UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PFENEX INC.,
Petitioner,

v.

GLAXOSMITHKLINE BIOLOGICALS S.A.,
Patent Owner.

U.S. Patent No. 9,422,345

Case IPR2019-01027

PETITION FOR *INTER PARTES* REVIEW

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1007	J. Zhou and R. Petracca, "Secretory expression of recombinant diphtheria toxin mutants in B. subtilis," *Journal of Tongji Medical University* 19:253-256 (1999)
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I. INTRODUCTION

Petitioner Pfenex Inc. ("Pfenex") seeks *inter partes* review of claims 1, 2, 4, 6, 8, 12, 13, 14, 17, 18, 19 and 21 of U.S. Patent No. 9,422,345 ("the '345 patent," EX1001). 35 U.S.C. § 311. This petition shows a reasonable likelihood that the prior art renders the challenged claims unpatentable.

II. MANDATORY NOTICES

A. Related Matters

Petitioner is concurrently filing another petition for *inter partes* review of the '345 patent on distinct grounds (i.e., IPR2019-01028). In addition, two petitions for *inter partes* review of the '345 patent were filed (IPR2019-00230 and IPR2019-00241) on November 7, 2018 by Merck Sharp & Dohme Corp ("Merck"), (hereinafter "Merck Petitions").

B. Discretionary Denial is Not Appropriate

Discretionary denial of institution of the present petition in view of the Merck Petitions is not appropriate. The Board outlined seven factors[1] for

[1] The seven factors are: (1) Whether the same petitioner previously filed a petition directed to the same claims of the same patent; (2) whether at the time of filing of the first petition the petitioner knew of the prior art asserted in the second petition or should have known of it; (3) whether at the time of filing of the second petition the petitioner already received the patent owner's preliminary response to the first

consideration in determining whether to exercise its discretion in *General Plastic Industrial Co., Ltd. v. Canon Kabushiki Kaisha*, IPR2016-01357, Paper 19 (PTAB Sept. 6, 2017). Five of the seven factors relate to follow-on petitions filed by the same petitioner, which is not the case here. Two of those factors are relevant here, and weigh against the Board exercising its discretion to deny institution. **First**, unlike *General Plastic*, here the Petitioner (Pfenex) has not previously filed a petition challenging the claims of the '345 patent. Rather, the previously filed petitions regarding the '345 patent (IPR2019-00230 and IPR2019-00241) were filed by Merck, and Pfenex is not a party (nor a real party-in-interest) to Merck's petitions.

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petition or received the Board's decision on whether to institute review in the first petition; (4) the length of time that elapsed between the time the petitioner learned of the prior art asserted in the second petition and the filing of the second petition; (5) whether the petitioner provides adequate explanation for the time elapsed between the filings of multiple petitions directed to the same claims of the same patent; (6) the finite resources of the Board; and (7) the requirement under 35 U.S.C. § 316(a)(11) to issue a final determination not later than 1 year after the date on which the Director notices institution of review.

Second, that Patent Owner, GlaxoSmithKline Biologicals S.A. ("GSK") already filed a Patent Owner's Preliminary Response to the Merck petitions does not weigh in favor of discretionary denial of the present petition for several reasons. The Board has not issued a Decision on Institution for either of the Merck petitions. Further, in each of its responses to the Merck petitions, GSK limited its remarks to a rebuttal of Merck's contention that the '345 patent is not entitled to the filing date of the priority application, and did not address the substantive grounds of invalidity. EX1057 (Patent Owner's Preliminary Response in IPR2019-00230) at pp. 16-31; EX1058 (same in IPR2019-00241 at pp. 16-31). The present petition is not based on the priority date contention raised in the Merck petitions. Accordingly, GSK is not prejudiced nor is Pfenex advantaged by the public availability of the Patent Owner's Preliminary Responses to the Merck petitions prior to the filing of the present petition.

C. Real Party-In-Interest

The real party-in-interest ("RPI") is Petitioner Pfenex Inc.

Merck filed four petitions for *inter partes* review of two other GSK-owned patents: U.S. Patent No. 8,753,645 (IPR2018-01229 and IPR2018-01236) and U.S. Patent No. 9,265,839 (IPR2018-01234 and IPR2018-01237). In those proceedings, the Board rejected GSK's contention that Pfenex was an RPI finding that "Pfenex need not be named as a real party-in-interest under 35 U.S.C. § 312(a)(2)." *Merck*

Sharp & Dohme Corp. v. GlaxoSmithKline Biologicals SA, IPR2018-01229, Paper 13 at 10 (PTAB Dec. 18, 2018); *see also* IPR2018-01236, IPR2018-01234, IPR2018-01237 (same). Notably, GSK does not contend that Pfenex is an RPI in its responses to Merck's petitions challenging the '345 patent (*i.e.*, IPR2019-00230 and IPR2019-00241).

D. Identification of Counsel and Service Information

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Please direct all correspondence to lead counsel and back-up counsel at the contact information above. Petitioner consents to electronic mail service at 38194.650.palib1@matters.wsgr.com and the email addresses above. A power of attorney accompanies this petition.

III. CERTIFICATIONS

Pfenex certifies the ’345 patent is available for IPR, and that it is not barred or estopped from requesting IPR on the identified grounds.

IV. IDENTIFICATION OF CHALLENGE; STATEMENT OF PRECISE RELIEF REQUESTED

Pfenex requests IPR and cancellation of claims 1, 2, 4, 6, 8, 12, 13, 14, 17, 18, 19 and 21 of the ’345 patent under pre-AIA 35 U.S.C. §§ 102 and 103, as the detailed statement of the reasons for relief requested sets forth, supported with exhibits and the Declaration of George Georgiou, Ph.D. (EX1002).

Claims 1, 2, 4, 6, 8, 12, 13, 14, 17, 18, 19 and 21 are unpatentable on the following grounds:

Ground	Claims	Basis
1	1, 2, 18, 19 and 21	Anticipated under §102 over Indian Patent Application No. 9745/DELNP/2007, published June 20, 2008 (EX1049, “Mekada”)
2	1, 2, 18, 19 and 21	Obvious under §103(a) over Mekada in view of the Novagen pET plasmid vector series (EX1043-EX1048)
3	4, 6, 8, 12, 13, 14 and 17	Obvious under §103(a) over Mekada in view of the Novagen pET plasmid vector series and Thie (EX1052)

V. STATEMENT OF REASONS FOR RELIEF REQUESTED

A. Summary of Argument

The ’345 patent relates to the expression of diphtheria toxins, including the diphtheria toxin mutant, CRM197, and broadly claims polynucleotides comprising a 5’ signal sequence[2] portion and a 3’ toxin portion, that encodes a polypeptide at least 90% identical to SEQ ID NO:32 (*i.e.,* CRM197). The 5’ signal sequence portion is only limited by requiring that (1) it encodes a polypeptide capable of directing transport of the 3’ toxin to the bacterial periplasm when expressed in a bacterial host, and (2) the signal sequence is not from *C. diphtheria*.

As discussed below in detail, prior to the earliest possible priority date of the ’345 patent, the sequence of CRM197 was known, as were methods and vectors for expression and secretion into the periplasm of *E. coli* bacterial host cells, as well as the advantages of such expression and secretion. As this Petition demonstrates, the challenged claims encompass embodiments taught in the art using common, well-established components and methods for an already-recognized, beneficial purpose.

[2] The term “signal sequence” is also referred to in the literature as a “signal peptide,” “leader peptide,” or “leader sequence.” EX1002, 16n.1.

B. The ’345 Patent

1. Background

The ’345 patent, entitled “Expression System,” relates to the expression of bacterial toxins, in particular diphtheria toxins (including mutant forms of diphtheria toxin, such as CRM197). EX1001, title, 1:9-13. Such *diphtheria* toxoids, including a mutant form with reduced toxicity, CRM197, are components in many vaccines providing immunity against *Corynebacterium diphtheria.* EX1001, 1:52-54. The patent purports to provide “an improved process for making a bacterial toxin by periplasmic expression” and “polynucleotides which are used in the process.” EX1001, 2:26-28, 2:34-35.

2. Challenged Claims

Claims 1, 2, 4, 6, 8, 12, 13, 14, 17, 18, 19 and 21 of the ’345 patent are challenged in the present petition. The claims of the ’345 patent are directed to DNA (polynucleotide) constructs that include:

(1) a 5’ signal sequence; and

(2) a sequence that encodes a polypeptide that is at least 90% identical to CRM197 (SEQ ID NO:32). EX1002, ¶¶37-48.

