UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MERCK SHARP & DOHME CORP.,
Petitioner
v.
GLAXOSMITHKLINE BIOLOGICALS S.A.,
Patent Owner

CASE IPR: Unassigned

PETITION FOR *INTER PARTES* REVIEW OF
U.S. PATENT NO. 9,422,345
Claims 1-2, 4-14, and 16-21
UNDER 35 U.S.C. §§ 311-319 and 37 C.F.R. §§ 42.1-.80, 42.100-.123
(Collier-Neville)

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TABLE OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
BRC	broadest reasonable construction
CRM	cross-reacting material
CSP	consensus signal peptide
DTx	diphtheria toxin
IPR	*Inter partes* review
MCS	multiple cloning site
mRNA	messenger RNA
ORF	open reading frame
POSA	person of ordinary skill in the art
Sec	Secretory
SRP	signal recognition particle

EXHIBIT LIST

Exhibit No.	Description
1001	United States Patent No. 9,422,345 (Blais et al.) (“the ’345 patent”)
1002	Prosecution History of United States Patent No. 9,422,345 (USSN 13/500,244)
1003	International Publication No. WO 2011/042516A2 published on April 14, 2011 (Blais, et al.)
1004	Great Britain Patent Application No. 0917647.0 (GlaxoSmithKline Biologicals S.A.)
1005	United States Patent No. 6,455,673 (Collier) (“Collier-1”)
1006	Declaration of Matthew P. DeLisa, Ph.D. dated November 2, 2018
1007	United States Patent Application Publication No. US2003/0157093A1 (Neville, Jr., et al) (“Neville”)
1008	Huber, et al., “Use of Thioredoxin as a Reporter to Identify a Subset of *Escherichia coli* Signal Sequences That Promote Signal Recognition Particle-Dependent Translocation,” *J. Bacteriol. 187*(9), 2983-91 (May 2005) (“Huber”)
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1011	Giannini, et al., “The Amino-Acid Sequence of Two Non-Toxic Mutants of Diphtheria Toxin: CRM45 and CRM197,” *Nucleic Acids Research 12*(10), 4063-69 (1984) (“Giannini-1”)
1012	pET-22B(+) Vector Map (https://web.archive.org/web/20030819085202/http://www.novagen.com:80/docs/NDIS/69744-000.pdf)
1013	Oh, et al., “Translocation of the Catalytic Domain of Diphtheria Toxin Across Planar Phospholipid Bilayers by Its Own T Domain,” *Proc. Natl. Acad. Sci. USA 96*, 8467-70 (July 1999)
1014	Uchida, et al., “Diphtheria Toxin and Related Proteins,” *J. Biological Chem. 248*(11), 3838-44 (June 10, 1973)
1015	Mekada, et al., “Binding Properties of Diphtheria Toxin to Cells Are Altered by Mutation in the Fragment A Domain,” *J. Biological Chem. 260*(22), 12148-53 (Oct. 5, 1985)

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1017	HibTITER®, *2007 Physicians' Desk Reference*, 61st ed., 3426-29 (Thomson PDR, Montvale, N.J., 2007)
1018	PREVNAR®, *2008 Physicians' Desk Reference*, 62nd ed., 3405-11 (Thomson Healthcare Inc., Montvale, N.J., 2007)
1019	Tweten, et al., "Molecular Cloning and Expression of Gene Fragments from Corynebacteriophage β Encoding Enzymatically Active Peptides of Diphtheria Toxin," *J. Bacteriology 156*(2), 680-85 (Nov. 1983)
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1021	Novagen, pET System Manual, 11th ed. (2006)
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1024	Lei, et al., "Characterization of the *Erwinia carotovora pelB* Gene and Its Product Pectate Lyase," *J. Bacteriol. 169*(9), 4379-83 (Sept. 1987)
1025	Sletta, et al., "The Presence of N-Terminal Secretion Signal Sequences Leads to Strong Stimulation of the Total Expression Levels of Three Tested Medically Important Proteins During High-Cell-Density Cultivations of *Escherichia coli*," *Applied & Environmental Microbiology 73*(3), 906-12 (Feb. 2007)
1026	Papini, et al., "Diphtheria Toxin and Its Mutant *crm* 197 Differ in Their Interaction with Lipids," *FEBS Letters 215*(1), 73-78 (May 1987)
1027	Ikemura, et al., "Requirement of Pro-Sequence for the Production of Active Subtilisin E in *Escherichia coli*," *J. Biological Chem. 262*(16), 7859-64 (June 5, 1987) ("Ikemura")

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1032	Kaczorek, et al., "Nucleotide Sequence and Expression of the Diphtheria *tox*228 Gene in *Escherichia coli*," *Science 221*, 855-58 (Aug. 26, 1983)
1033	Lees, et al., "Conjugation Chemistry," *Pneumococcal Vaccines: The Impact of Conjugate Vaccine*, Chap. 11, 163-74 (ASM Press, Washington, D.C., 2008)
1034	United States Patent Application Publication No. US2012/0289688A1 (Blais et al.)
1035	*Merriam-Webster's Collegiate Dictionary*, 10th ed., 168 ("capable") (Merriam-Webster, Incorporated, Springfield, Mass., 1997)
1036	Zhou, et al., "Secretory Expression of Recombinant Diphtheria Toxin Mutants in *B. Subtilis*," *J. Tongji Med. Univ. 19*(4), 253-56 (1999)
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1038	Lewin, *Genes IX*, 246-49 (Jones and Bartlett Publishers, Inc., Boston, Mass., 2008)
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1043	Tan, et al., "*E. coli* Selection of Human Genes Encoding Secreted and Membrane Proteins Based on cDNA Fusions to a Leaderless *β-Lactamase* Reporter," *Genome Research 13*, 1938-43 (Cold Spring Harbor Lab. Press, 2003)

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1051	United States Patent Application Publication No. US2008/0193974A1 (Coleman, et al.)
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1063	Prosecution History excerpt of European Patent Application No. 16192258.8, August 16, 2018 GlaxoSmithKline Response to April 18, 2018 European Patent Office Communication (https://register.epo.org/application?number=EP16192258&d-16544-o=2&Ing=en&tab=doclist&d-16544-s=1)
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1065	Schäffner, et al., "Cosecretion of Chaperones and Low-Molecular-Size Medium Additives Increases the Yield of Recombinant Disulfide-Bridged Proteins," *Applied & Environmental Microbiology 67*(9), 3994-4000 (Sept. 2001)
1066	Chatel, et al., "Expression of a Lipocalin in Prokaryote and Eukaryote Cells: Quantification and Structural Characterization of Recombinant Bovine β-Lactoglobulin," *Protein Expression and Purification 16*, 70-75 (1999)

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1068	Retallack, et al., "Transport of Heterologous Proteins to the Periplasmic Space of *Pseudomonas fluorescens* Using a Variety of Native Signal Sequences," *Biotechnol. Lett. 29*, 1483-91 (2007) ("Retallack (2007)")
1069	Horton et al., "Gene Splicing by Overlap Extension," Methods in Enzymology, 217, 270-279 (1993)
1070	Heckman, et al., "Gene Splicing and Mutagenesis by PCR-Driven Overlap Extension," Nature Protocols 2(4), 924-32 (2007)
1071	INTENTIONALLY NOT USED
1072	INTENTIONALLY NOT USED
1073	Declaration of Catharina J. Chin Eng dated November 5, 2018
1074	Declaration of John T. Haines dated October 25, 2018
1075	Merck Press Release, "Pfenex Grants Exclusive License to Merck & Co., Inc. for the Production of Proteins for Undisclosed Vaccine Program," dated December 14, 2009

<u>**37 C.F.R. § 42.8 MANDATORY NOTICES**</u>

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

a. ***Related Matters* (37 C.F.R. § 42.8(b)(2))**. Petitioner is concurrently filing another petition for *Inter Partes* Review against the '345 patent on distinct grounds. Petitioner has also filed two petitions for *Inter Partes* Review of U.S. Patent No. 8,753,645 (IPR Nos. 2018-01229 & -01236) and two petitions for *Inter Partes* Review of U.S. Patent No. 9,265,839 (IPR Nos. 2018-01234 & -01237).

b. ***Real Party-In-Interest* (37 C.F.R. § 42.8(b)(1))**. The real parties-in-interest ("RPI") are Petitioner Merck Sharp & Dohme Corp. and Merck & Co., Inc. Petitioner is not barred by operation of estoppel to submit this petition for *inter partes* review.

Patent Owner alleges in the related proceedings that Pfenex, Inc. ("Pfenex") is a RPI to the above-mentioned related matters merely because Patent Owner believes that Pfenex has some interest in Petitioner bringing its V114 product to market. This argument impermissibly expands the RPI requirement. The two questions lying at "the heart" of the RPI inquiry are whether a petition "has been filed at a nonparty's behest" and whether the non-party "desires review of the patent." *Applications in Internet Time, LLC v. RPX Corp.*, 897 F.3d 1336, 1351 (Fed. Cir. 2018). Here, neither factor weighs in favor of a finding that Pfenex is an RPI.

First, the lack of evidence that this petition was filed at the behest of Pfenex, as well as the "practical situation" here – *i.e.*, the *implausibility of* Merck filing this petition to serve Pfenex — make clear that the "RPI behest-of test" is not satisfied. *See AIT*, 897 F.3d at 1349 n.3, 1351. Merck is one of the largest pharmaceutical companies in the world (with $40 Billion in revenue in 2017), and has plans to seek regulatory approval for the sale of its V114 product. Merck is challenging the validity of the '345 patents in anticipation of a future launch of that product. Merck has not filed these petitions at the behest of Pfenex, a small company (with less than $30 Million in revenue in 2017) that has merely licensed Merck technology.

Second, Pfenex is in the business of licensing its Pfenex Expression Technology™. (Exh. 1075). Pfenex has not been, and will not be, involved in making, using, offering for sale, selling or importing Merck's proposed V114 product or any portion of that product. (Exh. 1074, ¶ 6). Licensing technology to Merck is not making, using, offering for sale, selling or importing that technology, and is not otherwise an act of infringement. *See* 35 U.S.C. § 271(a). Instead, it merely authorizes Merck to use that technology without fear of facing an infringement suit *from Pfenex*. Accordingly, Pfenex has no incentive to attack any claim of the challenged patents based on Merck's making, using, offering for sale, selling or importing Merck's proposed V114 product or any portion of that product.

These facts lay in stark contrast to those of *AIT*. The *AIT* Petitioner, RPX, was in the business of challenging patents for other companies. 897 F.3d at 1351-52. RPX filed its petition on behalf of a company, Salesforce.com, that was statutorily time-barred[1] from filing an IPR in its own name. *Id*. at 1353. This was exactly the type of end-run around the IPR estoppel/time bar provisions that the RPI requirement was designed to prevent.

In the related proceedings mentioned above, Patent Owner cites to other cases that are easily distinguished. Unlike *Sirius XM Radio* (POPR at 20), Merck and Pfenex have no similar common ownership, management overlap, or history of coordinating legal matters suggesting that Merck filed this Petition at Pfenex's behest. No. IPR2018-00681, Paper 12 at 6 (P.T.A.B. Sept. 6, 2018). As for *Worlds Inc. v. Bungie, Inc.*, (1) the alleged RPI was time barred from the IPR; (2) products made by that petitioner were being added to litigation against the RPI; and (3) the

[1] There are no time bar issues associated with Merck's petition. Moreover, "if a petition fails to identify all real parties in interest under § 312(a)(2), the Director can, and does, allow the petitioner to add a real party in interest." *Google LLC v. Seven Networks, LLC*, No. IPR2018-01047, Paper 11 at 4 (P.T.A.B. Sept. 25, 2018). Thus, not naming Pfenex as an RPI does not warrant denying institution even if GSK's RPI arguments are valid, which they are not.

same five patents were at issue in litigation and the IPR. 903 F.3d 1237, 1244 (Fed. Cir. 2018). No such fact pattern is posed here. Accordingly, Pfenex is not an RPI to this petition.

c. ***Designation of Lead and Back-Up Counsel and Service Information*** **(37 C.F.R. § 42.8(b)(3)-(4))**. Petitioner identifies the following:

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I. INTRODUCTION

Merck Sharp & Dohme Corp. ("Petitioner") requests *inter partes* review of independent claims 1 and 6, and dependent claims 2, 4-5, 7-14 and 16-21 of U.S. Patent No. 9,422,345 ("the '345 patent") (Exh. 1001), assigned to GlaxoSmithKline Biologicals S.A. ("Patent Owner" or "GSK"). This petition demonstrates that there is a reasonable likelihood that Petitioner will prevail since the prior art renders all the challenged claims anticipated or obvious, under 35 U.S.C. §§ 102 or 103, by a preponderance of the evidence.

