Filed on behalf of Junior Party
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
UNIVERSITY OF VIENNA, AND EMMANUELLE CHARPENTIER

Paper No. ___

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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.

TOOLGEN, INC.
Senior Party
(Application 14/685,510).

Patent Interference No. 106,127 (DK)

JUNIOR PARTY'S REPLY 1

(for priority benefit)

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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 is high, as shown by the speed and ease with which numerous groups successfully applied the claimed system in eukaryotes. A POSA reading P1 would immediately see that P1 describes how to successfuly apply the system in a fish cell (E1), human cell (E2), and fruit-fly cell (E3) using the well-known molecular biology techniques described in P1. That is all the count, and the law, requires to demonstrate a constructive reduction to practice.

In arguing estoppel, ToolGen ignores black letter law. Estoppel does not apply when the issues are different, precluding applicability of the '048 decision concerning obviousness as an estoppel. And estoppel does not arise from an interim decision or one that was not essential to the final judgment, precluding estoppel arising from the '115 interference decision on motions. Moreover, the record supporting this motion is vastly different, requiring whole-record review.

ToolGen'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. *Alcon Rsch. Ltd. v. Barr Labs., Inc*., 745 F.3d 1180, 1189 (Fed. Cir. 2014); *Ariad Pharms., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1352 (Fed. Cir. 2010) (en banc); *CFMT, Inc. v. Yieldup Int'l Corp.*, 349 F.3d 1333, 1338-39 (Fed. Cir. 2003). ToolGen insists that P1 must instruct a POSA how to overcome or rule out every hypothetical concern that ToolGen conjures up, as well as convey certainty of success. But that argument too fails because written description and enablement require nothing of the sort.

Even if the written description and enablement standards did require P1 to describe how to address ToolGen's hypothesized obstacles, P1 does so. There is no question that P1 describes how to make pre-assembled ribonucleoprotein complexes (RNPs) that function *in vitro*. There is

also no question that P1 describes "microinjection" as a "well known technique[]" for delivering nucleic acids and proteins into eukaryotic cells. A POSA would therefore immediately envisage microinjecting the pre-assembled RNPs or mRNAs encoding the system into the nucleus or into dividing cells. Indeed, ToolGen's own expert agreed that microinjecting pre-assembled RNPs of sgRNA CRISPR-Cas9 into dividing cells, such as embryos, would have been understood to eliminate any nuclear localization and degradation considerations. Ex. 2538, 161:17-162:2.

When using vector expression, the experts agree that a POSA would have known how to use strong promoters, a nuclear localization signal (NLS), and codon optimization, all of which are described in P1 and are established molecular biology techniques well-known to a POSA. Although ToolGen argues that a POSA would have believed more was needed, the evidence shows the contrary. The record in this case, as supported by admissions by ToolGen's experts and new evidence obtained during the priority phase of the '115 interference, shows that a POSA would not have expected to need any other adaptations, including with respect to chromatin. P1 nonetheless conveys the CVC inventors' understanding that chromatin is dynamic, and describes established techniques for mitigating chromatin inhibition, including targeting multiple sites.

ToolGen's argument that E1, E2, and E3 are "imaginary" because P1 includes "laundry lists" is likewise belied by the admissions of its expert, who testified that the cell types of E1, E2, and E3 were among the most popular for gene editing, and that a POSA would understand which techniques and optimizations apply to each. At bottom, ToolGen's argument is just another way of demanding a working example, which is not required for a constructive reduction to practice.

ToolGen invokes the "reasonable expectation of success" standard, but this has no place in the analysis. That there may be uncertainty or even doubt about success does not detract from the sufficiency of the description. "Acknowledgment 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). Regardless, there is overwhelming contemporaneous evidence showing that a POSA would have expected sgRNA CRISPR-Cas9 to have activity in eukaryotes. Indeed, the 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—which are invoked in the first paragraph of P1. None of the contemporaneous evidence is to the contrary.

While ToolGen argues that P1 conveys possession only of using CRISPR-Cas9 "in a test tube," the scientific community awarded Jennifer Doudna and Emmanuelle Charpentier the 2020 Nobel Prize in Chemistry for their "development of a method for genome editing." Ex. 2225, 2.

II. ARGUMENT

A. CVC is not estopped from seeking priority benefit.

ToolGen argues, at page 4 line 8 to page 22 line 12, that CVC is precluded from seeking priority benefit to P1 and P2. ToolGen's arguments are contrary to law. Decisions on different issues, or in non-final or non-essential determinations, cannot dictate resolution of this motion. The record supporting this motion is different, contains new evidence, and must be considered as a whole. Granting this motion would not require overturning or contradicting any prior findings.

1. The '048 proceeding has no preclusive or binding effect. First, that proceeding considered a different issue—obviousness—based on the claims alone, not written description or enablement. Issue preclusion does not apply when a "different legal standard" was applied in the prior proceeding, even to the same facts—which, on this record, are decidedly *not* the same. *B & B Hardware, Inc. v. Hargis Indus., Inc.*, 575 U.S. 138, 154 (2015) ("Issues are not identical if the second action involves application of a different legal standard, even though the factual setting of both suits may be the same."). CVC had no "opportunity or incentive to litigate" the facts and

law related to written description or enablement in the '048 proceeding, because neither P1's disclosure nor those issues were under consideration. *Power Integrations, Inc. v. Semiconductor Components Indus., LLC*, 926 F.3d 1306, 1312 (Fed. Cir. 2019). It would violate the law and basic principles of equity and fairness to give preclusive effect here based on the record in the '048 proceeding. *See Smith v. Bayer Corp.*, 564 U.S. 299, 312 n.9 (2011); *Fears v. Wilkie*, 843 F. App'x 256, 261 (Fed. Cir. 2021) (quoting and applying *Smith*). Second, ToolGen has made no effort to establish that the findings on which it relies were "essential" to the judgment. *B & B Hardware*, 575 U.S. at 148. Those findings therefore cannot have preclusive effect. *Bobby v. Bies*, 556 U.S. 825, 835 (2009) (barring preclusive effect for a "subsidiary finding that, standing alone, is not outcome determinative …"). Finally, the Federal Circuit's decision was "on *this record*" and "[i]n light of the *record* evidence …." *Regents of Univ. of California v. Broad Inst., Inc.*, 903 F.3d 1286, 1294, 1296 (Fed. Cir. 2018) (emphasis added). Here, the record is different.

2. Nor does the interlocutory decision on motions in the '115 proceeding have any preclusive or otherwise binding effect here. As the PTAB recognized in authorizing this motion, that decision is not final and cannot, as a matter of law, have any preclusive consequences. Paper 25, 2-3. The PTAB is still free to reconsider its decision on motions in view of the entire record. Indeed, CVC has requested that the PTAB revisit its priority benefit decision. Even should the '115 priority benefit decision become final, it would lack preclusive effect if, for example, it is not necessary to the judgment, *B & B Hardware, Inc.*, 575 U.S. at 148, or it becomes one of two independent bases for the ultimate judgment. Restatement (Second) of Judgments § 27(i) (1982).

The '115 decision on motions would not be "essential" to the overall judgment because priority of invention and accorded benefit are different issues. The PTAB's decision on accorded benefit, issued at the end of the motions phase, merely serves the purpose of determining who is the "[s]enior party" during the priority phase. 37 C.F.R. § 41.201. Ultimately, *either* party could

prevail on priority of invention. A decision regarding priority benefit is therefore non-essential.

Yet, even if the interlocutory decision on motions were final and also somehow essential to the judgment, the PTAB has discretion to decline to apply preclusion, even when its technical pre-requisites are met. Indeed "[n]onmutual issue preclusion is not available as a matter of right." 18A Fed. Prac. & Proc. Juris. § 4465 (3d ed.); *see also Blonder-Tongue Lab'ys, Inc. v. Univ. of Illinois Found.*, 402 U.S. 313, 333–34 (1971) ("[N]o one set of facts, no one collection of words or phrases, will provide an automatic formula for proper rulings on estoppel pleas. In the end, decision will necessarily rest on the trial courts' sense of justice and equity."). Tribunals should be particularly "cautious about applying offensive collateral estoppel than defensive collateral estoppel." *Intell. Ventures I LLC v. Cap. One Fin. Corp.*, 937 F.3d 1359, 1373 (Fed. Cir. 2019).

Preclusion is especially inappropriate because CVC offers significant new evidence that casts doubt on the basis of the '115 decision. This new evidence alters the landscape upon which this motion will be decided. The record here is indeed "substantially different." *J.E.T.S., Inc. v. United States*, 838 F.2d 1196, 1200 (Fed. Cir. 1988). CVC obtained evidence during the priority phase in the '115 interference, including from subpoenaed discovery (Ex. 2455) and Broad's witnesses (Ex. 2342, 2456). In this proceeding, CVC has also obtained admissions from ToolGen and offers fact witness testimony that addresses specific inaccuracies and misinterpretations that Broad presented during the '115 motions phase, upon which the PTAB relied. The presentation of this new evidence, which was not considered in the PTAB's '115 decision on motions, makes this case akin to those in which fact finding is ongoing. *Cf. AbbVie Deutschland GmbH & Co., KG v. Janssen Biotech, Inc.*, 759 F.3d 1285, 1296 (Fed. Cir. 2014) ("[W]hen additional evidence is presented, the district court makes a *de novo* finding of facts in light of the new evidence.").

