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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 January 2008 (03.01.2008)

PCT

(10) International Publication Number
WO 2008/002740 A3

(51) International Patent Classification:
C12Q 1/68 (2006.01) *C12P 19/34* (2006.01)

(21) International Application Number:
PCT/US2007/069976

(22) International Filing Date: 30 May 2007 (30.05.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11/476,273 28 June 2006 (28.06.2006) US
11/476,269 28 June 2006 (28.06.2006) US

(71) Applicant *(for all designated States except US)*: **SIGMA-ALDRICH CO.** [US/US]; 3050 Spruce Street, St. Louis, Missouri 63103 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MICHALIK, Steve, S.** [US/US]; 3050 Spruce Street, St. Louis, Missouri 63103 (US). **WEBER, Scott, A.** [US/US]; 3050 Spruce Street, St. Louis, Missouri 63103 (US). **KREADER, Carol, A.** [US/US]; 3050 Spruce Street, St. Louis, Missouri 63103 (US).

(74) Agents: **HILMERT, Laura, J.** et al.; Senniger Powers, One Metropolitan Square, 16th Floor, St. Louis, Missouri 63102 (US).

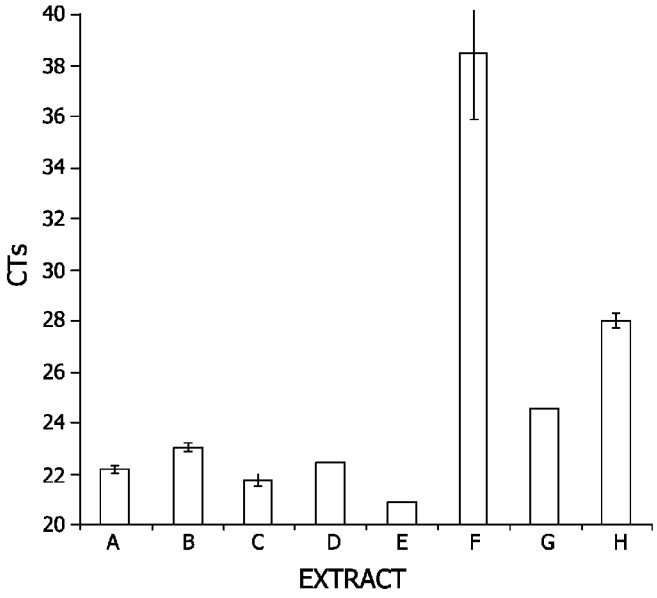
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, 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).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

[Continued on next page]

(54) **Title:** METHODS, COMPOSITIONS, AND KITS FOR RNA EXTRACTION



(57) **Abstract:** The present invention provides methods and compositions for extracting RNA from cells. The cellular extract may be directly used in a variety of reactions, such as reverse transcription and PCR.

WO 2008/002740 A3

— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(88) Date of publication of the international search report:
18 September 2008

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/69976

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C12Q 1/68**(2006.01);**C12P 19/34**(2006.01)

USPC: 435/6,91.5
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, 91.5

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	US 5,972,613 A (SOMACK et al) 26 October 1999 (26.10.1999), column 6, lines 35-46.	1-6
X --- Y	BRADY et al. Construction of cDNA libraries from single cells. Methods in Enzymology. September 1993, Vol. 225, pages 611-623, see entire document, especially pages 613-616.	1-6, 8-10, 13, 15-19 ---------- 21-31, 34, 36, 38
Y	WO 2004/104181 A2 (BRANDEIS UNIVERSITY) 2 December 2004 (02.12.2004), see entire document, especially pages 12 and 19-21.	1-6, 8, 10, 13-19, 21-23, 26, 27, 29-31, 33-36, 39, 40
Y	AHERN H. Biochemical, Reagent kits offer scientists good return on investment. The Scientist. July 1995, Vol. 9. No. 15, pages 20-24, see entire document.	21-31, 34, 36, 38

☒ 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 21 May 2008 (21.05.2008)	Date of mailing of the international search report 07 JUL 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 Angela Bertagna /CYNTHIA WILDER/ Patent Examiner, AU 1637 Telephone No. 571-272-1600

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/69976

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.:

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/US07/69976

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DULBECCO et al. Plaque formation and isolation of pure lines with poliomyelitis virus. Journal of Experimental Medicine. February 1954, Vol. 99. No. 2, pages 167-182, especially page 167.	1-6, 8, 10, 13-19, 21-23, 26, 27, 29-31, 33-36, 39, 40
Y	WEYANT et al. Effect of ionic and nonionic detergents on the Taq polymerase. BioTechniques. September 1990, Vol. 9. No. 3, pages 308-309, especially page 308.	1-6, 8, 10, 13-19, 21-23, 26, 27, 29-31, 33-36, 39, and 40
Y	GUT et al. One-tube fluorogenic reverse transcription-polymerase chain reaction for the quantitation of feline coronaviruses. Journal of Virological Methods. January 1999, Vol. 77. No. 1, pages 37-46, especially pages 41, 43, and 44.	11, 12
Y	US 5,310,652 A (GELFAND et al) 10 May 1994 (10.05.1994), column 3, line 65 - column 4, line 22.	7, 20, 37
Y	BASKARAN et al. Uniform amplification of a mixture of deoxyribonucleic acids with varying GC content. Genome Research. July 1996, Vol. 6, No. 7, pages 633-638, especially page 634.	32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/69976

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claim(s) 1-20, drawn to a method for RNA extraction and cDNA synthesis.

Group II, claim(s) 21-40, drawn to a kit comprising a detergent, a salt, and reverse transcriptase.

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 Somack et al. (US 5,972,613 A) teaches a method for extracting RNA from cells that comprises combining a cell population with an extraction medium to form a cellular extract containing extracted RNA, a salt, and a nonionic detergent (see column 6, lines 35-46). Somack teaches that the concentrations of the salt (NaCl) and nonionic detergent (Tween 20) in the extract are 200 mM and 2.5%, respectively (column 6, lines 35-41). These concentrations lie within the claimed ranges of about 10 mM to about 5 M (monovalent salt concentration) and about 0.1% to about 10% (amount of detergent by weight). Since Somack teaches all of the elements of 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:
patent databases (USPAT, USPGPUBS, EPO, JPO, DERWENT), medlince, caplus, embase, biosis
search terms: RNA, reverse transcriptase, detergent, NaCl, Tris