(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 January 2014 (03.01.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/004852 A3

(51) International Patent Classification:
C12P 19/34 (2006.01)

(21) International Application Number:
PCT/US2013/048229

(22) International Filing Date:
27 June 2013 (27.06.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
13/538,955 29 June 2012 (29.06.2012) US
13/538,962 29 June 2012 (29.06.2012) US

(71) Applicant: **GENERAL ELECTRIC COMPANY** [US/US]; 1 River Road, Schenectady, New York 12345 (US).

(72) Inventors: **NELSON, John Richard**; One Research Circle, Niskayuna, New York 12309 (US). **DUTHIE, Robert Scott**; One Research Circle, Niskayuna, New York 12309 (US). **GROSSMANN, Gregory Andrew**; One Research Circle, Niskayuna, New York 12309 (US). **HELLER, Ryan Charles**; One Research Circle, Niskayuna, New York 12309 (US).

(74) Agents: **KOHLI, Vineet** et al.; GE Healthcare Bio-Sciences Corp., 800 Centennial Avenue, Piscataway, New Jersey 08855 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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))*

[Continued on next page]

(54) Title: KIT FOR ISOTHERMAL DNA AMPLIFICATION STARTING FROM AN RNA TEMPLATE

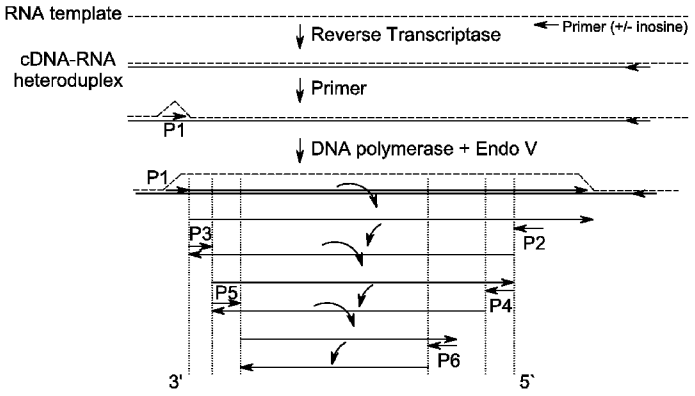


FIG. 1

(57) Abstract: A method of amplifying RNA template is provided. The method comprises reverse-transcribing a ribonucleic acid (RNA) template to form a cDNA using a first reaction mixture comprising RNA template, at least one primer capable of hybridizing to the RNA template, a reverse transcriptase and deoxynucleoside triphosphates (dNTPs); and amplifying the cDNA to form an amplified product using a second reaction mixture comprising at least one strand displacement DNA polymerase, at least one inosine-containing primer and a nuclease that is capable of nicking DNA 3' to an inosine residue of the primer. The method is accomplished under an isothermal condition without denaturing the cDNA template. A method of quantifying RNA template in a sample and a method of detecting RNA template in a sample are also provided. Kits for amplifying deoxynucleic acid (DNA) from ribonucleic acid (RNA) template are also provided.

WO 2014/004852 A3

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
24 April 2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/48229

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 19/34 (2014.01)
USPC - 435/91.2, 91.1, 89, 85, 84, 72, 41
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 (2014.01)
USPC: 435/91.2, 91.1, 89, 85, 84, 72, 41

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)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Patent/Google Scholar; PubMed; ProQuest; Search Terms: Amplifying; 'Nucleic acid'; 'Reverse transcribing'; 'Ribonucleic acid'; RNA; cDNA; 'Complementary Deoxyribonucleic acid'; 'Isothermal conditions'; pyrophosphate

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 2009/0011472 A1 (NELSON, JR et al.) January 8, 2009; abstract; paragraphs [0005], [0006], [0007], [0008], [0009], [0010], [0011], [0012], [0013], [0021], [0037], [0039], [0042], [0046], [0053], [0055], [0056], [0057], [0058], [0061], [0066], [0068], [0071], [0082], [0086], [0161]	1-3, 5-9, 11, 13-30, 32, 34-49, 53-57 ----- 4, 10, 12, 31, 33, 50-52, 58
Y	US 2012/0021461 A1 (MILLAR, DS et al.) January 26, 2012; paragraphs [0026], [0027], [0033], [0049]	4, 10, 31, 33, 52
Y	US 2006/0188893 A1 (KUMAR, G et al.) August 24, 2006; paragraphs [0089], [0163], [0164], [0220]	12, 51, 58
Y	Fluorescent Detection Reagent User Manual [online]. Mast Diagnostica. 2005 [retrieved on 2014-02-13]. Retrieved from the Internet; <URL: http://www.mast-diagnostica.com/MASTD_dt/PCR-Fly-67LMXXXX-Loopamp-Fluorescence-e.pdf>; page 2, paragraphs 1-2.	50

