



(51) International Patent Classification:

C12N 1/19 (2006.01) C12N 9/16 (2006.01)  
C12N 15/85 (2006.01) C07H 21/00 (2006.01)  
C12N 5/0735 (2010.01)

(21) International Application Number:

PCT/US2016/032049

(22) International Filing Date:

12 May 2016 (12.05.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/160,396 12 May 2015 (12.05.2015) US  
62/303,595 4 March 2016 (04.03.2016) US

(71) Applicant: SANGAMO BIOSCIENCES, INC. [US/US];  
501 Canal Blvd. Suite A100, Richmond, CA 94804 (US).

(72) Inventor: REIK, Andreas; c/o Sangamo Biosciences, Inc.,  
501 Canal Blvd. Suite A100, Richmond, CA 94804 (US).

(74) Agent: PASTERNAK, Dagna, S.; Pasternak Patent Law,  
1900 Embarcadero Rd. Suite 211, Palo Alto, CA 94303 (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, 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, KN, KP, KR, 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:  
29 December 2016

(54) Title: NUCLEASE-MEDIATED REGULATION OF GENE EXPRESSION

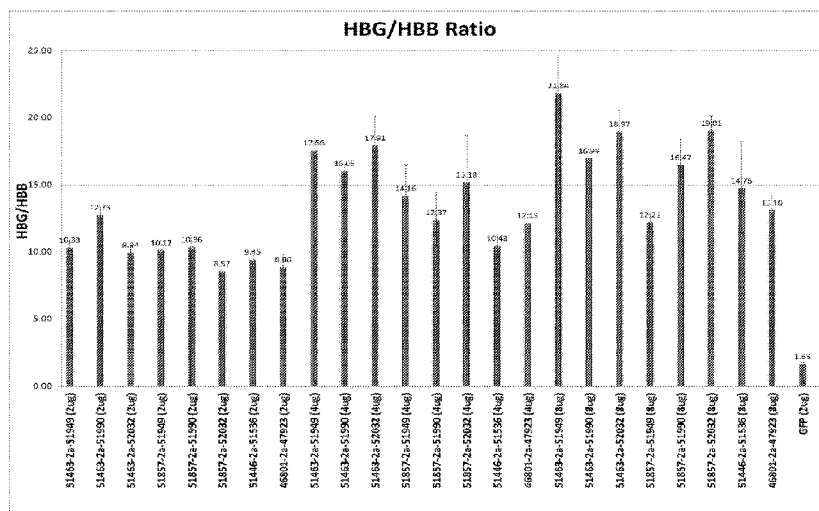


Figure 1

(57) Abstract: The present disclosure is in the field of genome engineering, particularly targeted modification of the genome of a hematopoietic cell.

