IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Fuqiang CHEN et al.  
Art Unit: 1636

Serial Nos.: 15/188,911; 15/188,924; & 15/456,204
Examiner: Jennifer Ann DUNSTON

Filed: June 21, 2016  
Conf. Nos.: 8930; 2753; & 1023

For: CRISPR-BASED GENOME MODIFICATION AND REGULATION  
October 17, 2019


TO THE DIRECTOR AND THE CHIEF ADMINISTRATIVE PATENT JUDGE:

This Renewed Petition renews Sigma-Aldrich’s original Petition, dated July 19, 2019, and responds to the Office’s Decision on Petition in each of the above-captioned applications, dated September 23, 2019, signed by the Vice Chief APJ. Sigma-Aldrich appreciates the attention given to this urgent matter by the Office, and thanks the Vice Chief APJ in particular for his consideration and analysis. Sigma-Aldrich notes that the Decision on Petition concludes that “Applicants’ petition is dismissed without prejudice” (Dec. on Pet. at 6 (emphasis in original)). Thus, Sigma-Aldrich believes that the Office expressly contemplates the filing of a follow-on petition, such as this Renewed Petition presented by Sigma-Aldrich here, to address the comments in the Decision onPetition.¹

¹ The Decision on Petition characterizes the original Petition as an “improper” filing of multiple petitions on different matters. Dec. on Pet. at 5 (citing 37 C.F.R. § 1.4(c)). Respectfully, Sigma-Aldrich’s original Petition was directed to a single basic issue with a single request for relief. Because the Office may address the petition and provide the requested relief under different rules and through different offices, Sigma-Aldrich believes that efficiency warrants a single petition, allowing the Office to determine its preferred manner and appropriate office for addressing the petition. Sigma-Aldrich’s approach is common in petition practice, and avoids needless redundacy. In any event, Sigma-Aldrich would be amenable to filing multiple (albeit redundant) petitions if the Office indicates that such an approach would be preferable and, in its view, more compliant with Rule 1.4(c).
For the sake of brevity, Sigma-Aldrich hereby incorporates by reference the entirety of its original Petition in this Renewed Petition. In addition, this Renewed Petition addresses the comments from the Office in its recent Decision on Petition.

I. SIGMA-ALDRICH’S LACK OF AN ALLOWED CLAIM IS THE FUNDAMENTAL INJUSTICE THAT SIGMA-ALDRICH’S PETITIONS SEEK TO REMEDY

The Decision on Petition provides two separate and distinct bases for dismissing Sigma-Aldrich’s original Petition: (1) Sigma-Aldrich’s lack of an allowed claim in its pending patent applications at issue; and (2) Sigma-Aldrich’s lack of a statement under 37 C.F.R. § 41.102 (“202 Statement”) in those applications’ prosecution records. Dec. at 5-6. With regard to the first basis, the decision states:

> Because at least one of Applicants’ claims has not been found patentable, and examination of the application has not been completed in accordance with 37 C.F.R. § 41.102, Applicants’ request to declare an interference is premature.

Dec. at 6.

Of course, it is the Examiner’s steadfast and repeated refusals to allow Sigma-Aldrich’s pending claims that compelled Sigma-Aldrich to file the original Petition. As the original Petition explains in considerable detail, the Examiner has repeatedly determined that Broad Inst.’s CRISPR-Cas9 eukaryotic claims are patentable over UC’s CRISPR-Cas9 prokaryotic P1 and P2 provisional applications. Pet. at 7-8. But the Examiner (including the same SPE) continues to reject Sigma-Aldrich’s substantively identical claims over the same UC disclosures. Id. Indeed, as Sigma-Aldrich noted at the outset of its original Petition:

> Sigma-Aldrich recognizes, of course, that its pending applications’ claims have not yet been allowed, and thus declaring a patent interference now would – in ordinary circumstances – be premature. However, the facts here are truly extraordinary, and Sigma-Aldrich feels compelled to apprise the Director and the CAPJ of the current situation and to briefly explain why the PTAB’s declaration of a parallel interference in this instance would be in the long-term best interests of everyone, including the USPTO, the parties, and the public.

_Id._ at 1-2.

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2 UC’s disclosures are of CRISPR-Cas9 in _in vitro_ cell-free and nucleus-free test tube environments, called a “prokaryotic environment” in the original Petition.
In fact, Sigma-Aldrich respectfully submits that examination of the applications at issue is, for all practical purposes, effectively “completed” under the meaning of 37 C.F.R. § 41.102 because the only remaining issues regarding the patentability of Sigma-Aldrich’s pending claims are the anticipation and obviousness rejections over the UC disclosures. And, as Sigma-Aldrich explained in detail in the original Petition, those rejections are entirely meritless. See Pet., passim. Accordingly, Sigma-Aldrich’s pending applications should be promptly allowed and an interference with UC should be declared.

Sigma-Aldrich therefore renews its request that the Director and/or the CAPJ address the fundamental inconsistency and unfairness of the Examiner’s actions in the prosecution of Sigma-Aldrich’s pending applications, which directly contravene the PTAB’s and the Federal Circuit’s decisions. Respectfully, simply noting that Sigma-Aldrich lacks any allowed claims fails to provide any reasoned analysis that addresses Sigma-Aldrich’s detailed discussion in its original Petition, and fails to provide any rationale for the Examiner’s diametrically opposite treatment of two similarly situated applicants (viz., Sigma-Aldrich and Broad Inst.) and purposeful straight-arming of binding legal authority.

II. SIGMA-ALDRICH HAS NOW PREPARED AND FILED A THOROUGH AND COMPELLING 202 STATEMENT, CITING EACH OF THE APPLICATIONS AT ISSUE

With regard to the second basis for dismissing Sigma-Aldrich’s original Petition, the decision states:

Specifically, Applicants’ petition does not comply with the requirements of 37 C.F.R. §§ 41.202(a) and 41.203(d) for suggestions regarding an interference or the addition of an application to an interference... Absent such information, the Board lacks a sufficient basis to entertain the suggestion of an interference.

Dec. at 6.

Sigma-Aldrich readily acknowledges the usefulness of a 202 Statement in evaluating whether to declare an interference. Indeed, Sigma-Aldrich offered to prepare such a statement at the conclusion of its original Petition:

The undersigned practitioners remain fully prepared to assist the PTAB in formalizing the declaration of that proposed interference in an
expedited manner, including promptly filing a complete statement under 37 C.F.R. § 41.202.

Pet. at 12.

Accordingly, in response to the Decision on Petition (and in particular to the above-noted dismissal “without prejudice”), Sigma-Aldrich has prepared and filed (concurrently herewith) a 202 Statement in each of the applications at issue. For the convenience of the Director, Chief APJ, and Vice Chief APJ, the 202 Statement is attached hereto as an Exhibit. A review of Sigma-Aldrich’s thorough 202 Statement reveals that Sigma-Aldrich has a compelling basis for the Office to declare an interference between Sigma-Aldrich’s applications and UC’s applications and/or patents.

III. REGULATORY AUTHORITY FOR THIS PETITION AND PAYMENT OF THE REQUISITE PETITION FEES

Sigma-Aldrich brings this Petition to the Director pursuant to 37 C.F.R. §§ 1.181-1.183, and to the CAPJ pursuant to 37 C.F.R. §§ 41.3 & 41.103. Those regulatory provisions provide, in relevant part:

1.181 Petition to the Director.

(a) Petition may be taken to the Director:

   (3) To invoke the supervisory authority of the Director in appropriate circumstances.

1.182 Questions not specifically provided for.

All situations not specifically provided for in the regulations of this part will be decided in accordance with the merits of each situation by or under the authority of the Director, subject to such other requirements as may be imposed, and such decision will be communicated to the interested parties in writing.

1.183 Suspension of rules.

In an extraordinary situation, when justice requires, any requirement of the regulations in this part which is not a requirement of the statutes may be suspended or waived by the Director or the Director’s designee, sua sponte, or on petition of the interested party, subject to such other requirements as may be imposed.
41.3 Petitions.

(a) Deciding official. Petitions must be addressed to the Chief Administrative Patent Judge.
(b) Scope. This section covers petitions on matters pending before the Board (§§ 41.35, 41.64, 41.103, and 41.205[.])

41.103 Jurisdiction over involved files.

The Board acquires jurisdiction over any involved file when the Board initiates a contested case.

In addition, the provisions of 37 C.F.R. §§ 1.181-1.183 state that "[a]ny petition seeking a decision under this section must be accompanied by the petition fee set forth in § 1.17(f)," and the provisions of 37 C.F.R. §§ 41.3 & 41.103 state that "[t]he fee set in § 41.20(a) must accompany any petition under this section." Accordingly, submitted herewith are both of these petition fees under 37 C.F.R. § 1.17(f) and 37 C.F.R. § 41.20(a). Sigma-Aldrich believes that no additional fees are due with the filing of this Petition. The Commissioner, however, is hereby authorized to charge any fees, or credit any overpayment, to Deposit Account 50-5915.

IV. CONCLUSION

In light of the foregoing, Sigma-Aldrich respectfully renews its request that, with the Director's consult and at the Director's direction, the PTAB declare a parallel UC v. Sigma-Aldrich interference.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Fuqiang CHEN et al.  Art Unit: 1636
Serial Nos.: 15/188,911 & 16/654,613  Examiner: Jennifer Ann DUNSTON
Filed: June 21, 2016 & Oct. 16, 2019  Conf. Nos.: 8930 & 6509
For: CRISPR-BASED GENOME MODIFICATION AND REGULATION

October 17, 2019

SUGGESTION OF INTERFERENCE PURSUANT TO 37 C.F.R. § 41.202

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicant Sigma-Aldrich Co. LLC respectfully requests that an interference be declared involving (1) at least Claim 1 of U.S. Patent Application No. 15/188,911 (“the Sigma ’911 Application”), which is concurrently filed herewith in an Amendment and Reply; and (2) at least Claim 1 of U.S. Patent Application No. 16/654,613 (“the Sigma ’613 Application), which Applicant filed on Wednesday, October 16, 2019.

Pursuant to 37 C.F.R. § 41.202, Applicant provides, inter alia: (1) an identification of the patents with which Applicant seeks an interference; (2) proposed counts and claim correspondence thereto; (3) an identification of claims that Applicants believe interfere; (4) an explanation of why Applicant will prevail on priority; (5) claim charts showing written description in Applicant’s specification for each claim added to provoke the interference; and (6) for each constructive reduction to practice for which the applicant wishes to be accorded benefit, provide a chart showing where the disclosure provides a constructive reduction to practice within the scope of the interfering subject matter.
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**APPENDIX III** – Support for Claim 1 of the Sigma ‘911 Application in the ‘911 Specification

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**APPENDIX VI** – Support for Claim 1 of the Sigma ‘613 Application in U.S. Provisional Application No. 61/734,256
I. 37 C.F.R. § 41.202(a)(1) – Identification of Patents With Which Applicant Seeks an Interference

Applicant requests that an interference be declared between the Sigma ‘911 Application and U.S. Patent Application No. 15/947,718 ("the UCB ‘718 Application").

Applicant also requests that an interference be declared between the Sigma ‘613 Application and U.S. Patent Application No. 15/981,809 ("the UCB ‘809 Application").

At filing, the Sigma ‘911 Application and the Sigma ‘613 Application listed Fuqiang Chen and Greg Davis as co-inventors. According to the USPTO's assignment records, Drs. Chen and Davis have assigned their interest in the Sigma ‘911 Application and the Sigma ‘613 Application to Sigma-Aldrich Co. LLC. The Sigma ‘911 Application and the Sigma ‘613 Application each claim priority benefit to U.S. Provisional Application No. 61/734,256 filed December 6, 2012, U.S. Provisional Application No. 61/758,624 filed January 30, 2013; U.S. Provisional Application No. 61/761,046 filed February 5, 2013, and U.S. Provisional Application No. 61/794,422 filed March 15, 2013.

With regard to the UCB ‘718 Application and the UCB ‘809 Application, each lists Jennifer A. Doudna, Martin Jinek, Krzysztof Chylinski, and Emmanuelle Charpentier as co-inventors. According to the USPTO’s assignment records, Jennifer A. Doudna and Martin Jinek assigned their rights in the UCB ‘718 Application and the UCB ‘809 Application to The Reagents of the University of California, and Krzysztof Chylinski assigned his interest in the UCB ‘718 Application and the UCB ‘809 Application to the University of Vienna. The UCB ‘718 Application and the UCB ‘809 Application claim priority benefit to U.S. Provisional Application No. 61/652,086 filed May 25, 2012; U.S. Provisional Application No. 61/716,256 filed October 19, 2012; U.S. 61/757,640 filed January 28, 2013; and U.S. Provisional Application No. 61/765,576 filed February 15, 2013.

II. 37 C.F.R. § 41.202(a)(2) and 37 C.F.R. § 41.202(a)(3) – Proposal of Counts, Claim Correspondence, and Identification of All Claims Applicant Believes Interfere

Pursuant to 37 C.F.R. § 41.202(a)(2), Applicant hereby proposes two Counts, sets forth the claims from the Sigma and UCB Applications that correspond to each Count, and identifies the claims Applicant believes interfere.
Proposed Count 1 is directed to methods and Proposed Count 2 is directed to compositions.

A. Proposed Count 1 – Methods

Proposed Count 1 is directed to methods of cleaving or editing a target DNA molecule or target chromosomal sequence in a eukaryotic cell by employing a Type-II CRISPR Cas system. Proposed Count 1 is an alternative (aka “McKelvey”) count: “Claim 1 of the Sigma ‘911 Application OR Claim 156 of the UCB ‘718 Application.” The following Table 1 presents each alternative of Proposed Count 1:

<table>
<thead>
<tr>
<th>Alternative Count 1A – Claim 1 of the Sigma ‘911 Application</th>
<th>Alternative Count 1B – Claim 156 of the UCB ‘718 Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising: introducing into the eukaryotic cell</td>
<td>156. A method of cleaving or editing a target DNA molecule or modulating transcription of at least one gene encoded thereon, the method comprising contacting a target DNA molecule having a target sequence with</td>
</tr>
<tr>
<td>(i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas type II protein is codon optimized for expression in the eukaryotic cell, and</td>
<td>(a) a Cas9 protein; and</td>
</tr>
<tr>
<td>wherein the CRISPR-Cas type II system protein is a Cas9 protein,</td>
<td></td>
</tr>
</tbody>
</table>
(1) a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and

(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and

(iii) at least one donor polynucleotide comprising the exogenous sequence; and

whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process leads to integration of the exogenous sequence into the chromosomal sequence.

(i) a targeter-RNA that hybridizes with the target sequence, and

(ii) an activator-RNA that hybridizes with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,

wherein said contacting takes place outside of a bacterial cell and outside an archaeal cell,

wherein the DNA-targeting RNA forms a complex with the Cas9 protein,

whereby said target DNA molecule is cleaved or edited or transcription of at least one gene encoded by the target DNA molecule is modulated,

wherein said contacting occurs in a eukaryotic cell.

1. Claims That Correspond to Proposed Count 1

“A claim corresponds to a count if the subject matter of the count, treated as prior art to the claim, would have anticipated or rendered obvious the subject matter of the claim.” 37 C.F.R. § 41.207(b)(2). The following Table 2 sets forth the claims from the Sigma Applications and Claims, and the claims from the UCB Applications and Claims, that correspond to Proposed Count 1:

**TABLE 2**

<table>
<thead>
<tr>
<th>Proposed Count 1 - Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigma Applications and Claims that Correspond</td>
</tr>
<tr>
<td>‘911 Application – Claim 1</td>
</tr>
</tbody>
</table>
2. Identification of Interfering Claims

At a minimum, Claim 1 of the Sigma ‘911 Application defines the same invention as Claim 156 of the UCB ‘718 Application. A detailed comparison of Claim 1 of the Sigma ‘911 Application with Claim 156 of the UCB ‘718 Application is provided in Appendices IA and IB.

B. Proposed Count 2 – Compositions

Proposed Count 2 is directed to compositions, more specifically eukaryotic cells that are modified using a Type-II CRISPR Cas system. Proposed Count 1 is an alternative (aka “McKelvey”) count: “Claim 1 of the Sigma ‘613 Application OR Claim 156 of the UCB ‘809 Application.” The following Table 3 presents each alternative of Proposed Count 2:

<table>
<thead>
<tr>
<th>Alternative Count 2A – Claim 1 of the Sigma ‘613 Application</th>
<th>Alternative Count 2B – Claim 156 of the UCB ‘809 Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A eukaryotic cell comprising</td>
<td>156. A eukaryotic cell comprising</td>
</tr>
<tr>
<td>a target chromosomal sequence; and</td>
<td>a target DNA molecule and</td>
</tr>
<tr>
<td>(i) at least one RNA-guided endonuclease comprising at least</td>
<td>an engineered and/or non-naturally occurring Type II Clusters</td>
</tr>
<tr>
<td>one nuclear localization signal or nucleic acid encoding at</td>
<td>Regularly Interspersed Short Palindromic Repeats (CRISPR)</td>
</tr>
<tr>
<td>least one RNA-guided endonuclease comprising at least one</td>
<td>(CRISPR)-CRISPR associated (Cas)</td>
</tr>
<tr>
<td>nuclear localization signal, wherein the at least one</td>
<td>(CRISPR-Cas) system comprising</td>
</tr>
<tr>
<td>RNA-guided endonuclease is a clustered regularly interspersed</td>
<td></td>
</tr>
<tr>
<td>short palindromic repeats (CRISPR)/CRISPR associated (Cas)</td>
<td></td>
</tr>
<tr>
<td>(CRISPR-Cas) type II system protein, wherein the nucleic</td>
<td></td>
</tr>
<tr>
<td>acid encoding the CRISPR-Cas9 type II</td>
<td></td>
</tr>
</tbody>
</table>
protein is codon optimized for expression in the eukaryotic cell, and

wherein the CRISPR-Cas type II system protein is a Cas9 protein, and

(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising

(1) a first region at the 5′ end that is capable of base pairing with a target site in the chromosomal sequence, and

(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and

whereby base pairing of the first region to the target site is capable of targeting the Cas9 protein to the target chromosomal sequence.

a Cas9 protein or a nucleic acid comprising a nucleotide sequence encoding the Cas9 protein, and

a DNA-targeting RNA or one or more nucleic acids comprising one or more nucleotide sequences encoding the DNA-targeting RNA; wherein the DNA-targeting RNA comprises

i) a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule, and

ii) an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,

whereby hybridization of the targeter-RNA to the target sequence is capable of targeting the Cas9 protein to the target DNA molecule.

1. Claims That Correspond to Proposed Count 2

“A claim corresponds to a count if the subject matter of the count, treated as prior art to the claim, would have anticipated or rendered obvious the subject matter of the claim.” 37 C.F.R. § 41.207(b)(2). The following Table 4 sets forth the claims from the Sigma Applications and Claims, and the claims from the UCB Applications and Claims, that correspond to Proposed Count 2:

| TABLE 4 |
|-----------------|-----------------|
| **Proposed Count 2 – Compositions** | |
| Sigma Applications and Claims that Correspond | UCB Applications and Claims that Correspond |
| '613 Application – Claim 1 | ‘809 Application – Claim 156 |
| | U.S. Patent Application No. 15/981,808 – Claim 156 |
2. Identification of Interfering Claims

At a minimum, Claim 1 of the Sigma ‘613 Application defines the same invention as Claim 156 of the UCB ‘809 Application. A detailed comparison of Claim 1 of the Sigma ‘613 Application with Claim 156 of the UCB ‘809 Application is provided in Appendices IIA and IIB.

C. Other UCB Patents and Patent Applications That May Correspond to Proposed Count 1 and/or Proposed Count 2

Applicant reserves the right to supplement its request to suggest an interference with any one or more of the following (or other) UCB patent applications and patents:

- U.S. Patent Application Nos. 15/947,680; 15/981,807; 16/136,159; 16/136,165; 16/136,168; and/or 16/136,175; and/or
- U.S. Patent Nos. 10,000,772; 10,113,167; 10,227,611; 10,266,850; 10,301,651; 10,308,961; 10,337,029; 10,351,878; 10,358,658; 10,358,659; 10,385,360; 10,400,253; 10,407,697; 10,415,061; 10,421,980; and/or 10,428,352.

Applicant notes that each of the aforementioned UCB patent applications and patents listed in Table 2, Table 4, and the first and second bullet points above are involved in presently pending Patent Interference No. 106,115 with several patents to The Broad Institute/Massachusetts Institute of Technology/President and Fellows of Harvard College.

IV. 37 C.F.R. § 41.202(a)(4) – Why Applicant Will Prevail on Priority

Applicant will prevail on priority because Applicant was the first to invent the subject matter of the Proposed Counts, namely, methods and compositions of CRISPR-Cas9 in eukaryotic cells. In particular, Applicant’s earliest-filed benefit application is as follows:

- U.S. Provisional Application No. 61/734,256, filed December 6, 2012 (“Chen P1”).
As set forth herein and in Appendices IV and VI, Chen P1 provides a constructive reduction to practice of the Proposed Counts (i.e., a described and enabled anticipation under 35 U.S.C. § 102(g)(1) of the subject matter of the Proposed Counts). Thus, Applicant has a constructive reduction to practice date of December 6, 2012, and an even earlier date for an actual reduction to practice.

UCB’s earliest-filed applications are as follows:

- U.S. Provisional App. No. 61/652,086, filed May 25, 2012 ("Doudna P1");
- U.S. Provisional Application No. 61/716,256, filed October 19, 2012 ("Doudna P2"); and
- U.S. Provisional Application No. 61/757,640, filed January 28, 2013 ("Doudna P3").

Importantly, while Doudna P1 and Doudna P2 pre-date Chen P1, those UCB provisional applications are directed to CRISPR-Cas9 in *in vitro* cell-free and nucleus-free test tube environments (a “prokaryotic environment”). The Declarations of Drs. Lambowitz, Loring, Urnov, and Cannon, which are of record in the involved Sigma-Aldrich patent applications, confirm the lack of any reasonable expectation of success that one of ordinary skill in the art would have had at the time of the invention to use the disclosed CRISPR-Cas9 prokaryotic system in eukaryotic cells. The Declarations also discuss in detail the failures and considerable barriers that were known to one of ordinary skill in the art at the time of the invention that contributed to the doubt and uncertainty of successfully using a CRISPR-Cas9 system in eukaryotic cells.

Furthermore, these Declarations confirm that the incomplete and insufficient disclosure in Doudna P1, Doudna P2, and Jinek 2012 would not have enabled one of ordinary skill in the art at the time of the invention to practice a CRISPR-Cas9 system adapted to function in a eukaryotic cell without undue experimentation, nor do they provide a written description demonstrating possession of a CRISPR-Cas9 system adapted to function in a eukaryotic cell:
19. In my considered opinion, set forth in detail below, the incomplete and insufficient disclosure in Doudna P1, Doudna P2, and Jinek 2012 does not enable one of ordinary skill in the art to practice a CRISPR-Cas9 system adapted to function in a eukaryotic cell without undue experimentation, nor do they provide a written description demonstrating possession of a CRISPR-Cas9 system adapted to function in a eukaryotic cell. Referring to Wands Factor (1), Doudna P1, Doudna P2, and Jinek 2012 provide no useful guidance for one of ordinary skill to adapt CRISPR-Cas9 for use in eukaryotic cells. Regarding Wands Factor (2), adapting CRISPR-Cas9 to function in eukaryotic cells required extensive experimentation, as shown by Dr. Doudna's own experiences, which she admitted resulted in "many frustrations." Regarding Factor (3), Doudna P1, Doudna P2, and Jinek 2012 contain no working examples of CRISPR-Cas9 functioning in eukaryotic cells. Regarding Wands Factors (4), (5), and (6), given the nascent state of CRISPR-Cas technology, the nature of the disclosure requires more details than may be needed in other instances. Finally, regarding Wands Factors (7) and (8), the field is highly unpredictable and even Dr. Doudna admitted that she "[wasn't] sure if CRISPR/Cas9 would work in eukaryotes.

20. Furthermore, in my considered opinion, set forth in detail below, based at least upon the incomplete and insufficient disclosure in Doudna P1, Doudna P2, and Jinek 2012 one of ordinary skill in the art would not have had a reasonable expectation of success that a CRISPR-Cas9 system could be adapted to function in a eukaryotic cell.


Notably, the Declaration evidence of record is even stronger than that considered by the PTAB and the Federal Circuit in the first UC v. Broad interference (Int'f No. 106,048) and appeal. See Broad Inst., Inc. v. Regents of the Univ. of Cal., Decision on Motions, Int’f No. 106,048 (DK), 2017 WL 657415, Paper 893 (PTAB Feb. 15, 2017); Regents of the Univ. of Cal. v. Broad Inst., Inc., 903 F.3d 1286, 1294 (Fed. Cir. 2018).

Accordingly, UCB’s earliest possible constructive reduction to practice of the Proposed Counts is January 28, 2013, nearly two months later than Applicants’ date.
And while it is possible that UCB has an earlier actual reduction to practice date, it is highly speculative and unlikely that UCB would be able to pre-date Applicant’s considerably earlier date of invention. This is particularly true given the PTAB’s stringent and exacting standards for proving conception, diligence, and reduction to practice. Thus, Applicant is likely to prevail on priority of invention for each of the two Proposed Counts.

V. 37 C.F.R. §§ 41.202(a)(5) & (a)(6) – Claim Charts Showing Written Description in Applicant’s Specification and Benefit Application

A. The Sigma ‘911 Application

The Sigma ‘911 Application is a 35 U.S.C. § 120 Continuation of U.S. Patent Application No. 14/649,777, which is a 35 U.S.C. § 371 National Stage application from PCT International Application No. PCT/US2013/073307. A chart is provided in Appendix III showing where exemplary written description support for Claim 1 of the Sigma ‘911 Application can be found in the Sigma ‘911 Specification.

The Sigma ‘911 Application also claims the benefit of, inter alia, U.S. Provisional Application No. 61/734,256 filed December 6, 2012. A chart is provided in Appendix IV showing where exemplary written description support for Claim 1 of Sigma ‘911 Application can be found in U.S. Provisional Application No. 61/734,256.

B. The Sigma ‘613 Application

The Sigma ‘613 Application is a 35 U.S.C. § 120 Continuation of the Sigma ‘911 Application, which is a 35 U.S.C. § 120 Continuation of U.S. Patent Application No. 14/649,777, which is a 35 U.S.C. § 371 National Stage application from PCT International Application No. PCT/US2013/073307. A chart is provided in Appendix V showing where exemplary written description support for Claim 1 of the Sigma ‘613 Application can be found in the Sigma ‘613 Specification.

