Filed on behalf of Junior Party Paper No. ___
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
UNIVERSITY OF VIENNA, AND EMMANUELLE CHARPENTIER

By: Eldora L. Ellison, Ph.D., Esq.
Eric K. Steffe, Esq.
David H. Holman, Ph.D., Esq.
Byron L. Pickard, Esq.
John Christopher Rozendaal, Esq.
Paul A. Ainsworth, Esq.
Michael E. Joffre, Ph.D., Esq.
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, NW
Washington, D.C. 20005
Tel: (202) 371-2600
Fax: (202) 371-2540
eellison-PTAB@sternekessler.com
esteffe-PTAB@sternekessler.com
dholman-PTAB@sternekessler.com
bpickard-PTAB@sternekessler.com
jcrozendaal-PTAB@sternekessler.com
painsworth-PTAB@sternekessler.com
mjoffre-PTAB@sternekessler.com

By: Li-Hsien Rin-Laures, M.D., Esq.
RINLAURES LLC
321 N. Clark Street, 5th floor
Chicago, IL 60654
Tel. (773) 387-3200; Fax (773) 929-2391
lily@rinlauresip.com

Sandip H. Patel, Esq.
MARSHALL GERSTEIN & BORUN LLP
6300 Willis Tower, 233 South Wacker Drive
Chicago, IL 60606
Tel: (312) 474-6300; Fax: (312) 474-0448
spatel@marshallip.com

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.

THE BROAD INSTITUTE, INC., MASSACHUSETTS INSTITUTE OF TECHNOLOGY, and PRESIDENT AND FELLOWS OF HARVARD COLLEGE
Senior Party

(Patents 8,697,359; 8,771,945; 8,795,965; 8,865,406; 8,871,445; 8,889,356; 8,895,308; 8,906,616; 8,932,814; 8,945,839; 8,993,233; 8,999,641; 9,840,713; and Application 14/704,551).

Patent Interference No. 106,115 (DK)
(Technology Center 1600)

CVC Reply 2 (for judgment based on priority)

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Cases

I. INTRODUCTION

The CVC inventors revolutionized the field of genome editing, giving the world a new system capable of cleaving and editing genes in eukaryotic cells. By identifying for the first time the necessary and sufficient components of the Type II CRISPR-Cas9 cleavage complex and further modifying the RNAs into a single-guide (sgRNA) format, the CVC inventors provided a simple, programmable tool for site-specific gene editing in eukaryotic cells. By the time Zhang learned of CVC's single-guide CRISPR-Cas9 system, the CVC inventors had already conceived multiple specific embodiments of the invention and were diligently working to reduce the invention to practice.

Count 1 recites a composition: a system or a eukaryotic cell comprising a sgRNA CRISPR-Cas9 system. Conception of that composition is complete "when the idea is so clearly defined in the inventor's mind that only ordinary skill would be necessary to reduce the invention to practice, without extensive research or experimentation." *Burroughs Wellcome Co. v. Barr Labs., Inc.*, 40 F.3d 1223 (Fed. Cir. 1994) (citing *Sewall v. Walters*, 21 F.3d 411 (Fed. Cir. 1994) and *Coleman v. Dines*, 754 F.2d 353 (Fed. Cir. 1985)). In accordance with that standard, the CVC inventors unquestionably conceived of the invention before Broad's alleged June 26 conception. By then, CVC had already documented at least two specific embodiments that they later reduced to practice: (1) vectors to express the sgRNA and Cas9 protein to cleave the CTLA6 gene in human cells, and (2) a pre-formed sgRNA/Cas9 ribonucleoprotein (RNP) complex that CVC had already demonstrated works in vitro and subsequently simply microinjected into fish cells to cleave DNA. During the critical period, CVC worked diligently to reduce to practice both embodiments of the invention, achieving multiple actual reductions to practice in a matter of months and requiring *no further inventive steps*.

Now that the record is developed, the facts and the law are clear that CVC is entitled to

favorable judgment on priority. Corroborated evidence shows that before Broad's conception, CVC had already documented the details of both embodiments that CVC then diligently reduced to practice. There is new testimony – from Barrangou, Sontheimer, and even Zhang's collaborator Marraffini – that scientists at the time expected it to be "straightforward" and "routine" to implement CVC's sgRNA CRISPR-Cas9 system in eukaryotic cells. Moreover, the record now shows that Zhang first learned of the sgRNA CRISPR-Cas9 system from CVC and exercised *no inventive skill* in reducing it to practice, confirming that CVC's conception was complete. Zhang merely used publicly known expression vectors and protocols to implement CVC's system in eukaryotic cells. Thus, CVC prevails for any of three independent reasons:

Reason 1: CVC conceived of the invention of Count 1 before Zhang's earliest alleged June 26 conception date, and exercised reasonable diligence during the critical period through CVC's reductions to practice. By June 26, CVC had not only conceived of a sgRNA CRISPR-Cas9 system for cleaving DNA in a eukaryotic cell, but had also identified the specific reagents and methods used in the working example described in its P3 application: chimera A sgRNA targeting the CLTA gene at a site adjacent a PAM, specific sgRNA and Cas9 vectors with specific promoters (U6 and CMV), a specific nuclear localization signal (NLS) added to Cas9, and human kidney (HEK293T) cells. The PTAB has already concluded this example is a constructive reduction to practice of Count 1. CVC's definite and permanent idea never changed, as the inventors used these same sgRNA, Cas9, vectors, promoters, and NLS successfully to cleave the CLTA target gene in HEK293T cells.

Reason 2: By June 26, CVC had also conceived of and documented a sgRNA CRISPR-Cas9 system in which the sgRNA and Cas9 components are assembled as a pre-formed RNP outside of the eukaryotic cell, and the RNP is delivered via microinjection into the cell nucleus.

Microinjection was a well-known technique for introducing RNA, DNA and/or protein into cells. This is precisely the embodiment the inventors reduced to practice on August 9, 2012, by microinjecting zebrafish embryos with a sgRNA-Cas9 RNP complex that resulted in an *rx*3 mutation. CVC's embodiment using a pre-formed RNP complex and nuclear microinjection obviated any obstacles that Broad imagines would have prevented a person of ordinary skill in the art from achieving CRISPR-Cas9-mediated target DNA cleavage.

The PTAB should enter judgment in CVC's favor because CVC has demonstrated a complete conception before Zhang, as shown by either of the foregoing embodiments, and reasonably continuous diligence from before Zhang's alleged conception date through each of CVC's multiple actual reductions to practice and CVC's constructive reduction to practice in P3. *See Rey-Bellet v. Englehardt*, 493 F.2d 1380, 1386 (C.C.P.A. 1974). Broad presented *no credible challenge* to CVC's diligence, neither disputing that CVC began its diligence just before June 26, nor pointing to *any* evidence to challenge CVC's diligence thereafter. Should the PTAB agree that CVC exercised reasonably continuous diligence from before Zhang's alleged conception date through the filing of CVC's P3 in January 2013, a finding that CVC's actual reductions to practice (ARTPs) succeeded is not required to grant CVC's motion. *Id.*

Reason 3: CVC is independently entitled to judgment on priority because, as fully explained in CVC's Opposition 5 (Paper 2567), Zhang derived the invention of Count 1 from the CVC inventors, via Marraffini. Zhang had no concept of a single-guide RNA CRISPR-Cas9 system for genome editing in eukaryotes until Marraffini relayed it to him, based on what Marraffini learned from CVC. Before then, Zhang had only toiled with a cumbersome RNA processing system, resulting in experiments that Zhang's graduate student summarized in June 2012 as having "all failed" before concluding that "[m]aybe other factors need to be identified."

See e.g., Ex. 5013, ¶¶145, 147, 156, A74. In contrast, as Marraffini testified, using CVC's system would be "straightforward." Recognizing a race to publish, Zhang dropped his RNA processing experiments and rushed to use well-known, published vectors and protocols to implement CVC's chimera A sgRNA system. That was not invention; that was derivation. *Applegate v. Scherer*, 332 F.2d 571, 573 (C.C.P.A. 1964) (1964).

In the face of such strong evidence in CVC's favor, Broad invites the PTAB to commit legal error by interpreting the law of conception to require a reasonable expectation of success. The PTAB should reject that interpretation. Conception has been consistently defined for over a century as "the formation, in the mind of the inventor of a definite and permanent idea of the complete and operative invention, as it is thereafter to be applied in practice." *Coleman*, 754 F.2d at 359 (citing *Mergenthaler v. Scudder*, 11 App.D.C. 264, 276 (1897)). It is a bedrock principle of conception law that "an inventor need not know that his invention will work for conception to be complete." *Burroughs*, 40 F.3d at 1228. Indeed, "[w]hether or not [the inventors] *believed* the invention[s] would in fact work … is *irrelevant." Id.* at 1229 (emphasis added). "[T]he discovery that an invention actually works is part of its reduction to practice." *Id.* at 1228. *Hitzeman v. Rutter*, 243 F.3d 1345 (Fed. Cir. 2001) does not support Broad's interpretation of conception law. *Hitzeman* at most held that conception requires an inventor to expect to *produce* the claimed invention, *not* have a reasonable expectation that the invention will work for its intended purpose. *Id.* at 1358. The evidence unambiguously shows that the CVC inventors not only expected to produce the invention of the Count, but did indeed produce it. Their subsequent demonstration that the system *worked* for its intended purpose was part of its reduction to practice, not conception. *Burroughs*, 40 F.3d at 1228.

Broad blatantly attempts to have the PTAB's '048 no interference-in-fact decision dictate

the outcome of this priority dispute. But that, again, would be an error, because that decision involved a different count (method), different legal issue (obviousness, not conception), and vastly different and limited evidentiary record. The facts and relevant law compel the PTAB to award priority to CVC here, and doing so is consistent with the PTAB's previous decisions.

II. ARGUMENT

A. CVC had a complete conception before Broad, coupled with CVC's uncontested reasonably continuous diligence through CVC's P3 filing in January 2013.

Conception requires a "definite and permanent idea" of the invention and its intended purpose, not a reasonable expectation that the inventor will succeed in achieving it, as Broad argues. *See Burroughs*, 40 F.3d at 1228. Broad concedes that its earliest possible conception date is June 26, 2012. Paper 2118, 42:9-10; MF201. Broad could not assert an earlier date because June 26 is the date Zhang first learned of the invention of Count 1 from CVC via Marraffini. Paper 2567, 33:12-41:24; Ex. 5265, 66:7-67:7; 68:13-21; Ex. 3713, 27-29; MF225-231.

