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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)

TOOLGEN OPPOSITION 1
(opposing CVC benefit)

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I. INTRODUCTION

In 2012, the Junior Party ("CVC") claims to have identified, for the first time, the necessary and sufficient components of the CRISPR/Cas9 system, which is found naturally only in prokaryotic cells. Ever since, and armed only with a cell-free experiment in a test tube with purified components, CVC has tried to convince this Board and the Federal Circuit that it also discovered how the CRISPR-Cas9 system works in eukaryotic cells. Although CVC has failed at every turn, in this Interference, CVC persists in its campaign. Yet CVC's Motion 1 is most notable for what it never provides – instructions in P1 and P2, or a discussion of the potential specific conditions needed, for CRISPR-Cas9 activity in a eukaryotic cell.

Instead, CVC and its experts use what is now known in 2021, and urge that those gaps could have been or would have been traversed in 2012. But that kind of hindsight reconstruction is not nearly enough. CVC needed to show that CRISPR-Cas9 activity in eukaryotic cells was taught as of 2012 by P1 and P2. All the *post hoc* creations of CVC, and its experts, cannot change the dispositive facts: Neither P1 nor P2 contained an adequate disclosure that would support eukaryotic activity when they were filed, and the knowledge of persons of ordinary skill in the art in 2012 cannot fill that gap. And significantly, even the CVC applicants themselves expressed serious doubts at the time about whether their work on bacteria could ever be translated to other eukaryotic cells. CVC's motion should be denied.

II. DESCRIPTION OF APPENDICES

Appendix 1 is a List of Exhibits Cited. Appendix 2 is the Statement of Material Facts (F_).

III. APPLICABLE LEGAL STANDARDS

A. Requirements To Be Accorded Benefit Of An Application

To be accorded benefit of an earlier-filed application, a party must show a constructive reduction to practice of an embodiment within the count. *Hunt v. Treppschuh*, 523 F.2d 1386,

1389 (C.C.P.A. 1975). The described and enabled embodiment within the count must satisfy the requirements of 35 U.S.C. § 112, first paragraph. To satisfy the written description requirement, "the applicant must 'convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention,' and demonstrate that by disclosure in the specification of the patent." *Carnegie Mellon Univ. v. Hoffmann-La Roche Inc.*, 541 F.3d 1115, 1122 (Fed. Cir. 2008) (quoting *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563–64 (Fed. Cir. 1991)). Where, as here, the field is unpredictable, and the applicants did not provide a complete description of the alleged invention, significantly more is required to demonstrate such possession. *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1357–58 (Fed. Cir. 2010) (en banc) (holding patent invalid for lack of written description where the patent provided no working or prophetic examples and the "state of the art at the time of filing was primitive and uncertain, leaving Ariad with an insufficient supply of prior art knowledge with which to fill the gaping holes in its disclosure"); *Capon v. Eshhar*, 418 F.3d 1349, 1357 (Fed. Cir. 2005) ("Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science."); *id.* at 1360 ("The predictability or unpredictability of the science is relevant to deciding how much experimental support is required to adequately describe the scope of an invention.").

To satisfy the enablement requirement, a person of ordinary skill in the art ("POSA") must be able to make and use the invention without undue experimentation. *In re Wands*, 858 F.2d 731, 736–37 (Fed. Cir. 1988). Factors for assessing whether any experimentation would be "undue" include: "(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or

unpredictability of the art, and (8) the breadth of the claims." *Id.* at 737.

B. Level Of Ordinary Skill In The Art

Dr. Bryan Cullen, Professor of Molecular Genetics and Microbiology at Duke University and the founding Director of the Duke University Center for Virology, who has over 30 years of research experience in gene expression and RNA-sequence mediated gene regulation in eukaryotic cells, explains that, as of the filing dates of P1 or P2, with respect to the subject matter of this Interference, a POSA would have had a life sciences Ph.D. (such as a Ph.D. in microbiology, genetics, virology, molecular biology, cell biology, etc.) and been actively involved for at least 1-2 years post-Ph.D. in research related to manipulation of gene expression in eukaryotes. Ex. 1403 (Cullen Decl.), ¶17.

CVC's level of skill should be rejected as too high and having no basis. CVC equips their POSA with the tools, skills, and knowledge of numerous disciplines in order to fill in the gaps in P1 and P2's disclosures. For example, CVC's expert, Dr. Phillip Zamore, opined that a POSA according to his definition could have had a Ph.D. in diverse subjects as "[c]hemistry, molecular biology, biophysics, computational biology, bioinformatics, immunology, biochemistry, [or] genetics." Ex. 1540 (Zamore Tr.), 57:6–9. And according to Dr. Cullen, it would be "very unusual for a POSA to have the wide set of molecular biological skills" in CVC's definition. Ex. 1403, ¶18; F_. *See, e.g.* Dr. Cullen's definition should be accepted and CVC's rejected.

IV. REASONS WHY CVC'S MOTION 1 SHOULD BE DENIED

A. CVC FAILS TO ALLEGE THAT P1 OR P2 ARE ENABLED IN ITS STATEMENT OF MATERIAL FACTS

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. F26. The Board has made clear that "[e]ach fact must be set out as a single, short, numbered

declaratory sentence that is capable of being admitted or denied" and that "[a] motion may be denied if the facts alleged in [the SOMF] are insufficient to state a claim for which relief may be granted." Standing Order at ¶121.5.2 Appendix: Statement of material facts. A constructive reduction to practice requires a described *and* enabled embodiment. Because CVC failed to allege enablement in its SOMF, the Board should deny CVC's request for benefit of P1 and P2.

B. CVC'S P1 AND P2 BENEFIT ARGUMENTS HAVE ALREADY BEEN CONSIDERED AND REJECTED.

In Motion 1 ("Mot."), CVC repeats its long-standing argument that as of May 25, 2012, applying the natural prokaryotic CRISPR/Cas9 system to achieve DNA cleavage in eukaryotes would have been predictable and merely a matter of applying ordinary skill in the art. *E.g.*, Mot. at 1:2–5. On substantially the same evidence, both the Board (twice) and Federal Circuit have found to the contrary. 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; F27. Now CVC's motion repeats the same arguments already found deficient to meet its burden, based on purportedly new arguments and evidence not considered by the Board. Mot. at 3:5–6:5. But the Board and the Federal Circuit have already heard and rejected CVC's purportedly "new arguments," like the use of direct injection techniques and ribonucleoprotein ("RNP") complexes, and the supposed similarity between zinc-finger nuclease ("ZFN") and transcription activator-like effector nuclease ("TALEN") technology and CRISPR/Cas9. *See, e.g.*, Ex. 2444 (CVC '115 Mot. for Benefit), 6, 12, 12, 15, 17, 21, 28, and claim charts. Moreover, CVC's "new evidence" consists of biased witness declarations describing 1) irrelevant events that occurred after the filing of CVC's P1 and 2) litigation-inspired retractions of contemporaneous statements by the inventors and their colleagues that they did not know based upon *in vitro* or prokaryotic data whether CRISPR-Cas9 would work in eukaryotic cells. CVC's "new" arguments and evidence

are unavailing. As the Board previously found, neither P1 nor P2 contains a sufficiently disclosed or enabled embodiment within Count 1. Nothing material has changed. The ordinarily skilled artisan of 2012 has not become clairvoyant and imbued with the knowledge of 2021. The Board should again deny CVC benefit to P1 and P2.

1. In The '048 Interference, The Board and the Federal Circuit Determined A POSA Would Have Had No Reasonable Expectation of Success Applying CRISPR/Cas9 To Eukaryotes.

The Board first found that a POSA would have had no reasonable expectation of success using CRISPR-Cas9 in eukaryotes in the '048 Interference. Ex. 2335, 2. 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; F28. The Board therefore terminated the interference. *Id.* at 2, 13–22, 28–45.

