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 REPLY 2
(opposing CVC benefit to P3)

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1 Reading CVC's Opposition 2 ("Opp."), one could be forgiven for misunderstanding which
2 document is at issue. To be clear, it is CVC's third provisional application, 61/757,640 ("P3")
3 (Ex. 1018). It is not Cong or Mali 2013. And it is certainly not Jinek 2013, which was published
4 *after* P3 and, unlike P3, contains sequencing data because the journal required it. But someone
5 else's successes, or even Jinek 2013, say nothing about whether *P3* demonstrates that *the*
6 *applicants* possessed a CRISPR-Cas9 system capable of functioning in a eukaryotic cell. Lacking
7 sufficient disclosure in P3, CVC pretends that ToolGen and Dr. Turchi considered only a working
8 example, repeatedly mischaracterizing Dr. Turchi's testimony in the process. To the contrary, Dr.
9 Turchi testified that a working example is one way to show possession and that he analyzed P3
10 and the pertinent art in the context of P3. And he showed that P3 contained aberrant methods and
11 unreliable results that belie *the applicants'* possession of the claimed invention. CVC fails to
12 explain these flaws, not least because CVC's expert analysis is just as unreliable as P3's data. Dr.
13 Doyon could not identify the origin of all the gels' unexpected bands, relied on papers published
14 after P3, and admitted he was unaware Jinek 2013 even contained sequencing data, despite relying
15 on Jinek 2013 and equating it to P3. The Board should deny CVC accorded benefit of its P3.

16 **I. CVC FAILS TO REBUT THE EVIDENCE THAT P3 LACKS A DESCRIBED**
17 **EMBODIMENT WITHIN COUNT 1**

18 **A. P3 and Jinek 2013 Are Not Interchangeable**

19 At Opp. 13:1-14:4, CVC argues that some post-Jinek 2013 papers "endorsed" the cleavage
20 experiments in Jinek 2013 (citing exhibits that reference Jinek 2013, *not P3*). Despite CVC's
21 desire to replace P3 with Jinek 2013, the written-description inquiry focuses on P3, which lacks
22 sequencing data of the Cas9-induced mutations in the experimental cells. Ex. 1604 (Doyon Tr.),
23 24:21-25:12; Ex. 2033, 4 (Figure Supplement 1); Ex. 1605; Ex. 1018; F44. This is the exact data
24 Dr. Turchi testified a POSA would need to confirm cleavage in view of P3's disclosure. Ex. 1410

1 (Turchi Decl.), ¶¶ 93-94; F46. To wit, the Jinek 2013 publisher *required* such data for publication:
2 "A few of the mutated targets *must be sequenced* (from cloned PCR products)[.]" Ex. 2499, 17
3 (emphasis added); F103. The inclusion of sequencing data in Jinek 2013—and its absence in P3—
4 makes later papers purportedly endorsing Jinek 2013 irrelevant to P3. That the publisher required
5 sequencing data over the authors' initial objections confirms, in real time, that POSAs found Jinek
6 2013's surveyor assay disclosure to be insufficient. Moreover, the Jinek 2013 sequencing data
7 themselves are suspect; the reported +65 nucleotide mutation is not human, but instead perfectly
8 matches sequences in Wild Yak, Bighorn Sheep and Red Deer. *See* Ex. 1605, 3; Ex. 1606; F104.
9 The Board also should accord no weight to Dr. Doyon's testimony equating P3 and Jinek
10 2013. Ex. 2476, ¶¶64-68. *First*, written-description law considers the application itself, not later-
11 published, extrinsic articles. *Second*, Dr. Doyon does not even testify that Jinek 2013 reported the
12 same data as P3, but only that the experiments "appear to correspond." Ex. 2476, ¶22; F105. And
13 *third*, Dr. Doyon did not even know Jinek 2013 had sequencing data. Ex. 1604, 21:3-23:10; F106.
14 **B. ToolGen And Its Expert Considered The Pertinent Art**
15 At Opp. 4:9-5:5, CVC argues that Dr. Turchi failed to consider art published by Jan. 2013.
16 The response is that CVC omits that Dr. Turchi reviewed Cong (Zhang), Ex. 2030, and Mali
17 (Church), Ex. 2345, both as "part of [his] initial review" and again in preparing for deposition. *See*
18 Ex. 2475 (Turchi Tr.), 31:11-34:17; F107. Dr. Turchi reviewed the pertinent art and properly
19 focused his inquiry on P3—not some extrinsic disclosure which says nothing about whether *the*
20 *P3 applicants* possessed the claimed invention. And, despite CVC now implying otherwise, Dr.
21 Doyon testified that neither Cong nor Mali 2013 "materially advanced the general knowledge in
22 the art" beyond May 2012. Ex. 1560 (Doyon Tr.), 47:13-19, 51:17-52:16, 54:8-55:9; F108. CVC
23 cannot have it both ways. The other two CVC-cited articles are similarly inconsequential because
24 both merely confirm the lack of success in eukaryotes. *See* Ex. 2215, 838 (CRISPR-Cas9 "will

1 require testing whether the crRNA-Cas systems can efficiently cleave chromatin DNA in vivo and
2 be readily transferred into organisms of interest [eukaryotic cells].”); Ex. 2497, 1527; F109.

3 **C. ToolGen Correctly Applied The Written Description Standard**

4 At Opp. 6:15-7:4, CVC claims that ToolGen applied the wrong standard because Dr.
5 Turchi analyzed only Example 2. Wrong. Dr. Turchi explained that analyzing written description
6 requires “looking at the entirety of . . . the specific description and the data[.]” Ex. 2475, 82:13-
7 83:3, 93:17-20; F110. Dr. Turchi also did not testify written description requires a working
8 example, as CVC contends. Opp. 7:14-8:2. Dr. Turchi testified that “a working example is one,
9 *but not the only*, route by which you could demonstrate possession.” Ex. 2475, 80:12-20 (emphasis
10 added); *see also id.*, 85:4-6; F111. Neither Dr. Turchi nor ToolGen has ever argued that written
11 description *requires* a working example, as CVC claims. Opp. 6:18-25. In fact, it is CVC that
12 argues (incorrectly) that it can pick-and-choose, using hindsight, from the specification by ignoring
13 Example 2’s unreliable results in favor of pieced together disparate laundry lists. This conflicts
14 with written-description law, which requires a POSA to assess the entire specification. *See Ariad*
15 *Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010). CVC admits as much but
16 does not practice what it preaches, instead discarding Example 2 when it suits its needs. Opp.
17 6:18-8:2 (*Binstead v. Littmann*, 242 F.2d 766, 768 (C.C.P.A. 1957)); Ex. 2476, ¶¶14, 26-27.

