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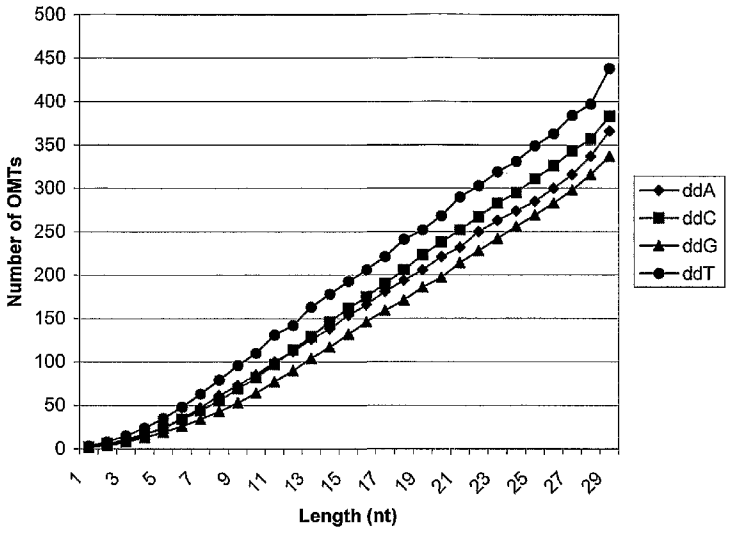
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12 April 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: QUANTIFICATION OF NUCLEIC ACIDS AND PROTEINS USING OLIGONUCLEOTIDE MASS TAGS



(57) Abstract: The invention provides a method for detecting and quantifying the amount of target molecules, such as nucleic acids or proteins in a sample. The target molecules are first recognized and bounded by target-specific probes, generally nucleic acids or proteins that bind specifically to the targets, each of which is labeled with a short single-stranded nucleic acid probe, either DNA or RNA, with distinct molecular weight. This label is called an oligonucleotide mass tag. One or several standard oligonucleotide sequences can be designed with similar sequence but distinct molecular weight to those oligonucleotide mass tags. Then the oligonucleotide mass tags associated with bounded probes and the standard sequences are co-amplified using a pair of common primers. The presence and/or amount of each oligonucleotide mass tag, which corresponds to the amount of corresponding target molecule, is determined by a primer extension reaction and quantification of the primer extension product.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/20470

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C12Q 1/68**(2007.01);**C12P 19/34**(2007.01);**G01N 33/53**(2007.01)

USPC: 435/6,7.1,91.2
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)
U.S. : 435/6, 7.1, 91.2

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)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2003/0119004 A1 (WENZ et al) 26 June 2003 (26.06.2003), see entire document, especially Figure 6, Figure 8, and paragraphs 133-138.	1-4 --- 5
Y	US 2004/0081993 A1 (CANTOR et al) 29 April 2004 (29.04.2004), see entire document, especially paragraphs 8-10 and 27-31.	5

☐ 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 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
27 October 2006 (27.10.2006)	05 DEC 2006
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 Angela Bertagna Telephone No. 571-272-1600

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Form PCT/ISA/210 (second sheet) (April 2005)

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:
Please 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 any 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.: 1-5

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.
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BOX 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.

Group I, claim(s) 1-5, drawn to methods of detecting a target nucleic acid using mass tag probes.

Group II, claim(s) 6-15, drawn to methods of detecting protein-protein interactions, DNA-binding protein specificities, and target proteins using mass tag probes.

The inventions listed as Groups I and II 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 prior art of Wenz et al. (US 2003/0119004; published June 26, 2003) teaches a method comprising the following steps: (1) designing two probes where the most 3' region of the first probe and the most 5' region of the second probe are target-specific, where the most 5' region of the first probe and the most 3' region of the second probe contains a primer binding site, and where the middle region of the probes contains a mass tag (see, for example, the probes depicted in Figures 6 & 8 and described in paragraphs 21 and 133-138; paragraph 67 teaches that one or both probes may contain a mobility modifier between the primer-specific and target-specific portions), (2) contacting the target in double-stranded form with the two probes where the target-specific portions anneal to the target (see Figure 8; paragraph 61 teaches double-stranded targets), (3) ligating the two probes (Figure 8), (4) amplification using primers specific to the primer sequences on the ligated probe (Figure 8 and paragraph 134), and (5) detecting the amplified product (Figure 8 and paragraphs 134-135). Since the prior art of Wenz anticipates the instant claim 1, the claims lack a special technical feature linking them over the prior art, and therefore, a lack of unity requirement is proper.

~~Continuation of B. FIELDS SEARCHED Item 3:~~

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/20470

patent databases: USPAT, USPGPUBS, EPO, JPO, DERWENT
search terms: mass tag, primer, ligate, amplification, extension