Filed on behalf of: **Junior Party, Broad** Paper No. ____
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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

THE BROAD INSTITUTE, INC., MASSACHUSETTS INSTITUTE OF TECHNOLOGY, and PRESIDENT AND FELLOWS OF HARVARD COLLEGE,
(Patents 8,697,359; 8,771,945; 8,795,965; 8,865,406; 8,871,445; 8,889,356; 8,895,308; 8,906,616; 8,932,814; 8,945,839; 8,993,233; 8,999,641; and Application 14/704,551),

Junior Party,

v.

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, UNIVERSITY OF VIENNA, and EMMANUELLE CHARPENTIER
Application 13/842,859,

Senior Party.

Patent Interference No. 106,048 (DK)

BROAD et al. SUBSTANTIVE MOTION 2
(for judgment of no interference-in-fact)

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I. PRECISE RELIEF REQUESTED

Junior Party, The Broad Institute, Inc., Massachusetts Institute of Technology, and President and Fellows of Harvard College (collectively "Broad"), moves under 37 CFR §41.121(a)(iii) for a judgment of No Interference-In-Fact between the involved claims of Application 13/842,859 ("the '859 application") (Ex. 1001) of The Regents of the University of California, University of Vienna, and Emmanuelle Charpentier (collectively "UC") and all of the involved claims of the Broad's Patents and application in the Interference (hereinafter collectively "Broad's involved claims").

According to 37 CFR §41.203(a), "An interference exists if the subject matter of a claim of one party would, if prior art, have anticipated or rendered obvious the subject matter of a claim of the opposing party and vice versa." That condition is not met here, because, *inter alia*, all Broad involved claims recite "eukaryotic" and explicitly require operability in a eukaryotic cell, whereas none of UC's involved claims recite, teach, or suggest "eukaryotic" or explicitly require operability in eukaryotic cells.

II. DESCRIPTION OF APPENDICES

Appendix 1 is a list of Exhibits cited in this motion. Appendix 2 is a Statement of Material Facts, cited herein as "Fact" (*e.g.*, "Fact 1" is a citation to the first material fact.)

III. REASONS WHY THIS MOTION SHOULD BE GRANTED

A. Summary Of The Argument

UC's involved claims, if treated as prior art, do not anticipate or render obvious Broad's involved claims, and, thus, there is no interference-in-fact. All of Broad's involved claims explicitly require operability of the CRISPR-Cas9 system in a eukaryotic cell. By way of example, claim 1 of Broad's U.S. Patent No. 8,697,359 ("'359 patent") (Ex. 1007) and claim 1 of Broad's U.S. Patent No. 8,771,945 ("'945 patent") (Ex. 1008) are each directed to a method of

"altering expression of at least one gene product" in a "eukaryotic cell" by way of CRISPR-Cas9-mediated action.

By contrast, as UC admits, "none of [its] claims require performance of the claimed method or application of the claimed system in a eukaryotic cell." Paper 27, 7:25-8:1. UC's involved claims do not recite any environment—and thus do not recite "in a eukaryotic cell." UC's involved claims merely call for "contacting" or "hybridizing" components of a CRISPR-Cas9 system with a target DNA molecule, thereby leaving the claims open to any environment, including cell-free, test-tube experiments. Accordingly, UC's involved claims do not anticipate Broad's involved claims which all recite eukaryotic claim limitations. See § D *infra*.

Nor do UC's involved claims, either alone or in combination with the known prior art, render the subject matter of Broad's involved claims obvious. One of ordinary skill in the art in the 2012 time frame would have understood that Broad's involved claims were directed to highly unpredictable, nascent technology. Thus, the person of ordinary skill would not have had any reasonable expectation that the CRISPR-Cas9 system, which is found naturally only in prokaryotic cells as an immune system, would successfully work for a different purpose, such as genome editing, in a different environment, such as a eukaryotic cell. The contemporaneous statements of Dr. Dana Carroll, UC's expert during prosecution of the '859 application (including UC's suggestion of interference), and one of its named inventors, Dr. Jennifer Doudna, corroborate the uncertainty and difficulty of implementing CRISPR-Cas9 technology in a eukaryotic environment in 2012. Indeed, even after UC's *in vitro* experiments in 2012, Dr. Doudna indicated that development of an adapted CRISPR system that functioned in eukaryotic cells would be a "profound discovery." Ex. 2230, Pandika at 3. Consistent with that view, the

successful invention of a CRISPR-Cas system that functioned in eukaryotic cells by the Broad's inventors has been recognized as a pioneering advance in the field. See § E *infra*.

Broad therefore respectfully requests judgment of no interference-in-fact.

B. Background Of Technology And Broad's Invention Of CRISPR-Cas Systems For Eukaryotic Cells

1. Discovery Of CRISPR In Prokaryotic Cells And The *In Vitro* Experiments In Jinek 2012

Clustered Regularly Interspaced Short Palindromic Repeats or "CRISPR"-Cas9 refers to a natural system found only in prokaryotic cells, where it provides an immune response to invading phages and plasmids; now the term also includes adapted CRISPR-Cas9 systems that are the subject of this interference. Fact 1; Ex. 2001, Simons ¶ 2.1.[1] UC's involved claims are directed to "contacting" or "hybridizing" certain components of a CRISPR-Cas9 system with a target DNA molecule. Fact 5. UC's involved claims are not limited to any particular environment and, specifically, do not recite any eukaryotic limitations. Facts 4–6.

The 2012 published experiments of UC's inventors, Doudna et al, as set forth in the Jinek 2012 reference, and included in the priority applications of UC's '859 application filed in 2012, only contacted isolated components of a CRISPR-Cas9 system with a naked DNA target in a cell-free environment in *in vitro* experiments. Fact 24; Jinek *et al*., SCIENCE 2012; 337:816–821 ("Jinek 2012") (Ex. 1155). This work did not demonstrate or involve the use of the CRISPR-Cas9 components in a eukaryotic cell. Ex. 2001, Simons ¶¶ 6.1-6.4, 6:29; *see also* Fact 23. This work also fails to disclose cleaving or editing a target DNA molecule, or gene editing, or

[1] A Declaration by Paul Simons, Ph.D. accompanies Broad's Motions, is Exhibit 2001 and is cited to herein as "Ex. 2001, Simons ¶."

modulating transcription of a gene and does not describe any experiments in any type of cell, including eukaryotic cells. *Id.; see also* Ex. 2001, Simons ¶ 6.4.

After publication of Jinek 2012, it remained unknown whether CRISPR-Cas9 would be useful in eukaryotic cells. Facts 23-25; Ex. 2001, Simons ¶¶ 6.4-6.5. Indeed, in January 2013, six months after the publication of Jinek 2012, Dr. Doudna and Dr. Jinek stated that "***it was not known whether such a bacterial system [the CRISPR-Cas9 system] would function in eukaryotic cells***." (emphasis added). Jinek et al., *RNA-programmed genome editing in human cells*, 2 eLIFE e00471 (2013) at 2 ("Jinek 2013") (Ex. 1057). Dr. Doudna made a similar observation in 2014, stating: "Our 2012 paper [Jinek 2012] was a big success, but ***there was a problem. We weren't sure if CRISPR/Cas9 would work in eukaryotes***—plant and animal cells." (emphasis added). Ex. 2207 at 3.[2]

2. Adapting A Prokaryotic System To Eukaryotic Cells Was Unpredictable As Of 2012

Dr. Doudna's observations are entirely consistent with the state of the art in 2012. At that time, a person of ordinary skill would not have had a reasonable expectation of successfully adapting the prokaryotic, CRISPR-Cas9 immune system to function as a gene editing tool in a eukaryotic cell. Facts 9-17, 21-36, 38-40; Ex. 2001, Simons ¶ 6.5. There is no corollary to the natural, bacterial Cas9 or the CRISPR-Cas9 system in eukaryotic cells; and, those of ordinary skill in 2012, would have considered the use of a CRISPR-Cas9 system in eukaryotic cells to be unpredictable at least because of the many differences between the cellular environments of

[2] These contemporaneous statements should be credited over UC's current litigation-induced theory that work in eukaryotic cells was obvious from and a routine extension of the *in vitro* work. *See, e.g., Manning v. Paradis*, 296 F.3d 1098, 1104 (Fed. Cir. 2002) (contemporaneous statements by the inventor outweigh later statements made for purposes of interference).

prokaryotic and eukaryotic cells. Facts 12-15; Ex. 2001, Simons ¶¶ 6.14-6.47. Indeed, there are even more differences between the cell-free, test-tube environment of UC's 2012 experiments and a eukaryotic cell. Facts 19-21, 23; Ex. 2001, Simons ¶¶ 6.5-6.27.

