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UNIVERSITY OF VIENNA, AND EMMANUELLE CHARPENTIER

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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 OPPOSITION 2
(for benefit of Application No. 61/757,640)

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1 ***I. INTRODUCTION***

2 ToolGen's Motion 2 should be denied because CVC's P3 application provides a
3 constructive reduction to practice of an embodiment within Count 1. ToolGen has not and cannot
4 meet its burden of proving that CVC should be denied the priority benefit of P3. To constitute a
5 constructive reduction to practice ("CRTP"), an application must provide a described and
6 enabled anticipation of at least one embodiment within the scope of the count. Although a CRTP
7 does not require working examples or an actual reduction to practice, P3's Example 2 does just
8 that. It discloses a working example showing target DNA cleavage by sgRNA-programmed Cas9
9 in eukaryotic cells. The scientific results are beyond any reasonable dispute as those same
10 experiments in Example 2 were the subject of the peer-reviewed *eLife* Jinek 2013 publication. As
11 the *eLife* editors prefaced, "Now Jinek et al. demonstrate the capability of RNA-programmed
12 Cas9 to introduce targeted double-strand breaks into human chromosomal DNA, thereby
13 inducing site-specific genome editing reactions." Ex. 2499, 3. Indeed, the PTAB has concluded
14 on three different occasion that P3 is a constructive reduction to practice of counts that each
15 included the same CVC claim 156. *See e.g.*, Paper 1, 10; Ex. 2400, 106:11-14; Ex. 2501, 10.
16 While this Opposition addresses ToolGen's criticisms of P3, CVC maintains its position that
17 CVC's P1 and P2 applications are also each a CRTP of Count 1. *See* Paper 368.

18 ToolGen premises its motion on a litigation-driven, revisionist history that by January 28,
19 2013 (P3's filing date) a POSA would have viewed using CRISPR-Cas9 in eukaryotic cells as
20 "highly unpredictable," and the artisan would have therefore required disclosure of an actual
21 reduction to practice in P3 to conclude that P3 is a CRTP of Count 1. But Turchi, ToolGen's
22 expert, admittedly failed to consider pertinent art such as Barrangou 2012, *Science* 2012, Mali
23 2013, and Cong 2013, each of which would have been available to a POSA by P3's filing date.
24 By then, these contemporaneous publications had confirmed that no special adaptations were

1 needed to apply CRISPR-Cas9 in eukaryotic cells and that the routine methods and reagents that
2 had been used previously to express RNA and other gene-editing nucleases had also been used to
3 implement CVC's CRISPR-Cas9 system disclosed in P3.

4 After misrepresenting the state of the art, ToolGen applies the wrong legal standard for a
5 CRTP. ToolGen demands that P3 disclose an actual reduction to practice while ignoring its
6 burden as movant to prove that *none of the disclosure in P3 described and enabled any*
7 *embodiment within Count 1.* CVC's half of Count 1 is directed to a eukaryotic cell comprising a
8 single-guide CRISPR-Cas9 system capable of cleaving or editing a target DNA or modulating
9 transcription of a gene encoded by the target DNA in the eukaryotic cell. ToolGen does not even
10 attempt to show that any of P3's embodiments fail to meet all the elements of Count 1. In fact,
11 Turchi *admitted* that the human cell embodiment described in Example 2 meets all the elements
12 of Count 1. The failure of proof and logic in ToolGen's arguments are fatal, and provide the
13 PTAB with more than sufficient reason to deny Motion 2 on this basis alone.

14 But the record affirmatively demonstrates that P3 discloses a working example of Count
15 1. CVC's expert Doyon explains that Example 2 clearly depicts successful target DNA cleavage
16 in eukaryotic cells. For example, both Doyon and Turchi agree that P3's Figure 36E shows that
17 the CRISPR-Cas9 and positive control zinc finger nuclease (ZFN) experiments produced DNA
18 cleavage bands of the expected size that are not present in any of the negative control lanes.

19 ToolGen provides only speculation regarding alleged inconsistencies with DNA cleavage
20 band sizes and concerns with cell lysis conditions disclosed in P3. But the exact same data and
21 lysing protocols were accepted by the scientific community as evidence of successful DNA
22 cleavage in human cells when the experiments published as Jinek 2013. Moreover, prior to this
23 interference, ToolGen's own inventor (Jin-Soo Kim) and its other expert (Bryan Cullen) each

1 independently endorsed the Jinek 2013 paper as showing target DNA cleavage in eukaryotic
2 cells. In 2015, Kim cited the Jinek 2013 paper when stating, "we *and others* have reported RNA-
3 guided *genome editing in human cells* in January, 2013." Ex. 2484, 479 (emphasis added)[1]. In
4 2016, Cullen cited the Jinek 2013 paper when referring to groups that obtained DNA cleavage
5 "*in cultured mammalian cells*." Ex. 2494, 402.

6 In addition to the experimental protocol in Example 2, P3 describes and enables multiple
7 embodiments that fall within the scope of Count 1, including, e.g. a fish cell, human cell, and
8 fruit fly cell. P3 describes in detail a sgRNA-Cas9 system comprising, e.g., a chimera A sgRNA
9 and a *S. pyogenes* Cas9 protein. P3 also describes methods of making the sgRNA-Cas9 system
10 outside of a eukaryotic cell, e.g., by pre-assembling the sgRNA and Cas9 as a ribonucleoprotein
11 (RNP) complex. And P3 describes routine methods of delivering the sgRNA-Cas9 system to
12 eukaryotic cells, such as direct microinjection into fish cells or fruit fly cells, or lipofection
13 transfection of human cells. ToolGen failed to address any of this disclosure in its motion.

14 As the movant, ToolGen bears the burden of establishing that P3 is not a CRTP of at least
15 one embodiment of Count 1. ToolGen has not met its burden here; Motion 2 should be denied.

16 ***II. STATEMENT OF MATERIAL FACTS AND EVIDENCE***

17 Appendix 1 is a list of exhibits cited. Appendix 2 is a Statement of Material Facts.

18 ***III. LEGAL STANDARD***

19 For a party to be accorded priority benefit of an earlier-filed application, all that must be
20 shown is a constructive reduction to practice—a described and enabled anticipation—"with
21 respect to at least one embodiment within the scope of the count." *Falkner v. Inglis*, 448 F.3d
22 1357, 1362 (Fed. Cir. 2006). It is well-settled that "[t]here is no requirement … that a party

[1] Emphasis is added throughout this paper unless otherwise noted.

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). Even in the so-called "unpredictable" arts, the "written description requirement does not demand either examples or an actual reduction to practice." *Ariad Pharmaceuticals, Inc. v. Eli Lilly and Co*, 598 F.3d 1336, 1352 (Fed. Cir. 2010) (en banc).

IV. ARGUMENT

A. ToolGen fails to show that P3 is not a constructive reduction to practice of at least one embodiment of Count 1.

1. ToolGen's portrayal of the state of the art is contradicted by contemporaneous, objective evidence.

ToolGen's Motion 2 is founded on the false premise that "[a]t the time the applicants filed P3, adapting the native prokaryotic CRISPR-Cas9 system to cleave DNA within eukaryotic cells was highly unpredictable." *See* Paper 171, 3:19-20; *see also*, Ex. 1410, ¶41. Specifically, on page 4, lines 14-16 of the motion, it is argued that, "[g]iven the inherent unpredictability and difficulties of adapting CRISPR-Cas9 to eukaryotic cells, a POSA would not have viewed the P3 applicants as having possession of an embodiment with [*sic*] Count 1 without a showing of convincing experimental results." Paper 171, at 4:14-16. The response is that long before P3's filing date, a POSA would have considered achieving cleavage in eukaryotic cells using the CRISPR-Cas9 system to be straightforward, as predicted, undermining ToolGen's and Turchi's assertions. And while ToolGen and Turchi had a burden to consider *all* the pertinent art, they failed to do so—instead selectively citing only art that purportedly supported their litigation-driven position. Turchi admitted that he failed to consider pertinent art such as Barrangou 2012 (Ex. 2215), *Science* 2012 (Ex. 2497), Mali 2013 (Ex. 2345; Ex. 2512), and Cong 2013 (Ex. 2030)—each of which was published before P3's January 28, 2013 filing date. Ex. 2475, 35:21-36:2; Ex. 1410, Appx. B; MF71-79. Discussed below, these references show that POSAs

1 expected and confirmed that applying CVC's sgRNA-Cas9 system in eukaryotic cells required
2 nothing more than routine skill and standard methods—i.e., there were no special adaptions
3 needed or obstacles encountered when using CRISPR-Cas9 in eukaryotic cells. ToolGen failed
4 to explain how a POSA could find the art "highly unpredictable" against the background of this
5 state of the art.

6 For example, in September 2012, Barrangou published a "News and Views" article in
7 *Nature Biotechnology* in response to CVC's landmark Jinek 2012 publication. Ex. 2215; Ex.
8 2031. Barrangou described CVC's sgRNA-Cas9 system as a "synthetic tour de force" that may
9 "outcompete ZFN and TALEN DNA scissors for precise genomic surgery." Ex. 2215, 837-838.
10 As Barrangou testified, "after the CVC inventors' disclosure, CRISPR-Cas9-mediated genome
11 editing in eukaryotes *was inevitable*." Ex. 2021, ¶¶19-21; MF71-72. By December 21, 2012,
12 *Science* published its annual "Breakthrough of the Year" issue describing CRISPR-Cas9 as a
13 "gene-targeting technology" that "may one day challenge zinc finger nucleases and TALENs as
14 the *core genome engineering technology*." *Id.*, Ex. 2497, 1526-1527; MF73-74.

15 Contemporaneous work published by two separate research groups from Harvard
16 University and the Broad Institute prior to January 28, 2013, further confirms that using
17 CRISPR-Cas9 to cleave DNA in eukaryotic cells required only routine techniques. *See* Ex. 2512
18 ("Mali 2013"); Ex. 2030 ("Cong 2013"); Ex. 2476, ¶¶18-22; MF75-78. Mali 2013 published on
19 January 3, 2013, and disclosed making expression vectors encoding a sgRNA and a Cas9
20 protein, delivering the vectors to mammalian cells via standard liposome transfection, and
21 reportedly cleaving a target DNA sequence in the mammalian cells. Ex. 2512, 826, Fig. 1, S7-
22 S8; Ex. 2476, ¶20; MF75-76. Mali 2013 reported that the authors' work "demonstrate[d] the
23 promise of CRISPR-mediated gene targeting for RNA-guided, robust, and multiplexable

mammalian genome engineering" and that "[t]he ease of retargeting our system to modify genomic sequences greatly exceeds that of comparable ZFNs and TALENs, while offering similar or greater efficiencies." Ex. 2512, 825; Ex. 2476, ¶20. Cong 2013 also published on January 3, 2013, and reported standard transfection of human cells with expression vectors encoding Cas9 and CVC's chimera A sgRNA. Ex. 2030, Fig. 2; Ex. 2476, ¶21; MF77-78. Cong 2013 reported that CVC's chimera A design "could facilitate cleavage of [its] genomic target[]." Ex. 2030, 820, Fig. 2C, Table S1; Ex. 2476, ¶21.

