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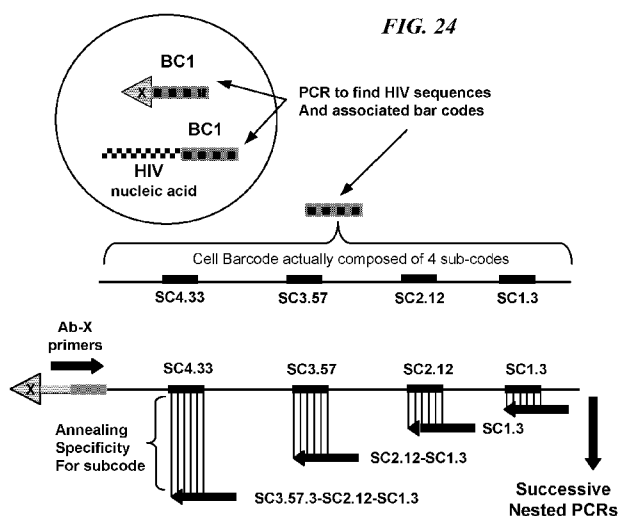
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(54) Title: METHODS FOR IDENTIFYING MULTIPLE EPITOPES IN SELECTED SUB-POPULATIONS OF CELLS



(57) Abstract: A method for identifying a sub-population within a mixed population of cells is disclosed. The method involves contacting the mixed population of cells with at least one unique binding agent, wherein the at least one unique binding agent is designed to bind to a target molecule present in the sub-population, and wherein the at least one unique binding agent is attached to an epitope specific barcode that represents the identity of the target molecule. The method further involves sequentially attaching two or more assayable polymer subunits to the epitope specific barcode to create unique cell origination barcodes that represent the identities of individual cells to which the at least one unique binding agent has bound; and decoding the epitope specific barcode and cell origination barcodes, thereby identifying the sub-population within the mixed population of cells.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/67147

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68; G01N 33/53; C12N 5/07; C07H 21/04 (2016.01)

CPC - C12Q 1/6806; C12Q 2563/159; G01N 2333/00; G01N 33/5308; G01N 33/536

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)

IPC(8) - C12Q 1/68; G01N 33/53; C12N 5/07; C07H 21/04 (2016.01); USPC - 435/6.1; 435/6.12; 435/6.19; 435/7.1; 435/347; 536/23.1

CPC - C12Q 1/6806; C12Q 2563/159; G01N 2333/00; G01N 33/5308; G01N 33/536

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

IPC(8) - C12Q 1/68; G01N 33/53; C12N 5/07; C07H 21/04 (2016.01); USPC - 435/6.1; 435/6.12; 435/6.19; 435/7.1; 435/347; 536/23.1

CPC - C12Q 1/6806; C12Q 2563/159; G01N 2333/00; G01N 33/5308; G01N 33/536 - see keyword below

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

PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google: barcode, unique, detect, identifying, cell, subpopulation, population, mixture, attach, coupling, conjugate, binding, antibody, agent, gene, genome, DNA, histone, housekeeping, protein, hybridization, PCR, amplify, sequencing, ligation, moiety, epitope, split-pool, primer, probe, oligonucleoti

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0203543 A1 (KRUTZIK et al.) 12 August 2010 (12.08.2010), Abstract, para [0009], [0012], [0017], [0020], [0030], [0048], and [0064]	1-8, 10-11
Y	US 2010/0240101 A1 (LIEBERMAN et al.) 23 September 2010 (23.09.2010), para [0007], [0012], [0015], [0021], [0037], [0071], [0125], [0130], [0139]-[0164], [0241], [0291], and [0294]	1-8, 10-11
Y	US 2011/0263688 A1 (BARANY et al.) 27 October 2011 (27.10.2011), para [0027], [0033], [0034], [0093], [0094], [0141], [0453], [0488], and [0547]	3
A	LAURING et al., Exploring the Fitness Landscape of an RNA Virus by Using a Universal Barcode Microarray. J Virol. 2011, Vol. 85(8), p. 3780-91. Entire documentation, especially Abstract; pg 3782, Fig 1; pg 3785, col 2, lower para; and pg 3786, Fig 7	7
A	US 2001/0041344 A1 (SEPETOV et al.) 15 November 2001 (15.11.2001), para [0004]	3
P, A	MACOSKO et al., Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. Cell. 2015 May 21, Vol. 161(5), p. 1202-14. Entire documentation, especially Abstract; and pg 1203, Fig 1	1-8, 10-11

☐ Further documents are listed in the continuation of Box C.