For decades, "carrier" proteins have been coupled to polysaccharides to create vaccines that boost human immune response. CRM197 protein is a diphtheria toxin ("DTx") mutant and one of the most well-known carrier proteins in prior art commercial vaccines. The '345 patent claims are directed to polynucleotides that make CRM197 (and related polypeptides at least 90% identical to CRM197) with well-known techniques of bacterial recombinant expression. There is nothing new or nonobvious about the '345 patent claims.

The '345 patent attempts to distinguish itself from the prior art by directing production of CRM197 (and related) proteins into the bacterial periplasm. But, the inventors of the '345 patent did not invent periplasmic expression of proteins (including DTx proteins). The prior art taught the benefit of directing protein transport to the favorable environment of the bacterial periplasm (*e.g.,* for properly

folded proteins). Indeed, the state of the art was so well developed in this area that commercial suppliers marketed their polynucleotides to POSAs based on their ability to direct transport of proteins to the periplasm. Furthermore, these polynucleotides were used for producing DTx proteins.

Claim 1 is directed to a polynucleotide with the following well-known features: (1) a "3' portion" (*i.e.*, downstream portion) encoding a polypeptide at least 90% identical to CRM197; and (2) a "5' portion" (*i.e.*, upstream portion) encoding a signal sequence that is capable of directing transport to the periplasm and is not derived from C. *diphtheriae*. As discussed below, claim 1 is separately anticipated by at least two references: Collier-1 (2002) (Exh. 1005) and Neville (2003) (Exh. 1007).

Collier-1 is a United States patent that discloses diphtheria toxin polypeptides as vaccines and their production in bacteria. Collier-1 discloses polynucleotides encoding polypeptides with at least 98% identity to CRM197, and a PelB signal sequence (which is not derived from C. *diphtheria*) that is capable of directing transport to the periplasm. Neville is a published U.S. Patent Application that also discloses polynucleotides meeting all the requirements of the '345 patent claims. Neville discloses polynucleotides encoding CRM197, and signal sequences (including PelB) that are capable of directing transport to the periplasm.

The other claims of the ’345 patent do not add any novel and nonobvious features. For example, claims 2 and 18, which limit the 3' portion of the polynucleotide to those that encode polypeptides having the amino acid sequence of CRM197, are anticipated by Neville (which discloses polynucleotides encoding CRM197). Also, claims 2 and 18 would have been obvious over Giannini-1 (Exh. 1011), in view of Collier-1. Giannini-1 directs POSAs to the therapeutic importance of CRM197 and appears on the face of the Collier-1 reference, demonstrating that its teachings are closely related to Collier-1. Moreover, POSAs would have been motivated by Collier-1’s disclosure of periplasmic transport in *E. coli* to generate polynucleotides that encode CRM197 for periplasmic transport with a reasonable expectation of success.

Likewise, claim 21, wherein the 5’ signal sequence portion is directly 5’ of the 3’ toxin portion, would have been obvious over Collier-1 in view of the state of the art, as exemplified by Sambrook (1989) (Exh. 1029) at 15.51-15.62, Horton (1993) (Exh. 1069) at 270-79, and Heckman (2007) (Exh. 1070) at 924-32. Collier-1 teaches that its DTx mutant polypeptides can be used as vaccines. Basic regulatory and vaccine related considerations would have motivated POSAs to make polynucleotides having this claimed feature in order to produce polypeptides that are as similar to the native polypeptides as possible (while preserving their non-toxic

nature). Also, routine techniques for preparing polynucleotides having this claimed feature were well-known.

Similarly, claims 4-14, 16-17 and 20 specify that the 5' portion encodes particularly recited, known signal sequences. Huber (2005) (Exh. 1008) teaches the same signal sequences recited in these claims and demonstrates they are capable of directing transport of a polypeptide to the periplasm. Consequently, POSAs would have been motivated to combine the teaching of either Collier-1 or Neville with Huber to arrive at the polynucleotides of claims 4-14, 16-17 and 20 with a reasonable expectation of success.

In view of the foregoing, Petitioner respectfully submits there is at least a reasonable likelihood that it will prevail in showing at least one of the challenged claims is unpatentable. In support of the proposed grounds for unpatentability, this Petition is accompanied by the declaration of Matthew P. DeLisa, Ph.D. (Exh. 1006), an expert in recombinant polypeptide production.

II. REQUIREMENTS FOR REVIEW

Pursuant to 37 C.F.R. § 42.104, Petitioner states as follows:

A. Grounds For Standing

Petitioner certifies that: (1) the '345 patent is available for IPR; and (2) Petitioner is not barred or estopped from requesting review of any claim on the

grounds identified in this Petition. 37 C.F.R. § 42.104(a). The Office is authorized to charge all fees due in connection with this matter to Deposit Account No. 50-3013.

B. Identification Of Challenge

Pursuant to 37 C.F.R. §§ 42.104(b) and 42.22(a)(1), Petitioner requests review and cancellation of claims 1-2, 4-14 and 16-21 of the '345 patent pursuant to the following statement of precise relief requested:

Ground	Claims	Basis	Reference(s)
I	1 and 19	Pre-AIA 102(b)	• Collier-1
II	1, 2, 18, 19 and 21	Pre-AIA 102(b)	• Neville
III	2 and 18	Pre-AIA 103	• Collier-1 • Giannini-1
IV	4-14, 16, 17 and 20	Pre-AIA 103	• Collier-1 • Huber
V	21	Pre-AIA 103	• Collier-1 • State of the art, as exemplified by Sambrook (1989), Horton (1993) and Heckman (2007)
VI	4-14, 16, 17 and 20	Pre-AIA 103	• Neville • Huber

III. PERSON OF ORDINARY SKILL IN THE ART ("POSA")

The field of the invention is directed to polypeptide expression in bacteria using recombinant DNA technology. As confirmed by Dr. DeLisa, as of either October 8, 2009 or October 7, 2010, POSAs would have: (a) possessed or have been

pursuing a post-undergraduate degree, *e.g.*, Ph.D., in Bioengineering, Biomedical Engineering, Biomolecular Engineering, Chemical Engineering, Biotechnology, Biochemistry, Microbiology, Molecular Biology, or a comparable discipline, and (b) had at least 2-3 years of research experience relating to recombinant protein expression in bacteria. (Exh. 1006, ¶ 22).

IV. STATE OF THE ART

A. Diphtheria Toxin And CRM197

For decades, CRM197 has been used as a carrier protein in glycoconjugate vaccines for increasing the immunogenicity of saccharides. (Exh. 1006, ¶ 30; Exh. 1016, 233; Exh. 1017, 3426; Exh. 1018, 3405; Exh. 1033, 164). This includes use in prior art pneumococcal vaccines made and sold in the United States and abroad. (*See, e.g.*, Exh. 1018).

CRM197, first identified 45 years ago, is a non-toxic form of diphtheria toxin ("DTx"), a protein secreted by *Corynebacterium diphtheriae* bacteria. (Exh. 1014, 3838). CRM197 differs from DTx by one amino acid. (Exh. 1011, 4063-64, 4067-68). Both DTx and CRM197 are made of two subunits joined by disulfide bonds. (Exh. 1015, 12148; Exh. 1026, 74, Fig. 1). Well before the '345 patent's earliest filing date, the amino acid sequences of native DTx and CRM197 were known and used, as were polynucleotide sequences encoding these polypeptides. (Exh. 1011, 4065-66; Exh. 1050, 6592; Exh. 1055, 6855; Exh. 1006, ¶¶ 28-29).

B. Bacterial Recombinant Polypeptide Production Systems Were Well-Known And Diphtheria Toxin Expression Was Routine

For decades before the ’345 patent’s earliest filing date, recombinant DNA technology—by which DNA sequences that do not exist in nature are made and used—facilitated the expression of polypeptides of interest in bacteria. (Exh. 1061, 405-33; Exh. 1029, 5.1-5.95, 17.1-17.44; s*ee also* Exh. 1020, 211; Exh. 1021, 3-5; Exh. 1031, 3240; Exh. 1069, 270-79; Exh. 1070, 924-32; Exh. 1006, ¶¶ 31-39, Appendix C). By the ’345 patent’s earliest filing date, commonly-used, undergraduate-level molecular biology textbooks described this technology. (Exh. 1037, 532; Exh. 1038, 246; Exh. 1006, ¶ 31).[2]

By the ’345 patent’s earliest filing date, bacterial recombinant expression systems routinely produced DTx mutants, including CRM197. (*See*, *e.g.,* Exh. 1032, 855; Exh. 1023, 15016; Exh. 1019, 680, 685; Exh. 1044, 1647-49; Exh. 1049, 5141, 5143; Exh. 1028, 343; Exh. 1036, 253; Exh. 1013, 8467; Exh. 1067, 370-71; Exh. 1030, 6:15-7:22; Exh. 1005, 4:64-6:3; Exh. 1006, ¶ 32).

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[2] One well-known recombinant DNA approach for producing polypeptides in bacterial host cells involved the use of a collection of polynucleotides organized into a "plasmid” DNA expression vector that bacteria recognize and process. (Exh. 1037, 540-41, 546-48; Exh. 1006, ¶ 35, Fig. 1).

It was common for recombinant expression of DTx mutants to involve transport of the toxin polypeptides to the bacterial periplasm, which was known to be favorable for the expression of many polypeptides. In particular, commercially available polynucleotide expression vectors[3] with signal sequences capable of directing periplasmic transport of polypeptides were used to produce DTx mutants with greater than 98% identity to CRM197. (Exh. 1013, 8467; Exh. 1067, 370-71; Exh. 1005, 4:64-6:3; Exh. 1006, ¶ 33).

C. POSAs Knew The Advantages Of Transporting Polypeptides Through The Cytoplasmic Membrane To The Bacterial Periplasm

Certain bacteria, referred to as "gram negative" bacteria, *e.g.*, *Escherichia coli* and *Pseudomonas fluorescens* (Exh. 1020, 211, 217-19), contain a lipid bilayer membrane, referred to as the cytoplasmic (also, "inner" or "plasma") membrane, adjacent to the cell cytoplasm and an outer membrane (composed of lipopolysaccharide and protein) adjacent to the extracellular milieu. (Exh. 1037,

[3] Terms such as "expression vector," "expression construct" and "translation vector" are used interchangeably in the literature (and herein) to refer to a polynucleotide designed to express a protein of interest. (Exh. 1006, ¶ 33 n.4). As used herein, reference to a polynucleotide "vector" or a polynucleotide "construct" refers to an expression vector, unless otherwise indicated.

617-18). The space created between the two membranes is referred to as the "periplasm" or "periplasmic space." (*See* Exh. 1038, 247, Fig. 10.44; Fig. 1 below; Exh. 1006, ¶ 40).

Polypeptide translation begins in the cytoplasm. Long before the '345 patent, POSAs understood that polypeptides produced by a bacterial cell could be transported[4] through the cytoplasmic membrane to the periplasm. (Exh. 1038, 247, Fig. 10.44; Exh. 1006, ¶ 41). As noted below, polypeptide transport to the periplasm can occur after the polypeptide has been fully translated or while the polypeptide is still being translated.

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[4] The art uses different terms to refer to the process of transport of a polypeptide through the cytoplasmic membrane to the periplasm, including "export," "secretion," "translocation" or "expression" of the polypeptide in or to the periplasm; and to "periplasmic expression." (*See, e.g.*, Exh. 1027, 7859 ("secreted into the periplasmic space"); Exh. 1008, 2983 ("export to the periplasm"; "translocation of these proteins across the cytoplasmic membrane"); Exh. 1020, 214 ("[p]eriplasmic expression")). Thus, unless otherwise noted, these terms and similar ones will be treated interchangeably.

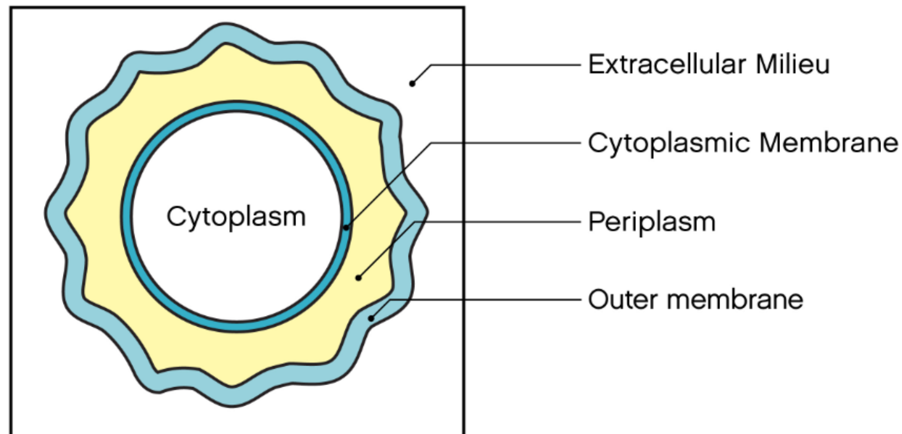

Figure 1. Representative bacterial periplasm in a gram-negative bacterium (derived from Exh. 1056, 68).

