

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;
8,889,418; 8,895,308; 8,906,616; 8,932,814; 8,945,839; 8,993,233; 8,999,641; and
9,840,713; and Applications 14/704,551 and 15/330,876,

Junior Party,

v.

SIGMA-ALDRICH CO., LLC,

Application 15/456,204,

Senior Party.

Patent Interference No. 106,133 (DK)
(Technology Center 1600)

BROAD OPPOSITION 1

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1 **I. PRECISE RELIEF REQUESTED**

2 Junior Party, The Broad Institute, Inc., Massachusetts Institute of Technology, and
3 President and Fellows of Harvard College (collectively, “Broad”), requests that the PTAB deny
4 Senior Party Sigma-Aldrich Co. LLC’s (“Sigma”) Motion 1 to Deny Broad Benefit of Application
5 61/736,527 (“Broad P1”).

6 If the PTAB grants Broad’s Motion 1 and changes the count to Proposed Count 3, Sigma’s
7 Motion 1 should be dismissed as moot as it only relates to benefit for Count 1.

8 **II. DESCRIPTION OF APPENDICES**

9 Appendix A is an Exhibit List, Appendix B is Broad’s responses to Sigma’s Material Facts
10 and Broad’s Statement of Material Facts.

11 **III. BACKGROUND**

12 At the time of the invention at issue here, it was well known in the art that zinc-finger
13 nucleases (“ZFNs”) and transcription activator-like effector nucleases (“TALENs”) could be used
14 to cleave DNA for precise genome engineering in eukaryotes, including altering expression of
15 genes. Ex. 2468, ¶¶ 277-282; MF14. In the early 2000s, well before 2012, ZFNs had been used for
16 site-specific, programmable gene editing to accomplish genome modifications in eukaryotic cells.
17 Ex. 2468, ¶ 278. Similarly, after first being discovered in 1989, by 2011, years of efforts to develop
18 TALENs culminated in demonstrations that TALENs could be programmed to cleave DNA
19 sequences of choice in a eukaryotic cell in a site-specific manner, and thereby, alter expression of
20 genes. *Id.*, ¶¶ 279-282; *see also* Ex. 2280.

21 POSAs in the field by 2011 therefore had no doubt that, based on the ability to achieve
22 programmable, site-specific cleavage of DNA, these systems could alter expression of gene
23 products. Ex. 2468, ¶¶ 279-282. POSAs knew that these systems could be used “to knock out or
24 knock in genes, to make allelic mutants, to change gene-regulatory control and to add reporters or

1 epitope tags, all in the endogenous genomic context.” Ex. 2280 at 1. And many DNA targets—
2 genes or their regulatory elements controlling expression—were known, as were methods for
3 identifying them in DNA. MF15; Ex. 2468, ¶¶ 269-276; Ex. 2269. But one of the key problems
4 with both ZFN and TALEN systems was their lack of scalability and the effort required to
5 reprogram them. Accordingly, the need remained for a gene-editing system that could be “*easily*
6 *and quickly designed*, in practice, to specifically modify any sequence of any genome; having
7 more than one technology available will help achieve this goal.” Ex. 2280 at 1.¹

8 The eukaryotic CRISPR-Cas9 invention, as for example set forth as Broad’s half of Count
9 1 (Broad’s 359 patent, claim 18), achieved that goal. Based on work commencing in 2011 and
10 continuing through 2012, Broad’s inventor Dr. Feng Zhang adapted the prokaryotic CRISPR-Cas9
11 system to function in eukaryotic cells as a programmable, generalizable system to achieve site-
12 specific cleavage of DNA. He used his system to achieve such cleavage, and also for donor
13 template integration and multiplexing (introducing multiple concurrent breaks in DNA to cause
14 large deletions). This system was described in detail in Application No. 61/736,527 (“Broad P1”
15 or “Zhang B1”), filed December 12, 2012. MF16-19.

16 The key disclosures of Zhang B1 were replicated in a manuscript that Dr. Zhang and his
17 colleagues submitted to *Science* on October 5, 2012 (“October 5 Manuscript”) reporting the
18 development of “a new class of precision genome engineering tools” based on RNA-guided
19 CRISPR-Cas9 nucleases. Ex. 2564; MF21.² The October 5 Manuscript compared the CRISPR-
20 Cas9 system it disclosed to prior systems that could “enable targeted genome perturbations,”
21 noting that while the CRISPR-Cas9 system shared their ability to mediate precise cleavage, its

¹ All emphases added unless otherwise noted.

² Zhang B1 also includes an additional multiplexing working example.

1 easy programmability meant it was “affordable, easy to set up, scalable, and can be multiplexed to
2 target multiple positions within the eukaryotic genome.” Ex. 2564 at 2. In specifically discussing
3 the same working examples, both Zhang B1 and the October 5 Manuscript state:

- 4 • “[t]hese results define a three-component system for efficient **CRISPR-mediated genome**
5 **modification** in mammalian cells” Ex. 2001, ¶ [00173];
- 6 • the disclosures “establish[] a set of components for achieving **CRISPR-mediated gene**
7 **editing** in mammalian cells through the error-prone NHEJ mechanism” *Id.*, ¶ [00183] and;
- 8 • “[t]hese results [on integration of an HR template] demonstrate the utility of CRISPR **for**
9 **facilitating targeted gene insertion** in the mammalian genome.” *Id.*

10 *See also* Ex. 2564 at 5, 6, 7. Zhang B1 and the manuscript conclude by stating that “the ability to
11 use RNA to program sequence-specific DNA cleavage defines **a new class of genome engineering**
12 **tools** for a variety of research and industrial applications.” *Id.* at 7; Ex. 2001, ¶ [00185]; MF22.

13 The impartial reviewers who reviewed this data for *Science* did not challenge these
14 conclusions as lacking proof, as Sigma does. *See* Ex. 2836. They, like others in the field, knew
15 that with eukaryotic experiments showing CRISPR-Cas9 allowed for programmable,
16 generalizable, site-specific, double-stranded DNA cleavage, including, for example, donor
17 template integration, one could achieve genome engineering similar to what had been done with
18 ZFNs and TALENs as the nucleases. The *Science* reviewers noted:

- 19 • One reviewer stated that experiments in the manuscript “now provide **experimental**
20 **evidence** that Cas9-crRNA complex can be employed for the **mammalian genome**
21 **engineering**” as with prior systems such as ZFNs and TALENs. *Id.* at 3. The reviewer
22 further concluded that the reported CRISPR-Cas9 work had “set a stage for genome editing
23 using Cas9-crRNA complexes.” *Id.* at 3-4.
- 24 • Another reviewer stated that “results show compellingly and thoroughly that the system
25 they developed based on Cas9, crRNA and tracrRNA, or on Cas9 and chimeric RNA **is**
26 **functional and efficient in vivo**, for **cleavage and inducing mutations at the target site.**”
27 *Id.* at 3.
- 28 • A third reviewer noted “[t]he authors show that Cas9 variants can be targeted to the nucleus
29 and loaded guide RNAs to enable programmed DNA cleavage **to disrupt genes** or induce
30 homologous recombination.” *Id.* at 4; MF23.

1 The experiments in the October 5 Manuscript are included, and elaborated upon, in Zhang B1.
2 Nevertheless, Sigma challenges the disclosure of Zhang B1 as insufficient to demonstrate
3 possession of a eukaryotic CRISPR-Cas9 system capable of altering expression of genes, as recited
4 in Broad's half of Count 1.

