

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
19 May 2023 (19.05.2023)



(10) International Publication Number
WO 2023/084099 A1

- (51) **International Patent Classification:**
C12N 9/22 (2006.01) C12N 15/10 (2006.01)
- (21) **International Application Number:**
PCT/EP2022/081868
- (22) **International Filing Date:**
15 November 2022 (15.11.2022)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
21208214.3 15 November 2021 (15.11.2021) EP
- (71) **Applicant:** TECHNISCHE UNIVERSITÄT DRESDEN
[DE/DE]; Helmholtzstr. 10, 01069 Dresden (DE).
- (72) **Inventors:** HOERSTEN, Jenna; Ermelstr. 34, Dresden
01277 (DE). LANSING, Felix; Koenigsbruecker Str. 48,
Dresden 01099 (DE). PROF. BUCHHOLZ, Frank; Schu-
bertstr. 40, 01307 Dresden (DE).
- (74) **Agent:** ZWICKER, Jörk; Zwicker Schnappauf & Partner
Patentanwälte PartG mbB, Hansastr. 32, 80686 München
(DE).

- (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, CV, 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, 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, CV,
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, ME, 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).

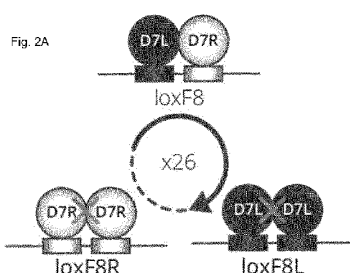
Declarations under Rule 4.17:

- as to non-prejudicial disclosures or exceptions to lack of
novelty (Rule 4.17(v))

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))

(54) **Title:** SITE-SPECIFIC RECOMBINASES FOR EFFICIENT AND SPECIFIC GENOME EDITING



(57) **Abstract:** The invention relates generally to the field of genome editing and provides a method of generating DNA recombinases and provides DNA recombinases, which efficiently and specifically recombine genomic target sequences as obligate DNA recombinase enzymes. More specifically, the invention provides genetically engineered DNA recombinases for efficient and specific genome editing, comprising an obligate complex of recombinases, wherein said complex comprises at least a first recombinase enzyme and at least a second recombinase enzyme, wherein said first recombinase enzyme and said second recombinase enzyme specifically recognize a first half-site and a second half-site of an upstream target site and/or a downstream target site of a DNA recombinase; wherein said first recombinase enzyme and said second recombinase enzyme each comprises at least one mutation in a catalytic site, wherein said first recombinase enzyme and said second recombinase enzyme carrying said at least one mutation in a catalytic site, when expressed in isolation, do not show the catalytic activity of a DNA recombinase; and wherein the catalytic activity as a DNA recombinase in said obligate complex of recombinases is complemented. The invention further relates to nucleic acid molecules encoding said genetically engineered DNA recombinases and obligate complexes, as well as to the use of said genetically engineered DNA recombinases and obligate complexes and nucleic acid molecules in a pharmaceutical composition.

WO 2023/084099 A1