Three basic components are responsible for the transport of most polypeptides across the cytoplasmic membrane to the periplasm: 1) transport machinery of the cell in which expression occurs; 2) a signal sequence attached at the N-terminus of the protein of interest,[5] and 3) exportability of the protein of interest, *i.e.*, whether the protein is intrinsically compatible with the cell's transport machinery, such that the polypeptide can be transported. (Exh. 1057, 4-5; Exh. 1064, 347; Exh. 1008, 2983-84; Exh. 1006, ¶ 42).

As the '345 patent discloses in a discussion of the prior art, POSAs knew the advantages of directing transport to the periplasm: (1) the oxidizing environment of

[5] A polypeptide has two ends: a leading end or amino terminus ("N-terminus"), and a carboxyl terminus ("C-terminus"). (Exh. 1037, 128).

the periplasm that "allows the formation of disulfide bonds" which "may help produce soluble, correctly folded proteins," (Exh. 1001, 2:39-41; *see also* Exh. 1015, 12148; Exh. 1021, 57; Exh. 1020, 215), (2) the presence of fewer proteases (enzymes that break down proteins) than the cytoplasm (Exh. 1001, 2:42-44; Exh. 1059, 1830-33), and (3) ease of polypeptide recovery (Exh. 1001, 2:45-46; Exh. 1005, 7:14-18). Moreover, it was known that periplasmic transport produced polypeptides with authentic N-termini, which is usually required for polypeptides used as human therapeutics. (Exh. 1064, 346). As a result, as of the '345 patent's earliest filing date, production and use of polynucleotides for "export of proteins from bacterial cytoplasm [was] widely employed." (Exh. 1020, 214; Exh. 1006, ¶ 43).

D. The Pathways That Cells Use For Polypeptide Transport Across Cytoplasmic Membranes Were Well-Known

The pathways for transporting polypeptides across the cytoplasmic membrane and into the periplasm were known before the earliest filing date of the '345 patent. The transport pathways have been maintained throughout evolutionary history (*i.e.,* are highly conserved) among species, including from bacteria to humans. (Exh. 1022, 1726-27; Exh. 1040, 36; Exh. 1060, 17; Exh. 1006, ¶ 44). The vast majority of polypeptides directed across the cytoplasmic membrane utilize the general secretory ("Sec") pathway (Exh. 1048, 6115), or the very similar SRP ("signal recognition particle") pathway, which is often regarded as a sub-category of the Sec pathway (Exh. 1022, 1726; Exh. 1060, 18-19; Exh. 1051, [0029]; Exh. 1006, ¶ 45).

Functionally, the main difference between the Sec and SRP pathways is that polypeptides transported via the Sec pathway are first fully translated in the cytoplasm (and, therefore, transport occurs post-translationally), while via the SRP pathway, a growing polypeptide is transported to the periplasm co-translationally, that is, while the polypeptide is still being translated. (Exh. 1057, 3, 8-9). Nonetheless, the Sec and SRP pathways feature the same basic components for protein transport across the cytoplasmic membrane discussed above—the transport machinery, a signal sequence, and an export-compatible protein of interest. Both pathways converge at the same core machinery (a complex of the SecY, SecE, and SecG proteins (collectively, "SecYEG translocon")) and require the presence of a signal sequence attached to the N-terminus of the polypeptide to be transported. (Exh. 1060, 19, Fig. 1; Exh. 1057, 3-5, 9-10; Exh. 1020, 215; Exh. 1022, 1726-27, 1741). The signal sequences used by the pathways are highly similar. (Exh. 1057, 4-5; Exh. 1008, 2988; Exh. 1006, ¶¶ 46-47).

E. The Purpose, Identity And Use Of Signal Sequences Were Well-Known

The purpose of a signal sequence is to direct polypeptides across the cytoplasmic membrane to the periplasm. (Exh. 1020, 215; Exh. 1022, 1741). When a signal sequence is attached to the N-terminus of a polypeptide intrinsically compatible with the host cell's transport machinery (*i.e.*, a protein that is "exportable"), the signal sequence directs transport of the polypeptide to the

periplasm. This ability of signal sequences to direct protein transport in cells had been known for decades prior to the earliest filing date of the '345 patent. (*See* Exh. 1023, 15016; Exh. 1024, 4380-82; Exh. 1006, ¶¶ 48 & 50).

1. POSAs Knew of a Variety of Signal Sequences Capable of Directing Transport of Polypeptides Through the Cytoplasmic Membrane

By the time of the '345 patent's earliest filing date, a variety of signal sequences[6] were commonly used for transport of polypeptides into the periplasm. (Exh. 1020, 215 (listing thirteen signal sequences "in use for secretion of heterologously expressed proteins"); Exh. 1008, 2988 (demonstrating use of signal sequences, including nine of the ten *E. coli* signal sequences recited in the '345 patent claims, to direct transport of a protein to the *E. coli* periplasm); Exh. 1045, 7038-39; Exh. 1025, 906-08 (demonstrating the use of PelB, OmpA and synthetic CSP ("consensus signal peptide") signal sequences, for periplasmic transport of human proteins in *E. coli*); Exh. 1068, 1486, 1489-90 (demonstrating periplasmic transport of a variety of proteins in both *E. coli* using a PelB signal sequence encoded by the pET22b vector (*see* below) and in *P. fluorescens* using seven different *P. fluorescens* signal sequences); Exh. 1006, ¶ 52).

[6] The signal sequence literature uses the terms "signal sequence," "leader sequence," and "signal peptide" interchangeably.

It was known that the signal sequence and polypeptide could originate from different species, and that signal sequences from one species could be used for polypeptide transport in a different species. (*See*, *e.g.*, Exh. 1013, 8467-68; Exh. 1067, 370-71; Exh. 1005, 4:64-6:3 (*Erwinia carotovora* PelB signal sequence directs transport of DTx mutants to *E. coli* periplasm); Exh. 1025, 906, 911 (*E. coli* OmpA, *E. carotovora* PelB, and synthetic CSP signal sequences transport human proteins to the bacterial periplasm; "total product yields of up to 2.3 g/liter . . . which is sufficiently high to be commercially interesting"); Exh. 1043, 1938 (human signal sequences direct transport of bacterial β-lactamase enzyme to the *E. coli* periplasm); Exh. 1020, 215 (the *E. carotovora* PelB signal sequence has been used extensively to direct periplasmic transport of diverse heterologous proteins of interest in *E. coli*); Exh. 1027, 7862 (signal sequence of subtilisin from *B. subtilis* directs periplasmic transport of recombinant proteins in *E. coli*); Exh. 1062, 854 (*Cellulomonas fimi* cellulose Cex leader (signal) sequence directs recombinant protein transport to the *E. coli* periplasm); Exh. 1023, 15016; Exh. 1044, 1647-49 (*C. diphtheriae* DTx signal sequence directs periplasmic transport of DTx mutants in *E. coli*); Exh. 1006, ¶ 53).

2. Commercially Available Bacterial Expression Kits Were Commonly Used to Direct Transport of DTx Mutant Polypeptides to the Bacterial Periplasm

By the time of the '345 patent's earliest filing date, commercially available

bacterial expression kits were readily available for directing transport of polypeptides to the periplasm. (Exh. 1021, 3, 5-24, 38, 42-43, 55-65; Exh. 1039; Exh. 1020, 212). These kits featured polynucleotide vectors designed to allow POSAs to easily insert DNA segments encoding polypeptides of interest, as well as detailed instructions for expressing and harvesting the polypeptides. (Exh. 1021, 3-24; Exh. 1006, ¶ 54).

Novagen[7] sold polynucleotide expression vectors as part of its widely used "pET System." That system was described as "the most powerful system yet developed for the cloning and expression of recombinant proteins in *E. coli*" and "designed for convenient cloning, expression, detection and purification of target proteins." (Exh. 1021, 3; Exh. 1006, ¶ 54). The pET System included vectors specifically designed to encode signal sequences capable of directing transport of polypeptides to the periplasm. (Exh. 1021, 26-27, 38, 40, 42-43). These vectors were marketed as a "strategy to obtain active, soluble proteins . . . export[ed] into the periplasm, which is a more favorable environment for folding and disulfide bond formation." (Exh. 1021, 38; Exh. 1006, ¶ 55).

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[7] Novagen (now EMD Millipore) was involved in, among other things, the marketing and sale of systems, reagents and other technology designed to facilitate recombinant expression of proteins in bacteria. (Exh. 1021, 3-5).

One such Novagen product was the pET22b vector. (Exh. 1021, 40, 42; Exh. 1006, ¶¶ 56-57; *see* Figs. 2A-2B below). The pET22b vector design facilitates production of polynucleotides encoding polypeptides of interest attached to a PelB signal sequence for transport to the *E. coli* bacterial periplasm. (Exh. 1021, 40, 42; Exh. 1006, ¶ 57).[8]

A well-known feature of vectors such as pET22b was the inclusion of a "multiple cloning site" ("MCS"). That site facilitated the easy insertion of a polynucleotide coding sequence into a specific position within the vector. (Exh. 1006, ¶ 59; Appendix C, ¶¶ 11-19). In the case of pET22b, insertion of a polynucleotide coding sequence into the MCS could be used to place it 3' of the

[8] As noted above, prior to the earliest filing date of the '345 patent the PelB signal sequence was known to be capable of directing transport of polypeptides to the bacterial periplasm, and was routinely utilized to do so. For example, the PelB signal sequence encoded by pET System vectors was known to direct polypeptide transport to the bacterial periplasm. (*See, e.g.,* Exh. 1065, 3995 (utilizing the pET20b vector); Exh. 1068, 1486 (utilizing the pET22b vector); Exh. 1066, 71 (utilizing the pET26b vector)).

PelB signal (leader) coding sequence.[9] (*See* Figs. 2A-2B). For ease of illustration, Figures 2A-2B show the PelB signal (leader) sequence in yellow, and the MCS region into which a polynucleotide could be inserted 3' (*i.e.*, downstream) of the PelB signal (leader) sequence in blue. The 5' to 3' direction of the polynucleotide coding sequence of pET22b is indicated, *e.g.*, by the arrow over the "T7 promoter" in Figure 2B below. (*See also* the thick black arrow in Fig. 2A below; Exh. 1006, ¶ 60).

Indeed, by the '345 patent's earliest filing date, the pET22b vector, with a polynucleotide encoding a PelB signal sequence placed N-terminal to a bacterial toxin, had produced mature bacterial toxins with greater than 98% sequence identity to CRM197, which were transported to the bacterial periplasm of *E. coli*. (*See* Exh. 1005, 4:64-6:3; Exh. 1006, ¶ 58).

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[9] A polynucleotide has a 5' end and a 3' end, corresponding to the N- and C-termini of a polypeptide, respectively. (Exh. 1006, Appendix C, ¶ 9).