5 Sigma stands alone in its position here. In related interferences, also involving counts that
6 included claim 18 of Broad's 359 patent, neither CVC nor ToolGen ever challenged that idea that
7 Zhang B1 demonstrated to a POSA possession of the ability to alter expression of gene products
8 using a CRISPR-Cas9 system able to cleave eukaryotic DNA. Moreover, the PTAB recently found
9 that experiments reported in the October 5 Manuscript were actual reductions to practice of claim
10 18 of the 359 patent. Ex. 2863. Based on those same disclosures in in Zhang B1 and more, a POSA
11 would have concluded that the inventor had possession of, and that Zhang B1 is a constructive
12 reduction to practice of, the Broad half of Count 1. Nothing more was needed.

13 **IV. ARGUMENT**

14 In declaring this Interference, the PTAB awarded Broad the benefit of Zhang B1, consistent
15 with its prior determinations of benefit in the 115 and 126 Interferences. Paper No. 1, Decl. at 16.
16 Sigma challenges Broad's benefit to Zhang B1, but its motion can be readily dismissed. To succeed
17 on its motion, Sigma has the double burden to show that Zhang B1 does not show possession of
18 either half of Count 1. 37 C.F.R. §§ 41.121(b), 41.208(b); S.O. ¶ 121.3; Bd.R. 201 ("Constructive
19 reduction to practice"); S.O. ¶ 208.4.2; *Kubota v. Shibuya*, 999 F.2d 517, 521 (Fed. Cir. 1993).
20 Sigma's motion fails with respect to both halves of Count 1 and so should be denied.

21 To demonstrate possession, Zhang B1 must disclose the invention "in sufficient detail that
22 one skilled in the art can clearly conclude that the inventor invented the claimed invention as of
23 the filing date sought." *Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997). The
24 standard for possession does not require a working example including every element of the count.

1 *Ariad Pharms., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1352 (Fed. Cir. 2010). Rather, all that is
2 required is that the specification “reasonably conveys to those skilled in the art that the inventor
3 had possession of the claimed subject matter as of the filing date.” *Id.* at 1351. Zhang B1 shows
4 possession of each half of Count 1, and so Sigma fails to meet its burden to show the opposite.

5 **A. Sigma Failed In Its Burden With Respect To The Broad Half Of Count 1**

6 Sigma challenges Broad’s benefit to the Broad half of Count 1 on only one ground, namely,
7 written description regarding one element—“whereby expression of the at least one gene product
8 is altered” (the “altering expression” element).

9 Critically, Sigma does not dispute that a POSA reading Zhang B1 would conclude that the
10 inventor was in possession of a eukaryotic CRISPR-Cas9 system capable of programmable,
11 generalizable, site-specific DNA cleavage, including for both donor template integration (which
12 can be used for inserting sequences into the DNA) and multiplexing (introducing multiple
13 concurrent breaks in DNA to cause large deletions). MF24. Zhang B1 discloses multiple working
14 examples of CRISPR-Cas9 systems functioning in eukaryotic cells with such capabilities.

15 Sigma also does not dispute that Zhang B1 discloses over and over again throughout the
16 application that the disclosed CRISPR-Cas9 systems can be used to alter expression of genes. *See*
17 *e.g.*, Ex. 2001, ¶¶ [0011], [0013], [0038], [00103]-[00111], [00173]-[00185].

18 Rather, Sigma’s argument is based on an overly narrow definition of “altering expression”
19 that restricts that claim element to “changes in the expression *level* of the gene product, and not
20 simply changes in the sequence of the gene.” Mot. at 7:11-14. From that flawed position, Sigma
21 argues Zhang B1 does not show possession because, Sigma contends, it lacks “any demonstrated
22 showing of DNA cleavage resulting in alteration of the targeted gene’s expression.” *Id.* at 5:3-12.

23 Sigma’s expert admitted at deposition that her entire analysis of possession was premised
24 on looking for working examples in Zhang B1 that *measured* changes in gene expression *levels*:

1 Q. Is it your understanding that in order to demonstrate possession of an
2 embodiment within the scope of the first part of Count 1, a working example is
3 necessary?

4 A. My understanding is that a working example is kind of a home run and is very
5 convincing. *So yes, that's what I was looking for in Broad P1, looking for actual*
6 *evidence that that analysis had been done and a working example.*

7 Ex. 1519 at 41:23-42:6. She further confirmed her view that measurements were necessary:

8 Q. If cleavage resulted in a decreased transcription of a target gene, that would meet
9 the idea of altering expression of at least one gene product; correct?

10 A Yes, *if measured*, yes.

11 Ex. 1519 at 59:9-13. Sigma's motion follows suit, contending (incorrectly) that Zhang B1 lacks a
12 "demonstrated showing of DNA cleavage resulting in alteration of the targeted gene's expression"
13 (Mot. at 5:3-12), which Sigma says must be shown by results of a specific set of assays ("Northern
14 blot and quantitative RT-PCR" or "immunoassays") to experimentally prove a "change in the
15 expression levels of RNA or protein" actually occurred—not just a change in "the sequence of a
16 gene by editing at a CRISPR-Cas9 targeted location." Mot. at 6:3-8, 7:17-20, 8:1-3.

17 Broad's first response is that this is not the correct standard for possession. It is dispositive
18 that a POSA would have concluded from Zhang B1's working examples and disclosures that the
19 inventor possessed the invention of a CRISPR-Cas9 system capable of achieving altered
20 expression of a gene product (under any definition), whether or not Zhang B1 provided a working
21 example of altered gene expression. This is so because Zhang B1 indisputably showed a POSA
22 that the CRISPR-Cas9 system could be adapted as a programmable, generalizable system that
23 would induce site-specific cleavage of DNA in a eukaryotic cell. MF16-19, 22. And, based on the
24 prior work with systems such as ZFNs and TALENs, the POSA would have understood that ability
25 meant that previously-identified targets affecting gene expression, as well as new targets identified
26 via known methods, could be chosen for cleavage. For that reason alone, Sigma's motion fails.

27 Broad's second response is that Zhang B1 includes multiple experiments meeting every

1 element of the Broad half of Count 1, including altered expression, even under Sigma’s flawed
2 interpretation. Broad’s third response is that Sigma never met its burden of showing that the
3 “whereby” clause reciting “altered expression” is a positive claim limitation, rather than simply
4 the intended result of the remainder of claim 18. Broad’s fourth response is that Sigma’s overly
5 narrow definition of altered expression is both erroneous, as it is not the broadest reasonable
6 interpretation of that language, and irrelevant to the dispositive question of possession.

7 By any reasonable definition of altered expression, a POSA would readily conclude that
8 Zhang B1 showed possession of the invention of Broad’s half of Count 1.

9 **1. A POSA Would Readily Conclude From Zhang B1 That The Inventor**
10 **Possessed The Invention Of Broad’s Half Of Count 1, Even Without**
11 **The Measurements Demanded By Sigma**

12 Sigma’s motion is based entirely on the flawed premise that a POSA would need working
13 examples with assays showing *measurements of the level* of altered gene expression, e.g., altered
14 levels of protein expression, to find possession. Sigma argues that based on the “inherent
15 complexities and unpredictability of the eukaryotic processes that impact gene expression,” a
16 POSA would not have found possession, “particularly without any *demonstrated* showing of DNA
17 cleavage resulting in alteration of the targeted gene’s expression.” Mot. at 5:7-12. However,
18 POSAs well knew how to target and cleave DNA to impact gene expression, including the precise
19 locations of potential targets and methods for identifying others. MF14-15. Thus, a POSA reading
20 Zhang B1, including the disclosed experiments—working examples of a specific assay or not—
21 would readily conclude that the inventor possessed the invention of Broad’s half of Count 1, even
22 under Sigma’s overly narrow definition of “altered expression.” Ex. 2468, ¶¶ 302-312, 328-356.