Turchi ignored these references when offering his opinions about the state of the art. Ex. 1410, Appx. B; Ex. 2475, 35:21-36:2; MF79. Unlike Turchi, *a POSA would not have ignored these references*. ToolGen's other expert Cullen admitted a POSA would have considered Mali 2013 and Cong 2013. Ex. 2474, 179:10-15, 182:4-11; Ex. 2476, ¶¶18-22; MF75, 77. Thus, a POSA would have been aware of these references and concluded that using CVC's sgRNA-Cas9 system in eukaryotic cells by January 28, 2013, required nothing more than ordinary skill. The "highly unpredictable" state of the art ToolGen portrays is a false premise. Ex. 2476, ¶¶17-24.

2. ToolGen and its expert Turchi wrongly focused exclusively on P3's Example 2 and ignored P3's extensive description and enablement of embodiments of Count 1.

In addition to its incredible view of the state of the art, ToolGen's motion asks the PTAB to reject decades of binding legal precedent by requiring a working example to satisfy CRTP. ToolGen limits its criticisms of P3 almost exclusively to the *data* in Example 2, and argues that "a POSA would not have viewed the P3 applicants as having possession of an embodiment with [*sic*] Count 1 without a showing of convincing experimental results." Paper 171, 4:15-16. But the law is clear: "[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, supra*, 222 F.2d at 278. Moreover, assessing whether an

1 application is a CRTP requires "[a] careful and reasonable reading of the *specification in its*
2 *entirety*." *Binstead v. Littmann*, 242 F.2d 766, 768 (C.C.P.A. 1957). ToolGen and Turchi failed
3 on both accounts. Paper 171, at 4:20-17:5; Ex. 1410, ¶¶63-110; Ex. 2475, 79:1-4, 80:21-81:13,
4 91:6-20, 92:7-16, 98:10-17; MF80-82.

5 Turchi's rubric that "a POSA would focus on the experiments in P3's Example 2" infects
6 his entire analysis. Ex. 1410, ¶43. Turchi's opinions focus *exclusively* on the *data* in Example 2,
7 failing to address Example 2's materials and methods for producing the eukaryotic cell of Count
8 1, or any of P3's embodiments of Count 1 outside of Example 2. For example, the disclosures in
9 P3 that form Example 2 are ¶¶[00408]-[00423] and Figs. 36-38. Throughout his declaration,
10 Turchi cites exclusively to these paragraphs and figures in Example 2, ignoring the rest of the
11 specification. *See generally,* Ex. 1410. Turchi's *only* reference to a paragraph or figure outside of
12 Example 2 is a single instance when he cites P3 ¶[00378] and Figure 21 for his comment that
13 magnesium is required for *in vitro* DNA cleavage reactions. Ex. 1410, ¶76; MF81.

14 On cross-examination, it became clear that Turchi wrongly believed or was led to believe
15 that P3 must disclose demonstration of actual DNA cleavage in a eukaryotic cell for P3 to
16 convey to a POSA that the inventors had possession of at least one embodiment of Count 1:

17 Q: Is the demonstration of cleavage the only way to demonstrate that
18 one has possession of the invention?

19 A: If the context of the written description describes an experiment
20 and the working example is one that is assessing cleavage in a
21 eukaryotic cell, for that example, the demonstration would be
22 required to support the description that they possess that invention.

23 Ex. 2475, 85:7-17 (objection omitted); *see also, id.*, 107:15-108:1; MF80-82. Turchi's analysis is
24 contrary to the law because CRTP does not require working examples or an actual reduction to
25 practice. *Lawson*, 222 F.2d at 278; *Falkner*, 448 F.3d at 1366. Unlike Turchi, a POSA would
26 have performed "[a] careful and reasonable reading of the *specification in its entirety*"—not just

1 Example 2—when assessing whether P3 provides a described and enabled anticipation of at least
2 one embodiment of Count 1. *Binstead*, 242 F.2d at 768.

3 Turchi's shortsighted analysis failed to address the fact that P3 describes how to make
4 and use different sgRNA-Cas9 systems comprising a chimera A sgRNA and *S. pyogenes* Cas9
5 protein. *See e.g.,* Ex. 2003, ¶¶[0030]-[0031], [0037]-[0040], [00169], [00196], [00364], [00401]-
6 [00405], Figs. 1-3, 17; Ex. 2476, ¶¶27-30; MF83. Turchi also failed to address the fact that P3
7 describes assembling chimera A sgRNA and Cas9 complexes outside of a eukaryotic cell as a
8 ribonucleoprotein (RNP) complex.[2] Ex. 2003, ¶¶[00366]-[00370], [00401], claim 98; Ex. 2476,
9 ¶27-30; MF84. And while Turchi admits that "direct microinjection" was a "straightforward and
10 effective" technique for delivering gene editing systems to eukaryotic cells, he failed to address
11 the fact that P3 describes delivering sgRNA-Cas9 systems to eukaryotic cells, such as fish cells
12 or fruit fly cells, using the same direct microinjection techniques. Ex. 2003, ¶¶[00108], [00251]-
13 [00252], [00261]; Ex. 2476, ¶¶27-30; MF85-86. Indeed, Turchi admitted that "I don't mention
14 drosophila [fruit flies] or zebra fish that I can find at this point in the declaration." Ex. 2475,
15 108:2-16; *see also*, *id.*, 108:17-19. Turchi also failed to address the fact that P3 describes
16 eukaryotic expression vectors for expressing sgRNA and Cas9, promoters to drive the expression
17 (including, e.g., Pol II promoters such as CMV and Pol I promoters such as U6 and H1), codon
18 optimization of the Cas9 gene, adding a nuclear localization signal (NLS) to Cas9, routine vector
19 transfection methods, and established cell lines suitable for transfection—all outside of P3's
20 Example 2. Ex. 2003, ¶¶[0090], [00206], [00243]-[00244], [00252], [00256]-[00259]; Ex. 2476,

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[2] Turchi admits that "pre-expressed Cas9 and sgRNA could also be transfected" into eukaryotic cells, but failed to discuss these aspects as they are described in P3. Ex. 1410, ¶47 n.2.

1 ¶¶27-30; MF87. And Turchi did not address a POSA's routine implementation of the expression
2 vectors disclosed in Example 2. Ex. 2003, ¶¶[00409]-[00410]; Ex. 2476, ¶29.
3 On page 4, lines 14-17 of the motion, it is argued that P3 would not have conveyed to a
4 POSA that CVC possessed an embodiment of Count 1 due to alleged "difficulties in adapting
5 CRISPR-Cas9 to eukaryotic cells." Paper 171, 4:14-17. The response is that neither ToolGen nor
6 Turchi point to *any* so-called CRISPR "adaptations" purportedly missing from the disclosures in
7 P3 that a POSA would have needed to make and use an embodiment of Count 1. Nor has
8 ToolGen pointed to any "adaptation" in its own application that is not also described in P3. Ex.
9 2476, ¶¶27-30. P3 discloses all of the routine materials and methods a POSA would have
10 needed, be it pre-assembled sgRNA-Cas9 RNP complexes and direct microinjection delivery or
11 eukaryotic expression vectors and routine liposome transfection. Ex. 2003, ¶¶[0090], [00206],
12 [00256]-[00257], [00259], [00409]-[00410]; Ex. 2476, ¶¶27-30; MF83-87. And to be clear,
13 ToolGen does not even attempt to address P3's enablement of embodiments of Count 1. *See* Ex.
14 2475, 106:20-22 (counsel for ToolGen stating that "ToolGen will stipulate that Dr. Turchi, in
15 Exhibit 1410, does not opine on enablement issues.").
16 As the movant, ToolGen bears the burden of showing that P3 is not a CRTP of at least
17 one embodiment of Count 1. Turchi's cursory view of the state of the art as of January 28, 2013,
18 and his application of the wrong legal standard for CRTP leave gaping holes in his analysis of
19 P3, which are fatal to ToolGen's Motion 2.

20 **3. P3's Example 2 indisputably meets all the elements of Count 1.**

21 Although a working example is not required for CRTP, P3's Example 2 describes and
22 enables a human cell embodiment that meets all the elements of Count 1. Ex. 2003, ¶¶[00408]-
23 [00423]; Ex. 2476, ¶¶31-33. ToolGen criticizes *the data* in Example 2, but *does not dispute* that
24 the human cell embodiment in Example 2 meets all the elements of Count 1. *See* Paper 171,

1 4:18-17:5. In fact, Turchi admitted that Example 2 satisfies each element of Count 1:

Element of Count 1	Disclosure in P3	Turchi admissions
A eukaryotic cell comprising a target DNA molecule and an engineered and/or non-naturally occurring Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) system comprising	Example 2 discloses using human embryonic kidney cells (HEK293T) comprising CLTA target DNA. *See* Ex. 2003, ¶¶[00410], [00412], [00416]-[00417], [00421]-[00422]; Fig. 36C	"I'm fairly confident that HEK stands for *human embryonic kidney cells*, so I'm fairly *confident that's what they are.*" Ex. 2475, 51:12-16. Q: Human cells contain a CLTA gene, correct? A: *That is correct*. Ex. 2475, 52:22-53:1 Q: Is the target DNA molecule that's depicted in figure 36C from the human genome of a human cell? A: So I believe the *sequence depicted in figure 36C represents* the human DNA sequence for the *CLTA gene in human cells*. Ex. 2475, 110:4-8. *See also*, Ex. 1410, ¶76.
a) a Cas9 protein, or a nucleic acid comprising a nucleotide sequence encoding said Cas9 protein; and	Example 2 discloses an expression vector encoding *S. pyogenes* Cas9 protein. *See* Ex. 2003, ¶¶[00409]-[00410]; Fig. 36C	"The experiments in *example 2 include* the definition or a *description of a plasmid coding the Cas9 gene* with GFP or mCherry and a second plasmid coding the sgRNA, which I believe in our footnote earlier encompasses the chimeric guide-RNA." Ex. 2475, 108:20-109:10. *See also*, Ex. 1410, ¶76.
b) a single molecule DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding said single molecule DNA-targeting RNA; wherein the single molecule DNA-targeting RNA comprises:	Example 2 discloses an expression vector encoding a chimera A sgRNA. *See* Ex. 2003, ¶¶[00409]-[00410]; Fig. 36C	"The experiments in *example 2 include* the definition or a *description of a* plasmid coding the Cas9 gene with GFP or mCherry and *a second plasmid coding the sgRNA*, which I believe in our footnote earlier encompasses the chimeric guide-RNA." Ex. 2475, 108:20-109:10. *See also*, Ex. 1410, ¶76.

