

**IN THE UNITED STATES DISTRICT COURT
FOR DISTRICT OF DELAWARE**

IMPOSSIBLE FOODS INC.,

Plaintiff,

V.

MOTIF FOODWORKS, INC.,

Defendant.

C.A. No. 22-311 (WCB)

DECLARATION OF HAL ALPER, PH.D.

TABLE OF CONTENTS

I.	Introduction and Background	1
II.	Qualifications and Experience	2
III.	Legal Principles	5
IV.	The Yeast Patents.....	6
A.	Relevant Background.....	6
B.	Overview of the '492 Patent	8
C.	Overview of the '656 Patent	9
D.	Shared Specification	11
V.	Analysis of the Disputed Claim Terms.....	13
A.	Terms 18-20: Activator Sequence Terms	13
1.	A POSA Would Understand Terms 18-20 To Refer to Different Molecular Components.....	13
2.	A POSA Would Not Interpret Terms 18-20 To Require “Native” Sequences.....	16
B.	Term 26: Promoter Element”	17
VI.	Conclusion	19

I. INTRODUCTION AND BACKGROUND

1. I, Hal Alper, Ph.D., have been retained as an expert witness by plaintiff Impossible Foods Inc. (“Impossible”) to provide testimony in the above-captioned litigation against defendant Motif FoodWorks, Inc. (“Motif”).

2. I have been asked to provide opinions as to the disputed terms of certain asserted claims of U.S. Patent Nos. 10,273,492 (“’492 patent”) and 10,689,656 (“’656 patent”) (collectively, the “Yeast Patents”) based on the understanding of a person of ordinary skill in the art (“POSA”) as of the May 11, 2015 priority date of the Yeast Patents. Specifically, counsel for Impossible has asked me to offer my opinions on the meaning of the terms “a Mxr1 transcriptional activator sequence from *P. pastoris*” (term 18), “a first exogenous nucleic acid encoding a methanol expression regulator 1 (Mxr1) transcriptional activator” (term 19), “a nucleic acid encoding a Mxr1 transcriptional activator sequence from *P. pastoris*” (term 20) (collectively, the “transcriptional activator” terms or terms 18-20), and “promoter element” (term 26). Throughout this declaration I refer to these terms based on the numbering and proposed constructions in Section II of the parties’ Joint Claim Construction Statement (“JCCS”) (D.I. 94-1). I have reproduced and adapted excerpts of the chart for ease of reference in my discussion below.

3. I have formed my opinions using my education, knowledge, and experience together with my review of the materials cited in this declaration. I have reviewed the Yeast Patents and their prosecution histories. I also have considered the Joint Claim Construction Statement and the parties’ proposed meanings for terms 18-20 and 26. I have based my opinions in this declaration on the information and evidence currently available to me.

4. Accordingly, I reserve the right to change, amend, and supplement my opinions in response to any new information or evidence made available to me; additional scientific analysis

that leads me to conclude that supplementation is necessary; new issues that may arise; and any additional discovery, arguments, evidence, or testimony presented in this case, including any evidence or testimony presented prior to or during trial.

5. I reserve the right to testify with regard to my qualifications, experience, and the materials I have reviewed. I also reserve the right to respond, as appropriate, to documents, information, or declarations that Motif produces. In addition to the materials I cite in this declaration, I may consider additional documents and information in forming any rebuttal opinions.

6. I have not provided either trial or deposition testimony in any cases in the past four years.

7. I have no financial interest in the outcome of this case. I am being compensated at my standard consulting rate, which is \$950 per hour. My compensation is not related in any way to the outcome of this lawsuit.

II. QUALIFICATIONS AND EXPERIENCE

8. The following is a brief summary of my qualifications and experience, the full extent of which is in my curriculum vitae, attached to this declaration as Exhibit A.

9. I am currently the Kenneth A. Kobe Endowed Professor of Chemical Engineering at The University of Texas at Austin in the Department of Chemical Engineering. I also serve as the Executive Director of the Center for Biomedical Research Support.