18 Worse, CVC’s argument does not apply to the present facts. P3 *did* contain a working
19 example—one that did *not* demonstrate successful cleavage. The question is not whether a
20 working example is required, but instead, where one is provided, whether a POSA would evaluate
21 its methods and results. The answer is “yes”: The specification always “must . . . show that the
22 inventor actually invented the invention claimed.” *Ariad*, 598 F.3d at 1351. As Dr. Turchi
23 testified, a POSA would consider Example 2 and its methods and results in concluding the
24 applicants lacked possession. *See* Ex. 2475, 85:12-17, 86:3-9; Ex. 1410, ¶¶65-92; F18, 38.

1 **D. P3 Does Not Disclose A CRISPR-Cas9 System Capable Of Functioning In A**
2 **Eukaryotic Cell**

3 P3 does not describe any embodiment within Count 1. Ex. 1410, ¶¶17, 111. Rather, CVC
4 imagines one by cobbling together disparate references to target cells, transfection techniques and
5 forms of Cas9 and guide RNA that P3 does not connect. Opp. 8:3-9; *cf.* ToolGen Opp. 1. E.g.,
6 CVC itself did not use a ribonucleoprotein in Example 2, Ex. 1018, ¶408-423; F112, yet it argues
7 P3 would lead a POSA to do so. Opp. 8:6-9. Example 2, which connects some but not all elements
8 of Count 1, also does not describe a CRISPR-Cas9 system capable of cleaving a eukaryotic cell.

9 **1. Example 2 Does Not Meet All The Elements of Count 1**

10 CVC claims Dr. Turchi admitted that Example 2 satisfies Count 1. Opp. 9:20-12:9. He
11 did not. As to Count 1, element [8] ("capable of cleaving or editing the target DNA molecule")
12 (Opp. 12, last row), Dr. Turchi *actually* testified that Fig. 36C would *not* satisfy that element. Ex.
13 2475, 114:13-22 ("I don't believe you could convince someone, based on the artist's rendition of
14 an interaction [in Fig. 36C], that it's capable of an enzymatic event."); F113. Dr. Turchi then
15 explained that a POSA would require additional testing, *which P3 lacks* (*See* Ex. 1018) and CVC
16 nowhere cites, such as x-ray crystallography and "[b]iochemical analyses that would assess
17 affinity, off rates, on rates, [and] binding parameters." *Id.* at 117:2-14; F114.

18 **2. A POSA Would Understand That The Lysing Method Used In Example 2**
19 **Allowed Cleavage To Occur Outside The Cell**

20 CVC argues that Example 2's lysing method, which left Cas9 active to cleave DNA
21 extracellularly, was of no concern because a POSA would not have expected SpCas9 to work at
22 4°C. Opp. 20:8-23; Ex. 2476, ¶¶55-56. CVC is doubly wrong. *First*, P3 does not disclose whether
23 the sample reached 4°C or, if it did, how long it took to do so. P3 discloses only that the sample
24 started at 37°C, was washed, then rocked for 10 minutes at 4°C in a lysis buffer, and then divided
25 for storage. Ex. 1018, ¶410; Ex. 1410, ¶101; F61. Factors such as sample volume, time between

1 lysis and storage, and method of cooling would all impact the speed of cooling, yet P3 is silent as
2 to these factors. Ex. 1018, ¶410; Ex. 1410, ¶101; F115. Dr. Turchi testified that a POSA would
3 understand the lysate could *not* have immediately dropped to 4°C, which is why the applicants
4 should have used a lysis buffer with, for instance, Proteinase K to degrade protein (Cas9) activity,
5 rather than one with magnesium, which *promotes* Cas9 activity, and protease inhibitor cocktail,
6 which *maintains* Cas9 activity. Ex. 1410, ¶¶48, 50, 96-101; F6, 63. Yet the applicants did not add
7 Proteinase K until an unspecified time later. Ex. 1018, ¶¶410, 415; F52, 116. And Dr. Doyon
8 admits the lysate contained active Cas9, as shown by the *extracellular* cleavage experiment with
9 the same lysate, reported in Fig. 37A. Ex. 1604, 86:4-8, 88:5-89:21; Ex. 1018, ¶¶415, 419-420;
10 Ex. 1410, ¶¶67, 72, 97; F56, 117. A POSA thus would not conclude the temperature in Example
11 2 prevented extracellular cleavage, which does not satisfy Count 1. Ex. 1410, ¶101; F66.
12 *Second*, a POSA could not possibly have ruled out Cas9 activity in the lysate cooling from
13 37°C to 4°C in an unspecified period of time. Dr. Doyon admitted neither he nor a POSA are
14 experts on the active temperature range of *S. pyogenes* or SpCas9. Ex. 1604, 63:18-65:9; F118.
15 He bases a POSA's alleged knowledge of SpCas9's active temperature range on papers published
16 years after P3. Ex. 2473 (2017); Ex. 2472 (2019); F119. But neither article rules out SpCas9
17 activity in Example 2's temperature range (4°–37°C), nor tests temperatures below 20°C. Ex.
18 2472, 23103 (20°–40°C); Ex. 2473, 6 (25°–44°C); F119.
19 CVC next argues that even if cleavage occurred in the lysate, the cleavage repair process
20 (non-homologous end joining, or "NHEJ") could not have occurred. Opp. 21:1-22:1. The
21 response is that CVC cites no "requirements" for NHEJ. F120. Dr. Doyon claims that one cell-
22 free NHEJ paper "required '2 mM ATP.'" Ex. 2476, ¶59 (citing Ex. 1229, 14067). However it
23 did not *require* but only *used* 2mM ATP. Ex. 1604, 126:2-7. That a reaction worked with 2mM
24 ATP but not without added ATP, says nothing about amounts between 0mM and 2mM, and Diggle

1 2003 successfully used 1mM. Ex. 2489, 3; F120. Moreover, CVC admits the cell-free NHEJ
2 papers separated and removed the nucleus from the cytosol, where cellular ATP would be, through
3 ultracentrifugation and dialysis. Opp. 21: 9-14; F121. P3 did not; therefore, as Dr. Turchi testified,
4 cytoplasmic and mitochondrial ATP would remain in the lysate. Ex. 1410, ¶98; F121. Similarly,
5 CVC argues that NHEJ requires hotter incubation than P3's lysates (Opp. 21:19-22), but Dr.
6 Doyon's references state no such requirement. *See* Ex. 2490, 858; F120. Finally, Dr. Doyon
7 asserts that P3 had fewer cells than used for cell extracts in cell-free NHEJ papers. Ex. 2476, ¶61.
8 But his conclusions are unreliable: Dr. Doyon miscalculated the number of cells per 6-well plate.
9 *Compare* Ex. 2476, ¶61 (1.2×10^6 cells per plate) *with* Ex. 1604, 118:4-14 (1.2×10^6 cells *per*
10 *well*); F122. He disregarded that the *in vitro* reactions in the cell-free references added exogenous
11 DNA, which would have required more contributing cells. He also failed to consider methods that
12 used fewer cells. Ex. 1604, 120:2-16; Ex. 1607; F123.