While claim 1 does not recite a specific type of 5’ signal sequence, this broad claim recites that: (1) the signal sequence is “capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a

baterial [sic] host cell," and (2) that the signal sequence is "not derived from *C. diphtheriae*," *i.e.*, not of the same origin as CRM197. EX1002, ¶37; EX1001, Claim 1. Independent claim 6 and the challenged dependent claims only vary from claim 1 by having (1) a narrower scope of the 5' signal sequence (claims 4, 6, and 8), (2) a narrower subset of CRM197 variants, as well as CRM197 itself (claims 2, 12-14, 18, and 19), or (3) that the signal sequence is directly 5' of the bacterial toxin (claims 17 and 21). EX1002, ¶¶42-46; EX1001, 49:65 to 52:42.

Claims 1 and 6 are independent. Claim 1 of the '345 patent is representative and recites:

> A polynucleotide comprising a 5' signal sequence portion and a 3' toxin portion wherein:
>
> (a) the 3' toxin portion encodes a mature bacterial toxin polypeptide having an amino acid sequence at least 90% identical to SEQ ID NO: 32; and
>
> (b) the 5' signal sequence portion encodes a polypeptide having an amino acid sequence capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a baterial [sic] host cell, and wherein the 5' signal sequence is not derived from *C. diphtheriae*.

EX1001, 49:54-64.

3. Prosecution History

The ’345 patent issued from Application 13/500,244 (“the ’244 application”), which is a national stage filing of PCT Application No. PCT/EP2010/065047, and claims priority to Great Britain Application No. 0917647.0 filed on October 8, 2009. EX1001, cover.

During prosecution, the claims were rejected as anticipated by a reference teaching expression of diphtheria toxin in the periplasmic space and a diphtheria toxin signal sequence modified by an insertion of an asparagine residue. EX1004, 547-548.[3] In response, Applicant amended the claims to recite “the 5’ signal sequence portion is not derived from *C. diphtheriae*” and to further recite that the 3’ toxin portion encodes a mature bacterial toxin polypeptide having an amino acid sequence at least 90% identical to SEQ ID NO:32. EX1004, 565, 570. Applicant argued that the applied reference discloses only the use of a modified native *C. diphtheriae* signal sequence. *Id.* at p. 570. Additional rejections for lack of written description and another ground of anticipation were overcome by Applicant’s deletion of claim language concerning B or T cell epitopes. EX1004, 569, 570. The claims were subsequently allowed. EX1004, 575, 579.

[3] Citations to the prosecution history of Exhibit 1004 refer to the page numbering added by Petitioner.

C. Level of Ordinary Skill

The relevant time is before October 8, 2009, the earliest priority date claimed in the '345 patent. A person of ordinary skill in the art ("POSA"), at the relevant time, would have held an M.S. or Ph.D. in microbiology, microbial genetics or molecular biology. EX1002, ¶27. The POSA would have had working knowledge of microbial genetics, including genetic engineering and recombinant DNA techniques to manipulate microbial DNA and induce bacterial host production of exogenous proteins and polypeptides. *Id.* The POSA would have had at least 3 years of experience with a M.S., or less with a Ph.D. *Id.* The experience may have come from the POSA's own experience, or through research or work collaborations with other individual(s) with experience in the biotechnology industry or in academia, *e.g.*, as members of a research team or group. For example, the POSA may have worked as part of a team or collaboration to develop or utilize genetic engineering and microbial process techniques, or research potential therapeutic or diagnostic molecules for expression in bacterial systems. *Id.* Further, a POSA would have known about the variety of research kits and recombinant tools, including commercially available products that could be used to improve protein expression in microbial systems, and would have known how to apply these available tools in order to, for example, optimize bacterial cell culture growth and purify a target protein. *Id.* ¶¶27-29.

D. Claim Construction

Claims should be given their ordinary and customary meaning, consistent with the specification, as a POSA understood them. 37 C.F.R. § 42.100(b) (as amended Nov. 13, 2018); *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312-13 (Fed. Cir. 2005) (*en banc*). Except as discussed below, for purposes of this IPR, the claim terms should be given their plain and ordinary meaning.[4]

1. "capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a bacterial host cell"

The independent claims of the '345 patent recite a 5' signal sequence portion encoding a polypeptide having an amino acid sequence "capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a baterial [sic] host cell." The '345 patent does not provide a

[4] Without taking a position on whether the claims are sufficiently definite, even when the metes and bounds of a claim are indefinite, the Board nevertheless can determine whether embodiments plainly within the scope of the claim would have been obvious. *Ex parte Tanksley*, 26 U.S.P.Q.2d (BNA) 1384, 1387 (B.P.A.I. 1991) (embodiment within scope despite indefiniteness); *Ex parte Sussman*, 8 U.S.P.Q.2d (BNA) 1443, 1445 n.* (B.P.A.I. 1988) (affirming obviousness despite indefinite claim format).

definition for this phrase, thus it should be understood to have the plain and ordinary meaning attributed to it by a POSA. *Teleflex, Inc. v. Ficosa N. Am. Corp*., 299 F.3d 1313, 1325 (Fed. Cir. 2002). Moreover, while the '345 patent provides examples of signal sequences capable of directing an expressed protein to the periplasm (*e.g.*, EX1001, 7:33-39); such examples of embodiments do not function as a definition of the phrase "capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a ba[c]terial host cell." *Liebel-Flarsheim Co. v. Medrad, Inc.*, 358 F.3d 898, 913 (Fed. Cir. 2004) (holding that it is "improper to read limitations from a preferred embodiment described in the specification—even if it is the only embodiment—into the claims absent a clear indication in the intrinsic record that the patentee intended the claims to be so limited."). Accordingly, the phrase must be given its ordinary and customary meaning.

The plain meaning of "capable of" is "having ability, capacity, or power to do something." EX1009, 75. Here the phrase is used in the context of a composition, therefore, the signal peptide merely has the ability to direct the bacterial toxin to the periplasm regardless of whether or not it actually does so, and regardless of the particular amount to be secreted to the periplasm. Accordingly, this phrase should be construed as meaning an amino acid sequence having the ability to direct transport of the bacterial toxin polypeptide to the bacterial

periplasm when expressed in a bacterial host cell.

E. State of the Art

Diphtheria toxin was identified over 100 years ago as a potent exotoxin produced by *Corynebacterium diphtheria*, and is responsible for the majority of symptoms experienced by patients with diphtheria, a life-threatening and highly contagious bacterial infection caused by *C. diphtheria*. EX1002, ¶¶49-50. Early diphtheria vaccines significantly reduced the incidence of and death due to diphtheria disease. *Id.,* ¶51. These vaccines were based on preparations of chemically modified diphtheria toxin proteins (*i.e.*, toxoids), which could contain incompletely inactivated toxin, posing a potential danger to patients. *Id*. Thus, safer vaccine preparations were sought using recombinant DNA technology. *Id*.

Recombinant DNA technology, in conjunction with diphtheria toxin structural properties, was employed by researchers in the field to improve safety of vaccines by genetically modifying the toxin protein to irreversibly inactivate it. *Id.,* ¶¶52-56. Diphtheria toxin was known to be synthesized in the cytoplasm of *C. diphtheriae* as a single-chain polypeptide containing a signal peptide, which is removed during secretion to yield a 535-residue mature toxin. *Id.,* ¶52. The mature toxin forms a loop, linked internally via two disulfide bonds. The 3-dimensional crystal structure revealed diphtheria toxin contains three discrete folding domains: the catalytic (C-domain; A-fragment), the translocation (T-domain; B-fragment)

and the receptor-binding (R-domain), which binds to the cell membrane anchored heparin binding epidermal growth factor precursor (HB-EGF). *Id.,* ¶53. Researchers in the field applied this knowledge to vaccine programs to isolate and identify recombinant diphtheria toxin mutants or toxoids (modified versions of diphtheria toxin that lack toxic activity yet still possesses antigenic activity). *Id.,* ¶¶52-57.

Such diphtheria toxin mutants, also known as "Cross Reacting Materials" or "CRMs," possessed reduced or eliminated toxicity, while maintaining or even enhancing immunogenic activity of the protein. *Id.,* ¶57. One such CRM was CRM197 developed by Tsuyoshi Uchida in 1971. *Id.,* ¶58. CRM197 is a genetically mutated diphtheria toxoid, containing a glycine to glutamic acid substitution at position 52. *Id.* CRM197 lacks diphtheria toxin activity, yet maintains antigenicity to the diphtheria toxin. *Id.* Because of the growing range of uses of diphtheria toxins, researchers well before the earliest possible priority date of the '345 patent sought to improve and secure reliable sources of diphtheria toxin mutant proteins, such as CRM197, using bacterial cell expression systems. *Id.,* ¶48.

