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

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

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

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,
Junior Party,

v.

SIGMA-ALDRICH, CO., LLC
Application 15/456,204

Senior Party.

Patent Interference No. 106,132 (DK)
(Technology Center 1600)

CVC SUBSTANTIVE MOTION 3
(to change the Count)

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1 ***I. Statement of Precise Relief Requested***

2 The PTAB should substitute Count 1 with Proposed Count 2:

Count 1	Proposed Count 2
CVC Application 15/981,807, claim 156 or Sigma Application 15/456,205, claim 31.	CVC Application 15/981,807, claim 156 or Sigma Application 15/456,205, claim 33.

3 This substitution changes Sigma's half of the count to its involved dependent claim 33,
4 which recites "wherein the guide RNA is a single molecule," but is otherwise identical to the
5 current Count. Substituting Sigma's claim 33 for claim 31 makes Sigma's half of the count more
6 symmetrical with CVC's half and avoids inconsistency with the counts in multiple other pending
7 interferences involving overlapping subject matter.

8 ***II. Statement of Material Facts and Evidence***

9 Appendix 1 is a list of Exhibits cited. Appendix 2 is a Statement of Material Facts.

10 ***III. Argument***

11 The PTAB should change the Count to better—and more consistently—reflect the
12 common invention being adjudicated in multiple pending interferences directed to CRISPR-Cas9
13 systems useful in eukaryotic cells. *See* SO ¶ 208.2. CVC's half of Count 1 aligns with the scope
14 of the counts in multiple ongoing interferences, including interferences between: CVC and Broad
15 (Interference No. 106,115), Ex. 2560, 12-13; CVC and ToolGen (Interference No. 106,127), Ex.
16 2561 at 5-6; and Broad and ToolGen (Interference No. 106,126), Ex. 2562, 12-13; MF 1. CVC's
17 half of the count and CVC's involved claims in these interferences, as well as this one, are the
18 same.

19 In each of these pending interferences, both halves of the count recite single-guide
20 ("sgRNA") CRISPR-Cas9 systems, whether or not the parties have claims that are generic as to

the format of the guide RNA (i.e., "generic-guide" claims). MF 2; MF 3. Sigma's half of the count is an outlier in this respect, with no apparent reason to treat Sigma differently than CVC, Broad, or ToolGen. Substituting the count to clearly recite single-guide RNA will coordinate and simplify the issues of priority of invention to be resolved among these multiple parties.

The PTAB has discretion over whether to substitute the count in an interference. *See Hitzman v. Rutter*, 243 F.3d 1345, 1359 (Fed. Cir. 2001) (finding no abuse of discretion in narrowing the scope of a count). Whatever action the PTAB takes across similar interferences must not be arbitrary or capricious. *In re Vivint, Inc.*, 14 F.4th 1342, 1351 (Fed. Cir. 2021) (noting that "[a]gency action is an abuse of discretion when it '(1) is clearly unreasonable, arbitrary, or fanciful'") (quoting *Motor Vehicle Mfrs. Ass'n of U.S., Inc. v. State Farm Mut. Auto. Ins. Co.*, 463 U.S. 29, 43 (1983)). Efficiency, uniformity, and clarity therefore compel changing Sigma's half of the Count as proposed herein. *See* 37 C.F.R. § 41.1(b) (providing the Board's responsibility "to secure the just, speedy, and inexpensive resolution of every proceeding"). There is no countervailing reason to administer this Interference with Count 1 instead of proposed Count 2.

Likewise, there are significant negative consequences of proceeding with this Interference if the Sigma side of the count is not conformed to the other pending interferences and continues to recite something other than a single guide system. *First*, proceeding with the existing count in this interference invites the parties to submit proofs that are different than those in the co-pending interferences. The inconsistency between the count in this interference and the co-pending interferences creates the possibility of irreconcilable decisions among interferences that involve the same claims for each party. Such inconsistency would be prejudicial to any party in CVC's position, and the public. The Board owes the parties and the public finality, not uncertainty, over who owns this ground-breaking invention.

1 *Second*, this uncertainty is magnified where the Sigma side of the count is subject to a
2 disputed construction and is, as discussed in detail below, ambiguous as to whether it covers
3 RNAs other than single-guide RNA. To avoid this ambiguity, the Board should substitute
4 dependent claim 33 because it clearly recites a single-guide RNA molecule. Alternatively, if the
5 Board declines to substitute claim 33 because claim 31 is properly interpreted as a single-guide
6 RNA, the Board should make this construction clear for the parties before the priority phase.

7 ***A. Substituting the count will better harmonize this interference with co-pending***
8 ***interferences involving the same invention***

9 The PTAB should substitute Count 1 of the present Interference to more consistently
10 align it with the counts of co-pending interferences involving the same invention. The PTAB
11 chose to declare multiple, co-pending two-party interferences as an alternative to managing a
12 single, multi-party interference. Ex. 2560; Ex. 2561; Ex. 2562. Unnecessary differences in scope
13 across the various counts could lead to inconsistent results, and will create uncertainty among the
14 parties and the public as to the significance and consequences of such differences. Both of these
15 negative consequences are avoided by ensuring that the counts are as consistent as practicable
16 across proceedings.

17 Count 1 is the alternative statement of CVC Application 15/981,807, claim 156 or Sigma
18 Application 15/456,204, claim 31. *See* Paper 1, Declaration of Interference, 5; MF 4. CVC claim
19 156 recites “a single molecule DNA-targeting RNA, or a nucleic acid comprising a nucleotide
20 sequence encoding said single molecule DNA-targeting RNA” and then goes on to define three
21 components of a CRISPR-Cas9 system. MF 5. Sigma’s claim 31 recites “a guide RNA or DNA
22 encoding the guide RNA, wherein the guide RNA comprises a first region that is complementary
23 to a target site in the chromosomal sequence . . . and a second region that interacts with the

1 CRISPR-Cas type-II protein, and wherein the guide RNA comprises a crRNA and a tracrRNA."
2 Paper 1, Declaration of Interference, 5-6; MF 6.
3 CVC's half of this count aligns with the scope of the counts in multiple pending
4 interferences (Interference Nos. 106,115, 106,127, and 106,126). MF 1. In each of these pending
5 interferences, both halves of the count are directed to single-guide RNA CRISPR-Cas9 systems,
6 thus coordinating and simplifying the issues of priority of invention to be resolved among these
7 multiple parties. CVC's half of the count (reciting "wherein the activator-RNA and the targeter-
8 RNA are covalently linked to one another with intervening nucleotides") is identical in the
9 present interference, the '115 Interference, and the '127 interference. Ex. 2560, 12-13; Ex. 2561,
10 5-6. Broad's half of the count (reciting "guide RNAs comprise a guide sequence fused to a tracr
11 sequence") is identical in the '115 Interference, the '126 Interference. Ex. 2560, 12-13; Ex. 2562,
12 12-13. ToolGen's half of the count (reciting "chimeric guide RNA comprising a CRISPR RNA
13 (crRNA) portion and a transactivating crRNA (tracrRNA) portion") is identical in the '126
14 Interference and the '127 Interference. Ex. 2561, 5-6; Ex. 2562, 12-13.
15 The same CVC claims are involved in the '115, '127 and present interferences. Ex. 2560,
16 14-16; Ex. 2561, 7-10. Yet in the '115 and '127 Interferences, the PTAB chose to define the
17 Count as single-guide subject matter.[1] Ex. 2560, 12-13; Ex. 2561, 5-6. Sigma's half of the Count
18 in this proceeding, if construed to be generic (not limited to a single-molecule) as to the structure
19 of the guide RNA, appears to be an outlier. There is no apparent reason that the Count in this

[1] Broad argued in the '115 Interference that it had generic-guide claims involved. CVC does not agree that Broad has any generic-guide claims. The PTAB did not find Broad's argument a compelling reason to adopt a generic-guide count in the '115 interference. *See* Ex. 2400, 31-33.

