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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; 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)

SIGMA REPLY IN SUPPORT OF SIGMA MOTION 1 (to Deny Broad Benefit of Application 61/736,527 (Broad P1))

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REPLY IN SUPPORT OF SIGMA MOTION 1
(to Deny Broad Benefit of Broad P1)

I. BROAD P1 DOES NOT DESCRIBE AN ANTICIPATED EMBODIMENT WITHIN THE SCOPE OF COUNT 1

A "[c]onstructive reduction to practice means a described and enabled anticipation under 35 U.S.C. § 102(g)(1), in a patent application of the subject matter of a count." 37 CFR § 41.201 (highlighting added). As the Federal Circuit has explained:

> "It is 'not a question of whether one skilled in the art *might* be able to construct the patentee's device from the teachings of the disclosure . . . . Rather, it is a question whether the application necessarily discloses that particular device.'"
>
> * * *
>
> This finding comports with the criterion not only of whether the description conveyed to the artisan the specific subject matter of the count, but also of whether the applicant established that this was the necessary construction of that description.

Hyatt v. Boone, 146 F.3d 1348, 1353-54, 1355 (Fed. Cir. 1998) (emphasis in original).

A. Broad P1 Does Not Demonstrate That Broad Possessed An Anticipated Embodiment In Which "Expression Of The At Least One Gene Product Is Altered"

In its Opposition, Broad does not dispute that its relevant analyses in Broad P1 were limited to demonstrating that the ***sequence*** of the target gene had been altered. *E.g.*, Opp'n 1 at 10-11. Indeed, Broad does not point to any evaluations of gene product ***expression***, either pre-cleavage or post-cleavage. *Id.* Instead, given that lack of evidence, Broad simply argues that demonstration of cleavage and editing "necessarily" (*i.e.*, inherently) demonstrates that some unspecified gene product had been altered. *E.g., id.* at 11 ("site-specific genome engineering . . . which necessarily demonstrates possession"). But altering the ***expression*** of a given gene product is not inherent in cleaving and editing a gene, and in fact would not likely occur "if those changes occurred distant from the promoter or the translation start site of the gene, for example towards the 3′ end of a gene, which is the location targeted by Broad P1's examples." Sigma Mot. 1 at 8. Put simply, in this admittedly fledgling and unpredictable field in December 2012, demonstration

of an anticipatory embodiment of the claimed invention in which "expression of the at least one gene product is altered" would need to show that, necessarily, the expression of the gene product was, in fact, altered. *Hyatt*, 146 F.3d at 1354. Broad's hand-waving and conclusory assumptions about what its disclosures ***might*** have been ***capable*** of doing cannot substitute for evidence that the inventors actually possessed an embodiment that accomplished that claim recital.

1. In The '048 And '115 Interferences, Broad Readily Acknowledged (And Affirmatively Argued) That Altering Gene Product Expression Is A Claim Limitation

Broad has repeatedly argued to the PTAB that Broad's claim limitation "expression of the at least one gene product is altered" is a "required" element of the counts containing Broad's similar claims. *E.g.*, Ex. 1550 (Mot. 2, '048 Int'f) at 1-2; Ex. 1551 (Mot. 3, '048 Int'f) at 3-4, 12; Ex. 1553 (Mot. 4, '115 Int'f) at 12, A3-10 – A3-13; Ex. 1530 (Mot. 5, '115 Int'f) at 30-31, 35-36; Ex. 1562 at 20, 22, 24, Appx. A at 2, Appx B. at 6. Broad's suggestion now, that "expression of the at least one gene product is altered" is not limiting (Opp'n 1 at 15), is directly inconsistent with Broad's previous representations to the PTAB in that regard. *Id.*

2. "Altering Gene Product Expression" Imparts A Further Functional Requirement, And Is Thus Limiting

Broad's new suggestion that "altering gene product expression" is not limiting (Opp'n 1 at 15) is contrary to well-established patent law. *Griffin v. Bertina*, 285 F.3d 1029, 1033-34 (Fed. Cir. 2002) (the Board did not err by giving limiting effect to the "wherein" clauses in an interference count because the wherein clauses "giv[e] meaning and purpose to the manipulative steps" rather than "merely stat[ing] the inherent result of performing the manipulative steps"). Here, because "whereby expression of the at least one gene product is altered" is not inherent in cleavage only and further narrows the claim, it is limiting. *Id.*; *see Air Liquide Large Indus. U.S. LP v. Praxair Tech., Inc.*, 2015 Pat. App. LEXIS 12765, *16 (PTAB Oct. 26, 2015) ("[T]he

whereby clause constrains the 'maintaining the stored hydrogen' limitation to only those pressures that result in the formation of a substantially impermeable barrier.").