* 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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/67147

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out 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).

2. ☐ 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.

3. Additional comments:

ISA/225 mailed on 08 February 2016. No approved electronic sequence listing was submitted in response to the ISA/225.

INTERNATIONAL SEARCH REPORT

International application No.

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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:
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.

Groups I+, Claims 1-20, directed to a method for identifying a sub-population within a mixed population of cells, the method comprising:
a) contacting the mixed population of cells with at least one unique binding agent. The unique binding agents will be searched to the extent that the unique binding agents encompasses wherein two or more unique binding agents are used to identify the subpopulation, and wherein the at least one unique binding agent is an antibody or antibody fragment. It is believed that claims 1-8, 10-11 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass wherein two or more unique binding agents are used to identify the subpopulation, and wherein the at least one unique binding agent is an antibody or antibody fragment. Additional unique binding agents will be searched upon the payment of additional fees.

*****Continued in the extra sheet*****

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.: 1-8, 10-11, limited to wherein two or more unique binding agents are used to identify the subpopulation, and wherein the at least one unique binding agent is an antibody or antibody fragment

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

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Continuation of:
Box No III (unity of invention is lacking)

(Continuation of Groups I+) Applicants must specify the claims that encompass any additionally elected unique binding agents. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be: 1) wherein two or more unique binding agents are used to identify the subpopulation, and wherein the at least one unique binding agent is a DNA intercalating molecule [claims (1-8, 10), 12]; or 2) wherein two or more unique binding agents are used to identify two or more subpopulations, and wherein at least one unique binding agent comprises a nucleic acid sequence that is capable of hybridizing to an intracellular nucleic acid sequence, or wherein the at least one unique binding agent is an antibody or antibody fragment [claims (1-7), 9, 14-20].

The inventions listed as Groups I+ 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 Feature

Among Groups I+, each unique binding agent, including an antibody (claim 11), a DNA intercalating molecule (claim 12), an amine-reactive probe (claim 13), or a nucleic acid sequence that is capable of hybridizing to an intracellular nucleic acid sequence (claim 15), is structurally different from all other binding agents; and wherein two or more unique binding agents are used to identify the subpopulation (claim 8) requires using a combination of two or more unique binding agents that is different from a combination of two or more unique binding agents used for wherein two or more unique binding agents are used to identify two or more subpopulations (claims 9, 14-20)

Common Technical Features

The inventions of Groups I+ share the technical feature of a method for identifying a sub-population within a mixed population of cells, the method comprising: a) contacting the mixed population of cells with at least one unique binding agent, wherein the at least one unique binding agent is designed to bind to a target molecule present in the sub-population, and wherein the at least one unique binding agent is attached to an epitope specific barcode that represents the identity of the target molecule; b) sequentially attaching two or more assayable polymer subunits to the epitope specific barcode to create unique cell origination barcodes that represent the identities of individual cells to which the at least one unique binding agent has bound; and c) decoding the epitope specific barcode and cell origination barcodes, thereby identifying the sub-population within the mixed population of cells (claim 1);
-- wherein the epitope specific barcode and the assayable polymer subunits comprise oligonucleotide sequences (claim 2);
-- wherein each occurrence of the epitope specific barcode and the two or more assayable polymer subunits of an associated cell origination barcode are linked to form a conjugate that can be amplified and sequenced (claim 4);
-- the method further comprising amplification of epitope specific barcode - cell origination barcode conjugates (claim 5);
-- wherein the decoding step comprises sequencing all or a portion of the amplified epitope specific barcode - cell origination barcode conjugates (claim 6);
-- wherein two or more unique binding agents are used to identify the subpopulation (claim 8); and
-- wherein at least one of the unique binding agents is designed to bind to a target molecule selected from the group consisting of DNA, and histones (claim 10);
-- Claims 11 and 18-20 of Groups I+ further share the special feature of wherein the at least one unique binding agent is an antibody or antibody fragment.