23 **a. The Impartial Reviewers’ Comments Show That Zhang B1’s**
24 **Disclosure Suffices From A POSA’s Point Of View**

25 Once Zhang B1 demonstrated experimentally that the CRISPR-Cas9 system was able to

1 be adapted to allow for programmable, generalizable, site-specific cleavage, donor template
2 integration, and multiplexing in a eukaryotic cell, a POSA would have known that system could
3 achieve the very same effects as achieved by prior cleavage systems such as ZFNs and TALENs,
4 including altering expression of gene products, even under Sigma’s restrictive definition. Dr.
5 Seeger identifies in his declaration the specific disclosures and examples in Zhang B1 from which
6 a POSA would have concluded that the inventor possessed the invention. Ex. 2468, ¶¶ 328-356.
7 That is all that is required to show possession of the Broad half of Count 1.

8 The comments of the *Science* peer reviewers of the October 5 Manuscript confirm that
9 conclusion. Ex. 2836. The peer reviewers—impartial experts in the field—essentially answered
10 the question presented here—namely, whether the disclosure of the manuscript (and Zhang B1)
11 convincingly demonstrated to a POSA the invention of an adapted, generalizable, programmable
12 CRISPR-Cas9 system that, like ZFNs and TALENs, was functional for site-specific cleavage of
13 DNA in eukaryotic cells and so able to accomplish genome engineering, including alteration of
14 the expression of gene products. The contemporaneous, unanimous answer was in the affirmative.

15 The October 5 Manuscript and Zhang B1 both state that CRISPR-Cas9 systems could be
16 put to the same tasks as systems such as ZFNs and TALENs for “selective perturbation of
17 individual genetic elements.” Ex. 2001, ¶ [0002]; Ex. 2836 at 2. The *Science* reviewers agreed
18 with that statement, stating that the experimental data showed CRISPR-Cas9 was a “quite *exciting*
19 *alternative to ZFNs and TALENs for genome engineering.*” Ex. 2836 at 3.

20 Indeed, one reviewer commented that the manuscript showed that “Cas9 variants can be
21 targeted to the nucleus and loaded guide RNAs to enable programmed DNA cleavage *to disrupt*
22 *genes*”—that is, to cleave them such that their expression is altered or eliminated (Ex. 2468, ¶
23 353)—thereby answering the precise question at issue here. Ex. 2836 at 4; MF23. Even under

1 Sigma’s definition, altering gene expression includes “disrupt[ing] genes”—disruption would
2 necessarily alter the level of gene product expression. Likewise, another reviewer evaluated the
3 experiments and concluded that the authors “now provide experimental evidence that Cas9-crRNA
4 complex *can be employed for the mammalian genome engineering.*” Ex. 2836 at 3. Yet another
5 reviewer concluded that the system “*can be used for* RNA-guided DNA cleavage and *genome*
6 *engineering*” in human cells and that the “*results show compellingly and thoroughly that the*
7 *system* they developed based on Cas9, crRNA and tracrRNA, or on Cas9 and chimeric RNA *is*
8 *functional and efficient in vivo, for cleavage and inducing mutations* at the target site.” *Id.*

9 The bottom line is that a POSA would not demand whatever tests to determine the level of
10 expression that Sigma asserts are missing for possession. The impartial reviewers reached their
11 conclusions based on experiments that Sigma now argues are lacking in some manner.

12 In considering these reviewer comments in the 115 Interference in the context of priority,
13 the PTAB noted that “[t]he reviewers’ comments indicate to us that not only did *the Broad*
14 *inventors recognize and appreciate* the positive results of an engineered Type II *CRISPR-Cas*
15 *system targeting DNA* in eukaryotic cells *to specifically cleave* DNA molecules *and alter gene*
16 *expression, others in the field confirmed the results.*” Ex. 2863 at 61:17-21. Here, the comments
17 of the impartial reviewers remove any possible doubt that the experiments in Zhang B1 suffice, in
18 the eyes of a POSA, to establish that the inventor possessed a eukaryotic CRISPR-Cas9 system
19 for genome engineering—i.e., a system for altering expression of gene products as in Count 1.

20 In sum, Zhang B1’s groundbreaking showing of site-specific genome editing with a
21 programmable, generalizable CRISPR-Cas9 system “reasonably conveys to those skilled in the art
22 that the inventor had possession of the claimed subject matter as of the filing date.” *Ariad*, 598
23 F.3d at 1351. In fact, even Sigma’s expert admitted that based on the successful working examples

1 of Zhang B1, “[a] person of ordinary skill in the art could have certainly *predicted or expected* that
2 expression might have been changed” as a result of the use of CRISPR-Cas9 and that a POSA
3 could have “*predicted or expected* the gene expression might have been altered” based on an
4 altered DNA sequence. Ex. 1519 at 41:6-10, 54:8-13; MF25. Once site-specific cleavage was
5 established, along with donor template integration, a POSA would have more than enough
6 information to find possession—whether or not the Zhang B1 experiments specifically targeted
7 those sites that would be used for the specific purpose of altering expression of gene products.

8 **b. The Experiments In Zhang B1 Demonstrating Possession**

9 Zhang B1 includes results showing successful use of CRISPR-Cas9 systems in eukaryotic
10 cells, including with a chimeric RNA SpCas9 system against mTh, hEMX1, and hPVALB targets.
11 Zhang B1 shows: (1) cleavage that introduces single- and multi-nucleotide indels into target DNA,
12 (2) two concurrent cleavage events (multiplexing) leading to a 118-bp deletion from the target
13 DNA (demonstrating a deletion of effectively any length is possible), and (3) insertion of an over
14 2000-nt donor template into the target DNA. Ex. 2468, ¶¶ 330-347; Ex. 1501, ¶¶ 29-32; MF17-19.

15 • **Experiments demonstrating cleavage and the introduction of indels into a gene** (*see*
16 Ex. 2001, ¶¶ [00154]-[00156], [00173], [00175]-[00178], [00180], [00182], Fig. 1D, Fig. 2, Figs.
17 3B-D, Fig. 5, Fig. 6B, Fig. 11D, Fig. 12C; MF17): Zhang B1 specifically states that “[t]hese results
18 define a three-component system for efficient CRISPR-mediated genome modification in
19 mammalian cells” (Ex. 2001, ¶ [00173]) and discloses “a set of components for achieving
20 CRISPR-mediated gene editing in mammalian cells through the error-prone NHEJ mechanism”
21 (*id.*, ¶ [00183]). Zhang B1 further teaches that in some embodiments, this “cleavage results in
22 *decreased* transcription of a target gene” (*id.*, ¶¶ [0011], [0013])—i.e., a change in its level of
23 expression—or can be used to inactivate regulatory sequences controlling gene expression. Ex.
24 2468, ¶¶ 333-338; Ex. 2001, ¶¶ [0011], [00103]-[00104]; MF20.

1 • **Experiments demonstrating concurrent double-stranded breaks (DSBs)**
2 **(“multiplexing”) deleting a 118-bp sequence from a gene:** Zhang B1 teaches that “the CRISPR
3 system can mediate multiplexed editing within a single genome”—introducing two concurrent
4 DSBs in the target DNA, thus deleting a sequence of any potential length. Ex. 2001, ¶ [00184],
5 Figs. 4F-G; MF18. Such a deletion can, for instance, disrupt expression of any gene excising its
6 regulatory sequences or by deleting an entire gene sequence itself. Ex. 2468, ¶¶ 339-341.

