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(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, SA, 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.

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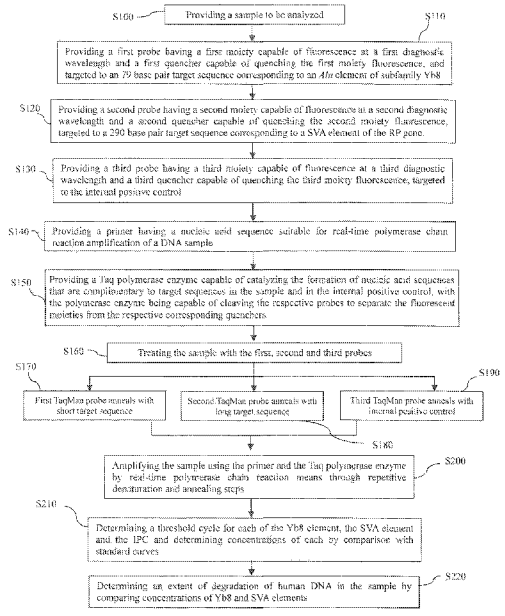
Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

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

[Continued on next page]

(54) **Title**: DEVELOPMENT OF A HIGHLY SENSITIVE QUANTIFICATION SYSTEM FOR ASSESSING DNA DEGRADATION AND QUALITY IN FORENSIC SAMPLES

Figure 1



(57) **Abstract**: A process of quantifying the extent of degradation present in a human DNA sample is described. The process makes use of a real time PGR system to separately quantitate within a sample a first, retrotransposon interspersed element and a relatively longer second retrotransposon interspersed element, where the longer element is expected to be disrupted at a faster pace than is the shorter element as the sample degrades. In one embodiment, the process makes use of the appearance of the relatively young (on an evolutionary scale) *Alu* Yb-lineage subfamily sequences appearing in every human genome and their virtual absence in non-human samples. In a preferred embodiment, the process quantifies longer 290 bp sequences of "SVA" elements and shorter 80 bp sequences of *Alu* Yb8-lineage. Newly designed primers and TaqMan probes that are useful in the process are presented. A related process additionally quantifies male specific human DNA.

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

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C07H 21/02; C12P 19/34 (2014.01)
USPC - 435/6.12, 91.21; 536/23.1
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) Classification(s): C12Q 1/68, 1/00; C07H 21/02, 21/04, 21/00; C12P 19/34, 19/30, 19/28, 19/26, 19/00 (2014.01)
USPC Classification(s): 435/6.12, 6.11, 6.1, 4, 91.21, 91.2, 91.1, 89, 85, 84, 72, 41; 536/23.1, 24.3, 22.1, 18.7, 1.11

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/Google Scholar; Pubmed/Pubmed central/NCBI Blast; UniProt; quantitating, DNA, 'nucleic acid', degradation, damage, SVA, element, AluYb8, assess, evaluate, determine, breakdown, real-time, PCR, qPCR, RT-PCR, retrotransposon, transposon

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	SWANGO, KL et al. A Quantitative PCR Assay for the Assessment of DNA Degradation in Forensic Samples. Forensic Sci Int. 03 June 2005, Vol. 158, No. 1, pp 14-26; abstract; page 14, second column, first paragraph; page 15, first column, second paragraph to second column, second paragraph; page 16, first column, second paragraph to second column, third paragraph; page 17, Table 2; page 18, first column, first paragraph; page 20, first column, second paragraph; page 22, second column, third paragraph DOI: 10.1016/j.forsciint.2005.04.034.	1-11, 14-16, 18, 22 -- 12, 13
Y -- A	HUDLOW, WR et al. A Quadruplex Real-time qPCR Assay For The Simultaneous Assessment Of Total Human DNA, Human Male DNA, DNA Degradation And The Presence of PCR Inhibitors In Forensic Samples: A Diagnostic Tool For STR Typing. Forensic Sci Int Genet. 26 November 2007, Vol. 2, No. 2, pp 108-125; page 110, first column, third paragraph. DOI: 10.1016/j.fsigen.2007.09.001	1, 17, 19-21, 23-28 -- 29, 30
Y	US 2008/0206755 A1 (SINHA, SK et al.) August 28, 2008; paragraphs [0006], [0032]-[0035]; Claims 6, 24	1-11, 14-28
Y	LIFE TECHNOLOGIES. Introduction To Gene Expression: Getting Started Guide. Manual [online]. Applied Biosystems by Life Technologies, May 2010; page 8, paragraphs 3-5; [retrieved on 21 March 2014]. Retrieved from internet: <URL: https://www.lifetechnologies.com/content/dam/LifeTech/migration/en/filelibrary/nucleic-acid-amplification-expression-profiling/pdfs.par.34366.file.dat/introduction%20to%20gene%20expression.pdf>.	5, 8-11
Y	WO 2007/100530 A2 (ANVERSA, P) September 7, 2007; page 91, lines 10-30	6, 26
Y	WO 2004/081186 A2 (CARGILL, M et al.) September 23, 2004; page 8, lines 6-13; page 15, line 25 to page 16, line 19	6, 26

