UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GLAXOSMITHKLINE BIOLOGICALS SA,
Petitioner,

v.

PFENEX INC.,
Patent Owner.

Case No. IPR2020-00890
Patent No. 8,530,171

PETITION FOR *INTER PARTES REVIEW*

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I. INTRODUCTION

U.S. Patent No. 8,530,171 ("the '171 patent") claims methods of producing a known protein, CRM197, in a known bacterial host, a *Pseudomonad* host cell, at yields of about 0.2 to about 12 g/L of soluble and/or active protein. '171 patent (Ex. 1001), 145:42-54 (claim 1). These methods would have been obvious over the prior art that was available as of Patent Owner's alleged priority date of March 30, 2010.[1]

Long before that time, those skilled in the art had been actively attempting to improve the expression of CRM197, a well-characterized protein routinely used in commercial vaccines, in different bacterial expression systems. In 2009, Blais achieved high-level expression of soluble and active CRM197 at yields of over 3 g/L in an *E. coli* host. *Pseudomonas fluorescens* ("*P. fluorescens*"), a *Pseudomonad* strain, was known to be unusually well suited for high-level production of soluble and active recombinant proteins, and had been proven to be superior to *E. coli* for high-level expression of a variety of therapeutic proteins. Squires further disclosed that *P. fluorescens* could tolerate a wide range of cultivating conditions and that large-scale fermentation conditions had been routinely optimized for high-level

[1] For purposes of this Petition, Petitioner does not challenge the alleged priority date of the '171 patent but reserves the right to do so.

expression of recombinant proteins in *P. fluorescens*. In view of Blais's high-level production of soluble and active CRM197 in *E. coli* and Squires's express teaching that *P. fluorescens* was "a compelling alternative" to *E. coli* and particularly suitable for high-level protein expression, a POSA would have had a strong motivation to use *P. fluorescens* as a host for expressing soluble and active CRM197 from Blais's successful FlgI-CRM197 expression vector, and would have had a reasonable expectation in achieving the yields recited in claim 1.

The dependent claims of the '171 patent recite well-known limitations that also would have been obvious to a POSA in view of the prior art, including signal sequences, tags, promoters, induction conditions, protease-deficient hosts, and codon optimization. Because these additional limitations were routinely used in the art, as reflected by Choi, Raimseier I, Raimseier II, and Maunsell, claims 2-36 of the '171 patent also would have been obvious.

In view of the foregoing, Petitioner respectfully submits there is a reasonable likelihood that it will prevail in showing the challenged claims are unpatentable. In support of the proposed grounds for unpatentability, this Petition is accompanied by a declaration of Dr. Collier (Ex. 1003), an expert in molecular cloning and expression of recombinant proteins, including diphtheria toxins, in bacterial host cells.

II. REQUIREMENTS FOR *INTER PARTES* REVIEW UNDER 37 C.F.R. 42.104

A. Grounds for Standing

Petitioner certifies that (1) the '171 patent is available for *inter partes* review ("IPR") based on the March 28, 2011 filing date of the '171 patent ('171 patent (Ex. 1001), (22)), and (2) Petitioner is not barred or estopped from requesting review on the grounds identified.

B. Identification of Challenges

Pursuant to 37 C.F.R. §§ 42.104(b) and 42.22(a)(1), Petitioner requests review and cancellation of claims 1-36 of the '171 patent pursuant to the following challenges:

Ground	Claims	Basis	References
1	1-3, 7, 9, 11, 12, 15, 17, 21-24, 27, 29, 32 and 33	103	Blais in view of Squires
2	13, 14, 30 and 31	103	Blais in view of Squires and Choi
3	4, 5, 8, 10, 16, 18-20, 25, 28 and 34-36	103	Blais in view of Squires and Ramseier I
4	6 and 26	103	Blais in view of Squires, Ramseier I, Ramseier II, and Maunsell

III. THE '171 PATENT

The '171 patent is entitled "High Level Expression of Recombinant Toxin Proteins." '171 patent, (54). The '171 patent states that the alleged invention "relates to the field of recombinant toxin protein production in bacterial hosts" and in particular "relates to production processes for obtaining high levels of a recombinant CRM197" and other recombinant toxin proteins "from a bacterial host." *Id.*, Abstract.

A. The Challenged Claims

Claim 1, the only independent claim of the '171 patent, recites:

> A method for producing a recombinant toxin protein in a *Pseudomonad* host cell, said method comprising:
> ligating into an expression vector a nucleotide sequence encoding the toxin protein;
> transforming the *Pseudomonad* host cell with the expression vector; and
> culturing the transformed *Pseudomonad* host cell in a culture media suitable for the expression of the recombinant toxin protein;
> wherein the recombinant toxin protein is CRM 197, and wherein the recombinant protein is produced at a yield of soluble or active CRM197 protein of about 0.2 grams per liter to about 12 grams per liter.

'171 patent, 145:42-54; Ex. 1003, ¶ 45. Dependent claim 2 recites ranges of CRM197 protein yield that entirely overlap with the range of "about 0.2 grams per liter to about 12 grams per liter" recited in claim 1. *Compare*, *id.*, 145:56 ("0.2 grams per liter to about 12 grams per liter") *with* 145:57 ("about 0.2 g/L").

The other challenged dependent claims additionally require:

- soluble and active CRM197 protein (claim 21 and its dependent claims)[2]
- secretion signal sequences that direct periplasmic expression of CRM197 (claims 3, 7, 9, 10, 23, 24, 27 and 29)
- a tag sequence adjacent to the coding sequence of the secretion signal sequence (claims 4 and 20)
- a host cell defective in the expression of protease(s) (claims 5, 6, 8, 25, 26 and 28)
- a lac promoter (claims 14 and 31)
- induction conditions (claims 13 and 30)

[2] Like dependent claim 2, dependent claim 22 recites ranges of CRM197 protein yield that entirely overlap with the range of "about 0.2 grams per liter to about 12 grams per liter" recited in claim 21. *Compare* '171 patent, 148:16-17 ("about 0.2 gram per liter to 12 grams per liter") *with* 148:24 ("about 12 g/L").

- activity levels measured in an activity assay (claims 11 and 12)
- a *Pseudomonas* or *Pseudomonas fluorescens* host cell (claims 16, 17, 32 and 33)
- codon optimized CRM197-encoding nucleotide sequences (claims 16, 18, 19 and 34-36)

'171 patent, claims 2-36; Ex. 1003, ¶ 46.

B. Patent Owner's Admissions in the Specification

"Admissions in the specification regarding the prior art are binding on the patentee for the purposes of a later inquiry into obviousness." *PharmaStem Therapeutics, Inc. v. ViaCell, Inc.*, 491 F.3d 1342, 1362 (Fed. Cir. 2007). The '171 patent discloses that multiple elements of the alleged invention were known in the art. These prior art admissions are binding on the Patent Owner.

The '171 patent admits that the claimed nucleotide sequence "may be prepared using known techniques of recombinant DNA technology." '171 patent, 11:24-35 (citing laboratory manuals published in 1989 and 1993). The '171 patent further admits that methods for optimizing the nucleic acid sequence to improve expression in a bacterial host "are known in the art and described in the literature" and that "optimization of codons for expression in a *Pseudomonas* host strain is described" in the prior art. *Id.*, 16:67-17:6. In addition, the '171 patent admits that the claimed activity assays used to measure the produced CRM197 "are known in

the art and described in the literature." *Id.*, 40:41-42. The '171 patent also admits that host cell proteases, including degP1 and AprA, "were known in the art." '171 patent, 25:33-26:9. These admissions are consistent with the common knowledge that multiple limitations recited in the '171 patent claims, such as codon optimization and activity assays, were known or routine as of the alleged priority date of the '171 patent. Ex. 1003, ¶ 44.

C. Prosecution of the '171 Patent

The original independent method claim of the '171 patent did not recite any CRM197 expression levels. Ex. 1002, 90. During prosecution, the Examiner rejected the claims over prior art references not relied on in this petition. *Id.*, 405-08. In response, Patent Owner amended claim 1 to recite a yield of soluble and/or active CRM197. *Id.*, 426.

IV. BACKGROUND

A. Expression of Diphtheria Toxin Proteins Including CRM 197

Decades before the '171 patent was filed, it was known that the diphtheria toxin protein, which is secreted by *Corynebacterium diphtheriae*, causes the symptoms of diphtheria infection. Ex. 1010, 69-70; Ex. 1003, ¶ 17. As "one of the most extensively studied and well understood bacterial toxins," diphtheria toxin had "occupied a central focus of the field of toxi[c]ology" since its discovery in the late 1800s. Ex. 1012, 1793; Ex. 1003, ¶ 19. The toxin molecule is synthesized as a

single-chain protein containing a signal peptide that directs the toxin to the bacterial periplasmic space. Ex. 1011, 343; Ex. 1003, ¶ 18. The toxin molecule has two subunits, an amino-terminal fragment A, and a carboxy-terminal fragment B, that are linked by a peptide bond and a disulfide bond. Ex. 1010, 70-71; Ex. 1012, 1795; Ex. 1003, ¶ 18. Fragment B is required for the recognition of specific surface receptors on cells, while fragment A is an NAD-dependent ADP-ribosyltransferase that shuts down protein synthesis in an infected cell, thereby causing the symptoms of diphtheria infection. Ex. 1012, 1795; Ex. 1010, 70-71; Ex. 1004, 1:15-19; Ex. 1003, ¶ 18.

Long before Patent Owner filed the '171 patent, several diphtheria toxin mutants had been isolated. Ex. 1010, 81; Ex. 1003, ¶ 19. Those mutated *tox* genes produced proteins that cross-reacted with diphtheria antitoxin (and therefore were termed "cross-reacting materials" or "CRMs"), but were either completely nontoxic or had greatly reduced toxicity. Ex. 1010, 81; Ex. 1003, ¶ 19. One such protein, CRM197, has a single amino acid mutation that eliminates the toxin's ADP-ribosyltransferase activity and toxicity. Ex. 1004, 1:30-38; Ex. 1012, 1795; Ex. 1010, 81-82; Ex. 1003, ¶ 20. CRM197 is otherwise immunogenically indistinguishable from the wild type diphtheria toxin protein. Ex. 1013, 560; Ex. 1012, 1795; Ex. 1010, 81-82; Ex. 1003, ¶ 20.

Due to its non-toxic nature, CRM197 became a preferred toxoid to use in diphtheria vaccines. Ex. 1013, 560; Ex. 1003, ¶ 21. CRM197 also enhances the immunogenicity of poorly-immunogenic molecules when chemically coupled to such molecules. Ex. 1013, 560; Ex. 1003, ¶ 21. Accordingly, CRM197 had been used in multiple commercially-available conjugate vaccines prior to 2010. Ex. 1004, 1:46:49; Ex. 1003, ¶ 21.