However, these shared technical features do not represent a contribution over prior art as being obvious over US 2010/0203543 A1 to KRUTZIK et al. (hereinafter 'Krutzik'), in view of US 2010/0240101 A1 to LIEBERMAN et al. (hereinafter 'Lieberman') as follows:

Krutzik discloses a method for identifying a sub-population within a mixed population of cells (para [0009] - 'distinguishing between cell types in a sample comprising a population of cells which comprises a plurality of different cell types... provide a unique barcode for a specified cell type', wherein 'distinguishing ... a specified cell type' 'between cell types in a sample comprising a population of cells' is 'identifying a sub-population within a mixed population of cells'; Please see Specification for the definition of sub-population; Specification: para [0013]; para [0014] - 'identifying a sub-population within a mixed population of cells ... with a unique binding agent... target molecule present in the sub-population') the method comprising:

a) contacting the mixed population of cells with at least one unique binding agent, wherein the at least one unique binding agent is designed to bind to a target molecule present in the sub-population, and wherein the at least one unique binding agent is attached to an epitope specific barcode that represents the identity of the target molecule (para [0009] - 'distinguishing between cell types in a sample comprising a population of cells which comprises a plurality of different cell types. ... combination of amount and type of signal markers provide a unique barcode for a specified cell type. ... detecting from at least one of the cell types the at least two different signal markers, whereby one of the plurality of cell types is distinguished', wherein 'combination of amount and type of signal markers provide a unique barcode for a specified cell type' is based on 'wherein the at least one unique binding agent is attached to an epitope specific barcode that represents the identity of the target molecule'; para [0012] - 'combining the cells with antibodies to the cell surface marker. The antibodies are conjugated to at least one of different fluorescers', wherein 'at least one of different fluorescers' serves as 'an epitope specific barcode that represents the identity of the target molecule'; Abstract - 'provide a barcode for each cell type ...labeled antibodies can be used to create the barcode and the cells analyzed with flow cytometry'),

and c) thereby identifying the sub-population within the mixed population of cells (Abstract - 'Mixtures of cell types can be analyzed by having at least two signal markers, with at least one at three different levels to provide a barcode for each cell type. ...identification of the cell by the barcode. ... labeled antibodies can be used to create the barcode and the cells analyzed with flow cytometry'; para [0009] - 'distinguishing between cell types in a sample comprising a population of cells which comprises a plurality of different cell types. ... combination of amount and type of signal markers provide a unique barcode for a specified cell type. ...whereby one of the plurality of cell types is distinguished').

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Continuation of:

The previous extra sheet - Box No III (unity of invention is lacking)

Krutzik further discloses wherein the cell type specific barcode is generated by cell marker specific antibodies conjugated to different detectable molecules (para [0017] - 'distinguishing between cell types in a sample comprising at least 6 different cell types... distinguishable by at least two different signal markers ... combination of amount and type of identifiable marker provide a unique barcode for a specified cell population. ...by flow cytometry, whereby one of the cell types is distinguished'; para [0020] - 'cells are combined with antibodies to the cell surface marker, wherein the antibodies are conjugated to at least one of different fluorescers'; para [0030] - 'Barcoding of five cell populations ... anti-CD19 antibody was conjugated to PerCPy5.5 and the anti-EGFR antibody to PE...cells were cloned based on expression of the introduced CD19 signal marker'; Abstract - 'provide a barcode for each cell type ...labeled antibodies can be used to create the barcode and the cells analyzed with flow cytometry').