☒ 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
21 March 2014 (21.03.2014)	14 APR 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/US13/54788

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/042920 A1 (HOLM, H et al.) April 14, 2011; page 13, lines 12-20	7
Y	US 2006/0199217 A1 (SINHA, SK et al.) September 7, 2006; paragraphs [0020]-[0022]	23-28
Y	US 2006/0099620 A1 (WALKER, J et al.) May 11, 2006; paragraphs [0011]-[0016]	26, 27
A	CALDWELL, R et al. Full-length cDNAs From Chicken Bursal Lymphocytes To Facilitate Gene Function Analysis. Genome Biol. 23 December 2004, Vol. 6, No. 1, pp R6.1-R6.9, Genbank supplement included; Genbank supplement, pages 1 and 2 Accession AJ721039. DOI:10.1186/gb-2004-6-1-r6	12, 13, 29
A	US 2009/0093620 A1 (KOVALIC, D et al.) April 9, 2009; SEQ ID NO: 295393	30
A	WO 2001/75067 A2 (DRMANAC, R et al.) October 11, 2001; SEQ ID NO: 8960	29

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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 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.:

Claims 1-30, SEQ ID NOs: 5-12, 16, 17, 20-22 and 24-27

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.:

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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-***-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 1-30 and SEQ ID NOs: 5-10, 16, 17, and 20; Exemplary Election: SEQ ID NO: 11, 12, 21, 22, and 24-27 are directed toward a process for quantitating human DNA in a sample in order to assess the extent of degradation of the DNA therein, the process comprising the steps of: providing a sample to be analyzed; using a real time polymerase chain reaction system to separately quantitate within the sample a first retrotransposon interspersed element and a second retrotransposon interspersed element, the first retrotransposon interspersed element being an Alu element, the second retrotransposon interspersed element being an SVA element of the RP gene; and calculating a ratio of an occurrence within the sample of the first retrotransposon interspersed element to an occurrence of the second retrotransposon interspersed element; and a process for quantitating total human DNA and male specific DNA in a sample in order to assess the extent of degradation of the DNA therein, the process comprising the steps of: (i) providing a sample to be analyzed; (ii) using a real time polymerase chain reaction system to separately quantitate within the sample a male specific DNA sequence, a first retrotransposon interspersed element and a second retrotransposon interspersed element, the male specific DNA sequence being a 90 bp Y-chromosome specific DNA sequence, the first retrotransposon interspersed element being an Alu element, the second retrotransposon interspersed element bein an SVA element of the RP gene; and (iii) calculating a ratio of an occurrence within the sample of the first retrotransposon interspersed element to an occurrence of the second retrotransposon interspersed element.

The process for quantitating human DNA or total human DNA in a sample in order to assess the extent of degradation of the DNA therein, the process comprising the steps of: providing a sample to be analyzed; using a real time polymerase chain reaction system to separately quantitate within the sample a first retrotransposon interspersed element and a second retrotransposon interspersed element will be searched to the extent it encompasses SEQ ID NOs: 5-10, 16, 17, and 20. It is believed that Claims 1-25 and 28 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass 5-10, 16, 17, and 20. 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: 11, 12, 21, 22, and 24-27.

Groups I+ share the technical features including a process for quantitating human DNA in a sample in order to assess the extent of degradation of the DNA therein, the process comprising the steps of: providing a sample to be analyzed; using a real time polymerase chain reaction system to separately quantitate within the sample a first retrotransposon interspersed element and a second retrotransposon interspersed element, the first retrotransposon interspersed element being an Alu element, the second retrotransposon interspersed element being an SVA element of the RP gene; and calculating a ratio of an occurrence within the sample of the first retrotransposon interspersed element to an occurrence of the second retrotransposon interspersed element; and a process for quantitating total human DNA and male specific DNA in a sample in order to assess the extent of degradation of the DNA therein, the process comprising the steps of: (i) providing a sample to be analyzed; (ii) using a real time polymerase chain reaction system to separately quantitate within the sample a male specific DNA sequence, a first retrotransposon interspersed element and a second retrotransposon interspersed element, the male specific DNA sequence being a 90 bp Y-chromosome specific DNA sequence, the first retrotransposon interspersed element being an Alu element, the second retrotransposon interspersed element bein an SVA element of the RP gene; and (iii) calculating a ratio of an occurrence within the sample of the first retrotransposon interspersed element to an occurrence of the second retrotransposon interspersed element.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Box-***-

However, these shared technical features are previously disclosed by US 2006/0199217 A1 to Sinha, et al. (hereinafter 'Sinha'217') in view of US 2008/0206755 A1 to Sinha, et al. (hereinafter 'Sinha'755').

