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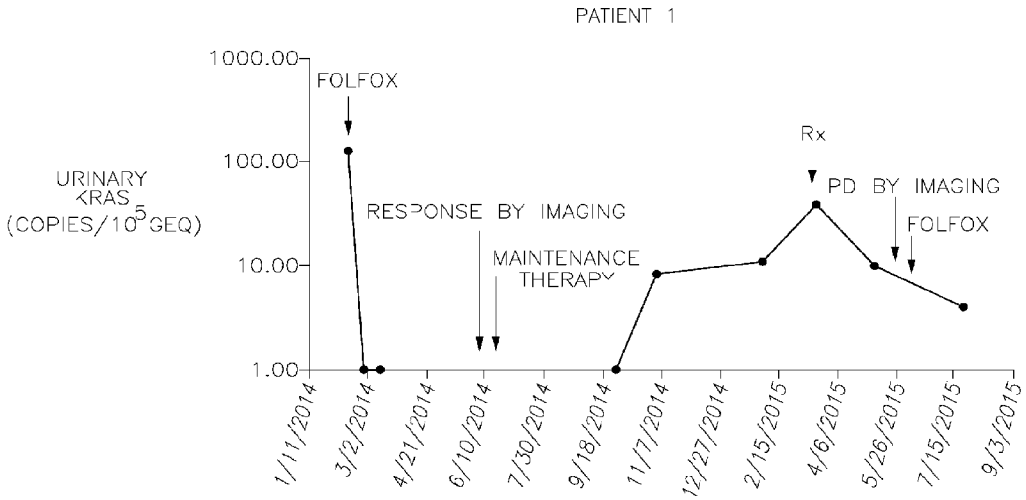
(54) Title: OLIGONUCLEOTIDE SEQUENCES FOR DETECTION OF LOW ABUNDANCE TARGET SEQUENCES AND KITS THEREOF

FIG. 13



(57) Abstract: The present disclosure relates to the detection of target nucleic acid sequences present in low-abundance relative to corresponding non-target or wild-type nucleic acid sequence in a sample. In particular, compositions comprising oligonucleotide sequences suitable for use in enrichment and amplification methods allow for a substantially greater level of detection sensitivity of mutant or target sequence within a high background of wild type sequence.

[Continued on next page]

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TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *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))*

(88) Date of publication of the international search report:
21 December 2017 (21.12.2017)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/33401

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/68; G01N 33/50, 33/574 (2017.01)

CPC - C12Q 1/6853, 1/6883, 1/6886, 1/6844; G01N 33/5011, 33/574

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.
A	(YO, J et al.) Detection of BRAF Mutations from Solid Tumors using TumorplexTM Technology. MethodsX. 12 June 2015; Vol. 2; pages 316-322; page 317, paragraph 2; DOI: 10.1016/j.mex.2015.06.002	1-15, 19-20
A	(ASCIERTO, PA et al.) The Role of BRAF V600 Mutation in Melanoma. Journal of Translational Medicine. 09 July, 2012; Vol. 10, No. 85; pages 1-9; page 4, column 2, paragraph 4; DOI: 10.1186/1479-5876-10-85	1-15, 19-20
A	US 2013/0005585 A1 (ANDERSON, M et al.) 03 January, 2013; page 48, table 6; page 54, table 6	1-15, 19-20

☐ 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
12 October 2017 (12.10.2017)	06 NOV 2017
Name and mailing address of the ISA/ 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 PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/33401

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:
-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 1-15 and 19-20

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
Information on patent family members

International application No.
PCT/US17/33401

-***-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.

Groups I+, Claims 1-20 and SEQ ID NOs: 3-5 are directed toward a composition comprising a set of two primers for detecting a sequence encoding a mutation; and methods associated therewith.

The composition and methods will be searched to the extent they encompass primers for the detection of a mutation at BRAF V600, wherein the primers encompass SEQ ID NO: 3 (first exemplary first primer) and SEQ ID NO: 4 (first exemplary second primer) and a blocker encompassing SEQ ID NO: 5 (first exemplary blocker). Applicant is invited to elect additional set(s) of primers, with specified set (s) of SEQ ID NOs: for each, and, where applicable, an associated blocker, with specified SEQ ID NO:, to be searched. Additional set(s) of primer and, where applicable, blocker sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 4 (in-part), 5 (in-part), 6 (in-part), 7 (in-part), 8 (in-part), 9 (in-part), 10 (in-part), 11 (in-part), 12 (in-part), 13 (in-part), 14 (in-part), 15 (in-part), 19 (in-part) and 20 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 3 (first primer), SEQ ID NO: 4 (second primer) and SEQ ID NO: 5 (blocker). 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/examined. An exemplary election would be a set of primers encompassing SEQ ID NO: 6 (first exemplary elected first primer) and SEQ ID NO: 7 (first exemplary elected second primer), with an associated blocker encompassing SEQ ID NO: 8 (first exemplary elected blocker).

