Filed on behalf of Junior Party Paper No. ___
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
UNIVERSITY OF VIENNA, AND EMMANUELLE CHARPENTIER

By: Eldora L. Ellison, Ph.D., Esq.
Eric K. Steffe, Esq.
David H. Holman, Ph.D., Esq.
Byron Pickard, Esq.
Paul A. Ainsworth, Esq.
John Christopher Rozendaal, Esq.
Michael E. Joffre, Ph.D., Esq.
Pauline M. Pelletier, Esq.
STERNE, KESSLER, GOLDSTEIN & FOX, P.L.L.C.
1100 New York Avenue, NW
Washington, DC 20005
Telephone: (202) 371-2600
Facsimile: (202) 371-2540
eellison-PTAB@sternekessler.com
esteffe-PTAB@sternekessler.com
dholman-PTAB@sternekessler.com
bpickard-PTAB@sternekessler.com
painsworth-PTAB@sternekessler.com
jcrozendaal-PTAB@sternekessler.com
mjoffre-PTAB@sternekessler.com
ppelletier-PTAB@sternekessler.com

By: Li-Hsien Rin-Laures, M.D., Esq.
RINLAURES LLC
321 N. Clark Street, 5th Floor
Chicago, IL 60654
Telephone: (773) 387-3200
Facsimile: (773) 929-2391
lily@rinlauresip.com

Sandip H. Patel, Esq.
MARSHALL GERSTEIN & BORUN LLP
233 South Wacker Drive
6300 Willis Tower,
Chicago, IL 60606
Telephone: (312) 474-6300
Facsimile: (312) 474-0448
spatel@marshallip.com

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 1 (for accorded benefit)

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Page(s)

Cases

Other Authorities

I. INTRODUCTION

CVC's P1, all of which is carried over into P2, describes and enables a eukaryotic cell comprising a sgRNA CRISPR-Cas9 system that is capable of modifying target DNA. The level of ordinary skill in the art was high, as numerous independent groups successfully and near-simultaneously demonstrated the claimed system in eukaryotes. A POSA reading P1 would have immediately understood that P1 explains how to apply the system in a fish cell (E1), human cell (E2), and fruit-fly cell (E3) using the well-known molecular biology techniques P1 described. To qualify as a constructive reduction to practice of Count 1, CVC's P1 did not need to include a working example or dispel the hypothetical concerns that CVC's opponents have alleged. It was wrong—as a matter of law—for the Board to have previously concluded otherwise and wrong also for Sigma to oppose CVC on that basis.

Sigma's arguments rest heavily on the premise that a constructive reduction to practice requires experiments, working examples, and optimization of the invention. But none of those things are required to prove written description or enablement. 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 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 arguments 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

previously 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. The first paragraph of P1 and all of the *contemporaneous* record evidence directly compares CRISPR-Cas9 with ZFNs and TALENs, without any mention of Group II introns. 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 doubts that 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 first paragraph. There is no contemporaneous contrary factual evidence.

Sigma's Opposition relies on and refers nearly 60 times to the Board's refusal to accord CVC benefit to P1 in its Decision on Motions from the '115 Interference. But that Decision 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 the understanding of a POSA that the described invention was straightforward to implement. 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 1.

Similarly, the '115 Priority Decision—which was also wrongly decided and is being appealed—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. Sigma does not contest any of the Material Facts from CVC's Motion 1.

Sigma failed to respond to CVC's Statement of Material Facts set forth in CVC's Motion 1 and therefore admits each fact. Paper 476, MF 1-29; Paper 735 (lacking Appendix 2). Rule 122 expressly states that "[a]ny material fact not specifically denied *shall be considered admitted*." 37 C.F.R. § 41.122(a) (emphasis added); Standing Order ¶¶ 121.5.2, 122.4.2.1. On this basis alone, CVC's Motion should be granted. *See Zhang v. Amsel*, Interference 105,550, Paper 26 at *10 (BPAI March 24, 2008) (Ex. 2664) ("Amsel did not file with its opposition a statement identifying any material facts in dispute … Since Amsel has not identified any material facts in dispute and has not specifically denied any material fact, Zhang's material facts are considered to be admitted by Amsel. *On that basis alone Zhang's motion is granted.*" (emphasis added)).

B. P1 provides a constructive reduction to practice of at least one embodiment of Count 1.

On page 2, line 8, to page 3, line 6, Sigma explains the basic legal standards for according benefit to an earlier-filed application, but fails to acknowledge that the 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."); *see also, Falkner v. Inglis,* 448 F.3d 1357, 1366 (Fed. Cir. 2006). 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, or how to make it work, which is an enablement issue." *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 P1 to "disclose … cleavage of a target DNA *in a eukaryotic cell*" (i.e., a working example). Paper 735, 40:25-41:1. Sigma further argues that P1 must "demonstrate that the CVC inventors *knew* how to make CRISPR-Cas9 work in eukaryotic cells." *Id.*, 42:19-21. Sigma's expert, Cannon, likewise uses a legally erroneous standard for written description requiring *actual possession* of the invention. *See e.g.,* Ex. 1080, ¶63 ("none of those disclosures demonstrate that CVC's inventors *actually possessed* that invention."); Ex. 2655, 24:17-25:3 ("possession means the inventors had *performed the experiment* 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 476, 17:23-38:1.

C. P1 describes all the elements of Count 1 and methods for its implementation.

CVC's Motion shows that P1 (or alternatively, P2) describes and enables at least one embodiment of Count 1, including a sgRNA CRISPR-Cas9 system "capable of cleaving or editing a target DNA molecule, or modulating transcription of a target DNA molecule." Paper 476, 15:12-26:2; MF 3-22, 27-28. P1 expressly describes a sgRNA CRISPR-Cas9 system that,

upon introduction into a fish (E1), human (E2), or fruit-fly (E3) cell, is capable of modifying target DNA. *Id.*, 15:12-38:16; MF 3-22, 27-28. These three cell types are among the most commonly used in biological research. Ex. 2543, ¶18; MF 7. P1 describes three specific ways to prepare and deliver the system: (i) as pre-assembled RNP complexes for E1 (Paper 476, 17:23-28:15; MF 9-13); (ii) as expression vectors encoding the system for E2 (*id.*, 28:16-36:14; MF 9-10, 15-16); or (iii) as RNA molecules encoding the system for E3 (*id.*, 36:15-38:1; MF 9-10, 13-14). Both parties' experts agree that these delivery techniques (microinjection and transfection) were well-established before 2012. Ex. 1080, ¶¶ 49-50; Ex. 2543, ¶¶ 65-89; MF 9-16. A POSA wanting to practice the invention would, upon reading P1, immediately recognize and understand how to implement E1, E2, and E3. Ex. 2543, ¶¶ 90-95, 100, 140-141. 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 Count 1. *Id.;* 37 C.F.R. § 41.201.

