



(51) International Patent Classification:

C12Q 1/6816 (2018.01) C12Q 1/6832 (2018.01)
C12Q 1/682 (2018.01) C12Q 1/6869 (2018.01)

(21) International Application Number:

PCT/US2023/021925

(22) International Filing Date:

11 May 2023 (11.05.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/340,730 11 May 2022 (11.05.2022) US

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(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, MG, MK, MN, MU, MW, MX, MY, MZ, NA,
NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO,

(54) Title: COMPOSITIONS AND METHODS FOR *IN SITU* SEQUENCING

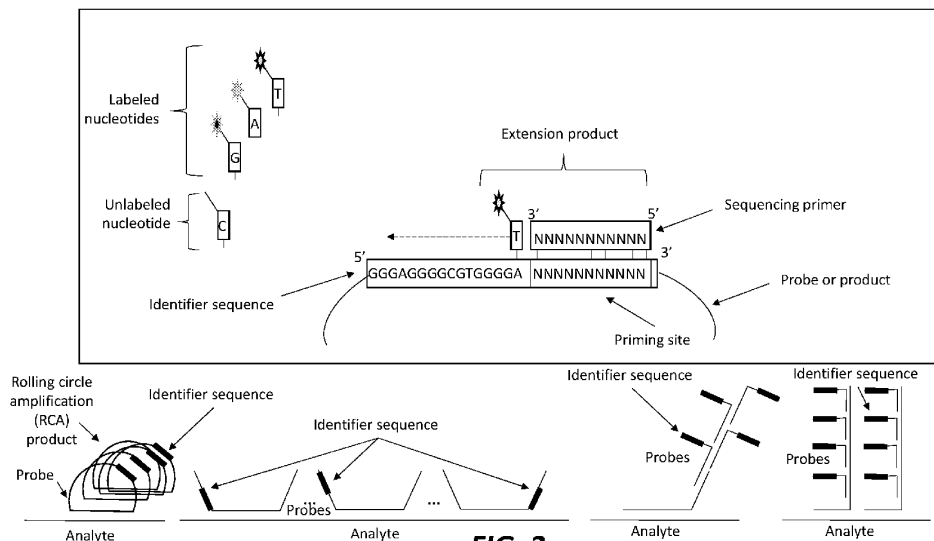


FIG. 2

(57) Abstract: Methods and compositions for performing base-by-base sequencing *in situ* in a cell or tissue sample that minimize optical crowding are described. In some embodiments, a sequencing primer hybridizes to a priming site 3' to an identifier sequence (e.g., a barcode sequence) in the sample such that the sequencing primer can be extended by a polymerase in a base-by-base fashion using the identifier sequence as a template. The sample can be contacted with nucleotides in a cyclic series of nucleotide incorporation or binding steps, and signals indicative of the incorporation or binding events can be detected to generate signal code sequences comprising a series of signal codes (corresponding to signals (ON signals), absence of signals (OFF signals), or a combination thereof) detected in the sequential cycles. Decoding of the identifier sequences based at least in part of the signal code sequences can be used to detect and locate the corresponding analytes.

[Continued on next page]

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, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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, ME, 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))