

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. IPR2023-00322

U.S. Patent No. 10,273,492

“Expression Constructs and Methods of
Genetically Engineering Methylophilic Yeast”

**PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 10,273,492
UNDER 35 U.S.C. §§ 311-319 AND 37 C.F.R. §§ 42.100 *et seq.***

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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”)
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EX-1016	Hartner et al., <i>Regulation of methanol utilisation pathway genes in yeasts</i> , MICROBIAL CELL FACTORIES, 5, 39 (2006) (“Hartner”)
EX-1017	Hartner et al., <i>Promoter library designed for fine-tuned gene expression in Pichia pastoris</i> , NUCLEIC ACID RESEARCH, 36(12), e76 (2008) (“Hartner_2008”)
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LIST OF CHALLENGED CLAIMS

Claim 1	
[1pre]	A methylotrophic <i>Pichia</i> yeast cell comprising:
[1a]	a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from <i>P. pastoris</i> and a Mxr1 transcriptional activator sequence from <i>P. pastoris</i> ; and
[1b]	a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis operably linked to a promoter element from <i>P. pastoris</i> and a Mxr1 transcriptional activator sequence from <i>P. pastoris</i> .
Claim 2	
The yeast cell of claim 1, wherein the heme-containing protein is Leghemoglobin (LegH).	
Claim 3	
The yeast cell of claim 2, wherein the LegH has the sequence shown in SEQ ID NO:6.	
Claim 4	
The yeast cell of claim 1, wherein the at least one polypeptide involved in heme biosynthesis is selected from the group consisting of ALA synthase, ALA dehydratase, porphobilinogen [sic] deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, PPG oxidase, and/or ferrochelatase.	
Claim 5	
The yeast cell of claim 1, wherein the promoter element from <i>P. pastoris</i> is a constitutive promoter element from <i>P. pastoris</i> or a methanol-inducible promoter element from <i>P. pastoris</i> .	
Claim 6	
The yeast cell of claim 5, wherein the constitutive promoter element from <i>P. pastoris</i> is a promoter element from a transcriptional elongation factor EF-1 (TEF1) gene.	
Claim 7	
The yeast cell of claim 5, wherein the methanol-inducible promoter element from <i>P. pastoris</i> is an alcohol oxidase I (AOX1) promoter element.	
Claim 8	
The yeast cell of claim 1, wherein the promoter element from <i>P. pastoris</i> comprises a constitutive promoter element from <i>P. pastoris</i> and a methanol-inducible promoter element from <i>P. pastoris</i> .	

Claim 9
The yeast cell of claim 8, wherein the constitutive promoter element from <i>P. pastoris</i> is a promoter element from a transcriptional elongation factor EF-1 (TEF1) gene.
Claim 10
The yeast cell of claim 8, wherein the methanol-inducible promoter element from <i>P. pastoris</i> is an alcohol oxidase I (AOX1) promoter element.
Claim 11
The yeast cell of claim 1, wherein the nucleic acid molecule encoding the heme-containing protein is present in the yeast cell in at least two copies.
Claim 12
The yeast cell of claim 1, wherein the nucleic acid molecule encoding the heme-containing protein operably linked to the promoter element from <i>P. pastoris</i> and the Mxr1 transcriptional activator sequence from <i>P. pastoris</i> further comprises a termination sequence.
Claim 13
The yeast cell of claim 1, wherein the nucleic acid encoding the at least one polypeptide involved in the heme biosynthesis operably linked to the promoter element from <i>P. pastoris</i> and the Mxr1 transcriptional activator sequence from <i>P. pastoris</i> further comprises a termination sequence.
Claim 14
The yeast cell of claim 1, further comprising a nucleic acid molecule encoding a Mxr1 transcriptional activator operably linked to a promoter element from <i>P. pastoris</i> and a Mxr1 transcriptional activator sequence from <i>P. pastoris</i> .

I. INTRODUCTION

Motif FoodWorks, Inc. (“Petitioner”) petitions for *inter partes* review (“IPR”) of U.S. Patent No. 10,273,492 (“’492 patent,” EX-1001). 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 ’492 patent.

II. STATEMENT OF RELIEF REQUESTED

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

Petitioner requests IPR and cancellation of claims 1-14 (“Challenged Claims”) of the ’492 patent.

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

Ground	Claims	Basis
1A	1, 4-11	§103, Mattoon and Vogl
1B	2-3, 12-13	§103, Mattoon, Vogl, and Crane
1C	14	§103, Mattoon, Vogl, and Lin-Cereghino
2A	1-13	§103, Crane and Vogl
2B	14	§103, Crane, Vogl, and Lin-Cereghino

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), ¶¶55-57. 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), ¶56; EX-1004(Lin-Cereghino), ¶¶53-54, 58.

Proteins are encoded by genetic sequence information, i.e., a “gene” sequence. EX-1003(Batt), ¶¶55-56; 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), ¶¶55, 62. For example, the gene coding for leghemoglobin is transcribed and translated to produce the protein leghemoglobin. EX-1003(Batt), ¶¶55, 58-59.

rDNA expression results in recombinant proteins, also known as “heterologous” proteins. EX-1003(Batt), ¶¶56-57; 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), ¶¶56-57; 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), ¶¶58-62, 68-77; EX-1028(Stryjewska), 1076-79. For example, a gene encoding a protein-of-interest can be obtained and inserted (“cloned”) into a DNA expression vector. EX-1003(Batt), ¶¶60-61; 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), ¶¶60-61; 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), ¶¶60-62; EX-1004(Lin-Cereghino), ¶55; EX-1028(Stryjewska), 1077-79; EX-1015(Lin-Cereghino_2000), 50. To select for cells into which the rDNA is successfully introduced, vectors include “selection markers,” which are typically genes that confer resistance to antibiotics. EX-1003(Batt), ¶¶63; 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 a medium containing the antibiotic. EX-1003(Batt), ¶63; 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), ¶¶63; EX-1028(Stryjewska), 1078-79.

B. *P. pastoris* Gene Expression Systems

POSITAs have used many different microbes to express rDNA. EX-1003(Batt), ¶¶64-66; EX-1022(Zhang), 145-46. Among eukaryotic expression systems, POSITAs have commonly used yeasts such as *P. pastoris* for rDNA expression. EX-1022(Zhang), 145-46; EX-1003(Batt), ¶¶65-67; 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), ¶¶65-67; 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), ¶¶65-67; 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), ¶¶65-67; 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), ¶¶65-67; 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.

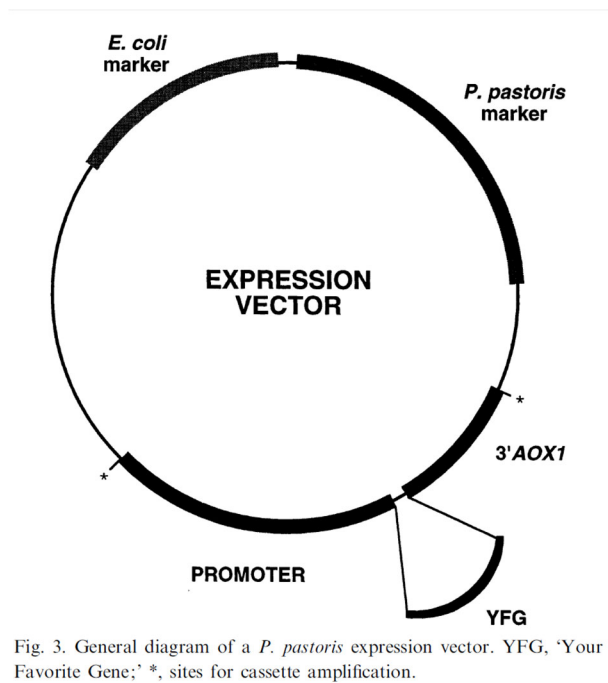
Transcription factors can activate or repress gene transcription. EX-1042(Alberts), 310-11; EX-1005(Vogl), 386; EX-1004(Lin-Cereghino), ¶¶67-69. They do so by binding the promoter in ways that either allow transcription to occur (i.e., they are “transcriptional activators”), or cause it not to occur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

In *P. pastoris*, native promoters can be constitutive or inducible. EX-1003(Batt), ¶70; 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 transcription factors that maintain a constant level of transcription under different conditions. EX-1003(Batt), ¶70; 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), ¶70; 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), ¶70; 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 included constitutive promoters P_{GAP} and P_{TEF1}, and methanol-inducible 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), ¶¶70-75; 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*:



EX-1015(Lin-Cereghino_2000), 47-48; EX-1004(Lin-Cereghino), ¶53; EX-1003(Batt), ¶76. 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), ¶¶76-77; 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), ¶¶78-82; 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 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), ¶¶70-73, 80; 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), ¶¶78, 80-81; EX-1004(Lin-Cereghino), ¶69. When glucose or 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, 7; EX-1003(Batt), ¶¶78-81; 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), ¶79.

Methanol-inducible promoters can promote high levels of recombinant protein expression. EX-1005(Vogl), 386-87; EX-1015(Lin-Cereghino_2000), 48; EX-1003(Batt), ¶¶78-81; 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), ¶¶79-80; 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), ¶¶79-80; EX-1021(Sreekrishna), 6-8; EX-1022(Zhang), 148.

To take advantage of the strength and inducible nature of the P_{AOX1} promoter, 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), ¶¶80-81; EX-1005(Vogl), 386-87; EX-1015(Lin-Cereghino_2000), 51-52; EX-1029(Ahmad), 5302. For example, to allow *P. pastoris* cells to grow to high numbers and densities, POSITAs “repress[]” methanol-inducible promoters in a medium that lacks methanol but contains glucose or glycerol. EX-1003(Batt), ¶¶78-80; 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), ¶¶79-82; 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 controlled by P_{AOX1} and to control the timing and yield of recombinant protein expression. EX-1003(Batt), ¶¶78-82; 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), ¶¶80-81.