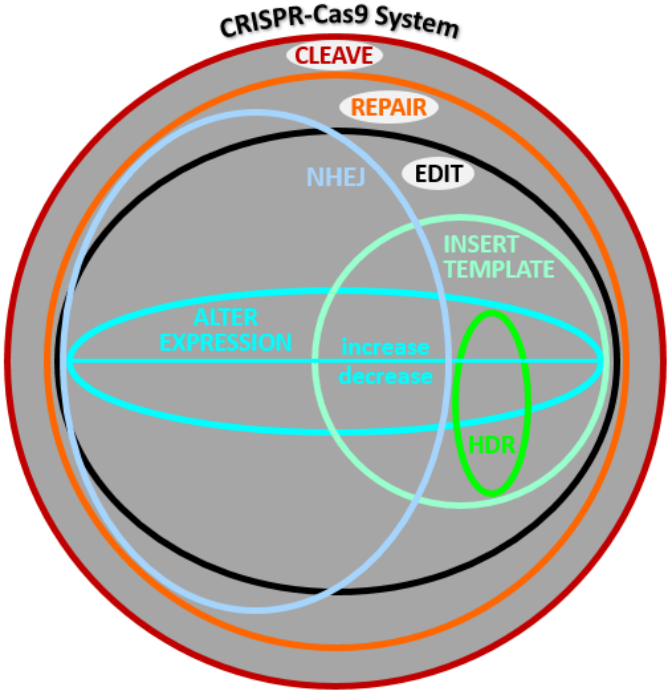
3. Altering Gene Product Expression Is A Further Functional Limitation Beyond Simply Cleaving (Generating A Double-Stranded Break) And Editing (Modifying A Sequence)

Broad P1 repeatedly teaches that altering the expression of a gene product is a subset (one possible outcome) of cleaving and editing a gene. Sigma Mot. 1 at 5-6. Broad's involved claims also demonstrate that cleavage is a broader functional result than altering gene expression, and that modifying a target gene's sequence is different than modifying its expression:

> 4. . . . wherein the Cas9 protein is a nuclease directing cleavage of both strands of the target sequence in the eukaryotic cell.
>
> * * *
>
> 13. . . . wherein the genome engineering comprises [a] modifying a target polynucleotide in a eukaryotic cell, [b] modifying expression of a polynucleotide in a eukaryotic cell, [c] generating a model eukaryotic cell comprising a mutated disease gene, or [d] knocking out a gene.

Ex. 1518, Appx. C at 81-82 (claims of involved App'n No. 14/704,551). As illustrated in concept below, Broad P1 and the involved claims further demonstrate other possible outcomes of cleaving and subsequent repair and editing, including insertion of a template, and even further by HDR:

> 15. . . . wherein the genome engineering comprises cleaving [the] target polynucleotide in a eukaryotic cell and editing said cleaved target polynucleotide by inserting an exogenous template polynucleotide, wherein said edit results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of said target polynucleotide.
>
> 16. The composition of claim 15 wherein the inserting is by homologous recombination.



Id. at 82.

4. In The '115 Interference, Broad Focused Solely On DNA Cleavage, And Did Not Demonstrate Any Alteration Of Gene Product Expression, Which CVC Did Not Challenge

Broad argues that the Board's Decision on Priority in the '115 interference demonstrates that Broad's Cong manuscript demonstrates an actual reduction to practice ("ARTP") in ***this*** interference. Opp'n 1 at 12. But in the '115 interference, Broad only demonstrated that its proofs of invention showed ***cleavage*** (generation of a double-stranded break), and then made a conclusory assertion that altering gene expression had therefore been shown. *E.g.*, Ex. 1557 at 3, 6; *see* Ex. 1552 at 13 ("Both of these methods verified cleavage of *EMX1* target DNA in E17.").

Importantly, CVC nowhere challenged Broad's assertion that demonstration of cleavage alone demonstrated the ***further*** limitation of altering gene product expression. Ex. 1555 at 3-4; Ex. 1556 at 5. And given that CVC's half of the Count only required cleavage alone, the '115 interference was effectively directed to CRISPR-Cas9-mediated cleavage in eukaryotic cells. Thus, in light of the parties' tacit agreement, the Board concluded that Broad had sufficiently carried its burden of demonstrating an ARTP, while explicitly recognizing that CVC had not challenged Broad's arguments in that regard. Ex. 1531 at 63-64.

5. Broad P1 Teaches That "Altering Expression" Of The Gene Product Means Increasing Or Decreasing Its Expression Level, *Not* Simply Modifying Its Sequence

In its opposition, Broad criticizes Sigma for distinguishing between ***modifying the sequence*** of the gene, and ***altering the expression*** of the gene, in which Sigma points to Broad P1's teachings and claims regarding changing the ***levels*** of expression in that regard. Opp'n 1 at 18-19; MF4 ("[A] change in the sequence of a gene by editing at a CRISPR-Cas9 targeted location translates to altering expression under the correct meaning of that term."). In the context of Broad P1's intrinsic teachings, however, altering expression is consistently discussed as increasing or decreasing the level of a gene product's expression:

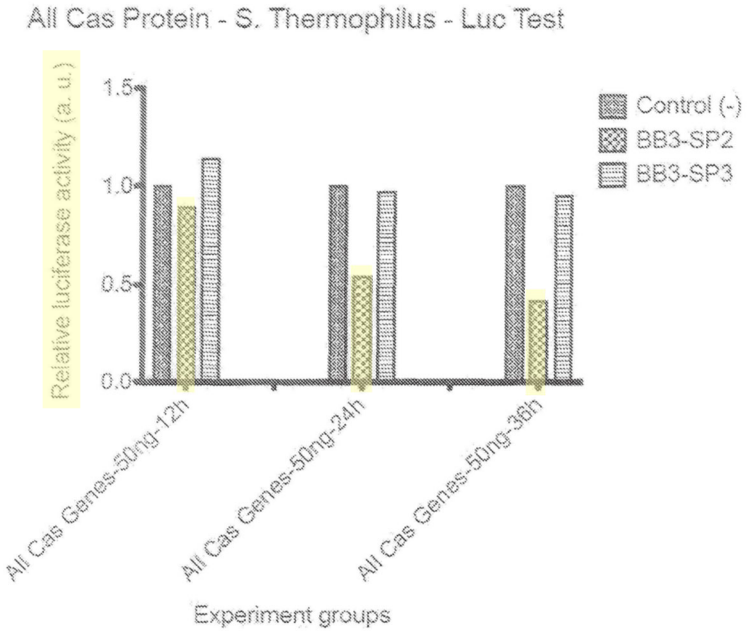
> In one aspect, the invention provides ***a method of modifying expression of a polynucleotide*** in a eukaryotic cell. In some embodiments, the method comprises allowing a CRISPR complex to bind to the polynucleotide such that said binding results in ***increased or decreased expression of said polynucleotide*** . . . ."

Ex. 1503 [Broad P1] ¶¶ [0012] & [0081] (emphases added); *see id.* ¶ [0012] (similar); *id.* ¶ [0100] (similar); *id.* at Claim 84 (similar); *see also* Ex. 1518 at Appx. C (Broad's involved claims) *passim* ("***wherein the expression of one or more gene products is increased***" or ". . . ***decreased***") (emphases added). Indeed, there is not a single disclosure in Broad P1 in which altering the expression of a gene product is equated to simply modifying the gene's sequence.

6. The Prosecution History Of Broad's Involved 8,697,359 Patent Demonstrates That "Altering Expression" Of The Gene Product Means Increasing Or Decreasing Its Expression Level

During prosecution of Broad's involved U.S. Patent No. 8,697,359, Broad submitted arguments and a 131 Declaration to contend that Broad completed the claimed invention, including "expression of the at least one gene product is altered", before CVC. Ex. 1558 at 9-15; *see* Ex. 1561 at 10. In that respect, Broad ***only*** pointed to earlier experiments (which are not contained in Broad P1) that purport to show the altered expression ***levels*** of luciferase:

> In particular, Page Z-27 of Exhibit 7 shows the quantification of data after the experiment was performed. A reduction of luciferase expression was seen . . .
>
> * * *
>
> It is clearly seen from the data of Exhibit 7 (also summarized in the panel set forth above) that there is a reduction in luciferase activity for the experiment conducted with Sp2 as compared to the control and Sp3. I submit that this provides support for methods of, and compositions and CRISPR-Cas systems for altering expression of at least one gene product by introducing into a eukaryotic cell a CRISPR-Cas system that functions in a eukaryotic cell as claimed in the present invention.



Ex. 1558 (Zhang 131 Decl.) at 12-13 ¶¶ 5.1.2.6, 5.1.3, & 5.1.4; Ex. 1559 (Exhibit 7) at Z-27.

These discussions—part of the intrinsic patent record here—confirm that the phrase "altering gene product expression" would be understood by a POSITA to mean altering the gene's level of expression by comparing pre-cleavage and post-cleavage expression levels.

7. The Ordinary Meaning Of "Altering Gene Expression" Means Increasing Or Decreasing A Gene's Expression Level

The art is replete with the use of the phrase "altering gene expression" to mean altering the ***level*** of expression. *E.g.,* Ex. 1563 at col. 9:45-47; Ex. 1564 at col. 14:7-15; Ex. 1565 ¶ [0139]; Ex. 1566 ¶ [0041]; Ex. 1567 ¶ [0038]; Ex. 1568 ¶ [0096]; Ex. 1569 ¶ [0024]; Ex. 1570 ¶ [0062]; Ex. 1571 ¶¶ [0043, 0055]; Ex. 1572 ¶ [0158]; Ex. 1573 ¶ [0028]; Ex. 1574 at 5; Ex. 1575 at 9:24-30; Ex. 1576 ¶ [0046]; Ex. 1577 ¶ [0064]; *see* Ex. 1579.2 (Ausubel (2003) (cited twice in Broad P1)) at 15.8.17 ("[T]he changes in gene expression or the levels of a specified transcript may be measured and described as an arbitrary unit relative to some control sample . . . ."); Ex. 1586 (Klipp (2000)) at 165 ("[A]ltering gene expression . . . [is measured by] changes in gene expression levels. . . ."); Ex. 1581 (Goeddel (1990) (cited three times in Broad P1)) at 508 ("[I]t is necessary to include a positive control in order to compare expression of the desired gene with a gene that is known to be expressed well."); Ex. 1591.2 (Sambrook (2001) (cited five times in Broad P1)) at 17.1, 17.30 ("Analysis of Gene Expression in Cultured Mammalian Cells": . . . "[S]elected mutations are introduced into the sequence under study, and its consequent activity . . . is measured compared to that of the wild-type or normal sequence."); *see also* Ex. 1587 (Lovén (2012)) at 476 ("Global gene expression analysis uses . . . methods to measure the levels of RNA species in biological systems."); Ex. 1578 (Applied Biosystems (2010)) at 5 ("When studying gene expression with [RT-PCR], scientists usually investigate changes – increases or decreases – in the expression of a particular gene or set of genes by measuring the abundance of the gene-specific transcript. . . . quantitating changes in expression levels . . . ."). Indeed, some of these

