

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
19 August 2021 (19.08.2021)



(10) International Publication Number
WO 2021/162642 A1

- (51) International Patent Classification:
C12Q 1/6844 (2018.01) C12Q 1/70 (2006.01)
- (21) International Application Number:
PCT/SG2021/050075
- (22) International Filing Date:
15 February 2021 (15.02.2021)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
10202001393W 15 February 2020 (15.02.2020) SG
- (71) Applicant: **AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH** [SG/SG]; 1 Fusionopolis Way, #20-10 Connexis North Tower, Singapore 138632 (SG).
- (72) Inventors: **YING, Jackie Y.**; c/o NanoBio Lab, 31 Biopolis Way, #09-01 The Nanos, Singapore 138669 (SG). **BIN ABDUL RAHIM, Muhammad Nadjad**; c/o NanoBio Lab, 31 Biopolis Way, #09-01 The Nanos, Singapore 138669 (SG).
- (74) Agent: **AMICA LAW LLC**; 77 Robinson Road, #22-01 Robinson 77, Singapore 068896 (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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, 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,

(54) Title: A METHOD TO AMPLIFY A NUCLEIC ACID

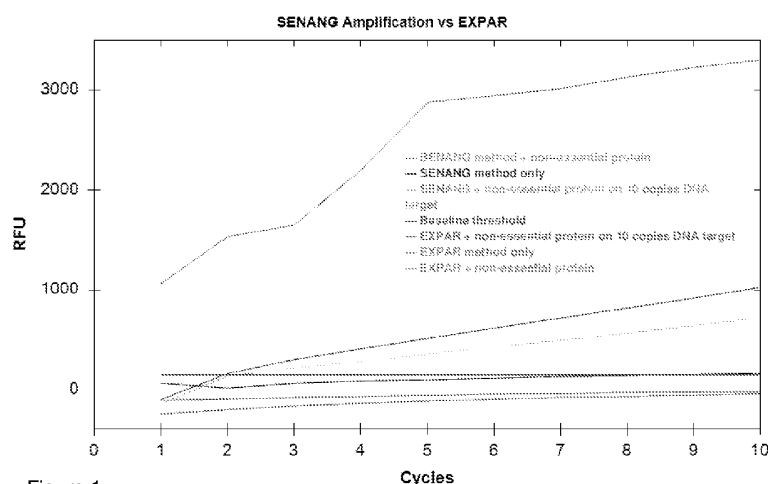


Figure 1

(57) **Abstract:** This invention relates to methods and compositions for amplifying nucleic acids, e.g., genomic DNA, using nicking agents. The method of amplifying nucleic acids comprising: (a) forming a reaction mixture comprising: (i) a first nucleic acid template comprising a strand having a first nicking agent recognition sequence; (ii) a second nucleic acid template comprising a strand having a second nicking agent recognition sequence; (iii) at least one primer for a target region on the first or second nucleic acid template; (iv) at least one protein having DNA polymerase domain function, wherein the domain function comprises a first domain function capable of strand displacement activity and a second domain function capable of high processivity activity, or at least one protein having DNA polymerase domain function capable of strand displacement activity and at least one protein having DNA polymerase domain function capable of high processivity activity; (v) at least one deoxynucleoside triphosphate; and (vi) a first nicking agent for recognizing the first nicking agent recognition sequence and a second nicking agent for recognizing the second nicking agent recognition sequence; (b) incubating the reaction mixture under conditions that amplifies the nucleic acid templates, wherein the domain functions capable of strand displacement activity and high processivity activity are separate from each other and capable of carrying out their activities simultaneously. In specific embodiments, the nicking agent is NB.BsrDI and the proteins having DNA polymerase domain functions are Bst 3.0 polymerase and Pfu polymerase.

[Continued on next page]

SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, 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))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*