Filed on behalf of Junior Party
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
UNIVERSITY OF VIENNA, AND EMMANUELLE CHARPENTIER

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, UNIVERSITY OF VIENNA, AND EMMANUELLE CHARPENTIER
Junior Party

Applications 15/947,680; 15/947,700; 15/947,718; 15/981,807; 15/981,808; 15/981,809; 16/136,159; 16/136,165; 16/136,168; 16/136,175; 16/276,361; 16/276,365; 16/276,368; and 16/276,374

v.

TOOLGEN, INC.
Application 14/685,510

Patent Interference No. 106,127 (DK)
(Technology Center 1600)

CVC RESPONSIVE MOTION 2 (for accorded benefit)

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As authorized by the Board's Order of June 10, 2021, and in response to ToolGen's Substantive Motion 2, Junior Party ("CVC") moves to be accorded benefit of the filing date of U.S. Appl. No. 13/842,859, filed March 15, 2013 ("the '859 application") (Ex. 2005), with respect to Count 1, contingent upon the PTAB granting ToolGen's Motion 2.

I. INTRODUCTION

The PTAB has already accorded CVC the benefit date of CVC's P3 application: January 28, 2013. *See* Paper 1, at 10; MF7. Indeed, in the CVC v. Broad Interference 106,115, the PTAB concluded that P3 is a constructive reduction to practice of Count 1 in part due to the working example (Example 2) disclosed in P3. The Board explained in its Decision on Motions in Interference 106,115:

> Because Example 2 provides the protocols necessary and results of a CRISPR-Cas9 system in eukaryotic, human cells, we are persuaded that P3 presents a sufficient written and enabled description of an embodiment of Count 1. Accordingly, P3 is a constructive reduction to practice of Count 1.

See Ex. 2400, 106:11-14; MF8.

ToolGen moved to deny CVC benefit to P3. *See* ToolGen Motion 2, Paper 171, 2:6-9, 6:8-16:23. As CVC will explain in its Opposition 2, ToolGen's Motion 2 should be denied because it fails to show that P3 is not a constructive reduction to practice of Count 1. Thus, the PTAB need not consider this Responsive Motion if the Board denies ToolGen's Motion 2, as it should. If, however, the PTAB were to grant ToolGen's Motion 2 and deny CVC the benefit of P3, CVC contingently moves to be accorded the benefit of the '859 application: March 15, 2013. Ex. 2005.

The '859 application shares a common specification with CVC's involved applications and thus provides the same constructive reduction to practice of at least one embodiment of the

count as the involved applications. Ex. 2466, ¶¶19-27; MF12-14; Appx. 6. CVC is entitled to the benefit of its earliest application sharing a specification with its involved applications.

II. STATEMENT OF PRECISE RELIEF REQUESTED

CVC moves to be accorded the benefit of the filing date of U.S. Application No. 13/842,859, filed March 15, 2013 ("the '859 application") (Ex. 2005). As alternatives, CVC moves to be accorded benefit of the filing date of U.S. Application Nos. 14/685,504, filed April 13, 2015 ("the '504 application") (Ex. 2006); or 15/138,604, filed April 26, 2016 ("the '604 application") (Ex. 2007).

III. ARGUMENT

A. CVC is entitled to be accorded benefit to its predecessor non-provisional applications in the priority chain that share the same specification as its involved applications

As a matter of law, CVC is presumptively entitled to the benefit of its earliest application sharing an identical specification with its involved applications—the '859 application filed on March 15, 2013. CVC's involved U.S. Application No. 15/981,807 ("the '807 application") is a continuation application that describes and enables its claim 156—which is one alternative of the Count—and is properly entitled to benefit of the earlier '859 application under 35 U.S.C. § 120. *See also, Transco Prod. Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 556–57 (Fed. Cir. 1994). CVC should therefore be accorded the benefit of the filing dates of the '504, '604, and '859 applications, because they share a common specification with the involved applications as a string of continuation applications and thus provide the *same disclosure* as the involved applications. *See* Appx. 6. This disclosure supports constructive reduction to practice. Ex. 2466, ¶¶9-13, 19-27; MF4-6, 12-14; Appx. 3-6.

B. Constructive reductions to practice in P1, P2, and P3 are present also in CVC's '859, '504, and '604 non-provisional applications

Moreover, the constructive reduction to practice in each of CVC's P1, P2 and P3 applications has been continuously disclosed and carried forward through a continuing chain of patent applications including CVC's involved applications (referred to in this discussion as "common specification"). 37 C.F.R. § 41.201; Ex. 2466, ¶¶19-22; MF9-11; Appx. 6. CVC's half of Count 1 is shown below, separated into eight elements:

Element of Count 1	Count 1
Element [1]	"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…"
Element [2]	"a Cas9 protein, or a nucleic acid comprising a nucleotide sequence encoding said Cas9 protein; and…"
Element [3]	"a single molecule DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding said single molecule DNA-targeting RNA; wherein the single molecule DNA-targeting RNA comprises…"
Element [4]	"a targeter-RNA that is capable of hybridizing with a target sequence in the target DNA molecule, and…"
Element [5]	"an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment…"
Element [6]	"wherein the activator-RNA and the targeter-RNA are covalently linked to one another with intervening nucleotides; and…"
Element [7]	"wherein the single-molecule DNA-targeting RNA is capable of forming a complex with the Cas9 protein, thereby targeting the Cas9 protein to the target DNA molecule…"
Element [8]	"whereby said system is capable of cleaving or editing the target DNA molecule or modulating transcription of at least one gene encoded by the target DNA molecule."

As CVC demonstrated in Substantive Motion 1, CVC's provisional application numbers 61/652,086 ("P1"), filed May 25, 2012, and 61/716,256, filed Oct. 19, 2012 ("P2") each provides a constructive reduction to practice of at least one embodiment of Count 1. *See* Paper 368, CVC Motion 1, 15:11-39:5; Ex. 2013, ¶¶90-245. Specifically, CVC showed that P1 and P2 each describes and enables three specific embodiments the meet all the elements [1]-[8] of the count:

E1 (fish cell), E2 (human cell), and E3 (fruit fly cell). *Id.* Additionally, the PTAB determined in Interference 106,115 that CVC's P3 "is a constructive reduction to practice of Count 1." Ex. 2400, 106:11-14; MF8, 16. And the PTAB accorded CVC the benefit of P3 in this proceeding. *See* Paper 1, at 10; MF7.

The disclosures in each of P1, P2, and P3 have been carried forward in the common specification. Ex. 2466, ¶¶20-22; Appx. 3-5; MF9-12. For example, the common specification describes in detail the same fish, human, and fruit fly cell embodiments taught in P1-P3 and methods of making and using them. Exs. 2005-2007, ¶¶[0035], [0046], [00213], [00215], [00275], [00283], [00285], [00515], [00555]-[00559], [00562]-[00577], Figs. 3, 14; Ex. 2466, ¶21; MF9-10. These embodiments meet all of elements [1]-[8] of Count 1. *Id.* The common specification also discloses the results from the same *in vitro* working example as in P1-P3 and the same eukaryotic cell working example from P3. Exs. 2005-2007, ¶¶[00555]-[00559], [00562]-[00577], Figs. 14, 29, 31; Ex. 2466, ¶21; MF11-12. These disclosures in the common specification meet all the elements [1]-[8] of Count 1. Exs. 2005-2007, ¶¶[0035], [0046], [00213], [00215], [00275], [00283], [00285], [00515], [00555]-[00559], [00562]-[00577], Figs. 3, 14, 29, 31; Ex. 2466, ¶¶19-22. Thus, the common specification provides a constructive reduction to practice of Count 1 for at least the same reasons that P1, P2, and P3 provide constructive reductions to practice of the count. Ex. 2466, ¶¶9-12, 19-22; MF9-12; Appx. 3-5.

As CVC will explain in its Opposition 2, ToolGen's criticisms of the working example disclosed in P3 (Example 2) fail to show that P3 is not a constructive reduction to practice of Count 1. Nonetheless, the common specification also provides supplementary disclosures beyond Example 2 that further bolster the §112 support for multiple embodiments of Count 1, including embodiments E1–E3.

C. The common specification of CVC's '859, '504, and '604 non-provisional applications includes additional disclosure compelling a conclusion that each application presents a constructive reduction to practice of the count

The common specification provides new Example 4, which describes a "method for controlling gene expression, based on Cas9, an RNA-guided DNA endonuclease from a Type II CRISPR system" called CRISPR interference (CRISPRi). Exs. 2005-2007, ¶[00606]; Ex. 2466, ¶23; MF16, 18. The common specification discloses that the CRISPRi system can repress transcription (i.e., modulate transcription) in human HEK293 cells. Exs. 2005-2007, ¶[00646]; Ex. 2466, ¶23; MF18. In fact, the common specification discloses that using the sgRNA design shown in Figure 40B targeting the non-template DNA strand of the green fluorescent protein (EGFP) coding region produced a "moderate but reproducible knockdown of gene expression…. (46% repression, Figure 45A)" in HEK293 cells. Exs. 2005-2007, ¶[00646]; Ex. 2466, ¶23; MF18. This example meets all the elements [1]-[8] of Count 1: element [1] (HEK293 cells comprising a Type II CRISPR-Cas system, CRISPRi); elements [2]-[6] (the CRISPRi system comprising a Cas9 and a sgRNA), and elements [7]-[8] (the CRISPRi Cas9 and sgRNA are capable of forming a functional complex and modulating transcription of the target DNA). Exs. 2005-2007, ¶¶[00646]-[00648]; Ex. 2466, ¶23. Further, ToolGen's criticisms in its Motion 2 regarding CVC's cell lysis protocol in P3 Example 2 are inapposite to Example 4 of the common specification because the HEK293T cells in Example 4 are analyzed intact (i.e., not lysed) via fluorescence activated cell sorting (FACS). Ex. 2005-2007, ¶¶[00617], [00646]-[00648], Fig. 45, 52; Ex. 2466, ¶23.

The common specification also bolsters § 112 support for Count 1 through new Example 5, which describes an experiment using "a fusion protein compr[i]sing a catalytically inactive Cas9 and an [] activator domain or a repressor domain" to "increase or decrease transcription from a target DNA, respectively." Exs. 2005-2007, ¶[00655]; Ex. 2466, ¶24; MF16, 19. The

common specification discloses specific results after fusing the humanized catalytically inactive Cas9 with either a "transcription activator domain VP64" or a "transcription repressor domain KRAB." Exs. 2005-2007, ¶[00656] and [00657]; Ex. 2466, ¶24; MF19. The common specification further discloses that the dCas9-VP64 fusion was able to "effectively activate GAL4 UAS by 20-fold in the presence of a cognate guide RNA that binds to the GAL4 UAS region." Exs. 2005-2007, ¶[00656]; Ex. 2466, ¶24; MF18. The common specification also describes a "20-fold and 15-fold repression" when using the dCas9-KRAB repressor domain fusion protein. Exs. 2005-2007, ¶[00657]; Ex. 2466, ¶24; MF19. This example also meets all the elements [1]-[8] of Count 1: element [1] (HEK293 cells comprising a Type II CRISPR-Cas system with a Cas9-fusion protein); elements [2]-[6] (the CRISPR-Cas system comprising a Cas9-fusion protein and a sgRNA), and elements [7]-[8] (the Cas9-fusion protein and sgRNA are capable of forming a functional complex and increasing or decreasing transcription of the target DNA). Exs. 2005-2007, ¶¶[00656]-[00657]; Ex. 2466, ¶24. ToolGen's criticisms of CVC's cell lysis protocol in P3 Example 2 are also inapposite to Example 5 of the common specification because the common specification discloses that the CRISPR-Cas system functionality in Example 5 was analyzed in intact, non-lysed HEK293T cells using flow cytometry. Ex. 2005-2007, ¶¶[00656]-[00657], Fig. 55, 56; Ex. 2466, ¶24.

