

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MOTIF FOODWORKS, INC.,

Petitioner,

v.

IMPOSSIBLE FOODS INC.,

Patent Owner.

Case No. IPR2023-00321

U.S. Patent No. 10,689,656

“Expression Constructs and Methods of
Genetically Engineering Methylophilic Yeast”

**PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 10,689,656
UNDER 35 U.S.C. §§ 311-319 AND 37 C.F.R. §§ 42.100 *et seq.***

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EX-1004	Declaration of Dr. Geoffrey Lin-Cereghino, dated January 27, 2023
EX-1005	Vogl et al., <i>Regulation of Pichia pastoris promoters and its consequences for protein production</i> , NEW BIOTECHNOLOGY, 30(4), 385-404 (2013) (“Vogl”)
EX-1006	Lin-Cereghino et al., <i>Regulator of the methanol utilization pathway and peroxisomal genes in Pichia pastoris</i> , MOLECULAR & CELLULAR BIOLOGY, 26(3), 883-897 (2006) (“Lin-Cereghino”)
EX-1007	U.S. Patent No. 8,143,023 B2 of Takagi et al., issued March 27, 2012 (“Takagi”)
EX-1008	U.S. Patent No. 5,824,511 of Mattoon et al., issued October 20, 1998 (“Mattoon”)
EX-1009	U.S. Patent No. 6,261,827 of Elrod et al., issued July 17, 2001 (“Elrod”)
EX-1010	U.S. Patent Publication No. 2011/0287467 A1, published November 24, 2011 (“Crane”)
EX-1011	Liu et al., <i>Balanced globin protein expression and heme biosynthesis improve production of human hemoglobin in Saccharomyces cerevisiae</i> , METABOLIC ENGINEERING, 21, 9-16 (2014) (“Liu”)
EX-1012	Vedvick et al., <i>High-level secretion of biologically active aprotinin from the yeast Pichia pastoris</i> , J. INDUSTRIAL MICROBIOLOGY, 7, 197-202 (1991) (“Vedvick”)
EX-1013	Engel et al., <i>Foods and food ingredients produced via recombinant DNA techniques</i> , GENETICALLY MODIFIED FOODS – ACS SYMPOSIUM SERIES, 605, Chapter 1, 1-10 (1995) (“Engel”)

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EX-1022	Zhang et al., <i>Heterologous protein expression in yeasts and filamentous fungi</i> , MANUAL OF INDUSTRIAL MICROBIOLOGY & BIOTECHNOLOGY, 145-156 (Bull et al. ed. 2010) (“Zhang”)
EX-1023	Kranthi et al., <i>Identification of Mxr1p-binding sites in the promoters of genes encoding dihydroxyacetone synthase and peroxin 8 of the methylotrophic yeast Pichia pastoris</i> , YEAST, 27, 705-711 (2010) (“Kranthi_2010”)
EX-1024	U.S. Patent Publication No. 2011/0129874 A1, issued June 2, 2011 (“Tsutsumi”)

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EX-1027	Rabert et al., <i>Recombinants proteins for industrial uses: utilization of Pichia pastoris expression system</i> , BRAZILIAN J. MICROBIOLOGY, 44(2), 351-356 (2013) (“Rabert”)
EX-1028	Stryjewska et al., <i>Biotechnology and genetic engineering in the new drug development. Part I. DNA technology and recombinant proteins</i> , PHARMACOLOGICAL REPORTS, 65, 1075-1085 (2013) (“Stryjewska”)
EX-1029	Ahmad et al., <i>Protein expression in Pichia pastoris: recent achievements and perspectives for heterologous protein production</i> , APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, 98, 5301-5317 (2014) (“Ahmad”)
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EX-1033	Katakura et al., <i>Effect of methanol concentration on the production of human β_2-glycoprotein I Domain V by a recombinant Pichia pastoris: a simple system for the control of methanol concentration using a semiconductor gas sensor</i> , J. FERMENTATION & BIOENGINEERING, 86(5), 482-487 (1998) (“Katakura”)

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EX-1045	Hargrove et al., <i>Characterization of Recombinant Soybean Leghemoglobin a and Apolar Distal Histidine Mutants</i> , J. MOLECULAR BIOLOGY, 266, 1032-1042 (1997) (“Hargrove”)

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EX-1063	Roggenkamp et al., <i>Transformation of the methylotrophic yeast Hansenula polymorpha by autonomous replication and integration vectors</i> , 202 MOLECULAR & GEN. GENETICS 302-08 (1986)

LIST OF CHALLENGED CLAIMS

Claim 1	
[1pre]	A methylotrophic yeast cell comprising
[1a]	a recombinant nucleic acid molecule, wherein the recombinant nucleic acid molecule comprises a first exogenous nucleic acid encoding a methanol expression regulator 1 (Mxr1) transcriptional activator operably linked to at least one methanol-inducible promoter element comprising a sequence to which the Mxr1 transcriptional activator binds,
[1b]	wherein the recombinant nucleic acid molecule comprises a second exogenous nucleic acid encoding a heterologous polypeptide operably linked to at least one methanol-inducible promoter element.
Claim 2	
The yeast cell of claim 1, wherein the methylotrophic yeast cell is of a yeast selected from the group consisting of <i>Candida</i> , <i>Hansenula</i> , <i>Pichia</i> and <i>Torulopsis</i> .	
Claim 3	
The yeast cell of claim 1, wherein the cell is a <i>Pichia</i> cell.	
Claim 4	
The yeast cell of claim 1, wherein the cell is a <i>Pichia pastoris</i> cell.	
Claim 5	
The yeast cell of claim 1, wherein the recombinant nucleic acid molecule is stably integrated into the genome of the methylotrophic yeast cell.	
Claim 6	
The yeast cell of claim 1, wherein the recombinant nucleic acid molecule is extrachromosomally expressed from a replication-competent plasmid.	
Claim 7	
The yeast cell of claim 1, wherein the exogenous nucleic acid encoding a transcriptional activator comprises a Mxr1 sequence from <i>Pichia pastoris</i> .	
Claim 8	
The yeast cell of claim 1, wherein the transcriptional activator comprises the sequence shown in SEQ ID NO: 59.	

Claim 9
The yeast cell of claim 8, wherein the transcriptional activator is encoded by the nucleic acid sequence shown in SEQ ID NO: 60.
Claim 10
The yeast cell of claim 1, wherein the at least one methanol-inducible promoter element is selected from the group consisting of an alcohol oxidase 1 (AOX1) promoter element from <i>Pichia pastoris</i> , an alcohol oxidase (AOD1) promoter element from <i>Candida boidinii</i> , a methanol oxidase (MOX) promoter element from <i>Hansenula polymorpha</i> , a methanolic alcohol oxidase (MOD1) promoter element from <i>Pichia methanolica</i> , a dihydroxyacetone synthase (DHAS) promoter element from <i>Pichia pastoris</i> , a formaldehyde dehydrogenase (FLD1) promoter element from <i>Pichia pastoris</i> , and a peroxin 8 (PEX8) promoter element from <i>Pichia pastoris</i> .
Claim 11
The yeast cell of claim 1, wherein the at least one methanol-inducible promoter element is an alcohol oxidase 1 (AOX1) promoter element from <i>Pichia pastoris</i> .
Claim 12
The yeast cell of claim 1, wherein the second exogenous nucleic acid encodes at least one heterologous polypeptide involved in biosynthesis of an iron co-factor.
Claim 13
The yeast cell of claim 12, wherein the iron co-factor is heme.
Claim 14
The yeast cell of claim 13, wherein the at least one polypeptide involved in the biosynthesis of heme is selected from the group consisting of aminolevulinic acid (ALA) synthase, ALA dehydratase, porphobilinogen deaminase, uroporphyrinogen (UPG) III synthase, UPG III decarboxylase, coproporphyrinogen (CPG) oxidase, protoporphyrin (PPG) oxidase, and ferrochelatase.
Claim 15
The yeast cell of claim 13, wherein the at least one polypeptide involved in the biosynthesis of heme comprises two or more polypeptides selected from the group consisting of ALA synthase, ALA dehydratase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, PPG oxidase, and ferrochelatase.

Claim 16
A method for expressing a heterologous polypeptide in a cell, the method comprising: providing the yeast cell of claim 1; and culturing the cell under conditions for expression of the recombinant nucleic acid molecule, thereby expressing the heterologous polypeptide.
Claim 17
The method of claim 16, where in the heterologous polypeptide is a member of globin family PF00042.
Claim 18
The method of claim 16, where in the heterologous polypeptide is a plant hemoglobin.
Claim 19
The method of claim 18, where in the plant hemoglobin is leghemoglobin.
Claim 20
The method of claim 19, where in the leghemoglobin has a sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:6.
Claim 21
The method of claim 16, where in the introducing step comprises a technique selected from the group consisting of transduction, electroporation, biolistic particle delivery, and chemical transformation.
Claim 22
The method of claim 16, where in the conditions under which the cell is cultured comprises addition of iron or a pharmaceutically or metabolically acceptable salt thereof.
Claim 23
The method of claim 16, where in the culturing step comprises culturing the cell in the presence of methanol.
Claim 24
The method of claim 16, where in the culturing step comprises culturing the cell in the absence of methanol.
Claim 25
The method of claim 16, where in the culturing step comprises culturing the cell in the absence of selection.

Claim 26	
[26pre]	A methylotrophic <i>Pichia</i> yeast cell comprising
[26a]	a recombinant nucleic acid molecule, wherein the recombinant nucleic acid molecule comprises:
[26b]	a nucleic acid encoding a Mxr1 transcriptional activator sequence from <i>P. pastoris</i> ;
[26c]	a nucleic acid encoding a member of globin family PF00042; and a nucleic acid encoding at least one polypeptide involved in heme biosynthesis, wherein each nucleic acid is operably linked to a methanol-inducible promoter element, wherein the methanol-inducible promoter element comprises at least one sequence to which a Mxr1 transcriptional activator binds.
Claim 27	
The yeast cell of claim 26, wherein the member of the globin family is leghemoglobin.	
Claim 28	
The yeast cell of claim 26, where in the methanol-inducible promoter element is an alcohol oxidase 1 (AOX1) promoter element from <i>P. pastoris</i> .	

I. INTRODUCTION

Motif Foodworks, Inc. (“Petitioner”) petitions for *inter partes* review (“IPR”) of U.S. Patent No. 10,689,656 (“’656 patent,” EX-1002). Patent Owner (“PO”), Impossible Foods, Inc., sued Petitioner for patent infringement in the District of Delaware, Case No. 22-cv-00311, on March 9, 2022. PO filed a Second Amended Complaint on September 7, 2022 alleging infringement of the ’656 patent.

II. STATEMENT OF RELIEF REQUESTED

A. Identification of Challenge (37 C.F.R. § 42.104(b))

Petitioner requests IPR and cancellation of claims 1-28 (“Challenged Claims”) of the ’656 patent.

B. Grounds of Challenge (37 C.F.R. § 42.204(b)(2))

Ground	Claims	Basis
1	1-17, 21, 23-25	§103, Tsutsumi, Lin-Cereghino, Mattoon
2	18-20	§103, Tsutsumi, Lin-Cereghino, Mattoon, Crane
3	22	§103, Tsutsumi, Lin-Cereghino, Mattoon, Krainer
4	26, 28	§103, Mattoon, Vogl
5	27	§103, Mattoon, Vogl, Crane

III. TECHNOLOGY BACKGROUND

A. Recombinant DNA Technology

The advent of recombinant DNA (“rDNA”) technology in the 1970s marked “one of the most important technologies developed in the 20th century” that allows the production of “a wide range of peptides, proteins, and biochemicals from naturally nonproducing cells.” EX-1028(Stryjewska), 1075-76; EX-1003(Batt), ¶¶54-56. rDNA is often the result of genetic engineering in which DNA from a “heterologous” source—a different cell type or a different species than the host in which it is being expressed—can be combined and used to express a protein of interest in a different host. EX-1003(Batt), ¶55; EX-1004(Lin-Cereghino), ¶¶53-54, 58.

Proteins are encoded by genetic sequence information—i.e., a “gene” sequence. EX-1003(Batt), ¶¶54-55; EX-1004(Lin-Cereghino), ¶¶53-54. In cells, a DNA gene sequence is transcribed into messenger-ribonucleic acid (“mRNA”) which is then translated into a sequence of amino acids called a protein or polypeptide. EX-1003(Batt), ¶¶54, 61. For example, the gene coding for leghemoglobin is transcribed and translated to produce the protein leghemoglobin. EX-1003(Batt), ¶¶54, 57-58.

rDNA expression results in recombinant proteins, also known as “heterologous” proteins. EX-1003(Batt), ¶¶55-56; EX-1004(Lin-Cereghino), ¶¶53-

54, 59. rDNA technology to produce such heterologous proteins has been widely utilized in the pharmaceutical, agricultural, and food industries. EX-1003(Batt), ¶¶55-56; EX-1028(Stryjewska), 1076; EX-1013(Engel), 1-6.

Production of a recombinant protein-of-interest typically involves creating rDNA by combining the gene that encodes the protein with other genetic elements. EX-1003(Batt), ¶¶57-61, 67-76; EX-1028(Stryjewska), 1076-79. *E.g.*, a gene encoding a protein-of-interest can be obtained and inserted (“cloned”) into a DNA expression vector. EX-1003(Batt), ¶¶59-60; EX-1004(Lin-Cereghino), ¶¶53-54; EX-1028(Stryjewska), 1076-79. This gene/vector combination is one form of rDNA that is routinely used by persons of skill in the art (“POSITAs”). EX-1003(Batt), ¶¶59-60; EX-1004(Lin-Cereghino), ¶¶53-54; EX-1028(Stryjewska), 1076-78.

To express recombinant proteins, rDNA vectors must first be introduced into host cells (*e.g.*, *P. pastoris*) whose machinery can be used to transcribe the rDNA and produce proteins—*e.g.*, via transformation. EX-1003(Batt), ¶¶59-61; EX-1004(Lin-Cereghino), ¶55; EX-1028(Stryjewska), 1077-79; EX-1015(Lin-Cereghino_2000), 50. To select for cells in which the rDNA is successfully introduced, vectors include “selection markers,” which are typically genes that confer resistance to antibiotics. EX-1003(Batt), ¶62; EX-1028(Stryjewska), 1078-79; EX-1015(Lin-Cereghino_2000), 48-50; EX-1029(Ahmad), 5304, 5310-11; EX-1004(Lin-Cereghino), ¶54. Transformed host cells can then be grown in medium

containing the antibiotic. EX-1003(Batt), ¶¶62; EX-1028(Stryjewska), 1078-79. Only cells that successfully incorporate the rDNA vector containing the selection marker will survive, and they can be used to express the recombinant protein. EX-1003(Batt), ¶¶62; EX-1028(Stryjewska), 1078-79.

B. *P. pastoris* Gene Expression System

POSITAs have used many different microbes to express rDNA. EX-1003(Batt), ¶¶63-64; EX-1022(Zhang), 145-46. Among eukaryotic expression systems, POSITAs have commonly used yeasts such as methylotrophic *P. pastoris* for rDNA expression. EX-1022(Zhang), 145-46; EX-1003(Batt), ¶¶64-66; EX-1004(Lin-Cereghino), ¶35. In fact, by 2015, *P. pastoris* had become “the premier example of yeast species used for the production of recombinant proteins.” EX-1030(Haon), 1-2; EX-1004(Lin-Cereghino), ¶60.