Diphtheria toxoid, including CRM197, had been produced in bacterial host cells, including Gram-negative bacteria, long before the '171 patent was filed. Ex. 1004, 1:51, Ex. 1003, ¶ 22. Gram-negative bacteria contain a periplasm, which is a space between the outer surface of the cytoplasmic membrane and the inner surface of the lipopolysaccharide membrane. Ex. 1014, 75, 68 (Fig. 3.29); Ex. 1003, ¶ 22. *E. coli* and *P. fluorescens* are Gram-negative bacterial cells that can express recombinant proteins in the cytoplasm or the periplasm. Ex. 1014, 68 (Fig. 3.29); Ex. 1004, 12:51-65; Ex. 1003, ¶ 22. Early studies had reported certain difficulties in producing high levels of diphtheria toxoid proteins in a soluble and active form in *E. coli*. Ex. 1003, ¶ 23. However, Blais solved these problems and achieved successful production of soluble and active CRM197 at high yields by generating an optimized CRM197-expression construct having an upstream FlgI signal sequence under the control of a *lac* promoter, transforming the construct into *E. coli* host cells, and achieving periplasmic expression of soluble and active

CRM197 at "unprecedented" levels of over 3 g/L. Ex. 1004, Examples 3 and 9; Ex. 1003, ¶ 23. Blais also taught that *Pseudomonad* cells could be used as suitable hosts instead of *E. coli*. Ex. 1004, 12:54, 14:39; Ex. 1003, ¶ 23.

Thus, before March 30, 2010, high-level expression of soluble and active CRM197 had been achieved in *E. coli*, which opened the door for those skilled in the art to further test the expression of Blais's successful construct in other bacterial host cells known to be suitable for high-level expression of heterologous proteins. Ex. 1003, ¶ 24.

B. *P. fluorescens* Was Known as A Superior Platform for High-Level Expression of Soluble and Active Recombinant Proteins

Before the alleged priority date of the '171 patent, those skilled in the art had developed robust bacterial host cell platforms to achieve high-level expression of recombinant proteins. Ex. 1003, ¶ 25 (citing Ex. 1015, 45-46). Among the existing bacterial hosts, *P. fluorescens* was known to have "an impressive capacity for producing heterologous proteins at high levels" and being "unusually well suited for high level expression." Ex. 1015, 60-61; Ex. 1003, ¶ 25.

It was understood that the superior performance of the *P. fluorescens* platform is "due to the combination of a robust host strain and the availability of extensive molecular biology and bioinformatics tools," and a "well-optimized, high-cell-density (HCD) fermentation process." Ex. 1015, 46-47; Ex. 1003, ¶ 26. It was also

known that *P. fluorescens* strains "are stable, amenable to genetic or molecular manipulations, and can be cultivated to high cell densities in fully defined mineral salts media in standard fermentors, without oxygen enrichment." Ex. 1015, 52; Ex. 1005, 54-55; Ex. 1003, ¶ 26.

Various promoters, including *tac* and *lac*UV5 promoters, had been used to drive the expression of heterologous proteins in *P. fluorescens*. Ex. 1015, 52; Ex. 1005, 55; Ex. 1003, ¶ 27. Derivatives of the *lac* promoter were also used to drive expression in *P. fluorescens*, which permits induction of expression by isopropyl-thiogalactopyranoside (IPTG) just like in *E. coli*. Ex. 1015, 52: Ex. 1005, 55; Ex. 1003, ¶ 27. *P. fluorescens* was known to "tolerate a wide range of conditions," and large scale culturing conditions for *P. fluorescens* were known to be simple and easy to control, without requiring additional animal components, antibiotics, organic nitrogen, or supplemental oxygen. Ex. 1015, 61; Ex. 1016, 2; Ex. 1005, 55, 58; Ex. 1003, ¶ 27. Additionally, recovery and downstream purification procedures for *P. fluorescens* were considered "standard, and consistent with those employed with *E. coli*." Ex. 1015, 61; Ex. 1005, 58; Ex. 1003, ¶ 27. Culturing conditions for *P. fluorescens* had been "successfully scaled up for the commercial production of a number of heterologous proteins," prior to 2010, and had been "routinely optimized in 20-L fermenters." Ex. 1015, 47; Ex. 1003, ¶ 27.

P. fluorescens was known to possess "many favorable properties" for "higher volume applications," including "rapid and efficient expression" of "high volumetric and specific expression of a broad range of therapeutic proteins," producing "[s]oluble and active protein yields in excess of 25g L^{-1} with a variety of protein types ranging from enzymes for industrial use to proteins for pharmaceutical applications . . . ." Ex. 1015, 47, 61; *see also* Ex. 1016, 2; Ex. 1005, 58; Ex. 1003, ¶ 28. *P. fluorescens* routinely achieved expression of recombinant proteins at high cell densities and high biomass levels of greater than 100 g/L, accounting for more than 30% of total cell protein, through "well characterized" fermentation techniques that could be scaled up "predictab[ly] and rapid[ly].". Ex. 1015, 47, 61; Ex. 1005, 55; Ex. 1016, 2; Ex. 1003, ¶ 28.

Comparing the production of various recombinant therapeutic proteins in *P. fluorescens* and *E. coli* side-by-side, POSAs had concluded before 2010 that "[i]n each case, *P. fluorescens* was equivalent to, or had some advantages over the commonly used T7 system in *E. coli*." Ex. 1015, 55; Ex. 1003, ¶ 29. In fact, "[i]n most cases the *P. fluorescens* strain produced up to five[-]fold more protein (on a g L^{-1} basis) than the *E. coli* strain." Ex. 1015, 60; Ex. 1003, ¶ 30. And in multiple cases, production of soluble and active recombinant proteins was only achieved by *P. fluorescens* but not *E. coli*. Ex. 1015, 59; Ex. 1005, 58; Ex. 1003, ¶ 31.

For instance, recombinant human growth hormone (rhGH) was expressed cytoplasmically in both *P. fluorescens* and *E. coli* at a 20-L fermentation scale, and *P. fluorescens* produced 1.6-fold more rhGH per gram dry biomass than *E. coli*. Ex. 1015, 55; Ex. 1003, ¶ 29. Indeed, cytoplasmic expression of rhGH in *E. coli* had led to formation of insoluble inclusion bodies, and so a *P. fluorescens* signal sequence, Pbp, was used to express soluble and active rhGH in the periplasm of *P. fluorescens*, reaching a yield of more than 5 g/L. Ex. 1005, 58; Ex. 1003, ¶ 29.

The comparative expression of human gamma interferon (γ-IFN) in *P. fluorescens* and *E. coli* likewise "revealed a significant advantage for recovery of the active cytokine in the *P. fluorescens* expression system." Ex. 1015, 56; Ex. 1003, ¶ 30. Compared to *E. coli*, which produced 2-4 g/L of insoluble γ-IFN protein at the 20-L scale, *P. fluorescens* produced about 4 g/L of γ-IFN in a 20-L fermentation, with 95% of the expressed protein being found in the soluble fraction. Ex. 1015, 56; Ex. 1003, ¶ 30. Additionally, γ-IFN protein expressed in *P. fluorescens* was as active as the commercially available protein in a viral inhibition assay. Ex. 1015, 56; Ex. 1003, ¶ 30.

Even when *P. fluorescens* was used to express antibody proteins, which were known to be challenging to produce in bacterial host cells, it still achieved superior results compared to *E. coli*. Ex. 1017, 350; Ex. 1015, 56; Ex. 1003, ¶ 31. For instance, a cytoplasmically-expressed anti-β-galactosidase single chain antibody,

Gal13, had been expressed at 8-fold greater levels in *P. fluorescens* than in *E. coli*. Ex. 1015, 56-57; Ex. 1003, ¶ 31. And whereas only 48% of the Gal13 produced in *E. coli* was soluble, 96% was soluble when produced in *P. fluorescens*. Ex. 1015, 56, 58 (Figure 3.5); Ex. 1003, ¶ 31. Thus, it was noted that "significant savings in purification costs can be achieved" by using *P. fluorescens* rather than *E. coli*. Ex. 1015, 60; Ex. 1003, ¶ 31.

Thus, it is unsurprising that prior to March 30, 2010, those skilled in the art had concluded that "[t]he combination of high volumetric and specific expression of a broad range of therapeutic proteins, coupled with a potential for soluble, active, and secreted products, makes *P. fluorescens* a compelling alternative for the microbial expression of biologicals for human health." Ex. 1015, 61; Ex. 1005, 58; Ex. 1003, ¶ 32.

V. ASSERTED ART

A. Blais

Blais is a PCT application claiming priority to a GB application filed Oct. 8, 2009. Ex. 1004, (30); Ex. 1031. Blais is entitled to an effective prior art date as of the filing date of the GB application because the GB application supports at least one claim of Blais. *See Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.*, 800 F.3d 1375, 1378-79 (Fed. Cir. 2015); *see also Amgen Inc. v. Sanofi*, 872 F.3d 1367, 1380 (Fed. Cir. 2017); *Amazon.com Inc. v. Customplay, LLC*, IPR2018-01496,

Paper 34 at 46-47 (PTAB March 4, 2020) ("Nothing in *Dynamic Drinkware* indicates a departure from" the reasoning that "if the patent is shown to have at least one claim to an invention that is supported by the disclosure of a provisional application, … the patent may be considered prior art as of the filing date of the provisional under § 102(e)(2)."); *Cox Commc'n, Inc. v. AT&T Intellectual Property LLP*, IPR2015-01227, Paper 70 at 34 (PTAB Nov. 15, 2016) ("a petitioner need only show support for one claim of a referenced patent, as opposed to all claims"). Accordingly, because the GB application provides support for at least claim 38 of Blais, as demonstrated in the following table, Blais is prior art to the '171 patent under 35 U.S.C. § 102(e).

