IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Jennifer Doudna et al.

Application Serial No.: 13/842,859

Filed: March 15, 2013

For: METHODS AND COMPOSITIONS FOR RNA-DIRECTED TARGET DNA MODIFICATION AND FOR RNA-DIRECTED MODULATION OF TRANSCRIPTION

MAIL STOP AMENDMENT

Group Art Unit: 1636

Examiner: Michele K. JOIKE

Confirmation Number: 8182

MAIL STOP AMENDMENT

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

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

Madam:

Applicants respectfully request that an interference be declared involving Claims 165-247 of U.S. Patent Application No. 13/842,859 (“the ‘859 Application”), which are concurrently filed herewith in an Amendment and Reply.

Pursuant to 37 C.F.R. § 41.202, Applicants provide, inter alia: (1) an identification of the patents with which Applicants seek an interference; (2) proposed counts and claim correspondence thereto; (3) an identification of claims that Applicants believe interfere; (4) an explanation of why Applicants will prevail on priority; and (5) claim charts showing written description in Applicants’ specification for each claim added to provoke the interference.
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APPENDIX I: EXAMPLES OF SUPPORT FOR PENDING CLAIMS -- U.S. PROVISIONAL APPLICATION NO. 61/716,256

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TABLE 3 - COMPARISON OF CLAIMS OF U.S. PATENT NO. 8,795,965 (EXHIBIT 3) AND U.S. PATENT APPLICATION NO. 13/842,859

TABLE 4 - COMPARISON OF CLAIMS OF U.S. PATENT NO. 8,865,406 (EXHIBIT 4) AND U.S. PATENT APPLICATION NO. 13/842,859

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TABLE 10 - COMPARISON OF CLAIMS OF U.S. PATENT NO. 8,871,445 (EXHIBIT 10) AND U.S. PATENT APPLICATION NO. 13/842,859
I. Summary of Proposed Interference

Applicants request that an interference be declared between the ‘859 Application and (1) U.S. Patent No. 8,697,359 ("the ‘359 Patent") (Exhibit 1); (2) U.S. Patent No. 8,771,945 ("the ‘945 Patent") (Exhibit 2); (3) U.S. Patent No. 8,795,965 ("the ‘965 Patent") (Exhibit 3); (4) U.S. Patent No. 8,865,406 ("the ‘406 Patent") (Exhibit 4); (5) U.S. Patent No. 8,906,616 ("the ‘616 Patent") (Exhibit 5); (6) U.S. Patent No. 8,895,308 ("the ‘308 Patent") (Exhibit 6); and (7) U.S. Patent No. 8,945,839 ("the ‘839 Patent") (Exhibit 7). Applicants also request that an interference be declared between the ‘859 Application and (8) U.S. Patent No. 8,889,356 ("the ‘356 Patent") (Exhibit 8); (9) U.S. Patent No. 8,932,814 ("the ‘814 Patent") (Exhibit 9); and (10) U.S. Patent No. 8,871,445 ("the ‘445 Patent") (Exhibit 10) if the Board interprets the claims of those patents as requiring the guide RNA to include both a guide sequence and a tracer sequence. The above-listed patents are collectively referred to as “the Broad/MIT Patents.”

Applicants propose a first count ("Proposed Count 1") directed to methods of cleaving or editing a target DNA molecule or modulating transcription of at least one gene encoded thereon by employing a Type-II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) - CRISPR associated ("Cas") ("CRISPR-Cas") system comprising a single molecule DNA-targeting RNA. Proposed Count 1 is an alternative Count: Claim 165 of the ‘859 Application OR Claim 168 of the ‘859 Application OR Claim 1 of the ‘965 Patent OR Claim 4 of the ‘359 Patent OR Claim 5 of the ‘945 Patent. See Appendix A for the full text of Proposed Count 1. A side-by-side comparison of Claim 168 of the ‘859 Application with claims in each of the Broad/MIT Patents is provided in Appendix B. An explanation of why claims from the ‘859 Application and claims from the Broad/MIT Patents correspond to Proposed Count 1 is provided in Appendix C.
A summary of the claim designation is provided below.

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<td>‘445 Patent</td>
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Applicants propose a second count (“Proposed Count 2”) directed to an engineered and/or non-naturally occurring Type-II CRISPR-Cas system having a single molecule DNA-targeting RNA or a nucleic acid comprising a nucleotide sequence encoding the single molecule DNA-targeting RNA. Proposed Count 2 is an alternative Count: Claim 166 of the ‘859 Application OR Claim 203 of the ‘859 Application OR Claim 26 of the ‘965 Patent OR Claim 18 of the ‘359 Patent OR Claim 2 of the ‘616 Patent. See Appendix D for the full text of Proposed Count 2. A side-by-side comparison of Claim 203 of the ‘859 Application with claims in each of the Broad/MIT Patents is provided in Appendix E. An explanation of why claims from the ‘859 Application and claims from the Broad/MIT Patents correspond to Proposed Count 2 is provided in Appendix F.
A summary of the claim designation is provided below.

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<tr>
<td>‘445 Patent</td>
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In addition to one other provisional application, the ‘859 Application claims priority to U.S. Provisional Application No. 61/757,640, filed on January 28, 2013 (“the Third Provisional”) (Exhibit 109); U.S. Provisional Application No. 61/716,256, filed on October 19, 2012 (“the Second Provisional”) (Exhibit 108); and U.S. Provisional Application No. 61/652,086, filed on May 25, 2012 (“the First Provisional”) (Exhibit 107).

A chart providing where examples of support are found in the specification of the ‘859 Application for each of the claims of the ‘859 Application is provided in Appendix G.

A chart providing where examples of support are found in the specification of the Third Provisional for each of the claims of the ‘859 Application is provided in Appendix H.

A chart providing where examples of support are found in the specification of the Second Provisional for each of the claims of the ‘859 Application is provided in Appendix I.

A chart providing where examples of support are found in the specification of the First Provisional for each of the claims of the ‘859 Application is provided in Appendix J.
II. 37 C.F.R. § 41.202(a)(1) – Identification of Patents With Which Applicants Seek an Interference

Applicants seek an interference with the Broad/MIT Patents. Copies of the Broad/MIT Patents are attached as exhibits:

<table>
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<th>Broad/MIT Patent</th>
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<tr>
<td>U.S. Patent No. 8,697,359</td>
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<td>U.S. Patent No. 8,771,945</td>
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<td>U.S. Patent No. 8,795,965</td>
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<td>U.S. Patent No. 8,865,406</td>
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<tr>
<td>U.S. Patent No. 8,906,616</td>
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<td>U.S. Patent No. 8,895,308</td>
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<tr>
<td>U.S. Patent No. 8,945,839</td>
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<tr>
<td>U.S. Patent No. 8,889,356</td>
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<tr>
<td>U.S. Patent No. 8,932,814</td>
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<tr>
<td>U.S. Patent No. 8,871,445</td>
<td>10</td>
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A. Summary of the Parties, Application, and Patents

At filing, the ‘859 Application listed Jennifer A. Doudna, Martin Jinek, Emmanuelle Charpentier, Krzysztof Chylinski, James Harrison Doudna Cate, Wendell Lim, and Lei Qi as co-inventors. According to the USPTO’s assignment records, Jennifer A. Doudna, Martin Jinek, James Harrison Doudna Cate, Wendell Lim, and Lei Qi assigned their interests in the ‘859 Application to The Regents of the University of California and Krzysztof Chylinski assigned his interest in the ‘859 Application to the University of Vienna. The ‘859 Application claims priority benefit to at least the First Provisional, Second Provisional, and Third Provisional.

As for the Broad/MIT Patents, the ‘359, ‘945, ‘965, ‘356, and ‘839 Patents list Feng Zhang as the sole inventor. The ‘814 and ‘445 Patents list Feng Zhang and Le Cong as co-inventors. The ‘308 and ‘406 Patents list Feng Zhang and Fei Ran as co-inventors. Finally, the ‘616 Patent lists Feng Zhang, Le Cong, Patrick Hsu, and Fei Ran as co-inventors. According to the USPTO’s assignment records, Feng Zhang assigned his interest in the Broad/MIT Patents to The Broad Institute, Inc. and the Massachusetts Institute of Technology; Le Cong, Fei Ran, and
Patrick Hsu assigned their interests in these patents to the President and Fellows of Harvard
College; and The Broad Institute, Inc. licensed some of their rights in the Broad/MIT Patents to
the National Institutes of Health, the U.S. Department of Health and Human Services, and the
U.S. Government. The Broad/MIT Patents claim priority benefit to U.S. Provisional Patent
Application No. 61/736,527, filed on December 12, 2012 ("the Broad/MIT Provisional").

B. Overview of Relevant Technology and Summary of the Invention

Before explaining Applicants’ invention, Applicants provide a brief review of previously-
known genetic engineering tools. This Suggestion of Interference is supported by the
Declaration of Dr. Dana Carroll. See Exhibit 53 at ¶¶ 1-244.

1. History of Genetic Engineering

Scientists have long sought the ability to precisely manipulate the genomes of organisms
because of the wide range of possible applications in biotechnology and medicine. See
Declaration of Dr. Carroll (Exhibit 53) at ¶ 27. Where they are possible, methods of genetic
engineering can be used to introduce desired character traits or remove deleterious traits in
organisms. Id. For example, bacteria or yeast used in industrial processes may be genetically
engineered to resist viral infection or produce larger titers of biofuels or therapeutic compounds.
Id. Plants may be genetically engineered to resist insect pests or herbicides. Many genetically-
inherited diseases may be prevented or treated by modifying the genomes of plants and animals.
Id.

It has been known of for many years that manipulation of genomic sequences by genetic
engineering is greatly facilitated and enhanced by the ability to make a double-strand break in

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1 This section provides a technical overview of the relevant technology, so citation of a reference in this section
should not be viewed as an indication that the reference was published prior to the filing date of the First
Provisional. Many of the cited references were in fact published after the First Provisional was filed. Throughout
this Suggestion and its associated appendices and exhibits, use and/or citation to a given reference is not an
indication that the reference published prior to the filing of the First Provisional or prior to Applicants’ date of
invention.
the intended target DNA. *Id.* at ¶ 28. Reagents and methods for making double-strand breaks were developed initially in the 1990s and into the early 2000s. *Id.*

Prior to Applicants’ invention, other genome engineering tools relied upon protein-guided targeting, *e.g.*, zinc-finger nucleases ("ZFNs") and transcription activator-like effector nucleases ("TALENs"), to cut at specific targeted points in a DNA strand. *Id.* at ¶ 29. The Type-II CRISPR-Cas system described by Applicants was immediately recognized as being analogous to the ZFNs and TALENs in its ability to make targeted double-strand breaks in DNA. *Id.* Therefore, manipulations and uses that had been applied to the prior platforms would have been obvious to apply to CRISPR-Cas and this was in fact done. *Id.* Such manipulations and uses include, but are not limited to, the addition of a nuclear localization signal ("NLS") to direct a protein to the cell nucleus; applications to many sorts of eukaryotic cells and organisms, including mammalian and human cells; addition of appropriate regulatory sequences to achieve expression; use of viral and non-viral vectors; reliance on cellular repair mechanisms, like non-homologous end joining ("NHEJ") for local mutagenesis and homologous recombination ("HR") for sequence insertion or replacement; mutation of one nuclease active site to create a nicking enzyme. *Id.*

a. **Zinc-Finger Nuclease ("ZFN") Protein-Guided Targeting Methods**

ZFNs stemmed from the observation that the natural type IIS restriction enzyme, *FokI*, has physically separable DNA-binding and cleavage activities. See D. Carroll, *Genome Engineering With Zinc-Finger Nucleases*, 188 *GENETICS* 773-782 (2011) (*Exhibit 86*) at 775; see also *Exhibit 53* at ¶ 30. Because the cleavage domain of *FokI* has no sequence specificity, researchers were able to *demonstrate* that DNA-cleavage activities could be redirected by
substituting alternative DNA-binding domains for the natural one. See Exhibit 86 at 775; see also Exhibit 53 at ¶ 30.

Accordingly, ZFNs are generated by fusing a zinc finger DNA-binding domain to the FokI DNA-cleaving domain. See Exhibit 86 at 775; see also Exhibit 53 at ¶ 31. Because the DNA-binding domains of individual ZFNs contain between three and six individual zinc “finger” repeats that each contact three nucleotide basepairs in a modular fashion, the DNA-cleavage domain of a ZFN can be targeted to a specific DNA sequence simply by selecting the type and order of “fingers” used in the ZFN. Id. The following diagram depicts the DNA-Binding Domains and DNA-Cleaving Domains of two ZFNs and how they interact with a target nucleotide sequence.

(obtained from http://www.sigmaaldrich.com/life-science/zinc-finger-nuclease-technology/learning-center/what-is-zfn.html (downloaded on Feb. 3, 2015) (Exhibit 87); the DNA-Cleaving Domain is the nuclease domain of restriction endonuclease FokI)

When a pair of ZFNs is brought into close proximity on a double-stranded target DNA sequence, they can introduce a break in both strands. See Exhibit 53 at ¶ 32. The double-stranded break is then repaired by the cell’s machinery either by nonhomologous end joining (“NHEJ”), which can lead to gene knockouts, or by homologous recombination (“HR”), which achieves precise gene/nucleotide replacement, insertion, or deletion. See G. Davis and X. Cui, Zinc Finger Nucleases for Genome Editing, 30 Gen. Eng. & Biotech. News. 1-2 (2010)
(Exhibit 88) at 1; see also Exhibit 53 at ¶ 32. The following diagram depicts the events that take place between the introduction of a ZFN-induced double-stranded break and gene knockout/modification.

(Exhibit 88 at Figure 1)

Not every nucleotide base pair triplet in an organism’s genome has a corresponding zinc finger. See Exhibit 53 at ¶ 33. Thus, not every sequence in a genome can be targeted by ZFNs. Further, “a substantial proportion of ZFN pairs [were known to] fail, whether they are produced by design or selection. Even scientists at Sangamo Biosciences and Sigma-Aldrich, who have access to the largest and best-characterized archive of ZFs, make multiple pairs [of ZFNs] for sequences within a single target gene and test them extensively.” See Exhibit 86 at 778; see also id..

b. Transcription Activator-Like Effector Nuclease (TALEN) Protein-Guided Targeting Methods

TALENs, like ZFNs, are dimeric proteins and are generated by fusing a DNA-binding domain to the FokI DNA-cleaving domain. See Exhibit 53 at ¶ 34. The TAL effector DNA-
binding domain recognizes specific individual base pairs in a target DNA sequence by using a known cipher involving two key amino acid residues (also referred to as the repeat variable di-residues (“RVDs”)). See C. Mussolino et al., *A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity*, 39(21) *NUCL. ACIDS RES.* 9283-9293 (2011) (*Exhibit 89*) at 9284; *see also Exhibit 53* at ¶ 34. RVDs that preferentially recognize each of the four nucleotide bases have been defined. *See Exhibit 53* at ¶ 34. Accordingly, the “one amino acid repeat to one base” cipher of TALENs enables the engineering of customized TAL effector repeat arrays that recognize a user-defined target sequence. *Id.* The following diagram depicts the DNA-Binding Domains (an array of TAL effector subunits) and DNA-Cleaving Domains of two TALENs and how they interact with a target nucleotide sequence.

(obtained from http://www.systembio.com/services_tales (downloaded on Feb. 3, 2015) (*Exhibit 90*); the DNA-Cleaving Domain is the nuclease domain of restriction endonuclease FokI)

When a pair of TALENs is brought into close proximity on a double-stranded target DNA sequence, it can introduce a break in both strands. *See Exhibit 53* at ¶ 35. Once a double-stranded break has been introduced into the target DNA sequence, the cell’s endogenous DNA repair machinery takes over and genes can then be knocked out or modified through NHEJ or HR. *Id.*

Although simpler and more reliable than for ZFNs, the process of designing TALENs for new or different genetic loci is tedious, time consuming, and expensive. *See Exhibit 53* at ¶ 36.
For each new individual target, two new proteins must be designed and expressed. *Id.* For whole genome interrogation, one would have to clone and express large-scale libraries of DNA-Binding Domains. *Id.*

2. Using A Type-II CRISPR-Cas System for Genomic Modification

As explained above, both ZFNs and TALENs require designing a novel enzyme comprised of two new proteins for each new genomic locus that is targeted. See **Exhibit 53** at ¶ 37. Unlike ZFNs and TALENs and all genome editing technology before it, Applicants’ invention provides a way to use a single enzyme to modify the genome at multiple locations. *Id.* The First Provisional, and ultimately the ‘859 Application, describes Applicants’ revolutionary approach to genome modification. The approach involves the use of an RNA-guided targeting Type-II CRISPR-Cas system. *Id.*

In 1987, Japanese researchers described a series of short direct repeats interspaced with short variable sequences in the genome of *Escherichia coli*. See Y. Ishino et al., *Nucleotide Sequence of the iap Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in Escherichia coli, and Identification of the Gene Product*, 169(12) J. Bacteriol. 5429–5433 (1987) (**Exhibit 54**); see also **Exhibit 53** at ¶ 38. These repeat sequences became known as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs). These CRISPRs were detected in several bacteria and archaea. See F. Mojica et al., *Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria*, 36(1) Mol. Microbiol. 244–246 (2000) (**Exhibit 55**); see also **Exhibit 53** at ¶ 38. It was established that many of the variable sequences, called spacers, in these CRISPRs were derived from viral and plasmid sources. See A. Bolotin et al., *Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin*, 151 Microbiol. Read. Engl. 2551–2561 (2005) (**Exhibit 56**); F. Mojica et al., *Intervening Sequences of Regularly Spaced Prokaryotic
Repeats Derive from Foreign Genetic Elements, 60(2) J. Mol. Evol. 174-182 (2005) (Exhibit 57); see also Exhibit 53 at ¶ 38. Additionally, it was determined that there are CRISPR-associated (Cas) genes that encode proteins with nuclease and helicase domains. See Exhibit 56; C. Pourcel et al., CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies, 151 Microbiol. Read. Engl. 653–663 (2005) (Exhibit 58); R. Jansen et al., Identification of genes that are associated with DNA repeats in prokaryotes, 43(6) Mol. Microbiol. 1565–1575 (2002) (Exhibit 60); D. Haft et al., A Guild of 45 CRISPR-Associated (Cas) Protein Families and Multiple CRISPR/Cas Subtypes Exist in Prokaryotic Genomes, 1(6) PLOS Comput. Biol. 1(6):474-483 (2005) (Exhibit 59); see also Exhibit 53 at ¶ 38. It was suggested that these CRISPR-Cas systems are adaptive defense systems in prokaryotes to protect against viral and plasmid infection. See K. Makarova et al., A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action, 1(7) Biol. Direct. (2006) (Exhibit 61); see also Exhibit 53 at ¶ 38.

There are three varieties of CRISPR-Cas systems that have been identified: Types I, II, and III. See Exhibit 53 at ¶ 39. Each of these systems uses different molecular mechanisms for nucleic acid cleavage, although all three were believed to rely in some way on the presence of crRNA as part of the complex responsible for cleavage of the target nucleic acid. See K. Makarova et al., Evolution and classification of the CRISPR-Cas systems, 9(6) Nat. Rev. Microbiol. 467–477 (2011) (Exhibit 62); see also id. The Type-I and Type-III systems use a large complex of Cas proteins to achieve nucleic acid cleavage. See S. Brouns et al., Small CRISPR RNAs guide antiviral defense in prokaryotes, 321 Science 960–964 (2008) (Exhibit
K. Nam et al., Cas5d Protein Processes Pre-crRNA and Assembles into a Cascade-like Interference Complex in Subtype I-C/Dvulg CRISPR-Cas system, 20 Struct. 1574–1584 (2012) (Exhibit 64); R. Haurwitz et al., Sequence- and structure-specific RNA processing by a CRISPR endonuclease, 329 Science 1355–1358 (2010) (Exhibit 65); A. Hatoum-Aslan et al., Mature clustered, regularly interspaced, short palindromic repeats RNA (crRNA) length is measured by a ruler mechanism anchored at the precursor processing site, 108(52) PNAS 21218-21222 (2011) (Exhibit 66); C. Rouillon et al., Structure of the CRISPR Interference Complex CSM Reveals Key Similarities with Cascade, 52(1) Mol. Cell. 124–134 (2013) (Exhibit 67); C. Hale et al., RNA-Guided RNA Cleavage by a CRISPR RNA-Cas Protein, 139(5) Cell 945-956 (2009) (Exhibit 68); see also Exhibit 53 at ¶ 39. In contrast, it is now known that the Type-II system uses only a single Cas protein for RNA-guided DNA cleavage. See M. Jinek et al., A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity, 337 Science 816-821 (2012) (“Jinek 2012”) (Exhibit 22) at 816 and G. Gasiunas et al., Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria, 109(39) PNAS E2579-E2586 (2012) (Exhibit 69); see also Exhibit 53 at ¶ 39.

