); and e. processing said generated first data set and said generated second data set, thereby quantifying said first and second nucleic acid (After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]; The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes, Para. [0064]; Similarly, the methods described in this disclosure may be applied in a real-time PCR assay....This additional data enables determination of the identity and starting quantity of each analyte, Para. [0157]).

Caltech fails to explicitly disclose wherein said processing uses reference quantification parameters derived from a reference data set(s) to generate quantification parameters of said generated first and second data set, wherein said reference data set(s) corresponds to a reference condition(s), wherein said reference condition(s) comprises a quantity of reference nucleic acid(s) to quantify said first and second nucleic acid.

Loeffert teaches using reference quantification data such as concentrations of reference nucleic acids for quantifying target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying one or more target nucleic acids.

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In case the space in any of the preceding boxes is not sufficient.
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Regarding Claim 2, modified Caltech discloses the method of claim 1. Caltech further discloses wherein the method does not comprise immobilization, separation, mass spectrometry, or melting curve analysis (In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis, Para. [0003]).

Regarding Claim 3, modified Caltech discloses the method of claim 1. Caltech further discloses wherein c) further comprises measuring an intensity of said first or second signal is (iii) a third range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; For example, 1, 2, 3,... or more second values that are values or ranges of values from a second component of a signal may be used, Para. [0130]).

Regarding Claim 4, modified Caltech discloses the method of claim 1. Caltech further discloses wherein subjecting said mixture to said reaction conditions comprises applying electromagnetic radiation to said mixture (In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum, Para. [0159]; A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal, Para. [0122]).

Regarding Claim 5, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said measuring comprises detecting said first or second signal using a multi-channel detector (In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths, Para. [0009]; Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel.", Para. [0123]).

Regarding Claim 6, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second signal comprises electromagnetic radiation (In some examples, the signal is an electromagnetic signal, Para. [0010]).

Regarding Claim 7, modified Caltech discloses the method of claim 6. Caltech further discloses wherein said electromagnetic radiation comprises a wavelength of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 8, modified Caltech discloses the method of claim 6. Caltech further discloses wherein said electromagnetic radiation comprises a plurality of wavelengths of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 9, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second signal is generated by fluorescence emission (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 10, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second signal is generated by chemiluminescence (For example, the at least one additional value may be selected from the group consisting of... chemiluminescence wavelength, Para. [0015]).

Regarding Claim 11, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]; If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme.... Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time, Para. [0160]).

Regarding Claim 12, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first range of wavelengths and said third range of wavelengths comprise a same wavelength (For example, using the method provided herein... 2,... analytes may be detected per fluorophore, Para. [0074]).

Regarding Claim 13, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths do not comprise a same wavelength (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]).

Regarding Claim 14, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first range of wavelengths and said third range of wavelengths do not comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]).

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Regarding Claim 15, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said detection probe is configured to anneal to said at least one nucleic acid (The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte, Para. [0066]).

Regarding Claim 16, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said detection probe is configured to generate said signal upon degradation of a portion of said detection probe (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 17, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said detection probe comprises a fluorophore or dye (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 18, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said mixture further comprises an amplification oligomer (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 19, modified Caltech discloses the method of claim 18. Caltech further discloses wherein said amplification oligomer comprises a sequence complementary to a portion of the sequence of said at least said nucleic acid (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 20, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said reaction conditions comprise conditions for a DNA extension reaction (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 21, modified Caltech discloses the method of claim 20. Caltech further discloses wherein said DNA extension reaction is a polymerase chain reaction (PCR) (Amplification may include PCR amplification, Para. [0108]).

Regarding Claim 22, modified Caltech discloses the method of claim 21. Caltech further discloses wherein said polymerase chain reaction is a quantitative polymerase chain reaction (qPCR) (The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, Para. [0156]).

Regarding Claim 23, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said processing comprises using a mathematical algorithm (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 25, modified Caltech discloses the method of claim 23. Caltech further discloses wherein said mathematical algorithm comprises a process parameter (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 26, modified Caltech discloses the method of claim 25. Caltech further discloses wherein said process parameter comprises a) a threshold cycle, b) an amplitude, or c) a slope (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 27, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said processing comprises fitting said first or second data set to a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 28, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second data set is plotted as a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 30, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said reference condition(s) comprises an amplification reaction condition.
Loeffert teaches using amplification reactions for reference conditions (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying a target nucleic acid.

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Regarding Claim 31, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said reference conditions comprise a) a temperature, b) a pH, c) a concentration of said reference nucleic acid, or a combination thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 32, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said reference data set corresponds with a data set generated by amplification of said reference nucleic acid under amplification parameters, wherein said reference data set is indicative of an amplification parameter comprising: a) a primer concentration, b) a polymerase concentration, c) polymerase type, d) a reference nucleic acid concentration, e) a number of thermocycles, f) a rate of thermocycling, g) a thermocycle time length, h) a probe sequence; i) a primer sequence, or combinations thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 33, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said quantifying comprises calculating an absolute quantification.
Loeffert teaches calculating absolute quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining levels of contamination in a sample such as from bacteria.

Regarding Claim 35, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said at least one nucleic acid is derived from a biological sample (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 36, modified Caltech discloses the method of claim 35. Caltech further discloses wherein the biological sample is blood or plasma (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 37, modified Caltech discloses the method of claim 35. Caltech further discloses wherein the biological sample is derived from a virus (In some cases, the polynucleotide is from a source selected from the group consisting of... a virus, Para. [0017]).

Regarding Claim 38, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second nucleic acid comprises DNA (In some cases, an analyte may be a polynucleotide, such as DNA, Para. [0107]).

Regarding Claim 39, modified Caltech discloses the method of claim 38. Caltech further discloses wherein said DNA comprises genomic DNA (Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art, Para. [0108]).

Regarding Claim 40, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second nucleic acid comprises RNA (In some cases, an analyte may be a polynucleotide, such as... RNA, Para. [0107]).

Regarding Claim 41, modified Caltech discloses the method of claim 40. Caltech further discloses wherein said RNA comprises mRNA (Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to... messenger RNA (mRNA), Para. [0107]).

Regarding Claim 42, modified Caltech discloses the method of claim 1. Caltech further discloses further comprising in c), measuring an intensity of said second signal in (iv) a fourth range of wavelengths, and further comprising in d) generating said second data derived from said intensity iii) and said intensity of said second signal in said fourth range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; Table 3 shows an exemplary coding scheme where each analyte is encoded by up to 4 colors. In the scheme shown in Table 3, the control color (blue) is generated in the presence of the control sequence and each of the other seven analytes (A-G). The other seven analytes are each encoded by the presence of one to three additional colors, Para. [0078]; Table 3 discloses analyte B is measured in a third and fourth range of wavelengths).

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Regarding Claim 43, modified Caltech discloses the method of claim 42. Caltech further discloses wherein said fourth range of wavelength is the same as said first range of wavelength or said second range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; Table 3 shows an exemplary coding scheme where each analyte is encoded by up to 4 colors. In the scheme shown in Table 3, the control color (blue) is generated in the presence of the control sequence and each of the other seven analytes (A-G). The other seven analytes are each encoded by the presence of one to three additional colors, Para. [0078]; Table 3 discloses analyte B is measured in a third and fourth range of wavelengths where the fourth range of wavelengths is the same as the first range of wavelengths).

Regarding Claim 44, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said third range of wavelengths is the same as said first range of wavelength or said second range of wavelengths (For example, using the method provided herein... 2,... analytes may be detected per fluorophore, Para. [0074]).

Regarding Claim 45, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said processing comprises identifying data points as corresponding to said first data set (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively). The color represents a fluorophore attached to an oligonucleotide probe that also comprises a quencher.... The chart on the left-hand side of FIG. 1 shows intensity versus color for a hypothetical sample containing two analytes: Seq 1 and Seq 3. The presence of these analytes is determined based on the measurement of a blue signal (corresponding to Seq 1) and a yellow signal (corresponding to Seq 3), Paras. [0070]-[0071]).

Regarding Claim 46, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said processing comprises identifying data points as corresponding to said second data set (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively). The color represents a fluorophore attached to an oligonucleotide probe that also comprises a quencher.... The chart on the left-hand side of FIG. 1 shows intensity versus color for a hypothetical sample containing two analytes: Seq 1 and Seq 3. The presence of these analytes is determined based on the measurement of a blue signal (corresponding to Seq 1) and a yellow signal (corresponding to Seq 3), Paras. [0070]-[0071]).

Regarding Claim 47, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said quantifying comprises calculating a relative quantification.
Loeffert teaches calculating relative quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining a target nucleic acid relative to other nucleic acids in a sample for determining the presence of, for example, copy number variations.

Regarding Claim 48, modified Caltech discloses the method of claim 47. Caltech fails to explicitly discloses wherein said relative quantification is generated by comparing said first data set and said second data set.
Loeffert teaches calculating relative quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]; For better comparison of the quantities of specific nucleic acids in two or more different (biological) samples or the comparison of the quantities of two or more different specific nucleic acids in a sample, a normalization of the quantities of the specific nucleic acids to the input nucleic acids or a specific class of input nucleic acid has to be performed, Para. [0003]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining a target nucleic acid relative to other nucleic acids in a sample for determining the presence of, for example, copy number variations.

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Regarding Claim 49, Caltech discloses a method of quantifying at least one nucleic acid in a sample volume (This disclosure provides methods, systems, compositions, and kits for the detection of multiple analytes in a sample.... The analyte may be any suitable analyte, such as a polynucleotide, Para. [0055]; In some cases, an assay provided herein is quantitative, Para. [0026]), the method comprising: a. providing a mixture comprising: i. said at least one nucleic acid; ii. a first detection probe configured to generate a signal when in the presence of said at least one nucleic acid and when subjected to reaction conditions (The compositions may comprise any... reaction mixture... described herein, Para. [0161]; This disclosure provides... compositions,... for the multiplexed detection of analytes, Para. [0003]; In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores, Para. [0012]; In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]); b. subjecting said mixture to said reaction conditions, thereby generating said signal (A series of reactions may be performed to label probes with at least one fluorophore and the reaction products may be mixed to generate a mixture of probes with different fluorophores, Para. [0143]; In some examples, the signal that is measured is generated during a polymerase chain reaction, Para. [0013]); c. measuring (i) an intensity of said signal in a first range of wavelengths and (ii) an intensity of said signal in a second range of wavelengths (For example, building on the traditional methods described above, one color (e.g., blue) may be used to encode the presence of a control analyte that is always present in the sample. The control analyte may be added to the sample, or may be inherently present in the sample. The remaining colors (e.g., green, yellow, red) may be used to encode the presence of additional analytes. Table 2 shows an example of one such method, Para. [0076]; Table 2 discloses analytes A and B, where A has a first color blue and a second color red, and B has a first color blue and and second color yellow); d. generating a data set derived from said intensities measured in c) (In some cases, at least two components of a signal (e.g., intensity and wavelength) are cumulatively measured for the sample. This measurement can be performed, for example, by measuring the intensity at a particular wavelength or the intensity within a particular range of wavelengths, Para. [0058]; The terms "dimension" and "component," as used herein when referring to a signal, generally refer to an aspect of the signal that may be quantified. For example, if a signal is generated by a fluorescent molecule, it may be quantified in terms of its wavelength (e.g., a first dimension or component) and its intensity (e.g., a second dimension or component), Para. [0063]); and e. processing said generated data set, thereby quantifying said at least one nucleic acid (After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]; The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes, Para. [0064]; Similarly, the methods described in this disclosure may be applied in a real-time PCR assay....This additional data enables determination of the identity and starting quantity of each analyte, Para. [0157]).
Caltech fails to explicitly disclose wherein said processing uses reference quantification parameters derived from a reference data set(s) to generate quantification parameters of said generated first and second data set, wherein said reference data set(s) corresponds to a reference condition(s), wherein said reference condition(s) comprises a quantity of reference nucleic acid(s) to quantify said first and second nucleic acid.
Loeffert teaches using reference quantification data such as concentrations of reference nucleic acids for quantifying target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying one or more target nucleic acids.

Regarding Claim 50, modified Caltech discloses the method of claim 49. Caltech further discloses wherein the method does not comprise immobilization, separation, mass spectrometry, or melting curve analysis (In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis, Para. [0003]).

Regarding Claim 51, modified Caltech discloses the method of claim 49. Caltech further discloses wherein c) further comprises measuring (iii) an intensity of said signal in a third range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; For example, 1, 2, 3,... or more second values that are values or ranges of values from a second component of a signal may be used, Para. [0130]).

Regarding Claim 52, modified Caltech discloses the method of claim 49. Caltech further discloses wherein subjecting said mixture to said reaction conditions comprises applying electromagnetic radiation to said mixture (In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum, Para. [0159]; A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal, Para. [0122]).

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Regarding Claim 53, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said measuring comprises detecting said signal using a multi-channel detector (In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths, Para. [0009]; Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel.", Para. [0123]).

Regarding Claim 54, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said signal comprises electromagnetic radiation (In some examples, the signal is an electromagnetic signal, Para. [0010]).

Regarding Claim 55, modified Caltech discloses the method of claim 54. Caltech further discloses wherein said electromagnetic radiation comprises a wavelength of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 56, modified Caltech discloses the method of claim 54. Caltech further discloses wherein said electromagnetic radiation comprises a plurality of wavelengths of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 57, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said signal is generated by fluorescence emission (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 58, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said signal is generated by chemiluminescence (For example, the at least one additional value may be selected from the group consisting of... chemiluminescence wavelength, Para. [0015]).

Regarding Claim 59, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]; If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme.... Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time, Para. [0160]).

Regarding Claim 60, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths do not comprise a same wavelength (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]).

Regarding Claim 61, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said detection probe is configured to anneal to said at least one nucleic acid (The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte, Para. [0066]).

Regarding Claim 62, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said detection probe is configured to generate said signal upon degradation of a portion of said detection probe (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 63, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said detection probe comprises a fluorophore or dye (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 64, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said mixture further comprises an amplification oligomer (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 65, modified Caltech discloses the method of claim 64. Caltech further discloses wherein said amplification oligomer comprises a sequence complementary to a portion of the sequence of said at least said nucleic acid (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

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Regarding Claim 66, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said reaction conditions comprise conditions for a DNA extension reaction (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 67, modified Caltech discloses the method of claim 66. Caltech further discloses wherein said DNA extension reaction is a polymerase chain reaction (PCR) (Amplification may include PCR amplification, Para. [0108]).

Regarding Claim 68, modified Caltech discloses the method of claim 67. Caltech further discloses wherein said polymerase chain reaction is a quantitative polymerase chain reaction (qPCR) (The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, Para. [0156]).

Regarding Claim 69, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said processing comprises using a mathematical algorithm (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 71, modified Caltech discloses the method of claim 69. Caltech further discloses wherein said mathematical algorithm comprises a process parameter (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 72, modified Caltech discloses the method of claim 71. Caltech further discloses wherein said process parameter comprises a) a threshold cycle, b) an amplitude, or c) a slope (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 73, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said processing comprises fitting said generated data set to a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 74, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said data set is plotted as a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 76, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said reference condition(s) comprises an amplification reaction condition.
Loeffert teaches using amplification reactions for reference conditions (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying a target nucleic acid.

Regarding Claim 77, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said reference conditions comprise a) a temperature, b) a pH, c) a concentration of said reference nucleic acid, or a combination thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 78, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said reference data set corresponds with a data set generated by amplification of said reference nucleic acid under amplification parameters, wherein said reference data set is indicative of an amplification parameter comprising: a) a primer concentration, b) a polymerase concentration, c) polymerase type, d) a reference nucleic acid concentration, e) a number of thermocycles, f) a rate of thermocycling, g) a thermocycle time length, h) a probe sequence; i) a primer sequence, or combinations thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

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Regarding Claim 79, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said quantifying comprises calculating an absolute quantification.
Loeffert teaches calculating absolute quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining levels of contamination in a sample such as from bacteria.

Regarding Claim 81, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said at least one nucleic acid is derived from a biological sample (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 82, modified Caltech discloses the method of claim 81. Caltech further discloses wherein said biological sample is blood or plasma (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 83, modified Caltech discloses the method of claim 81. Caltech further discloses wherein said biological sample is derived from a virus (In some cases, the polynucleotide is from a source selected from the group consisting of... a virus, Para. [0017]).

Regarding Claim 84, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said at least one nucleic acid comprises DNA (In some cases, an analyte may be a polynucleotide, such as DNA, Para. [0107]).

Regarding Claim 85, modified Caltech discloses the method of claim 84. Caltech further discloses wherein said DNA comprises genomic DNA (Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art, Para. [0108]).

Regarding Claim 86, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said at least one nucleic acid comprises RNA (In some cases, an analyte may be a polynucleotide, such as... RNA, Para. [0107]).

Regarding Claim 87, modified Caltech discloses the method of claim 86. Caltech further discloses wherein said RNA comprises mRNA (Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to... messenger RNA (mRNA), Para. [0107]).

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Regarding Claim 88, Caltech discloses a system (This disclosure provides... systems,... for the multiplexed detection of a plurality of analytes in a sample, Abstract), comprising a controller comprising or capable of accessing, computer readable media comprising non-transitory computer-executable instructions which, when executed by at least one electronic processor perform a method (Each of the elements described above may be connected to a controller that communicates with each of the elements and coordinates their actions. For example, a controller may initiate the encoding of a series of analytes into values of color and intensity.... Alternatively, the controller may operate an oligonucleotide synthesis instrument that can synthesize the appropriate probes. After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]) comprising: a. providing a mixture comprising: i. said at least one nucleic acid; ii. at least a first detection probe configured to generate a signal when in the presence of said at least one nucleic acid and when subjected to reaction conditions (The compositions may comprise any... reaction mixture... described herein, Para. [0161]; This disclosure provides... compositions,... for the multiplexed detection of analytes, Para. [0003]; In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores, Para. [0012]; In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]); b. subjecting said mixture to said reaction conditions, thereby generating said signal (A series of reactions may be performed to label probes with at least one fluorophore and the reaction products may be mixed to generate a mixture of probes with different fluorophores, Para. [0143]; In some examples, the signal that is measured is generated during a polymerase chain reaction, Para. [0013]); c. measuring (i) an intensity of said signal in a first range of wavelengths and (ii) an intensity of said signal in a second range of wavelengths (For example, building on the traditional methods described above, one color (e.g., blue) may be used to encode the presence of a control analyte that is always present in the sample. The control analyte may be added to the sample, or may be inherently present in the sample. The remaining colors (e.g., green, yellow, red) may be used to encode the presence of additional analytes. Table 2 shows an example of one such method, Para. [0076]; Table 2 discloses analytes A and B, where A has a first color blue and a second color red, and B has a first color blue and and second color yellow); d. generating a data set derived from said intensities measured in c) (In some cases, at least two components of a signal (e.g., intensity and wavelength) are cumulatively measured for the sample. This measurement can be performed, for example, by measuring the intensity at a particular wavelength or the intensity within a particular range of wavelengths, Para. [0058]; The terms "dimension" and "component," as used herein when referring to a signal, generally refer to an aspect of the signal that may be quantified. For example, if a signal is generated by a fluorescent molecule, it may be quantified in terms of its wavelength (e.g., a first dimension or component) and its intensity (e.g., a second dimension or component), Para. [0063]); and e. processing said generated data set, thereby quantifying said at least one nucleic acid (After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]; The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes, Para. [0064]; Similarly, the methods described in this disclosure may be applied in a real-time PCR assay....This additional data enables determination of the identity and starting quantity of each analyte, Para. [0157]).
Caltech fails to explicitly disclose wherein said processing uses reference quantification parameters derived from a reference data set(s) to generate quantification parameters of said generated first and second data set, wherein said reference data set(s) corresponds to a reference condition(s), wherein said reference condition(s) comprises a quantity of reference nucleic acid(s) to quantify said first and second nucleic acid.
Loeffert teaches using reference quantification data such as concentrations of reference nucleic acids for quantifying target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying one or more target nucleic acids.

Regarding Claim 89, modified Caltech discloses the system of claim 88. Caltech further discloses wherein the method does not comprise immobilization, separation, mass spectrometry, or melting curve analysis (In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis, Para. [0003]).

Regarding Claim 90, modified Caltech discloses the system of claim 88. Caltech further discloses wherein c) further comprises measuring an intensity of said signal is (iii) a third range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; For example, 1, 2, 3,... or more second values that are values or ranges of values from a second component of a signal may be used, Para. [0130]).

Regarding Claim 91, modified Caltech discloses the system of claim 88. Caltech further discloses wherein subjecting said mixture to said reaction conditions comprises applying electromagnetic radiation to said mixture (In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum, Para. [0159]; A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal, Para. [0122]).

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Regarding Claim 92, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said measuring comprises detecting said signal using a multi-channel detector (In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths, Para. [0009]; Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel.", Para. [0123]).

Regarding Claim 93, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said signal comprises electromagnetic radiation (In some examples, the signal is an electromagnetic signal, Para. [0010]).

Regarding Claim 94, modified Caltech discloses the system of claim 93. Caltech further discloses wherein said electromagnetic radiation comprises a wavelength of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 95, modified Caltech discloses the system of claim 93. Caltech further discloses wherein said electromagnetic radiation comprises a plurality of wavelengths of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 96, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said signal is generated by fluorescence emission (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 97, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said signal is generated by chemiluminescence (For example, the at least one additional value may be selected from the group consisting of... chemiluminescence wavelength, Para. [0015]).

Regarding Claim 98, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]; If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme.... Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time, Para. [0160]).

Regarding Claim 99, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths do not comprise a same wavelength (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]).

Regarding Claim 100, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said detection probe is configured to anneal to said at least one nucleic acid (The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte, Para. [0066]).

Regarding Claim 101, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said detection probe is configured to generate said signal upon degradation of a portion of said detection probe (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 102, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said detection probe comprises a fluorophore or dye (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 103, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said mixture further comprises an amplification oligomer (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 104, modified Caltech discloses the system of claim 103. Caltech further discloses wherein said amplification oligomer comprises a sequence complementary to a portion of the sequence of said at least said nucleic acid (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

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Regarding Claim 105, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said reaction conditions comprise conditions for a DNA extension reaction (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 106, modified Caltech discloses the system of claim 105. Caltech further discloses wherein said DNA extension reaction is a polymerase chain reaction (PCR) (Amplification may include PCR amplification, Para. [0108]).

Regarding Claim 107, modified Caltech discloses the system of claim 106. Caltech further discloses wherein said polymerase chain reaction is a quantitative polymerase chain reaction (qPCR) (The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, Para. [0156]).

Regarding Claim 108, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said processing comprises using a mathematical algorithm (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 110, modified Caltech discloses the system of claim 108. Caltech further discloses wherein said mathematical algorithm comprises a process parameter (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 111, modified Caltech discloses the system of claim 110. Caltech further discloses wherein said process parameter comprises a) a threshold cycle, b) an amplitude, or c) a slope (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 112, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said processing comprises fitting said generated data set to a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 113, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said data set is plotted as a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 115, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said reference condition(s) comprises an amplification reaction condition.
Loeffert teaches using amplification reactions for reference conditions (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying a target nucleic acid.

Regarding Claim 116, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said reference conditions comprise a) a temperature, b) a pH, c) a concentration of said reference nucleic acid, or a combination thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 117, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said reference data set corresponds with a data set generated by amplification of said reference nucleic acid under amplification parameters, wherein said reference data set is indicative of an amplification parameter comprising: a) a primer concentration, b) a polymerase concentration, c) polymerase type, d) a reference nucleic acid concentration, e) a number of thermocycles, f) a rate of thermocycling, g) a thermocycle time length, h) a probe sequence; i) a primer sequence, or combinations thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

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Regarding Claim 118, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said quantifying comprises calculating an absolute quantification.
Loeffert teaches calculating absolute quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining levels of contamination in a sample such as from bacteria.

Regarding Claim 120, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said at least one nucleic acid is derived from a biological sample (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 121, modified Caltech discloses the system of claim 120. Caltech further discloses wherein the biological sample is blood or plasma (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 122, modified Caltech discloses the system of claim 120. Caltech further discloses wherein the biological sample is derived from a virus (In some cases, the polynucleotide is from a source selected from the group consisting of... a virus, Para. [0017]).

Regarding Claim 123, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said at least one nucleic acid comprises DNA (In some cases, an analyte may be a polynucleotide, such as DNA, Para. [0107]).

Regarding Claim 124, modified Caltech discloses the system of claim 123. Caltech further discloses wherein said at least one nucleic acid comprises genomic DNA (Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art, Para. [0108]).

Regarding Claim 125, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said at least one nucleic acid comprises RNA (In some cases, an analyte may be a polynucleotide, such as... RNA, Para. [0107]).

Regarding Claim 126, modified Caltech discloses the system of claim 125. Caltech further discloses wherein said at least one nucleic acid comprises mRNA (Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to... messenger RNA (mRNA), Para. [0107]).

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Regarding Claim 127, Caltech discloses a system for the quantification of at least one nucleic acid in a sample (This disclosure provides... systems,... for the detection of multiple analytes in a sample.... The analyte may be any suitable analyte, such as a polynucleotide, Para. [0055]; In some cases, an assay provided herein is quantitative, Para. [0026]) comprising: a. said sample comprising said at least one nucleic acid (In some cases, this disclosure provides systems for detecting the presence or absence of each analyte of a plurality of analytes, comprising:... (b) providing a sample comprising, or potentially comprising, at least one of said analytes, Para. [0034]); b. a first detection probe configured to generate a signal when in the presence of said at least one nucleic acid and when subjected to reaction conditions ((c) contacting said sample with analyte-specific reagents that generate said at least one first value and said at least one second value, as represented in said coding scheme, when each of said analytes is present, Para. [0034]; In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores, Para. [0012]; In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]); c. a detector or plurality of detectors configured to measure (i) an intensity of said signal in a first range of wavelengths and (ii) an intensity of said signal in a second range of wavelengths (For example, the methods may be applied using an analytical technique that measures the wavelength and intensity of a fluorescent signal. This may be accomplished by measuring the intensity of a signal across a spectrum of wavelengths, or by using band pass filters that restrict the passage of certain wavelengths of light, thereby allowing only light of certain wavelengths to reach a photodetector, Para. [0153]); and d. a processor configured to: i. generate a data set derived from said measured intensities of c) (After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]; The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes, Para. [0064]; Similarly, the methods described in this disclosure may be applied in a real-time PCR assay....This additional data enables determination of the identity and starting quantity of each analyte, Para. [0157]).
Caltech fails to explicitly disclose a processor configured to ii. process said generated data set by using reference quantification parameters derived from a reference data set(s) to calculate quantification parameters of said generated data set, wherein said reference data set(s) corresponds to a reference condition(s), wherein said reference condition(s) comprises a quantity of reference nucleic acid.
Loeffert teaches systems configured to use reference quantification data such as concentrations of reference nucleic acids for quantifying target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]; Real-time PCR was carried out on a Applied Biosystems 7500 Real-time PCR System, Para. [0100]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying one or more target nucleic acids.

Regarding Claim 128, modified Caltech discloses the system of claim 127. Caltech further discloses wherein c) further comprises detectors configured to measure (iii) an intensity of said signal is a third range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; For example, 1, 2, 3,... or more second values that are values or ranges of values from a second component of a signal may be used, Para. [0130]).

Regarding Claim 129, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said reaction conditions comprises applying electromagnetic radiation to said mixture (In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum, Para. [0159]; A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal, Para. [0122]).

Regarding Claim 130, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said detector comprises a multi-channel detector (In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths, Para. [0009]; Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel.", Para. [0123]).

Regarding Claim 131, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said signal comprises electromagnetic radiation (In some examples, the signal is an electromagnetic signal, Para. [0010]).

Regarding Claim 132, modified Caltech discloses the system of claim 131. Caltech further discloses wherein said electromagnetic radiation comprises a wavelength of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

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Regarding Claim 133, modified Caltech discloses the system of claim 131. Caltech further discloses wherein said electromagnetic radiation comprises a plurality of wavelengths of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 134, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said signal is generated by fluorescence emission (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 135, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said signal is generated by chemiluminescence (For example, the at least one additional value may be selected from the group consisting of... chemiluminescence wavelength, Para. [0015]).

Regarding Claim 136, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]; If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme.... Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time, Para. [0160]).

Regarding Claim 137, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths do not comprise a same wavelength (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]).

Regarding Claim 138, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said detection probe is configured to anneal to said at least one nucleic acid (The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte, Para. [0066]).

Regarding Claim 139, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said detection probe is configured to generate said signal upon degradation of a portion of said detection probe (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 140, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said detection probe comprises a fluorophore or dye (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 141, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said mixture further comprises an amplification oligomer (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 142, modified Caltech discloses the system of claim 141. Caltech further discloses wherein said amplification oligomer comprises a sequence complementary to a portion of the sequence of said at least said nucleic acid (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 143, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said reaction conditions comprise conditions for a DNA extension reaction (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 144, modified Caltech discloses the system of claim 143. Caltech further discloses wherein said DNA extension reaction is a polymerase chain reaction (PCR) (Amplification may include PCR amplification, Para. [0108]).

Regarding Claim 145, modified Caltech discloses the system of claim 144. Caltech further discloses wherein said polymerase chain reaction is a quantitative polymerase chain reaction (qPCR) (The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, Para. [0156]).

Regarding Claim 146, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said processing comprises using a mathematical algorithm (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

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Regarding Claim 148, modified Caltech discloses the system of claim 146. Caltech further discloses wherein said mathematical algorithm comprises a process parameter (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 149, modified Caltech discloses the system of claim 148. Caltech further discloses wherein said process parameter comprises a) a threshold cycle, b) an amplitude, or c) a slope (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 150, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said processing comprises fitting said generated data set to a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 151, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said data set is plotted as a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 153, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said reference condition(s) comprises an amplification reaction condition.
Loeffert teaches using amplification reactions for reference conditions (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying a target nucleic acid.