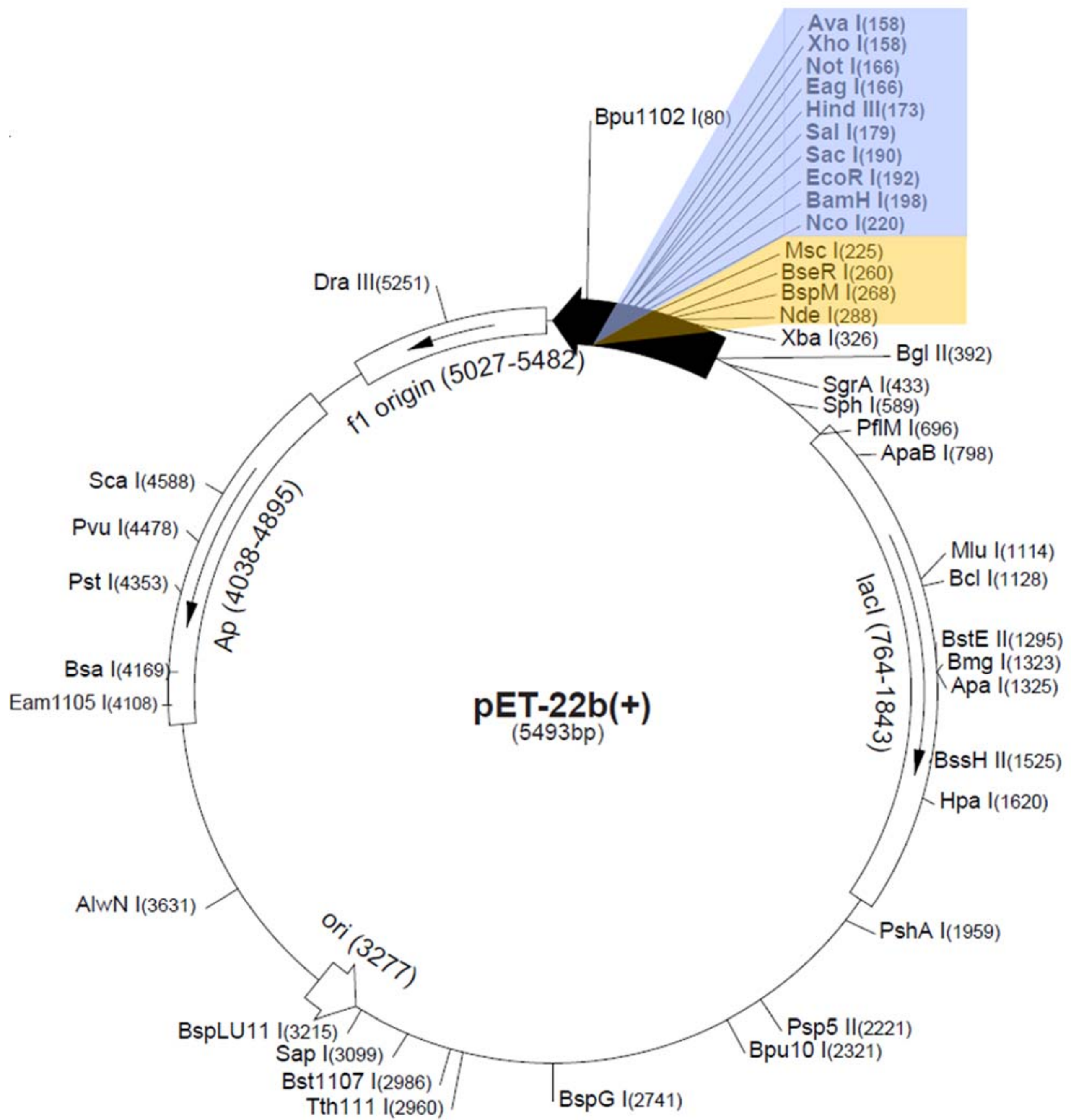

Figure 2A (derived from pET22b map (1998) (Exh. 1012))

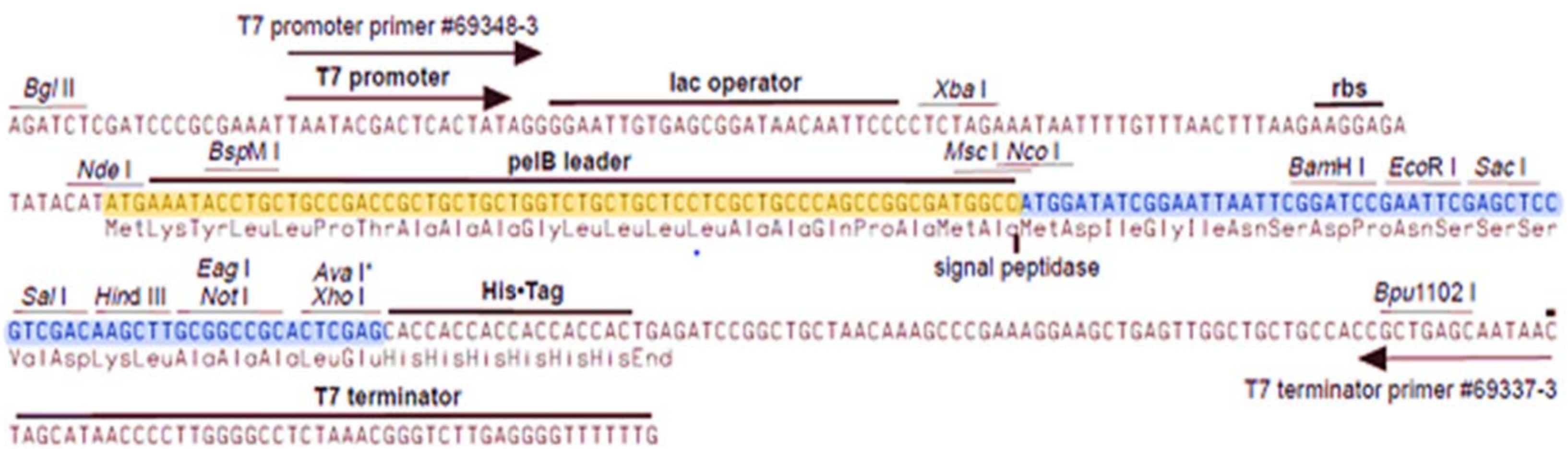

Figure 2B (derived from pET22b map (1998) (Exh. 1012)

F. POSAs Knew DTx Mutants, Including CRM197, Were Exportable To The Bacterial Periplasm

As of the ’345 patent’s earliest filing date, CRM197, and other DTx mutants were well-known exportable polypeptides; both native and non-native signal sequences transported such polypeptides to the periplasm of bacteria, during protein production. (Exh. 1005, 4:64-6:3 (periplasmic transport in *E. coli* of DTx polypeptides having greater than 98% identity to CRM197 attached to a PelB signal sequence); Exh. 1049, 5141, 5143 (periplasmic transport of CRM197 in *E. coli* with native *C. diphtheriae* signal sequence); Exh. 1023, 15016 (showing periplasmic transport in *E. coli* of a DTx fragment attached to the native *C. diphtheriae* signal sequence); Exh. 1007, [0178], [0182], Fig. 9 (reporting secretion in *E. coli* of a DTx mutant fusion protein expressed with the PelB signal sequence from *E. carotovora*)). Collier-1 is illustrative, demonstrating periplasmic transport in *E. coli* of a DTx mutant polypeptide in which the host cell, signal sequence, and DTx mutant were each from a different species. (Exh. 1005, 4:64-6:3; Exh. 1006, ¶¶ 62-63).

V. THE ’345 PATENT

The ’345 patent issued on August 23, 2016 and is assigned on its face to GlaxoSmithKline Biologicals S.A. (Exh. 1001, IPR1). This patent issued from U.S. Patent Application No. 13/500,244 (Exh. 1002), the national stage entry of International Patent Application No. PCT/EP2010/065047 (Exh. 1003), filed on

October 7, 2010, which claims priority to United Kingdom Patent Application No. GB0917647.0, filed on October 8, 2009 (Exh. 1004).

In general, the ’345 patent relates to polynucleotides encoding a signal sequence and a bacterial toxin, for the production of polypeptides in the periplasm of bacteria.

Claim 1 of the ’345 patent, one of two independent claims, is directed to a polynucleotide comprising a 5’ signal sequence portion and a 3’ toxin portion:

> 1. 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 bacterial host cell, and wherein the 5’ signal sequence is not derived from *C. diphtheriae*.

(Exh. 1001, 49:54-64). SEQ ID NO: 32 corresponds to the amino acid sequence of mature CRM197. (*Id.*, Fig, 9E).

Claims depending from claim 1 recite that the 3’ toxin portion encodes: a polypeptide having the amino acid sequence of SEQ ID NO: 32 (claim 2); CRM197 itself (claim 18); or a polypeptide having at least 95% sequence identity to SEQ ID

NO: 32 (claim 19). Other claims that depend from claim 1 recite that the 5' portion encodes specific signal sequences (claims 4-5), that the encoded polypeptide comprises specific (but known) signal sequences plus the first 30 amino acids of CRM197 (claim 20), or that the 5' signal sequence portion is directly 5' of the 3' toxin portion (claim 21).

Independent claim 6 combines the elements of claims 1 and 4:

> 6. A polynucleotide comprising a 5' signal sequence portion and a 3' toxin portion wherein:
>
> (i) 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
>
> (ii) the 5' signal sequence portion encodes a polypeptide having an acid sequence capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm when expressed in a bacterial host cell, and wherein the 5' signal sequence is not derived from *C. diphtheriae* and wherein the polypeptide has an amino acid sequence selected from:
>
> (a) SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26;
>
> (b) variants of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26, varying from the corresponding sequences by 1, 2 or 3 point mutations, amino acid insertions or amino acid deletions, which variants are capable of directing transport of said bacterial toxin polypeptide to the periplasm of said bacterial host cell; and
>
> (c) fragments of at least 10 amino acids of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26, which fragments are capable of

directing transport of said bacterial toxin polypeptide to the periplasm of said bacterial host cell.

(*Id*., 51:13-37).

Similar to the claims that depend from claim 1, claims depending from claim 6 recite that the 3' toxin portion encodes: a polypeptide having the amino acid sequence of SEQ ID NO: 32 (claim 14), CRM197 itself (claim 12), or a polypeptide having at least 95% sequence identity to SEQ ID NO: 32 (claim 13). Likewise, other claims that depend from claim 6 recite that the 5' portion encodes specific (but known) signal sequences (claims 7-11), that the encoded polypeptide comprises specific (but known) signal sequences plus the first 30 amino acids of CRM197 (claim 16), or that the 5' signal sequence portion is directly 5' of the 3' toxin portion (claim 17).

The '345 patent makes clear that its examples merely combined prior art elements to achieve predictable results. The patent describes using "standard molecular biology techniques" to combine signal sequences from a public database to express a decades-old "target gene" in *E. coli*. (*Id*., 19:63-67). Although the '345 patent refers to "low protein abundance" and a search for ways to "increase expression" (*id*., 2:7-25), significantly, none of the claims of the '345 patent require that the polynucleotide achieve any particular yield. Nor does the patent describe

any novel sequences that are superior to those in the prior art. Finally, the patent does not include any evidence of unexpected results.

VI. CLAIM CONSTRUCTION

In accordance with 37 C.F.R. § 42.100(b), the challenged claims must be given their broadest reasonable construction ("BRC") in light of the specification of the '345 patent.[10]

A. "encodes a mature bacterial toxin polypeptide"

The claim term "encodes a mature bacterial toxin polypeptide" should be construed to mean "***contains a polynucleotide that, when translated,***[11] ***yields a bacterial toxin polypeptide lacking a signal sequence***." This construction is the plain and ordinary meaning of the term and is supported by the intrinsic record.

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[10] On October 10, 2018, the USPTO changed the standard for construing claims from the BRC standard to the one articulated in *Phillips v. AWH Corp*., 415 F.3d 1303 (Fed. Cir. 2005). This rule change applies to IPR petitions filed on or after November 13, 2018. (83 Fed. Reg. 51,340 (Oct. 11, 2018) (to be codified at 37 C.F.R. pt. 42)).

[11] "Translation" is the process by which a cell expresses a polypeptide based on the coding sequence of a polynucleotide. (Exh. 1006, ¶ 37, Appendix C, ¶ 9).

POSAs at the time of the ’345 patent’s earliest filing date would have understood that a polynucleotide “encoding” a particular protein or polypeptide means a polynucleotide sequence that, when translated, yields that particular protein sequence. (Exh. 1037, 550-51). Likewise, POSAs would have understood that “mature” polypeptides do not include a signal sequence. (Exh. 1022, 1727; Exh. 1006, ¶ 95).

The intrinsic evidence is consistent with the plain and ordinary meaning of "mature bacterial toxin polypeptide." The specification describes the creation of a plasmid containing a “mature CRM197 sequence (SEQ ID NO:31)” which “contains no signal sequence.” (Exh. 1001, 21:8-10). Figure 2 refers to “mature CRM197” and specifies that this polypeptide contains “no signal sequence.” (*Id.*, Fig. 2). The specification also states that “the process for making the bacterial toxin involves removal of a signal peptide from the bacterial toxin within the bacterial host cell to obtain a mature bacterial toxin.” (*Id.*, 16:10-13; Exh. 1006, ¶ 96).

The prosecution history further confirms that a “mature” polypeptide is one lacking a signal sequence. During prosecution, in order to overcome a prior art reference that disclosed a DTx polypeptide with a signal sequence, Patent Owner amended the claim language to add the term “mature bacterial toxin.” (Exh. 1002, IPR522-23). Patent Owner stated that “‘[m]ature’ refers to a diphtheria toxin

polypeptide lacking the signal sequence, see e.g. paragraphs 0153 and 0204 of the present specification." (*Id.*, IPR527; Exh. 1006, ¶ 97).[12]

Consequently, the term "encodes a mature bacterial toxin polypeptide" should be construed to mean "contains a polynucleotide that, when translated, yields a bacterial toxin polypeptide lacking a signal sequence." (Exh. 1006, ¶ 98).

B. "an amino acid sequence at least 90% *identical to*" or "at least 95% sequence *identity to*"

The claim term "an amino acid sequence at least 90% identical to" should be construed to mean "an amino acid sequence with at least 90% identity to a reference sequence, as determined by known methods." Likewise, the term "at least 95% sequence identity to" should be construed to mean "a polypeptide with at least 95% sequence identity to a reference sequence as determined by known methods." (Exh. 1006, ¶ 99).

