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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:
14 October 2010

(54) Title: SEQUENCE-SPECIFIC DETECTION OF NUCLEOTIDE SEQUENCES

(57) Abstract: A method for detecting the presence of a target nucleotide sequence in a sample of DNA is described herein in which a test sample comprising single stranded DNA is exposed to a DNA probe and a nicking endonuclease under conditions that would permit sequence-specific hybridization of the probe to a complementary target sequence. The probe comprises a sequence complementary to the target sequence to be detected and this sequence also includes a recognition sequence for the nicking endonuclease. If the sample contains the target sequence, the probe hybridizes to the target and is cleaved by the nicking endonuclease, which leaves the target intact. Observing the presence of probe cleaved by the nicking endonuclease indicates the presence of the target nucleotide sequence in the sample of DNA.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/00527

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 19/34; C12Q 1/68 (2010.01)
USPC - 435/91.2; 435/6
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; C12Q 1/68 (2010.01)
USPC - 435/91.2; 435/6

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)
PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: Nicking endonuclease, NEase, N.BstNBI, N.AlwI, Nicking, endonuclease, DNA probe, cleavage, reverse transcribe, complementary DNA, amplify, hybridization, displacement amplification, multiple, viral RNA,genome, pathogen, plurality, unpurified contaminant, biological sample, cDNA, simultaneous, strain.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KIESLING et al. Sequence specific detection of DNA using nicking endonuclease signal amplification (NESA). Nucleic Acids Res. 2007, 35(18):e117; pg 1-9; Abstract; pg 1, col 2, last para; pg 2, col 1, top para, and col 2, top para; pg 3, col 1, last para, col 2, last para, and Fig 1; pg 6, Fig 5	1-4
Y	US 2006/0259249 A1 (SAMPATH et al.) 16 November 2006 (16.11.2006) para [0062], [0070], [0082], [0084], [0099], [0120], [0124], and [0270]	1-4
A	HIGGINS et al. The nicking endonuclease N.BstNBI is closely related to Type IIs restriction endonucleases MlyI and PleI. Nucleic Acids Res. 2001, 29(12):2492-2501	1-4
A	HOSODA et al. A novel sequence-specific RNA quantification method using nicking endonuclease, dual-labeled fluorescent DNA probe, and conformation-interchangeable oligo-DNA. RNA 2008, 14(3):584-592	1-4

☐ 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
04 August 2010 (04.08.2010)	11 AUG 2010
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: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/00527

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:

Group I: claims 1-5, drawn to a method for detecting the presence of an RNA sequence in a sample of biological material, said method comprising: (a) performing a reverse transcription procedure capable of reverse transcribing RNA into a complementary DNA nucleotide,
(b) performing multiple displacement amplification to amplify DNA in the sample of biological material to form an amplified sample product; (c) exposing all or part of the amplified sample product to a DNAprobe and a nicking endonuclease under conditions that would permit sequence-specific hybridization of the probe to a complementary target sequence, wherein the probe comprises a sequence complementary to a unique sequence known to be present in the transcript of the RNA sequencethat also includes a recognition sequence for the nicking endonuclease; and, (d) observing whether the probe is cleaved by the nicking endonuclease, wherein the presence of probe cleaved by the nicking endonuclease indicates the presence of the RNA sequence in the sample of biological material.
[NOTE 1: Claim 5 was excluded from group I as being drawn to a non-elected subject matter.]

**********Continued in the extra 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.:
1-4

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.

********** Supplemental Box **********

Continuation of: Box No. III (unity of invention is lacking)

Group II, claims 6-11, method for detecting the presence of a target nucleotide sequencein a sample of DNA comprising:
(a) exposing a test sample comprising single stranded DNA to a nicking endonuclease and a substrate surface onto which a DNA probe is affixed under conditions that would permit sequence-specific hybridization of the probe to a complementary target sequence, wherein the probecomprises a sequence complementary to the target sequence that alsoincludes a recognition sequence for the nicking endonuclease; and,
(b) observing whether the probe is cleaved by the nicking endonuclease, wherein the presence of probe cleaved by the nicking endonuclease indicates the presence of the target nucleotide sequence inthe sample DNA.
NOTE 2: Claim 5 was added to Group II to correct its mistaken placement into Group I.

Group III, claims 12-17, drawn to a DNA probe comprising a sequence complementary to a unique sequence of a target DNA molecule that also includes a recognition sequence for a nicking endonuclease, a fluorescent tag, and a fluorescence quencher, the tag and quencher being located on different sides of the recognition sequence for the nicking endonuclease, a first stem portion ofthe probe being capable of hybridizing to a second stem portion of the probe unless the probe is cleaved at a cut site of the nicking endonuclease, the first and second stem portions being separated by a loop portion, the tag and quencher being located in the probe such that the quencher is effective toquench fluorescent emissions of the tag when the stem portions are hybridized to each other, and its use for detecting the presence of a target nucleotide sequence in a sample of DNA.

Group IV, claims 18-24, drawn to a substrate comprising a unique surface onto which a DNA probe is affixed, wherein the DNA probe comprises a sequence complementary to a sequence of a target molecule sequence that includes a recognition sequence for a nicking endonuclease.

Group V+, claim 25, drawn to a kit for detection and/or identification of a dengue virus, said kit comprises a polynucleotide(s) of a specific sequence(s).

Group VI, claim 26, drawn to a method for making one or more NESA probes for the detection of a pathogen genome.