Cleavage of a target DNA by the natural Type-II CRISPR-Cas system requires the presence of three components: a CRISPR RNA (crRNA) molecule, a trans-activating CRISPR RNA (tracrRNA) molecule, and a single enzyme known as Cas9. See, e.g., First Provisional at Fig. 1A; Exhibit 22 at 818 and Fig. 3; see also Exhibit 53 at ¶ 40. However, this was not shown in the art until the First Provisional first identified the components that were both necessary and sufficient for Cas9 cleavage of target DNA, and demonstrated that those components could effect target DNA cleavage outside of the prokaryotic cellular environment. See Exhibit 53 at ¶ 40.
In 2007, Barrangou et al. reported that, in the natural environment of a prokaryotic cell, the Type-II CRISPR-Cas locus provided adaptive bacterial resistance or immunity to invading pathogens, and further determined that the variable spacer sequences and the Cas9 protein (at that time referred to as Cas5 and later referred to as Csn1) were involved in that adaptive immunity. See R. Barrangou et al., CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes, 325 SCIENCE 1709-12 (2007) (Exhibit 48); see also Exhibit 53 at ¶ 41. Various studies on the system continued until, in 2010, Garneau et al. reported that the Type-II system is guided by the short spacer sequences in the CRISPR locus to double-stranded DNA targets in the vicinity of a complementary sequence, termed the protospacer sequence. See J. Garneau et al., The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA, 468 NATURE 67-71 (2010) (Exhibit 47) see also Exhibit 53 at ¶ 41. This study again was performed in the natural environment of a prokaryotic cell. See Exhibit 53 at ¶ 41. These early studies demonstrated that Cas9 and the spacer sequences played a role in the molecular events that ultimately result in cleavage of DNA of invading pathogens within the natural environment of a prokaryotic cell. Id. But none of these early studies investigated the role of tracrRNA, defined the precise role of Cas9, or determined which components were needed for the system to cleave target DNA outside of the prokaryotic cell environment. Id.

In 2011, Deltcheva et al. published a study which “show[ed] that tracrRNA directs the maturation of crRNAs by the activities of the widely conserved endogenous RNase III and the CRISPR-associated Csn1 [Cas9] protein; all of these components are essential to protect S. pyogenes against prophage-derived DNA.” See E. Deltcheva et al., CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III, 471 NATURE 602-07 (2011) (“Deltcheva”) (Exhibit 31); see also Exhibit 53 at ¶ 42. This study did not investigate or draw any conclusions
about the role of tracrRNA in the complex that cleaves target DNA, nor did it determine the components that are necessary and sufficient for the Type-II CRISPR system to operate outside of the natural environment of a prokaryotic cell. See Exhibit 53 at ¶ 42. Instead, as illustrated by Figure 4 of Deltcheva, reproduced below, this study was focused solely on the process of producing a mature crRNA from a precursor molecule and the roles of tracrRNA, Cas9, and RNase III in that process. Id. Contrary to what is described in the ‘859 Application and its provisionals, Figure 4 of Deltcheva shows that the tracrRNA is involved with the processing of the precursor molecule, but is absent once the processing is complete. Id.

(Adapted from Exhibit 31 at p. 17, Figure 4)

In 2012, Sapranaukas et al. published results of a study in which they transported a natural *S. thermophilus* Type-II CRISPR locus into a different species of bacteria, *E. coli*, and demonstrated that the locus was still able to provide protection against infection of the bacteria. See R. Sapranaukas et al., *The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli*, 39(21) NUC. ACIDS RES. 9275-82 (2012) (“Sapranaukas”)(Exhibit 21); see also Exhibit 53 at ¶ 43. This study did not investigate much less draw any
conclusions about the role of tracrRNA, nor did it determine the components that are necessary and sufficient for the Type-II CRISPR system to operate outside of the environment of a prokaryotic cell. See Exhibit 53 at ¶ 43. In fact, since it was known from Deltcheva that RNase III was required for maturation of the crRNA, Sapranauskas was relying upon other endogenous proteins of the E. coli cells for operation of the heterologous CRISPR-Cas system. Id.

This all demonstrates that, prior to the invention set forth in the First Provisional, and ultimately in the ‘859 Application, the role of tracrRNA in the complex that cleaves the target DNA was not appreciated, and it was not known in the art if a Type-II CRISPR system could be operated outside of a prokaryotic cell environment or which components would be necessary and sufficient to do so. Id. at ¶ 44.

As shown for the first time in the First Provisional, tracrRNA, crRNA, and Cas9 form a complex which cleaves a target DNA at a specific sequence determined by the crRNA; these three components are both necessary and sufficient for operation of the system; and these three components can be expressed heterologously to apply the system outside of the prokaryotic cell environment. See, e.g., First provisional at ¶¶ [0045]-[0048], [00248]-[00252] and Fig. 1; see also Exhibit 53 at ¶ 44.

ELEMENTS OF AN EFFECTIVE TYPE-II CRISPR-CAS SYSTEM
The Type-II system relies on the Cas9 protein to cleave both strands of a target DNA molecule. See First Provisional at ¶1 [00248]-[00252] and Fig. 5; Exhibit 22 at 816; see also Exhibit 53 at ¶45. The Cas9 protein is directed to a specific sequence within the target DNA molecule by a complex of two separate RNA molecules: crRNA and tracrRNA. See Exhibit 22 at 816; see also Exhibit 53 at ¶45. These two RNA molecules associate with one another in the complex through corresponding regions of complementarity in the crRNA and the tracrRNA, which allows them to form a double-stranded or duplex region. See First Provisional at Figs. 1, 3; see also Exhibit 53 at ¶45. These duplexed RNAs associate with the Cas9 protein. See First Provisional at Fig. 1; Exhibit 22 at 818; see also Exhibit 53 at ¶45.

In addition to the region that hybridizes with tracrRNA, the crRNA also contains a sequence that hybridizes with, i.e., is complementary to, a sequence in the target DNA molecule. See First Provisional at Figs. 1 & 3C; see also Exhibit 53 at ¶46. This sequence in crRNA is sometimes referred to as the spacer sequence and the complementary sequence in the target DNA molecule is sometimes referred to as the protospacer sequence. See Exhibit 53 at ¶46. This spacer portion of the crRNA hybridizes to the protospacer region of the target DNA molecule, and the three-component complex (crRNA, tracrRNA, Cas9) is, therefore, targeted to the protospacer region of the target DNA. Id. The Cas9 enzyme then cleaves both strands of the target DNA molecule. Id. The following diagram depicts the interaction among the crRNA, tracrRNA, and Cas9 molecules and the target DNA.
Again, the First Provisional was the first document to disclose this critical association of crRNA and tracrRNA in the cleavage complex—prior to the invention of the First Provisional, this association was completely unknown. See Exhibit 53 at ¶47. Before the First Provisional, as evidenced by, e.g., Deltcheva, one of ordinary skill in the art believed that tracrRNA was only involved in the process of crRNA maturation, such that it would not be a necessary component to effectuate cleavage if a mature crRNA molecule was provided. Id. Such a (mis)understanding in the art was further supported by the knowledge of the operation of Type-I and Type-III CRISPR systems, which use a single RNA (not two separate RNAs) and utilize multiple protein components (not a single protein) to effectuate DNA targeting and cleavage. See, e.g., Exhibits 62-68; see also Exhibit 53 at ¶47. This aspect of the Type-I and Type-III CRISPR systems contributed to the belief in the art that tracrRNA was not a required component of the target DNA cleavage complex. See Exhibit 53 at ¶47.

As noted in the diagram above, the First Provisional, and ultimately the ‘859 Application, also refers to the crRNA, tracrRNA, and Cas9 protein as the “targeter-RNA,” the “activator-
RNA,” and the “site-directed modifying polypeptide,” respectively. Id. at ¶ 48. The “targeter-RNA” contains a “DNA-targeting segment” that hybridizes with the target DNA sequence. Id. Both the “targeter-RNA” and “activator-RNA” include nucleotide sequences that hybridize to form the “protein-binding segment.” Id.

With regard to the naming conventions in the Broad/MIT Patents, these indicate that the crRNA contains a “guide sequence” of about 20 basepairs in length that specifies the target site and may be used interchangeably with the terms “guide” or “spacer.” See, e.g., ’965 Patent, col. 19, ll. 44-48; see also Exhibit 53 at ¶ 49. This correspondence is reinforced by comparing figures in the relevant documents. See Exhibit 53 at ¶ 49.

Once Cas9 cleaves the target DNA in cells, any number of DNA modifications can then take place through various endogenous cellular DNA repair mechanisms, e.g., nucleotides can be inserted, deleted, or substituted within the target DNA molecule, as shown in experiments with ZFNs and TALENs. See Exhibit 53 at ¶ 50. Thus, Applicants’ system allows for site-specific DNA modification. Id. Applicants’ invention permits DNA modification at multiple target DNA loci by simply varying the DNA-targeting segment (the sequence that hybridizes with the target DNA) of the targeter-RNA, i.e., the spacer portion of the crRNA can be modified so that it is complementary to the desired target sequence (protospacer) of the target DNA molecule. Id. This is a much cheaper and simpler process than designing novel nuclease proteins as was required by prior art genome modification techniques. Id. Additionally, Applicants’ invention permits other DNA modifying activities by simply varying the catalytic domains of the Cas9 protein, e.g., site-specific nickase activity and/or by fusing heterologous protein domains to the Cas9 protein to generate chimeric Cas9 proteins. Id. The ease and efficiency of Applicants’
invention is a significant breakthrough over prior laborious genome editing tools, including ZFNs and TALENs. *Id.*

The First Provisional was the first document to disclose that the complex that binds and cleaves target DNA molecules in the Type-II CRISPR-Cas system comprises three essential components: (i) crRNA or crRNA-like molecule or targeter-RNA; (ii) tracrRNA or tracrRNA-like molecule or activator-RNA; and (iii) a Cas9 protein or a site-directed modifying polypeptide. *See First Provisional at Fig. 1; see also Exhibit 53 at ¶ 51.* Experiments conducted in connection with the invention showed that these components were capable of binding and cleaving target DNA even when they were removed from their normal environment in bacteria and reconstituted in a reaction mixture, demonstrating that the components disclosed in the First Provisional were both necessary and sufficient to achieve the critical result. *See, e.g., First Provisional at ¶¶ [00248]-[00252]: see also Exhibit 22 and Exhibit 53 at ¶ 51.* Thus, the First Provisional was the first document to disclose Applicants’ innovative approach to genetic manipulation. *See Exhibit 53 at ¶ 51.*

3. **Single Molecule DNA-Targeting RNA is Patentably Distinct From a Double Molecule DNA-Targeting RNA**

The First Provisional was also the first disclosure of combining crRNA and tracrRNA—two molecules that were until that time not even known to function together during cleavage of a target DNA—into a single molecule DNA-targeting RNA. *See, e.g., First Provisional at ¶ [0077] and Figs. 1, 3; see also Exhibit 53 at ¶ 52.* The single molecule DNA-targeting RNA of the First Provisional brings together in one molecule these two functional components, the “targeter-RNA” and the “activator-RNA,” to provide a single molecule that can effectively complex with Cas9 to bind and cleave DNA. *See Exhibit 53 at ¶ 52.* The single molecule
DNA-targeting RNA of the First Provisional includes a “DNA-targeting segment” that is analogous to the “guide sequence” of the Broad/MIT Patents. *Id.*

(Adapted from Figure 1 of the ‘859 Application)

The Broad/MIT Patents disclose the same RNA structure and refer to it as a “synthetic guide RNA,” “sgRNA,” “chimeric RNA,” “chimeric guide RNA,” or “single guide RNA.” *See, e.g., the ‘359 Patent at col. 12, ll. 6-10; see also Exhibit 53 at ¶ 53.* The diagram below depicts the single guide RNA of the Broad/MIT Patents. In some instances, the Broad/MIT Patents also refer to this RNA structure as having the guide sequence or cRNA “fused” to the tracr. *See, e.g., the ‘359 patent at col. 2, ll. 53-54; col. 47, l. 38-42; and Claim 4; see also Exhibit 53 at ¶ 53.*

(Adapted from Figure 1 of the ‘359 Patent)
The single guide RNA or single molecule DNA-targeting RNA is a unique format that is not found in nature. See Exhibit 53 at ¶ 54. The single molecule DNA-targeting RNA is separately patentable from the double-molecule format. Further, the fact that the single molecule DNA-targeting RNA is patentably distinct from a double molecule DNA-targeting RNA was recently recognized by the USPTO. See, e.g., Restriction Requirement mailed on February 6, 2015, in the ‘859 Application Prosecution History (the single molecule DNA-targeting RNA of Group II was distinguished from the double molecule DNA-targeting RNA of Group IV as being “independent and distinct”).

Type-II CRISPR-Cas systems that comprise the single molecule DNA-targeting RNA and methods that make use of the same are claimed in both the ‘859 Application and the Broad/MIT Patents and are the subject of the present Suggestion of Interference.

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

Pursuant to 37 C.F.R. § 41.202(a)(2), Applicants hereby propose two Counts, set forth the claims from the ‘859 Application and the Broad/MIT patents that correspond to each Count, and identify the claims Applicants believe interfere.

Applicants propose two Counts for this interference. Proposed Count 1 is directed to methods and Proposed Count 2 is directed to systems and compositions.

A. Proposed Count 1 – Methods

Proposed Count 1 is directed to methods of cleaving or editing a target DNA molecule, or modulating transcription of at least one gene encoded thereon by employing a Type-II CRISPR-Cas system. Proposed Count 1 is an alternative Count: Claim 165 of the ‘859 Application OR Claim 168 of the ‘859 Application OR Claim 1 of the ‘965 Patent OR Claim 4 of the ‘359 Patent OR Claim 5 of the ‘945 Patent. See Appendix A.
1. Claims That Correspond to Proposed Count 1

“A claim corresponds to a count if the subject matter of the count, treated as prior art to the claim, would have anticipated or rendered obvious the subject matter of the claim.” 37 C.F.R. § 41.207(b)(2). The following table sets forth the claims from the ‘859 Application and the Broad/MIT Patents that correspond to Proposed Count 1.

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<tr>
<th>Proposed Count 1</th>
<th>859 Application Claims That Correspond</th>
<th>Broad/MIT Claims That Correspond</th>
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<tbody>
<tr>
<td>USSN 13/842,859</td>
<td>USPN 8,697,359</td>
<td>Claims 1-7</td>
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<td>Claims 165, 168-202, and 236-242</td>
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A more detailed explanation of why the above-listed Broad/MIT Patent claims should be designated to correspond to Proposed Count 1 is provided in Tables 1-10 of Appendix C.

2. Identification of Interfering Claims


3. **Interference-In-Fact**


a. **Comparison of Claim 168 of the ‘859 Application and Claim 1 of the ‘965 Patent**

A side-by-side comparison of Claim 168 of the ‘859 Application and Claim 1 of the ‘965 Patent is provided in Table 1 of Appendix B. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘965 Patent is provided in Table 3 of Appendix K.

Claim 1 of the ‘965 Patent recites “[a] method of altering transcription of at least one gene product.” Claim 168 of the ‘859 Application also recites “[a] method of cleaving or editing
a target DNA molecule or *modulating transcription of at least one gene encoded thereon.*” See **Exhibit 53** at ¶¶ 25 and 240.

Claim 1 of the ‘965 Patent recites the use of “an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)—CRISPR associated (Cas) (CRISPR-Cas) system,” wherein the “Cas9 protein and the guide RNA do not naturally occur together.” Claim 168 of the ‘859 Application also recites the use of “an engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)—CRISP associated (Cas) (CRISPR-Cas) system.” See **Exhibit 53** at ¶¶ 25 and 229-231.

Claim 1 of the ‘965 Patent recites the use of a “CRISPR-Cas system guide RNA that hybridizes with the target sequence.” Claim 168 of the ‘859 Application also recites the use of a single molecule DNA-targeting RNA comprising “a targeter-RNA that hybridizes with the target sequence.” See **Exhibit 53** at ¶¶ 25 and 232.

Claim 1 of the ‘965 Patent recites “the guide RNA is comprised of a chimeric RNA and includes a guide sequence and a trans-activating cr (tracr) sequence.” See **Exhibit 53** at ¶¶ 25 and 233. Claim 168 of the ‘859 Application recites the “single molecule DNA-targeting RNA comprises i) a targeter-RNA that hybridizes with the target sequence, and ii) an activator-RNA that hybridizes with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment wherein the targeter-RNA and the activator-RNA are covalently linked to one another with intervening nucleotides.” The guide RNA of the ‘965 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. See, e.g., the ‘965 Patent at col. 19, ll. 44-48 with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also **Exhibit 53** at ¶¶ 25 and 233-235.
Claim 1 of the ‘965 Patent recites the use of a Cas9 protein. See Exhibit 53 at ¶¶ 229-231. Claim 168 of the ‘859 Application also recites the use of a Cas9 protein. Id.

Claim 1 of the ‘965 Patent recites “the guide RNA targets the target sequence and the Cas9 protein cleaves the DNA molecule.” See Exhibit 53 at ¶¶ 25 and 239. Claim 168 of the ‘859 Application also recites “the single molecule DNA-targeting RNA forms a complex with the Cas9 protein, thereby targeting the Cas9 to the target DNA molecule, whereby said target DNA molecule is cleaved.” Id.

Claim 1 of the ‘965 Patent recites “expression of the at least one gene product is altered.” See Exhibit 53 at ¶¶ 25 and 242. Claim 168 of the ‘859 Application also recites “whereby said target DNA molecule is cleaved or edited or transcription of at least one gene encoded by the target DNA molecule is modulated.” Id.

Claim 1 of the ‘965 Patent additionally recites “introducing [the CRISPR-Cas system] into a eukaryotic cell containing and expressing a DNA molecule having a target sequence and encoding the gene product,” “a) a first regulatory element operable in a eukaryotic cell operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA,” “b) a second regulatory element operable in a eukaryotic cell operably linked to a nucleotide sequence encoding a Type II Cas9 protein,” and that “components (a) and (b) are located on same or different vectors of the system.” Claim 168 of the ‘859 Application does not recite these limitations. While these differences exist, one of ordinary skill in the art would have readily realized these aspects to be obvious in view of Claim 168 of the ‘859 Application for the following reasons. See Exhibit 53 at ¶¶ 24 and 198-201.

Obviousness is a question of law based on the following factual inquiries:

(a) determining the scope and content of the prior art; (b) ascertaining the differences between
the claimed invention and the prior art; and (c) resolving the level of skill in the art. See Graham v. John Deere Co., 383 U.S. 1, 17-18 (1966). A person of ordinary skill in the art is a hypothetical person, presumed to know the relevant art at the time the invention was made and “is also a person of ordinary creativity, not an automaton.” KSR Int’l Co. v. Teleflex Inc., 550 U.S. 398, 421 (2007); see also Exhibit 53 at ¶¶ 63-67.

i) Using The Type-II CRISPR-Cas System In Eukaryotic Cells

The First Provisional was filed on May 25, 2012, and shortly before is considered “the time of the invention” for purposes of this Suggestion of Interference. See Exhibit 107; see also Exhibit 53 at ¶ 21. At the time of the invention, the state of the art of genetic engineering and genome editing was advanced. See Exhibit 53 at ¶ 64. Many techniques were well-known and had been established for years. Id. A person of ordinary skill in the art at the time of the invention would have possessed a strong understanding of molecular biology techniques used to clone and express proteins in different systems. Id. at ¶¶ 65-67. A person of ordinary skill in the art at that time would have also possessed a strong understanding of techniques for manipulating and analyzing nucleic acids, such as RNA and DNA, with much, if not all of this being routine laboratory work. Id. Moreover, many of the reagents used in these methods were commercially available from a wide variety of sources, which were all supplied with instructions on how to perform the methods. Id. These instructions were easily followed to achieve the intended results. Id. Accordingly, it would have been routine for one of ordinary skill in the art to use these known methods and materials to apply the Type-II CRISPR-Cas system to eukaryotic cells. Id.
Introducing The Type-II CRISPR-Cas System Into Eukaryotic Cells

Organisms may be sorted into one of two groups based upon the fundamental structure of their cells. Eukaryotic cells contain membrane-bound organelles, such as the nucleus, whereas prokaryotic cells do not.