However, technical challenges of expressing recombinant protein in the cytoplasm of *E. coli* expression systems and subsequent purification were well-known, including aggregation of the expressed protein in "inclusion bodies" and proteolytic degradation of the protein by bacterial proteases present in the

cytoplasm. EX1002, ¶59. Degradation of proteins expressed and secreted by gram positive bacteria such as *Bacillus subtilis* and *Staphylococcus carnosus* was also known. *E.g.*, *Id.*; Braun [EX1025]; Dilsen [EX1026]; and Zhou [EX1007], 255 (proteolytic degradation of expressed CRM197). Additionally, the reducing environment and the lack of the needed machinery for disulfide bond formation in the *E. coli* cytoplasm, does not allow the formation of disulfide bonds that are necessary for correct folding of disulfide-linked proteins, such as CRM197. EX1002, ¶¶60-61.

To circumvent these known issues with cytoplasmic expression of heterologous proteins, researchers regularly turned to *E. coli* expression systems that secreted proteins into the periplasmic space. *Id.,* ¶62. The advantages of secreting expressed protein into the periplasm of *E. coli* were well-known in the art before the earliest possible priority date of the ’345 patent and include increased stability of expressed proteins, presence of machinery and requisite oxidizing environment of the periplasm allowing disulfide bond formation, and ease of release from the periplasm. *Id*.; Duffaud [EX1029], 499; Mergulhao [EX1030], 178-179. Further, expressed proteins can be secreted in large amounts (even those proteins that are toxic to the host cell) to the periplasmic space, from which they can be easily purified with simple and efficient methods. EX1002, ¶¶62, 66 (citing Duffaud [EX1029], 501). Because of the advantages afforded by periplasmic

secretion, numerous proteins prior to October 8, 2009 have been expressed and purified from *E. coli* expression systems employing periplasmic secretion, including at least 19 commercial therapeutic products, including Food and Drug Administration (FDA)-approved biologics (*e.g.*, somatropin recombinant human growth hormone and ranibizumab (Lucentis)) that were produced by expression and secretion to the periplasmic space of *E. coli*, as well as certolizumab pegol (Cimzia) and parathyroid hormone (Preotact). EX1002, ¶¶63-65. Accordingly, expression and secretion of heterologous proteins into the periplasm was well-known and accepted throughout the biotherapeutic community, including the FDA, before the earliest possible priority date of the '345 patent. *Id*.

By October 8, 2009, and well before the filing of the '345 patent, the role of signal sequences located on the N-terminus of exported proteins in directing secreted proteins to export machinery was well-understood. *Id.,* ¶¶67-70. It was known that a common mechanism for prokaryotic protein and mammalian protein translocation across the cytoplasmic and endoplasmic reticulum membranes, respectively, exists between prokaryotic and eukaryotic cells. *Id.,* ¶67. Diphtheria toxin, for example, was known to be processed by the insertion of its signal sequence into the cytoplasmic membrane followed by export of the diphtheria toxin into the periplasmic space or cell medium, where it is folded and disulfide bonds formed. *Id.,* ¶68. The signal sequence is then cleaved and the diphtheria

toxin further processed. *Id.*

Prior to the earliest possible priority date of the ’345 patent, vectors enabling the expression of cloned proteins in *E. coli* bacterial host cells and secretion of the expressed proteins into the periplasmic space were readily available to a POSA. *Id.,* ¶¶66-70. These vectors contained signal sequences as already added components in commercially available vector constructs. For example, Novagen Inc. manufactured multiple prokaryotic expression vectors encoding signal sequences, including PelB and OmpT. *Id.,* ¶71; Novagen pET Vector Cloning and Expression Regions (archived November 19, 1997, EX1044); *see also* EX1043; EX1045; EX1046; EX1047; EX1048. In addition, in 2006 Mekada (EX1049) explicitly directed POSAs to clone CRM197 into pET-22b, a plasmid vector that included the nucleic acid sequence encoding the signal peptide sequence PelB for periplasmic secretion in *E. coli*. Other commercial biotechnology vendors (*e.g.*, Sigma-Aldrich, New England Biolabs, and Invitrogen) also supplied ready-to-use vectors for use by POSAs for expression and secretion of cloned proteins in host cell expression systems. EX1002, ¶71.

Prior to October 8, 2009, POSAs recognized the advantages of periplasmic secretion applied to CRM197, and thus designed polynucleotide constructs to express and secrete CRM197, using secretory signal sequences derived from other organisms, including PelB, OmpA, and others. EX1002, ¶¶72-76. In particular,

CRM197 was known to be a disulfide bond-containing protein, requiring complex folding for proper configuration to maintain activity. *Id.*, ¶¶72-73. Thus, an oxidizing environment, such as that of the periplasmic space, is necessary for disulfide bonds to form in CRM197. *Id.,* ¶72. With the reducing environment of the cytoplasm, which prevents disulfide bond formation, as well as difficulties due to inclusion-body formation, production of active, cytoplasmically-expressed proteins in *E. coli* often requires a complex denaturation-renaturation/refolding process, which leads to lower yield and higher processing costs. *Id.,* ¶¶59, 72. However, expressing protein and secreting into the periplasmic space bypassed this necessity. *Id.,* ¶72. The oxidizing and insulating environment of the periplasmic space allows disulfide-bond formation to occur, and shields expressed and secreted proteins from extensive protease degradation. *Id.*

Thus, before October 8, 2009, research groups studying diphtheria toxin mutants taught the use of vectors including secretory signal sequences for export of diphtheria toxin mutants into the periplasmic space of *E. coli*. *E.g.*, *id.*, ¶¶74-75, citing to Davis (EX1005), ¶324 ("vector systems … which include secretory leader sequences for export of DT into the periplasmic space of *E. coli*," including CRM197); Zhou (EX1007), 256 (taught the need to secrete protein out of the host cell and away from the cytoplasm in order to allow for correct folding of the mutant diphtheria toxins, including CRM197); Collier (EX1050), 6:18-40

(expression of the diphtheria toxin mutants as secreted protein into the periplasmic space of *E. coli*, and the use of commercial vectors, including pET vectors (Novagen, Inc.), which contain secretory leader sequences). *Id.*, ¶¶74-76. Indeed Collier's group in 1994 (*i.e.*, 15 years before the '345 patent's earliest possible priority date), utilized commercially available cassette-type vector constructs that allowed the quick and relatively simple option of swapping in and exchanging diphtheria toxin mutants for expression, purification and testing. *Id.,* ¶76. Accordingly, the acceptance of periplasmic-secreted heterologous proteins in *E. coli* expression systems was already widespread and in use well prior to the claimed priority date the '345 patent. *Id.,* ¶77.

1. Indian Patent Appl. No. 9745/DELNP/2007 "Therapeutic Agent for Cancer," Published June 20, 2008, Filed December 17, 2007 from PCT Application No. PCT/JP2006/312321 ("Mekada") (EX1049)

Mekada[5] disclosed the use of CRM197 as a therapeutic agent in the treatment of cancer, noting that the receptor binding domain of diphtheria toxin can inhibit the binding of HB-EGF to EGF receptor through its binding to HB-EGF.

[5] Mekada published on June 20, 2008 more than one year prior to the earliest possible priority date of the '345 patent, making it prior art under 35 U.S.C. § 102(b). Mekada was not before the Examiner during prosecution of the '345 patent.

EX1002, ¶93. Mekada disclosed that CRM197, which has a mutation from glycine to glutamic acid at position 52, is a preferred such agent. *Id*. An alignment of the amino acid sequence for Mekada CRM197 (SEQ ID NO:1 (without signal sequence amino acid 1-25)) ("Mekada CRM") and SEQ ID NO:32 in the '345 patent ("SEQ ID NO:32") shows 100% identity between the toxin polypeptides, including at mutated amino acid position 52. *Id*. Further, Mekada used a plasmid vector (pET-22b) from Novagen that included the nucleic acid sequence encoding the PelB signal peptide, which vector was designed for periplasmic secretion in *E. coli* host cells. EX1002, ¶94. Mekada stated:

> The mutant having the mutation in the catalytic action domain can be made as follows. A CRM197 region is synthesized by PCR with the gene (Pβ197) encoding CRM197 incorporated in the plasmid as the template using as a primer a portion to be mutated. The primer is synthesized by introducing a point mutation so as to be mutated, and used. The mutant can be made by incorporating the synthesized DNA into a gene expression vector (pET-22b) for *Escherichia coli*, transforming *Escherichia coli* with the vector to express the mutant in *Escherichia coli*.

EX1002, ¶94; Mekada (EX1049) at 6-7. Thus, Mekada disclosed the production of CRM197 (*i.e.*, SEQ ID NO:32) via expression of CRM197 in *E. coli* using a commercially available expression vector that included the nucleic acid

sequence encoding the PelB, a 5' signal sequence for periplasmic expression not derived from *C. diphtheria*. EX1002, ¶¶93-95.