Interference-in-fact was the question in the '048 Interference, but in terminating the interference, the Board made several factual findings essential to the Board's judgment that are relevant to both written description and enablement, which were then affirmed by the Federal Circuit. That the ultimate question may have been slightly different is not decisive if the underlying fact was determined and essential to the judgment. *B & B Hardware, Inc. v. Hargis Indus., Inc.*, 575 U.S. 138, 157, 135 S. Ct. 1293, 1308, 191 L. Ed. 2d 222 (2015) (citing to the Restatement (Second) of Judgments § 27, Comment c, at 252–253 that the "'issue' must be understood broadly enough 'to prevent repetitious litigation of what is essentially the same dispute'"). And, "[i]t is well established that collateral estoppel, also known as issue preclusion, applies in the administrative context. *MaxLinear, Inc. v. CF CRESPE LLC*, 880 F.3d 1373, 1376

(Fed. Cir. 2018. *See also SynQor, Inc v. Vicor Corp.*, 988 F.3d 1341, 1346, 1353-55 (Fed. Cir. 2021) (applying issue preclusion to inter partes reexaminations).

The following findings of fact preclude CVC from establishing benefit to P1 and P2 in this Interference. 37 C.F.R. § 41.127(a)(1). *See also A.B. Dick Co. v. Burroughs Corp.*, 713 F.2d 700, 702 (Fed. Cir. 1983), *cert. denied*, 464 U.S. 1042 (1984). First, 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); F29. Both the Board and the court recognized the unpredictability of the field as of December 2012—seven months *after* the filing date of P1; F29. If a POSA had no reasonable expectation of success before successful eukaryotic experiments were done, a POSA could not "clearly conclude" that the applicants actually invented a eukaryotic CRISPR/Cas9 system without experiments in eukaryotic cells—which neither P1 nor P2 provide. *See Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997).

Second, 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; F30.

Third, 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 1292–94 (discussing the Board's review of evidence related to ZFNs or TALENs, Cre, riboswitches, ribozyme systems, and group II introns); F31. *See also* Ex. 2335, 39. It is undisputed that P1 and P2 do not provide any tailoring of particular conditions, nor any "unique conditions" or "specific instructions relevant to

CRISPR/Cas9," in eukaryotes. To the contrary, CVC continues to assert that a POSA would not have expected to see any unique conditions or specific instructions discussed because a POSA would not have expected any challenges. Mot. at 2:10-11, 13:5–8.

These findings are relevant to P1 and P2's disclosures because benefit to a prior provisional application requires that the "prior application itself must describe an invention . . . in sufficient detail that one skilled in the art can clearly conclude that the inventor invented the claimed invention as of the filing date sought." *Trading Techs. Int'l Inc. v. eSpeed Inc.*, 595 F.3d 1340, 1350 (Fed. Cir. 2010). If a POSA had no reasonable expectation of success that CRISPR/Cas9 could be adapted to eukaryotes, and a POSA would have required either experimental results in eukaryotes or at least discussion of the "unique conditions" or "specific instructions" required, then a disclosure providing only cell-free, *in vitro* experiments and no specific instructions to adapt CRISPR/Cas9 to eukaryotes does not render a claimed invention obvious for the purposes of satisfying the requirements of § 112. *Regents of the Univ. of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1567 (Fed. Cir. 1997). CVC cannot now re-open these factual findings.

The Federal Circuit also discussed the Board's consideration of a contemporaneous article by Dr. Dana Carroll, which doubted whether CRISPR/Cas9 could be adapted to eukaryotes, citing access to chromatin and degradation by nucleases as potential problems. *Broad*, 903 F.3d at 1292. The court 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." *Id.* at 1293; F32. The court 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. *Id.* at 1293; F33. Despite CVC's arguments that the Board misinterpreted those statements (the same argument CVC now makes), the court upheld the Board's decision. Dr. Doudna's contemporaneous statements of doubt and frustration were then, and remain, compelling and conclusive evidence that she and her co-inventors lacked possession of, or any reasonable likelihood of success in, CRISPR/Cas9 in eukaryotes.

2. **The Board Already Found P1 and P2 Lack Written Description.**

The Board then declared a second interference between CVC and Broad. Ex. 1105 ('115 Declaration of Interference), 1. 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; F34. Reviewing materially the same evidence and arguments CVC recycles today, and which is also presented in the '048 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; F35.

As in the '048 Interference, 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 ("[A]bsent results of a successful working example, the lack of discussion of PAM sequences, or sample target DNA sequences, the lack of special instructions or conditions necessary to accommodate the eukaryotic cellular environment, and the lack of a discussion of whether access to chromatin could hinder CRISPR-Cas activity would have indicated to those of ordinary skill in the art that the P1 applicants were not in possession of an embodiment of Count 1."); F36. Therefore, given the lack of a working example,

a POSA would not have believed the applicants possessed a CRISPR/Cas9 system adapted to function in a eukaryotic cell if P1 did not show that the applicants appreciated the potential challenges a natural prokaryotic CRISPR/Cas9 system might face when used in a eukaryotic cell. Ex. 1101, 81, 102–03, 105. These potential challenges include the differences in prokaryotic and eukaryotic cell structure and environment, potential RNA degradation, the failure to adapt prior prokaryotic systems for eukaryotes, *id.* at 87–88, and structural characteristics of CRISPR systems, like the requirements of non-natural targets, PAM, and chromatin access. *Id.* at 86, 92–93.

Using the same logic, the Board also found that P2 lacked written description. *Id.* at 105; F_. The disclosure of P2 adds only "supplemental disclosures about expressing Cas9 protein in *E. coli* and information about what was known in the art about PAM sequences." *Id.*; 37. But these limited additional disclosures did not "cure[] the deficiencies" of P1. *Id.* F.37.

Granting CVC the benefit of the filing date of either P1 or P2 in this Interference would therefore contradict the Board's factual findings in the '048 Interference, its factual findings and reasoning in the '115 Interference, and its decisions in both. Again, CVC's alternative of the count here and in the '115 Interference is the same: claim 156 of CVC application 15/981,807. *Compare* Ex. 1105, 12 *with* '127 Declaration of Interference at 5–6. And the alternatives of the counts in both the '115 Interference and this one require a CRISPR system capable of functioning in a eukaryotic cell. *See* Ex. 1101, 95 ("Count 1 requires a system that is capable of cleaving or editing target DNA molecules and modulating transcription of at least one gene or altering the expression of at least one gene product."). This is exactly what the Board already determined CVC did not adequately describe in P1 or P2. *Id.* at 104–105. As explained in detail below, CVC provides nothing today that the Board has not already considered, or that would cause the Board to

reconsider its previous decision. P1 and P2 lacked a described or enabled embodiment of the count when they were filed and when the Board reviewed them in 2020, and they still do not today.

3. CVC's Supposedly "New" Evidence and Arguments Are Just Recycled Evidence and Arguments.

Despite this extensive history, CVC now argues that it "presents new evidence and highlights the specific description" in P1 allegedly not addressed in the Board's '115 Decision on Motions. Mot. at 3:5–7, 5:15–6:5. The response is that 1) the arguments are not new, but are all merely recycled from the '048 and '115 Interferences, 2) the "new" evidence does not change the disclosures of P1 or P2, nor the contemporaneous doubts and concerns of a POSA in 2012, and 3) litigation-inspired attempts to explain contemporaneous statements ten years later are to no avail. CVC's "new" arguments reveal CVC's motion for what it really is: an effort to get a second—no, a third—bite of the apple.

a) CVC's Comparison to ZFN/TALENs Is Not New.