1 Interference should have a different scope than the multiple other co-pending interferences
2 involving the same invention and the same CVC claims. There is also no reason that Sigma
3 should be treated differently than CVC, Broad, or ToolGen with respect to the structure of the
4 guide RNA recited in the count.

5 If there are multiple, related interferences where the parties and involved claims overlap,
6 and where each count is directed to the same patentable invention, it is reasonable to expect the
7 Board will ensure that the scope of all the interference counts is the same. *See Vivint,* 14 F.4th at
8 1351. Under these facts, divergent count scopes will likely lead to an inability to harmonize the
9 priority decisions from the different interferences and determine the first inventor of the
10 commonly claimed subject matter. For example, divergent count scopes will necessarily allow
11 consideration of relevant priority evidence in one interference that cannot be considered in
12 another interference. The more divergent the counts, the greater the risk of irreconcilable priority
13 judgments. Thus, interference policy should strive for harmonization in count scope among
14 related interferences where possible. More importantly, this motion offers the Board an
15 opportunity to avoid imposing this risk on the parties and the public.

16 Efficiency, uniformity, and clarity thus support amending Sigma's half of the count to
17 more clearly align its limitations with CVC's, Broad's, and ToolGen's halves of the counts of
18 other co-pending interferences. 37 C.F.R. § 41.1(b). Doing so will also provide the public a
19 clearer record as to the issues being resolved and the respective rights of the multiple parties
20 involved. The PTAB should therefore substitute Count 1 with Proposed Count 2 to recite the
21 following alternatives: claim 156 of CVC's '807 Application or claim 33 of Sigma's '204
22 Application.

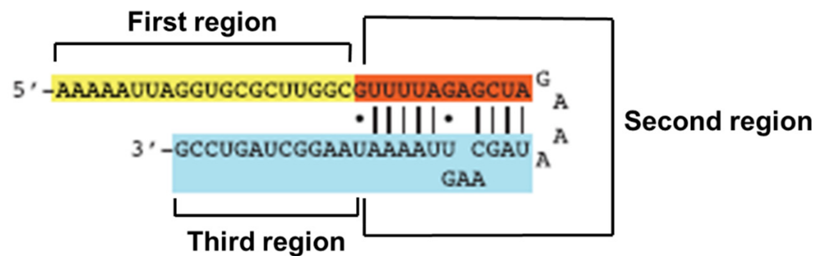
B. Substituting Count 2 for Count 1 as requested will simplify the issues in this Interference

Where a McKelvey count uses two claims in the alternative to define the subject matter for the determination of priority of invention, that purpose is best served by choosing claims that are as balanced and similar in scope as possible. MF 4. The PTAB has repeatedly determined that the Count in other interferences involving the same CVC claims is best represented by claims clearly reciting single-guide RNA. Ex. 2560, 12-13; Ex. 2561, 5-6. As one example, in the ’115 Interference, the PTAB selected Broad’s dependent claim 18 (“fused”) to represent Broad’s half of the count, thus avoiding any ambiguity or dispute as to whether Broad’s claim 15 encompassed generic-guide or single-guide subject matter. Ex. 2560, 12-13. At the very least, the PTAB should do the same here, selecting Sigma’s dependent claim 33 to represent Sigma’s half of the count and thus avoid any ambiguity.

There is “no per se rule that a count must be as broad as the broadest patentable claims designated as corresponding to the count.” *Lee v. McIntyre*, 55 U.S.P.Q.2d 1406 (B.P.A.I. 2000) (precedential). Indeed, at least some of CVC’s involved claims in the ’115 and ’127 Interferences and, according to the PTAB, Broad’s involved claims 15 and 26 of U.S. Patent No. 9,840,713 in the ‘115 and ‘126 Interferences are not limited to sgRNA, and yet the counts in those interferences recited claims that included sgRNA limitations. Ex. 2400, 47:12-13; MF 3. Defining the scope of Sigma’s half of the Count with claim 33 is thus a proper way to capture the duplicative subject matter claimed by the parties.

Here, substituting proposed Count 2 for Count 1 will clarify the scope of this proceeding and avoid potential disputes. 37 C.F.R. § 41.1(b). For example, substituting Count 1 with Count 2 will obviate any ambiguity regarding whether Sigma’s half of Count 1 already recites a single-guide format for the “guide RNA,” thus clarifying the scope of admissible proofs during the

1 priority phase. 37 C.F.R. § 41.1(b). Sigma's claim 31 recites "a guide RNA or DNA encoding
2 the guide RNA, wherein the guide RNA comprises a first region that is complementary to a
3 target site in the chromosomal sequence . . . and a second region that interacts with the CRISPR-
4 Cas type-II protein, and wherein the guide RNA comprises a crRNA and a tracrRNA." Paper 1,
5 5-6. Sigma's P1 application defines "guide RNA" as comprising a first region, "a second internal
6 region that forms a stem loop structure," and a third region. Ex. 2026, ¶¶ [0021], [0019]; MF 9.
7 These regions are illustrated below.



8

9 *See* Ex. 2031, Fig. 5A (annotated) (cited in Sigma's '204 application); Ex. 2549 ¶99; MF 9.

10 The "loop" of claim 31 refers to the linker loop in the stem-loop region shown in purple
11 in the diagram above, which joins the crRNA and the tracrRNA. Ex. 2549, ¶¶98-99, 101; MF 9.
12 The presence of the stem-loop in the second internal region necessarily denotes a single-guide
13 RNA in which the loop joins the crRNA and the tracrRNA. Ex. 2549, ¶¶98-99, 101; MF 9.
14 Additionally, claim 11 originally recited a "vector [that] further comprises a sequence encoding
15 the guide RNA that is operably linked to a promoter sequence." Ex. 2585, 77. This
16 grammatically implies a single-guide RNA because a dual molecule structure would require
17 multiple promoters. Ex. 2549, ¶100; MF 49. Accordingly, despite the language of dependent
18 claim 33 (reciting "wherein the guide RNA is a single molecule") and claim 34 (reciting
19 "wherein the guide RNA is two molecules"), the PTAB may conclude that "the guide RNA" of
20 claim 31 is a single structure that includes all the recited parts. Ex. 2459 ¶102.