☐ 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
14 February 2014 (14.02.2014)	03 MAR 2014
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: Shane Thomas 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/US13/48229

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☒ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/48229

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.:

Groups I+: Claims 1-58, SEQ ID NO: 1

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/US13/48229

-***-Continuation of 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 1-58 and SEQ ID NO: 1 (endonuclease V nucleic acid sequence) are directed toward a method for amplifying a nucleic acid molecule comprising: reverse-transcribing a ribonucleic acid (RNA) template to form a cDNA using a first reaction mixture comprising RNA template, at least one primer capable of hybridizing to the RNA template, a reverse transcriptase and deoxynucleoside triphosphates (dNTPs); and amplifying the cDNA to form an amplified product using a second reaction mixture comprising at least one strand displacement DNA polymerase, at least one inosine-containing primer and a nuclease that is capable of nicking DNA 3' to an inosine residue of the primer; wherein the method is accomplished under an isothermal condition without denaturing the cDNA template; a method of quantifying a ribonucleic acid (RNA) template from a sample comprising: reverse-transcribing the RNA to form a cDNA using a first reaction mixture comprising RNA template, at least one primer capable of hybridizing to the RNA template, a reverse transcriptase and deoxynucleoside triphosphates (dNTPs); and amplifying the cDNA to form an amplified product using a second reaction mixture comprising at least one strand displacement DNA polymerase, at least one inosine-containing primer and a nuclease that is capable of nicking DNA 3' to an inosine residue of the primer; analyzing a rate of synthesis of the amplification product to quantify the RNA template present in the sample, wherein the reverse transcribing and amplifying are achieved under isothermal condition without denaturing the cDNA template; a method of quantifying a ribonucleic acid (RNA) template from a sample comprising: reverse-transcribing the RNA to form a cDNA using a first reaction mixture comprising RNA template, at least one primer capable of hybridizing to the RNA template, a reverse transcriptase and deoxynucleoside triphosphates (dNTPs); and amplifying the cDNA to form an amplification product using a second reaction mixture comprising at least one strand displacement DNA polymerase, at least one inosine-containing primer and a nuclease that is capable of nicking DNA 3' to an inosine residue of the primer; and detecting the amplification product using a nucleic acid detection system, wherein the reverse transcribing and amplifying are achieved under isothermal condition without denaturing the cDNA template; a method of amplifying a ribonucleic acid (RNA) template, comprising: reverse-transcribing the RNA template to cDNA; and amplifying the cDNA to form an amplified product without denaturing the cDNA, wherein the reverse transcription and amplification occurs under an isothermal condition and in a single reaction mixture comprising the RNA template, at least one primer for capable of hybridizing to RNA, a reverse transcriptase, a strand displacement DNA polymerase, deoxyribonucleoside triphosphates (dNTPs), and at least one inosine-containing primer and a nuclease that is capable of nicking DNA 3' to an inosine residue of the primer; a kit for amplifying a deoxyribonucleic acid (DNA) from a ribonucleic acid (RNA) template, comprising: at least one inosine-containing primer; and at least one enzyme possessing: reverse transcriptase activity, strand displacement DNA polymerase activity, nuclease activity for nicking DNA 3' to an inosine residue of the primer or a combination thereof; a kit for amplifying deoxyribonucleic acids (DNA) from a ribonucleic acid (RNA) template, comprising: at least one inosine-containing primer; an enzyme comprising reverse transcriptase activity and strand displacement DNA polymerase activity, and a nuclease for nicking DNA 3' to an inosine residue of the primer; and a kit for amplifying deoxyribonucleic acids (DNA) from a ribonucleic acid (RNA) template, comprising: at least one inosine-containing primer; a reverse transcriptase; a strand displacement DNA polymerase; and a nuclease for nicking DNA 3' to an inosine residue of the primer; and a kit for quantifying a ribonucleic acid (RNA) template from a sample comprising: at least one inosine-containing primer, a reverse transcriptase, a strand displacement DNA polymerase; a nuclease for nicking DNA 3' to an inosine residue of the primer; and a nucleic acid detection system.

