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61/844,804	10 July 2013 (10.07.2013)	US
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(81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Published:
— with international search report (Art. 21(3))

[Continued on nextpage]

(54) **Title:** PARTITIONING AND PROCESSING OF ANALYTES AND OTHER SPECIES

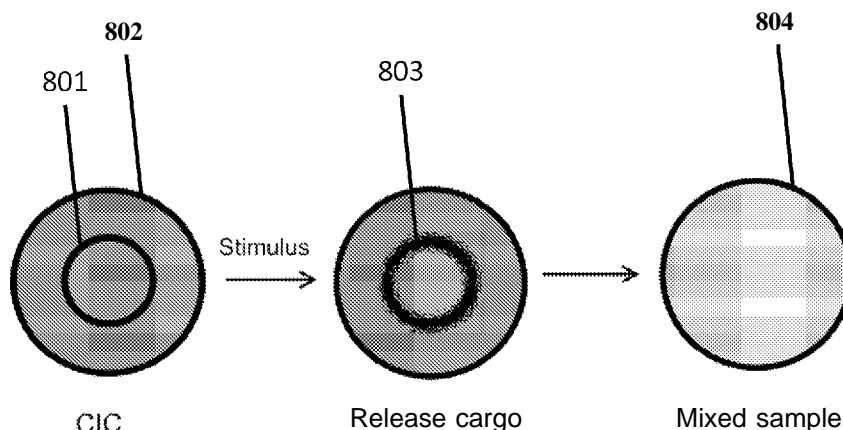


Fig. 8A

(57) **Abstract:** The present disclosure provides compositions, methods, systems, and devices for polynucleotide processing. Such polynucleotide processing may be useful for a variety of applications, including polynucleotide sequencing.



— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) **Date of publication of the international search report:**
23 October 2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/15424