The Sigma ‘613 Application also claims the benefit of, inter alia, U.S. Provisional Application No. 61/734,256 filed December 6, 2012. A chart is provided in Appendix VI showing where exemplary written description support for Claim 1 of the Sigma ‘613 Application can be found in U.S. Provisional Application No. 61/734,256.
VI. Conclusion

Applicant respectfully requests a declaration of interference between (1) the Sigma '911 Application and the UCB '718 Application with Proposed Count 1; and (2) the Sigma '613 Application and the UCB '809 Application with Proposed Count 2.

Respectfully submitted,

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## APPENDIX IA - COLOR

### SIDE-BY-SIDE COMPARISON OF CLAIM 1 OF SIGMA’S U.S. PATENT APPLICATION NO. 15/188,911
WITH CLAIM 156 OF UCB’S U.S. PATENT APPLICATION NO. 15/947,718

<table>
<thead>
<tr>
<th>UCB’S USSN 15/947,718</th>
<th>SIGMA’S USSN 15/188,911</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>156. A method of cleaving or editing a target DNA molecule or modulating transcription of at least one gene encoded thereon, the method comprising:</td>
<td>1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising:</td>
<td>Sigma’s “integrating” ≈ UCB’s “editing.” “Integration” of an exogenous sequence into a chromosomal sequence” requires “cleaving” the chromosomal sequence first.</td>
</tr>
<tr>
<td>contacting a target DNA molecule having a target sequence with an engineered and/or non-naturally-occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising:</td>
<td>introducing into the eukaryotic cell (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas type II system protein is codon optimized for expression in the eukaryotic cell, and</td>
<td>Sigma and UCB’s claims each require a Type II CRISPR/Cas system.</td>
</tr>
<tr>
<td>(a) a Cas9 protein; and</td>
<td>wherein the CRISPR-Cas type II system protein is a Cas9 protein;</td>
<td>Sigma and UCB’s claims each require a Cas9 protein.</td>
</tr>
<tr>
<td>(b) a DNA-targeting RNA comprising:</td>
<td>(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising</td>
<td>Sigma’s “guide RNA” ≈ UCB’s “DNA-targeting RNA.”</td>
</tr>
<tr>
<td>(i) a targeter-RNA that hybridizes with the target sequence, and</td>
<td>(1) a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and</td>
<td>Sigma’s “first region” ≈ UCB’s “targeter-RNA.” Sigma’s “base pairs with a target...” ≈ UCB’s “hybridizes with the target...”</td>
</tr>
<tr>
<td>(ii) an activator-RNA that hybridizes with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,</td>
<td>(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and</td>
<td>Sigma’s “second region” ≈ UCB’s “activator-RNA.”</td>
</tr>
<tr>
<td>Wherein said contacting takes place outside of a bacterial cell and outside an archaeal cell, wherein the DNA-targeting RNA forms a complex with the Cas9 protein,</td>
<td>(iii) at least one donor polynucleotide comprising the exogenous sequence; and Sigma’s claim requires a “donor polynucleotide” that is “integrated” into the eukaryotic chromosome thereby “editing” it.</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Whereby said target DNA molecule is cleaved or edited or transcription of at least one gene encoded by the target DNA molecule is modulated,</td>
<td>Sigma’s claim is directed to modifying a eukaryotic cell.</td>
<td></td>
</tr>
<tr>
<td>Wherein said contacting occurs in a eukaryotic cell.</td>
<td>The “second region” of Sigma’s “guide RNA” “interacts with the at least one RNA-guided endonuclease.”</td>
<td></td>
</tr>
<tr>
<td>Whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process leads to integration of the exogenous sequence into the chromosomal sequence.</td>
<td>Sigma’s “introduces a double-stranded break” ∋ UCB’s “cleaved.” Sigma’s “integrating” ∋ UCB’s “editing.”</td>
<td></td>
</tr>
<tr>
<td>Sigma’s claim is directed to modifying a eukaryotic cell.</td>
<td></td>
<td></td>
</tr>
</tbody>
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## APPENDIX IB - GRAYSCALE

### SIDE-BY-SIDE COMPARISON OF CLAIM 1 OF SIGMA’S U.S. PATENT APPLICATION NO. 15/188,911 WITH CLAIM 156 OF UCB’S U.S. PATENT APPLICATION NO. 15/947,718

<table>
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<tr>
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<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>156. A method of cleaving or editing a target DNA molecule or modulating transcription of at least one gene encoded thereon, the method comprising</td>
<td>1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising: <strong>introducing into the eukaryotic cell (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) system comprising:</strong></td>
<td>Sigma’s “integrating” ≈ UCB’s “editing.” “Integr[ation] of an exogenous sequence into a chromosomal sequence” requires “cleaving” the chromosomal sequence first.</td>
</tr>
<tr>
<td>contacting a target DNA molecule having a target sequence with an engineered and/or non-naturally-occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising:</td>
<td>wherein the CRISPR-Cas type II system protein is a Cas9 protein; (ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising</td>
<td>Sigma and UCB’s claims each require a Type II CRISPR/Cas system.</td>
</tr>
<tr>
<td>(a) a Cas9 protein; and</td>
<td>(i) a targeter-RNA that hybridizes with the target sequence, and</td>
<td>Sigma and UCB’s claims each require a Cas9 protein.</td>
</tr>
<tr>
<td>(b) a DNA-targeting RNA comprising:</td>
<td>(1) a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and</td>
<td>Sigma’s “guide RNA” ≈ UCB’s “DNA-targeting RNA.”</td>
</tr>
<tr>
<td>(1) a targeter-RNA that hybridizes with the target sequence, and</td>
<td></td>
<td>Sigma’s “first region” ≈ UCB’s “targeter-RNA.”</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sigma’s “base pairs with a target...” ≈ UCB’s “hybridizes with the target...”</td>
</tr>
<tr>
<td>(ii) an activator-RNA that hybridizes with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,</td>
<td>(ii) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and</td>
<td>Sigma’s “second region” ≈ UCB’s “activator-RNA”</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>(iii) at least one donor polynucleotide comprising the exogenous sequence; and</td>
<td>Sigma’s claim requires a “donor polynucleotide” that is “integrat[ed]” it into the eukaryotic chromosome thereby “editing” it.</td>
<td></td>
</tr>
<tr>
<td>wherein said contacting takes place outside of a bacterial cell and outside an archaeal cell.</td>
<td>Sigma’s claim is directed to modifying a eukaryotic cell.</td>
<td></td>
</tr>
<tr>
<td>wherein the DNA-targeting RNA forms a complex with the Cas9 protein.</td>
<td>The “second region” of Sigma’s “guide RNA” “interacts with the at least one RNA-guided endonuclease.”</td>
<td></td>
</tr>
<tr>
<td>whereby said target DNA molecule is cleaved or edited or transcription of at least one gene encoded by the target DNA molecule is modulated.</td>
<td>whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process leads to integration of the exogenous sequence into the chromosomal sequence.</td>
<td>Sigma’s “introduces a double-stranded break” ≈ UCB’s “cleaved.”</td>
</tr>
<tr>
<td>wherein said contacting occurs in a eukaryotic cell.</td>
<td>Sigma’s “integrating” ≈ UCB’s “editing.”</td>
<td>Sigma’s claim is directed to modifying a eukaryotic cell.</td>
</tr>
</tbody>
</table>
## APPENDIX II

### SIDE-BY-SIDE COMPARISON OF CLAIM 1 OF SIGMA’S U.S. PATENT APPLICATION NO. 16/654,613 WITH CLAIM 156 OF UCB’S U.S. PATENT APPLICATION NO. 15/981,809

<table>
<thead>
<tr>
<th>UCB’S ISSN 15/981,809</th>
<th>SIGMA’S ISSN 16/654,613</th>
<th>Comments</th>
</tr>
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<tr>
<td>156. A eukaryotic cell comprising a target DNA molecule and an engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising</td>
<td>1. A eukaryotic cell comprising a target chromosomal sequence; and (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II protein is codon optimized for expression in the eukaryotic cell, and (ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising (1) a first region at the 5’ end that is capable of base pairing with a target site in the chromosomal sequence, and (2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and</td>
<td>Sigma and UCB are each claiming a eukaryotic cell. Sigma’s “target chromosomal sequence” ≈ UCB’s “target DNA molecule.” Sigma and UCB’s claims each require a Type II CRISPR/Cas system. Sigma’s “guide RNA” ≈ UCB’s “DNA-targeting RNA.” Sigma’s “first region” ≈ UCB’s “targeter-RNA.” Sigma’s “second region” ≈ UCB’s “activator-RNA.”</td>
</tr>
<tr>
<td>a Cas9 protein or a nucleic acid comprising a nucleotide sequence encoding the Cas9 protein, and a DNA-targeting RNA or one or more nucleic acids comprising one or more nucleotide sequences encoding the DNA-targeting RNA; wherein the DNA-targeting RNA comprises i) a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule, and ii) an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,</td>
<td>wherein the CRISPR-Cas type II system protein is a Cas9 protein, and</td>
<td>Sigma and UCB’s claims each require a Cas9 protein.</td>
</tr>
<tr>
<td>whereby hybridization of the tageter-RNA is capable of targeting the Cas9 protein to the target DNA molecule.</td>
<td>whereby base pairing of the first region to the target site is capable of targeting the Cas9 protein to the target chromosomal sequence.</td>
<td>Sigma’s “base pairing . . . to the target site” = UCB’s “hybridization . . . to the target sequence”</td>
</tr>
</tbody>
</table>
APPENDIX IIB - GRAYSCALE

SIDE-BY-SIDE COMPARISON OF CLAIM 1 OF SIGMA’S U.S. PATENT APPLICATION NO. 16/654,613 WITH CLAIM 156 OF UCB’S U.S. PATENT APPLICATION NO. 15/981,809

<table>
<thead>
<tr>
<th>UCB’S USSN 15/981,809</th>
<th>SIGMA’S USSN 16/654,613</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>156. A eukaryotic cell comprising</td>
<td>1. A eukaryotic cell comprising</td>
<td>Sigma and UCB are each claiming a eukaryotic cell</td>
</tr>
<tr>
<td>a target DNA molecule and</td>
<td>a target chromosomal sequence; and</td>
<td>Sigma’s “target chromosomal sequence” ≈ UCB’s “target DNA molecule”</td>
</tr>
<tr>
<td>an engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising</td>
<td>(i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II protein is codon optimized for expression in the eukaryotic cell, and</td>
<td>Sigma and UCB’s claims each require a Type II CRISPR/Cas system.</td>
</tr>
<tr>
<td>a Cas9 protein or a nucleic acid comprising a nucleotide sequence encoding the Cas9 protein, and</td>
<td>wherein the CRISPR-Cas type II system protein is a Cas9 protein, and</td>
<td>Sigma and UCB’s claims each require a Cas9 protein</td>
</tr>
<tr>
<td>a DNA-targeting RNA or one or more nucleic acids comprising one or more nucleotide sequences encoding the DNA-targeting RNA; wherein the DNA-targeting RNA comprises</td>
<td>(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising</td>
<td>Sigma’s “guide RNA” ≈ UCB’s “DNA-targeting RNA.”</td>
</tr>
<tr>
<td>i) a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule, and</td>
<td>(1) a first region at the 5’ end that is capable of base pairing with a target site in the chromosomal sequence, and</td>
<td>Sigma’s “first region” ≈ UCB’s “targeter-RNA.”</td>
</tr>
<tr>
<td>ii) an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,</td>
<td>(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and</td>
<td>Sigma’s “second region” ≈ UCB’s “activator-RNA”</td>
</tr>
<tr>
<td>whereby hybridization of the targeter-RNA to the target sequence is capable of targeting the Cas9 protein to the target DNA molecule.</td>
<td>whereby base pairing of the first region to the target site is capable of targeting the Cas9 protein to the target chromosomal sequence.</td>
<td>Sigma’s “base pairing . . . to the target site” ≈ UCB’s “hybridization . . . to the target sequence”</td>
</tr>
</tbody>
</table>
### APPENDIX III

**EXAMPLES OF SUPPORT FOR PENDING CLAIM 1 OF U.S. PATENT APPLICATION NO. 15/188,911**  
**IN U.S. PATENT APPLICATION NO. 15/188,911 FILED JUNE 21, 2016**

<table>
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<tr>
<th>SIGMA’S USSN 15/188,911 (Proposed Claim for Interference)</th>
<th>SIGMA’S USSN 15/188,911 filed June 21, 2016 (hereinafter “Sigma ’911”)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising:</td>
<td>“Another aspect of the present invention encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease as defined herein, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs a RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. In one embodiment, the RNA-guided endonuclease can be derived from a Cas9 protein. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be mRNA. In a further embodiment, wherein the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be DNA. In a further embodiment, the DNA encoding the RNA-guided endonuclease can be part of a vector that further comprises a sequence encoding the guide RNA. In certain embodiments, the eukaryotic cell can be a human cell, a non-human mammalian cell, a stem cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism. In certain other embodiments, the embryo is a non-human one cell animal embryo. (Sigma ’911 at [0006]).”</td>
</tr>
</tbody>
</table>

“(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal. (ii) at least one guide RNA or DNA encoding at least one guide..."
RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryy such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '911 at [0064]). See also Sigma ‘911 at [0065]-[0067].

"(a) RNA-guided endonuclease
The method comprises introducing into a cell or embryo at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal. Such RNA-guided endonucleases and nucleic acids encoding RNA-guided endonucleases are described above in sections (I) and (III), respectively.” (Sigma ‘911 at [0068]). See also Sigma ‘911 at [0069]-[0070].

"(g) Cell and embryo types
A variety of eukaryotic cells and embryos are suitable for use in the method. For example, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism. In general, the embryo is non-human mammalian embryo. In specific embodiments, the embryos can be a one cell nonhuman mammalian embryo. Exemplary mammalian embryos, including one cell embryos, include without limit mouse, rat, hamster, rodent, rabbit, canine, ovine, porcine, bovine, equine, and primate embryos. In still other embodiments, the cell can be a stem cell. Suitable stem cells include without limit embryonic stem cells, ES-like stem cells, fetal stem cells, adult stem cells, pluripotent stem cells, induced pluripotent stem cells, multipotent stem cells, oligopotent stem cells, unipotent stem cells and others. In exemplary embodiments, the cell is a mammalian cell.” (Sigma ‘911 at [0104]). See also Sigma ‘911 at [0105].

“The term "exogenous," as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location.” (Sigma ‘911 at [0134]).

See also Examples 1-9 (Sigma ‘911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ‘911.

| introducing into the eukaryotic cell (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one | “Another aspect of the present invention encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided |
RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas type II system protein is codon optimized for expression in the eukaryotic cell, and endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease as defined herein, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs a RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. In one embodiment, the RNA-guided endonuclease can be derived from a Cas9 protein. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be mRNA In a further embodiment, wherein the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be DNA In a further embodiment, the DNA encoding the RNA-guided endonuclease can be part of a vector that further comprises a sequence encoding the guide RNA. In certain embodiments, the eukaryotic cell can be a human cell, a non-human mammalian cell, a stem cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism. In certain other embodiments, the embryo is a non-human one cell animal embryo. (Sigma ‘911 at [0006]).

*Provided herein are RNA-guided endonucleases, which comprise at least one nuclear localization signal, at least one nuclease domain, and at least one domain that interacts with a guide RNA to target the endonuclease to a specific nucleotide sequence for cleavage. Also provided are nucleic acids encoding the RNA-guided endonucleases, as well as methods of using the RNA-guided endonucleases to modify chromosomal sequences of eukaryotic cells or embryos. The RNA-guided endonuclease interacts with specific guide RNAs, each of which directs the endonuclease to a specific targeted site, at which site the RNA-guided endonuclease introduces a double-stranded break that can be repaired by a DNA repair process such that the chromosomal sequence is modified. Since the specificity is provided by the guide RNA, the RNA-based endonuclease is universal and can be used with different guide RNAs to target different genomic sequences. The methods disclosed herein can be used to target and modify specific chromosomal sequences and/or introduce exogenous sequences at targeted locations in the genome of cells or embryos. Furthermore, the targeting is specific with limited off target effects. (Sigma ‘911 at [0015]).

[(f) RNA-Guided Endonucleases]
One aspect of the present disclosure provides RNA-guided endonucleases comprising at least one nuclear localization signal, which permits entry of the
endonuclease into the nuclei of eukaryotic cells and embryos such as, for least one nuclease domain and at least one domain that interacts with a guide RNA. An RNA-guided endonuclease is directed to a specific nucleic acid sequence (or target site) by a guide RNA. The guide RNA interacts with the RNA-guided endonuclease as well as the target site such that, once directed to the target site, the RNA-guided endonuclease is able to introduce a double-stranded break into the target site nucleic acid sequence. Since the guide RNA provides the specificity for the targeted cleavage, the endonuclease of the RNA-guided endonuclease is universal and can be used with different guide RNAs to cleave different target nucleic acid sequences. Provided herein are isolated RNA-guided endonucleases, isolated nucleic acids (i.e., RNA or DNA) encoding the RNA-guided endonucleases, vectors comprising nucleic acids encoding the RNA-guided endonucleases, and protein-RNA complexes comprising the RNA-guided endonuclease plus a guide RNA. (Sigma ‘911 at [0017]).

“The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. Non-limiting examples of suitable CRISPR/Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas8e, Cas6f, Cas?, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.” (Sigma ‘911 at [0018]).

“In one embodiment, the RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In specific embodiments, the RNA-guided endonuclease is derived from a Cas9 protein. The Cas9 protein can be from Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Nocardiosis dassovillei, Streptomyces pristinae spiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Micrococcus marina, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas sp., Crocosphaera watsonii, Cyanobethea sp., Microcystis aeruginosa, Synechococcus sp., Acetohalobium arabaticum, Ammonifex degensii, Caldicellulosiruptor beecroii, Candidatus Desulfurodus, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus
caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter sp., Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Arthrosira maxima, Arthrosira platensis, Arthrosira sp., Lyngbya sp., Microcoleus chthonoplastes, Oscillatoria sp., Petrotoga mobilis, Thermosiphon africanus, or Acaryochloris marina." (Sigma '911 at [0019]). See also Sigma ‘911 at [0020]-[0023].

“The RNA-guided endonuclease disclosed herein comprises at least one nuclear localization signal. In general, an NLS comprises a stretch of basic amino acids. Nuclear localization signals are known in the art (see, e.g., Lange et al., J. Biol. Chem., 2007, 282:5101-51 05). For example, in one embodiment, the NLS can be a monopartite sequence, such as PKKRRK (SEQ ID NO:1) or PKKKRRV (SEQ ID NO:2). In another embodiment, the NLS can be a bipartite sequence. In still another embodiment, the NLS can be KRPAATKKGQAKKKK (SEQ ID NO:3). The NLS can be located at the N-terminus, the C-terminal, or in an internal location of the RNA-guided endonuclease.” (Sigma ‘911 at [0024]).

(III) Nucleic Acids Encoding RNA-Guided Endonucleases or Fusion Proteins
Another aspect of the present disclosure provides nucleic acids encoding any of the RNA-guided endonucleases or fusion proteins described above in sections (I) and (II), respectively. The nucleic acid can be RNA or DNA. In one embodiment, the nucleic acid encoding the RNA-guided endonuclease or fusion protein is mRNA. The mRNA can be 5' capped and/or 3' polyadenylated. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease or fusion protein is DNA. The DNA can be present in a vector (see below). (Sigma ‘911 at [0055]).

“The nucleic acid encoding the RNA-guided endonuclease or fusion protein can be codon optimized for efficient translation into protein in the eukaryotic cell or animal of interest. For example, codons can be optimized for expression in humans, mice, rats, hamsters, cows, pigs, cats, dogs, fish, amphibians, plants, yeast, insects, and so forth. Programs for codon optimization are available as freeware. Commercial codon optimization programs are also available.” (Sigma ‘911 at [0057]). See also Sigma ‘911 at [0058]-[0063].

(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease
Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided
endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma ‘911 at [0064]). See also Sigma ‘911 at [0065]-[0067].

“(a) RNA-guided endonuclease
The method comprises introducing into a cell or embryo at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal. Such RNA-guided endonucleases and nucleic acids encoding RNA-guided endonucleases are described above in sections (I) and (III), respectively.” (Sigma ‘911 at [0068]). See also Sigma ‘911 at [0069]-[0070].

“(e) Introducing into the cell or embryo
The RNA-targeted endonuclease(s) (or encoding nucleic acid), the guide RNA(s) (or encoding DNA), and the optional donor polynucleotide(s) can be introduced into a cell or embryo by a variety of means. In some embodiments, the cell or embryo is transfected. Suitable transfection methods include calcium phosphate-mediated transfection, nucleofection (or electroporation), cationic polymer transfection (e.g., DEAE-dextran or polyethylenimine), viral transduction, virosome transfection, virion transfection, liposome transfection, cationic liposome transfection, immunoliposome transfection, nonliposomal lipid transfection, dendrimer transfection, heat shock transfection, magnetofection, lipofection, gene gun delivery, impalefection, sonoporation, optical transfection, and proprietary agent-enhanced uptake of nucleic acids. Transfection methods are well known in the art (see, e.g., “Current Protocols in Molecular Biology” Ausubel et al., John Wiley & Sons, New York, 2003 or “Molecular Cloning: A Laboratory Manual” Sam brook & Russell, Cold Spring Harbor Press, Cold Spring Harbor, NY, 3rd edition, 2001). In other embodiments, the molecules are introduced into the cell or embryo by microinjection. Typically, the embryo is a fertilized one-cell stage embryo of the species of interest. For example, the molecules can be injected into the pronuclei of one cell embryos.” (Sigma ‘911 at [0094]).
“The RNA-targeted endonuclease(s) (or encoding nucleic acid), the guide RNA(s) (or DNAs encoding the guide RNA), and the optional donor polynucleotide(s) can be introduced into the cell or embryo simultaneously or sequentially. The ratio of the RNA-targeted endonuclease(s) (or encoding nucleic acid) to the guide RNA(s) (or encoding DNA) generally will be about stoichiometric such that they can form an RNA-protein complex. In one embodiment, DNA encoding an RNA-targeted endonuclease and DNA encoding a guide RNA are delivered together within the plasmid vector.” (Sigma '911 at [0095]).

“(g) Cell and embryo types
A variety of eukaryotic cells and embryos are suitable for use in the method. For example, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism. In general, the embryo is non-human mammalian embryo. In specific embodiments, the embryos can be a one cell nonhuman mammalian embryo. Exemplary mammalian embryos, including one cell embryos, include without limit mouse, rat, hamster, rodent, rabbit, feline, canine, ovine, porcine, bovine, equine, and primate embryos. In still other embodiments, the cell can be a stem cell. Suitable stem cells include without limit embryonic stem cells, ES-like stem cells, fetal stem cells, adult stem cells, pluripotent stem cells, induced pluripotent stem cells, multipotent stem cells, oligopotent stem cells, unipotent stem cells and others. In exemplary embodiments, the cell is a mammalian cell.” (Sigma '911 at [0104]). See also Sigma '911 at [0105].

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma '911 at [0115]). See also Sigma '911 at [0116]-[0130].

“The terms “nucleic acid” and “polynucleotide” refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate
backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Sigma ’911 at [0137]).

“The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.” (Sigma ’911 at [0139]).

See also Examples 1-9 (Sigma ’911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ’911.

wherein the CRISPR-Cas type II system protein is a Cas9 protein;

“The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. Non-limiting examples of suitable CRISPR/Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.” (Sigma ’911 at [0018]).

“In one embodiment, the RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In specific embodiments, the RNA-guided endonuclease is derived from a Cas9 protein. The Cas9 protein can be from Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Nocardiosis dassonvillei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Micrococcina marinia, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas sp., Crocosphaera watsonii, Cyanothece sp., Microcystis aeruginosa, Synechococcus sp., Aceothalobium arabaticum, Ammonifex degensii, Caldocelulosirauprt becsci, Candidatus Desulfuris, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermostrophium, Acidithiobacillus caldus, Acidithiobacillus ferroxidans, Allochromatium vinosum, Marinobacter sp., Nitrosococcus halophilus, Nitrosococcus watsonii, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Arthrosira maxima, Arthrosira platensis, Arthrosira sp., Lyngbya sp., Microcoleus chthonoplastes, Oscillatoria sp., Petrotroga mobilis, Thermosiphon africanus, or Acaryochloris marina.” (Sigma ’911 at [0019]). See also Sigma ’911 at [0020]-[0023].
(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising

"(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '911 at [0064]). See also Sigma '911 at [0065]-[0067].

"(b) Guide RNA

The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA. A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5' end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence." (Sigma '911 at [0071]).

"Each guide RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs." (Sigma '911 at [0072]). See also Sigma '911 at [0073]-[0077].

"In some embodiments, the guide RNA can be introduced into the cell or embryo as a RNA molecule. The RNA molecule can be transcribed in vitro. Alternatively, the RNA molecule can be chemically synthesized." (Sigma '911 at [0078]).
“In other embodiments, the guide RNA can be introduced into the cell or embryo as a DNA molecule. In such cases, the DNA encoding the guide RNA can be operably linked to promoter control sequence for expression of the guide RNA in the cell or embryo of interest. For example, the RNA coding sequence can be operably linked to a promoter sequence that is recognized by RNA polymerase III (Pol III).” (Sigma ‘911 at [0079]). See also Sigma ‘911 at [0080]-[0081].

See also Examples 1-9 (Sigma ‘911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ‘911. 

| (1) a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and | ‘(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease
Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma ‘911 at [0064]). See also Sigma ‘911 at [0065]-[0067]. |
| | ‘(b) Guide RNA
The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5’ end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.” (Sigma ‘911 at [0071]). |
| | “Each guide RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs.” (Sigma ‘911 at [0072]). |
“The first region of the guide RNA is complementary to sequence (i.e., protospacer sequence) at the target site in the chromosomal sequence such that the first region of the guide RNA can base pair with the target site. In various embodiments, the first region of the guide RNA can comprise from about 10 nucleotides to more than about 25 nucleotides. For example, the region of base pairing between the first region of the guide RNA and the target site in the chromosomal sequence can be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more than 25 nucleotides in length. In an exemplary embodiment, the first region of the guide RNA is about 19, 20, or 21 nucleotides in length.” (Sigma '911 at [0073]).

“(c) Target site

“An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGNG, and NNAGAAW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (IV)(b), the first region (at the 5' end) of the guide RNA is complementary to the protospacer of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5'-N<sub>19-21</sub>-NGG-3'. The PAM is in italics.” (Sigma '911 at [0082]).

“The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest.” (Sigma '911 at [0083]).

See also Examples 1-9 (Sigma '911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '911.