The method for amplifying a nucleic acid molecule; method of quantifying a ribonucleic acid (RNA) template from a sample; method of amplifying a ribonucleic acid (RNA) template; and kits for amplifying a deoxyribonucleic acid (DNA) from a ribonucleic acid (RNA) template will be searched to the extent that they encompass SEQ ID NO: 1 (endonuclease V nucleic acid sequence). It is believed that claims 1-58 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass this sequence. Applicants must indicate, if applicable, the claims which encompass the first named invention if different than what was indicated above for this group. 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. Additional SEQ ID NOs can be searched upon the payment of additional fees. An Exemplary Election would be: SEQ ID NO: 2 (endonuclease V nucleic acid sequence).

Groups I+ share the technical features including a method for amplifying a nucleic acid molecule comprising: reverse-transcribing a ribonucleic acid (RNA) template to form a cDNA using a first reaction mixture comprising RNA template, at least one primer capable of hybridizing to the RNA template, a reverse transcriptase and deoxynucleoside triphosphates (dNTPs); and amplifying the cDNA to form an amplified product using a second reaction mixture comprising at least one strand displacement DNA polymerase, at least one inosine-containing primer and a nuclease that is capable of nicking DNA 3' to an inosine residue of the primer; wherein the method is accomplished under an isothermal condition without denaturing the cDNA template; a method of quantifying a ribonucleic acid (RNA) template from a sample comprising: reverse-transcribing the RNA to form a cDNA using a first reaction mixture comprising RNA template, at least one primer capable of hybridizing to the RNA template, a reverse transcriptase and deoxynucleoside triphosphates (dNTPs); and amplifying the cDNA to form an amplified product using a second reaction mixture comprising at least one strand displacement DNA polymerase, at least one inosine-containing primer and a nuclease that is capable of nicking DNA 3' to an inosine residue of the primer; analyzing a rate of synthesis of the amplification product to quantify the RNA template present in the sample, wherein the reverse transcribing and amplifying are achieved under isothermal condition without denaturing the cDNA template; a method of quantifying a ribonucleic acid (RNA) template from a sample comprising: reverse-transcribing the RNA to form a cDNA using a first reaction mixture comprising RNA template, at least one primer capable of hybridizing to the RNA template, a reverse transcriptase and deoxynucleoside triphosphates (dNTPs); and amplifying the cDNA to form an amplification product using a second reaction mixture comprising at least one strand displacement DNA polymerase, at least one inosine-containing primer and a nuclease that is capable of nicking DNA 3' to an inosine residue of the primer; and detecting the amplification product using a nucleic acid detection system, wherein the reverse transcribing and amplifying are achieved under isothermal condition without denaturing the cDNA template; a method of amplifying a ribonucleic acid (RNA) template, comprising: reverse-transcribing the RNA template to cDNA; and amplifying the cDNA to form an amplified product without denaturing the cDNA, wherein the reverse transcription and amplification occurs under an isothermal condition and in a single reaction mixture comprising the RNA template, at least one primer for capable of hybridizing to RNA, a reverse transcriptase, a strand displacement DNA polymerase, deoxyribonucleoside triphosphates (dNTPs), and at least one inosine-containing primer and a nuclease that is capable of nicking DNA 3' to an inosine residue of the primer; a kit for amplifying a deoxyribonucleic acid (DNA) from a ribonucleic acid (RNA) template, comprising: at least one inosine-containing primer; and at least one enzyme possessing: reverse transcriptase activity, strand displacement DNA polymerase activity, nuclease activity for nicking DNA 3' to an inosine residue of the primer or a combination thereof; ... Continued on Next Supplemental Page ...