Regarding Claim 154, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said reference conditions comprise a) a temperature, b) a pH, c) a concentration of said reference nucleic acid, or a combination thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 155, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said reference data set corresponds with a data set generated by amplification of said reference nucleic acid under amplification parameters, wherein said reference data set is indicative of an amplification parameter comprising: a) a primer concentration, b) a polymerase concentration, c) polymerase type, d) a reference nucleic acid concentration, e) a number of thermocycles, f) a rate of thermocycling, g) a thermocycle time length, h) a probe sequence; i) a primer sequence, or combinations thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 156, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said quantification comprises calculating an absolute quantification.
Loeffert teaches calculating absolute quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining levels of contamination in a sample such as from bacteria.

Regarding Claim 158, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said at least one nucleic acid is derived from a biological sample (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 159, modified Caltech discloses the system of claim 158. Caltech further discloses wherein said biological sample is blood or plasma (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 160, modified Caltech discloses the system of claim 158. Caltech further discloses wherein said biological sample is derived from a virus (In some cases, the polynucleotide is from a source selected from the group consisting of... a virus, Para. [0017]).

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Regarding Claim 161, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said at least one nucleic acid comprises DNA (In some cases, an analyte may be a polynucleotide, such as DNA, Para. [0107]).

Regarding Claim 162, modified Caltech discloses the system of claim 161. Caltech further discloses wherein said DNA comprises genomic DNA (Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art, Para. [0108]).

Regarding Claim 163, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said at least one nucleic acid comprises RNA (In some cases, an analyte may be a polynucleotide, such as... RNA, Para. [0107]).

Regarding Claim 164, modified Caltech discloses the system of claim 163. Caltech further discloses wherein said RNA comprises mRNA (Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to... messenger RNA (mRNA), Para. [0107]).

Claims 24, 70, 109, and 147 lack an inventive step under PCT Article 33(3) as being obvious over California Institute of Technology (hereinafter Caltech) in view of Loeffert et al. (hereinafter Loeffert) and Two Pore Guys, Inc. (hereinafter Two Pore Guys).

Regarding Claim 24, modified Caltech discloses the method of claim 23. Caltech fails to explicitly disclose wherein said mathematical algorithm comprises expectation maximization, nearest neighbor analysis, basic model parameterization or Bayesian estimation.
Two Pore Guys teaches using an expectation maximization algorithm for use in quantifying target nucleic acids (In some embodiments, the quantity of first event signatures associated with said target analyte and said quantity of second event signatures associated with said reference analyte are identified according to a defined threshold. In some embodiments, the method of determining an improved estimate of a true relative abundance of a target analyte in a mixed unknown sample using a nanopore device further comprises optimizing said threshold to increase accuracy of detection of said reference analytes and/or said target analytes using... an expectation maximization algorithm, Para. [0022]; In some embodiments, the target analyte and said reference analyte each comprise a polynucleotide, Para. [0025]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Two Pore Guys for the purpose of increasing the accuracy of quantified nucleic acids by better discriminating signals correlated with target and reference nucleic acids.

Regarding Claim 70, modified Caltech discloses the method of claim 69. Caltech fails to explicitly disclose wherein said mathematical algorithm comprises expectation maximization, nearest neighbor analysis, basic model parameterization or Bayesian estimation.
Two Pore Guys teaches using an expectation maximization algorithm for use in quantifying target nucleic acids (In some embodiments, the quantity of first event signatures associated with said target analyte and said quantity of second event signatures associated with said reference analyte are identified according to a defined threshold. In some embodiments, the method of determining an improved estimate of a true relative abundance of a target analyte in a mixed unknown sample using a nanopore device further comprises optimizing said threshold to increase accuracy of detection of said reference analytes and/or said target analytes using... an expectation maximization algorithm, Para. [0022]; In some embodiments, the target analyte and said reference analyte each comprise a polynucleotide, Para. [0025]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Two Pore Guys for the purpose of increasing the accuracy of quantified nucleic acids by better discriminating signals correlated with target and reference nucleic acids.

Regarding Claim 109, modified Caltech discloses the system of claim 108. Caltech fails to explicitly disclose wherein said mathematical algorithm comprises expectation maximization, nearest neighbor analysis, basic model parameterization or Bayesian estimation.
Two Pore Guys teaches using an expectation maximization algorithm for use in quantifying target nucleic acids (In some embodiments, the quantity of first event signatures associated with said target analyte and said quantity of second event signatures associated with said reference analyte are identified according to a defined threshold. In some embodiments, the method of determining an improved estimate of a true relative abundance of a target analyte in a mixed unknown sample using a nanopore device further comprises optimizing said threshold to increase accuracy of detection of said reference analytes and/or said target analytes using... an expectation maximization algorithm, Para. [0022]; In some embodiments, the target analyte and said reference analyte each comprise a polynucleotide, Para. [0025]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Two Pore Guys for the purpose of increasing the accuracy of quantified nucleic acids by better discriminating signals correlated with target and reference nucleic acids.

Regarding Claim 147, modified Caltech discloses the system of claim 146. Caltech fails to explicitly disclose wherein said mathematical algorithm comprises expectation maximization, nearest neighbor analysis, basic model parameterization or Bayesian estimation.
Two Pore Guys teaches using an expectation maximization algorithm for use in quantifying target nucleic acids (In some embodiments, the quantity of first event signatures associated with said target analyte and said quantity of second event signatures associated with said reference analyte are identified according to a defined threshold. In some embodiments, the method of determining an improved estimate of a true relative abundance of a target analyte in a mixed unknown sample using a nanopore device further comprises optimizing said threshold to increase accuracy of detection of said reference analytes and/or said target analytes using... an expectation maximization algorithm, Para. [0022]; In some embodiments, the target analyte and said reference analyte each comprise a polynucleotide, Para. [0025]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Two Pore Guys for the purpose of increasing the accuracy of quantified nucleic acids by better discriminating signals correlated with target and reference nucleic acids.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/041239

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Claims 29, 75, 114, and 152 lack an inventive step under PCT Article 33(3) as being obvious over California Institute of Technology (hereinafter Caltech) in view of Loeffert et al. (hereinafter Loeffert) and The Regents of the University of Michigan (hereinafter University of Michigan).

Regarding Claim 29, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said first or second data set is a kinetic signature.
University of Michigan teaches using kinetic signatures (Provided herein is technology relating to detecting and identifying nucleic acids and particularly, but not exclusively, to compositions, methods, kits, and systems for detecting, identifying, and quantifying target nucleic acids with high confidence at single-molecule resolution, Pg. 1, Lns. 13-16; In some embodiments, kinetic signatures provide a correction mechanism to identify and correct false positive detections resulting from, e.g., deposit of a Capture/Query probe on the wrong part of the imaging surface, Pg. 48, Lns. 15-18; However, equilibrium binding of the probe to target yields a distinctive kinetic signature, or fingerprint, that can achieve ultra-high discrimination against background binding, Pg. 25, Lns. 23-25).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of University of Michigan for the purpose of improving quantitative methods by reducing false positives.

Regarding Claim 75, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said data set comprises a kinetic signature.
University of Michigan teaches using kinetic signatures (Provided herein is technology relating to detecting and identifying nucleic acids and particularly, but not exclusively, to compositions, methods, kits, and systems for detecting, identifying, and quantifying target nucleic acids with high confidence at single-molecule resolution, Pg. 1, Lns. 13-16; In some embodiments, kinetic signatures provide a correction mechanism to identify and correct false positive detections resulting from, e.g., deposit of a Capture/Query probe on the wrong part of the imaging surface, Pg. 48, Lns. 15-18; However, equilibrium binding of the probe to target yields a distinctive kinetic signature, or fingerprint, that can achieve ultra-high discrimination against background binding, Pg. 25, Lns. 23-25).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of University of Michigan for the purpose of improving quantitative methods by reducing false positives.

Regarding Claim 114, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said data set is a kinetic signature.
University of Michigan teaches using kinetic signatures (Provided herein is technology relating to detecting and identifying nucleic acids and particularly, but not exclusively, to compositions, methods, kits, and systems for detecting, identifying, and quantifying target nucleic acids with high confidence at single-molecule resolution, Pg. 1, Lns. 13-16; In some embodiments, kinetic signatures provide a correction mechanism to identify and correct false positive detections resulting from, e.g., deposit of a Capture/Query probe on the wrong part of the imaging surface, Pg. 48, Lns. 15-18; However, equilibrium binding of the probe to target yields a distinctive kinetic signature, or fingerprint, that can achieve ultra-high discrimination against background binding, Pg. 25, Lns. 23-25).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of University of Michigan for the purpose of improving quantitative methods by reducing false positives.

Regarding Claim 152, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said data set is a kinetic signature.
University of Michigan teaches using kinetic signatures (Provided herein is technology relating to detecting and identifying nucleic acids and particularly, but not exclusively, to compositions, methods, kits, and systems for detecting, identifying, and quantifying target nucleic acids with high confidence at single-molecule resolution, Pg. 1, Lns. 13-16; In some embodiments, kinetic signatures provide a correction mechanism to identify and correct false positive detections resulting from, e.g., deposit of a Capture/Query probe on the wrong part of the imaging surface, Pg. 48, Lns. 15-18; However, equilibrium binding of the probe to target yields a distinctive kinetic signature, or fingerprint, that can achieve ultra-high discrimination against background binding, Pg. 25, Lns. 23-25).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of University of Michigan for the purpose of improving quantitative methods by reducing false positives.

Claims 34, 80, 119, and 157 lack an inventive step under PCT Article 33(3) as being obvious over California Institute of Technology (hereinafter Caltech) in view of Loeffert et al. (hereinafter Loeffert) and Carrick.

Regarding Claim 34, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said reference data set is generated using predetermined concentrations of said reference nucleic acid.
Carrick teaches using predetermined concentrations of reference nucleic acids for quantifying target nucleic acids in a sample (More specifically, the invention relates to quantitation of analyte polynucleotides using nucleic acid amplification, and still more specifically relates to the use of factory-supplied calibration curves and adjustment calibrators for analyzing results from real-time nucleic acid amplification reactions, Para. [0002]; This preferably involves first calculating the values of the indicia of amplification for analyte polynucleotide standard and the indicia of amplification for internal calibrator at input analyte polynucleotide levels corresponding to the calibration standards used to create the master calibration curve, Para. [0074]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Carrick for the purpose of generating a standard curve of known quantities of reference nucleic acids for determining the concentration of a target nucleic acid in a sample.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/041239

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding Claim 80, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said reference data set is generated using predetermined concentrations of said reference nucleic acid.
Carrick teaches using predetermined concentrations of reference nucleic acids for quantifying target nucleic acids in a sample (More specifically, the invention relates to quantitation of analyte polynucleotides using nucleic acid amplification, and still more specifically relates to the use of factory-supplied calibration curves and adjustment calibrators for analyzing results from real-time nucleic acid amplification reactions, Para. [0002]; This preferably involves first calculating the values of the indicia of amplification for analyte polynucleotide standard and the indicia of amplification for internal calibrator at input analyte polynucleotide levels corresponding to the calibration standards used to create the master calibration curve, Para. [0074]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Carrick for the purpose of generating a standard curve of known quantities of reference nucleic acids for determining the concentration of a target nucleic acid in a sample.

Regarding Claim 119, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said reference data set is generated using predetermined concentrations of said reference nucleic acid.
Carrick teaches using predetermined concentrations of reference nucleic acids for quantifying target nucleic acids in a sample (More specifically, the invention relates to quantitation of analyte polynucleotides using nucleic acid amplification, and still more specifically relates to the use of factory-supplied calibration curves and adjustment calibrators for analyzing results from real-time nucleic acid amplification reactions, Para. [0002]; This preferably involves first calculating the values of the indicia of amplification for analyte polynucleotide standard and the indicia of amplification for internal calibrator at input analyte polynucleotide levels corresponding to the calibration standards used to create the master calibration curve, Para. [0074]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Carrick for the purpose of generating a standard curve of known quantities of reference nucleic acids for determining the concentration of a target nucleic acid in a sample.

Regarding Claim 157, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said reference data set is generated using predetermined concentrations of said reference nucleic acid.
Carrick teaches using predetermined concentrations of reference nucleic acids for quantifying target nucleic acids in a sample (More specifically, the invention relates to quantitation of analyte polynucleotides using nucleic acid amplification, and still more specifically relates to the use of factory-supplied calibration curves and adjustment calibrators for analyzing results from real-time nucleic acid amplification reactions, Para. [0002]; This preferably involves first calculating the values of the indicia of amplification for analyte polynucleotide standard and the indicia of amplification for internal calibrator at input analyte polynucleotide levels corresponding to the calibration standards used to create the master calibration curve, Para. [0074]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Carrick for the purpose of generating a standard curve of known quantities of reference nucleic acids for determining the concentration of a target nucleic acid in a sample.

Claims 1-164 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: NICHOLAS TIEE
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing *(day/month/year)*	30 SEP 2019
Applicant's or agent's file reference: 44158-719601	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No.: PCT/US2019/041239	International filing date *(day/month/year)*: 10 July 2019
Applicant: CHROMACODE, INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
   - ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
   - ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Within **22 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2017)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 44158-719601	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2019/041239	International filing date *(day/month/year)* 10 July 2019	(Earliest) Priority Date *(day/month/year)* 11 July 2018
Applicant CHROMACODE, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1A

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☒ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/041239

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/6851; G01N 21/64 (2019.01)
CPC - C12Q 1/6851; G01N 21/6486; G16B 25/20; G16B 40/10 (2019.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/6.12; 435/288.7; 702/19 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2015/0057178 A1 (CALIFORNIA INSTITUTE OF TECHNOLOGY) 26 February 2015 (26.02.2015) entire document	1-164
Y	US 2012/0252014 A1 (LOEFFERT et al) 04 October 2012 (04.10.2012) entire document	1-164
Y	WO 2018/081178 A1 (TWO PORE GUYS, INC.) 03 May 2018 (03.05.2018) entire document	24, 70, 109, 147
Y	WO 2017/139354 A1 (THE REGENTS OF THE UNIVERSITY OF MICHIGAN) 17 August 2017 (17.08.2017) entire document	29, 75, 114, 152
Y	US 2011/0171658 A1 (CARRICK) 14 July 2011 (14.07.2011) entire document	34, 80, 119, 157
A	US 2005/0250146 A1 (MCMILLAN) 10 November 2005 (10.11.2005) entire document	1-164

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 2019	30 SEP 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: NICHOLAS TIEE
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* 30 SEP 2019

Applicant's or agent's file reference
44158-719601

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2019/041239	10 July 2019	11 July 2018

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/6851; G01N 21/64 (2019.01)
CPC - C12Q 1/6851; G01N 21/6486; G16B 25/20; G16B 40/10 (2019.08)

Applicant CHROMACODE, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	11 September 2019	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/041239

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/041239

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-164	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-164	NO
Industrial applicability (IA)	Claims	1-164	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-23, 25-28, 30-33, 35-69, 71-74, 76-79, 81-108, 110-113, 115-118, 120-146, 148-151, 153-156, and 158-164 lack an inventive step under PCT Article 33(3) as being obvious over California Institute of Technology (hereinafter Caltech) in view of Loeffert et al. (hereinafter Loeffert).

Regarding Claim 1, Caltech discloses a method of quantifying at least a first and a second nucleic acid in a sample (This disclosure provides methods, systems, compositions, and kits for the detection of multiple analytes in a sample.... The analyte may be any suitable analyte, such as a polynucleotide, Para. [0055]; In some cases, an assay provided herein is quantitative, Para. [0026]), the method comprising: a. providing a mixture comprising: i. said first nucleic acid and said second nucleic acid; ii. a first detection probe configured to generate a first signal when in the presence of said first nucleic acid and when subjected to reaction conditions; iii. a second detection probe configured to generate a second signal when in the presence of said second nucleic acid and when subjected to reaction conditions (The compositions may comprise any... reaction mixture... described herein, Para. [0161]; This disclosure provides... compositions,... for the multiplexed detection of analytes, Para. [0003]; In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores, Para. [0012]; In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]); b. subjecting said mixture to said reaction conditions thereby generating said first and second signal (A series of reactions may be performed to label probes with at least one fluorophore and the reaction products may be mixed to generate a mixture of probes with different fluorophores, Para. [0143]; In some examples, the signal that is measured is generated during a polymerase chain reaction, Para. [0013]); c. measuring (i) an intensity of said first signal in first range of wavelengths, (ii) an intensity of said first signal in said second range of wavelengths, (iii) an intensity of said second signal in a third range of wavelengths (For example, building on the traditional methods described above, one color (e.g., blue) may be used to encode the presence of a control analyte that is always present in the sample. The control analyte may be added to the sample, or may be inherently present in the sample. The remaining colors (e.g., green, yellow, red) may be used to encode the presence of additional analytes. Table 2 shows an example of one such method, Para. [0076]; Table 2 discloses analytes A and B, where A has a first color blue and a second color red, and B has a first color blue and and second color yellow); d. generating a first data set derived from said intensity (i) and said intensity (ii) measured in c) and generating a second data set derived from said intensity (iii) measured in c) (In some cases, at least two components of a signal (e.g., intensity and wavelength) are cumulatively measured for the sample. This measurement can be performed, for example, by measuring the intensity at a particular wavelength or the intensity within a particular range of wavelengths, Para. [0058]; The terms "dimension" and "component," as used herein when referring to a signal, generally refer to an aspect of the signal that may be quantified. For example, if a signal is generated by a fluorescent molecule, it may be quantified in terms of its wavelength (e.g., a first dimension or component) and its intensity (e.g., a second dimension or component), Para. [0063]); and e. processing said generated first data set and said generated second data set, thereby quantifying said first and second nucleic acid (After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]; The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes, Para. [0064]; Similarly, the methods described in this disclosure may be applied in a real-time PCR assay....This additional data enables determination of the identity and starting quantity of each analyte, Para. [0157]).

Caltech fails to explicitly disclose wherein said processing uses reference quantification parameters derived from a reference data set(s) to generate quantification parameters of said generated first and second data set, wherein said reference data set(s) corresponds to a reference condition(s), wherein said reference condition(s) comprises a quantity of reference nucleic acid(s) to quantify said first and second nucleic acid.

Loeffert teaches using reference quantification data such as concentrations of reference nucleic acids for quantifying target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying one or more target nucleic acids.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/041239

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding Claim 2, modified Caltech discloses the method of claim 1. Caltech further discloses wherein the method does not comprise immobilization, separation, mass spectrometry, or melting curve analysis (In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis, Para. [0003]).

Regarding Claim 3, modified Caltech discloses the method of claim 1. Caltech further discloses wherein c) further comprises measuring an intensity of said first or second signal is (iii) a third range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; For example, 1, 2, 3,... or more second values that are values or ranges of values from a second component of a signal may be used, Para. [0130]).

Regarding Claim 4, modified Caltech discloses the method of claim 1. Caltech further discloses wherein subjecting said mixture to said reaction conditions comprises applying electromagnetic radiation to said mixture (In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum, Para. [0159]; A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal, Para. [0122]).

Regarding Claim 5, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said measuring comprises detecting said first or second signal using a multi-channel detector (In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths, Para. [0009]; Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel.", Para. [0123]).

Regarding Claim 6, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second signal comprises electromagnetic radiation (In some examples, the signal is an electromagnetic signal, Para. [0010]).

Regarding Claim 7, modified Caltech discloses the method of claim 6. Caltech further discloses wherein said electromagnetic radiation comprises a wavelength of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 8, modified Caltech discloses the method of claim 6. Caltech further discloses wherein said electromagnetic radiation comprises a plurality of wavelengths of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 9, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second signal is generated by fluorescence emission (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 10, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second signal is generated by chemiluminescence (For example, the at least one additional value may be selected from the group consisting of... chemiluminescence wavelength, Para. [0015]).

Regarding Claim 11, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]; If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme.... Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time, Para. [0160]).

Regarding Claim 12, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first range of wavelengths and said third range of wavelengths comprise a same wavelength (For example, using the method provided herein... 2,... analytes may be detected per fluorophore, Para. [0074]).

Regarding Claim 13, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths do not comprise a same wavelength (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]).

Regarding Claim 14, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first range of wavelengths and said third range of wavelengths do not comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]).

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Regarding Claim 15, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said detection probe is configured to anneal to said at least one nucleic acid (The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte, Para. [0066]).

Regarding Claim 16, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said detection probe is configured to generate said signal upon degradation of a portion of said detection probe (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 17, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said detection probe comprises a fluorophore or dye (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 18, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said mixture further comprises an amplification oligomer (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 19, modified Caltech discloses the method of claim 18. Caltech further discloses wherein said amplification oligomer comprises a sequence complementary to a portion of the sequence of said at least said nucleic acid (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 20, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said reaction conditions comprise conditions for a DNA extension reaction (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 21, modified Caltech discloses the method of claim 20. Caltech further discloses wherein said DNA extension reaction is a polymerase chain reaction (PCR) (Amplification may include PCR amplification, Para. [0108]).

Regarding Claim 22, modified Caltech discloses the method of claim 21. Caltech further discloses wherein said polymerase chain reaction is a quantitative polymerase chain reaction (qPCR) (The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, Para. [0156]).

Regarding Claim 23, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said processing comprises using a mathematical algorithm (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 25, modified Caltech discloses the method of claim 23. Caltech further discloses wherein said mathematical algorithm comprises a process parameter (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 26, modified Caltech discloses the method of claim 25. Caltech further discloses wherein said process parameter comprises a) a threshold cycle, b) an amplitude, or c) a slope (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 27, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said processing comprises fitting said first or second data set to a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 28, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second data set is plotted as a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 30, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said reference condition(s) comprises an amplification reaction condition.
Loeffert teaches using amplification reactions for reference conditions (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying a target nucleic acid.

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Regarding Claim 31, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said reference conditions comprise a) a temperature, b) a pH, c) a concentration of said reference nucleic acid, or a combination thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 32, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said reference data set corresponds with a data set generated by amplification of said reference nucleic acid under amplification parameters, wherein said reference data set is indicative of an amplification parameter comprising: a) a primer concentration, b) a polymerase concentration, c) polymerase type, d) a reference nucleic acid concentration, e) a number of thermocycles, f) a rate of thermocycling, g) a thermocycle time length, h) a probe sequence; i) a primer sequence, or combinations thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 33, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said quantifying comprises calculating an absolute quantification.
Loeffert teaches calculating absolute quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining levels of contamination in a sample such as from bacteria.

Regarding Claim 35, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said at least one nucleic acid is derived from a biological sample (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 36, modified Caltech discloses the method of claim 35. Caltech further discloses wherein the biological sample is blood or plasma (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 37, modified Caltech discloses the method of claim 35. Caltech further discloses wherein the biological sample is derived from a virus (In some cases, the polynucleotide is from a source selected from the group consisting of... a virus, Para. [0017]).

Regarding Claim 38, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second nucleic acid comprises DNA (In some cases, an analyte may be a polynucleotide, such as DNA, Para. [0107]).

Regarding Claim 39, modified Caltech discloses the method of claim 38. Caltech further discloses wherein said DNA comprises genomic DNA (Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art, Para. [0108]).

Regarding Claim 40, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said first or second nucleic acid comprises RNA (In some cases, an analyte may be a polynucleotide, such as... RNA, Para. [0107]).

Regarding Claim 41, modified Caltech discloses the method of claim 40. Caltech further discloses wherein said RNA comprises mRNA (Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to... messenger RNA (mRNA), Para. [0107]).

Regarding Claim 42, modified Caltech discloses the method of claim 1. Caltech further discloses further comprising in c), measuring an intensity of said second signal in (iv) a fourth range of wavelengths, and further comprising in d) generating said second data derived from said intensity iii) and said intensity of said second signal in said fourth range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; Table 3 shows an exemplary coding scheme where each analyte is encoded by up to 4 colors. In the scheme shown in Table 3, the control color (blue) is generated in the presence of the control sequence and each of the other seven analytes (A-G). The other seven analytes are each encoded by the presence of one to three additional colors, Para. [0078]; Table 3 discloses analyte B is measured in a third and fourth range of wavelengths).

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Regarding Claim 43, modified Caltech discloses the method of claim 42. Caltech further discloses wherein said fourth range of wavelength is the same as said first range of wavelength or said second range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; Table 3 shows an exemplary coding scheme where each analyte is encoded by up to 4 colors. In the scheme shown in Table 3, the control color (blue) is generated in the presence of the control sequence and each of the other seven analytes (A-G). The other seven analytes are each encoded by the presence of one to three additional colors, Para. [0078]; Table 3 discloses analyte B is measured in a third and fourth range of wavelengths where the fourth range of wavelengths is the same as the first range of wavelengths).

Regarding Claim 44, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said third range of wavelengths is the same as said first range of wavelength or said second range of wavelengths (For example, using the method provided herein... 2,... analytes may be detected per fluorophore, Para. [0074]).

Regarding Claim 45, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said processing comprises identifying data points as corresponding to said first data set (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively). The color represents a fluorophore attached to an oligonucleotide probe that also comprises a quencher.... The chart on the left-hand side of FIG. 1 shows intensity versus color for a hypothetical sample containing two analytes: Seq 1 and Seq 3. The presence of these analytes is determined based on the measurement of a blue signal (corresponding to Seq 1) and a yellow signal (corresponding to Seq 3), Paras. [0070]-[0071]).

Regarding Claim 46, modified Caltech discloses the method of claim 1. Caltech further discloses wherein said processing comprises identifying data points as corresponding to said second data set (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively). The color represents a fluorophore attached to an oligonucleotide probe that also comprises a quencher.... The chart on the left-hand side of FIG. 1 shows intensity versus color for a hypothetical sample containing two analytes: Seq 1 and Seq 3. The presence of these analytes is determined based on the measurement of a blue signal (corresponding to Seq 1) and a yellow signal (corresponding to Seq 3), Paras. [0070]-[0071]).

Regarding Claim 47, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said quantifying comprises calculating a relative quantification.
Loeffert teaches calculating relative quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining a target nucleic acid relative to other nucleic acids in a sample for determining the presence of, for example, copy number variations.

Regarding Claim 48, modified Caltech discloses the method of claim 47. Caltech fails to explicitly discloses wherein said relative quantification is generated by comparing said first data set and said second data set.
Loeffert teaches calculating relative quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]; For better comparison of the quantities of specific nucleic acids in two or more different (biological) samples or the comparison of the quantities of two or more different specific nucleic acids in a sample, a normalization of the quantities of the specific nucleic acids to the input nucleic acids or a specific class of input nucleic acid has to be performed, Para. [0003]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining a target nucleic acid relative to other nucleic acids in a sample for determining the presence of, for example, copy number variations.

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Regarding Claim 49, Caltech discloses a method of quantifying at least one nucleic acid in a sample volume (This disclosure provides methods, systems, compositions, and kits for the detection of multiple analytes in a sample.... The analyte may be any suitable analyte, such as a polynucleotide, Para. [0055]; In some cases, an assay provided herein is quantitative, Para. [0026]), the method comprising: a. providing a mixture comprising: i. said at least one nucleic acid; ii. a first detection probe configured to generate a signal when in the presence of said at least one nucleic acid and when subjected to reaction conditions (The compositions may comprise any... reaction mixture... described herein, Para. [0161]; This disclosure provides... compositions,... for the multiplexed detection of analytes, Para. [0003]; In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores, Para. [0012]; In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]); b. subjecting said mixture to said reaction conditions, thereby generating said signal (A series of reactions may be performed to label probes with at least one fluorophore and the reaction products may be mixed to generate a mixture of probes with different fluorophores, Para. [0143]; In some examples, the signal that is measured is generated during a polymerase chain reaction, Para. [0013]); c. measuring (i) an intensity of said signal in a first range of wavelengths and (ii) an intensity of said signal in a second range of wavelengths (For example, building on the traditional methods described above, one color (e.g., blue) may be used to encode the presence of a control analyte that is always present in the sample. The control analyte may be added to the sample, or may be inherently present in the sample. The remaining colors (e.g., green, yellow, red) may be used to encode the presence of additional analytes. Table 2 shows an example of one such method, Para. [0076]; Table 2 discloses analytes A and B, where A has a first color blue and a second color red, and B has a first color blue and and second color yellow); d. generating a data set derived from said intensities measured in c) (In some cases, at least two components of a signal (e.g., intensity and wavelength) are cumulatively measured for the sample. This measurement can be performed, for example, by measuring the intensity at a particular wavelength or the intensity within a particular range of wavelengths, Para. [0058]; The terms "dimension" and "component," as used herein when referring to a signal, generally refer to an aspect of the signal that may be quantified. For example, if a signal is generated by a fluorescent molecule, it may be quantified in terms of its wavelength (e.g., a first dimension or component) and its intensity (e.g., a second dimension or component), Para. [0063]); and e. processing said generated data set, thereby quantifying said at least one nucleic acid (After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]; The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes, Para. [0064]; Similarly, the methods described in this disclosure may be applied in a real-time PCR assay....This additional data enables determination of the identity and starting quantity of each analyte, Para. [0157]).
Caltech fails to explicitly disclose wherein said processing uses reference quantification parameters derived from a reference data set(s) to generate quantification parameters of said generated first and second data set, wherein said reference data set(s) corresponds to a reference condition(s), wherein said reference condition(s) comprises a quantity of reference nucleic acid(s) to quantify said first and second nucleic acid.
Loeffert teaches using reference quantification data such as concentrations of reference nucleic acids for quantifying target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying one or more target nucleic acids.

Regarding Claim 50, modified Caltech discloses the method of claim 49. Caltech further discloses wherein the method does not comprise immobilization, separation, mass spectrometry, or melting curve analysis (In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis, Para. [0003]).