2. Novagen pET Plasmid Vector Maps (EX1043-EX1048)

The Novagen pET Vector Maps[6] disclosed vectors developed by Novagen that included nucleic acid sequences encoding signal peptides, such as PelB and

[6] Novagen pET Vector Maps were available at least as early as 1997, more than 1 year prior to the earliest priority date of the '345 patent, making them prior art under 35 U.S.C. § 102(b). Detailed vector maps were archived by Wayback Machine at least as early as 2003. EX1045 (pET-22b, archived by WayBack Machine August 19, 2003 (https://web.archive.org/web/20030819085202/http://www.novagen.com/docs/NDIS/69744-000.pdf)); EX1046 (pET-25b, archived by WayBack Machine May 16, 2003 (https://web.archive.org/web/20030516011318/http://novagen.com/SharedImages/TechnicalLiterature/7_TB065.pdf)); EX1047 (pET-26b, archived by WayBack Machine June 20, 2003 (https://web.archive.org/web/20030620093207/http://www.novagen.com/SharedImages/TechnicalLiterature/7_TB071.pdf)); EX1048 (pET-27b, archived by WayBack Machine June 22, 2003

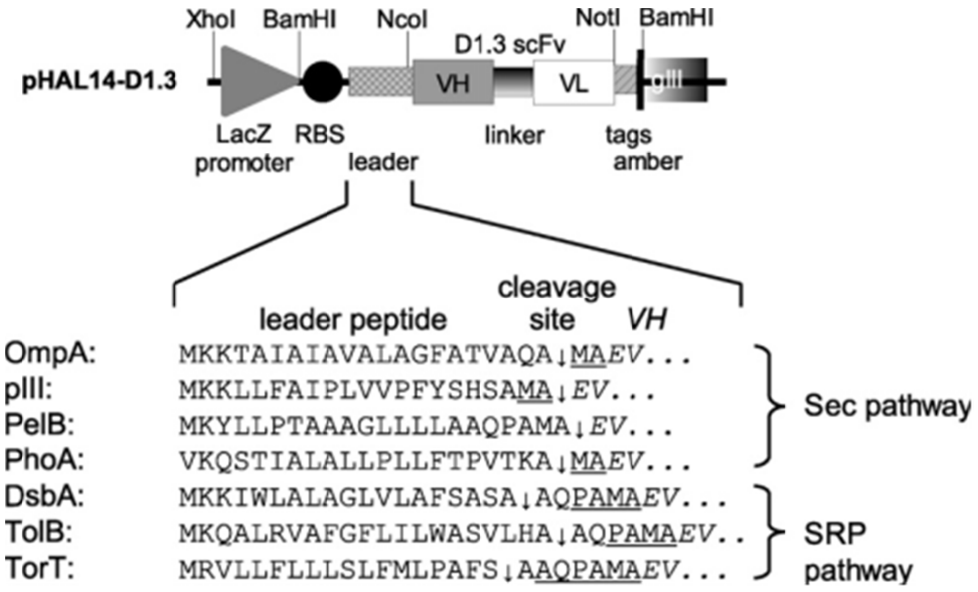
OmpT leader sequences, that exhibited "potential periplasmic localization" of the expressed and secreted heterologous protein. EX1002, ¶97; EX1043, 92. Novagen distributed detailed vector maps of each of the pET vectors in the series, including pET-22b. EX1002, ¶¶97-98. By 2002-2003, pET vectors had been used "to express thousands of different proteins." EX1002, ¶97; EX1043, 88.

3. Thie et al., "SRP and Sec pathway leader peptides for antibody phage display and antibody fragment production in *E. coli*," *New Biotechnol.* 25:49-54 (June 2008) ("Thie") (EX1052)

Thie[7] disclosed the generation of human recombinant antibody fragments and fusions thereof using leader peptides from the SRP pathway (DsbA, TorT, and TolB) and Sec pathways (PelB, OmpA, PhoA, and PIII). The SRP and Sec pathways are both "compatible with antibody phage display and the production of soluble antibody fragments." EX1002, ¶100; EX1052, Abstract. Thie discussed testing expression and secretion to the *E. coli* periplasmic space of antibody fragments and fusions thereof using the above leader peptides, which were cloned into a plasmid construct and located just 5' of the heterologous protein cassette:

(https://web.archive.org/web/20030622190457/http://www.novagen.com/SharedImages/TechnicalLiterature/7_TB073.pdf)).

[7] Thie was published in June 2008, more than one year before the earliest priority date of the '345 patent.



EX1002, ¶100. Thie demonstrated the ease with which different *E. coli* leader peptides, including leader peptides claimed in the ’345 patent (*e.g.*, OmpA, DsbA, TolB, and TorT), could be tested in a routine manner for production and secretion of heterologous protein in *E. coli*. EX1002, ¶101.

F. Ground 1: Claims 1, 2, 18, 19 and 21 are Unpatentable as Anticipated by Mekada

As detailed below, Mekada, which taught the use of diphtheria toxin mutants, including CRM197, as a therapeutic agent in the treatment of cancer, disclosed all elements of claims 1, 2, 18, 19, and 21 of the ’345 patent, rendering each of those claims invalid as anticipated. EX1002, ¶103. *Monsanto Tech. LLC v. E.I. DuPont de Nemours & Co.*, 878 F.3d 1336, 1342-43 (Fed. Cir. 2018) (“A prior art reference anticipates a patent’s claim under § 102(b) if it discloses each and every element of the claimed invention arranged or combined in the same way as in the claim.”) (internal quotations omitted, citation omitted).

In particular, Mekada taught the use of CRM197 protein in treating various cancers. EX1002, ¶103. Mekada also disclosed production of CRM197 in *E. coli* using a plasmid vector (pET-22b) that encodes the non-*C. diphtheriae* derived PelB signal peptide, which vector is designed for periplasmic secretion in *E. coli* host cells. EX1002, ¶104. Thus, the challenged claims are all anticipated by the disclosure of Mekada. *King Pharms., Inc. v. Eon Labs, Inc.*, 616 F.3d 1267, 1274 (Fed. Cir. 2010) ("Under 35 U.S.C. § 102 a claim is anticipated 'if each and every limitation is found either expressly or inherently in a single prior art reference.'") (citing *Celeritas Techs., Ltd. v. Rockwell Int'l Corp.*, 150 F.3d 1354, 1361 (Fed. Cir. 1998)).

1. Independent Claim 1

The polynucleotide of claim 1 requires the following elements:

(1) a 3' toxin portion encoding a mature bacterial toxin polypeptide having an amino acid sequence at least 90% identical to SEQ ID NO: 32; and

(2) a 5' signal sequence portion encoding a polypeptide having an amino acid sequence:

(a) capable of directing transport of the bacterial toxin polypeptide into the periplasmic space; and

(b) not derived from *C. diphtheriae*.

EX1002, ¶102. The below analysis demonstrates that each of these claim limitations was disclosed in the prior art patent application, Mekada.

a. [1] 3' toxin portion encodes a mature bacterial toxin polypeptide having an amino acid sequence at least 90% identical to SEQ ID NO:32 (i.e., CRM197)

Mekada taught that CRM197 (*i.e.*, SEQ ID NO: 32) is an agent for treating cancer, and exemplified in Examples 1-4 the administration of CRM197 for treating peritoneal cancers. EX1002, ¶103. Mekada noted that CRM197 is a mutant diphtheria toxin having a Gly to Glu substitution at position 52. EX1002, ¶103; EX1049, 6. Mekada further taught that "the amino acid (Gly) at position 26 was numbered as No. 1 by removing a signal sequence (1 to 25) in an amino acid sequence in SEQ ID NO:1" and that the "signal sequence of 25 amino acid residues may or may not be included." EX1002, ¶103; EX1049, 4. In his declaration, Dr. Georgiou confirms "[a] sequence alignment of SEQ ID NO:1 (minus the signal sequence of the 1st-25 amino acid residues) is identical to SEQ ID NO:32 [of the '345 patent]." EX1002, ¶¶93, 103. Accordingly, the claim limitation of claim 1 "a mature bacterial toxin polypeptide having an amino acid sequence at least 90% identical to SEQ ID NO:32" was disclosed in Mekada.