13 **3. Figure 36E Does Not Disclose A CRISPR-Cas9 System Capable Of**
14 **Functioning In A Eukaryotic Cell**

15 CVC argues that a POSA would have disregarded the unexpected bands in Fig. 36E as (1)
16 undigested heteroduplexes, that is, remnants of DNA with mismatched strands that the Cel-I
17 nuclease in the surveyor assay did not digest or cleave; and/or (2) evidence of "non-specific
18 cleavage" by the Cel-I nuclease. Opp. 16:14-16. Neither adequately explains the multiple
19 inconsistent bands in Fig. 36E. *First*, CVC's "undigested heteroduplex" argument misses the
20 mark. Dr. Doyon cites Guschin 2010 to explain bands above the PCR product. Ex. 2476, ¶40
21 (quoting Ex. 2479, 254). But Guschin, which looks nothing like the P3 results, only supports
22 undigested heteroduplexes *in lanes treated with a nuclease, like ZFN or sgRNA-Cas9*. F124.
23 SgRNA was not added to Lane B, so no sgRNA-Cas9 complex could cleave the DNA, and no
24 heteroduplex should appear. Ex. 1018, ¶¶417-418, Fig. 36E; F124. Yet Band 2 appears there, so,

1 Guschin is no help. Ex. 1410, ¶82. Dr. Doyon could not explain its presence, in contrast to Band
2 2 in Lane F. *Compare* Ex. 1604, 108:10-21 *with id.* at 107:17-108:6.
3 *Second*, far from stating he had no opinion, as CVC argues (Opp. 16:10-13), Dr. Turchi
4 explained that the P3 surveyor assay is designed to digest *all* heteroduplexes, and that to determine
5 whether a given band represented undigested heteroduplexes, "[y]ou would need an independent
6 determination of the heteroduplexes and the percentage of the DNA that is heteroduplexed." Ex.
7 2475, 65:13-66:1, 67:13-18, 68:4-6; F125. Therefore, even ignoring the P3 surveyor assay's
8 design, a POSA could only diagnose Bands 2 and 8 as "undigested heteroduplexes" by sequencing
9 the results. Ex. 2475, 67:6-68:7; F126.
10 *Third*, a POSA would recognize that Fig. 36E contains additional, unexpected bands
11 consistent with an origin other than targeted sgRNA-Cas9 cleavage that CVC cannot explain. Dr.
12 Doyon's only explanation for Bands 3 and 4 is that a POSA would ignore them because they
13 represent "low-level non-specific cleavage by the Cel-I nuclease that occur commonly in Surveyor
14 nuclease assay gels." Ex. 2476, ¶43; Opp. 16:14-21. This hand-waving does not address Dr.
15 Turchi's point that Bands 3 and 4 are discrete products and not merely "small amounts of
16 nonspecific breakdown products shorter than full-length substrate." Ex. 2513, 705; F36, 37. And
17 a POSA would have recognized such bands as a problem to be fixed with better methods, not a
18 way to explain aberrant results. *See* Ex. 2514, 25-26 (listing "High Background After Surveyor
19 Nuclease Treatment" as a "problem" in "Troubleshooting" section); F128. CVC does not even
20 address that Band 5, the supposed sgRNA-Cas9 cleavage band, appears in Lanes E *and* G, yet no
21 sgRNA-Cas9 was present in Lane G. Ex. 1410, ¶¶79, 84; *see* Ex. 1018, ¶417-418, Fig. 36E; F39.

22 **4. Figure 38B Does Not Disclose Target DNA Cleavage In A Eukaryotic Cell**

23 CVC argues that Fig. 38B discloses successful target DNA cleavage in a eukaryotic cell.
24 Opp. 16:22-17:2. The response is that, like Fig. 36E, CVC fails to address the inconsistent and

1 unexpected bands Dr. Turchi labeled in Fig. 38B. *See* Ex. 1410, ¶¶86-90. CVC cannot simply
2 red-line P3 to fit its argument, as Dr. Doyon does, Ex. 2476, ¶51, or irrationally assert that a POSA
3 would ignore certain bands in favor of those CVC likes. CVC does not dispute that the supposed
4 cleavage band (Band 2 in Dr. Turchi's annotated Fig. 38B), is far larger than expected in all the
5 Cas9-sgRNA lanes (Lanes F, H, and I). Opp. 17:1-10; Ex. 1410, ¶88; F21. Instead, CVC changes
6 the ruler because it does not like the measurements. Opp. 18:5-15. Admitting P3's bad data, CVC
7 claims a POSA would have known both that the scale used to measure band size in Fig. 38B was
8 wrong, and exactly which scale to replace it with *post hoc*. Ex. 2476, ¶¶49-51. But a POSA would
9 have known that the scale, called a molecular-weight marker, used known band sizes to measure
10 unknown experimental bands. Ex. 1604, 53:2-10; F129. A POSA would not reverse this
11 relationship and use unknown experimental data to craft the scale that fit. Nor would a POSA
12 know the "right" scale among the many available in 2012. Ex. 1604, 55:18-56:2; Ex. 2475, 56:9-
13 14; F130. And CVC does not even try to explain Bands 5, 6, and 7, *see* Opp. 16:22-20:1; F127,
14 which are unexpected bands—larger than the PCR band—of unknown origin. Ex. 1410, ¶90; F24.

15 **II. CONCLUSION**

16 CVC is not entitled to benefit of P3. The Board should grant Senior Party's motion.

17 Respectfully submitted,

18 Dated: August 27, 2021 /Timothy J. Heverin/
19 Timothy J. Heverin
20 Reg. No. 77,386
21 JONES DAY
22 Counsel for Senior Party ToolGen, Inc.