The common specification also provides new Example 7 (describing using CRISPR-Cas to generate transgenic organisms). Exs. 2005-2007, ¶[00661]; Ex. 2466, ¶25; MF16, 20; Appx. 3-5. Example 7 discloses generating "[a] transgenic mouse expressing Cas9," isolating embryonic stem cells from the transgenic mouse, and using the Cas9-expressing embryonic stem cells to rapidly generate "new knock-out or knock-in cells (and therefore mice) … at any desired locus in the genome by introducing an appropriately designed DNA-targeting RNA that targets

the Cas9 to a particular locus of choice." Exs. 2005-2007, ¶[00661]; Ex. 2466, ¶25; MF20. As Dr. Doyon explains, the technique of gene knock-in at a targeted locus, as described in the common specification, was well-known in the art prior to 2012. Ex. 2466, ¶25; Ex. 2468, 70-71; Ex. 2469, 503-504, 510-511; Ex. 2470, 576-578. This example also meets all the elements [1]-[8] of Count 1: element [1] (mouse cells comprising a Type II CRISPR-Cas system); elements [2]-[6] (the CRISPR-Cas system comprising a Cas9 and a sgRNA), and elements [7]-[8] (the Cas9 and sgRNA are capable of forming a functional complex and editing the target DNA to generate "knock-out or knock-in" mouse cells). Exs. 2005-2007, ¶[00661];. Ex. 2466, ¶25.

Thus, as Dr. Doyon explains, a person of ordinary skill in the art reading the common specification would have understood that each specification described and enabled multiple embodiments of Count 1, including embodiments E1, E2, and E3, and that each embodiment meets all the elements [1]-[8] of Count 1. Ex. 2466, ¶¶9-13, 19-27; MF9-12, 16-20; Appx. 3-5.

IV. CONCLUSION

CVC should be accorded the benefit of the '859 application's March 15, 2013, filing date.

Respectfully submitted,

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APPENDIX 1: LIST OF EXHIBITS

Exhibit No.	Description
2001	Prov. Appl. No. 61/652,086, filed May 25, 2012
2002	Prov. Appl. No. 61/716,256, filed October 19, 2012
2003	Prov. Appl. No. 61/757,640, filed January 28, 2013
2005	U.S. Appl. No. 13/842,859, filed March 15, 2013
2006	U.S. Appl. No. 14/685,504, filed April 13, 2015
2007	U.S. Appl. No. 15/138,604, filed April 16, 2016
2013	Declaration of Yannick Doyon, Ph.D.
2034	U.S. Appl. No. 15/947,680
2035	U.S. Appl. No. 15/981,807
2036	U.S. Appl. No. 16/136,168
2037	U.S. Appl. No. 16/136,175
2038	U.S. Appl. No. 15/947,700
2039	U.S. Appl. No. 15/947,718
2040	U.S. Appl. No. 15/981,808
2041	U.S. Appl. No. 15/981,809
2042	U.S. Appl. No. 16/136,159
2043	U.S. Appl. No. 16/276,361
2044	U.S. Appl. No. 16/276,365
2045	U.S. Appl. No. 16/276,368
2046	U.S. Appl. No. 16/276,374
2047	U.S. Appl. No. 16/136,165
2400	Decision on Motions 37 C.F.R. § 41.125(a), *The Regents of the University of California v. The Broad Institute, Inc.,* Patent Interference No. 106,115, Paper 877, (September 10, 2020)
2463	Redline Comparison of Provisional Application No. 61/652,086 and Non-Provisional Application No. 13/842,859
2464	Redline Comparison of Provisional Application No. 61/716,256 and Non- Provisional Application No. 13/842,859
2465	Redline Comparison of Provisional Application No. 61/757,640 and Non- Provisional Application No. 13/842,859
2466	Second Declaration of Yannick Doyon, Ph.D.
2468	Soriano, P., "Generalized lacZ expression with the ROSA26 Cre reporter strain," *Nature Genetics* 21: 70-71 (1999)
2469	Thomas, K.T., and Capecchi, M.R., "Site-Directed Mutagenesis by Gene Targeting in Mouse Embryo-Derived Stem Cells," *Cell* 51:503-512 (1987)
2470	Doetschman, T., *et al*., "Targetted correction of a mutant HPRT gene in mouse embryonic stem cells," *Nature* 330:576-578 (1987)

APPENDIX 2: STATEMENT OF MATERIAL FACTS

1. U.S. Provisional Patent Application No. 61/652,086 ("P1") was filed on May 25, 2012, and lists Martin Jinek, Jennifer Doudna, Emmanuelle Charpentier, and Krzysztof Chylinski as co-inventors. Ex. 2001, p. 195.

2. U.S. Provisional Patent Application No. 61/716,256 ("P2") was filed on October 19, 2012, and lists Martin Jinek, Jennifer Doudna, Emmanuelle Charpentier, Krzysztof Chylinski, and James Harrison Doudna Cate as co-inventors. Ex. 2002, p. 277.

3. U.S. Provisional Patent Application No. 61/757,640 ("P3") was filed on January 28, 2013, and lists Martin Jinek, Jennifer Doudna, Emmanuelle Charpentier, Krzysztof Chylinski, and James Harrison Doudna Cate as co-inventors. Ex. 2003, p. 377.

4. CVC's '859 application was filed within 12 months of the filing dates of P1, P2, and P3, and makes specific reference to P1, P2, and P3 applications. Ex. 2005, p. 5.

5. CVC's '504 application was filed during the '859 application's pendency and makes specific reference to the '859, P1, P2, and P3 applications. Ex. 2006, pp. 4-5.

6. CVC's '604 application was filed during the '504 application's pendency and makes specific reference to the '504, '859, and P1, P2, and P3 applications. Ex. 2007, pp. 356-360.

7. The PTAB accorded CVC the benefit filing date of P3: January 28, 2013. Paper 1, at 10.

8. In Interference 106,115, the PTAB determined that "P3 is a constructive reduction to practice of Count 1." Ex. 2400, 106:11-14**.**

9. The common specification discloses the same "chimera A" single-molecule DNA-targeting RNA taught in P1 and P2 (including the 3' extended chimera A), the same *S. pyogenes* Cas9 protein, and the same fish, human, and fruit fly target cells taught in P1-P3. Ex. 2463-2465;

Exs. 2005-2007, ¶¶[0035], [0046], [00213], [00215], [00275], [00515], Figs. 3, 14; Ex. 2466, ¶21.

10. The common specification describes the same microinjection and transfection techniques taught in P1-P3 for introducing the CRISPR-Cas9 system into eukaryotic cells. Ex. 2463-2465; Exs. 2005-2007, ¶¶[00283], [00285]; Ex. 2466, ¶21.

11. The common specification discloses the results from the same *in vitro* working example in P1-P3 which describes making and using a CRISPR-Cas9 RNP complex comprising a recombinant *S. pyogenes* Cas9 protein and a chimera A molecule to cleave a target DNA sequence. Ex. 2463-2465; Exs. 2005-2007, ¶¶[00555]-[00559], Fig. 14; Ex. 2466, ¶21.

12. The common specification discloses the same working example from P3 which describes transfecting human cells with nucleic acids encoding a *S. pyogenes* Cas9 and a chimera A RNA targeting the human CLTA locus to achieve cleavage of a target DNA molecule. Ex. 2465; Exs. 2005-2007, ¶¶[00562]-[00577], Figs. 29, 31; Ex. 2466, ¶22.

13. The ’859 application shares a common specification with CVC’s involved applications. Ex. 2005; Exs. 2034-2047.

14. The ’504 application shares a common specification with CVC’s involved applications. Ex. 2006; Exs. 2034-2047.

15. The ’604 application shares a common specification with CVC’s involved applications. Ex. 2007; Exs. 2034-2047.

16. The common specification provides multiple different examples of using CRISPR-Cas9 systems in eukaryotic cells. Exs. 2005-2007, ¶¶[00562]-[00577], [00606]-[00657], [00661]; Ex. 2466, ¶¶22-25.

17. Example 2 in the common specification describes using a Type II CRISPR-Cas system to cleave a target DNA in eukaryotic cells. Exs. 2005-2007, ¶¶[00562]-[00577], Figs. 29, 31; Ex. 2466, ¶22.

18. Example 4 in the common specification describes using a Type II CRISPR-Cas system named CRISPRi to repress transcription in eukaryotic cells. Exs. 2005-2007, ¶¶[00606]-[00654]; Ex. 2466, ¶23

19. Example 5 in the common specification describes using a Type II CRISPR-Cas system comprising a Cas9 fusion protein to modulate transcription in eukaryotic cells. Exs. 2005-2007, ¶¶[00655]-[00657]; Ex. 2466, ¶24

20. Example 7 in the common specification describes using a Type II CRISPR-Cas system to generate transgenic eukaryotic organisms. Exs. 2005-2007, ¶[00661]; Ex. 2466, ¶25.

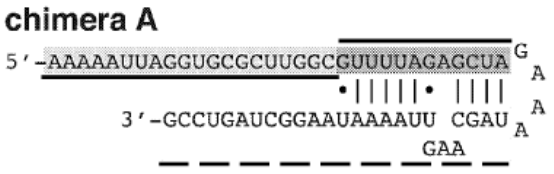
APPENDIX 3 – EXEMPLARY EVIDENCE OF CONSTRUCTIVE REDUCTION TO PRACTICE OF COUNT 1 IN THE '604 APPLICATION

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
[1] 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	"In some of the above applications, **the subject methods may be employed to induce DNA cleavage, DNA modification, and/or transcriptional modulation in mitotic or post-mitotic cells** *in vivo* and/or *ex vivo* and/or *in vitro* (e.g., to produce genetically modified cells that can be reintroduced into an individual). Because the DNA-targeting RNA provide specificity by hybridizing to target DNA, a mitotic and/or post-mitotic **cell of interest in the disclosed methods may include a cell from** any organism (e.g. a bacterial cell, an archaeal cell, **a cell of a single-cell eukaryotic organism, a plant cell, an algal cell** … **a fungal cell (e.g., a yeast cell), an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal, a cell from a rodent, a cell from a human,** etc.). Ex. 2007, ¶[00274]. "Any type of cell may be of interest (e.g. a stem cell, e.g. an embryonic stem (ES) cell, an induced **pluripotent stem (iPS) cell, a germ cell; a somatic cell, e.g. a fibroblast, a hematopoietic cell, a neuron, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell**; an in vitro or in vivo **embryonic cell** of an embryo at any stage, e.g., a 1-cell, 2-cell, 4-cell, 8-cell, etc. stage **zebrafish embryo**; etc.). Cells may be from **established cell lines** or they may be primary cells…." Ex. 2007, ¶[00275]. "In some embodiments a cell comprising a target DNA is *in vitro*. In some embodiments **a cell comprising a target DNA is *in vivo***." Ex. 2007, ¶[00217]. "In other aspects of the invention, the DNA-targeting RNA and/or site-directed modifying polypeptide and/or donor polynucleotide are employed to **modify cellular DNA in vivo**, again for purposes such as **gene therapy**, e.g. to **treat a disease or as an antiviral, antipathogenic, or anticancer therapeutic, for the production of genetically modified organisms in agriculture, or for biological research**." Ex. 2007, ¶[00310].

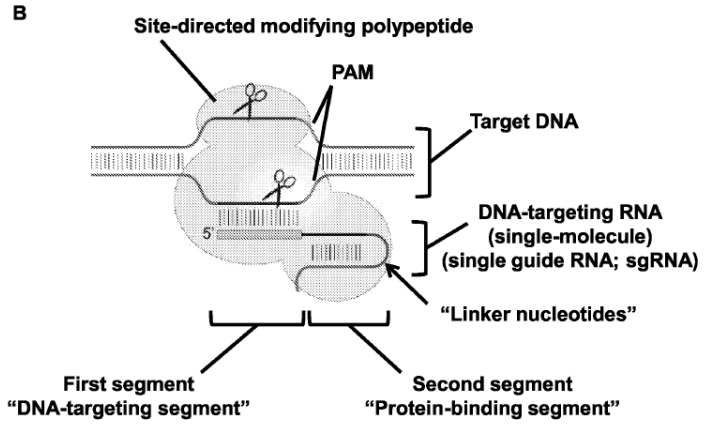
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	"The present disclosure provides **genetically modified host cells,** including isolated genetically modified host cells, where a subject genetically modified host cell comprises (has been genetically modified with: 1) an exogenous **DNA-targeting RNA**; 2) an exogenous nucleic acid comprising a nucleotide sequence encoding a DNA-targeting RNA; 3) an exogenous **site-directed modifying polypeptide** (e.g., a naturally occurring **Cas9**; a modified, i.e., **mutated or variant, Cas9**; a **chimeric Cas9**; etc.); 4) an exogenous nucleic acid comprising a nucleotide sequence encoding a site-directed modifying polypeptide; or 5) **any combination of the above**." Ex. 2007, ¶[000324]. All cells suitable to be a target cell are also suitable to be a genetically modified host cell. For example, **a genetically modified host cells of interest can be a cell from** any organism (e.g. a bacterial cell, an archaeal cell, a cell of a **single-cell eukaryotic organism, a plant cell, an algal cell … a fungal cell, an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal (e.g., a pig, a cow, a goat, a sheep, a rodent, a rat, a mouse, a non-human primate, a human**, etc.), etc." Ex. 2007, ¶[00325]. "In some embodiments, a subject genetically modified host cell is *in vitro*. In some embodiments, **a subject genetically modified host cell is *in vivo*** … In some embodiments, **a subject genetically modified host cell is a eukaryotic cell** or is derived from a eukaryotic cell. In some embodiments, a subject genetically modified host **cell is a plant cell** or is derived from a plant cell. In some embodiments, a subject genetically modified host **cell is an animal cell** or is derived from an animal cell. In some embodiments, a subject genetically modified host **cell is an invertebrate cell** or is derived from an invertebrate cell. In some embodiments, a subject genetically modified host **cell is a vertebrate cell** or is derived from a vertebrate cell. In some embodiments, a subject genetically modified host **cell is a mammalian cell** or is derived from a mammalian cell. In some embodiments, a subject genetically modified host **cell is a rodent cell** or is derived from a rodent cell. In some embodiments, a subject genetically modified host **cell is a**