Claim Language	**GB Application**
38. A process for periplasmic expression of a recombinant polypeptide	"the present application provides an improved process for making a bacterial toxin by periplasmic expression" (Ex. 1031, 3). "In a sixth aspect of the invention there is provided a process for making a bacterial toxin" (*d.*, 4). "In a further aspect of the invention there is provided a process for making a bacterial toxin" (*id.*, 16). "Periplasmic extraction was performed by osmotic shock using a procedure adapted from Chen et al. (Biochem. Eng. J. 19:211-215 (2004)). CRM197 content in the periplasmic and cytoplasmic fractions were assayed by Elisa." *Id.*, 33.

by A. Growing a culture of a gram-negative host cell;	"In a sixth aspect of the invention there is provided a process for making a bacterial toxin comprising the steps of a) growing a culture of the bacterial host cell of the invention" (*id.*, 4). "In a further aspect of the invention there is provided a process for making a bacterial toxin comprising the steps of a) growing a culture of the bacterial host cell of the invention" (*id.*, 16). "Representative examples of appropriate hosts include gram negative bacterial cells" (*id.*, 15). "A 20 litre fermenter (Biolafitte) was used. Nine litres of batch phase medium were aseptically transferred into the fermenter….Inoculation was achieved by the addition of 18 ml of pre-culture (prepared as described in Example 8)." *Id.*, 30.
B. Inducing expression of a polypeptide such that a protein is expressed periplasmically;	"In a sixth aspect of the invention there is provided a process for making a bacterial toxin comprising the steps of … b) inducing expression of the polypeptide a such that a bacterial toxoid is expressed periplasmically." *Id.*, 4. "In a further aspect of the invention there is provided a process for making a bacterial toxin comprising the steps of … b) inducing expression of the polypeptide such that a bacterial toxoid is expressed periplasmically." *Id.*, 16. "Once these conditions were achieved IPTG was added to a final concentration of 1 mM.…At the end of fermentation, periplasmic CRM197 productivity was assayed by Elisa." *Id.*, 33-34; *see also* Table 4 (reporting 3180 mg/L periplasmic expression yield).

wherein one or more of the following steps is actioned during expression: i. The pH of step a) is lower than the pH of step b); ii. The temperature of step a) is higher than the temperature of step b); or iii. The substrate feed rate of step a) is higher than the substrate feed rate of step b).	"In a second embodiment the pH of step a) is lower than the pH of step b)." *Id.*, 16. "In an embodiment the pH is increased such that the pH in step b) is higher than the pH of step a). Optionally this increase in pH is achieved by addition of sodium hydroxide or ammonia." *Id.*, 17. "During the fed-batch phase (15-46h), the pH was maintained at 6.8 by addition of base.…After 46h, the pH is increased to 7.5 by base addition.…Once these conditions were achieved IPTG was added a final concentration of 1 mM." *Id.*, 33.

Blais relates to "production processes for obtaining high levels of" recombinant bacterial toxins including CRM 197. *See* '171 patent (Ex. 1001), Abstract; Ex. 1003, ¶ 43. Blais acknowledges prior reported difficulties of expressing soluble and active diphtheria toxin proteins (including mutant forms, such as CRM197) and discloses improved processes for the expression and manufacture of bulk cultures of such toxins in bacterial hosts. Ex. 1004, 1:5-7, 2:4-18; Ex. 1003, ¶¶ 50-51. Blais discloses that representative examples of appropriate hosts include gram negative bacterial cells, such as *E. coli* and *Pseudomonas*. Ex. 1004, 12:51-54, 14:35-39. Example 1 describes the construction of periplasmic CRM197 expression vectors containing various signal sequences, and Example 3 describes the design of an optimized CRM197 expression vector that contains an FlgI signal sequence fused to the N-terminus of the CRM197-encoding

sequence. Ex. 1004, Examples 1 and 3; Ex. 1003, ¶ 52. Example 7 describes transforming BLR(DE3) *E. coli* cells with the construct produced in Example 3, culturing those transformed cells, and inducing production of CRM197 "at an easily detectable" level. Ex. 1004, 21:45-57; Ex. 1003, ¶ 52.

Example 8 describes pre-culturing the *E. coli* cells transformed with the FlgI-CRM197 construct for large-scale production. Ex. 1004, Example 8. Example 9 describes a detailed process for a 20-liter scale fermentation and production of CRM197, extracting soluble CRM197 from the periplasm of the *E. coli* host cells by osmotic shock, and measuring the yield of soluble and active CRM197 by ELISA. *Id.*, 22:15-24:12; 24:13-15; Ex. 1003, ¶ 53. Table 4 shows that periplasmic CRM197 expression reached yields of 3180 mg/L (3.18 g/L) and that cytoplasmic CRM197 expression reached yields of 394 mg/L (0.394 g/L). Ex. 1004, Table 4; Ex. 1003, ¶ 53. Blais concluded that its CRM197 production technique "demonstrated unprecedented levels of expression and efficiency of secretion." Ex. 1004, 24:20-21.

B. Squires

Squires, entitled "Heterologous Protein Production in *P. fluorescens*," is a scientific article that was published in December 2004. Ex. 1005, 54. Squires is prior art to the '171 patent under 35 U.S.C. § 102(b).

Squires describes *P. fluorescens* as a superior heterologous expression platform that is "amenable to genetic or molecular manipulations and can be

cultivated at high cell densities." Ex. 1005, 54; Ex. 1003, ¶ 54. Squires teaches that *P. fluorescens* "has many favorable properties" for "rapid, efficient expression of therapeutic molecules" and can "routinely achieve[]" high cell density and high-level protein production yield "in standard fermentation." Ex. 1005, 58; Ex. 1003, ¶ 56. Squires emphasizes that *P. fluorescens* "is unusually well suited for high-level expression and will tolerate a wide range of fermentation conditions" and that "[r]ecovery and downstream purification procedures are standard and consistent with those used for molecules expressed by *E. coli*." Ex. 1005, 58.

Squires discloses that, unlike *E. coli*, *P. fluorescens* can be cultivated to high densities using relatively simple conditions during fermentation and can produce heterologous protein biomass levels of greater than 100 g/L dry weight, which account for more than 50% of the total cell proteins. Ex. 1005, 54-55, 58; Ex. 1003, ¶ 54. Squires further emphasizes the advantages of periplasmic expression in *P. fluorescens* over cytoplasmic expression in *E. coli* and discloses that the native PbP *P. fluorescens* signal sequence "has been found effective at efficiently transporting" various disulfide-bonded heterologous proteins into the periplasm of *P. fluorescens* in their mature and properly folded forms. Ex. 1005, 55; Ex. 1003, ¶¶ 54-55.

Squires demonstrated successful expression of soluble and active heterologous disulfide-bonded proteins, including Gal2, interferon-γ, and hGH, in the periplasm (by using the Pbp signal sequence) and cytoplasm of *P. fluorescens* at

levels of 1.02 g/L to 5 g/L. Ex. 1005, 56, 58; Ex. 1003, ¶ 55. Squires concluded that "[h]igh-volumetric and specific expression of a range of therapeutic molecules as soluble, active, and secreted products makes *P. fluorescens* a compelling alternative for the microbial expression of biologicals for human health." *Id.*

C. Choi

Choi, entitled "Efficient secretory production of alkaline phosphate by high cell density culture of recombinant *Escherichia coli* using the *Bacillus* sp. endoxylanase signal sequence," is a scientific article published in June 2000. Ex. 1006, 640. Thus, Choi is prior art to the '171 patent under 35 U.S.C. § 102(b).

Choi describes using a *Bacillus* signal sequence for secretory production of soluble recombinant alkaline phosphatase in *E. coli* by high cell density cultivation. *Id.*, Abstract; Ex. 1003, ¶ 57. Choi discloses that whereas IPTG induction at low cell density (OD_{600}=50) led to formation of insoluble inclusion bodies, induction at high cell density (OD_{600}=150) produced soluble periplasmic alkaline phosphatase at concentrations of 5.2 g/L. Ex. 1006, 643-44; Ex. 1003, ¶ 58. Using a different *E. coli* strain, Choi also achieved periplasmic expression of soluble alkaline phosphatase at a level of 2.7 g/L when expression was induced at high cell density (OD_{600}=100). Ex. 1006, 644; Ex. 1003, ¶ 59.

Choi concluded that "IPTG induction at high cell density (OD_{600}=150) resulted in the formation of soluble proteins and a high efficiency of fractionation

(Fig. 4)," which "demonstrated that alkaline phosphatase could be efficiently secreted in high-cell- density fed-batch fermentation." Ex. 1006, Abstract, 645; Ex. 1003, ¶ 59.

D. Ramseier I

Ramseier I, entitled "Method for Rapidly Screening Microbial Hosts to Identify Certain Strains with Improved Yield And/or Quality in the Expression of Heterologous Proteins," is a U.S. Patent Application published on October 30, 2008. Ex. 1007, 1. Thus, Ramseier I is prior art to the '171 patent under 35 U.S.C. § 102(b).

Acknowledging that "the presence of specific host cell proteases may degrade the protein of interest and thus reduce the final yield" of recombinant proteins in a host cell, Ramseier I describes methods of generating "a population of *P. fluorescens* cells that has been genetically modified to reduce the expression of at least one target gene involved in protein degradation." Ex. 1007, [0008]; Ex. 1003, ¶ 60. Ramseier I discloses that the target gene in some embodiments is a protease selected from hslV, hslU, clpA, clpB and clpX. Ex. 1007, [0034]. Ramseier I further discloses that "[t]he modification can also be to more than one protease." *Id.*, [0049].

Example 5 of Ramseier I describes construction of *P. fluorescens* strains with genomic deletions of protease genes. *Id.*, Example 5; Ex. 1003, ¶ 61. Example 6 reported high-throughput growth of *P. fluorescens* strains that are defective in various proteases, including degP2 and prc1, for expression of the heterologous

protein Gal2. Ex. 1007, Example 6; Ex. 1003, ¶ 61. Ramseier I discloses that expression of Gal2 in strains including Δprcl and ΔdegP2 "were all 2.4-fold or more higher than the control strains, which was statistically significant ($p<0.5$)" and that fully assembled active Gal2 protein was detected in the soluble fraction of the Δprcl and ΔdegP2 strains but not in the control. Ex. 1007, [0211].

Ramseier I further discloses that expression vectors may include a polypeptide tag- encoding sequence adjacent to the coding sequence for the protein or polypeptide of interest to "facilitate[] identification, separation, purification, and/or isolation of an expressed polypeptide." *Id.*, [0124]; Ex. 1003, ¶ 62. Ramseier I also discloses optimizing the coding sequence of the heterologous proteins by synthesizing the gene of interest to reflect the codon use preferences of *P. fluorescens*. Ex. 1007, [0123], [0149]; Ex. 1003, ¶ 63.

E. Ramseier II

Ramseier II, entitled "Process for Improved Protein Expression by Strain Engineering," is a U.S. Patent Application published on May 25, 2006. Ex. 1008, 1. Thus, Ramseier II is prior art to the '171 patent under 35 U.S.C. § 102(b).