CVC argues that it presents a "new" argument that ZFN/TALENs and CRISPR/Cas9 were the most analogous art, which would have informed a POSA's expectations regarding use of CRISPR/Cas9 in eukaryotic cells.[1] Mot. 1 at 5:20–22, 9–15. The response is that this argument is not new; the Board has seen CVC's argument on this point twice already and found it lacking both times. Indeed, CVC argued that ZFN/TALENs are the most analogous genome editing art to CRISPR/Cas9, that the eukaryotic successes in ZFN/TALENs would translate to adapting

[1] CVC incorrectly claims that the Board found that neither party presented expert testimony on "the systems they used as comparisons to CRISPR-Cas systems." Mot. at 9 (citing Ex. 1101, 99). The response is that CVC twists the Board's words, which were directed only to the parties' evidence on *access to chromatin*. *See* Ex. 1101, 99.

CRISPR/Cas9 for eukaryotes, and that a POSA would not have considered evidence from other systems' failures relevant in both the '048 and '115 Interferences. *See* Ex. 1101, 97–99 (citing and CVC's expert opinion that regarding prior systems such as Cre, RecA, φC31 integrase); Ex. 2335, 39–41 (discussing and rejecting CVC's arguments and evidence that ZFN and TALEN systems are "relevant" and "analogous" and that other systems would not have informed a POSA's expectation of success). The Federal Circuit even cited the Board's '048 Decision on this point in affirming that a POSA would have lacked a reasonable expectation of success in eukaryotes. *Broad*, 903 F.3d at 1293 ("[The Board] found that the prior art TALEN and zinc finger nuclease ("ZFN") systems were not analogous to CRISPR-Cas9 . . . ."); F88.

None of CVC's evidence or arguments on this issue have materially changed since these decisions. CVC's comparison continues to rest largely on one commonality between ZFN/TALEN systems and CRISPR/Cas9—that they are nucleases guided by DNA binding domains. But highlighting this oversimplifies the reality that 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 with ZFN/TALENs would transfer to CRISPR/Cas9 in eukaryotes. Ex. 1403,¶¶ 109–119; F38. Even CVC's own witness Dr. Carroll did not consider them analogous during deposition. When 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; F39.

In particular, the DNA binding of ZFN and TALENs is very different than that of CRISPR/Cas9. 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; F40. The DNA binding domains of ZFN/TALENs also naturally act on eukaryotic DNA, while the CRISPR/Cas9 system is strictly prokaryotic. *Id.* at ¶¶112–115; F40.

To be sure, the nuclease domain in ZFN/TALENs is derived from Fok1 restriction enzyme domains, and therefore prokaryotic in origin. *Id.* at ¶114. But one of the key potential challenges in eukaryotes is access to tightly bound chromatin. *Id.* at ¶¶116–118; F_. In the eukaryotic chromatin context, it is not just the ability to cut DNA which is of concern. The system must find the target DNA molecule within a much larger eukaryotic genome, and then bind and act on the target DNA molecule within the tightly bound chromatin structure. *Id.* at ¶ 64, 72, 116, 123; F41.[2]

Unlike the prokaryotic CRISPR/Cas9 system, both ZFN and TALENs have binding domains evolved to function in eukaryotes. *Id.* at ¶ 111; F42. *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." *Id.* at ¶ 113; F42. 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)." *Id.* at ¶113; F_42. By focusing on the prokaryotic origin of the cleavage domains in these three systems, CVC ignores the eukaryotic adaptations of ZFN and TALENs which a POSA would have appreciated as setting them apart from the all-prokaryotic CRISPR/Cas9 system.

CVC also argues that "contemporaneous evidence confirms that the inventors viewed ZFNs and TALENs as the most analogous systems and as a model." Mot. at 9:1–2. The response is that what the inventors subjectively thought is not the question at hand; what a POSA objectively

[2] As seen below, despite Dr. Zamore and Dr. Doyon's declarations that chromatin was not a concern, Dr. Cullen disagrees, as did Dr. Mirkin in the '115 Interference and Dr. Carroll in September 2012. Ex. 2292, (Mirkin Decl.); Ex. 2339, (Carroll 2012).

would have understood from the specification is. *Ariad*, 598 F.3d at 1351 (explaining that written description "requires an objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art"). Similarly, whether others in the field recognized the need to improve on ZFN/TALEN systems, or even hoped that CRISPR/Cas9 would do so, says nothing about whether P1 or P2 provided the necessary disclosure to show possession of active CRISPR/Cas9 in eukaryotes. And, as noted elsewhere, the inventors were in fact expressing contemporaneous doubts and frustrations about any possibility of extending their prokaryotic discovery to eukaryotes.

Contrary to CVC's argument, comparison to Group II introns, ribozymes, and riboswitches is not inapposite. Mot. at 11:9–10. A POSA would have been aware that there had been numerous attempts to use prokaryotic-derived systems in eukaryotes and that success had been unpredictable, at best. Ex. 1403, ¶71, 73, 95 (discussing T7 RNA polymerase, ribozymes, riboswitches, and Cre recombinase). For example, 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; F43. Though bacterial group II introns work well in bacteria, their function in eukaryotic cells is inhibited by the chromatin in eukaryotic cells. *Id.;* F43. And, as the Board has previously found twice, examples of limited success in these systems were achieved only with the discovery of "specific tailoring of conditions" for that system. Ex. 2335, 39. *See also* Ex. 1101, 103–04.

b) CVC's Argument About Using RNPs Is Not New

CVC's second "new" argument is that P1 and P2 disclose direct injection of RNPs into fish and fly cells or transfection into human cells. Mot. at 5:17–19, 22:14–25:9. First, neither P1 nor P2 describe injecting or transfecting an RNP into any cell, let alone a eukaryotic one. Ex. 1403, ¶57, 58 Second, the Board is already well aware of CVC's contrived argument that P1 and P2

disclose methods of making RNAs, proteins, *and RNPs* and then introducing them into eukaryotic cells. Again, CVC argued as much throughout the '048 and '115 Interferences. *See* Ex. 2444, 12–13, 15, 17–18; Ex. 1107 ('048 CVC Mot. 4), 5, 7 (arguing that P1 Example 1 satisfies the count because it uses "a complex [that] was assembled from '[t]he DNA-targeting RNA' and the [Cas9] polypeptide"), 8, 20 (arguing that groups later used the methods of P1 Example 1, including "assembl[ing] the Cas9/RNA complexes as was done in Example 1); Appendix 2-2 (MF 11, 14, 17). The only thing new about this argument is the amount of space CVC now devotes to it in its briefing and declarations.

Implicitly conceding that their argument is not actually new, CVC claims that the real issue is that the Board did not specifically address whether microinjection of pre-assembled RNP complexes into an embryo "would trigger the same alleged concerns as embodiments relying on vector expression." Mot. at 5:17–19. As an initial matter, the Board's decision need not address every argument or piece of evidence offered by the parties. *See Synopsys, Inc. v. Mentor Graphics Corp.*, 814 F.3d 1309, 1322 (Fed. Cir. 2016), *overruled on other grounds by Aqua Prod., Inc. v. Matal*, 872 F.3d 1290 (Fed. Cir. 2017 (reasoning that "an agency [need not] . . . address every argument raised by a party or explain every possible reason supporting its conclusion"). CVC presented its RNP arguments, the Board ruled against CVC, and so the Board at least implicitly considered CVC's RNP.

And substantively, 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; F44. In fact, P1 and P2 discuss introducing the guide RNA and Cas9 in practically every format except an RNP. *See id.* at ¶58 (describing that P1 lists

introducing RNA and Cas9 into a cell as RNA, nucleotides encoding the guide RNA and/or Cas9, Cas9 as a polypeptide, and in vectors); Ex. 2001, ¶¶00167–00178. A POSA would not have chosen to include an RNP in the embodiment when P1 and P2 do not do so.