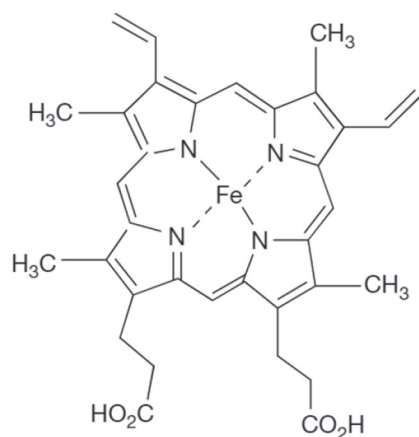
E. Mxr1 Regulation of Methanol-Inducible Genes

Before 2015, it was well-known that methanol-inducible promoters such as P_{AOX1}, 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-1003(Batt), ¶¶83-84. Natively, Mxr1p 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), ¶84.

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), ¶¶83-84; EX-1004(Lin-Cereghino), ¶¶43-45, 76-78.

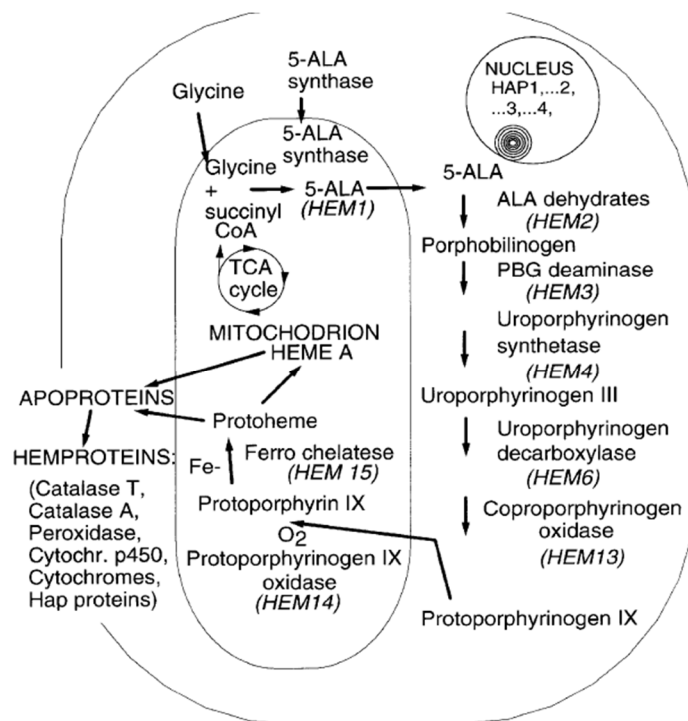
F. Expression of Heme-Containing Proteins

By 2015, POSITAs had routinely applied microbial-based rDNA technology to produce proteins known as heme-containing proteins (“hemoproteins”) in microbes, including yeasts. EX-1003(Batt), ¶¶85-86; 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), ¶¶85-86; 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), ¶86; 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), ¶89.

Typically, cells make heme through multi-step biosynthesis, involving enzymes that catalyze steps of the biosynthesis. EX-1003(Batt), ¶¶87-89; 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), ¶¶87.

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, Lin-Cereghino, and Vogl is prior art under AIA 35 U.S.C. §102(a)(1) based on their dates of public accessibility.

A. Mattoon

U.S. Patent No. 5,824,511 (“Mattoon”) issued October 20, 1998. Mattoon teaches “[a] method for increasing the production” of hemoproteins. EX-1008(Mattoon), Abstract; EX-1003(Batt), ¶90. 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; EX-1003(Batt), ¶90.

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; EX-1003(Batt), ¶91. 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.” EX-1008(Mattoon), 2:9-30. 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 required to form desired hemoproteins, thereby ending up with purer materials.” EX-1008(Mattoon), 6:3-8, 6:27-38; EX-1003(Batt), ¶91.

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), ¶92. 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), ¶92. 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), ¶92.

B. 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 ***ferrochelata****se protein*," a heme biosynthetic enzyme commonly known as HEM15. EX-1010(Crane), Abstract, [0009], [0056]; EX-1003(Batt), ¶¶100-102.

Crane teaches that heme-containing proteins, which Crane calls heme-binding proteins, include "hemoglobin, myoglobin, ... [and] ***leg****hemoglobin*." 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].

C. 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), ¶¶103-104; 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), ¶105. 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 and produced functional Mxr1p at “relatively high levels.” EX-1006(Lin-Cereghino), 893; EX-1004(Lin-Cereghino), ¶77; EX-1003(Batt), ¶105. Lin-Cereghino also reported that the 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.¹ 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.D), 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*

¹ 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).

expressing Mxr1p under P_{GAP} and a cutinase gene under P_{DAS} variants); EX-1004(Lin-Cereghino), ¶¶40-41.

D. 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 production.” EX-1005(Vogl), 386; EX-1003(Batt), ¶¶93-94. This includes reviewing “the most commonly used” promoter P_{AOX1} and its regulation. EX-1005(Vogl), 386-92; EX-1003(Batt), ¶94.

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), ¶95. 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), ¶¶96-97. 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; EX-1003(Batt), 96. 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 (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), ¶¶98-99; 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.”² 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 on Mxr1p. EX-1005(Vogl), 394 (citing EX-1024(Tsutsumi)); EX-1003(Batt), ¶99. 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],

² Takagi also teaches that P_{GAP} “can be replaced with another promoter,” such as P_{TEF1} or P_{PGK1} . EX-1007(Takagi), 6:18-26, 18:43-45.

[0040], [0041], [0145]. 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.*³

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

Vogl and Lin-Cereghino are printed publication prior art. As set forth in EX-1057(Hall-Ellis), ¶¶48-64, 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 May 2013 and February 2006, respectively. Indeed, these references were published by highly reputable scientific journals and leading publishers in the field of recombinant protein production, and

³ 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].

were available to POSITAs exercising reasonable diligence before 2015. EX-1003(Batt), ¶¶93, 103.

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. EX-1005(Vogl) and EX-1006(Lin-Cereghino) are authentic copies that would have been available to a POSITA as of the dates cited above, respectively. EX-1057(Hall-Ellis), ¶¶48-64.

V. OVERVIEW OF THE '492 PATENT

A. Claims and Specification

The '492 patent has fourteen claims. EX-1001, 77:49-79:17. The '492 patent is directed to “methods and materials for genetically engineering methylotrophic yeast.” EX-1001, 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-1001, 1:37-42. 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-1001, 1:42-46,

3:50-57. Similarly, it states that “[e]xpression of Mxr1 results in a significant increase in the amount of protein produced.” EX-1001, 1:46-48.

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-1001, 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-1001, 4:44-47. The ’492 patent states 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-1001, 4:47-51.

The patent’s examples describe *P. pastoris* strains that expressed the genes of Mxr1p, soybean leghemoglobin, and heme biosynthetic enzymes under the control of P_{AOX1}. EX-1001, 12:15-28:19, 38:46-40:8. 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-1001, 26:42-44. The specification also describes *P. pastoris* strains that used both inducible and constitutive promoters—placing the genes of Mxr1p, leghemoglobin, and heme biosynthetic enzymes under inducible promoter P_{AOX1} (as in the above examples), and additional copies of the

leghemoglobin gene under constitutive promoter P_{TEF1} , P_{GAP} , or P_{GCW14} . EX-1001, 28:21-67; 40:10-25.

The specification acknowledges that many elements and techniques were known by POSITAs prior to 2015. For example, the '492 patent acknowledges that (1) transcriptional activators including Mxr1p, (2) methanol-inducible promoters including P_{AOX1} , and (3) constitutive promoters including P_{TEF1} , were all “known in the art.” EX-1001, 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-1001, 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-1001, 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-1001, 7:11-18, 7:59-60, 10:10-24.

B. Prosecution History

During prosecution of the '492 patent, the Examiner did not make any prior art-based rejection. EX-1039. Rather, the Examiner rejected claims 1, 4-7, and 10-13 under §112 for failing the written description requirement and for indefiniteness,

objected to claims 2-3 and 8-9 as depending from a rejected base claim, and rejected claims 1-14 for non-statutory double patenting over co-pending Application No. 15/678,891 (“’891 Application”). EX-1039, 936-44, 968-73. Following claim amendments and a terminal disclaimer, the Examiner allowed the claims. EX-1039, 958-64, 990-91, 997-1001, 1005-09.

C. Priority Date

The ’492 patent claims priority to eight provisional applications, the earliest of which was filed on May 11, 2015. *See* EX-1001, Cover Page. Even assuming, for purposes of this IPR, the ’492 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* §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 ’492 patent is directed to the field of recombinant protein production. A person of ordinary skill in the art (“POSITA”) would have at least a Bachelor of Science in biochemistry, chemistry, biological sciences, or an equivalent area of study. EX-1003(Batt), ¶35; EX-1004(Lin-Cereghino), ¶29. 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. GROUNDS 1A-1C: MATTOON, VOGL, CRANE, LIN-CEREGHINO

A. Overview

Mattoon and Vogl render obvious Claims 1 and 4-11 (Ground 1A). Mattoon, Vogl, and Crane render obvious Claims 2-3 and 12-13 (Ground 1B). Mattoon, Vogl, and Lin-Cereghino render obvious claim 14 (Ground 1C).