Ramseier II relates to "the field of protein production, and in particular [discloses] a process for improving the production levels of recombinant proteins or peptides or improving the level of active recombinant proteins or peptides expressed in host cells." *Id.*, [0002]. Ramseier II discloses that known "approaches have been

taken to avoid degradation during recombinant protein production," including the use of previously described *E. coli* host strains that are deficient in proteases DegP and Prc. *Id.*, [0012]; Ex. 1003, ¶ 64.

F. Maunsell

Maunsell, entitled "Complex regulation of AprA metalloprotease in *Pseudomonas fluorescens* M114: evidence for the involvement of iron, the ECF sigma factor, PbrA and pseudobactin M114 siderophore," is a scientific article that was published in January 2006. Ex. 1009, 29. Thus, Maunsell is prior art to the '171 patent under 35 U.S.C. § 102(b). Maunsell discloses that *aprA*, a serralysin-type metalloprotease gene, "was identified and found to encode the major, if not only, extracellular protease produced by [*P. fluorescens* strain M114]." Ex. 1009, Abstract, 39; Ex. 1003, ¶ 65.

VI. LEVEL OF ORDINARY SKILL IN THE ART

A POSA working in the field of the '171 patent in March 30, 2010, would have possessed a Ph.D. in Biology, Microbiology, Molecular Biology, or Genetic Engineering, with several years of post-doctoral research experience focused on molecular cloning, designing expression constructs for recombinant proteins, including diphtheria toxins, and expression of recombinant proteins in a bacterial host cell. Ex. 1003, ¶ 48. The POSA may have worked as a team member or through collaborations with others to develop or utilize molecular cloning techniques, or to

research potential therapeutic or diagnostic molecules for expression in bacterial systems.

VII. GROUND 1: CLAIMS 1-3, 7, 9, 11, 12, 15, 17, 21-24, 27, 29, 32, AND 33 WOULD HAVE BEEN OBVIOUS OVER BLAIS IN VIEW OF SQUIRES

Each element of claims 1-3, 7, 9, 11, 12, 15, 17, 21-24, 27, 29, 32, and 33 is taught by Blais and Squires. Blais describes processes for the expression and manufacture of bulk cultures of bacterial toxins, including CRM197, in Gram-negative bacterial hosts, including *Pseudomonas*. Ex. 1004, 1:5-9, 12:54, 14:39. Blais created expression vectors comprising a CRM197-encoding sequence fused to an FlgI signal sequence, and successfully used those vectors to achieve periplasmic expression of soluble and active CRM197 in *E. coli* at levels over 3 g/L, as measured by ELISA. Ex. 1004, Example 9, Table 4; Ex. 1003, ¶ 67. Thus, Blais establishes a reasonable expectation of successfully achieving high levels of periplasmic expression of CRM197 in bacterial host cells. Ex. 1003, ¶ 67.

Squires discloses the *P. fluorescens* bacterial strain as being "unusually well suited for high-level expression" of recombinant proteins, making it a "compelling alternative" to *E. coli*. Ex. 1005, 54-55, 58; Ex. 1003, ¶¶ 56, 80. Squires further discloses using a Pbp signal sequence to successfully produce various soluble and active disulfide-bonded recombinant proteins at high levels in the periplasm of *P. fluorescens*, and discloses the routineness and predictability of achieving

reproducibly high yields of recombinant protein expression through well-characterized fermentation techniques. Ex. 1005, 56, 58; Ex. 1003, ¶¶ 88-89.

Thus, in view of the prior art's teachings as a whole, a POSA would have been motivated to express CRM197 in *P. fluorescens* host cells using Blais's FlgI-CRM197 construct or using the Pbp signal sequence disclosed by Squires. Ex. 1003, ¶ 85. Furthermore, Blais's expression of more than 3 g/L of CRM197 in *E. coli*, and Squires's expression of various recombinant proteins at similar levels in *P. fluorescens* would have provided a reasonable expectation of successfully achieving expression of soluble and active CRM197 at levels of 0.2-12 g/L in *P. fluorescens*, as claimed in the '171 patent. *Id.*, ¶ 90.

A. Claim 1 Would Have Been Obvious Over Blais in View of Squires

Claim 1, the only independent claim of the '171 patent, recites a method for producing CRM197 in a *Pseudomonad* host cell by (1) ligating a nucleotide sequence encoding CRM197 into an expression vector, (2) transforming a *Pseudomonad* host cell with the expression vector, and (3) culturing the transformed *Pseudomonad* host cell to produce a yield of soluble or active CRM197 protein of about 0.2 grams per liter to about 12 grams per liter. '171 patent, 145:41-54; Ex. 1003, ¶ 66. As discussed below, Blais discloses all the elements of claim 1. Ex. 1003, ¶¶ 13, 67. And although Blais does not demonstrate actual production of CRM197 in a *Pseudomonad* host cell, a POSA would have been motivated to

improve CRM197 production by expressing Blais's FlgI-CRM197 construct in the *Pseudomonad* host cell disclosed in Squires, and would have had a reasonable expectation of success in arriving at the method recited in claim 1. *Id.*, ¶¶ 14-15, 67.

1. Blais Discloses the Expression of Soluble and Active CRM197 at the Claimed Levels in An *E. coli* Bacterial Host

Methods of producing CRM197 were known in the art prior to the alleged priority date of the '171 patent. Ex. 1004, 1:51; Ex. 1003, ¶ 22. Although the field initially experienced difficulties in producing CRM197, Blais provided an efficient solution to that problem by describing an improved process for making bacterial toxin proteins, such as CRM197, by transforming bacterial host cells with a vector encoding a signal sequence fused to the bacterial toxin such that the bacterial toxin is expressed periplasmically. Ex. 1004, 2:12-18, 13:43; Ex. 1003, ¶ 23. Blais demonstrates high-level expression of CRM197 in *E. coli*, and specifically discloses *Pseudomonas* as a representative example of another suitable host that could be used in the improved process. Ex. 1004, 12:54, 14:39, 24:18-21; Ex. 1003, ¶ 67. Thus, Blais discloses all of the limitations of claim 1 aside from actual production of CRM197 in a *Pseudomonad* host.

a. ligating into an expression vector a nucleotide sequence encoding CRM197

Example 3 and Figure 3 of Blais disclose how to ligate an FlgI signal sequence to a CRM197-encoding nucleotide sequence and then insert this FlgI-CRM197

fragment into a pRIT 16669 expression vector. Ex. 1004, 20:22-34, Figure 3; Ex. 1003, ¶¶ 68-69. Specifically, Blais discloses that "[a] region of DNA containing the FlgI signal sequence fused to the N-terminal part of the CRM197 sequence was amplified using standard PCR techniques." Ex. 1004, 20:25-26. Blais also discloses the sequences of the primers, (SEQ ID NOs:29 and 30), and the PCR conditions, and states that the amplified CRM197-encoding fragment "was inserted into plasmid pRIT 16669 using standard molecular biology techniques through digestion of the PCR product and plasmid pRIT 16669 with the restriction enzymes NdeI and AatII." *Id.*, 20:26-30, 20:32-34. Blais discloses that "[t]he resulting plasmid contains the complete mature N-terminus of CRM197 (SEQ ID NO:31) and the FlgI signal sequence terminating at the signalase binding site (SEQ ID NO:23) and was named pRIT 16681." *Id.*, 20:34-36. As such, Blais expressly discloses the "ligating" step of claim 1 of the '171 patent and the challenged dependent claims. Ex. 1003, ¶ 68.

b. transforming the Pseudomonad host cell with the expression vector

Example 3 of Blais discloses that the pRIT 16681 plasmid containing the FlgI signal sequence and CRM197-encoding sequence was "transformed into Novablue chemically competent cells" having a Novagen catalog number of 70181-3 (corresponding to a K-12 *E. coli* strain). Ex. 1004, 20:38-39. Blais confirmed the presence of the FlgI-CRM197 sequence in the plasmids by sequencing, and then

transformed the confirmed plasmids into B834(DE) chemically competent cells (Novagen Cat. 69041-3) for expression. *Id.*, 20:41-42, 22:1-5. Thus, Blais discloses the "transforming" step of claim 1, with the only difference being the bacterial host cell was *E. coli* instead of the recited *Pseudomonad* host cell. Ex. 1003, ¶¶ 70-71.

c. *culturing the transformed Pseudomonad host cell in a culture media suitable for the expression of CRM197*

In Example 5, Blais discloses culturing the transformed *E. coli* cells in LBT medium supplemented with 1% glucose, as described in Example 4, and inducing expression of CRM197 by addition of 1mM IPTG. Ex. 1004, 20:47-48, 21:10-15. Example 7 of Blais discloses that the "BLR(DE3) *E. coli* cells were transformed with the construct produced in Example 3, cultured and expression [was] induced as described in the optimised protocol of example 5." *Id.*, 21:46-47. For large scale production, Blais further prepared the transformed CRM197-expressing *E. coli* cells for fermentation and then cultured the cells under specified conditions, including pH, temperature, dissolved oxygen level, induction time, and IPTG concentration. *Id.*, Examples 8 and 9. Thus, Blais expressly discloses the "culturing" step of claim 1, with the only difference being the bacterial host cell was *E. coli* instead of the recited *Pseudomonad* host cell. Ex. 1003, ¶¶ 72-73.

d. wherein the recombinant protein is produced at a yield of soluble or active CRM197 protein of about 0.2 grams per liter to about 12 grams per liter

After culturing the transformed *E. coli* cells and inducing the production of CRM197, Blais used osmotic shock to extract CRM197 from the cells

. Ex. 1004, 24:13-14; Ex. 1003, ¶ 74. As Dr. Collier explains, the osmotic shock method disclosed in Blais collected the supernatant fraction of the host cell lysate, which contains only soluble proteins. Ex. 1003, ¶ 74; Ex. 1024, 212.

Blais then measured the amounts of CRM197 in the periplasmic and cytoplasmic fractions by ELISA, which revealed a yield of 3180 mg/L (3.180 g/L) and 394 mg/L (0.394 g/L), respectively. Ex. 1004, 24:15-19; Ex. 1003, ¶ 74. As Dr. Collier explains, because the soluble protein retained its ability to bind to anti-CRM197 antibodies in the ELISA, a POSA would have understood that Blais's CRM197 protein was active, as that term is used in the '171 patent. Ex. 1003, ¶¶ 75-76; '171 patent, 41:42-22 ("Activity assays include … antibody binding assays, e.g., … ELISA"); Ex. 1005, 58 (using ELISA to measure the activity of the soluble periplasmic Gal2 in *P. fluorescens* and reporting that the purified soluble protein "was found to be active").