<table>
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<th>(2)</th>
<th>a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and</th>
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<th><em>(IV)</em> Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease</th>
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<tr>
<td>Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear</td>
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localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma ‘911 at [0064]). See also Sigma ‘911 at [0065]-[0067].

"(b) Guide RNA
The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA. A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5' end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence." (Sigma ‘911 at [0071]).

"Each guide RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs." (Sigma ‘911 at [0072]).

"The guide RNA also comprises a second region that forms a secondary structure. In some embodiments, the secondary structure comprises a stem (or hairpin) and a loop. The length of the loop and the stem can vary. For example, the loop can range from about 3 to about 10 nucleotides in length, and the stem can range from about 8 to about 20 base pairs in length. The stem can comprise one or more bulges of 1 to about 10 nucleotides. Thus, the overall length of the second region can range from about 16 to about 60 nucleotides in length. In an exemplary embodiment, the loop is about 4 nucleotides in length and the stem comprises about 12 base pairs." (Sigma ‘911 at [0074]).

See also Examples 1-9 (Sigma ‘911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ‘911.

(ii) at least one donor polynucleotide comprising the exogenous sequence; and

(iv) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease
Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises
introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '911 at [0084]). See also Sigma '911 at [0065]-[0067].

"(d) Optional donor polynucleotide
In some embodiments, the method further comprises introducing at least one donor polynucleotide into the embryo. A donor polynucleotide comprises at least one donor sequence. In some aspects, a donor sequence of the donor polynucleotide corresponds to an endogenous or native chromosomal sequence. For example, the donor sequence can be essentially identical to a portion of the chromosomal sequence at or near the targeted site, but which comprises at least one nucleotide change. Thus, the donor sequence can comprise a modified version of the wild type sequence at the targeted site such that, upon integration or exchange with the native sequence, the sequence at the targeted chromosomal location comprises at least one nucleotide change. For example, the change can be an insertion of one or more nucleotides, a deletion of one or more nucleotides, a substitution of one or more nucleotides, or combinations thereof. As a consequence of the integration of the modified sequence, the cell or embryo/animal can produce a modified gene product from the targeted chromosomal sequence." (Sigma '911 at [0084]).

"In other aspects, the donor sequence of the donor polynucleotide corresponds to an exogenous sequence. As used herein, an "exogenous" sequence refers to a sequence that is not native to the cell or embryo, or a sequence whose native location in the genome of the cell or embryo is in a different location. For example, the exogenous sequence can comprise protein coding sequence, which can be operably linked to an exogenous promoter control sequence such that, upon integration into the genome, the cell or embryo/animal is able to express the protein coded by the integrated sequence. Alternatively, the exogenous sequence can be integrated into the chromosomal sequence such that its expression is regulated by an endogenous promoter control sequence. In other iterations, the exogenous sequence can be a transcriptional control sequence, another expression control sequence, an RNA coding sequence, and so forth. Integration of an exogenous
sequence into a chromosomal sequence is termed a "knock in."" (Sigma '911 at [0085]).

“As can be appreciated by those skilled in the art, the length of the donor sequence can and will vary. For example, the donor sequence can vary in length from several nucleotides to hundreds of nucleotides to hundreds of thousands of nucleotides.” (Sigma '911 at [0086]). See also Sigma '911 at [0087]-[0093].

“The term "exogenous," as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location.” (Sigma '911 at [0134]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma '911 at [0115]). See also Sigma '911 at [0116]-[0130].

“The terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Sigma '911 at [0137]).

See also Examples 1-9 (Sigma '911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '911.

| whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process |

| "(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease
Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide |
leads to integration of the exogenous sequence into the chromosomal sequence. RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma ‘911 at [0064]). See also Sigma ‘911 at [0065]-[0067].

*(c) Target site*

“An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protoscaler adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGNG, and NNAGAW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (IV)(b), the first region (at the 5’ end) of the guide RNA is complementary to the protoscaler of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5’-N19-21-NGG-3’. The PAM is in italics.” (Sigma ‘911 at [0082]).

“The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest.” (Sigma ‘911 at [0083]).

*(d) Optional donor polynucleotide*

In some embodiments, the method further comprises introducing at least one donor polynucleotide into the embryo. A donor polynucleotide comprises at least one donor sequence. In some aspects, a donor sequence of the donor polynucleotide corresponds to an endogenous or native chromosomal sequence. For example, the donor sequence can be essentially identical to a portion of the chromosomal sequence at or near the targeted site, but which comprises at least one nucleotide change. Thus, the donor sequence can comprise a modified version of the wild type sequence at the targeted site such that, upon integration or exchange with the native sequence, the sequence at the targeted chromosomal location comprises at least one nucleotide change. For example, the change can be an insertion of one or more nucleotides, a deletion of one or more nucleotides, a substitution of one or
more nucleotides, or combinations thereof. As a consequence of the integration of the modified sequence, the cell or embryo/animal can produce a modified gene product from the targeted chromosomal sequence.” (Sigma '911 at [0084]). 

"In other aspects, the donor sequence of the donor polynucleotide corresponds to an exogenous sequence. As used herein, an "exogenous" sequence refers to a sequence that is not native to the cell or embryo, or a sequence whose native location in the genome of the cell or embryo is in a different location. For example, the exogenous sequence can comprise protein coding sequence, which can be operably linked to an exogenous promoter control sequence such that, upon integration into the genome, the cell or embryo/animal is able to express the protein coded by the integrated sequence. Alternatively, the exogenous sequence can be integrated into the chromosomal sequence such that its expression is regulated by an endogenous promoter control sequence. In other iterations, the exogenous sequence can be a transcriptional control sequence, another expression control sequence, an RNA coding sequence, and so forth. Integration of an exogenous sequence into a chromosomal sequence is termed a "knock in."" (Sigma '911 at [0085]). 

"As can be appreciated by those skilled in the art, the length of the donor sequence can and will vary. For example, the donor sequence can vary in length from several nucleotides to hundreds of nucleotides to hundreds of thousands of nucleotides." (Sigma '911 at [0086]). See also Sigma '911 at [0087]-[0093]. 

"(f) Culturing the cell or embryo
The method further comprises maintaining the cell or embryo under appropriate conditions such that the guide RNA(s) directs the RNA-guided endonuclease(s) to the targeted site(s) in the chromosomal sequence, and the RNA-guided endonuclease(s) introduce at least one double-stranded break in the chromosomal sequence. A double-stranded break can be repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.” (Sigma ‘911 at [0096]).

"In embodiments in which no donor polynucleotide is introduced into the cell or embryo, the double-stranded break can be repaired via a non-homologous end-joining (NHEJ) repair process. Because NHEJ is error-prone, deletions of at least one nucleotide, insertions of at least one nucleotide, substitutions of at least one nucleotide, or combinations thereof can occur during the repair of the break. Accordingly, the sequence at the chromosomal sequence can be modified such that
the reading frame of a coding region can be shifted and that the chromosomal sequence is inactivated or "knocked out." An inactivated protein-coding chromosomal sequence does not give rise to the protein coded by the wild type chromosomal sequence. (Sigma '911 at [0097]).

"In embodiments in which a donor polynucleotide comprising upstream and downstream sequences is introduced into the cell or embryo, the double-stranded break can be repaired by a homology-directed repair (HDR) process such that the donor sequence is integrated into the chromosomal sequence. Accordingly, an exogenous sequence can be integrated into the genome of the cell or embryo, or the targeted chromosomal sequence can be modified by exchange of a modified sequence for the wild type chromosomal sequence." (Sigma '911 at [0098]). See also Sigma '911 at [0069]-[0103].

"The term "exogenous," as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location." (Sigma '911 at [0134]).

See also Examples 1-9 (Sigma '911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '911.
## APPENDIX IV

**EXAMPLES OF SUPPORT FOR PENDING CLAIM 1 OF U.S. PATENT APPLICATION NO. 15/188,911 IN U.S. PROVISIONAL APPLICATION NO. 61/734,256 FILED DECEMBER 6, 2012**

<table>
<thead>
<tr>
<th>SIGMA’S USSN 15/188,911 (Proposed Claim for Interference)</th>
<th>SIGMA’S USSN 61/734,256 filed December 6, 2012 (hereinafter “Chen P1”)</th>
</tr>
</thead>
</table>
| 1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising: | “(II) Method for Modifying a Chromosomal Sequence

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell. The method comprises introducing into the eukaryotic cell (i) an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease, wherein the RNA-guided endonuclease comprises a nuclear localization signal, (ii) at least one guiding RNA or at least one DNA molecule encoding a guiding RNA, wherein each guiding RNA guides the RNA-guided endonuclease to a targeted site in the chromosomal sequence, and optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell such that the RNA-guided endonuclease introduces a double-stranded break at the targeted site in the chromosomal sequence and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof." (Chen P1 at [0016]). |
<p>| | “A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism.&quot; (Chen P1 at [0045]). |
| | “The term &quot;exogenous,&quot; as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location.&quot; (Chen P1 at [0050]). |
| | See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1. |
| introducing into the eukaryotic cell (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear | “One aspect of the present disclosure provides RNA-guided endonucleases that comprise at least one nuclear localization signal, which permits entry of the endonuclease into the nuclei of eukaryotic cells.&quot; (Chen P1 at [0004]). |</p>
<table>
<thead>
<tr>
<th><strong>localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas type II system protein is codon optimized for expression in the eukaryotic cell, and</strong></th>
<th><strong>“The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. In some embodiments, the RNA-guided endonuclease can be derived from a type II CRISPR/Cas system.” (Chen P1 at [0004]).</strong></th>
</tr>
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<tbody>
<tr>
<td><strong>In some embodiments, the RNA-guided endonuclease can be derived from a wild type Cas9 protein(s) or fragment(s) thereof. In other embodiments, the RNA-guided endonuclease can be derived from modified Cas9 protein(s). For example, the amino acid sequence of the Cas9 protein can be modified such that one or more properties (e.g., nuclease activity, affinity, stability, etc.) of the protein is improved. Alternatively, domains of the Cas9 protein not involved in RNA-guided cleavage can be eliminated from the protein such that the modified Cas9 protein is smaller than the wild type Cas9 protein. In still other embodiments, the RNA-guided endonuclease can be a fusion protein comprising domains of wild type Cas9 proteins, modified Cas9 proteins, and/or other proteins. For example the RNA-guided endonuclease could comprise a marker, such as GFP or another fluorescent protein.” (Chen P1 at [0006]).</strong></td>
<td><strong>The Cas9-derived endonucleases disclosed herein comprise at least one nuclear localization signal (NLS) for transport into the nuclei of eukaryotic cells. In general, an NLS comprise a stretch of basic amino acids. Nuclear localization signals are known in the art (see, e.g., Lange et al., J. Biol. Chem., 2007, 282:5101-5105). For example, examples of classical NLSs included monopartite sequences such as PKKKRKV (SEQ ID NO:1) or PKKKRRV (SEQ ID NO:13), as well as bipartite sequences. The NLS can be located at the N-terminus, the C-terminal, or in an internal location of the endonuclease. In an exemplary embodiment, the NLS is located at the C-terminus of the endonuclease.” (Chen P1 at [0006]).</strong></td>
</tr>
<tr>
<td><strong>The disclosure also provides nucleic acids encoding the RNA-guided endonuclease. The nucleic acid encoding the RNA-guided endonuclease can be RNA or DNA. The DNA encoding the RNA-guided endonuclease can be present in a vector (see below).” (Chen P1 at [0011]). See also Chen P1 at [0012]-[0015].</strong></td>
<td><strong>“The method comprises introducing into the cell an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease.” (Chen P1 at [0018]).</strong></td>
</tr>
<tr>
<td><strong>(d) Introducing into the cell</strong> The RNA-targeted endonuclease (or its encoding nucleic acid), the guiding RNA(s) (or DNAs encoding the guiding RNA), and the optional donor polynucleotide(s) can</td>
<td></td>
</tr>
</tbody>
</table>
be introduced into the cell by a variety of means. In some embodiments, the cell is transfected. Suitable transfection methods include calcium phosphate-mediated transfection, nucleofection (or electroporation), cationic polymer transfection (e.g., DEAE-dextran or polyethyleneimine), viral transduction, virosome transfection, virion transfection, liposome transfection, cationic liposome transfection, immunoliposome transfection, nonliposomal lipid transfection, dendrimer transfection, heat shock transfection, magnetofection, lipofection, gene gun delivery, impalefection, sonoporation, optical transfection, and proprietary agent-enhanced uptake of nucleic acids. Transfection methods are well known in the art (see, e.g., "Current Protocols in Molecular Biology" Ausubel et al., John Wiley & Sons, New York, 2003 or "Molecular Cloning: A Laboratory Manual" Sambrook & Russell, Cold Spring Harbor Press, Cold Spring Harbor, NY, 3rd edition, 2001). In other embodiments, the molecules are introduced into the cell by microinjection. For example, the molecules can be injected into the pronuclei of one cell embryos." (Chen P1 at [0037]). See also Chen P1 at [0038].

“A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism.” (Chen P1 at [0045]).

“The terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Chen P1 at [0053]).

“The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.” (Chen P1 at [0055]).

See also Examples 1-5 (Chen P1 at [0058]-0068] and accompanying Figures; Claims 12-21 of Chen P1.

wherein the CRISPR-Cas type II system protein is a Cas9 protein;  

“In exemplary embodiments, the endonuclease can be derived from a Cas9 protein of a type II system.” (Chen P1 at [0004]).

“The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.” (Chen P1 at [0055]).
(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising

<table>
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<tr>
<th>See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.</th>
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<tbody>
<tr>
<td>“The present disclosure provides RNA-guided endonucleases that are engineered for use in eukaryotic cells. The RNA-guided endonuclease can be used to modify the genome of eukaryotic cells. For this, the endonuclease is guided to a specific chromosomal sequence by a specific guiding RNA.” (Chen P1 at [0003]).</td>
</tr>
<tr>
<td>“The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell.” (Chen P1 at [0019]).</td>
</tr>
<tr>
<td>See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.</td>
</tr>
</tbody>
</table>

(1) a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and

| “(a) Target site
The RNA-guided endonuclease in conjunction with the guiding RNA is directed to a target site in the chromosomal sequence and cleaves the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG and NGGNG (wherein N is defined as any nucleotide). The target site can be in the coding region of a gene, in an intron of a gene, in a control region between genes, etc. The gene can be a protein coding gene or an RNA coding gene.” (Chen P1 at [0017]). |
| “The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell. Each guiding RNA interacts with the RNA-guided endonuclease to guide the endonuclease to a specific target site, wherein the endonuclease cleaves the target site. Each guiding RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guiding RNA is different such that each guiding RNA guides the endonuclease to a specific target site. The second and third regions of each guiding RNA can be the same in all guiding RNAs.” (Chen P1 at [0019]). |
| “The first region of the guiding RNA is complementary to the target site in the chromosomal sequence such that the first region of the guiding RNA can base pair with the target site. In various embodiments, the first region of the guiding RNA can comprise from about 10 nucleotides to more than about 25 nucleotides.” (Chen P1 at [0020]). |
(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and

"The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell. Each guiding RNA interacts with the RNA-guided endonuclease to guide the endonuclease to a specific target site, wherein the endonuclease cleaves the target site. Each guiding RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guiding RNA is different such that each guiding RNA guides the endonuclease to a specific target site. The second and third regions of each guiding RNA can be the same in all guiding RNAs."

(Chen P1 at [0019]).

"The guiding RNA also comprises a second region that forms a secondary structure. In some embodiments, the secondary structure comprises a stem (or hairpin) and a loop. The length of the loop and the stem can vary. For example, the loop can range from about 3 to about 10 nucleotides in length, and the stem can range from about 6 to about 20 base pairs in length. The stem can comprise one or more bulges of 1 to about 10 nucleotides. Thus, the overall length of the second region can range from about 16 to about 80 nucleotides in length. In an exemplary embodiment, the loop is about 4 nucleotides in length and the stem comprises about 12 base pairs."

(Chen P1 at [0021]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

(iii) at least one donor polynucleotide comprising the exogenous sequence; and

"(c) Optional donor polynucleotide"

The method optionally also comprises introducing at least one donor polynucleotide comprising a donor sequence into the cell. As detailed below, the donor polynucleotide can comprise additional sequence elements."

(Chen P1 at [0026]).

See also Chen P1 at [0027]-[0036]).

"The term "exogenous," as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location."

(Chen P1 at [0050]).

"The terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms
can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Chen P1 at [0053]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process leads to integration of the exogenous sequence into the chromosomal sequence.

"[T]he endonuclease is guided to a specific chromosomal sequence by a specific guiding RNA." (Chen P1 at [0003]).

"The double-stranded break can be repaired by a cellular DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof." (Chen P1 at [0003]).

(a) Target site
The RNA-guided endonuclease in conjunction with the guiding RNA is directed to a target site in the chromosomal sequence and cleaves the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG and NGGNG (wherein N is defined as any nucleotide). The target site can be in the coding region of a gene, in an intron of a gene, in a control region between genes, etc. The gene can be a protein coding gene or an RNA coding gene." (Chen P1 at [0017]).

(e) Culturing the cell
The method further comprises maintaining the cell under appropriate conditions such that the guiding RNA guides the endonuclease to the targeted site in the chromosomal sequence, and the endonuclease introduces a double-stranded break in the targeted site in the chromosomal sequence. The double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof." (Chen P1 at [0039]).

"A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism." (Chen P1 at [0045]).
“The term "exogenous," as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location.” (Chen P1 at [0050]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.
APPENDIX V
EXAMPLES OF SUPPORT FOR PENDING CLAIM 1 OF U.S. PATENT APPLICATION NO. 16/654,613
IN U.S. PATENT APPLICATION NO. 16/654,613 FILED OCTOBER 16, 2019

<table>
<thead>
<tr>
<th>SIGMA’S USSN 16/654,613 (Proposed Claim for Interference)</th>
<th>SIGMA’S USSN 16/654,613 filed October 16, 2019 (hereinafter “Sigma ‘613”)</th>
</tr>
</thead>
</table>
| 1. A eukaryotic cell comprising                             | *(g) Cell and embryo types
A variety of eukaryotic cells and embryos are suitable for use in the method. For example, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism. In general, the embryo is non-human mammalian embryo. In specific embodiments, the embryos can be a one cell nonhuman mammalian embryo. Exemplary mammalian embryos, including one cell embryos, include without limit mouse, rat, hamster, rodent, rabbit, feline, canine, ovine, porcine, bovine, equine, and primate embryos. In still other embodiments, the cell can be a stem cell. Suitable stem cells include without limit embryonic stem cells, ES-like stem cells, fetal stem cells, adult stem cells, pluripotent stem cells, induced pluripotent stem cells, multipotent stem cells, oligopotent stem cells, unipotent stem cells and others. In exemplary embodiments, the cell is a mammalian cell.” (Sigma ‘613 at [0104]). See also Sigma ‘613 at [0105]. |
|                                                            | *(v) Genetically Modified Cells and Animals
The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ‘613 at [0114]). |
|                                                            | *(The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an |
altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein." (Sigma '613 at [0115]). See also Sigma '613 at [0116]-[0130].

See also Examples 1-9 (Sigma '613 at [0142]-[0160]) and accompanying Figures; Claims 1-27 of Sigma '613.

<table>
<thead>
<tr>
<th>a target chromosomal sequence; and</th>
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**'(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease**

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '613 at [0064]). See also Sigma '613 at [0065]-[0067].

**'(b) Guide RNA**

The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5' end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.” (Sigma '613 at [0071]).

"Each guide RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs.” (Sigma '613 at [0072]).

"The first region of the guide RNA is complementary to sequence (i.e., protospacer sequence) at the target site in the chromosomal sequence such that the first region of the guide RNA can base pair with the target site. In various embodiments, the first region of the guide RNA can comprise from about 10 nucleotides to more than about 25 nucleotides. For
example, the region of base pairing between the first region of the guide RNA and the target site in the chromosomal sequence can be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more than 25 nucleotides in length. In an exemplary embodiment, the first region of the guide RNA is about 19, 20, or 21 nucleotides in length.” (Sigma ‘613 at [0073]).

“(c) Target site

“An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGNG, and NNAGAAW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (IV)(b), the first region (at the 5’ end) of the guide RNA is complementary to the protospacer of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5’-N19-21-NGG-3’. The PAM is in italics.” (Sigma ‘613 at [0082]).

“The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest.” (Sigma ‘613 at [0083]).

“(VI) Genetically Modified Cells and Animals

The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ‘613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an
altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA-guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein." (Sigma '613 at [0115]). See also Sigma '613 at [0116]-[0130].

See also Examples 1-9 (Sigma '613 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '613.

<table>
<thead>
<tr>
<th>(i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II protein is codon optimized for expression in the eukaryotic cell, and</th>
</tr>
</thead>
</table>
| "Another aspect of the present invention encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease as defined herein, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs a RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. In one embodiment, the RNA-guided endonuclease can be derived from a Cas9 protein. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be mRNA in a further embodiment, wherein the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be DNA. In a further embodiment, the DNA encoding the RNA-guided endonuclease can be part of a vector that further comprises a sequence encoding the guide RNA. In certain embodiments, the eukaryotic cell can be a human cell, a non-human mammalian cell, a stem cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism. In certain other embodiments, the embryo is a non-human one cell animal embryo. (Sigma '613 at [0006]).

"Provided herein are RNA-guided endonucleases, which comprise at least one nuclear localization signal, at least one nuclease domain, and at least one domain that interacts with a guide RNA to target the endonuclease to a specific nucleotide sequence for cleavage. Also provided are nucleic acids encoding the RNA-guided endonucleases, as well as methods of using the RNA-guided endonucleases to modify chromosomal sequences of eukaryotic cells or embryos. The RNA-guided endonuclease interacts with specific guide RNAs, each of which directs the endonuclease to a specific targeted site, at which site the RNA-guided endonuclease introduces a double-stranded break that can be repaired by a DNA repair process such that the chromosomal sequence is modified. Since the specificity is provided by the guide RNA, the RNA-based endonuclease is universal and can be used with different guide RNAs to target different genomic sequences. The methods disclosed herein can be used to target and modify specific chromosomal sequences and/or
introduce exogenous sequences at targeted locations in the genome of cells or embryos. Furthermore, the targeting is specific with limited off target effects. (Sigma ‘613 at [0015]).

“(l) RNA-Guided Endonucleases
One aspect of the present disclosure provides RNA-guided endonucleases comprising at least one nuclear localization signal, which permits entry of the endonuclease into the nuclei of eukaryotic cells and embryos such as, for least one nuclease domain and at least one domain that interacts with a guide RNA. An RNA-guided endonuclease is directed to a specific nucleic acid sequence (or target site) by a guide RNA. The guide RNA interacts with the RNA-guided endonuclease as well as the target site such that, once directed to the target site, the RNA-guided endonuclease is able to introduce a double-stranded break into the target site nucleic acid sequence. Since the guide RNA provides the specificity for the targeted cleavage, the endonuclease of the RNA-guided endonuclease is universal and can be used with different guide RNAs to cleave different target nucleic acid sequences. Provided herein are isolated RNA-guided endonucleases, isolated nucleic acids (i.e., RNA or DNA) encoding the RNA-guided endonucleases, vectors comprising nucleic acids encoding the RNA-guided endonucleases, and protein-RNA complexes comprising the RNA-guided endonuclease plus a guide RNA.” (Sigma ‘613 at [0017]).

“The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. Non-limiting examples of suitable CRISPR/Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, CasF, CasG, CasH, CasY1, CasY2, Cas3, Cas5 (or CasA), Cas2 (or CasB), Cas4e (or CasE), Cas4 (or CasC), Cas1c, Cas2, Cas5, Cas2, Casm2, Casm3, Casm4, Casm5, Casm6, Cmr1, Cmr2, Cmr4, Cmr5, Cmr6, Cas1b, Cas2b, Cas3, Cas17, Cas14, Cas10, Cas16, CasX, Cas3, Cas1, Cas15, Cas1f, Cas2, Cas3, Cas4, and Cm166.” (Sigma ‘613 at [0018]).

“In one embodiment, the RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In specific embodiments, the RNA-guided endonuclease is derived from a Cas9 protein. The Cas9 protein can be from Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Nocardiosis dassonvillei, Streptomyces pristinae spiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseu domycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Microscilla marina, Burkholderiales bacteria, Poloromonas napthalenivorans, Poloromonas sp., Crocosphaera watsonii, Cyanothecace sp., Microcystis aeruginosa, Synechococcus sp., Acetohalobium arabaticum, Ammoniformegens degensii, Caldicellulosiruptor becscii, Candidatus Desulfuridis, Clostridium botulinum,
Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter sp., Nitrosooccus halophilus, Nitrosooccus watsonii, Pseudoalteromonas haloplanktis, Kledonobacter racemifer, Methanolobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Arthrosira maxima, Arthrosira platensis, Arthrosira sp., Lyngbya sp., Microcoleus chthonoplastes, Oscillatoria sp., Petrotya mobilis, Thermosiphon africanus, or Acaryochloris marina."  (Sigma '613 at [0019]).  See also Sigma '613 at [0020]-[0023].

"The RNA-guided endonuclease disclosed herein comprises at least one nuclear localization signal. In general, an NLS comprises a stretch of basic amino acids. Nuclear localization signals are known in the art (see, e.g., Lange et al., J. Biol. Chem., 2007, 282:5101-51 05). For example, in one embodiment, the NLS can be a monopartite sequence, such as PKKRRKV (SEQ ID NO:1) or PKKRRKV (SEQ ID NO:2). In another embodiment, the NLS can be a bipartite sequence. In still another embodiment, the NLS can be KRPAATKKAGQAKKKK (SEQ ID NO:3). The NLS can be located at the N-terminus, the C-terminal, or in an internal location of the RNA-guided endonuclease."  (Sigma '613 at [0024]).

(III) Nucleic Acids Encoding RNA-Guided Endonucleases or Fusion Proteins

Another aspect of the present disclosure provides nucleic acids encoding any of the RNA-guided endonucleases or fusion proteins described above in sections (I) and (II), respectively. The nucleic acid can be RNA or DNA in one embodiment, the nucleic acid encoding the RNA-guided endonuclease or fusion protein is mRNA The mRNA can be 5' capped and/or 3' polyadenylated. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease or fusion protein is DNA The DNA can be present in a vector (see below).  (Sigma '613 at [0056]).

"The nucleic acid encoding the RNA-guided endonuclease or fusion protein can be codon optimized for efficient translation into protein in the eukaryotic cell or animal of interest. For example, codons can be optimized for expression in humans, mice, rats, hamsters, cows, pigs, cats, dogs, fish, amphibians, plants, yeast, insects, and so forth. Programs for codon optimization are available as freeware. Commercial codon optimization programs are also available."  (Sigma '613 at [0057]).  See also Sigma '613 at [0058]-[0063]).