Krutzik neither specifically teaches wherein the epitope specific barcode is a nucleotide sequence, as defined by the specification (Specification: para [0015] - 'epitope specific barcode ... comprise oligonucleotide sequences'), nor teaches the method comprising b) sequentially attaching two or more assayable polymer subunits to the epitope specific barcode to create unique cell origination barcodes that represent the identities of individual cells to which the at least one unique binding agent has bound; and c) decoding the epitope specific barcode and cell origination barcodes. Lieberman discloses a method for detecting cell specific targets by using different barcode conjugated antibodies (para [0241] - 'Verification that the SNAPPLE biochemistry ...using: i) EGFR conjugated with a His tag ...EGFR conjugated with both a His tag and a FLAG tag at the N and C termini.... a first unique nucleotide barcode is conjugated to a His monoclonal antibody (mAb), and a second unique nucleotide barcode is conjugated to a FLAG monoclonal antibody. ... three protein samples are individually incubated with each of the two barcoded monoclonal antibodies. Quantitative polymerase chain reaction (qPCR) ... asymmetric junctions resulting from a ligation of the first barcode and the second barcode. ...a chimeric barcode comprising the first and second nucleic acid barcodes are formed only with the double-tagged EGFR', wherein the barcode comprising nucleotide (para [0007] - 'a binding partner attached to a unique nucleotide barcode sequence. ... binding partner is ...an antibody'), and further the method comprising [b]) sequentially attaching two or more assayable polymer subunits to the epitope specific barcode to create unique cell origination barcodes that represent the identities of individual cells to which the at least one unique binding agent has bound (para [0241], quotations as above; para [0130] - 'two different unique nucleotide barcodes derived from two different SNAPPLE probes ...amplifying each asymmetric nucleotide barcode with a different single primer pair. ...to identify the unique nucleotide barcodes that were ligated together.... the sequencing data creates a 'contact map' identifying paired intracellular components ...wherein each binding partner is conjugated to a unique nucleic acid barcode sequence', wherein 'a different single primer pair' is part of 'two or more assayable polymer subunits to the epitope specific barcode', and wherein 'amplifying each asymmetric nucleotide barcode with a different single primer pair' indicating 'sequentially attaching two or more assayable polymer subunits to the epitope specific barcode to create unique cell origination barcodes that represent the identities of individual cells to which the at least one unique binding agent has bound'; and [c]) decoding the epitope specific barcode and cell origination barcodes (para [0241], quotations as above; para [0021] - 'asymmetric nucleotide barcode sequence... refers to a joining (i.e., for example, by ligation) of two unique nucleotide barcode sequences. Sequencing of the asymmetric nucleotide barcode sequence provides information related to the proximal location of specific intracellular components', wherein 'Sequencing of the asymmetric nucleotide barcode sequence' is 'decoding the epitope specific barcode and cell origination barcodes' based on the definition of the Specification; Specification: para [0015] - 'decoding step comprises sequencing').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Krutzik and Lieberman, to obtain a method for identifying a sub-population within a mixed population of cells, the method comprising: a) contacting the mixed population of cells with at least one unique binding agent, wherein the at least one unique binding agent is designed to bind to a target molecule present in the sub-population, and wherein the at least one unique binding agent is attached to an epitope specific barcode that represents the identity of the target molecule; and c) thereby identifying the sub-population within the mixed population of cells, based on the teaching of Krutzik, and further wherein the epitope specific barcode is a nucleotide sequence, and the method comprising b) sequentially attaching two or more assayable polymer subunits to the epitope specific barcode to create unique cell origination barcodes that represent the identities of individual cells to which the at least one unique binding agent has bound; and c) decoding the epitope specific barcode and cell origination barcodes for identifying the sub-population within the mixed population of cells, based on the combination of Lieberman and Krutzik, in order to combine the technologies and methods available in the art for facilitating identifying a sub-population within a mixed population of cells with an expected success and without undue experimentation.