For at least 30 years, prokaryotic proteins have been robustly expressed in eukaryotic cells. See Exhibit 53 at ¶ 93. For example, the prokaryotic cre-lox site-specific recombination system has been used successfully in eukaryotic cells since the late 1980s. See, e.g., B. Sauer, Functional Expression of the cre-lox Site-Specific Recombination System in the Yeast Saccharomyces cerevisiae, 7(6) MOL. CELL. BIOL. 2087-2096 (1987) (Exhibit 38); B. Sauer and N. Henderson, Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1, 85 PNAS 5166-70 (1988) (Exhibit 39); see also Exhibit 53 at ¶ 93. It was known from these studies that a prokaryotic protein could be expressed in a eukaryotic cell using a promoter functional in the eukaryotic cell, i.e., a regulatory element operable in a eukaryotic cell. See, e.g., Exhibit 38 at 2088-90, Figure 1; Exhibit 39 at 5166-67 and Figure 1; see also Exhibit 53 at ¶ 93.

Additionally, the prokaryotic RecA protein has been used successfully in eukaryotic cells from at least the mid-1990s. See, e.g., B. Reiss et al., RecA protein stimulates homologous recombination in plants, 93 PNAS 3094-98 (1996) (Exhibit 11); see also Exhibit 53 at ¶ 94. It was shown in this study that, to be effective, the RecA protein had to be targeted to the eukaryotic nucleus by including a nuclear localization signal, or nuclear targeting sequence. See Exhibit 11 at 3094-95 and Figure 1; see also Exhibit 53 at ¶ 94. Additionally, a promoter that was suitable for expression of the RecA protein in eukaryotic cells was used. See Exhibit 11 at 3094-95 and Figure 1; see also Exhibit 53 at ¶ 94. The plasmids containing the RecA protein
were introduced into the plant cells by electroporation. See Exhibit 11 at 3095; see also Exhibit 53 at ¶ 94.

Other prokaryotic DNA site-specific proteins, such as the φC31 recombinase from Streptomyces lividans, were also well-known to display activity in mammalian cells. See C.S. Raymond and P. Soriano, High-Efficiency FLP and φC31 Site-Specific Recombination in Mammalian Cells, 2(1) PLoS One e162 (2007) (Exhibit 94); see also Exhibit 53 at ¶ 95. In this study, the φC31 recombinase gene was optimized to include more common mouse codons to improve translation efficiency in mammalian cells. See Exhibit 53 at ¶ 95. Further, a number of sequence motifs were avoided, including internal TATA-boxes, ribosomal entry sites, stretches of AT- and GC- rich sequence, repeat sequences, RNA secondary structure, and cryptic splice and polyadenylation sites. Id. The codon-optimized φC31 gene was synthesized with a reduced number of CpG dinucleotides to avoid gene silencing associated with DNA methylation at such sites. Id.

Similarly, long before the First Provisional was filed, numerous research groups had successfully employed bacterial restriction endonucleases in mammalian cells. See, e.g., J. Carney & W. Morgan, Induction of DNA Double-Strand Breaks by Electroporation of Restriction Enzymes into Mammalian Cells, 113 METHODS IN MOL. BIOL. 465-471 (1999) (Exhibit 100); W. Morgan et al., Inducible Expression and Cytogenetic Effects of the EcoRI Restriction Endonuclease in Chinese Hamster Ovary Cells, 8(10) MOL. CELL. BIOL. 4204-4211 (1988) (Exhibit 101); see also Exhibit 53 at ¶ 96. As all of this demonstrates, at the time the First Provisional was filed, it was well-known in the art how to employ bacterial nucleases and other DNA-modifying proteins in mammalian cells, and was quite common to do so. See, e.g., Exhibit 39 at 5166; see also Exhibit 53 at ¶¶ 96-97.
Perhaps most relevant to the Type-II CRISPR-Cas system, all of the methods discussed above and below were used in the applications of ZFNs and TALENs, the prior genome engineering technologies. *Id.* at ¶ 98. For example, ZFNs and TALENs are hybrids of protein derived from eukaryotes and prokaryotes; they were expressed in eukaryotic cells through the use of vectors of the types mentioned below, but also without vectors; and they were typically endowed with nuclear localization signals. *Id.*

(2) **Use of Viral Vectors to Deliver Cas9- or RNA-Encoding Nucleotides Into Eukaryotic Cells**

et al., A simple method for the rapid generation of recombinant adenovirus vectors, 7 GENE THER. 1034-1038 (2000) (Exhibit 19); and K. Sato et al., Generation of Adeno-Associated Virus Vector Enabling Functional Expression of Oxytocin Receptor and Fluorescence Marker Genes Using the Human eIF4G Internal Ribosome Entry Site Element, 73(9) BIOSCI. BIOTECHNOL. BIOCHEM. 2145-2148 (2009) (Exhibit 20); see also Exhibit 53 at ¶ 83. And, in fact, these well-known viral vectors had been used to express ZFNs in eukaryotic cells. See, e.g., A. Lombardo, et al., Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery, 25(11) NATURE BIOTECH. 1298-1306 (2007) (Exhibit 105) (introducing ZFNs using lentiviral vectors); H. Li, et al., In vivo genome editing restores haemostasis in a mouse model of haemophilia, 475 NATURE 217-223 (2011) (Exhibit 106) (introducing ZFNs using adeno-associated viral vectors); see also Exhibit 53 at ¶ 83. Thus, it would have been obvious for one of ordinary skill in the art to use viral vectors to deliver Cas9- or RNA-encoding nucleotides into eukaryotic cells at the time of the invention. See Exhibit 53 at ¶ 83.

(3) Use of Codon-Optimization

Prokaryotic proteins had been successfully processed and folded in eukaryotic cells as early as the late 1980s. See, e.g., Exhibit 38 and Exhibit 39; see also Exhibit 53 at ¶ 91. Furthermore, those of ordinary skill in the art would have been aware of the usefulness of codon optimization when expressing a prokaryotic protein in a eukaryotic cell. See, e.g., C. Gustafsson et al., Codon bias and heterologous protein expression, 22(7) TRENDS IN BIOTECH. 346-353 (2004) (Exhibit 15); see also Exhibit 53 at ¶ 91. In fact, by at least the mid-1990s it was routine to provide codon-optimized versions of genes in conjunction with a nuclear localization signal to provide high-level expression of a protein in the nucleus of eukaryotic cells. See, e.g., W. Chiu et al., Engineered GFP as a vital reporter in plants, 6(3) CURR. BIOL. 325-330 (1996) (Exhibit
83); see also Exhibit 53 at ¶91. Thus, it would have been obvious for one of ordinary skill in the art to optimize codons in Cas9-encoding sequences at the time of the invention. See Exhibit 53 at ¶ 89-92.

(4) Nucleotide Sequence Encoding Cas9 Linked to Promoter That is Operable in Eukaryotic Cells

Techniques for expressing prokaryotic proteins were well-known in the art at the time of the invention. Id. at ¶ 86. For example, a number of studies made use of the yeast GAL1 promoter—a regulatory element operable in eukaryotic cells. See Exhibit 38 and Exhibit 39; see also Exhibit 53 at ¶ 86. As another example, the enhancer and promoter of the human cytomegalovirus immediately early gene was known to be a highly effective transcription control element for expression in mammalian cells. See, e.g., M.K. Foecking and H. Hofstetter, Powerful and versatile enhancer-promoter unit for mammalian expression vectors, 45(1) GENE 101-105 (1986) (Exhibit 84); see also Exhibit 53 at ¶ 86. As a further example, over 30 years ago, researchers expressed two bacterial resistance genes in mouse fibroblast cells using a known expression vector containing the SV40 promoter and other control elements. See K. O’Hare et al., Transformation of mouse fibroblasts to methotrexate resistance by a recombinant plasmid expressing a prokaryotic dihydrofolate reductase, 78(3) PNAS 1527-1531 (1981) (Exhibit 85); see also Exhibit 53 at ¶ 86. These publications evidence that linking a prokaryotic protein-encoding sequence to a promoter that is operable in eukaryotic cells would have been obvious to one of ordinary skill in the art at the time of the invention. See Exhibit 53 at ¶ 86.

(5) Nucleotide Sequence Encoding Guide RNA Linked to Promoter That is Operable in Eukaryotic Cells

The use of a promoter—a regulatory element operable in a eukaryotic cell—for expression of non-coding RNA molecules in eukaryotic cells was also well known. See, e.g., L.

Linking a non-coding RNA-encoding sequence to a promoter that is operable in eukaryotic cells would have been obvious to one of ordinary skill in the art at the time of the invention. *See Exhibit 53 at ¶ 85.*

Thus, the process of using a Type-II CRISPR-Cas system in eukaryotic cells would have been obvious to one of ordinary skill in the art in view of Claim 168 of the ‘859 Application at the time of the invention. *Id. at ¶¶ 24 and 198-201.*

**ii) Other Scientists Applied the Type-II CRISPR-Cas System to Eukaryotic Cells Within Months of the First Published In Vitro Experiments**

Once the Type-II CRISPR-Cas system was demonstrated to function *in vitro*, *i.e.*, outside of a cell, due to its ease and applicability one of ordinary skill in the art would certainly have wanted to use the system in eukaryotic cells. *See Exhibit 53 at ¶¶ 100-114.* Applying the system to eukaryotic cells would have been obvious to one of ordinary skill in the art based on the discussion provided above. *Id.* This is evidenced by the fact that multiple research groups quickly confirmed that the Type-II CRISPR-Cas system could function in eukaryotic cells shortly after Jinek 2012 disclosed the basic components required for the Type-II CRISPR-Cas system to operate and demonstrated its effectiveness outside of a cell. *Id.* Despite the fact that Jinek 2012 lacked a eukaryotic example, within a few months of the Jinek 2012 publication several separate groups of scientists were able to successfully perform experiments showing that the Type-II CRISPR-Cas system worked in eukaryotic cells and draft and submit manuscripts
describing the same. See, e.g., M. Jinek et al., *RNA-programmed genome editing in human cells*, 2 eLife e00471 (2013) (“Jinek 2013”) (Exhibit 91); L. Cong et al., *Multiplex Genome Engineering Using CRISP/Cas Systems*, 339 Science 819-823 (2013) (“Cong”) (Exhibit 51); P. Mali et al., *RNA-guided Human Genome Engineering via Cas9*, 339 Science 823-826 (2013) (“Mali”) (Exhibit 72); S. Cho et al., *Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease*, 31(3) Nature Biotech 230-232 (2013) (“Cho”) (Exhibit 71); W. Hwang et al., *Efficient In Vivo Genome Editing Using RNA-Guided Nucleases*, 31(3) Nature Biotech 227-229 (2013) (“Hwang”) (Exhibit 73); and B. Shen et al., *Generation of gene-modified mice via Cas9/RNA-mediated gene targeting*, 23 Cell Res. 720-723 (2013) (“Shen”) (Exhibit 74); see also Exhibit 53 at ¶ 105-108. As Dr. Carroll astutely points out, “all of these articles, from independent research groups at different institutions, specifically cited the Jinek 2012 Science paper as a basis of their work.” See Exhibit 53 at ¶ 107. In fact, “all of the studies conducted by the various groups used a single chimeric RNA that includes a truncation of the double stranded RNA duplex (i.e., truncation of the crRNA at the 3’ end and truncation of the tracrRNA at the 5’ end), just as was disclosed in the Jinek [2012] publication.” *Id.* at ¶ 109.

All of the researchers “used molecular biology techniques and reagents that were well-known in the art to apply [the system of Jinek 2012] to eukaryotic cells.” *Id.* at ¶ 112. For example, Jinek 2013, Cong, and Mali all used the well-known U6 promoter to express the single chimeric RNA in eukaryotic cells. *See Exhibit 91* at Materials and Methods; *Exhibit 51* at Figure 4A; *Exhibit 72* at Figure 1A; and *Exhibit 53* at ¶ 112. Cho, Hwang, and Shen used the well-known T7 promoter to express the single chimeric RNA outside of cells before introducing the transcribed RNA into eukaryotic cells. *See Exhibit 71* at Supplementary Methods 2; *Exhibit 73* at Methods; *Exhibit 74* at 720; and *Exhibit 53* at ¶ 112.
Jinek 2013, Mali, and Cho all used the well-known CMV promoter to express Cas9 in eukaryotic cells. See Exhibit 91 at Materials and Methods; Exhibit 72 at Figure 1A; Exhibit 71 at Supplementary Methods 2; and Exhibit 53 at ¶ 112. Shen and Hwang used the T7 promoter to produce Cas9 mRNA outside of cells, which was then introduced into eukaryotic cells. See Exhibit 73 at Methods; Exhibit 74 at 720; and Exhibit 53 at ¶ 112. Cong used the well-known Ef1α promoter to express Cas9 in eukaryotic cells. See Exhibit 51 at Figure 1B; see also Exhibit 53 at ¶ 112.

Further, all of the groups used the well-known technique of codon optimization to more efficiently express Cas9 in eukaryotic cells. See Exhibit 91 at Materials and Methods; Exhibit 51 at 819 and Figure 1A; Exhibit 72 at 823 and Figure 1A; Exhibit 71 at Supplementary Methods 2 and Supplementary Figure 1; Exhibit 73 at Methods; Exhibit 74 at 720-722; and Exhibit 53 at ¶ 113. Finally, all of the groups made use of conventional nuclear localization signals to target Cas9 to eukaryotic cell nuclei. See Exhibit 91 at Materials and Methods; Exhibit 51 at Figure 1A; Exhibit 72 at Figure 1A; Exhibit 71 at Supplementary Methods 2; Exhibit 73 at Methods; and Exhibit 74 at 720-722; see also Exhibit 53 at ¶ 113. However, as discussed in Dr. Carroll’s Declaration, these studies also demonstrated that neither codon optimization nor nuclear localization signals were necessary for cleavage of target DNA in a eukaryotic cell. See Exhibit 53 at ¶ 113.

The fact that these several separate groups of scientists were able to readily apply the Type-II CRISPR-Cas system to eukaryotes so quickly after Jinek 2012 published is further evidence that Broad/MIT’s additional claim limitations amount to nothing more than routine processes and components known to those of ordinary skill in the art. Id. at ¶ 114.

b. **Comparison of Claim 168 of the ‘859 Application and Claim 4 of the ‘359 Patent**

A side-by-side comparison of Claim 168 of the ‘859 Application and Claim 4 of the ‘359 Patent is provided in Table 2 of Appendix B. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘359 Patent is provided in Table 1 of Appendix K.

Claim 4 of the ‘359 Patent differs from Claim 1 of the ‘965 Patent in that it recites “the guide RNAs [sic] comprise a guide sequence fused to a trans-activating cr (tracr) sequence.” However, the guide RNA of Claim 4 of the ‘359 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. *Compare, e.g., the ‘359 Patent at col. 12, ll.6-10; col. 47, ll. 38-48; and Figure 11B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶¶ 25 and 233-235.*

c. Comparison of Claim 168 of the ‘859 Application and Claim 5 of the ‘945 Patent

A side-by-side comparison of Claim 168 of the ‘859 Application and Claim 5 of the ‘945 Patent is provided in Table 3 of Appendix B. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘945 Patent is provided in Table 2 of Appendix K.

Claim 5 of the ‘945 Patent differs from Claim 1 of the ‘965 Patent in that it recites “the guide RNAs [sic] comprise a guide sequence fused to a tracr sequence” and the use of viral vectors. However, the guide RNA of Claim 5 of the ‘945 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. Compare, e.g., the ‘945 Patent at col. 12, ll. 8-12; col. 54, l. 64-col. 55, l. 7 and Figure 11B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶¶ 25 and 233-235. Further, as discussed above in Section III(A)(3)(a)(i)(2), it would have been obvious for one of ordinary skill in the art to use viral vectors to deliver Cas9- or RNA-encoding nucleotides into eukaryotic cells at the time of the invention. Additionally, the ‘859 Application also includes a claim that recites the use of viral vectors. See Claim 173 of the ‘859 Application (“the vector is selected from the group consisting of . . . viral vectors.”).

d. Comparison of Claim 168 of the ‘859 Application and Claim 5 of the ‘406 Patent

A side-by-side comparison of Claim 168 of the ‘859 Application and Claim 5 of the ‘406 Patent is provided in Table 4 of Appendix B. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘406 Patent is provided in Table 4 of Appendix K.

Claim 5 of the ‘406 Patent differs from Claim 1 of the ‘965 Patent in that it recites “the guide RNA comprise a guide sequence and a tracer sequence” and the use of a \textit{Staphylococcus aureus} Cas9 protein. However, the guide RNA of Claim 5 of the ‘406 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. \textit{Compare}, e.g., the ‘406 Patent at col. 22, ll. 11-15 and col. 27, ll. 60-66 with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶¶ 25 and 233-235.

