



(51) International Patent Classification:  
C12N 9/22 (2006.01)

(21) International Application Number:  
PCT/US2023/084258

(22) International Filing Date:  
15 December 2023 (15.12.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
63/387,767 16 December 2022 (16.12.2022) US

(71) Applicant: PAIRWISE PLANTS SERVICES, INC.  
[US/US]; 807 East Main Street, Suite 4-100, Durham, North Carolina 27701 (US).

(72) Inventor: KIM, Yongjoo; 114 Sir Richard Ln., Chapel Hill, North Carolina 27517 (US).

(74) Agent: BOBAY, Erin Regal; Myers Bigel, P.A., 4130 Park-lake Avenue, Ste. 250, Raleigh, North Carolina 27612 (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, 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, MU, 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).

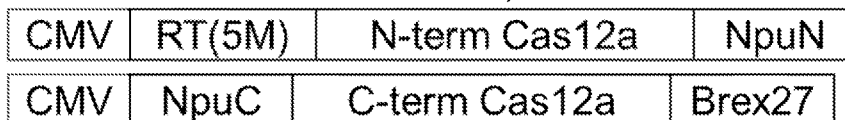
**Published:**

- with international search report (Art. 21(3))
- 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:  
08 August 2024 (08.08.2024)

(54) Title: FUSION PROTEINS COMPRISING A CAS12A POLYPEPTIDE AND AN INTEIN AND METHODS OF USE THEREOF

**FIG. 3**



Split site: Cas12a residue: 175, 310, 406, 441, 550

(57) Abstract: Described herein are fusion proteins that include an intein polypeptide along with methods of using of such proteins. Fusion proteins described herein may include a Cas12a polypeptide and an intein polypeptide or a polypeptide of interest (e.g., a reverse transcriptase polypeptide) and an intein polypeptide. Also described herein are compositions and systems for modifying or editing a target nucleic acid.



# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2023/084258

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> INV. C12N9/22 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) C07K C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  EPO-Internal, Sequence Search		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/158658 A1 (UNIV LELAND STANFORD JUNIOR [US]) 12 August 2021 (2021-08-12)	1-12, 15-33, 35, 38-41, 58-73, 76,79-82
Y	the whole document in particular paragraphs [0061] to [0072]; figure 11; paragraph [0290]; claims 1-71	13,14, 34,36, 37, 42-44, 77,78
----- - / - -		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.		
<input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents :		
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
Date of the actual completion of the international search	Date of mailing of the international search report	
29 April 2024	08/07/2024	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  <b>Strobel, Andreas</b>	

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2023/084258

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JAMES W BRYSON: "Multiplexed activation in mammalian cells using a split-intein CRISPR/Cas12a based synthetic transcription factor", NUCLEIC ACIDS RESEARCH, vol. 50, no. 1, 15 December 2021 (2021-12-15), pages 549-560, XP093155429, GB ISSN: 0305-1048, DOI: 10.1093/nar/gkab1191 Retrieved from the Internet: URL:https://academic.oup.com/nar/article-pdf/50/1/549/42149230/gkab1191.pdf>	1-12, 15-33, 35, 38-41, 58-76, 79-82
Y	the whole document	13,14, 34,36, 37, 42-44, 77,78
Y	----- US 2017/233703 A1 (XIE ZHEN [CN] ET AL) 17 August 2017 (2017-08-17)  the whole document	13,14, 34,36, 37, 42-44, 77,78
A	----- Y. Bill Kim: "A novel mechanistic framework for precise sequence replacement using reverse transcriptase and diverse CRISPR-Cas systems", bioRxiv, 13 December 2022 (2022-12-13), XP093155410, DOI: 10.1101/2022.12.13.520319 Retrieved from the Internet: URL:https://www.biorxiv.org/content/10.1101/2022.12.13.520319v1 the whole document -----	1-44, 58-82

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2023/084258

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

**see additional sheet**

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:  
**1 - 44, 58 - 82**

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2023/084258

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2021158658	A1	12-08-2021	NONE
-----			
US 2017233703	A1	17-08-2017	CN 106011104 A 12-10-2016
		US 2017233703 A1	17-08-2017
-----			

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-44, 58-82

A fusion protein comprising a Cas12a polypeptide fused to an intein polypeptide

---

2. claims: 45-57

A fusion protein comprising a polypeptide of interest fused to an intein polypeptide

---