Interference No. 106,127

Filed on behalf of Senior Party ToolGen, Inc. 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

Application No. 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 TO CVC RESPONSIVE MOTION 2
(opposing CVC's request for benefit)

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OTHER AUTHORITIES

Senior Party, ToolGen, Inc. ("ToolGen"), requests that the Board deny CVC's Responsive Motion 2 ("Mot.") seeking to be accorded benefit of the filing date of U.S. Appl. No. 13/842,859, filed March 15, 2015 (the '859 application") (Ex. 2005), or alternatively U.S. Application Nos. 14/685,504, filed April 7, 2015 ("the '504 application") (Ex. 2006), or 15/138,604, filed April 26, 2016 ("the '604 application") (Ex. 2007) (collectively, "CVC's Non-Provisional Applications"). CVC's Non-Provisional Applications, in particular Examples 4, 5, and 7, do not establish a constructive reduction to practice of an embodiment of the Count.

I. DESCRIPTION OF APPENDICES

Appendix A is a List of Exhibits Cited. Appendix B is the Statement of Material Facts (F_).

II. CVC's NON-PROVISIONAL APPLICATIONS DO NOT CONSTRUCTIVELY REDUCE TO PRACTICE AN EMBODIMENT WITHIN COUNT 1

For its Motion to be successful, CVC has the burden to show a constructive reduction to practice of an embodiment within the count. *Hunt v. Treppschuh*, 523 F.2d 1386, 1389 (CCPA 1975). A "*[c]onstructive reduction to practice* means a described and enabled anticipation under 35 U.S.C. § 102(g)(1), in a patent application of the subject matter of a count." 37 C.F.R. § 41.201 (emphasis original).

To satisfy Section 112's written description requirement, the application must "reasonably convey[] to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date." *Ariad Pharm., Inc. v. Eli Lilly & Co*., 598 F.3d 1336, 1351 (Fed. Cir. 2010). "When determining whether a specification contains adequate written description, one must make an 'objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art.'" *Boston Sci. Corp. v. Johnson & Johnson*, 647 F.3d 1353, 1366 (Fed. Cir. 2011). A prior application "must describe an invention, and do so 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." *Lockwood v. Am. Airlines, Inc*., 107 F.3d 1565, 1572 (Fed. Cir. 1997).

For the reasons set forth below, CVC should not be accorded benefit of the filing date of the CVC Non-Provisional Applications because the new examples relied upon by CVC do not compel the POSA to conclude that an embodiment of Count 1 is constructively reduced to practice, alone or in view of earlier embodiments E1-E3.

A. Neither P1, P2, Nor P3 Constructively Reduce To Practice An Embodiment Within Count 1

In its Motion, CVC argues that the "constructive reduction to practice in each of P1, P2, and P3 applications has been continuously disclosed and carried forward through a continuing chain of patent applications including CVC's involved applications (referred to in this discussion as 'common specification')." Mot. at 3. In doing so, CVC refers back to its Motion 1 for support "that P1 and P2 each describes and enables three specific embodiments the [*sic*] meet all the elements [1]-[8] of the count: E1 (fish cell), E2 (human cell), and E3 (fruit fly cell)."[1] *Id*. at 3-4. But CVC's Motion 1 merely repeats its long-standing argument that as of 2012, CRISPR-Cas9 DNA cleavage would have been predictable in eukaryotic cells. CVC Mot. 1 (Paper 368, 24:18–22. On substantially the same evidence, both the Board (twice) and Federal Circuit have found

[1] CVC improperly incorporates its Motion 1 and its Opposition to ToolGen Motion 2, Paper 171, in support of the arguments made herein. *See* Standing Order (Paper 2) ¶ 106.2 ("Incorporation by reference and combined papers are prohibited…."); *DeSilva v. DiLeonardi*, 181 F.3d 865, 866-67 (7th Cir 1999). To the extent the Board permits such, ToolGen incorporates its Opposition to CVC's Motion 1 and ToolGen's Motion 2, Paper 171.

the opposite. *Regents of Univ. of California v. Broad Inst., Inc.*, 903 F.3d 1286, 1291–92 (Fed. Cir. 2018); Ex. 1101, 102 ('115 Decision on Motions). As discussed fully in ToolGen's Opposition to CVC's Motion 1, and as the Board previously held, neither P1 nor P2 contains a sufficiently disclosed or enabled embodiment within the Count.

