Filed on behalf of Junior Party Paper No. ___

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

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

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

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

v.

SIGMA-ALDRICH CO., LLC.
Senior Party

(Application 15/456,204).

Patent Interference No. 106,132 (DK)

CVC REPLY IN SUPPORT OF RESPONSIVE MOTION 1
(for accorded benefit to Proposed Count 2)

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Page(s)

Cases

Other Authorities

I. INTRODUCTION

CVC's P1, all of which is carried over into P2 and P3, describes and enables the method of Proposed Count 2. The level of ordinary skill in the art was high, as numerous independent groups successfully and near-simultaneously demonstrated the claimed invention. A POSA reading P1 would have immediately understood P1 explains how to apply the system in a fish cell (E4), human cell (E5), and fruit-fly cell (E6) using the well-known molecular biology techniques P1 described. A POSA would have known that after DNA is cut by any method—ZFNs, TALENs, meganucleases or even radiation—the cell's natural repair processes will incorporate a donor polynucleotide via homology-directed repair (HDR). To qualify as a constructive reduction to practice of Proposed Count 2, CVC's P1 did not need to include a working example or dispel hypothetical concerns posited by Sigma.

P1 describes how to make pre-assembled ribonucleoprotein complexes (RNPs) that function *in vitro*. P1 also describes "microinjection" as a "well known technique[]" for delivering nucleic acids and proteins into eukaryotic cells. A POSA, who would approach P1 with knowledge of these routine techniques, would immediately recognize microinjecting the pre-assembled RNPs into a nucleus or into dividing cells. And, when using vector expression, a POSA would recognize strong promoters, a nuclear localization signal (NLS), and codon optimization, all of which are described in P1 and were established, routine molecular biology techniques well known to a POSA.

Nothing supports Sigma's argument that a POSA would have needed more for P1 to convey possession. To the contrary, the factual record here shows the speed and ease with which the system was implemented by multiple independent groups, using the same platforms used for ZFNs and TALENs. A POSA would have considered ZFNs and TALENs—not Group II introns—to be the analogous gene editing systems for comparison to CRISPR-Cas9. Placing the

prior art in the proper context, it is clear that a POSA would approach P1 not as the overly skeptical person plagued with doubt as Sigma portrays, but as someone who would look to ZFNs and TALENs as a model for implementing CRISPR-Cas9 in eukaryotes.

An objective inquiry into the four corners of P1 reveals only confidence—and no uncertainty—about use of the system in a wide variety of eukaryotic cells. "[A]cknowledgment of the complexities of the science does not negate the disclosure," where the disclosure itself does not "fail[] to teach any essential step," but rather describes how to obtain the subject matter by "following the general procedure disclosed." *Frazer v. Schlegel*, 498 F.3d 1283, 1288-89 (Fed. Cir. 2007). There is uncontroverted contemporaneous factual evidence showing that persons skilled in the art expected sgRNA CRISPR-Cas9 to have activity in eukaryotes. Indeed, the *only* question posed immediately after disclosure of CVC's sgRNA CRISPR-Cas9 system was not whether it would *function* in eukaryotes, but whether it would *outperform* the incumbent genome-editing technologies, ZFNs and TALENs, identified in P1's opening paragraphs. There is no contemporaneous contrary factual evidence.

The Board's Decision on Motions in the '115 Interference was wrong and did not address the new CVC declaration evidence, new testimony from Sigma's expert agreeing with CVC, or key facts such as P1's description of the RNP-microinjection embodiment or P1's direct analogy of CRISPR-Cas9 to ZFNs and TALENs. There is new evidence from six different declarants, including subpoenaed testimony from a collaborator of Zhang, showing persons skilled in the art understood the described invention was straightforward to implement. Nor did the Decision address the fact that others in the field, including scientists from Broad, ToolGen, and Sigma, all implemented CRISPR-Cas9 by incorporating it into existing ZFNs or TALENs gene editing platforms. The Board may not simply recycle its '115 Decision on Motions here. The Board must review the different record in this interference and consider whether CVC's P1 is a

constructive reduction to practice of Count 2.

Similarly, the '115 Priority Decision has no relevance here because it decided a different legal question, relied on evidence that was not available to a POSA, and explicitly avoided considering the viewpoint of a POSA.

