



(51) International Patent Classification:

C12N 15/86 (2006.01) C12N 15/09 (2006.01)
C12N 15/90 (2006.01)

(21) International Application Number:

PCT/US2017/024369

(22) International Filing Date:

27 March 2017 (27.03.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/314,163 28 March 2016 (28.03.2016) US

(71) Applicant: **THE CHARLES STARK DRAPER LABORATORY, INC.** [US/US]; 555 Technology Square, Cambridge, Massachusetts 02139 (US).

(72) Inventors: **HOLDER, Jason W.**; 11 Bay View Drive, Swampscott, Massachusetts 01907 (US). **MCBRINE, Connor**; 344 Summer Street, Unit 1, Somerville, Massachusetts 02144 (US). **GRUSZKA, Sarah**; 285 Harvard Street, Apartment 404, Cambridge, Massachusetts 02139 (US). **ROGERS, Miles**; 8 Unity Street, Apartment 23, Boston, Massachusetts 02113 (US). **BILLINGS, Nicole**; 288 Baldwin Avenue, Framingham, Massachusetts 01701 (US).

(74) Agent: **EWING, James F.** et al.; Foley & Lardner LLP, 3000 K Street NW, Suite 600, Washington, District of Columbia 20007 (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, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, 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:

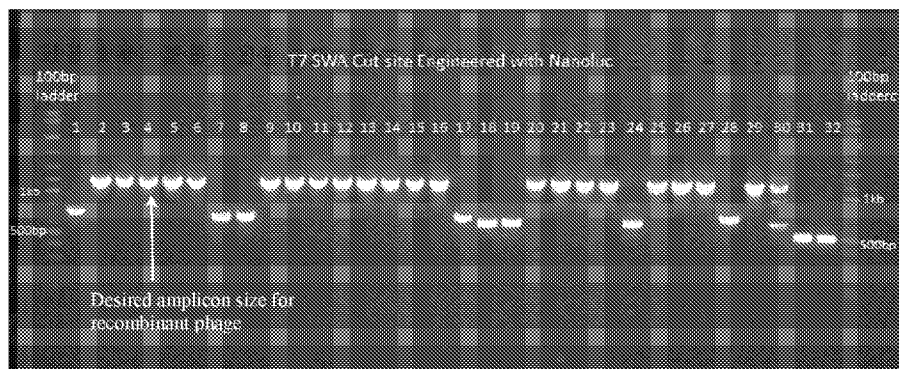
- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

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

23 November 2017 (23.11.2017)

(54) Title: BACTERIOPHAGE ENGINEERING METHODS

Figure 2



(57) Abstract: The present disclosure provides methods and kits for generating recombinant bacteriophage genomes.



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/024369

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/86 C12N15/90 C12N15/09
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C12N C12Q
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, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ying-Ta Lai ET AL: "In Vitro Repair of Gaps in Bacteriophage T7 DNA", Journal of Bacteriology, 1 December 1998 (1998-12-01), pages 6193-6202, XP055385229, UNITED STATES Retrieved from the Internet: URL:http://jb.asm.org/content/180/23/6193. full.pdf#page=1&view=FitH tables 2, 4, 5 figure 3 page 6195, right-hand column, paragraph 2 ----- -/--	1-10,19, 20,33-39

Further documents are listed in the continuation of Box C.

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 28 June 2017	Date of mailing of the international search report 09/10/2017
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 Brouns, Gaby

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/024369

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.:

1-10, 39(completely); 19, 20, 33-38(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-10, 39(completely); 19, 20, 33-38(partially)

Relating to a method of generating a recombinant bacteriophage genome comprising contacting the genome with a sgRNA-CRISPR enzyme conjugate in vitro and recombining the cleaved bacteriophage genome in vitro with a heterologous nucleic acid in the presence of a recombination system.

2. claims: 11-18(completely); 19, 20, 33-38, 40-42(partially)

Relating to a method of generating a recombinant bacteriophage genome comprising contacting the genome with a restriction enzyme in vitro and recombining the cleaved bacteriophage genome in vitro with a heterologous nucleic acid in the presence of a recombination system.

3. claims: 21-25(completely); 28-38, 40-42(partially)

Relating to a method of generating a recombinant bacteriophage genome comprising contacting the genome with a sgRNA-CRISPR enzyme conjugate or restriction enzyme in vitro and recombining the cleaved bacteriophage genome in vivo with a heterologous nucleic acid in the presence of a non-endogenous recombination system.