P. pastoris is a methylotrophic yeast “able to use methanol as [its] sole carbon source.” EX-1021(Sreekrishna), 1; EX-1003(Batt), ¶¶65-66; EX-1004(Lin-Cereghino), ¶¶35, 62. POSITAs widely adopted *P. pastoris* as an rDNA expression system because it “grows to high cell density, provides tightly controlled methanol-inducible transgene expression and efficiently secretes heterologous proteins in defined media.” EX-1018(De_Schutter), 561; EX-1003(Batt), ¶¶64-66; EX-1004(Lin-Cereghino), ¶¶70, 84. Before 2015, POSITAs routinely used *P. pastoris* for “industrial scale production of proteins via recombinant DNA technology” and

also “employed [it] in laboratories around the world to produce proteins for basic research and medical applications.” EX-1012(Vedvick), 197; EX-1018(De_Schutter), 561. By 2008, “[s]everal hundred proteins from almost all kingdoms of life ha[d] been expressed in this yeast.” EX-1017(Hartner_2008), 1.

By 2015, the *P. pastoris* expression technology had been commercially available for many years, the full genomic sequences of several strains of *P. pastoris* had been published, and the FDA had approved recombinant proteins expressed by *P. pastoris* as safe for use in humans and animals. EX-1003(Batt), ¶¶64-66; EX-1029(Ahmad), 5301-02; EX-1018(De_Schutter), 561, 565; EX-1021(Sreekrishna), 1-2; EX-1022(Zhang), 148. *P. pastoris* became “the most commonly used yeast species” and “a highly successful system” for the production of recombinant proteins. EX-1018(De_Schutter), 561; EX-1015(Lin-Cereghino_2000), 45; EX-1005(Vogl), 386.

POSITAs recognized that *P. pastoris* has “several advantages over other eukaryotic and prokaryotic expression systems,” including:

(i) rapid growth rate, coupled with ease of high cell-density fermentation; (ii) high levels of productivity...; (iii) elimination of...contamination; (iv) ease of genetic manipulation of well-characterized yeast expression vectors; (v) absence of known human pathogenicity...; (vi) diverse post-translational modifications that include polypeptide folding, glycosylation, methylation, acylation, proteolytic adjustment, and targeting to subcellular compartments; and (vii) the ability to engineer secreted proteins that can be purified from growth medium without harvesting the yeast cells themselves.”

EX-1027(Rabert), 351-52; EX-1003(Batt), ¶¶64-66; EX-1004(Lin-Cereghino), ¶¶60, 82-83; EX-1005(Vogl), 385-86; EX-1017(Hartner_2008), 1; EX-1022(Zhang), 149; EX-1015(Lin-Cereghino_2000), 51-52; EX-1018(De_Schutter), 561; EX-1021(Sreekrishna), 6.

C. Promoters and Transcriptional Activators

A promoter is typically a non-coding region of DNA upstream of the gene’s coding region. EX-1042(Alberts), 306; EX-1004(Lin-Cereghino), ¶55. Elements of the cell’s transcription machinery—transcription factors—bind to promoters and regulate transcription, largely determining the timing of gene expression and amount of protein that will be produced. EX-1042(Alberts), 306-07; 1004(Lin-Cereghino), ¶¶55-56.

Transcription factors can activate or repress gene transcription. EX-1042(Alberts), 310-11; EX-1005(Vogl), 386; EX-1004(Lin-Cereghino), ¶¶67-69. They do so by binding the promoter in ways that either allow transcription to occur (e.g., they are “transcriptional activators”), or cause it not to occur (“repressors”). EX-1042(Alberts), 310-11; EX-1005(Vogl), 386; EX-1003(Batt), ¶68; EX-1004(Lin-Cereghino), ¶¶67-69.

In *P. pastoris*, native promoters can be constitutive or inducible. EX-1003(Batt), ¶69; EX-1004(Lin-Cereghino), ¶57; EX-1005(Vogl), 393; EX-1029(Ahmad), 5302-04. Constitutive promoters are active regardless of external stimuli (*i.e.*, constitutively active); they bind to transcription factors that maintain a constant level of transcription under different conditions. EX-1003(Batt), ¶69; EX-1004(Lin-Cereghino), ¶57; EX-1005(Vogl), 386, 392; EX-1029(Ahmad), 5304. Inducible promoters can be made more or less active in response to external stimuli, such as temperature, oxygen, or chemicals. EX-1003(Batt), ¶69; EX-1004(Lin-Cereghino), ¶57; EX-1005(Vogl), 386; EX-1029(Ahmad), 5302-03.

By 2015, various *P. pastoris* expression systems for rDNA expression had been constructed that included constitutive and/or inducible promoters to control heterologous protein expression. EX-1017(Hartner_2008), 1; EX-1003(Batt), ¶69; EX-1005(Vogl), 385-87, 392-96; EX-1015(Lin-Cereghino_2000), 46-48; EX-1016(Hartner), 2; EX-1021(Sreekrishna), 7; EX-1029(Ahmad), 5302-04. Examples

of routinely used promoters alcohol oxidase I gene promoter (“P_{AOX1}”), the dihydroxyacetone synthase promoter (“P_{DAS}”), and the peroxisomal matrix protein promoter (“P_{PEX8}”), among others. EX-1003(Batt), ¶69-74; EX-1004(Lin-Cereghino), ¶40; EX-1005(Vogl), 385-87, 392-96; EX-1015(Lin-Cereghino_2000), 46-48; EX-1021(Sreekrishna), 7; EX-1029(Ahmad), 5302-04.

Below is an illustration of a typical vector engineered to express a gene-of-interest (“YFG”) in *P. pastoris*:

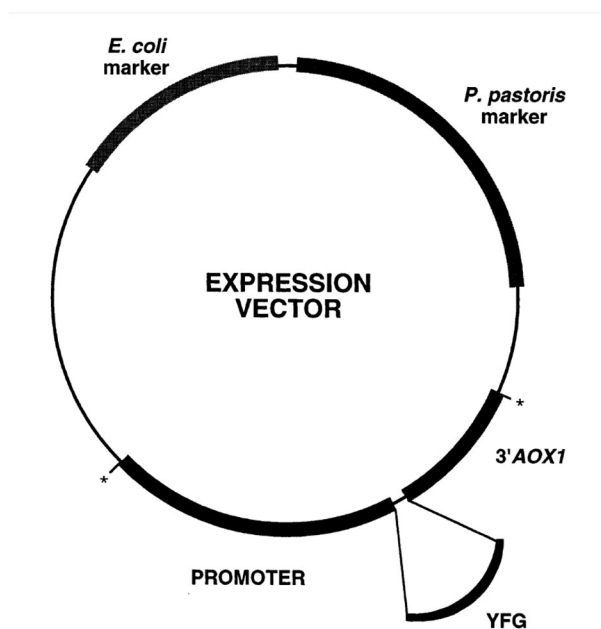


Fig. 3. General diagram of a *P. pastoris* expression vector. YFG, ‘Your Favorite Gene;’ *, sites for cassette amplification.

EX-1015(Lin-Cereghino_2000), 47-48; EX-1003(Batt), ¶75; EX-1004(Lin-Cereghino), ¶53. Adjacent to the promoter is a site into which the gene-of-interest can be cloned, so that after insertion the promoter operates to control the expression

of the gene-of-interest following transformation in *P. pastoris*. EX-1003(Batt), ¶¶75-76; EX-1004(Lin-Cereghino), ¶¶55, 58.

D. Carbon Source-Mediated Protein Expression

POSITAs understood that the expression of certain genes in methylotrophic yeast is regulated by carbon sources (*e.g.*, glucose, glycerol, or methanol) in the growth medium. EX-1003(Batt), ¶¶77-81; EX-1005(Vogl), 386-87; EX-1015(Lin-Cereghino_2000), 46-47; EX-1029(Ahmad), 5302; EX-1004(Lin-Cereghino), ¶¶66-67. Natively, when methanol is the only carbon source, methanol-inducible promoters “drive[s] the production of enzymes” needed for methanol metabolism by “produc[ing] extremely high levels” of the transcripts of those enzymes. EX-1018(De_Schutter), 564; EX-1003(Batt), ¶¶69-72, 77-81; EX-1004(Lin-Cereghino), ¶¶66-67. Other carbon sources such as glucose or glycerol “repress” transcription from these promoters. EX-1005(Vogl), 386; EX-1003(Batt), ¶¶77, 79-80; EX-1004(Lin-Cereghino), ¶69. When glucose/glycerol is depleted or at low concentrations, transcription is “derepressed” (*i.e.*, less repressed), though at lower levels than when methanol is the sole carbon source. EX-1017(Hartner_2008), 1; EX-1003(Batt), ¶¶77-80; EX-1005(Vogl), 387; EX-1015(Lin-Cereghino_2000), 47, 51-52; EX-1029(Ahmad), 5302. Methanol-inducible promoters are “ideal tools for heterologous protein production” because they afford control over gene expression. EX-1005(Vogl), 386; EX-1003(Batt), ¶78.

Methanol-inducible promoters can promote high levels of recombinant protein expression. EX-1005(Vogl), 386-87; 50-51, EX-1003(Batt), ¶¶77, 79-80; EX-1004(Lin-Cereghino), ¶¶60, 67-68. But high levels of transcription alone are not enough to express a recombinant protein at a high yield. *Id.* POSITAs must also take into account the growth rate of the host cells. EX-1003(Batt), ¶¶78-79; EX-1021(Sreekrishna), 6-8; EX-1022(Zhang), 148-49. For example, high expression of certain recombinant proteins may interfere with the growth of the host cells in which they are expressed. EX-1003(Batt), ¶¶78-79; EX-1021(Sreekrishna), 6-8; EX-1022(Zhang), 148.

To take advantage of the strength and inducible nature of methanol-inducible promoters, and produce large amounts of a recombinant protein without interfering with growth of the host cells, POSITAs regulate the available carbon sources in the growth medium. EX-1003(Batt), ¶¶79-80; EX-1005(Vogl), 386-87; EX-1015(Lin-Cereghino_2000), 51-52; EX-1029(Ahmad), 5302. To allow *P. pastoris* cells to grow to high numbers and densities, e.g., POSITAs “repress[]” methanol-inducible promoters in medium that lacks methanol but contains glucose or glycerol. EX-1003(Batt), ¶¶77-79; EX-1021(Sreekrishna), 7-8; EX-1022(Zhang), 149; EX-1005(Vogl), 386-87. Once the cells have grown to a significant mass, methanol-inducible promoters can be “derepressed” by depleting the glucose or glycerol and then “induced” by adding methanol to the medium, which induces protein expression

from a large number of cells. EX-1003(Batt), ¶¶78-81; EX-1005(Vogl), 387; EX-1015(Lin-Cereghino_2000), 51-52; EX-1021(Sreekrishna), 7-8; EX-1022(Zhang), 149.

In this way, POSITAs use carbon sources to regulate transcription and to control the timing and yield of recombinant protein expression. EX-1003(Batt), ¶¶77-81; EX-1005(Vogl), 386-87; EX-1015(Lin-Cereghino_2000), 51-52; EX-1029(Ahmad), 5302. This allows for production of a wide variety of recombinant proteins in *P. pastoris*, including even proteins that have been characterized as “toxic” to *P. pastoris*. EX-1029(Ahmad), 5302; EX-1021(Sreekrishna), 7; EX-1003(Batt), ¶¶79-80.

E. Mxr1 Regulation of Methanol-Inducible Genes

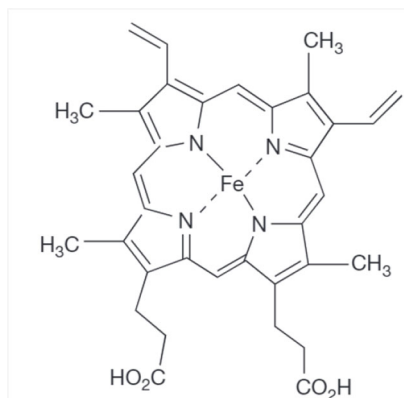
Before 2015, it was well-known that methanol-inducible promoters such as P_{AOXI} , P_{DAS} , and P_{PEX8} are regulated by a transcriptional activator called methanol expression regulator 1 (“Mxr1” or “Mxr1p,” encoded by the *MXR1* gene), which is “an important transcription factor” and “global regulator” of genes involved in methanol utilization in *P. pastoris*. EX-1026(Parua), 282, 288; EX-1019(Kranthi), 460; EX-1005(Vogl), 390; EX-1016(Hartner), 13; EX-1004(Lin-Cereghino), ¶¶35-36, 73-74; EX-1006(Lin-Cereghino), 883; EX-1023(Kranthi_2010), 705; EX-1003(Batt), ¶¶82-83. Natively, Mxr1 is produced constitutively at a low level in *P. pastoris* and its activity is regulated by the carbon source present in the growth

medium. EX-1016(Hartner), 13; EX-1026(Parua), 282-84, 287-88; EX-1005(Vogl), 390; EX-1003(Batt), ¶83.

Before 2015, POSITAs understood that genetically modifying *P. pastoris* cells to increase Mxr1 expression—for example, “by controlling the expression of Mxr1 [under a promoter] or by increasing the copy number of the gene encoding Mxr1”—can provide greater levels of Mxr1p-regulated recombinant protein expression. EX-1024(Tsutsumi), [0007], [0038], [0075]; EX-1003(Batt), ¶¶82-83; EX-1004(Lin-Cereghino), ¶¶43-45, 76-78.

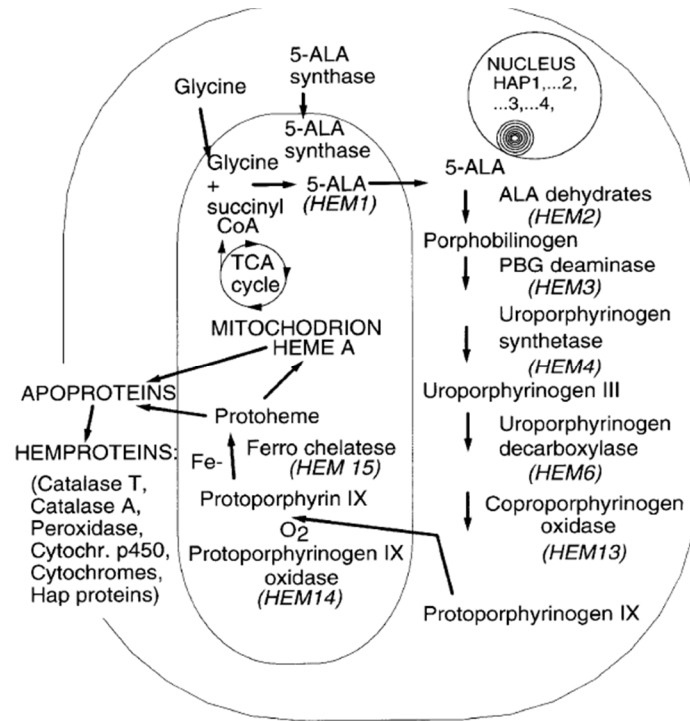
F. Expression of Heme-Containing Proteins

By 2015, POSITAs routinely applied microbial-based rDNA technology to produce proteins known as heme-containing proteins (“hemoproteins”) in microbes including yeasts. EX-1003(Batt), ¶¶84-85; EX-1008(Mattoon); EX-1010(Crane); EX-1011(Liu). Hemoproteins bind heme, an iron-containing compound (structure below), and are involved in many cellular processes. EX-1003(Batt), ¶¶84-85; EX-1037(Ferreira), 539-40; EX-1008(Mattoon), 1:39-48; EX-1010(Crane), [0021]; EX-1011(Liu), 9; EX-1014(Garrocho), 78-79; EX-1037(Ferreira), 539-40.



Hemoproteins include hemoglobin, myoglobin, and leghemoglobin (a plant hemoglobin). EX-1003(Batt), ¶85; EX-1010(Crane), [0021]; EX-1014(Garrocho), 78-79. Leghemoglobin is known to have a similar structure to myoglobin. EX-1044(Nicola), 133, 139; EX-1045(Hargrove), 1032; EX-1003(Batt), ¶88.