APPENDIX 1: LIST OF EXHIBITS CITED

Ex. No.	Description
1018	U.S. Provisional Application No. 61/757,640, filed January 28, 2013.
1229	Baumann and West, DNA end-joining catalyzed by human cell-free extracts, *Proc. Natl. Acad. Sci.*, 95, 14066–14070 (1998).
1410	May 20, 2021 Declaration of John J. Turchi, Ph.D.
1560	Deposition Transcript of Yannick Doyon, Ph.D., The Regents of the University of California v. ToolGen, Inc., Interference No. 106, 127, July 25, 2021.
1604	Second Deposition Transcript of Dr. Yannick Doyon The Regents of the University of California v. ToolGen, Inc., Interference No. 106,127, August 12, 2021.
1605	Figures and Figure Supplements from Jinek *et al.*, RNA-Programmed Genome Editing in Human Cells, 2 *ELIFE* e00471 (2013).
1606	NCBI BLAST Results for Nucleotide Sequence of the +65nt Mutated Allele Reported in Jinek 2013.
1607	Smeaton *et al.*, Small-scale extracts for the study of nucleotide excision repair and non-homologous end joining, *Nucleic Acid Research*, 35(22), e152, 1–6 (2007).
1609	Hockemeyer *et al.*, Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases, *Nature Biotechnology*, 27(9), 851–859 (2009) with Supplemental Material.
1610	Urnov *et al.*, Highly efficient endogenous human gene correction using designed zinc-finger nucleases, *Nature*, 435(2), 646–651 (2005) with Supplemental Material.
2030	Cong *et al.*, Multiplex Genome Engineering Using CRISPR/Cas Systems, *Science*, 339(6121), 819–823 (2013) with Supplemental Material.
2033	Jinek, *et al.*, RNA-programmed genome editing in human cells, *eLife*, 2:e00471, 1–9 (2013).
2215	Barrangou, RNA-mediated programmable DNA cleavage, *Nature Biotechology*, 30(9), 836–838 (2012).
2227	Miller *et al.*, A TALE nuclease architecture for efficient genome editing, *Nat. Biotechnology*, 29, 143–148, Supplementary Information (2010).
2345	Mali *et al.*, RNA-Guided Human Genome Engineering via Cas9, *Science*, 339(6121), 823–826 (2013).

Ex. No.	Description
2472	Schmidt *et al.*, Nucleic acid cleavage with a hyperthermophilic Cas9 from an uncultured Ignavibacterium, *PNAS*, 116(46), 23100–23105 (2019).
2473	Mougiakos *et al.*, Characterizing a thermostable Cas9 for bacterial genome editing and silencing, *Nature Communs.*, 8(1647), 1–11 (2017).
2475	Deposition Transcript of John Turchi, Ph.D, The Regents of the University of California v. ToolGen, Inc., Interference No. 106, 127, June 29, 2021.
2476	Third Declaration of Yannick Doyon, Ph.D.
2479	Guschin *et al.*, Chapter 15: A Rapid and General Assay for Monitoring Endogenous Gene Modification, Mackay, J.P. and Segal, D.J., (eds.) Engineered Zinc Finger Proteins, Methods in Molecular Biology, 649, 247–256 (2010).
2489	Diggle *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 and Chu, Processing of DNA for nonhomologous end-joining by cell-free extract, *The EMBO Journal*, 24, 849–860 (2005).
2497	Genomic Cruise Missiles, *Science*, 338, 1526–1527 (2012).
2499	Jinek 2013 Manuscript Author Response
2513	Qiu, P., *et al.*, "Mutation detection using SurveyorTM nuclease," BioTechniques 36(4):702-707 (2004).
2514	Integrated DNA Technologies User Guide: Surveyor® Mutation Detection Kit for Standard Gel Electrophoresis, available at https://sfvideo.blob.core.windows.net/sitefinity/docs/default-source/user-guidemanual/surveyor-kit-for-gel-electrophoresis-user-guide.pdf?sfvrsn=a9123407_6 (last visited March 26, 2012).

APPENDIX 2: STATEMENT OF MATERIAL FACTS

Senior Party ToolGen's Material Facts 1-70 and Junior Party's Answers

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

Response: Admitted.

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

Response: Denied.

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

Response: Admitted.

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

Response: Admitted.

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

Response: Admitted.

6. In P3's experiments testing for cleavage, forty-eight hours after transfection the cells were lysed using a gentle lysing method, which included a lysis buffer that was supplemented with a Roche Protease Inhibitor cocktail—which retains protein and enzyme activity—and magnesium, which is required for the cleavage reaction. Ex. 1018 ¶¶ [00378 and Fig. 21, 00410]; Ex. 1410 ¶ 75.

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

1 7. A POSA would understand that the cell lysates used in the experiments depicted in Figures
2 38B, 36E, and 37A were all prepared using the same gentle lysing method. Ex. 1018 ¶ [00410];Ex.
3 1410 ¶ 97.

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

6 8. Figures 38B and 36E portray the results of Surveyor assays using extracted genomic DNA from
7 cell lysates that were amplified by PCR with primers targeted to the human CLTA gene containing
8 the target sequence. Ex. 1018 ¶¶ [00408, 00410, 00412, 00416-00417, 00421- 00422]; Ex. 1410 ¶¶
9 58-60, 77-79.

10 **Response: Admitted that P3's Example 2 describes a procedure for Surveyor assay in, e.g.,**
11 **¶[00412] and Surveyor assay data in Figures 36E and 38B; otherwise denied.**

12 9. The Surveyor assays in Example 2 were performed on an unpurified PCR product. Ex. 1018 ¶
13 [00412].

14 **Response: Admitted that P3's Example 2 describes a procedure for Surveyor assay in**
15 **¶[00412]; otherwise denied.**

16 10. Surveyor assays detect mismatched base pairs resulting from non-homologous end joining
17 ("NHEJ") DNA repair, which occurs after cleavage by the Cas9-sgRNA complex, and cleave DNA
18 into fragments at the site of the mismatched base pairs. Ex. 1018 ¶ [00412]; Ex. 1235; Ex. 1218; Ex.
19 1225.

20 **Response: Admitted.**

21 11. Figures 38B and 36E portray the results of Surveyor assay products subjected to gel
22 electrophoresis to separate the digestion products by size into visible bands. Ex. 1018 ¶¶ [00412,
23 00417, 00422].

1 **Response: Admitted that P3's Example 2 describes a procedure for Surveyor assay in, e.g.,**
2 **¶[00412] and Surveyor assay data in Figures 36E and 38B; otherwise denied.**
3 12. A POSA would not have considered off-target cleavage of the DNA (that is, cleavage at sites
4 other than the intended site) to successfully demonstrate DNA cleavage by a CRISPR-Cas9 system.
5 Ex. 1410 ¶ 85; Ex. 1210.
6 **Response: Denied.**
7 13. The figure titled "P3 (Ex. 1018) Example 2 - PCR Amplicon Sequence" at Page 7, Line 6 above
8 depicts the sequence of the PCR amplicon generated in Example 2's experiments that resulted in
9 Figures 38B and 36E. Ex. 1410 ¶ 77.
10 **Response: Denied.**
11 14. The figure titled "P3 (Ex. 1018) Example 2 - PCR Amplicon Sequence" at Page 7, Line 6 above
12 depicts the primers, target sequence, predicted Cas9-sgRNA cleavage site, ZFN binding sites, and
13 ZFN predicted cleavage sites for Example 2's experiments that resulted in Figures 38B and 36E. Ex.
14 1410 ¶ 77.
15 **Response: Unable to admit or deny.**
16 15. If Cas9-sgRNA cleavage at the target site was successful, a POSA would expect a 369 bp PCR
17 product band; ~183 and ~186 bp Cas9-sgRNA cleavage bands; and/or ~162-169 and ~200-207bp ZFN
18 cleavage bands in the Surveyor assays depicted in Figures 38B and 36E. Ex. 1410 ¶¶78, 80.
19 **Response: Admitted that a POSA would expect to see a cleavage band in the sgRNA-Cas9**
20 **samples; otherwise denied.**
21 16. A POSA would not expect any bands larger than the PCR product band in the Surveyor assays
22 depicted in Figures 38B and 36E. Ex. 1410 ¶¶ 78, 80.
23 **Response: Denied.**

