

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MOTIF FOODWORKS, INC.,
Petitioner,

v.

IMPOSSIBLE FOODS, INC.,
Patent Owner.

Case IPR2023-00321
Patent No. 10,689,656

**PATENT OWNER'S PRELIMINARY RESPONSE
PURSUANT TO 37 C.F.R. § 42.107**

TABLE OF CONTENTS

I.	INTRODUCTION	1
II.	CLAIM CONSTRUCTION	4
III.	OVERVIEW OF THE TECHNOLOGY.....	4
IV.	GROUND 1 FAILS	8
A.	Petitioner fails to show that the combined references teach “a first exogenous nucleic acid encoding a methanol expression regulator 1 (Mxr1) transcriptional activator operably linked to at least one methanol-inducible promoter element comprising a sequence to which the Mxr1 transcriptional activator binds” of claim 1	9
1.	Petitioner’s proposed combination conflicts with Tsutsumi’s teachings.....	11
2.	Petitioner’s alleged motivation to “further overexpress” Mxr1 expression is contrary to Lin-Cereghino’s teachings.....	14
3.	Petitioner’s analysis further mischaracterizes Tsutsumi as allegedly suggesting a methanol-inducible promoter to express Mxr1	18
4.	Petitioner fails to establish that a POSA would have been motivated to make the proposed modification with a reasonable expectation of success.....	22
B.	Petitioner fails to establish that the combined references teach “a recombinant nucleic acid molecule” having the claimed first and second nucleic acids	23
1.	Petitioner’s analysis mischaracterizes Tsutsumi’s claim 15.....	25
2.	Petitioner’s reliance on Mattoon is insufficient.....	28
C.	Ground 1 with respect to dependent claims 2-17, 21, and 23-25 fails	30
V.	GROUND 2 FAILS	31
VI.	GROUND 3 FAILS	31
VII.	GROUND 4 FAILS	31

A.	Petitioner fails to establish that the combination of Mattoon and Vogl discloses each and every limitation of claim 26	32
1.	Petitioner fails to establish that the combination of Mattoon and Vogl discloses a recombinant nucleic acid molecule containing all three claimed nucleic acids	32
2.	Petitioner fails to show the combination of Mattoon and Vogl disclose that “each nucleic acid is operably linked to a methanol-inducible promoter element” that “comprises at least one sequence to which a Mxr1 transcriptional activator binds”	34
B.	Petitioner’s combination of Mattoon and Vogl is transparent hindsight	36
1.	Petitioner’s motivation to combine Mattoon and Vogl is deficient.....	37
2.	Petitioner fails to establish that a POSA would have been motivated to combine Mattoon and Vogl with a reasonable expectation of success.....	41
C.	Ground 4 with respect to dependent claim 28 fails.....	43
VIII.	GROUND 5 FAILS	43
IX.	INSTITUTION SHOULD BE DENIED UNDER § 325(D)	43
A.	Petitioner Presents Substantially the Same Art and Arguments Previously Considered by the Office	44
B.	Petitioner Fails to Demonstrate that the Office Materially Erred	47
X.	CONCLUSION.....	48

I. INTRODUCTION

The Board should not institute *inter partes* review of claims 1-28 of U.S. Patent No. 10,689,656 (EX1002, “’656 patent”) because Petitioner Motif FoodWorks, Inc., fails to show that it has a reasonable likelihood of prevailing. Petitioner’s grounds engage in impermissible hindsight and provide no supportable motivation or rationale to combine the cited references to achieve what is claimed, while instead combining references in a manner that conflicts with the express content of the cited references.

Each of the challenged claims recites, in relevant part, a methylotrophic yeast cell comprising a recombinant nucleic acid molecule that includes a nucleic acid encoding an Mxr 1 transcriptional activator that is operably linked to a methanol-inducible promoter element. Attempting to reach this particularly claimed combination, the petition improperly disregards what its very own references teach about recombinant protein expression systems. For example, in its Ground 1 challenge, Petitioner proposes using a methanol-inducible promoter for expression of Mxr1, all the while ignoring that the primary reference (Tsutsumi) it seeks to modify to incorporate such a promoter expressly teaches that it is desirable to **avoid** reliance on methanol. Petitioner then turns to Lin-Cereghino for using a methanol-inducible promoter to “further overexpress” Mxr1 beyond the overexpressed Mxr1 production that Tsutsumi already achieved. But

Lin-Cereghino expressly discusses that increasing Mxr1 expression levels should be avoided because of its toxicity to host *P. pastoris* cells, causing a growth defect in the cells. Petitioner’s conclusory assertions and failure to account for the teachings of the very references it relies upon for the proposed combination, which teachings undermine its proposed combination, provides ample reason to deny institution.

In addition to ignoring what its references say, Petitioner’s analysis attempts to manufacture teachings simply not present. For example, Petitioner asserts that Tsutsumi’s *constitutive*¹ GAP promoter somehow suggests a methanol-inducible system. It does not. For Ground 4, Petitioner asserts that its proposed combination discloses all three nucleic acids recited in the claim in the same recombinant nucleic acid molecule and all “operably linked to a methanol-inducible promoter element” that includes “at least one sequence to which a Mxr1 transcriptional activator binds.” It does not. These deficiencies are fatal to all grounds of challenge.

The petition also asserts that a POSA ***could*** have made the claimed combination, without ever acknowledging the complexity and unpredictability

¹ A constitutive promoter is an unregulated promoter—that is, the opposite of an inducible promoter.

underlying the recombinant technology reported in its own cited references, and without demonstrating that a POSA **would** have done so with a reasonable expectation of success. For example, Ground 4 depends on a review article (Vogl) describing numerous activators and promoters. Petitioner plucks out the pieces it desires from Vogl, while ignoring other aspects, including Vogl's description on the known disadvantages to methanol-inducible promoters, uncertainties as to expression of Mxr1 in yeast, and the need to carefully consider the particular aspects of the recombinant expression system. Contrary to the petition's conclusory assertions, recombinant genetic expression systems are reported in the literature as complex and unpredictable, not routinely interchanged like simple components in the mechanical arts. Designing recombinant protein expression systems at the time required more than haphazardly combining of any available transcriptional activator with any available promoter for any protein of interest. Indeed, Petitioner's own cited references report that the regulatory mechanisms underlying the numerous transcriptional activators and promoter elements were complex and still not understood at the critical date. Accordingly, engineering recombinant expression systems with an appropriate transcriptional activator and promoter element required a deliberative, evidence-based analysis specific to the protein of interest. The petition fails to provide such an analysis, much less

demonstrate that the claimed combination would have been obvious to a POSA before the critical date.

For the reasons detailed below, institution of *inter partes* review should be *denied*.

II. CLAIM CONSTRUCTION

A claim subject to *inter partes* review receives its plain and ordinary meaning, interpreted in light of the specification of the patent in which it appears. *See* 37 C.F.R. § 42.100(b). Petitioner proposes no constructions. Pet., 28. Patent Owner submits that no constructions are needed for the Board to reach a decision denying institution.

III. OVERVIEW OF THE TECHNOLOGY

Petitioner's deficient analysis of the challenged claims relies on an oversimplification and lack of understanding of the technology at issue. Petitioner wrongly treats recombinant yeast protein expression systems as if they were predictable and readily interchangeable during the relevant timeframe of May 11, 2015. *See* Pet., 44 (alleging recombinant technologies "only require[s] routine techniques, known methods, and available materials"). However, as Petitioner's own cited art demonstrates, recombinant systems for the expression of heterologous proteins using yeast transcriptional activators, particularly the Mxr1 transcriptional activator, and promoter elements in recombinant systems were

recognized as complex and not fully understood. In fact, “[t]he majority of molecular mechanisms investigated up to now, are still far from being completely understood.” EX1005, 400-401. Indeed, Petitioner’s own cited art emphasizes the complexity of yeast protein expression systems, particularly methanol-inducible systems, including then-known methanol-responsive yeast transcriptional activators. *Id.* When considering the art in its totality, work in this field was not predictable as noted by Petitioner’s own references, further highlighting Petitioner’s failure to set forth an articulated rationale that is supported by the references. *Id.*

For instance, Vogl (EX1005) explains that the control and expression of yeast transcriptional activators, including Mxr1, was not fully understood and therefore unpredictable. *See* EX1005, 400. In discussing the work of Takagi (EX1007), for example, Vogl specifically highlights uncertainties surrounding work by Takagi regarding the control and mechanism of action of two transcriptional activators, PRM1 and Mxr1. *See id.*, 391 (noting Takagi’s experiments failed to provide for “comparison to methanol induction or comparative biomass production”). Vogl also particularly noted the disconnect of Takagi’s findings with Lin-Cereghino (EX1006), which showed that high levels of expression of Mxr1 using the strong methanol-inducible promoter AOX1 was toxic to *P. pastoris* cells, “caus[ing] a growth defect on methanol and inhibited

expression of MUT genes.”² *Id.* Vogl concluded from these studies, both of which were cited by Petitioner, the data available at the time was “not entirely consistent and would require further investigation to clarify the activating mechanism of Mxr1p.” *Id.* Vogl’s message, which was closest in time to the critical date, reinforced the position that overexpression of Mxr1 was harmful to yeast cells and therefore cautioned those in the art that the effects of incorporating Mxr1 in protein expression systems were unpredictable, not well understood, and potentially harmful to its host.