Dr. Carroll, UC's expert declarant for the suggestion of interference, made that precise observation in September 2012. Fact 26; *see* Carroll, *A CRISPR Approach to Gene Targeting*, 20 Molecular Therapy 1658-1660 (2012) ("Carroll 2012") (Ex. 1152). Dr. Carroll observed that "[a]ll the experiments described [in Jinek 2012] were performed *in vitro* with purified components," *id.* at 1659, and expressed his doubt about the operability of the CRISPR-Cas system in eukaryotic cells:

> What about activity of the system in eukaryotic cells? Both zinc fingers and TALE modules come from natural transcription factors that bind their targets in a chromatin context. This is not true of the CRISPR components. There is no guarantee that Cas9 will work effectively on a chromatin target or that the required DNA–RNA hybrid can be stabilized in that context … Only attempts to apply the system in eukaryotes will address these concerns …

Id. at 1660. Dr. Carroll's contemporaneous statement recognized that one of skill in this art would not have had any reasonable expectation of success in adapting the system to have it function in eukaryotic cells due to a plethora of unknowns. Fact 26; Ex. 2001, Simons ¶ 6.4. A skilled person would have recognized important differences between the eukaryotic cellular environment and that of a cell-free test tube experiment. One of ordinary skill in the art would not have believed that the behavior of an RNA-protein complex system, native only to prokaryotic cells, in a test tube assay would reliably predict or reflect activity in eukaryotic cells. Fact 23; Ex. 2001, Simons ¶ 6.10.

One significant difference, as Dr. Carroll noted, relates to eukaryotic chromosomes which are composed of chromatin. Chromatin is a complex and tightly-packed structure composed of

genomic DNA associated with proteins (primarily histones). Prokaryotic cells, in contrast, do not have a nucleus and generally have a single chromosome that is not complexed with histones to form chromatin. Fact 20; Ex. 2001, Simons ¶ 6.29.

In addition to this significant difference, prokaryotic and eukaryotic cells differ in many other important respects. For example, the gene expression machinery in eukaryotic cells involves proteins and complexes not present in prokaryotic cells. Cellular compartmentalization is prominent in eukaryotic cells but not in prokaryotic cells. Intracellular metal ion concentrations (which are involved in proper function for certain proteins) and intracellular pH also differ between prokaryotic and eukaryotic organisms. Modifications to DNA and RNA differ between prokaryotic and eukaryotic cells as do the nucleases and other molecules present in each type of cell such that proteins involved in DNA or RNA modification native to one would not necessarily be present in the other. Thus, different interactions of the CRISPR-Cas9 complex and components thereof with proteins or nucleic acids that are present in eukaryotic cells, and not in prokaryotic cells, are inevitable. One of skill in the art in 2012 would have had no reasonable expectation that a multi-component RNA-protein complex system that is uniquely found only in prokaryotic cells would be compatible with eukaryotic cells and capable of adaptation as a genomic DNA editing tool. Fact 14; Ex. 2001, Simons ¶¶ 6.28-6.35.

Because prokaryotic proteins like Cas9 evolved in the context of prokaryotic cells and their different protein folding environment, the folding of a Cas9 protein in a eukaryotic cell was unpredictable. Facts 12-15; Ex. 2001, Simons ¶¶ 6.13, 6.33. One simply could not predict whether a prokaryotic protein would properly fold in a eukaryotic cell, or whether a CRISPR-Cas9 system that functioned *in vitro* would function *in vivo* in a eukaryotic cell. Adapting a bacterial system involving a multi-component RNA-protein complex such that it would be fully

functional in the eukaryotic environment would also involve temporal and spatial requirements. Thus, all of the system's components (including the target) must be together in the same place at the same time for it to work successfully. Facts 16; Ex. 2001, Simons ¶ 6.32. This is especially complicated in eukaryotic cells where translation of the Cas9 protein takes place in the cytoplasm, whereas transcription of the RNA component of the CRISPR-Cas9 complex takes place in the nucleus.

A person of ordinary skill in the art likewise could not have predicted whether the components of a CRISPR-Cas9 system could be sufficiently stable to interact and function in eukaryotic cells. One of ordinary skill in the art could not have predicted whether intracellular degradation pathways, for both protein and/or RNA components would degrade the molecules or otherwise inhibit formation of a functional complex of the components in eukaryotic cells. Fact 18; Ex. 2001, Simons ¶ 6.30. A skilled person also would have recognized that components unique to bacterial cells could be deleterious to eukaryotic cells. Bacterial protein and RNA can present issues of cellular toxicity when expressed *in vivo* in the eukaryotic cell. Facts 22, 31; Ex. 2001, Simons ¶¶ 6.22-6.27, 6.35, 6.60.

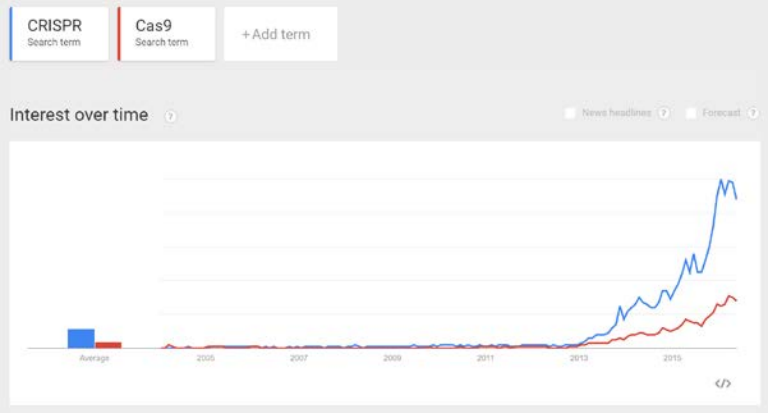
Given the complex cellular milieu of a eukaryotic cell and the packaging of DNA in chromatin in the nucleus of eukaryotic cells, one of ordinary skill considering the purified *in vitro* experiments disclosed in Jinek 2012 would not have had a reasonable expectation that *in vitro* data would be predictive of CRISPR-Cas function in a eukaryotic cell. Ex. 2001, Simons ¶ 2.11; *see also* Fact 23. Thus, in 2012, in the absence of positive experimental results in eukaryotic cells, one skilled in the art would have had no reasonable expectation that a CRISPR-Cas9 system could function to cleave or edit the nuclear DNA of a eukaryotic cell. Facts 13-20; Ex. 2001, Simons ¶¶ 6.26-6.27, 6.30, 6.36, 6.43, 6.46, 6.47, 6.71.

Indeed, after publication of Jinek 2012, Dr. Doudna herself indicated that she experienced "many frustrations" trying to get CRISPR to work in human cells. Fact 25; Ex. 2230, Pandika at 3. She concluded that "if the system could be made to work in human cells, it would be a really profound discovery." Fact 25; Ex 2230, Pandika at 2.