The new evidence requires *de novo* consideration of the entire record as a whole. When evaluating the sufficiency of a specification, the "[f]ailure to consider the totality of the record in

assessing written description constitutes legal error." *In re Tropp*, 748 F. App'x 1022, 1023 (Fed. Cir. 2018) (*citing In re Alton*, 76 F.3d 1168, 1176 (Fed. Cir. 1996)). This requirement includes declaration evidence, such as that being offered here. *In re Alton*, 76 F.3d at 1174-76. Agencies are obligated to examine "the record as a whole, taking into account evidence that both justifies and detracts from the agency's decision." *OSI Pharms. v. Apotex*, 939 F.3d 1375, 1384 (Fed. Cir. 2019). This principle accords with the "whole record" requirement. 5 U.S.C. § 706 (postamble).

Independent of these factors, a final decision in the '115 proceeding should not be given preclusive effect until after the decision is either affirmed or the parties waive their appeal rights. *Vardon Golf Co. v. Karsten Mfg. Corp.*, 294 F.3d 1330, 1333 (Fed. Cir. 2002) (holding that the judgment must be "immune, as a practical matter, to reversal or amendment"); *In re Stampa*, 65 U.S.P.Q.2d 1942 (B.P.A.I. 2002) (following *Vardon Golf*). Indeed, it is impossible to identify all the reasons that preclusion would or should not apply in advance of a final affirmation on appeal.

B. P1 describes the system and well-established techniques for delivering it into model cell types, which a POSA would immediately recognize as capable of demonstrating activity in eukaryotes, with or without special adaptations.

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. Paper 368, 15:11-38:12; MF 3-20. These three cell types are among the most popular in biological research, and both parties' experts agree that the techniques described in P1 for delivering the system (e.g., microinjection and transfection) were well-established before 2012. Ex. 1403, ¶¶ 59-61, 151; Ex. 1412, ¶¶ 19-21, 39-48; Ex. 2013, ¶¶ 69-85; MF 9-16. P1 describes three specific ways to prepare and deliver the system: (i) as pre-assembled RNP complexes (Paper 368, 17:12-28:7; MF 9, 10, 11, 12, 13); (ii) as expression vectors encoding the system (Paper 368, 28:8-36:6; MF 9, 10, 15, 16); or (iii) as RNA molecules encoding the system (Paper 368, 36:7-37:17; MF 9, 10, 13, 14). A POSA would immediately envisage E1, E2, and E3 reading P1's logically-arranged, successive,

and interrelated paragraphs. Ex. 2013, ¶¶ 90-94, 100, 141. P1 provides a "described and enabled anticipation" of at least one embodiment within the scope of the count. 37 C.F.R. § 41.201.

1. P1 provides description of PAMs and a POSA would understand that a PAM is required, including when applying the system in eukaryotes.

ToolGen argues, at page 7 lines 8-14, that P1 is missing adaptations for using the system in eukaryotes, alleging: "[T]here is absolutely no mention of a PAM sequence in P1." Paper 718, 30:7. The response is that P1 discloses the PAM sequences "GGG" and "TGG" immediately adjacent to the 3' end of each target in Figure 3C, including for a *non-natural* target ("Target DNA A"). Paper 368, 37:18-38:12; MF 17-18, 22, 89; Ex. 2539. As ToolGen's expert testified, a POSA would have recognized that these sequences satisfy the PAM requirement. Ex. 2538, 76:20-77:5. P1 need not explain the role of PAMs, as PAMs were understood to be a "common theme for the most diverse CRISPR systems," applicable "irrespective of the spacer carrier or the proto-spacer holder." Ex. 1210, 733, 734. A POSA also understood as of P1 that CRISPR-Cas9 requires a PAM adjacent to the target outside of its natural environment. Ex. 2132, 9278; MF 89. Consistent with this, Dr. Sternberg 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. 2221, ¶¶ 17-18.

2. Delivering pre-assembled RNPs via microinjection, as in E1, is not subject to the alleged concerns or obstacles ToolGen hypothesizes.

ToolGen argues, at page 7 lines 8-14, that P1 is missing adaptations to address alleged "concerns" and "obstacles." The response is that ToolGen's alleged concerns are irrelevant when microinjecting pre-assembled RNP complexes into the nucleus or into dividing cells. Paper 368, 25:19-28:7. 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. Microinjection of pre-assembled RNP complexes into embryos *obviates all ToolGen's alleged concerns and obstacles*. MF 90-96. No prior decision addressed CVC's arguments or evidence on this point.

ToolGen argues, at page 15 line 20, that microinjecting pre-assembled RNP complexes into fish embryos is not a "panacea." But ToolGen's own expert conceded that considerations including RNA degradation (Ex. 2474, 161:9-162:7), nuclear localization (Ex. 2474, 159:21-160:11), and codon optimization (Ex. 2538, 52:15-53:7) would not apply when microinjecting pre-assembled RNP complexes into the nucleus or into dividing cells. For example, ToolGen's expert testified that: "I would predict that, if you microinject the protein into the nucleus, that it would be active without an NLS." Ex. 2474, 160:9-11. And while ToolGen insists that chromatin would be a "concern," ToolGen's expert disclaimed that any adaptation would be applicable to chromatin.[1] Regardless, there is no factual dispute that eukaryotic DNA would be accessible in dividing cells and that embryos are rapidly dividing cells. For example, Mastroianni 2008, upon which both parties' experts rely, explains that: "The effects of chromatinization are expected to be mitigated during DNA replication, as occurs in developing embryos." Ex. 2150, 8; Ex. 2013, ¶¶ 70, 123; Ex. 2017, ¶ 32; Ex. 1403, ¶¶ 59, 107. ToolGen's expert agrees that Mastroianni 2008 describes "successful" introduction of pre-formed RNPs into fish cells (zebrafish embryos) by microinjection. Ex. 2538, 80:8-81:2. Mastroianni 2008 states that chromatin is not "expected" to be a problem because the cells are dividing. Ex. 2150, Abstract, 8, 11; Ex. 2348, ¶¶ 13, 14.

[1] Indeed, ToolGen's expert concedes that ToolGen's provisional does not address adaptations for chromatin. Ex. 2474, 161:6-8 ("The adaptations in the ToolGen applications do not address the question of chromatinized versus non-chromatinized DNA."). Neither did the numerous groups who reported success in eukaryotes within a year of Jinek 2012. Ex. 1520, 113:21-114:20.

In response, ToolGen baldly speculates that pre-assembled RNPs "may disassociate, be degraded or diluted, or bind other molecules." Paper 718, 16:5-6. However, ToolGen's expert testified that delivering pre-assembled RNP complexes of sgRNA CRISPR-Cas9 is "designed to avoid the problem of sgRNA degradation"—an expectation premised on the underlying principle (later confirmed experimentally) that pre-assembly into an complex with Cas9 prior to delivery stabilizes the sgRNA and makes it "protected from degradation." Ex. 2474, 161:9-162:7. The CVC inventors shared this expectation—regarding protection of the guide RNA by Cas9—based on what they understood about the interactions between Cas9 and the RNA. Ex. 2023, ¶¶ 12, 13, 26, 33. And ToolGen does not deny that microinjecting pre-formed RNP complexes would, at a minimum, have been expected to co-localize the system's components within the cell. *Id.*, ¶ 31.

As with its litany of other hypothetical "concerns," ToolGen does not cite any evidence, contemporaneous or otherwise, that the pre-assembled RNPs, once introduced, would have been expected to disassociate. Nor does ToolGen argue that anyone in the field was, in fact, concerned about RNP disassociation and degradation. Rather, ToolGen argues that using RNPs does not "[e]liminate" every hypothetical concern imaginable. Paper 718, 15:18. The law does not require this. 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.*, 349 F.3d at 1338-39; *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").

In this regard, ToolGen's expert testified that by "activity" he means a "*phenotypic* effect that was *measurable*." Ex. 2538, 78:1-2 (emphasis added). But this is far from what the count requires. And where, as here, the count does not require a degree of activity or effectiveness—let alone a "phenotypic effect"—applying this arbitrary threshold for activity was improper. *CFMT,*

Inc., 349 F.3d at 1338-39. His opinions applying this flawed standard should carry no weight.

In consecutive paragraphs, P1 discloses using "direct injection" to introduce the sgRNA "into cells as RNA" and Cas9 "as a polypeptide." Ex. 2001, [00177]-[00178]. Even though NLSs are not required, particularly in embryos which undergo frequent nuclear breakdown (Ex. 2474, 114:16-22; Ex. 2013, ¶ 120; Ex. 2017, ¶ 38), the very next paragraph states that the polypeptide may be fused to permeant domains that are NLSs. Ex. 2001, [00179]; Ex. 2013, ¶ 186; MF 20.