In fact, despite continually arguing that there were no potential challenges to eukaryotic use of CRISPR/Cas9, CVC now reverses course and argues that P1 and P2 disclose use of RNPs, which a POSA would have recognized overcome the (supposedly nonexistent) challenges to adapting CRISPR/Cas9 to eukaryotes. Mot. at 3:17–19, 17:18–24, 25:19–28:7. Assuming all of the potential challenges could have been overcome by injecting RNPs, which ToolGen disputes, P1 and P2 do not discuss any of the potential challenges, let alone how using an RNP would solve these challenges. Tellingly, CVC still can only identify five articles prior to P1 or P2 in which the researchers either injected or transfected an RNP into a eukaryotic cell. Ex. 1403, ¶59; s*ee* Ex. 2013 (Doyon Decl.), ¶ 70–71 (citing Ex. 2149; Ex. 2150; Ex. 2148; Ex. 2174; Ex. 2175). Four of these involve RNPs from viruses that naturally infect eukaryotic cells or were transferred from one eukaryote to another. *Id.* And 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.); F45. Not even Jinek 2013—the work of the P1 and P2 applicants themselves—used an RNP. Ex. 2033; F45.

c) Use Of RNPs Does Not Eliminate The Potential Challenges Of Chromatin Access, Degradation, And Toxicity

The use of RNPs is also not the panacea CVC claims it to be. A POSA would have known that, at the least, the potential challenges of chromatin access, degradation, and toxicity still remain, even with the use of RNPs. Ex. 1403, ¶ 64, 104-108.

One of the Board's justified concerns in the '115 Interference was whether a POSA would expect that CRISPR/Cas9 could access the target DNA molecule in the eukaryotic chromatin

structure. The use of RNPs has no impact on the potential concerns for chromatin access by CRISPR/Cas9 in eukaryotes. *Id.* at ¶122. The same uncertainty about whether CRISPR/Cas9 could access target DNA in chromatin exists regardless of how the system's components are delivered to the cell. In fact, a POSA would have expected an additional potential challenge with using 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; F46.

Degradation of the guide RNA would also still be a concern when using RNPs. A POSA in 2012 would have known that the guide RNA and Cas9 are not covalently associated. *Id.* at ¶¶ 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. *Id.* at ¶¶102; F47. But 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. *Id.* at ¶93, 106; F48 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. F48.

Therefore, in the unbound state, the guide RNA and Cas9 could still be subject to degradation or modification. RNA is a labile nucleic acid and subject to nuclease catalyzed degradation. Ex. 1403, ¶88–91. And even when it is complexed with Cas9, it is not immune from degradation. *Id.* at ¶88–89, 105; F49. CVC cites no evidence to the contrary, other than speculation in Deltcheva 2011 that Cas9 *may* protect the guide RNA. Ex, 1403, ¶105; Ex. 2013, ¶117; Ex. 1203 (Deltcheva 2011), 604. Further, 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. Similarly, 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. F50.

Finally, even with the use of an RNP, a POSA would also have been concerned that toxicity remained an issue for CRISPR/Cas9 use in eukaryotic cells. 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 also 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; F51.

4. No "New" Evidence From The '115 Interference Could Change The Inadequate Disclosures Of P1 and P2

In this motion, CVC seeks to capitalize not only on the benefit of another bite at the apple, having seen the Board's reasoning on P1 and P2, but also on evidence supposedly unearthed in the ongoing priority phase of the '115 Interference. Mot. at 5:23–6:1. The response is that regardless of whether it would be proper to consider such evidence—which it would not be—no such evidence "has since come to light." *Id.* CVC cites only post-P1 and P2 articles that have always been available. Mot. 25:10–18. More importantly, none of this post-filing evidence does, or even could, have any bearing on the question at hand: whether the four corners of P1 and P2 describe and enable an embodiment of the count as of May 25 and October 19, 2012, respectively.

5. CVC's "New" Fact Declarants Provide Only Irrelevant and Biased Information

In a futile attempt to undo scientific publications and discussions at the relevant time that contradict CVC's litigation-driven view, CVC submits the declarations of five biased fact witnesses—Dr. Sontheimer, Dr. Barrangou, Dr. Sternberg, Dr. Carroll and Dr. Doudna—all of whom purport to attest to the expectations of those in the field for CRISPR/Cas9 in eukaryotes. Mot. 6:2–5. The response it that all of these witnesses have significant professional or personal investments in CVC's success in these Interferences. *E.g.*, Ex. 1530, 40:2–41:2 (Barrangou Tr.) Their statements also provide no new information that would change the Board's reasoning that CVC is not entitled to the benefit of P1 or P2.

First, all five are offered by CVC as fact witnesses and not as experts testifying as to the ultimate conclusions of sufficient written description or enablement, or to the relevant underlying technical questions. *See* Fed. R. Evid. 702; *Sundance, Inc. v. DeMonte Fabricating Ltd.*, 550 F.3d 1356, 1362 (Fed. Cir. 2008) (holding that an abuse of discretion occurred where a witness was permitted to testify as an expert, in part because the witness "was never offered as a technical expert"). None claim to have reviewed or analyzed P1 or P2. *E.g.* 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; F52.

Second, the inquiry for written description and enablement focuses on the specifications of P1 and P2, and what they told the hypothetical POSA in 2012. *Ariad*, 598 F.3d at 1351. What five intensely interested fact witnesses now say about events almost ten years ago, which are unrelated to the disclosures of P1 and P2, is entirely irrelevant to the question of what P1 and P2 disclosed to a POSA in May and October of 2012, respectively. And a declaration cannot patch the holes in P1 and P2. *See Application of Smyth*, 189 F.2d 982, 990 (C.C.P.A. 1951). ("[N]or

would it be either proper or permissible to accept affidavits in order to establish facts which the specification itself should recite in order to conform to the mandatory provisions of the pertinent statutes."). Of note, none of these fact declarants can now change the message created by Dr. Doudna, or voiced by Dr. Carroll, that in 2012 those in the field doubted whether CRISPR/Cas9 could be successfully used in eukaryotes.

a) **Dr. Sontheimer and Dr. Barrangou**

The declarations Drs. Sontheimer and Barrangou submit are irrelevant to CVC's motion for benefit to P1 or P2, and much of their declarations is inadmissible hearsay. Both declarations focus on observations from the 2012 Annual CRISPR Conference and CVC's presentation there, which occurred after P1's filing date, and is notably not P1 or P2. Ex. 2021, (Barrangou Decl.), ¶11; Ex. 2019 (Sontheimer Decl.), ¶ 10–11.

Dr. Sontheimer's notes from the CVC presentation, which are inadmissible hearsay, achieve nothing more that confirming that even a month after P1, the CVC applicants had presented no experimental evidence that they had used a CRISPR/Cas9 system in a eukaryotic cell or that they had considered the kinds of adaptations a POSA would have expected to see for one claiming to have adapted a prokaryotic system for use in eukaryotic cells. *See* Ex. 2019 (Sontheimer Decl.), ¶12–18.