3. The Broad Scientists' Breakthrough Discovery

The Broad scientists made that profound discovery. Dr. Feng Zhang and his colleagues confronted the problem head-on and developed engineered CRISPR-Cas9 systems that function in eukaryotic cells. Fact 40. By October 5, 2012, Dr. Zhang and his group submitted their first manuscript describing use of an adapted CRISPR-Cas9 system in eukaryotic cells, which published on January 3, 2013. Cong et al., *Multiplex Genome Engineering Using CRISPR/Cas Systems*, 339 Science 819-823 (2013) (Ex. 1055). And, they filed their first priority application, 61/736,527, on December 12, 2012 Ex. 2101 ("Zhang B1" or "B1"), disclosing detailed methods for adapting CRISPR-Cas9 systems to function in eukaryotic cells. *See* Fact 8.

Google provides real-time data on internet searches, known as Google Trends. A search as to CRISPR or Cas9 demonstrates that January 2013—coinciding with the first published demonstrations of the successful adaption of the CRISPR-Cas system for gene editing in eukaryotic cells—appears to be the beginning of significant rise in interest in the CRISPR field:



Fact 42; Ex. 2403; *see also* Ex. 2001, Simons ¶ 2.14. The direct correlation between the significant increase in the level of interest in CRISPR-Cas systems and the publications announcing the adaption for function in eukaryotic cells evidences the profound importance of this key breakthrough. *Id.*; *see also* Exs. 1055 and 2258, and Fact 43.

On January 7, 2013, Dr. Doudna was interviewed about those publications on the first demonstrations (Ex. 1055 and Ex. 2258) of successful function of CRISPR-Cas9 systems in eukaryotic cells (human and mouse). She stated that this work would remove a "huge bottleneck" relating to "techniques for making these modifications in animals and humans":

> "The ability to modify specific elements of an organism's genes has been essential to advance our understanding of biology, including human health," said Doudna, a professor … at UC Berkeley. "However, the techniques for making these modifications in animals and humans have been a huge bottleneck in both research and the development of human therapeutics."… "This is going to remove a major bottleneck in the field, because it means that essentially anybody can use this kind of genome editing or reprogramming to introduce genetic changes into mammalian or, quite likely, other eukaryotic systems." … "[I]t's possible that this technique will completely revolutionize genome engineering in animals and plants."

Sanders, 2013 (Ex. 2259). As Dr. Doudna predicted, this early work has indeed launched a new revolution.

As of May 2016, the Zhang laboratory (including through Addgene, a nonprofit plasmid repository), has distributed more than 30,000 CRISPR-Cas9 reagents, stemming from the eukaryotic work set forth in the Zhang B1 priority application and disclosed in Cong *et al*. (with more than 1650 acknowledging citations as of May 2016, Cong *et al*. is currently the most highly-cited CRISPR publication). Fact 44; Ex. 2001, Simons ¶ 2.14. This level of intense interest further confirms that the eukaryotic subject matter of Broad's involved claims would not have been obvious over the prior *in vitro* work.

Thus, far from being a routine advancement over the UC work set forth in Jinek 2012, the invention of CRISPR-Cas systems for function in eukaryotic cells was recognized as a pioneering breakthrough, consistent with Dr. Doudna's view that it would be a "profound discovery." Ex. 2230, Pandika at 3; Ex. 2001, Simons ¶ 6.50. Now, however, recognizing that others achieved this accomplishment first, UC is trying to use its cell-free *in vitro* experiments to stake a claim to the subject matter of Count 1—the use of CRISPR-Cas9 systems in eukaryotic cells. But, in view of the complexity of the eukaryotic environment and the inherent unpredictability in attempting to adapt the bacterial immune CRISPR-Cas9 system for eukaryotic cells, the Broad's invention would not have been obvious.

C. The Two-Way Test

An interference in-fact exists when an application and a patent or another application each have at least one claim directed to patentably indistinct subject matter. Interference in-fact is established by applying the "two-way test." *See Noelle v. Lederman,* 355 F.3d 1343, 1351 (Fed. Cir. 2004); *Medichem, S. A. v. Rolabo, S. L.,* 353 F.3d 928, 934 (Fed. Cir. 2003). The "two-way test" is set forth in the Board's rules:

> An interference exists if the subject matter of a claim of one party would, if prior art, have anticipated or rendered obvious the subject matter of a claim of the opposing party and vice versa.

37 C. F. R. § 41.203(a)(2004).

Thus, the subject matter of a claim of one party is assumed to be prior art with respect to the claimed subject matter of the opponent. An evaluation is made to determine if the opponent's claimed subject matter is anticipated by or obvious over the subject matter of the other party's claims. Failure of one party's claims to anticipate or render obvious the other party's claims will defeat a finding of an interference in-fact regardless of whether the test is met in the other direction. *Noelle,* 355 F.3d at 1351.

D. UC's Involved Claims Do Not Anticipate Broad's Claims

Anticipation is established only when a single prior art reference discloses all limitations of a claimed invention. *Celeritas Technologies, Ltd. v. Rockwell International Corporation*, 150 F.3d 1354, 1360, 47 USPQ2D 1516, 1521 (Fed. Cir. 1998). An "anticipating" reference must also enable one of skill in the field of the invention to make and use the claimed invention. *Bristol-Myers Squibb Co. v. Ben Venue Labs., Inc*., 246 F.3d 1368, 1378-79, 58 USPQ2D 1508, 1515-16 (Fed. Cir. 2001).

As noted above, all of Broad's involved claims recite "eukaryotic" limitations that require performance of the claimed method or application of the claimed system in a eukaryotic cell. Facts 2-3; Ex. 2001, Simons ¶ 6.1. UC's involved claims, if treated as prior art to Broad's involved claims, do not anticipate those claims at least because UC's involved claims do not recite or require that the CRISPR-Cas9 system operate within a eukaryotic cell. Facts 4-5. Indeed, UC's involved claims do not recite any environment at all.

UC has conceded in this interference that its claims do not anticipate the Broad involved claims. Facts 6–7, Paper 27, p. 7, l. 25-p. 8, l. 1 ("none of Senior Party's claims require performance of the claimed method or application of the claimed system in a eukaryotic cell"); *see also* Paper 27, p. 21 ("because [the interference is] limited to a count that is – that has a eukaryotic environment limitations…then there should have been no interference declared."); *and* Facts 4-6. Instead, UC's argument appears to be that the eukaryotic subject matter of Broad's involved claims would have been obvious over UC's claims in view of the prior art showing CRISPR-Cas performed *in vitro*. However, as discussed below, contemporaneous comments by the UC Inventors and expert belie any such contention—consistent with the plain truth in the field at that time.

E. UC's Involved Claims Do Not Render Broad's Claims Obvious

1. Law of Obviousness

UC's involved claims, if treated as prior art, do not render Broad's involved claims obvious, either alone or in view of any available prior art. In considering whether prior art renders the claims obvious, consideration is given to the four *Graham* factors: (1) the scope and content of the prior art, (2) the level of ordinary skill in the art, (3) the differences between the claimed invention and the prior art and (4) any applicable secondary considerations. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). The Supreme Court summarized the analysis that is relevant to the obviousness analysis presented here:

> When there is a design need or market pressure to solve a problem and there are a finite number of identified, *predictable solutions*, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the *anticipated success*, it is likely the product not of innovation but of ordinary skill and common sense.

KSR Int'l Co. v. Teleflex Inc., 550 U.S. 398, 421 (2007) (emphasis added). As the Federal Circuit has pointed out:

> The Supreme Court's reference to 'predictable solutions' and 'anticipated success' accords with this court's longstanding focus on whether a person of ordinary skill in the art would, at the relevant time, have had a 'reasonable expectation of success' in pursuing the possibility that turns out to succeed and is claimed.

Institut Pasteur v. Focarino, 738 F.3d 1337, 1344 (Fed. Cir. 2013).

Therefore, a party seeking to prove that the subject matter of a claim would have been obvious must demonstrate "that a skilled artisan would have had reason to combine the teaching of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success from doing so." In *In re Cyclobenzaprine*

Hydrochloride Extended-Release Capsule Patent Litig., 676 F.3d 1063, 1068-1070 (Fed. Cir. 2012).