² Petitioner attempts to negate Lin-Cereghino’s explicit teachings of Mxr1 toxicity through the *post hoc* testimony of present-day Dr. Lin-Cereghino (EX1004). See Pet., 19-20 (citing EX1004, ¶¶37-39) (alleging “we did not perform any additional experiments to test or confirm this hypothesis” and a POSA “would recognize that further investigation would be needed to determine what caused this strain not to grow”). Dr. Lin-Cereghino’s hindsight testimony ignores that subsequent experiments were performed using lower Mxr1 expression levels with the weak promoter PEX8 that rescued yeast cells, with no indication that culture conditions differed (see EX1006, 884 (Materials & Methods)), providing strong support for what Lin-Cereghino unequivocally concluded: “Apparently, high levels of Mxr1p are toxic to *P. pastoris*.” EX1006, 893.

Further, utilizing various genetic elements such as transcriptional promoters for driving expression in methylotrophic yeast cells was far from routine. Even limiting consideration to transcriptional promoters, the cited references report that a POSA would have been faced with many different promoters and would have had to first determine the compatibility of inducible and constitutive promoters with heterologous protein expression and transcriptional activators. For example, Vogl provides a table listing over twenty different promoters known at the time. As Vogl evidences, a promoter that works for a particular protein may not work for a different protein. *See* EX1005, 392 (“the strong expression from P_{AOX1} and P_{GAP} is not always the most favorable condition for heterologous protein production”); *see also* EX1042, 4-5 (“Thus, we are no longer confident as we were 18 years ago that simplicity will eventually emerge from complexity. The extreme sophistication of cellular mechanisms will challenge cell biologists throughout the new century....”); EX1022, 145 (“Each protein expression system offers a unique set of properties....”). Using a transcriptional promoter for the purpose of increasing expression may not even work, and may even provide deleterious effects, such as toxicity. *See* EX1005, 391; EX1017, Abstract, e76 (“little is known about the regulation of the AOX1 promoter at the molecular level”); *id.*, e76 (“the regulatory properties of the promoter variant, the target protein and the production process have to be seen as a whole,” noting certain promoters can cause

toxic effects). In other words, the art showed that it was not a matter of simple substitution with predictable results. Petitioner's own references report that genetic expression systems including recombinant insertion of a transcriptional promoter for expression of a given protein was complex and challenging, not a mere matter of simple and predictable substitutions of mechanical components.

IV. GROUND 1 FAILS

Petitioner's Ground 1 challenges claims 1-17, 21, and 23-25 based on the combination of Tsutsumi (EX1024), Lin-Cereghino (EX1004), and Mattoon (EX1008). Pet., 1, 29. As detailed below, Petitioner fails to show a reasonable likelihood that it will prevail on the asserted ground.

Petitioner presents a three-reference combination that relies on Tsutsumi as teaching the "overexpression of Mxr1 to enhance production of a recombinant polypeptide in *P. pastoris*," Lin-Cereghino for its disclosure of "expressing Mxr1 using methanol-inducible promoters to which Mxr1 binds, such as P_{PEX8} and P_{AOX1}," and Mattoon for its teaching of "recombinant techniques to produce heterologous polypeptides, including expression of multiple nucleic acid sequences on one vector." Pet., 29. As detailed below, however, Petitioner presents a conclusory and misplaced analysis for the proposed combination that is not logically supported by the teachings of the applied references. Given the uncertainties in the field at the relevant time, and teachings in the art that counsel

against the proposed combination, Petitioner's obviousness challenge fails to satisfy its burden.

A. Petitioner fails to show that the combined references teach “a first exogenous nucleic acid encoding a methanol expression regulator 1 (Mxr1) transcriptional activator operably linked to at least one methanol-inducible promoter element comprising a sequence to which the Mxr1 transcriptional activator binds” of claim 1

Petitioner alleges that “Tsutsumi in view of Lin-Cereghino discloses methylotrophic yeast including a recombinant nucleic acid molecule comprising a first exogenous nucleic acid encoding Mxr1 operably linked to a methanol-inducible promoter to which Mxr1 binds.” Pet., 33. To reach this limitation, Petitioner begins with Tsutsumi's Example 5, which discloses the expression of Mxr1 under the control of the constitutive promoter P_{GAP}, and proposes “substitut[ing] Tsutsumi's GAP promoter controlling Mxr1 expression with a methanol-inducible promoter such as P_{PEX8}, P_{DAS} or P_{AOX1}” based on Lin-Cereghino. *Id.*, 41; *see also id.*, 36, 38. Petitioner alleges that a POSA would have been motivated to make this modification “to tightly control and **further overexpress** Mxr1.” *Id.*, 38 (emphasis added); *see also id.*, 40 (arguing POSA would have been motivated to substitute Tsutsumi's GAP promoter “to increase the expression of the *MXR1* gene by placing it under the control of a methanol-inducible promoter”). But Petitioner's stated rationale is contradicted by the very references it cites.

To start, Petitioner ignores Tsutsumi's express objective: to provide a recombinant protein expression system that reduces the need to rely on methanol for induction of gene expression. EX1024, [0006], [0042]. Yet Petitioner's proposed modification would contradict the very teachings of Tsutsumi by abandoning the methanol-independent constitutive promoter of Tsutsumi (GAP promoter) and substituting it with a promoter that relies on methanol as its source for transcription induction.

Next, Petitioner relies on Lin-Cereghino for motivation to "further overexpress" Mxr1 by replacing Tsutsumi's GAP promoter with Lin-Cereghino's methanol-inducible promoters. Pet., 38. But this proposal contradicts Lin-Cereghino's own teachings that high levels of Mxr1 "are toxic to *P. pastoris*." EX1006, 893.

In addition to advancing a rationale that finds no support in the applied references, Petitioner attempts to manufacture a teaching in Tsutsumi to use a methanol-inducible promoter for Mxr1 expression. Pet., 34-35. But Petitioner mischaracterizes the reference and fails to show that a POSA would have been motivated to make the proposed modification with a reasonable expectation of success.

Simply put, the petition fails to substantiate its proposed motivation for its proposed modifications. For these reasons, Petitioner fails to establish a reasonable likelihood that the challenged claims are unpatentable under Ground 1.

1. Petitioner’s proposed combination conflicts with Tsutsumi’s teachings

Petitioner’s proposed modification to replace Tsutsumi’s constitutive GAP promoter with a methanol-inducible promoter is not sufficiently explained and contradicts Tsutsumi’s own teachings to provide a system that is less dependent on methanol. EX1024, [0006], [0042]. To achieve this end, Tsutsumi discloses the use of variant DAS promoters that induce expression of a polypeptide of interest without the need for methanol. To further achieve the aim of avoiding methanol as an inducer, Tsutsumi expressly teaches using ***constitutive or non-methanol inducible promoters*** for inducing expression of Mxr1. *Id.*, [0042], [0076]-[0077].

Specifically, Tsutsumi relates to “improved variants of the *Pichia* DAS promoter for increased expression of a polypeptide of interest.” EX1024, [0007]. As acknowledged by Tsutsumi, the wild-type DAS promoter is a methanol-inducible promoter, which normally requires the presence of methanol for induction. *See id.*, [0040]-[0041] (“expression of heterologous polypeptides can be controlled by the addition of methanol to the growth medium[,] [s]uch promoters include . . . DAS promoter”). However, Tsutsumi explains that the DAS promoter variants of its invention are designed so that expression of the polypeptide is “***less***

dependent on the substrate” methanol, and transcription can even be induced “*without the need for methanol.*” See, *id.*, [0006], [0042] (emphasis added).