Further, CVC is not entitled to the benefit of the filing date of P3. While the Board has initially accorded CVC the benefit date of CVC's P3 application, it did so without considering ToolGen's argument that P3 does not constructively reduce to practice an embodiment within Count 1. As discussed more fully in ToolGen's Motion 2, Paper 171, the experimental results reported in P3 do not disclose to a POSA successful target site cleavage. F51–58. P3 contains a single example (i.e., Example 2) which purports to show (by way of two figures, numbers 38B and 36E) cleavage of DNA by CRISPR-Cas9 in eukaryotic cells. F51. The single example, however, fails for at least the following reasons:

- Figure 38B shows alleged cleavage bands at positions where there should be no bands, and in some instances no bands where there should be bands. F54-55.
- Figure 36E contains many unexplained bands such that a POSA would not view it as showing possession of an embodiment within Count 1. F52, 56. For example, 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). F56. There are also unexplained larger bands (~477 bp) in the control lanes lacking nuclease. F57. These bands disappear in the presence of nuclease. *Id*.
- Even if Figures 38B and 36E did show cleavage at the target sites, cleavage could have occurred extracellularly instead of *within a eukaryotic cell* because the gentle lysing method allowed for CRISPR-Cas9 cleavage to occur in the cell lysate, as opposed to

within the eukaryotic cell as required by Count 1. F58.

Thus, for the very same reasons, the disclosures carried forward from P1, P2, and P3 in the common specification of CVC's Non-Provisional Applications do not provide a constructive reduction to practice of an embodiment within Count 1. As discussed below, the common specification's new examples 4, 5, and 7 do not cure the common specification's inadequate disclosure.

B. The Additional Disclosures In CVC's Non-Provisional Applications Do Not Demonstrate That Each Application Presents A Constructive Reduction To Practice Of The Count

CVC points to Examples 4, 5, and 7 in the common specification for purported "supplementary disclosures beyond Example 2 that further bolster the §112 support for multiple embodiments of Count 1, including embodiments E1–E3." CVC Responsive Motion 2 ("Mot.") 4:21–23. As discussed below and in Dr. Turchi's accompanying declaration (Ex. 1412), the new examples, alone or in combination with the previous disclosure, do not disclose a constructive reduction to practice of an embodiment within Count 1, alone or in view of the previous disclosure.

Examples 4, 5, and 7 lack definitive evidence that CRISPR-Cas9 modulates transcription of at least one gene encoded by the target DNA molecule in eukaryotic cells. Those examples also lack the proper experiments and controls—which were conducted in the very same examples in E. coli cells *but not* in the human cells—necessary to attribute the reported change in fluorescence to the RNA-guided targeting of dCas9 to GFP. Experimental controls are critical to eliminate alternate explanations of the observed results and to infer causality in an experimental system, but proper controls were noticeably absent for the HEK293 experiments and thus doom the conclusions. Ex. 1412, ¶¶54, 56, 60; F21. In fact, CVC's own expert defined experimental failure as "an experiment that couldn't be interpreted because the appropriate controls weren't included."

Ex. 1540 (Zamore Tr.), 47:10-14; F22.. Dr. Zamore further testified that "improper control design" and "lack of control experiments" was one main reason grant proposals and peer-reviewed articles were rejected. *Id*. at 45:2-46:6; F23. By CVC's expert's own definition and as discussed below, Examples 4, 5, and 7 fail to demonstrate constructive reduction to practice of Count 1.

1. Example 4

Example 4 describes a purported "method for controlling gene expression, based on Cas9, an RNA-guided DNA endonuclease from a Type II CRISPR system" called CRISPR interference (CRISPRi). Exs. 2005-2007, ¶00606. The Cas9 used in the CRISPRi system is catalytically inactive ("dCas9") such that it is incapable of cleaving DNA. Ex. 1412, ¶51; F28.

According to CVC, "using the sgRNA design shown in Figure 40B targeting the non-template DNA strand of the green fluorescent protein (EGFP) coding region produced a 'moderate but reproducible knockdown of gene expression…. (46% repression, Figure 45A)' in HEK293 cells." Mot. at 5. The common specification further notes that the "repression was dependent on both the dCas9 protein and sgRNA, ***implying*** that repression was due to the dCas9-sgRNA complex and RNA-guided targeting." Ex. 2005-2007, ¶00646 (emphasis added). Implications, however, are not proof, and a POSA could have drawn other inferences from the data reported.