7 • **Experiments demonstrating donor template integration:** Zhang B1 expressly teaches a
8 POSA that the insertion of a donor template “demonstrate[s] the utility of CRISPR *for facilitating*
9 *targeted gene insertion* in the mammalian genome.” Ex. 2001, ¶ [00183], Figs. 4C-E; MF19. That
10 is, Zhang B1 teaches that by this type of insertion, new genes can be expressed in the cell entirely.
11 Ex. 2468, ¶¶ 342-344; Ex. 2001, ¶¶ [0093]-[0095] (“Thus, the sequence for integration may be
12 operably linked to an appropriate control sequence or sequences. Alternatively, the sequence to be
13 integrated may provide a regulatory function.”).

14 Incredibly, Sigma and Dr. Cannon even contest that Zhang B1’s disclosure of insertion of
15 a donor template that “facilitates targeted gene insertion” shows possession of the Broad half of
16 Count 1. But a POSA would have understood the importance of showing that a 2000-bp section of
17 the genomic DNA could be replaced by an engineered donor template. As Dr. Seeger explains, a
18 POSA would have understood these particular showings of site-specific genome engineering by a
19 programmable system would mean, at a minimum, that known (and easily identified) regulatory
20 elements which control gene expression could be added, deleted, or modified in genes of interest,
21 and that entire genes could be knocked in or out—just as they had been done with ZFNs and
22 TALENs. *See* Ex. 2468, ¶¶ 342-344. Indeed, a POSA could hardly miss this conclusion, which
23 necessarily demonstrates possession: Zhang B1 specifically teaches that the CRISPR-Cas9 system

1 in these examples can be used for “targeted gene insertion” or to inactivate a control sequence in
2 a DNA template “such that it no longer functions as a control sequence.” Ex. 2001, ¶ [00103].

3 In sum, a POSA would conclude, as to the dispositive question here, that the disclosures
4 of Zhang B1 show possession of the invention of Broad’s half of Count 1, including the altering
5 expression term “in sufficient detail that one skilled in the art can clearly conclude that the inventor
6 invented the claimed invention as of the filing date sought.” *Lockwood*, 107 F.3d at 1572.

7 **2. Although A Working Example Of All Claim Elements Is Not**
8 **Required For Possession, Zhang B1 Discloses Working Examples**
9 **Showing Altered Expression Under Either Party’s Definition**

10 For the reasons discussed above, the PTAB need not even determine whether cleavage of
11 the specific targets selected for Zhang B1 resulted in altered gene expression. The targets were
12 selected to show that the system was programmable and generalizable for site-specific cleavage to
13 establish CRISPR-Cas9 as a tool that one could readily use for eukaryotic genome engineering of
14 all sorts, including altering expression of gene products. But even if experiments showing altered
15 expression were required, Zhang B1 would satisfy that requirement.

16 In fact, in the 115 Interference, the PTAB awarded Broad priority based on an ARTP of
17 that Count 1, the Broad half of which was also claim 18 of the 359 patent, citing the October 5
18 Manuscript and the same data from Zhang B1. Ex. 2863 at 64:11-14. Given that the data in the
19 manuscript reflects an ARTP, the same data in Zhang B1 must be a working example of Count 1
20 here as well (and, at a minimum, is sufficient to be a constructive reduction to practice).

21 Sigma presents no reason why that result should be changed. But the PTAB need not rest
22 on its prior conclusions; it is unequivocally clear on a fresh evaluation that Zhang B1’s experiments
23 disclose every element of claim 18. As Dr. Seeger testifies, Zhang B1 discloses multiple working
24 examples of a CRISPR-Cas9 system within the scope of the Broad half of Count 1 under either
25 party’s view of the altering expression term, including cleavage introducing indels, a 118-bp

1 deletion, and insertion of an over 2000-nt template into target DNA. Ex. 2468, ¶¶ 357-385.

2 As just one example, Zhang B1 discloses what Sigma and Dr. Cannon refer to as “a gene
3 editing outcome where a 12-bp sequence comprising two restriction enzyme sites replaced the stop
4 codon in the *emx1* gene,” referring to Figure 4C and paragraphs [00183] and [00201] of Zhang
5 B1. Ex. 1501, ¶ 32; MF19. This refers to the donor template embodiment in Zhang B1 (“E17+” in
6 Broad Motion 1), which, as Dr. Seeger explains, demonstrates not just a 12-bp change to the *EMX1*
7 sequence, but insertion of an over 2000-nt template into the *EMX1* gene. Ex. 2468, ¶ 379.

8 The donor template embodiment includes experiments demonstrating “the ability of
9 CRISPR to stimulate homologous recombination (HR), a high fidelity gene repair pathway for
10 making precise edits in the genome.” Ex. 2001, ¶ [00183]. These experiments successfully
11 integrated into the *EMX1* gene a donor template that included a 12-bp restriction site not present
12 in the natural cellular DNA (“wild-type” DNA): “SpCas9... indeed catalyzed integration of the
13 HR template into the *EMX1* locus,” which was verified by a restriction fragment length
14 polymorphism gel analysis,” Figure 4D, as well as Sanger sequencing of genomic amplicons, an
15 example is Figure 4E. *Id.* The sequence of the HR template that was integrated is provided in
16 paragraph [00201] (showing “hEMX1-HR Template-HindII-NheI”) and is over 2000-nt, with an
17 addition of 12-nt over the sequence of the wild-type *EMX1* gene. And as Dr. Cannon
18 acknowledges, “[t]he human *emx1* gene produces the *EMX1* protein.” Ex. 1501, ¶ 30.

19 Based on this data, a POSA would have understood there had been a modification of the
20 sequence of the *EMX1* gene (Ex. 1501, ¶ 30, citing Ex. 1505), and a corresponding modification
21 to the transcription, splicing, and translation steps for producing the *EMX1* protein—i.e., that
22 expression of a gene product would be altered. Ex. 2468, ¶¶ 375-385; Ex. 2001, ¶ [0038]; Ex.
23 1501, ¶ 32. The inserted template added 12-bps to the original wild-type DNA sequence, replacing

1 the stop codon in the EMX1 gene. As Dr. Cannon acknowledges, a POSA would have understood
2 that this would change the process of translation into the EMX1 protein by “continu[ing] the open
3 reading frame with an additional 101 amino acids at the C-terminus” of the EMX1 protein as
4 expressed—i.e., a mutated protein was produced. Ex. 1501, ¶ 11 (citing to her Appendix C); Ex.
5 2468, ¶¶ 375-385. In other words, expression of the final protein gene product would be altered.

6 Sigma contests that “whether this modification actually occurred is unknown and was not
7 shown.” Mot. at 11:9-18. Sigma contends that further testing via, for instance, Western blot, could
8 have been done. As Dr. Seeger explains, however, a POSA would not find any need for such
9 testing, as the process of transcription, splicing, and translation was well understood as of 2012,
10 as were the effects of editing DNA on those processes given the use of prior systems for DNA
11 cleavage. *See* Ex. 2468, ¶¶ 381-385. The data demonstrates the expression processes were altered.

