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

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(54) Title: DETECTION OF SEQUENCE VARIANTS IN THE HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) GENE

(57) Abstract: Provided herein are methods for detecting and identifying sequence variants in the human epidermal growth factor receptor (EGFR) gene, and compositions and kits for performing such methods. In particular, nucleic acid amplification and fluorescence detection methods are provided for the detection and identification of EGFR sequence variants.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/39594

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/50, C12Q 1/68, C07H 21/04 (2012.01)
USPC - 436/63, 436/64, 43/6.14, 435/6.11, 536/24.53
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 – 436/63, 436/64, 435/6.14, 435/6.11, 536/24.53, 536/24.3, 536/24.1

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)
Pat Base; PubWEST -- PGPB,USPT,USOC,EPAB,JPAB; Dialog Classic Files – 654, 652, 349, 348, 340, 35, 65, 155; Google Scholar; Search terms -- fluorescent probe, quencher, FRET, primer, amplification, LATE-PCR, EGFR mutation, exons 18-21, cancer diagnosis, melting, annealing, signatures, computer analysis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>–<br>Y | US 2011/0081651 A1 (HILLAN) 07 April 2011 (07.04.2011) para [0002], [0010], [0015], [0036], [0037], [0041], [0043], [0046], [0057], Fig 2, abstract | 74<br>—<br>1-24, 26, 39-51, 68-73, 75, 76 |
| Y | WO 2011/050173 A1 (WANGH et al.) 28 April 2011 (28.04.2011) pg 6, ln 1-17; pg 7, ln 1-32; pg 8, ln 26-35; pg 9, ln 1 -- pg 10, ln 5; pg 10, ln 18 -- pg 12, ln 5; pg 12, ln 27 -- pg 13, ln 12; pg 13, ln 13 -- pg 14, ln 2; pg 17, ln 31 -- pg 18, ln 6; pg 19, ln 32-34; pg 20, ln 18 -- pg 21, ln 24; pg 22, ln 3-23; pg 23, ln 5-17; pg 28, ln 17 -- pg 29, ln 4; pg 33, ln 11-23; pg 34, ln 2-11; pg 54, ln 18 -- pg 55, ln 13; pg 61, ln 5-16, Fig 1, 10B | 1-24, 26, 39-51, 68-73 |
| Y | US 2008/0026393 A1 (MINDRINOS et al.) 31 January 2008 (31.01.2008) para [0005], [0019], [0037], abstract, SEQ ID NO: 393 | 24, 26, 50, 51, 70, 72, 73, 75, 76 |
| Y | US 2005/0272083 A1 (SESHAGIRI) 08 December 2005 (08.12.2005) para [0010], [0011], SEQ ID NO: 4 | 24, 26, 50, 51, 72, 73, 76 |
| A | US 2007/0281896 A1 (MORRIS et al.) 06 December 2007 (06.12.2007) para [0050], [0309], SEQ ID NO: 26 | 24, 26, 50, 51, 72, 73, 76 |

☐ 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 | Date of mailing of the international search report |
|---|---|
| 30 October 2012 (30.10.2012) | 28 NOV 2012 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (July 2009)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/39594

**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 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 1-76 directed to a method for detecting mutations in the human EGFR gene, a reagent kit for identifying one or more mutations in the human EGFR gene and a method for detecting mutations in the human EGFR gene, and a reagent kit comprising primer pairs a probe sets.

SEE CONTINUATION 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.:
1-76, restricted to the probe set designated as SEQ ID NO.:9, SEQ ID NO.:10, SEQ ID NO.:11, SEQ ID NO.:12, and SEQ ID NO.:13 and the primer pair designated as SEQ ID NO.:1 and SEQ ID NO.:2 (specifically Claims 1-24, 39-51, 68-76)