1 As the PTAB has noted, the doctrine of claim differentiation is only "a guide, not a rigid
2 rule" for claim interpretation. *See* Int. No. 106,115, Paper 877, 31-33; *Marine Polymer Techs.,*
3 *Inc. v. HemCon, Inc.*, 672 F.3d 1350, 1359 (Fed. Cir. 2012) (en banc); *Nomos Corp. v. BrainLAB*
4 *USA, Inc.*, 357 F.3d 1364, 1368 (Fed. Cir. 2004); *Fantasy Sports Props. v. Sportsline.com*, 287
5 F.3d 1108, 1115-16 (Fed. Cir. 2002) (finding that claim differentiation "cannot broaden claims
6 beyond their correct scope"). The PTAB thus construed the term "guide RNA" in the '115
7 Interference to refer to sgRNA, notwithstanding dependent claims more specifically reciting the
8 guide RNA structure. *See* Ex. 2400, 31-33 (noting, e.g., dependent claims reciting that the guide
9 RNA components are "fused"); MF 7.

10 Substituting Proposed Count 2, as requested herein, will simplify the issues in this
11 interference by, for example, obviating any dispute over the scope of "guide RNA," and thus
12 clarifying the scope of proofs on priority that will be admissible in the priority phase of the
13 interference. 37 C.F.R. §41.201; *see also Papayannopoulou* (indicating that simplifying the
14 issues and clarifying the scope of the interference and applicable proofs is appropriate for the
15 motions phase). Further, substituting the Count as requested herein will better balance the two
16 halves of the Count.[2]

[2] The parties and public are entitled to clarity in the scope of the count, which at best is ambiguous. Eliminating that uncertainty necessarily reduces the burdens on the parties and the Board in assessing priority during the next phase of the interference. As an alternative relief, if Count 2 is not adopted, the Board should construe the Count's claim 31 as directed to a single chimeric RNA.

1 ***C. Sigma will suffer no prejudice and the public will benefit from a Count based***
2 ***on Sigma's '204 Application claim 33***

3 Substituting the Count as requested will provide greater clarity to the public regarding the
4 scope of this interference relative to the co-pending interferences without any prejudice to
5 Sigma. Sigma's first provisional specification discloses data from a single experiment: a gel that
6 has lanes for both single-guide RNA and dual-guide RNA molecules. Ex. 2549, ¶103; MF 10.
7 Sigma also cannot claim any prejudice due to its earliest proofs falling outside the scope of a
8 sgRNA Count, as Sigma's proofs appear to be identical either way. *Id.* Prejudice to Sigma or loss
9 of priority benefit therefore do not provide reasons to deny this motion. Additionally, changing
10 the Count as requested herein does not change the designations, patentability, or CVC's
11 entitlement to priority benefit as provided further below.

12 ***1. Designations of claims corresponding to proposed Count 2***

13 The substitution to the Count proposed in this Motion does not de-designate any of the
14 involved claims identified in the Declaration of Interference. As the PTAB has previously found,
15 all claims regarding the structure of the guide RNA are rendered obvious in view of sgRNA, and
16 therefore correspond to the count. *See* Ex. 2400, 31-33.

17 Additionally, the analysis and relief requested in CVC Substantive Motion 4 to Add the
18 Claims of Sigma Patent No. 10,731,181 ("the '181 patent) and No. 10,745,716 ("the '716
19 patent") this Interference are equally applicable if Count 1 were substituted with Count 2.
20 Sigma's '181 and '716 patents (collectively, "Sigma's Patents") both use the same ambiguous
21 claim term "guide RNA" that is addressed above. Count 1 and Count 2 differ only in clarifying
22 the scope of this guide RNA as a single RNA molecule, and thus all of the analysis in Motion 4
23 applies equally to Count 2.

1 Sigma's Patents and Sigma's half of proposed Count 2 all encompass (1) methods for
2 modifying a chromosomal sequence in a eukaryotic cell by introducing and integrating a donor
3 sequence via a CRISPR/Cas9 system (2) using a codon-optimized nucleic acid encoding Cas9
4 tagged with a nuclear localization signal ("NLS"), and (3) a guide RNA with a DNA-targeting
5 region and a region for interacting with Cas9. The nominal differences between the Sigma
6 Patents' claims and Count 2 are the recitation of the well-established Cas9 protein (from *S.*
7 *pyogenes*), a well-known nuclear localization signal (the SV40 NLS) attached to the C-terminus,
8 and the natural and previously disclosed location for the DNA-targeting region (at the 5' end of
9 the guide RNA). MF 39; Ex. 2549 ¶¶ 9, 29-32. These are not patentable differences. These
10 would have been obvious to a skilled artisan, as Dr. Bailey's declaration explains in detail. *Id.*
11 ¶¶ 29-96. These claims should correspond to Count 2 just as Sigma's involved claims reciting
12 the same *S. pyogenes* Cas9 and the same NLS sequences already correspond to Count 1—and
13 Sigma has not requested de-designating such claims. MF 39, 50.

14 Sigma's claims should be added to this proceeding and designating as corresponding to
15 the count because "the subject matter of the count, if treated as prior art, would have anticipated
16 or rendered obvious the subject matter of [each] claim." Standing Order, ¶ 208.3.1; 37 C.F.R. §
17 41.207(b)(2). As part of this analysis, "additional references…may be relied upon to establish the
18 obviousness of the differences between the count and the claims." *Desjardins v Wax*,
19 Interference No. 105,915, Paper 127, 17-20 (P.T.A.B. Jan. 21, 2014) (granting motion to add
20 claims to the count where the prior art disclosed the only limitation that was "the difference
21 between" the count and claims).

22 All of the nominal differences between the claims of Sigma's Patents and Count 2 either
23 had been disclosed in Jinek 2012 (Ex. 2031) or had been well known for years. Ex. 2549 ¶¶ 33-
24 45; Ex. 2031, Figs. 1-5; Ex. 2023, 285, 289-290; MF 40-45. Jinek 2012 discloses *S. pyogenes*

1 Cas9 and mimicking the natural arrangement of the CRISPR system by placing the DNA-
2 targeting region at the 5' end of the guide RNA. Ex. 2549 ¶¶ 33-37; Ex. 2031, 818, Figs. 1E, 3C,
3 5B; MF 41-42. In view of Count 2, which describes using CRISPR-Cas9 systems in eukaryotic
4 cells for chromosomal modification, a person of ordinary skill in the art would have been
5 motivated to combine the gene-editing system of Count 2 with Jinek 2012 because Jinek 2012
6 recommends using its CRISPR-Cas9 systems for gene editing applications. Ex. 2549 ¶¶ 46-50;
7 Ex. 2031, 820. As to the selection of an NLS, a skilled artisan would have had reason to tag Cas9
8 on its C-terminus with an NLS of SEQ ID NO: 1 or SEQ ID NO: 2, as disclosed in Krebber 2000
9 (Ex. 2023) and Lange 2007 (Ex. 2221), because these NLSs were the most widely used tags for
10 targeting proteins to the nucleus and were even part of commercially-available tools. Ex. 2549
11 ¶¶ 51-55; Ex. 2550, 451-452; Ex. 2023, 285; Ex. 2348, 478; MF 43-47. A skilled artisan would
12 have had a reasonable expectation of success in modifying Count 2 to apply the known NLS
13 sequences to the C-terminus of *S pyogenes* Cas9 according to Jinek 2012, Krebber 2000, and
14 Lange 2007 because Count 2 is presumed to be operable as prior art as to the claims, and because
15 the modifications involved no more than applying standard and proven details to the general
16 components listed in Count 2. 37 C.F.R. § 41.207(b)(2); Ex. 2549 ¶¶ 56-62; Ex. 2550, 451-452;
17 Ex. 2023, 285; Ex. 2348, 478; MF 44, 46-47. Moreover, there are no objective indicia of non-
18 obviousness that would distinguish the additional limitations of Sigma's claims from Count 2.
19 Ex. 2549 ¶ 97.