12 A POSA would thus have understood that Zhang B1, including the working examples,
13 discloses altering expression of gene products even under Sigma’s restrictive definition. The
14 working examples showing CRISPR-Cas9 systems capable of programmable, generalizable, site-
15 specific cleavage of DNA in a eukaryotic cell, including for donor template integration and
16 multiplexing, together with the multiple disclosures that the systems are capable of altering gene
17 expression, constitute embodiments of the Broad half of Count 1 and demonstrate constructive
18 reductions to practice of the Broad half of Count 1, including the altering expression element,
19 under either (a) Broad’s view of the term or (b) Sigma’s restrictive definition that requires “a
20 change in the expression level of RNA or protein of a targeted DNA.” *Id.*, ¶¶ 357-385. Dr. Seeger
21 further explains why multiple other experiments in Zhang B1 similarly demonstrate possession
22 and a constructive reduction of practice of the subject matter of the Broad half of Count 1. *Id.*

1 **3. Sigma Fails To Address Whether “whereby expression of the at least**
2 **one gene product is altered” Is A Positive Claim Limitation**

3 Additionally, Sigma failed to carry its burden because it never addresses the claim
4 construction issue of whether the language of the whereby clause—“whereby expression of the at
5 least one gene product is altered”—states an intended result of the remainder of the claim or
6 whether it is a positive limitation that also needs to be met to show possession. Having failed to
7 address this threshold issue, Sigma’s motion is deficient.

8 Whether a whereby clause is limiting is a context and fact specific inquiry. *See Minton v.*
9 *Nat’l Ass’n. of Sec. Dealers, Inc.*, 336 F.3d 1373, 1381, (Fed. Cir. 2003). “A whereby clause in a
10 method claim is not given weight when it simply expresses the intended result of a process step
11 positively recited.” *Id.*; *see also Lockheed Martin Corp. v. Space Systems/Loral, Inc.*, 324 F.3d
12 1308, 1319 (Fed. Cir. 2003) (language only stated an inherent result); *In re Kubin*, 561 F.3d 1351,
13 1357 (Fed. Cir. 2009) (irrelevant whether prior art disclosed a feature of “wherein the polypeptide
14 binds CD48” as feature was necessarily present in protein). “Whereby” language must provide
15 structure or acts necessary to define the invention to be a positive limitation. *In re Kubin*, 561 F.3d
16 at 1353.

17 It is clear why Sigma did not address this point. At her deposition, Dr. Cannon admitted
18 that altering expression was, in fact, an intended result of the cleavage of the DNA:

19 Q. So you read [Broad] claim [18] as that the cleavage of the DNA results in the
20 expression of at least one gene product being altered; is that right?

21 A. Yes.

22 Ex. 1519 at 69:25-70:3; MF26. In any event, Sigma failed to address whether the altering
23 expression whereby clause, the only limitation Sigma challenges, was a positive limitation
24 required to show possession. For this additional reason, the PTAB can deny Sigma’s motion.

1 **4. Sigma’s Motion Is Premised On An Improper Attempt To Restrict**
2 **Altering Gene Expression To Altering Gene Expression Levels**

3 As stated above, Sigma’s motion fails no matter the definition of “altered expression” and
4 fails because it is premised on an overly narrow and erroneous understanding of what altering
5 expression means. At 5:13-8:11, Sigma argues that “altered expression of a gene product means
6 changes in the *level* of expression.” Mot. at 5:13-8:11. The response is that Sigma’s conclusions
7 are not supported by Zhang B1 and Sigma can in no way justify restricting “whereby expression
8 of the at least one gene product is altered” to “whereby *the* expression *level* of the at least one gene
9 product is altered,” particularly as the broadest reasonable interpretation of that claim language.
10 Nor does Zhang B1 support Sigma’s further requirement that the altering expression claim term
11 *requires* a specific set of assays to measure the supposedly required changed levels of expression.

12 Zhang B1 unequivocally defines “expression” and “gene products” and makes it clear that
13 expression refers to processes, including splicing of the mRNA:

14 As used herein, ‘*expression*’ *refers to the process* by which a polynucleotide is
15 transcribed from a DNA template (such as into and [sic] mRNA or other RNA
16 transcript) and/or the process by which a transcribed mRNA is subsequently
17 translated into peptides, polypeptides, or proteins. Transcripts and encoded
18 polypeptides may be collectively referred to as ‘gene product.’ If the polynucleotide
19 is derived from genomic DNA, *expression may include splicing of the mRNA* in
20 a eukaryotic cell.

21 Ex. 2001, ¶ [0038]; MF27. Taking these statements and the usage in the prior art together, a POSA
22 would have understood that the broadest reasonable interpretation of “whereby the *expression* of
23 the at least one gene product *is altered*” as used in Count 1 includes any alteration in the multi-
24 step *process* by which a protein is expressed from a gene in a eukaryotic cell—including altering
25 any step thereof. *See also* Ex. 2468, ¶¶ 313-327.

26 Dr. Cannon, Sigma’s expert, acknowledged multiple times that expression in Zhang B1 is
27 the transcriptional and translational processes: “Q. As we saw before, expression refers to a

1 process; correct? A. Yes, transcription or translation.” Ex. 1519 at 66:16-18. Dr. Cannon further
2 admitted changes to the sequence of DNA would “change the process by which a polynucleotide
3 is transcribed from a DNA template.” *Id.* at 36:4-12. She disputed that this “would not necessarily
4 mean that expression had been altered”; however, under the paragraph [0038] definition of
5 expression, “chang[ing] the process by which a polynucleotide is transcribed from the DNA
6 template” (Dr. Cannon’s answer) is necessarily altering “the process by which a polynucleotide is
7 transcribed from the DNA template” (the definition of “expression”). *Id.* at 55:7-13, 36:4-12.

8 Sigma primarily relies on paragraph [00110] to claim that altering expression requires
9 assaying for a change in expression *levels*. But paragraph [00100] does not define altering
10 expression—the cited passage is just a permissive example of one way of determining altered
11 expression of a gene product in a highly-context specific example—in this case, assaying the final
12 protein product while “developing a biologically active agent that modulates a cell signaling event
13 associated with a disease gene.” Ex. 2001, ¶ [00108].

14 Even Sigma admits all that paragraph [00110] states is that “‘altered expression’ *can be*
15 ‘determined by assaying for a difference in the mRNA levels of the corresponding genes.’” Mot. at
16 6:3-8. Yet from that permissive language, Sigma leaps to the conclusion that “a [POSA] would
17 have understood that the claim term ‘expression of at least one gene product is altered’ means a
18 change in the expression *levels* of RNA or protein.” *Id.* But nothing in paragraph [00110] amounts
19 to “words of manifest exclusion or restriction.” *Liebel-Flarsheim Co. v. Medrad, Inc.*, 358 F.3d
20 898, 906 (Fed. Cir. 2004) (“[T]he claims of the patent will not be read restrictively unless the
21 patentee has demonstrated a clear intention to limit the claim scope using ‘words or expressions
22 of manifest exclusion or restriction.’”).

23 Accordingly, even if everything else Sigma conjures from paragraph [00110] were true—

1 it is not—paragraph [00110] merely lists assaying for the level of gene product as one possible
2 way to determine “altered expression” in the specific context given, and in no way would it have
3 been understood by a POSA to work as some kind of manifest exclusion or restriction on the scope
4 of the broadest reasonable interpretation of the altering expression element, which includes
5 changes in any step in the process of transcription and translation. Ex. 2001; *Genentech v Chiron*,
6 112 F3d 495, 500 (Fed. Cir. 1997) (language of a count “given the broadest reasonable
7 interpretation” not “a contrived, artificial, or narrow interpretation”) (citation omitted). Certainly,
8 Sigma’s definition is not the broadest reasonable interpretation of the claim language at issue.

