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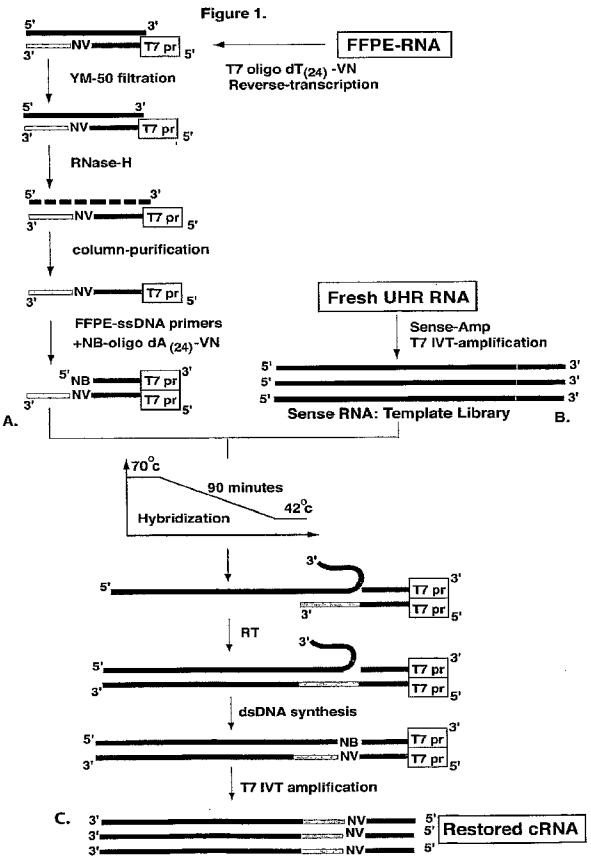
[Continued on next page]

(54) Title: RESTORATION OF NUCLEIC ACID FROM DEGRADED OR FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUE AND USES THEREOF



(57) Abstract: This invention provides methods, primers and kits for restoration of nucleic acid from tissue, in particular degraded tissue and formalin-fixed and paraffin-embedded (FFPE) tissue, where the methods involve complementary-template reverse-transcription (CT-RT) where short single-stranded DNA sequences reverse-transcribed from mRNA are used for reverse-transcription of complementary sense-RNA templates. The methods can be used to determine patterns of gene expression and chromosomal alterations in archived tissue samples, and may be used to identify expression of disease-related genes.

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FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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20 November 2008

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/04892

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **G06F 15/00**(2006.01)

USPC: 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)
U.S. : 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)
Google, Google Scholar, term searched - dTVn primer, 1st strand synthesis, column, capture

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Schlingemann J et al., Effective Transcriptome Amplication for Expression Profiling on Sense-oriented Oligonucleotide Microarrays Nucleic Acids Research, 2005, vol. 33, no. 5, e29, p. 1-12	1-6, 13-15, 52-68
Y	Phillips J et al., Antisense RNA Amplification: A Linear Amplification Method for Analyzing the mRNA Populaton from Single Living Cells. Methods: A companion to methods in enzymology, 1996, vol. 10, p. 283-288	1-6, 13-15, 52-68

☐ 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
31 August 2008 (31.08.2008)	19 SEP 2008
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 STEPHANIE K. MUMMERT Telephone No. 571-272-0872

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/04892

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.: 7-12,16-51,59,63-65 and 69-77
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.: 1-6,13-15 and 52-68

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.

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) 1-6, 13-15, 52-68 drawn to a method of restoring nucleic acid sequences recovered fragmented or degraded from a tissue.

Group II, claim(s) 78-80, drawn to a method of identifying the expression of disease-related genes in a subject.

Group III, claim(s) 81-93, drawn to a method of size exclusion and size selection of duplex DNA and RNA obtained from degraded or formalin-fixed and paraffin embedded tissues (FFPE) tissue.

Group IV, claim(s) 94-97, drawn to an oligonucleotide.

Group V, claim(s) 98-124, drawn to a pool of single stranded cDNA oligonucleotide primers.

Group VI, claim(s) 125-137, drawn to a kit for restoring nucleic acid from tissue.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

SEQ ID NO:17
SEQ ID NO:18
SEQ ID NO:19
SEQ ID NO:20.
SEQ ID NO:21
SEQ ID NO:22

The claims are deemed to correspond to the species listed above in the following manner:

Claims 90 and 110 correspond to SEQ ID NO:17
Claims 91 and 111 correspond to SEQ ID NO:18
Claim 94 corresponds to SEQ ID NO:19
Claim 95 corresponds to SEQ ID NO:20
Claim 115 corresponds to SEQ ID NO:21
Claim 116 corresponds to SEQ ID NO:22

The following claim(s) are generic: 1-6, 13-15, 52-68, 78-89, 92-93, 96-109, 112-114, 117-137

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 claims are not linked by a special technical feature which distinguishes over the prior art. The first broadest product, the pool of cDNA oligonucleotide primers of Group V as claimed comprising reverse transcription with random primers or 5' promoter-oligo-dT primers, followed by removal of the RNA from the RNA:DNA duplex and purifying the single stranded cDNA does not distinguish over the teaching of the prior art. Alexandersson et al. (Plant Molecular Biology, 2005, vol. 59, p. 469-484) in view of ProtoScript Manual, 2005 (obtained from New England Biolabs, p. 1-18) teaches first strand synthesis using random hexamer primers, followed by degradation of the RNA from the RNA/DNA duplex to yield a single stranded cDNA, followed by purification through amplification (see p. 473, col. 1, where cDNA was synthesized with oligo dT and then the template RNA was degraded by RNase H; see 5, where the oligo dT and random hexamer primers are used in first strand synthesis, followed by degradation using RNAse H and the first strand cDNA is processed). Therefore, the combination of Alexandersson in view of ProtoScript minimally renders the pool of cDNA of Group V obvious and therefore the claims do not share a special technical feature that distinguishes over the art.