Sinha'217 discloses a process for the quantitation of DNA in a sample (a process for the quantitation of DNA in a sample; paragraphs [0017]) the process comprising the steps of: providing a sample to be analyzed (providing a sample to be analyzed; paragraph [0019]), using a real time polymerase chain reaction system (using a real time polymerase chain reaction system; paragraph [0031]), to quantitate within the sample a first retrotransposon interspersed element, the first retrotransposon interspersed element being an Alu element (to quantitate within the sample a first retrotransposon interspersed element, the first retrotransposon interspersed element being an Alu element; paragraph [0022]) and comparing an occurrence within the sample of the first retrotransposon interspersed element to a standard (and comparing an occurrence within the sample of the first retrotransposon interspersed element to a standard; paragraph [0022]); and a process to separately quantitate (separately quantitate; paragraphs [0007], [0022]) within a sample (within a sample; paragraph [0022]) a first retrotransposon interspersed element, and a second retrotransposon interspersed element (AluSTYa and AluSTXa (a first retrotransposon interspersed element, and a second retrotransposon interspersed element); paragraph [0022]), and comparing the quantity within the sample of the retrotransposon interspersed elements to a standard (comparing the quantity within the sample of the retrotransposon interspersed elements to a standard; paragraph [0022]); and a process for quantitating total human DNA (a process for quantitating total human DNA; paragraph [0017]) and male specific DNA in a sample (and male specific DNA in a sample; paragraph [0022]), the process comprising the steps of: (i) providing a sample to be analyzed (providing a sample to be analyzed; paragraph [0019]); (ii) using a real time polymerase chain reaction system (using a real time polymerase chain reaction system; paragraph [0031]) to separately quantitate within the sample (to separately quantitate within the sample; paragraphs [0007], [0022]) a male specific DNA sequence (Y90 (a male specific DNA sequence; paragraph [0022]), a first retrotransposon interspersed element and a second retrotransposon interspersed element (a first retrotransposon interspersed element and a second retrotransposon interspersed element; paragraph [0022]), the male specific DNA sequence being a 90 bp Y-chromosome specific DNA sequence (Y90 (the male specific DNA sequence being a 90 bp Y-chromosome specific DNA sequence); paragraph [0022]), and the first retrotransposon interspersed element being an Alu element (AluSTXa, AluSTYa (the first retrotransposon interspersed element being an Alu element); paragraph [0022]).

Sinha'217 does not disclose in order to assess the extent of degradation of the DNA therein the second retrotransposon interspersed element being an SVA element of the RP gene, and calculating a ratio of an occurrence within the sample of the first retrotransposon interspersed element to an occurrence of the second retrotransposon interspersed element.

Sinha'755 discloses a process for testing for the amount of an interspersed genetic element (a process for testing for the amount of an interspersed genetic element; abstract) in degraded DNA (degraded DNA; paragraph [0067]) the retrotransposon interspersed element being an SVA element (retrotransposon interspersed element being an SVA element; paragraphs [0006]; Claim 24) of the RP gene (SINE, VNTR and Alu combination; paragraph [0006]; (see instant application, paragraph [0008] of the instant PCT application, regarding the components of the SVA element of the RP gene). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Sinha'217 regarding the quantitation of total human DNA, for providing a second measure of the quantity using a second interspersed element measurement, such as an SVA element, as disclosed by Sinha'755 as an internal control. 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 previous disclosure of Sinha'217 regarding the evaluation of the quantity of one or more interspersed elements in a sample by comparing the amounts to a control to assess the extent of degradation of DNA in a sample, based on the disclosure of Sinha'755, for varying amounts of detected elements from a plurality of elements, which would have been reflective of the degree of degradation of the sample, enabling an assessment of the amount of degradation of the DNA in the sample based on said comparative quantitation.

Moreover, although Sinha'217 does not disclose calculating a ratio of an occurrence within the sample of the first retrotransposon interspersed element to an occurrence of the second retrotransposon interspersed element, Sinha'217 does disclose the comparison of a quantity of an element from a sample to a control standard, and it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have utilized a ratio of the amounts of more than one quantitated interspersed elements in a sample as means of quantitatively determining the amount of a further assessed element, provided the previous disclosure by Sinha'217, and to have utlized the quantity of at least one interspersed element as an internal control for the quantitation and comparison of the amounts of elements within the sample, without undue experimentation or testing.

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 Sinha'217 and Sinha'755 references, unity of invention is lacking.