The '345 patent does not define the term "an amino acid sequence at least 90% *identical to*" or "at least 95% sequence *identity to*" a reference sequence, however the intrinsic evidence demonstrates that Patent Owner used the terms

[12] These paragraph numbers refer to the published application that issued as the '345 patent and correspond to the disclosures regarding mature toxins in the '345 patent specification discussed above. (Exh. 1001, 16:10-16, 21:4-10; Exh. 1034, [0153], [0204]).

"identical to" and "sequence identity" interchangeably. For example, independent claim 1 is directed to a polynucleotide encoding, *inter alia*, an amino acid sequence "at least 90% ***identical to*** SEQ ID NO: 32," and claim 19, which depends from claim 1, is directed to a polynucleotide "having at least 95% sequence ***identity to*** SEQ ID NO: 32." (Exh. 1001, 49:56-58, 52:36-38 (emphases added)). This relationship exists between independent claim 6 ("90% ***identical to*** SEQ ID NO: 32") and its dependent claim 13 ("95% sequence ***identity to*** SEQ ID NO: 32"). (*Id*., 51:15-17, 52:22-24 (emphases added); Exh. 1006, ¶ 100).

Also, the '345 patent describes standard methods for determining an amino acid sequence's "identity" to a reference sequence. (Exh. 1001, 6:15-48). The specification's disclosure regarding sequence "identity" makes clear that "'[i]dentity' can be readily calculated by known methods," identity is "determined by the match between strings of such sequences," "[m]ethods to determine identity are designed to give the largest match between the sequences tested," and that such methods "are codified in publicly available computer programs" such as BLAST.[13] (*Id*., 6:18-47; Exh. 1006, ¶ 101).

In view of these disclosures, the claim term "an amino acid sequence at least 90% identical to" should be construed to mean "an amino acid sequence with at least

[13] *See, e.g.*, https://blast.ncbi.nlm.nih.gov/Blast.cgi.

90% identity to a reference sequence, as determined by known methods." Likewise, the term "at least 95% sequence identity to" should be construed to mean "a polypeptide with at least 95% sequence identity to a reference sequence as determined by known methods." (Exh. 1006, ¶ 102).

C. "capable of directing transport of said bacterial toxin polypeptide to the bacterial periplasm"

Claims 1 and 6 recite that 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 bacterial host cell." (Exh. 1001, 49:59-62, 51:18-21). This term is not defined in the specification of the '345 patent and should be given its plain and ordinary meaning in light of the specification.[14]

The plain and ordinary meaning of the "capable of" portion of this term is

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[14] The inventor must provide a clear indication of their intent to act as their own lexicographer, which they may do by placing quotation marks around the terms in question. *See Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576, 1582 (Fed. Cir. 1996). The applicants for the '345 patent clearly identified the terms they wished to define in this manner. (Exh. 1001, 7:5-25). Their decision not to define the "capable of" term (*e.g.*, not to put it in quotations) manifests their intent to give this term its well understood ordinary meaning, and not to act as their own lexicographers.

well-known. *See Ricoh Americas Corp. v. MPHJ Tech. Invs., LLC*, No. IPR2014-00538, Paper 8 at 9-10 (P.T.A.B. Aug. 25, 2014); (*see also* Exh. 1047, 207 ("[h]aving capacity or ability . . .[; h]aving the ability required for a specific task or accomplishment"); Exh. 1035, 168 ("having attributes . . . required for performance or accomplishment . . .[;] having traits conducive to or features permitting"); Exh. 1006, ¶ 104). The remainder of this term is directed to basic biological terminology well-known to POSAs. Accordingly, no construction of this term is required. *See Phillips*, 415 F.3d at 1314 ("[i]n some cases, the ordinary meaning of claim language as understood by a person of skill in the art may be readily apparent even to lay judges"); *U.S. Surgical Corp. v. Ethicon, Inc.*, 103 F.3d 1554, 1568 (Fed. Cir. 1997) (Courts "need not repeat or restate every claim term," as claim construction "is not an obligatory exercise in redundancy."); *Syntheon, LLC v. Covidien AG*, No. 16-10244-ADB, 2017 WL 4225994, at *9 (D. Mass. Sept. 22, 2017) ("The parties' suggested constructions are merely **synonyms** for readily understood claim language. Accordingly, the Court declines to construe this term.").

The Board should reject any attempt by Patent Owner to improperly incorporate limitations from the specification, such as a particular amount of transport to the periplasm, and/or a specific type of bacteria. Patent Owner might point to a passage of the specification where it states that "[a] signal sequence is capable of directing an expressed protein to the periplasm if, when it is attached to a

polypeptide of interest, during translation of the polypeptide in a gram negative bacteria, more of said polypeptide is found in the periplasm of a gram negative bacteria than in the absence of the signal sequence." (Exh. 1001, 7:33-39). But this statement does not meet the exacting standard for disavowal of claim scope. The inclusion of a sentence identifying ***one example*** of "a signal sequence [] capable of directing an expressed protein to the periplasm" that relates to a particular amount of transport to the periplasm and/or expression in a specific type of bacteria does not mean that it "limits all subsequent claims." *Unwired Planet, LLC v. Apple Inc.*, 829 F.3d 1353, 1358 (Fed. Cir. 2016); *see Thorner v. Sony Computer Entm't Am. LLC*, 669 F.3d 1362, 1366 (Fed. Cir. 2012). The specification "is not a substitute for, nor can it be used to rewrite, the chosen claim language." *SuperGuide Corp. v. DirecTV Enters., Inc.,* 358 F.3d 870, 875 (Fed. Cir. 2004).

There is no clear intention in the specification to limit the scope of the claims to that embodiment. Indeed, the Federal Circuit has emphasized that "[e]ven when the specification describes only a single embodiment, the claims of the patent will not be read restrictively unless the patentee has demonstrated a clear intention to limit the claim scope using 'words or expressions of manifest exclusion or restriction.'" *Liebel-Flarsheim Co. v. Medrad, Inc.,* 358 F.3d 898, 906 (Fed. Cir. 2004). And even though the inventors plainly understood how to describe such an embodiment in the specification (Exh. 1001, 7:33-38), the claims notably do not

limit the claimed compositions to expression in a particular type of bacteria, or require any particular amount of transport to the periplasm. Accordingly, well established principles of patent law prohibit reading those (or any other) limitations from the specification into the claims.

D. "wherein the 5' signal sequence is not derived from *C. diphtheriae*"

Claims 1 and 6 recite "wherein the 5' signal sequence is not derived from *C. diphtheriae*." (*Id.*, 49:63-64, 51:22-23). This term should be construed to mean "wherein the signal sequence of a polypeptide is different in amino acid sequence to the signal sequence of the mature bacterial toxin polypeptide found in native (not recombinant) *C. diphtheriae*." (Exh. 1006, ¶ 105).

This construction is supported by the claim language and specification. POSAs would understand the "not derived from *C. diphtheriae*" claim language, based on the specification, to capture signal sequences that are different than found in native (not recombinant) *C. diphtheriae*. While this exact claim term is not defined in the specification, a similar term "polypeptide **not derived from** *C. diphtheriae*" is defined as "a polypeptide **which is different in sequence** to a polypeptide **found in native (not recombinant)** *C. diphtheriae*." (Exh. 1001, 7:22-24 (emphases added); Exh. 1006, ¶ 106). Petitioner has adopted the relevant portions of this definition into its proposed construction of this term. Accordingly, the specification supports Petitioner's proposed construction. *See Phillips*, 415 F.3d at

1315 ; *see also Vitronics*, 90 F.3d at 1582.

Although Patent Owner argued during prosecution that "not derived from *C. diphtheriae*" excluded "***modified*** native *C. diphtheriae* signal sequence[s]," that argument is irrelevant to the clear guidance supplied by the specification. (Exh. 1002, IPR527 (emphasis added)). Because the prosecution history "often lacks the clarity of the specification and thus is less useful for claim construction purposes," *Phillips*, 415 F.3d at 1317, the claim term should be construed in accordance with the definitions in the specification. "[W]hen the specification and prosecution history appear in conflict, any ambiguity must be resolved in favor of the specification and claims . . . ." *Lydall Thermal/Acoustical, Inc. v. Fed. Mogul Corp.*, 566 F. Supp. 2d 602, 614 (E.D. Mich. 2008), *aff'd*, 344 F. App'x 607 (Fed. Cir. 2009). For these reasons, this term should be construed in accordance with the claim language and the specification to mean "wherein the signal sequence of a polypeptide is different in amino acid sequence to the signal sequence of the mature bacterial toxin polypeptide found in native (not recombinant) *C. diphtheriae*."

The remaining terms of the challenged claims are explicitly defined by the specification or have a well understood ordinary meaning to POSAs and require no further construction for the purposes of this Petition.

VII. GROUNDS FOR INSTITUTION

A. Ground I: Claims 1 And 19 Of The ’345 Patent Are Anticipated By Collier-1

Collier-1 is directed to DTx polypeptide mutants with greater than 98% identity to CRM197, polynucleotides encoding such polypeptides and their use in vaccines. (Exh. 1005, 2:14-52, 4:64-6:3; Exh. 1006, ¶ 109). Collier-1 is prior art under pre-AIA Section 102(b) because it published on September 24, 2002, which is more than one year before the earliest U.S. filing date.[15] It was not considered by the Examiner, much less cited in a claim rejection.

Collier-1 teaches that a signal sequence capable of transporting its DTx mutant polypeptides to the bacterial periplasm can be advantageous for "facilitating recovery of the protein." (Exh. 1005, 7:14-18). The examples in Collier-1 carry out this teaching by using a commercially available polynucleotide vector, pET22b (described above), with routine recombinant DNA technology procedures. Specifically, Collier-1 discloses polynucleotide constructs comprising a 5’ signal sequence portion encoding the PelB signal sequence and 3’ toxin portion encoding a DTx mutant polypeptide falling within the scope of the challenged claims. (*Id.*,

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[15] Collier-1 was also published more than one year before the earliest foreign priority date of October 8, 2009.

4:64-6:3; Exh. 1006, ¶ 109).[16]

In view of the foregoing and as detailed below, Collier-1 discloses each element of the '345 patent's claims 1 and 19. (Exh. 1006, ¶ 110).

1. Claim 1 Is Anticipated

a. "A polynucleotide comprising a 5' signal sequence portion and a 3' toxin portion"

Collier-1 teaches a polynucleotide comprising a 5' signal sequence portion and a 3' toxin portion.

The examples in Collier-1 describe the placement of polynucleotides encoding mutant DTx polypeptides into the pET22b vector. (Exh. 1005, 4:64-6:3;

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[16] Collier-1 is representative of work the Collier laboratory published well prior to the earliest filing date of the '345 patent. (*See also* Exh. 1013, 8467-70 & Exh. 1067, 370-76, which also disclose use of the pET22b vector to generate polynucleotides encoding non-toxic DTx polypeptides with greater than 98% identity to CRM197 attached to the PelB signal sequence, and demonstrate transport of the polypeptides to the *E. coli* bacterial periplasm). Although this Petition focuses on Collier-1 for the sake of simplicity, the disclosures of either Oh (1999) (Exh. 1013) or Paoletti (2002) (Exh. 1067) could be utilized in place of Collier-1, throughout.

see also Section VII.A. above). The pET22b vector (Novagen), as noted above, encodes polypeptides attached to a PelB signal sequence for transport of the polypeptide to the *E. coli* periplasm. (Exh. 1021, 40). The Collier-1 examples teach the insertion of polynucleotides encoding the mutant DTx polypeptides 3' of the portion of the vector encoding the PelB signal sequence.[17] In other words, the portion of the Collier-1 polynucleotide construct encoding the PelB signal sequence is 5' to the portion encoding the bacterial toxin polypeptide. Thus, the polynucleotide constructs of Collier-1 comprise a 5' signal sequence portion and a 3' toxin portion. (Exh. 1006, ¶ 111).

b. "wherein (a) the 3' toxin portion encodes a mature bacterial toxin polypeptide"

As explained above, the claim term "encodes a mature bacterial toxin polypeptide" means "contains a polynucleotide that, when translated, yields a bacterial toxin polypeptide lacking a signal sequence." (Exh. 1006, ¶ 112).

With its description of how DTx polynucleotide coding sequences were generated for insertion into the pET22b vector, Collier-1 discloses amplification reactions and provides the amplification primer sequences used. (Exh. 1005, 5:13-17). By providing the sequences of the amplification primers, Collier-1 identifies

[17] Briefly, the mutant DTx coding sequences were introduced into pET22b in place of the BamHI-XhoI region of the MCS. (*See also* Fig. 2A-2B above).

the particular DTx polynucleotide coding sequence that was amplified and inserted into the vector. (*Id.*; *see* Fig. 3 below). The inserted sequence lacks a sequence that encodes a signal sequence. (Exh. 1005, 4:64-5:46; Exh. 1006, ¶ 113). Thus, the 3' toxin portion of the Collier-1 polynucleotides contains a polynucleotide that, when translated, yields a bacterial toxin polypeptide lacking a signal sequence.