The question presented here is whether a person of ordinary skill in the art would have had a reasonable expectation of success in adapting the bacterial-based CRISPR-Cas9 system to function in a eukaryotic cell to cleave DNA, in view of the generic subject matter in UC's involved claims and the known art, which was limited to published studies of the natural bacterial system and the system's ability to cleave DNA *in vitro*.

In its suggestion of interference, UC primarily relied on Jinek 2012's *in vitro* experiments as the known art. UC argued that "performing the method in a eukaryotic cell would have been obvious to a person of ordinary skill in the art in view of a disclosure of performing the method *in vitro*." UC's obviousness argument is contradicted by Dr. Doudna's "many frustrations" in her attempts after publication of Jinek 2012 to get CRISPR-Cas9 to work in human cells and her contemporaneous acknowledgement that development of a CRISPR system for use in eukaryotic cells would be a "profound discovery." Fact 25; Ex. 2230, Pandika at 3.

The contradiction between UC's litigation position and inventor Dr. Doudna's contemporaneous rationale is understandable. Having realized that the UC scientists failed to get CRISPR-Cas9 to work in eukaryotic cells prior to the Broad scientists, UC now attempts to equate the breakthrough invention that is the subject of Count 1—the use of CRISPR-Cas9 systems in eukaryotic cells—with its *in vitro* activities. However, given the differences between prokaryotes and eukaryotes and the inherent unpredictability in attempting to adapt the bacterial immune CRISPR-Cas9 system for use in eukaryotic cells, the subject matter of Broad's involved claims would not have been obvious over UC's claims and the "test tube" work in Jinek 2012.

2. The Level Of One Having Ordinary Skill In The Art

During the relevant time period, a person of ordinary skill in the art would have a broad background that includes a strong understanding of the molecular biology and biochemistry techniques needed to clone, express, isolate, purify, and manipulate proteins and nucleic acids in the context of both in vitro and in vivo experiments in both prokaryotes and eukaryotes; a Ph.D. degree in a life sciences discipline, e. g., chemistry, biochemistry, neurobiology; and at least one year of relevant post-doctoral experience. Fact 9; Ex. 2001, Simons ¶ 4.1.

3. Generic Recitation Of CRISPR-Cas9 System Cleaving DNA Does Not Teach Or Suggest Successful Implementation In A Eukaryotic System

The "eukaryotic" limitation recited in all the involved Broad claims renders the Broad claims patentable over the UC claim's generic recitation of CRISPR-Cas9 systems, at least because there was no reasonable expectation that the CRISPR-Cas9 system, native only to prokaryotic cells, would successfully cleave or edit a target DNA molecule or modulate transcription of a gene in a eukaryotic cell. This is so at least because, as of 2012, one of ordinary skill would not have known: (a) whether a CRISPR-Cas9 complex could be introduced successfully into eukaryotic cells; (b) whether a CRISPR-Cas9 system could be adapted from a bacterial immune system to a system that cleaves DNA *in vivo* in eukaryotic cells such that editing could result; and (c) whether such an adapted CRISPR-Cas9 system could be made to function in such a different cellular milieu as in eukaryotic cells and to cleave the DNA in the nucleus of eukaryotic cells, which is packaged in chromatin, such that editing could result. Facts 12-19; Ex. 2001, Simons ¶¶ 2.11–2.12.

There were multiple unknowns in the nascent CRISPR-Cas9 field, and many aspects that could not be predicted with any reasonable certainty, including due to the many differences between prokaryotic and eukaryotic cells and the inherent unpredictability in attempting to adapt

a bacterial immune system to function in eukaryotic cells. As discussed in more detail in Section B.2 above, these differences include: gene expression, protein folding, cellular compartmentalization, chromosome structure, cellular nucleases, intracellular temperature, intracellular ion concentrations, intracellular pH, and different types of molecules present in the eukaryotic cell that are not native to the bacterial cell.

Eukaryotic chromosomes are composed of chromatin, which is a complex and tightly-packed structure composed of genomic DNA complexed with proteins (primarily histones). Prokaryotic cells, in contrast, do not have a nucleus and generally have a single chromosome that is not complexed with histones to form chromatin. Thus, it was uncertain whether a Cas9 protein could access target DNA in a eukaryotic cell. Fact 48; Ex. 2001, Simons ¶ 6.29. In addition, the components of a CRISPR-Cas9 system must be delivered to the cell such that temporal and spatial requirements are met, i.e., the components must be together in the same place at the same time for the system to work successfully. Fact 16; Ex. 2001, Simons ¶ 6.32.

The different cellular conditions could also impact protein folding, which is of particular importance given that CRISPR-Cas9 systems must undergo significant conformational changes both when binding the guide RNA, and when binding and cleaving the target DNA. Fact 49; Ex. 2001, Simons ¶¶ 6.13, 6.33. In addition, a eukaryotic cell might detect the RNA of the CRISPR-Cas9 system as virus and degrade it, thereby preventing CRISPR-Cas9 complex formation. Eukaryotic cells express a number of enzymes that cleave RNA. A person of ordinary skill in the art during the relevant time period could not have predicted whether eukaryotic enzymes would cleave the RNA molecules critical for the functioning of the CRISPR-Cas9 system. Fact 50; Ex. 2001, Simons ¶ 6.15. Bacterial protein and RNA could also present issues of cellular

toxicity when expressed *in vivo* in eukaryotic cells. Fact 31; Ex. 2001, Simons ¶¶ 6.22-6.27, 6.35, 6.60.

A person skilled in the art also would have been aware of the obstacles previously encountered in attempts to transfer other prokaryotic, RNA-based protein systems into a eukaryotic environment, even after successful *in vitro* experiments. As discussed below, the prior art provided examples of obstacles in transferring prokaryotic RNA-based systems (such as ribozymes and riboswitches), and transferring prokaryotic nucleic acid manipulation systems (such as Group II introns), into eukaryotic environments.

For example, the prior art included failed attempts to transfer prokaryotic, RNA-based riboswitch systems to eukaryotic cells. Riboswitches are usually found in bacteria where they commonly regulate expression of enzymes. (Link et al., *Engineering ligand-responsive gene-control elements: lessons learned from natural riboswitches*, 16 Gene Therapy 1189-1201 (2009), p. 1190, sec. col., first para.) (Ex. 2223). Despite great interest in the use of riboswitches as a means of controlling gene expression in non-bacterial environments, the creation of riboswitches that function as desired in mammalian systems has proven intractable to date. Fact 46. As Link *et al*. note, "…a few TPP riboswitches are the only validated metabolite-binding riboswitches in eukaryotes." Fact 47; Ex. 2223, Link at 1192. Further, many of the engineered riboswitches that function well *in vitro* fail to provide useful function when translated to eukaryotic cells. Fact 47; Ex. 2001,Simons ¶ 6.47. Similarly, in 1999, Koseki *et al*. reported that the efficacy of RNA ribozymes molecules *in vitro* is not predictive of functional activity *in vivo*, for a variety of reasons, including degradation of the ribozyme in the eukaryotic cell and inability to colocalize with its target in the eukaryotic cell. Fact 45; Ex. 2001, Simons ¶¶ 6.44-6.46; Ex.

2221, Koseki et al., *Factors governing the activity in vivo of ribozymes transcribed by RNA polymerase III*, 73 J. Virology 1868-1877 (1999) at 1875-76.

In addition, the prior art further included failed attempts to replicate prokaryotic DNA editing techniques into eukaryotes. For example, it was known in the prior art that RNA-based, self-splicing Group II introns are found in prokaryotic organisms and even in the mitochondria and chloroplasts of lower eukaryotes. Fact 33; Ex. 2001, Simons ¶¶6.37-6.38; Ex. 1276, Lambowitz at 14; Ex. 2260, Romani *et al.*, (2002); Ex. 2261, Mastroianni *et al.*, (2008). This system has been used in prokaryotes for gene targeting applications. However, the requirement for high concentrations of monovalent salt and/or magnesium ions, as found in prokaryotic cells, as well as the separation of transcription and translation by the presence of the nuclear membrane, has rendered the system so very inefficient in higher eukaryotic cells, it is at the point where it is unusable. *Id*.