10. I received a B.Sc. in Chemical Engineering from the University of Maryland, College Park in 2002. I received my Ph.D. in Chemical Engineering from the Massachusetts Institute of Technology in 2006. The topic of my dissertation and the focus of my research was the “Development of Systematic and Combinatorial Approaches for the Metabolic Engineering of Microorganisms.” After earning my Ph.D., I was a Postdoctoral Research Associate from

2006-2008 at the Whitehead Institute for Biomedical Research, where my research specifically focused on yeast genetics, including engineering yeast systems for biofuel production. From 2007-2008, I also held a postdoctoral position in mammalian cell engineering at Shire Human Genetic Therapies, Inc., in Cambridge, MA.

11. In 2008, I joined the Department of Chemical Engineering at the University of Texas at Austin. I was an Assistant Professor from 2008-2014, an Associate Professor from 2014-2017, and then became a full Professor in 2017.

12. While a professor at University of Texas at Austin, I have held various other leadership positions. From 2013-2018, I was a Graduate Advisor in the Department of Chemical Engineering, and from 2018-2020, I was Associate Chair for the program. In addition to these responsibilities, I have been a Member of the Institute for Cell and Molecular Biology since 2008 and a member of the Graduate Studies Committees in the Departments of Cellular and Molecular Biology, Chemistry and Biochemistry (Biochemistry Division), and Microbiology, since 2008, 2010, and 2014, respectively.

13. I have extensive teaching experience and have taught undergraduate courses on engineering biology, computing, chemical reactor analysis and design, process stoichiometry and energy balances, and biochemical, cellular, and metabolic engineering. I have published extensively on topics that include synthetic promoter design and bioproduction of proteins in engineered microbial organisms in such journals as *Science*, *Nature Communications*, *ACS Synthetic Biology*, *Biotechnology Advances*, *Bioresource Technology*, *Microbial Cell Factories*, *PNAS*, *FEMS Yeast Research*, *Metabolic Engineering*, *Molecular Systems Design & Engineering*, and *Fungal Genetics and Biology*. Collectively, I have published over 150 peer-reviewed papers and 10 book chapters on the topic of microbial and fungal biotechnology.

14. I have received numerous awards and honors, including a Wiley Biosciences Award, a *Biochemical Engineering Journal* Young Investigator Award, and a Division 15 Service Award, recognizing dedication and service to the profession of food engineering, pharmaceutical engineering, and bioengineering, from the American Institute of Chemical Engineers. I also was inducted as a Fellow of the National Academy of Inventors in 2019 and elected to the College of Fellows for The American Institute for Medical and Biological Engineering in 2018, a distinction only given to the top 1% of researchers in the field.

15. I am an editor for multiple journals, including *Biotechnology Advances*, *Metabolic Engineering*, *Synthetic Biology*, and *Discover Chemical Engineering*, among others. As part of my work for these journals, as well as for other journals that I have been involved with, I critically review and analyze papers and have reviewed over 100 papers submitted for publication and edited multiple published articles.

16. I am a named inventor on several patents issued by the United States Patent & Trademark Office, including U.S. Patent Nos. 8,110,672 (“Promoter engineering and genetic control”), 9,273,307 (“Global transcription machinery engineering”), and 9,951,344 (“Synthetic terminators for controlling fungal gene expression”), among others.

17. In addition to my educational training, professional experiences, and research activities described above, I keep abreast of new developments relating to my fields of expertise by reading scientific literature, conferring with colleagues in the field, and attending and presenting at scientific conferences and invited lectures. I have given invited talks at multiple national and international conferences in both academic and industry spheres, including the American Institute of Chemical Engineers Annual Meeting, the International Metabolic

Engineering Conference held every two years, and the International Conference on Biomolecular Engineering, as well as many others.

18. Based on my qualifications and experience, I am an expert in genetic engineering, synthetic promoter design, yeast engineering, and synthetic biotechnology and have been since well before the relevant timeframe for the Yeast Patents.

III. LEGAL PRINCIPLES

19. Counsel for Impossible has asked me to offer my opinions on the meaning of the disputed terms of the Yeast Patents. I have no formal legal training. In order to form my opinions on these questions, counsel explained to me the legal standards and general guidelines relevant to determining the meaning of patent claims. Below, I set forth my understanding of these standards, which provide the framework for my analysis.

