Filed on behalf of Junior Party Paper No. ___

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

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

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

v.

TOOLGEN, INC.
Senior Party

(Application 14/685,510).

Patent Interference No. 106,127 (DK)

CVC REPLY IN SUPPORT OF RESPONSIVE MOTION 2 (for accorded benefit)

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I. INTRODUCTION

Contingent upon the Board granting ToolGen's Substantive Motion 2 and denying CVC the benefit of P3, CVC should be accorded the benefit date of its '859 application: March 15, 2013. "The plain and unambiguous meaning of section 120 is that any application fulfilling the requirements therein 'shall have the same effect' as if filed on the date of the application upon which it claims priority. The courts have repeatedly recognized this principle." *Transco Prods. Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 556 (Fed. Cir. 1994). CVC's Responsive Motion 2 showed that, as a matter of law, CVC is entitled to at least a March 15, 2013 benefit date because all of CVC's involved applications share the same specification with, and properly claim priority to, the '859 application. *See* Paper 660, 2:10-23 and Appx. 6; MFs 4-6, 13-15. ToolGen's opposition completely and conveniently ignores CVC's entitlement to benefit under Section 120. On this basis alone, the PTAB should grant CVC's Responsive Motion 2.

On the merits, the disclosures in the '859 application compel a finding that it provides a constructive reduction to practice of at least one embodiment of Count 1. ToolGen's opposition is built on a false proposition that the previous PTAB and Federal Circuit decisions from the *CVC v. Broad* interferences (Nos. 106,048 and 106,115) were based on "substantially the same evidence" as here. Paper 717, 2:17-3:2. But the evidence supporting this motion involves additional disclosures, including at least the '859 application's Examples 4, 5, and 7, which were not at issue in the '048 or '115 interferences, as well as different expert testimony and a later state of the art, March 15, 2013—by which time numerous peer-reviewed publications had reported using sgRNA CRISPR-Cas9 in eukaryotic cells.

ToolGen does not dispute that the '859 application's text and figures *describe* at least one embodiment of the count. Its expert, Turchi, conceded this on cross examination. And as ToolGen concedes, P3's Example 2 "purports to show (by way of two figures, numbers 38B and

36E) cleavage of DNA by CRISPR-Cas9 in eukaryotic cells." Paper 717, 3:10-11. ToolGen also concedes that the '859 application's Example 4 "demonstrates that CRISPRi modulates GFP," and Example 5 reports "20-fold activation of the GAL4 UAS gene based on GFP fluorescence." *Id.*, 8:8-9, 9:3-4. And, ToolGen's opposition does not challenge enablement at all.

These facts and concessions compel granting CVC's motion. ToolGen nevertheless argues that the PTAB should disregard the literal disclosure in CVC's applications because, in ToolGen's view, the working examples imply but "are not proof" that the sgRNA CRISPR-Cas9 systems actually worked—i.e., an actual reduction to practice (ARTP). No authority supports ToolGen's argument that priority benefit requires an ARTP. To the contrary, "[a]ll that is necessary for a party to be entitled to benefit of an earlier filed application for priority purposes is compliance with 35 U.S.C. § 112 with respect to at least one embodiment within the scope of the count." *Falkner v. Inglis*, 448 F.3d 1357, 1362 (Fed. Cir. 2006); *see also*, 37 C.F.R. § 41.201.

Further, even if an ARTP were required for priority benefit—which it is not—the '859 application contains several. Example 2 describes experiments in which a sgRNA CRISPR-Cas9 system was used to cleave a target DNA molecule in human cells. Example 4 describes experiments in which a sgRNA CRISPR-dCas9 system was used to repress transcription of the EGFP target gene in human cells, and Example 5 describes experiments in which a sgRNA CRISPR-chimeric-Cas9 system was used to induce or repress transcription of the EGFP target gene in human cells. ToolGen raises speculative criticisms of selected data from Examples 4 and 5, but those criticisms lack merit because ToolGen mischaracterized the results, applied an incorrect and onerous burden of proof, and ignored other data and experiments.

II. ARGUMENT

A. CVC is entitled to the benefit of the '859 application as a matter of law.

ToolGen never disputes the mandate of 35 U.S.C. § 120 that "any application fulfilling

the requirements therein '*shall have the same effect*' as if filed on the date of the application upon which it claims priority." *Transco*, 38 F.3d at 556 (quoting 35 U.S.C. § 120 (emphasis added)); MF59. 37 C.F.R. § 41.201 provides that "[e]arliest constructive reduction to practice means the first constructive reduction to practice that has been continuously disclosed through a chain of patent applications including in the involved application or patent. For the chain to be continuous, each subsequent application must comply with the requirements of 35 U.S.C. §119–121, 365, or 386." 37 C.F.R. § 41.201. CVC's '859 application meets these statutory and regulatory requirements because the '859 application shares a common specification with CVC's involved applications as a string of continuation applications and thus provides the same disclosure as the involved applications. *See* Paper 660, 2:10-23 and Appx. 6.