T7 promoter primer #69348-3

T7 promoter

lac operator

Bgl II

Xba I

rbs

```
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
```

Nde I

*Bsp*M I

pelB leader

Msc I *Nco* I

*Bam*H I

DTx corresponding to AA1-535 of Collier's SEQ ID NO:2

```
TATACATATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGCTCCTCGCTGCCCAGCCGGCGATGGCCATGGATATCGGAATTAATTCGGATCCGGGCCTGGATGATGTTGTTGATTCTTCTAAA...
       MetLysTyrLeuLeuProThrAlaAlaAlaGlyLeuLeuLeuLeuAlaAlaGlnProAlaMetAlaMetAspIleGlyIleAsnSerAspProGlyLeuAspAspValValAspSerSerLys...
```

signal peptidase

Ava I*

Xho I

His•Tag

*Bpu*1102 I

```
...AAGCTATCGCTATTTTTTGAAATCAAAAGCCTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAAC
...LysLeuSerLeuPhePheGluIleLysSerLeuGluHisHisHisHisHisHisEnd
```

T7 terminator primer #69337-3

T7 terminator

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TAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
```

Figure 3 (Polynucleotide sequence generated by Collier-1 and polypeptide sequence encoded thereby)

c. “having an amino acid sequence at least 90% identical to SEQ ID NO: 32”

The ’345 patent specifies that SEQ ID NO: 32 is the amino acid sequence of mature CRM197. (Exh. 1001, Fig. 9E; Exh. 1006, ¶ 114).

The examples in Collier-1 describe the generation of two polynucleotides which encode mutant DTx polypeptides. (Exh. 1005, 4:64-5:46). One mutant DTx polypeptide sequence differs from CRM197 by six amino acids and the second differs by seven amino acids.[18] As such, one mutant DTx polypeptide has an amino acid sequence that is 98.8% identical to SEQ ID NO: 32 ($\frac{535-6}{535} \times 100 = 98.8\%$)

[18] In particular, the mutants contain a 535 amino acid sequence that aligns with SEQ ID NO: 32 (CRM197), wherein one contains five amino acid mutations relative to DTx (Ala2Leu, Glu148Ser, Glu349Lys, Lys516Ala, and Phe530Ala), and the second contains six (Ala2Leu, Glu148Ser, Arg190Ser, Arg192Gly, Arg193Ser, and Glu349Lys). (Exh. 1005, 4:64-5:17). As CRM197 differs from DTx by one amino acid (position 52), these sequences differ from CRM197 by six and seven amino acids, respectively.

and the second has an amino acid sequence that is 98.6% identical to SEQ ID NO: 32 ($\frac{535-7}{535} \times 100 = 98.6\%$).[19] (Exh. 1006, ¶ 114).

The Collier-1 examples also disclose generating polynucleotides encoding mutant DTx polypeptides with a subset of the above-described mutations. (Exh.

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[19] The polypeptides encoded by the polynucleotides in the Collier-1 examples include eleven amino acids encoded by the pET22b MCS as part of the mature bacterial DTx mutant polypeptide (nine at the amino end and two at the carboxyl end). (*Id.*, 5:8-17; *infra* note 21). Even taking these amino acids into account the polypeptides encoded by the 3' portions of the polynucleotides in the Collier-1 examples are greater than 90% identical to SEQ ID NO:32 (($\frac{546-6-11}{535+11=546} \times 100 = 96.8\%$) and ($\frac{546-7-11}{546} \times 100 = 96.7\%$), respectively). The polypeptides encoded by the Collier-1 examples also included a hexa-histidine (six histidine amino acids) tag at their C-termini to aid in purification. (Exh. 1005, 5:8-11; *see also* Fig. 3 above). Even taking these additional six amino acids into account, the polypeptides encoded by the 3' portions of the polynucleotides in the Collier-1 examples are greater than 90% identical to SEQ ID NO:32 (($\frac{552-6-11-6}{535+11+6=552} \times 100 = 95.8\%$) and ($\frac{552-7-11-6}{552} \times 100 = 95.6\%$).

1005, 5:2-46). One such polynucleotide encodes a DTx containing a Glu148Ser mutation, in addition to the Ala2Leu mutation (present in each of the polypeptides of the Collier-1 examples). (*Id.*). This polynucleotide, therefore, encodes a polypeptide having a 535 amino acid sequence that aligns with the reference sequence SEQ ID NO: 32 (CRM197) that differs from CRM197 by three amino acids.[20] As such, this amino acid sequence is 99.4% identical to SEQ ID NO:32 ($\frac{535-3}{535} \times 100 = 99.4\%$).[21] (Exh. 1006, ¶ 116).

Accordingly, Collier-1 discloses this claim limitation.

d. "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 bacterial host cell"

As discussed above, Collier-1 generated polynucleotides comprising a 5' signal sequence portion encoding the PelB signal sequence. The PelB signal sequence was known to transport polypeptides to the bacterial periplasm (Exh. 1021, 38, 42), and the pET22b vector used to generate the Collier-1 polynucleotides was

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[20] In particular, these differences are at amino acid positions 2, 52 and 148.

[21] Even taking both the eleven MCS-encoded amino acids and the hex-histidine tag into account, the polypeptide encoded by this polynucleotide is greater than 96% identical to SEQ ID NO:32 ($\frac{552-3-11-6}{535+11+6=552} \times 100 = 96.4\%$).

specifically designed to take advantage of that characteristic of PelB (Exh. 1006, ¶ 118; Exh. 1005, 4:64-6:3, 7:14-18). Indeed, Collier-1 introduced the polynucleotides into *E. coli*, where the mutant DTx polypeptides were expressed, transported to the periplasm and purified from periplasmic extracts in good yield. (Exh. 1005, 5:47-6:3).

Collier-1's disclosure of transport of DTx mutant polypeptides to the periplasm confirms that the PelB signal sequence encoded in Collier-1 is capable of directing transport of the claimed polypeptide to the periplasm. (Exh. 1006, ¶ 119).

Therefore, Collier-1 discloses this limitation of claim 1.

e. "and wherein the 5' signal sequence is not derived from *C. diphtheriae*"

As explained above, this claim limitation requires that the 5' signal sequence is different from the signal sequence found in native (not recombinant) *C. diphtheriae*. Collier-1 discloses polynucleotide constructs encoding PelB signal sequences, which is native to *E. carotovora*, not *C. diphtheriae*. (Exh. 1006, ¶ 121; Exh. 1005, 4:64-5:41; Exh. 1024, 4380-82). As such, the PelB sequence is not derived from *C. diphtheriae.*[22] Moreover, the PelB signal sequence encoded by the

[22] This is consistent with Patent Owner's statements during prosecution. In arguing for the patentability of claims reciting the specific signal sequences found

Collier-1 polynucleotide constructs is not found in *C. diphtheriae*. (Exh. 1006, ¶ 121). Accordingly, Collier-1 discloses this limitation.

Thus, each and every element of claim 1 is found in Collier-1, which therefore anticipates claim 1.

2. Claim 19 Is Anticipated

Claim 19 recites "[t]he polynucleotide of claim 1 wherein the 3' toxin portion encodes a polypeptide having at least 95% sequence identity to SEQ ID NO: 32." (Exh. 1001, 52:36-38). As discussed above, Collier-1 discloses 3' toxin portions that encode a mature bacterial toxin polypeptides having amino acid sequences 98.6% and 98.8% identical to the '345 patent SEQ ID NO: 32. (*See also* supra note 21; Exh. 1006, ¶ 123).

Thus, Collier-1 anticipates claim 19.

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in Table 2 of the '345 patent, which identifies the signal sequences by bacterial origin (*S. pneumoniae, E. coli,* etc.), Patent Owners stated "these claims recite signal sequences that are not derived from *C. diphtheriae*." (Exh. 1002, IPR527). Likewise, during prosecution of a European counterpart of the '345 patent, in characterizing prior art teaching the FlgI signal sequence, Patent Owner stated that "FlgI . . . is not derived from *C. diphtheria* . . . . Instead it is derived from *E. coli* as shown in Table 2 of Example 1." (Exh. 1063, IPR006; Exh. 1073).

B. Ground II: Claims 1-2, 18-19 And 21 Of The ’345 Patent Are Anticipated By Neville

Neville is directed to, *inter alia*, immunotoxins comprising mutant DTx polypeptides fused to antibodies, and methods of treating autoimmune diseases using the same, either alone or co-administered with a non-toxic DTx mutant such as CRM197. (Exh. 1007, [0047], [0050]). Neville is prior art under pre-AIA Section 102(b) because it published on August 21, 2003, which is more than one year before the earliest U.S. filing date. It was not considered by the Examiner, much less used by the Examiner in a claim rejection.

Neville discloses constructs encoding CRM197 or a mutant with at least 99% identity to CRM197, *i.e.*, the non-toxic DTM2 that is identical to CRM197 except for two amino acids at the C-terminus. (*Id.*, [0178]-[0179], [0184]). Neville also teaches that all of its constructs can be expressed in *E. coli* using a signal sequence such as Pe1B. (*Id.*, [0185]). As detailed below, Neville discloses each and every element of the ’345 patent’s claims 1-2, 18-19 and 21.

1. Claim 1 Is Anticipated

a. “A polynucleotide comprising a 5’ signal sequence portion and a 3’ toxin portion”

Neville teaches the use of a polynucleotide comprising a 5’ signal sequence portion and a 3’ toxin portion, as required by claim 1 of the ’345 patent. Specifically, Example 11 of Neville describes production of a polynucleotide construct that encodes DTM2, a non-toxic DTx mutant containing a PelB signal (leader) sequence.

This example teaches the addition of the sequence encoding the “pelB leader sequence” directly to the 5’ end of the DTx mutant coding sequence through the use of the polymerase chain reaction and specific primers. (*Id.*, [0178]). The resulting polynucleotide construct, therefore, comprises a 5’ signal sequence portion and a 3’ toxin portion. (Exh. 1006, ¶ 126).

Accordingly, Neville discloses this claim limitation.

b. “wherein (a) the 3’ toxin portion encodes a mature bacterial toxin polypeptide”

As explained above, the claim term “encodes a mature bacterial toxin polypeptide” means “contains a polynucleotide that, when translated, yields a bacterial toxin polypeptide lacking a signal sequence.” Example 11 describes production of a polynucleotide with a 3’ portion that encodes the DTM2 DTx mutant polypeptide (polypeptide positions 376 to 1983). (Exh. 1007, [0177]-[0179]; Exh. 1006, ¶ 128). This 3’ portion encodes a mature bacterial toxin polypeptide (DTM2) that lacks a signal sequence. Hence, the 3’ DTM2 coding sequence contains a polynucleotide that, when translated, yields a bacterial toxin polypeptide that lacks a signal sequence. (Exh. 1006, ¶ 128).

Thus, Neville discloses this limitation.

c. “having an amino acid sequence at least 90% identical to SEQ ID NO: 32”

The ’345 patent specifies that SEQ ID NO: 32 is the amino acid sequence of mature CRM197. (Exh. 1001, Fig. 9E). Among the mature bacterial toxin polypeptides disclosed in Neville is the DTx mutant DTM2. (Exh. 1007, [0047], [0050], [0176]-[0180]). As explained, below, the amino acid sequence of DTM2 is 99.6% identical to ’345 patent SEQ ID NO: 32.

Neville Example 11 describes a polynucleotide with a 3’ toxin portion that encodes the DTx mutant DTM2, which Neville teaches “has the same mutation as CRM197 plus two mutations in the C-terminus.” (*Id.*, [0184]).[23] Thus, the 3’ toxin portion of polynucleotide of Neville Example 11 encodes a mature bacterial toxin polypeptide (DTM2) that differs from ’345 patent SEQ ID NO: 32—the 535 amino acid sequence of CRM197—by only two amino acids. As such, DTM2 is 99.6% identical to SEQ ID NO: 32 ($\frac{535-2}{535} \times 100 = 99.6$). (Exh. 1006, ¶ 131).

Accordingly, Neville discloses this claim limitation.

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[23] Specifically, the two additional mutations are Ser (serine) to Phe (phenylalanine) mutations at positions 508 and 525, respectively. (*Id.*, [0178]-[0179]).

d. "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 bacterial host cell"

Example 11 of Neville discloses a polynucleotide coding sequence with a 5' portion that encodes the PelB signal (leader) sequence and a 3' end that encodes the mutant DTx polypeptide DTM2. (Exh. 1007, [0178]). Neville teaches that "DTM2 is made for high level production by including the pelB secretory signal for production in *E. coli*." (*Id.*, [0184]). Neville also teaches that "[a]ll of the constructs reported here can be expressed in *E. coli*, using pelB signal sequences." (*Id.*, [0185]).

The PelB signal sequence was a well-known signal sequence, routinely used prior to the earliest filing date of the '345 patent, and known to direct transport of polypeptides, including DTx mutant polypeptides, to the bacterial periplasm. (Exh. 1021, 38; Exh. 1005, 4:64-6:3; Exh. 1013, 8467; Exh. 1030, 6:15-7:22). Moreover, the presence of a signal sequence that directs transport of a protein of interest to the periplasm results in more of the protein being found in the periplasm than in the absence of the signal sequence. (Exh. 1006, ¶ 49).