Element of Count 1	Disclosure in P3	Turchi admissions
i) a targeter-RNA that is capable of hybridizing with a target sequence in the target DNA molecule, and	The chimera A sgRNA in Example 2 is capable of hybridizing with the CLTA target sequence. *See e.g.,* Ex. 2003, Fig. 36C	Q: Does the single guide-RNA that's depicted in 36C include a nucleic acid sequence that functions as a targeter-RNA? A: *Yes.* There is DNA -- there's *RNA sequences within the guide-RNA that serve as a targeter-RNA*. Ex. 2475, 112:7-12.
ii) an activator-RNA that is capable of hybridizing with the targeter-RNA to form a double-stranded RNA duplex of a protein-binding segment,	The chimera A sgRNA in Example 2 comprises a double-stranded RNA duplex. *See e.g.,* Ex. 2003, Fig. 36C	Q: Does the single guide-RNA that's depicted in figure 36C include a nucleic acid sequence that functions as an activator-RNA? A: *Yes.* There are s*equences within the guide-RNA depicted that function as transactivator-RNA*. In this case, it wouldn't be trans, but as an activator-RNA. Ex. 2475, 112:13-19. Q: In figure 36C of CVC's P3 application, does the single guide-RNA form a double-stranded RNA duplex? *** A: *there is, in fact, RNA/RNA duplex structure* in that single guide-RNA depicted in figure 36C. Ex. 2475, 110:9-111:7.
wherein the activator-RNA and the targeter-RNA are covalently linked to one another with intervening nucleotides; and	The chimera A sgRNA in Example 2 comprises targeter-RNA and activator RNA covalently linked with intervening nucleotides. *See e.g.,* Ex. 2003, Fig. 36C	Q: In this single guide-RNA that's depicted in figure 36C, are the nucleic acid sequences that function as the targeter-RNA covalently linked with intervening nucleotides to the nucleic acid sequence that functions as the activator-RNA? A: That *interpretation is consistent with the figure*. Ex. 2475, 112:20-113:4
wherein the single molecule DNA-targeting RNA is capable of forming a complex with the Cas9 protein, thereby targeting the Cas9	The chimera A sgRNA in Example 2 is capable of forming a complex with Cas9 and targeting Cas9 to the CLTA target.	Q: Does figure 36C depict a single guide-RNA that forms a complex with the Cas9 protein? A: *Yeah.* From the artist's rendition of the complex, *one could reasonably conclude that the single guide-RNA somehow binds to the Cas9 protein*. Ex. 2475, 113:6-10.

Element of Count 1	Disclosure in P3	Turchi admissions
protein to the target DNA molecule	*See e.g.,* Ex. 2003, Fig. 36C	Q: And does figure 36C depict targeting the Cas9 protein to the target DNA? A: Figure 36C depicts *hydrogen bonds* occurring *between the targeter region of the single guide-RNA and the target DNA*. Ex. 2475, 113:11-15.
whereby said system is capable of cleaving or editing the target DNA molecule or modulating transcription of at least one gene encoded by the target DNA molecule.	The sgRNA-Cas9 system in Example 2 is capable of cleaving or editing the CLTA target DNA molecule. *See e.g.,* Ex. 2003, Fig. 36C, 36E, 38B	Ex. 2475, 116:20-118:1 (testifying that biochemical assays – such as the *in vitro* cleavage assays in P3 Example 1 – would give a POSA confidence that the system depicted in Figure 36C is capable of cleaving a target DNA.)

1 For CRTP, the "application must contain a written description of the subject matter of the
2 interference count, and must meet the enablement requirement." *Hyatt v. Boone,* 146 F.3d 1348,
3 1352 (Fed. Cir. 1998) (citation omitted). Turchi's admissions compel a conclusion that P3
4 describes an embodiment that meets all the elements of Count 1. Ex. 2476, ¶¶31-33; MF88-89.
5 Turchi never addresses whether the same expression vectors, cells, and methods disclosed in
6 Example 2 convey a system *capable of cleaving or editing* a target DNA in a eukaryotic cell.
7 And neither ToolGen nor Turchi attempts to contest enablement of this embodiment. Ex. 2475,
8 106:20-22. P3 is thus unquestionably a CRTP of at least one embodiment of Count 1, and
9 ToolGen's Motion 2 fails to show otherwise.

10 ***B. P3's Example 2 discloses successful CRISPR-Cas9-mediated target DNA***
11 ***cleavage in eukaryotic cells.***

12 The fatal errors discussed above provide the PTAB with ample basis to deny ToolGen's
13 Motion 2, and thus the PTAB need not even consider whether the data in P3's Example 2
14 demonstrate an *actual reduction to practice*. But, even when considering the data in Example 2,
15 P3 clearly discloses successful target DNA cleavage in eukaryotic cells. Ex. 2476, ¶¶34-52.

1 **1. The same experiments described in P3's Example 2 were peer-reviewed**
2 **and published in the Jinek 2013 *eLife* paper.**

3 Turchi criticizes the cell lysis procedure and Surveyor gels disclosed in P3's Example 2,
4 but he failed to consider the fact that the *same cell lysis procedure* and *same Surveyor gels* were
5 peer-reviewed and published in the journal *eLife* on January 29, 2013. Ex. 2033 ("Jinek 2013"),
6 7-8, Figs. 1, 3; Ex. 2003, ¶[00410], Figs. 36, 38; Ex. 2476, ¶¶64-69. After reviewing the Jinek
7 2013 manuscript (described as an "excellent paper" by the reviewers), no reviewer commented
8 on the cell lysis procedure, the DNA band sizes in the Surveyor gels, or the possibility of
9 unidentified bands in the gels. Ex. 2499, 16-17. Turchi admitted he "did not use [Jinek 2013] to
10 form my opinions presented in the declaration." Ex. 2475, 143:19-144:3, 144:20-145:2.

11 Further, ToolGen's own inventor, Jin-Soo Kim, endorsed Jinek 2013 as showing
12 "genome editing in human cells," stating:

> 13 Since we and *others have reported RNA-guided genome editing in*
> 14 *human cells in January, 2013* (Cho et al., 2013a; Cong et al., 2013;
> 15 *Jinek et al., 2013*; Mali et al., 2013b), the CRISPR-Cas9 system has
> 16 been widely used in many labs all around the world to modify
> 17 genomes in various organisms and cells.

18 Ex. 2484, 479. ToolGen's expert Cullen has also acknowledged Jinek 2013 as disclosing target
19 DNA cleavage "in cultured mammalian cells":

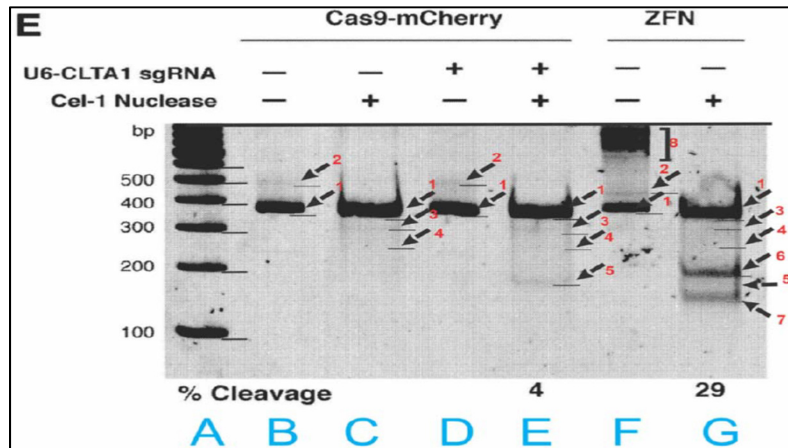
> 20 The key step toward utilizing Cas9 as a programmable DNA
> 21 endonuclease was the realization that a synthetic crRNA, containing
> 22 a novel spacer sequence linked to the constant region of the crRNA,
> 23 could be used to confer a novel DNA sequence specificity on Cas9.
> 24 This result, obtained first in vitro (6) and *soon after in cultured*
> 25 *mammalian cells (7–9)*….

26 Ex. 2494, 402 (citing Jinek 2013 as Reference 9). In addition to Kim and Cullen, numerous
27 others in the field have likewise endorsed Jinek 2013 as evincing targeted DNA cleavage *in*
28 *eukaryotic cells*. *See e.g.,* Ex. 2482, 7430; Ex. 2485, 1; Ex. 2506, 489, 497; Ex. 2486, 6; Ex.
29 2492, 402; Ex. 2487, 14523-14524; Ex. 2496, 688-689; Ex. 2214, 1; Ex. 2495, 974; Ex. 2483,

1 1181; Ex. 2493, 1173, 1175; Ex. 2509, 348; Ex. 2502, 2; Ex. 2488, 2; Ex. 2507, 404; Ex. 2508,
2 139; Ex. 2476, ¶¶64-69. These references—ignored by ToolGen and Turchi—all serve as
3 objective, contemporaneous evidence that a POSA would have viewed the experiments described
4 in P3 as showing successful target DNA cleavage in eukaryotic cells. Ex. 2476, ¶¶64-69.

5 **2. Figure 36E discloses successful target DNA cleavage in a eukaryotic cell.**

6 Just as reviewers of Jinek 2013 understood, a POSA would have understood that P3's
7 Figure 36E shows target DNA cleavage in a eukaryotic cell. Ex. 2476, ¶¶35-44; Ex. 2003, Fig.
8 36E. Discussed below, Turchi agrees with Doyon that (1) the PCR amplicons, sgRNA-Cas9-
9 cleaved DNA, and ZFN-cleaved DNA are all consistent with the expected DNA fragment sizes;
10 and (2) the sgRNA-Cas9-cleaved DNA and ZFN-cleaved DNA are *not* present in any of the
11 negative control lanes. Turchi's annotated copy of Figure 36E is shown below for reference:

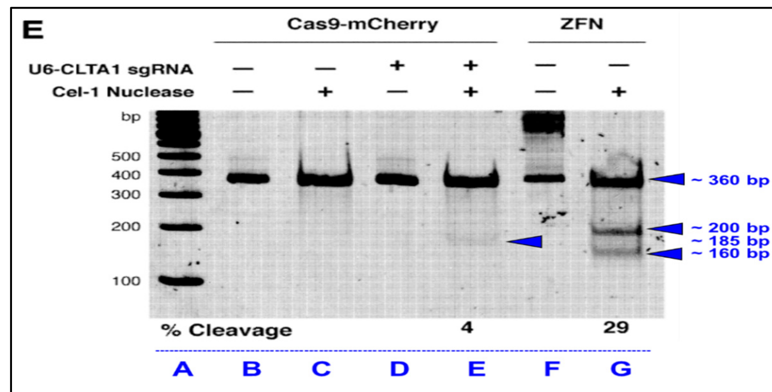
12 

13 Ex. 1410, ¶79; Ex. 2003, Fig. 36E.

14 P3 discloses that the PCR amplicon for the CLTA target region is "360 bp," which is
15 exactly what Figure 36E shows (see band 1 in Turchi's image above) and what Turchi admits is
16 "consistent" with his expected size. Ex. 2003, ¶[00412], Fig. 36E; Ex. 1410, ¶82; Ex. 2476,
17 ¶¶36-38; MF90. P3 also discloses that the sizes of the expected cleavage products are "160-200
18 bps," which again are precisely as depicted in Figure 36E (see bands 5, 6, and 7 above) and

1 consistent with Turchi's estimates of "approximately 183 and 186 bp" for Cas9 cleavage
2 products and "approximately 162-169 and 200-207 bp" for ZFN cleavage products. Ex. 2003,
3 ¶[00412], Fig. 36E; Ex. 1410, ¶80; Ex. 2476, ¶¶36-38; MF91. As Doyon explains, a POSA
4 would have understood that the Cas9-cleaved bands are close to the same size (about 185 bps)
5 and thus would appear as a single band in a polyacrylamide gel, in contrast to the ZFN-cleaved
6 bands which are more different in size and appear as two distinct bands in the gel. Ex. 2476,
7 ¶¶36-38. Turchi did not dispute this at deposition. Ex. 2475, 53:18-55:19.