20 Jinek 2012 discloses using both sgRNA and dual-molecule guide RNA ("dgRNA")
21 structures that have a 5' DNA-targeting region as recited in the claims of Sigma's patents. Ex.
22 2031, Figs. 1E, 3C, 5B; MF 41. A POSA would have had reason to use a 5' DNA-targeting
23 region in a system as described in proposed Count 2 because Jinek 2012 demonstrated success
24 with both the sgRNA and dgRNA, and because the natural CRISPR system uses a 5' DNA-

1 targeting region. Ex. 2549 ¶¶ 34-37, 46-50; Ex. 2031, Figs. 1-5; MF 41-42. Substituting a
2 sgRNA count would not diminish the expectation of success because Jinek 2012 demonstrated
3 that both sgRNA and dgRNA were effective in targeting Cas9-mediated DNA cleavage *in vitro*.
4 Ex. 2031, Figs. 1-5; MF 41.

5 Accordingly, the claims in Sigma's patents correspond to Count 2, and the full list of
6 claims that correspond to Count 2 as proposed herein are:

CVC

App. 15/947,680 – Cl. 156-185	App. 16/136,165 – Cl. 156-184
App. 15/947,700- Cl. 156-185	App. 16/136,168 – Cl. 156-184
App. 15/947,718 – Cl. 156-185	App. 16/136,175 – Cl. 156-184
App. 15/981,807 – Cl. 156-185	App. 16/276,361 – Cl. 3-31
App. 15/981,808 – Cl. 156-170, 172-185	App. 16/276,365 – Cl. 3-32
App. 15/981,809 – Cl. 156-170, 172-185	App. 16/276,368 – Cl. 3-31
App. 16/136,159 – Cl. 156-184	App. 16/276,374 – Cl. 3-32

Sigma

App. 15/456,204 – Cl. 31-63	Pat. 10,745,716 – Cl. 2-4, 11, 14, 21-22
Pat. 10,731,181 – Cl. 1-17	

7 The PTAB should grant this motion, substitute the Count as proposed herein, and add the Sigma
8 patents as reflected above.

9 ***2. CVC is entitled to priority benefit under proposed Count 2***

10 CVC's half of proposed Count 2 is the same as CVC's half of Count 1. It recites a
11 eukaryotic cell comprising a target DNA and a Type II CRISPR-Cas9 system, wherein the guide
12 RNA is a single RNA molecule ("sgRNA"). CVC's U.S. Provisional Application 61/652,086
13 ("P1") filed May 25, 2012 and, in the alternative, U.S. Provisional Application 61/716,256
14 ("P2") filed October 19, 2012, describe and enable this invention. P1 and P2 describe eukaryotic
15 cell types [1] and a sgRNA CRISPR-Cas9 system capable of modifying target DNA [2]-[8].

Element of Count 1	Embodiment 1 (E1)	Embodiment 2 (E2)	Embodiment 3 (E3)
[1]	Fish cell	Human cell	Fruit fly cell
[2] – [8]	*S. pyogenes* Cas9 and chimera A of Example 1	*S. pyogenes* Cas9 and a 3' extended chimera A comprising GUUUUAGAGCUAG AAA and tracrRNA (23-89) of Example 1 (see Fig. 3B) joined by a GAAA tetraloop.	*S. pyogenes* Cas9 and a 3' extended chimera A comprising GUUUUAGAGCUAG AAA and tracrRNA (23-89) of Example 1 (see Fig. 3B) joined by a GAAA tetraloop.

1 Taking into account general knowledge in the art and the POSA's high level of skill, P1
2 conveys possession of at least three embodiments within the scope of Count 2, including all the
3 detail needed for a POSA to practice each: (1) a fish cell comprising a sgRNA CRISPR-Cas9
4 system made by microinjecting a pre-assembled ribonucleoprotein ("RNP") complex into a fish
5 embryo ("**E1**"), Ex. 2543, ¶¶ 96-168, 303; (2) a human cell comprising a sgRNA CRISPR-Cas9
6 system made by transfecting human cells with expression vectors encoding the system ("**E2**"),
7 Ex. 2543, ¶¶ 169-221, 303; and (3) a fruit fly cell comprising sgRNA CRISPR-Cas9 made by
8 microinjecting Cas9 mRNA and sgRNA into a fruit fly embryo ("**E3**"), Ex. 2543, ¶¶ 222-239,
9 303. These embodiments are summarized below, in relation to the elements of Count 2.

10 P1's Figure 1A illustrates the components of the Type II CRISPR-Cas9 system—Cas9,
11 "targeter-RNA" (crRNA), and "activator-RNA" (tracrRNA). Ex. 2009, Fig. 1A. Figure 1B
12 illustrates how the targeter-RNA and the activator-RNA can be linked together by intervening
13 nucleotides to form the sgRNA. *Id.* at Fig. 1B. P1 contemplates the broad utility of the system, as
14 illustrated by the exemplified "target cells," including fish, human, and fruit fly cells, and cell
15 types, including "embryonic" cells, that can be derived from various organisms. *See, e.g.*, *id.*
16 ¶¶ [00165], [00216], [00050], [00051], [00052], [00174]. P1's working example discloses
17 making a pre-assembled RNP complex of sgRNA and recombinant Cas9 protein that is shown to

1 cleave target DNA cell-free *in vitro*. *Id.* ¶¶ [00248]-[00251], Fig. 3. P1 discloses established
2 techniques for delivering sgRNA CRISPR-Cas9 into a target cell, including microinjection and
3 transfection. *Id.* ¶¶ [0039], [00154], [00165], [00173]-[00175], [00177]. P1 contemplates that
4 features and elements of the disclosed embodiments and identified methods can be performed
5 separately or in any suitable combination. *See, e.g.*, *id.* ¶ [0070]. Further, publications cited in
6 P1 (e.g., Beumer 2008) demonstrate the state of the art. *Id.* ¶¶ [0067]-[0068]; Ex. 2543, ¶¶ 93,
7 94, 222, 224, 225.

8 Methods of making RNAs, proteins, and RNPs for microinjection into fish embryos were
9 well-known and routine by May 25, 2012. Ex. 2009, ¶¶ [0039], [00173]-[00175], [00177],
10 [00178]; Ex. 2543, ¶¶ 66-72; MF 18, 20, 22, 24, 25. A POSA reading P1, in view of general
11 knowledge in the art, could therefore have practiced these methods to make and use the
12 embodiment of E1 (e.g., to generate mutant fish embryos) without undue experimentation. Ex.
13 2543, ¶¶ 130-168.