Indeed, CVC's involved Application No. 15/981,807 is a grandchild continuation of the '859 application and contains claim 156, which is one alternative of Count 1. ToolGen does not contest written description or enablement of the '807 application's claim 156. MF60. Nor does ToolGen dispute that each CVC application in the chain of continuation applications satisfies 35 U.S.C. § 120. Paper 660, 2:10-23 and Appx. 6. In fact, ToolGen admitted that the '859 application shares the same specification with CVC's involved applications and is part of a chain of continuing applications that include CVC's involved applications. *See* MFs 1-6, 13-15.

These undisputed facts, ToolGen's failure to contest description support in CVC's involved '807 application (MF60), and ToolGen's failure to dispute the law compel the PTAB to grant CVC's Responsive Motion. *See Dreyfus v. Lilienfeld*, 49 F.2d 1062, 1064 (C.C.P.A. 1931); *Renz v. Jacob*, 326 F.2d 792, 799 (C.C.P.A. 1964). Even though the PTAB need not evaluate the '859 application's disclosures, as discussed below, it too clearly provides a CRTP of at least one embodiment of Count 1.

B. CVC is entitled to the benefit of the '859 application because it describes and enables at least one embodiment of Count 1.

1. The human cell embodiments described in the '859 application's working examples meet all the elements of Count 1 and are enabled

CVC showed in its Responsive Motion that the mammalian cell embodiments described in Examples 4, 5, and 7 meet all of elements [1]-[8] of Count 1, arranged as in the count. Paper 660, 5:4-7:12; Ex. 2466, ¶¶23-25. While ToolGen questions selected experimental *results* in Examples 4 and 5, its expert Turchi admitted that the human cell embodiment in Example 4 meets all the elements of Count 1. Ex. 2537, 43:9-60:15, 61:8-16, 67:17-68:1. In particular, Turchi admitted that Example 4 describes a eukaryotic cell (element **[1]**), transfected with a Cas9 expression vector (element **[2]**) and a sgRNA expression vector (elements **[3]-[6]**), and further includes a figure depicting the sgRNA and Cas9 forming a "protein-RNA complex" (element **[7]**), and a figure depicting the sgRNA CRISPR-Cas9 system invoking a "mechanism … to abrogate transcription" (element **[8]**). *Id*. Thus, there is no question that the '859 application describes at least one embodiment of Count 1. And ToolGen does not dispute that the '859 application enables embodiments of Count 1. *See generally*, Paper 717; Ex. 1412; *see also,* Ex. 2537, 41:20-42:4; MF62. Putting aside any potential disputes over the '859 application's experimental data, it describes and enables an embodiment of Count 1. Paper 660, 5:4-7:12.

2. ToolGen's opposition fails to undermine the '859 application's CRTP.

ToolGen's opposition is based on an erroneous legal theory, a disingenuous view of the art, and mischaracterizations of the experimental data in the '859 application. The PTAB should reject these attempts to rewrite the law and mischaracterize the evidence.

a. ToolGen uses the wrong legal standard to assess CRTP.

ToolGen argues that Examples 4, 5, and 7 of the '859 application must provide "*definitive evidence* that CRISPR-Cas9 modulates transcription of at least one gene encoded by

the target DNA molecule in eukaryotic cells" to qualify as a CRTP of Count 1. *See e.g.*, Paper 717, 4:16-17 (emphasis added); *see also,* Ex. 1412, ¶¶66 and 69 (Turchi requiring "experimental proof"). ToolGen and its expert not only apply a highly improper "definitive evidence" standard, but they conflate CRTP with ARTP.

ToolGen's position contradicts the long-accepted standard for CRTP, that "[t]here is *no requirement* … that a party relying on a constructive reduction to practice to establish priority of invention must *show a specific working example* to support the compound claimed." *Lawson v. Bruce*, 222 F.2d 273, 278 (C.C.P.A. 1955) (emphasis added); *see also, Falkner*, 448 F.3d at 1366 ("the written description standard may be met … even where actual reduction to practice of an invention is absent."); *In re Borkowski*, 422 F.2d 904, 908 (C.C.P.A. 1970) ("a specification need not contain a working example" for enablement). Under the correct legal standard, the '859 application describes and enables embodiments of Count 1, irrespective of whether the application provides "definitive evidence" that the sgRNA CRISPR-Cas9 system worked. Paper 660, 3:1-7:12; Ex. 2537, 41:20-42:4, 43:9-60:15, 61:8-16, 67:17-68:1. In any event, the '859 application provides proof of working sgRNA CRISPR-Cas9 systems, as discussed below.

b. Turchi's opinions are based on an erroneous view of the art and should be given no weight.