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C12P 19/34 (2014.01) USPC - 435/91.2, 91.1, 89, 85, 84, 72, 41 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>: C12P 19/34 (2014.01) USPC: 435/91.2, 91.1, 89, 85, 84, 72, 41 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-CB, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; Dialog ProQuest; droplets, capsules, emulsions, release, analyte, reagentt, Oligonucleotide barcode,' 'retriction enzyme,' 'compound droplets,' 'flow path,' sequence coverage, sequencing, partitions, wells, spots							
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th style="width:10%;">Category*</th> <th style="width:60%;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="width:30%;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td style="vertical-align: top;"> X — Y </td> <td style="vertical-align: top;"> US 2011/0217736 A1 (HINDSON, BJ) September 8, 2011, abstract; figure 4; paragraphs [0009], [0013], [0015], [0016], [0023], [0024], [0029], [0030], [0036], [0038], [0040], [0041], [0056], [0057], [0068]-[0070], [0072] </td> <td style="vertical-align: top;"> 1-3, 4/2, 4/3, 8/2, 8/3, 9/8/2, 9/8/3, 10/9/8/2, 10/9/8/3, 14-17, 19, 20, 22-27, 30-33, 34/32, 34/33, 38/32, 38/33, 39/38/32, 39/38/33, 40/39/38/32, 40/39/38/33, 44-47, 49, 50, 52-57, 78, 89, 110-112, 118-120, 156-161 ----- 5/4/2, 5/4/3, 6/5/4/2, 6/5/4/3, 7/4/2, 7/4/3, 11/9/8/2, 11/9/8/3, 12/9/8/2, 12/9/8/3, .13/8/2, 13/8/3, 18, 21, 28, 29, 35/34/32, 35/34/33, 36/35/34/32, 36/35/34/33, 37/35/34/32, 37/35/34/33, 41/39/38/32, 41/39/38/33, 42/39/38/32, 42/39/38/33, 43/38/32, 48/38/33, 48, 51, 58-60, 79-88, 90-99, 113, 114, 115/111, 115/112, 116/115/111, 116/115/112 ... See Next Page ... </td> </tr> </tbody> </table>		Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X — Y	US 2011/0217736 A1 (HINDSON, BJ) September 8, 2011, abstract; figure 4; paragraphs [0009], [0013], [0015], [0016], [0023], [0024], [0029], [0030], [0036], [0038], [0040], [0041], [0056], [0057], [0068]-[0070], [0072]	1-3, 4/2, 4/3, 8/2, 8/3, 9/8/2, 9/8/3, 10/9/8/2, 10/9/8/3, 14-17, 19, 20, 22-27, 30-33, 34/32, 34/33, 38/32, 38/33, 39/38/32, 39/38/33, 40/39/38/32, 40/39/38/33, 44-47, 49, 50, 52-57, 78, 89, 110-112, 118-120, 156-161 ----- 5/4/2, 5/4/3, 6/5/4/2, 6/5/4/3, 7/4/2, 7/4/3, 11/9/8/2, 11/9/8/3, 12/9/8/2, 12/9/8/3, .13/8/2, 13/8/3, 18, 21, 28, 29, 35/34/32, 35/34/33, 36/35/34/32, 36/35/34/33, 37/35/34/32, 37/35/34/33, 41/39/38/32, 41/39/38/33, 42/39/38/32, 42/39/38/33, 43/38/32, 48/38/33, 48, 51, 58-60, 79-88, 90-99, 113, 114, 115/111, 115/112, 116/115/111, 116/115/112 ... See Next Page ...
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.					
X — Y	US 2011/0217736 A1 (HINDSON, BJ) September 8, 2011, abstract; figure 4; paragraphs [0009], [0013], [0015], [0016], [0023], [0024], [0029], [0030], [0036], [0038], [0040], [0041], [0056], [0057], [0068]-[0070], [0072]	1-3, 4/2, 4/3, 8/2, 8/3, 9/8/2, 9/8/3, 10/9/8/2, 10/9/8/3, 14-17, 19, 20, 22-27, 30-33, 34/32, 34/33, 38/32, 38/33, 39/38/32, 39/38/33, 40/39/38/32, 40/39/38/33, 44-47, 49, 50, 52-57, 78, 89, 110-112, 118-120, 156-161 ----- 5/4/2, 5/4/3, 6/5/4/2, 6/5/4/3, 7/4/2, 7/4/3, 11/9/8/2, 11/9/8/3, 12/9/8/2, 12/9/8/3, .13/8/2, 13/8/3, 18, 21, 28, 29, 35/34/32, 35/34/33, 36/35/34/32, 36/35/34/33, 37/35/34/32, 37/35/34/33, 41/39/38/32, 41/39/38/33, 42/39/38/32, 42/39/38/33, 43/38/32, 48/38/33, 48, 51, 58-60, 79-88, 90-99, 113, 114, 115/111, 115/112, 116/115/111, 116/115/112 ... See Next Page ...					
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <u> 1 </u>							
<table style="width:100%; border-collapse: collapse;"> <tr> <td style="width:50%; vertical-align: top;"> * 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 </td> <td style="width:50%; vertical-align: top;"> "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 </td> </tr> </table>		* 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				
* 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 01 August 2014 (01.08.2014)	Date of mailing of the international search report <div style="text-align: center; font-size: 1.5em; font-weight: bold;">20 AUG 2014</div>						
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 PCT OSP: 571-272-7774						

INTERNATIONAL SEARCH REPORT

International application No.