Mattoon, Vogl, Crane, and Lin-Cereghino are each analogous art to the '492 patent because each discloses genetically modifying *P. pastoris* yeast cells to express recombinant proteins. EX-1003(Batt), ¶¶108-120. Mattoon discloses all limitations of claim 1 except “a Mxr1 transcriptional activator sequence from *P. pastoris*” (which is taught by Vogl). Vogl discloses commonly used constitutive and methanol-inducible promoters and transcriptional activators from *P. pastoris* such as P_{AOX1} and Mxr1p, a key transcriptional activator that regulates methanol-inducible promoters. Lin-Cereghino discloses placing Mxr1 under the control of promoters to express high levels of Mxr1p, and incorporating multiple copies of the *MXR1* gene in the yeast genome. As discussed below, a POSITA would have been motivated to combine these references with a reasonable expectation of success (“REOS”).

B. Ground 1A: Mattoon and Vogl

1. Claim 1

(a) 1[pre]

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

Mattoon discloses “[a] methylotrophic *Pichia* yeast cell.” EX-1003(Batt), ¶¶121-122. Mattoon teaches that *P. pastoris* yeast cells are the most preferred host cells for expressing hemoproteins. EX-1008(Mattoon), 4:32-37, 9:20-10:4.

(b) 1[a]

(“a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from *P. pastoris* and a Mxr1 transcriptional activator sequence from *P. pastoris*”)

Mattoon discloses “a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from *P. pastoris*.” EX-1003(Batt), ¶123. Mattoon is directed to “[a] method for increasing the production of desired hemoproteins.” EX-1008(Mattoon), Abstract. Mattoon teaches “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), 2:18-22, 2:60-63. Mattoon describes 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. Mattoon discloses “transforming yeast cells with CTT1 [the gene encoding catalase T, a cytoplasmic hemoprotein] to produce catalase T.”

EX-1008(Mattoon), 7:37-41, 7:56-8:10, Figs. 6-7. A POSITA would understand that yeast cells transformed with *CTTI* would comprise “a nucleic acid molecule encoding a heme-containing protein.” EX-1003(Batt), ¶124.

Mattoon explains that “*preferred* yeast strains include *Pichia pastoris*” due to its “exceptionally high yields.” EX-1008(Mattoon), 9:20-10:4; *see also id.*, 4:32-37. It was also well-understood in the art that *P. pastoris* was “the most commonly used yeast species” and “a highly successful system” for recombinant protein production. EX-1018(De_Schutter), 561; EX-1015(Lin-Cereghino_2000), 45; EX-1005(Vogl), 385-86; EX-1003(Batt), ¶126.; EX-1004(Lin-Cereghino), ¶¶50, 63. Thus, a POSITA would have had numerous reasons to use *P. pastoris* as host cells for producing recombinant hemoproteins including but not limited to catalase.

Mattoon discloses that vectors used to transform *P. pastoris* yeast cells are “controllable by inducible or constitutive *promoters*” that are “*operatively linked*” to the nucleic acid sequences encoding proteins-of-interest. EX-1008(Mattoon), 2:41-44, 5:4-10, 5:46-59, 6:9-63. And, as discussed above, Mattoon teaches that “[p]referred host cells” are “yeast of the genus *Pichia*, and *even more preferably, Pichia pastoris.*” EX-1008(Mattoon), 4:29-37. Thus, a POSITA would understand that Mattoon discloses “a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from *P. pastoris.*” EX-1003(Batt), ¶126-127.

The only element of limitation 1[a] not expressly disclosed by Mattoon is “a Mxr1 transcriptional activator sequence from *P. pastoris*.” EX-1003(Batt), ¶128. Vogl discloses this limitation. EX-1003(Batt), ¶¶128-132; EX-1005(Vogl), 390-91. And, a POSITA would have been motivated to combine Mattoon with Vogl to engineer *P. pastoris* with “a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from *P. pastoris* and a Mxr1 transcriptional activator sequence from *P. pastoris*.” EX-1003(Batt), ¶128.

A POSITA would have naturally looked to Vogl when engineering *P. pastoris* to express a hemoprotein. EX-1003(Batt), ¶¶128-134. Vogl discloses that “methylophilic 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. Vogl, reviews commonly used *P. pastoris* constitutive and methanol inducible promoters, regulation of inducible promoters by known transcriptional activator Mxr1p, and “their application to improve heterologous protein production,” which is consistent with Mattoon’s teaching of using inducible or constitutive promoters. EX-1005(Vogl), 385-92; EX-1008(Mattoon), 2:41-44, 5:4-10, 5:46-59, 6:9-63; EX-1003(Batt), ¶¶131-133.

For example, Mattoon teaches that vectors used to transform host cells are “operably linked” and “*controllable by inducible or constitutive promoters*.” EX-

1008(Mattoon), 2:41-49, 4:65-5:10, 5:46-64, 6:9-63, Fig. 4. Mattoon further teaches that “[i]t is *preferred to have promoters*, whether inducible or constitutive,” to control expression of genes encoding *both* hemoprotein *and* heme biosynthetic enzymes, so as to regulate and adjust the amounts of heme-binding apoprotein and heme produced by yeast cells. EX-1008(Mattoon), 8:27-32.

Moreover, Vogl describes over twenty well-known promoters that are used in *P. pastoris* to regulate exogenous protein expression. EX-1005(Vogl), 385-401, Table 1. For each promoter, Vogl discloses its category (inducible or constitutive) and strength (*e.g.*, strong or weak). EX-1005(Vogl), 393. A POSITA would have readily combined Mattoon with Vogl to select suitable promoters to control the expression of the nucleic acid encoding hemoprotein in *P. pastoris* and would have had a REOS in doing so using Vogl’s teachings, routine techniques, and available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶¶133-139.

For example, Vogl teaches that strong constitutive promoters such as P_{TEF1} and P_{GAP}, or strong methanol-inducible promoters such as P_{AOX1} and P_{DAS}, have been used in *P. pastoris* expression systems to provide high levels of protein expression. EX-1005(Vogl), 392-95. Vogl explains that P_{GAP} and P_{AOX1} “provide similar expression levels under the respective optimal growth conditions.” EX-1005(Vogl), 392. Vogl additionally teaches that “[o]ne of the most favorable properties of *P. pastoris*” are its “methanol inducible promoters.” EX-1005(Vogl), 386. Vogl

explains that because “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. Thus, a POSITA would have been motivated to select promoters such as P_{AOX1} and P_{GAP}, among other promoters, to control the expression of the nucleic acid encoding hemoprotein in *P. pastoris*. EX-1003(Batt), ¶¶135-138; EX-1004(Lin-Cereghino), ¶¶77-79. A POSITA would have had a REOS in doing so because both Mattoon and Vogl provide numerous examples of using promoters such as P_{AOX1} and P_{GAP} to regulate the expression of recombinant proteins. EX-1003(Batt), ¶139; EX-1008(Mattoon), 5:60-62 (explaining that “high yield promoters” “can also be used”).

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)).

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.⁴ EX-1005(Vogl), 391; EX-1003(Batt), 133; EX-1004(Lin-Cereghino), ¶¶47-48, 51. Accordingly, a POSITA would have been motivated to incorporate Mxr1p in Mattoon's nucleic acid construct to express a heme-containing protein in *P. pastoris*, for example, by expressing Mxr1p under P_{GAP} , to regulate P_{AOX1} -controlled expression of hemoprotein, and would have had a REOS in doing so using Vogl's teachings, routine techniques, and readily available materials that PO admits were well-known. See §V.A; EX-1003(Batt), ¶¶137-139.

In particular, a POSITA would have been motivated to (1) select a promoter from *P. pastoris* such as P_{AOX1} to control the expression of a heme-containing

⁴ While Takagi used glucose-based (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), ¶73; EX-1036(Bawa), 1, 3 (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.").

protein, thus providing “*a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from P. pastoris*,” and (2) include in the same nucleic acid molecule “*a Mxr1 transcriptional activator sequence from P. pastoris*” that is expressed under the control of a promoter, such as P_{GAP}, where the expressed Mxr1p further regulates a methanol-inducible promoter such as P_{AOX1} in *P. pastoris*, including any methanol-inducible promoter that drives the expression of heme-containing protein. EX-1003(Batt), ¶¶137-139; EX-1004(Lin-Cereghino), ¶¶76-79; EX-1005(Vogl), 391; EX-1008(Mattoon), 5:43-46 (explaining 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.”).

A POSITA would have had a REOS in incorporating such “a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from *P. pastoris* and a Mxr1 transcriptional activator sequence from *P. pastoris*” into a methylotrophic *Pichia* yeast cell. 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), 385-92. A POSITA would have had a REOS in following the well-known techniques disclosed in these prior studies to construct similar *P. pastoris* expression systems. EX-1003(Batt), ¶139; EX-1004(Lin-Cereghino), ¶¶76-86. Moreover, as explained in

the '492 patent, incorporating the claimed nucleic acid molecule into *P. pastoris* yeast cells only requires routine techniques, known methods, and readily available materials for genetic engineering and recombinant protein production. See §V.A; EX-1003(Batt), ¶139.

(c) 1[b]

(“a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis operably linked to a promoter element from *P. pastoris* and a Mxr1 transcriptional activator sequence from *P. pastoris*.”)

Mattoon discloses “a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis operably linked to a promoter element from *P. pastoris*.” EX-1003(Batt), ¶140. For example, Mattoon teaches “simultaneous production of heme and heme binding agents (*e.g.*, apoprotein)” in *P. pastoris* yeast cells.⁵ EX-1008(Mattoon), 2:22-26.