Tsutsumi accomplishes this methanol-independent expression by adding multiple Upstream Activating Sequences (UASs) to the variant DAS promoter, which the inventors postulate comprises a binding region for a positive regulator (*e.g.*, transcriptional activator) encoded by the Prm1 gene. See *id.*, [0042]-[0043], [0046], [0054].

To induce expression of the transcriptional activator (*e.g.*, Prm1 or Mxr1) intended to bind to the variant DAS promoter for polypeptide expression, and consistent with its stated goal of providing a system that is not dependent on methanol, Tsutsumi provides two options: the transcriptional activator “is expressed *constitutively* from a suitable promoter” or is expressed “from an inducible promoter which is *not methanol inducible.*” *Id.*, [0076]-[0077] (emphasis added). Thus, Tsutsumi describes a yeast comprising a transcriptional activator (*e.g.*, the Prm1 or Mxr1 transcriptional activator) under the control of a *constitutive or non-methanol inducible promoter*, where the activator binds to UASs of a variant DAS promoter operably connected to a sequence encoding a polypeptide. This is indeed confirmed in the example relied on by Petitioner, Example 5 of Tsutsumi, which provides an example yeast comprising: (i) the Prm1 or Mxr1 transcriptional activator under the control of the *constitutive GAP*

promoter, and (ii) a gene of a protein of interest (cutinase gene) controlled by a variant DAS promoter. *Id.*, [0137]-[0145].

That Tsutsumi deliberately avoids the use of a methanol-inducible promoter for expression of the transcriptional activator is further illustrated by its background discussion. There, Tsutsumi expressly acknowledges methanol-inducible promoters and the transcriptional activator Mxr1. *See* EX1024, [0003]-[0005]. Tsutsumi even specifically discusses the teachings of Lin-Cereghino. *See id.*, [0003]-[0005], [0041]. Upon considering Lin-Cereghino's discussion of methanol-inducible promoters to express Mxr1, Tsutsumi teaches to induce Mxr1 through a constitutive or non-methanol inducible promoter, in full support of its overall teachings to decrease reliance on methanol as a source for induction of gene expression. *Id.*, [0006], [0042], [00076]-[0077]. The avoidance of methanol as an inducer is consistent with the art's general teaching that the use of methanol was "undesirable" as a "highly flammable and hazardous substance." EX1029, 5302.

Petitioner makes no attempt to acknowledge or resolve the conflict between the teachings of the Tsutsumi reference it cites and its proposed modification. *See* Pet., 38-41. Instead, Petitioner ignores Tsutsumi's own teachings and simply contends that a POSA would have sought to substitute the constitutive GAP promoter used in Tsutsumi with the methanol-inducible promoter of Lin-

Cereghino, despite the fact that such a modification would make the system *more* dependent on methanol to drive expression, contrary to Tsutsumi’s stated teachings. *See* Pet., 36-38. Because Petitioner provides no explanation or evidence as to why a POSA would ignore the teachings of Tsutsumi and make a system more reliant on methanol, Petitioner’s rationale for combining Tsutsumi and Lin-Cereghino is contradicted by its own evidence and insufficient to establish the obviousness of the claims.

2. Petitioner’s alleged motivation to “further overexpress” Mxr1 expression is contrary to Lin-Cereghino’s teachings

Furthermore, Petitioner’s reason why a POSA allegedly would have pursued the modification—to “further overexpress” Mxr1 (Pet., 38)—is also contrary to Lin-Cereghino’s teachings, which disclose toxic effects when Mxr1 is expressed at high levels. EX1006, 893.

Lin-Cereghino is a scientific article describing studies designed “to understand, at the molecular level, how *P. pastoris* coordinately regulates the expression of *AOX1* and other genes necessary for methanol utilization,” where the gene *MXR1* (whose product is Mxr1p) was identified and characterized. EX1006, 884. “Because Mxr1p is produced at only low levels in wild-type *P. pastoris*, it was necessary to construct a *P. pastoris* strain that overexpressed the protein.” *Id.*, 893. Lin-Cereghino discloses the construction of “a strain that produced Mxr1p under the control of the strong methanol-regulated *AOX1* promoter with the vector

pGC215,” but Lin-Cereghino found that the strain “did not grow on methanol and did not induce Mxr1p or other methanol pathway components in response to methanol” because “[a]pparently, high levels of Mxr1p are toxic to *P. pastoris*.”

Id. As a result, Lin-Cereghino pursued the weaker promoter PEX8 to express Mxr1 at lower levels in order to avoid the toxic effects observed at high expression levels of Mxr1. *Id.* (“*P_{PEX8}* is methanol regulated...but not nearly as strongly induced or repressed as *AOX1*.”).

Thus, rather than motivating a POSA to **further overexpress** Mxr1 as Petitioner claims, Lin-Cereghino undermines Petitioner’s proposed rationale because “high levels of Mxr1p are toxic to *P. pastoris*.” EX1006, 893; *see Depuy Spine, Inc. v. Medtronic Sofamor Danek, Inc.*, 567 F.3d 1314, 1326 (Fed. Cir. 2009) (“An inference of nonobviousness is especially strong where the prior art’s teachings undermine the very reason being proffered as to why a person of ordinary skill would have combined the known elements.”). Lin-Cereghino thus contradicts Petitioner’s argument that a POSA would have been motivated “to substitute Tsutsumi’s GAP promoter controlling Mxr1 with a fully methanol-inducible promoter in order to tightly control and further overexpress Mxr1.” Pet., 38. Again, Petitioner fails to address how its own evidence, here Lin-Cereghino, undermines Petitioner’s proposed motivation. Moreover, arguing instead that a POSA “would have options (e.g., *P_{PEX8}*, *P_{DAS}* and *P_{AOX1}*)” if she wanted to “try” a

methanol-inducible promoter is conclusory and unsupported. *Id.* This “obvious-to-try” argument is insufficient to satisfy Petitioner’s burden of showing a motivation that has a rational underpinning in the applied references. Even if a POSA would have wanted to try to modify Tsutsumi’s system to have one of various methanol-inducible promoter options—an assertion Petitioner fails to justify—Petitioner still fails to demonstrate that the options represented “a finite number of identified, predictable solutions.” *KSR Int’l Co. v. Teleflex Inc.*, 500 U.S. 398, 421 (2006).

Moreover, despite Petitioner’s assertions, Tsutsumi itself does not indicate a desire or need for improving control or increasing expression of ***Mxr1*** beyond the expression provided for by the disclosed constitutive and non-methanol inducible promoters. See EX1024, [0055], [0076]-[0077]. Petitioner cites to an alleged teaching from Tsutsumi that “[c]ontrolled expression is often desirable” (Pet., 38 (citing EX1024, [0005])), but Petitioner overlooks that Tsutsumi’s reference to a desire for “[c]ontrolled expression” in the background section is in relation to the expression of a *heterologous polypeptide of interest*, not a transcriptional activator. EX1024, [0004]-[0005]. Specifically, Tsutsumi states that, in the preceding paragraph, “[c]onventionally, maximal expression of a gene in a eukaryotic organism is achieved by amplifying in the chromosome an expression cassette containing a single promoter operably linked to a gene encoding ***the polypeptide of***

interest and an amplifier selective marker.” *Id.*, [0004]. Tsutsumi continues by explaining that “[c]ontrolled expression is often desirable” through control of the respective promoter of the polypeptide of interest. *Id.* However, nothing in Petitioner’s citation to Tsutsumi supports its alleged motivation to “tightly control” or “further overexpress” **Mxr1** beyond expression via constitutive or non-methanol inducible promoters. Tsutsumi goes on to state that while “induction by methanol requires the presence of additional factors,” specifically including Mxr1p, Tsutsumi recognized that “the exact mechanism of action for such factors have not been elucidated,” emphasizing the unpredictability of “controlling” factors such as transcriptional activators. *Id.*, [0005]. And whatever the case, a POSA certainly would not have found Lin-Cereghino as supporting Petitioner’s proffered rationale, as it counsels against high levels of expression, much less increasing overexpression of Mxr1. *See* EX1006, 893.