14 P1 also enables a POSA to make and use the sgRNA CRISPR-Cas9 system in a human
15 cell (E2) routine transfection techniques that had been used with ZFNs and TALENs, to transfer
16 the components of the system into human cells, e.g., established cell lines. Ex. 2543, ¶¶ 194-213;
17 MF 15, 20, 26, 27. These transfection techniques are disclosed in P1 and include "lipofection,
18 electroporation, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection,
19 DEAE-dextran mediated transfection, liposome-mediated transfection, and the like." Ex. 2009,
20 [00129]; *id.* ¶¶ [0039], [00154], [00173]-[00175], [00177]; Ex. 2543, ¶¶ 73, 199. Publications
21 like Ausubel 1995 illustrate that transfection techniques for human cells in culture
22 (electroporation and liposome mediated transfection) were generally known. Ex. 2124, 9.3-9.4;
23 Ex. 2543, ¶¶ 199-200. Transfecting nucleic acids, proteins, or RNPs into eukaryotic cells using
24 electroporation or liposomes required only ordinary skill. Ex. 2543, ¶¶ 199-200; MF 27. Further,

there are several examples of applying such transfection techniques in mammalian cells, including human cells: transfecting RNA by electroporation (Ex. 2143, Suppl.Methods; Ex. 2167, 5563) or liposomes (Ex. 2168, 2; Ex. 2169, 497); transfecting protein by electroporation (Ex. 2170, 65; Ex. 2171, 8367) or liposomes (Ex. 2172, 10189-10190; Ex. 2173, 2026); transfecting RNPs by DEAE-dextran (Ex. 2174, 2862) or liposomes (Ex. 2175, 3974). Ex. 2543, ¶¶ 199-200; MF 26, 27.

A POSA reading P1 in view of general knowledge in the art would have been able use routine transfection methods to implement the 3' extended chimera A CRISPR-Cas system in an established human cell line culture to make E2 without undue experimentation. Ex. 2543, ¶¶ 199-200; Ex. 2009, ¶¶ [00039], [00129], [00154], [00173], [00175]. Adjustments to protocols and methods to enhance expression, improve efficiency, or assign appropriate cellular conditions were well within the knowledge and understanding of a POSA. Ex. 2543, ¶¶ 83-89, 246-297.

P1 discloses microinjection methods for introducing the sgRNA CRISPR-Cas9 system into a fruit fly cell. Ex. 2009, ¶¶ [0039], [00154], [00165], [00173]; Ex. 2543, ¶¶ 66-72; MF 24. Microinjection was routine by May 25, 2012. Ex. 2543, ¶¶ 66-72; Ex. 2123; Ex. 2187, 210; Ex. 2188; Ex. 2145; Ex. 2148; Ex. 2189; MF 25. In 2007, Fish described microinjection techniques used for "introduce[ing] mRNAs, double-stranded RNAs, proteins or other biologically active molecules directly into *Drosophila* embryos." Ex. 2188, 2325, 2326-2330; Ex. 2543, ¶ 225. P1 also incorporates Beumer 2008 (Ex. 2123) for the state of the art, which describes methods for *Drosophila* (fruit fly) "direct embryo injection." Ex. 2123, 19821, 19824; Ex. 2009, ¶¶ [0068], [00174]; Ex. 2543, ¶ 225; MF 24, 25. Beumer 2008 states: "Embryo injection has been the method of choice for introducing transgenes into *Drosophila* for >25 years." Ex. 2123, 19822.

While not recited in the count, P1 describes and enables the selection of targets adjacent to a protospacer-adjacent motif ("PAM"), including for non-natural DNA targets, consistent with

1 what a POSA would have understood to be required when applying the system in a eukaryotic
2 cell. Ex. 2543, ¶¶ 249-259; MF 28, 29, 33, 37. P2 provides additional discussion and citations
3 regarding the PAM requirement. Ex. 2010, ¶ [00350]; Ex. 2543, ¶¶ 54-64, 249-259; Ex. 2548, ¶¶
4 13-19; MF 33. While not recited in CVC's half of the count, P1 describes and enables optional
5 optimizations, including adding nuclear localization signals and replacing codons for improved
6 expression. Ex. 2543, ¶¶ 186-189, ¶ 190, 262-297; MF 30, 31. A POSA would also have been
7 aware of suitable promoters for expressing the sgRNA and Cas9 at high levels in eukaryotic
8 cells, as P1 expressly states "[s]election of the appropriate vector and promoter is well within the
9 level of ordinary skill in the art," and suitable promoters were well-known in the art, for proteins
10 in the ZFN context and for RNAs in shRNA context. Ex. 2009,¶ [00127]; Ex. 2543, ¶ 196.
11 With respect to Sigma's half of Count 2, CVC's P1 describes and enables modifying a
12 chromosomal sequence in a eukaryotic cell by integrating a donor sequence, which occurs when
13 the cell's own homology-directed repair ("HDR") process repairs DNA cut by any nuclease,
14 whether a sgRNA CRISPR-Cas9 system, ZFN, or TALEN. Ex. 2009, ¶¶ [0058], [0059], [00157],
15 [00189]-[00193], Fig. 4, claims 77, 88, 99; Ex. 2543, ¶¶ 126-128, 303; MF 38. Before May 25,
16 2012, skilled artisans had been exploiting natural HDR to incorporate donor sequences into DNA
17 after the target DNA had been cleaved by a ZFN or a TALEN. Ex. 2543, ¶¶ 126-128, 303.
18 Thus, in light of general knowledge in the art combined with the POSA's high degree of
19 skill, P1 fully describes and enables E1, E2, and E3. Ex. 2543, ¶¶ 90-245, 303; MF 12-38.
20 All the disclosures of P1 appear in P2, and in CVC's U.S. Provisional Application No.
21 61/757,640 ("P3"), and in CVC's involved '859 application. MF 48. While not necessary for a
22 constructive reduction to practice, CVC's P3 and '859 application include a working example
23 ("Example 2") that describes successful genome-editing experiments performed in human cells

1 using the same sgRNA CRISPR-Cas9 system that is described in P1. Ex. 2011, ¶¶ [00408]-
2 [00450]; Ex. 2015, ¶¶ [00562]-[00577]; Ex. 2033, Jinek 2013, Fig. 1E; Ex. 2543, ¶¶ 219, 316.
3 For the foregoing reasons, CVC's entitlement to priority benefit to P1 or P2, as discussed
4 in CVC's co-pending Substantive Motion 1, is equally applicable to proposed Count 2.

5 ***3. Proposed Count 2 is patentable over the prior art***

6 "A party seeking to substitute an existing count must show that the new count is
7 patentable over the prior art." 37 C.F.R. § 41.208(c)(2). Here, Proposed Count 2 is narrower than
8 Count 1, and therefore does not raise any issues regarding patentability over the prior art. There
9 is no dispute that Proposed Count 2 is patentable over the art as it existed *before* CVC's May 25,
10 2012 P1 date.[3]

11 ***IV. Conclusion***

12 The PTAB should grant CVC's Motion 3 and Substitute Proposed Count 2 for Count 1.
13
14 Respectfully submitted,

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15

[3] While there is no dispute that Count 2 is patentable over the prior art before May 25, 2012, CVC does not agree that any of Sigma's claims are patentable to Sigma over the prior art.