Further, the use of a \textit{Staphylococcus aureus} Cas9 protein in a Type-II CRISPR-Cas system would have been obvious to one of ordinary skill in the art in view of Claim 168 of the ‘859 Application. \textit{See} Exhibit 53 at ¶¶ 24 and 202-205. The amino acid sequence of the \textit{Staphylococcus aureus} Cas9 protein was publicly available on the National Center for Biotechnology Information (“NCBI”) website on or before September 7, 2012. \textit{See} http://www.ncbi.nlm.nih.gov/protein/403411236?sat=16&satkey=13804560 (downloaded on Jan. 21, 2015) (Exhibit 24); see also Exhibit 53 at ¶ 203. Because the protein sequences and domain structures of \textit{Streptococcus thermophilus} and \textit{Streptococcus pyogenes} Cas9 proteins were already known, these sequences could have easily been aligned by one of ordinary skill in the art to determine the domain structure of \textit{Staphylococcus aureus} Cas9 as of September 7, 2012, the NCBI public release date of the \textit{Staphylococcus aureus} Cas9 protein sequence. \textit{See} Exhibit 21; see also Exhibit 53 at ¶¶ 203-204. Once it was demonstrated that \textit{Streptococcus thermophilus} and \textit{Streptococcus pyogenes} Cas9, in combination with guide/tracer RNA, could
clease DNA, one of ordinary skill in the art would have been motivated to use Cas9 proteins from other bacterial species, e.g., *Staphylococcus aureus*. See Exhibit 53 at ¶¶ 203-204. And because it was known that the *Staphylococcus aureus* Cas9 protein sequence is shorter than the Cas9 protein sequences of *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Listeria innocua*, and *Streptococcus mutans*, one of ordinary skill in the art would have been motivated to use the shorter *Staphylococcus aureus* Cas9 protein in a Type-II CRISPR-Cas system because it was generally known that some vectors have limitations for the size of protein-encoding genes that they can deliver to cells. See Z. Wu et al., *Effect of Genome Size on AAV Vector Packaging*, 18(1) MOL. THER. 80-86 (2010) (Exhibit 25); see also Exhibit 53 at ¶¶ 203-204. Thus, the use of the *Staphylococcus aureus* Cas9 protein sequence would have been obvious to one of ordinary skill in the art in view of Claim 168 of the ‘859 Application in combination with the first public disclosure of the *Staphylococcus aureus* protein sequence and Sapranauskas. See Exhibit 53 at ¶ 24 and 205.


e. **Comparison of Claim 168 of the ‘859 Application and Claim 10 of the ‘308 Patent**

A side-by-side comparison of Claim 168 of the ‘859 Application and Claim 10 of the ‘308 Patent is provided in Table 5 of Appendix B. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘308 Patent is provided in Table 6 of Appendix K.
Claim 10 of the ‘308 Patent differs from Claim 1 of the ‘965 Patent in that it recites “the guide sequence and tracr sequence are chimeric,” the use of a *Staphylococcus aureus* Cas9 protein, “one or more nuclear localization signal(s),” and “inserting DNA into a cleaved strand of the DNA molecule.” However, the guide RNA of Claim 10 of the ‘308 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. *Compare, e.g., the ‘308 Patent at col. 22, ll. 23-27 and col. 28, ll. 5-11; col. 56, l. 62-col. 57, l. 7 and Fig. 13B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶¶ 25 and 233-235. The use of a *Staphylococcus aureus* Cas9 protein would have been obvious based on the reasons provided above with respect to Claim 5 of the ‘406 Patent.*

The use of one or more nuclear localization signals in a Type-II CRISPR-Cas system would also have been obvious to one of ordinary skill in the art in view of Claim 168 of the ‘859 Application. Researchers have been using nuclear localization signals to target prokaryotic peptides expressed in eukaryotic cells to the cells’ nucleus for decades. *See, e.g., A. Fieck et al., Modifications of the E. coli Lac repressor for expression in eukaryotic cells: effects of nuclear signal sequences on protein activity and nuclear accumulation, 29(7) *Nucl. Acids Res.* 1785-1791 (1992) (Exhibit 82); Exhibit 11 at 3094-95, Figure 1; L. Fischer-Fantuzzi and C. Vesco, Cell-Dependent Efficiency of Reiterated Nuclear Signals in a Mutant Simian Virus 40 Oncoprotein Targeted to the Nucleus, 8(12) *Mol. Cell. Biol.* 5495-5503 (1988) (Exhibit 12); see also Exhibit 53 at ¶ 91. Indeed, commercial vectors that incorporate one or more nuclear localization signals for targeting proteins of interest to cell nuclei were known as of the early 2000s. *See, e.g., S. Planey et al., Inhibition of Glucocorticoid-induced Apoptosis in 697 Pre-B Lymphocytes by the Mineralocorticoid Receptor N-terminal Domain, 277(44) J. Biol. Chem. 42188-42196 (2002) (Exhibit 13) and Y.S. Dai et al., The Transcription Factors GATA4 and*
dHAND Physically Interact to Synergistically Activate Cardiac Gene Expression through a  
p300-dependent Mechanism, 277(27) J. BIOL. CHEM. 24390-24398 (2002) (Exhibit 14)  
(describing the use of Invitrogen’s pShooter vector); see also Exhibit 53 at ¶ 92. Thus, the use  
of one or more nuclear localization signals would have been obvious to one of ordinary skill in  
the art in view of Claim 168 of the ‘859 Application. See Exhibit 53 at ¶ 24. The ‘859  
Application also includes a claim that recites the use of nuclear localization domains. See Claim  
182 of the ‘859 Application (“one or more PTD(s) comprises an amino acid sequence selected  
from the group consisting of SEQ ID NOs:268 and 269”).  

Further, the additional step of “inserting DNA into a cleaved strand of the DNA  
molecule,” would have been obvious to one of ordinary skill in the art in view of Claim 168 of  
the ‘859 Application. It was well known in the art that when DNA strand breaks arise in cells,  
there are two major categories of DNA repair mechanisms that restore the duplex structure, i.e.,  
NHEJ and HR. See M. Lieber, The Mechanism of Double-Strand DNA Break Repair by the  
Nonhomologous DNA End Joining Pathway, 79 ANNU. REV. BIOCHEM. 181-211 (2010) (Exhibit  
27) at 1; Exhibit 27 at 2; see also N. Jensen et al., An update on targeted gene repair in  
mammalian cells: methods and mechanisms, 18 J. BIOMED. SCI. 10 (2011) (Exhibit 29) at  
Figure 1; Exhibit 53 at ¶¶ 214-220; see also supra Section II(B)(1). Further, it was known that  
“[t]he most common form of homology-directed repair is called homologous recombination  
[HR].” See Exhibit 27 at 2. One of ordinary skill in the art also knew that if sufficient  
appropriately designed homologous recombination nucleotide template was present at the time  
the DNA strand break was induced, then HR was an option for DNA break repair. Id; see also  
Exhibit 53 at ¶¶ 214-220. Additionally, it was also known that the HR repair mechanisms  
require a homologous recombination template that shares sequence homology with the target
DNA sequence. See Exhibit 27 at 2; Exhibit 29 at Figure 1; L. Symington & J. Gautier, *Double-Strand Break End Resection and Repair Pathway Choice*, 45 ANNU. REV. GENETC. 247-271 (2011) (Exhibit 52); M. Porteus & D. Carroll, *Gene Targeting Using Zinc Finger Nucleases*, 23(8) NAT. BIOTECH. 967-973 (2005) (Exhibit 50); see also Exhibit 53 at ¶ 214-220. Thus, it would have been obvious to one of ordinary skill in the art that HR repair would result in the incorporation of a homologous recombination template at the DNA strand break site. See Exhibit 53 at ¶ 24 and 214-220. Additionally, the ‘859 Application also includes a claim that recites inserting a donor polynucleotide sequence into the cleaved strand of a target DNA molecule. See Claim 242 of the ‘859 Application (“the method comprises editing the target DNA by insertion of a sequence of a donor polynucleotide into the cleaved strand of the target DNA molecule”).


f. Comparison of Claim 168 of the ‘859 Application and Claim 1 of the ‘839 Patent

A side-by-side comparison of Claim 168 of the ‘859 Application and Claim 1 of the ‘839 Patent is provided in Table 6 of Appendix B. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘839 Patent is provided in Table 7 of Appendix K.

Claim 1 of the ‘839 Patent differs from Claim 1 of the ‘965 Patent in that it recites “the guide RNA comprises a tracr sequence,” “one or more nuclear localization signals,” “a tracr sequence which is 30 or more nucleotides in length,” a “Cas9 protein compris[ing] one or more
mutations in a catalytic domain,” and does not recite “the Cas9 protein cleaves the DNA molecule.” However, the guide RNA of Claim 1 of the ‘839 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. Compare, e.g., the ‘839 Patent at col. 12, ll. 15-19; col. 55, ll. 11-15 and Fig. 11B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶ 25. The use of one or more nuclear localization signals would have been obvious based on the reasons provided above with respect to Claim 10 of the ‘308 Patent. Additionally, the ‘859 Application includes a claim that recites the use of nuclear localization domains. See Claim 182 of the ‘859 Application (“one or more PTD(s) comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:268 and 269”).

The use of a Cas9 protein with one or more mutations in a catalytic domain would also have been obvious to one of ordinary skill in the art in view of Claim 168 of the ‘859 Application. See Exhibit 53 at ¶¶ 24 and 206-211. Cas9 proteins were known to contain two types of endonuclease catalytic domains: HNH and RuvC. See Exhibit 21 at 9276 and 9280; see also Exhibit 53 at ¶ 207. Additionally, mutations in the HNH and RuvC endonuclease domains had already been generated. See Exhibit 21 at 9280 and 9281; see also Exhibit 53 at ¶ 207. In fact, certain mutations in HNH and RuvC endonuclease motifs were known to disrupt Cas9 double-stranded cleavage activity. See Exhibit 21 at 9280-9281; see also Exhibit 53 at ¶ 207. For example, it was known that HNH and RuvC mutations that disrupt double-stranded cleavage activity included D31A, H868A, N882A, and N891A in the Streptococcus thermophilus Cas9 protein. See Exhibit 21 at 9280; see also Exhibit 53 at ¶ 207. Moreover, one skilled in the art would have been motivated to make such mutations within the catalytic domain(s) of Cas9 because it was well known in the art that one could mutate catalytic sites of proteins in order to
alter their functions. See e.g., K.H. Zavitz and K.J. Marians, ATPase-deficient mutants of the
Escherichia coli DNA replication protein PriA are capable of catalyzing the assembly of active
primasomes, 267(10) J. BIOL. CHEM. 6933-6940 (1992) (Exhibit 103); A. Saito et al.,
Identification of four acidic amino acids that constitute the catalytic center of the RuvC Holliday
junction resolvase, 92 PNAS 7470-7474 (1995) (Exhibit 104); see also Exhibit 53 at ¶ 208. For
example, it was known that mutations to the catalytic domains of ZFNs and homing
endonucleases could produce nickases with certain desirable properties. See, e.g., J. Wang et
al., Targeted gene addition to a predetermined site in the human genome using a ZFN-based
nicking enzyme, 22 GEN. RES. 1316-1326 (2012) (Exhibit 23) (zinc finger nuclease-based
nicking enzymes were shown to insert a gene into a predetermined site in the human genome); E.
Kim et al., Precision Genome Engineering with Programmable DNA-Nicking Enzymes, 22 GEN.
RES. 1327-1333 (2012) (Exhibit 102); A. Smith et al., Generation of a Nicking Enzyme that
Stimulates Site-Specific Gene Conversion from the 1-Anil LAGLIDADG Homing Endonuclease,
106(13) PNAS USA 5099-5104 (2009) (Exhibit 75); Ramirez et al., Engineered zinc finger
nickases induce homology-directed repair with reduced mutagenic effects, 40(12) NUCLEIC
ACIDS RES. 5560-5568 (2012) (Exhibit 28); see also Exhibit 53 at ¶ 208. Further, because of
those precedents, one skilled in the art would have been motivated to make such mutations to the
Cas9 catalytic domains, for example, to produce Cas9 proteins with nonfunctioning catalytic
sites to allow for site specific binding but without DNA cleavage. See Exhibit 21; see also
Exhibit 53 at ¶ 208. Accordingly, it would have been obvious for one of ordinary skill in the art
to use any of the mutated Cas9 proteins described in the art. See Exhibit 53 at ¶ 210.
Additionally, the ‘859 Application includes a claim that recites one or more mutations in a Cas9
catalytic domain. See Claim 240 of the ‘859 Application (“the Cas9 protein comprises a
mutation selected from D10A and H840A with reference to the numbering of *Streptococcus pyogenes* Cas9 protein").

The use of a guide RNA that includes a tracr sequence which is 30 or more nucleotides in length in a Type-II CRISPR-Cas system would also have been obvious to one of ordinary skill in the art in view of Claim 168 of the ‘859 Application. Wild-type tracr sequences from *Streptococcus pyogenes* were known in the art to be greater than 30 nucleotides in length. See, e.g., Exhibit 31 at 602; see also Exhibit 53 at ¶¶ 221-228. Thus, it would have been obvious for one of ordinary skill in the art to use the wild-type tracr sequence, e.g., a tracr sequence of *Streptococcus pyogenes*—a tracrRNA sequence of 30 or more nucleotides—in a single molecule DNA-targeting RNA in view of Claim 168 of the ‘859 Application. See Exhibit 53 at ¶¶ 24 and 221-228. Additionally, the ‘859 Application includes a claim that recites the activator-RNA comprises “the 88 nucleotide tracrRNA sequence set forth in SEQ ID NO:433,” which is plainly a tracrRNA sequence of 30 or more nucleotides. See Claim 239 of the ‘859 Application.


g. Comparison of Claim 168 of the ‘859 Application and Claim 4 of the ‘356 Patent

A side-by-side comparison of Claim 168 of the ‘859 Application and Claim 4 of the ‘356 Patent is provided in Table 7 of Appendix B. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘356 Patent is provided in Table 8 of Appendix K.
Claim 4 of the ‘356 Patent recites “[a] method of altering expression of at least one gene product.” Claim 168 of the ‘859 Application also recites “[a] method of cleaving or editing a target DNA molecule or modulating transcription of at least one gene encoded thereon.” See Exhibit 53 at ¶ 25 and 240.

Claim 4 of the ‘356 Patent recites the use of “an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) system,” wherein the “Cas9 protein and the guide RNA do not naturally occur together.” Claim 168 of the ‘859 Application also recites the use of “an engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)—CRISPR associated (Cas) (CRISPR-Cas) system.” See Exhibit 53 at ¶¶ 25 and 229-231.

Claim 4 of the ‘356 Patent recites the use of a “CRISPR-Cas system guide RNA that hybridizes with the target sequence” and “wherein the CRISPR-Cas system comprises a trans-activating cr (tracr) sequence.” Claim 168 of the ‘859 Application recites the use of a “single molecule DNA-targeting RNA comprising i) a targeter-RNA that hybridizes with the target sequence, and ii) an activator-RNA that hybridizes with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment wherein the targeter-RNA and the activator-RNA are covalently linked to one another with intervening nucleotides.” Should the claims of this patent be interpreted such that the “trans-activating cr (tracr) sequence” of Claim 4 is a portion of the guide RNA molecule recited in independent Claim 1, then the system of the ‘356 Patent includes a molecule having essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. Compare, e.g., the ‘356 Patent at col. 19, ll. 41-45; col. 63, ll. 12-23 and Figure 13B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶¶ 25 and 232.

Claim 4 of the ‘356 Patent recites “the guide RNA targets the target sequence.” Claim 168 of the ‘859 Application also recites “the single molecule DNA-targeting RNA forms a complex with the Cas9 protein, thereby targeting the Cas9 protein to the target DNA molecule.” See Exhibit 53 at ¶¶ 25 and 239.

Claim 4 of the ‘356 Patent recites “expression of at least one gene product is altered.” Claim 168 of the ‘859 Application also recites “whereby said target DNA molecule is cleaved or edited or transcription of at least one gene encoded by the target DNA molecule is modulated.” See Exhibit 53 at ¶¶ 25 and 242.

Claim 4 of the ‘356 Patent additionally recites “introducing [the CRISPR-Cas system] into a eukaryotic cell containing and expressing a DNA molecule having a target sequence and encoding the gene product,” “a) a first regulatory element operable in a eukaryotic cell operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA,” “b) a second regulatory element operable in a eukaryotic cell operably linked to a nucleotide sequence encoding a Type-II Cas9 protein,” and that “components (a) and (b) are located on same or different vectors of the system.” Claim 168 of the ‘859 Application does not recite these limitations. However, one of ordinary skill in the art would have readily realized these aspects to be obvious in view of Claim 168 of the ‘859 Application for the same reasons discussed with respect to Claim 4 of the ‘965 Patent in Section III(A)(3)(a).

Claim 4 of the ‘356 Patent also recites “the Cas9 protein comprises one or more mutations in a catalytic domain whereby the Cas9 protein is a nickase that cleaves only one strand of the DNA molecule.” Claim 168 of the ‘859 Application also does not recite this
limitation. However, the use of a Cas9 protein with nickase activity would also have been obvious to one of ordinary skill in the art in view of Claim 168 of the ‘859 Application. As discussed above in Section III(A)(3)(f), particular mutations in the HNH and RuvC endonuclease catalytic domains were known to disrupt Cas9 activity. See Exhibit 21 at 9280-9281; see also Exhibit 53 at ¶ 24 and 206-210. In fact, it had been previously suggested that alanine-substituting mutations at amino acid residues D31, H868, N882, and N891 of *Streptococcus thermophilus* Cas9 could produce proteins with nickase activity. See Exhibit 21 at 9281; see also Exhibit 53 at ¶ 212. In addition, as discussed above, it was known that mutations to catalytic domains of DNA cleaving enzymes could produce nickases with certain desirable properties. See, e.g., Exhibits 23, 75, and 102; see also Exhibit 53 at ¶ 212. Accordingly, it would have been obvious for one of ordinary skill in the art to use any of the Cas9 nickases described in the art. See Exhibit 53 at ¶ 212. Further, on June 28, 2012, Jinek et al. reported disrupting double-stranded cleavage activity with the *Streptococcus pyogenes* Cas9 protein when either the D10 amino acid residue in the RuvCI motif or the H840 amino acid residue in the HNH motif was substituted with alanine. See Exhibit 22 at 817; see also Exhibit 53 at ¶ 213. Thus, Cas9 mutations that result in nickase activity were already known to those of ordinary skill in the art. See Exhibit 53 at ¶¶ 24 and 212.

Accordingly, it would have been obvious for one of ordinary skill in the art to use any of known Cas9 nickase proteins described in the art. *Id.* Additionally, the ‘859 Application also includes a claim that recites the use of a Cas9 nickase enzyme. See Claim 185 of the ‘859 Application (“said Cas9 protein comprises an activity portion having an amino acid sequence that is modified compared to an amino acid sequence of a corresponding wild-type Cas9 protein and cleaves only one strand of DNA”).

h. Comparison of Claim 168 of the ‘859 Application and Claim 3 of the ‘445 Patent

A side-by-side comparison of Claim 168 of the ‘859 Application and Claim 3 of the ‘445 Patent is provided in Table 8 of Appendix B. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘445 Patent is provided in Table 10 of Appendix K.

Claim 3 of the ‘445 Patent differs from Claim 4 of the ‘356 Patent in that it recites “two or more nuclear localization signals” and does not require the Cas9 protein to have nickase activity. However, the use of two or more nuclear localization signals would have been obvious based on the reasons provided above with respect to Claim 10 of the ‘308 Patent. See Exhibit 53 at ¶ 24. Additionally, the ‘859 Application includes a claim that recites the use of nuclear localization domains. See Claim 182 of the ‘859 Application (“one or more PTD(s) comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:268 and 269”).

i. **Comparison of Claim 168 of the ‘859 Application and Claim 4 of the ‘814 Patent**

A side-by-side comparison of Claim 168 of the ‘859 Application and Claim 4 of the ‘814 Patent is provided in Table 9 of Appendix B. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘814 Patent is provided in Table 9 of Appendix K.

Claim 4 of the ‘814 Patent differs from Claim 4 of the ‘356 Patent in that it recites “two or more nuclear localization signals.” However, the use of two or more nuclear localization signals would have been obvious based on the reasons provided above with respect to Claim 10 of the ‘308 Patent. See Exhibit 53 at ¶ 24. Additionally, the ‘859 Application includes a claim that recites the use of nuclear localization domains. See Claim 182 of the ‘859 Application (“one or more PTD(s) comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:268 and 269”).


B. **Proposed Count 2 – Systems/Compositions**

Proposed Count 2 is directed to an engineered and/or non-naturally occurring CRISPR-Cas system comprising a Type-II Cas9 protein and a single molecule DNA-targeting RNA, whereby the single molecule DNA-targeting RNA targets a DNA molecule having a target sequence, and wherein the Type-II Cas9 protein and the single molecule DNA-targeting RNA do not naturally occur together. Proposed Count 2 is an alternative Count: Claim 166 of the ‘859

1. **Claims That Correspond to Proposed Count 2**

The following table sets forth the claims from the ‘859 Application and the Broad/MIT patents that correspond to Proposed Count 2.

<table>
<thead>
<tr>
<th>Proposed Count 2</th>
<th>'859 Application Claims That Correspond</th>
<th>Broad/MIT Claims That Correspond</th>
</tr>
</thead>
<tbody>
<tr>
<td>USSN 13/842,859</td>
<td>Claims 166, 167, 203-235, and 243-247</td>
<td>USPN 8,697,359 Claims 8-20</td>
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<tr>
<td></td>
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<td>USPN 8,771,945 Claims 19-29</td>
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<td></td>
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<td>USPN 8,795,965 Claims 17-30</td>
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<td>USPN 8,889,356 Claims 24-30</td>
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<td>USPN 8,865,406 Claims 24-30</td>
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<td>USPN 8,871,445 Claims 18-30</td>
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<td>USPN 8,906,616 Claims 1-18, 20, 23-28</td>
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<td>USPN 8,895,308 Claims 25-28 and 30</td>
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<td>USPN 8,932,814 Claims 25-30</td>
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<td>USSN 8,945,839 Claims 11-28</td>
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A more detailed explanation of why the above-listed Broad/MIT Patent claims should be designated to correspond to Tables 1-10 of Appendix F.

2. **Identification of Interfering Claims**


3. Interference-In-Fact


A side-by-side comparison of Claim 203 of the ‘859 Application and Claim 26 of the ‘965 Patent is provided in Table 1 of Appendix E. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘965 Patent is provided in Table 3 of Appendix K.

Claim 26 of the ‘965 Patent recites “[a]n engineered, programmable, non-naturally occurring Type II CRISPR-Cas system,” wherein “the Cas9 protein and the guide RNA do not naturally occur together.” Claim 203 of the ‘859 Application also recites “[a]n engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system.” See Exhibit 53 at ¶¶ 25 and 229-231.
Claim 26 of the ‘965 Patent recites “at least one guide RNA that targets and hybridizes to a target sequence of a DNA molecule.” Claim 203 of the ‘859 Application also recites “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: i) a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule.” See Exhibit 53 at ¶¶ 25 and 232.