references also include a definition of "expression" similar to that set forth in Broad P1. *E.g.*, Ex. 1564 at col. 14:18-27; Ex. 1565 ¶ [0146]; Ex. 1566 ¶ [0031]; Ex. 1568 ¶ [0110]; Ex. 1576 ¶ [0059]. While the patent law permits an inventor to be his or her own lexicographer, there is nothing in Broad P1 that demonstrates any attempt by the Broad inventors to change the ordinary meaning of "altering gene expression". Indeed, Broad P1's usage of that phrase is entirely consistent with its ordinary meaning, and nowhere consistent with Broad's arguments here.

8. Broad Claimed The Genome Engineering Result Of Altering The Cell's Gene Product Expression, Not Modifying The DNA Molecule's Encoding Process (*i.e.*, Transcription, Translation, Or Splicing)

Broad argues that "the broadest reasonable interpretation of 'whereby the ***expression*** of the at least one gene product ***is altered***' as used in Count 1 includes any alteration in the multi-step ***process*** by which a protein is expressed from a gene in a eukaryotic cell—including altering any step thereof." Opp'n 1 at 16 (citing Ex. 2001 ¶ [0038]) (emphases in original). But Broad's sole reliance on Broad P1's textbook discussion of the basic cellular processes involved in gene expression (*e.g.*, transcription, translation, and splicing) is unenlightening here because the claim limitation is directed to ***the cell's*** "express[ion]" of the gene product, not ***the DNA molecule's*** "encod[ing]" process (*i.e.*, transcription, translation, or splicing). But Broad P1 ¶ [0038]'s general discussion of "expression" is wholly consistent, and nowhere contradicts, Broad P1's directly pertinent teachings that the result of altering the cell's gene product expression is a measurable quantity – *i.e.*, a change in the level of expression of the gene product. *See supra* Parts I.A.5 – I.A.7. And Broad's system claim is directed to that measurable result—the "expression of the at least one gene product is altered", not the DNA molecule's underlying cellular processes of transcription, translation, or splicing. Moreover, ¶ [0038] does not include any reference to ***comparing*** the expression of a gene product, particularly pre- and post-cleavage, nor does it address what is meant by the language "expression of the at least one gene product is altered".

9. Broad's Claim Expressly Requires That The Gene Product "*Is*" Altered, Not Simply Is "*Capable*" Of Being Altered

Broad argues that Broad P1 "demonstrate[s] possession of a eukaryotic CRISPR-Cas9 system ***capable*** of altering expression of genes . . . ." Opp'n 1 at 4 (emphasis added); *see id. passim* ("capable", "could", "can", and "ability"). Broad's arguments attempt to entirely re-write the express claim recital to simply require cleavage that is ***capable*** of altering expression. *Id*. But the express claim language cannot be ignored: "whereby the expression of the at least one gene product ***is*** altered". Further, the claim recites that pre-cleavage, the cell expresses ***a*** ("at least one") gene product, and post-cleavage, the ***expression*** of ***that*** ("***the*** at least one") gene product is altered, *i.e.*, a comparison of the expression of a gene product pre-cleavage and post-cleavage.

10. Modifying The Target DNA Is Not The Same As Altering The Expression Of The Gene Product

Broad argues that the claim limitation "expression of the at least one gene product is altered" is broad enough to encompass simply modifying the sequence of the target DNA itself. Opp'n 1 at 16-19. But the claim differentiates between "a target ***sequence*** of a DNA molecule," and a "***gene product*** expressed from the eukaryotic cell". And Broad ignores that, pre-cleavage, "the eukaryotic cell expresses at least one gene product", and post-cleavage, the "expression of the at least one gene product is altered". Accordingly, the claim requires that the expression of ***the*** gene product be altered as a consequence of the CRISPR-Cas9 mediated cleavage, which is not the same as simply modifying the target gene, as shown by comparing these teachings:

> In one aspect, the invention provides for ***methods of modifying a target polynucleotide*** in a eukaryotic cell. In some embodiments, the method comprises allowing a CRISPR complex to bind to the target polynucleotide to ***effect cleavage of said target polynucleotide thereby modifying the target polynucleotide*** . . . ."
>
> In one aspect, the invention provides ***a method of modifying expression of a polynucleotide*** in a eukaryotic cell. In some embodiments, the method comprises allowing a CRISPR complex to bind to the polynucleotide such that said binding results in ***increased or decreased expression of said polynucleotide*** . . . ."

Ex. 1503 [Broad P1] ¶¶ [0080]-[0081] (emphases added); *see id.* ¶¶ [0092] & [00100] ("one embodiment . . . comprises ***modifying a target polynucleotide***" and "other embodiments . . . provide[] . . . . ***modifying expression of a polynucleotide***") (emphases added). Broad's involved claims further clarify this distinction between modifying a gene and altering the expression of the gene product. *E.g.*, Ex. 1518, Appx. C at 49-51 (claims 13, 16, 21, & 22), 82 (claims 13 & 15).

