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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 June 2014 (26.06.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/100755 A3

(51) International Patent Classification:
A61K 38/54 (2006.01)

(21) International Application Number:
PCT/US2013/077290

(22) International Filing Date:
20 December 2013 (20.12.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/740,356 20 December 2012 (20.12.2012) US

(71) Applicant: **BIOMATRICA, INC.** [US/US]; 5627 Oberlin Drive, Suite 120, San Diego, CA 92121 (US).

(72) Inventors: **WHITNEY, Scott, E.**; 7615 Canyon Point Lane, San Diego, CA 92126 (US). **DE LOS RIOS, Stephanie**; 750 Kalamath Drive, Del Mar, CA 92014 (US). **GHIRMAI, Senait**; 5335 Repecho Drive, B102, San Diego, CA 92124 (US). **MULLER, Rolf**; 12744 Via Donada, Del Mar, CA 92014 (US). **LIBERAL, Vasco**; 12768 Via Nieve, San Diego, CA 92130 (US). **WILKINSON, Steven, P.**; 14542 Kittery Street, Poway, CA 92064 (US).

(74) Agent: **ELAMRANI, Samir**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304 (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, IR, 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.

(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).

Published:

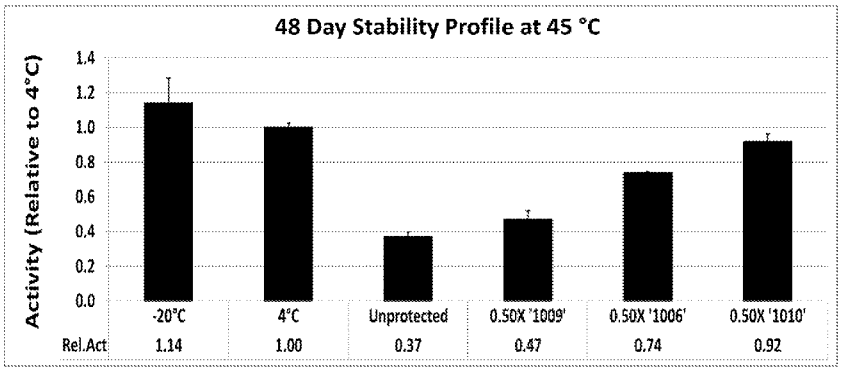
— *with international search report (Art. 21(3))*

— *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:
21 August 2014

(54) Title: FORMULATIONS AND METHODS FOR STABILIZING PCR REAGENTS

FIG. 8



(57) Abstract: Described herein are stabilized polymerase compositions comprising a polymerase and an polymerase stabilizing agent, such as a non-detergent zwitterionic stabilizer or a cationic ester disclosed, for use in nucleic acid amplification or nucleic acid sequencing. Compositions are provided for the stabilization of one or more polymerases in a single stabilized liquid formulation. Also disclosed are methods for making and using stabilized polymerase compositions and kits for nucleic acid amplification and sequencing comprising the stabilized polymerase compositions provided.

WO 2014/100755 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/077290

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/54 (2014.01)
USPC - 435/6.12
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) - A61K 38/54, 47/18; C12Q 1/68 (2014.01)
USPC - 424/94.3; 435/6.12; 436/18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 38/54, 47/186; C12Q 1/68 (2014.02)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Orbit, PubChem, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,787,305 B1 (LI et al) 07 September 2004 (17.09.2004) entire document	1, 2, 4, 8-10, 20-25, 28-30, 32-34
---		---
Y		3, 7, 11-15, 26
Y	GOWRISHANKAR, J. Osmoregulation in Enterobacteriaceae: Role of Proline/Betaine Transport Systems. Current Science, 57(5):225-234, 1988. entire document	3, 7
Y	US 2012/0142070 A1 (BATTRELL et al) 07 June 2012 (07.06.2012) entire document	11, 15
Y	NATALE et al. Sensitivity of bovine blastocyst gene expression patterns to culture environments assessed by differential display RT–PCR. Reproduction, 122:5:687-693, 2001. entire document	12, 13
Y	US 5,834,254 A (SHEN et al) 10 November 1998 (10.11.1998) entire document	14
Y	HENKE et al. Betaine improves the PCR amplification of GC-rich DNA sequences" Nucleic Acids Research. 25(19):3957–3958, 1997. entire document	26
A	US 2010/0099150 A1 (FANG et al) 22 April 2010 (22.04.2010) entire document	1-37
A	WO 99/67371 A1 (SHULTZ et al) 29 December 1999 (29.12.1999) entire document	1-37

☐ 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
05 June 2014	23 JUN 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: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/077290

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:

See 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.:
1-37

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.

<Continued from 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 need to be paid.

Group I: claims 1-37 are drawn to a liquid composition comprising a polymerase and storage stabilizing agent, wherein the storage stabilizing agent comprises a zwitterionic compound.

Group II: claims 38-49 are drawn to a liquid composition comprising a polymerase and storage stabilizing agent, wherein the storage stabilizing agent comprises a non-surfactant cationic ester.

Group III: claims 50-56 are drawn to a liquid composition comprising a polymerase and dipeptide zwitterion.

Group IV: claims 57-65 are drawn to a liquid composition comprising a polymerase and a cationic imidazolium compound.

The inventions listed in Groups I through IV 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 special technical features of Group I, liquid compositions wherein the storage stabilizing agent comprises a zwitterionic compound and does not contain sulfate and is a non-detergent zwitterionic compound, are not present in Groups II through IV; the special technical features of Group II, liquid compositions wherein the storage stabilizing agent comprises a non-surfactant cationic ester, are not present in Groups I, III, and IV; the special technical features of Group III, liquid compositions comprising a polymerase and dipeptide zwitterion, are not present in Groups I, II, and IV; and the special technical features of Group IV, liquid compositions comprising a polymerase and a cationic imidazolium compound, are not present in Groups I through III.

The Groups I through IV share the technical features of a liquid composition comprising a polymerase and storage sabilizing agent, wherein the storage stabilizing agent is a zwitterionic or cationic compound. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2010/0099150 to Fang et al. teach a liquid composition comprising a polymerase and storage sabilizing agent, wherein the storage stabilizing agent is a zwitterionic compound (Para. [0001], protein stabilization, particularly the stabilization of polymerases in aqueous solutions containing ionic, particularly zwitterionic detergents and an inert protein; Para. [0017], ...(a) an enzyme with nucleic acid polymerase activity, (b) an inert protein and, (c) an ionic detergent...).

Additionally, WO 1999/67371 to Shultz et al. teach a liquid composition comprising a polymerase and storage sabilizing agent, wherein the storage stabilizing agent is a cationic compound (Abstract, ...compositions for protein stabilization, particularly the stabilization of polymerases in aqueous solutions with cationic surfactants...).

Since none of the special technical features of the Groups I through IV inventions are found in more than one of the inventions, unity is lacking.

<End Box III: Observations where unity of invention is lacking>