APPENDIX A: EXHIBIT LIST

Exhibit No.	Description
2006	U.S. Appl. No. 14/685,504, filed April 13, 2015
2007	U.S. Appl. No. 15/138,604, filed April 16, 2016
2009	Prov. Appl. No. 61/652,086, filed May 25, 2012
2010	Prov. Appl. No. 61/716,256, filed October 19, 2012
2011	Prov. Appl. No. 61/757,640, filed January 28, 2013
2015	U.S. Appl. No. 13/842,859, filed March 15, 2013
2023	Krebber, H. and Silver, P.A., “Directing Proteins to Nucleus by Fusion to Nuclear Localization Signal Tags,” *Methods in Enzymology* 327: 283-296 (2000)
2026	Prov. Appl. No. 61/734,256, filed December 6, 2012
2031	Jinek, M., *et al*., “A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity,” *Science* 337(6096):816-821, with Supplementary Information (2012)
2033	Jinek, M., *et al*., “RNA-programmed genome editing in human cells,” *eLife* 2:e00471, 1-9 (2013)
2123	Beumer, K.J., *et al.*, “Efficient gene targeting in *Drosophila* by direct embryo injection with zinc-finger nucleases,” *Proc. Natl. Acad. Sci,* 105(50):19821-19826 (2008)
2124	Ausubel, F.M., *et al*., Eds., “Chapter 9: Introduction of DNA into Mammalian Cells,” *Short Protocols in Molecular Biology,* pp. 9-1 – 9-57, Third Edition, John Wiley & Sons, Inc., United States (1995)
2125	Deveau, H., *et al*., “Phage Response to CRISPR-Encoded Resistance in *Streptococcus thermophilus*,” *J. Bacteriol*. 190(4):1390-1400 (2008)
2127	Mojica, F.J.M., *et al*., “Short motif sequences determine the targets of the prokaryotic CRISPR defence system,” *Microbiology* 155:733-740 (2009)
2130	Makarova, K.S., *et al*., “Evolution and classification of the CRISPR-Cas systems,” *Nature Reviews Microbiology* 9:467-477 (2011)
2132	Sapranauskas, R., *et al*., “The *Streptococcus thermophilus* CRISPR/Cas system provides immunity in *Escherichia coli*,” *Nucl. Acids Res*. 39(21):9275-9282, Supplementary Figures (2011)
2134	Wiedenheft, B., *et al*., “RNA-guided genetic silencing systems in bacteria and archaea,” *Nature* 482:331-338 (2012)
2143	Tesson, L., *et al*., “Knockout rats generated by embryo microinjection of TALENs,” *Nat. Biotechnol*. 29(8):695-696, Supplementary Information (2011)
2145	Buchenau, P., *et al*., “The Dynamic Nuclear Redistribution of an hnRNP K-homologous Protein during *Drosophila* Embryo Development and Heat Shock. Flexibility of Transcription Sites In Vivo,” *J. Cell Biol*. 137(2):291-303 (1997)
2148	Eickbush, D.G., *et al*., “Integration of *Bombyx mori* R2 Sequences into the 28S Ribosomal RNA Genes of *Drosophila melanogaster*,” *Mol. Cell. Biol*. 20(1):213-223 (2000)

Exhibit No.	Description
2167	Zou, J., *et al*., "Oxidase-deficient neutrophils from X-linked chronic granulomatous disease iPS cells: functional correction by zinc finger nuclease-mediated safe harbor targeting," *Blood* 117(21):5561-5572 (2011)
2168	Donze, O. and Picard, D., "RNA interference in mammalian cells using siRNAs synthesized with T7 RNA polymerase," *Nucl. Acids Res*. 30(10): e46, pp. 1-4 (2002)
2169	Elbashir, S.M., *et al*., "Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells," *Nature* 411:494-498 (2001)
2170	Berglund, D.L. and Starkey, J.R., "Introduction of Antibody Into Viable Cells Using Electroporation," *Cytometry* 12:64-67 (1991)
2171	Koken, S.E.C., *et al*., "Intracellular Analysis of *in Vitro* Modified HIV Tat Protein," *J. Biol. Chem*., 269(11):8366-8375 (1994)
2172	Debs, R.J., *et al*., "Regulation of Gene Expression *in Vivo* by Liposome-mediated Delivery of a Purified Transcription Factor," *J. Biol. Chem*. 265(18):10189-10192 (1990)
2173	Baubonis, W. and Sauer, B., "Genomic targeting with purified Cre recombinase," *Nucl. Acids Res*. 21(9):2025-2029 (1993)
2174	Luo, G., *et al*., "The Polyadenylation Signal of Influenza Virus RNA Involves a Stretch of Uridines Followed by the RNA Duplex of the Panhandle Structure," *J. Virol*. 65(6):2861-2867 (1991)
2175	Lopez, N., *et al*., "The L Protein of Rift Valley Fever Virus Can Rescue Viral Ribonucleoproteins and Transcribe Synthetic Genome-Like RNA Molecules," *J. Virol*. 69(7):3972-3979 (1995)
2187	Liu, J., *et al*., "Efficient and Specific Modifications of the *Drosophila* Genome by Means of an Easy TALEN Strategy," *J. Genet. And Genomics* 39:209-215 (2012)
2188	Fish, M., *et al*., "Creating transgenic *Drosophila* by microinjecting the site-specific ϕC31 integrase mRNA and a transgene-containing donor plasmid," *Nat. Protocols* 2(10):2325-2331 (2007)
2189	Methods in Molecular Biology, Transgenesis Techniques: Principles and Protocols, Second Edition, Ed., Clarke, A.R., Humana Press, Inc., United States (2002)
2221	Lange, A., *et al*., "Classical Nuclear Localization Signals: Definition, Function, and Interaction with Importin α," *J. Biol. Chem.* 282(8): 5101–5105 (2007)
2348	Doudna, J.A., and Lorsch, J.R., "Ribozyme catalysis: not different, just worse," *Nature Structure & Molecular Biology* 12(5):395-402 (2005)
2400	Decision on Motions 37 C.F.R. § 41.125(a), *The Regents of the University of California v. The Broad Institute, Inc.,* Patent Interference No. 106,115, Paper 877, (September 10, 2020)
2543	Declaration of Yannick Doyon, Ph.D.
2548	Declaration of Samuel Sternberg, Ph.D.
2549	Declaration of Scott Bailey, Ph.D.

Exhibit No.	Description
2550	van der Aa, M.A.E.M., *et al*., "The Nuclear Pore Complex: The Gateway to Successful Nonviral Gene Delivery," *Pharmaceutical Research* 23(3): 447-459 (2006)
2560	Declaration 37 C.F.R. § 41.203(b), Count and claims of the parties, *The Regents of the University of California v. The Broad Institute, Inc*., Patent Interference No. 106,115, Paper 1, (June 24, 2019)
2561	Declaration 37 C.F.R. § 41.203(b), Count and claims of the parties, *The Regents of the University of California v. ToolGen, Inc.,* Patent Interference No. 106,127, Paper 1, (December 14, 2020)
2562	Declaration 37 C.F.R. § 41.203(b), Count and claims of the parties, *The Broad Institute, Inc. v. ToolGen, Inc*., Patent Interference No. 106,126, Paper 1, (December 14, 2020)
2585	U.S. Appl. No. 15/456,204 with current claims appended

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

2 **1.** CVC's half of Count 1 aligns with the scope of the counts in multiple ongoing
3 interferences, including interferences between: CVC and Broad (Interference No. 106,115), Ex.
4 2560 at 12-13; CVC and ToolGen (Interference No. 106,127), Ex. 2561 at 5-6; and Broad and
5 ToolGen (Interference No. 106,126), Ex. 2562 at 12-13.