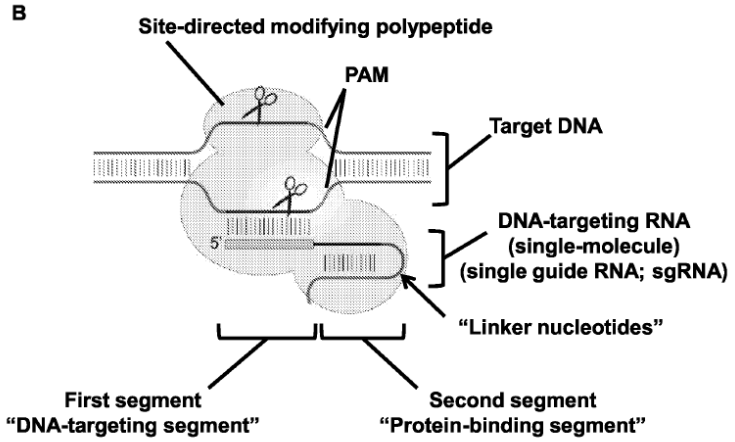
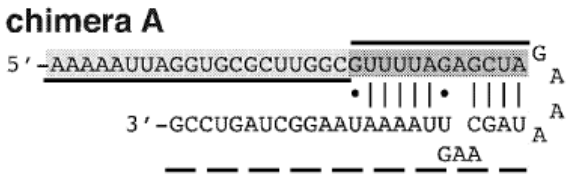
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	human cell or is derived from a human cell." Ex. 2007, ¶[00327]. "The CRISPR loci belong to the **Type II (Nmeni/CASS4) CRISPR/Cas system**." Ex. 2007, ¶[0040]. "Figure 15 depicts the **type II RNA-mediated CRISPR/Cas** immune pathway." Ex. 2007, ¶[00535]. "Data provided below demonstrate that **Cas9 can be expressed and localized to the nucleus of human cells, and that it assembles with single-guide RNA** ('sgRNA'; encompassing the features required for both Cas9 binding and DNA target site recognition) **in a human cell**." Ex. 2007, ¶[00562]. *See also*, Ex. 2007, ¶¶[00123], [00188], [00224]-[00225], [00253], [00283]-[00284], [00287], [00326], [00360]-[00381], [00504]-[00560] (Example 1), [00562]-[00577] (Example 2), Figs. 3, 29, 31, claims 40, 70, 95, 101, 104.
[2] a) a Cas9 protein, or a nucleic acid comprising a nucleotide sequence encoding said Cas9 protein; and	"Figures 3A-B depict the amino acid sequence of a **Cas9/Csn1 protein** from *Streptococcus pyogenes* (SEQ ID NO:8). Cas9 has domains homologous to both HNH and RuvC endonucleases...." Ex. 2007, ¶[0035]. "Figures 4A-B depict the percent identity between the **Cas9/Csn1 proteins** from multiple species. (A) Sequence identity relative to *Streptococcus pyogenes.*" Ex. 2007, ¶[0036]. Exemplary naturally-occurring site-directed modifying polypeptides are set forth in SEQ ID NOs:1-255 as a non-limiting and non-exhaustive list of naturally occurring **Cas9/Csn1 endonucleases**…." Ex. 2007, ¶[00188] "A subject DNA-targeting RNA and/or a **site-directed modifying polypeptide** and/or a chimeric site-directed modifying polypeptide may instead be used to contact DNA or **introduced into cells as RNA**." Ex. 2007, ¶[00287]. "A subject **site-directed modifying polypeptide** may instead be **provided to cells as a polypeptide**." Ex. 2007, ¶[00288].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	"The **site-directed modifying polypeptides** may be prepared by in vitro synthesis, using conventional methods as known in the art." Ex. 2007, ¶[00293]. "The **site-directed modifying polypeptides** may also be isolated and purified in accordance with conventional methods of recombinant synthesis. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique." Ex. 2007, ¶[00295]. "The sequence encoding **Cas9** (residues 1-1368) was PCR amplified from the genomic **DNA of S. pyogenes** SF370 and inserted into a custom pET-based expression vector using ligation-independent cloning (LIC)." Ex. 2007, ¶[00518]. "The **sequence encoding *Streptococcus pyogenes* Cas9** (residues 1-1368) fused to an HA epitope … a nuclear localization signal … was codon optimized for human expression and synthesized by GeneArt." Ex. 2007, ¶[00563]. "Suitable promoters can be used to drive expression by any RNA polymerase (e.g., pol I, pol II, pol III). Exemplary promoters include, but are not limited to the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, a human U6 small nuclear promoter (U6) (Miyagishi et al. , Nature Biotechnology 20, 497 - 500 (2002)), an enhanced U6 promoter (e.g., Xia et al., Nucleic Acids Res. 2003 Sep 1;31(17)), a human HI promoter (HI), and the like" Ex. 2007, ¶[00477]. *See also* Ex. 2007, ¶¶[0037], [00102], [00192], [00242], [00283], [00451], Figs. 3, 4, 5, 29, 31, Example 1 ([00518]), claims 5, 15-17, 19, 39, 42, 48, 73, 103.
[3] b) a single molecule DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding said single molecule DNA-targeting	"In other embodiments, the subject **DNA-targeting RNA is a single RNA molecule** (single RNA polynucleotide) and is referred to herein as a '**single-molecule DNA-targeting RNA**,' a 'single-guide RNA,' or an 'sgRNA.'" Ex. 2007, ¶[00136].

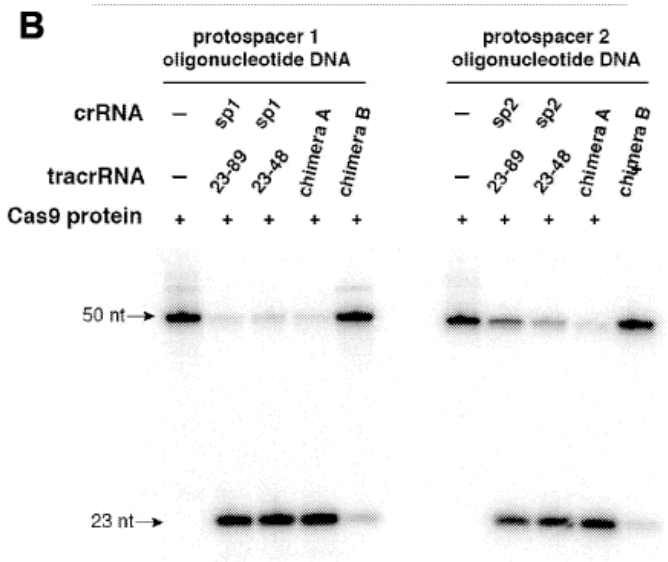
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
RNA; wherein the single molecule DNA-targeting RNA comprises:	"In some embodiments, a **DNA-targeting RNA** and/or a site-directed modifying polypeptide **can be provided as RNA**. In such cases, the DNA-targeting RNA and/or the RNA encoding the site-directed modifying polypeptide can be produced by direct chemical synthesis or may be **transcribed *in vitro*** from a DNA encoding the DNA-targeting RNA. Methods of synthesizing RNA from a DNA template are well known in the art. In some cases, the DNA-targeting RNA and/or the RNA encoding the site-directed modifying polypeptide will be synthesized *in vitro* **using an RNA polymerase enzyme (e.g., T7 polymerase, T3 polymerase, SP6 polymerase, etc.)**. Once synthesized, the RNA may directly contact a target DNA or may be introduced into a cell by any of the well-known techniques for introducing nucleic acids into cells (e.g., microinjection, electroporation, transfection, etc)." Ex. 2007, ¶[00283].  Ex. 2007, Fig. 14A. "We designed two versions of a **chimeric RNA** containing a target recognition sequence at the 5′ end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA (Figure 14A). This single transcript effectively **fuses the 3′ end of crRNA to the 5′ end of tracrRNA**, thereby mimicking the dual-RNA structure required to guide site-specific DNA cleavage by Cas9." Ex. 2007, ¶[00555]. "Suitable promoters can be used to drive expression by any RNA polymerase (e.g., pol I, pol II, pol III). Exemplary promoters include, but are not limited to the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, a human U6 small nuclear promoter (U6) (Miyagishi et al. , Nature Biotechnology 20, 497 - 500 (2002)), an enhanced U6 promoter (e.g., Xia et al., Nucleic Acids Res. 2003 Sep

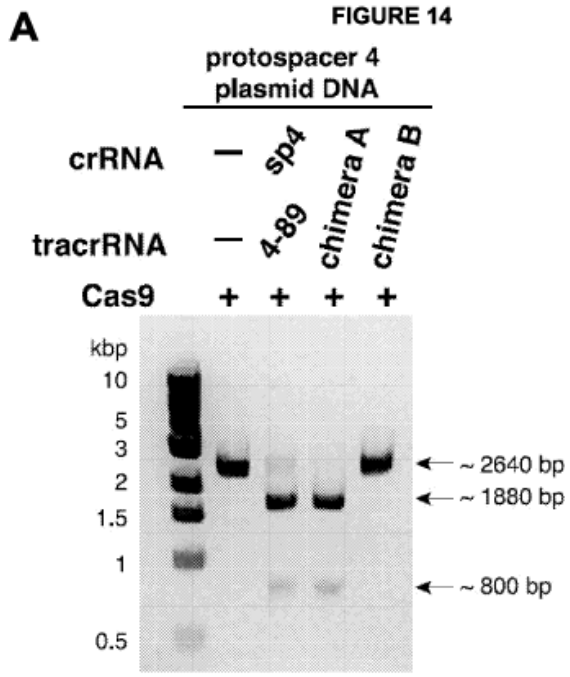
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	1;31(17)), a human HI promoter (HI), and the like" Ex. 2007, ¶[00477]. *See also*, Ex. 2007, ¶¶[0033], [00136], [00177], [00215], [00389], [00391], [00515], [00517], [00555]-[00559], Figs. 1, 9, 14, 27, 28, 29, 31.
[4] i) a targeter-RNA that is capable of hybridizing with a target sequence in the target DNA molecule, and	"A site-directed modifying polypeptide as described herein is targeted to a specific DNA sequence by the RNA molecule to which it is bound. **The RNA molecule comprises a sequence that is complementary to a target sequence within the target DNA**, thus targeting the bound polypeptide to a specific location within the target DNA (the target sequence)." Ex. 2007, ¶[00126]. "A subject DNA-targeting RNA and a subject site-directed modifying polypeptide (i.e., site-directed polypeptide) form a complex (i.e., bind via non-covalent interactions). The **DNA-targeting RNA provides target specificity to the complex by comprising a nucleotide sequence that is complementary to a sequence of a target DNA**. The site-directed modifying polypeptide of the complex provides the site-specific activity." Ex. 2007, ¶[00135]. "A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a **targeter-RNA** and an activator-RNA) that are complementary to one another, are covalently linked by intervening nucleotides ("linkers" or "linker nucleotides"), and hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment, thus resulting in a stem-loop structure (Figure 1B)." Ex. 2007, ¶[00177]. "The DNA-targeting segment of a subject DNA-targeting RNA comprises a **nucleotide sequence that is complementary to a sequence in a target DNA**. In other words, the DNA-targeting segment of a subject DNA-targeting **RNA interacts with a target DNA in a sequence-specific manner via hybridization** (i.e., base pairing)." Ex. 2007, ¶[00165]. "In some embodiments, the DNA-targeting RNA is a single-molecule DNA-targeting RNA and comprises the sequence 5'-GUUUUAGAGCUA-linker-UAGCAAGUUAAAAUAAGGCUAGUCCG-3' **linked at its**