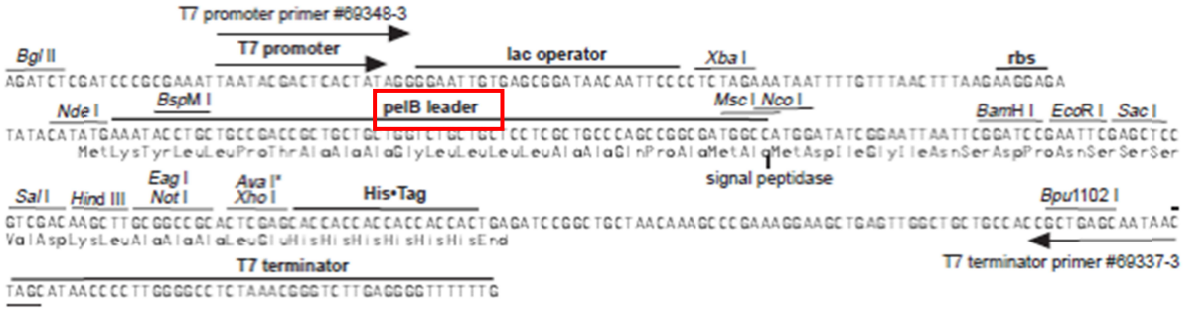
b. [2a] a 5' signal sequence portion encodes a polypeptide having an amino acid sequence capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a bacterial host cell

Examples 1-4 of Mekada taught the production of CRM197 (*i.e.*, SEQ ID NO: 32) for use as a therapeutic agent. EX1002, ¶104. Mekada further disclosed:

> The mutant can be made by incorporating the synthesized DNA into a **gene expression vector (pET-22b) for *Escherichia coli***, transforming *Escherichia coli* with the vector to express the mutant in *Escherichia coli*.

EX1049, 7 (emphasis added); EX1002, ¶104.

pET-22b is an expression vector that (i) was commercially available prior to 2009; (ii) was manufactured and sold by Novagen; and (iii) encoded the signal peptide sequence from *Erwinia carotovora* PelB ("pelB leader"):



pET-22b(+) cloning/expression region

EX1002, ¶104; EX1045. Moreover, Mekada taught the use of the expression vector pET-22b, which is capable of directing transport of an expressed bacterial toxin polypeptide to the bacterial periplasm when expressed in *E. coli*. EX1002,

¶104. Because the periplasmic secretion function of pET-22b is an inherent property, regardless of whether this function is discussed in Mekada, the disclosure of Mekada anticipates this element of claim 1. *SmithKline Beecham Corp. v. Apotex Corp.*, 403 F.3d 1331, 1343-44 (Fed. Cir. 2005); *In re Cruciferous Sprout Litig.*, 301 F.3d 1343, 1350 (Fed. Cir. 2002).

In any event, as Dr. Georgiou explains in his declaration, prior to the earliest possible priority date of the '345 patent "[a] POSA would have known that the use of a secretion cloning vector such as pET-22b would have been 'capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a ba[c]terial host cell,' for example, as Mekada directs into *Escherichia coli*." EX1002, ¶104; *In re LeGrice*, 301 F.2d 929, 936 (C.C.P.A. 1962) ("such that a skilled artisan could take its teachings in combination with his own knowledge of the particular art and be in possession of the invention.").

c. [2b] the 5' signal sequence is not derived from C. diphtheriae

The signal peptide, PelB, encoded by the pET-22b vector (disclosed in Mekada and shown above) is derived from PelB of *E. carotovora*. EX1002, ¶104. Accordingly, this signal peptide is "not derived from *C. diphtheriae*," and reads on the third portion of claim 1. *Id*.

Accordingly, Mekada taught each and every element of claim 1 and thus, anticipates this claim.

2. Claims 2, 18, 19, and 21

Claims 2, 18, 19 and 21 depend from claim 1 and recite additional requirements of the 3' toxin portion of the claimed polynucleotide. Each of the additional limitations of claims 2, 18, 19 and 21 are anticipated by Mekada. Claim 2 recites "wherein the 3' toxin portion encodes a polypeptide having the amino acid sequence of SEQ ID NO:32." Claim 18 recites "wherein the 3' toxin portion encodes CRM197." Claim 19 recites "wherein the 3' toxin portion encodes a polypeptide having at least 95% sequence identity to SEQ ID NO:32." As discussed above, Dr. Georgiou confirms that SEQ ID NO:1 (minus the signal sequence of the 1st-25 amino acid residues) (*i.e.*, CRM197 of Mekada) is identical to SEQ ID NO:32 [of the '345 patent]. EX1002, ¶¶93, 107. Accordingly, CRM197 (*i.e.*, SEQ ID NO:32)—and sequences at least 95% identical to SEQ ID NO:32—are disclosed by Mekada. *Id.*, ¶¶107-108.

Claim 21 recites "wherein the 5' signal sequence portion is directly 5' of the 3' toxin portion." *Id.,* ¶106. Mekada taught CRM197 (*i.e.*, SEQ ID NO:32) can be produced by cloning "into a gene expression vector (pET-22b) for *Escherichia coli*, transforming *Escherichia coli* with the vector to express the mutant in *Escherichia coli*." EX1049, 7; EX1002, ¶104. As Dr. Georgiou explains, "a skilled artisan would know that cloning into the pET-22b vector is accomplished by inserting the desired protein to be expressed, in this case CRM197 (*i.e.*, SEQ ID

NO:32) directly 3' of the pelB signal sequence, *e.g.*, pET-22b Vector Map [EX1045], rendering the pET-22b pelB signal peptide portion "directly 5' of the 3' toxin portion." EX1002, ¶107; *In re Cruciferous Sprout Litig.*, 301 F.3d at 1350.

Accordingly, for at least these reasons, claims 2, 18, 19 and 21 are also anticipated by Mekada.

G. Ground 2: Claims 1, 2, 18, 19 and 21 are Unpatentable as Obvious Over Mekada in View of Novagen pET Plasmid Vectors

As discussed with respect to Ground 1, claims 1, 2, 18, 19, and 21 of the '345 patent are anticipated by the disclosure of Mekada. To the extent that the Board finds that Mekada does not explicitly or inherently disclose any element of these claims, such element is at least rendered obvious by the disclosure of Mekada in view of the Novagen pET Plasmid Vectors.

Well before the 2009 earliest possible priority date of the '345 patent, POSAs conducted extensive research to improve *E. coli* expression systems for production of complex and properly folded heterologous proteins. EX1002, ¶110. The signal peptide field developed rapidly to the point where, by 2005, expression of heterologous secreted proteins in *Escherichia coli* was widely employed for laboratory and preparative purposes such that many mammalian proteins were produced routinely in secreted form. EX1002, ¶111; EX1041, Abstract; *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1368 (Fed. Cir. 2007) ("The experimentation needed, then, to arrive at the subject matter claimed in the [] patent was nothing

more than routine application of a well-known problem-solving strategy, and we conclude, the work of a skilled artisan, not of an inventor.") (internal quotations and citations omitted).

Further, the advantages of expressing and secreting heterologous proteins in the periplasmic space of *E.coli* were well-known prior to the '345 patent. EX1002, ¶112. Such advantages include the stabilizing environment of the periplasmic space, separated from detrimental factors (*e.g.*, proteases) present in the cytoplasm. *Id*. Further, the presence of molecular machinery and an oxidizing environment in the periplasm facilitates proper disulfide bond formation (contrast with the reducing environment of the cytoplasm preventing disulfide bond formation), which is particularly important for proteins, like diphtheria toxin proteins, that require disulfide bond formation for correct folding and secretion of an active protein. *Id*. Finally, relatively simple protocols allowed soluble proteins secreted into the periplasmic space to be easily released and purified by standardized techniques. *Id.*; *Ecolab, Inc. v. FMC Corp.*, 569 F.3d 1335, 1350 (Fed. Cir. 2009), *amended on reh'g in part*, 366 F. App'x 154 (Fed. Cir. 2009) (reversing finding of nonobviousness where there was "an apparent reason to combine the known elements in the fashion claimed by the patent at issue" as the advantages of and methods for doing so were well known) (citing *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007)).

Moreover, by the earliest possible priority date for the ’345 patent, a POSA was well-versed and trained in the use of the multitude of widely-available, commercial products (from companies such as Novagen, Sigma-Aldrich, Invitrogen, New England Biolabs and others) to assist in constructing secretion vectors for expressing and secreting heterologous proteins into the periplasmic space of *E. coli*. EX1002, ¶¶113-114. For example, Novagen marketed secretion cloning vectors encoding signal peptides, including OmpT and PelB. EX1002, ¶113; Novagen pET System Tutorial, Vector Cloning/Expression Regions, and Vector Maps (EX1043-EX1048).

As discussed above (§ V.F.1), the nucleotide sequences claimed by the ’345 patent and those of at least the Mekada reference are identical. However, to the extent that the Board considers the Mekada reference as not including information regarding vector sequences encoding a signal peptide not of *C. diphtheria* origin as claimed, the challenged claims of the ’345 patent would have been obvious to a POSA in view of commercially available plasmid constructs specifically targeting periplasmic secretion, all of which included a signal peptide not of *C. diphtheria* origin, as explained in detail below. EX1002, ¶115.