II. ARGUMENT

A. P1 provides a constructive reduction to practice of at least one embodiment of Proposed Count 2.

Sigma's Opposition does not acknowledge the basic legal standards for according benefit to an earlier-filed application or that an application does not need to include a working example. *Ariad Pharms., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1352 (Fed. Cir. 2010) (en banc) ("written description requirement does not demand either examples or an actual reduction to practice."); *Falkner v. Inglis,* 448 F.3d 1357, 1366 (Fed. Cir. 2006) ("Examples are not required [for written description].") Nor does the application have to *prove* that the embodiment works to satisfy §112. *Alcon Rsch. Ltd. v. Barr Labs., Inc.*, 745 F.3d 1180, 1189 (Fed. Cir. 2014). "[W]ritten description is about whether the skilled reader of the patent disclosure can recognize that what was claimed corresponds to what was described; *it is not about whether the patentee has proven* to the skilled reader that the invention works…." *Id.*, 1191; *see also, id.*, 1189 ("a patent does not need to guarantee that the invention works for a claim to be enabled.").

Contrary to these binding legal precedents, Sigma invokes a written description standard requiring that "CVC's inventors *actually possessed* that invention." Paper 718, 8:8-9 (emphasis added). Sigma's expert, Cannon, likewise uses the same legally erroneous standard for written description requiring *actual possession*. *See e.g.,* Ex. 1080, ¶63 ("none of those disclosures demonstrate that CVC's inventors *actually possessed* that invention.") (emphasis added); Ex. 2655, 24:17-25:3 ("[p]ossession means the inventors had *performed the experiments* or the

protocol … or the technique that they are describing in their patent.") (emphasis added); *see also* Ex. 2616, 86:1−11, 96:9−97:3. Under the proper application of the law, P1 provides a constructive reduction to practice of at least one embodiment of Count 1. Paper 588, 5:17-21:19.

B. P1 describes all the elements of Proposed Count 2 and methods for its implementation.

CVC's Responsive Motion 1 shows that P1 (or alternatively, P2 or P3) describes and enables at least one embodiment of Proposed Count 2 (PC2), including a sgRNA CRISPR-Cas9 system "capable of cleaving or editing a target DNA molecule, or modulating transcription of a target DNA molecule," and further incorporating a donor polynucleotide using HDR. Paper 588, 5:17-21:19; MF 4-21, 26-28. P1 expressly describes a sgRNA CRISPR-Cas9 system that, upon introduction into a fish (E4), human (E5), or fruit-fly (E6) cell along with a donor polynucleotide, is capable of modifying target DNA. *Id.* These three cell types are among the most standard in biological research. Ex. 2612, ¶ 19. P1 describes three specific ways to prepare and deliver the system: (i) as pre-assembled RNP complexes (Paper 588, 7:10-16:14; MF 12-13); (ii) as expression vectors encoding the system (*id.*, 16:15-20:3; MF 14-15); or (iii) as RNA molecules (*id.*, 20:4-18; MF 14-15). Both parties' experts agree that these delivery techniques (microinjection and transfection) were well-established before 2012. Ex. 1080, ¶¶ 49-50; Ex. 2543, ¶¶ 65-89; Ex. 2612, ¶¶ 66-89; MF 12-15. P1 also discloses PAM sequenecs for Cas9, a fact Sigma failed to substantively address in its Opposition. Ex. 2009, Fig. 3C; Ex. 2612, ¶¶55-65, 267-277; MF 17, 25; Ex. 2548, ¶¶13-19. A POSA reading P1 would immediately recognize and understand how to implement E4, E5, and E6. Ex. 2612, ¶¶ 91-96, 102, 145-146.

As the Federal Circuit correctly stated in *Alcon*, "written description is about whether the skilled reader of the patent disclosure can recognize that *what was claimed* corresponds to *what was described*." *Alcon*, 1191 (emphasis added). Here, P1 describes and enables "what was

claimed" – in this case, an embodiment of PC2. *Id.;* 37 C.F.R. § 41.201.

C. Sigma admits that P1 discloses using CRISPR-Cas9 with a donor polynucleotide and homology directed repair.

Sigma admits that P1's Figure 4 depicts CRISPR-Cas9 target DNA cleavage in combination with donor integration and homology-directed repair in a eukaryotic cell. Paper 718, 8:6-8; Ex. 2009, Fig. 4. Sigma only criticisms are that Figure 4 shows how CRISPR-Cas9 "might" invoke a DNA-damage repair process and "might" permit donor polynucleotide integration via homology directed repair. *Id.* Sigma fails to realize that that is enough for written description of PC2 because working examples are not required, nor does an application need to *prove* that the invention works. *Ariad*, 1352; *Alcon*, 1189, 1191.