Finally, Petitioner turns to a third reference that is not part of the ground, Vogl, to allege that there were “major advantages” to the use of methanol-inducible promoters for the “production of toxic or detrimental heterologous proteins.” *Pet.*, 41 (citing EX1005, 392). Petitioner, however, mischaracterizes Vogl’s statement as it applies to Mxr1 expression. The cited portion of Vogl relates to the second phase production of a “heterologous protein” **after** a first phase where cultures have “grown to high cell densities.” *Id.*; EX1005, 392. This

is irrelevant in view of Lin-Cereghino, which teaches “[p]romoters of methanol-responsive genes require MXR1 function for transcriptional induction.” EX1006, FIG. 3. However, by choosing to overexpress toxic Mxr1, the culture environment and the resulting yeast would not be able to support production of the heterologous protein.³

Accordingly, Petitioner fails to establish that a POSA would have been motivated to “increase the expression of the *MXR1* gene by placing it under the control of a methanol-inducible promoter” in view of the teachings of Tsutsumi and Lin-Cereghino.

3. Petitioner’s analysis further mischaracterizes Tsutsumi as allegedly suggesting a methanol-inducible promoter to express Mxr1

Finally, perhaps recognizing the issues that Tsutsumi provides with its emphasis on decreasing reliance on methanol as an inducer of transcriptional activation, Petitioner tries another tact by twisting Tsutsumi’s teachings into a

³ To the extent Petitioner uses the quote to suggest that methanol-inducible promoters would have “major advantages” for the production of toxic Mxr1, Petitioner contradicts itself elsewhere with its *post hoc* testimony of Dr. Lin-Cereghino and Dr. Batt, alleging that Mxr1 is not toxic. See footnote 1; EX1003, ¶289.

disclosure of “a methylotrophic yeast cell with Mxr1 under the control of a promoter element, which may be constitutive or methanol-inducible.” Pet., 35. However, in doing so, Petitioner mischaracterizes Tsutsumi.

For example, Petitioner first argues that “Tsutsumi teaches using a methanol-inducible system” because Tsutsumi teaches that “promoters suitable for the above activation may in particular be...induced by methanol” and that “expression of the positive regulator Mxr1 is increased by controlling the expression of Mxr1.” Pet., 34 (citing EX1024, [0108], claim 15). However, Petitioner’s argument mischaracterizes Tsutsumi and paragraph [0108].

Tsutsumi’s discussion of suitable promoters that may be “induced by methanol” (*i.e.*, the variant DAS promoter) is in relation to expression of the polypeptide of interest, **not** the transcriptional activator. See EX1024, [0100]-[0108] (describing “a use of an UAS for increasing transcription from a promoter”). With respect to expression of the transcriptional activator, Tsutsumi expressly discloses using a constitutive or non-methanol inducible promoter, as noted above. See *id.*, [0076]-[0077]; *supra*, section IV.A.1. Furthermore, Petitioner’s reliance on claim 15 as reciting that expression of Mxr1 “is increased by controlling the expression of Mxr1” (Pet., 34) does not support a conclusion that Tsutsumi teaches using a methanol-inducible system for such Mxr1 expression since Tsutsumi consistently uses this phrase in relation to the constitutive or non-methanol inducible promoters

it discloses for expression of Mxr1. See EX1024, [0076]-[0077] (“the expression of the positive regulator is controlled from an inducible promoter which is not methanol inducible”), [0140] (“Both plasmids have the Zeocin resistant gene as a selection marker gene and the regulator gene is controlled by the GAP promoter.”).

In addition, Petitioner falsely argues that the GAP promoter of Tsutsumi’s Example 5 “could be at least partly methanol-induced” because “‘there is a clear influence of the carbon source on the expression strength’ of the *GAP* promotor and ‘studies in *P. pastoris* indicate that P_{GAP} driven expression is not entirely constitutive but regulated under certain conditions.’” Pet., 34-35 (citing EX1005) (original alterations omitted). Petitioner either misunderstands or grossly misrepresents the cited teachings in Vogl related to carbon source and expression:

GAP is principally constitutively expressed, although there is a clear influence of the carbon source on the expression strength. Namely Northern blots showed, that the *GAP* mRNA levels were highest in cells grown on glucose, and decreased approximately to two-thirds on glycerol, half on oleic acid and *one-third on methanol*.

EX1005, 392 (citations omitted) (emphasis added).

Instead of supporting that GAP is “methanol-inducible,” Vogl teaches that use of methanol as a carbon source with GAP actually **reduced** expression of a polypeptide significantly, from its preferred carbon source of glucose where expression is strongest, to **one-third** of the expression level with methanol. In

other words, a POSA would have understood Vogl as reporting use of GAP in cells grown on methanol as having the *opposite* effect—that is, it would significantly decrease expression of the transcriptional activator.

Moreover, Petitioner also ignores, as noted above, that Tsutsumi explicitly categorizes the GAP promoter as a *constitutive* promoter and the only “inducible promoters” contemplated by Tsutsumi for Mxr1 expression are *non-methanol* inducible. EX1024, [0074]-[0077] (“In one embodiment the positive regulator is expressed constitutively from a suitable promoter.... In one particular embodiment the promoter is selected from the group consisting of the GAP promoter.... In another embodiment the expression of the positive regulator is controlled from an inducible promoter which is not methanol inducible.”). Even Petitioner’s cited reference, Vogl, categorizes P_{GAP} as a constitutive promoter and Petitioner in fact agrees with this general understanding elsewhere in its petition. EX1005, 393 (Table 1); Pet., 39 (“Tsutsumi discloses over-expressing the MXR1 gene under the strong constitutive promoter P_{GAP} ”).

Petitioner’s attempts to manufacture a teaching in Tsutsumi of using a methanol-inducible system for Mxr1 expression rest on mischaracterizations that are simply contrary to Tsutsumi’s (and Vogl’s) express teachings. Petitioner’s Ground 1 analysis is thus further unsupported for this additional reason.

4. Petitioner fails to establish that a POSA would have been motivated to make the proposed modification with a reasonable expectation of success

Discussion of motivation with reasonable expectation of success in the petition is conclusory and illogical. Pet. 41-42. For example, Petitioner further contends a POSA would have had a reasonable expectation of success in “engineering methylotrophic yeast that overexpress Mxr1 under a methanol-inducible promoter to which Mxr1 binds (e.g., *PEX8*) in order to produce higher levels of heterologous protein expression” and “could do so using routine techniques.” Pet., 41-42. Petitioner’s modification, however, is premised on the replacement of a particular constitutive promoter, pGAP, for a methanol-inducible promoter, such as pPEX8, pDAS, or pAOX1, to express a particular transcriptional activator, Mxr1, which is ultimately used to drive expression of a particular polypeptide of interest, cutinase. See EX1024, Example 5; Pet., 33, 38. Petitioner, however, provides no evidence or explanation that such a modification would have been considered routinely interchangeable in the manner suggested by Petitioner.

As explained above, the art, including the relied-upon references, acknowledges significant complexities of engineering into a recombinant system, *inter alia*, a promoter for expression of a particular protein. See EX1022, 145; EX1042, 4-5; *supra*, section III. Even expression of Mxr1 using a methanol-inducible promoter was reported as not well-understood and unpredictable, as

evidenced by Lin-Cereghino. See EX1006, 893; EX1005, 391 (noting inconsistent results regarding Mxr1 expression that “require further investigation”). As such, the petition’s conclusory argument regarding “routine techniques” is unsubstantiated and even contradicted by Petitioner’s own cited references. Moreover, even establishing that “routine” techniques could be used to insert a given promoter without any evidence or justification does not establish that a POSA would have been motivated to make the proposed modification with a reasonable expectation of success. Pet., 44; see *Belden Inc. v. Berk-Tek LLC*, 805 F.3d 1064, 1073 (Fed. Cir. 2015) (obviousness requires showing whether a POSA “not only could have made but would have been motivated to make the combinations”).

Accordingly, for these reasons, Petitioner fails to establish that a POSA would have been motivated to combine Tsutsumi and Lin-Cereghino with a reasonable expectation of success.