1. Independent Claim 1

The polynucleotide of claim 1 requires the following elements:

(1) a 3' toxin portion encoding a mature bacterial toxin polypeptide having an amino acid sequence at least 90% identical to SEQ ID NO: 32; and

(2) a 5' signal sequence portion encoding a polypeptide having an amino acid sequence:

(a) capable of directing transport of the bacterial toxin polypeptide into the periplasmic space; and

(b) not derived from *C. diphtheriae*.

EX1002, ¶116. The below analysis demonstrates that each of these claim limitations would have been obvious over the disclosures of Mekada in view of the Novagen pET Plasmid Vectors.

a. [1] a 3' toxin portion encodes a mature bacterial toxin polypeptide having an amino acid sequence at least 90% identical to SEQ ID N O:32 (i.e. CRM197)

Mekada taught that CRM197 (*i.e.*, SEQ ID NO: 32) is an agent for treating cancer, and exemplified in Examples 1-4 the administration of CRM197 for treating peritoneal cancers. EX1002, ¶¶117-118. Mekada noted that CRM197 is a mutant diphtheria toxin having a Gly to Glu substitution at position 52. EX1002, ¶117; EX1049 at 6. Mekada further taught that "the amino acid (Gly) at position 26 was numbered as No. 1 by removing a signal sequence (1 to 25) in an amino acid sequence in SEQ ID NO:1" and that the "signal sequence of 25 amino acid

residues may or may not be included." EX1002, ¶117; EX1049, 4. In his declaration, Dr. Georgiou confirms "[a] sequence alignment of SEQ ID NO:1 (minus the signal sequence of the 1st-25 amino acid residues) is identical to SEQ ID NO:32 [of the '345 patent]." EX1002, ¶¶93, 117. Accordingly, the claim limitation of claim 1 "a mature bacterial toxin polypeptide having an amino acid sequence at least 90% identical to SEQ ID NO:32" was disclosed in Mekada.

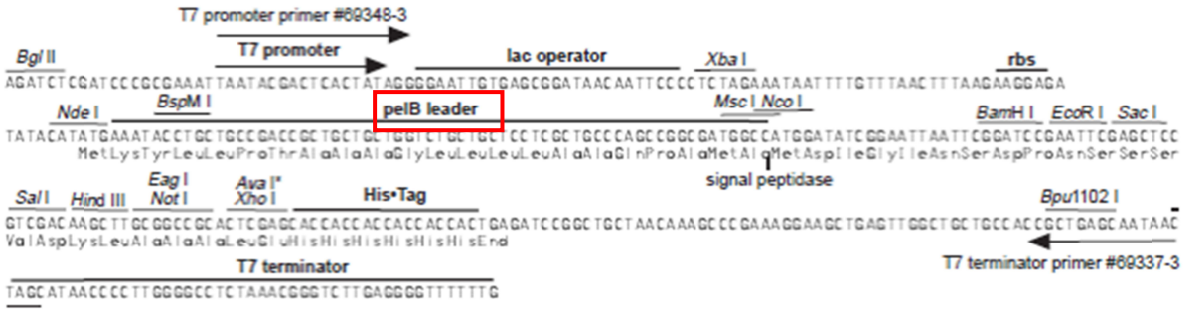
b. [2a] a 5' signal sequence portion encodes a polypeptide having an amino acid sequence capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a bacterial host cell

Examples 1-4 of Mekada taught the production of CRM197 (*i.e.*, SEQ ID NO: 32) for use as a therapeutic agent. EX1002, ¶118. Mekada further disclosed:

> The mutant can be made by incorporating the synthesized DNA into a **gene expression vector (pET-22b) for *Escherichia coli***, transforming *Escherichia coli* with the vector to express the mutant in *Escherichia coli*.

EX1049, 7 (emphasis added); EX1002, ¶118.

pET-22b is an expression vector that (i) was commercially available prior to 2009; (ii) was manufactured and sold by Novagen; and (iii) encoded the signal peptide sequence from *Erwinia carotovora* PelB ("pelB leader"):



pET-22b(+) cloning/expression region

EX1002, ¶118; EX1045. Moreover, Mekada taught the use of the expression vector pET-22b, which is capable of directing transport of an expressed bacterial toxin polypeptide to the bacterial periplasm when expressed in *E. coli*. EX1002, ¶118. Because the periplasmic secretion function of pET-22b is an inherent property, regardless of whether that function is discussed in Mekada, the disclosure of Mekada anticipates this element of claim 1. *SmithKline Beecham*, 403 F.3d at 1343-44; *In re Cruciferous Sprout Litig.*, 301 F.3d at 1350.

In any event, as Dr. Georgiou explains in his declaration, prior to the earliest possible priority date of the '345 patent "[a] POSA would have known that the use of a secretion cloning vector such as pET-22b would have been 'capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a ba[c]terial host cell,' for example, as Mekada directs into *Escherichia coli*." EX1002, ¶118; *In re LeGrice*, 301 F.2d at 936 ("such that a skilled artisan could take its teachings in combination with his own knowledge of the particular art and be in possession of the invention."). Nevertheless, Novagen

confirms this capability of the leader sequence in describing the pET-22b as having "potential periplasmic localization" when used to express heterologous protein. EX1002, ¶118; EX1043, 92.

c. [2b] the 5' signal sequence is not derived from C. diphtheriae

To the extent that the Board considers the Mekada reference as not including information regarding vector sequences encoding a signal peptide not of *C. diphtheriae* origin as claimed, the challenged claims of the '345 patent would nonetheless have been obvious to a POSA in view of commercially available vectors targeting periplasmic secretion, all of which included a signal peptide not of *C. diphtheria* origin. EX1002, ¶118. For example, the signal peptide, PelB, encoded by the pET-22b vector from Novagen shown above was derived from PelB of *E. carotovora,* and as such is "not derived from *C. diphtheriae*." *Id*.

d. Rationale to combine and reasonable expectation of success

At the time of filing the '345 patent, a POSA would have had the motivation as well as a reasonable expectation of success of producing and secreting correctly folded CRM197 diphtheria toxin protein in *E. coli* with the commercially-available pET-22b periplasmic-directed plasmid vector. EX1002, ¶119*;* EX1049, 7; EX1043, 88; Novagen pET-22b Vector Map [EX1045]. Commercial vectors like pET-22b were routinely used well before 2009 to produce and secrete complex

heterologous proteins to the periplasmic space. EX1002, ¶119. A POSA, thus, had motivation, as explicitly directed by Mekada, and a reasonable expectation of success, given the successful use of the pET vector series to express "thousands of different proteins" and confirmed by the Novagen System Tutorial [EX1043 and EX1044], to clone CRM197 into a vector encoding a non-*C. diphtheriae* origin signal sequence for production and secretion into the periplasmic space of an *E. coli* cell which is a bacterial host cell as claimed in claim 1. EX1002, ¶119; EX1043, 88; *E.g.*, *Pfizer, Inc.*, 480 F.3d at 1368; *see generally Kinetic Concepts, Inc. v. Smith & Nephew, Inc.*, 688 F.3d 1342, 1360 (Fed. Cir. 2012). Therefore, claim 1 is at least obvious over Mekada in view of the pET vector series by Novagen.

2. Claims 2, 18, 19, and 21

Claims 2, 18, 19 and 21 depend from claim 1 and recite additional properties of the 3' toxin portion of the claimed polynucleotide. Each of the additional limitations of claims 2, 18, 19 and 21 are obvious over Mekada in view of the Novagen pET vector series. Claim 2 recites "wherein the 3' toxin portion encodes a polypeptide having the amino acid sequence of SEQ ID NO:32." Claim 18 recites "wherein the 3' toxin portion encodes CRM197." Claim 19 recites "wherein the 3' toxin portion encodes a polypeptide having at least 95% sequence identity to SEQ ID NO:32." Mekada disclosed the use of CRM197, which, as discussed

above, Dr. Georgiou confirms that CRM197 or SEQ ID NO:1 (minus the signal sequence of the first 25 amino acid residues) of Mekada is identical to SEQ ID NO:32 of the '345 patent. EX1002, ¶¶93, 121. Accordingly, CRM197 (*i.e.*, SEQ ID NO:32) and sequences at least 95% identical to SEQ ID NO:32 are disclosed by Mekada. EX1002, ¶121.