P1 refers to microinjection as a "well known technique[]" for delivering nucleic acids and polypeptides into cells. Ex. 2013, ¶ 141; MF 13, 14, 95. ToolGen does not dispute that the known "advantages of microinjection include the precision of delivery dosage and timing, high efficiency of transduction as well as low cytotoxicity." Ex. 2136, Abstract; MF 96. Regarding off-target activity, ToolGen's expert testified that, when using a 20-nucleotide sequence, "perfect matches other than the desired targeted sequence" are unlikely. Ex. 2474, 155:6-12. MF 93.

While ToolGen attempts to characterize microinjection as an extraordinary or specialized skill, its expert testified that microinjection was "a fairly common technique" that a POSA "would generally use" for introducing nucleic acids, proteins, and RNPs into fish and fruit fly cells, among other genetic systems (e.g., oocytes). Ex. 2538, 40:1-11, 43:16-45:18; *see also* Ex. 2150, Abstract; Ex. 2538, 44:10-45:7; Ex. 1403, ¶ 107. Indeed, ToolGen's expert authored a commentary in 2003 that alone cites *seven* articles using microinjection to deliver biomolecules into eukaryotic cells. Ex. 2530, 588, 592, 593. As of P1, microinjection had been used to deliver diverse molecules and complexes of molecules, ranging from RNPs and DNA-protein complexes to recombinant proteins, prokaryotic proteins, and multichain antibodies. Ex. 2013, ¶¶ 70-71.

As to P1's disclosure of the pre-assembled RNP itself, ToolGen does not argue that P1 lacks such description; rather, ToolGen asserts that the disclosure is limited to giving instructions for preparing the RNP in a "dual guide" format. Paper 718, 14:21-22. This is simply wrong. P1's

[00248]-[00249], including the referenced figures and descriptions, unambiguously describe how to make pre-assembled RNPs of CRISPR-Cas9 having "single-molecule DNA targeting RNAs." Paragraph [00248] specifically describes the procedure for making sgRNA RNPs and refers to Figure 3, which provides experimental results using the pre-assembled RNP showing "efficient" target cleavage by chimera A sgRNA CRISPR-Cas9, as shown in Figure 3B. *Id.*, Fig. 3, [00006].

3. P1 expressly refers to microinjection as a "well known technique[]" for delivering biomolecules into cells, including fruit fly embryos.

P1 also cites and incorporates Beumer 2008 (Ex. 2123), entitled "Efficient gene targeting in Drosophila by direct embryo injection with zinc-finger nucleases." Ex. 2001, [00174]. A POSA would select fruit fly cells because, as ToolGen concedes, it is "a system that's widely used." Ex. 2474, 144:17-22. A POSA would microinject fruit fly embryos because, as ToolGen's expert testified: "If you want to get something into a drosophila larva you would generally use microinjection." Ex. 2538, 40:9-11. P1's description of fruit fly cells and its citation to Beumer provides a start-to-finish roadmap for using this cell type and delivery method. Ex. 2013, ¶ 94.

4. Although not required for a constructive reduction to practice, P1 in fact addresses each of the supposed adaptations that ToolGen raises.

ToolGen's expert (like Broad's) has conceded that the purported adaptations apply only to vector expression and amount, in essence, to using: (1) strong promoters to achieve high RNA and protein expression; (2) adding an NLS; and (3) codon optimizing. Ex. 2538, 103:1-105:14; Ex. 2456, 69:8-97:3-9. P1 describes each of these aspects. Paper 368, 31:18-36:6; MF 16, 19, 20. Even if P1 did not describe these enhancements, the experts agree that a POSA would know how to design vectors, including by selecting common promoters for enhancing expression, including U6 for short RNAs. Ex. 2538, 59:9-14, 63:2-9, 64:5-17; Ex. 2456, 69:8-70:2; Ex. 1412, ¶ 44; Ex. 2013, ¶¶ 182, 196, 197, 322. The experts agree that a POSA would have understood how to add NLSs to enhance nuclear localization. Ex. 2474, 61:10-21; Ex. 2013, ¶ 84-86; Ex. 2017, ¶ 36.

The experts agree that a POSA would have known how to use codon optimization to improve expression in a given host. Ex. 2474, 63:5-64:12, 65:15-66:13, 74:8-16, 88:14-22; Ex. 2013, ¶¶ 87, 88. This testimony is consistent with the high level of ordinary skill, which ToolGen itself argues includes at least one year "post-Ph.D." doing "research related to manipulation of gene expression in eukaryotes." Paper 718, 3:3-10; MF 97. While ToolGen argues that "Dr. Cullen's definition should be accepted and CVC's rejected," ToolGen's definition, if anything, endows a POSA with *more* experience and knowledge of genetic manipulation techniques—e.g., "post-Ph.D." experience. Paper 718, 3:18. 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 368, 12:20-15:10; *Regents of Univ. of California*, 903 F.3d at 1295-96 (recognizing that this simultaneity was "evidence of the level of skill in the art" as well as "objective evidence that persons of ordinary skill in the art understood the problem and a solution to that problem").

While ToolGen's expert did not identify purported adaptations for addressing chromatin, P1 recognizes that chromatin is dynamic and that "structural changes in the surrounding DNA" control "the accessibility of potentially large portions of DNA to interacting factors." Ex. 2001, [00164]. ToolGen does not deny that P1 conveys this appreciation of chromatin, or that P1 refers to the CCR5 locus, which was known to be accessible to ZFNs, or that P1 describes how to use multiple sgRNAs to target different sequences in ways that mitigate the potential for chromatin impedance, or that there were established techniques for identifying open regions of chromatin. Paper 368, 35:2-24. ToolGen's expert also does not dispute that mapping the accessibility of target genes was a known strategy in the art, and one that had been used successfully with ZFNs. Ex. 1403, ¶ 126. Additionally, the count does not require targeting a specific sequence, and thus encompasses guides targeted to well-known, accessible, eukaryotic genomic loci (e.g., CCR5).

Even the literature ToolGen cites shows that bacterial systems delivered into eukaryotes, e.g., T7 polymerase, were, in fact, quite *capable* of acting on DNA notwithstanding chromatin. *See, e.g.*, Ex. 1270, 76 (concluding "[c]learly, T7 RNA polymerase must have the ability to transcribe through several nucleosome cores in an array, as transcript bands corresponding to the reading through of arrays of at least ten nucleosome cores can be seen in this gel."); Ex. 1272, 577; Ex. 2337, Abstract; Ex. 1271, Abstract; Ex. 2150, Abstract, 8, 11. Rather than suggest that chromatin was believed to be a barrier, this literature shows several strategies for addressing it. Ex. 2013, ¶¶ 262-270, 309-311; Ex. 2017, ¶¶ 26-32; Ex. 2023, ¶¶ 28-30; Ex. 2348, ¶¶ 13-15.

5. The teachings in P1 are arranged logically and in specific successive paragraphs. A POSA would immediately envisage E1, E2, and E3.

At page 23 lines 5-16, ToolGen criticizes P1 on the theory that its teachings are not all in one location in the specification. Neither the law nor the facts support ToolGen's argument.

First, ToolGen distorts the law to impose requirements not found in precedent. It is well-established that "the specification *as a whole* must be considered in determining its sufficiency." *Application of Long*, 368 F.2d 892, 895 (C.C.P.A. 1966) (emphasis added). Furthermore, written description does not "require identical descriptions" to what is being claimed; it merely requires enough disclosure to convey that the inventor had possession of it. *Union Oil Co. of California v. Atl. Richfield Co.*, 208 F.3d 989, 1001 (Fed. Cir. 2000); *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1570 (Fed. Cir. 1996) ("*ipsis verbis* disclosure is not necessary"). With respect to enablement, "[a] patent need not teach, and preferably omits, what is well known in the art." *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1534 (Fed. Cir. 1987). Under the "described and enabled anticipation" standard, there is no requirement that all relevant teachings appear in one place, or even proximate each other. 37 C.F.R. § 41.201. Rather, multiple distinct teachings can properly anticipate if a POSA reading the disclosure would "immediately envisage" their arrangement or

combination. *Kennametal, Inc. v. Ingersoll Cutting Tool Co.*, 780 F.3d 1376, 1381 (Fed. Cir. 2015). This is particularly true where the disclosure expressly contemplates that "the disclosed components or functionalities may be combined and [a POSA] would be able to implement the combination." *Blue Calypso, LLC v. Groupon, Inc.*, 815 F.3d 1331, 1344 (Fed. Cir. 2016).

Second, ToolGen alleges, at page 23 line 8, that the teachings in P1 appear "hundreds of paragraphs apart." This is incorrect. P1's teachings are continuous and logically arranged. 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. 2001, [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. 2001, [00171]. In the same section, P1 cites to Angel 2010 (Ex. 2235) and Beumer 2008 (Ex. 2123). Ex. 2001, [00174]. Paragraphs [00177]-[00179] describe "direct injection" as a method to introduce the sgRNA "into cells as RNA" and Cas9 "as a polypeptide," followed by description that an NLS can be added. *Id.*, [00177]-[00179]. Additionally, P1 provides description of how to make pre-assembled RNP complexes in a single working example. Ex. 2001, [00248]-[00252].