A skilled person would have known that high levels of ions, as in the prokaryotic environment, which includes a high concentration of magnesium, might be essential for the operation of CRISPR systems *in vivo*. Fact 51; Ex. 2001, Simons ¶¶6.13, 6.38. In contrast, eukaryotic cells do not inherently include, and cannot be readily provided, such high levels of magnesium. Thus, persons skilled in the art would have doubted the ability to transfer the prokaryotic CRISPR-Cas9 immune system to eukaryotic cells. Fact 51; Ex 2001, Simons ¶6.39.

In sum, the prior art taught one of ordinary skill in the art that obstacles existed when attempting to transfer prokaryotic RNA-based systems into eukaryotic environments and attempting to transfer prokaryotic nucleic acid manipulation systems into eukaryotic environments. Given the knowledge of these prior failures, one of skill in the art would not have

had a reasonable expectation of success in obtaining successful functioning of a CRISPR-Cas9 system in eukaryotes.

4. The Contemporaneous Evidence Shows That Skilled Artisans Did Not Have A Reasonable Expectation Of Success In Applying CRISPR-Cas9 To Eukaryotic Cells.

The contemporaneous evidence confirms that, given the nature of the bacterial-derived CRISPR system and the substantial differences between the prokaryotic and eukaryotic environments, skilled artisans would not have reasonably predicted that CRISPR systems would function in eukaryotes, even after successful *in vitro* DNA cleavage experiments such as reported in Jinek 2012. Fact 52; Ex. 2001, Simons ¶¶ 6.48-6.51. As discussed above, after publication of Jinek 2012, UC's expert Dr. Carroll expressed doubt that CRISPR-Cas9 systems would be operable in eukaryotic cells. Fact 26; Ex. 1152, Carroll 2012 at 1660. Similarly, Dr. Doudna stated that "[w]e weren't sure if CRISPR/Cas9 would work in eukaryotes—plant and animal cells," Fact 53; Ex. 2207 at 3, after publication of Jinek 2012 and that, as of January 29, 2013, "it was not known whether such a bacterial system [the CRISPR-Cas9 system] would function in eukaryotic cells." Ex. 1057, Jinek 2013 at 2. Dr. Doudna further elaborated on the unique difficulties of adapting the CRISPR-Cas9 system to eukaryotes, noting that "[u]nlike bacteria, plant and animal cells have a cell nucleus, and inside, DNA is stored in a tightly wound form, bound in a structure called chromatin." Ex. 2207 at 3.

Another scientist in the field noted, "[i]t's not trivial to make CRISPR/Cas systems work in eukaryotic cells … One thing is to have them *in silico* and have a sequence … and another thing is to do the experiments and make it work. " Fact 54; Ex. 2213, Grens at 2 (Grens et al., *Enzyme Improves CRISPR A smaller Cas9 protein enables in vivo genome engineering via viral vectors,* The Scientist (April 1, 2015) (quoting Luciano Marraffini)). Later experimentation with CRISPR-Cas9 systems confirmed the opinions of Drs. Carroll, Doudna and Marraffini that it was

non-obvious to go from an *in vitro* system to an *in vivo* eukaryotic system. These statements provide further evidence that, as of 2012, when Broad's inventors were doing their initial work, applying a CRISPR-Cas9 system in eukaryotic cells required overcoming uncertainty regarding whether such a system might function in eukaryotic cells, especially for genome editing, and what means of delivery might work for proper expression, folding and assembly into a CRISPR-Cas9 complex, with function thereafter, e. g., for genome editing. Fact 23; Ex. 2001, Simons Dec. ¶ 6.51.

Thus, the CRISPR-Cas9 systems adapted for functional use in eukaryotic cells, the subject matter claimed in Broad's involved claims, were nonobvious over UC's involved clams alone or in view of any prior art, including because there was no reasonable expectation of success as to that subject matter as of December 12, 2012.

5. The Known Prior Art Did Not Provide The Information Necessary To Provide A Reasonable Expectation Of Success In Eukaryotic Cells

None of the art available prior to the Broad's disclosures would have led a person of ordinary skill to have any reasonable expectation of success of implementing a CRISPR-Cas system in eukaryotic cells. In order to qualify as prior art, the reference must have been known at least prior to the December 12, 2012, filing date of the Zhang B1 priority provisional application (in which each and every example disclosed is directed specifically to the use of CRISPR-Cas in eukaryotic cells). Dr. Simons has reviewed the relevant prior art and found no basis for the person of ordinary to have had any reasonable expectation of successfully implanting CRISPR-Cas in a eukaryotic environment. Facts 9-17, 21-36, 38-40; Ex. 2001, Simons ¶ ¶ 2.10-2:12; *see also id*. ¶¶ 6.3-6.5, 6.13, 6.26-6.27, 6.36, 6.46. As Dr. Simons details in his accompanying declaration (Ex. 2001), there was no prior art that could have been

combined with UC's involved claims such that the combined teachings would have suggested or rendered obvious the "eukaryotic" subject matter of Broad's involved claims.

UC pointed to nothing in its suggestion of interference, and the accompanying declaration of Dr. Dana Carroll, to indicate that a person of ordinary skill in the art would have reasonably expected the CRISPR-Cas system to function in a eukaryotic cell. UC simply argued that "it would have been routine for one of ordinary skill in the art to use [] known methods and materials to apply the Type-II CRISPR Cas system to eukaryotic cells." Ex. 1529, Suggestion filed April 13, 2015 at 27; *see also* Ex. 1476, Carroll declaration filed April 13, 2015 in USSN 13/842,859 ¶¶ 65-67. UC's argument misses the mark. Nothing in UC's suggestion of interference provides any argument or evidence showing that a person of ordinary skill in the art as of the relevant time would have had a reasonable expectation of successfully implementing the bacterial based CRISPR-Cas system as a DNA cleavage tool in a eukaryotic cell. Fact 16; Ex. 2001, Simons ¶6.52-6.72. On pages 28-30 of the suggestion of interference, UC discusses proteins and enzymes that had been shown in the art to be expressed in eukaryotic cells and argues that they support the obviousness of Broad's invention even though none of these proteins or enzymes resemble a Type-II CRISPR-Cas system. Ex. 1529, Suggestion filed April 13, 2015 at 28–30.

UC argues that ZFNs and TALENs are "[p]erhaps most relevant" to the inquiry. Ex. 1529, Suggestion filed April 13, 2015 at 30. However, the contemporaneous statements of UC's expert Dr. Carroll contradict UC's present position. As Dr. Carroll noted in 2012, "zinc fingers and TALE modules come from natural transcription factors that bind their targets in a chromatin context. **This is not true of the CRISPR components**." Ex. 1152, Carroll 2012 at 1660 (emphasis added). As Dr. Carroll recognized, ZFNs and TALENs are not purely prokaryotic in

nature. TAL effectors are proteins secreted by bacteria that were known to infect plants and are expressed in eukaryotic cells in nature. Furthermore, zinc fingers are nucleases that are naturally expressed in prokaryotic and eukaryotic cells. Fact 36; Ex. 2001, Simons ¶¶ 6.69-6.70. These systems are therefore very different from CRISPR-Cas9, which consists of a protein and RNA components, neither one of which had ever been expressed or shown to function in eukaryotic cells. Fact 37; Ex. 2001, Simons ¶ 6.68.