20. I understand from counsel that patent claims are interpreted from the perspective of a POSA, in the context of the patent as a whole, as of the critical date. I further understand that a POSA is presumed to have knowledge of all pertinent prior art.

21. Counsel has informed me that the critical date for the Yeast Patents is May 11, 2015. I have formed my opinions based on the knowledge and understanding of a POSA as of the May 11, 2015.

22. In forming my opinions, I have considered the qualifications of a POSA based on my review of the Yeast Patents and their file histories.

23. In my view, a POSA would have a graduate degree in chemical engineering, biochemistry, microbiology, or a similar discipline, along with 2 years of experience with recombinant protein expression systems.

24. I understand that the claim terms of the Yeast Patents are to receive their plain and ordinary meaning, *i.e.*, the meaning that the terms would have had to a POSA as of the

critical date. Counsel has advised me that this analysis focuses on the patent claims, the specification, and the prosecution history (referred to as “intrinsic evidence”). I also understand that dictionaries or other sources can be helpful in determining the plain and ordinary meaning of a term but cannot override a meaning that is clear from the intrinsic evidence.

25. Counsel has informed me that each claim of a patent defines a separate invention with a distinct scope. I understand that a claim may be considered indefinite if, in the context of the patent specification and prosecution history, it fails to inform a POSA about the scope of the claimed invention with reasonable certainty.

26. I have followed the above principles in my analysis throughout this declaration.

IV. THE YEAST PATENTS

A. Relevant Background

27. Nucleic acid, including deoxyribonucleic acid (“DNA”), encodes protein molecules necessary for the function of living organisms. DNA contains genes, each with a specific nucleic acid code that corresponds to a protein. Cellular machinery first transcribes the DNA “code” into messenger ribonucleic acid (“mRNA”) and then translates the mRNA into an amino acid sequence, *i.e.*, a peptide or protein.

28. Through advances in genetic engineering, it is possible to introduce the genetic sequence for a protein of interest into a different cell type or organism (a “host”) that may lack its own such sequence. This process is known as genetic recombination. The transcription and translation of such genetic material to produce proteins is known as recombinant protein expression. Recombinant protein expression requires the insertion of exogenous nucleic acid (containing the gene(s) of interest) into host cells, which may be from any one of a variety of organisms, including bacteria (*e.g.*, *Escherichia coli*) and yeast (*e.g.*, *Saccharomyces cerevisiae*, *Pichia pastoris*).

29. In yeast, transcription factor proteins regulate gene expression, meaning that they control the timing or degree of transcription by interacting with DNA at certain target sequences residing upstream, *i.e.*, attached in front, of the gene of interest. This upstream region is known as the promoter. Transcriptional activators, including Mxr1, promote transcription, while transcriptional repressors do the opposite, as their names would suggest. *See* Ex. B,¹ at IF_0013553 (describing “DNA elements required for initiating gene transcription,” including “elements that play positive roles in transcription” by “providing the binding sites for transcriptional activators that increase the levels of gene transcription”). For example, the transcriptional activator Mxr1 interacts with the alcohol oxidase I (“AOX1”) promoter (“P_{AOX1}”) or promoter element through binding sites known as “motifs” or “consensus sequences” and enhances the rate of transcription of downstream genes. *See generally* Ex.C²; *see also* Ex. D.³ It is important to emphasize two components of this interaction: activation requires (1) an Mxr1 binding target, which consists of a consensus sequence or motif, and (2) Mxr1 protein, which itself is encoded by a nucleic acid sequence.

30. The Yeast Patents relate to the field of recombinant protein expression and the use of transcriptional activators and promoter elements, including Mxr1 and P_{AOX1}, to enhance the production of certain proteins of interest, including heme-containing proteins and heme biosynthesis proteins.

¹ J. Ma, “Transcriptional activators and activation mechanisms,” *Protein Cell*, 2(11):879–88 (2011) (IF_013553-562).

² Zhan, et al., “Transcription factor Mxr1 promotes the expression of Aox1 by repressing glycerol transporter 1 in *Pichia pastoris*,” *FEMS Yeast Res.*, 17(4):1–10 (2017) (IF_0013972-981) (“Zhan”).