Typically, cells make heme through multi-step biosynthesis, involving enzymes that catalyze steps of the biosynthesis. EX-1003(Batt), ¶¶86-88; EX-1037(Ferreira), 539-40; EX-1008(Mattoon), Fig. 1; EX-1011(Liu), 11. Below is a scheme of the heme biosynthesis pathway:



See EX-1008(Mattoon), Fig. 1; EX-1003(Batt), ¶86.

Before 2015, one strategy for hemoprotein production was to co-express hemoprotein and enzymes involved in heme biosynthesis in host cells. EX-1008(Mattoon), 2:17-26; EX-1010(Crane), [0020], EX-1011(Liu), 9-10. This strategy ensures that the appropriate amount of heme is produced by the host cells to bind the recombinant heme-binding apoprotein produced by the same cells to form the hemoprotein. *Id.*

IV. OVERVIEW OF PRIOR ART

As discussed below, each of Mattoon, Crane, Tsutsumi, Lin-Cereghino, Vogl, and Krainer is prior art under AIA 35 U.S.C. §102(a)(1).

A. Tsutsumi

U.S. Patent Application Publication No. 2011/0129874 (“Tsutsumi”) published on June 2, 2011. Tsutsumi teaches a methylotrophic yeast expression system for expressing any exogenous protein under the control of a methanol-inducible promoter, such as P_{DAS}, and the transcriptional activator Mxr1 under the control of a promoter, where “overexpressed” Mxr1 drives expression of the exogenous protein. EX-1024(Tsutsumi), [0002], [0005], [0038], [0040]-[0041], [0145]; EX-1003(Batt), ¶¶89-91. Tsutsumi discloses that “it has been known for long that certain promoters are dependent on the presence of methanol in the growth medium for the induction of transcription” and that methanol “[c]ontrolled expression is often desirable.” EX-1024(Tsutsumi), [0005], [0041]. Tsutsumi further teaches that “induction by methanol requires the presence of...Mxr1...a key positive regulator required for methanol utilization in *Pichia pastoris*.” *Id.*¹

B. Mattoon

U.S. Patent No. 5,824,511 (“Mattoon”) issued October 20, 1998. Mattoon teaches “[a] method for increasing the production” of hemoproteins. EX-

¹ Tsutsumi previously discovered that controlled expression of the Prm1 gene can induce transcription “without the presence of methanol in the growth medium.” EX-1024(Tsutsumi), [0042].

1008(Mattoon), Abstract; EX-1003(Batt), ¶¶92-95. Mattoon’s claimed methods of heme-binding protein production are directed towards a “nucleic acid sequence compris[ing] a HEM2 gene,” a heme biosynthetic enzyme also known as ALA dehydrate. EX-1008(Mattoon), 11:46-48. Mattoon explains that there was a considerable interest in producing “cell-free hemoglobin as a substitute” for blood, and “[m]any commercial ventures [we]re working to enhance the biosynthesis of heme protein[s]” in “new strains” of yeast. EX-1008(Mattoon), 1:14-30.

Mattoon explains that “[f]or efficient industrial production of hemoproteins, appropriate ratios of heme and apoproteins must be simultaneously produced in a host cell.” EX-1008(Mattoon), 3:21-23. To accomplish this, Mattoon teaches “a method to produce hemoproteins by transforming a host cell with a nucleic acid sequence encoding a rate-limiting *enzyme in a heme biosynthetic pathway*” (e.g., HEM2), “coupled with the expression of a gene encoding a *heme-binding agent*” (e.g., heme-binding apoprotein), to “prevent[] undesired feedback inhibition that would otherwise be caused by excess concentrations of free heme.”² EX-1008(Mattoon), 2:9-30; EX-1003(Batt), ¶94. Mattoon also teaches that “[i]t is preferable to have an adequate supply of heme present while a cell is expressing a particular apoprotein, so as to provide an adequate amount of the constituents

² Emphasis added unless otherwise specified in the Petition.

required to form desired hemoproteins, thereby ending up with purer materials.”
EX-1008(Mattoon), 6:3-8, 6:27-38; EX-1003(Batt), ¶¶94-95.

According to Mattoon, “[p]referred host cells are eukaryotic,” “[i]n particular, yeast of the genus *Pichia*, and *even more preferably, Pichia pastoris*.” EX-1008(Mattoon), 4:29-37; EX-1003(Batt), ¶95. Mattoon teaches that vectors used to transform host cells are “controllable by *inducible or constitutive promoters*” that are “operatively linked” to the nucleic acid sequences encoding the proteins-of-interest. EX-1008(Mattoon), 2:41-44, 5:51-59, 6:9-63; EX-1003(Batt), ¶95. As an example, Mattoon discloses increased production of catalase, a hemoprotein, by co-expressing the catalase apoprotein and HEM2. EX-1008(Mattoon), 7:26-8:10; EX-1003(Batt), ¶95.

C. Crane

U.S. Patent Application Publication No. 2011/0287467 (“Crane”) published on November 24, 2011. Crane teaches “methods of producing recombinant functional heme-binding proteins with complete heme incorporation.” EX-1010(Crane), Abstract. Crane’s claimed methods of heme-binding protein production are directed to “co-expressing a recombinant *heme-binding protein*” with a “recombinant *ferrochelataase protein*,” a heme biosynthetic enzyme commonly known as HEM15. EX-1010(Crane), Abstract, [0009], [0056]; EX-1003(Batt), ¶¶96-98.

Crane teaches that heme-containing proteins, which Crane calls heme-binding proteins, include “hemoglobin, myoglobin, ... [and] *leghemoglobin*.” EX-1010(Crane), [0021]. Crane notes that “[y]east strains and yeast-derived vectors are used commonly” for heterologous protein expression and “*Pichia pastoris* expression systems[] may be used to co-express a recombinant heme-binding protein of interest with ferrochelatase.” EX-1010(Crane), [0044].

D. Lin-Cereghino

Lin-Cereghino is an article published and publicly available in February 2006. Lin-Cereghino is an early paper that characterized Mxr1p as a transcriptional activator for methanol-inducible genes in *P. pastoris*. EX-1006(Lin-Cereghino), 883; EX-1003(Batt), ¶¶99-101; EX-1004(Lin-Cereghino), ¶¶33-35.

Lin-Cereghino observes that in *P. pastoris* most foreign genes had been expressed under P_{AOX1} in cells grown on methanol. EX-1006(Lin-Cereghino), 883. Lin-Cereghino notes that “derepressed (carbon-starved) cells display levels of *AOX1* transcriptional activity that are **approximately 2%** of that seen on methanol, whereas methanol-grown cells display activity that is **more than 1,000-fold** higher than that observed in fully repressed (glucose-grown) cells.” EX-1006(Lin-Cereghino), 884; EX-1004(Lin-Cereghino), ¶¶35-36.

Lin-Cereghino characterizes Mxr1p as “mostly cytoplasmic in glucose-grown cells, but it was mostly localized” to the nucleus in methanol-grown cells, and it was

also localized to the nucleus in glycerol-grown cells. EX-1006(Lin-Cereghino), 890, 893-94; EX-1004(Lin-Cereghino), ¶51.

Lin-Cereghino describes *P. pastoris* strains with increased expression of Mxr1p under control of either P_{AOX1} (a strong methanol-inducible promoter) or P_{PEX8} (a weaker methanol-inducible promoter). EX-1006(Lin-Cereghino), 884-86; EX-1004(Lin-Cereghino), ¶¶77-79; EX-1003(Batt), ¶¶100-101. The yeast strains were tested for their ability to grow on a medium containing 0.5% methanol. EX-1006(Lin-Cereghino), 884-86.

Lin-Cereghino reported that the yeast strains that produced Mxr1p under the weaker methanol-inducible promoter P_{PEX8} (pGC217) grew slowly on methanol but “contained relatively high levels of the product.” EX-1006(Lin-Cereghino), 893; EX-1003(Batt), ¶101. Lin-Cereghino also reported that yeast strains that produced Mxr1p under the strong methanol-inducible promoter P_{AOX1} (pGC215) “did not grow on methanol and did not induce Mxr1p or other methanol pathway components in response to methanol.” EX-1006(Lin-Cereghino), 893; EX-1004(Lin-Cereghino), ¶¶37-38. Based on this, Lin-Cereghino hypothesized that: “Apparently, high levels of Mxr1p are toxic to *P. pastoris*.” EX-1006(Lin-Cereghino), 893; EX-1004(Lin-Cereghino), ¶¶37-41.

Notably, however, Lin-Cereghino did not explore other growth conditions for pGC215, such as first growing the yeast in glucose or glycerol to increase cell mass

and then switching the carbon source to methanol to induce high levels of protein production, as taught by other prior art.³ EX-1004(Lin-Cereghino), ¶¶37-41; EX-1005(Vogl), 386-87; EX-1015(Lin-Cereghino_2000), 51-52; EX-1021(Sreekrishna), 7-8; EX-1022(Zhang), 149; EX-1029(Ahmad), 5302. Indeed, as discussed herein (§IV.E), subsequently published prior-art references, such as Vogl, disclose *P. pastoris* yeast strains that expressed high levels of Mxr1p under the strong promoter P_{GAP} to regulate P_{AOX1}-driven expression of cells grown in glucose-based medium, where the cells did grow and produce protein. EX-1005(Vogl), 391; *see also* EX-1024(Tsutsumi), [0137]-[0145] (methanol-grown *P. pastoris* expressing Mxr1p under P_{GAP} and a cutinase gene under P_{DAS} variants); EX-1004(Lin-Cereghino), ¶¶40-41.

E. Vogl

Vogl is an article published and publicly available in May 2013. Vogl reviews prior publications on *P. pastoris* promoters and transcription factors, including EX-1006(Lin-Cereghino), and “their application to improve heterologous protein

³ Moreover, Lin-Cereghino also did not explore different methanol concentrations or the monitoring or control of methanol concentrations in the growth medium, which would have affected *P. pastoris* cell growth. EX-1004(Lin-Cereghino), ¶¶37-41; EX-1032(Guarna); EX-1033(Katakura); EX-1034(Hong); EX-1035(Trinh).

production.” EX-1005(Vogl), 386; EX-1003(Batt), ¶¶102-103. This includes reviewing “the most commonly used” promoter P_{AOX1} and its regulation. EX-1005(Vogl), 386-92; EX-1003(Batt), ¶¶102-103.

Vogl teaches the effect of carbon source on P_{AOX1} , explaining that P_{AOX1} is “repressed” when *P. pastoris* cells are grown on a “repressing carbon source,” such as glucose or glycerol, and strongly induced when cells are grown on methanol. EX-1005(Vogl), 386; EX-1003(Batt), ¶104. Vogl explains that this is a useful tool “for heterologous protein expression in *P. pastoris*,” as it allows “cell growth to high densities on a repressing carbon source without heterologous protein expression hampering the cellular metabolism.” EX-1005(Vogl), 386.

Vogl also provides an overview of other promoters, including constitutive promoters P_{GAP} and P_{TEF1} . EX-1005(Vogl), 392-95; EX-1003(Batt), ¶¶105-106. Vogl explains that promoters have different strengths. EX-1005(Vogl), 392-95. Vogl further explains that P_{GAP} and P_{AOX1} are both strong promoters that “provide similar expression levels under the respective optimal growth conditions.” EX-1005(Vogl), 392. Vogl explains, however, that “the strong expression from P_{AOX1} and P_{GAP} is not always the most favorable condition for heterologous protein production”; rather, “a weaker expression level can be more desirable” in some cases. EX-1005(Vogl), 392. Weaker promoters such as P_{PEX8} (inducible) and P_{YPT1} (constitutive) are “alternatives in case P_{AOX1} or P_{GAP} driven expression is too strong.”

EX-1005(Vogl), 393. In Table 1, Vogl lists over 20 promoters that can be used to provide varying levels of protein expression in *P. pastoris* as desired by POSITAs. EX-1005(Vogl), 393.

Vogl explains that methanol-inducible promoters are regulated by the transcriptional activator Mxr1p, and that Mxr1p binds to specific sites of methanol-inducible promoters (such as P_{AOX1}, P_{DAS} and P_{PEX8}) to activate transcription. EX-1005(Vogl), 390-91 (citing EX-1019(Kranthi) and EX-1023(Kranthi_2010)); EX-1004(Lin-Cereghino), ¶68.

Vogl reviews several prior studies involving increased expression of Mxr1p in *P. pastoris*. EX-1003(Batt), ¶¶107-108; EX-1005(Vogl), 391, 394. In one study, Takagi co-expressed (1) Mxr1p under the strong constitutive promoter P_{GAP} and (2) a phytase under P_{AOX1} regulated by Mxr1p in *P. pastoris* cells grown on glucose, which “resulted in a **7.6-fold** increased activity of the phytase expressed from P_{AOX1} compared to the non *MXR1* coexpressing strain.” EX-1005(Vogl), 391 (citing EX-1007(Takagi)); EX-1007(Takagi), 12:43-44, 14:40-41, 17:30-18:40; EX-1004(Lin-Cereghino), ¶¶47-48, 51. Vogl notes that this “7.6-fold increase might still account only for a low absolute expression level, as P_{AOX1} is increased ~1000-fold from glucose to methanol.” EX-1005(Vogl), 391; EX-1004(Lin-Cereghino), ¶51. However, Takagi is described as a method for producing exogenous protein with “no addition of methanol.” EX-1007(Takagi), Abstract, 2:4-5. In light of Takagi’s

findings, Vogl expressly invites “further investigation” into overexpressing Mxr1 under P_{AOX1} and other promoters to regulate expression of heterologous proteins under the control of methanol-inducible promoters to which Mxr1p binds. EX-1005(Vogl), 391.; EX-1004(Lin-Cereghino), ¶52.

Vogl also reviews Tsutsumi, another study of Mxr1p discussed above. EX-1005(Vogl), 394 (citing EX-1024(Tsutsumi)); *see* §IV.A.

F. Krainer

Krainer is an article published and publicly available on January 13, 2015. Krainer describes certain *P. pastoris* strains that co-expressed (1) the hemoprotein horseradish peroxidase (“HRP”) and (2) one of the eight heme biosynthetic enzymes. EX-1031(Krainer), 2-4. Krainer reports that on a microscale, co-expressing HEM1 or HEM3 with HRP resulted in increased production of HRP. EX-1031, 2-4. Krainer further discloses “medium supplementation with iron, heme precursors or hemin (the ferric chloride species of heme) was assessed as alternative strategy to effectively saturate recombinant apo-species of heme proteins [13-17].” EX-1031(Krainer), 2. Krainer concludes “FeSO₄ and hemin...to be useful medium supplements to increase the yield of active heme protein.” EX-1031(Krainer), 6.

G. Cited Non-Patent References Are Printed Publications Publicly Available Before the Priority Date of the Challenged Claims

Lin-Cereghino, Vogl, and Krainer are printed publication prior art. As set forth in EX-1057(Hall-Ellis), ¶¶48-64, 208-217, these references were disseminated or otherwise made available, including being cataloged and indexed by a library, such that a person interested in and ordinarily skilled in the relevant subject matter could locate them through the exercise of ordinary diligence on or about February 2006, May 2013 and January 13, 2015, respectively. Indeed, these references were published by highly reputable scientific journals and leading publishers in the field of recombinant protein production, and were available to POSITAs exercising reasonable diligence before May 11, 2015. EX-1003(Batt), ¶¶99, 102, 109.

Additionally, the corresponding publication was publicly available on or around that same date through libraries with access to the OCLC bibliographic database or through an individual library. EX-1057(Hall-Ellis), ¶¶48-64, 208-217. EX-1006(Lin-Cereghino), EX-1005(Vogl), and EX-1031(Krainer) are authentic copies that would have been available to a POSITA as of the dates cited above, respectively. EX-1057(Hall-Ellis), ¶¶48-64, 208-217.