D. P1 discloses PAM, and a POSA would understand that Cas9 requires PAM, including in a eukaryotic environment.

Sigma does not dispute that P1 discloses PAM sequences in Figure 3C. Paper 476, 38:2-16; Ex. 2543, ¶¶ 249-259; MF 17-18, 22, 26. Instead, Sigma argues that P1 does not disclose the *role* of PAM sequences "in eukaryotic cells." Paper 735, 19:17-20:21. The response is that P1 need not explain the "role" of PAMs, as PAMs were understood in the prior art to be a "common theme for the most diverse CRISPR systems," applicable "irrespective of the spacer carrier or the proto-spacer holder." Ex. 2127, 733, 734. Sternberg (a fact witness with a skill set in 2012 similar to that of a POSA) testified that, when he reviewed a CVC notebook entry dated March 1, 2012, discussing applications of the CRISPR-Cas9 system in eukaryotes, he understood that a

PAM would likewise be required in those eukaryotic applications. Ex. 2548, ¶¶ 17-18. Sigma chose not to cross-examine Sternberg, leaving his testimony unchallenged. MF 30.

E. No element of Count 1 is missing from P1's description.

Sigma cannot explain what element of Count 1 is missing from the description in P1– because nothing is. *See* Paper 735, 32:20-37:16. In fact, Sigma *admits* that P1 discloses all the elements of Count 1. MF 1-29. Unable to dispute the facts, Sigma resorts to relying on extrinsic evidence to conjure up supposed "challenges and uncertainties" that it then alleges P1 does not address. Paper 735, 10:3-4. The response is, first, "[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). This caution is especially important here because, in P1, the CVC inventors *claimed the subject matter* of Count 1. Paper 476, 1:3-5, 15:15-16:1; Ex. 2009, claims 1 61-69, 93-96, 102-109. Second, as shown below, Sigma's "challenges and uncertainties" (i) were not relevant for CVC's RNP fish microinjection embodiment—a fact unaddressed in the '115 Interference decision, and (ii) are addressed in P1 nonetheless.

1. Delivering pre-assembled RNPs via microinjection, as in E1, is not subject to Sigma's alleged "challenges and uncertainties".

P1 describes making pre-assembled RNPs of sgRNA CRISPR-Cas9 *in vitro*. MF 9, 11. P1 describes introducing the system into eukaryotic cells using microinjection, which P1 also states was a "well known technique[]" for delivering various biomolecules into cells. MF 13, 14. P1 expressly identifies fish cells and embryos as target cells of interest. MF 7. As CVC explains in its Motion, Sigma's alleged challenges and uncertainties would not have been relevant (to a POSA) for CVC's RNP microinjection embodiments. Paper 476, 26:4-28:15, 32:4-36:14, 38:2-

16; Ex. 2543, ¶¶115-129; Ex. 2542, ¶¶39-42; Ex. 2545, ¶¶ 10-15; Ex. 2544, ¶¶ 28-30. Indeed, microinjection of pre-assembled RNPs into embryos *obviates all of Sigma's alleged concerns*. MF 31-32. And no prior Board decision addressed CVC's arguments or evidence on this point.

Sigma's expert Cannon admitted that RNPs can obviate Sigma's concerns, testifying that (i) microinjection is a technique suited for "early stage embryos" (like fish embryos); (ii) "[m]icroinjection of an RNP complex can get around issues of suboptimal protein expression"; and (iii) "RNPs can indeed shield their associated RNAs from cleavage." Ex 1080, ¶¶49-50; *see also*, Paper 735, 30:4-5 ("microinjection could obviate some potential challenges a POSITA would have anticipated."); Ex. 2543, ¶¶115-131.

Sigma offers three criticisms of CVC's RNP fish microinjection embodiment, none of which are supported by the evidence. Paper 735, 30:4-32:1. *First*, relying on disclosures in Mastroianni 2008 (Ex. 2150), which pertains to Group II inrons, Sigma alleges that a POSA would have expected that microinjecting a CRISPR-Cas9 RNP into fish embryos would require addition of Mg^{2+} at concentrations "deleterious to eukaryotic cells" in order to "make the RNP work in [a] eukaryotic environment." Paper 735, 30:15-17. The response is that Sigma's "deleterious" argument should be rejected because it lacks contemporaneous supporting evidence. Sigma and Cannon both rely on a 2017 declaration from Alan Lambowitz (Ex. 1092), which was prepared on behalf of Sigma as a 1.132 declaration during *ex parte* prosecution. The Lambowitz declaration contains only a bald assertion with no underlying evidence. Ex. 1092, ¶ 26. In fact, Mastroianni 2008 did not disclose that Mg^{2+} concentrations were "deleterious to eukaryotic cells." Quite the contrary – Zamore noted "even in the absence of injected magnesium, [Mastroianni] were able to detect some integration." Ex.1081, 100:7-17. Moreover, Mastroianni reported no "deleterious effects" when stating, "we show efficient Mg2+-dependent group II intron integration" in zebrafish and *Drosophila melanogaster* embryos. Ex. 2150,

Abstract. The word "deleterious" is from Lambowitz's unsupported declaration. Ex. 1092, ¶ 6. Drs. Lambowitz's and Cannon's statements are thus entitled to no weight. 37 C.F.R. § 41.158(a) ("Expert testimony that does not disclose the underlying facts or data on which the opinion is based is entitled to little or no weight.").

Moreover, the artisan would not have considered Mastroianni's Group II introns to be analogous to CRISPR-Cas9 anyway, rendering Mastroianni's Mg^{2+} concentrations irrelevant. Paper 476, 11:10-12:19. Group II introns are enormous RNAs (six to ten times larger than a sgRNA CRISPR-Cas9 complex) that undergo complicated folding and *require specific concentrations of* Mg^{2+}, higher than those usually found in eukaryotic cells. *Id.*; Ex. 2542, ¶ 21; Ex. 2543, ¶¶ 307-308. And, unlike CRISPR-Cas9 in which the Cas9 protein is the component that cuts the target DNA, in Group II introns it is the *RNA component* that makes the first cut on the target DNA. *Id.* Cannon admitted this critical difference at deposition:

> Q. So the RNA carries out the catalytic reaction; correct?
>
> THE WITNESS: The RNA within the context of an RNP.
>
> ***
>
> Q. It's the RNA component of an RNP that carries out a hydrophilic attack in the script in Zhuang that we've been discussing; correct?
>
> A. Yes, the RNA component of an RNP carries out the hydrophilic attack.