CVC's evidence of conception before June 26 expressly meets all the elements of Count 1. *See* Ex. 4381, 63-65; Ex. 4382, 7; Ex. 4349, ¶¶124-128, 155, 167-172; Ex. 4603; Ex. 5105, 16-29; Ex. 4345, ¶¶61, 158-163, 167-174; Paper 1579, 7:17-19:9; MF26-30, 32-39. In addition to the March 1 notebook entry, by April 11, 2012, CVC's invention disclosure form described attaching an NLS to Cas9, confirmed that the system should target a DNA sequence adjacent a PAM, confirmed that a chimera A sgRNA system worked *in vitro*, and described approaches for using the system in eukaryotic cells, including (i) microinjecting the system's components into zebrafish embryos and (ii) producing the system's components from expression vectors in cells, both of which approaches CVC later used in its reductions to practice. Paper 1579, 14:2-17:10; Ex. 4382, 4, 7; Ex. 4349, ¶¶72-74; Ex. 5105, 16-29; Ex. 4345, ¶¶158-163; MF32-36. By May 28, the CVC inventors had produced a pre-formed complex of chimera A sgRNA and *S. pyogenes*

Cas9, and demonstrated it worked *in vitro* to cleave multiple eukaryotic DNA targets. Paper 1579, 17:11-19:9; Ex. 4382, 4-7; Ex. 4603; Ex. 4349, ¶¶124-128; Ex. 4345, ¶¶167-174. Also by May 28, the CVC inventors had documented a specific way to make their invention with expression vectors, and had documented the specifics of the sgRNA and Cas9 vectors, promoters (U6 and CMV), sgRNA (CLTA6 chimera A), target sequence (CLTA site adjacent to PAM), Cas9 (*S. pyogenes*), target cell (human kidney cells), and delivery method (transfection)—i.e., the very details Broad alleges were lacking for CVC's conception on March 1. *See id.;* Paper 2569, 10:5-8, 36:12-17; MF37-39, 202-208, 211-214.

CVC's inventors documented each of the approaches discussed above, corroborating their complete conception of at least two specific embodiments of Count 1 before June 26. *See e.g.*, Paper 1579, 7:17-19:9; Paper 2567, 31:9-33:11. CVC prevails on priority by showing conception of either embodiment before Broad, coupled with CVC's reasonably continuous diligence to a reduction to practice. Broad failed to raise any plausible challenge to CVC's diligence through CVC's P3 filing date. Thus, the law compels judgment in CVC's favor.

Broad nitpicks CVC's March 1 notebook entry, arguing "descriptions of delivery technique, promoter selection, NLS selection and placement, codon optimization approach, vector selection … [or] planned experimental conditions…." and "other parameters, such as the U6 promoter, are not found…." Paper 2569, 10:5-8, 36:12-17. The response is that Count 1 neither recites nor requires a specific delivery technique, promoter, NLS, codon optimization, or vector. These aspects are routine and involve exercising only ordinary skill; none are required for complete conception of the Count. MF5-21. Indeed, complete conception does not require that "the final size and shape of every part and the location of every nut, screw, and bolt must be exactly foreseen before the conception of an apparatus can be said to be complete." *In re Tansel*,

253 F.2d 241, 243 (C.C.P.A. 1958). Even if such details were required for conception, CVC had those, too—*all before June 26. See e.g.*, Paper 1579, 17:11-19:9; MF32-39, 202-208, 211-214.

1. Reason 1: CVC had a complete conception of Count 1 evinced by CVC's specific embodiment of delivering the sgRNA and Cas9 components to human cells using specific expression vectors and transfection

By June 26, CVC had documented conception of a specific embodiment of Count 1 that includes the *same* sgRNA sequence, Cas9 sequence, promoters, NLS, cell type, and methods described in Example 2 in CVC's P3 application, which the PTAB concluded is a constructive reduction to practice of Count 1. Ex. 3004, ¶¶408-450; Paper 877, 106:1-14; MF49, 206-210. If CVC's disclosure of those elements in its P3 application was sufficient to establish *constructive reduction to practice*, CVC's description of that same combination of elements by May 28 is necessarily sufficient to demonstrate *conception*. *Haskell v. Colebourne*, 671 F.2d 1362, 1365-66 (C.C.P.A. 1982) (disclosure "adequate to establish a constructive reduction to practice" is sufficient to establish conception); *Spero v. Ringold*, 377 F.2d 652, 660 (C.C.P.A. 1967) (same).

By May 28, 2012, the CVC inventors had constructed the pMJ874 vector, which uses the *U6 promoter* to drive expression of the *CLTA6 sgRNA* targeting a *specific CLTA target gene sequence* next to a *PAM*. *See e.g.*, Paper 1579, 17:11-19:9; Ex. 4382, 4-7; Ex. 4349, ¶¶124-128; Ex. 4603; Ex. 4345, ¶¶167-172; Ex. 3004, ¶¶408-450; MF37-39, 202. This is the *same pMJ874 vector* that Jinek and East would later transfect into human HEK293T cells with *S. pyogenes* Cas9 to successfully target and cleave the CLTA target DNA, and the *same* vector used in Example 2 of CVC's P3 application. *Id*. And, as the contemporaneous evidence shows, CVC specifically chose the CLTA locus because their colleagues Drubin and Cheng had already shown successful genome editing using zinc finger nucleases (ZFNs) targeting the same locus. *See e.g.*, Paper 1579, 27:20-28:17; Ex. 4475; Ex. 4775; Ex. 4776; Ex. 4350, ¶62; Ex. 4349, ¶¶75, 78; Ex. 4352, ¶12; Ex. 5013, ¶¶45-47; MF23-24. Also by May 28, CVC had constructed an *S.*

pyogenes Cas9 expression vector with the same elements in P3's Example 2, *i.e.*, a strong *CMV promoter*, *NLSs*, and a *GFP tag*, and CVC had *successfully expressed* the Cas9 protein in *human HEK293T cells* and *visualized nuclear localization* of Cas9. *See e.g.*, Paper 1579, 18:17-19:4; Ex. 4349, ¶¶109, 123-127, 155; Ex. 4444; Ex. 4484; Ex. 4536; Ex. 4345, ¶173; Ex. 3004, ¶¶408-450. The CVC inventors were also *codon optimizing the Cas9 gene* to improve expression levels. *Id.* CVC's specific vector embodiment undeniably meets all the elements of Count 1:

CVC's Specific embodiment	**Elements of Count 1**
• Human HEK293T cells with CLTA target gene • Type II CRISPR System • *S. pyogenes* Cas9 • CLTA6 chimera A sgRNA • System is capable of cleaving or editing CLTA locus	✓ Eukaryotic cell comprising target DNA molecule ✓ Type II CRISPR System comprising: ✓ - Cas9 ✓ - sgRNA ✓ System is capable of cleaving or editing a target DNA molecule or modulating transcription of a gene encoded by the target DNA molecule

See Paper 1579, 7:17-19:9; Ex. 4345, ¶¶167-174; MF32-39, 202-207.

Before June 26, CVC also understood how to obviate Broad's theoretical "obstacles" to implementing the invention in eukaryotic cells. MF3-24. According to Broad's expert, Breaker, Zhang allegedly overcame theoretical obstacles in eukaryotic cells, such as RNA degradation, localization, complex formation, and chromatin, by making certain "adaptations" to CRISPR – using strong promoters to express the sgRNA and Cas9 protein, adding an NLS to Cas9, and codon optimization. Ex. 5264, 69:8-70:2, 77:18-79:8, 84:21-85:13, 97:3-9. The contemporaneous evidence summarized below shows that CVC had already made each of Zhang's so-called "adaptations" before June 26 – i.e., before Zhang ever learned of CVC's sgRNA CRISPR-Cas9 system – and understood that these were routine methods to address the purported obstacles.

Broad's theoretical obstacle	**CVC's evidence of conception *before June 26***	**Citations to record**
RNA degradation	• CVC made the **pMJ874 vector** using the strong Pol III promoter, **U6**, to drive **expression of CLTA6 sgRNA**.	Paper 1579, 17:11-19:9; Ex. 4382, 1-7; Ex. 4349, ¶¶124-128; Ex. 4603; Ex. 4345, ¶¶167-172; Ex. 5264, 69:8-70:2.
Localization and complex formation	• CVC made an expression vector using the strong Pol II promoter, **CMV**, to express ***S. pyogenes* Cas9** with two **NLSs** and a **GFP** tag. • CVC demonstrated **nuclear localization** of NLS-Cas9 in **human HEK293T cells**. • CVC was **codon optimizing the Cas9 gene** sequence.	Paper 1579, 18:17-19:4; Ex. 4349, ¶155; Ex. 4444; Ex. 4484; Ex. 4536; Ex. 4345, ¶173; Ex. 5264, 77:18-79:8, 79:11-80:2, 82:17-84:5, 84:21-85:13.
Chromatin	• CVC designed a **series of sgRNA guides** to target Cas9 to the **human CLTA locus**. • CVC selected the **CLTA locus** because the same locus had been previously shown to be **accessible** by ZFNs, indicating to the CVC inventors that **chromatin was not a concern**.	Paper 1579, 27:20-28:17; Ex. 4382, 1-5; Ex. 4469; Ex. 5040; Ex. 4350, ¶¶33-34; Ex. 4384; Ex. 5264, 97:3-9.

"An idea is definite and permanent when the inventor has a specific, settled idea…." *Burroughs*, 40 F.3d at 1228. CVC's contemplated expression vector embodiment was definite and permanent, and its design did not change over time. Paper 1579, 12:9-13:2, 17:11-19:9, 29:8-32:10. CVC's vector using the U6 promoter to express CLTA chimera A sgRNA (pMJ874) was constructed by May 28 and is the *same chimera A vector* Jinek and East used when reducing CVC's invention to practice in October 2012, only a few months later. *Id.*, 29:8-32:10; MF37-39, 202-208. Likewise, CVC's *S. pyogenes* Cas9 vector was constructed by May 28 and uses the *same CMV promoter*, encodes the *same Cas9 amino acid sequence*, and contains the *same NLS* that Jinek and East used to reduce the invention to practice in October 2012. *Id.* CVC's target cell (human HEK293T cells) and vector delivery method (transfection) described by May 28 were also the same as what CVC used in its October actual reductions to practice. *Id.*

Broad argues on page 36, lines 18-20, that CVC's conception *by March 1* was incomplete

because the chimera A sgRNA in Jinek's *March 1* notebook entry is "not what results when expressed from a U6 promoter." Paper 2569, 36:18-20. That argument is irrelevant. By *May 28*—before Broad's earliest alleged conception—CVC had already made the vector that uses the U6 promoter to express the CLTA sgRNA (pMJ874). MF37-39, 202-208. Moreover, Broad's argument is scientifically wrong. Dr. Doyon explained that adding four U's to the 3' end of an RNA transcript made from the U6 promoter was a well-known inherent aspect of using the U6 promoter and is not a substantive change in structure or function. Paper 2567, 22:11-24:16; Ex. 5013, ¶¶74-76; MF20. Even Broad's evidence confirms this. Zhang's internal emails and the Cong 2013 publication repeatedly refer to the sgRNA sequences with no mention of additional U's on the 3' end because it was so well understood that the U's would result from the promoter. *See e.g.*, Ex. 3751; Ex. 3537, 3; Ex. 3829; Ex. 3201, Fig. 2B; Ex. 3425, ¶24; Ex. 5013, ¶75.