Turchi's opinions are generally premised on an erroneous view of the state of the art as of March 15, 2013. He alleges in his first declaration that "[i]t was uncertain in 2013" whether a CRISPR-Cas9 system could perform targeted DNA cleavage in a eukaryotic cell, and that a POSA would view any data pertaining to the use of CRISPR-Cas9 in eukaryotes with "professional skepticism." Ex. 1410, ¶¶41-42. At deposition, Turchi testified that he saw no "distinction" in the state of the art between October 2012 and March 15, 2013, and, as a consequence, he "considered a POSA from the position of the 2012 date in light of the data in

the non-provisional listed in 2013." Ex. 2537, 41:4-19. This error is highly material for this motion because by March 15, 2013—when the '859 application was filed—the art was replete with reports of researchers using sgRNA CRISPR-Cas9 in eukaryotic cells. The art included Mali 2013 (Ex. 2512), Cong 2013 (Ex. 2030), Jinek 2013 (Ex. 2033), Cho 2013 (Ex. 2154), Hwang 2013 (Ex. 2028), and Qi 2013 (Ex. 2254), each of which published before March 15, 2013, and each reporting implementing a sgRNA CRISPR-Cas9 system in eukaryotic cells to modify a target DNA using routine laboratory methods. Yet Turchi ignored these references. Ex. 2537, 27:21-28:11; Ex. 1412, Appx. 1; MF61. Thus, the true state of the art by March 15, 2013 contradicts Turchi's alleged "uncertainty" in the art and "skeptical" POSA. Ex. 1410, ¶¶41-42.

In addition, Turchi's opinions in the record regarding the '859 application should be afforded no weight under 37 C.F.R. § 41.158(a). ToolGen's arguments rest on Turchi's opinions in paragraphs 51, 54-57, 59-65, and 67-69 of Exhibit 1412, but Turchi failed to cite *any* underlying support for those opinions outside of the '859 application (Ex. 2005). *See* Paper 717, 4:7-9:22; Ex. 1412, ¶¶51, 54-57, 59-65, 67-69.

c. ToolGen's criticisms of the '859 application lack merit.

None of ToolGen's criticisms of the '859 application has merit. First, ToolGen improperly demands that the examples show more than Count 1 requires. *See Kroekel v. Shah*, 558 F.2d 29, 32 (C.C.P.A. 1977) ("limitations not clearly included in a count will not ordinarily be read into it.") For example, at page 7, lines 12-14 of the opposition, it is argued that "Example 4 does not demonstrate that dCas9-sgRNA can repress *endogenous* genes originating from within the system, as opposed to the EGFP gene that was artificially introduced into the cell." Paper 717, 7:12-14 (emphasis in original). The response is that Count 1 does not require targeting *endogenous* genes. MF63. Count 1 recites that the CRISPR-Cas9 system be capable of cleaving or editing or modulating transcription of *a target DNA molecule*, nothing more. MF63. Examples

4 and 5 each disclose a sgRNA CRISPR-Cas9 system capable of modulating transcription of a target DNA, satisfying all the elements of Count 1. Ex. 2466, ¶¶23-26; Ex. 2005, ¶¶[00646]-[00648], [00655]-[00657], Figs. 45, 52, 55, 56; Ex. 2537, 43:9-60:15, 61:8-16, 67:17-68:1.

Second, disclosures in the '859 application contradict ToolGen's accusations that it is missing necessary controls and experiments. Turchi alleges that the CRISPR-dCas9 experiment in Example 4 lacked "critical controls" and that the EGFP gene repression disclosed in Figure 45A *could have* been the result of "an indirect effect, such as a global repression of protein expression." Ex. 1412, ¶¶56, 60; Paper 717, 6:7-7:11. However, Turchi deliberately ignores the experiment in Figure 45B, which demonstrates that there was no global repression of protein expression. Ex. 2005, ¶¶[00616], [00646]-[00647], Fig. 45B. The '859 application discloses that the sgRNA expression vector "co-expresses mCherry from a CMV promoter" as an internal control, and Figure 45B shows that target gene expression (EGFP) was repressed in samples eNT2 and eNT5, while the internal control mCherry expression remained *unaffected*. *Id*. Turchi ignored this data in his declaration, but admitted at deposition that the eNT2 and eNT5 samples showed decreases in EGFP fluorescence compared to mCherry. Ex. 1412, ¶¶51-62; Ex. 2537, 72:5-74:4; MF64. Thus, the '859 application suffices even under ToolGen's ARTP standard.