P1 is arranged such that, after selecting a "target cell of interest," the POSA would select from among the disclosed methods of delivery. As Dr. 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. *Id.*, ¶¶[0001]-[0002]." Ex 2013, ¶94; Ex. 2017, ¶ 24. And as ToolGen concedes, 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. Ex. 2538, 44:10-45:7 (E1); *id.*, 37:1-38:16 (E2); *id.*, 40:1-11 (E3).

P1's invocation of ZFNs and TALENs *in the very first paragraph* as well as its citation to

Beumer 2008 (Ex. 2123) mean that a POSA reading P1 would have the ZFN and TALEN art in mind. Ex. 2017, ¶ 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. 2013, ¶¶ 66-82.

P1 also states that all "sub-combinations" are contemplated as if set forth "in combination in a single embodiment." Ex. 2001, [00070]. A POSA reading P1 would therefore immediately see E1, E2, and E3 as straight-forward combinations of the disclosed structures, target cells, and delivery methods, and would readily understand how to implement them. Ex. 2013, ¶¶ 70-82.

While ToolGen asserts, at page 24 lines 16-22, that using an sgRNA with a less truncated tracrRNA is an "imagined" guide, ToolGen does not dispute that Figures 3A-3B depict the same less truncated tracrRNA sequence that ToolGen criticizes, and demonstrates its functionality. Ex. 2001, [0087], [00117], Figs. 3A-3B, 6A-C; Ex. 2013, ¶¶ 175-179. A POSA would immediately see that P1 describes how to make and use such a sgRNA in the system. Ex. 2013, ¶¶ 175-179.

At bottom, ToolGen's argument that E1, E2, and E3 are "imaginary" is just another way of demanding a working example, which the law does not require. *Ariad*, 598 F.3d at 1352.

C. 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.

Beyond this, ToolGen focuses on amplifying alleged doubts regarding whether CRISPR-Cas9 would succeed as a genome-editing tool in eukaryotes. Paper 718, 26-30. But a reasonable expectation of success applies only in the context of *obviousness*. *Allergan, Inc. v. Sandoz Inc.*, 796 F.3d 1293, 1310 (Fed. Cir. 2015). Even if a reasonable expectation of success were required, ToolGen fails to establish that a POSA would have had any of the purported concerns or doubts. The contemporaneous evidence, including admissions by ToolGen and new testimony obtained during the priority phase of the '115 interference, completely undermines ToolGen's arguments.

1. CVC has proffered a wealth of contemporaneous evidence confirming 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 368, 39:14-42:14. ToolGen leaves substantively unrebutted the testimony of multiple fact witnesses having first-hand knowledge as well as contemporaneous evidence. For example, 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.

ToolGen's expert admitted that he did not consider any of this evidence when forming his opinions. Ex. 2538, 114:2-20, 14:9-15:16. ToolGen and its expert also categorically disregard CVC's post-filing evidence, asserting it is "irrelevant." Page 718, 35:3-14, 17:21-23. The Federal Circuit, however, regards post-filing evidence as relevant to enablement and written description. *Amgen Inc. v. Sanofi*, 872 F.3d 1367, 1379 (Fed. Cir. 2017). Here, it confirms CVC's possession, contradicts ToolGen's arguments, and must be considered. Paper 368, 25:10-18, 30:10-31:17.

ToolGen argues post-filing evidence is irrelevant because it comes from outside the specification. Paper 718, 12:17-13:8. ToolGen cannot have it both ways. If all extrinsic evidence must be disregarded, then so too must the unsworn statements ToolGen cites as evidence of doubt or concern, including from Doudna and Carroll. On the other hand, if extrinsic evidence, including CVC inventor statements, *is* considered, then the sworn testimony of Doudna, Carroll, Barrangou, Sontheimer, Sternberg, and Marraffini, and other extrinsic evidence must also be considered. That testimony contradicts ToolGen'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. 2023, ¶¶ 41-50 (identifying inaccuracies); Ex. 2348, ¶¶ 10 (noting the decision in the '115 interference "misinterprets my article"), 11-15.

Neither can ToolGen manufacture doubt from statements that have been attributed to Doudna. Paper 718, 21:3-35. Doudna discredits the Pandika article (Ex. 2032). Ex. 2023, ¶¶ 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. 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 for biotechnological, biomedical and gene-therapeutic purposes." Ex. 2303, 1.

What the evidence as a whole confirms is that CVC's invention of sgRNA CRISPR-Cas9 was immediately regarded as transcending use of CRISPR-Cas9 "in a test tube" (Paper 718, 1:4), consistent with the contemporaneous evidence showing that those in the field saw immediately how to apply it in eukaryotes. Paper 368, 12:20-10. It is also confirmed by the fact that Doudna and Charpentier were awarded the Nobel Prize for their "development of a method for genome editing." Ex. 2225, 2. CVC's biochemical work, which is described both in Jinek 2012 and more extensively in P1, is not "meaningless," as ToolGen's expert incredibly asserts. Ex. 1403, ¶ 100.

ToolGen makes allegations regarding personal financial holdings and institutional affiliations, but offers no evidence that any fact witness failed to provide a truthful account of the facts based on their first-hand knowledge—much of which is corroborated by authenticated, contemporaneous documentary evidence that pre-dates the alleged financial interests. *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 ToolGen's alleged concerns and obstacles rely on extrapolation from Group II introns, T7 polymerase, ribozymes, and riboswitches. *See* Paper 718, 27:7-30:18. ToolGen's extrapolation of doubt and concern from these disparate systems is critically flawed.

i. The contemporaneous evidence is uniform that CRISPR-Cas9 was compared to ZFNs and TALENs. Paper 368, 9:1-15:10. ToolGen blatantly distorts Dr. Carroll's testimony on this point, suggesting that he testified at deposition that he did not think ZFNs and TALENs were analogous to CRISPR-Cas9. Paper 718, 11:16-18. In fact, Dr. Carroll was stating, based on the question he was asked, whether he regarded ZFNs and TALENs as analogous to "Cas9"—the *unguided nuclease*. Ex. 1520, 87:14-19. He later clarified that he did, indeed, regard ZFNs and TALENs as directly analogous to the *CRISPR-Cas9 system*, because "if an endonuclease domain of the ZFNs and TALENs worked, that it could easily be that the endonuclease domain in the CRISPR system would also work in eukaryotic cells." Ex. 1520, 111:14-112:7. Setting aside ToolGen's gross mischaracterization, Dr. Carroll's direct testimony stands unrebutted. Ex. 2348.

ii. ToolGen has also not substantively refuted any of Dr. Zamore's testimony that Group II introns, T7 polymerase, ribozymes, and riboswitches are all large catalytic RNAs that undergo complicated tertiary folding and thus, unlike CRISPR-Cas9, were understood to require significant magnesium levels. Ex. 2017, ¶¶ 21-23. CRISPR-Cas9 is different because the *protein* is the catalytic part. *Id.*, ¶ 21. Because magnesium requirements for catalytic proteins are lower, a POSA would not have understood high magnesium concentrations to be necessary for CRISPR-Cas9 to have activity. *Id.*, ¶ 22. Moreover, unlike these catalytic RNAs, the Cas9 and the RNA components of the CRISPR-Cas9 system were expected to function within the pH, temperature, and ionic ranges found in eukaryotic cell types. Ex. 2013, ¶¶ 290-294; Ex. 2023, ¶¶ 21-22.

iii. None of the catalytic RNAs ToolGen cites target DNA analogously to CRISPR-Cas9. Unlike T7 polymerase, CRISPR-Cas9 does not translocate, processively, along DNA. Ex. 2538, 83:12-84:16. Therefore, ToolGen's argument that the CRISPR-Cas9 system must "scan" the eukaryotic genome to find its target is unfounded. *See* Paper 718, 28:18-20. Rather, a POSA would have known that, like other guided endonucleases, CRISPR-Cas9 would "locate … target

sites primarily by multiple dissociation/reassociation events" enabled by facilitated diffusion. Ex. 2532a, Abstract; *see also* Ex. 2535, Abstract; Ex. 2536, Abstract, 15888. And while ToolGen's expert offered opinions regarding this alleged "scanning" process, when asked whether he was "familiar with the concept of facilitated diffusion in the context of DNA binding proteins finding their DNA targets," he stated: "Only in very loose terms, not particular, I am not a biophysicist." Ex. 2538, 117:2-7. When he was asked whether this involves the DNA-binding protein randomly colliding with the DNA, he stated: "[T]his is outside of my expertise, I am sorry, I am not going to be able to give you authoritative answers." *Id.*, 117:17-22. ToolGen's expert's opinions on CRISPR-Cas9 finding its target are admittedly uninformed and should be given no weight.

iv. Even assuming catalytic RNAs are seen as predictive of CRISPR-Cas9 function, Group II introns, T7 polymerase, ribozymes, and riboswitches were not "failures" and would not have led a POSA to doubt that sgRNA CRISPR-Cas9 would have activity in eukaryotes. Ex. 2017, ¶¶ 24-25. All the evidence that ToolGen cites reports at least *some* activity in eukaryotes, nothwithstanding chromatin, exonucleolytic factors, and differing cellular conditions. Ex. 2150, Abstract; Ex. 1270, Abstact; Ex. 1271, Abstract; Ex. 1272, Abstract; Ex. 2337, Abstract; Ex. 1273, 1192; Ex. 1274, Abstract. It is irrelevant whether the activity reported was optimal, robust, or competitive with alternatives. The count does not require that. *CFMT*, 349 F.3d at 1338-39.