⁵ Mattoon teaches coordinating the amount of hemoprotein produced by *P. pastoris* yeast cells with the amount of heme synthesized by the same cells, so that the heme-binding apoprotein would readily bind heme to form stable heme-containing protein, thereby improving the yield and purity of the hemoprotein. EX-1008(Mattoon), 1:13-2:40. To achieve this, Mattoon teaches yeast strains that co-express (1) a gene

Mattoon further discloses “transforming a host cell with a nucleic acid sequence encoding a rate-limiting enzyme in a heme biosynthetic pathway,” such as HEM2, an enzyme (*i.e.*, a polypeptide) involved in heme biosynthesis. EX-1008(Mattoon), 2:9-30. 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), 7:62-65, Figs. 6-7. A POSITA would understand that the 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), ¶¶141-144.

As discussed in §VI.B.1(b), Mattoon also teaches vectors “controllable by inducible or constitutive promoters” from *P. pastoris* that are “operatively linked” to the nucleic acid encoding proteins-of-interest. EX-1003(Batt), ¶145; EX-1008(Mattoon), 2:41-44, 5:51-59, 6:9-63; EX-1008(Mattoon), 4:29-40. Thus, Mattoon discloses the “operably linked to a promoter element from *P. pastoris*” element of 1[b]. EX-1003(Batt), ¶145.

encoding recombinant hemoprotein and (2) one or more genes encoding heme biosynthetic enzymes. *Id.*

Moreover, for the same reasons discussed in §VI.B.1(b), a POSITA would have been motivated to and naturally looked to Vogl to incorporate an Mxr1 sequence in the nucleic acid construct to regulate P_{AOX1}-controlled expression of heme biosynthetic enzymes and would have had a REOS in doing so. EX-1003(Batt), ¶¶146-147; EX-1004(Lin-Cereghino), ¶76; EX-1005(Vogl), 385-87, 391; EX-1008(Mattoon), 2:41-44, 5:51-59, 6:9-63. A POSITA would understand such a *P. pastoris* construct would comprise “***a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis operably linked to a promoter element from P. pastoris,***” and (2) in the same rDNA construct “***a Mxr1 transcriptional activator sequence from P. pastoris.***” EX-1003(Batt), ¶¶146-148; EX-1005(Vogl), 391; EX-1008(Mattoon), 5:34-46 (explaining 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.”).

For the same reasons discussed in §VI.B.1(b), a POSITA would have had a REOS in incorporating such a construct into a methylotrophic *Pichia* yeast cell using routine techniques and readily available materials that PO admits were well-known. See §V.A; EX-1003(Batt), ¶¶148-150; EX-1004(Lin-Cereghino), ¶¶82-86.

2. Claim 4

(“polypeptide involved in heme biosynthesis is selected from the group consisting of...ALA dehydratase...”)

As discussed in §VI.B.1, Mattoon and Vogl disclose every limitation of claim

1. Figure 1 of Mattoon expressly discloses all eight heme biosynthetic enzymes recited by claim 4 and, as discussed in §VI.B.1(c), teaches yeast cells transformed with HEM2, HEM3, HEM13, and/or HEM15 for co-expression of at least one polypeptide involved in heme biosynthesis. EX-1008(Mattoon), Fig. 1. Mattoon discloses examples in which “the at least one polypeptide involved in heme biosynthesis” is HEM2, ALA dehydratase. EX-1008(Mattoon), 7:63-65; EX-1003(Batt), ¶¶151-152.

3. Claim 5

(“promoter element from *P. pastoris* is a constitutive...or a methanol-inducible promoter”)

As discussed in §VI.B.1, Mattoon teaches vectors “controllable by inducible or constitutive promoters” from *P. pastoris* that are “operatively linked” to the nucleic acids encoding proteins-of-interest. EX-1003(Batt), ¶¶153-157; EX-1008(Mattoon), 2:41-44, 4:29-40, 5:51-59, 6:9-63. For this reason, Mattoon expressly discloses the limitations of this claim.

To the extent specific promoters are required, as discussed in §VI.B.1(b), a POSITA would have been motivated to combine Mattoon with Vogl. Vogl discloses

commonly used methanol-inducible and constitutive promoters from *P. pastoris* for heterologous protein expression. EX-1005(Vogl), 385-401, Table 1. For example, Vogl teaches that “[o]ne of the most favorable properties of *P. pastoris* and the other methylotrophic yeasts are the strong methanol inducible promoters present in these organisms,” such as P_{AOX1}, P_{DAS} and P_{PEX8}. EX-1005(Vogl), 386, 393. Vogl also explains that because “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. Thus, a POSITA would have been motivated to select a methanol-inducible promoter from *P. pastoris* such as P_{AOX1}, among others, to control the expression of hemoprotein and one or more heme biosynthetic enzymes in *P. pastoris*. EX-1003(Batt), ¶¶155-157; EX-1004(Lin-Cereghino), ¶¶63-67.

Vogl also teaches that the promoter P_{GAP} is “strong and ***constitutive***, reaching similar expression levels as P_{AOX1},” and that “P_{GAP} has emerged as the most commonly used alternative to P_{AOX1}.” EX-1005(Vogl), 392. Vogl further teaches that “[e]xpression from P_{GAP} has been studied with respect to optimal vector design, the regulatory pattern and [high cell density] bioreactor cultivations,” which would motivate a POSITA to use the constitutive promoter P_{GAP}, among others, as recited in this claim. EX-1003(Batt), ¶¶155-157.

As explained in §VI.B.1, a POSITA would have had a REOS in following Vogl's teaching to use promoters such as P_{AOX1} or P_{GAP} , among others, to practice the claim using routine techniques and readily available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶157.

4. Claim 6

(claim 5's "constitutive promoter element from *P. pastoris* is a promoter element from a transcriptional elongation factor EF-1 (TEF1) gene")

As explained in §VI.B.1, a POSITA would have been motivated to combine Mattoon with Vogl, which teaches that strong constitutive promoters have been used in *P. pastoris* to provide high levels of protein expression. EX-1005(Vogl), 392-93; EX-1008(Mattoon), 5:60-62 (explaining that the use of "high yield promoters" "can also be used"). Vogl explains that "[f]or nontoxic proteins, which do not hamper cell growth, a strong constitutive expression system can help to minimize cultivation efforts and lead to a higher space/time yield." EX-1005(Vogl), 392. Thus, a POSITA would have been motivated to select a strong constitutive promoter to control the expression of hemoprotein and/or heme biosynthesis enzymes. EX-1003(Batt), ¶¶158-160.

Vogl teaches that P_{TEF1} is a strong constitutive promoter from *P. pastoris* that is "similar to P_{GAP} ," another well-characterized constitutive promoter. EX-1005(Vogl), 393. Vogl describes P_{TEF1} as "a crucial component of the eukaryotic

translation machinery,” and teaches that under certain conditions, “the activity levels of P_{TEF1} driven expression exceeded P_{GAP} almost twofold.” EX-1005(Vogl), 395. Thus, a POSITA would have been motivated to select P_{TEF1} to control the expression of hemoprotein and one or more heme biosynthesis enzymes. EX-1003(Batt), ¶¶158-160.

As explained in §VI.B.1, a POSITA would have had a REOS in following Vogl’s teaching to use P_{TEF1} to practice the claim using routine techniques and readily available materials that PO admits were well-known. See §V.A; EX-1003(Batt), ¶¶159-160.

5. Claim 7

(claim 5’s “methanol-inducible promoter element from *P. pastoris*” is an alcohol oxidase I (AOX1) promoter element)

As explained in §VI.B.1, a POSITA would have been motivated to combine Mattoon with Vogl, which teaches that methanol inducible “ P_{AOX1} is [] remarkably strong” and “*the most commonly used promoter* for heterologous protein expression in *P. pastoris*.” EX-1005(Vogl), 386. Thus, a POSITA would have been motivated to select P_{AOX1} to control the expression of hemoprotein and one or more heme biosynthetic enzymes in *P. pastoris*. EX-1003(Batt), ¶¶161-167; EX-1004(Lin-Cereghino), ¶¶63-67, 76-79.

As explained in §VI.B.1, a POSITA would have had a REOS in following Vogl's teaching to use P_{AOX1} to practice the claim using routine techniques and readily available materials that PO admits were well-known. See §V.A; EX-1003(Batt), ¶168.

6. Claim 8

(“promoter element from *P. pastoris* comprises a constitutive promoter...and a methanol-inducible promoter”)

As discussed in §VI.B.1, Mattoon teaches vectors that are “controllable by inducible or constitutive promoters” from *P. pastoris* that are “operatively linked” to the nucleic acid encoding the proteins-of-interest. EX-1003(Batt), ¶¶169-170; EX-1008(Mattoon), 2:41-44, 4:29-40, 5:51-59, 6:9-63. Here, claim 1 recites two promoters, one controlling the expression of a heme-containing protein, and the other controlling the expression of at least one polypeptide involved in heme biosynthesis. EX-1001, 77:49-57. A POSITA would understand that the two promoters can be different, for example, one being methanol-inducible and the other being constitutive. EX-1003(Batt), ¶171. A POSITA would have been motivated to select suitable constitutive and methanol-inducible promoters for the expression of different proteins with a REOS by applying Mattoon's teachings, routine techniques, and readily available materials that PO admits were well-known. See §V.A; EX-1003(Batt), ¶¶171-172.

