

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
03 February 2022 (03.02.2022)



(10) International Publication Number
WO 2022/023753 A1

- (51) International Patent Classification:
C12Q 1/6869 (2018.01)
- (21) International Application Number:
PCT/GB2021/051957
- (22) International Filing Date:
29 July 2021 (29.07.2021)

- (25) Filing Language: English
- (26) Publication Language: English

- (30) Priority Data:
- | | | |
|------------|-------------------------------|----|
| 63/058,712 | 30 July 2020 (30.07.2020) | US |
| 63/061,093 | 04 August 2020 (04.08.2020) | US |
| 63/105,860 | 26 October 2020 (26.10.2020) | US |
| 63/106,566 | 28 October 2020 (28.10.2020) | US |
| 63/152,976 | 24 February 2021 (24.02.2021) | US |
| 63/178,386 | 22 April 2021 (22.04.2021) | US |
| 63/210,927 | 15 June 2021 (15.06.2021) | US |
| 63/212,500 | 18 June 2021 (18.06.2021) | US |
| 63/213,626 | 22 June 2021 (22.06.2021) | US |
| 63/215,752 | 28 June 2021 (28.06.2021) | US |

- (71) Applicant: **CAMBRIDGE EPIGENETIX LIMITED**
[GB/GB]; B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB).

- (72) Inventors: **BALASUBRAMANIAN, Shankar**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **FULLGRABE, Jens**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **GOSAL, Walraj Singh**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **HOLBROOK, Joanna Dawn**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **LIU, Sidong**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **MORLEY, David**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **NENTWICH, Oliver**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **OST, Tobias**; Cambridge

Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **STEWART, Michael**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **VILELLA, Albert**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **WALKER, Nicholas James**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **YU, Shirong**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **BIGNELL, Helen Rachel**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB). **SAN-BENTO, Rita Santo**; Cambridge Epigenetix Limited, B400, The Trinity Building, Chesterford Business Park, Little Chesterford, Saffron Walden Essex CB10 1XL (GB).

- (74) Agent: **J A KEMP LLP**; 80 Turnmill Street, London EC1M 5QU (GB).

- (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).

- (54) Title: COMPOSITIONS AND METHODS FOR NUCLEIC ACID ANALYSIS

- (57) Abstract: Provided herein are methods, systems, and compositions for determining a base in a polynucleotide. In various aspects, the methods, systems, and compositions presented herein are useful for performing 4-base, 5 -base, or 6-base sequencing of polynucleotide molecules, for example, from liquid biopsy samples or wherein the base is a low frequency mutation.



WO 2022/023753 A1

[Continued on next page]

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))*