D. No element of Proposed Count 2 is missing from P1's description.

Sigma cannot explain what element of PC2 is purportedly missing from the description in P1—because nothing is. *See* Paper 718, 7:1-10:18. In fact, Sigma *admits* that P1 discloses all the elements of PC2. MF 3-5, 38. Unable to dispute the facts, Sigma resorts to relying on extrinsic evidence to conjure up supposed "concerns" that Sigma alleges are not addressed in P1. Paper 718, 8:10-10:18. The response is, "[w]here the disclosure in a patent's specification plainly corresponds to what is claimed, extrinsic evidence should not be used to cast doubt on … what is disclosed." *Biogen Int'l GmbH v. Mylan Pharms., Inc.,* No. 2020-1933, 2022 WL 791426, at *7 (Fed. Cir. March 16, 2022) (Lourie, J., Moore, J. and Newman, J., dissenting from order denying rehearing), *accord*, *Alcon*, 1181. This caution is especially important here because, in P1, the CVC inventors *claimed the subject matter* of PC2. Paper 588, 1:11-12, 5-22-23; Ex. 2009, claims 77-78, 88-89, 99-100. Nonetheless, P1 addresses Sigma's "concerns." Paper 588, 6:10-22, 10:20-11:4, 12:11-22, 15:1-16:14, 19:12-20:3; Ex. 2612, ¶¶ 125-134, 189-201. As Doyon explains, "a POSA would expect microinjecting CRISPR-Cas9 systems [to] retain capability of cleaving

DNA in eukaryotic cells and using the cell's own HDR machinery to integrate a donor polynucleotide *because the same techniques had been used successfully for analogous ZFN and TALEN* systems." Ex. 2612, ¶ 161 (emphasis added); Paper 588, 6:10-22. Cannon admitted that microinjection is an established technique and that RNPs circumvent these concerns, e.g., by shielding RNAs. Ex 1080, ¶¶49-50; *see also*, Paper 735, 30:4-7; Ex. 2543, ¶¶115-131.

E. Sigma's fabricated post-hoc "concerns" regarding HDR either have nothing to do with CRISPR-Cas9 or are premised on faulty assumptions.

On page 8, line 14, to page 10, line 18, Sigma provides a 2-page string citation to Cannon's deposition testimony that Sigma purports shows a POSA's "concerns" about donor integration via HDR. The response is that the so-called "concerns" are either not related to CRISPR-Cas9 or lack scientific merit. First, Sigma's Opposition improperly incorporates by reference arguments from other briefing, in contravention to clear rules prohibiting such practices. *See* Paper 718, 1:12, 4:13-15, 5:6-8; Standing Order, ¶ 106.2. Specifically, Sigma incorporates its arguments from Sigma Motion 1. *Id.*, 8:14-9:3. The Board should disregard the incorporated material.

In any event, Sigma's 12 alleged concerns incorporated from Sigma Motion 1 lack scientific merit. Even if there were concerns that [1] the mechanism of HDR was not fully characterized, [2] that in higher eukaryotes HDR occurs at a lower frequency than NHEJ, [3] that HDR is available only during specific phases of the cell cycle, [4] that NHEJ makes errors preventing target recognition and cleavage, and [5] that NHEJ was generally more likely to occur than HDR, all of these considerations *also* apply to meganucleases, ZFNs, and TALENs, which were all routinely and reliably used for donor integration via HDR by 2012. Ex. 2619, ¶¶ 97−99, 108−125; MF 26-27. In 2012, a POSA would have understood that the cell's DNA repair machinery cares not *how* the DNA was cleaved (e.g., by which nuclease), the cell's natural repair

processes will incorporate a donor polynucleotide via HDR whether the DNA is cut by radiation, ZFN, TALEN, or Cas9. *Id.* Thus, Sigma's concerns were not real concerns.

Sigma's concerns [6]-[7] and [10]-[12] relate to whether CRISPR-Cas9 will cleave a target DNA molecule in a eukaryotic cell. As Doyon previously explained, however, the POSA would have been confident that CRISPR-Cas9 would cleave its target DNA. Ex. 2543, ¶¶ 113-129; Ex. 2612, ¶¶115-134. In addition, Sigma's alleged concerns over [10] specificity and [11] off-target activity would have also applied to ZFNs and TALENs, which were routinely used for donor integration via HDR. Thus, these were not real concerns. Ex. 2619, ¶¶ 152−154.