³ U.S. Patent No. 8,114,974, issued Feb. 14, 2012 (IF_0013569-971).

B. Overview of the '492 Patent

31. I understand that Impossible and Motif disagree about the meaning of certain terms in the claims of the '492 patent.

32. The '492 patent is entitled "Expression Constructs and Methods of Genetically Engineering Methylophilic Yeast" and generally relates to *Pichia* yeast cells capable of recombinant protein expression. '492 patent, Abstract.

33. Specifically, the '492 patent claims and describes a methylophilic *Pichia* yeast cell comprising: 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*; and 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*.

34. Dependent claim 4 of the '492 patent is directed to the yeast cell of claim 1, wherein the heme biosynthesis polypeptide is selected from: ALA synthase, ALA dehydratase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, PPG oxidase, and/or ferrochelatase.

35. Dependent claim 5 of the '492 patent is directed to the yeast cell of claim 1, wherein the promoter element from *P. pastoris* is a constitutive or methanol-inducible promoter element.

36. Dependent claim 7 of the '492 patent is directed to the yeast cell of claim 5, wherein the methanol-inducible promoter element from *P. pastoris* is an alcohol oxidase I (AOX1) promoter element.

37. Dependent claim 14 of the '492 patent is directed to the yeast cell of claim 1, 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*.

C. Overview of the '656 Patent

38. I understand Impossible and Motif disagree about the meaning of certain terms in the claims of the '656 patent.

39. The '656 patent is entitled "Expression Constructs and Methods of Genetically Engineering Methylophilic Yeast" and, like the '492 patent, generally relates to *Pichia* yeast cells capable of recombinant protein expression. '656 patent, Abstract. The '656 patent further relates to protein expression methods using the yeast cell constructs of the claims.

40. Specifically, the '656 patent claims and describes an invention comprising a methylophilic yeast cell comprising a recombinant nucleic acid molecule, wherein the recombinant nucleic acid molecule comprises a first exogenous nucleic acid encoding a methanol expression regulator 1 (Mxr1) transcriptional activator operably linked to at least one methanol-inducible promoter element comprising a sequence to which the Mxr1 transcriptional activator binds, wherein the recombinant nucleic acid molecule comprises a second exogenous nucleic acid encoding a heterologous polypeptide operably linked to at least one methanol-inducible promoter element. The '656 patent also claims and describes a method for expressing a heterologous polypeptide in a cell by providing the methylophilic yeast cell described above and culturing the cell under conditions for expression of the recombinant nucleic acid molecule, thereby expressing the heterologous polypeptide.

41. The '656 patent has two independent claims, claims 1 and 26, which I describe below.

42. Claim 1 recites "[a] methylophilic yeast cell comprising a recombinant nucleic acid molecule, wherein the recombinant nucleic acid molecule comprises a first exogenous

nucleic acid encoding a methanol expression regulator 1 (Mxr1) transcriptional activator operably linked to at least one methanol-inducible promoter element comprising a sequence to which the Mxr1 transcriptional activator binds, wherein the recombinant nucleic acid molecule comprises a second exogenous nucleic acid encoding a heterologous polypeptide operably linked to at least one methanol-inducible promoter element.”

43. Claim 26 recites “[a] methylotrophic *Pichia* yeast cell comprising a recombinant nucleic acid molecule, wherein the recombinant nucleic acid molecule comprises: a nucleic acid encoding a Mxr1 transcriptional activator sequence from *P. pastoris*; a nucleic acid encoding a member of globin family PF00042; and a nucleic acid encoding at least one polypeptide involved in heme biosynthesis, wherein each nucleic acid is operably linked to a methanol-inducible promoter element, wherein the methanol-inducible promoter element comprises at least one sequence to which a Mxr1 transcriptional activator binds.”