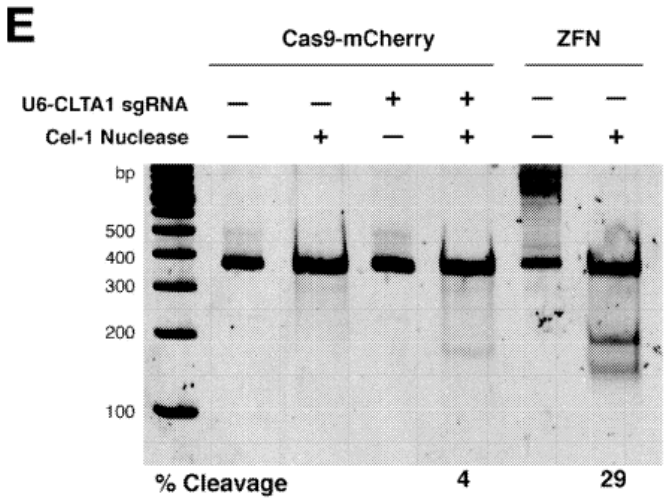
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	5' end to a stretch of nucleotides that are complementary to a target DNA (where "linker" denotes any a linker nucleotide sequence that can comprise any nucleotide sequence) (SEQ ID NO://). Other exemplary single-molecule DNA-targeting RNAs include those set forth in SEQ ID NOs: 680-682." Ex. 2007, ¶[00215].  Ex. 2007, Fig. 1B. See also, Ex. 2007, ¶¶[00131]-[00132], [00136], [00138], [00164]-[00169], [00171]-[00172], [00179]-[00180], [00185], [00255], [00385], [00391], [00393], [00215].
[5] 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,	"A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a targeter-RNA and an **activator-RNA**) that are complementary to one another, are covalently linked by intervening nucleotides ("linkers" or "linker nucleotides"), and **hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment**, thus resulting in a stem-loop structure (Figure 1B)." Ex. 2007, ¶[00177].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	 Ex. 2007, Fig. 1B. "We designed two versions of a **chimeric RNA** containing a target recognition sequence at the 5′ end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA (Figure 14A). This single transcript effectively **fuses the 3′ end of crRNA to the 5′ end of tracrRNA**, thereby mimicking the dual-RNA structure required to guide site-specific DNA cleavage by Cas9." Ex. 2007, ¶[00555].  Ex. 2007, Fig. 14A. *See also*, Ex. 2007, ¶¶[00131]-[00132], [00136], [00138], [00164]-[00169], [00171]-[00172], [00179]-[00180], [00393], [00434], [00435], Figs. 1, 9, 14, 27, 28, 29, 31.
[6] wherein the activator-RNA and the targeter-RNA are covalently linked to one another with intervening nucleotides; and	"A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a targeter-RNA and an activator-RNA) that are complementary to one another, are **covalently linked by intervening nucleotides** ("linkers" or "linker nucleotides"), and hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment, thus resulting in a stem-loop structure (Figure 1B)." Ex. 2007, ¶[00177].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	"In some embodiments, the DNA-targeting RNA is a single-molecule DNA-targeting RNA and comprises the sequence 5'-GUUUUAGAGCUA-linker-UAGCAAGUUAAAAUAAGGCUAGUCCG-3' **linked at its 5' end to a stretch of nucleotides that are complementary to a target DNA** (where "linker" denotes any a linker nucleotide sequence that can comprise any nucleotide sequence) (SEQ ID NO://). Other exemplary single-molecule DNA-targeting RNAs include those set forth in SEQ ID NOs: 680-682." Ex. 2007, ¶[00215]. See also, Ex. 2007, ¶¶[0033], [0041], [0046], [0061], [0063], [00178], [00215], [00556], Figs. 1, 9, 14, 27, 28, 29, 31.
[7] wherein the single molecule DNA-targeting RNA is capable of forming a complex with the Cas9 protein, thereby targeting the Cas9 protein to the target DNA molecule,	"A subject DNA-targeting RNA and a subject site-directed modifying polypeptide (i.e., site-directed polypeptide) **form a complex** (i.e., bind via non-covalent interactions). The DNA-targeting RNA **provides target specificity to the complex** by comprising a nucleotide sequence that is complementary to a sequence of a target DNA." Ex. 2007, ¶[00135]. "Generally, a subject method involves **contacting a target DNA with a complex (a "targeting complex")**, which complex comprises a DNA-targeting RNA and a site-directed modifying polypeptide." Ex. 2007, ¶[00254]. "As discussed above, a subject DNA-targeting RNA and a subject site-directed modifying polypeptide **form a complex**. The DNA-targeting RNA provides target specificity to the complex by comprising a nucleotide sequence that is complementary to a sequence of a target DNA." Ex. 2007, ¶[00255]. "A site-directed polypeptide as described herein **is targeted to a specific DNA sequence** by the RNA molecule to which it is bound. The RNA molecule comprises a sequence that is complementary to a target sequence within the target DNA, thus **targeting the bound polypeptide to a specific location within the target DNA** (the target sequence)." Ex. 2007, ¶[00385]. "To test whether Cas9 could be programmed to cleave genomic DNA in living cells, **Cas9 was co-expressed together with an sgRNA designed to target the human clathrin light chain (CLTA) gene**. The CLTA genomic locus has previously been

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	targeted and edited using ZFNs [10]. We first tested the expression of a human-codon-optimized version of the *Streptococcus pyogenes* Cas9 protein and sgRNA in human HEK293T cells." Ex. 2007, ¶[00297], [00423], [00570]. *See also*, Ex. 2007, ¶¶[00169], [00185], [00255], [00388], [00449], Fig. 1.
[8] whereby said system is capable of cleaving or editing the target DNA molecule or modulating transcription of at least one gene encoded by the target DNA molecule.	"Exemplary naturally-occurring site-directed modifying polypeptides are set forth in SEQ ID NOs:1-255 as a non-limiting and non-exhaustive list of naturally occurring Cas9/Csn1 endonucleases. These **naturally occurring polypeptides, as disclosed herein, bind a DNA-targeting RNA**, are thereby directed to a specific sequence within a target DNA, and cleave the target DNA to generate a double strand break." Ex. 2007, ¶[00188].  Ex. 2007, Fig. 27B. "In some embodiments, a subject complex modifies a target DNA, leading to, for example, **DNA cleavage**, DNA methylation, DNA damage, DNA repair, etc." Ex. 2007, ¶[00255]. "In some of the above applications, the subject methods may be employed **to induce DNA cleavage, DNA modification**, and/or **transcriptional modulation** in mitotic or post-mitotic cells…." Ex. 2007, ¶[00274].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	"As such, a complex comprising a DNA-targeting RNA and a site-directed modifying polypeptide is useful in any *in vitro* or *in vivo* application in which it is desirable to modify DNA in a site-specific, i.e. "targeted", way, for example **gene knock-out, gene knock-in, gene editing**, gene tagging, etc., as used in, for example, gene therapy, e.g. to treat a disease or as an antiviral, antipathogenic, or anticancer therapeutic, the production of genetically modified organisms in agriculture, the large scale production of proteins by cells for therapeutic, diagnostic, or research purposes, the induction of iPS cells, biological research, the targeting of genes of pathogens for deletion or replacement, etc." Ex. 2007, ¶[00261]. "In some cases, the site-directed modifying polypeptide has activity that **modulates the transcription** of target DNA (e.g., in the case of a chimeric site-directed modifying polypeptide, etc.)." Ex. 2007, ¶[00270].  Ex. 2007, Fig. 14A. "Figure 29 demonstrates that co-expression of Cas9 and guide RNA in human cells generates double-strand DNA breaks at the target locus." Ex. 2007, ¶[00571].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '604 Application (Ex. 2007)**
	 Ex. 2007, Fig. 29.E. *See also*, Ex. 2007, ¶¶[0046], [0059], [0061], [00127], [00186], [00189], [00226], [00227], [00231], [00254]-[00268], [00270], [00296], [00298], [00300], [00304], [00342]-[00344], [00352], [00354], [00555]-[00559], [00571]-[00577], [00606]-[00657], [00661], Fig. 14, 27, 29, 31.

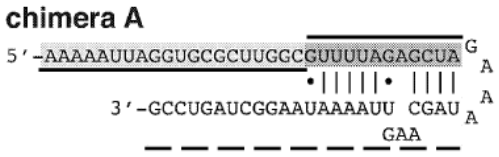
APPENDIX 4 – EXEMPLARY EVIDENCE OF CONSTRUCTIVE REDUCTION TO PRACTICE OF COUNT 1 IN THE '504 APPLICATION

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
[1] 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	"In some of the above applications, **the subject methods may be employed to induce DNA cleavage, DNA modification, and/or transcriptional modulation in mitotic or post-mitotic cells** *in vivo* and/or *ex vivo* and/or *in vitro* (e.g., to produce genetically modified cells that can be reintroduced into an individual). Because the DNA-targeting RNA provide specificity by hybridizing to target DNA, a mitotic and/or post-mitotic **cell of interest in the disclosed methods may include a cell from** any organism (e.g. a bacterial cell, an archaeal cell, **a cell of a single-cell eukaryotic organism, a plant cell, an algal cell** … **a fungal cell (e.g., a yeast cell), an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal, a cell from a rodent, a cell from a human,** etc.). Ex. 2006, ¶[00274]. "Any type of cell may be of interest (e.g. a stem cell, e.g. an embryonic stem (ES) cell, an induced **pluripotent stem (iPS) cell, a germ cell; a somatic cell, e.g. a fibroblast, a hematopoietic cell, a neuron, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell**; an in vitro or in vivo **embryonic cell** of an embryo at any stage, e.g., a 1-cell, 2-cell, 4-cell, 8-cell, etc. stage **zebrafish embryo**; etc.). Cells may be from **established cell lines** or they may be primary cells…." Ex. 2006, ¶[00275]. "In some embodiments a cell comprising a target DNA is *in vitro*. In some embodiments **a cell comprising a target DNA is *in vivo***." Ex. 2006, ¶[00217]. "In other aspects of the invention, the DNA-targeting RNA and/or site-directed modifying polypeptide and/or donor polynucleotide are employed to **modify cellular DNA in vivo**, again for purposes such as **gene therapy**, e.g. to **treat a disease or as an antiviral, antipathogenic, or anticancer therapeutic, for the production of genetically modified organisms in agriculture, or for biological research**." Ex. 2006, ¶[00310].

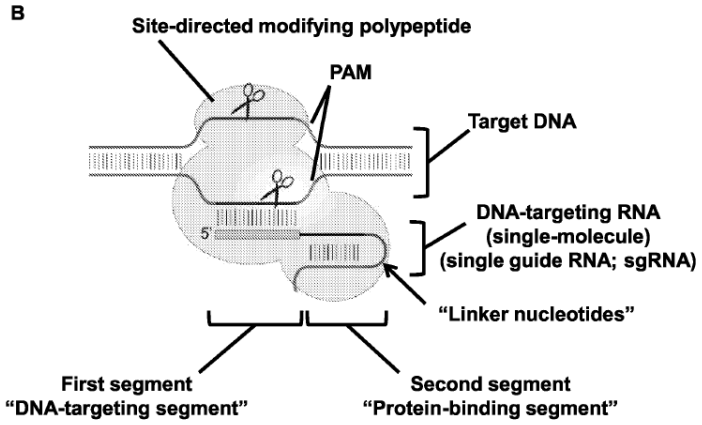
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	"The present disclosure provides **genetically modified host cells,** including isolated genetically modified host cells, where a subject genetically modified host cell comprises (has been genetically modified with: 1) an exogenous **DNA-targeting RNA**; 2) an exogenous nucleic acid comprising a nucleotide sequence encoding a DNA-targeting RNA; 3) an exogenous **site-directed modifying polypeptide** (e.g., a naturally occurring **Cas9**; a modified, i.e., **mutated or variant, Cas9**; a **chimeric Cas9**; etc.); 4) an exogenous nucleic acid comprising a nucleotide sequence encoding a site-directed modifying polypeptide; or 5) **any combination of the above**." Ex. 2006, ¶[000324]. All cells suitable to be a target cell are also suitable to be a genetically modified host cell. For example, **a genetically modified host cells of interest can be a cell from** any organism (e.g. a bacterial cell, an archaeal cell, a cell of a **single-cell eukaryotic organism, a plant cell, an algal cell … a fungal cell, an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal (e.g., a pig, a cow, a goat, a sheep, a rodent, a rat, a mouse, a non-human primate, a human**, etc.), etc." Ex. 2006, ¶[00325]. "In some embodiments, a subject genetically modified host cell is *in vitro*. In some embodiments, **a subject genetically modified host cell is *in vivo*** … In some embodiments, **a subject genetically modified host cell is a eukaryotic cell** or is derived from a eukaryotic cell. In some embodiments, a subject genetically modified host **cell is a plant cell** or is derived from a plant cell. In some embodiments, a subject genetically modified host **cell is an animal cell** or is derived from an animal cell. In some embodiments, a subject genetically modified host **cell is an invertebrate cell** or is derived from an invertebrate cell. In some embodiments, a subject genetically modified host **cell is a vertebrate cell** or is derived from a vertebrate cell. In some embodiments, a subject genetically modified host **cell is a mammalian cell** or is derived from a mammalian cell. In some embodiments, a subject genetically modified host **cell is a rodent cell** or is derived from a rodent cell. In some embodiments, a subject genetically modified host **cell is a**