Turchi also speculates that Example 4 does not "demonstrate that the dCas9 expression plasmid *functions to express dCas9 protein* in the HEK293 cells." Ex. 1412, ¶59; Paper 717, 5:17-19 (emphasis added). However, a POSA would have viewed protein expression data as superfluous because the experiments in Example 4 included negative controls (no dCas9 or no sgRNA), demonstrating that "the [EGFP] repression was dependent on both *the dCas9 protein* and sgRNA." Ex. 2005, ¶[00646] (emphasis added). Further, by March 15, 2013, it was routine to transfect CRISPR-Cas9 expression vectors in eukaryotic cells without directly measuring

Cas9 protein expression. Mali 2013 disclosed analyzing GFP reporter fluorescence in CRISPR-Cas9-modified HEK293T cells without directly measuring Cas9 protein expression, and Hwang 2013 disclosed analyzing CRISPR-Cas9-generated zebrafish mutants without directly measuring Cas9 protein expression. Ex. 2512, Fig. 1; Ex. 2028, Table 1. Even *Turchi himself* published experiments analyzing results from CRISPR-Cas9-mediated DNA modification in eukaryotic cells without directly measuring Cas9 protein expression. *See e.g.*, Ex. 2526, Fig. 8, Table 2.

Turchi also argues that Example 4 does not identify the genome integration site of the EGFP reporter gene used in Figure 45A, alleging that this somehow weakens the data in the experiment. Ex. 1412, ¶57; Paper 717, 7:17-23. But a POSA would have considered the integration site of the EGFP reporter gene to be irrelevant to whether the gene was repressed. And, in fact, ToolGen admits that Example 4 "demonstrates that CRISPRi can reduce GFP fluorescence." Paper 717, 8:5-6. The art shows that researchers routinely generated stable cell lines (such as the EGFP-reporter cell line in Figure 45) without identifying the transgene's integration site. For example, the *Molecular Cloning* laboratory manual – a reference Turchi praised as the "go-to" laboratory resource and a "very popular manual" – provides a detailed protocol on generating stably transfected cell lines and nowhere mentions the need for identifying the transgene integration site. *See e.g.*, Ex. 1243, 73; Ex. 2475, 157:19-160:3; *see also*, Ausubel's *Short Protocols in Molecular Biology*, Ex. 2124, 37, 63-67. Additionally, Turchi completely ignores the experiment described in paragraph [00648] and depicted in Figure 52, in which a sgRNA-dCas9 system was used to repress the EGFP target gene *on a plasmid reporter* instead of an integrated site in the genome. Ex. 2005, ¶[00648], Fig. 52; MF65. Ex. 2537, 75:18-77:7. Turchi's "integration site" argument is misleading and simply has no merits.

Finally, Turchi's criticisms of the experimental results in Example 4 are contrary to how

a skilled artisan and the scientific community viewed the experiments. The same experiments, methods, and data in Example 4—including the human cell experiments—were published by Qi et al. in the peer-reviewed journal *Cell* on February 28, 2013. Ex. 2516, Fig. 7, Fig. S7; Ex. 2537, 88:10-18. Turchi, however, did not consider the Qi 2013 publication when forming the opinions in his declaration. Ex. 1412, Appx. 1; Ex. 2537, 87:2-5, 88:10-18; MF61.

d. ToolGen's challenges to CVC's P1-P3 applications fail.

At page 3, lines 3 to 4 of the opposition,[1] it is argued that the PTAB's decision on motions in the '115 interference, which did not address the '859 application's Examples 4, 5, and 7, forecloses CVC's reliance on its P1 or P2 applications for CRTP. This argument is plainly erroneous in view of the '859 application's disclosures beyond P1 and P2. Additionally, as briefed fully in CVC's Motion 1, the '115 decision was based on a different and significantly less-developed evidentiary record, while the more fully-developed record here shows that P1 and P2 unquestionably provide a CRTP of Count 1. *See* Paper 368, 9:1-15:10.

At page 3, line 5, to page 4, line 6 of the opposition, ToolGen parrots its arguments from its Motion 2, criticizing Example 2 in CVC's P3 application, which is carried forward in the '859 application. Paper 717, 3:5-4:6. The response is that ToolGen's challenges to Example 2 fail here, just as they do in ToolGen's Motion 2. *See* Paper 722; Ex. 2476, ¶¶17-70. ToolGen again focuses only on the experimental data in Example 2, ignoring the disclosures in the rest of the application that describe and enable embodiments of Count 1. *See* Paper 717, 3:5-4:6. Nor do ToolGen's technical criticisms of Example 2 rebut its CRTP. As Dr. Doyon previously

[1] At page 2 of the opposition, ToolGen wrongly accuses CVC of incorporating arguments by reference. CVC did no such thing. CVC's Responsive Motion explained that the disclosures CVC's P1, P2, and P3 applications are the subject of separate briefing. *See* Paper 660, 3:8-4:4.

explained, a POSA would have understood that the CRISPR-Cas9-mediated DNA cleavage products disclosed in Figures 36E and 38B of P3 were the correct size, and the artisan would not have had any concerns with the cell lysis protocol in Example 2. Ex. 2476, ¶¶34-69. Indeed, the same data and protocol were published in the Jinek 2013 paper that has been cited by numerous other researchers—including ToolGen's own inventor Kim and expert Cullen—as showing target DNA cleavage in eukaryotic cells. Ex. 2476, ¶¶64-68; Ex. 2484, 479; Ex. 2494, 402.