Accordingly, Neville discloses this limitation.

e. "and wherein the 5' signal sequence is not derived from *C. diphtheriae*"

As explained above, this claim limitation requires that the 5' signal sequence

is different from the signal sequence found in native (not recombinant) *C. diphtheriae*. Neville discloses polynucleotide constructs encoding PelB signal sequences. PelB signal sequence is native to *E. carotovora*, not *C. diphtheriae*. (Exh. 1006, ¶ 136). As such, the PelB sequence is not derived from *C. diphtheriae*. (*See supra* note 22). Moreover, the PelB signal sequence encoded by the Neville polynucleotide constructs is not found in *C. diphtheriae*. (Exh. 1006, ¶ 136). Therefore, Neville discloses this limitation.

Thus, each and every element of claim 1 is found in Neville, which therefore anticipates claim 1.

2. Claim 2 Is Anticipated

Claim 2 recites "[t]he polynucleotide of claim 1 wherein the 3' toxin portion encodes a polypeptide having the amino acid sequence of SEQ ID NO: 32." (Exh. 1001, 49:65-67). SEQ ID NO: 32 recites the amino acid sequence of mature CRM197. (*Id*., Fig. 9E).

Neville teaches use of CRM197: "a non-toxic DT mutant such as DTM2 or CRM197 can first be administered followed by the immunotoxin" (Exh. 1007, [0047]); "[t]he non-toxic mutant of diphtheria toxin for use in the above [co-administration] method can be DTM2 or CRM197" (*id.*, [0050]). Neville additionally teaches that all of its constructs can be expressed in *E. coli* using PelB

signal sequences. (*Id*., [0185]). As such, Neville satisfies each of the limitations of claim 2.

Accordingly, Neville anticipates claim 2.

3. Claim 18 Is Anticipated

Claim 18 recites "[t]he polynucleotide of claim 1 wherein the 3' toxin portion encodes CRM197." (Exh. 1001, 52:34-35).

Neville teaches use of CRM197. (Exh. 1007, [0047], [0050]). Neville also teaches that all of its constructs can be expressed in *E. coli* using PelB signal sequences. (*Id.*, [0185]). As such, Neville satisfies each of the limitations of claim 18.

Accordingly, Neville anticipates claim 18.

4. Claim 19 Is Anticipated

Claim 19 recites "[t]he polynucleotide of claim 1 wherein the 3' toxin portion encodes a polypeptide having at least 95% sequence identity to SEQ ID NO: 32." (Exh. 1001, 52:36-38). As discussed above, Neville Example 11 discloses a 3' toxin portion that encodes a mature bacterial toxin polypeptide having an amino acid sequence (DTM2) 99.6% identical to the '345 patent SEQ ID NO: 32.

Thus, Neville anticipates claim 19.

5. Claim 21 Is Anticipated

Claim 21 recites "[t]he polynucleotide of claim 1 wherein the 5' signal sequence portion is directly 5' of the 3' toxin portion." (Exh. 1001, 52:41-42).

Neville Example 11 discloses a polynucleotide wherein the 5' signal sequence portion (PelB) is directly 5' to the 3' toxin portion (DTM2). (Exh. 1006, ¶ 145).

In particular, in producing the polynucleotide construct of Neville Example 11, "[t]he pelB leader sequence . . . was added to the 5' end of the DT coding sequence." (Exh. 1007, [0178]). As such, the portion of the resulting polynucleotide encoding the PelB signal sequence is upstream of and contiguous with—*i.e.,* is directly 5' of the 3' toxin portion encoding the DTM2 bacterial toxin. (Exh. 1006, ¶¶ 146-148).

Accordingly, Neville discloses this element and anticipates claim 21.

C. Ground III: Claims 2 And 18 Would Have Been Obvious Over Giannini-1 In View Of Collier-1 And The State Of The Art

Claims 2 and 18 of the '345 patent recite "[t]he polynucleotide of claim 1 wherein the 3' toxin portion encodes" "a polypeptide having the amino acid sequence of SEQ ID NO:32" (claim 2) or "CRM197" (claim 18). (Exh. 1001, 49:65-67, 52:34-35). The '345 patent specifies that SEQ ID NO: 32 is the amino acid sequence of mature CRM197. (*Id.*, Fig. 9E).

CRM197 was a well-known DTx mutant, commonly used well before the priority date of the '345 patent. For example, Giannini-1 (published in 1984) teaches that CRM197 has "attracted in the last few years the attention of laboratories interested in studying the properties of chimeric toxins, e.g. as specific cytocidal agents (4), or new vaccine development (5,6,7)." (Exh. 1011, 4063; Exh. 1016, 233).

Giannini-1 also provides the sequence of CRM197, and it is 100% identical to SEQ ID NO: 32. (Exh. 1011, 4066, Fig. 2; Exh. 1006, ¶ 151).

Moreover, as of the '345 patent's earliest filing date, POSAs would have appreciated the research and therapeutic utility and commercial value of CRM197. For nearly three decades, various vaccines featured CRM197 as a carrier protein for boosting an immune response to antigen. (Exh. 1016, 233; Exh. 1017, 3426; Exh. 1018, 3405; Exh. 1033, 164; Exh. 1006, ¶ 152).

Thus, POSAs would have been motivated to generate polynucleotides designed to express CRM197 in the *E. coli* periplasm. In fact, as discussed above, as of the '345 patent's earliest filing date, bacterial recombinant expression systems were well-known, and their use in expressing DTx mutant polypeptides, including CRM197, was routine. (*See* Section IV.B; Exh. 1006, ¶ 153).

As of the '345 patent's earliest filing date, POSAs would have also known of the advantages of transporting a polypeptide such as CRM197 to the bacterial periplasm. As discussed above, Section IV.C., these known advantages included: an oxidizing environment conducive to the formation of disulfide bonds (CRM197 contains two disulfide bonds), fewer proteases than the cell cytoplasm and ease of polypeptide recovery. Moreover, POSAs would have been well acquainted with signal sequences for directing transport of polypeptides of interest, including DTx mutants (including CRM197) into the bacterial periplasm. (Exh. 1006, ¶ 154).

The polynucleotides of Collier-1 are discussed at length above. Collier-1 teaches that expression of the polynucleotides in *E. coli* resulted in periplasmic transport of DTx mutant polypeptides having amino acid sequences greater than 98% identical to CRM197, as evidenced by their purification from periplasmic extracts. (Exh. 1005, 5:47-6:3). Although Collier-1 does not specifically disclose CRM197, POSAs would have immediately understood the relevance of Collier-1 to the expression of CRM197; indeed, Giannini-1 is listed on the face of the Collier-1 reference (*id.*, IPR1). (Exh. 1006, ¶ 155).

The well-established importance of CRM197 and the benefit of its transport to the periplasm, coupled with the results in Collier-1 would have motivated POSAs to use the expression strategy of Collier-1 to generate the polynucleotides of claims 2 and 18. Moreover, the state of the art at the time, as exemplified, for example, by Sambrook (1989) (Exh. 1029) at 15.51-15.62, Horton (1993) (Exh. 1069) at 270-79 and Heckman (2007) (Exh. 1070) at 924-32, included molecular biology techniques that POSAs could routinely use with a reasonable expectation of success to modify the Collier-1 polynucleotides to encode CRM197 in place of the Collier-1 mutant DTx polypeptides. (Exh. 1006, ¶ 156).

For these reasons, claims 2 and 18 would have been obvious over Giannini-1 in view of the state of the art and Collier-1.

D. Ground IV: Claims 4-14, 16, 17 And 20 Would Have Been Obvious Over Collier-1 In View Of Huber

Claims 4-14, 16-17 and 20 recite polynucleotides comprising a 5' signal sequence portion that encodes one of the following, specifically recited signal sequences, each of which were well-known as of the '345 patent's earliest filing date: PhtE (2), SipA (4), OmpA (6), NspA (8), TorT (10), SfmC (12), FocC (14), CcmH (16), YraI (18), TolB (20), NikA (22), FlgI (24), and DsbA (26). (SEQ ID NOs. in parentheses; *see*, *e.g.*, Exh 1001, claims 4, 6, 8, 12-14 & 17). Claim 10 recites specific nucleic acid sequences encoding these signal sequences. The 5' signal sequence in these claims can be any one of the recited signal sequences, except in claims 5, 7, and 9, which are directed specifically to SEQ ID NO: 24 for the FlgI signal sequence, variants or fragments, and claim 11, which recites a nucleic acid sequence encoding FlgI. Finally, claims 16 and 20 recite polynucleotides of claims 6 and 1, respectively, that encode "a polypeptide comprising any one of SEQ ID NOs: 33-45." The SEQ ID NOs in claims 16 and 20 correspond to the same specifically-disclosed 5' signal sequences noted above plus the first 30 amino acids of CRM197. (Exh. 1001, 50:55-58, 51:13-26, 52:30-31, 52:39-40).

1. Huber Teaches the Specifically Recited Signal Sequence Limitations

As discussed above, Collier-1 anticipates claims 1 and 19 of the '345 patent. Collier-1 teaches that "the coding sequence [encoding a non-toxic (toxoid) DTx

polypeptide] can contain, at its 5' end, a sequence encoding any of the known signal sequences capable of effecting secretion of the expressed protein into the periplasmic space of the host cell, thereby facilitating recovery of the protein." (Exh. 1005, 7:14-18).

As discussed above, Collier-1 demonstrated the use of the PelB signal sequence directing transport of DTx mutant polypeptides to the periplasm; Collier-1 discloses the purification of polypeptides from periplasmic extracts, at approximately 3 mg of purified protein per liter of culture. (*Id.*, 5:47-6:3). The teaching in Collier-1 of "other known signal sequences", however, would have motivated POSAs to try signal sequences in addition to PelB. Substitution of one signal sequence for a different one was a routine approach for optimizing protein expression and/or yield. (Exh. 1025, 911 (signal sequences found to "strongly stimulat[e] the levels of expression"); *id.*, 909 (substitution of PelB signal sequence for OmpA or CSP signal sequence resulted in 2-fold increase in protein expression); Exh. 1068, 1490). For example, while the 3 mg per liter polypeptide expression result obtained in Collier-1 using Pe1B was substantial, POSAs would have been motivated to use other signal sequences to optimize and, therefore, increase this yield. (Exh. 1006, ¶ 161).

Like Collier-1, Huber discloses polynucleotide constructs that encode a polypeptide with signal sequences for use in bacterial host systems. (Exh. 1008,

2984). Huber discloses nine of the thirteen signal sequences recited in the '345 patent claims, including FlgI (which is present in each of claims 4-14, 16-17 and 20), and demonstrates that each of these signal sequences is capable of directing transport of a normally cytoplasmic polypeptide (thioredoxin) to the *E. coli* periplasm. (*Id.*, 2983-84, 2987, Fig. 3).[24] Notably, it is these precise nine signal sequences that Huber demonstrates can successfully transport the normally cytoplasmic thioredoxin reporter polypeptide to the periplasm. (*See id.*, 2988, Table 2). Huber thus supplies "other known signal sequences" as specifically recited in these claims, and provides POSAs with motivation to combine its teachings with Collier-1. (Exh. 1006, ¶ 162).

The positive results in Huber were obtained with thioredoxin, a protein that is not normally transported to the periplasm, but is exported to the periplasm in *E. coli* when modified at the N-terminus with a signal sequence. As such, POSAs would

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[24] In particular, signal sequences DsbA, TolB, SfmC, FocC, CcmH, YraI, TorT, NikA and FlgI (*id.*, 2988, Table 2) correspond to nine of the ten *E. coli* signal sequences disclosed and claimed in the '345 patent. Huber states that the sequences it utilized were *E. coli* sequences obtained from the publicly accessible SwissProt databank. (*Id.*, 2984). The Patent Owner, too, obtained the amino acid sequences for these nine *E. coli* signal sequences from a public database (GenEMBL). (Exh. 1001, 19:65-67 & Table 2).

have expected that the same signal sequences that can transport thioredoxin would also be able to transport proteins already known to be exportable to the periplasm. Thus, given that DTx mutants, including CRM197, were known to be exportable to the bacterial periplasm, POSAs would have understood that Huber's outcome would apply to DTx mutants, including CRM197. (Exh. 1006, ¶ 163).

As such, POSAs would have also had a reasonable expectation that combining the teaching of Collier-1 and Huber would successfully produce the polynucleotides of claims 4-14, 16-17 and 20. (Exh. 1006, ¶ 164).

2. The Claim 11 Nucleotide Sequence, Encoding the FlgI Signal Sequence, Would Have Also Been Obvious