(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at
least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '613 at [0064]). See also Sigma '613 at [0065]-[0067].

“(a) RNA-guided endonuclease
The method comprises introducing into a cell or embryo at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal. Such RNA-guided endonucleases and nucleic acids encoding RNA-guided endonucleases are described above in sections (i) and (iii), respectively.” (Sigma '613 at [0068]). See also Sigma '613 at [0069]-[0070].

“(e) Introducing into the cell or embryo
The RNA-targeted endonuclease(s) (or encoding nucleic acid), the guide RNA(s) (or encoding DNA), and the optional donor polynucleotide(s) can be introduced into a cell or embryo by a variety of means. In some embodiments, the cell or embryo is transfected. Suitable transfection methods include calcium phosphate-mediated transfection, nucleofection (or electroporation), cationic polymer transfection (e.g., DEAE-dextran or polyethylenimine), viral transduction, virosome transfection, virion transfection, liposome transfection, cationic liposome transfection, immunoliposome transfection, nonliposomal lipid transfection, dendrimer transfection, heat shock transfection, magnetofection, lipofection, gene gun delivery, impalement, sonoporation, optical transfection, and proprietary agent-enhanced uptake of nucleic acids. Transfection methods are well known in the art (see, e.g., “Current Protocols in Molecular Biology” Ausubel et al., John Wiley & Sons, New York, 2003 or “Molecular Cloning: A Laboratory Manual” Sambrook & Russell, Cold Spring Harbor Press, Cold Spring Harbor, NY, 3rd edition, 2001). In other embodiments, the molecules are introduced into the cell or embryo by microinjection. Typically, the embryo is a fertilized one-cell stage embryo of the species of interest. For example, the molecules can be injected into the pronuclei of one cell embryos.” (Sigma '613 at [0094]).

“The RNA-targeted endonuclease(s) (or encoding nucleic acid), the guide RNA(s) (or DNAs encoding the guide RNA), and the optional donor polynucleotide(s) can be introduced into the cell or embryo simultaneously or sequentially. The ratio of the RNA-targeted
endonuclease(s) (or encoding nucleic acid) to the guide RNA(s) (or encoding DNA) generally will be about stoichiometric such that they can form an RNA-protein complex. In one embodiment, DNA encoding an RNA-targeted endonuclease and DNA encoding a guide RNA are delivered together within the plasmid vector.” (Sigma ’613 at [0095]).

“(g) Cell and embryo types
A variety of eukaryotic cells and embryos are suitable for use in the method. For example, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism. In general, the embryo is non-human mammalian embryo. In specific embodiments, the embryos can be a one cell nonhuman mammalian embryo. Exemplary mammalian embryos, including one cell embryos, include without limit mouse, rat, hamster, rodent, rabbit, feline, canine, ovine, porcine, bovine, equine, and primate embryos. In still other embodiments, the cell can be a stem cell. Suitable stem cells include without limit embryonic stem cells, ES-like stem cells, fetal stem cells, adult stem cells, pluripotent stem cells, induced pluripotent stem cells, multipotent stem cells, oligopotent stem cells, unipotent stem cells and others. In exemplary embodiments, the cell is a mammalian cell.” (Sigma ’613 at [0104]). See also Sigma ’613 at [0105].

“(VI) Genetically Modified Cells and Animals
The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ’613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma ’613 at [0115]). See also Sigma ’613 at [0116]-[0130].
wherein the CRISPR-Cas type II system protein is a Cas9 protein, and

<p>| The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. Non-limiting examples of suitable CRISPR/Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, CasF, CasG, CasH, CasI, Cas2, Cas3, Cas6c (or CasA), Cas2 (or CasB), CasE (or CasC), Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas10d, CasF, CasG, CasH, CasI, CasJ, and CasK. | The RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In specific embodiments, the RNA-guided endonuclease is derived from a Cas9 protein. The Cas9 protein can be from Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Nocardiosis dasonvillii, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Microscilla marina, Burkholderiales bacterium, Polaromonas napththalenivorans, Polaromonas sp., Crocosphaera watsonii, Cyanobacteria sp., Microcystis aeruginosa, Synechococcus sp., Acetohalobium arabaticum, Ammonifex degensii, Caldioceulosiruptor beccii, Candidatus Desulforudis, Clostridium botulinum, Clostridium difficile, Finegaldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus caldus, Acidithiobacillus ferrooxidans, Allochroamatum vinosum, Marinobacter sp., Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudomonas haloclines, Ktedonobacter racemifer, Methanoclostridium estelgatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Arthospira maxima, Arthospira |</p>
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<th>(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising</th>
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<tr>
<td><strong>(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease</strong></td>
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| Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a
| targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '613 at [0064]). See also Sigma '613 at [0065]-[0067].

**“(b) Guide RNA**
The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA. A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5’ end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.” (Sigma '613 at [0071]).

“Each guide RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs.” (Sigma '613 at [0072]). See also Sigma '613 at [0073]-[0077].

“In some embodiments, the guide RNA can be introduced into the cell or embryo as a RNA molecule. The RNA molecule can be transcribed *in vitro*. Alternatively, the RNA molecule can be chemically synthesized.” (Sigma '613 at [0078]).

“In other embodiments, the guide RNA can be introduced into the cell or embryo as a DNA molecule. In such cases, the DNA encoding the guide RNA can be operably linked to promoter control sequence for expression of the guide RNA in the cell or embryo of interest. For example, the RNA coding sequence can be operably linked to a promoter sequence that is recognized by RNA polymerase III (Pol III).” (Sigma '613 at [0079]). See also Sigma '613 at [0080]-[0081].

**“(VI) Genetically Modified Cells and Animals**
The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an
RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ’613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma ’613 at [0115]). See also Sigma ’613 at [0116]-[0130].

See also Examples 1-9 (Sigma ’613 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ’613.

(1) a first region at the 5’ end that is capable of base pairing with a target site in the chromosomal sequence, and

(IV) **Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease**  
Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma ’613 at [0064]). See also Sigma ’613 at [0065]-[0067].

(b) **Guide RNA**  
The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA. A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5’ end of the guide RNA base pairs with a specific protoscaler sequence in the chromosomal sequence.” (Sigma ’613 at [0071]).

“Each guide RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides...
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<th>a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs.” (Sigma ‘613 at [0072]).</th>
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<td>“The first region of the guide RNA is complementary to sequence (i.e., protospacer sequence) at the target site in the chromosomal sequence such that the first region of the guide RNA can base pair with the target site. In various embodiments, the first region of the guide RNA can comprise from about 10 nucleotides to more than about 25 nucleotides. For example, the region of base pairing between the first region of the guide RNA and the target site in the chromosomal sequence can be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more than 25 nucleotides in length. In an exemplary embodiment, the first region of the guide RNA is about 19, 20, or 21 nucleotides in length.” (Sigma ‘613 at [0073]).</td>
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| “(c) Target site

“An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGNG, and NNAGAAW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (iv)(b), the first region (at the 5’ end) of the guide RNA is complementary to the protospacer of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5’-N\textsubscript{19-21}-NGG-3’. The PAM is in italics.” (Sigma ‘613 at [0082]). |
| “The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest.” (Sigma ‘613 at [0083]). |
| “(vi) Genetically Modified Cells and Animals

The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an |
RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ‘613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA-guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma ‘613 at [0115]). See also Sigma ‘613 at [0116]-[0130].

See also Examples 1-9 (Sigma ‘613 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ‘613.

(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and

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<td>Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma ‘613 at [0064]). See also Sigma ‘613 at [0065]-[0067].</td>
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<td>The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5’ end of the guide RNA base pairs with a specific protosparse sequence in the chromosomal sequence.” (Sigma ‘613 at [0071]).</td>
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“Each guide RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides
a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs.” (Sigma '613 at [0072]).

“The guide RNA also comprises a second region that forms a secondary structure. In some embodiments, the secondary structure comprises a stem (or hairpin) and a loop. The length of the loop and the stem can vary. For example, the loop can range from about 3 to about 10 nucleotides in length, and the stem can range from about 6 to about 20 base pairs in length. The stem can comprise one or more bulges of 1 to about 10 nucleotides. Thus, the overall length of the second region can range from about 16 to about 60 nucleotides in length. In an exemplary embodiment, the loop is about 4 nucleotides in length and the stem comprises about 12 base pairs.” (Sigma '613 at [0074]).

*(IV) Genetically Modified Cells and Animals*

The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma '613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma '613 at [0115]). See also Sigma '613 at [0116]-[0130].

See also Examples 1-9 (Sigma '613 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '613.

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least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '613 at [0064]). See also Sigma '613 at [0065]-[0067].

"(c) Target site
"An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGNG, and NNAGAAW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (IV)(b), the first region (at the 5’ end) of the guide RNA is complementary to the protospacer of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5’-N19-21-NGG-3’. The PAM is in italics." (Sigma '613 at [0082]).

"The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest." (Sigma '613 at [0083]).

"(d) Optional donor polynucleotide
In some embodiments, the method further comprises introducing at least one donor polynucleotide into the embryo. A donor polynucleotide comprises at least one donor sequence. In some aspects, a donor sequence of the donor polynucleotide corresponds to an endogenous or native chromosomal sequence. For example, the donor sequence can be essentially identical to a portion of the chromosomal sequence at or near the targeted site, but which comprises at least one nucleotide change. Thus, the donor sequence can comprise a modified version of the wild type sequence at the targeted site such that, upon integration or exchange with the native sequence, the sequence at the targeted chromosomal location comprises at least one nucleotide change. For example, the change can be an insertion of one or more nucleotides, a deletion of one or more nucleotides, a substitution of one or more nucleotides, or combinations thereof. As a consequence of the
integration of the modified sequence, the cell or embryo/animal can produce a modified
gene product from the targeted chromosomal sequence." (Sigma '613 at [0084]).

“In other aspects, the donor sequence of the donor polynucleotide corresponds to an
exogenous sequence. As used herein, an "exogenous" sequence refers to a sequence that
is not native to the cell or embryo, or a sequence whose native location in the genome of
the cell or embryo is in a different location. For example, the exogenous sequence can
comprise protein coding sequence, which can be operably linked to an exogenous
promoter control sequence such that, upon integration into the genome, the cell or
embryo/animal is able to express the protein coded by the integrated sequence.
Alternatively, the exogenous sequence can be integrated into the chromosomal sequence
such that its expression is regulated by an endogenous promoter control sequence. In other
iterations, the exogenous sequence can be a transcriptional control sequence, another
expression control sequence, an RNA coding sequence, and so forth. Integration of an
exogenous sequence into a chromosomal sequence is termed a "knock in."” (Sigma '613
at [0085]).

“As can be appreciated by those skilled in the art, the length of the donor sequence can
and will vary. For example, the donor sequence can vary in length from several nucleotides
to hundreds of nucleotides to hundreds of thousands of nucleotides.” (Sigma '613 at
[0086]). See also Sigma '613 at [0087]-[0093].

“(f) Culturing the cell or embryo
The method further comprises maintaining the cell or embryo under appropriate conditions
such that the guide RNA(s) directs the RNA-guided endonuclease(s) to the targeted site(s)
in the chromosomal sequence, and the RNA-guided endonuclease(s) introduce at least one
double-stranded break in the chromosomal sequence. A double-stranded break can be
repaired by a DNA repair process such that the chromosomal sequence is modified by a
deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of
at least one nucleotide, or a combination thereof.” (Sigma '613 at [0096]).

“In embodiments in which no donor polynucleotide is introduced into the cell or embryo, the
double-stranded break can be repaired via a non-homologous end-joining (NHEJ) repair
process. Because NHEJ is error-prone, deletions of at least one nucleotide, insertions of at
least one nucleotide, substitutions of at least one nucleotide, or combinations thereof can
occur during the repair of the break. Accordingly, the sequence at the chromosomal
sequence can be modified such that the reading frame of a coding region can be shifted
and that the chromosomal sequence is inactivated or "knocked out." An inactivated protein-
coding chromosomal sequence does not give rise to the protein coded by the wild type
chromosomal sequence. (Sigma '613 at [0097]).
“In embodiments in which a donor polynucleotide comprising upstream and downstream sequences is introduced into the cell or embryo, the double-stranded break can be repaired by a homology-directed repair (HDR) process such that the donor sequence is integrated into the chromosomal sequence. Accordingly, an exogenous sequence can be integrated into the genome of the cell or embryo, or the targeted chromosomal sequence can be modified by exchange of a modified sequence for the wild type chromosomal sequence.” (Sigma ’613 at [0098]). See also Sigma ’613 at [0099]-[0103].

*(VI) Genetically Modified Cells and Animals*

The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ’613 at [0114]).

“*The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma ’613 at [0115]). See also Sigma ’613 at [0116]-[0130].

See also Examples 1-9 (Sigma ’613 at [0142]-[0160]) and accompanying Figures, Claims 1-16 of Sigma ’613.
### APPENDIX VI

**EXAMPLES OF SUPPORT FOR PENDING CLAIM 1 OF U.S. PATENT APPLICATION NO. 16/654,613 IN U.S. PROVISIONAL APPLICATION NO. 61/734,256 FILED DECEMBER 6, 2012**

<table>
<thead>
<tr>
<th>SIGMA’S U.S. IN 16/654,613</th>
<th>SIGMA’S U.S. IN 61/734,256</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Proposed Claim for Interference)</td>
<td>filed December 6, 2012</td>
</tr>
<tr>
<td>(hereinafter “Chen P1”)</td>
<td>(hereinafter “Chen P1”)</td>
</tr>
</tbody>
</table>

1. A eukaryotic cell comprising

```
“(II) Method for Modifying a Chromosomal Sequence

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell. The method comprises introducing into the eukaryotic cell (i) an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease, wherein the RNA-guided endonuclease comprises a nuclear localization signal, (ii) at least one guiding RNA or at least one DNA molecule encoding a guiding RNA, wherein each guiding RNA guides the RNA-guided endonuclease to a targeted site in the chromosomal sequence, and optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell such that the RNA-guided endonuclease introduces a double-stranded break at the targeted site in the chromosomal sequence and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.”  
(Chen P1 at [0016]).

“A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism.”  
(Chen P1 at [0045]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.
```

2. a target chromosomal sequence; and

```
“The present disclosure provides RNA-guided endonucleases that are engineered for use in eukaryotic cells. The RNA-guided endonuclease can be used to modify the genome of eukaryotic cells. For this, the endonuclease is guided to a specific chromosomal sequence by a specific guiding RNA.”  
(Chen P1 at [0003]).

“(II) Method for Modifying a Chromosomal Sequence

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell. The method comprises introducing into the
```
eukaryotic cell (i) an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease, wherein the RNA-guided endonuclease comprises a nuclear localization signal, (ii) at least one guiding RNA or at least one DNA molecule encoding a guiding RNA, wherein each guiding RNA guides the RNA-guided endonuclease to a targeted site in the chromosomal sequence, and optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell such that the RNA-guided endonuclease introduces a double-stranded break at the targeted site in the chromosomal sequence and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.” (Chen P1 at [0016]).

“The RNA-guided endonuclease in conjunction with the guiding RNA is directed to a target site in the chromosomal sequence and cleaves the chromosomal sequence.” (Chen P1 at [0017]).

See also Examples 1-5 (Chen P1 at [0056]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

(i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II protein is codon optimized for expression in the eukaryotic cell, and

“One aspect of the present disclosure provides RNA-guided endonucleases that comprise at least one nuclear localization signal, which permits entry of the endonuclease into the nuclei of eukaryotic cells.” (Chen P1 at [0004]).

“The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. In some embodiments, the RNA-guided endonuclease can be derived from a type II CRISPR/Cas system.” (Chen P1 at [0004]).

“In some embodiments, the RNA-guided endonuclease can be derived from a wild type Cas9 protein(s) or fragment(s) thereof. In other embodiments, the RNA-guided endonuclease can be derived from modified Cas9 protein(s). For example, the amino acid sequence of the Cas9 protein can be modified such that one or more properties (e.g., nuclease activity, affinity, stability, etc.) of the protein is improved. Alternatively, domains of the Cas9 protein not involved in RNA-guided cleavage can be eliminated from the protein such that the modified Cas9 protein is smaller than the wild type Cas9 protein. In still other embodiments, the RNA-guided endonuclease can be a fusion protein comprising domains of wild type Cas9 proteins, modified Cas9 proteins, and/or other proteins. For example, the RNA-guided endonuclease could comprise a marker, such as GFP or another fluorescent protein.” (Chen P1 at [0006]).
The Cas9-derived endonucleases disclosed herein comprise at least one nuclear localization signal (NLS) for transport into the nuclei of eukaryotic cells. In general, an NLS comprise a stretch of basic amino acids. Nuclear localization signals are known in the art (see, e.g., Lange et al., J. Biol. Chem., 2007, 282:5101-5105). For example, examples of classical NLSs included monopartite sequences such as PKKRRK (SEQ ID NO:1) or PKKRRV (SEQ ID NO:13), as well as bipartite sequences. The NLS can be located at the N-terminus, the C-terminal, or in an internal location of the endonuclease. In an exemplary embodiment, the NLS is located at the C-terminus of the endonuclease. (Chen P1 at [0008]).

The disclosure also provides nucleic acids encoding the RNA-guided endonuclease. The nucleic acid encoding the RNA-guided endonuclease can be RNA or DNA. The DNA encoding the RNA-guided endonuclease can be present in a vector (see below). (Chen P1 at [0011]).

In embodiments in which the nucleic acid encoding the RNA-guided endonuclease is DNA, the DNA coding sequence can be codon optimized for efficient translation into protein in the eukaryotic cell of interest. For example, the codons can be optimized for expression in humans, mice, rats, hamsters, cows, pigs, cats, dogs, fish, amphibians, plants, yeast, insects, and so forth (see Codon Usage Database at www.kazusa.or.jp/codon/). Programs for codon optimization are available as freeware (e.g., OPTIMIZER at http://genomes.urv.es/OPTIMIZER; OptimumGeneTM from GenScript at http://www.genscript.com/codon_opt.html). Commercial codon optimization programs are also available. In an exemplary embodiment, the DNA encoding a modified Cas9 protein consisting of SEQ ID NO.2 is codon optimized for translation in human cells and consists of SEQ ID NO.3. In various iterations, the DNA encoding sequence can be at least about 75%, 80%, 85%, 95%, or 99% identical to SEQ ID NO.3. (Chen P1 at [0012]). See also Chen P1 at [0013]-[0015].

The method comprises introducing into the cell an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease. (Chen P1 at [0018]).

(d) Introducing into the cell
The RNA-targeted endonuclease (or its encoding nucleic acid), the guiding RNA(s) (or DNAs encoding the guiding RNA), and the optional donor polynucleotide(s) can be introduced into the cell by a variety of means. In some embodiments, the cell is transfected. Suitable transfection methods include calcium phosphate-mediated transfection, nucleofection (or electroporation), cationic polymer transfection (e.g., DEAE-dextran or polyethylenimine), viral transduction, virosome transfection, virion transfection, liposome
transfection, cationic liposome transfection, immunoliposome transfection, nonliposomal lipid transfection, dendrimer transfection, heat shock transfection, magnetofection, lipofection, gene gun delivery, impalefection, sonoporation, optical transfection, and proprietary agent-enhanced uptake of nucleic acids. Transfection methods are well known in the art (see, e.g., "Current Protocols in Molecular Biology" Ausubel et al., John Wiley & Sons, New York, 2003 or "Molecular Cloning: A Laboratory Manual" Sambrook & Russell, Cold Spring Harbor Press, Cold Spring Harbor, NY, 3rd edition, 2001). In other embodiments, the molecules are introduced into the cell by microinjection. For example, the molecules can be injected into the pronuclei of one cell embryos." (Chen P1 at [0037]). See also Chen P1 at [0038].

“A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism.” (Chen P1 at [0045]).

“The terms “nucleic acid” and “polynucleotide” refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Chen P1 at [0053]).

“The terms “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues.” (Chen P1 at [0055]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

wherein the CRISPR-Cas type II system protein is a Cas9 protein, and

“in exemplary embodiments, the endonuclease can be derived from a Cas9 protein of a type II system.” (Chen P1 at [0004]).

“The terms “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues.” (Chen P1 at [0055]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.
| (ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising | “The present disclosure provides RNA-guided endonucleases that are engineered for use in eukaryotic cells. The RNA-guided endonuclease can be used to modify the genome of eukaryotic cells. For this, the endonuclease is guided to a specific chromosomal sequence by a specific guiding RNA.” (Chen P1 at [0003]).

“The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell.” (Chen P1 at [0019]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1. |
|---|---|
| (1) a first region at the 5’ end that is capable of base pairing with a target site in the chromosomal sequence, and | “(a) Target site
The RNA-guided endonuclease in conjunction with the guiding RNA is directed to a target site in the chromosomal sequence and cleaves the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG and NGGNG (wherein N is defined as any nucleotide). The target site can be in the coding region of a gene, in an intron of a gene, in a control region between genes, etc. The gene can be a protein coding gene or an RNA coding gene.” (Chen P1 at [0017]).

“The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell. Each guiding RNA interacts with the RNA-guided endonuclease to guide the endonuclease to a specific target site, wherein the endonuclease cleaves the target site. Each guiding RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guiding RNA is different such that each guiding RNA guides the endonuclease to a specific target site. The second and third regions of each guiding RNA can be the same in all guiding RNAs.” (Chen P1 at [0019]).

“The first region of the guiding RNA is complementary to the target site in the chromosomal sequence such that the first region of the guiding RNA can base pair with the target site. In various embodiments, the first region of the guiding RNA can comprise from about 10 nucleotides to more than about 25 nucleotides.” (Chen P1 at [0020]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1. |
| (2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and | “The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell. Each guiding RNA interacts with the RNA-guided endonuclease to guide the endonuclease to a specific target site, wherein the |
| whereby base pairing of the first region to the target site is capable of targeting the Cas9 protein to the target chromosomal sequence. | endonuclease cleaves the target site. Each guiding RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guiding RNA is different such that each guiding RNA guides the endonuclease to a specific target site. The second and third regions of each guiding RNA can be the same in all guiding RNAs." (Chen P1 at [0019]).

"The guiding RNA also comprises a second region that forms a secondary structure. In some embodiments, the secondary structure comprises a stem (or hairpin) and a loop. The length of the loop and the stem can vary. For example, the loop can range from about 3 to about 10 nucleotides in length, and the stem can range from about 6 to about 20 base pairs in length. The stem can comprise one or more bulges of 1 to about 10 nucleotides. Thus, the overall length of the second region can range from about 16 to about 60 nucleotides in length. In an exemplary embodiment, the loop is about 4 nucleotides in length and the stem comprises about 12 base pairs." (Chen P1 at [0021]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1. |
| --- | --- |
| “[T]he endonuclease is guided to a specific chromosomal sequence by a specific guiding RNA." (Chen P1 at [0003]). | “The double-stranded break can be repaired by a cellular DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.” (Chen P1 at [0003]).

The first region of the guiding RNA is complementary to the target site in the chromosomal sequence such that the first region of the guiding RNA can base pair with the target site.” (Chen P1 at [0020]). |
| “(e) Culturing the cell
The method further comprises maintaining the cell under appropriate conditions such that the guiding RNA guides the endonuclease to the targeted site in the chromosomal sequence, and the endonuclease introduces a double-stranded break in the targeted site in the chromosomal sequence. The double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.” (Chen P1 at [0039]). |

6
| “A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism.” (Chen P1 at [0045]).

“*The terms “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues.*” (Chen P1 at [0055]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.
SUGGESTION OF INTERFERENCE PURSUANT TO 37 C.F.R. § 41.202

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicant Sigma-Aldrich Co. LLC respectfully requests that an interference be declared involving (1) at least Claim 1 of U.S. Patent Application No. 15/188,911 (“the Sigma ‘911 Application”), which is concurrently filed herewith in an Amendment and Reply; and (2) at least Claim 1 of U.S. Patent Application No. 16/654,613 (“the Sigma ‘613 Application”), which Applicant filed on Wednesday, October 16, 2019.

Pursuant to 37 C.F.R. § 41.202, Applicant provides, inter alia: (1) an identification of the patents with which Applicant seeks an interference; (2) proposed counts and claim correspondence thereto; (3) an identification of claims that Applicants believe interfere; (4) an explanation of why Applicant will prevail on priority; (5) claim charts showing written description in Applicant’s specification for each claim added to provoke the interference; and (6) for each constructive reduction to practice for which the applicant wishes to be accorded benefit, provide a chart showing where the disclosure provides a constructive reduction to practice within the scope of the interfering subject matter.
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APPENDIX VI – Support for Claim 1 of the Sigma ’613 Application in U.S. Provisional Application No. 61/734,256
I. 37 C.F.R. § 41.202(a)(1) – Identification of Patents With Which Applicant Seeks an Interference

Applicant requests that an interference be declared between the Sigma ‘911 Application and U.S. Patent Application No. 15/947,718 ("the UCB ‘718 Application").

Applicant also requests that an interference be declared between the Sigma ‘613 Application and U.S. Patent Application No. 15/981,809 ("the UCB ‘809 Application").

At filing, the Sigma ‘911 Application and the Sigma ‘613 Application listed Fuqiang Chen and Greg Davis as co-inventors. According to the USPTO’s assignment records, Drs. Chen and Davis have assigned their interest in the Sigma ‘911 Application and the Sigma ‘613 Application to Sigma-Aldrich Co. LLC. The Sigma ‘911 Application and the Sigma ‘613 Application each claim priority benefit to U.S. Provisional Application No. 61/734,256 filed December 6, 2012, U.S. Provisional Application No. 61/758,624 filed January 30, 2013; U.S. Provisional Application No. 61/761,046 filed February 5, 2013, and U.S. Provisional Application No. 61/794,422 filed March 15, 2013.

With regard to the UCB ‘718 Application and the UCB ‘809 Application, each lists Jennifer A. Doudna, Martin Jinek, Krzysztof Chylinski, and Emmanuelle Charpentier as co-inventors. According to the USPTO’s assignment records, Jennifer A. Doudna and Martin Jinek assigned their rights in the UCB ‘718 Application and the UCB ‘809 Application to The Reagents of the University of California, and Krzysztof Chylinski assigned his interest in the UCB ‘718 Application and the UCB ‘809 Application to the University of Vienna. The UCB ‘718 Application and the UCB ‘809 Application claim priority benefit to U.S. Provisional Application No. 61/652,086 filed May 25, 2012; U.S. Provisional Application No. 61/716,256 filed October 19, 2012; U.S. 61/757,640 filed January 28, 2013; and U.S. Provisional Application No. 61/765,576 filed February 15, 2013.