WO 2016/183298 A3

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/US 16/32049

<p><b>A. CLASSIFICATION OF SUBJECT MATTER</b>                  IPC(8) - C12N 1/19, 15/85, 5/0735, 9/16; C07H 21/00 (2016.01)                  CPC - C07K 2319/81, 14/4702; C12N 15/85, 9/22                  According to International Patent Classification (IPC) or to both national classification and IPC</p>																				
<p><b>B. FIELDS SEARCHED</b></p> <p>Minimum documentation searched (classification system followed by classification symbols)                  IPC(8): C12N 1/19, 15/85, 5/0735, 9/16; C07H 21/00 (2016.01)                  CPC: C07K 2319/81, 14/4702; C12N 15/85, 9/22</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched                  CPC: C07K 2319/81, 14/4702; C12N 15/85, 9/22 (text search)                  USPC: 435/196, 372, 375, 69.7; 530/350; 536/23.4 (text search)</p> <p>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)                  Electronic data bases: PatBase; Google Patents; Google Scholar                  Search terms: Genome engineering, zinc finger protein, fusion protein, functional domain (e.g. cleavage domain, transcriptional activation domain, hematopoietic cell, stem cell, BCL11a enhancer sequence, targeted modification)</p>																				
<p><b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b></p> <table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th style="width:10%;">Category*</th> <th style="width:70%;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="width:20%;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td align="center">A</td> <td>US 2011/0082093 A1 (GREGORY et al.) 7 April 2011 (07.04.2011). Especially para [0016]</td> <td align="center">1-4</td> </tr> <tr> <td align="center">A</td> <td>WO 2013/179211 A1 (Evogene Ltd) 5 December 2013 (05.12.2013). Especially SEQ ID NO 8186</td> <td align="center">1-4</td> </tr> <tr> <td align="center">A</td> <td>US 2003/0068675 A1 (LIU et al.) 10 April 2003 (10.04.2003). Especially SEQ ID NO: 3030</td> <td align="center">1-4</td> </tr> <tr> <td align="center">A</td> <td>US 2012/0192301 A1 (JAENISCH et al.) July 26 2012 (07.26.2012). Especially SEQ ID NO: 26</td> <td align="center">1-4</td> </tr> <tr> <td align="center">A</td> <td>US 2014/0161873 A1 (Moderna Therapeutics Inc.) 12 June 2014 (12.06.2014). Especially SEQ ID NO: 6756</td> <td align="center">1-4</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	A	US 2011/0082093 A1 (GREGORY et al.) 7 April 2011 (07.04.2011). Especially para [0016]	1-4	A	WO 2013/179211 A1 (Evogene Ltd) 5 December 2013 (05.12.2013). Especially SEQ ID NO 8186	1-4	A	US 2003/0068675 A1 (LIU et al.) 10 April 2003 (10.04.2003). Especially SEQ ID NO: 3030	1-4	A	US 2012/0192301 A1 (JAENISCH et al.) July 26 2012 (07.26.2012). Especially SEQ ID NO: 26	1-4	A	US 2014/0161873 A1 (Moderna Therapeutics Inc.) 12 June 2014 (12.06.2014). Especially SEQ ID NO: 6756	1-4
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																		
A	US 2011/0082093 A1 (GREGORY et al.) 7 April 2011 (07.04.2011). Especially para [0016]	1-4																		
A	WO 2013/179211 A1 (Evogene Ltd) 5 December 2013 (05.12.2013). Especially SEQ ID NO 8186	1-4																		
A	US 2003/0068675 A1 (LIU et al.) 10 April 2003 (10.04.2003). Especially SEQ ID NO: 3030	1-4																		
A	US 2012/0192301 A1 (JAENISCH et al.) July 26 2012 (07.26.2012). Especially SEQ ID NO: 26	1-4																		
A	US 2014/0161873 A1 (Moderna Therapeutics Inc.) 12 June 2014 (12.06.2014). Especially SEQ ID NO: 6756	1-4																		
<p><input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/></p>																				
<p>* Special categories of cited documents:</p> <table style="width:100%;"> <tr> <td style="width:50%;"> <p>“A” document defining the general state of the art which is not considered to be of particular relevance</p> <p>“E” earlier application or patent but published on or after the international filing date</p> <p>“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)</p> <p>“O” document referring to an oral disclosure, use, exhibition or other means</p> <p>“P” document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width:50%;"> <p>“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</p> <p>“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</p> <p>“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</p> <p>“&amp;” document member of the same patent family</p> </td> </tr> </table>			<p>“A” document defining the general state of the art which is not considered to be of particular relevance</p> <p>“E” earlier application or patent but published on or after the international filing date</p> <p>“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)</p> <p>“O” document referring to an oral disclosure, use, exhibition or other means</p> <p>“P” document published prior to the international filing date but later than the priority date claimed</p>	<p>“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</p> <p>“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</p> <p>“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</p> <p>“&amp;” document member of the same patent family</p>																
<p>“A” document defining the general state of the art which is not considered to be of particular relevance</p> <p>“E” earlier application or patent but published on or after the international filing date</p> <p>“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)</p> <p>“O” document referring to an oral disclosure, use, exhibition or other means</p> <p>“P” document published prior to the international filing date but later than the priority date claimed</p>	<p>“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</p> <p>“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</p> <p>“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</p> <p>“&amp;” document member of the same patent family</p>																			
<p>Date of the actual completion of the international search</p> <p>17 October 2016</p>		<p>Date of mailing of the international search report</p> <p align="center"><b>16 NOV 2016</b></p>																		
<p>Name and mailing address of the ISA/US</p> <p>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents                  P.O. Box 1450, Alexandria, Virginia 22313-1450                  Facsimile No. 571-273-8300</p>		<p>Authorized officer:</p> <p align="center">Lee W. Young</p> <p>PCT Helpdesk: 571-272-4300                  PCT OSP: 571-272-7774</p>																		