In fact, UC does not cite or point to any prokaryotic RNA-based systems that successfully transferred to eukaryotes. The Cre, RecA, EcoRI, and *Φ*C31 "systems" cited by UC are far less complicated than the Type-II CRISPR-Cas9 system at issue in the instant matter. In each example cited by UC, the proteins are not required to form a complex with a DNA-targeting RNA to function successfully. None of the prior systems cited by Dr. Carroll or relied upon by UC involve a bacterial protein that is RNA-guided and hence involved protein and RNA components. The bacterial protein and the RNA can present issues of cellular toxicity in the eukaryotic environment, and when produced *in vivo* in the eukaryotic cell, the protein is produced in the cytoplasm and the RNA is transcribed in the nucleus—aspects of the bacterial CRISPR-Cas9 system that made modifying it for functioning in the environment of a eukaryotic cell wholly unpredictable at the December 12, 2012 filing date of Zhang B1, Ex. 2101. Facts 30–37; Ex. 2001, Simons ¶ 6.67-6.68.

As shown in Ex. 1335, Sauer 1987 and Ex. 1336, Sauer 1988, prokaryotic systems cannot be presumed to be able to be effectively transferred to eukaryotes. In Ex. 1336, Sauer 1988, the authors explicitly stated that they did not know whether Cre would recombine such chromosomal sequences: "[T]he ability of the Cre protein to access a lox site placed on a chromosome and then to perform site specific synapsis of DNA and reciprocal recombination may be highly dependent

on surrounding chromatin structure and on the particular location within the genome of the lox site. Some regions of the genome may be inaccessible to a bacterial recombinase." Fact 32; Ex. 1336, Sauer 1988 at 5170; Ex. 2001, Simons Dec. ¶ 6.59.

Likewise, while the authors of the Reiss *et al*. publication (Reiss et al., *RecA protein stimulates homologous recombination in plants*, 93 Proc. Nat'l Acad. Sci. USA 3094-3098 (1996)) (Ex. 1329, Reiss) were able to successfully use a prokaryotic protein (RecA) in eukaryotic cells (tobacco plant cells) (Ex. 1529, Suggestion filed April 13, 2015 at 28, ll. 17-22), their success was unpredictable and the authors were awarded a patent for their accomplishment (US Patent No. 6,583,336) (Ex. 2104). Moreover, a person of skill in the art would understand that RecA has a corresponding homologous protein present in eukaryotic cells. In contrast, Cas9 has no equivalent in a eukaryotic cell. Thus, the work of Reiss *et al*. with RecA would not provide any basis for a reasonable expectation of success with respect to adaptation of a CRISPR-Cas system for eukaryotes. Fact 34–35; Ex. 2001, Simons Dec. ¶ 6.65.

Because CRISPR-Cas9 is very different, both structurally and functionally, from the proteins and enzymes discussed by UC in the suggestion of interference, the proteins noted by UC cannot be relied upon to demonstrate, and do not demonstrate, that the application of a Type-II CRISPR-Cas9 system in a eukaryotic cell was routine or predictable. Facts 30-36; Ex. 2001, Simons ¶ 6.71.

IV. CONCLUSION

For the forgoing reasons, a judgment of no interference in-fact should be entered.

Dated: May 23, 2016

Respectfully submitted,

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APPENDIX 1: LIST OF EXHIBITS

Exhibit Number	Description
1001	U.S. Patent Application No. 13/842,859, filed March 15, 2013.
1007	Zhang, U.S. Patent 8,697,359 B1, April 15, 2014.
1008	Zhang, U.S. Patent 8,771,945 B1, July 8, 2014.
1055	Cong et al., *Multiplex Genome Engineering Using CRISPR/Cas Systems*, 339 Science 819-823 (2013) and Supplementary Materials.
1057	Jinek et al., *RNA-programmed genome editing in human cells*, 2 eLIFE e00471 (2013).
1152	Carroll, *A CRISPR Approach to Gene Targeting*, 20 Molecular Therapy 1658-1660 (2012).
1155	Jinek et al., *A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity*, 337 Science 816-821 (2012) and Supplementary Materials.
1276	Lambowitz et al., *Group II introns: Mobile Ribozymes that invade DNA*, 3 Cold Spring Harbor Perspectives Biology a003616 (2011).
1329	Reiss et al., *RecA protein stimulates homologous recombination in plants*, 93 Proc. Nat'l Acad. Sci. USA 3094-3098 (1996).
1335	Sauer, *Functional expression of the cre-lox site-specific recombination system in the yeast Saccharomyces cerevisiae*, 7 Molecular Cell Biology 2087-2096 (1987).
1336	Sauer et al., *Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1*, 85 Proc. Nat'l Acad. Sci. USA 5166-5170 (1988).
1476	Declaration of Dr. Carroll filed April 13, 2015, In U.S. Patent Application No. 13/842,859.
1529	Suggestion of Interference, filed April 13, 2015, In U.S. Patent Application No. 13/842,859.
2001	Declaration of Dr. Paul Simons, executed May 23, 2016.
2101	U.S. Provisional Patent Application No. 61/736,527, filed December 12, 2012.
2104	Reiss et al., U.S. Patent 6,583,336 B1, June 4, 2003.
2207	College of Chemistry, University of California, Berkeley, 9 Catalyst 1-32 (Spring/Summer 2014).
2213	Grens et al., *Enzyme Improves CRISPR A smaller Cas9 protein enables in vivo genome engineering via viral vectors*, The Scientist (April 1, 2015), http://www.the-scientist.com//?articles.view/articleNo/42580/title/Enzyme-Improves-CRISPR/.
2221	Koseki et al., *Factors governing the activity in vivo of ribozymes transcribed by RNA polymerase III*, 73 J. Virology 1868-1877 (1999).
2223	Link et al., *Engineering ligand-responsive gene-control elements: lessons learned from natural riboswitches*, 16 Gene Therapy 1189-1201 (2009).
2230	Pandika, *Rising Stars: Jennifer Doudna, CRISPR Code Killer*, OZY (Jan. 7, 2014), http://www.ozy.com/rising-stars/jennifer-doudna-crispr-code-killer/4690.
2258	Mali et al., *RNA-Guided Human Genome Engineering via Cas9*, 339 Science 823-826 (2013)

2259	Sanders, *Cheap and easy technique to snip DNA could revolutionize gene therapy*, Berkeley News (Jan. 7, 2013), http://news.berkeley.edu/2013/01/07/cheap-and-easy-technique-to-snip-dna-could-revolutionize-gene-therapy/.
2260	Romani and Maguire, *Hormonal regulation of Mg2+ transport and homeostasis in eukaryotic cells*, BioMetals 15: 271-283 (2002).
2261	Mastroianni *et al.*, *Group II Intron-Based Gene Targeting Reactions in Eukaryotes*, PLoS ONE 3(9):e3121 (2008).
2403	Google Trends, https://www.google.com/trends/explore#q=CRISPR%2C%20Cas9&cmpt=q&tz=Etc%2FGMT%2B4.

APPENDIX 2: STATEMENT OF MATERIAL FACTS

1. "Clustered Regularly Interspaced Short Palindromic Repeats" or "CRISPR" refers to several different microbial systems that were discovered to be involved in a bacterial immune response to invading phages and plasmids. Ex. 2001, Simons ¶ 2.1.

2. All of Broad's involved claims recite language that explicitly requires operability of the CRISPR-Cas9 system in a eukaryotic cell. Ex. 2001, Simons ¶ 6.1; Paper 43, Broad Clean Claims.

3. Claim 1 of the Broad '359 patent is directed to "A method of altering expression of at least one gene product comprising introducing into a eukaryotic cell containing and expressing a DNA molecule having a target sequence and encoding the gene product an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)--CRISPR associated (Cas) (CRISPR-Cas) system comprising one or more vectors comprising: 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 that hybridizes with the target sequence, and b) a second regulatory element operable in a eukaryotic cell operably linked to a nucleotide sequence encoding a Type-II Cas9 protein, wherein components (a) and (b) are located on same or different vectors of the system, whereby the guide RNA targets the target sequence and the Cas9 protein cleaves the DNA molecule, whereby expression of the at least one gene product is altered; and, wherein the Cas9 protein and the guide RNA do not naturally occur together." Paper 43, Broad Clean Claims at p. 3, ll. 2–15.