11. Cleaving And Editing Alone, Particularly At The Target DNA's 3′ End, Would Be Unlikely To Alter Gene Product Expression

A POSITA would have understood that altering the ***expression*** of a given gene product is not inherent in simply cleaving and editing a gene, and in fact would not likely occur "if those changes occurred distant from the promoter or the translation start site of the gene, for example towards the 3′ end of a gene, which is the location targeted by Broad P1's examples." Ex. 1501 ¶ 25. Indeed, given that the Broad P1 applicants targeted cleavage at the 3′ end, and near or even after the stop codon of the encoded protein product, a POSITA would have understood that their objective was to ***not*** alter gene expression. *See* Ex. 1549 (Cannon Tr.) at 65:21 – 66:10 ("You could insert a sequence, for example, at the [3′] end of the gene or the C-terminus of the protein. So it would not alter the expression levels of that protein."); Ex. 1593 (Shalem (2015) (F. Zhang)) ("Early exons are preferred for targeting, as indels in these exons have a higher probability of introducing an early stop codon or a frameshift of a larger portion of the protein."); *see also* Ex. 1589 (Ranjan (2010)) at 165 ("For many purposes, C-terminal tagging [added amino acids] is advantageous because the possibility for the tag to affect normal folding of polypeptide chain during translation is minimized and only full length protein could be detected."); Ex. 1584 (Gurvich (2003)) at 5948 ("Frameshift errors near the end of genes should generally be less harmful, since they affect only protein C-terminal heterogeneity."). Broad's expert, Dr. Seeger, agrees. Ex. 2469 (Seeger Tr.) at 148:18-22 ("[R]egulatory regions [of the EMX1, PVALB, and

mTH genes] are upstream – 5[′] upstream of a gene, and so one would be very surprising if the regulatory regions were in the areas indicated in Figure 2 [of Broad P1].”). Indeed, even “some frameshifting indels could be functionally neutral.” Ex. 1585 (Hu (2012)) at 1; *id.* at 3-4 (for a frameshifting indel where no bases or amino acids are deleted or changed except the loss of the stop codon (like the example in Broad P1 Fig. 4E), and the frameshift occurs at “the end of the cDNA sequence,” “then very likely the indel will have no significant effect on gene function.”).

12. Cleavage And Editing Of Only One Allele Would Be Unlikely To Alter Gene Product Expression Of “The Eukaryotic Cell”

Broad argues that if “there had been a modification of the sequence of the EMX1 gene . . . [then] expression of a [EMX1 protein] gene product would be altered.” Opp’n 1 at 13. But a POSITA would have known that most genes are expressed from two homologous chromosomes (*i.e.*, two alleles), and that not all genes, or alleles of genes, are expressed in all eukaryotic cells. *See* Ex. 1582 (Gordon (2001)) at 2 (“[E]ach locus has two genes or alleles (which may be the same, or may be different) at that particular location.”); Ex. 2469 (Seeger Tr.) at 155:17 – 156:13 (“[I]n most cases, both alleles are expressed.”). Broad Claim 18 recites that “the eukaryotic cell expresses at least one gene product”, and is thus directed to the bi-allelic expression product of the cell, not the mono-allelic expression of a single gene. Ex. 1560 at 4-5; Ex. 1561; *see* Ex. 1503 (Broad P1) ¶ [0105] (“[A] method of the invention may be used to create [a] . . . cell in which the expression of one or more nucleic acid sequences associated with a disease are altered.”). Therefore, even if the sequence of one gene copy were modified, and even if that modification altered the expression of that gene (which Broad P1 does not show), the unmodified copy can compensate for cellular gene expression, and thus the expression level of the gene product may remain unaltered. *See, e.g.*, Ex. 1583 (Gross (2006)) at 2173 (“If one allele is damaged, the activity of the other can often compensate.”); Ex. 1582 (Gordon (2001)) at 2 (“[I]f the mutation

leads to a loss of function, the gene product from the other allele at this locus would provide the missing function . . . ."). A POSITA would have also known that eukaryotic cells have various mechanisms to ensure homeostasis of gene expression, even though those mechanisms were not well understood in late 2012. *See, e.g.,* Ex. 1594 (Villa (2012)) at 647 ("The term 'dosage compensation' subsumes vital regulatory processes through which all individuals of a species control equality of gene expression product levels despite varying gene copy numbers."); Ex. 1588 (Prestel (2010) ("[T]he measured expression levels do not reflect the actual copy number, as compensatory mechanisms aimed at re-establishing homeostasis take effect."); Ex. 1590 (Robinson (2003)) at 4 ("Usually a cell can compensate for this loss [of allele function] through excess production from the good allele or other means . . . ."). Therefore, even in a situation with, for example, one wildtype and one mutant (or edited) allele in a cell, there can often be no effect on the overall level of gene product expression in that cell. *See* Ex. 1549 (Cannon Tr.) at 18:10-18, 20:7-23, 92:24 – 93:14. Thus, a POSITA would have understood that it is not possible to predict gene expression of a eukaryotic cell simply by looking at the DNA sequence. *Id.*