44. Dependent claim 10 of the '656 patent is directed to the yeast cell of claim 1, wherein the methanol-inducible promoter element(s) is selected from: an alcohol oxidase 1 (AOX1) promoter element from *Pichia pastoris*, an alcohol oxidase (AOD1) promoter element from *Candida boidinii*, a methanol oxidase (MOX) promoter element from *Hansenula polymorpha*, a methanolic alcohol oxidase (MOD1) promoter element from *Pichia methanolica*, a dihydroxyacetone synthase (DHAS) promoter element from *Pichia pastoris*, a formaldehyde dehydrogenase (FLD1) promoter element from *Pichia pastoris*, and a peroxin 8 (PEX8) promoter element from *Pichia pastoris*.

45. Dependent claim 11 of the '656 patent is directed to the yeast cell of claim 1, wherein the methanol-inducible promoter element(s) is an alcohol oxidase 1 (AOX1) promoter element from *Pichia pastoris*.

46. Dependent claim 28 of the '656 patent is directed to the yeast cell of claim 26, wherein the methanol-inducible promoter element is an alcohol oxidase 1 (AOX1) promoter element from *P. pastoris*.

D. Shared Specification

47. I am informed by counsel that the Yeast Patents have a common specification. For simplicity, I will reference the '656 patent in the discussion that follows.

48. The Yeast Patents describe a recombinant expression system using yeast cells, including *Pichia pastoris*, "other species of the *Pichia* genus," and "species from any of the *Candida*, *Hansenula*, . . . and *Torulopsis* genera." '656 patent, 3:60-63. The patent provides yeast cells that comprise "nucleic acid constructs . . . that allow for genetically engineering a cell to increase the recombinant expression of a polypeptide from an inducible promoter." *Id.* at 3:46-50. For example, a methylotrophic *P. pastoris* expression system can utilize constructs where a promoter or promoter element is operably linked to protein-encoding nucleic acid "in such a way as to direct or regulate expression." *Id.* at 4:50-51. The Yeast Patents discuss many types of promoters and promoter elements, including the methanol-inducible AOX1 promoter ("P_{AOX1}"), which "is strongly transcribed in response to methanol," as well as the transcriptional elongation factor EF-1a (TEF1) promoter, "which is strongly transcribed in a constitutive manner," and the glyceraldehyde-3-phosphate dehydrogenase ("GAP") promoter. *Id.* at 4:56-60, 5:58-60, 6:37-44; *see also id.* at cl. 28. The shared specification further states that "the methanol-regulated transcriptional activators in *Pichia* can bind to the AOX1 promoter and act cooperatively with Mxrl to activate transcription from the AOX1 promoter." *Id.* at 5:18-21.

49. The Yeast Patents state that "a recombinant nucleic acid molecule typically includes an exogenous nucleic acid that encodes a transcriptional activator . . . [.]" '656 patent, 3:66-4:2. The Yeast Patents describe such "[t]ranscriptional activators, and nucleic acids

encoding transcriptional activators (e.g., exogenous nucleic acids encoding transcriptional activators),” including Mxr1 in *Pichia pastoris* as well as “suitable transcriptional activators” in multiple other genera and species of yeast. *Id.* at 4:23-43. The shared specification clarifies the nucleic acid and polypeptide sequences of “transcriptional activators” through references to GenBank Accession Nos. as follows:

Transcriptional activators, and nucleic acids encoding transcriptional activators (e.g., exogenous nucleic acids encoding transcriptional activators), are known in the art. For example, a transcriptional activator from *Pichia pastoris* is the Mxr1 sequence, but suitable transcriptional activators also can be found in *Hansenula polymorpha* (the Adrl sequence; see, for example, GenBank Accession No. AE0102000005, bases 858873 to 862352, for the nucleic acid sequence and GenBank Accession No. ESX01253 for the amino acid sequence) . . . [.] A representative *P. pastoris* Mxr1 nucleic acid sequence can be found, for example, in GenBank Accession No. DQ395124, while a representative *P. pastoris* Mxr1 polypeptide sequence can be found, for example, in GenBank Accession No. ABD57365.

Id.

50. Importantly, the Yeast Patents indicate that “‘exogenous’ refers to any nucleic acid sequence that is introduced into the genome of a cell from an external source, where the external source can be the same or a different organism or a nucleic acid generated synthetically.” ’656 patent, 4:6-9. The specification also states:

[A]n exogenous nucleic acid can be a nucleic acid from one microorganism (e.g., one genus or species of methylotrophic yeast) that is introduced into a different genus or species of methylotrophic yeast, however, an exogenous nucleic acid also can be a nucleic acid from a methylotrophic yeast that is introduced recombinantly into a methylotrophic yeast as an additional copy despite the presence of a corresponding native nucleic acid sequence.