As an initial matter, Example 4 provides no evidence demonstrating that dCas9 is even expressed in the cell, and if it is, that it is localized to the nucleus. Ex. 1412, ¶59; F24, F25. There is also no evidence that dCas9 forms a complex with sgRNA, let alone that dCas9 directly binds to the target gene. Ex. 1412, ¶59; F26. Moreover, a critical failure of Example 4 is the lack of any direct measurement of gene *transcription* in eukaryotic cells, which is required by the Count (i.e., "modulating transcription of at least one gene encoded by the target DNA molecule"). Ex. 1412, ¶62; F27. To determine whether transcription is being modulated, RNA must be directly measured

using any number of well-known methods (e.g., RNA-seq, northern blot, and RT-PCR). Ex. 1412, ¶62; F29. In eukaryotic cells, however, only the presence of GFP protein was analyzed using flow cytometry to detect fluorescence (as opposed to in bacterial cells, where RNA-seq was conducted to measure RNA). Ex. 1412, ¶62; F30. Given the lack of any measurement of gene transcription, a POSA would not have concluded that Example 4 discloses the constructive reduction to practice of an embodiment of the Count. Ex. 1412, ¶62.

Assuming, arguendo, that modulation of transcription were properly demonstrated, the decrease in GFP fluorescence—which is the only reported measure suggesting repression—could be attributed to reasons other than dCas9 binding the target gene that were neither studied nor controlled for. Ex. 1412, ¶60. A POSA would have understood that 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; F31. For example, 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; F32. Example 4, however, does not demonstrate targeting of the Cas9 protein to the target DNA molecule or control for any of these other possibilities. Ex. 1412, ¶60.

Moreover, Example 4 does not even show specific repression of the EGFP gene in the HEK293 cells. Ex. 1412, ¶60; F33. That is, the example does not show that non-targeted genes were not "modulated" by the Cas9/sgRNA system. Ex. 1412, ¶60. A POSA would have considered that to be a critical control before concluding that the CRISPR-Cas9 complex bound the target sequence in a DNA molecule and modulated gene expression. To demonstrate

specificity, RNA-Seq, a technique which uses sequencing to reveal the presence and quantity of RNA in a biological sample, should have been performed. Ex. 1412, ¶62; F34. Indeed, these critical control experiments were performed in the E. coli studies reported in Example 4, but were absent for the human HEK293 cells. Ex. 1412, ¶60; F35. In E. coli, RNA-Seq was performed on dCas9 transformed cells with and without sgRNA co-expression. In the dCas9-sgRNA sample, only the mRFP gene expression was repressed, with no other significant changes to expression observed in other tested genes. Ex. 1412, ¶54. This same method was used with other sgRNAs targeting different E. coli genes as well, showing similar results. *Id.* Without explanation, however, target specificity was not tested in HEK293 cells using this or any other method. Thus, a POSA could not conclude that the CRISPRi system was achieving its intended, targeted effect of repressing only EGFP in eukaryotic cells. Ex. 1412, ¶60.

Additionally, 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. Ex. 1412, ¶61; F36. At the very least, a POSA would have tested a number of genes—not just one—to show that repression was not just affecting GFP expression and fluorescence. Ex. 1412, ¶61; F38. Importantly, at least one of the genes tested should have been an endogenous gene. *Id*. However, the stable HEK293 reporter cell line in Example 4 utilized only an exogenous EGFP gene that was randomly integrated into the HEK293 genome, at an unknown (or undisclosed) site. Ex. 1412, ¶61; F36. The location of gene integration could have transcriptional effects on gene expression that are independent of the dCas9-sgRNA effects. Ex. 1412, ¶61; F39. 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, ¶57; F40. In fact, applicants conducted this critical control in their E. coli

system (Figure 44), but failed to do the same control in HEK293 cells. Ex. 1412, ¶¶55, 61; F37. The disparity between E. coli and HEK293 cells with respect to the controls conducted would raise warning flags to a POSA regarding the experimental design, and ultimately the conclusions drawn about the effects of CRISPRi in eukaryotic cells.