6 **2.** In each of these Interferences 106,115, 106,127 and 106,126, both halves of the count
7 recite single-guide ("sgRNA") CRISPR-Cas9 systems, whether or not the parties have claims
8 that are generic as to the format of the guide RNA (i.e., "generic-guide" claims). Ex. 2560, 12-
9 13; Ex. 2561 at 5-6; Ex. 2562, 12-13; *see* Ex. 2400, 31-33.

10 **3.** CVC has involved claims directed to both generic guide RNA and single-guide RNA
11 embodiments. *See, e.g.*, CVC Application Nos. 15/947,700; 15/947,718; 15/981,808; 15/981,809
12 (reciting CRISPR-Cas9 systems in eukaryotic cells that are not limited to single-molecule RNA).

13 **4.** Count 1 in this Interference is defined as CVC Application 15/981,807, claim 156 or
14 Sigma Application 15/456,204 claim 31 (colloquially referred to as a "McKelvey count"). *See*
15 Paper 1, Declaration of Interference, 5.

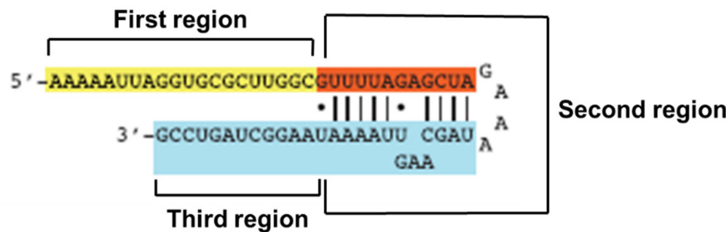
16 **5.** CVC claim 156 recites "a single molecule DNA-targeting RNA, or a nucleic acid
17 comprising a nucleotide sequence encoding said single molecule DNA-targeting RNA" and then
18 goes on to define the three essential components of a single-molecule guide RNA. *See* Paper 1,
19 Declaration of Interference, 5.

20 **6.** Sigma's claim 31 recites "a guide RNA or DNA encoding the guide RNA, wherein the
21 guide RNA comprises a first region that is complementary to a target site in the chromosomal
22 sequence . . . and a second region that interacts with the CRISPR-Cas type-II protein, and
23 wherein the guide RNA comprises a crRNA and a tracrRNA." *See* Paper 1, Declaration of
24 Interference, 6.

7. The PTAB construed the term “guide RNA” in the ’115 Interference to refer to sgRNA. Ex. 2400, 31-33.

8. Claim 33 of Sigma’s ’204 Application adds the limitation “wherein the guide RNA is a single molecule.” Ex. 2585, 104.

9. Sigma’s P1 application defines “guide RNA” as comprising a first region, “a second internal region that forms a stem loop structure,” and a third region. Ex. 2026, ¶¶ [0021], [0019]. These regions can be illustrated according to the figure below:



Jinek, *Science* 2012 Fig. 5A (annotated) (cited in Sigma’s ’204 application). Ex. 2549, ¶ 99. The “loop” of claim 31 refers to the linker loop in the stem-loop region shown in purple in the diagram above, which joins the crRNA and the tracrRNA. Ex. 2549, ¶¶ 98-99, 101.

10. Sigma’s ’204 application discloses data from a single experiment: a gel that has lanes for both single-guide RNA and dual-guide RNA molecules. Ex. 2585, 11-75; Ex. 2549, ¶103.

11. CVC’s co-pending motion for priority benefit (CVC’s Substantive Motion 1) supports priority benefit based on CVC’s half of the count (claim 156 of CVC’s ’807 Application).

12. Application No. 61/652,086 (“P1”), filed on May 25, 2012, lists Martin Jinek, Jennifer Doudna, Emmanuelle Charpentier, and Krzysztof Chylinski as co-inventors. Ex. 2009, 195.

13. Application No. 61/716,256 (“P2”), filed on October 19, 2012, lists Jinek, Doudna, Charpentier, Chylinski, and James Harrison Doudna Cate as co-inventors. Ex. 2010, 277.

14. CVC’s P1 describes CRISPR-Cas systems comprising a) a Cas9 protein and b) a single molecule DNA-targeting RNA. Ex. 2009, ¶¶ [00248]-[00251], Figs. 1-3; Ex. 2543, ¶¶ 90-242.

1 **15.** CVC's P1 describes a sgRNA comprising i) a targeter RNA capable of hybridizing with a
2 target sequence in the target DNA and ii) an activator-RNA capable of hybridizing with the
3 targeter RNA to form a double-stranded duplex, wherein the activator-RNA and the targeter-
4 RNA are covalently linked to one another with intervening nucleotides. Ex. 2009, ¶¶ [0079],
5 [00119], [00248], Figs. 1, 3, 9; Ex. 2543, ¶¶ 90-95, 106-108, 175-179, 223.

6 **16.** CVC's P1 describes a sgRNA capable of forming a complex with Cas9 and thereby
7 targeting the Cas9 protein to the target DNA molecule. Ex. 2009, ¶¶ [0046], [0048], [0076],
8 [0089], [00155]-[00156], [00248]-[00251], Figs. 1, 3; Ex. 2543, ¶¶ 90-95, 110-112, 180, 223.

9 **17.** CVC's P1 describes CRISPR-Cas9 systems capable of cleaving or editing target DNA or
10 modulating transcription of at least one gene encoded by the target DNA. Ex. 2009, ¶¶ [00155]-
11 [00159], [00248]-[00251], Figs. 3, 4; Ex. 2543, ¶¶ 90-95, 113-114, 180, 223.

12 **18.** CVC's P1 describes target cells including a fish, a human, and a fruit fly cell, and that a
13 target cell may be "embryonic." Ex. 2009, ¶¶ [00165], [00216], [00218], [00050]-[00052],
14 [00174].

15 **19.** CVC's P1 describes making and using a single-molecule DNA-targeting RNA and a
16 Cas9 RNA. Ex. 2009, ¶¶ [00173], [00248]; Ex. 2543, ¶¶ 90-95, 100, 170-173, 222.

17 **20.** CVC's P1 describes that Cas9 can be delivered into a eukaryotic cell "as a polypeptide,"
18 as a nucleic acid encoding Cas9, or in a pre-formed RNP complex. Ex. 2009, ¶¶ [00120],
19 [00126]-[00128], [00167]-[00172], [00177]-[00178]; Ex. 2543, ¶¶ 92, 96-99, 115, 132-135, 140.

20 **21.** CVC's P1 describes that the sgRNA can be delivered into a eukaryotic cell "directly as
21 RNA" or as a nucleic acid "comprising a nucleotide sequence encoding a subject DNA-targeting
22 RNA." Ex. 2009, ¶¶ [00120], [00167], [00170]-[00173], [00177]; Ex. 2543, ¶¶ 92, 96-99, 137-
23 140.

24 **22.** CVC's P1's working example describes incubating a recombinant Cas9 protein with the

1 sgRNA to make an RNP complex. Ex. 2009, ¶¶ [00248]-[00251]; Ex. 2543, ¶¶ 92, 96-99, 137-
2 140.