PCT7US14/15424

C (Continuation). DOCUMENTS CONSIDERED \emptyset BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	... Continued from Previous Page Continued from Previous Page ... 117/1 16/1 15/1 11, 117/1 16/1 15/1 12, 121, 122, 123/1 19, 123/120, 124/123/1 19, 124/123/120, 125/124/123/1 19. 125/124/123/120
Y	US 2012/0316074 A1 (SAXONOV, S) December 13, 2012; abstract; paragraphs [0004], [0017], [0022], [0026], [0034], [0071], [0078], [01 12], [01 15], [01 17], [0143], [0156], [0159], [0160], [0172], [0210], [0240]	5/4/2, 5/4/3, 6/5/4/2, 6/5/4/3, 7/4/2, 7/4/3, 11/9/8/2, 11/9/8/3, 12/9/8/2, 12/9/8/3, 13/8/2, 13/8/3, 21, 35/34/32, 35/34/33, 36/35/34/32, 36/35/34/33, 37/35/34/32, 37/35/34/33, 41/39/38/32, 41/39/38/33, 42/39/38/32, 42/39/38/33, 43/38/32, 48/38/33, 51, 81, 82, 92, 93, 113, 114, 115/1 11, 115/1 12, 116/1 15/1 11, 116/1 15/112, 117/1 16/115/1 11, 117/1 16/115/1 12, 121, 122, 123/1 19, 123/120, 124/123/119, 124/123/120, 125/124/123/1 19, 125/124/123/120
Y	US 2012/0000777 A1 (GARRELL, RL et al.) January 05, 2012; abstract; paragraphs [0025], [0029], [0063]	18, 28, 29, 48, 58-60
Y	US 2007/0154903 A1 (MARLA, SS et al.) July 05, 2007; figure 22; paragraphs [0035], [0061], [0078], [0155], [0163]	79, 84, 85, 90, 95, 96
Y	US 2007/0207060 A1 (ZOU, F et al.) September 06, 2007; paragraphs [0046], [0101], [0109]-[0118], [0127], [0234], [0270]	80, 83, 86-88, 91, 94, 97-99

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 14/1 5424

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.: **175**
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:

- " *-Please See Supplemental Page- " -

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.:

Group 1: Claims 1-60, 78-99, 110-125, 156-161

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/US14/15424

-*-Continued from Box No. III: Lack of Unity of Invention:

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 1-60, 78-99, 110-125 and 156-161 are directed toward compositions, kits, methods and devices comprising a plurality of capsules situated within droplets, or within outer capsules.

Group II: Claims 61-77, 100-109, 126-133, 162 and 163 are directed toward a composition, kit, method and device comprising a plurality of discrete spots disposed on a surface within a well.

Group III: Claims 134-155 are directed toward a method comprising: a. providing a sample comprising a cell; b. lysing said cell, thereby generating a lysate; and c. partitioning said lysate into a capsule.

Group IV: Claims 164-174 are directed toward a method comprising generating fragmented polynucleotides.

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 capsules situated within droplets in an emulsion, wherein said capsules are configured to release their contents into said droplets upon the application of a stimulus, which are not present in any other Groups, the special technical features of Group II including a plurality of discrete spots disposed on a surface within a well, which not present in any other Groups, the special technical features of Group III including a cell lysate, which are not present in any other Groups, the special technical features of Group IV including fragmented polynucleotides.

Groups I-IV share the technical features including partitions; Groups I, III and IV share the technical features including capsules or encapsulation; Groups I and II share the technical features including a composition, release of a species upon application of a stimulus, a device and a kit comprising instructions. Groups III and IV share the technical features including a method comprising partitioning.

However, these shared technical features are previously disclosed by US 2011/0217736 A1 (HINDSON). Hindson discloses partitions, capsules or encapsulation (droplets and encapsulation, including compound droplets or capsules (partitions, capsules and encapsulation); paragraphs [0029], [0030]); a composition (a composition; paragraph [0023]); release of a species upon application of a stimulus (release of a reagent upon heating of an emulsion (release of a species upon application of a stimulus); paragraph [0016]); a device (a system for forming droplets (a device); paragraph [0068]); a kit (a kit; paragraph [0020]) for nucleic acid amplification (for nucleic acid amplification; paragraph [0020]); and a method comprising partitioning (a method of making compound droplets (a method comprising partitioning); paragraph [0015]). Hindson does not disclose a kit comprising instructions. However, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have provided instructions with a kit, as previously disclosed by Hindson, for enabling a user to process samples appropriately using the partitioning methods and release system previously disclosed by Hindson.

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 Hindson reference, unity of invention is lacking.