9 At 6:18-7:25, Sigma cites paragraphs [00105], [0099], and [00100], which Sigma says
10 “expressly distinguish” between “modifying a sequence and altering expression.” Mot. at 6:18-
11 7:25. As to [0099] and [00100], the entire basis for Sigma’s claim is that paragraph states “[i]n an
12 exemplary method for modifying a target polynucleotide” which Sigma claims is mutually
13 exclusive with paragraph [00100], which states “[i]n other embodiments, this invention provides
14 a method of modifying expression of a polynucleotide.” *Id.* Paragraph [00105] uses similar
15 language. Taken alone or in context, however, paragraphs [0099], [00100], and [00105] do not
16 “expressly distinguish” anything, let alone make any statement about “modifying a target
17 nucleotide” not showing “modifying expression of a polynucleotide.” They simply refer to two
18 embodiments, which are not mutually exclusive—as Sigma’s expert, Dr. Cannon, admitted,
19 explaining that “modifying a DNA sequence could lead to changes in expression.” Ex. 1519 at
20 54:23-55:6 (“outcomes are not mutually exclusive” in [0099] and [00100]).

21 Tellingly, Sigma ignores the context including the paragraphs that follow (and others),
22 which teach that cleavage leads to alterations in expression. For instance, paragraphs [00103]-
23 [00104] teach that the system can cleave and modify a control sequence in a DNA template “such

1 that it no longer functions as a control sequence” to control gene expression, and that “deletion of
2 one or more nucleotides” or “insertion of one or more nucleotides” can result in “inactivation of a
3 target sequence” resulting in “knock-out” of the target sequence. Ex. 2001, ¶¶ [00103], [00104];
4 *see also* ¶ [0011], [0013] (“cleavage results in decreased transcription of a target gene”; “said
5 mutation results in one or more amino acid changes in a protein expressed from a gene comprising
6 the target sequence”). Similarly, paragraphs [0093]-[0095] teach that cleavage and integration of
7 a donor template can be used to delete a disease gene and replace it with a new gene, including a
8 regulatory sequence for expressing it. In both cases, cleavage translates to alteration of gene
9 expression, exactly as a POSA would understand in the context of genome engineering.

10 Accordingly, Sigma is wrong in its definition of altering expression, and a POSA would
11 not have needed assays to measure levels of expression to conclude Zhang B1 showed possession
12 of the subject matter of the Broad half of Count 1. *See also* Ex. 2468, ¶¶ 313-327.

13 For all of these reasons, Sigma has not shown that the PTAB was in error in awarding
14 Broad benefit to Zhang B1. Zhang B1 shows that the inventor had possession of the invention
15 recited in Broad’s half of Count 1. Sigma’s motion should be denied.

16 **B. Zhang B1 Demonstrates Possession Of A CRISPR-Cas9 System Including A**
17 **Cas9 With Only One NLS Within The Sigma Half Of Count**

18 Sigma’s motion fails for a second, independent reason—Zhang B1 also demonstrates
19 possession and a constructive reduction to practice of the Sigma half of Count 1.

20 In its motion at 15:5-18:9, Sigma argues Zhang B1 does not provide adequate written
21 description support for a single element of the Sigma half of Count 1—a Cas9 protein “linked to
22 only one” NLS. Mot. at 15:5-18:9. The response is that, like its argument on the altered expression
23 language in Broad’s half of Count 1, Sigma’s argument fails at the outset as Sigma once again
24 improperly bases its argument solely on the lack of a working example; but the dispositive question

1 is whether a POSA would have understood from the *entire disclosure* of Zhang B1 that the
2 inventor possessed the invention of the subject matter of Sigma’s half of Count 1. As shown below,
3 to a POSA, Zhang B1 clearly showed possession of an embodiment within the Sigma half of Count
4 1. Sigma did not and cannot carry its burden to attack Broad’s benefit.

5 A POSA reviewing Zhang B1 would understand that Zhang B1 established the ability of a
6 CRISPR-Cas9 system (with a Cas9 linked to two NLSs) to perform donor template integration in
7 eukaryotic cells—which it did through a working example (which Sigma does not contest). Zhang
8 B1 also showed that a Cas9 with only one NLS achieved nuclear localization, although at a
9 diminished level compared to a Cas9 with two NLSs. As a result, as explained below, Zhang B1
10 demonstrates possession of the subject matter of the Sigma half of Count 1.

11 **1. Zhang B1 Discloses A CRISPR System With A Cas9 Construct**
12 **Including Only One NLS Within The Sigma Half Of Count 1**

13 Sigma does not contest that Zhang B1 discloses working examples with a two NLS Cas9
14 system that meet all of the non-NLS elements of the Sigma half of Count 1 (and this concession
15 highlights one of the problems with Count 1 (*see* Broad Mot. 1)). As discussed above, the donor
16 template integration experiments (reflected, *inter alia*, in Figures 4C-E and paragraphs [00183]
17 and [00201]) include the use of a CRISPR-Cas9 system to integrate a donor template into the target
18 site in the EMX1 gene in a eukaryotic cell. Ex. 2001, Figs. 4C-E, ¶¶ [00183], [00201]; Ex. 1501,
19 ¶ 32 (Dr. Cannon acknowledging system’s function); Mot. at 11:9-11 (referring to embodiment
20 and acknowledging “two restriction enzyme sites replaced the stop codon in the *emx1* gene.”).

21 Sigma’s only argument with regard to its half of Count 1 is that, because that working
22 example of donor template integration used a system with a Cas9 linked to two NLSs, not one
23 NLS, a POSA supposedly would have concluded that Zhang B1 did not show the inventor had
24 possession of, and taught away from, use of a system with one NLS. Mot. at 13:9-14:4. The

1 response is that a working example with one NLS was not necessary once Zhang B1 disclosed: (1)
2 a functional eukaryotic CRISPR-Cas9 system to accomplish donor template integration, and (2)
3 the use of a Cas9 with “one or more nuclear localization sequences” with disclosure of numerous
4 embodiments in which the Cas9 “comprises one or more nuclear localization sequences of
5 sufficient strength to drive accumulation of said CRISPR enzyme in a detectable amount in the
6 nucleus of a eukaryotic cell.” Ex. 2001, ¶¶ [0004]-[0007], [0062]. To be clear, “one or more” NLSs
7 includes both one NLS and more than one NLS, such as two.

8 Zhang B1 also provides experimental data comparing the localization of two specific Cas9-
9 NLS combinations to a third NLS-Cas9-NLS combination that a POSA would have further found
10 support possession. This experiment is described in paragraph [00170] and depicted in Figure 1B.
11 Zhang B1 concludes that the use of a Cas9 with NLSs on both the N- and C-terminus of the protein
12 is superior, and that—for the particular single NLSs tested—nuclear localization of the Cas9 with
13 one NLS was not adequate comparatively: “attachment of a single copy of *these particular* NLS’s
14 to either the N- or C-terminus of SpCas9 was unable to achieve adequate nuclear localization in
15 this system.” Ex. 2001, ¶ [00170]. It also states, again limited to the specific versions of Cas9 with
16 NLSs tested, that “[*o*f the versions of SpCas9 tested, only 2xNLS-SpCas9 exhibited nuclear
17 localization (Figure 1B).” *Id.* Both of these statements evince a clear intention to cabin the
18 conclusions of [00170] to the particular NLSs used.

19 While Sigma relies heavily on those statements out of context, a POSA would also look at
20 these conclusions in light of the entire disclosure, including Figure 1B itself, and see localization
21 by a Cas9 construct with one NLS. Ex. 2001, Fig. 1B (color version of same data in Cong 2013,
22 Ex. 2201); MF28. As Dr. Seeger testified, a POSA would have concluded from the
23 “immunofluorescence data from the three engineered Sp-Cas9 proteins” in Figure 1B that “two

1 NLSs ha[d] better nuclear localization than one NLS,” but that this data, along with the
2 experiments showing functional CRISPR-Cas9 systems with two NLSs, “*showed that the system*
3 *functioned* in the eukaryotic cells with *both* one and two NLS.” Ex. 2467, 76:7-14, 77:10-17;
4 MF29. Sigma gives no reason why a POSA would ignore the data in *the second row of Figure 1B*
5 *showing that a Cas9 construct with one NLS achieved localization*—albeit at a lower efficiency
6 than the two-NLS Cas9. This disclosure of one NLS achieving localization is consistent with the
7 disclosure of Zhang B1 as a whole. Zhang B1 recites numerous embodiments in which the Cas9
8 “comprises one or more nuclear localization sequences” to achieve localization of Cas9 “in a
9 detectable amount in the nucleus of a eukaryotic cell.” Ex. 2001, ¶¶ [0004]-[0007], [0062].