Broad fabricates an illusion of doubt in the inventors' minds by cataloging snippets from various CVC documents. *See* Paper 2569, 24:22-29:11. These simply reflect that the inventors understood and considered these routine implementation issues during the process and, at all stages, had a plan to address them. The inventors all testified as much. Ex. 4350, ¶40; Ex. 4349, ¶¶28, 36, 230; Ex. 4351, ¶26; Ex. 4348, ¶¶22-24; MF3-24. At most, the snippets reflect that the inventors did not have absolute *certainty* that every single experiment would work. But complete conception does not require certainty that the invention will work; discovery that the invention *actually works* is part of its reduction to practice. *Burroughs*, 40 F.3d at 1228.

Moreover, "the extent of testing or other research done after the mental formulation of an invention is not a reliable indicator" of a complete conception—instead, it is the "nature of [the] research" that matters. *Rey-Bellet*, 493 F.2d at 1387; *Sewall*, 21 F.3d at 415 n.3 ("the existence of research or experimentation does not necessarily indicate, by itself, that complete conception did

not exist."). CVC's research after June 26 required nothing more than ordinary skill and routine methods, and does not undermine conception. *See Rey-Bellet*, 493 F.2d at 1387; *Summers v. Vogel*, 332 F.2d 810, 815-16 (C.C.P.A. 1964).

Even for the more demanding standard of enablement, supposed failure by POSAs—or even the *inventors themselves*—does not disprove invention. *Trumbull v. Kirschbraun*, 67 F.2 974, 980 (C.C.P.A. 1933); *Field v. Knowles*, 183 F.2d 593, 604-605 (C.C.P.A. 1950). It certainly does not do so where, as here, the inventors as well as multiple POSAs actually reduced the invention to practice using information the CVC inventors disclosed by June 26, "mak[ing] such adjustments and corrections as would occur to those naturally skilled in the art." *Trumbull*, 67 F.2d at 980; *Field*, 183 F.2d at 601. "Conception is complete when *one* of ordinary skill in the art could construct the apparatus without unduly extensive research or experimentation." *Sewall*, 21 F.3d at 415 (emphasis added); *see also*, *Mergenthaler v. Scudder*, 11 App. D.C. 264, 277 (D.C. Cir. 1897) ("'The true date of invention is at the point where the work of the inventor ceases and the work of the mechanic begins.'"); *Applegate*, 332 F.2d at 573.

2. Reason 2: CVC had a complete conception of Count 1 before June 26, evinced by CVC's specific embodiment of delivering the sgRNA and Cas9 components to zebrafish cells as a pre-formed sgRNA-Cas9 ribonucleoprotein complex using nuclear microinjection.

CVC's alternative second specific embodiment also proves CVC's complete conception of Count 1 before June 26. Before that date, CVC had conceived of all the elements of Count 1 and contemplated delivering a pre-formed sgRNA-Cas9 ribonucleoprotein (RNP) complex to zebrafish embryos using nuclear microinjection. Paper 1579, 14:2-18:4; Ex. 4382, 7; Ex. 4349, ¶¶124-128; Ex. 4603; Ex. 5105, 16-29; MF32-39, 211-214. CVC's pre-formed RNP complex is the very "system capable of cleaving … the target DNA molecule," required by the Count. MF32-39, 211-214.

For example, before June 26, CVC had conceived of, and indeed already *constructed, a working sgRNA and Cas9 system as a pre-formed RNP complex* and confirmed that it was capable of cleaving at least *five different eukaryotic target DNA sequences* within the GFP gene *in vitro*. *See e.g.*, Paper 1579, 17:11-19:9; Ex. 4382, 2, 7; Ex. 4349, ¶¶124-128; Ex. 4345, ¶¶167-172; MF32-39, 211-214. By April 11, CVC had identified "Example references" for introducing their "RNA-guided DNA endonuclease" into eukaryotic cells for "gene targeting in zebrafish," citing multiple articles published for ZFN and TALENs that describe nuclear *microinjection into zebrafish embryos*. *See e.g.*, Ex. 5105, 2-3, 8; Ex. 4349, ¶¶72-74; Ex. 4350, ¶¶57-61; Ex. 4345, ¶¶158-164; MF32-36. And the CVC inventors had already agreed to contact Raible to microinject CVC's sgRNA CRISPR-Cas9 system into zebrafish. *See e.g.*, Paper 1579, 19:19-24, 22:2-6; Paper 2567, 32:21-33:3; Ex. 4799; Ex. 4348, ¶115; Ex. 4351, ¶¶56-58; Ex. 4294, ¶¶9-14; Ex. 5013, ¶¶45-47. CVC's specific RNP embodiment undeniably meets all the elements of Count 1:

CVC's Specific embodiment	**Elements of Count 1**
• Zebrafish embryo with target DNA • Type II CRISPR System • *S. pyogenes* Cas9 • Chimera A sgRNA • System is capable of cleaving or editing the target DNA	✓ Eukaryotic cell comprising target DNA molecule ✓ Type II CRISPR System comprising: ✓ - Cas9 ✓ - sgRNA ✓ System is capable of cleaving or editing a target DNA molecule or modulating transcription of a gene encoded by the target DNA molecule

See Paper 1579, 7:17-19:9; Ex. 4345, ¶¶167-174; Ex. 4343, ¶¶60-70; MF32-39, 211-214.

CVC's RNP specific embodiment further shows the inventors' idea was definite and permanent, as the sgRNA CRISPR-Cas9 system never changed from conception through the actual reductions to practice in zebrafish on August 9. *See e.g.*, Paper 1579, 12:9-13:2, 17:11-19:9; MF45, 217. For example, Raible prepared CVC's sgRNA and Cas9 components as a pre-formed RNP complex comprising a *chimera A sgRNA* and *S. pyogenes Cas9* – the same chimera

A sgRNA-Cas9 system CVC had already demonstrated to be capable of cleaving eukaryotic target DNA sequence *in vitro*. Ex. 4294, ¶¶8, 54; Ex. 4382, 1-7; Ex. 4349, ¶¶124-128; Ex. 4603; Paper 1579, 14:2-17:10; MF45, 217. Raible's routine microinjection of the sgRNA-Cas9 RNP complex to the nucleus of the zebrafish embryo was the same technique CVC had already described, e.g., in their invention disclosure form. Ex. 4294, ¶¶8, 54; Ex. 5105, 2-3, 8. Indeed, the PTAB has previously acknowledged that "*direct injection*, codon optimization, and targeting of proteins and RNA to the cell nucleus" were "*routine and known to be useful* in achieving activity of prokaryotic proteins in eukaryotic cells" Ex. 3110, 35:3-7 (emphasis added). By 2008, microinjection was "widely used in the studies of transduction-challenged cells, transgenic animal production, and in vitro fertilization to mechanically transfer DNAs, RNA interferences, sperms, proteins, peptides, and drugs. The advantages of microinjection include the precision of delivery dosage and timing, high efficiency of transduction as well as low cytotoxicity." Ex. 4053, Abstract.

Chylinski, Charpentier and Raible all "expected" that the experiments would result in success. Ex. 4348, ¶¶11, 25, 115; Ex. 4351, ¶¶23, 26, 56; Ex. 4294, ¶¶10, 13, 74. Charpentier in particular was "convinced" the system would work, and it did, as Raible observed and documented an eyeless zebrafish by August 9. Ex. 4351, ¶60; Ex. 4294, ¶14. Paper 1579, 22:1-27:15; Ex. 4294, ¶¶50-58, 70-71; Ex. 4338, 79-80; Ex. 4343, ¶¶59-72; MF45, 215-221.

Tellingly, Broad's opposition barely addresses CVC's pre-formed RNP complex microinjection evidence. Paper 2569, 46:13-14. This is undoubtedly because microinjecting a pre-formed RNP complex directly to the nucleus *completely obviates* Broad's theoretical barriers. *See* Paper 871, 17:3-12 (*Judge Katz:* "But is there something different about microinjection that sort of negates any of the issues that we talked about in the prior opinion"

and "which overcomes any of those problems that were known in the art about using this kind of a complex system in a eukaryotic cell?"); *see also*, Ex. 4036, ¶¶136-137; Ex. 4343, ¶¶93-106; Ex. 4345, ¶87. As Dr. Moens explained, when microinjecting a pre-formed RNP complex:

> There is *no need for codon optimization, RNA or protein expression, concomitant folding, and co-localization of RNA and protein*, because *the complex is already formed* before injection. The use of pre-formed complexes also *minimizes RNA and protein degradation*, because the complex is protected from cellular factors, as it is in bacterial host cells.

Ex. 4343, ¶95 (emphasis added); *see also*, Ex. 4345, ¶87. Microinjection of a pre-formed RNP complex into zebrafish also negates any potential impact of chromatin because, e.g., the zebrafish cells are rapidly dividing. MF23-24. As Doyon explains, "cells have time periods when the DNA is unwrapped from chromatin, and this happens more often during cell division. Opening of access to chromatin happens more frequently *when cells are dividing frequently, such as zebrafish embryos*." Ex. 4345, ¶113 (emphasis added); *see also*, Ex. 4343, ¶103 ("chromatinization changes dynamically in rapidly dividing cells and across developmental time."). Further, Dr. Zamore testified, "DNA is constantly alternating between states where it's wrapped around the nucleosome and states where it's not completely wrapped around the nucleosome," and "nucleosome[s] slide" along the DNA "leaving areas open for processes like gene expression." Ex. 5349, 196:2-198:2. Breaker admitted he did not consider publications disclosing nucleosome sliding and the open and closed dynamics of chromatin. Ex. 5347, 213:22-223:11, 226:20-228:18; Ex. 5323, Ex. 4570; Ex. 5325.

The inventors knew that microinjection rendered Broad's "obstacles" irrelevant. For example, in a June email to Charpentier, Chylinski stated that injecting RNPs into zebrafish meant there was "no need for in vivo expression of neither RNA nor protein, codon bias optimization and so on." Ex. 4796; Ex. 4348, ¶116; Ex. 4351, ¶¶58-61. Charpentier also understood that RNA degradation was not an issue due to stability of the RNP complex and that

chromatin was not an issue in rapidly dividing cells. Ex. 4351, ¶¶26, 32; MF3-4, 23-24, 218-219.