II. 37 C.F.R. § 41.202(a)(2) and 37 C.F.R. § 41.202(a)(3) – Proposal of Counts, Claim Correspondence, and Identification of All Claims Applicant Believes Interfere

Pursuant to 37 C.F.R. § 41.202(a)(2), Applicant hereby proposes two Counts, sets forth the claims from the Sigma and UCB Applications that correspond to each Count, and identifies the claims Applicant believes interfere.
Proposed Count 1 is directed to methods and Proposed Count 2 is directed to compositions.

**A. Proposed Count 1 – Methods**

Proposed Count 1 is directed to methods of cleaving or editing a target DNA molecule or target chromosomal sequence in a eukaryotic cell by employing a Type-II CRISPR Cas system. Proposed Count 1 is an alternative (aka “McKelvey”) count: “Claim 1 of the Sigma ‘911 Application OR Claim 156 of the UCB ‘718 Application.” The following Table 1 presents each alternative of Proposed Count 1:

<table>
<thead>
<tr>
<th>Alternative Count 1A – Claim 1 of the Sigma ‘911 Application</th>
<th>Alternative Count 1B – Claim 156 of the UCB ‘718 Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising:</td>
<td>156. A method of cleaving or editing a target DNA molecule or modulating transcription of at least one gene encoded thereon, the method comprising</td>
</tr>
<tr>
<td>introducing into the eukaryotic cell</td>
<td>contacting a target DNA molecule having a target sequence with</td>
</tr>
<tr>
<td>(i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas type II protein is codon optimized for expression in the eukaryotic cell, and</td>
<td>an engineered and/or non-naturally-occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising:</td>
</tr>
<tr>
<td>wherein the CRISPR-Cas type II system protein is a Cas9 protein,</td>
<td>(a) a Cas9 protein; and</td>
</tr>
<tr>
<td>(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising</td>
<td>(b) a DNA-targeting RNA comprising:</td>
</tr>
</tbody>
</table>
(1) a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and

(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and

(iii) at least one donor polynucleotide comprising the exogenous sequence; and

whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process leads to integration of the exogenous sequence into the chromosomal sequence.

(i) a targeter-RNA that hybridizes with the target sequence, and

(ii) an activator-RNA that hybridizes with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,

wherein said contacting takes place outside of a bacterial cell and outside an archaeal cell,

wherein the DNA-targeting RNA forms a complex with the Cas9 protein,

whereby said target DNA molecule is cleaved or edited or transcription of at least one gene encoded by the target DNA molecule is modulated,

wherein said contacting occurs in a eukaryotic cell.

1. Claims That Correspond to Proposed Count 1

“A claim corresponds to a count if the subject matter of the count, treated as prior art to the claim, would have anticipated or rendered obvious the subject matter of the claim.” 37 C.F.R. § 41.207(b)(2). The following Table 2 sets forth the claims from the Sigma Applications and Claims, and the claims from the UCB Applications and Claims, that correspond to Proposed Count 1:

**TABLE 2**

<table>
<thead>
<tr>
<th>Proposed Count 1 - Methods</th>
<th>Sigma Applications and Claims that Correspond</th>
<th>UCB Applications and Claims that Correspond</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘911 Application – Claim 1</td>
<td>’718 Application – Claim 156</td>
<td></td>
</tr>
</tbody>
</table>
2. Identification of Interfering Claims

At a minimum, Claim 1 of the Sigma '911 Application defines the same invention as Claim 156 of the UCB '718 Application. A detailed comparison of Claim 1 of the Sigma '911 Application with Claim 156 of the UCB '718 Application is provided in Appendices IA and IB.

B. Proposed Count 2 – Compositions

Proposed Count 2 is directed to compositions, more specifically eukaryotic cells that are modified using a Type-II CRISPR Cas system. Proposed Count 1 is an alternative (aka “McKelvey”) count: “Claim 1 of the Sigma ‘613 Application OR Claim 156 of the UCB ‘809 Application.” The following Table 3 presents each alternative of Proposed Count 2:

<table>
<thead>
<tr>
<th>Alternative Count 2A – Claim 1 of the Sigma ‘613 Application</th>
<th>Alternative Count 2B – Claim 156 of the UCB ‘809 Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A eukaryotic cell comprising a target chromosomal sequence; and (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II</td>
<td>156. A eukaryotic cell comprising a target DNA molecule and an engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising</td>
</tr>
</tbody>
</table>
protein is codon optimized for expression in the eukaryotic cell, and

wherein the CRISPR-Cas type II system protein is a Cas9 protein, and

(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising

(1) a first region at the 5’ end that is capable of base pairing with a target site in the chromosomal sequence, and

(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and

whereby base pairing of the first region to the target site is capable of targeting the Cas9 protein to the target chromosomal sequence.

a Cas9 protein or a nucleic acid comprising a nucleotide sequence encoding the Cas9 protein, and

a DNA-targeting RNA or one or more nucleic acids comprising one or more nucleotide sequences encoding the DNA-targeting RNA; wherein the DNA-targeting RNA comprises

i) a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule, and

ii) an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,

whereby hybridization of the targeter-RNA to the target sequence is capable of targeting the Cas9 protein to the target DNA molecule.

1. Claims That Correspond to Proposed Count 2

“A claim corresponds to a count if the subject matter of the count, treated as prior art to the claim, would have anticipated or rendered obvious the subject matter of the claim.” 37 C.F.R. § 41.207(b)(2). The following Table 4 sets forth the claims from the Sigma Applications and Claims, and the claims from the UCB Applications and Claims, that correspond to Proposed Count 2:

**TABLE 4**

<table>
<thead>
<tr>
<th>Proposed Count 2 – Compositions</th>
<th>Sigma Applications and Claims that Correspond</th>
<th>UCB Applications and Claims that Correspond</th>
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<tbody>
<tr>
<td>'613 Application – Claim 1</td>
<td>‘809 Application – Claim 156</td>
<td>‘809 Application – Claim 156</td>
</tr>
<tr>
<td></td>
<td>U.S. Patent Application No. 15/981,808 – Claim 156</td>
<td>U.S. Patent Application No. 15/981,808 – Claim 156</td>
</tr>
</tbody>
</table>

7
2. Identification of Interfering Claims

At a minimum, Claim 1 of the Sigma ‘613 Application defines the same invention as Claim 156 of the UCB ‘809 Application. A detailed comparison of Claim 1 of the Sigma ‘613 Application with Claim 156 of the UCB ‘809 Application is provided in Appendices IIA and IIB.

C. Other UCB Patents and Patent Applications That May Correspond to Proposed Count 1 and/or Proposed Count 2

Applicant reserves the right to supplement its request to suggest an interference with any one or more of the following (or other) UCB patent applications and patents:

- U.S. Patent Application Nos. 15/947,680; 15/981,807; 16/136,159; 16/136,165; 16/136,168; and/or 16/136,175; and/or
- U.S. Patent Nos. 10,000,772; 10,113,167; 10,227,611; 10,266,850; 10,301,651; 10,308,961; 10,337,029; 10,351,878; 10,358,658; 10,358,659; 10,385,360; 10,400,253; 10,407,697; 10,415,061; 10,421,980; and/or 10,428,352.

Applicant notes that each of the aforementioned UCB patent applications and patents listed in Table 2, Table 4, and the first and second bullet points above are involved in presently pending Patent Interference No. 106,115 with several patents to The Broad Institute/Massachusetts Institute of Technology/President and Fellows of Harvard College.

IV. 37 C.F.R. § 41.202(a)(4) – Why Applicant Will Prevail on Priority

Applicant will prevail on priority because Applicant was the first to invent the subject matter of the Proposed Counts, namely, methods and compositions of CRISPR-Cas9 in eukaryotic cells. In particular, Applicant’s earliest-filed benefit application is as follows:

- U.S. Provisional Application No. 61/734,256, filed December 6, 2012 ("Chen P1").
As set forth herein and in Appendices IV and VI, Chen P1 provides a constructive reduction to practice of the Proposed Counts (i.e., a described and enabled anticipation under 35 U.S.C. § 102(g)(1) of the subject matter of the Proposed Counts). Thus, Applicant has a constructive reduction to practice date of December 6, 2012, and an even earlier date for an actual reduction to practice.

UCB’s earliest-filed applications are as follows:

- U.S. Provisional App. No. 61/652,086, filed May 25, 2012 (“Doudna P1”);
- U.S. Provisional Application No. 61/716,256, filed October 19, 2012 (“Doudna P2”); and
- U.S. Provisional Application No. 61/757,640, filed January 28, 2013 (“Doudna P3”).

Importantly, while Doudna P1 and Doudna P2 pre-date Chen P1, those UCB provisional applications are directed to CRISPR-Cas9 in *in vitro* cell-free and nucleus-free test tube environments (a “prokaryotic environment”). The Declarations of Drs. Lambowitz, Loring, Urnov, and Cannon, which are of record in the involved Sigma-Aldrich patent applications, confirm the lack of any reasonable expectation of success that one of ordinary skill in the art would have had at the time of the invention to use the disclosed CRISPR-Cas9 prokaryotic system in eukaryotic cells. The Declarations also discuss in detail the failures and considerable barriers that were known to one of ordinary skill in the art at the time of the invention that contributed to the doubt and uncertainty of successfully using a CRISPR-Cas9 system in eukaryotic cells.

Furthermore, these Declarations confirm that the incomplete and insufficient disclosure in Doudna P1, Doudna P2, and Jinek 2012 would not have enabled one of ordinary skill in the art at the time of the invention to practice a CRISPR-Cas9 system adapted to function in a eukaryotic cell without undue experimentation, nor do they provide a written description demonstrating possession of a CRISPR-Cas9 system adapted to function in a eukaryotic cell:
19. In my considered opinion, set forth in detail below, the incomplete and insufficient disclosure in Doudna P1, Doudna P2, and Jinek 2012 does not enable one of ordinary skill in the art to practice a CRISPR-Cas9 system adapted to function in a eukaryotic cell without undue experimentation, nor do they provide a written description demonstrating possession of a CRISPR-Cas9 system adapted to function in a eukaryotic cell. Referring to Wands Factor (1), Doudna P1, Doudna P2, and Jinek 2012 provide no useful guidance for one of ordinary skill to adapt CRISPR-Cas9 for use in eukaryotic cells. Regarding Wands Factor (2), adapting CRISPR-Cas9 to function in eukaryotic cells required extensive experimentation, as shown by Dr. Doudna’s own experiences, which she admitted resulted in “many frustrations.” Regarding Factor (3), Doudna P1, Doudna P2, and Jinek 2012 contain no working examples of CRISPR-Cas9 functioning in eukaryotic cells. Regarding Wands Factors (4), (5), and (6), given the nascent state of CRISPR-Cas technology, the nature of the disclosure requires more details than may be needed in other instances. Finally, regarding Wands Factors (7) and (8), the field is highly unpredictable and even Dr. Doudna admitted that she “[wasn’t] sure if CRISPR/Cas9 would work in eukaryotes.”

20. Furthermore, in my considered opinion, set forth in detail below, based at least upon the incomplete and insufficient disclosure in Doudna P1, Doudna P2, and Jinek 2012 one or ordinary skill in the art would not have had a reasonable expectation of success that a CRISPR-Cas9 system could be adapted to function in a eukaryotic cell.


Notably, the Declaration evidence of record is even stronger than that considered by the PTAB and the Federal Circuit in the first UC v. Broad interference (Int’l No. 106,048) and appeal. See Broad Inst., Inc. v. Regents of the Univ. of Cal., Decision on Motions, Int’l No. 106,048 (DK), 2017 WL 657415, Paper 893 (PTAB Feb. 15, 2017); Regents of the Univ. of Cal. v. Broad Inst., Inc., 903 F.3d 1286, 1294 (Fed. Cir. 2018).

Accordingly, UCB’s earliest possible constructive reduction to practice of the Proposed Counts is January 28, 2013, nearly two months later than Applicants’ date.
And while it is possible that UCB has an earlier actual reduction to practice date, it is highly speculative and unlikely that UCB would be able to pre-date Applicant’s considerably earlier date of invention. This is particularly true given the PTAB’s stringent and exacting standards for proving conception, diligence, and reduction to practice. Thus, Applicant is likely to prevail on priority of invention for each of the two Proposed Counts.

V. 37 C.F.R. §§ 41.202(a)(5) & (a)(6) – Claim Charts Showing Written Description in Applicant’s Specification and Benefit Application

A. The Sigma ‘911 Application

The Sigma ‘911 Application is a 35 U.S.C. § 120 Continuation of U.S. Patent Application No. 14/649,777, which is a 35 U.S.C. § 371 National Stage application from PCT International Application No. PCT/US2013/073307. A chart is provided in Appendix III showing where exemplary written description support for Claim 1 of the Sigma ‘911 Application can be found in the Sigma ‘911 Specification.

The Sigma ‘911 Application also claims the benefit of, *inter alia*, U.S. Provisional Application No. 61/734,256 filed December 6, 2012. A chart is provided in Appendix IV showing where exemplary written description support for Claim 1 of Sigma ‘911 Application can be found in U.S. Provisional Application No. 61/734,256.

B. The Sigma ‘613 Application

The Sigma ‘613 Application is a 35 U.S.C. § 120 Continuation of the Sigma ‘911 Application, which is a 35 U.S.C. § 120 Continuation of U.S. Patent Application No. 14/649,777, which is a 35 U.S.C. § 371 National Stage application from PCT International Application No. PCT/US2013/073307. A chart is provided in Appendix V showing where exemplary written description support for Claim 1 of the Sigma ‘613 Application can be found in the Sigma ‘613 Specification.

The Sigma ‘613 Application also claims the benefit of, *inter alia*, U.S. Provisional Application No. 61/734,256 filed December 6, 2012. A chart is provided in Appendix VI showing where exemplary written description support for Claim 1 of the Sigma ‘613 Application can be found in U.S. Provisional Application No. 61/734,256.
VI. Conclusion

Applicant respectfully requests a declaration of interference between (1) the Sigma ‘911 Application and the UCB ‘718 Application with Proposed Count 1; and (2) the Sigma ‘613 Application and the UCB ‘809 Application with Proposed Count 2.

Respectfully submitted,

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## APPENDIX IA - COLOR

**SIDE-BY-SIDE COMPARISON OF CLAIM 1 OF SIGMA’S U.S. PATENT APPLICATION NO. 15/188,911 WITH CLAIM 156 OF UCB’S U.S. PATENT APPLICATION NO. 15/947,718**

<table>
<thead>
<tr>
<th>UCB’S USSN 15/947,718</th>
<th>SIGMA’S USSN 15/188,911</th>
<th>Comments</th>
</tr>
</thead>
</table>
| 156. A method of cleaving or editing a target DNA molecule or modulating transcription of at least one gene encoded thereon, the method comprising: | 1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising: | Sigma’s “integrating” ≈ UCB’s “editing.”  
“Integration” of an exogenous sequence into a chromosomal sequence requires “cleaving” the chromosomal sequence first. |
| contacting a target DNA molecule having a target sequence with an engineered and/or non-naturally-occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising: | introducing into the eukaryotic cell (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas type II system protein is codon optimized for expression in the eukaryotic cell, and | Sigma and UCB’s claims each require a Type II CRISPR/Cas system. |
| (a) a Cas9 protein; and | wherein the CRISPR-Cas type II system protein is a Cas9 protein; | Sigma and UCB’s claims each require a Cas9 protein. |
| (b) a DNA-targeting RNA comprising: | (ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising | Sigma’s “guide RNA” ≈ UCB’s “DNA-targeting RNA.” |
| (i) a targeter-RNA that hybridizes with the target sequence, and | (1) a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and | Sigma’s “first region” ≈ UCB’s “targeter-RNA.”  
Sigma’s “base pairs with a target...” ≈ UCB’s “hybridizes with the target...” |
| (ii) an activator-RNA that hybridizes with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment, | (2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and | Sigma’s “second region” ≈ UCB’s “activator-RNA.” |
(iii) at least one donor polynucleotide comprising the exogenous sequence; and

wherein said contacting takes place outside of a bacterial cell and outside an archaean cell,

Sigma’s claim requires a “donor polynucleotide” that is “integrated” into the eukaryotic chromosome thereby “editing” it.

wherein the DNA-targeting RNA forms a complex with the Cas9 protein,

Sigma’s claim is directed to modifying a eukaryotic cell.

whereby said target DNA molecule is cleaved or edited or transcription of at least one gene encoded by the target DNA molecule is modulated,

whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process leads to integration of the exogenous sequence into the chromosomal sequence.

The “second region” of Sigma’s “guide RNA” “interacts with the at least one RNA-guided endonuclease.”

Sigma’s “introduces a double-stranded break” ≈ UCB’s “cleaved.”

Sigma’s “integrating” ≈ UCB’s “editing.”

wherein said contacting occurs in a eukaryotic cell.

Sigma’s claim is directed to modifying a eukaryotic cell.
## APPENDIX IB - GRAYSCALE

**SIDE-BY-SIDE COMPARISON OF CLAIM 1 OF SIGMA’S U.S. PATENT APPLICATION NO. 15/188,911 WITH CLAIM 156 OF UCB’S U.S. PATENT APPLICATION NO. 15/947,718**

<table>
<thead>
<tr>
<th>UCB’S USSN 15/947,718</th>
<th>SIGMA’S USSN 15/188,911</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>156. A method of cleaving or editing a target DNA molecule or modulating transcription of at least one gene encoded thereon, the method comprising</td>
<td>1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising:</td>
<td>Sigma’s “integrating” = UCB’s “editing.” “Integr[jon] of an exogenous sequence into a chromosomal sequence” requires “cleaving” the chromosomal sequence first.</td>
</tr>
<tr>
<td>contacting a target DNA molecule having a target sequence with an engineered and/or non-naturally-occurring <strong>Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising:</strong></td>
<td><strong>Introducing into the eukaryotic cell</strong> (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II protein is codon optimized for expression in the eukaryotic cell, and</td>
<td>Sigma and UCB’s claims each require a Type II CRISPR/Cas system.</td>
</tr>
<tr>
<td>(a) a Cas9 protein; and</td>
<td>wherein the CRISPR-Cas type II system protein is a Cas9 protein;</td>
<td>Sigma and UCB’s claims each require a Cas9 protein.</td>
</tr>
<tr>
<td>(b) a DNA-targeting RNA comprising:</td>
<td>(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising</td>
<td>Sigma’s “guide RNA” = UCB’s “DNA-targeting RNA.”</td>
</tr>
<tr>
<td>(i) a targeter-RNA that hybridizes with the target sequence, and</td>
<td>(1) a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and</td>
<td>Sigma’s “first region” = UCB’s “targeter-RNA.” Sigma’s “base pairs with a target...” = UCB’s “hybridizes with the target...”</td>
</tr>
<tr>
<td>(ii) an activator-RNA that hybridizes with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,</td>
<td>(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and</td>
<td>Sigma’s “second region” ≈ UCB’s “activator-RNA”</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>wherein said contacting takes place outside of a bacterial cell and outside an archaeal cell,</td>
<td>(iii) at least one donor polynucleotide comprising the exogenous sequence; and</td>
<td>Sigma’s claim requires a “donor polynucleotide” that is “integrated” into the eukaryotic chromosome thereby “editing” it.</td>
</tr>
<tr>
<td>wherein the DNA-targeting RNA forms a complex with the Cas9 protein,</td>
<td></td>
<td>Sigma’s claim is directed to modifying a eukaryotic cell.</td>
</tr>
<tr>
<td>whereby said target DNA molecule is cleaved or edited or transcription of at least one gene encoded by the target DNA molecule is modulated,</td>
<td>whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process leads to integration of the exogenous sequence into the chromosomal sequence.</td>
<td>Sigma’s “introduces a double-stranded break” ≈ UCB’s “cleaved.” Sigma’s “integrating” ≈ UCB’s “editing.”</td>
</tr>
<tr>
<td>wherein said contacting occurs in a eukaryotic cell.</td>
<td></td>
<td>Sigma's claim is directed to modifying a eukaryotic cell.</td>
</tr>
</tbody>
</table>
### APPENDIX II

**SIDE-BY-SIDE COMPARISON OF CLAIM 1 OF SIGMA’S U.S. PATENT APPLICATION NO. 16/654,613 WITH CLAIM 156 OF UCB’S U.S. PATENT APPLICATION NO. 15/981,809**

<table>
<thead>
<tr>
<th>UCB’S USPN 15/981,809</th>
<th>SIGMA’S USPN 16/654,613</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>156. A eukaryotic cell comprising a target DNA molecule and an engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising</td>
<td>1. A eukaryotic cell comprising a target chromosomal sequence; and (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II protein is codon optimized for expression in the eukaryotic cell, and (ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising a) a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule; and b) an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,</td>
<td>Sigma and UCB are each claiming a eukaryotic cell Sigma’s “target chromosomal sequence” ≈ UCB’s “target DNA molecule” Sigma and UCB’s claims each require a Type II CRISPR/Cas system. Sigma and UCB’s claims each require a Cas9 protein Sigma’s “guide RNA” ≈ UCB’s “DNA-targeting RNA.” Sigma’s “first region” ≈ UCB’s “targeter-RNA.” Sigma’s “second region” ≈ UCB’s “activator-RNA”</td>
</tr>
<tr>
<td>whereby hybridization of the targeter-RNA to the target sequence is capable of targeting the Cas9 protein to the target DNA molecule.</td>
<td>whereby base pairing of the first region to the target site is capable of targeting the Cas9 protein to the target chromosomal sequence.</td>
<td>Sigma’s “base pairing . . . to the target site” = UCB’s “hybridization . . . to the target sequence”</td>
</tr>
</tbody>
</table>
### APPENDIX IIB - GRAYSCALE

**SIDE-BY-SIDE COMPARISON OF CLAIM 1 OF SIGMA’S U.S. PATENT APPLICATION NO. 16/654,613 WITH CLAIM 156 OF UCB’S U.S. PATENT APPLICATION NO. 15/981,809**

<table>
<thead>
<tr>
<th>UCB’S USSN 15/981,809</th>
<th>SIGMA’S USSN 16/654,613</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>156. A eukaryotic cell comprising a target DNA molecule and an engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising a Cas9 protein or a nucleic acid comprising a nucleotide sequence encoding the Cas9 protein, and a DNA-targeting RNA or one or more nucleic acids comprising one or more nucleotide sequences encoding the DNA-targeting RNA; wherein the DNA-targeting RNA comprises a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule, and an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment.</td>
<td>1. A eukaryotic cell comprising a target chromosomal sequence; and (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas)/CRISPR-Cas type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II protein is codon optimized for expression in the eukaryotic cell, and wherein the CRISPR-Cas type II system protein is a Cas9 protein, and (ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising (1) a first region at the 5' end that is capable of base pairing with a target site in the chromosomal sequence, and (2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and</td>
<td>Sigma and UCB are each claiming a eukaryotic cell Sigma’s “target chromosomal sequence” ≈ UCB’s “target DNA molecule” Sigma and UCB’s claims each require a Type II CRISPR/Cas system. Sigma and UCB’s claims each require a Cas9 protein Sigma’s “guide RNA” ≈ UCB’s “DNA-targeting RNA.” Sigma’s “first region” ≈ UCB’s “targeter-RNA.” Sigma’s “second region” ≈ UCB’s “activator-RNA.”</td>
</tr>
<tr>
<td>whereby hybridization of the targeter-RNA to the target sequence is capable of targeting the Cas9 protein to the target DNA molecule.</td>
<td>whereby base pairing of the first region to the target site is capable of targeting the Cas9 protein to the target chromosomal sequence.</td>
<td>Sigma’s “base pairing . . . to the target site” ≈ UCB’s “hybridization . . . to the target sequence”</td>
</tr>
</tbody>
</table>
APPENDIX III

EXAMPLES OF SUPPORT FOR PENDING CLAIM 1 OF U.S. PATENT APPLICATION NO. 15/188,911
IN U.S. PATENT APPLICATION NO. 15/188,911 FILED JUNE 21, 2016

<table>
<thead>
<tr>
<th>SIGMA’S USSN 15/188,911 (Proposed Claim for Interference)</th>
<th>SIGMA’S USSN 15/188,911 filed June 21, 2016 (hereinafter “Sigma ’911”)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising:</td>
<td>“Another aspect of the present invention encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease as defined herein, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs a RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. In one embodiment, the RNA-guided endonuclease can be derived from a Cas9 protein. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be mRNA in a further embodiment, wherein the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be DNA. In a further embodiment, the DNA encoding the RNA-guided endonuclease can be part of a vector that further comprises a sequence encoding the guide RNA. In certain embodiments, the eukaryotic cell can be a human cell, a non-human mammalian cell, a stem cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism. In certain other embodiments, the embryo is a non-human one cell animal embryo. (Sigma ’911 at [0006]).”</td>
</tr>
</tbody>
</table>

“(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide
RNA, and, optionally, (iii) at least one donor polynucleotid comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '911 at [0064]). See also Sigma '911 at [0065]-[0067].

“(a) RNA-guided endonuclease
The method comprises introducing into a cell or embryo at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal. Such RNA-guided endonucleases and nucleic acids encoding RNA-guided endonucleases are described above in sections (I) and (III), respectively.” (Sigma ‘911 at [0068]). See also Sigma ‘911 at [0069]-[0070].

“(g) Cell and embryo types
A variety of eukaryotic cells and embryos are suitable for use in the method. For example, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism. In general, the embryo is non-human mammalian embryo. In specific embodiments, the embryos can be a one cell nonhuman mammalian embryo. Exemplary mammalian embryos, including one cell embryos, include without limit mouse, rat, hamster, rodent, rabbit, feline, canine, ovine, porcine, bovine, equine, and primate embryos. In still other embodiments, the cell can be a stem cell. Suitable stem cells include without limit embryonic stem cells, ES-like stem cells, fetal stem cells, adult stem cells, pluripotent stem cells, induced pluripotent stem cells, multipotent stem cells, oligopotent stem cells, unipotent stem cells and others. In exemplary embodiments, the cell is a mammalian cell.” (Sigma ‘911 at [0104]). See also Sigma ‘911 at [0105].

“the term “exogenous,” as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location.” (Sigma ‘911 at [0134]).

See also Examples 1-8 (Sigma ‘911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ‘911.

| introducing into the eukaryotic cell (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one | *Another aspect of the present invention encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided |
RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas type II system protein is codon optimized for expression in the eukaryotic cell, and endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease as defined herein, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs a RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. In one embodiment, the RNA-guided endonuclease can be derived from a Cas9 protein. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be mRNA. In a further embodiment, wherein the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be DNA. In a further embodiment, the DNA encoding the RNA-guided endonuclease can be part of a vector that further comprises a sequence encoding the guide RNA. In certain embodiments, the eukaryotic cell can be a human cell, a non-human mammalian cell, a stem cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism. In certain other embodiments, the embryo is a non-human one cell animal embryo. (Sigma ‘911 at [0006]).