To the extent specific promoters are required, as discussed in §VI.B.1, a POSITA would have been motivated to combine Mattoon with Vogl, which discloses well-known constitutive and methanol-inducible promoters from *P. pastoris* to control recombinant protein expression in *P. pastoris*. EX-1005(Vogl), 386, 392-393; EX-1003(Batt), ¶172. For example, Vogl teaches that “P_{AOX1} is a remarkably strong” and “*most commonly used promoter* for heterologous protein expression in *P. pastoris*.” EX-1005(Vogl), 386; EX-1003(Batt), ¶173; EX-1004(Lin-Cereghino), ¶50. Vogl also teaches that “[f]or nontoxic proteins, which do not hamper cell growth, a strong constitutive expression system can help to *minimize cultivation efforts and lead to a higher space/time yield*.” EX-1005(Vogl), 392; EX-1003(Batt), ¶174. Vogl provides examples of strong constitutive promoters such as P_{GAP} and P_{TEF1}. EX-1005(Vogl), 392-93.

Moreover, as discussed in Vogl, Takagi discloses using two different promoters to express two different proteins—phytase under the methanol-inducible promoter P_{AOX1} and Mxr1p under the constitutive promoter P_{GAP}. EX-1005(Vogl), 391; EX-1003(Batt), ¶175. Thus, for the same reasons discussed in §VI.B.1, a POSITA would have been motivated to combine Mattoon’s and Vogl’s teachings to use both types of promoters in the same *P. pastoris* cells with a REOS using prior art teachings, routine techniques, and readily available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶176.

7. Claim 9

(claim 8's "constitutive promoter element from *P. pastoris* is a promoter element from a transcriptional elongation factor EF-1 (TEF1) gene")

For the same reasons discussed above for claims 6 and 8, a POSITA would have been motivated to combine Mattoon with Vogl and select P_{TEF1} as one of the promoters recited by claim 1 with a REOS. EX-1003(Batt), ¶177.

8. Claim 10

(claim 8's "methanol-inducible promoter element from *P. pastoris* is an alcohol oxidase I (AOX1) promoter element")

For the same reasons discussed above for claims 7 and 8, a POSITA would have been motivated to combine Mattoon with Vogl and select P_{AOX1} as one of the promoters recited by claim 1 with a REOS. EX-1003(Batt), ¶178.

9. Claim 11

("nucleic acid molecule encoding the heme-containing protein is present in the yeast cell in at least two copies")

Mattoon expressly discloses "double transformant yeast cells simultaneously harboring the multicopy plasmids pCTT1" encoding catalase T, a cytoplasmic hemoprotein. EX-1008(Mattoon), 7:62-8:2, Figs. 6-7. A POSITA would understand that such double transformant yeast cells comprise multiple copies of the nucleic acid molecule encoding the heme-containing protein of claim 1. EX-1003(Batt), ¶¶179-180.

C. Ground 1B: Mattoon and Vogl further in view of Crane

1. Claim 2

(“heme-containing protein” is “Leghemoglobin (LegH)”)

Selecting “Leghemoglobin (LegH)” as the heme-containing protein of claim 1 would have been obvious to a POSITA based on Mattoon and Vogl alone, or further in view of Crane. EX-1003(Batt), ¶181.

Mattoon teaches “a method to produce hemoproteins.” EX-1008(Mattoon), 2:9-10. As discussed in §VI.B.1, a POSITA would have been motivated to combine Mattoon with Vogl to add the “Mxr1” element with a REOS to practice all elements of claim 1. Although Mattoon does not expressly disclose leghemoglobin, Mattoon discloses that “[u]sing the present method, desired hemoproteins, *such as* hemoglobin, myoglobin . . . can be produced.” EX-1008(Mattoon), 1:39-41, 2:34-40. A POSITA would have understood that Mattoon’s disclosures are not limited to hemoglobin and myoglobin, but may apply to other hemoproteins, such as leghemoglobin. Indeed, a POSITA would have been motivated to select leghemoglobin, a well-known plant hemoglobin with a known sequence, because it is a member of the hemoprotein family and would be expected to yield a similar desired hemoprotein. EX-1003(Batt), ¶¶181-187; EX-1004(Lin-Cereghino), ¶¶83-85; 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), 78-79, Fig. 1; *see also* EX-1001, 6:12-16 (acknowledging that soybean leghemoglobin was known and “naturally found in root modules of soybean”). A POSITA further would have been motivated to select leghemoglobin as the heme-containing protein of choice based on the finite number of hemoglobins to choose from. EX-1003(Batt), ¶¶181, 185. Additionally, a POSITA would have been motivated to select leghemoglobin for applications that required plant-based heme-containing proteins, for example, to add to plant-based products or to appeal to individuals who have concerns about or who otherwise abstain from using proteins with an animal provenance. EX-1003(Batt), ¶¶185-187. A POSITA also would have had a REOS in recombinantly expressing leghemoglobin, using prior art teachings, routine techniques, and readily available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶188.

The recombinant expression of leghemoglobin in *P. pastoris* as recited in claim 2 is also expressly disclosed by Crane. EX-1003(Batt), ¶184. Crane teaches that the “heme-binding proteins ... encompassed by [its] methods ... include, without limitation, globins (e.g.,...**leghemoglobin**).” Ex-1010(Crane), [0021]. A POSITA would have been motivated to combine Mattoon and Vogl with Crane’s leghemoglobin and would have had a REOS. EX-1003(Batt), ¶¶185-188. Mattoon,

Vogl 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), ¶¶181-184; *see* §IV.A-B. 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]; *see also* §IV.B. For the same reasons explained above for Mattoon, a POSITA would be motivated to select leghemoglobin for use in the Mattoon/Vogl system because leghemoglobin is a member of the hemoprotein family, it is one of a finite number of hemoglobins, and it is desirable for plant-based applications. A POSITA also would have a REOS in expressing leghemoglobin in the Mattoon/Vogl expression system, using prior art teachings, routine techniques, and readily available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶¶188.

2. Claim 3

(“LegH” has a sequence as shown in “SEQ ID NO:6”)

The sequence shown in SEQ ID NO:6 of the '492 patent is the *known amino-acid* sequence of soybean (*Glycine max*) leghemoglobin. EX-1001, cols. 49-51; EX-1003(Batt), ¶¶189-191; EX-1046(GenBank). As discussed in §VI.C,1, Crane

discloses methylotrophic *P. pastoris* expression systems expressing leghemoglobin. A POSITA could have readily obtained the amino-acid sequence of *P. pastoris* leghemoglobin as shown in SEQ ID NO: 6 prior to 2015.

The sequence shown in SEQ ID NO:6 is available in the National Center for Biotechnology Information's (NCBI) GenBank database. The sequence shown in SEQ ID NO:6 is available under GenBank Accession Number AAA33980, which first appeared in the database in April 1993. EX-1046(GenBank), 1-2; EX-1003(Batt), ¶190. The GenBank database is publicly available and contains "the most up-to-date and comprehensive DNA sequence information" from multiple databases around the world. EX-1053(NCBI); EX-1003(Batt), ¶190. Thus, a POSITA desiring to know the sequence for soybean leghemoglobin prior to 2015 would have naturally turned to GenBank and found these sequences. EX-1003(Batt), ¶190.⁶

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 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).

3. Claim 12 and 13

(“nucleic acid molecule encoding [the heme-containing protein / the at least one polypeptide involved in heme biosynthesis] operably linked to the promoter element from *P. pastoris* and the Mxr1 transcriptional activator sequence from *P. pastoris* further comprises a termination sequence”)

Termination sequences were routinely used in *P. pastoris* expression vectors to terminate rDNA transcription at the 3'-end of the DNA sequence encoding a protein-of-interest. EX-1003(Batt), ¶¶192-193, 195-196; EX-1015(Lin-Cereghino_2000), 48 (cited by EX-1005(Vogl)). A POSITA would understand that the *P. pastoris* expression systems disclosed by Mattoon and Vogl would include termination sequences to ensure that all mRNAs will have a uniform 3'-end (last nucleotide in the mRNA), which promotes polyadenylation, stability, translation, and ultimately production of the recombinant protein. EX-1003(Batt), ¶¶193, 196; e.g., EX-1006(Lin-Cereghino), 886 (discussing “termination sequence”) (cited by EX-1005(Vogl)).

Moreover, Crane expressly discloses an expression system “wherein the one or more expression constructs further comprise ... a nucleic acid encoding a **termination sequence.**” EX-1010(Crane), Claim 22; EX-1003(Batt), ¶¶193, 196. As explained in §VI.C.1, a POSITA would have been motivated to combine Mattoon, Vogl, and Crane, and would have readily incorporated Crane’s teachings on termination sequences—an element routinely used in expression systems—in the

Mattoon/Vogl system, and would have had REOS in doing so, using prior art teachings, routine techniques, and readily available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶¶193, 196.

D. Ground 1C: Mattoon and Vogl further in view of Lin-Cereghino

1. Claim 14

(“further comprising a nucleic acid molecule encoding a Mxr1 transcriptional activator operably linked to a promoter element from *P. pastoris* and a Mxr1 transcriptional activator sequence from *P. pastoris*”)

As discussed in §VI.B.1, Mattoon discloses all limitations of claim 1 except “a Mxr1 transcriptional activator sequence from *P. pastoris*,” which is taught by Vogl, and Mattoon and Vogl together render claim 1 obvious. Also as discussed in §VI.B.1, Vogl describes studies such as Takagi in which Mxr1p expression was regulated by a strong promoter (P_{GAP}) resulting in expression of a recombinant protein under the control of P_{AOX1} that was 7.6-fold higher than that produced by *P. pastoris* strains without the P_{GAP} -*MXR1* construct. EX-1005(Vogl), 391; EX-1004(Lin-Cereghino), ¶¶47-48, 51.