Sigma's alleged concern [8] over blunt-end cuts from Cas9 also lacks merit. Sigma alleges that "CRISPR-Cas9 cleavage would create blunt-ended DNA," which "are not amenable to HDR absent the subsequent activation of DNA end resection." Paper 718, 15:13-18. The response is that P1 and the CVC inventors' publication Jinek 2012 each state that Cas9 also produces DNA cuts with overhang (i.e., staggered ends), yet Sigma and its expert failed to consider this. Ex. 2616, 116:23-118:18; MF 42-43. CVC's P1 discloses that "DNA cleavage [by CRISPR-Cas9] can result in the production of either blunt ends *or staggered ends*." Ex. 2009, ¶ [0042] (emphasis added); *id.*, Fig. 4 (depicting a "DSB" having "staggered" ends); Ex. 2619, ¶ 137; MF 43. Similarly, Jinek 2012 reported that CRISPR-Cas9 generates double-strand breaks, some blunt *and some with overhangs*. Ex. 2619, ¶¶ 136-140; Ex. 2618, ¶ 32; Ex. 2031, 816, Fig. 1E, Fig. S4B; MF 42. Cannon's own publication also acknowledges that Cas9 induces "staggered DSB ends." Ex. 2662, Fig. 6; Ex. 2655, 86:1-24. Moreover, a POSA would have known that blunt-ends *can indeed* undergo HDR-mediated donor polynucleotide integration because the first step of the HDR process resects the cut ends of the DNA to create an end suitable for HDR. Ex. 2619, ¶¶ 61, 63, 66, 85, 138-139. Finally, Sigma cites no prior art to support its argument, relying instead on later publications that were not available to a POSA as

of P1's filing date. Paper 718, 15:19-16:14 (citing Exs. 1126, 1127, 1141, 1145, and 1147).

Sigma's concern [9] alleges that Cas9 "remains tightly bound to the ends of the target DNA … thereby blocking HDR." Paper 718, 16:15-19. Sigma's concern is premised on its blatant mischaracterization of the Gasiunas paper (Ex. 1031), which Barrangou coauthored. Barrangou explains Gasiunas 2012 reported, in passing, that *some* Cas9-crRNA remained bound to the reaction products in a specific experiment. Ex. 2618, ¶ 29 (discussing Fig. S11, which Cannon did not consider). Barrangou explains that his article does not state or imply that Cas9 is "sticky" or that it interferes with HDR. *Id.* In addition, Sigma again fails to cite any prior art evidence to support its position, relying on later publications that were not available to a POSA as of P1's filing date. Paper 718, 16:20-18:16 (citing Exs. 1126, 1133, 1142, 1145, 1155, 1158, 1165, and 1166). A POSA reading P1 would not have shared Sigma's post hoc concerns about Cas9 remaining bound to the target DNA because the POSA knew that HDR-mediated donor integration occurs even when a nuclease *remains covalently bound* at the site of the DSB, as is the case with the protein Spo11. Ex. 2619, ¶ 138; MF 39. Similarly, the targeted nucleases I-SceI "meganuclease" and ZFNs were known to remain bound to the ends of the DSBs, and yet they routinely induced HDR-mediated donor integration. Ex. 2619, ¶ 104; MF 39. Given this, the POSA would not have expected Cas9 to interfere with HDR or otherwise preclude HDR-mediated donor integration.

F. The Decision on Motions in the '115 interference is not controlling.

The Board's analysis for this motion is different than that in the '115 Interference Decision on Motions because this is a different count, on a different evidentiary record. Paper 588, 15:1-16:14, 19:12-20:3. As CVC explained, the following evidence was not considered by the Board in the '115 Interference, or only came to light during the priority phase: ***(1)*** P1's description of the RNP fish microinjection embodiment. *Id.* This unaddressed evidence is