Regarding Claim 51, modified Caltech discloses the method of claim 49. Caltech further discloses wherein c) further comprises measuring (iii) an intensity of said signal in a third range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; For example, 1, 2, 3,... or more second values that are values or ranges of values from a second component of a signal may be used, Para. [0130]).

Regarding Claim 52, modified Caltech discloses the method of claim 49. Caltech further discloses wherein subjecting said mixture to said reaction conditions comprises applying electromagnetic radiation to said mixture (In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum, Para. [0159]; A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal, Para. [0122]).

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Regarding Claim 53, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said measuring comprises detecting said signal using a multi-channel detector (In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths, Para. [0009]; Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel.", Para. [0123]).

Regarding Claim 54, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said signal comprises electromagnetic radiation (In some examples, the signal is an electromagnetic signal, Para. [0010]).

Regarding Claim 55, modified Caltech discloses the method of claim 54. Caltech further discloses wherein said electromagnetic radiation comprises a wavelength of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 56, modified Caltech discloses the method of claim 54. Caltech further discloses wherein said electromagnetic radiation comprises a plurality of wavelengths of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 57, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said signal is generated by fluorescence emission (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 58, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said signal is generated by chemiluminescence (For example, the at least one additional value may be selected from the group consisting of... chemiluminescence wavelength, Para. [0015]).

Regarding Claim 59, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]; If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme.... Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time, Para. [0160]).

Regarding Claim 60, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths do not comprise a same wavelength (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]).

Regarding Claim 61, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said detection probe is configured to anneal to said at least one nucleic acid (The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte, Para. [0066]).

Regarding Claim 62, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said detection probe is configured to generate said signal upon degradation of a portion of said detection probe (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 63, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said detection probe comprises a fluorophore or dye (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 64, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said mixture further comprises an amplification oligomer (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 65, modified Caltech discloses the method of claim 64. Caltech further discloses wherein said amplification oligomer comprises a sequence complementary to a portion of the sequence of said at least said nucleic acid (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

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Regarding Claim 66, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said reaction conditions comprise conditions for a DNA extension reaction (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 67, modified Caltech discloses the method of claim 66. Caltech further discloses wherein said DNA extension reaction is a polymerase chain reaction (PCR) (Amplification may include PCR amplification, Para. [0108]).

Regarding Claim 68, modified Caltech discloses the method of claim 67. Caltech further discloses wherein said polymerase chain reaction is a quantitative polymerase chain reaction (qPCR) (The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, Para. [0156]).

Regarding Claim 69, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said processing comprises using a mathematical algorithm (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 71, modified Caltech discloses the method of claim 69. Caltech further discloses wherein said mathematical algorithm comprises a process parameter (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 72, modified Caltech discloses the method of claim 71. Caltech further discloses wherein said process parameter comprises a) a threshold cycle, b) an amplitude, or c) a slope (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 73, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said processing comprises fitting said generated data set to a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 74, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said data set is plotted as a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 76, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said reference condition(s) comprises an amplification reaction condition.
Loeffert teaches using amplification reactions for reference conditions (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying a target nucleic acid.

Regarding Claim 77, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said reference conditions comprise a) a temperature, b) a pH, c) a concentration of said reference nucleic acid, or a combination thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 78, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said reference data set corresponds with a data set generated by amplification of said reference nucleic acid under amplification parameters, wherein said reference data set is indicative of an amplification parameter comprising: a) a primer concentration, b) a polymerase concentration, c) polymerase type, d) a reference nucleic acid concentration, e) a number of thermocycles, f) a rate of thermocycling, g) a thermocycle time length, h) a probe sequence; i) a primer sequence, or combinations thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

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Regarding Claim 79, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said quantifying comprises calculating an absolute quantification.
Loeffert teaches calculating absolute quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining levels of contamination in a sample such as from bacteria.

Regarding Claim 81, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said at least one nucleic acid is derived from a biological sample (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 82, modified Caltech discloses the method of claim 81. Caltech further discloses wherein said biological sample is blood or plasma (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 83, modified Caltech discloses the method of claim 81. Caltech further discloses wherein said biological sample is derived from a virus (In some cases, the polynucleotide is from a source selected from the group consisting of... a virus, Para. [0017]).

Regarding Claim 84, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said at least one nucleic acid comprises DNA (In some cases, an analyte may be a polynucleotide, such as DNA, Para. [0107]).

Regarding Claim 85, modified Caltech discloses the method of claim 84. Caltech further discloses wherein said DNA comprises genomic DNA (Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art, Para. [0108]).

Regarding Claim 86, modified Caltech discloses the method of claim 49. Caltech further discloses wherein said at least one nucleic acid comprises RNA (In some cases, an analyte may be a polynucleotide, such as... RNA, Para. [0107]).

Regarding Claim 87, modified Caltech discloses the method of claim 86. Caltech further discloses wherein said RNA comprises mRNA (Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to... messenger RNA (mRNA), Para. [0107]).

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Regarding Claim 88, Caltech discloses a system (This disclosure provides... systems,... for the multiplexed detection of a plurality of analytes in a sample, Abstract), comprising a controller comprising or capable of accessing, computer readable media comprising non-transitory computer-executable instructions which, when executed by at least one electronic processor perform a method (Each of the elements described above may be connected to a controller that communicates with each of the elements and coordinates their actions. For example, a controller may initiate the encoding of a series of analytes into values of color and intensity.... Alternatively, the controller may operate an oligonucleotide synthesis instrument that can synthesize the appropriate probes. After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]) comprising: a. providing a mixture comprising: i. said at least one nucleic acid; ii. at least a first detection probe configured to generate a signal when in the presence of said at least one nucleic acid and when subjected to reaction conditions (The compositions may comprise any... reaction mixture... described herein, Para. [0161]; This disclosure provides... compositions,... for the multiplexed detection of analytes, Para. [0003]; In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores, Para. [0012]; In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]); b. subjecting said mixture to said reaction conditions, thereby generating said signal (A series of reactions may be performed to label probes with at least one fluorophore and the reaction products may be mixed to generate a mixture of probes with different fluorophores, Para. [0143]; In some examples, the signal that is measured is generated during a polymerase chain reaction, Para. [0013]); c. measuring (i) an intensity of said signal in a first range of wavelengths and (ii) an intensity of said signal in a second range of wavelengths (For example, building on the traditional methods described above, one color (e.g., blue) may be used to encode the presence of a control analyte that is always present in the sample. The control analyte may be added to the sample, or may be inherently present in the sample. The remaining colors (e.g., green, yellow, red) may be used to encode the presence of additional analytes. Table 2 shows an example of one such method, Para. [0076]; Table 2 discloses analytes A and B, where A has a first color blue and a second color red, and B has a first color blue and and second color yellow); d. generating a data set derived from said intensities measured in c) (In some cases, at least two components of a signal (e.g., intensity and wavelength) are cumulatively measured for the sample. This measurement can be performed, for example, by measuring the intensity at a particular wavelength or the intensity within a particular range of wavelengths, Para. [0058]; The terms "dimension" and "component," as used herein when referring to a signal, generally refer to an aspect of the signal that may be quantified. For example, if a signal is generated by a fluorescent molecule, it may be quantified in terms of its wavelength (e.g., a first dimension or component) and its intensity (e.g., a second dimension or component), Para. [0063]); and e. processing said generated data set, thereby quantifying said at least one nucleic acid (After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]; The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes, Para. [0064]; Similarly, the methods described in this disclosure may be applied in a real-time PCR assay....This additional data enables determination of the identity and starting quantity of each analyte, Para. [0157]).
Caltech fails to explicitly disclose wherein said processing uses reference quantification parameters derived from a reference data set(s) to generate quantification parameters of said generated first and second data set, wherein said reference data set(s) corresponds to a reference condition(s), wherein said reference condition(s) comprises a quantity of reference nucleic acid(s) to quantify said first and second nucleic acid.
Loeffert teaches using reference quantification data such as concentrations of reference nucleic acids for quantifying target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying one or more target nucleic acids.

Regarding Claim 89, modified Caltech discloses the system of claim 88. Caltech further discloses wherein the method does not comprise immobilization, separation, mass spectrometry, or melting curve analysis (In some cases, this disclosure provides assays that are capable of unambiguously detecting the presence or absence of each of at least 7 analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis, Para. [0003]).

Regarding Claim 90, modified Caltech discloses the system of claim 88. Caltech further discloses wherein c) further comprises measuring an intensity of said signal is (iii) a third range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; For example, 1, 2, 3,... or more second values that are values or ranges of values from a second component of a signal may be used, Para. [0130]).

Regarding Claim 91, modified Caltech discloses the system of claim 88. Caltech further discloses wherein subjecting said mixture to said reaction conditions comprises applying electromagnetic radiation to said mixture (In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum, Para. [0159]; A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal, Para. [0122]).

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Regarding Claim 92, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said measuring comprises detecting said signal using a multi-channel detector (In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths, Para. [0009]; Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel.", Para. [0123]).

Regarding Claim 93, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said signal comprises electromagnetic radiation (In some examples, the signal is an electromagnetic signal, Para. [0010]).

Regarding Claim 94, modified Caltech discloses the system of claim 93. Caltech further discloses wherein said electromagnetic radiation comprises a wavelength of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 95, modified Caltech discloses the system of claim 93. Caltech further discloses wherein said electromagnetic radiation comprises a plurality of wavelengths of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 96, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said signal is generated by fluorescence emission (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 97, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said signal is generated by chemiluminescence (For example, the at least one additional value may be selected from the group consisting of... chemiluminescence wavelength, Para. [0015]).

Regarding Claim 98, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]; If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme.... Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time, Para. [0160]).

Regarding Claim 99, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths do not comprise a same wavelength (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]).

Regarding Claim 100, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said detection probe is configured to anneal to said at least one nucleic acid (The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte, Para. [0066]).

Regarding Claim 101, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said detection probe is configured to generate said signal upon degradation of a portion of said detection probe (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 102, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said detection probe comprises a fluorophore or dye (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 103, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said mixture further comprises an amplification oligomer (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 104, modified Caltech discloses the system of claim 103. Caltech further discloses wherein said amplification oligomer comprises a sequence complementary to a portion of the sequence of said at least said nucleic acid (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

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Regarding Claim 105, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said reaction conditions comprise conditions for a DNA extension reaction (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 106, modified Caltech discloses the system of claim 105. Caltech further discloses wherein said DNA extension reaction is a polymerase chain reaction (PCR) (Amplification may include PCR amplification, Para. [0108]).

Regarding Claim 107, modified Caltech discloses the system of claim 106. Caltech further discloses wherein said polymerase chain reaction is a quantitative polymerase chain reaction (qPCR) (The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, Para. [0156]).

Regarding Claim 108, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said processing comprises using a mathematical algorithm (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 110, modified Caltech discloses the system of claim 108. Caltech further discloses wherein said mathematical algorithm comprises a process parameter (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 111, modified Caltech discloses the system of claim 110. Caltech further discloses wherein said process parameter comprises a) a threshold cycle, b) an amplitude, or c) a slope (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 112, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said processing comprises fitting said generated data set to a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 113, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said data set is plotted as a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 115, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said reference condition(s) comprises an amplification reaction condition.
Loeffert teaches using amplification reactions for reference conditions (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying a target nucleic acid.

Regarding Claim 116, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said reference conditions comprise a) a temperature, b) a pH, c) a concentration of said reference nucleic acid, or a combination thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 117, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said reference data set corresponds with a data set generated by amplification of said reference nucleic acid under amplification parameters, wherein said reference data set is indicative of an amplification parameter comprising: a) a primer concentration, b) a polymerase concentration, c) polymerase type, d) a reference nucleic acid concentration, e) a number of thermocycles, f) a rate of thermocycling, g) a thermocycle time length, h) a probe sequence; i) a primer sequence, or combinations thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

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Regarding Claim 118, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said quantifying comprises calculating an absolute quantification.
Loeffert teaches calculating absolute quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining levels of contamination in a sample such as from bacteria.

Regarding Claim 120, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said at least one nucleic acid is derived from a biological sample (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 121, modified Caltech discloses the system of claim 120. Caltech further discloses wherein the biological sample is blood or plasma (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 122, modified Caltech discloses the system of claim 120. Caltech further discloses wherein the biological sample is derived from a virus (In some cases, the polynucleotide is from a source selected from the group consisting of... a virus, Para. [0017]).

Regarding Claim 123, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said at least one nucleic acid comprises DNA (In some cases, an analyte may be a polynucleotide, such as DNA, Para. [0107]).

Regarding Claim 124, modified Caltech discloses the system of claim 123. Caltech further discloses wherein said at least one nucleic acid comprises genomic DNA (Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art, Para. [0108]).

Regarding Claim 125, modified Caltech discloses the system of claim 88. Caltech further discloses wherein said at least one nucleic acid comprises RNA (In some cases, an analyte may be a polynucleotide, such as... RNA, Para. [0107]).

Regarding Claim 126, modified Caltech discloses the system of claim 125. Caltech further discloses wherein said at least one nucleic acid comprises mRNA (Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to... messenger RNA (mRNA), Para. [0107]).

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Regarding Claim 127, Caltech discloses a system for the quantification of at least one nucleic acid in a sample (This disclosure provides... systems,... for the detection of multiple analytes in a sample.... The analyte may be any suitable analyte, such as a polynucleotide, Para. [0055]; In some cases, an assay provided herein is quantitative, Para. [0026]) comprising: a. said sample comprising said at least one nucleic acid (In some cases, this disclosure provides systems for detecting the presence or absence of each analyte of a plurality of analytes, comprising:... (b) providing a sample comprising, or potentially comprising, at least one of said analytes, Para. [0034]); b. a first detection probe configured to generate a signal when in the presence of said at least one nucleic acid and when subjected to reaction conditions ((c) contacting said sample with analyte-specific reagents that generate said at least one first value and said at least one second value, as represented in said coding scheme, when each of said analytes is present, Para. [0034]; In some cases, a detecting step is performed with reagents comprising hybridization probes. In some examples, the number of the hybridization probes is greater than the number of analytes. In some cases, the set of hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores, Para. [0012]; In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]); c. a detector or plurality of detectors configured to measure (i) an intensity of said signal in a first range of wavelengths and (ii) an intensity of said signal in a second range of wavelengths (For example, the methods may be applied using an analytical technique that measures the wavelength and intensity of a fluorescent signal. This may be accomplished by measuring the intensity of a signal across a spectrum of wavelengths, or by using band pass filters that restrict the passage of certain wavelengths of light, thereby allowing only light of certain wavelengths to reach a photodetector, Para. [0153]); and d. a processor configured to: i. generate a data set derived from said measured intensities of c) (After analysis of the sample on the instrument (which may also be automated), the controller may process the results of the analysis using the decoding matrix, Para. [0169]; The term "decoding," as used herein, generally refers to a method of determining which analytes are present based on the cumulative signal and a coding scheme or decoding matrix that enables the conversion of a cumulative signal to information concerning the presence or absence of one or more analytes, Para. [0064]; Similarly, the methods described in this disclosure may be applied in a real-time PCR assay....This additional data enables determination of the identity and starting quantity of each analyte, Para. [0157]).
Caltech fails to explicitly disclose a processor configured to ii. process said generated data set by using reference quantification parameters derived from a reference data set(s) to calculate quantification parameters of said generated data set, wherein said reference data set(s) corresponds to a reference condition(s), wherein said reference condition(s) comprises a quantity of reference nucleic acid.
Loeffert teaches systems configured to use reference quantification data such as concentrations of reference nucleic acids for quantifying target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]; Real-time PCR was carried out on a Applied Biosystems 7500 Real-time PCR System, Para. [0100]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying one or more target nucleic acids.

Regarding Claim 128, modified Caltech discloses the system of claim 127. Caltech further discloses wherein c) further comprises detectors configured to measure (iii) an intensity of said signal is a third range of wavelengths (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]; For example, 1, 2, 3,... or more second values that are values or ranges of values from a second component of a signal may be used, Para. [0130]).

Regarding Claim 129, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said reaction conditions comprises applying electromagnetic radiation to said mixture (In some cases, instruments may be modified or constructed, for example, to provide additional excitation light sources, at multiple wavelength, and/or to provide additional band pass filters or a capability of determining a complete spectrum, Para. [0159]; A signal may be an electromagnetic signal. An electromagnetic signal may be a sound, a radio signal, a microwave signal, an infrared signal, a visible light signal, an ultraviolet light signal, an x-ray signal, or a gamma-ray signal, Para. [0122]).

Regarding Claim 130, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said detector comprises a multi-channel detector (In some examples, the coding scheme comprises at least five wavelengths or ranges of wavelengths, Para. [0009]; Intensity may be measured with a photodetector. A range of wavelengths may be referred to as a "channel.", Para. [0123]).

Regarding Claim 131, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said signal comprises electromagnetic radiation (In some examples, the signal is an electromagnetic signal, Para. [0010]).

Regarding Claim 132, modified Caltech discloses the system of claim 131. Caltech further discloses wherein said electromagnetic radiation comprises a wavelength of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

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Regarding Claim 133, modified Caltech discloses the system of claim 131. Caltech further discloses wherein said electromagnetic radiation comprises a plurality of wavelengths of electromagnetic radiation (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 134, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said signal is generated by fluorescence emission (In some examples, the signal is an electromagnetic signal. In some cases, the electromagnetic signal is a fluorescence emission signal. In some examples, the intensity of the fluorescence emission signal is measured at at least four wavelengths or ranges of wavelengths, Para. [0010]).

Regarding Claim 135, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said signal is generated by chemiluminescence (For example, the at least one additional value may be selected from the group consisting of... chemiluminescence wavelength, Para. [0015]).

Regarding Claim 136, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths comprise a same wavelength (The left-hand side of FIG. 1 shows an encoding method where four analytes (Seq 1, Seq 2, Seq 3, and Seq 4) are each encoded by a single color (blue, green, yellow, and red, respectively), Para. [0070]; If there is amplification of the analyte by the maximal cycle number, the result for that analyte is positive and the saturation intensity in each color may be used to decode the result using the coding scheme.... Although PCR has been used to illustrate this quantitative method, one of ordinary skill in the art will readily recognize that similar principles could be applied in any catalytic system where the starting amount of an analyte limits the rate of reaction, and the rate of reaction can be measured and reported by an evolution in a signal intensity over time, Para. [0160]).

Regarding Claim 137, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said first range of wavelengths and said second range of wavelengths do not comprise a same wavelength (In some examples, each analyte is encoded as a first value in a first component of a signal at each of a plurality of second values in a second component of a signal (e.g., a signal intensity or range of signal intensities at each of a plurality of wavelengths or ranges of wavelengths), Para. [0005]).

Regarding Claim 138, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said detection probe is configured to anneal to said at least one nucleic acid (The sequence of the oligonucleotide probe is designed to be complementary to a polynucleotide sequence present in an analyte, and therefore capable of hybridizing to the polynucleotide sequence present in the analyte, Para. [0066]).

Regarding Claim 139, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said detection probe is configured to generate said signal upon degradation of a portion of said detection probe (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 140, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said detection probe comprises a fluorophore or dye (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 141, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said mixture further comprises an amplification oligomer (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 142, modified Caltech discloses the system of claim 141. Caltech further discloses wherein said amplification oligomer comprises a sequence complementary to a portion of the sequence of said at least said nucleic acid (In some examples, a detecting step is performed with reagents comprising at least one pair of primers, Para. [0012]).

Regarding Claim 143, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said reaction conditions comprise conditions for a DNA extension reaction (Upon extension of the primers by a DNA polymerase, the 5' to 3' exonuclease activity of the polymerase degrades the probe, releasing the fluorophore and the quencher into the medium. The proximity between the fluorophore and the quencher is broken and the signal from the fluorophore is no longer quenched. Thus, the amount of fluorescence detected is a function of the amount of analyte present, Para. [0066]).

Regarding Claim 144, modified Caltech discloses the system of claim 143. Caltech further discloses wherein said DNA extension reaction is a polymerase chain reaction (PCR) (Amplification may include PCR amplification, Para. [0108]).

Regarding Claim 145, modified Caltech discloses the system of claim 144. Caltech further discloses wherein said polymerase chain reaction is a quantitative polymerase chain reaction (qPCR) (The methods described in this disclosure are compatible with a variety of amplification methods, including quantitative PCR (qPCR) methods, Para. [0156]).

Regarding Claim 146, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said processing comprises using a mathematical algorithm (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

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Regarding Claim 148, modified Caltech discloses the system of claim 146. Caltech further discloses wherein said mathematical algorithm comprises a process parameter (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 149, modified Caltech discloses the system of claim 148. Caltech further discloses wherein said process parameter comprises a) a threshold cycle, b) an amplitude, or c) a slope (The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 150, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said processing comprises fitting said generated data set to a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 151, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said data set is plotted as a curve (The end-point values may then be decoded to indicate the presence or absence of analytes. The individual cycle thresholds (Ct) values may be analyzed for each color by detecting the maxima of the second derivative of the real-time curve in that color, Para. [0157]).

Regarding Claim 153, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said reference condition(s) comprises an amplification reaction condition.
Loeffert teaches using amplification reactions for reference conditions (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR. Furthermore, the invention is related to normalized quantification of nucleic acids and to the normalization of quantities of nucleic acids in samples, e.g. mixtures of nucleic acids, Para. [0001]; The present invention also relates to the use of the methods of the invention or the kit of the invention for the normalization of the quantity of a specific nucleic acid to the quantity of a reference nucleic acid, Para. [0088]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of generating a sufficient quantities of target and reference nucleic acids for use in quantifying a target nucleic acid.

Regarding Claim 154, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said reference conditions comprise a) a temperature, b) a pH, c) a concentration of said reference nucleic acid, or a combination thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 155, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said reference data set corresponds with a data set generated by amplification of said reference nucleic acid under amplification parameters, wherein said reference data set is indicative of an amplification parameter comprising: a) a primer concentration, b) a polymerase concentration, c) polymerase type, d) a reference nucleic acid concentration, e) a number of thermocycles, f) a rate of thermocycling, g) a thermocycle time length, h) a probe sequence; i) a primer sequence, or combinations thereof.
Loeffert teaches a reference condition could be the concentration of a reference nucleic acid (The present invention provides (a) robust and improved method(s) for the normalization of the quantity of a (specific) nucleic acid (i.e. a "target nucleic acid") in a sample or in a plurality of samples to the total quantity of nucleic acid in the sample(s), Para. [0008]; The "total quantity of nucleic acid in the sample" or the "total quantity of a specific class of nucleic acid in the sample" refer to the reference quantities (reference nucleic acid) for normalization of the quantity of the nucleic acid to be quantified, Para. [0033]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of using the concentration of a reference nucleic acid for determining the concentration of a target nucleic acid.

Regarding Claim 156, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said quantification comprises calculating an absolute quantification.
Loeffert teaches calculating absolute quantities of target nucleic acids (More particular, the invention is in the field of quantification of nucleic acids and real-time PCR, Para. [0001]; In quantification methods, the concentrations and/or the relative or absolute amounts of specific nucleic acids in samples are determined, Para. [0002]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Loeffert for the purpose of determining levels of contamination in a sample such as from bacteria.

Regarding Claim 158, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said at least one nucleic acid is derived from a biological sample (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 159, modified Caltech discloses the system of claim 158. Caltech further discloses wherein said biological sample is blood or plasma (In some instances, an analyte may be obtained from bodily fluids which may include blood, Para. [0115]).

Regarding Claim 160, modified Caltech discloses the system of claim 158. Caltech further discloses wherein said biological sample is derived from a virus (In some cases, the polynucleotide is from a source selected from the group consisting of... a virus, Para. [0017]).

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Regarding Claim 161, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said at least one nucleic acid comprises DNA (In some cases, an analyte may be a polynucleotide, such as DNA, Para. [0107]).

Regarding Claim 162, modified Caltech discloses the system of claim 161. Caltech further discloses wherein said DNA comprises genomic DNA (Analytes comprising genomic DNA may be obtained from any source and using any methods known in the art, Para. [0108]).

Regarding Claim 163, modified Caltech discloses the system of claim 127. Caltech further discloses wherein said at least one nucleic acid comprises RNA (In some cases, an analyte may be a polynucleotide, such as... RNA, Para. [0107]).

Regarding Claim 164, modified Caltech discloses the system of claim 163. Caltech further discloses wherein said RNA comprises mRNA (Polynucleotides may be any suitable polynucleotide for which one or more reagents (or probes) as described herein may be produced, including but not limited to... messenger RNA (mRNA), Para. [0107]).

Claims 24, 70, 109, and 147 lack an inventive step under PCT Article 33(3) as being obvious over California Institute of Technology (hereinafter Caltech) in view of Loeffert et al. (hereinafter Loeffert) and Two Pore Guys, Inc. (hereinafter Two Pore Guys).

Regarding Claim 24, modified Caltech discloses the method of claim 23. Caltech fails to explicitly disclose wherein said mathematical algorithm comprises expectation maximization, nearest neighbor analysis, basic model parameterization or Bayesian estimation.
Two Pore Guys teaches using an expectation maximization algorithm for use in quantifying target nucleic acids (In some embodiments, the quantity of first event signatures associated with said target analyte and said quantity of second event signatures associated with said reference analyte are identified according to a defined threshold. In some embodiments, the method of determining an improved estimate of a true relative abundance of a target analyte in a mixed unknown sample using a nanopore device further comprises optimizing said threshold to increase accuracy of detection of said reference analytes and/or said target analytes using... an expectation maximization algorithm, Para. [0022]; In some embodiments, the target analyte and said reference analyte each comprise a polynucleotide, Para. [0025]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Two Pore Guys for the purpose of increasing the accuracy of quantified nucleic acids by better discriminating signals correlated with target and reference nucleic acids.

Regarding Claim 70, modified Caltech discloses the method of claim 69. Caltech fails to explicitly disclose wherein said mathematical algorithm comprises expectation maximization, nearest neighbor analysis, basic model parameterization or Bayesian estimation.
Two Pore Guys teaches using an expectation maximization algorithm for use in quantifying target nucleic acids (In some embodiments, the quantity of first event signatures associated with said target analyte and said quantity of second event signatures associated with said reference analyte are identified according to a defined threshold. In some embodiments, the method of determining an improved estimate of a true relative abundance of a target analyte in a mixed unknown sample using a nanopore device further comprises optimizing said threshold to increase accuracy of detection of said reference analytes and/or said target analytes using... an expectation maximization algorithm, Para. [0022]; In some embodiments, the target analyte and said reference analyte each comprise a polynucleotide, Para. [0025]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Two Pore Guys for the purpose of increasing the accuracy of quantified nucleic acids by better discriminating signals correlated with target and reference nucleic acids.

Regarding Claim 109, modified Caltech discloses the system of claim 108. Caltech fails to explicitly disclose wherein said mathematical algorithm comprises expectation maximization, nearest neighbor analysis, basic model parameterization or Bayesian estimation.
Two Pore Guys teaches using an expectation maximization algorithm for use in quantifying target nucleic acids (In some embodiments, the quantity of first event signatures associated with said target analyte and said quantity of second event signatures associated with said reference analyte are identified according to a defined threshold. In some embodiments, the method of determining an improved estimate of a true relative abundance of a target analyte in a mixed unknown sample using a nanopore device further comprises optimizing said threshold to increase accuracy of detection of said reference analytes and/or said target analytes using... an expectation maximization algorithm, Para. [0022]; In some embodiments, the target analyte and said reference analyte each comprise a polynucleotide, Para. [0025]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Two Pore Guys for the purpose of increasing the accuracy of quantified nucleic acids by better discriminating signals correlated with target and reference nucleic acids.

Regarding Claim 147, modified Caltech discloses the system of claim 146. Caltech fails to explicitly disclose wherein said mathematical algorithm comprises expectation maximization, nearest neighbor analysis, basic model parameterization or Bayesian estimation.
Two Pore Guys teaches using an expectation maximization algorithm for use in quantifying target nucleic acids (In some embodiments, the quantity of first event signatures associated with said target analyte and said quantity of second event signatures associated with said reference analyte are identified according to a defined threshold. In some embodiments, the method of determining an improved estimate of a true relative abundance of a target analyte in a mixed unknown sample using a nanopore device further comprises optimizing said threshold to increase accuracy of detection of said reference analytes and/or said target analytes using... an expectation maximization algorithm, Para. [0022]; In some embodiments, the target analyte and said reference analyte each comprise a polynucleotide, Para. [0025]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Two Pore Guys for the purpose of increasing the accuracy of quantified nucleic acids by better discriminating signals correlated with target and reference nucleic acids.

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Claims 29, 75, 114, and 152 lack an inventive step under PCT Article 33(3) as being obvious over California Institute of Technology (hereinafter Caltech) in view of Loeffert et al. (hereinafter Loeffert) and The Regents of the University of Michigan (hereinafter University of Michigan).

Regarding Claim 29, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said first or second data set is a kinetic signature.
University of Michigan teaches using kinetic signatures (Provided herein is technology relating to detecting and identifying nucleic acids and particularly, but not exclusively, to compositions, methods, kits, and systems for detecting, identifying, and quantifying target nucleic acids with high confidence at single-molecule resolution, Pg. 1, Lns. 13-16; In some embodiments, kinetic signatures provide a correction mechanism to identify and correct false positive detections resulting from, e.g., deposit of a Capture/Query probe on the wrong part of the imaging surface, Pg. 48, Lns. 15-18; However, equilibrium binding of the probe to target yields a distinctive kinetic signature, or fingerprint, that can achieve ultra-high discrimination against background binding, Pg. 25, Lns. 23-25).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of University of Michigan for the purpose of improving quantitative methods by reducing false positives.