8 Doyon's annotation of Figure 36E is below:

9 

10 Ex. 2003, Fig. 36E; Ex. 2476, ¶36. It is undisputed that the sgRNA-Cas9 cleaved band only
11 appears in Lane E and is not present in any of the negative control lanes. Ex. 2476, ¶37; Ex.
12 2003, Fig. 36E; Ex. 2475, 126:4-127:14; MF92. It is also undisputed that the sgRNA-Cas9
13 cleaved band in Lane E migrates to a position in between the two ZFN cleavage bands in Lane
14 G. Ex. 2476, ¶¶37-38; Ex. 2003, Fig. 36E; Ex. 2475, 125:15-126:3; MF93.

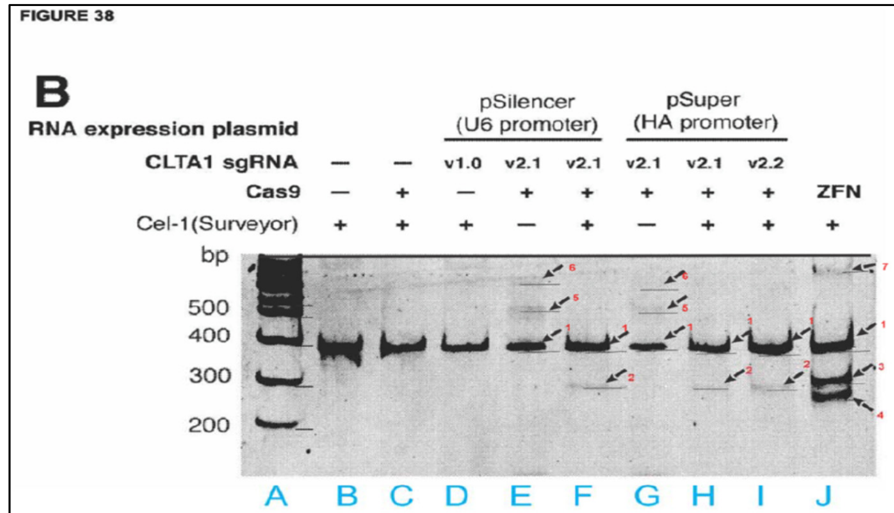
15 Further, P3 expressly discloses that the ZFN positive control in Figure 36E was "[a] ZFN
16 construct previously used to target the CLTA locus." Ex. 2003, ¶[00417]. A POSA would have
17 known that the ZFN positive control is consistent with the *same CLTA-targeted ZFN* previously
18 published in Surveyor assays in Doyon 2011. *See* Ex. 2114, Fig. S1(b); Ex. 2003, ¶[00417], Fig.
19 36E; Ex. 2476, ¶38. Unlike a POSA, Turchi failed to consider Doyon 2011 for the purposes of

1 his declaration, admitting that he was "not familiar with this paper." Ex. 2475, 40:8-20.

2 Turchi's only complaint for Figure 36E is his allegation of "unexpected bands" with
3 "unclear" origin that appear to be "nuclease sensitive." Ex. 1410, ¶82. But Turchi failed to
4 account for the likelihood of undigested heteroduplexes in the gel, which were common
5 occurrences in Surveyor assays. Ex. 2476, ¶¶39-43; Ex. 2479; Ex. 2480, or Ex. 2481. A POSA
6 would not have been concerned by the potential presence of such bands in Figure 36E because
7 this phenomenon was well known in the art. For example, Guschin 2010 discloses that "[i]n
8 some instances, a ZFN-treated lane will contain *visibly obvious undigested heteroduplex running*
9 *above the parent amplicon* (e.g., the *faint band above the parent band …*)" Ex. 2479, 254.
10 Turchi failed to discuss undigested heteroduplexes in his declaration, and when cross-examined,
11 he testified that he "couldn't speak to that or have an opinion without looking at it more deeply."
12 Ex. 2475, 66:13-18. Indeed, Turchi failed to consider any of Exhibits 2479, 2480, or 2481 when
13 offering the opinions in his declaration. Ex. 1410, Appx. B; Ex. 2475, 35:21-36:2; MF94.
14 Further, a POSA would have recognized that Turchi's additional "unexpected" bands 3 and 4
15 are, at most, a result of low-level non-specific cleavage by the Cel-1 nuclease—not off-target
16 Cas9 cleavage. Ex. 2476, ¶44. Regardless, even in view of allegedly unidentified bands in the
17 gel, a POSA knew that the most important observation is the sgRNA-Cas9 band of the expected
18 size in Lane E (reflecting the Cas9-cleavage products) that is not present in any of the negative
19 control lanes. Ex. 2476, ¶¶36-38; Ex. 2479, 254; Ex. 2480, Abs; Ex. 2481, 1611; Ex. 2475,
20 126:4-127:14; MF92. As Doyon explains, these are the data that show successful CRISPR-Cas9
21 cleavage. Ex. 2476, ¶¶36-38.

22 **3. Figure 38B discloses successful target DNA cleavage in a eukaryotic cell.**

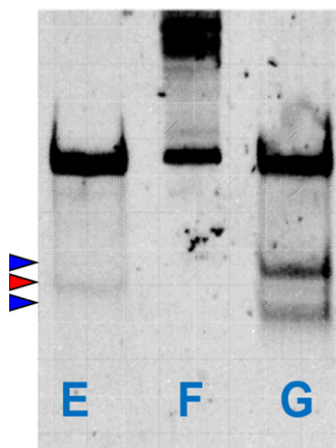
23 A POSA would have also understood that the same positive results are depicted in Figure
24 38B. Ex. 2476, ¶¶45-52; Ex. 2003, Fig. 38B. Turchi's annotated Figure 38B is shown below:



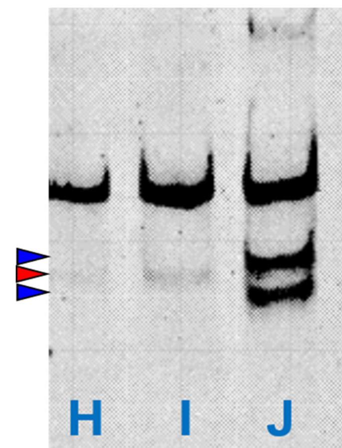
1

2 Ex. 1410, ¶86; Ex. 2003, Fig. 38B.

3 It is undisputed that Figure 38B and 36E used *the same ZFN positive control*. Ex. 2003,
4 ¶¶[00417], [00422], Figs. 36E, 38B; Ex. 2476, ¶46; Ex. 2475, 136:19-21; MF95. It is also
5 undisputed that the sgRNA-Cas9-cleaved bands in Figure 38B (Turchi's band 2 above) appear
6 only in Lanes F, H, and I and *do not appear in any of the negative control lanes*. Ex. 2003, Fig.
7 38B; Ex. 2476, ¶¶47-48; Ex. 2475, 137:22-138:15; MF96. Additionally, it is undisputed that the
8 sgRNA-Cas9-cleaved bands (red arrows below) migrate at a position in between the two ZFN-
9 cleaved bands (blue arrows below), just as they did in Figure 36E. Ex. 2003, Fig. 38B; Ex. 2476,
10 ¶¶47-48; Ex. 2475, 137:12-21; MF97. This is clear in Doyon's annotation of the figures:



Ex. 2003, Fig. 36E



Ex. 2003, Fig. 38B

1 Ex. 2476, ¶47. The relative position of the sgRNA-Cas9-cleaved bands is thus completely
2 consistent with what was expected. Turchi merely criticizes the calculated band sizes based on
3 the molecular weight marker, without giving any credible consideration to the possibility that the
4 marker was inadvertently mislabeled. Ex. 2476, ¶¶49-51; Ex. 1410, ¶¶91-92.
5 Because the Cas9-cleaved bands migrated at their expected position in between the two
6 ZFN-cleaved bands, a POSA would have understood and concluded that Figure 38B
7 demonstrates positive sgRNA-Cas9 cleavage results. The only difference between Figures 36E
8 and 38B might be the molecular weight markers. Ex. 2476, ¶¶49-51. But here, that is irrelevant.
9 Doyon explains there were molecular weight markers available in 2012-2013 that would have
10 produced the ladder depicted in Figure 38B. *Id.* Turchi also admitted that "in 2012, you could
11 purchase molecular weight markers that were not uniform differences between the individual
12 DNAs … It didn't necessarily have to be equal increments." Ex. 2475, 57:2-21. At any rate, the
13 potential for an inadvertently mislabeled marker in Figure 38B would not have undermined the
14 POSA's understanding of clear, positive results in the figure, especially when viewed in the
15 context of Figure 36E. Ex. 2476, ¶¶45-50.
16 Turchi baldly alleges that a POSA would "look for an explanation of these
17 inconsistencies from the applicants in order to rule out unexpected sources of the purported
18 target cleavage bands." Ex. 1410, ¶85. As discussed above, however, the results disclosed in
19 Figures 36E and 38B are *not inconsistent*. Ex. 2476, ¶¶35-50. Moreover, any conclusion with
20 respect to Figure 38B would not detract from the positive results of Figure 36E because the data
21 are from two independent experiments—neither of which is required for a CRTP. Ex. 2476,
22 ¶¶35-50. Nor would such a view change the fact that P3 describes the sgRNA-Cas9 system of
23 Count 1 and all the information a POSA needs to use the system in eukaryotic cells. *Id.*, ¶¶27-30.

ToolGen's argument that a POSA would require confirmatory sequencing also lacks merit. Paper 171, 13:16-17. A POSA would have known at the time that the Surveyor assay was a validated, routine method for detecting target DNA cleavage in the absence of any sequencing data. Ex. 2476, ¶¶51-52. For example, Miller 2007 (Ex. 1225), Doyon 2010 (Ex. 1216), and Miller 2010 (Ex. 2227) each discloses using the Surveyor assay *as a standalone technique* to detect various genome modifications induced by ZFNs or TALENs. Ex. 1225, Fig. 6b and p. 784 ("In control studies comparing gene modification rates deduced by the Surveyor assay and direct sequencing the two methods have generally agreed to within a factor of 0.05."); Ex. 1216, 459; Ex. 2227, 145; Ex. 2476, ¶51; MF98. Doudna and Jinek also explained this well-known concept in their 2013 comments to the *eLife* reviewers, "the Surveyor assay is a well-established stand-alone method of detecting the presence of mutations introduced by NHEJ events as a consequence of double-stranded DNA breaks." Ex. 2499, 17.

On page 6, lines 17-19, ToolGen speculates that the positive DNA cleavage results disclosed in P3's Example 2 could be "off-target cleavage of the DNA (that is, cleavage at sites other than the intended site)." But ToolGen provides no *evidence* to support its speculation. And even if some off-target cleavage existed, as it did for predecessor technologies ZFNs and TALENs, that does not undermine the conclusion that site-specific DNA cleavage occurred, as evidenced by positive cleavage bands of the expected sizes that are not present in the negative control lanes. In other words, even if there were some low level of off-target cleavage in the experiments of Example 2, the targeted cleavage data would still fall within the scope of Count 1. P3 discloses, and the POSA would have known, that prior ZFN and TALENs technologies exhibited some off-target effects, but were still useful gene-editing technologies because off-target effects are of little or no concern outside of the human therapeutic context. Ex. 2003,

1 ¶[0001]-[0002]. Ex. 2476, ¶¶43-44. The PTAB should reject ToolGen's speculations.