Claim 26 of the ‘965 Patent recites “a Cas9 protein.” Claim 203 of the ‘859 Application also recites “a Cas9 protein, or a nucleic acid comprising a nucleotide sequence encoding said Cas9 protein.” See Exhibit 53 at ¶¶ 25 and 229-231.

Claim 26 of the ‘965 Patent recites “the guide RNA is comprised of a chimeric RNA and includes a guide sequence and a tracr sequence.” Claim 203 of the ‘859 Application recites “the single molecule DNA-targeting RNA comprises: i) a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule, and ii) an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein binding segment, wherein the activator-RNA and the targeter-RNA are covalently linked to one another with intervening nucleotides.” As discussed above in Section III(A)(3)(a), the guide RNA of the ‘965 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. Compare, e.g., the ‘965 Patent at col. 19, ll. 44-48 with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶¶ 25 and 233-235.

Claim 26 of the ‘965 Patent recites “the Cas9 protein cleaves the DNA molecule.” Claim 203 of the ‘859 Application also recites “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, whereby said system is capable of cleaving.” See Exhibit 53 at ¶¶ 25 and 239.
Claim 26 of the ‘965 Patent recites “the DNA molecule encodes . . . at least one gene product” and “expression of the at least one gene product is altered.” Claim 203 of the ‘859 Application also recites “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.” See Exhibit 53 at ¶¶ 25 and 242.

Claim 26 of the ‘965 Patent additionally recites “a target sequence of a DNA molecule in a eukaryotic cell” and “the eukaryotic cell expresses at least one gene product.” Claim 203 of the ‘859 Application does not recite these limitations. While these differences exist, one of ordinary skill in the art would have readily realized these aspects to be obvious in view of Claim 203 of the ‘859 Application for the same reasons provided in Section III(A)(3)(a).


A side-by-side comparison of Claim 203 of the ‘859 Application and Claim 18 of the ‘359 Patent is provided in Table 2 of Appendix E. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘359 Patent is provided in Table 1 of Appendix K.

Claim 18 of the ‘359 Patent differs from Claim 26 of the ‘965 Patent in that it recites “the guide RNAs [sic] comprise a guide sequence fused to a tracer sequence.” As discussed above in Section III(A)(3)(b), the guide RNA of Claim 18 of the ‘359 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. Compare, e.g.,
the ‘359 Patent at col. 12, ll. 6-10; col. 47, ll. 38-48 and Figure 11B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶ 25.


A side-by-side comparison of Claim 203 of the ‘859 Application and Claim 30 of the ‘308 Patent is provided in Table 3 of Appendix E. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘308 Patent is provided in Table 6 of Appendix K.

Claim 30 of the ‘308 Patent differs from Claim 26 of the ‘965 Patent in that it recites “the guide sequence and tracer sequence are chimeric,” the use of a Staphylococcus aureus Cas9 protein, “one or more nuclear localization signal(s),” and “DNA for insertion into a cleaved strand of the DNA molecule.” However, the guide RNA of Claim 30 of the ‘308 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. Compare, e.g., the ‘308 Patent at col. 22, ll. 23-27 and col. 28, ll. 5-11; col. 56, l. 62-col. 57, l. 7 and Fig. 13B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶¶ 25 and 233-235. The use of a Staphylococcus aureus Cas9 protein would have been obvious based on the reasons provided above with respect to Claim 5 of the ‘406 Patent. The use of one or more nuclear localization signals and DNA for insertion into a cleaved strand of the DNA molecule would also have been obvious based on the reasons provided above with respect to Claim 10 of the ‘308 Patent.
The ‘859 Application also includes a claim that recites the use of nuclear localization domains. See Claim 208 of the ‘859 Application ("one or more PTD(s) comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:268 and 269"). Additionally, the ‘859 Application also includes a claim that recites inserting a donor polynucleotide sequence into the cleaved strand of a target DNA molecule. See Claim 247 of the ‘859 Application ("the system comprises a donor polynucleotide and the system is capable of editing the target DNA molecule by inserting a sequence of the donor polynucleotide into a cleaved strand of the target DNA molecule").


A side-by-side comparison of Claim 203 of the ‘859 Application and Claim 21 of the ‘839 Patent is provided in Table 4 of Appendix E. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘839 Patent is provided in Table 7 of Appendix K.

Claim 21 of the ‘839 Patent differs from Claim 26 of the ‘965 Patent in that it recites "the guide RNA comprises a tracr sequence," "one or more NLSs," "a tracr sequence which is 30 or more nucleotides in length," a "Cas9 protein comprising one or more mutations in a catalytic domain," and does not recite "the Cas9 protein cleaves the DNA molecule." As discussed above in Section III(A)(3)(f), the guide RNA of Claim 21 of the ‘839 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. Compare, e.g.,
the ‘839 Patent at col. 12, ll. 15-19; col. 55, ll. 11-15 and Fig. 11B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B. Further, all of the other additional limitations in Claim 21 of the ‘839 Patent would have been obvious to one of ordinary skill in the art for the reasons provided in Sections III(A)(3)(c)-(f). The ‘859 Application includes a claim that recites the activator-RNA comprises “the 88 nucleotide tracrRNA sequence set forth in SEQ ID NO:433,” which is plainly a tracrRNA sequence of 30 or more nucleotides. See Claim 244 of the ‘859 Application. The ‘859 Application also includes a claim that recites the use of nuclear localization domains. See Claim 208 of the ‘859 Application (“one or more PTD(s) comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:268 and 269”). Finally, the ‘859 Application includes a claim that recites one or more mutations in a Cas9 catalytic domain. See Claim 245 of the ‘859 Application (“the Cas9 protein comprises a mutation selected from D10A and H840A with reference to the numbering of Streptococcus pyogenes Cas9 protein”).


e. Comparison of Claim 203 of the ‘859 Application and Claim 2 of the ‘616 Patent

A side-by-side comparison of Claim 203 of the ‘859 Application and Claim 2 of the ‘616 Patent is provided in Table 5 of Appendix E. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘616 Patent is provided in Table 5 of Appendix K.
Claim 2 of the ‘616 Patent recites “[a]n engineered, non-naturally occurring composition comprising a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system.” Claim 203 of the ‘859 Application also recites “[a]n engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system.” See Exhibit 53 at ¶¶ 25 and 229-231.

Claim 2 of the ‘616 Patent recites “guide RNA polynucleotide sequence, wherein the polynucleotide sequence comprises (a) a guide sequence capable of hybridizing to a target sequence . . . (b) a tracr mate sequence, and (c) a tracr sequence wherein (a), (b), and (c) are arranged in a 5’ to 3’ orientation,” “the polynucleotide sequence, one or more of the guide, tracr, and tracr mate sequences are modified,” and “the modified guide RNA comprises a chimeric guide sequence and a tracr sequence.” Claim 203 of the ‘859 Application recites “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: i) a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule, and ii) an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein binding segment, wherein the activator-RNA and the targeter-RNA are covalently linked to one another with intervening nucleotides.” The guide RNA of Claim 2 of the ‘616 Patent has essentially the same structure as the single molecule DNA-targeting RNA of the ‘859 Application. Compare, e.g., the ‘616 Patent at col. 17, ll. 33-37; col. 59, ll. 48-52 and Fig. 8B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶¶ 25 and 236.
Claim 2 of the ‘616 Patent recites “when transcribed, the tracr mate sequence hybridizes to the tracr sequence.” Claim 203 of the ‘859 Application recites “activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein binding segment.” As discussed above in Section II(B)(2), the targeter-RNA of the ‘859 Application comprises a tracr mate sequence which hybridizes with the tracr sequence present in the activator-RNA of the ‘859 Application. See Exhibit 53 at ¶¶ 25 and 237.

Claim 2 of the ‘616 Patent recites “the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, wherein the CRISPR complex comprises a Type II Cas9 protein complexed with (1) the guide sequence that is hybridized to the target sequence, and (3) the tracr mate sequence that is hybridized to the tracr sequence.” Claim 203 of the ‘859 Application also recites “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.” See Exhibit 53 at ¶¶ 25 and 238.

Claim 2 of the ‘616 Patent differs from Claim 203 of the ‘859 Application in that it does not recite a Cas9 protein. Claim 2 also additionally requires the “guide sequence [to be] capable of hybridizing to a target sequence in a eukaryotic cell.” Claim 203 of the ‘859 Application does not recite this latter limitation. While this difference exists, one of ordinary skill in the art would have readily realized these aspects to be obvious in view of Claim 203 of the ‘859 Application for the same reasons provided in Section III(A)(3)(a).

Patent. Thus, there is an interference-in-fact between Claim 203 of the ‘859 Application and Claim 2 of the ‘616 Patent.


A side-by-side comparison of Claim 203 of the ‘859 Application and Claim 25 of the ‘356 Patent is provided in Table 6 of Appendix E. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘356 Patent is provided in Table 8 of Appendix K.

Claim 25 of the ‘356 Patent recites “[a]n engineered, programmable, non-naturally occurring Type II CRISPR-Cas system,” wherein “the Cas9 protein and the guide RNA do not naturally occur together.” Claim 203 of the ‘859 Application also recites “[a]n engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system.” See Exhibit 53 at ¶¶ 25 and 229-231.


Claim 25 of the ‘356 Patent recites “at least one guide RNA that targets and hybridizes to a target sequence of a DNA molecule” and “wherein the CRISPR-Cas system comprises a tracer sequence.” Claim 203 of the ‘859 Application also recites “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: i) a targeter-RNA that is capable of hybridizing with a target sequence in a target DNA molecule.” Should the claims of this patent be interpreted such that the “trans-activating cr (tracr) sequence” of Claim 25 is a portion of the guide RNA molecule recited in independent Claim 24, then the system of the ‘356 Patent includes a molecule having essentially the same structure as the single molecule
DNA-targeting RNA of the ‘859 Application. Compare, e.g., the ‘356 Patent at col. 19, ll. 41-45; col. 63, ll. 12-23 and Figure 13B with the ‘859 Application at ¶¶ [130-31]; [136]; [177-80] and Figure 1B; see also Exhibit 53 at ¶ 25.

Claim 25 of the ‘356 Patent recites “the DNA molecule encodes . . . at least one gene product” and “expression of the at least one gene product is altered.” Claim 203 of the ‘859 Application also recites “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.” See Exhibit 53 at ¶¶ 25 and 242.

Claim 25 of the ‘356 Patent additionally recites “the eukaryotic cell expresses at least one gene product.” Claim 203 of the ‘859 Application does not recite this limitation. However, one of ordinary skill in the art would have readily realized this aspect to be obvious in view of Claim 203 of the ‘859 Application for the same reasons provided in Section III(A)(3)(a).

Claim 25 of the ‘356 Patent also recites “the Cas9 protein comprises one or more mutations in a catalytic domain whereby the Cas9 protein is a nickase that cleaves only one strand of the DNA molecule.” Claim 203 of the ‘859 Application also does not recite this limitation. However, the use of a Cas9 protein with nickase activity would also have been obvious to one of ordinary skill in the art in view of Claim 203 of the ‘859 Application as discussed above in Section III(A)(3)(g). See also Exhibit 53 at ¶ 24. Additionally, the ‘859 Application also includes a claim that recites the use of a Cas9 nickase enzyme. See Claim 213 of the ‘859 Application (“said Cas9 protein comprises an activity portion having an amino acid sequence that is modified compared to an amino acid sequence of a corresponding wild-type Cas9 protein and is capable of cleaving only one strand of DNA”).

g.  **Comparison of Claim 203 of the ‘859 Application and Claim 28 of the ‘445 Patent**

A side-by-side comparison of Claim 203 of the ‘859 Application and Claim 28 of the ‘445 Patent is provided in Table 7 of Appendix E. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘445 Patent is provided in Table 10 of Appendix K.

Claim 28 of the ‘445 Patent differs from Claim 25 of the ‘356 Patent in that it recites “two or more nuclear localization signals” and does not require the Cas9 protein to have nickase activity. However, the use of two or more nuclear localization signals would have been obvious based on the reasons provided above with respect to Claim 10 of the ‘308 Patent. *See also Exhibit 53 at ¶ 24.* Additionally, the ‘859 Application includes a claim that recites the use of nuclear localization domains. *See Claim 208 of the ‘859 Application (“one or more PTD(s) comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:268 and 269”).*

h. Comparison of Claim 203 of the ‘859 Application and Claim 26 of the ‘814 Patent

A side-by-side comparison of Claim 203 of the ‘859 Application and Claim 26 of the ‘814 Patent is provided in Table 8 of Appendix E. A more exhaustive comparison of claims of the ‘859 Application to claims of the ‘814 Patent is provided in Table 9 of Appendix K.

Claim 26 of the ‘814 differs from Claim 25 of the ‘356 Patent in that it recites “two or more nuclear localization signals.” However, the use of two or more nuclear localization signals would have been obvious based on the reasons provided above with respect to Claim 10 of the ‘308 Patent. See also Exhibit 53 at ¶ 24. Additionally, the ‘859 Application includes a claim that recites the use of nuclear localization domains. See Claim 208 of the ‘859 Application (“one or more PTD(s) comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:268 and 269”).


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

There are several reasons why Applicants will prevail on priority. These reasons include Applicants’ status as Senior Party, the strength of its First Provisional, and Broad/MIT’s inability to antedate Applicants.

A. Applicants’ Earliest Benefit Date Entitles Them to Senior Party Status

As discussed above, the ‘859 Application was filed on March 15, 2013, and claims a priority benefit from the First Provisional, filed on May 25, 2012; the Second Provisional, filed
on October 19, 2012; the Third Provisional, filed on January 28, 2013; and U.S. Provisional Application No. 61/765,576, filed on February 15, 2013. Even if Broad/MIT were granted the benefit of its earliest U.S. Provisional Application—U.S. Provisional Application No. 61/736,527, filed on December 12, 2012—Applicants would still be designated Senior Party in the interference, because both the First and Second Provisionals were filed months before the Broad/MIT Provisional. Because Applicants’ First Provisional describes and enables not only an embodiment within each Proposed Count, but also supports Claims 165-247, Applicants should retain Senior Party status. See Appendices G-J; see also Exhibit 53 at ¶¶ 68-74.

B. Applicants’ Constructive Reduction To Practice Predates Broad/MIT’s First Disclosure

A constructive reduction to practice means a described and enabled anticipation under § 102(g)(1) in a patent application of the subject matter of the counts. 37 C.F.R. § 41.201. As discussed above, the ‘859 Application claims priority to the First Provisional, filed on May 25, 2012. Pursuant to 37 C.F.R. § 41.202(a)(6), Appendices G-J provide charts illustrating where exemplary support may be found for Claims 165-247 of the ‘859 Application in the First Provisional, the Second Provisional, and the Third Provisional. See also Exhibit 53 at ¶¶ 68-74. Because Claims 168 and 203 are each an alternative of Proposed Count 1 and Proposed Count 2, respectively, the First Provisional provides a constructive reduction to practice for each proposed Count. As a result, Applicants have a constructive reduction to practice prior to the earliest filing date of the Broad/MIT patents.

C. Broad/MIT’s Evidence Submitted in Its Applications Does Not Show a Conception or Reduction to Practice That Antedates Applicants’ Constructive Reduction to Practice

The documents relied upon by Broad/MIT in its applications to attempt to show priority do not evidence conception or a reduction to practice that antedates Applicants’ constructive
reduction to practice on May 25, 2012, the filing date of the First Provisional. During prosecution of the Broad/MIT Patents, a declaration was submitted by putative Broad/MIT inventor Feng Zhang (“the Zhang Declaration”). See Declaration Under 37 C.F.R. §§ 1.132 and 1.131, filed in U.S. Patent Application No. 14/054,414 on January 30, 2014 (Exhibit 70). The Zhang Declaration was accompanied by several exhibits, including excerpts from laboratory notebooks, designated as Exhibit 7 to the Zhang Declaration (“the Zhang Experimental Data”), and a manuscript. In his Declaration, Dr. Zhang asserted that the exhibits evidence that the subject-matter claimed in the Broad/MIT Patents had been actually reduced to practice before Applicants constructively reduced their invention to practice. Dr. Zhang is wrong. Dr. Zhang’s referenced work was not an actual reduction to practice of the claimed invention. Additionally, as shown in detail below, Dr. Zhang’s attempts to demonstrate that the First and Second Provisionals fail to teach, suggest, describe, or enable Type-II CRISPR-Cas systems and methods of using the same in eukaryotic cells also are incorrect. See Exhibit 70 at ¶¶3-4.10; see also Exhibit 53 at ¶¶128-197.

1. Exemplary Faults of the Zhang Declaration Showing Why the Referenced Work Was Not an Actual Reduction to Practice

Dr. Zhang states that “I submit that Exhibit 7 shows that prior to May 2012, I conceived and reduced to practice the presently claimed subject matter.” Exhibit 70 at ¶ 5.1.5. To further clarify, Dr. Zhang then sets forth the independent claims that were currently pending at that time. Id. These claims require certain elements to be present. As shown in the table below, and as discussed in detail in the sections that follow, many of these elements are lacking from the Zhang Experimental Data and Zhang Declaration. See Exhibit 53 at ¶¶141-142.
<table>
<thead>
<tr>
<th>Claimed Component or Step</th>
<th>What is Shown in Zhang’s Declaration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introducing a CRISPR-Cas system into a eukaryotic cell</td>
<td>Not shown</td>
</tr>
<tr>
<td>A first regulatory element operable in a eukaryotic cell operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA</td>
<td>Not shown</td>
</tr>
<tr>
<td>Guide RNA that hybridizes with the target sequence in a eukaryotic cell</td>
<td>Not shown</td>
</tr>
<tr>
<td>Cas9 protein</td>
<td>Cas9 is only used in combination with other Cas proteins; Cas9 is not utilized as the sole Cas protein in any of the Zhang experiments</td>
</tr>
<tr>
<td>Cas9 protein cleaves the target DNA</td>
<td>Not shown</td>
</tr>
<tr>
<td>tracr sequence</td>
<td>Not shown</td>
</tr>
<tr>
<td>Guide RNA comprising a guide sequence fused to a tracr sequence</td>
<td>Not shown</td>
</tr>
</tbody>
</table>

**a. Cas9 DNA Cleavage Activity Is Not Disclosed in the Laboratory Notebook**

There is no evidence in the Zhang Declaration or the associated notebook to support a claim in which Cas9 cleaves DNA. See Exhibit 53 at ¶ 142. To demonstrate actual reduction to practice of the claimed invention, Dr. Zhang’s experiments would need to demonstrate cleavage of the target DNA by Cas9 and alteration of gene expression of at least one gene product on the target DNA molecule. Id. at ¶ 143. To demonstrate conception of the claimed invention, the notebook pages included in Zhang’s Declaration would need to show that a method of cleaving DNA and altering gene expression using Cas9 was fully conceived by Dr. Zhang. Id. However,
the Zhang Experimental Data and Declaration do not demonstrate either cleavage or alteration of
gene expression by Cas9. *Id.*

In his Declaration, Dr. Zhang cites to the results shown on page Z-27 of the Zhang
Experimental Data and concludes from those results that “[a] reduction of luciferase expression
was seen at each subsequent time point from 12 hr, 24 hr and 36 hr at which the relative
luciferase activity was measured for Sp2 at a concentration of 50 ng.” Exhibit 70 at ¶ 5.1.2.6.
Page 13 of the Zhang Declaration also provides a “summary” panel of the results to which Dr.
Zhang refers, which has been reproduced below. However, a review of the results presented in
the Zhang Experimental Data demonstrates that Dr. Zhang is misstating the results of that study
and is presenting misleading conclusions. *See Exhibit 53 at ¶ 144.*

![Graph showing relative luciferase activity](image)

*All Cas Protein - S. Thermophilus - Luc Test*

- Control (-)
- BB3-SP2
- BB3-SP3

Experiment groups

(Adapted from Exhibit 70 at 13)
i) The Full Data Do Not Show a Consistent Change in Luciferase Activity

As shown in this “summary” data, Dr. Zhang extracted a single subset of data from the results to demonstrate that the CRISPR-Cas system caused a reduction in luciferase activity, and thereby infers that the Cas9 protein cleaved the target luciferase gene. See Exhibit 53 at ¶ 145. The extracted data set includes only reactions labeled “All Cas Genes-50ng” across three time points: 12, 24, and 36 hours. Id. Utilizing just that narrow data set, Dr. Zhang asserts that, when the Sp2 spacer (targeting sequence) was used, there was a reduction in luciferase activity versus control, which Dr. Zhang asserts demonstrates that Cas9 is cleaving the target molecule. Id.