Ex. 2655, 30:14-18, 31:15-19 (objections omitted); Ex. 2658, Abstract, 18194. Group II introns were simply not relevant or analogous to CRISPR-Cas9.

Second, Sigma argues that a POSA would have been concerned about "the stability of guide RNA and a CRISPR-Cas9 complex." Paper 735, 31:14-15. Again, Sigma's criticisms fall flat. As Doyon previously explained, a POSA would have known that the RNP complex protects the sgRNA guide from nuclease degradation. Ex. 2543, ¶ 117. And Cannon admitted that "RNPs can indeed shield their associated RNAs from cleavage." Ex. 1080, ¶ 50. On cross-exam, Cannon

further admitted that there were known mechanisms to protect RNA from "degradation in eukaryotic cells," such as "chemical modifications you can introduce if the RNA is being synthesized chemically." Ex. 2655, 61:21-62:4. Cannon also admitted that P1 describes such chemical modifications to protect RNA from degradation:

> Q. Are the modifications listed in Paragraph [97] of Exhibit 2009 examples of modifications used in the art to protect RNAs from degradation?
>
> A. I'd have to read the full context of why these modifications are being described, but *that seems reasonable*.

Ex. 2655, 64:16-22 (emphasis added); *see also, id.*, 65:2-8 ("it looks like [P1 paragraph [0098]] could encompass modification to RNAs that could help protect against degradation.").

Third, Sigma argues that "the RNPs would need to be introduced at high concentrations," which, according to Sigma, would "exacerbate off-target effects" and cause toxicity. Paper 735, 31:20-24. The response is that a POSA would not have been concerned about "toxicity" due to "high concentrations" of CRISPR-Cas9 RNPs because the POSA would have known that the *amount* of RNPs is easily adjusted to mitigate toxicity. Ex. 2543, ¶117; Ex. 2009, ¶¶ [00201]-[00214]; Paper 476, 27:24-28:13. Indeed, Cannon admitted on cross-exam that "before 2012 persons of ordinary skill in the art *knew how to … optimize* the concentration of materials micro injected into eukaryotic cells." Ex. 2655, 65:8-13 (emphasis added).

Finally, while Sigma alleges that a POSA would have been concerned whether a CRISPR-Cas9 RNP could recognize target DNA in the presence of chromatin, Sigma fails to cite any evidence of such concerns. Paper 735, 30:7-11. Mastroianni 2008, the same reference Sigma portrays as casting doubt on a POSA's expectations, explains that: "The effects of chromatinization are expected to be mitigated during DNA replication, as occurs in developing embryos." Ex. 2150, 8, 11, Abstract; Ex. 2543, ¶¶ 70, 123; Ex. 2542, ¶ 32. And Sigma admits

that "success was observed" in Mastroianni 2008. Paper 735, 30:13-14. Sigma is therefore left with no credible basis for criticizing CVC's RNP fish microinjection embodiment.

The law of written description does not require an application to rule out all "potential" challenges and uncertainties. Nor would a POSA have needed such disclosures in P1 to understand that P1 described and enabled the RNP fish microinjection embodiment of Count 1.

2. Although not required for a constructive reduction to practice, P1 in fact addresses each of Sigma's "challenges and uncertainties."

Although not required for constructive reduction to practice, P1 directly addresses Sigma's so-called "challenges and uncertainties." Paper 476, 26:4-28:15, 32:4-36:14, 38:2-16. Nearly all of the alleged challenges and uncertainties relate to vector expression embodiments, not RNP microinjection. Paper 476, 5:13-15. While Sigma argues that injection of an RNP complex into an embryo "would not obviate most of the concerns a POSITA would have had," the law does not require that an application rule out all possible concerns. Paper 735, 6:12-14. The law does not even require "verification that an invention actually works." *Dana-Farber Cancer Inst., Inc. v. Ono Pharm. Co., Ltd.*, 964 F.3d 1365, 1372 (Fed. Cir. 2020). Nor does the law require that the description guarantee a degree of activity where, as here, the count does not specify one. *CFMT, Inc. v. Yieldup Int'l Corp.*, 349 F.3d 1333, 1338-39 (Fed. Cir. 2003); *Newkirk v. Lulejian*, 825 F.2d 1581, 1583 (Fed. Cir. 1987) (holding that "limitations not clearly included in a count should not be read into it").

Regardless, P1 describes methods of mitigating RNA degradation, molecular crowding, adjusting cellular conditions, adding nuclear localization signals to Cas9, codon optimizing Cas9, mitigating toxicity, and aspects of chromatin dynamics in the eukaryotic cell. *Id.*, 32:4-36:14; Ex. 2009, ¶¶[0033], [0048], [00107], [0015], [00158], [00164], [00174], [00179], [00204], [00248]-[00252], Fig. 3; Ex. 2543, ¶¶182-193, 247, 271; Ex. 2542, ¶¶26-38; Ex. 2545, ¶¶ 10-15; Ex.

2544, ¶¶ 28-30; MF 5, 12, 16, 19-20. Thus, even if a POSA required ways to mitigate these "potential" challenges—which is *not* the standard for written description—P1 had them. *Id.*

P1 recognizes that chromatin is dynamic and that "structural changes" in the DNA control "the accessibility of potentially large portions of DNA to interacting factors." Ex. 2009, ¶ [00164]; Paper 476, 35:10-36:8; Ex. 2543, ¶¶ 191-92; Ex. 2542, ¶¶ 26-32; Ex. 2545, ¶¶ 10-15; Ex. 2544, ¶¶ 28-30. Sigma effectively concedes that P1 conveys this appreciation of chromatin. Paper 735, 19:3-4. And Sigma does not deny that P1 refers to the eukaryotic CCR5 locus (known to be accessible to ZFNs), or that P1 describes how to use multiple sgRNAs to target different regions and mitigate potential chromatin impedance, or that there were established techniques for identifying open regions of chromatin. Paper 476, 35:10-36:8. Additionally, Count 1 does not require targeting a specific sequence, and thus encompasses sgRNAs targeting known, accessible eukaryotic genomic loci (e.g., CCR5). Ex. 2009, ¶ [00158]; Ex. 2543, ¶¶ 171-173.