Dr. Barrangou's declaration attempts to walk back comments he made regarding the unproven nature of CRISPR-Cas9 in eukaryotes in a "News and Views" article he authored in September 2012. Ex. 2021, ¶¶19–20. He expressed in that article that CRISPR-Cas9 was only an "intriguing possibility" in eukaryotes that "will require testing [to determine] whether crRNA-Cas systems can efficiently cleave chromatin in DNA *in vivo* and be readily transferred into organisms of interest[.]" *Id.,* Ex. 2215 (Barrangou 2012), 838. The Board should give no credence to his belated attempts to rewrite the contemporaneous record nearly ten years later. *See United States*

v. U.S. Gypsum Co., 333 U.S. 364, 395–96 (1948) (noting that oral testimony deserves little weight when it contradicts contemporaneous written evidence).

b) **Dr. Sternberg**

Dr. Sternberg's declaration states that he witnessed a private lab notebook entry by Dr. Jinek in March 2012. Ex. 2221 (Sternberg Decl.). Like Drs. Sontheimer and Barrangou, Dr. Sternberg did not analyze P1 or P2. F52. His declaration is irrelevant to of the sufficiency of their written description or enablement.

c) **Dr. Carroll**

Dr. Carroll's declaration similarly attempts to rewrite history by explaining what he believes was the Board's misinterpretation of his September 2012 article. *See* Ex. 2348 (Carroll Decl.). In the article, Dr. Carroll expressed his skepticism about whether "the new CRISPR reagents" could create targeted double strand breaks. Ex. 2339, 1659. He 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." *Id.* And 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.*; F53. His skepticism was not limited to the "versatility" or "efficiency" of the CRISPR/Cas9 system, as he now claims. *See* Ex. 2348 (Carroll Decl.), ¶¶10– 12; s*ee also* Ex. 1251 (Carroll 2014). In the '115 Interference, the Board viewed Dr. Carroll's statements as contemporaneous evidence that those in the field doubted whether CRISPR/Cas9 could work in eukaryotes. Ex. 1101, 101. As a result, Dr. Carroll now effectively wants to rewrite the article. But 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 (conveniently) changed view he asserts today. F54. In fact, despite his bold and certain assertions now about what he actually meant when he wrote that article ten years ago, he admitted

at deposition that even things that happened "five or six years ago" are fuzzy, because it's "hard to remember that long ago." Ex. 1520, 15:8–11.

d) **Dr. Doudna**

Finally, Dr. Doudna's latest declaration is nothing more than an applicant's self-serving testimony offered to rewrite the existing narrative of the events of 2012—that Dr. Doudna and her colleagues doubted whether CRISPR-Cas9 could be adapted for use in eukaryotes. Ex. 2023 (Doudna Decl.). Dr. Doudna created and repeated this narrative herself in multiple interviews, stating (F55):

- "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 (emphasis added).

- 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 (emphasis added).

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

- Dr. Doudna and her colleagues "experienced '*many frustrations*' getting CRISPR to work in human cells." *Id.* at 3 (emphasis added).

- "These findings [detailing research in bacterial CRISPR/Cas systems, including Jinek 2012] 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).

In the ’115 Interference, the Board credited such statements with showing that “even the CVC inventors, who could be considered to have had more skill than the ordinary artisans, were not sure if the eukaryotic chromatin would allow for a functional CR[I]S[]PR-Cas9 system in a eukaryotic cell. The CVC inventors’ comments tend to indicate that they did not have possession of a functional CRISPR-Cas9 system in eukaryotic cells until such experiments had been done.” Ex. 1101, 102.

To the extent the Board considers Dr. Doudna’s current declaration as proof of what a POSA would have understood in 2012 with regard to CRISPR/Cas9 in eukaryotic cells, little weight should be given now to statements re-crafted with the benefit of ten years of hindsight, the course of litigation, and the tremendous self-interest in claiming such an important advancement as her own. Instead, the Board should rely on Dr. Doudna’s contemporaneous statements. *See* Ex. 2335, 14; *United States v. U.S. Gypsum Co.*, 333 U.S. 364, 395–96 (1948).

C. CVC AGAIN FAILS TO SHOW ENTITLEMENT TO BENEFIT OF P1 AND P2.

CVC is still not entitled to the benefit of the filing dates of P1 or P2, even if viewing the recycled arguments and evidence without the benefit of two prior Board decisions and a Federal Circuit appeal. Neither P1 nor P2 contain a described or enabled embodiment of Count 2. Even the three imagined embodiments (E1, E2, and E3) CVC claims appear in P1 and P2—are not disclosed, not complete with respect to the eukaryotic system of Count 1, nor adequately described or enabled under 35 U.S.C. § 112. For at least these reasons, the Board should again deny CVC the benefit of P1 and P2.

1. **P1 and P2 Do Not Describe An Embodiment of Count 1.**

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; F56.

The best CVC can offer in its effort to satisfy the written-description requirement is to imagine three embodiments purportedly disclosed in P1 and P2, Mot. at 15–16 (citations omitted), but those imagined embodiments only arise by piecing together, with the benefit of hindsight, disparate disclosures that are hundreds of paragraphs apart in the specification, and an entirely cell-free, *in vitro* example with prokaryotic target DNA. CVC's imagined embodiments are (1) a fish cell comprising a sgRNA CRISPR-Cas9 system made by microinjecting a pre-assembled RNP complex into a fish embryo ("E1"); (2) a human cell comprising a sgRNA CRISPR-Cas9 system made by transfecting human cells with expression vectors ("E2"); and (3) a fruit fly cell comprising sgRNA CRISPR-Cas9 made by microinjecting Cas9 mRNA and sgRNA into a fruit fly embryo ("E3")." Mot. at 16:5–10. The response is that these *post hoc*, imagined-after-the-fact embodiments are not disclosed in P1 or P2, and their creation in 2021 does not show possession of an embodiment of Count 1 in 2012.

To start, CVC's embodiments are incomplete because CVC uses cell-free, *in vitro* experiments to satisfy Elements [1], [4], [7] and [8]. *See* Mot. at Appendix 2 at xxii–xxiii; Appendix 3 at xxx–xxxii. Element [4] requires "a targeter-RNA that is capable of hybridizing with a target sequence in the target DNA molecule," Elements [1] and [7] requires a eukaryotic "target DNA molecule," and Element [8] requires a CRISPR-Cas9 system "capable of cleaving or editing the target [eukaryotic] DNA molecule or modulating transcription of at least one gene encoded by the target DNA molecule." *Id.* Yet the experimental results in P1 and P2 do not show that the system is capable of acting on a *eukaryotic* target molecule *in a eukaryotic cell* as required.

Ex. 1403, ¶¶39–44. Both 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. *Id.*; F57.

Further, a POSA would not have picked any of these disparate pieces out of the laundry lists of information that P1 and P2 provide regarding the types of cells to be used ("a cell from any organism," Ex. 1016 (CVC P1), ¶00165), the method of delivery (*e.g.* "viral infection, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate precipitation, direct micro injection, and the like," *Id.* at ¶0039, the items to be introduced into the cell (*e.g.* RNA, DNA, vectors, Cas9 as a polypeptide), and the specific conditions necessary for each kind of cell (*e.g.* "The choice of delivery method is generally dependent on the type of cell being transformed and the circumstances under which transformation is taking place[.]" *Id.* Instead, the embodiments were picked and pieced together from various unrelated aspects of the 2012 disclosures with the benefit of hindsight of how CRISPR/Cas9 ultimately succeeded in eukaryotes.

Perhaps the most egregious example is that CVC's expert, Dr. Doyon, claims that the imagined E2 and E3 embodiments include a single guide RNA which is 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. Ex. 1403, ¶48–50; Ex. 2001, Fig. 3. Yet P1 contains no such single guide RNA; Dr. Doyon literally pieces it together by juxtaposing portions of a single guide RNA next to a dual guide RNA. *See* Ex. 2013, ¶80. And there is no guidance in P1 or P2 for a POSA to do the same. Ex. 1403, ¶48–50. F58. Similarly, CVC argues that a POSA would have chosen to inject preformed sgRNA-Cas9 RNP complexes into fish (E1) cells because this would have obviated any of the potential

challenges of degradation, co-localization, toxicity, and chromatin.[3] Mot. at 25:20–28:5. But CVC cannot point to where P1 or P2 discusses microinjecting an RNP into any cell, much less the particular cells in CVC's imagined embodiments, or discusses why or how it should be done.

P1 and P2's failure to link the elements of Count 1 means that CVC's expert-crafted embodiments are not a constructive reduction to practice, which requires "a described and enabled anticipation under 35 U.S.C. § 102(g)(1)." Anticipation "requires the presence in a single prior art disclosure of all elements of a claimed invention arranged as in the claim." *Connell v. Sears, Roebuck & Co.*, 722 F.2d 1542, 1548 (Fed. Cir. 1983).

