

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
04 July 2019 (04.07.2019)



(10) International Publication Number
WO 2019/129239 A1

- (51) International Patent Classification:
C12Q 1/68 (2018.01)
- (21) International Application Number:
PCT/CN2018/125053
- (22) International Filing Date:
28 December 2018 (28.12.2018)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
62/611,698 29 December 2017 (29.12.2017) US
- (71) Applicant: **ACT GENOMICS CO., LTD.** [CN/CN]; 1F.,
No.280, Xinhui 2nd Rd., Neihu Dist., Taipei City 114, Tai-
wan (CN).
- (72) Inventors: **WU, Ko-wen**; 3F., No.345, Xinhui 2nd Rd., Nei-
hu Dist., Taipei City, Taiwan 114 (CN). **LI, Kun-lin**; 3F.,
No.345, Xinhui 2nd Rd., Neihu Dist., Taipei City, Taiwan
114 (CN).
- (74) Agent: **SHANGHAI DANRONG & ZONGDE INTEL-
LECTUAL PROPERTY AGENCY CO., LTD.**; YU,
Danrong, Room 1708-1709, 2nd Building, No.335 Guoding
Road, Yangpu District, Shanghai 200433 (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, 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, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, 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,

(54) Title: METHOD AND SYSTEM FOR SEQUENCE ALIGNMENT AND VARIANT CALLING

(a)
Misalignment: 1 11 21 31
Reference Sequence: AACAAGCTCTTTCTTTCTCTCTGTTTAAAGATCT
Sequencing Read: -----AACAAGCTCTTAAAGATCT

(b)
Misalignment with soft-clipping:
Reference Sequence: AACAAGCTCTTTCTTTCTCTCTGTTTAAAGATCT
Sequencing Read: AACAAGCTCTTAAAGATCT
Soft-clipped region

(c)
Correct alignment:
Reference Sequence: AACAAGCTCTTTCTTTCTCTCTGTTTAAAGATCT
Sequencing Read: AACAAGCTC-----TTAAGATCT

Figure 1

(57) Abstract: A method to align a next generation sequencing read to a reference sequence includes: (a) receiving a sequencing read; (b) performing a first alignment of the sequencing read to a reference sequence so as to identify a target sequence within the reference sequence where to the sequencing read maps; (c) selecting a first and a second anchor sequence; (d) attaching the first anchor sequence to the upstream region of the sequencing read and the second anchor sequence to the downstream region of the sequencing read so as to generate an extended sequencing read; (e) attaching the first anchor sequence to the upstream region of the target sequence and the second anchor sequence to the downstream region of the target sequence, so as to generate an extended target sequence; (f) performing a second alignment of the extended sequencing read to the extended target sequence, so that the second alignment is more correctly mapped to the target sequence than the first alignment; (g) identifying a position where one or more bases between the extended sequencing read and the extended target sequence are different based on the second alignment result; and (h) calling a variant based on the identification in step (g).

[Continued on next page]

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:

- *as to the identity of the inventor (Rule 4.17(i))*
- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*

Published:

- *with international search report (Art. 21(3))*
- *with sequence listing part of description (Rule 5.2(a))*