B. Petitioner fails to establish that the combined references teach “a recombinant nucleic acid molecule” having the claimed first and second nucleic acids

As Petitioner recognizes, claim 1 requires that the *same* recombinant nucleic acid molecule comprises both the claimed first exogenous nucleic acid and the claimed second exogenous nucleic acid. See EX1001, claim 1 (“[a] methylotrophic yeast cell comprising *a* recombinant nucleic acid molecule,

wherein **the** recombinant nucleic acid molecule comprises a first exogenous nucleic acid..., wherein **the** recombinant nucleic acid molecule comprises a second exogenous nucleic acid”). Petitioner alleges Tsutsumi teaches “a methylotrophic yeast including a single recombinant nucleic acid molecule comprising both of the nucleic acids required by limitations 1[a] and 1[b]” based on a reference to a “DNA construct” recited in a dependent claim of Tsutsumi. Pet., 30-31. However, when Tsutsumi’s claim is read in its entirety, nowhere is there an indication that the DNA construct as claimed contains **both** a nucleic acid encoding a Mxr1 transcriptional activator and a nucleic acid encoding a heterologous polypeptide. Indeed, Tsutsumi only discloses embodiments where the nucleic acid encoding the transcriptional activator is on a separate plasmid. EX1024, [0137] (“co-expression of transcription factors”); [0140] (Example 5).

Perhaps recognizing this lack of disclosure, Petitioner then turns to Mattoon for allegedly teaching the “expression of multiple nucleic acid sequences on one vector.” Pet., 29, 32. Apart from Mattoon itself failing to teach providing both the nucleic acid encoding the heterologous polypeptide and the nucleic acid encoding Mxr1 on the same vector, however, Petitioner fails to present any reasoned basis for why a POSA would have sought to combine the references. Petitioner instead relies on conclusory assertions that Mattoon’s teachings “could be” readily applied

to Tsutsumi. *Id.*, 32. For these reasons, Petitioner fails to establish that the applied references disclose or suggest the recombinant nucleic acid molecule of the claims.

1. Petitioner’s analysis mischaracterizes Tsutsumi’s claim 15

Petitioner’s sole basis for arguing that Tsutsumi discloses a single recombinant nucleic acid molecule having the claimed nucleic acids of claim 1 is a citation to Tsutsumi’s dependent claim 15, which incorporates the limitations of claims 1, 10, 12, and 13. Pet., 30-31. Petitioner argues that Tsutsumi’s claim 15 “claims a ‘DNA construct’ that includes Mxr1 under control of a promoter and a polypeptide under control of a DAS promoter,” but Petitioner’s assertion is not supported by the claim language of claim 15. *Id.*, 30. The full text of the limitations included in claim 15 is reproduced below:

Claim	Recited Elements
1	An isolated polynucleotide comprising: i) a nucleotide sequence consisting of the DAS promoter sequence from <i>Pichia</i> or a functional part thereof, wherein the said DAS promoter is comprised in SEQ ID NO: 1; and ii) at least one additional UAS, wherein the said UAS is comprised in SEQ ID NO: 2.
10	<i>A DNA construct comprising a polynucleotide sequence of claim 1</i> operably linked to a structural gene encoding a polypeptide of interest and a terminator.
12	A <i>Pichia</i> host cell comprising an expression vector of claim 10.
13	A method of producing a polypeptide of interest comprising: (a) cultivating the host cell of claim 12, under conditions conducive for the production of the polypeptide of interest; and (b) recovering the polypeptide.
15	The method according to claim 13, wherein expression of the positive regulator Mxr1 is increased by controlling the expression of Mxr1 or by increasing the copy number of the gene encoding Mxr1.

As shown above, the “DNA construct” referred to by Petitioner is first recited in dependent claim 10. According to the claim, that DNA construct comprises the “polynucleotide sequence of claim 1” (which is the variant DAS promoter of Tsutsumi’s invention) that is “operably linked to a structural gene encoding a polypeptide of interest and a terminator.” The claimed construct, in turn, may be further included in a *Pichia* host cell, as recited in claim 12, which may be cultivated “under conditions conducive for the production of the polypeptide of interest” using the method of claim 13. Claim 15 adds that “expression of the positive regulator Mxr1 is increased by controlling the expression of Mxr1 or by increasing the copy number of the gene encoding Mxr1.”

Nowhere does claim 15 state that the “**DNA construct**” of claim 10 (which, by its terms, only comprises the variant DAS promoter of claim 1, the gene encoding the polypeptide of interest and a terminator) further comprises an “exogenous nucleic acid encoding a methanol expression regulator (Mxr1) transcriptional activator.” *See also* EX1024, [0064]-[0077] (providing similar description). Aside from its conclusory assertion that the “DNA construct” of claim 10 includes it, Petitioner provides no additional evidence or explanation supporting its erroneous reading of Tsutsumi’s claim 15. Pet., 30-31; *see also* EX1003, ¶126 (Petitioner’s expert advancing same conclusory assertion). Indeed, Tsutsumi’s detailed description further confirms that Petitioner’s reading of claim

15 is unsupported: the only sequence comprising Mxr1 disclosed in Tsutsumi is on a construct that is **separate** from the one comprising the nucleic acid encoding the polypeptide of interest. *See* EX1024, [0140] (SEQ ID NO: 45 disclosing an Mxr1 expression cassette consisting of a GAP promoter, Mxr1 coding sequence, and a terminator; and SEQ ID NO: 43 disclosing a plasmid containing the Mxr1 expression cassette, which was used to transform cells already containing an enzyme expression plasmid for cutinase controlled by a DAS promoter).

Thus, Petitioner's arguments that Tsutsumi teaches "a single recombinant nucleic acid molecule comprising both of the nucleic acids required by limitations 1[a] and 1[b]" fail because they are premised on a mischaracterization of Tsutsumi's dependent claim 15.

2. Petitioner's reliance on Mattoon is insufficient

Petitioner's combination with Mattoon to reach this limitation is also insufficient because it rests on conclusory assertions that fail to address the actual language of the claims.

The totality of Petitioner's motivation to combine Tsutsumi with Mattoon depends on Petitioner's assertion that "Mattoon teaches that 'both 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.'" Pet., 32 (emphasis and alterations

omitted). From this teaching, Petitioner argues that a POSA “would understand that Mattoon’s teachings ***could be*** readily applied to Tsutsumi/Lin-Cereghino.” *Id.* (emphasis added). And, based on Tsutsumi’s teaching that its variant DAS promoters are “useful for expression of any polypeptide of interest in *Pichia*,” Petitioner concludes that a POSA “would thus be motivated to combine Mattoon’s teaching (reflecting general knowledge in the art) with Tsutsumi/Lin-Cereghino with a REOS.” *Id.* This limited analysis is insufficient because it fails to provide a reason ***why*** a POSA would apply Mattoon’s teachings to modify Tsutsumi to include a single recombinant nucleic acid having both a nucleic acid encoding a *Mxr1 transcriptional activator* and a nucleic acid encoding a heterologous polypeptide.

As an initial matter, Mattoon itself does not suggest the modification that Petitioner proposes. Petitioner’s citation to Mattoon is related to two genes encoding *two heme-associated polypeptides*—a heme enzyme and a heme-binding agent—present on one expression vector. *See* EX1008, 5:34-46; Pet., 32. Mattoon *does not* disclose the expression of a *transcriptional activator* and a heterologous polypeptide in a single recombinant nucleic acid as is required by the present claims. Thus, Petitioner’s claims that a POSA would understand that Mattoon’s teachings “could be” readily applied to Tsutsumi/Lin-Cereghino—which itself is facially insufficient to support a motivation to combine—at best results in two

genes encoding two heme-associated polypeptides of interest on the same vector, which does not meet the requirements of the claims.

Petitioner's remaining analysis also does not explain why a POSA would have been motivated to combine Tsutsumi and Mattoon in such a way that both the nucleic acid encoding the Mxr1 transcriptional activator and the nucleic acid encoding the heterologous polypeptide would be present on the same vector. Pet., 32. Petitioner cites only to Tsutsumi's use of its modified DAS promoters. *Id.*; see EX1024, [0068]. But Tsutsumi's variant DAS promoters are not used to express Mxr1 (*see id.*, [0076]-[0077], [0137]-[0140]), instead they are used to express the polypeptide of interest (*e.g.*, cutinase). Petitioner provides no explanation as to how this teaching somehow leads to a motivation to include a gene encoding the Mxr1 transcriptional activator on the same vector as that containing the gene encoding a heterologous polypeptide. Given the complexity of the field at the time (*see supra*, section III), Petitioner's conclusory statements that fail to actually address the claim requirements are legally insufficient to demonstrate obviousness. Thus, for this additional reason, Petitioner's Ground 1 fails.