V. OVERVIEW OF THE '656 PATENT

A. Claims and Specification

The '656 patent has twenty-eight claims, two independent claims (1 and 26) and twenty-six dependent claims. EX-1002, 87:31-90:18. The '656 patent is

directed to “methods and materials for genetically engineering methylotrophic yeast.” EX-1002, Abstract. The specification “describes the use of *P. pastoris* strains that overexpress the transcriptional activator, Mxr1, from the AOX1 promoter to increase expression of transgenes that also are expressed from the AOX1 promoter, which significantly improves the recombinant production of one or more proteins.” EX-1002, 1:38-43. Like the prior art discussed herein, it states that “expression of Mxr1 from the AOX1 promoter creates a positive feedback loop that allows for expression of other transgenes from the AOX1 promoter in the absence of methanol ... when repressing carbon sources are depleted.” EX-1002, 1:43-47, 3:50-57. Similarly, it states that “[e]xpression of Mxr1 results in a significant increase in the amount of protein produced.” EX-1002, 1:47-49.

The specification states that “*P. pastoris* contains an endogenous nucleic acid encoding a Mxr1 transcriptional activator,” and “an additional *P. pastoris* Mxr1 nucleic acid (e.g., introduced recombinantly into *P. pastoris*).” EX-1002, 4:18-22. It further reports that “[t]ranscriptional activators such as Mxr1 may be normally expressed at low levels,” and “it is desirable to place the exogenous nucleic acid (i.e., the transcriptional activator) under control of a promoter that is inducible.” EX-1002, 4:44-47. The specification further provides that “‘operably linked’ means that a promoter or other expression element(s) are positioned relative to a nucleic acid

coding sequence in such a way as to direct or regulate expression of the nucleic acid (e.g., in-frame).” EX-1002, 4:47-51.

The patent’s examples describe *P. pastoris* strains that expressed the genes of Mxr1, soybean leghemoglobin, and heme biosynthetic enzymes under the control of P_{AOX1}. EX-1002, 12:15-28:48, 39:24-40:34. The specification notes that “[t]he presence of an additional copy of Mxr1 under the control of the AOX1 promoter in these strains was confirmed by PCR.” EX-1002, 27:1-3. The specification describes *P. pastoris* strains that used both inducible and constitutive promoters—placing the genes of Mxr1, leghemoglobin, and heme biosynthetic enzymes under inducible promoter P_{AOX1} (as in the above examples), and additional copies of the leghemoglobin gene under a constitutive promoter such as P_{TEF1}, P_{GAP}, or P_{GCW14}. EX-1002, 28:50-29:24; 40:36-50.

The specification acknowledges that many elements and techniques were known by POSITAs prior to 2015. For example, the ’656 patent acknowledges that (1) transcriptional activators including Mxr1, (2) methanol-inducible promoters including P_{AOX1}, and (3) constitutive promoters including P_{TEF1}, were all “known in the art.” EX-1002, 4:23-38, 4:55-5:11, 6:36-51. It also acknowledges that soybean leghemoglobin was known and “naturally found in root modules of soybean.” EX-1002, 6:11-17. Additionally, it acknowledges that methods of “stably integrat[ing]” the recombinant nucleic acid molecules “into the genome of the methylotrophic

yeast cells,” or “extrachromosomally express[ing]” them “from a replication-competent plasmid,” were “well known and routinely used in the art.” EX-1002, 5:12-17. It further acknowledges that (1) methods of “introducing nucleic acids into methylotrophic yeast cells,” (2) methods of “culturing methylotrophic yeast cells,” and (3) methods of “purifying polypeptides,” were all “known in the art.” EX-1002, 7:11-18, 7:59-60, 10:9-23.

B. Prosecution History

During prosecution of the '656 patent, the Examiner rejected claims 1-5, 7, 12-14, and 19 as anticipated by Zhou (WO2014/008729); claims 1-19 and 30-38 as obvious over Zhou, Varadan (WO2015/153666) and Lin-Cereghino (EX-1006(Lin-Cereghino)), or Zhou/Varadan/Lin-Cereghino in view of Liu (EX-1011(Liu)); and all the claims for non-statutory double patenting over U.S. Patent Nos. 9,938,327 and 10,273,492 and co-pending Application No. 16/123,121. EX-1040, 1260-74. The Applicant filed terminal disclaimers to overcome the double-patenting rejections, amended the claims to overcome the Zhou-based anticipation rejection by specifying Mxr1 as the required transcriptional activator, and argued that Varadan cannot support obviousness rejections because the subject application and Varadan were both owned by Impossible Foods Inc. EX-1040, 1293-1300, 1399-1410 (citing 35 U.S.C. §102(b)(2)(C)). The Applicant did not substantively distinguish the

claims from Varadan, Lin-Cereghino, or Liu. *Id.* Thereafter, the Examiner allowed the claims. EX-1040, 1427-34, 1445-61, 1479-91.

C. Priority Date

The '656 patent claims priority to eight provisional applications, the earliest of which was filed on May 11, 2015. *See* EX-1002, Cover Page. Even assuming, for purposes of this IPR, the '656 patent is entitled to the May 11, 2015 filing date of the earliest provisional application, each prior art reference applied in this petition predates that date and qualifies as prior art. *See supra* §IV; EX-1057(Hall-Ellis), ¶320.

D. Claim Construction

No construction is necessary for purposes of this IPR. *See Wellman, Inc. v. Eastman Chem. Co.*, 642 F.3d 1355, 1361 (Fed. Cir. 2011) (“[C]laim terms need only be construed to the extent necessary to resolve the controversy.”).

E. Person of Ordinary Skill in the Art

The '656 patent is directed to the field of recombinant protein production. A person of ordinary skill in the art would have at least a Bachelor of Science in biochemistry, chemistry, biological sciences, or an equivalent area of study. EX-1003(Batt), ¶36. A POSITA would also have at least three years of graduate study or professional experience with recombinant techniques for protein production or general genetic modification experience. *Id.*

VI. GROUND 1: TSUTSUMI, LIN-CEREGHINO AND MATTOON

A. Overview

Tsutsumi and Lin-Cereghino and, optionally, Mattoon, render obvious Claims 1-17, 21, and 23-25. EX-1003(Batt), §IX.A-V.

Tsutsumi, Lin-Cereghino and Mattoon are analogous art to the '656 patent because each discloses genetically modifying *P. pastoris* yeast cells to express recombinant proteins. Tsutsumi teaches overexpression of Mxr1 to enhance production of a recombinant polypeptide in *P. pastoris*. Lin-Cereghino discloses expressing Mxr1 using methanol-inducible promoters to which Mxr1 binds, such as P_{PEX8} and P_{AOX1}. Mattoon generally teaches recombinant techniques to produce heterologous polypeptides, including expression of multiple nucleic acid sequences on one vector. As discussed below, a POSITA would have been motivated to combine these references with a reasonable expectation of success (“REOS”); EX-1003(Batt), ¶¶112-116.

B. Claim 1

1. 1[pre]

(“A methylotrophic yeast cell comprising a recombinant nucleic acid molecule...”)

Tsutsumi discloses that its “invention relates to the controlled expression of polypeptides...in several yeast cells belonging to the group of yeast known as **methylotrophic yeast**...defined as a group of yeast which can utilize methanol as a

sole carbon source for their growth.” EX-1024(Tsutsumi), [0038]. Lin-Cereghino and Mattoon also disclose the use of methylotrophic *Pichia pastoris* as an expression system. EX-1003(Batt), ¶¶117-121; EX-1006(Lin-Cereghino), 883; EX-1008(Mattoon), 4:29-40 (“[p]referred host cells are...yeast of the genus *Pichia*, and even more preferably, *Pichia pastoris*”). As explained below, the methylotrophic yeast disclosed in Tsutsumi, Lin-Cereghino, and Mattoon each comprise a “recombinant nucleic acid molecule” (expression vector). EX-1003(Batt), ¶¶122-125.

Claim 1 recites that the claimed recombinant nucleic acid molecule “comprises a first exogenous nucleic acid” and “comprises a second exogenous nucleic acid.” As a POSITA would recognize, the claimed recombinant nucleic acid molecule (*e.g.*, an expression vector) may be integrated into the yeast genome. EX-1003(Batt), ¶¶125-126. As explained below (limitation 1[a]), Tsutsumi teaches *Pichia* transformed with multiple expression vectors, which a POSITA would understand are integrated into the *Pichia* genome. For example, Tsutsumi claims a “DNA construct” that includes Mxr1 under control of a promoter and a polypeptide under control of a DAS promoter, *i.e.*, a DNA construct comprising first and second nucleic acids encoding a heterologous polypeptide operably linked to a methanol-inducible promoter, *e.g.*, the DAS promoter, and Mxr1 operably linked to a promoter, *e.g.*, a methanol-inducible promoter, to which it can bind. EX-

1024(Tsutsumi), Claim 15 (incorporating Claims 1, 10, 12, and 13); EX-1003(Batt), ¶126. Tsutsumi therefore teaches a methylotrophic yeast including a single recombinant nucleic acid molecule comprising both of the nucleic acids required by limitations 1[a] and 1[b]. EX-1003(Batt), ¶126.

To the extent that the Patent Owner argues that Tsutsumi does not expressly disclose a recombinant nucleic acid molecule comprising two exogenous nucleic acid sequences, it would have been obvious to a POSITA to prepare such a construct. EX-1003(Batt), ¶¶126-127. There are ample teachings in the art of this recombinant technique, and a POSITA would have been able to engineer a single expression vector comprising multiple gene sequences of interest using the familiar Sambrook Laboratory Manual. EX-1003(Batt), ¶¶126-130.⁴ Similar techniques could be used

⁴ Crane also teaches an example of this approach. EX-1003(Batt), ¶131; EX-1010(Crane), [0034], [0035] (“In another embodiment of the invention, both nucleotide sequences are inserted into one expression vector and the single expression vector encodes both the recombinant heme-binding protein and the recombinant ferrochelatase.”), [0074] (describing successful transformation with two nucleic acids in one expression vector).

to prepare such a vector for use in methylotrophic yeast with no more than ordinary skill in the art. EX-1003(Batt), ¶130.

In this context, Mattoon teaches that vectors used to transform host cells are “controllable by inducible or constitutive promoters” that are “operatively linked” to the nucleic acid sequences. EX-1008(Mattoon), 2:41-44, 5:51-59, 6:9-63; EX-1003(Batt), ¶132. Mattoon teaches that “[b]oth the nucleic acid sequence encoding the [heme] rate-limiting enzyme as well as the heme-binding agent can be present on one expression vector or, alternatively, can be on separate vectors, such as separate multi-copy plasmids.” EX-1008(Mattoon), 5:34-46; EX-1003(Batt), ¶132. A POSITA would understand that Mattoon’s teachings could be readily applied to Tsutsumi/Lin-Cereghino. Tsutsumi teaches that “[t]he modified *DAS* promoters...will be useful for expression of any polypeptide of interest in *Pichia*.” EX-1024(Tsutsumi), [0068]; EX-1006(Lin-Cereghino), 883 (“Over 550 such proteins have been synthesized in [*P. pastoris*].”); EX-1003(Batt), ¶¶133-134. A POSITA would thus be motivated to combine Mattoon’s teaching (reflecting general knowledge in the art) with Tsutsumi/Lin-Cereghino with a REOS. See §V.A; EX-1003(Batt), ¶134.

2. 1[a]

(“recombinant nucleic acid molecule comprises a first exogenous nucleic acid encoding...Mxr1[] transcriptional activator operably linked to at least one methanol-inducible promoter element comprising a sequence to which the Mxr1 transcriptional activator binds”)

Tsutsumi in view of Lin-Cereghino discloses methylotrophic yeast including a recombinant nucleic acid molecule comprising a first exogenous nucleic acid encoding Mxr1 operably linked to a methanol-inducible promoter to which Mxr1 binds. EX-1003(Batt), ¶¶135-158.

As explained above, Tsutsumi teaches a methylotrophic yeast system for expressing exogenous protein and the transcriptional activator Mxr1. *See* §IV.A; EX-1003(Batt), ¶139. Tsutsumi’s Example 5 is titled “Expression of *Humicola Insolens* Cutinase from Modified DAS Promoter with...Mxr1 Over-Expression.” EX-1024(Tsutsumi), [0137]; EX-1003(Batt), ¶140. Tsutsumi discloses that the “aim of this [Example 5] experiment was to check whether the expression of a protein of interest, exemplified by the *Humicola insolence* [sic] cutinase gene controlled by an improved DAS promoter according to the invention,...could be improved by co-expression of transcription factors, such as...Mxr1 in *Pichia pastoris*.” *Id.*

Tsutsumi discloses co-expressing Mxr1 in the same cells under the control of a promoter element. Specifically, “**re-transformation of low-expressing *Humicola insolens* cutinase transformants...controlled by the improved DAS**

promoter...was carried out with the plasmids harboring...Mxr1...and the regulator gene [Mxr1] is controlled by the *GAP* promoter.” EX-1024(Tsutsumi), [0140]; EX-1003(Batt), ¶141. Tsutsumi further discloses advantages of this approach: “The obtained data [from Example 5] indicate an improved expression of the test protein [cutinase] when Mxr protein was over-expressed.” EX-1024(Tsutsumi), [0145]; EX-1003(Batt), ¶141.

Tsutsumi teaches, consistent with the ’656 patent specification, that “operably linked” means “a configuration in which a control sequence is placed at an appropriate position relative to the coding sequence of the polynucleotide sequence such that the control sequence directs the expression of the coding sequence of a polypeptide.” EX-1024(Tsutsumi), [0037]; EX-1003(Batt), ¶141; *see also* §IV.A. A POSITA would understand from these disclosures that, in Example 5, Tsutsumi’s Mxr1 transcriptional activator is operably linked (*i.e.*, controlled) by the *GAP* promoter. EX-1003(Batt), ¶141-142.

While Tsutsumi’s Example 5 utilizes the *GAP* promoter to control Mxr1, Tsutsumi teaches using a methanol-inducible system. Tsutsumi teaches, *e.g.*, that “[p]romoters suitable for the above activation may in particular be...induced by methanol,” EX-1024(Tsutsumi), [0108], and that “expression of the positive regulator Mxr1 is increased by **controlling** the expression of Mxr1.” *Id.*, Claim 15; EX-1003(Batt), ¶143. Moreover, a POSITA would understand that *GAP* promoter-

driven expression of Mxr1 in Tsutsumi Example 5 could be at least partly methanol-induced “there is a clear influence of the carbon source on the expression strength” of the *GAP* promoter and “studies in *P. pastoris*...indicat[e] that P_{GAP} driven expression is not entirely constitutive but regulated under certain conditions.” EX-1005(Vogl), 392; EX-1003(Batt), ¶144. A POSITA would thus understand that Tsutsumi discloses a methylotrophic yeast cell with Mxr1 under the control of a promoter element, which may be constitutive or methanol-inducible. EX-1003(Batt), ¶144.

In light of the benefits observed in Tsutsumi Example 5 from Mxr1 expression driven by the *GAP* promoter, a POSITA would recognize expressing Mxr1 under the control of a strong promoter is advantageous, and would reasonably be expected to succeed at producing a protein of interest. EX-1003(Batt), ¶144; EX-1004(Lin-Cereghino), 76-79. Indeed, Tsutsumi teaches that “over-expressing the Mxr 1 protein may have an effect on the modified *DAS* promoter activity” by enhancing recombinant protein expression. EX-1024(Tsutsumi), [0074], [0137]-[0145]. EX-1003(Batt), ¶145. Tsutsumi also teaches that methanol “[c]ontrolled expression is often desirable” and methanol-inducible promoters are “particularly strong” and “generally used to control the heterologous expression of proteins in yeast.” EX-1024(Tsutsumi), [0005]-[0006] (citing EX-1006(Lin-Cereghino) as support); EX-1003(Batt), ¶145.