Regarding Claim 75, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said data set comprises a kinetic signature.
University of Michigan teaches using kinetic signatures (Provided herein is technology relating to detecting and identifying nucleic acids and particularly, but not exclusively, to compositions, methods, kits, and systems for detecting, identifying, and quantifying target nucleic acids with high confidence at single-molecule resolution, Pg. 1, Lns. 13-16; In some embodiments, kinetic signatures provide a correction mechanism to identify and correct false positive detections resulting from, e.g., deposit of a Capture/Query probe on the wrong part of the imaging surface, Pg. 48, Lns. 15-18; However, equilibrium binding of the probe to target yields a distinctive kinetic signature, or fingerprint, that can achieve ultra-high discrimination against background binding, Pg. 25, Lns. 23-25).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of University of Michigan for the purpose of improving quantitative methods by reducing false positives.

Regarding Claim 114, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said data set is a kinetic signature.
University of Michigan teaches using kinetic signatures (Provided herein is technology relating to detecting and identifying nucleic acids and particularly, but not exclusively, to compositions, methods, kits, and systems for detecting, identifying, and quantifying target nucleic acids with high confidence at single-molecule resolution, Pg. 1, Lns. 13-16; In some embodiments, kinetic signatures provide a correction mechanism to identify and correct false positive detections resulting from, e.g., deposit of a Capture/Query probe on the wrong part of the imaging surface, Pg. 48, Lns. 15-18; However, equilibrium binding of the probe to target yields a distinctive kinetic signature, or fingerprint, that can achieve ultra-high discrimination against background binding, Pg. 25, Lns. 23-25).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of University of Michigan for the purpose of improving quantitative methods by reducing false positives.

Regarding Claim 152, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said data set is a kinetic signature.
University of Michigan teaches using kinetic signatures (Provided herein is technology relating to detecting and identifying nucleic acids and particularly, but not exclusively, to compositions, methods, kits, and systems for detecting, identifying, and quantifying target nucleic acids with high confidence at single-molecule resolution, Pg. 1, Lns. 13-16; In some embodiments, kinetic signatures provide a correction mechanism to identify and correct false positive detections resulting from, e.g., deposit of a Capture/Query probe on the wrong part of the imaging surface, Pg. 48, Lns. 15-18; However, equilibrium binding of the probe to target yields a distinctive kinetic signature, or fingerprint, that can achieve ultra-high discrimination against background binding, Pg. 25, Lns. 23-25).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of University of Michigan for the purpose of improving quantitative methods by reducing false positives.

Claims 34, 80, 119, and 157 lack an inventive step under PCT Article 33(3) as being obvious over California Institute of Technology (hereinafter Caltech) in view of Loeffert et al. (hereinafter Loeffert) and Carrick.

Regarding Claim 34, modified Caltech discloses the method of claim 1. Caltech fails to explicitly disclose wherein said reference data set is generated using predetermined concentrations of said reference nucleic acid.
Carrick teaches using predetermined concentrations of reference nucleic acids for quantifying target nucleic acids in a sample (More specifically, the invention relates to quantitation of analyte polynucleotides using nucleic acid amplification, and still more specifically relates to the use of factory-supplied calibration curves and adjustment calibrators for analyzing results from real-time nucleic acid amplification reactions, Para. [0002]; This preferably involves first calculating the values of the indicia of amplification for analyte polynucleotide standard and the indicia of amplification for internal calibrator at input analyte polynucleotide levels corresponding to the calibration standards used to create the master calibration curve, Para. [0074]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Carrick for the purpose of generating a standard curve of known quantities of reference nucleic acids for determining the concentration of a target nucleic acid in a sample.

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Regarding Claim 80, modified Caltech discloses the method of claim 49. Caltech fails to explicitly disclose wherein said reference data set is generated using predetermined concentrations of said reference nucleic acid.
Carrick teaches using predetermined concentrations of reference nucleic acids for quantifying target nucleic acids in a sample (More specifically, the invention relates to quantitation of analyte polynucleotides using nucleic acid amplification, and still more specifically relates to the use of factory-supplied calibration curves and adjustment calibrators for analyzing results from real-time nucleic acid amplification reactions, Para. [0002]; This preferably involves first calculating the values of the indicia of amplification for analyte polynucleotide standard and the indicia of amplification for internal calibrator at input analyte polynucleotide levels corresponding to the calibration standards used to create the master calibration curve, Para. [0074]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Carrick for the purpose of generating a standard curve of known quantities of reference nucleic acids for determining the concentration of a target nucleic acid in a sample.

Regarding Claim 119, modified Caltech discloses the system of claim 88. Caltech fails to explicitly disclose wherein said reference data set is generated using predetermined concentrations of said reference nucleic acid.
Carrick teaches using predetermined concentrations of reference nucleic acids for quantifying target nucleic acids in a sample (More specifically, the invention relates to quantitation of analyte polynucleotides using nucleic acid amplification, and still more specifically relates to the use of factory-supplied calibration curves and adjustment calibrators for analyzing results from real-time nucleic acid amplification reactions, Para. [0002]; This preferably involves first calculating the values of the indicia of amplification for analyte polynucleotide standard and the indicia of amplification for internal calibrator at input analyte polynucleotide levels corresponding to the calibration standards used to create the master calibration curve, Para. [0074]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Carrick for the purpose of generating a standard curve of known quantities of reference nucleic acids for determining the concentration of a target nucleic acid in a sample.

Regarding Claim 157, modified Caltech discloses the system of claim 127. Caltech fails to explicitly disclose wherein said reference data set is generated using predetermined concentrations of said reference nucleic acid.
Carrick teaches using predetermined concentrations of reference nucleic acids for quantifying target nucleic acids in a sample (More specifically, the invention relates to quantitation of analyte polynucleotides using nucleic acid amplification, and still more specifically relates to the use of factory-supplied calibration curves and adjustment calibrators for analyzing results from real-time nucleic acid amplification reactions, Para. [0002]; This preferably involves first calculating the values of the indicia of amplification for analyte polynucleotide standard and the indicia of amplification for internal calibrator at input analyte polynucleotide levels corresponding to the calibration standards used to create the master calibration curve, Para. [0074]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caltech with the teaching of Carrick for the purpose of generating a standard curve of known quantities of reference nucleic acids for determining the concentration of a target nucleic acid in a sample.

Claims 1-164 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

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First Named Inventor/Applicant Name:	Emil P. Kartalov
Customer Number:	21971
Filer:	Nicholas Shaofeng Tiee/Christina Campbell
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	ISR-WO-6-22-2017-PCTUS2017-24933.PDF	2753488 cdaf294d08eeb4cf2b66e4487c0750a7e84635c6	no	20

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2	Non Patent Literature	ISR-WO-11-16-17-US1737682.PDF	1047446 4593a50ed9bcd7a6df9c082fbd2b9b42f3bb3dbc	no	17
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3	Non Patent Literature	PCTUS2018039846-IPRP-31DEC2019.PDF	637369 38171aba048f34a4784310de8fe3618d34596c40	no	9
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4	Non Patent Literature	PCTUS2018055927-IPRP-21APR2020.PDF	710341 6b9e35c2dd173fe2db0da5d3bf15c1a40ece6af2	no	10
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5	Non Patent Literature	ISRWO-PCTUS1839846-14SEP2018.PDF	1465658 49020e052296d3be6ea2a5f996e40f94c57b9db4	no	17
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6	Non Patent Literature	PCTUS2019019906-IPRP-01SEP2020.PDF	889706 c7e4715139f801df38f2c95cc6c3bf6d417f2819	no	14
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7	Non Patent Literature	PCTUS2019-019906-ISRWO-06MAY2019.PDF	1056563 cb1e493a0bcbea58b93c6414e4a5f12acb161021	no	17
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9	Non Patent Literature	PCTUS2019-027751-ISRWO-26JUNE2019.PDF	1241856 d8b9dfb6ae5bb1cc54ead4e3b1cc8caeb6261cf1	no	18
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12	Non Patent Literature	PCTUS2019040392-IPRO-05JAN2021.PDF	1457620 550e55e187769e090d26b8da7cb4b5f7efff3632	no	18
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34	Non Patent Literature	URDEA-REQUIREMENTS-FOR-73.PDF	351004 77a0ce4a832c001ccf89fb620517aea1a54f4310	no	8
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35	Non Patent Literature	NOA-6-2-2014-US13756760.PDF	439635 63df3d2975604d72aa18e740b9a5a4c8d49f3356	no	8
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39	Non Patent Literature	OA-11-23-2016-US14451876.PDF	453805 b577db979f36b6cb54c70955efa03ac4f1e58b13	no	14
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42	Non Patent Literature	OA-US15677772-07JUNE2018.PDF	745251 9209597effc71cd116eb6d711279d4f8cf85a4ba	no	13
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44	Non Patent Literature	OA-US15892245-19JULY2018.PDF	126610 f7c4cb8d6e2c9878680a9692d7a3532e0874bf18	no	4
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49	Non Patent Literature	NOA-06-14-2018-US14-451876.PDF	444780 b30a597a0ea6db36c0703e4d13c0808bf7eb126b	no	9
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52	Non Patent Literature	US16128343-OA-16JUN2020.PDF	1553317 d23071e3d853c18723b26a4c6890084f2b7c7295	no	38
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57	Non Patent Literature	OA-08-21-2020-US16051736.PDF	221419 b61c179aea111eebf9d9c4be0f6456e1582fdba5	no	6
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58	Non Patent Literature	US16128343_1STACTIONINTERVIEW_18APRIL2019.PDF	870144 a767b76712efeb1ceb8ec25d9af36a9ad8e5702f	no	25
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Warnings:					
Information:					
		Total Files Size (in bytes):	51200046		

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<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-13)
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NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	16/864,744
Filing Date	2020-05-01
First Named Inventor	Emil P. KARTALOV
Title	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
Art Unit	
Examiner Name	
Attorney Docket Number	30KJ-330783-US4

SIGNATURE of Applicant or Patent Practitioner

Signature	/Jesse A. Salen/	Date (Optional)	
Name	Jesse A. Salen	Registration Number	72209
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)	California Institute of Technology		

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Doc Code: PA..
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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
16/864,744	2020-05-01

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 30764

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

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OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

California Institute of Technology

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/Fred Farina/	Date (Optional)	03/29/21
Name	Fred Farina		
Title	Chief Innovation Officer, OTTCP		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Electronic Acknowledgement Receipt

EFS ID:	42339676
Application Number:	16864744
International Application Number:	
Confirmation Number:	9929
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil P. Kartalov
Customer Number:	21971
Filer:	Jesse A. Salen/Jamie Barber
Filer Authorized By:	Jesse A. Salen
Attorney Docket Number:	44158-727.304
Receipt Date:	31-MAR-2021
Filing Date:	01-MAY-2020
Time Stamp:	21:31:09
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	POA03_30KJ330783US4.pdf	265735 6a0572d2e69249a01109be8765a607d42bf937cc	no	2
Warnings:					

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Total Files Size (in bytes):	265735

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/864,744	05/01/2020	Emil P. Kartalov	44158-727.304

CONFIRMATION NO. 9929

21971
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

PUBLICATION NOTICE

OC000000124555233

Title:SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

Publication No.US-2021-0098076-A1
Publication Date:04/01/2021

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

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IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4363

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UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/864,744	05/01/2020	Emil P. Kartalov	30KJ-330783-US4

CONFIRMATION NO. 9929

30764
SHEPPARD, MULLIN, RICHTER & HAMPTON LLP
650 Town Center Drive, 10th Floor
Costa Mesa, CA 92626

POA ACCEPTANCE LETTER

OC000000124606992

Date Mailed: 04/06/2021

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 03/31/2021.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/864,744	05/01/2020	Emil P. Kartalov	44158-727.304

CONFIRMATION NO. 9929

21971
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

POWER OF ATTORNEY NOTICE

OC000000124606976

Date Mailed: 04/06/2021

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 03/31/2021.

- The Power of Attorney to you in this application has been revoked by the applicant. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/sleutchit/

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-22)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	16864744
Filing Date	2020-05-01
First Named Inventor	Emil P. Kartalov
Art Unit	1672
Examiner Name	NEGIN, RUSSELL SCOTT
Attorney Docket Number	30KJ-330783-US4

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	1	20040248082		2004-12-09	SCALLON	
	2	20110183884		2011-07-28	MILLER ET AL.	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16864744
	Filing Date	2020-05-01
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	Art Unit	1672
	Examiner Name	NEGIN, RUSSELL SCOTT
	Attorney Docket Number	30KJ-330783-US4

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1		

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16864744
	Filing Date	2020-05-01
	First Named Inventor	Emil P. Kartalov
	Art Unit	1672
	Examiner Name	NEGIN, RUSSELL SCOTT
	Attorney Docket Number	30KJ-330783-US4

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/David E. Heisey/	Date (YYYY-MM-DD)	2022-12-27
Name/Print	David E. Heisey	Registration Number	42651

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	47281381
Application Number:	16864744
International Application Number:	
Confirmation Number:	9929
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil P. Kartalov
Customer Number:	30764
Filer:	DAVID E. HEISEY/Krystle Mestressat
Filer Authorized By:	DAVID E. HEISEY
Attorney Docket Number:	30KJ-330783-US4
Receipt Date:	27-DEC-2022
Filing Date:	01-MAY-2020
Time Stamp:	19:59:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	30KJ-330783-US4_IDS_9929.pdf	1263237 d9c60943c85816b2c5ea4c5bad044ed8daf a7d18	no	4

Warnings:

Information:	
Total Files Size (in bytes):	1263237

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	05/01/2020	Emil P. Kartalov	30KJ-330783-US4	9929

30764 7590 12/28/2022

SHEPPARD, MULLIN, RICHTER & HAMPTON LLP
650 Town Center Drive, 10th Floor
Costa Mesa, CA 92626

EXAMINER	
NEGIN, RUSSELL SCOTT	

ART UNIT	PAPER NUMBER
1672	

NOTIFICATION DATE	DELIVERY MODE
12/28/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DOCKETING@SHEPPARDMULLIN.COM
SheppardMullin_Pair@firsttofile.com

Office Action Summary	Application No. 16/864,744	Applicant(s) Kartalov et al.	
	Examiner RUSSELL S NEGIN	Art Unit 1672	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 21-40 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 21-40 is/are rejected.

8) ☑ Claim(s) 33 and 37 is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 01 May 2020 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Comments

The present application is being examined under the pre-AIA first to invent provisions. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

Claims 21-40 are pending and examined in the instant Office action.

Information Disclosure Statement

The IDS filed on 3/25/2021 has been considered.

Claim Objections

Claims 33 and 37 are objected to because of the following informalities:

Claim 33 does not end with a period.

Claim 37 recites "The kit of any of claims 34" which should read "The kit of claim 34".

Appropriate correction is required.

Claim Rejections - 35 USC § 112(b) - Indefiniteness

The following is a quotation of 35 U.S.C. 112(b):

> (b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.
>
> The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 30 and 32-40 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

Claim 30 recites the limitation "said analyte" in line 2. There is insufficient antecedent basis for this limitation in the claim. While the claim previously recites the plural term of analytes, it is unclear as to which of the plurality of analytes is “said analyte.” For the purpose of examination, any analyte is interpreted to be “said analyte.”

Claim 32 recites the limitation "said coding scheme" in line 1. There is insufficient antecedent basis for this limitation in the claim. The phrase “coding scheme” is not previously precited in the claim. For the purpose of examination, “said coding scheme” is interpreted to correspond to the presence/absence pattern of analytes.

Claim 33 recites the limitation "said coding scheme" in line 1. There is insufficient antecedent basis for this limitation in the claim. The phrase “coding scheme” is not previously precited in the claim. For the purpose of examination, “said coding scheme” is interpreted to correspond to the presence/absence pattern of analytes.

Claim 34 is recites the limitation "the first value" in the final line of the claim. There is insufficient antecedent basis for this limitation in the claim. For the purpose of

examination, “the first value” is interpreted to correspond to a value of one component of a fluorescent signal.

Claim 36 recites the limitation "F" in line 2. There is insufficient antecedent basis for this limitation in the claim. The variable “F” is not previously recited or defined in the claim. For the purpose of examination, it is interpreted that “F” corresponds to a number of wavelength components.

Claim 39 recites the limitation "the number of analytes" in lines 1 and 2. There is insufficient antecedent basis for this limitation in the claim. The phrase “the number of analytes” is not previously recited in the claim. For the purpose of examination, the phrase is interpreted to correspond to M analytes.

Claim 39 recites the limitation "the plurality of analytes" in line 2. There is insufficient antecedent basis for this limitation in the claim. The phrase “the plurality of analytes” is not previously recited in the claim. The claim does not require the variable M in M analytes to be more than one.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter

of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

35 U.S.C. 103 Rejection #1:

Claims 21-25, 27, 29-30, and 32-40 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Han et al. [Nature Biotechnology, volume 19, 2001, pages 631-835; on IDS] in view of Lin et al. [WO 2012/058628 A2; on IDS].

Claim 21 is drawn to an assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes. The assay requires that each of the M analytes is encoded as a value of one component of a fluorescent signal. The assay requires that the fluorescent signal comprises F wavelength components. The assay is capable of detecting the presence or absence of each of the at least M analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis, based on detection of the F wavelength components. M is greater than F.

Claim 32 is further limiting requiring the coding scheme to be innately non-degenerate or non-degenerate by construction.

Claims 34-36 are drawn to similar subject matter as claim 32, except as a kit.

The document of Han et al. studies quantum-dot tagged microbeads for multiplexed optical coding of biomolecules [title]. Figure 5 of Han et al. illustrates four oligonucleotides analytes (M=4) attached to microbeads with different combinations of 3 types of quantum dots- red, green, and blue (F=3). The type of optical code as a result of spectroscopy indicates the type of analyte being examined. Han et al. does not use immobilization, separation, mass spectrometry, or melting curve analysis.

Han et al. does not teach detecting cumulative non-degenerate combinations of analytes.

The document of Lin et al. studies nucleic acid nanostructure barcode probes [title]. Figure 2 of Lin et al. illustrates the cumulative non-degenerate combination of each of 27 different combinations of the three fluorophores (R, G, and B) within an image. Pages 7 and 8 of Lin et al. describe Figure 2 of Lin et al.

With regard to claims 22-23, Han et al. and Lin et al. teach red, green, and blue components to the signal. Figure 6 of Han et al. illustrates a range of intensities associated with each component.

With regard to claims 24-25 and 37-39, Figure 5 of Han et al. illustrates hybridization probes for different analytes wherein each analyte comprises a different combination of fluorophores. Figure 2 of Lin et al. teaches that the number of hybridization probes is greater than the number of analytes.

Wirth regard to claim 27, Figure 5 of Han et al. illustrates three components of the signals used to detect each analyte.

With regard to claim 29, Figure 5 of Han et al. teaches that each analyte comprises a polynucleotide.

With regard to claim 30, page 31 of Lin et al. teaches the presence of a computer to analyze spectral data.

With regard to claims 32-33, Figure 2 of Lin et al. teaches a histogram of non-degenerate counts of presence the 27 different combinations of fluorophores in an image.

With regard to claim 40, the different sizes of the bars in the histogram in Figure 2 of Lin et al. teaches that the analyte specific reagents are each present at a different amount.

It would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the quantum dot hybridization assay of Han et al. by use of the cumulative non-degenerate fluorophore assay of Lin et al. wherein the motivation would have been that the cumulative and non-degenerate nature of the assay of Lin et al. adds mathematical and empirical tools to the assay of Han et al. [Figure 2 of Han et al.]. There would have been a reasonable expectation of success in combining Han et

al. and Lin et al. because both studies analogously pertain to assays involving colored fluorophores or quantum dots.

35 U.S.C. 103 Rejection #2:

Claims 26 and 31 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Han et al. in view of Lin et al. as applied to claims 21-25, 27, 29-30, and 32-40 above, in further view of Albani et al. [US PGPUB 2006/0039918 A1].

Claim 26 is further limiting wherein the signal is generated during PCR.

Claim 31 is further limiting wherein the assay is performed using a computer and thermal cycler.

Han et al. and Lin et al. make obvious fluorophore and quantum dot assays to detect presence and absence of analytes, as discussed above.

Han et al. and Lin et al. do not teach PCR, computers, and thermal cyclers.

The document of Albani et al. studies stress proteins and peptides [title]. Paragraph 184 of Albani et al. relates fluorophores, PCR, and thermal cyclers.

It would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the quantum dot hybridization assay of Han et al. and the cumulative non-degenerate fluorophore assay of Lin et al. by use of the PCR, computers, and thermal cyclers of Albani et al. wherein the motivation would have been that Albani et al. adds empirical tools that facilitate the analysis of fluorophores [paragraph 184 of Albani et al.].

35 U.S.C. 103 Rejection #3:

Claim 28 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Han et al. in view of Lin et al. as applied to claims 21-25, 27, 29-30, and 32-40 above, in further view of Hardin et al. [US PGPUB 2010/0317005 A1].

Claim 28 is further limiting wherein the fluorescence signals are used to generate chromatograms.

Han et al. and Lin et al. make obvious fluorophore and quantum dot assays to detect presence and absence of analytes, as discussed above.

Han et al. and Lin et al. do not teach using fluorescence signals to generate chromatograms.

The document of Hardin et al. studies modified nucleotides [title]. Paragraph 143 of Hardin et al. relates fluorescence signals to chromatograms.

It would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the quantum dot hybridization assay of Han et al. and the cumulative non-degenerate fluorophore assay of Lin et al. by use of the chromatograms of Hardin et al. wherein the motivation would have been that Hardin et al. adds empirical tools that facilitate the analysis of fluorophores [paragraph 143 of Hardin et al.].

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at

least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For

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www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Double Patenting Rejection #1:

Claims 21, 23, 26, 27, and 29-34 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 7-8, 11-12, 15-17, and 20, respectively, of U.S. Patent No. 8,838,394. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims are drawn to assays for detection cumulative presence/absence patterns of analytes associated with fluorophores without using immobilization or mass spectrometry. While the claims of '394 recite more limitations than the instantly rejected claims, the claims of '394 anticipate the instantly rejected claims.

Double Patenting Rejection #2:

Claims 21, 26, and 30-31 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 4-5, and 9, respectively, of U.S. Patent No. 10,068,051 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims are drawn to assays for detection cumulative presence/absence patterns of analytes associated with fluorophores without using immobilization or mass spectrometry. While the claims of '394 recite more limitations than the instantly rejected claims, the claims of '051 anticipate the instantly rejected claims.

Double Patenting Rejection #3:

Claims 21, 26, and 30 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 7, and 13, respectively, of U.S. Patent No. 10,770,170 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims are drawn to assays for detection cumulative presence/absence patterns of analytes associated with fluorophores without using immobilization or mass spectrometry. While the claims of '394 recite more limitations than the instantly rejected claims, the claims of '170 anticipate the instantly rejected claims.

Related Prior Art

The prior art of Geiss et al. [WO 2008/124847; on IDS] teach targeting sequences using coding via labeling. However, Geiss et al. does not teach the technique in the recited claims.

E-mail Communications Authorization

Per updated USPTO Internet usage policies, Applicant and/or applicant's representative is encouraged to authorize the USPTO examiner to discuss any subject matter concerning the above application via Internet e-mail communications. See MPEP 502.03. To approve such communications, Applicant must provide written authorization for e-mail communication by submitting the following statement via EFS-Web (using PTO/SB/439) or Central Fax (571-273-8300):

> Recognizing that Internet communications are not secure, I hereby authorize the USPTO to communicate with the undersigned and practitioners in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application by video conferencing,

> instant messaging, or electronic mail. I understand that a copy of these communications will be made of record in the application file.

Written authorizations submitted to the Examiner via e-mail are **NOT** proper. Written authorizations must be submitted **via EFS-Web** (using PTO/SB/439) **or Central Fax** (571-273-8300). A paper copy of e-mail correspondence will be placed in the patent application when appropriate. E-mails from the USPTO are for the sole use of the intended recipient, and may contain information subject to the confidentiality requirement set forth in 35 USC § 122. See also MPEP 502.03

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Russell Negin, whose telephone number is (571) 272-1083. This Examiner can normally be reached from Monday through Thursday from 9:00 am to 5 pm and variable hours on Fridays.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's Supervisor, Karlheinz Skowronek, Supervisory Patent Examiner, can be reached at (571) 272-9047.

Information regarding the status of the application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

/RUSSELL S NEGIN/
Primary Examiner, Art Unit 1672
22 December 2022

Notice of References Cited	Application/Control No. 16/864,744	Applicant(s)/Patent Under Reexamination Kartalov et al.	
	Examiner RUSSELL S NEGIN	Art Unit 1672	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20060039918-A1	02-2006	Albani; Salvatore	A61P25/00	424/185.1
*	B	US-20100317005-A1	12-2010	Hardin; Susan H.	C12Q1/6869	435/6.14
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/864,744	Kartalov et al.
	Examiner	Art Unit
	RUSSELL S NEGIN	1672

CPC - Searched*		
Symbol	Date	Examiner
C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or G06F17/10 or G06F17/11	12/16/2022	RSN

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
see attached search history printouts	12/16/2022	RSN
PE2E Search prior art search	12/16/2022	RSN
PE2E Search inventor search on all inventors	12/16/2022	RSN
PALM inventor search on all inventors	12/16/2022	RSN
PE2E Search CPC code search with modified terms	12/16/2022	RSN

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1672	

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PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	3	("10770170," "10068051," "8838394").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/16 09:21 AM
L2	140	kartalov.in. NEAR emil.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2022/12/16 11:12 AM
L3	286	rajagopal.in. NEAR aditya.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2022/12/16 11:12 AM
L4	1077	scherer.in. NEAR axel.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2022/12/16 11:12 AM
L5	166506	(C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU,	OR	ON	ON	2022/12/16 11:13 AM

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		G06F17/10 or G06F17/11).cpc.	CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L6	1264	L2 L3 L4	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:13 AM
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L8	1900960	PCR	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN,	OR	ON	ON	2022/12/16 11:14 AM

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			WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L9	113706	thermal ADJ cycler	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L10	339872	fluorophores	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L11	25989671	computers	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L12	64751	L8 WITH L9	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB,	OR	ON	ON	2022/12/16 11:15 AM

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			HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L13	448	L10 WITH L12	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L14	1	L13 WITH L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L15	519	L13 andL11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2022/12/16 11:15 AM

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			IBM_TDB)				
L16	26534951	L13 andL 11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L17	278	L13 AND L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L18	439599	chromatogram	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:21 AM
L19	125	L10 WITH L18	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS,	OR	ON	ON	2022/12/16 11:21 AM

			IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /RSN/

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	001	US-5079351	01-07-1992	SNINSKY; John J. et al.	
	002	US-5538848	07-23-1996	LIVAK; Kenneth J. et al.	
	003	US-5618711	04-08-1997	GELFAND; David H. et al.	
	004	US-5677152	10-14-1997	BIRCH; David Edward et al.	
	005	US-5723591	03-03-1998	LIVAK; Kenneth J. et al.	
	006	US-5773258	06-30-1998	BIRCH; David Edward et al.	
	007	US-5789224	08-04-1998	GELFAND; David H. [Us] et al.	
	008	US-5804375	09-08-1998	GELFAND; David H. et al.	
	009	US-5834203	11-10-1998	KATZIR; Nir et al.	
	010	US-5876930	03-02-1999	LIVAK; Kenneth J. et al.	
	011	US-5882856	03-16-1999	SHUBER; Anthony P.	
	012	US-5928862	07-27-1999	MORRISON; Larry E.	
	013	US-5948360	09-07-1999	RAO; Prabhakar P. et al.	
	014	US-5981180	11-09-1999	CHANDLER; Van S. et al.	
	015	US-5994056	11-30-1999	HIGUCHI; Russell G.	
	016	US-6030787	02-29-2000	LIVAK; Kenneth J. et al.	
	017	US-6103463	08-15-2000	CHETVERIN; Alexander B. et al.	
	018	US-6127155	10-03-2000	GELFAND; David H. et al.	
	019	US-6171785	01-09-2001	HIGUCHI; Russell G.	
	020	US-6258569	07-10-2001	LIVAK; Kenneth J. et al.	
	021	US-6534266	03-18-2003	SINGER; Robert H.	

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	12/16/2022

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /RSN/

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	022	US-6534274	03-18-2003	BECKER; Michael M. et al.	
	023	US-6548259	04-15-2003	WARD; David C. et al.	
	024	US-6642062	11-04-2003	KAUVAR; Lawrence M. et al.	
	025	US-6893875	05-17-2005	TSUJI; Yasuhiro et al.	
	026	US-7101663	09-05-2006	GODFREY; Tony E. et al.	
	027	US-7141377	11-28-2006	GELFAND; David H. et al.	
	028	US-7348141	03-25-2008	FRENCH; David John et al.	
	029	US-7385043	06-10-2008	KRAMER; Fred R.	
	030	US-7410764	08-12-2008	GOCKE; Christopher et al.	
	031	US-7413708	08-19-2008	MAYRAND; Paul E.	
	032	US-7473767	01-06-2009	DIMITROV; Krassen	
	033	US-7507575	03-24-2009	BEDINGHAM; William [Us] et al.	
	034	US-7575864	08-18-2009	BEDZYK; Laura A. et al.	
	035	US-7667024	02-23-2010	MAO; FEI et al.	
	036	US-7671184	03-02-2010	HAENER; Robert et al.	
	037	US-7709249	05-04-2010	BEDINGHAM; William et al.	
	038	US-7767423	08-03-2010	KOPRESKI; Michael [Us] et al.	
	039	US-7771949	08-10-2010	KRAMER; Fred R.	
	040	US-7919237	04-05-2011	DIMITROV; Krassen et al.	
	041	US-7919244	04-05-2011	MADEJON; Seiz Antonio et al.	