C. Ground 1 with respect to dependent claims 2-17, 21, and 23-25 fails

Ground 1 relies on Tsutsumi, Lin-Cereghino, and Mattoon, as allegedly rendering obvious dependent claims 2-17, 21, and 23-25. The petition challenge to

these dependent claims relies on the petition challenge to claim 1 and fails for the same reasons described above with respect to claim 1.

V. GROUND 2 FAILS

Ground 2 relies on Tsutsumi, Lin-Cereghino, Mattoon, and Crane (EX1010) as allegedly rendering obvious dependent claims 18-20. The petition challenge to these dependent claims relies on the petition challenge to claim 1 and fails for the same reasons described above with respect to claim 1.

Accordingly, for this reason, Petitioner's Ground 2 fails.

VI. GROUND 3 FAILS

Ground 3 relies on Tsutsumi, Lin-Cereghino, Mattoon, and Krainer (EX1031) as allegedly rendering obvious dependent claim 22. The petition challenge to this dependent claim relies on the petition challenge to claim 1 and fails for the same reasons described above with respect to claim 1.

Accordingly, for this reason, Petitioner's Ground 3 fails.

VII. GROUND 4 FAILS

In Ground 4, Petitioner alleges that claims 26 and 28 are obvious based on the combination of Mattoon (EX1008) and Vogl (EX1005). Pet., 1, 67. Petitioner fails to establish a reasonable likelihood of prevailing on this ground.

As explained below, Petitioner's Ground 4 fails against claim 26 for several reasons. First, Petitioner fails to establish that its proposed combination discloses each and every limitation of the claims, specifically, that all three nucleic acids

recited in the claim are included in the same recombinant nucleic acid molecule and that all three are “operably linked to a methanol-inducible promoter element” that includes “at least one sequence to which a Mxr1 transcriptional activator binds.” Second, Petitioner’s proposed combination is premised on a conclusory rationale that selectively picks and chooses disclosure from Vogl to reach the claimed invention. For these reasons, Petitioner’s Ground 4 fails.

A. Petitioner fails to establish that the combination of Mattoon and Vogl discloses each and every limitation of claim 26

1. Petitioner fails to establish that the combination of Mattoon and Vogl discloses a recombinant nucleic acid molecule containing all three claimed nucleic acids

Claim 26 recites a methylotrophic yeast cell comprising “a recombinant nucleic acid molecule” that includes three nucleic acids: (1) “encoding a Mxr1 transcriptional activator sequence from *P. pastoris*”; (2) “encoding a member of globin family PF00042”; and (3) “encoding at least one polypeptide involved in heme biosynthesis.” While Petitioner asserts that “Mattoon discloses all limitations of claim 26 except ‘a Mxr1 transcriptional activator sequence from *P. pastoris*,’” (Pet., 67), Petitioner’s analysis overlooks that the claim requires all three nucleic acids to be present on the same recombinant nucleic acid molecule.

Petitioner admits that Mattoon fails to disclose a nucleic acid that encodes an Mxr1 transcriptional activator sequence from *P. pastoris*, and turns to Vogl as teaching “a Mxr1 transcriptional activator sequence from *P. pastoris*.” Pet., 68.

But Petitioner improperly focuses on only a single element of the limitation—the Mxr1 transcriptional activator sequence—to the exclusion of the rest of the claim limitation—that the sequence must be on the same recombinant nucleic acid molecule as the other two claimed nucleic acids. *See* Pet., 68.

Vogl, as a review article, does not disclose or suggest including a nucleic acid encoding an Mxr1 transcriptional activator on the same recombinant nucleic acid molecule that contains the nucleic acids designed to encode the polypeptides of interest. *See* EX1005, 391. Indeed, the portion of Vogl that Petitioner relies upon describes providing the nucleic acid encoding Mxr1 on a vector that is *separate from* the one having the nucleic acid encoding a single polypeptide of interest. Pet., 68-69; citing EX1005, 391, which cites to EX1007, which describes “coexpression” of Mxr1 with a heterologous protein. Thus, Vogl fails to disclose placing a nucleic acid encoding an Mxr1 transcriptional activator along with the nucleic acids encoding the polypeptides of interest on a single “recombinant nucleic acid molecule.”

Petitioner argues for later limitations that Mattoon teaches “that more than one nucleic acid ‘can be present on one expression vector’” and a POSA “would thus understand that the nucleic acids” of claim 26 “can be present on one recombinant nucleic acid molecule,” relying on its Ground 1 analysis of Tsutsumi. Pet., 70-71. As discussed above, however, Petitioner fails to show *why* a POSA

would incorporate into a single recombinant nucleic acid a nucleic acid encoding a *Mxr1 transcriptional activator* and two additional nucleic acids encoding a member of the globin family and a polypeptide involved in heme biosynthesis (*see* section IV.B.2.), especially in view of Petitioner’s reliance on Vogl, which instead teaches providing the nucleic acid encoding Mxr1 on a *separate* vector than the vector encoding the heterologous protein of interest. EX1005, 391.

Accordingly, Petitioner fails to establish that the combination of Mattoon and Vogl renders obvious claim 26.

2. Petitioner fails to show the combination of Mattoon and Vogl disclose that “each nucleic acid is operably linked to a methanol-inducible promoter element” that “comprises at least one sequence to which a Mxr1 transcriptional activator binds”

Claim 26 requires that *each* of the three nucleic acids are “operably linked to a methanol-inducible promoter element” that “comprises at least one sequence to which a Mxr1 transcriptional activator binds.” Petitioner has failed to establish that the combination of Mattoon and Vogl disclose this limitation.

Petitioner first states, in conclusory fashion, that “Mattoon discloses ‘each nucleic acid is operably linked to a methanol-inducible promoter’ with at least one sequence to which Mxr1 binds, citing only to its expert who parrots the same statement. Pet., 72 (citing EX1003, ¶281). Petitioner then references only general statements in Mattoon related to inducible promoters (*see id.*), but this does not

establish the use of a promoter that is “methanol-inducible” and “comprises at least one sequence to which a Mxr1 transcriptional activator binds,” much less the use of such a promoter operably linked to *each* of the claimed nucleic acids.

Petitioner next argues that “Vogl would have motivated a POSITA to select methanol-inducible promoters that comprise at least one sequence to which a Mxr1 transcriptional activator binds...to control expression of nucleic acids encoding a member of the globin protein family, a polypeptide involved in heme biosynthesis, and Mxr1.” Pet., 72. Petitioner’s reliance on Vogl is premised on taking citations from the reference out of context and ignoring what the reference actually discloses.

Specifically, Petitioner’s reliance on Vogl for teaching the use of a methanol-inducible promoter as claimed incorrectly focuses on Vogl’s discussion of Takagi (EX1007), which describes an example where “a protein-of-interest [is] controlled by a promoter [that is] activated by Mxr1p.” *Id.*, 73 (citing EX1005, 391). Yet, as noted above, the claim requires that *three different nucleic acids* encoding three different proteins are each expressed under a methanol-inducible promoter with the Mxr1 binding sequence. The example of Takagi utilized a methanol-inducible promoter to express the nucleic acid encoding the protein of interest (phytase), while a *different* promoter type, namely, the *constitutive* promoter P_{GAP}, was used to express the nucleic acid encoding the transcriptional

activator. *See* EX1005, 391. Petitioner fails to point to any evidence teaching the use of the **same** regulatory mechanism (methanol-inducible promoters with a Mxr1 binding sequence) to express each of the nucleic acids in a recombinant protein expression system. Indeed, as Petitioner’s own citation demonstrates, the art taught the use of **different** regulatory mechanisms for controlling and expressing different nucleic acids in the system. *Id.*

Petitioner’s reliance on Vogl’s description of the use of methanol-inducible promoters generally is thus insufficient to establish that the reference discloses or teaches that “**each nucleic acid** is operably linked to a methanol-inducible promoter element” in a recombinant nucleic acid molecule as claimed.

B. Petitioner’s combination of Mattoon and Vogl is transparent hindsight

Petitioner’s purported reasoning why a POSA would have been motivated to combine Mattoon and Vogl to arrive at the claimed invention is premised on impermissible hindsight. Petitioner’s analysis starts with Mattoon (Pet., 70-71), which provides an example where two constructs, pCTT1 expressing catalase T from CTT1 and pHEM2 expressing ALA dehydratase from HEM2, are co-expressed. EX1008, 7:51-64. Petitioner then turns to Vogl to further include a nucleic acid sequence encoding “a Mxr1 transcriptional activator sequence from *P. pastoris*” and further operably link Mxr1, CTT1, **and** HEM2 to a “methanol-inducible promoter element. . . compris[ing] at least one sequence to which a Mxr1

transcriptional activator binds.” Pet., 67, 72. As detailed below, Petitioner fails to establish why a POSA would have been motivated to express Mxr1, CTT1, and HEM2 in Mattoon’s system using a methanol-inducible promoter with a reasonable expectation of success.