Id. at 4:9-17. The Yeast Patents also refer to heterologous nucleic acid as follows:

Similar to the **exogenous nucleic acid** encoding the transcriptional activator, a heterologous nucleic acid refers to any nucleic acid sequence that is **not native** to the genome or in the genome of an organism (e.g., a heterologous nucleic acid can be a nucleic acid from one microorganism (e.g., one genus or species of methylotrophic yeast) that is introduced into a different genus or species of methylotrophic yeast).

Id. at 5:31-37.

51. The Yeast Patents teach the use of nucleic acid constructs for co-expression of heme biosynthesis proteins and heme-containing proteins, including leghemoglobin (“LegH”). ’656 patent, cls. 19, 27. The “polypeptide[s] involved in the biosynthesis of heme” include ALA synthase, ALA dehydratase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, PPG oxidase, and/or ferrochelatase. *Id.* at cl. 14.

V. ANALYSIS OF THE DISPUTED CLAIM TERMS

A. Terms 18-20: Activator Sequence Terms

1. A POSA Would Understand Terms 18-20 To Refer to Different Molecular Components

Term	Claim Term	Impossible’s Proposed Construction	Motif’s Proposed Construction
18.	“a Mxr1 transcriptional activator sequence from <i>P. pastoris</i> ” (’492 patent, cl. 1, 14)	Plain and ordinary meaning.	“the sequence of the native <i>P. pastoris</i> Mxr1 transcriptional activator”
19.	“a first exogenous nucleic acid encoding a methanol expression regulator 1 (Mxr1) transcriptional activator” (’656 patent, cl. 1)	Plain and ordinary meaning.	“a first exogenous nucleic acid sequence of a native Mxr1 transcriptional activator”
20.	“a nucleic acid encoding a Mxr1 transcriptional activator sequence from <i>P. pastoris</i> ” (’656 patent, cl. 26)	Plain and ordinary meaning.	“a nucleic acid encoding the sequence of the native <i>P. pastoris</i> Mxr1 transcriptional activator”

52. I understand that Motif has proposed a similar meaning for each of disputed terms 18-20, as indicated in the above chart. All three of Motif’s proposed constructions refer to the sequence of the Mxr1 transcriptional activator, *i.e.*, the sequence encoding the protein Mxr1. However, term 18 does not refer to the sequence of Mxr1 but rather the sequence that Mxr1

targets to promote transcription. As I discuss in greater detail below, a POSA would not understand term 18 to refer to the same component as terms 19 and 20.

53. In my opinion, the plain and ordinary meaning of “a Mxr1 transcriptional activator sequence from *P. pastoris*” (term 18) is a nucleic acid sequence to which Mxr1 binds, *e.g.*, a motif or consensus sequence.

54. A POSA would understand, in the context of the specification and claim language, that “a Mxr1 transcriptional activator sequence from *P. pastoris*” (term 18) does not refer to the sequence encoding Mxr1, in contrast to Motif’s proposed construction. Rather, the Yeast Patents use the phrase “a nucleic acid molecule **encoding** a Mxr1 transcriptional activator” when defining the sequence of Mxr1. *E.g.*, ’492 patent, cl. 14 (emphasis added).

55. I also note that dependent claim 14 of the ’492 patent is directed to the yeast cell of claim 1 and further requires **both** “a nucleic acid molecule encoding a Mxr1 transcriptional activator” and “a Mxr1 **transcriptional activator sequence** from *P. pastoris*.” (emphasis added). In my view, Motif’s proposed meaning would duplicate the phrase “a nucleic acid molecule encoding a Mxr1 transcriptional activator” while erasing the requirement for an Mxr1 target sequence. For convenience I include the following illustration:

'992 Patent Claims 1 and 14	Plain and Ordinary meaning	Motif's Construction
A methylotrophic <i>Pichia</i> yeast cell comprising: [a] 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 [b] 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>.	Mxr1 protein binds here Mxr1 protein binds here	Sequence encoding native <i>P. pastoris</i> Mxr1 Sequence encoding native <i>P. pastoris</i> Mxr1
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>.	Sequence encoding Mxr1 Mxr1 protein binds here	Sequence encoding Mxr1 Sequence encoding native <i>P. pastoris</i> Mxr1

Motif's proposed construction would render two limitations in claim 14 duplicative.