Further, Doyon explains that a POSA would have recognized common expression vectors in P1, and would have known common promoters like the U6 promoter for enhancing expression of short RNAs. Ex. 2543, ¶¶ 182, 196, 197, 322. Doyon also explains that a POSA would have understood how to add NLSs to enhance nuclear localization, including the many different NLS disclosed in P1 as protein transduction domains. *Id.*, ¶¶ 84-86, 120-121, 186-189; Ex. 2009, ¶¶[00115], [00179]; Ex. 2542, ¶ 36; MF 20. And the POSA would have known how to use codon optimization as taught in P1, to improve expression in a given host. Ex. 2543, ¶¶ 87, 88, 190; MF 19. Doyon's testimony is consistent with the high level of ordinary skill, which Cannon argues includes "at least two years of relevant post-doctoral experience." Ex. 1080, ¶17; Paper 735, 3:14-17. The high level of skill is also consistent with the speed and ease with which several groups simultaneously reported success in eukaryotes using known vectors, promoters, and reagents, and repurposing existing ZFN and TALEN platforms. Paper 476, 12:20-15:11; *Regents*

of Univ. of California v. Broad Inst., Inc., 903 F.3d 1286, 1295-1296 (Fed. Cir. 2018) (recognizing that this simultaneity was "evidence of the level of skill in the art").

P1 also discloses that CRISPR-Cas9 cleavage reactions occur at standard pH, temperature, and ion concentrations. Ex. 2009, [00248]-[00252], Fig. 3; MF 12; Paper 476, 33:14-34:5; Ex. 2543, ¶¶ 184-185. And P1 discloses that the only "special conditions" needed for applying CRISPR-Cas9 in eukaryotic cells are the same conditions used for ZFNs and TALENs by making a direct analogy between CRISPR-Cas9 and ZFNs/TALENs in the very first paragraph. Ex. 2009, ¶¶[0001]-[0003], [00158], [00174]; Ex. 2543, ¶¶ 85, 93-94, 120, 171-173, 222, 307. P1 describes conditions for cell culture and transfection, just as those that were used with the predecessor nucleases. Ex. 2009, ¶ [00189] (describing "culture media" and "culture conditions" for "[c]ontacting the cells with a DNA-targeting RNA and/or site-directed modifying polypeptide."). P1's description is consistent with the CVC inventors' views at the time that ZFN and TALENs were the most analogous systems. Paper 476, 9:21-11:9; Ex. 2544, ¶¶ 11, 17, 37-40; Ex. 2114; Ex. 2250; Ex. 2543, ¶¶ 219, 316; Ex. 2399.

Sigma and other groups around that time also viewed ZFNs and TALENs as the most analogous. Paper 476, 10:15-11:9; Ex. 2585, ¶¶ [0146], [0149]; Ex. 2543, ¶ 261. Despite being intimately familiar with Group II introns via Sigma's commercial "targetron" technology, Sigma made no comparison with Group II introns in its own patent application, instead directly comparing CRISPR-Cas9 to ZFNs and TALENs, just like CVC. *Id.* ToolGen makes the same comparison in its patent filing. Ex. 2008, Abstract, 6-17.

Sigma argues that the Board previously considered and rejected the notion that CRISPR-Cas9 was analogous to ZFNs and TALENs, but Sigma mischaracterizes the procedural history and record. Paper 735, 6:24-7:5. The Board only addressed the CRISPR-Cas9/ZFNs/TALENs analogy in the context of *obviousness* in the '048 interference and *did not address P1's*

disclosures on this point. Indeed, Sigma only cites the Board's '048 decision of no interference-in-fact and the Federal Circuit's affirmance, neither of which dealt with P1. *Id.*, 23:20-25. In its Decision on Motions in the '115 Interference, the Board never addressed P1's teaching that applying CRISPR-Cas9 in eukaryotic cells would be analogous to applying ZFNs or TALENs. Paper 476, 5:16-18, 8:20-9:20.

3. *In vitro* studies have predictive value.

At page 37, line 17, to page 39, line 11, Sigma argues that P1's *in vitro* working example demonstrating target DNA cleavage would not have conveyed to a POSA that the CVC inventors had "possession of a eukaryotic CRISPR-Cas9 system." The response is that Sigma's argument is contrary to the law, and again relies on the grossly inapposite Group II introns. The law clearly recognizes that *in vitro* studies have "predictive" value as to broader applicability. *Cross v. Iizuka*, 753 F.2d 1040, 1050-51 (Fed. Cir. 1985) (recognizing that *in vitro* testing can be "generally predictive of *in vivo* test results, i.e., there is a reasonable correlation therebetween"); *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1566 (Fed. Cir. 1996); Paper 476, 25:10-13.

Cannon argues that P1's *in vitro* experiments would not have been informative to a POSA because there could "*possibl[y]*" be additional components that "*may*" be required for CRISPR-Cas9 to function in eukaryotes. Ex. 1080, ¶¶56-57. Cannon relies on Makarova (Ex. 2130) and Bhaya (Ex. 2131), both of which published before CVC disclosed in P1 the requisite components for a functional sgRNA CRISPR-Cas9 DNA cleavage complex. A POSA reading P1 would have known that the *in vitro* experiments were the gold standard for showing that no other components were necessary for DNA cleavage to occur. Paper 476, 16:18-17:8; Ex. 2009, Fig. 1. And the POSA would have understood that those same components—demonstrated in P1 to cut a target DNA molecule *in vitro*—would function the same way in a eukaryotic cell. *Id.*; Ex. 2655, 51:17-52:13. Indeed, Cannon admitted on cross-exam that *biochemists* do indeed perform *in*

vitro studies to understand biological systems. Ex. 2655, 66:24-67:4. And the POSA has a background in biochemistry Ex. 1080, ¶ 17.

Sigma also relies on Koseki (Ex. 2574)—another ribozymes reference—to argue that P1's *in vitro* biochemical studies hold no value. Paper 735, 37:25-38:7. But as Zamore explained, ribozymes and riboswitches were irrelevant to a POSA's views on CRISPR-Cas9 as disclosed in P1. Ex. 2542, ¶¶ 21-25; Ex. 2655, 46:9-15, 49:7-50:6. Cannon failed to address or even consider Zamore's testimony. Ex. 1080, Appendix A.

4. A POSA reading P1 would immediately envisage E1, E2, and E3.

At page 41, line 21, to page 43, line 8, Sigma criticizes P1 on the theory that it includes "anything and everything in its disclosure" and amounts to a "wish or plan." The response is that neither the law nor the facts support Sigma's argument.