The inventions listed as Groups I-VI 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, IV-VI do not include the inventive concept of a DNA probe that also includes a fluorescent tag, and a fluorescence quencher, the tag and quencher being located on different sides of the recognition sequence for the nicking endonuclease, a first stem portion of the probe being capable of hybridizing to a second stem portion of the probe unless the probe is cleaved at a cut site of the nicking endonuclease, the first and second stem portions being separated by a loop portion, the tag and quencher being located in the probe such that the quencher is effective to quench fluorescent emissions of the tag when the stem portions are hybridized to each other, as required by Group III.

The inventions of Groups I-V+ do not include the inventive concept of a method for making one or more NESA probes for the detection of a pathogen genome, as required by group VI.

The inventions of Groups I-IV and VI do not include the inventive concept of a kit for detection and/or identification of a dengue virus comprising a specific sequence(s), as required by group V+.

The inventions of Group II do not include the inventive concept of (a) performing a reverse transcription procedure capable of reverse transcribing RNA into a complementary DNA nucleotide,(b) performing multiple displacement amplification to amplify DNA in the sample of biological material to form an amplified sample product, as required by Group I.

The inventions of Group I-III share the technical feature of a method for detecting the presence of a target nucleotide sequence in a sample of DNA comprising:
(a) exposing a test sample comprising single stranded DNA to a nicking endonuclease and a substrate surface onto which a DNA probe is affixed under conditions that would permit sequence-specific hybridization of the probe to a complementary target sequence, wherein the probe comprises a sequence complementary to the target sequence that also includes a recognition sequence for the nicking endonuclease; and,
(b) observing whether the probe is cleaved by the nicking endonuclease, wherein the presence of probe cleaved by the nicking endonuclease indicates the presence of the target nucleotide sequence in the sample DNA. However, this shared technical feature does not represent a contribution over prior art. Specifically, an article entitled 'The nicking endonuclease N.BstNBI is closely related to Type IIs restriction endonucleases MlyI and PleI' to Higgins et al. (hereinafter 'Higgins') that discloses a method for detecting the presence of an DNA sequence in a sample of biological material (Abstract - 'a new method for identifying specific single- or double-stranded DNA sequences called nicking endonuclease signal amplification (NESA)'), the method comprising:
(b) performing multiple displacement amplification to amplify DNA in the sample of biological material to form an amplified sample product (Abstract - 'B. anthracis genomic DNA can be detected and specifically differentiated from DNA of other Bacillus species. When combined with multiple displacement amplification'; pg 2, col 2, top para - 'Genomic DNA was amplified by multiple displacement amplification (MDA)'; pg 6, Fig 5);
(c) exposing all or part of the amplified sample product to a DNA probe and a nicking endonuclease under conditions that would permit sequence-specific hybridization of the probe to a complementary target sequence (pg 3, col 1, last para - 'The method involves the hybridization of a complementary oligonucleotide probe to the target DNA'),
--- wherein the probe comprises a sequence complementary to a unique sequence known to be present in the transcript of the DNA sequence that also includes a recognition sequence for the nicking endonuclease (pg 2, col 1, top para - 'We have used the single-strand cleavage activities of Nt.Alw1 to develop a sensitive method for detecting the presence of unique DNA sequences that contain a nicking endonuclease recognition site. The presence of a restriction site within the probe increases specificity since hybridization alone is not enough for enzyme recognition'; pg 3, Fig 1A); and,

********** Continued in the next Supplemental Box **********

********** Supplemental Box **********

Continuation of: The previous Supplemental Box and Box No III (unity of invention is lacking)

(d) observing whether the probe is cleaved by the nicking endonuclease, wherein the presence of probe cleaved by the nicking endonuclease indicates the presence of the DNA sequence in the sample of
biological material (pg 1, col 2, last para - 'We have developed a hybridizationbased nucleic acid detection method that is specific and, when combined with multiple displacement amplification, is sensitive and tolerant to contaminants commonly found in biological samples'; pg 3, col 2, last para - 'NESA cleaves the probe into two pieces, one of which is fluorescently labeled. Since the resulting pieces are smaller than the full-length probe, the rate of the reaction can be measured by any method that can both detect fluorescent molecules and distinguish such oligonucleotides by size'; pg 3, Fig 3B). As said method was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The inventions of Groups I-III do not include the inventive concept of a substrate comprising a surface onto which a DNA probe is affixed, wherein the DNA probe comprises a sequence complementary to a unique sequence of a target molecule sequence that includes a recognition sequence for a nicking endonuclease, as required by group IV.

The inventions of Group I-IV share the technical feature of a DNA probe comprising a sequence complementary to a unique sequence of a target DNA molecule. However, this shared technical feature does not represent a contribution over prior art. Specifically, Higgins discloses a DNA probe (pg 3, col 1, last para - 'The method involves the hybridization of a complementary oligonucleotide probe to the target DNA'),
said probe comprises a sequence complementary to a unique sequence known to be present in the transcript of the DNA sequence that also includes a recognition sequence for the nicking endonuclease (pg 2, col 1, top para - 'We have used the single-strand cleavage activities of Nt.Alw1 to develop a sensitive method for detecting the presence of unique DNA sequences that contain a nicking endonuclease recognition site. The presence of a restriction site within the probe increases specificity since hybridization alone is not enough for enzyme recognition'; pg 3, Fig 1A). As said probe was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The special technical feature of the inventions listed as Group V+ is the specific nucleic acid sequence recited therein. The inventions do not share a special technical feature, because 1) no significant structural similarities can readily be ascertained among the amino acid sequences, 2) US 2006/ 0062803 A1 to Kinney, et al., in the context of avirulent, immunogenic flavivirus chimeras, discloses a nucleotide comptising the claimed first nucleotide of claim 25, i.e. the claimed SEQ ID NO:1 (Kinney, et al., SEQ ID NO 7, nucleotides 1689-1710). Without a shared special technical feature, the inventions lack unity with one another.

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