Thus, whether assessing the periplasmic or cytoplasmic fractions (or adding the fractions together), the yields of soluble and active CRM197 protein obtained in Blais fall within the range of about 0.2 grams per liter to about 12 grams per liter

recited in claim 1. Ex. 1003, ¶ 74. In view of Blais's successful expression of soluble and active CRM197 in *E. coli*, a POSA would have had a reasonable expectation of achieving similar success with the other bacterial host strains described therein, including *Pseudomonas*. Ex. 1003, ¶ 77.

2. A POSA Would Have Been Motivated to Modify Blais Based on the Teachings of Squires

As discussed above, Blais discloses using *E. coli* as a host cell to perform every step recited in claim 1. However, as Dr. Collier explains and as Squires highlights, a POSA at the relevant time would have been strongly motivated to switch to *P. fluorescens* host cells. *PGS Geophysical AS v. Iancu*, 891 F.3d 1354, 1365 (Fed. Cir. 2018) ("The motivation to modify a reference can come from the knowledge of those skilled in the art, from the prior art reference itself, or from the nature of the problem to be solved."); *see In re Urbanski*, 809 F.3d 1237, 1244 (Fed. Cir. 2016) (finding a claimed method obvious when the prior art provided the motivation to modify and suggested the desirability of such modification); Ex. 1003, ¶¶ 78, 85.

As exemplified by the prior art, *P. fluorescens* was developed and used as a powerful manufacturing platform for high-yield production of recombinant proteins years before the earliest possible priority date of the '171 patent. Ex. 1015, 46, 52; Ex. 1003, ¶ 79. As Dr. Collier explains, *P. fluorescens* was known to be particularly

suitable for high-yield production of therapeutic proteins because it was known to have a robust protein production capacity. *Id.*, ¶ 79; Ex. 1015, 46. In addition to being stable and amenable to genetic and molecular manipulations, *P. fluorescens* strains were also known to be superior to *E. coli* in that they could "be cultivated to high cell densities." Ex. 1015, 52; Ex. 1003, ¶¶ 26, 79. Extensive molecular biological tools were available to engineer and optimize *P. fluorescens* strains for recombinant protein expression, and production conditions had been optimized for expressing recombinant proteins in *P. fluorescens* in 20-liter fermenters, routinely generating yields above 25 g/L for a variety of protein types. Ex. 1003, ¶¶ 27-28, 79; Ex. 1015, 45, 46.

Indeed, Squires expressly teaches that *P. fluorescens* "has many favorable properties" for "rapid, efficient" and "higher-volume" production of therapeutic molecules because this bacterial strain can be cultivated to high densities in bioreactors using simple culturing medium and conditions. Ex. 1005, 54, 58; Ex. 1003, ¶ 80. Squires's emphasizes multiple advantages of *P. fluorescens* over *E. coli* as a high-yield bacterial host for recombinant protein production, including no accumulation of undesired acetate during fermentation. Ex. 1005, 55; Ex. 1003, ¶ 80. Squires concludes that *P. fluorescens* "is ***unusually well suited for high-level expression*** and will tolerate a wide range of fermentation conditions." Ex. 1005, 58 (emphasis added).

Squires compares production of various heterologous proteins in *E. coli* and *P. fluorescens* and demonstrates that problems associated with *E. coli* expression can be overcome by using *P. fluorescens*. Ex. 1003, ¶ 80. For example, Squires reports that whereas interferon-γ produced from *E. coli* was insoluble and inactive, *P. fluorescens* successfully produced interferon-γ in a soluble form, with a total yield of about 4 g/L. Ex. 1005, 58; Ex. 1003, ¶ 81. Squires concludes that "[t]he increased overall yield and dramatically improved expression of soluble and active protein seen in *P. fluorescens* ***clearly highlights the advantages*** it can offer in producing recombinant proteins." Ex. 1005, 58 (emphasis added).

Squires further demonstrates that whereas the recombinant disulfide-bonded human growth hormone (hGH) formed insoluble inclusion bodies when expressed in *E. coli*, fusing a Pbp signal sequence to hGH achieved more than 5 g/L of soluble hGH in the periplasm of *P. fluorescens* that was properly processed and active. Ex. 1005, 55; Ex. 1003, ¶ 83. Squires also discloses that when the Pbp signal sequence was fused to an anti-β-galactosidase single chain antibody, Gal2, a properly-processed Gal2 protein was expressed at levels of 3 g/L in *P. fluorescens*, with 34% of the protein being soluble and active in an anti-β-galactosidase ELISA. Ex. 1005, 56, 58. Thus, Squires achieved about 1 g/L of soluble, fully processed, and active Gal2. Ex. 1003, ¶ 82. Based on those successful results, Squires concluded that "[h]igh-volumetric and specific expression of a range of therapeutic

molecules as ***soluble, active, and secreted*** products makes *P. fluorescens* a ***compelling alternative*** for the microbial expression of biologicals for human health." Ex. 1005, 58. (emphasis added); Ex. 1003, ¶ 84.

In view of the express conclusion in Squires that *P. fluorescens* was "unusually well-suited" for high level protein production and "a compelling alternative" to *E. coli*, combined with Blais's identification of *Pseudomonas* as a suitable host for improved CRM197 production, a POSA would have been motivated to express Blais's FlgI-CRM197 construct in *P. fluorescens* for high level expression of soluble and active CRM197. Ex. 1003, ¶ 85. *See Acorda Therapeutics, Inc. v. Roxane Labs., Inc.*, 903 F.3d 1310, 1331 (Fed. Cir. 2018) (finding a motivation to pursue a claimed method when the prior art taught the desirability of the claimed method).

3. The Combination of Blais and Squires Would Have Provided A Reasonable Expectation of Achieving the Claimed Method

Claim 1 requires a "yield of soluble or active CRM197 protein of about 0.2 grams per liter to about 12 grams per liter." Blais's successful production in the *E. coli* periplasm of over 3 g/L of soluble and active CRM197, combined with Squires's demonstration of the superiority of *P. fluorescens* over *E. coli* in achieving high-level expression of multiple soluble and active proteins, would have provided a reasonable expectation of success in achieving expression of soluble and active

CRM197 at levels of about 0.2 to about 12 g/L, as recited in the claims of the '171 patent. *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1364 (Fed. Cir, 2007) (finding it obvious to substitute amlodipine maleate with amlodipine besylate when prior art taught superiority of amlodipine besylate and expectation that besylate salt would show improved physicochemical characteristics over maleate salt); Ex. 1003, ¶¶ 86, 90.

As Blais explains, once the expression construct is built, recombinant CRM197 protein "may be prepared by processes well known to those skilled in the art from genetically engineered host cells comprising expression systems." Ex. 1004, 12:28-30. Blais also acknowledges that methods for introducing CRM197-encoding constructs into host cells had been described in many standard laboratory manuals. *Id.*, 12:44-50 (citing laboratory manuals published in the 1980s). Thus, it would have been routine for a POSA to introduce Blais's FlgI-CRM197 construct into the *P. fluorescens* host cell. Ex. 1003, ¶ 87.

As Dr. Collier explains, it was common knowledge as of 2010 that soluble and active protein yields greater than 25 g/L had been achieved for a variety of recombinant proteins using *P. fluorescens* as the bacterial host. Ex. 1003, ¶ 89. In 2004, production conditions in *P. fluorescens* had been "routinely optimized in 20-L fermentors" and successfully scaled up for commercial production of various heterologous proteins. Ex. 1015, 47; Ex. 1016, 1; Ex. 1003, ¶¶ 27-28

Squires similarly taught that its fed-batch fermentation process "is well characterized, and scale-up is predictable and rapid to thousands of liters." Ex. 1005, 58. Squires emphasized that *P. fluorescens* "will tolerate a wide range of fermentation conditions" and "[r]ecovery and downstream purification procedures are standard and consistent with those used for molecules expressed by *E. coli*." *Id.*, 58; Ex. 1003, ¶ 88. Thus, achieving the claimed high-level production of CRM197 by expressing Blais's construct in *P. fluorescens* would have involved no more than routine optimization and, therefore, would have been obvious. *Senju Pharm. Co. v. Lupin Ltd.*, 780 F.3d 1337, 1353 (Fed. Cir. 2015) (invalidating a claim that was directed to "a product of routine optimization that would have been obvious to one of skill in the art.").

Indeed, Squires discloses actual successful expression of multiple soluble and active recombinant proteins at levels of 1-5 g/L, which is well within the range recited in the claims of the '171 patent. Ex. 1005, 56, 58. Such successes would have further boosted the confidence of those skilled in the art to achieve high-level expression of soluble and active CRM197 in *P. fluorescens* by using the construct described in Blais, which had already produced over 3 g/L of soluble and active CRM197 in *E. coli*. Ex. 1003, ¶ 89.

Accordingly, a POSA considering the teachings of Blais and Squires, alone or in combination, would have had a strong motivation to express Blais's FlgI-

CRM197 construct in a *P. fluorescens* host cell with a reasonable expectation of achieving soluble and active CRM197 with a yield of about 0.2 to about 12 g/L. *See Agrizap, Inc. v. Woodstream Corp.*, 520 F.3d 1337, 1344 (Fed. Cir. 2008) (finding asserted claims obvious when they simply substituted a resistive electrical switch for a prior art mechanical pressure switch known to solve the same problem); *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988) (holding that it would have been obvious to replace a prior art gene with another gene known to lead to protein production, because one of ordinary skill in the art would have been able to carry out such a substitution, and the results were reasonably predictable.); Ex. 1003, ¶¶ 86, 90 For at least these reasons, claim 1 would have been unpatentable as obvious over Blais in view of Squires as of the earliest possible priority date of the '171 patent.

B. Claim 21 Would Have Been Obvious

Whereas claim 1 recites producing soluble ***or*** active CRM197, dependent claim 21 recites producing soluble ***and*** active CRM197. *Compare* '171 patent 145:53 *with* 148:12-15. As discussed above, Blais discloses production of soluble and active CRM197 with a yield of 3.18 g/L. *Supra*, §VII.A.1.d; Ex. 1003, ¶¶ 91-92. Squires discloses expression of various soluble and active recombinant proteins in *P. fluorescens* at yield levels of 1-5 g/L. *Supra*, §VII.A.3; Ex. 1003, ¶ 93. Thus, as discussed above, the teachings of Blais and Squires would have provided a strong motivation to express Blais's FlgI-CRM197 construct in a *P. fluorescens* host cell,

with a reasonable expectation of achieving soluble and active CRM197 at a yield of 0.2-12 g/L, thereby rendering claim 21 unpatentable as obvious under 35 U.S.C. § 103 for the same reasons as discussed above for claim 1. *Supra*, §VII.A; Ex. 1003, ¶ 93.