The lack of predictability is even worse when the sequence information is of the type presented in Broad P1, *i.e.*, it comes from PCR amplicons derived from a ***population*** of cells treated with CRISPR-Cas9. *E.g.*, Ex. 1503 (Broad P1) ¶ [00175] ("Figure 1F illustrates mutated alleles identified from sequencing analysis of 43 clonal amplicons showing a variety of micro insertions and deletions."). A POSITA would have understood that these methods provide no information about the actual number of edited cells, or specifically whether any edited cells contained modifications at one or two alleles. *See* Ex. 2469 (Seeger Tr.) at 112:10-11 ("[Y]ou can have different editing events in one single cell."). Indeed, the very low frequency of gene editing described in Broad P1 means that even those cells that contained an edited allele were highly likely to be heterozygous (*i.e.*, to also contain a wild-type allele). *See* Ex. 1593 (Shalem

(2015)) (F. Zhang) ("As DSB induction and NHEJ-mediated repair occur independently at each allele in diploid cells, targeting by Cas9 results in a range of biallelic and heterozygous target gene lesions in different cells."). Broad P1 describes no examples of cells where both copies of the gene were known to be edited. As a result, a POSITA would have concluded that even if an edited DNA sequence were present in the population of PCR amplicons that suggested an effect *might* have happened to alter gene expression, it would not be possible to conclude that the cell's bi-allelic expression of the gene product had been altered without actually measuring gene expression. *See* Ex. 1510 (Holt (2010)) at 8 ("ZFN treatment results in disruption of the CCR5 open-reading frame and thus has the ***potential*** to completely eliminate this important HIV-1 co-receptor from the surface of cells that are bi-allelically modified.") (emphasis added); *see also* Ex. 1549 (Cannon Tr.) at 20:24 – 21:3 ("[W]e went to quite a lot of trouble to demonstrate an effect on CCR5 gene expression. We didn't just rely on the indel sequence information.").

13. Broad P1's Boilerplate Discloses How To Evaluate Altered Gene Expression, But Broad P1 Nowhere Conducted Any Of Those Evaluations

In its boilerplate discussions, Broad P1 discloses the multiple ways that an investigator could determine whether the expression of a gene product had been altered:

> An altered expression of one or more genome sequences . . . can be determined by ***assaying for a difference in the mRNA levels of the corresponding genes between the test model cell and a control cell*** . . . . Alternatively, the differential expression of the sequences . . . is determined by ***detecting a difference in the level of the encoded polypeptide or gene product***.

Ex. 1503 (Broad P1) ¶ [00110] (emphases added). Broad P1 elaborates on these tests and assays:

Evaluating Altered Gene Expression by Examining Changes in Expression Levels	Test/Assay For Evaluating Altered Expression of Gene Product
"To assay for an agent-induced alteration in the ***level*** of mRNA transcripts or corresponding polynucleotides . . ." *Id.* ¶ [0111] (emphasis added).	"amplification procedures or conventional hybridization assays (e.g. Northern blot analysis)" *Id.* ¶ [0111].
". . . to quantify the expression ***level*** of a sequence associated with a signaling	"A preferred amplification method is PCR. . . . a reverse transcription assay that is coupled with a

biochemical pathway." *Id.* ¶ [0112] (emphasis added).	quantitative polymerase chain reaction (RT-PCR)" *Id.* ¶ [0112].
"Detection of the gene expression ***level*** can be conducted in real time in an amplification assay." *Id.* ¶ [0113] (emphasis added).	"quantifying the fluorescence of the intercalated dye using conventional optical systems in the art" *Id.* ¶ [0113]. "other fluorescent labels . . . to facilitate the detection and quantification of the amplified products. . . . utiliz[ing] fluorescent, target-specific probes (e.g., TaqMan® probes) . . . ." *Id.* ¶ [0114]. "conventional hybridization assays using hybridization probes" *Id.* ¶ [0115]. "The hybridization assay can be formed using probes immobilized on any solid support . . . ." *Id.* ¶ [0116]. "the nucleotide probes are conjugated to a detectable label" *Id.* ¶ [0117]. "For example, radiolabels . . . [f]luorescent markers . . . [e]nzymatic labels . . . and finally colorimetric labels . . . ." *Id.* ¶ [0118].
"An agent-induced change in expression of sequences associated with a signaling biochemical pathway can also be determined by examining the corresponding gene product." *Id.* ¶ [0119]	"Determining the protein ***level*** typically involves a) contacting the protein . . . with an agent that specifically bind to a protein . . . and (b) identifying any agent:protein complex so formed." *Id.* ¶ [0119] (emphasis added). "The formation of the complex can be detected directly or indirectly according to standard procedures in the art." *Id.* ¶ [0120]. "A wide variety of labels suitable for detecting protein levels are known in the art." *Id.* ¶ [0121]. "The amount of agent:polypeptide complexes formed during the binding reaction can be quantified by standard quantitative assays." *Id.* ¶ [0122]. "A number of techniques for protein analysis . . . are available in the art. . . . radioimmunoassays, ELISA . . . , 'sandwich' immunoassays, immunoradiometric assays, in situ immunoassays . . . , western blot analysis, immunoprecipitation assays, immunofluorescent assays, and SDS-PAGE." *Id.* ¶ [0123].