A POSITA would have several well-established promoter options to choose from in order to express Mxr1 using a recombinant nucleic acid molecule. EX-1003(Batt), ¶146; EX-1004(Lin-Cereghino), ¶¶63-67. *E.g.*, Tsutsumi provides that “promoters...from which the expression of heterologous polypeptides can be controlled by the addition of methanol to the growth medium...include but are not limited to e.g. the **AOX1 promoter** (Alcohol Oxidase promoter), **DHAS promoter (or DAS promoter)** (dihydroxyacetone synthase promoter),...**PEX8-...promoter.**” EX-1024(Tsutsumi), [0040]. “The promoters controlling expression of the enzymes involved in methanol metabolism in these organisms are particularly strong, and these promoters are generally used to control the heterologous expression of proteins in yeast.” EX-1024(Tsutsumi), [0006]; EX-1003(Batt), ¶146; EX-1005(Vogl), 386 (“One of the most favorable properties of *P. pastoris*...are the strong methanol inducible promoters....”).

To the extent PO argues that Tsutsumi does not expressly disclose Mxr1 expression under control of a “methanol-inducible promoter element comprising a sequence to which the Mxr1 transcriptional activator binds,” it would be obvious to combine Tsutsumi with Lin-Cereghino, which discloses the use of a methanol-inducible promoter, *e.g.*, *PEX8*, to drive expression of Mxr1 in *P. pastoris*. EX-1003(Batt), ¶¶137, 147-148; EX-1004(Lin-Cereghino), ¶¶76-79; EX-1006(Lin-

Cereghino), 885, 893-94 (“[W]e expressed *MXR1* under the control of the promoter from the *P. pastoris* *PEX8* gene.... *PPEX8* is methanol regulated....”).

As discussed in §IV.D, Lin-Cereghino discloses that *P. pastoris* yeast cells are “best known as a popular system for the production of recombinant proteins.” EX-1006(Lin-Cereghino), 883. Lin-Cereghino describes *P. pastoris* strains with Mxr1p under control of either P_{AOX1} (a strong methanol-inducible promoter) or P_{PEX8} (a weaker methanol-inducible promoter). EX-1006(Lin-Cereghino), 885 (“pGC215, in which *MXR1* transcription is regulated by the *AOX1* promoter. pGC216 [with] the *MXR1* gene under the regulation of the *PEX8* promoter”); EX-1003(Batt), ¶148; EX-1005(Vogl), 393, Table 1. Lin-Cereghino further discloses that the *P. pastoris* strain with Mxr1p under regulation of the methanol inducible promoter P_{PEX8} (pGC217)⁵ grew on methanol and produced “relatively high levels of the product.” EX-1006(Lin-Cereghino), 893; EX-1004(Lin-Cereghino), ¶77; EX-1003(Batt), ¶148.

⁵ Lin-Cereghino discloses that pGC217 was created by mutagenizing the stop codon of P_{PEX8}-MXR1 construct in pGC216 to “provide an in-frame fusion with the c-myc epitope and His₆ tag.” EX-1006(Lin-Cereghino), 885; EX-1003(Batt), ¶147.

A POSITA would understand that, as taught in Lin-Cereghino Mxr1 binds to at least one sequence in the *PEX8* and *AOX1* promoters controlling Mxr1. EX-1003(Batt), ¶149; EX-1004(Lin-Cereghino), ¶¶74-75. Lin-Cereghino teaches that “Mxr1p specifically bound to one or more sites within the *AOX1* promoter,” EX-1006(Lin-Cereghino), 893, and “systematic analysis for Mxr1p binding sites” before 2015 revealed Mxr1 binding sites in, among others, “the promoters of dihydroxyacetone synthase (DAS) and peroxin 8 (PEX8).” EX-1005(Vogl), 390. For example, the art taught “[i]dentification of Mxr1p-binding sites in the promoters of genes encoding dihydroxyacetone synthase [DHAS] and peroxin 8 [PEX8] of the methylotrophic yeast *Pichia pastoris*.” EX-1023(Kranthi), Title, 706-07. Consequently, *e.g.*, a POSITA would recognize that the *PEX8* promoter-driven expression of Mxr1 taught in Lin-Cereghino reflects the methanol-inducible expression of Mxr1 by a promoter element (*PEX8*) to which Mxr1 itself binds. EX-1003(Batt), ¶149; EX-1004(Lin-Cereghino), ¶77.

A POSITA would therefore be motivated to substitute Tsutsumi’s GAP promoter controlling Mxr1 with a fully methanol-inducible promoter in order to tightly control and further overexpress Mxr1 that would bind to the methanol-inducible promoter and would have a REOS in doing so. EX-1003(Batt), ¶¶150-153; EX-1004(Lin-Cereghino), ¶¶76-79. For example, as Tsutsumi teaches, “[c]ontrolled expression is often desirable. In methylotrophic yeast it has been

known for long that certain promoters are dependent on the presence of methanol in the growth medium for the induction of transcription. This induction by methanol requires...Mxr1 p, described as a key positive regulator required for methanol utilization in *Pichia pastoris* ([citing EX-1006] Lin-Cereghino *et al.*, 2006, Mol Cell Biol 26(3): 883-897).” EX-1024(Tsutsumi), [0005]; EX-1003(Batt), ¶153; EX-1004(Lin-Cereghino), ¶67.

As discussed above, Tsutsumi discloses over-expressing the *MXR1* gene under the strong constitutive promoter P_{GAP} in turn increased the P_{DAS}-driven expression of the protein-of-interest (cutinase). EX-1024(Tsutsumi), [0145]. Tsutsumi also teaches that methanol-inducible promoters are “particularly strong” and “generally used to control the heterologous expression of proteins in yeast.” EX-1024(Tsutsumi), [0005]-[0006] (citing EX-1006(Lin-Cereghino) as support); EX-1003(Batt), ¶146. A POSITA would therefore know that an expression system constructed with Mxr1p controlled by a strong promoter is desirable and reasonably expected to succeed. EX-1003(Batt), ¶¶150-158; EX-1004(Lin-Cereghino), ¶¶76-79.

Tsutsumi’s teachings are consistent with Lin-Cereghino, which provides that “since most foreign genes expressed in *P. pastoris* are transcribed under the control of the...AOX1 gene, transcription in response to methanol is a key feature of this expression system.” EX-1006(Lin-Cereghino), 883; EX-1003(Batt), ¶¶149-151.

Lin-Cereghino further teaches that Mxr1 is “essential for the large accumulations of the AOX1 and DHAS [DAS] transcripts,” such as the DAS promoter controlling the protein-of-interest in Tsutsumi, and discloses placing the *MXR1* gene under the control of methanol-inducible *P. pastoris* promoter, P_{PEX8} or P_{AOX1}, to increase Mxr1p expression. EX-1006(Lin-Cereghino), 889, 893-94; EX-1003(Batt), ¶151.

Accordingly, a POSITA would have been motivated to increase the expression of the *MXR1* gene by placing it under the control of a methanol-inducible promoter, which would allow a POSITA to modulate expression using carbon sources and increase expression of a recombinant protein under the control of a methanol-inducible promoter (e.g., P_{DAS} or P_{AOX1}) that Mxr1 binds. EX-1003(Batt), ¶152; EX-1004(Lin-Cereghino), ¶¶76-79. A POSITA would have options (e.g., P_{PEX8}, P_{DAS} and P_{AOX1}), which are natural alternatives to Mxr1 expression controlled under P_{GAP}. EX-1003(Batt), ¶¶153-156; EX-1004(Lin-Cereghino), ¶¶63-65; EX-1005(Vogl), 392 (“P_{GAP} has emerged as the most commonly used alternative to P_{AOX1}” and “provide[s] similar expression levels under the respective optimal growth conditions”), 393 (“P_{DAS} is a promoter...showing a similar regulatory pattern and expression level as P_{AOX1}.”); Table 1 (listing promoters). A POSITA would recognize the benefits of trying a variety of methanol-inducible promoters to achieve the most favorable conditions for heterologous protein production. EX-1003(Batt), ¶154; EX-1005(Vogl), 392-93 (“[T]he strong expression from P_{AOX1} and P_{GAP} is not

always the most favorable condition for heterologous protein production,” and “P_{PEX8} and P_{YPT1} are weak promoters and considered alternatives in case P_{AOX1} or P_{GAP} driven expression is too strong.”).

A POSITA would further understand that methanol-inducible promoters have “major advantages” due to their “strength and the tight regulation...[e]specially for the production of toxic or detrimental heterologous proteins, the inducibility with methanol is favorable. Cultures can at first be grown to high cell densities under repressing conditions and in a second phase, heterologous protein production can be started by methanol induction.” EX-1005(Vogl), 392; EX-1003(Batt), ¶152; EX-1004(Lin-Cereghino), ¶¶67-71. For all these reasons, a POSITA would be motivated to substitute Tsutsumi’s GAP promoter controlling Mxr1 expression with a methanol-inducible promoter such as P_{PEX8}, P_{DAS} or P_{AOX1}, with a REOS. EX-1003(Batt), ¶153; EX-1004(Lin-Cereghino), ¶¶76-79.

A POSITA would reasonably expect success engineering methylotrophic yeast that overexpress Mxr1 under a methanol-inducible promoter to which Mxr1 binds (e.g., *PEX8*) in order to produce higher levels of heterologous protein expression. EX-1003(Batt), ¶157; EX-1004(Lin-Cereghino), ¶¶76-79. Moreover, a POSITA could do so using routine techniques and readily available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶¶157-158. A POSITA looking to implement a P_{PEX8}-*MXR1*, P_{DAS}-*MXR1* or P_{AOX1}-*MXR1* construct in *P.*

pastoris (including strains with multiple copies) would employ well-known strategies to control protein expression in *P. pastoris* with carbon sources. For example, a POSITA would first grow the cells to high densities while repressing recombinant protein expression in a medium that contained glucose or glycerol. EX-1005(Vogl), 386-87 (describing cultivation methods). A POSITA would then derepress expression by depleting the glucose or glycerol and adding methanol. *Id.* This would in turn strongly induce recombinant protein expression driven by methanol-inducible promoters (*e.g.*, P_{PEX8}, P_{DAS} or P_{AOX1}) from a large number of cells. *Id.* A POSITA would understand that growing the cells to a high density and only then inducing recombinant gene expression with the addition of methanol would result in high levels of protein expression. EX-1003(Batt), ¶158; EX-1004(Lin-Cereghino), ¶70.

3. 1[b]

(“...wherein the recombinant nucleic acid molecule comprises a second exogenous nucleic acid encoding a heterologous polypeptide operably linked to at least one methanol-inducible promoter element”)

Tsutsumi discloses, alone or in view of Mattoon, methylotrophic yeast comprising the recombinant nucleic acid molecule of limitation 1[a] also comprising a second exogenous nucleic acid encoding a heterologous polypeptide operably linked to at least one methanol-inducible promoter element. To the extent not

disclosed in Tsutsumi, it would be obvious to create such a recombinant nucleic acid molecule as explained above. EX-1003(Batt), ¶159. For example, using a methanol-inducible system to express both Mxr1 and a heterologous polypeptide would afford a POSITA control over the expression of both. EX-1003(Batt), ¶160; EX-1004(Lin-Cereghino), ¶¶77-78.

As discussed in §VI.B(2), Tsutsumi's Example 5 discloses methylotrophic *P. pastoris* yeast expressing a cutinase polypeptide operably linked to (under the control of) a methanol-inducible *DAS* promoter. EX-1024(Tsutsumi), [0040], [0137] ("The aim...was to check whether the expression of...**cutinase gene controlled by an improved DAS promoter**...could be improved by co-expression of transcription factors, such as...Mxr1...."); Tsutsumi also claims a "DNA construct" that includes Mxr1 under control of a promoter and a polypeptide under control of a DAS promoter. EX-1024(Tsutsumi), Claim 15 (incorporating Claims 1, 10, 12, and 13). EX-1003(Batt), ¶¶162-165; *see also* EX-1005(Vogl), 386, Table 1. Tsutsumi teaches use of other methanol-inducible promoters, "e.g. the AOX1 promoter...DHAS promoter (or DAS promoter)...PEX8-, ...promoter." EX-1024(Tsutsumi), [0040]; EX-1003(Batt), ¶166.

A POSITA would thus understand from these teachings that Tsutsumi discloses a recombinant nucleic acid molecule comprising an exogenous nucleic acid encoding a heterologous polypeptide operably linked to at least one methanol-

inducible promoter element such as P_{DAS}. EX-1003(Batt), ¶163-164. A POSITA would further understand and it would be obvious to try a variety of methanol-inducible promoters, including others identified by Tsutsumi, to achieve the most favorable conditions for heterologous protein production. EX-1003(Batt), ¶166-167; EX-1005(Vogl), 392, Table 1.

Accordingly, using the teachings of Tsutsumi, Lin-Cereghino and Mattoon, as well as the routinely used techniques and readily available materials that were well-known in the art, a POSITA would have a reasonable expectation of success engineering methylotrophic yeast that overexpress Mxr1 under the control of a methanol-inducible promoter to which Mxr1 binds (e.g., in order to produce higher levels of heterologous protein expression), when the latter is also expressed under the control of a methanol-inducible promoter. EX-1003(Batt), ¶167; EX-1004(Lin-Cereghino), ¶¶76-79. As explained in the '656 patent, incorporating the claimed nucleic acid molecule into *P. pastoris* yeast cells only requires routine techniques, known methods, and available materials for genetic engineering and recombinant protein production. EX-1002, 5:13-17, 9:29-55 (“[E]xpression constructs or vectors[] are commercially available or can be produced by recombinant DNA techniques routine in the art.”); EX-1003(Batt), ¶167.

C. Claims 2-4

(“the...cell is...*Pichia* [*pastoris*] [cell]”)

Tsutsumi discloses that its methylotrophic yeast cells “belong to the genera...*Pichia*, *Hansenula*, *Candida*, *Torulopsis*.” Tsutsumi teaches that “**the *Pichia* host cell can in one embodiment be selected from...*P. pastoris*.**” EX-1024, [0039]; EX-1003(Batt), §§IX.C-E, ¶169. As discussed in §§VI.B(2)-(3), Lin-Cereghino and Mattoon also both disclose methylotrophic yeast expression systems, including *P. pastoris*. EX-1003(Batt), §§IX.C-E.

D. Claims 5-6

Claim 5 (“...the recombinant nucleic acid molecule is stably integrated into the genome of the methylotrophic yeast cell”)

Claim 6 (“...the recombinant nucleic acid molecule is extrachromosomally expressed from a replication-competent plasmid”)

Tsutsumi discloses or renders obvious, alone or in view of Mattoon or the knowledge of a POSITA, methylotrophic yeast comprising a recombinant nucleic acid molecule that is either stably integrated into the genome or extrachromosomally expressed from a replication-competent plasmid. EX-1003(Batt), §§IX.F-G.

For example, Tsutsumi teaches stable integration of recombinant nucleic acids into the genome: “[c]onventionally, maximal expression of a gene in a eukaryotic organism is achieved by **amplifying in the chromosome an expression cassette containing a single promoter operably linked to a gene encoding the**

polypeptide of interest.” EX-1024(Tsutsumi), [0004]; EX-1003(Batt), ¶177. Tsutsumi teaches an “expression cassette” is synonymous with “nucleic acid construct,” which a POSITA would understand is a recombinant nucleic acid molecule, when the nucleic acid construct contains the control sequences required for expression of a coding sequence. EX-1024(Tsutsumi), [0037]; EX-1003(Batt), ¶177. Tsutsumi further discloses screening transformants for the presence of stable integration of the gene sequence of interest at a particular locus using conventional techniques. EX-1003(Batt), ¶178-179; *see also* EX-1024(Tsutsumi), [0069]-[0070], [0115]-[0117] (“Screening for His4 Locus Integration”), [0124], [0136]; EX-1008(Mattoon), 4:41-48 (teaching “use of a vector that integrates into the genum [sic] of the host cell”).