D. CVC's statement of material facts is not deficient.

ToolGen incorrectly asserts, at page 3 line 20 to page 4 line 5, that CVC's statement of material facts is deficient for not arguing enablement. Enablement is a question of "law" based on underlying "facts." *In re Vaeck*, 947 F.2d 488, 495 (Fed. Cir. 1991). CVC's statement lists material "facts" and CVC's motion presented enablement arguments referring to those "facts."

III. CONCLUSION

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

Respectfully submitted,

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IV. APPENDIX 1 – LIST OF EXHIBITS

Exhibit No.	Description
2001	Prov. Appl. No. 61/652,086, filed May 25, 2012
2013	Declaration of Yannick Doyon, Ph.D.
2017	Declaration of Phillip Zamore, Ph.D.
2019	Declaration of Erik Sontheimer, Ph.D.
2021	Declaration of Rodolphe Barrangou, Ph.D.
2023	Declaration of Jennifer Doudna, Ph.D.
2063	U.S. Patent Publication No. 2010/0076057, published on March 25, 2010 to Sontheimer et al.
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)
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)
2136	Zhang, Y. and Yu, L-C., "Microinjection as a tool of mechanical delivery," *Curr. Opin. Biotechnol*., 19:506-510 (2008)
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
2221	Declaration of Samuel Sternberg
2225	The Royal Swedish Academy of Science, Scientific background on the Nobel Prize in Chemistry 2020 "A Tool for Genome Editing," 14 page (Octobr 7, 2020)
2232	Martin Jinek, Ph.D., laboratory notebook excerpt
2235	Decision on Motions 37 C.F.R. § 41.125(a), *The Broad Institute, Inc., v. The Regents of the University of California,* Patent Interference No. 106,048, Paper 893, (February 15, 2017)
2250	Email from Jennifer Doudna to Martin Jinek, dated April 14, 2012, with attachments, 33 pages
2337	Wirtz, E., *et al*., "Regulated processive transcription of chromatin by T7 RNA polymerase in Trypanosoma brucei," *Nucleic Acids Res*. 26:4626 (1998)
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
2342	Declaration of Le Cong, dated December 17, 2020
2348	Declaration of Dana Carroll, Ph.D.
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)
2456	Deposition transcript of Ronald Breaker, Ph.D., *The Regents of the University of California v. The Broad Institute, Inc*., Patent Interference No.106,115 (March 5, 2021)
2263	Email from Martin Jinek to Jennifer Doudna, dated April 11, 2012, with attachment, 33 pages
2474	Deposition Transcript of Bryan Cullen, Ph.D., with errata, Patent Interference No. 106,127 (June 22, 2021)
2528	Kennedy, E.M., *et al*., "Inactivation of the Human Papillomavirus E6 or E7 Gene in Cervical Carcinoma Cells by Using a Bacterial CRISPR/Cas RNA-Guided Endonuclease," *J Virol* 88(10): 11965–11972 (2014)
2530	Cullen, B.R., Nuclear RNA Report," *J Cell Science* 116(4): 587-597 (2003)
2532a	Halford, S.E., "An end to 40 years of mistakes in DNA–protein association kinetics?," *Biochem. Soc. Trans.* 37: 343-348 (2009)
2535	Bonnet, I., *et al*., "Sliding and jumping of single EcoRV restriction enzymes on non-cognate DNA," *Nucleic Acids Research* 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: 15883-15888 (2005)
2539	BLASTN search for Target DNA A from Prov. Appl. No. 61/652,086, filed May 25, 2012 (search last performed August 26, 2021)
1210	Mojica et al., Short Motif Sequences Determine the Targets of the Prokaryotic CRISPR Defence System, Microbiology, 155, 733–740 (2009), with Supplementary Material.
1270	O'Neill et al., Nucleosome Arrays Inhibit Both Initiation and Elongation of Transcripts by Bacteriophage T7 RNA Polymerase, J. Mol. Biol., 223, 67–78 (1992).
1271	Jenuwein et al., The immunoglobulin μ enhancer core establishes local factor access in nuclear chromatin independent of transcriptional stimulation, Genes & Development, 7, 2016–2032 (1993).
1272	McCall and Bender, Probes for chromatin accessibility in the Drosophila bithorax complex respond differently to Polycomb-mediated repression, The EMBO Journal, 15(3), 569–580 (1996).
1273	Link and Breaker, Engineering ligand-responsive gene-control elements: lessons learned from natural riboswitches, Gene Therapy, 16, 1189–1201 (2009).
1274	Koseki et al., Factors Governing the Activity In Vivo of Ribozymes Transcribed by RNA Polymerase III, J. Virol., 73(3), 1868–1877 (1999).
1403	July 15, 2021 Declaration of Bryan R. Cullen, Ph.D.
1412	July 15, 2021 Declaration of John J. Turchi, Ph.D.
1520	Deposition Transcript of Dana Carroll, Ph.D., The Regents of the University of California v. ToolGen, Inc., Interference No. 106,127, June 17, 2021
1570	Deposition Transcript of Jennifer Doudna, Ph.D., The Regents of the

	University of California v. ToolGen, Inc., Interference No. 106,127, July 8, 2021.

V. APPENDIX 2 – STATEMENT OF MATERIAL FACTS

1. No. 61/652,086 ("P1"), filed on May 25, 2012, lists Martin Jinek, Jennifer Doudna, Emmanuelle Charpentier, and Krzysztof Chylinski as co-inventors. Ex. 2001, p. 195. Broad

ToolGen Response: Admitted.

2. No. 61/716,256 ("P2"), filed on October 19, 2012, lists Jinek, Doudna, Charpentier, Chylinski, and James Harrison Doudna Cate as co-inventors. Ex. 2001, p. 277.

ToolGen Response: Admitted, to the extent the filing reference in MF 2 refers to Exhibit 2002, p. 277.

3. P1 describes CRISPR-Cas systems comprising a) a Cas9 protein and b) a single molecule DNA-targeting RNA. Ex. 2001, [00248-251], Figs. 1-3; Ex. 2013, ¶¶90-242, Appx2.

ToolGen Response: Denied.

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. 2001, [0079], [00119], [00248], Figs. 1, 3, 9; Ex. 2013, ¶¶90-95, 106-108, 175-179, 223, Appx2.

ToolGen Response: Denied.

5. P1 describes a sgRNA capable of forming a complex with Cas9 and thereby targeting the Cas9 protein to the target DNA molecule. Ex. 2001, [0046], [0048], [0076], [0089], [00155]-[00156], [00248]-[00251], Figs. 1, 3; Ex. 2013, ¶¶90-95, 110-112, 180, 223, Appx2.

Response: Denied.

6. P1 describes CRISPR-Cas9 systems capable of cleaving or editing a target DNA molecule or modulating transcription of at least one gene encoded by the target DNA molecule. Ex. 2001, [00155]-[00159], [00248]-[00251], Figs. 3, 4; Ex. 2013, ¶¶90-95, 113-114, 180, 223, Appx2.

ToolGen 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. 2001, [00165], [00216], [00218]; [00050-52], [00174].

ToolGen Response: Admitted that the words fish, human, and fruit fly appear in P1 and that the word "embryonic" appears in a section titled "Target cells of interest"; otherwise, denied.

8. P1 describes making and using a single-molecule DNA-targeting RNA and a Cas9 RNA. Ex. 2001, [00173], [00248]; Ex. 2013, ¶¶90-95, 100, 170-173, 222, Appx2.

ToolGen Response: Denied.

9. P1 describes that Cas9 can be delivered into a eukaryotic cell "as a polypeptide," as a nucleic acid encoding Cas9, or as part of a pre-formed RNP complex. Ex. 2001, [00120], [00126]-[00128], [00167]-[00172], [00177-178]; Ex. 2013, ¶¶92, 96-99, 115, 132-135, 140, Appx2.

ToolGen Response: Admitted that the words in the partial, cropped, quote appears in P1; otherwise, 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. 2001, [00120], [00167], [00170-173], [00177]; Ex. 2013, ¶¶92, 96-99, 137-140, Appx2.

ToolGen Response: Admitted that the words in the partial, cropped, quotes appear in P1; otherwise, denied.

11. P1's working example describes incubating a recombinant Cas9 protein with the sgRNA to make an RNP complex. Ex. 2001, [00248]-[00251]; Ex. 2013, ¶¶92, 96-99, 137-140, Appx2.