1 17. Figures 38B and 36E depict the only experimental results in P3 purporting to show cleavage of
2 target eukaryotic DNA by a CRISPR-Cas9 system in a eukaryotic cell. Ex. 1018 ¶¶ [00417, 00422].
3 **Response: Admitted that Figures 36E and 38B show cleavage of target DNA by a sgRNA-**
4 **Cas9 system in a eukaryotic cell.**
5 18. Figure 38B would not have shown successful CRISPR-Cas9 cleavage at the target site to a
6 POSA. Ex. 1410 ¶¶ 86-92.
7 **Response: Denied.**
8 19. Figure 38B shows bands where there should be none. Ex. 1410 ¶¶ 86-92.
9 **Response: Denied.**
10 20. Figure 38B shows a ~378 bp PCR band (band 1) that is consistent in size with the predicted
11 369 bp full-length PCR band. Ex. 1410 ¶ 88.
12 **Response: Admitted that Figure 38B shows a full-length PCR amplicon band in each lane;**
13 **otherwise denied.**
14 21. Figure 38B shows digestion products of ~280 bp (band 2) that are not the expected ~185 bp size
15 of a Cas9-sgRNA cleavage band in all the Cas9-sgRNA lanes (Lanes F, H, and I). Ex. 1410 ¶ 88.
16 **Response: Admitted that Figure 38B shows successful sgRNA-Cas9-mediated target DNA**
17 **cleavage in Lanes F, H, and I; otherwise denied.**
18 22. Figure 38B shows no band at all close to the expected ~185 bp size of a Cas9-sgRNA cleavage
19 band. Ex. 1410 ¶ 88.
20 **Response: Denied.**
21 23. Figure 38B's ~280 bp digestion band (band 2) is irreconcilable with its ~378 bp PCR band
22 (band 1) because there would be an unaccounted for ~98 bp DNA fragment. Ex. 1410 ¶ 88.
23 **Response: Denied.**

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

4 **Response: Denied.**

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

8 **Response: Denied.**

9 26. Figure 38B's Lane A is not simply mislabeled; the PCR band is the correct size, and any
10 "corrections" to Lane A would only serve to shift the PCR band away from its expected size. Ex.
11 1410 ¶¶ 91-92.

12 **Response: Denied.**

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

15 **Response: Denied.**

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

17 **Response: Denied.**

18 29. Based on the experimental protocols, a POSA would expect the purported cleavage bands to be
19 identical in Figures 38B and 36E, and if they were not, a POSA would question both results. Ex. 1410
20 ¶¶ 82-85, 87.

21 **Response: Denied.**

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

1 **Response: Denied.**

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

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

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

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

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

12 **Response: Denied.**

13 34. Figure 36E's alleged cleavage band (band 5) could be a digestion product of the unexpected
14 ~477 bp band (band 2). Ex. 1410 ¶ 83.

15 **Response: Denied.**

16 35. Figure 36E has an unexpected digestion band at ~297/312 bp (band 3) in Lanes E and G. Ex.
17 1410 ¶ 83.

18 **Response: Denied.**

19 36. Figure 36E's unexpected ~477 bp band (band 2) could have been digested into the ~173 and
20 ~297/312 bp digestion product bands (bands 5 and 3, respectively). Ex. 1410 ¶ 83.

21 **Response: Denied.**

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

1 **Response: Denied.**

2 38. A POSA would be unconvinced of the applicants' possession of an embodiment within Count1
3 based on the results in Figure 36E. Ex. 1410 ¶¶ 83, 85.

4 **Response: Denied.**

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

7 **Response: Denied.**

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

11 **Response: Denied.**

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

13 **Response: Denied.**

14 42. In view of Figures 38B and 36E's results, a POSA would have considered the well-known
15 procedure of sequencing the PCR product as an appropriate way to confirm and characterize any
16 potential cleavage in the PCR-amplified region of DNA surrounding the target sequence. Ex. 1410
17 ¶¶ 62, 93-94.

18 **Response: Denied.**

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

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

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

2 **Response: Admitted.**

3 45. Assays that use cleavage to affect a directly measurable result can be more reliable at detecting
4 cleavage because—unlike the Surveyor assay—they do not require genomic DNA extraction,
5 amplification, digestion, and gel electrophoresis, all of which can be highly error prone. Ex. 1410 ¶¶
6 61, 95; Ex. 1236; Ex. 1219.

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

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

12 **Response: Denied.**

13 47. The method used to prepare the cell lysates in the experiments in Example 2 allowed for
14 CRISPR-Cas9 cleavage activity outside of the cells in the cell lysate. Ex. 1410 ¶¶ 72, 97.

15 **Response: Denied.**

16 48. Any cleavage shown in Figures 38B and 36E could have occurred outside the cells, in the lysate.
17 Ex. 1410 ¶¶ 66, 72, 101-103.

18 **Response: Denied.**

19 49. Extracellular cleavage in the cell lysate does not satisfy Count 1. Ex. 1410 ¶¶ 66, 72, 101- 103;
20 Paper 1 at 5-7.

21 **Response: Admitted that Count 1 requires a CRISPR-Cas9 system capable of cleaving or**
22 **editing a target DNA molecule or modulating transcription of at least one gene encoded by a**
23 **target DNA molecule in a eukaryotic cell; otherwise denied.**

1 50. Figure 37A depicts the results of an experiment that assessed extracellular Cas9-sgRNA
2 cleavage of a donor plasmid. Ex. 1018 ¶¶ [00415, 00419, 00420]; Ex. 1410 ¶¶ 67; 71-72.

3 **Response: Admitted that Figure 37A discloses results from an *in vitro* cleavage assay;**
4 **otherwise denied.**

5 51. Figure 37A's experiment introduced the target sequence to the lysate with abundant donor
6 plasmid and a "cleavage buffer" to promote Cas9-sgRNA activity in the lysate. Ex. 1018 ¶¶ [00410,
7 00412, 00415, 00419-00420].