It is black-letter law that a reference is not anticipatory where it discloses the elements of the claimed invention, but not in a manner that links the disclosures together in the claimed order or otherwise "provide[s] specific guidance that would lead a [POSA] to an embodiment within" the count. *Otonomy, Inc. v. Auris Med., AG,* 743 F. App'x 430, 439 (Fed. Cir. 2018); *see also Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1370–71 (Fed. Cir. 2008) ("[I]t is not enough that the prior art reference discloses part of the claimed invention, which an ordinary artisan might supplement to make the whole, or that it includes multiple, distinct teachings that the artisan might somehow combine to achieve the claimed invention."); *Ecolochem, Inc. v. Southern California Edison Co.*, 227 F.3d 1361, 1367–69 (Fed. Cir. 2000 (reversing the district court's finding of anticipation by references where there was no suggestion of a link between the claimed elements, which the authors intentionally separated); *In re Arkley*, 455 F.2d 586, 587 (CCPA 1972) (holding that an anticipatory reference "must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without *any* need for picking, choosing, and combining various disclosures not directly related to each other by the teachings of the cited

[3] ToolGen disagrees, as does its expert. Ex. 2013, ¶¶ 104–108.

reference") (emphasis in original). Again, there is no such link or guidance in P1 or P2 that would lead a POSA to the embodiments imagined in hindsight by CVC. Ex. 1403, ¶ 45–51. As discussed in the previous section, P1 and P2 provide only unconnected disclosures separated by hundreds of paragraphs together with a cell-free, *in vitro* experiment with purified and highly concentrated components, which a POSA would not have pieced together in 2012.

2. CVC's Imagined Embodiments Still Do Not Demonstrate Possession of the Count

Putting aside the specific failings of E1, E2, and E3, the reality is that *no embodiment* CVC now imagines could demonstrate possession of the count because P1 and P2 omit any recognition of the potential challenges a POSA might face in adapting the native prokaryotic CRISPR/Cas9 for eukaryotes—the same kinds of challenges that caused Dr. Doudna to express pessimism and doubts in 2012. Faced only with *in vitro*, cell-free data in P1 and P2 and the knowledge of prior difficulties and failures adapting other prokaryotic systems to eukaryotes, a POSA in 2012 would have recognized P1 and P2 for what they are—mere speculation and hope that CRISPR/Cas9 could someday be adapted for eukaryotes. But that day was not when P1 or P2 were filed.

a) P1 and P2 Still Do Not Address Why a POSA Would Doubt a Successful Adaptation to Eukaryotic Cells.

In the summer and fall of 2012, when P1 and P2 were filed, the CRISPR/Cas9 field was still in a nascent stage. The basics of CRISPR/Cas9 were still being researched, as evidenced by the surprise CVC's fact witnesses expressed when they learned that tracrRNA was a component of mature guide RNA at the June 2012 CRISPR Conference. Ex. 2019, ¶¶13–15. No one had yet shown use of CRISPR/Cas9 systems in eukaryotic cells—not *in vitro*, and not *in vivo*. F59. A POSA would have been well aware of the nascence of the field, as well as past challenges in adapting prokaryotic systems for eukaryotes. As a result, a POSA would have needed to see relevant indicia that an applicant claiming to be in possession of a CRISPR/Cas system

successfully adapted for use in eukaryotes had more than a mere hope or plan for eukaryotic CRISPR/Cas9. *See Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341, 1348 (Fed. Cir. 2011). Specifically, absent a working example, a POSA at the time of filing of P1 and P2, would have expected to see that the applicants considered, or at least recognized, the potential challenges and uncertainties inherent in adapting a prokaryotic system for targeted genome editing in eukaryotes. *See* Ex. 1101, 91.

(1) Degradation

A POSA would have been aware that the cytoplasm and nuclei of eukaryotic cells contain exonucleases capable of rapidly degrading RNA. Ex. 1403, ¶88. Moreover, a POSA would have known 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. *Id.*; F60. In fact, 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. *Id.* at ¶89. A POSA in 2012 would not have known whether these enzymes would cleave the guide RNA, rendering the system nonfunctional. *Id.*; F61. Neither P1 nor P2 mentions the potential for degradation. Ex. 2001; Ex. 2002; F62.

(2) Cellular Differences

P1 and P2 do not address potential needs for differing ion concentrations of CRISPR/Cas9 in a eukaryotic cell. Ex. 2001; Ex. 2002; F63. Yet a POSA would have known that the intracellular ion concentration "can significantly impact activity of bacterial enzymes in eukaryotic cells." *Id.* at ¶80, 98; F64. Other cellular conditions that could differ in eukaryotic cells include temperature and pH. 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; F65.

(3) Cellular Toxicity

P1 and P2 also do not address the potential for cellular toxicity. Yet 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. This is particularly true given that 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)); "[O]ne of ordinary skill in the art at the time would have had no way of knowing whether the presence of guide RNA, Cas9 protein or both would, in fact, be toxic to eukaryotic cells, thus adding a further layer of uncertainty for the use of a Type II CRISPR/Cas system in eukaryotic cells. *Id.* at ¶97; F66. Neither P1 nor P2 mentions the potential for cellular toxicity. F67.

(4) Chromatin Access

A POSA in 2012 would have recognized numerous potential concerns with chromatin access by CRISPR/Cas9 in eukaryotic cells. First, 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; F68. 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; F69. *See also* Ex. 1570, 162:3–10.

Second, 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; F70. 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. F71. CVC's superficial comparison between prokaryotic and eukaryotic DNA glosses over the details. The fact that bacterial DNA contains proteins says nothing about how similar those proteins are to histones, or any other specific comparison between the two. [Cullen Dec?] In fact, at a keynote address two months *after* P1, Dr. Doudna was asked in the question and answer session afterwards 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. F72. Moreover, 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; F73. 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. F74.

Finally, 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; F75. Eukaryotic cells contain Ribonuclease H (RNase H), a nuclease that effectively recognizes and cleaves the RNA strand of RNA:DNA hybrids. *Id.*; F76. In fact, Dr. Dana Carroll voiced this exact concern in his September 2012 article which questioned whether the system could function in eukaryotes. Ex. 2239, 1660 (questioning whether the "required DNA-RNA hybrid can be stabilized" and whether it "may be a substrate for RNA hydrolysis by ribonuclease H and/or FEN1, both of which function in the removal of RNA primers during DNA replication."); F77. Neither P1 nor P2 mentions the issue of chromatin access. Ex. 2001; Ex. 2002; F78.

(5) PAM

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); F79. 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. Ex. 1403; F80. Yet there is absolutely no mention of a PAM sequence in P1, which a POSA would undoubtedly have expected a discussion about for the applicants to demonstrate possession. Ex. 1403, ¶41, 68; F81.

(6) Past Failures

A POSA in 2012 would have known of a number of failures in attempting to transfer non-eukaryotic systems to eukaryotic cells. Again, 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, 72–87. 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. ¶87; F82. Therefore, one of ordinary skill in the art would have expected that, if CRISPR/Cas9 could be made to work in eukaryotic cells, it would require its own unique set of conditions. *Id.*

b) Cell-Free Experiments Are Not Predictive Of Success In Eukaryotic Cells

CVC argues that "[d]emonstration of targeted DNA cleavage by sgRNA CRISPR-Cas9 outside of its natural prokaryotic environment shows that the system is functional independent of its environment, and thus provided a POSA with reason to expect the microinjected CRISPR-Cas9 system to be capable of cleaving target DNA in a fish cell." Mot. 1 at 24:19–22. The response is

that a POSA would not agree that experiments using only bacteria or *in vitro*, test tube conditions. conditions with purified components at high concentrations not present in cells were predictive of success in a eukaryotic cell. Ex. 1403, ¶100–102. In fact, in his own patent application, CVC's expert 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; F83. And 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; Ex. 2013, ¶ F84. CVC's cell-free *in vitro* results are indicative of no more than cell-free *in vitro* success; a POSA would not have made the leap to success in eukaryotic cells from these results.