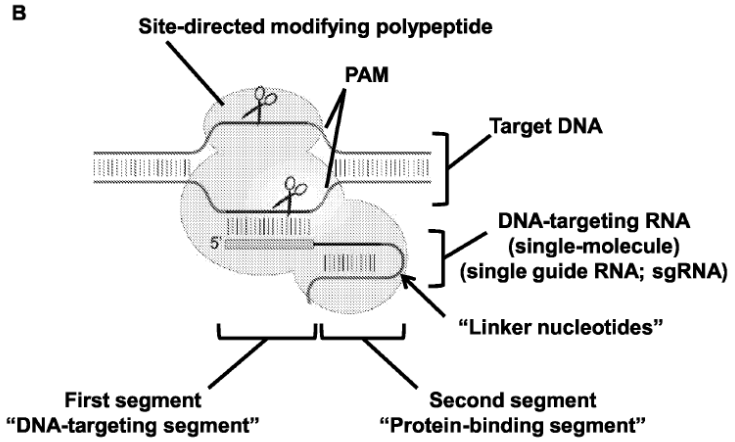
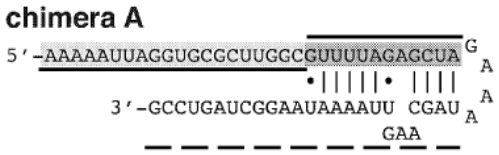
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	human cell or is derived from a human cell." Ex. 2006, ¶[00327]. "The CRISPR loci belong to the **Type II (Nmeni/CASS4) CRISPR/Cas system**." Ex. 2006, ¶[0040]. "Figure 15 depicts the **type II RNA-mediated CRISPR/Cas** immune pathway." Ex. 2006, ¶[00535]. "Data provided below demonstrate that **Cas9 can be expressed and localized to the nucleus of human cells, and that it assembles with single-guide RNA** ('sgRNA'; encompassing the features required for both Cas9 binding and DNA target site recognition) **in a human cell**." Ex. 2006, ¶[00562]. *See also*, Ex. 2006, ¶¶[00123], [00188], [00224]-[00225], [00253], [00283]-[00284], [00287], [00326], [00360]-[00381], [00504]-[00560] (Example 1), [00562]-[00577] (Example 2), Figs. 3, 29, 31, claims 40, 70, 95, 101, 104.
[2] a) a Cas9 protein, or a nucleic acid comprising a nucleotide sequence encoding said Cas9 protein; and	"Figures 3A-B depict the amino acid sequence of a **Cas9/Csn1 protein** from *Streptococcus pyogenes* (SEQ ID NO:8). Cas9 has domains homologous to both HNH and RuvC endonucleases...." Ex. 2006, ¶[0035]. "Figures 4A-B depict the percent identity between the **Cas9/Csn1 proteins** from multiple species. (A) Sequence identity relative to *Streptococcus pyogenes.*" Ex. 2006, ¶[0036]. Exemplary naturally-occurring site-directed modifying polypeptides are set forth in SEQ ID NOs:1-255 as a non-limiting and non-exhaustive list of naturally occurring **Cas9/Csn1 endonucleases**…." Ex. 2006, ¶[00188] "A subject DNA-targeting RNA and/or a **site-directed modifying polypeptide** and/or a chimeric site-directed modifying polypeptide may instead be used to contact DNA or **introduced into cells as RNA**." Ex. 2006, ¶[00287]. "A subject **site-directed modifying polypeptide** may instead be **provided to cells as a polypeptide**." Ex. 2006, ¶[00288].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	"The **site-directed modifying polypeptides** may be prepared by in vitro synthesis, using conventional methods as known in the art." Ex. 2006, ¶[00293]. "The **site-directed modifying polypeptides** may also be isolated and purified in accordance with conventional methods of recombinant synthesis. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique." Ex. 2006, ¶[00295]. "The sequence encoding **Cas9** (residues 1-1368) was PCR amplified from the genomic **DNA of S. pyogenes** SF370 and inserted into a custom pET-based expression vector using ligation-independent cloning (LIC)." Ex. 2006, ¶[00518]. "The **sequence encoding *Streptococcus pyogenes* Cas9** (residues 1-1368) fused to an HA epitope … a nuclear localization signal … was codon optimized for human expression and synthesized by GeneArt." Ex. 2006, ¶[00563]. "Suitable promoters can be used to drive expression by any RNA polymerase (e.g., pol I, pol II, pol III). Exemplary promoters include, but are not limited to the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, a human U6 small nuclear promoter (U6) (Miyagishi et al. , Nature Biotechnology 20, 497 - 500 (2002)), an enhanced U6 promoter (e.g., Xia et al., Nucleic Acids Res. 2003 Sep 1;31(17)), a human HI promoter (HI), and the like" Ex. 2006, ¶[00477]. *See also* Ex. 2006, ¶¶[0037], [00102], [00192], [00242], [00283], [00451], Figs. 3, 4, 5, 29, 31, Example 1 ([00518]), claims 5, 15-17, 19, 39, 42, 48, 73, 103.
[3] b) a single molecule DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding said single molecule DNA-targeting	"In other embodiments, the subject **DNA-targeting RNA is a single RNA molecule** (single RNA polynucleotide) and is referred to herein as a '**single-molecule DNA-targeting RNA**,' a 'single-guide RNA,' or an 'sgRNA.'" Ex. 2006, ¶[00136].

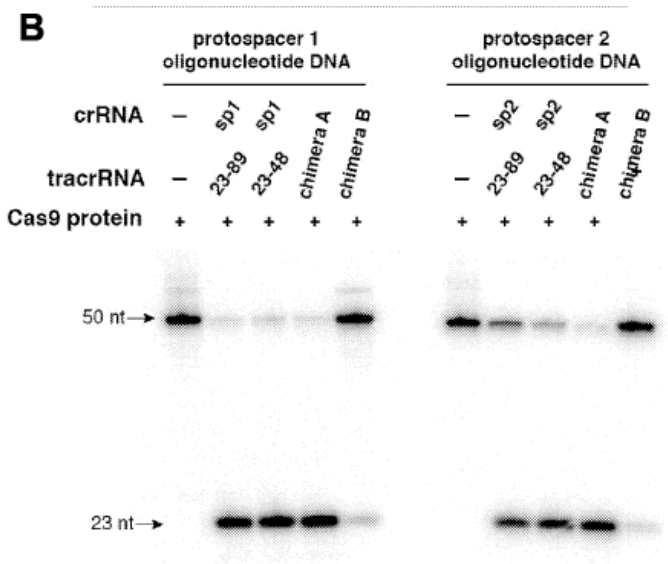
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
RNA; wherein the single molecule DNA-targeting RNA comprises:	"In some embodiments, a **DNA-targeting RNA** and/or a site-directed modifying polypeptide **can be provided as RNA**. In such cases, the DNA-targeting RNA and/or the RNA encoding the site-directed modifying polypeptide can be produced by direct chemical synthesis or may be **transcribed *in vitro*** from a DNA encoding the DNA-targeting RNA. Methods of synthesizing RNA from a DNA template are well known in the art. In some cases, the DNA-targeting RNA and/or the RNA encoding the site-directed modifying polypeptide will be synthesized *in vitro* **using an RNA polymerase enzyme (e.g., T7 polymerase, T3 polymerase, SP6 polymerase, etc.)**. Once synthesized, the RNA may directly contact a target DNA or may be introduced into a cell by any of the well-known techniques for introducing nucleic acids into cells (e.g., microinjection, electroporation, transfection, etc)." Ex. 2006, ¶[00283].  Ex. 2006, Fig. 14A. "We designed two versions of a **chimeric RNA** containing a target recognition sequence at the 5′ end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA (Figure 14A). This single transcript effectively **fuses the 3′ end of crRNA to the 5′ end of tracrRNA**, thereby mimicking the dual-RNA structure required to guide site-specific DNA cleavage by Cas9." Ex. 2006, ¶[00555]. "Suitable promoters can be used to drive expression by any RNA polymerase (e.g., pol I, pol II, pol III). Exemplary promoters include, but are not limited to the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, a human U6 small nuclear promoter (U6) (Miyagishi et al. , Nature Biotechnology 20, 497 - 500 (2002)), an enhanced U6 promoter (e.g., Xia et al., Nucleic Acids Res. 2003 Sep

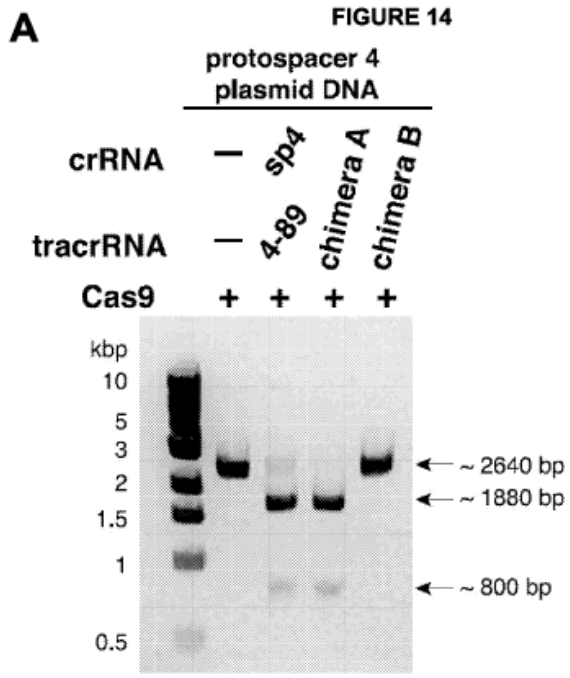
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	1;31(17)), a human HI promoter (HI), and the like" Ex. 2006, ¶[00477]. *See also*, Ex. 2006, ¶¶[0033], [00136], [00177], [00215], [00389], [00391], [00515], [00517], [00555]-[00559], Figs. 1, 9, 14, 27, 28, 29, 31.
[4] i) a targeter-RNA that is capable of hybridizing with a target sequence in the target DNA molecule, and	"A site-directed modifying polypeptide as described herein is targeted to a specific DNA sequence by the RNA molecule to which it is bound. **The RNA molecule comprises a sequence that is complementary to a target sequence within the target DNA**, thus targeting the bound polypeptide to a specific location within the target DNA (the target sequence)." Ex. 2006, ¶[00126]. "A subject DNA-targeting RNA and a subject site-directed modifying polypeptide (i.e., site-directed polypeptide) form a complex (i.e., bind via non-covalent interactions). The **DNA-targeting RNA provides target specificity to the complex by comprising a nucleotide sequence that is complementary to a sequence of a target DNA**. The site-directed modifying polypeptide of the complex provides the site-specific activity." Ex. 2006, ¶[00135]. "A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a **targeter-RNA** and an activator-RNA) that are complementary to one another, are covalently linked by intervening nucleotides ("linkers" or "linker nucleotides"), and hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment, thus resulting in a stem-loop structure (Figure 1B)." Ex. 2006, ¶[00177]. "The DNA-targeting segment of a subject DNA-targeting RNA comprises a **nucleotide sequence that is complementary to a sequence in a target DNA**. In other words, the DNA-targeting segment of a subject DNA-targeting **RNA interacts with a target DNA in a sequence-specific manner via hybridization** (i.e., base pairing)." Ex. 2006, ¶[00165]. "In some embodiments, the DNA-targeting RNA is a single-molecule DNA-targeting RNA and comprises the sequence 5'-GUUUUAGAGCUA-linker-UAGCAAGUUAAAAUAAGGCUAGUCCG-3' **linked at its**