8 **Response: Admitted that P3 describes incubating the CLTA-RFP donor plasmid with cell**
9 **lysates for one hour at 37°C, followed by digestion with XhoI for 30 minutes at 37°C; otherwise**
10 **denied.**

11 52. During Figure 37A's experiment, the addition of Proteinase K was the first step that inactivated
12 the Cas9. Ex. 1018 ¶¶ [00410, 00415].

13 **Response: Denied.**

14 53. Figure 37A reveals two Cas9-sgRNA cleavage bands of similar sizes and two ZFN control
15 cleavage bands also of similar sizes, which confirms the proximity of their target sequences. Ex. 1410
16 ¶¶ 70, 105.

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

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

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

23 55. The presence of two bands in Figure 37A's experiment indicates to a POSA that the plasmid
24 was successfully cleaved extracellularly at the predicted Cas9-sgRNA or ZFN target site and the

1 donor plasmid restriction enzyme site, while one band would indicate only the restriction site was
2 cleaved Ex. 1410 ¶¶ 66, 70, 105.

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

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

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

9 57. Because the applicants preserved the activity of Cas9 in the lysate, Figures 38B and 36E's
10 results might show extracellular cleavage that occurred in cell lysates prepared using the gentle lysing
11 method. Ex. 1410 ¶¶ 96-108.

12 **Response: Denied.**

13 58. The Cas9-sgRNA and ZFN efficiency data is consistent with cleavage occurring in the lysate,
14 not in the cells. Ex. 1410 ¶¶ 72, 104-106.

15 **Response: Denied.**

16 59. The efficiency of the plasmid-expressed Cas9-sgRNA complex in Lane G is comparable to that
17 of the ZFN construct in Lane H of Figure 37A, as assessed by band intensity. Ex. 1410 ¶¶ 105-106.

18 **Response: Admitted that Lanes G and H in Figure 37A each shows successful target DNA**
19 **cleavage; otherwise denied.**

20 60. In Figure 36E, the Cas9-sgRNA efficiency is drastically lower than that of the ZFN control
21 compared to their comparable efficiency in Figure 37A. Ex. 1410 ¶¶ 104-106.

22 **Response: Admitted that Figure 36E discloses successful target DNA cleavage in a**
23 **eukaryotic cell; otherwise denied.**

1 61. The transfected cells in the experiments depicted in Figures 38B and 36E were incubated at
2 37°C and were then gently lysed by rocking for ten minutes in a 4°C environment, but P3 does not
3 provide the lysate temperature at the end of the ten minutes or the subsequent storage conditions. Ex.
4 1018 ¶ [00410]; Ex. 1410 ¶ 101.
5 **Response: Admitted that P3 does not expressly disclose lysate storage temperatures;**
6 **otherwise denied.**
7 62. Rocking is considered a gentle method of physical agitation that would not disrupt genomic
8 DNA or protein structure. Ex. 1410 ¶¶ 48, 98.
9 **Response: Denied.**
10 63. Use of a protease such as Proteinase K would have been critical in the Figure 38B and 36E
11 experiments to stop Cas9 activity because the lysate could not have immediately dropped from 37°C
12 to 4°C. Ex. 1410 ¶¶ 48, 96-101; Ex. 1233.
13 **Response: Denied.**
14 64. NHEJ repair of DNA damage from Cas9-sgRNA cleavage could have occurred in the lysate.
15 Ex. 1410 ¶¶ 48, 100-102; Ex. 1227.
16 **Response: Denied.**
17 65. The low cleavage efficiencies reported by the applicants in Figure 36E (4% in purported Cas9
18 cleavage Lane E) are consistent with cleavage only occurring in the lysate. Ex. 1410 ¶¶ 103- 104.
19 **Response: Denied.**
20 66. A POSA cannot rely on Figures 38B and 36E as showing intracellular cleavage because the
21 applicants failed to "turn off" the Cas9 in the lysate before extracting the DNA. Ex. 1410 ¶¶ 101, 103,
22 107-108; Ex. 1233; Ex. 1227.
23 **Response: Denied.**

1 67. Initial research in CRISPR-Cas9 systems had focused on their structure and function in
2 prokaryotic cells—the only cells where CRISPR-Cas9 systems naturally occur. Ex. 1410 ¶ 41;Ex.
3 1202.

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

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

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

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

12 **Response: Denied.**

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

17 **Response: Denied.**

18 **Junior Party's Alleged Facts 71-102 and Senior Party ToolGen's Answers**

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

21 **Response: Admitted.**

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

1 **Response: Admitted that Barrangou 2012 includes the excerpted phrase "synthetic tour**
2 **de force" in a sentence describing Jinek 2012 (Ex. 1206) and the excerpted phrases "molecular**
3 **scalpel" and "outcompete ZFN and TALEN DNA scissors for precise genomic surgery" in**
4 **sentences describing the need for testing CRISPR-Cas9 to determine whether it can act on**
5 **chromatin and/or in eukaryotes. Ex. 2215, 837-38 ("This will require testing whether crRNA-**
6 **Cas systems can efficiently cleave chromatin DNA in vivo and be readily transferred into**
7 **organisms of interest, notably yeast and fungi, but also plants, for crop and agricultural**
8 **applications, and human cells, for medical purposes. Only the future will tell whether this**
9 **programmable molecular scalpel can outcompete ZFN and TALEN DNA scissors for precise**
10 **genomic surgery"). Otherwise, denied.**
11 73. The *Science* 2012 article published in December 2012 and would have been known to a POSA
12 before January 28, 2013. Ex. 2497, 1526; Ex. 2476, ¶¶18-19.
13 **Response: Admitted.**
14 74. The *Science* 2012 article states that CRISPR-Cas9 "may one day challenge zinc finger
15 nucleases and TALENs as the core genome engineering technology." Ex. 2497, 1527.
16 **Response: Admitted that the *Science* 2012 article states that "Now, those researchers are**
17 **trying this approach in organisms other than bacteria, and other genome engineers are quite**
18 **excited about their prospects, suggesting that it may one day challenge zinc finger nucleases**
19 **and TALENs as the core genome engineering technology." Ex. 2497, 1527. Otherwise, denied.**
20 75. The Mali 2013 article first published online on January 3, 2013 and would have been known to
21 a POSA before January 28, 2013. Ex. 2345; Ex. 2474, 179:10-15; Ex. 2476, ¶20.
22 **Response: Admitted.**

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

4 **Response: Admitted this is what Mali 2013 reports; otherwise, denied.**

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

7 **Response: Admitted.**

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

10 **Response: Admitted this is what Cong 2013 reports; otherwise, denied.**

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

14 **Response: Admitted that Dr. Turchi did not consider Ex. 2215 or Ex. 2497. Otherwise**
15 **denied.**

16 80. Turchi's declaration focuses on the disclosures in P3's Example 2. Ex. 1410; Ex. 2476, ¶¶25-
17 26; Ex. 2003, ¶¶[00408]-[00423], Figs. 36-38.