However, as shown below in the reproduction of page Z-27, there are far more data actually included on page Z-27. Id. at ¶ 146. This additional data shows Dr. Zhang’s conclusion based on his selected data is wrong. Id. Rather, when the more complete data are analyzed, they actually demonstrate that any change in luciferase activity that is observed appears to not be the result of cleavage by Cas9. Id.
Looking at page Z-27, in addition to the “All Cas Genes” reaction mix that Dr. Zhang relies upon, there are two other reaction mixes that were tested, labeled as “Triple” and “Double,” which Dr. Zhang fails to discuss in his Declaration. See Exhibit 53 at ¶ 147. Further, in addition to the “50ng” data set on which Dr. Zhang relies, there is also a “250ng” data set that Dr. Zhang again ignores.² *Id.* This can be clearly seen in the annotated excerpt from page Z-27 shown below.

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² Paragraph 5.1.2.6 of the Zhang Declaration (Exhibit 70) states that a reduction in luciferase activity “was measured for Sp2 at a concentration of 50ng,” which Dr. Carroll has taken as an indication that the “50ng” and “250ng” figures in the results refer to the amount of spacer nucleic acid added. If this is incorrect, then there is no indication of the component to which these numbers refer. Exhibit 53 at footnote 2.
Beginning with the 250ng data set, an analysis of the “All Cas Genes” subset in these panels demonstrates that the reduction in luciferase activity to which Dr. Zhang cites is not consistent, i.e., no such reduction in luciferase activity can be seen when the amount of spacer nucleic acid is increased from 50ng to 250ng. Id. at ¶ 148. As shown below in the annotated excerpts from page Z-27, if one compares the “All Cas Genes” portions of the “250ng” panels on page Z-27, it is clear that the Sp2 samples not only failed to show a reduction in luciferase activity but they actually showed a large increase in luciferase activity as compared to control over time, increasing from approximately 140% of control at 12 hr to about 240% of control at 36 hr. Id.

**Comparison of 250ng “All Cas Genes” Results for Control and Sp2 Guide Sequences**
Normally, one expects a monotonic dose response when the concentration of a critical reagent is changed, meaning that one would expect a greater decrease in luciferase activity in the 250ng data set than in the 50ng data set. *Id.* at ¶ 149. At least, one would expect some response, *i.e.*, some decrease in luciferase activity, yet no such decrease was shown for any of the 250 ng data set. *Id.* Thus, even if one were to accept that the “All Cas Genes” data set were the most relevant data, which they are not (as discussed in detail below), even these data do not appear to show that the CRISPR-Cas complex is able to consistently decrease luciferase activity. *Id.* This alone brings the accuracy of Zhang’s conclusion into question. *Id.*

**ii) The Data Do Not Show That Cas9 Is the Cause of Any Change in Luciferase Activity That Is Present**

Perhaps even more important is the fact that Dr. Zhang relies upon data that included not just Cas9, but *four different Cas proteins*. *Id.* at ¶ 150. And, when one of those *other* Cas proteins was eliminated from the reaction, the reduction in luciferase activity to which Dr. Zhang cites disappears. *Id.* This is a clear indication that, if any cleavage or reduction in luciferase activity were occurring, it was not Cas9, but a different Cas protein that was responsible. *Id.*

In reaching his conclusion, Dr. Zhang cites the “All Cas Genes” data while ignoring the “Triple” and “Double” reaction data. *Id.* at ¶ 151. As shown below in the annotated excerpts from page Z-27, an analysis of the results for the “Triple” and “Double” reaction results demonstrates that those reactions do not produce any noticeable decrease in luciferase activity. *Id.* Looking at the “Triple” reactions in the “50ng” data set, shown below, the results for the Sp2 spacer (which allegedly targets the luciferase gene) show no clear reduction in luciferase activity over time, though there is a slight trend of increased luciferase activity from 12 to 36 hours as compared to control. *Id.*
Comparison of 50ng “Triple” Cas Genes Results for Control and Sp2 Guide Sequences

The “Double” reaction data in the same “50ng” data set, shown below, shows the control and Sp2 samples to have nearly identical luciferase activity throughout the time course. *Id.* at ¶ 152.

Comparison of 50ng “Double” Cas Genes Results for Control and Sp2 Guide Sequences

In the “250ng” “Triple” and “Double” data sets, there is again essentially no change in luciferase activity as compared to control over the three time points. *Id.* at ¶ 153.
Because the Triple and Double reactions show no noticeable change in luciferase activity as compared to control, any decrease in luciferase activity observed in the All Cas Genes reactions should be attributable to a component that is present in the All Cas Genes reactions but absent from the Triple and Double reactions. *Id.* at ¶ 154. Put another way, if whatever caused the reduction in luciferase activity in the All Cas Genes reactions was also present in the Triple and/or Double reactions, it would also be expected to cause a reduction in luciferase activity in those reactions. *Id.* Because of that, any purported reduction in luciferase activity cannot be the result of the activity of Cas9. *Id.*
The Zhang Experimental Data indicate that the difference between the All Cas Genes reactions and the Triple and Double reactions is the set of Cas proteins that was included in the reaction.\(^3\) Id. at ¶ 155. Page Z-2 indicates that all of the reactions contain a master mix that contains luciferase (“Luc 1ng/uL”), green fluorescent protein (“GFP 10ng/uL”), Cas5 (“Cas5 120 ng/uL”), and Cas2 (“Cas2 120 ng/uL”). Id. The Zhang Declaration also indicates that Cas5 is another name for Cas9. Exhibit 70 at ¶ 5.1.2.1. Thus, all reactions in the cited experiment contained both Cas9 and Cas2. See Exhibit 53 at ¶ 155. In addition, the chart at the top of the page indicates that the “Triple” reactions further contained Cas1, while the “All Cas Genes” reactions further contained both Cas1 and Cas7. Id. This aligns well with the naming convention utilized in the experiments: the “Double” reactions contained two Cas proteins (Cas9 and Cas2), the “Triple” reactions contained three Cas proteins (Cas9, Cas2, and Cas1), and the “All Cas Genes” reactions contained all four of the Cas proteins being evaluated (Cas9, Cas2, Cas1, and Cas7). Id.

A similar, but somewhat different version of this experimental design is provided in graphic form on page Z-25. Id. at ¶ 156. In the chart labeled “S. thermophiles,” which is also reproduced below, page Z-25 indicates that the two-protein (i.e., Double) reaction included just Cas5 (Cas9) and Cas2; the three-protein (i.e., Triple) reaction included Cas9, Cas2, and Cas7; and the four-protein (i.e., All Cas Genes) reaction included Cas9, Cas2, Cas7, and Cas1. Id.

\(^3\) Dr. Carroll states “[i]n the absence of information that clearly indicated what experiments were used to derive the results shown on page Z-27, [he is] forced to make the assumption that the data on page Z-27 derive from the experimental setup shown on page Z-2.” See Exhibit 53 at ¶ 155 n. 3. However, as discussed in detail below, there are certain discrepancies between the experimental setup on page Z-2 and the results on page Z-27 that bring into question whether these two pages relate to the same set of experiments.
Thus, regardless of which experimental set-up is accepted as correct, the Zhang Experimental Data provide a clear indication that the Double reactions include Cas9 and Cas2; the Triple reactions include Cas9 and Cas2 plus one additional Cas protein (either Cas7 or Cas1); and the All Cas Genes reactions include Cas9, Cas2, Cas1, and Cas7. See Exhibit 53 at ¶ 157. Because all of the samples include Cas9, if Cas9 were responsible for the reduction in luciferase activity that Dr. Zhang cites in the 50ng data set, that reduction in luciferase activity would be expected to be seen in all of the samples. Id. at ¶ 158. Yet, it is not. Id. As discussed above, the Double and Triple data do not show a reduction in luciferase activity. Id. Because of that, the purported reduction in luciferase activity appears to be caused not by Cas9, but by the additional Cas protein that is included in the All Cas Genes reactions. Id. The additional Cas9 protein is either Cas7 or Cas1, depending on which page of the Zhang Experimental Data shows the set up that represents the experiments reported on page Z-27. Id.

At best, Dr. Zhang demonstrated reduction in luciferase activity possibly caused by Cas1 or Cas7. Id. at ¶ 159. Nothing in the Zhang Experimental Data or Zhang Declaration demonstrates cleavage of the target DNA by the Cas9 protein. Id. Nor does the Zhang Declaration demonstrate alteration of gene expression by Cas9. Id. As such, the Zhang Declaration and Experimental Data do not demonstrate actual reduction to practice or even conception of the claimed invention. Id.
iii) **The Data Include No Indication That Any Change in Luciferase Activity Is Caused by DNA Cleavage**

It should also be noted that the results presented on page Z-27 of the Zhang Experimental Data allegedly measure the luciferase activity in various experiments, though neither the notebook nor the Zhang Declaration describe how this luciferase activity is measured. *Id.* at ¶ 160. In fact, the experimental set-up shown on page Z-2 demonstrates that there were two different luciferases included in the experiment, “Gluc” and “Cluc,” which most likely indicate the use of *Gaussia* luciferase and *Cypridina* luciferase, as well as a green fluorescent protein (“GFP”). *Id.*

There is nothing else in the experimental protocol or results that indicate how these were used, or even which luciferase was being analyzed. *Id.* Additionally, close inspection of the experimental design provided on page Z-2 and the results shown on page Z-27 indicates that other discrepancies exist between the description on page Z-2 and the results on page Z-27. *Id.* On page Z-2, it is stated “All Sp are in 100ng/ul,” and “G/C Luc MM = Luc 1 ng/ul + GFP 10ng/ul + Cas5 120ng/ul + Cas2 120 ng/ul.” *Id.* All reaction mixtures described in the table on Z-2 contain 5 μl of the G/C Luc/GFP mix and 6 μl of spacer Sp2, Sp3, or Jk, along with 6 μl of additional Cas protein, if included. *Id.* Thus, the table on page Z-2 describes an experiment in which the quantity of all components is the same for each reaction, except for the Cas proteins used. *Id.* Yet the results of page Z-27 demonstrate that some reactions included “50ng” of a component, while other reactions contained “250ng.” *Id.* Deficiencies such as these in the laboratory notebook materials bring question to the quality and trustworthiness of the data being presented. *Id.*

However, regardless of which luciferase was analyzed and how the luciferase activity was measured, it is clear that cleavage of the luciferase gene, or any target DNA for that matter, was not being analyzed in that experiment. *Id.* at ¶ 161. To determine if DNA is cleaved, one
generally demonstrates that a specific size of direct cleavage product is produced (such as by visualizing cleavage products on a gel) or demonstrates that DNA repair took place at the proposed cleavage site (such as by using the SURVEYOR assay or DNA sequencing to look for indels). \textit{Id.} No such experiments are disclosed in the notebook or the Zhang Declaration. \textit{Id.} The notebook does disclose a proposed experiment on pages Z-25 and Z-26 that contemplates DNA extraction and the SURVEYOR assay, but there is no indication that this experiment was ever conducted. \textit{Id.} Furthermore, there are no results of such an experiment included in either the notebook pages or the Zhang Declaration. \textit{Id.}

Thus, even if Cas9 was shown to have an impact on luciferase activity, which it was not, the data still fail to demonstrate that the impact was due to \textit{cleavage} by Cas9 as opposed to some other action by Cas9. \textit{Id.} at ¶ 162. This further demonstrates that the Zhang Experimental Data and Zhang Declaration fail to demonstrate actual reduction to practice or even conception of method claims that require that Cas9 cleaves the target DNA molecule. \textit{Id.}

\textbf{b. Lack of Cas9 Cleavage Can Be Explained by the Absence of tracrRNA in Laboratory Notebook Experiments}

The claims require that tracrRNA, or a tracr sequence, be present for the invention to operate as claimed. There, however, is no evidence that there was any tracrRNA or tracr sequence present in any of the experiments disclosed in the Zhang Declaration or notebook. \textit{See Exhibit 53} at ¶¶ 163-164.

Dr. Carroll states that “[a] thorough analysis of the Zhang Experimental Data not only demonstrates that Cas9 is not cleaving the target DNA, but it also suggests a reason why.” \textit{Id.} As was stated in the First Provisional and the Jinek 2012 publication, the Type II CRISPR-Cas system is a three-component system. \textit{See First Provisional at ¶¶ [00248]-[00252] and Fig. 1A; Exhibit 22; see also Exhibit 53} at ¶ 163. The Cas9 protein is but one of those components. \textit{See}
Exhibit 22; see also Exhibit 53 at ¶ 163. Directing Cas9 to the target DNA and inducing cleavage of that target DNA by Cas9 also requires two separate stretches of RNA: a targeting RNA (crRNA) and a tracrRNA. See Exhibit 53 at ¶ 163. In the absence of either of these RNAs, the system will not operate effectively. Id. And, in fact, Dr. Zhang’s research group eventually found this to be true, and indicated this in their 2013 publication in the journal Science. See Exhibit 51 at 820 (stating that “[r]emoving any of the remaining RNA or Cas9 components abolished the genome cleavage activity of the CRISPR/Cas system (Fig. 1D). These results define a minimal three-component system for efficient RNA-guided genome modification in mammalian cells.”); see also Exhibit 53 at ¶ 163.

Nothing in the Zhang Experimental Data or Zhang Declaration indicates that tracrRNA was included. Id. at ¶ 164. In fact, page Z-2 includes a rather detailed recipe of the reaction components included, and that list does not have anything that suggests that a tracrRNA was included. Id. The absence of tracrRNA, which is an element of Broad/MIT’s patent claims, would explain why Cas9 was not able to cleave the luciferase target and in turn reduce luciferase activity in Dr. Zhang’s experiment. Id.

c. Eukaryotic Cell Claim Limitations Not Disclosed in the Laboratory Notebook

Dr. Zhang makes numerous unsupported assertions in paragraphs 5.1.2.1 and 5.1.2.2 of his Declaration that certain limitations relating to eukaryotic cells are shown in the appended experimental data. Id. at ¶ 165. A review of the cited notebook pages demonstrates that they contain no such information. Id.

i) Introducing a CRISPR-Cas System Into a Eukaryotic Cell Is Not Disclosed in the Laboratory Notebook

Dr. Zhang states that page Z-2 shows the setup of an experiment in which numerous components “are transfected into a eukaryotic cell (human embryonic kidney cells, 293T).” See
Exhibit 70 at ¶ 5.1.2.1. Similarly, in paragraph 5.1.2.5, Dr. Zhang states that “Pages Z-25 to Z-26 of Exhibit 7 shows the experimental protocol followed to transfect eukaryotic cells . . . with the various vectors.” These pages provide no such teachings. See Exhibit 53 at ¶ 166.

Page Z-2, reproduced below, merely shows the amount of various components that were included in certain reactions. Id. at ¶ 167. There is nothing on page Z-2 that indicates that any of the stated components were even vectors, much less that they were transfected or otherwise introduced into eukaryotic cells. Id.

Similarly, pages Z-25 and Z-26, reproduced below, do not provide an experimental protocol that has anything to do with transfection. Instead, the only protocol shown on those pages is a DNA extraction procedure. Id. at ¶ 168.
Thus, there is nothing in the cited pages, nor anywhere else in the Zhang Experimental Data, that demonstrates introduction of components into a eukaryotic cell. *Id.* at ¶ 169. Yet all of the Broad/MIT Patents claim a method wherein the first step is “introducing into a eukaryotic cell . . . an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)—CRISPR associated (Cas) (CRISPR-Cas) system.” This limitation is not shown by the Zhang Experimental Data and Declaration, making the Zhang Declaration and data insufficient to demonstrate actual reduction to practice of all the method claims in the Broad/MIT Patents that recite this limitation. *Id.*

ii) **A First Regulatory Element Operable in Eukaryotic Cell Is Not Disclosed in the Laboratory Notebook**

Nowhere in the Zhang Declaration is a first regulatory element operable in a eukaryotic cell linked to at least one guide RNA disclosed. *See Exhibit 53* at ¶¶ 170-171. The Zhang Declaration does not make any specific statement that indicates that this limitation of the claims
was shown in the cited experiments. *Id.* at ¶ 170. Nor does the Zhang Experimental Data provide any such showing—there is nothing in the laboratory notebook pages that demonstrates that a guide RNA is somehow operably linked to a regulatory element that is operable in a eukaryotic cell. *Id.* Dr. Zhang states that page Z-24 of the notebook “shows the backbone vector into which the spacers (guide sequences) were cloned.” *See Exhibit 70 at ¶ 5.1.2.4.* Page Z-24 has been reproduced below.

(Adapted from *Exhibit 70 at Exhibit 7, Z-24*)
The “backbone vector” sequence shown is not a complete vector (e.g., it is not a plasmid and does not contain an origin of replication or a selectable marker), and in fact it does not contain a regulatory element that is operable in a eukaryotic cell. See Exhibit 53 at ¶ 171. For example, a nucleotide BLAST® search reveals that the “Leader” sequence disclosed on page Z-24, which is just upstream (5’) of the sequences of the supposed guide sequence, shares 100% homology with the CRISPR repeat sequence of S. thermophilus strain JIM 1293—it is the naturally occurring Leader sequence from Streptococcus thermophilus CRISPR locus 1. See Exhibit 43; see also Exhibit 53 at ¶ 171. The “Leader” sequence is, therefore, in no way a promoter that is operable in a eukaryotic cell. See Exhibit 53 at ¶ 171. And there is nothing else in the Zhang Experimental Data that demonstrates the use of a regulatory element operable in a eukaryotic cell with a guide RNA. Id. The Zhang Experimental Data and Declaration have therefore also failed to demonstrate this limitation. Id.

d. Guide RNA That Hybridizes With or Targets the Target DNA Sequence is Not Disclosed in the Laboratory Notebook

Nowhere in the Zhang Declaration or associated notebook is a guide RNA that hybridizes with a target DNA disclosed. See Exhibit 53 at ¶¶ 172-174. As noted above, the claims require that the guide RNA hybridizes with the target sequence in the DNA molecule.

For such hybridization and/or targeting to occur, the guide RNA should have a targeting portion that is complementary to the target sequence of interest. Id. at ¶ 172. In his Declaration, Dr. Zhang indicates that luciferase was to be transfected into the eukaryotic cells and that activity of the CRISPR-Cas system was to be measured as a reduction in luciferase activity. See Exhibit 70 at ¶¶ 5.1.2.1, 5.1.2.6. This implies that the intended target sequence was the luciferase gene. See Exhibit 53 at ¶ 172. Dr. Zhang also mentions selection of guide sequences used in the experiment, which are identified as “Sp2” and “Sp3,” as well as a control indicated as a junk

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spacer, termed “Jk.” *See Exhibit 70* at ¶¶ 5.1.2.1, 5.1.4. Nowhere does the Zhang Experimental Data or Declaration indicate what the Sp2 and Sp3 guide sequences are or even that they would hybridize to, and therefore target, the luciferase gene. *See Exhibit 53* at ¶ 172.