C. Claims 2 and 22 Would Have Been Obvious

As explained above in Section III.A., claims 2 and 22 recite ranges of yields that entirely overlap with the ranges recited in claims 1 and 21. Thus, claims 2 and 22 would have been obvious for at least the reasons discussed above for claims 1 and 21. Specifically, Blais discloses expressing CRM197 in the periplasm of *E. coli* at levels over 3 g/L, and Squires discloses expressing various recombinant proteins in the periplasm of *P. fluorescens* at levels of 1-5 g/L. *Supra*, §VII.A.3. Thus, the teachings of Blais and Squires would have provided a strong motivation to express Blais's FlgI-CRM197 construct in a *P. fluorescens* host with a reasonable expectation of achieving soluble and active CRM197 with a yield of about 0.2 g/L to about 12 g/L, thereby rendering claims 2 and 22 unpatentable as obvious under 35 U.S.C. § 103; Ex. 1003, ¶¶ 16, 97.

D. Claims 3, 7, 9, 23, 24, 27, and 29 Would Have Been Obvious

Claims 3 and 23, which depend from claims 1 and 21, respectively, recite that the CRM197-encoding sequence is fused to a secretion signal coding sequence that directs transfer of CRM197 to the periplasm when expressed. These claims would

have been obvious over Blais in view of Squires as of the alleged priority date of the '171 patent. Ex. 1003, ¶¶ 16, 98, 104.

As discussed above, Blais expressly discloses fusing a CRM197-encoding sequence to an FlgI signal sequence, ligating the FlgI-CRM197 fragment into an expression vector, transforming *E. coli* cells with the CRM197 expression vector, and achieving periplasmic expression of soluble and active CRM197 at a level of 3.18 g/L. *Supra*, VII.A.1; Ex. 1003, ¶ 99. Squires encouraged expressing recombinant proteins in the periplasm of *P. fluorescens*, stating that disulfide-bonded proteins, like CRM197, "should be secreted at least to the periplasmic space" to facilitate the proper formation of the disulfide bonds, consistent with the common knowledge of those skilled in the art at the time. Ex. 1005, 55; Ex. 1003, ¶¶ 33-36, 100. Squires also discloses that expression in the periplasm of *P. fluorescens* has the advantage of producing proteins "in a soluble and active form," unlike cytoplasmic expression in *E. coli.* Ex. 1005, 58. Thus, Blais and Squires would have motivated a POSA to use Blais's FlgI-CRM197 construct to express soluble and active CRM197 in the periplasm of *P. fluorescens*. Ex. 1003, ¶ 100.

The combination of Blais and Squires also would have provided a reasonable expectation of success in expressing soluble and active CRM197 in the periplasm of *P. fluorescens* at levels within the claimed ranges. Ex. 1003, ¶ 101. As discussed above, Blais discloses achieving periplasmic expression of soluble and active

CRM197 at levels over 3 g/L from its FlgI-CRM197 construct. *Supra*, VII.A.1. Squires further highlighted the routineness and predictability of achieving high-level periplasmic production in *P. fluorescens*. Ex. 1003, ¶ 101. Particularly, Squires taught that several recombinant proteins had been successfully secreted to the periplasm of *P. fluorescens* using signal sequences, which "led to discovery of disulfide-bonded proteins in their native, active conformations." Ex. 1005, 55; Ex. 1003, ¶ 103. Squires further acknowledged that Blais's osmotic shock technique effectively releases periplasmic proteins at "commercially relevant scales." Ex. 1005, 55. As explained above in Section VII.A.3., Squires additionally discloses using a signal sequence to express soluble and active hGH and Gal2 at levels of 1-5 g/L in the periplasm of *P. fluorescens*. Thus, the teachings of Blais and Squires would have rendered claims 3 and 23 unpatentable as obvious under 35 U.S.C. § 103. Ex. 1003, ¶ 101.

Dependent claims 7, 9, 24, 27, and 29 further recite that the secretion leader is Azu, Pbp, IbpS31A, CupA2, or PbpA20V. Ex. 1003, ¶ 102. As the '171 patent admits, Pbp was a well-known native signal sequence from *P. fluorescens*. '171 patent, 42:53-55, Table 8. Squires explicitly discloses that the *P. fluorescens* Pbp signal sequence "in particular has been found effective at efficiently transporting" various recombinant proteins into the periplasm of *P. fluorescens* in their "native and active conformations." Ex. 1005, 55; Ex. 1003, ¶ 103. Thus, Squires would

have motivated a POSA to improve CRM197 production in *P. fluorescens* by substituting the *E. coli* FlgI signal sequence used in Blais's construct with the *P. fluorescens* Pbp signal sequence that had already been shown to be effective for guiding periplasmic expression of soluble and active proteins in *P. fluorescens*, with a reasonable expectation of likewise achieving successful periplasmic expression of CRM197. Ex. 1003, ¶ 104.

Accordingly, claims 7, 9, 24, 27, and 29 would have been obvious over Blais in view of Squires. *Allergan, Inc. v. Apotex Inc.*, 754 F.3d 952, 963 (Fed. Cir. 2014) (the claimed genus is obvious when any of the compounds in the genus were obvious at the time of the invention).

E. Claims 15, 17, 32, and 33 Would Have Been Obvious

Claims 15 and 32, which depend from claims 1 and 21, respectively, specify a *Pseudomonas* cell. Claims 17 and 33, which depend from claims 1 and 21, respectively, specify a *Pseudomonas fluorescens* cell. As discussed above, Blais identified *Pseudomonas* as an appropriate host for the expression of CRM197, and Squires discussed successful examples of high-level protein expression in *Pseudomonas fluorescens*. *Supra*, VII.A. Thus, the teachings of Blais and Squires would have provided a strong motivation to express Blais's FlgI-CRM197 construct in a *P. fluorescens* host with a reasonable expectation of achieving soluble and active

CRM197 with a yield of 0.2-12 g/L, thereby rendering claims 15, 17, 32, and 33 obvious. *Supra*, VII.A.; Ex. 1003, ¶¶ 16, 105; *Allergan*, 754 F.3d at 963.

F. Claims 11 and 12 Would Have Been Obvious

Claim 11 recites measuring the activity of CRM197 in an activity assay, wherein about 40% to about 100% of the soluble toxin protein produced is determined to be active. Claim 12 depends from claim 11 and further recites using an activity assay such as an immunological assay. Ex. 1003, ¶ 94. The '171 patent explains that the term "soluble and/or active" "refers to protein that is determined to be soluble, active, or both soluble and active, by methods known to those of skill in the art and described herein." '171 patent, 40:31-34. The '171 patent further explains that "[t]he 'activity' of a given protein can include binding activity, e.g., that represented by binding to a receptor, a specific antibody, or to another known substrate, or by enzymatic activity if relevant." *Id.*, 40:34-37.

The '171 patent acknowledges that "[a]ctivity assays for evaluating toxins are known in the art and described in the literature," which "include immunological or antibody binding assays, e.g., Western Blot analysis and ELISA…." *Id.*, 40:41-44. As Dr. Collier explains, an ELISA measures activity of a protein by detecting its ability to bind to its antibody. Ex. 1003, ¶ 95. Because only a properly folded and soluble protein can bind to its antibody, a POSA would consider it more likely than not that the protein detected in an ELISA is properly folded and soluble. *Id.*

Blais used osmotic shock to release soluble CRM197 from the periplasmic fraction, and measured the activity of CRM197 in an ELISA to determine "periplasmic CRM197 productivity." Ex. 1004, 24:13-15, 24:19. As Dr. Collier explains, a POSA would have understood that the CRM197 detected in Blais's ELISA is representative of the total soluble and active CRM197 produced in the *E. Coli* cells. Ex. 1003, ¶ 95. Indeed, Squires similarly used an ELISA to measure the activity of the soluble Gal2 protein expressed in the periplasm of *P. fluorescens* and reported that the purified soluble protein "was found to be active" in the ELISA. Ex. 1005, 58.

Thus, a POSA would have been motivated to produce soluble and active CRM197 in *P. fluorescens* and use an ELISA to measure its activity, as recited in claims 11 and 12. And because Blais's ELISA demonstrated that the CRM197 protein produced therein was soluble and active, a POSA would have had a reasonable expectation of success in determining that about 40% to about 100% of the soluble protein produced by expressing Blais's CRM197 construct in *P. fluroescens* is active, as recited in claims 11 and 12. Ex. 1003, ¶¶ 16, 94, 96. Accordingly, claims 11 and 12 would have been obvious over Blais in view of Squires.

VIII. GROUND 2: CLAIMS 13, 14, 30, AND 31 WOULD HAVE BEEN OBVIOUS OVER BLAIS IN VIEW OF SQUIRES AND CHOI

Claims 13 and 30, which depend from claims 1 and 21, respectively, recite that the expression vector comprises a lac promoter operatively linked to the CRM197 protein coding sequence, and that the culturing comprises induction using IPTG at a concentration of about 0.02 to about 1.0 mM, the cell density at induction is an optical density of about 40 to about 200 absorbance units (AU), the pH of the culture is from about 6 to about 7.5, and the growth temperature is about 20 to about 35° C. Claims 14 and 31 depend from claims 13 and 30, respectively, and recite a lac promoter selected from tac, trc, Ptac16, Ptac17, PtacII, PlacUV5 and T7lac. These claims would have been obvious in view of Blais, Squires, and Choi. Ex. 1003, ¶¶ 16, 106, 115.

Blais discloses generating a FlgI-CRM197 construct in Example 3 and illustrated the cloning process in Figure 3. Ex. 1004, Example 3, Fig. 3. Figure 3 of Blais shows that the expression vector has a lac promoter. *Id.*; Ex. 1003, ¶ 107. As discussed in Section VIII.A.1.d, Example 9 of Blais discloses that after the *E. coli* cells were transformed by this FlgI-CRM197 expression vector, high-level production of soluble and active CRM197 was achieved. Ex. 1004, Example 9. Similarly, Squires discloses that " transcription promoters of varying strengths, such as the *tac* and *lac*UV5 promoters derived from *E. coli* sequences" can be used to

drive expression of various recombinant proteins in *P. fluorescens*. Ex. 1005, 55. Thus, a POSA would have been motivated to use the lac promoter in Blais's FlgI-CRM197 expression vector to achieve high-level expression of CRM197 in *P. fluorescens*, with a reasonable expectation of success. Ex. 1003, ¶¶ 106, 112.