For the same reasons discussed above, P2 does not disclose a described embodiment of the count. Therefore, P1 and P2 do not disclose a described embodiment of the Count.

3. **P1 And P2 Do Not Disclose An Enabled Embodiment.**

P1 and P2 also do not contain an enabled embodiment within the scope of Count 1. CVC's enablement arguments rely on the idea that a POSA, armed with the disclosures in P1 or P2, could use only ordinary skill and knowledge to practice the invention of CRISPR/Cas9 in eukaryotic cells. But the disclosure itself must enable the novel aspects of the invention, and P1 and P2 fail to do so. Moreover, the *Wands* factors support that a POSA could not have made or used a CRISPR-Cas9 system in eukaryotes without undue experimentation. And, CVC's citation to articles published after the filing dates of P1 and P2 cannot retroactively provide enablement.

a) P1 and P2 Fail To Meet The Requirements of 35 U.S.C. § 112.

P1 and P2 fail to meet the requirements of 35 U.S.C. § 112 under *Rasmusson v. SmithKline Beecham Corp.*, 413 F.3d 1318, 1323 (Fed. Cir. 2005). Where a POSA would question an

application's statements regarding the invention's utility and the application provides no evidence showing the invention has the claimed effect, an application does not demonstrate utility and is therefore not enabled. *Id.* at 1323. Here, the utility of either alternative of the Count is targeted gene editing or modulation by CRISPR-Cas9 in eukaryotic cells. As discussed in detail throughout this brief, a POSA at the time of filing of either P1 or P2 would have had ample reason to question the applicants' claim that the CRISPR/Cas9 system (purportedly) described in P1 or P2 would be effective in achieving such a result. CVC cannot dispute that P1 and P2 provide no data beyond *in vitro* testing of purified components in a cell-free environment. F_. And again, cell-free, *in vitro* experiments of this type would be inadequate to demonstrate or predict whether a CRISPR/Cas9 system as described in P1 or P2 could act on a target DNA molecule once introduced into the complexities of the eukaryotic cell. P1 and P2 therefore fail to meet the requirements of § 112 under *Rasmusson*, and are therefore not enabled.

b) Purported Skill In The Art Is Not A Substitute For An Enabling Disclosure

Given P1 and P2's lack of written description and CVC's repeated reliance a POSA's knowledge to chart a course through its multiple generic disclosures, P1 and P2 do not contain an enabled embodiment. CVC cannot simply rely on laundry lists of procedures, cells types and other "knowledge of a POSA" to enable its alleged invention. Mot. 2:1–2, 17–23. "Although 'a specification need not disclose what is well known in the art,' that rule is 'not a substitute for a basic enabling disclosure.'" *Enzo Life Scis., Inc. v. Roche Molecular Sys., Inc.*, 928 F.3d 1340, 1348 (Fed. Cir. 2019), *cert. denied*, 140 S. Ct. 2634, 206 L. Ed. 2d 513 (2020) (internal citations omitted). Here, the specification must disclose how to adapt CRISPR-Cas9 to eukaryotes, the alleged novelty, and CVC cannot do so by simply averring to what was known in the art. *See Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1366 (Fed. Cir. 1997) ("It is the specification,

not the knowledge of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement."). This is particularly true where the Board and the Federal Circuit have held that a POSA would expect a working example or at least discussion of the unique conditions required for CRISPR/Cas9 in eukaryotes. Ex. 1101, 86, 102–103

For example, CVC argues that that it would have been obvious for a POSA to choose microinjection of RNPs because a POSA 1) would have known of the advantages of microinjection, 2) would have known that would have known that potential concerns to adaptation in eukaryotes would be obviated, 3) would have known to purify Cas9 to mitigate toxicity, and 4) could have adjusted the amount of RNP injected into the cell to minimize potential toxicity. Mot. 25:19–28:7. *See also* Mot. 29:7–9 (arguing a POSA would have known how to adjust "the protocols and methods to enhance expression, improve efficiency, or assign appropriate cellular conditions" but providing no citation to P1 or P2); 17 (arguing that a POSA would know that microinjection into the nucleus was a preferred technique); 24:16–25:5 (arguing a POSA would believe cell-free *in vitro* results predict results in cells); 28:15-19 (arguing a POSA would know how to select a human cell line, make sgRNA-CRISPR system, and apply "well-known techniques" to get human cell embodiment). Yet none of this information specific to enabling a POSA to achieve a functioning CRISPR/Cas9 system in eukaryotic cells is disclosed in P1 or P2.

c) Wands Factors

An analysis of the Wands factors shows that, given the information disclosed in P1 and P2, a POSA would have had to conduct undue experimentation to adapt a CRISPR/Cas9 system for use in eukaryotic cells. Ex. 1403, ¶144. Given the Board and the Federal Circuit's past decisions that a POSA would have had no reasonable expectation that a CRISPR-Cas9 system would succeed in a eukaryotic environment, Wands factor 7 (predictability or unpredictability of the art) weighs heavily in favor of lack of enablement and informs the six other factors. *See Broad*, 903

F.3d at 1294 (affirming the "Board's finding that the success in applying similar prokaryotic systems in eukaryotes was unpredictable").

CVC admits that the applicants themselves "set out to apply sgRNA CRISPR-Cas9 in a eukaryotic cell, prior to the filing date of P1" using a target that "had been successfully targeted using a ZFN[.]" Mot. at 2:16–19, 16–17; Ex. 1402, ¶145. That the applicants could not even claim to have achieved successful target cleavage until approximately seven months later is evidence that Wands factor 1 (quantity of experimentation necessary) also weighs heavily in favor of the need for undue experimentation. *Novo Nordisk Pharms., Inc. v. Bio-Tech. Gen. Corp.*, 424 F.3d 1347, 1362 (Fed. Cir. 2005) ("[A]n inventor's failed attempts to practice an invention are relevant evidence of non-enablement.").

The remaining factors also support that a POSA would have to conduct undue experimentation. 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; F86. The omission of this information informs Wands Factor 2 (amount of guidance presented). *Id.* Also absent from P1 and P2 is a working example (Wands factor 3); P1 and P2 contain only *in vitro* testing of purified components in a cell-free environment. *Id.*; Ex. 2001, ¶248–52; F87. As to Wands Factors 4, 5, and 6, the nature of the invention, the relative skill of those in the art, and the predictability or unpredictability of the art all weigh in favor of a lack of enablement where, as here, the field CRISPR-Cas9 field was still developing—so much so that CVC claims that the three components that make up the CRISPR-Cas9 complex for DNA cleavage were not revealed until June 2012. See Ex. 2019, ¶12. Such a nascent field, especially in the unpredictable biological arts, requires relatively more disclosure. D. Chisum, Patents § 7.03[4][d][i] (2005). Finally, a POSA would expect more disclosure in a claim to CRISPR/Cas9 in all

eukaryotic cells (Wands factor 8 "breadth of the claims"), given that neither P1 nor P2 provides any data of any successful activity in even one type of eukaryotic cell.

d) CVC's Post-Filing Evidence Is Irrelevant

CVC cites a number of articles published after the filing dates of P1 and P2 to show that its imagined embodiments were enabled. *E.g.*, Mot. 25:10–18. The response is that these articles are at best irrelevant, and worse (for CVC), demonstrate the absence of enablement in the 2012 disclosures. They are irrelevant because an applicant cannot use post-filing evidence to show that the art was predictable and the invention was enabled. *See In re Wright*, 999 F.2d 1557, 1562–63 (Fed. Cir. 1993). The articles also prove the opposite of what CVC claims. All were published in well-regarded journals, Ex. 1403, ¶158, which demonstrates that if the experiments "set forth in these articles, especially those successes in eukaryotes, were mere routine experimentation based on the written descriptions in the patent specifications, it is unlikely that they would have been published in such prestigious journals." *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1376 (Fed. Cir. 1999).