18 **Response: Denied, at least because Dr. Turchi's declaration is based on whether a POSA**
19 **reading P3 at the time of filing would view the applicants as having possession of an embodiment**
20 **of Count 1. Ex. 1410, ¶29.**

21 81. The only disclosure in P3 that Turchi cites in his declaration that is not part of P3's Example 2
22 is a single citation to P3 ¶[00378] and Figure 21. Ex. 1410, ¶76.

23 **Response: Admitted that Dr. Turchi's declaration cites to Example 2 and ¶ [00378].**
24 **Denied that Dr. Turchi did not review and consider the entirety of P3 in his analysis.**

1 82. Turchi testified that "demonstration of [DNA cleavage] would be required to support the
2 description that [the inventors] possess that invention." Ex. 2475, 85:7-17.

3 **Response: Admitted that when asked if "[T]he demonstration of cleavage [is] the only way**
4 **to demonstrate that one has possession of the invention?", Dr. Turchi testified that "If the**
5 **context of the written description describes an experiment and the working example is one that**
6 **is assessing cleavage in a eukaryotic cell, for that example, the demonstration would be required**
7 **to support the description that they possess that invention." Ex. 2475, 85:7-17. Otherwise,**
8 **denied.**

9 83. P3 discloses making and using sgRNA-Cas9 systems comprising a chimera A sgRNA and the
10 *S. pyogenes* Cas9 protein. Ex. 2003, ¶¶[0030]-[0031], [0037]-[0040], [00169], [00196], [00364],
11 [00401]-[00405], Figs. 1-3, 17; Ex. 2476, ¶27.

12 **Response: Admitted that P3 discloses making and using sgRNA-Cas9 systems outside of**
13 **eukaryotic cells. Denied that P3 discloses successfully making and using sgRNA-Cas9 systems**
14 **within eukaryotic cells.**

15 84. P3 discloses assembling chimera A sgRNA-Cas9 ribonucleoprotein complexes outside of a
16 eukaryotic cell. Ex. 2003, ¶¶[00366]-[00370], [00401], claim 98; Ex. 2476, ¶28.

17 **Response: Admitted.**

18 85. P3 discloses delivering CRISPR-Cas9 systems to eukaryotic cells using "direct microinjection."
19 Ex. 2003, ¶¶[00108], [00261]; Ex. 2476, ¶28.

20 **Response: Admitted that "direct micro injection" appears in a laundry list of delivery**
21 **methods in P3 ¶[00108]; otherwise, denied.**

22 86. By January 28, 2013, direct micro injection was known to be "straightforward and effective."
23 Ex. 1410, ¶46; Ex. 2476, ¶28; Ex. 2003, ¶¶[00108], [00261].

1 **Response: Admitted that Dr. Turchi's declaration states that "physical transfection**
2 **methodologies were straightforward and effective but sometimes required extensive**
3 **instrumentation," Ex. 1410 ¶46; otherwise, denied.**

4 87. P3 discloses eukaryotic expression vectors for expressing sgRNA and Cas9, eukaryotic
5 promoters (e.g., CMV, U6, and H1), codon optimization of the Cas9 gene, adding a NLS to Cas9,
6 routine vector transfection methods, and established cell lines suitable for transfection. Ex. 2003,
7 ¶¶[0090], [00206], [00243]-[00244], [00252], [00256]-[00259]; Ex. 2476, ¶29.

8 **Response: Denied.**

9 88. P3 discloses a human cell embodiment in Example 2 that meets all the elements of Count 1. Ex.
10 2003, ¶¶[00408]-[00423], Fig. 36; Ex. 2475, 51:12-16, 52:22-53:1, 110:4-8, 108:20- 109:10, 110:9-
11 111:7, 112:7-19, 112:20-113:4, 113:6-15, 116:20-118:1; Ex. 2476, ¶¶31-33.

12 **Response: Denied.**

13 89. Turchi admitted that Example 2 meets all elements of Count 1. Ex. 2475, 51:12-16, 52:22-53:1,
14 110:4-8, 108:20-109:10, 110:9-111:7, 112:7-19, 112:20-113:4, 113:6-15, 116:20-118:1.

15 **Response: Denied.**

16 90. Turchi described the PCR amplicon band shown in P3 Figure 36E as "consistent" with his
17 predicted size. Ex. 1410, ¶83; Ex. 2003, Fig. 36E; Ex. 2476, ¶¶36-38.

18 **Response: Admitted that the quoted word appears in Ex. 1410, ¶82; otherwise, denied.**

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

22 **Response: Denied.**

23 92. The DNA band Turchi labeled as "band 5" in Figure 36E appears only in Lane E. Ex. 1410,
24 ¶79; Ex. 2475, 126:4-127:14; Ex. 2476, ¶¶36-38; Ex. 2003, Fig. 36E.

1 **Response: Denied.**

2 93. The DNA band Turchi labeled as "band 5" in Figure 36E migrates at a position in between the
3 DNA bands Turchi labeled as "band 6" and "band 7." Ex. 1410, ¶79; Ex. 2475, 125:15-126:3; Ex.
4 2476, ¶¶36-38; Ex. 2003, Fig. 36E.

5 **Response: Admitted.**

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

9 **Response: Admitted that Dr. Turchi did not consider Exs. 2479, 2480 or 2481.**

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

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

13 96. The DNA band Turchi labeled as "band 2" in Figure 38B appears only in Lanes F, H, and I. Ex.
14 1410, ¶86; Ex. 2475, 137:22-138:15; Ex. 2476, ¶¶47-48; Ex. 2003, Fig. 38B.

15 **Response: Admitted that Dr. Turchi labeled "band 2" in Lanes F, H, and I of Figure 38B;**
16 **otherwise, denied.**

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

20 **Response: Admitted.**

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

1 **Response: Denied at least because, of the three papers CVC argues show surveyor assays**
2 **alone, one contains sequencing data and a surveyor assay, Ex. 2227 (Miller 2010), 145 (Figure**
3 **3), and the other two were not using a nascent technology, as ZFNs already had been confirmed**
4 **to cleave DNA, so are not comparable to using CRISPR-Cas9 in P3. *See* Ex. 1609 (Hockemeyer**
5 **(2009)); Ex. 1610 (Urnov (2005)).**

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

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

8 100. Cell-free non-homologous end-joining (NHEJ) requires addition of ATP. Ex. 1229, 14067; Ex.
9 2489, 4; Ex. 2490, 850; Ex. 2491, 1; Ex. 2476, ¶¶59-62.