III. CONCLUSION

CVC should be accorded benefit of the '859 application: March 15, 2013.

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APPENDIX 1: LIST OF EXHIBITS

Exhibit No.	Description
1018	U.S. Provisional Application No. 61/757,640, filed January 28, 2013.
1243	Molecular Cloning: A Laboratory Manual (3rd ed. Cold Spring Harbor Laboratory Press 2001), Chapter 16 [Substitute]
1410	May 20, 2021 Declaration of John J. Turchi, Ph.D.
1412	July 15, 2021 Declaration of John J. Turchi, Ph.D.
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.
2001	Prov. Appl. No. 61/652,086, filed May 25, 2012
2002	Prov. Appl. No. 61/716,256, filed October 19, 2012
2003	Prov. Appl. No. 61/757,640, filed January 28, 2013
2005	U.S. Appl. No. 13/842,859, filed March 15, 2013
2006	U.S. Appl. No. 14/685,504, filed April 13, 2015
2007	U.S. Appl. No. 15/138,604, filed April 16, 2016
2028	Hwang, W.Y., *et al*., "Efficient genome editing in zebrafish using a CRISPR-Cas system," *Nature Biotechnology* 31(3):227-229, Supplementary Information (2013)
2030	Cong, L. *et al*., "Multiplex Genome Engineering Using CRISPR/Cas Systems," *Science* 339(6121):819-823 (2013) with Supplemental Material.
2033	Jinek, M., *et al*., "RNA-programmed genome editing in human cells," *eLife* 2:e00471, 1-9 (2013)
2034	U.S. Appl. No. 15/947,680
2035	U.S. Appl. No. 15/981,807
2036	U.S. Appl. No. 16/136,168
2037	U.S. Appl. No. 16/136,175
2038	U.S. Appl. No. 15/947,700
2039	U.S. Appl. No. 15/947,718
2040	U.S. Appl. No. 15/981,808
2041	U.S. Appl. No. 15/981,809
2042	U.S. Appl. No. 16/136,159
2043	U.S. Appl. No. 16/276,361
2044	U.S. Appl. No. 16/276,365
2045	U.S. Appl. No. 16/276,368
2046	U.S. Appl. No. 16/276,374
2047	U.S. Appl. No. 16/136,165
2124	Ausubel, F.M., *et al*., Eds., Chapter 9: Introduction of DNA into Mammalian Cells, *Short Protocols in Molecular Biology*, pp. 9-1 – 9-57, Third Edition, John Wiley & Sons, Inc., United States (1995)
2154	Cho, S.W., *et al*., "Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease," *Nature Biotechnol.* 31(3):230-232, Supplementary Information (2013)

Exhibit No.	Description
2254	Qi, S., *et al*., "Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression," *Cell* 152:1173-1183 (2013)
2400	Decision on Motions 37 C.F.R. § 41.125(a), *The Regents of the University of California v. The Broad Institute, Inc.*, Patent Interference No. 106,115, Paper 877, (September 10, 2020)
2463	Redline Comparison of Provisional Application No. 61/652,086 and Non-Provisional Application No. 13/842,859
2464	Redline Comparison of Provisional Application No. 61/716,256 and Non-Provisional Application No. 13/842,859
2465	Redline Comparison of Provisional Application No. 61/757,640 and Non-Provisional Application No. 13/842,859
2466	Second Declaration of Yannick Doyon, Ph.D.
2475	Deposition Transcript of John Turchi, Ph.D., Patent Interference No. 106,127 (June 29, 2021)
2476	Third Declaration of Yannick Doyon, Ph.D.
2484	Koo, T., *et al*., "Measuring and Reducing Off-Target Activities of Programmable Nucleases Including CRISPR-Cas9," *Mol. Cells* 38(6): 475-481 (2015)
2494	Kennedy, E.M. and Cullen, B.R., "Gene Editing: A New Tool for Viral Disease," *Annu. Rev. Med*. 68:401–11 (2017)
2512	Mali, P., *et al*., "RNA-Guided Human Genome Engineering via Cas9," *Science* 339(6121): 823-826 (2013), with Supplemental Materials
2516	Qi, S., *et al*., "Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression," *Cell* 152:1173-1183 (2013)
2526	Gavande, N.S., *et al*., "Discovery and development of novel DNA-PK inhibitors by targeting the unique Ku–DNA interaction," *Nucleic Acids Research* 48(20): 11536–11550 (2020)
2532b	Second Supplemental Declaration of Yannick Doyon, Ph.D.
2537	Deposition Transcript of John Turchi, Ph.D., with errata, Patent Interference No. 106,127 (August 11, 2021)

APPENDIX 2: STATEMENT OF MATERIAL FACTS

CVC's Facts 1-20

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

ToolGen's Response: Admitted.

2. U.S. Provisional Patent Application No. 61/716,256 ("P2") was filed on October 19, 2012, and lists Martin Jinek, Jennifer Doudna, Emmanuelle Charpentier, Krzysztof Chylinski, and James Harrison Doudna Cate as co-inventors. Ex. 2002, p. 277.