2 **4. Turchi's "gentle lysis" argument has no basis in fact.**

3 On page 17, lines 1-3 of the motion, it is argued that the cell lysis procedure in Example 2
4 "foreclosed any ability for a POSA to rely on Figures 38B and 36E as showing intracellular
5 cleavage by failing to 'turn off' the Cas9 in the lysate before extracting the DNA studied in those
6 figures." Paper 171, 17:1-3. The response is that a POSA would have understood the target DNA
7 cleavage in Figures 36 and 38 to have occurred *in the cells*, not in the lysates, for several reasons.

8 *First*, ***S. pyogenes*** **Cas9 is not active at 4°C (i.e., ice cold conditions)**. A POSA would
9 have known that *S. pyogenes* is the pathogen that causes strep throat in humans and thus
10 naturally grows at *warm temperatures* (e.g., 37 °C). Ex. 2223, 3928; Ex. 2476, ¶54. A POSA
11 thus would not have expected *S. pyogenes* Cas9 to be active at cold temperatures that do not
12 permit *S. pyogenes* bacteria growth (e.g., 4 °C). *Id.* Indeed, later publications confirmed that the
13 active temperature for *S. pyogenes* Cas9 is between 20 and 44 °C. *See e.g.,* Ex. 2472, 23103; Ex.
14 2473, 4, 6; Ex. 2476, ¶54; MF99. But Turchi did not consider those publications when forming
15 the opinions in his declaration. Ex. 2475, 140:10-12, 142:4-6. And P3 expressly discloses that
16 the transfected cells were first washed in "PBS" and then lysed in "250 µl lysis buffer" (i.e., a
17 very small volume) while being "rocked for 10 min at 4°C." Ex. 2003, ¶[00410]. P3's reference
18 to rocking the lysates at 4 °C is consistent with a POSA's knowledge that it was standard
19 practice to keep all lysis conditions at 4°C or lower, such as using *ice cold PBS* for the wash and
20 *ice cold lysis buffer* to begin the cell lysis, in addition to carrying out the lysis itself at 4 °C. Ex.
21 2476, ¶¶55-56. Thus, a POSA would have expected that the cell lysates in Example 2 would
22 have immediately dropped to 4°C, preventing any Cas9-mediated DNA cleavage in the ice-cold
23 lysates. *Id.*; MF99.

1 *Second*, **cell-free non-homologous end-joining (NHEJ) would not have occurred in**
2 **the lysate**. *See* Ex. 1229, 14067; Ex. 2489, 4; Ex. 2490, 850; Ex. 2491, 1; Ex. 2476, ¶¶57-60. For
3 a Surveyor assay to detect target DNA cleavage (as in P3's Figure 36E and 38B), NHEJ must
4 occur at the cleavage site to create insertions or deletions in the target DNA while repairing the
5 cleaved DNA. Ex. 2476, ¶57. Turchi speculates that NHEJ *could* have occurred in the cell
6 lysates described in P3, but a POSA would not have believed his speculations because Turchi
7 relies on references disclosing preparation of *nuclear extracts* for cell-free NHEJ reactions. A
8 POSA would not have found cell-free NHEJ reactions informative for assessing the biochemistry
9 of whole cell lysates for several reasons. (1) A POSA would have known that cell-free NHEJ
10 reactions require very specific *nuclear extraction method*s (e.g., special buffers, multiple
11 centrifugation steps including ultracentrifugation, and in some cases, dialysis) in order to
12 concentrate the active NHEJ components for the reaction. Ex. 2476, ¶¶57-58; Ex. 1234. 4147;
13 Ex. 1229, 14066; Ex. 2490, 858; Ex. 2489, 2. P3's whole cell lysate procedure does not use these
14 techniques. *Id.* (2) A POSA also would have known that cell-free NHEJ reactions *require adding*
15 *ATP* to the reactions. Ex. 1229, 14067; Ex. 2489, 3-4; Ex. 2490, 850; Ex. 2491, 1; Ex. 2476, ¶59;
16 MF100. Yet, P3 does not disclose adding any ATP during the lysis procedure. Ex. 2003,
17 ¶[00410]; Ex. 2476, ¶59. Turchi makes a single conclusory assertion that "ATP within the cell—
18 for example cytoplasmic and mitochondrial ATP" would be "available for use in the NHEJ
19 reactions," but he again fails to provide any basis for this assertion. Ex. 1410, ¶98. (3) A POSA
20 also would have known that cell-free NHEJ reactions require incubating the reactions at *much*
21 *warmer temperatures* (typically 37°C), which again is far different from P3's whole cell lysates
22 kept at ice-cold 4°C. Ex. 2476, ¶60; Ex. 2490, 858; Ex. 2489, 2. Thus, a POSA would have
23 understood that any NHEJ that occurred in Example 2 (and thus caused indels detected by the

1 Surveyor assay), occurred *in the cells, before lysis. Id.*, ¶¶57-62.

2 ToolGen also alleges that the sgRNA-Cas9 cleavage efficiency "as measured by band
3 intensity" indicates that DNA cleavage occurred only in the lysates, not in the cells. Paper 171,
4 16:5-11. But as Doyon explains, a POSA would not attempt to draw any conclusions regarding
5 relative DNA cleavage efficiency by comparing the "band intensity" from completely different
6 experiments performed under different conditions. Ex. 2476, ¶63; Ex. 2003, ¶¶[00412], [00415].
7 The PTAB should reject Turchi's apples-to-oranges comparison. *Id.*

8 ***V. CONCLUSION***

9 P3 is unquestionably a CRTP of Count 1. Toolgen's Motion 2 should be denied.

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10

APPENDIX 1: LIST OF EXHIBITS

Exhibit No.	Description
1018	U.S. Provisional Application No. 61/757,640, filed January 28, 2013
1201	Barrangou et al., CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes, Science, 315, 1709–12 (2007), with Supplementary Material
1202	Brouns et al., Small CRISPR RNAs Guide Antiviral Defense in Prokaryotes, Science, 321, 960–964 (2008)
1216	Doyon *et al.*, Transient cold shock enhances zinc-finger nuclease-mediated gene disruption, *Nature Methods*, 7, 459–460 (2010)
1218	Mashal et al., Detection of Mutations by Cleavage of DNA Heteroduplexes with Bacteriophage Resolvases, Nature, 9, 177–183 (1995)
1225	Miller *et al*., An improved zinc-finger nuclease architecture for highly specific genome editing, *Nature Biotechnology*, 25, 778–785 (2007)
1227	Li and Kelly, Simian Virus 40 DNA Replication In Vitro: Specificity of Initiation and Evidence for Bidirectional Replication, Molecular and Cell Biology, 5, 1238–1246 (1985)
1229	Baumann and West, DNA end-joining catalyzed by human cell-free extracts, *Proc. Natl. Acad. Sci*., 95, 14066–14070 (1998)
1233	Cole-Strauss et al., Targeted gene repair directed by the chimeric RNA/DNA oligonucleotide in a mammalian cell-free extract, Nucleic Acids Research, 27(5), 1323–1330 (1999)
1234	Fairman et al., Multiple Components Are Involved in The Efficient Joining of Double Stranded DNA Breaks in Human Cell Extracts, Nucleic Acids Research, 20(16), 4145–4152 (1992)
1235	Takata et al., Homologous Recombination and Non-Homologous End-Joining Pathways of DNA Double-Strand Break Repair Have Overlapping Roles in the Maintenance of Chromosomal Integrity in Vertebrate Cells, The EMBO Journal, 17(18), 5467–5508 (1998)
1236	Yang et al., Optimized Codon Usage and Chromophore Mutations Provide Enhanced Sensitivity with the Green Fluorescent Protein, Nucleic Acids Research, 24(22), 4592–93 (1996)
1410	May 20, 2021 Declaration of John J. Turchi, Ph.D.
2003	U.S. Provisional Appl. No. 61/757,640, filed January 28, 2013
2021	Declaration of Rodolphe Barrangou, Ph.D.
2030	Cong, L. *et al*., “Multiplex Genome Engineering Using CRISPR/Cas Systems,” *Science* 339(6121):819-823 (2013) with Supplemental Material.
2031	Jinek, M., *et al*., “A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity,” *Science* 337(6096):816-821 (2012) with Supplementary Information
2033	Jinek, M., *et al*., “RNA-programmed genome editing in human cells,” *eLife*, 2:e00471, 1-9 (2013)
2114	Doyon, J.B., *et al*., “Rapid and efficient clathrin-mediated endocytosis revealed in genome-edited mammalian cells,” *Nat. Cell Biol*., 13(3):331-337 (2011)
2214	Mali, P., *et al*., “CAS9 transcriptional activators for target specificity screening

Exhibit No.	Description
	and paired nickases for cooperative genome engineering," *Nat Biotechnol.*, 31(9):833-838, pp.1-17 (2013)
2215	Barrangou, R., "RNA-mediated programmable DNA cleavage," *Nature Biotechology*, 30(9):836-838 (2012)
2223	Dalton, T.L., and Scott, J.R., "CovS Inactivates CovR and Is Required for Growth under Conditions of General Stress in Streptococcus pyogenes," *Journal of Bacteriology*, 186(12):3928-3937 (2004)
2227	Miller, J.C., *et al*., "A TALE nuclease architecture for efficient genome editing," *Nat. Biotechnology*, 29:143-148, Supplementary Information (2010)
2345	Mali, P. *et al*., "RNA-Guided Human Genome Engineering via Cas9," *Science* 339(6121): 823-826 (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)
2473	Mougiakos, I., *et al*., "Characterizing a thermostable Cas9 for bacterial genome editing and silencing," *Nature Communs*., 8(1647):1-11 (2017)
2474	Deposition Transcript of Byran Cullen, Ph.D., with errata, Patent Interference No. 106,127 (June 22, 2021)
2475	Deposition Transcript of John Turchi, Ph.D., with errata, Patent Interference No. 106,127 (June 29, 2021)
2476	Third Declaration of Yannick Doyon, Ph.D.
2479	Guschin, D.Y., *et al*., Chapter 15: A Rapid and General Assay for Monitoring Endogenus Gene Modification, Mackay, J.P. and Segal, D.J., (eds.) Engineered Zinc Finger Proteins, Methods in Molecular Biology, vol. 649, pp. 247-256, Springer Science+Business Media, LLC, Germany (2010)
2480	Delwart, E.L., *et al*., "Genetic Relationships Determined by a DNA Heteroduplex Mobility Assay: Analysis of HIV-1 *env* Genes," *Science* 262: 1257-1261 (1993)
2481	Espejo, R.T., *et al*., "PAGE analysis of the heteroduplexes formed between PCR-amplified 165 rRNA genes: estimation of sequence similarity and rDNA complexity," *Microbiology* 144: 1611-1617 (1998)
2482	Bikard, D., *et al*., "Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas System," *Nucleic Acids Research*, 41(15): 7429–7437 (2013)
2483	Tzur, Y.B., *et al*., "Heritable Custom Genomic Modifications in *Caenorhabditis elegans* via a CRISPR–Cas9 System," *Genetics*, 195: 1181–1185 (2013)
2484	Koo, T., *et al*., "Measuring and Reducing Off-Target Activities of Programmable Nucleases Including CRISPR-Cas9," *Mol. Cells* 38(6): 475-481 (2015)
2485	Ding, K., *et al*., "Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs," *Cell Stem Cell*. 12(4): 393–394 (2013)
2486	Musunuru, K., "Genome editing of human pluripotent stem cells to generate