Of the 22 quotes Broad highlights, only two involve microinjecting a pre-formed RNP into a zebrafish embryo. *See* Paper 2569, 27 (citing Exs. 4799 and 4916). Neither quote indicates the inventors encountered any perplexing difficulties or unduly extensive experimentation. In Exhibit 4799, Raible merely states that "off target sites" and "toxicity" are relevant to "*human applications*" (i.e., clinical therapeutics). *Id.* (emphasis added). Broad crops out the remainder of Raible's sentence, which says these are "*less of an issue in zebrafish or medaka*." *Id.* (emphasis added). Exhibit 4916 merely acknowledges that CVC "observed" toxicity in some fish, far from a "perplexing intricate difficulty" undermining conception. Raible stated that toxicity from the *E. coli*-produced protein preparation was not unexpected and that there were "conventional way[s] of proceeding" including using "lower concentrations" or a "fresh preparation with more highly concentrated Cas9" – which was exactly what he did. Ex. 4294, ¶¶39-40.

Broad's witness, Mourrain, attempts to brush aside CVC's RNP microinjection approach, opining with no support that "no one was injecting gene editing nuclease proteins in fish at the time." Ex. 3447, ¶115. But Mourrain is contradicted by evidence he did not consider: that artisans were routinely injecting gene-editing proteins such as I-*Sce*I meganucleases into zebrafish and medakafish embryos. *See* Ex. 4328; Ex. 4622; Ex. 5344; Ex. 4343, ¶87; MF222-224; Ex. 5348, 182:20-183:4; Ex. 4053, 507 ("direct microinjection of proteins is most powerful to deliver cytosolic and nuclear proteins."); MF222-224.

Before June 26, the CVC inventors conceived of the sgRNA CRISPR-Cas9 system of Count 1, had made a pre-formed RNP complex containing a chimera A sgRNA and *S. pyogenes* Cas9 and demonstrated its ability to cleave DNA *in vitro*, and had a settled approach for microinjecting the sgRNA-Cas9 system as a pre-formed RNP complex into zebrafish embryos.

Paper 1579, 7:17-19:9; MF32-39, 211-214. Given the "stunning efficiency" *in vitro*, Raible predicted that he would have results within 1-2 months. MF215. Within six weeks, as predicted, Raible successfully reduced the invention to practice. Paper 1579, 22:1-27:15; MF45-47, 215-217. East repeated that success by November 1, 2012, by introducing a pre-formed RNP into human cells with a commercially available transfection reagent. Paper 1579, 34:1-13; Ex. 4353, ¶102; Ex. 4366, 38; Ex. 4349, ¶252; MF50. Thus, CVC's CRISPR "system" was not only "capable of cleaving or editing," as Count 1 recites, but it indeed cleaved and edited the target DNA. *Id.*

3. Multiple lab groups used CVC's system with only ordinary skill and routine techniques, further confirming CVC's conception.

The record now includes testimony from multiple scientists about the state of the art in 2012. *See* Exs. 4343 (Moens), 4345 (Doyon), 4348 (Chylinski), 4349 (Jinek), 4350 (Doudna), 4351 (Charpentier), 4354 (Sternberg), 5013 (Doyon), 5014 (Zamore), 5016 (Barrangou), 5018 (Sontheimer), and 5265 (Marraffini). *See* Paper 1579, 36:5-42:4; Paper 2567, 10:1-15:20; *see also*, Exs. 4036, 4189, and 4193. For example, Sontheimer stated, "scientists would be able to quickly apply [CVC's system] for genome editing in eukaryotic cells, because the process for doing so was straightforward and required only routine genome-editing techniques." Barrangou and Marraffini agreed that it would be "straightforward." Ex. 5016, ¶17; Ex. 5265, 31:8-19. And Barrangou stated, "[t]he question at the time was not whether [CVC's] CRISPR-Cas9 system would work in eukaryotes—my colleagues and I all expected it would work and someone would get that first paper—the real question was whether it could *outcompete* the existing genome-editing technologies, such as TALENs and ZFNs." Ex. 5016, ¶17; *see also*, *id.*, ¶¶18-20; Ex. 5018, ¶21; Ex. 4294, ¶74. But, outcompeting existing technologies or any particular efficiency are not part of the Count.

The state of the art in 2012 was confirmed by the fact that numerous lab groups rapidly applied CVC's sgRNA-Cas9 system in eukaryotic cells using ordinary skill and the same reagents and methods they had previously published for use with ZFNs or TALENs. Paper 1579, 35:10-36:4; Ex. 4076, Fig. 1A; Ex. 4079, Fig. 1B; Ex. 3623, Fig. 1A; Ex. 4084, Fig. 1C; Ex. 4233, Fig. 1; Ex. 5020; Ex. 4075; Ex. 4080; Exs. 4082-4087; Ex. 4089; Ex. 4090; Ex. 4093; Ex. 4106; *see also*, Ex. 5350, 54:3-58:1; MF232-237. This is also discussed in CVC's Opposition 5. *See* Paper 2567, 19:14-20:19. As the Federal Circuit noted in *Regents of Univ. Calif. v. Broad Inst., Inc.*, evidence of near simultaneous ARTPs can be used to show "the level of skill in the art" and "constitutes objective evidence that persons of ordinary skill in the art understood the problem and a solution to that problem." 903 F.3d 1286, 1295 (Fed. Cir. 2018).

CVC's complete conception is further bolstered by the new evidence in the record regarding Zhang's purported reductions to practice. Once he learned of CVC's sgRNA system for gene editing in eukaryotes, he quickly used it, along with published reagents and protocols, exercising just ordinary skill. Paper 2567, 20:20-30:15; Ex. 5013, ¶¶64-104; MF232-233. Zhang made no contributions to Count 1. As Marraffini testified, once he told Zhang about CVC's sgRNA CRISPR-Cas9 system for genome editing in eukaryotic cells, the next steps of applying CVC's system were "pretty straightforward." Ex. 5265, 31:8-19. The PTAB may not ignore the copious objective evidence showing that CVC and the rest of the field understood the alleged "problem" and knew how to solve it. *Regents* at 1295. That neither CVC nor others encountered "perplexing intricate difficulties arising every step of the way" (*Rey-Bellet*, 493 F.2d at 1386) or "unduly extensive research or experimentation" (*Sewall*, 21 F.3d at 415) when applying CVC's sgRNA CRISPR-Cas9 system in eukaryotic cells confirms the completeness of CVC's conception.

It is irrelevant whether, as Broad argues on page 33, lines 23-24, some experiments performed by CVC's colleagues in other eukaryotic cell types (e.g., nematodes) had not yet succeeded, whether there was doubt that the positive results in medakafish ("less green" embryos) "might be heterozygotes, might be unspecific," or whether collaborations in yeast, mice, or plants had not yet started. Paper 2569, 33:23-24; *id.*, 12:16-14:11. The question is not whether some colleagues' experiments succeeded or failed, but rather whether the inventors' conception of Count 1 was complete. The law does not require a 100% success rate for attempts at reduction to practice. *See Trumbull* at 980. In fact, the law of conception does not require *any* success rate for reductions to practice. *See Rey-Bellet*, 493 F.2d at 1389. There is no evidence that any of CVC's colleagues engaged in unduly extensive research or experimentation. *See Sewall*, 21 F.3d at 415. Broad also improperly infers activities not in the record, and which CVC does not rely upon for conception or reduction to practice. *See Agilent Tech. Inc. v. Dionex Softron GMBH*, Interference 106,087, Paper 267 (P.T.A.B., Feb. 25. 2021) (non-precedential) (finding "evidence that may have been, but was not, relied upon" by junior party irrelevant to "whether [junior party] has met its burden of proof.") Rather, CVC cites correspondence with its colleagues as e*vidence of CVC's reasonable diligence*, which, as discussed below, Broad barely challenged.

4. CVC's reasonably continuous diligence during the critical period through CVC's P3 filing went unchallenged by Broad

CVC proved its inventors exercised reasonably continuous diligence from before Broad's alleged June 26 conception date through CVC's actual reductions to practice in August, October, and November 2012, and through CVC's constructive reduction to practice in January 2013. *See* Paper 1579, 3:11-12, 32:11-33:6, 49:5-21, Appx 3, 3-85. Broad's opposition only cites pages 44-45 of CVC's priority motion, which discusses CVC's diligence activities in *March and April*

2012, outside of the critical period. *Id.* Broad made no attempt to challenge the fact that CVC was acting diligently from just before Broad's earliest asserted conception date of June 26. MF37-39, 252-254. Broad provides *no expert testimony* and cites *no evidence* to challenge CVC's diligence during the critical period, instead providing only conclusory attorney argument. Paper 2569, 39:17-24.

CVC had a complete conception of Count 1 *before June 26*, coupled with *uncontested diligence* from at least just before June 26 through CVC's P3 filing, which the PTAB has already deemed a constructive reduction to practice of Count 1. Ex. 3004, ¶¶408-450; Paper 877, 106:1-14. Thus, whether CVC has proven its ARTPs in the Fall of 2012 does not matter for priority. At a minimum, ARTP efforts constitute diligence. *See Rey-Bellet*, 493 F.2d at 1389 (even repeated attempts and failures at reduction to practice count as diligence).

5. Broad's attempt to rewrite conception law fails: conception does not require a reasonable expectation of success.

Broad's attempt to import a "reasonable expectation of success" into conception law lacks any credible basis. Binding precedent in *Applegate*, 332 F.2d at 573, *Burroughs*, 40 F.3d at 1230-1231 and, more recently, *Dana-Farber Cancer Inst., Inc. v. Ono Pharm. Co.*, 964 F.3d 1365, 1372 (Fed. Cir. 2020), have made it clear that conception does *not* require a reasonable expectation of success. *See also*, Paper 2567, 37:14-39:14; Paper 1579, 42:5-43:21. Rather, it requires a specific, settled idea of *the invention*, which the CVC inventors possessed as evinced by their documentation of the specific embodiments discussed above. *Coleman*, 754 F.2d at 359.

Broad mischaracterizes *Hitzeman v. Rutter* as requiring both the inventors *and* a POSA to have a reasonable expectation of success for *the inventors* to have had a complete conception. Paper 2569, 19:1-4. Broad's theory would turn conception on its head, failing to recognize that inventors are persons of *extraordinary creativity*, in contrast to POSAs, who merely have

ordinary creativity. Instead of assessing whether a complete conception has formed in the mind *of the inventor*, Broad would require a complete conception *in the mind of a POSA*. This is not the law, nor does it even make sense. The Federal Circuit explicitly rejected Broad's theory: "[f]or conception, we look *not to whether one skilled in the art* could have thought of the invention, but whether *the … inventors* actually had in their minds the required definite and permanent idea." *Burroughs*, 40 F.3d at 1232. Broad's theory is found nowhere in *Hitzeman*.