-***-Continued Within the Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page:

... Continued from Previous Supplemental Page ... a kit for amplifying deoxyribonucleic acids (DNA) from a ribonucleic acid (RNA) template, comprising: at least one inosine-containing primer; an enzyme comprising reverse transcriptase activity and strand displacement DNA polymerase activity, and a nuclease for nicking DNA 3' to an inosine residue of the primer; and a kit for amplifying deoxyribonucleic acids (DNA) from a ribonucleic acid (RNA) template, comprising: at least one inosine-containing primer; a reverse transcriptase; a strand displacement DNA polymerase; and a nuclease for nicking DNA 3' to an inosine residue of the primer; and a kit for quantifying a ribonucleic acid (RNA) template from a sample comprising: at least one inosine-containing primer, a reverse transcriptase, a strand displacement DNA polymerase; a nuclease for nicking DNA 3' to an inosine residue of the primer; and a nucleic acid detection system.

However, these shared technical features are previously disclosed by US 2009/0011472 A1 to Nelson, et al. (hereinafter 'Nelson') in view of US 2012/0021461 A1 to Millar, et al (hereinafter 'Millar'). Nelson discloses a method for amplifying a nucleic acid molecule (a method for amplifying a nucleic acid molecule; abstract) comprising: a ribonucleic acid (RNA) template (RNA amplicon (RNA template); paragraph [0037]) using a first reaction mixture (reaction mixture; paragraph [0071]) comprising RNA template (RNA amplicon (RNA template); paragraph [0037]), at least one primer (primer; paragraph [0046]) capable of hybridizing to RNA (capable of hybridizing to RNA; paragraph [0046]), a DNA polymerase (a DNA polymerase; paragraph [0056]) and deoxynucleoside triphosphates (dNTPs) (deoxyribonucleoside triphosphates (dNTPs); paragraphs [0042], [0068]); and amplifying the DNA to form an amplified product (amplifying the DNA to form an amplified product; paragraph [0056]) using a reaction mixture (reaction mixture; paragraph [0071]) comprising at least one strand displacement DNA polymerase (a strand displacement DNA polymerase; paragraph [0056]), deoxyribonucleoside triphosphates (dNTPs) (deoxyribonucleoside triphosphates (dNTPs); paragraphs [0042], [0068]), at least one inosine-containing primer (inosine-containing primer; paragraph [0037]) and a nuclease (endonuclease V (a nuclease); paragraph [0055]) that is capable of nicking DNA 3' to an inosine residue of the primer (capable of nicking DNA 3' to an inosine residue of the primer; paragraph [0056]) wherein the amplification occurs under an isothermal condition (reaction temperatures can be held at 46 degrees C (wherein the amplification occurs under an isothermal condition); paragraph [0058]); a method of quantifying (quantify; paragraph [0061]) a ribonucleic acid (RNA) template (RNA amplicon (RNA template); paragraph [0037]) from a sample (sample; paragraph [0039]) comprising: using a reaction mixture (reaction mixture; paragraph [0071]) comprising RNA template (RNA amplicon (RNA template); paragraph [0037]), at least one primer capable of hybridizing to the RNA template (capable of hybridizing to RNA; paragraph [0046]), a DNA polymerase (a DNA polymerase; paragraph [0056]) and deoxynucleoside triphosphates (dNTPs) (deoxyribonucleoside triphosphates (dNTPs); paragraphs [0042], [0068]); and amplifying the DNA to form an amplified product (amplifying the DNA to form an amplified product; paragraph [0056]) using a reaction mixture (reaction mixture; paragraph [0071]) comprising at least one strand displacement DNA polymerase (a strand displacement DNA polymerase; paragraph [0056]), deoxyribonucleoside triphosphates (dNTPs) (deoxyribonucleoside triphosphates (dNTPs); paragraphs [0042], [0068]), at least one inosine-containing primer (inosine-containing primer; paragraph [0037]) and a nuclease (endonuclease V (a nuclease); paragraph [0055]) that is capable of nicking DNA 3' to an inosine residue of the primer (capable of nicking DNA 3' to an inosine residue of the primer; paragraph [0056]), wherein the amplification occurs under an isothermal condition (reaction temperatures can be held at 46 degrees C (wherein the amplification occurs under an isothermal condition); paragraph [0058]); a method of quantifying (quantify; paragraph [0061]) a ribonucleic acid (RNA) template (RNA amplicon (RNA template); paragraph [0037]) from a sample (sample; paragraph [0039]) comprising: amplifying (producing an amplification product (amplifying); paragraph [0005]) the RNA (RNA amplicon (RNA template); paragraph [0037]) using a reaction (reaction mixture; paragraph [0071]) comprising RNA template (RNA amplicon (RNA template); paragraph [0037]), at least one primer capable of hybridizing to the RNA template (capable of hybridizing to RNA; paragraph [0046]), a DNA polymerase (a DNA polymerase; paragraph [0056]) and deoxynucleoside triphosphates (dNTPs) (deoxyribonucleoside triphosphates (dNTPs); paragraphs [0042], [0068]); and amplifying the DNA to form an amplified product (amplifying the DNA to form an amplified product; paragraph [0056]) using a reaction mixture (reaction mixture; paragraph [0071]) comprising at least one strand displacement DNA polymerase (a strand displacement DNA polymerase; paragraph [0056]), deoxyribonucleoside triphosphates (dNTPs) (deoxyribonucleoside triphosphates (dNTPs); paragraphs [0042], [0068]), at least one inosine-containing primer (inosine-containing primer; paragraph [0037]) and a nuclease (endonuclease V (a nuclease); paragraph [0055]) that is capable of nicking DNA 3' to an inosine residue of the primer (capable of nicking DNA 3' to an inosine residue of the primer; paragraph [0056]; and detecting the amplification product (detecting the amplification product; paragraph [0021]) using a nucleic acid detection system (nucleic acid detection scheme (detection system); paragraph [0021]), wherein the amplification occurs under an isothermal condition (reaction temperatures can be held at 46 degrees C (wherein the amplification occurs under an isothermal condition); paragraph [0058]); a method of amplifying (method of producing an amplification product (amplifying); paragraph [0005]) a ribonucleic acid (RNA) template (RNA amplicon (RNA template); paragraph [0037]), comprising: amplifying the DNA to form an amplified product (amplifying the DNA to form an amplified product; paragraph [0056]), wherein the amplification occurs under an isothermal condition (reaction temperatures can be held at 46degrees C (wherein the amplification occurs under an isothermal condition); paragraph [0058]) and in a reaction mixture (reaction mixture; paragraph [0071]) comprising the RNA template, (RNA amplicon (RNA template); paragraph [0037]), at least one primer (primer; paragraph [0046]) capable of hybridizing to RNA (capable of hybridizing to RNA; paragraph [0046]), a strand displacement DNA polymerase (a strand displacement DNA polymerase; paragraph [0056]), deoxyribonucleoside triphosphates (dNTPs) (deoxyribonucleoside triphosphates (dNTPs); paragraphs [0042], [0068]), at least one inosine-containing primer (inosine-containing primer; paragraph [0037]) and a nuclease (endonuclease V (a nuclease); paragraph [0055]) that is capable of nicking DNA 3' to an inosine residue of the primer (capable of nicking DNA 3' to an inosine residue of the primer; paragraph [0056]); a kit (kit; paragraph [0005]) for amplifying a deoxyribonucleic acid (DNA) (for amplifying a deoxyribonucleic acid (DNA); paragraph [0005]) from a ribonucleic acid (RNA) template (RNA amplicon (RNA template); paragraph [0037]), comprising: at least one inosine-containing primer (inosine-containing primer; paragraph [0037]); and at least one enzyme possessing: reverse transcriptase activity, strand displacement DNA polymerase activity (a strand displacement DNA polymerase; paragraph [0056]), nuclease activity (endonuclease V (nuclease activity); paragraph [0055]) for nicking DNA 3' to an inosine residue of the primer (for nicking DNA 3' to an inosine residue of the primer; paragraph [0056]) or a combination thereof; a kit (kit; paragraph [0005]) for amplifying a deoxyribonucleic acid (DNA) (for amplifying a deoxyribonucleic acid (DNA); paragraph [0005]) from a ribonucleic acid (RNA) template (RNA amplicon (RNA template); paragraph [0037]), comprising: at least one inosine-containing primer (inosine-containing primer; paragraph [0037]); a strand displacement DNA polymerase (a strand displacement DNA polymerase; paragraph [0056]), and a nuclease (endonuclease V (a nuclease); paragraph [0055]) for nicking DNA 3' to an inosine residue of the primer (for nicking DNA 3' to an inosine residue of the primer; ... Continued on Next Supplemental Page ...

