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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 June 2021 (24.06.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/127436 A3

(51) International Patent Classification:
C12N 15/10 (2006.01)

(21) International Application Number:
PCT/US2020/066013

(22) International Filing Date:
18 December 2020 (18.12.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/950,670 19 December 2019 (19.12.2019) US

(71) Applicants: **ILLUMINA, INC.** [US/US]; 5200 Illumina Way, San Diego, California 92122 (US). **UNIVERSITY OF WASHINGTON** [US/US]; 4545 Roosevelt Way NE, Suite 400, Seattle, Washington 98105 (US).

(72) Inventors; and
(71) Applicants: **SHENDURE, Jay** [US/US]; 4545 Roosevelt Way NE, Suite 400, Seattle, Washington 98105 (US). **CUSANOVICH, Darren** [US/US]; 4545 Roosevelt Way NE, Suite 400, Seattle, Washington 98105 (US). **DAZA, Riza** [US/US]; 4545 Roosevelt Way NE, Suite 400, Seattle, Washington 98105 (US).

(72) Inventor: **STEEMERS, Frank**; 5200 Illumina Way, San Diego, California 92122 (US).

(74) Agent: **PROVENCE, David** et al.; 111 Washington Ave S., Suite 700, Minneapolis, Minnesota 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, 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))*
— *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))*

(88) Date of publication of the international search report:
29 July 2021 (29.07.2021)

(54) Title: HIGH-THROUGHPUT SINGLE-CELL LIBRARIES AND METHODS OF MAKING AND OF USING

(57) Abstract: Provided herein are methods for preparing a sequencing library that includes nucleic acids from a plurality of single cells. In one embodiment, the sequencing library includes nucleic acids that represent the chromatin accessibility from the plurality of single cells. In one embodiment, the nucleic acids include three index sequences. In another embodiment, the present disclosure provides methods for characterizing rare events in isolated cells and nuclei.

WO 2021/127436 A3