The "wish or plan" phrase Sigma relies upon was originally coined in *Regents of University of Cal. v. Eli Lilly & Co*., 119 F.3d 1559, 1566 (Fed. Cir. 1997). In that case, the claims at issue required a human insulin-encoding cDNA. *Id.*, 1563, 1567. The appellant's specification, however, did not disclose the human insulin cDNA sequence. *Id.* The court concluded that the appellant's claim amounted to "a mere wish or plan for obtaining the claimed chemical invention," and that "what is required is a *description of the DNA itself*." *Id.*, 1566-1567. Sigma would have the Board believe that P1 provides nothing but a wish to someday be able use CRISPR-Cas9 in eukaryotic cells. A fair reading of P1 shows that Sigma's position is untenable. Unlike the specification in *Lilly*, P1 *describes the claimed invention*—e.g., P1 describes the sgRNA CRISPR-Cas9 system and eukaryotic cell of Count 1, and it describes *precisely how to make and use it*. The only thing missing in P1 is a working example in eukaryotic cells, and that is not required for written description or enablement.

First, a POSA reading two consecutive paragraphs of P1 would immediately envisage

microinjection of an RNP containing sgRNA and cas9 protein according to E1. Paragraphs [00177]-[00178] describe "direct injection" as a method to introduce the sgRNA "into cells as RNA" and Cas9 "as a polypeptide," followed by description in the next paragraph [0179] that an NLS can be added. Ex. 2009, ¶¶ [00177]-[00179]. Additionally, P1 provides description of how to make pre-assembled RNP complexes in a single working example. *Id.*, ¶¶ [00248]-[00252].

Second, P1's teachings are continuous and logically arranged, providing detailed workings of the sgRNA CRISPR-Cas9 system, and how to use it in eukaryotic cells. For example, Paragraphs [00165]-[00166] describe "[t]arget cells of interest." Paragraphs [00167]-[00179] describe how to deliver the system into target cells, and refers to "eukaryotic host cells." Ex. 2009, ¶ [00170]. P1 describes expression vectors, reagents, and promoters "functional in either a eukaryotic cell, e.g., a mammalian cell, or a prokaryotic cell (e.g., bacterial or archaeal cell)." Ex. 2009, ¶ [00171]. In the same section, P1 cites to Angel 2010 (Ex. 2235) and Beumer 2008 (Ex. 2123). Ex. 2009, ¶ [00174].

P1 is arranged such that, after selecting a "target cell of interest," the POSA would select from the most appropriate method of delivery to the target cell. As Doyon explains: "[A] POSA would have no difficulty immediately discerning, e.g., which delivery methods best match which eukaryotic cell types … a POSA would already have this knowledge based on general experience, e.g., with ZFNs and TALENs—the two systems P1 identifies as comparable. [Ex. 2009], ¶¶[0001]-[0002]." Ex 2543, ¶ 94; Ex. 2542, ¶ 24. And as Sigma concedes in Material Facts 13-16, the techniques applicable to each of the fish (E1), human (E2), and fruit-fly (E3) embodiments would be apparent to a POSA because they are routine in the field. MF 13-14 (microinjecting RNPs), MF 15-16 (transfecting expression vectors).

P1's invocation of ZFNs and TALENs in its opening paragraphs as well as its citation to Beumer's 2008 article, "Efficient gene targeting in Drosophila by direct embryo injection" (Ex.

2123), mean that a POSA reading P1 would have the ZFN and TALEN as context. Ex. 2542, ¶ 24. The combinations of target cells and delivery methods that are disclosed in the ZFN and TALEN art are the *same* as those reflected in E1, E2, and E3. Ex. 2543, ¶¶ 66-82. P1 also states that all "sub-combinations" are contemplated as if set forth "in combination in a single embodiment." Ex. 2009, ¶[0070]. A POSA reading P1 would therefore immediately see E1, E2, and E3 as straightforward combinations of the disclosed structures, target cells, and delivery methods, and would readily understand how to implement them. Ex. 2543, ¶¶ 70-82.

F. A reasonable expectation of success is not required, but even if it were, the contemporaneous evidence overwhelmingly shows that those in the field expected sgRNA CRISPR-Cas9 to function in a eukaryotic environment.

Sigma emphasizes alleged doubts regarding whether CRISPR-Cas9 would succeed as a genome-editing tool in eukaryotes. Paper 735, 10:12-23:14. But a reasonable expectation of success applies to *obviousness*, not written description or enablement. *Allergan, Inc. v. Sandoz Inc.*, 796 F.3d 1293, 1310 (Fed. Cir. 2015). Even if a reasonable expectation of success were required for accorded benefit, the contemporaneous evidence undermines Sigma's arguments.

(1) The contemporaneous evidence confirms that those in the field in 2012 expected sgRNA CRISPR-Cas9 to function in eukaryotes, and that doing so would be "pretty straightforward." Ex. 2455, 31:8-19; Paper 476, 39:9-42:17. The record now includes unrebutted testimony from a third party witness, Luciano Marraffini, confirming that in June of 2012, he understood immediately how to apply CVC's system in eukaryotes. Ex. 2455, 66:7-67:7, 68:13-21. Cannon did not consider any of this evidence when forming her opinions. Ex. 1080, ¶ 9, Appendix A; Ex. 2655, 18:5-18. Sigma also failed to rebut CVC's post-filing evidence of enablement. Paper 735, 43:9-25; *Amgen Inc. v. Sanofi*, 872 F.3d 1367, 1379 (Fed. Cir. 2017). Here, the evidence confirms CVC's possession, contradicts Sigma's arguments, and must be considered. *Id.*; Paper 476, 25:18-26:2, 30:18-32:3.

Sigma argues that CVC's inventor emails and post-filing references showing that ZFNs and TALENs were analogous to CRISPR are "irrelevant" because they published after P1's filing date or come from outside the specification. Paper 735, 28:1-30:1, 44:8-11. Sigma cannot have it both ways. If all extrinsic evidence must be disregarded, then so too must the unsworn statements Sigma cites as evidence of doubt or concern, including from Doudna and Carroll. *Id.*., 39:12-40:20. On the other hand, if extrinsic evidence *is* considered, then the sworn testimony of Doudna, Carroll, Barrangou, Sontheimer, Sternberg, and Marraffini, must also be considered. That testimony contradicts Sigma's arguments regarding what a POSA would have thought in 2012. Doudna and Carroll also identify specific inaccuracies in how their views and understandings have been characterized. Ex. 2544, ¶¶ 41-50 (identifying inaccuracies); Ex. 2545, ¶¶ 10 (noting the decision in the '115 interference "misinterprets my article"), 11-15.