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	12/16/2022

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 3 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	042	US-7930106	04-19-2011	CARRICK; James M. [Us] et al.	
	043	US-7941279	05-10-2011	HWANG; Jenq-Neng et al.	
	044	US-8039215	10-18-2011	HIGUCHI; Russell [Us] et al.	
	045	US-8148512	04-03-2012	DIMITROV; Krassen et al.	
	046	US-8426132	04-23-2013	LI; BIN [Us] et al.	
	047	US-8455184	06-04-2013	ATCHLEY; Daniel H. [Us] et al.	
	048	US-8492094	07-23-2013	DIMITROV; Krassen [Us] et al.	
	049	US-8519115	08-27-2013	WEBSTER; Philippa J. [Us] et al.	
	050	US-8614061	12-24-2013	BRABETZ; Werner [De] et al.	
	051	US-8771955	07-08-2014	REED; Michael W. [Us] et al.	
	052	US-8838394	09-16-2014	KARTALOV; Emil [Us] et al.	
	053	US-8877464	11-04-2014	BABIEL; Reiner [De] et al.	
	054	US-8962250	02-24-2015	STANLEY; Keith [Au] et al.	
	055	US-9133506	09-15-2015	KATZIR; NIR [Il] et al.	
	056	US-9222128	12-29-2015	SAXONOV; Serge et al.	
	057	US-9260761	02-16-2016	TYAGI; Sanjay [Us] et al.	
	058	US-9366632	06-14-2016	LINK; Darren R. et al.	
	059	US-9422593	08-23-2016	ROTHMANN; Thomas [De] et al.	
	060	US-9441266	09-13-2016	LARSON; Jonathan William et al.	

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Sheet 4 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	061	US-9447457	09-20-2016	CHUN; Jong Yoon [Kr] et al.	
	062	US-9458497	10-04-2016	HASSIBI; Arjang et al.	
	063	US-9791372	10-17-2017	MALIK; Imran R. et al.	
	064	US-10066263	09-04-2018	RAJAGOPAL; Aditya et al.	
	065	US-10068051	09-04-2018	KARTALOV; Emil et al.	
	066	US-10068051	09-04-2018	KARTALOV; Emil et al.	
	067	US-10301668	05-28-2019	JACKY; Lucian A.E.	
	068	US-10770170	09-08-2020	KARTALOV; Emil P. et al.	
	069	US-20020022273	02-21-2002	EMPEDOCLES; Stephen A. [Us] et al.	
	070	US-20020146734	10-10-2002	ORTYN; William E. et al.	
	071	US-20030134320	07-17-2003	BARRUS; Jeffrey K. et al.	
	072	US-20030148280	08-07-2003	HARRIS; Barbara J. et al.	
	073	US-20030148544	08-07-2003	NIE; Shuming et al.	
	074	US-20040053230	03-18-2004	SCHAFFER; Michael Edward [Us] et al.	
	075	US-20040191794	09-30-2004	WEINDEL; Kurt et al.	
	076	US-20050053950	03-10-2005	ZUDAIRE UBANI; Enrique et al.	
	077	US-20050064435	03-24-2005	SU; Xing et al.	
	078	US-20050106607	05-19-2005	YIN; Li-Te et al.	
	079	US-20050164264	07-28-2005	SHIPWASH; Edward	
	080	US-20050214753	09-29-2005	SHULTZ; John W. et al.	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 5 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

U. S. PATENT DOCUMENTS

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	081	US-20050250146	11-10-2005	MCMILLAN; William A.	
	082	US-20050260640	11-24-2005	ANDERSEN; Mark R. et al.	
	083	US-20060216708	09-28-2006	VENEMA; Fokke	
	084	US-20070072211	03-29-2007	NEWTON; Nick et al.	
	085	US-20070178485	08-02-2007	EL-DEIRY; Wafik S. et al.	
	086	US-20070231824	10-04-2007	CHEE; Mark S. et al.	
	087	US-20080050737	02-28-2008	ARIELI; Boaz et al.	
	088	US-20080069733	03-20-2008	MALTEZOS; George et al.	
	089	US-20080096767	04-24-2008	KOHN; Heinz G.	
	090	US-20080124705	05-29-2008	KRAMER; Fred R.	
	091	US-20090042735	02-12-2009	BLAIR; Steven M. et al.	
	092	US-20090048785	02-19-2009	KATZIR; NIR et al.	
	093	US-20090062129	03-05-2009	MCKERNAN; Kevin [Us] et al.	
	094	US-20100015607	01-21-2010	GEISS; Gary K. et al.	
	095	US-20100041092	02-18-2010	LIN; Michael Z. et al.	
	096	US-20100047924	02-25-2010	WEBSTER; Philippa J. et al.	
	097	US-20100112710	05-06-2010	GEISS; Gary K. et al.	
	098	US-20100120043	05-13-2010	SOOD; Anup et al.	
	099	US-20100151443	06-17-2010	XIANG; Qing et al.	
	100	US-20100159447	06-24-2010	LI; BIN et al.	
	101	US-20100210472	08-19-2010	EMPEDOCLES; Stephen A. et al.	

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Sheet 6 of 27

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Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

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	102	US-20100233686	09-16-2010	HIGUCHI; Russell et al.	
	103	US-20100248257	09-30-2010	JACOBSEN; Kivin et al.	
	104	US-20100261026	10-14-2010	FERREE; Sean M. et al.	
	105	US-20100267064	10-21-2010	KARTALOV; Emil P. et al.	
	106	US-20100273173	10-28-2010	HIRAI; Mitsuharu et al.	
	107	US-20100324834	12-23-2010	TREPTOW; Rainer [De] et al.	
	108	US-20110104684	05-05-2011	HOOPER; Dennis G.	
	109	US-20110151459	06-23-2011	ROTHMANN; Thomas et al.	
	110	US-20110151550	06-23-2011	SAGNER; Gregor et al.	
	111	US-20110171658	07-14-2011	CARRICK; James M.	
	112	US-20110207623	08-25-2011	DIMITROV; Krassen et al.	
	113	US-20110223602	09-15-2011	WHITMAN; Douglas F. [Us] et al.	
	114	US-20110237459	09-29-2011	NOVA; Michael P. et al.	
	115	US-20120003646	01-05-2012	JOO; Sunghae A. et al.	
	116	US-20120040349	02-16-2012	VON; Lode Piia et al.	
	117	US-20120040352	02-16-2012	WANGH; Lawrence J. [Us] et al.	
	118	US-20120045756	02-23-2012	ROTHMANN; Thomas et al.	
	119	US-20120077195	03-29-2012	LI; Qingge et al.	
	120	US-20120101740	04-26-2012	ORPANA; Arto [Se] et al.	
	121	US-20120122704	05-17-2012	ATCHLEY; Daniel H. et al.	

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Sheet 7 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

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	122	US-20120141995	06-07-2012	LI; Qingge et al.	
	123	US-20120171677	07-05-2012	LUDOWISE; Peter D.	
	124	US-20120184017	07-19-2012	CHATTERJEE; DEB K.	
	125	US-20120190030	07-26-2012	CHUN; Jong Yoon et al.	
	126	US-20120196283	08-02-2012	BABIEL; Reiner et al.	
	127	US-20120244534	09-27-2012	CHING; Jesus et al.	
	128	US-20120252014	10-04-2012	LOEFFERT; Dirk et al.	
	129	US-20120252017	10-04-2012	REED; Michael W. et al.	
	130	US-20120258457	10-11-2012	JAROSCH; Florian et al.	
	131	US-20120302448	11-29-2012	HUTCHISON; John Brian et al.	
	132	US-20130017971	01-17-2013	GEISS; Gary K. [Us] et al.	
	133	US-20130040841	02-14-2013	SAXONOV; Serge et al.	
	134	US-20130078626	03-28-2013	WASSERSTROM; Adam et al.	
	135	US-20130116780	05-09-2013	MILLER; Eran et al.	
	136	US-20130261019	10-03-2013	LIN; Chenxiang [Us] et al.	
	137	US-20130288244	10-31-2013	DECIU; Cosmin et al.	
	138	US-20140004520	01-02-2014	MOHAPATRA; Shyam S. et al.	
	139	US-20140038195	02-06-2014	MALIK; Imran R. [Us] et al.	
	140	US-20140171341	06-19-2014	JOUVENOT; Yann et al.	
	141	US-20140213471	07-31-2014	RAJAGOPAL; Aditya et al.	

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	12/16/2022

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Sheet 8 of 27

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Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

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	142	US-20140274774	09-18-2014	LI; Kelly et al.	
	143	US-20150057178	02-26-2015	KARTALOV; Emil et al.	
	144	US-20150140554	05-21-2015	SNYDER; Richard O. et al.	
	145	US-20150211054	07-30-2015	KOSTEM; Emrah et al.	
	146	US-20150275295	10-01-2015	WANG; Linfa et al.	
	147	US-20160040256	02-11-2016	CHEN; Caifu et al.	
	148	US-20160108464	04-21-2016	SAXONOV; Serge et al.	
	149	US-20160201122	07-14-2016	BUSHKIN; Yuri et al.	
	150	US-20160273048	09-22-2016	ROPERCH; Jean-Pierre	
	151	US-20170145490	05-25-2017	CHIU; Daniel T. et al.	
	152	US-20170314073	11-02-2017	GRÖMMINGER; Sebastian et al.	
	153	US-20170362636	12-21-2017	RAJAGOPAL; Aditya et al.	
	154	US-20180030551	02-01-2018	RAJAGOPAL; Aditya et al.	
	155	US-20180052110	02-22-2018	MALIK; Imran R. et al.	
	156	US-20180057864	03-01-2018	JACKY; Lucien A. E. et al.	
	157	US-20190002963	01-03-2019	RAJAGOPAL; Aditya	
	158	US-20190032112-A1	01-31-2019	RAJAGOPAL; Aditya et al.	
	159	US-20190112636	04-18-2019	RAJAGOPAL; Aditya et al.	
	160	US-20190233882	08-01-2019	JACKY; Lucien et al.	
	161	US-20200010876-A1	01-09-2020	MACDONALD; Christopher	

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	12/16/2022

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 9 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	001	CN-101570782-A	11-04-2009	CHUNHUA YANG [CN]	Abstract:	☒
	002	CN-1936019-A	03-28-2007	UNIV XIAMEN [CN]	Abstract:	☒
	003	EP-0519338-A1	12-23-1992	HOFFMANN LA ROCHE [CH]		☐
	004	EP-1448581-B1	11-12-2008	INST SYSTEMS BIOLOGY [US]		☐
	005	EP-1629108-B1	12-31-2014	PHRI PROPERTIES INC [US]		☐
	006	EP-1963531-B1	09-21-2011	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	007	EP-2224017-A1	09-01-2010	ARKRAY INC [JP]		☐
	008	WO-0107640-A2	02-01-2001	SEC DEP HOME DEPT [GB], et al.		☐
	009	WO-02056014-A2	07-18-2002	GLAXO GROUP LTD [GB], et al.		☐
	010	WO-02056014-A3	10-09-2003	GLAXO GROUP LTD [GB], et al.		☐

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	011	WO-03002979-A2	01-09-2003	ILLUMINA INC [US], et al.		☐
	012	WO-03020967-A1	03-13-2003	ROCHE DIAGNOSTICS GMBH [DE], et al.		☐
	013	WO-2004087950-A2	10-14-2004	ROCHE DIAGNOSTICS GMBH [DE], et al.		☐
	014	WO-2006079049-A2	07-27-2006	THIRD WAVE TECH INC [US], et al.		☐
	015	WO-2007076128-A2	07-05-2007	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	016	WO-2007076128-A3	11-29-2007	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	017	WO-2007076129-A2	07-05-2007	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	018	WO-2007076129-A3	03-06-2008	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	019	WO-2007076132-A2	07-05-2007	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	020	WO-2007076132-A3	09-07-2007	NANOSTRING TECHNOLOGIES INC [US], et al.		☐

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	021	WO-2007139766-A2	12-06-2007	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	022	WO-2007139766-A3	12-11-2008	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	023	WO-2008118998-A2	10-02-2008	PRIMERA BIOSYSTEMS INC [US], et al.		☐
	024	WO-2008124847-A2	10-16-2008	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	025	WO-2008124847-A3	02-19-2009	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	026	WO-2009036514-A2	03-26-2009	MOLECULAR PLANT BREEDING NOMINEES LTD [AU], et al.		☐
	027	WO-2010007355-A1	01-21-2010	LUMORA LTD [GB], et al.		☐
	028	WO-2010013017-A1	02-04-2010	OXITEC LTD [GB], et al.		☐
	029	WO-2010017543-A1	02-11-2010	SMITHS DETECTION INC [US], et al.		☐
	030	WO-2010019826-A1	02-18-2010	NANOSTRING TECHNOLOGIES INC [US], et al.		☐

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	031	WO-2010128206-A1	11-11-2010	EXPRESSION ANALYTICS OY [FI], et al.		☐
	032	WO-2011047087-A2	04-21-2011	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	033	WO-2011047087-A3	08-18-2011	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	034	WO-2011100541-A2	08-18-2011	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	035	WO-2011100541-A3	01-05-2012	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	036	WO-2011116088-A2	09-22-2011	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	037	WO-2011116088-A3	02-02-2012	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	038	WO-2012058638-A2	05-03-2012	HARVARD COLLEGE [US], et al.		☐
	039	WO-2012058638-A3	12-20-2012	HARVARD COLLEGE [US], et al.		☐
	040	WO-2012106428-A2	08-09-2012	UNIV FLORIDA [US], et al.		☐

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	041	WO-2012135340-A2	10-04-2012	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	042	WO-2012135340-A3	12-20-2012	NANOSTRING TECHNOLOGIES INC [US], et al.		☐
	043	WO-2013096851-A1	06-27-2013	HARVARD COLLEGE [US]		☐
	044	WO-2013116780-A1	08-08-2013	CALIFORNIA INST OF TECHN [US], et al.		☐
	045	WO-2014022827-A1	02-06-2014	CALIFORNIA INST OF TECHN [US]		☐
	046	WO-2014116884-A1	07-31-2014	CALIFORNIA INST OF TECHN [US]		☐
	047	WO-2015147370-A1	10-01-2015	SEEGENE INC [KR]		☐
	048	WO-2016154600-A1	09-29-2016	MASSACHUSETTS GEN HOSPITAL [US]		☐
	049	WO-2017139354-A1	08-17-2017	UNIV MICHIGAN REGENTS [US]		☐
	050	WO-2017173035-A1	10-05-2017	CHROMACODE INC [US]		☐

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	051	WO-2017218777-A1	12-21-2017	CALIFORNIA INST OF TECHN [US]		☐
	052	WO-2018081178-A1	05-03-2018	TWO PORE GUYS INC [US]		☐
	053	WO-2019006023-A1	01-03-2019	CHROMACODE INC [US]		☐
	054	WO-2019079204-A1	04-25-2019	CHROMACODE INC [US]		☐
	055	WO-2019169043-A1	09-06-2019	CHROMACODE INC [US]		☐
	056	WO-9746714-A1	12-11-1997	UNIV UTAH RES FOUND [US], et al.		☐
	057	WO-99/52708	10-21-1999	LUMINEX CORP [US]		☐
	058	WO-9919515-A1	04-22-1999	LUMINEX CORP [US]		☐

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NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	001	ARYA, et al., Basic principles of real-time quantitative PCR, 2005, Expert Rev. Mol. Diagn., 5(2), pg. 209-219.	☐
	002	BEIGE, et al. Clinical evaluation of a Mycobacterium tuberculosis PCR assay. J Clin Microbiol. 1995 Jan;33(1):90-5.	☐
	003	BLACKET et al. Universal primers for fluorescent labeling of PCR fragments - an efficient and cost-effective approach to genotyping by fluorescence. Moleular Ecology Resources 12(3):456-463 (2012) Epub 2012 Jan 24.	☐
	004	CHAMBERLAIN, et al. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res. 1988 Dec 9;16(23):11141-56.	☐
	005	CHAPIN et al., Rapid microRNA profiling on encoded gel microparticles. Angewandte Chemie International Edition, 50(10):2289-2293, 2011.	☐
	006	CHEN, et al. A Homogeneous, ligase-mediated DNA diagnostic test. Genome Research, 1998, volume 8, pages 549-556.	☐
	007	CHONG, et al. Single-tube multiplex-PCR screen for common deletional determinants of alpha-thalassemia. Blood. 2000 Jan 1;95(1):360-2.	☐
	008	Chromatogram, 2011, 2 pages. Dorland's illustrated medical dictionary. Retrieved online on 22 January 2014 from «http://www.credoreference.com».	☐
	009	CHUN et al. Dual priming oligonucleotide system for the multiplex detection of respiratory viruses and SNP genotyping of CYP2C19 gene. Nucleic Acids Res. 35(6):e40 (2007).	☐

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Sheet 16 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

NON-PATENT LITERATURE DOCUMENTS

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	010	CRAIG, et al. Ordering of cosmid clones covering Herpes simplex virus type I (HSV-I) genome: a test case for fingerprinting by hybridisation. Nucleic Acids Research, 1990, volume 18, pages 2653-2660.	☐
	011	DOS SANTOS, et al. A simple one-step real-time RT-PCR for diagnosis of dengue virus infection. J Med Virol. 2008 Aug;80(8):1426-33. .	☐
	012	EL-HAJJ, et al. Detection of rifampin resistance in Mycobacterium tuberculosis in a single tube with molecular beacons. J Clin Microbiol. 2001 Nov;39(11):4131-7.	☐
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	014	EMBL-Bank: GQ395623. http://www.ebi.ac.uk/Tools/dbfetch/emblfetch?db=embl&id=GQ395623&format=default&style=default&Retrieve=Retrieve. Accessed February 2012.	☐
	015	EP17814096.8 Extended European Search Report dated December 2, 2019.	☐
	016	European Office Action for Patent Application No. EP13824744.0 dated November 6, 2018.	☐
	017	European Patent Application No. 17776626 Office Action dated October 18, 2019.	☐
	018	EVANS et al. Digital PCR for Noninvasive Detection of Aneuploidy: Power Analysis Equations for Feasibility. Fetal Diagn. Ther. 31:244-247(2012).	☐
	019	Extended European Search Report for Patent Application No. 13744261.2 dated May 3, 2016.	☐

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	020	Extended European Search Report for Patent Application No. EP13824744.0 dated February 26, 2016.	☐
	021	FODOR, et al. Multiplexed biochemical assays with biological chips. Nature. 1993 Aug 5;364(6437):555-6.	☐
	022	FORTINA, et al. Digital mRNA profiling. Nat Biotechnol. 2008 Mar;26(3):293-4.	☐
	023	FU et al. Multiplex detection and SNP genotyping in a single fluorescence channel. PLoS One 7(1):Article No. e30340 (January 2012).	☐
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	025	GenBank: M93130.1. Dengue type 3 virus complete genome RNA, complete cds. http://www.ncbi.nlm.nih.gov/nuccore/M93130. Accessed February 2012.	☐
	026	HAN, et al., Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. Nature Biotechnology 19.99 (July 2001): 631-635.	☐
	027	HARTMAN, et al. Development of a novel internal positive control for Taqman based assays. Mol Cell Probes. 2005 Feb;19(1):51-9. Epub 2004 Dec 10.	☐
	028	HAUSTEIN et al. Fluorescence correlation spectroscopy: novel variations of an established technique. Ann Rev Biophys Biomol Struct 36:151-69 (2007).	☐
	029	HEIDARI, et al. Detection of Plasmodium falciparum Directly from Blood Samples Using the Polymerase Chain Reaction. Journal of Sciences, Islamic Republic of Iran. 2005 16(1):21-24.	☐

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	030	HENEGARIU, et al. Multiplex PCR: critical parameters and step-by-step protocol. Biotechniques. 1997 Sep;23(3):504-11.	☐
	031	HIV databases. http://www.hiv.lanl.gov/content/index. Accessed February 2012.	☐
	032	Holland, et al., Detection of specific polymerase chain reaction product by utilizing the 5´ to 3´ exonuclease activity of Thermus aquaticus DNA polymerase. PNAS (USA) 88:7276–7280, 1991.	☐
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	038	JOTHIKUMAR, et al. Design of FRET-TaqMan probes for multiplex real-time PCR using an internal positive control. Biotechniques. 2009 Jun;46(7):519-24. .	☐
	039	KLOSTRANEC et al., Convergence of quantum dot barcodes with microfluidics and signal processing for multiplexed high-throughout infectious disease diagnostics. Nano Letters, 7(9):2812-2818, 2007.	☐

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	040	KUHN et al. Hybridization of DNA and PNA molecular beacons to single-stranded and double-stranded DNA targets. J Am Chem Soc. 124(6):1097-103 (2002).	☐
	041	LAO, et al. Multiplexing RT-PCR for the detection of multiple miRNA species in small samples. Biochem Biophys Res Commun. 2006 Apr 28;343(1):85-9. Epub 2006 Feb 28.	☐
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	046	LIN et al., Self-assembled combinatorial encoding nanoarrays for multiplexed biosensing. Nano Letters, 7(2):507-512, 2007.	☐
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	050	MORGAN et al., A commercial line probe assay for the rapid detection of rifampicin resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis. BMC Infectious Diseases. 5:62 doi:10.1186/1471-2334-5-62 (2005).	☐
	051	MORRISON, et al. Two-color ratio-coding of chromosome targets in fluorescence in situ hybridization: quantitative analysis and reproducibility. Cytometry. 1997 Apr 1;27(4):314-26.	☐
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	053	NAVARRO et al., Real-time PCR detection chemistry. Clin Chim Acta. 439:231-250 (2015).	☐
	054	NOORDHOEK, et al. Sensitivity and specificity of PCR for detection of Mycobacterium tuberculosis: a blind comparison study among seven laboratories. J Clin Microbiol. 1994 Feb;32(2):277-84.	☐
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	056	OU, et al. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science. 1988 Jan 15;239(4837):295-7.	☐
	057	PATON, et al. Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E. coli hlyA, rfbO111, and rfbO157. J Clin Microbiol. 1998 Feb;36(2):598-602.	☐
	058	PATTERSON, et al. Detection of HIV-1 DNA and messenger RNA in individual cells by PCR-driven in situ hybridization and flow cytometry. Science. 1993 May 14;260(5110):976-9.	☐

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	059	PCT/US19/40392 International Search Report and Written Opinion dated November 06, 2019.	☐
	060	PCT/US2013/024509 International search report and written opinion dated April 12, 2013 .	☐
	061	PCT/US2013/053512 International Search Report and Written Opinion dated October 16, 2013.	☐
	062	PCT/US2017/24933 International search report and written report dated June 22, 2017.	☐
	063	PCT/US2017/37682 International Search Report and Written Opinion dated November 16, 2017.	☐
	064	PCT/US2018/039846 International Preliminary Report on Patentability dated December 31, 2019.	☐
	065	PCT/US2018/055927 International Preliminary Report on Patentability dated April 21, 2020.	☐
	066	PCT/US2018/39846 International Search Report and Written Opinion Mailed September 14, 2018.	☐
	067	PCT/US2019/019906 International Preliminary Report on Patentability September 01, 2020.	☐
	068	PCT/US2019/019906 International Search Report and Written Opinion dated May 06, 2019.	☐
	069	PCT/US2019/027751 International Preliminary Report on Patentability dated October 20, 2020.	☐
	070	PCT/US2019/027751 International Search Report and Written Opinion dated June 26, 2019.	☐
	071	PCT/US2019/035129 International Search Report and Written Opinion dated November 01, 2019.	☐

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	072	PCT/US2019/035129 Invitation to Pay Additional Fees dated August 19, 2019.	☐
	073	PCT/US2019/040392 International Preliminary Report on Patentability dated January 05, 2021.	☐
	074	PCT/US2019/041239 International Preliminary Report on Patentability dated January 21, 2021.	☐
	075	PCT/US2019/041239 International Search Report and Written Opinion dated September 30, 2019.	☐
	076	PETERSEN, et al. Short PNA molecular beacons for real-time PCR allelic discrimination of single nucleotide polymorphisms. Mol Cell Probes. 2004 Apr;18(2):117-22.	☐
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	081	RAJAGOPAL et al., Significant Expansion of Real-Time PCR Multiplexing with Traditional Chemistries using Amplitude Modulation. Scientific Reports. 9(1): 1053 (2019). .	☐

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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 23 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	082	Rickert et al., "Multiplexed Real-Time PCR Using Universal Reporters," Clin. Chem., 50(9):1680- 1683, 2004.	☐
	083	ROSENSTRAUS, et al. An internal control for routine diagnostic PCR: design, properties, and effect on clinical performance. J Clin Microbiol. 1998 Jan;36(1):191-7.	☐
	084	ROTH, et al. Feasibility and efficacy of routine PCR screening of blood donations for hepatitis C virus, hepatitis B virus, and HIV-1 in a blood-bank setting. Lancet. 1999 Jan 30;353(9150):359-63.	☐
	085	SAIKI, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science, 239(4839):487-491 (January 29, 1988).	☐
	086	SAMBROOK, et al. Molecular Cloning: A Laboratory Manual. 2nd Edition, 1989.	☐
	087	SANGER et al. DNA sequencing with chain-terminating inhibitors. PNAS USA 1977 Dec;74(12):5463-7 (1977).	☐
	088	Second Office Action for Chinese Patent Application No. 201380049844.9 dated September 19, 2016 (w/translation).	☒
	089	SPEICHER, et al. Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet. 1996 Apr;12(4):368-75.	☐
	090	ThermoFisher Scientific Fluorescence SpectraViewer. Retrieved from http://www.thermofisher.com/us/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html on September 25, 2015.	☐
	091	TIRASOPHON, et al. A novel detection of a single Plasmodium falciparum in infected blood. Biochem Biophys Res Commun. 1991 Feb 28;175(1):179-84.	☐

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	12/16/2022

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 24 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

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	092	TYAGI, et al. Multicolor molecular beacons for allele discrimination. Nat Biotechnol. 1998 Jan;16(1):49-53.	☐
	093	TYAGI, et al. Wavelength-shifting molecular beacons. Nat Biotechnol. 2000 Nov;18(11):1191-6.	☐
	094	United States Application No. 16/128,343 Final Office Action dated March 16, 2021.	☐
	095	URDEA, et al. Requirements for high impact diagnostics in the developing world. Nature. 2006 Nov 23;444 Suppl 1:73-9.	☐
	096	U.S. Application No. 13/756,760 Notice of allowance dated June 2, 2014.	☐
	097	U.S. Application No. 13/756,760 Office action dated January 24, 2014 .	☐
	098	U.S. Application No. 13/958,479 Office Action dated March 1, 2016.	☐
	099	U.S. Application No. 14/451,876 Office action dated June 6, 2017.	☐
	100	U.S. Application No. 14/451,876 Office action dated November 23, 2016.	☐
	101	U.S. Application No. 15/623,974 Notice of Allowance dated July 3, 2018.	☐
	102	U.S. Application No. 15/623,974 Office Action dated February 22, 2018.	☐
	103	U.S. Application No. 15/677,772 Final Office Action Mailed June 7, 2018.	☐
	104	U.S. Application No. 15/677,772 Non-Final Office Action Mailed February 8, 2018.	☐
	105	U.S. Application No. 15/892,245 First Action Interview - Office Action mailed July 19, 2018.	☐

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	12/16/2022

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 25 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author(in CAPITAL LETTERS),title of the article(when appropriate), title of the item (book,magazine,journal,serial,symposium,catalog,etc.),date,page(s),volume-issue number(s),publisher, city and/or country where published.	T[2]
	106	U.S. Application No. 15/892,245 Office Action dated December 31, 2018.	☐
	107	U.S. Application No. 15/892,245 Preinterview First Interview Office Action Mailed May 11, 2018.	☐
	108	U.S. Application No. 16/020,673 Non-Final Office Action dated November 27, 2020.	☐
	109	U.S. Application No. 14/451,876 Notice of Allowance dated July 11, 2018.	☐
	110	U.S. Application No. 14/451,876 Notice of Allowance dated June 14, 2018.	☐
	111	U.S. Patent Application No. 15/892,245 Office Action dated December 11, 2019.	☐
	112	U.S. Patent Application No. 15/892,245 Office Action dated June 17, 2019.	☐
	113	U.S. Patent Application No. 16/128,343 Non-Final Office Action dated June 16, 2020.	☐
	114	U.S. Patent Application No. 16/128,343 Office Action dated October 15, 2019.	☐
	115	U.S. Serial No. 15/701,014 Office Action dated January 24, 2020.	☐
	116	U.S. Serial No. 15/914,356 Office Action dated July 22, 2019.	☐
	117	U.S. Serial No. 15/914,356 Office Action dated March 8, 2019.	☐
	118	U.S. Serial No. 16/051,736 Office Action dated August 21, 2020.	☐
	119	U.S. Serial No. 16/128,343 First Action Interview dated April 18, 2019.	☐

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	12/16/2022

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 26 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

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	120	VET, et al. Multiplex detection of four pathogenic retroviruses using molecular beacons. Proc Natl Acad Sci U S A. 1999 May 25;96(11):6394-9.	☐
	121	VOGELSTEIN, et al., Digital PCR, PNAS August 3, 1999 96 (16) 9236-9241; https://doi.org/10.1073/pnas.96.16.9236.	☐
	122	WANG, et al. Locked nucleic acid molecular beacons. J Am Chem Soc. 2005 Nov 16;127(45):15664-5.	☐
	123	WATERS, et al. Microchip device for cell lysis, multiplex PCR amplification, and electrophoretic sizing. Anal Chem. 1998 Jan 1;70(1):158-62.	☐
	124	WEIDMANN, et al. Rapid detection of herpes simplex virus and varicella-zoster virus infections by real-time PCR. J Clin Microbiol. 2003 Apr;41(4):1565-8.	☐
	125	WIESE, et al. Simultaneous multianalyte ELISA performed on a microarray platform. Clin Chem. 2001 Aug;47(8):1451-7.	☐
	126	XU et al., Multiplexed SNP genotyping using the Qbead system: a quantum dot-encoded microspere-based assay. Nucleic Acids Research, 31(8):e43, 2003.	☐
	127	YANG, L. et al. A novel universal real-time PCR system using the attached universal duplex probes for quantitative analysis of nucleic acids. BMC Molecular Biology, 9:54 (1-13) June 4, 2008.	☐
	128	ZHANG, et al. A novel real-time quantitative PCR method using attached universal template probe. Nucleic Acids Res. 2003 Oct 15;31(20):e123(pp. 1-8).	☐

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	12/16/2022

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 27 of 27

Complete if Known	
Application Number	16864744
Filing Date	05-01-2020
First Named Inventor	KARTALOV EMIL; P.
Art Unit	1631
Examiner Name	NA
Attorney Docket Number	44158-727.304

NON-PATENT LITERATURE DOCUMENTS

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	129	ZHANG, et al. Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome mec Types I to V in Methicillin-Resistant Staphylococcus aureus. J Clin Microbiol. 2005 Oct;43(10):5026-33.	☐
	130	ZHAO et al. Comprehensive Algorithm for Quantitative Real-Time Polymerase Chain Reaction. Journal of Computational Biology 12(8):1047-1064 (2005).	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /RSN/

Examiner Signature	/RUSSELL S NEGIN/	Date Considered	12/16/2022

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16864744
Google Scholar Search
/RSN/ 12/16/2022

analytes fluorescence
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analytes fluorescence wavelengths intensities signals degenerate nondegenerate

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California Institute of Technology - Ex. 2003 Page 4421

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/864,744	Filing Date 05/01/2020	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	* 0		x $100 =	0
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	* 0		x $460 =	0
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	0

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	03/28/2023	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 20	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/CASSANDRA B DOWNS/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INVENTORS :	Emil KARTALOV *et al.*	CONFIRMATION NO. :	9929
SERIAL NUMBER :	16/864,744	EXAMINER :	Russell Scott Negin
FILING DATE :	May 1, 2020	ART UNIT :	1672
FOR :	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS		

RESPONSE TO NON-FINAL OFFICE ACTION

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Non-Final Office Action mailed **December 28, 2022**, please amend the above-identified application as follows:

Listing of Claims begin on page **2** of this paper.