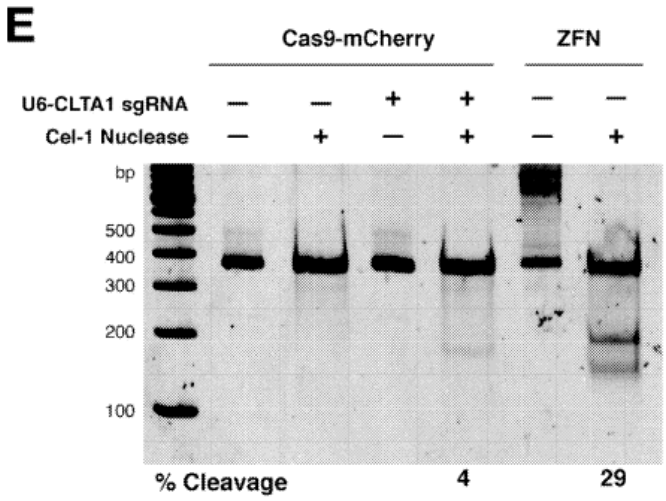
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	5' end to a stretch of nucleotides that are complementary to a target DNA (where "linker" denotes any a linker nucleotide sequence that can comprise any nucleotide sequence) (SEQ ID NO://). Other exemplary single-molecule DNA-targeting RNAs include those set forth in SEQ ID NOs: 680-682." Ex. 2006, ¶[00215].  Ex. 2006, Fig. 1B. See also, Ex. 2006, ¶¶[00131]-[00132], [00136], [00138], [00164]-[00169], [00171]-[00172], [00179]-[00180], [00185], [00255], [00385], [00391], [00393], [00215].
[5] 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,	"A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a targeter-RNA and an **activator-RNA**) that are complementary to one another, are covalently linked by intervening nucleotides ("linkers" or "linker nucleotides"), and **hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment**, thus resulting in a stem-loop structure (Figure 1B)." Ex. 2006, ¶[00177].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	 Ex. 2006, Fig. 1B. "We designed two versions of a **chimeric RNA** containing a target recognition sequence at the 5′ end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA (Figure 14A). This single transcript effectively **fuses the 3′ end of crRNA to the 5′ end of tracrRNA**, thereby mimicking the dual-RNA structure required to guide site-specific DNA cleavage by Cas9." Ex. 2006, ¶[00555].  Ex. 2006, Fig. 14A. See also, Ex. 2006, ¶¶[00131]-[00132], [00136], [00138], [00164]-[00169], [00171]-[00172], [00179]-[00180], [00393], [00434], [00435], Figs. 1, 9, 14, 27, 28, 29, 31.
[6] wherein the activator-RNA and the targeter-RNA are covalently linked to one another with intervening nucleotides; and	"A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a targeter-RNA and an activator-RNA) that are complementary to one another, are **covalently linked by intervening nucleotides** ("linkers" or "linker nucleotides"), and hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment, thus resulting in a stem-loop structure (Figure 1B)." Ex. 2006, ¶[00177].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	"In some embodiments, the DNA-targeting RNA is a single-molecule DNA-targeting RNA and comprises the sequence 5'-GUUUUAGAGCUA-linker-UAGCAAGUUAAAAUAAGGCUAGUCCG-3' **linked at its 5' end to a stretch of nucleotides that are complementary to a target DNA** (where "linker" denotes any a linker nucleotide sequence that can comprise any nucleotide sequence) (SEQ ID NO://). Other exemplary single-molecule DNA-targeting RNAs include those set forth in SEQ ID NOs: 680-682." Ex. 2006, ¶[00215]. *See also*, Ex. 2006, ¶¶[0033], [0041], [0046], [0061], [0063], [00178], [00215], [00556], Figs. 1, 9, 14, 27, 28, 29, 31.
[7] wherein the single molecule DNA-targeting RNA is capable of forming a complex with the Cas9 protein, thereby targeting the Cas9 protein to the target DNA molecule,	"A subject DNA-targeting RNA and a subject site-directed modifying polypeptide (i.e., site-directed polypeptide) **form a complex** (i.e., bind via non-covalent interactions). The DNA-targeting RNA **provides target specificity to the complex** by comprising a nucleotide sequence that is complementary to a sequence of a target DNA." Ex. 2006, ¶[00135]. "Generally, a subject method involves **contacting a target DNA with a complex (a "targeting complex")**, which complex comprises a DNA-targeting RNA and a site-directed modifying polypeptide." Ex. 2006, ¶[00254]. "As discussed above, a subject DNA-targeting RNA and a subject site-directed modifying polypeptide **form a complex**. The DNA-targeting RNA provides target specificity to the complex by comprising a nucleotide sequence that is complementary to a sequence of a target DNA." Ex. 2006, ¶[00255]. "A site-directed polypeptide as described herein **is targeted to a specific DNA sequence** by the RNA molecule to which it is bound. The RNA molecule comprises a sequence that is complementary to a target sequence within the target DNA, thus **targeting the bound polypeptide to a specific location within the target DNA** (the target sequence)." Ex. 2006, ¶[00385]. "To test whether Cas9 could be programmed to cleave genomic DNA in living cells, **Cas9 was co-expressed together with an**

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	sgRNA designed to target the human clathrin light chain (CLTA) gene. The CLTA genomic locus has previously been targeted and edited using ZFNs [10]. We first tested the expression of a human-codon-optimized version of the *Streptococcus pyogenes* Cas9 protein and sgRNA in human HEK293T cells." Ex. 2006, ¶[00297], [00423], [00570]. *See also*, Ex. 2006, ¶¶[00169], [00185], [00255], [00388], [00449], Fig. 1.
[8] whereby said system is capable of cleaving or editing the target DNA molecule or modulating transcription of at least one gene encoded by the target DNA molecule.	"Exemplary naturally-occurring site-directed modifying polypeptides are set forth in SEQ ID NOs:1-255 as a non-limiting and non-exhaustive list of naturally occurring Cas9/Csn1 endonucleases. These **naturally occurring polypeptides, as disclosed herein, bind a DNA-targeting RNA**, are thereby directed to a specific sequence within a target DNA, and cleave the target DNA to generate a double strand break." Ex. 2006, ¶[00188].  Ex. 2006, Fig. 27B. "In some embodiments, a subject complex modifies a target DNA, leading to, for example, **DNA cleavage**, DNA methylation, DNA damage, DNA repair, etc." Ex. 2006, ¶[00255].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	"In some of the above applications, the subject methods may be employed **to induce DNA cleavage, DNA modification**, and/or **transcriptional modulation** in mitotic or post-mitotic cells…." Ex. 2006, ¶[00274]. "As such, a complex comprising a DNA-targeting RNA and a site-directed modifying polypeptide is useful in any *in vitro* or *in vivo* application in which it is desirable to modify DNA in a site-specific, i.e. "targeted", way, for example **gene knock-out, gene knock-in, gene editing**, gene tagging, etc., as used in, for example, gene therapy, e.g. to treat a disease or as an antiviral, antipathogenic, or anticancer therapeutic, the production of genetically modified organisms in agriculture, the large scale production of proteins by cells for therapeutic, diagnostic, or research purposes, the induction of iPS cells, biological research, the targeting of genes of pathogens for deletion or replacement, etc." Ex. 2006, ¶[00261]. "In some cases, the site-directed modifying polypeptide has activity that **modulates the transcription** of target DNA (e.g., in the case of a chimeric site-directed modifying polypeptide, etc.)." Ex. 2006, ¶[00270].  Ex. 2006, Fig. 14A.

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '504 Application (Ex. 2006)**
	"Figure 29 demonstrates that co-expression of Cas9 and guide RNA in human cells generates double-strand DNA breaks at the target locus." Ex. 2006, ¶[00571].  Ex. 2006, Fig. 29.E. *See also*, Ex. 2006, ¶¶[0046], [0059], [0061], [00127], [00186], [00189], [00226], [00227], [00231], [00254]-[00268], [00270], [00296], [00298], [00300], [00304], [00342]-[00344], [00352], [00354], [00555]-[00559], [00571]-[00577], [00606]-[00657], [00661], Fig. 14, 27, 29, 31.

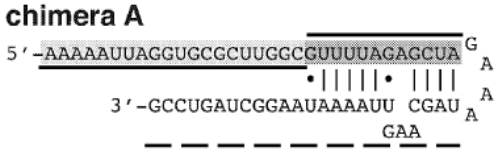
APPENDIX 5 – EXEMPLARY EVIDENCE OF CONSTRUCTIVE REDUCTION TO PRACTICE OF COUNT 1 IN THE '859 APPLICATION

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
[1] 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	"In some of the above applications, **the subject methods may be employed to induce DNA cleavage, DNA modification, and/or transcriptional modulation in mitotic or post-mitotic cells** *in vivo* and/or *ex vivo* and/or *in vitro* (e.g., to produce genetically modified cells that can be reintroduced into an individual). Because the DNA-targeting RNA provide specificity by hybridizing to target DNA, a mitotic and/or post-mitotic **cell of interest in the disclosed methods may include a cell from** any organism (e.g. a bacterial cell, an archaeal cell, **a cell of a single-cell eukaryotic organism, a plant cell, an algal cell** … **a fungal cell (e.g., a yeast cell), an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal, a cell from a rodent, a cell from a human,** etc.). Ex. 2005, ¶[00274]. "Any type of cell may be of interest (e.g. a stem cell, e.g. an embryonic stem (ES) cell, an induced **pluripotent stem (iPS) cell, a germ cell; a somatic cell, e.g. a fibroblast, a hematopoietic cell, a neuron, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell**; an in vitro or in vivo **embryonic cell** of an embryo at any stage, e.g., a 1-cell, 2-cell, 4-cell, 8-cell, etc. stage **zebrafish embryo**; etc.). Cells may be from **established cell lines** or they may be primary cells…." Ex. 2005, ¶[00275]. "In some embodiments a cell comprising a target DNA is *in vitro*. In some embodiments **a cell comprising a target DNA is *in vivo***." Ex. 2005, ¶[00217]. "In other aspects of the invention, the DNA-targeting RNA and/or site-directed modifying polypeptide and/or donor polynucleotide are employed to **modify cellular DNA in vivo**, again for purposes such as **gene therapy**, e.g. to **treat a disease or as an antiviral, antipathogenic, or anticancer therapeutic, for the production of genetically modified organisms in agriculture, or for biological research**." Ex. 2005, ¶[00310].

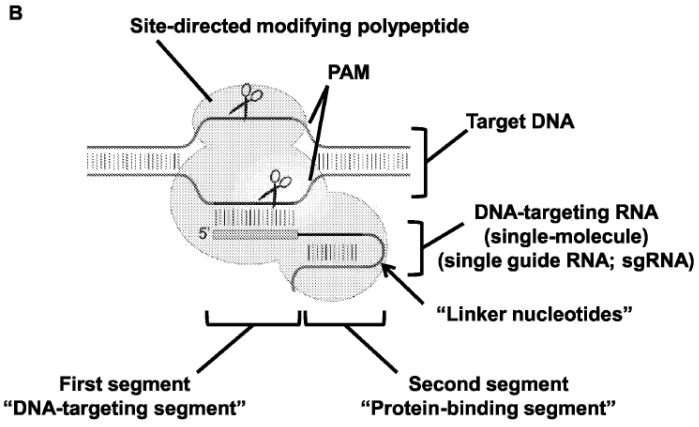
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	"The present disclosure provides **genetically modified host cells,** including isolated genetically modified host cells, where a subject genetically modified host cell comprises (has been genetically modified with: 1) an exogenous **DNA-targeting RNA**; 2) an exogenous nucleic acid comprising a nucleotide sequence encoding a DNA-targeting RNA; 3) an exogenous **site-directed modifying polypeptide** (e.g., a naturally occurring **Cas9**; a modified, i.e., **mutated or variant, Cas9**; a **chimeric Cas9**; etc.); 4) an exogenous nucleic acid comprising a nucleotide sequence encoding a site-directed modifying polypeptide; or 5) **any combination of the above**." Ex. 2005, ¶[000324]. All cells suitable to be a target cell are also suitable to be a genetically modified host cell. For example, **a genetically modified host cells of interest can be a cell from** any organism (e.g. a bacterial cell, an archaeal cell, a cell of a **single-cell eukaryotic organism, a plant cell, an algal cell … a fungal cell, an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal (e.g., a pig, a cow, a goat, a sheep, a rodent, a rat, a mouse, a non-human primate, a human**, etc.), etc." Ex. 2005, ¶[00325]. "In some embodiments, a subject genetically modified host cell is *in vitro*. In some embodiments, **a subject genetically modified host cell is *in vivo*** … In some embodiments, **a subject genetically modified host cell is a eukaryotic cell** or is derived from a eukaryotic cell. In some embodiments, a subject genetically modified host **cell is a plant cell** or is derived from a plant cell. In some embodiments, a subject genetically modified host **cell is an animal cell** or is derived from an animal cell. In some embodiments, a subject genetically modified host **cell is an invertebrate cell** or is derived from an invertebrate cell. In some embodiments, a subject genetically modified host **cell is a vertebrate cell** or is derived from a vertebrate cell. In some embodiments, a subject genetically modified host **cell is a mammalian cell** or is derived from a mammalian cell. In some embodiments, a subject genetically modified host **cell is a rodent cell** or is derived from a rodent cell. In some embodiments, a subject genetically modified host **cell is a**