In fact, the only spacer sequence that is provided in the Zhang Declaration and Zhang Experimental Data is on page Z-24, where the “Thermophilus_Backbone3” sequence is included. *See Exhibit 53* at ¶ 173. However, a review of that Thermophilus_Backbone3 sequence demonstrates that it could not target the luciferase gene and does not contain the Sp2 and Sp3 spacers discussed elsewhere in the Zhang Declaration and Zhang Experimental Data. *Id.* The two spacer sequences identified as “spacer1” and “spacer2” on page Z-24 are identical to one another, and comparisons of “spacer1” and “spacer2” to the complete luciferase gene sequences from numerous species of firefly (*Photinus pyralis*, *Photuris pennsylvanica*, *Luciola cruciata*, and *Luciola lateralis*; GenBank Accession Nos. M15077, D25416, D25415, M26194, X66919, Z69619, and Z49891) as well as *Gaussia* (GenBank Accession No. AY015993) and *Cypridina* (GenBank Accession Nos. AB159608 and U89490) demonstrate that the sequence of these spacers is not found in the luciferase gene, and therefore it would not target the luciferase gene. *See Exhibit 49; Exhibit 44* (BLAST results of the spacer 1/2 sequence against the NCBI “nr” database); *see also Exhibit 53* at ¶ 173. Thus, there is nothing in the Zhang Experimental Data or Zhang Declaration that provides a guide RNA that hybridizes with and/or targets a particular target sequence. *See Exhibit 53* at ¶ 174.

The Zhang Declaration and associated exhibits, therefore, do not show that there was an actual reduction to practice prior to the Applicants’ constructive reduction to practice. *See Exhibit 53* at ¶¶ 23 and 175. Accordingly, Applicants will prevail on priority.
2. **Dr. Zhang’s Arguments Regarding Lack of Enablement of Eukaryotic Cells Are Incorrect**

In his Declaration, Dr. Zhang alleges that the First Provisional is not enabling for compositions comprising a Type-II CRISPR-Cas system operable in eukaryotic cells and methods of altering gene expression or genome editing using a Type-II CRISPR-Cas system. See Exhibit 70 at ¶ 4.1-4.10. However, paragraphs 4.1-4.10 of the Zhang Declaration simply provide a series of unsupported assertions. See Exhibit 53 at ¶ 132. Paragraphs 4.1-4.9 of the Zhang Declaration—which encompass nearly every paragraph in this section of the Zhang Declaration—essentially state “I understand that the Examiner alleges that at least paragraphs [X, Y, and Z] of the ‘086 priority application discloses [a certain feature of the CRISPR-Cas system in eukaryotic cells]. I submit that in the ‘086 application there is no teaching whatsoever that enables and describes [that feature].” Id. These paragraphs are just a series of unsupported assertions that the First Provisional fails to teach various aspects of the invention in eukaryotic cells. Id. Dr. Zhang provides no discussion or rationale as to why the specific paragraphs of the First Provisional cited by the Examiner, or the application as a whole, fail to teach the stated features. Id. And, as discussed in Dr. Carroll’s Declaration and below, those paragraphs of the First Provisional indeed do teach what the Examiner asserts is taught. Thus, paragraphs 4.1-4.9 of the Zhang Declaration provide no support for Dr. Zhang’s conclusion that the applications fail to describe and enable the CRISPR-Cas system in eukaryotic cells. Id.

With regard to the other two paragraphs of this section of the Zhang Declaration, paragraphs 4 and 4.10, these merely state that the First and Second Provisionals do not include a working example of the use of the systems and methods in eukaryotic cells. Id. at ¶ 133. Dr. Zhang then provides an unsupported assertion that “[i]n the instance of the CRISPR-Cas system working in a eukaryotic cell . . . , I assert that there must be simultaneous conception and
reduction to practice for there to be invention, and hence an actual working example in a previously-filed patent application for that patent application to have an enabling written description to anticipate or destroy novelty as to the CRISPR-Cas system working in a eukaryotic cell . . .” See Exhibit 70 at ¶ 4. This again fails to provide support for Dr. Zhang’s assertion that the First and Second Provisionals fail to describe and enable systems and methods in eukaryotic cells. See Exhibit 53 at ¶ 133.

Dr. Zhang appears to suggest that “simultaneous conception and reduction to practice” is required because there is a high level of unpredictability associated with utilizing prokaryotic proteins in eukaryotic cells. Id. at ¶ 134. Some areas of unpredictability that Dr. Zhang suggested relate to expressing the proteins “at a level necessary to achieve activity” and ensuring “that the protein and RNAs are appropriately processed and folded into the proper conformation, and that they are efficiently transported into the desired intracellular location (e.g. organelle), and are not degraded by endogenous protein recycling machineries.” See Exhibit 70 at ¶ 4. No such unpredictability existed. Id. at ¶ 134. Expressing a prokaryotic protein in a eukaryotic cell at levels sufficient for activity without premature degradation, in properly folded conformation, and transported to the appropriate cellular compartment (for example, the nucleus) was quite routine for persons of ordinary skill in this art at the time of the invention, as evidenced by the numerous prokaryotic proteins that have been successfully applied to eukaryotic cells to effectuate genetic manipulation, such as ZFNs, TALENs, RecA, ΦC31 integrase, and Cre/Lox systems, and even simple restriction endonucleases. Id. Moreover, the evidence provided above also demonstrates that there was no great difficulty in utilizing the Type-II CRISPR-Cas systems and methods in eukaryotic cells—it was accomplished by multiple independent research groups in just a few short months after publication of the Jinek 2012 paper. Id.
Even Dr. Zhang’s specific argument regarding the difficulties arising from different ion concentrations is misguided. *Id.* at ¶ 135. In paragraph 4 of his Declaration, Dr. Zhang cites articles by Lambowitz & Zimmerly (2011) and Mastroianni et al. (2008) as evidence of the difficulties that can arise from differing ion concentrations between prokaryotic and eukaryotic cells. *See Exhibit 70* at ¶ 4. However, as noted above, the magnesium ion concentration in eukaryotic cells is perfectly adequate to support the activities of many prokaryotic DNA-modifying proteins. *See Exhibit 53* at ¶ 135. In fact, the Group II intron is the exception to this rule. *Id.*

Moreover, the Jinek 2012 paper provides data that demonstrate that the relatively low magnesium ion concentration in eukaryotic cells would not cause an issue with the Type-II CRISPR-Cas system in eukaryotic cells. *Id.* at ¶ 136. As discussed above, Mastroianni et al. indicates that the magnesium ion concentration in eukaryotic nuclei is estimated to be 1-2 mM. *See Exhibit 97* at 2; *see also Exhibit 53* at ¶ 136. Supplementary Figure S4 of Jinek 2012 demonstrates that Cas9 can cleave target DNA even when the magnesium ion concentration is only 1 mM, which is the bottom of the range that Mastroianni et al. provides. *See Exhibit 22,* Supplementary Figure S4; *see also Exhibit 53* at ¶ 136. Thus, the Jinek 2012 paper plainly indicates to one skilled in the art that the relatively low magnesium ion concentration of eukaryotic cells would not cause a problem for the Type-II CRISPR-Cas system. *See Exhibit 53* at ¶ 136. This further demonstrates that Dr. Zhang’s arguments regarding unpredictability in moving prokaryotic systems into eukaryotic cells are incorrect. *Id.*

As all of this demonstrates, Dr. Zhang has not provided any sound evidence of unpredictability in applying the Type-II CRISPR-Cas system described in the First Provisional to eukaryotic cells. *Id.* at ¶ 137. Indeed, the results that numerous research groups obtained when
applying the invention of the First Provisional to eukaryotic cells were not surprising or unexpected. *Id.* To the contrary, the fact that this system was readily applicable to a variety of eukaryotic cell types would have been predicted and expected by persons of ordinary skill in the art at the time the invention was made. *Id.*

3. **The Disclosures of the ‘859 Application and the First and Second Provisionals Describe and Enable Use of the Type-II CRISPR-Cas System and Claimed Methods in Eukaryotic Cells**

Not only is at least one embodiment of Proposed Count 1 and of Proposed Count 2 described and enabled in the ‘859 Application and the First and Second Provisionals, all of these patent applications describe and enable *all* of the ‘859 Application claims. *See Exhibit 53* at ¶¶ 21, 22, and 76-99. First and foremost, the applications specifically state that the claimed system can be applied in eukaryotic cells. For example, the First Provisional states:

In some of the above applications, the subject methods may be employed to induce DNA cleavage and DNA modification in mitotic or post-mitotic cells *in vitro* [sic: *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 [sic] 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 animal cell, a cell from an invertebrate animal . . ., a cell from a vertebrate animal . . ., a cell from a mammal, a cell from a rodent, a cell from a human, etc.).

*First Provisional* at ¶ [00165]; *see also Second Provisional* at ¶ [00203] and *‘859 Application* at ¶ [00274]; *see also Exhibit 53* at ¶ 77. Similarly, the First Provisional indicates that genetically modified cells, including eukaryotic cells, that contain the components of the Type-II CRISPR-Cas system are part of the disclosure:

A subject genetically modified cell is generated by genetically modifying a host cell with, for example: 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; 4) an exogenous nucleic acid comprising a nucleotide sequence encoding a site-directed modifying polypeptide; or 5) any combination of the above. . . . In some embodiments, a subject genetically modified host cell is a eukaryotic cell or is derived from a eukaryotic cell.

First Provisional at ¶ [00215]-[00216]; see also Second Provisional at [00254]-[00257] and ‘859 Application at ¶ [00324]-[00327]; see also Exhibit 53 at ¶ 77.

Not only do the patent applications provide this clear disclosure of the use of the compositions in eukaryotic cells, they also provide detailed descriptions of numerous steps that could be taken to apply the Type-II CRISPR-Cas system to a eukaryotic cell environment. See Exhibit 53 at ¶ 78. It should be noted that many of the steps are optional as they are not necessarily required to apply the Type-II CRISPR-Cas system to eukaryotic cells. Id. To the contrary, depending on the specific approach one takes, the Type-II CRISPR-Cas system (the DNA-targeting RNA and Cas9 protein) can be introduced into a eukaryotic cell, for example, without vectors that include promoters operable in a eukaryotic cell. See, e.g., Exhibit 71 (DNA-targeting RNA was transcribed in vitro using a T7 transcription system and then was transfected into cells); Exhibit 73 (in vitro transcribed Cas9 mRNA and DNA-targeting RNA were injected into cells); Exhibit 74 (in vitro transcribed Cas9 mRNA and DNA-targeting RNA were injected into cells); see also Exhibit 53 at ¶ 78. As one example, in the Zhang Declaration, Dr. Zhang cites a study by Mastroianni et al. See Exhibit 70 at ¶ 4; Exhibit 97; see also Exhibit 53 at ¶ 78. In that study, the researchers directly injected a pre-assembled prokaryotic protein/RNA complex (ribonucleoprotein particles) into eukaryotic cells to cause genome modification in those cells, thereby avoiding the need to introduce the individual components to the eukaryotic cells in the form of vectors containing appropriate promoters. See Exhibit 97 at 12; see also Exhibit 53 at ¶ 78. This demonstrates that such components are not a requirement to apply a prokaryotic system to a eukaryotic cell. See Exhibit 53 at ¶ 78. Moreover, such a
disclosure in Mastroianni et al. would suggest to one skilled in the art that the assembled Cas9 protein:RNA complex, which is both necessary and sufficient to support cleavage of targeted DNA, could be directly introduced into eukaryotic cells to effect target nucleic acid binding and cleavage without undue experimentation. *Id.*

Similarly, it has been shown that Cas9 can be successfully utilized in eukaryotic cells without an NLS signal and without codon optimization, demonstrating that these components and techniques are also not necessary to apply the Type-II CRISPR-Cas system to eukaryotic cells. *See, e.g.*, Exhibit 73 (Cas9 was not codon optimized); Exhibit 74 (supplemental Figure S7 shows that the Cas9 protein was functional in the cell without an NLS signal); *see also* Exhibit 53 at ¶ 79. However, even if these steps were required for the system to be operable in eukaryotic cell, the ‘859 Application and its priority documents provide ample support for these limitations. *See Exhibit 53 at ¶ 80.*

The patent applications indicate that the Cas9 protein and/or the DNA-targeting RNAs can be provided in the form of a nucleic acid containing a nucleotide sequence encoding those components, and further indicate that the nucleotide sequences can be contained on an expression vector, such as a viral vector, as would be commonly done when performing the method in a eukaryotic cell. *Id.* at ¶ 81. For example, the applications state as follows:

> The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a subject DNA-targeting RNA and/or a subject site-directed modifying polypeptide. . . . Suitable nucleic acids comprising nucleotide sequences encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide include expression vectors . . . . Suitable expression vectors include, but are not limited, viral vectors . . . and the like.

*First Provisional at ¶¶ [00120]-[00123]; Second Provisional at ¶¶ [00157]-[00160]; and ‘859 Application at ¶¶ [00216]-[00219]; see also Exhibit 53 at ¶ 81.*
The patent applications then go on to provide a list of examples of suitable expression vectors, including commercially-available expression vectors that are suitable for use in eukaryotic cells: “The following vectors are provided by way of example; for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia).” See First Provisional at ¶¶ [00123]-[00124]; Second Provisional at ¶¶ [00160]-[00161]; and ‘859 Application at ¶¶ [00219]-[00220]; see also Exhibit 53 at ¶¶ 82-83.

The patent applications further indicate that “[i]n some embodiments, a nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide is operably linked to a control element, e.g., a transcriptional control element, such as a promoter,” and that the “transcriptional control element may be functional in [] a eukaryotic cell.” See First Provisional at ¶ [00126]; see also Second Provisional at ¶ [00163] and ‘859 Application at ¶ [00222]; see also Exhibit 53 at ¶ 84. The applications also provide numerous examples of promoters that are known to be functional in eukaryotic cells. See First Provisional at ¶ [00127]; see also Second Provisional at ¶ [00164] and ‘859 Application at ¶ [00223]; see also Exhibit 53 at ¶ 84.

The patent applications also indicate that the methods can involve “introducing into a cell (or a population of cells) one or more nucleic acids comprising nucleotide sequences encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide,” and disclose that there are numerous well-known methods that can be used to accomplish this introduction. See First Provisional at ¶¶ [00121], [00129]; see also Second Provisional at ¶¶ [00158], [00165] and ‘859 Application at ¶¶ [00217], [00225]; see also Exhibit 53 at ¶ 87. For example, the applications state: “Suitable methods include, e.g., infection, lipofection, electroporation, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran
mediated transfection, liposome-mediated transfection, and the like.” See First Provisional at ¶ [00129]; Second Provisional at ¶ [00165]; ‘859 Application at ¶ [00225]; see also First Provisional at ¶¶ [00154], [00174]-[00175]; see also Exhibit 53 at ¶ 87.

Additionally, the patent applications discuss the use of elements to target the Cas9 protein to a desired cellular component. For example, the applications disclose that the Cas9 protein can include a conjugate to facilitate traversing a cell or organelle membrane, referred to as a Protein Transduction Domain (“PTD”). See First Provisional at ¶ [00115]; Second Provisional at ¶ [00152]; and ‘859 Application at ¶ [00211]; see also Exhibit 53 at ¶ 89. Moreover, some of the PTDs discussed in the applications are known nuclear localization signals, including the sequences RQIKIWFQNRRMKWKK and RKKRRQRRR, which are provided as SEQ ID NOs:268 and 269, respectively, in the ‘859 Application. See ‘859 Application at ¶¶ [00211], [00289]; see also First Provisional at ¶¶ [00115], [00179] and Second Provisional at ¶¶ [00152], [00218]; see also Exhibit 53 at ¶ 89. The applications also provide examples of expression vectors that contain a nuclear localization signal, such as the vector pSVK3. See First Provisional at ¶ [00124]; see also Exhibit 53 at ¶ 89.

Moreover, the applications disclose that standard recombinant nucleic acid manipulation techniques, such as codon optimization (i.e., replacing a codon with a codon encoding the same amino acid), may be used. See, e.g., First Provisional at ¶ [0033]; Second Provisional at ¶ [0066]: ‘859 Application at ¶ [00118]; see also Exhibit 53 at ¶ 90.

In view of the detailed disclosure in the ‘859 Application and First and Second Provisionals describing the methods to apply the Type-II CRISPR-Cas system to eukaryotic cells, the knowledge in the art of the various possible steps available for applying a system to eukaryotic cells, and the knowledge in the art of other methods of manipulating DNA in
eukaryotic cells using proteins from prokaryotic cells that had been successfully employed, the ‘859 Application and the First, Second, and Third Provisionals would have demonstrated to one skilled in the art that the inventors were in possession of the use of the Type-II CRISPR-Cas system in eukaryotic cells. See Exhibit 53 at ¶ 99. Moreover, the disclosures of the ‘859 Application and the First, Second, and Third Provisional would have allowed persons skilled in the art to readily make and use the invention in eukaryotic cells without undue experimentation. Id. The applications therefore describe and enable methods employing the Type-II CRISPR-Cas system in eukaryotic cells and systems/compositions for carrying out those methods. Id.

4. Multiple Research Groups Applied the CRISPR-Cas System to Eukaryotic Cells Within Just a Few Months of Jinek’s Initial Publication

Further evidence of the adequacy of the information provided in the application can be seen in the fact that numerous independent research groups from around the world were able to apply the system disclosed in the First Provisional to a variety of different eukaryotic cell types within just a few months of the initial publication of the Applicants’ invention. See Exhibit 53 at ¶¶ 100-114.

The ‘859 Application did not publish until March 6, 2014. However, on June 28, 2012, approximately one month after the First Provisional was filed, the Jinek SCIENCE Paper was first published online, and contained much of the same information as the First Provisional, albeit in simplified form. See Exhibit 22; see also Exhibit 53 at ¶ 101.

Jinek 2012 established the components that are necessary and sufficient for cleavage by a Type-II CRISPR-Cas system and demonstrated successful reconstitution of the system outside of its natural environment, i.e., reconstitution outside of a prokaryotic cell. See Exhibit 53 at ¶ 102. Jinek 2012 disclosed that Cas9 is an endonuclease that is guided by two RNA molecules, a 42 nucleotide crRNA and a 75 nucleotide tracrRNA. See Exhibit 22 at Figure 1; see also id. The
article also disclosed that DNA cleavage resulted when a tracrRNA having 86 nucleotides (nucleotides 4 to 89 of the tracrRNA in Figure 3C of Exhibit 22) was present with a crRNA and Cas9. See Exhibit 22 at Figure 3C; see also Exhibit 53 at ¶ 102. Jinek 2012 further disclosed experiments directed to determining whether the entire length of tracrRNA and/or crRNA sequences are required for cleavage, demonstrating that cleavage of DNA was still supported by a 32 nucleotide crRNA truncated at the 3’ end (see, e.g., “1-32” in Figure 3B and shaded region in Figure 3C of Exhibit 22) and by a variety of different truncated versions of tracrRNA (truncations at both the 5’ and 3’ ends of tracrRNA; see, e.g., Figure 1A of Exhibit 22). See Exhibit 22 at Figures 1 and 3; see also Exhibit 53 at ¶ 102.

Additionally, Jinek 2012 disclosed that a single chimeric RNA, in which the 3’ end of a truncated crRNA is fused to the 5’ end of a truncated tracrRNA to form a hairpin structure, is able to direct Cas9 DNA cleavage. See Exhibit 22 at 819-20 (compare Figure 5 to Figure 3C); see also Exhibit 53 at ¶ 103. The dual molecule and single molecule versions can be seen in the Figure below.
This single chimeric RNA included a 20 nucleotide segment that hybridized with the target sequence and a 42 nucleotide segment that formed the truncated RNA duplex. See Exhibit 22 at Figure 5; see also Exhibit 53 at ¶ 104. Jinek 2012 concluded that “Cas9 endonuclease can be programmed with guide RNA engineered as a single transcript to target and cleave any dsDNA sequence of interest . . . . We propose an alternative methodology [to zinc-finger nucleases and transcription-activator-like effector nucleases] based on RNA-programmed Cas9 that could offer considerable potential for gene-targeting and genome-editing.” See Exhibit 22 at 820; see also Exhibit 53 at ¶ 104. Jinek 2012, therefore, identified the components of the system and suggested that this system could be used as an alternative to zinc-finger nucleases and transcription-activator-like effector nucleases for genome editing. See Exhibit 53 at ¶ 104.

Thus, Jinek 2012 provided the basic components required for the system to operate, demonstrated its effectiveness outside of a cell, and discussed its usefulness within a eukaryotic
cell. *Id.* at ¶ 105. The success of numerous other research groups in applying the system to eukaryotic cells immediately after that initial publication demonstrates that even the less detailed disclosure from the Jinek 2012 paper was sufficient to allow persons of ordinary skill in the art to utilize the CRISPR-Cas system in eukaryotic cells. *Id.*

Within just a few months after Jinek 2012 published on-line in June 2012, several groups from around the world had successfully performed and published the results of experiments showing that the system disclosed in Jinek 2012 worked in eukaryotic cells. *Id.* at ¶ 106.