relevant here because, if a POSA had any of Sigma's so-called "concerns," P1 describes an embodiment of PC2 to mitigate them. *Id.* ***(2)*** P1 directly analogizes CRISPR-Cas9 to ZFNs and TALENs. *Id.*, 2:21-4:16. This unaddressed evidence is relevant because it places the state of the art in the proper context within the four corners of P1 – that is, ZFNs and TALENs were the apt comparison, not Group II introns or riboswitches. *Id.* ***(3)*** The use of existing ZFN/TALEN platforms by others in the field, including scientists from Broad, ToolGen, and Sigma, when they implemented CRISPR-Cas9 in eukaryotes. *Id*. This evidence is relevant because it shows no "special conditions" were needed to implement CRISPR-Cas9 in eukaryotic cells. *Id.* ***(4)*** The sworn testimony of Doudna and Carroll clarifying their statements, and testimony from Sternberg, Barrangou, Marraffini, and Sontheimer that it would have been "straightforward" and "routine" to implement the system in eukaryotic cells. *Id.*, 22:6-16. This evidence is relevant because it demonstrates what those in the field with first-hand knowledge were thinking at the time. *Id.* Moreover, Sigma opted not to cross-examine Drs. Doudna, Sontheimer, and Sternberg, leaving their testimony regarding P1 and the state of the art unrebutted. Ex. 2544; Ex. 2548; Paper 588, 22:6-16. ***(5)*** Sigma and its expert have made admissions that agree with CVC on many relevant issues. Paper 718, 86:-9; Ex 1080, ¶¶49-50; Paper 735, 30:4-7; MF 3-5, 38.

G. The Board's Judgment in the '115 interference is wrong and has been appealed.

CVC has appealed the Board's Judgment in the '115 interference. That judgment and underlying Decision on Priority are not dispositive here because (i) the Decision focused on a different legal issue (conception and reduction to practice), not written description; (ii) the Decision relied on documents that were not available to a POSA as of P1's filing date; (iii) the Decision explicitly avoided considering the viewpoint of a POSA (Ex. 2656, 46:11-14), which is highly relevant to description and enablement; (iv) the Decision is not final until all appeals have

been exhausted, and CVC has yet to exhaust all appeals; and (v) the Federal Circuit can be expected to conclude the Decision reached an incorrect legal conclusion on conception.

H. CVC's Responsive Motion is proper.

On page 3, lines 1-22, Sigma argues that the parties' briefings on "CVC Motion 1 and Sigma Motion 1 effectively moot CVC's Responsive Motion 1." Sigma's argument has no merit. CVC's Motion 1 addresses *Count 1*. The Board authorized this Motion, which addresses Sigma's *Proposed Count 2* (proposed in Sigma Motion 1). Paper 579 (Order); Standing Order, ¶ 208.5. CVC's Motion may become moot if the Board denies Sigma Motion 1.

III. CONCLUSION

CVC should be accorded the benefit of the filing date of P1 or, in the alternative, P2/P3.

Respectfully submitted,

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APPENDIX 1 – LIST OF EXHIBITS

Exhibit No.	Description
1001	Cannon Declaration
1031	Gasiunas (2012
1080	Cannon Supp'l Decl.
2006	U.S. Appl. No. 14/685,504, filed April 13, 2015
2007	U.S. Appl. No. 15/138,604, filed April 16, 2016
2009	Prov. Appl. No. 61/652,086, filed May 25, 2012
2010	Prov. Appl. No. 61/716,256, filed October 19, 2012
2011	Prov. Appl. No. 61/757,640, filed January 28, 2013
2015	U.S. Appl. No. 13/842,859, filed March 15, 2013
2031	Jinek, M., *et al*., "A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity," *Science* 337(6096):816-821, with Supplementary Information (2012)
2125	Deveau, H., *et al*., "Phage Response to CRISPR-Encoded Resistance in *Streptococcus thermophilus*," *J. Bacteriol*. 190(4):1390-1400 (2008)
2130	Makarova, K.S., *et al*., "Evolution and classification of the CRISPR-Cas systems," *Nature Reviews Microbiology* 9:467-477 (2011)
2132	Sapranauskas, R., *et al*., "The *Streptococcus thermophilus* CRISPR/Cas system provides immunity in *Escherichia coli*," *Nucl. Acids Res*. 39(21):9275-9282, Supplementary Figures (2011)
2134	Wiedenheft, B., *et al*., "RNA-guided genetic silencing systems in bacteria and archaea," *Nature* 482:331-338 (2012)
2543	Declaration of Yannick Doyon, Ph.D.
2544	Declaration of Jennifer Doudna, Ph.D.
2548	Declaration of Samuel Sternberg, Ph.D.
2612	Second Declaration of Yannick Doyon, Ph.D.
2616	Deposition Transcript of Paula Cannon, Ph.D., (January 27, 2022)
2618	Second Declaration of Rodolphe Barrangou, Ph.D.
2619	Third Declaration of Yannick Doyon, Ph.D.
2640	Suppl. Information from Gasinuas, G., *et al*., "Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria," *PNAS* E2579–E2586 (2012)
2643	Neale, M.J., *et al*., "Endonucleolytic processing of covalent protein-linked DNA double-strand breaks," *Nature* 436: 1053-1057 (2005)
2655	Deposition Transcript of Paula Cannon, Ph.D., (March 28, 2022)
2662	Tatiossian, K.J., *et al*., "Rational Selection of CRISPR-Cas9 Guide RNAs for Homology-DirectedGenomeEditing," *Molecular Therapy* 29(3): 1057-1069 (2021)
2656	Decision on Priority, *The Regents of the University of California v. The Broad Institute, Inc.,* Patent Interference No. 106,115 (February 28, 2022)