56. Accordingly, I disagree that a POSA would understand “a Mxr1 transcriptional activator sequence from *P. pastoris*” (term 18) to mean “the sequence of the native *P. pastoris* Mxr1 transcriptional activator.” Rather, term 18 plainly refers to the nucleic acid sequence that Mxr1 recognizes and binds as part of its function as a transcriptional activator. I further note that this interaction does not require any full-length sequences but depends simply on the transcriptional activator binding to a consensus sequence for Mxr1. *See generally* Ex. C.

2. A POSA Would Not Interpret Terms 18-20 To Require “Native” Sequences

57. In my opinion, a POSA would understand that “a Mxr1 transcriptional activator sequence from *P. pastoris*” (term 18), “a first exogenous nucleic acid encoding a methanol expression regulator 1 (Mxr1) transcriptional activator” (term 19), and “a nucleic acid encoding a Mxr1 transcriptional activator sequence from *P. pastoris*” (term 20) need not be native sequences.

58. Claim 1 of the '656 patent does not contain the genus and species restrictions of claim 26 and, with respect to the host cell requires only “[a] methylotrophic yeast cell.” The plain language does not include the term “native.” The claim refers to exogenous nucleic acid sequences, which may or may not be native to the host cell. As the specification indicates, “‘exogenous’ refers to any nucleic acid sequence that is introduced into the genome of a cell from an external source, where the external source can be the same or a different organism or a nucleic acid generated synthetically.” '656 patent, 4:6-9.

59. Even claim 26 of the '656 patent, which includes genus and species limitations, does not require a “native” transcriptional activator sequence. As I described above, claim 26 of the '656 patent recites a methylotrophic yeast cell of the *Pichia* genus where the nucleic acid sequence encoding the Mxr1 transcriptional activator is from *Pichia pastoris*. The Yeast Patents indicate that the yeast cell may belong to any one of several genera and species. *See, e.g.*, '656 patent, 3:59-63 (“[O]ther species of the *Pichia* genus can be used or species from any of the *Candida*, *Hansenula*, *Pichia* and *Torulopsis* genera.”). In other words, the nucleic acid sequence encoding the Mxr1 transcriptional activator could be from *Pichia pastoris*, while the yeast cell host could belong to a different *Pichia* species.

60. For the same reasons, claims 1 and 14 of the '492 patent also do not require a "native" transcriptional activator sequence. Though the transcriptional activator sequence is from *P. pastoris*, the claims recite a methylotrophic *Pichia* yeast cell and **not** a methylotrophic *Pichia pastoris* yeast cell. Adding the term "native" introduces restrictions that go beyond the plain language of the claims.

61. My understanding is consistent with that of the Yeast Patents themselves, which state:

Similar to the **exogenous nucleic acid** encoding the transcriptional activator, a heterologous nucleic acid refers to any nucleic acid sequence that is **not native** to the genome or in the genome of an organism (e.g., a heterologous nucleic acid can be a nucleic acid from one microorganism (e.g., one genus or species of methylotrophic yeast) that is introduced into a different genus or species of methylotrophic yeast).

'656 patent, 5:31-37 (emphasis added).

62. Accordingly, I disagree that a POSA would understand "a Mxr1 transcriptional activator sequence from *P. pastoris*" (term 18) to mean "the sequence of the native *P. pastoris* Mxr1 transcriptional activator." For the same reasons, I disagree that a POSA would understand terms 19 and 20 to mean, respectively, "a first exogenous nucleic acid sequence of a native Mxr1 transcriptional activator" and "a nucleic acid encoding the sequence of the native *P. pastoris* Mxr1 transcriptional activator."