**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/US 12/39594

Continuation of Box III

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

For Group I+ the shared technical features of the Groups is a method for detecting mutations in the human EGFR gene, a reagent kit for identifying one or more mutations in the human EGFR gene and a method for detecting mutations in the human EGFR gene, and a reagent kit comprising primer pairs a probe sets. However, this is not an improvement over the prior art of WO 2011/050173 A1 to Wangh et al. (hereinafter "Wangh") that teaches a method for detecting mutations in target genes (pg 22, ln 3-23), comprising
a) providing:
i) a sample suspected of comprising the gene of interest (pg 6, ln 3-17), and
ii) detection reagents (reagent kit, pg 7, ln 28-32) comprising at least one pair of primers (col 7, ln 1-13) and at least one detectably distinguishable (pg 6, ln 3-17) probe set (pg 7, ln 14-27; pg 8, ln 26-35) of two or more hybridization probes (pg 6, ln 3-17) which hybridize to adjacent target nucleic acid sequences (pg 30, ln 15 to pg 31, ln), each probe set comprising:
A) one or more quencher probe(s) labeled with a non-fluorescent quencher (pg 9, ln 1-32), and
B) one or more signaling probes labeled with a fluorescence-emitting moiety (pg 9, ln 1-32) and a non-fluorescent quencher (pg 9, ln 1-32), wherein said signaling probes do not emit fluorescence above background when not hybridized to the target sequence (pg 20, ln 30 to pg 21, ln 24), but emit a fluorescence signal above background upon hybridization to the target sequence in the absence of adjacently bound quencher probe, wherein, if both a signaling probe and an adjacently-bound quencher probe are hybridized to their target nucleic acid sequences, the non-fluorescent quencher of the quencher probe quenches the signal from the signaling probe (pg 6, ln 1-17);
b) amplifying all or a portion of said target with said primers (pg 6, ln 30-33);
c) detecting the fluorescence of said fluorescence-emitting fluorophore from each detectably distinguishable probe set over a range of temperatures (pg 13, ln 13 to pg 14, ln 2);
d) generating temperature-dependent composite fluorescence curves for each fluorescence-emitting fluorescence; (pg 16, ln 1-25) and
e) analyzing said temperature-dependent fluorescence curves (pg 16, ln 1-25) to detect the presence or absence of one or more mutations in the target gene (pg 22, ln 3-23). Wangh does not specifically teach that detection of mutations in an EGFR target gene. US 2011/0081651 A1 to Hillan, published 7 April 2011, teaches detection of mutations in human (para [0049]) EGFR (para [0002], [0010], abstract) using fluorescent hybridization probes (para [0036], [0041]). It would have been obvious to one of ordinary skill in the art to combine the teachings of Wangh and Hillan to provide that the steps detailed above for detecting mutations in a target gene in a sample would apply to samples comprising a human EGFR as taught by Hillan, based on the common procedures of detecting hybridization of fluorescent probes to the target sequences. Hillan further teaches that the probe sets (para [0011], [0036]) are configured to hybridize to exon 18, exon 19, exon 20, or exon 21 (para [0015], [0057, Fig 2) of the human (para [0049]) EGFR gene (para [0002], [0010], [0036], abstract). The Groups are different because the different polynucleotide sequences represented by the different probe sets SEQ ID NO.:9, SEQ ID NO.:10, SEQ ID NO.:11, SEQ ID NO.:12, and SEQ ID NO.:13; SEQ ID NO.:14, SEQ ID NO.:15, SEQID NO.:16, SEQ ID NO.:17, and SEQ ID NO.:18; SEQ ID NO.:19, SEQ ID NO.:20, SEQ ID NO.:21, and SEQ ID NO.:22; and SEQ ID NO.:23, SEQ ID NO.:24, SEQ ID NO.:25, SEQ Id NO.:26, SEQ ID NO.27, SEQ ID NO.:28, and SEQ ID NO.:29 are different structures that are not common to one another but are different because they are composed of unique nucleic acid sequences. Likewise, the primer pairs represented by SEQ ID NO.:1 and SEQ ID NO.:2; SEQ ID NO.:3 and SEQ ID NO.:4; SEQ ID NO.:5 and SEQ ID NO.:6; and SEQ ID NO.:7 and SEQ ID NO.:8 are different structures that are not common to one another but are different because they are composed of unique nucleic acid sequences. Therefore the inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-76 restricted to the probe set designated as SEQ ID NO.:9, SEQ ID NO.:10, SEQ ID NO.:11, SEQ ID NO.:12, and SEQ ID NO.:13 and the primer pair designated as SEQ ID NO.:1 and SEQ ID NO.:2 (specifically Claims 1-24, 39-51, 68-76) without additional fees. In order for all inventions to be examined, applicants must designate with specificity the particular probe set and primer pairs to be searched and the appropriate examination fees must be paid for each additional set to be searched.