“Provided herein are RNA-guided endonucleases, which comprise at least one nuclear localization signal, at least one nuclease domain, and at least one domain that interacts with a guide RNA to target the endonuclease to a specific nucleotide sequence for cleavage. Also provided are nucleic acids encoding the RNA-guided endonucleases, as well as methods of using the RNA-guided endonucleases to modify chromosomal sequences of eukaryotic cells or embryos. The RNA-guided endonuclease interacts with specific guide RNAs, each of which directs the endonuclease to a specific targeted site, at which site the RNA-guided endonuclease introduces a double-stranded break that can be repaired by a DNA repair process such that the chromosomal sequence is modified. Since the specificity is provided by the guide RNA, the RNA-based endonuclease is universal and can be used with different guide RNAs to target different genomic sequences. The methods disclosed herein can be used to target and modify specific chromosomal sequences and/or introduce exogenous sequences at targeted locations in the genome of cells or embryos. Furthermore, the targeting is specific with limited off target effects. (Sigma ‘911 at [0015]).

“(f) RNA-Guided Endonucleases
One aspect of the present disclosure provides RNA-guided endonucleases comprising at least one nuclear localization signal, which permits entry of the
endonuclease into the nuclei of eukaryotic cells and embryos such as, for least one nuclease domain and at least one domain that interacts with a guide RNA. An RNA-guided endonuclease is directed to a specific nucleic acid sequence (or target site) by a guide RNA. The guide RNA interacts with the RNA-guided endonuclease as well as the target site such that, once directed to the target site, the RNA-guided endonuclease is able to introduce a double-stranded break into the target site nucleic acid sequence. Since the guide RNA provides the specificity for the targeted cleavage, the endonuclease of the RNA-guided endonuclease is universal and can be used with different guide RNAs to cleave different target nucleic acid sequences. Provided herein are isolated RNA-guided endonucleases, isolated nucleic acids (i.e., RNA or DNA) encoding the RNA-guided endonucleases, vectors comprising nucleic acids encoding the RNA-guided endonucleases, and protein-RNA complexes comprising the RNA-guided endonuclease plus a guide RNA.” (Sigma ‘911 at [0017]).

“The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. Non-limiting examples of suitable CRISPR/Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas8e, Cas6f, Cas?, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Csa2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr2, Cmr5, Cmr6, Csb1, Csb2, Csb3,Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.” (Sigma ‘911 at [0018]).

“In one embodiment, the RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In specific embodiments, the RNA-guided endonuclease is derived from a Cas9 protein. The Cas9 protein can be from Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Nocardiosis dassonvilliei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Microcilla marina, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas sp., Crocosphaera watsonii, Cyanotheroe sp., Microcystis aeruginosa, Synechococcus sp., Acetohalobium arabaticum, Ammonifex degensii, Caldicelulosiruptor becsonii, Candidatus Desulfurococcus, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaeobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus
caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter sp., Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Arthroseira maxima, Arthroseira platensis, Arthroseira sp., Lyngbya sp., Microcoleus chthonoplastes, Oscillatoria sp., Petrotricha mobilis, Thermosiphon africanus, or Acaryochloris marina.” (Sigma ’911 at [0019]). See also Sigma ’911 at [0020]-[0023].

“The RNA-guided endonuclease disclosed herein comprises at least one nuclear localization signal. In general, an NLS comprises a stretch of basic amino acids. Nuclear localization signals are known in the art (see, e.g., Lange et al., J. Biol. Chem., 2007, 282:5101-5105). For example, in one embodiment, the NLS can be a monopartite sequence, such as PKKKRRK (SEQ ID NO:1) or PKKKRRV (SEQ ID NO:2). In another embodiment, the NLS can be a bipartite sequence. In still another embodiment, the NLS can be KRPAATKAGQAKKKK (SEQ ID NO:3). The NLS can be located at the N-terminus, the C-terminal, or in an internal location of the RNA-guided endonuclease.”  (Sigma ’911 at [0024]).

(III) Nucleic Acids Encoding RNA-Guided Endonucleases or Fusion Proteins
Another aspect of the present disclosure provides nucleic acids encoding any of the RNA-guided endonucleases or fusion proteins described above in sections (I) and (II), respectively. The nucleic acid can be RNA or DNA In one embodiment, the nucleic acid encoding the RNA-guided endonuclease or fusion protein is mRNA. The mRNA can be 5’ capped and/or 3’ polyadenylated. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease or fusion protein is DNA. The DNA can be present in a vector (see below). (Sigma ’911 at [0055]).

“The nucleic acid encoding the RNA-guided endonuclease or fusion protein can be codon optimized for efficient translation into protein in the eukaryotic cell or animal of interest. For example, codons can be optimized for expression in humans, mice, rats, hamsters, cows, pigs, cats, dogs, fish, amphibians, plants, yeast, insects, and so forth. Programs for codon optimization are available as freeware. Commercial codon optimization programs are also available.” (Sigma ’911 at [0057]). See also Sigma ’911 at [0058]-[0063]).

“(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease
Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided
endonuclease comprising at least one nuclear localization signal or nucleic acid
encoding at least one RNA-guided endonuclease comprising at least one nuclear
localization signal, (ii) at least one guide RNA or DNA encoding at least one guide
RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor
sequence. The method further comprises culturing the cell or embryo such that
each guide RNA directs an RNA-guided endonuclease to a targeted site in the
chromosomal sequence where the RNA-guided endonuclease introduces a double-
stranded break in the targeted site, and the double-stranded break is repaired by a
DNA repair process such that the chromosomal sequence is modified. (Sigma ’911
at [0064]). See also Sigma ’911 at [0065]-[0067].

(a) RNA-guided endonuclease
The method comprises introducing into a cell or embryo at least one RNA-guided
endonuclease comprising at least one nuclear localization signal or nucleic acid
encoding at least one RNA-guided endonuclease comprising at least one nuclear
localization signal. Such RNA-guided endonucleases and nucleic acids encoding
RNA-guided endonucleases are described above in sections (I) and (III),
respectively.” (Sigma ’911 at [0068]). See also Sigma ’911 at [0069]-[0070].

(e) Introducing into the cell or embryo
The RNA-targeted endonuclease(s) (or encoding nucleic acid), the guide RNA(s)
(or encoding DNA), and the optional donor polynucleotide(s) can be introduced into
a cell or embryo by a variety of means. In some embodiments, the cell or embryo is
transfected. Suitable transfection methods include calcium phosphate-mediated
transfection, nucleofection (or electroporation), cationic polymer transfection (e.g.,
DEAE-dextran or polyethyleneimine), viral transduction, virosome transfection, virion
transfection, liposome transfection, cationic liposome transfection, immunoliposome
transfection, nonliposomal lipid transfection, dendrimer transfection, heat shock
transfection, magnetofection, lipofection, gene gun delivery, impalefection,
sonoporation, optical transfection, and proprietary agent-enhanced uptake of
nucleic acids. Transfection methods are well known in the art (see, e.g., "Current
or "Molecular Cloning: A Laboratory Manual" Sam brook & Russell, Cold Spring
Harbor Press, Cold Spring Harbor, NY, 3rd edition, 2001). In other embodiments,
the molecules are introduced into the cell or embryo by microinjection. Typically, the
embryo is a fertilized one-cell stage embryo of the species of interest. For example,
the molecules can be injected into the pronuclei of one cell embryos.” (Sigma ’911
at [0094]).
“The RNA-targeted endonuclease(s) (or encoding nucleic acid), the guide RNA(s) (or DNAs encoding the guide RNA), and the optional donor polynucleotide(s) can be introduced into the cell or embryo simultaneously or sequentially. The ratio of the RNA-targeted endonuclease(s) (or encoding nucleic acid) to the guide RNA(s) (or encoding DNA) generally will be about stoichiometric such that they can form an RNA-protein complex. In one embodiment, DNA encoding an RNA-targeted endonuclease and DNA encoding a guide RNA are delivered together within the plasmid vector.” (Sigma ‘911 at [0095]).

“(g) Cell and embryo types
A variety of eukaryotic cells and embryos are suitable for use in the method. For example, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism. In general, the embryo is non-human mammalian embryo. In specific embodiments, the embryos can be a one cell nonhuman mammalian embryo. Exemplary mammalian embryos, including one cell embryos, include without limit mouse, rat, hamster, rodent, rabbit, feline, canine, ovine, porcine, bovine, equine, and primate embryos. In still other embodiments, the cell can be a stem cell. Suitable stem cells include without limit embryonic stem cells, ES-like stem cells, fetal stem cells, adult stem cells, pluripotent stem cells, induced pluripotent stem cells, multipotent stem cells, oligopotent stem cells, unipotent stem cells and others. In exemplary embodiments, the cell is a mammalian cell.” (Sigma ‘911 at [0104]). See also Sigma ‘911 at [0105].

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma ‘911 at [0115]). See also Sigma ‘911 at [0116]-[0130].

“The terms “nucleic acid” and “polynucleotide” refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate
backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Sigma ‘911 at [0137]).

“The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.” (Sigma ‘911 at [0139]).

See also Examples 1-9 (Sigma ‘911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ‘911.

wherein the CRISPR-Cas type II system protein is a Cas9 protein;

“The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. Non-limiting examples of suitable CRISPR/Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas11, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.” (Sigma ‘911 at [0018]).

“In one embodiment, the RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In specific embodiments, the RNA-guided endonuclease is derived from a Cas9 protein. The Cas9 protein can be from *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus sp.*, *Nocardiopsis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *Allicyclacobillus acidocaldarius*, *Bacillus pseudomyxoides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Micrococcus marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas sp.*, *Crocosphaera watsonii*, *Cyanothece sp.*, *Microcystis aeruginosa*, *Synechococcus sp.*, *Acetohalobium abaraticum*, *Ammonifex degensii*, *Caldicellulosiruptor bescii*, *Candidatus Desulfurodus*, *Clostridium botulinum*, *Clostridium difficile*, *Finnegoldia magna*, *Natrananaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter sp.*, *Nitrososoccus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium eustigmatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc sp.*, *Arthropsira maxima*, *Arthropsira platensis*, *Arthropsira sp.*, *Lyngbya sp.*, *Microcoleus chthonoplastes*, *Oscillatoria sp.*, *Petrotoga mobilis*, *Thermosiphon africanus*, or *Acaryochloris marina.” (Sigma ‘911 at [0019]). See also Sigma ‘911 at [0020]-[0023].
| **(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising** |
| **(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease** |
| Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '911 at [0064]). See also Sigma '911 at [0065]-[0067]. |
| **(b) Guide RNA** |
| The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA. A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5' end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.” (Sigma '911 at [0071]). |
| “Each guide RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs.” (Sigma '911 at [0072]). See also Sigma '911 at [0073]-[0077]. |
| “In some embodiments, the guide RNA can be introduced into the cell or embryo as a RNA molecule. The RNA molecule can be transcribed in vitro. Alternatively, the RNA molecule can be chemically synthesized.” (Sigma '911 at [0078]). |
(1) a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and

"(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma ‘911 at [0064]). See also Sigma ‘911 at [0065]-[0067].

"(b) Guide RNA

The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5’ end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.” (Sigma ‘911 at [0071]).

"Each guide RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs." (Sigma ‘911 at [0072]).
“The first region of the guide RNA is complementary to sequence (i.e., protospacer sequence) at the target site in the chromosomal sequence such that the first region of the guide RNA can base pair with the target site. In various embodiments, the first region of the guide RNA can comprise from about 10 nucleotides to more than about 25 nucleotides. For example, the region of base pairing between the first region of the guide RNA and the target site in the chromosomal sequence can be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more than 25 nucleotides in length. In an exemplary embodiment, the first region of the guide RNA is about 19, 20, or 21 nucleotides in length.” (Sigma ‘911 at [0073]).

(c) Target site

“An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGNG, and NNAGA WW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (IV)(b), the first region (at the 5’ end) of the guide RNA is complementary to the protospacer of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5’-N19-21-NGG-3’. The PAM is in italics.” (Sigma ‘911 at [0082]).

“The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest.” (Sigma ‘911 at [0083]).

See also Examples 1-9 (Sigma ‘911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ‘911.

(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and

(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear
localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '911 at [0064]). See also Sigma '911 at [0065]-[0067].

"(b) Guide RNA"
The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA. A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which the 5' end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence." (Sigma '911 at [0071]).

"Each guide RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs." (Sigma '911 at [0072]).

"The guide RNA also comprises a second region that forms a secondary structure. In some embodiments, the secondary structure comprises a stem (or hairpin) and a loop. The length of the loop and the stem can vary. For example, the loop can range from about 3 to about 10 nucleotides in length, and the stem can range from about 6 to about 20 base pairs in length. The stem can comprise one or more bulges of 1 to about 10 nucleotides. Thus, the overall length of the second region can range from about 16 to about 60 nucleotides in length. In an exemplary embodiment, the loop is about 4 nucleotides in length and the stem comprises about 12 base pairs." (Sigma '911 at [0074]).

See also Examples 1-9 (Sigma '911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '911.

(iii) at least one donor polynucleotide comprising the exogenous sequence; and

"(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease"

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises
introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '911 at [0084]). See also Sigma '911 at [0065]-[0067].

“(d) Optional donor polynucleotide
In some embodiments, the method further comprises introducing at least one donor polynucleotide into the embryo. A donor polynucleotide comprises at least one donor sequence. In some aspects, a donor sequence of the donor polynucleotide corresponds to an endogenous or native chromosomal sequence. For example, the donor sequence can be essentially identical to a portion of the chromosomal sequence at or near the targeted site, but which comprises at least one nucleotide change. Thus, the donor sequence can comprise a modified version of the wild type sequence at the targeted site such that, upon integration or exchange with the native sequence, the sequence at the targeted chromosomal location comprises at least one nucleotide change. For example, the change can be an insertion of one or more nucleotides, a deletion of one or more nucleotides, a substitution of one or more nucleotides, or combinations thereof. As a consequence of the integration of the modified sequence, the cell or embryo/animal can produce a modified gene product from the targeted chromosomal sequence.” (Sigma ‘911 at [0084]).

“In other aspects, the donor sequence of the donor polynucleotide corresponds to an exogenous sequence. As used herein, an “exogenous” sequence refers to a sequence that is not native to the cell or embryo, or a sequence whose native location in the genome of the cell or embryo is in a different location. For example, the exogenous sequence can comprise protein coding sequence, which can be operably linked to an exogenous promoter control sequence such that, upon integration into the genome, the cell or embryo/animal is able to express the protein coded by the integrated sequence. Alternatively, the exogenous sequence can be integrated into the chromosomal sequence such that its expression is regulated by an endogenous promoter control sequence. In other iterations, the exogenous sequence can be a transcriptional control sequence, another expression control sequence, an RNA coding sequence, and so forth. Integration of an exogenous
sequence into a chromosomal sequence is termed a "knock in." (Sigma '911 at [0085]).

"As can be appreciated by those skilled in the art, the length of the donor sequence can and will vary. For example, the donor sequence can vary in length from several nucleotides to hundreds of nucleotides to hundreds of thousands of nucleotides." (Sigma '911 at [0086]). See also Sigma '911 at [0087]-[0093].

"The term "exogenous," as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location." (Sigma '911 at [0134]).

"The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein." (Sigma '911 at [0115]). See also Sigma '911 at [0116]-[0130].

"The terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Sigma '911 at [0137]).

See also Examples 1-9 (Sigma '911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '911.

| whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process | ‘(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease' Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide |
leads to integration of the exogenous sequence into the chromosomal sequence.

RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma ‘911 at [0064]). See also Sigma ‘911 at [0065]-[0067].

"(c) Target site

"An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGG, and NNAGAAW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (IV)(b), the first region (at the 5’ end) of the guide RNA is complementary to the protospacer of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5’-N<sub>19-21</sub>-NGG-3’. The PAM is in italics.” (Sigma ‘911 at [0082]).

"The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest.” (Sigma ‘911 at [0083]).

"(d) Optional donor polynucleotide

In some embodiments, the method further comprises introducing at least one donor polynucleotide into the embryo. A donor polynucleotide comprises at least one donor sequence. In some aspects, a donor sequence of the donor polynucleotide corresponds to an endogenous or native chromosomal sequence. For example, the donor sequence can be essentially identical to a portion of the chromosomal sequence at or near the targeted site, but which comprises at least one nucleotide change. Thus, the donor sequence can comprise a modified version of the wild type sequence at the targeted site such that, upon integration or exchange with the native sequence, the sequence at the targeted chromosomal location comprises at least one nucleotide change. For example, the change can be an insertion of one or more nucleotides, a deletion of one or more nucleotides, a substitution of one or
more nucleotides, or combinations thereof. As a consequence of the integration of
the modified sequence, the cell or embryo/animal can produce a modified gene
product from the targeted chromosomal sequence.” (Sigma ‘911 at [0084]).

“In other aspects, the donor sequence of the donor polynucleotide corresponds to
an exogenous sequence. As used herein, an “exogenous” sequence refers to a
sequence that is not native to the cell or embryo, or a sequence whose native
location in the genome of the cell or embryo is in a different location. For example,
the exogenous sequence can comprise protein coding sequence, which can be
operably linked to an exogenous promoter control sequence such that, upon
integration into the genome, the cell or embryo/animal is able to express the protein
coded by the integrated sequence. Alternatively, the exogenous sequence can be
integrated into the chromosomal sequence such that its expression is regulated by
an endogenous promoter control sequence. In other iterations, the exogenous
sequence can be a transcriptional control sequence, another expression control
sequence, an RNA coding sequence, and so forth. Integration of an exogenous
sequence into a chromosomal sequence is termed a “knock in.”” (Sigma ‘911 at
[0065]).

“As can be appreciated by those skilled in the art, the length of the donor sequence
can and will vary. For example, the donor sequence can vary in length from several
nucleotides to hundreds of nucleotides to hundreds of thousands of nucleotides.”
(Sigma ‘911 at [0066]). See also Sigma ‘911 at [0087]-[0093].

“(f) Culturing the cell or embryo
The method further comprises maintaining the cell or embryo under appropriate
conditions such that the guide RNA(s) directs the RNA-guided endonuclease(s) to
the targeted site(s) in the chromosomal sequence, and the RNA-guided
endonuclease(s) introduce at least one double-stranded break in the chromosomal
sequence. A double-stranded break can be repaired by a DNA repair process such
that the chromosomal sequence is modified by a deletion of at least one nucleotide,
an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a
combination thereof.” (Sigma ‘911 at [0096]).

“In embodiments in which no donor polynucleotide is introduced into the cell or
embryo, the double-stranded break can be repaired via a non-homologous end-
joining (NHEJ) repair process. Because NHEJ is error-prone, deletions of at least
one nucleotide, insertions of at least one nucleotide, substitutions of at least one
nucleotide, or combinations thereof can occur during the repair of the break.
Accordingly, the sequence at the chromosomal sequence can be modified such that
the reading frame of a coding region can be shifted and that the chromosomal sequence is inactivated or "knocked out." An inactivated protein-coding chromosomal sequence does not give rise to the protein coded by the wild type chromosomal sequence. (Sigma ’911 at [0097]).

"In embodiments in which a donor polynucleotide comprising upstream and downstream sequences is introduced into the cell or embryo, the double-stranded break can be repaired by a homology-directed repair (HDR) process such that the donor sequence is integrated into the chromosomal sequence. Accordingly, an exogenous sequence can be integrated into the genome of the cell or embryo, or the targeted chromosomal sequence can be modified by exchange of a modified sequence for the wild type chromosomal sequence." (Sigma ’911 at [0098]). See also Sigma ’911 at [0099]-[0103].

"The term "exogenous," as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location." (Sigma ’911 at [0134]).

See also Examples 1-9 (Sigma ’911 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ’911.
## APPENDIX IV

### EXAMPLES OF SUPPORT FOR PENDING CLAIM 1 OF U.S. PATENT APPLICATION NO. 15/188,911

**IN U.S. PROVISIONAL APPLICATION NO. 61/734,256 FILED DECEMBER 6, 2012**

<table>
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<tr>
<th>SIGMA’S USSN 15/188,911 (Proposed Claim for Interference)</th>
<th>SIGMA’S USSN 61/734,256 filed December 6, 2012 (hereinafter “Chen P1”)</th>
</tr>
</thead>
</table>
| 1. A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising: | “(II) Method for Modifying a Chromosomal Sequence

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell. The method comprises introducing into the eukaryotic cell (i) an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease, wherein the RNA-guided endonuclease comprises a nuclear localization signal, (ii) at least one guiding RNA or at least one DNA molecule encoding a guiding RNA, wherein each guiding RNA guides the RNA-guided endonuclease to a targeted site in the chromosomal sequence, and optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell such that the RNA-guided endonuclease introduces a double-stranded break at the targeted site in the chromosomal sequence and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.” (Chen P1 at [0016]).

“A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism.” (Chen P1 at [0045]).

“The term "exogenous," as used here, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location.” (Chen P1 at [0050]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1. |

introducing into the eukaryotic cell (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal |

“One aspect of the present disclosure provides RNA-guided endonucleases that comprise at least one nuclear localization signal, which permits entry of the endonuclease into the nuclei of eukaryotic cells.” (Chen P1 at [0004]).
localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas type II system protein is codon optimized for expression in the eukaryotic cell, and

“The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. In some embodiments, the RNA-guided endonuclease can be derived from a type II CRISPR/Cas system.” (Chen P1 at [0004]).

“In some embodiments, the RNA-guided endonuclease can be derived from a wild type Cas9 protein(s) or fragment(s) thereof. In other embodiments, the RNA-guided endonuclease can be derived from modified Cas9 protein(s). For example, the amino acid sequence of the Cas9 protein can be modified such that one or more properties (e.g., nuclease activity, affinity, stability, etc.) of the protein is improved. Alternatively, domains of the Cas9 protein not involved in RNA-guided cleavage can be eliminated from the protein such that the modified Cas9 protein is smaller than the wild type Cas9 protein. In still other embodiments, the RNA-guided endonuclease can be a fusion protein comprising domains of wild type Cas9 proteins, modified Cas9 proteins, and/or other proteins. For example the RNA-guided endonuclease could comprise a marker, such as GFP or another fluorescent protein.” (Chen P1 at [0006]).

“The Cas9-derived endonucleases disclosed herein comprise at least one nuclear localization signal (NLS) for transport into the nuclei of eukaryotic cells. In general, an NLS comprise a stretch of basic amino acids. Nuclear localization signals are known in the art (see, e.g., Lange et al., J. Biol. Chem., 2007, 282:5101-5105). For example, examples of classical NLSs included monopartite sequences such as PKKKRKV (SEQ ID NO:1) or PPKKR (SEQ ID NO:13), as well as bipartite sequences. The NLS can be located at the N-terminus, the C-terminal, or in an internal location of the endonuclease. In an exemplary embodiment, the NLS is located at the C-terminus of the endonuclease.” (Chen P1 at [0006]).

“The disclosure also provides nucleic acids encoding the RNA-guided endonuclease. The nucleic acid encoding the RNA-guided endonuclease can be RNA or DNA. The DNA encoding the RNA-guided endonuclease can be present in a vector (see below).” (Chen P1 at [0011]). See also Chen P1 at [0012]-[0015].

“The method comprises introducing into the cell an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease.” (Chen P1 at [0018]).

“(d) Introducing into the cell
The RNA-targeted endonuclease (or its encoding nucleic acid), the guiding RNA(s) (or DNAs encoding the guiding RNA), and the optional donor polynucleotide(s) can
be introduced into the cell by a variety of means. In some embodiments, the cell is transfected. Suitable transfection methods include calcium phosphate-mediated transfection, nucleofection (or electroporation), cationic polymer transfection (e.g., DEAE-dextran or polyethylenimine), viral transduction, virosome transfection, virion transfection, liposome transfection, cationic liposome transfection, immunoliposome transfection, nonliposomal lipid transfection, dendrimer transfection, heat shock transfection, magnetofection, lipofection, gene gun delivery, impalefection, sonoporation, optical transfection, and proprietary agent-enhanced uptake of nucleic acids. Transfection methods are well known in the art (see, e.g., "Current Protocols in Molecular Biology" Ausubel et al., John Wiley & Sons, New York, 2003 or "Molecular Cloning: A Laboratory Manual" Sambrook & Russell, Cold Spring Harbor Press, Cold Spring Harbor, NY, 3rd edition, 2001). In other embodiments, the molecules are introduced into the cell by microinjection. For example, the molecules can be injected into the pronuclei of one cell embryos." (Chen P1 at [0037]). See also Chen P1 at [0038].

“A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism.” (Chen P1 at [0045]).

“The terms "nucleic acid" and "polynucleotide" refer to a deoxyribo nucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Chen P1 at [0053]).

“The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.” (Chen P1 at [0055]).

See also Examples 1-5 (Chen P1 at [0058]-0068) and accompanying Figures; Claims 12-21 of Chen P1.

wherein the CRISPR-Cas type II system protein is a Cas9 protein;

"In exemplary embodiments, the endonuclease can be derived from a Cas9 protein of a type II system.” (Chen P1 at [0004]).

“The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.” (Chen P1 at [0055]).
See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising

“The present disclosure provides RNA-guided endonucleases that are engineered for use in eukaryotic cells. The RNA-guided endonuclease can be used to modify the genome of eukaryotic cells. For this, the endonuclease is guided to a specific chromosomal sequence by a specific guiding RNA.” (Chen P1 at [0033]).

“The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell.” (Chen P1 at [0019]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

(1) a first region at the 5' end that base pairs with a target site in the chromosomal sequence, and

“(a) Target site

The RNA-guided endonuclease in conjunction with the guiding RNA is directed to a target site in the chromosomal sequence and cleaves the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG and NGGNG (wherein N is defined as any nucleotide). The target site can be in the coding region of a gene, in an intron of a gene, in a control region between genes, etc. The gene can be a protein coding gene or a RNA coding gene.” (Chen P1 at [0017]).

“The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell. Each guiding RNA interacts with the RNA-guided endonuclease to guide the endonuclease to a specific target site, wherein the endonuclease cleaves the target site. Each guiding RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guiding RNA is different such that each guiding RNA guides the endonuclease to a specific target site. The second and third regions of each guiding RNA can be the same in all guiding RNAs.” (Chen P1 at [0019]).

