

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
05 January 2023 (05.01.2023)



(10) International Publication Number
WO 2023/278409 A9

- (51) International Patent Classification:
C12Q 1/6841 (2018.01) *C12Q 1/6804* (2018.01) **GY** [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US).
- (21) International Application Number:
PCT/US2022/035271
- (22) International Filing Date:
28 June 2022 (28.06.2022)
- (25) Filing Language:
English
- (26) Publication Language:
English
- (30) Priority Data:
63/216,315 29 June 2021 (29.06.2021) US
- (71) Applicants: **THE BROAD INSTITUTE, INC.** [US/US];
415 Main Street, Cambridge, MA 02142 (US).
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
- (72) Inventors: **WANG, Xiao**; c/o The Broad Institute, Inc., 415 Main Street, Cambridge, MA 02142 (US). **ZENG, Hu**; c/o The Broad Institute, Inc., 415 Main Street, Cambridge, MA 02142 (US). **REN, Jingyi**; c/o Massachusetts Institute Of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139 (US).
- (74) Agent: **GODDARD, Tyler, N.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (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: SINGLE-CELL PROFILING OF RNA TRANSLATION STATUS

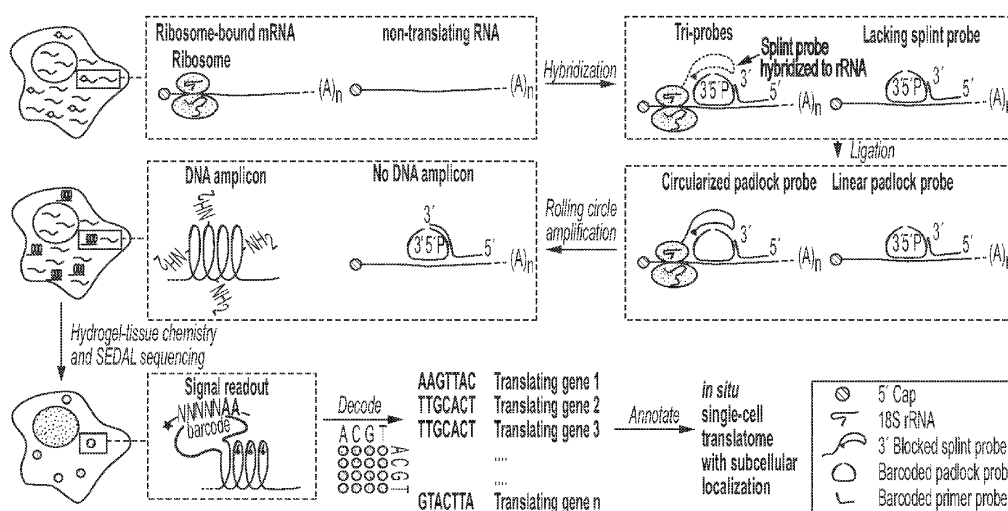


FIG. 9A

(57) **Abstract:** The present disclosure provides methods and systems for profiling RNAs being translated in a cell. Also provided by the present disclosure are methods for diagnosing a disease or disorder in a subject based on a profile of the RNAs being translated in a cell, including cells within an intact tissue. Methods of screening for or testing a candidate agent capable of modulating translation of one or more RNAs are also provided by the present disclosure. The present disclosure also provides methods for treating a disease or disorder in a subject in need thereof. Pairs of probes and sets of probes comprising oligonucleotide portions, which may be useful for performing the methods described herein, are also described by the present disclosure. Additionally, the present disclosure provides kits comprising any of the probes described herein.

[Continued on next page]

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, 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).

Published:

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

(48) Date of publication of this corrected version:

23 March 2023 (23.03.2023)

(15) Information about Correction:

see Notice of 23 March 2023 (23.03.2023)