ToolGen's Response: Admitted.

3. U.S. Provisional Patent Application No. 61/757,640 ("P3") was filed on January 28, 2013, and lists Martin Jinek, Jennifer Doudna, Emmanuelle Charpentier, Krzysztof Chylinski, and James Harrison Doudna Cate as co-inventors. Ex. 2003, p. 377.

ToolGen's Response: Admitted.

4. CVC's '859 application was filed within 12 months of the filing dates of P1, P2, and P3, and makes specific reference to P1, P2, and P3 applications. Ex. 2005, p. 5.

ToolGen's Response: Admitted.

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

ToolGen's Response: Admitted.

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

ToolGen's Response: Admitted.

7. The PTAB accorded CVC the benefit filing date of P3: January 28, 2013. Paper 1, at 10.

ToolGen's Response: Admitted.

8. In Interference 106,115, the PTAB determined that "P3 is a constructive reduction to practice of Count 1." Ex. 2400, 106:11-14.

ToolGen's Response: Admitted, but only to the extent the PTAB did not consider ToolGen's argument that P3 does not constructively reduce to practice an embodiment within Count 1, otherwise denied.

9. The common specification discloses the same "chimera A" single-molecule DNA-targeting RNA taught in P1 and P2 (including the 3' extended chimera A), the same *S. pyogenes* Cas9 protein, and the same fish, human, and fruit fly target cells taught in P1-P3. Ex. 2463-2465; Exs. 2005-2007, ¶¶[0035], [0046], [00213], [00215], [00275], [00515], Figs. 3, 14; Ex. 2466, ¶21.

ToolGen's Response: Denied.

10. The common specification describes the same microinjection and transfection techniques taught in P1-P3 for introducing the CRISPR-Cas9 system into eukaryotic cells. Ex. 2463-2465; Exs. 2005-2007, ¶¶[00283], [00285]; Ex. 2466, ¶21.

ToolGen's Response: Denied.

11. The common specification discloses the results from the same in vitro working example in P1-P3 which describes making and using a CRISPR-Cas9 RNP complex comprising a recombinant *S. pyogenes* Cas9 protein and a chimera A molecule to cleave a target DNA sequence. Ex. 2463-2465; Exs. 2005-2007, ¶¶[00555]-[00559], Fig. 14; Ex. 2466, ¶21.

ToolGen's Response: Denied.

12. The common specification discloses the same working example from P3 which describes transfecting human cells with nucleic acids encoding a *S. pyogenes* Cas9 and a chimera A RNA

targeting the human CLTA locus to achieve cleavage of a target DNA molecule. Ex. 2465; Exs. 2005-2007, ¶¶[00562]-[00577], Figs. 29, 31; Ex. 2466, ¶22.

ToolGen's Response: Denied.

13. The '859 application shares a common specification with CVC's involved applications. Ex. 2005; Exs. 2034-2047.

ToolGen's Response: Admitted.

14. The '504 application shares a common specification with CVC's involved applications. Ex. 2006; Exs. 2034-2047.

ToolGen's Response: Admitted.

15. The '604 application shares a common specification with CVC's involved applications. Ex. 2007; Exs. 2034-2047.

ToolGen's Response: Admitted.

16. The common specification provides multiple different examples of using CRISPR-Cas9 systems in eukaryotic cells. Exs. 2005-2007, ¶¶[00562]-[00577], [00606]-[00657], [00661]; Ex. 2466, ¶¶22-25.

ToolGen's Response: Denied.

17. Example 2 in the common specification describes using a Type II CRISPR-Cas system to cleave a target DNA in eukaryotic cells. Exs. 2005-2007, ¶¶[00562]-[00577], Figs. 29, 31; Ex. 2466, ¶22.

ToolGen's Response: Denied.

18. Example 4 in the common specification describes using a Type II CRISPR-Cas system named CRISPRi to repress transcription in eukaryotic cells. Exs. 2005-2007, ¶¶[00606]-[00654]; Ex. 2466, ¶23.

ToolGen's Response: Denied.

19. Example 5 in the common specification describes using a Type II CRISPR-Cas system comprising a Cas9 fusion protein to modulate transcription in eukaryotic cells. Exs. 2005-2007, ¶¶[00655]-[00657]; Ex. 2466, ¶24.

ToolGen's Response: Denied.

20. Example 7 in the common specification describes using a Type II CRISPR-Cas system to generate transgenic eukaryotic organisms. Exs. 2005-2007, ¶[00661]; Ex. 2466, ¶25.

ToolGen's Response: Denied.

ToolGen's Additional Facts 21-58

21. Experimental controls are critical to eliminate alternate explanations of the observed results and to infer causality in an experimental system. Ex. 1412, ¶54, ¶56.

CVC's Response: Admitted as a general statement; denied to the extent ToolGen implies that the working examples in the common specification lack experimental controls.