Claim 11 recites "wherein the 5' signal sequence portion comprises SEQ ID NO: 23," which is identified as the "[n]ucleotide sequence of the FlgI signal sequence." (Exh. 1001, Fig. 9C). The amino acid sequence of the FlgI polypeptide was known, as was the fact that the FlgI signal sequence was composed of the first nineteen amino acids of the FlgI polypeptide. (Exh. 1053, 1-2; Exh. 1073; Exh. 1058, 34; Exh. 1006, ¶¶ 166-167). The FlgI polynucleotide coding sequence was also known, the first fifty-seven nucleotides of which encode the signal sequence (*i.e.*, the first nineteen FlgI amino acids). (Exh. 1052, 3; Exh. 1073). This polynucleotide encoding this FlgI signal sequence is identical to SEQ ID NO: 23 as recited in claim 11, with the exception of the first base. (Exh. 1006, ¶ 168).

This single base difference, however, does not alter the FlgI signal sequence.

Both resulting codons encode methionine. In terms of *E. coli* codon usage, the single base change that yields SEQ ID NO: 23 merely replaces a "suboptimal" codon (GTG) with an "optimal" one (ATG) (AUG in RNA). POSAs would have been motivated to exchange a suboptimal codon for an optimal one using standard codon optimization techniques that were well-known at the time of the '345 patent's earliest filing date. (Exh. 1037, 367; Exh. 1046, W126-27). Because the amino acid sequence would not change after codon optimization, POSAs would have reasonably expected to preserve the capability of directing transport to the periplasm as described by Huber. (Exh. 1006, ¶ 169).

Consequently, for the reasons set forth herein, claims 4-14, 16-17 and 20 would have been obvious. *KSR Int'l Co. v. Teleflex Inc*, 550 U.S. 398, 416 (2007).

E. Ground V: Claim 21 Would Have Been Obvious Over Collier-1 In View Of The State Of The Art

Claim 21 recites "[t]he polynucleotide of claim 1 wherein the 5' signal sequence portion is directly 5' of the 3' toxin portion." (Exh. 1001, 52:41-42). Claim 21 depends from claim 1, which requires 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.

The polynucleotides of Collier-1 are discussed above. As explained, Collier-1 introduces polynucleotides into the pET22b vector 3' of the PelB coding sequence; such that the resulting polynucleotides encode mutant DTx polypeptides with an

amino acid sequence at least 90% identical to SEQ ID NO: 32 attached to an N-terminal PelB signal sequence. Collier-1 teaches the benefit of expressing its DTx mutant polypeptides in the periplasm (Exh. 1005, 4:64-5:46, 7:14-18) and, in fact, uses its polynucleotides to express and transport the DTx mutant polypeptides to the bacterial periplasm. (*Id*., 5:47-6:3).

As also explained above, for ease of cloning, the polynucleotides produced in the Collier-1 examples encode DTx mutant polypeptides that begin with several N-terminal amino acids encoded by the vector (pET22B) MCS sequence. (*Id*., 5:7-17).[25] Nevertheless, the polynucleotides of claim 21 would have been obvious over Collier-1 in view of the state of the art at the time of the earliest filing date of the '345 patent. (Exh. 1006, ¶ 172).

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[25] For the purpose of argument, it is assumed that the presence of these N-terminal amino acids indicates that the 5' signal sequence portion of the Collier-1 polynucleotides is not directly 5' of the 3' toxin portion. It should be noted however that upon cleavage of the PelB signal sequence (*i.e*., the polypeptide region encoded by the 5' signal sequence portion) what remains of the polypeptides in Collier-1 is the portion encoded by the 3' toxin portion of the polynucleotide, the 5' (PelB) signal sequence portion of the Collier-1 polynucleotides is already directly 5' of the 3' toxin portion.

Collier-1 teaches use of its DTx mutant polypeptides as vaccines. (Exh. 1005, 2:14-44). Basic regulatory and vaccine related considerations would favor administration of polypeptides that are as similar to the native polypeptide as possible (while, in this case, preserving the non-toxic nature of the DTx mutants). (*See* Exh. 1033, 166, 171 (recognizing that changes to proteins used in vaccines can change the immune response); *see also* Exh. 1064, 346). Moreover, a recombinant polypeptide having additional amino acids at the N-terminus of the native polypeptide would likely entail scientific justification that the recombinant polypeptide is equivalent to the native polypeptide. Thus, POSAs would have been motivated to modify the teaching of the Collier-1 examples to produce polynucleotides wherein the 5' signal sequence portion is directly 5' of a 3' toxin portion that encodes a DTx mutant polypeptide exhibiting an authentic N-terminus (rather than one encoded by the vector MCS). (Exh. 1006, ¶ 173).

The state of the art at the time of the earliest filing date of the '345 patent exemplified, for example, by Sambrook (1989) (Exh. 1029) at 15.51-15.62, Horton (1993) (Exh. 1069) at 270-79 and Heckman (2007) (Exh. 1070) at 924-32, included molecular biology techniques that POSAs could routinely use with a reasonable expectation of success to modify the Collier-1 polynucleotides to specifically remove the portion giving rise to the MCS-encoded amino acid sequences. (Exh. 1006, ¶ 174).

Accordingly, claim 21 would have been obvious over Collier-1 in view of the state of the art.

F. Ground VI: Claims 4-14, 16, 17 And 20 Would Have Been Obvious Over Neville In View Of Huber

Claims 4-14, 16-17 and 20 are summarized above. For the reasons set forth herein, these claims would have been obvious over Neville in view of Huber.

1. Huber Teaches the Specifically Recited Signal Sequence Limitations

As discussed above, Neville anticipates claims 1-2, 18-19, and 21 of the ’345 patent. While Neville does not disclose the signal sequences specifically recited in claims 4-14, 16-17 and 20, Neville teaches that “[a]ll of the constructs reported here can be expressed in *E. coli* using pelB signal sequences or other appropriate signal sequences.” (Exh. 1007, [0185]).

This teaching of “other appropriate signal sequences” would have motivated POSAs to try signal sequences in addition to PelB. Substitution of one signal sequence for a different one was a routine approach for optimizing protein expression and/or yield. (Exh. 1025, 911 (signal sequences found to “strongly stimulat[e] the levels of expression”; *id.*, 909 (substitution of PelB signal sequence for OmpA or CSP signal sequence resulted in 2-fold increase in protein expression); Exh. 1006, ¶ 178).

Both Neville and Huber disclose polynucleotide constructs that encode a

polypeptide with signal sequences for use in bacterial host systems. (Exh. 1008, 2984). Huber demonstrated that nine of the thirteen signal sequences recited in the ’345 patent claims, including FlgI (which is present in each of claims 4-14, 16-17 and 20), are capable of directing transport of a normally cytoplasmic polypeptide (thioredoxin) to the *E. coli* periplasm. By supplying “other appropriate signal sequences” as specifically recited in the claims, Huber provides POSAs with motivation to combine its teachings with Neville. (Exh. 1006, ¶ 179).

As also discussed above, the knowledge that DTx mutants, including CRM197, were exportable to the bacterial periplasm, coupled with the fact that Huber’s positive results were obtained with a protein (thioredoxin) that is normally cytoplasmic, but is capable of being exported when modified at the N-terminus with a signal sequence, would have provided POSAs with a reasonable expectation that combining the teaching of Neville and Huber would successfully produce the polynucleotides of claims 4-14, 16-17 and 20. (*Id.*, ¶ 180).

2. The Claim 11 Nucleotide Sequence, Encoding the FlgI Signal Sequence, Would Have Also Been Obvious

As explained above, the specifically recited polynucleotide (SEQ ID NO:23) of claim 11 encodes the FlgI signal sequence. The amino acid of the FlgI polypeptide was known, as was the fact that the FlgI signal sequence was composed of the first nineteen amino acids of the FlgI polypeptide. (Exh. 1053, 1-2). The first nineteen amino acids of this UniProt entry, identified as the signal sequence, are

100% identical to the amino acid sequence of FlgI signal sequence in the ’345 patent. (Exh. 1001, Fig. 9C, SEQ ID NO: 24). The FlgI polynucleotide coding sequence was also known, the first fifty-seven nucleotides of which encode the signal sequence (*i.e.*, the first nineteen FlgI amino acids). (Exh. 1052, 3). This polynucleotide encoding this FlgI signal sequence is identical to SEQ ID NO: 23 as recited in claim 11, with the exception of the first base. (Exh. 1006, ¶ 181).

Further, the single base difference, does not alter the FlgI signal sequence and merely replaces a suboptimal (in terms of expression) codon (GTG) with an optimal one (ATG) (AUG in RNA); such standard codon optimization techniques that were well-known at the time of the ’345 patent’s earliest filing date. (Exh. 1037, 367; Exh. 1046, W126-27). POSAs would have been motivated to exchange the suboptimal codon for an optimal one. Because the amino acid sequence would not change after codon optimization, POSAs would have reasonably expected to preserve the capability of directing transport to the periplasm as described by Huber. (Exh. 1006, ¶ 182).

Consequently, for the reasons set forth herein, claims 4-14, 16-17 and 20 would have been obvious. *KSR*, 550 U.S. at 416.

VIII. THERE IS NO PROBATIVE EVIDENCE OF SECONDARY CONSIDERATIONS

Patent Owner has the burden of establishing secondary considerations of nonobviousness to overcome Petitioner’s strong *prima facie* showing of obviousness

above. Although secondary considerations must be taken into account, they do not necessarily control the obviousness conclusion. *See Newell Cos., Inc. v. Kenney Mfg.*, 864 F.2d 757, 768 (Fed. Cir. 1988). And in cases such as this one where a strong *prima facie* obviousness showing exists, the Federal Circuit has repeatedly held that secondary considerations may not dislodge the primary conclusion of obviousness. *See, e.g., Leapfrog Enters., Inc. v. Fisher-Price, Inc.*, 485 F.3d 1157, 1162 (Fed. Cir. 2007). Petitioner is not aware of any relevant evidence of secondary considerations. Notably, there is no evidence of the claimed invention achieving unexpected results: POSAs would have expected to achieve periplasmic expression using the claimed polynucleotides. *Galderma Labs., L.P. v. Tolmar, Inc.*, 737 F.3d 731, 739 (Fed. Cir. 2013) (test results, showing the continuation of a trend already known in the prior art, only establish a difference in degree, not a difference in kind needed to demonstrate unexpected results that are probative of nonobviousness).

Moreover, to the extent that Patent Owner attempts to point to alleged superior results achieved by specific examples, those results lack the necessary nexus to the claims to overcome a *prima facie* case of obviousness. *In re GPAC Inc*., 57 F.3d 1573, 1580 (Fed. Cir. 1995) ("For objective evidence to be accorded substantial weight, its proponent must establish a nexus between the evidence and the merits of the claimed invention."); *In re Paulsen*, 30 F.3d 1475, 1482 (Fed. Cir. 1994) (Even "impressive" evidence of secondary considerations is not "entitled to weight" unless

"it is relevant to the claims at issue.").

IX. CONCLUSION

Based on the foregoing and the accompanying expert declaration of Dr. DeLisa submitted herewith, claims 1-2, 4-14, and 16-21 of the '345 patent are anticipated and/or rendered obvious over the prior art cited herein. Petitioner has established a reasonable likelihood of prevailing on each Ground, and favorable resolution of this Petition in favor of Petitioner is respectfully requested.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this proceeding by this firm) to Jones Day Deposit Account No. 50-3013, ref: 122350-610012.

Respectfully Submitted,

Date: November 7, 2018

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CERTIFICATE OF COMPLIANCE

I, the undersigned, certify that the above Petition complies with the type-volume limitations of 37 C.F.R. § 42.24(a)(1)(i). Exclusive of the portions exempted by 37 C.F.R. § 42.24(a)(1), this Petition, including footnotes, contains 12,759 words as counted by the word count function of Microsoft Word.

Dated: November 7, 2018

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CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the foregoing Petition for *Inter Partes* Review of U.S. Patent No. 9,422,345, along with all exhibits supporting and filed with the Petition, were served on November 7, 2018, via UPS overnight courier delivery directed to the attorneys of record for the patents at the following addresses:

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