No technical features are shared between the primer and/or blocker sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a composition comprising a set of two primers for detecting a sequence encoding a mutation at BRAF V600; EGFR Exon 19, Exon 20, T790, or L858; or KRAS G12 or G13, wherein at least one of the two primers comprises an oligonucleotide; a method of detecting a specific mutant nucleic acid sequence in a sample of a bodily fluid, wherein the sample comprises a nucleic acid comprising a specific wild-type nucleic acid sequence that differs from the specific mutant nucleic acid sequence by at least one nucleotide, the method comprising preparing a reaction mixture comprising nucleic acids from the sample, and the composition; subjecting the reaction mixture to one or more cycles of an amplification reaction to create amplified sample nucleic acids; and detecting the specific mutant nucleic acid in the amplified sample nucleic acids, wherein the specific mutant nucleic acid sequence encodes a mutation at BRAF V600; EGFR Exon 19, Exon 20, T790, or L858; or KRAS G12 or G13; a method of detecting a secondary mutation in a cancer in a subject, wherein the cancer has a first mutation, the method comprising obtaining a sample of a bodily fluid from the subject; and testing for the presence of the second mutation in the cancer by the method.

However, these shared technical features are previously disclosed by US 2014/0170662 A1 to Montagut Viladot et al. (hereinafter 'Montagut')

Montagut discloses a composition (a reaction mixture (a composition); paragraphs [0061], [0086]) comprising a set of two primers for detecting a sequence encoding a mutation (comprising a set of two primers for detecting a sequence encoding a mutation; paragraphs [0020], [0086]) at BRAF V600 (at BRAF V600; paragraph [0068]); or KRAS G12 or G13 (or KRAS G12 or G13; paragraph [0067]), wherein at least one of the two primers comprises an oligonucleotide (wherein at least one of the two primers comprises an oligonucleotide; paragraphs [0022], [0023]); a method of detecting a specific mutant nucleic acid sequence in a sample of a bodily fluid (a method of detecting a specific mutant nucleic acid sequence in a sample; paragraph [0031]), wherein the sample comprises a nucleic acid comprising a specific wild-type nucleic acid sequence that differs from the specific mutant nucleic acid sequence by at least one nucleotide (wherein the sample comprises a nucleic acid comprising a specific wild-type nucleic acid sequence that differs from the specific mutant nucleic acid sequence by at least one nucleotide; paragraph [0031]), the method comprising preparing a reaction mixture comprising nucleic acids from the sample, and the composition (the method comprising preparing a reaction mixture comprising nucleic acids from the sample, and the composition; paragraphs [0031], [0061], [0086]); subjecting the reaction mixture to one or more cycles of an amplification reaction to create amplified sample nucleic acids (subjecting the reaction mixture to one or more cycles of an amplification reaction to create amplified sample nucleic acids; paragraphs [0061], [0086]); and detecting the specific mutant nucleic acid in the amplified sample nucleic acids (and detecting the specific mutant nucleic acid in the amplified sample nucleic acids; paragraph [0061]), wherein the specific mutant nucleic acid sequence encodes a mutation at BRAF V600 (wherein the specific mutant nucleic acid sequence encodes a mutation at BRAF V600; paragraph [0068]); or KRAS G12 or G13 (or KRAS G12 or G13; paragraph [0067]); a method of detecting a secondary mutation in a cancer in a subject (a method of detecting a mutation associated with resistance to a therapeutic (a secondary mutation) in a cancer in a subject; abstract, paragraph [0031]), wherein the cancer has a first mutation (wherein the cancer has a first mutation; paragraph [0002]), the method comprising obtaining a sample from the subject (the method comprising obtaining a sample from the subject; paragraph [0031]); and testing for the presence of the second mutation in the cancer by the method (testing for the presence of the second mutation in the cancer by the method; paragraphs [0031], [0061], [0086]).

Montagut does not disclose wherein the sample is a sample of a bodily fluid. However, It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Montagut to have specified wherein the sample obtained from a subject with cancer was a body fluid sample, such as a peripheral blood sample, or plasma, lymph, or serum sample, in order to enable the detection of circulating cancer cells in a subject, wherein the cells were resistant to treatment with a first therapeutic, in order to better enable the selection of a treatment for the subject with a different therapeutic in order to prevent metastases.

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