Claim 21 recites "wherein the 5' signal sequence portion is directly 5' of the 3' toxin portion." Mekada taught CRM197 (*i.e.*, SEQ ID NO:32) can be produced by cloning "into a gene expression vector (pET-22b) for *Escherichia coli*, transforming *Escherichia coli* with the vector to express the mutant in *Escherichia coli*." EX1002, ¶121; Mekada [EX1049] at 7. Moreover, as Dr. Georgiou explains, "a skilled artisan would know that cloning into the pET-22b vector is accomplished by inserting the desired protein to be expressed, in this case CRM197 (*i.e.*, SEQ ID NO:32) directly 3' of the pelB signal sequence. *E.g.*, pET-22b Vector Map [EX1045], rendering the pET-22b pelB signal portion "directly 5' of the 3' toxin portion." EX1002, ¶121.

Accordingly, for at least these reasons, claims 2, 18, 19 and 21 also would have been obvious in over Mekada in view of the Novagen pET plasmid vectors.

H. Ground 3: Claims 4, 6, 8, 12-14, and 17 are Unpatentable as Obvious Over Mekada in View of Novagen pET Plasmid Vectors and Thie

1. Independent Claim 6

Independent claim 6 differs from independent claim 1 in that claim 6 limits the identity of the peptides to variants or modifications (at least 10 amino acids) of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26. EX1002, ¶126. For the same reasons as with claim 1 above (§ V.G.), claim 6 also would have been obvious over the combination of Mekada and Novagen pET plasmid vectors in further view of Thie. EX1002, ¶¶126-131.

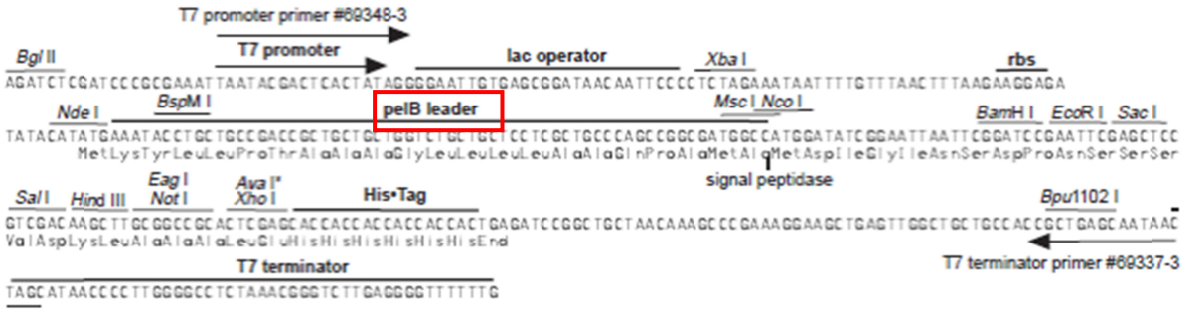
As discussed above (§ V.G.), Mekada taught a "3' toxin portion encodes a mature bacterial toxin polypeptide having an amino acid sequence at least 90% identical to SEQ ID NO:32" (*i.e.*, CRM197). In particular, Mekada taught CRM197 as an agent for treating cancer, and exemplified the administration of CRM197 for treating several cancers in Examples 1-4. EX1002, ¶127. Mekada taught that CRM197 is the mutant diphtheria toxin having a Gly to Glu substitution at position 52. EX1002, ¶127; EX1049, 6. Mekada further taught that "the amino acid (Gly) at position 26 was numbered as No. 1 by removing a signal sequence (1 to 25) in an amino acid sequence in SEQ ID NO:1" and that the "signal sequence of 25 amino acid residues may or may not be included." EX1002, ¶127; EX1049, 4. As Dr. Georgiou explains "[a] sequence alignment of SEQ ID NO:1 (minus the

signal sequence of the 1st-25 amino acid residues) is identical to SEQ ID NO:32 [of the '345 patent]." EX1002, ¶127. Accordingly, CRM197 (*i.e.*, SEQ ID NO:32) was disclosed and used in Mekada.

Further, Mekada taught "a 5' signal sequence portion encoding a polypeptide having an amino acid sequence capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a bacterial host cell." Specifically, examples 1-4 of Mekada taught the production of CRM197 (*i.e.*, SEQ ID NO: 32) for use as a therapeutic agent. EX1002, ¶128. Mekada further disclosed:

> The mutant can be made by incorporating the synthesized DNA into a **gene expression vector (pET-22b) for *Escherichia coli***, transforming *Escherichia coli* with the vector to express the mutant in *Escherichia coli*.

EX1049, 7 (emphasis added); EX1002, ¶128. pET-22b is an expression vector that (i) was commercially available prior to 2009; (ii) was manufactured and sold by Novagen; and (iii) encoded the signal peptide sequence from *Erwinia carotovora* PelB ("pelB leader"):



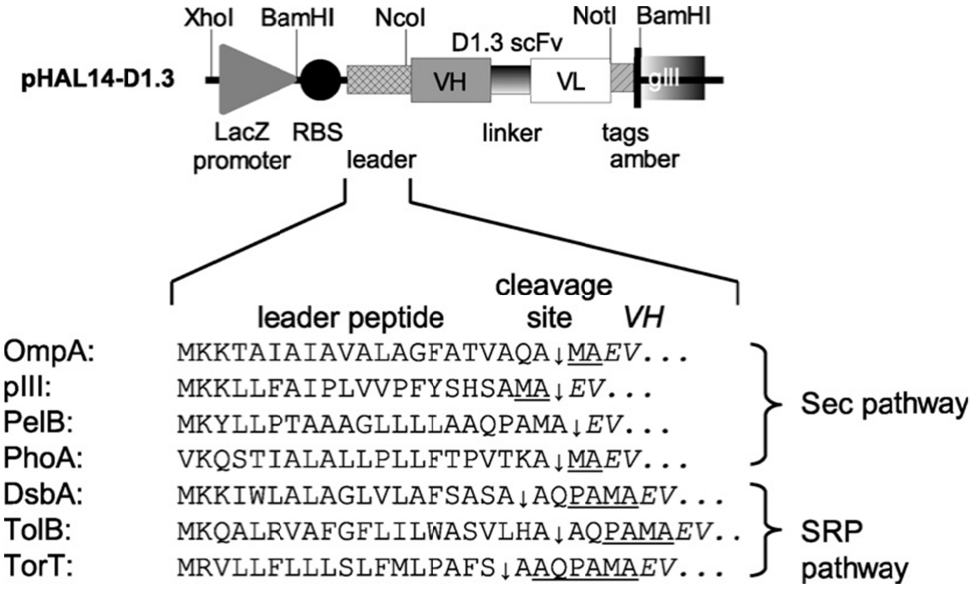
pET-22b(+) cloning/expression region

EX1002, ¶128; EX1045. Moreover, Mekada taught the use of the expression vector pET-22b, which is capable of directing transport of an expressed bacterial toxin polypeptide to the bacterial periplasm when expressed in *E. coli*. EX1002, ¶128. Because the periplasmic secretion function of pET-22b is an inherent property, regardless of whether that function is discussed in Mekada, the disclosure of Mekada anticipates this element of claim 1. *SmithKline Beecham*, 403 F.3d at 1343-44; *In re Cruciferous Sprout Litig.*, 301 F.3d at 1350.

In any event, as Dr. Georgiou explains in his declaration, prior to the earliest possible priority date of the '345 patent "[a] POSA would have known that the use of a secretion cloning vector such as pET-22b would have been 'capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a ba[c]terial host cell,' for example, as Mekada directs into *Escherichia coli*." EX1002, ¶128; *In re LeGrice*, 301 F.2d at 936 ("such that a skilled artisan could take its teachings in combination with his own knowledge of the particular art and be in possession of the invention."). Nevertheless, Novagen

confirms this capability of the leader sequence in describing the pET-22b as having "potential periplasmic localization" when used to express heterologous protein. EX1002, ¶128; EX1043, 92.

Furthermore, a POSA at the time of filing of the '345 patent would have had knowledge of many signal peptides in addition to those leader peptides disclosed in the Novagen pET plasmid vectors that also could have been used to express and secrete heterologous protein into the periplasmic space of *E. coli*. EX1002, ¶129. Thie is one publication that disclosed this, exemplifying a list of signal sequences from the SRP and Sec secretory pathways in *E. coli*, and thus exemplifying the extensive molecular biological tools that were available well prior to 2009 to express and secrete heterologous protein into the periplasmic space. EX1002, ¶129; EX1052, Abstract. Specifically, Thie disclosed the generation of human recombinant antibody fragments and fusions thereof using leader peptides from the SRP and Sec pathways in *E. coli*, including PelB, OmpA, PhoA and PIII (Sec pathway) and DsbA, TorT and TolB (SRP pathway). EX1002, ¶129. Thie tested expression and secretion to the *E. coli* periplasmic space of antibody fragments and fusions thereof using the above leader peptides, which were cloned into a plasmid construct and located just 5' of the heterologous protein cassette:



EX1002, ¶129; EX1052, 51. Thie included in its experiments many of the listed leader peptides that are recited in claim 6 of the '345 patent, including OmpA (SEQ ID NO:6), DsbA (SEQ ID NO: 26), TolB (SEQ ID NO:20) and TorT (SEQ ID NO: 10). Thie thus demonstrated the interchangeability of the PelB leader peptide used in the Novagen pET plasmid vector series, including pET-22b, with other leader peptides, including the claimed OmpA, DsbA, TolB and TorT leader peptides in claim 6 of the '345 patent. EX1002, ¶129.