In summary, Example 4 merely demonstrates that CRISPRi can reduce GFP fluorescence in one cell type, but lacks both the necessary experiment to show that transcription is being modulated and the necessary control to demonstrate that the reduction is due to direct binding of a sgRNA/Cas9 complex to the gene. Ex. 1412, ¶¶59, 62. Moreover, Example 4 demonstrates that CRISPRi modulates GFP, but not more generally that it is capable of modulating any other gene, much less endogenous genes in eukaryotic cells. Ex. 1412, ¶61. And a POSA would not have reasonably expected that the system disclosed in Example 4 achieved targeted modulation of gene expression in eukaryotic cells without evidence of at least one additional reporter cell line to assess gene expression regulation. *Id*.

Accordingly, Example 4 does not demonstrate constructive reduction to practice of an embodiment of Count 1, or further bolster the §112 support for embodiments E1–E3.

2. Example 5

Example 5, which appears to report a follow-up experiment to Example 4, describes the addition of protein domains onto dCas9 to impart additional activities (i.e., activation or repression). Example 5 suffers all of the same issues as Example 4 discussed above, including the absence of any evidence that a complex is indeed formed and then binds the target gene, the lack of proper controls to demonstrate specificity in modulating the target genes, the absence of studies on endogenous genes to dispel concerns arising from gene integration, and the absence of any

measurements to detect gene transcription instead of just GFP fluorescence. Ex. 1412, ¶¶64–65; F42–45.

Moreover, Example 5 reports a mere 20-fold activation of the GAL4 UAS gene based on GFP fluorescence. Ex. 1412, ¶63, F45. The specification provides no explanation for why the reported activation is so low. *Id*. Further, 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; F46.

Accordingly, Example 5 does not demonstrate constructive reduction to practice of an embodiment of Count 1, or further bolster the §112 support for embodiments E1–E3.

3. Example 7

Example 7, which is strictly prophetic, discloses a plan to generate transgenic mice expressing Cas9. Ex. 1412, ¶67; F47. Example 7 suggests making Cas9 transgenic mice, and then isolating embryonic stem ("ES") cells from the transgenic mice that can then be altered in cell culture, and perhaps subsequently used to generate altered mice. Ex. 1412, ¶67. This example, however, provides no guidance that would lead a POSA to determine how to make such mice. Ex. 1412, ¶¶67–69; F48. Moreover, 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, but the ability to cleave DNA in a eukaryotic cell upon introduction of Cas9/sgRNA has not been demonstrated in the common specification. Ex. 1412, ¶69; F49.

Further, there are no experimental results reported in Example 7, 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, ¶67; F50.

Accordingly, Example 7 does not demonstrate constructive reduction to practice of an embodiment of Count 1, or further bolster the §112 support for embodiments E1–E3.

III. CONCLUSION

For the foregoing reasons, CVC should not be accorded benefit of the filing date of CVC's Non-Provisional Applications because it has not shown a constructive reduction to practice of an embodiment within Count 1.

Respectfully submitted,

Dated: July 15, 2021

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APPENDIX A: LIST OF EXHIBITS CITED

Ex.	Description
1018	U.S. Provisional Application No. 61/757,640, filed January 28, 2013.
1101	Paper 877, Decision on Motions 37 C.F.R. § 41.125(a), Interference 106,115, September 10, 2020.
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
2005	U.S. Patent Application No. 13/842,859, filed March 15, 2013
2006	U.S. Patent Application No. 14/685,504, filed April 13, 2015
2007	U.S. Patent Application No. 15/138,604, filed April 26, 2016

APPENDIX B: STATEMENT OF MATERIAL FACTS

Junior Party's Alleged Facts 1-20 and Senior Party's Answers

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. U.S. Provisional Patent Application No. 61/716,256 ("P2") was filed on October 19, 2012, 6 and lists Martin Jinek, Jennifer Doudna, Emmanuelle Charpentier, Krzysztof Chylinski, and James Harrison Doudna Cate as co-inventors. Ex. 2002, p. 277.

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.

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.

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.

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.

Response: Admitted.

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

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**.**

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.

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.

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.

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.

Response: Denied.

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

Response: Admitted.

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

Response: Admitted.

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

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.

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.

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.

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.

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.

Response: Denied.

ToolGen, Inc's Additional Material Facts

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.

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.

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.

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

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

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.

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

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

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.

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

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.

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.

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

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

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

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.

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

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.

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

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.

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

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

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

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

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.

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.

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

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

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.

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.

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.

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

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

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

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

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.

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.

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.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **TOOLGEN OPPOSITION TO CVC RESPONSIVE MOTION 2** was filed via the Interference Web Portal on July 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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