ToolGen Response: Denied.

12. P1's working example describes a sgRNA complexed with a Cas9 protein cleaving a target DNA. Ex. 2001, [00248]-[00251], Fig. 3A; Ex. 2013, ¶¶92, 96-99, 137-140, Appx2.

ToolGen Response: Denied.

13. P1 describes microinjection as a method of delivering the Type II CRISPR-Cas system into a cell. Ex. 2001, [0039], [00154], [00173]-[00175]; Ex. 2013, ¶¶141-146, 225, Appx2.

ToolGen Response: Denied.

14. By May 25, 2012, microinjecting protein, RNA, or RNPs into eukaryotic cells were well-known, routine laboratory techniques. Ex. 2013, ¶¶66-72.

ToolGen Response: Denied.

15. P1 describes transfection as a method for delivering the Type II CRISPR-Cas system into a cell. Ex. 2001 [00129], [0039], [00154], [00173-175], [00177]; Ex. 2013, ¶¶199-200, Appx2.

ToolGen Response: Denied.

16. By May 25, 2012, transfecting proteins, RNA, and RNPs into eukaryotic cells human cell lines were well-known, routine laboratory techniques. Ex. 2013, ¶¶73-82.

ToolGen Response: Denied.

17. By May 25, 2012, the art disclosed that a PAM must be adjacent to the target sequence for Type II CRISPR systems to cleave target DNA. Ex. 2013, ¶¶54-64, 249-259.

ToolGen Response: Admitted, to the extent that the target DNA is a natural prokaryotic target of Type II CRISPR.

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. 2001, Fig. 3C; Ex. 2013, ¶¶249-259.

ToolGen Response: Admitted that the quoted sequences appear in Target DNA A, B, and C.

19. P1 describes "replac[ing] a codon with a codon encoding the same amino acid." Ex. 2001, [0033]; Ex. 2013, ¶¶190, 285-289.

ToolGen Response: Admitted that the words in the partial, cropped, quote appears in P1;

otherwise, denied.

20. P1 describes peptide that can be added to Cas9, including a polypeptide that facilitates traversing an organelle membrane. Ex. 2001, [00115]; Ex. 2013, ¶¶120-121, 277-284.

ToolGen Response: Denied.

21. All of the disclosures in P1 are in P2. Ex. 2001; Ex. 2002; Ex. 2013, ¶¶ 243-245.

ToolGen Response: Denied.

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. 2002, [00103], [00350]-[00352], [00359]; Ex. 2013, ¶¶243-245.

ToolGen Response: Admitted to the extent that P2 cites the listed articles; otherwise denied.

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. 2005, p. 5.

ToolGen Response: 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, pp. 4-5.

ToolGen Response: 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, pp. 356-360.

ToolGen Response: Admitted.

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26. CVC includes only twenty-five facts in its Statement of Material Facts (SOMF). None allege that P1 and P2 are enabled, or provide material facts that would support this conclusion. Mot. CVC Statement of Material Facts.

CVC Response: Denied.

27. The Board (twice) and Federal Circuit have found that applying the natural prokaryotic CRISPR/Cas9 system to achieve DNA cleavage in eukaryotes would not have been predictable and merely a matter of applying ordinary skill in the art. Ex. 1101 ('115 Decision on Motions), 104; Regents of Univ. of California v. Broad Inst., Inc., 903 F.3d 1286, 1291 (Fed. Cir. 2018); Ex. 2335 ('048 Decision on Motions), 45–46.

CVC Response: Denied.

28. Given the substantial differences in eukaryotic and prokaryotic cellular environments, contemporaneous evidence from skilled artisans in the field, and statements made by the CVC applicants themselves supporting that success in eukaryotes was unpredictable, the Board found that a POSA would not have had a reasonable expectation of success in applying the prokaryotic CRISPR/Cas9 system in eukaryotes. Ex. 2335, 48–49.

CVC Response: Denied.

29. The Board found, and the Federal Circuit affirmed that "one of ordinary skill in the art would not have reasonably expected success before experiments in eukaryotic cells were done." Ex. 2335, 23; Regents of Univ. of California v. Broad Inst., Inc., 903 F.3d 1286, 1291–92 (Fed. Cir. 2018). In so deciding, both the Board and the court recognized the unpredictability of the field as of December 2012—seven months after the filing date of P1.

CVC Response: Admitted to the extent the quoted language is accurately

quoted, otherwise denied.

30. The Board found, and the Federal Circuit affirmed, that "the prior art TALEN and zinc finger nuclease ("ZFN") systems were not analogous to CRISPR-Cas9 . . . and that the adaptability of small prokaryotic protein systems like Cre would not have informed the expectation of success" of CRISPR in eukaryotes. Broad, 903 F.3d at 1293. See also Ex. 2335, 41–43.

CVC Response: Admitted to the extent the quoted language is accurately quoted, otherwise denied.

31. The Board found, and the Federal Circuit affirmed, that "the success in applying similar prokaryotic systems in eukaryotes was unpredictable and had relied on tailoring particular conditions to the technology." Broad, 903 F.3d at 1294 (discussing the Board's review of evidence related to ZFNs or TALENs, Cre, riboswitches, ribozyme systems, and group II introns). See also Ex. 2335, 39.

CVC Response: Admitted to the extent the quoted language is accurately quoted, otherwise denied.

32. The Federal Circuit affirmed the Board's conclusion that Dr. Carroll's article was "substantial evidence that skilled artisans believed many problems could arise in implementing the CRISPR-Cas9 system in eukaryotes, which the Board viewed as indicating that an ordinarily skilled artisan would have lacked a reasonable expectation of success." Broad, 903 F.3d at 1293.

CVC Response: Admitted to the extent the quoted language is accurately quoted, otherwise denied.

33. The Federal Circuit also reviewed Dr. Doudna's contemporaneous statements—like that the applicants' "2012 paper was a big success, but . . . [they] weren't sure if CRISPR/Cas9 would work in eukaryotes" and that the applicants had faced "many frustrations" in getting CRISPR to work in eukaryotes. Broad, 903 F.3d at 1293.

CVC Response: Admitted to the extent the quoted language is accurately quoted, otherwise denied.

34. In the '115 Interference, the CVC alternative of the count was the same as the CVC alternative here, claim 156 of CVC application 15/981,807, both requiring eukaryotes. Ex. 1105, 12.

CVC Response: Admitted.

35. In the '115 interference. the Board found that P1 and P2 lacked written description because neither showed that the applicants possessed an embodiment of the count, which requires "a eukaryotic cell with a CRISPR-Cas system capable of cleaving or editing a target DNA or of modulating transcription[.]" Ex. 1101, 91.

CVC Response: Denied.

36. The Board's reasoning in the '115 Interference rested on the unpredictability of the field and the fact that a POSA would have been aware of the potential challenges for using CRISPR/Cas9 in eukaryotes, and would require more than the cell-free, in vitro experiments of P1 and P2. Ex. 1101, 86, 102–103.

CVC Response: Denied.

37. In the ’115 interference, CVC asserted that P2 contained “supplemental disclosures about expressing Cas9 protein in E. coli and information about what was known in the art about PAM sequences” and the Board found that this did not “cure[] the deficiencies” of P1. Ex. 1101 at 105.

CVC Reponse: Admitted to the extent the quoted language is accurately quoted, otherwise denied.

38. ZFNs and TALENs differ in several ways that would have led a POSA at the time that P1 and P2 were filed to doubt whether successes ith ZFN/TALENs would transfer to CRISPR/Cas9 in eukaryotes. Ex. 1403,¶¶ 109–119.

CVC Response: Denied.

39. When CVC’s own witness Dr. Carroll was asked whether “back in 2012, you thought that the zinc finger nucleases and TALENs were analogous to Cas9,” Dr. Carroll responded “I wouldn’t say that, no.” Ex. 1520, (Carroll Tr.), 87:14–19.

CVC Response: Admitted to the extent the quoted language is accurately quoted, otherwise denied.

40. The DNA binding domains of ZFN/TALENs are made up of amino acids, while DNA binding in CRISPR/Cas9 occurs by Watson-Crick base pairing between nucleotides. Ex. 1403, ¶¶118–119. The DNA binding domains of ZFN/TALENs also naturally act on eukaryotic DNA, while the CRISPR/Cas9 system is strictly prokaryotic. Ex. 1403 at ¶¶112–115.

CVC Response: Denied.

41. In the chromatin context, one of the key potential challenges is access to tightly

bound chromatin, where a system must first find the target DNA molecule within a much larger eukaryotic genome than prokaryotes, and then bind and act on the target DNA molecule within the tightly bound chromatin structure. Ex. 1403 at ¶ 64, 72, 116, 123.

CVC Response: Denied.