A POSITA would have understood from Vogl (and its discussion of Takagi) that expressing *MXR1* gene under a strong promoter (*e.g.*, P_{GAP}) increased the amount of Mxr1p in yeast cells, which in turn increased the P_{AOX1} -driven recombinant protein expression that is regulated by Mxr1p. EX-1003(Batt), ¶¶198-199. In view of this, a POSITA would have been motivated to increase the

expression of *MXR1* gene by placing it under the control of a promoter, which would increase expression of a protein (*e.g.*, a hemoprotein or a heme biosynthetic enzyme) under the control of a methanol-inducible promoter (*e.g.*, P_{AOX1}) that is regulated by Mxr1p in *P. pastoris*. EX-1003(Batt), ¶199.

To increase Mxr1p expression, a POSITA would have naturally looked to Lin-Cereghino (cited and discussed by Vogl). EX-1003(Batt), ¶¶200-201; EX-1005(Vogl), 389-90. Lin-Cereghino discloses placing the *MXR1* gene under the control of a *P. pastoris* promoter to increase expression of Mxr1p. EX-1006(Lin-Cereghino), 885, 893-94 (“[W]e expressed *MXR1* under the control of the promoter from the *P. pastoris* *PEX8* gene (32). *P_{PEX8}* is methanol regulated...”); EX-1003(Batt), ¶201. For example, Lin-Cereghino discloses that the *P. pastoris* strain with Mxr1p under regulation of the methanol inducible promoter P_{PEX8} (pGC217)⁷ grew on methanol and produced “relatively high levels of” Mxr1p. EX-1006(Lin-Cereghino), 893.

Therefore, a POSITA would have been motivated to design rDNA constructs that not only co-express genes encoding hemoprotein and one or more heme

⁷ 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.

biosynthetic enzymes (as set forth in claim 1) under methanol-inducible promoters such as P_{AOX1} , but also to increase Mxr1p expression, for example, by expressing Mxr1p under P_{GAP} in glucose-grown cells, or under P_{PEX8} in methanol-grown cells, or by increasing the copy number of *MXR1* gene, as taught by Vogl and Lin-Cereghino. EX-1003(Batt), ¶¶201-203; EX-1004(Lin-Cereghino), ¶¶77-79. A POSITA would have had a REOS in view of the higher levels of protein expression reported by Vogl and Lin-Cereghino, and the known methods and routine techniques used by these references to construct such expression systems. EX-1003(Batt), ¶¶203; EX-1004(Lin-Cereghino), ¶¶77-79.

Moreover, in view of the well-known strategy to control protein expression in *P. pastoris* with carbon sources, a POSITA looking to implement a methanol-inducible P_{AOX1} -*MXR1* construct, among other inducible constructs, in *P. pastoris* (including strains with multiple copies of P_{AOX1} -*MXR1*) would first grow the cells to high densities while repressing recombinant protein expression in a medium that contained glucose or glycerol. EX-1003(Batt), ¶203; EX-1004(Lin-Cereghino), ¶¶69-72. Once the cells have grown to the desired density, a POSITA would derepress expression by depleting the glucose or glycerol and adding methanol. EX-1003(Batt), ¶203; EX-1004(Lin-Cereghino), ¶¶69-72. This would in turn induce recombinant protein expression driven by methanol-inducible promoters (e.g., P_{AOX1}) from a large number of cells. EX-1003(Batt), ¶203; EX-1004(Lin-

Cereghino), ¶¶70-71. A POSITA would understand that growing the cells to a high density and then inducing recombinant gene expression with the addition of methanol would result in high levels of protein expression. EX-1003(Batt), ¶203; EX-1004(Lin-Cereghino), ¶¶70-71.

Vogl provides further support for a POSITA. Vogl explains that expressing the *MXR1* gene under the control of a strong promoter results in high levels of protein expression. EX-1005(Vogl), 391 (citing EX-1007(Takagi)). In particular, Vogl teaches that high levels of protein expression can be achieved by co-expressing a protein-of-interest under the control of P_{AOX1} and *Mxr1p* under the control of P_{GAP} , a strong constitutive promoter in a glucose-based medium. EX-1003(Batt), ¶200, 202; EX-1005(Vogl), 391. Vogl teaches that expression of the protein-of-interest under P_{AOX1} was higher than the strain that expressed the protein under P_{AOX1} without co-expressing *MXR1* under P_{GAP} . EX-1005(Vogl), 391; EX-1007(Takagi), 18:29-40; EX-1003(Batt), ¶¶200, 202. Based on these teachings, a POSITA would understand that an expression system constructed with *Mxr1p* expression controlled by strong promoters such as P_{GAP} or P_{AOX1} is desirable and reasonably expected to succeed. EX-1004(Lin-Cereghino), ¶¶76-79.

Therefore, a POSITA would have been motivated to incorporate into the *P. pastoris* cells of claim 1 “a nucleic acid molecule encoding a *Mxr1* transcriptional activator operably linked to a promoter element from *P. pastoris* [*e.g.*, P_{AOX1} , P_{PEX8} ,

or P_{GAP}] and a Mxr1 transcriptional activator sequence from *P. pastoris*,” and would have had REOS. EX-1003(Batt), ¶203.

VII. Ground 2A-2B: Crane, Vogl, Lin-Cereghino

A. Overview

Crane and Vogl render obvious claims 1-13 (Ground 2A). Crane, Vogl, and Lin-Cereghino render obvious claim 14 (Ground 2B).

Crane, Vogl, and Lin-Cereghino are each analogous art to the '492 patent because each discloses genetically modifying *P. pastoris* yeast cells to express recombinant proteins. EX-1003(Batt), ¶¶204-208. Crane expressly discloses every limitation of claim 1 except “a Mxr1 transcriptional activator sequence from *P. pastoris*” (which is taught by Vogl). As explained in Ground 1, Vogl discloses commonly used promoters and transcription factors from *P. pastoris*, including Mxr1. And Lin-Cereghino discloses placing Mxr1 under the control of promoters to express high levels of Mxr1p, and incorporating multiple copies of the *MXR1* gene in the yeast genome. As discussed below, a POSITA would have been motivated to combine these references with a REOS.

B. Ground 2A: Crane and Vogl

1. Claim 1

(a) 1[pre]

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

Crane discloses “[a] methylotrophic *Pichia* yeast cell.” EX-1003(Batt), ¶¶209-211. Crane teaches that “[y]east strains and yeast-derived vectors are used commonly for the expression of heterologous proteins.” EX-1010(Crane), [0043]-[0044]; EX-1003(Batt), ¶210. More specifically, Crane discloses that “*Pichia pastoris* expression systems[] may be used to co-express a recombinant heme-binding protein of interest with ferrochelatase,” in accordance with the methods it discloses. EX-1010(Crane), [0009], [0043]-[0044]; EX-1003(Batt), ¶210.

(b) 1[a]

(“a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from *P. pastoris* and a Mxr1 transcriptional activator sequence from *P. pastoris*”)

Crane discloses “a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from *P. pastoris*.” EX-1003(Batt), ¶212. Crane discloses “methods of producing recombinant functional heme-binding proteins with complete heme incorporation.” EX-1010(Crane), Abstract, [0003]. Crane’s methods include an expression system with “one or more expression constructs encoding a recombinant heme-binding protein.” EX-1010(Crane),

[0010]. Crane further teaches that “[e]xpression vectors include (among other elements) regulatory sequences (*e.g.*, *promoters*) that can be *operably linked* to the desired protein-encoding nucleic acid molecule” (*i.e.*, a heme-containing protein) “to cause the expression of such nucleic acid molecule in the host cell.” EX-1010(Crane), [0033]. As discussed in §VII.B.1(a), Crane’s expression system host cell can be *P. pastoris*. EX-1010(Crane), [0043]-[0044]. Hence, a POSITA would understand that Crane’s methods for recombinant heme-containing protein expression comprise a nucleic acid molecule encoding a heme-containing protein that is operably linked to a promoter element from *P. pastoris*. EX-1003(Batt), ¶213.

The only aspect of limitation 1[a] not expressly disclosed by Crane is “a Mxr1 transcriptional activator sequence from *P. pastoris*.” EX-1003(Batt), ¶214. However, Vogl discloses this limitation. EX-1003(Batt), ¶¶214-215; EX-1005(Vogl), 390-91. And, as discussed in §VI.B.1(b), a POSITA would have been motivated to combine Crane with Vogl to engineer *P. pastoris* with “a nucleic acid molecule encoding a heme-containing protein operably linked to a promoter element from *P. pastoris* and a Mxr1 transcriptional activator sequence from *P. pastoris*.” EX-1003(Batt), ¶¶216-217.

Crane teaches that it is “desirable to use strong promoters” to direct “high level[s]” of expression of desired recombinant heme-binding proteins. EX-1010(Crane), [0040]. While Crane teaches that “[s]uitable promoting sequences”

include for example glyceraldehyde-3-phosphate dehydrogenase (P_{GAP}) (EX-1010(Crane), [0047]), a POSITA would understand that Crane was ripe for modifications that would yield “a high level of transcription” of heme-containing proteins. EX-1003(Batt), ¶216; EX-1010(Crane), [0040], [0087].

As discussed more fully in §VI.B.1(b), a POSITA desiring to select suitable promoters for a recombinant protein expression system in *P. pastoris* would have been motivated to and naturally looked to Vogl. EX-1003(Batt), ¶216. Vogl discloses commonly used constitutive and methanol-inducible *P. pastoris* promoters, regulation of inducible promoters by Mxr1p, and “their application to improve heterologous protein production.” See EX-1005(Vogl), 385-87; EX-1003(Batt), ¶216. Indeed, Vogl details over twenty well-known promoters that can be used in *P. pastoris* expression systems, including strong constitutive promoters, such as P_{TEF1} and P_{GAP} , and strong methanol inducible promoters, such as P_{AOX1} and P_{DAS} . EX-1005(Vogl), 393.