1. Petitioner’s motivation to combine Mattoon and Vogl is deficient

Furthermore, Petitioner’s arguments that a POSA would have been motivation to incorporate the use of methanol-inducible promoters and the Mxr1 transcriptional activator in Mattoon’s expression system based on Vogl’s teachings rely on misleadingly combining together snippets of Vogl’s text. See Pet., 68-69, 72-73.

Mattoon relates to methods for “enhancing the production of hemoproteins” by (1) “increasing the production of heme by eliminating the rate-limiting step or steps in the heme biosynthetic pathway” (*e.g.*, increase ALA dehydratase encoded by the HEM2 gene), and (2) “reducing the concentration of free heme within the cell by ‘trapping’ such heme using the ability of apoproteins” (*e.g.*, heme-binding agents such as a catalase encoded by CTT1), “thereby reducing the occurrence of heme-feedback inhibition.” EX1008, 3:33-40; 2:9-30; *see also id.*, Abstract.

Mattoon provides an example where yeast cells are transformed simultaneously with a plasmid encoding ALA dehydratase (pHEM2) and a plasmid encoding a catalase (pCTT1). *See id.*, 7:62-65.

Petitioner relies on Vogl to modify Mattoon to incorporate a nucleic acid encoding “a Mxr1 transcriptional activator sequence from *P. pastoris*” and further operably link each of the nucleic acids to a methanol-inducible promoter. Pet., 68. When taken as a whole, Petitioner fails to provide sufficient evidence as to why a POSA would have been motivated to specifically select, among the many options for promoters, a methanol-inducible promoter for each of the three nucleic acids in the proposed combination. *See Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (“[T]he petition has the burden from the onset to show with particularity why the patent it challenges is unpatentable.”).

As Petitioner acknowledges, “Vogl provides a comprehensive review of prior studies using promoters and transcription factors from *P. pastoris* to regulate recombinant protein production.” Pet., 69. While Petitioner provides out-of-context quotes describing the purported benefits to using methanol-inducible promoters (*see* Pet., 72-73), Vogl instead teaches that the selection of promoters is context-specific and many factors go into guiding the choice for a particular promoter element. For example, Vogl notes that “the combination of promoter and protein of interest can have a strong influence on the expression levels” and provides a table of numerous different constitutive or inducible *P. pastoris* promoters that can be utilized to express a protein of interest. EX1005, 396, Table 1. Consistent with Vogl, Hartner 2008 explains that “[t]here is no general optimal

promoter: the regulatory properties of the promoter variant, the target protein and the production process have to be seen as a whole.” EX1017, 13. Further, “production strateg[ies]” are tailored to be “specific to the needs of the individual protein.” *Id.* For example, “stronger variants would be useful for the expression of non-toxic proteins” and “[w]eaker variants could be favourable for the expression of proteins, which cause deleterious effects to the host cell.” *Id.* Thus, while Petitioner selects, with the benefit of hindsight, methanol-inducible promoters in its proposed combination, citing to general statements in Vogl describing such promoters, Petitioner conveniently ignores the art’s acknowledgement that such selection should be made with the protein of interest in mind. *See id.* In this regard, Petitioner provides no analysis as to why a POSA would have been motivated to pluck methanol-inducible promoters from among the listed promoters to express the *specific* proteins in the proposed combination (*i.e.*, Mxr1, CTT1, and HEM2).

Furthermore, Petitioner ignores that regulation via Mxr1 was not well understood at the time. *See* EX1005, 400. For example, with respect to the AOX1 promoter, P_{AOX1}, Vogl describes the identification of activators Mxr1 and Prm1, but makes clear that “there exist also additional, so far uncharacterized regulators of Mxr1p” and “other aspects of P_{AOX1} regulation are only partially understood,” indicating “a highly complex regulation pattern of P_{AOX1} and other MUT genes,

that involves multiple activating and repressing factors.” *Id.* Ignoring this clear teaching that regulation via Mxr1 was not well understood at the time, Petitioner selectively relies on one experiment from Takagi (EX1007) to support an alleged motivation for combining a “Mxr1 transcriptional activator sequence” with the pCTT1 or pHEM2 of Mattoon. Pet., 69. In the described experiment, Takagi co-expressed Mxr1 under the control of the constitutive GAP promoter with a phytase under the control of the methanol-inducible promoter AOX1. EX1005, 391.

Although the co-expression purportedly resulted in a 7.6-fold increased activity of the phytase, Petitioner fails to acknowledge that Vogl found these results inconsistent with an experiment performed in Lin-Cereghino (EX1006). EX1005, 391. Specifically, Vogl referenced Lin-Cereghino for overexpressing Mxr1 under the control of the AOX1 promoter, which “caused a growth defect on methanol and inhibited expression of MUT genes.” *Id.*, 391. Vogl summarized the findings of Takagi and Lin-Cereghino as “***not entirely consistent*** and would require further investigation to ***clarify the activating mechanism of Mxr1p.***” *Id.* (emphasis added). Petitioner fails to recognize and reconcile the inconsistencies between Takagi and Lin-Cereghino referenced by Vogl.

Furthermore, Vogl teaches that the use of methanol is both complex and dangerous. EX1005, 392 (methanol induction “increases the process time and handling efforts” and “the induction with methanol (a toxic and flammable

compound) was considered problematic for safety reasons, especially for large-scale production”). Indeed, Petitioner’s own cited art suggested that “[c]onstitutive expression eases process handling, omits the use of potentially hazardous inducers and provides continuous transcription of the gene of interest.” EX1029, 5304.

Thus, while Petitioner focuses on the supposed “advantages of methanol-inducibility” mentioned in Vogl, Petitioner ignores its own disclosure, including in Vogl, related to the known disadvantages of methanol-inducible promoters. Pet., 73. Petitioner’s conclusory statement that a POSA “would have been motivated to incorporate methanol-inducible promoters...in Mattoon’s *P. pastoris* expression system” in the manner claimed rests on cherry-picked citations to Vogl’s disclosure and fails to address disclosure counseling against the modification.

Accordingly, for this reason, Petitioner’s rationale for combining Mattoon and Vogl fails to satisfy its burden in establishing a reasonable likelihood of success on the merits.

2. Petitioner fails to establish that a POSA would have been motivated to combine Mattoon and Vogl with a reasonable expectation of success

Petitioner argues that a POSA would have reasonably expected success with the proposed modifications because they would have involved “routine techniques.” Pet., 69-70, 73. As noted above, however, protein expression systems are not predictable: “[e]ach protein expression system offers a unique set of

properties, and for successful production of a heterologous protein, it is often required to tailor a process specifically based on the properties of the protein to be produced and the expression system used.” EX1022, 145; *supra*, section III.

For example, overexpression of certain proteins can be toxic to a cell, and thus render that expression system unsuitable for use with that particular protein. See EX1006, 893 (“high levels of Mxr1p are toxic to *P. pastoris*”). However, because Mattoon teaches increased co-expression of HEM2 (to increase heme production) and CTT1 (to trap free heme) (EX1008, 3:33-56), Petitioner’s combination relies on Mxr1 to drive expression of **both** HEM2 and CTT1. Yet, the art taught that high levels of Mxr1 “are toxic to *P. pastoris*,” thus undermining Petitioner’s assertions regarding a POSA’s motivation to use Mxr1 with a reasonable expectation of success. EX1006, 893.

With respect to production of heme-containing proteins, it was known that improving recombinant protein production was not straightforward, and that simply “co-overexpress[ing] genes of the endogenous heme biosynthesis pathway d[oes] not improve the recombinant production of active heme protein.” EX1031, Abstract. Neither Mattoon nor Vogl provide any reason for a POSA, who would have been aware of the uncertain aspects of recombinant protein production, to reasonably expect success in selecting the particular promoter elements picked out by Petitioner from Vogl’s review for the successful production of a globin family

member and a polypeptide involved in heme biosynthesis, absent further affirmative evidence. Instead, a POSA would recognize the “potential pitfalls connected to metabolic pathway engineering, such as the additional metabolic burden upon overexpression of multiple genes as well as an accumulation of free intracellular porphyrin intermediates which can lead to the formation of reactive oxygen species.” *Id.*, 1.