In *Hitzeman*, the count required a DNA expression vector capable of replicating in yeast such that it (i) expresses hepatitis B virus S protein in yeast and (ii) the yeast assembles the S protein into virus particles with a sedimentation rate identical to authentic HBsAg 22nm particles. *Hitzeman*, 243 F.3d at 1352. *Hitzeman* held that the purported inventors had not yet conceived the invention because they had no "reasonable expectation that they would *produce* the claimed invention" in the first place– particularly the recited 22 nm particles. *Id.* at 1358. *See Burroughs*, 40 F.3d at 1229 (conception requires only (1) inventors have in their minds "the specific structure of the compound" and (2) "an operative method of making it"). CVC's conception satisfies *Hitzeman* because before June 26, CVC had already produced the sgRNA CRISPR-Cas9 system of Count 1 as a pre-formed RNP complex outside of a cell and had a way of delivering the complex into a eukaryotic cell via microinjection. Paper 1579, 14:2-17:10; Ex. 4382, 7; Ex. 4349, ¶¶124-128; Ex. 4603; Ex. 5105, 16-29; MF214. A pre-formed RNP complex obviates any issues related to expressing or assembling the sgRNA or Cas9 in the eukaryotic cell, because the complex is *already formed* outside the eukaryotic cell *prior to delivery*. Ex. 4343, ¶95; MF214. And there is no dispute that CVC's pre-formed sgRNA-Cas9 RNP complex, demonstrated to be "capable of" cleaving eukaryotic DNA, could be delivered into a eukaryotic cell using routine techniques and ordinary skill, such as microinjection into a zebrafish embryo

as Raible did. MF16, 217-219. In contrast to the inventors in *Hitzeman*, the CVC inventors *expected to easily produce the invention of Count 1*. The same holds true for CVC's expression vector embodiment. Before June 26, the inventors had made specific vectors with specific promoters, specific CLTA sgRNA, and specific Cas9, and *expected to produce the invention* using those same elements, and that is all that *Hitzeman* requires.

Broad also argues that the court's disposition of priority on the sixth patent in *Burroughs* supports its reasonable expectation of success theory. Paper 2569, 17:24-18:22. But Broad overlooks critical facts in *Burroughs*. The sixth patent in *Burroughs* claimed a "method of increasing the number of T-lymphocytes in a human infected with the [HIV] virus comprising administering to said human an effective amount of AZT." *Burroughs*, 40 F.3d at 1231. In contrast to the inventions in the first through fifth patents, there was a factual dispute as to whether the inventors *even contemplated* that AZT would cause the increased T-cell numbers claimed in the sixth patent. *Id.* As the court noted, "the record [was] *devoid of any statement* that the inventors thought AZT could raise a patient's T-cell levels." *Id.* (emphasis added). The court mentioned reasonable expectation of success only because such an expectation might imply that the inventors themselves likely contemplated that claimed element. This is in marked contrast to the CVC inventors, who clearly documented before June 26 their idea for a sgRNA-Cas9 system that was capable of cleaving a target DNA in a eukaryotic cell. Paper 1579, 7:17-19:9. And, the CVC inventors expected it to work based on their *in vitro* successes, and later demonstrated the use of this same system in their actual reductions to practice. *Id*., 22:1-35:9. There is no dispute that eukaryotic cells comprising CVC's sgRNA-CRISPR Cas9 system "capable of" cleaving DNA could be produced, and no evidence to support Broad's argument that POSAs and the inventors would expect a system with "stunning efficiency" to lose all of its activity upon

microinjection.

For example, in emails to the editors of *Science* and *Nature*, Doudna and Charpentier each stated that the inventors expected this gene-editing capability, stating "[w]e *foresee considerable exploitation of this system for targeted genome editing in cells of the three kingdoms of life* for biotechnological biomedical and gene-therapeutic purposes." Ex. 4598 and Ex. 4583 (emphasis added). Similarly, Charpentier stated in a June 28 draft email to Raible that the CVC inventors were "indeed *convinced*" that the system would work in a zebrafish model, and were eager to test it. Ex. 4351, ¶60; Ex. 4807; Ex. 4294, ¶14; *see also*, Ex. 4469 (Doudna email to Jinek stating, "a lot of the pieces are already in place … we could compare efficiencies [between Cas9 and ZFN]"). Thus, the facts here are nothing like the sixth patent in *Burroughs*; rather, the facts here mirror *Burroughs* patents 1-5, where the court said:

> The question is not whether Burroughs Wellcome reasonably believed that the inventions would work for their intended purpose…, *but whether the inventors had formed the idea of their use for that purpose in sufficiently final form* that only the exercise of ordinary skill remained to reduce it to practice.

Burroughs, 40 F.3d at 1231 (emphasis added). Here, the inventors' idea of Count 1 was "in sufficiently final form," as evinced by their documented specific embodiments discussed above.

Moreover, evidence in the record from Raible, Sontheimer, Barrangou, and Marraffini show that POSAs expected success *with CVC's sgRNA CRISPR-Cas9 system specifically* once they learned of it. Ex. 5016, ¶¶17-20; Ex. 2018, ¶21; Ex. 4294, ¶74; Ex. 5265, 31:8-19; MF268, 271-275. Broad's reasonable expectation of success theory is premised on supposed issues with non-analogous systems: group II introns, ribozymes, and riboswitches, all of which Broad admits are RNA enzymes. Paper 2569, 22:27-24:21, 26:1-29:11. As Zamore and Doyon explain, such systems differ from CRISPR-Cas9 because the *RNA, rather than protein, is the catalytic component* that cuts the DNA, and that RNA is vastly larger and more complex than the simple

stem-loop structure of sgRNA. Ex. 4345, ¶114; Ex. 5349, 193:3-194:18; MF238-239. For example, Breaker testified that the catalytic RNA of group II introns is *800-1000 nucleotides*. Ex. 5347, 177:22-178:9. Additionally, the protein component of group II introns is required to help the RNA fold so that it can cut DNA. Ex. 5349, 193:3-194:18; MF238-239. In contrast, *protein* folding occurs much more readily; therefore, systems in which the catalytic component is a protein, e.g., ZFN, TALEN, and Cas9, are more straightforward to use. *Id.* Breaker admitted he failed to consider that group II introns are larger and more complex than sgRNA, or that they use RNA as the catalytic component unlike CRISPR-Cas9, ZFNs, TALENs, and meganucleases. Ex. 5347, 169:12-171:16, 172:19-177:11, 177:22-183:18; Ex. 5318; MF238-239. Moreover, Breaker's testimony is unreliable. He failed to consider relevant art and evidence related to using prokaryotic RNPs in eukaryotic cells and siRNA/shRNA successes that contradict his opinions in his declaration. Ex. 5347, 158:5-165:7, 192:5-198:22, 233:9-238:7, 242:17-245:5; Ex. 5316; Ex. 5317; Exs. 5319-5322; Ex. 4097; Ex. 5328; Ex. 5321; Ex. 5327; MF242-246. Thus, Breaker's views and Broad's arguments lack scientific merit.

On the contrary, the evidence shows that the field at the time considered *protein nucleases* like ZFNs, TALENs, and meganucleases to be the relevant art for CRISPR-Cas9:

Evidence	**Description**
Ex. 3521, 3	Zhang's February 2011 invention disclosure memorandum stating "related art include *anything pertaining to Zinc Finger or TAL Effector*-based genome targeting."
Ex. 3424, ¶50	Zhang's 2020 interference declaration stating "[ZFNs] and TALENs ... were the predominant methods used for eukaryotic genome editing as of 2011"
Ex. 4381, 63	Jinek March 1 lab notebook comparing CRISPR-Cas9 with ZFNs and TALENs
Ex. 4598 and Ex. 4583	Doudna's and Charpentier's May 27 emails to *Science* and *Nature* stating that "RNA-programmed Cas9 offers significant advantages over zinc-finger nucleases or TALEN proteins."
Ex. 5255, 2	Jinek 2012 reviewer comments stating that CRISPR-Cas9 is "desirable for a number of genome editing applications that is impossible to obtain with TALENs or ZFNs."

Evidence	Description
Ex. 5324, 1527	*Science* December 2012 publication stating that CRISPR-Cas9 "may one day challenge zinc finger nucleases and TALENs as the core genome engineering technology."

See also, Ex. 3001, ¶2; Ex. 3201, 819; Ex. 3233, 5-6; Ex. 3832, 1; Ex. 3623, 825; Ex. 4233, 229; Ex. 4085, 1; Ex. 4075, 1179; Ex. 4076, 230; Ex. 3655, 2; Ex. 4257, 838; Ex. 4343, ¶¶12, 85, 93; Ex. 4345, ¶¶64, 149; Ex. 5016. ¶¶17-20; Ex. 5018, ¶21; Ex. 5105, 3, 19; Ex. 5314, 1; Ex. 5347, 223:12-226:17. And ZFNs, TALENs, and meganucleases all had been shown to work in eukaryotic cells, notwithstanding the prokaryotic origin of the FokI nuclease included within ZFNs and TALENs. MF260-262.

Broad cannot rely on unsworn, improperly submitted statements, filed as Exs. 3435-3443, 3446, 3449-3452, from scientists who have not offered testimony in this proceeding. These statements should be afforded no weight if they are admitted into evidence. *First,* the statements are not sworn testimony. *See* Standing Order, ¶157.2. *Second,* Broad refused to make any of the 12 scientists available for cross-examination. Paper 2706, 2:2-6. *Third*, the PTAB refused to order Broad to make the scientists available. *Fourth*, the PTAB denied CVC's request to counter the statements with new declarations. Paper 2708, 2. Broad's 14 statements were submitted in USPTO *ex parte* examination or in EP oppositions of uninvolved applications, where the scientists were *never* cross-examined. Giving any weight to these statements would therefore be highly prejudicial to CVC. *Fifth*, Broad in presenting these statements, failed to inform the PTAB or even its own expert that there were counter-statements from other scientists. *See* Ex. 5347, 140:7-148:9. In other words, Breaker's "selection" of the witness statements was not *objective*; it was one-sided and deliberately spoon-fed to him by Broad's counsel. *Id*. In contrast, CVC provides sworn testimony from numerous experts and fact witnesses explaining the state of the art as of 2012. *See* Exs. 4343, 4345, 4348, 4349, 4350, 4351, 4354, 5013, 5014, 5016, 5018,

and 5265; *see also*, Paper 1579, 36:5-42:4; Paper 2567, 10:1-15:20.