Exhibit No.	Description
	human cellular disease models," *Dis Model Mech*. 6(4): 896–904 (2013)
2487	Richter, H., *et al*., "Exploiting CRISPR/Cas: Interference Mechanisms and Applications," *Int. J. Mol. Sci.* 14: 14518-14531 (2013)
2488	Ousterout, D. G., *et al*., "Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy," *Nat. Commun.* 6(6244): 1-13 (2014)
2489	Diggle, C.P., *et al*., "Development of a rapid, small-scale DNA repair assay for use on clinical samples," *Nucleic Acids Research* 31(15): e83 (2003)
2490	Budman, J. and Chu, G., "Processing of DNA for nonhomologous end-joining by cell-free extract," *The EMBO Journal* 24: 849-860 (2005)
2491	Deshpande, R.A., *et al*., "DNA-dependent protein kinase promotes DNA end processing by MRN and CtIP," *Sci. Adv*. 6 :1-12 (2020)
2492	Gaj, T., *et al*., "ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering," *Trends in Biotechnology* 31(7): 397-405 (2013)
2493	Katic, I. and Groβhans, H., "Targeted Heritable Mutation and Gene Conversion by Cas9-CRISPR in *Caenorhabditis elegans*," *Genetics* 195: 1173–1176 (2013)
2494	Kennedy, E.M. and Cullen, B.R., "Gene Editing: A New Tool for Viral Disease," *Annu. Rev. Med*. 68:401–11 (2017)
2495	Perez-Pinera, P., *et al*., "RNA-guided gene activation by CRISPR-Cas9-based transcription factors," *Nat. Meth*. 10(10): 973-978 (2013)
2496	Li, J-F., *et al*., "Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9," *Nat. Biotechnol.* 31(8): 688-691 (2013)
2497	Genomic Cruise Missiles, *Science,* 338:1526-1527 (2012)
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2502	Wu, X., *et al*., "Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells," *Nat. Biotechnol*. 32(7): 670-676 (2014)
2507	Nelson, C.E., *et al*., "In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy," *Science*, 351(6271):403-407 (2016)
2508	Nishimasu, H., *et al*., "Structural Basis for the Altered PAM Recognition by Engineered CRISPR-Cpf1," *Molecular Cell*, 67:139–147 (2017)
2509	Sander, J.D. and Joung, J.K., "CRISPR-Cas systems for editing, regulating and targeting genomes," *Nat. Biotechnol*., 32(4):347-355 (2014)
2512	Mali, P. *et al*., "RNA-Guided Human Genome Engineering via Cas9," *Science* 339(6121): 823-826 (2013), with Supplemental Materials

1 ***APPENDIX 2: STATEMENT OF MATERIAL FACTS***

2 **ToolGen's Facts 1-70:**

3 **1.** Count 1 requires a CRISPR-Cas9 system alternatively "capable of cleaving or editing the target
4 DNA molecule or modulating transcription of at least one gene encoded by the target DNA
5 molecule" or which "mediate double stranded cleavage at the [DNA] target sequence." Paper
6 1 at 5-7.

7 **Response:** Admitted.

8 **2.** At the time the applicants filed P3, adapting the native prokaryotic CRISPR-Cas9 system to
9 cleave DNA within eukaryotic cells was highly unpredictable. Ex. 1410 ¶¶ 41-42; Ex. 1201;
10 Ex. 1202.

11 **Response:** Denied.

12 **3.** Example 2 describes P3's only experiments attempting DNA cleavage in a eukaryotic cell. Ex.
13 1018 ¶¶ [00408–00423]; Ex. 1410 ¶¶ 66, 74.

14 **Response:** Admitted.

15 **4.** The P3 applicants conducted the Example 2 experiments on HEK293T human kidney cells.
16 Ex. 1018 ¶¶ [00410, 00411, 00416–00418].

17 **Response:** Admitted.

18 **5.** In P3's experiments testing for cleavage, the applicants transfected the cells with plasmids
19 coding Cas9 protein and a single guide RNA molecule ("sgRNA") targeting a sequence in the
20 human CLTA gene. Ex. 1018 ¶¶ [00410, 00411, 00416–00418]; Ex. 1410 ¶¶ 68, 76.

21 **Response:** Admitted.

22 **6.** In P3's experiments testing for cleavage, forty-eight hours after transfection the cells were
23 lysed using a gentle lysing method, which included a lysis buffer that was supplemented with
24 a Roche Protease Inhibitor cocktail—which retains protein and enzyme activity—and

1 magnesium, which is required for the cleavage reaction. Ex. 1018 ¶¶ [00378 and Fig. 21,
2 00410]; Ex. 1410 ¶ 75.

3 **Response:** Admitted that P3's Example 2 describes a procedure for cell lysis in ¶[00410];
4 otherwise denied.

5 **7.** A POSA would understand that the cell lysates used in the experiments depicted in Figures
6 38B, 36E, and 37A were all prepared using the same gentle lysing method. Ex. 1018 ¶ [00410];
7 Ex. 1410 ¶ 97.

8 **Response:** Admitted that P3's Example 2 describes a procedure for cell lysis in ¶[00410];
9 otherwise denied.

10 **8.** Figures 38B and 36E portray the results of Surveyor assays using extracted genomic DNA
11 from cell lysates that were amplified by PCR with primers targeted to the human CLTA gene
12 containing the target sequence. Ex. 1018 ¶¶ [00408, 00410, 00412, 00416-00417, 00421-
13 00422]; Ex. 1410 ¶¶ 58-60, 77-79.

14 **Response:** Admitted that P3's Example 2 describes a procedure for Surveyor assay in, e.g.,
15 ¶[00412] and Surveyor assay data in Figures 36E and 38B; otherwise denied.

16 **9.** The Surveyor assays in Example 2 were performed on an unpurified PCR product. Ex. 1018 ¶
17 [00412].

18 **Response:** Admitted that P3's Example 2 describes a procedure for Surveyor assay in
19 ¶[00412]; otherwise denied.

20 **10.** Surveyor assays detect mismatched base pairs resulting from non-homologous end joining
21 ("NHEJ") DNA repair, which occurs after cleavage by the Cas9-sgRNA complex, and cleave
22 DNA into fragments at the site of the mismatched base pairs. Ex. 1018 ¶ [00412]; Ex. 1235;
23 Ex. 1218; Ex. 1225.

1 **Response:** Admitted.

2 **11.** Figures 38B and 36E portray the results of Surveyor assay products subjected to gel
3 electrophoresis to separate the digestion products by size into visible bands. Ex. 1018 ¶¶
4 [00412, 00417, 00422].

5 **Response:** Admitted that P3's Example 2 describes a procedure for Surveyor assay in, e.g.,
6 ¶[00412] and Surveyor assay data in Figures 36E and 38B; otherwise denied.

7 **12.** A POSA would not have considered off-target cleavage of the DNA (that is, cleavage at sites
8 other than the intended site) to successfully demonstrate DNA cleavage by a CRISPR-Cas9
9 system. Ex. 1410 ¶ 85; Ex. 1210.

10 **Response:** Denied.

11 **13.** The figure titled "P3 (Ex. 1018) Example 2 - PCR Amplicon Sequence" at Page 7, Line 6
12 above depicts the sequence of the PCR amplicon generated in Example 2's experiments that
13 resulted in Figures 38B and 36E. Ex. 1410 ¶ 77.

14 **Response:** Denied.

15 **14.** The figure titled "P3 (Ex. 1018) Example 2 - PCR Amplicon Sequence" at Page 7, Line 6
16 above depicts the primers, target sequence, predicted Cas9-sgRNA cleavage site, ZFN binding
17 sites, and ZFN predicted cleavage sites for Example 2's experiments that resulted in Figures
18 38B and 36E. Ex. 1410 ¶ 77.

19 **Response:** Unable to admit or deny.

20 **15.** If Cas9-sgRNA cleavage at the target site was successful, a POSA would expect a 369 bp PCR
21 product band; ~183 and ~186 bp Cas9-sgRNA cleavage bands; and/or ~162-169 and ~200-207
22 bp ZFN cleavage bands in the Surveyor assays depicted in Figures 38B and 36E. Ex. 1410 ¶¶
23 78, 80.

1 **Response:** Admitted that a POSA would expect to see a cleavage band in the sgRNA-Cas9
2 samples; otherwise denied.
3 **16.** A POSA would not expect any bands larger than the PCR product band in the Surveyor assays
4 depicted in Figures 38B and 36E. Ex. 1410 ¶¶ 78, 80.
5 **Response:** Denied.
6 **17.** Figures 38B and 36E depict the only experimental results in P3 purporting to show cleavage
7 of target eukaryotic DNA by a CRISPR-Cas9 system in a eukaryotic cell. Ex. 1018 ¶¶ [00417,
8 00422].
9 **Response:** Admitted that Figures 36E and 38B show cleavage of target DNA by a sgRNA-
10 Cas9 system in a eukaryotic cell.
11 **18.** Figure 38B would not have shown successful CRISPR-Cas9 cleavage at the target site to a
12 POSA. Ex. 1410 ¶¶ 86-92.
13 **Response:** Denied.
14 **19.** Figure 38B shows bands where there should be none. Ex. 1410 ¶¶ 86-92.
15 **Response:** Denied.
16 **20.** Figure 38B shows a ~378 bp PCR band (band 1) that is consistent in size with the predicted
17 369 bp full-length PCR band. Ex. 1410 ¶ 88.
18 **Response:** Admitted that Figure 38B shows a full-length PCR amplicon band in each lane;
19 otherwise denied.
20 **21.** Figure 38B shows digestion products of ~280 bp (band 2) that are not the expected ~185 bp
21 size of a Cas9-sgRNA cleavage band in all the Cas9-sgRNA lanes (Lanes F, H, and I). Ex.
22 1410 ¶ 88.
23 **Response:** Admitted that Figure 38B shows successful sgRNA-Cas9-mediated target DNA

1 cleavage in Lanes F, H, and I; otherwise denied.

2 **22.** Figure 38B shows no band at all close to the expected ~185 bp size of a Cas9-sgRNA cleavage
3 band. Ex. 1410 ¶ 88.

4 **Response:** Denied.

5 **23.** Figure 38B's ~280 bp digestion band (band 2) is irreconcilable with its ~378 bp PCR band
6 (band 1) because there would be an unaccounted for ~98 bp DNA fragment. Ex. 1410 ¶ 88.

7 **Response:** Denied.