Accordingly, Petitioner’s Ground 4 fails for this additional reason.

C. Ground 4 with respect to dependent claim 28 fails

Ground 4 relies on Mattoon and Vogl as allegedly rendering obvious dependent claim 28. The petition challenge to this dependent claim relies on the petition challenge to claim 26 and fails for the same reasons described above with respect to claim 26.

VIII. GROUND 5 FAILS

Ground 5 relies on Mattoon, Vogl, and Crane (EX1010) as allegedly rendering obvious dependent claim 27. The petition challenge to this dependent claim relies on the petition challenge to claim 26 and fails for the same reasons described above with respect to claim 26.

Accordingly, for this reason, Petitioner’s Ground 5 fails.

IX. INSTITUTION SHOULD BE DENIED UNDER § 325(D)

Separately, the Board should deny institution under § 325(d) because Petitioner relies on substantially the same prior art or arguments previously

considered by the Office, and Petitioner fails to show that the Office materially erred.

A two-part analysis applies to the Board's authority to deny institution under § 325(d). *Advanced Bionics, LLC v. Med-El Elektromedizinische Geräte GMBH*, IPR2019-01469, Paper 6 at 9-10 (precedential). First, the Board considers whether "the same or substantially the same" art or arguments were previously presented to the Office. *Id.*, 8. If either is met, the Board then considers whether the petition demonstrates that the Office "erred in a manner material to the patentability of the challenged claims." *Id.* In applying the two-part framework, the Board considers several nonexclusive factors set forth in *Becton, Dickinson & Co. v. B. Braun Melsungen AG*, IPR2017-01586, Paper 8 at 17-18 (PTAB Dec. 15, 2017) (precedential as to § III.C.5, first paragraph).

The Board should exercise its discretionary powers under § 325(d) for two reasons: 1) the art asserted in the petition is substantially the same as what was asserted by the Examiner during prosecution; and 2) the Examiner pursued the same line of arguments asserted by Petitioner, which Patent Owner overcame through extensive declarations and evidence presented during prosecution.

A. Petitioner Presents Substantially the Same Art and Arguments Previously Considered by the Office

The Examiner rejected the application on several grounds, including under 35 U.S.C. § 102 in view of Zhou *et al.* (PCT Patent Application Publication No.

WO2014/008729) (“Zhou”) and under 35 U.S.C. § 103 in view of Zhou in combination with Varandan *et al.* (PCT Publication No. WO2015/153666), Liu (Metabol. Engineering (2014)), cited by Petitioner as EX1011, and Lin-Cereghino *et al.* (Mol. Cell. Biol. 883-897 (2006)), cited by Petitioner in Grounds 1, 2 and 3 of the Petition as EX1006. EX1040, 1266-1270; Pet., 1. Upon allowance of the application, the Examiner specifically noted in its “Reasons for Allowance” that the “prior art fails to disclose or suggest a methylotrophic yeast cell comprising a methanol expression regulator 1 (Mxr1) transcriptional activator, a methanol inducible promoter element to which the Mxr1 transcriptional activator binds, and a nucleic acid encoding a heterologous polypeptide”. *Id.*, 1488.

While Petitioner admits that EX1006 (Lin-Cereghino) was before the Examiner, Petitioner asserts that “Applicant did not substantively distinguish the claims from Varandan, Lin-Cereghino, or Liu.” Pet, 76. Petitioner’s arguments, however, are substantially similar, if not identical to the Examiner’s use of Lin-Cereghino during prosecution. The Examiner, for example, cited to Lin-Cereghino as “disclos[ing] plasmids comprising the Mxr1 transcriptional activator under the control of the AOX1 promoter or the PEX8 promoter.” *Id.* (citing EX1006, pg. 885, col. 1, final full paragraph). This is the same disclosure that Petitioner relies on, including that “Lin-Cereghino . . . discloses the use of a methanol-inducible promoter, *e.g.*, *PEX8*, to drive expression of Mxr1 in *P. pastoris*.” Pet., 36-37

(citing EX1006, 885 and 893-894 (“[W]e expressed *MXR1* under the control of the promoter from the *P. pastoris PEX8* gene *PPEX8* is methanol regulated”)).

The Examiner additionally stated that Lin-Cereghino provided the full sequences of Mxr1, and furthermore recognized “that *Pichia pastoris* is a major system for the production of recombinant proteins.” *Id.* (citing EX1006, pg. 896, col. 2, final paragraph). However, despite the Examiner’s thorough knowledge of the disclosure of Lin-Cereghino, including that the yeast strain *Pichia pastoris* was a well-known system for producing recombinant and heterologous polypeptides, the Examiner allowed the case, specifically stating that the “prior art fails to disclose or suggest a methylotrophic yeast cell comprising a methanol expression regulator 1 (Mxr1) transcriptional activator, a methanol inducible promoter element to which the Mxr1 transcriptional activator binds, and a nucleic acid encoding a heterologous polypeptide.” EX1040, 1488. Petitioner’s arguments that the Examiner did not substantively apply Lin-Cereghino is disingenuous.

Petitioner’s use of Tsutsumi, moreover, is farther afield from Zhou, which was cited by the Examiner for its disclosure of a methylotrophic yeast comprising an exogenous nucleic acid encoding a transcriptional activator (citing to the transcriptional activator Mit1 in Zhou) “operably linked to a methanol-inducible promoter” including “AOX1 promoter, a DHAS promoter, a MOX promoter, an

AOD1 promoter, a PEX8 promoter from methylotrophic yeasts . . .” *Id.*, 1267.

The Examiner also stated that “Zhou discloses that an exogenous (*i.e.*, heterologous) polypeptide can be expressed in the methylotrophic yeast (paragraph [0066]).” *Id.* Tsutsumi, in contrast, explicitly made the choice to express its transcriptional activator (either Mxr1 or Prm1) under a constitutive promoter or a non-methanol inducible promoter (EX1024, [0076]-[0077]), *i.e.*, **not** “operably linked to a methanol-inducible promoter” as claimed. Petitioner’s combination of Tsutsumi and Lin-Cereghino, thus, are simply a farther removed argument than what was already considered by the Examiner.

Thus, the first part of the *Advanced Bionics* test is satisfied because “the same or substantially the same” art and arguments were previously presented to the Office.

B. Petitioner Fails to Demonstrate that the Office Materially Erred

Petitioner does not allege that the Office materially erred, only that “Applicant did not substantively distinguish the claims from Varandan, Lin-Cereghino, or Liu.” Pet, 76. Yet even with Lin-Cereghino before the Examiner, the Examiner did not apply Lin-Cereghino in a subsequent rejection, instead finding that the “prior art fails to disclose or suggest a methylotrophic yeast cell comprising a methanol expression regulator 1 (Mxr1) transcriptional activator, a

methanol inducible promoter element to which the Mxr1 transcriptional activator binds, and a nucleic acid encoding a heterologous polypeptide.” EX1040, 1488.

Petitioner’s obviousness grounds under Tsutsumi and Lin-Cereghino are just a farther removed re-hash of the same rationale to combine Zhou and Lin-Cereghino as was advanced by the Examiner. For these reasons, this Board should exercise its discretionary powers under § 325(d) and deny institution.

X. CONCLUSION

Accordingly, for at least the reasons set forth above, institution of *inter partes* review should be *denied*.

Respectfully submitted,

Date: May 9, 2023

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182

CERTIFICATE OF COMPLIANCE

Pursuant to §42.24(d), the undersigned certifies that this paper contains no more than 14,000 words, not including the portions of the paper exempted by §42.24(b). According to the word-processing system used to prepare this paper, the paper contains 10,023 words.

Respectfully submitted,

Date: May 9, 2023

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182

CERTIFICATE OF SERVICE

I certify that the foregoing Patent Owner's Preliminary Response was served on this 9th day of May, 2023, on the Petitioner at the following electronic service addresses:

Joseph M. Paunovich
James Glass
Sandra Haberny
Mikayla Wasiri
Jihong Lou
QUINN EMANUEL URQUHART & SULLIVAN, LLP
qeimpossibleipr@quinnemanuel.com

Respectfully submitted,

Date: May 9, 2023

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182