4. claims: 26, 27(completely); 28-38, 40-42(partially)

Relating to a method of generating a recombinant bacteriophage genome comprising transforming an intact genome into a host cell which expresses a heterologous nucleic acid and recombining the genome and nucleic acid in vivo in the presence of a non-endogenous recombination system.

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/024369

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>LAURA J. MARINELLI ET AL: "BRED: A Simple and Powerful Tool for Constructing Mutant and Recombinant Bacteriophage Genomes", PLOS ONE, vol. 3, no. 12, 17 December 2008 (2008-12-17), page e3957, XP055385275, DOI: 10.1371/journal.pone.0003957 table 3 page 7, left-hand column, paragraph 5 page 7, right-hand column, paragraphs 1, 2</p>	1-10,19, 20,33-39
A	<p>ALLISON M. BOX ET AL: "Functional analysis of bacteriophage immunity through a type I-E CRISPR-Cas system in Vibrio cholerae and its application in bacteriophage genome editing", JOURNAL OF BACTERIOLOGY, vol. 198, no. 3, 23 November 2015 (2015-11-23), pages 578-590, XP055385261, US ISSN: 0021-9193, DOI: 10.1128/JB.00747-15 page 586, paragraph 1 page 587, right-hand column, lines 4-7 figure 7</p>	1,3,19, 33
A	<p>BRUNO MARTEL ET AL: "CRISPR-Cas: an efficient tool for genome engineering of virulent bacteriophages", NUCLEIC ACIDS RESEARCH, vol. 42, no. 14, 24 July 2014 (2014-07-24), pages 9504-9513, XP055311115, ISSN: 0305-1048, DOI: 10.1093/nar/gku628 page 9510, left-hand column, paragraph 2</p>	1,3,19
A	<p>-& Bruno Martel ET AL: "CRISPR-Cas: an efficient tool for genome engineering of virulent bacteriophages Supplementary Data", Nucleic Acids Research, 24 July 2014 (2014-07-24), pages 9504-9513, XP055386011, DOI: 10.1093/nar/gku628 Retrieved from the Internet: URL:https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gku628 [retrieved on 2017-06-28] p.6, legend figure S1c</p>	1,3,19

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/024369

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RUTH KIRO ET AL: "Efficient engineering of a bacteriophage genome using the type I-E CRISPR-Cas system", RNA BIOLOGY, vol. 11, no. 1, 1 January 2014 (2014-01-01), pages 42-44, XP055385263, US ISSN: 1547-6286, DOI: 10.4161/rna.27766 -----	1,3,19
A	JIA-WANG WANG ET AL: "CRISPR/Cas9 nuclease cleavage combined with Gibson assembly for seamless cloning", BIOTECHNIQUES RAPID DISPATCHES, vol. 58, no. 4, 1 April 2015 (2015-04-01), pages 161-170, XP055385711, US ISSN: 0736-6205, DOI: 10.2144/000114261 page 164, left-hand column, paragraph 2 - middle column, paragraph 1 page 168, left-hand column, paragraph 2 page 169, middle column, paragraph 1 -----	1,6,20, 39
A	Jia-Wang Wang ET AL: "Supplementary material for: CRISPR/Cas9 nuclease cleavage combined with Gibson assembly for seamless cloning", BioTechniques, 1 April 2015 (2015-04-01), pages 1-2, XP055213775, England DOI: 10.2144/000114261 Retrieved from the Internet: URL: http://www.ncbi.nlm.nih.gov/pubmed/25861928 [retrieved on 2015-09-16] -----	1,6,20, 39
A	WENJUN JIANG ET AL: "Cas9-Assisted Targeting of CHromosome segments CATCH enables one-step targeted cloning of large gene clusters", NATURE COMMUNICATIONS, vol. 6, 1 September 2015 (2015-09-01), page 8101, XP055213782, DOI: 10.1038/ncomms9101 page 2, left-hand column, paragraph 3 - right-hand column, paragraph 1 page 6, right-hand column, paragraph 3 -----	1,6,20, 39
X,P	WO 2016/100389 A1 (SYNTHETIC GENOMICS INC [US]) 23 June 2016 (2016-06-23) examples I, XI figures 1, 2, 11 -----	1-6,19, 20,33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/024369

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016100389	A1	23-06-2016	
		AU 2015362618 A1	13-07-2017
		CA 2971205 A1	23-06-2016
		EP 3234136 A1	25-10-2017
		US 2016186147 A1	30-06-2016
		WO 2016100389 A1	23-06-2016