Neither can Sigma manufacture doubt from statements that have been attributed to Doudna. Paper 735, 39:12-40:20. Doudna discredits the Pandika article (Ex. 2032). Ex. 2544, ¶¶ 47-50. Doudna also explains that any statements indicating she was not "sure," or that it was not yet "known," merely reflect "the fact that we had not yet performed experiments to test the system—not that we did not expect it to work or had doubts." *Id.*, ¶ 45. Quite the contrary, as Doudna said in an email to her co-inventor dated April 14, 2012, she believed that by leveraging an existing ZFN platform that worked on the CLTA locus "a lot of the pieces are already in place" and "we could compare efficiencies to what they observed." Ex. 2250, 1; *see also,* Ex. 2263, 24-25. And Charpentier conveyed the unqualified confidence of all of the inventors in a May 28, 2012 email addressed to *Nature*: "We foresee considerable exploitation of this system for targeted genome editing in cells of the three kingdoms of life…." Ex. 2303, 1. CVC's fact witnesses, who were not cross-examined, provide their accounts of the facts based on their first-hand knowledge—much of which is corroborated by authenticated, contemporaneous

documentary evidence. *See, e.g.*, Ex. 2232; Ex. 2263; Ex. 2250; Ex. 2303; Ex. 2304; Ex. 2399; Ex. 2063; Ex. 2220; Ex. 2215.

(2) Virtually all of Sigma's alleged concerns and obstacles rely on extrapolation from Group II introns and riboswitches, a critically flawed argument. *See* Paper 735, 11:1-14: 16.

(i) The contemporaneous evidence is uniform that CRISPR-Cas9 was compared to ZFNs and TALENs. Paper 476, 9:1-15:11; Ex. 2009, ¶¶ [0001]-[0003]; Ex. 2544, ¶¶ 11-17, 37-40; Ex. 2250; Ex. 2399; Ex. 2585, ¶¶ [0003], [0146], [0149]; Ex. 2543, ¶ 261; Ex. 2008, Abstract, 6-17.

(ii) Nor has Sigma substantively refuted any of Zamore's testimony that Group II introns and riboswitches are large catalytic RNAs that undergo complicated tertiary folding and thus, unlike CRISPR-Cas9, were understood to require significant magnesium levels. Ex. 2542, ¶¶ 21-23; Ex. 2543, ¶¶ 290-294; Ex. 2544, ¶¶ 21-22. Cannon ignores Zamore's testimony, and instead focuses on a single, specific type of Group II intron called LtrA. Ex. 1080, ¶¶ 24-26. Cannon admitted, however, that for Group II introns, it is still the *catalytic RNA* that performs the first cut on the target DNA. Ex. 2655, 30:14-18, 31:15-19.

(iii) Sigma's argument that the CRISPR-Cas9 system must "scan" the eukaryotic genome to find its target is unfounded because none of the catalytic RNAs Sigma relies on targets DNA analogously to CRISPR-Cas9. *See* Paper 735, 26:12-13, 31:17-19. Rather, a POSA would have known that, like other targeted endonucleases, CRISPR-Cas9 would "locate … target sites primarily by multiple dissociation/reassociation events" enabled by facilitated diffusion. Ex. 2532, Abstract; *see also* Ex. 2535, Abstract; Ex. 2536, Abstract, 15888.

(iv) Even assuming catalytic RNAs were seen as predictive of CRISPR-Cas9 function, Group II introns and riboswitches were not "failures" and would not have led a POSA to doubt that sgRNA CRISPR-Cas9 would have activity in eukaryotes. Ex. 2542, ¶¶ 24-25. All the evidence that Sigma cites reports at least *some* activity in eukaryotes, notwithstanding chromatin,

exonucleolytic factors, and differing cellular conditions. Ex. 2150, Abstract; Ex. 2574, 1192; Ex. 2575, Abstract; *CFMT*, 349 F.3d at 1338-39.

G. 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, in view of evidence that was either not addressed in the '115 Interference or new evidence uncovered during the priority phase. Paper 476, 5:10-6:1. As CVC explained, the following evidence was not addressed in the '115 Interference: ***(1)*** P1's description of the RNP fish microinjection embodiment. Paper 476, 5:13-15, 17:23-28:15. ***(2)*** P1's direct analogy between CRISPR-Cas9 and ZFNs/TALENs. *Id.*, 5:16-18, 7:22-15:11. ***(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.*, 5:19-21, 12:20-15:11. ***(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.*, 5:22-6:1, 39:9-42:17.

On page 6, lines 5, Sigma wrongly argues that "the Board has already rejected CVC's purportedly 'new' arguments in the Decision in the '115 Interference." Throughout its Opposition, Sigma relies on the Board's decision *from the '048 Interference* and the Federal Circuit's affirmance when alleging that CVC's argument was previously "rejected." *See e.g.*, Paper 735, 7:2-5, 8:20-24, 10:15-16, 32:24-33:144:4-7. But the '048 Interference dealt with *obviousness*—an entirely different legal issue than here, as well as *different evidentiary record*. Sigma does not cite a single instance in the '115 Decision on Motions addressing any of the four points above. Paper 735, 6:8-7:11, 23:15-27:6, 30:4-32:19, 44:3-17. This unaddressed evidence changes the Board's analysis for this Motion and must be considered. Paper 476, 2:21-4:17.

H. 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.

III. CONCLUSION

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

Respectfully submitted,

By /Eldora L. Ellison/
Eldora L. Ellison, Ph.D., Esq.
Lead Attorney for UC and UV
Registration No. 39,967
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, NW
Washington, D.C. 20005

Date: April 7, 2022

By /Li-Hsien Rin-Laures/
Li-Hsien Rin-Laures, M.D., Esq.
Lead Attorney for EC
Registration No. 33,547
RINLAURES LLC
321 N. Clark Street, 5th floor
Chicago, IL 60654

