

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
13 September 2018 (13.09.2018)



(10) International Publication Number
WO 2018/165593 A1

(51) International Patent Classification:

C07H 21/04 (2006.01) *C12Q 1/686* (2018.01)
C12P 19/34 (2006.01) *C12Q 1/6811* (2018.01)
C12Q 1/6806 (2018.01) *G01N 30/02* (2006.01)

(21) International Application Number:

PCT/US2018/021816

(22) International Filing Date:

09 March 2018 (09.03.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/469,309 09 March 2017 (09.03.2017) US

(71) Applicant: **IREPERTOIRE, INC.** [US/US]; 601 Genome Way, Suite 3005, Huntsville, AZ 35806 (US).

(72) Inventors: **PAN, Wenjing**; 316 Dovington Drive, Huntsville, AL 35806 (US). **BYRNE-STEELE, Miranda**; 107 Saint Anne Road, Madison, AL 35758 (US). **HOU,**

Xiaohong; 114 Horizon View Court, Madison, AL 35758 (US). **BROWN, Brittany**; 225 Postwood Court, Madison, AL 35758 (US). **HAN, Jian**; 7712 Donegal Drive, Huntsville, AL 35802 (US).

(74) Agent: **PARKER, Matthew J.** et al.; Maynard Cooper & Gale, P.C., 655 Gallatin Street, SW, Huntsville, AL 35801 (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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(54) Title: DIMER AVOIDED MULTIPLEX POLYMERASE CHAIN REACTION FOR AMPLIFICATION OF MULTIPLE TARGETS

Step 1: Reverse transcription--cDNA synthesis from reverse primer mix.

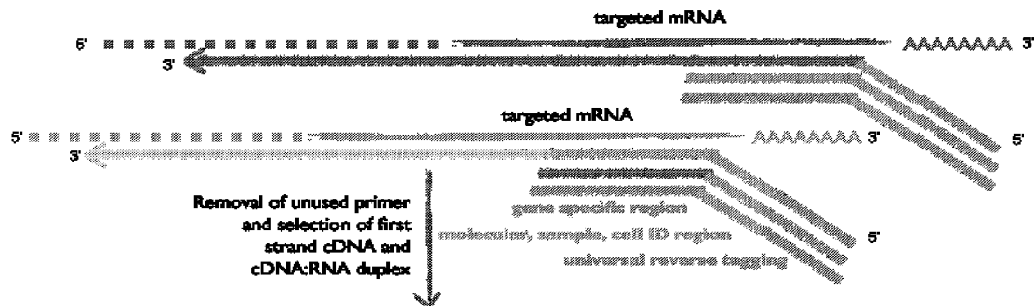


FIG. 1A

(57) Abstract: The present disclosure relates to methods for amplifying nucleic acids that avoid problems associated with primer-dimer formation. The present methods are referred to herein as dimer avoided multiplex polymerase chain reaction (dam-PCR). The methods disclosed herein generally comprise the steps of reverse transcribing at least one first strand of DNA, for example cDNA from an RNA sample, wherein each first strand of DNA incorporates a reverse common primer binding site; selecting each first strand of DNA; synthesizing at least one second strand of DNA from each of the at least one first strand of DNA, wherein each second strand of DNA incorporates a forward common primer binding site; selecting each second strand of cDNA; and amplifying the DNA strands using common primers. Alternatively, the method may be performed using a gDNA template. The methods described herein, due to the selection of DNA strands and removal of unused primers prior to amplification, avoid primer-dimer formation and allow for greater sensitivity and efficiency compared with conventional multiplex PCR methods.

[Continued on next page]

(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).

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))*