6. CVC proved multiple actual reductions to practice of Count 1.

CVC achieved successful ARTP in zebrafish. By August 9, as expected, Raible detected the specific *chokh* phenotype, which is diagnostic of a fish having a mutation in its *rx3* gene, and Raible conveyed that finding to Chylinski. Paper 1579, 21:1-34:9; MF45-47, 240. CVC showed that this was an ARTP, meeting all the elements of Count 1, and that the inventors appreciated the invention worked for its intended purpose. *See e.g.*, Paper 1579, 23:8-26:24; Ex. 4294, ¶¶55-58; Ex. 4338, 79-80; Exs. 4912-4915; Ex. 4916, 10; Ex. 4343, ¶¶45-58; Ex. 4351, ¶70; Ex. 4348, ¶127; MF45-47, 240. Indeed, Chylinski testified that he "recall[ed] having the conversation with Dr. Raible and him describing [the fish embryo] – him describing the phenotypes he observed" and that he "remember[ed] seeing pictures" of fish embryos. Ex. 6202, 101:14-102:3. And Raible testified that "I also communicated with Dr. Chylinski [about the result]." Ex. 6211, 146:1-4. Under a rule of reason, the CVC inventors' testimony is corroborated by contemporaneous documentary evidence, e.g., Raible's lab notebook and photographs of the mutant fish, and testimony from non-inventor Raible. *See e.g.*, Ex. 4294, ¶¶55-58; Ex. 4338, 79-80; Exs. 4912-4915; Ex. 4916, 10; MF45-46. Raible wanted to publish data comparing efficiencies to ZFNs or TALENs, but "believed that other labs with more resources" were likely to generate those data first. Ex. 4294, ¶74-75. MF276.

Mourrain fails to undermine CVC's successful ARTP. The loss of the pigmented retina and the reduced eye lens Raible observed are sufficient to diagnose an *rx3* mutant fish, because they are the hallmarks of the mutant. Ex. 4294, ¶¶55-56; Ex. 4343, ¶¶3-40, 45-48. Ex. 4343, ¶52; MF46. Indeed, these two unique characteristics were used to identify and propagate the mutant zebrafish line before scientists even determined the gene where the mutation was located, and thus before scientists could even perform any molecular assays. Ex. 4306. In other words,

scientists have long used the same criteria Raible used to identify fish as mutants, without requiring molecular confirmation. Mourrain admitted that the remaining characteristics were all identified after publication of the reference identifying the mutated gene, and thus could not have been *required* for identification of an *rx3* mutant. Ex. 4306; Ex. 4343, ¶¶32-39; MF247-250. And as Mourrain acknowledged, a 2019 article reported CRISPR-Cas9 cleavage of the *rx3* gene based solely on the visual phenotype, *without molecular assays*. Ex. 5348, 179:6-21 (they "did a good job documenting the phenotypes."); Ex 5342. Mourrain admittedly failed to consider prior art publications in which *phenotypes alone* were commonly used to conclude successful mutagenesis in zebrafish, including the *rx3* mutant phenotype. Ex. 5348, 173:3-174:22; 178:6-179:21 (admitting he failed to consider Ex. 5340 or Ex. 5342).

While Mourrain noted that Raible did not obtain molecular *confirmation* of the rx3 mutant, Raible's molecular analysis was simply inconclusive because it was not sensitive enough to detect all possible mutations. As Mourrain admitted, Raible's 2% agarose gel would not have "distinguish[ed] [mutations] of eight base pairs or fewer," even though a single base mutation had been shown to disrupt the *rx3* gene. Ex. 5348, 120:19-123:7. And while Mourrain criticized Raible's fish as showing some signs of developmental delay, he admitted that a mutant could show some toxicity-related developmental delay and successful targeted mutagenesis at the same time. Ex. 5348, 40:2-11, 185:4-186:16; Ex. 5345; MF251. Moreover, Moens, who has personally injected tens of thousands of zebrafish embryos with gene targeting reagents, testified that in over 25 years of zebrafish injections, she has "*never* seen a fish that looked like a *rx3/chokh* mutant result from nonspecific mutation or toxicity." Ex. 4343, ¶54. And Mourrain admitted that he was unaware of any literature disclosing a *chokh* phenotype occurring spontaneously or from nonspecific toxicity or microinjection injury. Ex. 5348, 49:3-52:18, 61:20-64:21.

CVC achieved successful ARTPs in mammalian cells in Fall 2012. CVC also achieved multiple successful ARTPs of Count 1 in mammalian cells in October and November 2012, when non-inventor corroborator East obtained CRISPR-Cas9-mediated DNA cleavage products in human HEK293T cells, and Doudna and Jinek appreciated those results. Paper 1579, 27:17-35:9; Ex. 4345, ¶¶87-101, 201-218; Ex. 4349, ¶¶245-251; Ex. 4353, ¶¶87-142; Ex. 4366, 31-53; 48-52, 241. East's October 31 and November 20 ARTPs are described in Example 2 of CVC's P3 application, which is a constructive reduction to practice of Count 1. Ex. 3004, ¶¶416-423, Fig. 36E; Paper 1579, 32:11-33:6; Paper 877, 106:11-14; MF49, 209-210.

Breaker attempted to cast doubt on CVC's experiments through his conjecture about CVC's cell lysis protocol, but his criticisms lack merit. CVC's same lysis protocol was reviewed and accepted by multiple peer-reviewers of the Jinek 2013 publication, which the reviewers described as an "excellent paper," and has since been cited numerous times by others (including Zhang) as demonstrating CRISPR-Cas9 cleavage in human cells. *See e.g.*, Ex. 5329; Ex. 5335, 348; Ex. 5336, 404; Ex. 5337, 139; Ex. 5347, 251:21-257:19; MF255. And, Breaker's "gentle lysis buffer" theory that CVC's CRISPR-Cas9-mediated DNA cleavage occurred in the cell lysate rather than in the cells is completely debunked by the record evidence clearly showing the lysates were maintained at 4°C or kept frozen. *See e.g.*, Ex. 4382, 26-27 (cells were lysed "at 4°C" and separated using a "refrigerated centrifuge"); Ex. 4366, 26 (cells lysed by "shak[ing] at 4°"); EX. 4366, 66 (lysate "place[d] @ -20 [°C]"). *S. pyogenes* Cas9 is only active between 20 and 44°C and *cannot cleave DNA* at the cold temperatures Jinek and East kept the lysates, meaning that CVC's DNA cleavage occurred *in the cells* before the cells were lysed. *See* Ex. 5334, 4, 6; Ex. 5333, 23103; MF256-257. Breaker admitted that he did not know Cas9's permissible temperature range and did not consider references that explicitly disclose the

temperature range for Cas9 activity (Exs. 5333 and 5334) when forming his opinions Ex. 5347, 264:7-269:4; Ex. 5333; Ex. 5334. Moreover, after DNA cleavage, the NHEJ process (required to detect indels in a Surveyor assay) *requires* the addition of *ATP*. Ex. 6335, 14067; Ex. 6336, 4; Ex. 6343, 850; Ex. 6347, 1; MF258-259. Breaker admitted that neither Jinek nor East added any ATP to the lysates, again proving that NHEJ could not have occurred in the lysates. Ex. 5347, 276:7-16; Ex. 4382, 26-27; Ex. 4366, 26. Breaker also admitted he did not consider the fact that Jinek sonicated the genomic DNA in the lysates, shearing the DNA into countless fragments with non-homologous ends that would "swamp" any NHEJ system theoretically active in the lysate. Ex. 5347, 276:21-279:7; Ex. 5338, Ex. 5339. Breaker's conjecture should not be credited, and his testimony is entitled to little or no weight under 37 C.F.R. § 41.158, because he repeatedly fails to provide *any underlying evidence* to support his speculations. *See e.g.*, Ex. 3448, ¶¶269-271, 278-280, 301-302. In sum, more than a preponderance of the evidence establishes that CVC conceived the invention before Broad and exercised reasonably continuous diligence during the critical period. Broad's priority motion throws out a fallback argument (Paper 2118, 46:18-22), hoping it would stick. But it, too, lacks any credible foundation in law and should be rejected. *See* Paper 2567, 53:21-59:19.

B. Reason 3: CVC prevails under derivation law.

As shown in CVC's Opposition 5, Zhang derived the invention of Count 1 from CVC, compelling judgment in CVC's favor. *See* Paper 2567, 30:19-37:13. CVC's priority motion could not have raised derivation, because Broad had hidden the fact that Marraffini provided Zhang CVC's sgRNA CRISPR-Cas9 system, including instructions to use it in eukaryotic cells. After CVC's cross-examination of Marraffini, CVC promptly notified the PTAB of CVC's intent to raise derivation in its Opposition 5. *See* Paper 2474, 4; Paper 2567. And the PTAB instructed CVC not to file a separate motion. *Id.* Regardless, the facts amply show derivation.

The CVC inventors had a complete, corroborated conception of Count 1 before June 26, 2012. *See* Sections A.1-A.3, *supra* and Paper 1579, 7:17-19:9; *see also,* Paper 2567, 30:17-33:11. And, CVC communicated the entirety of Count 1, including the eukaryotic element, to Zhang via Zhang's collaborator, Marraffini. Paper 2567, 33:12-34:19; MF225-231, 263-270. Marraffini—an independent third party witness whom Broad elected not to question at deposition—testified that he first learned about CVC's sgRNA-Cas9 genome-editing system and its projected use for genome editing in eukaryotic cells by serving as a reviewer of the confidential Jinek 2012 draft manuscript. Ex. 5265, 23:19-24:9, 24:18-25:3, 27:4-16, 29:20-30:3, 64:9-18, 66:7-67:7, 68:13-21; *see also*, Ex. 5013, ¶¶48-55; Ex. 5254, 2, 12; Ex. 4768, 27; Ex. 5016, ¶¶11-15; Ex. 5018, ¶¶10-18; Ex. 5266, 187:21-188:13; Ex. 5255, 2; Ex. 3713, 27-29; Paper 2567, 13:5-18:17, 33:12-37:13; MF270. Promptly following the June 21 CRISPR Research Conference where CVC disclosed its invention, Marraffini relayed CVC's invention to Zhang on June 26. MF225-231, 263-269. Before then, Zhang admittedly had no concept of the Count's sgRNA CRISPR-Cas9 system, which is unsurprising since Zhang did not yet know that tracrRNA was a necessary component of the DNA-cleavage complex—a concept that had been unknown even to the CRISPR community before CVC disclosed it at the June 21 conference. MF263-267. (Zhang's assertions to the contrary are unsupported by the contemporaneous evidence and are scientifically unfounded. Ex. 5014, ¶¶56-76; *see also,* Ex. 5265, 23:6-18, 24:17-25:3, 29:20-30:3; Ex. 5018, ¶16; Ex. 5016, ¶14.) On June 26, Marraffini instructed Zhang to change course from his prior RNA processing experiments and use "pre-processed RNAs" instead as CVC did. Marraffini testified unequivocally that he communicated to Zhang the "single-guide RNA system described in Jinek 2012" and that "it would be an important tool for genome editing in eukaryotes, specifically." Ex. 5265, 68:13-21; MF267. CVC's communication

