

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
07 September 2023 (07.09.2023)



(10) International Publication Number
WO 2023/166029 A1

(51) International Patent Classification:

C12N 9/22 (2006.01) C12N 15/82 (2006.01)
C12N 15/10 (2006.01) C12N 15/85 (2006.01)
C12N 15/75 (2006.01)

(21) International Application Number:

PCT/EP2023/055130

(22) International Filing Date:

01 March 2023 (01.03.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

22159465.8 01 March 2022 (01.03.2022) EP
22202125.5 18 October 2022 (18.10.2022) EP

(71) Applicants: **BASF AGRICULTURAL SOLUTIONS SEED US LLC** [US/US]; 100 Park Avenue, FLORHAM PARK, New Jersey 07932 (US). **WAGENINGEN UNIVERSITEIT** [NL/NL]; Droevendaalsesteeg 4, 6708 PB WAGENINGEN (NL).

(72) Inventors: **VAN DER OOST, John**; Droevendaalsesteeg 4, 6708 PB Wageningen (NL). **VILLEGAS WAR-**

REN, Ricardo; Droevendaalsesteeg 4, 6708 PB Wageningen (NL). **LUTEIJN, Maartje Janneke**; Droevendaalsesteeg 4, 6708 PB WAGENINGEN (NL). **STAALS, Raymond Hubert Josèphe**; Droevendaalsesteeg 4, 6708 PB Wageningen (NL). **WU, Wen Ying**; Droevendaalsesteeg 4, 6708 PB Wageningen (NL). **DE VLEESSCHAUWER, David**; c/o BASF Belgium Coordination Center, Innovation Center Gent, Technologiepark 101, 9052 GENT ZWIJNAARDE (BE). **D'HALLUIN, Katelijn**; c/o BASF Belgium Coordination Center, Innovation Center Gent, Technologiepark 101, 9052 GENT ZWIJNAARDE (BE). **MEULEWAETER, Frank**; c/o BASF Belgium Coordination Center, Innovation Center Gent, Technologiepark 101, 9052 GENT ZWIJNAARDE (BE).

(74) Agent: **EISENFÜHR SPEISER PATENTANWÄLTE RECHTSANWÄLTE PARTGMBB**; P.O. Box 31 02 60, 80102 Munich (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,

(54) Title: CAS12A NICKASES

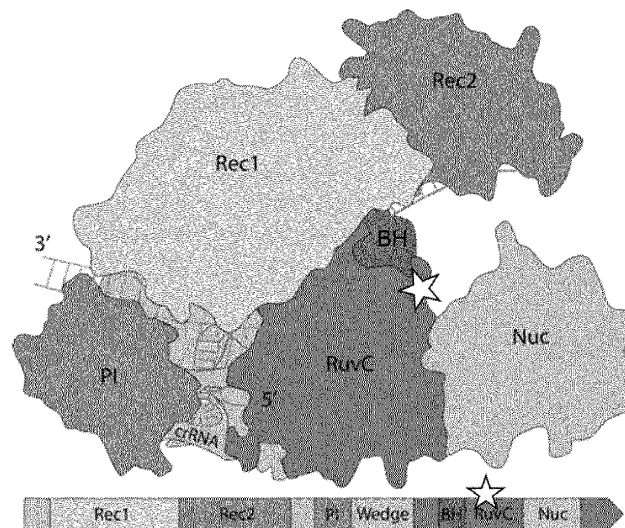


Fig. 2

(57) Abstract: The present invention relates to the field of gene genome editing. In particular, it relates to the provision of a Cas12a enzyme having nickase activity, as well as the means and methods for the modification of a genomic locus of interest with a Cas12a enzyme having nickase activity and uses thereof.

[Continued on next page]

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, SC, 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).

Published:

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