These types of assays for measuring mRNA or protein levels were well known in the art:

> In addition to Northern blot tests and SAGE analyses, there are several other techniques for analyzing gene expression. Most of these techniques, including microarray analysis and reverse transcription polymerase chain reaction (RT-PCR), work by measuring mRNA levels. However, researchers can also analyze gene expression by directly measuring protein levels with a technique known as a Western blot.

Ex. 1592 (Scitable (2011)) at 4; *see* Ex. 1580 (Ferec (2012)) at 3 ("Mutations that permit protein to be expressed invariably require experimental studies to assess their effect on the protein.").

But in the context of its gene editing experiments, Broad P1 nowhere conducts ***any*** of those well-known and conventional analyses to evaluate gene product expression. *See* Ex. 1503 (Broad P1) ¶¶ [0173]-[0185]. Indeed, in Broad P1's examples, Broad never even ***mentions***

altering expression of a gene product. *See id.* ¶¶ [0149]-[0203]. Broad does not dispute that this basic scientific evaluation is absent from Broad P1's analyses, and instead simply argues that "no such assays were necessary". *E.g.*, Opp'n 1 at MF8. But Broad P1 teaches that such assays were integral to evaluating altered gene expression, and Broad P1 expressly differentiates an "assay for altered gene expression activity affected by CRISPR complex formation" from an "assay for DNA cleavage or mutation at the target sequence". Ex. 1503 (Broad P1) ¶ [0063].

B. Broad P1 Does Not Demonstrate That Broad Possessed An Anticipated Embodiment In Which A Template Was Inserted Using A Cas9 With "Only One NLS"

1. Broad P1's "Laundry Lists" Do Not Demonstrate Actual Possession Of A "Unified Whole" Embodiment

In its Opposition 1, Broad argues that Broad P1 discloses a Cas9 construct linked to "only one NLS", relying on—and twice citing to—"Ex. 2001, ¶¶ [0004]-[0007], [0062]." Opp'n 1 at 20-22. But, repurposing Broad's own earlier criticism of CVC regarding a "eukaryotic cell", those textbook discussions of "fictitious embodiments" in Broad P1 do not demonstrate that the Broad inventors actually possessed an anticipatory embodiment within the scope of the count:

> [N]one of the fictitious embodiments are actually disclosed in [Broad] P1 . . . and certainly none are there as a unified whole. They are, instead, post-hoc creations of [Broad]'s expert, manufactured by stitching together disparate disclosures in [Broad] P1 . . . , using the Count as a roadmap.

Ex. 1554 (Broad Opp'n 1, '115 Int'f); *see* Ex. 1501 (Cannon Decl.) ¶¶ 47-48. Indeed, those disclosures are the same kinds of "laundry list disclosures" that Broad has previously (and successfully) criticized in CVC P1 and P2 relating to the "eukaryotic cell" limitation. Ex. 1557, Appx. 2 (Broad's Demonstratives, '115 Int'f) at 29 (identifying CVC's "generic lists").

2. Broad's Attempt To "Mix And Match" Different Embodiments Do Not Demonstrate Any Single Anticipated Embodiment

In its Opposition 1, Broad attempts to mix-and-match different embodiments in Broad P1, arguing that "Zhang B1 demonstrates [1] ***nuclear localization*** of Cas9 with a ***single NLS***, as well

as [2] a ***working system*** with the more efficient ***Cas9-2NLS*** construct." Opp'n 1 at 22 (emphases added). But, as Broad implicitly concedes, neither of these embodiments ***alone*** anticipates Sigma Claim 31. Indeed, again repurposing Broad's own earlier criticism of CVC, combining these disparate embodiments is the same kind of "mixing and matching" that Broad has (successfully) criticized in CVC P1 and P2 relating to the "eukaryotic cell" limitation:

> [Broad] creates i[t]s fictitious embodiments by "mixing and matching" various parts of [Broad P1]. Such stitched-together embodiments are not "as arranged" in the Count in [Broad P1] – it was [Broad's] expert who arranged them as such.

Ex. 1554 (Broad Opp'n 1, '115 Int'f) at 32; *see* Ex. 1501 (Cannon Decl.) ¶¶ 37-46.

Further, Broad's assertion that "Dr. Cannon, did not interpret Figure 1B" (Opp'n 1 at 22) is flatly contradicted by Dr. Cannon's detailed discussion of that figure in her declaration. Ex. 1501 (Cannon Decl.) ¶ 39 (agreeing with the applicants' own contemporaneous conclusions from reviewing their own Figure 1B that a single NLS "was ***unable*** to achieve adequate nuclear localization" and "***only*** 2xNLS-SpCas9 exhibited nuclear localization") (emphases added).

3. Broad Has Acknowledged That A Single NLS Does Not Anticipate Two NLSs

In Broad Motion 3, Broad distinguished two NLSs from only one NLS, arguing:

> With respect to anticipation, the Sigma half of Count 1 specifically recites that the Cas9 has "only one NLS," not two or more. . . . Thus, Count 1 does not anticipate these claims that require two or more NLSs.

Broad Mot. 3 at 13. In elaborating further on those differences, Broad relied on "a declaration from Dr. Fyodor Urnov arguing that 'protein functional domains are not mix-and-match interchangeable. . . .'" *Id.* at 15-16. These arguments reveal Broad's about-face positions on this Motion, in which Broad now endeavors to blur the distinctions between those two constructs.