42. Unlike the prokaryotic CRISPR/Cas9 system, both ZFN and TALENs have binding domains evolved to function in eukaryotes. Ex. 1403, ¶ 111. See also Ex. 2339, at 1660. In TALENs, the DNA binding domains derive from bacteria which "naturally access, scan, recognize and bind to eukaryotic, plant genomic DNA in a chromatin context." Ex. 1403, ¶ 113. Similarly, the DNA binding portion of the ZFN "is derived from eukaryotic transcription factors, such as TFIIIA, that naturally recognize, scan, and bind DNA in the chromatin context of higher eukaryotes (vertebrates)." Ex. 1403, ¶113.

CVC Response: Denied.

43. The bacterial group II introns are a bacterial site-specific gene targeting system which functions, like the CRISPR/Cas system, as an RNA component complexed with a protein complex. Ex. 1403, ¶77. Though bacterial group II introns work well in bacteria, their function in eukaryotic cells is inhibited by the chromatin in eukaryotic cells. Ex. 1403, ¶77.

CVC Response: Denied.

44. Neither P1 nor P2 disclose the use of an RNP outside of the cell-free, in vitro experiments on natural prokaryotic targets presented in P1 Example 1. Ex. 1403,

¶51; Ex. 2001, ¶00249. And there it appears that only experiments included steps to create an RNP when using dual guide RNA. Ex. 1403, ¶56.

CVC Response: Denied.

45. When several research groups published papers in January 2013 purporting to show successful cleavage in eukaryotic cells, not one group used an RNP. Mot. at 13:5–15:9, 30:10-17; Ex. 2013, ¶205–10; Ex. 1560 174:9–178:1 (Doyon Tr.). Not even Jinek 2013—the work of the P1 and P2 applicants themselves—used an RNP. Ex. 2033.

CVC Response: Admitted that the papers published in January 2013 did not not appear to report such data, otherwise denied.

46. A POSA would have expected an additional potential challenge with introducing RNPs because the preformed complex may disassociate, be degraded or diluted, or bind other molecules before it is able to reach the nucleus and the chromatin within, and there is no way for the cell to make more guide RNA or Cas9 protein to replace it. Ex. 1403, ¶46, 93, 106.

CVC Response: Denied.

47. A POSA in 2012 would have known that the guide RNA and Cas9 are not covalently associated. Ex. 1403, ¶¶ 93, 106. Instead, they exist in a state of equilibrium binding between bound and unbound. Id. In a highly concentrated, purified solution, like the cell-free, in vitro conditions of P1 Example 1, there is no competitive binding by other molecules, and the complex may be stable. Ex. 1403, ¶102.

CVC Response: Denied.

48. Once the guide RNA and Cas9 enter the eukaryotic cell, the solution is diluted, and numerous molecules within the cell can compete to bind with both the guide RNA and the protein. Ex. 1403, ¶¶93, 106. Because no studies were available when P1 or P2 were filed regarding the affinity or kinetics of modified guide RNA and Cas9, id. at ¶106; Ex. 1560 (Doyon Tr.), 108:9–18, a POSA would not have been able to rule this out as a concern.

CVC Response: Denied.

49. RNA is a labile nucleic acid and subject to nuclease catalyzed degradation. Ex. 1403, ¶88–91. Even when it is complexed with Cas9, it is not immune from degradation. Id. at ¶88–89, 105.

CVC Response: Denied.

50. Guide RNA differs from other double stranded RNAs in that it, by definition, must have a single-strand RNA exposed in order to hybridize with the target DNA molecule. This exposed single strand of RNA is susceptible to degradation as well, even when complexed with the Cas9. Id. at ¶88–89, 105. A POSA would have been aware that the stability of Cas9 within a eukaryotic cell at the relevant time period here was unknown, id. at ¶105, 106, and therefore without experiments could not have known how long the RNP would last inside a eukaryotic cell.

CVC Response: Denied.

51. A POSA as of May 25, 2012 would have known that RNA molecules containing a

5’ triphosphate, such as the guide RNA of a CRISPR/Cas9 complex, can activate the eukaryotic cell’s interferon response. Ex. 1403, ¶95. By using an RNP instead of a vector, a POSA would have known that an RNP would need to be introduced at high concentrations to account for natural and eukaryotic cell degradation, as well as dilution by cell division, and so the possibility of toxicity remained a concern. Id. at ¶108.

CVC Response: Denied.

52. Drs. Sternberg, Sontheimer, Barrangou, and Carroll did not analyze P1 or P2. Ex. 1520 (Carroll Tr.), 107:13–108:16; Ex. 1510 (Sternberg Tr.), 23:12–24:8; Ex. 1530 (Barrangou Tr.), 12:22–13:10; Ex. 1500 (Sontheimer Tr.), 24:18-19, 25:4-10, 26:5-10; Ex. 2001; Ex. 2002.

CVC Response: Admitted with respect to Sternberg, Sontheimer, and Barrangou and admitted with respect to Carroll to the extent P1 and P2 are not listed in the appendix to his declaration, otherwise denied.

53. Dr. Carroll characterized the claims in Jinek 2012 as a “bold prediction” that the CRISPR/Cas9 “system can potentially be used . . . for targeted genomic cleavage in higher organisms.” Ex. 2339, 1659. The diagram in his article shows question marks next to CRISPR to indicate that “perhaps” CRISPR could make double-stranded DNA breaks like other gene editing system. Id.

CVC Response: Denied.

54. There is no indication that Dr. Carroll attempted to withdraw the article, Ex. 1520, 90:15–91:5, and he cites no other evidence contemporaneous to P1 or P2 to

support the changed view he asserts today.

CVC Response: Denied.

55. Dr. Doudna made the following statements:

• "Our 2012 paper [Jinek 2012] was a big success, but there was a problem. We weren't sure if CRISPR/Cas9 would work in eukaryotes—plant and animal cells." Ex. 2279 (Catalyst Magazine), 3.

• After Jinek 2012, her lab, along with labs at Harvard and MIT were all "working hard to see if they could get CRISPR/Cas9 to function in eukaryotic cells." Id. at 3.

• "[I]f the system could be made to work in human cells, it would be a really profound discovery." Ex. 2032 (Pandika 2014), 2.

• Dr. Doudna and her colleagues "experienced 'many frustrations' getting CRISPR to work in human cells." Id. at 3.

• "These findings suggested the exciting possibility that Cas9:sgRNA complexes might constitute a simple and versatile RNA-directed system for generating DSBs that could facilitate site-specific genome editing. However, it was not known whether such a bacterial system would function in eukaryotic cells." Ex. 2033 (Jinek 2013), 1–2.

• "People have asked me over and over again: Did you know that it was going to work? But until you do an experiment, you don't know--that's science. I've been lambasted for this in the media, but I have to be true to who I am as a scientist. We certainly had an hypothesis, and it certainly seemed like a very good guess that it would." Ex. 1570, (Doudna Tr.), 207:12–21 (quoting Ex. 1593).

• Cas9 "is a protein that has evolved over time in bacteria. And so it has to deal with bacterial genomes, which are a lot smaller than eukaryotic genomes like the human genome, and also don't have the kind of highly compacted structures that we see in chromatin in eukaryotic cells." Id. at 161:5–13 (Doudna Tr.) (quoting Ex. 1576, Breakthrough Prize Symposium on November 9th, 2015).

CVC Response: Admitted to the extent the quoted language is accurately quoted, otherwise denied.

56. Count 1 requires a Type II CRISPR/Cas9 system with a single guide RNA molecule and a Cas9 polypeptide that is capable of forming a complex and acting on the target DNA molecule in a eukaryotic cell. See Declaration of '127 Interference (Dec. 14, 2020), Paper 1 at 5-6.

CVC Response: Admitted to the extent some individual words above appear in the count, otherwise denied.

57. P1 and P2 report only cell-free, in vitro experiments, none of which would have informed a POSA that P1 or P2 disclosed a CRISPR/Cas9 system capable of acting on a target DNA molecule in a eukaryotic cell, particularly given the numerous uncertainties a POSA would have been aware of in 2012. Ex. 1403, ¶¶39–44

CVC Response: Denied.

58. P1 contains no single guide RNA disclosing a version of chimera A in which the 3' end of the tracrRNA is less truncated than the chimera A depicted in P1's Fig. 3B, and no guidance to do the same. Ex. 2001, Fig. 3.

CVC Response: Denied.

59. In the summer and fall of 2012, the basics of CRISPR/Cas9 were still being researched. Ex. 2019, ¶¶13–15. No one had yet shown use of CRISPR/Cas9 systems in eukaryotic cells.

CVC Response: Denied.

60. A POSA would have been aware that the cytoplasm and nuclei of eukaryotic cells contain exonucleases capable of rapidly degrading RNA and that exonuclease-mediated degradation of eukaryotic mRNA transcripts is mitigated by, for example, 5' capping and 3' poly-adenylation of the mRNA, but that bacterial DNA contains no such mechanisms to mitigation exonuclease degradation. Ex. 1403, ¶88.

CVC Response: Denied.

61. It would be counterproductive for a guide RNA to have such capping and poly-adenylation because these features serve to recruit ribosomes and other protein structures, while the guide RNA needs to stay available to complex with the Cas9 protein. Ex. 1403, ¶89. A POSA in 2012 would not have known whether these enzymes would cleave the guide RNA, rendering the system nonfunctional. Id.