Finally, CVC also claims that these post-filing papers use "materially the same methods taught in P1." Mot. 13–15, 30. CVC's claim here is reductive; these papers disclose experimental variables that were not disclosed in P1 and P2, such as the tracr length of the guide RNA. Based both on their timing and their content, CVC's post-filing papers do nothing to alleviate P1 and P2's lack of an enabling disclosure. Ex. 1403, ¶158

V. CONCLUSION

CVC is not entitled to benefit of P1 or P2. The Board should deny Junior Party's motion.

Respectfully submitted,

Dated: July 15, 2021

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APPENDIX 1: LIST OF EXHIBITS CITED

Ex. No.	Description
1540	Deposition Transcript of Phillip Zamore, Ph.D., The Regents of the University of California v. ToolGen, Inc., Interference No. 106,127, June 21, 2021.
1403	July 15, 2021 Declaration of Bryan R. Cullen, 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.
2339	Carroll, A CRISPR Approach to Gene Targeting, *Molec. Therapy*, 20, 1658 (2012).
2013	Declaration of Yannick Doyon, Ph.D.
1560	Deposition Transcript of Yannick Doyon, Ph.D., The Regents of the University of California v. ToolGen, Inc., Interference No. 106, 127, July 2, 2021.
2348	Declaration of Dana Carroll, Ph.D.
1101	Paper 877, Decision on Motions 37 C.F.R. § 41.125(a), Interference 106,115, September 10, 2020.
2335	Paper 893, Decision on Motions 37 C.F.R. § 41.125(a), Interference 106,048, February 15, 2017.
2444	Paper 212, CVC Substantive Motion 1 (for accorded benefit), Interference 106,115, October 14, 2019.
1105	Paper 1, Declaration – 37 C.F.R. § 41.203(b), Interference 106,115, June 24, 2019.
1106	Paper 1, Declaration – 37 C.F.R. § 41.203(b), Interference 106,048, January 11, 2016.
2292	January 9, 2019 Declaration of Chad Mirkin, Ph.D., Interference 106, 115
1107	Paper 57, UC *et al.* Substantive Motion 4, Interference 106,048, May 23, 2016.
1203	Deltcheva *et al.*, CRISPR RNA Maturation by *Trans*-Encoded Small RNA and Host Factor RNase III, *Nature*, 471, 602–607 (2011), with Supplementary Information.
2215	Barrangou, RNA-mediated programmable DNA cleavage, *Nature Biotechology*, 30(9), 836-838 (2012).
1251	Carroll, Genome Engineering with Targetable Nucleases, *Annu. Rev. Biochem.*, 83 409–439 (2014).

Ex. No.	Description
2279	The CRISPR Revolution, CATALYST MAGAZINE, College of Chemistry, University of California, Berkeley (July 9, 2014), http://catalyst.berkeley.edu/slideshow/the-crispr-revolution/.
2032	Pandika, Rising Stars: Jennifer Doudna, CRISPR Code Killer, OZY (Jan. 7, 2014), http://ozy.com/rising-stars/jennifer-doudna-crispr-codekiller/.
2033	Jinek, *et al.*, RNA-programmed genome editing in human cells, *eLife*, 2:e00471, 1–9 (2013).
1016	U.S. Provisional Application No. 61/652,086, filed May 25, 2012.
2139	Foley, *et al.*, Targeted Mutagenesis in Zebrafish Using Customized Zinc Finger Nucleases, *Nat. Protoc*., 4(12), 1855, 1–40 (2009).
2300	U.S. Patent Application Publication No. 2009/0111119 A1.
2019	Declaration of Erik Sontheimer, Ph.D.
2125	Deveau *et al.*, Phage response to CRISPR-encoded resistance in *Streptococcus thermophilus*, *J. Bacteriology*, 190, 1390-1400 (2008).
1210	Mojica *et al.*, Short Motif Sequences Determine the Targets of the Prokaryotic CRISPR Defence System, *Microbiology*, 155, 733–740 (2009), with Supplementary Material.
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.
2149	Thornton, *et al.*, Microinjection of Vesicular Stomatitis Virus Ribonucleoprotein Into Animal Cells Yields Infectious Virus, *Biochem. & Biophys. Res. Communs*., 116(3), 1160–1167 (1983).
2150	Mastroianni, *et al*., Group II Intron-Based Gene Targeting Reactions in Eukaryotes," *PloS ONE*, 3(9):e3121, 1–15 (2008).
2148	Eickbush, *et al*., Integration of *Bombyx mori* R2 Sequences into the 28S Ribosomal RNA Genes of *Drosophila melanogaster*,*Mol. Cell. Biol*., 20(1), 213–223 (2000).
2174	Luo, *et al*., The Polyadenylation Signal of Influenza Virus RNA Involves a Stretch of Uridines Followed by the RNA Duplex of the Panhandle Structure, *J. Virol*., 65(6), 2861–2867 (1991).
2175	Lopez, *et al*., The L Protein of Rift Valley Fever Virus Can Rescue Viral Ribonucleoproteins and Transcribe Synthetic Genome-Like RNA Molecules, *J. Virol*., 69(7), 3972–3979 (1995).

Ex. No.	Description
1593	Doudna Interview, "I have to be true to who I am as a scientist." The Guardian
1576	Doudna, The CRISPR-Cas 9 Genome Engineering Revolution – Lecture at 2016 Breakthrough Prize Symposium.
1294	Schmidt *et al.*, Sensing of viral nucleic acids by RIG-I: From translocation to translation, *Eur. J. Cell. Biol.*, 91, 78–85 (2012).
1275	Karpala *et al.*, Immune responses to dsRNA: Implications for gene silencing technologies, *Immunology and Cell Biology*, 83, 211–216 (2005).
2001	Prov. Appl. No. 61/652,086, filed May 25, 2012.
2002	Prov. Appl. No. 61/716,256, filed October 19, 2012.
2021	Declaration of Rodolphe Barrangou, Ph.D.
2023	Declaration of Jennifer Doudna, Ph.D.
1530	Deposition Transcript of Samuel H. Sternberg, Ph.D., The Regents of the University of California v. ToolGen, Inc., Interference No. 106,127, June 16, 2021.
2221	Declaration of Samuel Sternberg, Ph.D.
1510	Deposition Transcript of Samuel H. Sternberg, Ph.D., The Regents of the University of California v. ToolGen, Inc., Interference No. 106,127, June 16, 2021.
1500	Deposition Transcript of Erik Sontheimer, Ph.D., The Regents of the University of California v. ToolGen, Inc., Interference No. 106,127, June 15, 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.

APPENDIX 2: STATEMENT OF MATERIAL FACTS

Junior Party's Alleged Facts 1-25

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.

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.

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.

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.

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.

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].

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

Response: Denied.

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

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.

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.

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.

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.

Response: Admitted.

Senior Party ToolGen's Additional Material Facts 26-88

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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

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.

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.

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.

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.

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.*

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.

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).

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.

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

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.

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.

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.

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.*

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

63. P1 and P2 do not address potential needs for differing ion concentrations of CRISPR/Cas9 in a eukaryotic cell. Ex. 2001; Ex. 2002.

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.

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.

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)).

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

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.

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.

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.

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.

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.

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.

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.

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.

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

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.

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

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).

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.*

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

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.

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.

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.

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

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.

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

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.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **TOOLGEN OPPOSITION 1** was filed via the Interference Web Portal on June 15, 2021 by 5:00 PM ET, and thereby served on the attorneys of record for the Junior Party pursuant to ¶ 105.3 of the Standing Order. Pursuant to agreement of the parties, service copies are being sent by email by 11:00 pm ET to counsel for Junior Party as follows:

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