8 **24.** Figure 38B shows several unexpected bands—larger than the PCR band—of unknown origin
9 that disappear upon digestion (bands 5 and 6) that could have been the origin of the ~280 bp
10 digestion band (band 2). Ex. 1410 ¶ 90.

11 **Response:** Denied.

12 **25.** Figure 38B's ZFN construct bands at ~292 bp and ~256 bp (bands 3 and 4) are not the expected
13 sizes, and it is not possible to get these two digestion product bands from the ~378 bp PCR
14 band (band 1). Ex. 1410 ¶ 89.

15 **Response:** Denied.

16 **26.** Figure 38B's Lane A is not simply mislabeled; the PCR band is the correct size, and any
17 "corrections" to Lane A would only serve to shift the PCR band away from its expected size.
18 Ex. 1410 ¶¶ 91-92.

19 **Response:** Denied.

20 **27.** A POSA at the time P3 was filed would find Figure 38B to be unreliable and would have
21 disregarded it in its entirety. Ex. 1410 ¶ 92.

22 **Response:** Denied.

23 **28.** Figure 36E does not show successful CRISPR-Cas9 cleavage at the target site. Ex. 1410 ¶ 85.

1 **Response:** Denied.

2 **29.** Based on the experimental protocols, a POSA would expect the purported cleavage bands to
3 be identical in Figures 38B and 36E, and if they were not, a POSA would question both results.
4 Ex. 1410 ¶¶ 82-85, 87.

5 **Response:** Denied.

6 **30.** A POSA would view the data in Figure 36E as too sloppy and unexplained to be reliable
7 because almost every lane inexplicably has more than one unexpected band, making it
8 impossible to determine the origin of each digestion product band. Ex. 1410 ¶¶ 82-85.

9 **Response:** Denied.

10 **31.** Figure 36E's PCR band (band 1) measures ~352 bp, which is consistent with its expected size
11 of 369 bp. Ex. 1410 ¶ 82.

12 **Response:** Admitted that Figure 36E shows a full-length PCR amplicon band in each lane;
13 otherwise denied.

14 **32.** The applicants rely on a single ~173 bp band (band 5) in Lane E of Figure 36E to indicate
15 CRISPR-Cas9 cleavage at the target site. Ex. 1018 ¶ [00418]; Ex. 1410 ¶ 83.

16 **Response:** Admitted that Figure 36E shows successful sgRNA-Cas9-mediated target DNA
17 cleavage in Lane E; otherwise denied.

18 **33.** Figure 36E has an unexpected ~477 bp band (band 2) in Lanes B, D, and F that disappears
19 upon digestion in the Surveyor assay Lanes E and G. Ex. 1410 ¶ 83.

20 **Response:** Denied.

21 **34.** Figure 36E's alleged cleavage band (band 5) could be a digestion product of the unexpected
22 ~477 bp band (band 2). Ex. 1410 ¶ 83.

23 **Response:** Denied.

35. Figure 36E has an unexpected digestion band at ~297/312 bp (band 3) in Lanes E and G. Ex. 1410 ¶ 83.

Response: Denied.

36. Figure 36E's unexpected ~477 bp band (band 2) could have been digested into the ~173 and ~297/312 bp digestion product bands (bands 5 and 3, respectively). Ex. 1410 ¶ 83.

Response: Denied.

37. Figure 36E has an unexpected digestion product band at ~252 bp (band 4) in Lanes E and G, which could reflect the unexpected ~477 bp band (band 2) being digested into two equal DNA fragments. Ex. 1410 ¶¶ 83, 85.

Response: Denied.

38. A POSA would be unconvinced of the applicants' possession of an embodiment within Count 1 based on the results in Figure 36E. Ex. 1410 ¶¶ 83, 85.

Response: Denied.

39. There is a digestion band in Lane G—between the two ZFN cleavage bands—of similar size and intensity as the purported cleavage band (band 5) in Lane E. Ex. 1410 ¶ 84.

Response: Denied.

40. A POSA would conclude that band 5 in Lane E is simply a digestion product of one of the larger unexpected bands (band 2) in the undigested lanes (Lanes, B, D, and F), and not indicative of successful Cas9 cleavage at the target site. Ex. 1410 ¶¶ 84-85.

Response: Denied.

41. A POSA would find the Figure 36E gel inconclusive. Ex. 1410 ¶ 85.

Response: Denied.

42. In view of Figures 38B and 36E's results, a POSA would have considered the well-known

1 procedure of sequencing the PCR product as an appropriate way to confirm and characterize
2 any potential cleavage in the PCR-amplified region of DNA surrounding the target sequence.
3 Ex. 1410 ¶¶ 62, 93-94.

4 **Response:** Denied.

5 **43.** The presence or absence of insertions or deletions at the target sequence in the sequencing
6 results would have definitively indicated whether or not there was successful cleavage, but not
7 where that cleavage occurred. Ex. 1410 ¶ 62.

8 **Response:** Admitted that sequencing can be used to identify specific insertions or deletions
9 resulting from targeted DNA cleavage, but sequencing is not necessary to determine that
10 targeted DNA cleavage occurred.

11 **44.** P3 has no sequencing results for its experiments in Example 2. Ex. 1018.

12 **Response:** Admitted.

13 **45.** Assays that use cleavage to affect a directly measurable result can be more reliable at detecting
14 cleavage because—unlike the Surveyor assay—they do not require genomic DNA extraction,
15 amplification, digestion, and gel electrophoresis, all of which can be highly error prone. Ex.
16 1410 ¶¶ 61, 95; Ex. 1236; Ex. 1219.

17 **Response:** Admitted that Surveyor assays typically require genomic DNA extraction,
18 DNA amplification, and gel electrophoresis; otherwise denied.

19 **46.** A POSA would require confirmatory sequencing, or more accurate assay results, to conclude
20 that the applicants possessed a CRISPR-Cas9 system that achieved target DNA cleavage in a
21 eukaryotic cell. Ex. 1410 ¶¶ 85, 92, 93.

22 **Response:** Denied.

1 **47.** The method used to prepare the cell lysates in the experiments in Example 2 allowed for
2 CRISPR-Cas9 cleavage activity outside of the cells in the cell lysate. Ex. 1410 ¶¶ 72, 97.
3 **Response:** Denied.
4 **48.** Any cleavage shown in Figures 38B and 36E could have occurred outside the cells, in the
5 lysate. Ex. 1410 ¶¶ 66, 72, 101-103.
6 **Response:** Denied.
7 **49.** Extracellular cleavage in the cell lysate does not satisfy Count 1. Ex. 1410 ¶¶ 66, 72, 101- 103;
8 Paper 1 at 5-7.
9 **Response:** Admitted that Count 1 requires a CRISPR-Cas9 system capable of cleaving or
10 editing a target DNA molecule or modulating transcription of at least one gene encoded by
11 a target DNA molecule in a eukaryotic cell; otherwise denied.
12 **50.** Figure 37A depicts the results of an experiment that assessed extracellular Cas9-sgRNA
13 cleavage of a donor plasmid. Ex. 1018 ¶¶ [00415, 00419, 00420]; Ex. 1410 ¶¶ 67; 71-72.
14 **Response:** Admitted that Figure 37A discloses results from an *in vitro* cleavage assay;
15 otherwise denied.
16 **51.** Figure 37A's experiment introduced the target sequence to the lysate with abundant donor
17 plasmid and a "cleavage buffer" to promote Cas9-sgRNA activity in the lysate. Ex. 1018 ¶¶
18 [00410, 00412, 00415, 00419-00420].
19 **Response:** Admitted that P3 describes incubating the CLTA-RFP donor plasmid with cell
20 lysates for one hour at 37°C, followed by digestion with XhoI for 30 minutes at 37°C;
21 otherwise denied.
22 **52.** During Figure 37A's experiment, the addition of Proteinase K was the first step that inactivated
23 the Cas9. Ex. 1018 ¶¶ [00410, 00415].

1 **Response:** Denied.

2 **53.** Figure 37A reveals two Cas9-sgRNA cleavage bands of similar sizes and two ZFN control
3 cleavage bands also of similar sizes, which confirms the proximity of their target sequences.
4 Ex. 1410 ¶¶ 70, 105.

5 **Response:** Admitted that Figure 37A shows successful target DNA cleavage bands in the
6 sgRNA-Cas9 and ZFN lanes; otherwise denied.

7 **54.** Figure 37A's assay could not have detected intracellular cleavage, and was only meant to
8 assess Cas9 activity by detection of extracellular cleavage of the donor plasmid. Ex. 1410 ¶
9 71.

10 **Response:** Admitted that the experiment in Figure 37A discloses detection of extracellular
11 cleavage of a plasmid target DNA; otherwise denied.

12 **55.** The presence of two bands in Figure 37A's experiment indicates to a POSA that the plasmid
13 was successfully cleaved extracellularly at the predicted Cas9-sgRNA or ZFN target site and
14 the donor plasmid restriction enzyme site, while one band would indicate only the restriction
15 site was cleaved Ex. 1410 ¶¶ 66, 70, 105.

16 **Response:** Admitted that Figure 37A shows successful target DNA cleavage bands in the
17 sgRNA-Cas9 and ZFN lanes; otherwise denied.

18 **56.** Figure 37A's results prove the gentle lysing method successfully preserved Cas9 activity in
19 the cell lysate. Ex. 1410 ¶ 70.

20 **Response:** Admitted that Figure 37A shows successful target DNA cleavage bands in the
21 sgRNA-Cas9 and ZFN lanes; otherwise denied.

22 **57.** Because the applicants preserved the activity of Cas9 in the lysate, Figures 38B and 36E's
23 results might show extracellular cleavage that occurred in cell lysates prepared using the gentle

1 lysing method. Ex. 1410 ¶¶ 96-108.

2 **Response:** Denied.

3 **58.** The Cas9-sgRNA and ZFN efficiency data is consistent with cleavage occurring in the lysate,

4 not in the cells. Ex. 1410 ¶¶ 72, 104-106.

5 **Response:** Denied.

6 **59.** The efficiency of the plasmid-expressed Cas9-sgRNA complex in Lane G is comparable to

7 that of the ZFN construct in Lane H of Figure 37A, as assessed by band intensity. Ex. 1410 ¶¶

8 105-106.

9 **Response:** Admitted that Lanes G and H in Figure 37A each shows successful target DNA

10 cleavage; otherwise denied.

11 **60.** In Figure 36E, the Cas9-sgRNA efficiency is drastically lower than that of the ZFN control

12 compared to their comparable efficiency in Figure 37A. Ex. 1410 ¶¶ 104-106.

13 **Response:** Admitted that Figure 36E discloses successful target DNA cleavage in a

14 eukaryotic cell; otherwise denied.

15 **61.** The transfected cells in the experiments depicted in Figures 38B and 36E were incubated at

16 37°C and were then gently lysed by rocking for ten minutes in a 4°C environment, but P3 does

17 not provide the lysate temperature at the end of the ten minutes or the subsequent storage

18 conditions. Ex. 1018 ¶ [00410]; Ex. 1410 ¶ 101.

19 **Response:** Admitted that P3 does not expressly disclose lysate storage temperatures;

20 otherwise denied.

21 **62.** Rocking is considered a gentle method of physical agitation that would not disrupt genomic

22 DNA or protein structure. Ex. 1410 ¶¶ 48, 98.

23 **Response:** Denied.

