

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
20 September 2018 (20.09.2018)



(10) International Publication Number
WO 2018/170164 A1

(51) International Patent Classification:

C40B 30/06 (2006.01) *C12N 5/071* (2010.01)
C40B 40/08 (2006.01) *C12N 5/10* (2006.01)
C40B 50/02 (2006.01) *C12Q 1/68* (2018.01)
C40B 50/06 (2006.01) *A61K 31/711* (2006.01)
C12N 15/66 (2006.01)

(21) International Application Number:

PCT/US2018/022487

(22) International Filing Date:

14 March 2018 (14.03.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/471,723	15 March 2017 (15.03.2017)	US
62/578,326	27 October 2017 (27.10.2017)	US

(71) Applicant: **TWIST BIOSCIENCE CORPORATION**
[US/US]; 455 Mission Bay Blvd. South, Suite 545, San Francisco, California 94158 (US).

(72) Inventors: **COX, Anthony**; 455 Mission Bay Blvd. South, Suite 545, San Francisco, California 94158 (US). **CHEN, Siyuan**; 455 Mission Bay Blvd. South, Suite 545, San Francisco, California 94158 (US). **LEDOGAR, Charles**; 455 Mission Bay Blvd. South, Suite 545, San Francisco, California 94158 (US). **TOPPANI, Dominique**; 455 Mission Bay Blvd. South, Suite 545, San Francisco, California 94158 (US).

(74) Agent: **HARBURGER, David**; WILSON SONSINI GOODRICH & ROSATI, 650 Page Mill Road, Palo Alto, California 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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

(54) Title: DE NOVO SYNTHESIZED COMBINATORIAL NUCLEIC ACID LIBRARIES

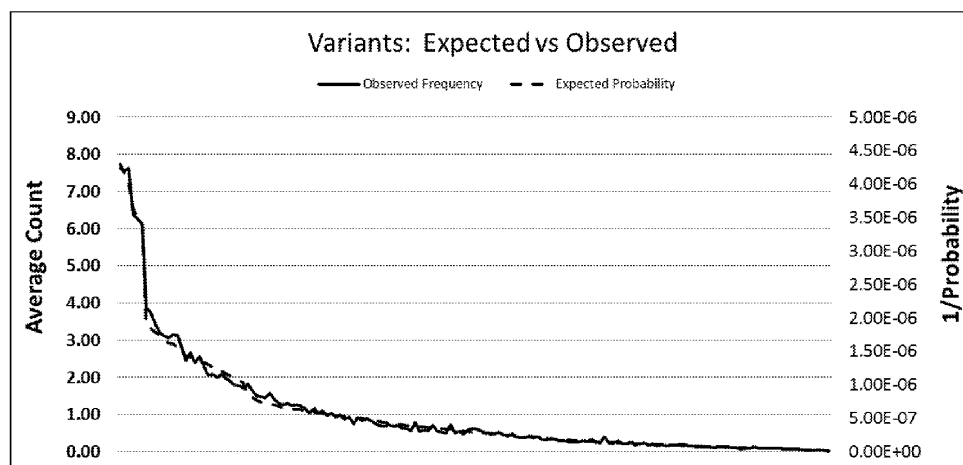


FIG. 22

(57) **Abstract:** Disclosed herein are methods for the generation of highly accurate nucleic acid libraries encoding for predetermined variants of a nucleic acid sequence. The degree of variation may be complete, resulting in a saturated variant library, or less than complete, resulting in a non-saturating library of variants. The variant nucleic acid libraries described herein may be designed for further processing by transcription or translation. The variant nucleic acid libraries described herein may be designed to generate variant RNA, DNA and/or protein populations. Further provided herein are method for identifying variant species with increased or decreased activities, with applications in regulating biological functions and the design of therapeutics for treatment or reduction of disease.

[Continued on next page]

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.

(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:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

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))*
- *with sequence listing part of description (Rule 5.2(a))*