to Zhang was sufficiently enabling for "one of ordinary skill in the art to construct and successfully operate the invention." *Meade v. McKirnan,* 585 F.2d 504, 507 (C.C.P.A. 1978). As Marraffini testified, the next steps of putting CVC's system into eukaryotic cells would be "pretty straightforward," and Zhang purportedly obtained results with CVC's system in a matter of weeks. Ex. 5265, 31:8-19; Paper 2118, 42:9-10. Ex. 5013, ¶¶56-58; Paper 2567, 18:18-20:19; MF232-233, 267-268. Binding precedent in *Applegate* and *Alexander v. Williams,* 342 F.2d 466 (C.C.P.A. 1965), compels judgment against Broad; *see also, Hedgewick v. Akers,* 497 F.2d 905, n.4 (C.C.P.A. 1974); *Polye v Uhl,* 328 F.2d 893 (C.C.P.A. 1964). Finally, Broad implies, but never expressly argues, that a simultaneous conception and reduction to practice standard applies, but that rare exception cannot apply in cases such as this "where the issue is originality or derivation." *MacMillan v. Moffett,* 432 F.2d 1237, 1240 (C.C.P.A. 1970).

III. CONCLUSION

The law rewards *inventors*, such has CVC, not those who merely derive and then reduce to practice using ordinary skill. The CVC inventors, not Zhang, invented the subject matter of Count 1. Whether applying priority of invention law or derivation law, the PTAB should recognize what the scientific community recognized in awarding the Nobel Prize to Charpentier and Doudna: it was their teamwork that made the tremendous scientific leap forward to allow the world to reap the benefits of a single-guide RNA CRISPR-Cas9 system for genome editing in eukaryotes. The extensive corroborated evidence of record shows this to be the case, and the PTAB should award priority to CVC.

Respectfully submitted,

By /Eldora L. Ellison/
Eldora L. Ellison, Ph.D., Esq.
Lead Attorney for UC and UV

By /Li-Hsien Rin-Laures/
Li-Hsien Rin-Laures, M.D., Esq.
Lead Attorney for EC

Registration No. 39,967
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, NW
Washington, D.C. 20005

Registration No. 33,547
RINLAURES LLC
321 N. Clark Street, 5th floor
Chicago, IL 60654

Date: May 6, 2021

Date: May 6, 2021

1

163. To conduct this in vitro assay experiment, he changed to a gentle lysate method designed to keep proteins active in the lysate. Ex. 3448, Breaker 3rd Dec. ¶¶ 254-261, 380-395.

CVC Response: Denied.

164. Gentle lysing keeps the Cas9 and the DNA repair-mechanism proteins active, meaning that it is possible for cleavage to occur outside the cell during and after the lysing step. Id. at ¶¶ 254-309.

CVC Response: Denied

165. For the gentle lysing, Jinek eliminated any proteinase enzyme from the lysing process, added a Roche protease inhibitor, and used a buffer that approximates the pH and ionic conditions present in cells, suitable for maintaining enzymes and other protein factors intact. Ex. 4382 at 26-27; Ex. 3448, Breaker 3rd Dec. ¶¶ 299-304.

CVC Response: Denied.

166. With a gentle lysing process, one cannot know whether any positive PCR resulted from cleavage and NHEJ that occurred in the cell. Ex. 3448, Breaker 3rd Dec. ¶¶ 254-309.

CVC Response: Denied

167. A typical lysing procedure for genomic DNA testing includes deactivating the Cas9 protein as the cells are lysed, which precludes Cas9-mediated cleavage in the lysate. Id. at 268-286.

CVC Response: Denied

168. DNA released from the cell by lysing can be cleaved in the lysate thereby producing a "positive" in the Surveyor assay that cannot distinguish between cleavage that happened in the cell and cleavage that occurred in the lysate. Id.

CVC Response: Denied

169. On October 19, Dr. Doudna advised Jinek and East that "doing the experiment with cell extracts to test whether the transfected Cas9 is active is a critical control." Ex. 5053.

CVC Response: Denied but acknowledged that the exhibit contains the partially quoted text.

170. Jinek gave a portion of the gentle lysate to East to conduct PCR Surveyor assays to determine if cleavage of the target DNA had occurred in the cell.

CVC Response: Unable to admit or deny due to lack of evidence cited in the statement.

171. Dr. Jinek explained that he gave East a portion of the lysate for her analysis of genomic DNA cleavage in part "because I had more lysate than I needed…." Ex. 4349, Jinek Dec. ¶¶238-39.

CVC Response: Denied but acknowledged that the exhibit contains the partially quoted text.

172. The potential cleavage activity in lysate created confounding problems for determining whether targeted cleavage had occurred in the cell prior to lysing. Ex. 3448, Breaker 3rd Dec. ¶¶ 287-312.

CVC Response: Denied

173. Jinek showed that CRISPR-Cas9 systems expressed in the transfected human cells were able to cleave genomic DNA after the cells were lysed. Ex 4349, Jinek Dec. ¶253.

CVC Response: Denied

174. The gentle lysing protocol also preserves the activity of the enzymes that are responsible for NHEJ and so those repairs/edits can likewise occur in the active lysate. Id. at ¶¶ 267-277.

CVC Response: Denied

175. Examples of these lysis and extract preparation methods are described in numerous papers cited in a 2010 review entitled "Nonhomologous DNA End Joining in Cell-Free Extracts." Ex. 6312.

CVC Response: Denied

176. Dr. Breaker explains that a cell lysis and extract preparation protocol similar to that prepared by Jinek detailed below demonstrated NHEJ activity in cell free systems. Ex. 3448, Breaker 3rd Dec. ¶¶ 299-304.

CVC Response: Denied

177. CVC's first alleged ARTP on October 31 was based on a PCR test of sample where Jinek had used the gentle lysing method. Id. at ¶¶ 313-379.

CVC Response: Admitted that CVC achieved a successful ARTP on October 31, otherwise denied.

178. All of the cell extracts given to East for PCR Surveyor assay analysis for CVC's alleged ARTPs retained active Cas9 protein and sgRNA for some period of time after the cells were gently lysed. Id. ¶¶ 313-379.

CVC Response: Denied

179. On September 14, Cheng used a chimeric RNA with U6 promoter to attempt to cleave the CLTA6 target in U2-OS cells but reported, after following standard lysing protocols, that "there was no evidence of genomic cleavage." Ex. 4352, Cheng Dec. ¶102; Ex. 6201, Cheng Tr. 92:1-10 (confirming his use of a U6 promoter in his U2-OS experiments).

CVC Response: Denied but acknowledged that the exhibit contains the partially quoted text.

180. On November 1, 2012 East used a system with chimeric RNA with U6 promoter to target CLTA6 in U2-OS cell) and reports a success after using the gentle lysing method. Ex, 4353, East Dec. ¶102.

CVC Response: Admitted that East used a system with chimeric RNA with U6 promoter to target CLTA6 in U2-OS cell and achieved a successful ARTP on November 1.

181. CVC's earliest alleged ARTP in human cells is based on an October 29 email from East reporting an "unexpected singleton band," and stating that she needed to "repeat both the PCR and nuclease cleavage steps to confirm this isn't a weird PCR byproduct." Ex. 4488, see also Ex. 5242.

CVC Response: Admitted that CVC's October 31 ARTP is based in part on the October 29 email Ex. 4488.

182. Doudna's recent biography states as follows:

"When [East] showed me the data, it was immediately clear to me that she had beautiful evidence of genome editing by Cas9 in the human cells," Doudna says. "This is a classic difference between a student who is in training and someone like me who's been doing this for a while. I knew what I was looking for, and when I saw the data she had, it just clicked and I thought, 'Yes, she's got it.' Whereas she was unsure and thought she might have to do the experiments again, I was saying, 'Oh my gosh, this is huge! This is so exciting!'"

Ex. 3681 at 189.

CVC Response: Admitted.

183. In an October 29 email, Doudna posed a question to East regarding whether the band was due to Cas9-cleaved DNA:

> Hi Alex - this looks interesting! Is it possible that you would get a singleton band due to the way the Cas9-cleaved DNA is repaired? The +/- nuclease control will be good to see.

Ex. 5072.

CVC Response: Admitted.

184. On Tuesday October, 30, at Dr. Doudna's request, Ms. East performed a further Surveyor assay with a new PCR product that she made using her gDNA from Dr. Jinek's lystates (she also reran the sample from Monday October 29). Ex. 4366 at 34-35.

CVC Response: Admitted.

185. East summarized her results to Doudna and Jinek, the same day:

> Here are the results from this afternoon, which are not particularly conclusive. The singleton band was reproducible from the PCR reaction I performed yesterday, but only a smear appears in the PCR I performed today.

Ex. 5083.

CVC Response: Admitted.

186. Dr. Doudna's response was that the PCR results "might be consistent with the position of the Cas9 cleavage site being shifted relative to the ZFN cleavage site." Id.

CVC Response: Admitted.

187. Ms. East ran a subsequent experiment showing signs of a weak band, which led Dr. Doudna to respond on November 5: "Woo hoo, looks good Alex! Can't wait to see RFP and protein transfection data." Ex. 5171.

CVC Response: Admitted that CVC achieved a successful ARTP on November 5, and that the partially quoted text appears in Ex. 5171; otherwise denied.

188. On November 7, Doudna advised Charpentier:

> We are making some progress here with human cells, and should have more data to discuss soon. I'd like to get a couple more replicate experiments done to confirm what we are seeing, and then will be in touch to set up a phonecall or Skype.

Ex. 5092 at 1; see also Ex. 4351, Charpentier Dec. ¶75.

CVC Response: Admitted.

189. On November 11, 2012 after further tests, Dr. Doudna inquired on the status of further testing. Ex. 5096.

CVC Response: Unable to admit or deny.

190. Dr. Jinek responded to Dr. Doudna as follows:

> I have processed the lysates over the weekend but have not had time to run the final gel. I will do first thing tomorrow morning. Alex has done the Surveyor assay over the weekend and should have sent you her results by now. Basically, it seems that the original U6-promoter constructs give us some hints of activity while the new CMV constructs did not really work.

Id.

CVC Response: Admitted that the partially quoted text appears in Ex. 5096.