1 **63.** Use of a protease such as Proteinase K would have been critical in the Figure 38B and 36E
2 experiments to stop Cas9 activity because the lysate could not have immediately dropped from
3 37°C to 4°C. Ex. 1410 ¶¶ 48, 96-101; Ex. 1233.

4 **Response:** Denied.

5 **64.** NHEJ repair of DNA damage from Cas9-sgRNA cleavage could have occurred in the lysate.
6 Ex. 1410 ¶¶ 48, 100-102; Ex. 1227.

7 **Response:** Denied.

8 **65.** The low cleavage efficiencies reported by the applicants in Figure 36E (4% in purported Cas9
9 cleavage Lane E) are consistent with cleavage only occurring in the lysate. Ex. 1410 ¶¶ 103-
10 104.

11 **Response:** Denied.

12 **66.** A POSA cannot rely on Figures 38B and 36E as showing intracellular cleavage because the
13 applicants failed to "turn off" the Cas9 in the lysate before extracting the DNA. Ex. 1410 ¶¶
14 101, 103, 107-108; Ex. 1233; Ex. 1227.

15 **Response:** Denied.

16 **67.** Initial research in CRISPR-Cas9 systems had focused on their structure and function in
17 prokaryotic cells—the only cells where CRISPR-Cas9 systems naturally occur. Ex. 1410 ¶ 41;
18 Ex. 1202.

19 **Response:** Admitted that CRISPR-Cas9 naturally exists in prokaryotic cells; otherwise
20 denied.

21 **68.** There are significant differences between the structures of prokaryotes and eukaryotes, such
22 as eukaryotes containing a nucleus and tightly bound genomic DNA (chromatin) within it. Ex.
23 1410 ¶ 41.

1 **Response:** Admitted that the eukaryotic nucleus contains chromatin; otherwise denied.

2 **69.** Prokaryotes and eukaryotes also differ significantly in their intracellular functions, for example
3 in the necessary components for eukaryotic protein transcription, translation, folding and
4 complexing. Ex. 1410 ¶ 41.

5 **Response:** Denied.

6 **70.** There was uncertainty at the time of filing P3 as to whether for any given experiment the
7 CRISPR-Cas9 system could be expressed properly, survive eukaryotic cellular defense
8 mechanisms, undergo correct protein folding, enter the nucleus, access the highly organized
9 genetic material (chromatic) therein, and ultimately cleave the eukaryotic DNA in the target
10 location. Ex. 1410 ¶ 41.

11 **Response:** Denied.

1 **CVC's Additional Facts 71-102:**

2 **71.** Barrangou 2012 published in *Nature Biotechnology* in September 2012 and would have been
3 known to a POSA before January 28, 2013. Ex. 2215, 836; Ex. 2476, ¶¶18-19.

4 **72.** Barrangou 2012 described CVC's sgRNA-Cas9 system as a "synthetic tour de force" and a
5 "molecular scalpel" that may "outcompete ZFN and TALEN DNA scissors for precise
6 genomic surgery." Ex. 2215, 837-838.

7 **73.** The *Science* 2012 article published in December 2012 and would have been known to a
8 POSA before January 28, 2013. Ex. 2497, 1526; Ex. 2476, ¶¶18-19.

9 **74.** The *Science* 2012 article states that CRISPR-Cas9 "may one day challenge zinc finger
10 nucleases and TALENs as the core genome engineering technology." Ex. 2497, 1527.

11 **75.** The Mali 2013 article first published online on January 3, 2013 and would have been known
12 to a POSA before January 28, 2013. Ex. 2345; Ex. 2474, 179:10-15; Ex. 2476, ¶20.

13 **76.** Mali 2013 disclosed making expression vectors encoding a sgRNA and a Cas9 protein,
14 delivering the vectors to mammalian cell lines via liposome transfection, and reported
15 cleaving a target DNA sequence in a eukaryotic cell. Ex. 2512, Fig. 1, S7-S8; Ex. 2476, ¶20.

16 **77.** The Cong 2013 article first published online on January 3, 2013 and would have been known
17 to a POSA before January 28, 2013. Ex. 2030; Ex. 2474, 182:4-11; Ex. 2476, ¶21.

18 **78.** Cong 2013 disclosed transfecting eukaryotic cells with expression vectors encoding Cas9 and
19 a sgRNA and reported cleaving target DNA therein. Ex. 2030, Fig. 2; Ex. 2476, ¶21.

20 **79.** Turchi did not consider Ex. 2215, Ex. 2497, Ex. 2345, or Ex. 2030 in forming the opinions in
21 his declaration as reflected by the fact that he did not list these exhibits in his Appendix B.
22 Ex. 1410, Appx. B; Ex. 2475, 35:21-36:2.

23 **80.** Turchi's declaration focuses on the disclosures in P3's Example 2. Ex. 1410; Ex. 2476, ¶¶25-
24 26; Ex. 2003, ¶¶[00408]-[00423], Figs. 36-38.

1 **81.** The only disclosure in P3 that Turchi cites in his declaration that is not part of P3's Example
2 2 is a single citation to P3 ¶[00378] and Figure 21. Ex. 1410, ¶76.
3 **82.** Turchi testified that "demonstration of [DNA cleavage] would be required to support the
4 description that [the inventors] possess that invention." Ex. 2475, 85:7-17.
5 **83.** P3 discloses making and using sgRNA-Cas9 systems comprising a chimera A sgRNA and
6 the *S. pyogenes* Cas9 protein. Ex. 2003, ¶¶[0030]-[0031], [0037]-[0040], [00169], [00196],
7 [00364], [00401]-[00405], Figs. 1-3, 17; Ex. 2476, ¶27.
8 **84.** P3 discloses assembling chimera A sgRNA-Cas9 ribonucleoprotein complexes outside of a
9 eukaryotic cell. Ex. 2003, ¶¶[00366]-[00370], [00401], claim 98; Ex. 2476, ¶28.
10 **85.** P3 discloses delivering CRISPR-Cas9 systems to eukaryotic cells using "direct micro
11 injection." Ex. 2003, ¶¶[00108], [00261]; Ex. 2476, ¶28.
12 **86.** By January 28, 2013, direct micro injection was known to be "straightforward and effective."
13 Ex. 1410, ¶46; Ex. 2476, ¶28; Ex. 2003, ¶¶[00108], [00261].
14 **87.** P3 discloses eukaryotic expression vectors for expressing sgRNA and Cas9, eukaryotic
15 promoters (e.g., CMV, U6, and H1), codon optimization of the Cas9 gene, adding a NLS to
16 Cas9, routine vector transfection methods, and established cell lines suitable for transfection.
17 Ex. 2003, ¶¶[0090], [00206], [00243]-[00244], [00252], [00256]-[00259]; Ex. 2476, ¶29.
18 **88.** P3 discloses a human cell embodiment in Example 2 that meets all the elements of Count 1.
19 Ex. 2003, ¶¶[00408]-[00423], Fig. 36; Ex. 2475, 51:12-16, 52:22-53:1, 110:4-8, 108:20-
20 109:10, 110:9-111:7, 112:7-19, 112:20-113:4, 113:6-15, 116:20-118:1; Ex. 2476, ¶¶31-33.
21 **89.** Turchi admitted that Example 2 meets all elements of Count 1. Ex. 2475, 51:12-16, 52:22-
22 53:1, 110:4-8, 108:20-109:10, 110:9-111:7, 112:7-19, 112:20-113:4, 113:6-15, 116:20-118:1.
23 **90.** Turchi described the PCR amplicon band shown in P3 Figure 36E as "consistent" with his

1 predicted size. Ex. 1410, ¶83; Ex. 2003, Fig. 36E; Ex. 2476, ¶¶36-38.

2 **91.** P3 Figure 36E discloses DNA bands that are consistent with Turchi's estimated DNA band
3 sizes of "approximately 183 and 186 bp" for Cas9 cleavage products and "approximately
4 162-169 and 200-207 bp" for ZFN cleavage products. Ex. 2003, Fig. 36E; Ex. 1410, ¶80.

5 **92.** The DNA band Turchi labeled as "band 5" in Figure 36E appears only in Lane E. Ex. 1410,
6 ¶79; Ex. 2475, 126:4-127:14; Ex. 2476, ¶¶36-38; Ex. 2003, Fig. 36E.

7 **93.** The DNA band Turchi labeled as "band 5" in Figure 36E migrates at a position in between
8 the DNA bands Turchi labeled as "band 6" and "band 7." Ex. 1410, ¶79; Ex. 2475, 125:15-
9 126:3; Ex. 2476, ¶¶36-38; Ex. 2003, Fig. 36E.

10 **94.** Turchi did not consider any of Ex. 2479, Ex. 2480, or Ex. 2481 when forming the opinions in
11 his declaration as reflected by the fact that he did not list these exhibits in his Appendix B.
12 Ex. 1410, Appx. B; Ex. 2475, 35:21-36:2.

13 **95.** A POSA would have understood P3 Figures 36E and 38B use the same ZFN positive control.
14 Ex. 2003, ¶¶[00417], [00422], Figs. 36E, 38B; Ex. 2476, ¶46; Ex. 2475, 136:19-21.

15 **96.** The DNA band Turchi labeled as "band 2" in Figure 38B appears only in Lanes F, H, and I.
16 Ex. 1410, ¶86; Ex. 2475, 137:22-138:15; Ex. 2476, ¶¶47-48; Ex. 2003, Fig. 38B.

17 **97.** The DNA band Turchi labeled as "band 2" in Figure 38B migrates at a position in between
18 the DNA bands Turchi labeled as "band 3" and "band 4." Ex. 1410, ¶86; Ex. 2475, 137:12-
19 21; Ex. 2476, ¶¶47-48; Ex. 2003, Fig. 38B.

20 **98.** Miller 2007 (Ex. 1225), Doyon 2010 (Ex. 1216), and Miller 2010 (Ex. 2227) each discloses
21 using the Surveyor assay as a standalone technique to detect genome modifications induced
22 by ZFNs or TALENs. Ex. 2476, ¶51.

23 **99.** *S. pyogenes* Cas9 is not active at 4°C. Ex. 2472, 23103; Ex. 2473, 4, 6; Ex. 2476, ¶54.

1 **100.** Cell-free non-homologous end-joining (NHEJ) requires addition of ATP. Ex. 1229,
2 14067; Ex. 2489, 4; Ex. 2490, 850; Ex. 2491, 1; Ex. 2476, ¶¶59-62.
3 **101.** Jin-Soo Kim is a co-author on Koo 2015, which cites the Jinek 2013 paper along with
4 three other papers when stating, "we and others have reported RNA-guided genome editing
5 in human cells in January, 2013." Ex. 2484, 479; Ex. 2033.
6 **102.** Bryan Cullen is a co-author on Kennedy 2016, which cites the Jinek 2013 paper along with
7 two other papers when referring to groups that obtained DNA cleavage "in cultured
8 mammalian cells." Ex. 2494, 402; Ex. 2033.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **CVC OPPOSITION 2 (in support of benefit of Application No. 61/757,640)** was filed via the Interference Web Portal by 8:00 PM Eastern Time on July 15, 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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