APPENDIX 2 – STATEMENT OF MATERIAL FACTS

1. P1 lists Jinek, Doudna, Charpentier, and Chylinski as co-inventors. Ex. 2009, 195.

Sigma's response: *Admitted*.

2. P2 lists Jinek, Doudna, Charpentier, Chylinski, and Cate as co-inventors. Ex. 2010, 277.

Sigma's response: *Admitted*.

3. P1 describes CRISPR-Cas systems comprising a) a Cas9 protein and b) a single molecule DNA-targeting RNA. Ex. 2009, [00248]-[00251], Figs. 1-3; Ex. 2612, ¶¶91-252, Appx3.

Sigma's response: *Admitted*.

4. P1 describes a sgRNA comprising i) a targeter RNA that hybridizes with a target sequence and ii) an activator-RNA that hybridizes with the targeter RNA to form a double-stranded duplex, wherein (i) and (ii) are covalently linked to one another with intervening nucleotides. Ex. 2009, [0079], [00119], [00248], Figs. 1, 3, 9; Ex. 2612, ¶¶91-96, 103-110, 181-184, 232, Appx3.

Sigma's response: *Admitted*.

5. P1 describes a sgRNA that forms a complex with Cas9 and targets the Cas9 protein to the target DNA molecule. Ex. 2009, [0046], [0048], [0076], [0089], [00155]-[00156], [00248]-[00251], Figs. 1, 3; Ex. 2612, ¶¶91-96, 112-114, 185, 232, Appx3.

Sigma's response: *Admitted*.

6. P1 describes CRISPR-Cas9 systems that cleave or edit target DNA or modulate transcription of at least one gene encoded by the target DNA. Ex. 2009, [00155]-[00159], [00248]-[00251], Figs. 3, 4; Ex. 2612, ¶¶91-96, 115-116, 185, 232, Appx3.

Sigma's response: *Denied*.

7. P1 describes target cells including a fish, a human, and a fruit fly cell, and that a target cell may be "embryonic." Ex. 2009, [00165], [00216], [00218], [00050]-[00052], [00174].

Sigma's response: *Denied*.

8. P1 describes making and using a single-molecule DNA-targeting RNA and a Cas9 RNA. Ex. 2009, [00173], [00248]; Ex. 2612, ¶¶91-96, 102, 177-180, 231, Appx3.

Sigma's response: *Denied.*

9. P1 describes that Cas9 can be delivered into a eukaryotic cell as "a polypeptide," as a nucleic acid encoding Cas9, or in a pre-formed RNP complex. Ex. 2009, [00120], [00126]-[00128], [00167]-[00172], [00177]-[00178]; Ex. 2612, ¶¶93, 97-101, 137-140, 145, Appx3.

Sigma's response: *Denied*.

10. P1 describes that the sgRNA can be delivered into a eukaryotic cell "directly as RNA" or as a nucleic acid "comprising a nucleotide sequence encoding a subject DNA-targeting RNA." Ex. 2009, [00120], [00167], [00170]-[00173], [00177]; Ex. 2612, ¶¶93, 97-101, 142-145, Appx3.

Sigma's response: *Denied*.

11. P1's Example 1 describes incubating a Cas9 protein with the sgRNA to make an RNP to cleave a target DNA. Ex. 2009, [00248]-[00251]; Ex. 2612, ¶¶93, 97-101, 142-145, Appx3.

Sigma's response: *Admitted*.

