

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
09 February 2023 (09.02.2023)



(10) International Publication Number
WO 2023/014303 A3

(51) International Patent Classification:

C12Q 1/686 (2018.01) C12Q 1/6848 (2018.01)
C12Q 1/6851 (2018.01) C12Q 1/6806 (2018.01)
C12Q 1/6853 (2018.01)

(21) International Application Number:

PCT/SG2022/050560

(22) International Filing Date:

05 August 2022 (05.08.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

10202108592Y 05 August 2021 (05.08.2021) SG

(71) Applicant: **AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH** [SG/SG]; 1 Fusionopolis Way, #20-10 Connexis North Tower, Singapore 138632 (SG).

(72) Inventors: **KOH, Lian Chye Winston**; c/o Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673 (SG). **KONG, Kiat Whye**; c/o Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673 (SG).

(74) Agent: **DONALDSON & BURKINSHAW LLP**; 24 Raffles Place, #15-00 Clifford Centre, Singapore 048621 (SG).

(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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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, ST, SV, SY, TH,

(54) Title: A METHOD OF AMPLIFICATION OF A NUCLEIC ACID

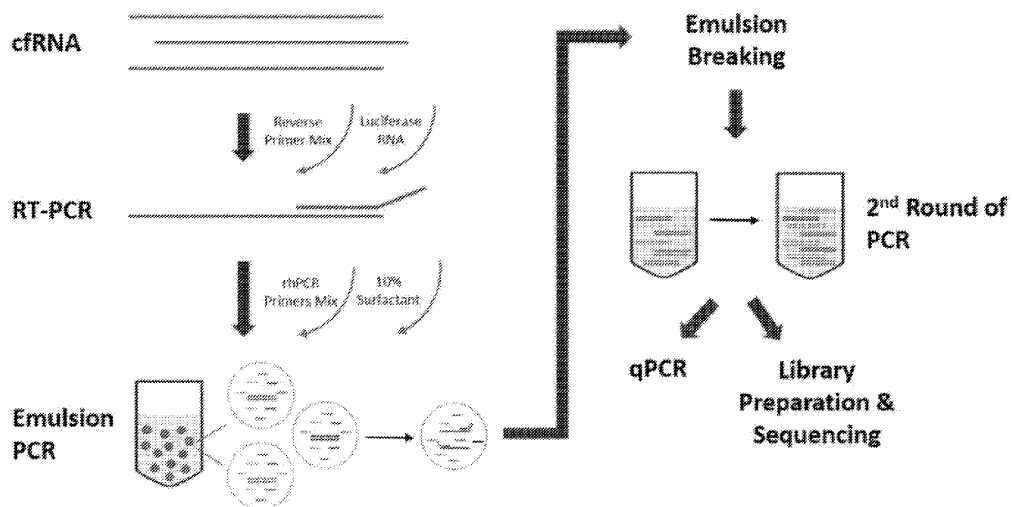


Fig. 1

(57) Abstract: This disclosure relates to a method of amplification of a target nucleic acid, the method comprising subjecting the target nucleic acid to one or more amplification step in the presence of a mixture comprising a control nuclei acid, a surfactant and an oligonucleotide primer and/or probe capable of hybridizing with the target nucleic acid, wherein the oligonucleotide primer and/or probe comprises a cleavage site and a cleavable 3' end. In one embodiment, the target nucleic acid is cell-free RNA and the method comprises annealing the target nucleic acid and performing a reverse transcription prior to the one or more amplification step. Also disclosed are nucleic acid amplification mixtures, kits for amplifications, and methods of detecting and/or determining the presence and/or the amount of a target nucleic acid.

[Continued on next page]

TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS,
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, ST, 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:

- *of inventorship (Rule 4.17(iv))*

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:

23 March 2023 (23.03.2023)