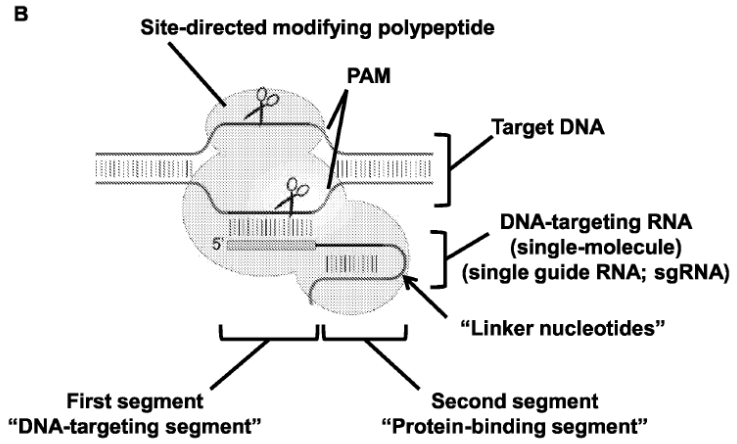
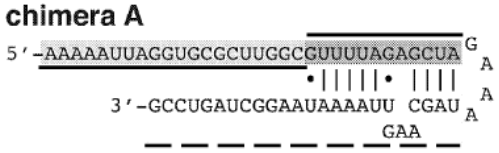
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	human cell or is derived from a human cell." Ex. 2005, ¶[00327]. "The CRISPR loci belong to the **Type II (Nmeni/CASS4) CRISPR/Cas system**." Ex. 2005, ¶[0040]. "Figure 15 depicts the **type II RNA-mediated CRISPR/Cas** immune pathway." Ex. 2005, ¶[00535]. "Data provided below demonstrate that **Cas9 can be expressed and localized to the nucleus of human cells, and that it assembles with single-guide RNA** ('sgRNA'; encompassing the features required for both Cas9 binding and DNA target site recognition) **in a human cell**." Ex. 2005, ¶[00562]. *See also*, Ex. 2005, ¶¶[00123], [00188], [00224]-[00225], [00253], [00283]-[00284], [00287], [00326], [00360]-[00381], [00504]-[00560] (Example 1), [00562]-[00577] (Example 2), Figs. 3, 29, 31, claims 40, 70, 95, 101, 104.
[2] a) a Cas9 protein, or a nucleic acid comprising a nucleotide sequence encoding said Cas9 protein; and	"Figures 3A-B depict the amino acid sequence of a **Cas9/Csn1 protein** from *Streptococcus pyogenes* (SEQ ID NO:8). Cas9 has domains homologous to both HNH and RuvC endonucleases...." Ex. 2005, ¶[0035]. "Figures 4A-B depict the percent identity between the **Cas9/Csn1 proteins** from multiple species. (A) Sequence identity relative to *Streptococcus pyogenes.*" Ex. 2005, ¶[0036]. Exemplary naturally-occurring site-directed modifying polypeptides are set forth in SEQ ID NOs:1-255 as a non-limiting and non-exhaustive list of naturally occurring **Cas9/Csn1 endonucleases**…." Ex. 2005, ¶[00188] "A subject DNA-targeting RNA and/or a **site-directed modifying polypeptide** and/or a chimeric site-directed modifying polypeptide may instead be used to contact DNA or **introduced into cells as RNA**." Ex. 2005, ¶[00287]. "A subject **site-directed modifying polypeptide** may instead be **provided to cells as a polypeptide**." Ex. 2005, ¶[00288].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	"The **site-directed modifying polypeptides** may be prepared by in vitro synthesis, using conventional methods as known in the art." Ex. 2005, ¶[00293]. "The **site-directed modifying polypeptides** may also be isolated and purified in accordance with conventional methods of recombinant synthesis. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique." Ex. 2005, ¶[00295]. "The sequence encoding **Cas9** (residues 1-1368) was PCR amplified from the genomic **DNA of S. pyogenes** SF370 and inserted into a custom pET-based expression vector using ligation-independent cloning (LIC)." Ex. 2005, ¶[00518]. "The **sequence encoding Streptococcus pyogenes Cas9** (residues 1-1368) fused to an HA epitope … a nuclear localization signal … was codon optimized for human expression and synthesized by GeneArt." Ex. 2005, ¶[00563]. "Suitable promoters can be used to drive expression by any RNA polymerase (e.g., pol I, pol II, pol III). Exemplary promoters include, but are not limited to the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, a human U6 small nuclear promoter (U6) (Miyagishi et al. , Nature Biotechnology 20, 497 - 500 (2002)), an enhanced U6 promoter (e.g., Xia et al., Nucleic Acids Res. 2003 Sep 1;31(17)), a human HI promoter (HI), and the like" Ex. 2005, ¶[00477]. *See also* Ex. 2005, ¶¶[0037], [00102], [00192], [00242], [00283], [00451], Figs. 3, 4, 5, 29, 31, Example 1 ([00518]), claims 5, 15-17, 19, 39, 42, 48, 73, 103.
[3] b) a single molecule DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding said single molecule DNA-targeting	"In other embodiments, the subject **DNA-targeting RNA is a single RNA molecule** (single RNA polynucleotide) and is referred to herein as a '**single-molecule DNA-targeting RNA**,' a 'single-guide RNA,' or an 'sgRNA.'" Ex. 2005, ¶[00136].

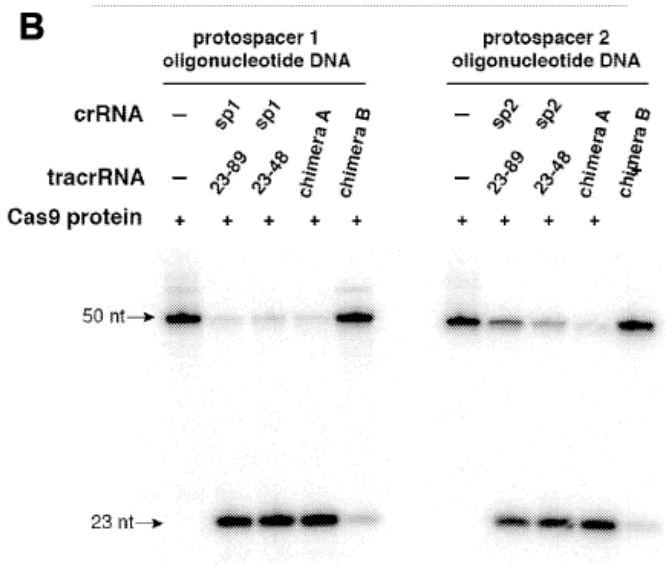
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
RNA; wherein the single molecule DNA-targeting RNA comprises:	"In some embodiments, a **DNA-targeting RNA** and/or a site-directed modifying polypeptide **can be provided as RNA**. In such cases, the DNA-targeting RNA and/or the RNA encoding the site-directed modifying polypeptide can be produced by direct chemical synthesis or may be **transcribed *in vitro*** from a DNA encoding the DNA-targeting RNA. Methods of synthesizing RNA from a DNA template are well known in the art. In some cases, the DNA-targeting RNA and/or the RNA encoding the site-directed modifying polypeptide will be synthesized *in vitro* **using an RNA polymerase enzyme (e.g., T7 polymerase, T3 polymerase, SP6 polymerase, etc.)**. Once synthesized, the RNA may directly contact a target DNA or may be introduced into a cell by any of the well-known techniques for introducing nucleic acids into cells (e.g., microinjection, electroporation, transfection, etc)." Ex. 2005, ¶[00283].  Ex. 2005, Fig. 14A. "We designed two versions of a **chimeric RNA** containing a target recognition sequence at the 5′ end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA (Figure 14A). This single transcript effectively **fuses the 3′ end of crRNA to the 5′ end of tracrRNA**, thereby mimicking the dual-RNA structure required to guide site-specific DNA cleavage by Cas9." Ex. 2005, ¶[00555]. "Suitable promoters can be used to drive expression by any RNA polymerase (e.g., pol I, pol II, pol III). Exemplary promoters include, but are not limited to the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, a human U6 small nuclear promoter (U6) (Miyagishi et al. , Nature Biotechnology 20, 497 - 500 (2002)), an enhanced U6 promoter (e.g., Xia et al., Nucleic Acids Res. 2003 Sep

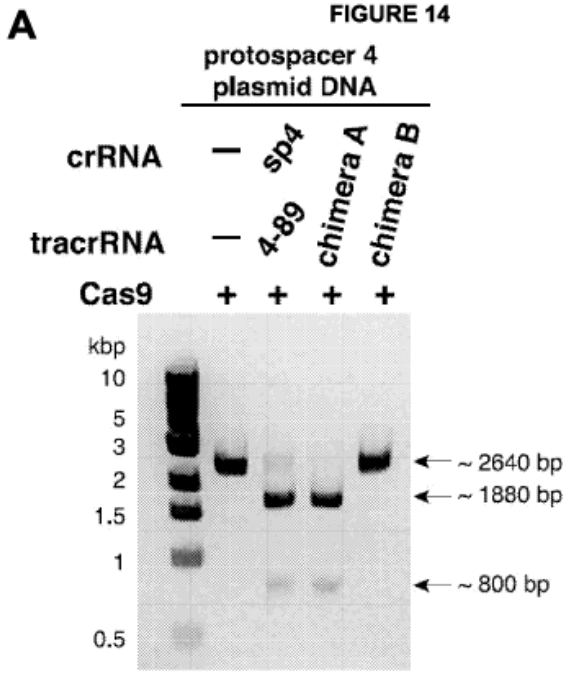
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	1;31(17)), a human HI promoter (HI), and the like" Ex. 2005, ¶[00477]. *See also*, Ex. 2005, ¶¶[0033], [00136], [00177], [00215], [00389], [00391], [00515], [00517], [00555]-[00559], Figs. 1, 9, 14, 27, 28, 29, 31.
[4] i) a targeter-RNA that is capable of hybridizing with a target sequence in the target DNA molecule, and	"A site-directed modifying polypeptide as described herein is targeted to a specific DNA sequence by the RNA molecule to which it is bound. **The RNA molecule comprises a sequence that is complementary to a target sequence within the target DNA**, thus targeting the bound polypeptide to a specific location within the target DNA (the target sequence)." Ex. 2005, ¶[00126]. "A subject DNA-targeting RNA and a subject site-directed modifying polypeptide (i.e., site-directed polypeptide) form a complex (i.e., bind via non-covalent interactions). The **DNA-targeting RNA provides target specificity to the complex by comprising a nucleotide sequence that is complementary to a sequence of a target DNA**. The site-directed modifying polypeptide of the complex provides the site-specific activity." Ex. 2005, ¶[00135]. "A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a **targeter-RNA** and an activator-RNA) that are complementary to one another, are covalently linked by intervening nucleotides ("linkers" or "linker nucleotides"), and hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment, thus resulting in a stem-loop structure (Figure 1B)." Ex. 2005, ¶[00177]. "The DNA-targeting segment of a subject DNA-targeting RNA comprises a **nucleotide sequence that is complementary to a sequence in a target DNA**. In other words, the DNA-targeting segment of a subject DNA-targeting **RNA interacts with a target DNA in a sequence-specific manner via hybridization** (i.e., base pairing)." Ex. 2005, ¶[00165]. "In some embodiments, the DNA-targeting RNA is a single-molecule DNA-targeting RNA and comprises the sequence 5'-GUUUUAGAGCUA-linker-UAGCAAGUUAAAAUAAGGCUAGUCCG-3' **linked at its**