Thie demonstrated the ease with which different leader peptides, including claimed leader peptides OmpA, DsbA, TolB, and TorT, could have been tested in a routine manner for production and secretion of heterologous protein in *E. coli*, and therefore motivated POSAs to seek leader peptides for purposes of expression and secretion by insertion into the vector of other leader peptides, such as OmpA, DsbA, TolB and TorT. EX1002, ¶¶101, 129. Commercial vectors like pET-22b

were routinely used and modified in the laboratory, including by insertion of different leader peptides, such as OmpA, DsbA, TolB and TorT, well prior to 2009 to produce and secrete complex heterologous proteins in *E. coli*, such as disulfide bond containing proteins. EX1002, ¶130; *Pfizer, Inc.*, 480 F.3d at 1368.

Further, given the successful expression of "thousands of different proteins" using the pET vector series, and the extensive teaching in the art regarding the use of various leader peptides, such as the claimed OmpA, DsbA, TolB and TorT leaders to secrete heterologous protein in *E. coli* expression systems exemplified in Thie, a POSA would have had a reasonable expectation of success of producing and secreting into the periplasmic space correctly folded CRM197 diphtheria toxin protein in *E. coli* using at least OmpA, DsbA, TolB and TorT leader peptides. EX1002, ¶130; *see generally KSR*, 550 U.S. at 416 ("The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.").

For at least the reasons above, a POSA would have been motivated based on the explicit direction of Mekada, to clone CRM197 into a vector encoding a non-*C. diphtheriae* origin signal sequence including OmpA, DsbA, TolB and TorT for production and secretion into the periplasmic space of the *E. coli* host cell. EX1002, ¶131. Further a POSA would have had a reasonable expectation of success in achieving a properly folded and secreted CRM197 protein, given the

POSA's familiarity and experience with these vectors and methods and confirmed by the Novagen pET plasmid vectors and Thie. EX1002, ¶131. Accordingly, claim 6 is also obvious over Mekada in view of the Novagen pET plasmid vectors and Thie.

2. Dependent Claims 4 and 8

Claims 4 and 8 include the same claim limitations as in claim 6 for selection of the signal peptide, *i.e.*, the signal peptide list including SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 and 26. For the same reason as above as for claim 6, claims 4 and 8 also would have been obvious over Mekada in view of the Novagen pET plasmid vectors and Thie.

As discussed above, Thie specifically taught many of the leader peptides recited in claims 4 and 8 of the '345 patent, including OmpA (SEQ ID NO:6), DsbA (SEQ ID NO: 26), TolB (SEQ ID NO:20), and TorT (SEQ ID NO: 10). EX1002, ¶133. Thie further demonstrated the interchangeability of the PelB leader peptide used in the Novagen pET plasmid vector series, including pET-22b, with other leader peptides, including the claimed OmpA, DsbA, TolB and TorT leader peptides in claims 4 and 8 of the '345 patent. EX1002, ¶133.

Further, a POSA would have been motivated to use other signal peptides, such as OmpA, DsbA, TolB and TorT, for expression and secretion of CRM197 into the *E. coli* periplasmic space, given the ease with which the signal peptide

leaders could be tested in a routine manner for production and secretion of the heterologous protein in *E. coli*. EX1002, ¶134. *See generally KSR*, 550 U.S. at 416 ("The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.").

A POSA would have had a reasonable expectation of success in achieving a properly folded and secreted CRM197 protein, given the POSA's familiarity and experience with these vectors and methods and confirmed by the Novagen pET plasmid vectors and Thie. EX1002, ¶134. *Pfizer, Inc.*, 480 F.3d at 1364 ("[T]he expectation of success need only be reasonable, not absolute.")

For at least the foregoing reasons, a POSA would have been motivated, by the explicit direction of Mekada, to use pET-22b containing a non-*C. diphtheriae* origin signal sequence including OmpA, DsbA, TolB and TorT as exemplified by Thie for production and secretion into the periplasmic space of the *E. coli* host cell of properly folded CRM197. EX1002, ¶¶133-134. Thus, claims 4 and 8 also would have been obvious over Mekada in view of the Novagen pET plasmid vectors and Thie.

3. Dependent Claims 12, 13, 14 and 17 are Obvious Over Mekada in View of Novagen pET Plasmid Vectors and Thie

Claims 12, 13, 14 and 17 each depend from claim 6, and further recite CRM197 (SEQ ID NO:32) variations. Claim 12 recites "wherein the 3' toxin portion encodes CRM197." Claim 13 recites "wherein the 3' toxin portion encodes

a polypeptide having at least 95% sequence identity to SEQ ID NO: 32." Claim 14 recites "wherein the 3' toxin portion encodes a polypeptide having the amino acid sequence of SEQ ID NO: 32." Claim 17 recites "wherein the 5' signal sequence portion is directly 5' of the 3' toxin portion." EX1002, ¶135.

For the same reasons as in § V.H.1., above, each of the additional limitations of claims 12, 13, 14 and 17 also would have been obvious over Mekada in view of the Novagen pET plasmid vectors and Thie. EX1002, ¶¶135-136. Mekada disclosed the use of CRM197, which as discussed above, Dr. Georgiou confirms that CRM197 or SEQ ID NO:1 (minus the signal sequence of the 1st-25 amino acid residues) of Mekada is identical to SEQ ID NO:32 [of the '345 patent]. Accordingly, CRM197 (i.e., SEQ ID NO:32) and sequences at least 95% identical to SEQ ID NO:32 were disclosed by Mekada. EX1002, ¶136.

Mekada further taught CRM197 (i.e., SEQ ID NO:32) can be produced by cloning "into a gene expression vector (pET-22b) for *Escherichia coli*, transforming *Escherichia coli* with the vector to express the mutant in *Escherichia coli*." EX1002, ¶136; Mekada [EX1049] at 7. Moreover, as Dr. Georgiou explains, "a skilled artisan would know that cloning into the pET-22b vector is accomplished by inserting the desired protein to be expressed, in this case CRM197 (*i.e.*, SEQ ID NO:32) directly 3' of the pelB signal sequence. *E.g.*, pET-22b Vector Map [EX1045], rendering the pET-22b pelB signal portion "directly 5'

of the 3' toxin portion." EX1002, ¶136. As detailed above for Thie, a POSA would have had the knowledge and motivation to utilize other leader peptides, such as OmpA (SEQ ID NO:6), DsbA (SEQ ID NO: 26), TolB (SEQ ID NO:20) and TorT (SEQ ID NO: 10), for expression and secretion of heterologous protein into the *E. coli* periplasmic space. EX1002, ¶136; EX1052, 51.

Accordingly, for these reasons, claims 12, 13, 14 and 17 also would have been obvious over Mekada in view of the Novagen pET plasmid vectors and Thie.

I. Secondary Considerations

Pfenex is unaware of any objective evidence of nonobviousness that would outweigh a conclusion of obviousness of the claims.

VI. CONCLUSION

The challenged claims are unpatentable. Pfenex respectfully requests that IPR be instituted.

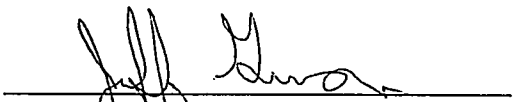
Dated: May 6, 2019

Jeffrey W. Guise, Reg. No. 34,613

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

Under the provisions of 37 C.F.R. §42.24(d), the undersigned hereby certifies that the word count for the foregoing Petition for Inter Partes Review totals 10,245, which is less than the 14,000 allowed under 37 C.F.R. 42.24(a)(i). In accordance with 37 C.F.R. 42.24(a), this word count does not include table of contents, table of authorities, mandatory notices under §42.8, certificate of service or word count, or appendix of exhibits or claim listing.

Dated: May 6, 2019



Jeffrey W. Guise, Reg. No. 34,613

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§42.6(e) and 42.105, I certify that I caused to be served a true and correct copy of the foregoing: **PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 9,422,345 and Exhibits 1001-1061** by *Federal Express Next Business Day Delivery* on May 6, 2019 on the Patent Owner's correspondence address of record for the subject patent as follows:

Respectfully submitted,

Dated: May 6, 2019

Jeffrey W. Guise, Reg. No. 34,613