Example 9 of Blais further discloses that during the CRM197 production stage, "the pH of the medium was readjusted to 6.8" and "maintained at 6.8," which is within the pH range recited in claims 13 and 30 of "about 6 to about 7.5." Ex. 1004, 22:17, 24:3; Ex. 1003, ¶ 108. Blais also discloses that IPTG "was added to a final concentration of 1 mM to induce the bacteria." Ex. 1004, 24:6. Thus, the IPTG concentration used in Blais was also within the range recited in claims 13 and 30 of "about 0.02 to about 1.0 mM." Ex. 1003, ¶ 108. In addition, Blais discloses that the growth temperature during CRM197 production "was regulated at 28° C" and "decreased to 23° C" upon induction, which is also within the range recited in claims 13 and 30 of "about 20° C to about 35° C." Ex. 1004, 24:3-4, 24:6-8; Ex. 1003, ¶ 108. Because Blais's production conditions including pH, temperature, and IPTG concentration successfully produced high yields of soluble and active CRM197, those skilled in the art would have been motivated to use the same promoter and similar conditions in *P. fluorescens*. Ex. 1003, ¶ 108. And because Squires taught that growth conditions suitable for *E. coli* are consistent with those for *P. fluorescens* and should be tolerated by *P. fluorescens*, a POSA would have had a reasonable

expectation of success in achieving high-level production of CRM197 using Blais's construct and induction conditions in *P. fluorescens*. Ex. 1005, 58 (*P. fluorescens* "is unusually well suited for high-level expression and will tolerate a wide range of fermentation conditions. Recovery and downstream purification procedures are standard and consistent with those used for molecules expressed by *E. coli*."); Ex. 1003, ¶¶ 112-113.

The recited cell density at induction of an optical density of about 40 to about 200 absorbance units (AU) also would have been obvious. Ex. 1003, ¶¶ 109-111. Specifically, Choi taught efficient periplasmic production of alkaline phosphatase by high cell density culture of recombinant *E. coli*, noting that when the cells were induced at a density (OD_{600}=50) close to the lower end of the range recited in claims 13 and 30, the alkaline phosphatase formed inclusion bodies in the periplasm, but when the cells were induced at a density (OD_{600}=150) closer to the upper limit of the range recited in claims 13 and 30, most of the alkaline phosphatase secreted into the periplasm was soluble. Ex. 1006, Abstract, 243-244; Ex. 1003, ¶¶ 58, 109-110. Choi concludes that "IPTG induction at high cell density (OD_{600}=150) resulted in the formation of soluble proteins and a high efficiency of fractionation." Ex. 1006, 645. According to Choi, "[t]hese results demonstrate the possibility of efficient secretory production of recombinant proteins in *E. coli* by high cell density cultivation." *Id.*, Abstract.

Choi's teachings would have motivated a POSA to perform IPTG induction at a high cell density within the claimed range to achieve high-level production of soluble and active proteins in bacterial host cells. Ex. 1003, ¶ 111. And Squires's disclosure that *P. fluorescens* "can be cultivated at high cell densities" would have provided a reasonable expectation of success in achieving high-level expression of CRM197 in *P. fluorescens* by using Blais's construct and induction conditions, as modified by Choi. Ex. 1005, 54 (noting that *P. fluorescens* is a superior recombinant protein expression platform because it is "amenable to genetic or molecular manipulations and can be cultivated at high cell densities."), 55 ("As with other *Pseudomonads*, *P. fluorescens* can be cultivated to high densities"); Ex. 1003, ¶¶ 106, 114-115.

Accordingly, claims 13, 14, 30, and 31 would have been obvious over Blais in view of Squires and Choi. *Alcon Research, Ltd. v. Apotex Inc.*, 687 F.3d 1362, 1368 (Fed. Cir. 2012) ("if prior art discloses a portion of the claimed range, the entire claim is invalid.")

IX. GROUND 3: CLAIMS 4, 5, 8, 10, 16, 18-20, 25, 28, AND 34-36 WOULD HAVE BEEN OBVIOUS OVER BLAIS IN VIEW OF SQUIRES AND RAMSEIER I

Claims 4, 5, 8, 10, 16, 18-20, 25, 28, and 34-36, which all depend directly or indirectly from claim 1, recite additional elements such a tag sequence adjacent to the coding sequence for the secretion signal, a host cell being defective in the

expression of at least one protease, or an optimized CRM197-encoding sequence. All of these additional limitations would have been obvious over Blais in view of Squires and Ramseier I. Ex. 1003, ¶¶ 16, 116-132.

A. Claims 4, 10, and 20 Would Have Been Obvious

Claims 4, 10, and 20 recite that the expression vector further comprises a tag sequence adjacent to the coding sequence for the secretion signal. This additional feature would have been obvious as of the alleged priority date of the '171 patent. Ex. 1003, ¶ 116.

As discussed above in Section VIII,A., it would have been obvious to improve expression of CRM197 from Blais's FlgI-CRM197 construct by using Squires's *P. fluorescens* host cells to achieve the claimed yield of soluble and active CRM197 in *P. fluorescens*. Ramseier I, which relates to identifying host cells for large-scale production of recombinant proteins including CRM197, discloses that "heterologous protein or polypeptide can be expressed in a manner in which it is linked to a tag protein and the 'tagged' protein can be purified from the cell." Ex. 1007, [0092]. Ramseier I further discloses that the expression vector may include additional elements including "tag sequences, such as nucleotide sequence 'tags' and 'tag' polypeptide coding sequences, which facilitates identification, separation, purification, and/or isolation of an expressed polypeptide." *Id.*, [0124]; Ex. 1003¶117. According to Ramsier I, in some embodiments:

> [T]he expression vector further comprises a tag sequence adjacent to the coding sequence for the protein or polypeptide of interest. In one embodiment, this tag sequence allows for purification of the protein. The tag sequence can be an affinity tag, such as a hexa-histidine affinity tag…a glutathione-5-transferase molecule…[or] a fluorescent molecule, such as YFP or GFP, or analogs of such fluorescent proteins.

Id., [0125]. Ramseier I's disclosures reflect the common understanding at the time of the alleged invention that it would have been both desirable and routine to add a tag sequence adjacent to the coding sequence of a heterologous protein for expression in a host cell. Ex. 1003, ¶ 118.

Thus, a POSA would have been motivated by Ramseier I's teachings to add a tag sequence to Blais's successful CRM197 expression vector with a reasonable expectation of arriving at the methods recited in claims 4, 10, and 20. Ex. 1003, ¶ 119. Accordingly, claims 4, 10, and 20 would have been obvious over Blais in view of Squires and Ramseier I.

B. Claims 5, 8, 25 and 28 Would Have Been Obvious

Claims 5 and 25, which depend from claims 1 and 21, respectively, recite the use of a host cell that is defective in the expression of at least one protease or a host cell that overexpresses at least one folding modulator, or a combination thereof.

Claims 8 and 28, which also depend from claims 1 and 21, respectively, recite a host cell that is defective in the expression of a protease that is Serralysin, HslU, HslV, Prc1, DegP1, DegP2, or AprA, or a combination thereof, or wherein the host cell overexpresses folding modulators DsbA, DsbB, DsbC, and DsbD, and further wherein the recombinant toxin protein is fused to the Azu, Pbp, or native secretion leader. These claims also would have been obvious over Blais, Squires, and Ramseier I. Ex. 1003, ¶¶ 120-127.

As discussed above in Section VII.A., it would have been obvious to improve CRM197 production by expressing the Blais FlgI-CRM197 construct in Squires's *P. fluorescens* host cells and achieve the claimed yield of soluble and active CRM197. Moreover, as Dr. Collier explained, protease-deficient bacterial host strains had been routinely generated to optimize recombinant protein production decades before March 30, 2010. Ex. 1003, ¶¶ 37-40, 121; Ex. 1021, 81 (reporting in 1998 that the use of protease-deficient strains had been "shown to be a successful approach to improve the yield of fully active, expressed proteins"), 1; Ex. 1020, Abstract, Ex. 1008, [0012]. Indeed, Blais successfully used a protease-deficient *E. coli* strain, B834(DE), to achieve high-level expression of soluble and active CRM197 from its FlgI-CRM197 construct. Ex. 1004, 22:1-5; Ex. 1027, 662, Table 3; Ex. 1003, ¶ 122.

A POSA would have been motived to likewise reduce or eliminate the activity of one or more proteases in the *P. fluorescens* strains disclosed in Squires to achieve improved yield of soluble and active CRM197 based on Ramsier I's disclosure of various protease-deficient *P. fluorescens* strains. Ex. 1003, ¶¶ 122-123. Specifically, Ramsier I acknowledges that "the presence of specific host cell proteases may degrade the protein of interest and thus reduce the final yield." Ex. 1007, [0008]. Ramseier I identifies exemplary protein folding modulators in *P. fluorescens* in Table 1 and exemplary *P. fluorescens* proteases in Table 2, and "provides an array for rapidly identifying a host cell population capable of producing a heterologous protein with improved yield and/or quality." Ex. 1007, Abstract, [0033], [0034], Tables 1 and 2. Using this array, Ramseier I generated populations of *P. fluorescens* cells that have been genetically modified to reduce the expression of at least one target gene involved in protein degradation and/or to increase the expression of at least one target gene involved in protein production. Ex. 1007, [0024]; Ex. 1003, ¶¶ 61, 123.

Ramseier I provided further guidance on generating protease-deficient *P. fluorescens* strains, and demonstrated that reducing the activity of proteases or overexpressing protein folding modulators in *P. fluorescens* cells improved the yield and quality of various heterologous proteins. Ex. 1007, Abstract; Ex. 1003, ¶ 124. In Example 5, Ramseier I describes the construction of various *P. fluorescens* strains

that have genomic deletions of different protease genes. Ex. 1007, Example 5. Example 6 of Ramseier I describes expressing a recombinant protein, Gal2, in sixty-three different *P. fluorescens* strains "carrying either a directed gene deletion or pDOW2247 carrying a folding modulator for co-expression," and reports that expression of soluble Gal2 in multiple protease-deficient strains (including Δprcl, ΔdegP2, ΔLa2, ΔclpP, Δprc2, Δtig, ΔclpX, and Δlon strains) and in the grpEdnaKJ co-expression strain "were all 2.4-fold or more higher than the control strains" that express wild type proteases and that the increase in expression was statistically significant. *Id.* [0211]

As Dr. Collier explained, Blais's successful production of soluble and active CRM197 in a protease-deficient *E. coli* host and Ramseier I's improved yield of various heterologous proteins in protease-deficient *P. fluorescens* strains would have provided a reasonable expectation of achieving improved yield of soluble and active CRM197 in a protease-deficient *P. fluorescens* strain, as recited in claims 5 and 25. Ex. 1003, ¶ 125. Accordingly, claims 5 and 25 would have been obvious over Blais in view of Squires and Ramseier I.

