

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
20 January 2022 (20.01.2022)



(10) International Publication Number
WO 2022/012504 A1

(51) International Patent Classification:
C12Q 1/6883 (2018.01)

(21) International Application Number:
PCT/CN2021/105939

(22) International Filing Date:
13 July 2021 (13.07.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/051,268 13 July 2020 (13.07.2020) US

(71) Applicants: **THE CHINESE UNIVERSITY OF HONG KONG** [CN/CN]; Office of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN). **GRAIL, INC.** [US/US]; 1525 O'Brien Drive, Menlo Park, California 94025 (US).

(72) Inventors: **LO, Yuk-Ming, Dennis**; c/o The Chinese University of Hong Kong, Office of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN). **CHIU, Rossa, Wai Kwun**; c/o The Chinese University of Hong Kong, Office of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN). **CHAN, Kwan Chee**; c/o The Chinese University of Hong Kong, Office of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN). **JIANG, Peiyong**; c/o The Chinese University of Hong Kong, Office of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN). **CHAN, Wing Yan**; c/o The Chinese University of Hong Kong, Office of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN). **LAM, Wai Kei**; c/o The Chinese University of Hong Kong, Office of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN). **HAN, Diana, Siao Cheng**; c/o The Chinese University of Hong Kong, Office of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN). **PENG, Wenlei**; c/o The Chinese University of Hong Kong, Of-

fice of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN). **DING, Chen**; c/o The Chinese University of Hong Kong, Office of Research And Knowledge Transfer Services, Room 301, Pi Ch'iu Building, Shatin, New Territories, Hong Kong (CN).

(74) Agent: **INSIGHT INTELLECTUAL PROPERTY LIMITED**; Floor 7, China Overseas Plaza Central Tower, No. 8 Guanghua Dongli, Jianwai Avenue, Chaoyang, Beijing 100020 (CN).

(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))

(54) Title: NUCLEASE-ASSOCIATED END SIGNATURE ANALYSIS FOR CELL-FREE NUCLEIC ACIDS

(57) Abstract: Various embodiments are performed to using nuclease expression in tissues that influences cell-free DNA end signatures/motifs and size of overhang between DNA strands. Embodiments can identify a nuclease that is being differentially regulated in abnormal cells relative to normal cells. Embodiments can determine that the nuclease preferentially cuts DNA into DNA molecules having: (i) a particular sequence end signature; or (ii) a specified length of overhang between a first strand and a second strand. A parameter can be determined for a biological sample based on an amount of DNA molecules that include an end sequence corresponding to the particular sequence end signature and/or a measured property correlating to the specified length of overhang. The parameter can be used to determine a characteristic of a tissue type, a fractional concentration of clinically-relevant DNA molecules, or a level of abnormality of a tissue type in the biological sample.



WO 2022/012504 A1