The said combination further discloses wherein the epitope specific barcode and the assayable polymer subunits comprise oligonucleotide sequences (Krutzik: para [0009] - 'distinguishing between cell types in a sample comprising a population of cells which comprises a plurality of different cell types. ... a unique barcode for a specified cell type. ...detecting ... signal markers, whereby one of the plurality of cell types is distinguished'; Lieberman: para [0241] - 'Verification that the SNAPPLE biochemistry ... a first unique nucleotide barcode is conjugated to a His monoclonal antibody (mAb), and a second unique nucleotide barcode is conjugated to a FLAG monoclonal antibody ... asymmetric junctions resulting from a ligation of the first barcode and the second barcode. ...a chimeric barcode comprising the first and second nucleic acid barcodes are formed only with the double-tagged EGFR'; para [0130] - 'two different unique nucleotide barcodes derived from two different SNAPPLE probes ...amplifying each asymmetric nucleotide barcode with a different single primer pair. ...to identify the unique nucleotide barcodes that were ligated together...wherein each binding partner is conjugated to a unique nucleic acid barcode sequence', wherein 'a different single primer pair' is a part of 'the assayable polymer subunits' and comprising 'oligonucleotide sequences'; para [0071] - 'term "primer" refers to an oligonucleotide'; para [0021] - 'asymmetric nucleotide barcode sequence... refers to a joining ... two unique nucleotide barcode sequences'; para [0037] - 'Nucleic acid sequence...nucleotide sequence...refer to an oligonucleotide'); and

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Continuation of:

The previous extra sheet - Box No III (unity of invention is lacking)

Lieberman further discloses wherein each occurrence of the epitope specific barcode and the two or more assayable polymer subunits of an associated cell origination barcode are linked to form a conjugate that can be amplified and sequenced (para [0241] - 'Verification that the SNAPPLE biochemistry a first unique nucleotide barcode is conjugated to a His monoclonal antibody (mAb), and a second unique nucleotide barcode is conjugated to a FLAG monoclonal antibody... Quantitative polymerase chain reaction (qPCR) ... asymmetric junctions resulting from a ligation of the first barcode and the second barcode. ...a chimeric barcode comprising the first and second nucleic acid barcodes are formed only with the double-tagged EGFR', wherein 'Quantitative polymerase chain reaction (qPCR) ... asymmetric junctions resulting from a ligation of the first barcode and the second barcode' is the indication of 'wherein each occurrence of the epitope specific barcode and the two or more assayable polymer subunits of an associated cell origination barcode are linked to form a conjugate that can be amplified and sequenced, see the quotations that follow; para [0130] - 'two different unique nucleotide barcodes derived from two different SNAPPLE probes ...amplifying each asymmetric nucleotide barcode with a different single primer pair. ...to identify the unique nucleotide barcodes that were ligated together.... the sequencing data creates a 'contact map' identifying paired intracellular components ...wherein each binding partner is conjugated to a unique nucleic acid barcode sequence'; para [0021] - 'asymmetric nucleotide barcode sequence... refers to a joining ... by ligation... of two unique nucleotide barcode sequences. Sequencing of the asymmetric nucleotide barcode sequence provides information related to the proximal location of specific intracellular components'); and

--the method further comprising amplification of epitope specific barcode-cell origination barcode conjugates (para [0241] - 'Verification that the SNAPPLE biochemistry a first unique nucleotide barcode is conjugated to a His monoclonal antibody (mAb), and a second unique nucleotide barcode is conjugated to a FLAG monoclonal antibody... Quantitative polymerase chain reaction (qPCR) ... asymmetric junctions resulting from a ligation of the first barcode and the second barcode. ...a chimeric barcode comprising the first and second nucleic acid barcodes are formed only with the double-tagged EGFR'; para [0130] - 'two different unique nucleotide barcodes derived from two different SNAPPLE probes ...amplifying each asymmetric nucleotide barcode with a different single primer pair. ...to identify the unique nucleotide barcodes that were ligated together.... the sequencing data creates a 'contact map' identifying paired intracellular components ...wherein each binding partner is conjugated to a unique nucleic acid barcode sequence'; para [0291] - 'amplifying an asymmetric nucleotide barcode ...identifying an intracellular component associated with the SNAPPLE probe unique nucleotide barcode'); and