As further discussed in §VI.B.1(b), Vogl also teaches that Mxr1p is essential for recombinant protein expression in methylotrophic yeast and that high levels of Mxr1p expression results in higher expression of a protein-of-interest controlled by a promoter regulated by Mxr1p, such as P_{AOX1} , “the most commonly used promoter in *P. pastoris*.” EX-1005(Vogl), 391, 400. Vogl discloses that one way to regulate Mxr1p expression (and thus further activate promoters to which Mxr1p binds) can

be to express the *MXR1* gene under the control of a promoter, such as the constitutive promoter P_{GAP} . EX-1005(Vogl), 391. Vogl describes studies, such as Takagi, in which Mxr1p expression under P_{GAP} increased the production of recombinant protein sequences controlled by P_{AOX1} 7.6 fold. EX-1005(Vogl), 391; EX-1004(Lin-Cereghino), ¶¶47-48, 51.

Based on Vogl's teachings, a POSITA would have been motivated to incorporate an Mxr1 sequence in Crane's *P. pastoris* expression system to produce recombinant heme-containing proteins (for example, by expressing Mxr1 under P_{GAP}) to improve P_{AOX1} -controlled expression of hemoprotein. EX-1003(Batt), ¶¶216-217; EX-1004(Lin-Cereghino), ¶61. A POSITA would understand that this would further Crane's goals of using "strong promoters" in order "to obtain a high level of transcription and, hence, expression." EX-1010(Crane), [0040]; EX-1003(Batt), ¶216; EX-1004(Lin-Cereghino), ¶¶63-67.

A POSITA would also have had a REOS in combining Crane's and Vogl's teachings. EX-1003(Batt), ¶217. Crane teaches that "[t]here are several fungal species which have been used as hosts for the expression of recombinant proteins, including, without limitation, yeast (e.g., *Pichia pastoris*[])." EX-1010(Crane), [0043]. Crane further teaches that "[a]ny plasmid vector containing yeast-compatible promoter, origin of replication and termination sequences is suitable." EX-1010(Crane), [0047]. Moreover, only ordinary skill would have been required

to modify the expression system in Crane to include the Mxr1 sequence using prior art teachings, routine techniques, and available materials that PO admits were well-known. *See* §§V.A, VI.B.1(b); EX-1003(Batt), ¶217. Thus, a POSITA would have had a REOS in expressing recombinant heme-containing proteins in *P. pastoris* when adding a transcriptional activator Mxr1 sequence from *P. pastoris* to Crane's methods of recombinant heme-containing protein production. EX-1003(Batt), ¶¶217-218.

(c) 1[b]

(“a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis operably linked to a promoter element from *P. pastoris* and a Mxr1 transcriptional activator sequence from *P. pastoris*.”)

Crane discloses “a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis and operably linked to a promoter element from *P. pastoris*.” EX-1003(Batt), ¶219. Specifically, Crane teaches methods to achieve complete heme incorporation in recombinant heme-containing proteins by “co-expressing a recombinant heme-binding protein and [a] recombinant *ferrochelatase protein*,” which is a heme biosynthetic enzyme (*i.e.*, a polypeptide involved in heme-biosynthesis). EX-1010(Crane), [0009]; EX-1003(Batt), ¶220. Further, as discussed in §VII.B.1(a), Crane teaches that its expression system host cell can be *P. pastoris*, and that the protein-encoding nucleic acid molecules in its expression

vectors can be “operably linked” to “promoters.” EX-1010(Crane), [0033], [0043]-[0044]. Thus, a POSITA would understand that Crane discloses an expression system comprising a nucleic acid molecule encoding at least one polypeptide involved in heme biosynthesis operably linked to a promoter from *P. pastoris*. EX-1003(Batt), ¶221.

For reasons discussed in §§VI.B.1 and VII.B.1(b), a POSITA would have been motivated to combine Crane with Vogl and include a Mxr1 transcriptional activator sequence from *P. pastoris* in vectors encoding a heme biosynthesis enzyme under Crane’s methods. EX-1003(Batt), ¶¶222-223. A POSITA would have naturally looked to Vogl when engineering *P. pastoris* expression systems using Crane’s methods to select strong promoters to achieve Crane’s goal of obtaining “high level[s]” of recombinant protein expression. EX-1003(Batt), ¶222; EX-1010(Crane), [0040]. Moreover, as discussed in §VII.B.1(b), a POSITA would have understood from Vogl’s teachings that the inclusion of an Mxr1 sequence from *P. pastoris* in Crane’s expression system would further maximize recombinant protein production. EX-1003(Batt), ¶222. For the same reasons discussed in §VI.B.1, a POSITA would have had a REOS in combining Crane’s and Vogl’s teachings. See §V.A; EX-1003(Batt), ¶222.

2. Claim 2

(“heme-containing protein” is Leghemoglobin (LegH))

As discussed in §VII.B.1, Crane and Vogl disclose every limitation of claim 1. Selecting leghemoglobin as the heme-containing protein of claim 1 would have been obvious over Crane. EX-1003(Batt), ¶224.

Crane expressly discloses constructing a *P. pastoris* expression system with genes encoding leghemoglobin. Ex-1010(Crane), [0021] (“[H]eme-binding proteins ... encompassed by the methods of the present invention include, without limitation, globins (e.g.,...*leghemoglobin*)”). In view of this, and for reasons discussed in §VI.C.1, a POSITA would have been motivated to use the *P. pastoris* cells of claim 1 to produce leghemoglobin and would have had a REOS. See §V.A; EX-1003(Batt), ¶¶225-226.

3. Claim 3

(“LegH” has a sequence as shown in “SEQ ID NO:6”)

The LegH sequence shown in SEQ ID NO:6 of the '492 patent is the *known amino-acid* sequence of soybean (*Glycine max*) leghemoglobin. EX-1001, 32:7-13; EX-1003(Batt), ¶227; EX-1046(GenBank). As discussed in §VII.B.2, Crane discloses the expression of leghemoglobin in methylotrophic *P. pastoris* expression systems. For the same reasons discussed in §VI.C.2, a POSITA desiring to

recombinantly produce leghemoglobin could have readily obtained the amino-acid sequence found in SEQ ID NO:6 prior to 2015. EX-1003(Batt), ¶¶228-229.

The sequence shown in SEQ ID NO:6 is available in the NCBI's GenBank database under Accession Number AAA33980, which first appeared in the database in April 1993. EX-1046(GenBank), 1-2; EX-1003(Batt), ¶¶229. As explained in §VI.C.2, a POSITA desiring to know the sequence of leghemoglobin prior to 2015 would have naturally turned to GenBank and found the sequence. EX-1003(Batt), ¶¶229.

4. Claim 4

(“polypeptide involved in heme biosynthesis is selected from the group consisting of...ferrochelatase”)

As explained in §VII.B.1(c), Crane expressly discloses a *P. pastoris* expression system that “co-express[es] a recombinant heme-binding protein and [a] recombinant *ferrochelatase protein*.” EX-1010(Crane), [0009]; EX-1003(Batt), ¶¶231-233.

5. Claim 5

(“promoter element from *P. pastoris* is a constitutive...or a methanol-inducible promoter”)

As discussed in §VII.B.1(b)-(c), Crane discloses a methylotrophic *P. pastoris* expression system comprising nucleic acid molecules encoding a heme-containing protein and at least one polypeptide involved in heme biosynthesis that are operably

linked to promoter elements from *P. pastoris*. Crane further teaches that to obtain a “high level” of recombinant protein and polypeptide expression, “it is desirable to use strong promoters.” EX-1010(Crane), [0040].

For the same reasons discussed in §§VI.B.1, VI.B.3, and VII.B.1, a POSITA would have been motivated to combine Crane with Vogl to choose suitable *P. pastoris* promoter elements. EX-1003(Batt), ¶¶234-237; EX-1004(Lin-Cereghino), ¶¶63-67. Vogl discloses the advantages and disadvantages of a variety of constitutive and methanol-inducible promoters commonly used in *P. pastoris* expression systems. EX-1005(Vogl), 386-96; EX-1003(Batt), ¶235. Moreover, Vogl specifically discloses the use and advantages of strong constitutive promoters, such as P_{TEF1} and P_{GAP}, and strong methanol-inducible promoters, such as P_{AOX1} and P_{DAS}. EX-1005(Vogl), 392-93, Table 1.

A POSITA would have been motivated to follow Vogl’s teachings to select a methanol-inducible or a constitutive promoter from *P. pastoris* to control the expression of hemoprotein and one or more heme biosynthetic enzymes in Crane’s expression system. See §§VI.B.1, VI.B.3, VII.B.1; EX-1003(Batt), ¶¶235-236. A POSITA would also have had a REOS in following Vogl’s teachings to use these promoters to practice the claim using routine techniques and readily available materials that PO admits were well-known. See §§V.A, VI.B.1, VII.B.1; EX-1003(Batt), ¶236.