A POSITA would also be aware that recombinant nucleic acid molecules—such as the nucleic acid sequences encoding Mxr1 and a heterologous polypeptide—could be expressed via extrachromosomal expression, using replication-competent (autonomously-replicating) expression vectors. EX-1003(Batt), ¶¶181-184. Such vectors were available for use in methylotrophic yeast, *e.g.*, *Candida* and *Hansenula* species. EX-1003(Batt), ¶184; *see generally* EX-1062(Kurtz); EX-1063(Roggenkamp). It would be obvious to a POSITA to prepare replication-competent constructs—*e.g.*, vectors containing a familiar origin of replication (*ori*)

sequence—in order to replicate and propagate the vectors during proliferation of host cells. EX-1003(Batt), ¶183.

In view of the finite number of predictable ways for engineering a methylotrophic yeast cell with recombinant nucleic acid molecules—either stably integrated or extrachromosomally expressed—a POSITA would find it obvious to try both options, and would have reasonably expected successful incorporation of the nucleic acids into the yeast. EX-1003(Batt), ¶¶173-174, 183-184. Indeed, the '656 patent acknowledges that methods of “stably integrat[ing]” recombinant nucleic acids “into the genome of the methylotrophic yeast cells,” or “extrachromosomally express[ing]” them “from a replication-competent plasmid,” were “well known and routinely used in the art.” EX-1002, 5:12-17.

To the extent Tsutsumi does not expressly disclose or render obvious a construct using either technique, the art taught both approaches. EX-1003(Batt), ¶¶173-184. As exemplified in Mattoon, “transfection includes any means for introducing a nucleic acid sequence or expression vector into a host cell, including, but not limited to,...expression of a vector, such as...the use of a vector that integrates into the genum [sic] of the host cell.” EX-1008(Mattoon), 4:41-48; EX-1003(Batt), ¶180. Mattoon further teaches that “the vector may be an autonomously replicating vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal [sic] replication, such as a plasmid.

Alternatively, a vector may be one in which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosomes into which it has been integrated.” EX-1008(Mattoon), 4:60-5:4; EX-1003(Batt), ¶184. As discussed in §§VI.B(2)-(3), a POSITA would be motivated to combine Mattoon with Tsutsumi/Lin-Cereghino and would have a REOS in doing so.

E. Claim 7

(“the exogenous nucleic acid encoding a transcriptional activator comprises a Mxr1 sequence from *Pichia pastoris*”)

As discussed in §VI.B(2), Lin-Cereghino discloses a methylotrophic *P. pastoris* expression system constructed with the sequence that encodes Mxr1p(Mxr1)—namely, the *P. pastoris MXR1* gene. EX-1003(Batt), §IX.H. Lin-Cereghino teaches that Mxr1p has its origins in *P. pastoris*. EX-1006(Lin-Cereghino), 883 (“MXR1 is the *P. pastoris* homologue of...*S. cerevisiae* ADR1....”); EX-1003(Batt), ¶186; *see also* EX-1005(Vogl), 389. Accordingly, a POSITA would understand that Lin-Cereghino’s *MXR1* sequence is derived from *P. pastoris*. EX-1003(Batt), ¶¶185-186; EX-1004(Lin-Cereghino), ¶¶76-85.

F. Claims 8-9

(the transcriptional activator “comprises the sequence shown in SEQ ID NO: 59”/“is encoded by the nucleic acid sequence shown in SEQ ID NO: 60”)

Claims 8-9 are obvious in light of the knowledge of a POSITA, in addition to the teachings of at least Tsutsumi and Lin-Cereghino. EX-1003(Batt), §§IX.I-J. The

sequence shown in SEQ ID NO: 59 is the **known amino acid** sequence of transcriptional activator Mxr1p (protein) found in *P. pastoris*. EX-1002, 77-84; EX-1003(Batt), ¶188; EX-1051(GenBank). Likewise, the sequence shown in SEQ ID NO: 60 is the **known nucleic acid** sequence of the *MXR1* gene in *P. pastoris*. EX-1002, 83-88; EX-1003(Batt), ¶192; EX-1052(GenBank).

As discussed in §VI.B(2), Lin-Cereghino discloses methylotrophic *P. pastoris* expression systems expressing *P. pastoris* Mxr1p (Mxr1). A POSITA could have readily obtained the amino acid sequence of *P. pastoris* Mxr1p as shown in SEQ ID NO: 59 prior to 2015, *e.g.*, as a POSITA would be able to derive this sequence from the known nucleic acid sequence encoding Mxr1 (SEQ ID NO: 60) using routine methods in the art. EX-1003(Batt), ¶188.

Additionally, the SEQ ID NOS: 59 and 60 sequences were available to a POSITA in the National Center for Biotechnology Information's (NCBI) GenBank database (including GenPept), which was publicly accessible and contains “the most up-to-date and comprehensive DNA sequence information” from multiple databases around the world. EX-1053(NCBI); EX-1003(Batt), ¶189.⁶ SEQ ID No: 59 is

⁶ Judicial notice of the sequence entries in GenBank is appropriate, because they are made publicly available and maintained by a government entity, and the

available under Accession Number ABD57365, which first appeared in GenBank in March 2006. EX-1051(GenBank), 1-3; EX-1003(Batt), ¶¶187-191. SEQ ID No: 60 is available under Accession Number DQ395124, which first appeared in GenBank in March 2006. EX-1052, 1-3; EX-1003(Batt), ¶¶192-194. Thus, a POSITA desiring to know the Mxr1 and *MXR1* sequences prior to 2015 would have been motivated to obtain these sequences from GenBank, with a REOS. EX-1003(Batt), ¶¶189-194.

G. Claims 10-11

(the methanol-inducible promoter element is an alcohol oxidase 1 (AOX1) promoter element...a dihydroxyacetone synthase (DHAS) promoter element...or a peroxin 8 (PEX8) promoter element from *Pichia pastoris*)

As described in §VI.B(2), Tsutsumi and Lin-Cereghino both teach the selection and use of a variety of methanol-inducible promoters from *P. pastoris* for heterologous protein production. EX-1024(Tsutsumi), [0040]; EX-1006(Lin-Cereghino), 893-94; EX-1004(Lin-Cereghino), §§VI.A-B; EX-1003(Batt), §§IX.K-

authenticity and accuracy of its entries is not subject to reasonable dispute. *See Pickett v. Sheridan Health Care Ctr.*, 664 F.3d 632, 648 (7th Cir. 2011); *Daniels-Hall v. Nat'l Educ. Ass'n*, 629 F.3d 992, 998-99 (9th Cir. 2010); *New Mexico ex rel. Richardson v. Bureau of Land Mgmt.*, 565 F.3d 683, 703 (10th Cir. 2009).

L; *see also* EX-1005(Vogl), 392-93, Table 1. Specifically, Tsutsumi and Lin-Cereghino disclose that a POSITA would select and use methanol-inducible promoters P_{DAS} , P_{PEX8} , and P_{AOX1} , among others, depending on whether higher or lower levels of heterologous protein production is desired. EX-1024(Tsutsumi), [0040]; EX-1006(Lin-Cereghino), 893-94; EX-1003(Batt), ¶¶195-197; EX-1005(Vogl), 392-93. P_{DAS} and P_{AOX1} , for example, are strong methanol inducible promoters that provide for high levels of recombinant protein expression, whereas P_{PEX8} , provides for lower levels of expression. EX-1003(Batt), ¶¶195-197; EX-1005(Vogl), 392-93.

Moreover, a POSITA would understand that ‘656 patent claim 1 requires two promoters (one for Mxr1 and one for the heterologous polypeptide) but does not require that they be the same promoter. EX-1003(Batt), ¶198. In this context, Tsutsumi discloses using two different promoters to express two different proteins—a polypeptide (e.g., cutinase) under the methanol-inducible promoter P_{DAS} and Mxr1p under the constitutive promoter P_{GAP} . EX-1024(Tsutsumi), [0042]; EX-1003(Batt), ¶199. Thus, based on Tsutsumi’s teachings, a POSITA would also be motivated to use two different promoters in the same *P. pastoris* cells, e.g., a heterologous polypeptide under a methanol-inducible promoter such as P_{DAS} or P_{AOX1} and Mxr1p under a methanol-inducible promoter such as P_{PEX8} , with a REOS

using prior art teachings, routine techniques, and available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶200.

H. Claims 12-13

(“second exogenous nucleic acid encodes at least one heterologous polypeptide involved in biosynthesis of [an iron co-factor / heme]”)

As discussed in §VI.B, Tsutsumi, Lin-Cereghino and Mattoon disclose every limitation of the yeast cell of claim 1. Mattoon further discloses “a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis” of “heme,” which a POSITA would understand is an iron co-factor. EX-1003(Batt), §§IX.M-N, ¶208; EX-1002, 2:15-20 (providing examples of iron co-factors). For example, Mattoon teaches the “simultaneous production of **heme** and heme binding agents (*e.g.*, apoprotein)” in yeast cells.⁷ EX-1008(Mattoon), 2:22-26. Mattoon further discloses “transforming a host cell with a nucleic acid sequence encoding a

⁷ Mattoon teaches coordinating the amount of apoprotein produced by yeast cells with the amount of heme synthesized by the same cells, so that the apoprotein would readily bind heme to form stable heme-containing protein, thereby improving yield and purity. EX-1008(Mattoon), 1:14-2:38. To achieve this, Mattoon teaches yeast strains that co-express (1) a gene encoding recombinant apoprotein and (2) one or more genes encoding heme biosynthetic enzymes. *Id.*

rate-limiting enzyme in a heme biosynthetic pathway,” such as HEM2, an enzyme (*i.e.*, a polypeptide) involved in heme biosynthesis. EX-1008(Mattoon), 2:9-12, 2:27-30. For example, Mattoon discloses “double transformant yeast cells simultaneously harboring the multicopy plasmid pCCT1 and pHEM2,” and “double transformant yeast cultures simultaneously harboring the multicopy plasmid pCCT1 and one of the following multicopy plasmids: pHEM2, pHEM3, pHEM13, and pHEM15.” EX-1008(Mattoon), 7:62-8:2, Figs. 6-7. A POSITA would understand that yeast cells transformed with HEM2, HEM3, HEM13, or HEM15 would comprise “a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis.” EX-1003(Batt), ¶¶209-211. Mattoon further teaches that “HEM2 over-expression...when used in combination with hemoglobin expression plasmids, can be used to increase the production of hemoglobin.” EX-1008 (Mattoon), 7:45-50.

A POSITA would also understand that these polypeptides are involved in the biosynthesis of heme, an iron co-factor. EX-1003(Batt), ¶211; EX-1008(Mattoon), 6:9-12 (“[T]he present invention therefore involves the use of yeast transformed with particular genes encoding enzymes that are involved in the production of heme and porphyrin...”). A POSITA would be motivated to combine Mattoon with Tsutsumi/Lin-Cereghino because “proteins carrying a heme prosthetic group” are “[a]n important class of...heterologous proteins produced in genetically engineered

cells”—for example, for therapeutic purposes. EX-1008(Mattoon), 1:13-16. Mattoon discloses that “[m]any commercial ventures are working to enhance the biosynthesis of the heme protein family...in yeast.” EX-1008(Mattoon), 1:17-30. EX-1003(Batt), ¶212.

As discussed in §VI.B(2)-(3) and above, a POSITA would be motivated to combine Mattoon with Tsutsumi / Lin-Cereghino and would have a REOS in doing so using prior art teachings, routine techniques, and available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶213.

I. Claims 14-15

(“wherein the at least one polypeptide involved in the biosynthesis of heme [is / comprises two or more polypeptides] selected from the group consisting of [HEM1] aminolevulinic acid (ALA) synthase, [HEM2] ALA dehydratase, [HEM3] porphobilinogen deaminase,...[HEM13] coproporphyrinogen (CPG) oxidase,...and [HEM15] ferrochelatase”)

As discussed in §VI.H, Tsutsumi, Lin-Cereghino and Mattoon disclose every limitation of claims 12-13. Mattoon further discloses expression of two or more of the claimed polypeptides involved in heme biosynthesis. EX-1003(Batt), §§IX.O-P. As explained in §VI.H, Mattoon discloses “double transformant yeast cultures simultaneously harboring the multicopy plasmid pCCT1 and one of the following multicopy plasmids: pHEM2, pHEM3, pHEM13, and pHEM15.” EX-1008(Mattoon), 7:62-8:2, Figs. 6-7. Mattoon teaches that heme biosynthesis

enzymes (polypeptides) “includ[e] **ALA synthase** (encoded by *HEM1*), **ALA dehydratase** (encoded by *HEM2*), and **PBG [porphobilinogen] deaminase** (encoded by *HEM3*).” EX-1008(Mattoon), 1:54-58, Fig. 1; EX-1003(Batt), ¶¶215-216.

Moreover, Mattoon expressly discloses transformation of yeast cells with more than one of these plasmids. EX-1008(Mattoon), 7:62-65 (double transformants); EX-1003(Batt), ¶¶217-220. A POSITA would be motivated to do so, as taught in Mattoon, in order to improve the yield of the hemoprotein of interest. EX-1008(Mattoon), 6:45-63 (“By transforming cells with a *HEM2*-containing plasmid, the present inventors are able to ... increase the production of hemoproteins such as hemoglobin, myoglobin, various cytochromes and hemoprotein enzymes such as catalase.”). A POSITA would reasonably expect success doing so in light of Mattoon and knowledge in the art concerning recombinant protein expression. EX-1003(Batt), ¶¶217-218.

As discussed in §VI.B(2)-(3) and §VI.H, a POSITA would be motivated to combine Mattoon with Tsutsumi / Lin-Cereghino and would have a REOS in doing so for all the reasons explained above.

J. Claim 16

(“A method for expressing a heterologous polypeptide in a cell, the method comprising: providing the yeast cell of claim 1; and culturing the cell under conditions for expression of the recombinant nucleic acid molecule, thereby expressing the heterologous polypeptide”)

As discussed in §VI.B, Tsutsumi, Lin-Cereghino and Mattoon disclose every limitation of the yeast cell of claim 1. Tsutsumi further discloses culturing methylotrophic *P. pastoris* yeast cells under conditions for expression of the recombinant nucleic acid molecule to produce the heterologous polypeptide. Specifically, Tsutsumi discloses that the yeast cells of Example 5 were cultured under conditions for expression of *H. insolens* cutinase and Mxr1 of the DNA construct, including small-scale and large-scale culture conditions. EX-1024(Tsutsumi), [0142]-[0143]; EX-1003(Batt), ¶¶221-224.

Under these conditions, Tsutsumi discloses expression of the “test protein” (cutinase)—a heterologous polypeptide—and that cutinase expression was improved when Mxr protein was “over-expressed.” EX-1024(Tsutsumi), [0144]-[0145]; EX-1003(Batt), ¶225. Moreover, Lin-Cereghino also discloses culturing methylotrophic *P. pastoris* yeast cells under conditions for over-expression of Mxr1 under the control of methanol-inducible promoters. EX-1006(Lin-Cereghino), 884 (see “Media, strains, and microbial techniques” and “Induction on carbon sources”),

893 (describing results of over-expression); EX-1004(Lin-Cereghino), ¶¶69-72; EX-1003(Batt), ¶226.

K. Claim 17

(“wherein the heterologous polypeptide is a member of globin family PF00042”)

As discussed in §VI.B(3) and §VI.H, a POSITA would be motivated to combine Mattoon with Tsutsumi/Lin-Cereghino and would have a REOS in doing so for reasons explained above. EX-1003(Batt), §IX.R.