Date: April 7, 2022

APPENDIX 1 – LIST OF EXHIBITS

Exhibit No.	Description
1080	Cannon Supp'l Decl.
1092	Lambowitz Decl. (2017)
2006	U.S. Appl. No. 14/685,504, filed April 13, 2015
2007	U.S. Appl. No. 15/138,604, filed April 16, 2016
2008	Prov. Appl. No. 61/717,324, October 23, 2012
2009	Prov. Appl. No. 61/652,086, filed May 25, 2012
2010	Prov. Appl. No. 61/716,256, filed October 19, 2012
2015	U.S. Appl. No. 13/842,859, filed March 15, 2013
2032	Pandika, Rising Stars: Jennifer Doudna, CRISPR Code Killer, OZY (Jan. 7, 2014) (Ex. 2230), http://ozy.com/rising-stars/jennifer-doudna-crispr-code-killer/4690
2063	U.S. Patent Publication No. 2010/0076057, published on March 25, 2010 to Sontheimer et al.
2114	Doyon, J.B., *et al*., "Rapid and efficient clathrin-mediated endocytosis revealed in genome-edited mammalian cells," *Nat. Cell Biol*. 13(3):331-337 (2011)
2123	Beumer, K.J., *et al.*, "Efficient gene targeting in *Drosophila* by direct embryo injection with zinc-finger nucleases," *Proc. Natl. Acad. Sci,* 105(50):19821-19826 (2008)
2125	Deveau, H., *et al*., "Phage Response to CRISPR-Encoded Resistance in *Streptococcus thermophilus*," *J. Bacteriol*. 190(4):1390-1400 (2008)
2127	Mojica, F.J.M., *et al*., "Short motif sequences determine the targets of the prokaryotic CRISPR defence system," *Microbiology* 155:733-740 (2009)
2130	Makarova, K.S., *et al*., "Evolution and classification of the CRISPR-Cas systems," *Nature Reviews Microbiology* 9:467-477 (2011)
2131	Bhaya, D., *et al*., "CRISPR-Cas Systems in Bacteria and Archaea: Versatile Small RNAs for Adaptive Defense and Regulation," *Annu. Rev. Genet*. 45:273-297 (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)
2150	Mastroianni, M., *et al*., "Group II Intron-Based Gene Targeting Reactions in Eukaryotes," *PloS ONE* 3(9):e3121, pp. 1-15 (2008)
2215	Barrangou, R., "RNA-mediated programmable DNA cleavage," *Nature Biotechology* 30(9):836-838 (2012)
2220	Erik Sontheimer handwritten notes from Martin Jinek and Krzysztof Chylinski presentation at the CRISPR 2012: 5th Annual CRISPR Research Meeting held at the University of California, Berkeley, CA (June 2012), 1 page
2232	Martin Jinek, Ph.D., laboratory notebook excerpt
2235	Angel, M. and Yanik, M., "Innate Immune Suppression Enables Frequent Transfection with RNA Encoding Reprogramming Proteins," *PLoS ONE* 5(7): e11756 (2010)

2250	Email from Jennifer Doudna to Martin Jinek, dated April 14, 2012, with attachments, 33 pages
2263	Email from Martin Jinek to Jennifer Doudna, dated April 11, 2012, with attachment, 33 pages
2303	Email from Emmanuelle Charpentier to Claudia Lupp, Angela Eggleston and Jennifer Doudna, dated May 28, 2012, 2 pages
2304	Email from Guy Riddihough to Jennifer Doudna and Emmanualla Charpentier, dated May 29, 2012, 2 pages
2399	Email from Jennifer Doudna to Emmanuelle Charpentier, dated June 28, 2012, 4 pages
2455	Deposition Transcript of Luciano Marraffini, Ph.D., *The Regents of the University of California v. The Broad Institute, Inc*., Patent Interference No. 106,115 (March 11, 2021)
2535	Bonnet, I., *et al*., "Sliding and jumping of single EcoRV restriction enzymes on non-cognate DNA," *Nucleic Acids Res.* 36(12): 4118-4127 (2008)
2536	Gowers, D.M., *et al*., "Measurement of the contributions of 1D and 3D pathways to the translocation of a protein along DNA," *PNAS* 102(44): 15883-15888 (2005)
2542	Declaration of Philip Zamore, Ph.D.
2543	Declaration of Yannick Doyon, Ph.D.
2544	Declaration of Jennifer Doudna, Ph.D.
2545	Declaration of Dana Carroll, Ph.D.
2548	Declaration of Samuel Sternberg, Ph.D.
2574	Link, K.H. and Breaker, R.R., "Engineering ligand-responsive gene-control elements: lessons learned from natural riboswitches," *Gene Therapy* 16:1189-1201 (2009)
2575	Koseki, S., *et al*., "Factors Governing the Activity In Vivo of Ribozymes Transcribed by RNA Polymerase III," *J. Virol.* 73(3): 1868-1877 (1999)
2585	U.S. Appl. No. 15/456,204 with current claims appended
2616	Deposition Transcript of Paula Cannon, Ph.D., (January 27, 2022)
2655	Deposition Transcript of Paula Cannon, Ph.D., (March 28, 2022)
2656	Decision on Priority, *The Regents of the University of California v. The Broad Institute, Inc.,* Patent Interference No. 106,115 (February 28, 2022)
2658	Zhuang, F., *et al*., "Linear group II intron RNAs can retrohome in eukaryotes and may use nonhomologous end-joining for cDNA ligation," *PNAS* 106(43): 18189–18194 (2009)
2664	*Zhang v. Amsel*, Interference No. 105,550, Paper 26 (B.P.A.I., March 24, 2008)

APPENDIX 2 – STATEMENT OF MATERIAL FACTS

1. Application No. 61/652,086 ("P1"), filed on May 25, 2012, lists Martin Jinek, Jennifer Doudna, Emmanuelle Charpentier, and Krzysztof Chylinski as co-inventors. Ex. 2009, 195.

Sigma Response: Sigma failed to respond, so **admitted**.

2. Application No. 61/716,256 ("P2"), filed on October 19, 2012, lists Jinek, Doudna, Charpentier, Chylinski, and James Harrison Doudna Cate as co-inventors. Ex. 2010, 277.

Sigma Response: Sigma failed to respond, so **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. 2543, ¶¶ 90-242, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

4. P1 describes a sgRNA comprising i) a targeter RNA capable of hybridizing with a target sequence in the target DNA and ii) an activator-RNA capable of hybridizing with the targeter RNA to form a double-stranded duplex, wherein the activator-RNA and the targeter-RNA are covalently linked to one another with intervening nucleotides. Ex. 2009, [0079], [00119], [00248], Figs. 1, 3, 9; Ex. 2543, ¶¶ 90-95, 106-108, 175-179, 223, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

5. P1 describes a sgRNA capable of forming a complex with Cas9 and thereby targeting the Cas9 protein to the target DNA molecule. Ex. 2009, [0046], [0048], [0076], [0089], [00155]-[00156], [00248]-[00251], Figs. 1, 3; Ex. 2543, ¶¶ 90-95, 110-112, 180, 223, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

6. P1 describes CRISPR-Cas9 systems capable of cleaving or editing target DNA or modulating transcription of at least one gene encoded by the target DNA. Ex. 2009, [00155]-[00159], [00248]-[00251], Figs. 3, 4; Ex. 2543, ¶¶ 90-95, 113-114, 180, 223, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

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 Response: Sigma failed to respond, so **admitted**.