II. CONCLUSION

For the foregoing reasons and the reasons set forth in Sigma Motion 1, Sigma respectfully requests that the Board grant Sigma Motion 1.

Respectfully submitted,

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APPENDIX 1

LIST OF EXHIBITS CITED

Exhibit No.	Description
1501	Cannon Decl.
1503	Broad P1
1510	Holt (2010)
1518	Cannon Supp'l Decl.
1530	106,115 [2118] – Broad Mot. 5
1531	106,115 [2863] – Decision on Priority
1549	Cannon Depo. Tr. 2022-04-13
1550	106,048 [77] – Broad Mot. 2
1551	106,048 [66] – Broad Mot. 3
1552	106,048 [570] – Broad Cont. Mot. 6
1553	106,115 [269] – Broad Mot. 4
1554	106,115 [596] – Broad Opp'n 1
1555	106,115 [821] – Broad Reply 4
1556	106,115 [2745] – Broad Reply 5
1557	106,115 [2862] – Hearing Transcript
1558	14054414 (Zhang 131 Declaration)
1559	14054414 (Exhibit 7)
1560	14054414 (Claims 15 & 18)
1561	14054414 (Notice of Allowance)
1562	14054414 (Emergency Petition)
1563	US8065014
1564	US9725723
1565	US20030166227A1
1566	US20050026290A1
1567	US20090077687A1
1568	US20090293148A1
1569	US20100048723A1
1570	US20100196929A1
1571	US20100255486A1
1572	US20110281756A1
1573	US20110321190A1
1574	WO1999061631A1
1575	WO2013001055A1
1576	EP1681347A1
1577	EP2112235A1
1578	Applied Biosystems (2010)
1579	Ausubel (2003)
1580	Ferec (2012)
1581	Goeddel (1990)
1582	Gordon (2001)
1583	Gross (2006)
1584	Gurvich (2003)

Exhibit No.	Description
1585	Hu (2012)
1586	Klipp (2000)
1587	Lovén (2012)
1588	Prestel (2010)
1589	Ranjan (2010)
1590	Robinson (2003)
1591	Sambrook (1989)
1592	Scitable (2011)
1593	Shalem (2015)
1594	Villa (2012)
2001	A Complete Copy of the 61/736,537 Provisional Patent Application
2469	Seeger Depo. Tr. 2022-04-21

APPENDIX 2

APPENDIX 2: STATEMENT OF MATERIAL FACTS

Sigma's Material Facts:

To a POSITA in mid-December 2012:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

RESPONSE: Denied as set forth in Section IV.B. 18

Broad's Material Facts:

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

RESPONSE: Admitted.

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

RESPONSE: Admitted.

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

RESPONSE: Admitted.

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

RESPONSE: Denied.

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

RESPONSE: Denied.

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

RESPONSE: Denied.

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

RESPONSE: Denied.

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

RESPONSE: Denied.

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

- "[t]hese results define a three-component system for efficient CRISPR-mediated genome modification in mammalian cells" and "establish[] a set of components for achieving CRISPR-mediated gene editing in mammalian cells through the error-prone NHEJ mechanism" Ex. 2001, ¶ [00173], [00183]; Ex. 2564 at 5, 6;

- "[t]hese results demonstrate the utility of CRISPR for facilitating targeted gene insertion in the mammalian genome." Ex. 2001, ¶ [00183]; Ex. 2564 at 7; and

- "the ability to use RNA to program sequence-specific DNA cleavage defines a new class of genome engineering tools for a variety of research and industrial applications." Ex. 2001, ¶ [00185]; Ex. 2564 at 7.

RESPONSE: Denied.

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

> "[t]he authors show that Cas9 variants can be targeted to the nucleus and loaded Guide RNAs to enable programmed DNA cleavage to disrupt genes or induce homologous recombination."

RESPONSE: Denied (*see* forthcoming Sigma Motion to Exclude).

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

RESPONSE: Denied.

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

RESPONSE: Denied.

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

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

RESPONSE: Admitted.

27. Paragraph [0038] of Zhang B1 (Ex. 2001) defines "expression" and "gene products."

RESPONSE: Denied.

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

RESPONSE: Denied.

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

RESPONSE: Denied.

CERTIFICATE OF FILING AND SERVICE

I hereby certify that:

I. The following paper is being filed April 26, 2022, with the Patent Trial and Appeal Board via:

✓ Interference Web Portal at https://acts.uspto.gov/filing/. Under SO ¶ 105.3, a paper filed through the Interference Web Portal is considered served. The web portal e-filing system is to send email notification of the filing to counsel for Junior Party THE BROAD INSTITUTE, INC., MASSACHUSETTS INSTITUTE OF TECHNOLOGY, and PRESIDENT AND FELLOWS OF HARVARD COLLEGE.

SIGMA REPLY IN SUPPORT OF SIGMA MOTION 1
(to Deny Broad Benefit of Application 61/736,527 (Broad P1))

II. A courtesy copy of the above paper is being sent to counsel for Junior Party THE BROAD INSTITUTE, INC., MASSACHUSETTS INSTITUTE OF TECHNOLOGY, and PRESIDENT AND FELLOWS OF HARVARD COLLEGE at the address(es) below on April 26, 2022, via e-mail:

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