4. UC's involved claims do not recite or require that the CRISPR-Cas 9 system operate within a eukaryotic cell. Ex. 2001, Simons ¶ 6.1; Paper 12, Senior Party's Clean Claims.

5. UC's involved claims are directed to "contacting" certain components of a CRISPR-Cas9 system and a target DNA molecule. *Id.*

6. UC admits, "none of [its] claims require performance of the claimed method or application of the claimed system in a eukaryotic cell." Paper 27, UC Proposed Motions List at p. 7, line 25-p. 8, line 1.

7. UC's involved claims, if treated as prior art, do not anticipate Broad's involved claims. Ex 2001, Simons ¶¶ 6.1–6.2.

8. Application 61/736,527, Exhibit 2101, Zhang B1, was filed on December 12, 2012. Ex. 1007, "B1" (Cover Page Certificate).

9. During the relevant time period, a person of ordinary skill in the art would have a broad background that includes a strong understanding of the molecular biology and biochemistry techniques needed to clone, express, isolate, purify, and manipulate proteins and nucleic acids in the context of both in vitro and in vivo experiments in both prokaryotes and eukaryotes; a Ph.D. degree in a life sciences discipline, e. g., chemistry, biochemistry, neurobiology; and at least one year of relevant post-doctoral experience. Ex. 2001, Simons ¶ 4.1.

10. Prokaryotic proteins, like Cas9, have evolved in the context of prokaryotic cells. Ex. 2001, Simons ¶ 6.33.

11. There is no corollary to the natural, bacterial Cas9 or the CRISPR-Cas9 system in eukaryotic cells. Ex. 2001, Simons ¶ 6.13.

12. The folding of a Cas9 protein in a eukaryotic cell is unpredictable. Ex. 2001, Simons ¶¶ 6.6–6.9.

13. The unpredictability is due at least to the different protein folding environment in a eukaryotic cell compared to a prokaryotic cell. Ex. 2001, Simons ¶¶ 6.9, 6.13.

14. As of December 2012, a person of ordinary skill in this art would have considered the use of a CRISPR-Cas9 system in eukaryotic systems to be unpredictable at least because of

the many differences between the cellular environment of a prokaryotic or bacterial cell and the cellular environment of a eukaryotic cell. Ex. 2001, Simons ¶¶ 6.14-6.47.

15. Differences between prokaryotic systems and eukaryotic systems include gene expression, cellular compartmentalization, cellular nucleases, intracellular ion concentrations, intracellular pH, different types of molecules present in eukaryotic cells that are not native to bacterial cells, and different interactions of the CRISPR-Cas9 complex and components thereof with proteins or nucleic acids that are present in eukaryotic cells and not native to bacterial cells. Ex. 2001, Simons ¶ 6.28.

16. Engineering a delivery system for proper expression or presence of the CRISPR-Cas9 system would involve temporal and spatial requirements, i.e., the components must be together at the same time and in the same place as each other and the target for the system to work successfully. Ex. 2001, Simons ¶ 6.32.

17. In a eukaryotic cell, translation of the Cas9 protein takes place in the cytoplasm, whereas transcription of the RNA component of the CRISPR-Cas9 complex takes place in the nucleus. Ex. 2001, Simons ¶ 6.68.

18. As of 2012, one of ordinary skill in the art could not have predicted whether intracellular degradation pathways, for both protein and / or RNA components would degrade the molecules or otherwise inhibit complexing of the components. Ex. 2001, Simons ¶ 6.30.

19. Eukaryotic chromosomes are composed of chromatin. Ex. 2001, Simons ¶ 6.29.

20. Chromatin is a complex and tightly-packed structure composed of genomic DNA complexed with proteins (primarily histones). Ex. 2001, Simons ¶ 6.29.

21. Prokaryotic cells do not have a nucleus and generally have a single chromosome that is not complexed with histones to form chromatin. Ex. 2001, Simons ¶ 6.29.

22. A skilled person in this art on December 12, 2012, would have recognized that components unique to bacterial cells may not function in a eukaryotic cell and could be deleterious to a eukaryotic cell. Ex. 2001, Simons ¶ 6.35.

23. The known disclosures as of December 12, 2012, did not provide sufficient information for one of skill in this art to engineer a CRISPR-Cas9 system for use in eukaryotic cells with any reasonable expectation of success. Ex. 2001, Simons ¶ 2.11.

24. The 2012 published experiments of UC's inventors, Doudna et al, as set forth in the Jinek 2012 reference and included in the priority applications of UC's '859 application filed in 2012, only contacted isolated components of a CRISPR-Cas9 system with a naked DNA target in a cell-free environment in *in vitro* experiments. Ex. 2001, Simons ¶¶ 6.1-6.4, 6.29

25. Even after UC's *in vitro* experiments in 2012, Dr. Doudna experienced "many frustrations" getting CRISPR to work in human cells and believed that development of a CRISPR system for use in eukaryotic cells would be a "profound discovery " Ex. 2230, Pandika at 3; Ex. 2001, Simons ¶ 6.50.

26. Dr. Dana Carroll's contemporaneous expression of doubt about the operability of the CRISPR-Cas9 system in eukaryotic cells from the experiments of Jinek 2012 (Ex. 1152, Carroll 2012) recognizes that one of skill in this art could not have had any reasonable expectation of success in adapting CRISPR-Cas9 to function eukaryotic cells. Ex. 1152, Carroll 2012 at 1660; Ex. 2001, Simons ¶ 6.4.

27. Neither UC nor Dr. Carroll points to any "methods and materials" that would have made it routine to apply a CRISPR-Cas system in a eukaryotic cell. Ex. 2001, Simons ¶¶ 6.57-6.72.

28. In the suggestion of interference (Ex. 1529, Suggestion filed April 13, 2015 at 27, ll. 22-23), the arguments and examples relied upon by UC fail to support UC's conclusion that

introduction of the Type-II CRISPR-Cas System in eukaryotic cells would have been routine or that one of ordinary skill in the art would have had a reasonable expectation of success. Ex. 2001, Simons ¶ 6.57.

29. The Cre, RecA, EcoRI, *Φ*C31, TALEN, and ZFN "systems" cited by UC in its suggestion of interference are far less complicated than the Type-II CRISPR-Cas system at issue in the instant matter; in each example cited by UC, the proteins are not required to form a complex with a DNA-targeting RNA to function successfully. Ex. 2001, Simons ¶ 6.67.

30. None of the prior systems cited by Dr. Carroll or relied upon by UC in its suggestion of interference involve a bacterial protein that is RNA-guided and hence involved protein and RNA components. Ex. 2001, Simons ¶ 6.68.

31. The bacterial protein and the RNA can present issues of cellular toxicity in the eukaryotic environment, and when expressed in vivo in the eukaryotic cell, the protein is expressed in the cytoplasm and the RNA is in the nucleus—aspects of the bacterial CRISPR-Cas9 system that made modifying it for functioning in the environment of a eukaryotic cell wholly unpredictable at the December 12, 2012 filing date of Zhang B1, Ex. 2101. Ex. 2001, Simons ¶ 6.68.

32. Ex. 1335, Sauer 1987 and Ex. 1336, Sauer 1988 teach that prokaryotic systems cannot be presumed to be able to be effectively transferred to eukaryotes, stating that "the ability of the Cre protein to access a lox site placed on a chromosome and then to perform site specific synapsis of DNA and reciprocal recombination may be highly dependent on surrounding chromatin structure and on the particular location within the genome of the lox site; some regions of the genome may be inaccessible to a bacterial recombinase, for example." Ex. 1336, Sauer 1988 at 5170.