As discussed in Section VII.D., it would have been obvious to improve CRM197 periplasmic production in *P. fluorescens* by substituting the FlgI signal sequence in the Blais FlgI-CRM197 construct with the *P. fluorescens* Pbp signal sequence disclosed in Squires. And as explained above, in view of Ramseier I's

success in improving the yield of multiple recombinant proteins by expressing those proteins in protease-deficient *P. fluorescens* strains, including a ΔdegP2 strain, a POSA would have been motivated to further improve CRM197 yields by expressing a Pbp-CRM197 construct in the periplasm of a *P. fluorescens* host cell that is defective in at least the expression of DegP2, with a reasonable expectation of successfully achieving improved yields of about 0.2 to about 12 g/L, as recited in claims 8 and 28. Ex. 1003, ¶¶ 126-127.

Accordingly, claims 5, 8, 25 and 28 would have been obvious over Blais in view of Squires and Ramseier I as of the alleged priority date of the '171 patent.

C. Claims 16, 18, 19, and 34-36 Would Have Been Obvious

Claims 16, 18, 19, and 34-36, which depend from claims 1 and 21 directly or indirectly, recite an optimized nucleotide sequence for expressing CRM197 in the host cell. These claims also would have been obvious over Blais in view of Squires and Ramsier I. Ex. 1003, ¶ 128.

As discussed above in Section VII.A., it would have been obvious to transform Squires's *P. fluorescens* host cells with Blais's FlgI-CRM197 construct to achieve the claimed yield of soluble and active CRM197 in *P. fluorescens*. Ramseier I, which relates to improving the expression of recombinant proteins in modified *P. fluorescens* strains, discloses that the coding sequence for the to-be-expressed recombinant protein "will more preferably be a coding sequence that has been

selected, improved, or optimized for use in an expressible form in the strains of the array: for example, by optimizing the gene to reflect the codon use bias of a *Pseudomonas* species such as *P. fluorescens*." Ex. 1007, [0149]; Ex. 1003, ¶ 129. Ramseier I then provides specific guidance on how to optimize the codons of a recombinant protein, stating that

> For gene optimization, one or more rare codons may be removed to avoid ribosomal stalling and minimize amino acid misincorporation. One or more gene-internal ribosome binding sites may also be eliminated to avoid truncated protein products. Long stretches of C and G nucleotides may be removed to avoid RNA polymerase slippage that could result in frame-shifts. Strong gene internal stem-loop structures, especially the ones covering the ribosome binding site, may also be eliminated.

Ex. 1007, [0149]; Ex. 1003, ¶ 131.

As Dr. Collier explained, codon optimization for protein expression in *P. fluorescens* had been routine as of 2010. Ex. 1003, ¶¶ 41-42, 130, 132. Multiple tools, including various software programs, had been designed and were available to researchers for generating codon-optimized sequences for any gene based on knowledge of highly expressed genes in the host of interest. Ex. 1022, 247; Ex. 1023, [0045]-[0047], [0055], [0059]; Ex. 1003, ¶ 132. And as the '171 patent admits, those skilled in the art had published and claimed methods of codon optimization and

heterologous expression in *P. fluorescens* years before the patent's priority date. '171 patent, 16:67-17:6 (citing Ex. 1023); Ex. 1023, Abstract, claim 36; Ex. 1003, ¶ 132.

In view of this common knowledge and Ramseier I's teachings, a POSA seeking to improve CRM197 expression in *P. fluorescens* would have been motivated to optimize the CRM197-coding sequence in Blais's construct to adapt to the codon use bias of *P. fluorescens* and improve expression with a reasonable expectation of success. Ex. 1003, ¶¶ 130, 132.

Accordingly, claims 16, 18, 19, and 34-36 would have been obvious over Blais in view of Squires and Ramseier I as of the alleged priority date of the '171 patent.

X. GROUND 4: CLAIMS 6 AND 26 WOULD HAVE BEEN OBVIOUS OVER BLAIS IN VIEW OF SQUIRES, RAMSEIER I, RAMSEIER II, AND MAUNSELL

Claims 6 and 26, which depend from claims 1 and 21, respectively, recite a host cell that is defective in the expression of the proteases HslU, HslV, Prc1, DegP1, DegP2, and AprA. These claims would have been obvious over Blais in view of Squires, Ramseier I, Ramseier II, and Maunsell. Ex. 1003, ¶¶ 16, 133, 141.

As explained above in Section IX.B., Ramseier I provided express guidance on generating protease-deficient *P. fluorescens* strains for improved expression of recombinant proteins. Ramseier I discloses that because proteases can negatively

affect protein yield and/or quality, *P. fluorescens* host cell populations "have been genetically engineered to decrease the expression of one or more those protease enzymes." Ex. 1007, [0049]. Ramseier I expressly discloses that the proteases "can be selected from hslV, hslU, clpA, clpB and clpX." *Id.*, [0034]. As such, Ramseier I would have motivated a POSA to delete multiple proteases in *P. fluorescens* simultaneously to increase the likelihood of reducing degradation and improving yield. Ex. 1003, ¶¶ 134, 139. Indeed, Ramseier I reported that expression of soluble Gal2 in multiple protease-deficient strains including ΔdegP2 "were all 2.4-fold or more higher than the control strains" that express wild type proteases and that the increase in expression was statistically significant. Ex. 1007, [0211]; Ex. 1003, ¶ 135.

Although Ramseier I does not specifically disclose deleting degP1 and AprA, the '171 patent admits that degP1, along with the other recited proteases, "were known in the art and described in e.g., U.S. Pat. App. Pub. No. 2006/01107 47 [Ramseier II]." '171 patent, 25:33-26:2. Ramseier II not only discloses that degP proteases were known, but also describes that *E. coli* strains deficient in degP proteases had been generated as an approach "to avoid degradation during recombinant protein production." Ex. 1008, [0012]; Ex. 1003, ¶ 136. As Dr. Collier explained, it was common knowledge as of 2010 that the degP protease family contains two related proteases named degP1 and degP2. Ex. 1003, ¶ 137; Ex. 1028,

429. Thus, a POSA would have understood Ramseier II's disclosure of degP-deficient strains as referring to bacterial strains that are deficient in both degP1 and degP2. Ex. 1003, ¶ 137. Because Ramseier I had successfully produced degP2-deficient *P. fluorescens* strains and Ramseier II discloses degP1- and degP2-deficient *E. coli* strains, a POSA would been motivated to improve production of CRM197 from Blais's construct in a *P. fluorescens* host cell by similarly deleting both degP1 and degP2 from the *P. fluorescens* host cell, with a reasonable expectation of success. Ex. 1007, [0211]; Ex. 1003, ¶ 137.

The '171 patent further admits that "Apr A, an extracellular serralysin-type metalloprotease metalloproteinase, is described by, e.g., Maunsell, et al., 2006." '171 patent, 26:2-9. As Manusell reported, "[a] serralysin-type metalloprotease gene, *aprA*, was identified and found to encode the major, if not only, extracellular protease produced by [the *P. fluorescens*] strain." Ex. 1009, Abstract. Thus, based on the teachings of Manusell, a POSA would have been motivated to delete this "major, if not only," extracellular protease from *P. fluorescens* host cells and would have reasonably expected to effectively reduce degradation of CRM197 expressed from Blais's construct in those cells. Ex. 1003, ¶ 138.

In view of (1) Blais's use of a *E. coli* strain that is deficient in multiple proteases, (2) Ramseier I's express disclosure that proteases including HslU, HslV

may be deleted to improve yield in *P. fluorescens*, (3) Ramseier I's successful improvement of yield using Prc1-deficient and DegP2-deficient *P. fluorescens* strains, (4) Ramseier I's recommendation to delete one or more proteases in *P. fluorescens* to further improve yield, and (5) the prior knowledge of the proteases HslU, HslV, Prc1, DegP1, DegP2, and AprA and their roles in protein degradation as reflected in Ramseier II and Maunsell, a POSA would have been motivated to express the Blais FlgI-CRM197 construct in a *P. fluorescens* host cell that is defective in the expression of the proteases HslU, HslV, Prc1, DegP1, DegP2, and AprA as recited in claims 6 and 26 and would have reasonably expected to achieve improved expression of soluble and active CRM197 within the yield range recited in those claims. Ex. 1003, ¶¶ 140-141.

Accordingly, claims 6 and 26 would have been obvious over Blais in view of Squires, Ramseier I, Ramseier II, and Maunsell as of the alleged priority date of the '171 patent.

XI. SECONDARY CONSIDERATIONS

Petitioner is not aware of any objective evidence of nonobviousness that would outweigh a conclusion of obviousness of the challenged claims.

XII. THE EVIDENCE SUBMITTED IN THIS PETITION WAS NOT PREVIOUSLY CONSIDERED BY THE OFFICE

The evidence identified in this Petition was either not before the Examiner or not fully considered during prosecution. The Examiner did not cite Blais, Squires, Choi, Ramseier I, Ramseier II, or Maunsell in any Office Action. *See generally* Ex. 1002. The Examiner also did not have the benefit of Dr. Collier's declaration, which explains what a POSA would have understood from the prior art as of March 30, 2010.

Accordingly, Petitioner submits that any argument for noninstitution under 35 U.S.C. § 325(d) is misplaced, and respectfully requests that the Board institute *inter partes* review on the grounds presented in this Petition and cancel claims 1-36 of the '171 patent as unpatentable.

XIII. MANDATORY NOTICES UNDER 37 C.F.R. § 42.8

Pursuant to 37 C.F.R. § 42.8, Petitioner states as follows:

A. Real Parties-in-Interest (37 C.F.R. § 42.8(b)(1))

GlaxoSmithKline Biologicals SA is the real party-in-interest.

B. Related Matters (37 C.F.R. § 42.8(b)(2))

Petitioner is not currently aware of any related matter involving the '171 patent.

C. Lead and Backup Counsel (37 C.F.R. § 42.8(b)(3))

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XIV. CERTIFICATION UNDER 37 C.F.R § 42.24(d)

Pursuant to 37 C.F.R. § 42.24(a)(1)(i), the foregoing PETITION FOR *INTER PARTES* REVIEW contains 13,432 words, excluding parts of this Petition exempted under § 42.24(a), as measured by the word-processing system used to prepare this paper.

Respectfully submitted,

Dated: May 7, 2020

By: /Charlies E. Lipsey/
Charles E. Lipsey, Lead Counsel
Registration No. 28,165

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§ 42.6(e) and 42.105(a), the undersigned certifies that on May 7, 2020, a copy of the foregoing PETITION FOR *INTER PARTES* REVIEW and associated Power of Attorney were served by FedEx on the correspondence address of record indicated in the Patent Office's public PAIR system for U.S. Patent No. 8,530,171:

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