“The first region of the guiding RNA is complementary to the target site in the chromosomal sequence such that the first region of the guiding RNA can base pair with the target site. In various embodiments, the first region of the guiding RNA can comprise from about 10 nucleotides to more than about 25 nucleotides.” (Chen P1 at [0020]).
| (2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and | "The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell. Each guiding RNA interacts with the RNA-guided endonuclease to guide the endonuclease to a specific target site, wherein the endonuclease cleaves the target site. Each guiding RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guiding RNA is different such that each guiding RNA guides the endonuclease to a specific target site. The second and third regions of each guiding RNA can be the same in all guiding RNAs." (Chen P1 at [0019]).

"The guiding RNA also comprises a second region that forms a secondary structure. In some embodiments, the secondary structure comprises a stem (or hairpin) and a loop. The length of the loop and the stem can vary. For example, the loop can range from about 3 to about 10 nucleotides in length, and the stem can range from about 6 to about 20 base pairs in length. The stem can comprise one or more bulges of 1 to about 10 nucleotides. Thus, the overall length of the second region can range from about 16 to about 80 nucleotides in length. In an exemplary embodiment, the loop is about 4 nucleotides in length and the stem comprises about 12 base pairs." (Chen P1 at [0021]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

| (iii) at least one donor polynucleotide comprising the exogenous sequence; and | "(c) Optional donor polynucleotide
The method optionally also comprises introducing at least one donor polynucleotide comprising a donor sequence into the cell. As detailed below, the donor polynucleotide can comprise additional sequence elements." (Chen P1 at [0026]).

See also Chen P1 at [0027]-[0036]).

"The term "exogenous," as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location." (Chen P1 at [0050]).

"The terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms
can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Chen P1 at [0053]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process leads to integration of the exogenous sequence into the chromosomal sequence.

"[T]he endonuclease is guided to a specific chromosomal sequence by a specific guiding RNA." (Chen P1 at [0003]).

"The double-stranded break can be repaired by a cellular DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof." (Chen P1 at [0003]).

"(a) Target site
The RNA-guided endonuclease in conjunction with the guiding RNA is directed to a target site in the chromosomal sequence and cleaves the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG and NGNG (wherein N is defined as any nucleotide). The target site can be in the coding region of a gene, in an intron of a gene, in a control region between genes, etc. The gene can be a protein coding gene or an RNA coding gene." (Chen P1 at [0017]).

"(e) Culturing the cell
The method further comprises maintaining the cell under appropriate conditions such that the guiding RNA guides the endonuclease to the targeted site in the chromosomal sequence, and the endonuclease introduces a double-stranded break in the targeted site in the chromosomal sequence. The double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof." (Chen P1 at [0039]).

"A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism." (Chen P1 at [0045]).
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>“The term &quot;exogenous,&quot; as used herein, refers to a sequence that is not native to the cell, or a chromosomal sequence whose native location in the genome of the cell is in a different chromosomal location.” (Chen P1 at [0050]).</td>
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<tr>
<td>See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.</td>
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APPENDIX V

EXAMPLES OF SUPPORT FOR PENDING CLAIM 1 OF U.S. PATENT APPLICATION NO. 16/654,613
IN U.S. PATENT APPLICATION NO. 16/654,613 FILED OCTOBER 16, 2019

<table>
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<th>SIGMA’S USSN 16/654,613 (Proposed Claim for Interference)</th>
<th>SIGMA’S USSN 16/654,613 filed October 16, 2019 (hereinafter “Sigma ’613”)</th>
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<tr>
<td>1. A eukaryotic cell comprising</td>
<td>*(g) Cell and embryo types</td>
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<td>A variety of eukaryotic cells and embryos are suitable for use in the method. For example, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism. In general, the embryo is non-human mammalian embryo. In specific embodiments, the embryos can be a one cell nonhuman mammalian embryo. Exemplary mammalian embryos, including one cell embryos, include without limit mouse, rat, hamster, rodent, rabbit, feline, canine, ovine, porcine, bovine, equine, and primate embryos. In still other embodiments, the cell can be a stem cell. Suitable stem cells include without limit embryonic stem cells, ES-like stem cells, fetal stem cells, adult stem cells, pluripotent stem cells, induced pluripotent stem cells, multipotent stem cells, oligopotent stem cells, unipotent stem cells and others. In exemplary embodiments, the cell is a mammalian cell.” *(Sigma ’613 at [0104]). See also Sigma ’613 at [0105].</td>
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</table>

*(VI) Genetically Modified Cells and Animals
The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” *(Sigma ’613 at [0114]).

*The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an
altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein." (Sigma '613 at [0115]). See also Sigma '613 at [0116]-[0130].

See also Examples 1-9 (Sigma '613 at [0142]-[0160]) and accompanying Figures; Claims 1-27 of Sigma '613.

| a target chromosomal sequence; and |
|                                   |

**(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease**

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '613 at [0064]). See also Sigma '613 at [0065]-[0067].

**(b) Guide RNA**

The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5' end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence." (Sigma '613 at [0071]).

"Each guide RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs." (Sigma '613 at [0072]).

"The first region of the guide RNA is complementary to sequence (i.e., protospacer sequence) at the target site in the chromosomal sequence such that the first region of the guide RNA can base pair with the target site. In various embodiments, the first region of the guide RNA can comprise from about 10 nucleotides to more than about 25 nucleotides. For
example, the region of base pairing between the first region of the guide RNA and the target site in the chromosomal sequence can be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more than 25 nucleotides in length. In an exemplary embodiment, the first region of the guide RNA is about 19, 20, or 21 nucleotides in length.” (Sigma ‘613 at [0073]).

“(c) Target site

“An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGNG, and NNAGAAW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (IV) (b), the first region (at the 5’ end) of the guide RNA is complementary to the protospacer of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5’-N_{19-21}-NGG-3’. The PAM is in italics.” (Sigma ‘613 at [0082]).

“The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest.” (Sigma ‘613 at [0083]).

“(VI) Genetically Modified Cells and Animals

The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ‘613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an
altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma '613 at [0115]). See also Sigma '613 at [0116]-[0130].

See also Examples 1-9 (Sigma '613 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '613.

(i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II protein is codon optimized for expression in the eukaryotic cell, and

“Another aspect of the present invention encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease as defined herein, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs a RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. In one embodiment, the RNA-guided endonuclease can be derived from a Cas9 protein. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be mRNA in a further embodiment, wherein the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be DNA. In a further embodiment, the DNA encoding the RNA-guided endonuclease can be part of a vector that further comprises a sequence encoding the guide RNA. In certain embodiments, the eukaryotic cell can be a human cell, a non-human mammalian cell, a stem cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism. In certain other embodiments, the embryo is a non-human one cell animal embryo. (Sigma '613 at [0006]).

*Provided herein are RNA-guided endonucleases, which comprise at least one nuclear localization signal, at least one nuclease domain, and at least one domain that interacts with a guide RNA to target the endonuclease to a specific nucleotide sequence for cleavage. Also provided are nucleic acids encoding the RNA-guided endonucleases, as well as methods of using the RNA-guided endonucleases to modify chromosomal sequences of eukaryotic cells or embryos. The RNA-guided endonuclease interacts with specific guide RNAs, each of which directs the endonuclease to a specific targeted site, at which site the RNA-guided endonuclease introduces a double-stranded break that can be repaired by a DNA repair process such that the chromosomal sequence is modified. Since the specificity is provided by the guide RNA, the RNA-based endonuclease is universal and can be used with different guide RNAs to target different genomic sequences. The methods disclosed herein can be used to target and modify specific chromosomal sequences and/or
introduce exogenous sequences at targeted locations in the genome of cells or embryos. Furthermore, the targeting is specific with limited off target effects. (Sigma '613 at [0015]).

**“(I) RNA-Guided Endonucleases**

One aspect of the present disclosure provides RNA-guided endonucleases comprising at least one nuclear localization signal, which permits entry of the endonuclease into the nuclei of eukaryotic cells and embryos such as, for least one nuclease domain and at least one domain that interacts with a guide RNA. An RNA-guided endonuclease is directed to a specific nucleic acid sequence (or target site) by a guide RNA. The guide RNA interacts with the RNA-guided endonuclease as well as the target site such that, once directed to the target site, the RNA-guided endonuclease is able to introduce a double-stranded break into the target site nucleic acid sequence. Since the guide RNA provides the specificity for the targeted cleavage, the endonuclease of the RNA-guided endonuclease is universal and can be used with different guide RNAs to cleave different target nucleic acid sequences. Provided herein are isolated RNA-guided endonucleases, isolated nucleic acids (i.e., RNA or DNA) encoding the RNA-guided endonucleases, vectors comprising nucleic acids encoding the RNA-guided endonucleases, and protein-RNA complexes comprising the RNA-guided endonuclease plus a guide RNA.” (Sigma ‘613 at [0017]).

“The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. Non-limiting examples of suitable CRISPR/Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, CasF, CasG, CasH, CasI, CasJ, CasK, CasL, CasM, CasN, CasO, CasP, CasQ, CasR, CasS, CasT, CasU, CasV, CasW, CasX, CasY, CasZ, and Cu1966.” (Sigma ‘613 at [0018]).

“In one embodiment, the RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In specific embodiments, the RNA-guided endonuclease is derived from a Cas9 protein. The Cas9 protein can be from *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus sp.*, *Nocardiosis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *Alicyclobacillus acidocaldarius*, *Bacillus pseudomycoide*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas napththalenivorans*, *Polaromonas sp.*, *Crocosphaera watsonii*, *Cyanothece sp.*, *Microcystis aeruginosa*, *Synechococcus sp.*, *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicellulosiruptor bescii*, *Candidatus Desulforudis*, *Clostridium botulinum*, *Candidatus Desulforudis*, *Clostridium botulinum*,
**Clostridium difficile**, *Finegoldia magna*, *Natraneraebiis thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter sp.*, *Nitrosococcus halophilus*, *Nitrosococcus watsonii*, *Pseudoalteromonas haloplanktis*, *Kledonobacter racemifer*, *Methanoallobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc sp.*, *Arthrospira maxima*, *Arthrospira platensis*, *Arthrospira sp.*, *Lyngbya sp.*, *Microcoleus chthonoplastes*, *Oscillatoria sp.*, *Petrotoya mobiliis*, *Thermosiphon africanus*, or *Acaryochloris marina.* (Sigma '613 at [0019]). See also Sigma '613 at [0020]-[0023].

“The RNA-guided endonuclease disclosed herein comprises at least one nuclear localization signal. In general, an NLS comprises a stretch of basic amino acids. Nuclear localization signals are known in the art (see, e.g., Lange et al., J. Biol. Chem., 2007, 282:5101-51 05). For example, in one embodiment, the NLS can be a monopartite sequence, such as PKKKRKV (SEQ ID NO:1) or PKKRRRV (SEQ ID NO:2). In another embodiment, the NLS can be a bipartite sequence. In still another embodiment, the NLS can be KRPAATKKAGQAKKKK (SEQ ID NO:3). The NLS can be located at the N-terminus, the C-terminal, or in an internal location of the RNA-guided endonuclease.” (Sigma '613 at [0024]).

***Nucleic Acids Encoding RNA-Guided Endonucleases or Fusion Proteins***

Another aspect of the present disclosure provides nucleic acids encoding any of the RNA-guided endonucleases or fusion proteins described above in sections (I) and (II), respectively. The nucleic acid can be RNA or DNA in one embodiment, the nucleic acid encoding the RNA-guided endonuclease or fusion protein is mRNA. The mRNA can be 5' capped and/or 3' polyadenylated. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease or fusion protein is DNA. The DNA can be present in a vector (see below). (Sigma '613 at [0056]).

“The nucleic acid encoding the RNA-guided endonuclease or fusion protein can be codon optimized for efficient translation into protein in the eukaryotic cell or animal of interest. For example, codons can be optimized for expression in humans, mice, rats, hamsters, cows, pigs, cats, dogs, fish, amphibians, plants, yeast, insects, and so forth. Programs for codon optimization are available as freeware. Commercial codon optimization programs are also available.” (Sigma '613 at [0057]). See also Sigma '613 at [0058]-[0063].

***Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease***

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at
least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '613 at [0064]). See also Sigma '613 at [0065]-[0067].

**(a) RNA-guided endonuclease**
The method comprises introducing into a cell or embryo at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal. Such RNA-guided endonucleases and nucleic acids encoding RNA-guided endonucleases are described above in sections (i) and (iii), respectively.” (Sigma ‘613 at [0068]). See also Sigma ‘613 at [0069]-[0070].

**(b) Introducing into the cell or embryo**
The RNA-targeted endonuclease(s) (or encoding nucleic acid), the guide RNA(s) (or encoding DNA), and the optional donor polynucleotide(s) can be introduced into a cell or embryo by a variety of means. In some embodiments, the cell or embryo is transfected. Suitable transfection methods include calcium phosphate-mediated transfection, nucleofection (or electroporation), cationic polymer transfection (e.g., DEAE-dextran or polyethyleneimine), viral transduction, virosome transfection, virion transfection, liposome transfection, cationic liposome transfection, immunoliposome transfection, nonliposomal lipid transfection, dendrimer transfection, heat shock transfection, magnetofection, lipofection, gene gun delivery, impalefection, sonoporation, optical transfection, and proprietary agent-enhanced uptake of nucleic acids. Transfection methods are well known in the art (see, e.g., “Current Protocols in Molecular Biology” Ausubel et al., John Wiley & Sons, New York, 2003 or “Molecular Cloning: A Laboratory Manual” Sam brook & Russell, Cold Spring Harbor Press, Cold Spring Harbor, NY, 3rd edition, 2001). In other embodiments, the molecules are introduced into the cell or embryo by microinjection. Typically, the embryo is a fertilized one-cell stage embryo of the species of interest. For example, the molecules can be injected into the pronuclei of one cell embryos.” (Sigma ‘613 at [0084]).

*The RNA-targeted endonuclease(s) (or encoding nucleic acid), the guide RNA(s) (or DNAs encoding the guide RNA), and the optional donor polynucleotide(s) can be introduced into the cell or embryo simultaneously or sequentially. The ratio of the RNA-targeted
endonuclease(s) (or encoding nucleic acid) to the guide RNA(s) (or encoding DNA) generally will be about stoichiometric such that they can form an RNA-protein complex. In one embodiment, DNA encoding an RNA-targeted endonuclease and DNA encoding a guide RNA are delivered together within the plasmid vector.” (Sigma ‘613 at [0095]).

“(g) Cell and embryo types
A variety of eukaryotic cells and embryos are suitable for use in the method. For example, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism. In general, the embryo is non-human mammalian embryo. In specific embodiments, the embryos can be a one cell nonhuman mammalian embryo. Exemplary mammalian embryos, including one cell embryos, include without limit mouse, rat, hamster, rodent, rabbit, canine, ovine, porcine, bovine, equine, and primate embryos. In still other embodiments, the cell can be a stem cell. Suitable stem cells include without limit embryonic stem cells, ES-like stem cells, fetal stem cells, adult stem cells, pluripotent stem cells, induced pluripotent stem cells, multipotent stem cells, oligopotent stem cells, unipotent stem cells and others. In exemplary embodiments, the cell is a mammalian cell.” (Sigma ‘613 at [0104]). See also Sigma ‘613 at [0105].

“(VI) Genetically Modified Cells and Animals
The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ‘613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma ‘613 at [0115]). See also Sigma ‘613 at [0116]-[0130].
The terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Sigma '613 at [0137]).

The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.* (Sigma '613 at [0139]).

See also Examples 1-9 (Sigma '613 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '613.

wherein the CRISPR-Cas type II system protein is a Cas9 protein, and

The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. Non-limiting examples of suitable CRISPR/Cas proteins include Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, CasF, CasG, CasH, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), Cse4 (or CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966.” (Sigma '613 at [0018]).

In one embodiment, the RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In specific embodiments, the RNA-guided endonuclease is derived from a Cas9 protein. The Cas9 protein can be from Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Nocardiosis dassonvillei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Microscilla marina, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas sp., Crocosphaera watsonii, Cyanobacteria sp., Microcystis aeruginosa, Synechococcus sp., Acetohalobium arabaticum, ammonifex degensii, Caldocellulosiruptor beccii, Candidatus Desulforudis, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus caldus, Acidithiobacillus ferroxidans, Allochrofulum vinosum, Marinobacter sp., Nitrosococcus halophilus, Nitrosococcus watsonii, Pseudomonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Anthospira maxima, Anthospira
(VI) Genetically Modified Cells and Animals

The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma '613 at [0114]).

*(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a
targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '613 at [0064]). See also Sigma '613 at [0065]-[0067].

“(b) Guide RNA
The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA. A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5′ end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.” (Sigma '613 at [0071]).

*Each guide RNA comprises three regions: a first region at the 5′ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3′ region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs.” (Sigma '613 at [0072]). See also Sigma '613 at [0073]-[0077].

“In some embodiments, the guide RNA can be introduced into the cell or embryo as a RNA molecule. The RNA molecule can be transcribed in vitro. Alternatively, the RNA molecule can be chemically synthesized.” (Sigma '613 at [0078]).

“In other embodiments, the guide RNA can be introduced into the cell or embryo as a DNA molecule. In such cases, the DNA encoding the guide RNA can be operably linked to promoter control sequence for expression of the guide RNA in the cell or embryo of interest. For example, the RNA coding sequence can be operably linked to a promoter sequence that is recognized by RNA polymerase III (Pol III).” (Sigma '613 at [0079]). See also Sigma '613 at [0080]-[0081].

“(VI) Genetically Modified Cells and Animals
The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an
RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma '613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma '613 at [0115]). See also Sigma '613 at [0116]-[0130]. See also Examples 1-9 (Sigma '613 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma '613.

| (1) a first region at the 5’ end that is capable of base pairing with a target site in the chromosomal sequence, and |
| (IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease |
| Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '613 at [0064]). See also Sigma '613 at [0065]-[0067]. |
| (b) Guide RNA |
| The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA and RNA enzyme with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5’ end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.” (Sigma '613 at [0071]). |
| “Each guide RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides |
a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs.” (Sigma ’613 at [0072]).

“The first region of the guide RNA is complementary to sequence (i.e., protospacer sequence) at the target site in the chromosomal sequence such that the first region of the guide RNA can base pair with the target site. In various embodiments, the first region of the guide RNA can comprise from about 10 nucleotides to more than about 25 nucleotides. For example, the region of base pairing between the first region of the guide RNA and the target site in the chromosomal sequence can be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more than 25 nucleotides in length. In an exemplary embodiment, the first region of the guide RNA is about 19, 20, or 21 nucleotides in length.” (Sigma ’613 at [0073]).

“(c) Target site
*An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGNG, and NNAGAAW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (IV)(b), the first region (at the 5’ end) of the guide RNA is complementary to the protospacer of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5’-N_{19-21}-NGG-3’. The PAM is in italics.” (Sigma ’613 at [0082]).

“The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest.” (Sigma ’613 at [0083]).

“(VI) Genetically Modified Cells and Animals
The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an
RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ‘613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma ‘613 at [0115]). See also Sigma ‘613 at [0116]-[0130].

See also Examples 1-9 (Sigma ‘613 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ‘613.

(2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and

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<th>(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease</th>
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<td>Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma ‘613 at [0064]). See also Sigma ‘613 at [0065]-[0067].</td>
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<td>The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5’ end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.” (Sigma ‘613 at [0071]).</td>
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| “Each guide RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3’ region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides |
a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs.” (Sigma ‘613 at [0072]).

“The guide RNA also comprises a second region that forms a secondary structure. In some embodiments, the secondary structure comprises a stem (or hairpin) and a loop. The length of the loop and the stem can vary. For example, the loop can range from about 3 to about 10 nucleotides in length, and the stem can range from about 6 to about 20 base pairs in length. The stem can comprise one or more bulges of 1 to about 10 nucleotides. Thus, the overall length of the second region can range from about 16 to about 60 nucleotides in length. In an exemplary embodiment, the loop is about 4 nucleotides in length and the stem comprises about 12 base pairs.” (Sigma ‘613 at [0074]).

*(IV) Genetically Modified Cells and Animals*

The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ‘613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma ‘613 at [0115]). See also Sigma ‘613 at [0116]-[0130].

See also Examples 1-9 (Sigma ‘613 at [0142]-[0160]) and accompanying Figures; Claims 1-16 of Sigma ‘613.

whereby base pairing of the first region to the target site is capable of targeting the Cas9 protein to the target chromosomal sequence. *(IV) Method for Modifying a Chromosomal Sequence Using an RNA-Guided Endonuclease*

Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at
least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. (Sigma '613 at [0064]). See also Sigma '613 at [0065]-[0067].

"(c) Target site

"An RNA-guided endonuclease in conjunction with a guide RNA is directed to a target site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG, NGGNG, and NNAGAAW (wherein N is defined as any nucleotide and W is defined as either A or T). As detailed above in section (IV)(b), the first region (at the 5’ end) of the guide RNA is complementary to the protospacer of the target sequence. Typically, the first region of the guide RNA is about 19 to 21 nucleotides in length. Thus, in certain aspects, the sequence of the target site in the chromosomal sequence is 5’-N<sub>19-21</sub>-NGG-3’. The PAM is in italics." (Sigma ‘613 at [0082]).

"The target site can be in the coding region of a gene, in an intron of a gene, in a control region of a gene, in a non-coding region between genes, etc. The gene can be a protein coding gene or an RNA coding gene. The gene can be any gene of interest." (Sigma ‘613 at [0083]).

"(d) Optional donor polynucleotide

In some embodiments, the method further comprises introducing at least one donor polynucleotide into the embryo. A donor polynucleotide comprises at least one donor sequence. In some aspects, a donor sequence of the donor polynucleotide corresponds to an endogenous or native chromosomal sequence. For example, the donor sequence can be essentially identical to a portion of the chromosomal sequence at or near the targeted site, but which comprises at least one nucleotide change. Thus, the donor sequence can comprise a modified version of the wild type sequence at the targeted site such that, upon integration or exchange with the native sequence, the sequence at the targeted chromosomal location comprises at least one nucleotide change. For example, the change can be an insertion of one or more nucleotides, a deletion of one or more nucleotides, a substitution of one or more nucleotides, or combinations thereof. As a consequence of the
integration of the modified sequence, the cell or embryo/animal can produce a modified gene product from the targeted chromosomal sequence." (Sigma '613 at [0084]).

"In other aspects, the donor sequence of the donor polynucleotide corresponds to an exogenous sequence. As used herein, an "exogenous" sequence refers to a sequence that is not native to the cell or embryo, or a sequence whose native location in the genome of the cell or embryo is in a different location. For example, the exogenous sequence can comprise protein coding sequence, which can be operably linked to an exogenous promoter control sequence such that, upon integration into the genome, the cell or embryo/animal is able to express the protein coded by the integrated sequence. Alternatively, the exogenous sequence can be integrated into the chromosomal sequence such that its expression is regulated by an endogenous promoter control sequence. In other iterations, the exogenous sequence can be a transcriptional control sequence, another expression control sequence, an RNA coding sequence, and so forth. Integration of an exogenous sequence into a chromosomal sequence is termed a "knock in." (Sigma '613 at [0085]).

"As can be appreciated by those skilled in the art, the length of the donor sequence can and will vary. For example, the donor sequence can vary in length from several nucleotides to hundreds of nucleotides to hundreds of thousands of nucleotides." (Sigma '613 at [0086]). See also Sigma '613 at [0087]-[0093].

"(f) Culturing the cell or embryo
The method further comprises maintaining the cell or embryo under appropriate conditions such that the guide RNA(s) directs the RNA-guided endonuclease(s) to the targeted site(s) in the chromosomal sequence, and the RNA-guided endonuclease(s) introduce at least one double-stranded break in the chromosomal sequence. A double-stranded break can be repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof." (Sigma '613 at [0096]).

"In embodiments in which no donor polynucleotide is introduced into the cell or embryo, the double-stranded break can be repaired via a non-homologous end-joining (NHEJ) repair process. Because NHEJ is error-prone, deletions of at least one nucleotide, insertions of at least one nucleotide, substitutions of at least one nucleotide, or combinations thereof can occur during the repair of the break. Accordingly, the sequence at the chromosomal sequence can be modified such that the reading frame of a coding region can be shifted and that the chromosomal sequence is inactivated or "knocked out." An inactivated protein-coding chromosomal sequence does not give rise to the protein coded by the wild type chromosomal sequence. (Sigma '613 at [0097]).
“In embodiments in which a donor polynucleotide comprising upstream and downstream sequences is introduced into the cell or embryo, the double-stranded break can be repaired by a homology-directed repair (HDR) process such that the donor sequence is integrated into the chromosomal sequence. Accordingly, an exogenous sequence can be integrated into the genome of the cell or embryo, or the targeted chromosomal sequence can be modified by exchange of a modified sequence for the wild type chromosomal sequence.” (Sigma ‘613 at [0098]). See also Sigma ‘613 at [0099]-[0103].

*(VI) Genetically Modified Cells and Animals*

The present disclosure encompasses genetically modified cells, non-human embryos, and non-human animals comprising at least one chromosomal sequence that has been modified using an RNA-guided endonuclease-mediated or fusion protein-mediated process, for example, using the methods described herein. The disclosure provides cells comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest or a fusion protein, at least one guide RNA, and optionally one or more donor polynucleotide(s). The disclosure also provides non-human embryos comprising at least one DNA or RNA molecule encoding an RNA-guided endonuclease or fusion protein targeted to a chromosomal sequence of interest, at least one guide RNA, and optionally one or more donor polynucleotide(s).” (Sigma ‘613 at [0114]).

“The present disclosure provides genetically modified non-human animals, non-human embryos, or animal cells comprising at least one modified chromosomal sequence. The modified chromosomal sequence may be modified such that it is (1) inactivated, (2) has an altered expression or produces an altered protein product, or (3) comprises an integrated sequence. The chromosomal sequence is modified with an RNA guided endonuclease-mediated or fusion protein-mediated process, using the methods described herein.” (Sigma ‘613 at [0115]). See also Sigma ‘613 at [0116]-[0130].