B. Term 26: Promoter Element"

Term	Claim Term	Impossible's Proposed Construction	Motif's Proposed Construction
26.	"promoter element" ('492 patent, cls. 1, 5, 7, 14) ('656 patent, cls. 1, 10, 11, 26, 28)	Plain and ordinary meaning.	Indefinite.

63. It is my opinion that a POSA would understand the meaning of "promoter element" based on the specification, file history, and claim language of the Yeast Patents.

64. I understand that Motif has asserted that a POSA “would not be reasonably certain of the meaning of the term ‘promoter element’” and that “[i]t is not apparent . . . whether the claimed ‘promoter element contains an entire endogenous promoter or merely some portion thereof.” Ex. 4⁴ at 87–88. I disagree. As a preliminary matter, I note that a POSA would not characterize the “promoter element” of the Yeast Patent claims as “endogenous” but would understand that the promoter element to be a component—either endogenous or exogenous—of a “DNA construct[] to genetically engineer methylotrophic yeast.” ’656 patent, 1:25-27. That is, the “promoter element” is a nucleic acid sequence present in genetically engineered yeast cells and capable of “efficiently express[ing] one or more polypeptides.” *Id.* at 1:32-34. This is consistent with the language of the claims, where the words “at least one methanol-inducible” precede “promoter element,” indicating one mechanism by which a promoter can regulate expression. *See, e.g.*, ’656 patent, cls. 1, 10, 11, 26, 28. I note that this also is consistent with the prosecution history of the ’656 patent. In the Notice of Allowance for the claims of the ’656 patent, which issued from Application No. 15/573300, the Examiner stated that “[t]he prior art fails to disclose or suggest a methylotrophic yeast cell comprising a methanol expression regulator 1 (Mxrl) transcriptional activator, **a methanol-inducible promoter element** to which the Mxrl transcriptional activator binds, and a nucleic acid encoding a heterologous polypeptide.” Ex. E at IF_0007678 (emphasis added).

65. A POSA would understand that a “promoter element” could be a complete promoter or could be a portion of a promoter, *i.e.*, an element, so long as the “promoter or other expression element(s) [is] 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).” ’656 patent, 4:47-50

⁴ Attached to Plaintiff Impossible Foods’ Opening Claim Construction Brief.

(emphasis added); *see also id.* at 6:57-61 (“It would be understood by the skilled artisan that a combination of inducible (e.g., methanol-inducible) and constitutive promoters (or promoter elements therefrom) can be combined to further increase the expression of any of the nucleic acids operably linked thereto.”). The specification of the Yeast Patents states that “[e]xpression elements . . . can include introns, enhancer sequences, response elements, or inducible elements that **modulate expression** of a nucleic acid.” *Id.* at 10:3-5 (emphasis added); *see also id.* at 9:66-10:1 (“Expression elements include nucleic acid sequences that **direct and regulate expression** of nucleic acid coding sequences.”) (emphasis added).

66. The specification provides a series of examples with citations to the GenBank sequences of both methanol-inducible and constitutive promoter elements. *See* ’656 patent, 4:60-5:11, 6:37-51; *see also, e.g., id.* at 6:36-41 (“Constitutive promoters and constitutive promoter elements are known in the art. For example, a commonly used constitutive promoter from *P. pastoris* is the promoter, or a portion thereof, from the transcriptional elongation factor EF-1 α gene (TEF1), which is strongly transcribed in a constitutive manner.”). For these reasons, I also disagree with Motif’s contention that “[t]he patents’ specification . . . does not provide any definition or description of a ‘promoter element’ or an ‘element’ of a promoter.” Ex. 4 at 87–88.

67. Based on the context of the specification and the purpose of the claimed invention, a POSA would understand the term “promoter element” to refer to a nucleic acid sequence that regulates transcription of downstream genes.

68. Accordingly, I disagree with the Motif’s assertion that the term “promoter element” is indefinite.

VI. CONCLUSION

69. For the reasons stated above, it is my opinion that each of the disputed terms of the Yeast Patents should be accorded its plain and ordinary meaning.

Dated: June 14, 2023

A handwritten signature in dark ink, appearing to read 'H Alper', is positioned above a horizontal line.

/s/ Hal Alper, Ph.D.