Remarks begin on page **5** of this paper.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned for under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to Deposit Account No. 504561 (**Ref. No. 88DG-330783-US4**).

LISTING OF CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1.– 20. (Canceled)

21. (Currently Amended) An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,

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

wherein said fluorescent signal comprises F wavelength components, and

wherein said assay is capable of detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume without immobilization, separation, mass spectrometry, or melting curve analysis, based on detection of said F wavelength components, when M is greater than F.

22. (Previously Presented) The assay of claim 21, wherein each of said at least M analytes is encoded as at least one first value in a first component of said fluorescent signal and at least one second value in a second component of said fluorescent signal.

23. (Previously Presented) The assay of claim 22, wherein said first value is an intensity or range of intensities.

24. (Previously Presented) The assay of claim 21, wherein said detecting is performed with reagents comprising hybridization probes.

25. (Previously Presented) The assay of claim 24, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.

26. (Previously Presented) The assay of claim 21, wherein said signal is generated during a polymerase chain reaction.

27. (Previously Presented) The assay of claim 21, wherein at least one of said analytes is encoded by at least one additional value, wherein said at least one additional value is selected from the group

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California Institute of Technology - Ex. 2003 Page 4424

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

28. (Currently Amended) The assay of ~~claim 21~~ claim 24, wherein said fluorescence signal from said hybridization probes are used to construct a chromatogram.
29. (Previously Presented) The assay of claim 21, wherein at least one of said analytes comprises a polynucleotide.
30. (Currently Amended) The assay of claim 21, further comprising transmitting information concerning the presence or absence of at least one of said ~~analyte~~ analytes through a computer network.
31. (Previously Presented) The assay of claim 21, wherein at least one step of said assay is performed using a computer and wherein said computer is connected to a thermal cycler.
32. (Previously Presented) The assay of claim 21, wherein said coding scheme is innately non-degenerate or non-degenerate by construction.
33. (Currently Amended) The assay of claim 22, wherein said coding scheme is made non-degenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.
34. (Currently Amended) A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising analyte-specific reagents, wherein:
    a. each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy; and
    b. each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the ~~first~~ value.
35. (Currently Amended) The kit of claim 34, wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes ~~the plurality of analytes~~ in a single sample volume, in any combination of presence or absence, without immobilization, separation, or melting curve analysis.

36. (Currently Amended) The kit of claim 34, wherein said fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, when M is greater than F.
37. (Currently Amended) The kit of ~~any of claims 34~~ claim 34, wherein said analyte-specific reagents comprise hybridization probes.
38. (Previously Presented) The kit of claim 37, wherein said hybridization probes comprise a fluorophore, and wherein said plurality of hybridization probes are specific for different analytes.
39. (Currently Amended) The kit of claim 38, wherein said number of hybridization probes in the kit is greater than M ~~the number of the analytes of the plurality of analytes~~.
40. (Currently Amended) The kit of ~~any of claims 34~~ claim 34, wherein the analyte-specific reagents are each present at a different amount in the kit.

REMARKS

Claims 21-40 are pending. Claims 21, 30, 34-36 and 39 are amended for clarity. Claims 28, 33, 37 and 40 are amended to correct claim dependency and/or to correct minor typographical issues. No new matter is added. In view of the amendments and following remarks, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, Applicant respectfully requests that the Examiner reconsider all outstanding rejections and allow the claims.

Objection to Claims 33 and 37

Applicant respectfully submits that the amendments to claims 33 and 37 render moot the objections to these claims.

Rejection of Claims 22-31 under 35 U.S.C. § 112, second paragraph

Claims 30 and 32-40 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite for the reasons described below.

Claim 30 is rejected as indefinite for allegedly lacking antecedent for the recitation "said analyte". Applicant respectfully submits that this rejection is rendered moot by the amendment of claim 30 to recite "at least one of said analytes", for which antecedent can be found in claim 21.

Claims 32 and 33 are rejected as indefinite for allegedly lacking antecedent for the recitation "said coding scheme". Reconsideration and withdrawal of this rejection respectfully is requested in view of the amendment of claim 21 to add the recitation "thereby generating a coding scheme", thus providing antecedent.

Claim 34 is rejected as indefinite for allegedly lacking antecedent for the recitation "the first value". Reconsideration and withdrawal of this rejection respectfully is requested in view of the amendment of claim 34 to delete recitation of "first" before "value".

Claim 36 is rejected as indefinite for allegedly lacking antecedent for the recitation of variable "F". Reconsideration and withdrawal of this rejection respectfully is requested in view of the amendment of claim 36 to add the recitation "said fluorescent signal comprises F wavelength components", thus describing, and providing antecedent for, variable "F".

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Claim 39 is rejected as indefinite for allegedly lacking antecedent for the recitations "the number of analytes" and "the plurality of analytes". As amended, claim 39 does not recite "the number of the analytes of the plurality of analytes", thus rendering this rejection moot.

Claims Rejections Under 35 U.S.C. §103

Rejection of Claims 21-25, 27, 29-30, and 32-40 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin

Claims 21-25, 27, 29-30, and 32-40 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han et al. (Nature Biotechnology, volume 19, 2001, pages 631-635) ("Han") in view of International Patent Publication No. 2012/058628 ("Lin").

This rejection is respectfully traversed. Han teaches a method of multiplexing in which "Optical readout is accomplished by measuring the fluorescence spectra of ***single beads***." (Han at Figure 1, page 632, emphasis added). Furthermore, Han teaches the ***immobilization of single-color signal beads*** on a polylysine-coated glass slide, as expressly illustrated in Figure 2 (Han, at Figure 2, Page 632). Han's **single-bead** multiplex approach is again emphasized in Fig. 5, where Han describes the necessary coding scheme to identify the presence and abundance **of each individual bead** (i.e., single targets). Specifically, Han on page 634 teaches:

> To demonstrate the use of QD-tagged beads for biological assays, we designed a model DNA hybridization system using oligonucleotide probes and triple-color encoded beads, as shown in Figure 5. **Target DNA molecules** are either directly labeled with a fluorescent dye or with a biotin (for binding to fluorescently tagged avidin). **Optical spectroscopy at the single-bead level** yields **both the coding and the target signals**. The coding signals identify **the DNA sequence**, whereas the target signal **indicates the presence and the abundance** of **that sequence.** [emphasis added]

Han does **not** teach or suggest an assay, as recited in claim 21, that is capable of detecting the presence or absence of each of said at least M analytes, **in any combination of presence or absence**, wherein each of said M analytes is encoded as a value of **one component of a fluorescent signal**, **without immobilization, separation, mass spectrometry, or melting curve analysis**. Han also

does **not** teach or suggest a kit, as recited in claim 34, for detecting the presence or absence of at least M analytes by generating **a single cumulative measurement, in a manner that eliminates degeneracy**, wherein each of said M analytes is encoded as a value of one component of a fluorescent signal. The Office Action acknowledges that "Han et al. does not teach detecting cumulative non-degenerate combinations of analytes." *Office Action*, p. 6.

Lin does not cure the deficiencies in the teachings of Han. Lin teaches DNA nanostructure barcode probes to detect a nucleic acid. Lin teaches that "it is preferable for the barcode probe and/or target to remain stationary during image acquisition". Page 20, ll. 27-28. Figure 2 of Lin, which is cited in the Office Action as describing "the cumulative non-degenerate combination of each of 27 different combinations of the three fluorophores (R, G, and B) within an image", illustrates how identification is based, at least in part, on the **positions** of barcodes. The immobilized barcodes taught by Lin produce **multiple separate** fluorescent signals that are analyzed, at least in part, based on their position. Thus, Lin does not teach or suggest an assay, as recited in claim 21, that is capable of detecting the presence or absence of each of said at least M analytes, **in any combination of presence or absence**, wherein each of said M analytes is encoded as a value of **one component of a fluorescent signal**, **without immobilization, separation, mass spectrometry, or melting curve analysis**. Similarly, Lin does not teach or suggest a kit, as recited in claim 34, for detecting the presence or absence of at least M analytes by generating **a single cumulative measurement, in a manner that eliminates degeneracy**, wherein each of said M analytes is encoded as a value of one component of a fluorescent signal.

Therefore, for at least this reason, there is no teaching or suggestion in Lin that would have led one of ordinary skill in the art to modify Han to result in the claimed assays and kits. Thus, the combination of Han and Lin fails to teach or suggest all elements of claim 21 or claim 34. Claims 22-25, 27, 29-30, 32-33 and 35-40 depend on claim 21 or claim 34 and incorporate the limitations therein. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 21-25, 27, 29-30, and 32-40 under 35 U.S.C. § 103.

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Rejection of Claims 26 and 31 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin and Albani

Claims 26 and 31 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han in view of Lin and U.S. Patent Publication No. 2006/0039918 ("Albani").

The Examiner states that the combination of Han and Lin teach or suggest all features of claim 22, but does not teach or suggest a signal generated during PCR, or using a computer connected to a thermal cycler to perform at least one step of said assay, as recited in claims 26 and 31, respectively. The Examiner states that this deficiency is cured by Albani, which allegedly "relates fluorophores, PCR, and thermal cyclers".

As described above, the combination of Han and Lin does not teach or suggest the method of claim 21, upon which claims 26 and 31 depend. Albani does not cure this deficiency. Albani teaches peptides that modulate, block, or inhibit inflammatory responses by altering T cell functional phenotype. Paragraph 184 of Albani, which is cited by the Examiner, teaches **quantifying** gene expression by measuring relative mRNA for a target gene. Albani does not teach or suggest an assay, as recited in claim 21, that is capable of detecting the presence or absence of each of said at least M analytes, **in any combination of presence or absence**, wherein each of said M analytes is encoded as a value of **one component of a fluorescent signal**. Therefore, for at least this reason, the combination of Han, Lin and Albani does not teach or suggest the method of claim 26 or 31. Therefore, the Examiner has failed to set forth a prima facie rejection for obviousness.

Furthermore, any *prima facie* rejection for obviousness is rebutted by the unexpectedly advantageous results in the application, demonstrating unambiguous detection of polynucleotide markers from pathogens, in which the number of analytes is greater than the number of wavelength components. *See*, *e.g.*, Example 3, and, for example, ¶¶ [0188]-[0190] and Figure 7.

Therefore, for at least the above reasons, Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 26 and 31 under 35 U.S.C. § 103.

Rejection of Claim 28 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin and Hardin

Claim 28 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han in view of Lin and U.S. Patent Publication No. 2010/0317005 ("Hardin").

The Examiner states that the combination of Han and Lin teach or suggest all features of claim 22, but do not teach or suggest using the fluorescent signal from to construct a chromatogram, as recited in dependent claim 28. The Examiner states that this deficiency is cured by Hardin, which allegedly teaches use of a chromatogram to monitor the mobility of fluorescently labeled molecules.

As described above, the combination of Han and Lin does not teach or suggest the method of claim 21, upon which claim 28 depend. Hardin does not cure this deficiency. Hardin teaches gamma phosphate labeled nucleotides, and their use in labeling target biomolecules. Hardin does not teach or suggest an assay, as recited in claim 21, that is capable of detecting the presence or absence of each of said at least M analytes, **in any combination of presence or absence**, wherein each of said M analytes is encoded as a value of **one component of a fluorescent signal**. Therefore, for at least this reason, the combination of Han, Lin and Hardin does not teach or suggest the method of claim 28.

Obviousness-type Double Patenting

Rejection of claims 21, 23, 26, 27 and 29-34

Claims 21, 23, 26, 27 and 29-34 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 7-8, 11-12, 15-17, and 20, respectively, of U.S. Patent No. 8,838,394. Obviousness-type double patenting cannot be finally determined until there is allowable subject matter. Accordingly, Applicant requests deferral of this issue until there is an indication of allowable claims.

Rejection of claims 21, 26, and 30-31

Claims 21, 26, and 30-31 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 4-5, and 9, respectively, of U.S. Patent No. 10,068,051. Obviousness-type double patenting cannot be finally determined until there is allowable subject matter. Accordingly, Applicant requests deferral of this issue until there is an indication of allowable claims.

CONCLUSION

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number

provided. Prompt and favorable consideration of this Response is respectfully requested. The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit account No. 50-4561 (Attorney Docket No. 88DG-330783-US4)

Respectfully submitted,

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Dated: March 28, 2023

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Electronic Acknowledgement Receipt

EFS ID:	47756963
Application Number:	16864744
International Application Number:	
Confirmation Number:	9929
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil P. Kartalov
Customer Number:	30764
Filer:	James McKay Turner
Filer Authorized By:	
Attorney Docket Number:	88DG-330783-US4
Receipt Date:	28-MAR-2023
Filing Date:	01-MAY-2020
Time Stamp:	23:01:08
Application Type:	Utility under 35 USC 111(a)

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Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		330783-US4--Response_to_12-28-22_Non-Final_OA.pdf	206902 f17345a505b37301e3267f0805cd281970b25ce2	yes	10

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Request for Reconsideration-After Non-Final Rejection	1	1
	Claims	2	4
	Applicant Arguments/Remarks Made in an Amendment	5	10
Warnings:			
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National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/864,744	Kartalov et al.
	Examiner	Art Unit
	RUSSELL S NEGIN	1672

CPC - Searched*		
Symbol	Date	Examiner
C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or G06F17/10 or G06F17/11	05/06/2023	RSN

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

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Search Notes		
Search Notes	Date	Examiner
see attached search history printouts	05/06/2023	RSN
PE2E Search prior art search	05/06/2023	RSN
PE2E Search inventor search on all inventors	05/06/2023	RSN
PDP inventor search on all inventors	05/06/2023	RSN
PE2E Search CPC code search with modified terms	05/06/2023	RSN

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1672	

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16864744
Google Scholar Search
/RSN/ 5/6/2023

analytes presence absence
analytes presence absence wavelength
analytes presence absence wavelength components
analytes presence absence wavelength fluorophores
analytes presence absence wavelength fluorescent
analytes presence absence wavelength hybridization
analytes presence absence wavelength hybridization intensities

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Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16864744
Filing Date	2020-05-01
First Named Inventor	Emil P. Kartalov
Art Unit	1672
Examiner Name	NEGIN, RUSSELL SCOTT
Attorney Docket Number	30KJ-330783-US4

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/RSN/	1	20040248082		2004-12-09	SCALLON	
/RSN/	2	20110183884		2011-07-28	MILLER ET AL.	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16864744
	Filing Date	2020-05-01
	First Named Inventor	Emil P. Kartalov
	Art Unit	1672
	Examiner Name	NEGIN, RUSSELL SCOTT
	Attorney Docket Number	30KJ-330783-US4

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
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Examiner Signature	/RUSSELL S NEGIN/	Date Considered	05/06/2023

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[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	3	("10770170," "10068051," "8838394").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/16 09:21 AM
L2	140	kartalov.in. NEAR emil.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2022/12/16 11:12 AM
L3	286	rajagopal.in. NEAR aditya.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2022/12/16 11:12 AM
L4	1077	scherer.in. NEAR axel.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2022/12/16 11:12 AM
L5	166506	(C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU,	OR	ON	ON	2022/12/16 11:13 AM

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		G06F17/10 or G06F17/11).cpc.	CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L6	1264	L2 L3 L4	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:13 AM
L7	119	L5 AND L6	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L8	1900960	PCR	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN,	OR	ON	ON	2022/12/16 11:14 AM

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			WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L9	113706	thermal ADJ cycler	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L10	339872	fluorophores	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L11	25989671	computers	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L12	64751	L8 WITH L9	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB,	OR	ON	ON	2022/12/16 11:15 AM

			HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L13	448	L10 WITH L12	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L14	1	L13 WITH L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L15	519	L13 andL11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2022/12/16 11:15 AM

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			IBM_TDB)				
L16	26534951	L13 andL 11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L17	278	L13 AND L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L18	439599	chromatogram	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:21 AM
L19	125	L10 WITH L18	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV,	OR	ON	ON	2022/12/16 11:21 AM

			MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
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L21	3	("10770170," "10068051," "8838394").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/05/06 09:11 AM
L22	145	kartalov.in. NEAR emil.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2023/05/06 09:11 AM
L23	293	rajagopal.in. NEAR aditya.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2023/05/06 09:11 AM
L24	1089	scherer.in. NEAR axel.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA,	OR	ON	ON	2023/05/06 09:11 AM

			VN); FPRS; EPO; JPO; DERWENT)				
L25	176265	(C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or G06F17/10 or G06F17/11).cpc.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L26	1284	L2 L3 L4	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L27	122	L5 AND L6	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L28	1966331	PCR	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS,	OR	ON	ON	2023/05/06 09:11 AM

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			IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L29	116375	thermal ADJ cycler	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L30	352655	fluorophores	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L31	26957183	computers	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM

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L32	66099	L8 WITH L9	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L33	471	L10 WITH L12	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L34	1	L13 WITH L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L35	543	L13 andL11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL,	OR	ON	ON	2023/05/06 09:11 AM

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			NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L36	27502615	L13 andL 11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L37	295	L13 AND L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L38	454840	chromatogram	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L39	133	L10 WITH L18	(US-PGPUB; USPAT; USOCR; FIT (AP, AT,	OR	ON	ON	2023/05/06 09:11 AM

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			AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L40	2	("20040248082" OR "20110183884").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/05/06 09:11 AM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	05/01/2020	Emil P. Kartalov	88DG-330783-US4	9929

30764 7590 05/10/2023

SHEPPARD, MULLIN, RICHTER & HAMPTON LLP
650 Town Center Drive, 10th Floor
Costa Mesa, CA 92626

EXAMINER	
NEGIN, RUSSELL SCOTT	

ART UNIT	PAPER NUMBER
1672	

NOTIFICATION DATE	DELIVERY MODE
05/10/2023	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DOCKETING@SHEPPARDMULLIN.COM
SheppardMullin_Pair@firsttofile.com

Office Action Summary	Application No. 16/864,744	Applicant(s) Kartalov et al.	
	Examiner RUSSELL S NEGIN	Art Unit 1672	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 28 March 2023.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 21-40 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 21-40 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Comments

The present application is being examined under the pre-AIA first to invent provisions. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

Claims 21-40 are pending and examined in the instant Office action.

Information Disclosure Statement

The IDS filed on 12/27/2022 has been considered.

Withdrawn Objections/Rejections

The objections to the claims are withdrawn in view of amendments filed to the instant set of claims on 28 March 2023.

The rejections of the claims under 35 U.S.C. 112 are withdrawn in view of amendments filed to the instant set of claims on 28 March 2023.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

The following rejection is reiterated:

35 U.S.C. 103 Rejection #1:

Claims 21-25, 27, 29-30, and 32-40 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Han et al. [Nature Biotechnology, volume 19, 2001, pages 631-835; on IDS] in view of Lin et al. [WO 2012/058628 A2; on IDS].

Claim 21 is drawn to an assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes. The assay requires that each of the M analytes is encoded as a value of one component of a fluorescent signal. The assay requires that the fluorescent signal comprises F wavelength components. The assay is capable of detecting the presence or absence of each of the at least M analytes, in any combination of presence or absence, in a single sample volume without

immobilization, separation, mass spectrometry, or melting curve analysis, based on detection of the F wavelength components. M is greater than F.

Claim 32 is further limiting requiring the coding scheme to be innately non-degenerate or non-degenerate by construction.

Claims 34-36 are drawn to similar subject matter as claim 32, except as a kit.

The document of Han et al. studies quantum-dot tagged microbeads for multiplexed optical coding of biomolecules [title]. Figure 5 of Han et al. illustrates four oligonucleotides analytes (M=4) attached to microbeads with different combinations of 3 types of quantum dots- red, green, and blue (F=3). The type of optical code as a result of spectroscopy indicates the type of analyte being examined. Han et al. does not use immobilization, separation, mass spectrometry, or melting curve analysis.

Han et al. does not teach detecting cumulative non-degenerate combinations of analytes.

The document of Lin et al. studies nucleic acid nanostructure barcode probes [title]. Figure 2 of Lin et al. illustrates the cumulative non-degenerate combination of each of 27 different combinations of the three fluorophores (R, G, and B) within an image. Pages 7 and 8 of Lin et al. describe Figure 2 of Lin et al.

With regard to claims 22-23, Han et al. and Lin et al. teach red, green, and blue components to the signal. Figure 6 of Han et al. illustrates a range of intensities associated with each component.

With regard to claims 24-25 and 37-39, Figure 5 of Han et al. illustrates hybridization probes for different analytes wherein each analyte comprises a different combination of fluorophores. Figure 2 of Lin et al. teaches that the number of hybridization probes is greater than the number of analytes.

Wirth regard to claim 27, Figure 5 of Han et al. illustrates three components of the signals used to detect each analyte.

With regard to claim 29, Figure 5 of Han et al. teaches that each analyte comprises a polynucleotide.

With regard to claim 30, page 31 of Lin et al. teaches the presence of a computer to analyze spectral data.

With regard to claims 32-33, Figure 2 of Lin et al. teaches a histogram of non-degenerate counts of presence the 27 different combinations of fluorophores in an image.

With regard to claim 40, the different sizes of the bars in the histogram in Figure 2 of Lin et al. teaches that the analyte specific reagents are each present at a different amount.

It would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the quantum dot hybridization assay of Han et al. by use of the cumulative non-degenerate fluorophore assay of Lin et al. wherein the motivation would have been that the cumulative and non-degenerate nature of the assay of Lin et al. adds mathematical and empirical tools to the assay of Han et al. [Figure 2 of Han et al.]. There would have been a reasonable expectation of success in combining Han et al. and Lin et al. because both studies analogously pertain to assays involving colored fluorophores or quantum dots.

Response to arguments:

Applicant's arguments filed 28 March 2023 have been fully considered but they are not persuasive.

Applicant argues that the beads of Han et al. are immobilized on a glass slide. While a subset of beads are localized on a glass slide for the purpose of experimental measurement, the common state of the glass particles is immobilized in solution.

In response to applicant's argument that the references fail to show certain features of the invention, it is noted that the features upon which applicant relies (i.e. a single cumulative measurement) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Applicant argues that the prior art does not teach eliminating degeneracy. This argument is not persuasive because Figure 2 of Lin et al. illustrates the cumulative non-

degenerate combination of each of 27 different combinations of the three fluorophores (R, G, and B) within an image.

Applicant argues that Lin et al. does not teach a technique to measure presence and absence combination without immobilization, mass spectrometry, or melting curve analysis. This is an obviousness prior art rejection, and Han et al. is relied upon to teach this limitation.

The following rejection is reiterated:

35 U.S.C. 103 Rejection #2:

Claims 26 and 31 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Han et al. in view of Lin et al. as applied to claims 21-25, 27, 29-30, and 32-40 above, in further view of Albani et al. [US PGPUB 2006/0039918 A1].

Claim 26 is further limiting wherein the signal is generated during PCR.

Claim 31 is further limiting wherein the assay is performed using a computer and thermal cycler.

Han et al. and Lin et al. make obvious fluorophore and quantum dot assays to detect presence and absence of analytes, as discussed above.

Han et al. and Lin et al. do not teach PCR, computers, and thermal cyclers.

The document of Albani et al. studies stress proteins and peptides [title]. Paragraph 184 of Albani et al. relates fluorophores, PCR, and thermal cyclers.

It would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the quantum dot hybridization assay of Han et al. and the cumulative non-degenerate fluorophore assay of Lin et al. by use of the PCR,

computers, and thermal cyclers of Albani et al. wherein the motivation would have been that Albani et al. adds empirical tools that facilitate the analysis of fluorophores [paragraph 184 of Albani et al.].

Response to arguments:

Applicant's arguments filed 28 March 2023 have been fully considered but they are not persuasive.

Applicant argues that Albani et al. does not overcome the alleged deficiencies of the initial obviousness prior art rejection. Since the initial obviousness prior art rejection is not deficient, this argument is not persuasive.

The following rejection is reiterated:

35 U.S.C. 103 Rejection #3:

Claim 28 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Han et al. in view of Lin et al. as applied to claims 21-25, 27, 29-30, and 32-40 above, in further view of Hardin et al. [US PGPUB 2010/0317005 A1].

Claim 28 is further limiting wherein the fluorescence signals are used to generate chromatograms.

Han et al. and Lin et al. make obvious fluorophore and quantum dot assays to detect presence and absence of analytes, as discussed above.

Han et al. and Lin et al. do not teach using fluorescence signals to generate chromatograms.

The document of Hardin et al. studies modified nucleotides [title]. Paragraph 143 of Hardin et al. relates fluorescence signals to chromatograms.

It would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the quantum dot hybridization assay of Han et al. and the cumulative non-degenerate fluorophore assay of Lin et al. by use of the chromatograms of Hardin et al. wherein the motivation would have been that Hardin et al. adds empirical tools that facilitate the analysis of fluorophores [paragraph 143 of Hardin et al.].

Response to arguments:

Applicant's arguments filed 28 March 2023 have been fully considered but they are not persuasive.

Applicant argues that Hardin et al. does not overcome the alleged deficiencies of the initial obviousness prior art rejection. Since the initial obviousness prior art rejection is not deficient, this argument is not persuasive

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have

been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

The following rejection is reiterated:

Double Patenting Rejection #1:

Claims 21, 23, 26, 27, and 29-34 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 7-8, 11-12, 15-17, and 20, respectively, of U.S. Patent No. 8,838,394. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims are drawn to assays for detection cumulative presence/absence patterns of analytes associated with fluorophores without using immobilization or mass spectrometry. While the claims of '394 recite more limitations than the instantly rejected claims, the claims of '394 anticipate the instantly rejected claims.

Response to arguments:

Applicant requests the double patenting rejection be held in abeyance.

The following rejection is reiterated:

Double Patenting Rejection #2:

Claims 21, 26, and 30-31 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 4-5, and 9, respectively, of U.S. Patent No. 10,068,051 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims are drawn to assays for detection cumulative presence/absence patterns of analytes associated with fluorophores without using immobilization or mass spectrometry. While the claims of

‘394 recite more limitations than the instantly rejected claims, the claims of ‘051 anticipate the instantly rejected claims.

Response to arguments:

Applicant requests the double patenting rejection be held in abeyance.

The following rejection is reiterated:

Double Patenting Rejection #3:

Claims 21, 26, and 30 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 7, and 13, respectively, of U.S. Patent No. 10,770,170 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims are drawn to assays for detection cumulative presence/absence patterns of analytes associated with fluorophores without using immobilization or mass spectrometry. While the claims of ‘394 recite more limitations than the instantly rejected claims, the claims of ‘170 anticipate the instantly rejected claims.

Response to arguments:

Applicant requests the double patenting rejection be held in abeyance.

Related Prior Art

The prior art of Geiss et al. [WO 2008/124847; on IDS] teach targeting sequences using coding via labeling. However, Geiss et al. does not teach the technique in the recited claims.

E-mail Communications Authorization

Per updated USPTO Internet usage policies, Applicant and/or applicant's representative is encouraged to authorize the USPTO examiner to discuss any subject matter concerning the above application via Internet e-mail communications. See MPEP 502.03. To approve such communications, Applicant must provide written authorization for e-mail communication by submitting the following statement via EFS-Web (using PTO/SB/439) or Central Fax (571-273-8300):

> Recognizing that Internet communications are not secure, I hereby authorize the USPTO to communicate with the undersigned and practitioners in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application by video conferencing, instant messaging, or electronic mail. I understand that a copy of these communications will be made of record in the application file.

