

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

(19) World Intellectual Property
Organization

International Bureau

(43) International Publication Date
29 September 2022 (29.09.2022)



(10) International Publication Number
WO 2022/204032 A1

(51) International Patent Classification:

C12Q 1/6806 (2018.01) C12Q 1/6837 (2018.01)

(21) International Application Number:

PCT/US2022/021153

(22) International Filing Date:

21 March 2022 (21.03.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/164,163 22 March 2021 (22.03.2021) US

(71) Applicants: **ILLUMINA CAMBRIDGE LIMITED**

[GB/GB]; 19 Granta Park, Great Abington, Cambridge CB21 6DF (GB). **ILLUMINA, INC.** [US/US]; 5200 Illumina Way, San Diego, CA 92122 (US).

(72) Inventors: **SKINNER, Gary, Mark**; 19 Granta Park, Great

Abington, Cambridge Cambridgeshire CB21 6DF (GB). **EVANS, Geraint**; 19 Granta Park, Great Abington, Cambridge Cambridgeshire CB21 6DF (GB). **GORMLEY, Niall**; 19 Granta Park, Great Abington, Cambridge Cambridgeshire CB21 6DF (GB). **BOUTELL, Jonathan**; 19 Granta Park, Great Abington, Cambridge Cambridgeshire CB21 6DF (GB). **KELLINGER, Matthew, W.**; 5200 Illumina Way, San Diego, CA 92122 (US). **PREVITE, Michael**; 5200 Illumina Way, San Diego, CA 92122 (US). **HE, Molly**; 5200 Illumina Way, San Diego, CA 92122 (US).

(74) Agent: **CAMPBELL, Keith, M.**; 111 Washington Avenue

South, Ste. 700, Minneapolis, MN 55401 (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, 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))



WO 2022/204032 A1

(54) Title: METHODS FOR IMPROVING NUCLEIC ACID CLUSTER CLONALITY

(57) Abstract: A method for seeding and amplifying target nucleic acids derived from a sample in a cluster at a site on a surface of a substrate includes retaining at least a portion of the target nucleic acids in an inactive form that cannot seed to provide a relatively low concentration of active form target nucleic acids available for seeding. As the active form target nucleic acids seed on the surface of the substrate, they may be amplified. Because the concentration of active form target nucleic acids is low, the likelihood is low that a second active form target nucleic acid will seed at the same site within the same cluster before the first active form target nucleic acid is sufficiently amplified to dominate. Accordingly, the likelihood that the cluster will pass filters is increased relative to traditional seeding and amplification methods employing a higher concentration of active form target nucleic acids.