12. P1 describes microinjection as a method of delivering Type II CRISPR-Cas9 into a cell. Ex. 2009, [0039], [00154], [00173]-[00175]; Ex. 2612, ¶¶146-151, 234, Appx3.

Sigma's response: *Denied*.

13. By May 25, 2012, microinjecting protein, RNA, or RNPs into eukaryotic cells were well-known, routine laboratory techniques. Ex. 2009, [00173]; Ex. 2612, ¶¶67-73.

Sigma's response: *Denied*.

14. P1 describes transfection as a method for delivering Type II CRISPR-Cas9 systems into a cell. Ex. 2009, [00129], [0039], [00154], [00173-175], [00177]; Ex. 2612, ¶¶207-208,

Appx3.

Sigma's response: *Denied*.

15. By May 25, 2012, transfecting proteins, RNA, and RNPs into human cell lines were well-known, routine laboratory techniques. Ex. 2009, [00173]; Ex. 2612, ¶¶74-83.

Sigma's response: *Denied*.

16. By May 25, 2012, the art disclosed that a PAM must be adjacent to the target sequence for Type II CRISPR-Cas9 systems to cleave target DNA. Ex. 2612, ¶¶55-65, 267-277.

Sigma's response: *Denied*.

17. P1 discloses a PAM sequence adjacent to the target in Target DNA A ("GGG"), Target DNA B ("GGG"), and Target DNA C ("TGG"). Ex. 2009, Fig. 3C; Ex. 2612, ¶¶267-277.

Sigma's response: *Denied*.

18. P1 describes "replac[ing] a codon with a codon encoding the same amino acid." Ex. 2009, [0033]; Ex. 2612, ¶¶198, 303-307.

Sigma's response: *Denied*.

19. P1 describes peptides that can be added to Cas9, including a polypeptide that facilitates traversing an organelle membrane. Ex. 2009, [00115]; Ex. 2612, ¶¶129-130, 295-302.

Sigma's response: *Denied*.

20. All of the disclosures in P1 are in P2 and P3. Ex. 2009-2011; Ex. 2612, ¶¶253-259.

Sigma's response: *Denied*.

21. P2 describes PAMs and cites references that discuss PAMs. Ex. 2010, [00103], [00350]-[00352], [00359]; Ex. 2132; Ex. 2125; Ex. 2127; Ex. 2130; Ex. 2134; Ex. 2612, ¶¶253-255.

Sigma's response: *Denied*.

22. CVC's '859 application was filed within 12 months of the filing dates of P1-P3, and makes specific reference to P1, P2, and P3 applications. Ex. 2015, 5.

Sigma's response: *Admitted*.

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

Sigma's response: *Admitted*.

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

Sigma's response: *Admitted*.

25. Target DNA A, disclosed in Figure 3C of P1 and P2, is a non-natural target and P1 discloses Target DNA A as including a PAM. Ex. 2009, Fig. 3C; Ex. 2612, ¶¶267-277.

Sigma's response: *Denied*.

26. P1 describes and enables modification of a target DNA molecule in a eukaryotic cell by integrating a donor sequence, which occurs when the cell's own HDR process repairs DNA cut by a nuclease, whether Cas9, ZFN, or TALEN. Ex. 2009, [0058], [0059], [00157], [00189]-[00193], Fig. 4, claims 77-78, 88-89, 99-100; Ex. 2612, ¶¶118-124.

Sigma's response: *Denied*.

27. It was known that a donor sequence could be incorporated at the target site by a cell's natural HDR process after target cleavage by any nuclease. Ex. 2612, ¶¶118-124, 152-156, 324.

Sigma's response: *Denied*.

28. P3's Example 2 provides an actual reduction to practice of a human cell embodiment meeting all of elements of [1]-[9] of PC2, and P3 discloses codelivering a donor polynucleotide to integrate at the target site via HDR (element [10]). Ex. 2011, [00133], [00237]-[00239], [00275]-[00281], [00416]-[00423]; Ex. 2612, ¶¶256-259.

Sigma's response: *Denied*.

Sigma's Additional Facts 29-37

29. Sigma Proposed Count 2, like current Count 1, is directed to using CRISPR-Cas9 in a eukaryotic cell.

CVC's Response: *Admitted.*

30. With respect to CVC P1 and CVC P2, the parties' respective briefings on CVC Motion 1 address whether CVC P1 and/or CVC P2 demonstrate that the CVC inventors possessed a CRISPR-Cas9 system in a eukaryotic cell. Sigma Opp'n 1; Ex. 1080.

