



- (51) **International Patent Classification:**
C12Q 1/6869 (2018.01) *C12Q 1/6886* (2018.01)
G01N 33/48 (2006.01)
- (21) **International Application Number:**
PCT/SG2022/050367
- (22) **International Filing Date:**
30 May 2022 (30.05.2022)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
10202105843Q 02 June 2021 (02.06.2021) SG
- (71) **Applicant:** LUCENCE LIFE SCIENCES PTE. LTD.
[SG/SG]; 211 Henderson Road, #04-02 Henderson Industrial Park, Singapore 159552 (SG).
- (72) **Inventors:** CHOUDHURY, Yukti; c/o LUCENCE LIFE SCIENCES PTE. LTD., 211 Henderson Road, #04-02 Henderson Industrial Park, Singapore 159552 (SG). LIM, Jing Shan; c/o LUCENCE LIFE SCIENCES PTE. LTD., 211 Henderson Road, #04-02 Henderson Industrial Park, Singapore 159552 (SG). LEE, Jin Wee; c/o LUCENCE LIFE SCIENCES PTE. LTD., 211 Henderson Road, #04-02 Henderson Industrial Park, Singapore 159552 (SG). GUP-

TA, Chaitanya; c/o LUCENCE LIFE SCIENCES PTE. LTD., 211 Henderson Road, #04-02 Henderson Industrial Park, Singapore 159552 (SG). TAN, Min-Han; c/o LUCENCE LIFE SCIENCES PTE. LTD., 211 Henderson Road, #04-02 Henderson Industrial Park, Singapore 159552 (SG). CHEN, Hao; c/o LUCENCE LIFE SCIENCES PTE. LTD., 211 Henderson Road, #04-02 Henderson Industrial Park, Singapore 159552 (SG). MADAN MOHAN, Aravind; c/o LUCENCE LIFE SCIENCES PTE. LTD., 211 Henderson Road, #04-02 Henderson Industrial Park, Singapore 159552 (SG).

(74) **Agent:** SPRUSON & FERGUSON (ASIA) PTE LTD;
P.O. Box 1531, Robinson Road Post Office, Singapore 903031 (SG).

(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, IQ, 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,

(54) **Title:** METHOD FOR DETECTION AND QUANTIFICATION OF METHYLATED DNA

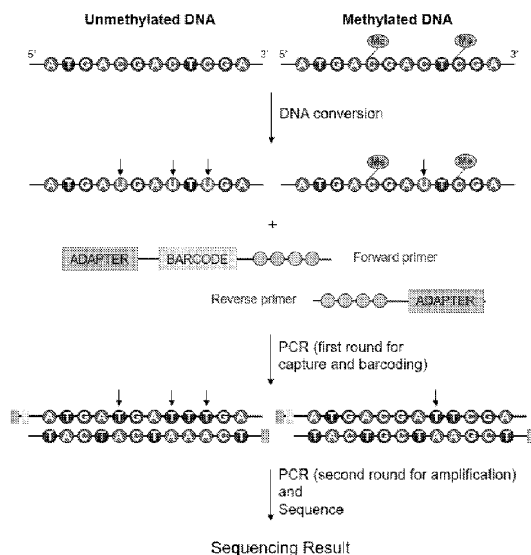


Fig. 1

(57) **Abstract:** The invention relates to a method of detecting methylated pattern in DNA in a biological sample, comprising (a) converting unmethylated cytosine of the DNA to uracil by deamination; (b) purifying the converted DNA; (c) tagging a barcode sequence on the converted DNA, by performing a first PCR amplification using a plurality of forward and reverse primer pairs specific to one or more CpG sites, wherein each forward primer of the plurality comprises a barcode sequence on its 5' end, and wherein the barcode of each forward primer is different from each other; (d) subjecting the tagged converted DNA to a second PCR amplification with universal indexed primers to create a sequencing library; (e) subjecting the sequencing library to multiplex sequencing; (f) assigning each DNA sequence to an original parental DNA molecule by (i) performing cluster reassignment of sequencing reads with the same barcode sequence and (ii) performing consensus calling to obtain consensus reads; and (g) reconstructing the methylated pattern of the DNA by (i) comparing the DNA sequence to a reference genome using a sequence alignment tool and (ii) conducting variant analysis by comparing the consensus reads to the reference genome.

[Continued on next page]

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).

Declarations under Rule 4.17:

- *of inventorship (Rule 4.17(iv))*

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))*
— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:

12 January 2023 (12.01.2023)