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/32049

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a.  forming part of the international application as filed:
    - in the form of an Annex C/ST.25 text file.
    - on paper or in the form of an image file.
  - b.  furnished together with the international application under PCT Rule 13ter. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c.  furnished subsequent to the international filing date for the purposes of international search only:
    - in the form of an Annex C/ST.25 text file (Rule 13ter. 1(a)).
    - on paper or in the form of an image file (Rule 13ter. 1(b) and Administrative Instructions, Section 713).
2.  In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

GenCore ver 6.4.1 SEQ ID NOs: 2, 5, 6, 7 were specifically examined as part of the International Search & Opinion.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/32049

**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.: 5-22, 25  
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:  
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claim 1-4, drawn to a zinc finger protein comprising 4, 5 or 6 fingers designated F1 to F4, F1 to F5 or F1 to F6, each finger comprising a recognition helix region that recognizes a target subsite.

The zinc finger protein will be searched to the extent that the zinc finger recognition helix encompasses the first named zinc finger recognition helix (claim 1(i)), F1: STGNLTN (SEQ ID NO:7); F2: TSGSLTR (SEQ ID NO:5); F3: DQSNLRA (SEQ ID NO:2); and F4: AQCCLFH (SEQ ID NO:6). It is believed that claims 1-4 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass the recognition helices defined as SEQ ID NOS: 7, 5, 2, 6. Additional zinc finger recognition helices will be searched upon payment of additional fees.

----Go to Extra Sheet for continuation----

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 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.: 1-4, limited to SEQ ID NOS: 7, 5, 2, 6

**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

International application No.

PCT/US 16/32049

-----continuation of Box III (Lack of Unity of Invention)-----

Applicant must specify the claims that encompass any zinc finger recognition helix(s). Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: recognition helix (claim 1 (ii)) F1: DQSNLRA (SEQ ID NO:2); F2: RPYTLRL (SEQ ID NO:3); F3: SRGALKT (SEQ ID NO:8); F4: TSGSLTR (SEQ ID NO:5); F5: DQSNLRA (SEQ ID NO:2); and F6: AQCCLFH (SEQ ID NO:6); (claims 1-4).

Group II: Claims 23, 24, drawn to a method of producing a genetically modified cell comprising a genomic modification within an endogenous BCL11A enhancer sequence.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ has the special technical feature of a composition of zinc finger comprising a specific recognition helix region, not required by Group II.

Group II has the special technical feature of a method for genomic modification within an endogenous BCL11A enhancer sequence, not required by Group I+.

Among the inventions listed as Groups I+ are the specific sequences recited therein and their specific consecutive appearance to define a recognition helix. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained among sequences or the recognition helices.

Common Technical Feature:

Groups I+ share the common technical feature of claim 1, and Group II shares the common technical feature of Group I+ claims 2 or 3.

However, said common technical feature do not represent a contribution over the prior art, and is anticipated by US 2011/0082093 A1 to GREGORY et al. (hereinafter "Gregory").

As to claim 1, Gregory teaches a zinc finger protein comprising 4, 5 or 6 fingers designated F1 to F4, F1 to F5 or F1 to F6, each finger comprising a recognition helix region that recognizes a target subsite (para [0016]; "Engineered zinc finger proteins are non-naturally occurring zinc finger proteins whose recognition helices have been altered (e.g., by selection and/or rational design) to bind to a pre-selected target site. Any of the zinc finger proteins described herein may include 1, 2, 3, 4, 5, 6 or more zinc fingers, each zinc finger having a recognition helix that binds to a target subsite in the selected sequence").

As to claim 2, Gregory further teaches a fusion protein comprising the zinc finger protein of claim 1 and a functional domain (para [0017]; In certain embodiments, the zinc finger proteins (ZFPs) as described herein can be placed in operative linkage with a regulatory domain (or functional domain) as part of a fusion protein. By selecting either an activation domain or repression domain for fusion with the ZFP, such fusion proteins can be used either to activate or to repress gene expression")

As to claim 3, Gregory further teaches that the fusion protein of claim 2, wherein the functional domain is a transcriptional activation domain, a transcriptional repression domain (para [0017]).

As the common technical feature was known in the art at the time of the invention, this cannot be considered a common special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I+ and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning item 4: Claims 5-22 and 25 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).