For example, Cho submitted an article to Nature Biotechnology on November 20, 2012 – just five months after Jinek 2012 published–that demonstrated the use of the CRISPR-Cas system of Jinek 2012 in eukaryotic cells. *See Exhibit 71; see also Exhibit 53* at ¶ 107. Similarly, the Cong *Science* article was submitted October 5, 2012. *See Exhibit 51.* This article demonstrated successful application of Jinek’s system to eukaryotic cells. The same is also true for the Mali *Science* article, which was submitted October 26, 2012. *See Exhibit 72; see also Exhibit 53* at ¶ 107. And, in fact, Jinek and colleagues published another paper shortly after this initial 2012 *Science* publication which demonstrated the laboratory group’s success at applying the system in eukaryotic cells. *See Exhibit 91; see also Exhibit 53* at ¶ 107.

Importantly, all of these articles, from independent research groups at different institutions, specifically cited the Jinek 2012 *Science* paper as a basis of their work. *See Exhibit 53* at ¶ 107.

Additionally, the CRISPR-Cas studies that published within the first year after the initial Jinek 2012 publication demonstrate that persons skilled in the art were effectively able to design experiments to apply the system in cells from multiple types of eukaryotic organisms. *See Exhibit 53* at ¶ 108. For example, Cong *et al.*, Cho *et al.*, and Mali *et al.* utilized human cells for their CRISPR-Cas studies (*see Exhibits 51, 71, and 72*), while Hwang *et al.* utilized
zebrafish cells (see Exhibit 73), Shen et al. used both zebrafish and mouse cells (see Exhibit 74); DiCarlo et al. used yeast (see J. DiCarlo et al., Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems, 41(7) Nucl. Acids Res. 4336-4343 (2013) (Exhibit 96)); and Gratz et al. used fruit fly cells (see S. Gratz et al., Genome Engineering of Drosophila with the CRISPR RNA-Guided Cas9 Nuclease, 194 Genetics 1029-1035 (2013) (Exhibit 95)); see also Exhibit 53 at ¶ 108. This provides further evidence that even the disclosure in the Jinek 2012 Science paper was sufficient to allow persons of ordinary skill in the art to utilize the CRISPR-Cas system in eukaryotic cells, and that it was obvious for persons skilled in the art to do so. See Exhibit 53 at ¶ 108.

Further, the experiments performed by these groups also demonstrate their reliance on the Jinek 2012 Science paper when conducting their eukaryotic cell experiments. Id. at ¶ 109. For example, all of the studies conducted by the various groups used a single chimeric RNA that includes a truncation of the double stranded RNA duplex (i.e., truncation of the crRNA at the 3’ end and truncation of the tracrRNA at the 5’ end), just as was disclosed in the Jinek publication (and the First Provisional). See, Exhibit 51 at Figure 2B; Exhibit 72 at Figure 1B; Exhibit 71 at Figure 1A; Exhibit 73 at Figure 1B; Exhibit 74 at Figure 1B; see also Exhibit 53 at ¶ 109. A comparison of the single molecule DNA-targeting RNAs from Jinek 2012 and these other publications is shown in the figure below:
Exhibit 22 Figure 5, Jinek et al 2012

Chimera A

Exhibit 51 Figure 2B

Chimeric RNA

guide sequence (20 bp)

Exhibit 71 Figure 1A

Chimeric RNA

Exhibit 72 Figure 1A

Targeting/guide sequence

Exhibit 73 Figure 1C

Targeting/guide sequence

Exhibit 74 Figure 1B

Targeting/guide sequence
In fact, the majority of these studies conducted by the various groups used a single chimeric RNA with a 20 nucleotide segment that hybridizes with the target sequence and a 42 nucleotide segment that is identical to the 42 nucleotide segment of the single chimeric RNA disclosed by Jinek. *Compare Exhibit 22 at 819-20, Figure 5 with Exhibit 51 at Figure 2B; Exhibit 71 at Figure 1A; B; Exhibit 74 at Figure 1B; see also Exhibit 53 at ¶ 110.*

The fact that all of these groups used a single molecule DNA-targeting RNA—which had never been disclosed prior to the First Provisional and the Jinek 2012 paper—and the majority used a single-molecule DNA targeting RNA that was nearly identical to the one Jinek disclosed provides strong evidence of those groups’ reliance on the teachings of Jinek in applying the CRISPR-Cas system to eukaryotic cells. *See Exhibit 53 at ¶ 111.*

Beyond the information from the Jinek 2012 *Science* paper, all of the groups merely used molecular biology techniques and reagents that were well-known in the art to apply Jinek’s system to eukaryotic cells. *Id. at ¶112.* For example, all of the studies conducted by the various groups used well-known promoters specific for the system in which they wished to express the Cas9 protein. *See Exhibit 51 at Figure 1B; Exhibit 72 at Figure 1A; Exhibit 71 at Supplementary Methods 2; Exhibit 73 at Methods; Exhibit 74 at 720; see also Exhibit 53 at ¶ 112.* Additionally, all of the studies conducted by the various groups used well-known promoters specific for the system in which they wished to express the single chimeric RNA. *See Exhibit 51 at Figure 4A; Exhibit 72 at Figure 1A; Exhibit 71 at Supplementary Methods 2; Exhibit 73 at Methods; Exhibit 74 at 720; see also Exhibit 53 at ¶ 112.* In fact, all of the studies used commercially available vectors for expression of CRISPR-Cas components. *See Exhibit 53 at ¶ 112.*
Also, the studies used well-known techniques such as codon optimization and nuclear localization signals. See Exhibit 51 at 819 and Figure 1A; Exhibit 72 at 823 and Figure 1A; Exhibit 71 at Supplementary Methods 2 and Supplementary Figure 1; Exhibit 73 at Methods; Exhibit 74 at 720-722; see also Exhibit 53 at ¶ 113. However, as noted above, these studies also demonstrated that neither codon optimization (see Exhibit 73 at Methods) nor a nuclear localization signal (see Exhibit 74 at Supplementary Figure S7) is necessary for cleavage of target DNA in a eukaryotic cell. See Exhibit 53 at ¶ 113.

By using well-known methods, which had been shown to express functional prokaryotic proteins in eukaryotic cells, the scientists conducting the studies in eukaryotic cells were not conducting extensive research, nor were they engaged in undue experimentation. Id. at ¶ 114. Rather, they were performing routine experiments with a new technology that had been fully described a few months previous and which had been suggested could be used in a similar way to other genome editing tools. Id. These multiple successes at applying the system disclosed in Jinek 2012 for use in eukaryotic cells in such a short period of time using components and methods that were well-known in the art provides strong evidence that even the disclosure of the Jinek 2012 SCIENCE paper—which is shorter and much less detailed than the Provisional applications—was sufficient to enable one skilled in the art to make and use the CRISPR-Cas system in eukaryotic cells, which further demonstrates the sufficiency of the disclosures of the much more detailed patent applications. Id.

5. The Examples of Zhang’s Earliest Issued Patents Further Demonstrate that Applicants’ First Provisional Would Enable One Skilled in the Art to Apply the CRISPR-Cas System to Eukaryotic Cells

The earliest examples provided in the Zhang patent applications similarly demonstrate that one skilled in the art would be able to utilize the disclosure of Applicants’ First Provisional,
coupled with general knowledge in the art of available molecular biology techniques, to apply the CRISPR-Cas system to eukaryotic cells without undue experimentation. See Exhibit 53 at ¶ 115-127.

Dr. Carroll reviewed Example 1 of Zhang’s earliest provisional patent application, U.S. App. No. 61/736,527 (“Zhang ‘527 Application”) (Exhibit 32), which is reiterated in numerous Broad Patents, e.g. the ‘359 and ‘945 Patents, and found that the techniques employed in this example were disclosed in the First Provisional and known in the art. See Exhibit 53 at ¶ 116.

Example 1 states that:

CRISPR locus elements, including tracrRNA, Cas9, and leader were PCR amplified from Staphylococcus pyogenes SF370 genomic DNA with flanking homology arms for Gibson Assembly. Two BsaI type IIS sites were introduced in between two direct repeats to facilitate easy insertion of spacers (FIG. 8). PCR products were cloned into EcoRV-digested pACYC184 downstream of the tet promoter using Gibson Assembly Master Mix (NEB). Other endogenous CRISPR system elements were omitted, with the exception of the last 50 bp of Csn2. Oligos (Integrated DNA Technology) encoding spacers with complimentary overhangs were cloned into the BsaI-digested vector pDC000 (NEB) and then ligated with T7 ligase (Enzymatics) to generate pCRISPR plasmids. Challenge plasmids containing spacers with PAM sequences (also referred to herein as “CRISPR motif sequences”) were created by ligating hybridized oligos carrying compatible overhangs (Integrated DNA Technology) into BamHI-digested pUC19. Cloning for all constructs was performed in E. coli strain JM109 (Zymo Research).

pCRISPR-carrying cells were made competent using the Z-Competent E. coli Transformation Kit and Buffer Set (Zymo Research, T3001) according to manufacturer's instructions. In the transformation assay, 50µL aliquots of competent cells carrying pCRISPR were thawed on ice and transformed with 1 ng of spacer plasmid or pUC19 on ice for 30 minutes, followed by 45 second heat shock at 42°C and 2 minutes on ice. Subsequently, 250ul SOC (Invitrogen) was added followed by shaking incubation at 37°C for 1 hr, and 100 uL of the post-SOC outgrowth was plated onto double selection plates (12.5
ug/ml chloramphenicol, 100 ug/ml ampicillin). To obtain cfu/ng of DNA, total colony numbers were multiplied by 3.

Exhibit 32 at ¶¶ 168-69.

Applicants’ First Provisional discloses insertion of nucleic acids encoding the DNA-targeting RNA and Cas9 protein into plasmids to form recombinant vectors and transformation of cells with those recombinant vectors, including disclosure of techniques such as cloning, cleaving with restriction endonucleases, polymerase chain reaction (PCR) amplification, and ligation, as is done in this example. See, e.g., First Provisional at ¶¶ [0033], [0035]-[0039], [00120]-[00129], [00145]-[00154], [00167]-[00176]; see also Exhibit 53, ¶ 118. Moreover, the techniques utilized in this portion of the example—PCR amplification, insertion of restriction sites, cloning, ligation, preparation of competent cells, and transforming cells with plasmid DNA—were all well-known in the art, and had been for many years. See, e.g., Introducing Cloned Genes into Cultured Mammalian Cells, Molecular Cloning: A Laboratory Manual, Chapter 16 (Joseph Sambrook & David W. Russell eds., 3d ed. 2001) (Exhibit 81); see also Exhibit 53 at ¶ 118.

The example goes on to state:

To improve expression of CRISPR components in mammalian cells, two genes from the SF370 locus1 of Streptococcus pyogenes (S. pyogenes) were codon-optimized, Cas9 (SpCas9) and RNase III (SpRNaseIII). To facilitate nuclear localization, a nuclear localization signal (NLS) was included at the amino (N)- or carboxyl (C)-termini of both SpCas9 and SpRNase III (Figure 1B). To facilitate visualization of protein expression, a fluorescent protein marker was also included at the N- or C-termini of both proteins (Figure 1B). A version of SpCas9 with an NLS attached to both N- and C-termini (2xNLS-SpCas9) was also generated. Constructs containing NLS-fused SpCas9 and SpRNase III were transfected into 293FT human embryonic kidney (HEK) cells, and the relative positioning of the NLS to SpCas9 and SpRNase III was found to affect their nuclear localization efficiency. Whereas the C-terminal NLS was sufficient to target SpRNaseIII to the nucleus, attachment
of a single copy of these particular NLS's to either the N- or C-terminus of SpCas9 was unable to achieve adequate nuclear localization in this system. In this example, the C-terminal NLS was that of nucleoplasmin (KRPAATKKAGQAKKKK), and the C-terminal NLS was that of the SV40 large T-antigen (PKKKRKV). Of the versions of SpCas9 tested, only 2xNLS-SpCas9 exhibited nuclear localization (Figure 1B).

Exhibit 32 at ¶ 170; see also Exhibit 53 at ¶ 119.

As discussed in detail above, codon optimization and use of one or more nuclear localization signals to facilitate nuclear localization was described in Applicants' First Provisional and was well-known in the art and routine. See, e.g., First Provisional at ¶¶ [0033], [00115], [00179], Exhibits 13, 14, 15, 30, 82, and 83; see also Exhibit 53 at ¶ 120. The use of fluorescent protein markers was also well-known in the art and routine. See, e.g., R. Tsien, The Green Fluorescent Protein, 67 ANNU. REV. BIOCHEM. 509-44 (1998) (Exhibit 45); see also Exhibit 53 at ¶ 120. It should also be noted that, although this example states that only the Cas9 with two NLSs attached exhibited nuclear localization, multiple studies demonstrated that no NLS is required for Cas9 to operate in eukaryotic cells, as discussed in detail above. See, e.g., Exhibit 74; see also Exhibit 53 at ¶ 120.

Next, the example states:

The tracrRNA from the CRISPR locus of S. pyogenes SF370 has two transcriptional start sites, giving rise to two transcripts of 89-nucleotides (nt) and 171nt that are subsequently processed into identical 75nt mature tracrRNAs. The shorter 89nt tracrRNA was selected for expression in mammalian cells (expression constructs illustrated in Figure 6A, with functionality as determined by results of Surveyor assay shown in Figure 6B). Transcription start sites are marked as +1, and transcription terminator and the sequence probed by northern blot are also indicated. Expression of processed tracrRNA was confirmed by Northern blot. FIG. 6C shows results of a Northern blot analysis of total RNA extracted from 293FT cells transfected with U6 expression constructs carrying long or short tracrRNA, as well as SpCas9 and DR-EMX1(1)-DR. Left and right panels are from 293FT cells transfected without
or with SpRNase III, respectively. U6 indicate loading control blotted with a probe targeting human U6 snRNA. Transfection of the short tracrRNA expression construct led to abundant levels of the processed form of tracrRNA (~75 bp). Very low amounts of long tracrRNA are detected on the Northern blot.

To promote precise transcriptional initiation, the RNA polymerase III-based U6 promoter was selected to drive the expression of tracrRNA (FIG. 2C). Similarly, a U6 promoter-based construct was developed to express a pre-crRNA array consisting of a single spacer flanked by two direct repeats (DRs, also encompassed by the term "tracr-mate sequences"; FIG. 2C). The initial spacer was designed to target a 33-base-pair (bp) target site (30-bp protospacer plus a 3-bp CRISPR motif (PAM) sequence satisfying the NGG recognition motif of Cas9) in the human EMX1 locus (FIG. 2C), a key gene in the development of the cerebral cortex.

Exhibit 32 at ¶ 171-72; see also Exhibit 53 at ¶ 121.

In this example, Zhang utilizes a 89 nucleotide ("nt") tracr precursor molecule, which is in turn cleaved to form a tracr that is ~75 nt in length. See Exhibit 53 at ¶ 122. The same 89 nt tracr precursor and ~75 nt processed tracr molecule were also disclosed in Applicants’ First Provisional and in the Jinek 2012 Science paper. See, e.g., First Provisional at Figures 6A and 9A; Exhibit 22 at Figure 3 and Supplementary Figure S1; see also Exhibit 53 at ¶ 122.

Moreover, the use of U6 promoter constructs to express short, non-messenger RNAs was well-known in the art and routine, as described in detail above. See, e.g., Exhibits 42, 98, and 99; see also Exhibit 53 at ¶ 122.

The example continues on as follows:

To test whether heterologous expression of the CRISPR system (SpCas9, SpRNase III, tracrRNA, and pre-crRNA) in mammalian cells can achieve targeted cleavage of mammalian chromosomes, HEK 293FT cells were transfected with combinations of CRISPR components. Since DSBs in mammalian nuclei are partially repaired by the non-homologous end joining (NHEJ) pathway, which leads to the formation of indels, the Surveyor assay was used to detect potential cleavage activity at the target EMX1 locus (FIG. 7)
Co-transfection of all four CRISPR components was able to induce up to 5.0% cleavage in the protospacer (see FIG. 2D). Co-transfection of all CRISPR components minus SpRNase III also induced up to 4.7% indel in the protospacer, suggesting that there may be endogenous mammalian RNases that are capable of assisting with crRNA maturation, such as for example the related Dicer and Drosha enzymes. Removing any of the remaining three components abolished the genome cleavage activity of the CRISPR system (FIG. 2D). Sanger sequencing of amplicons containing the target locus verified the cleavage activity: in 43 sequenced clones, 5 mutated alleles (11.6%) were found. Similar experiments using a variety of guide sequences produced indel percentages as high as 29% (see FIGS. 3-6, 10, and 11). These results define a three-component system for efficient CRISPR-mediated genome modification in mammalian cells.

Exhibit 32 at ¶ 173; see also Exhibit 53 at ¶ 123.

As discussed in detail above, methods of transfecting mammalian cells, such as HEK293FT cells, were disclosed in Applicants’ First Provisional, well-known in the art and routine. See, e.g., First Provisional at ¶¶ [00121], [00129]; Exhibits 76, 78-81; see also Exhibit 53 at ¶ 124. The example acknowledges that it was well-known in the art that DSBs are partially repaired by the NHEJ pathway, which is also discussed in detail above. See Exhibit 53 at ¶ 124. The example reports that co-transfection of all of the CRISPR components edited the genome, and that the S. pyogenes RNase III was not necessary. Id. The example confirms that these results define a three-component system for genome modification in mammalian cells. Id.

The three components described here, i.e., Cas9, tracrRNA and crRNA, are the same three components described in the First Provisional and in the Jinek 2012 SCIENCE paper. See, e.g., First Provisional at Figure 1; Exhibit 22 at Figure 1; id.

Additionally, the example states:

To further simplify the three-component system, a chimeric crRNA-tracrRNA hybrid design was adapted, where a mature crRNA (comprising a guide sequence) may be fused to a partial
tracrRNA via a stem-loop to mimic the natural crRNA:tracrRNA duplex. To increase co-delivery efficiency, a bicistronic expression vector was created to drive co-expression of a chimeric RNA and SpCas9 in transfected cells. . . . The efficiency of chimeric RNA-mediated cleavage was tested by targeting the same EMX1 locus described above. Using both Surveyor assay and Sanger sequencing of amplicons, Applicants confirmed that the chimeric RNA design facilitates cleavage of human EMX1 locus with approximately a 4.7% modification rate (FIG. 3).

Exhibit 32 at ¶ 176; see also Exhibit 53 at ¶ 125.

This design of a chimeric crRNA-tracrRNA hybrid, where the mature crRNA is fused to a partial tracrRNA via a stem-loop linker, is the same as what is described in the First Provisional and in the Jinek 2012 Science paper. See, e.g., First Provisional at ¶¶ [0077], [0079], and Figure 1B; Exhibit 22 at Figure 5; see also Exhibit 53 at ¶ 126.

Thus, this example from Zhang’s earliest provisional application again demonstrates that a person skilled in the art could utilize the disclosure of Applicants’ First Provisional and molecular biology techniques well-known in the art to apply the CRISPR-Cas system to eukaryotic cells without undue experimentation. See Exhibit 53 at ¶ 127. As such, this provides further evidence that Applicants’ First Provisional, as well as Applicants’ subsequent applications, provide an enabling disclosure for applying the CRISPR-Cas system to eukaryotic systems. Id.

So, Dr. Zhang’s assertion that the ‘859 Application and the First and Second Provisionals fail to describe and enable systems and methods in eukaryotic cells fails.

D. 37 C.F.R. § 41.202(a)(5) – Claim Charts Showing Written Description in Applicants’ Specification For Each Claim Added to Provoke the Interference

Claims 165-247 of the ‘859 Application were added to provoke the interference. A chart is provided in Appendix G showing where exemplary written descriptive support for these claims can be found in the ‘859 Application. And, as discussed above, a chart provided in
Appendix J shows where exemplary written descriptive support for these claims can be found in the First Provisional.

V. Conclusion

An interference between the '859 Application and the Broad/MIT Patents with the two Proposed Counts is respectfully requested.

Respectfully submitted,
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