

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
15 December 2022 (15.12.2022)



(10) International Publication Number
WO 2022/261122 A1

(51) International Patent Classification:
C12N 15/63 (2006.01) C12N 15/113 (2010.01)
C12N 9/22 (2006.01)

TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(21) International Application Number:
PCT/US2022/032541

(22) International Filing Date:
07 June 2022 (07.06.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/197,889 07 June 2021 (07.06.2021) US
63/211,631 17 June 2021 (17.06.2021) US
63/236,337 24 August 2021 (24.08.2021) US
63/284,837 01 December 2021 (01.12.2021) US

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

(71) Applicant: **THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK**
[US/US]; 412 Low Memorial Library, 535 West 116th Street, New York, New York 10027 (US).

(72) Inventors: **STERNBERG, Samuel Henry**; 412 Low Memorial Library, 535 West 116th Street, New York, New York 10027 (US). **LAMPE, George Davis**; 412 Low Memorial Library, 535 West 116th Street, New York, New York 10027 (US). **KING, Rebeca**; 412 Low Memorial Library, 535 West 116th Street, New York, New York 10027 (US). **CHAVEZ, Alejandro**; 412 Low Memorial Library, 535 West 116th Street, New York, New York 10027 (US). **KLOMPE, Sanne Eveline**; 412 Low Memorial Library, 535 West 116th Street, New York, New York 10027 (US).

(74) Agent: **BARTON, Kelly A.**; Casimir Jones, S.C., 2275 Deming Way, Suite 310, Middleton, Wisconsin 53562 (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, 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: CRISPR-TRANSPOSON SYSTEMS FOR DNA MODIFICATION

(57) Abstract: The present disclosure provides systems, kits, and methods for nucleic acid integration utilizing engineered Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated transposon (CRISPR-Tn) system. More particularly, the present disclosure provides systems comprising: an engineered CRISPR-Tn system or one or more nucleic acids encoding the engineered CRISPR-Tn system, wherein the CRISPR-Tn system comprises at least one or both of: a) at least one Cas protein (e.g., Cas6, Cas7, Cas5, and/or Cas8); and b) one or more transposon-associated proteins (e.g., TnsA, TnsB, TnsC, TnsD, and/or TniQ). The present disclosure also provides systems, kits, and methods for nucleic acid integration in a eukaryotic cell.



WO 2022/261122 A1