Written authorizations submitted to the Examiner via e-mail are **NOT** proper. Written authorizations must be submitted **via EFS-Web** (using PTO/SB/439) **or Central Fax** (571-273-8300). A paper copy of e-mail correspondence will be placed in the patent application when appropriate. E-mails from the USPTO are for the sole use of the intended recipient, and may contain information subject to the confidentiality requirement set forth in 35 USC § 122. See also MPEP 502.03

Conclusion

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Russell Negin, whose telephone number is (571) 272-1083. This Examiner can normally be reached from Monday through Thursday from 8 am to 3 pm and variable hours on Fridays.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's Supervisor, Larry Riggs, Supervisory Patent Examiner, can be reached at (571) 270-3062.

Information regarding the status of the application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

/RUSSELL S NEGIN/
Primary Examiner, Art Unit 1672
6 May 2023

PTO/SB/06 (09-11)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/864,744	Filing Date 05/01/2020	☐ To be Mailed

ENTITY: ☐ LARGE ☑ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $50 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $230 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	07/10/2023	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 19	Minus	** 20	= 0		x $40 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $192 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/TINA J BARDEN/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PATENT
Docket No. 88DG-330783-US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INVENTORS :	Emil KARTALOV *et al.*	CONFIRMATION NO. :	9929
SERIAL NUMBER :	16/864,744	EXAMINER :	Russell Scott NEGIN
FILING DATE :	May 1, 2020	ART UNIT :	1672
FOR :	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS		

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO FINAL OFFICE ACTION

Dear Sir:

In response to the Non-Final Office Action mailed **May 10, 2023**, please amend the above-identified application as follows:

Listing of Claims begin on page **2** of this paper.

Remarks begin on page **6** of this paper.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned for under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to Deposit Account No. 504561 (**Ref. No. 30KJ-330783-US4**).

LISTING OF CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

1.–20. (Canceled).

21. (Currently Amended) An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,
wherein each of said M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,
wherein said fluorescent signal comprises F wavelength components, and
wherein said assay is capable of detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume ~~without immobilization, separation, mass spectrometry, or melting curve analysis~~, based on detection of said F wavelength components, when M_is greater than F,
wherein the assay does not require the use of mass spectrometry or immobilization of said M analytes, and
wherein the analytes are amplified in the single sample volume.

22. (Previously Presented) The assay of claim 21, wherein each of said at least M analytes is encoded as at least one first value in a first component of said fluorescent signal and at least one second value in a second component of said fluorescent signal.

23. (Previously Presented) The assay of claim 22, wherein said first value is an intensity or range of intensities.

24. (Previously Presented) The assay of claim 21, wherein said detecting is performed with reagents comprising hybridization probes.

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25. (Previously Presented) The assay of claim 24, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.

26. (Previously Presented) The assay of claim 21, wherein said signal is generated during a polymerase chain reaction.

27. (Previously Presented) The assay of claim 21, wherein at least one of said analytes is encoded by at least one additional value, wherein said at least one additional value is selected from the group consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal, and combinations thereof.

28. (Previously Presented) The assay of claim 24, wherein said fluorescence signal from said hybridization probes are used to construct a chromatogram.

29. (Previously Presented) The assay of claim 21, wherein at least one of said analytes comprises a polynucleotide.

30. (Previously Presented) The assay of claim 21, further comprising transmitting information concerning the presence or absence of at least one of said analytes through a computer network.

31. (Previously Presented) The assay of claim 21, wherein at least one step of said assay is performed using a computer and wherein said computer is connected to a thermal cycler.

32. (Previously Presented) The assay of claim 21, wherein said coding scheme is innately nondegenerate or non-degenerate by construction.

33. (Previously Presented) The assay of claim 22, wherein said coding scheme is made nondegenerate by enumerating every legitimate result that can be obtained from said coding

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scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.

34. (Currently Amended) A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising analyte-specific reagents, wherein:
a. each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy; and
b. each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the value;
wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single sample volume, in any combination of presence or absence, and
wherein the kit does not require the use of mass spectrometry or immobilization of said M analytes, and
wherein the analytes are simplified in the single sample volume.

35. (Canceled).

36. (Previously Presented) The kit of claim 34, wherein said fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, when M is greater than F.

37. (Previously Presented) The kit of claim 34, wherein said analyte-specific reagents comprise hybridization probes.

38. (Previously Presented) The kit of claim 37, wherein said hybridization probes comprise a fluorophore, and wherein said plurality of hybridization probes are specific for different analytes.

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39. (Previously Presented) The kit of claim 38, wherein said number of hybridization probes in the kit is greater than M.

40. (Previously Presented) The kit of claim 34, wherein the analyte-specific reagents are each present at a different amount in the kit.

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REMARKS

Claims 21-42 are pending. Claims 21 and 34 are currently amended. Support can be found throughout the Specification and at least at paragraphs **[0014]**, **[0043]-[0044]**, **[0058]**, **[0068]**, **[0110]**, **[0117]**, **[0142]**, **[0147]-[0148]**, and **[0158]-[0168]**. No new matter is added. Claim 35 is cancelled. In view of the amendments and following remarks, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, Applicant respectfully requests that the Examiner reconsider all outstanding rejections and allow the claims.

Claims Rejections Under 35 U.S.C. §103

Rejection of Claims 21-25, 27, 29-30 and 32-40 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin

Claims 21-25, 27, 29-30 and 32-40 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han et al. (Nature Biotechnology, volume 19, 2001, pages 631-635) ("Han") in view of WO 2012/058628 [sic, WO 2012/058638] ("Lin"). Applicant respectfully disagrees for at least the reasons previously submitted.

Additionally, contrary to the Office Action, as alleged at page 4, the Han assay describes not one, but at least three steps of immobilization.

First, the Han assay, at the centrifugation step, describes that "[b]efore fluorescence measurement, the beads were cleaned by two rounds of centrifugation" (see Han at page 635). Thus, the Han assay clearly requires separating the mobile solution phase from the immobile fluorescence beads that were collecting as a pellet. This is in stark contrast to the claimed assay and kit, as amended, clearing stating that wherein the assay or kit "does **not** require … **immobilization of said M analytes**" (emphasis added). In fact, the Specification of this Application does not mention the terms "centrifuge," "centrifugation," "centrifuging," "spin," "spinning," and "spung" even once. Instead, the Specification of this Application clearly distinguishes the novel and non-obvious assay and kit disclosed here to not require "immobilization, separation, mass spectrometry, or melting curve analysis" (see for example, paragraphs [0005], [0033], [0039]-[0041], [0121]). Moreover, the Specification at paragraph **[0155]** specifically states that "[i]mportantly, the methods and compositions provided herein may

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be used to detect multiple analytes by obtaining a cumulative measurement on a single solution. **No separation is necessary**. **The invention does not require the use of beads or a solid phase**" (emphasis added). Thus, a person of ordinary skill in the art would not have considered the separation and immobilization bead technology of Han, whether alone or in combination with any cited references, to arrive at the claimed assay and kit. For at least this reason, reconsideration and withdrawal of the rejection are respectfully requested.

Second, the Han assay, at the imaging step, describes that "[t]he beads were spread and immobilized on a polylysine-coated glass slide." It is well known to a person of ordinary skill in the art that polylysine, here, is specifically **used to attach and immobilize the fluorescence beads** to the slide (see Fisher Scientific Product No. 50-279-88 describing the poly-l-lysine coated slides to "improve the adhesion of tissues to the slide.") Applicant respectfully points out that the specific choice of using polylysine-coated slides was **not** representative of a necessary and ordinary step for experimental observation. A person of ordinary skill in the art would have recognized that glass slides **not** coated with polylysine are more commonly available, less expensive, and are commonly used for experimental observation, including fluorescence imagining, that does not require immobilization or strong adhesion to the slides. Indeed, a person of ordinary skill in the art would have understood that the Han assay (1) specifically chose a polylysine-coated glass slide, as opposed a non-coated slide to immobilize the fluorescence beads; (2) specifically covalently attached streptavidin molecule to the surface of the beads, to enhance the immobilization of the beads (see Han at for example page 635); and (3) specifically packed the nonspecific binding sites on the surface of the beads with the protein BSA to enhance immobilization of the beads to the polylysine-coated glass slide. This, again, is in stark contrast to the claimed assay and kit, clearing stating that it "does **not** require … **immobilization of said M analytes**" (emphasis added). In fact, the Specification of this Application does not mention the terms "slide," "coat," "coated," and "polylysine," even once. Thus, a person of ordinary skill in the art would not have arrived at the claimed assay or kit in view of Han, whether alone or in combination with any cited references. For at least this reason, reconsideration and withdrawal of the rejection are respectfully requested.

Third, a person of ordinary skill in the art would have viewed attaching the target oligonucleotides of Han to QD-tagged beads through cross-linked probes would be a step of

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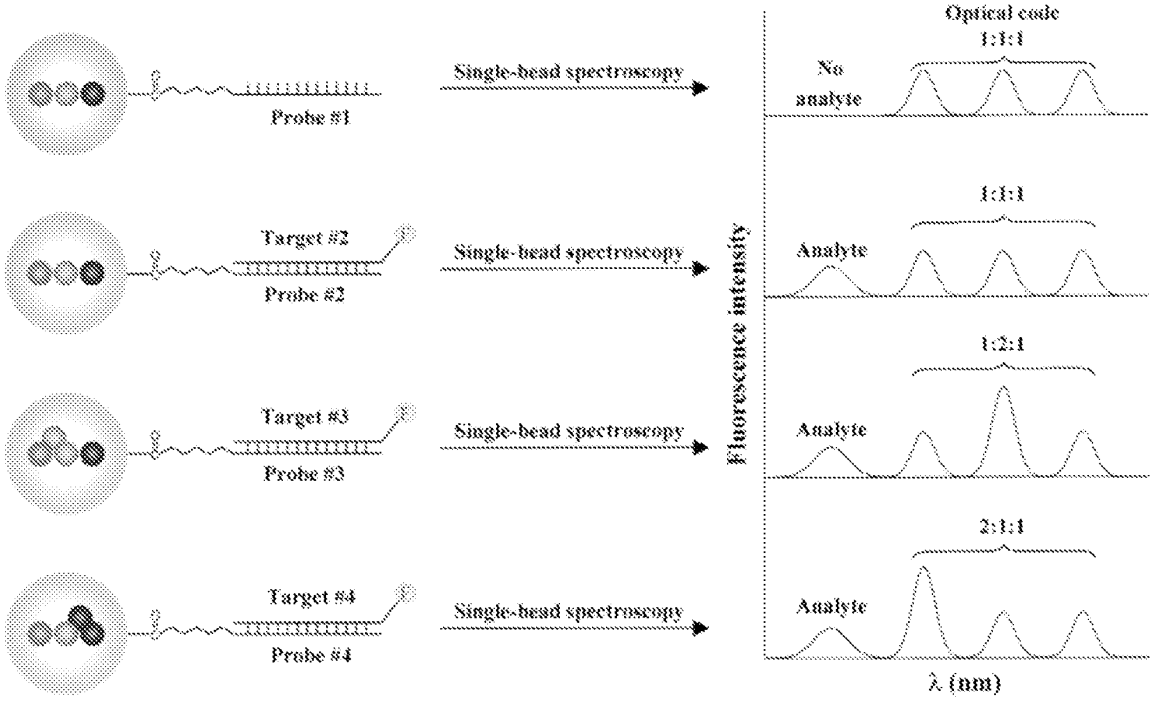
immobilization as the term is used in the Specification. Indeed, the term "immobilization" as used in the Specification requires no additional definition and the implications of using the term "immobilization," requires no additional explanation. A person of ordinary skill in the art would have understood "immobiliz[ing] a target gene onto insoluble [bead] particles" have been described in the context of PCR methods. See, for example, Andreadis et. al, Nucleic Acids Research, volume 28, e5 i-viii, 1999 entitled "Use of immobilized PCR primers to generate **covalently immobilized DNAs** for *in vitro* transcription/translation reactions" ("Andreadis"), where the authors describe covalently attaching, through chemical crosslinkers, two primers to a bead particle during or after DNA synthesis to achieve immobilization. The Abstract of Andreadis, for example, states as follows, with emphasis added:

> We have developed a novel biochemical method to simultaneously amplify and **immobilize** a target gene onto **insoluble particles** using PCR. This method employs the covalent attachment of one of two PCR primers to a particle surface either directly during DNA synthesis of the primer or post-DNA synthesis, through the use of chemical crosslinkers. **Immobilization** of the target gene can be achieved directly during PCR amplification, with one **bead-bound** primer and one soluble primer. Alternatively, this can be achieved post-PCR, through covalent attachment of a chemically modified primer incorporated into the amplicon to **an activated particle**. All of the **immobilized DNA templates** containing appropriate regulatory regions were fully competent for transcription and translation reactions and several could be re-used in serial reactions. The most successful strategy utilized **amino-silanized controlled pore glass beads**, which were coupled to phosphorylated primers using carbodiimide chemistry. These **bead-bound** primers were used during PCR to generate attached DNA templates that could be collected and re-used for at least seven sequential transcription reactions without significant loss in efficiency. This method has also been successfully applied to the amplification, transcription and translation of multiple DNA templates using a single, **immobilized** primer. The combined PCR-based amplification/**immobilization** method was shown to be more durable than post-PCR chemical **immobilization** and affords the convenience of performing sequential PCR amplification, transcription and translation reactions in a single tube.

Thus, a person of ordinary skill in the art would have understood that binding of the target to the probe oligos and conjugating the probe oligos "to the beads by cross-linking" in Han would have

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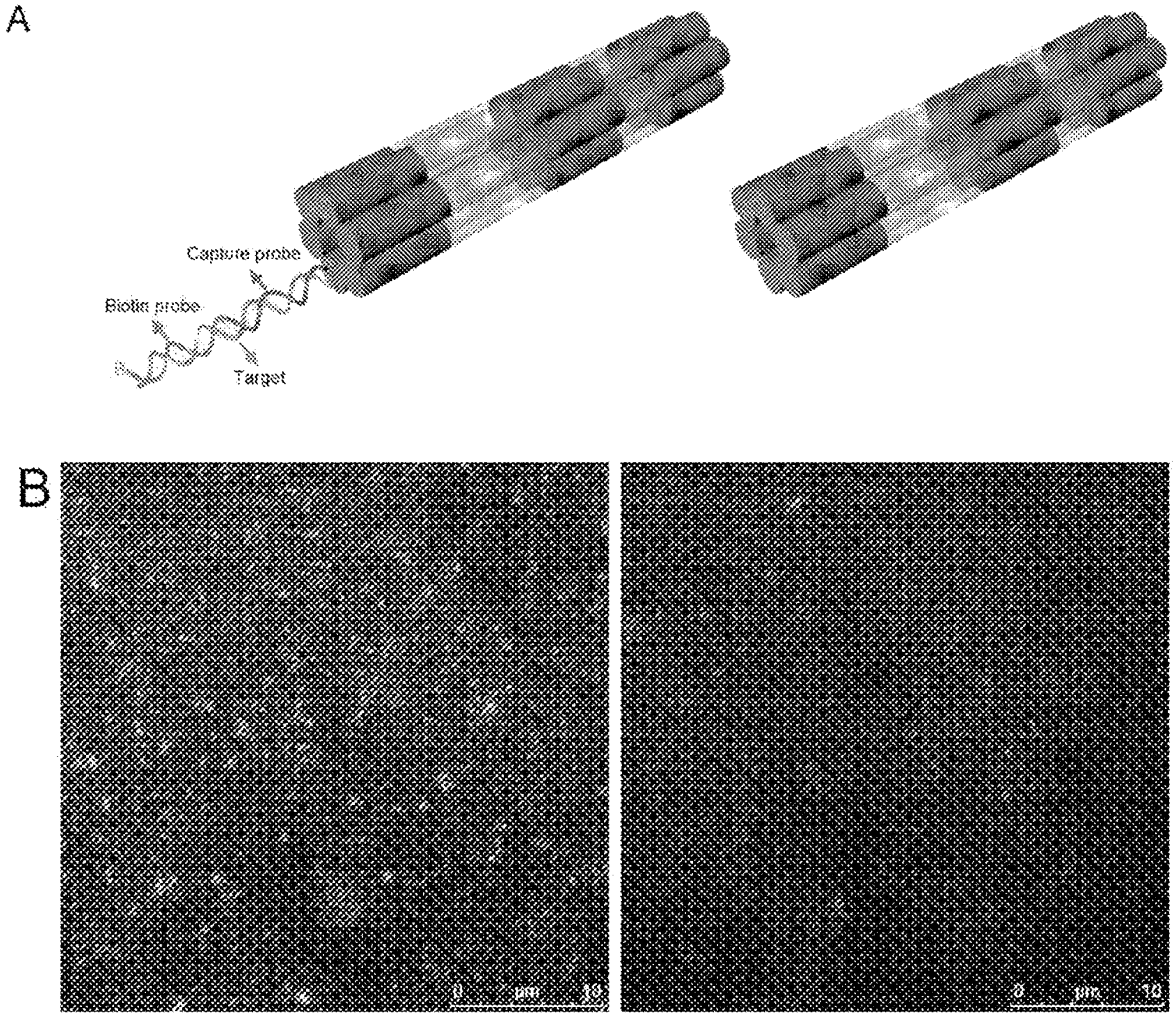
immobilized the target analyte, as the term "immobilization" is used in the Specification of this Application and understood by persons of ordinary skill in the art. Figure 5 of Han clearly show immobilization of the target analyte on the nanobeads.



(Reproduced from Han at Figure 5).

For at least this reason, a person of ordinary skill in the art would not have arrived at the claimed assay or kit in view of Han, whether alone or in combination with any cited references. Reconsideration and withdrawal of the rejection are respectfully requested.

Lin does not cure the deficiencies in the teachings of Han, whether alone or in combination with any other cited reference. Like Han, Lin discloses immobilization of target analyte onto nanotubes (see Lin at FIG. 4A, reproduced below). Also like Han, Lin describes "immobiliz[ation]" of "nano-barcodes" on "streptavidin coated glass cover-slips (see Lin at FIG. 4B, reproduced below).



Thus, neither Han nor Lin, whether alone or in combination, would have led one of ordinary skill in the art to arrive at the claimed assay or kit, which does not require immobilization of the analytes, without undue experimentation. The combination of Han and Lin fails to teach or suggest all elements of claims 21 and 34, as amended. Claims 22-25, 27, 29-30, 32-34, and 36-40, depend directly or indirectly on claim 21 or claim 34, and incorporate the limitations therein. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 22-25, 27, 29-30, 32-34, and 36-40 under 35 U.S.C. § 103.

Moreover, claims 21 and 34, as amended recite "and wherein the analytes are amplified in the single sample volume." Han and Lin, whether alone or in combination, fail to teach or

even suggest an assay or a kit wherein the analytes are amplified in the single sample volume. Applicant respectfully points out neither Han nor Lin mention "PCR" or "amplification" even once. Thus, for at least these reasons, the claims of this Application are novel and non-obvious over Han and Lin, whether alone or in combination with any other cited reference. Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 22-25, 27, 29-30, 32-34, and 36-40 under 35 U.S.C. § 103.

Rejection of Claims 26 and 31 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin, and in further view of Albani

Claims 26 and 31 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han in view of Lin and in further view of US Published Application No. 2006/0039918 ("Albani"). Applicant respectfully disagrees for at least the reasons previously submitted.

Additionally, as explained above, the Han assay describes at least the three steps of immobilization. Thus, a person of ordinary skill in the art would not have arrived at the claimed assay in view of Han, whether alone or in combination with any cited references. Lin does not cure the deficiencies in the teachings of Han, whether alone or in combination with any other cited reference. Albani, which discloses the use of HLA pan DR-binding peptides to modulate, block, or inhibit immune responses to effect treatment of immune-mediated diseases and conditions, such as inflammatory and autoimmune diseases, cancer, and microbial infections (see Albani at abstract). Thus, for at least these reasons reconsideration and withdrawal of the rejection are respectfully requested.

Moreover, claim 21, as amended recite "and wherein the analytes are amplified in the single sample volume." As discussed above, Han and Lin, whether alone or in combination, fail to teach or even suggest an assay or a kit wherein the analytes are amplified in the single sample volume. Albani does not cure the deficiencies in the teachings of Han and Lin, whether alone or in combination with any other cited reference. In fact, Albani only gloss over the use of existing TaqMan qPCR technology and the store-purchased Perkin-Elmer ABI PRISM 7700 thermal cycler to conduct standard analysis and quantifications of mRNA levels. Applicant respectfully request Examiner to explain how the analyte, having being bound to the nanobeads of Han, the nanotubes of Lin, or the peptides of Albani could be "amplified in the single sample volume", without undue experimentation. Thus, for at least these reasons, the claims of this Application

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are novel and non-obvious over Han, Lin, and Albani, whether alone or in combination with any other cited reference. Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 26 and 31 under 35 U.S.C. § 103.

Rejection of Claim 28 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin and Hardin

Claim 28 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han in view of Lin and U.S. Patent Publication No. 2010/0317005 ("Hardin"). Applicant respectfully disagrees for at least the reasons previously submitted.

Additionally, as explained above, Han assay describes at least the three steps of immobilization. Thus, a person of ordinary skill in the art would not have arrived at the claimed assay in view of Han, whether alone or in combination with any cited references. Lin does not cure the deficiencies in the teachings of Han, whether alone or in combination with any other cited reference. Hardin, which describes its invention as "[t] he present invention provides a method for screening kinases including the step of providing one **immobilized** substrate or a plurality of immobilized substrates on a support" (see Hardin at least at paragraph **[0070]**) also fails to cure the deficiencies of Han and Lin. Thus, for at least these reasons reconsideration and withdrawal of the rejection are respectfully requested.

Moreover, claim 21, as amended recite "and wherein the analytes are amplified in the single sample volume." As discussed above, Han and Lin, whether alone or in combination, fail to teach or even suggest an assay or a kit wherein the analytes are amplified in the single sample volume. Albani does not cure the deficiencies in the teachings of Han and Lin, whether alone or in combination with any other cited reference. Applicant respectfully request Examiner to explain how the analyte, having being bound to the nanobeads of Han or the nanotubes of Lin could be combined with the labeled nucleotide triphosphates of Albani to arrive at the claimed embodiment of "wherein the analytes are amplified in the single sample volume", without undue experimentation. Thus, for at least these reasons, the claims of this Application are novel and non-obvious over Han, Lin, and Hardin, whether alone or in combination with any other cited reference. Applicant respectfully requests reconsideration and withdrawal of the rejection of claim 28 under 35 U.S.C. § 103.

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Obviousness-Type Double Patenting

Rejection of Claims 21, 23, 26, 27, and 29-34

Claims 21, 23, 26, 27, and 29-34 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 7-8, 11-12, 15-17 and 20, respectively, of U.S. Patent No. 8,838,394. Applicant respectfully disagree for at least the reasons previously submitted. Moreover, for at least the reason that obviousness-type double patenting cannot be finally determined until there is allowable subject matter, Applicant requests deferral of this issue until there is an indication of allowable claims.

Rejection of Claim 21, 26, 30-31

Claim 21, 26, 30-31 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 4-5, and 9 of U.S. Patent No. 10,068,051. Applicant respectfully disagree for at least the reasons previously submitted. Moreover, for at least the reason that obviousness-type double patenting cannot be finally determined until there is allowable subject matter, Applicant requests deferral of this issue until there is an indication of allowable claims.

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CONCLUSION

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided. Prompt and favorable consideration of this Response is respectfully requested. The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit account No. 50-4561 (Attorney Docket No. 88DG-330783-US4)

Respectfully submitted,

SHEPPARD MULLIN RICHTER & HAMPTON LLP

By____/ Jesse A. Salen /_____
Jesse A. Salen
Registration No.: 72,209

Dated: July 10, 2023

12275 El Camino Real, Suite 100
San Diego, CA 92130-4092
Telephone: (858) 720-8900
Facsimile: (858) 509-3691

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4479

Electronic Acknowledgement Receipt

EFS ID:	48273115
Application Number:	16864744
International Application Number:	
Confirmation Number:	9929
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil P. Kartalov
Customer Number:	30764
Filer:	Jesse A. Salen/Annie Wong
Filer Authorized By:	Jesse A. Salen
Attorney Docket Number:	88DG-330783-US4
Receipt Date:	10-JUL-2023
Filing Date:	01-MAY-2020
Time Stamp:	16:58:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		FOA_Response_16864744.pdf	1760543 79d9253f75097e1191093e5d8e4189780b3a624d	yes	14

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response After Final Action	1	1
	Claims	2	5
	Applicant Arguments/Remarks Made in an Amendment	6	14
Warnings:			
Information:			
	Total Files Size (in bytes):	1760543	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY.DOCKET NO./TITLE	REQUEST ID
16/864,744	05/01/2020	Emil P. Kartalov	88DG-330783-US4	192364

Acknowledgement of Change to Small Entity Status

The entity status change request below filed through Private PAIR on 07/13/2023 has been accepted.

CERTIFICATIONS:

Change of Entity Status:

X Applicant asserting small entity status. See 37 CFR 1.27.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

This portion must be completed by the signatory or signatories making the entity status change in accordance with 37 CFR 1.4(d)(4).

Signature:	/Jesse A. Salen/
Name:	Jesse A. Salen
Registration Number:	42651

Doc Code: DIST.E.FILE **Document Description: Electronic Terminal Disclaimer - Filed**	PTO/SB/26 U.S. Patent and Trademark Office Department of Commerce

Electronic Petition Request	**TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT**
Application Number	16864744
Filing Date	01-May-2020
First Named Inventor	Emil Kartalov
Attorney Docket Number	88DG-330783-US4
Title of Invention	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

☒ Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action

☒ This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.

Owner	Percent Interest
CALIFORNIA INSTITUTE OF TECHNOLOGY	100%

The owner(s) with percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior patent number(s)

10770170

10068051

8838394

as the term of said prior patent is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later:
- expires for failure to pay a maintenance fee;
- is held unenforceable;
- is found invalid by a court of competent jurisdiction;
- is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
- has all claims canceled by a reexamination certificate;
- is reissued; or
- is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

(●) Terminal disclaimer fee under 37 CFR 1.20(d) is included with Electronic Terminal Disclaimer request. ( ) I certify, in accordance with 37 CFR 1.4(d)(4), that the terminal disclaimer fee under 37 CFR 1.20(d) required for this terminal disclaimer has already been paid in the above-identified application.	
Applicant claims the following fee status: (●) Small Entity ( ) Micro Entity ( ) Regular Undiscounted I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.	
THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES I certify, in accordance with 37 CFR 1.4(d)(4) that I am: (●) An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application Registration Number 72209 ( ) A sole inventor ( ) A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application ( ) A joint inventor; all of whom are signing this request	
Signature	/Jesse A. Salen/
Name	Jesse A. Salen

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	Your fee transmittal sheet cannot be generated at this time. Please complete your submission. After completing your submission, please contact the Electronic Business Center (EBC) to request the fee transmittal sheet for this submission. We apologize for the inconvenience. Electronic Business Center (EBC) 1-866-217-9197 (toll-free) or 571-272-4100 6 a.m. to 12 Midnight Eastern Time Monday -Friday
First Named Inventor/Applicant Name:	
Filer:	
Attorney Docket Number:	

Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Total in USD ($)				

Doc Code: DISQ.E.FILE
Document Description: Electronic Terminal Disclaimer – Approved

Application No.: 16864744

Filing Date: 01-May-2020

Applicant/Patent under Reexamination: Kartalov

Electronic Terminal Disclaimer filed on July 26, 2023

☒ APPROVED

This patent is subject to a terminal disclaimer

☐ DISAPPROVED

Approved/Disapproved by: Electronic Terminal Disclaimer automatically approved by EFS-Web

U.S. Patent and Trademark Office

Electronic Acknowledgement Receipt	
EFS ID:	48355614
Application Number:	16864744
International Application Number:	
Confirmation Number:	9929
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil P. Kartalov
Customer Number:	30764
Filer:	Jesse A. Salen/Annie Wong
Filer Authorized By:	Jesse A. Salen
Attorney Docket Number:	88DG-330783-US4
Receipt Date:	26-JUL-2023
Filing Date:	01-MAY-2020
Time Stamp:	21:21:22
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$170
RAM confirmation Number	E20237PL21217231
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Terminal Disclaimer-Filed (Electronic)	eTerminal-Disclaimer.pdf	41693 5099909fa18a617c6dd92d740cd688e8a05ea146	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30061 769380a49c92bc8e06d1c1dfb5959d2273810ce5	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	71754		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Examiner-Initiated Interview Summary	**Application No.** 16/864,744	**Applicant(s)** Kartalov et al.		
	Examiner RUSSELL S NEGIN	**Art Unit** 1672	**AIA (First Inventor to File) Status** No	**Page** 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	**Title**	**Type**
RUSSELL S NEGIN	Primary Examiner	Telephonic
Jesse A. Salen	Attorney	

Date of Interview: 26 July 2023

Issues Discussed:

Non-statutory Double Patenting

The Examiner agreed to allow the application if Terminal Disclaimers are filed with US Patents 8838394, 10068051, and 10770170.