8. P1 describes making and using a single-molecule DNA-targeting RNA and a Cas9 RNA. Ex. 2009, [00173], [00248]; Ex. 2543, ¶¶ 90-95, 100, 170-173, 222, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

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. 2543, ¶¶ 92, 96-99, 115, 132-135, 140, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

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. 2543, ¶¶ 92, 96-99, 137-140, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

11. P1's working example describes incubating a recombinant Cas9 protein with the sgRNA to make an RNP complex. Ex. 2009, [00248]-[00251]; Ex. 2543, ¶¶ 92, 96-99, 137-140, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

12. P1's working example describes a sgRNA complexed with a Cas9 protein cleaving a target DNA. Ex. 2009, [00248]-[00251], Fig. 3A; Ex. 2543, ¶¶ 92, 96-99, 137-140, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

13. P1 describes microinjection as a method of delivering Type II CRISPR-Cas9 into a cell. Ex. 2009, [0039], [00154], [00173]-[00175]; Ex. 2543, ¶¶ 141-146, 225, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

14. By May 25, 2012, microinjecting protein, RNA, or RNPs into eukaryotic cells were well-known, routine laboratory techniques. Ex. 2009, [00173]; Ex. 2543, ¶¶ 66-72.

Sigma Response: Sigma failed to respond, so **admitted**.

15. 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. 2543, ¶¶ 199-200, Appx2.

Sigma Response: Sigma failed to respond, so **admitted**.

16. By May 25, 2012, transfecting proteins, RNA, and RNPs into eukaryotic cells human cell lines were well-known, routine laboratory techniques. Ex. 2009, [00173]; Ex. 2543, ¶¶ 73-82.

Sigma Response: Sigma failed to respond, so **admitted**.

17. 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. 2543, ¶¶ 54-64, 249-259.

Sigma Response: Sigma failed to respond, so **admitted**.

18. 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. 2543, ¶¶ 249-259.

Sigma Response: Sigma failed to respond, so **admitted**.

19. P1 describes "replac[ing] a codon with a codon encoding the same amino acid." Ex. 2009, [0033]; Ex. 2543, ¶¶ 190, 285-289.

Sigma Response: Sigma failed to respond, so **admitted**.

20. P1 describes peptide that can be added to Cas9, including a polypeptide that facilitates traversing an organelle membrane. Ex. 2009, [00115]; Ex. 2543, ¶¶ 120-121, 277-284.

Sigma Response: Sigma failed to respond, so **admitted**.

21. All of the disclosures in P1 are in P2. Ex. 2009; Ex. 2010; Ex. 2543, ¶¶ 243-245.

Sigma Response: Sigma failed to respond, so **admitted**.

22. P2 describes PAMs and cites Sapranauskas (Ex. 2132), Deveau (Ex. 2125), Mojica (Ex. 2127), Makarova (Ex. 2130), and Wiedenheft (Ex. 2134), which discuss PAMs in CRISPR-Cas systems. Ex. 2010, [00103], [00350]-[00352], [00359]; Ex. 2543, ¶¶ 243-245.

Sigma Response: Sigma failed to respond, so **admitted**.

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

Sigma Response: Sigma failed to respond, so **admitted**.

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

Sigma Response: Sigma failed to respond, so **admitted**.

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

Sigma Response: Sigma failed to respond, so **admitted**.

26. Target DNA A, disclosed in Figure 3C of P1 and P2, is a non-natural target and P1 and P2 disclose Target DNA A as including a PAM. Ex. 2009, Fig. 3C; Ex. 2543, ¶¶ 255-259.

Sigma Response: Sigma failed to respond, so **admitted**.

27. P1 describes and enables modification of a chromosomal sequence in a eukaryotic cell by integrating a donor sequence, which occurs when the cell's own homology-directed repair process repairs DNA cut by a nuclease, whether a sgRNA-CRISPR-Cas9, a ZFN, or a TALEN. Ex. 2009, [0058], [0059], [00157], [00189]-[00193], Fig. 4, claims 77, 88, 99; Ex. 2543, ¶¶ 126-128, 303.

Sigma Response: Sigma failed to respond, so **admitted**.

28. It was known that a donor sequence could be incorporated into a chromosome by a cell's natural HDR process following target cleavage by a ZFN or TALEN. Ex. 2543, ¶¶ 126-128, 303.

Sigma Response: Sigma failed to respond, so **admitted**.

Sigma's Additional Facts:

None

CVC's Additional Facts 29-34

29. Sigma failed to respond to CVC's Material Facts 1-28. *See* Paper 735, Paper 718 (lacking Appendix 2); Standing Order ¶¶ 121.5.2, 122.4.2; 37 C.F.R. § 41.122(a).

30. Sigma did not cross-examine Sternberg. Ex. 2548; Paper 735.

31. Sigma did not cross-examine Doudna. Ex. 2544; Paper 735.

32. Before 2012, microinjection had been used to deliver nucleic acids, polypeptides, and RNPs into fruit fly and fish cells. Ex. 2150, Abstract; Ex. 2123, Abstract; Ex. 2543, ¶¶66-72, 94.

33. "[A]dvantages of microinjection include the precision of delivery dosage and timing, high efficiency of transduction as well as low cytotoxicity." Ex. 2136, Abstract; Ex. 2543, ¶ 66.

34. Mastroianni 2008 states with respect to fish and fruit fly embryos that "[t]he effects of chromatinization are expected to be mitigated during DNA replication, as occurs in developing embryos." Ex. 2150, 8; *see also* Ex. 2543, ¶ 123; Ex. 2542, ¶ 32.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **JUNIOR PARTY'S REPLY 1** was 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 agreement between the parties, the foregoing were also served via email by 11:00 pm ET on counsel for the Senior Party at:

Brenton R. Babcock
Dan Liu, Ph.D.
LOEB & LOEB LLP
10100 Santa Monica Blvd., Ste. 2200
Los Angeles, CA 90067
bbabcock@loeb.com
dliu@loeb.com
BoxSigma132@loeb.com

Benjamin J. Sodey
SIGMA-ALDRICH CORP.
3050 Spruce St.
Saint Louis, MO 63103
benjamin.sodey@milliporesigma.com

Benjamin I. Dach, Ph.D.
LOEB & LOEB LLP
345 Park Ave.
New York, NY 10154
bdach@loeb.com

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C

/Eldora L. Ellison/
Eldora L. Ellison, Ph.D., Esq.
Lead Attorney for UC and UV
Registration No. 39,967

Date: April 7, 2022
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, NW
Washington, DC 20005-3934