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Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

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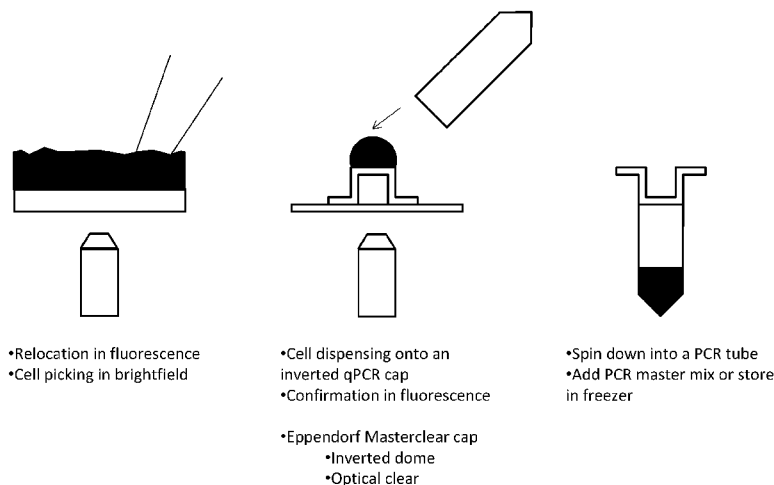
— with international search report (Art. 21(3))

— 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))

[Continued on next page]

(54) Title: METHODS FOR OBTAINING SINGLE CELLS AND APPLICATIONS OF SINGLE CELL OMICS

Figure 1



(57) Abstract: The present application provides methods for obtaining single cells from a sample. Methods for isolating and analyzing molecular features obtained from a single cell are also disclosed herein. For example, individual circulating tumor cells (CTCs) from a sample such as a patient's blood sample can be identified and obtained using methods disclosed herein, and picked for further analysis.



(88) Date of publication of the international search report:
20 September 2012

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/22248

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/574 (2012.01)

USPC - 435/7.23

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)

USPC: 435/7.23

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

USPC: 435/7.23; 424/277.1 (text search)

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

Electronic data bases: PubWEST (USPT, EPAB, JPAB, PGPB); Google Scholar

Search terms: circulating tumor cell (CTC), isolation, single cell, tumor marker, cytotokeratin, epithelial cell adhesion molecule (EpCAM), isolation, detection, antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0317836 A1 (KUHN et al.) 24 December 2009 (24.12.2009). Especially para [0010], [0011], [0014], [0018], [0032], [0033].	1-8
X	US 2010/0028915 A1 (GUALBERTO et al.) 4 February 2010 (04.02.2010). Especially para [0041], [0044], [0045].	1, 4-8/1

☐ 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

Date of the actual completion of the international search

5 July 2012 (05.07.2012)

Date of mailing of the international search report

19 JUL 2012

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/22248

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.: 9-18, 24-28 and 31-35
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:

Group I: claims 1-8, drawn to a method for obtaining individual circulating tumor cells (CTCs) in blood, comprising: providing a blood sample from a patient; identifying one or more CTCs in the blood sample; and obtaining single CTCs.

Group II: claims 19-23 and 29-30, drawn to a method for assessing cancer progression in a patient suffering from cancer, comprising: providing a circulating tumor cell (CTC) or a substantially pure population of CTCs from the patient; and performing one or more cellular or molecular analyses on the CTCs to determine cancer progression in the patient.

- Please see extra sheet for continuation -

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

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.

Continuation of:

Box NO III. Observations where unity of invention is lacking

Group III: claims 36-38, drawn to a method for assessing response of a patient suffering from cancer to a treatment, comprising: providing a circulating tumor cell (CTC) or a substantially pure population of CTCs from the patient; and performing one or more cellular or molecular analyses to determine treatment response in the patient.

The inventions listed as Groups I through III 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 inventions of Groups I-II do not include the inventive concept of assessing response of a patient suffering from cancer to a treatment, as required by Group III.

The inventions of Groups I and III do not include the inventive concept of assessing cancer progression in a patient suffering from cancer, as required by Group II.

The inventions of Groups II-III do not include the inventive concept of obtaining individual circulating tumor cells (CTCs), as required by Group I.

The inventions of Groups I-III share the technical feature of providing a circulating tumor cell (CTC) or a substantially pure population of CTCs from the patient. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2010/0028915 A1 to Gualberto et al. (hereinafter 'Gualberto'). Gualberto discloses a method for providing a circulating tumor cell (CTC) or a substantially pure population of CTCs from the patient (para [0018], a kit for screening a patient sample for the presence of CTCs; para [0039] and [0044], 7.5 ml of blood is mixed with ferrofluids coated with antibodies directed against the tumor-associated antigen EpCAM followed by immunomagnetic enrichment). As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Groups II and III is to perform one or more cellular or molecular analyses on the CTCs from a cancer patient. The inventions do not share a special technical feature, because Gualberto teaches a method of performing one or more cellular or molecular analyses on the CTCs (para [0044], FITC-labeled antibodies recognizing cytokeratins 4, 5, 6, 8, 10, 12, 18 and 19, and PE-labeled antibodies recognizing IGF-1R were added) from a cancer patient (para [0029]). Without a shared special technical feature, the inventions lack unity with one another.

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