CVC Response: Denied.

62. Neither P1 nor P2 mentions the potential for degradation. Ex. 2001; Ex. 2002.

CVC Response: Denied.

63. P1 and P2 do not address potential needs for differing ion concentrations of

CRISPR/Cas9 in a eukaryotic cell. Ex. 2001; Ex. 2002.

CVC Response: Denied.

64. A POSA would have known that the intracellular ion concentration "can significantly impact activity of bacterial enzymes in eukaryotic cells." Ex. 1403, at ¶90, 98.

CVC Response: Denied.

65. A POSA would have appreciated that these cellular differences could prevent CRISPR-Cas9 from functioning in eukaryotic cells. Ex. 1403, ¶¶64, 79, 98. Neither P1 nor P2 mentions cellular differences that may be problematic. Ex. 2001; Ex. 2002.

CVC Response: Denied.

66. A POSA in 2012 would have understood that the presence of the guide RNA can trigger an interferon response, leading to toxicity. Ex. 1403, ¶95–97. RNA with a 5' trisphosphate, as the guide RNA in a CRISPR-Cas9 complex would have, "can activate pattern recognition receptors, such as RIG-I, in turn leading to a cellular interferon response. Id. at ¶95 (citing Ex. 1294 (Schmidt 2012) and Ex. 1275 (Karpala 2005)).

CVC Response: Denied.

67. Neither P1 nor P2 mentions the potential for cellular toxicity. Ex. 2001; Ex. 2002.

CVC Response: Denied.

68. A POSA would have known that the eukaryotic genomic is significantly larger

than the bacterial genome¬ (approx. 1,800 times different between the human genome and S. pyogenes genome). Id. at ¶67.

CVC Response: Denied.

69. A POSA would therefore have doubted whether the CRISPR/Cas9 system could scan such a larger genome and successfully identify target DNA molecules. Id. at ¶64, 70–72, 75. See also Ex. 1570, 162:3–10.

CVC Response: Denied.

70. A POSA would have known that eukaryotic DNA is condensed into a tightly bound chromatin structure and the eukaryotic cell division is a complex and tightly controlled process that was not fully understood. Ex. 1403, ¶123–125.

CVC Response: Denied.

71. In 2012, a POSA would also have known that the majority of methods used to map chromatin accessibility do not provide information on "how higher-order [chromatin] structure relates to access of enzymes to the DNA template." Id. at 125.

CVC Response: Denied.

72. At a keynote address two months after P1, Dr. Doudna was asked whether. "[i]f you chromatinized your plasm[ids], would they still cut? If you . . . put histones in there?" Ex. 1570, 136:19–137:1 (Doudna Tr.). Dr. Doudna responded that "we have not done that experiment[.]" Id. at 137:2–3.

CVC Response: Admitted to the extent the words are partially quoted accurately, otherwise denied.

73. CRISPR/Cas9 evolved to function within the structure of prokaryotic DNA. But CRISPR/Cas9 did not exist in eukaryotic cells and therefore would not have encountered chromatin. Ex. 1403, ¶71.

CVC Response: Denied.

74. A POSA would also have known that chromatin had prevented other bacterial systems, like T7 RNA polymerase, Id. at ¶72–76, and group II introns, Id. at ¶79, from working successfully in eukaryotes.

CVC Response: Denied.

75. A POSA would have known that even if the guide RNA and Cas9 formed a complex, did not induce cellular toxicity and was not degraded, and could access, scan, recognize, and bind DNA in chromatin, the ensuing RNA:DNA hybrid still might not be stable. Id. at ¶91, 120.

CVC Response: Denied.

76. Eukaryotic cells contain Ribonuclease H (RNase H), a nuclease that effectively recognizes and cleaves the RNA strand of RNA:DNA hybrids. Id.

CVC Response: Denied.

77. Dr. Dana Carroll voiced this exact concern in his September 2012 article which questioned whether the system could function in eukaryotes. Ex. 2239, 1660.

CVC Response: Denied.

78. Neither P1 nor P2 mentions the issue of chromatin access. Ex. 2001; Ex. 2002

CVC Response: Admitted that the phrase "chromatin access" does not

appear in P1 or P2, otherwise denied.

79. As of 2012, a POSA would have known that a PAM sequence played a role in the ability of a bacterial CRISPR system to cleave natural DNA targets. Ex. 1403, ¶41, 68; Ex. 2125 (Deveau 2008); Ex. 1210 (Mojica 2009).

CVC Response: Admitted.

80. While the role of the PAM in CRISPR/Cas9's natural prokaryotic targets was known, a POSA on May 25, 2012 would not have known what role the PAM might play, if any, in eukaryotic cells, and would have expected the applicants to discuss it. Id.

CVC Response: Denied.

81. There is no mention of a PAM sequence in P1. Ex. 2001.

CVC Response: Denied.

82. Failures with T7 RNA polymerase, Group II introns, riboswitches, ribozymes, and cre recombinase would have informed a POSA's doubt that CRISPR/Cas9 would be successful in eukaryotes. Ex. 1403, ¶ 68, 72–79, 85–87. These systems demonstrate the unpredictability a POSA would have recognized in 2012 that adapting systems for eukaryotes either failed or required specific tailoring of conditions unique to each system. Id. at ¶87.

CVC Response: Denied.

83. In his patent application, Dr. Doyon stated that with respect to ZFNs, "previously-described assays do not predict in vivo functionality . . . . Nor do these assays

accurately determine which nucleases are least toxic to the host cell." Id.; Ex. 2300 (Doyon Patent Application), ¶ 0005.

CVC Response: Admitted to the extent the partially quoted language is quoted accurately, otherwise denied.

84. Dr. Doyon cites to an article about ZFN/TALENs systems which details that "some of the engineered ZFNs . . . may fail to induce mutations in the target sites in vivo," despite showing success in bacterial two-hybrid assays. Ex. 2139 (Foley 2009) 2, ¶4.

CVC Response: Admitted to the extent the partially quoted language is quoted accurately, otherwise denied.

85. P1 and P2 provide no data beyond in vitro testing of purified components in a cell-free environment. Ex. 2001; Ex. 2002.

CVC Response: Denied.

86. Based on experiences with prior systems, without a working example, a POSA would have expected to see a discussion of the "unique conditions" for CRISPR-Cas9 in P1 and P2. Ex. 2013 ¶144.

CVC Response: Denied.

87. P1 and P2 contain only in vitro testing of purified components in a cell-free environment. Id.; Ex. 2001, ¶248–52.

CVC Response: Denied.

88. In the '048 Interference the Board found and the Federal Circuit affirmed "that

the prior art TALEN and zinc finger nuclease ("ZFN") systems were not analogous to CRISPR-Cas9 . . . ." Broad, 903 F.3d at 1293.

CVC Response: Admitted to the extent the partially quoted language is quoted accurately, otherwise denied.

CVC's Additional Facts 89-97

89. The complete sequence of Target DNA A, shown in Figure 3C of P1, is not found in the NCBI nucleotide collection (nr/nt) database and is not a natural sequence. Ex. 2539.

90. Dr. Cullen testified that: "[E]xperiments that used preformed Cas9 sgRNA complexes were, in part, designed to avoid the problem of sgRNA degradation" because when "bound by the Cas9 protein, [sgRNA] would be protected from degradation." Ex. 2474, 161:13-162:7.

91. Dr. Cullen testified: "if you deliver the protein directly into the nucleus, then you don't need a nuclear localization signal," "I would predict that, if you microinject the protein into the nucleus, that it would be active without an NLS," and "I would expect that Cas9 would enter the nucleus of a eukaryotic cell during cell division." Ex. 2474, 159:21-160:6, 160:9-11, 114:16-22.

92. Dr. Cullen testified: "[I]f we make the assumption you have an ample supply of pure protein in hand, then additional issues of codon optimization do not arise." Ex. 2538, 52:15-53:7.

93. Dr. Cullen testified that when targeting a 20-nucleotide sequence using CRISPR-Cas9 "perfect matches other than the desired targeted sequence" are unlikely. Ex. 2474, 155:6-12.

94. 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. 2013, ¶ 123; Ex. 2017, ¶ 32; Ex. 1403, ¶¶ 59, 107.

95. Before 2012, microinjection had been used to deliver nucleic acids, polypeptides, and pre-formed RNPs into fruit fly and fish cells. Ex. 2150, Abstract; Ex. 2123, Abstract; Ex. 2538, 40:1-11, 43:16-47:7, 80:8-81:2; Ex. 1403, ¶ 59; Ex. 2530, 588-593; Ex. 2013, ¶¶ 66-72, 94.

96. "[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. 2013, ¶ 66.

97. ToolGen's proposed definition of a POSA "is consistent, although not identical, with the definition of a POSA espoused by the CVC's expert, Dr. Peterson." Ex. 1412, ¶¶ 21, 39-48.

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 August 27, 2021, pursuant to an agreement between the parties, 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:

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