See also Examples 1-9 (Sigma ‘613 at [0142]-[0160]) and accompanying Figures, Claims 1-16 of Sigma ‘613.
**APPENDIX VI**

**EXAMPLES OF SUPPORT FOR PENDING CLAIM 1 OF U.S. PATENT APPLICATION NO. 16/654,613 IN U.S. PROVISIONAL APPLICATION NO. 61/734,256 FILED DECEMBER 6, 2012**

<table>
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<tr>
<th>SIGMA’S USSN 16/654,613 (Proposed Claim for Interference)</th>
<th>SIGMA’S USSN 61/734,256 filed December 6, 2012 (hereinafter “Chen P1”)*</th>
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| 1. A eukaryotic cell comprising                           | *(II) Method for Modifying a Chromosomal Sequence*
|                                                           | Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell. The method comprises introducing into the eukaryotic cell (i) an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease, wherein the RNA-guided endonuclease comprises a nuclear localization signal, (ii) at least one guiding RNA or at least one DNA molecule encoding a guiding RNA, wherein each guiding RNA guides the RNA-guided endonuclease to a targeted site in the chromosomal sequence, and optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell such that the RNA-guided endonuclease introduces a double-stranded break at the targeted site in the chromosomal sequence and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.” (Chen P1 at [0016]). |

*“A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism.” (Chen P1 at [0045]).*  

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

| **a target chromosomal sequence; and**                   | *(II) Method for Modifying a Chromosomal Sequence*
|                                                           | Another aspect of the present disclosure encompasses a method for modifying a chromosomal sequence in a eukaryotic cell. The method comprises introducing into the |
(i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas9 type II protein is codon optimized for expression in the eukaryotic cell, and

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- eukaryotic cell (i) an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease, wherein the RNA-guided endonuclease comprises a nuclear localization signal, (ii) at least one guiding RNA or at least one DNA molecule encoding a guiding RNA, wherein each guiding RNA guides the RNA-guided endonuclease to a targeted site in the chromosomal sequence, and optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell such that the RNA-guided endonuclease introduces a double-stranded break at the targeted site in the chromosomal sequence and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.” (Chen P1 at [0016]).

“The RNA-guided endonuclease in conjunction with the guiding RNA is directed to a target site in the chromosomal sequence and cleaves the chromosomal sequence.” (Chen P1 at [0017]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

- “One aspect of the present disclosure provides RNA-guided endonucleases that comprise at least one nuclear localization signal, which permits entry of the endonuclease into the nuclei of eukaryotic cells.” (Chen P1 at [0004]).

- “The RNA-guided endonuclease can be derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. The CRISPR/Cas system can be a type I, a type II, or a type III system. In some embodiments, the RNA-guided endonuclease can be derived from a type II CRISPR/Cas system.” (Chen P1 at [0004]).

- “In some embodiments, the RNA-guided endonuclease can be derived from a wild type Cas9 protein(s) or fragment(s) thereof. In other embodiments, the RNA-guided endonuclease can be derived from modified Cas9 protein(s). For example, the amino acid sequence of the Cas9 protein can be modified such that one or more properties (e.g., nuclease activity, affinity, stability, etc.) of the protein is improved. Alternatively, domains of the Cas9 protein not involved in RNA-guided cleavage can be eliminated from the protein such that the modified Cas9 protein is smaller than the wild type Cas9 protein. In still other embodiments, the RNA-guided endonuclease can be a fusion protein comprising domains of wild type Cas9 proteins, modified Cas9 proteins, and/or other proteins. For example the RNA-guided endonuclease could comprise a marker, such as GFP or another fluorescent protein.” (Chen P1 at [0006]).
The Cas9-derived endonucleases disclosed herein comprise at least one nuclear localization signal (NLS) for transport into the nuclei of eukaryotic cells. In general, an NLS comprise a stretch of basic amino acids. Nuclear localization signals are known in the art (see, e.g., Lange et al., J. Biol. Chem., 2007, 282:5101-5105). For example, examples of classical NLSs included monopartite sequences such as PKKKRKV (SEQ ID NO:1) or PPKKRRV (SEQ ID NO:13), as well as bipartite sequences. The NLS can be located at the N-terminus, the C-terminal, or in an internal location of the endonuclease. In an exemplary embodiment, the NLS is located at the C-terminus of the endonuclease.” (Chen P1 at [0008]).

“The disclosure also provides nucleic acids encoding the RNA-guided endonuclease. The nucleic acid encoding the RNA-guided endonuclease can be RNA or DNA. The DNA encoding the RNA-guided endonuclease can be present in a vector (see below).” (Chen P1 at [0011]).

“In embodiments in which the nucleic acid encoding the RNA-guided endonuclease is DNA, the DNA coding sequence can be codon optimized for efficient translation into protein in the eukaryotic cell of interest. For example, the codons can be optimized for expression in humans, mice, rats, hamsters, cows, pigs, cats, dogs, fish, amphibians, plants, yeast, insects, and so forth (see Codon Usage Database at www.kazusa.or.jp/codon/). Programs for codon optimization are available as freeware (e.g., OPTIMIZER at http://genomes.urv.es/OPTIMIZER; OptimumGeneTM from GenScript at http://www.genscript.com/codon_opt.html). Commercial codon optimization programs are also available. In an exemplary embodiment, the DNA encoding a modified Cas9 protein consisting of SEQ ID NO:2 is codon optimized for translation in human cells and consists of SEQ ID NO:3. In various iterations, the DNA encoding sequence can be at least about 75%, 80%, 85%, 95%, or 99% identical to SEQ ID NO:3.” (Chen P1 at [0012]). See also Chen P1 at [0013]-[0015].

“The method comprises introducing into the cell an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease.” (Chen P1 at [0018]).

“(d) Introducing into the cell
The RNA-targeted endonuclease (or its encoding nucleic acid), the guiding RNA(s) (or DNAs encoding the guiding RNA), and the optional donor polynucleotide(s) can be introduced into the cell by a variety of means. In some embodiments, the cell is transfected. Suitable transfection methods include calcium phosphate-mediated transfection, nucleofection (or electroporation), cationic polymer transfection (e.g., DEAE-dextran or polyethylamine), viral transduction, virosome transfection, virion transfection, liposome
transfection, cationic liposome transfection, immunoliposome transfection, nonliposomal lipid transfection, dendrimer transfection, heat shock transfection, magnetofection, lipofection, gene gun delivery, impalefection, sonoporation, optical transfection, and proprietary agent-enhanced uptake of nucleic acids. Transfection methods are well known in the art (see, e.g., "Current Protocols in Molecular Biology" Ausubel et al., John Wiley & Sons, New York, 2003 or "Molecular Cloning: A Laboratory Manual" Sambrook & Russell, Cold Spring Harbor Press, Cold Spring Harbor, NY, 3rd edition, 2001). In other embodiments, the molecules are introduced into the cell by microinjection. For example, the molecules can be injected into the pronuclei of one cell embryos.” (Chen P1 at [0037]). See also Chen P1 at [0038].

“A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism.” (Chen P1 at [0045]).

“The terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. (Chen P1 at [0053]).

“The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.” (Chen P1 at [0055]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

| wherein the CRISPR-Cas type II system protein is a Cas9 protein, and | “In exemplary embodiments, the endonuclease can be derived from a Cas9 protein of a type II system.” (Chen P1 at [0004]).

“The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues.” (Chen P1 at [0055]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1. |
| (ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising | “The present disclosure provides RNA-guided endonucleases that are engineered for use in eukaryotic cells. The RNA-guided endonuclease can be used to modify the genome of eukaryotic cells. For this, the endonuclease is guided to a specific chromosomal sequence by a specific guiding RNA.” (Chen P1 at [0003]).

“The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell.” (Chen P1 at [0019]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1. |
| --- | --- |
| (1) a first region at the 5’ end that is capable of base pairing with a target site in the chromosomal sequence, and | “(a) Target site
The RNA-guided endonuclease in conjunction with the guiding RNA is directed to a target site in the chromosomal sequence and cleaves the chromosomal sequence. The target site has no sequence limitation except that the sequence is immediately followed (downstream) by a consensus sequence. This consensus sequence is also known as a protospacer adjacent motif (PAM). Examples of PAM include, but are not limited to, NGG and NGGNG (wherein N is defined as any nucleotide). The target site can be in the coding region of a gene, in an intron of a gene, in a control region between genes, etc. The gene can be a protein coding gene or an RNA coding gene.” (Chen P1 at [0017]).

“The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell. Each guiding RNA interacts with the RNA-guided endonuclease to guide the endonuclease to a specific target site, wherein the endonuclease cleaves the target site. Each guiding RNA comprises three regions: a first region at the 5’ end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3′ region that remains essentially single-stranded. The first region of each guiding RNA is different such that each guiding RNA guides the endonuclease to a specific target site. The second and third regions of each guiding RNA can be the same in all guiding RNAs.” (Chen P1 at [0019]).

“The first region of the guiding RNA is complementary to the target site in the chromosomal sequence such that the first region of the guiding RNA can base pair with the target site. In various embodiments, the first region of the guiding RNA can comprise from about 10 nucleotides to more than about 25 nucleotides.” (Chen P1 at [0020]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1. |
| (2) a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and | “The method also comprises introducing at least one guiding RNA or at least one DNA molecule encoding a guiding RNA into the cell. Each guiding RNA interacts with the RNA-guided endonuclease to guide the endonuclease to a specific target site, wherein the |
| addToSequence | endonuclease cleaves the target site. Each guiding RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guiding RNA is different such that each guiding RNA guides the endonuclease to a specific target site. The second and third regions of each guiding RNA can be the same in all guiding RNAs." (Chen P1 at [0019]).

“The guiding RNA also comprises a second region that forms a secondary structure. In some embodiments, the secondary structure comprises a stem (or hairpin) and a loop. The length of the loop and the stem can vary. For example, the loop can range from about 3 to about 10 nucleotides in length, and the stem can range from about 6 to about 20 base pairs in length. The stem can comprise one or more bulges of 1 to about 10 nucleotides. Thus, the overall length of the second region can range from about 16 to about 60 nucleotides in length. In an exemplary embodiment, the loop is about 4 nucleotides in length and the stem comprises about 12 base pairs." (Chen P1 at [0021]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.

| deleteLine | whereby base pairing of the first region to the target site is capable of targeting the Cas9 protein to the target chromosomal sequence.

| deleteLine | “[T]he endonuclease is guided to a specific chromosomal sequence by a specific guiding RNA." (Chen P1 at [0003]).

“The double-stranded break can be repaired by a cellular DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof." (Chen P1 at [0003]).

The first region of the guiding RNA is complementary to the target site in the chromosomal sequence such that the first region of the guiding RNA can base pair with the target site.” (Chen P1 at [0020]).

“(e) Culturing the cell
The method further comprises maintaining the cell under appropriate conditions such that the guiding RNA guides the endonuclease to the targeted site in the chromosomal sequence, and the endonuclease introduces a double-stranded break in the targeted site in the chromosomal sequence. The double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified by a deletion of at least one nucleotide, an insertion of at least one nucleotide, a substitution of at least one nucleotide, or a combination thereof.” (Chen P1 at [0039]).
| "A variety of eukaryotic cells are suitable for use in the method. In various embodiments, the cell can be a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, an insect cell, a plant cell, a yeast cell, or a single cell eukaryotic organism." (Chen P1 at [0045]).

"The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues." (Chen P1 at [0055]).

See also Examples 1-5 (Chen P1 at [0058]-[0068]) and accompanying Figures; Claims 12-21 of Chen P1.
REMARKS

With this amendment, claims 1, 6-11, and 13-27 are pending. Claims 6, 7, and 27 are cancelled, and claims 2-5 and 12 were previously cancelled.

I. PRIORITY

Applicant previously amended the specification and claim 18 to replace “YP_005388840.1” with “WP_014407541.1” in the Amendment and Response filed April 29, 2019. As previously indicated by the Office, the GenBank Accession number record for the Streptococcus pyogenes MGAS15252 has changed from YP_005388840.1 to WP_014407541.1.\(^1\) Applicant stated that no new matter was introduced with these amendments because, as noted in the webpage cited by the Office, “the sequence YP_005388840.1 is identical to WP_014407541.1.” The Office now denies priority for Claim 18 to U.S. Provisional Application No. 61/734,256 on the ground that the priority application does not provide support for Accession number WP_014407541.1 or the complete content of the database entry and states that claim 18 has an effective filing date of June 21, 2016, the filing date of the present application.\(^2\)

Without conceding the propriety of the objection and to advance prosecution, the specification is amended to replace “WP_014407541.1” with the original “YP_005388840.1,” which is supported and enabled by U.S. Provisional Application No. 61/734,256 at paragraph [0059] and the application as filed at paragraph [0143]. Claim 18 has been amended to change the dependency from claim 17 to claim 19, to delete "(Accession number WP_014407541.1)" and to add “and comprises SEQ ID NO:9”. The amendments to claim 18 are supported in the application as filed by Example 1 at paragraph [0143], Table 1 and SEQ ID NO: 9, and are supported and enabled in U.S. Provisional Application No. 61/734,256 based on paragraph [0059] and Table 1. Claim 18 is entitled to priority from U.S. Provisional Application No. 61/734,256 and has an effective filing date of December 6, 2012.

\(^1\) Final Office Action dated December 14, 2018, page 5.
\(^2\) Non-Final Office Action dated July 18, 2019, pages 2-3.
II. SPECIFICATION

The specification has been objected to as adding new matter. Without conceding the propriety of the objection that replacing “YP _005388840.1” with “WP_014407541.1” added new matter and to advance prosecution, paragraph [0143] of the specification has been amended to replace “WP_014407541.1” with the original “YP_005388840.1.” This amendment is supported and enabled by the application as originally filed.

III. 35 U.S.C. § 112, FIRST AND SECOND PARAGRAPH, REJECTIONS

Claim 18 is rejected under 35 U.S.C. §, second paragraph, as being indefinite. Claim 18 is also rejected under 35 U.S.C. §§, first paragraph, as failing to comply with the written description requirement and based on a non-enabling disclosure.

With the amendment to claim 18 herein (and the corresponding amendment to the specification at paragraph [0143]), it is believed that the 35 U.S.C. § 112, first and second paragraph, rejections are moot and can be withdrawn. Specifically, claim 18 has been amended to change the dependency from claim 17 to claim 19, to delete “(Accession number WP_014407541.1)” and to add “and comprises SEQ ID NO:9”. The amendments to claim 18 are supported in the application as filed by Example 1 at paragraph [0143], Table 1 and SEQ ID NO: 9. No new matter is introduced with these amendments. SEQ ID NO:9, which appears in Table 1, provides the amino acid sequence for a Cas9 protein from Streptococcus pyogenes strain MGAS15252 with a nuclear localization signal. Claim 18 as amended is therefore definite, enabled and complies with the written description requirement.

Reconsideration and withdrawal of these grounds of rejection under 35 U.S.C. § 112, first and second paragraphs, is respectfully requested.

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3 Id., pages 3-4.
4 Id., pages 5-6.
5 Non-Final Office Action dated July 18, 2019, pages 6-9.
IV. GROUNDS OF REJECTION UNDER 35 U.S.C. §§ 102(e)(1) AND 103

Claims 1, 8, 11, 13-17, 19 and 22-26 stand rejected under 35 U.S.C. §§ 102(e)(1) and 103, as follows:


ii. Claim 9 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna ’797, as evidenced by Järver, in view of Cost et al., U.S. Pub. No. 2013/0326645 (“Cost ‘645”).

iii. Claim 18 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna ’797, as evidenced by Järver, in view of GenBank Accession No. YP_005388840.1 (“GenBank”).


v. Claim 21 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna ’797, as evidenced by Järver, in view of Eloit et al., U.S. Pub. No. 2013/0345400 (“Eloit”).

vi. Claims 1, 8, 11, 13-17, and 22-26 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna ’797, as evidenced by Järver, in view of Sontheimer et al., U.S. Pub. No. 2010/00706057 (“Sontheimer ’057”).

vii. Claim 9 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna ’797, as evidenced by Järver, in view of Sontheimer ’057 and further in view of Cost ‘645.

viii. Claim 18 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna ’797, as evidenced by Järver, in view of Sontheimer ’057 and further in view of GenBank.

ix. Claims 1, 8, 11, 13-17, and 22-26 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna ’797, as evidenced by Järver, in view of Gustafsson et al., TRENDS In Biotechnology, Vol. 22, No. 7, p. 346-353 (2004) (“Gustafsson”).
x. Claim 9 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna '797, as evidenced by Järver, in view of Gustafsson and further in view of Cost '645.

xi. Claim 18 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna '797, as evidenced by Järver, in view of Gustafsson and further in view of GenBank.

xii. Claims 1, 8, 11, 13-17, 19, and 22-26 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna '797, as evidenced by Järver, in view of Rozwadowski et al., U.S. Patent No. 7,947,874 ("Rozwadowski '874").

xiii. Claim 18 is rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Doudna '797, as evidenced by Järver, in view of Rozwadowski '874 and further in view of GenBank.


The Examiner also references the following Doudna '797 priority applications: U.S. Provisional App. No. 61/652,086, filed May 25, 2012 ("Doudna P1"); U.S. Provisional Application No. 61/716,256, filed October 19, 2012 ("Doudna P2"); and U.S. Provisional Application No. 61/757,640, filed January 28, 2013 ("Doudna P3").

Reconsideration and withdrawal of each of these grounds of rejection is respectfully requested.

V. THE REJECTIONS UNDER 35 U.S.C. §§ 102(e)(1) AND 103 SHOULD BE WITHDRAWN

As detailed previously, the disclosure of the various references cited above (Doudna '797; Järver; Cost '645; Sontheimer '057; Gustafsson; Dong; Eloit; Rozwadowski '874; Hu; Smith), whether considered alone or in combination, do not disclose or suggest each and every element of the claim and/or would not have
provided one of ordinary skill in the art as of December 6, 2012 (the filing date of Chen P1), with a reasonable expectation that CRISPR/Cas9 would function in eukaryotic cells.

Furthermore, the Declarations of record confirm the lack of reasonable expectation of success that one of ordinary skill in the art (such as Drs. Lambowitz, Loring, Urnov and Cannon) would have had, and discuss the failures and considerable barriers that were known to one of ordinary skill in the art that contributed to the doubt and uncertainty of successfully using a CRISPR/Cas9 system in eukaryotic cells.

Still further, these Declarations confirm that the incomplete and insufficient disclosure in Doudna P1, Doudna P2, and Jinek 2012 does not enable one of ordinary skill in the art to practice a CRISPR-Cas9 system adapted to function in a eukaryotic cell without undue experimentation, nor do they provide a written description demonstrating possession of a CRISPR-Cas9 system adapted to function in a eukaryotic cell.

The Examiner cites no credible scientific evidence to refute the considered opinions of the Declarants, who have decades of combined experience in genome editing. Instead, and incredibly, the Examiner now relies upon attorney argument in connection with Applicant’s corresponding granted European patent in European Patent Office proceedings. In any event, these “arguments” have been addressed in detail by Dr. Cannon in that entirely separate proceeding.

The Examiner also brushes off the Applicant’s Section VII of the RCE and Response filed April 29, 2019, entitled THE EXAMINER’S ANTICIPATION AND OBVIOUSNESS REJECTIONS DIRECTLY CONTRAVENE THE JUDGMENTS OF BOTH THE PTAB AND THE FEDERAL CIRCUIT, and totaling over 30 pages of relevant argument and patent law jurisprudence. Here, the Examiner devotes barely a page (of a 145-page Office action) to incorrectly and inappropriately dismiss this controlling and highly relevant precedent.

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7 Id., pages 56 and 142.
It is respectfully submitted that, based upon the current record, the Applicant’s claims are novel and nonobvious over the cited art and should be moved immediately to allowance.

VI. **SUGGESTION OF AN INTERFERENCE**

Submitted contemporaneously herewith is a Suggestion of Interference Pursuant to 37 C.F.R. § 41.202, with at least several of the Doudna et al. references employed in the Grounds of Rejection (U.S. Pub. Nos. 2018/0251794; 2018/0251795; 2018/0230496; and 2018/0230497). The instant application is cited as an involved application under Proposed Count 1 in Table 2 of Section II (37 C.F.R. § 41.202(a)(2) and 37 C.F.R. § 41.202(a)(3) – Proposal of Counts, Claim Correspondence, and Identification of All Claims Applicant Believes Interfere).
CONCLUSION

All of the pending issues have been addressed. However, the absence of a reply to a specific rejection, issue, or comment does not signify agreement with or concession of that rejection, issue, or comment. In addition, because the arguments made above may not be exhaustive, there may be reasons for patentability of any or all pending claims (or other claims) that have not been expressed. Finally, nothing in this response should be construed as an intent to concede any issue with regard to any claim, except as specifically stated herein.

In light of the foregoing, the Applicant requests entry of the claim amendments, withdrawal of the claim rejections, and solicit allowance of the pending claims. The Examiner is invited to contact the undersigned practitioner should any issues remain unresolved.

Respectfully submitted,

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AMENDMENTS TO THE CLAIMS:

1. (currently amended) A method for integrating an exogenous sequence into a chromosomal sequence of a eukaryotic cell, the method comprising:

\[[\text{a}]\] introducing into the eukaryotic cell

(i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or codon-optimized nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the at least one RNA-guided endonuclease is a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR associated (Cas) (CRISPR-Cas) type II system protein, wherein the nucleic acid encoding the CRISPR-Cas type II system protein is codon optimized for expression in the eukaryotic cell, and wherein the CRISPR-Cas type II system protein is a Cas9 protein;\[[\text{b}]\]

(ii) at least one engineered guide RNA or DNA encoding at least one engineered guide RNA, each guide RNA comprising

1. a first region at the 5’ end that base pairs with a target site in the chromosomal sequence, and

2. a second region that forms a secondary structure which interacts with the at least one RNA-guided endonuclease; and

(iii) at least one donor polynucleotide comprising the exogenous sequence; and

b) culturing the eukaryotic cell such that whereby the at least one guide RNA guides the at least one RNA-guided endonuclease to \[[\text{a}]\] the target site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break, the target site in the chromosomal sequence is immediately followed by a protospacer adjacent motif (PAM), and repair of the double-stranded break by a DNA repair process leads to integration of the exogenous sequence into the chromosomal sequence.

2-5. (previously cancelled).

6-7. (cancelled).
8. (original) The method of claim 1, wherein the exogenous sequence in the donor polynucleotide is flanked by sequences having substantial sequence identity to sequences on either side of the target site in the chromosomal sequence.

9. (previously presented) The method of claim 1, wherein the exogenous sequence in the donor polynucleotide further comprises a targeted cleavage site that is recognized by the at least one RNA-guided endonuclease.

10. (previously presented) The method of claim 1, wherein the nucleic acid encoding the at least one RNA-guided endonuclease is mRNA.

11. (previously presented) The method of claim 1, wherein the nucleic acid encoding the at least one RNA-guided endonuclease is DNA.

12. (previously cancelled).

13. (previously presented) The method of claim 1, wherein the eukaryotic cell is a human cell, a nonhuman mammalian cell, or a plant cell.

14. (original) The method of claim 1, wherein the eukaryotic cell is in vitro.

15. (original) The method of claim 1, wherein the eukaryotic cell is in vivo.

16. (original) The method of claim 1, wherein the at least one guide RNA is at least partially chemically synthesized.

17. (previously presented) The method of claim 1, wherein the Cas9 protein is from Streptococcus pyogenes.
18. (currently amended) The method of claim [[17]] 19, wherein the Cas9 protein is from Streptococcus pyogenes strain MGAS15252 (Accession—number WP_04407541.1) and comprises SEQ ID NO:9.

19. (previously presented) The method of claim 1, wherein the Cas9 protein comprises one nuclear localization signal at its C-terminus.

20. (previously presented) The method of claim 19, wherein the nuclear localization signal comprises SEQ ID NO:1.

21. (previously presented) The method of claim 1, wherein the at least one RNA-guided endonuclease comprising the at least one nuclear localization signal comprises SEQ ID NO:2.

22. (previously presented) The method of claim 1, wherein the nucleic acid encoding the at least one RNA-guided endonuclease is mRNA and the at least one guide RNA is comprised of two non-covalently bound RNA molecules.

23. (previously presented) The method of claim 1, wherein the nucleic acid encoding the at least one RNA-guided endonuclease is mRNA and the at least one guide RNA is comprised of a single RNA molecule.

24. (previously presented) The method of claim 1, wherein the nucleic acid encoding the at least one RNA-guided endonuclease is DNA, the at least one guide RNA is encoded by DNA, and the at least one guide RNA is comprised of a single RNA molecule.

25. (previously presented) The method of claim 1, wherein the at least one donor polynucleotide is double stranded DNA.
26. (previously presented) The method of claim 1, wherein the at least one donor polynucleotide is single stranded DNA.

27. (cancelled).
AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0143] of the as-filed specification with the following amended paragraph:

[0143] A Cas9 gene from *Streptococcus pyogenes* strain MGAS 15252 (Accession number [YP_005388840.1](https://www.ncbi.nlm.nih.gov/nuccore/YP_005388840.1)) was optimized with *Homo sapiens* codon preference to enhance its translation in mammalian cells. The Cas9 gene also was modified by adding a nuclear localization signal PKKKRKV (SEQ ID NO: 1) at the C terminus for targeting the protein into the nuclei of mammalian cells. Table 1 presents the modified Cas9 amino acid sequence, with the nuclear localization sequence underlined. Table 2 presents the codon optimized, modified Cas9 DNA sequence.
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Fuqiang CHEN et al.          Art Unit:     1636
Serial No.:      15/188,911                  Examiner: Jennifer Ann DUNSTON
Filed:           June 21, 2016                Conf. No.:      8930
For:            CRISPR-BASED GENOME MODIFICATION AND REGULATION

October 17, 2019

AMENDMENT AND RESPONSE TO NON-FINAL OFFICE ACTION

TO THE COMMISSIONER FOR PATENTS:

In response to the non-final Office action dated July 18, 2019, please consider the amendments and remarks below in accordance with the Rules of Practice. It is believed that no additional fee is due with the filing of this paper. The Commissioner, however, is hereby authorized to charge any fees, or credit any overpayment, to Deposit Account 50-5915 referencing the docket number above.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 7 of this paper.
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Fuqiang CHEN et al.  
Serial No.: 15/188,911  
Filed: June 21, 2016  
For: CRISPR-BASED GENOME MODIFICATION AND REGULATION

Art Unit: 1636  
Examiner: Jennifer Ann DUNSTON  
Conf. No.: 8930

SUPPLEMENTAL DECLARATION OF PAULA CANNON UNDER 37 C.F.R. § 1.132

I, Paula Cannon, Ph.D., declare and state as follows:

1. Paragraphs 1-9 from my prior Declaration executed April 24, 2019 (hereinafter, “Cannon Declaration I”), are incorporated by reference herein in their entirety.

2. I have reviewed the claim amendments filed in the above-referenced application on October 17, 2019.

3. My analyses and opinions in Cannon Declaration I, paragraphs 9-174 and all exhibits thereto, apply equally to the newly-amended claims. Stated another way, my analyses and opinions in Cannon Declaration I do not change in view of the claim amendments.

4. I am being compensated for my work at my customary consulting rate. I have no personal or financial interest in the outcome of this or any other CRISPR patent matter.

5. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.