33. It was known in the prior art as of 2012, that RNA-based, self-splicing Group II introns are found in prokaryotic organisms and even in the mitochondria and chloroplasts of lower eukaryotes, but that obstacles include nuclear accessibility of RNPs and suboptimal Mg^{2+} concentrations. Ex. 2001, Simons ¶¶ 6.37–6.38; Ex. 1276, Lambowitz et al. 2011 at 14; Romani *et al.*, (2002); Ex. 2261, Mastroianni *et al.*, (2008).

34. While the authors of the Reiss et al. publication (Ex. 1329, Reiss) were able to successfully use a prokaryotic protein (RecA) in eukaryotic cells (tobacco plant cells) (Ex. 1529, Suggestion filed April 13, 2015 at 28, ll. 17-22), their success was unpredictable and the authors were awarded a patent for their accomplishment (US Patent No. 6,583,336) (Ex. 2104).

35. A person of skill in the art would understand that RecA has a corresponding homologous protein present in eukaryotic cells, while Cas9 has no equivalent in a eukaryotic cell and, the work of Reiss et al. with RecA would not provide any basis for a reasonable expectation of success with respect to adaptation a CRISPR-Cas9 system for eukaryotes. Ex. 2001, Simons ¶ 6.65.

36. The success with ZFNs and TALENs in eukaryotes would not provide any basis for a reasonable expectation of success with respect to adaptation of a CRISPR-Cas9 system for eukaryotes because, unlike CRISPR-Cas9, ZFNs and TALENs are not purely prokaryotic in nature (the eukaryote-derived portion of ZFNs comprise DNA-binding domains critical to the protein's function in a eukaryote. while the equivalently critical DNA binding domains of TALENs originate in bacteria, the TALE proteins from which they are derived have their function in plants, i.e. eukaryotes). Ex. 2001, Simons ¶¶ 6.69–6.70.

37. ZFN and TALEN systems are very different from CRISPR-Cas, which includes an RNA-guided protein that had never been previously known to express or function in a eukaryotic cell . Ex. 2001, Simons ¶¶ 6.67-6.70.

38. As Dr. Carroll noted in 2012 that "zinc fingers and TALE modules come from natural transcription factors that bind their targets in a chromatin context. This is not true of the CRISPR components." Ex. 1152, Carroll 2012 at 1660.

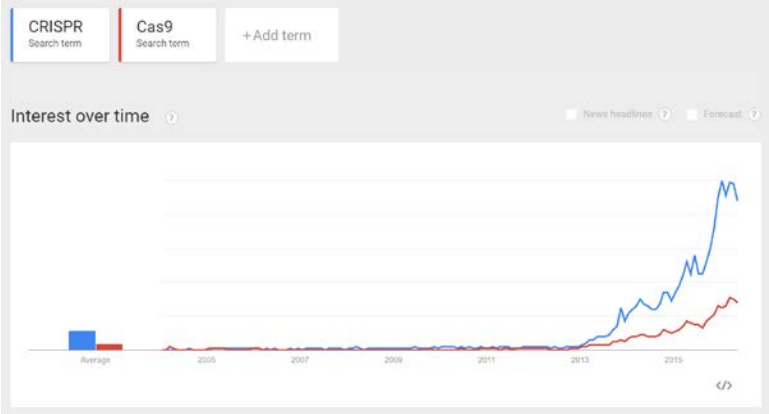
39. Prior to the Broad's publication of the work reflected in its first two provisional applications in Ex. 1055, Cong, no group had shown that the RNA constructs and Cas9 protein of the CRISPR-Cas system could function in a eukaryotic cell. Ex. 2001, Simons ¶ 2.10.

40. Dr. Feng Zhang and his colleagues invented engineered CRISPR-Cas9 systems that function in eukaryotic cells. Ex. 2001, Simons ¶ 2.13.

41. The invention of CRISPR-Cas systems for eukaryotic cells, as in Ex. 2101, Zhang B1; Ex. 1055, Cong, and the eukaryotic subject matter claims of the Broad patents was recognized as a pioneering. Ex. 2001, Simons ¶¶ 2.13-2.15.

42. Google provides real-time data on internet searches, known as Google Trends, and that such a search as to CRISPR or Cas9 is available from the following link: https://www.google.com/trends/explore#q=CRISPR%2C%20Cas9&cmpt=q&tz=Etc%2FGMT%2B4. Ex. 2403.

43. This real-time data, as shown by the following Google Trends graph understood to reflect inclusion of search terms CRISPR or Cas9 over time, demonstrates that January 2013—coinciding with the publications on work in eukaryotic cells, (Exs. 1055 and 2258)—appears as the beginning of significant interest in the CRISPR field. Ex. 2403.



44. As of May 2016, the Zhang laboratory, including through Addgene, has distributed more than 30,000 CRISPR-Cas9 reagents, stemming from the eukaryotic work of the Zhang lab, and Cong et al. (Ex. 1055) has more than 1650 acknowledging citations to date and is the most highly cited CRISPR publication. Ex. 2001, Simons ¶ 2.14.

45. In 1999, Koseki *et al*. reported that the efficacy of RNA ribozymes molecules *in vitro* is not predictive of functional activity *in vivo*, for a variety of reasons, including degradation of the ribozyme in the eukaryotic cell and inability to colocalize with its target in the eukaryotic cell. Ex. 2221, Koseki at 1875–76.

46. Despite great interest in the use of riboswitches as a means of controlling gene expression in other contexts, the creation of riboswitches that function as desired in mammalian systems has proved intractable to date. Ex. 2223, Link.

47. Link et al. (Ex. 2223, Link at 1192) note, "a few TPP riboswitches are the only validated metabolite-binding riboswitches in eukaryotes" and many of the engineered riboswitches that function well *in vitro* fail to provide useful function when translated to eukaryotic cells. Ex. 2001, Simons ¶ 6.47.

48. It was uncertain whether a Cas protein could access target DNA in a eukaryotic cell. Ex. 2001, Simons ¶ 6.29.

49. Different cellular conditions can impact protein folding, which is of particular importance given that CRISPR-Cas9 systems must undergo significant conformational changes both when binding the guide RNA, and when binding and cleaving the target DNA. Ex. 2001, Simons ¶¶ 6.13, 6.33.

50. A person of ordinary skill in the art during the relevant time period could not have predicted whether eukaryotic enzymes would cleave the RNA molecules critical for the functioning of the CRISPR-Cas9 system. Ex. 2001, Simons ¶ 6.15.

51. The CRISPR-Cas9 system's requirement for magnesium would have suggested to a skilled person that the prokaryotic environment, which includes a high concentration of magnesium, might be essential for the operation of CRISPR systems *in vivo*. Ex. 2001, Simons ¶¶ 6.13, 6.38-6.39.

52. The contemporaneous evidence confirms that, given the nature of the bacterial-derived CRISPR system and the substantial differences between the prokaryotic and eukaryotic environments, skilled artisans would not have reasonably predicted that CRISPR systems would function in eukaryotes, even after successful *in vitro* DNA cleavage experiments such as reported in Jinek 2012. Ex. 2001, Simons ¶¶ 6.48-6.51.

53. Dr. Doudna stated that "[w]e weren't sure if CRISPR/Cas9 would work in eukaryotes—plant and animal cells." Ex. 2207 at 3.

54. Another scientist in the field noted, "[i]t's not trivial to make CRISPR/Cas systems work in eukaryotic cells … One thing is to have them *in silico* and have a sequence … and another thing is to do the experiments and make it work. " Ex. 2213, Grens at 2 (quoting Luciano Marraffini).

CERTIFICATE OF FILING AND SERVICE

I hereby certify that on the 23rd day of May, 2016, a true and complete copy of the foregoing **BROAD et al. SUBSTANTIVE MOTION 2 (for judgment of no interference-in-fact)** is being filed by 5:00 pm EST via the Interference Web Portal. Pursuant to agreement of the parties, service copies are being sent by e-mail by 8:00 pm EST, to counsel for Senior Party as follows:

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