PF00042 is a reference number to the heme-binding family of globin containing proteins. EX-1048; EX-1003(Batt), ¶228. It would be obvious to a POSITA to utilize a nucleic acid encoding a hemoprotein to encode the claimed heterologous polypeptide in light of the teachings of Mattoon. EX-1003(Batt), ¶229; *see also* claims 12-13. Mattoon discloses “recombinant cells” comprising “a gene encoding a heme binding agent” (*e.g.*, apoprotein) “that when expressed, is capable of” binding to heme. EX-1008(Mattoon), 2:17-22, 2:57-63. Mattoon further discloses yeast cells that produce the hemoprotein catalase “as a model for producing other hemoproteins, such as hemoglobin [and] myoglobin.” EX-1008(Mattoon), 7:26-33. A POSITA would understand that hemoproteins such as catalase, hemoglobin, and myoglobin are members of the globin family PF00042. EX-1003(Batt), ¶¶229-230.

L. Claim 21

(“The method of claim 16, wherein the introducing step comprises a technique selected from...electroporation”)

As discussed in §VI.J, claim 16 is obvious over Tsutsumi, Lin-Cereghino and Mattoon. Tsutsumi further discloses an introducing step for integrating recombinant nucleic acid molecules into methylotrophic yeast cells. EX-1003(Batt), §IX.S. For example, Tsutsumi describes the technique used to “transform” or introduce recombinant DNA to *P. pastoris* strains by “**electroporation.**” EX-1024(Tsutsumi), [0115] (describing how the host cell strain was mixed with “linearized plasmid DNA” and transferred to an electroporation cuvette where transformation was performed); EX-1003(Batt), ¶¶231-233.

M. Claims 23-24

(“The method of claim 16, wherein the culturing step comprises culturing the cell in the [presence / absence] of methanol”)

As discussed in §VI.J, claim 16 is obvious over Tsutsumi, Lin-Cereghino and Mattoon. Tsutsumi further discloses cell culturing steps in the absence and presence of methanol. EX-1003(Batt), §§IX.T-U.

For example, Tsutsumi describes “expression in large-scale” culturing processes with or without methanol: “inoculation in shake flasks with...**Buffered Sorbitol Complex media.... After ~24 h of growth, the cultures were induced with 100% methanol...**was carried out every 24 h...for 3 days with induction and

without induction for 2.5 days.” EX-1024(Tsutsumi), [0143]; EX-1003(Batt), ¶¶237-238. A POSITA would understand that Buffered Sorbitol Complex media does not contain methanol. EX-1003(Batt), ¶237.

A POSITA would understand from these disclosures that Tsutsumi describes conventional methods for culturing *P. pastoris* yeast cells with and without methanol where the cells contain methanol-inducible promoters. EX-1003(Batt), ¶239. POSITA would recognize that culturing in methanol could be desirable at certain times during the recombinant protein production process but not at other times. EX-1003(Batt), ¶237; EX-1004(Lin-Cereghino), ¶¶69-72; EX-1005(Vogl), 386 (describing how expression can be “repressed under growth on repressing carbon sources (e.g. glucose)” and “strongly induced on methanol”). Cells can be grown to high densities while repressing recombinant protein expression in glucose/glycerol medium, e.g., in the absence of methanol, to prevent interference by recombinant protein expression and maximize cell growth. EX-1003(Batt), ¶¶237, 240; EX-1004(Lin-Cereghino), ¶¶69-72. A POSITA would then derepress expression by depleting glucose/glycerol and adding methanol, thereby inducing recombinant protein expression driven by methanol-inducible promoters from a large number of cells. EX-1003(Batt), ¶237; EX-1004(Lin-Cereghino), ¶¶69-72; EX-1005(Vogl), 386.

N. Claim 25

(“The method of claim 16, wherein the culturing step comprises culturing the cell in the absence of selection”)

As discussed in §VI.J, claim 16 is obvious over Tsutsumi, Lin-Cereghino and Mattoon. Moreover, Tsutsumi’s large-scale culturing discussed above does not involve any selection. EX-1003(Batt), §IX.V, ¶243. Specifically, Tsutsumi discloses transformants expressing the heterologous polypeptide were initially grown without selection (without the antibiotic). EX-1024(Tsutsumi), [0142] (“[T]he cells were...re-suspended in 1 ml YPDS w/o Zeocin”); EX-1003(Batt), ¶241. Tsutsumi’s large-scale culturing phase also does not involve any selection. EX-1003(Batt), §IX.V. A POSITA would understand from these disclosures that Tsutsumi discloses culturing the cell in the absence of selection. EX-1003(Batt), ¶243.

VII. GROUND 2: TSUTSUMI, LIN-CEREGHINO, MATTOON AND CRANE

A. Overview

Tsutsumi, Lin-Cereghino, Mattoon and Crane render obvious Claims 18-20. Tsutsumi, Lin-Cereghino, Mattoon and Crane are analogous art to the ’656 patent because each discloses genetically modifying *P. pastoris* yeast cells to express recombinant proteins. EX-1003(Batt), ¶243; *see* §IV.A-D. As discussed in Ground

1, claims 1 and 16 are obvious over Tsutsumi, Lin-Cereghino, and, optionally, Mattoon.

Similar to Mattoon, Crane discloses “methods of producing recombinant functional heme-binding proteins with complete heme incorporation” by “co-express[ing] a recombinant heme-binding protein of interest with ferrochelatase,” a polypeptide involved in heme biosynthesis. EX-1010(Crane), Abstract, [0044]. The recombinant heme-binding proteins can be produced in “*Pichia pastoris* expression systems.” *Id.* Crane teaches that it is “desirable to use strong promoters ... to direct high levels” of expression of the recombinant heme-binding protein. *Id.*, [0040]; EX-1003(Batt), ¶243.

B. Claims 18-19

(“The method of claim 16, wherein the heterologous polypeptide is [a plant hemoglobin / leghemoglobin]”)

As discussed in §VI.J, claim 16 is obvious over Tsutsumi, Lin-Cereghino and Mattoon. Selecting a “plant hemoglobin”—and specifically, “leghemoglobin”—as the heterologous polypeptide of claim 16 would have been obvious to a POSITA based on Tsutsumi, Lin-Cereghino and Mattoon alone, or further in view of Crane. EX-1003(Batt), §§X.A-B, ¶244.

Mattoon teaches “a method to produce hemoproteins.” EX-1008(Mattoon), 2:9-10. Mattoon discloses that “[u]sing the present method, desired hemoproteins,

such as hemoglobin, myoglobin . . . can be produced.” EX-1008(Mattoon), 1:39-41, 2:34-37. A POSITA would have understood that Mattoon’s disclosures are not limited to hemoglobin and myoglobin and would apply to other hemoproteins, such as plant hemoglobins, which were known in the art. EX-1003(Batt), ¶¶245-247; EX-1014(Garrocho), 81 & Table 1 (“Hemoglobin sequences identified in land plants”). Recombinant expression of plant hemoglobins (*e.g.*, leghemoglobin) in *P. pastoris* is also expressly disclosed by Crane. EX-1003(Batt), ¶¶248-249; EX-1010(Crane), [0021] (“[H]eme-binding proteins ... encompassed by the methods ... include, without limitation, globins (*e.g.*, ...**leghemoglobin**)”).

A POSITA would have been motivated to select leghemoglobin, a well-known plant hemoglobin with a known sequence, because it has a structure similar to other hemoglobins, red pigmentation, and would be expected to yield a similar desired hemoprotein. EX-1003(Batt), ¶ 250; EX-1044(Nicola), 133 (“Leghaemoglobins are plant haemoproteins which resemble animal myoglobin....”); EX-1045(Hargrove), 1032 (“[T]ertiary folding patterns of ... soybean Lb are very similar to those of mammalian myoglobins, and the α and β subunits of human hemoglobin.”); EX-1014(Garrocho), 79 (Fig. 1); *see also* EX-1002, 6:11-17 (acknowledging that leghemoglobin was “naturally found in root nodules of soybean”).

Leghemoglobin was one of a finite number of hemoglobins (plant or otherwise) to a POSITA to choose from. EX-1003(Batt), ¶250. A POSITA would have been motivated to select leghemoglobin for applications that required plant-based heme-containing proteins, for example, as a colorant or to appeal to individuals who have concerns about or who otherwise abstain from using proteins with an animal provenance. EX-1003(Batt), ¶¶249-250.

A POSITA also would have had REOS in recombinantly expressing plant hemoglobins such as leghemoglobin, using prior art teachings, routine techniques, and available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶¶250-251. Tsutsumi, Lin-Cereghino, Mattoon and Crane all provide interrelated teachings on expressing proteins in *P. pastoris*, and Mattoon and Crane specifically on co-expressing hemoproteins and heme biosynthetic enzymes in *P. pastoris*. EX-1003(Batt), ¶248; *see* §IV.A-D. Indeed, like Mattoon, Crane teaches “methods of producing recombinant functional heme-binding proteins with complete heme incorporation and purified preparations” in “*Pichia pastoris* expression systems,” which are “completely functional” and suitable for “commercial applications.” EX-1010(Crane), Abstract, [0021], [0024], [0044], [0060]-[0062]; EX-1003(Batt), ¶248.

C. Claim 20

(“The method of claim 19, where in the leghemoglobin has a sequence...consisting of SEQ ID NO:4”)

As discussed in §VII.B, claim 19 is obvious over Tsutsumi, Lin-Cereghino, Mattoon, and Crane. For reasons discussed in §VII.A-B, it would also be obvious to use the sequence of SEQ ID NO: 4, which is the *known amino acid* sequence of soybean (*Glycine max*) leghemoglobin. EX-1002, 51-52; EX-1003(Batt), §X.C; EX-1046(GenBank). For example, Crane discloses methylotrophic *P. pastoris* expression systems expressing leghemoglobin.

As explained in §VI.F, a POSITA desiring to recombinantly produce leghemoglobin could have readily obtained the amino acid sequence of SEQ ID No: 4 prior to 2015. EX-1003(Batt), ¶253-254. This sequence is available in the NCBI's GenBank database under Accession Number AAA33980, which first appeared in GenBank in April 1993. EX-1046(GenBank), 1-2; EX-1003(Batt), ¶254. A POSITA would also know how to locate the nucleic acid sequence encoding *G. max* leghemoglobin and would be able to derive the amino acid sequence using routine methods in the art. EX-1003(Batt), ¶254-255; EX-1014(Garrocho), 79, 81.

VIII. GROUND 3: TSUTSUMI, LIN-CEREGHINO, MATTOON AND KRAINER

A. Overview

Tsutsumi, Lin-Cereghino, Mattoon and Krainer render obvious Claim 22. EX-1003(Batt), §XI.A. Tsutsumi, Lin-Cereghino, Mattoon and Krainer are analogous art to the '656 patent because each discloses genetically modifying *P. pastoris* yeast cells to express recombinant proteins. EX-1003(Batt), ¶¶256-257; *see* §§IV.A-B, D-E. As discussed in Ground 1, claims 1 and 16 are obvious over Tsutsumi, Lin-Cereghino, and, optionally, Mattoon. Adding “iron or a pharmaceutically or metabolically acceptable salt thereof” to the culture medium of claim 16 would have been obvious based on Tsutsumi, Lin-Cereghino and Mattoon in further in view of Krainer. EX-1003(Batt), ¶257.

B. Claim 22

(“The method of claim 16, wherein the conditions under which the cell is cultured comprises addition of iron or a pharmaceutically or metabolically acceptable salt thereof”)

Similar to Mattoon, Krainer discloses using methylotrophic *P. pastoris* yeast to co-express (1) the hemoprotein horseradish peroxidase (“HRP”) and (2) one of the eight heme biosynthetic enzymes (*i.e.*, co-expressing a nucleic acid molecule encoding a heme-containing protein with a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis). EX-1031(Krainer), 3-4. Krainer expressly discloses “medium supplementation with **iron**....to effectively saturate

recombinant apo-species of heme proteins.” EX-1031(Krainer), 1-2. Krainer further discloses an iron salt, “FeSO₄...to be [a] useful medium supplement[] to increase the yield of active heme protein.” *Id.*, 6; EX-1003(Batt), ¶¶259-260.

In light of Krainer’s teachings, a POSITA would have been motivated to supplement the culture medium of *P. pastoris* cells expressing a heme-containing protein (*e.g.*, as taught in Mattoon) with iron. EX-1003(Batt), ¶¶261-262. Indeed, like Mattoon, Krainer teaches “optimizing heme availability...[to] boost the amount of recombinant active heme protein...in the yeast *P. pastoris*” and evaluates both biosynthetic “pathway engineering” and “medium supplementation.” EX-1031(Krainer), 1-2; EX-1003(Batt), ¶¶260-262. Krainer discloses that “medium supplementation” with FeSO₄ proved to be an efficient strategy to increase the yield of active enzyme and further Krainer’s goal of producing proteins saturated with heme. EX-1031(Krainer), 4 (“[A]ddition of...FeSO₄ boosted the amount of active HRP up to 7-fold”). A POSITA would thus be motivated to add FeSO₄ to the culture medium of Tsutsumi/Lin-Cereghino/Mattoon because Krainer teaches that it is a “strategy to effectively saturate recombinant...heme proteins” and “useful medium supplements to increase the yield of active heme protein.” EX-1031(Krainer), 2, 6; EX-1003(Batt), ¶¶259-261; EX-1004(Lin-Cereghino), ¶72.

Moreover, a POSITA would have had a REOS in doing so in light of the teachings of Krainer, routine techniques, and available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶262.

IX. GROUND 4: MATTOON AND VOGL

A. Overview

Mattoon and Vogl render claims 26 and 28 obvious. EX-1003(Batt), §§XII.A-C. Mattoon and Vogl are analogous art to the '656 patent because each discloses genetically modifying *P. pastoris* yeast cells to express recombinant proteins. EX-1003(Batt), ¶263. Mattoon discloses all limitations of claim 26 except “a Mxr1 transcriptional activator sequence from *P. pastoris*.” Vogl discloses commonly used constitutive and methanol-inducible promoters and transcriptional activators from *P. pastoris* and Mxr1p, a key transcriptional activator that regulates methanol-inducible promoters. As discussed below, a POSITA would have been motivated to combine these references with a REOS.

B. Claim 26

1. 26[pre]

(“A methylotrophic *Pichia* yeast cell comprising”)

Mattoon and Vogl both disclose “[a] methylotrophic *Pichia* yeast cell.” EX-1003(Batt), ¶265. Mattoon expressly teaches that “[p]referred host cells” are “yeast of the genus *Pichia*, and *even more preferably, Pichia pastoris*.” EX-

1008(Mattoon), 4:32-37, 9:20-10:4. Vogl discloses that “methylotrophic yeast *Pichia pastoris* is a widely used host for heterologous protein production” because it has “favorable properties such as growth to high cell density and high capacities for protein.” EX-1005(Vogl), 385; EX-1003(Batt), ¶265.

2. 26[a]

(“a recombinant nucleic acid molecule, wherein the recombinant nucleic acid molecule comprises a nucleic acid encoding a Mxr1 transcriptional activator sequence from *P. pastoris*”)

Vogl teaches “a Mxr1 transcriptional activator sequence from *P. pastoris*” and the advantages of utilizing Mxr1-driven expression of gene sequences of interest in a recombinant nucleic acid molecule. EX-1003(Batt), ¶266. The nucleic acid sequence encoding Mxr1 was available to a POSITA as explained above. *See, e.g.*, §VI.F.