3 **23.** CVC's P1's working example describes a sgRNA complexed with a Cas9 protein
4 cleaving a target DNA. Ex. 2009, ¶¶ [00248]-[00251], Fig. 3A; Ex. 2543, ¶¶ 92, 96-99, 137-140.

5 **24.** CVC's P1 describes microinjection as a method of delivering Type II CRISPR-Cas9 into
6 a cell. Ex. 2009, ¶¶ [0039], [00154], [00173]-[00175]; Ex. 2543, ¶¶ 141-146, 225.

7 **25.** By May 25, 2012, microinjecting protein, RNA, or RNPs into eukaryotic cells were well-
8 known, routine laboratory techniques. Ex. 2009, ¶ [00173]; Ex. 2543, ¶¶ 66-72.

9 **26.** CVC's P1 describes transfection as a method for delivering Type II CRISPR-Cas9
10 systems into a cell. Ex. 2009, ¶¶ [00129], [0039], [00154], [00173-175], [00177]; Ex. 2543, ¶¶
11 199-200.

12 **27.** By May 25, 2012, transfecting proteins, RNA, and RNPs into eukaryotic cells human cell
13 lines were well-known, routine laboratory techniques. Ex. 2009, ¶ [00173]; Ex. 2543, ¶¶ 73-82.

14 **28.** By May 25, 2012, the art disclosed that a PAM must be adjacent to the target sequence
15 for Type II CRISPR-Cas9 systems to cleave target DNA. Ex. 2543, ¶¶ 54-64, 249-259.

16 **29.** CVC's P1 discloses a PAM sequence adjacent to the target in Target DNA A ("GGG"),
17 Target DNA B ("GGG"), and Target DNA C ("TGG"). Ex. 2009, Fig. 3C; Ex. 2543, ¶¶ 249-259.

18 **30.** CVC's P1 describes "replac[ing] a codon with a codon encoding the same amino acid."
19 Ex. 2009, ¶ [0033]; Ex. 2543, ¶¶ 190, 285-289.

20 **31.** CVC's P1 describes peptide that can be added to Cas9, including a polypeptide that
21 facilitates traversing an organelle membrane. Ex. 2009, ¶ [00115]; Ex. 2543, ¶¶ 120-121, 277-
22 284.

23 **32.** All of the disclosures in CVC's P1 are in P2. Ex. 2009; Ex. 2010; Ex. 2543, ¶¶ 243-245.

24 **33.** CVC's P2 describes PAMs and cites Sapranauskas (Ex. 2132), Deveau (Ex. 2125),

1 Mojica (Ex. 2127), Makarova (Ex. 2130), and Wiedenheft (Ex. 2134), which discuss PAMs in
2 CRISPR-Cas systems. Ex. 2010, ¶¶ [00103], [00350]-[00352], [00359]; Ex. 2543, ¶¶ 243-245.

3 **34.** CVC's '859 application was filed within 12 months of the filing dates of P1 and P2, and
4 makes specific reference to CVC's P1 and P2 applications. Ex. 2015, 5.

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

7 **36.** CVC's '604 application was filed during the '504 application's pendency and makes
8 specific reference to CVC's '504, '859, and P1 and P2 applications. Ex. 2007, 356-360.

9 **37.** Target DNA A, disclosed in Figure 3C of CVC's P1 and P2, is a non-natural target and P1
10 and P2 disclose Target DNA A as including a PAM. Ex. 2009, Fig. 3C; Ex. 2543, ¶¶ 255-259.

11 **38.** CVC's P1 describes and enables modification of a chromosomal sequence in a eukaryotic
12 cell by integrating a donor sequence, which occurs when the cell's own homology-directed repair
13 process repairs DNA cut by a nuclease, whether a sgRNA-CRISPR-Cas9, a ZFN, or a TALEN.
14 Ex. 2009, ¶¶ [0058], [0059], [00157], [00189]-[00193], Fig. 4, claims 77, 88, 99; Ex. 2543, ¶¶
15 126-128, 303.

16 **39.** The differences between claim 1 of the '181 or '716 patent and Sigma's half of Count 1,
17 are that the claims specify which Cas9 protein to use (from *S. pyogenes*), where to locate the DNA-
18 targeting region within the guide RNA (at the 5' end), and which NLS to use (C-terminal SEQ ID
19 NO: 1 or SEQ ID NO: 2). Ex. 2017, 71:34-72:39; Ex. 2019, 71:14-51; Ex. 2549, ¶¶9, 30.

20 **40.** Jinek 2012 discloses *in vitro* experiments that used *S. pyogenes* Cas9 to cleave target DNA,
21 including GFP, a sequence from a eukaryote. Ex. 2031, Figs. 1-5; Ex. 2549, ¶¶47, 57.

22 **41.** Jinek 2012 discloses *in vitro* experiments using guide RNAs comprising a DNA-targeting
23 region at the 5' end that base pairs with a target site in the chromosomal sequence. Ex. 2031, Figs.
24 1E, 3C, 5B; Ex. 2549, ¶¶34-37.

1 **42.** The natural location for the DNA-targeting region of a guide RNA in a CRISPR system is
2 at the 5' end. Ex. 2031, 818; Ex. 2549, ¶34.
3 **43.** Krebber 2000 discloses methods of using the NLS listed as SEQ ID NO: 1. Ex. 2023, 285;
4 Ex. 2549, ¶¶39.
5 **44.** Krebber 2000 discloses attaching an NLS at either the N-terminus or C-terminus of the
6 tagged protein. Ex. 2023, 289-290; Ex. 2549, ¶39.
7 **45.** Lange 2007 discloses methods of using the NLS listed as SEQ ID NO: 2. Ex. 2221, 3; Ex.
8 2549, ¶39.
9 **46.** Before December 2012, the SV40 NLS was the most commonly used NLS peptide for
10 tagging proteins. Ex. 2550, 451-452; Ex. 2023, 285; Ex. 2549, ¶39.
11 **47.** Before December 2012, the SV40 NLS was "the model" for NLSs. Ex. 2348, 478; Ex.
12 2549, ¶39.
13 **48.** All the disclosures of CVC's P1 application appear in CVC's P2 application, and in CVC's
14 U.S. Provisional Application No. 61/757,640, as well as in CVC's U.S. Appl. No. 13/842,859. Ex.
15 2009; Ex. 2010; Ex. 2011; Ex. 2015; Ex. 2543, ¶¶ 219, 243-245, 316.
16 **49.** Expressing a dual molecule guide RNA would require multiple promoters. Ex. 2549, ¶100.
17 **50.** Sigma has not requested de-designating any claims from this proceeding. *See* Order
18 Authorizing Motions and Setting Times 37 C.F.R. §§ 104(c) and 121.

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CERTIFICATE OF SERVICE

I hereby certify that the foregoing **JUNIOR PARTY'S SUBSTANTIVE MOTION 3,** is being filed via the Interference Web Portal by 8:00 PM Eastern Time on November 19, 2021, pursuant to the Order Authorizing Motions and Setting Times ("Order"; Paper 30), and thereby served on the attorney of record for the Senior Party pursuant to ¶ 105.3 of the Standing Order. Pursuant to the Order, the foregoing was also served via email by 11:00 PM Eastern Time on counsel for the Senior Party at:

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