6. Claim 6

(claim 5’s “constitutive promoter element from *P. pastoris* is a promoter element from a transcriptional elongation factor EF-1 (TEF1) gene”)

For the reasons discussed in §§VI.B.1, VI.B.4, and VII.B.1, a POSITA would have been motivated to combine Crane with Vogl, which teaches that strong constitutive promoters have been used in *P. pastoris* to provide high levels of protein expression. EX-1005(Vogl), 392-93. Vogl further teaches that P_{TEF1} is a strong constitutive promoter from *P. pastoris* that is “similar to P_{GAP},” (EX-1005(Vogl), 393, 395), which Crane discloses is a “[s]uitable promot[er].” EX-1010(Crane), [0047]. Vogl describes P_{TEF1} as “a crucial component of the eukaryotic translation machinery.” EX-1005(Vogl), 393, 395; EX-1003(Batt), ¶¶239-240. Vogl further discloses that under certain conditions, “the activity levels of P_{TEF1} driven expression exceeded P_{GAP} almost twofold.” *Id.* Thus, a POSITA would have been motivated to modify Crane’s expression system to select P_{TEF1} to control the expression of hemoprotein or one or more heme biosynthesis enzymes. EX-1003(Batt), ¶¶240.

Further, as explained in §§VI.B.1 and VII.B.1, a POSITA would have had a REOS in following Vogl’s teaching to use P_{TEF1} to practice the claim using routine techniques and readily available materials that PO admits were well-known. *See* §V.A; EX-1003(Batt), ¶¶238-241.

7. Claim 7

(claim 5's "methanol-inducible promoter element from *P. pastoris*" is an alcohol oxidase I (AOX1) promoter element)

For the reasons discussed in §§VI.B.1, VI.B.5, and VII.B.1, a POSITA would have been motivated to combine Crane with Vogl, which discloses constructing a *P. pastoris* expression system with P_{AOX1}. For example, Vogl teaches that because "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, 400. Thus, a POSITA would have been motivated to select P_{AOX1} to control the expression of hemoprotein and one or more heme biosynthetic enzymes in *P. pastoris*. EX-1003(Batt), ¶¶243-248; EX-1004(Lin-Cereghino), ¶¶66-67.

As explained in §§VI.B.1 and VII.B.1, a POSITA would have had a REOS in following Vogl's teaching to use P_{AOX1} to practice the claim using routine techniques and readily available materials that PO admits were well-known. See §V.A; EX-1003(Batt), ¶¶249-250.

8. Claim 8

("promoter element from *P. pastoris* comprises a constitutive promoter...and a methanol-inducible promoter")

For the reasons discussed in §§VI.B.1, VI.B.6, and VII.B.1, a POSITA would have been motivated to combine Crane with Vogl, which discloses using strong

constitutive promoters and methanol-inducible promoters from *P. pastoris* to control recombinant protein expression in *P. pastoris*. EX-1005(Vogl), 386-96; EX-1003(Batt), ¶¶251-253. For example, Vogl describes a *P. pastoris* expression system constructed by Takagi, which uses two different *P. pastoris* promoters to express two different proteins—phytase under the methanol-inducible promoter P_{AOX1} and Mxr1p under the constitutive promoter P_{GAP}. EX-1005(Vogl), 391. Thus, for the same reasons discussed in §§VI.B.1 and VII.B.1, a POSITA would have been motivated to use both types of promoters in the same *P. pastoris* cells and would have had a REOS in using prior art teachings, routine techniques, and readily available materials that PO admits were well-known. See §V.A; EX-1003(Batt), ¶¶251-253.

9. Claim 9

(claim 8’s “constitutive promoter element from *P. pastoris* is a promoter element from a transcriptional elongation factor EF-1 (TEF1) gene”)

For the same reasons discussed above for claims 6 and 8, a POSITA would have been motivated to combine Crane with Vogl and select P_{TEF1} as one of the promoters recited by claim 1 and would have had a REOS in doing so. EX-1003(Batt), ¶254.

10. Claim 10

(claim 8's "methanol-inducible promoter element from *P. pastoris* is an alcohol oxidase I (AOX1) promoter element")

For the same reasons discussed above for claims 7 and 8, a POSITA would have been motivated to combine Crane with Vogl and select P_{AOX1} as one of the promoters recited by claim 1 and would have had a REOS in doing so. EX-1003(Batt), ¶255.

11. Claim 11

("nucleic acid molecule encoding the heme-containing protein is present in the yeast cell in at least two copies")

Crane expressly teaches using "*one or more* expression vectors encoding the recombinant heme-binding protein." EX-1010(Crane), claim 8. A POSITA would understand that Crane teaches multiple copies of "nucleic acid molecule encoding the heme-containing protein" present in the yeast cells. EX-1003(Batt), ¶¶256-258.

12. Claim 12 and 13

("nucleic acid molecule encoding [the heme-containing protein / at least one polypeptide involved in heme biosynthesis] operably linked to the promoter element from *P. pastoris* and the Mxr1 transcriptional activator sequence from *P. pastoris* further comprises a termination sequence")

Crane expressly discloses an expression construct comprising "a nucleic acid encoding *a termination sequence*." EX-1010(Crane), claim 22; EX-1003(Batt), ¶¶259, 262. Moreover, a POSITA would understand that termination sequences were routinely used in expression vectors for *P. pastoris*. EX-1003(Batt), ¶¶260,

263; EX-1015(Lin-Cereghino_2000), 48. Thus, a POSITA would understand that Crane teaches nucleic acid molecules encoding the heme-containing protein and at least one polypeptide involved in heme biosynthesis of claim 1 further comprise termination sequences. EX-1003(Batt), ¶¶259-264.

C. Ground 2B: Crane and Vogl in further view of Lin-Cereghino

1. Claim 14

(“further comprising a nucleic acid molecule encoding a Mxr1 transcriptional activator operably linked to a promoter element from *P. pastoris* and a Mxr1 transcriptional activator sequence from *P. pastoris*”)

As discussed in §VII.B.1, Crane discloses all limitations of claim 1 except “a Mxr1 transcriptional activator sequence from *P. pastoris*,” which is taught by Vogl, and Crane and Vogl together render claim 1 obvious. As discussed in §VI.B.1, Vogl describes studies such as Takagi in which Mxr1p expression was regulated by a strong 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 *P. pastoris* strains without the P_{GAP} -*MXR1* construct. EX-1005(Vogl), 391.

As discussed in §§VI.B.1, VI.D.1, and VII.B.1., a POSITA would have understood from Vogl’s discussion of Takagi that expressing *MXR1* under a strong promoter (*e.g.*, P_{GAP}) increased the amount of Mxr1p in yeast cells, which in turn increased the P_{AOX1} -driven recombinant protein expression that is regulated by Mxr1p. EX-1003(Batt), ¶¶265-271. In view of this, a POSITA would have been

motivated to increase the expression of *MXR1* by placing the gene under the control of a promoter and/or controlling expression by adjusting carbon sources in growth medium in order to increase expression of a protein (*e.g.*, a hemoprotein or a heme biosynthetic enzyme) under the control of a methanol-inducible promoter (*e.g.*, P_{AOX1}) that is regulated by Mxr1p in *P. pastoris*. EX-1003(Batt), ¶271.

As discussed in §VI.D.1, to increase Mxr1p expression, a POSITA would have naturally looked to Lin-Cereghino (cited and discussed by Vogl). EX-1003(Batt), ¶268; EX-1005(Vogl), 390-91. Lin-Cereghino describes *P. pastoris* strains with increased expression of Mxr1p by controlling the *MXR1* gene under P_{AOX1} or P_{PEX8}. EX-1006(Lin-Cereghino), 885, 893; EX-1004(Lin-Cereghino), ¶¶77-79; EX-1003(Batt), ¶268.

Accordingly, as discussed in §VI.D.1, a POSITA would have been motivated to incorporate into the *P. pastoris* cells of claim 1 “a nucleic acid molecule encoding a Mxr1 transcriptional activator operably linked to a promoter element from *P. pastoris* [*e.g.*, P_{AOX1}, P_{PEX8}, or P_{GAP}] and a Mxr1 transcriptional activator sequence from *P. pastoris*,” and would have had a REOS in doing so. EX-1003(Batt), ¶¶268-272; EX-1004(Lin-Cereghino), ¶¶76-79.

VIII. NO OBJECTIVE EVIDENCE OF NONOBVIOUSNESS

Petitioner is unaware of any objective indicia of nonobviousness for the '492 patent. EX-1003(Batt), ¶¶273; 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.

IX. 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 considered during prosecution, nor cumulative of art evaluated during prosecution. The present grounds are based on combinations involving Mattoon, Vogl, Crane, and/or Lin-Cereghino. Mattoon and Vogl, alone or in combination, were *not* cited in any IDS or otherwise considered during prosecution of the '492 patent. Crane and Lin-Cereghino were cited in the IDS but they 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, the Examiner did not make any prior-art rejections during prosecution of the '492 patent. Thus, neither the Applicant nor the Examiner substantively discussed any disclosures from Mattoon, Vogl, Lin-Cereghino, or

Crane 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 '492 patent has not been challenged by any IPR before this Petition. The district court litigation is in the very early stage. *See Impossible Foods Inc. v. Motif Foodworks, Inc.*, No. 22-cv-00311 (D. Del.).

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

Petitioner certifies that the '492 patent is available for *Inter Partes* Review and that Petitioner is not barred or estopped from requesting this proceeding.

XI. CONCLUSION

Petitioner has demonstrated a reasonable likelihood that at least one of claims 1-14 of the '492 patent would have been obvious in view of the prior art and should be cancelled. Accordingly, Petitioner respectfully requests institution.

XII. 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., IPR2023-00321 (concerning U.S. Patent No. 10,689,656).

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,

By: /Joseph M. Paunovich/

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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 13,299 words as counted by the word processing program used to prepare the paper.

Date: January 27, 2023

Respectfully submitted,

By: /Joseph M. Paunovich/
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*Lead Attorney for Petitioner Motif
FoodWorks, Inc.*

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. 10,273,492** 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
Fish & Richardson P.C.
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:

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