Vogl teaches that Mxr1p is “a key regulator of methanol inducible genes,” and at the time of Vogl’s publication was the only well-characterized transcriptional activator of methanol-inducible promoters from *P. pastoris*. EX-1005(Vogl), 390-91, 400. Vogl explains that “*MXR1* was shown to be crucial for the activation of numerous genes involved in MUT [methanol utilization pathway].” EX-1005(Vogl), 390. In fact, Vogl discloses that deletion of the *MXR1* gene causes “highly reduced

protein” expression from genes controlled by methanol-inducible promoters. EX-1005(Vogl), 390 (citing Ex-1006(Lin-Cereghino)); EX-1003(Batt), ¶267.

Vogl also describes studies such as Takagi in which Mxr1p was expressed under the strong constitutive promoter P_{GAP} , resulting in production of a recombinant protein under the control of P_{AOX1} that was 7.6-fold higher than that produced by yeast cells without the P_{GAP} -*MXR1* construct.⁸ EX-1005(Vogl), 391; EX-1003(Batt), ¶268; EX-1004(Lin-Cereghino), ¶¶47-48, 51. In view of Vogl’s teachings, a POSITA would have been motivated to incorporate a nucleic acid encoding Mxr1 into the construct to regulate P_{AOX1} -controlled expression of heterologous proteins. EX-1003(Batt), ¶268, 270-273; EX-1004(Lin-Cereghino), ¶76. Indeed, as discussed above, Vogl provides a comprehensive review of prior studies using promoters and transcription factors from *P. pastoris* to regulate recombinant protein production. EX-1005(Vogl), 385-86. A POSITA also would have had a REOS in following the

⁸ While Takagi used glucose (a repressing carbon source) growth medium in its experiments, a POSITA would have understood that glucose does not completely shut off P_{AOX1} -driven transcription. EX-1003(Batt), ¶268; EX-1036(Bawa), 1 (describing “leaky expression” from P_{AOX1} in both glucose- and glycerol-grown cells); EX-1006(Lin-Cereghino), 893 (“Mxr1p was *mostly* cytoplasmic in glucose-grown cells....”).

techniques disclosed in Vogl and the prior studies it cites to construct similar *P. pastoris* expression systems. EX-1003(Batt), ¶269; EX-1004(Lin-Cereghino), ¶¶78-79. As explained in the '656 patent, incorporating the claimed nucleic acid molecule into *P. pastoris* yeast cells only requires routine techniques, known methods, and available materials for genetic engineering and recombinant protein production. See §V.A; EX-1003(Batt), ¶269.

3. 26[b]

(“a nucleic acid encoding a member of globin family PF00042”)

As discussed in §VI.K, PF00042 is a reference number to the heme-binding family of globin containing proteins. EX-1048; EX-1003(Batt), ¶274. Mattoon discloses yeast cells that produce the hemoprotein catalase “as a model for producing other hemoproteins, such as **hemoglobin** [and] **myoglobin**.” EX-1008(Mattoon), 7:26-33. A POSITA would understand that such hemoproteins are members of the globin family PF00042. EX-1003(Batt), ¶¶274-276.

As discussed in §VI.B, Mattoon also teaches that more than one nucleic acid “can be present on one expression vector.” EX-1008(Mattoon), 5:34-46. A POSITA would thus understand that the nucleic acids of limitations 26[a] and 26[b] can be present on one recombinant nucleic acid molecule. EX-1003(Batt), ¶277.

4. 26[c]

(“a nucleic acid encoding at least one polypeptide involved in heme biosynthesis”)

As discussed in §VI.H, Mattoon discloses “a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis” and advantages of co-expressing such polypeptide with a globin family member. EX-1003(Batt), ¶¶278-279. For example, Mattoon teaches “transforming a host cell with a nucleic acid sequence encoding **a rate-limiting enzyme in a heme biosynthetic pathway,**” such as HEM1, HEM2, and HEM3—enzymes (*i.e.*, a polypeptide) involved in heme biosynthesis. EX-1008(Mattoon), 2:9-12, 2:27-30. Mattoon teaches that “HEM2 over-expression...when used in combination with hemoglobin expression plasmids, can be used to increase the production of hemoglobin.” EX-1008 (Mattoon), 7:45-50; EX-1003(Batt), ¶¶278-279.

Moreover, as discussed in §VI.B(1) and §IX.B, Mattoon teaches that more than one nucleic acid “can be present on one expression vector,” and a POSITA would understand that the nucleic acids of limitations 26[a], 26[b] and 26[c] can be present on one recombinant nucleic acid molecule. EX-1003(Batt), ¶280.

5. 26[d]

(“each nucleic acid is operably linked to a methanol-inducible promoter element,...compris[ing] at least one sequence to which a Mxr1 transcriptional activator binds”)

Mattoon discloses “each nucleic acid is operably linked to a methanol-inducible promoter” with at least one sequence to which Mxr1 binds. EX-1003(Batt), ¶281. As discussed in §IV.B and §VI.B, Mattoon teaches vectors “controllable by inducible or constitutive promoters” that are “operatively linked” to the nucleic acid encoding the proteins-of-interest. EX-1003(Batt), ¶281; EX-1008(Mattoon), 2:41-44, 5:51-59, 6:9-63.

Vogl would have motivated a POSITA to select methanol-inducible promoters that comprise at least one sequence to which a Mxr1 transcriptional activator binds—*e.g.*, P_{AOX1}, P_{DAS} or P_{PEX8} (*see* §VI.B)—to control the expression of nucleic acids encoding a member of the globin protein family, a polypeptide involved in heme biosynthesis, and Mxr1. EX-1003(Batt), ¶282; EX-1005(Vogl), 385-87, 391; EX-1008(Mattoon), 2:41-44, 5:51-59, 6:9-63. Vogl teaches that Mxr1p plays an important role in the expression of recombinant proteins as Mxr1p is a “positive regulator of methanol inducible genes” in methylotrophic *P. pastoris*. EX-1005(Vogl), 387, 390. Vogl also teaches that Mxr1p is a DNA-binding protein that binds to specific sites of methanol-inducible promoters such as P_{AOX1}, P_{DAS} or P_{PEX8} which activates downstream protein expression. EX-1005(Vogl), 393 (citing

EX-1019(Kranthi)); EX-1006(Lin-Cereghino), 893. Vogl further teaches that high levels of Mxr1p expression results in higher expression of a protein-of-interest controlled by a promoter activated by Mxr1p. EX-1005(Vogl), 391 (discussing Takagi). Vogl also teaches advantages of methanol-inducibility—*e.g.*, “tight regulation” of gene expression, and “controllable promoters” which “are a crucial tool for efficient heterologous protein production.” EX-1005(Vogl), 386; EX-1003(Batt), ¶¶282-283.

Based on Vogl’s teachings, a POSITA would have been motivated to incorporate methanol-inducible promoters (*e.g.*, P_{AOX1}, P_{DAS} or P_{PEX8}) in Mattoon’s *P. pastoris* expression system to produce recombinant heme-containing proteins. EX-1003(Batt), ¶284; EX-1004(Lin-Cereghino), ¶¶63-67. For the same reasons discussed in §VI.B, a POSITA would have had a REOS in operably linking each nucleic acid to a methanol-inducible promoter using routine techniques and available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶284; EX-1004(Lin-Cereghino), ¶¶63-67.

C. Claim 28

(“The yeast cell of claim 26, where in the methanol-inducible promoter element is an alcohol oxidase 1 (AOX1) promoter element from *P. pastoris*”)

As discussed in VI.B(1), Vogl discloses constructing a *P. pastoris* expression system with P_{AOX1}, and a POSITA would understand that it would be feasible to

drive expression of the claimed Mxr1, globin, and heme biosynthesis polypeptide nucleic acid sequences using P_{AOX1}. EX-1003(Batt), ¶¶285-290; EX-1004(Lin-Cereghino), ¶¶63-67. For example, Vogl teaches that because the methanol-inducible promoter “P_{AOX1} is a remarkably strong promoter and at the same time directly controllable by simply changing the inexpensive carbon source, it is the *most commonly used promoter* for heterologous protein expression in *P. pastoris*.” EX-1005(Vogl), 386. EX-1003(Batt), ¶288.

X. GROUND 5: MATTOON, VOGL AND CRANE

A. Overview

Mattoon and Vogl in further view of Crane render claim 27 obvious. Mattoon, Vogl and Crane are analogous art to the '656 patent because each discloses genetically modifying *P. pastoris* yeast cells to express recombinant proteins. EX-1003(Batt), ¶¶291; *see* §IX.B. As discussed in Ground 4, Mattoon discloses all limitations of claim 26 except for “a Mxr1 transcriptional activator sequence from *P. pastoris*” (which is taught by Vogl). As discussed in §VII.B, Crane discloses “methods of producing recombinant functional heme-binding proteins,” such as leghemoglobin. EX-1010(Crane), Abstract, [0044].

B. Claim 27

(“The yeast cell of claim 26, wherein the member of the globin family is leghemoglobin”)

Crane discloses constructing a *P. pastoris* expression system with genes encoding Leghemoglobin. EX-1010(Crane), Abstract, [0009], [0044]; *see also id.*, [0021] (“[H]eme-binding proteins ... encompassed by the methods ... include, without limitation, globins (e.g., ...*leghemoglobin*)”); EX-1003(Batt), ¶¶292-294. For all the reasons discussed in §VII.B-C, a POSITA would understand selecting “leghemoglobin” as the member of the globin family of claim 26 would have been obvious to a POSITA based on Mattoon and Vogl alone, or further in view of Crane. EX-1003(Batt), ¶¶292-294.

XI. NO OBJECTIVE EVIDENCE OF NONOBVIOUSNESS

Petitioner is unaware of any objective indicia of nonobviousness for the ’656 patent. EX-1003(Batt), ¶295; EX-1004(Lin-Cereghino), ¶86. Should Patent Owner come forward with any objective indicia evidence, Petitioner reserves the right to respond and rebut such evidence.

XII. INSTITUTING *INTER PARTES* REVIEW WOULD BE EQUITABLE

A. No Discretionary Denial Under 35 U.S.C. § 325(d)

None of the grounds in this Petition was substantively considered during prosecution. The present grounds are based on combinations involving Tsutsumi, Vogl, and Mattoon, which were not cited in any IDS or otherwise presented during

prosecution of the '656 patent. Crane and Krainer were cited in IDS but not relied upon or discussed substantively in any office action, and while the secondary reference Lin-Cereghino was cited by the Examiner it was only as part of a different combination of references. The specific combinations here were not relied upon or discussed substantively in any office action for this patent. *See PEAG LLC v. Varta Microbattery GmbH*, IPR2020-01212, Paper 8 at 11-12 (Jan. 6, 2021) (finding the “specific combinations” of references in the Petition not the same or substantially the same as the art previously presented to the Office, where one reference in each combination was cited in an IDS); *FireKing Security Prods., LLC v. Ellenby Techs., Inc.*, IPR2021-00531, Paper 15 at 10-14 (Aug. 23, 2021) (same); *see also Thorne Research, Inc. v. Trs. of Dartmouth College*, IPR2021-00268, Paper 21 at 9 (June 10, 2021).

As discussed in §V.B, during prosecution of the '656 patent, the Examiner rejected the claims as obvious over combinations involving Zhou, Varadan, and Lin-Cereghino. EX-1040, 1260-74. In response, the Applicant did not substantively address any of the cited references, but rather amended the claims and invoked the 35 U.S.C. §102(b)(2)(c) prior-art exception for Varadan, and the Examiner allowed the claims thereafter. EX-1040, 1427-34, 1445-61, 1479-91. Thus, the Examiner did not rely on Crane or Krainer, which were only cited in IDS, and did not

substantively consider Lin-Cereghino as part of the combinations of references presented herein.

Neither the Applicant nor the Examiner substantively discussed any of Tsutsumi, Vogl or Mattoon during prosecution, much less the specific combinations raised herein. There is no substantial overlap between the Examiner's rejections and the Petitioner's arguments. Discretionary denial is not warranted.

B. No Discretionary Denial Under 35 U.S.C. § 314(a)

The '656 patent has not been challenged by any IPR before this Petition. The district court litigation is still in an early stage. *See Impossible Foods Inc. v. Motif FoodWorks, Inc.*, No. 22-cv-00311 (D. Del.).

XIII. GROUNDS FOR STANDING UNDER 37 C.F.R. § 42.104

Petitioner certifies that the '656 patent is available for *Inter Partes* Review and that Petitioner is not barred or estopped from requesting this proceeding.

XIV. CONCLUSION

Petitioner has demonstrated a reasonable likelihood that at least one claim of the '656 patent would have been obvious in view of the prior art and should be cancelled. Accordingly, Petitioner respectfully requests institution.

XV. MANDATORY NOTICES

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

Motif FoodWorks, Inc.

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

Impossible Foods Inc. v. Motif FoodWorks, Inc., No. 22-cv-00311 (D. Del.).

Motif FoodWorks, Inc. v. Impossible Foods Inc., IPR2023-00322 (concerning U.S. Patent No. 10,273,492).

C. Lead and Back-Up Counsel Under 37 C.F.R. § 42.8(b)(3) and Service Information Under 37 C.F.R. § 42.8(b)(3)-(4)

Petitioner provides the following counsel and service information. Pursuant to 37 C.F.R. § 42.10(b), a Power of Attorney accompanies this Petition.

LEAD COUNSEL	BACK-UP COUNSEL
Joseph M. Paunovich (Reg. No. 59,044) qeimpossibleipr@quinnemanuel.com <u>Postal and Hand Delivery Address:</u> QUINN EMANUEL URQUHART & SULLIVAN, LLP 865 S. Figueroa St., 10th Floor Los Angeles, California 90017 Tel: (213) 443-3000 Fax: (213) 443-3100	James Glass (Reg. No. 46,729) Sandra Haberny (Reg. No. 64,150) Jihong Lou (Reg. No. 64,074) qeimpossibleipr@quinnemanuel.com Sarah Cork (<i>pro hac vice</i> to be requested upon grant authorization) Mikayla Wasiri (<i>pro hac vice</i> to be requested upon grant authorization) qeimpossibleipr@quinnemanuel.com <u>Postal and Hand Delivery Address:</u> QUINN EMANUEL URQUHART & SULLIVAN, LLP 865 S. Figueroa St., 10th Floor Los Angeles, California 90017 Tel: (213) 443-3000 Fax: (213) 443-3100

D. Service Information (37 C.F.R. § 42.8(b)(3)-(4))

Please send all correspondence to the lead and backup counsel at the addresses shown above. Petitioner consents to service by e-mail at the addresses of lead and back-up counsel shown above.

E. Payment of Fees Under 37 C.F.R. § 42.103

The undersigned authorizes the Patent Office to charge Deposit Account No. No. 50-5708 for the fee set in 37 C.F.R. § 42.15(a) for this Petition and further authorizes payment for any additional fees to be charged to this Deposit Account.

DATED: January 27, 2023

Respectfully submitted,

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CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. § 42.24(a) and (d), the undersigned hereby certify that the **PETITION FOR *INTER PARTES* REVIEW** complies with the type-volume limitation of 37 C.F.R. § 42.24(a)(i) and (b)(i) permitting a petition of up to 14,000 words because, exclusive of the exempted portions, it contains 13,809 words as counted by the word processing program used to prepare the paper.

Date: January 27, 2023

Respectfully submitted,

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CERTIFICATE OF SERVICE

In accordance with 37 C.F.R. §§ 42.6(e) and 42.105, the undersigned certifies that the foregoing **PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 10,689,656** and supporting materials will be served on Patent Owner by USPS Overnight Express Mail at the following address of record for the subject patent:

Dr. M. Angela Parsons
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P.O. Box 1022
Minneapolis, MN 55440-1022
Attorney Docket No. 38767-0019002

Courtesy copies of the same documents will be served on Patent Owner by USPS Overnight Express Mail at the following address of record for litigation counsel:

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Date: January 27, 2023 By: /Joseph M. Paunovich/
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