10 Notably, Dr. Cannon, did not interpret Figure 1B; instead she carefully limited her opinion
11 to the statements in [00170] in isolation. Ex. 1501, ¶¶ 38-39. Similarly, Sigma contends Zhang B1
12 teaches Cas9-1NLS constructs do not localize, but does not address Figure 1B. Mot. at 14:7-15:2.

13 Zhang B1 demonstrates nuclear localization of Cas9 with a single NLS, as well as a
14 working system with the more efficient Cas9-2NLS construct. Zhang B1 thus demonstrates to a
15 POSA possession of a system within the Sigma half of Count 1.

16 **2. Sigma Provides No Support For Its Argument That A Working** 17 **Example With One NLS Is Required**

18 At 15:2-18:9, Sigma reviews the working examples in Zhang B1, concluding that all of the
19 working examples used a Cas9 construct with two NLSs. Mot. at 15:2-18:9. Sigma thus concludes
20 at 18:5-9 that “[t]his analysis further shows that the Broad P1 applicants did not demonstrate
21 introducing a Cas9 protein with a single NLS into a eukaryotic cell to perform gene editing at the
22 time of filing Broad P1” and thus argues that a POSA “would have concluded that the Broad P1
23 applicants did not possess the second part of Count 1.” *Id.* at 18:5-9.

24 The response is that, despite Sigma’s burden, it provided no evidence that a POSA would

1 find Zhang B1’s working examples using a two NLSs Cas9, combined with the disclosures relating
2 to an actual working example with a Cas9-1NLS construct, to be insufficient to show possession.

3 It is well settled that the “written description requirement does not demand either examples
4 or an actual reduction to practice” in every case. *Ariad*, 598 F.3d at 1352; *see also, Falkner*, 448
5 F.3d at 1366. It was the case that, at the time of the invention, actual working examples of CRISPR-
6 Cas9 systems sufficient to overcome the hurdles and obstacles to eukaryotic genome editing were
7 necessary to show possession of such a system. Ex. 2121. But Sigma offers *no* evidence or
8 argument for its apparent contention that a POSA would have concluded, after Zhang B1 provided
9 actual working examples of CRISPR-Cas9 systems using Cas9 constructs with two NLSs and
10 disclosed the nuclear localization tests with one NLS, that a POSA would have believed that an
11 actual working example showing cleavage with a Cas9 construct with “only one” NLS was
12 necessary to show possession of the invention of the Sigma half of Count 1.

13 To the contrary, a POSA would have understood that this system could be used in the same
14 manner as taught for the working examples—albeit at likely lower efficiencies, given the lower
15 rate of nuclear localization. *See also* Ex. 2001, ¶ [0062] (disclosing 13 other specific NLS
16 sequences), ¶¶ [00194]-[00195], [00198]-[00199] (disclosing specific Cas9-1NLS constructs).

17 Regardless, it was Sigma’s burden to prove that a working example with a single NLS Cas9
18 construct was necessary, once an example with two NLSs was proven and data was presented
19 showing that one NLS achieved nuclear localization. Sigma did not address this at all, and so its
20 motion fails to meet its burden.

21 **V. CONCLUSION**

22 For the foregoing reasons, Broad respectfully requests the PTAB deny Sigma Motion 1.
23

1 Dated: March 16, 2022

Respectfully submitted,

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APPENDIX A: LIST OF EXHIBITS CITED

Ex.	Description
1501	Declaration of Paula M. Cannon, Ph.D., executed December 3, 2021.
1519	Deposition of Paula M. Cannon, Ph.D., dated February 24, 2022.
2001	U.S. Application 61/736,527, Zhang et al., December 12, 2012.
2121	Paper 877, Decision on Motions, Interference 106,115, September 10, 2020.
2201	Cong et al., “Multiplex Genome Engineering Using CRISPR/Cas Systems,” <i>Science</i> , 339(6121):819-823 (2013) with Supplemental Material.
2269	Maston et al., “Transcriptional Regulatory Elements in the Human Genome,” <i>Annu. Rev. Genomics Hum. Genet.</i> , 7:29–59 (2006).
2280	“Method of the Year 2011,” <i>Nat. Methods</i> , 9(1):1 (2012).
2467	Deposition of Christoph Seeger, dated March 1, 2022.
2468	Declaration of Christoph Seeger, executed March 16, 2022.
2564	Cong et al., “CRISPR-Assisted Mammalian Genome Engineering,” <i>Science</i> , Manuscript (October 5, 2012) (“Oct. 5 Manuscript”).
2836	Reviewers’ Comments of Cong et al., “CRISPR-Assisted Mammalian Genome Engineering,” <i>Science</i> , Manuscript (October 5, 2012).
2863	Decision on Priority, Interference 106,115, February 28, 2022.

1 **APPENDIX B: STATEMENT OF MATERIAL FACTS**

2 **Broad’s Responses to Sigma’s Material Facts:**

3 To a POSITA in mid-December 2012:

4 **1.** Broad P1 does not demonstrate that Broad’s applicants possessed a CRISPR-Cas9
5 system that cleaved a target DNA molecule and altered the expression of the gene product of that
6 cleaved molecule. Ex. 1501 ¶¶ 15, 17, 27-36.

7 **RESPONSE:** Denied, as shown herein, Broad P1 (Zhang B1) did demonstrate
8 possession.

9 **2.** In the context of Broad P1, a POSITA would have understood that the phrase
10 “expression of at least one gene product” means the expression of RNA or protein from a target
11 DNA molecule. *Id.* ¶ 20.

12 **RESPONSE:** Admitted that in paragraph [0038] of Zhang B1 it is stated that “[a]s used
13 herein, ‘expression’ refers to the process by which a polynucleotide is transcribed from a DNA
14 template (such as into and mRNA or other RNA transcript) and/or the process by which a
15 transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts
16 and encoded polypeptides may be collectively referred to as ‘gene product.’ If the polynucleotide
17 is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic
18 cell.”; otherwise denied.

19 **3.** In the context of Broad P1, a POSITA would have understood that the claim term
20 “expression of at least one gene product is altered” means changes in the expression level of the
21 gene product, and not simply changes in the sequence of the gene. *Id.* ¶¶ 21-26.

22 **RESPONSE:** Denied as this is not the broadest reasonable interpretation of the claim
23 term and not supported by the specification as set forth in Section IV.A.4.

1 **4.** A POSITA would have understood that merely changing the sequence of a gene
2 by editing at a CRISPR-Cas9 targeted location would not be expected to alter the expression of
3 the gene product. *Id.* ¶ 25.

4 **RESPONSE:** Denied, a change in the sequence of a gene by editing at a CRISPR-Cas9
5 targeted location translates to altering expression under the correct meaning of that term.

6 **5.** Broad P1 does not demonstrate that Broad’s applicants evaluated the expression
7 of the gene product of any target DNA molecule. *Id.* ¶¶ 27-36.

8 **RESPONSE:** Denied, Broad P1 (Zhang B1) does demonstrate evaluation of expression
9 of the gene product as set forth in Section IV.A.2.