CVC's Response: *Admitted.*

31. Sigma Proposed Count 2 is directed to integration of a donor polynucleotide by HDR following cleavage by a CRISPR-Cas9 system in a eukaryotic cell.

CVC's Response: *Admitted that Sigma's PC2 (and Sigma's half of Count 1) recites integration of a donor polynucleotide.*

32. With respect to CVC P3, the parties' respective briefings on Sigma Motion 1 address whether the CVC inventors possessed integration of a donor polynucleotide by HDR following cleavage by a CRISPR-Cas9 system in a eukaryotic cell. Sigma Mot. 1; Ex. 1001.

CVC's Response: *Admitted that CVC's Opposition 1 addresses CVC's P3 application, denied to the extent Sigma implies this Responsive Motion is redundant or moot.*

33. Not all of CVC's involved claims in this interference contain limitations to CRISPR-Cas9 induced cleavage of DNA in a eukaryotic cell. Ex. 1001, Appx. C.

CVC's Response: *Denied.*

34. Urnov (2010) is directed to DDR processes in ZFN systems, not in CRISPR-Cas9 systems. Ex. 1080 ¶¶ 62-63.

CVC's Response: *Admitted.*

35. Figure 4 in CVC P1-P3 illustrates CRISPR-Cas9 cleavage-only (as illustrated in CVC P1-P3

Fig. 1), combined with Urnov (2010)'s ZFN-specific DDR diagram from 2010. *Id*.

CVC's Response: *Admitted that Figure 4 illustrates CRISPR-Cas9 cleavage and donor polynucleotide integration via homology-directed repair.*

36. In late 2012, a POSITA would have had many concerns about whether doublestranded breaks ("DSBs") created by CRISPR-Cas9 cleavage in eukaryotic cells could be repaired by donor integration via HDR. Ex. 1001 ¶¶ 100-157, Summary; Ex. 1080 ¶ 58.

CVC's Response: *Denied.*

37. Post-2012 publications reveal the continuing uncertainty a POSITA would have had regarding donor integration via HDR in eukaryotic cells following cleavage by a CRISPR-Cas9 system. Ex. 1080 ¶¶ 64-75.

CVC's Response: *Denied.*

CVC's Additional Facts 38-43

38. Sigma admits that Figure 4 of CVC's P1, P2, and P3 shows "how CRISPR-Cas9 might function to enable cleavage plus donor-integration via HDR in a eukaryotic cell." Paper 718, 8:7-8.

39. Before May 25, 2012, the art taught that DSBs created by nucleases that remained bound after cleavage (e.g., Spo11, I-SceI) induce HDR. Ex. 2619, ¶¶ 62, 104, 138, 142; Ex. 2643, Fig. 4.

40. Supplemental Fig. S11 of Gasiunas 2012 (Ex. 1031, Ex. 2640) shows free DNA products (unbound from Cas9) migrating at the bottom of the gel after DNA cleavage. Ex. 2618, ¶¶ 28−31; Ex. 2619, ¶ 144.

41. Jinek 2012 states that Cas9 is a "multiple-turnover enzyme." Ex. 2031, 816; Ex. 2619, ¶ 143.

42. The ends of DNA cleaved by sgRNA CRISPR-Cas9 can have an overhang. Ex. 2619, ¶ 137; Ex. 2618, ¶ 32; Ex. 2031, 816, Fig. 1E, Fig. S4B; Ex. 2009, ¶ [0042], Fig. 4.

43. CVC's P1, P2, and P3 applications each discloses that "DNA cleavage [by CRISPR-Cas9] can result in the production of either blunt ends *or staggered ends*." Ex. 2009, ¶ [0042], Fig. 4; Ex. 2010, ¶ [0076], Fig. 4; Ex. 2011, ¶ [00112], Fig. 4; Ex. 2619, ¶ 137.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **CVC REPLY IN SUPPORT OF RESPONSIVE MOTION 1** is being filed via the Interference Web Portal by 8:00 PM Eastern Time on April 7, 2022, pursuant to the Order Authorizing Motions and Setting Times ("Order"; Paper 30), and thereby served on the attorney of record for the Senior Party pursuant to ¶ 105.3 of the Standing Order. Pursuant to the Order, the foregoing was also served via email by 11:00 PM Eastern Time on counsel for the Senior Party at:

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