/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1672	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4490

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/864,744	Kartalov et al.
	Examiner	Art Unit
	RUSSELL S NEGIN	1672

CPC - Searched*		
Symbol	Date	Examiner
C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or G06F17/10 or G06F17/11	08/05/2023	RSN

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
see attached search history printouts	08/05/2023	RSN
PE2E Search prior art search	08/05/2023	RSN
PE2E Search inventor search on all inventors	08/05/2023	RSN
PDP inventor search on all inventors	08/05/2023	RSN
PE2E Search CPC code search with modified terms	08/05/2023	RSN

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	see attached interference search history printouts	08/05/2023	RSN

/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1672	

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4491

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/864,744	Kartalov et al.
	Examiner	Art Unit
	RUSSELL S NEGIN	1672

CPC							
Symbol						Type	Version
C12Q	/	1	/	6825		F	2013-01-01
G16B	/	25	/	00		I	2019-02-01
G16B	/	5	/	00		I	2019-02-01
C12Q	/	1	/	6851		I	2013-01-01
G16B	/	25	/	20		I	2019-02-01
G16B	/	40	/	10		I	2019-02-01
C12Q	/	1	/	68		I	2013-01-01
G01N	/	21	/	6486		I	2013-01-01
G06F	/	17	/	10		A	2013-01-01
G06F	/	17	/	11		A	2013-01-01
G16B	/	40	/	00		A	2019-02-01
Y02A	/	50	/	30		A	2018-01-01

CPC Combination Sets								
Symbol						Type	Set	Ranking	Version
C12Q	/	1	/	6851		I	1	1	2013-01-01
C12Q	/	2537	/	101		I	1	2	2013-01-01
C12Q	/	2537	/	143		I	1	3	2013-01-01
C12Q	/	2537	/	165		I	1	4	2013-01-01
C12Q	/	2563	/	107		I	1	5	2013-01-01

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	19	
/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1672	05 August 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	21	1

U.S. Patent and Trademark Office — Part of Paper No.: 20230805

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4492

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/864,744	Kartalov et al.
	Examiner	**Art Unit**
	RUSSELL S NEGIN	1672

INTERNATIONAL CLASSIFICATION					
CLAIMED					
G01N	/	33	/	48	
G01N	/	33	/	50	

NON-CLAIMED					
	/		/		

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**
702	19

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner) (Date)	**Total Claims Allowed:** 19	
/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1672 (Primary Examiner) 05 August 2023 (Date)	O.G. Print Claim(s) 21	O.G. Print Figure 1

U.S. Patent and Trademark Office Part of Paper No.: 20230805

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4493

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/864,744	Kartalov et al.
	Examiner	Art Unit
	RUSSELL S NEGIN	1672

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☑ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	21	10	29	19	40										
2	22	11	30												
3	23	12	31												
4	33	13	32												
5	24	14	34												
6	25	15	36												
7	28	16	37												
8	26	17	38												
9	27	18	39												

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	19	
/RUSSELL S NEGIN/ Primary Examiner, Art Unit 1672	05 August 2023	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	21	1

U.S. Patent and Trademark Office — Part of Paper No.: 20230805

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4494

PATENT
Docket No. 88DG-330783-US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INVENTORS :	Emil KARTALOV *et al.*	CONFIRMATION NO. :	9929
SERIAL NUMBER :	16/864,744	EXAMINER :	Russell Scott NEGIN
FILING DATE :	May 1, 2020	ART UNIT :	1672
FOR :	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS		

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

OK TO ENTER
/RSN/ 8/5/2023

RESPONSE TO FINAL OFFICE ACTION

Dear Sir:

In response to the Non-Final Office Action mailed **May 10, 2023**, please amend the above-identified application as follows:

Listing of Claims begin on page **2** of this paper.

Remarks begin on page **6** of this paper.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned for under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to Deposit Account No. 504561 (**Ref. No. 30KJ-330783-US4**).

Bibliographic Data

Application No: 16/864,744

Foreign Priority claimed: ○ Yes ◉ No

35 USC 119 (a-d) conditions met: ☐ Yes ☑ No ☐ Met After Allowance

Verified and Acknowledged: /Russell Negin/ (Examiner's Signature) /RSN/ (Initials)

Title: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
05/01/2020	702	1672	88DG-330783-US4
RULE			

APPLICANTS

California Institute of Technology, Pasadena, CA,

INVENTORS

Emil P. Kartalov, Pasadena, CA, UNITED STATES

Aditya Rajagopal, Orange, CA, UNITED STATES

Axel Scherer, Pasadena, CA, UNITED STATES

CONTINUING DATA

This application is a CON of 15914356 03/07/2018 PAT 10770170

15914356 is a CON of 14451876 08/05/2014 PAT 10068051

14451876 is a CON of 13756760 02/01/2013 PAT 8838394

13756760 has PRO of 61703093 09/19/2012

13756760 has PRO of 61594480 02/03/2012

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

05/21/2020

**** SMALL ENTITY ****

STATE OR COUNTRY

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fluorescent analytes
fluorescent analytes components
fluorescent analytes components immobilization
fluorescent analytes components immobilization coding
fluorescent analytes components immobilization coding wavelength
fluorescent analytes components immobilization coding hybridization
fluorescent analytes components immobilization coding "mass spectrometry"

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PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	3	("10770170," "10068051," "8838394").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/16 09:21 AM
L2	140	kartalov.in. NEAR emil.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2022/12/16 11:12 AM
L3	286	rajagopal.in. NEAR aditya.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2022/12/16 11:12 AM
L4	1077	scherer.in. NEAR axel.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2022/12/16 11:12 AM
L5	166506	(C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU,	OR	ON	ON	2022/12/16 11:13 AM

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		G06F17/10 or G06F17/11).cpc.	CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L6	1264	L2 L3 L4	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:13 AM
L7	119	L5 AND L6	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L8	1900960	PCR	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN,	OR	ON	ON	2022/12/16 11:14 AM

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			WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L9	113706	thermal ADJ cycler	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L10	339872	fluorophores	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L11	25989671	computers	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:14 AM
L12	64751	L8 WITH L9	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB,	OR	ON	ON	2022/12/16 11:15 AM

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			HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L13	448	L10 WITH L12	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L14	1	L13 WITH L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L15	519	L13 andL11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2022/12/16 11:15 AM

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			IBM_TDB)				
L16	26534951	L13 andL 11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L17	278	L13 AND L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:15 AM
L18	439599	chromatogram	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/16 11:21 AM
L19	125	L10 WITH L18	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV,	OR	ON	ON	2022/12/16 11:21 AM

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			MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L20	2	("20040248082" OR "20110183884").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/05/06 09:06 AM
L21	3	("10770170," "10068051," "8838394").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/05/06 09:11 AM
L22	145	kartalov.in. NEAR emil.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2023/05/06 09:11 AM
L23	293	rajagopal.in. NEAR aditya.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2023/05/06 09:11 AM
L24	1089	scherer.in. NEAR axel.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA,	OR	ON	ON	2023/05/06 09:11 AM

			VN); FPRS; EPO; JPO; DERWENT)				
L25	176265	(C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or G06F17/10 or G06F17/11).cpc.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L26	1284	L2 L3 L4	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L27	122	L5 AND L6	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L28	1966331	PCR	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS,	OR	ON	ON	2023/05/06 09:11 AM

			IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L29	116375	thermal ADJ cycler	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L30	352655	fluorophores	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L31	26957183	computers	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM

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L32	66099	L8 WITH L9	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L33	471	L10 WITH L12	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L34	1	L13 WITH L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L35	543	L13 andL11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL,	OR	ON	ON	2023/05/06 09:11 AM

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			NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L36	27502615	L13 andL 11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L37	295	L13 AND L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L38	454840	chromatogram	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/05/06 09:11 AM
L39	133	L10 WITH L18	(US-PGPUB; USPAT; USOCR; FIT (AP, AT,	OR	ON	ON	2023/05/06 09:11 AM

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			AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L40	2	("20040248082" OR "20110183884").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/05/06 09:11 AM
L41	3	("10770170," "10068051," "8838394").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:58 AM
L42	145	kartalov.in. NEAR emil.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2023/08/05 09:58 AM
L43	297	rajagopal.in. NEAR aditya.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2023/08/05 09:58 AM
L44	1096	scherer.in. NEAR axel.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS,	OR	ON	ON	2023/08/05 09:58 AM

			IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)				
L45	179566	(C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or G06F17/10 or G06F17/11).cpc.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L46	1294	L2 L3 L4	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L47	123	L5 AND L6	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L48	1994726	PCR	(US-PGPUB; USPAT;	OR	ON	ON	2023/08/05

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			USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				09:58 AM
L49	117415	thermal ADJ cycler	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L50	357378	fluorophores	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L51	27387141	computers	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL,	OR	ON	ON	2023/08/05 09:58 AM

			PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L52	66649	L8 WITH L9	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L53	479	L10 WITH L12	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L54	1	L13 WITH L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L55	551	L13 andL11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY,	OR	ON	ON	2023/08/05 09:58 AM

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			CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L56	27932605	L13 andL 11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L57	299	L13 AND L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L58	460237	chromatogram	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH,	OR	ON	ON	2023/08/05 09:58 AM

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			TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L59	131	L10 WITH L18	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L60	2	("20040248082" OR "20110183884").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:58 AM
L61	3	("10770170," "10068051," "8838394").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:58 AM
L62	145	kartalov.in. NEAR emil.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2023/08/05 09:58 AM
L63	297	rajagopal.in. NEAR aditya.in.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2023/08/05 09:58 AM
L64	1096	scherer.in. NEAR	(US-PGPUB; USPAT;	OR	ON	ON	2023/08/05

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		axel.in.	USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)				09:58 AM
L65	179566	(C12Q1/6825 or C12Q1/68 or C12Q1/6851 or G01N21/6486 or G06F17/10 or G06F17/11).cpc.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L66	1294	L2 L3 L4	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L67	123	L5 AND L6	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE,	OR	ON	ON	2023/08/05 09:58 AM

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			SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L68	1994726	PCR	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L69	117415	thermal ADJ cycler	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L70	357378	fluorophores	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L71	27387141	computers	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU,	OR	ON	ON	2023/08/05 09:58 AM

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			CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L72	66649	L8 WITH L9	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L73	479	L10 WITH L12	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L74	1	L13 WITH L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN,	OR	ON	ON	2023/08/05 09:58 AM

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			WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L75	551	L13 andL11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L76	27932605	L13 andL 11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L77	299	L13 AND L11	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L78	460237	chromatogram	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB,	OR	ON	ON	2023/08/05 09:58 AM

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			HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				
L79	131	L10 WITH L18	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/08/05 09:58 AM
L80	2	("20040248082" OR "20110183884").pn.	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:58 AM

PE2E SEARCH - Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
N1	799182	fluorescent	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:58 AM
N2	179751	analytes	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:58 AM
N3	82386	N1 AND N2	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:59 AM
N4	139840	immobilization	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:59 AM
N5	23036	N3 AND N4	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:59 AM
N6	275243	mass ADJ spectrometry	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:59 AM
N7	8116	N5 AND N6	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:59 AM
N8	1556767	wavelength	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:59 AM
N9	4567	N7 AND N8	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 09:59 AM
N10	1439277	encoded	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 10:00 AM

N11	2864	N9 AND N10	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 10:00 AM
N12	126025	fluorophores	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 10:00 AM
N13	1984	N11 AND N12	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 10:00 AM
N14	62546	sample ADJ volume	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 10:00 AM
N15	550	N13 AND N14	(US-PGPUB; USPAT)	OR	ON	ON	2023/08/05 10:00 AM

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NOTICE OF ALLOWANCE AND FEE(S) DUE

30764 7590 08/10/2023

SHEPPARD, MULLIN, RICHTER & HAMPTON LLP
650 Town Center Drive, 10th Floor
Costa Mesa, CA 92626

EXAMINER	
NEGIN, RUSSELL SCOTT	
ART UNIT	PAPER NUMBER
1672	

DATE MAILED: 08/10/2023

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	05/01/2020	Emil P. Kartalov	88DG-330783-US4	9929

TITLE OF INVENTION: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0.00	$0.00	$480	11/13/2023

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PTOL-85 (Rev. 02/11)

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PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

30764 7590 08/10/2023
SHEPPARD, MULLIN, RICHTER & HAMPTON LLP
650 Town Center Drive, 10th Floor
Costa Mesa, CA 92626

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	05/01/2020	Emil P. Kartalov	88DG-330783-US4	9929

TITLE OF INVENTION: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0.00	$0.00	$480	11/13/2023

EXAMINER	ART UNIT	CLASS-SUBCLASS
NEGIN, RUSSELL SCOTT	1672	702-019000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1_____
2_____
3_____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required)

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via Patent Center or EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	05/01/2020	Emil P. Kartalov	88DG-330783-US4	9929

30764 7590 08/10/2023

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EXAMINER	
NEGIN, RUSSELL SCOTT	
ART UNIT	**PAPER NUMBER**
1672	

DATE MAILED: 08/10/2023

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 02/11)

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OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 16/864,744	Applicant(s) Kartalov et al.	
	Examiner RUSSELL S NEGIN	Art Unit 1672	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the communications of 7/10/2023 and 7/26/2023.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 21-34 and 36-40 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☑ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/RUSSELL S NEGIN/
Primary Examiner, Art Unit 1672

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TERMINAL DISCLAIMERS

The terminal disclaimers filed on 26 July 2023 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of US Patents 8838394, 10068051, and 10770170 have been reviewed and are accepted. The terminal disclaimer has been recorded.

REASONS FOR ALLOWANCE

The following is an examiner's statement of reasons for allowance:

The claims recite an empirical assay for detecting the presence and absence of M analytes with F wavelength components wherein M is greater than F without using mass spectrometry or immobilization.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

CONCLUSION

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Russell Negin, whose telephone number is (571) 272-1083. This Examiner can normally be reached from Monday through Thursday from 8 am to 3 pm and variable hours on Fridays.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's Supervisor, Larry Riggs, Supervisory Patent Examiner, can be reached at (571) 270-3062.

Information regarding the status of the application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

/RUSSELL S NEGIN/
Primary Examiner, Art Unit 1672
5 August 2023

PATENT
Attorney Docket No.: 88DG-330783-US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Emil P. KARTALOV *et al.*	Allowed: August 10, 2023
Application No.: 16/864,744	Confirmation No.: 9929
Filed: May 1, 2020	Art Unit: 1672
For: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS	Examiner: Russell Scott NEGIN

AMENDMENT AFTER ALLOWANCE UNDER 37 C.F.R. § 1.312

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

INTRODUCTORY COMMENTS

Prior to issuance of the patent, applicant respectfully requests entry of this amendment under 37 C.F.R. 1.312 for the above-captioned patent application.

Amendments to the Claims begin on page 2 of this paper.

Remarks follow the amendments section of this paper.

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application:

1.-20. (Canceled)

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

22. (Previously Presented) The assay of claim 21, wherein each of said at least M analytes is encoded as at least one first value in a first component of said fluorescent signal and at least one second value in a second component of said fluorescent signal.

23. (Previously Presented) The assay of claim 22, wherein said first value is an intensity or range of intensities.

24. (Previously Presented) The assay of claim 21, wherein said detecting is performed with reagents comprising hybridization probes.

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25. (Previously Presented) The assay of claim 24, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.

26. (Previously Presented) The assay of claim 21, wherein said signal is generated during a polymerase chain reaction.

27. (Previously Presented) The assay of claim 21, wherein at least one of said analytes is encoded by at least one additional value, wherein said at least one additional value is selected from the group consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal, and combinations thereof.

28. (Previously Presented) The assay of claim 24, wherein said fluorescence signal from said hybridization probes are used to construct a chromatogram.

29. (Previously Presented) The assay of claim 21, wherein at least one of said analytes comprises a polynucleotide.

30. (Previously Presented) The assay of claim 21, further comprising transmitting information concerning the presence or absence of at least one of said analytes through a computer network.

31. (Previously Presented) The assay of claim 21, wherein at least one step of said assay is performed using a computer and wherein said computer is connected to a thermal cycler.

32. (Previously Presented) The assay of claim 21, wherein said coding scheme is innately nondegenerate or non-degenerate by construction.

33. (Previously Presented) The assay of claim 22, wherein said coding scheme is made nondegenerate by enumerating every legitimate result that can be obtained from said coding

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scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.

34. (Previously Presented) A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising analyte-specific reagents, wherein:
a. each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy; and
b. each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the value;
wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single sample volume, in any combination of presence or absence, and
wherein the kit does not require the use of mass spectrometry or immobilization of said M analytes, and
wherein the analytes are amplified in the single sample volume.

35. (Cancelled).

36. (Previously Presented) The kit of claim 34, wherein said fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, when M is greater than F.

37. (Currently Amended) The kit of claim 34, wherein said analyte-specific reagents comprise a plurality of hybridization probes.

38. (Currently Amended) The kit of claim 37, wherein said plurality of hybridization probes comprise a fluorophore, and wherein said plurality of hybridization probes are specific for different analytes.

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39. (Previously Presented) The kit of claim 38, wherein said number of hybridization probes in the kit is greater than M.

40. (Previously Presented) The kit of claim 34, wherein the analyte-specific reagents are each present at a different amount in the kit.

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REMARKS

Claims 21, 37 and 38 are amended to improve the form. No new matter is being added. Applicant believes no fee is due with this response. In the event the U.S. Patent and Trademark Office determines that a fee or an extension is required with this submission, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 50-4561 referencing docket no. 88DG-330783-US4.

Dated: October 10, 2023

Respectfully submitted,

By: /Jesse A. Salen/
Jesse A. Salen
Registration No.: 72209
Attorney for Applicant

SHEPPARD MULLIN RICHTER & HAMPTON LLP
12275 El Camino Real, Suite 100
San Diego, CA 92130
(858) 720-8900

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Electronic Acknowledgement Receipt

EFS ID:	48703183
Application Number:	16864744
International Application Number:	
Confirmation Number:	9929
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil P. Kartalov
Customer Number:	30764
Filer:	Jesse A. Salen/Annie Wong
Filer Authorized By:	Jesse A. Salen
Attorney Docket Number:	88DG-330783-US4
Receipt Date:	10-OCT-2023
Filing Date:	01-MAY-2020
Time Stamp:	19:40:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		312_Amendment_16864744.pdf	78211 7bd0b2c023debc37a1e3b74b1b28e6dbea e055a8	yes	6

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment after Notice of Allowance (Rule 312)	1	1
	Claims	2	5
	Applicant Arguments/Remarks Made in an Amendment	6	6
Warnings:			
Information:			
	Total Files Size (in bytes):	78211	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT
Docket No.: 88DG-330783-US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	Group Art Unit: 1672
Emil P. KARTALOV *et al.*	)	
	)	Examiner: Russell Scott NEGIN
Application No.: 16/864,744	)	
	)	
Filed: May 1, 2020	)	Confirmation No.: 9929
	)	
For: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS	)	***VIA EFS-Web***

MAIL STOP ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

INFORMATION DISCLOSURE STATEMENT

REFERENCES AND LISTING

Pursuant to 37 CFR 1.56, an Information Disclosure Statement listing references is provided herewith. Copies of any listed foreign and non-patent literature references are being submitted. Any foreign references may also include English abstract(s) and/or machine translation(s), but no representation is made as to their accuracy.

Pursuant to 37 C.F.R § 1.97(g) and (h), Applicant makes no representation that the information is considered to be material to patentability. Additionally, inclusion on this list is not an admission that any of the cited documents are prior art in this application. Further, Applicant makes no representation regarding the completeness of this list, or that better art does not exist. If the Examiner would like additional information regarding these references or if anything is unclear, the Examiner is invited to contact the undersigned for assistance.

NO DISCLAIMERS

To the extent that anything in the Information Disclosure Statement or the listed references could be construed as a disclaimer of any subject matter supported by the present application, Applicant hereby rescinds and retracts such disclaimer.

TIMING OF DISCLOSURE

This Information Disclosure Statement is being filed after the mailing date of a final action or after the mailing date of a Notice of Allowance. Please place these references in the file in accordance with 37 CFR 1.97(i).

Respectfully submitted,

SHEPPARD MULLIN RICHTER & HAMPTON LLP

Dated: October 11, 2023

By: /Jesse A. Salen/
Jesse A. Salen
Reg. No. 72209
(858) 720-8900

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4537

Doc code: IDS PTO/SB/08a (01-10)

.Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)* Sheet 1 of 1	***Complete if Known***	
	Application Number	16/864,744
	Filing Date	May 1, 2020
	First Named Inventor	Emil P. KARTALOV
	Art Unit	1672
	Examiner Name	Russell Scott NEGIN
	Attorney Docket No.	88DG-330783-US4

U.S. PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear	T[5]

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	Joanne D. ANDREADIS et al., "Use of immobilized PCR primers to generate covalently immobilized DNAs for *in vitro* transcription/translation reactions", Nucleic Acids Research, Vol. 28. No. 2. 2000.	
	2	Electron Microscopy Sciences Poly-L-Lysine Coated Slides, Fisher Scientific Product No. 50-279-88; https:/www.fishersci.com/shop/products/poly-l-lysine-coated-slides/5027988, retrieved July 6. 2023.	

EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Electronic Acknowledgement Receipt

EFS ID:	48709334
Application Number:	16864744
International Application Number:	
Confirmation Number:	9929
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil P. Kartalov
Customer Number:	30764
Filer:	Jesse A. Salen/Annie Wong
Filer Authorized By:	Jesse A. Salen
Attorney Docket Number:	88DG-330783-US4
Receipt Date:	11-OCT-2023
Filing Date:	01-MAY-2020
Time Stamp:	23:18:13
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	IDS_197i_16864744.pdf	100868 c4183872a619936a2a227007759fe6726cc5b92d	no	3

Warnings:

Information:					
This is not an USPTO supplied IDS fillable form					
2	Non Patent Literature	ANDREADIS.pdf	905101 a6a77fd22819b41d0c5caab0ffab9d6360ba84d2	no	8
Warnings:					
Information:					
3	Non Patent Literature	Fisher_Scientific.pdf	364515 2df2170e6dd410b6315fe7ca336b6ea348f5d8a3	no	3
Warnings:					
Information:					
			Total Files Size (in bytes):	1370484	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

30764 7590 08/10/2023
SHEPPARD, MULLIN, RICHTER & HAMPTON LLP
650 Town Center Drive, 10th Floor
Costa Mesa, CA 92626

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	05/01/2020	Emil P. Kartalov	88DG-330783-US4	9929

TITLE OF INVENTION: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0.00	$0.00	$480	11/13/2023

EXAMINER	ART UNIT	CLASS-SUBCLASS
NEGIN, RUSSELL SCOTT	1672	702-019000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

Sheppard Mullin Richter & Hampton LLP
1
2 12275 El Camino Real, Suite 100
3 San Diego, CA 92130

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: California Institute of Technology

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Pasadena, CA

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ☒ Corporation or other private group entity ❑ Government

4a. Fees submitted: ☒ Issue Fee ❑ Publication Fee (if required)

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

☒ Electronic Payment via Patent Center or EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 50-4561

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Jesse A. Salen/ Date October 13, 2023

Typed or printed name Jesse A. Salen Registration No. 72209

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4541

Electronic Patent Application Fee Transmittal

Application Number:	16864744
Filing Date:	01-May-2020
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil P. Kartalov
Filer:	Jesse A. Salen/Annie Wong
Attorney Docket Number:	88DG-330783-US4

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	2501	1	480	480

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**480**

Electronic Acknowledgement Receipt	
EFS ID:	48719869
Application Number:	16864744
International Application Number:	
Confirmation Number:	9929
Title of Invention:	SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS
First Named Inventor/Applicant Name:	Emil P. Kartalov
Customer Number:	30764
Filer:	Jesse A. Salen/Annie Wong
Filer Authorized By:	Jesse A. Salen
Attorney Docket Number:	88DG-330783-US4
Receipt Date:	13-OCT-2023
Filing Date:	01-MAY-2020
Time Stamp:	21:14:33
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$480
RAM confirmation Number	E20230CL15256218
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	IF_Transmittal_16864744.pdf	123998 4526e747fb61d8f77076f0607811e11763e2da26	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	38069 ee1ac9a3f5a9890532ed5aa271d8957d1bfbe0c1	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	162067		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	05/01/2020	Emil P. Kartalov	88DG-330783-US4	9929

7590 10/18/2023

SHEPPARD, MULLIN, RICHTER & HAMPTON LLP
650 Town Center Drive, 10th Floor
Costa Mesa, CA 92626

EXAMINER	
NEGIN, RUSSELL SCOTT	
ART UNIT	PAPER NUMBER
1672	
NOTIFICATION DATE	DELIVERY MODE
10/18/2023	ELECTRONIC

NOTICE OF NON-COMPLIANT INFORMATION DISCLOSURE STATEMENT

An Information Disclosure Statement (IDS) filed 10/11/2023 in the above-identified application fails to meet the requirements of 37 CFR 1.97(d) for the reason(s) specified below. Accordingly, the IDS will be placed in the file, but the information referred to therein has not been considered.

The IDS is not compliant with 37 CFR 1.97(d) because:

☑ The IDS lacks a statement as specified in 37 CFR 1.97(e).

☐ The IDS lacks the fee set forth in 37 CFR 1.17(p).

☐ The IDS was filed after the issue fee was paid. Applicant may wish to consider filing a petition to withdraw the application from issue under 37 CFR 1.313(c) to have the IDS considered. See MPEP 1308.

C. Garner, for

571-272-4200 or 1-888-786-0101
Application Assistance Unit
Office of Data Management

FORM PTOM327-B (Rev. 02/08)

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4546

Response to Rule 312 Communication	Application No.	Applicant(s)	
	16/864,744	Kartalov et al.	
	Examiner	Art Unit	AIA (FITF) Status
	RUSSELL S NEGIN	1672	No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

1. ☑ The amendment filed on 10 October 2023 under 37 CFR 1.312 has been considered, and has been:
   - a) ☑ entered.
   - b) ☐ entered as directed to matters of form not affecting the scope of the invention.
   - c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
     Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.
   - d) ☐ disapproved. See explanation below.
   - e) ☐ entered in part. See explanation below.
   - f) ☐ not entered because the supplemental or corrected Application Data sheet (ADS)
     - ☐ was not accompanied by a petition to accept an unintentionally delayed claim under 37 CFR 1.55 or 27 CFR 1.78;
     - ☐ did not identify the information being changed in accordance with 37 CFR 1.76(c)(2);
     - ☐ was not properly signed in accordance with 37 CFR 1.76(e) (or 37 CFR 1.33(b) for applications filed prior to September 16, 2012).

/RUSSELL S NEGIN/
Primary Examiner, Art Unit 1672

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4547

Doc code: IDS PTO/SB/08a (01-10)

.Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/864,744
				Filing Date	May 1, 2020
				First Named Inventor	Emil P. KARTALOV
(Use as many sheets as necessary)				Art Unit	1672
				Examiner Name	Russell Scott NEGIN
Sheet	1	of	1	Attorney Docket No.	88DG-330783-US4

U.S. PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear	T[5]

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	Joanne D. ANDREADIS et al., "Use of immobilized PCR primers to generate covalently immobilized DNAs for *in vitro* transcription/translation reactions", Nucleic Acids Research, Vol. 28. No. 2. 2000.	
	2	Electron Microscopy Sciences Poly-L-Lysine Coated Slides, Fisher Scientific Product No. 50-279-88; https:/www.fishersci.com/shop/products/poly-l-lysine-coated-slides/5027988, retrieved July 6. 2023.	

EXAMINER SIGNATURE			
Examiner Signature	/RUSSELL S NEGIN/	Date Considered	10/17/2023

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

PATENT
Attorney Docket No.: 88DG-330783-US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Emil P. KARTALOV *et al.*	Allowed: August 10, 2023
Application No.: 16/864,744	Confirmation No.: 9929
Filed: May 1, 2020	Art Unit: 1672
For: SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS	Examiner: Russell Scott NEGIN

AMENDMENT AFTER ALLOWANCE UNDER 37 C.F.R. § 1.312

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

INTRODUCTORY COMMENTS

Prior to issuance of the patent, applicant respectfully requests entry of this amendment under 37 C.F.R. 1.312 for the above-captioned patent application.

Amendments to the Claims begin on page 2 of this paper.

Remarks follow the amendments section of this paper.

OK TO ENTER
/RSN/ 10/17/2023

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	05/01/2020	Emil P. Kartalov	88DG-330783-US4	9929

30764 7590 10/23/2023

SHEPPARD, MULLIN, RICHTER & HAMPTON LLP
650 Town Center Drive, 10th Floor
Costa Mesa, CA 92626

EXAMINER	
NEGIN, RUSSELL SCOTT	

ART UNIT	PAPER NUMBER
1672	

NOTIFICATION DATE	DELIVERY MODE
10/23/2023	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DOCKETING@SHEPPARDMULLIN.COM
SheppardMullin_Pair@firsttofile.com

Notice of Allowability	Application No. 16/864,744	Applicant(s) Kartalov et al.	
	Examiner RUSSELL S NEGIN	Art Unit 1672	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the communication of 10/11/2023.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 21-34 and 36-40 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☑ Other PTO-271 form.

/RUSSELL S NEGIN/
Primary Examiner, Art Unit 1672

IPR2024-01451
California Institute of Technology - Ex. 2003 Page 4551

INFORMATION DISCLOSURE STATEMENT

The information disclosure statement filed 11 October 2023 fails to comply with 37 CFR 1.97(d) because it lacks a statement as specified in 37 CFR 1.97(e). It has been placed in the application file, but the information referred to therein has not been considered.

The information disclosure statement filed 11 October 2023 fails to comply with 37 CFR 1.97(d) because it lacks the fee set forth in 37 CFR 1.17(p). It has been placed in the application file, but the information referred to therein has not been considered.

CONCLUSION

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Russell Negin, whose telephone number is (571) 272-1083. This Examiner can normally be reached from Monday through Thursday from 8 am to 3 pm and variable hours on Fridays.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's Supervisor, Larry Riggs, Supervisory Patent Examiner, can be reached at (571) 270-3062.

Information regarding the status of the application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

/RUSSELL S NEGIN/
Primary Examiner, Art Unit 1672
17 October 2023

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	11/28/2023	11827921	88DG-330783-US4	9929

30764 7590 11/08/2023

SHEPPARD, MULLIN, RICHTER & HAMPTON LLP
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ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at https://patentcenter.uspto.gov/. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

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Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 580 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Center (https://patentcenter.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

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California Institute of Technology - Ex. 2003 Page 4553

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/864,744	05/01/2020	Emil P. Kartalov	88DG-330783-US4	9929

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EXAMINER	
NEGIN, RUSSELL SCOTT	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DOCKETING@SHEPPARDMULLIN.COM
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