10 **6.** In Broad P1, the applicants’ endeavors focused only on examining changes to a
11 target DNA sequence in a eukaryotic cell. *Id.* ¶¶ 15, 17, 27-36.

12 **RESPONSE:** Denied as set forth in Section IV.A.1-2.

13 **7.** A POSITA would not be able to rely upon any disclosure in Broad P1 to conclude
14 that the expression of any gene product of a target DNA molecule had been altered. *Id.* ¶¶ 35-36.

15 **RESPONSE:** Denied as set forth in Section IV.A.1-2.

16 **8.** In the context of Broad P1, without using an assay to demonstrate an alteration in
17 the level of a gene product, a POSITA would not be able to conclude whether gene editing has
18 had any effect on the levels of expression of the gene product. *Id.* ¶ 29.

19 **RESPONSE:** Denied as set forth in Section A.1-2; no such assays were necessary.

20 **9.** Broad P1 does not demonstrate that Broad’s applicants possessed a CRISPR-Cas9
21 system that introduced a Cas9 protein into a eukaryotic cell with only one linked NLS to perform
22 gene editing. *Id.* ¶¶ 16-17, 38-46.

23 **RESPONSE:** Denied as set forth in Section IV.B.

1 **10.** Broad P1 does not demonstrate that Broad’s applicants introduced a Cas9 protein
2 with only one NLS into a eukaryotic cell to perform gene editing. *Id.*

3 **RESPONSE:** Admitted that there is no working example of a Cas9 protein with only one
4 NLS in the working examples of Zhang B1; otherwise denied.

5 **11.** Broad P1 demonstrates that for each of the two Cas9 proteins Broad’s applicants
6 introduced into a eukaryotic cell to perform gene editing, each Cas9 protein had two NLSs. Ex.
7 2001 ¶¶ [0194]-[0197], [0200], [0202]; Ex. 1501 ¶ 46.

8 **RESPONSE:** Admitted that in the cited locations of the cited exhibits the experiments
9 included Cas9 with two NLSs; otherwise denied.

10 **12.** While Broad P1’s general descriptions discuss altering the expression of a gene
11 product of a cleaved target DNA molecule, Broad performed no experimental analysis and
12 provided no evidence to demonstrate possession of that altering expression step. Ex. 1501 ¶ 47.

13 **RESPONSE:** Denied as set forth in Section IV.A.

14 **13.** While Broad P1’s general descriptions discuss introducing into a eukaryotic cell a
15 Cas9 protein linked to only one NLS, Broad performed no experimental analysis and provided no
16 evidence to demonstrate possession of such a construct used for genome editing. *Id.* ¶ 48.

17 **RESPONSE:** Denied as set forth in Section IV.B.

18

1 **Broad's Additional Material Facts:**

2 **14.** By December 2012, it was known that ZFNs and TALENs could cleave DNA for genome
3 engineering in eukaryotes, including altering expression of genes. *See, e.g.*, Ex. 2280; Ex. 2271.

4 **15.** By December 2012, a POSA would have known of DNA targets affecting gene expression,
5 including genes themselves or their regulatory elements. *See, e.g.* Ex. 2001, ¶ [00103]; Ex. 2269.

6 **16.** Zhang B1 disclosed a CRISPR-Cas9 system that was programmable to achieve site-specific
7 DNA cleavage in eukaryotic cells. Ex. 2001, ¶¶ [00149]-[00185].

8 **17.** Zhang B1 includes example experiments demonstrating cleavage and the introduction of
9 indels into an endogenous gene expressed in a eukaryotic cell. *See* Ex. 2001, ¶¶ [00154]-[00156],
10 [00173], [00175]-[00178], [00180], [00182], Fig. 1D, Fig. 2, Figs. 3B-D, Fig. 5, Fig. 6B, Fig. 11D,
11 Fig. 12C; Ex. 1501, ¶ 29.

12 **18.** Zhang B1 includes example experiments demonstrating concurrent double-stranded breaks
13 deleting a 118-bp sequence from a gene. *See* Ex. 2001, ¶ [00184], Figs. 4F-G; Ex. 1501, ¶ 30.

14 **19.** Zhang B1 includes example experiments demonstrating donor template integration. *See* Ex.
15 2001, ¶ [00183], Figs. 4C-E; Ex. 1501, ¶ 32.

16 **20.** Zhang B1 teaches that, in some embodiments, “cleavage results in decreased transcription of
17 a target gene” or can be used to inactivate regulatory sequences controlling gene expression. *See*
18 Ex. 2001, ¶¶ [0011], [0013].

19 **21.** The October 5, 2012 Manuscript includes the same example experiments and disclosures
20 referenced in MFs 16, 17, and 19. Ex. 2564 at 3-7, 12-18, 20-34.

21 **22.** Both Zhang B1 and the October 5 Manuscript state:

- 22 • “[t]hese results define a three-component system for efficient CRISPR-mediated genome
23 modification in mammalian cells” and “establish[] a set of components for achieving

1 CRISPR-mediated gene editing in mammalian cells through the error-prone NHEJ
2 mechanism” Ex. 2001, ¶ [00173], [00183]; Ex. 2564 at 5, 6;

- 3 • “[t]hese results demonstrate the utility of CRISPR for facilitating targeted gene insertion
4 in the mammalian genome.” Ex. 2001, ¶ [00183]; Ex. 2564 at 7; and
- 5 • “the ability to use RNA to program sequence-specific DNA cleavage defines a new class
6 of genome engineering tools for a variety of research and industrial applications.” Ex.
7 2001, ¶ [00185]; Ex. 2564 at 7.

8 **23.** One of the reviewers of the October 5 Manuscript noted (Ex. 2836 at 4) that:

9 “[t]he authors show that Cas9 variants can be targeted to the nucleus and loaded
10 guide RNAs to enable programmed DNA cleavage to disrupt genes or induce
11 homologous recombination.”

12 **24.** Sigma does not dispute that a POSA reading Zhang B1 would conclude that the inventor was
13 in possession of a programmable CRISPR-Cas9 system capable of site-specific, cleavage,
14 including to accomplish both donor template integration and multiplexing.

15 **25.** Dr. Cannon testified that as the result of the use of CRISPR-Cas9, “[a] person of ordinary
16 skill in the art could have certainly predicted or expected that expression might have been changed”
17 and that a POSA “could have predicted or expected the gene expression might have been altered”
18 based on an altered DNA sequence. Ex. 1519 at 41:6-10, 54:8-13.

19 **26.** At her deposition, Dr. Cannon testified (Ex. 1519 at 69:25-70:3):

20 Q. So you read [Broad] claim [18] as that the cleavage of the DNA results in the
21 expression of at least one gene product being altered; is that right?
22 A. Yes.

23 **27.** Paragraph [0038] of Zhang B1 (Ex. 2001) defines “expression” and “gene products.”

24 **28.** Figure 1B of Zhang B1 shows that one NLS achieves nuclear localization. Ex. 2001.

25 **29.** A POSA would have concluded from the “immunofluorescence data from the three
26 engineered Sp-Cas9 proteins” in Figure 1B that “two NLSs ha[d] better nuclear localization than
27 one NLS” but that this data “showed that the system functioned in the eukaryotic cells with both
28 one and two NLS.” Ex. 2467 at 76:7-14, 77:10-17.

CERTIFICATE OF FILING AND SERVICE

I hereby certify that on March 16, 2022, a true and complete copy of the foregoing **BROAD OPPOSITION 1** is being filed and served by 8:00 pm ET via the Interference Web Portal (SO ¶ 105.3; Paper 27 at 11). By agreement, service copies are being sent by email by 11:00 pm ET to counsel for Senior Party as follows:

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