22. Dr. Phillip Zamore testified on cross-examination that a failed experiment is one where the experiment cannot be interpreted due to lack of appropriate controls. Ex. 1540, 47:10-14.

CVC's Response: Admitted.

23. Dr. Phillip Zamore testified on cross-examination that improper control design is a primary reason grant proposals and peer-reviewed articles are rejected. Ex. 1540, 45:2-46:6.

CVC's Response: Admitted that Dr. Zamore testified that "improper control design" is one of several reasons a grant proposal or peer-review article may be rejected.

24. Example 4 provides no evidence demonstrating that dCas9 is expressed in the cell. Ex. 1412, ¶24.

CVC's Response: Denied.

25. Example 4 provides no evidence that dCas9 is localized to the nucleus. Ex. 1412, ¶25.

CVC's Response: Denied.

26. Example 4 provides no evidence that dCas9 forms a complex with sgRNA, let alone that dCas9 directly binds to the target gene. Ex. 1412, ¶26.

CVC's Response: Denied.

27. Example 4 does not provide any direct measurement of gene transcription in eukaryotic cells. Ex. 1412, ¶62.

CVC's Response: Admitted.

28. The Cas9 used in the CRISPRi system is catalytically inactive ("dCas9") such that it is incapable of cleaving DNA. Ex. 1412, ¶51.

CVC's Response: Admitted.

29. To measure gene transcription, RNA must be directly measured using any number of well-known methods (e.g., RNA-seq, northern blot, and RT-PCR). Ex. 1412, ¶62.

CVC's Response: Denied.

30. In Example 4, only the presence of GFP protein was analyzed in eukaryotic cells using flow cytometry to detect fluorescence. Ex. 1412, ¶62.

CVC's Response: Denied.

31. The moderate repression reported in Figure 45A could instead be the result of indirect repression events, driven by DNA methylating events and/or endogenous repressors activated by stresses on the cell, such as the transfection and expression of dCas9 and sgRNA. Ex. 1412, ¶60.

CVC's Response: Denied.

32. In the absence of RNA-guided targeting of dCas9 to a target sequence, repression of transcription could indirectly be due to inactivation of a transcriptional activator or epigenetic modifications that affect the chromatin structure. Ex. 1412, ¶60.

CVC's Response: Denied.

33. Example 4 does not show specific repression of the EGFP gene specifically in the HEK293 cells. Ex. 1412, ¶ 60.

CVC's Response: Denied.

34. RNA-Seq is a common technique used to reveal the presence and quantity of RNA in a biological sample Ex. 1412, ¶62.

CVC's Response: Admitted.

35. Applicants did not perform RNA-seq in their experiments with HEK293 cells, but did perform RNA-seq in E. coli cells.

CVC's Response: MF35 lacks any citation to evidence, so denied. *See* SO ¶122.4.2.1.

36. Example 4 does not demonstrate that dCas9-sgRNA can repress endogenous genes originating from within the system, as opposed to the EGFP gene that was artificially introduced into the cell at an undisclosed site. Ex. 1412, ¶61.

CVC's Response: Denied.

37. Applicants tested endogenous genes, as the critical control, in their E. coli system (Figure 13 44), but failed to do the same control in HEK293 cells. Ex. 1412, ¶¶55, 61.

CVC's Response: Admitted that Example 4 describes and enables testing genes in bacteria; otherwise denied.

38. A POSA would have tested a number of genes—not just one—to show that repression was not just affecting GFP expression and fluorescence, including an endogenous gene. Ex. 1412, ¶61.

CVC's Response: Denied.

39. The location of gene integration could have transcriptional effects on gene expression that are independent of the dCas9-sgRNA effects.

CVC's Response: MF39 lacks any citation to evidence, so denied. *See* SO ¶122.4.2.1.

40. A POSA would have tested repression of endogenous genes to rule out the possibility that GFP repression was simply a function of issues stemming from integration into the genome. Ex. 1412, ¶64.

CVC's Response: Denied.

41. Example 5 provides no evidence that a complex is formed and then binds the target gene. Ex. 1412, ¶¶64–65.

CVC's Response: Denied.

42. Example 5 lacks proper controls to demonstrate specificity in modulating the target genes. Ex. 1412, ¶¶64–65.

CVC's Response: Denied.

43. Example 5 provides no studies on endogenous genes to dispel concerns arising from gene integration. Ex. 1412, ¶¶64–65.

CVC's Response: Denied.

44. Example 5 does not contain measurements to detect gene transcription instead of GFP flouroscence.[*sic*] Ex. 1412 at ¶¶64–65.

CVC's Response: Admitted that Example 5 describes detecting GFP fluorescence; otherwise denied.

45. Example 5 provides no explanation for why the reported 20-fold activation of the GAL4 UAS gene based on GFP fluorescence is so low. Ex. 1412, ¶¶64–65.