-- wherein the decoding step comprises sequencing all or a portion of the amplified epitope specific barcode - cell origination barcode conjugates (para [0241] - 'Verification that the SNAPPLE a first unique nucleotide barcode is conjugated to a His monoclonal antibody (mAb), and a second unique nucleotide barcode is conjugated to a FLAG monoclonal antibody... Quantitative polymerase chain reaction (qPCR) ... asymmetric junctions resulting from a ligation of the first barcode and the second barcode. ...a chimeric barcode comprising the first and second nucleic acid barcodes are formed only with the double-tagged EGFR'; para [0130] - 'two different unique nucleotide barcodes derived from two different SNAPPLE probes ...amplifying each asymmetric nucleotide barcode with a different single primer pair. ...to identify the unique nucleotide barcodes that were ligated together.... the sequencing data creates a 'contact map' identifying paired intracellular components ...wherein each binding partner is conjugated to a unique nucleic acid barcode sequence'; para [0021] - 'asymmetric nucleotide barcode sequence... refers to a joining... by ligation... of two unique nucleotide barcode sequences. Sequencing of the asymmetric nucleotide barcode sequence provides information related to the proximal location of specific intracellular components'; para [0012] - 'amplifying said asymmetric nucleotide barcode sequence, thereby forming amplicons. ... further comprises sequencing said amplicons, thereby identifying said ligated first and second unique nucleotide barcode sequences').

Furthermore, the said combination further discloses wherein two or more unique binding agents are used to identify the subpopulation (Krutzik: para [0017] - 'distinguishing between cell types in a sample comprising at least 6 different cell types, wherein the cell types are distinguishable by at least two different signal markers ...combination of amount and type of identifiable marker provide a unique barcode for a specified cell population. ...detecting from at least one of the cell types at least two different signal markers by flow cytometry, whereby one of the cell types is distinguished'; para [0009] - 'distinguishing between cell types in a sample comprising a population of cells which comprises a plurality of different cell types. ... combination of amount and type of signal markers provide a unique barcode for a specified cell type. ...detecting from at least one of the cell types the at least two different signal markers, whereby one of the plurality of cell types is distinguished'; para [0020] - 'cells produce at least two surface markers,...the cells are combined with antibodies to the cell surface marker'; Abstract - 'Mixtures of cell types can be analyzed by having at least two signal markers, with at least one at three different levels to provide a barcode for each cell type. ...identification of the cell by the barcode... labeled antibodies can be used to create the barcode and the cells analyzed with flow cytometry'; Lieberman: para [0241] - 'Verification that the SNAPPLE biochemistry a first unique nucleotide barcode is conjugated to a His monoclonal antibody (mAb), and a second unique nucleotide barcode is conjugated to a FLAG monoclonal antibody... Quantitative polymerase chain reaction (qPCR) ... asymmetric junctions resulting from a ligation of the first barcode and the second barcode. ...a chimeric barcode comprising the first and second nucleic acid barcodes are formed only with the double-tagged'); and

-- Lieberman further discloses wherein at least one of the unique binding agents is designed to bind to a target molecule selected from the group consisting of DNA, histones (para [0241] - 'Verification that the SNAPPLE a first unique nucleotide barcode is conjugated to a His monoclonal antibody (mAb), and a second unique nucleotide barcode is conjugated to a FLAG monoclonal antibody... Quantitative polymerase chain reaction (qPCR) ... asymmetric junctions resulting from a ligation of the first barcode and the second barcode. ...a chimeric barcode comprising the first and second nucleic acid barcodes are formed only with the double-tagged'); para [0139]-[0164] - 'Such nucleic acid structure determinations may be accomplished by SNAPPLE probes comprising binding partners selected from commercially available chromatin-immunoprecipitating antibodies (NimbleGen), for example... Histone H3').

Furthermore, Lieberman discloses wherein the at least one unique binding agent is an antibody or antibody fragment (para [0241] - 'EGFR conjugated with both a His tag and a FLAG tag at the N and C termini ...a first unique nucleotide barcode is conjugated to a His monoclonal antibody (mAb), and a second unique nucleotide barcode is conjugated to a FLAG monoclonal antibody').

Without a shared special technical feature, the inventions lack unity with one another.

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