10 **Response: Denied.**

11 101. Jin-Soo Kim is a co-author on Koo 2015, which cites the Jinek 2013 paper along with three
12 other papers when stating, "we and others have reported RNA-guided genome editing in human cells
13 in January, 2013." Ex. 2484, 479; Ex. 2033.

14 **Response: Admitted.**

15 102. Bryan Cullen is a co-author on Kennedy 2016, which cites the Jinek 2013 paper along with two
16 other papers when referring to groups that obtained DNA cleavage "in cultured mammalian cells."
17 Ex. 2494, 402; Ex. 2033.

18 **Response: Admitted that Dr. Cullen is a co-author on Kennedy 2016 (Ex. 2494), that**
19 **Kennedy 2016 cites Jinek 2013, and that the quoted language appears on page 402; otherwise,**
20 **denied.**

1 **Senior Party ToolGen's Additional Material Facts 103-130**

2 103. The Jinek 2013 publisher required sequencing data before publication, stating: "A few of the
3 mutated targets must be sequenced (from cloned PCR products)[.]" Ex. 2499, 17.

4 104. The reported +65 nucleotide mutation is not human, but perfectly matches sequences in Wild
5 Yak, Bighorn Sheep and Red Deer. See Ex. 1605, 3; Ex. 1606

6 105. Dr. Doyon does not testify that Jinek 2013 reported the same data as P3, but only that the
7 experiments "appear to correspond." Ex. 2476, ¶22.

8 106. Dr. Doyon did not know Jinek 2013 contained sequencing data. Ex. 1604, 21:3-23:10.

9 107. Dr. Turchi considered Cong (Ex. 2030) and Mali (Ex. 2345). *See* Ex. 2475, 31:11-34:17.

10 108. Dr. Doyon testified neither Cong 2013 nor Mali 2013 "materially advanced the general
11 knowledge in the art" beyond May 2012. Ex. 1560 (Doyon Tr.), 47:13-19, 51:17-52:16, 54:8-55:9.

12 109. Barrangou 2012 stated CRISPR-Cas9 "will require testing whether the crRNA-Cas systems can
13 efficiently cleave chromatin DNA in vivo and be readily transferred into organisms of interest
14 [eukaryotic cells][,]" Ex. 2215, 838, and Science 2012 stated "researchers are trying this [CRISPR-
15 Cas9] approach in organisms other than bacteria," Ex. 2497, 1527.

16 110. Dr. Turchi testified that analyzing written description requires "looking at the entirety of . . .
17 the specific description and the data[.]" Ex. 2475, 82:13-83:3, 93:17-20.

18 111. Dr. Turchi testified that "a working example is one, *but not the only*, route by which you could
19 demonstrate possession." Ex. 2475, 80:12-20 (emphasis added); *see also id.*, 85:4-6.

20 112. CVC did not use a ribonucleoprotein in Example 2. Ex. 1018, ¶408-423.

21 113. Dr. Turchi testified: "I don't believe you could convince someone, based on the artist's rendition
22 of an interaction [in Fig. 36C], that it's capable of an enzymatic event. Ex. 2475, 114:13-22.

23 114. Dr. Turchi testified that a POSA would require additional testing not present in P3, such as x-
24 ray crystallography and "[b]iochemical analyses that would assess affinity, off rates, on rates, [and]
25 binding parameters." *Id.* at 117:2-14.

1 115. Sample volume, time between lysis and storage, and method of cooling would all impact the
2 speed of lysate cooling; P3 is silent about these factors. Ex. 1018, ¶410; Ex. 1410, ¶101.
3 116. P3 does not specify the time between cell lysis and adding Proteinase K. Ex. 1018, ¶¶410, 415.
4 117. Dr. Doyon admitted Figure 37 shows Cas9 remained active in the lysate. Ex. 1604, 86:4-8.
5 118. Dr. Doyon admitted neither he nor a POSA are experts on the active temperature range of *S.*
6 *pyogenes* or SpCas9. Ex. 1604, 63:18-65:9.
7 119. Dr. Doyon based a POSA's knowledge of SpCas9's active temperature range on post-P3 papers
8 which test activity only between 20°–44°C. Ex. 2472, 23103; Ex. 2473, 6.
9 120. None of Dr. Doyon's cited references state or determine the required amount of ATP or the
10 required temperature for NHEJ. Ex. 2489, 3; Ex. 1229, 14067; Ex. 2490, 858.
11 121. CVC's cited cell-free NHEJ papers describe removing the nucleus from the cytosol, where
12 cellular ATP is. Opp. 21: 9-19. P3 did not do so; so cytoplasmic and mitochondrial ATP would
13 remain in the P3 lysate. Ex. 1410, ¶98.
14 122. Dr. Doyon miscalculated the number of cells per 6-well plate. *Compare* Ex. 2476 ¶61 (1.2 x
15 10^6 cells per plate) *with* Ex. 1604, 118:4-14 (1.2 x 10^6 cells *per well*).
16 123. Dr. Doyon did not consider NHEJ papers using fewer cells. Ex. 1604, 120:2-16; Ex. 1607.
17 124. Guschin's surveyor assay does not explain P3 bands in lanes where no ZFN or sgRNA-Cas9 was
18 added, like Fig. 36E Lane B that had no sgRNA. Ex. 2476 ¶ 40; Ex. 2479, 254; Ex. 1018 ¶¶417-418.
19 125. Dr. Turchi testified that to determine whether a given band is undigested heteroduplexes, "[y]ou
20 would need an independent determination of the heteroduplexes and the percentage of the DNA that
21 is heteroduplexed." Ex. 2475, 65:13-66:1, 67:13-18, 68:4-6.
22 126. A POSA could only diagnose Bands 2 and 8 as "undigested heteroduplexes" by sequencing the
23 results. Ex. 2475, 67:6-68:7.
24 127. CVC does not explain Bands 5, 6, and 7 in Fig. 38B. *See* Opp. 16:22-20:1.

1 128. A POSA would see unexpected bands as a problem to be fixed with better methods, not a way
2 to explain aberrant results. *See* Ex. 2514, 25-26
3 129. A POSA would know that a molecular-weight marker used known band sizes to measure
4 unknown experimental bands, not the other way around. Ex. 1604, 53:2-10.
5 130. A POSA would not know the "right" scale among the many available in 2012. Ex. 1604, 55:18-
6 56:2; Ex. 2475, 56:9-14.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **TOOLGEN REPLY 2** was filed via the Interference Web Portal on August 27, 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 to counsel for Junior Party as follows:

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