191. On November 17, 2012 Dr. Doudna stated in Ex. 5106:

> Hi Martin - thanks for the update, although too bad about the assay. Hopefully the next round and/or next transfections work out better. Seems like the cells are particularly sensitive to these constructs, perhaps? Something to investigate in future experiments…

Ex. 5106.

CVC Response: Admitted that the partially quoted text appears in Ex. 5106.

192. As of mid-November, Doudna, heard from a colleague, that CRISPR-Cas9 could function in eukaryotic cells:

> "I hope you're sitting down," an excited colleague told Doudna in an unexpected phone call. "CRISPR is turning out to be absolutely spectacular in [Harvard geneticist] George Church's hands." He had even gotten it to work in human cells. Thrilled, Doudna immediately contacted Church.

Ex. 3203 at 3.

CVC Response: Denied but acknowledged that the exhibit contains the partially quoted text.

193. On November 14, Dr. Doudna wrote to Dr. Church stating "We will be very interested to hear how your experiments progress. And yes, there is a lot of interest in Cas9 at the moment - we are hopeful that it will turn out to be useful for genome editing and regulation in various cell types." Ex. 5235.

CVC Response: Admitted that the partially quoted text appears in Ex. 5235.

194. When Dr. Doudna learned of Church's progress, "she gave him a call." Ex. 3681 at 197, ¶2 and "He was gracious and explained the experiments he had done and the paper he had submitted" and he also shared that Zhang also had submitted a manuscript. Id.

CVC Response: Denied but acknowledged that the exhibit contains the partially quoted text.

195. On December 8, Dr. Church sent Dr. Doudna a draft of Mali et al., stating "I look forward to exchanging ideas about possible practical applications." Ex. 6060 at 1. When Doudna reviewed the manuscript that Church had sent, Doudna "was deflated." Ex. 3681 at 197, ¶3.

CVC Response: Denied but acknowledged that the exhibit contains the partially quoted text.

196. On December 10, Dr. Doudna wrote to Dr. Jinek about submitting a short paper to eLife "focused on the results in extracts." Ex. 5132.

CVC Response: Denied but acknowledged that the exhibit contains the partially quoted text.

197. Dr. Jinek responded:

> We could show all this and perhaps say that this prompted us to redesign the RNAs. As a result, we see now detectable DSB formation in vivo using 2nd generation RNAs with the Surveyor assay as our final figure/panel.

Ex. 5132.

CVC Response: Admitted that the partially quoted text appears in Ex. 5132.

198. Doudna states her biography regarding the eLife experiments that Jinek "felt what we had wasn't worth publishing." Ex. 3681 at 198, ¶4.

CVC Response: Admitted that the partially quoted text appears in Ex. 3681.

199. Dr. Jinek stated "If we publish this work, we're going to look like amateurs in the genome editing field." Id. ¶6.

CVC Response: Admitted that the partially quoted text appears in Ex. 3681.

200. Dr. Doudna, however, "put her foot down," rushing experiments with 2nd generation RNA—not relied on here—to eLife, and daily hounding the main scholar who was doing the peer review for her paper, Detlef Weigel at the Max Planck Institute, to prompt acceptance on January 3, 2013, coinciding with online publication in Science of the Cong and Mali articles. Id. at 198-202.

CVC Response: Denied.

CVC Additional Facts 201 - 276

201. The earliest date Broad alleges for Zhang's conception of Count 1 is June 26, 2012. Paper 2118, 42:9-10; Ex. 3424, ¶¶126, 168.

202. Before June 26, 2012, Jinek had already constructed the pMJ874 vector. *See e.g.*, Ex. 4382, 4-7; Ex. 4349, ¶¶124-128; Ex. 4603; Ex. 4345, ¶¶167-172; Ex. 3004, ¶¶408-450.

203. The pMJ874 vector uses the U6 promoter to drive expression of the CLTA6 sgRNA targeting a specific CLTA target gene sequence next to a PAM. *See e.g.*, Ex. 4382, 4-7; Ex. 4349, ¶¶124-128; Ex. 4603; Ex. 4345, ¶¶167-172; Ex. 3004, ¶¶408-450.

204. Before June 26, 2012, Jinek had constructed a *S. pyogenes* Cas9 expression vector with a strong CMV promoter, NLSs, and a GFP tag, and had successfully expressed the Cas9 protein in human HEK293T cells and visualized nuclear localization of Cas9. *See e.g.*, Ex. 4349, ¶¶109, 123-127, 155; Ex. 4444; Ex. 4484; Ex. 4536; Ex. 4345, ¶173.

205. Before June 26, 2012, the CVC inventors were codon optimizing the Cas9 gene. Paper 1579, 18:17-19:4; Ex. 4349, ¶¶109, 123-127, 155; Ex. 4444; Ex. 4484; Ex. 4536; Ex. 4345, ¶173.

206. The pMJ874 vector that CVC constructed by May 28 is the same vector Jinek and East used in their October and November 2012 experiments in HEK293T cells. *See e.g.*, Paper 1579, 17:11-19:9; Ex. 4382, 4-7; Ex. 4349, ¶¶124-128; Ex. 4603; Ex. 4345, ¶¶167-172; Ex. 3004, ¶¶408-450.

207. CVC's *S. pyogenes* Cas9 vector constructed by May 28 uses the same CMV promoter, encodes the same Cas9 amino acid sequence, and the same NLS that Jinek and East used when reducing the invention to practice in October 2012. Ex. 4349, ¶¶109, 123-127, 155; Ex. 4444; Ex. 4484; Ex. 4536; Ex. 4345, ¶173.

208. CVC's vector transfection embodiment comprises a eukaryotic cell (HEK293T cells) and a Type II CRISPR-Cas system comprising a Cas9 protein (*S. pyogenes* Cas9) and sgRNA (CLTA6 sgRNA). *See* Paper 1579, 7:17-19:9; Ex. 4345, ¶¶167-174.

209. The experiments East and Jinek performed using CLTA sgRNA CRISPR-Cas9 in HEK293T cells in October and November 2012 are the same experiments described in Example 2 of CVC's P3 application. Ex. 3004, ¶¶408-450; Ex. 4382, 4-7; Ex. 4349, ¶¶124-128; Ex. 4603; Ex. 4345, ¶¶167-172.

210. The PTAB stated in its Decision on Motions that CVC's P3 "is a constructive reduction to practice of Count 1," "[b]ecause Example 2 provides the protocols necessary and results of a CRISPR-Cas9 system in eukaryotic, human cells." Paper 877, 106:1-14.

211. Before June 26, 2012, CVC contemplated delivering a pre-formed sgRNA-Cas9 ribonucleoprotein (RNP) complex to zebrafish embryos using nuclear microinjection. Paper 1579, 14:2-18:4; Ex. 4382, 7; Ex. 4349, ¶¶124-128; Ex. 4603; Ex. 5105, 16-29.

212. Before June 26, 2012. CVC constructed a working sgRNA and Cas9 system as a pre-formed RNP complex and confirmed that it was capable of targeting and cleaving at least five different eukaryotic target DNA sequences (within the jellyfish GFP gene) in vitro. Paper 1579, 17:11-19:9; Ex. 4382, 2, 7; Ex. 4349, ¶¶124-128; Ex. 4345, ¶¶167-172.

213. Before June 26, 2012, CVC described using microinjection for introducing their sgRNA CRISPR-Cas9 system into eukaryotic cells, citing multiple articles published for ZFN and TALENs that describe nuclear microinjection into zebrafish embryos. Ex. 5105, 2-3, 8; Ex. 4349, ¶¶72-74; Ex. 4350, ¶¶57-61; Ex. 4345, ¶¶168-164.

214. The pre-formed RNP complex CVC demonstrated to target and cleave eukaryotic gene sequences (GFP) in vitro before June 26, 2012, comprised a chimera A sgRNA and a *S.*

pyogenes Cas9 protein. Paper 1579, 17:11-19:9; Ex. 4382, 2, 7; Ex. 4349, ¶¶124-128; Ex. 4345, ¶¶167-172.

215. Raible's first impression upon discussing the project with Chylinski was that the reduction to practice "should be a quite rapid procedure, to be done within the next 1-2 months" based on the "stunning efficiency of the CRISPR/CAS system … in vitro." Ex. 4799; Ex. 4294, ¶¶14-16.

216. Chylinski, Charpentier and Raible all "expected" that injecting CVC's sgRNA CRISPR-Cas9 system as a pre-formed RNP complex into zebrafish embryos would result in successful target DNA cleavage. Ex. 4348, ¶¶11, 25, 115; Ex. 4351, ¶¶23, 26, 56, 60; Ex. 4294, ¶¶10, 13, 14, 74.

217. Raible prepared the sgRNA and Cas9 components for his August 9, 2012 experiment in zebrafish as a pre-formed RNP complex comprising a chimera A sgRNA and *S. pyogenes* Cas9 protein. Ex. 4294, ¶¶8, 54; Ex. 4382, 1-7; Ex. 4349, ¶¶124-128; Ex. 4603; Paper 1579, 14:2-17:10.

218. Microinjecting a pre-formed sgRNA-Cas9 RNP complex directly to the nucleus of a eukaryotic cell avoids any issues with RNA degradation, complex localization, complex formation, and chromatin. Ex. 4036, ¶¶136-137; Ex. 4343, ¶¶93-106; Ex. 4345, ¶87.

219. Microinjection of zebrafish embryos with a pre-formed RNP complex negates any potential impact of chromatin because the zebrafish cells are rapidly dividing. Ex. 4345, ¶113; Ex. 4343, ¶103.

220. Breaker testified that, if making recombinant protein outside of a eukaryotic cell ("premade" protein), "then codon optimization would have no effect." Ex. 5264, 88:15-89:1.

CERTIFICATE OF SERVICE

I hereby certify that the foregoing **CVC'S REPLY 2** is being filed via the Interference Web Portal by 8:00 PM Eastern Time on May 6, 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 ET on counsel for the Senior Party at:

Raymond N. Nimrod
Quinn Emanuel Urquhart & Sullivan, LLP
51 Madison Avenue, 22nd Floor
New York, NY 10010
(212) 849-7000
raynimrod@quinnemanuel.com
matthewrobson@quinnemanuel.com
michaelmitrayon@quinnemanuel.com

Steven R. Trybus
Locke Lord LLP
353 North Clark Street
Chicago, IL 60606
(312) 433-0699
Steven.Trybus@lockelord.com
patent@lockelord.com
Interference106115@lockelord.com

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C

/Eldora L. Ellison/
Eldora L. Ellison, Ph.D., Esq.
Lead Attorney for UC and UV
Registration No. 39,967

Date: May 6, 2021

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, NW
Washington, DC 20005-3934