-***-Continued on Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page-***-

... Continued from Previous Supplemental Page ... a kit (kit; paragraph [0005]) for amplifying a deoxyribonucleic acid (DNA) (for amplifying a deoxyribonucleic acid (DNA); paragraph [0005]) from a ribonucleic acid (RNA) template (RNA amplicon (RNA template), comprising: at least one inosine-containing primer (inosine-containing primer; paragraph [0037]); a strand displacement DNA polymerase (a strand displacement DNA polymerase; paragraph [0056]), and a nuclease (endonuclease V (a nuclease); paragraph [0055]) for nicking DNA 3' to an inosine residue of the primer (for nicking DNA 3' to an inosine residue of the primer; paragraph [0056]); and a kit (kit; paragraph [0005]) for quantifying (quantifying; paragraph [0061]) a ribonucleic acid (RNA) (RNA amplicon (RNA template); paragraph [0037]) from a sample (sample; paragraph [0039]) comprising: at least one inosine-containing primer (inosine-containing primer; paragraph [0037]), (a strand displacement DNA polymerase; paragraph [0056]), and a nuclease (endonuclease V (a nuclease); paragraph [0055]) for nicking DNA 3' to an inosine residue of the primer (for nicking DNA 3' to an inosine residue of the primer; paragraph [0056]); and a nucleic acid detection system (nucleic acid detection scheme (nucleic acid detection system); paragraph [0021]).

Nelson does not disclose a method comprising: reverse-transcribing a ribonucleic acid (RNA) template to form a cDNA using a reverse transcriptase; and amplifying the cDNA using a second reaction mixture; analyzing a rate of synthesis of the amplification product to quantify the RNA template present in the sample, wherein the reverse transcribing is achieved under isothermal condition without denaturing the cDNA template: amplifying the cDNA to form an amplified product without denaturing the cDNA, wherein the reverse transcription and amplification occurs under an isothermal condition and in a single reaction mixture, a reverse transcriptase; a kit comprising an enzyme comprising reverse transcriptase activity and strand displacement DNA polymerase activity.

Millar discloses a reverse transcriptase (M-MuLV reverse transcriptase; paragraph [0033]), having strand-displacement activity (having strand displacement capability (activity); paragraph [0033]) reverse-transcribing an RNA template to cDNA (reverse-transcribing the RNA template to cDNA; paragraph [0049]), and amplifying the cDNA to form an amplified product (amplifying the cDNA to form an amplified product; paragraph [0049]) without denaturing the cDNA (isothermal amplification (without denaturing the cDNA); abstract, paragraph [0005]); and analyzing the amount of synthesis of the amplification product over time (figure 5).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the methods of Nelson, in order to have produced a method comprising: reverse-transcribing a ribonucleic acid (RNA) template to form a cDNA using a reverse transcriptase, as previously disclosed by Millar, with amplifying the cDNA using a second reaction mixture, based on the methods disclosed by Nelson, and using a DNA polymerase having a higher fidelity than a reverse transcriptase, for improving the accuracy in producing an amplification product. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the methods, as previously disclosed by Nelson, in order to have included analyzing a rate of synthesis of the amplification product from the amplification vs. time data collected by Millar (figure 5), for quantifying the RNA template present in the sample, according to the methods of Nelson, by performing the same measurement of the amount of product produced over time for each amplification cycle. Also, it would have been obvious to a person of ordinary skill in the art to have modified the methods of Nelson, in order to have conducted the reverse transcription and amplification under an isothermal condition, and in a single reaction mixture, based upon the use of a strand-displacing reverse transcriptase capable of polymerizing DNA, as previously disclosed by Millar (paragraph [0033]). In addition, it would have been obvious to a person of ordinary skill in the art to have modified a kit, as previously disclosed by Nelson, in order to have included an enzyme comprising reverse transcriptase activity and strand displacement DNA polymerase activity, such as M-MuLV reverse trancriptase, as previously disclosed by Millar (paragraph [0033]), for the isothermal amplification of an RNA template, and subsequent amplification of the cDNA, as previously disclosed by Millar (paragraph [0049]), when amplifying an RNA template from a sample, as previously disclosed by Nelson.

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 a combination of the Nelson and Millar references, unity of invention is lacking.