

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
14 July 2022 (14.07.2022)



(10) International Publication Number
WO 2022/150790 A3

(51) International Patent Classification:

C12N 15/11 (2006.01) C12N 9/12 (2006.01)
C12N 15/10 (2006.01) C12N 9/22 (2006.01)

(21) International Application Number:

PCT/US2022/012054

(22) International Filing Date:

11 January 2022 (11.01.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/136,194	11 January 2021 (11.01.2021)	US
63/176,180	16 April 2021 (16.04.2021)	US
63/176,202	16 April 2021 (16.04.2021)	US
63/194,865	28 May 2021 (28.05.2021)	US
63/194,913	28 May 2021 (28.05.2021)	US
63/231,230	09 August 2021 (09.08.2021)	US
63/255,897	14 October 2021 (14.10.2021)	US

(71) Applicants: **THE BROAD INSTITUTE, INC.** [US/US];
415 Main Street, Cambridge, MA 02142 (US). **PRESI-
DENT AND FELLOWS OF HARVARD COLLEGE**
[US/US]; 17 Quincy Street, Cambridge, MA 02138 (US).

THE TRUSTEES OF PRINCETON UNIVERSITY
[—/US]; 619 Alexander Road, Suite 102, Princeton, NJ
08540 (US). **THE REGENTS OF THE UNIVERSITY
OF CALIFORNIA** [US/US]; 1111 Franklin Street, 12th
Floor, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(71) Applicants: **LIU, David, R.** [US/US]; c/o The Broad In-
stitute, Inc., 415 Main Street, Cambridge, MA 02142 (US).
CHEN, Peter, J. [US/US]; c/o The Broad Institute, Inc.,
415 Main Street, Cambridge, MA 02142 (US).

(72) Inventors: **ADAMSON, Brittany**; 38 Longview Drive,
Princeton, NJ 08540 (US). **HUSSMANN, Jeffrey**; c/o The
Regents of the University of California, 1111 Franklin
Street, 12th Floor, Oakland, CA 94607-5200 (US).

(74) Agent: **MCCOOL, Gabriel, J.**; Wolf, Greenfield & Sacks,
P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (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,

(54) Title: PRIME EDITOR VARIANTS, CONSTRUCTS, AND METHODS FOR ENHANCING PRIME EDITING EFFICIENCY AND PRECISION

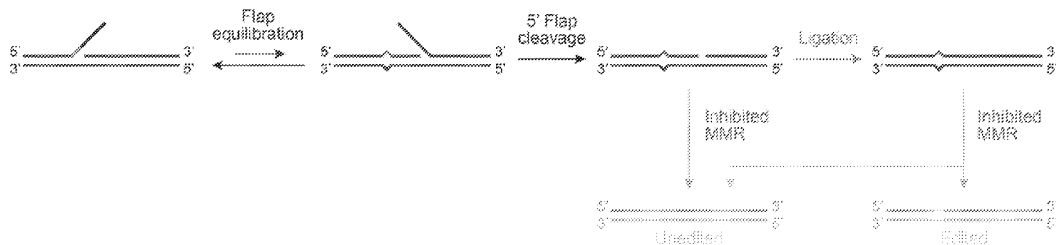


FIG. 9B

(57) Abstract: The present disclosure provides compositions and methods for prime editing with improved editing efficiency and/or reduced indel formation by inhibiting the DNA mismatch repair path way while conducting prime editing of a target site. Accordingly, the present disclosure provides a method for editing a nucleic acid molecule by prime editing that involves contacting a nucleic acid molecule with a prime editor, a pegRNA, and an inhibitor of the DNA mismatch repair pathway, thereby installing one or more modifications to the nucleic acid molecule at a target site with increased editing efficiency and/or lower indel formation. The present disclosure further provides polynucleotides for editing a DNA target site by prime editing comprising a nucleic acid sequence encoding a napDNABp, a polymerase, and an inhibitor of the DNA mismatch repair pathway, wherein the napDNABp and polymerase is capable in the presence of a pegRNA of installing one or more modifications in the DNA target site with increased editing efficiency and/or lower indel formation. The disclosure further provides, vectors, cells, and kits comprising the compositions and polynucleotides of the disclosure. The present disclosure also provides compositions and methods for prime editing with improved editing efficiency and/or reduced indel formation with modified prime editor fusion proteins. The disclosure further provides, vectors, cells, and kits comprising the compositions and polynucleotides of the disclosure.

[Continued on next page]

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

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

11 August 2022 (11.08.2022)