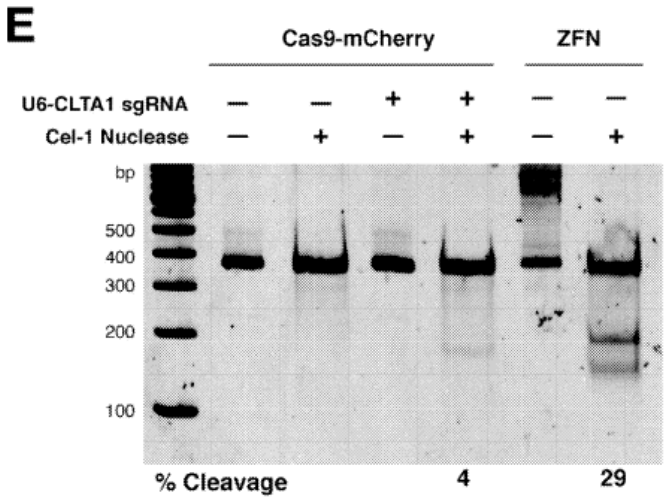
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	5' end to a stretch of nucleotides that are complementary to a target DNA (where "linker" denotes any a linker nucleotide sequence that can comprise any nucleotide sequence) (SEQ ID NO://). Other exemplary single-molecule DNA-targeting RNAs include those set forth in SEQ ID NOs: 680-682." Ex. 2005, ¶[00215].  Ex. 2005, Fig. 1B. *See also*, Ex. 2005, ¶¶[00131]-[00132], [00136], [00138], [00164]-[00169], [00171]-[00172], [00179]-[00180], [00185], [00255], [00385], [00391], [00393], [00215].
[5] 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,	"A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a targeter-RNA and an **activator-RNA**) that are complementary to one another, are covalently linked by intervening nucleotides ("linkers" or "linker nucleotides"), and **hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment**, thus resulting in a stem-loop structure (Figure 1B)." Ex. 2005, ¶[00177].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	 Ex. 2005, Fig. 1B. "We designed two versions of a **chimeric RNA** containing a target recognition sequence at the 5′ end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA (Figure 14A). This single transcript effectively **fuses the 3′ end of crRNA to the 5′ end of tracrRNA**, thereby mimicking the dual-RNA structure required to guide site-specific DNA cleavage by Cas9." Ex. 2005, ¶[00555].  Ex. 2005, Fig. 14A. *See also*, Ex. 2005, ¶¶[00131]-[00132], [00136], [00138], [00164]-[00169], [00171]-[00172], [00179]-[00180], [00393], [00434], [00435], Figs. 1, 9, 14, 27, 28, 29, 31.
[6] wherein the activator-RNA and the targeter-RNA are covalently linked to one another with intervening nucleotides; and	"A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a targeter-RNA and an activator-RNA) that are complementary to one another, are **covalently linked by intervening nucleotides** ("linkers" or "linker nucleotides"), and hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment, thus resulting in a stem-loop structure (Figure 1B)." Ex. 2005, ¶[00177].

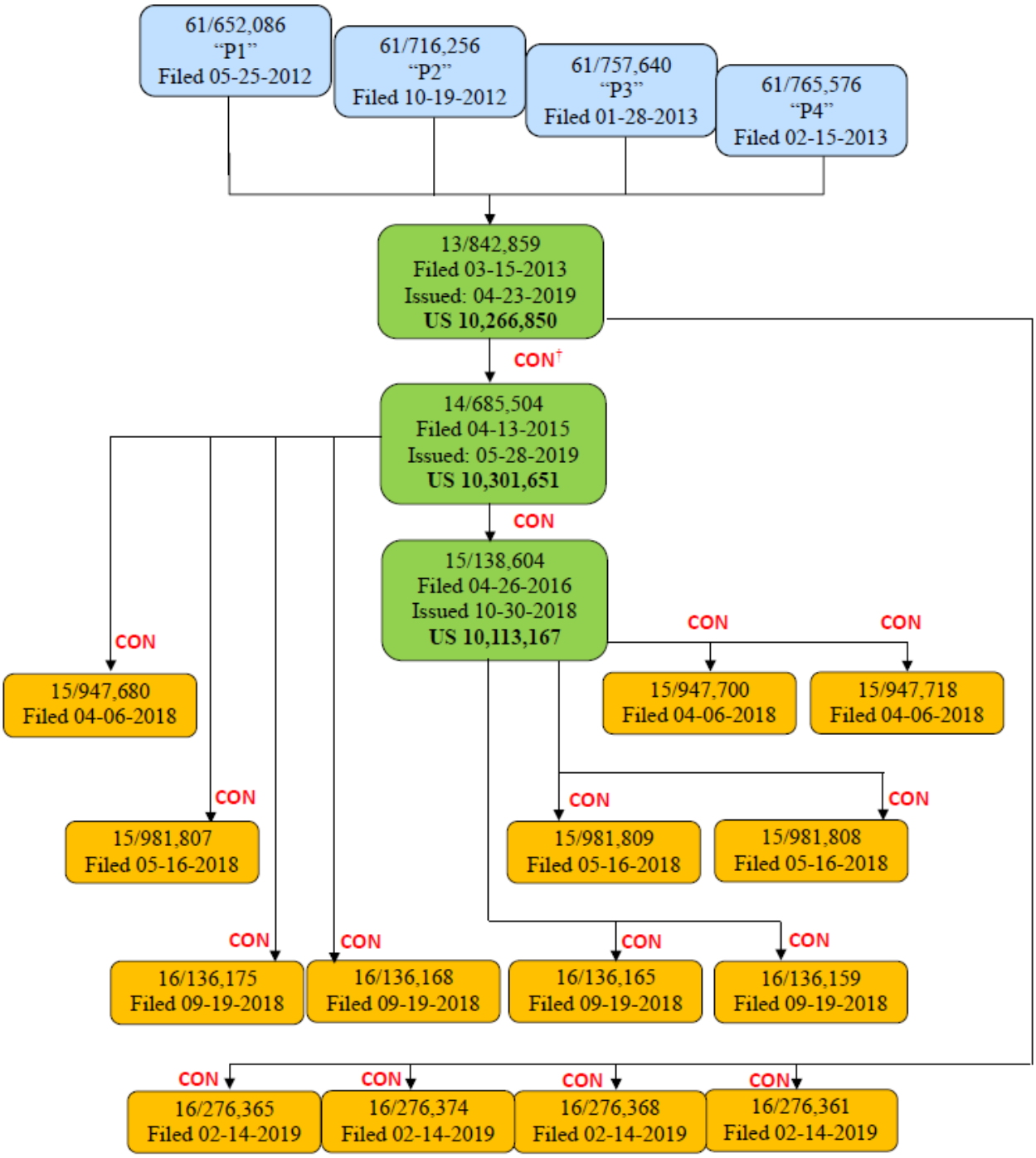
Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	"In some embodiments, the DNA-targeting RNA is a single-molecule DNA-targeting RNA and comprises the sequence 5'-GUUUUAGAGCUA-linker-UAGCAAGUUAAAAUAAGGCUAGUCCG-3' **linked at its 5' end to a stretch of nucleotides that are complementary to a target DNA** (where "linker" denotes any a linker nucleotide sequence that can comprise any nucleotide sequence) (SEQ ID NO://). Other exemplary single-molecule DNA-targeting RNAs include those set forth in SEQ ID NOs: 680-682." Ex. 2005, ¶[00215]. *See also*, Ex. 2005, ¶¶[0033], [0041], [0046], [0061], [0063], [00178], [00215], [00556], Figs. 1, 9, 14, 27, 28, 29, 31.
[7] wherein the single molecule DNA-targeting RNA is capable of forming a complex with the Cas9 protein, thereby targeting the Cas9 protein to the target DNA molecule,	"A subject DNA-targeting RNA and a subject site-directed modifying polypeptide (i.e., site-directed polypeptide) **form a complex** (i.e., bind via non-covalent interactions). The DNA-targeting RNA **provides target specificity to the complex** by comprising a nucleotide sequence that is complementary to a sequence of a target DNA." Ex. 2005, ¶[00135]. "Generally, a subject method involves **contacting a target DNA with a complex (a "targeting complex")**, which complex comprises a DNA-targeting RNA and a site-directed modifying polypeptide." Ex. 2005, ¶[00254]. "As discussed above, a subject DNA-targeting RNA and a subject site-directed modifying polypeptide **form a complex**. The DNA-targeting RNA provides target specificity to the complex by comprising a nucleotide sequence that is complementary to a sequence of a target DNA." Ex. 2005, ¶[00255]. "A site-directed polypeptide as described herein **is targeted to a specific DNA sequence** by the RNA molecule to which it is bound. The RNA molecule comprises a sequence that is complementary to a target sequence within the target DNA, thus **targeting the bound polypeptide to a specific location within the target DNA** (the target sequence)." Ex. 2005, ¶[00385]. "To test whether Cas9 could be programmed to cleave genomic DNA in living cells, **Cas9 was co-expressed together with an**

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	sgRNA designed to target the human clathrin light chain (CLTA) gene. The CLTA genomic locus has previously been targeted and edited using ZFNs [10]. We first tested the expression of a human-codon-optimized version of the *Streptococcus pyogenes* Cas9 protein and sgRNA in human HEK293T cells." Ex. 2005, ¶[00297], [00423], [00570]. *See also*, Ex. 2005, ¶¶[00169], [00185], [00255], [00388], [00449], Fig. 1.
[8] whereby said system is capable of cleaving or editing the target DNA molecule or modulating transcription of at least one gene encoded by the target DNA molecule.	"Exemplary naturally-occurring site-directed modifying polypeptides are set forth in SEQ ID NOs:1-255 as a non-limiting and non-exhaustive list of naturally occurring Cas9/Csn1 endonucleases. These **naturally occurring polypeptides, as disclosed herein, bind a DNA-targeting RNA**, are thereby directed to a specific sequence within a target DNA, and cleave the target DNA to generate a double strand break." Ex. 2005, ¶[00188].  Ex. 2005, Fig. 27B. "In some embodiments, a subject complex modifies a target DNA, leading to, for example, **DNA cleavage**, DNA methylation, DNA damage, DNA repair, etc." Ex. 2005, ¶[00255].

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	"In some of the above applications, the subject methods may be employed **to induce DNA cleavage, DNA modification**, and/or **transcriptional modulation** in mitotic or post-mitotic cells…." Ex. 2005, ¶[00274]. "As such, a complex comprising a DNA-targeting RNA and a site-directed modifying polypeptide is useful in any *in vitro* or *in vivo* application in which it is desirable to modify DNA in a site-specific, i.e. "targeted", way, for example **gene knock-out, gene knock-in, gene editing**, gene tagging, etc., as used in, for example, gene therapy, e.g. to treat a disease or as an antiviral, antipathogenic, or anticancer therapeutic, the production of genetically modified organisms in agriculture, the large scale production of proteins by cells for therapeutic, diagnostic, or research purposes, the induction of iPS cells, biological research, the targeting of genes of pathogens for deletion or replacement, etc." Ex. 2005, ¶[00261]. "In some cases, the site-directed modifying polypeptide has activity that **modulates the transcription** of target DNA (e.g., in the case of a chimeric site-directed modifying polypeptide, etc.)." Ex. 2005, ¶[00270].  Ex. 2005, Fig. 14A.

Elements of Count 1 *(claim 156 in Appl. No. 15/981,807)*	**Exemplary evidence of constructive reduction to practice in the '859 Application (Ex. 2005)**
	"Figure 29 demonstrates that co-expression of Cas9 and guide RNA in human cells generates double-strand DNA breaks at the target locus." Ex. 2005, ¶[00571].  Ex. 2005, Fig. 29.E. *See also*, Ex. 2005, ¶¶[0046], [0059], [0061], [00127], [00186], [00189], [00226], [00227], [00231], [00254]-[00268], [00270], [00296], [00298], [00300], [00304], [00342]-[00344], [00352], [00354], [00555]-[00559], [00571]-[00577], [00606]-[00657], [00661], Fig. 14, 27, 29, 31.

APPENDIX 6: CVC INVOLVED APPLICATION ABBREVIATED FAMILY TREE



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[1] *Involved applications shown in orange.*
† *CON, continuation application.*

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **CVC RESPONSIVE MOTION 2 (for accorded benefit)** and the related exhibits were filed via the Interference Web Portal by 8:00 PM Eastern Time on June 11, 2021, pursuant to an agreement between the parties, and thereby served on the attorney of record for the Senior Party pursuant to ¶ 105.3 of the Standing Order. Pursuant to the agreement between the parties, the foregoing was also served via email by 11:00 PM Eastern Time on counsel for the Senior Party at:

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