CVC's Response: Admitted that Example 5 demonstrates a 20-fold activation of GAL4 UAS based on GFP fluorescence; otherwise denied.

46. The specification provides no evidence that the reported increase or decrease in fluorescence is due to RNA-mediated targeting of dCas9 to the GFP target sequence. Ex. 1412, ¶¶64–65.

CVC's Response: Denied.

47. Example 7, which is strictly prophetic, discloses a plan to generate transgenic mice expressing Cas9. Ex. 1412, ¶67.

CVC's Response: Admitted that Example 7 describes generating a transgenic mouse; otherwise denied.

48. Example 7 provides no guidance that would lead a POSA to determine how to make such mice. Ex. 1412, ¶¶67–68.

CVC's Response: Denied.

49. The generation of knock-in and knock-out mice from Cas9 ES cells necessarily requires DNA cleavage in order to remove DNA or introduce DNA into the mouse genome. Ex. 1412, ¶69.

CVC's Response: Admitted.

50. Example 7 provides no experimental results reported and no reported studies looking at the properties of any gene of choice (or any expression product of choice, or any genomic locus of choice) in a transgenic mouse. Ex. 1412, ¶69.

CVC's Response: Admitted that Example 7 does not describe experimental results; otherwise denied.

51. Figures 38B and 36E depict the only experimental results in P3 purporting to show cleavage of target eukaryotic DNA by a CRISPR-Cas9 system in a eukaryotic cell. Ex. 1018, ¶¶00417, 00422.

CVC's Response: Admitted that P3's Figure 36E and 38B show sgRNA-Cas9-mediated cleavage of target DNA in eukaryotic cells, otherwise denied.

52. Figure 36E would not have shown show [*sic*] successful CRISPR-Cas9 cleavage at the target site to a POSA. Ex. 1410, ¶¶82-85, 87.

CVC's Response: Denied.

53. Figure 38B would not have shown successful CRISPR-Cas9 cleavage at the target site to a POSA. Ex. 1410, ¶¶ 86-92.

CVC's Response: Denied.

54. Figure 38B shows alleged cleavage bands at positions where there should be no bands. Ex. 1410, ¶¶86-92;

CVC's Response: Denied.

55. Figure 38B shows, in some instances, no bands where there should be bands. Ex. 1410, 7 ¶88.

CVC's Response: Denied.

56. Figure 36E contains many unexplained bands; in the presence of sgRNA and Cel-1 nuclease, the alleged cleavage product (~173 bp) is accompanied by two unexplained bands (~297/312 and ~252 bp). Ex. 1018, ¶00418; Ex. 1410, ¶83.

CVC's Response: Denied.

57. Figure 36E contains unexplained larger bands (~477 bp) in the control lanes lacking nuclease, which disappear in the presence of nuclease. Ex. 1410, ¶83, 85.

CVC's Response: Denied.

58. Any cleavage shown in Figures 38B and 36E could have occurred outside the cells, in the lysate, instead of within the cell as required by Count 1. Ex. 1410, ¶¶66, 72, 101-103.

CVC's Response: Denied.

CVC's Additional Facts 59-65

59. ToolGen's opposition does not address CVC's entitlement to the benefit date of the '859 application under 35 U.S.C. § 120. *See* Paper 660, 2:10-23 and Appx. 6; Paper 717.

60. ToolGen did not contest written description or enablement of claim 156 in CVC's Application No. 15/981,807 or any other involved claims in that application. Paper 717.

61. Turchi did not consider any of Mali 2013, Cong 2013, Jinek 2013, Cho 2013, Hwang 2013, or Qi 2013 when forming the opinions in his declaration. Ex. 1412, Appx. 1; Ex. 2537, 27:21-28:11, 87:2-5, 88:10-18; Ex. 2512; Ex. 2030; Ex. 2033; Ex. 2154; Ex. 2028; Ex. 2254.

62. ToolGen did not contest enablement in the '859 application. Paper 717; Ex. 1412.

63. Count 1 does not require that the target DNA molecule be an endogenous gene. Paper 1, 5-6.

64. Turchi's declaration does not discuss the mCherry control experiment in Example 4 depicted in Figure 45B of the '859 application. Ex. 1412, ¶¶51-62; Ex. 2005, ¶[00647], Fig. 45B.

65. Turchi's declaration does not discuss the experiment in Example 4 depicted in Figure 52 of the '859 application. Ex. 1412, ¶¶51-62; Ex. 2005, ¶[00648], Fig. 52.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **CVC REPLY IN SUPPORT OF CVC RESPONSIVE MOTION 2 (for accorded benefit)** was filed via the Interference Web Portal by 8:00 PM Eastern Time on August 27, 2021, pursuant to an agreement between the parties, and thereby served on the attorney of record for the Senior Party pursuant to ¶ 105.3 of the Standing Order. Pursuant to the agreement between the parties, the foregoing was also served via email by 11:00 PM Eastern Time on counsel for the Senior Party at:

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